UNIVERSITY OF IOANNINA

SCHOOL OF PHYSICAL SCIENCES

DEPARTMENT OF CHEMISTRY

DEVELOPMENT OF ANALYTICAL METHODS BASED ON SAMPLE PREPARATION AND LIQUID CHROMATOGRAPHY-HIGH RESOLUTION MASS SPECTROMETRY FOR THE DETERMINATION OF EMERGING CONTAMINANTS

Maria C. Kalampoka

CHEMIST, MSc

DOCTORAL THESIS

Ioannina 2020

«This research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Strengthening Human Resources IKY)»

Ευρωπαϊκή Ένωση
European Social Fund

Operational Programme
Human Resources Development,
Education and Lifelong Learning
Co-financed by Greece and the European Union

ΕΣΠΑ 2014-2020
ανάπτυξη - εργασία - αλληλεγγύη

UNIVERSITY OF IOANNINA

SCHOOL OF PHYSICAL SCIENCES

DEPARTMENT OF CHEMISTRY

DEVELOPMENT OF ANALYTICAL METHODS BASED ON SAMPLE PREPARATION AND LIQUID CHROMATOGRAPHY-HIGH RESOLUTION MASS SPECTROMETRY FOR THE DETERMINATION OF EMERGING CONTAMINANTS

Maria C. Kalampoka

CHEMIST, MSc

DOCTORAL THESIS

Ioannina 2020

«This research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Strengthening Human Resources IKY)»

Ευρωπαϊκή Ένωση
European Social Fund

Operational Programme
Human Resources Development,
Education and Lifelong Learning
Co-financed by Greece and the European Union

ΕΣΠΑ 2014-2020
ανάπτυξη - εργασία - αλληλεγγύη

ΠΑΝΕΠΙΣΤΗΜΙΟ ΙΩΑΝΝΙΝΩΝ

ΣΧΟΛΗ ΘΕΤΙΚΩΝ ΕΠΙΣΤΗΜΩΝ

ΤΜΗΜΑ ΧΗΜΕΙΑΣ

ΑΝΑΠΤΥΞΗ ΑΝΑΛΥΤΙΚΩΝ ΜΕΘΟΔΩΝ ΠΡΟΣΔΙΟΡΙΣΜΟΥ ΑΝΑΔΥΟΜΕΝΩΝ ΡΥΠΩΝ ΜΕ ΤΗ ΧΡΗΣΗ ΥΓΡΗΣ ΧΡΩΜΑΤΟΓΡΑΦΙΑΣ ΣΥΖΕΥΓΜΕΝΗΣ ΜΕ ΦΑΣΜΑΤΟΜΕΤΡΙΑ ΜΑΖΑΣ ΥΨΗΛΗΣ ΔΙΑΚΡΙΤΙΚΗΣ ΙΚΑΝΟΤΗΤΑΣ

Μαρία Χ. Καλαμπόκα

ΧΗΜΙΚΟΣ, MSc

ΔΙΔΑΚΤΟΡΙΚΗ ΔΙΑΤΡΙΒΗ

Ιωάννινα 2020

«Το έργο συγχρηματοδοτείται από την Ελλάδα και την Ευρωπαϊκή Ένωση (Ευρωπαϊκό Κοινωνικό Ταμείο) μέσω του Επιχειρησιακού Προγράμματος «Ανάπτυξη Ανθρώπινου Δυναμικού, Εκπαίδευση και Διά Βίου Μάθηση», στο πλαίσιο της Πράξης «Ενίσχυση του ανθρώπινου ερευνητικού δυναμικού μέσω της υλοποίησης διδακτορικής έρευνας» (MIS-5000432), που υλοποιεί το Ίδρυμα Κρατικών Υποτροφιών (ΙΚΥ)»

Ευρωπαϊκή Ένωση
Ευρωπαϊκό Κοινωνικό Ταμείο

Επιχειρησιακό Πρόγραμμα
Ανάπτυξη Ανθρώπινου Δυναμικού,
Εκπαίδευση και Διά Βίου Μάθηση
Με τη συγχρηματοδότηση της Ελλάδας και της Ευρωπαϊκής Ένωσης

ΕΣΠΑ 2014-2020
ανάπτυξη - εργασία - αλληλεγγύη

«Η έγκριση της διδακτορικής διατριβής από το Τμήμα Χημείας της Σχολής Θετικών Επιστημών, του Πανεπιστημίου Ιωαννίνων δεν υποδηλώνει αποδοχή των γνωμών του συγγραφέα Ν. 5343/32, άρθρο 202, παράγραφος 2»

Ορισμός Τριμελούς Συμβουλευτικής Επιτροπής από τη Συνέλευση: **930^{A}/17-06-2016**

Μέλη Τριμελούς Συμβουλευτικής Επιτροπής:

Επιβλέπων: Βασίλειος Σακκάς, Αναπληρωτής Καθηγητής, Τμήμα Χημείας, Πανεπιστήμιο Ιωαννίνων

Μέλη:

Τριαντάφυλλος Αλμπάνης, Καθηγητής, Τμήμα Χημείας, Πανεπιστήμιο Ιωαννίνων

Κωνσταντίνος Σταλίκας, Καθηγητής, Τμήμα Χημείας, Πανεπιστήμιο Ιωαννίνων

Ημερομηνία ορισμού θέματος: 17-06-2016
Ημερομηνία τροποποίησης θέματος: 27-5-2020

Θέμα: **«Development of analytical methods based on sample preparation and Liquid Chromatography-High Resolution Mass Spectrometry for the determination of emerging contaminants»**

ΟΡΙΣΜΟΣ ΕΠΤΑΜΕΛΟΥΣ ΕΞΕΤΑΣΤΙΚΗΣ ΕΠΙΤΡΟΠΗΣ από τη Συνέλευση: **1025/23-10-20**

1. κ. Σακκά Βασίλειο, Αναπληρωτή Καθηγητή Παν/μίου Ιωαννίνων

2. κ. Αλμπάνη Τριαντάφυλλο, Καθηγητή Παν/μίου Ιωαννίνων

3. κ. Κωνσταντίνο Σταλίκα, Καθηγητή Παν/μίου Ιωαννίνων

4. κ. Βλεσσίδη Αθανάσιο, Καθηγητή Παν/μίου Ιωαννίνων

5. κ. Γκιώκα Δημοσθένη, Αναπληρωτή Καθηγητή Παν/μίου Ιωαννίνων

6. κ. Βασίλειο Σταθόπουλο, Καθηγητή Εθνικού και Καποδιστριακού Πανεπιστημίου Αθηνών

7. κ. Τσόγκα Γεώργιο, Επίκουρο Καθηγητή Αριστοτέλειου Πανεπιστημίου Θεσσαλονίκης

Έγκριση Διδακτορικής Διατριβής με βαθμό «……» στις ……-……-……

Η Πρόεδρος του Τμήματος Χημείας
Λουλούδη Μαρία, Καθηγήτρια

Η Γραμματέας του Τμήματος
Ξανθή Τουτουνζόγλου

Στους πιο υπέροχους γονείς
του κόσμου Χρήστο και Ευαγγελία...

''Ανέβα... Ανέβα...
Πάντα ανέβαινε
Ακόμη πιο ψηλά...

Ανέβα ...
Όλο μπρος ... Όλο ψηλά.
Κι αν δε βρεις δρόμο
Φτιάξε
...
Ανέβα...
Και πες ''ευχαριστώ''
....
Πες ευχαριστώ στη δύναμη,
που σ' έκανε να ανέβεις

Μ. ΛΟΥΝΤΕΜΗΣ

Ευχαριστίες

Η πρώτη σελίδα αυτής της διδακτορικής διατριβής αποτελεί μια ελάχιστη ένδειξη ευγνωμοσύνης σε όλους τους ανθρώπους που συνετέλεσαν στην ολοκλήρωσή της.

Η παρούσα διδακτορική διατριβή, εκπονήθηκε στο Εργαστήριο Αναλυτικής Χημείας του Τμήματος Χημείας του Πανεπιστημίου Ιωαννίνων υπό από την επίβλεψη του Αναπληρωτή Καθηγητή κ. Βασίλειου Σακκά στον οποίο οφείλω τη διαμόρφωση της επιστημονικής μου σκέψης και τον τρόπο διαχείρισης επιστημονικών ζητημάτων χάρις σε όλα αυτά τα χρόνια συνεργασίας από τις μεταπτυχιακές μου σπουδές έως σήμερα. Ευχαριστώ ειλικρινά για τη συνεχή επιστημονική καθοδήγηση κατά τη διεξαγωγή της έρευνας, τις εύστοχες επιστημονικές παρατηρήσεις καθώς και τις ανθρώπινες συμβουλές του που δεν περιορίζονταν σε αυστηρά επιστημονικά πλαίσια. Τέλος τον ευχαριστώ για τις πολύτιμες εμπειρίες που αποκόμισα καθ' όλη τη διάρκεια των σπουδών μου οι οποίες θα αποτελέσουν εφόδιο για τη μετέπειτα επαγγελματική μου πορεία. Ευχαριστώ για την εμπιστοσύνη που μου έδειξε και την αμέριστη στήριξη του.

Ιδιαίτερες ευχαριστίες οφείλω στον Καθηγητή του τμήματος Χημείας αλλά και Πρύτανη του Πανεπιστημίου Ιωαννίνων κ. Αλμπάνη Τριαντάφυλλο για το αδιάλειπτο και συνεχές ενδιαφέρον που αφορούσε την εξέλιξη της ερευνητικής μου δραστηριότητας. Τον ευχαριστώ θερμά για την καθοδήγηση, τις εύστοχες υποδείξεις του, την ποικιλόμορφη στήριξή του, εντός και εκτός εργαστηρίου, και την συμπαράστασή του καθ' όλη τη διάρκεια των μεταπτυχιακών και διδακτορικών μου σπουδών. Τον ευχαριστώ για την τιμή να με θεωρεί μέλος του εργαστηρίου του καθώς και για τη δυνατότητα να εργάζομαι σ αυτό. Τέλος ευχαριστώ για την αποδοχή του να αποτελεί μέλος της τριμελούς επιτροπής και την αξιολόγηση της διδακτορική μου διατριβής.

Επίσης ένα μεγάλο ευχαριστώ στον Καθηγητή κ. Κωνσταντίνο Σταλίκα, μέλος της τριμελούς επιτροπής για την άριστη συνεργασία και για τις καίριες υποδείξεις του όλα αυτά τα χρόνια. Ευχαριστώ πολύ για τη διάθεση του να επιλύσει κάθε μου απορία καθώς και την έμπρακτη βοήθεια του σε κομβικά σημεία αυτής της διατριβής.

Θα ήθελα επίσης να εκφράσω τις ευχαριστίες μου στα μέλη της επταμελούς Εξεταστικής Επιτροπής, κ. Αθανάσιο Βλεσσίδη, κ. Δημοσθένη Γκιώκα, .κ. Τσόγκα Γεώργιο, η πόρτα των εργαστήριων τους ήταν πάντα ανοιχτή και με διάθεση για βοήθεια καθώς και τον κ. Βασίλειο Σταθόπουλο για την αποδοχή να αξιολογήσουν τη Διδακτορική Διατριβή.

Θα ήθελα να ευχαριστήσω τον Καθηγητή του τμήματος Χημείας Ιωάννη Κωνσταντίνου για τις πολύτιμες γνώσεις του για τη διάθεση του να επιλύσει οποιαδήποτε απορία μου δημιουργήθηκε, την πρόσβαση στο εργαστήριο του και τη χρήση υλικοτεχνικού εξοπλισμού για τη διενέργεια μετρήσεων BOD.

Ιδιαίτερες ευχαριστίες ανήκουν στη Δρ. Βάσια Μπότη, μέλος ΕΔΙΠ στο Τμήμα Χημείας του Πανεπιστημίου Ιωαννίνων, για την ευκαιρία να συνεργαστώ μαζί της, για τη διάθεση να μεταδώσει τις γνώσεις της πάνω στη φασματομετρία μάζας και ειδικότερα τον αναλυτή του Orbitrap. Την ευχαριστώ για την παροχή επιλεγμένων αναλυτών για την υλοποίηση της

παρούσας διατριβής και τη διάθεση της για επίλυση οποιονδήποτε επιστημονικών ζητημάτων. Ευχαριστώ επίσης τη Μονάδα περιβαλλοντικής, οργανικής και βιοχημικής ανάλυσης υψηλής ευκρίνειας Orbitrap–LC–MS του Πανεπιστημίου Ιωαννίνων και τον Δρ. Αθανάσιο Καρκαμπούνα για την πρόσβαση στις υπηρεσίες της Μονάδας.

Οι γραμμές αυτές είναι λίγες για να εκφράσω την ευγνωμοσύνη μου στη Δρ. Χριστίνα Κοσμά. Είχα την τύχη να βρίσκομαι ανάμεσα στους πολλούς ανθρώπους που έχουν βοηθηθεί από το συγκεκριμένο άτομο. Οι εις βάθος γνώσεις της και επιστημονικές της υποδείξεις παράλληλα με τη συνεχή της υποστήριξη, βοήθησε σημαντικά στην ολοκλήρωση αυτής της διατριβής. Η συμβολή της στην εξέλιξη των γνώσεων μου ήταν καθοριστική και η ίδια ως άνθρωπος και επιστήμονας αποτελούν πρότυπο για κάθε εργαστήριο και συνεργάτη. Δεν μπορώ να παραλείψω από τις ευχαριστίες τη Δρ. Χριστίνα Νάννου για το συνεχές και απρόσκοπτο ενδιαφέρον της, τις χρήσιμες γνώσεις της και την απλόχερη βοήθεια της έως και σήμερα.

Ιδιαίτερες αναφορές αξίζουν στους Δρ. Δημήτρη Μοσχόβα, και το εργαστήριο του κ. Αυγερόπουλου, τον υποψήφιο διδάκτορα του Εργαστήριου Βιομηχανικής Φειδία Μπαϊράμη για τη λήψη των εικόνων SEM, καθώς και την κ. Παπαχριστοδούλου Χριστίνα για την μελέτη των δειγμάτων με Περίθλαση Ακτίνων Χ.

Ιδιαίτερες ευχαριστίες στον Δρ. Θεόδωρο Χατζημητάκο για τις απεριόριστες και πολύτιμες γνώσεις του στη σύνθεση και χαρακτηρισμό των προσροφητικών, την παροχή για μελέτη διάφορων υλικών, τη γενικότερη βοήθεια του καθώς και την ανιδιοτελή στήριξη μαζί με τη Δρ. Αθανασία Κασούνη, οι οποίοι αποτέλεσαν πολλές φορές από μηχανής θεός αυτής της διατριβής.

Θα ήθελα επίσης να ευχαριστήσω τον κ. Χρήστο Παπαγεωργίου Χημικό Μηχανικό στις εγκαταστάσεις του Βιολογικού καθαρισμού Αμαλιάδας για τις πληροφορίες σχετικά με τη μονάδα και την ανταπόκριση του σε οποιαδήποτε απορία. Ευχαριστώ τον πατέρα μου για το διττό του ρόλο σε αυτή τη διατριβή. Η μελέτη παρακολούθησης των αναδυομένων ρύπων διάρκειας ενός έτους σε δύο περιοχές της Ελλάδας θα ήταν ανέφικτη χωρίς τη συνδρομή του πατέρα μου ο οποίος φρόντιζε με συνέπεια για τη συλλογή των δειγμάτων, τη διατήρησή τους και την αποστολή τους.

Ευχαριστώ για τη συνεργασία μας στο Εργαστήριο Βιομηχανικής το Δρ. Νίκο Πετρίδη, τη Δρ. Τσούτση Χαρούλα, τους υποψήφιους διδάκτορες Έμη Γότση, Επαμεινώνδα Τραντόπουλο Αγγελική Καλογεροπούλου, τους Έλσα Μπούση, Βασιλική Τόλη, Μαρτιναίου Παναγιώτα, Δάφνη Κιφοκέρη, Στέφανο Πατέρα, τον πάντα φιλότιμο Γιάννη Μπουκουβάλα. Δεν θα μπορούσα να μην αναφερθώ στους συναδέλφους μου όλα αυτά τα χρόνια στο εργαστήριο Αναλυτικής Χημείας, τους Άννα Στεφανίτση, Ειρήνη Σιώζου, Λία Κουταλιά, Βίκυ Κακαέ, Μάγκου Καίτη, Θάλεια Βλάχου και Λουκία Λυπηρού. Ευχάριστες επίσης εμπειρίες αποκόμισα χάρη στους Debora Calarrotta, Victor Candelario Leal, και Bruno Caram. Τέλος ιδιαίτερες ευχαριστίες οφείλω στην υποψήφια διδάκτωρ Cristina Jimenez Holgado και το μεταπτυχιακό φοιτητή Χριστόφορο Χρηματόπουλο, του οποίου η διάθεση για δουλειά και

το ενδιαφέρον του στη διεξαγωγή πειραμάτων συνέβαλαν σημαντικά. Τέλος τους ευχαριστώ για το ευχάριστο κλίμα.

Ευχαριστώ την φίλη και συνάδελφο Δρ. Μαργαρίτα Καψή, συνοδοιπόρο από τα πρώτα φοιτητικά έδρανα μέχρι τους τελευταίους εργαστηριακούς πάγκους. Αποτέλεσε το στήριγμα μου σε όλη αυτή τη διαδρομή του διδακτορικού, χάρις στις γνώσεις τις και τις πολύτιμες συμβουλές της. Ευχαριστώ πάρα πολύ την παλιά μου συνεργάτιδα στο εργαστήριο Αναλυτικής Χημείας, τον καταλληλότερο άνθρωπο που μπορεί κάποιος να συνεργαστεί, την Ελίνα Μπάρδα, φίλη και συνάδελφο και χορηγό έμπρακτης εμψύχωσης όλο αυτό το διάστημα. Ευχαριστώ το Δημήτρη Μαμμή για την πολύτιμη εφαρμογή των γνώσεων προγραμματισμού στην ερμηνεία κάποιων επιστημονικών δεδομένων, καθώς και για τη στήριξη του όλα αυτά τα χρόνια. Τέλος πολλά ευχαριστώ ανήκουν στους φίλους μου Χριστίνα, Μαρκέλλα, Χρύσα, Φωτεινή, Σούλα, Θύμιο και Αλέξη για την ένθερμη και ουσιαστική στήριξή τους όλα αυτά τα χρόνια.

Τέλος αυτές οι ευχαριστήριες γραμμές είναι λίγες για να περιγράψουν την ευγνωμοσύνη μου στους γονείς και τη γιαγιά μου. Ευχαριστώ ιδιαίτερα τη μητέρα μου και τον πατέρα μου, τους πιο ένθερμους υποστηρικτές μου σε κάθε στάδιο της ζωής μου. Τους ευχαριστώ για την αμέριστη συμπαράσταση τους, την ηθική υποστήριξη και την ευκαιρία να εκπληρώνω κάθε μου όνειρο. Μου δίνουν δύναμη να προσπαθώ σε κάθε μου βήμα. Το αποτέλεσμα αυτής της μεγάλης προσπάθειας αφιερώνεται σε αυτούς.

Μ. Χ. Καλαμπόκα
Νοέμβριος 2020, Ιωάννινα

Table of Contents

Development of Analytical Methods Based on Sample Preparation and Liquid Chromatography-High Resolution Mass Spectrometry for The Determination of Emerging Contaminants

Maria C. Kalampoka

PhD Thesis

Summary

A new class of contaminants has emerged an environmental issue, as a result of technology manufacturing or anthropogenic activities regarding medicine, agriculture, food, and domestic conveniences. The new class of contaminants the so-called ''emerging contaminants'' (ECs) are discovered to have been released to the environment and pose potential threats to environmental ecosystems and human health and safety. They are commonly not regulated and can be detected in low or very low concentrations by analytical techniques, raising special concern due to their long-term adverse effects. These emerging contaminants include Pharmaceutical Active Compounds (PhACs), Personal Care products (PCPs), pesticides, industrial chemicals and some of them are released in the environment via excretion and metabolism. The main receptors for these compounds are the WWTPs which are not designed to efficient remove these contaminants at trace levels and eventually, treated effluents still contain ECs in considerable concentrations. Therefore, data concerning their existence is required. Detection of these contaminants in environmental matrices such as wastewater is particularly challenging because of the low detection limits required, the complex nature of the samples, and difficulty in separating these compounds from interferences. For this reason, rapid, inexpensive, efficient and environmentally friendly sample preparation techniques have been developed along with a sensitive selective chromatographic system coupled with orbitrap mass analyzer (UHPLC-LTQ Orbitrap MS), for the determination of selected Emerging Contaminants (ECs), namely sulfacetamide, sulfadiazine, olanzapine, sulfathiazole, sulfapyridine, trimethoprim, sulfamethizole, sulfamethazine, sulfamethoxy-pyridazine, sulfamethoxazole, risperidone, venlafaxine, sulfaquinoxaline, oxolinic acid, paroxetine, cyclobenzaprine, erythromycin, amitriptyline, fluoxetine, carbamazepine, clomipramine, acesulfame, saccharin, sucralose, aspartame, florfenicol, salicylic acid, indomethacin, gemfibrozil, mefenamic acid, triclosan and

tolfenamic acid, in wastewater effluent. The sample preparation techniques involved in this study were: Magnetic Solid-Phase Extraction (MSPE), Fabric Phase Sorptive Extraction (FPSE) and Solid-Phase Extraction (SPE).

Specifically, the high mass resolution capabilities of the Orbitrap MS were exploited for the determination of 33 emerging contaminants belonging to pharmaceutical active compounds (PhACs), personal care products (PCPs), artificial sweeteners (ASs), allowing facile discrimination between analytes and matrix. Operational parameters of the Orbitrap MS (ESI parameters, mass resolving power, AGC target, tube lens, injection time) were evaluated along with the chromatographic conditions (chromatographic column, mobile phase etc.) for the confirmation of analytes at trace concentration levels. Mass accuracy for target contaminants using this method was less than 5 ppm in both positive and negative ionization modes investigated.

Magnetic solid Phase Extraction (MSPE) with the use of silica-based (C18) and graphene-based (mrGO) magnetic nanoparticles (MNPs) was developed, optimized and validated for the determination of 16 and 19 ECs respectively in effluent and tap water. Magnetic nanoparticles were successfully synthesized and characterized with XRD, SEM, FT-IR providing the desired morphological and structural characteristics. Analytical performance of the method was excellent providing good linearity with coefficients of determination (R^2) greater than 0.99, high repeatability and reproducibility and recoveries ranged from 58.4% to 102.6% for both MSPEs. MSPEs employed with C18 and mrGO MNPs provided high sensitivity with minimum MDLs and MQLs up to 0.6 and 1.8ng/L, respectively. In addition, MSPE technique proved to be efficient for elimination of sample matrix.

A novel FPSE protocol has been developed and optimized to follow the green analytical chemistry demands for the extraction and determination of 21 selected emerging contaminants in wastewater and tap water. FPSE was combined with UHPLC-MS-Orbitrap to take advantage the utilities of the new sample pretreatment for the enrichment of the analytes of interest and the powerful mass analyzer of Orbitrap. Fabric medium consisted of a fiber glass substrate and a sol-gel coating of PEG, was synthesized, characterized, and employed as an FPSE device. Under optimized conditions validation assays were followed for two aqueous matrices, tap water and effluent wastewater. FPSE exhibited excellent analytical characteristics in terms of linearity, intra-day, and inter-day precision for both tap water and effluent. MDLs and MQLs for effluent water ranged from 3.1-149.4 ng/L and 9.3-447.7 ng L/L, low enough considering the enrichment factor, but rather high for assessing an environmental monitoring survey in WWTPs.

For this reason, a Solid-Phase Extraction (SPE) followed by UHPL-LTQ-Orbitrap-MS was applied for the determination of 33 ECs in effluent water. SPE was optimized and validated with excellent analytical characteristics including low MDLs and MQLs up to 0.3 ng/L and 1.0 ng/L, respectively. Afterwards, it was employed for the analysis of effluent waters collected during a one-year monitoring study from municipal and hospital WWTPs located in different cities of Greece, Ioannina and Amaliada. The analysis of the samples revealed the occurrence of 28 compounds of different therapeutic classes and chemical categories. The most abundant compounds in terms of mean concentrations were acesulfame (4862.5 ng/L), sucralose (993.8 ng/L) and salicylic acid (981.8 ng/L). Concentration occurrence, frequency of detection, temporal and seasonal variations were investigated and compared with relevant studies. Consumption patterns and explanations about the occurrence of emerging contaminants were estimated. Finally, the presence of target compounds was further confirmed with the association of fragment ions (MS/MS) and precursor ions providing high mass accuracy less than 5ppm.

Ανάπτυξη αναλυτικών μεθόδων προσδιορισμού αναδυόμενων ρύπων με τη χρήση υγρής χρωματογραφίας συζευγμένης με φασματομετρία μάζας υψηλής διακριτικής ικανότητας

Μαρία Χ. Καλαμπόκα

Διδακτορική Διατριβή

Περίληψη

Τα νέα τεχνολογικά επιτεύγματα καθώς και οι ανθρωπογενείς δραστηριότητες που σχετίζονται με την ιατρική, τη γεωργία, την κτηνοτροφία, τη βιομηχανία τροφίμων και τις οικιακές ανέσεις έχουν αναδείξει ένα νέο περιβαλλοντικό ζήτημα που αφορά την καινούρια κατηγορία ρύπων των αποκαλούμενων αναδυόμενων ρύπων. Οι αναδυόμενοι ρύποι μπορούν να απελευθερωθούν στο περιβάλλον και θεωρούνται ως εν δυνάμει επιβλαβείς για το περιβάλλον και στην ανθρώπινη υγεία. Ως τώρα δεν περιλαμβάνονται στους υπάρχοντες κανονισμούς της ποιότητας των υδάτων και μπορούν να ανιχνευθούν σε χαμηλές ή πολύ χαμηλές συγκεντρώσεις (ng/L) με τη χρήση αναλυτικών τεχνικών προκαλώντας ιδιαίτερη ανησυχία, λόγω των μακροπρόθεσμων αρνητικών τους επιπτώσεων. Στην κατηγορία των αναδυόμενων ρύπων περιλαμβάνονται χημικές ενώσεις όπως φαρμακευτικές δραστικές ουσίες, προϊόντα προσωπικής φροντίδας, φυτοφάρμακα, βιομηχανικά χημικά, και κάποια από αυτά καταλήγουν στο περιβάλλον μέσω διεργασιών απέκκρισης και μεταβολισμού. Οι κύριοι αποδέκτες αυτών των χημικών ενώσεων είναι οι μονάδες επεξεργασίας υγρών αποβλήτων (ΜΕΥΑ), οι οποίες δεν είναι σχεδιασμένες να απομακρύνουν αποτελεσματικά τέτοιους ρύπους σε τόσο χαμηλές συγκεντρώσεις με αποτέλεσμα τα επεξεργασμένα λύματα να περιέχουν αυτές τις ενώσεις σε σημαντικά επίπεδα. Για το λόγο αυτό, απαιτούνται δεδομένα σχετικά με την ύπαρξή τους. Η ανίχνευση αυτών των ρύπων σε περιβαλλοντικά υποστρώματα όπως τα υγρά απόβλητα είναι ιδιαίτερα δύσκολη εξαιτίας των χαμηλών ορίων ανίχνευσης που απαιτούνται, την πολυπλοκότητα της φύσης του υποστρώματος και τη δυσκολία διαχωρισμού αυτών των ουσιών από τυχόν παρεμποδίσεις. Για αυτό το λόγο αναπτύχθηκαν τεχνικές προκατεργασίας γρήγορες, οικονομικές, αποτελεσματικές και φιλικές προς στο περιβάλλον σε συνδυασμό με ένα χρωματογραφικό σύστημα συζευγμένο με αναλυτή μάζας Orbitrap (UHPLC-Orbitrap MS) με υψηλή ευαισθησία και εκλεκτικότητα για

τον προσδιορισμό επιλεγμένων αναδυόμενων ρύπων σε υγρά απόβλητα. Οι τεχνικές προκατεργασίας που μελετήθηκαν στην παρούσα διδακτορική διατριβή περιλαμβάνουν : τη μαγνητική εκχύλιση δια της στερεάς φάσης (MSPE), την εκχύλιση με προσρόφηση σε ύφασμα (FPSE) και την εκχύλιση δια της στερεάς φάσης (SPE).

Συγκεκριμένα, αξιοποιήθηκαν οι δυνατότητες υψηλής ακρίβειας μάζας του υβριδικού τροχιακού αναλυτή Orbitrap, για τον προσδιορισμό 33 αναδυόμενων ρύπων των αντιπροσωπευτικών κατηγοριών των φαρμακευτικών ουσιών, των προϊόντων προσωπικής φροντίδας καθώς και των τεχνητών γλυκαντικών. Ονομαστικά οι ενώσεις που επιλέχθηκαν ήταν οι: σουλφακεταμίδη, σουλφαδιαζίνη, ολανζαπίνη, σοθλφαθιαζόλη, σουλφαπυριδίνη, τριμεθοπρίμη, σουλφαμεθιζόλη, σουλφαμεθαζίνη, σουλφαμεθόξυ-πυριδαζίνη, σουλφαμεθοξαζόλη, ρισπεριδόνη, βενλαφαξίνη, σουλφακινοξαλίνη, οξολινικό οξύ, παροξετίνη, κυκλομπενζαπρίνη, ερυθρομυκίνη, αμιτριπτυλίνη, φλουοξετίνη, καρμπαμαζεπινη, κλομιπραμίνη, ακεσουλφάμη, σακχαρίνη, σοθκραλόζη, ασπαρτάμη, φλορφενικόλη, σαλικυλικό οξύ, ινδομεθακίνη, γεμφιβροζίλη, μεφεναμικό οξύ, τρικλοζάνη και τολφαιναμικό οξύ.

Για την ταυτοποίηση των μελετώμενων αναλυτών σε χαμηλές συγκεντρώσεις αξιολογήθηκαν οι παράμετροι λειτουργίας του υβριδικού τροχιακού αναλυτής μάζας Orbitrap όπως παράμετροι ηλεκτροψεκασμού (ESI), διακριτική ικανότητα, ελέγχος αυτόματης απολαβής ιόντων (AGC), δυναμικό δακτυλιοειδούς φακού εστίασης (tube lens), χρόνος έγχυσης (IT) καθώς και οι χρωματογραφικές συνθήκες (χρωματογραφική στήλη, κινητή φάση κτλ.). Η ακρίβεια μάζας των επιλεγμένων αναλυτών χρησιμοποιώντας αυτή τη μέθοδο ήταν μικρότερη από 5ppm και στις δύο λειτουργίες ιονισμού, θετική και αρνητική.

Αναπτύχθηκε μαγνητική εκχύλιση δια της στερεάς φάσης με χρήση C18 υλικών με βάση την πυριτία, καθώς και μαγνητικών νανοϋλικών με βάση το γραφένιο για τον προσδιορισμό 16 και 19 αναδυόμενων ρύπων, αντίστοιχα, σε νερό βρύσης και υγρά απόβλητα. Η τεχνική αυτή βελτιστοποιήθηκε και επικυρώθηκε. Τα μαγνητικά νανοϋλικά συντέθηκαν επιτυχώς και χαρακτηρίστηκαν με τεχνικές περίθλασης ακτινών Χ (XRD), με φασματοσκοπία υπερύθρου (FT-IR), και μικροσκόπιο ηλεκτρονικής σάρωσης (SEM), παρέχοντας τα επιθυμητά μορφολογικά και δομικά χαρακτηριστικά. Η μέθοδος MSPE-UHPLC-LTQ-Orbitrap-MS χρησιμοποιώντας ως προσροφητικά μαγνητικά υλικά τα νανοσωματίδια των C18 και mrGO, επέδειξε εξαιρετικές αναλυτικές επιδόσεις με συντελεστές γραμμικότητας (R^2) μεγαλύτερους από 0.99, υψηλή επαναληψιμότητα και αναπαραγωγιμότητα και ανακτήσεις που κυμάνθηκαν από 58.4% έως 102.6% και για τις δύο τεχνικές. Οι μαγνητικές εκχυλίσεις με χρήση C18 και mrGO μαγνητικά νανοϋλικά παρείχε υψηλή ευαισθησία με ελάχιστα όρια ανίχνευσης και ποσοτικοποίησης έως

0.6 και 1.8 ng/L, αντίστοιχα. Επιπλέον η MSPE αποδείχθηκε μια αποτελεσματική τεχνική για τη μείωση της επίδρασης του υποστρώματος (matrix effect) σε ένα πολύπλοκο δείγμα όπως είναι τα υγρά απόβλητα.

Ένα νέο πρωτόκολλο βασισμένο στην εκχύλιση με προσρόφηση σε ύφασμα (FPSE) αναπτύχθηκε και βελτιστοποιήθηκε στο πλαίσιο των πράσινων περιβαλλοντικών απαιτήσεων για τον προσδιορισμό 21 αναδυόμενων ρύπων σε υγρά απόβλητα και νερό βρύσης. Η FPSE σε συνδυασμό με το χρωματογραφικό σύστημα και τον υβριδικό τροχιακό αναλυτή μάζας Orbitrap αξιοποιεί στο έπακρο τα οφέλη αυτής της καινούριας τεχνολογίας προκατεργασίας δείγματος για την προσυγκέντρωση των επιλεγμένων αναλυτών, μαζί με τις δυνατότητες για υψηλή ακρίβεια μάζας του ισχυρού αναλυτή. Ως κύριο προσροφητικό υλικό της FPSE χρησιμοποιήθηκε ένα υφασμάτινο μέσο αποτελούμενο από ένα υπόστρωμα από υαλοϊνες και μία sol-gel επίστρωση πολυμερούς PEG, το οποίο συντέθηκε και χαρακτηρίστηκε. Υπό τις βέλτιστες συνθήκες πραγματοποιήθηκαν πειράματα επικύρωσης για δύο υδατικά υποστρώματα, νερό βρύσης και υγρά επεξεργασμένα απόβλητα και η μέθοδος παρουσίασε εξαιρετική γραμμικότητα, επαναληψιμότητα και αναπαραγωγιμότητα και για τα δυο υποστρώματα. Τα όρια ανίχνευσης και ποσοτικοποίησης της μεθόδου κυμάνθηκαν από 3.1-149.4 ng/L και 9.3-447.7 ng L/, τιμές αρκετά χαμηλές αν λάβουμε υπόψιν τον παράγοντα προσυγκέντρωσης αλλά σχετικά υψηλές για έναν συστηματικό περιβαλλοντικό έλεγχο αναδυόμενων ρύπων σε μονάδες επεξεργασίας αποβλήτων.

Για το λόγο αυτό επιλέχθηκε η ευρύτατα διαδεδομένη εκχύλιση δια της στερεάς φάσης (SPE) για τον προσδιορισμό και των 33 επιλεγμένων αναδυόμενων ρύπων σε υγρά επεξεργασμένα απόβλητα στο πλαίσιο ενός περιβαλλοντικού ελέγχου διάρκειας ενός έτους. Για τη βελτιστοποίηση, ελέγχθηκαν διαφορετικά αναλυτικά πρωτόκολλα εξετάζοντας έτσι διαφορετικές μικροστήλες εκχύλισης, διαφορετικά pH δείγματος και διαφορετικούς διαλύτες. Η επικύρωση της μεθόδου επέδειξε υψηλή ευαισθησία με ελάχιστα όρια ανίχνευσης και ποσοτικοποίησης έως 0.3 ng/L και 1.0 ng/L, αντίστοιχα. Η εφαρμογή της μεθόδου σε δείγματα νερού από τις εξόδους των ΜΕΥΑ Ιωαννίνων (πόλης και νοσοκομείου) και Αμαλιάδας επιβεβαίωσε την παρουσία 28 ενώσεων από διαφορετικές χημικές και θεραπευτικές κατηγορίες. Ενδεικτικά οι ενώσεις με τις υψηλότερες συγκεντρώσεις ήταν η ακεσουλφάμη (4862.5 ng/L), η σουκραλόζη (993.8 ng/L) και το σαλικυλικό οξύ (981.8 ng/L). Τα επίπεδα συγκεντρώσεων, η συχνότητα ανίχνευσης, οι χρονικές και εποχιακές διακυμάνσεις ερευνήθηκαν και συγκρίθηκαν με σχετικές μελέτες. Παράλληλα, πραγματοποιήθηκε μια προσπάθεια εκτίμησης της παρουσίας των αναδυόμενων ρύπων ανάλογα με τα καταναλωτικά πρότυπα ή τις εκάστοτε ανθρωπογενείς δραστηριότητες. Τέλος η

επιβεβαίωση των θετικών ανιχνεύσεων βασίστηκε επίσης σε θραυσματοποίηση MS/MS για την περεταίρω ταυτοποίηση τους.

A. Theoretical Framework

..

CHAPTER 1: THEORETICAL FRAMEWORK

1.1 Emerging Contaminants

The development of modern technologies and the underlying science that makes them possible is the foundation of human progress. Technological achievements have improved human welfare in terms of health, longevity, and general living conditions both at the global as well as individual scale. Technology has provided around the years clean water, advanced medicines, improved techniques for agriculture, renewable sources of energy, more durable materials for housing, and an increased standard of living for nearly everyone around the globe. During the first half of the twentieth century, the continuous expansion of the chemical industry and the use of chemicals in many aspects of our life contributed toward creating a positive image of chemistry in our society. Nowadays, the chemical industry plays an essential role of the economy in developed countries, being considered a strategic sector, and contributing significantly to the gross domestic product. These advancements are often surrounded by peripheral consequences that are not always immediately understood but must be managed as they emerge. According to the World Health Organization (WHO), more than 100,000 chemicals are released into the global environment every year as a consequence of their production, use, and disposal. Non-polar hazardous substances, i.e. persistent organic pollutants (POPs) and heavy metals, were the subject of attention and recognition as priority pollutants until the beginning of the 1990s and were also part of extensive surveillance programs. Nevertheless, nowadays these compounds are less relevant since profuse legislation has been adopted worldwide to set the acceptable levels of pollutants in water, air, or soil, while appropriate measures and elimination of the dominant pollution sources have been implemented to protect the environment and human health.

However, a new class of contaminants has emerged as an environmental issue, as a result of technology manufacturing or human use, and there is a widespread agreement that this kind of contamination may require legislative intervention. The new class of contaminants the so-called ''emerging contaminants'' (ECs) are discovered to have been released to the environment and pose potential threats to environmental ecosystems and human health and safety. They are commonly not regulated and can be detected in low or very low concentrations by analytical techniques, raising special concern because their long-term adverse effects. Alternatively, the term "constituents/chemicals/compounds/contaminants of emerging concern" (CECs) is also

used. International organizations and national agencies of specific countries have developed many definitions of ''emerging contaminants'', with some examples presented in Fig.1.1.

Figure 1.1. Examples of definitions of emerging contaminants from various organisms [1–6]

One thing that is common among them is that the term "emerging" reflects the overall developing state in our understanding of this class of compounds. The term emerging contaminants does not necessarily correspond to "new substances", i.e., newly introduced chemicals and their degradation products/metabolites or by-products, but also refers to compounds with previously unrecognized adverse effects on the ecosystems, including naturally occurring compounds. They may substances which have been long used, but only recently found in the environment. We may just be beginning to understand their effect on the environment and on human health, or we may only now have the ability to detect them in the environment [7]. Areas of developing understanding may include routine monitoring programs, determining whether compound(s) have the potential to be harmful to humans or the environment and consequently to what extent they should be regulated, their occurrence in the environmental compartments and techniques for treating environmental media to remove or transform them [6].

From all the above discussions, ECs may be potentially considered in the next few years as priority substances and subject to regulation They derive from several industrial activities and scientific research and are related to lifestyle habits, and development of new materials. Moreover, massive use of household chemicals or pharmaceutical products during daily household activities is a source of ECs as well. In other cases, use of antibiotics, antiseptics, biocides, pesticides as common products for the prevention of diseases and elimination of pests in livestock and crops is getting expanded [8]. In some cases, the release of a chemical or microbial contaminant into the environment has probably occurred for a period but may have not been detected since no sensitive analytical methods existed. Moreover, the synthesis of new chemicals or changes in the use and disposal of existing chemicals can create new sources of emerging contaminants.

Emerging contaminants encompass diverse groups of compounds belonging in different classes depending on their origin, use, potential effects, or environmental fate, although sufficient knowledge for all the various ECs is still not available. Main ECs are divided in the following categories:

- Biological toxins
- Microorganisms
- Brominated flame retardants
- Disinfection by-products
- Industrial Chemicals
- Illicit drugs
- Personal care products and other lifestyle products
- Food additives
- Hormones and other endocrine disrupting compounds
- Organometallics
- Organophosphate flame retardants
- Per fluorinated compounds
- Pharmaceuticals and veterinary products
- Pesticides and their degradation/transformation products
- Surfactants and their metabolites
- Nanomaterials
- Ionic Liquids
- Plasticizers

Some of the most common terms used to categorize ECs along with some examples are listed in Table 1.1.

Table 1.1 Representative list of ECs

Category	Compound Examples
Per fluorinated Compounds	Perfluoroctane sulfonates (PFOS), perfluoroctanoic acid
Pesticides	Organophosphorus pesticides, thiocarbamates
Food additives	Sucralose, triacetin
Nanoparticles	Titanium dioxide, fullerenes
Algal toxins	Microcystine
Antifouling compounds	Organotins (dibutyltin and triphenyltin ions), cybutryne
Insect repellents	N,N-diethyl-m-toluamide (DEET).
Flame retardants	Tetrabromobisphenol A, Hexabromocyclododecane
Surfactants	Alkylphenol ethoxylates, alkylphenols (nonylphenol and octylphenol), alkylphenol carboxylates
Antiseptic additives	Triclosan, triclocarban
Plasticizers	Bisphenol A
Steroids & hormones	Estradiol, estrone, estriol, diethylstilbestrol
Water (disinfection)	2,2,2-Trichloroacetamide, chloroacetaldehyde
Fragrances	Nitro, polycyclic, and macrocyclic musks
Drugs of abuse	Morphine, dihydrocodeine, cocaine
Sunscreen agents	2-ethyl-hexyl-4-trimethoxycinnamate, octocrylene
Analgesics, anti-inflammatory drugs	Ibuprofen, acetaminophen, codeine, ,aspirin, diclofenac
Veterinary and human antibiotics	Trimethoprim, sulfamethoxazole, erythromycin
Psychiatric drugs	Risperidone, carbamazepine, lorazepam, bromazepam
β-Blockers	Metoprolol, propranolol, timolol, bisoprolol
Lipid regulators	Gemfibrozil, bezafibrate, clofibric acid, fenofibric acid,
X-ray contrast agent	Iopromide, iothalamic acid, diatrizoic acid
Gasoline additives	*tert*-Butyl methyl ether, dialkyl ethers

1.1.1 Sources of Emerging Contaminants

Sources of Emerging Contaminants (ECs) from which originate before their transport to surface waters, groundwater, sediments, and drinking water are varied and include discharges or leaks of

domestic, hospital or industrial wastewater containing pharmaceutical or personal care compounds, pesticide applications to agricultural land, parks, gardens, urban infrastructure, and the transport network, discharges or leaks of domestic, sewage sludge application to land, pharmaceutical and pesticides used to treat animals and solid waste disposal [9]. The sources of ECs can be divided into two types: point source pollution and non-point source pollution (Fig. 1.2).

Point source pollution: This is a single identifiable source which originates from separate locations and can be calculated in mathematical modeling [10]. For instance, industrial effluents, hospital effluents, and STPs as well as septic tanks, resource extraction (mining), and land disposal sites (landfill sites, industrial impoundments are the major point sources for soil zone and water resource pollution.

non-point-source pollution or diffuse pollution: This source is hard to identify and occurs over broad geographical areas [10]. One example is the runoff, including agricultural runoff from animal waste and manure (pesticides, pathogens, and fertilizers), urban runoff from domestic waste, and the leakage from waste treatment systems and plants, atmospheric deposition, wet and dry deposition of persistent organic pollutants such as PCBs [11]. In contrast to point-source pollution, in this case lower environmental loading occurs because it has higher potential for natural attenuation in the soil and subsurface [12].

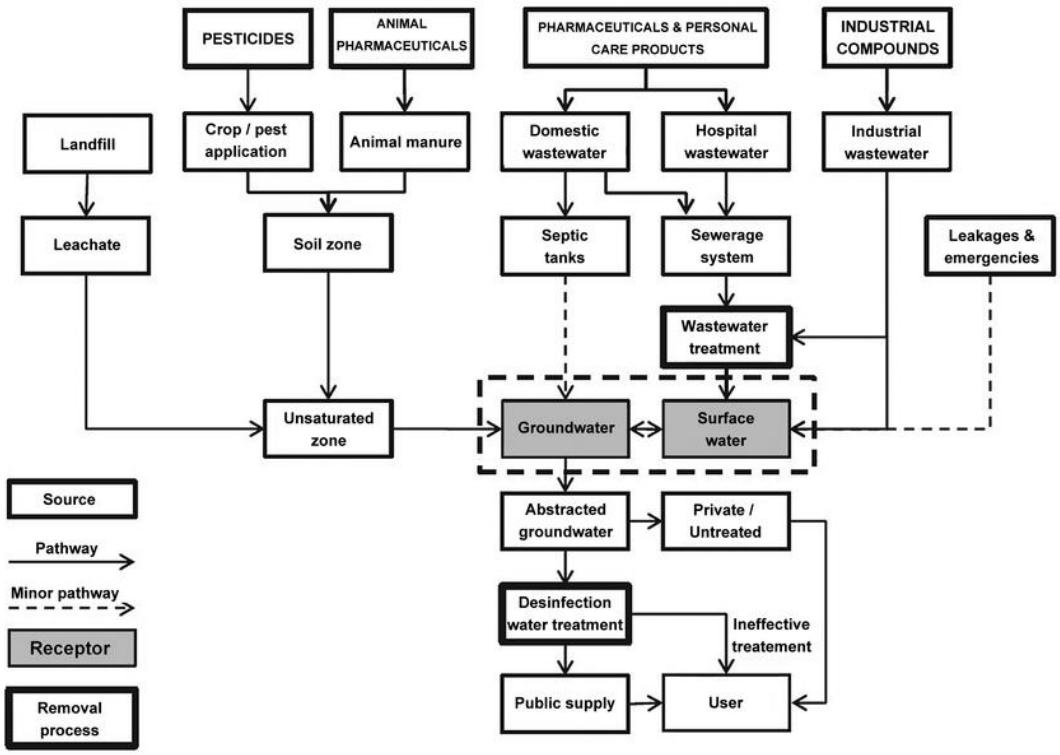


Figure 1.2 Sources of Emerging Contaminants [13]

1.1.2 Effects of ECs in human health and environment

Effects of CECs on human and ecosystem health are largely unknown, and relatively little is known about the fate and the transformation or degradation of these compounds during the pathway they follow. Human health, environmental waters, living organisms such as invertebrates and fish, are mainly affected from ECs. Even at very low exposure to certain ECs they can have impacts on biological systems. Effects of ECs depend upon the hazards related to the studied contaminant as well as the frequency and concentration of exposure to that substance. The hazards associated with specific types of contaminants are discussed in following sections. Table 1.2 presents several examples of ECs categories with associated effects. It is illustrated that ECs have many different potential health impacts on humans and other species. The potential to cause cancer or have toxic effects in animals and humans is noted, but endocrine disruption is the most frequently concern.

Table 1.2 Examples of ECs along with corresponding effects [14]

Use Category	Matrix detected	Suspected health effects from environmental exposure
Antibiotics	Groundwater, surface water, wastewater treatment plant effluent, land applied biosolids, potable water, recycled water	Antibiotic resistance in disease causing bacteria complicating treatment of infections
Disinfectants	Wastewater treatment plant effluent, treated potable water, ground and surface waters, recycled water	Genotoxicity, cytotoxicity, carcinogenicity
Fire retardants	Rivers down gradient of landfills and PBDE manufacturing sites, sewage sludge, natural waterways, sediments, bioaccumulation in fish, whales and other aquatic organisms	Endocrine disruption, indications of increased risk for cancer
Industrial chemicals	Industrial and household waste, soil	Can be toxic to animals, ecosystems, and humans
Life-style products (nicotine, caffeine)	Potable water, groundwater and surface waters affected by sewage or wastewater treatment plant effluent	Can cause cellular stress, negative effects on reproductive activity in animals
Non-prescription drugs	Wastewater treatment plant effluent, surface water, potable water, recycled water	Unknown health effects
Other prescription drugs	Potable water, recycled water, groundwater, surface water, wastewater treatment plant effluent, land applied biosolids	Increased cancer rates, organ damage

Personal care products	Ground-waters, surface waters, sewage, wastewater treatment plant effluent, biosolids, aquatic sediments, biological samples (bioaccumulated in fish tissues)	Bacterial resistance, endocrine disruption
Pesticides	Groundwater, surface water, potable water, recycled water	Endocrine disruption
Plasticizers	Surface water	Endocrine disruption, increased risk of cancer
Reproductive hormones	Surface waters, potable water, recycled water, wastewater	Endocrine disruption
Solvents	Groundwater, soil, potable water	Endocrine disruption, liver and kidney damage, respiratory impairment, cancer
Steroids	Surface waters, potable water, recycled water, wastewater Groundwater, soil, potable water	Endocrine disruption

1.1.2.1 Endocrine disruption

Endocrine disruptors are substances which may not be directly toxic but may have the ability to interfere with the natural functioning of the system responsible for regulating hormones (endocrine system). Endocrine system disruption may lead to cancerous tumors, birth defects and developmental disorders. The Environmental Protection Agency (EPA) defines environmental endocrine disrupting compounds as exogenous agents that interfere with the "synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior" [15]. A diverse range of chemicals can cause disruption of the endocrine system in some species, even at very low concentrations (ng/L). Endocrine substances which may be disrupted include:

- Oestrogens .They provide the stimulus for reproduction, development, and function of the female sex. The principal oestrogen in vertebrates is 17β-oestrodiol. Oestrogenic endocrine disrupting substances include: alkylphenol polyethoxylates, alkyl phenols, phthalates, and bisphenolic compounds. Pesticides may also have androgenic and hypothalmic activity.
- androgens – produce masculine characteristics, the development of skeletal muscle
- and bone and the development of the male reproductive organs

- thyroid hormones – regulate all functions related to the development and metabolic activity
- neurohormones – released from the hypothalamus and maintain the functioning of the endocrine system

1.1.2.2 Toxicity

The adverse effects of chemicals may be evaluated with several ways. As a result of a single short exposure, the acute toxicity of a chemical refers to its potential to do harm. For accidents and spillages where the health effect is rapid, this is likely to be important. Chronic toxicity refers to a chemical's ability to cause harm due to frequent or prolonged exposure, e.g. in an industrial environment or by food or drinking water. The chronic effects of a substance can be classified into various types, such as:

- toxicity (ability to cause unspecified harm)
- carcinogenicity (ability to produce tumors)
- mutagenicity (ability to cause alteration of genetic material)
- teratogenicity (effects on the fetus)

Genotoxic carcinogens, which are considered to pose the greatest risk to humans, cause cancer by interfering with genetic information in the affected cells. Other potential effects are allergies and disruption of the immune and nervous systems. Toxic effects can be used in order to prioritize pollutants in monitoring studies.

1.2 Pharmaceuticals and Personal Care Products

Pharmaceuticals are chemicals used for diagnosis, treatment (cure/mitigation), alteration, and prevention of disease and to address problems related to health conditions or the structure/function of the human and/or animal body. Pharmaceuticals, a milestone in human scientific development, have lengthened life spans, cured millions from deadly diseases, and improved the quality of life. They are designed either to be highly active and interact with receptors in humans and animals or to be toxic for many infectious organisms. Because of the nature, they can also bioaccumulate and have unintended effects on animals and microorganisms in the environment. Although the effects of the pharmaceuticals are investigated through safety and toxicology studies, the potential environmental impacts of their production and use are less understood and have recently become a topic of research interest. These compounds do not consist of a consistent group of substances with similar chemical, structural, biological, or

toxicological properties. For this reason, are classified according to their purpose and biological action (e.g., antibiotics, analgesics, anti-neoplastics, anti-inflammatory substances, antihistamines, X-ray contrast media, surface disinfectants, etc.). In this context can be analyzed according to their classification in therapeutic classes. The need for classification was fulfilled in 1981, by WHO Collaborating Centre for Drug Statistics Methodology establishing the Anatomic Therapeutic and Chemical (ATC) classification system. According to ATC classification system, the active substances are classified in a hierarchy with five different levels. The system has fourteen main anatomical/pharmacological groups or 1st levels (Table 1.3). Each ATC main group is divided into 2nd levels which could be either pharmacological or therapeutic groups. The 3rd and 4th levels are chemical, pharmacological, or therapeutic subgroups and the 5th level is the chemical substance. The 2nd, 3rd and 4th levels are often used to identify pharmacological subgroups when that is considered more appropriate than therapeutic or chemical subgroups.

Table 1.3 Classes and codes of anatomic therapeutic

Code	Classes	Code	Classes
A	Alimentary tract and metabolism	**L**	Antineoplastic and immunomodulating agents
B	Blood and blood forming organs	**M**	Musculo-skeletal system
C	Cardiovascular system	**N**	Nervous system
D	Dermatologicals	**P**	Antiparasitic products, insecticides, and repellents
G	Genito-urinary system and sex hormones	**R**	Respiratory system
H	Systemic hormonal preparations, excluding sex hormones and insulins	**S**	Sensory organs
J	Anti-infectives for systemic use	**V**	Vrious

The classification of PhACs by their chemical structure is used mainly in the analysis of active substances within subgroups of medicines, for example, within the group of antibiotic subgroups such as β-lactams, cephalosporins, penicillins, or quinolones. In these subgroups, the compounds might be expected to have similar chemical behavior. However, even minor changes in the chemical structure may have a significant impact on other properties that govern their environmental fate [16]. The therapeutic groups most commonly detected in water are (Fig. 1.3) [17,18]:

- Antibiotics (tetracyclines, macrolides, β-lactams, penicillins, quinolones, sulfonamides,

fluoroquinolones, chloramphenicol, and imidazole derivatives).

- Anti-inflammatories and analgesics (paracetamol, acetylsalicylic acid, ibuprofen, and diclofenac).
- Antidepressants (benzodiazepines).
- Lipid-lowering drugs (fibrates).
- β-Blockers (atenolol, propranolol, and metoprolol).
- Veterinary products
- Antipsychotics
- Antiepileptics (carbamazepine).
- Estrogens and hormonal compounds

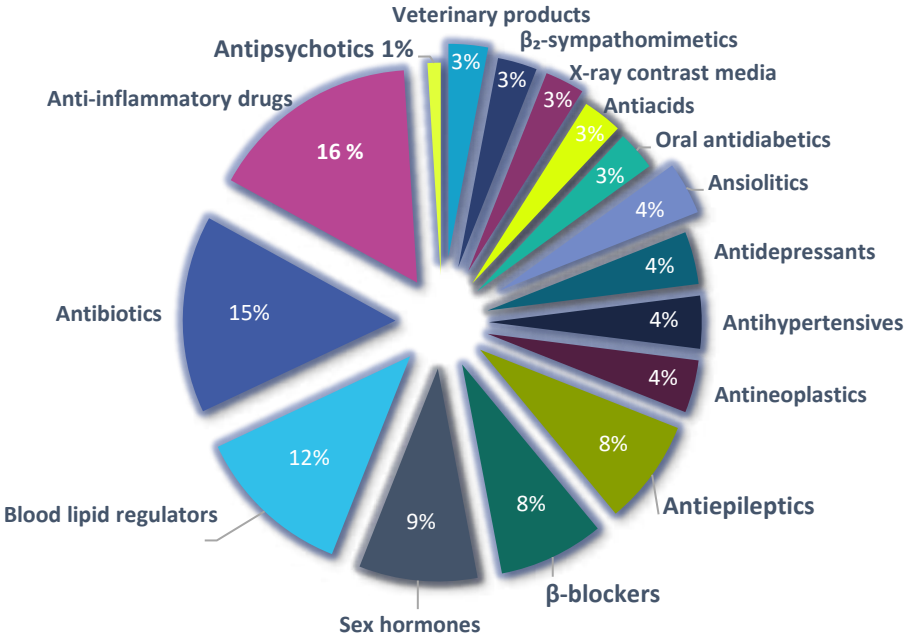


Figure 1.3 Relative percentage of therapeutic classes detected in the environment [19]

Pharmaceuticals are also mentioned as Pharmaceutically Active Compounds (PhACs) a term to describe that is about complex compounds with different physicochemical and biological properties and functionalities, that are pharmacologically active, resistant to degradation, highly persistent in aqueous medium, and potentially able to exhibit harmful effects in water organisms and have a negative impact on human health [17]. According to Rivera-Utrilla et al., [17] pharmaceutical contaminants differ from most other contaminants based on: (a) having molecular masses <500 Da, although larger for some compounds, (b) containing chemically complex molecules with a large variety of structures, shapes, molecular masses, and

functionalities, and (c) consisting of polar compounds having more than one ionizable group. Pharmaceuticals can also (d) exhibit properties and a degree of ionization that depends on the medium's pH, and (e) have lipophilic properties, while some may also have moderate water solubility. They also share (f) the ability to persist in nature, accumulate in life forms, and remain biologically active. For example, naproxen, sulfamethoxazole, and erythromycin can persist for almost one year while clofibric acid can remain unchanged for multiple years. Finally, (g) these molecules tend to adsorb and be distributed in a living body, which metabolically modifies their chemical structure [20]. Metabolites as well as environmental transformation products (TPs), along with parent PhACs, pose threats to the aquatic and terrestrial environment.

Similarly, Personal care products (PCPs) are yet another class of emerging contaminants that incorporate health products such as supplements, over-the-counter drugs prescribed and non-prescribed veterinary and human pharmaceuticals and the agile and inert elements for individual care purposes. A few PCPs to name are cosmetic products, engineered hormones, steroids, perfumes, shampoos, etc. UV filters, known to have estrogenic activity, are reported to be one of the most commonly found PCPs in aquatic environment. PCPs are released into wastewater and advance toward WWTPs, in their native or biologically transformed structures [21].

Hundreds of tons of pharmaceuticals and personal care products are dispensed and consumed annually world-wide. The usage and consumption of these products have been increasing consistently due to the discoveries of new drugs, the expanding population, and the inverting age structure in the general population, as well as due to expiration of patents with resulting availability of less-expensive generics [2].

Irrational use of medicines is a major problem worldwide. World Health Organization estimates that more than half of all medicines are prescribed, dispensed or sold inappropriately, and that half of all patients fail to take them correctly. The overuse, underuse or misuse of medicines results in wastage of scarce resources and widespread health hazards. Examples of irrational use of medicines include [22]:

- use of too many medicines per patient ("poly-pharmacy")
- inappropriate use of antimicrobials, often in inadequate dosage, for non-bacterial infections
- over-use of injections when oral formulations would be more appropriate
- failure to prescribe in accordance with clinical guidelines
- inappropriate self-medication with prescription-only medicines

1.2.1 Veterinary drugs

Veterinary pharmaceuticals, on the other hand, were traditionally less covered in environmental and human health toxicity studies. However, they are being increasingly used in many regions to improve the time of the lifetime of livestock, poultry, pets, aquatic animals, silkworms, bees etc. [23]. Current livestock and aquaculture production practices include the use of a wide variety of pharmaceuticals to enhance animal health and prevent the animals from diseases including antimicrobials (antibiotics), and other medicinal products. In addition, dietary enhancing feed additives including growth enhancers, feed supplements, are also incorporated into the feed of animals reared for food in order to improve their growth rates [9].

Specifically, veterinary medicines include substances used to kill or control a range of infections due to microorganisms (antimicrobials), internal and external parasites (ectoparasitides and endectocides) and fungi (antifungals). Other substances used include hormones, anaesthetics, tranquilisers, euthanasia products and anti-inflammatories. Many of these compounds are also used as pesticides (e.g. cypermethrin and diazinon) and as human medicines (e.g. oxytetracycline)[9] . Although there is a wide spectrum of therapeutic classes, veterinary practice tends to use mostly antibiotics, antiparasitic drugs and steroidal hormones. These veterinary compounds are followed by substances employed for treatment of alimentary tract and metabolism, compounds used on the central nervous system and other pharmaceuticals.

A large number of antibiotics employed in animal food production are inefficiently adsorbed in the animal's gut, and, as a result, almost 30–90% of these drugs are excreted Moreover, VA additives can be active and converted back to the prototype after excretion. Thus, a considerable percentage of the veterinary antibiotics may spread into the surroundings in bioactive forms, which will cause long-term adverse effects on the soil, water, microorganisms, plants, and animals and finally affect human health through the food chain [23]. These products must be assessed for their quality, efficacy, and safety (to both humans and the environment). Release occurs both directly, e.g. in fish farms, and indirectly via the application of animal manure to land. The frequent use of VAs has captured the attention about the potential increasing populations of new resistant strains of bacteria. Detected bacterial populations from gut of animals given antibiotics were about five times to be resistant to common antibiotic resistant microbial strains [23].

1.2.2 Global Pharmaceutical market

The world pharmaceutical market was worth an estimated € 845,235 million ($ 998,223 million) at ex-factory prices in 2018. The North American market (USA & Canada) remained the world's largest market with a 48.9% share, well ahead of Europe and Japan (Fig.1.4) [24].

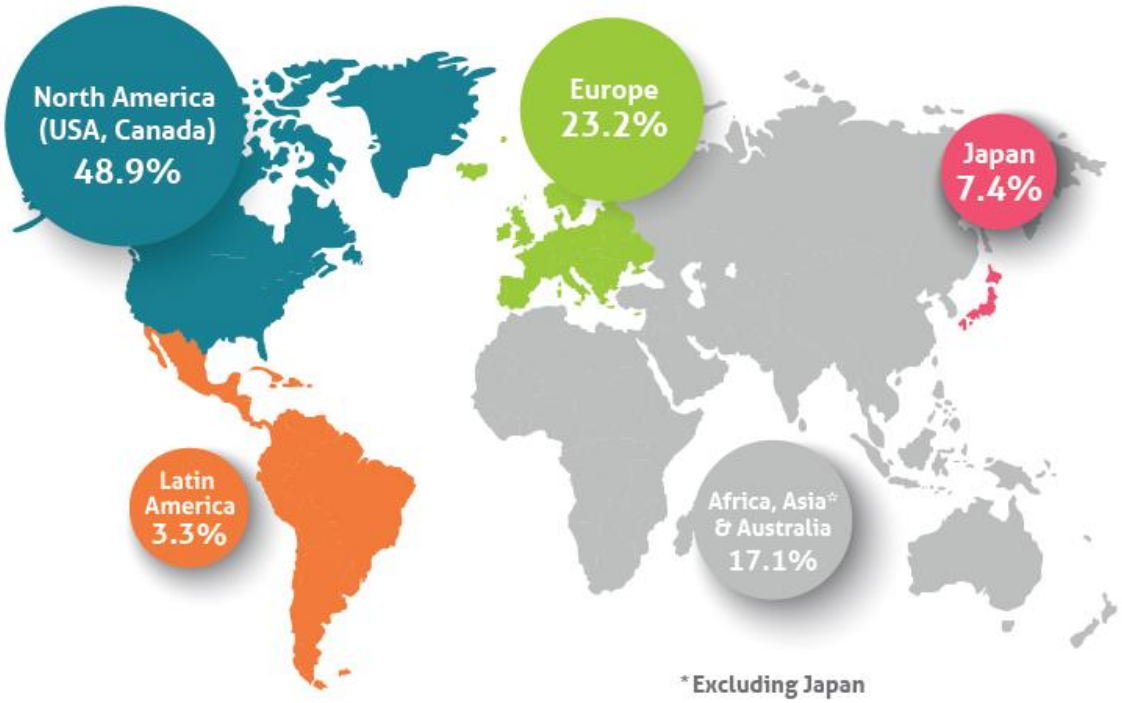


Figure 1.4 World Pharmaceutical Market – 2018 Sales, [Source: IQVIA (MIDAS), May 2019 (data relate to the 2018 audited global retail and hospital pharmaceutical market at ex-factory prices)]

1.2.3 Sales of pharmaceuticals in Greece

Sales of pharmaceutical products to pharmacies & wholesalers (in values) amounted to €4.1 bil. in 2018, showing an increase of +2.5% compared to 2017. Similarly, sales to hospitals & EOPYY pharmacies amounted to €2.1 bil. in 2018 presenting a higher increase of +16.7% compared to previous year. Approximately, 66.1% of total sales supplied to wholesalers and private pharmacies, while the remaining 33.9% to hospitals and EOPYY pharmacies. Regarding the number of packages, an increase of +1.0% was recorded in 2018 compared to 2017 (567.7 mil. packages) with an increase of 1.0% in pharmacies/wholesalers and an increase of 1.2% in hospitals/EOPYY pharmacies was depicted (Fig.1.5) [25].

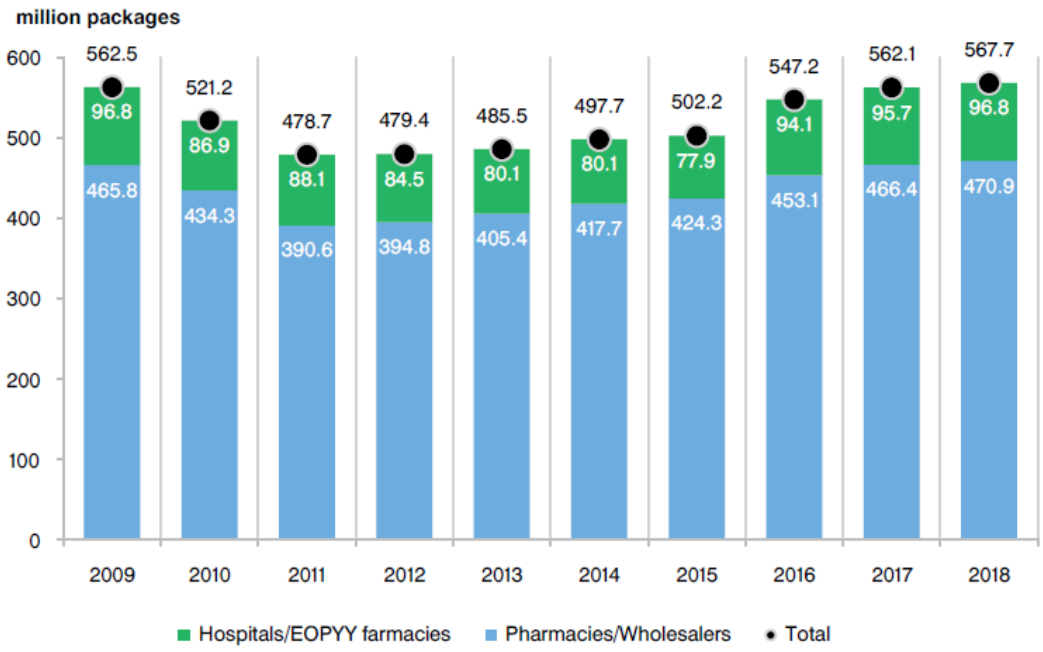


Figure 1.5. Sales of pharmaceutical products in volume (mil. packages) – Greece [25]

In addition, self-care is a major public health resource and the fundamental level of resilient health care systems. Approximately 9.7 billion packs of non-prescription medicines (over the counter, OTC) and 1 billion packs of minerals and vitamins were bought by Europeans in 2019 to improve their health and contribute to their well-being. Regarding the market of Over the Counter drugs (OTC) in Greece, followed an upward trend from 2013 onwards from €122 mil. in 2013 to €165 mil. in 2017, an increase of 35.2% (Fig.1.6).

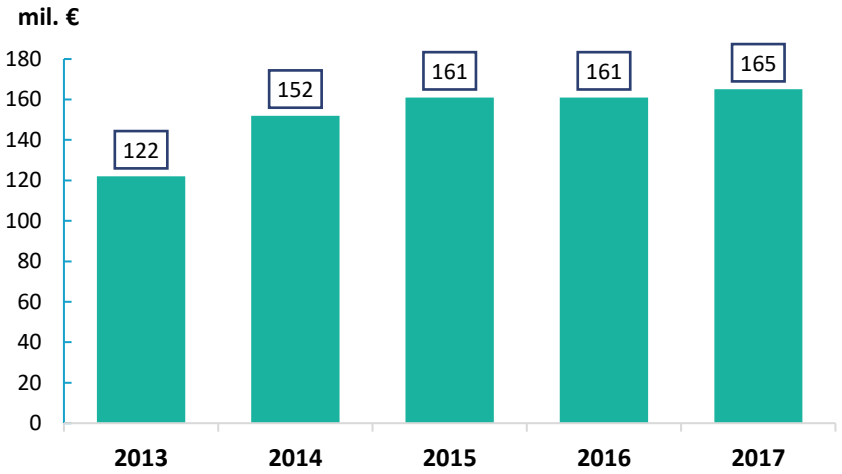


Figure.1.6 OTC sales in value 2013-2017 (mil. €) in Greece [25]

The general Distribution Medicines a subset of OTC are available outside pharmacies and concern analgesics, antipyretics, antipruritic, topical medications, laxatives (to treat constipation) and

mouthwashes. Of the self-medication products, analgesics, cough and cold products, digestive products, dermatological products and vitamins recorded the largest sales.

1.2.4 Consumption of Antibiotics in Europe

Antimicrobial consumption data are collected using the Anatomical Therapeutic Chemical (ATC) classification system and defined daily dose (DDD) methodology developed by the WHO Collaborating Centre for Drug Statistics Methodology (Oslo, Norway) [26]. In 2018, the EU/EEA population-weighted mean consumption of antibacterials for systemic use in the community (i.e. outside hospitals) was 18.4 DDD per 1 000 inhabitants per day, ranging from 8.9 in the Netherlands to 32.4 in Greece [27], (Fig.1.7).

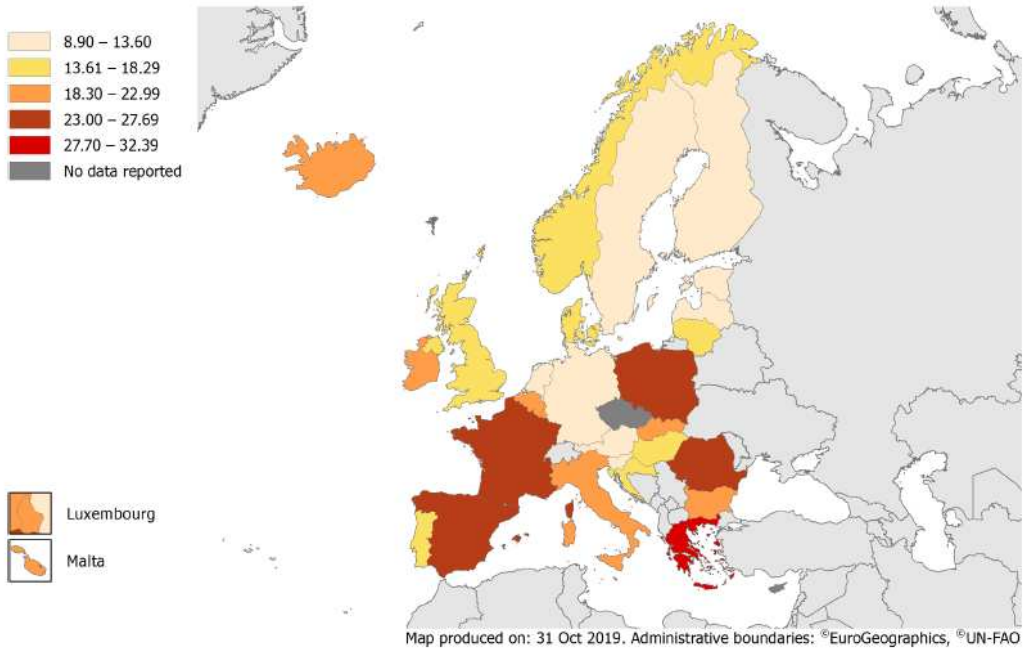


Figure 1.7 Consumption of antibacterials for systemic use (ATC group J01) in the community in EU/EEA countries in 2018 (expressed as DDD per 1 000 inhabitants per day) [27]

Consumption of major subgroups of antibacterials for systemic use (ATC group J01) in the community in 2018 is presented in Fig.1.8. As in previous years, penicillins (ATC group J01C) were the most frequently used antibacterials in all countries, ranging from 25% in Slovakia to 67% in Denmark of the total consumption in the community. The proportion of other antibacterial groups varied more widely between countries. For example, cephalosporins and other beta-lactams (ATC group J01D ranged from 0.2% in Denmark to 31% in Slovakia; macrolides, lincosamides and

streptogramins (ATC group J01F) ranged from 5% in Sweden to 26% in Poland; and quinolone antibacterials (ATC group J01M), from 2% in Norway to 17% in Hungary.

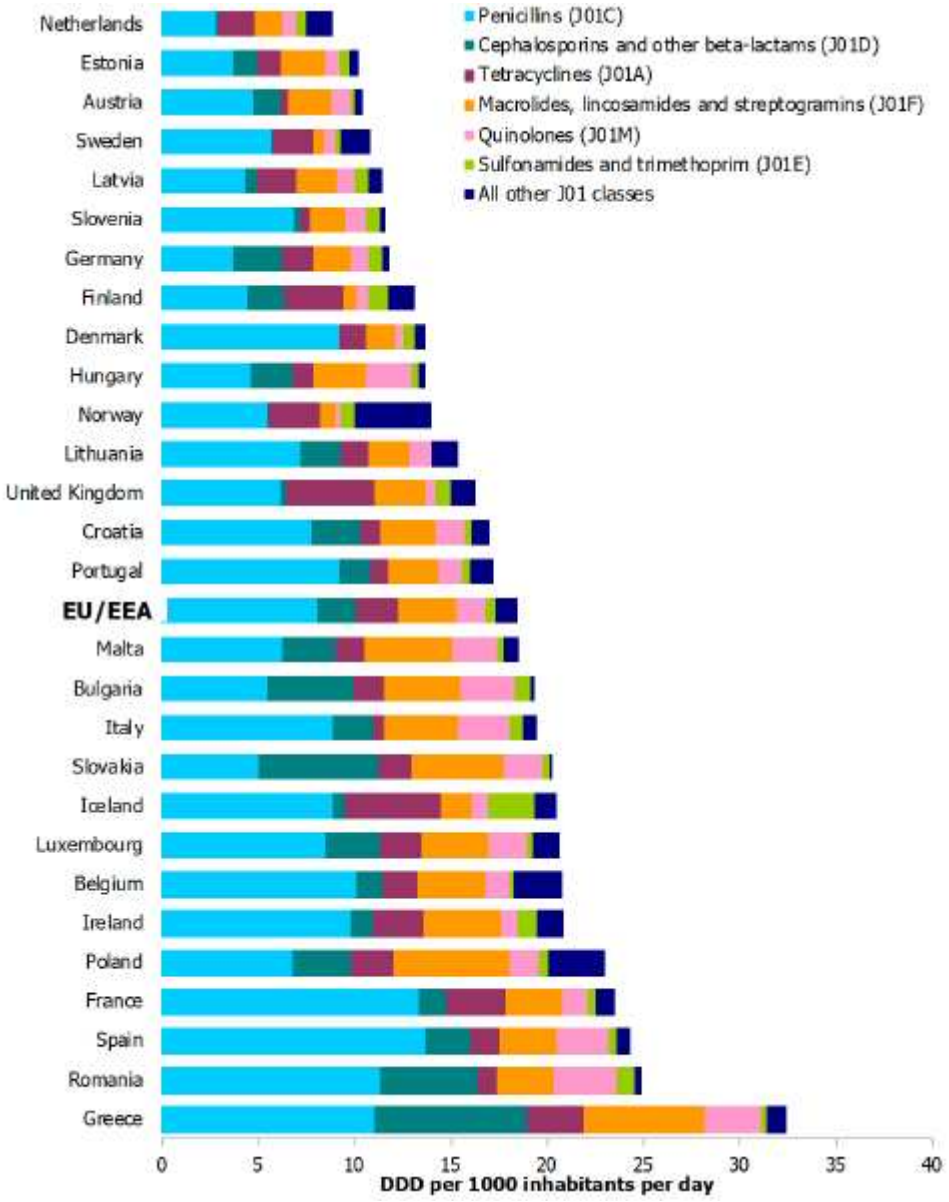


Figure 1.8 Consumption of antibacterials for systemic use (ATC group J01) by country and ATC group level 3 in the community in EU/EEA countries in 2018 (expressed as DDD per 1 000 inhabitants per day) [27]

1.2.5 Metabolism of PhACs

Since active pharmaceutical ingredients are generally ingested, they may be extensively metabolized. Drug metabolism refers to chemical alterations of a drug in vivo in the human body. In general, active pharmaceutical ingredients are metabolized and form more polar and water-soluble derivatives that have reduced pharmacological activity com- pared to the active pharmaceutical ingredient and are rapidly excreted. In some cases, however, the administered

compound may be a pro-drug, and must first be metabolized to the active pharmaceutical ingredient before being further metabolized to less active forms. The extent of drug metabolism in the human body varies among the pharmaceutical compounds, ranging from undetectable (e.g., X-ray contrast media iopromide and diatrizoate) to almost complete biotransformation (e.g., carbamazepine and diazepam). The metabolism of drugs in the human body can be divided into Phase I and Phase II reactions (Fig. 2). The first type comprises hydrolysis, along with oxidations (e.g., aliphatic hydroxylation of ibuprofen and diclofenac, ring oxidation of propranolol, epoxidation of carbamazepine, N-oxidation of trimethoprim, N-oxidation of acetaminophen), reductions (e.g., ketone reduction in prednisone), alkylations (e.g., O-methylation of norepinephrine) and dealkylations (e.g., O-demethylation of naproxen and diazepam, O-deethylation of phenacetin). The second type refers to conjugation reactions, in which a usually polar group or molecule is transferred to the parent drug or a metabolite formed previously in a Phase I reaction. The most prominent Phase II reaction is glucuronidation. Because of the general availability of glucose in biological systems, glucuronide formation is one of the more common routes of drug metabolism and quantitatively may account for a major share of metabolites. The reaction involves the condensation of the drug or its biotransformation product with D-glucuronic acid. Several types of drugs tend to form glucuronides, including alcohols, phenols, carboxylic acids, amines and certain thiols as well as normally occurring substrates such as steroids. In general, glucuronide formation diminishes the biological and pharmacological activity of a drug. Less frequent are sulfation (e.g., of hydroxylated diclofenac), N-acetylation (e.g., of sulfamethoxazole) and amino-acid conjugation (e.g., of salicylic acid with glycine) [28].

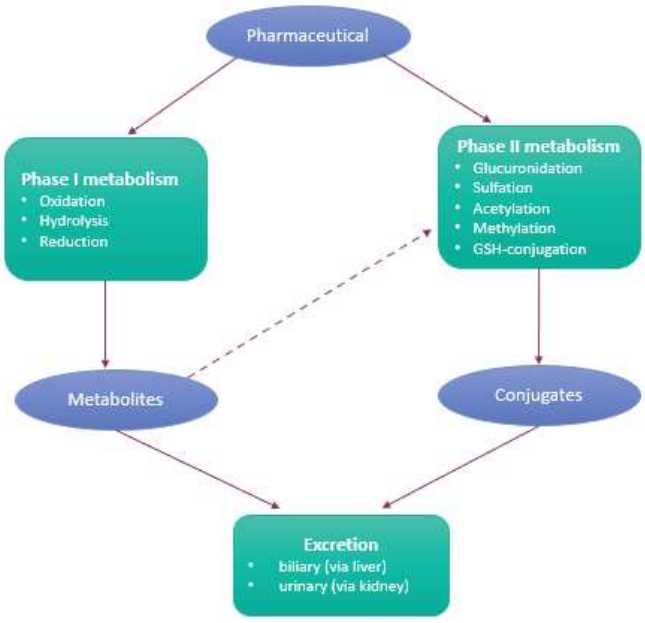


Figure 1.9 Metabolism of pharmaceutical in human body [28]

Administered pharmaceutical active compound may be excreted:

- unchanged
- as a glucuronide or sulfate conjugate
- as a "major" metabolite
- as a complex mixture of many metabolites

In general, metabolism may occur in several ways, including relatively simple conjugation to more polar glucuronides or sulfates, transformation to structurally similar metabolites which may have partial activity or be inactive, or transformation to a number of structurally related as well as unrelated species[29]. There is evidence that glucuronides, which are the simplest and most common form of conjugated pharmaceutical compounds excreted by humans, are capable of being reverted back to original compound during wastewater treatment plant The glucuronidase enzyme is present to such an extent in the fecal coliform bacteria that are so prevalent in WWTPs that its occurrence can be used as an indicator of fecal coliforms in environmental waters. Published research has demonstrated such deconjugation in oestrogenic compounds, antidepressant drugs, anti-inflammatory drugs, some antibiotics etc. [16,30–32]

1.2.6 Sources and fate of Pharmaceutical Active Compounds in the environment

Emerging pollutants can reach the environment by being transported and distributed via different routes. The physicochemical properties of chemicals (e.g., water solubility, vapor pressure and polarity) determine their behavior in the environment [33]. Then, biological and ecological processes such as bioavailability, biodegradation, and bioconcentration need to take place [34].

All these processes determine the life cycle of a pharmaceutical product after its release in the environment Wastewater treatment plant (WWTP) processes are not able to remove a pharmaceutical substance that enters the environment due to its physicochemical characteristics. For the same reason the pharmaceutical active compounds may resist photodegradation, chemical degradation, or biodegradation, persist in the soil, accumulate. Next after minimal degradation or transformation in sewage or soil this substance may be washed into groundwater resulting to direct or indirect explosion to living organisms. Synergistically to these phenomena can be the trophic dilution, zero-order metabolism, bioconcentration, and eventually high susceptibility or harmful impacts in nontarget organisms [35].

Additional to the molecular structure and physicochemical properties of PhACs, the properties and conditions of each environmental territory are important as well. Climate conditions,

temperature, light intensity, water composition (e.g., salt concentration), pH, microbial contribution and turbidity are some of the parameters that influence the fate of a contaminant in water [36], [37]. Concerning the distribution of PhACs in soil and sediment, organic matter and clay content, microbial diversity, pH, and the hydrogeological conditions, are directly correlated to assess soil retention time, persistence, and potential groundwater contamination [38]. Aqueous solubility, partition coefficient n-octanol/water (Kow), vapor pressure, and adsorption coefficient (Koc) are the most characteristic properties related to environmental toxicology of a drug. Additional to these, Kd, represents distribution between two phases of water and sludge or water and soil (LogKd). All these properties are responsible of the partition or the so call environmental mobility which means the distribution and displacement of a pharmaceutical compound in different environmental matrices. PhACs with high water solubility, they are very polar substances with high environmental mobility since they are most likely to be degraded by chemical hydrolysis [39]. On the contrary, PhACs with high Kow values tend to be adsorbed in soil and accumulate in living organisms [39]. Furthermore, a substance with a high Koc value tend to be distributed in the soil and will consequently have a high potential to contaminate surface waters. As far as degradation concerns, a drug released in the environment can undergo chemical degradation mainly through hydrolysis (in the aqueous phase) or by photolysis (in the air but also in water or soil), as well as aerobic or anaerobic biodegradation with the aid of microorganisms [38,40]. In Table 2 are presented the pharmaceutical properties mainly related to the corresponding environmental fate processes regarding persistence, bioaccumulation, and toxic potential in organisms.

Table 1.4 Environmental fate and transformation of drugs according to their physicochemical properties [39]

Environmental fate process	Contributing pharmaceutical properties
Mobility in water	Water solubility (high) Koc (low) pKa value(s)
Mobility in soil	Koc (low) Soil sorption behavior
Mobility in the air	Vapor pressure (low)
Sorption in sewage sludge	Koc (high) Kd LogKow, LogP (large)

Table 1.3 Environmental fate and transformation of drugs according to their physicochemical properties *(continued)*	
Leach into groundwater	Low sorption behavior pKa value(s)
Soil sorption, suspended solids, and sediment	Water solubility (low) Vapor pressure (low) LogKow, LogP (large) Koc (high) Kd (high) Redox potential Stereochemical structure Acidic and basic groups within the same molecule Planar aromatic structures Positively charged species
Volatilization from water surfaces (partitioning to the air)	Water solubility Vapor pressure (low) Henry's law constant (greater than 10^{-4} atm-m^3/mol) Amphoteric compounds
Volatilization from dry soil surfaces (partition in the air)	Vapor pressure (low) Henry's law constant (greater than10^{-4} atm-m^3/mol)
Photodegradation	Aromatic centers or conjugated double bonds in the chemical structure
Chemical degradation (abiotic degradation)	Presence of functional groups that hydrolyze under environmental conditions
Microbial degradation (biodegradation)	Water solubility (high) Structural attributes (e.g., minimal number of halogens, polycyclic residues and nitro, azo and arylamino groups) Antimicrobial activity (e.g., antibiotic)
Bioaccumulation (biota uptake)	pKa LogKow, LogP (large) Koc (high)
Bioconcentration (biota uptake)	Water solubility (low) pKa LogKow, LogP (large) Bioconcentration factor (BCF) (high)

The main sources for their entrance to the aquatic and terrestrial bodies are described below along with their fate which is also illustrated in Fig.1.10

- **Pharmaceutical production industry**

Because of the Good Manufacturing Practice (GMP) regulations required for the manufacturing of pharmaceuticals, the frequently high value of the active substance emissions during manufacturing has been thought to be negligible. Indeed, such emissions are low in Europe and the North Americas. Only recently it has been found, however, that in Asian countries concentrations up to several mg/l can be found in effluents for single compounds [41–43].

- **Private Household and WWTPs**

After the administration of pharmaceuticals and absorption by humans, PhACs are metabolized, although substantial fractions of the original compounds are often excreted in unchanged forms or as metabolites via urine or feces. PhACs can be excreted through biochemical reaction by two pathways, already mentioned : a) oxidation, reduction, hydrolysis, and alkylation reactions and b) excretion of more polar and hydrophilic derivatives as glucuronide or sulfate conjugates.

After the excretion, flushed PhACs, PCPs and the corresponding metabolites end up in the sewage system and pass through a WWTP. In WWTPs, the removal efficiency depends heavily on the chemical characteristics of the compounds. The conventional wastewater treatment plants (WWTP) are not prepared for the treatment of complex pharmaceuticals, as they were designed with the principal aim of removing biodegradable carbon, as well as nitrogen and phosphorus compounds [44]. Therefore, these pharmaceuticals residues may pass through the treatment plants In WWTPs, converted to CO_2 and water; mixing with the receiving water bodies either as the original or mineralized product and reach surface water such as rivers, seawater, streams and lakes or even groundwaters and drinking water. If the compound or the biologically moderated transformation product is lipophilic sorption by the solids like sludge/biosolids, may occur. Once in the environment, pharmaceuticals and their metabolites undergo natural attenuation by adsorption, dilution or degradation in the environment (e.g. photolysis, biodegradation, other chemical reactions [33,45].

PCPs such as vitamins, OTC-drugs, follow the same route in the environment with pharmaceuticals by their excretion in the human body and discharge in sewer network. PCPs incorporated in toothpastes, mouthwashes, detergents etc., are flushed through sink and drains, while shampoos, cosmetic creams, fragrances etc. through shower waste. Dermal contact for consumers may occur through clothing or personal care products, or inhalation may occur from building materials at home or in the workplace too [46].

Another household source of pharmaceuticals and personal care products is their disposal directly to the waste. The disposal of pharmaceuticals by sewer drain system is still the most common method in many countries with the absence of the proper disposal of expired medications from the patient side [47]. Despite the directive 2004/27/EC regarding the medication waste [48], flushing unused medications down the toilet or sink, especially liquid dosage forms, has been reported in a lot of studies from a number of countries such as United States [49], England [50], New Zealand [51], and Iraq [52].

- **Animal Husbandry and Veterinary Medicine**

There are a number of potential routes for veterinary drugs to reach the aqueous environment depending on how these drugs are emitted during the treatment process . For instance, it depends on whether the animal received the treatment topically, in feed or as an injection or bolus, and on the methods of husbandry. The most important routes for entry into the environment are likely to be the direct discharge of aquaculture products, the excretion of substances in the urine and faeces of livestock animals and the wash-off of topical treatments. There is limited or no evidence for significant contributions from the other routes [53].

Specifically, veterinary drugs and their metabolites are excreted with manure. Farmers use manure and sewage sludge to fertilize fields, thus the drug residues are introduced into the soil. Veterinary pharmaceuticals may reach surface water as run-off from the soil after rain [16]. The wash off from topical treatment may enter soil or ambient water environment. The route of release of pharmaceuticals to the environment by animal breeding and subsequent excretion by livestock, has been less studied but can by no means be neglected due to an extensive list of compounds registered as veterinary drugs. Moreover, some of the medications used currently in the livestock industry had been previously approved for humans but banned due to adverse effects. It is noteworthy to mention that as long as pharmaceuticals excreted by humans most of them reach wastewater treatment plants and their concentrations can be decreased to some extent during treatment processes, dislike to veterinary drugs which are more likely to reach the aquatic environment directly [48]. Typical example is the application of pharmaceuticals in aquaculture that results in direct input into water and sediments.

- **Hospitals**

Hospital wastewater is the other main source of contamination, although the dilution of hospital effluents by municipal wastewaters will lower the concentration of pharmaceuticals only moderately, because the latter also contain pharmaceuticals from households and veterinary

sources. Hospital wastewaters contain wider spectrum and higher quantity of pharmaceuticals than urban wastewaters, but they are generally discharged in sewers without pretreatment. Since traditional urban wastewater treatment plants (WWTP) are not designed to treat hospital treated effluents may still contain pollutants that could impair receiving aquatic environments [54].

The contribution from hospital wastewater appears to be lower than from household wastewater but some pharmaceuticals used in hospital may present an elevated toxicity risk because of their low no-observed-effect level (NOEL) and high level of consumption [55]

Furthermore, hospital effluents also play an important role in the introduction of pathogens into public wastewaters, especially concerning multi-resistant bacteria, contributing to the spread of antibiotic resistance into the environment [56].

- **Landfills**

Directive 2004/27/ EC (relating to medicinal products for human use) introduces the obligation of Member States to have "specific precautions relating to the disposal of unused medicinal products" as well as the obligation to implement appropriate collection systems [48]. However, several surveys have noted that the implementation of these systems and their efficiency varies widely across Member States [29,57]. As a result of discarding expired and unwanted drugs along with household waste, landfills are expected to be a source of pharmaceuticals products. Since many of the disposal sites are still open dumps without protective barriers and leachate collection systems, there is a danger of infiltration of contaminated leachates into the soil influencing the quality of groundwater near landfills [37].

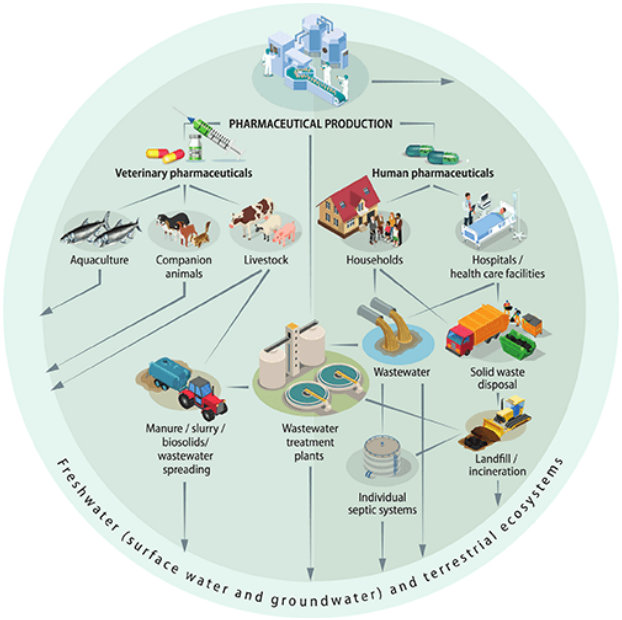


Figure 1.10 Environmental fate of Pharmaceuticals in ecosystems [58]

1.2.7 Effects of Pharmaceutical Active Compounds (PhACs)

The wide variety in pharmaceutical chemical structure associated with the different biological activities, their surface-active nature and their persistence, not only of the parent compounds but also of their metabolites, provide toxic effects in the environment followed by potential risks to human health. Once they enter the environment, chemical compounds become the target of diverse biotic factors (living organisms) and abiotic factors (sunlight, pH, temperature, salts, metals, other chemical compounds, and so on) that can potentially modify their properties and structure. After undergoing a series of physical, chemical, and biological processes, these compounds are transformed to metabolites which are often more persistent and more hazardous than the parent compound [59].

1.2.7.1 Effects of PhACs in environment

As pharmaceuticals do not occur individually in the environment, but as complex mixtures, the interaction of these compounds with wildlife that might have high similarity with the molecular targets, the so-called non-target organisms [60] may occur a relevant environmental concentrations, due to combined and synergistic effects called the “cocktail effect’’ [61]. In addition, the fact that pharmaceutical formulations may also incorporate adjuvants, and in some cases pigments and dyes which are commonly considered of minor importance in terms of environmental significance and impact, highlights the concern about the potential effects of these compounds when released in the environment. Low-level exposure due to their continual release to the environment is likely to lead to chronic effects. For some pharmaceuticals, mode of action is associated with a potentially harmful effect (e.g. cytostatic or endocrine modulating). Taxonomic variation may lead to inadequacy in metabolic, excretory or detoxification systems in some species. Age, sex, population, and species-specific differences may affect susceptibility. Finally, direct, and indirect effects may occur (the latter via the food chain).

Several examples of evidence of pharmaceutical effects in non-target organisms are reported. For instance, the major metabolite of the antibiotic amoxicillin, i.e. amoxicilloic acid, has been found to be toxic, acting as a compound capable of inducing hypersensitivity reactions by rising the immune system [62].

Hormones and steroids are another major example of the hazard that pharmaceuticals pose for the environment. Several studies have reported that exposure to exogenous natural or synthetic hormones can have an adverse effect on the normal reproductive physiology of diverse species, inducing direct effects on gonads such as feminization, demasculinization, or size reduction. It can

also affect reproductive fitness and sexual differentiation, steering it toward the female phenotype during early development in some species. The main sex hormones reported to be present in water include 17α-ethinyl estradiol, 17α-estradiol, 17β-estradiol, estrone, 17α-testosterone, and 17β-testosterone or androstenedione [63,64].

Furthermore, NSAIDs have also been reported to induce oxidative stress, cyto-/genotoxicity, and even teratogenesis in diverse aquatic species such as Hyalella azteca, Danio rerio, Cyprinus carpio, and Xenopus laevis among others [65–67]. A well-documented example of unanticipated toxicity of NSAIDs and in particular diclofenac, in population level is the deaths of large numbers of vultures in Central Asia caused to the accidental ingestion of diclofenac by feeding on a putrefied body of cattle which before was treated with the specific drug [35]. The foraging mode of Asian vultures and their sensitivity to certain NSAIDs resulted in the near extinction of three keystone species [68,69]. Vulture mortality causes an ecological imbalance becoming perhaps a potential threat to human health.

Additionally, the occurrence of pharmaceuticals in the environment has been related to a wide range of toxic effects such as: mortality, molting errors, hatching, variation in the rhythm of life, anatomical deformities, sublethal changes in plant growth, changes in biogeochemical cycles, changes in the sexual ratio of higher organisms, damage to microbial communities by disinfectants, trophic relationships by anesthetics, fertility issues, alteration in sexual status by hormones, and toxic reproductive effects by cytostatic drugs [70–72].

1.2.7.2 Effects of PhACs in human health

For humans, risks are therefore less clear than for the environment, but there are still concerns notably regarding certain types of molecules – in particular stemming from the results of European studies (BIO IS, 2014) – even if to date there is no clear evidence of short-term health effects on humans. The biological activity of antibiotics, antiparasiticides, anti-mycotics and anti-cancer pharmaceuticals, which are pharmaceutical groups that are especially intended to kill their target organism or target cells, might notably affect human health via environmental exposure. The mode of action of pharmaceuticals with endocrine-disrupting properties is also of particular concern.

A possible route for human exposure of pharmaceutical metabolites is via drinking water. Although the compounds of drugs in drinking water are at doses far below the ones used in therapy, drinking water standards have not yet been established for most pharmaceuticals.

Nevertheless, adverse health impacts to humans are very unlikely from exposure to the trace concentrations of pharmaceuticals that could potentially be found in drinking-water. It is suggested that appreciable risks to health arising from exposure to trace levels of pharmaceuticals in drinking water are extremely unlikely [73]. However, risks through long-term consumption of drinking water containing trace levels of pharmaceuticals should be evaluated.

Moreover, pharmaceutical effects can act indirectly on populations through the food chain, for example, if a key prey species is negatively affected [35]. Several studies indicate that PhACs can accumulate in fish and other aquatic organisms [74] and thus exposure to these PhACs through consumption by organisms at higher trophic levels including human must be considered. The examined compounds include fluoxetine, sertraline, ibuprofen, naproxen, diclofenac, ketoprofen, gemfibrozil, diphenhydramine, diltiazem carbamazepine and paroxetine [75–77]. A summary of research on bioaccumulation of pharmaceuticals in aquatic organisms highlighted the need to understand thresholds of drug accumulation along with potential risks [72]. Unfortunately, an understanding of human pharmaceuticals accumulating in terrestrial wildlife is poorly evaluated but has been recently recognized as a major research question.

1.2.7.3 Antimicrobial resistance

A potential indirect risk from the high consumption of medication and the eventual release of pharmaceuticals in environment is the development of microbial strains that are resistant to antibiotics or disinfectants. Antimicrobial resistance (AMR) is the ability of a microorganism to resist the action of one or more antimicrobial agents [78]. The consequences can be severe, as prompt treatment with effective antimicrobials is the most important intervention to reduce the risk of poor outcome of serious infections. Their presence in the environment can lead to the emergence and prevalence of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) [79].

Antibiotic resistance is a natural property of bacteria. Many environmental bacteria can not only cope with natural antimicrobial substances but also benefit from their presence. For instance, the use of antibiotics by bacteria as biochemical signals, modulators of metabolic activity or even carbon sources have been demonstrated. In other cases, antibiotics can be tolerated because they have structures similar to the natural substrates of bacterial housekeeping enzymes and thus are inactivated, leading to a natural form of resistance [80]. Before the massive introduction of antibiotics in human activities, the concentrations of these compounds were low and confined to the site of their production. However, use, misuse and inappropriate antibiotic prescriptions has

accelerated the occurrence of ARB and ARGs in the environment [81] especially in wastewater [82,83]. Concern is growing that humans could be exposed to strains of bacterial pathogens that have developed resistance and therefore cannot be killed by existing antibiotics. Some bacteria, owning given genes and physiological functions, are intrinsically resistant to one or more classes of antibiotics. This is an ancestral property within a group and thus is common to most or all representatives of a genus or species [84,85]. In contrast, acquired resistance is observed only in some representatives of a species, in which most of the representatives are susceptible to that antimicrobial agent. Acquired antibiotic resistance may result from gene mutation or genetic recombination. Gene mutations occur randomly in the genome, often potentiated by mutagens. Examples of resistance phenotypes emerging by mutation include altered targets for an antimicrobial agent (e.g. quinolones, rifampin, linezolid, clarithromycin, amoxicillin, and streptomycin) [80] limited access of the antimicrobial agent to the intracellular target (e.g. penicillin, cephalosporins, glycopeptides, and tetracyclines) or transformation and further broadening of the range of antimicrobial agents that can be inactivated (e.g. extended spectrum b-lactamases) [80].

Previously known diseases and infections with well-established means of treatment can re-emerge as significant public health problems because the microorganisms that cause them, have become resistant to the currently used drugs. Resistance to established treatments has been observed with respect to malaria and tuberculosis throughout the world [14]. The widespread use of antibiotics and disinfectants in many products such as detergents and soaps is contributing to the increase in antimicrobial resistance. When an antibiotic is used, a few resistant organisms in the target bacterial population may survive, and the new microbial community may end up containing a higher number of resistant bacteria. The incidence of infections due to drug resistant bacteria in hospitals has increased. Hospitals, which are ‘hotspots’ for antibiotic and antimicrobial use, have seen the rise of two resistant bacterial species: Methicillin- resistant Staphylococcus aureus (MRSA) and Vancomycin-resistant Enterococcus. They are known to cause infections originating in hospitals or nursing homes and can be very difficult to treat. This suggests that the concentrated use of antimicrobial agents in such facilities may be causing the rise in resistance . Moreover, Escherichia coli and Klebsiella pneumoniae, and extended-spectrum beta-lactamase (ESBL) are common pathogens prone to antimicrobial resistance in Europe [86].

Today, the evolution of microbial pathogens able to resist antibiotics treatments is seen as one of the most pressing public health crises [87–89]. The European Centre for Disease Prevention and Control estimates that each year, 25,000 people in Europe die directly from drug-resistant

bacterial infections [90] while recent estimates provided by the British government suggest a worldwide mortality of half a million people [88]. Antibiotic resistance is a serious threat to public health in Europe, leading to increased healthcare costs, prolonged hospital stays, treatment failures. Antibiotic resistance also imposes a significant financial burden on world economies, with the USA alone spending an estimated $35 billion per annum on the treatment of resistant infections [81,87].

In Table1.5 are summarized the adverse effects caused from specific pharmaceutical compounds on organism population and human health.

Table 1.5 Examples of adverse effects of certain pharmaceuticals in the environment on aquatic organisms, and human and animal health [58]

Therapeutic group	Examples of Pharmaceutical	Impact and effected organisms
Endocrine disrupting pharmaceuticals	17β-; EE2, 17α-ethinylestradiol	Disruption with hormones- reproduction toxicity (fish, frogs) Increased risk of breast or prostate cancer (humans)
Analgesics	Diclofenc, Ibuprofen	Organ damage, reduced hatching success (fish) Genotoxicity, neurotoxicity and oxidative stress (mollusk) Disruption with hormones (frog)
Anti-cancer	Mitomycin C, Fluorouracil	Genotoxicity Mutagenicity, carcinogenicity, toxicity to foetus
Antibiotics	-	Reduced growth (environmental bacteria, algae, plants) Indirect effects of antibiotic resistance (humans and animals)
Antidiabetics	Metformin	Potential endocrine-disrupting effects (fish)
Anti-convulstant	Carbamazepine, Phenytoin, valproic acid	Reduced growth (algae, fish), reduced algae growth Disruption with hormones (mammals including humans)
Antihistamines	Hydroxyzine, Fexofenadine, Diphenhydramine	Behaviour changes, growth and feeding rate (fish) Behaviour changes and reproduction toxicity (invertebrates)
Antifungal	Ketoconazole, Clotrimazole Triclosan	Reduced growth (algae, fish), reduced algae growth Disruption with hormones (mammals including humans)
β-blockers	Propanolol	Reproduction behavior (fish), Reproduction toxicity (invertebrates)
Psychiatric drugs	Fluoxetine, Sertraline, Oxazepam, Citalopram, Chlorpromazine	Behaviour changes-feeding, boldness, activity, sociality (fish) Disruption with hormones (fish) Behaviour change -swimming and cryptic (invertebrates) Reproduction toxicity and disruption with hormones (invertebrates)

1.3 Artificial Sweeteners

The sensory properties of food are highly influenced by the sensory properties like taste smell texture and appearance. The selection and consumption of food plays a crucial role in the regulation of human appetite and nutrient intake. A sweetener is a food additive, which mimics the effect of sugar on taste [91].

Artificial sweeteners (ASs)are a class of food additives that provide a sweet flavor with zero or low calories. They may be derived from plant extracts or manufactured by chemical synthesis. They are also named as non-nutritive sweeteners (NNS), high-intensity sweeteners, and low caloric sweeteners (LCS). The increasing prevalence of obesity and diabetes mellitus has led to an increased production and consumption of artificially sweetened foods as a dietary option for the management of these disorders over the world [92]. In contrast to sugar, they do not cause any glycemic effect/insulin response or calorie intake once digested, insulin levels is not affected by their consumption making them suitable for diabetics. In addition, ASs sweeteners are not metabolized as carbohydrates like sugars or only fermented slightly, thus can prevent potential dental plaque by the mouth microflora [93]. Nowadays, saccharin, sucralose, cyclamate, aspartame, acesulfame alitame neotame and neohesperidin dihydrochalcone are the most popular artificial sweeteners consumed by public. Cyclamate was banned in the United States in 1970 due to concern about carcinogenicity [94] but is registered in the European Union (EU) and many other countries. On the other hand, the use of neotame in foodstuffs is not allowed, in the European Union (EU) contrary to the USA, where neohesperidin dihydro-chalcone (NHDC) is additionally approved [95]. ASs exhibit countless applications with their use not limited to tabletop packets and diet soft drinks but also found in grain products including breads, cereals, and granola bars, dairy products including sugar-free or low fat yogurts, no-sugar added ice cream, and flavored milk. They can develop an artificial, metallic, or licorice-like aftertaste. Therefore, they often can be found blended in food to overcome this disadvantage [96]. In addition to reduced-calorie food, ASs use is reported also to drugs or medications, such as multivitamins, and sanitary products (e.g. flavored toothpaste and mouthwash). Furthermore, some sweeteners like saccharin is not used only for human consumption, but is also authorized for use as an additive in animal feed for piglets, pigs, bovines and calves in the European Union (E.U.) [97].

Artificial sweeteners are highly consumed, particularly in the U.S., with increasing trends in consumption, especially after the introduction of sucralose in 1998. The global market for artificial sweeteners reaches $5.1 billion, of which the U.S. and Europe currently make up 65% [98].

Production volumes of artificial sweeteners vary between reports. ASP represents the largest artificial sweetener product segment globally, it is the most popular artificial sweetener in the U.S., and it is used in more than 6000 food products. Around 16 000 t of ASP are produced annually in U.S. for worldwide consumption. The U.S. is also currently the largest market for SCL, making use of more than 1500 t per year, followed by Europe, with around 400 t per year ASP and ACS are the leading products in diet soft drinks, while SCL has become the leader in the key tabletop sweetener market [93]. Although these foods may be sold with a “healthy” or “diet” labeling, increasing the consumption of ASs, however, there have been lots of controversy regarding their safety and adverse health effects. The side effects that have been mentioned in experimental and epidemiological studies are: obesity and metabolic syndrome, alteration in gut microbiota, cancer and the hepatotoxic, nephrotoxic and neurobehavioral effects [92].

Concerns have been raised, however, regarding their growing presence and potential toxicity in environmental aquatic bodies. The approval of the ASs in combination with a simultaneous rise in diet programs and a following increase in marketing of ASs and ASs-containing products, led to a dramatic increase in their availability and consumption. After ingestion, some sweeteners pass through the human metabolism largely unaffected, are quantitatively excreted via urine and feces, and thus reach the environment associated with domestic wastewater.

1.3.1 Sources and fate of artificial sweeteners (ASs) in the environment

The major route of entry for ASs into the environment is as unchanged human excretion product after the consumption that flows down the drain and is ultimately discharged from wastewater treatment plants (WWTPs), which were not originally designed to remove these compounds [99]. Another source of artificial sweeteners in the environment is the leaking from animal farms where ASs are used as ingredient in animal feed for pigs, calves and bovines. For instance, saccharine is approved for use as additive in animal feed in European Union (E.U) [93]. Furthermore, other studies showed that saccharine is the major degradation product of certain sulfonylurea herbicides [100–102]. In addition, direct discharges from industry, households, animal farming and agriculture into surface waters can be another source of pollution of ASs [103]. The water ecosystem is the principal recipient of primary emissions and fate processes taking place in water, including photochemical and biochemical degradation, hydrolysis, partitioning with dissolved and suspended organic matter and settling with particles deposition. From households and industries, all artificial sweeteners enter into wastewater treatment plants and, from effluents, they eventually reside in the receiving environmental bodies. [103].

The sweeteners have been found in wastewaters, ground waters and surface waters in concentrations up to several tens of mg/l, but consistent with their low Kows [104] no absorption to various types of sediments has been observed [105]. Artificial sweeteners and environment Concentrations reported in the literature of artificial sweeteners in the environment are listed in Table 1.6.

Table 1.6 Reported concentrations of several artificial sweeteners in the environment

Environmental Sample	Concentration		Ref.
	Acesulfame	Sucralose	
WWTP Influents	Up to 81 µg/L	Up to 1 mg/L	[106],[98]
WWTP Effluents	Up to 2500 µg/L	0.8-1.8 mg/L	[106], [98]
Surface waters	Up to 53.7 µg/L	Up to 1.8 mg/L	[106], [98]
Groundwater	Up to 9.7 µg/L	0.6-0.4 mg/L	[106], [98]
WWTP sludge	Up to 190 ng/g	Up to 117 µg/g	[106], [98], [107]

Finally, concerning the ecotoxicology of ASs, the existing ecotoxicology studies mention that no bioaccumulation occurs, and most end points indicate no or only small environmental effects. Nevertheless, transformation or/and degradation may follow, by forming toxic substances. For instance, SCL in the presence of glycerol may generate toxic chloropropanols [108].

1.3.2 Artificial Sweeteners as Pollution Markers

When the concentration of a contaminant is measured at different spatial locations, it often exhibits concentration values that differ between the locations. This phenomenon is known as ‘‘spatial variability of the contaminant’’ and this is due to the existence of complex sources of contamination within an urbanized watershed. Thus, it is particularly difficult to trace the fate of a pollutant in a given urban setting [109–111]. For water-quality control purposes, the development of pollution indicators or pollution markers specific to wastewater effluents aids in determining the source contributions in recreational waters or potable-supply sources. The characteristics of an ideal wastewater marker were described by Oppenheimer et al. [109]. An ideal marker should be source specific, released to the environment in sufficient quantities, reflect contamination in a quantitative sense, and should be amenable to rapid and sensitive analysis [112]. Bacterial markers are commonly used to trace domestic wastewater but have disadvantages such as limited source specificity, time-consuming analysis, and relatively short

survival in natural waters. Alternatively, several chemical indicators have been suggested for domestic wastewater [113–116] including constituents and metabolites of pharmaceuticals, personal care products, household chemicals, food, and beverages. For application in groundwater, where minimal sorption to subsurface material and sufficient stability are other important criteria, currently proposed markers have their limitations [112].

The two artificial sweeteners acesulfame (ACE) and sucralose (SUC) were found to appear in the aquatic environment in much higher concentrations than most PPCPs and other wastewater-specific anthropogenic organic chemicals. The high environmental concentrations of ACE and SUC combined with their persistence, high water solubility, low sorption to solids, and the high sensitivity of modern trace analytical methods for the detection of artificial sweetener traces make them virtually ideal anthropogenic wastewater markers [97].

1.4 Legislation of Emerging Contaminants

1.4.1 Regulation of Pharmaceuticals

Profuse legislation has been issued worldwide to set the acceptable levels of pollutants in water, air, or soil, while strong control mechanisms have been implemented to protect the environment and human health. Regulations and controls are focused on traditional pollutants, and different organizations and governmental institutions around the world have normative and directives to preserve environmental quality, especially related to waters, whether surface or underground waters, which may be potentially used for human consumption. However, Emerging Contaminants are by definition, compounds that are not subject to regulation. Although numerous research projects around the world have reported on the occurrence, toxicity, and risk assessment of diverse pharmaceutical compounds, no federal regulations establishing limits for these products have been issued to date.

European Union (EU) in association with European Environment Agency (EEA) provide informative decisions about improving the environment and integrating environmental considerations into economic policies. The EEA works under the Water Framework Directive (WFD), with the aim to improve the quality of water in all members of EU. According to the WFD, to achieve the good status of surface water, ecological and chemical aspects are considered. The ecological aspect indicates ecosystem health through the evaluation of aquatic plant life and fish, while the chemical aspect concerns the presence of specific chemicals in sediment, water, and biota [72].

Directive 2000/60/EC laid down a framework for the protection of inland surface water, transitional waters, coastal waters, and groundwater, which prevents further deterioration and protects and enhances the status of aquatic ecosystems, promotes sustainable water use based on long-term protection, and aims at enhanced protection and improvement of the aquatic environment through specific measures targeting the progressive reduction of discharges, emissions, and leakages of priority substances [117]. Directive 2000/60/EC also discusses emission limit values and environmental quality standards for Hg, Cd, and hexachlorocyclohexane as well as other hazardous substances [72], [117]. In 2008, Directive 2008/105/EU, amending Directive 2000/60/EC set out the first list of 33 priority substances/group of substances (PSs) that should be monitored [118]. The list consisted of a list of 33 substances including herbicides, pesticides, metals, alkylphenols, organic compounds, polycyclic aromatic hydro- carbons, benzo(a)pyrene, and polycyclic aromatic hydrocarbons, as well as another list of substances subject to review for possible identification as priority substances or priority hazardous substances, including polychlorinated biphenyls (PCBs), glyphosate, bisphenol A, dicofol, bentazon, EDTA, musk xylene, AMPA, free cyanide, perfluorooctane, quinoxyfen, sulfonic acid, mecoprop, and dioxins. Still, no pharmaceutical compounds were included in these regulations.

A breakthrough in the regulation of pharmaceuticals in the environment occurred in 2013, in the form of Directive 2013/39/EU [119] on priority substances in the field of water policy, amending Directives 2000/60/EC and 2008/105/EU. The Directive 2013/39/EU updated the previous documents, recommending the monitoring of 45 pollutants and highlighting the demand to develop new water treatment solutions [119]. The new directive, was very useful, since it allowed the inclusion of emerging contaminants to the list of priority substances, based on the results obtained by diverse studies, and, specifically as regards pharmaceuticals, these agents are mentioned for the first time as contaminants of emerging concern. Furthermore, the Directive 2013/39/EU took a step forward in the field of water policy, proposing a first Watch List as a guideline of substances for which Union-wide monitoring data need to be gathered for the purpose of supporting future prioritization exercises in the EU, the complete list being published in the Decision 2015/495/EU. The referred Watch List encompassed 10 substances/group of substances (a total of17 organic substances). The importance of these provisions lied in the fact that three pharmaceuticals were incorporated into the first watch list, diclofenac, 17β-estradiol, and 17α-ethynylestradiol, in order to gather monitoring data to facilitate determination of appropriate measures to address the risk posed by these substances [120]. The matrices that should be monitored, as well as the possible methods of analysis for each substance/group of

substances, are also pointed out in the Decision 2015/495/EU [120]. Liquid chromatography tandem mass spectrometry (LC–MS/MS) is the suggested analytical technique for most Watch List CECs in the Decision 2015/495/EU., the maximum acceptable method detection limits for diclofenac and erythromycin (target analytes in this study) were 10 and 90 ng/L, respectively [120]. Recently, a second Watch List has been proposed by the European Union in the Decision 2018/840/EU. In the second Watch List, five substances were excluded from the first Watch List (diclofenac, oxadiazon, triallate, EHMC and BHT) and three new substances were included: two antibiotics, amoxicillin and ciprofloxacin and the pesticide metaflumizone [121], [117].

The urban wastewater treatment is one of the core elements of EU water policy. Council Directive 91/271/CEE regulates discharges of municipal wastewater from cities, towns and larger villages (called agglomerations) and explicitly specifies what kind of treatment should be applied. It concerns the collection, treatment, and discharge of domestic wastewater, mixtures of wastewaters originating from different sources, and wastewater from certain industrial sectors. Hospital effluents are included in this regulation [122]. The aim of this regulation is to protect the environment against the adverse effects of wastewater discharges.

The regulatory aspects surrounding the accumulation of pharmaceutical waste are implied by Directive 2004/27/ EC relating to medicinal products for human use [48]. This directive introduces the obligation of Member States to have “specific precautions relating to the disposal of unused medicinal products” as well as the obligation to implement appropriate collection systems [48].

Further regulation concerning human and veterinary medicinal products are set by Directives 2001/83/EU and 2001/82/EU, respectively [123,124].

In the US, the Drinking Water Contaminant Candidate List also contains several PhACs and EDCs, including antibiotics, and hormones (Environmental Protection Agency U.S., 2012) [125]. Other PhACs, such as carbamazepine, sulfamethoxazole, diclofenac, ibuprofen, naproxen, bezafibrate, atenolol, erythromycin and gemfibrozil have been classified as high priority pharmaceuticals to the water cycle by the GWRC, Global Water Research Coalition [126]

1.4.2 Regulation of Artificial Sweeteners

Artificial sweeteners saccharin, cyclamate, acesulfame, and sucralose, aspartame are regarded as although they are detected worldwide in a variety of environmental media, the monitoring of their presence is still not required by any existing regulations [127]. Artificial sweeteners are regulated according to food additives legislation rules. According to these guidelines, food additives are tested for their acute, subchronic, and chronic toxicity, as well as for carcinogenicity,

mutagenicity, teratogenicity, and biochemical activity. As a matter of principle, a food additive should not be pharmacologically active at the concentration at which it is used. Clinical trials are conducted to determine the concentration to which an additive remains nontoxic. Expert WHO committees use this value to define the acceptable daily intake (ADI) by reducing it by a factor of 100 [128]. The safety of all food additives that are currently authorized has been assessed by the Scientific Committee on Food (SCF) and/or the European Food Safety Authority (EFSA). Only additives for which the proposed uses are considered safe are on the EU list. EU requires listing of the food additives in the appropriate regulation once the safety assessment is complete, and the addition of additives to these regulations is handled by the European Commission. Currently, EU food additive uses are governed in regulations EU 1330/2008 (Common Procedures); EU 1333/2008 (Approved Food Additives); EU 231/2012 (Specifications and Limitations); EU 1332/2008 (Approved Food Enzymes); EU 1334/2008 (Approved Food Flavors). The regulation EU 1333/2008 established that the toxicity of food additives evaluated before 20th January 2009 must be re-evaluated by European Food Safety Authority (EFSA) [98,129,130].

All regulations related to water quality and pharmaceutical compound lists as well as artificial sweeteners are summarized in Table 1.7.

Table 1.7 Main Directives and Regulation of environmental interest from EU related to target Emerging Contaminants

Regulation	Content	Ref.
2000/60/EC	Establishing a framework for Community action in the field of water policy	[117]
2008/105/EU	Establishing environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC	[118]
2013/39/EU	Establishing a framework as regards priority substances in the field of water policy amending Directives 2000/60/EC and 2008/105/EC	[119]
2015/495/EU	Establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council	[120]

2018/840/EU	Establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council and repealing Commission Implementing Decision (EU) 2015/495	[121]
2001/83/EU	Establishing a Community code relating to medicinal products for human use	[124]
2001/82/EU	Establishing a Community code relating to veterinary medicinal products	[123]
2004/27/ EC	Establishing a Community code relating to medicinal products for human use, amending Directive 2001/83/EC . Introduces specific precautions relating to the disposal of unused medicinal products	[48]
91/271/CEE	Establishing a framework concerning urban wastewater treatment	[122]
2008/1330 EU	Harmonizes the use of food additives in foods in the Community	[95]

1.5 Wastewater Treatment

According to the discussion related to the main sources and pathways of pharmaceuticals and artificial sweeteners in the environment mentioned in previous sections, wastewater treatment plants (WWTP) are the major receptors of these compounds. The massive use of pharmaceuticals for both human and veterinary purposes as well as the consumption of artificial sweeteners leads to the introduction of tons of these compounds in wastewaters. After treatment processes in WWTPs, significant amounts can be released to surface waters due to eliminated removal efficiencies or even to high mass loadings. The concentration levels of these compounds have benn reported up to ng/L and μg/L or even mg/L for some influent waters depending on the compounds [17],[131]. Surface water is an important source for anthropogenic activities. It serves as a source of drinking water after treatment and as a source of domestic water without treatment particularly in rural areas in developing countries. It has been used for irrigation purposes in agricultural and generally in primary sector. The release of household and industrial wastewater has resulted to negative impacts in aquatic bodies and depletion of clean water resources. There are countries that their wastewater is not submitted to any treatment process. In few urban centers, various forms of wastewater treatment facilities (WWTFs) exist but most of them are producing ill-treated effluents, which are disposed of onto freshwater courses [132].

Consequently, the control of this type of substances in surface waters is crucial, because it may affect water quality and cause potential impacts on drinking water resources, ecosystems, and human health [133].

Wastewater treatment plants are designed to reduce the risk of adverse impact to the environment through oxygen depletion of receiving waters, or ecosystem eutrophication by the increase in nitrogen and phosphates. Wastewater treatment plants can be classified into primary, secondary and tertiary classes. Some northern European WWTPs include tertiary wastewater treatments, however in other countries primary and secondary treatments are performed. Secondary treatment is usually based on conventional activated sludge, and tertiary treatments are rarely implemented in treatment facilities. Limited degradability of pharmaceuticals under conventional treatments applied in the WWTPs was reported [134] implying the need for upgrade and implementation of advanced treatment technologies in order to achieve high-quality treated effluents [132].

The primary class of treatment is either chemical or physicochemical (such as ozonation, electrocoagulation) and is concerned with the removal of suspended solids from the untreated influent sewage using screening and sedimentation by gravity techniques. Secondary treatment includes biological processes such as microbial digestion that promotes aerobic degradation of organic matter to CO_2 and water by pumping air throughout the wastewater. The treated water is separated from the residual sludge and is suitable for discharge into the environment, while the sludge layer requires further clean-up. This may include liming or dewatering, producing a treated sludge supposed to be suitable for agricultural application as fertilizer or used as feedstock for energy production. A disinfection step by means of UV or chlorination with NaOCl is followed to destroy any pathogenic micro-organism and finally the effluent is discharged back into the water course or subjected to further treatment to produce drinking water in several countries [135]. Tertiary treatment is designed to filter out nutrients and waste particles that might damage sensitive ecosystems. Figure 1.11 illustrates the schematic process of wastewater treatment.

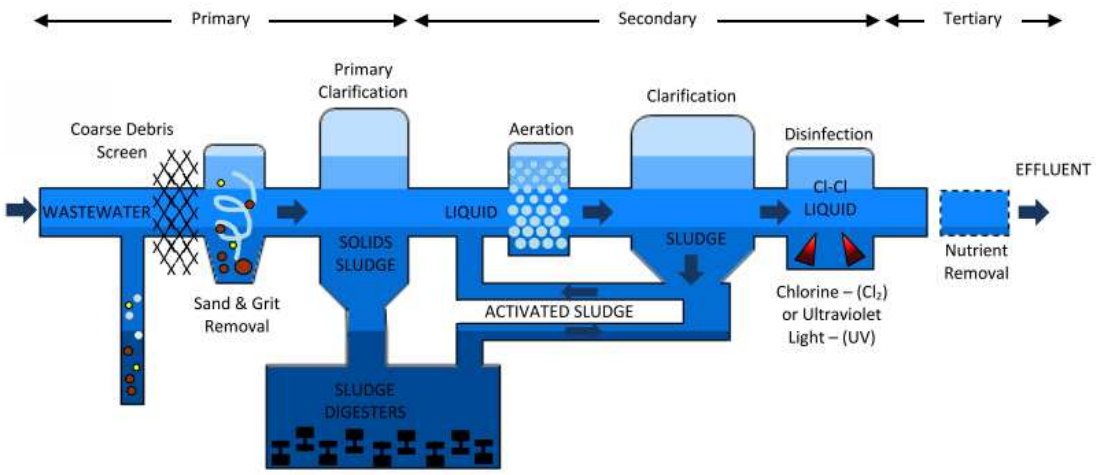


Figure 1.11 Schematic process of wastewater treatment [109]

1.6 Hospital Wastewater

Besides urban wastewaters, hospital wastewaters (HWW) have also stood up as an important environmental exposure pathway of pharmaceuticals [136]. Hospital effluents consisting of pathogens, faecal coliforms, Escherichia coli, etc, including phenols, detergents, toxic elements like cyanide and heavy metals such as copper (Cu), iron (Fe), gadolinium (Gd), nickel (Ni), platinium (Pt), among others have been detected The pollutant can be classified as micropollutants (10^{-6} to 10^{-3} mg/L) and micropollutants (>10^{-3} mg/L) based on their concentrations [137]. HWW have captured the attention of scientific community, although they present a small fraction <10% volume in the WWTP influent in total. [138]. Hospital effluents are generated from all activities of the hospital, including medical (diagnosis, surgeon operations, clinical laboratories, emergency and first aid, psychiatric clinics, radiology etc.) and non-medical activities and these can be classified into two main categories:

- Domestic discharges from kitchens, laundries and toilets of normal wards.
- Specific discharges generated by care, laboratory analysis and research activities. These discharges can contain antibiotics, psychiatric drugs, disinfectants, detergents, contagious faeces/excreta, biological liquids such as blood waste, drug residues, metal radioelements, and many other chemicals (acids, alkalis, solvents, benzene, hydrocarbons, colourants, etc.) [139].

Hospitals generate different loadings of wastewaters depending on factors like number of beds, number and types of wards and units, hospital age, general services present inside the structure (kitchen, laundry, etc.), institution management policies, cultural and geographical factors, among others [136]. Therefore, hospital effluents may differ from conventional urban wastewater due

to particular and complex composition. Compared to urban wastewaters (UWW), HWW contain wider spectrum and higher concentrations of pharmaceuticals, mainly antibiotics and analgesics [56,140]. Actually, according to Verlicchi et al. (2010) concentrations of micropollutants in HWWs are reported around 4 and 150 times higher than in UWW depending on the therapeutic class of corresponding compounds [136]. Micropollutants commonly found in HWW apart from pharmaceutical residues (parent compounds and metabolites), include iodinated X-ray contrast media, disinfectants, heavy metals, detergents and other substances [138].

Usually hospital effluents are directly discharged into public sewer network, being co-treated with household wastewaters in municipal WWTPs. Only a few countries have reference standards and specific treatment strategies to manage these effluents. Many countries consider hospital effluents as domestic effluents by discharging them directly in the municipal sewer network without any pretreatment or imposed quality limits. Reference standards and quality control are usually imposed only after the treatment of the WWTP effluents. In only few countries, hospital effluents are considered to be industrial and are pretreated before their transport to the municipal plant [139]. The common practice of co-treatment of hospital and urban wastewaters at the same municipal sewage network is characterized inappropriate by many researchers [141–143] because it is based on dilution of different discharge sources and does not provide a segregation/separation of pollutants, and in particular of emerging contaminants and toxic substances from the liquid phase which is then released into freshwater sources.

Taking into consideration the above discussion, hospital effluents can potentially contain some hazardous substances with a genotoxic or cytotoxic activity, toxic or hazardous chemicals or pharmaceutical residues, and radioactive and/or infectious agents [137,144]. Furthermore, hospital effluents also play an important role in the introduction of pathogens into public wastewaters, especially concerning multi-resistant bacteria, contributing to the spread of antibiotic resistance into the environment [37].

Although there is a discrepancy in the literature concerning weather the hospital is a major source of pharmaceutical compounds in the environment, given the great number of compounds measured in HWW [137,141,145,146] it is necessary to evaluate the corresponding contribution of effluents discharged, in ecotoxicity and human health. Monitoring studies of HWW for the identification of pharmaceutical compounds provide specific information on which compounds hospital managers should focus their efforts in order to eliminate their presence into the environment and their potential impacts on aquatic ecosystems and/or on the activated sludges of WWTP.

1.7 Selection of target emerging contaminants

The emerging contaminants (ECs) investigated in this study include a large variety of substance families and physicochemical properties, providing the opportunity to investigate the pathways of chemically different compounds through the wastewater treatment process Target compounds were selected according to the following criteria:

(i) high consumption in Greece and worldwide, **ii)** prescription and consumption patterns in the Psychiatric Department of the University hospital in case of psychiatric drugs, **(iii)** their proven occurrence in the aquatic environment, according to the data found in the scientific literature ,**(iv)** their frequency of detection in WWTPs and the difficult removal during treatment **(v)** their known fate and behaviour in the environment (e.g. water solubility or persistence), **(vi)** their environmental and toxic relevance (such as their endocrine disrupting potential) and **(vii)** on priority pollutant lists developed by European Union (E.U), under the water framework directive (WFD) as well as the United States Environmental Protection Agency (USEPA) (diclofenac, erythromycin, carbamazepine).

Thirty-three compounds belonging to 6 different classes (4 sweeteners, 5 analgesics-Non-steroidal anti-inflammatory drugs, 9 psychiatric drugs, 13 antibiotics, 1 lipid regulator and 1 disinfectant) were selected as target emerging contaminants in this study.

1.8 Sulfonamides

Sulfonamides (SAs) constitute one of the most consumed antimicrobial families. They are synthetic antimicrobial agents, derivatives of sulfanilamide, active against a broad spectrum of Gram-positive and many Gram-negative bacteria including species of the genus Streptococcus, Staphylococcus, Escherichia, Neisseria, Shigella, Salmonella, Nocardia, Chlamydia and Clostridium. Moreover, SNs have used against protozoa (e.g., Toxoplasma gondii), parasites (e.g., Plasmodium malaria), and fungi (e.g., Pneumocystis carinii) [147]. SAs possess antibacterial, anti-carbonic anhydrase, diuretic, hypoglycemic and antithyroid activity, among others. They are used in aquaculture, in animal husbandry [148,149], they are the second most widely used family of veterinary antibiotics, after tetracyclines, accounting for 11–23% of the sales in several other European countries [147]. Nevertheless, they are also used in human therapies, to treat many kinds of infection caused by these bacteria and certain other microorganisms (urinary tract infections, ear infections, bronchitis, bacterial meningitis, certain eye infections, Pneumocystis carinii pneumonia, traveler's diarrhea, and more) [150–153]. Many SAs derivatives have also been used as herbicides [154] and complexes of SAs with Ag^{+} and Zn^{2}+ have been used as antifungals

[155]. Sulfamethoxazole, or sulfasalazine belong to SAs that are frequently used in human medicine while sulfamethazine, sulfathiazole, sulfaquinoxaline, are commonly used in veterinary medicine, depending the countries. Moreover, SAs have been used as additives to animal feed premix applied in young animals, and as commercial beekeeping (protection of honeybees against bacterial diseases) as well. Usually SAs have been associated with trimethoprim [147].

The mechanism of action of SAs is presented in the following figure (Fig.1.12). SAs act as competitive inhibitors of the enzyme dihydropteroate synthease (DHPS) which catalyses the conversion of para-aminobenzoate (PABA) to dihydropteroate (AHHMD), a precursor of folate synthesis. Tetrahydrofolic acid (THF) participates in the synthesis of nucleic acids that are essentials as building blocks of DNA and RNA.

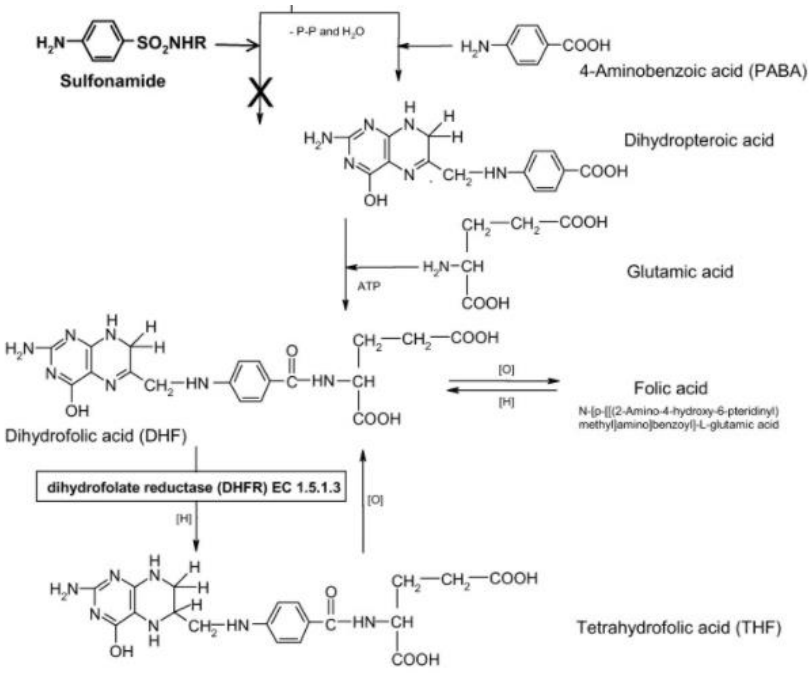


Figure 1.12 Mechanism of action of Sulfonamides [156]

SAs are polar molecules with amphoteric properties, they consist of sulfonamide group (R-S(=O)$_2$-NR$_2$) connected to a benzene ring that contains an amino group in the para position. The formula of structure is presented in Fig. 2 Their amino nitrogen (N4) is protonated at pH 2–3, while the amide nitrogen (N1) is deprotonated at pH 4.5–11.

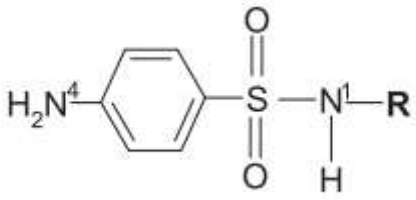


Figure 1.2. General chemical structure of sulfonamide [156]

SAs are usually small molecules, with high solubility and weakness to be adsorbed by soil resulted in their characterization as mobile contaminants in the environment. Due to their high mobility, and after their release of their main pathway of WWTP, they can reach the surface water during run off episodes or percolate with entirely biologically active. In this way they can also remain for a long time and could spread easily and penetrate groundwater. Although they have a very low toxicity to higher organisms (vertebrates) and are highly toxic to microorganisms, algae and certain plants the main environmental concern focuses on bacterial resistance acquired against antibiotics in aquatic and soil bacterial communities. Concentrations of SAs in the environment occur incidentally (in manure from livestock), but due to a gene transfer process, their relevance to the global change of drug resistance may be much larger than expected. The risk caused by the generation of drug resistance by anti-infectives drugs is much higher than the risk caused by their [147,150,156] In this study nine sulfonamides were investigated including sulfacetamide, sulfadiazine, sulfathiazole, sulfapyridine, sulfamethizole, sulfamethazine, sulfamethoxy-pyridazine, sulfamethoxazole and sulfaquinoxaline.

1.8.1 Sulfacetamide

Sulfacetamide, is a synthetic sulfanylacetamide derivative belonging to the class of organic compounds known as aminobenzenesulfonamides. These are organic compounds containing a benzenesulfonamide moiety with an amine group attached to the benzene ring. Sulfacetamide is a sulfonamide antibiotic with bacteriostatic actions and broad-spectrum activity against most gram-positive and many gram-negative organisms, with some restriction for individual species which can be resistant. Sulfacetamide inhibits multiplication of bacteria by acting as competitive inhibitors of p-aminobenzoic acid (PABA), an essential component for bacterial growth [157]. The inhibited reaction is necessary in these organisms for the synthesis of folic acid in metabolism cycle. It is used as an anti-infective topical agent to treat skin infections and as an oral agent for urinary tract infections. Specifically, is applied for the treatment of bacterial vaginitis, keratitis, acute conjunctivitis, and blepharitis. Sulfacetamide exists as a solid, slightly soluble (in water), and

a weakly acidic compound (based on its pKa). Sulfacetamide has been detected in multiple biofluids, such as urine and blood. Sulfacetamide is the N-acetyl derivative which is biosynthesized from sulfanilamide. The sodium salt of sulfacetamide, because of its effectiveness and low toxicity, continues to be the most widely prescribed sulfonamide in the form of eye-drops and ointment for ophthalmic infections. It is used mainly in the treatment of acute conjunctivitis and in the prophylaxis of ocular infections after injuries or burns [158]. Also, it is applied associated with sulfur in creams, lotions, foams, etc. for the treatment of acne rosacea [159]. The side effects of its use consist of ophthalmic burning, skin irritation, allergic reactions like tightness in the chest, swelling in the face, mouth, lips, and tongue. Rarely effects such as liver problems, blood problems, and bad skin reactions (Stevens-Johnson syndrome/toxic epidermal necrolysis) are mentioned. In addition, this kind of drug has been associated with lupus health issue [160].

1.8.2 Sulfadiazine

Sulfadiazine is one of the short-acting sulfonamides used in conjuction with pyrimethamine to treat toxoplasmosis in patients with acquired immunodeficiency syndrome such as Toxoplasma gondii encephalitis frequently n occurs in HIV patients as opportunistic infection. It is also used in newborns with congenital infections and for the for the prevention and treatment of certain types of bacterial infections, including the treatment of chancroid, treatment for bacterial otitis media caused by Haemophilus influenza and recurrent rheumatic fever. Unlike with other sulfonamides sulfadiazine is not recommended for the treatment of urinary tract infections because of its relatively lower urine solubility and the increased chance of crystalluria. Additional therapeutic uses reported for trachoma, inclusion conjunctivitis, nocardiosis, malaria, meningococcal meningitis, meningitis (adjunctive). Sulfadiazine is a competitive inhibitor of the bacterial enzyme dihydropteroate synthetase. This enzyme is needed for the proper processing of para-aminobenzoic acid (PABA) which is essential for folic acid synthesis. Common side effects may include allergic reaction nausea, headache, dizziness, insomnia, numbness, loss of appetite, spinning sensation, misfunction of thyroid, depressed mood. Also, its use is avoided from people related to liver and kidney health issues [161].

1.8.3 Sulfathiazole

Sulfathiazole is a sulfonamide antibiotic extensively used in veterinary medicine and especially in aquaculture and livestock production. It is applied in human medicine as well to treat bacterial, protozoal and fungal infections [160]. It is used in association with sulfacetamide and

sulfabenzamide for the treatment of vaginal infections and for disinfecting home aquariums . Sulfathiazole is commonly used in cattle for the treatment of bovine respiratory disease complex (shipping fever complex), bacterial pneumonia; calfdiphtheria and necrotic pododermatitis (foot rot) and acute metritis [162]. It is applied in swine farms for the treatment of bacterial pneumonia; porcine colibacillosis (bacterial scours); and, in combination with chlortectracyline and penicillin, for increased rate of weight gain and improved feed efficiency, reduction of the incidence of cervical abscesses, and treatment of bacterial swine enteritis. Sulfathiazole is also used in wound powders to prevent invasion of burn wounds by many gram-negative and gram-positive organisms [163–165].

1.8.4 Sulfapyridine

Sulfapyridine is a sulfonamide antibiotic no longer prescribed for treatment of infections in humans except from linear IgA disease and dermatitis herpetiformis (Duhring's disease). Nevertheless, it is applied in veterinary medicine . Sulfapyridine has not the therapeutic action of the other sulfonamides and may cause some serious side effects such as blood problems or kidney diseases. Moreover, use of sulfapyridine may cause an attack of porphyria, patients with lack of G6PD enzyme may have an increase in side effects affecting the blood. Moderate symptoms of nausea, continuous headache, skin rash, increased sensitivity to sunlight , diarrhea, fever is also common [166–168].

1.8.5 Sulfamethizole

Sulfamethizole is a sulfonamide derivative used as an antimicrobial drug for the prevention and cure of bacterial infections in both humans and animals Sulfamethizole is specialized in the treatment of urinary tract infections because it produces low plasma levels and is rapidly eliminated, making it suitable drug for urinary infections in comparison to systemic infections [169]. Sulfamethizole is effective with the susceptible strains of the following organisms Escherichia coli, Klebsiella-Enterobacter, Staphylococcus aureus, Proteus mirabilis, and Proteus vulgaris which are responsible for pyelonephritis, pyelitis, and cystitis in the absence of obstructive uropathy or foreign bodies [162,170]. Sulfamethizole blocks bacterial growth by inhibiting folic acid synthesis via enzyme called dihydropteroate synthase. The drug is no longer marketed in the USA. The use of sulfamethizole has been associated with hematologic toxicity, including methemoglobinemia, leukopenia, granulocytopenia, eosinophilia, hemolytic anemia,

aplastic anemia, purpura, clotting disorder, thrombocytopenia, hypofibrinogenemia, and hypoprothrombinemia [170,171].

1.8.6 Sulfamethazine

Sulfamethazine is a sulfonamide used to treat a variety of bacterial diseases in humans and other species. It has been used since the late 1950s to treat respiratory disease and promote growth in food-producing animals (cattle, sheep, pigs and poultry) [172]. It is a short-acting sulfonamide drug with similar properties to those of sulfamethoxazole. Sulfamethazine extended-release tablets are indicated in the treatment of pneumonia and bovine respiratory disease complex caused by susceptible Pasteurella species [173]. In chicken Sulfamethazine is administered for the control of acute fowl cholera caused by susceptible P. multocida, while in calves and cattle is indicated in the treatment of enteritis (colibacillosis, scours) caused by susceptible E. coli [174–176].

1.8.7 Sulfamethoxy-pyridazine

Sulfamethoxy-pyridazine is a sulfonamide antibiotic mainly used to prevent infections as well as conditions diarrhea and gastroenteritis. This drug possesses antibacterial activity with respect to a few cocci and colon bacillus. Sulfamethoxy-pyridazine contributes to reducing the number of oocysts shed but may not alter the clinical course of a susceptible coccidial infection [177] . Moreover, is the primary treatment for enteritis in many cases, including those involving colibacillosis in calves, is aggressive fluid replacement. Treatment of enteritis with antimicrobials is relied on specific diagnosis of pathogen susceptibility with the aid of sulfamethoxy-pyridazine [178]. It is a long-lasting drug. It is also used for treating pneumonia, bronchitis, tonsillitis, purulent otitis and meningitis, purulent infections of the urinary tract, dysentery, and eye infections [175,179].

1.8.8 Sulfamethoxazole

Sulfamethoxazole is a drug which is used for the treatment bacterial infections causing bronchitis, prostatitis and urinary tract infections [180,181]. Sulfamethoxazole is indicated in combination with trimethoprim, working synergistically to block two consecutive steps in the biosynthesis of nucleic acids and proteins which are necessary for bacterial growth and division, and using them in conjunction helps to slow the development of bacterial resistance [182,183] . This combination is used in various formulations, for the following infections caused by bacteria with reported

susceptibility: urinary tract infections, acute otitis media in pediatric patients (when clinically indicated), acute exacerbations of chronic bronchitis in adults, enteritis caused by susceptible Shigella, prophylaxis and treatment of Pneumocystis jiroveci pneumonia, and travelers' diarrhea caused by enterotoxigenic E. coli [173,175,184]. In Canada, additional indications include the adjunctive treatment of cholera, treatment of bacillary dysentery, nocardiosis, and second-line treatment of brucellosis in combination with gentamicin or rifampicin [185,186].

Fatalities associated with the administration of sulfamethoxazole, have been implicated. In hypersensitivity reactions the drug should be discontinued at the first sign of a developing rash, as this may be the signal for more severe reaction such as Stevens-Johnson syndrome or toxic epidermal necrolysis, hepatic necrosis, and serious blood disorders [187–190] . Sulfamethoxazole treatment may contribute to folate deficiency and should therefore be used with caution. Hemolysis has been observed in patients with glucose-6-phosphate dehydrogenase deficiency who are using sulfamethoxazole/trimethoprim [191,192].

1.8.9 Sulfaquinoxaline

Sulfaquinoxaline is a medicine exclusively for animal use which can be applied to cattle and sheep to treat coccidiosis as well as to chicken and turkeys as an aid in the control of acute fowl cholera caused by *pasteurella multocida* owl and Typhoid caused by salmonella gallinarum. It is a protective agent against upper respiratory infections of poultry and a prophylactic against coccidiosis. Sulfaquinoxaline, was synthesized during the war, and proved too toxic to be used in human malaria but was found to be a superior agent against another sporozoan parasite, Eimeria spp., the agent that causes coccidiosis in domestic chickens [193]. In 1948 sulfaquinoxaline was introduced commercially as a poultry coccidiostat and is widely used in meat industry [194]. Sulfaquinoxaline has been shown to be a freely reversible inhibitor of the dithiothreitol-dependent reduction of both vitamin K epoxide and vitamin K quinone [193].

1.8.10 Trimethoprim

Trimethoprim is an antibiotic used mainly in the treatment of urinary tract infections, although it may be used against any susceptible aerobic bacterial species. It is also used for the treatment of bladder infection, pneumocystis pneumonia, prevention of bladder infection and travelers' diarrhea. As individual administration trimethoprim is indicated for the treatment of acute episodes of uncomplicated urinary tract infections caused by susceptible bacteria, including E.

coli., K. pneumoniae, Enterobacter spp., P. mirabilis, and coagulase-negative Staphylococcus species [195].

In various formulations along with sulfamethoxazole, trimethoprim is indicated for the following infections caused by bacteria with documented susceptibility: urinary tract infections, acute otitis media in pediatric patients, acute exacerbations of chronic bronchitis in adults, enteritis caused by susceptible Shigella, prophylaxis and treatment of Pneumocystis jiroveci_pneumonia [190,196]. The use of co-trimoxazole was initially promoted following the development of antimicrobial resistance to older treatments, such as β-lactam antibiotics. Co-trimoxazole is also effective at treating nocardiosis, an infection that is commonly associated with patients who have a compromised immune system such as HIV patients [191]. Trimethoprim is available as an ophthalmic solution in combination with [polymyxin B] for the treatment of acute bacterial conjunctivitis, blepharitis, and blepharoconjunctivitis caused by susceptible bacteria. Common side effects from its use are vomiting, nausea, diarrhea, whereas more rarely thrombocytopenia (low levels of platelets) by lowering folic acid levels and hyperkalemia (increased potassium levels) can occur. Trimethoprim is a bacteriostatic antibiotic derived from trimethoxybenzylpyrimidine that belongs to a group of chemotherapeutic agents known as inhibitors of dihydrofolate reductase. Trimethoprim is a first line antibiotic in many countries although resistance to it is increasing. Trimethoprim binds to dihydrofolate reductase and inhibits the reduction of dihydrofolic acid (DHF) to tetrahydrofolic acid (THF). THF is an essential precursor in the thymidine synthesis pathway and interference with this pathway inhibits bacterial DNA synthesis. Trimethoprim's affinity for bacterial dihydrofolate reductase is several thousand times greater than its affinity for human dihydrofolate reductase [197].

1.9 Quinolones

The quinolones are a family of antibiotics containing a bicyclic core structure related to the compound 4-quinolone. There are 6 important positions for modifications to improve the activity of the drug: R1, R5, R6, R7, R8, and X. X = C defines quinolones, X = N defines naphthyridones (Fig.1.13). Since their discovery in the early 1960s, they have gained increasing importance as key therapies to treat both community-acquired and severe hospital-acquired infections [198]. The coverage of the quinolone class was expanded significantly by the breakthrough development of fluoroquinolones, which show a much broader spectrum of activity and improved pharmacokinetics compared to the first-generation quinolone [199]. Those fluoroquinolones, such as ciprofloxacin and ofloxacin, are active against both Gram-negative and Gram-positive

pathogens. The quinolone class of antibiotics inhibits the DNA synthesis of bacteria by disrupting the bacterial topoisomerase type II; inhibiting the catalytic activity of DNA gyrase and topoisomerase IV [200]. These two enzymes are critical bacterial enzymes that regulate the chromosomal supercoiling required for DNA synthesis [200]. Quinolones have been preferred as antibiotics for more than five decades because of their high potency, broad spectrum of activity, favorable bioavailability, convenient formulations, and high serum concentrations, as well as a comparatively low incidence of side effects [200]. Quinolones have a high bioavailability, good tissue penetration, long half-lives, high efficacy, and low incidence of adverse effects. Because of these characteristics, they are largely used against a wide range of parasitic diseases in humans and animals [201,202] including gastrointestinal reactions, CNS reactions, genotoxicity, phototoxicity [203].

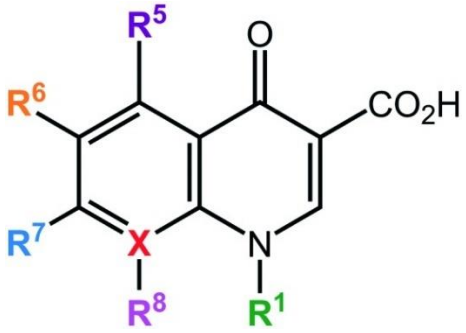


Figure 1.13 Core structure of quinolone antibiotics

1.9.1 Oxolinic acid

Oxolinic acid is a synthetic quinolone antibiotic targeting gram-negative bacteria, especially those responsible for causing urinary tract infections. It is also widely used in veterinary medicine, in particular in aquaculture [204]. Specifically oxolinic acid, sulfonamides are used in fish in order to treat bacterial diseases that pose a major problem in intensive culture systems [204]. Furthermore, are widely administered in farms to cattle and swine, either orally or in injectable solutions, to prevent and remedy several respiratory and gastrointestinal infections, as well as to promote growing [201], [205]. It has also been reported to be effective against Burkholderia glumae, a bacterium causing grain rot, sheath rot, seedling rot, and bacterial panicle blight. It has been useful in studying transmissible resistance mechanisms of E. coli and S. enterica. It can be used to isolate Gardnerella vaginalis and as a dopamine reuptake inhibitor in neurological research.

Oxolinic acid functions by inhibiting DNA gyrase or topoisomerase II, enzymes essential for DNA synthesis. In human oxolinic acid is metabolized to at least 8 urinary metabolites, 37.5% glucoronides and 18% non-glucoronides.

1.10 Macrolides

The term “macrolide” is used to describe drugs with a macrocyclic lactone ring of 12 or more elements [206]. This class of compounds includes a variety of bioactive agents, including antibiotics, antifungal drugs, prokinetics, and immunosuppressants. The 14-, 15-, and 16-membered macrolides are a widely used family of antibiotics. They have excellent tissue penetration and antimicrobial activity, mainly against Gram-positive cocci and atypical pathogens [206]. Antibiotic macrolides are used to treat infections caused by Gram-positive bacteria (e.g., Streptococcus pneumoniae) and limited Gram-negative bacteria (e.g., Bordetella pertussis, Haemophilus influenzae), and some respiratory tract and soft-tissue infections [207]. The antimicrobial spectrum of macrolides is slightly wider than that of penicillin, and, therefore, macrolides are a common substitute for patients with a penicillin allergy. Beta-hemolytic streptococci, pneumococci, staphylococci, and enterococci are usually susceptible to macrolides. Unlike penicillin, macrolides have been shown to be effective against Legionella pneumophila, mycoplasma, mycobacteria, some rickettsia, and chlamydia. Common side effects related to macrolide antibiotics include gastrointestinal upset, dizziness, headache, hearing impairment, insomnia, visual disturbance and skin reactions [208]. The first macrolide antibiotic was erythromycin discovered at 1950s.

1.10.1 Erythromycin

Erythromycin is the first macrolide antibiotic with antibacterial activity commonly used for the treatment of upper respiratory tract and skin and soft tissue infections caused by susceptible by susceptible strains of various bacteria especially in patients who are allergic to penicillin [209]. Erythromycin acts by inhibition of protein synthesis by binding to the 23S ribosomal RNA molecule in the 50S subunit of ribosomes in susceptible bacterial organisms [207]. It stops bacterial protein synthesis by inhibiting the transpeptidation step of protein synthesis and by inhibiting the assembly of the 50S ribosomal subunit.

Erythromycin is efficient with Streptococcus pyogenes or Staphylococcus aureus which are responsible for mild to moderate skin or skin structure infections however, the possibility of resistant staphylococcal organisms can occur. Additionally, erythromycin may aid in the prevention of pertussis infection [209]. Moreover, erythromycin is indicated to treat diphtheria and other infections due to Corynebacterium diphtheriae, as an adjunct to antitoxin, to prevent carrier status and to eradicate the organism in existing carriers. Other alternative uses for patients that have intolerance or hypersensitivity to penicillin, could be the treatment of acute pelvic

inflammatory disease caused by N gonorrheae in female patients. Moreover, erythromycin is an approved drug to treat chlamydial infections that cause conjunctivitis of the newborn, pneumonia of infancy, and urogenital infections occurring in pregnancy [210]. Several drawbacks, however, have limited the use of erythromycin, including frequent gastrointestinal intolerance and a short serum half-life [211]. Erythromycin may be bacteriostatic or bactericidal in action, depending on the concentration of the drug at the site of infection and the susceptibility of the organism involved. Erythromycin is concentrated in the liver and is then excreted in the bile. Erythromycin is partially metabolized to N-desmethylerythromycin and is commonly hydrolyzed to anhydroerythromycin [212].

1.11 Psychiatric Pharmaceuticals

One important class of pharmaceuticals which has received recent consideration is psychiatric compounds. Psychiatric drugs are a group of pharmaceuticals commonly prescribed because of the worldwide prevalence of psychiatric disorders. Psychiatric drugs are categorized according to their chemical structure, pharmacological actions on specific biological processes or by their therapeutic actions [213]. According to their therapeutic use, the four main classes of the most prescribed psychoactive drugs are: antidepressants, anxiolytics, sedatives and hypnotics, antipsychotics and mood stabilizers [61]. Consumption of anti-depressant drugs doubled in OECD countries between 2000 and 2017 (Fig.1.14). This may reflect improved recognition of depression, availability of therapies, evolving clinical guidelines and changes in patient and provider attitudes [214]. In addition, their high consumption could be also attributed to the insecurity created by the increased financial European crisis, which might lead to various neurological disorders [215–217]. Greece is among the most affected countries by the severe economic crisis plaguing Europe and studies have ,mentioned increase in the use of antipsychotics and antidepressants.

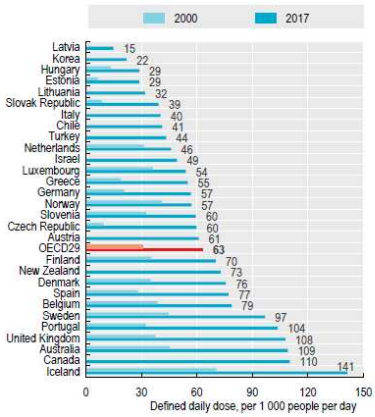


Figure 1.14 Anti-depressant drug consumption, 2000 and 2017, SOURCE: OECD Health Statistics 2019 [218]

1.11.1 Antidepressants

Antidepressants are used in the symptomatic treatment of depression and act through the action on various neurotransmitter systems, namely serotonin, norepinephrine and dopamine. The most important classes of antidepressants are the selective serotonin reuptake inhibitors (SSRI), tricyclic antidepressants (TCA) and monoamine oxidase inhibitors (MAOI). Tricyclic antidepressants were the first agents successfully used [61]. However, they exhibit neuro-pharmacological effects in addition to their original action. Nowadays, SSRI have emerged as a major therapeutic advance in psychopharmacology. The SSRI acts by modulating the levels of the neurotransmitter serotonin, specifically by blocking the function of the serotonin transporter on cell membranes leading to elevated levels of serotonin [61]. This class of antidepressants is largely prescribed to treat depression, anxiety, panic disorder, obsessive-compulsive disorder, eating disorders and social phobia. When the SSRIs are not effective, other antidepressants such as venlafaxine, duloxetine (selective serotonin and norepinephrine re-uptake inhibitors—SSNRIs), bupropion, tricyclic and tetracyclic antidepressants—amitriptyline and mianserin, respectively—are prescribed [218]. The antidepressants investigated in this study include fluoxetine, paroxetine (SSRIs), venlafaxine (SNRI), clomipramine, amitriptyline, cyclobenzaprine (TCA).

1.11.2 Antipsychotics

The term antipsychotic is applied to a group of drugs used to treat a number of psychiatric disorders, including schizophrenia, mania, dementia and delusional disorder. This group of pharmaceuticals is also known as neuroleptics. There are two types of antipsychotics drugs, the typical antipsychotics and the atypical antipsychotics. The typical antipsychotics pharmaceuticals (first generation of antipsychotics) are chlorpromazine, haloperidol, flupenthixol, perphenazine, thioridazine, thiothixene and trifluoperazine. The atypical antipsychotics (second generation of antipsychotics) pharmaceuticals such as olanzapine, quetiapine risperidone, aripiprazole, clozapine and ziprasidone, represent a new generation of drugs prescribed for schizophrenia and delusional disorders [219]. The antipsychotics pharmaceuticals act by regulating serotonin and dopamine levels in the brain [220]. Representative compounds of this category studied in this work were olanzapine and risperidone.

1.11.3 Mood Stabilizers

These pharmaceuticals are used in the treatment of acute phase of mania and depressive phase of bipolar disorder. Drugs classified as mood stabilizers include carbamazepine, oxcarbamazepine,

valproate and lithium [219]. Antiepileptics and anticonvulsants such as carbamazepine (commonly known as Tegretol) are central nervous system (CNS) pharmaceuticals that help to decrease the abnormal firings of nerves in the brain and in the CNS [220]. Carbamazepine is also investigated in this research.

1.11.4 Olanzapine

Olanzapine belongs to a class of drugs called atypical antipsychotics. It works by helping to restore the balance of certain natural substances in the brain. Olanzapine is a drug which is used for the acute and maintenance treatment of schizophrenia and related psychotic disorders, as well as acute treatment of manic or mixed episodes of bipolar 1 disorder [221]. Combined with other drugs olanzapine is used for treatment of depression. This medication can help to decrease hallucinations and help you to think more clearly and positively about yourself, feel less agitated, and take a more active part in everyday life. Intramuscular administration of olanzapine is indicated for the rapid control of agitated patients. Side effects associated with use of olanzapine include: Dizziness, low blood pressure upon standing, weakness, restlessness, Parkinsonism reactions, weight gain, high levels of cholesterol and triglycerides, insomnia [222].

Olanzapine, also known by its commercial brand as Zyprexa or Lanzac, belongs to benzodiazepines. These are organic compounds containing a benzene ring fused to isomers of diazepine(unsaturated seven-member heterocycle with two nitrogen atoms replacing two carbon atoms) [223]. As a selective monoaminergic antagonist, olanzapine binds with high affinity binding to the following receptors: serotoninergic, dopaminergic, muscarinic M1-5, histamine H1, and alpha-1-adrenergic receptors; it binds weakly to gamma-aminobutyric acid type A, benzodiazepine, and beta-adrenergic receptors. Although its exact mechanism of action in schizophrenia is unknown, it has been proposed that olanzapine's antipsychotic activity is mediated through antagonism to dopamine D2 receptors with rapid ligand-receptor dissociation kinetics that help to minimize extrapyramidal symptoms (EPS). The antinausea and antiemetic effects of this agent appear to be due to the blockade of 5-HT2 and 5-HT3 receptors for serotonin [224].

1.11.5 Risperidone

Risperidone is a medication used for treatment of schizophrenia (a mental illness that causes disturbed or unusual thinking, loss of interest in life, and strong or inappropriate emotions) in adults and children who are at least 13 years old. It is also known as a second-generation

antipsychotic (SGA) or atypical antipsychotic and its action is to rebalance dopamine and serotonin to improve thinking, mood, and behavior. Specifically, schizophrenia and various mood disorders are thought to be caused by an excess of dopaminergic D2 and serotonergic 5-HT2A activity, resulting in overactivity of central mesolimbic pathways and mesocortical pathways, respectively. Risperidone binds transiently and with loose affinity to the dopaminergic D2 receptor, with an ideal receptor occupancy of 60-70% for optimal effect.7,10 Rapid dissociation of risperidone from the D2 receptors contributes to decreased risk of extrapyramidal symptoms (EPS), which occur with permanent and high occupancy blockade of D2 dopaminergic receptors.
Risperidone is also approved by FDA (Food & Drug administration) as monotherapy, or combined with lithium or valproic acid, for the treatment of acute mania or mixed episodes associated with bipolar I disorder in adults and children who are at least 10 years old [225]. Risperidone is also used to treat behavior problems such as aggression, self-injury, and sudden mood changes in teenagers and children 5 to 16 years old with autistic disorders. Furthermore, when used combined with other drugs (antidepressants) risperidone may also be helpful for adjunctive treatment of major depression disorder, delusional parasitosis, post-traumatic stress disorder (PTSD), Tourette syndrome, and other mental health conditions. These therapeutic uses regard an “off-label” prescription. Risperidone is additionally indicated in Canada for the short-term symptomatic management of aggression or psychotic symptoms in patients with severe dementia of the Alzheimer type unresponsive to non-pharmacological approaches [226]. Side effects of the use of risperidone include nausea, vomiting, diarrhea, hurt burn, insomnia, late or missed menstrual periods, anxiety, agitation, vision problems, weight gain etc. [227]
Risperidone is extensively metabolized to 9-hydroxyrisperidone (i.e. paliperidone), and to a lesser extent also undergoes N-dealkylation [228].

1.11.6 Venlafaxine

Venlafaxine is an antidepressant belonging to a group of drugs called selective serotonin and norepinephrine reuptake inhibitors (SSNRIs). It exerts its effects primarily by blocking the transporters involved in the reuptake of the neurotransmitter’s serotonin and norepinephrine, therefore leaving more active neurotransmitter in the synapse [229].
Its main use is to treat depression but also treats generalized anxiety disorder (GAD, excessive worrying that is difficult to control), panic disorder (sudden, unexpected attacks of extreme fear and worry about these attacks), social phobia (extreme fear of interacting with others or performing in front of others that interferes with normal life). Venlafaxine is also used off-label

for prophylaxis of migraine headaches 10, for reduction of vasomotor symptoms associated with chemotherapies and menopause and for management of neuropathic pain (although there is only minimal evidence of efficacy for this condition) [230,231]. Common side effects of venlafaxine include drowsiness, dizziness, headache, nausea, heartburning, loss of appetite and weight, nightmares, uncontrollable shaking of a part of the body, muscle tightness etc. [232]

The metabolism of venlafaxine occurs in liver to its active metabolite, O-desmethylvenlafaxine ODV, and two minor, less active metabolites, N-desmethylvenlafaxine and N,O-didesmethylvenlafaxine. ODV possesses antidepressant activity that is comparable to that of venlfaxine. Renal elimination of venlafaxine and its metabolites is the primary route of excretion. Approximately 87% of a venlafaxine dose is recovered in the urine within 48 hours as either unchanged venlafaxine (5%), unconjugated ODV (29%), conjugated ODV (26%), or other minor inactive metabolites (27%) [233].

1.11.7 Paroxetine

Paroxetine is an antidepressant of selective serotonin reuptake inhibitor (SSRI) drug category commonly known as Paxil. Except of being a serotonin reuptake inhibitor, it also had mild to moderate noradrenergic effects through inhibiting reuptake of norepinephrine (NRI or NARI) and could be activating, which often helped depressed patients with lethargy. A unique feature of this drug is that it is highly potent and selective in its inhibition of serotonin reuptake and has little effect on other neurotransmitters. Because of its potent inhibition of serotonin reuptake, paroxetine is more likely to cause withdrawal effects upon cessation. Paroxetine is well tolerated in most patients with a similar adverse effect profile compared to other members of the same drug class, including citalopram, fluoxetine, and fluvoxamine. The controlled release formulation was designed to decrease the likelihood of nausea that is sometimes associated with paroxetine. Paroxetine is indicated for in the management of generalized anxiety, panic, social phobia, posttraumatic stress, premenstrual dysphoric disorder, and obsessive-compulsive spectrum disorders [234]. Currently, it has received FDA indications for managing mild to moderate vasomotor symptoms of menopause. However, according to literature paroxetine was proved that has serious side and adverse drug effects ranging from congenital birth defects and heart abnormalities to breast and other possible cancers. It also may, along with other SSRIs and SNRIs, increase suicidality, aggression, and akathisia in pediatric patients with incidence outcomes which initially may have been significantly underestimated in clinical trials. Off-label, paroxetine may be used for the treatment of premature ejaculation or irritable bowel syndrome (IBS). Paroxetine is

metabolized in the liver to a catechol metabolite that is subsequently converted to both glucuronide and sulfate metabolites via methylation and [234,235].

1.11.8 Cyclobenzaprine

Cyclobenzaprine is a centrally acting skeletal muscle relaxant for the treatment of muscle spasm associated with acute, painful musculoskeletal conditions. Cyclobenzaprine has antidepressant action and is a part of a group of medications referred to as cyclical antidepressants [236]. It was initially studied for use as antidepressant given its structural similarity to tricyclic antidepressants - it differs from Amitriptyline by only a single double bond. These cyclical antidepressants have roles in the treatment of depression, neuropathic pain, migraine prophylaxis, attention deficit hyperactive disorder as well as potential muscle relaxation properties. As an off-label prescription drug cyclobenzaprine is occasionally used for reducing pain and sleep disorders in patients with fibromyalgia [237]. Cyclobenzaprine is a tricyclic amine salt that works in the central nervous system (CNS) as a depressant and sedative and has associated effects of reducing muscle hyperactivity. It is a centrally-acting muscle relaxant that reduces tonic somatic motor activity, which may influence alpha and gamma motor neurons at the level of the spinal cord [234]. It helps in this way to relieve pain, stiffness, or discomfort caused by strains or injuries to the muscles. Its actions on the CNS may also cause some of this medicine's side effects such as dizziness, xerostomia, drowsiness, fatigue, headache, nervousness, and confusion, tachycardia, mydriasis, and hallucinations. Cyclobenzaprine is extensively metabolized in the liver via both oxidative and conjugative pathways. Oxidative metabolism, mainly N-demethylation occurs, responsible for the major metabolite desmethyl cyclobenzaprine. Cyclobenzaprine also undergoes N-glucuronidation with water-soluble glucuronide conjugates as metabolites excreted from the urine [237].

1.11.9 Amitriptyline

Amitriptyline is a tricyclic antidepressant (TCA)., widely used to treat depression. In addition, it has analgesic action and is used for neuropathic pain (neuaralgia, back pain) and prevention of migrain atracks [238]. It has anticholinergic and sedative properties helping diabetic neuropathic pain, and neurogenic pain syndromes that are frequently unresponsive to narcotic analgesics. Amitriptyline has also shown efficacy in diverse groups of patients with chronic non-malignant pain. Amitriptyline plays a special role in treatment of fibromyalgia as a first- line medication where tricyclic antidepressants are strongly recommended. Fibromyalgia is a complex chronic

condition characterized by pain, physical fatigue, sleep disorder and cognitive impairment. Amitriptyline evokes a preferential reduction in pain and fatigue of fibromyalgia improving the quality of life for these patients. Finally, prophylactic treatment of chronic tension-type headache (CTTH) in adults has been also reported. As an off-label use is considered for irritable bowel syndrome, diabetic neuropathy, agitation, and insomnia [239–242].

The mechanism of action of this drug is not fully elucidated. It is suggested that amitriptyline inhibits the membrane pump mechanism responsible for the re-uptake of transmitter amines, such as norepinephrine and serotonin, thereby increasing their concentration at the synaptic clefts of the brain. These amines are important in regulating mood. The monoamine hypothesis in depression, one of the oldest hypotheses, postulates that deficiencies of serotonin (5-HT) and/or norepinephrine (NE) neurotransmission in the brain lead to depressive effects . This drug counteracts these mechanisms, and this may be the mechanism of amitriptyline in improving depressive symptoms [240].

Amitriptyline and its metabolites are mainly excreted in the urine as glucuronide or sulfate conjugate of metabolites, with approximately 2% of unchanged drug appearing in the urine 25-50% of a single orally administered dose is excreted in urine as inactive metabolites within 24 hours. Lesser amounts are excreted in feces via. The metabolism of amitriptyline occurs mainly by demethylation as well as hydroxylation followed by conjugation with glucuronic acid. Its main active metabolite is the secondary amine, nortriptyline [243].

1.11.10 Fluoxetine

Fluoxetine is a diphenhydramine derivative and second generation antidepressant classified to selective serotonin reuptake inhibitors (SSRIs). It is known with the commercial name of Prozac, Sarafem widely used for the treatment of depression. Specifically, it is prescription medicine used to treat major depressive disorder, bulimia nervosa (an eating disorder), obsessive-compulsive disorder, panic disorder, and premenstrual dysphoric disorder (PMDD). Fluoxetine may also be used in associated with olanzapine to treat depression related to Bipolar I Disorder, and treatment resistant depression. Other uses of fluoxetine may occur to treat alcoholism, attention-deficit disorder, borderline personality disorder, sleep disorders, headaches, mental illness, posttraumatic stress disorder, Tourette's syndrome, obesity, sexual problems, and phobias.

Fluoxetine acts by inhibiting the presynaptic reuptake of the neurotransmitter serotonin. As a result, levels of 5-hydroxytryptamine (5-HT) are increased in various parts of the brain. Further, fluoxetine has high affinity for 5-HT transporters, weak affinity for noradrenaline transporters and

no affinity for dopamine transporters indicating that it is 5-HT selective. Fluoxetine interacts to a degree with the 5-HT2C receptor and it has been suggested that through this mechanism, it is able to increase noradrenaline and dopamine levels in the prefrontal cortex.

Fluoxetine therapy can be associated with transient asymptomatic elevations in serum aminotransferase levels and has been linked to rare instances of clinically apparent acute liver injury. Fluoxetine is primarily eliminated in the urine and its main metabolite is norfluoxetine. Both fluoxetine and norfluoxetine undergo glucuronidation to facilitate excretion [244–246].

1.11.11 Carbamazepine

Carbamazepine is an anticonvulsant drug used individually or in combination with other medications to control certain types of seizures in people with epilepsy. Carbamazepine is indicated for the pain associated with true trigeminal neuralgia (a condition that causes facial nerve pain), or nerve pain caused by diabetes (peripheral neuropathy). Carbamazepine has shown efficacy in treating mixed seizures, partial seizures with complex symptoms, and generalized tonic-clonic seizures. Carbamazepine is also indicated for the treatment of manic episodes and mixed manic-depressive episodes caused by bipolar I disorder [247]. Some off-label, unapproved uses of carbamazepine include the treatment of alcohol withdrawal syndrome and restless leg syndrome [248]. The suggestion of its action is summarized to reduction of abnormal electrical activity in the brain, inhibiting sodium channel firing, treating seizure activity. Carbamazepine is mainly excreted as hydroxylated and conjugated metabolites, and minimal amounts of unchanged drug. [248]

1.11.12 Clomipramine

Clomipramine, the 3-chloro analog of imipramine, is a dibenzazepine-derivative tricyclic antidepressant. It is used to treat symptoms of obsessive-compulsive disorder (OCD), such as persistent/unwanted thoughts or it helps to reduce the urge to perform repeated tasks such as handwashing, counting, checking. Clomipramine may also be used for purposes of depression, panic disorder, chronic pain (e.g. central pain, idiopathic pain disorder, tension headache, diabetic peripheral neuropathy, premature ejaculation, and premenstrual syndrome . Clomipramine is a strong, but not completely selective serotonin reuptake inhibitor (SRI), as the active main metabolite desmethyclomipramine acts preferably as an inhibitor of noradrenaline reuptake. α1-receptor blockage and β-down-regulation have been noted and most likely play a role in the short-term effects of clomipramine. Critical manifestations of side effects include skin rash, fever, mood

or behavior changes, muscle aches, trouble sleeping severe weakness, unusual bruising, anxiety, panic attacks [249,250].

The main active metabolite is desmethylclomipramine, mainly excreted in urine (51-60%) and feces via biliary elimination (24-32%). Other metabolites and their glucuronide conjugates are also produced [251].

1.12 Analgesics-Non-steroidal anti-inflammatory drugs (NSAIDs)

In general, NSAIDs are the most frequently and ubiquitously environmentally detected drugs worldwide. They are detected in surface water (river, lake, sea, ocean), groundwater, wastewater (municipal, industrial, hospital), soils, bottom sediments, drinking water, snow and the Antarctic glaciers [252–254]. They provide wide application treatment range because of their chemical structures with anti-inflammatory, antipyretic and analgesic properties. The fact that most of them are provided without prescription contributes to their uncontrolled use and release to the environment. Clinically, they are useful in relieving pain in many conditions, ranging from menstrual and postoperative pain to arthritic pain. These drugs are well-known anti-inflammatory agents, and they exert their effects through the inhibition of prostaglandin synthesis by blocking the enzyme cyclooxygenase (COX) [255]. Pharmaceuticals from the NSAID group are of a diverse and complex chemical nature. They include derivatives of indolacetic (indomethacin, sulindac, etodolac), phenylacetic (diclofenac), and propionic (ibuprofen, naproxen, flurbiprofen, ketoprofen) acids, salicylates (acetylsalicylic acid), pyrazolidines (metamizole), oxycames (meloxicam), alkanones (nabumeton), and sulfanomide derivatives (nimesulide) [256]. With the reactive groups (in particular, hydroxyl, amide), NSAID molecules have both high reactivity and stability. This determines their resistance to biodegradation, ecotoxicity, persistence and therefore threat to the environment [257]. NSAIDs enter the environment mainly with industrial and municipal wastewater [258] since are not totally metabolized in the human body, and unutilized compounds are excreted unchanged or in complexes with glucuronic acid [258]. Removal efficiency of some NSAIDs not exceeding 30% [259–261] demonstrates that treatment facilities fail to eliminate their occurrence too. The lack of strict control in the drug turnover including NSAIDs, leads to unused or expired medicines that enter the sewer systems without pretreatment [262].

In this study five drugs belonging to analgesics-NSAIDs category, salicylic acid, indomethacin, diclofenac, mefenamic acid and tolfenamic acid were investigated.

1.12.1 Salicylic acid

Salicylic is a cosmetic and pharmacologic additive in many skin-care products for the treatment of skin associated diseases. It is the main metabolite of acetylsalicylic acid (aspirin) a popular OTC non-steroidal anti-inflammatory drug. Acetyl salicylic acid is easily degraded by deacetylation into salicylic acid and two other metabolites, ortho-hydroxyhippuric acid and the hydroxylated metabolite gentisic acid [263]. Salicylic acid is in a class of medications called keratolytic agents. Salicylic acid treats acne by causing skin cells to slough off more readily, preventing pores from clogging up. This effect on skin cells also makes salicylic acid an active ingredient in several shampoos meant to treat dandruff. Use of straight salicylic solution may cause hyperpigmentation on unpretreated skin for those with darker skin types (Fitzpatrick phototypes IV, V, VI), as well as with the lack of use of a broad-spectrum sunblock. Topical salicylic acid is also used to treat skin conditions that involve scaling or overgrowth of skin cells such as psoriasis (a skin disease in which red, scaly patches form on some areas of the body), ichthyoses (inborn conditions that cause skin dryness and scaling). Subsalicylate in combination with bismuth form the popular stomach relief aid known commonly as Pepto-Bismol. When combined the two key ingredients help control diarrhea, nausea, heartburn, and even gas. It is also very mildl antibiotic [249,264].

1.12.2 Indomethacin

Indomethacin is a nonsteroidal anti-inflammatory drug (NSAID) with, analgesic, anti-inflammatory and antipyretic properties [265]. It is similar to ibuprofen and naproxen. Indomethacin works by reducing the production of prostaglandins. Prostaglandins are chemicals that the body produces, and which cause the fever and pain that are associated with inflammation [266]. Indomethacin blocks the enzymes that make prostaglandins (cyclooxygenase 1 and 2) and thereby reduces the levels of prostaglandins. As a result, fever, pain, and inflammation are reduced. Indomethacin reliefs the symptoms of pain, swelling, and joint stiffness caused by rheumatoid arthritis, ankylosing spondylitis, osteoarthritis, acute shoulder pains, and acute gouty arthritis, tendinitis. Indomethacin is excreted in urine, approximately 60 % as drug and metabolites, (26 % as indomethacin and its glucuronide), and 33 % in the feces (1.5 % as indomethacin) [265].

1.12.3 Diclofenac

Diclofenac is a nonsteroidal anti-inflammatory drug (NSAID), first approved by the FDA in 1988 under the trade name Voltaren, marketed by Novartis. It reduces inflammation and by extension reduces nociceptive pain and confronts fever [267]. Diclofenac is indicated for use in the

treatment of pain and inflammation from varying sources including inflammatory conditions such as osteoarthritis, rheumatoid arthritis, and akylosing spondylitis, as well as injury-related inflammation due to surgery and physical trauma [268]. Diclofenac inhibits cyclooxygenase-1 and -2, the enzymes responsible for production of prostaglandin (PG) G_2 which is the precursor to other PGs [269]. These molecules have broad activity in pain and inflammation and the inhibition of their production is the common mechanism linking each effect of diclofenac. Long term use of diclofenac or overdose can increase the risk of fatal heart attack or stroke and may also cause stomach or intestinal bleeding. 60-70% is eliminated in the urine and 30% is eliminated in the feces. Diclofenac undergoes oxidative metabolism to hydroxy metabolites as well as conjugation to glucuronic acid, sulfate, and taurine. The primary metabolite is 4'-hydroxy diclofenac [270].

1.12.4 Mefenamic acid

Mefenamic acid is in a class of medications called NSAIDs. Mefenamic It exhibits anti-inflammatory, analgesic, and antipyretic activities for the short-term treatment of mild to moderate pain from various conditions. It is in oral formulations as the brand-name drug Ponstan [271]. Similar to other NSAIDs, mefenamic acid inhibits the activity of the enzymes cyclo-oxygenase I and II, resulting in a decreased formation of precursors of prostaglandins and thromboxanes [272]. The resulting decrease in prostaglandin synthesis, by prostaglandin synthase, is responsible for the therapeutic effects of mefenamic acid. Common uses of mefenamic acid regard painful conditions such as arthritis, pain associated with heavy menstrual bleeding, and pain after surgical operations. Drug effects that may be dangerous concern heart risks and stomach problems. Mefenamic acid undergoes metabolism to 3-hydroxymethyl mefenamic acid, and further oxidation to a 3-carboxymefenamic acid may occur. The metabolites and conjugates are primarily excreted by the kidneys, although renal and hepatic excretion are significant pathways of elimination. Mefenamic acid is also glucuronidated directly [273].

1.12.5 Tolfenamic acid

Tolfenamic acid, belongs to NSAIDs pharmaceuticals and is effective in treating the pain associated with the acute attack of migraines in adults. The pharmacologic effects of tolfenamic acid are similar to those of aspirin. The mechanism of action of tolfenamic acid is based on the major mechanism of NSAIDs : inhibits the biosynthesis of prostaglandins, and it also presents inhibitory actions on the prostaglandin receptors and thus, exerts its anti-inflammatory and pain-blocking action. Tolfenamic Acid is used to relieve pain and swelling associated with

musculoskeletal conditions such as rheumatoid arthritis, osteoarthritis, ankylosing spondylitis. It is efficient to relief symptoms such as swelling, stiffness, joint pain, etc. associated with the above diseases. After prolonged usage of tolfenamic acid severe gastrointestinal bleeding and perforation may occur. This medicine should be used with extreme caution in patients suffering from hypertension, asthma, Systemic Lupus Erythematosus due to the increased risk of severe adverse effects [274].

Most of the elimination occurs by extrarenal mechanisms in which the unchanged drug together with its glucuronide in urine accounts for only 8.8% of the administered dose. The first pass metabolism accounts for 20% of the administered dose of tolfenamic acid. Urine metabolite studies have demonstrated the identification of five metabolites [275].

1.13 Personal Care Products

Personal care products are a wide variety of items applied to the human body for the purposes of cleaning, beautifying, promoting attractiveness or changing its appearance. Some personal care products meet the definitions of both cosmetics and drugs because of two intended uses [53]. Health products such as supplements, over-the-counter drugs, and prescription pharmaceuticals, cosmetic products comprise the diverse range of PCPs which also include [23,53]:

- UV filters/sunscreen
- DEET – N,N-diethyl-meta-toluamide, the most common active ingredient in insect repellents
- parabens – alkyl esters of p-hydroxybenzoic acid, used as bacteriostatic and fungistatic agents in drugs, cosmetics, and foods
- polycyclic musks – tonalide and galaxolide are used as fragrances in a wide range of washing and cleaning agents and personal care products
- triclosan – bacteriocide and antifungal agent widely used in household products, such as toothpaste and soap

1.13.1 Triclosan

Triclosan is a broad-spectrum antimicrobial, antiseptic, disinfectant or preservative since it is effective against many different bacteria as well as some fungi and protozoa. It is widely used in clinical settings and in various personal care products (PCPs), such as toothpaste, soap, shampoo, deodorant, mouthwash, cosmetics, typically in a concentration range of 0.1–0.3%. Triclosan can

also be incorporated in kitchen utensils, toys, and textiles, plastic materials, paints from which it might slowly leach for a long period of time during their use, to perform its biocidal action [276]. Triclosan binds to enoyl-acyl carrier protein reductase enzyme (ENR). This complex has increased affinity for NAD+ and forms a ternary complex. This complex is unable to participate in fatty acid synthesis, weakening the cell membrane and causing cell death.

Due to the widespread use of triclosan in all these applications, as antimicrobial agent, it may result in the development of cross-resistance to antibiotics, and thereby the emergence of bacterial strains resistant to both triclosan and antibiotics [277]. Human exposure can occur through oral or dermal contact with these PCPs or through the consumption of contaminated food or drinking water [278]. Triclosan can also reach the systemic circulation after skin application with absorption up to 9% of the administered amount [279]. Once absorbed, TCS is readily metabolized to its glucuronide and sulfate conjugates and primarily eliminated through urinary excretion. Important pathways of triclosan release into the environment include WWTP effluent discharge into surface waters and the land application of biosolids where can have adverse impacts on the growth of aquatic organisms.

1.14 Lipid regulators

The regulatory lipids are referred to as lipid chemical mediators which regulate various important biological processes such as cell proliferation, apoptosis, tissue repair, blood clotting, blood vessel permeability, inflammation, immune cell behavior, and other biological functions [280]. Cholesterol, for example, is an essential component of the human cell membrane and a precursor for steroid hormones and bile acids. Triglycerides also play an important role in transferring energy from food into body cells. However, any biomolecule in excess is not good for human health. Similarly, elevation of different forms of lipids in the bloodstream, a condition generally termed hyperlipidemia, causes a constant health problem. Because lipids are carried in the bloodstream, hyperlipidemia is always a threat to coronary arteries and the most important risk factor for coronary heart disease. Lipid regulatory drugs, or lipid-lowering drugs can confront these problems. They are classified mainly into two groups the statins and fibrates. Statins act by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and, thereby, suppress cholesterol biosynthesis, whereas fibrates stimulate β-oxidation of fatty acids lowering in this way the plasma levels of fatty acid and triacylglycerol [281]. The lipid regulator drug investigated in this study classified to the latter category, was gemfibrozil.

1.14.1 Gemfibrozil

Gemfibrozil is a lipid regulator drug that belongs in the subclass of fibrates. It is prescribed along with a proper diet to lower plasma triglycerides. This pharmaceutical, also reported to lower very low-density lipoproteins and total cholesterol and to increase high-density lipoproteins [281], has also been clinically prescribed in patients at high risk of coronary heart disease. For this reason, is indicated to treat patients with Types IV and V hyperlipidemia who have elevated serum triglycerides and VLDL cholesterol, fasting chylomicrons, with high risk of pancreatitis development. In addition, is indicated to reduce the risk of developing coronary heart disease in patients with Type IIb hyperlipidemia without history or symptoms of coronary heart disease. Gemfibrozil is not a first line treatment and is prescribed to patients who do not adequately respond to weight loss, diet, exercise, and other medications; and have low HDL, raised LDL, and raised triglycerides [282].

Approximately 70% of gemfibrozil is eliminated in the urine as a glucuronide conjugate and <2% is excreted unchanged. 11 6% of a dose is excreted in the feces [282,283].

1.15 Artificial Sweeteners

The category of artificial sweeteners (ASs) as emerging contaminants was described previously in section 1.3. This study focuses on the occurrence of four ASs including acesulfame, aspartame, sucralose, and saccharin in wastewater effluents.

1.15.1 Aspartame

Aspartame is a linear dipeptide methyl ester of l-aspartyl-l-phenylalanine, was discovered in 1965 and got its initial approval from FDA in 1974. Aspartame was approved as a general-purpose sweetener by FDA in 2006. It is a white, odorless powder, 180 times sweeter than sucrose [92]. The stability of aspartame in aqueous media is not entirely satisfactory, especially in liquids such as carbonated beverages, is not stable and degrades in liquids when stored over a longer period of time [105]. In addition is unstable during prolonged heating, therefore, it is not suitable for cooking. The acceptable daily intake (ADI) of aspartame is 50 and 40 mg/kg bw/ day, based on the United States (FDA) and European Union (JECFA1) recommendations, respectively [284,285]. Once ingested, aspartame is metabolized to aspartic acid, phenylalanine and methanol in the ratio of 50:40:10 %, respectively. For people with a seldom genetic disorder, the generated phenylalanine does carry some risk as their body cannot metabolize the degradation product. As a consequence, all products containing aspartame have to be labeled to point out the presence

of a phenylalanine source. Following the metabolic path of methanol, methanol is oxidized to formaldehyde in the liver, which is accompanied by the formation of superoxide anion and hydrogen peroxide [284,286]. Therefore, aspartame intake can increase the production of pro-oxidants in body and cause adverse health effects [287,288] especially in some conditions like diabetes, aging and intensive physical activity which there are innately increased production of free radicals. Numerous publications with contrary results about possible adverse effects of aspartame like neurological disturbances [289,290] or even cancer in rats [291,292] are available. Nevertheless, FDA and the European Union consider the compound as safe based on toxicological and clinical studies [96].

1.15.2 Acesulfame-K

Acesulfame-K is an acidic cyclic sulphonamide derivative. It was discovered in 1967 and approved by FDA in 1998 [92]. Acesulfame-potassium was approved for use in a variety of foods and beverages by the United States Food and Drug Administration (USFDA) in 1988 [91] and is often blended with other ASs (e.g., aspartame, sucralose) [106].Acesulfame-K is a white crystalline powder,120 times sweeter than sucrose and has high water solubility and heat stability, so it can be used in cooking and baking [286,293]. Acesulfame-K may cause a bitter taste when used alone; therefore, it is mostly used in combination with other sweeteners to mask this taste [293], [91] whereby each sweetener masks the other's after taste and exhibit a synergistic effect by which the blend is sweeter than its components [91]

It is not metabolized in the body, thus it provides no calories. ADI for acesulfame-K is set as 15 mg/kg of body weight/day based on the FDA and JECFA recommendations .Finally, it has been reported to be one of the ASs more resistant to efficient removal in WWTPs [96].

1.15.3 Saccharin

Saccharin began to be produced in 1878 and it is one of the oldest artificial sweeteners. Saccharin is a common name for the corresponding acid, 1,2-benzoisothiazol-3(2H)- one-1,1-dioxide, its sodium, potassium, and calcium salts. It is 200 to 700 times sweeter than table sugar (sucrose), and it does not contain any calories [105]. Saccharin is currently approved for use, under certain conditions, in beverages, fruit juice drinks, and bases or mixes when prepared for consumption in accordance with directions, as a sugar substitute for cooking or table use, and in processed foods. Saccharin is also approved for use for certain technological purposes [105]. The ADI established

by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is 15 milligrams per kilogram body weight per day (mg/kg bw/d) [294].

In the human body it is excreted unchanged in the urine, it is not metabolized and the unabsorbed portion is excreted with faeces [105]. Saccharin is very stable, its solutions buffered at pH ranging from 3.3 to 8.0 were essentially unchanged. Saccharin occurs in groundwater due to old landfills, application of fertilizers in agriculture, degradation of sulfonylurea herbicides, irrigation, soil water management, use of sludge as a fertilizer and leaks in the ducts. Saccharin and its salts are found in municipal wastewater and sewage. It serves not only as a sweetener for human consumption, but also it is registered as an additive for piglets. Smaller amounts of saccharin are used in industry as a galvanic brightener [105].

Saccharin was discussed for adverse health effect such as genotoxicity, however [91]considering the weight of evidence from all the genotoxicity studies, the European Committee considers that these indicate saccharin is not a direct acting genotoxin. Support for this view comes also from the fact that it has been shown to be a carcinogen at only one site in only one sex of one species of animal, whereas genotoxic carcinogens tend to be active at more than one site [295].

1.15.4 Sucralose

Sucralose is a synthetic tri-chlorinated disaccharide which was discovered in 1976 and approved some decades later by FDA in 1998. Sucralose is a sweet, white crystalline powder and its taste profile is very close to that of sucrose [92] although it is 450–650 times sweeter than sucrose [296,297]. Today sucralose is permitted for use in more than 80 countries worldwide and is present in excess of 4,000 different products [298]. It has a moderate synergy with other nutritive and non-nutritive sweeteners, it is very soluble in water and has high stability over a wide range of pH and temperature [299], although it liberates HCl when stored at high temperature and produce some kind of discoloration [300].

The ADI for sucralose is 5mg/kg bw/day in the United States (FDA) and 15 mg/kg/d in the European Union as recommended by Scientific Committee on Food of the European Commission (SCF) [296]. Most of the ingested sucralose (65%- 95%) is excreted relatively unchanged in feces and just negligible amounts can be absorbed; therefore, it provides no calories [298,301].

1.2 Analytical Methods-Sample preparation techniques

Taking into consideration the discussion about possible sources of emerging contaminants as well as their fate in the environment through physicochemical and metabolic processes, the ECs can be found in low concentrations even in ng/L levels in both natural and anthropogenic media, for example, in wastewater, soil, air, or tissues of living organisms. As described in previous sections, even in low concentrations ECs and especially pharmaceuticals can cause potential adverse effect across a range of species. In order to have a complete knowledge of the problems presented by ECs it is necessary to identify and quantify the types of the compounds that are present. To address this issue sampling, sample preparation methods along with analytical techniques which can confirm the identity of targeted analytes at trace concentrations in complex environmental samples are employed and consist all the steps of an analytical method. In fact, the advances in analytical methods have enabled the detection of molecules in so low quantities . For a long time ago such medication products or personal care products were off concern because of inappropriate detection techniques which did not provide sensitivity in the ranges of ppb or ppt either in environmental samples or in biological samples. Developing reliable analytical methods for the determination of ECs is a great analytical challenge taking into consideration the complexity of the studying media. For example, wastewater sample is a complex environmental matrix rich in lipids and dissolved organic matter. Sample preparation techniques for the determination of ECs along with analytical methods are presented in following sections. Analytical residual methods of determination are classified in single-residue method, when the analysis of one pollutant or a few pollutants from the same chemical family is performed and multi-residue methods when large number of pollutants or different classes of pollutants are included.

In this chapter section a theoretical background of the sample preparation techniques of choice in current investigation, is reported. More specifically, principles and strategies of Solid-Phase Extraction (SPE), Magnetic Solid Phase Extraction (MSPE) as well as Fabric Phase Sorptive Extraction (FPSE) are presented.

1.2.1 Sample preparation techniques

In order for a multiresidue analysis to be effective, sample pretreatment regardless the type and the nature of the sample should fulfill three main objectives: (a) modification of studied matrix in order to be feasible for analysis, e.g. injection in a chromatographic column without causing problems in analytical system (b) minimize interferences in order to improve accuracy and selectivity of the determination method but also to protect indirectly the instrument by reducing

the cost of the analytical assays (c) Substance-specific preconcentration to improve the sensitivity of the method. In multi-residue methods the optimum conditions vary depending on the physicochemical properties of the target analytes and compromise has to be taken in order develop a suitable and equally effective method for the analysis of a large number of compounds. The selection of a sample pretreatment method depends on the complexity of the matrix, its properties and the expectable concentration level of the analyte of interest. Sample pretreatment usually is a laborious and time-consuming step , as it has been calculated that requires almost 60-80% of the total time of sample analysis [302]. Moreover, insufficient, or erroneous pretreatment can introduce errors in the analysis which consist up to 30% of the total experimental errors. Typical pretreatment steps include:

- Sampling and homogenization of the sample in order to be representative
- Extraction and isolation of unwanted substances
- Clean-up for the removal of interferences
- Evaporation if preconcentration is necessary
- convert the analytes to instrument-compatible derivatives

All these steps are sequential and interdependent, therefore in case that one of these is not completed correctly, errors may be involved and transferred from one step to another leading to inaccurate results.

The development of simple, fast, low-cost, and environmentally friendly methods that provide analytical characteristics such as high accuracy, sensitivity in terms of low detection limits and repeatability is of crucial concern. Recently, several techniques have been developed that belong to the so-called modern techniques replacing in many cases the traditional extraction techniques. In case that no extraction technique, either traditional or modern, does not full fill the above conditions, the most suitable it should be chosen each time. The suitability of a method depends on the type of sample, the physicochemical properties of the target analytes, the accuracy of the determination etc. Parameters that influence the effectiveness of the method include the use of small volumes or mass of initial sample and volumes of organic solvents as well as the laboratory equipment which such as the automation ability, the on-line extraction techniques etc.

1.2.1.1 Extraction techniques of organic micropollutants

Extraction technique is defined the transfer of a substance from one phase (liquid, solid or suspension) to another phase (liquid) and is used for the separation, isolation and recovery of a substance from a chemical system. The extraction of compounds from liquid and solid

environmental matrices is based on their partition in one immiscible with water solvent and aqueous phase or on the retain of the compounds onto a column or a solid phase substrate or even more seldom on the evaporation to dry and the selective reconstitution of the analyzed compounds.

Traditional extraction techniques including liquid-liquid extraction (LLE) and Soxhlet extraction, for instance, were developed decades ago and have been since applied to sample processing. However, these traditional techniques show major drawbacks related to sample preparation including: (a) high solvent consumption and waste generation, (b)the laborious routine, (c) frequent source of sample contamination, and (d) analytical errors due to the operator's handling required to perform these techniques. For this reason, nowadays the scientific concern is focused to analytical techniques with the use of small amounts of organic solvents, alternative non-chlorinated solvents and with the ability of automation in order to provide accurate, selective and repeatable results. The evolution of classic extraction technique resulted to a series of extraction techniques with the use of a solid phase sorbent material. The principle of all these techniques is the selective retain of the target analyte onto the sorbent and the following selective recovery from it with an organic solvent. Developments in sample-preparation techniques, design of new extraction devices and their applications were employed depending the target analytes and the nature of the studied sample. Many approaches to sample preparation, for the extraction of multi-residue compounds from either water and wastewater samples or from terrestrial samples such as soil and sludge include: Liquid-liquid extraction (LLE), dispersive liquid-liquid microextraction (DLLME), (SBSE), solid phase extraction (SPE), supercritical fluid extraction (SFE), pressurized fluid extraction (PFE), microwave-assisted extraction (MAE), QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe), Ultra-sound –assisted extraction, UAE matrix solid-phase dispersion (MSPD), accelerated solvent extraction [303,304].

Solid phase extraction (SPE) was introduced to overcome the main disadvantages and limitations of LLE, especially the use of large amounts of organic solvent and low selectivity related. SPE can be considered as a simple technique presenting satisfactory extraction efficiency as well as a good pre-concentration capacity for most matrices. SPE is one of the most employed techniques for sample preparation especially in liquid samples. SPE was used as a theoretical base for the development of solid phase microextraction (SPME) and now is considered as the precursor of all miniaturized sample preparation techniques such as in-tube solid phase microextraction (in-tube SPME), stir-bar sportive extraction (SBSE), magnetic SPE (MSPE), microextraction by packed syringe (MEPS), and dispersive solid-phase extraction (d-SPE). Those miniaturized techniques

based upon the use of sorbent materials are the most utilized for sample preparation, once they considerably decrease the organic solvent consumption during the extraction and preconcentration steps [302,305,306].

In this context, several new materials that enable the extraction efficiency, have been developed. The selection of the most appropriate sorbent able to extract a wide range of analytes present in complex matrices is crucial for the susceptibility of the method. Recently novel materials for extraction purposes were developed including(a) molecularly imprinted polymers (MIPs), (b) ionic liquids (ILs), (c) immunosorbents (IS), (d) carbon-based materials, and (e) sol-gel-based compounds, among others. The synthesis of these novel compounds permits the assessment of suitable miniaturized extraction technique along with the most selective sorbents [302,305,306]. Currently, this doctoral thesis focuses on the development of Solid-Phase Extraction for the determination of pharmaceutical active compounds and artificial sweeteners. At the same time, metal oxides, polymer-based composites and carbon based nanomaterials (NMs) have been integrated in magnetic solid phase extraction (MSPE) and fabric phase sorptive extraction respectively for the efficient extraction and preconcentration the target emerging contaminants from wastewater samples.

1.3 Solid-Phase Extraction (SPE)

SPE has been accepted for more than 20 years as the alternative sample preparation method to LLE, due to its simplicity and economy in terms of time and solvent [307]. SPE has gained wide acceptance because of the inherent disadvantages of LLE, whose drawbacks include [308]

(1) inability to extract polar compounds; (2) being laborious and time-consuming; (3) expense; (4) tendency to form emulsions; (5) need for evaporation of large volumes of solvents; and, (6) disposal of toxic or flammable chemicals.

Moreover, recent regulations concerning to the use of organic solvents have made LLE techniques unacceptable. LLE procedures that require several successive extractions to recover more than 99% of the analyte can often be replaced by SPE methods [309]. Due to the fact that SPE is a more efficient separation process than LLE, it is easier to obtain a higher recovery of the analyte by using a reduced volume of solvents in the elution step. Furthermore, SPE does not require the phase separation required for LLE, and that eliminates errors associated with variable or inaccurately-measured extract volumes [309].

Solid-phase extraction (SPE) is the most popular sample pretreatment technique used for extraction and preconcentration of target analytes applied in environmental, biological, clinical

as well as food analysis [310–312]. Concerning forensics and clinical sciences, SPE has been successfully used for the determination of a wide range of substances such as antipsychotics, illicit drugs, drugs of abuse, stimulants etc. in a variety of biological samples and tissues [313,314]. It is also used as clean-up technique clean-up [315], class fractionation [316]. SPE is also useful for storing micro-pollutants from environmental samples [317] and desalting proteins and sugar samples [318]. Other uses of SPE are derivatization [307], concentration of pigments, and changing of solvents [319].

Moreover, SPE is a recommended procedure by Environmental Protection Agency (EPA) for the pretreatment of organic pollutants in several methodologies proposed. In fact, EPA method 1694 includes SPE with the use of Oasis HLB cartridges for the clean-up and extraction of pharmaceuticals and personal care products in waters and sediments [320].

Solid-phase extraction (SPE) can be employed in many formats, on discs, cartridges, and multi-well-plates [321], has still been the most important and used technique in the environmental research area due to the high simplicity and flexibility SPE procedure consists of four main steps (Fig.1.15):

1. Conditioning of the packing material:
2. Loading of the sample
3. Washing off undesired components (interferences)
4. Elution of target analytes and recovery into a collection tube

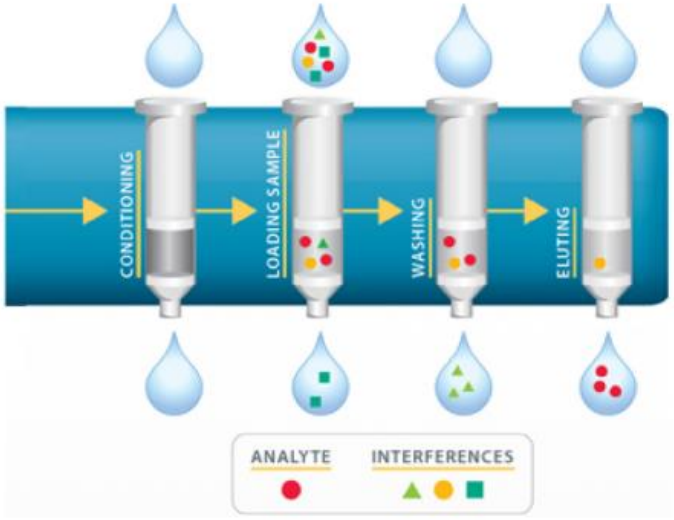


Figure 1.15 Typical schematic procedure of SPE associated with cartridges [307]

In case of SPE process with cartridges the sorbent material of the cartridge is activated with suitable organic solvent followed by the addition of water in order to be conditioned for the addition of the sample. Conditioning the solid-phase materials consists of passing organic solvents or water through the column to increase the effective surface area and reduce interferences. Next, real water sample is loaded on the cartridge causing strong retention of the analytes. Flow rates of 2 – 4 mL/min are usually recommended. High flow rates may not allow equilibrium to take place and therefore allowing some (or all) of the analyte to pass unretained. Slow flow rates may greatly increase the time of the SPE experiment. Next step of washing involves the removal of matrix and/or interferences by rinsing the cartridge with a solvent chosen for its intermediate strength. Water or buffered water is used as the wash solvent usually with a controlled amount of organic in order to enhance the removal of more hydrophobic interferences. Finally, elution of retained analyte is employed with the addition of organic solvent suitable to recover the target compounds. After drying the sorbent and possibly removing interferences, the interactions between analytes and the solid-phase material are disrupted by flushing small volumes of organic solvents, which leads to desorption of the target analytes from the solid phase [322].

Experimental parameters that affect the recoveries of solid phase extraction are:

1. Conditioning solvent
2.Flow rate during extraction
3.Physicochemical properties of the sample (pH, temperature, ionic strength etc.)
4.Time of drying under vacuum of sorbent material
5. Type of washing solution
6.Type and volume of elution solvent

A common mechanism to describe the SPE process is partition. In this case, the stationary phase can be considered a liquid that is immobilized on a solid support and the principle is similar to that of LLE, involving a partitioning of solutes or distribution processes between the liquid sample and the liquid immobilized on a stationary phase [323].

Liquid/solid adsorption and ion exchange are also possible mechanisms in various separations. In this case, instead of two immiscible liquid phases (as in LLE), SPE involves partitioning between a liquid (sample matrix) and solid (sorbent) phase. The simplest approach consists of considering the stationary phase as a liquid immobilized into the sorbent. The extraction is a result of competitive interactions of ionic forces retaining the solute compounds in water and dispersion

forces which generate the transfer of the target compounds from aqueous phase to the retained organic molecules onto the sorbent surface.

The retain of these compounds is achieved by strong and reverse interactions between analytes of interest and the surface of the sorbent material. Typical interactions include hydrophobic interactions van der Waals forces, polar interactions (hydrogen-bonding , dipole-dipole) as well as ion -exchange interactions.

A large number of SPE formats include SPE disks, cartridges as well as 96 well-plate for large number of samples. Various sorbent materials are commercially available covering a wide range of analytes and consequently a large variety of applications . Typical SPE sorbents include alkyl-bonded silicas, carbon-based sorbents, polymer sorbents etc. Polymer-based sorbents consist the latest trend appeared to replace silica-based and have remained one of the main developments in SPE, with continuous progress being made over recent decades. Polymeric sorbents combine outstanding morphological properties that promote capacity and retention with tuned chemical properties that allow suitable interactions with many types of compounds and show enhanced stability under several SPE conditions [324]. Indeed, although these materials are long-established and have been applied over the years, research in this area is continuously growing. Evidence of this is the fact that several polymeric sorbents are commercially available and widely applied among the scientific community for various applications, including a wide range of compounds extracted from different fields (food, biological, environmental, etc.). Table 1.8 shows some selected examples of the applications of polymeric-based sorbents [324].

Before selecting the suitable sorbent, the physicochemical properties such as the functional groups pf the target compounds, the nature of sorbent material, the energy bonds, the sorbent interactions between target analytes and sample matrix should be considered. The target analytes are eluted from sorbents with the aid of small quantity of organic solvent. The choice of suitable organic solvent depends on the polarity of the solvent along with the nature and properties of target analyte desorbed.

The disposable SPE cartridge (syringe-barrel format) are by far, the most widely used formats, providing systematization, repeatability, a wide range of reversed-phase and ion-exchange sorbent materials as well as various mechanisms of retention of target analytes. SPE cartridges generally used a single time and discarded to avoid sample cross-contamination.

Solid phase extraction has numerous advantages compared to liquid-liquid extraction processes such as (a) higher recoveries, (b) more efficient separation of interferences from analytes, (c) reduced organic solvent consumption, (d) no emulsion formation, (e) easier collection of analytes,

(f) more convenient manual procedures, (g) possibility of automation, (h) high preconcentration factor [309].

SPE sample preparation allows multi-residue analysis for compounds with a wide range of physicochemical properties and polarities.

Table 1.8 Applications of different sorbents employed in solid-phase extraction

Sorbent Material	Analytes	Sample matrix	Instrumental technique	Ref.
PLRP-S	Drugs of Abuse	Wastewater	LC-MS/MS	[325]
Strata X	Pharmaceuticals	Wastewater	LC-MS/MS	[326]
Oasis HLB	Sweeteners	Wastewater	LC-MS/MS	[327]
Lichrolut EN	EDCs	Urine, blood, milk	GC-MS	[328]
MAA-EDMA	Nerve agents & Organophosphorus	Organic matrix	GC-MS	[329]
HXLPP	Pesticides	River, wastewater	HPLC-UV	[330]
HXLPP (HEMA)	Pesticides	River, wastewater	HPLC-UV	[331]
Oasis MCX	Drugs of Abuse	Wastewater, urine	LC-MS/MS	[332]
Strata-X-WC	Toxins	Urine	LC-MS-TOF	[333]
Oasis WAX	PFCAs	River, groundwater, drinking Water	LC-MS/MS	[334]
Bond Elut Plexa SAX	Herbicides	Stormwater	LC-MS/MS	[335]
DEAEMA-DVB-SAX	Pharmaceuticals	Urine	HPLC-UV	[336]
GMA-DVB-SCX 13	Alkylate Purines	River, wastewater	HPLC-UV	[337]
HXLPP-WAX	Pharmaceuticals	River, wastewater	HPLC-UV	[338]
HXLPP-WCX	Pharmaceuticals	River, wastewater	LC-MS/MS	[339]

EDCs: endocrine disrupting compounds; MAA-EDMA: methacrylic acid-ethyldimethacrylate; HXLPP: hyper-crosslinked sorbent prepared by precipitation polymerization; HXLPP (HEMA): HXLPP including hydroxyethylene dimethacrylate: polybrominated diphenyl ethers; 10 TOF: time-of-flight; 11 PFCAs: polyfluorinated carboxylic acids; DEAEMA: 2-(diethylamino)ethyl methacrylate; GMA: glycidyl methacrylate

Polymer SPE sorbents as mentioned before they might nowadays be described as the most well-established technology for SPE, however recently, other developments in sorbent technology apart from those involving polymer-based materials have also taken place. These novel materials include organic-inorganic hybrid materials, metallic nanoparticles, metal-organic frameworks, carbon nanomaterials. Metallic nanoparticles (NPs), including Fe_3O_4, TiO_2, ZrO_2, Al_2O_3, bare form or coated, are particularly characterized with high sorption capacity due to high specific surface area. Among them magnetic NPs (MNPs) that mainly contain iron, nickel, and cobalt and their oxides, with magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) seem to be the most popular since they can be easily isolated using an external magnetic field. When MNPs were used as sorbents in d-SPE, the introduction of a new technique named magnetic SPE (MSPE) was a fact making them the main SPE mode used.

1.4 Magnetic Solid Phase Extraction

SPE is a popular technique in environmental, clinal and food analysis, however there is a need for improving and creating even better more modern and greener solutions for sample preparation, to overcome some drawbacks such as:

(1) perceived difficulty in handling its usage especially in method development

(2) cumbersome and time consuming

(3) requiring several steps before achieving a concentrated extract suitable for instrumental analysis

(4) greater cost per sample than simple LLE

(5) lack of selectivity

(6) non-reusable extraction cartridges

(7) higher amounts of a solvent when compared to the miniaturized techniques

To overcome these limitations of conventional magnetic or magnetically modified adsorbents were introduced, developed, and used for bio separation and chemical analysis. The introduction of these materials in SPE technique resulted in a considered environmentally friendly new technique the Magnetic Solid Phase Extraction (MSPE). The to the sample solution MSPE term was first introduced for analytical purposes by Šafaríková and Šafarík in 1999 [340] . A typical MSPE protocol, involves the addition of magnetic sorbent particles to the sample solution. The target compound is retained onto the magnetic material, and the magnetic particle (containing the analyte) is then separated from the sample solution by the application of an external magnetic

field. Finally, the analyte is recovered from the adsorbent by elution with the appropriate solvent, and the eluent is then used for further processing. Fig. 1.6 shows the steps in MSPE .

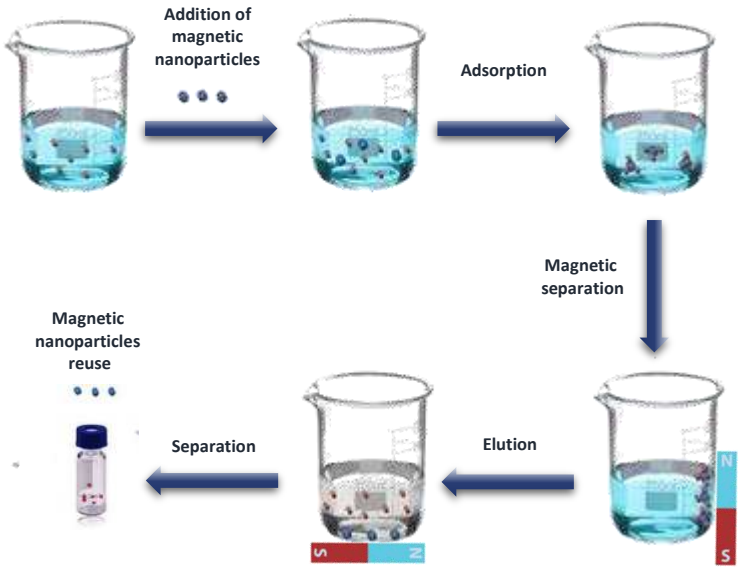


Figure 1.16 Schematic procedure of MSPE [302]

The separation mechanism of MSPE depends on the type of sorbent and is connected with the interaction of analyte molecules with the surface functional groups immobilized on the magnetic core, as in classical extraction in the solid phase. These interactions involve ionic, dipole-dipole, dipole-induced dipole, hydrogen bonding, and dispersion forces. Some authors reported that hydrophobic and Van der Waals interactions are presented in reversed phase systems while hydrogen bonding, dipole-dipole, and π-π interactions exist in normal-phase separations. These interactions are related to the functional group employed for coating the magnetic particles [341]. The functional groups mainly employed in MSPE in the silica phase are amine, thiol, carboxylic acid, alkyl, and aryl [341]. Fig. 1.17 shows a representation of possible functional compounds used to promote different interactions.

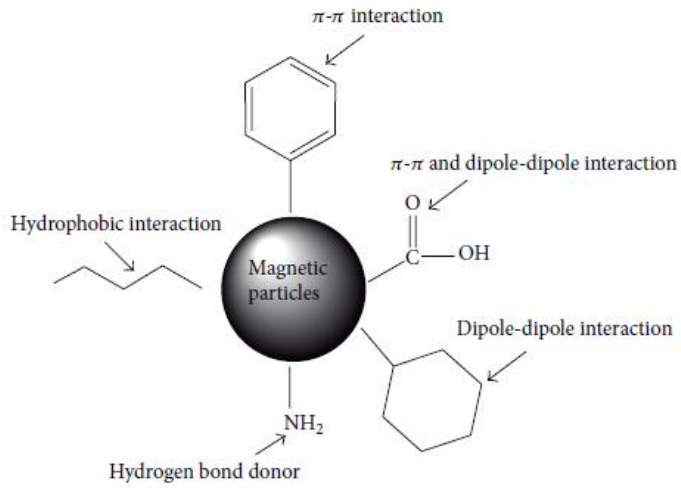


Figure 1.17 Magnetic nanoparticles of silica-coated Fe_3O_4 modified with different functional groups and corresponding interactions [341]

The successful isolation of analytes depends on the sorbent selected. The selection of the sorbent is executed depending the nature of analyte of interest. The polarity of the analytes, their solubility and the complex matrix composition are also important. In addition, the expected concentration of the analytes and matrix interferences of the sample should be considered. These properties can affect the interaction strength between the sorbent and the analyte retained. Moreover, in order to select a suitable type of solvent for the desorption of target compounds, the adequate elution strength and its role in the extraction process and the final determination should be taken into consideration.

Diverse magnetic sorbents have been developed and used in various applications for a wide scope of sample matrices and analytes. Nanoparticles and nanocomposites are preferred as sorbents in MSPE and d-SPE, due to their high surface area, high chemical activity, high adsorption capacity, fast adsorption dynamics, and good mechanical and chemical stability. The possibility for their surface functionalization is another advantage that can lead to the development of effective d-SPE procedures suitable for separation/preconcentration of various analytes in a broad range of analytical applications such as (Fig1.18):

(1) biomedicine, to separate cells and to isolate proteins, enzymes or peptides

(2) environmental science, for the isolation of metal ions, pesticides, dyes, surfactants, PAHs, drugs, antibiotics, and carcinogenic, and mutagenic compounds in water and sewage samples and,

(3) food analysis, to extract pesticides, antibiotics, metals and drugs from different kinds of food sample

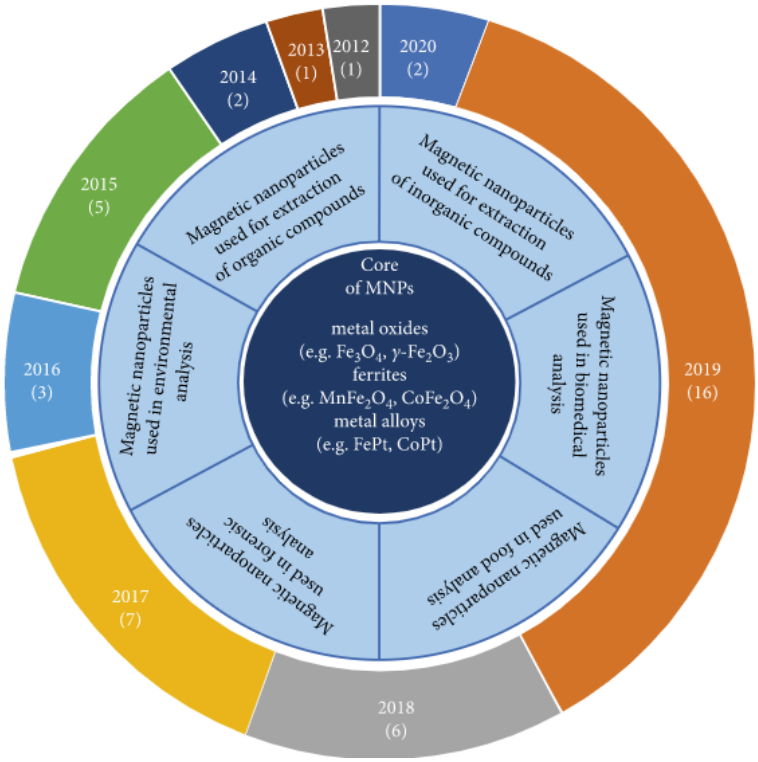


Figure 1.18 Overview of the applications of magnetic nanoparticles (MNPs) in scientific fields together with an illustration showing the number of reviews that had been annually published on this topic between January 2012 and April 2020 (according to the Web of Science database) [342]

Among nanomaterials, metallic nanoparticles, metal oxide nanoparticles, silica-based nanomaterials, and carbon-based nanomaterials have attracted increasing interest [343,344]. The use of nanomaterials as solid sorbents has several benefits compared to solid-phase extraction since, the contact area between the target analytes and the sorbent is higher than that in traditional SPE, the equilibrium rate increases, and higher extraction yields are obtained. As a solution to this restriction, magnetic nanomaterials (MNPs) were proposed since phase separation is performed in a simple way by the application of an external magnetic field. This approach has several advantages over traditional SPE including:

(1) Faster procedures. MSPE significantly reduces the time consumed for analysis by reducing the number of steps in the extraction procedure

(2) Easy analyte separation with no need for centrifugation and/or filtration. The separation of the sorbent with analytes adsorbed on the surface is achieved simply by using an external magnetic field.

(3) High selectivity of magnetic sorbents used as there is the possibility of functionalization of magnetic materials

(4) Fewer interferences since the majority of sample impurities are diamagnetic

(5) The possibility for automation of the entire process

Moreover, with magnetic sorbents there is no need to be packed into SPE cartridges, thus minimizing problems of column blocking and high pressure that are often observed in SPE . Finally, MSPE can reduce the use of organic solvents, and thus decrease the amounts of toxic and dangerous wastes – in accordance with the principles of green chemistry [345]

All these benefits have as a result the development of rapid, selective, and repeatable methods for routine analysis [346]. Excellent analytical performance by means of accuracy and precision is highlighted in all published works, thus demonstrating the great potential of MSPE procedures that can be used for determination of a wide range of analytes in different matrices. Examples of their applications in MSPE procedure can be found in Table 1.9.

Table 1.9 Examples of magnetic nanoparticle materials applied in MSPE

Type	Sorbent material	Analyte	Sample matrix	Instrumental technique	Ref.
Silica-based	Fe_3O_4@SiO_2-C18	Corticosteroid	Plasma	HPLC-UV	[347]
	Fe_3O_4@SiO_2-C18	PCBs	Milk	GC-MS	[348]
	Fe_3O_4@SiO_2-MIP	Codeine	Urine	HPLC-UV	[349]
	C16- Fe3O4@SiO2	Ochratoxin A	Wine, beer	HPLC-FLD	[350]
LDH	Fe@Mg-Al-LDH	Bisphenol A, nonylphenol	River, wastewater	HPLC-UV	[351]
MOF	Fe_3O_4@COF@Zr^4	Organophosphorus Pesticides	Vegetables	GC-FPD	[352]
	Fe_3O_4@TMU-10	Tricyclic Antidepressants	Biological Samples	HPLC-UV	[353]
Polymer-based	Fe_3O_4@PS-DVB	Fenitrothion	Water, urine	UV	[354]
	Fe_3O_4@PDA	Heterocyclic Aromatic Amines	Meat products	UPLC-MS/MS	[355]
	Fe_3O_4@polyaniline	Benzodiazepines	Plasma, urine	LC-UV	[356]
	Fe_3O_4@polypyrrole	Antiseptic Ingredients	Surface water	HPLC-MS/MS	[357]
Carbon-based	Fe_3O_4@C60 fullerenes	Azo dyes	Wastewater	CE-UV	[358]
	Fe_3O_4@MWCNTs	Aconitites	Serum	HPLC-DAD	[359]
	Fe_3O_4@GO	PAHs	Urine	LC-MS	[360]
	Fe_3O_4@SiO_2@GO@IL	Chlorophenols	Tap water	LC-MS/MS	[361]
	Fe_3O_4@GO@hemimicelles	PFAs	River, wastewater	LC-MS/MS	[362]

1.5 Magnetic sorbents in MSPE

Different types of coating have been applied to the surface of magnetic nanoparticles (MNPs), including polymer-based, silica-based, MOFs, multiwalled carbon nanotubes (MWCNTs), and graphene (G) or graphene oxide (GO). One of the most frequently used is coated with silica (Fe3O4@SiO2) and functionalized with different moieties, such as the C18 groups ($Fe_3O4@SiO_2$-C18) [305,321]. In the current study, we focus on the preparation and application of two magnetic materials, Fe_3O_4@SiO_2-C18 and Fe_3O_4@GO, silica and carbon-based, respectively. For this reason, the section of MSPE functionalization sorbents is divided in two parts: (A) silica-based materials and (B) graphene-based materials.

The selection of suitable magnetic materials is essential in order to achieve high extraction efficiency, high preconcentration, selectivity, and elimination of interferences. Magnetic particles with core-shell structures are widely used in MSPE procedures. They contain a magnetic core and a coating. Magnetic core is usually composed by elements such as Fe, Co, Ni or their oxides which can provide ferromagnetic or superparamagnetic properties. Upon magnetized, the particles behave like small permanent magnets, so that they form lattice or aggregates depending on the magnetic interaction. Ferromagnetic particles have a permanent magnetism and result in a lattice form when the magnetic field is removed. On the other hand, superparamagnetic particles are attracted to a magnetic field but retain no magnetism after the absence of the field.

Another important characteristic that affects adsorption efficiency is the size of magnetic particles. Magnetic particles are available in a wide range of sizes from nano-size to to microsize. A nanoparticle is a small particle that ranges between 1 to 100 nanometres in size. Nanoparticles (NPs), (1–100 nm), have attracted particular interest in the scientific community and are preferably used due to a plethora of physicochemical properties. They are superparamagnetic, allowing their easy manipulation under the influence of an external magnetic field. In addition, they possess unique physical and chemical properties such as high dispersibility, relative large surface area and the high ratio of surface-to-volume resulting in a higher adsorption capacity [345], thereby high extraction efficiency [342]. The simple synthesis of magnetic nanoparticles (MNPs) allows the production in large quantity, which along with flexibility of surface modification enable their potential for environmental, biological and food analysis. Furthermore, the MNPs can be reused or recycled.

1.6 Preparation of magnetic materials

The preparation of a magnetic material usually involves three steps, including (a) the synthesis of the magnetic particle (magnetite or maghemite), (b) the coating of the magnetic core and (c) the modification of the resultant core–shell structure [363].

1.7 Synthetic strategy of magnetic particles

Among MPs, iron oxides like magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3), are widely used as magnetic cores in magnetic particles due to the following characteristics [364]:

(1) Simple fabrication(easy co-precipitation methods, fast and easy to fit in large-scale production)

(2) Flexibility in surface modification (the abundant hydroxyls on the surface of iron oxide enhances the coating modification with functional groups)

(3) Ease of operation (due to their superparamagnetic properties, magnetic particles can be easily recovered by applying an external magnetic field without additional centrifugation or filtration)

(4) Reuse ability (magnetic particles can usually be reused after appropriate washing)

(5) High dispersibility in aqueous solution (iron-oxide particles benefit dispersion and can reach the extraction equilibrium rapidly)

There are various popular routes to obtain monodisperse, shape-controllable, and stable magnetic nanoparticles including Coprecipitation technique, microemulsion-based synthesis, sol-gel synthesis, electrochemical method, sonochemical reactions, hydrothermal synthesis, flow injection synthesis, thermal decomposition technique, solvothermal method (polyol), aerosol/vapor-phase-based synthesis.

1.7.1 Co-precipitation method

Coprecipitation technique is a facile and effective approach for the preparation of iron oxides with either γ-Fe_2O_3 or Fe_3O_4 structure (particle size = 2-17 nm). One of the main advantages of the coprecipitation approach is that magnetic iron oxide nanoparticles can successfully be obtained in a large scale. The method is based on the chemical reactions carried out in an aqueous monophasic liquid medium, allowing both the nucleation and growth of iron hydroxide nuclei to be controlled. The synthesis process consists of the co-precipitation of Fe(III) and Fe(II) salts at ambient temperature under alkaline conditions (e.g., NH_4OH or NaOH) [365]. Chemical reactions that carried out in the synthesis are expressed by Eq.1

$$\mathbf{Fe^{2+} + 2Fe^{3+} + 8OH \rightarrow Fe_3O_4 + 4H_2O} \qquad \textbf{(Eq.1)}$$

According to thermodynamics, when the reaction is carried out at anaerobic conditions it is completed in a pH range between 8-14 when the molar ration of the salts (Fe^{3+}/Fe^{2+}) is 2:1. In the presence of oxygen magnetite is oxidized to maghemite according to the following equation:

$$Fe_3O_4+2H^+ \rightarrow \gamma\text{-}Fe_2O_3 + Fe^{2+} + H_2O \qquad \textbf{(Eq.2)}$$

Massart was the firs that fabricated superparamagnetic magnetite with co-precipitation of FeCl3, $FeCl_2$ salts under alkaline conditions. According to Massart the synthesis of magnetic iron oxide nanoparticles with suitable diameter, magnetic responsiveness, and surface properties can successfully be achieved by the optimization of experimental conditions (pH, temperature, ionic strength, and the stoichiometric mixture of ferrous and ferric salts in the aqueous medium, concentration of the alkali medium etc.) [366]. Specifically, pH and ionic strength affect the chemical composition of the surface of the particles by affecting the electrostatic charge. The magnetic nanoparticles produced with co-precipitation method are characterized by wide particle size distribution >50%. With the addition of suitable electrolytes depending the studied medium they are decreased up to 5%. Under optimum conditions nanoparticles with average size of 4-4nm can be fabricated. Other parameters that affects the size of the particles include stirring rate of the reaction; with high stirring rate the size of nanoparticles tend to be reduced. In a similar way, small particle sizes along with high dispersion have been observed with the addition of base in the reagent system. Furthermore, by increasing the temperature of the solution the formation of magnetite is decreased whereas inert atmosphere (bubbling with N_2) not only guarantees the formation of magnetite by preventing oxidation but also results to smaller particle size [367].

In general the co-precipitation method is a multiplicate technique not only due to the complexity during hydrolysis in chemical equilibrium of ferrous and ferric ions, but also because the shape, the size and the distribution of the particles depend on several experimental conditions already mentioned. Nevertheless, co-precipitation method is preferred because of the simplicity of the procedure. Compared to other methods such as thermal decomposition or other hydrothermal methods the temperature and the time of the reaction is significantly lower. Major benefits of the method consist the use of environmentally friendly solution (aqueous) as well as the high controllable yield of the reaction and the repeatable results. Main drawbacks of the co-precipitation method are the formation of aggregates, the wide particle size distribution and the difficulty to control the shape of nanoparticles [346,367].

1.8 Functionalization of magnetic nanoparticles

Among MPs, iron oxides like magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3), are widely used as magnetic cores in magnetic particles. However, pure inorganic magnetic particles (Fe_3O_4 or γ-Fe_2O_3), which consist the core of the magnetic material, are prone to the form large aggregates resulting to alterations of their magnetic properties. Moreover, pure inorganic metallic oxides have lack of selectivity thus they cannot be applied in complex matrices or in multi-residue methods. To overcome these limitations a suitable modification of the magnetic core (coating) with specific active groups is usually employed. The coating of the surface of MPs which can be considered as a shell, is achieved by the attachment of inorganic components (e.g., silica or alumina) or organic molecules (e.g., modified with polymer or surfactant, etc.) improving their chemical stability, preventing their oxidation and, also, providing specific functionalities like selectivity for ion uptake. Moreover, modification or functionalization of the inorganic coating can be achieved with organic or inorganic functional groups in order to improve the sorption characteristics (Fig.1.19).

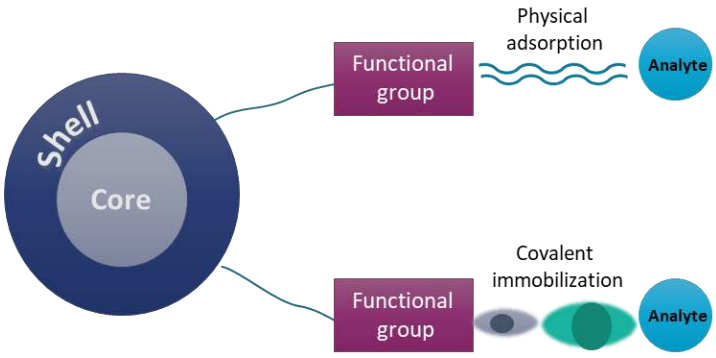


Figure 1.19 Functionalization of a core–shell type structure of MPs [363]

The modification of nanoparticle surface is one method to adjust the physical and chemical properties by functional molecules/particles/polymers to target applications. This approach is adopted in order to complete the following objectives [368]:

(a) stabilize the nanoparticles in solution by controlling the size and the morphology

(b) provide functional groups at the surface for further derivatization

(c) surface modification can change the optical, spectroscopic, electronic and chemical properties of the particles, providing a a wide range of controllable applications in nanotechnology

(d) modify the capability to agglomerate or the ability to be applied in desired chemical, physical, or biological environments

(e) in some cases, a decrease of their toxicity is achieved (e.g. cadmium-based quantum dots)

(f) enhance the solubility of nanoparticles in different solvents in order to extend their application capabilities

(g) improve the mechanical and chemical performance of the nanoparticle surface, e.g. prevent oxidation

There is a wide range of shells and functional groups which can be anchored to the surface of MPs and modify the material in order to be used , either in analytical extraction procedures or in removal processes as demonstrated in Fig.1.20.

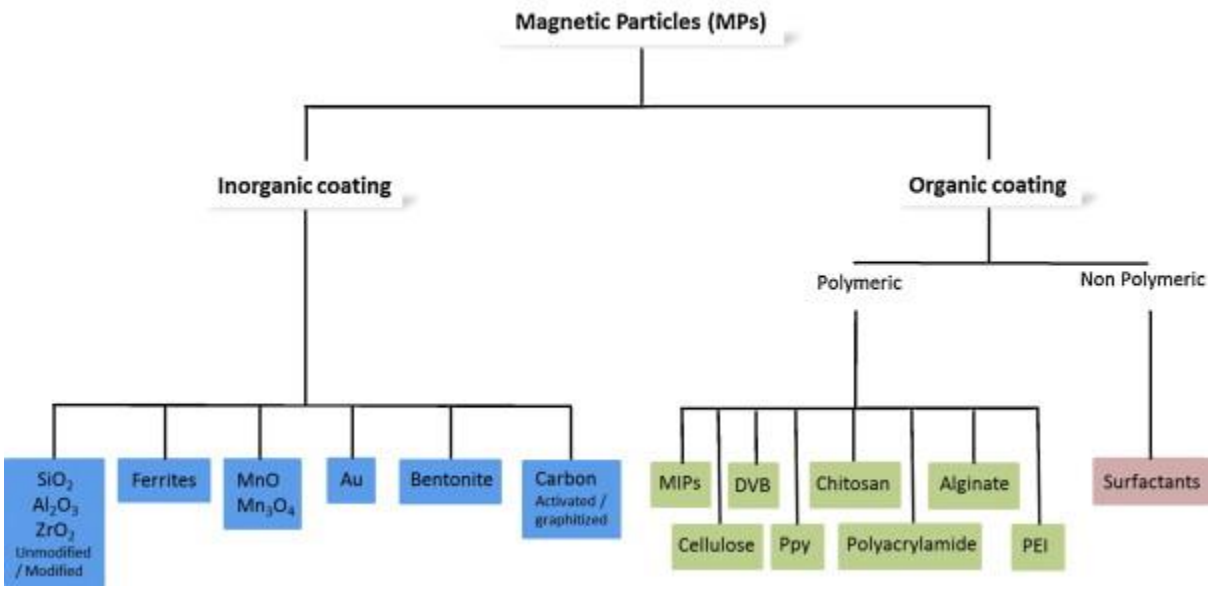


Figure 1.20 Classification of MPs according to the shell type [363]

1.8.1 Silica based materials

Iron oxide particles can be used for the isolation and enrichment of several analytes, but usually their surface is modified with functional groups for further applications. Iron oxide based on Lewis acid/base interaction can be easily modified with specific functional groups. Modification with polymers provides a variety of chemical properties and advantages for magnetic materials such as: high surface area, many active adsorption centers, high stability, pH and modification flexibility [369].

Also, silane-coupling agent can be directly immobilized on the surface of iron oxides. Silica is a very appealing material for analytical applications because it is relatively inexpensive, chemically inert, thermally stable, and biocompatible. Several advantages of its use include [370–373] :

(1)Silica can easily be coated onto the surface of iron oxide with sol-gel process

(2)Silca magnetic materials can be easily modified with coupling agents with silane or titanium dioxide etc

(3) Silica coated magnetic materials are more stable compared to ''bare'' iron oxides. In addition, silica protects them from leaching under acidic conditions.

(4) Silica provides a chemically inert surface area for applications in biological systems and protects iron oxide from agglomerating

(5) Silica enables the dispersion of magnetic materials

(6) Silica has high surface silanol concentration which enhances a wide range of superficial reactions as well as the bioconjugation of molecules such as antigen-antibody, peptides, proteins, nucleic acids, enzymes etc.

The physicochemical mechanism of the silane agent modifying on the surface of iron oxide NPs according to Arkles [374] is depicted in Fig.1.21

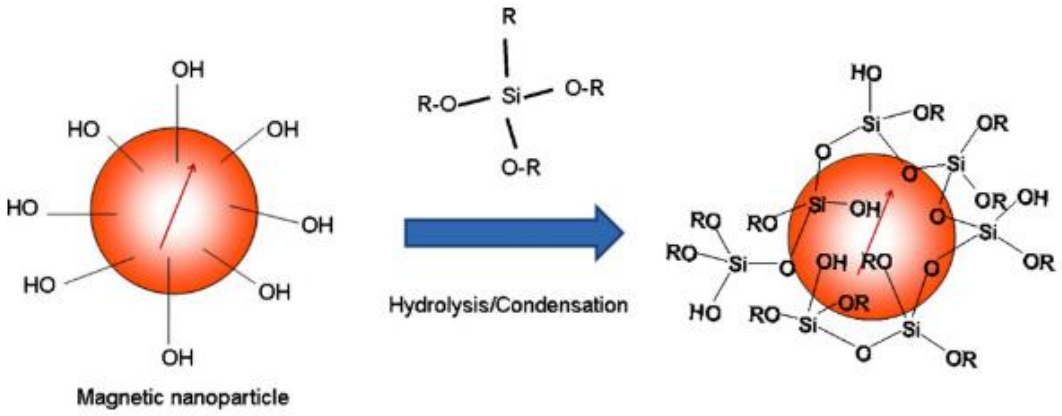


Figure 1.21 Silane surface coating of a magnetic nanoparticle [370]

The hydroxyl groups on the iron oxide NPs surface reacts with the alkoxy groups of the silane molecules leading to the formation of Si–O bonds and leaving the terminal functional groups available for further immobilization of other molecules. In general, the nature of silica coated MNPs depends on thickness of their corresponding silica shells. Theoretically, a thicker silica shell reduces the inter-particle interaction and super paramagnetism is preserved although a decrease

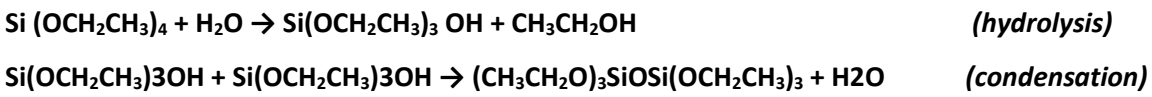
in saturation magnetization (Ms) is occurred. The reduction in Ms value could be attributed to the lower density of the magnetic component in the silanized nanoparticle sample [374]. For instance, saturation magnetization of a 15nm silica coating MNPs is decreased from 81.2 $emug^{-1}$ (pure Fe_3O_4) to 49.7 $emug^{-1}$ [374].

1.8.1.1 Synthesis of silica coated MNPs

Two methods are commonly used for preparation of magnetic nanoparticles with silica coating: (a) one is the Stöber method based in hydrolysis and condensation of alkoxy silane (usually tetraethyl orthosilicate, TEOS) on the surface of the iron-oxide particles through a sol-gel procedure [375,376] and, (b) another strategy is to introduce a magnetic source into porous silica [377]. In this study the Stöber method was followed for the modification of MNPs with silica.

1.8.1.1.1 Stöber method – Sol-gel process

Stöber method is a sol-gel process based on two main reactions for the preparation of monodisperse spherical silica nanoparticles: hydrolysis of tetraethyl orthosilicate (TEOS) followed by condensation (polymerization) of the dispersed phase material in ethanol in the presence of ammonia as a catalyst. The method provides silica nanoparticles with a wide range of size.



Silanol groups (Si-OH) are formed by hydrolysis and siloxane bridges (Si-O-Si) are formed by a condensation polymerization. Stöber process is regarded as the simplest and most effective route to prepare monodisperse silica spheres because the reactants are normal and reaction condition is controllable and is easy to be carried out[377].

Several experimental conditions affect the size distribution of silica particles such as (a) concentration of TEOS, (b) concentration of water, (c) concentration of ammonia catalyst, (d) type of alcohol used as the solvent, (e) temperature of the reaction [377]. One drawback of the the method is that all the above variables interact with each other, causing effects on the final particle properties [378].

The silanol groups of the coated surface of MNPs allow the easy functionalization of a plethora of chemical groups such as amino groups (-NH2), thioles (-SH), carboxylates (COOH) and C18 alkyl chains.

1.8.2 Modification of MNPS with C18

In the magnetic core-shell Fe_3O_4@SiO_2, amorphous silica shells have surfaces decorated with hydroxyl groups that provide hydrophilic character to the material. The hydrophilic surfaces easily adhere to each other through hydrogen bonding and form irregular agglomerations. In order to decrease these agglomerations of particles, the surface of the silica shell modifies with the coupling agents, in the case of this study trimethoxy octadecyl silane in order to functionalize with C18 alkyl group. The modification process is described as a hydrolysis and a condensation reaction between the coupling agents and the silica shell. Consequently, the surface modification removes the silanol groups on the surface of the core-shell particles and changes the hydrophilic surface into a hydrophobic one (Fig.1.22).

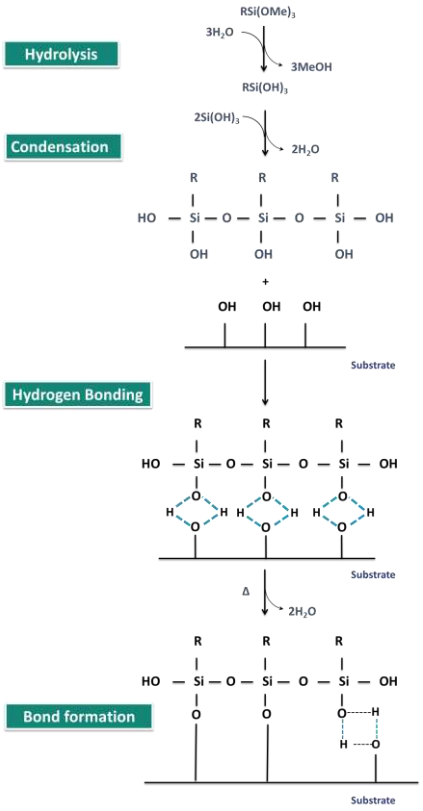


Figure 1.22 Deposition of silanes[374]

C18 MNPs are widely used for the enrichment of environmental pollutants due to their easy isolation, their stability and their long lifetime. They are usually applied for the adsorption and removal of medium- polar and non-polar analytes from environmental, biological and food analysis.

Table 1.10 Applications of C18-based MNPs

Analytes	Magnetic sorbent	Sample Matrix	Ref.
Fluorinated compounds(PFCs)	Fe_3O_4@C18@chitosan MNPs	Tap Water Rainwater Wastewater	[379]
Triclosan	γ-Fe_2O_3@SiO_2@C18	Seawater	[380]
Microcystine-LR (MC-LR)	Fe_3O_4@SiO_2@C18	Reservoir Water	[381]
Mycotoxins	MWCNTs@C18@SiO_2	Maize samples	[382]
4 antidepressants and 1 metabolite	Fe_3O_4@SiO_2@C18 MNPs	Human urine Clinical plasma	[383]
Alkylphenols	Fe_3O_4@PDA@C18 MNPs	Tap Water	[384]
Organophosphorus pesticides	Fe_3O_4@SiO_2@C18 MNPs	Environmental Waters	[385]
Industrial petroleum compounds	Fe_3O_4@SiO_2@C18 MNPs	Groundwater Soil	[386]
Endogenous volatile metabolites (EVOMs)	Fe_3O_4@SiO_2@C18 MNPs	Human urine	[387]
Phthalic acid esters (PAEs)	C18@FS MNPs	Herb preparation	[388]
Estrogens	C18@NH_2@ Fe_3O_4@mSiO_2 MNPs	Pork and chicken samples	[389]
Oxodiazon, profenofos	Fe_3O_4@SiO_2@C18 MNPs	Environmental waters	[390]

1.8.3 Graphene based materials

Graphene based materials, consist one allotropic form of carbon, and along with fullerenes and carbon nanotubes (CNTs) are the most applied for analytical purposes [391]. Graphene can be defined as a carbon allotrope composed by a structure containing sp2 hybridized atoms arranged in hexagons or also called a honeycomb pattern, which is the core for other widely-known allotropic forms ([392]). Graphene is regarded a blocking material which by stacking can form graphite, by rolling can form a carbon nanotube, or even wrapped to can become a fullerene like C_{60}, as shown in Fig.1.23. Since the confirmation of the existence of a single-layered graphene sheet in 2004 [392] it is considered a wonder material due to its nanosheet structure, which has strong σ-orbitals in the 2D plane, ensuring its stiffness. At the same time, the un-hybridized π-

orbitals are hinged outwards, superimposing one by one to form the long-range delocalized π electron system, responsible for its outstanding optical, and electrical properties [392]. In short, unmodified graphene sheets have a homogenous and large theoretical surface area distributed along with the thinnest structure of the negligible mass, enhancing the interaction between the sheets and polymer material. Likewise their charge carriers have high mobility, possibly traveling micrometers without scattering, allowing the use of graphene as an ideal material for electronic applications [393]. Moreover, the thermal and electrical conductivity transparency, and impermeability to gases as well as their mechanical strength is also highlighted [393]. All the above properties suggest that graphene would be an ideal material with several different potential applications, however, the manufacturing (especially in industrial scale) still represents a hurdle to its wide establishment. The most utilized manufacturing approach is laborious, non-reproducible, and highly dependent on human handling [308]. Nevertheless, intermediary graphene-based compounds such as the graphene oxide (GO) or reduced graphene oxide (rGO) have similarly captured the attention for a wide range of applications in electronics, biomedicine, analytical chemistry etc. Due to ultra-high surface area, the π-delocalized electron system, the easy synthesis and low-cost procedure, are widely used as sorbent materials in extraction techniques. In addition, they provide the possibility of surface modification and finally due to the planar structure, sorption interactions can occur in both inner and external surface [308].

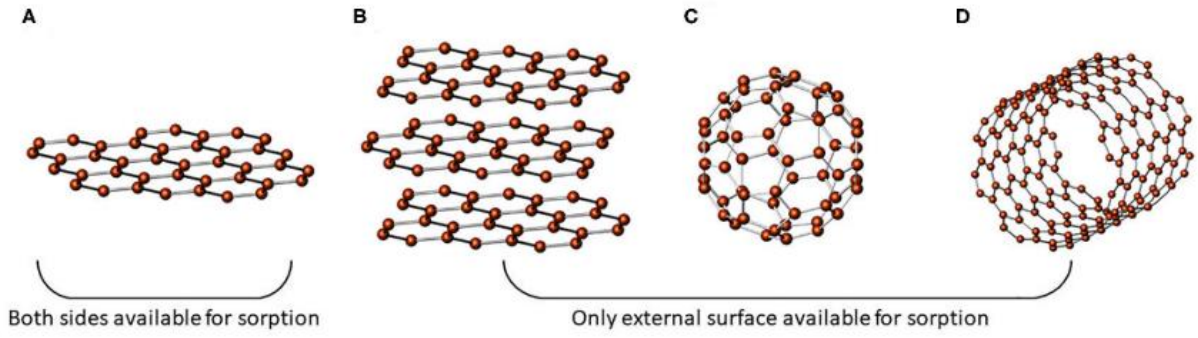


Figure 1.23 Schematic illustration of main carbon allotropes: (A) graphene; (B) graphite; (C) fullerene; (D) carbon nanotubes [308].

In analytical chemistry, GO has been successfully employed for the sample preparation of a wide range of samples including biological, food and environmental [304,394–396]. Graphene oxide consists of one-atom-thick two-dimensional layers of sp2-bonded carbon and the material is rich in oxygen-containing groups which assist the interaction between the sorbent and organic molecules through strong π-π stacking, hydrophobic interaction and hydrogen (Fig.1.24) [397–399]. Graphene oxide (GO) is like a graphene sheet functionalized on both sides with several

oxygenated functions such as hydroxyl, carboxyl, and epoxy. These functions impose a hydrophilic character to GO, consequently, the interaction between layers is weaker compared to graphene, making GO an easily exfoliated material. GO structure depends principally on the purification methods [400].

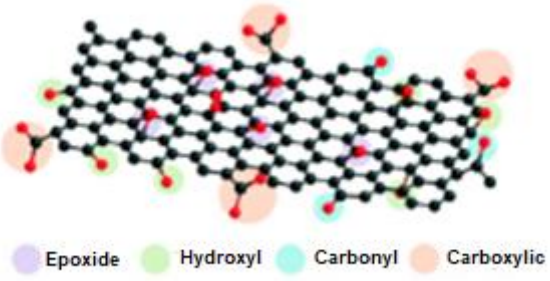


Figure1.24 Graphene oxide (GO) structure [324]

The most attractive property of GO is that it can be (partly) reduced to graphene-like sheets by removing the oxygen-containing groups with the recovery of a conjugated structure. The reduced GO (rGO) sheets are usually considered as one kind of chemically derived graphene. However, the most straight- forward goal of any reduction protocol is to produce graphene-like materials similar to the pristine graphene obtained from direct mechanical exfoliation (i.e. the ‘‘Scotch tape method’’) of individual layers of graphite both in structure and properties. Though numerous efforts have been made, the final product cannot be considered a pristine graphene. Residual functional groups and defects dramatically alter the structure of the carbon plane, therefore, it is not appropriate to refer to rGO as graphene since the properties are substantially different.

Reduced graphene oxide (rGO) is a nanomaterial obtained by chemical reduction of graphene oxide that contains less oxygen groups and has properties closer to those of graphene [400–402]. Reduced graphene oxide (rGO) has various applications such as extraction sorbent material, removal of metals and dyes [403,404] catalysts, [405], electroanalytical sensors [406] etc. Magnetic nanocomposites of reduced graphene oxide have been successfully applied for the MSPE of various analytes from different sample matrices. Due to the combination of the magnetic Fe_3O_4 nanoparticles and the graphene sheets, the magnetic RGO sorbent shows distinguished properties including good dispersity, high surface area, high adsorption efficiency and good super-paramagnetism [407]. In current study magnetic rGO (mrGO) was used as sorbent material in the applied MSPE for the determination of analytes of interest in wastewaters.

1.8.3.1 Synthesis of Graphene-based materials

Two different approaches can be used to obtain graphene: (i) the top-down, in which nanostructures are produced from larger dimensions, and (ii) the bottom-up, starting from atoms or small molecules to produce materials of larger dimensions. Among these micro-mechanical exfoliation, chemical vapour deposition (CVD), liquid phase reduction of graphene oxide and epitaxial growth have been proposed to improve the quality, size, and homogeneity of graphene. Among these methods, low cost and high throughput material are obtained through direct liquid phase exfoliation. Hummers and Offeman 1958 had defined a method to produce graphene oxide from graphene which is still used as a major method [407]. The graphene and their derivatives are produced via major techniques as shown in Fig.1.25.

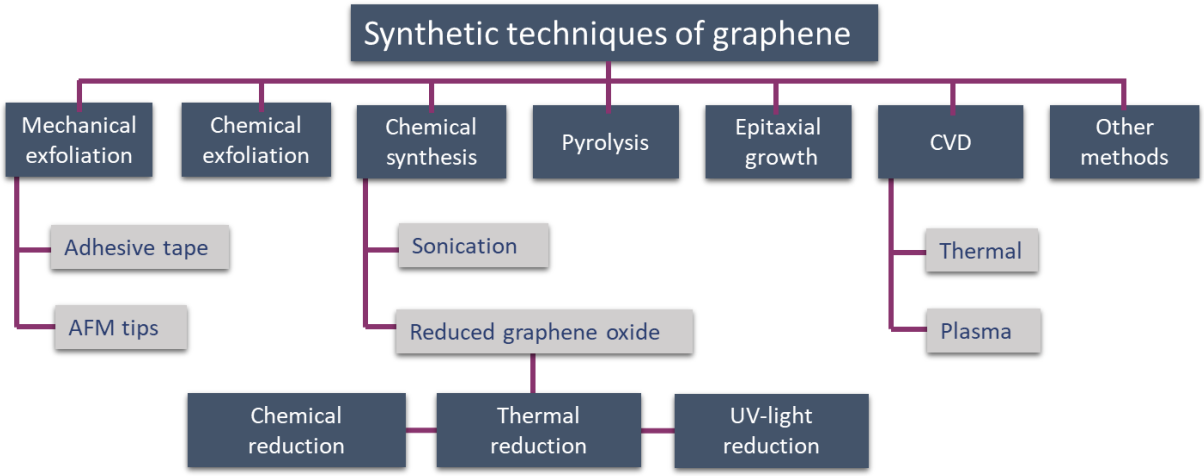


Figure 1.25 Synthetic methods for graphene and graphene oxide [408]

Chemical reduction of graphene oxide (GO) is the most popular method to obtain graphene. As shown in Figure 1.26, GO can be obtained by the oxidation of graphite powder and then exfoliated further to obtain single GO layers, which is subsequently chemically reduced to obtain rGO. Although the final product obtained from this pathway is rGO, its properties are very similar to graphene but are structurally different [409], [410].

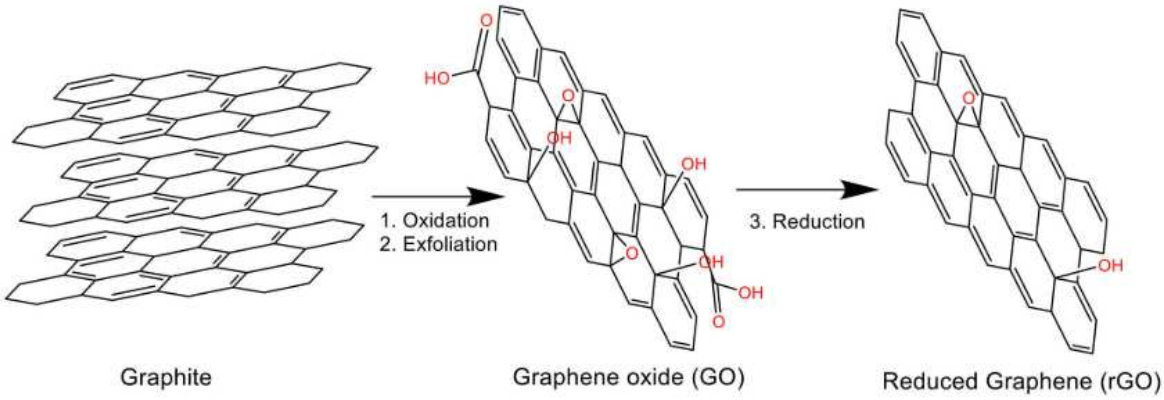


Figure 1.26 Chemical reduction of Graphene oxide (GO)

1.8.3.1.1 Synthesis of GO-Hummers and Offeman method

Currently, GO is prepared mostly according to the method proposed by Hummers and Offeman in 1958 [411], which is based on liquid phase exfoliation synthetic technique of Brodie and Staudenmaier [412,413]. In this method graphite powder is oxidized to graphite oxide by treating graphite with anhydrous mixture of concentrated sulfuric acid(H_2SO_4), followed by alkali metal compounds sodium nitrate ($NaNO_3$) and potassium permanganate ($KMnO_4$). These metals interact into the graphitic layers enabling the breaking of graphitic layers into small piece. The oxidation was carried out around 45 °C for two hours with mild stirring. The resultant material had a higher degree of oxidation than that of the Staudenmaier's method. Unfortunately, GO developed by Hummer's method generally have a non-oxidised graphite core with GO molecular structure. A pre-treatment was further required for improved oxidation with Hummer's method, which was first reported by Kovtyukhova [414] which involves adding graphite to the mixture of concentrated H_2SO_4, potassium persulfate ($K_2S_2O_8$) and phosphorus pentoxide (P_2O_5) at 80 °C for prolonged period [397], [414]. The pre-oxidized graphite was then washed thoroughly, filtrated and then washed with water followed by air drying, which produced much better GO with little non-oxidized GO. Despite the modifications proposed, [415], [397] the main strategy is unchanged. As a result, these methods are usually named modified Hummers methods. Differences with other modified methods are principally on the type and toxicity of the oxidant reagents, and the quality of the obtained product [416].

1.8.3.1.2 Synthesis of reduced Graphene Oxide (rGO)

The GO reduction never runs at 100% efficiency, so that on the surface, the sp^3 hybridized atoms remain connected to oxygen or slightly nitrogen. Of course, the number of oxygen atoms and

other elements in the rGO structure is smaller in comparison with their content in the GO structure and therefore their adsorption properties are significantly different [392]. There are several reduction strategies of GO including : thermal reduction, UV-light reduction and chemical reduction.

Thermal reduction: Thermal reduction of graphene oxide provides high-performance rGO powders. In this method, the GO is reduced under higher temperature (above or around 1000 °C) in order to evaporate and burn the water molecules and the oxygen functional groups. This is an efficient reduction method but it cannot retain a film form of GO [408].

UV light reduction: Graphite powder is dispersed in a solvent and exposed to UV-light which absorbs more in the liquid state of GO. In this way reduced GO is produced. This method can be applied either in powder for of GO, film or suspension [408].

Chemical reduction: It is about a method that produces graphene oxide and reduced graphene oxide in large scale, by the addition of a chemical reducing agent for a particular period of time and a temperature range. Adding the liquid reagents to a GO aqueous dispersion, results in agglomerated graphene-based nanosheets due to the increase of hydrophobicity. It is an effective and simple technique [416] as does not requires equipment and environment is not as critical as that of thermal annealing treatment. That makes chemical reduction a lower cost and user-friendly way for the mass production of graphene compared to thermal reduction process. The reduction in this process is performed by the removal the excessive functional groups, such as COOH and OH [417]. Chemical reduction for the transformation of graphene oxide to reduced graphene oxide was also used in current study.

Among the wide variety of chemical reduction agents that can be employed, hydrazine is the most often used because of its high reductive efficiency, even though it is highly environmental toxic.

The reduction of graphite oxide by hydrazine was used before the discovery of graphene [418] while the use of hydrazine to prepare chemically derived graphene was first reported by Stankovich et al. [419,420]. The reaction mechanism of hydrazine in te reduction is based in the the removal of oxygen atoms which results to precipitation. Hydrazine takes part in ring-opening reaction with epoxides and forms hydrazino alcohols [421]. This initial derivative reacts further via the formation of an aminoaziridine moiety which undergoes thermal elimination of diimide to form a double bond [410].

Because of the toxicity of hydrazine, metal hydrides, e.g. sodium hydride, sodium borohydride ($NaBH_4$) and lithium aluminium hydride, have been suggested as strong reducing reagents[422]

but unfortunately, these reductants have a slight to very strong reactivity with water, which is the main solvent for the exfoliation and dispersion of GO.

As an alternative, the use of greener reduction agents such as ascorbic acid [423] for GO reduction are also employed. However the mild reduction activity it yields a final product containing several structural defects on the sheets, which lead to low-quality materials with variable sizes and edges [409,424].

1.8.3.2 Reduction mechanism

The difference in structure of GO and graphene is located to the large amount of functional oxygen groups attached to the carbon plane and structural defects within the plane, both of which can significantly decrease the electrical conductivity. Therefore, the efforts of understanding the mechanism of reduction are focused in two pathways: the elimination of functional groups and the restoration of structural defects. For the elimination of functional groups, there are also two parameters that should be considered: whether the oxygen functional groups can be removed and whether the areas after removal can be restored to a long-range conjugated structure, so that there are pathways for carrier transport within the rGO sheet. For the restoration of structural defects, there are two possibilities, graphitization at high temperature and epitaxial growth or CVD in the defective area with an extra carbon supply [422].

Reduction of GO with chemical agents mainly relies in the elimination of functional groups. Specifically, the reduction of GO must be mainly aimed at eliminating epoxy and hydroxyl groups on the plane, while other groups, e.g. carboxyl, carbonyl and ester groups, present at the edges or defective areas only have a limited influence on the conductivity of an rGO sheet [422].

1.8.3.3 Synthesis of magnetic reduced nanocomposites mrGO

The characteristic of graphene oxide-based materials to stack between graphene oxide nanosheets due to π–π interactions, leads to eventual aggregation and restacking of the nanosheets. This has as result to a potential block of the active adsorption sites of the sorbent and a decrease of its specific surface area. In order to overcome this limitation modification of the sorbent with different functional groups that can enter between the GO nanosheet and prevent them from aggregation and restacking can be performed [425].

Due to the combination of metallic nanocomposites such as magnetic Fe_3O_4, and the graphene sheets, the magnetic rGO sorbent seems to be provide interesting properties including good dispersity, high surface area, high adsorption efficiency and good super-paramagnetism [407].

Owing to the superparamagnetic nature of the modified material, when dispersed into an aqueous solution it can be easily collected from water by applying an external magnetic field, hence showing the potential to be exploited in magnetic solid phase extraction. Magnetic graphene-based materials used in MSPE are summarized in Table 1.11 at the end of the section.

Different synthetic procedures for the production of rGO/Fe_3O_4 materials have been employed. The most common are the co-precipitation method, the solvothermal method and the hydrothermal method. Magnetic GO can be prepared by mixing GO and Fe_3O_4 under ultrasonic irradiation, stirring or mechanical shaking [324].

The co-precipitation approach is the most common synthetic route for the preparation of magnetic GO. In this method graphene oxide is dispersed in water and salts of Fe(III) and Fe (II) are added in the mixture under heating and ammonia solution as similarly described in the fabrication of magnetite. This approach is simple and fast with the only concern that the shape of the magnetic graphene-based materials is difficult to control [425].

Solvothermal method is another synthetic pathway where magnetic graphene particles are distributed with more graphene exposed adsorption sites and then, improved adsorption capacity [425]. For the solvothermal approach, graphite oxide is exfoliated in an organic solvent like diethylene glycol along with ferric chloride ($FeCl_3$) and sodium acetate (CH_3COONa) under sonication to produce graphene oxide. In this case, the role of diethylene glycol was double as it acted both as a solvent and reducing agent. Subsequently, the dispersion of GO was added into the second solution and the mixture was sonicated and finally heated at 190 ◦C in an autoclave for 12 hours [415].

Accordingly, rGO/Fe_3O_4 nanoparticles can be also prepared through, hydrothermal method which is similar to the solvothermal with the only difference that organic solvents have been replaced with water in the hydrothermal approach. For this purpose, a salt of Fe(III) and sodium hydroxide (NaOH) is added to an aqueous dispersion of GO and the mixture is heated at high temperature in an autoclave for several period of time [415].

Finally, the magnetic graphene oxide in a second step is chemically reduced with the methods already mentioned and magnetic rGO is obtained. Applications of magnetic graphene-based sorbent materials is presented in Table 1.11.

Table1.11 Applications of graphene-based magnetic sorbents

Sorbent material	Analyte	Sample matrix	Instrumental technique	Ref.
Fe_3O_4@GO	PAHs	Urine	LC-MS	[360]
Fe_3O_4@SiO2@GO@IL	Chlorophenols	Tap Water	LC-MS/MS	[361]
Fe_3O_4@GO@hemimicelles	PFOs	River, Wastewater	LC-MS/MS	[362]
Fe_3O_4@GO	Pseudoephedrine	Urine	HPLC-UV	[426]
Fe_3O_4@GO	Psychoactive Drugs	Urine	UHPLC-MS/MS	[427]
Fe_3O_4@GO	PCB 28	Water	GC-MS	[428]
Fe_3O_4@GO	Atrazine	Water	GC-MS	[429]
Fe_3O_4@GO	Sulfonamides	Water	HPLC-DAD	[430]
Fe_3O_4/rGO@β-CD	Phytohormones	Tomatoes	HPLC-DAD	[431]
Fe_3O_4@GO	Sulfadiazine	Milk, honey, water	Spectrophotometry	[432]
Fe_3O_4@GO	Sulfonamides	Milk	HPLC-MS/MS	[433]
Fe_3O_4@GO	Flavors, fragrances	Orange juice, chocolate, fruit sugar	HPLC-DAD	[434]
Fe_3O_4@GO	Flavonoids	Tea, wine, urine	HPLC-DAD	[350]
Fe_3O_4@rGO-CNTs	Sulfonamides	Milk	HPLC-DAD	[435]
Fe_3O_4@N-rGO	Bisphenol ECDs	Carbonated Beverages	HPLC-DAD	[435]
Fe_3O_4@rGO	Carbamate pesticides	Environmental waters	HPLC-DAD	[436]

1.9 Fabric Phase Sorptive Extraction

The limitations of SPE (laborious, time-consuming, and multistep sample preparation technique that demands clean, particle-free samples and often requires solvent evaporation and sample reconstitution in a suitable solvent, resulting in potential analyte loss of analyte) are decreased or even overpassed with the use of microextraction techniques like magnetic solid phase extraction (MSPE). Magnetic solid phase extraction avoided the limitations such as blocking cartridges and high pressure, the need to pack the cartridges, high sample and organic solvent consumption, low selectivity, filtration steps. Moreover, MSPE overpassed the limited options for polar sorbents in case of SPE. However, the utilization of MNPs in sample preparation has some drawbacks, since their selectivity is still low as well as their stability in strong acidic aqueous media and their low dispersion ability in many sample matrices. The need for surface modification of MNPs with special functional groups is usually required to enhance their stability and selectivity. In addition, although iron oxides were preferred for their low toxicity, low price, high magnetization, still their modification with some functional groups can introduces procedure with the use of toxic reagents (such as hydrazine). Following the trends of miniaturized techniques of sorbent microextraction such as magnetic solid phase extraction (MSPE), solid phase microextraction (SPME) which was the pioneer of all sorbent based micro extractions as well as stir-bar sorptive extraction (SBSE) [437] thin film microextraction (TFME) [438], Fabric Phase Sorptive extraction was also developed in 2014 by Kabir and Furton [439]. Kabir and Furton overstepped the aforementioned micro-extraction sorbent-based techniques in the concept of suggesting a sample preparation approach that coincides closest with the of Green Analytical Chemistry (GAC) principles. Fabric phase sorptive extraction (FPSE) has been developed as a universal sample preparation technique that innovatively combines the extraction mechanism of SPE and SPME into a single sample preparation platform [440].

FPSE successfully combines the advantages of sol–gel derived sorbents used in microextraction and the wide variety of fabric substrates, resulting in a highly efficient and green sample pretreatment technique [441,442]. FPSE sustains its evolutionary development to the fact that was able to overcome two major limitations of other sorptive extraction techniques: the low sorbent capacity and the long sample preparation time. These two parameters depend on the thermodynamics of the sorbent while the latter on the kinetics. Thermodynamic properties of the sorbent determine the maximum amount of analytes that can be extracted by unit mass of sorbent under a given set of extraction conditions. Since higher sorbent loading allows

accumulation of a higher mass of analyte under equilibrium extraction conditions, sorbent loading should be maximized. However, the substrate type and geometry determine the maximum sorbent loading capacity for a microextraction device [443]. On the other hand, kinetics controls the rate of extraction and therefore the time needed to reach the extraction equilibrium.
faster the extraction equilibrium, the shorter is the sample preparation time. These parameters are interrelated since with augmentation of surface area, direct interaction with the analytes during the extraction process is available and higher sorbent loading without any change in the coating technology is feasible. Therefore, more target analytes are adsorbed by the sorbent and reduction of the extraction equilibrium time is achieved. To address this issue sol-gel coating technology was recruited. The strong interaction between the sol-gel coated sorbent proposed by Malik and co-workers [444] and the substrate leads to high solvent and chemical stability. Due to the inherent porosity of the fabric used, short equilibrium time, high sensitivity is accomplished. In addition, the plethora of sorbent materials with significant analyte retention capacity that can be used, makes sol-gel technology a flexible and convenient coating technique.

FPSE utilizes a natural or synthetic fabric substrate (cellulose, polyester, or fiberglass) which is chemically coated with sol-gel organic-inorganic hybrid sorbent as the extraction medium. Generally, the FPSE procedure consists of the following steps: first, the sol-gel sorbent coated FPSE media is submerged into a mixture of appropriate solvents to clean any undesirable impurities from the material and then it is rinsed with deionized water to remove residual organic solvents. Afterward, the FPSE media can be immersed directly into a An amount of sample containing the target analytes is taken into a screw-capped glass vial that contains the sample matrix such as blood, urine, whole milk, environmental water without any sample pretreatment in order to extract the target analytes. The extraction can be facilitated by using a Teflon coated magnetic stir bar or sonication for a certain time. After the extraction, the FPSE media is removed and elution of the analytes takes place into another vial containing where desorption occurs, and the retained analytes are back-extracted to this eluting system. Afterwards, the extract is centrifuged and/or filtered to remove any particulate matter prior to further analysis in a chromatographic system. Step-by step the FPSE process is described schematically in Figure 1.27
The FPSE medium can be reused by washing with the solvent system or it can be left to dry on a watch glass and stored in an air-tight glass container for future use. [440,445,446].

As mentioned before, FPSE combines the extraction mode of SPME and SPE into a single device. Specifically, at the beginning , the FPSE media is in contact with the sample solution along with the target compounds which are transferred in the sorbent until an equilibrium between the sorbent and the sample matrix is established. This mode of extraction mimics in a way the direct-immersion SPME (equilibrium extraction mode). The Sponge-like porous architecture of the sol-gel sorbents and the permeability of the substrate leads to the existing flow-through system, which mimics the solid phase extraction (exhaustive extraction mode) [447].

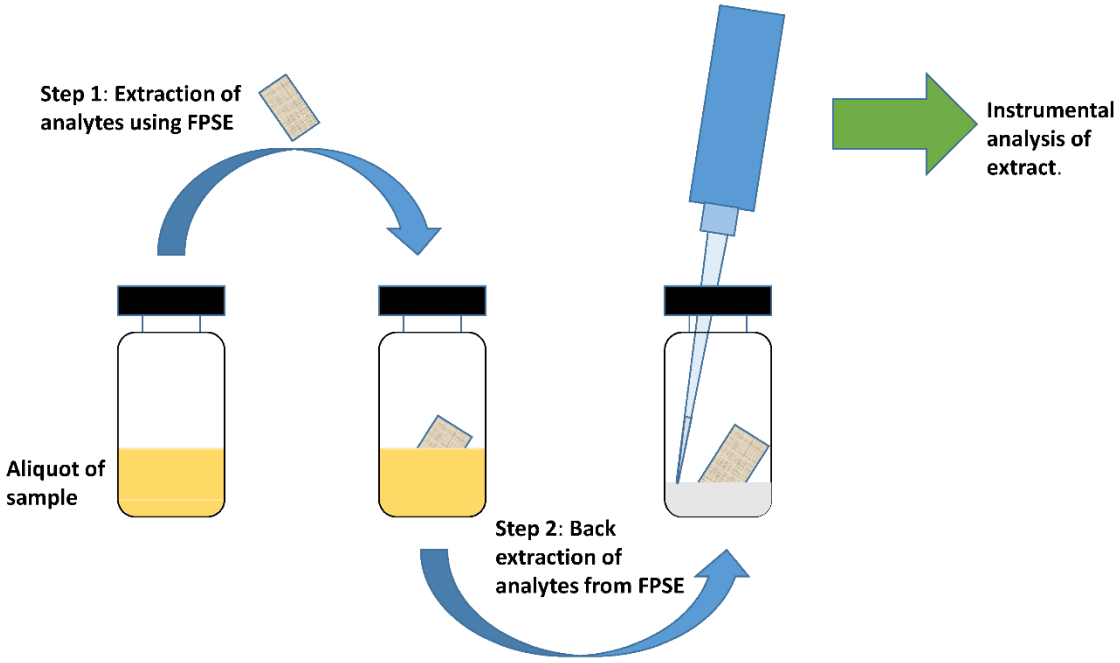


Figure 1.27 Schematical procedure of FPSE [446]

The implementation of FPSE has confronted the majority of the problems often encountered in sorbent-based sample preparation practices. Among its advantages include: (a) sample preparation can be performed by direct immersion of the FPSE medium into the vessel containing the sample matrix; (b) simplicity, minimum consumption of solvents, low cost; (c) flexibility in the selection of organic solvents that can be used as eluent (d) enhanced efficiency by magnetic stirring sonication; (e) minimization of sample preparation steps, reducing the time of sample pretreatment and the potential sources of errors; (f) a variety of effective sol–gel coatings can be employed as sorbent; (g) high chemical resistance of the FPSE media thanks to a strong chemical bonding between the sorbent phase and the substrate [445].

1.9.1 Fabric Phase Sorptive Extraction Media

The framework of Fabric Phase Extraction media is consisted of main three parts : (a) a fabric substrate; (b) a sol-gel inorganic precursor/organically modified inorganic precursor; (c) a sol-gel active inorganic/inorganic polymer. Fig. 1.28 presents a schematic representation of sol-gel sorbent-coated FPSE medium. Each individual part of FPSE medium is described below.

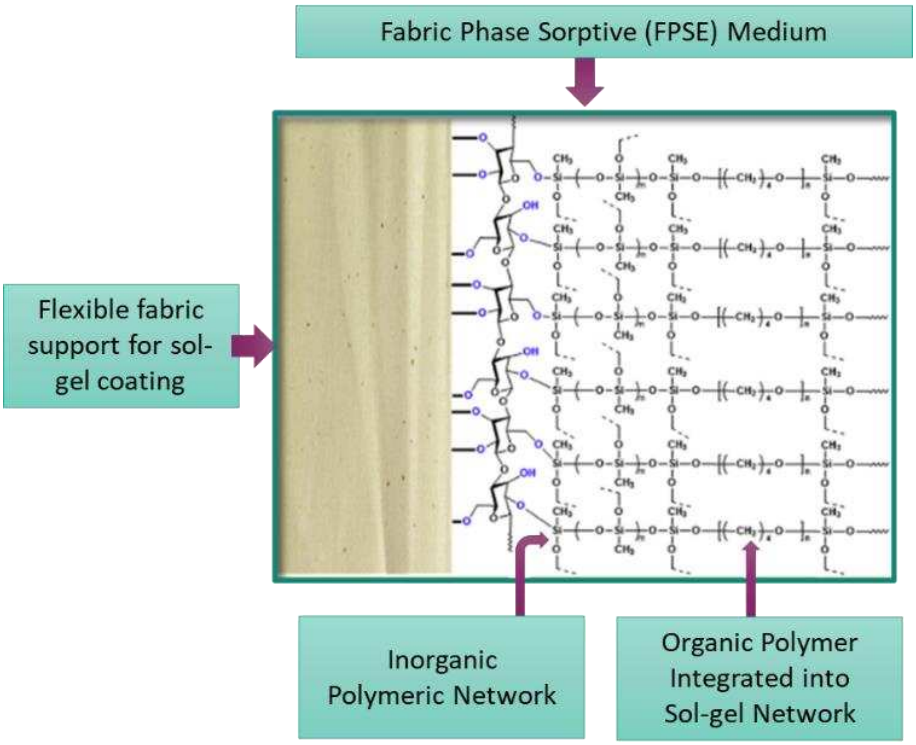


Figure 1.28 Schematic representation of FPSE medium and its different coating parts [440]

(a) Fabric substrate

Fabric substrate is a key part on the fabrication of FPSE medium. Unlike other extraction and microextraction techniques, the substrate in FPSE not only consists a receptor of the sorbent but also actively contributes to the overall selectivity of the FPSE medium via hydrophilic/hydrophobic interactions. Until today, various fabric substrates, including cellulose, polyester, and fiberglass fabrics are primarily used as the substrates for FPSE media. All these fabrics either contain readily available sol–gel active functional groups or may have the capability to contain sol–gel active functional groups obtained with surface modification. Cellulose fabric is known to be hydrophilic and polyester fabric hydrophobic [442]. If solvent mediated back-extraction is used, fabric substrates such as cellulose, polyester are preferred. On the other hand, if thermal desorption is used, glass fiber-based fabric is the best option. All these fabrics are dominated of sol-gel active

functional groups on which the sol-gel sorbent network is chemically bonded during the sol-gel coating process. The sorbent loading on the FPSE medium depends on the concentration of sol-gel active functional groups as well as on the amount of sorbent loading per unit area. For this reason, it is expected that the sorbent loading on cellulose fabric is substantially higher than polyester fabric. A remarkable fact is that the sol-gel sorbent networks chemically bind to the sol-gel active functional group, allowing the interaction with target compounds since the most part of substrate surface remains exposed. Due to the porous architecture of the fabric, FPSE media mimic a solid phase extraction disk. During the extraction process, sample solution passes through the FPSE medium accomplishing rapid and near exhaustive extraction. In Fig. 1.29 chemical structures of different fabric substrates are illustrated [440].

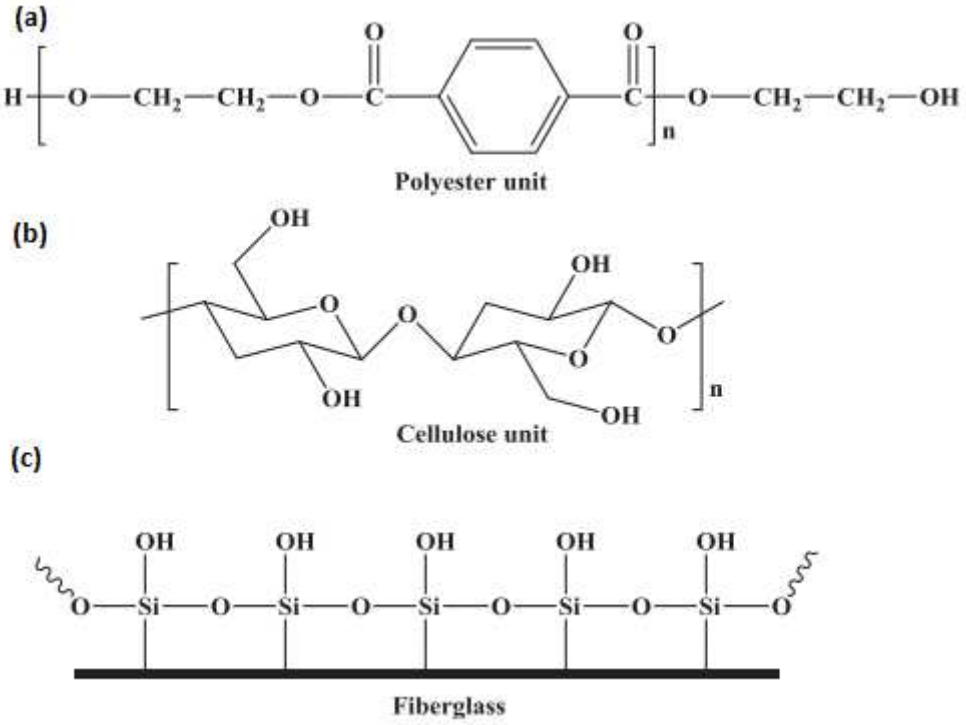


Figure 1.29 Chemical structures of various fabric substrates (a) a polyester unit; (b) a cellulose unit; and (c) fiberglass fabric [440]

(b) Inorganic/organically modified sol-gel precursor:

Another crucial factor in the sol-gel sorbent coating process is the inorganic/organically modified sol-gel precursor. This precursor is responsible for the generation of 3D networks of sol-gel sorbent by incorporating the inorganic/organic polymer into the networks in random positions. Moreover, it has the role of a ligand in the sol-gel sorbent networks with the fabric substrate.

Upon organically modified inorganic sol-gel precursors are used, the organic pendant moiety actively contributes to the overall selectivity of the FPSE medium. For this reason, the selection of sol-gel precursor affects the polar character and the selectivity of the FPSE medium. Various available sol-gel precursors, have been reported including : methyl-trimethoxysilane (MTMOS), tetra-ethoxysilane (TEOS), tetra-methoxysilane (TMOS), octyl-trimethoxysilane (C8- TMOS), 3-octadecyl-trimethoxysilane (C18-TMOS), aminopropyl-trimethoxysilane (3-APTMOS), phenyl-trimethoxysilane (PTMOS), tetra-methoxygermane, titanium isoproxide, zirconium isopropoxide [440].

Various available organically modified sol–gel precursors with a wide polarity range can be used in the sol solution design in order achieve the desired polarity character [448]. The characteristics and the chemical properties of sol–gel hybrid organic-inorganic sorbents are affected by several parameters, including the nature and type of precursors, the type of catalyst and its concentration, the organic solvent, the pH of sol-solution, the precursor to water ratio, the temperature and humidity during reactions, as well as the post-gelation aging conditions. The chemical structure of the produced sol–gel coating depends on the type of the catalyst, used in the sol solution [445]. The most commonly used precursors, as well as several catalysts reported in sol–gel processes are presented in Figure 1.30.

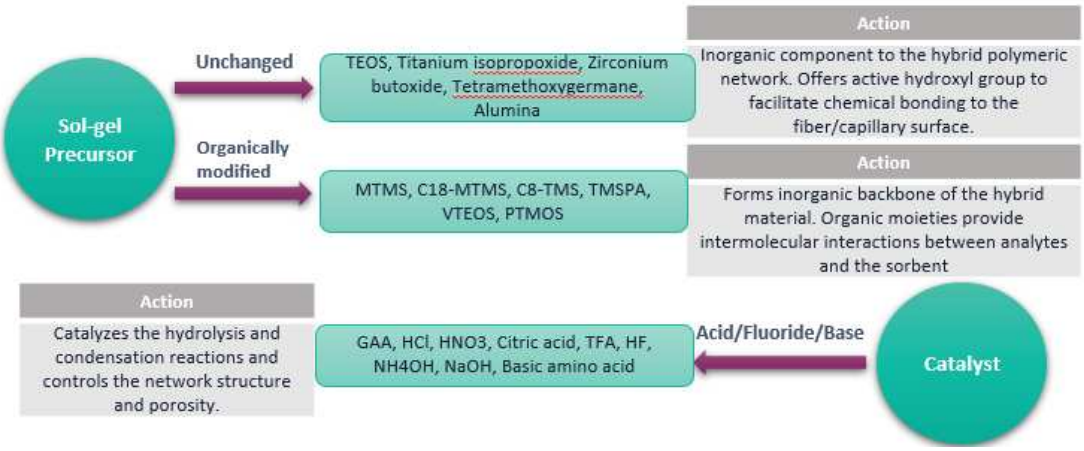


Figure 1.30 Types of precursors and catalysts used in a sol–gel process [440]

(c) Sol-gel active inorganic/organic polymer:

Sol-gel active organic polymers such as poly-ethylene glycol, poly-tetrahydrofuran polymers or inorganic polymers like poly-dimethylsiloxane, poly-dimethyldiphenylsiloxane are incorporated into the sol-gel networks via sol-gel process and consist the major source of the selectivity and extraction affinity toward the analytes of interest. Each polymer used in the sol-gel sorbent

coating process has different structure and other characteristics providing different intermolecular interactions from one polymer to the other. Since both the substrate and the sol–gel coating contribute to the final selectivity and polarity of the resulting extraction media, a relatively non-polar organic polymer PDMDPS are typically used as the organic polymer to coat hydrophobic polyester substrate in order to create a non-polar extraction media. Poly-THF is medium polar, whereas, PEG–PPG–PEG triblock and Carbowax 20M polymers are considered as highly polar polymers[449] . A wide range of sol–gel sorbent materials available for fabric coating are summarized in Table 1.12.

Table 1.12 Sol-gel sorbent materials available for fabric coating [440]

FPSE sorbent	Polarity	Sorbent loading (mg/cm^2)
Sol-gel polydimethylsiloxane	Non-polar	2.30
Sol-gel polydimethyldiphenylsiloxane	Non-polar	1.93
Sol-gel ppolytetrahydrofurane	Medium polar	3.96
Sol-gel polyethylene glycol-polypropylene glycol-polyethylene glycol	Medium polar	5.68
Sol-gel polypropylene glycol-polyethylene glycol-polypropylene glycol	Medium polar	5.25
Sol-gel methacrylate	Medium polar	4.51
Sol-gel C4	Medium polar	1.90
Sol-gel C8	Medium polar	2.33
Sol-gel C18	Non-polar	4.88
Sol-gel graphene	Non-polar	N/A
Sol-gel multiwalled carbon nanotubes	Non-polar	N/A
Sol-gel chitosan	Polar	N/A
Sol-gel carbowax 20M	Polar	8.64
Sol-gel polyethylene glycol 10,000	Polar	6.36
Sol-gel polyethylene glycol 300	Polar	4.45

1.9.1.1 Preparation of sol-gel sorbent coated FPSE media

The preparation of FPSE media involves two main steps: (1) the selection and pretreatment of fabric substrates for sol–gel coating and (2) design and preparation of the sol solution for sol–gel

coating process on the substrate. The sol-gel coating of the sorbent is processed via immersion coating technology is followed along with the cleaning, aging and conditioning of the prepared medium. Finally cutting the appropriate size of FPSE media is the final step before use [441]. The total design of the sorbent preparation is schematically presented in Figure 1.31.

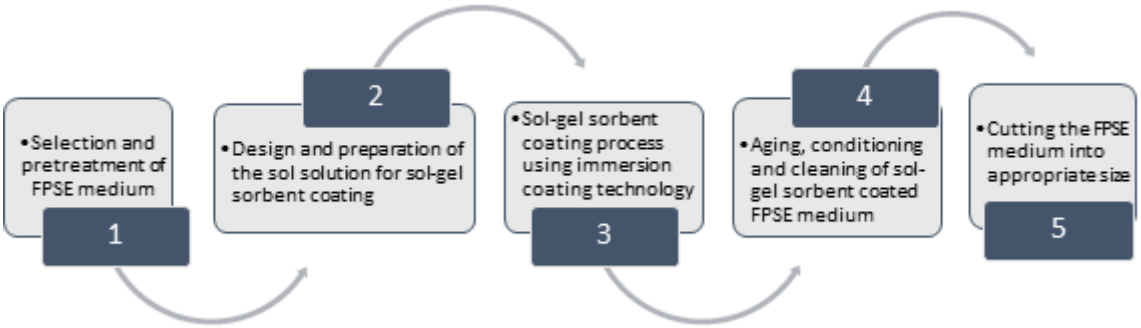


Figure 1.31 Preparation process of FPSE media

1.9.1.2 Selection and pretreatment of FPSE medium

An appropriate substrate determined for fabric phase sorptive extraction medium should fulfill the following criteria: (a) presence of abundant surface hydroxyl groups; and (b) surface permeability in order for sample solution to pass through the FPSE medium during the adsorption of target compounds as well as for organic solvent to desorb efficiently during back extraction/elution. Many fabrics that meet these criteria are commercially available for use as a substrate, including cellulose, fiberglass, polyester, nylon, polyamide fabric. The commercial fabric segments often contain residual finishing chemicals on their surface used to provide glossiness and protect the fiber from dust and other environmental conditions. These physically adsorbed residuals may block the direct access of the sol solution to form homogenous coating on the media. One objective of surface pretreatment is to maximize the number of available surface functional groups in order to facilitate strong bonding. Therefore, a washing procedure for cleaning the fabric and activate the hydroxyl groups onto the surface was adopted [449]. This procedure is described in detail in *''Chapter: Materials & Methods, Section 2.7.1''.*

1.9.1.3 Preparation of the Sol Solution for the Sol–gel Coating Process Designing

A key parameter in the development of sol–gel sorbent is the sol solution due to the fact that its composition and the relative ratio of the constituents, define the porosity as well as the selectivity and specificity of the resulting sorbent [450]. The design of the sol solution primarily depends on the nature of the target analytes and the sample matrix. For an effective sol–gel sorbent, the

selection of the sol–gel active organic polymer, the inorganic or organically modified inorganic sol–gel precursor, the solvent/solvent system, the catalyst, the amount of water, as well as an appropriate relative molar ratio of the constituents must be considered. For example, if the analytes are hydrogen bond donors or acceptors, the organic polymer should possess hydrogen bond acceptor or donor groups for complementary interactions with the analytes.

1.9.1.4 Sol-gel sorbent coating process using

The sol-gel process is an interesting approach for the preparation of advanced inorganic and organic- inorganic hybrid porous systems, of various sizes, shapes and formats like films, fibers, particles and monoliths. Performing mild reaction conditions results to materials that are pure, homogenous, chemically and thermally stable, selective with tunable porosity [450].

Sol–gel technology is an interesting approach for the synthesis of inorganic polymers and organic–inorganic hybrid porous materials. The main benefits of sol–gel technology for sorbent-based microextractions are the strong retention of the coating onto the substrate due to chemical bonding as well as the significant decrease of the extraction equilibrium time and the fast mass transfer thanks to the inherent porous structure [441].

Sol-gel process concerns the transfer of a liquid colloidal solution known as ‘‘sol’’ into a solid ‘‘gel’’ substrate. The following major sets of reactions take place during this procedure [445,451]:

(a) Catalytic hydrolysis of sol–gel precursor.

(b) Polycondensation of hydrolyzed precursors leading to a growing sol–gel network.

(c) Random integration of sol–gel active organic polymer into the growing sol–gel network.

(d) Immobilization of the growing sol–gel network on the substrate surface via polycondensation.

Sol-gel reactions are generally carried out under ambient conditions. Typically, methyltrimethoxysilane (MTMS) is used as the sol-gel precursor and trifluoroacetic acid (TFA) (95%, 5% H_2O) as the sol-gel catalyst. During the hydrolysis, the three methoxy groups of MTMS are hydrolyzed to form hydroxyl groups and subsequently begin forming a 3D network via polycondensation. During the formation of 3D network, sol-gel active organic polymer randomly enters the network. Finally, the sol-gel sorbent network chemically bonds to the fabric substrate through the fabric hydroxyl groups via polycondensation. The schematic presentation of sol-gel reactions is shown in Fig. 1.33 as well as the schematic representation of sol-gel coating on fabric medium (Fig.1.34).

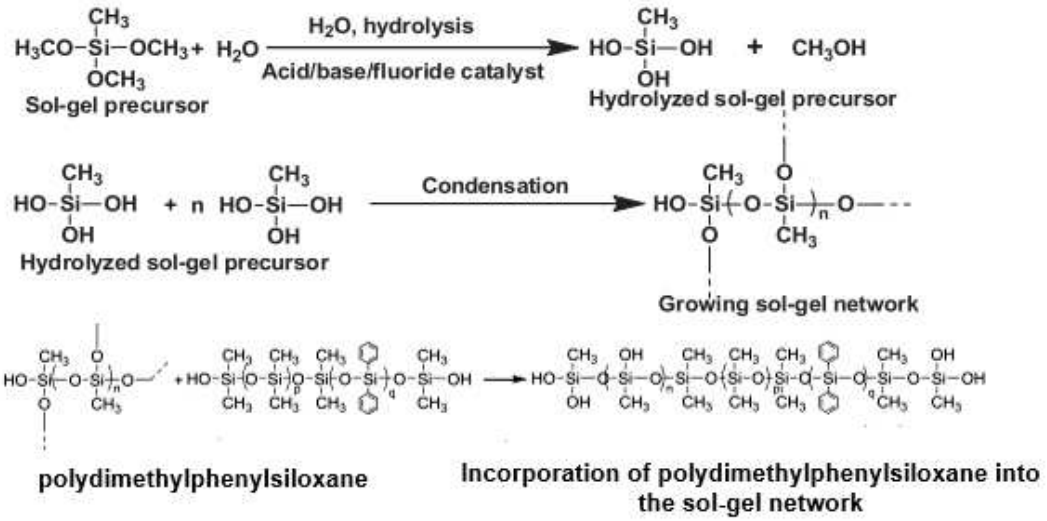


Figure 1.33 Chemical reactions involved in the sol-gel coating (poly-dimethyldiphenylsiloxane) [441]

The morphology of the synthesized sol-gel coating depends on the type of catalyst used in the sol solution. Acid-catalyzed hydrolysis yields a linear or randomly- branched sol-gel network. However, base-catalyzed hydrolysis generates highly branched and condensed network. The condensation rate depends on the sol- solution pH and is highest at intermediate pH [441].

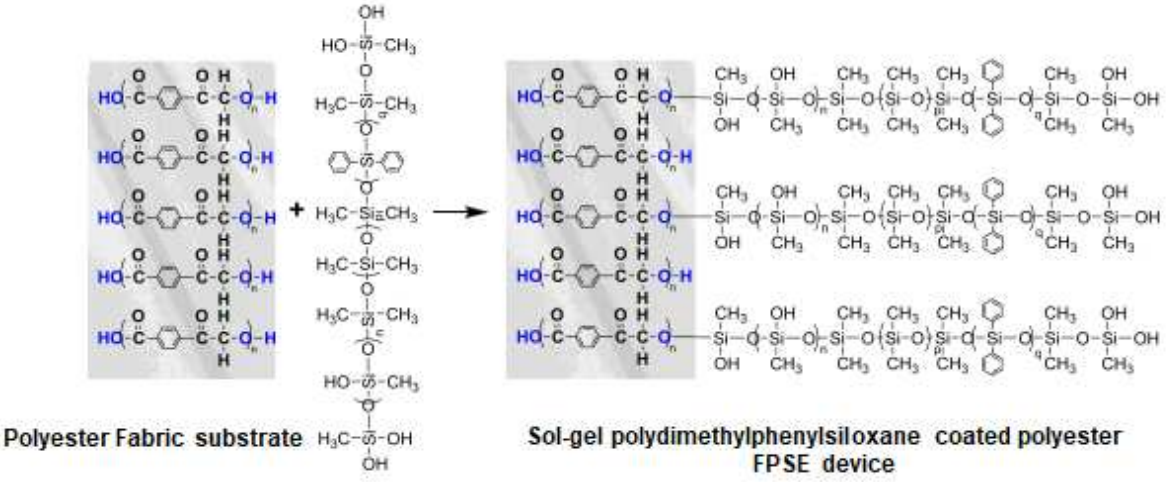


Figure 1.34 Schematic representation of sol-gel poly(dimethyldiphenylsiloxane) coating on a polyester fabric [440]

1.9.1.5 Post gelation-treatment

This step includes the aging, conditioning, and cleaning of sol-gel sorbent-coated FPSE medium prior to use. The aim of aging and conditioning of this procedure is to ensure that the condensation reaction is completed. The aging and conditioning is carried out inside a homemade conditioning unit at 50°C under continuous flow of helium gas for 24 h. The conditioning is

followed by cleaning the sol-gel sorbent- coated FPSE membrane with methanol/dichloromethane (50:50 v/v). The purpose of the cleaning step is to remove any unreacted precursor, polymer, reaction intermediates from the substrate in order to achieve a final product of clean surface bonded, porous, crack-free, 3D, hybrid, organic-inorganic sol-gel extraction phase. After cleaning, the FPSE membrane is conditioned again for overnight at 50^{o}C. [440,441].

1.9.1.6 Cutting the FPSE membrane into appropriate size

The flexibility of selecting the type and the size of the fabric medium consists one of the major advantages of FPSE. Depending the type of the sample in which FPSE is addressed the variable size of fabric medium can be tailored to specific applications. For example, in the extraction of biological samples such as blood, plasma, typically small volumes are used, thus FPSE media of 1cm^2 are employed. For environmental samples, an FPSE medium of 2.5 cm^2 is typically used. Cutting the fabric into circular disks ensures better reproducibility according to several studies[452,453]. The sol-gel sorbent-coated FPSE membranes are cut into sizes using an in-house custom-make cutting device [440].

1.9.2 Applications of FPSE

Since the first publication of FPSE in 2014 as a novel sample pretreatment for the determination of selected estrogens, a plethora of applications for organic and inorganic analytes have been reported in the literature. Taking into account the number of publications using FPSE so far, it is concluded that most of them concern environmental applications, (57.14%), followed by food samples analysis (25%) and finally of biological samples (17.86%) [447]. A brief review of the most recent applications of FPSE are presented in Table 1.13.

.

Table 1.13 Applications of FPSE technique

Fabric Substrate	Sol-gel Coating	Target compound	Sample matrix	Instrumental technique	Ref.
Cellulose	PEG	Substituted phenols	Tap-Pond-Reclaimed water	HPLC-UV	[446]
Glass Fiber	PEG	Pharmaceuticals, Sweeteners	Wastewater	UHPLC-Orbitrap-MS	[450]
Cellulose	PEG	Parabens	Cosmetics	HPLC-UV	[454]
Cellulose	PCL-PDMS-PCL	Pirimicarb, Fenitrothion	Environmental water	HPLC-PDA	[455]
Cellulose	Carbowax 20 M	Fungicides	Surfacewater, Tap Water	GC-MS-MS	[456]
Glass Fiber	PEG	Antidepressants	Wastewater, Lakewater	HPLC-DAD	[457]
Cellulose	PTHF	NSAIDs	Saliva	HPLC-DAD	[458]
Glass Fiber	PDMS	PAHs	Environmental waters	HPLC-FLD	[441]
Cellulose	PTHF	Amphetamines	Wastewaters	HPLC-MS	[459]
Polyester	PDMDPS	UV stabilizers in personal care products	Wastewaters	UHPLC-MS/MS	[460]
Polyester	PDMDPS	UV stabilizers in personal care products	Seawater	UHPLC-MS/MS	[461]
Cellulose	PTHF	Androgens, Progestogens	Wastewater, Tap water	UHPLC-MS/MS	[462]
Cellulose	PTHF	Parabens	Human Urine	HPLC-DAD	[463]
Polyester	PCL-PDMS-PCL	Antidepressants	Human Blood Serum	HPLC-DAD	[464]
Cellulose	PTHF	Penicillin Antibiotics	Human Blood Serum	HPLC-PDA	[465]
Cellulose	PEG-PPG-PEG	Organochloride Pesticides	Fruit Juice	GC-MS	[466]
Cellulose	PEG	Fungicides	Wine	UHPLC-MS/MS	[467]
Cellulose	M-PEG	Cytostatic Drugs	Wastewaters	UHPLC-MS/MS	[468]
Cellulose	Carbowax 20M	Pharmaceuticals Personal care products	River water, wastewaters	LC-MS/MS	[449]
Cellulose	PTHF	Bisphenol A	Milk	LC-MS/MS	[469]
Cellulose	PEG	Sulfonamides	Milk	HPLC-UV	[451]

1.10 Liquid Chromatography

Emerging contaminants (ECs) as already reported they can be found in several media, both natural and anthropogenic, like in wastewater, rivers, lakes, soil, air, or living organisms at very low concentration levels of ng/L. In fact, the complete understanding of the problems presented by ECs is attributed to the advances in analytical methods that have enabled the detection of compounds in very low quantities. Detection techniques the previous decades, were not able to measure concentrations in the range of μg/L (ppb) to ng/L (ppt) in environmental or biological samples having as a result most of the contaminants to be unnoticed and not classified as emerging pollutants. One fundamental technique for conducting environmental analysis has been Liquid chromatography usually coupled with mass spectrometry (LC-MS) [470].

In general, chromatography is a method in which the components of a mixture are separated based on their differential interactions with two chemical or physical phases: a mobile phase and a stationary phase. According to the physical state of mobile phase employed, it can be classified to gas chromatography (GC) and liquid chromatography (LC), respectively. Liquid chromatography consists a powerful analytical tool capable of identifying both polar and non-polar organic compounds, in levels of ng /L without the need for derivatization, in all kinds of matrices including water bodies (wastewater, surface water, groundwater, and drinking water) or in solid samples (sewage sludge, manure, soil, or sediments) , biological fluids (blood, plasma, urine) and foodstuff.

Conventional LC is commonly used in preparative scale work to purify and isolate some components of a mixture. Nowadays liquid chromatography generally utilizes very small packing particles and a relatively high pressure for analytical separations of solutions, detection & quantification, referred to as high performance liquid chromatography (HPLC). HPLC can provide a very high resolution and a fast analysis time [471]. The basic components and operation of a typical chromatographic system are illustrated in Fig. 1.35.

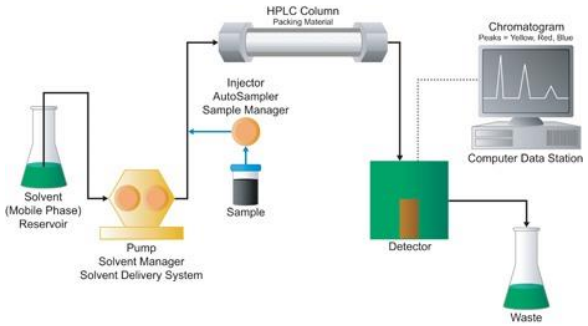


Figure 1.35 Liquid chromatographic system

Liquid chromatography is usually carried out with a packed chromatographic column composed of irregularly or spherically shaped particles as stationary phase. The mobile phase travels through the system carrying the sample compounds once the sample is injected. As the sample passes through this system, the compounds interact with the stationary phase due to the differences in ion-exchange, adsorption, partitioning, or size, different solutes having as a result the retention by this phase. This leads to a difference in the rate of travel for these components and therefore the separation of the compounds can be achieved. Besides needing a difference in retention for a separation to occur, the peaks for two neighboring compounds must be sufficiently narrow to allow this difference to be observed. Several parameters that affect the chromatographic efficiency are [472]:

- Column length, tube diameter and particle size of the column
- Uniformity in size, shape, and packing material of the column
- Flow rate and linear velocity
- Temperature and rate of solute diffusion
- Mobile phase
- Initial injection volume

The most widely used separation technique used for quantitative analysis is reversed-phase liquid chromatography, which uses a non-polar (hydrophobic) stationary phase, like a C18 sorbent and a polar (aqueous) mobile phase such as water. Considering this reversed-phase chromatography appeared to be more flexible for a broad range of compounds with solvents of low cost which are also safer compared to the ones used in normal-phase. Typical columns are consisted of an alkylsilica-based, non-polar sorbent linked with carbon-18 (C18) that allows separation based on hydrophobic interactions between the compound solute in mobile and the immobilized hydrophobic functional groups attached onto the sorbent.

1.10.1 Mobile Phase

Mobile phases usually consist of a water solution and a miscible polar solvent, such as acetonitrile or methanol, which solubilizes the analyte retained on the stationary phase resulting in elution from the column. For compounds with poor retention on column, the adjustment of the pH of the mobile phase is necessary with the addition of weak acids/bases or buffers improving in this way the

retention in the column. This adjustment determines the ionized or neutral form, of the target analyte with ionized species to be eluted earlier from the column. The mobile phase flow can be delivered either with isocratic mode where there is a fixed mobile phase composition (i.e. 50% mobile phase A:50% mobile phase B) throughout the entire run, or using a gradient elution, where the proportion of the organic mobile phase solvent is gradually increased throughout the analytical run. Gradient elution is typically used when high levels of resolution are required for separation of a complex mixture to ensure elution of all compounds of interest, as analytes will be eluted sequentially in order of hydrophobicity.

1.10.2 Chromatographic Column

Shortening the column analysis time is requested for the practical and efficient analysis especially in monitoring studies. This objective can be achieved by shortening the columns and increasing the flow rate decreasing the particle size of the stationary phase. The adjustment of the temperature, could also address to this issue, enabling the diffusivity so that higher flow rates can be used. Modern column technology for HPLC has produced columns having various dimensions, with a trend towards smaller internal volumes [473]. These small volume columns are useful in combining LC with other methods, such as mass spectrometry, to produce hyphenated techniques. In general, better efficiencies and lower detection limits are achieved with HPLC columns that have longer lengths and smaller inner diameters [472]. In recent years, fused core columns (also named core–shell columns), consisting of sub-3µm superficial porous particles made of a solid fused core and a 0.5µm porous shell, have been proposed as a notable alternative to UHPLC columns to generate fast separations. This technology exhibits efficiency close to that offered by sub-2µm fully porous particles and high speed of analysis, offering the advantage of modest backpressures, typically within the limits of conventional HPLC equipment [473]. Also, monolithic columns have emerged as a valid alternative to traditional packed columns for high efficiency separations in LC. Monolithic supports consist of a single piece of porous material, with good permeability and fast mass transfer, obtaining lower backpressure also at high flow rates (up to 10 ml min−1) in conventional column length and high resolution[473].

1.10.3 Temperature Control

The control of column temperature can be an important factor in determining the reproducibility and efficiency of an LC separation [472]. Temperature control of an LC column can be usually achieved with heating/cooling blocks ore chambers in the instrumental system. During an analytical run in liquid chromatography, the column temperature has to be stable in order to assess reproducible retention times. Furthermore, increasing the column temperature has as result to (a) lower the mobile phase viscosity, (b) increase the amounts of mass transfer between mobile phase and stationary phase, and (c) higher flow rates are feasible, which sequentially result to a shorter analysis time. The degree to which the temperature can be increased is determined by parameters such as the vapor pressure and the boiling point of the mobile phase, as well as the thermal stability of the target compounds in the injected samples and the nature of the sample matrix. There are cases that the target compounds may require separations to be carried out at lower temperature conditions [472].

1.10.4 Liquid Chromatography Detectors

A main advantage in liquid chromatography is that consists a compatible platform for many types of detectors. Typical LC detectors are (a) UV-Vis or diode-array detectors (DAD), (b) fluorescence detectors, (c) electrochemical detectors, (d) refractive index detectors, and (e) mass spectrometric detectors [472]. The detectors of a chromatographic system have the task to record the sample compounds that have been already separated in chromatographic column and transfer this information to a data output system. The progress in chromatographic systems due to the development of new instrumentation has established the combination of LC techniques with mass spectrometry (MS). Coupling mass spectrometry to chromatographic techniques has always been desirable due to the sensitive and highly specific nature of MS compared to other chromatographic detectors The combination of LC and MS exploits the benefits of both LC as a powerful and versatile separation technique and MS as a valuable and sensitive and selective detection and identification technology The intrinsic properties of these two techniques result in the advanced development of a powerful tool in analytical chemistry with applications in environmental chemistry, biomedical and clinical toxicology, forensic science, food analysis etc.

1.11 Mass Spectrometry

Mass spectrometry (MS) is one of the most powerful analytical tools and its use is increasing in various scientific fields due to its high sensitivity, selectivity, high mass accuracy, and high throughput capability. It has wide applications, including analytical and environmental chemistry [474,475], food authentication and food safety [476,477], biomedical and clinical research [478–480], in forensic science [481,482] in sports doping control [483] as well as in pharmaceutical manufacture industries [484]. Mass spectrometry is an analytical technique that can determine precisely the atomic or the molecular weight of atoms or molecules once they have been ionized expressed as mass-to-charge ratio (m/z) values. A typical mass spectrometer consists of four main components: the sample introduction, the source where ionization occurs, the mass analyzer, and the detector. It is also been associated with a computer recorder for data output. (Fig.1.36).

1. Inlet: Introducing samples from ambient room pressure into ion source
2. Ion source: Converting sample molecules to ions
3. Mass analyzer: Separating ions according to their m/z
4. 4.Detector: Detecting ions and amplifying the signal
5. Data Output: Receiving signal form detector, further amplifying, recording, creating mass spectrum

Mass spectrometry can analyze many different types of samples that range from solid, liquid, or gases. First, the molecules have to be ionized either under vacuum or at atmospheric pressure. Depending on the ionization technique, either molecular ions ($M^{+\bullet}$) with an odd electron number or protonated ions ($[M+H]^+$ with an even electron number are formed in the positive mode and M^-, $M^{-\bullet}$ or ($[M-H]^-$ in the negative mode. Ionization techniques are often classified into soft ionization, where little or no fragmentation occurs, and hard ionization, where fragmentation is extensive. Electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI) are considered as soft ionization techniques while electron impact (EI) is considered as a hard ionization technique. Over the years, many different types of ion sources and mass analyzers have been developed. Most mass analyzers operate under high vacuum conditions to ensure that charged particles do not deviate from their trajectories due to collision with residual gas. Mass spectrometers can be grouped into different types of operation mode: continuous mode (magnetic sector, quadrupole), pulsed mode (time of flight), and ion trapping mode (quadrupole traps, Fourier transform ion cyclotron, orbitrap) [485]

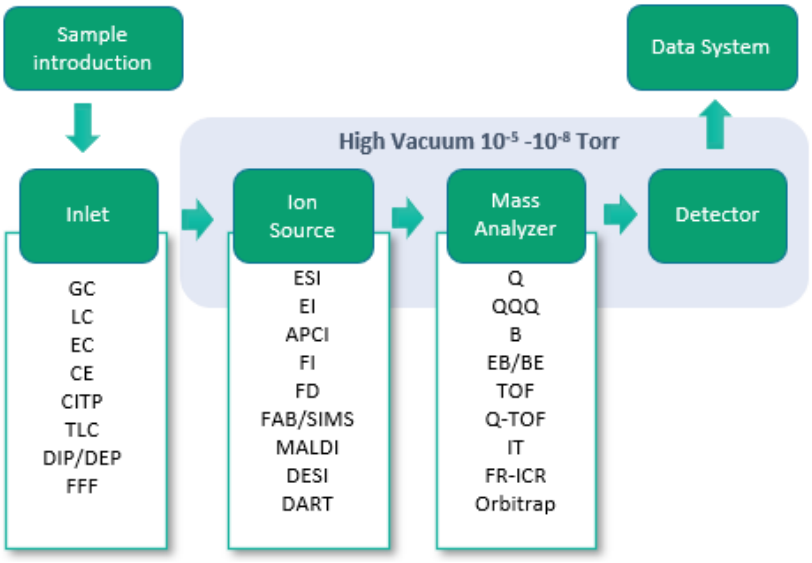


Figure1.36 Basic Components of a Mass Spectrometer

1.11.1 Basic Principle of Mass Spectrometry

''The basic principle of mass spectrometry (MS) is to generate ions from either inorganic or organic compounds by any suitable method, to separate these ions by their mass-to-charge ratio (m/z) and to detect them qualitatively and quantitatively by their respective m/z and abundance. The analyte may be ionized thermally, by electric fields or by impacting energetic electrons, ions or photons. The ions can be single ionized atoms, clusters, molecules or their fragments or associates. Ion separation is effected by static or dynamic electric or magnetic fields"[486]. Although this definition of mass spectrometry dates back to 1968 when organic mass spectrometry was in its first steps, it is still accurate. However, some additions according to the evolutions of mass spectrometers have been included. First, ionization of a sample can be effected not only by electrons, but also by (atomic) ions or photons, energetic neutral atoms, electronically excited atoms, massive cluster ions, and even electrostatically charged microdroplets can also be used to effect. Second, as demonstrated with great success by the time-of-flight analyzer on separation by m/z can also be effected in field-free regions, provided the ions possess a well-defined kinetic energy at the entrance of the flight path. The large variety of ionization techniques and their key applications can be roughly classified by their relative hardness or softness and (molecular) mass of suitable analytes.

1.11.2 Ion Sources

In the ion sources, the analyzed samples are ionized prior to analysis in the mass spectrometer.

A variety of ionization techniques are used for mass spectrometry. The most important considerations are the internal energy transferred during the ionization process and the physicochemical properties of the analyte that can be ionized. Some ionization techniques are very energetic and cause extensive fragmentation. Other techniques are softer and only produce ions of the molecular species. Electron ionization, chemical ionization and field ionization are only suitable for gas-phase ionization and thus their use is limited to compounds sufficiently volatile and thermally stable. However, a large number of compounds are thermally labile or do not have sufficient vapor pressure. Molecules of these compounds must be directly extracted from the condensed to the gas phase. These direct ion sources exist under two types: liquid-phase ion sources and solid-state ion sources. In liquid-phase ion sources the analyte is in solution. This solution is introduced, by nebulization, as droplets into the source where ions are produced at atmospheric pressure (API) and focused into the mass spectrometer through some vacuum pumping stages (Fig.1.37). Electrospray (ESI), atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization sources(APPI) correspond to this type. In solid-state ion sources, the analyte is in an involatile deposit. It is obtained by various preparation methods which frequently involve the introduction of a matrix that can be either a solid or a viscous fluid. This deposit is then irradiated by energetic particles or photons that desorb ions near the surface of the deposit. These ions can be extracted by an electric field and focused towards the analyzer. Matrix-assisted laser desorption (MALDI), secondary ion mass spectrometry, plasma desorption and field desorption sources all use this strategy to produce ions. Fast atom bombardment (FAB) uses an involatile liquid matrix. The ion sources produce ions mainly by ionizing a neutral molecule in the gas phase through electron ejection, electron capture, protonation, deprotonation, adduct formation or by the transfer of a charged species from a condensed phase to the gas phase. Ion production often implies gas-phase ion–molecule reactions [486].

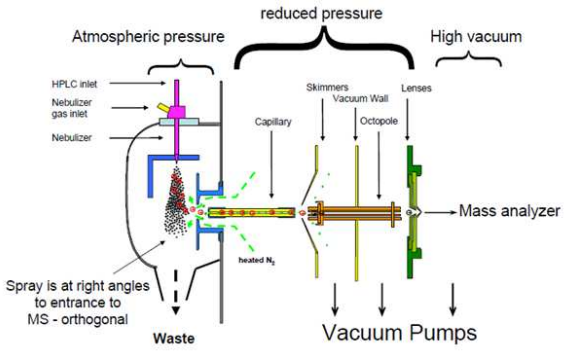


Figure 1.37 Atmospheric Pressure Ionization mode (API)

Nowadays, the two most widely used API for LC is Electro spray Ionization (ESI) and Atmospheric Pressure Chemical Ionization (APCI). ESI occurs in the liquid phase and its application is mainly on the polar compounds. While APCI occurs in the gas phase and can be employed to ionized less polar molecules. Atmospheric Pressure Photo ionization (APPI) is a relatively less popular ionization technology for LC-MS. Compared to the ESI and APCI, APPI is the last soft ionization technique that cans ionize less polar and nonpolar molecules which are poorly amenable to ESI and APCI. On the basis of the polarity and molecular weight of target compounds, the application of different ionization technology is shown in Fig.1.38.

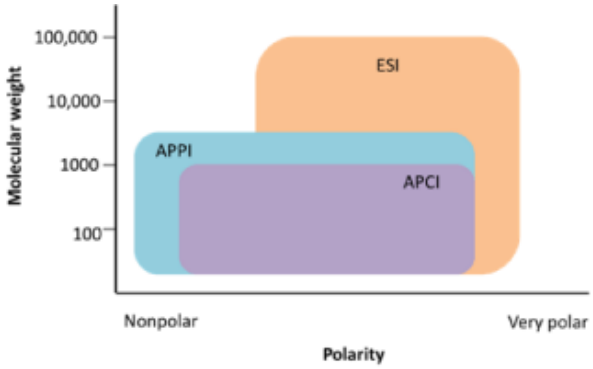


Figure 1.38 Application of atmospheric pressure ionization (API) techniques depending of polarity and molecular weight [487]

1.11.2.1 Electrospray Ionization (ESI)

ESI is a robust ion source capable of interfacing to LC and demonstrated its application to a number of important classes of biological molecules.[1] ESI works well with moderately polar molecules and is thus well suited to the analysis of many metabolites, xenobiotics and peptides

Under normal conditions, ESI is considered a “soft” ionization source, meaning that relatively little energy is imparted to the analyte, and hence little fragmentation occurs. This is in contrast to other MS ion sources, for example the electron impact source commonly used in GC-MS, which causes extensive fragmentation. It is possible to increase ESI “in-source” fragmentation by increasing voltages within the source to increase collisions with nitrogen molecules [484].

Electrospray Ionization (ESI), (Fig. 1.39) is a process where a high potential is applied onto a liquid to generate a fine aerosol. Typically, a sample in a liquid form is introduced with a typical flow rate of 1–1,000 µL/min into a spray needle/capillary. In this capillary a high voltage 3–6 kV applied in order to generate a gradient electric field. The voltage, which can be either negative or positive based on the nature of the analyte, produces charged droplets that are sprayed out from the needle tip into the atmosphere (nebulization) into a conical shape called Talylor cone. The presence of a sheath gas (nitrogen) flowing around the needle during the nebulization process at atmospheric pressure assists in size reduction of the droplets due to evaporation. As the solvent evaporates, the charge intensity on the surface of the droplet gradually increases, and finally the droplet split into one or a plurality of charged ions. The charged droplets pass either through a curtain of heated inert gas (nitrogen) or through a heated capillary allowing the analyte to enter the gas phase in the form of a single charge or multiple charges and become a gas phase ion. Electrospray works best within a flow rate range from 1 to 50 µL/min and with high organic solutions (methanol or acetonitrile) [485].

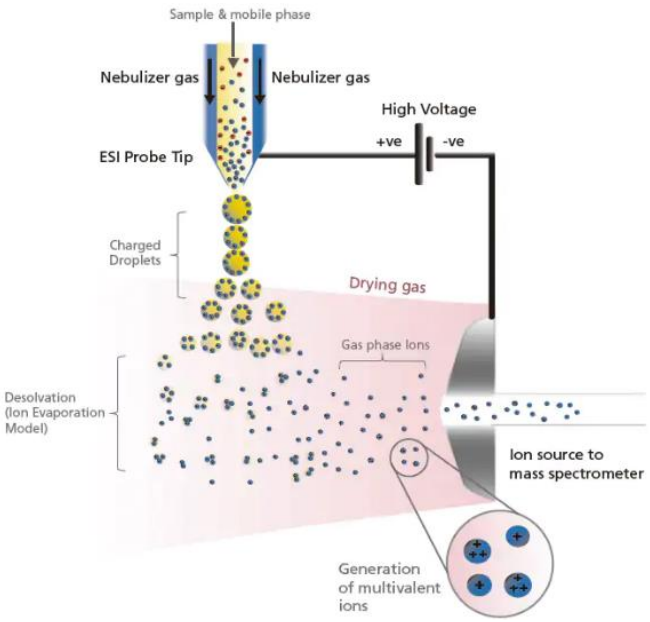


Figure 1.39 Schematic illustration of an Electrospray Ionization (ESI) [488]

Two different mechanisms were proposed for ion generation in ESI, as illustrated in Scheme 2 (8, 28–30). The ion evaporation mechanism (IEM) involves solvent evaporation and Coulombic fissions of the charged droplets, forming smaller droplets. The gas-phase ions are directly released/desorbed from the surface of the small droplets when the repulsion between charges at the droplet surface overcomes the cohesive force of the surface tension. In the charge residual model (CRM), the molecule will not desorb from the charged droplet, but it will be freed by complete evaporation of the solvent, and this is more likely to happen with large molecules .

The formation of ions in ESI is highly dependent on the pH value of the mobile phase and the pKa value of the analyte, as electrospray requires preformed ions in solution. This follows the well-known principles of acid-base theory to produce either positive or negative ions which are detected in the positive or negative mode respectively. Most mass spectrometers have acceptable ranges for mass detection of compounds typical of the pharmaceutical and bio- pharmaceutical industries. With large molecules such as proteins and peptides, typically carrying multiple ionizable sides, multiply-charged ions are produced. Since the detector monitors the mass/charge, multiple charges allow these large molecules to be detected in a MS such as a single quadrupole LC/MS system with a mass range of (for example) 2000 Daltons even if their mass is in the range of 10,000 Dalton [489]

1.11.2.2 Advantages and Disadvantages of ESI

Electrospray provides a relatively simple way to ionize non-volatile solutions, allowing the mass spectrometer to provide a sensitive direct detection. Electrospray mass spectrometry can be used not only for the detection and analysis of inorganic substances, but also for the analysis of organometallic ion complexes and biomacromolecules covering a mass range of up to 70,000 [490]. In electrospray mass spectrometry, high molecular weight molecules typically carry multiple charges, and the distribution of charge states accurately quantifies molecular weight, providing both accurate molecular mass and structural information. In addition, since ESI is soft ionization method, it has the ability to generate noncovalent complexes in the gas phase. ESI is combined adapted of chromatographs for complex system analysis and especially is easily adapted with LC-systems. The flexibility of ESI is applied also in tandem mass analyzers such as ion traps and triple quadrupole instruments. Finally, ESI provides the possibility of both positive and negative ionization mode [484,491].

The main negative concern about ESI is that ionization is susceptible to matrix suppression since adduct ions formed during fragmentation can interfere with sample matrix. Thus, for adduct formation through proton ionization compounds with the greatest proton affinity, will be enhanced in contrast with molecules with a lower affinity. For this reason, the levels of matrix effects when using this ionization technique should be evaluated in particular with complex matrices such as wastewater.

Some other shortcomings of the technology of ESI include [490]:

1. Complex matrices can interfere with ESI reducing sensitivity

2.The experimental parameters or technical conditions must be carefully selected based on the problem to be solved.

3.There is a limit to the choice of solvent and the range of solutions that can be used. 4. The presence of salts can reduce sensitivity

5. At the same time, the response of the mass spectrometer to different complexes varies widely, which prevents accurate quantitative analysis.

Since the solution parameters control the spray process, there is fluctuation in the ion signal even under good conditions.

6.Sample purity is important

1.11.3 Mass analyzers

Most mass analyzers operate under high vacuum or at low pressure to ensure that the charged particles do not significantly deviate from their trajectories due to collision with residual gas. Depending on the operation of mass analysis, analyzers could belong to continuous mode (i.e., magnetic sector, quadrupole), pulsed mode (i.e., time of flight), and ion trapping mode (i.e., quadrupole traps, ion cyclotron, orbitrap). Apart from a single MS, there are tandem/hybrid arrangements, also known as MS/MS systems. Single mass analyzers such as the magnetic sector, quadrupole, and time-of-flight (TOF) were commonly used for measuring organic compounds, but the quadrupole model has gradually been increasing their share due to its relatively lower cost. Besides these mass analyzers, an ion trap MS system that temporarily accumulates ions of a selected range before separating them by mass, and a tandem/hybrid MS system that combines multiple MS units have been developed as well. These types of MS systems each take advantage of their respective

features and are used according to various analytical objectives. The hybrid of Orbitrap MS was used in current survey and is described in following section.The basic types of mass analyzers are summarized in Table1.14.

Table 1.14 Common mass analyzers [486]

Mass analyzer	Acronym	Principle
Time-of-flight	TOF	Time dispersion of a pulsed ion beam; separation by time-of-flight
Magnetic sector	B	Deflection of a continuous ion beam; separation by momentum in magnetic field due to Lorentz force
Linerar quadropole	Q	Continuous ion beam in linear radio frequency quadrupole field; s due to instability of ion trajectories
Linear quadrupole ion trap	LIT	Continuous ion beam delivers ions for trapping; storage, and eventually separation in linear radio frequency quadrupole field by resonant excitation
Quadrupole ion trap	QIT	Trapped ions; separation in three-dimensional radio frequency quadrupole field by resonant excitation
Fourier transform-ion cyclotron resonance	FT-ICR	Trapped ions in magnetic field (Lorentz force); separation by cyclotron frequency, image current detection and Fourier transformation of a transient signal
Orbitrap	Orbitrap	Axial oscillation in inhomogeneous electric field; detection of frequency after Fourier transformation of a transient signal

1.11.3.1 Performance of mass analyzers

The performance of mass analyzers is typically quantitated in terms of resolution and mass accuracy. At a minimum, the resolution of the mass analyzer should be sufficient to separate two ions differing by one mass unit anywhere in the mass range scanned. Such resolution or a little bit higher is provided by quadrupole and ion trap analyzers and it is sometimes referred as unit mass resolution.

1.11.3.1.1 Resolution and Resolving Power

The ability of an instrument to resolve neighboring peaks in a mass spectrum is called mass resolving power or simply resolving power or resolution (R). It is obtained from the peak width at a specific percentage of the peak height expressed as a function of mass. Mass resolution is given as the

smallest difference in m/z , i.e., Δ(m/z) that can be separated for a given signal, i.e., at a given m/z value:

$$R = \frac{m}{\Delta m} = \frac{m/z}{\Delta(m/z)} \qquad \text{Eq.1}$$

Two neighboring peaks are assumed to be sufficiently separated when the valley separating their maxima has decreased to 10% of their intensity. Hence, this is known as 10% valley definition of resolution, R10%. The 10% valley conditions are fulfilled if the peak width at 5% relative height equals the mass difference of the corresponding ions, because then the 5% contribution of each peak to the same point of the m/z axis adds up to 10% (Fig.1.40). Usually, the peak width is measured as the full width at half maximum (FWHM) of the peak, which is nowadays accepted as a general definition of mass spectrometric resolution [486].

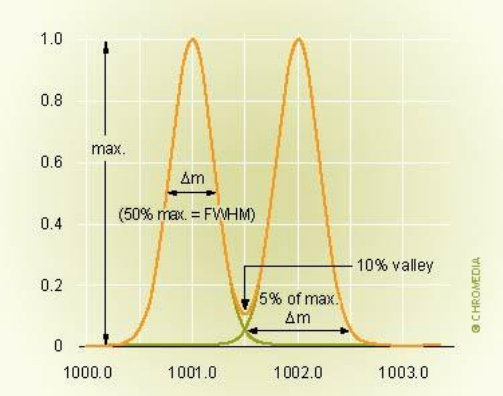


Figure 1.40 The 10% valley and full width at half maximum (FWHM) definitions of resolution [492]

In practice, high resolution designates a mass analyzer with resolving power m/Δm50% > 10 000, thereby excluding quadrupole mass filter, triple quadrupole, and quadrupole ion trap mass analyzers[493]. High resolving power is particularly important for experiments involving complex mixtures, such as biological, environmental, and food samples generated from a matrix, since these will contain a significant number of background ions in addition to the analytes of interest. In such cases, high resolving power will make the difference between detecting and not detecting analytes at low concentrations due to the masking effect of isobaric interferences. In other words, accurate quantitation relies on high selectivity, which is the ability to resolve compounds of interest from background interferences [494].

1.11.3.1.2 Mass Accuracy

High resolving power and accurate mass measurements are definitions closely related and interdependent because mass accuracy tends to improve as peak resolution is improved. Nevertheless, they should not be confused, as performing a measurement at high resolution alone does not equally imply measuring the accurate mass. High resolution separates neighboring signals, accurate mass can deliver molecular formulas. Until the early 1980s, accurate mass measurements were limited to electron ionization, a forgotten technique. However, new options introduced by FT-ICR instruments reestablished accurate mass measurements. The newly developed Orbitrap and a new generation of oaTOF analyzers contributed to an increasing need for accurate mass data. Nowadays, formula accurate mass measurements can be adopted to be any ionization method [486]. The absolute mass accuracy, Δ(m/z), is defined as the difference between measured accurate mass and calculated exact mass:

$$\Delta\,(m/z) = m/z\ experimental - m/z\ calculated \qquad \text{Eq.2}$$

Instead of stating the absolute mass accuracy at mmu, (1 millimass unit) it can also be given as relative mass accuracy, Δm/m, i.e., absolute mass accuracy divided by the mass it is determined for:

$$\delta\, m/m = \Delta(m/z)/(m/z) \qquad \text{Eq.3}$$

The relative mass accuracy, δm/m, is normally given in parts per million (ppm).

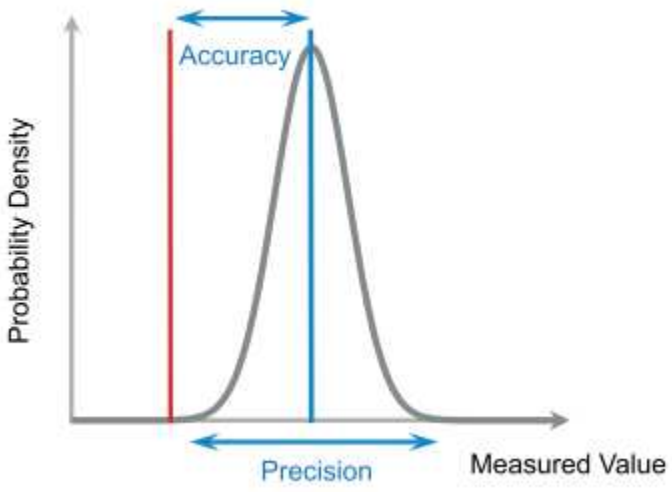


Figure 1.41 Accuracy and precision of mass determination [495]

The principal advantage of high mass accuracy is the possibility to determine the elemental composition of individual molecular or fragment ions, which is a powerful tool for the structural

elucidation or confirmation. For exact mass measurements, the minimum acceptable mass accuracy is 5 ppm. For higher m/z values or when the presence of many different elements in a given ion is suspected, the mass accuracy <5 ppm is not sufficient for a clear indication of the elemental composition.

Table 1 shows the performance characteristics of typical mass analyzers including both Resolution and Mass Accuracy.

Table 1.15 Typical characteristics of main mass analyzers [496]

Mass Analyzer	Resolving Power (FWHM)	Mass Accuracy (ppm)	Sensitivity (g)
Quadrupole (Q)	Up to 5000	50	10^{-15} (SRM)
Quadrupole ion trap (IT)	10000	50	10^{-15}
Time of flight (TOF)	20000	3	10^{-12} (Full Scan)
Magnetic Sector (B)	50000	≈1	10^{-12}
Orbitrap	100000	2	10^{-15} (Full Scan)
FT-ICR	1000000	≈1	10^{-12} (Full Scan)

1.11.4 High Resolution Mass Spectrometry

The tremendous success of LC-MS/MS with the association of quadrupole-based mass analyzers was able even to progress the detection technique, since instrument producers were able to significantly increase the sensitivity by almost orders of magnitude. However, this development was not accompanied by a significant increase in selectivity. Analysts are satisfied with clear analytical chromatograms, free of intense background with a flat baseline and a single, easy to recognize compound peak. Nevertheless, analysis performed by even high sensitivity instruments, often present chromatograms with the targeted analyte peak surrounded by several matrix peaks. This has as a result not only to make intricate the detection process but also to be necessary the use of one or even two confirmatory transitions. These issues become increasingly consistent when analyzing low concentrations of target compounds in complex matrices. The second critical aspect related to simple MS/MS is the need for individual evaluation of instrumental settings such as the precursor and product ion masses, as well as the corresponding collision energies for each compound. However, the need to include wide range of analytes into a single chromatographic run (multiresidue methods)

demands the use of short dwell times. Moreover, the introduction of chromatographic columns with small particle size material <2 μm which provide narrow chromatographic peaks, require short MS cycle times to obtain enough data points across a chromatographic peak. These two contradicting requirements led to sophisticated time window-based acquisition modes. Such acquisition windows need to be carefully defined and have a limited life span. Failing to fulfill these aspects, significant errors may occur such as the loss of analyte signals, since peaks can shift out of such predefined retention time windows. The mentioned MS/MS limitations along with the scarce use in semi-target and non-target approaches, were the main reasons for exploring alternative multiresidue approaches based on full scan, High-Resolution MS (HRMS) [497].

1.11.5 Orbitrap Mass Analyzer

The Orbitrap, a new type of mass analyzer, has drawn attention due to its analytical performance in terms of resolution, mass accuracy, space charge capacity and linear dynamic range, relatively small size, and cost. Invented by Makarov, the orbitrap mass analyzer is based on the Kingdom trap which uses a wire stretched along the axis of an outer cylinder enclosing the trapping volume. Hybrid LTQ orbitrap is a hybrid Orbitrap mass analyzer combined with a linear ion trap LTQ. The produced ions from ion source are trapped into the LTQ and analyzed with scan type method of MS and MSn. The instrumentation of Orbitrap mass analyzer follows the principles of a typical spectrometer. (Fig.1.42)

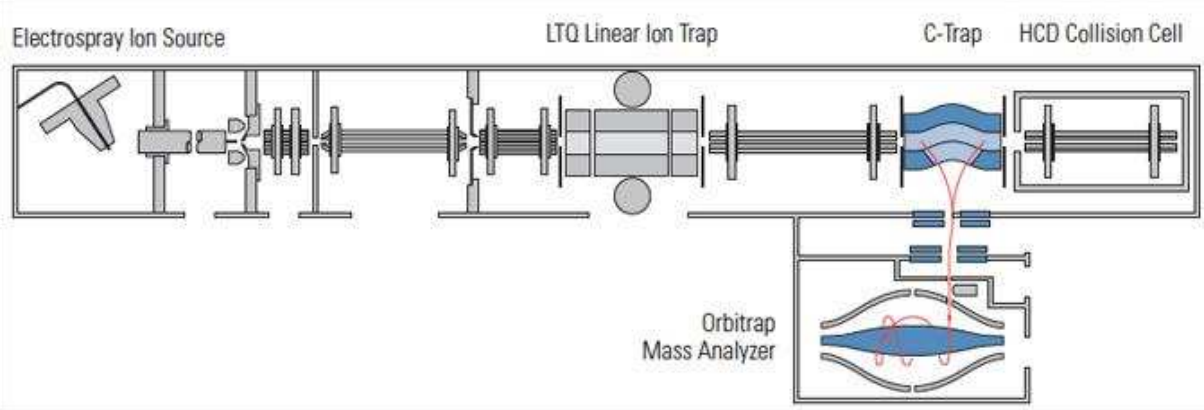


Figure 1.42 Schematic layout and picture of a linear trap-Orbitrap hybrid mass spectrometer[498]

Specifically, the hybrid LTQ Orbitrap consists of three main components, (a) a linear ion trap (Thermo Scientific LTQ XL) for sample ionization, selection, fragmentation, and AGC, (b) an intermediate

storage device (curved linear trap) that is required for short pulse injection, and (c) an Orbitrap analyzer for Fourier based analysis.

The hybrid mass spectrometer combines a linear ion trap MS and the Orbitrap mass analyzer. Comprised of two outer electrodes and a central electrode, the Orbitrap mass analyzer acts both as an analyzer and a detector. Ions generated by ESI are collected in the LTQ XL followed by axial ejection to the curved linear storage trap (C-trap) which is used to store and collisionally cool ions before injection into the orbital trap. The ions transferred from the C-Trap are captured in the orbital trap by rapidly increasing the electric field("electrodynamic squeezing"). Once captured, the ions oscillate around the central electrode and in between the two outer electrodes. Different ions oscillate at different frequencies resulting in mass separation. Signals from each of the orbital trap outer electrodes are amplified and transformed into a frequency spectrum by fast Fourier transformation which is finally converted into a mass spectrum. In this way, the Orbitrap mass analyzer works as a Fourier Transform (FT) mass analyzer analog of well-known FT-ion cyclotron resonance (ICR) technology, with the advantage of allowing smaller instrument size and easier instrument operation. Additionally, the LTQ Orbitrap XL features a new collision cell to provide additional flexibility to any MS/MS experiment. Ions can be selected in the linear ion trap and fragmented either in the ion trap or in the new collision cell (HCD). For HCD (Higher Energy Collisional Dissociation) ions are passed through the C-trap into the gas-filled collision cell [499], [500].

Th exceptional high resolution of Orbitrap mass analyzer is attributed to three factors: a) nanometer-range accuracy Orbitrap electrodes, b) high voltage supplies, and c) mass-to-charge measurements delivered as a function of oscillation frequency.

1.11.5.1 Working principle of Orbitrap mass analyzer

The working principle of the orbital mass analyzer is based on the orbital trapping of ions. Injected ions cycle around the central electrode and at the same time oscillate along the horizontal axis [498],[501]. The Orbitrap's axially symmetric electrodes create a combined 'quadro-logarithmic' electrostatic potential:

$$U\,(r,z) = \frac{k}{2}\left(z^2 - \frac{r^2}{2}\right) + \frac{k}{2}(R_m)^2\, Ln\left(\frac{r}{R_m}\right) + C \qquad \text{(Eq.1)}$$

Here, r and z are cylindrical coordinates, k is a constant, k is field curvature, and Rm is the characteristic radius. Stable ion trajectories involve both orbiting motion around the central electrode (r, φ-motion, where φ is the angular co-ordinate) and simultaneous oscillations in the z-direction. As is evident from Eq.1, the specially shaped electrodes produce an electrostatic potential containing no cross-terms in r and z. Thus, the potential in the z-direction is exclusively quadratic. When ions start their motion at the correct energy and radius, stable trajectories are formed which combine three cyclic motions (Fig.1.43):

- rotational motion around the central electrode with a frequency of rotation ω_ϕ
- radial motion with a frequency ω_r (between the maximum and minimum radii inside the 'ditch')
- axial oscillations along the central electrode with a frequency ω_z

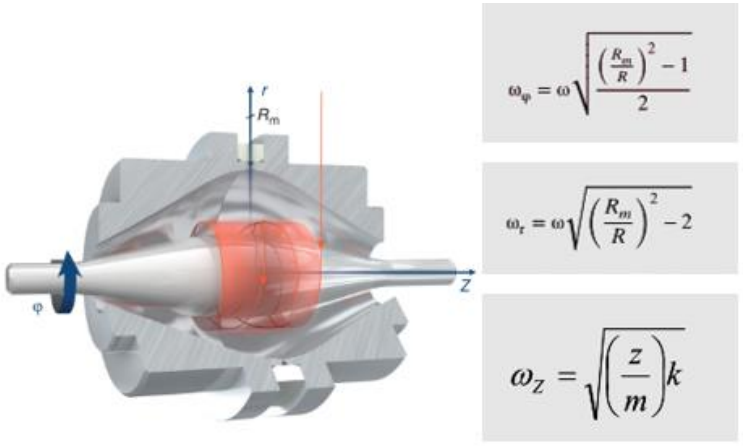


Figure 1.43 Diagram of the Orbitrap mass analyzer showing a stable spiral trajectory of an ion between the central electrode and the split outer electrodes [502]

Out of the three characteristic frequencies ω_ϕ, ω_r, and ω_z only the ion motion along the z-axis may be described as a harmonic oscillator and is completely independent of r, φ motion. therefore, only this frequency can be used for mass analysis.

$$\omega = \sqrt{\left(\frac{z}{m}\right)k} \qquad \text{Eq.2}$$

The abundance of a given ion is reflected by the amplitude of the given frequency ω_z of this ion. The information in the Orbitrap device with many different m/z values moving along the central electrode contains therefore both, the m/z information by different frequencies ω_z and the intensity

information by the amplitude of the individual frequency ω_z. This is called the transient signal. Outer electrodes are then used as receiver plates for image current detection of these axial oscillations. The digitized image current in the time domain is Fourier-transformed (FT) into the frequency domain in the same way as in FTICR . FT translates these frequencies into m/z values and their amplitudes into intensities. The longer the transient signal is recorded, the higher is the resolution of the mass spectrum obtained. The FT of a transient signal yields a highly-resolved, accurate-mass Orbitrap mass spectrum [498,501].

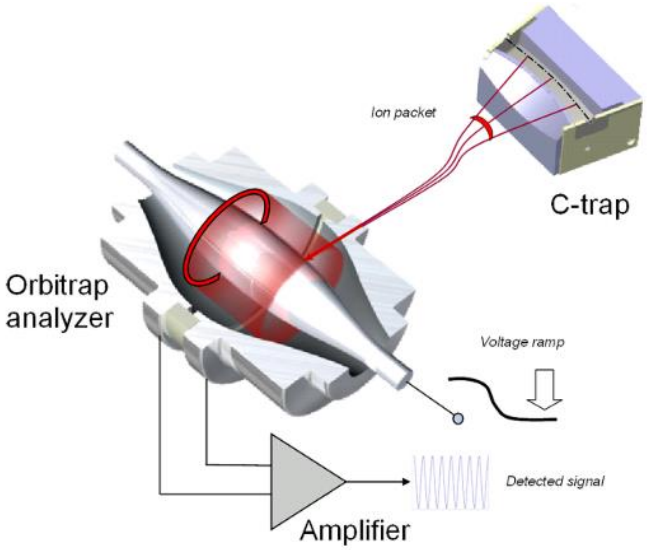


Figure 1.44 Orbitrap mass analyzer connected with C-trap [501]

Mass analysis can be carried out in one of two modes (a) Fourier Transform (FT) mode: Measuring coherent oscillations in the axial direction (image current detection) or (b) Mass Selective Instability (MSI) mode: Involving ion ejection and collection onto a detector.

1.11.5.2 Advantages of Orbitrap Mass Analyzer

High resolution setups based on Orbitrap mass analyzer proved to be capable of handling tasks that were considered to be the exclusive domain of tandem quadrupole technology [497]. This refers to precision, accuracy, sensitivity, dynamic range, and selectivity. The sensitivity of Orbitrap analyzer is currently slightly inferior compared to MS/MS methods, however, this is only a limitation in cases of determination at low concentrations of several analytes in some matrices. Orbitrap mass analyzer provides mass accuracy at or below ppm levels, eliminating the background interference and false

positive results that can occur with SRM methods. In that way, also improves the detection limit and sensitivity in a various range of sample matrices. Full scan of the product ions is possible without the need to select the ion pair and optimize the fragmentation energy, facilitating the establishment of the method. Moreover, Orbitrap mass analyzer possesses a wider linear dynamic range increased by 5 orders of magnitude compared to triple quadrupoles. When the Orbitrap mass spectrometer is operating in a screening mode, it is examining at the same time four criteria: exact mass, isotopes and fragment ions and retention time. Once the scan is complete, the mass spectra with isotopic pattern can be imported into a library search to identify the compound. With a high-resolution system like Orbitrap it is possible to look at multiple peaks at the same time and inspect multiple spectra related to different compounds that exist as mixture in the solution. This option is not feasible in a single quadrupole or triple quadrupole system where one peak is recognized in detection. Therefore, high resolution is of crucial importance since the identification of similar analytes with slight differentiations in their structures is possible. The main benefit of this is the ability to determine the elemental composition of individual molecular or fragment ions, creating a powerful informative tool for structural elucidation or confirmation. Moreover, using this technique the analysis and identification of compounds even at extremely low concentrations is achieved. In addition to this, an unknown peak can also undergo retrospective analysis. Once the data acquisition has been completed the development of compound libraries and databases can be used to find the analytes based on the experimental fragmentation of the compound of interest. This provides a powerful knowledge for discovering unknown compounds or metabolites existing in the sample [494,500,503]. Finally, Orbitrap mass analyzer can operate in full scan, MS/MS, SIM, or parallel reaction monitoring (PRM) modes. PRM can collect data performing a full scan of each transition by a precursor ion, in other words, parallel monitoring of all fragments from the precursor ion, unlike selected-reaction monitoring which only performs one transition at a time. The Orbitrap mass analyzer replaces the Q3 from the triple quadrupole set-up and performs scans for all product ions with high resolution and accuracy. This has as result that parallel reaction monitoring technology has the same selected-reaction monitoring quantitative analysis abilities, but at the same time it can perform qualitative analysis [503].

Table 1.16 Performance characteristics of Orbitrap mass analyzer [504]

Hybrid LTQ Orbitrap MS	
Mass Range	m/z 15-200 (for ion trap) m/z 50-2000 (for ion trap and Orbitrap) m/z 200-4000 (for ion trap and Orbitrap)
Resolving Power	60000 (Min: 7500, Max: 100000 *m/z*> 400)
Mass Accuracy	<3ppm
Sensitivity	sub-ppt
Dynamic Range	>4000 per scan
MS^n	n=1-10

1.11.5.3 Scan Modes in Mass Spectrometry

Mass spectrometric systems use various scan modes and different scan types to filter, fragment, or transmit ions into the mass analyzer. Along with the ionization and ion polarity modes, the ability to vary the scan mode and scan type provides instrumental flexibility in order to confront complex analytical tasks. The mass analyzer as mentioned in previous sections separates ions according to their mass-to-charge ratio and then passes them to the ion detection system. In order to explain the scan modes and scan types of mass spectrometric techniques, the set-up of a triple quadrupole mass analyzer of three quadrupole rod assemblies (Q1, Q2, and Q3) was recruited [504].

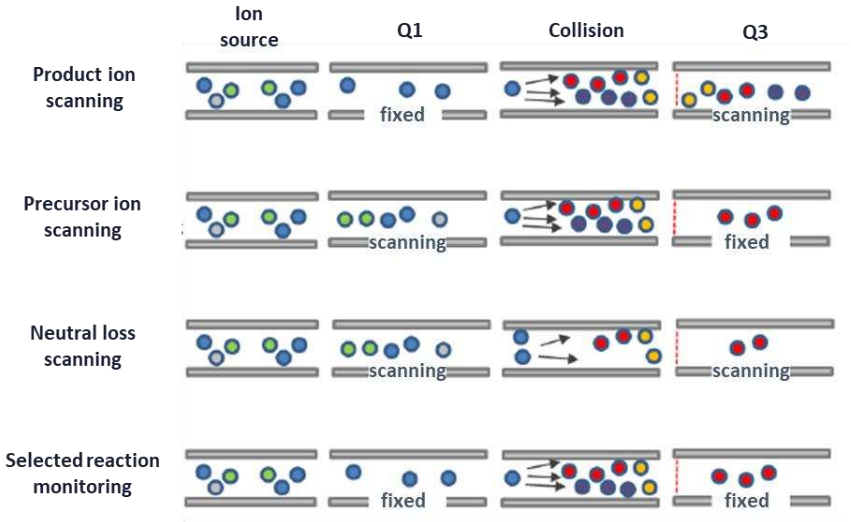


Figure 1.45 Schematic representation of scan modes and types. (a)Product ion scanning; (b) Precursor ion scanning sets the second analyzer (Q3) to transmit only one specific fragment ion to the detector; (c) Neutral loss scanning scans both analyzers in a synchronized manner, so that the mass difference of ions passing through Q1 and Q3

remains constant;(d) a series of short experiments in which one precursor ion and one specific fragment characteristic are selected by Q1 and Q3 respectively [505].

1.11.5.3.1 Product Scan Mode

Product scan mode operation is accomplished in two steps. In the first step ions formed in the ion source enter Q1, which is set to transmit ions of one m/z ratio. These ions are called parent ions and eventually Q1 is the parent mass analyzer. The m/z ratio of ions transmitted by the parent mass analyzer is referred to as the parent set mass. Parent ions selected by Q1 then enter to the collision cell where they undergo fragmentation for further generation of product ions. The product ions are produces through two processes: by unimolecular decomposition of metastable ions or by interaction with inert collision gas present in the collision cell, also known as collision-induced dissociation (CID). These ions enter Q3, which consists the product mass analyzer for the second step of mass analysis. Finally, Q3 is scanned to obtain a mass spectrum that presents the product ions generated from the fragmentation of the selected parent ion. A mass spectrum obtained in the product scan mode is the mass spectrum of a selected parent ion [506].

1.11.5.3.2 Parent Scan Mode

The parent scan mode also involves two analytical steps. Firstly, ions produced in the ion source are transferred into the parent mass analyzer, which is scanned to transmit parent ions sequentially into the collision cell. Next in the second step occurring in the collision cell, parent ions can fragment to produce product ions by unimolecular decomposition of metastable ions or by collision-induced dissociation. These ions after the collision cell are directed to the product mass analyzer, which transmits a selected product ion. In the final spectrum are presented all the parent ions which undergo to fragmentation for the generation of the selected product ions. Mass spectrum obtained in the parent scan mode, is obtained from Q1, whereas data for the ion intensity axis is obtained from Q3. Parent scan mode can be used in survey analyses of mixtures as well as in structure and fragmentation studies. Generally, parent scan mode is responsible for the detection of all compounds that decompose to a common fragment. The experiments are useful for the rapid detection of a series of structural homologs [504].

1.11.5.3.3 Neutral Loss Scan Modes

In the neutral loss scan mode, the two mass analyzers, Q1 and Q3, are linked together to perform scan events at the same rate over mass ranges of the same width. The corresponding mass ranges, however, are locked by a selected mass for the mass analyzer to scan lower than the parent mass analyzer. For this reason, the mass analysis is performed in two steps. In the first step the parent mass analyzer separates ions formed in the ion source by m/z ratio. Then the ions are introduced sequentially into the collision cell. Secondly, ions admitted to the collision cell can undergo further fragmentation by metastable ion decomposition or by CID to form product ions. These product ions are separated by mass product analyzer according to their m/z ratio. For ion detection, it has to lose a neutral moiety whose mass is equal to the difference in the mass ranges scanned by the two mass analyzers between the time the ion leaves Q1 and enters Q3. Therefore, a neutral loss mass spectrum presents all the parent ions that lose a neutral species of a selected mass. Experiments associated with neutral loss scan mode are useful in investigations with large number of compounds with common functionality. Neutral moieties are frequently lost from substituent functional groups (for example, H_2O from alcohols CO_2 from carboxylic acids) [506].

1.11.5.3.4 Selected Reaction Monitoring

Selected reaction monitoring (SRM) monitors a particular reaction or set of reactions, such as the fragmentation of an ion or the loss of a neutral moiety. SRM monitors a limited number of parent or product ion pairs. In product-type experiments, a parent ion is selected as usual, but generally only one product ion is monitored. SRM experiments are normally conducted with the product scan mode. As does SIM, SRM is suggested for fast analysis of trace compound analysis in complex mixtures. However, because SRM selects two sets of ions, it obtains specificity that is much greater than what SIM can obtain. Any possible interference would not only have to form a parent ion of the same m/z ratio but also have to fragment in order to form a product ion of the same m/z ratio as the selected product ion from the target compound [506].

1.11.6 Applications of Orbitrap mass analyzer

With approximately 6,000 peer-reviewed publications, Orbitrap mass spectrometer technology is well established as a powerful LC-MS platform. Its high-resolution, accurate-mass measurements,

along with its high dynamic range, have enhanced the analysis of complex mixtures. To date, an orbitrap mass analyzer consists one of the most powerful tools that can be applied for characterization, identification, and quantification of components in biological systems. Having as main advantage the high mass accuracy and the extremely high resolution performed at the same time within a short period of time, adjusted for nano-LC separations, the orbitrap has become an instrument of choice for many proteomics, lipidomic and metabolomic applications [507,508].

Orbitrap mass spectrometer-based HRMS analysis is ideally suited for screening and quantification of hundreds or thousands of contaminants in a single run for food safety field. Ensuring the safety of food supplies is of crucial importance for safety and public health. Screening and quantification of foodstuffs for known and unknown contaminants, such as pesticides, herbicides, antibiotics, additives, allergens in complex matrices is adopted for fast and simple data analysis. Relative studies are presented in Table 1.17.

Ultimately the goal of any environmental analysis is the quantitative determination of contaminants. Water, soil, and air are included to directives about the nominal limits of contaminants in order to protect human health. These levels are based on the degree of toxicity along with prevalence and cost of cleanup. The need for monitoring and removing toxic compounds from the environment, especially from anthropogenic sources like drinking water, also increases costs. For this reason, the quantification of exact concentration is necessary to protect human health and lower the costs of monitoring surveys. In this concept the study of non-regulated emerging contaminants is also important in order to establish possible future regulations about their determination. Ensuring the safety of drinking water consists a priority environmental issue. The intense anthropogenic activities, affect rivers, streams, lakes, and seawater and increase the demand for monitoring these resources. High resolution mass spectrometry analysis associated with Orbitrap mass analyzer is ideally suited to rapid screening and quantitation of water samples for targeted contaminants such as pesticides, herbicides, and other pharmaceuticals, industrial chemicals etc. (Table 1.17.)

Orbitrap mass spectrometers have the ability to deliver from low-ppm accurate mass, possess high dynamic range, and high resolution. These parameters are responsible for the successful quantification of both endogenous and exogenous compounds in biological substrate such as plasma, serum, whole blood, urine, cerebrospinal fluid, and oral fluid. This renders Orbitrap analyzer a powerful tool for clinical research and forensic toxicology applications where quantification of multiple target and non-target compounds is required. Accurate mass measurements help to

eliminate the risk of over- and under estimations, decrease the cases of false-positive results, and improve sensitivity by suppressing the background noise. Moreover, the ability to scan and quantify in the same analytical run, along with the option for retrospective data analysis, makes Orbitrap mass spectrometers an ideal technology approach for drug monitoring research in forensic toxicology, and sports anti-doping control applications (Table 1.17).

Current use of Orbitap MS in forensic toxicology and doping control, has gained special regard. Quantiification of drugs in body fluids is challenging due to varying concentrations, various chemical and physical properties of drugs, interferences and the presence of many homolog compounds. Further, the constant evolution of drugs and their analogs makes it harder to identify and quantify them. The performance characteristics of Orbitrap mass analyzer address excellently in forensic toxicology applications (Table 1.17)

The discovery of new successful drug medications and agricultural chemicals pass through trace level metabolite profiling in both early steps of discovery and regulatory phases. Therefore, there is a need for quantitative in vitro and in vivo analysis at each step. Orbitrap mass analyzer responds to the requirement for LC-MS assays that provide sensitivity, accuracy and ruggedness [509–511].

Table 1.17 Applications of Orbitrap Mass Analyzer

Analytes	Sample type	Ref.
Acrylamide	Biscuits	[512]
Veterinary Drugs	Honey, Veal Muscle, Egg and Milk	[513]
β-Lactams	Chicken muscles	[514]
Cephalosporins	Meat	[515]
Pesticide Residues	Fruit and vegetables	[516]
Steviol Glycosides	Commercial beverages	[517]
Perfluorinated Compounds	Drinking Water, Tap Water, River	[518]
Per- and Polyfluoroalkyl Substances (PFASs)	Drinking Water	[519]
Pharmaceuticals and PPCPs	Hospital Wastewater	[146]
Pharmaceuticals and Sweeteners	Municipal and Hospital Wastewater	[450]
Pesticides	Sediments	[520]
Cyanotoxins	Lake Water	[521]
Emerging Pollutants	River	[522]
Synthetic Dyes	Herbal Medicines	[523]
Imidacloprid And Metabolites	Tomato	[524]
Drugs and Metabolites	Blood Plasma	[525]
Substances with Anabolic Effects	Human Urine	[526]
Drugs of Abuse	Human Blood and Urine	[527]
Amphetamine-type Stimulants and Cocaine	Human Hair	[528]
Xenobiotics	Food	[529]
Antifungal residues	Honey	[530]
Phenolic Antioxidants	Chocolate	[531]
Pesticides	Tea	[532]
Antibiotics	Cosmetics	[533]
Veterinary antibiotics	livestock farming watersheds	[534]
Pesticides	aquaculture	[535]
Pesticides	bee specimens	[536]
Antibiotics	Rural streams, drinking wells	[537]
Environmental contaminants	Human blood	[538]
Psychiatrics and metabolites	Wastewater	[30]
Pharmaceuticals and Personal Care Products	Wastewater	[539]
Pharmaceuticals	Sediments	[540]
Drugs	Urine	[541]
Emerging contaminants	Freshwater invertebrates	[542]
Naphthenic acids	Groundwaters	[543]
Pharmaceuticals	Surface water	[544]
Steroidal endocrine disrupting compounds	Seawater	[545]
Drugs of abuse and metabolites	Surface water, wastewater	[546]
Emerging Contaminants	Urine and nails	[547]

B. Aim and Objectives

B. Aim and Objectives

The development of modern technologies and the underlying science that makes them possible is the foundation of human progress. However, these advancements often come with peripheral consequences that are not always immediately understood but must be managed as they emerge. The unlimited sources of production use and disposal of numerous chemicals commonly employed in medicine, industry, agriculture, food, and even common household comforts result to the widespread occurrence of pollutants. The new class of emerging contaminants, (ECs) including chemicals like pharmaceuticals, disinfectants, personal care products, food additives remain unregulated and have captured the attention of scientific committee and environmental organizations. The uncontrolled discharge of such substances into the environment, even at trace concentrations (ng/L, μg/L) contributes to the accumulation of some of them in the aquatic compartments, with potentially adverse effects to both aquatic ecosystems and human health. The main pathway in the environment for several emerging contaminants like pharmaceuticals, personal care products and artificial sweeteners is the urban or hospital wastewater treatment plants which receive these compounds through the sewage network after metabolism and excretion. Considering the global large use and the well-known partial or incomplete removal of these organic micropollutants from WWTPs they are frequently detected in effluents, surface waters and tap waters.

For this reason, the first objective of the current doctoral thesis was to select a wide range of emerging contaminants belonging to different chemical groups based on systematic literature survey with regards to their environmental fate, widespread occurrence and toxic relevance. A representative group of ECs, including antibiotics, antidepressants, NSAIDs, and artificial sweeteners was selected.

The detection of emerging contaminants in environment and particularly in complex matrix, such as wastewater, requires sensitive analytical methods in terms of sample pretreatment and chromatographic analysis, capable for trace determination (ng/L) and matrix elimination. Trying to respond to analytical challenges posed for sensitivity and accuracy in environmental analysis, a step-by step evaluation and optimization of the operational parameters (ESI parameters, AGC control, Injection Time, resolution) of Orbitrap mass analyzer was carried out, along with the relative chromatographic conditions.

A significant if not the most critical part of an analytical methodology concerns sample preparation. Although being time-consuming in the analytical workflow, sample preparation plays a key role in the improvement of the analytical parameters to enhance detectability as well as to protect the analytical equipment. However, because most of sample pretreatment techniques until now are time and solvent consuming and prone to error, the need for novel extraction technologies to overcome these drawbacks is ever-increasing.

Following the need for modern sample preparation techniques, this doctoral research aims to develop novel extraction strategies which focus on improving the efficiency of extraction, decreasing the use of solvents, reducing the time for extraction and the associated costs.

Specifically, a Magnetic Solid Phase Extraction coupled with UHPLC–LTQ/Orbitrap HRMS was developed, optimized, and validated for two aqueous matrices, wastewater effluent and tap water. Attaining that, silica-based nanoparticles coated with C18 as well as magnetic reduced graphene oxide were used as magnetic extraction sorbents. The materials were successfully synthesized and characterized.

Moreover, a Fabric Phase Sorptive Extraction (FPSE) combined with UHPLC–LTQ/Orbitrap HRMS was developed for effluent and tap water. In pursuit of this, fabric medium using as a substrate fiber glass coated with polymer of PEG was synthesized and characterized. The method was optimized and validated in both aqueous matrices.

After evaluating the developed analytical methodologies, the final objective was to investigate the concentration levels of the target Emerging Contaminants (ECs) including pharmaceutical active compounds, personal care products and artificial sweeteners in real effluent samples. To attain this, a one-year study took place at selected urban and hospital WWTPs of Greece, in order to characterize the occurrence (levels, temporal variation and correlations) of 33 multiresidue multiclass ECs in effluent samples, by means of SPE and UHPLC–LTQ/Orbitrap HRMS.

C. Experimental Part

CHAPTER 2: MATERIALS & METHODS

2.1 Standard Solutions

For the present study, a set of 29 multiclass pharmaceutical active compounds and 4 artificial sweeteners were selected. All pharmaceutical standards as well as artificial sweeteners were of high purity (≥98%) and (>95%) respectively and purity grade of the standard was considered for the preparation of standard solutions. Their physicochemical characteristics and chemical structures are listed in Table 2.1 and 2.2.

Sulfacetamide, sulfadiazine, sulfathiazole, sulfapyridine, trimethoprim, sulfamethizole, sulfamethazine, sulfamethoxy-pyridazine, sulfamethoxazole, sulfaquinoxaline, acesulfame-K, saccharin, sucralose, aspartame, erythromycin, diclofenac, oxolinic acid, indomethacin, gemfibrozil, triclosan, mefenamic acid, tolfenamic acid were purchased from Sigma-Aldrich (Athens, Greece), salicylic acid was obtained from Merck KGaA (Darmstadt, Germany), florfenicol was purchased from Dr. Ehrenstorfer company (Augsburg, Germany), olanzapine, paroxetine, risperidone, venlfafaxine, cyclobenzaprine, carbamazepine, amitriptyline, fluoxetine, clomipramine were obtained from TCI Tokyo Chemical Industry (Zwijndrecht, Belgium).

Individual stock solutions of each compound, were prepared on a weight basis solution in methanol, (at a concentration of 1000 mg/L) with exception of sulfaquinoxaline that was prepared in acetonitrile since this compound is slightly soluble in pure methanol but freely soluble in acetonitrile. Moreover, oxolinic acid was prepared at 0.1M NaOH solution [548], because of low solubility in high concentrations. Furthermore, since erythromycin is easily degraded in the aquatic environment and converted into anhydroerythromycin (ERY-H_2O) is always detected as this metabolite [549,550]. Therefore, to prepare an ERY-H_2O standard solution the pH of an erythromycin solution was adjusted to 3 with 3 M H_2SO4. The solution was stirred for 4 h at room temperature to complete conversion. The acidic solution was readjusted to pH 6 with 1M NaOH and was stored at 4 ◦C up to one week [549].

Labeled internal standards olanzapine-d_3, fluoxetine-d_5 hydrochloride, carbamazepine-d_{10}, and amitriptyline-d_6 hydrochloride were supplied from A2S (Saint Jean d'Illac, France). Individual isotopically labeled internal standard solution, were prepared in methanol, and stored at −20 °C.

Table 2.1. Structures and physicochemical characteristics of target analytes in positive ionization mode(PI)

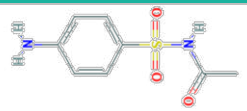
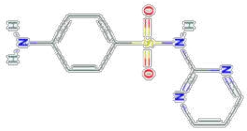
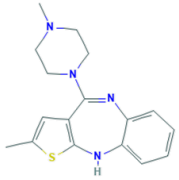
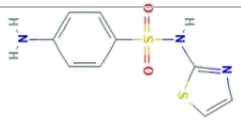
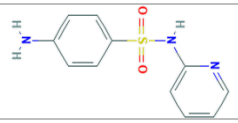
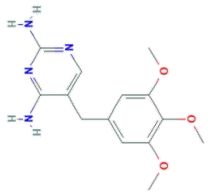
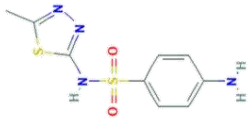
Compound	Chemical Structure	Molecular Formula	MW (g/mol)	pKa	logP	Therapeutic class
Sulfacetamide		$C_8H_{10}N_2O_3S$	214.24	4.3/2.14	-0.300	Sulfonamide Antibiotic
Sulfadiazine		$C_{10}H_{10}N_4O_2S$	250.28	6.99/2.01	0.387	Sulfonamide Antibiotic
Olanzapine		$C_{17}H_{20}N_4S$	312.44	6.24/2.14	1.009	Psychiatric- Antidepressant Drugs
Sulfathiazole		$C_9H_9N_3O_2S_2$	255.31	6.93/2.04	0.975	Sulfonamide Antibiotic
Sulfapyridine		$C_{11}H_{11}N_3O_2S$	249.29	6.24/2.14	1.009	Sulfonamide Antibiotic
Trimethoprim		$C_{14}H_{18}N_4O_3$	290.32	7.16	1.284	Sulfanilamide Antibiotic
Sulfamethizole		$C_9H_{10}N_4O_2S_2$	270.33	6.71/1.95	0.214	Sulfonamide Antibiotic

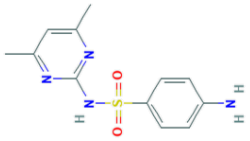
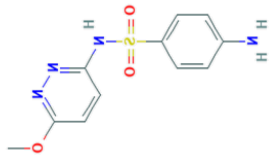
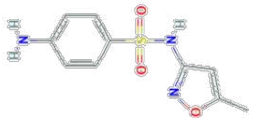
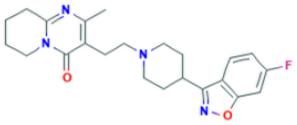
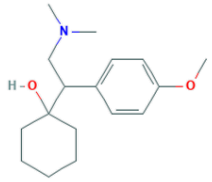
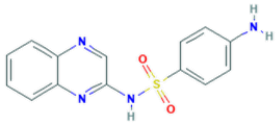
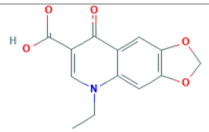
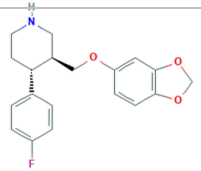
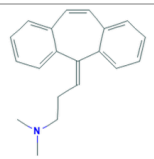
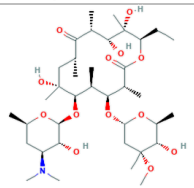
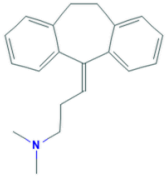
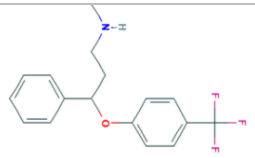
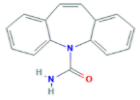
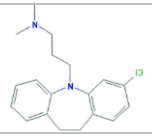
Table 2.1 Structures and physicochemical characteristics of target analytes (PI), *(continued)*						
Sulfamethazine		$C_{12}H_{14}N_4O_2S$	278.33	6.99/2.0	0.650	Sulfonamide Antibiotic
Sulfamethoxy-pyridazine		$C_{11}H_{12}N_4O_3S$	280.30	6.84/2.02	0.466	Sulfonamide Antibiotic
Sulfamethoxazole		$C_{10}H_{11}N_3O_3S$	253.28	6.16/1.97	0.791	Sulfonamide Antibiotic
Risperidone		$C_{23}H_{27}FN_4O_2$	410.49	8.76	2.628	Psychiatric -Antidepressant Drugs
Venlafaxine		$C_{17}H_{27}NO_2$	277.41	14.42/8.91	2.739	Psychiatric- Antidepressant Drugs
Sulfaquinoxaline		$C_{14}H_{12}N_4O_2S$	300.34	6.79/2.13	1.552	Sulfonamide Antibiotic
Oxolinic Acid		$C_{13}H_{11}NO_5$	261.23	5.39	1.353	Quinolone Antibiotic

Table 2.1 Structures and physicochemical characteristics of target analytes (PI), *(continued)*

Paroxetine		$C_{19}H_{20}O_3NF$	329.37	9.77	3.148	Psychiatric- Antidepressant Drugs
Cyclobenzaprine		$C_{20}H_{21}N$	275.39	9.76	4.613	Psychiatric -Antidepressant Drugs
Erythromycin		$C_{37}H_{66}NO_{12}$	733.93	12.45/9.00	2.596	Macrolide Antibiotic
Amitriptiline		$C_{20}H_{23}N$	277.41	9.76	4.810	Psychiatric- Antidepressant Drugs
Fluoxetine		$C_{17}H_{18}F_3NO$	309.33	9.8	4.173	Antidepressant Drugs
Carbamazepine		$C_{15}H_{12}N_2O$	263.27	15.96	2.766	Psychiatric -Antiepileptic Drugs
Clomipramine		$C_{19}H_{23}ClN_2$	314.86	9.2	4.883	Psychiatric -Antidepressant Drugs

Data concerning chemical structures, obtained from international database https://pubchem.ncbi.nlm.nih.gov/

Data concerning pKa and logP obtained from international database https://chemaxon.com/

Table 2.2. Structures and physicochemical characteristics of target analytes in negative ionization mode (NI)

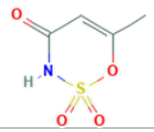
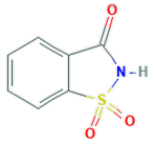
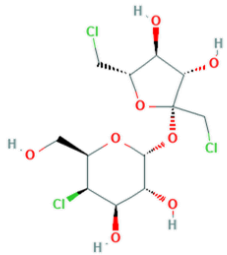
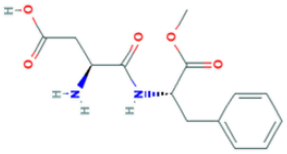
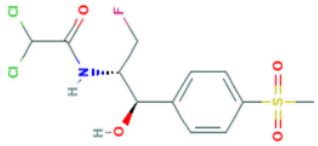
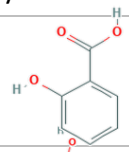
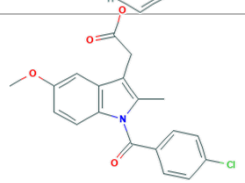
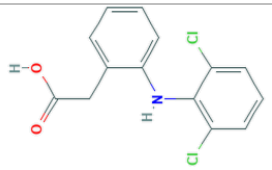
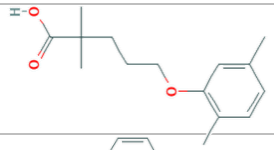
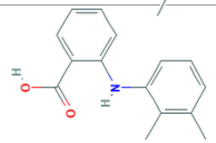
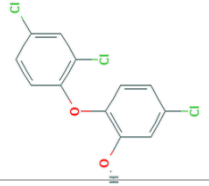
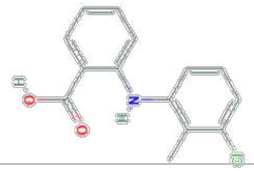
Compound	Chemical Structure	Molecular Formula	MW (g/mol)	PKa	logP	Therapeutic class
Acesulfame		$C_4H_5NO_4S$	163.15	3.02	-0.552	Artificial Sweetener
Saccharin		$C_7H_5NO_3S$	250.28	1.94	0.449	Artificial Sweetener
Sucralose		$C_{12}H_{19}Cl_3O_8$	312.44	11.91	-0.465	Artificial Sweetener
Aspartame		$C_{14}H_{18}N_2O_5$	294.30	3.53/8.53	-2.218	Artificial Sweetener
Florfenicol		$C_{12}H_{14}Cl_2FNO_4S$	358.21	8.49	0.670	Fluoroquinolone Antibiotic

Table 2.2 Structures and physicochemical characteristics of target analytes (NI), *(continued)*

Salicylic acid		$C_7H_6O_3$	138.12	2.79	1.977	Non-steroidal anti-inflammatory drug (NSAID)
Indomethacin		$C_{19}H_{16}ClNO_4$	330.40	3.8	3.530	Analgesic-anti-inflammatory drug
Diclofenac		$C_{14}H_{11}Cl_2NO_2$	296.15	4.0	4.259	Non-steroidal anti-inflammatory drug (NSAID)
Gemfibrozil		$C_{15}H_{22}O_3$	250.33	4.39/-4.8	4.42	Lipid regulator
Mefenamic Acid		$C_{15}H_{15}NO_2$	241.29	3.89/-1.58	5.398	Non-steroidal anti-inflammatory drug (NSAID)
Triclosan		$C_{12}H_7Cl_3O_2$	284.54	7.68	4.982	Disinfectant
Tolfenamic Acid		$C_{14}H_{12}ClNO_2$	261.71	3.88	5.488	Non-steroidal anti-inflammatory drug (NSAID)

Data concerning chemical structures, obtained from international database https://pubchem.ncbi.nlm.nih.gov/

Data concerning pKa and logP obtained from international database https://chemaxon.com/

2.1.1 Stability of analytes in a standard solution

Often, low recoveries in the analysis, lack of linearity and/or large RSDs are due to losses and low stability of analytes in the aqueous solutions and in organic solvents (i.e. extracts, standard solutions). Preparation of fresh stock and working standard solutions is time-consuming (and expensive), especially for multi-residue methods involving several tens or more than 100 analytes. Therefore, it is common practice to prepare standard stock solutions at the beginning of the study and store them refrigerated or frozen before use. Stock solutions in methanol, acetonitrile, and aqueous solution in case of oxolinic acid, and erythromycin, were analyzed after storage for one month, three months, six months, and one year in a refrigerator, and in a freezer. Some of the compounds investigated, such as oxolinic acid, erythromycin, olanzapine, paroxetine, florfenicol, proved to be unstable after three months of storage in the fridge at 4 °C showing 42%, 27%, 19 % and 32% deviation from the target value according to the equation Eq.1:

$$\frac{(Area\ new - Area\ old)}{Area\ old} \times 100\% \qquad \text{(Eq. 1)}$$

, whereas lower deviations <20% were observed for the same compounds in case of refrigerator storage (-20 °C). After the period of 6 months deviations of <18% were observed for sulfonamides stored at 4°C, whereas at -20°C no degradation occurred. After one year of storage at 4°C sulfonamides, proved to be unstable showing deviation of almost 50% and <20% at -20 °C, while the other compounds show deviations lower than 23 and 12 % at 4°C and -20°C, respectively.

For this reason, all stock standard solutions were stored at −20 ◦ C and refreshed every six months apart from antibiotics oxolinic acid, erythromycin, olanzapine and paroxetine and florfenicol that were prepared monthly because of their limited stability [551]. The mix of working solution containing all target compounds was prepared in methanol: ultrapure water (10:90, v/v) with 0.1% f. a v/v by diluting appropriate volume of stock solution. Working solutions were prepared before each analytical run.

2.1.2 Stability of analytes in matrix

Analysis of the samples was performed immediately in this study when possible, since the shorter the time that elapses between collection of a sample and its analysis, the more reliable will be the analytical results. However, this was often difficult or impossible to realize since sampling campaigns

covered areas that did not belong in the same region. Moreover, the samples have to be transported to the laboratory and analyzed, but often due to logistical constraints such as time of pretreatment, availability of SPE device or Orbitrap MS, the actual processing of the samples starts several days after its recollection. During that period and depending on the matrix, a different degree of alteration may occur, especially in wastewater samples [99]. Therefore, according to literature, if the analysis cannot be performed immediately, wastewater should be preserved by adding a stabilizing agent such as sodium azide [552,553] or by acidification to extremely low values of pH [554]. However, taking into consideration that acidification might influence partitioning of certain compounds between aqueous and particulate phase and the other preservatives can interfere with some of the analytes, freezing of water samples at -20 ^{o}C was selected instead. Low temperatures such as -20 ^{o}C proved to be safe for at least 1 month for all target compounds [99] . In addition, as an alternative, immediate preconcentration on SPE cartridges, is generally accepted as the best option. In fact, according to Carlson et al., storing the SPE cartridges frozen at -20 ^{o}C, no significant recovery losses were occurred over 20 months [555].

2.1.3 Stability of analytes under evaporation

Evaporation stage could be a critical step, especially for the most volatile compounds which may be degraded. In this work, the following different evaporation conditions have been used for the evaporation of analytes of interest: evaporation to dryness under a gentle stream of nitrogen, evaporation to dryness under nitrogen stream at 35◦C, and partial evaporation under nitrogen stream. To investigate recoveries of compounds after evaporation, 5 mL of a standard solution containing all compounds in MeOH was evaporated under the three mentioned conditions and reconstituted to 90:10 H_2O/MeOH. In the first two cases, regarding evaporation to dryness, reconstitution was performed with 1mL solvent, while in the last case, evaporation stopped when approximately 100μl of solution remained in the tube and then adjusted to 1 mL with water. Areas of target molecules were compared to areas obtained for a non-evaporated solution. Results showed that all compounds had the same overall behavior and resisted well to evaporation since recoveries obtained were above 85% for 25 out of 33 target compounds. A higher loss was noticed for antibiotics erythromycin, oxolinic acid, regardless the evaporation technique, nevertheless the recovery loss did not exceed 30 %. As there were no significant differences among these different techniques,

evaporation to dryness under gentle stream of nitrogen at 35°C was chosen, to concentrate the analytes in the shortest time possible.

2.2 Solvents, reagents, and sorbent materials

Methanol (MeOH), acetonitrile (AcN), formic acid (f.a) (all MS grade), were purchased from Fisher Scientific (Leicester, UK). Ultrapure water (resistivity of 18.2 MΩ-cm) was obtained by using an Evoqua purification system (Evoqua, Pittsburg, USA). Ethylenediaminetetraacetic acid disodium salt 2-hydrate (Na_2EDTA) (assay 99.9–101.0%) was obtained from Panreac (Barcelona, Spain), sodium chloride (NaCl), and ammonium hydroxide (NH_4OH) from Riedel de Haën (Hannover, Germany). The starting material used to be coated as FPSE media were Whatman microfiber glass filters 110 mm (Boston, Massachusetts, USA). Organic polymer polyethylene glycol (PEG 300) was purchased from Sigma-Aldrich (Athens, Greece). Trimethoxymethylsilane (MTMS), trifluoroacetic acid (TFA) and sodium hydroxide(NaOH) and hydrochloric acid (HCl, 37%) were supplied from Merck (Darmstadt, Germany).

For the synthesis of magnetic nanoparticles, Ferric chloride ($FeCl_3$) was purchased from Fluka (Milwaukee, WI, USA) and ferrous chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$) from Fischer Scientific (Berlin, Germany). Tetraethyl orthosilicate (TEOS) and trimethoxy(octadecyl)silane (99%) were purchased from Sigma-Aldrich. Sulfuric acid (96%), phosphoric acid (85%), potassium permanganate (99%), hydrogen peroxide (30% w/w), hydrochloric acid (37%), ethanol (99%), sodium nitrate (99%), were provided from Sigma-Aldrich (Athens, Greece). Hydrazinium hydroxide (~ 100% for synthesis) was purchased from Merck (Merck, Darmstadt, Germany). Natural graphite (99.99% purity, 20 μm, from Merck) was used as precursor of graphene oxide (GO).

The cartridges used for solid phase extraction were Oasis HLB (200 mg, 6 mL) and Oasis MCX (150 mg, 6 mL), both from Waters Corporation (Milford, MA, U.S.A.), while CNW bond HC-C18(200 mg, 3mL) from CNW Technologies (Duesseldorf, Germany). Glass fiber filters (110mm, 0.7μm) were purchased from Whatman. PTFE membrane filters (0.45μm) were purchased from Kinesis (U.K.).

2.3 Determination of physicochemical parameters

Physicochemical parameters of the effluent samples during the sampling campaigns have been determined by applying standard methods, as follows: The temperature, salinity, conductivity and total dissolved solids (TDS) were measured by a WTW LF 3215 conductivity meter with TetraCon 325

Probe (WTW, Weilheim, Germany). The pH was directly measured using a Consort C932 analyzer (Constort NT, Turnhout, Belgium) with a HI-1230 pH electrode (Hanna Instruments, Woonsocket, RI, USA). Five-day biochemical oxygen demand (BOD_5) was measured with a WTW OxiTop OC 110 system and a WTW TS 606-G/2-i thermostat cabinet (WTW, Weilheim, Germany). The chemical oxygen demand (COD), total Nitrogen concentration and total phosphorous concentration were measured by a WTW Thermoreactor 3200 and a WTW pHotoFlex portable photometer, by following the corresponding set test for each application (WTW, Weilheim, Germany). The methods in which commercial set test were based, are described below.

2.3.1 Chemical Oxygen Demand (COD)

The chemical oxygen demand (COD) is a measure of water and wastewater quality. The COD test is often used to monitor water treatment plant efficiency. The method used in this work is based on the fact that a strong oxidizing agent, under acidic conditions, can fully oxidize almost any organic compound to carbon dioxide. The COD is the amount of oxygen consumed to chemically oxidize organic water contaminants to inorganic end products.

Specifically, the wastewater sample is oxidized with a hot sulfuric solution of potassium dichromate, with silver sulfate as the catalyst. Chloride is masked with mercury sulfate. The concentration of unconsumed yellow $Cr_2O_7^{2-}$ ions is then determined photometrically. The COD expresses the amount of oxygen originating from potassium dichromate that reacts with the oxidizable substances contained in 1 L of water under the working conditions of the specified procedure. 1 mol $K_2Cr_2O_7$ is equivalent to 1.5 mol O_2.Results are expressed as mg/L COD (= mg/L O_2). COD determination with cell tests is performed at 148 °C for 120 min [556].

2.3.2 Total Phosphorous

Phosphorus compounds – in particular ortho-phosphate PO_4^{3-}– are considered to be the limiting nutrients in most stagnant and flowing waters. An increase in their concentration caused by higher input (wastewater, avulsion etc.) results directly in increasing eutrophication of the water with known effects such as increased growth of algae, oxygen depletion as far as anoxia in the deeper regions, etc.

Phosphorus occurs in three compounds in natural waters:

- Inorganic, dissolved ortho-phosphate
- Dissolved organic phosphorus compounds
- Particulate phosphorus (bound in biomass or attached to particles)

They add up to the total of phosphorus content P_{Total}, an important parameter in monitoring wastewater treatment plant effluents [557].

The method is intended for the determination of orthophosphoric and total phosphorus. To determine total phosphorous, a digestion step at 120 °C for 30 min is necessary. In a solution acidified with sulfuric acid, ortho-phosphate ions react with (Ammonium) molybdate ions to form molybdophosphoric acid. Ascorbic acid reduces this to phosphomolybdenum blue (PMB) which is then determined photometrically at or near 690 nm [558].

2.3.3 Total Nitrogen

Wastewaters generally contain various physical forms of nitrogen. The total nitrogen household is determined by measuring the parameters ammonium, nitrite, and nitrate nitrogen. The measurement of organically bound nitrogen requires the additional decomposition of the sample according to Kjeldahl [559,560]

The content of total inorganic nitrogen can be rationally transformed into nitrate and measured after decomposition according to the Koroleff method in the thermoreactor with alkaline peroxodisulfate solution. In concentrated sulfuric acid, this nitrate reacts with a benzoic acid derivative to form a red nitro compound that is determined photometrically [560].

2.3.4 Biochemical Oxygen demand (BOD)

Biochemical oxygen demand, or BOD, is a chemical procedure for determining the amount of dissolved oxygen required by aerobic biological organisms in a body of water to degrade organic matter. The respirometric method for determining the BOD is the classic self-monitoring method for BOD determination. The main application of respirometric BOD determination lies in the analysis of wastewater in WWTPs. Respirometric measurement in a bottle corresponds to the processes in a wastewater treatment plant, but on a greatly reduced scale. For the classification and evaluation of the degradation performance of a wastewater treatment plant it is customary to specify the BOD_5. In this case, the analysis time is 5 days. During this time, the measurement solution must be incubated

at 20°C, i.e. the sample bottle is thermostatted to (20 ± 1)°C in an incubator for the entire measurement duration. Nitrification inhibitor is added into the sample since the so-called nitrificants (typically Nitrosomonas and Nitrobacter bacteria) also consume oxygen in the conversion of ammonium to nitrite and then to nitrate. This consumption is not included in the BOD_5 value. In addition, sodium hydroxide tablets are inserted in the neck of the bottles before incubation [561,562].

The microorganisms draw oxygen to degrade organic substances from the amount of air remaining in the closed system. The carbon dioxide formed by this process is absorbed. Due to the reduction in the amount of oxygen, the pressure in the bottle sinks. This change is detected and stored by the measuring head. A mol of oxygen, i.e. 6.022·1023 molecules, has a volume of 22.4 liters. A mol of carbon dioxide, also with 6.022·1023 molecules, has a volume of 22.4 liters, too. If the oxygen is now converted to carbon dioxide by respiration, there is no direct change in pressure. At this point, the role of the sodium hydroxide in the neck of the bottle is important. Sodium hydroxide and carbon dioxide react chemically to form sodium carbonate : **$2\ NaOH + CO_2\uparrow \rightarrow Na_2CO_3\downarrow + H_2O$.**

This causes the carbon dioxide that was formed to be removed from the gas phase and results in a measurable negative pressure due to the respiration of oxygen. The measured negative pressure is converted into the BOD value [561].

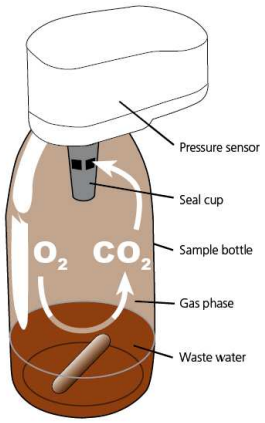


Figure 2.1. BOD_5 measurement

2.4 Characterization of materials -Instrumentation

The surface morphologies, properties and structures of graphene, graphene oxide nanosheets and their magnetic analogs were characterized by scanning electron microscopy (SEM) (JSM-7001F, Japan). The corresponding SEM images of C18- magnetic nanoparticles were obtained by a JOEL

microscope (JSM-5600, JEOL, Tokyo, Japan) after gold coating. Fourier transform infrared spectroscopy (FT-IR) measurements were obtained to identify the functionalities of the sorbents using an attenuated total reflectance accessory on a Perkin Elmer Spectrum Two FT-IR (Perkin Elmer Inc., MA, USA). The X-ray powder diffraction (XRD) measurements were carried out on a Bruker D8-advance X-ray diffractometer at 40 kV and 40 mA using Cu Kα (λ = 1.5406 Å) radiation and the sample was scanned from 5° to 60°, in steps of 0.02° (2ϑ), at a rate of 2 s per step. The magnetic properties of the respective nanomaterials were examined on a vibrating magnetometer (LakeShore 7300, Westerville, OH, USA), at room temperature.

2.5 UHPLC–LTQ Orbitrap MS Chromatographic Conditions

Chromatographic conditions were evaluated for positive (PI) and negative (NI) ionization. Chromatographic separation was conducted using an Accela UHPLC system (Thermo Fisher Scientific, Bremen, Germany) consisting of an Accela autosampler (model 2.1.1) and an Accela quaternary gradient UHPLC pump (model 1.05.0900). Separation of target analytes was carried out on a reversed-phase Hypersil Gold C18 analytical column (100 mm, 2.1 mm, 1.9 µm), purchased from Thermo Scientific, and maintained at 35 ◦C. The mobile phase consisted of water (A) and methanol (B), both containing 0.1% v/v formic acid.

The gradient program concerning positive ionization (PI) started at 95% mobile phase A and was maintained for 1 min; the next minute the amount of mobile phase B increased to 70% followed by an increase to 100 % within 3 min, where it stayed stable for additional 2 min. Afterwards, the mobile phase was restored to the initial conditions of 95% A and maintained over 3 min for re-equilibration of the column. The total running time was 10 min with a flow rate of 250µL min^{-1} and injection volume set at 10 µL. A gradient program with slight modifications was used for the separation of compounds ionized in negative mode: 90% of mobile phase (A) was used from 0- 0.5min, followed by consecutive linear declines to 30% (A) from 0.5 to 2.0 min, to 10% (A) from 2.0 to3.0 and 5% (A) from 3.0-3.9. In the 4.5 min of total run the percentage of methanol (B) increased to 100% and this composition was maintained for half a minute. Finally, the column was re-equilibrated with 90% (A) from 5.1 to 8.0 minutes. The mobile phase was delivered at the flow rate of 200 µL min^{-1} in a 35°C of thermostatted column. 20 µL aliquot of sample was injected. Water-Methanol (30:70, v/v) mixture was employed as the solvent system for washing the sample loop and injector's needle.

The LC system was coupled to a hybrid LTQ Orbitrap XL Fourier transform mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). The linear ion trap (LTQ) part of the hybrid MS system was equipped with an Ion Max electrospray ionization probe, operating in the positive and negative ionization mode. The qualification and quantification analyses were performed in full scan accurate mass spectra at high resolution as profile data mode in two separate runs for negative and positive ionization. For PI mode the following ionization parameters were applied: tube lens voltage, 90 V, ; spray voltage, 4.0 kV; capillary temperature, 320^{o}C; capillary voltage, 50V;flow rates for the sheath (N_2) and auxiliary (N_2) gas, 35 arbitrary units (au) and 10 (au) respectively. In full-scan MS mode the following parameters were used: resolution was set at 60,000; mass range, 120–1000; automatic gain control target (AGC), 5x10^{05}, .and the maximum injection time (IT) was set to 100ms, and the number of microscans to be performed was set at 1 scan s^{-1}

In negative ionization mode (NI) the following operational parameters were used: tube lens voltage, -90 V, ; spray voltage, 2.7 kV; capillary temperature, 320^{o}C; capillary voltage, -30V;flow rates for the sheath (N_2) and auxiliary (N_2) gas, 10 arbitrary units (au) and 7 (au) respectively. In full-scan MS mode resolution was set at 60,000 and the m/z scan range was 120–600; automatic gain control target (AGC) was set at target value of 4x10^{04,} and maximum injection time (IT) at 80ms. The number of microscans to be performed was set at 1 scan s^{-1}.

In data-dependent MS/MS mode, the precursor quadrupole isolation window was set to 1 m/z, the default charge state was set to 1 and -1 for PI and NI, respectively. The resolution was lower (15,000) both in the positive and negative modes. The ion fragmentation technique used was collision-induced dissociation with normalized collision energies (NCE) specified in the inclusion list of the software. The NCE energies were optimized for each target compound by injecting the working mix standard solution at a concentration of 10 µg L^{-1}.

Furthermore, the MS/MS scans were applied by targeting the automatic gain control (AGC) at 2 x 10^5 and 2 x10^4 ions for PI and NI, respectively, while maximum injection time (IT) was set at 50 ms for both polarity modes. The mass tolerance window was set to 5 ppm.

For external mass and quadrupole calibration, a mixture of peptide MRFA, caffeine, and Ultramark 1621 was used for PI mode, while for NI a mixture containing SDS, sodium taurocholate and Ultramark 1621 has been used every three days. The total instrument control and data processing was done with Xcalibur 2.1 (Thermo Electron, San Jose, CA, USA)

2.6 Magnetic Solid Phase Extraction- MSPE

2.6.1 MSPE-Fe_3O_4@GO

2.6.1.1 Synthesis of Graphene Oxide (GO)

In typical procedure, graphene oxide (GO) was produced from pure graphite powder according to the modified method reported by Hummers and Offerman [411,563]. Briefly, 1.0 g of graphite and 0.5 g $NaNO_3$ were added into 23 mL of 0°C concentrated H_2SO_4. Next, 3 g of KMnO4 was added gradually with continuous stirring and cooling, while at the same time the temperature of the mixture was maintained below 20 °C. Then, the ice-bath was removed, and the obtained mixture was stirred at 25°C for 30 min. After that time, 46 mL of distilled water was added slowly to reach temperature of 98 °C and the mixture was maintained at that temperature for 15 min. The resultant reaction was terminated with the addition of 140 mL of distilled water followed by 10mL of 30 % H_2O_2 aqueous solution. The GO was collected by centrifugation. The solid materials were washed consecutively with water and ethanol. The resultant materials were dried under vacuum overnight at 25 °C to obtain GO [405].

2.6.1.2 Synthesis of magnetic Graphene Oxide (mGO)

Synthesis of magnetic Graphene oxide was based on a previous study of Chatzimitakos et al.[564]. Magnetic graphene oxide was prepared by co-precipitation of Fe^{2+} and Fe^{3+} in the presence of GO in alkaline solution according to the following procedure [564]. 0.5 g of GO were exfoliated to 100 mL of ultrapure water with ultrasonication for 1 h to obtain a dispersion solution. The dispersion was heated at 80 °C and degassed under nitrogen flow, for 10 min. Then, 20 mL of ultrapure water, containing 0.7 g of ferric chloride ($FeCl_3$) and 0.42 g of ferrous chloride ($FeCl_2$4 H_2O) was added slowly to the above dispersion, and the mixture was stirred vigorously under continuous nitrogen flow. Next, 6 mL of concentrated ammonium hydroxide solution of 25% v/v was added instantly, and the mixture was further stirred for 30 min. The mixture was let to cool down in room temperature. Then, the magnetic GO was isolated using a neodymium magnet and washed three times with ultrapure and three times with ethanol. The nanomaterial was dried in an oven at 70 °C, overnight, ground to a fine powder with the aid of a mortar and stored at room temperature [405,564].

2.6.1.3 Synthesis of magnetic reduced Graphene Oxide (mrGO)

In order to obtain Fe_3O_4@rGO (mrGO) a typical procedure with direct addition of a reducing agent was followed [564] . 50 mg of synthesized mGO t was dispersed in 20 mL ultrapure water using ultrasonication. Afterwards, 0.25 mL of hydrazinium hydroxide (N_2H_5OH) was added to the dispersion (final concentration: 0.1 mol L^{-1}) with constant stirring to result in a black solution. Vigorous stirring of the dispersion was continued for 24 h at 70 °C, under reflux. Finally, the precipitate was isolated using a neodymium magnet (Nd–Fe–B), washed several times with deionized water followed by three times with ethanol and was dried under vacuum at 60 °C for 12 h [565].

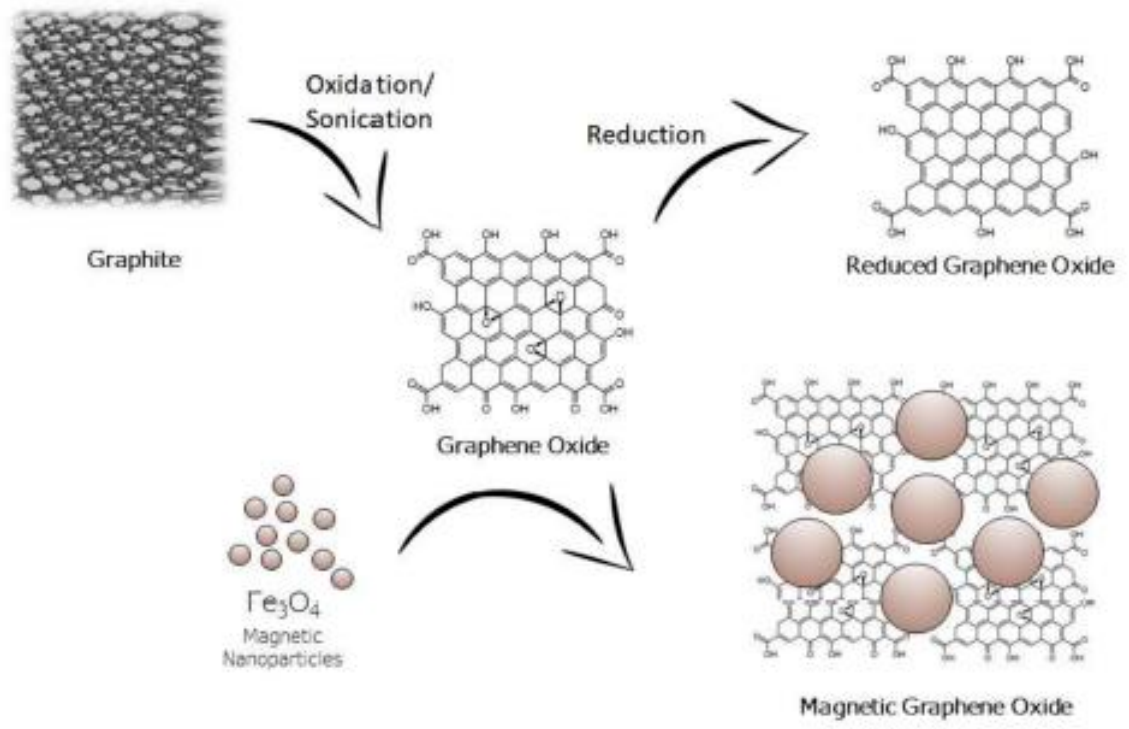


Figure 2.2. Structure of graphite, graphene oxide, reduced graphene oxide, and magnetic graphene oxide [425]

2.6.1.4 MSPE-mrGO- Experimental procedure

Firstly, 15mg of the Fe_3O_4@GO was rinsed and activated in 5mL of methanol, and then dispersed in 50 mL fortified/nonfortified aqueous sample whose pH had been adjusted to 3.0-3.5 with 1 M HCl. The mixture was stirred for 15 min to accomplish the extraction of target analytes. Subsequently, an Nd–Fe–B strong magnet was deposited at the bottom of the beaker to hold the magnetic graphene composites which had already extracted the analytes. After about 5min, the solution became clear

and the supernatant was discarded, while the target analytes were desorbed in two replicates from the Fe_3O_4@GO MNPs with 2.0 mL methanol containing 1% (v/v) ammonia by sonication for 1.0 min. Afterwards the magnet was placed again to the outside bottom of the beaker and the desorption solution was collected using a micropipette. The collected desorption solution was evaporated to dryness under a gentle N_2 flow at 30◦C and reconstituted to 250μL with initial mobile phase.

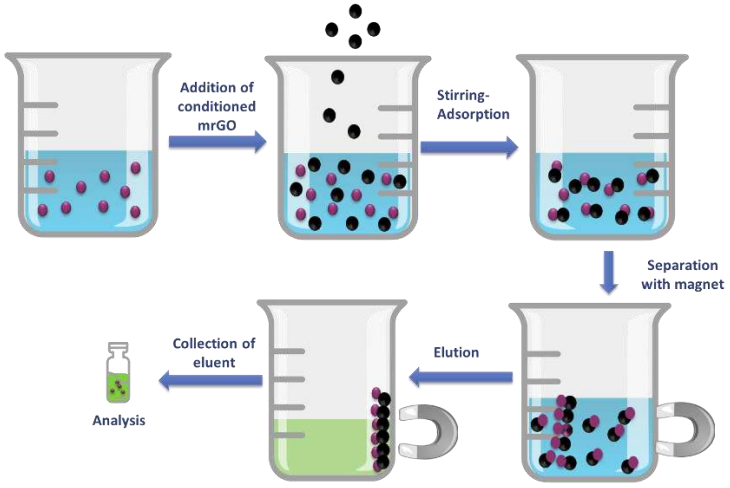


Figure 2.3 Schematic procedure of MSPE-mrGO

2.6.2 MSPE-Fe_3O_4@SiO_2@C18

2.6.2.1 Synthesis of Fe_3O_4 nanoparticles

The synthesis of magnetic nanoparticles of Fe_3O_4 was achieved by co-precipitation of Fe^{2+} and Fe^{3+} ions, at alkaline conditions and under hydrothermal treatment [94,348,566]. Specifically, 0.7 g of $FeCl_3$ and 0.42 g of $FeCl_2{\cdot}4H_2O$ were dissolved in 100 mL deionized water, prior degassed with N_2 to prevent the oxidation of Fe^{2+} ions. After 10 min of intense bubbling, 5 mL of 25% (w/w) NH_3 were added and the mixture was held at 80 ◦C for 30 min, with vigorous magnetic stirring (5000 rpm) under inert atmosphere of nitrogen. After cooling down to room temperature, the black iron oxide nanoparticles were washed several times with deionized water and ethanol.

2.6.2.2 Fabrication of silica gel-modified magnetic nanocomposites Fe_3O_4@SiO_2

Stöber process with minor modifications was followed for the preparation of Fe_3O_4@SiO_2 nanoparticles [348], [567–569]. First, 0.5 g Fe_3O_4 NPs were dispersed in a mixture containing 12.5 mL ethanol and 4 mL deionized water with the aid of ultrasonication, for 5 min. Then, 250 μL

tetraethoxysilane (TEOS) and 500 µL ammonia 25% (w/w) were added dropwise under vigorous stirring (5000 rpm) in a period of 10 min. The reaction was processed for 12 h. Finally, the nanoparticles were washed several times with ethanol and dried under vacuum for 24 h thus obtaining a gray–black powder of Fe_3O_4@SiO_2 nanocomposite.

2.6.2.3 Preparation of octadecylsilane nanoparticles (Fe_3O_4@SiO_2@C18)

The C18 was selected as the bonded phase, due to its lipophilic character that enables retention of lipophilic species while high recoveries can be obtained minimizing the presence of interferences. The C18 alkyl chain was efficiently bonded to the surface of magnetite silica nanoparticles through the Si-OH active sites [570]. Initially, 0.2 g silica-coated NPs were dispersed in 70 mL toluene with the aid of ultrasonication. Next, 200 µL of trimethoxy(octadecyl) silane were added under vigorous stirring (5000 rpm). The mixture was then refluxed for 12 h, at 80 ◦C. After cooling down to room temperature, the black product (Fe_3O_4@SiO_2@C18) was washed with toluene and ethanol several times and dried under vacuum at room temperature, before use.

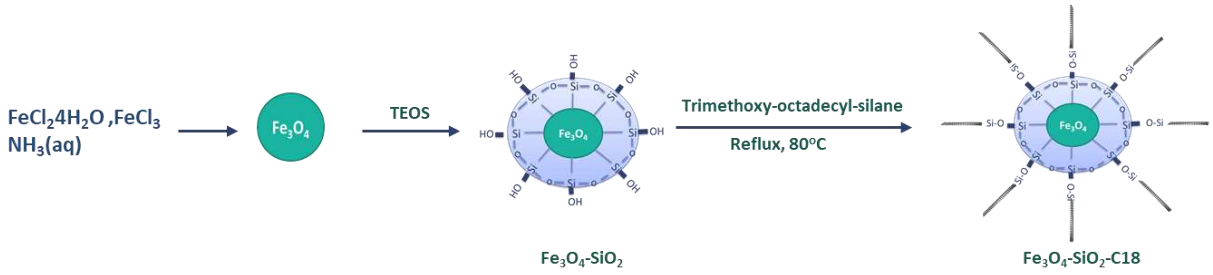


Figure 2.4. Schematic process of C18-MNPs synthesis

2.6.2.4 MSPE-C18

The MSPE procedure with the use of C18 MNPs was carried out as follows: 10 mg of C18 nanocomposites was introduced into a beaker and activated with 5 ml of MEOH. Then, 50 mL of standard solution or water sample was added. The mixture was stirred for 20 min to reach adsorption equilibrium, and once the extraction was complete, the target analytes adsorbed onto the magnetic nanocomposites were separated from the liquid phase with a neodymium magnet placed at the bottom of the beaker. Next, the supernatant was poured out and finally, the adsorbed analytes were eluted and desorbed from the sorbent by a 1 min sonication while applying 1 mL methanol containing 1% (v/v) formic acid over two cycles (2 × 1 mL). Then, placing again the magnet, the eluate was

obtained and transferred to a vial to be dried with a stream of nitrogen and reconstituted with 250µL with initial mobile phase.

2.7 Fabric Phase Sorptive Extraction

Fabric phase Sorptive extraction (FPSE), [439] is a novel, sensitive, efficient, and solvent minimized sample preparation technique that integrates the advantages of advanced material properties of sol–gel derived microextraction sorbents and high surface chemistry of natural or synthetic fabrics [571]. Sol-gel coating technology involves several steps:

(1) Surface pretreatment of the substrate

(2) Design and preparation of the sol solution

(3) Sol-gel coating process

(4) Conditioning the sorbent

2.7.1 Pretreatment of Fabric Substrates

Before the process of sol-gel coating, a systematic cleaning protocol of the commercial glass fiber used as FPSE media was applied for a twofold purpose. First to remove remaining finishing chemicals, dust or other contaminants that accumulate over the time on fabric surface. These physically adsorbed residuals may block the direct access of the sol solution to form homogenous coating on the media. Second, to activate the fabric media by increasing the available functional hydroxyl groups of the surface providing in this way more activation sites for the sol–gel inorganic–organic network to react. Hence a successful sol–gel sorbent loading is accomplished during the polymer sorbent coating phase. To address this issue, a 95 cm^2 (110 mm diameter) circular piece of commercial Whatman fiber glass filter was immersed in deionized water for 15 min, under constant sonication. The fabric was then cleaned with an abundant amount of deionized water and then was treated with 1M solution of NaOH treatment or 1h, under sonication. The fabric was then washed several times with deionized water, followed by treating with 0.1 M HCl solution for 1 h under sonication to neutralize any residual NaOH. The sequential base and acid-treated fabric was then thoroughly washed with excess of deionized water and finally was left overnight to dry in ambient air at room temperature. The dried fabric media was stored in a clean airtight glass container until it is coated with sol–gel sorbent.

2.7.2 Preparation of the Sol Solution for Coating on the FPSE substrate

Sol–gel solutions consist of a solvent system, a sol–gel precursor, an organic polymer, and an aqua solution of the catalyst. In this study, PEG polymer solution was developed using methyltrimethoxysilane (MTMS) as the sol–gel precursor. MTMS has been used in the sol–gel coating technology [451] in order to minimize cracking and shrinking of sol–gel coating. Thanks to its methyl pendant group attributes to the overall selectivity of the FPSE media and exerts London dispersion type intermolecular interaction towards the target compounds [572]. Trifluoroacetic acid (TFA) containing 5% water was used as catalyst, and a combination of acetone and dichloromethane (50:50 v/v) as the organic solvent. The molar ratio between polymer: precursor: organic solvent: catalyst was 0.5:0.2:1.0:0.5, respectively. Specifically, according to the protocol adopted [573], [442], 5 g of PEG 300 was dissolved in 5 mL of MTMS .The mixture was then vortexed for 1 min. Following this, 10 mL of the mixture of organic solvents acetone: dichloromethane in ratio 50:50 v/v (solvent) was added, and then was vortexed again for 1 min. After that, 2 mL of TFA catalyst was added. MTMS is hydrolyzed by the addition of the catalyst, and a network of the hydrolyzed MTMS molecules is created through the polycondensation. While the sol–gel network is growing, the PEG polymer incorporates randomly into it.

The entire mixture was then vortexed for 5 min to ensure the formation of a homogeneous sol solution and then centrifuged for 5 min at 5000 rpm to remove particulates from it. Finally, the transparent sol solution supernatant was moved to another clean flask for further application to the surface for creation of sol-gel coating.

2.7.3 Sol–gel PEG Coating Process

The coating procedure is integrated by immersing gently the treated fabric into the flask containing the sol solution, resulting to the formation of three-dimensional network of sol–gel PEG on the surface of the substrate as well as throughout the porous matrix. The fabric was kept inside the sol solution for 4 h. After completing the coating time, the fabric is removed from the sol solution, dried, and placed in a desiccator overnight for solvent evaporation. The objective of this step is to complete the condensation reaction and remove solvents and unreacted residuals from the sol–gel matrix, ensuring a clean, surface bonded sol–gel sorbent free of structural deformation and internal stress [445]. Next, as final step the coated FPSE media is rinsed with a mix of organic solvents (acetone:

dichloromethane, 50:50 v/v) under constant sonication for 30 min to remove unreacted and unbonded residual coating ingredients from the surface. The sol–gel-coated FPSE media is cut into circular disks of 1-cm diameter to provide identical surface area ensuring better reproducibility. The fabric coated disks were stored in closed glass container to prevent unwanted analytes from the atmosphere to be accumulated onto the surface. The successful synthesis of fabric media of fiber glass substrate coated with PEG300 polymer (FG@PEG) is confirmed with SEM, FT-IR techniques. Characterization of the material is described in detail in Section 4.3.

The creation of the sol–gel PEG coating on the fiber glass substrate involved a number of reactions including: (1) controlled catalytic hydrolysis of the sol– gel precursor, MTMS; (2) polycondensation of hydrolyzed MTMS, resulting in a growing three-dimensional inorganic silica network; (3) random incorporation of sol–gel active polyethylene glycol polymer into the evolving sol–gel network; and (4) chemical anchorage of the growing sol–gel network via condensation to the flexible cellulose substrate[574]. A tentative reaction scheme for the creation of the sol–gel PEG coating on the substrate is shown in Fig.2.5.

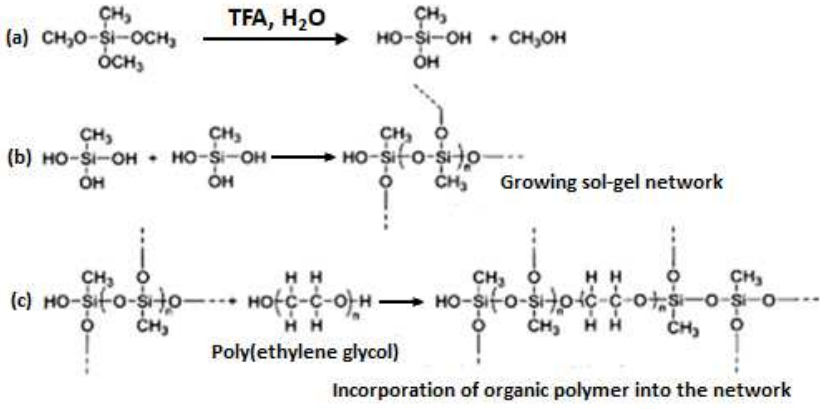


Figure 2.5. Chemical reactions involved in the synthesis of sol–gel short-chain PEG sorbent [574]

During polycondensation, the growing sol–gel PEG network reacts with the available surface hydroxyl groups of the cellulose microfibrils, resulting in an ultra-thin film of covalently bonded sol–gel PEG coating, uniformly distributed throughout the substrate with a characteristically high solvent and chemical stability, as well as highly accessible active sites for efficient and fast analyte extraction [574]. A schematic representation of sol–gel PEG coated FPSE media is shown in Fig. 2.6

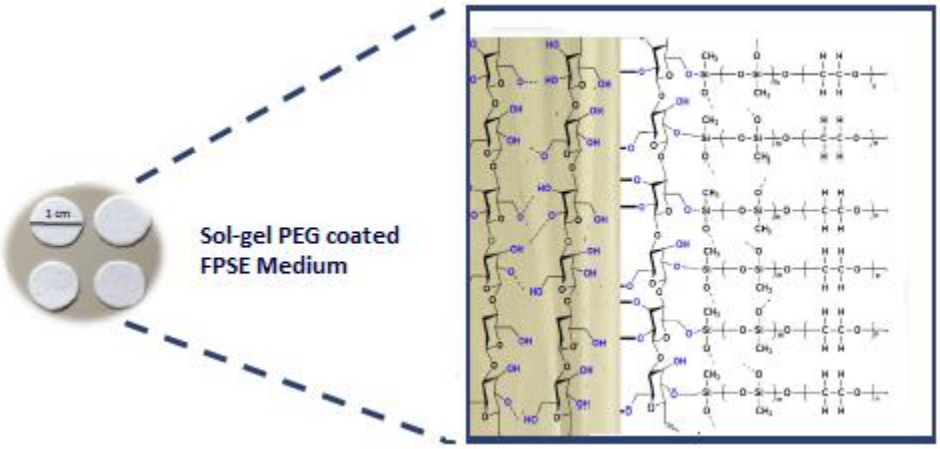


Figure 2.6 Schematic representation of sol–gel short-chain PEG coated Fabric Phase Sorptive extraction media [574]

2.7.4 Fabric Phase Sorptive Extraction (FPSE) -procedure

The primary material that was used for the extraction of target analytes was a Whatman microfiber glass filter (FG) coated with short-chain poly (ethylene glycol) (PEG) by sol-gel process. Based on previous study [573], with some modifications the procedure was applied for the extraction of 21 contaminants from tap water samples and effluent wastewaters.

Prior to the extraction two circle-shaped (FG)@ PEG300 with a diameter of 1 cm material were soaked using tweezers (avoiding possibility of contamination) in 5 mL of methanol: acetonitrile (50:50 v/v) solution for 5 min. This is necessary to activate the FPSE media and remove any unwanted residue deposited during the storage. Afterwards, a conditioning step is followed by soaking the material into 5mL of deionized water for 5 min, disposing in this way the previous organic solvents. Next, the FPSE media was transferred to a 12 mL screw-capped glass tube vial with 10mL of aqueous sample along with a clean PTFE magnetic stir bar. The magnetic stirrer was set at medium level for 30min to achieve adequate transfer of target analytes within the aqueous sample. After that time, the FPSE media was removed from the water sample, and let it dry to remove residual water. Then the FPSE media was inserted in a clean vial with 1 mL MeOH (acidic/alkaline) and the analytes were eluted with the aid of stirring for 10 min. The extracts were collected and evaporated to dryness under gentle stream of nitrogen. Finally, they were reconstituted to the initial conditions of mobile phase (H2O: MeOH, 90:10 v/v acidified with 0.1%f. a v/v) for further analysis in UHPLC-Orbitrap-MS. FPSE extraction steps are illustrated in Fig. 2.7.

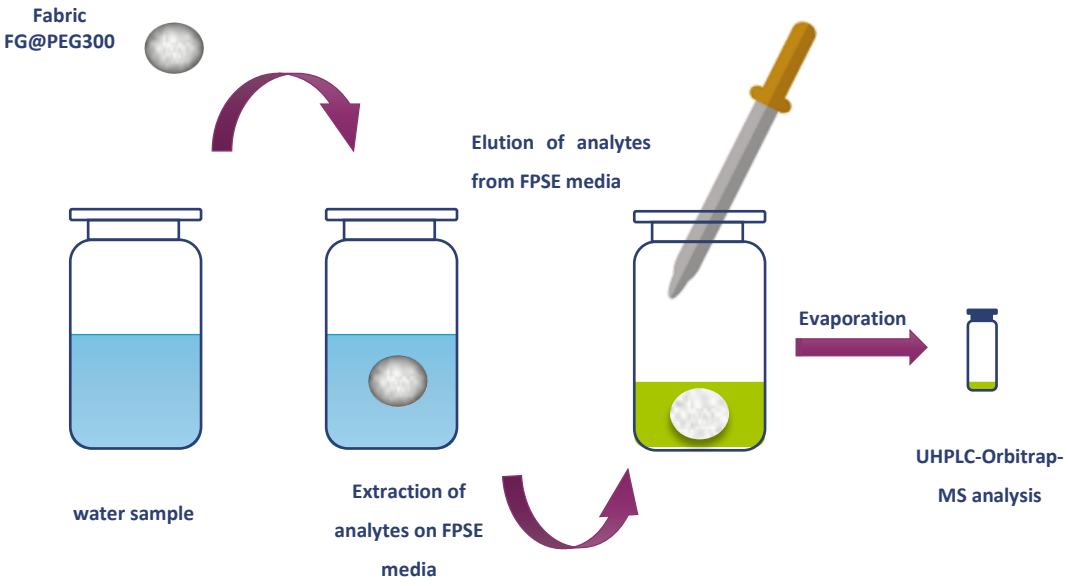


Figure 2.7. Schematic representation of fabric phase sorptive extraction (FPSE) procedure

2.8 Solid Phase Extraction (SPE)

Extraction of analytes of interest was carried out under the optimal conditions investigated, using an off-line SPE 16-port glass block vacuum extraction manifold (HyperSep, ThermoFischer Scientific). Oasis HLB (200 mg, 6 mL) cartridges were selected as extraction sorbent material. Prior to extraction 100 mL of wastewater sample and 250 ml of bottled were adjusted -if necessary- at natural pH 6.5-7.0 . Subsequently, an appropriate volume of 5% Na_2EDTA solution was added in in water sample to achieve final concentration of 0.1% (g solute/g solvent) . Addition of Na_2EDTA has been reported to increase recoveries of antibiotics and other pharmaceutical therapeutic groups[56]. Samples were spiked with the appropriate volume of internal standard mixture. The HLB cartridges were activated with 5 mL of methanol followed by 5 mL of ultrapure water as conditioning step. Next, samples were loaded and percolated onto the cartridges with a flow rate of 1 mL/min. After the extraction, the cartridges were washed with 5 mL of ultrapure water containing 5% MeOH and dried under vacuum for 15 min to remove water excess. Finally, elution of the analytes was performed twice with 5 mL of LC-MS grade methanol at 1 mL/min, then the extracts were collected in glass vials and evaporated to dryness under a gentle stream of nitrogen by means of Techne Dri-Block heater Model DB-3D. The

last step was the reconstitution, with 500 µL of methanol: water 10:90 (v/v) with 0.1% formic acid and the samples were stored at -20 °C until analysis.

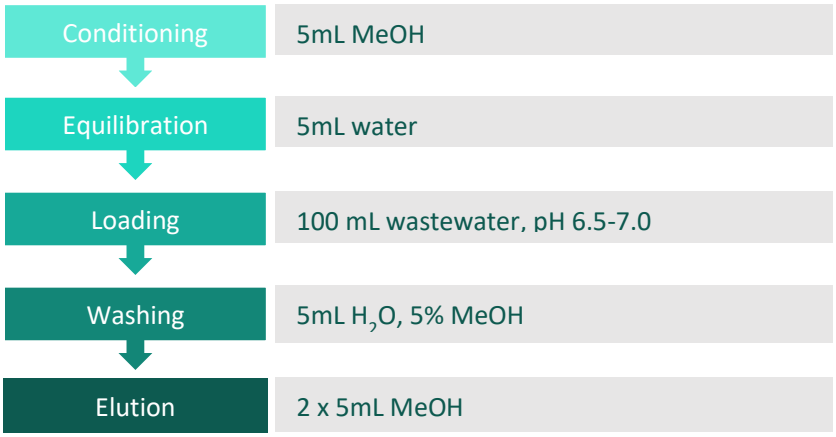


Figure 2.8. SPE procedure for the determination of target compounds in wastewaters

2.9 Quantification

Quantification of target analytes was based on peak area of protonated molecule, $[M+H]^+$ for PI and deprotonated one $[M-H]^-$ for NI mode, which were selected as precursor ions. Quantification strategy was performed by matrix matched calibration curves, together with the internal standard approach by adding the corresponding labeled compounds at a concentration of 50 ng L^{-1} . The internal standard (IS) approach was used only in the SPE method with the use of IS: (a) olanzapine d3; (b) carbamazepine d10; (c) fluoxetine d5; (d) amitriptyline d6. Calibration curves with internal standard were constructed by plotting the analyte response factor (peak area of analyte divided by peak area of internal standard) on the y-axis versus analyte concentration factor on the x-axis (concentration of analyte divided by concentration of internal standard). Normally, the use of analyte isotope-labelled IS is the preferred way to quantify complex matrices, but it can be limited because of commercial unavailability of reference standards and because of economic reasons, making their use in multi-residue methods more problematic. When the isotope-labelled internal standard (IS) of the analyte is not available, other compounds eluting at similar retention times or being structurally analogues have been tested, but no satisfactory data are always assured.

As concerns quantification with matrix matched calibration curves, bottled water and effluent extracts were used in each case to prevent slight variations in the signal for some analytes and possible enhancement or suppression of the signal. Since wastewater samples already contained

target compounds, first blank samples of effluent wastewaters were analyzed to determine their concentrations, which were afterwards subtracted from the spiked samples. With every batch of samples, matrix matched calibration curves were performed, as well as two quality control (QC) samples, which were blank wastewater samples spiked at MQL and 100 times the MQL level, respectively. Calibration standards were measured at the beginning and at the end of each sequence and possible fluctuation in signal intensity was checked by injecting a standard solution at two concentration levels after each 8-10 injections throughout the sequence.

For calculation of the concentrations of the compounds in the samples, the levels measured in the extracts were corrected by the corresponding QC recoveries. For statistical analysis, the concentrations that were below the MDL and MQL were substituted with ½ MDL and ½ MQL values, respectively.

2.10 Identification

For confirmatory purposes, a procedure was developed that established the characteristic fragments for each compound. Full data dependent acquisition (full MS/dd-MS2 mode) allowed to automatically obtain a product ion spectrum with accurate mass measurement. The identification process was based to the criteria for both screening and confirmatory analytical methods for pharmaceutical residues according to the identification points proposed by EU Commission Decision 2002/657/EC with combination of FDA guidelines that takes full advantage of the capabilities of modern HRMS instruments [575,576] and also the last update exploring the means of identification of small molecules [577].The criteria used included the presence of the protonated molecule at accurate mass, fragment ion(s) at accurate mass, retention time and isotopic pattern. To avoid reporting false positives and/or false negatives the below criteria should be fulfilled:

1. For LC-HRMS data, the presence of two ions, is required preferably the precursor ion protonated or deprotonated measured with a mass accuracy of ≤ 5ppm or 1mDa in case of m/z<200 , and a fragment ion with a mass accuracy of ≤ 10ppm. There is no requirement for an ion ratio unless the mass accuracy requirement cannot be achieved

If the measured exact mass from two or more ions match the mass accuracy criterion, it is not necessary to calculate and report ion abundance ratios. If, however, the measured mass error is greater than the mass accuracy criterion, the ion ratio criteria for nominal mass data as described

should be applied. The mandatory requirement for an ion ratio is removed, since with the added value of accurate mass measurement, the need for matching ion ratios is less critical. Nonetheless, it is recommended that ion ratios should be used as indicative with deviations exceeding 30% to be further investigated and judged with care. Thereby returning the onus back to the analyst to monitor the ratios and, where necessary, provide evidence for confident identification such as additional accurate mass fragments, isotope pattern, adduct ions, etc. Since the liquid chromatography (LC)-Orbitrap technology can produce the additional mass spectrometric data; full compliance for identification is easily achieved

2. The retention time must be in agreement with standard within one of the following limits: (a) ≤ 0.2 min; or (b) within ±2.5%, not to exceed 0.5 min; or (c) within experimental error (multiples of standard deviation) established in the validation method, not to exceed 0.5 min. Matrices may shift analyte retention times in which case matrix matched standards or standard additions might be necessary.

3.In high-resolution mass spectrometry (HRMS), the resolution shall typically be greater than 10 000 for the entire mass range at 10 % valley.

4. A Signal to Noise S/N threshold ≥ 3 is recommended. Extracted Ion Chromatogram from HRMS may produce baselines free of any background noise. Calculation of S/N ratio is not feasible under such conditions. When there is no noise, relative signal intensity acquired from the test sample vs. a comparison standard can be used to set up the threshold to recognize an XIC peak. Chromatographic peaks from different selective ions for the analyte must fully overlap.

2.11 Validation studies

Methods used in an analytical chemistry laboratory must be evaluated and tested to ensure that they produce valid results suitable for their intended purpose. Method validation is basically the process of defining an analytical requirement and confirming that the method under consideration has capabilities consistent with what the application requires. Inherent in this is the need to evaluate the method's performance. The judgement of method suitability is important; in the past method validation tended to concentrate only on evaluating the performance characteristics. Method validation is usually considered to be very closely tied to method development. For the validation of the analytical method there are some method performance characteristics to be evaluated. In this

study the validation of the analytical methods was carried out in two different aqueous matrices (bottled water, effluent WWTP) following the 2002/657/EC European Commission Decision [578].

2.11.1 Accuracy

«Accuracy» measurement expresses the closeness of a single result to a reference [579,580]. The accuracy of a measurement is defined as the closeness of the measured value to the true value. In a method with high accuracy, a sample (whose "true value" is known) is analyzed and the measured value is identical to the true value. Typically, accuracy is represented and determined by recovery studies. Recoveries were calculated through the matrix matched calibration curve prepared at each analytical session. For SPE validation, internal standards for specific compounds were also included. Three replicates of spiked bottled water and effluent sample at three concentration levels low, medium, high) were analyzed under the optimum conditions. The recovery was evaluated by comparing each level experimental concentration divided by the theoretical spiked concentration. In the case of effluent samples, due to the fact that unspiked effluent samples already contained some of the compounds, the concentration of the respective unspiked sample (blank) was subtracted from the concentration in the spiked sample and then divided by the spiked level. (Eq.2)

$$R\% = \frac{Cspiked\ exp - Cblank}{Cspiking\ level} \text{x } 100 \qquad \text{(Eq.2)}$$

In FPSE method, relative recoveries were used in real water samples. They were estimated from absolute recoveries of the Milli-Q water samples. Relative recovery is defined as the % concentration of target analytes recovered from the wastewater and bottled water with reference to the concentration found at spiked Milli-Q water.

Recoveries experiments in all cases were carried out in three replicates (n=3) for each concentration level in effluent wastewater and bottled water. Mean percentage Recovery values (R %) express the accuracy of the method combined with the respective RSD % value.

2.11.2 Precision

Precision is a measure of how close results are to one another [579], [581]. It is usually expressed by statistical parameters which describe the spread of results, typically the standard deviation (or relative standard deviation, RSD), calculated from results obtained by carrying out replicate

measurements on a suitable sample under specified conditions. Repeatability and reproducibility represent the two measures of precision which can be obtained. Evaluation of precision requires sufficient replicate measurements to be made on suitable samples.

Repeatability, also referred as intra-day precision was evaluated from the analysis of five spiked samples (n=5) for each concentration level within the same day, for bottled and effluent water. Intra-laboratory reproducibility or inter-day precision was performed in five spiked samples over three days of validation (n=15) in each concentration level. Intra-day and inter-day precision were expressed in terms of RSD_r% and RSD_R % respectively. RSDs % are estimated by dividing the standard deviation by the mean value obtained for each set of concentrations and multiplying it by 100.

2.11.3 Sensitivity

The sensitivity of the method was determined by estimation of the Method Detection Limit (MDL) and Method Quantification Limit (MQL). Limit of Detection or Method Limit Detection (MDL or MDL) is determined by the analysis of samples with known concentration of analyte and by establishing the minimum level at which the analyte can reliably detected, but not necessarily quantitated, under the stated experimental conditions. The detection limit is generally expressed in the concentration of analyte in the sample. As concerns Limit of quantification or Method Quantification Limit (MQL, MQL) is the least concentration of an analyte in a sample which is estimated with appropriate precision and accuracy under the validated experimental conditions.

Several approaches are recommended for determining the detection limit of sample, depending on instrument used for analysis, nature of analyte and suitability of the method. The acceptable approaches are: Visual evaluation, signal-to-noise ratio, standard deviation of the response, standard deviation of the slope of linearity plot. In this work the signal-to noise approach for the estimation of MDL and MQL was applied.

The MDL and MQL values were determined based on the signal-to-noise (S/N) ratio of fortified samples in each matrix (bottled water and effluent wastewater). The MDL and MQL were set as the concentration of analyte at which the chromatographic peak produces signal-to-noise ratio equal to 3 and 10, respectively (Fig.2.9).

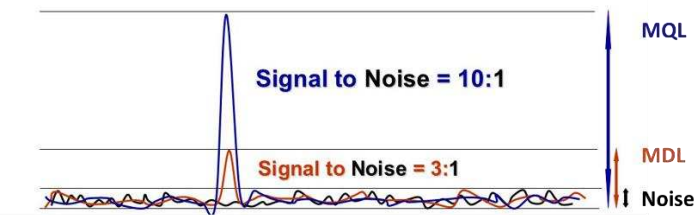


Figure 2.9 Determination of MDL and MQL

2.11.4 Linearity

The linearity of a method is a measure of how well a calibration plot of response versus concentration approximates a straight line. Linearity can be assessed by performing single measurements at several analyte concentrations. The data is then processed using a linear least-squares regression. The resulting plot slope, intercept and correlation coefficient provide the desired information on linearity. The linearity of the proposed method was investigated using bottled water and effluent wastewater spiked with ten to twelve different concentrations of the target compounds. The calibration curve was constructed using the peak area (Y-axis) of each standard compound versus its corresponding concentration (X- axis) and the regression equation was described as y = a x + b. The linearity was assessed by the coefficient of correlation (R^2).

In case of the internal standard approach a calibration curve was constructed in the specific range for every target analyte from the ratio of the analyte response to the specific internal standard response in every measured sample (bottled water, effluent wastewater).

In all cases the adequate lineal range for each compound was established from MQL to approximately 100xMQL.

2.11.5 Matrix effect

When the analysis of a complex real sample is performed, the matrix effect is one of the most relevant drawbacks that the analyst can expect. In analytical chemistry the matrix effect is defined by IUPAC as “the combined effect of all components of the sample other than the analyte on the measurement of the quantity. If a specific component can be identified as causing an effect, then this is referred to as interference. The definition, even if expressed in general terms, suggests the different and complex aspects of the phenomenon [582].

Even in the early times of chromatography with conventional detectors (i.e. UV/VIS, FID) it became evident that different sample matrices present peculiar interfering compounds, and the importance of using appropriate spiked matrix calibrators in order to get reliable quantitative results was recognized. In these conditions, however, the main concern was the presence of coeluting compounds giving similar detector responses, while the risk to alter the detector response of the analyte was not yet an issue [583].

Liquid chromatography coupled with tandem mass spectrometry, due to its high sensitivity and selectivity, is one of the most popular and appropriate techniques for trace analysis of pharmaceutical residues. However, in the case of very complex matrices (like environmental samples), this technique is highly susceptible to the influence of co-extracted components [584]. In mass spectrometry detection interference effects lead to ion suppression or ion enhancement phenomena, induced by the presence in the matrix of volatile compounds able to change the efficiency of analyte droplet formation or evaporation as well as the amount of the analyte ions formed in the gas phase that reaches the detector [585]. The different phenomena potentially induced by the matrix components dramatically affect the method performance in terms of detection capability, selectivity, repeatability, accuracy, linearity of response (signal vs. concentration) and limit of quantification.

2.11.5.1 Causes of Matrix Effect

Since, matrix effects were first observed for ESI-MS in 1990s, several mechanisms have been proposed [586–589].These include matrix components preventing analyte from gaining access to the charge, competing with analytes to gain charge, interfering with analyte's ability to remain charged in the gas phase, increasing surface tension of droplet or increasing electric resistance. Although, the exact mechanisms of matrix effects are still not fully understood, it has been widely accepted that the co-eluted matrix can alter ionization efficiency of target analytes and influence signal intensity due to the competition for the available charges and for the access to the droplet surface for gas-phase emission during the electrospray process [590,591]. Therefore, any process that changes the ionization efficiency and occurs in the liquid phase and gas phase, will cause matrix effects [591].

Matrix effects are caused by compounds which can be subdivided into two groups: endogenous and exogenous components. Endogenous species, naturally occurring in environmental samples they already existed as components of the sample and they are still present after its pre-treatment or

extraction. Potential ion suppressors are ionic species (inorganic electrolytes, salts), polar compounds (phenols, arylsulfonates) and organic molecules as carbohydrates, amines, urea, lipids, peptides and, in general, compounds or metabolites [582,591]. This applies especially to those compounds or metabolites which chemical structure is like the target analyte, as well as to the compounds which are present in analyzed samples extracts at high concentrations. The physicochemical properties of the analytes can also influence the degree of enhancement or suppression of their ionization. Bonfiglio et al. reported that highly polar compounds generally appeared to be affected to a greater ion suppression than less polar molecules [584]. Co-extracted substances present in the injected sample can cause relevant determination problems, especially when they are present at high concentration and co-elute with the analyte, so modifying its signal. Co-extracted species may also affect ion intensity of the analyte when form adducts or react with the analyte in the HPLC–MS interface.

Exogenous components may be components that are introduced during sample collection or during the pretreatment /extraction processes or also reagents added to the mobile phase to improve chromatographic resolution [592]. For example, the extraction process can bring into the extract interfering materials as polymer residues and phthalates released from plastic tubes or from packing materials used in solid phase extraction (SPE) or LC columns [593]. Furthermore, reagents added to the mobile phase to improve the chromatographic peak shape, as salts, ion-pairing agents, buffers and organic acids are potential responsible of ion suppression [585,594–598]. In this case, since the additives are continuously introduced into the interface with the mobile phase, the signal suppression can be observed throughout the entire chromatographic run.

The elution flow-rate can also play a role: low flow-rates and nano-spray systems may reduce the effects. Being the initial droplet diameter lower for flow-rate in nanoliter range, a lower number of subsequent coulombic explosions occur, that results in a lower concentration of salts in the droplet and then a lower suppression [599,600]. The level of signal suppression also depends on the hydrophobicity of the analyte and its affinity for the stationary phase. When using RP stationary packings, the effect is generally lower for the more hydrophobic compounds. The degree of ion suppression varies not only from sample to sample, but also from compound to compound and depends on the sample preparation[601]. In addition, for the same analyte, ion suppression entity may depend, even on the analyte concentration as well as on the matrix to analyte concentration ratio [599].

2.11.5.2 Evaluation of matrix effect

Matrix effects can dramatically influence analysis performance for both identification and quantification of an analyte. For instance, mass accuracy may deviate due to the matrix effects using high-resolution MS The evaluation of matrix effect in MS detection and possible methods for its overcoming should therefore be included in the validation process of new methods. There are two main protocols to evaluate the matrix effect: post column infusion protocol and post extraction addition protocol [586,602,603].

Post-column infusion protocol. This allows the analyte signal suppression or enhancement, by different coeluting matrix components, to be assessed throughout the duration of an entire chromatographic run, i.e., independent of a specific retention time.

Post-extraction addition protocol. In the case of the post-extraction addition method, the matrix effect can be quantitatively evaluated by comparing the response of the analyte in standard solution to that of a post-extract spiked with the analyte at the same concentration [591].

In this study a post-extraction addition approach was adopted combined with the comparison of standard line slopes, with the respective matrix-matched calibration slopes. When both curves are parallel and totally overlapped, compounds are not subjected to ion suppression. When curves obtained in spiked matrix extracts have lower or greater slope, indicates that compounds are susceptible to signal suppression or enhancement, respectively. To assess the ME in bottled water matrix-matched calibration curve was prepared with the spiked extracts of the respective method used in each case. For the evaluation of ME in effluent wastewater, matrix-matched calibration curves were prepared using pooled samples of three similar matrices (effluents from WWTP of Amaliada, Ioannina and hospital effluent of University of Ioannina). The slopes of the respective matrix-matched calibration curves were compared with the slope of the calibration curve prepared in solvent [methanol: ultrapurewater+0.1% f. a (10:90, v/v)]. A blank sample (no addition of standards) was simultaneously assayed to subtract the concentration of the target analytes present in the sample. Specifically, Matrix Effect (ME%) was calculated according to Eq. 3 [259,604,605].

$$Matrix\ effect\ (\%) = \left(\frac{Slope\ matrix-matched}{Slope\ standard\ solution} - 1\right) x100 \qquad \text{(Eq.3)}$$

A value of zero indicates that there is no ME, while for a positive value there is an ion enhancement signal and for a negative value an ion suppression signal. When the values of matrix effect are between +20% and −20% is considered a low matrix effect; when are between −50% and −20% or +20% and +50% is considered a medium matrix effect; lastly, when the values of matrix effect are less than −50% or higher than +50%, it is considered high matrix effect [259], [606,607].

2.11.5.3 Strategies to minimize matrix effect

Although, matrix effects cannot be completely avoided during LC–ESI-MS analysis, some actions can be suggested to minimize or compensate ion suppression/enhancement. They include modifications of the MS conditions, of the clean-up process, of the chromatographic conditions and of the calibration techniques.

Modification of mass spectrometric conditions

To overcome ion suppression/enhancement problems, it can be advantageous to modify MS conditions, especially when this operation does not require any other change in the already developed and optimized analytical procedure, as it concerns both sample preparation and chromatographic separation. From literature examples, it can be concluded that, with some exceptions, [593],[608–610], ESI is more subjected to ion suppression than APCI [594], [603], [611–614]. Desorption APPI source has even better tolerance over ESI and desorption ESI because the desorption process of Desorption APPI is thermal and only volatile or semi volatile compounds are efficiently evaporated to the gas phase [594].

As it regards the choice of ionization polarity, it can be tried to work, when the species can be ionized both in PI and NI modes. As mentioned, NI mode is usually considered less subjected to ion suppression because is more selective, being relatively low the number of compounds undergoing NI mode.

Pre-treatment and extraction process

Large differences in the matrix effect were observed after different sample preparation techniques [611]. Since the preconcentration process not only increases the concentration of the target analyte but often eliminates the potential of interfering substances. It is also true that the omission of a pre-concentration step generally lowers analysis sensitivity.

It is evident that one approach that only consists in the dilution of the sample or in the injection of a lower volume should be advantageous in term of work and time, but it is also inappropriate for trace analysis when low detection limits are required. A dilution could be critical for the analyte present at ultra-trace level and without significant effects for interfering substances present at high concentration.

A feasible way to remove co-eluting components is to perform suitable sample preparation and cleanup procedures. Widely used sample preparation methods include partition, filtration, centrifugation, sonication, precipitation, dilution, and various forms of extraction. Depending on sample type and analyte properties, liquid– liquid extraction (LLE), solid phase extraction (SPE), are popular sample preparation procedures. With an appropriate absorbent, an SPE procedure may lead to less matrix effects as the interfering matrix can be reduced or eliminated. An extra clean- up step in the procedures may be required to eliminate ion suppression effects. Improving sample preparation and purification, taking into account that the chemistry of the analyte affects the ESI response more than the sample pretreatment [601], is a way to compensate matrix effect problems.

Modification of chromatographic conditions

Improving HPLC separation efficiency is a good approach to overcome or at least to decrease matrix effect. Chromatographic conditions can be modified in order to shift the retention time of the analytes away from the time windows more affected by matrix effect [615,616]. When the concentration of target analyte is high, reducing the injection volume and/or diluting the sample are the simplest ways to decrease the amount of co-eluting components and minimize the matrix effects. Changing LC conditions, such as, using a lower flow rate, slower gradient program could be useful.

A special attention should be paid towards the analytes eluting in the solvent front (highly polar and non-retained compounds) or during the end of an elution gradient (or in the washing step of the chromatographic column where the strongly retained compounds are eluted). Since generally the areas of these peaks are more affected by interferences, the retention of the analytes can be adjusted in order that they elute in the time window between these two regions.

Different stationary phase packings can be used with characteristics suitable to the analytes of interest, as for example C18 packings containing polar moieties or HILIC stationary phases [613], [617]. When the change of the stationary phase does not provide the expected results, column switching technology (two-dimensional chromatography) may improve resolution [618,619]

Modifications of the mobile phase composition can also be useful. Low concentrations of weak acids (such as formic, acetic, trifluoroacetic acids), ammonium formate, ammonium acetate or ammonium hydroxide can be added to the mobile phase [585], [595]. It must be also noted that mobile phase additives used to improve separation may also affect the ionization process and suppress the signal.

Selection of the optimal calibration strategy

If matrix suppression/enhancement phenomena cannot be eliminated or made negligible through the application of the strategies described, appropriate calibration techniques can be used to compensate, as possible, the matrix effects. The following options can be experimented.

(a) The addition of internal standards(I.S.) is widely used. To achieve an efficient compensation of the matrix effects I.S. must be characterized by chemical structure and chromatographic retention similar as possible to those of the analyte. The co-eluting matrix components are expected to affect at comparable extent both analyte and I.S. In particular, isotopically labelled internal standards are the most powerful strategy for diminishing suppression effects and improve quantitation accuracy [618], [31,620,621]. Unfortunately, their use is rather expensive, especially in a multicomponent analysis, where an I.S. for each analyte is theoretically required. Not only due to economic reasons but mainly to the lack of available commercial standards, the use of isotopically labelled standards is generally more utilized in single residue analysis [622], [623]. Species of structure analogue to the target analyte can be added to the eluate coming from the LC separation column and entering the MS detector (post-column addition). But also, this strategy is not of easy application for multiclass multicomponent analysis [582].

(b) Echo-peak technique represents an interesting alternative to the I.S. approach. With this technique each analysis comprises two injections into the LC–MS system. The unknown sample and a standard solution are injected consecutively within a brief period, under the same chromatographic conditions. As a result, the peak of the standard elutes close to the peak of the analyte and is called the “echo-peak’’. Provided that the retention times of these two peaks are close enough to be affected in the same manner by the co-eluting undesired species, matrix effects are compensated.

(c) Calibration using external matrix-matched standards. If standards with the same or similar matrix composition of the sample to be analyzed are used, a practically full compensation of matrix effects is achieved. But unfortunately, this approach is not easy, mainly because an appropriate blank (i.e. material free of residues of target analyte) is generally not available.

(d) The standard addition method can be usefully employed. The actual samples are used to create a calibration plot individually. The main advantage of this method is that it can correct the matrix effect because exactly the sample matrix is present both in calibration standards and the sample itself. A drawback of the method is, for example, that the amount of sample needed is much higher than in other methods because the sample is needed also for calibration standards. Moreover, is more tedious to prepare the calibration plot for each sample separately.

D. Results and Discussion

CHAPTER 3: ULTRA-HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED WITH LTQ ORBITRAP MS FOR THE ANALYSIS OF EMERGING CONTAMINANTS

Introduction

The recent trend of analytical methods is focused on the use of powerful high-resolution MS detectors like Orbitrap with modern chromatographic systems. Such approaches allow the development of multi-analyte methods for the detection of a wide range of analytes in a single analytical run. With the introduction of new hybrid high resolution mass spectrometers such as Orbitrap, is allowed the combination of non-targeted screening in full scan mode and targeted MS/MS analysis. Recent studies are focused on the detection and quantification of pharmaceuticals and their metabolites in environmental samples with high resolution techniques, representing an excellent tool for fast and convenient environmental screening and confirmation of drugs and other micropollutants.

When establishing an instrumental method, it is interesting to evaluate the best chromatographic analytical conditions for the analytes, as well as the most relevant variables that can favor its ionization and subsequent detection. Chromatographic conditions have a significant impact on mass spectrometric analysis and can be optimized and adjusted to accomplish higher sensitivity and resolution. In this chapter the optimization of the selected instrument method is described and is divided in two parts: the selection of HPLC chromatographic conditions and subsequently the mass spectrometry parameters including the high-resolution detector of LTQ Orbitrap-MS.

3.1 Evaluation of chromatographic conditions

3.1.1 Mobile Phase

Selecting the LC mobile phase with proper pH and modifier is an effective way of achieving proper retention, peak shape, LC-MS/MS response, and chromatographic resolution. Controlling these

conditions, improvement of chromatographic performance such as best separation and effective retention of the compounds is achieved. First, several experiments were performed on different mobile phases consisting of acetonitrile (AcN) or methanol (MeOH) as organic phase and ultrapure HPLC water as polar phase with different concentrations of acetic and formic acid (from 0.05 to 0.5% v/v), ammonium formate, ammonium acetate (from 1 mM to10 mM). Methanol was chosen due to the observed overall reduction of ESI signal intensity when using acetonitrile compared to methanol. This may be attributed to the reduced charge status of ionized species in the electrospray droplets by the neutral vapor of acetonitrile in the atmospheric region of the source [624]. MeOH achieved a better resolution and sensitivity. Ammonium acetate modifier exhibited higher peak shape especially in the compounds with high retention times, however it also provided high signal suppression. Moreover, in negative ion analysis, formic acid (f.a) is observed as deprotonated molecule with the advantage being that lower molecular weights cause less interference. The addition of formic acid enhanced the formation of $[M+H]^+$, $[M-H]^-$, as dominant molecular ions for polar and negative ionization, respectively. Higher concentrations of formic acid resulted in reduction of the signal for most analytes of interest as well as to a shift in the retention time. Finally, the best results were obtained when MeOH and water consisted the mobile phase with the addition of 0.1% v/v formic acid in both solutions.

3.1.2 Gradient elution

Gradient elution is best suited to analyses conducted using reversed phase chromatography. The initial composition is chosen so that the strength is appropriate to retain and resolve early eluting analytes. The elution strength is then increased in a predetermined way to elute compounds with optimum resolution. Several gradient profiles were studied, obtaining good response to ensure elution of all compounds of interest from the column within a reasonable time. The gradient profile was optimized for analyzing 21 compounds for positive ionization (PI) and 12 for negative ionization (NI), within 10 and 8 minutes, respectively and consisted of (A) an aqueous solution of formic acid (0.1%, v/v) and (B) methanol (also containing f.a at the same concentration).

For the analysis in positive mode, the optimized chromatographic method held the initial mobile phase. The gradient program for the elution of target compounds in positive ionization mode started at 95% mobile phase A and was maintained for 1 min; the next minute the amount of mobile phase

B increased to 70% followed by an increase to 100 % within 3 min, where it stayed stable for additional 2 min. Afterwards, the mobile phase was restored to the initial conditions of 95% A and maintained over 3 min for re-equilibration of the column. The total running time was 10 min with a flow rate of 250 µLmin^{-1} and injection volume set at 10 µL. Compounds analyzed in negative mode were separated using a different gradient program that was applied as follows: 90% of mobile phase (A) was used from 0- 0.5min, followed by consecutive linear declines to 30%A from 0.5 to 2.0 min, to 10% A from 2.0 to3.0 and 5%A from 3.0-3.9. In the 4.5 min of total run the percentage of methanol (B) increased to 100% and this composition was maintained for half a minute. Finally, the column was re-equilibrated with 90%A from 5.1 to 8.0minutes.

At the beginning of the analysis, when the mobile-phase strength is low, with the excess of aqueous phase (A) the analytes were partitioned wholly into the stationary phase at the head of the column and were not moving through the column at all. As the mobile-phase strength increases, with the increasing of phase B the analytes begun to partition into the mobile phase and move along the column. As the mobile-phase strength increases continuously, the rate at which the analyte moves along the column subsequently increases and the analyte "accelerates" through the column. The elution depends on the nature of the analyte and its interaction with the stationary phase material. Thus, the polar analytes elute earlier than the non-polar ones which elute with the increase of the organic mobile phase. The retention times of the selected compounds for corresponding compounds ionized in positive and negative mode are presented at the end of the chapter, Tables 3.3 and 3.4, including the mass spectrometry data obtained after optimization was achieved.

3.1.3. Analytical Column

It was essential to have a chromatographic separation of the target analytes to minimize any interference during quantitation induced by similar structures for the analytes. Columns of smaller dimension (50 or100 mm×2 or 3 mm, ID) have been frequently used for liquid chromatography with tandem mass spectrometric detection (LC–MS–MS) methods for analysis of pharmaceuticals providing shorter run time, and selectivity. Smaller columns have much lower dead volume and enable, in a relatively short time period, the resolution of analytes from most endogenous matrix compounds, which elute at or near the dead volume. Such resolution is essential for avoiding detrimental matrix effects. C18 reversed-phase silica gel columns were proved to endow a highly

efficient separation of pharmaceutical compounds Three chromatographic columns were surveyed to optimize suitable chromatographic conditions: Speed-core- Diphenyl (50 mm x2.1, 2.6 µm), Speed-Core C18 (100 mmx 2.1, 2.6 µm) provided by Fortis Technologies and Hypersil Gold C18 (100 mm x2.1, 1.9 µm) from Thermo Fischer Scientific. Between all the mobile phase combinations mentioned, and the three examined columns, the best peak shapes and responses in both positive and negative ionization modes were achieved using the Speed-core- Diphenyl (50mmx2.1, 2.6µm) and Hypersil GOLD (1.9 µm) columns with acidified water and methanol as mobile phases, while the Speed-Core C18 (100mmx2.1, 2.6µm) was overruled because of the absence of chromatographic peaks especially in the sulfonamide group. Finally, Hypersil GOLD C18 yielded the best results by means of peak shape and area of chromatographic peak. Characteristic examples of the obtained chromatograms and response are depicted at Fig. 3.1 and Fig.3.2.

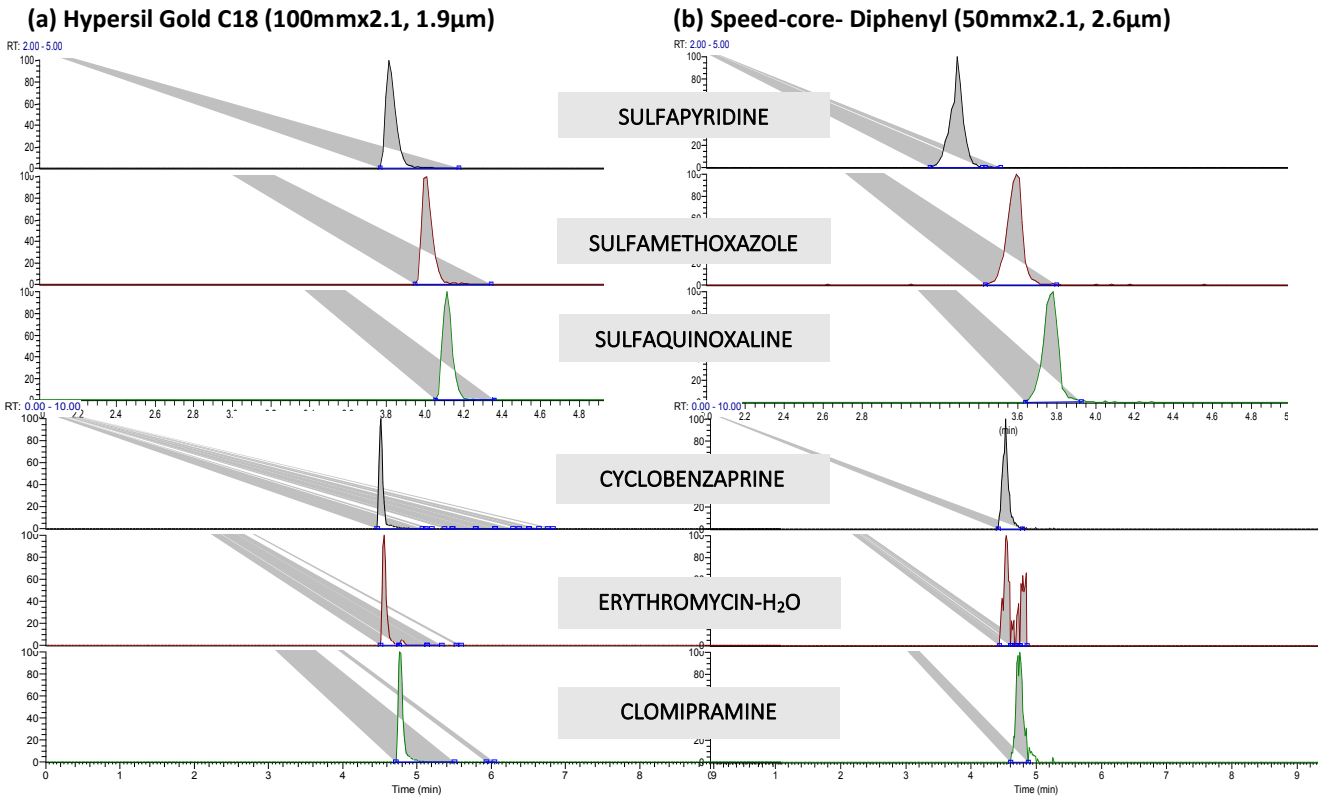


Figure 3.1. Chromatograms of selected pharmaceuticals of standard solution at concentration of 5 µg/L. (a) Hypersil Gold C18 (100mmx2.1, 1.9µm), (b) Speed-core-Diphenyl (50mmx2.1, 2.6µm)

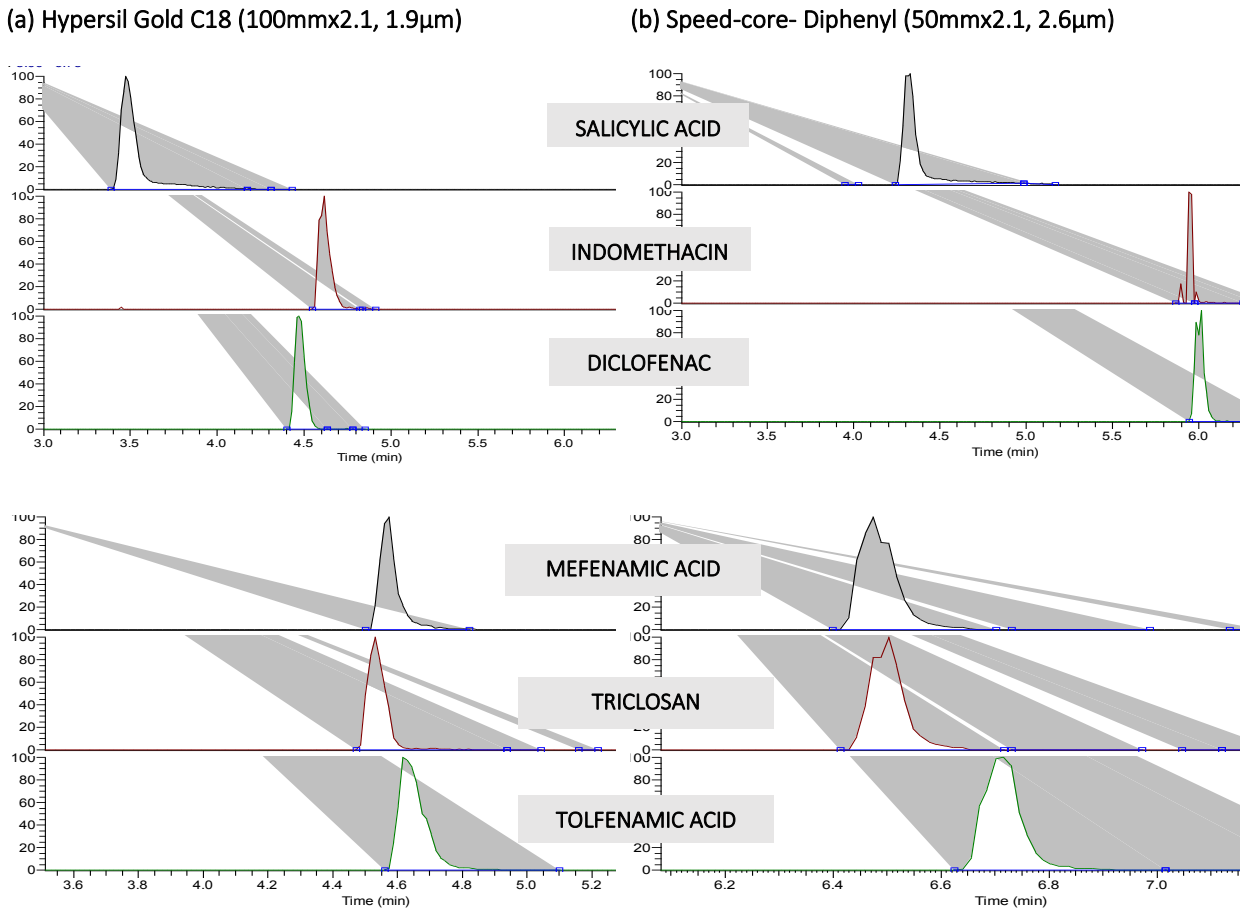


Figure 3.1.*(continued),* Chromatograms of selected pharmaceuticals of standard solution at concentration of 5 µg/L. (a) Hypersil Gold C18 (100mmx2.1, 1.9µm), (b) Speed-core-Diphenyl (50mmx2.1, 2.6µm)

Hypersil Gold Column provided narrow symmetrical chromatographic peaks that ensure the optimum resolution. Obtaining narrow peak widths is especially challenging for basic pharmaceutical compounds. The reduced silanol activity on Hypersil Gold columns reduces tailing for basic analytes, improving resolution.

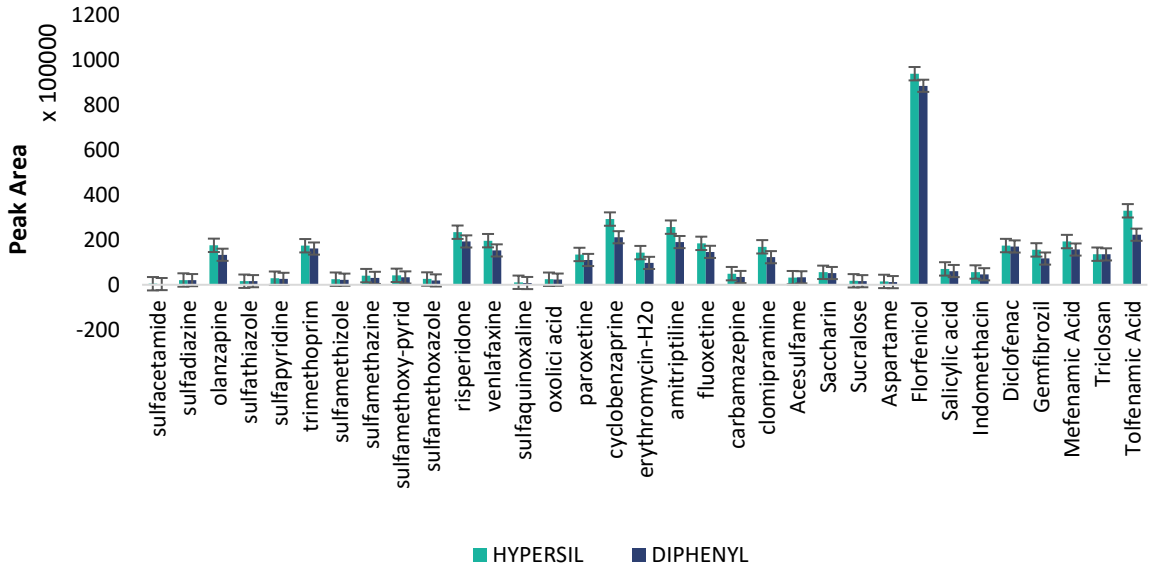


Figure 3.2 Response of target compounds in the examined columns

3.1.4 Injection Solution

The influence of the injection solution composition on the quality of LC–MS methods, in terms of column efficiency and peak shape, was investigated. Standard mixtures of the target compounds were prepared in different solutions ranging from 100% water to 100% MeOH. On silica column with aqueous/organic mobile phases, the retention mechanism is relatively straightforward. Water is the stronger elution solvent, thus as expected, by increasing the composition of methanol in the injection solution, chromatography efficiency decreases, particularly for the early eluting compounds such as sulfonamides and sweeteners. On the other hand, the non-polar analytes with higher retention times were favored with the increase of methanol. The optimum composition for the efficient separation of all analytes was 90:10 water: methanol. The addition of acid in this system of solvents was also investigated. Specifically, the addition of formic acid in the same concentration of the mobile phase (0.1% v/v) provided better peak shapes and also prevent analytes from being adsorbed onto the glass surface according to some studies [625,626]. Taking into consideration the efficiency of chromatographic separation as well as the stability of the analytes in the injection solvent, 90:10 water: methanol with 0.1% v/v formic acid was selected as optimum. Because of much lower quantities of the packing material in the smaller columns and because of lower retention of analytes on the column, it is more likely that chromatography can be disturbed, particularly by the mismatch of the elution strength between the injection solution and the mobile phase. The maximum

chromatography efficiency was achieved by using the injection solution of the weakest elution strength. Analytes were initially stacked on the top of the analytical column upon injection and were then eluted by the mobile phase [626]. This theoretical fact was very useful for selecting an appropriate injection solution under well-defined reversed-phase chromatographic conditions.

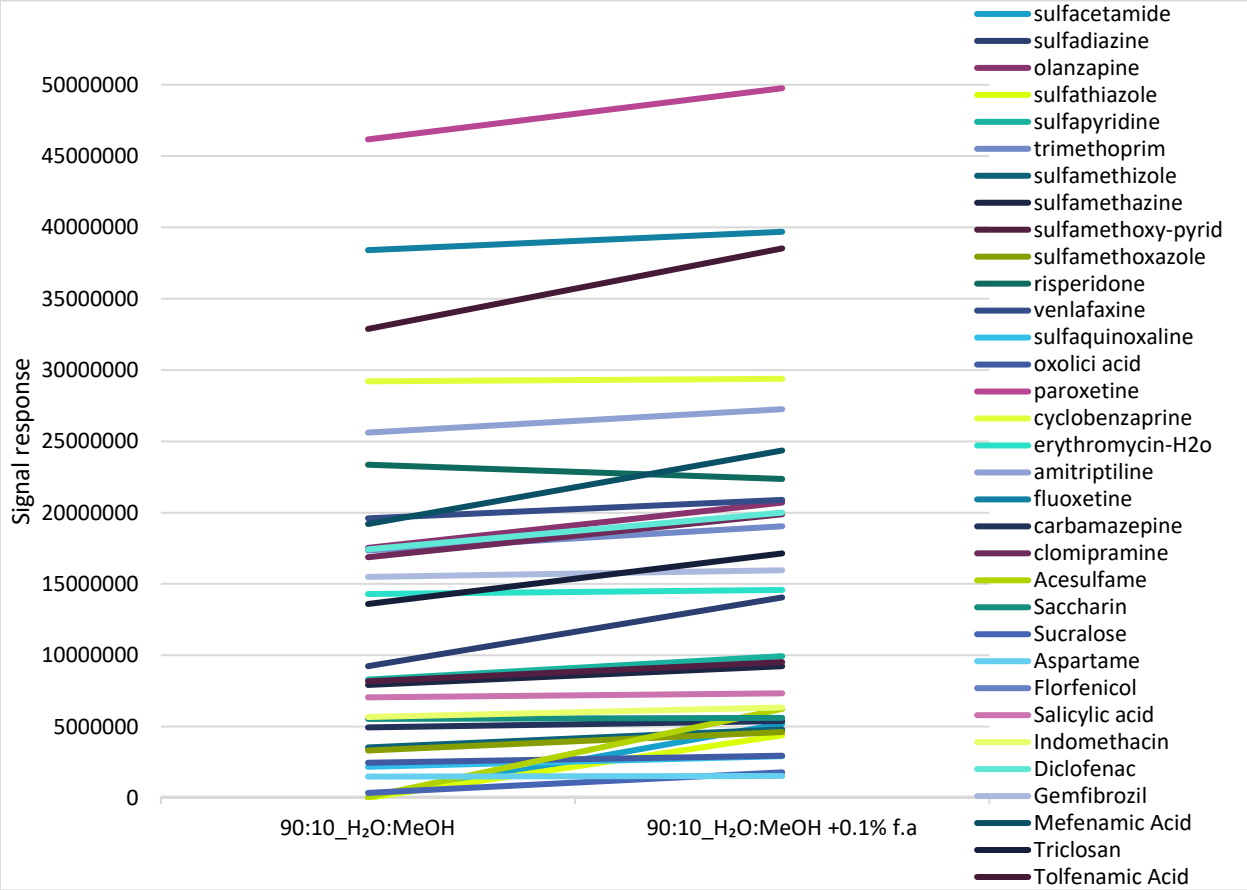


Figure 3.3 Effect of formic acid (0.1% v/v) on the response of the detector

3.1.5 Injection Volume

It was observed that injection volume could also influence the efficiency of chromatography. Different injection volumes of 2.5µl, 5 µl, 10 µl, 15 µl and 20 µl were investigated. Chromatographic peaks with 2.5µL of injection volume proved to have low response, while 5 µl provided sharp and narrow peaks with high intensity. A larger injection volume leaded to a longer tail of the peak (>15µL). As the injection volume increases the peak height and area grow in proportion to the injection volume up to the point when the injection volume is too large. At that point, the peak broadens and appears

rounded on the top, and this peak broadening reduces resolution between two adjacent peaks. This is due to the fact that when volume is increased, column performance decreases because the injected volume covers a more significant fraction of the column. In addition, other species in the mixture may overload the column and interfere with the detection of the compounds of interest. For this reason, injection volume of 5 µl was selected for compounds ionized in positive mode. On the other hand, concerning the negative ionization mode, small injection volume of sample provided small peaks in height and area and were not sufficient for all the analytes. Thus, the minimum amount of sample required for excellent chromatographic efficiency was 20µL .

3.1.6 Other parameters

The oven temperature of the UHPLC system was also evaluated. Firstly, the column oven was thermostatted in ambient temperature of 25^{o}C to have the temperature under control in all the process of the chromatographic analysis. Higher temperatures of 30 ^{o}C, 35 ^{o}C,40 ^{o}C, 45 ^{o}C was evaluated. At elevated temperatures >45^{o}C the silica backbone of the stationary phase or its bonding are prone to decompose, resulting in a possible column bleeding. Increasing the separation temperature provided shorter analysis time, improved separation efficiency. In addition, with elevated temperatures a lower system backpressure was achieved, and the separation of polar compounds seemed to be favored. The value of 35 ^{o}C was the optimum with narrower peaks, indicating higher separation efficiency, while 40 ^{o}C and 45 had no difference. Narrower and taller peaks in comparison to its equivalent at the lower temperature, resulted in lower detection limits. As the column temperature rises, the mobile phase viscosity decreases, thereby the flow resistance in the column, and the system backpressure as well.

Thus, the next step was to optimize the flow rate of the mobile phase. The right flow rate is dependent on column dimension. Since the selected column for the separation of pharmaceuticals was Hypersil Gold C18 (100 mm x 2.1, 1.9µm), that means material with small particle size, the recommended flow rates varied from 150 µL-400 µL. The best results in terms of time analysis and separation efficiency were achieved with a flow rate of 250µL min^{-1} and 200 µL min^{-1} for positive and negative ionization compounds, respectively.

Finally, an additional temperature control is necessary in the autosampler tray. The long sample batches and the two ionization modes of analysis, positive and negative, demand long time remaining

inside the autosampler tray. The variations of room temperature that occur can influence the chromatographic conditions and also can cause evaporation or degradation of the target analytes. To ensure the stability of the chromatographic system and the quality of the samples, the temperature in the autosampler tray was set at 15°C.

The final chromatographic conditions for the target compounds for positive and negative ionization mode are summarized in Table 3.1. Fig.3.4 a-b displays the extracted ion chromatogram (XIC) of standard solutions of pharmaceuticals at a concentration of 5 μg/ L.

Table 3.1. Final chromatographic conditions for target analytes

	POSITIVE IONIZATION	NEGATIVE IONIZATION
Analytical Column	*Hypersil Gold C18 100 x 2.1*mm, 1.9 μm	
Mobile Phase	(A): H_2O, 0,1 % f.a (v/v) (B): MeOH, 0,1 % f.a (v/v	
Pump Pressure	~7000psi	~6000psi
Flow rate	250μL^{-1}	200μL^{-1}
Column oven temperature	35°C	
Tray temperature	15°C	
Injection solvent	H_2O/MeOH 90/10+ 0,1 % f.a (v/v)	
Injection volume	5μL	20μL

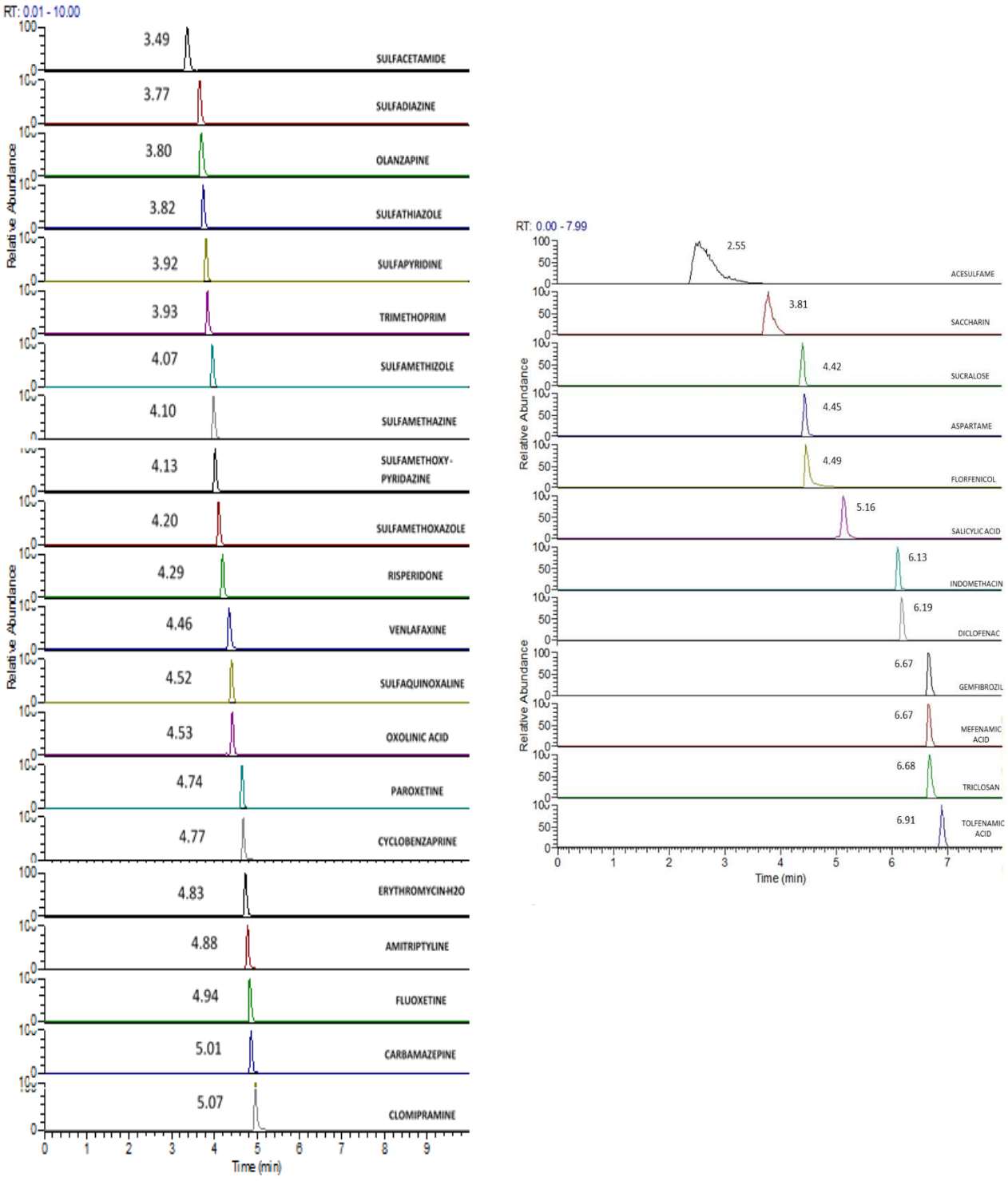


Figure 3.4 Extracted Ion Chromatogram, XIC of standard solution of 5 µg/L in UHPLC–LTQ/Orbitrap of target analytes a) positive ionization, b) negative ionization

3.2 Optimization of Mass Spectrometric Settings

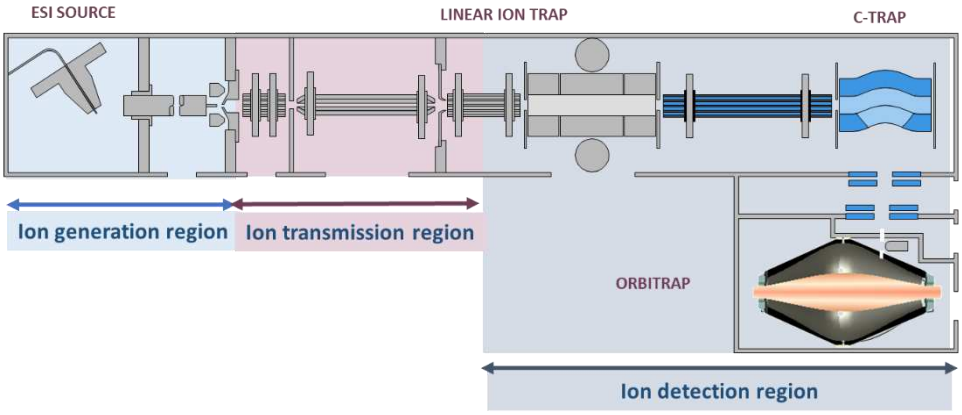


Figure 3.5 Illustration of Orbitrap mass spectrometer[627]

3.2.1 Ion generation- ESI Conditions

For the evaluation of instrumental conditions in mass spectrometry it is interesting to study the parameters as well that can favor the ionization of target analytes and their further detection. Optimization of both the ionization process and ion transportation in the mass spectrometer is of crucial importance to achieve high sensitivity, low detection limits and acceptable accuracy. The ESI source factors were identified through simultaneous batch screening and optimization experiments. Initially, the electrospray source generates ions employing a high voltage electric field with the aid of two gas flows. Thus, the main source parameters that are considered as critical were the sheath gas and auxiliary gas. Many other parameters also contribute to the efficient ion generation such as the capillary voltage and the capillary temperature or to the initial ion transmission from the source, such as the tube lens voltage.

The optimization was carried over in both ionization modes, positive and negative. A spiked effluent water sample with a concentration of 25μgL^{-1} was used to effectively study the influence of the ESI-MS parameters on both sensitivity and matrix effect. Full scan data both in the positive and negative modes were acquired and the mass range was set at 120-1000 and 120-600 m/z for positive and negative, respectively.

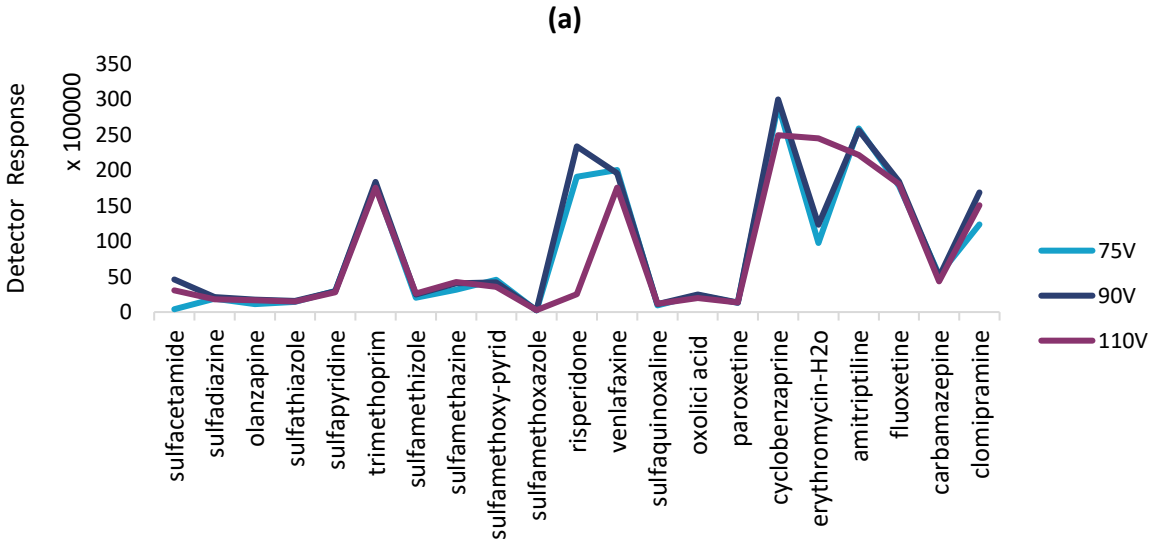
The sheath gas flow assists in the drying of the drop emitted by the capillary, to which it was assigned moderate relevance and its values were ranged between 5-40 au for both polarity modes. The sheath gas flow executed best results by means of signal intensity in the range of 25 and 35 au, and

specifically the value of 35 and 30 au was chosen as optimal for PI and NI, respectively. A capillary temperature, that helps the analytes to be emitted in solution form, was also tested and varied between 260, 280, 300, 320, 340, 360 and 380 °C. Capillary temperatures between 280-320°C provided similar and satisfactory results with some differentiations for more acidic compounds which exhibited higher sensitivity in high capillary temperatures. This observation is in accordance with another published work [628]. There is no ideal capillary temperature that fits equally for all the analytes of interest, so 320°C was selected as optimal. The results of optimization of the parameters of the ESI source are presented in Table 3.2. Sweep gas flow and capillary voltage were set by the auto tuning mode

Finally, to optimize spray stability and sensitivity in the ESI source, the ESI probe geometrical position is a critical parameter too. Front-to-back position (0-2μm), side to side (+1 to -1 marks) and depth positions B, C, D were tested. Farther away from the ion interface concerns depth C or D and conversely, B or C positions concern probe depth so that the nozzle is closer to the ion interface.

3.2.1.1 Tube Lens

The tube lens voltage directs ions into the ion guide which is offset from the orifice of the detector. The redirection of ions prevents neutral species from accumulating in the MS. Tube lens values evaluated were 70, 90, and 110 V, with the best results accomplished with 90 V and −90 V for positive and negative polarity, respectively. Variations of tube lens voltage for target analytes seem to be influenced by the molecular mass (Fig.3.5a-b). For example, a tube lens value of 110 V in the case of Erythromycin-H_2O (MW 715.93) provided a higher response compared to tube lens value of 90 for the same compound. However, most target analytes molecular masses ranged from 200–350.



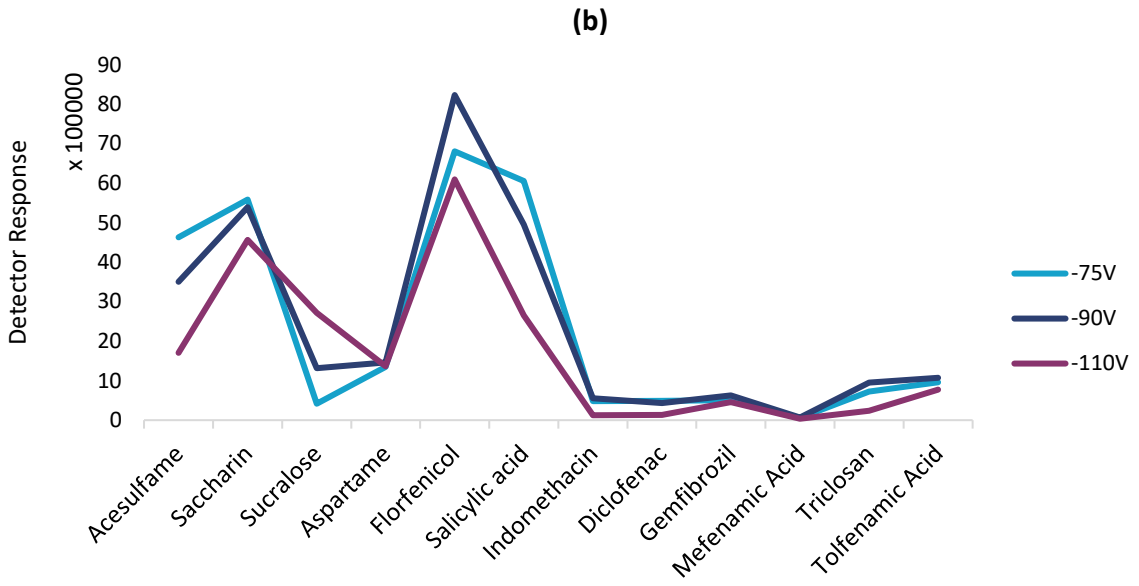


Figure 3.4 Response variance with different voltage of Tube Lens **(a)** Positive Ionization, **(b)** Negative Ionization

3.2.2 Ion transmission region (Ion Optics)

The investigation of the possible effects regarding the ion transmission region properties encompassed all the parameters of the orbitrap optics that can be changed by the user namely the multipole 00, lens 0, multipole 0, lens 1, gate lens, multipole 1, multipole RF and front lens. These parameters due to the large number of target analytes were selected based on the experience of the laboratory as well as guided by the parameters defined by the auto-tuning procedure.

3.2.3 Orbitrap parameters-Ion detection region

The last step of the optimization was the investigation of the parameters affecting the ion trapping / detection region on the signal intensity of pharmaceuticals. Thus, the AGC target and the maximum injection time values of the linear ion trap (IT) were evaluated.

3.2.3.1 AGC (Automatic Gain Control) Target Value

The AGC target value refers to the ion population in the linear ion trap or Orbitrap mass analyzer. The idea behind the AGC is to regulate the number of ions in the mass analyzer in order to avoid or minimize space charge effects to improve mass accuracy. The goal of the optimization has been set to maximize the response concerning the pharmaceuticals in both polarity modes. Four AGC target

settings were investigated for their effects on quantitation. of $3x10^5$, $5x10^5$, 10^6,$3x10^6$ for positive ionization while for negative ionization the corresponding test values were $2x10^4$, $4x10^4$, 10^5, $5x10^5$.The effects of AGC target are presented in Fig. 3.6 a-b.

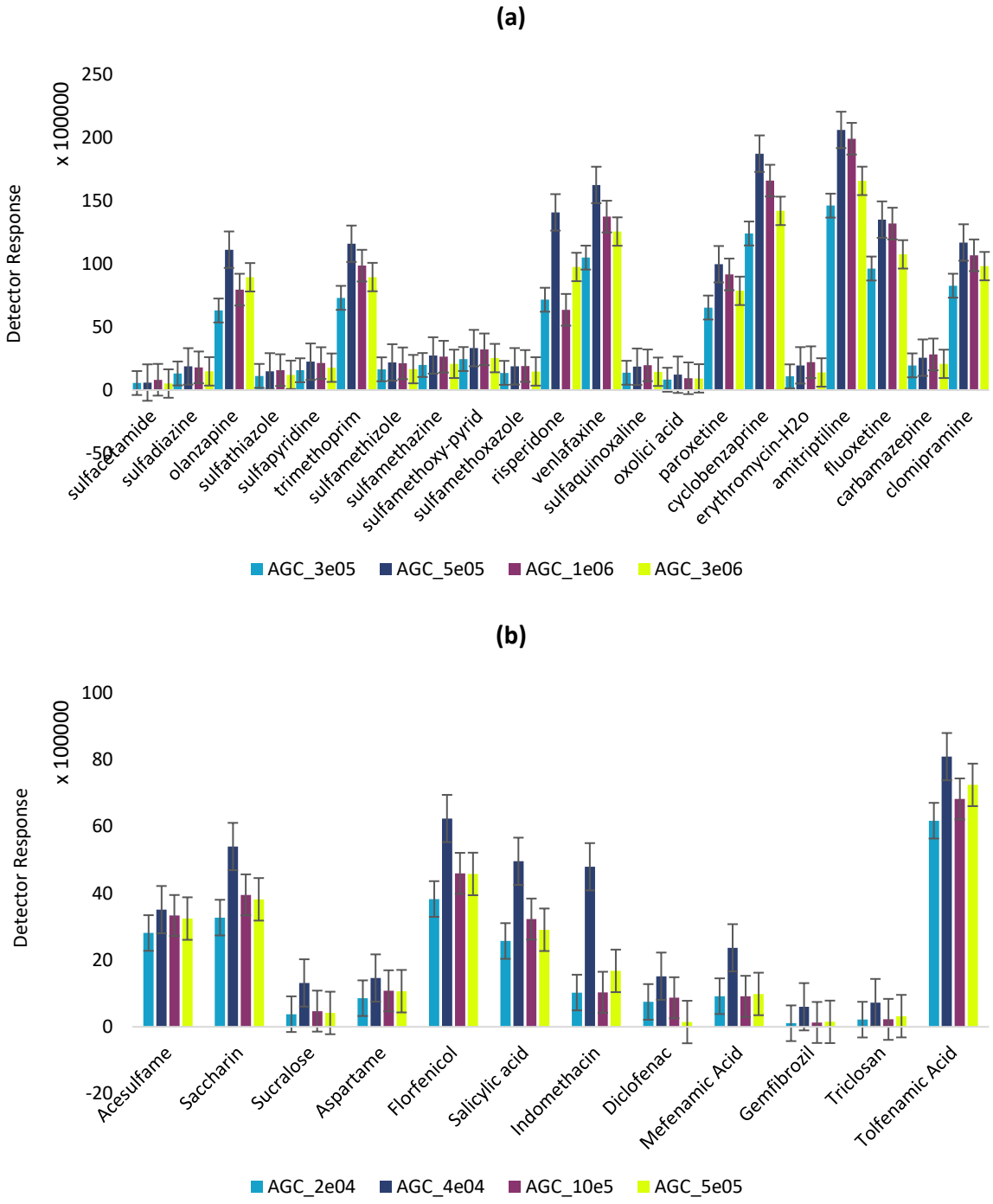


Figure 3.6 AGC effects of Mass Orbitrap analyzer on target analytes (a) Positive Ionization, (b) Negative Ionization

3.2.3.2 Injection Time

It is important to notice that the AGC target value and maximum ion injection time are dependent parameters. Either the maximum ion injection or AGC target value is responsible for the scan events of MS or MS/MS depending on which parameter is reached first. As an example, if maximum ion injection time is 100 ms while the AGC target value is set at 1 × 104 but it needs more time than 100 ms to accumulate 1 × 10^4 ions, therefore the MS events will be performed anyway within 100 ms regardless of the set of AGC target value. If it takes less time (<100 ms) to accumulate 1 × 104 ions, then the MS events will be executed with the already set AGC target. Increasing the AGC target values should follow an increase of the maximum ion injection time since it takes longer to accumulate more ions set [629]. Optimized injection time for AGC target value 5x10,^{5} was 100ms in positive method ionization, while in negative method 80ms for AGC target value 4x10^4 was selected.

AGC MS/MS and Injection Time MS/MS values were set based on common laboratory practices in the lab and literature [450,522,630–632]. By increasing the AGC target value for MS/MS, the MS/MS scan rates decrease as it takes longer to accumulate a higher ion population, resulting in a lower number of acquired MS/ MS. In addition, setting the AGC target value very low at 1 × 10^4 ions, also results in lower identification rates, presumably due to the lower quality of MS/MS spectra. Finally, the optimized AGC for positive method was 2x10^5 while for negative was 2x10^4. Maximum injection time in both polarities was 50ms. The optimum MS conditions for Emerging Contaminants studied, are summarized in Table 3.2.

Table 3.2 Optimum MS conditions for the analysis of target compounds

	POSITIVE IONIZATION	NEGATIVE IONIZATION
Sheath gas	35	30
Auxiliary gas	10	7
Sweep gas	0	0
Spray Voltage	4.00	2.7
Capillary temperature	320°C	320 °C
Capillary Voltage	50V	-30V
Tube Lens	90V	-90V
ESI probe position	Depth: C, side-to-side:0, front -to -back:2μm	

AGC Target MS	$5x10^5$	$4x10^4$
AGC Target MS/MS	$2x10^5$	$2x10^4$
Max. Injection Time	100ms	80ms
Max. Injection Time MS/MS	50ms	50ms

3.3 Data-Dependent Acquisition (DDA) Settings

Data-dependent scans were introduced as a source of fragmentation criteria and used to further reduce the chance of false positive results. The $ddMS^2$ events occur when there is a precursor ion detected in the full scan measurement. A DDA experiment consists of several MS and MS/MS parameters for which their values need to be defined by the user. These include, but are not limited to mass resolution, monoisotopic precursor selection, ion population (AGC target value), and maximum ion injection time for the full MS scan. MS/MS parameters include dynamic exclusion, minimal signal threshold, number of microscans per MS/MS scan, number of MS/MS events, AGC target value and maximum ion injection time for MS/MS.

3.3.1 Resolving Power (Resolution)

The resolving power is one of the most critical parameters in the analysis of difficult matrices by HRMS techniques. High resolving power helps to avoid false results. When the MS or MS/MS scans are detected in the Orbitrap mass analyzer, the mass resolving power to be used during the scan event needs to be defined by the user. To optimize the Resolving Power (R), the system operating in full-scan mode (120- 1000 and 120-600 m/z) was tested at the R of 15,000; 30,000; 60,000 and 100,000 FWHM. Six blank effluent wastewaters spiked with a mixed standard solution (50 ngL^{1-}) of 21 and 12 target pharmaceuticals for PI and NI, respectively were analyzed. The Resolving Power was evaluated measuring the peak area (signal response)

For the Orbitrap mass analyzer, the resolving power influences the acquisition time. The higher the resolution, the longer the time required for performing a single scan or mass spectral experiment. An increased mass resolution brought a higher mass accuracy and thereby better selectivity, however excessively high resolution (such as 100 000 FWHM) would significantly affect the sensitivity due to the reduced scanning speed and fewer data points (Fig. 3.7). To eliminate matrix interferences, the optimum resolving power was evaluated and the value of 60 000 FWHM (15 000 FWHM for $dd\text{-}MS^2$)

was found sufficient in order to distinguish the possible interfering endogenous matrix components from the analytes of interest.

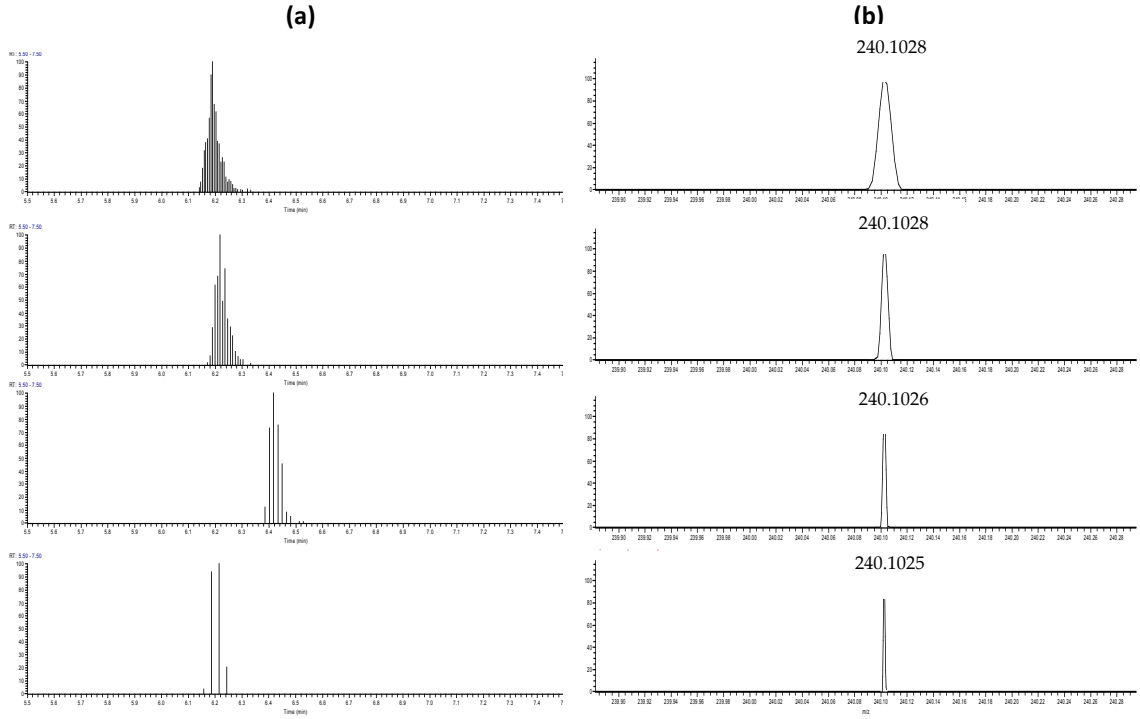


Figure 3.7 (**a**) Peak chromatogram (presented as data points) and (**b**) mass spectrum of Mefenamic acid (post-spiked sample at concentration 50 µg/L) with the resolution of 15,000 FWHM, 30,000 FWHM, 60,000 FWHM, and 100,000 FWHM (from top to the bottom).

3.3.1.1 Resolution in MS/MS

Mass resolution decreases with decreasing detection transient time for Orbitrap mass spectrometry because it is a Fourier transform detector. Consequently, the chromatographic peak width restricts the detection transient duration and, therefore, the maximum mass resolution. The higher resolution modes do not provide sufficient scan rates to accommodate the chromatographic peaks so scan cycle times can be increased in the Orbitrap MS by decreasing the mass resolution. Specifically, mass resolution for MS/MS mode was set at 15,000FWHM.

3.3.2 Fragmentation Optimization

For confirmatory purposes, a targeted MS/MS analysis was performed using the mass inclusion list which contains product ion mass, collision energies, and the expected retention times of analytes. This type of method includes a full MS scan performed by the Orbitrap analyzer with a defined

isolation window set by the quadrupole mass analyzer, followed by the application of the fragmentation energy and detection of the daughter ions by the high resolution Orbitrap analyzer. All the parameters were applied to find the optimum conditions ensuring the highest intensities of the instrumental response for the selected analytes. High accuracy mass spectra were recorded for all precursor and transition ions of the measured compounds and the optimum collision energy was selected by several optimization experiments. Different Normalized Collision Energies (NCE) values of 15, 20, 25, 30, 35, 40, 45 eV were evaluated. The collision energies were optimized for each target compound by injecting the working standard solution mix at a concentration of 25µg/L. The fragment ions with the corresponding collision energies are summarized in Tables 3.3 and 3.4.

3.4 Additional MS Parameters during DDA Mode

3.4.1 The isolation width

The isolation width is the m/z range that the MS detector uses to isolate the precursor ions. Setting the isolation width too narrow will result in loss of sensitivity, and in extreme cases cut out a portion of the ion packets, thus resulting in inaccurate measurements. In contrast, a wide isolation window may result in co-isolation and co-fragmentation of neighboring analytes resulting in unidentifiable or low scoring spectra. The isolation width of the developed method was set at 2 m/z and it has been suggested that that these typically used values (2 to 4 m/z) are optimal in practice [630,633,634].

3.4.1.1 Number of Microscans per MS/MS scan

The number of average microscans to produce one MS/MS scan is another variable during DDA. Increasing the number of microscans improves the signal to noise ratio but also increases the scan cycle time resulting in the collection of less MS/MS spectra. Employing one micro scan provides an MS/MS spectrum of sufficient quality of peak shape signal and intensity and subsequent target analysis identification.

Average mass accuracy was also estimated by injecting matrix-matched standards (n = 10) at 50µg/L of the selected compounds. In general, it can be noted that average mass error was <2 ppm, except for salicylic acid, gemfibrozil, triclosan and indomethacin which presented mass errors ranging from -2.881 to 4.252 ppm. Nevertheless, mass errors > 5 ppm were not observed for any of the assayed compounds.

The instrumental detection limits (IDL) and quantification limits (IQL) were determined as the minimum detectable amount of analyte with a signal-to-noise ratio of 3:1 and 10:1, respectively, by injection of decreasing concentrations of the standard mixture in positive and negative ionization. Linearity of the instrument for each analyte was evaluated from 0.05-1000 μgL^{-1} by injecting standard solutions in solvent. All analytes provided excellent detector response linearity with R^2>0.999. IQL was the lowest concentration of the linear range of each target compound and ranged from 0.06-3.13 μgL^{-1} The instrumental repeatability was evaluated in three concentration levels: 0,5 $\mu g\ L^{-1}$, 5 $\mu g\ L^{-1}$ and 25 $\mu g\ L^{-1}$ (n=5 per level) with RSD lower than 12% in all cases.

The carryover evaluation generated positive results as no peaks were found in blank runs after the analysis of standard mixture of 50μgL^{1-}, with the maximum a mass error set in 5ppm. Equally encouraged were the results for evaluation of retention time drift since the maximum time drift was almost absent (0.2min drift time).

Table 3.3. Parameters for full MS/dd-MS^2 Orbitrap analysis

COMPOUND	Rt(min)	Elemental formula	Theoretical mass (m/z)	Empirical mass (m/z)	RDB	Δ (ppm)	IQL (µgL-1)	FRAGM. ION	ELEMENTAL FORMULA	NCE
Sulfacetamide	3.49	$C_8H_{11}N_2O_3S+$	215.0485	215.0486	4.5	0.514	0.44	108.0448 92.0500 156.0112	C_6HNO+ C_6H_6N+ $C_6H_6NO_2S+$	30
Sulfadiazine	3.77	$C_{10}H_{11}N_4O_2S+$	251.0597	251.0598	7.5	0.307	0.22	156.0112 92.0489	$C_6H_6NO_2S+$ $C_6H_6NO_2S+$	30
Olanzapine	3.80	$C_{17}H_{21}N_4S+$	313.1481	313.1481	9.5	-0.140	0.23	256.0902 230.0745	$C_{14}H_{14}N_3S+$ $C_{12}H_{12}N_3S+$	35
Sulfathiazole	3.82	$C_9H_{10}N_3O_2S_{2+}$	256.0209	256.0211	6.5	0.021	0.24	156.0112 108.0440	C_6HNO_2S+ C_6H_6NO+	30
Sulfapyridine	3.92	$C_{11}H_{12}N_3O_2S+$	250.0645	250.0645	7.5	0.105	0.07	156.0112 108.0440 184.0867	C_6HNO_2S+ C_6H_6NO+ $C_{11}H_{10}N_3+$	30
Trimethoprim	3.93	$C_{14}H_{19}N_4O_{3+}$	291.1452	291.1453	7.5	0.457	0.18	230.1161 261.0980	$C_{12}H_{14}N_4O+$ $C_{12}H_{13}N_4O_3+$	30
Sulfamethizole	4.07	$C_9H_{11}N_4O_2S_{2+}$	271.0318	271.0319	6.5	0.393	0.17	156.0113 108.0447 65.0393 92.050	$C_6H_6NO_2+$ C_6H_6NO+ C_5H_5+ C_6H_6N+	35
Sulfamethazine	4.10	$C_{12}H_{15}N_4O_2S+$	279.0910	279.0909	7.5	-0.441	0.16	123.0662	$C_6H_9N_3+$	30
Sulfamethoxy-pyridazine	4.13	$C_{11}H_{13}N_4O_3S+$	281.0703	281.0703	7.5	0.045	0.23	156.0113 108.0430 92.0486	$C_6H_6NO_2S+$ C_6H_6NO+ C_6H_6N+	20

Table 3.3. Parameters for full MS/dd-MS^2 Orbitrap analysis, *(continued)*

Sulfamethoxazole	4.20	$C_{10}H_{12}N_3O_3S+$	254.0594	254.0592	6.5	-0.742	0.22	188.0818 156.0113 147.0789	$C_{10}H_{10}N_3O+$ $C_6H_6NO_2S+$ $C_8H_9N_3+$	20
Risperidone	4.29	$C_{23}H_{28}FN_4O_{2+}$	411.2191	411.2190	11.5	-0.197	0.44	191.1171	$C_{11}H_{15}N_2O+$	35
Venlafaxine	4.46	$C_{17}H_{28}NO_{2+}$	278.2115	278.2117	4.5	0.878	0.42	121.0643 260.2006	C_8H_9O+ $C_{17}H_{26}NO+$	25
Sulfaquinoxaline	4.52	$C_{14}H_{13}N_4O_2S+$	301.0754	301.0754	10.5	0.090	0.44	156.0113 108.0440	C_6HNO_2S+ C_6H_6NO+	30
Oxolinic Acid	4.53	$C_{13}H_{12}NO_{5+}$	262.0710	262.0709	8.5	-0.378	0.07	244.1905	$C_{13}H_{10}NO_4+$	25
Paroxetine	4.74	$C_{19}H_{21}O_3NF+$	330.1500	330.1502	9.5	0.611	0.06	192.1180 151.0387	$C_{12}H_{15}NF+$ $C_8H_7O_3+$	35
Cyclobenzaprine	4.77	$C_{20}H_{22}N+$	276.1747	276.1749	10.5	0.810	0.16	58.0659 84.0814	C_3H_8N+ $C_5H_{10}N+$	30
Erythromycin-H_2O	4.83	$C_{37}H_{66}NO_{12}$	716.4580	716.4591	5.5	1.601	0.14	158.1175 558.3635	$C_8H_{16}O_2N+$ $C_{26}H_{54}O_{12}$	40
Amitriptiline	4.88	$C_{20}H_{24}N+$	278.1903	278.1905	9.5	0.624	0.09	233.1322 191.0854 155.0854 117.0695	$C_8H_{17}+$ $C_{15}H_{11}+$ $C_{12}H_{11}+$ C_9H_9+	30
Fluoxetine	4.94	$C_{17}H_{19}F_3NO+$	310.1413	310.1415	7.5	0.563	0.06	148.1119 247.0918	$C_{10}H_{14}N+$ $C_4H_{18}FN_3+$	15
Carbamazepine	5.01	$C_{15}H_{13}N_2O$	237.1022	237.1024	10.5	0.677	0.16	194.0964 220.0756 192.0808	$C_{14}H_{12}N+$ $C_{15}H_{10}NO+$ $C_{14}H_{10}N+$	35
Clomipramine	5.07	$C_{19}H_{24}ClN_{2+}$	315.1623	315.1627	8.5	1.418	0.10	86.0940 58.059	$C_5H_{12}N+$ C_3H_8N+	30

Table 3.4 Parameters for full MS/dd-MS^2 Orbitrap analysis

COMPOUND	Rt(min)	Elemental formula	Theoretical mass (m/z)	Empirical mass (m/z)	RDB	Δ (ppm)	IDL (μgL^{-1})	IQL (μgL^{-1})	FRAGM. ION	ELEMENTAL FORMULA	NCE
Acesulfame	2.55	$C_4H_4NO_4S^-$	161.9867	161.9868	3.5	0.916	0.94	3.13	77.9642 82.0285	NO_2S^- $C_4H_4NO^-$	30
Saccharin	3.79	$C_7H_4NO_3S^-$	181.9917	181.9919	6.5	0.896	0.07	0.24	105.9592 61.9693	CNO_3S^- NOS^-	30
Sucralose	4.41	$C_{12}H_{18}Cl_3O_8^-$	395.0073	395.0072	2.5	-0.187	0.19	0.63	359.0299	$C_{12}H_{17}Cl_2O_8$	30
Aspartame	4.44	$C_{14}H_{17}N_2O_5^-$	293.1143	293.1141	7.5	-0.665	0.07	0.24	261.0880	$C_{13}H_{13}N2O_4^-$	15
Florfenicol	4.47	$C_{12}H_{13}Cl_2FNO_4S^-$	355.9932	355.9936	5.5	1.164	0.02	0.06	335.9869 218.9890	$C_{12}H_{12}Cl_2NO_4S^-$ $C_8H_8ClO_3S^-$	35
Salicylic acid	5.14	$C_7H_5O_3^-$	137.0244	137.0250	5.5	4.252	0.05	0.16	93.00334 65.0384	$C_6H_5O^-$ $C_5H_5^-$	30
Indomethacin	6.12	$C_{19}H_{15}ClNO_4^-$	356.0659	356.0675	12.5	-2.281	0.30	0.91	312.0796	$C_{18}H_{15}NO_2Cl^-$	
Diclofenac	6.19	$C_{14}H_{10}Cl_2NO_2^-$	294.0094	294.0089	9.5	-1.725	0.22	0.74	250.0193	$C_{13}H_{10}Cl_2N^-$	35
Gemfibrozil	6.67	$C_{15}H_{21}O_3^-$	249.1496	249.1489	5.5	-2.881	0.38	1.25	121.0659	$C_8H_9O^-$	25
Mefenamic Acid	6.67	$C_{15}H_{14}NO_2^-$	240.103	240.1026	9.5	-1.674	0.07	0.23	196.1133 240.1029	$C_{14}H_{14}N^-$ $C_{15}H_{14}NO_2^-$	20
Triclosan	6.68	$C_{12}H_6Cl_3O_2^-$	286.9439	286.9433	8.5	-2.042	0.18	0.59	161.2632	$C_6H_3Cl_2O^-$	25
Tolfenamic Acid	6.91	$C_{14}H_{11}ClNO_2^-$	260.0484	260.0480	9.5	-1.460	0.23	0.77	216.0580	$C_{14}H_{11}ClNO_2^-$	20

CHAPTER 4: CHARACTERIZATION OF SYNTHESIZED MATERIALS

The use of sorbent materials in Magnetic Solid Phase Extraction (MSPE), as well as in Fabric Phase Sorptive Extraction were in-house synthesized. The synthesis included the magnetic nanoparticles (MNPs) of graphene oxide and silica-C18, determined for MSPE application. Moreover, a fabric medium coated with PEG polymer was designed for FPSE sample preparation. To obtain information about their structural and morphologic characteristics, several characterization techniques were employed including: X-ray Diffraction analysis (XRD), Fourier Transform Infrared spectroscopy (FT-IR), Scanning electron spectroscopy.

4.1 Graphene based magnetic nanocomposites (GO-MNPs)

The synthesized composites of reduced graphene oxide modified by magnetic iron oxide, (mrGO), the intermediates materials of graphene oxide (GO), reduced graphene oxide (rGO) and magnetic composite of graphene oxide were characterized with X-ray Diffraction technique (XRD), Scanning Electron Microscopy (SEM) and Fourier transform-infrared spectroscopy (FT-IR).

4.1.1 X-Ray Diffraction analysis

XRD measurements were employed to investigate crystalline phase and structure of the synthesized materials. Figure 4.2 shows the XRD patterns of GO, rGO, mGO and mrGO. The XRD pattern of graphite powder provided from literature is also presented (Fig. 4.2). The peak observed at 2ϑ=10.19◦ of GO was attributed to the introduction of oxygen-containing functional groups into the graphite sheets in the formation of GO [635,636]. These functional groups facilitated the hydration and exfoliation of GO in water. This peak along with the disappearance of the intensive diffraction peak at 25.5^{o} of graphite (Fig. 4.2) is indicative of the total oxidation of graphite to graphene oxide. For rGO pattern a weak and broad reflection peak was observed at 24.44° which can be ascribed to the

relative short-range order structures in disordered stacked rGO [637,638] which implies the successful reduction of GO. Five diffraction lines were observed in the representative XRD patterns of mGO and mrGO at 2ϑ 30.14◦, 35.84◦, 43.73◦, 53.63◦, 57.26◦ and 30.11◦, 35.75◦, 43.55◦, 53.96◦, 57.35◦, respectively.

These characteristic diffraction peaks match the cubic spine crystal structure of iron oxide suggesting the existence of Fe_3O_4 [639,640].

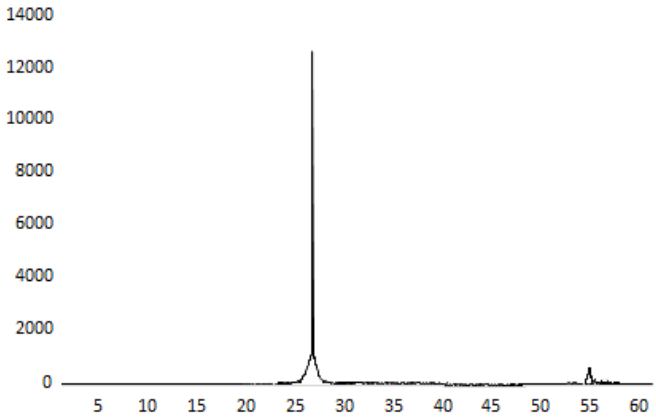
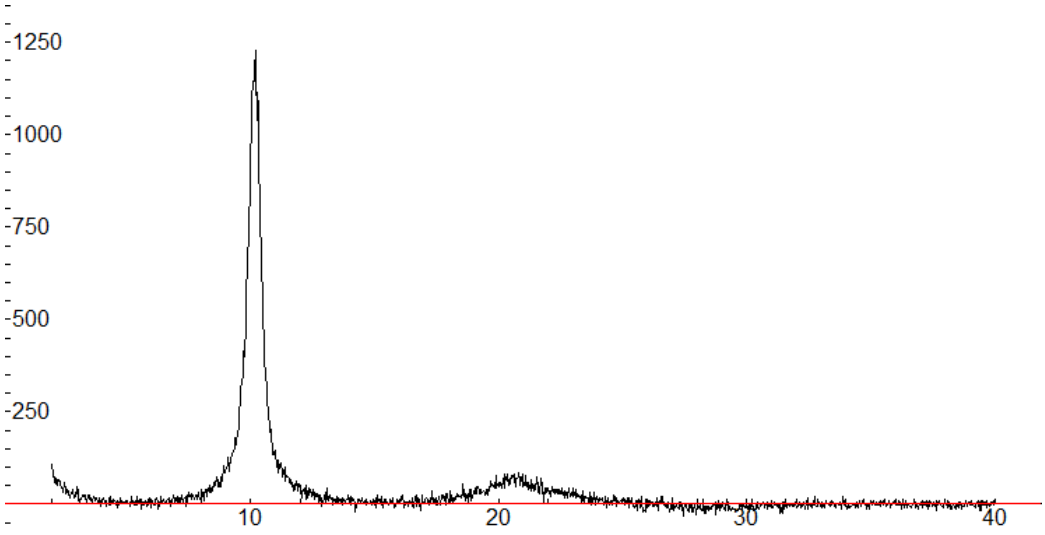
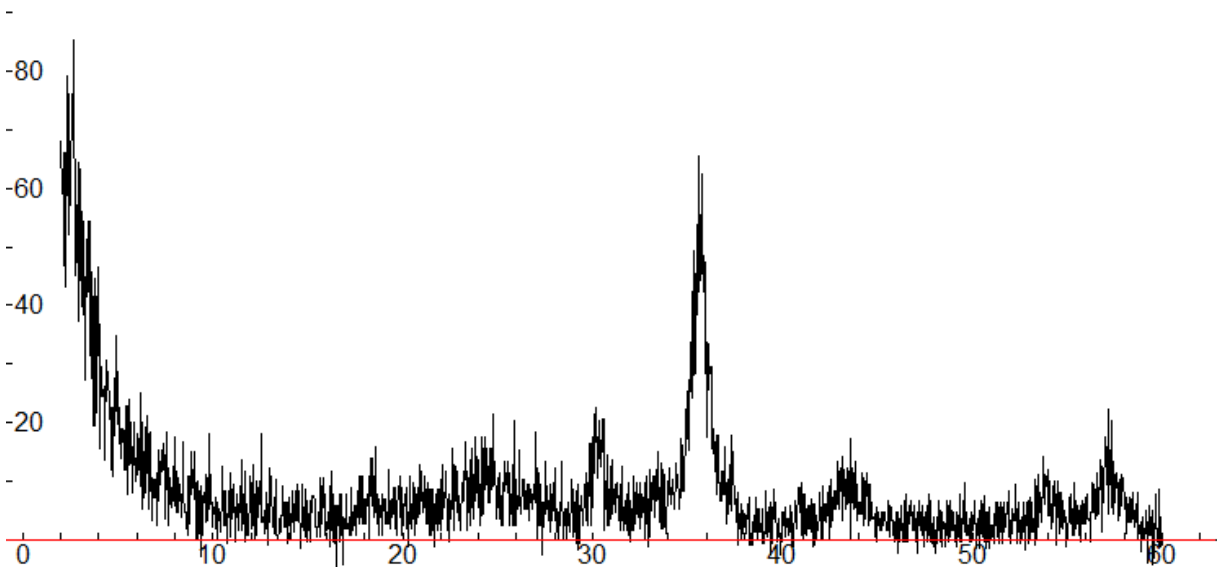


Figure 4.1. XRD pattern of graphite

(a) GO



(b) rGO



(c) mrGO

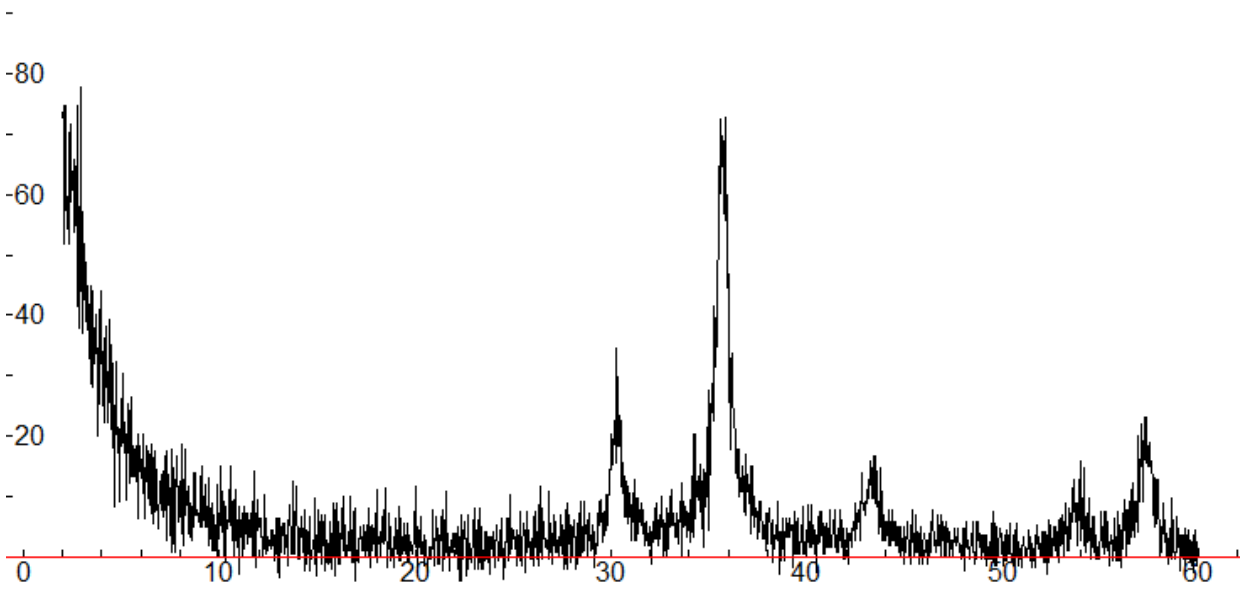


Figure 4.1. XRD patterns of (a) GO, (b) rGO, (c) mGO and (d) mrGO

The mean crystallite size (D, nm) of the particles was estimated using the Scherrer equation

$$D=(K\lambda)/(\beta\cos\vartheta),$$

D: mean crystallite size (nm)

λ (nm): wavelength (CuKα) = 0.15405 nm

K: constant, depended on crystallite shape. The most common value for K is 0.94 for crystals with cubic symmetry

b: is the full width at half-maximum (in radians) of the diffraction peak

***ϑ*:** is the diffraction angle (in radians).

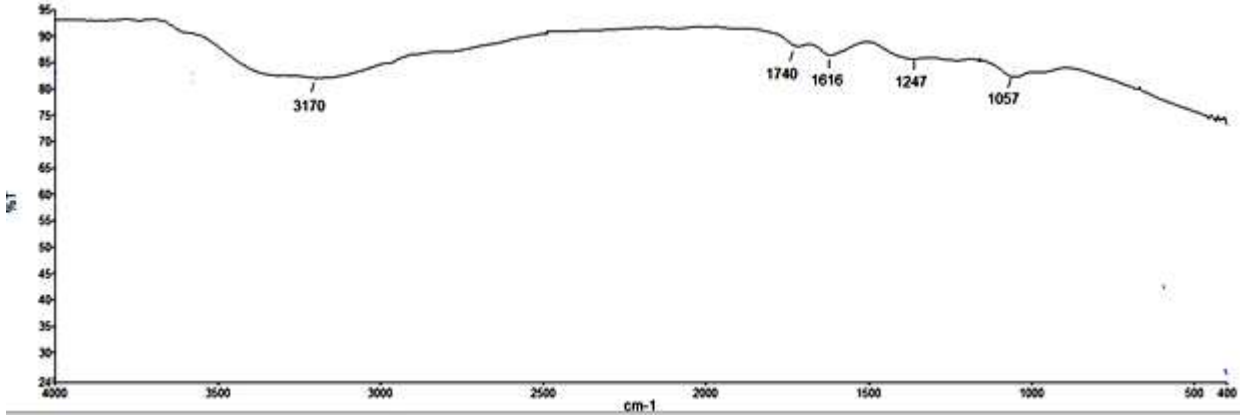
For the whole pattern fitting, a pseudo-Voight peak profile was used, after background subtraction. After the calculations, the mean crystallite size of mrGO was estimated at 3±1nm.

4.1.2 FT-IR Spectroscopy of magnetic Graphene based nanocomposites

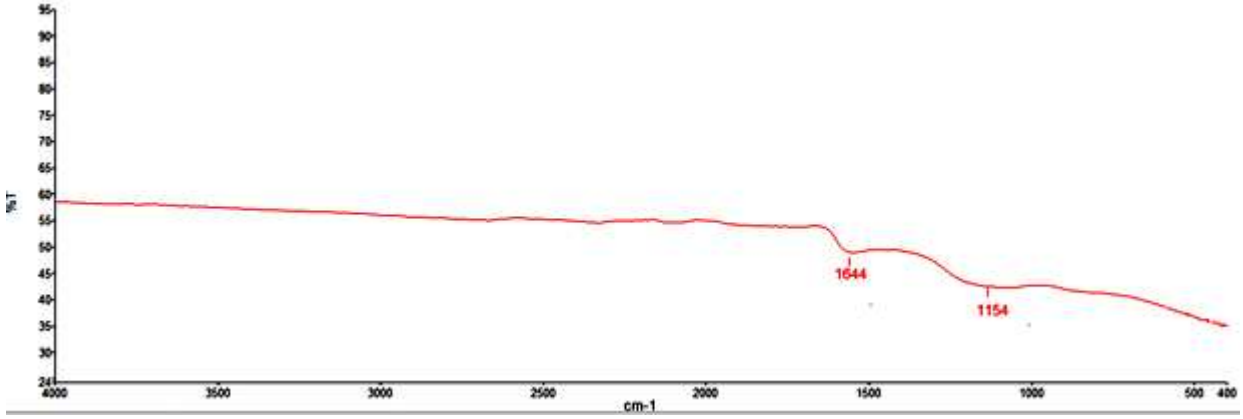
Fourier Transform Infrared spectroscopy (FT-IR) spectra provide information about the functional groups of the synthesized materials. The FT-IR spectra of the prepared GO based materials are shown in Fig.5. GO and mGO spectra are characterized by a broad peak at 3170 cm^{-1} and 3210 cm^{-1} respectively, which is assigned to the O-H stretching vibrations of the hydroxyl group. The peak at 1740 cm^{-1} correspond to the carbonyl or carboxyl groups (C=O) and that at 1616 cm^{-1} refers to the aromatic C=C bonds. Moreover, the C–O stretching vibrations of epoxy group and alkoxy are observed at 1247 cm^{-1} and 1057 cm^{-1} respectively [415]. These characteristic bands in GO spectrum are attributed to the oxidation process which has introduced strong oxygen containing functional groups in the initial graphite. On the other hand, all these absorption bands related to oxidized groups are significantly diminished or even disappeared in the FT-IR spectrum of rGO (Fig.4.3b), indicating the successful reduction of graphene oxide. In the FT-IR spectrum of mGO (Fig.4.3c), adsorption bands that characterized GO spectrum were also observed but the positions of the bonds are slightly shifted and sharpness of the peaks is changed indicating the change in the coordination environment of various functional groups in mGO [641]. Additional peak observed at 1396 cm^{-1} can be assigned to O-C=O carboxyl bonds (COO-symmetric vibration). Finally, in the low frequency region, a new peak appeared at 555 cm^{-1} which corresponds to the stretching vibration of the Fe–O, implying that Fe_3O_4 is attached with the COO— on the edge of the GO [564]. As far as concerns FT-IR spectrum of mrGO

(Fig.4.3c), in accordance with rGO, the decrease of peaks attributed to oxygen containing functional groups confirms the reduction. The peaks at 1227 cm^{-1} and 1121 cm^{-1} correspond to the epoxy and alkoxy groups, while the peak at 1574 cm^{-1} is attributed to the aromatic C=C bonds. Finally, like mGO, the existence of Fe_3O_4 is confirmed from the strong band at 557 cm^{-1}, characteristic for Fe–O bond.

(a) GO



(b) rGO



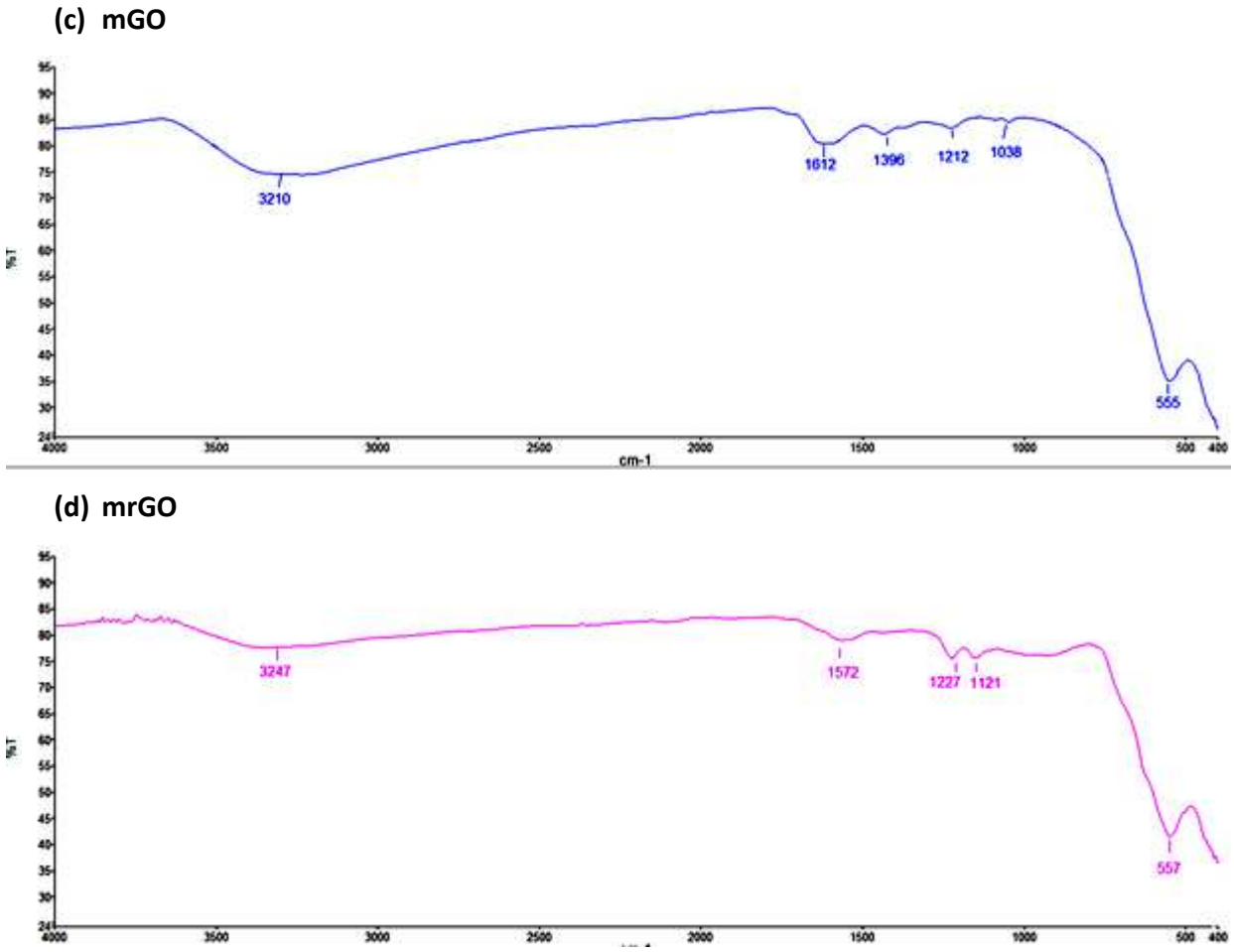


Figure 4.3. FT-IR spectra of (a) GO, (b) rGO, (c) mGO, (d) mrGO

4.1.3 Scanning electron microscopy (SEM) of GO and mrGO

Figure 4.4 portrays the SEM images of Graphene Oxide (GO) at two magnitudes. Morphology of GO is observed as thin randomly orientated crumpled sheets reflecting its layered structure. The characteristic wrinkled sheets of GO maintain a large surface area providing many adsorption sites. No amorphous or other kinds of crystallized phase particles is observed. On the other hand, in SEM image of mrGO (Fig.4.5), some particles are distinguishable on graphene layers and are attributed to the attached Fe_3O_4. Their shape is irregular spherical and decorate heterogeneously in bunches on graphene layers due to some agglomeration occurred. In any case microspheres are well integrated with GO sheets, showing the successful synthesis of mrGO.

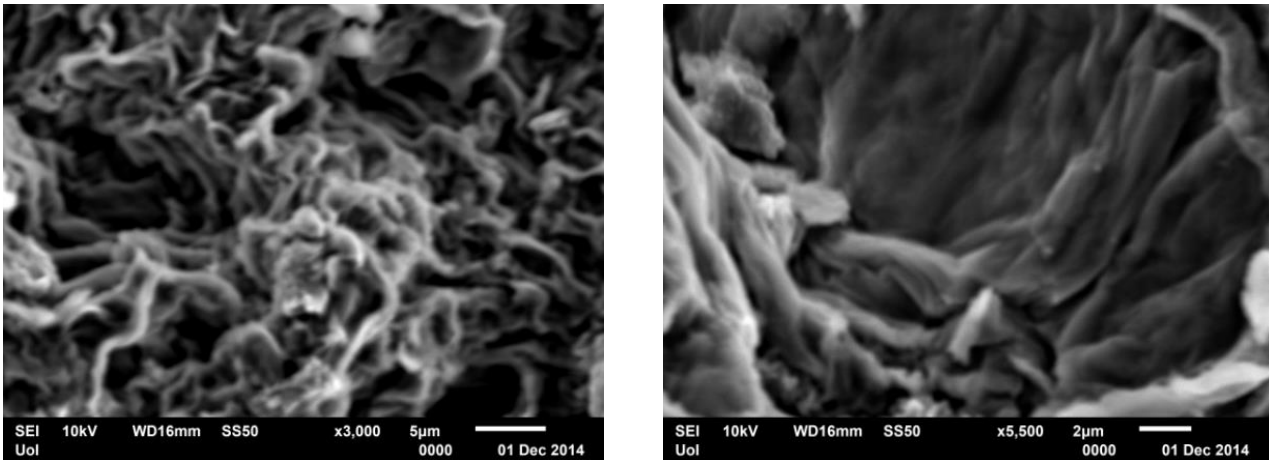


Figure 4.4. SEM Images of Graphene Oxide (GO) at two magnitudes

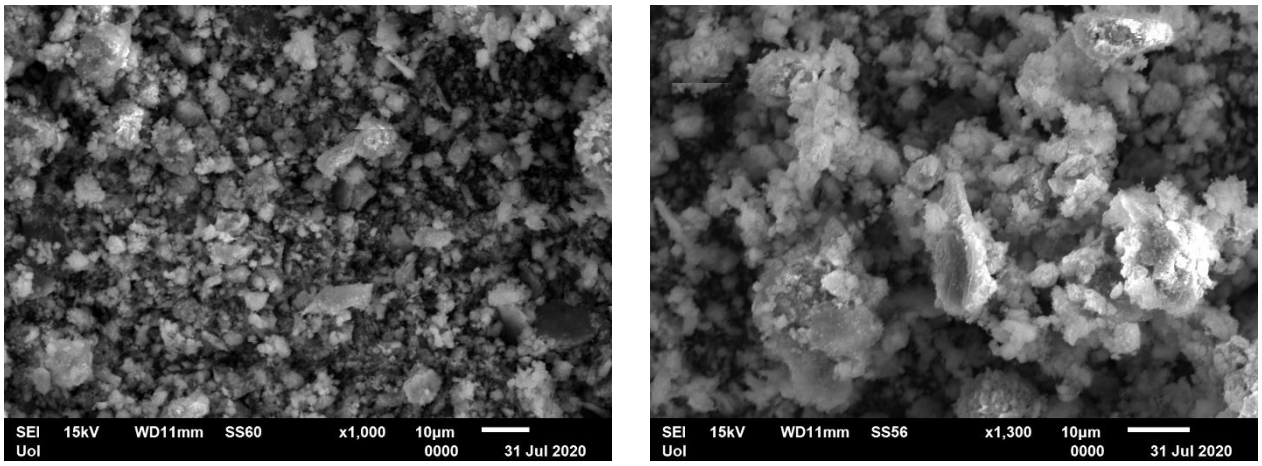


Figure 4.5. SEM Images of magnetic reduced Graphene Oxide (mrGO) at wo magnitudes

4.2 Fe_3O_4@SiO_2@C18 Magnetic nanoparticles (C18-MNPs)

The synthesized MNPs (Fe_3O_4, Fe_3O_4@SiO_2 and Fe_3O_4@SiO_2@C18) were characterized in terms of surface morphology ,crystalline structure and chemical modification of the surface including XRD, SEM, Elemental analysis (EA) and FT-IR. Magnetic properties were also evaluated.

4.2.1 Scanning electron microscopy (SEM) of MNPs

Figure 4.6 illustrates the SEM images of MNPs (Fe_3O_4, Fe_3O_4@SiO_2 and Fe_3O_4@SiO_2@C18). In the SEM image of bare Fe_3O_4 (Fig.4.6a) an aggregation of particles is occurred since the specific surface area is large resulting in a high surface energy [642]. After salinization of Fe_3O_4, and coating with C18 group the

aggregation was reduced and the nanoparticle dispersion was improved resulting in a nearly spherical shape (Fig. 4.6 b,c). Elemental analysis of Fe_3O_4@SiO_2@C18 NPs showed a carbon content of 7.05%

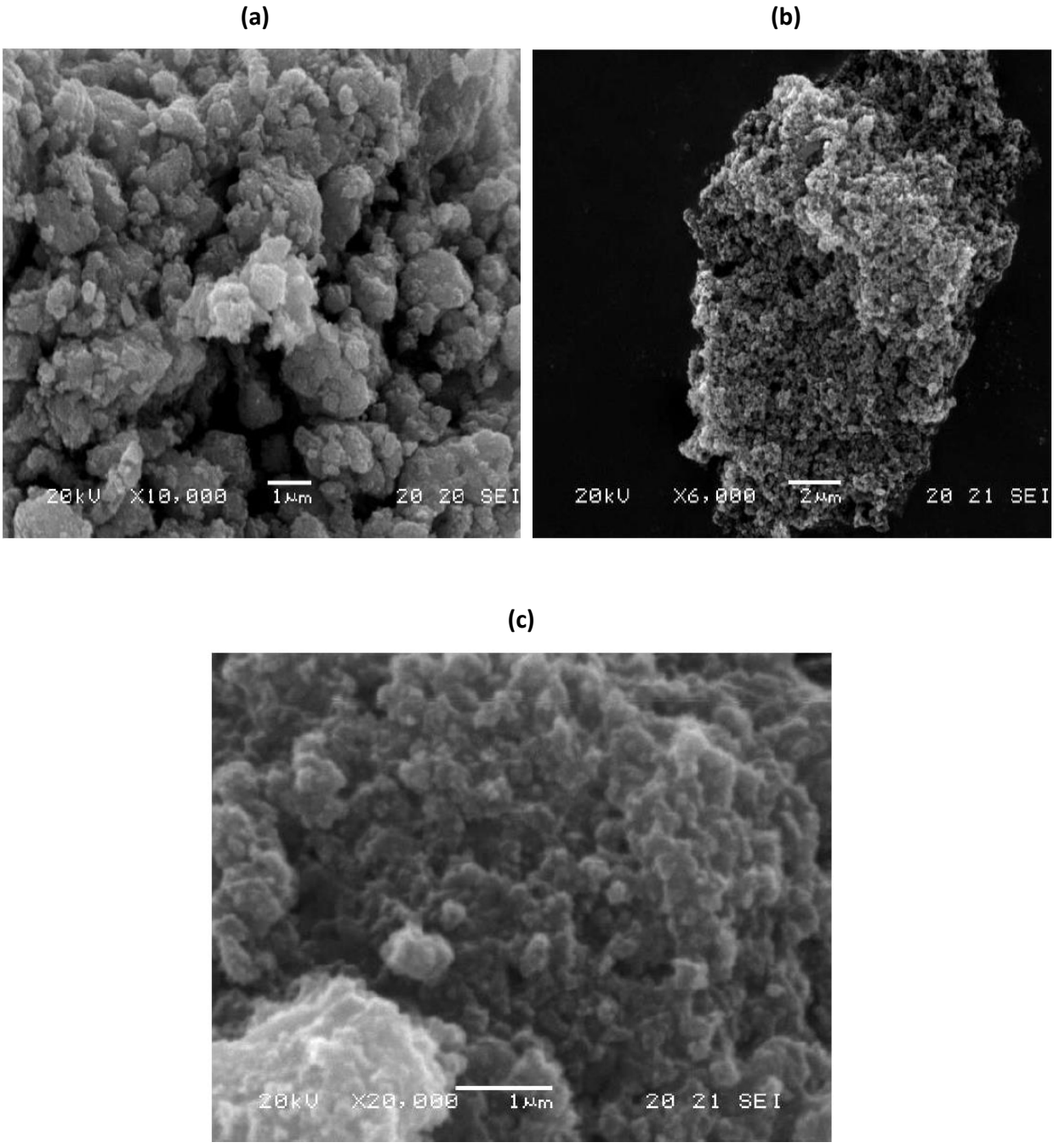
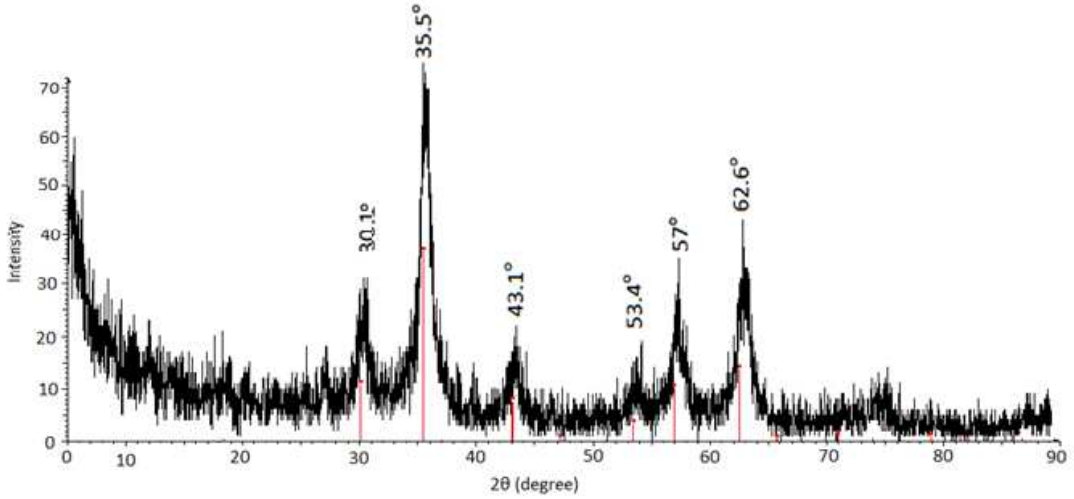


Figure 4.6. SEM images of (a) Fe_3O_4, (b) Fe_3O_4@SiO_2 and (c) Fe_3O_4@SiO_2@C18

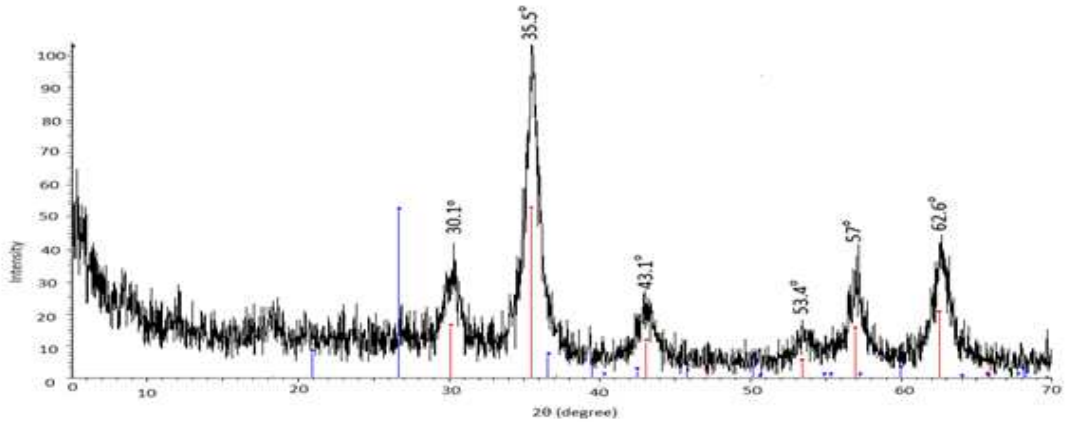
4.2.2 X-Ray Diffraction analysis

Figure 4.7 shows the X-ray Diffraction analysis of MNPs. The XRD patterns of Fe_3O_4@SiO_2@C18 present characteristic peaks at 2ϑ of 30.1◦, 35.5◦, 43.1◦, 53.4◦,57.0◦, and 62.6◦ .These peaks are identical to all synthesized composites and match the structure of cubic spinel phase of Fe_3O_4. That means that spinel structure of Fe_3O_4 remains unchanged with the introduction of silica coating and modification with C18 group.

(a)



(b)



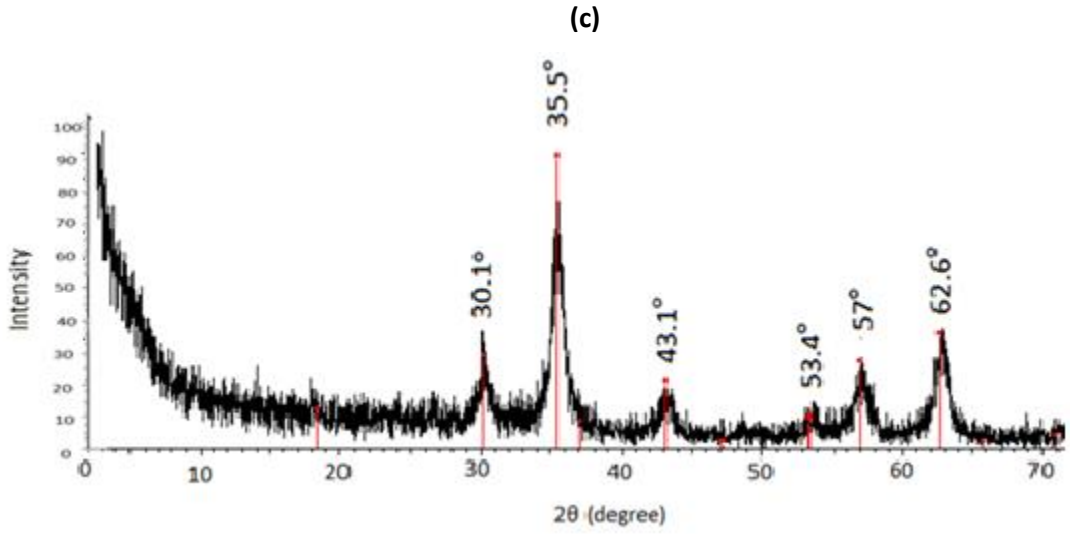


Figure 4.7 XRD spectra of (a) (Fe_3O_4, (b) Fe_3O_4@SiO_2 and (c) Fe_3O_4@SiO_2@C18)

4.2.3 FT-IR Spectroscopy of magnetic nanocomposites

The successful fabrication of silica coating and functionalization with C18 was ensured with FT-IR spectroscopy. The band at 570 cm^{-1} (Fig. 4.8a) represents the stretching vibration Fe- O- Fe of pure magnetic iron oxide. However, on FT-IR spectra of modified magnetite the absorption intensity of Fe–O group decreases with the addition of silica portion and a strong adsorption peak appeared at 1068 cm^{-1} (Fig. 4.8b and c) , attributed to the Si -O- Si vibration. Peaks at 1634 and 3400 cm−1 may be assigned to absorbed water or the silanol groups (Si- OH) of the silica. These peaks indicate the formation of a silica layer on the surface of Fe_3O_4. The characteristic peaks at 2928 and 2854 cm^{-1} (Fig. 2c) differentiate the Fe_3O_4@SiO_2@C18 NPs from Fe_3O_4@SiO_2. More specifically, they represent the asymmetric and the symmetric extension vibration of CH_2 in the $(CH_2)_{17}CH_3$ chain, respectively, corroborating the introduction of C18 groups on the surface of the magnetite silica nanoparticles [348,570,643].

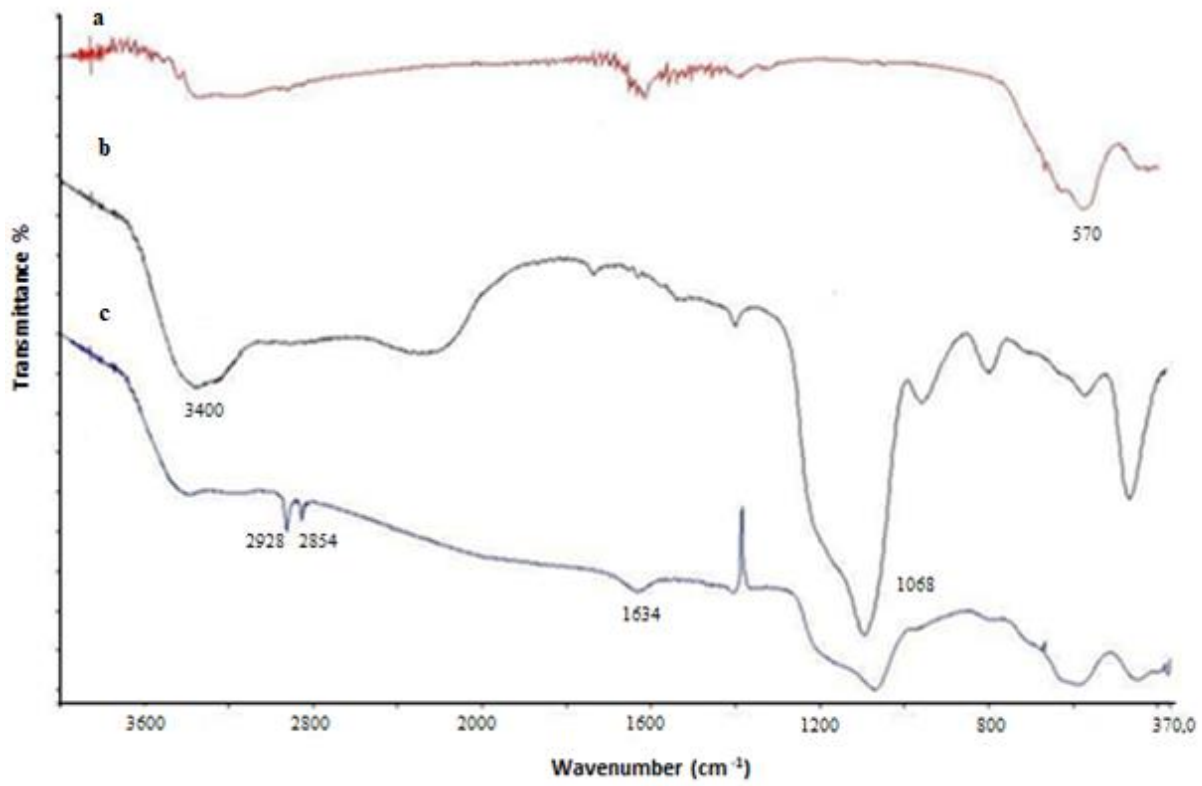


Figure 4.8. FT-IR spectra of (a) Fe_3O_4, (b) Fe_3O_4@SiO_2, (c) Fe_3O_4@SiO_2@C18

4.2.4 Magnetic Properties

The magnetization properties of the MNPs were investigated at room temperature by measuring the magnetization curves of Fe_3O_4 and Fe_3O_4@SiO_2@C18 (Fig.4.9). The magnetization curves were S-like curves and presented zero coercivity and permanence indicating this way the superparamagnetic properties of the synthesized nanocomposites. The saturation magnetization values were almost 60 Am^2/kg for Fe_3O_4 and 52 Am^2/kg for Fe_3O_4@SiO_2@C18, respectively. The value of Fe_3O_4@SiO_2@C18 is considerably lower than the value of the pure Fe_3O_4 due to silica coating with C18 group and smaller percentage of magnetite. However, the saturation magnetization was strong enough to ensure a convenient magnetic separation from aqueous solutions, under an external magnetic field within a few seconds.

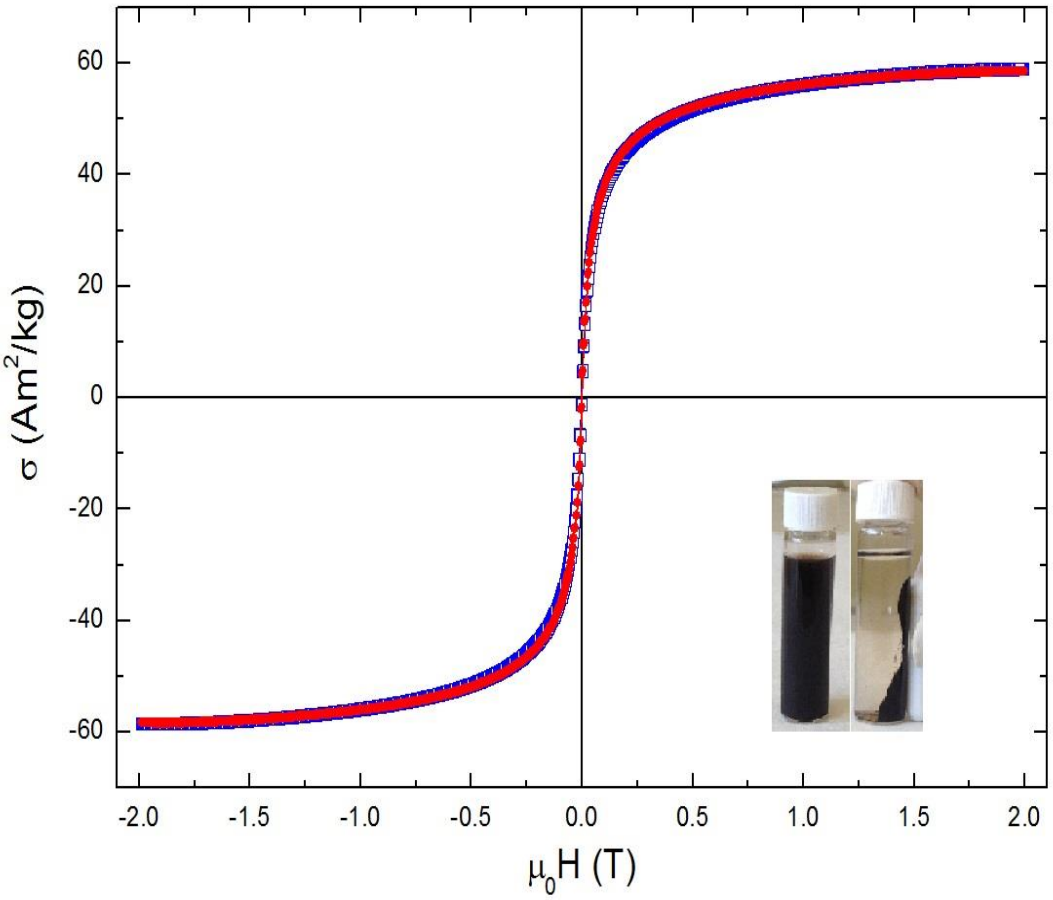


Figure 4.9. Magnetization curves of Fe_3O_4 and Fe_3O_4@SiO_2@C18

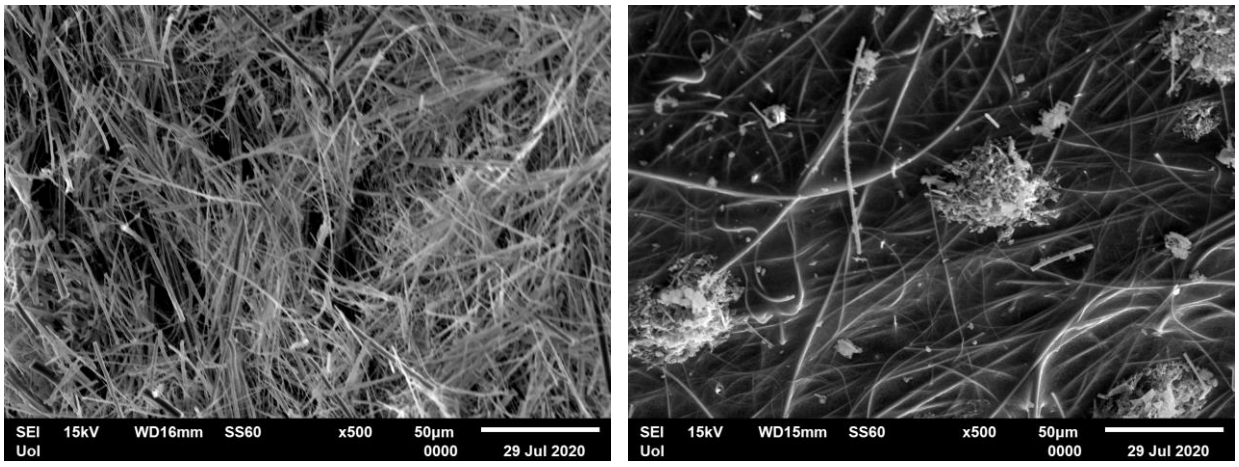
4.3 Sol–gel PEG coated Fiber Glass FPSE media

Techniques such as scanning electron microscopy (SEM), Fourier transform-infrared spectroscopy (FT-IR), elemental analysis were employed for the characterization of sol-gel sorbent-coated FPSE media. SEM provides details of the surface morphology of the fabric substrate, before and after the sol-gel sorbent coating. FT-IR spectra provides information on the chemical incorporation of different sol solution ingredients into the sol-gel sorbent network as well as integration of sol-gel sorbent network with the fabric substrate.

4.3.1 Scanning electron microscopy (SEM) of the substrate and the sol–gel PEG FPSE media

Figure 4.10 represents scanning electron micrographs of the commercial uncoated material of glass fiber (FG) in two magnitudes of x 500 and x 150 (Fig 4.10 a, c), and the sol-gel PEG coated FPSE media at x 500 and x 100 magnitude respectively (Fig. 1b,d). It can be observed that uncoated media has characteristic fibroid structure which is well preserved even after the sol-gel coating. The presence of sol-gel coating is visible and is described as a thin blurred film that covers the fibers of the pristine material. Excess of PEG particles is also observed. Moreover, this fact is confirmed from the energy dispersive X-ray (EDX) analysis of sol–gel PEG coated which identified the presence of Carbon. Specifically, in Fig.4.11a are observed peaks of carbon (C), oxygen (O) and silicon (Si) with atomic percent values of 44.98, 18.64 and 36.37, respectively. From this data it is obvious that organic components (in the form of methyl and polyethylene moieties from MTMS precursor and PEG organic polymer) has been successfully integrated into the inorganic silica network. EDX spectrum of uncoated fiber glass is also presented (Fig.4.11b) for comparison reasons showing the expected composition of uncoated fabric (O, Si, with atomic percent values of 50.05 and 49.95 respectively).

(a) **(b)**



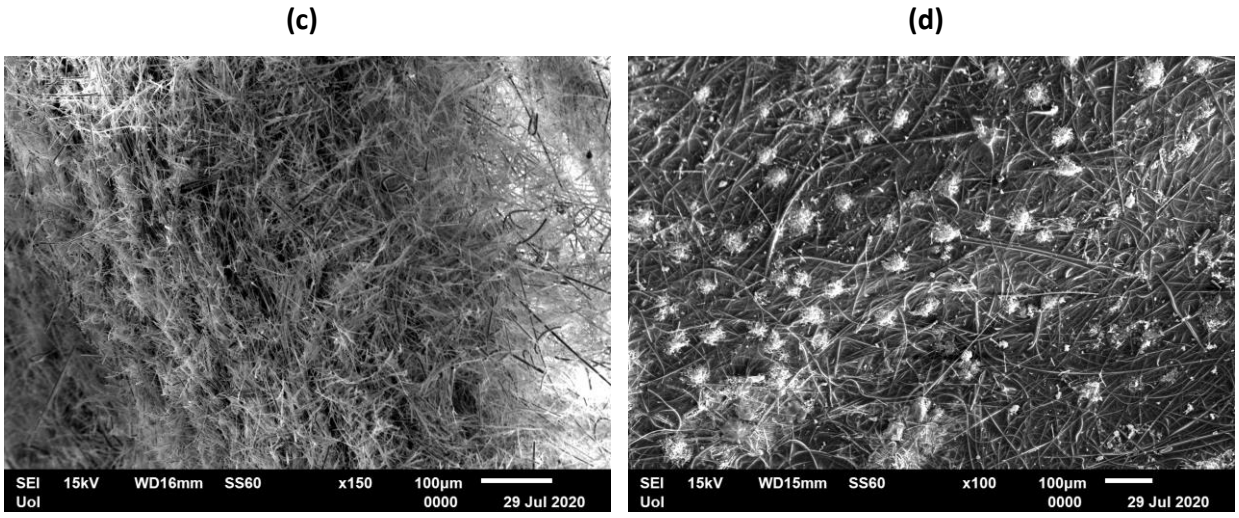


Figure 4.10. SEM mages of (a)uncoated fiber glass at 500 x magnifications; (b) PEG coated FPSE media at 500 x magnifications; (c) uncoated fiber glass at 150 x magnifications; (d) PEG coated FPSE media at 100 x magnifications

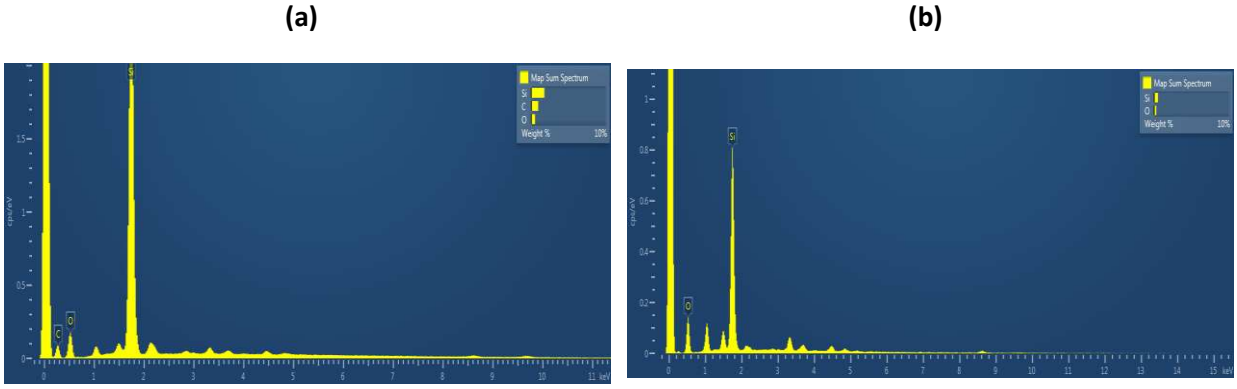


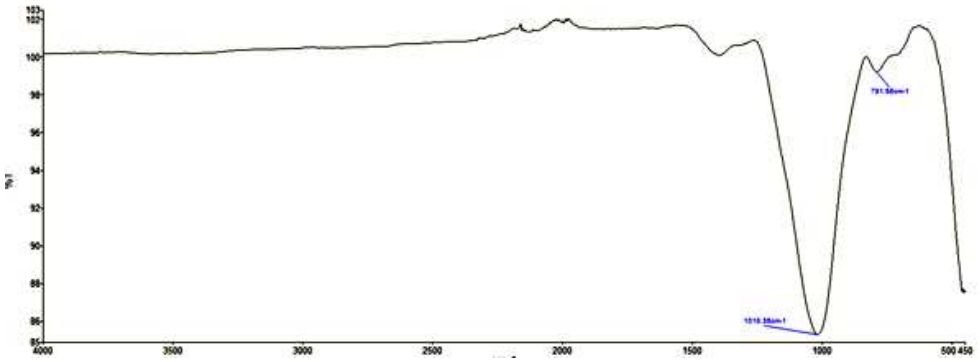
Figure 4.11. Energy Dispersion X-ray (EDS) analysis of (a) sol-gel PEG coated fiber glass, (b)uncoated fiber glass

4.3.2 Fourier transform-infrared spectroscopy (FT-IR)

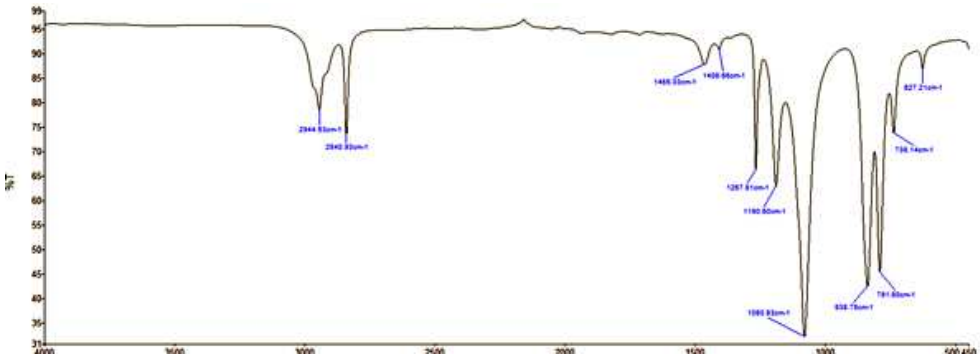
The FT-IR spectra of uncoated Whatman fiber glass substrate, sol–gel precursor methyltrimethoxysilane (MTMS), commercial short-chain poly (ethylene glycol) polymer (PEG300) and sol–gel PEG coated FPSE media are presented in Fig.4.12. The uncoated fiber glass presented a characteristic peak at 1016.3 cm^{-1}, which corresponds to Si—O—Si bending vibration, confirming the siloxane nature of the fabric [644]. On the MTMS FT-IR spectrum the sharp peak observed at 2944.53 cm^{-1} is attributed to Si—O—C—H band of the precursor. The peaks at 2840.93 cm^{-1}, 1190.60 $cm^{-1}$and 1080.83 cm^{-1} were associated to Si-O-CH_3 bonds, while the bands at 1267.81 cm^{-1}, 838.78 cm^{-1}, 791.80 cm^{-1} correspond to the vibration of CH_3 group of methyltrimethoxysilane [572,574,645]. From the FT-

IR spectrum of polyethylene glycol the band appeared at 3431.37 cm^{-1} is characteristic of O—H stretching mode [646]. Peaks of poly (ethylene glycol) also included 2866.05 cm^{-1}, 1454.70 cm^{-1}1, and 1349.25 cm^{-1}, which represent different vibration modes of C—H bonds while the peak at 1249.93 is assigned to C—C double bond [647], [574]. Finally, the FT-IR spectrum obtained from the sol–gel PEG coated fabric, demonstrated a systematic decrease in their absorption bands, around 3400 cm^{-1} and 2800 cm^{-1}, which may be attributed to the presence of sol–gel PEG [442,648] Moreover, several bands (e.g. symmetrical deformation vibration of Si—CH_3, at 1271 cm^{-1}, 3401.05 cm^{-1}, 2875.83 cm^{-1}, stretching vibration of Si—CH_3 at 858.14 cm^{-1}) appear simultaneously in PEG polymer and MTMS precursor as well as in the final sol-gel coated material, indicating the inclusion of both blocking materials into the fabric substrate via covalent bonding [574]. Moreover the displacement of bands in sol– gel poly(ethylene glycol) coated FPSE media towards higher wavenumber values (2866.05 cm^{-1}->2875.83 $cm^{-1}$and 1267.81 cm^{-1}-> 1271.14 cm^{-1}) compared with the other spectra, implies that the resulting sol–gel structure is more compact than its pristine polymer PEG and precursor MTMS [574], [649]. Finally, the shoulder of the band at 1018.77 cm^{-1} (CO stretching) may originate from SiO_2 network formation [650]. Any of the evidence above, implies the successful incorporation of sol–gel PEG network to the glass fiber fabric.

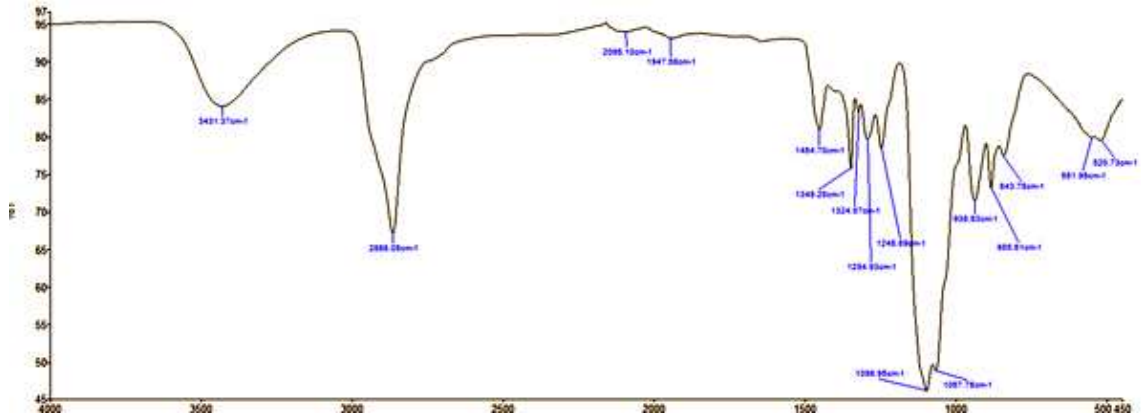
(a) Uncoated Fiber Glass



(b) MTMS Precursor



(c) PEG Polymer



(d) FG sol-gel PEG coated

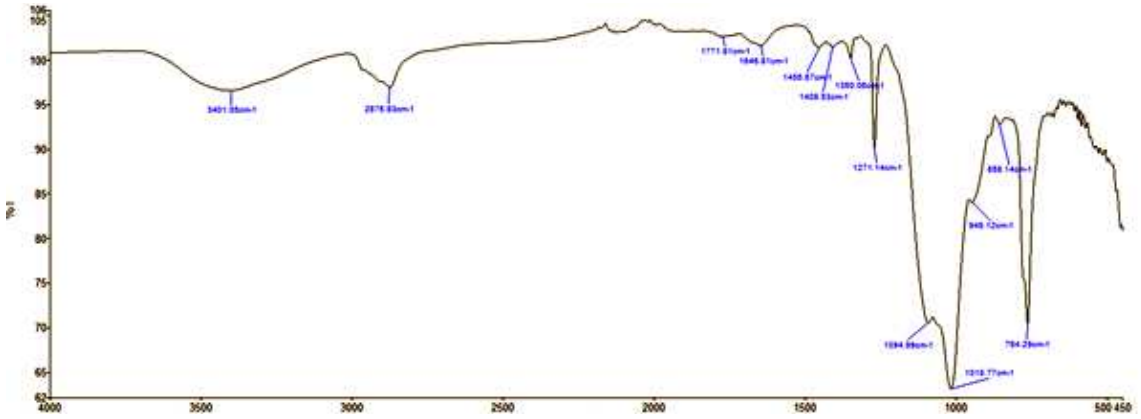


Figure 4.12. FT-IR spectra of **(a)** uncoated Whatman fiber glass substrate, **(b)** sol–gel precursor methyltrimethoxysilane (MTMS), **(c)** commercial short-chain poly (ethylene glycol) polymer (PEG300) and **(d)** sol–gel PEG coated FPSE media

CHAPTER 5 : OPTIMIZATION & VALIDATION OF MAGNETIC SOLID- PHASE EXTRACTION (MSPE)

A. Optimization of Magnetic Solid phase Extraction

Some preliminary absorption experiments were conducted to investigate the sorption capacity of the synthesized materials on the target Emerging Contaminants (ECs). All adsorption experiments were performed in a series of 15-mL tubes containing 10 mg of each magnetic sorbent (Fe_3O_4@GO and C18@SiO_2@Fe_3O_4). Ten (10) mL of aqueous pharmaceutical standard solution with initial concentration of 50 mg/L at neutral pH was used. All mixtures were placed in a shaker platform and were mixed for one hour in room temperature. After one hour the adsorbents were isolated from the solution using a strong magnet, and an aliquot of 5µL and 10 µL was injected in a UHPLC-Orbitrap-MS system. Fig.5.1 shows the adsorption performances of the magnetic nanoparticles (MNPs), Fe_3O_4@GO and Fe_3O_4@SiO_2@C18, towards selected analytes. All the experiments were conducted in triplicate. The average values were used.

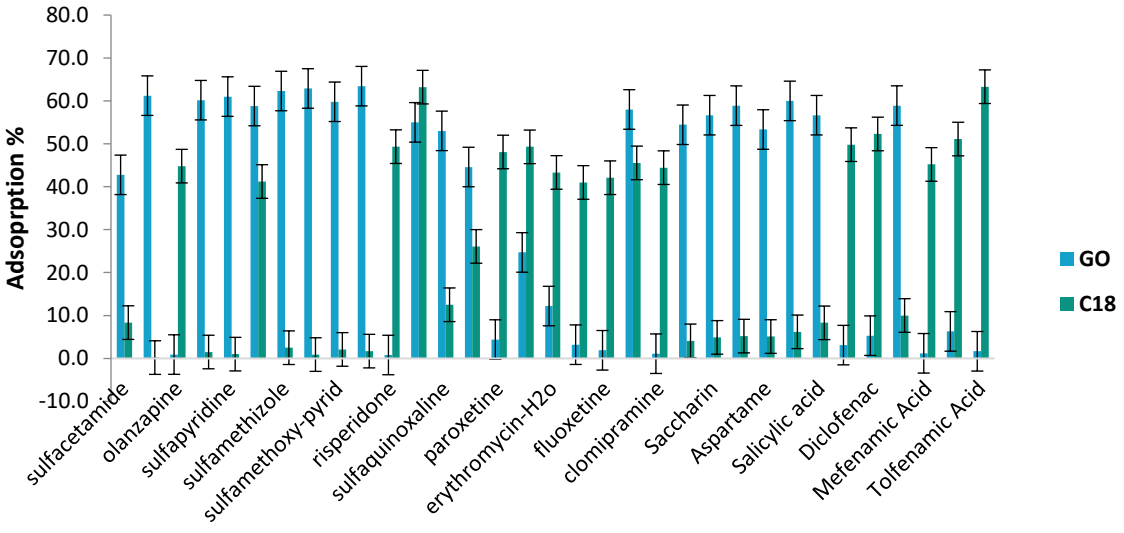


Figure 5.1 Adsorption of target analytes in Fe3O4@GO and Fe3O4@SiO2@C18

$Fe_3O4@SiO_2@C18$ favors the adsorption of non-polar compounds due to the function of hydrophobic octadecyl group while Fe_3O_4@GO provided mostly disappointing results. On the contrary, Fe_3O_4@GO favors the adsorption of polar compounds. Hydrophilic functional groups, such as hydroxyl and carbonyl, increase the affinity of graphene with polar compounds [651]. The extraction performance of these sorbents in relation to the polar analytes was in line with the relative polarity of the sorbents ("like dissolves like" principle). Only two compounds, trimethoprim and carbamazepine had adequate adsorption efficiency in both materials.

For this reason, two different protocols were established for the determination of emerging contaminants in wastewater using two varied materials as sorbents in the Magnetic Solid Phase Extraction. In this Chapter, the development, optimization, and validation of the MSPE for the two diverse groups of analytes is described. Table 5.1 presents the sorbents and the corresponding analytes for each MSPE technique.

Table 5.1. Selection of magnetic sorbents for the extraction of investigated analytes

Compounds	MSPE_Fe_3O_4@GO	MSPE_Fe_3O_4@SiO_2@C18
Acesulfame	✓	
Amitriptyline		✓
Aspartame	✓	
Carbamazepine	✓	✓
Clomipramine		✓
Cyclobenzaprine		✓
Diclofenac		✓
Erythromycin-H2O		✓
Florfenicol	✓	
Fluoxetine		✓
Gemfibrozil	✓	
Indomethacin		✓
Mefenamic acid		✓
Olanzapine		✓
Oxolinic acid	✓	

Paroxetine		✓
Risperidone		✓
Saccharin	✓	
Salicylic acid	✓	
Sucralose	✓	
Sulfacetamide	✓	
Sulfadiazine	✓	
Sulfamethazine	✓	
Sulfamethizole	✓	
Sulfamethoxazole	✓	
Sulfamethoxy-pyridazine	✓	
Sulfapyridine	✓	
Sulfaquinoxaline	✓	
Sulfathiazole	✓	
Tolfenamic acid		✓
Triclosan		✓
Trimethoprim	✓	✓
Venlafaxine		✓

5. 1 Optimization of MSPE-Fe_3O_4@GO

One-variable-at-a-time optimization approach was employed for the optimization of MSPE conditions. All optimization experiments were conducted in aqueous solutions fortified with nineteen pharmaceuticals of 200ng/L. The optimum conditions established were subsequently applied and tested in waste waters. Parameters such as the sample pH, sorbent amount, elution solvent, extraction and elution times, sample volume were investigated to achieve optimal extraction efficiencies for the nineteen selected pharmaceuticals. Optimal conditions are displayed in Table 5.2.

5.1.1 Effect of the pH

The pH of the working solution is a critical parameter because it affects the existing forms of the analytes and the surface charge of the sorbents and thus affects the extraction efficiency. To evaluate

the effect of sample pH on the Efficiency Recoveries (ERs) for target analytes it was examined in a range of 2.0–11.5. The studied emerging contaminants have different properties (pKa) and belong in different therapeutic and chemical classes, so they follow different adsorption rules according to pH variations. Most analytes studied with graphene oxide were sulfonamide antibiotics, then sweeteners, quinolones and macrolides, psychiatric drugs, and analgesics.

Concerning sulfonamide antibiotics, they contain two different ionizable functional groups, one aromatic amine (able to gain a proton) and a sulfonamide group with an acidic nitrogen atom (able to release a proton), thus alteration to the pH alters the form of these ionizable groups [652]. As shown in Fig.5.2 the ERs gradually rise with increasing pH from 2.0 to 3.0 and then decrease with the further increase of pH from 4.0 to 6.0. At pH 6.5-7.0 a minor increase is presented while the adsorption recovery of sulfonamides (SAs) decreased considerably when the pH peaked the range from 8.5 to 11.5. Optimum pH for SAs adsorption is 3.0-3.5; At pH values close to 3.0-3.5, the neutral and positively charged form of SAs co-exist according to their dissociation constant and speciation diagrams The respective MQLS for the analyzed compounds presented variations from 1.2 and 90.5 ng/L for tap ater (Appendix). In addition, the speciation of GO functional group changes as well with pH variations. Typically, alkyl carboxyl (COOH) functional group has p*K*a of 4.5. Thus, at pH of 3.0-3.5, almost all carboxyl groups are protonated with natural charge (-COOH) [653]. Considering the above, electrostatic interactions do not play principle role in the adsorption of SAs in this case. At pH values where the neutral form dominates, the adsorption is significantly higher, indicating hydrophobic interactions between the neutral form of SAs and GO. The surface of magnetic graphene oxide is rich in hydroxyl and carboxyl groups, which assist the interaction between the sorbent and the SAs through strong stacking π-π hydrophobic interaction [397,398,425,654–656]. At pH values higher than the pKa (pH>8.5) mostly negative charged form of SAs exists in aqueous solution. The deprotonation decreases pronouncedly the π-withdrawing ability of p-amino sulfonamide ring and further suppresses the π–π electron interactions with the graphene π-donor structures [653]. As a result, the SAs could not be efficiently adsorbed onto the sorbent and consequently the extraction recoveries were decreased. The pH effect on recoveries of ECs is depicted in Fig.5.2. The adsorption of gemfibrozil, carbamazepine, sucralose on the GO@Fe3O4 is not affected by pH and recovery value is almost constant in the wide pH range, while oxolinic acid, aspartame, acesulfame, saccharin had higher retention on the sorbent when the neutral form existed in aqueous solution at pH 3.

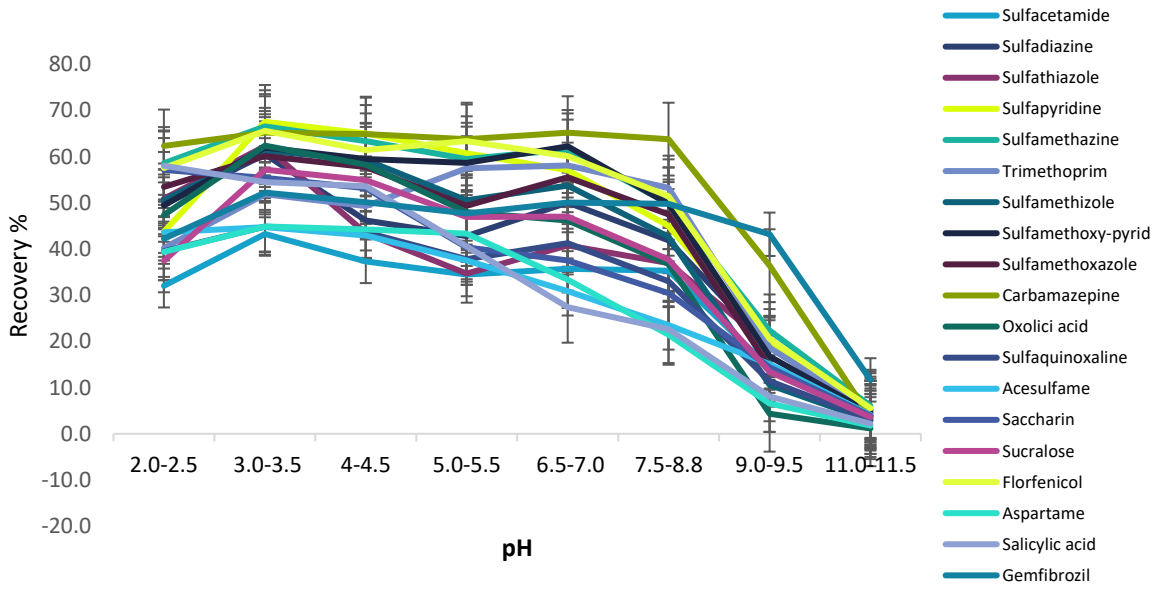


Figure 5.2. Effect of pH solution on recoveries of ECs, (n=3)

5.1.2 Amount of GO@Fe_3O_4

To acquire the maximum extraction efficiency of the target analytes, different amounts of the magnetic graphene (2.5, 5, 10, 15, 20, 30 mg) were assessed. As shown in Fig.5.3, the recoveries of the analytes increased with increasing sorbent doses from 2.5 to 15 mg, due to enhancement in the surface area and active sites, and then stabilized with no further increases. Large amounts of sorbent are useful for extraction but are inconvenient for the removal of the desorption solvent. Thus, 15 mg was employed in the next experiments.

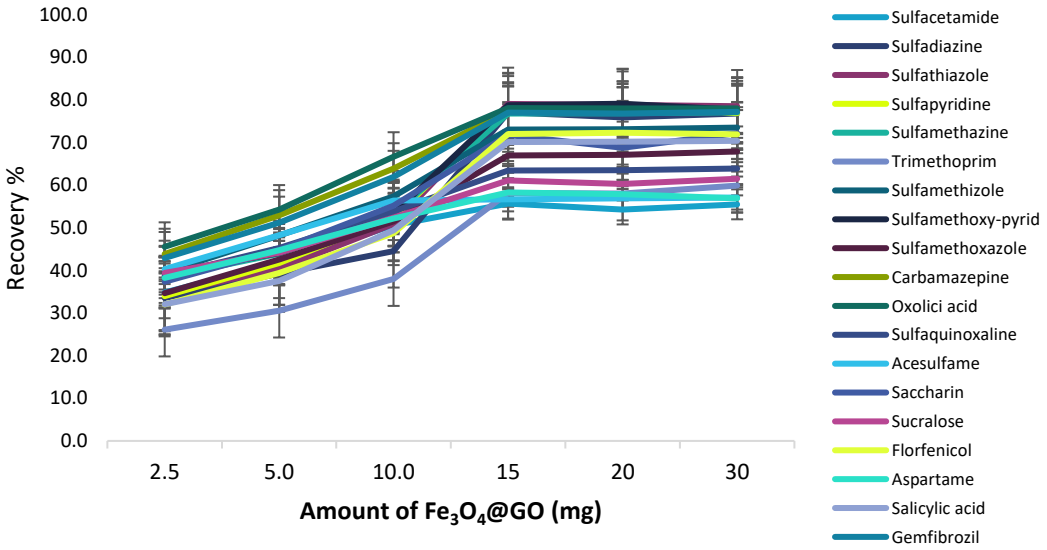


Figure 5.3. Effect of the amount of Fe_3O_4 @GO on recoveries of ECs

5.1.3 Effect of extraction time on Fe_3O_4 @GO-MSPE

To enhance extraction efficiency, the selection of extraction time is important after the sorbents are dispersed into the solution. A good dispersion of adsorbent is beneficial to the improvement of adsorption efficiency in the least time, due to the large contact surface area between the adsorbent and analytes in water sample. In this study, magnetic stirring was firstly used to assist the extraction of target analytes. The effect of stirring time on the extraction efficiency was investigated in the range of 1–50 min at ambient temperature. The results in Fig. 5.4 showed that the extraction efficiency increased by increasing extraction time from 1 to 10 min, and then the upward trend became slower gradually in the following five minutes. The extraction reached equilibrium and the recoveries of analytes nearly reached a maximum value when extraction time was 15min. Therefore, 15min of extraction time was selected for subsequent experiments.

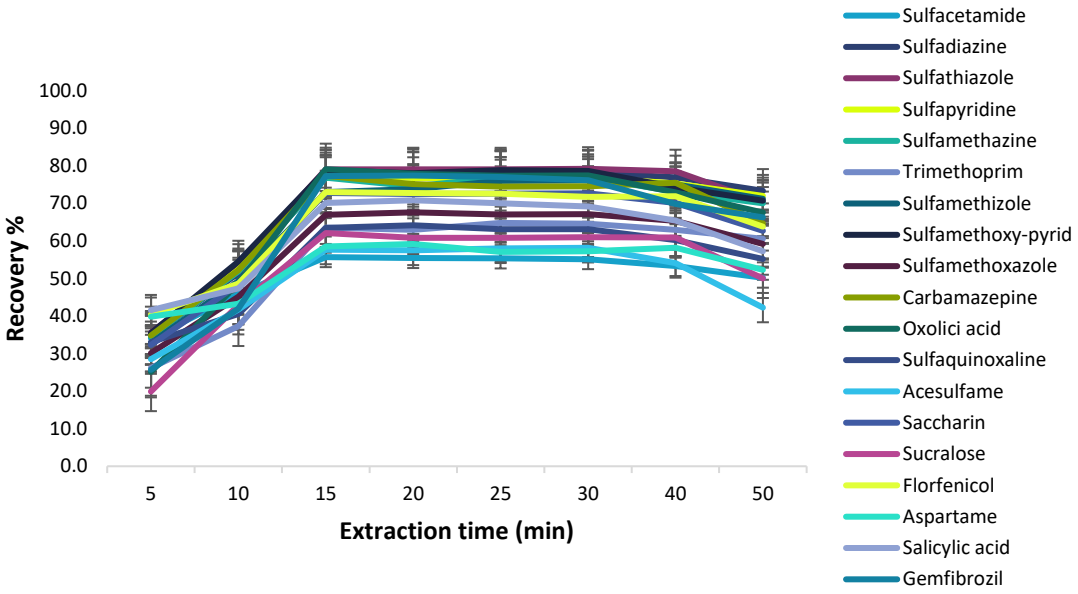


Figure 5.4 Effect of the extraction time on Fe_3O_4 @GO-MSPE

5.1.4 Type of desorption solvent

Following the adsorption, the implication of a proper organic solvent for the elution and desorption of the analytes from the sorbent is a crucial factor in MSPE. As the adsorption of target analytes onto the sorbent is based on π-π and hydrophobic interactions, several elution solvents of different polarities were considered (methanol, acetone, and acetonitrile) to break down the above

interactions. The results in Fig.5.5 showed that the efficiency of MSPE reached the maximum when methanol was used as the elution solvent under the same extraction and desorption conditions. To increase the desorption efficiency, taking into consideration the pH dependency on sorbent, formic acid or ammonia was added at various percentages (1–5% v/v) to increase the acidity or alkalinity. The addition of formic acid (f.a) was not more efficient than pure methanol in most of the compounds while on the other hand the addition of ammonia significantly increased the recoveries of the extraction. This is reasonable and in accordance with the results from the pH optimization of adsorption process as well as with the physicochemical properties (PKa, logD) of the compounds, since it was found that alkaline environment does not favor the adsorption, resulting in an increase in the desorption efficiency. The effect of ammonia percentage in methanol was also studied in the range of 0–5 % (v/v). The result indicated that the desorption efficiency of methanol containing 1% ammonia was superior to pure methanol. Increasing the percentage of ammonia higher than 1 %, the ERs of SAs decreased slightly. This is probably because SAs will be less positive charged in an alkaline medium, resulting in weakening the affinity with sorbent [657]. Thus, methanol containing 1% ammonia (v/v) was selected as the optimum elution solvent.

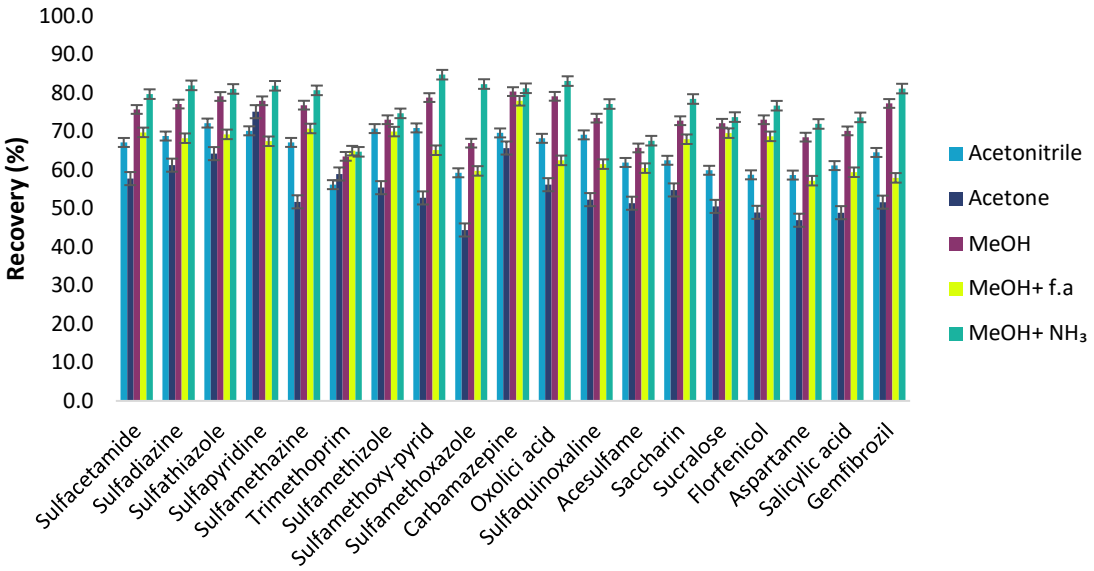


Figure 5.5. Effect of the desorption solvent on recoveries of ECs

5.1.5 Elution volume and desorption time

The volume of elution solvent and elution time are also principal factors to obtain reliable and reproducible analytical results. The desorption efficiency of target analytes increased with the increase of the desorption solvent volume in range of 0.5–5.0 mL, and no obvious changes were observed when the volume was further increased from 4.0 mL. It was observed that all the analytes could be nearly completely desorbed from the sorbent by sonication for 1 min with 2.0 mL desorption solvent for two times. The desorption time from 60 s to 240 s under sonication was studied. The result indicated that the sonication time had no clear effect on the eluting efficiency after 120sec. Thus, the desorption time selected was 120min (2x60sec) and the solvent volume was 4 mL (2x2mL).

5.1.6 Effect of sample volume

In order to obtain the maximal extraction efficiency and lower MQLs the effect of the sample volume on the extraction efficiency was examined under the optimal conditions (10 mg of adsorbent, pH 3.0, 15 min of extraction time, 2 mL of methanol containing 1% NH_3 and 60 s sonication twice. The different sample volumes (10–100 mL) investigated were containing 1 µg of target analytes. As shown in Fig.5.6, the target analytes provide satisfactory recoveries in sample volume from 10.0 to 50.0 mL, however when the sample volume was above 50 mL the extraction efficiency was reduced significantly. This may occur since larger sample volume results to more adsorbent loss during the recovery process. Consequently, 50 ml of sample solution volume was selected.

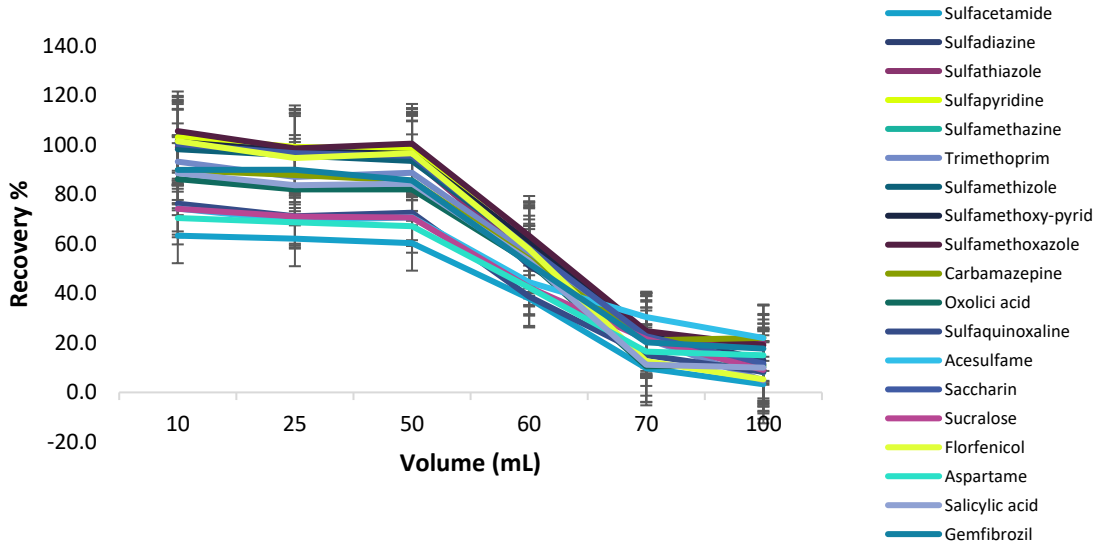


Figure 5.6. Effect of sample volume on Fe_3O_4 @GO-MSPE

5.1.7 Ionic strength

Generally, increasing the ionic strength of a solution results in an improvement in the extraction efficiency when salting-out effect plays a crucial role in the procedure. The effect of ionic strength on the adsorption of target analytes was examined using NaCl at concentrations ranging between 0 and 10% (w/v). Fig. 5.7 shows the effects of increasing ionic strength (via the addition of NaCl). Contrarily with the general speculation, a pronounced decrease in the extraction capability was noticed when NaCl was used. In this case, as the salt content increases, cation- π interactions between sodium cations and the aromatic rings of the majority of the compounds and especially SAs or the aromatic rings of the graphene may contribute to the overall efficiency. Moreover, an increase in the viscosity of the solution is probable (due to an increase in the electrostatic attraction between water layers) decelerating the percolation of the solution into the pores of graphene's sheets resulting in lower mass transfer[658,659]. Another reason that high ionic strength reduced the sorption of target analytes on Fe_3O_4@GO, could be attributed to the aggregation of GO sheets in the solution. It has been reported that high ionic strength can promote the aggregation of GO sheets [660],[661] and thus reduction of sorption analytes can be expected [653]. As a result, no adjustment of ionic strength of the media was undertaken in subsequent analyses.

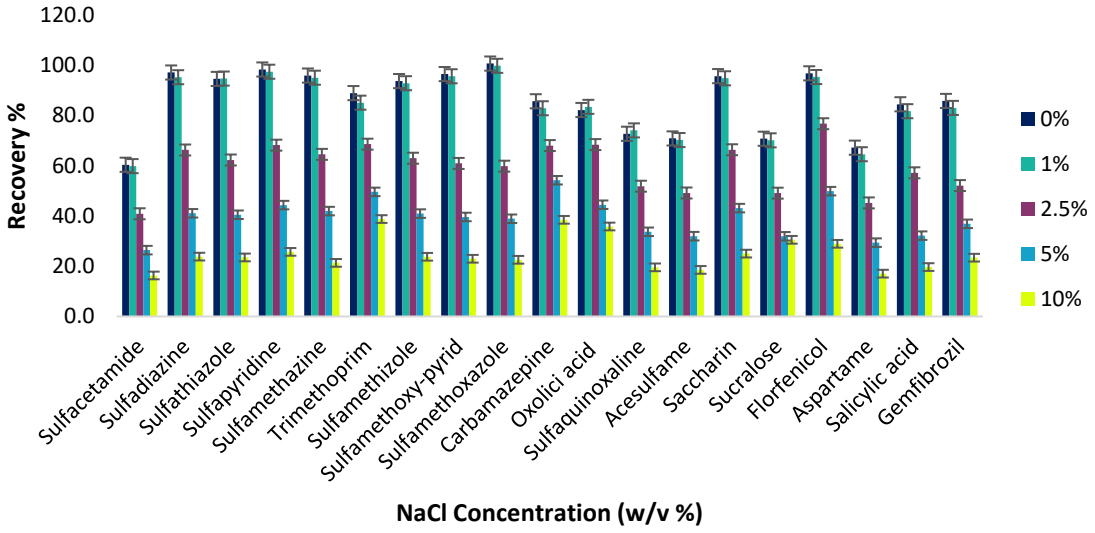


Figure 5.7. Effect of ionic strength on Fe_3O_4 @GO-MSPE

5.2 Optimization of MSPE @C18

5.2.1 Effect of pH

The investigated range of pH, from 2.0 to 12.5 ,was studied by setting the extraction time at 60 min and using 1 ml of MeOH as desorption solvent for 30 min. The target analytes selected for the adsorption on C18@SiO_2@Fe_3O_4 have different pKa ranging from 3.88- 15.52 and it is expected that the pH would have different effect on them. Most analytes are basic compounds with amine groups in structure, and pKa values from 8.0-9.0. Extraction efficiency starts to increase above pH 2.0, while above pH 7.0 the compounds are almost constant indicating increasing partition on C18-MNPs due to the increasing van der Waals forces with C18 groups. The analytes are supposed to show decreasing ionization tendency with the increase of pH, especially at extremely alkaline pH. However, as seen in Fig.5.8 adsorption of analytes is possible even in lower and neutral pH, probably due to the possible residual silanol groups on sorbent, which can exhibit hydrogen bond or dipolar interaction with the analytes in weak acidic or neutral medium, and cationic exchange interactions in strong acidic medium. Concerning the analytes with pKa 3.8-4.0 (Diclofenac, Indomethacin, Mefenamic acid, Triclosan, Tolfenamic acid), showed similar sorption trends on sorbent. Specifically, they presented maximum adsorption at pH 3.0-3.5 as expected from the pKa values, since the analytes exist in molecular form in acidic pH facilitating the extraction process. The ERs seem to be constant when pH 7.0 is reached, with slight decrease and minimum recovery of 58.1% (Indomethacin) in this area. On contrary, we observe important decreasing ERs in alkaline pH, due to the total ionization of the analytes and the less hydrophobic character of the compounds which does not promote the adsorption on the C18 sorbent. In view of the normal pH circumstance of wastewater close to neutral, pH 7.0 was chosen for the following experiments since the majority of analytes can maintain the original states and the recoveries of the extraction are satisfactory as well.

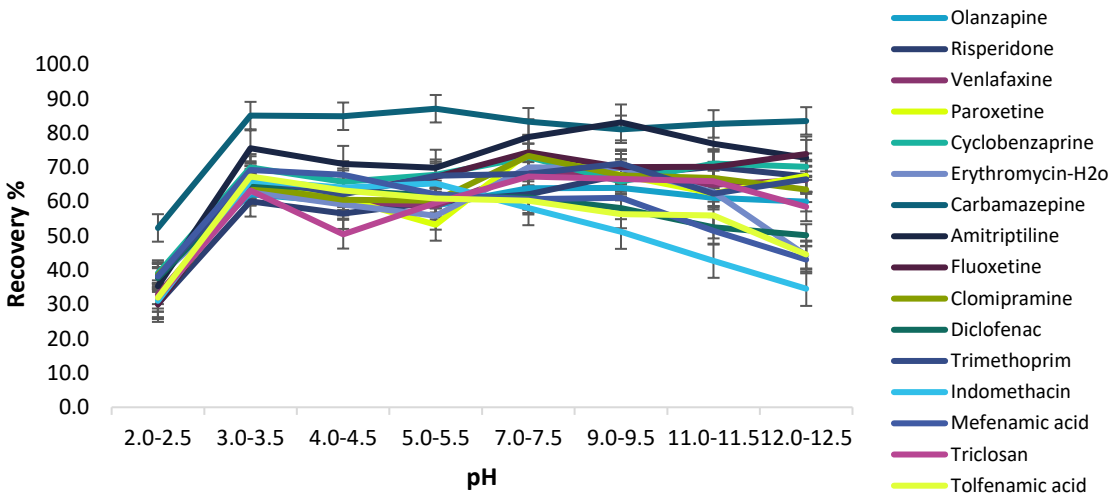


Figure 5.8. Effect of pH solution on Fe_3O_4 @SiO_2@C18-MSPE

5.2.2 Amount of Fe_3O_4@SiO_2@C18

The adsorbent amount was also investigated. Different amounts of the MNPs-C18 from 2.5 to 30 mg, were tested to optimize the amount of sorbent. As can be seen in the extraction efficiency profile shown in Fig.5.9, the mean recovery for all the analytes increased with an increase in the amount of sorbent from 10 up to 30 mg, while mefenamic acid kept a stable trend and thereafter leveled off. This could be explained by the fact that by increasing the amount of sorbent, the area required for the adsorption of the analytes is increased as well, up to a certain level. As can be deduced from Fig.5.9, the application of 10 mg sorbent provided the most satisfactory recoveries for all the analytes. Therefore, 10 mg was the optimum amount and used for the next studies.

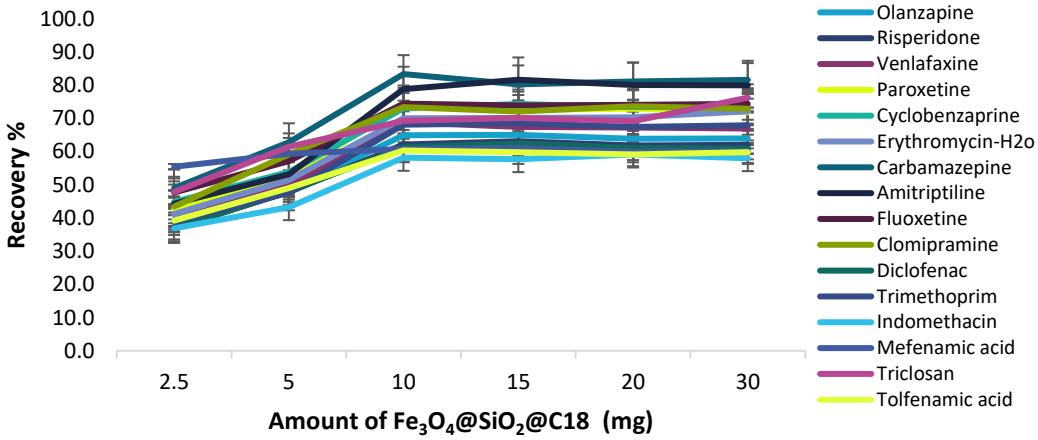


Figure 5.9. Effect of the amount of Fe_3O_4@SiO_2@C18 on the recoveries of ECs

5.2.3 Effect of extraction time on Fe_3O_4@SiO_2@C18 -MSPE

A short extraction time leads to incomplete adsorption of the target substance in the solution onto the adsorbent, nevertheless a long extraction time makes the MSPE process unnecessarily lengthy. Therefore, several experimental tests were performed in the range of 5-50 min. As can be seen in Fig.5.10 the extraction efficiency was significantly increased with the rise in stirring time from 5to 20 min, after which as the time prolonged, no remarkable increase of extraction efficiency was observed. Satisfactory extraction recoveries for the analytes were obtained at 20 min. These experimental data indicate that the adsorption equilibriums were achieved quickly. It may be interpreted that the large surface area of the C18- sorbent and the numerous C18 groups anchored in the magnetic nanoparticles, resulted in a strong hydrophobic interaction between analytes and sorbent, making the distribution equilibrium easy to be achieved in a short time. Therefore, 20 min was chosen as the optimal extraction time and employed for the subsequent tests.

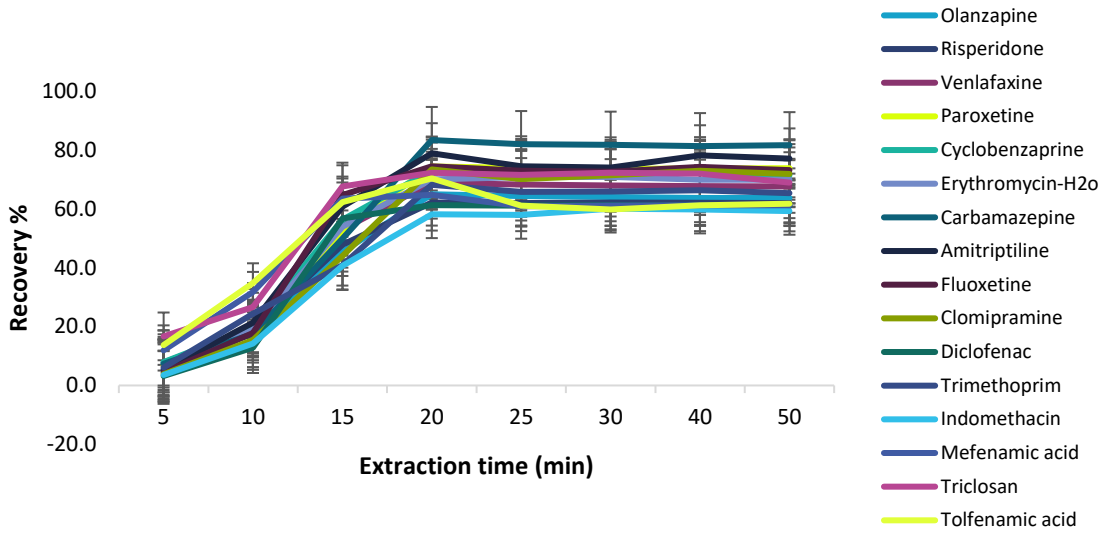


Figure 5.10. Effect of the extraction time on Fe_3O_4@SiO_2@C18 -MSPE

5.2.4 Desorption conditions

A suitable desorbing solvent should effectively elute the adsorbed analytes with the minimum volume without damaging the nature of the adsorbent surface and with less interfering impurities coeluted. Several eluents, such as acetonitrile, methanol, acetone, ethyl acetate, were evaluated to select the best eluent solvent for eluting pharmaceutical compounds from the C18-MNPs. Their recovery

efficiencies were evaluated under the same extraction and elution conditions and depicted in Fig.5.11. Methanol exhibited the highest recoveries and acidity or alkalinity in desorption solvent was further evaluated with the addition of formic acid and ammonium hydroxide solution in various concentrations (0 -5% v/v). The results indicated that methanol acidified with 1.0% v/v formic acid (f.a) provided the highest elution capability for almost all the compounds in comparison with other solvents. Most analytes are in ionic state in acidic FA solution, so reduced interaction occurs with non-polar C18 groups. Therefore, methanol containing 1% v/v f.a was chosen as the elution solvent.

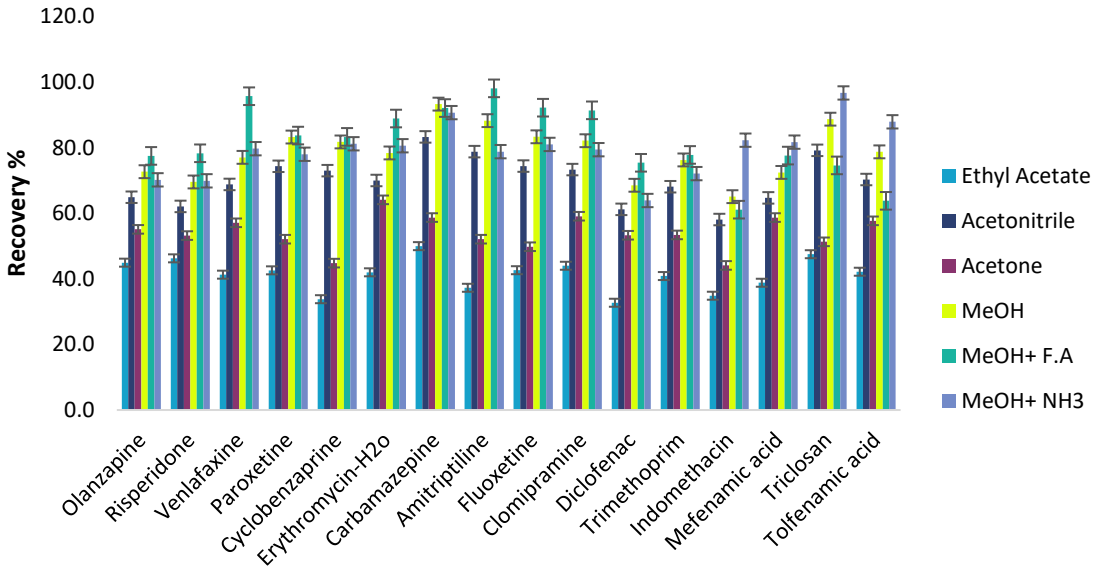


Figure 5.11. Effect of desorption solvent on recoveries of ECs

When methanol containing f.a 1.0% v/v was used for MSPE elution, the elution time and solvent volume was further optimized to achieve the optimal elution effect. The solvent volume increased from 0.5 mL to 2 mL exhibiting two successive elutions each time under 10 min stirring (2x5 over two cycles). Finally, two successive elutions with 1 mL of the solvent were adequate to completely desorb the target analytes from C18 MNPs.

To speed up the desorption efficiency, ultrasonication was applied instead of magnetic stirring. When ultrasonication applied, the maximum recoveries where accomplished within 1min. It was obvious that the ultrasonic-assisted elution was superior to magnetic stirring. Thus, the desorption time selected, was 1 minute.

5.2.5 Effect of Sample Volume

The possibility of enriching low concentrations of target analytes from large volumes of samples was examined by studying the effect of sample volume on the recovery of the analytes. Sample solution volume effect was tested by treating 15 mg magnetic C18 nanoparticles with different sample volumes (10-100 mL) of the standard solutions, each of them containing 1 µg of analytes. As shown in Fig. 5.12, quantitative recoveries of target analytes were obtained with up to 50 mL of sample solution while above 50 mL, the recoveries decreased. Hence, a sample volume of 50 mL was selected as the ideal volume for the extraction of target analytes from C18 MNPs.

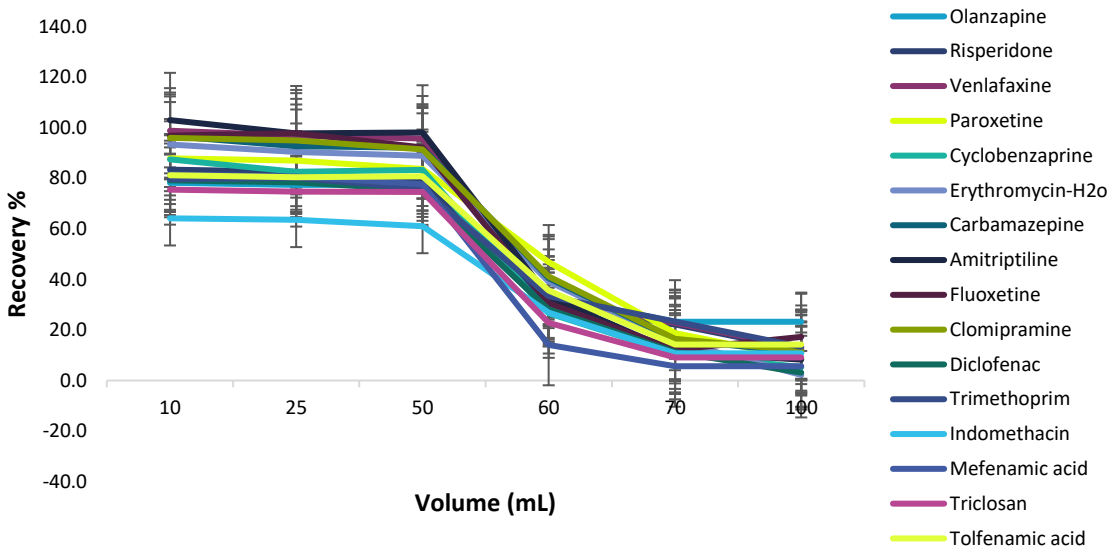


Figure 5.12. Effect of sample volume on Fe_3O_4@SiO_2@C18 -MSPE

5.2.6 Ionic strength

Mass transfer of analytes in MSPE can be highly affected by the ionic strength of the sample solution. The hydrophobic interaction between C18 group and organic compounds could be enhanced by increased ionic strength of the solution. On the other hand, high ionic strength may also weaken electrostatic interactions that may occur. To investigate the effect of ionic strength on the ERs for target analytes, different concentrations of NaCl (0–10%, w/v) were added into the sample solution. The results in Fig.5.13 showed that no obvious improvement was observed for the ERs when the concentration of NaCl is in the range 0-5% w/v. Since the hydrophobic interaction is the main

contributor to the adsorption, the ionic strength has little influence in extraction efficiency of target analytes. Higher concentrations of NaCl resulted to the reduction of the adsorption of analytes onto the sorbent. This can be explained by the fact that by increasing the content of NaCl, the aqueous solution becomes more viscous, and consequently, the transfer of the analytes into the sorbent is hindered. Hence, the ionic strength of water sample was not adjusted in the MSPE process.

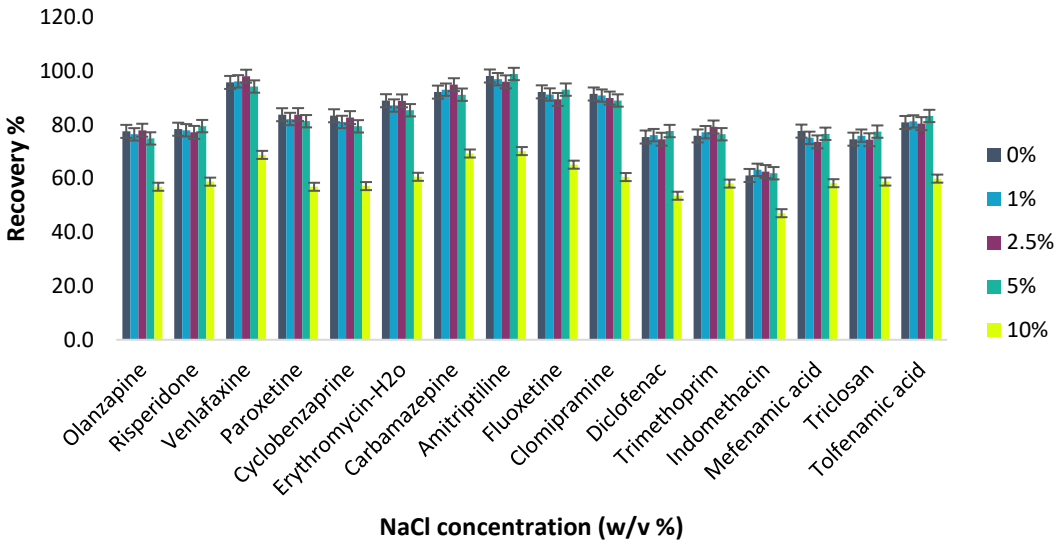


Figure 5.13. Effect of ionic strength on Fe_3O_4@SiO_2@C18 -MSPE

The optimized extraction parameters (Table 5.2) where applied in a pool sample of wastewater from three different sampling stations (WWTP of Ioannina, WWTP of University Hospital of Ioannina and WWTP of Amaliada) as well as in tap water, in order to evaluate the analytical performance of the two methods.

Table 5.2. Optimal parameters of MSPE with Fe_3O_4@GO and Fe_3O_4@SiO_2@C18

Optimized Extraction Conditions	MSPE -Fe_3O_4@GO	MSPE -Fe_3O_4@SiO_2@C18
pH	3.0	7.0
Sorbent Amount	15mg	10mg
Extraction time	15min	20min
Elution Solvent	MeOH +1%NH3	MeOH + 1% f.a
Elution volume-Desorption Time	2x2mL-2x60sec	2x1mL-2x30sec
Ionic Strength	0% NaCl	0% NaCl

B. Validation of Magnetic Solid Phase Extraction (MSPE)

The two methods (MSPE@GO and MSPE@C18) developed for the determination of the selected emerging contaminants in aqueous media were finally validated. Validation studies as well as identification and confirmation of the target compounds were based on the quality control procedures established by the European Union (EU) regulations (EU Commission Decision, 2002)[578]. Validation procedures were carried out in two different samples tap water and effluent wastewater providing different performance criteria such as sensitivity, linearity, precision and reproducibility, accuracy. Matrix effect studies were also evaluated for both aqueous matrices.

5.3. Accuracy

Accuracy of the developed method was checked by recovery experiments. For this purpose, three replicates of spiked tap water and effluent sample at three concentration levels 50 ng/L, 250 ng/L, 500 ng/L (low, medium, high) were analyzed under the optimum conditions. Blank samples (unspiked) were analyzed as well. The concentration levels were selected based on those estimated in wastewater effluent samples from previous studies in Greece [260,662–664]. In the case of effluent samples, due to the fact that unspiked effluent samples already contained some of the compounds, the concentration of the respective unspiked sample (blank) was subtracted from the concentration in the spiked sample and then divided by the spiked level. (Eq.1)

$$R\% = \frac{Cspiked\ exp - Cblank}{Cspiking\ level} \text{x } 100 \qquad \text{Eq.1}$$

Recoveries in the MSPE@GO method ranged from 58.4%-102.6% for effluent water while for tap water recoveries varied from 53.8 to 99.8% for all concentration levels (Table 5.3). Recoveries below 60% where considered in the correction of the concentration in the quantification procedure.

The evaluation of the accuracy experiments in MSPE@C18 method concluded to satisfactory recoveries >60% in all cases for the two matrices. In effluent water recoveries ranged from 61.1% for indomethacin to 98.5% for venlafaxine while tap water provided similar results concerning the minimum and the maximum recovery value (64.9% for indomethacin and 99.5% for venlafaxine) (Table 5.4).

5.4 Sensitivity

Regarding sensitivity, method detection limits (MDLs) and method quantification limits (MQLs) were determined, for tap water and effluent samples, as the minimum detectable and quantifiable amount of analyte with a signal-to-noise of 3 and 10, respectively. Spiked tap water and effluent samples (n = 3) as indicated above, were used for their calculation. Levels of MDLs and MQLs varied depending on the aquatic matrix, and higher values were achieved for WWTP effluent water.

Regarding MSPE@GO extraction, MDLs in tap water ranged between 0.4 and 29.4 ng/L while in effluent wastewaters between 0.6 and 31.2 ng/L The respective MQLS for the analyzed compounds presented variations from 1.2 and 90.5 ng/L for tap water and 1.8-98.7 ng/L for effluent wastewater with higher value for sucralose.

In MSPE@C18, MDLs and MQLs for tap water ranged from 0.3-6.6 ng/L and 0.9-19.8 $ngL,^{-1}$ respectively with lower detection limit for oxolinic acid. Concerning the effluent water, the applied method provided MDLs and MQLs in the range of 0.38-10.9 and 1.1-33.6 $ngL,^{-1}$ respectively with lowest detection limit for oxolinic acid as well.

5.5 Linearity

The linearity of the proposed method was investigated by setting a 10-point calibration curve using linear regression analysis, establishing the adequate lineal range for each compound from MQL to approximately 100xMQL, for tap water and effluent wastewater (covered range 0.5-1000ng/L) In each concentration range, all target compounds exhibited good linearity and calibration curves showed, in all cases, coefficients of determination (R^2) greater than 0.99.

5.6 Precision

The precision expressed as repeatability (intra-day precision), and reproducibility (inter-day precision) in terms of Relative Standard Deviation (RSD_r and RSD_R respectively) was evaluated in tap water samples and effluent wastewater spiked at three concentration levels of MQL, 10 times MQL and 100 times MQL. For intraday precision, results were obtained from the analysis of five spiked samples (n=5) for each concentration level within the same day, while for inter-day precision, spiked samples were analyzed over three days (n=15). RSDs were estimated by dividing the standard deviation by the mean value obtained for each set of concentrations and multiplying it by 100.

Regarding MSPE@GO method, RSDr (n=5) values for intra-day analyses were in the range of 2.1–16.7% and the RSD_R for inter-day (n = 15) values were between 2.6% and 18.1% for tap water. As regards effluent water, the RSDs of the spiked samples were lower than 14% for all the target analytes with only exception salicylic acid (Table 5.3). The higher RSD of salicylic acid observed at the lowest concentration level can be attributed to the elevated concentrations found in the blank samples.

MSPE@C18 method showed good precision for tap water, since RSD_r values (n=5) observed for intra-day analyses, were in the range of 3.8-17.6% in all cases, while RSD_R values (n=15) over different days were below 18.2%. Slightly higher RSDs were displayed for effluent water with intra-day precision (n=5) ranged from 3.9-23.1 and inter-day precision (n=15) below 24.8% for all cases.

5.7 Matrix effect

Matrix effect (ME) is one significant drawback in ESI MS quantitative analysis, because the ESI source is highly susceptible to other components present in the matrix. The ME could be defined as the change in HPLC–orbitrap-MS response of an analyte, by suppression or enhancement of the signal, caused by coeluting matrix compounds, relative to an injection of a pure standard [259],[35]. Natural organic matter, salts, ion-pairing agents, non- target contaminants have shown to be responsible for ion suppression. Therefore, a study to evaluate matrix effects should be included in the method validation, to ensure the reliability of the results obtained. ME, was evaluated for the different aquatic matrices and results are shown in Fig. 5.14 and Fig. 5.15.

To assess the ME in all types of waters, the slope of the matrix matched calibration curve was compared with the slope of the calibration curve prepared in solvent [methanol: ultrapurewater+0.1% f.a (10:90, v/v)]. A blank sample (no addition of standards) was simultaneously assayed to subtract the concentration of the target analytes present in the sample. ME was calculated according to Eq. (2), [259,604,605] respectively.

$$Matrix\ effect\ (\%) = \left(\frac{slope\ matrix{-}matched}{slope\ standard\ solution} - 1\right) x100 \qquad \text{Eq.2}$$

A value of zero indicates that there is no ME, while for positive value there is an ion enhancement signal and for negative value an ion suppression signal. When the values of matrix effect are between +20% and −20% is considered as low matrix effect; when are between −50% and −20% or +20% and

+50% is considered as medium matrix effect; lastly, when the values of matrix effect are less than −50% or higher than +50%, it is considered as high matrix effect [259],[606,607].

From the calculated matrix effect results of the samples extracted with MSPE@GO, it can be assumed that in all studied matrices there is evidence of matrix effect. In general, the matrix effect in tap water was not significant or negligible and expressed in most cases as signal enhancement (-20<ME<20). On the other hand, in effluent water from the total 19 target compounds studied, 14 of them presented positive matrix effect expressed as signal enhancement and 5 of them, signal suppression. However, for the majority of analytes low matrix effect was observed (-20<ME<20) with only exceptions five pharmaceutical compounds oxolinic acid, florfenicol, salicylic acid, carbamazepine and sulfamethoxy-pyridazine which presented medium matrix effect. Positive matrix values of carbamazepine (21.1%) and sulfamethoxy-pyridazine (24.3%) indicated enhancement of the signal, while negative values of oxolinic acid, florfenicol, salicylic acid, implied signal suppression. Salicylic acid showed the strongest matrix effect in this studied data set, with maximum signal suppression of -33.7%.

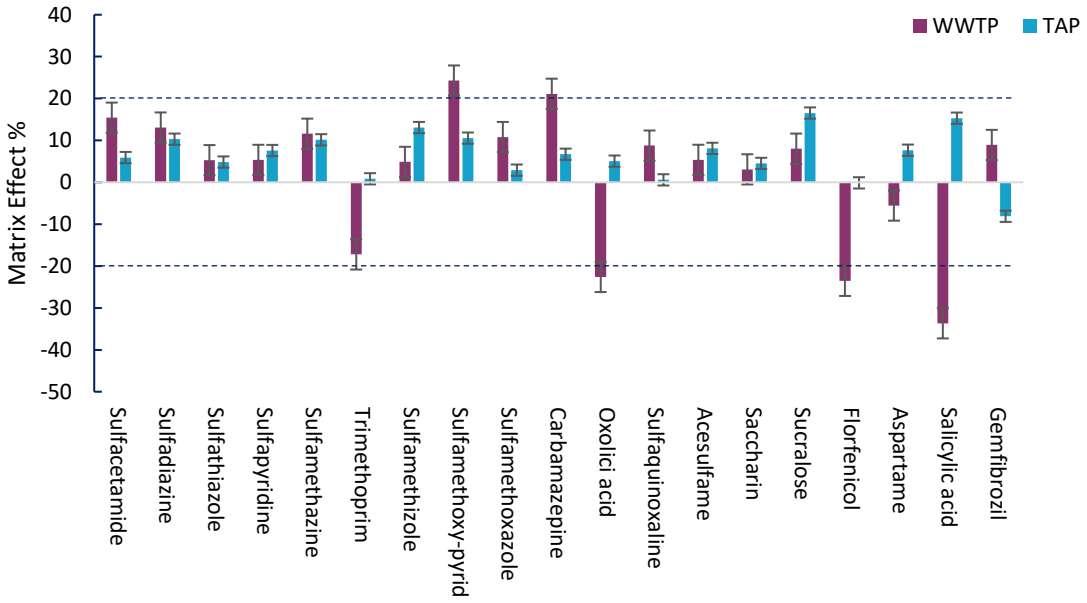


Figure 5.14. Matrix effect for the selected pharmaceuticals in the different aqueous matrices after MSPE@Fe_3O_4@GO

The results of matrix effect for MSPE@C18 showed variations concerning the aqueous matrix as well as the signal enhancement/signal suppression. In tap water, the majority of the tested compounds exhibited low matrix effect, with only exceptions risperidone and mefenamic acid that showed

medium matrix effect expressed as ion suppression with ME values of -20.3% and -25.3% respectively. These high percentages of ME for drinking water still are remaining lower compared with the corresponding ones of effluent water. The values of the matrix effect for effluent water are higher than 20% or less than -20% for 9 out of 16 compounds under study. 5 of them are submitted into ion suppression (venlafaxine, paroxetine, clomipramine, tolfenamic acid), while 3 of the target analytes showed ion enhancement (risperidone, indomethacin, and diclofenac). Specifically, risperidone, diclofenac, venlafaxine, paroxetine, fluoxetine, clomipramine, and tolfenamic acid are subjected to medium matrix effect, while mefenamic acid is being suppressed with a negative value of -55.8% , indicating high matrix effect.

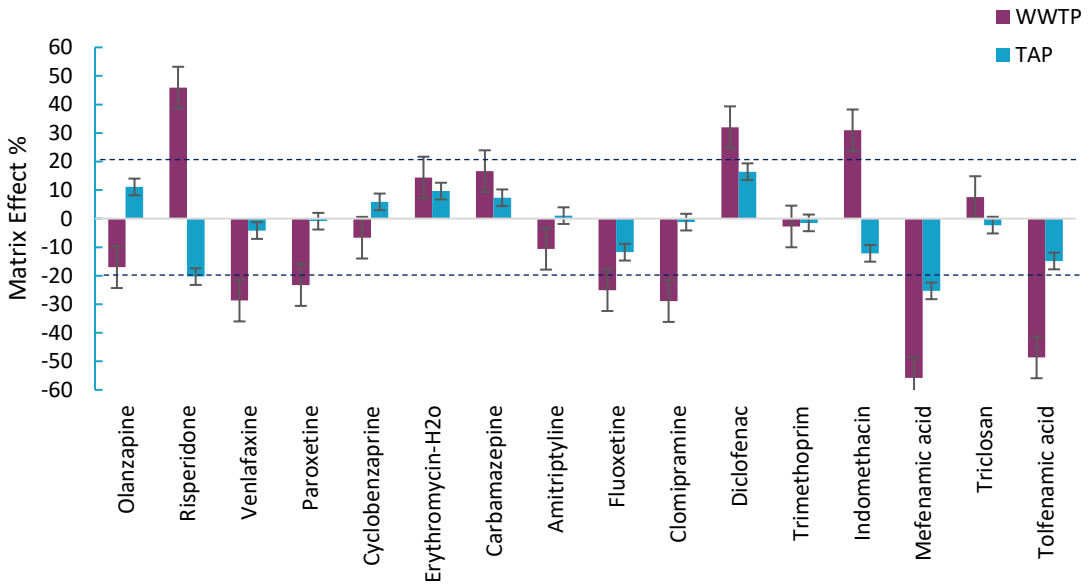


Figure 5.15. Matrix effect for the selected pharmaceuticals in the different aqueous matrices after MSPE Fe_3O_4@SiO_2@C18

Evaluating the general ME observed for the different aquatic matrices in both methods developed, it was noticed that ME were less pronounced (<20%) in drinking water for all of the selected pharmaceuticals while for WWTP effluent, more than 50% of the pharmaceuticals had ME higher than 20%. In fact, due to the high complexity of wastewater, matrix effects were considerably more pronounced in this category. In addition, it can also be assumed that matrix effect is a compound dependent phenomenon, since there are compounds that belong in the same class of pharmaceuticals but still present diverse matrix effects.

5.8 Quality control

Internal quality control was applied in every batch of samples to check if the system is under control. This quality control implies a matrix-matched calibration, a matrix blank and, one spiked blank at 250ng/L. The latter was injected repeatedly throughout the sequence to check the signal level and evaluate stability of the proposed method with time. Quantification of target analytes, based on peak area of $[M+H]^+$ for PI, and $[M-H]^-$ for NI mode, was performed by matrix matched calibration curves, by using effluent water of the corresponding sampling WWTP and tap water. With each batch of 12 samples, a nine-point calibration curve was prepared for analytes concentrations between the method limit of quantification (MQLs) and 100 MQLs. Calibration standards were measured at the beginning and at the end of each sequence. In addition, concerning the monitoring study two quality control (QC) samples were injected in every batch of samples. The QC samples were blank wastewater sample fortified at MQL and10 x MQL of target analytes. Blanks were subtracted and recoveries were considered for concentration calculations. Method performance parameters for both MSPE methods evaluated in two aqueous matrices (WWTP effluent and tap water) are listed in Table 5.5.

Table 5.3. Mean recoveries (%), RSD_r and RSD_R (%) in tap water, and effluent wastewater at three concentration levels with MSPE@GO

	TAP WATER									WWTP EFFLUENT								
COMPOUND	MQL			10 x MQL			100 x MQL			MQL			10 x MQL			100 x MQL		
	R% n=3	RSD_r% n=5	RSD_R% n=15	R% n=3	RSD_r% n=5	RSD_R% n=15	R% n=3	RSD_r% n=5	RSD_R% n=15	R% n=3	RSD_r% n=5	RSD_R% n=15	R% n=3	RSD_r% n=5	RSD_R% n=15	R% n=3	RSD_r% n=5	RSD_R% n=15
Sulfacetamide	57.9	6.9	9.1	58.8	5.1	7.2	59.0	5.8	9.2	59.4	7.6	9.0	60.3	7.1	7.9	61.2	8.9	11.0
Sulfadiazine	92.8	5.8	6.5	93.7	3.1	4.1	94.9	9.0	9.5	92.5	6.1	7.9	97.0	5.8	6.2	97.9	4.2	5.1
Sulfathiazole	95.8	3.5	4.0	96.2	4.5	5.9	100.0	2.8	3.6	93.6	5.3	6.2	94.4	5.4	7.0	99.3	5.9	6.2
Sulfapyridine	97.3	7.9	8.3	98.8	8.4	10.3	99.1	3.6	4.5	94.8	7.2	7.8	98.2	6.9	8.1	102.3	6.4	7.0
Sulfamethazine	84.1	11.2	12.0	84.2	6.9	9.1	84.3	10.8	11.0	93.5	10.1	12.3	95.8	12.3	13.0	97.7	11.0	11.9
Trimethoprim	93.4	2.1	2.6	93.3	3.5	6.3	95.1	3.5	4.1	89.4	2.5	3.9	88.8	1.8	3.0	90.6	2.6	3.2
Sulfamethizole	94.9	5.8	6.2	95.1	6.3	7.8	97.1	6.1	7.2	65.1	5.0	6.1	93.6	6.7	8.1	95.5	5.5	6.1
Sulfamethoxy-pyrid	90.2	6.0	9.1	89.9	2.8	3.9	89.1	4.1	5.9	94.2	3.2	4.0	96.4	4.1	6.3	98.3	5.9	6.7
Sulfamethoxazole	92.1	3.5	6.9	98.7	4.1	5.5	95.8	6.9	7.7	91.2	5.4	6.8	100.6	6.2	6.1	102.6	4.4	4.8
Carbamazepine	85.5	4.1	7.1	90.1	5.6	6.4	92.1	7.0	9.0	84.1	5.9	7.0	85.6	3.1	4.2	89.6	3.6	3.9
Oxolici acid	79.7	5.1	8.1	83.6	5.2	5.8	88.1	5.3	6.5	80.1	3.8	5.5	82.1	2.8	3.5	79.7	2.9	4.0
Sulfaquinoxaline	69.4	4.9	5.5	70.1	4.8	6.9	72.3	6.2	8.2	67.9	6.1	7.2	72.6	9.1	11.2	74.1	8.4	9.2
Acesulfame	70.1	7.5	10.2	72.3	9.0	12.2	75.1	8.0	9.1	68.3	7.0	8.9	70.8	7.8	9.1	66.2	7.2	9.4
Saccharin	92.8	4.1	6.2	94.3	5.3	6.1	92.1	4.9	6.6	90.9	6.5	9.0	95.6	5.4	5.9	97.5	5.5	6.1
Sucralose	77.1	6.8	7.9	79.5	8.1	9.0	73.1	7.0	8.6	71.2	3.2	4.4	70.7	6.9	7.7	70.1	8.9	10.5
Florfenicol	92.5	12.3	15.2	99.8	7.7	8.2	98.4	10.1	13.2	58.4	10.1	8.6	96.7	7.2	8.0	99.6	6.1	7.3
Aspartame	60.0	5.1	5.6	63.3	3.5	4.4	60.2	4.2	5.5	79.6	7.5	25.1	67.1	5.8	6.2	64.5	9.4	10.0
Salicylic acid	95.1	14.5	15.2	96.9	9.5	12.0	96.4	12.9	14.3	77.8	22.4	16.9	84.4	18.4	19.2	86.1	18.9	20.1
Gemfibrozil	84.3	16.7	18.1	88.1	13.5	16.6	86.3	13.1	20.0	59.4	15.8	9.0	85.7	12.8	13.3	80.4	9.2	10.4

Table 5.4. Mean recoveries (%), RSD_r and RSD_R (%) in tap water, and effluent wastewater at three concentration levels with MSPE@C18

	TAP WATER									WWTP EFFLUENT								
Compound	MQL			10 x MQL			100 x MQL			MQL			10 x MQL			100 x MQL		
	R% n=3	RSD_r% n=5	RSD_R% n=15	R% n=3	RSD_r% n=5	RSD_R% n=15	R% n=3	RSD_r% n=5	RSD_R% n=15	R% n=3	RSD_r% n=5	RSD_R% n=15	R% n=3	RSD_r% n=5	RSD_R% n=15	R% n=3	RSD_r% n=5	RSD_R% n=15
Olanzapine	87.1	10.4	11.0	88.4	10.8	12.6	89.8	13.8	15.8	70.1	13.3	15.1	77.5	9.1	12.1	76.1	14.8	18.1
Risperidone	69.5	11.8	14.6	72.1	9.3	10.9	72.6	7.4	7.9	69.2	12.1	13.4	78.3	13.4	14.1	77.3	10.1	11.3
Venlafaxine	99.5	15.6	17.1	99.8	11.2	12.5	99.1	8.9	9.6	98.5	15.1	16.0	95.7	8.4	9.2	92.4	8.7	9.4
Paroxetine	85.7	7.1	8.1	85.7	6.1	6.8	84.1	3.8	4.5	79.5	8.1	9.8	83.7	5.0	6.8	79.9	6.9	7.2
Cyclobenzaprine	82.8	8.5	9.2	84.8	5.8	6.9	83.6	4.9	5.1	81.3	9.4	10.0	83.3	5.2	6.9	84.1	9.2	9.8
Erythromycin-H2o	90.1	4.1	4.6	87.6	4.9	5.1	83.8	5.9	6.9	83.6	5.2	5.9	88.9	8.0	11.0	89.9	5.8	6.8
Carbamazepine	92.4	3.9	4.9	95.8	3.4	4.4	93.8	4.0	6.1	89.4	5.8	7.1	92.1	6.2	6.4	90.1	6.9	11.2
Amitriptyline	90.2	5.8	6.2	94.4	6.9	8.0	96.8	9.8	12.3	96.9	6.3	7.2	98.1	4.1	4.9	93.1	11.1	13.4
Fluoxetine	89.8	5.9	6.9	95.8	6.1	6.9	97.8	5.7	6.8	89.6	4.1	5.9	92.2	10.7	12.3	87.1	4.8	5.5
Clomipramine	92.1	13.4	14.0	96.8	12.8	13.5	99.2	10	10.4	86.7	10.8	13.1	91.4	3.9	4.5	79.8	12.1	13.1
Diclofenac	79.8	10.4	12.0	76.9	9.4	11.0	82.3	6.7	7.7	77.3	15.8	16.4	75.4	9.6	11.2	80.2	16.9	18.0
Trimethoprim	75.1	5.4	5.9	79.5	3.2	3.9	81.1	4.5	5.8	75.1	6.9	7.0	75.8	5.9	6.9	75.9	5.8	7.8
Indomethacin	64.1	3.9	4.2	65.3	5.8	6.1	71.8	7.2	7.6	63.1	7.4	8.1	61.1	15.8	17.2	63.8	4.9	5.3
Mefenamic acid	75.1	14.4	15.0	81.1	10.4	12.3	80.4	8.4	8.9	75.1	19.8	20.3	77.6	17.6	19.3	70.4	17.2	19.0
Triclosan	69.8	9.1	12.8	73.8	11.5	12.5	75.0	8.9	9.8	72.8	8.6	9.5	74.6	7.6	10.8	76.0	5.4	6.9
Tolfenamic acid	79.8	17.6	18.2	87.6	11.2	15.1	82.9	13.1	14.6	79.3	20.2	21.0	80.8	18.0	20.9	83.4	23.1	24.8

Table 5.5. Parameters indicating the performance of the analytical methods MSPE-GO, MSPE-C18. Method detection and quantification limits (MDL, MQL), linearity(R^2) and matrix effect in all matrices studied.

	MSPE@Fe_3O_4@GO									MSPE@Fe_3O_4@SiO_2@C18							
	TAP WATER				EFFLUENT					TAP WATER				EFFLUENT			
Compound	**R^2**	**MDL ng/L**	**MQL ng/L**	**ME %**	**R^2**	**MDL ng/L**	**MQL ng/L**	**ME %**		**R^2**	**MDL ng/L**	**MQL ng/L**	**ME %**	**R^2**	**MDL ng/L**	**MQL ng/L**	**ME %**
Sulfacetamide	0.9991	3.8	12.9	5.9	0.9973	9.8	32.8	15.4	Olanzapine	0.9991	1.5	4.4	11.1	0.9948	1.80	5.8	-17.0
Sulfadiazine	0.9987	3.2	10.4	10.3	0.9967	5.1	12.9	13.1	Risperidone	0.9951	0.6	1.8	-20.3	0.9912	1.45	4.9	45.9
Sulfathiazole	0.9990	3.9	13.9	4.8	0.9970	4.3	14.6	5.3	Venlafaxine	0.9990	0.3	0.9	-4.2	0.9988	0.65	1.8	-28.7
Sulfapyridine	0.9994	4.1	11.3	7.6	0.9975	3.9	13.5	5.3	Paroxetine	0.9968	1.9	5.8	-0.9	0.9933	3.84	11.2	-23.3
Sulfamethazine	0.9997	1.1	3.4	10.1	0.9949	1.3	3.5	11.6	Cyclobenzaprine	0.9992	0.5	1.5	5.9	0.9987	0.49	1.6	-6.7
Trimethoprim	0.9998	0.4	1.2	0.8	0.9978	1.1	2.1	-17.2	Ery-H2O	0.9998	2.3	7.0	9.7	0.9991	2.35	7.2	14.4
Sulfamethizole	0.9989	1.9	6.4	13.1	0.9959	2.0	6.8	4.9	Carbamazepine	1.0000	0.3	0.9	7.3	0.9994	0.38	1.1	16.6
Sulfamethoxy-pyrid	0.9995	0.8	2.5	10.5	0.9928	1.2	3.3	24.3	Amitriptyline	0.9958	1.3	3.8	1.1	0.9948	1.39	4.1	-10.6
Sulfamethoxazole	0.9998	0.9	2.7	2.9	0.9978	0.9	2.9	10.8	Fluoxetine	0.9981	1.3	3.8	-11.8	0.9969	1.68	5.1	-25.1
Carbamazepine	1.0000	0.9	2.6	6.7	0.9993	1.0	3.1	21.1	Clomipramine	0.9992	1.4	4.1	-1.2	0.998	2.01	6.1	-28.9
Oxolici acid	0.9993	1.2	3.0	5.0	0.9981	0.6	1.8	-22.6	Diclofenac	0.9993	6.6	19.8	16.4	0.9928	10.9	33.6	32.0
Sulfaquinoxaline	0.9987	0.7	2.0	0.6	0.9931	0.9	2.9	8.8	Trimethoprim	0.9998	0.7	2.1	-1.5	0.999	0.71	1.9	-2.7
Acesulfame	0.9992	10.8	32.4	8.1	0.9941	10.4	34.8	5.4	Indomethacin	0.9987	5.1	15.4	-12.2	0.9954	7.0	21.8	30.9
Saccharin	0.9993	11.2	30.7	4.5	0.9964	10.0	35.1	3.1	Mefenamic acid	0.9994	5.6	16.9	-25.3	0.9977	5.9	19.8	-55.8
Sucralose	0.9990	29.4	90.5	16.5	0.9973	31.2	98.7	8.0	Triclosan	0.9987	2.3	6.8	-2.3	0.9952	2.5	7.1	7.6
Florfenicol	0.9996	0.7	1.9	-0.1	0.9944	0.6	2.0	-23.5	Tolfenamic acid	0.9999	3.7	11.0	-14.8	0.9991	4.9	15.4	-48.6
Aspartame	0.9981	22.4	69.9	7.7	0.9964	30.2	93.9	-5.6									
Salicylic acid	0.9933	10.3	29.4	15.3	0.9913	11.8	37.8	-33.7									
Gemfibrozil	0.9900	5.9	18.8	-8.1	0.9922	9.4	29.4	8.9									

CHAPTER 6: OPTIMIZATION & VALIDATION OF FABRIC PHASE SORPTIVE EXTRACTION (FPSE)

Introduction

A sample strategy for the determination of twenty-one emerging contaminants was associated using the novel generation extraction protocol of Fabric Phase Sorptive Extraction (FPSE). All the parameters involved in the extraction, such as sample volume, extraction and desorption times, desorption solvent volume and sample pH values have been optimized. The optimal conditions were applied for validation studies in tap and effluent water. Evaluation of Matrix effects employed with FPSE-UHPLC-Orbitrap MS was also assessed.

A. Optimization of Fabric Phase Sorptive Extraction

Preliminary experiments were assessed for the selection of suitable FPSE media, in terms of type and shape, for the determination of selected analytes in aqueous samples. According to preliminary tests, some analytes of concern provided negligible efficiency, in terms of recovery, therefore 21 out of 33 contaminants were chosen for further application and optimization of FPSE. The substrates used for the evaluation of FPSE sorbent were two Whatman filters of cellulose (CF) and fiber glass (FG). Physicochemical characteristics and especially polarity properties of target analytes were considered. For this purpose, two different sol-gel coatings were selected, including polyethylene glycol 300 (PEG 300), a polymer of high polarity and poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) 5.800 (PEG-PPG-PEG 5.800), a medium polarity polymer. However (PEG-PPG-PEG 5.800) provided poor adsorption yields (below 5%) in both substrates (CF and FG) and for this reason was rejected for further optimization study. On the other hand, absolute recoveries of PEG 300 were satisfactory for further evaluation (20.1-58.7 %) and best results were achieved with FG media circle-shaped with a diameter of 1cm. This combination of substrate and coating was used for further protocol development and optimization.

Several FPSE parameters affecting extraction efficiency and desorption performance such as sample volume and pH, extraction time, ionic strength as well as elution solvent and desorption volume and time were studied.

6.1 Effect of pH

Initial conditions of 1 mL aqueous sample volume with a fixed elution volume of 1 mL MeOH solvent was chosen for the evaluation of pH effect (pH values of 3.0,7.0,12.0 -taking into consideration pKa of basic compounds- were evaluated). Results are depicted in Fig.6.1. The target analytes have different pKa and belong in different therapeutic and chemical classes, so they follow different adsorption rules according to pH variations. Antibiotics classified in sulfonamide category, NSAIDs and artificial sweetener (acesulfame) displayed higher adsorption in pH 3.0. In this pH the specific compounds exist in their neutral form according to pKa and their speciation charts [665], enabling in this way the extraction yield. On the other hand, when pH increases to 7.0 and 12.0 the absorption decreases significantly since the mostly ionizable charged form of these compounds exists in aqueous solution. In alkaline region, the highest absorptions were obtained for psychiatric drugs with basic pKa. However, the adsorption of psychiatric drugs was satisfactory also in other pH values. Carbamazepine provided high adsorption in all pH values indicating no pH effect. Different pKa of analytes of interest do not allow to apply a pH value that would be equally efficient for all, therefore pH 3.0 was selected as a satisfactory value for most analytes.

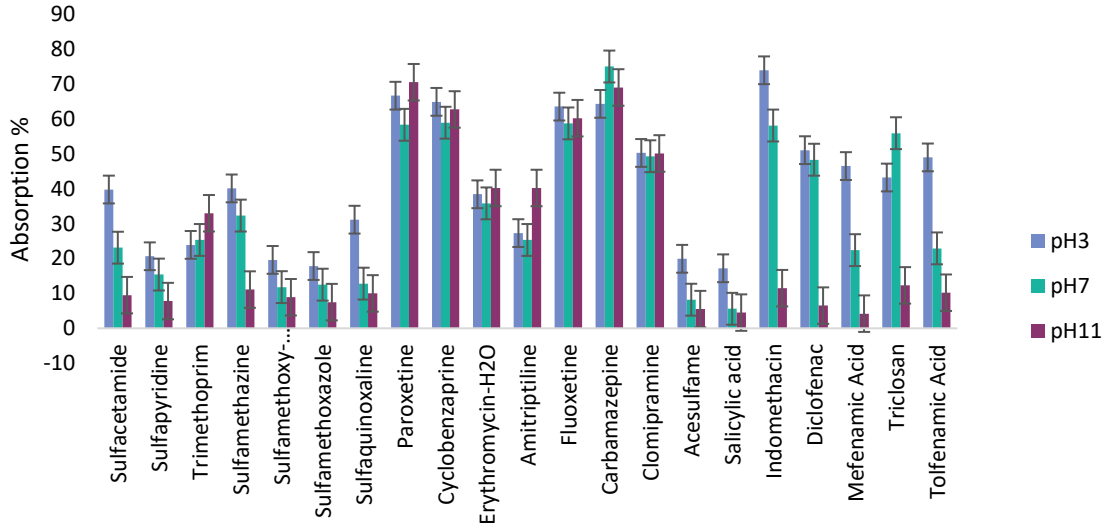


Figure 6.1. Effect of pH on target analytes

6.2 Effect of sample volume

After the evaluation of pH, the next step was to determine the sample volume that could be loaded in the FPSE sorbent accomplishing at the same time high preconcentration factor. To address this issue, two circles of 1 cm FG@PEG were used and three volumes of 5, 10, 20 mL of ultrapure water spiked with the target analytes were assessed. The lowest absorption rates were achieved with 20 mL of sample, while 5 mL and 10 mL provided satisfactory rates. When the sample volume was increased from 5 mL to 10 mL, absorption decreased slightly (~5% on average) for most analytes. Taking into consideration the preconcentration factor and making a compromise between the adsorption efficiency and the sensitivity of the method, 10 mL of sample was selected for further analysis.

6.3 Effect of Extraction time

A series of extraction times of 10, 20, 25, 30, 35 ,40 min was studied under two stirring speeds (250 and 350 rpm). The scale of 350 rpm was by far more effective in all cases. From Figure 3b it is obvious that the equilibrium of target analytes onto the sorbent is accomplished within 35 minutes, while further stirring time did not significantly improve the absorption rates. For this reason, 35 min was chosen as the optimal extraction time and employed for the subsequent tests.

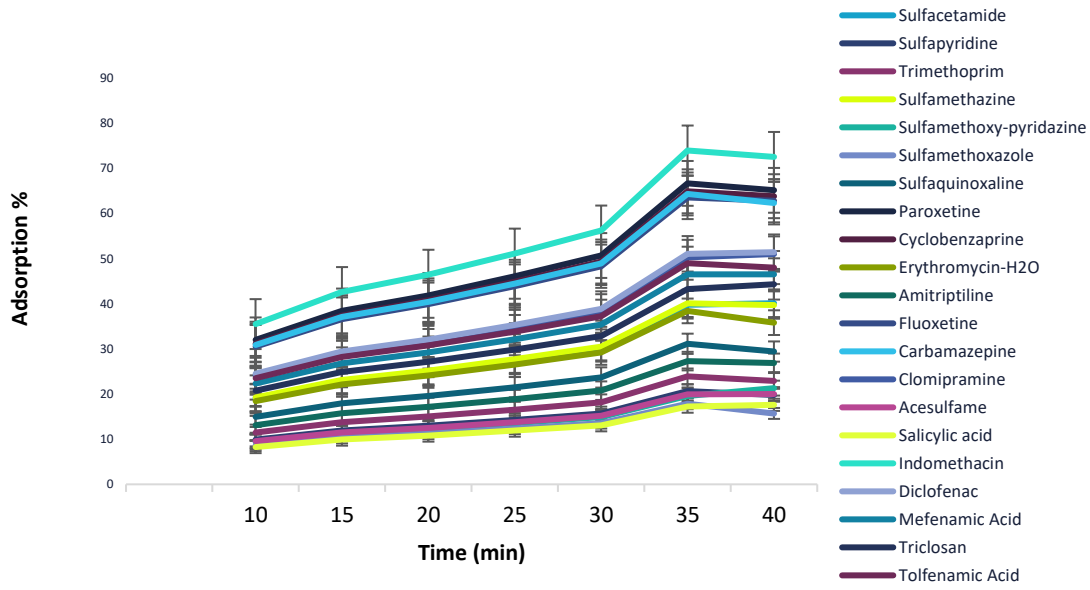


Figure 6.2. Effect of extraction time on target analytes

6.4 Ionic strength

Generally, increasing the ionic strength of a solution results in an improvement in the extraction efficiency when salting-out effect plays a crucial role in the procedure. The effect of ionic strength on the adsorption of target analytes was examined using NaCl at concentrations ranging between 0%, 5% and 10% (w/v). With the addition of 5% w/v of NaCl no significant improvement in the extraction efficiency was assessed. On the other hand, higher salt content of 10% w/v decreases the adsorption of several analytes and has a negative impact especially for non-polar ones (Figure 6.3). Target compounds with logP>3.5 like the studied NSAIDs, present the lowest adsorption rate with the highest salt content. This finding is in accordance to other reports [666–668].

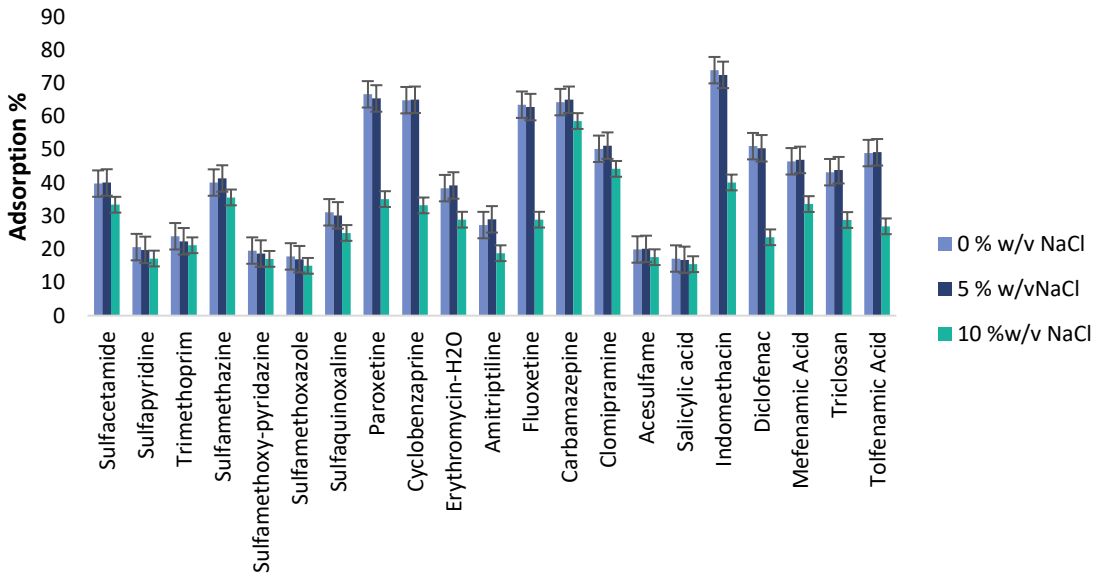


Figure 6.3. Effect of Ionic strength on FPSE extraction efficiency

Desorption Conditions

6.5 Elution solvent

Following the optimization of the adsorption step of FPSE, desorption conditions of extraction technique such as elution solvent, volume as well as elution time were evaluated. Different solvents such as acetone, acetonitrile methanol were tested. Acetone, methanol acetonitrile were used as elution solvents and their recovery efficiencies were evaluated under the same extraction and desorption conditions (extraction time 35 min, 10 mL of sample, 1 mL of elution solvent by stirring for 15 min). Acetone presented the lowest recoveries especially for polar analytes (logP<3), followed by acetonitrile while methanol exhibited

the higher. To increase the desorption efficiency, taking into consideration the pH dependency on sorbent, formic acid or ammonia was added at various percentages (1–5% v/v) to increase the acidity or alkalinity. The results in Fig.6.4 showed that the extraction efficiency reached the maximum when two elution systems of MeOH with 5% f.a v/v and MeOH with 5% v/v NH_3 separately was used. Analytes with maximum recovery in acidified methanol showed the lowest recovery in alkaline methanol and vice versa. This may be attributed to the different physicochemical properties of the analytes and the variation in pKa. During desorption process, should be promoted the ionic state of each compound since the interaction with sorbent should be reduced. As an example, considering the pKa of fluoxetine, in alkaline pH, the compound exists in its neutral form, maintaining its adsorption onto the FPSE media, thus an acidified solvent would be more suitable for the successful desorption. For sulfonamide compounds the same vice versa phenomenon is occurred. In low pH values sulfonamides exist in their neutral form which enables the retention onto the sorbent, indicating that a desorption solvent in alkaline media would be more efficient. To overcome this controversy the elution of the target analytes was accomplished with consecutive elution steps by adding aliquots of MeOH 5%NH_3 v/v and MeOH 5% f.a v/v. In this way, we take advantage the optimal elution conditions for all target analytes by avoiding the performance of two different extraction processes, simplifying FPSE.

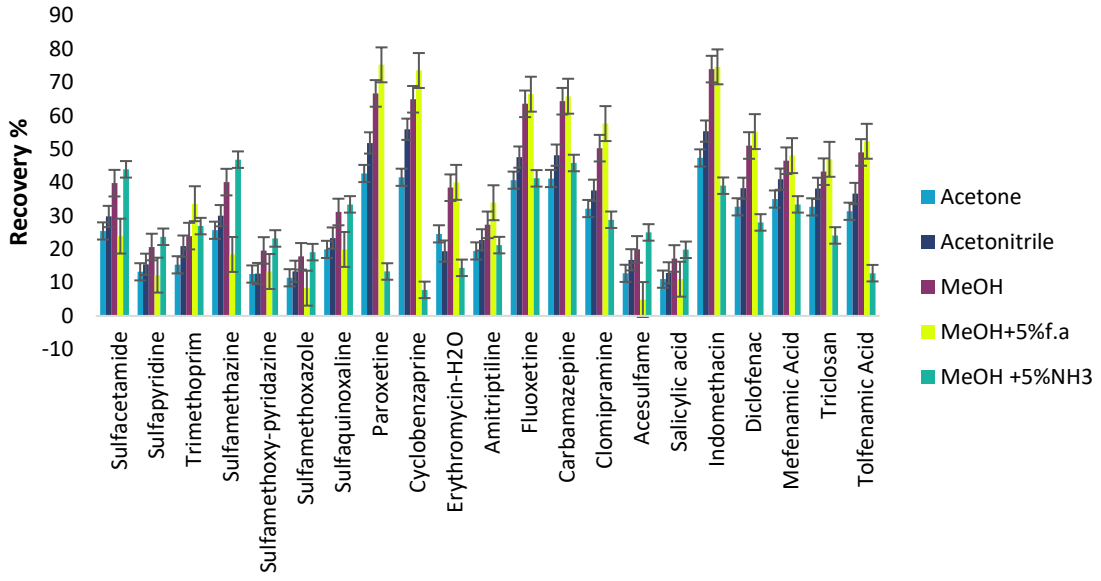


Figure 6.4. Effect of elution solvent on ECs recoveries

6.6 Elution volume and desorption time

For determining the volume of the elution solvent two fractions of 1 mL and 5 mL was investigated. The results demonstrated similar recoveries for both volumes selected, thus 1mL was selected as optimal. To increase preconcentration factor, the final acidified/alkaline methanolic extract was evaporated until dryness and reconstituted in 150 μL of mobile phase initial conditions. Finally, regarding desorption time 5, 10, 15, 20 min under stirring (350 rpm) was investigated. From Fig.6.5
it is noticed that recoveries increase with time of exposure of the FPSE media. Specifically, the maximum recoveries were achieved within 10 and 15 min, while 5 min of stirring was not long enough to achieve complete desorption. On the other hand, 20 min of stirring provided low desorption efficiency, probably due to the back re-sorption of analytes onto the coated media [650,669]

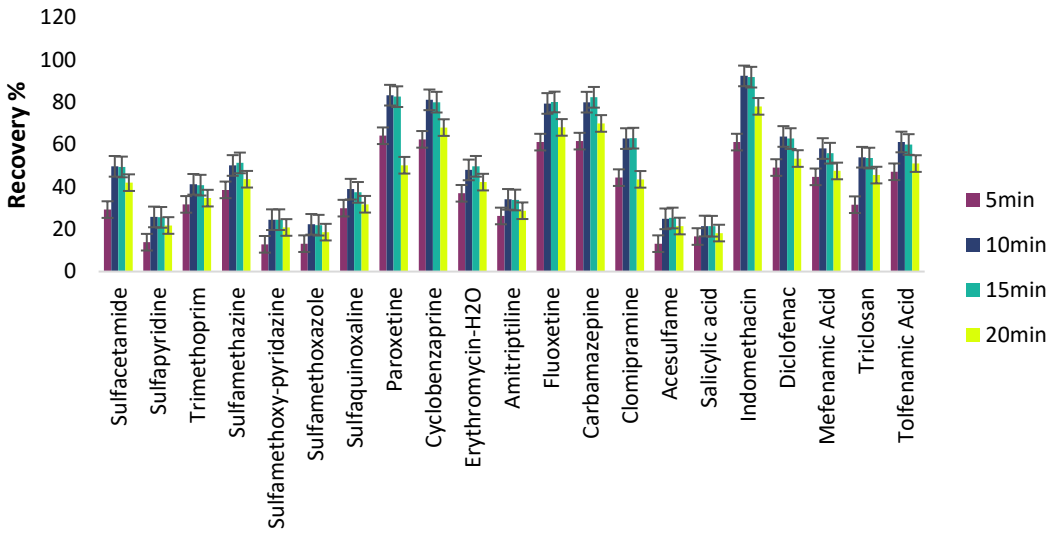


Figure 6.5. Effects of the desorption time on the recoveries of target analytes

In accordance with the obtained results, the optimum conditions for FPSE procedure were as follows: 10 mL of sample adjusted to pH 3 with no addition of NaCl was extracted for 35 min under stirring (350 rpm). Desorption occurred with 1 mL of methanol (acidified/alkaline) within 10 min of stirring at 350 rpm. The optimal conditions were applied to wastewater effluents for the determination of target analytes.

B. Validation of Fabric Phase Sorptive Extraction (FPSE)

The FPSE method developed for the determination of the selected pharmaceuticals and sweeteners in aqueous media was finally validated. Validation studies as well as identification and confirmation of the

target compounds were based on the quality control procedures established by the European Union (EU) regulations (EU Commission Decision, 2002) [8]. Validation procedure was conducted in pooled samples of effluent wastewaters of Ioannina city, Amaliada city as well as effluent wastewater from University hospital of Ioannina. Validation studies were also conducted for tap water.

6.7 Accuracy

Accuracy of the developed method was expressed as the percentage of Relative recovery. They were estimated from absolute recoveries of ultra-pure spiked water samples. Relative recovery is defined as the % concentration of target analytes recovered from the wastewater with reference to the concentration found at spiked ultra-pure water. Absolute recoveries are presented in Table 6.1. For this purpose, three replicates of spiked bottled water and effluent sample at three concentration levels of MQL, 10 times MQL, 100 times MQL (low, medium, high) were analyzed under the optimum conditions. Blank samples (unspiked) were analyzed as well. Due to the fact that unspiked effluent samples already contained some of the compounds, the concentration of the respective unspiked sample (blank) was subtracted from the concentration in the spiked sample and then divided by the spiked level.

Recoveries experiments in all cases were conducted in three replicates (n=3) for each concentration level in tap water and effluent wastewater. For the medium spiking level relative recoveries ranged from 79.2% -113.1% for tap water while for effluent water from 83.7% to 114.0%, (Table 6.1).

Table 6.1. Absolute recoveries (%) for and their RSDs% at ultra-pure water

COMPOUND	Rabs% n=3	RSD_r % n=5	RSD_R % n=15	Rabs% n=3	RSD_r% n=5	RSD_R % n=15	Rabs% n=3	RSD_r% n=5	RSD_R % n=15
	MQL			10 x MQL			100 x MQL		
Sulfacetamide	44.8	1.2	2.3	52.3	1.5	1.8	55.0	2.5	3.3
Sulfapyridine	22.9	0.9	1.9	24.8	0.6	1.2	21.5	1.8	2.4
Sulfamethazine	48.4	0.7	2.1	51.6	1.3	1.6	49.4	2.1	3.5
Trimethoprim	51.2	1.0	3.4	50.0	0.9	2.1	48.9	1.8	2.7
Sulfamethoxy-pyrid	29.8	1.3	4.1	31.8	0.8	1.5	33.5	1.4	2.1
Sulfamethoxazole	26.3	3.1	3.5	25.1	1.8	3.5	30.0	3.2	4.2
Sulfaquinoxaline	30.1	1.9	2.8	33.8	2.6	3.8	35.4	4.5	6.0
Paroxetine	80.1	5.2	6.0	86.9	4.1	5.4	82.3	5.2	7.1
Cyclobenzaprine	82.6	4.0	5.7	84.8	3.5	4.3	84.8	5.4	7.5
Erythromycin-H_2O	49.8	0.9	3.1	51.2	2.8	3.9	47.5	3.9	5.3
Carbamazepine	33.4	2.1	2.8	35.1	4.9	6.2	34.5	6.4	8.4
Amitriptiline	83.2	3.4	4.6	80.1	7.2	9.0	79.5	3.4	5.2
Fluoxetine	84.0	1.8	3.0	83.6	3.4	5.1	86.1	2.4	5.0
Clomipramine	68.4	3.5	5.2	70.0	4.1	6.1	65.8	3.0	4.8
Acesulfame	29.4	1.9	3.1	32.5	2.9	3.8	33.4	5.1	6.9
Diclofenac	58.3	5.7	5.9	64.9	7.0	8.9	65.8	4.9	6.5
Salicylic Acid	27.9	7.7	8.1	25.9	6.4	7.7	30.1	5.8	8.0
Indomethacin	84.5	3.8	4.2	93.0	5.8	7.0	87.8	6.5	8.9
Mefenamic Acid	60.0	11.2	12.0	61.2	9.7	11.6	54.8	11.2	14.6
Triclosan	55.4	10.0	10.5	59.4	8.5	11.0	65.9	10.2	13.2
Tolfenamic Acid	57.9	4.9	5.5	64.8	7.2	8.6	66.9	6.4	8.3

6.8 Precision

Intra-day precision (n=5) and intra-laboratory reproducibility (n=15) expressed as Relative standard deviation percentage (RSD_r and RSD_R) were lower than 12% and 14% for tap water and 13%and 14% respectively for effluent water (Table 6.2).

6.9 Sensitivity and Linearity

Method limit of detection (MDL) was calculated as the lowest concentration of compounds that provides a signal-to-noise equal to 3 (S/N=3.) Similarly, MQL, was determined as the concentration that generates a S/N =10.In tap water MDLs and MQLs varied from 1.4-105.9 ng $L^{-1}$and 4.3-317.8 ng L^{-1}, while in effluent water MDLs and MQLs were ranged from 3.1- 149.4 ng/L and 9.3-447.7 ng/L, respectively. Linearity of the method was investigated by triplicate analysis in both aqueous matrices by constructing a 11-point method calibration curve covering the range of MQL to approximately 100 times MQL for each target analyte. Coefficients of determination (R^2) were greater than 0.99 for both aqueous matrices indicating that linearity is satisfactory for all target analytes. The analytical parameters as well as matrix effect values (ME %) of effluent water is presented in Table 6.2.

6.10 Matrix Effect

To evaluate the degree of ion suppression or enhancement and to what extent target compounds were sensitive to them, matrix effects in tap water and effluent wastewater were evaluated by comparing the slopes in calibration solutions prepared in matrix and in solvent. Matrix-matched calibration solutions were prepared in pooled effluent samples consisted of effluents WWTP of Ioannina and Amaliada as well as effluent wastewater from hospital of Ioannina. Matrix effect (ME%) was evaluated for the different aquatic matrices and results are shown in Fig. 6.6

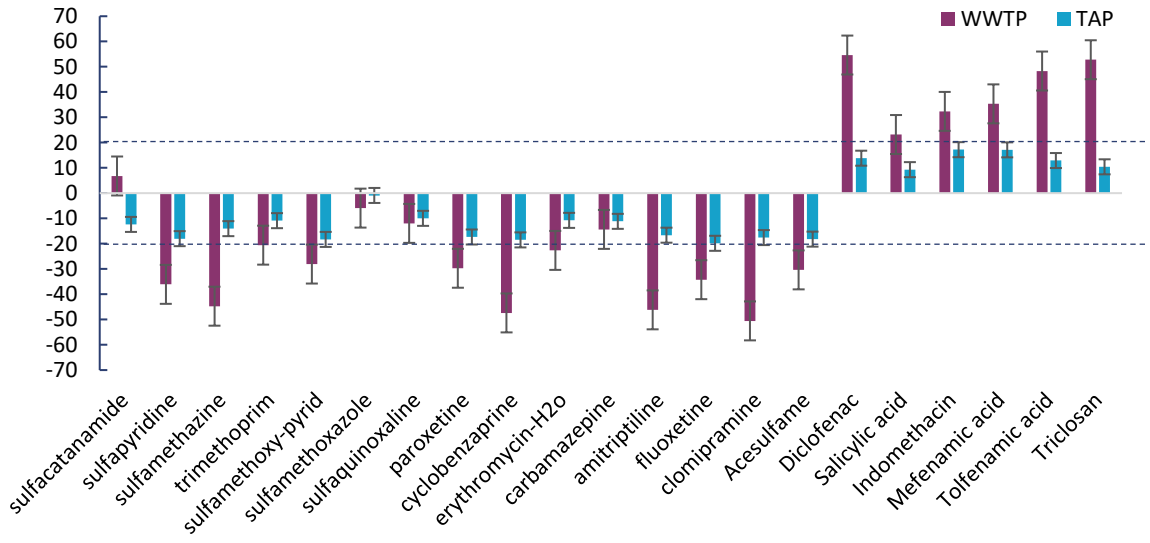


Figure 6.6. Matrix effect for the selected compounds in different aqueous matrices after FPSE

Results evaluated for tap water showed low influence of the matrix in signal suppression/enhancement. The ME ranged from-0.9 to 17.2 % for all the pharmaceutical compounds studied, indicating negligible or low matrix effect. There was a variance concerning the signal enhancement/suppression of the analytes since 15 out of 21 showed ion suppression and the rest of them ion enhancement. ME of WWTP effluent waters showed similar trend concerning the signal suppression/enhancement variations. Specifically, pharmaceuticals analyzed in positive ionization mode were noticed to be subjected to ion suppression while those in negative polarity ionization showed ion enhancement (except acesulfame) However the values seemed to be higher and exceeded the level of acceptance (-20%<ME<+20%).

Most of the target pharmaceuticals in WWTP effluent displayed medium matrix effect, with only exceptions sulfacetamide, trimethoprim sulfamethoxazole, sulfaquinoxaline, carbamazepine (-20<ME<+20). On the other hand, diclofenac and triclosan were the only compounds that displayed high matrix effect expressed as signal enhancement 54.7% and 52.8%, respectively. The results were expected taking into consideration the complex matrix of effluent water and the high content of organic matter. In any case matrix matched-calibration curves were used for the quantification of target analytes to avoid inaccurate results.

Table 6.2. Mean Recoveries and precision results expressed as RSD_r and RSD_R: within each spiking level for tap and effluent water

	TAP WATER									WWTP EFFLUENT WATER								
COMPOUND	RR% n=3	RSD_r% n=5	RSD_R% n=15	RR% n=3	RSD_r% n=5	RSD_R% n=15	RR% n=3	RSD_r% n=5	RSD_R% n=15	RR% n=3	RSD_r% n=5	RSD_R% n=15	RR% n=3	RSD_r% n=5	RSD_R% n=15	RR% n=3	RSD_r% n=5	RSD_R% n=15
	MQL			10 xMQL			100 x MQL			MQL			10 xMQL			100 x MQL		
Sulfacetamide	105.2	1.4	1.6	110.3	1.1	2.4	107.1	0.7	1.2	111.1	1.3	1.8	111.1	1.3	1.8	105.3	1.8	2.5
Sulfapyridine	99.9	0.5	1.2	98.5	0.4	1.0	96.7	0.6	1.0	99.8	1.2	1.9	99.8	1.1	1.9	95.6	1.0	1.9
Sulfamethazine	84.4	0.9	1.6	86.7	1.0	1.9	88.8	0.7	1.8	83.7	1.1	2.1	83.7	1.1	2.1	81.2	1.2	2.0
Trimethoprim	79.8	1.2	2.0	79.2	1.2	1.8	77.6	1.6	2.1	85.6	1.7	3.1	85.6	1.7	3.1	84.7	1.4	2.0
Sulfamethoxy-pyrid	77.6	1.5	2.4	81.8	1.1	2.3	79.1	2.0	3.5	90.2	1.9	2.4	90.2	1.1	2.4	90.3	1.2	1.9
Sulfamethoxazole	83.8	0.7	2.3	85.3	0.9	2.5	80.1	1.3	1.5	88.6	1.7	2.8	88.6	1.0	1.8	87.5	1.0	1.4
Sulfaquinoxaline	105.3	0.8	1.8	100.1	1.0	3.8	100.3	0.8	1.3	100.2	1.2	2.0	100.2	1.2	2.0	112.3	2.0	2.8
Paroxetine	106.1	4.1	5.0	113.1	3.8	4.2	99.8	4.5	6.4	85.3	2.1	3.1	85.3	2.1	3.1	84.6	3.1	3.9
Cyclobenzaprine	100.2	3.2	5.5	99.4	2.5	3.1	100	1.9	2.6	100.1	3.7	4.0	100.1	3.7	4.0	98.7	2.8	3.2
Erythromycin-H2o	95.8	1.8	3.2	96.3	1.9	3.0	94.2	1.1	2.0	95.6	2.5	2.8	95.6	2.5	2.8	94.1	1.7	2.5
Carbamazepine	97.8	3.6	4.7	100.0	4.0	5.7	93.8	5.0	6.2	98.6	2.3	3.3	98.6	2.3	3.3	97.1	1.9	3.3
Amitriptiline	88.2	5.2	6.5	90.4	6.0	6.5	84.8	3.7	4.9	86.8	4.7	5.2	86.8	4.7	5.2	82.5	3.9	4.2
Fluoxetine	97.7	2.9	3.0	99.8	2.7	3.8	93.5	2.1	2.7	99.1	1.8	2.8	99.1	1.8	2.8	97.8	2.2	2.9
Clomipramine	93.8	3.0	4.3	93.0	2.8	4.0	92.1	2.5	2.8	94.3	2.0	2.5	94.3	2.0	2.5	91.9	1.8	2.5
Acesulfame	84.4	1.0	1.7	84.8	1.3	2.6	92.8	1.7	3.0	92	1.9	2.7	92.0	1.9	2.7	93.0	3.1	4.0
Diclofenac	92.7	7.0	7.9	93.8	6.2	6.5	96.9	5.5	6.7	99.4	7.8	8.3	99.4	7.8	8.3	97.7	9.0	9.5
Salicylic acid	86.3	8.6	9.2	89.4	9.0	9.8	90.7	7.2	7.9	95.3	9.4	11.0	95.3	9.4	11.0	92.1	10.0	10.9
Indomethacin	100.8	4.9	5.1	99.2	6.0	7.5	101.3	5.2	7.0	114	5.8	6.9	114.0	5.8	6.9	101.3	5.4	6.2
Mefenamic acid	99.7	10.0	13.4	97.6	11.3	11.9	97.8	8.7	9.5	98.5	12.0	12.7	98.5	12.0	12.7	97.9	9.7	11.0
Triclosan	82.6	9.2	11.0	89.8	10.5	10.9	93.1	8.9	11.5	85.3	10.2	11.0	85.3	10.2	11.0	88.1	13.0	13.4
Tolfenamic acid	98.8	5.8	6.9	99.3	8.4	9.0	96.8	11.0	12.0	104	13.0	13.8	104.0	13.0	13.8	99.7	12.1	12.8

Table 6.3. Linearity as coefficient of determination (R^2), limits of detection and quantification of FPSE for tap water and effluent water

COMPOUND	R^2	MDL ng/L	MQL ng/L	ME %	R^2	MDL ng/L	MQL ng/L	ME %
	TAP WATER				WWTP EFFLUENT WATER			
Sulfacetamide	0.9982	39.4	122.8	-12.4	0.9994	100.8	300.8	6.8
Sulfapyridine	0.9957	34.3	102.8	-18.0	0.9918	40.1	119.7	-36.1
Sulfamethazine	0.9970	48.2	147.2	-14.0	0.9934	53.2	157.8	-44.7
Trimethoprim	0.9991	12.0	36.0	-10.9	0.9958	17.7	52.3	-20.6
Sulfamethoxy-pyrid	0.9971	2.9	8.8	-18.3	0.9979	5.0	14.2	-28.1
Sulfamethoxazole	0.9988	15.4	46.4	-0.9	0.9959	18.7	55.4	-5.9
Sulfaquinoxaline	0.9945	32.0	97.0	-10.0	0.9972	34.5	100.6	-12.0
Paroxetine	0.9932	4.1	12.3	-17.3	0.9935	4.5	13.3	-29.7
Cyclobenzaprine	0.9990	4.4	13.9	-18.5	0.9928	5.2	14.4	-47.4
Erythromycin-H2o	0.9983	13.4	38.1	-10.8	0.9988	7.4	45.8	-22.7
Carbamazepine	0.9999	1.4	4.3	-11.2	0.9974	3.1	9.3	-14.3
Amitriptiline	0.9966	6.1	18.3	-16.6	0.9989	9.4	28.2	-46.2
Fluoxetine	0.9959	13.4	41.1	-19.8	0.9952	15.7	47.1	-34.2
Clomipramine	0.9997	13.9	42.0	-17.6	0.9947	16.6	49.9	-50.5
Acesulfame	0.9951	105.0	317.8	-18.2	0.9987	149.4	444.7	-30.4
Diclofenac	0.9933	52.8	159.3	13.8	0.9932	68.5	204.5	54.7
Salicylic acid	0.9935	100.9	300.8	9.3	0.9912	119.9	354.7	23.2
Indomethacin	0.9951	63.3	192.9	17.2	0.9922	106.0	320.0	32.4
Mefenamic acid	0.9977	69.8	210.0	17.1	0.9934	80.1	236.9	35.3
Triclosan	0.9993	37.0	111.2	10.4	0.9961	49.5	146.9	52.8
Tolfenamic acid	0.9962	35.2	106.6	12.9	0.9980	43.0	128.0	48.3

CHAPTER 7: OPTIMIZATION & VALIDATION OF SOLID PHASE EXTRACTION (SPE)

A. Optimization of Solid Phase Extraction (SPE)

Sample preparation is a major step in the analytical process, and because it precedes analysis, it is necessary to optimize it in detail to achieve optimal analytical results. Solid phase extraction (SPE) remains the most widely used mean of extraction and pre-concentration of organic contaminants in aqueous matrices especially in multi-residue analytical methods. During the sample preparation, it is imperative to remove as many interferences, as possible, to concentrate the sample and to translate the analytes into more appropriate form for further analysis.

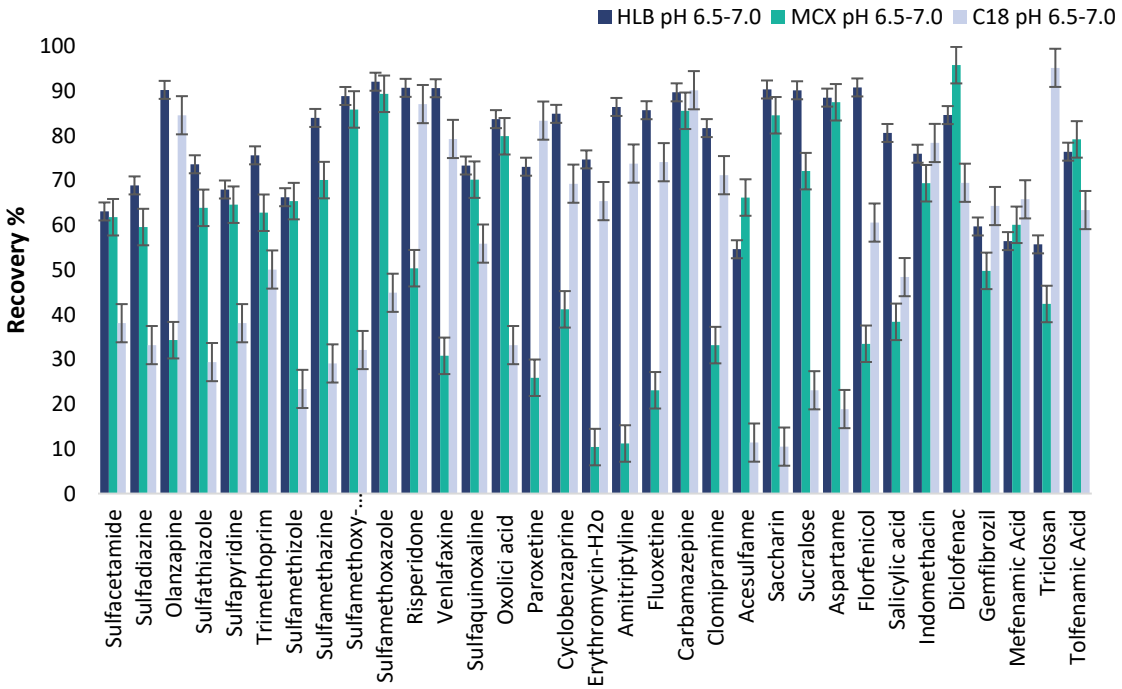
The SPE optimization starts with selection of adequate sorbent taking into consideration physicochemical properties of the analyte and matrix together with their possible interactions with the sorbent. Octanol-water partition coefficient (log Kow) and dissociation constant (Ka) are the properties of the analytes that help to predict the type of interactions between the sorbent and the analyte. Besides choosing the appropriate sorbent, it is important to choose adequate eluent that will efficiently break the bonds between the sorbent and the analyte.

7.1 SPE cartridge and sample pH

Due to the wide spectrum of target ECs with diverse physicochemical characteristics, it is challenging to extract all the analytes with adequate analytical characteristics. The choice of SPE materials suitable for all the analytes plays a crucial role in the method development. Regarding polymeric cartridges, Oasis HLB and MCX sorbents were tested while with regards to silica-based cartridges, CNW bond HC-C18 was also assessed. The polymeric cartridges are the mostly recommended in literature reviews for the analysis of different classes of emerging contaminants such as pharmaceuticals and artificial sweeteners [93,259,620] while a cartridge with non-polar material should be also evaluated taking into consideration the wide polar-non polar characteristics of the analytes of interest.

Besides sorbent material of the cartridge, the pH of the sample solution plays a significant role in the extraction efficiency of target compounds. In the majority of multi-residue methods published so far, sample pH is generally adjusted within the range from 2.5 to 4.0 [93,670,671] to achieve

good extraction recoveries for the majority of chemical groups included. The acidification of at least 2 units under pKa values of target analytes in water samples is recommended, in order to obtain their neutral or acidic forms, which may significantly improve their retention onto the SPE sorbent [672]. For this reason, in this work, the efficiency of three cartridges Oasis HLB (200mg, 6mL), MCX (150mg, 6mL) and CNWBOND HC-C18 (200mg, 3mL) was tested under acidified pH range of 2.5-3.0 and neutral pH conditions of 6.5-7.0. To evaluate which of these experiments yielded higher extraction yields of target analytes, preliminary experiments were performed with ultrapure water. In all cases, water samples were spiked with appropriate concentrations of a standard mixture containing all target analytes and surrogate standards. For this set of experiments 200 mL was used as sample volume and methanol as elution solvent. In the experiments where water samples were acidified, cartridges were conditioned with methanol followed by ultrapure water acidified with hydrochloric acid at pH 2.5, while in the experiments carried out with neutral pH adjustment, SPE cartridges were conditioned with methanol and ultrapure water. Recovery results obtained for all target analytes with different SPE protocols are illustrated in Fig.7.1.



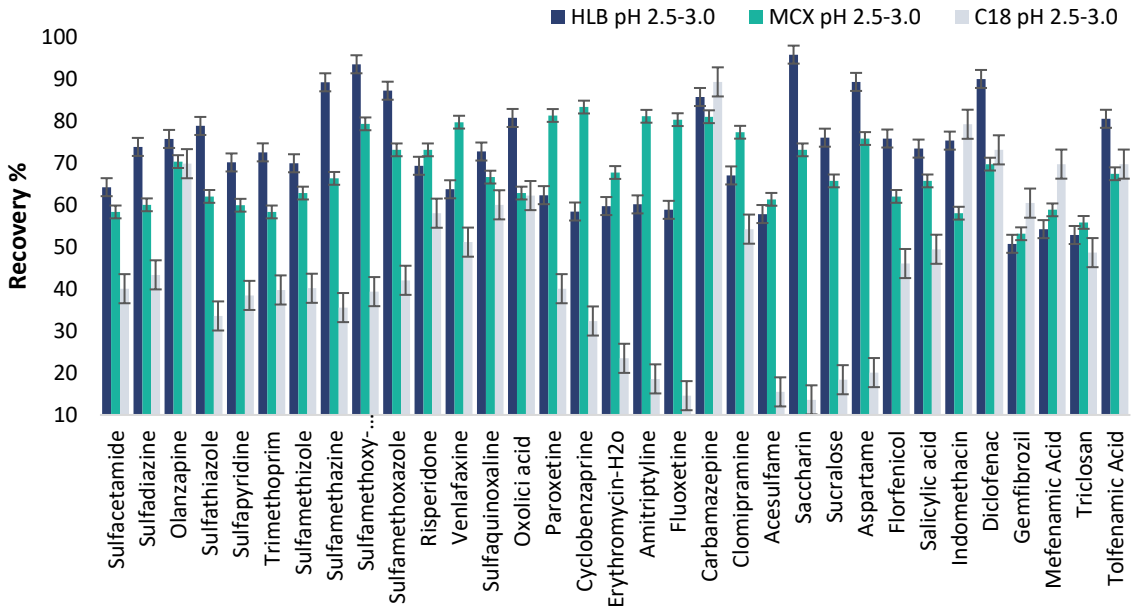


Figure 7.1 Recoveries of target analytes using Oasis HLB, MCX and CNW bond HC-C18 cartridges at different pH ranges

As it is depicted in Fig.7.1, in general terms, the methodology using Oasis HLB cartridges in both pH conditions, was the one that yielded higher recoveries for most target compounds. Oasis HLB cartridges retained successfully compounds belonging either in polar range such as sulfathiazole with logP 0.86 or in non-polar groups such as amitriptyline with logP 4.81.

The Oasis HLB sorbent consists of hydrophilic N vinylpyrrolidone monomers and hydrophobic divinylbenzene monomers, which enable retention of contaminants with a wide range of polarities and sample pH (Fig. 7.2). Due to the absence of free silanol groups which can interfere during extraction, they are very efficient in the extraction of organic contaminants [670].

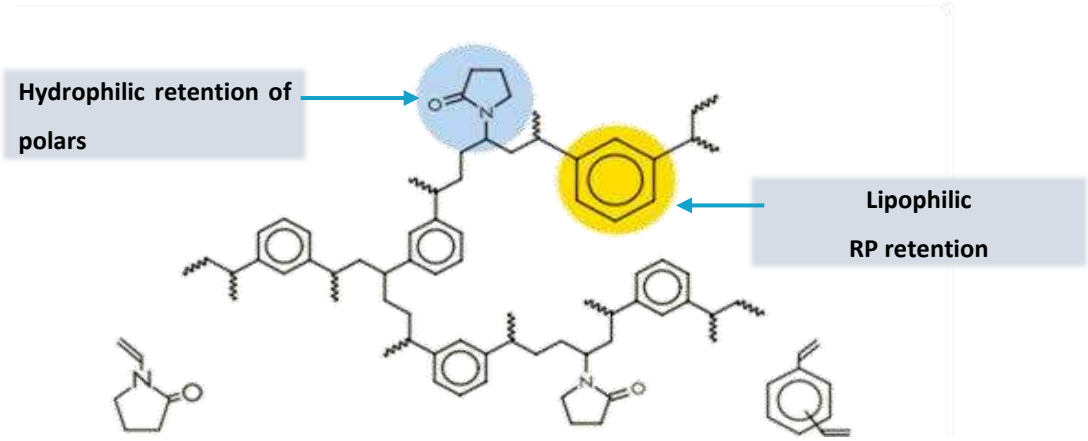


Figure 7.2 Chemical structure of HLB sorbent [673]

Using HLB sorbent sulfonamide antibiotics exhibited high performances, in pH range of 2.5-3.0, while in neutral pH the recoveries were slightly decreased. Sulfonamides, as mentioned in previous chapters they contain one basic amine group(-NH_2) and one acidic sulfonamide group (SO_2NH-). They are ampholytes with weakly basic and acidic characteristics, having two pKa values, pKa1 (2–2.5) and pKa2 (5–8), respectively [652].Thus, sulfonamides are in their neutral form at pH 2 and 5, and negatively charged in pH>5. This is the reason why in MCX cartridges good retention occurred under all conditions tested. In addition, similarities were observed for most of acidic compounds extracted either with Oasis HLB or Oasis MCX under neutral conditions. These results can be explained by presence of acidic functional groups in the molecular structure of these compounds, therefore lowering pH under their pKa values enhances the presence of neutral forms and their interaction with the reversed-phase sorbent. On the contrary, basic, and neutral compounds, extracted with MCX cartridges in pH ranges 6.5-7.0 were poorly recovered compared to HLB under any conditions. This could be attributed to the fact that Oasis MCX is a mixed reversed phase-cation exchange cartridge which, at low pH values, it can efficiently extract acidic, basic and neutral compounds, since the cation exchanger binds the basic compounds and the reversed phase can retain both acidic and neutral ones (Fig. 7.3). Therefore, the experiment set that was performed at neutral pH, provided satisfactory recoveries only for acidic and neutral compounds, whereas for basic analytes the performances were significantly lower (Rec%~10-50%).

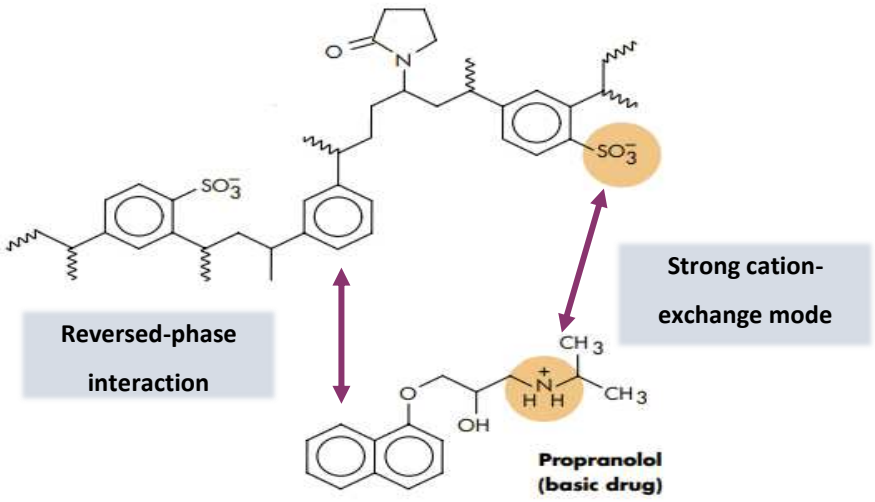


Figure 7.3 Chemical structure of MCX cartridge

On the other hand, for the same reason, basic analytes such as antidepressant drugs, the macrolide antibiotic erythromycin, showed improved recoveries with the protocol with MCX cartridges when acidification of the sample was performed. This could be explained by the fact that at the pH values used (2.5–3.0) basic drugs are positively charged and therefore, strongly bound to the column by the cation exchanger. Despite of the good recoveries found for the basic compounds using MCX cartridges, good recoveries for most compounds were still achieved using OASIS HLB cartridges. Major differences were observed for CNW HC-C18 cartridges. Non-polar compounds with logP> 3.0 presented higher recoveries with C18 cartridges, and especially recoveries of indomethacin, mefenamic acid, triclosan, gemfibrozil, carbamazepine and paroxetine were even higher than HLB cartridges. However, more polar compounds like sulfonamides, and artificial sweeteners were not retained to this sorbent which resulted in low recoveries regardless the pH of the solution. Most artificial sweeteners showed satisfactory recoveries when HLB cartridges were used, due to the hydrophilic intermolecular forces. Artificial sweeteners contain nitrogen and/or oxygen in their molecular structure, thus, when the sorbent possesses hydrophilic chemical moieties, retention is favored, and the recovery values increase. In case of acesulfame efficient retention occurred as well in MCX cartridge under neutral conditions because of the strong-cation interactions. Considering the number of compounds that exhibited recoveries >70%,(Fig. 7.4), CNW HC-C18 was not selected for further optimization. HLBac, MCXac and C18ac were the protocols with acidified pH 2.5-3.0 before sample extraction while HLBneu, MCXneu, C18neu were the neutral ones with pH values of 6.5-7.0 (Fig. 7.4).

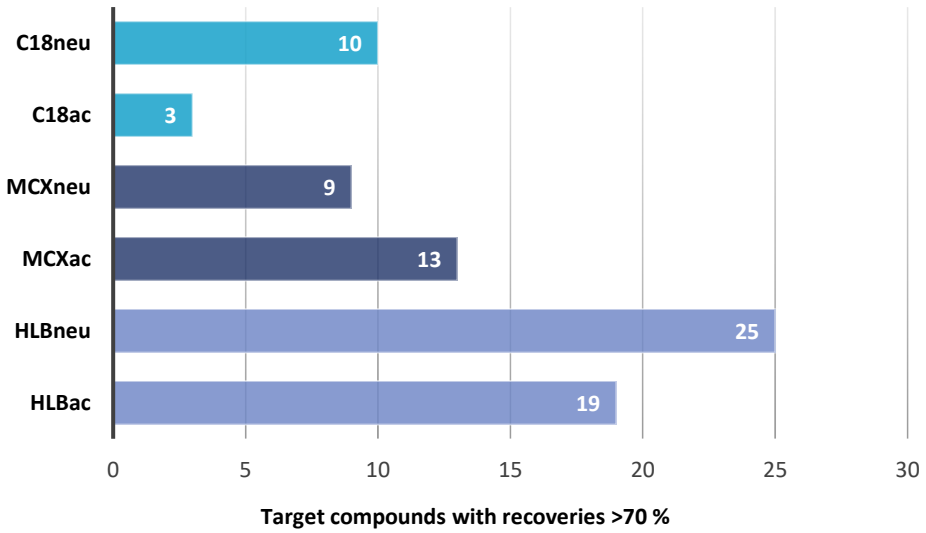


Figure 7.4. Target compounds with recoveries >70% under the different protocols investigated

7.2 Elution Solvent

Solvents like methanol and acetonitrile were investigated for analyte elution from SPE cartridges. The results showed that the application of MeOH performed better results for antidepressants drugs, NSAIDs and triclosan whereas for sulfonamides recoveries were slightly improved with acetonitrile. Considering that methanol can be dried more easily than acetonitrile due to its lower boiling point, methanol was selected as the desorption solvent and further optimization by adding acidic or alkaline modifier was assessed. To address this issue methanol containing 1% (v/v) formic acid (f.a) and methanol with 1% ammonia solution (NH_3) (v/v) along with pure methanol was evaluated as elution solvents in the aforementioned SPE protocols (HLBac, HLBneu and MCXac, MCXneu). The effect of elution solvent on the recoveries of SPE procedure are summarized in Figure 7.5.

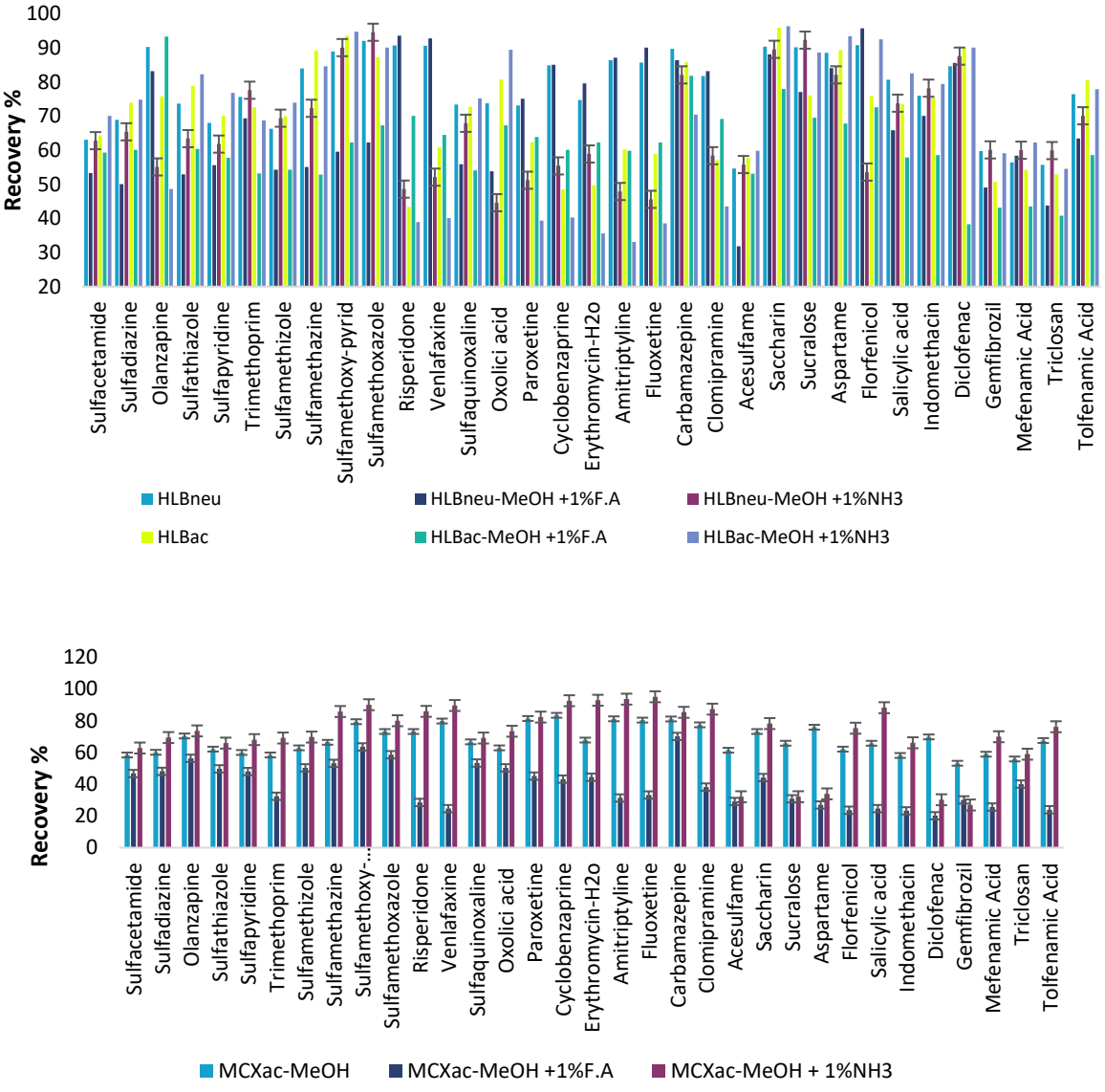


Figure 7.5. Effect of elution solvents on recoveries of different SPE protocols

Variances on recoveries of compounds were observed when different elution solvents were used. The alkalinity and acidity of methanol was also evaluated. Specifically, the addition of formic acid in protocols using HLB cartridges, was not more efficient than pure methanol for sulfonamides, acidic antibiotics such as oxolinic acid, NSAIDs and triclosan. These compounds are in their neutral form when formic acid is used as buffer modifier, reducing in this way the efficiency of the elution by enabling the adsorption of the target analytes onto the sorbent of the cartridge. On the other hand, according to this, the basic analytes are more favored when formic acid is used during the elution step. Basic analytes such as olanzapine, venlafaxine, risperidone, amitriptyline, erythromycin tend to be in their ionic form in acidic conditions, facilitating the transfer of target compounds to elution solvent. Similarly, ammonia solution as modifier in elution solvent, promotes the recoveries of acidic compounds (pKa<4.0) such as sulfonamides, trimethoprim, artificial sweeteners which in alkaline conditions they have no interaction with the sorbent material, since they are in their ionic form. Acidification prior to extraction combined with alkaline modifier in the elution solvent provided the highest recoveries for acidic analytes. On the contrary, ammonia solution with methanol provided low elution capability for basic analytes such as psychiatric drugs with recoveries ranging from 38.9-43.5 %. It was expectable since these compounds tend to be in their molecular in alkaline environment.

With respect to MCX cartridges, ammonia solution proved to be superior to pure methanol in acidified extraction conditions. Oasis MCX is a mixed reversed phase-cation exchange cartridge which, at low pH values, it can efficiently extract basic compounds through cation exchanger part of the sorbent material. To recover efficiently these compounds, strong cation interactions should be undermined with the use of ammonia solution. Due to the strong electrostatic interaction bond between positively charged basic compounds and the negatively charged sulfonyl group a strong elution was required to elute the basic drugs retained on the stationary phase. Therefore, adding ammonia in the elution step helps to neutralize the target compounds and leads to their elution. Indeed, high recoveries were exhibited for basic analytes with the use of MCXac-ammonia protocol ranging from 85.9-95.1 %. In addition, sulfonamides provided satisfactory recoveries with MCXac-ammonia, like HLBneu protocol for some compounds. However, this protocol was not simultaneously efficient for acidic NSAIDS such as diclofenac, artificial sweeteners like acesulfame, sucralose, aspartame and gemfibrozil which presented remarkably low recoveries (26.9-33.9 %). Finally, MCXac protocol with formic acid modifier in elution solvent provided the most discouraging results for most of the compounds. The use of formic acid enables the

retention of the analytes in the cation exchanger part of the polymer, resulting to low desorption capability.

The main objective with the use of SPE method, was to develop an extraction procedure that enables the simultaneous analysis of the widest range of pharmaceutical compounds in one single extraction step. Having that in mind and comparing the results obtained, (Fig.7.6) the extraction method based on Oasis HLB cartridges, with no acidification and pure methanol as elution solvent was selected as the optimum one, since better recoveries for a major number of substances were achieved.

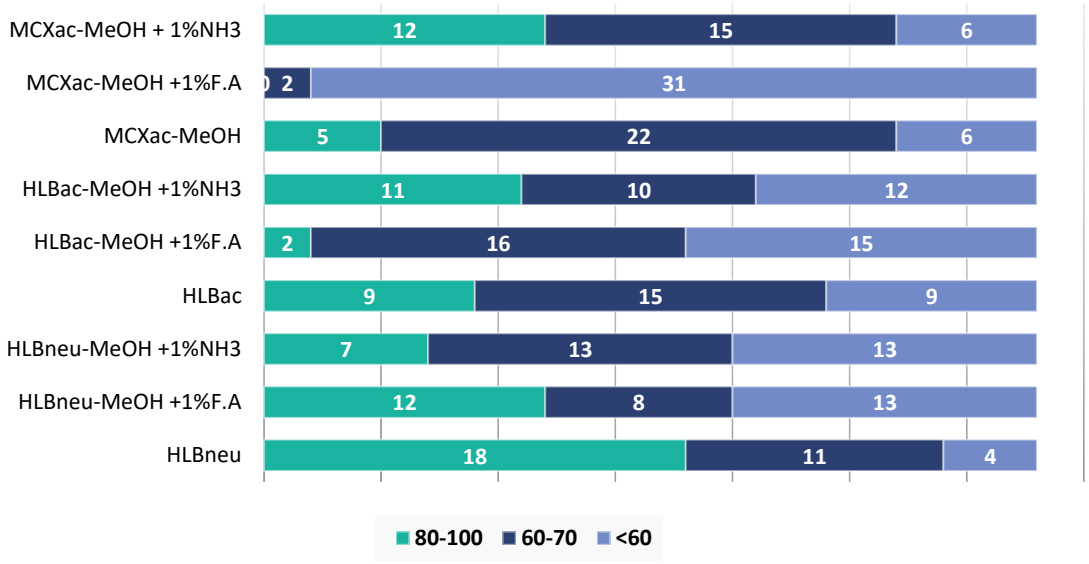


Figure 7.6. Number of target compounds and corresponding recovery ranges exhibited under different SPE protocols investigated

7.3 Addition of chelating agent Na_2EDTA

After the first optimization step mentioned above, SPE method was applied in effluent waters. In this case, an additional optimization step was necessary since most analytes of interest belong to the therapeutic class of antibiotics. According to several studies, antibiotics such as quinolones, macrolides and sulfonamides have a high tendency to react with divalent metallic ions like calcium and magnesium or polyvalent cations in the matrix, and form stable complexes which can bind irreversibly to the cartridge during the loading step. This results in low recoveries and inefficiency [620,674–676]. Therefore, chelating agent Na_2EDTA, which was highly recommended [672], [676,677], to chelate residual metal ions in the environmental samples, was evaluated for the extraction efficiency of selected antibiotics. The chelating agent Na_2EDTA was used prior to

extraction with HLBneu protocol and was compared with no addition of Na_2EDTA with the same protocol. The results of this comparison are summarized in Fig.7.7.

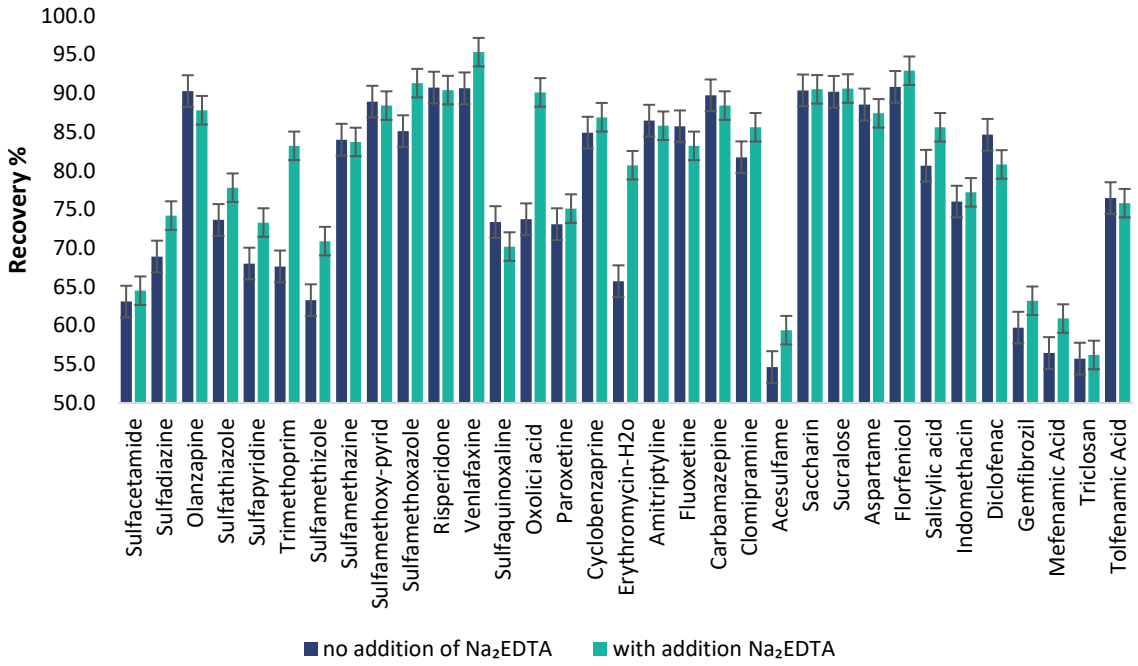


Figure 7.7. Effect of Na_2EDTA addition on the recoveries of target compounds in wastewater effluent

The overall recoveries of the analytes of interest were similar or improved with the addition of Na_2EDTA. The family groups though that were enabled more by the addition of the chelating agent were sulfonamide antibiotics. Furthermore, remarkable improvement was observed for trimethoprim, oxolinic acid and erythromycin, since these compounds have many functional groups to form complexes. A decrease was observed in the extraction efficiency of a group of psychiatric drugs, nevertheless it was negligible. Therefore, the addition of the chelating agent Na_2EDTA prior to extraction was included in the HLBneu protocol.

7.4 Sample volume

The study of the optimal volume was performed using wastewater samples (pooled effluents). Volumes from 50 to 150 mL were loaded to SPE cartridges and extracted with the above protocol. Wastewater volumes higher than 150 mL of clogged the cartridge, thus were not included in the analytical performance evaluation. Results of recoveries with different volumes tested are shown in Fig. 7.8.

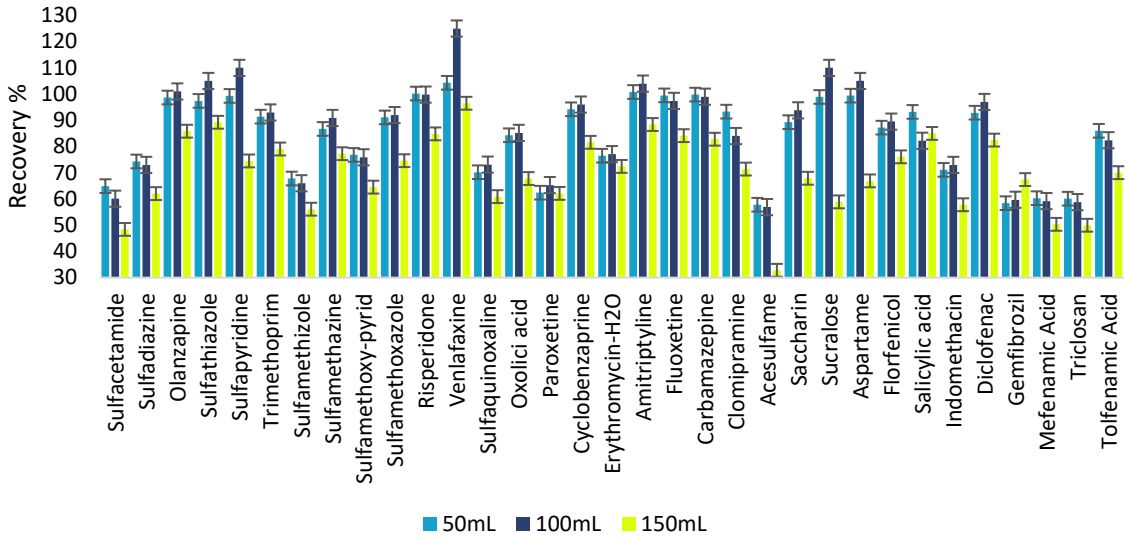


Figure 7.8. Evaluation of sample volume on extraction of wastewater by SPE

Similar mean recoveries were obtained for the majority of the compounds when 50 mL and 100 mL were loaded onto the cartridge, whereas by increasing the volume up to 150 mL the corresponding recoveries were dramatically decreased for sulfacetamide, sulfamethizole and artificial sweeteners. This result is in agreement with other study indicated that method recoveries for saccharin and sucralose decreased with an increase of interfering substances, if larger sample volumes were extracted [622]. Gómez et al. [678] along with other studies confirms this assumption, demonstrating that by reducing the sample volume of complex samples, such as wastewaters, matrix effects may be decreased [604,678] Consequently, the sample volume of effluent water applied in SPE method, considering matrix effect as well as the preconcentration factor, was set at 100 mL.

All the experiment sets for the optimization of SPE procedure are presented in Table 7.1. To sum up the optimal protocol used HLB cartridges, loaded with 100 mL wastewater sample with the addition of Na_2EDTA 0.1% and without acidification prior to extraction. Finally, methanol was used as the most effective elution solvent in these conditions.

Table 7.1 Experiments tested for the SPE procedure optimization

	HLB-1	HLB-2	HLB-3	HLB-4	HLB-5	HLB-6	HLB-7	HLB-8	HLB-9	HLB-10
Sample Pretreatment	pH 6.5-7.0	pH 2.5-3.0	pH 2.5-3.0	pH 6.5-7.0	pH 6.5-7.0	pH 2.5-3.0	pH 2.5-3.0	pH 6.5-7.0	pH 2.5-3.0	**pH 6.5-7.0 +EDTA 0.1%**
Cartridge conditioning	2x5 mL MeOH	2x5 mL MeOH	2x5 mL MeOH	2x5 mL MeOH	2x5 mL MeOH	2x5 mL MeOH	2x5 mL MeOH	2x5 mL MeOH	2x5 mL MeOH	**2x5 mL MeOH**
Washing	5mL H_2O	5mL H_2O	5mL H_2O	5mL H_2O	5mL H_2O	5mL H_2O	5mL H_2O	5mL H_2O	5mL H_2O	**5mL H_2O**
Elution	2x5 mL MeOH	2x5 mL MeOH	2x5 mL MeOH	2x5 mL MeOH +1% F.A	2x5 mL MeOH +1% NH_3	2x5 mL MeOH +1% F.A	2x5 mL MeOH +1% NH_3	2x5 mL AcN	2x5 mL AcN	**2x5 mL MeOH**
	HLB-11	**MCX-1**	**MCX-2**	**MCX-3**	**MCX-3**	**MCX-4**	**MCX-5**	**MCX-6**	**C18-HC-1**	**C18-HC-2**
Sample Pretreatment	pH 2.5-3.0 +EDTA 0.1%	pH 6.5-7.0	pH 6.5-7.0	pH 2.5-3.0	pH 2.5-3.0	pH 2.5-3.0	pH 2.5-3.0	pH 2.5-3.0	pH 6.5-7.0	pH 2.5-3.0
Cartridge conditioning	2x5 mL MeOH	2x5 mL MeOH	2x5 mL MeOH	2x5 mL MeOH	2x5 mL MeOH	2x5 mL MeOH H_2O+1% F.A	2x5 mL MeOH + H_2O+1% F.A	2x5 mL MeOH	2x5 mL MeOH	2x5 mL MeOH
Washing	5mL H_2O +5% MeOH	5mL H_2O +5% MeOH	5mL H_2O +5% MeOH	5mL H_2O +5% MeOH	5mL H_2O +5% MeOH	5mL H_2O +5% MeOH	5mL H_2O +5% MeOH	5mL H_2O +5% MeOH	5mL H_2O +5% MeOH	5mL H_2O +5% MeOH
Elution	2x5 mL MeOH	2x5 mL MeOH	2x5 mL MeOH	2x5 mL MeOH	2x5 mL MeOH	2x5 mL MeOH +1% F.A	2x5 mL MeOH +1% NH_3	2x5 mL AcN	2x5 mL MeOH	2x5 mL MeOH

B. Validation of Solid Phase Extraction (SPE)

The SPE method developed for the determination of the selected emerging contaminants in aqueous media was finally validated. The study of analytical performance were based on the quality control procedures established by the European Union (EU) regulations (EU Commission Decision, 2002)[578]. Validation procedures were carried out in two different samples, tap water and effluent wastewater providing different performance criteria such as sensitivity, linearity, precision and reproducibility, accuracy. Matrix effect studies were also evaluated for both aqueous matrices. Characteristics of analytical performance of SPE method are illustrated at the end of the Chapter in Tables 7.2 and 7.3.

7.5 Accuracy

Accuracy of the developed method was checked by recovery experiments. For this purpose, three replicates of spiked tap water and effluent sample at three concentration levels of approximately 50, 250 and 500 ng/L (low, medium, high) were analyzed under the optimum conditions. Blank samples (unspiked) were analyzed as well. The concentration levels were selected based on those estimated in wastewater effluent samples from previous studies in Greece [260,662–664]. In the case of effluent samples, due to the fact that unspiked effluent samples already contained some of the compounds, the concentration of the respective unspiked sample (blank) was subtracted from the concentration in the spiked sample and then divided by the spiked level. (Eq.1)

$$R\% = \frac{Cspiked\ exp - Cblank}{Cspiking\ level} \text{x } 100 \qquad \text{Eq.1}$$

Recoveries in the SPE method ranged from 52.6%-119.0% for effluent water while for tap water recoveries varied from 63.9 to 101.0% for all concentration levels (Table 7.2). Recoveries below 60% where considered in the correction of the concentration in the quantification procedure.

7.6 Sensitivity

Regarding sensitivity, method detection limits (MDLs) and method quantification limits (MQLs) were determined, for tap water and effluent samples, as the minimum detectable and quantifiable amount of analyte with a signal-to-noise of 3 and 10, respectively. Spiked tapwater and effluent samples (n = 3) as indicated above, were used for their calculation. Levels of MDLs and MQLs varied depending on the aquatic matrix, and higher values were achieved for WWTP effluent water.

MDLs in tap water ranged between 0.1 and 12.5ng/L while in effluent wastewaters between 0.3 and 34.7ng/L The respective MQLS for the analyzed compounds varied from 0.3 and 38.7 ng/L for tap water and 1.0-101.0 ng/L for effluent wastewater with higher value for sucralose (Table 7.3).

7.7 Linearity

The linearity of the proposed method was investigated by setting a 10-point calibration curve using linear regression analysis, establishing the adequate lineal range for each compound from MQL to approximately 100xMQL, for tap water and effluent wastewater (covered range 0.3-10,000 ng/L) In each concentration range, all target compounds exhibited good linearity and calibration curves showed, in all cases, coefficients of determination (R^2) greater than 0.99 (Table 7.3).

7.8 Precision

The precision expressed as repeatability (intra-day precision), and reproducibility (inter-day precision) in terms of Relative Standard Deviation (RSD_r and RSD_R respectively) was evaluated in tap water samples and effluent wastewater spiked at three concentration levels of 50, 250 and 500 ng/L. For intraday precision, results were obtained from the analysis of five spiked samples (n=5) for each concentration level within the same day, while for inter-day precision, spiked samples were analyzed over three days (n=15). RSDs were estimated by dividing the standard deviation by the mean value obtained for each set of concentrations and multiplying it by 100. Regarding tap water, RSDr (n=5) values for intra-day analyses were in the range of 1.1–16.3% and the RSD_R for inter-day (n = 15) values were between 2.4% and 17.0% . For effluent water, the RSDs of the spiked samples were lower than 14% for all the target analytes with only exception salicylic acid (Table 7.2). The higher RSD of salicylic acid observed at the lowest concentration level (50 ng/L) can be attributed to the elevated concentrations found in the blank samples.

7.9 Matrix Effect

Matrix effects from the water samples mentioned above, tap water (BW) and effluent wastewater, (WWTP) were evaluated for the 33 selected pharmaceutical compounds. To estimate the degree of ion suppression or enhancement, Eq. (2) was used. Fig.7.9 gives an overview of the obtained results.

$$Matrix\ effect\ (\%) = \left(\frac{Slope\ matrix-matched}{Slope\ standard\ solution} - 1\right) x100 \qquad \text{Eq.2}$$

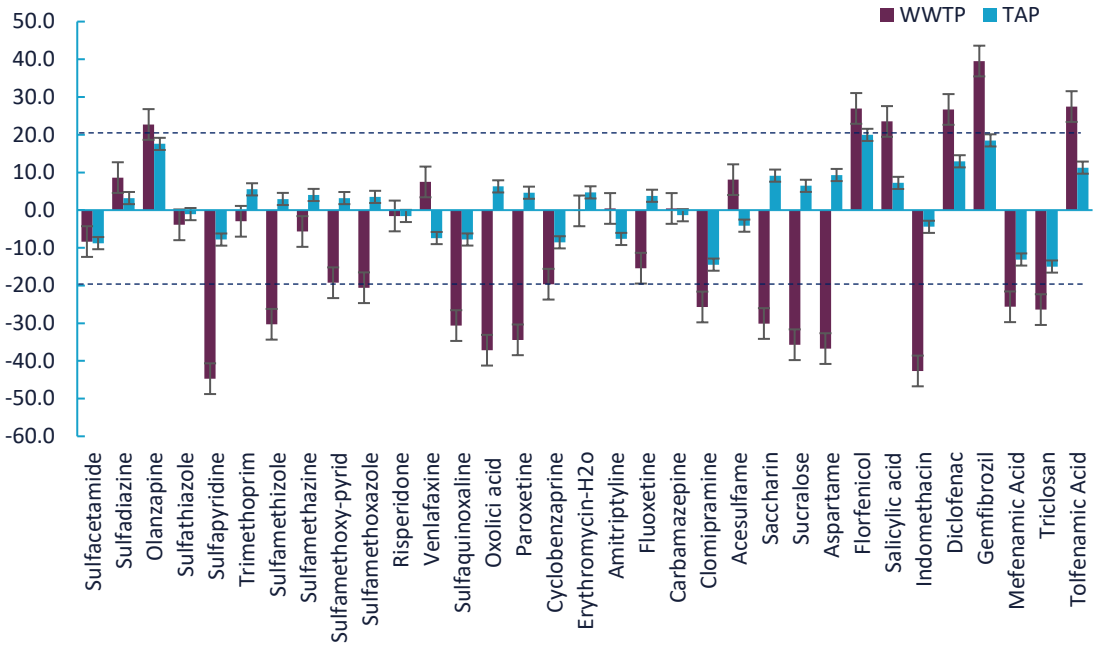


Figure 7.9. Matrix effect for the selected pharmaceuticals in the different aqueous matrices after SPE

For almost half of the studied samples (analyzed by LC-ESI-MS-Orbitrap technique), matrix composition caused signal suppression of target analytes. This effect was observed especially in the case of wastewater samples. The lowest values of ME were determined for analysis of tap water samples (from -1.05 to −18.49 %). The results obtained in effluent wastewater were different since matrix effect is more present in the these samples with 19 compounds out of 33 showing medium matrix effect (−50% and −20% >ME>+20% and +50%), while 14 compounds display low or negligible matrix effect, expressed in most of the cases as ion suppression. As it was expected, the highest signal suppression was observed in effluent wastewater with maximum negative values for sulfapyridine (-44.7%) and indomethacin (-42.6%). This is not a surprise as analyzed wastewater samples contain very high amounts of organic and inorganic matter that are recognized as enhancers of ME.

The overall greater signal suppression in the wastewater samples also shows that the wastewater organic matter exerts a strong matrix effect. This phenomenon may be caused by several reasons. Co-extracted organic materials (humic acids) cause signal suppression in ESI-MS detection as they may reduce ionization efficiency of the analytes by taking up some of the limited number of excess charged sites on the surfaces of electrosprayed droplets [584,678,679]. Schmitt-Kopplin et al. [680]. showed that antibiotics may sorb to organic matters in the samples. Furthermore,

especially polar analytes in the positive ionization mode are more susceptible to undergo ion suppression [582,621]

Some compounds were influenced by an enhancement of the signal (>20%) in effluent matrix with maximum enhancement for Gemfibrozil (39.54%). In general, the ion enhancement can be caused by matrix components which act as a dopant, increasing the ionization efficiency of analytes with high ionization energy [681].

Although there is evidence of Matrix effect in most of the compounds, the range of signal decrease in wastewater samples didn't exceeded the accepted level indicating the efficiency of Solid Phase Extraction as pretreatment technique. Unfortunately, in the case of complex aquatic matrices such as effluent waste waters, the complete elimination of interferents from samples is not normally possible. However, the introduction of the washing step, of 5% aqueous methanol in SPE procedure played an important role in the decrease of matrix effect. Furthermore, the neutral conditions of extraction procedure (pH 6.5-7.0) as well as the non-acidification in elution step of SPE, are parameters associated with low matrix effects. In fact when MeOH is used in acidic conditions as elution solvent, effects of co-extracted components may probably occur [682]. Specifically, according to studies, impurities such as organic matters and humic acids in the matrix might have interactions with target compounds forming conjugates which are co-extracted and influence the detection results [683,684]. As a matter of fact, Ben et al. [684] found that MeOH could elute the antibiotics along with a significant amount of interfering organics in wastewater samples when the pH was adjusted to 2.5– 3.0, resulting to severe matrix effects.In any case the use of matrix-matched calibration curves is recommended to ensure acceptable accuracy in the quantification of target compounds in wastewater.

Table 7.2. Mean recoveries (%), RSD_r and RSD_R (%) in tap water, and effluent wastewater at three concentration levels with SPE

	TAP WATER									WWTP EFFLUENT								
Compound	50 ng/L			250 ng/L			500 ng/L			50 ng/L			250 ng/L			500 ng/L		
	R% n=3	RSD_r% n=5	RSD_R% n=15	R% n=3	RSD_r% n=5	RSD_R% n=15	R% n=3	RSD_r% n=5	RSD_R% n=15	R% n=3	RSD_r% n=5	RSD_R% n=15	R% n=3	RSD_r% n=5	RSD_R% n=15	R% n=3	RSD_r% n=5	RSD_R% n=15
Sulfacetamide	63.9	3.9	5.8	69.4	3.4	5.8	70.1	3.9	4.5	52.6	5.4	6.0	60.1	6.5	7.2	58.7	7.0	7.2
Sulfadiazine	72.9	3.8	4.9	75.8	4.2	6.2	82.3	5.1	6.1	57.9	4.3	5.4	73.0	4.8	5.8	79.4	6.9	8.2
Olanzapine	95.8	4.2	5.8	99.3	3.9	4.1	99.0	2.1	3.1	94.8	5.8	6.9	101.0	7.3	8.2	111.3	1.6	2.4
Sulfathiazole	77.3	2.9	3.8	81.0	2.5	3.3	92.1	1.8	3.8	72.3	3.8	6.1	105.0	5.4	6.3	115.3	4.8	5.5
Sulfapyridine	73.9	4.5	6.5	74.8	4.9	5.2	82.8	3.2	4.8	74.8	5.2	5.9	110.0	4.9	6.5	118.8	6.0	7.9
Trimethoprim	81.2	1.2	4.1	83.2	2.3	3.8	88.9	1.9	5.1	74.8	1.8	3.9	93.0	2.1	5.5	83.0	1.4	3.4
Sulfamethizole	70.4	3.0	5.1	72.9	2.4	4.7	80.1	2.1	6.3	64.8	3.5	6.9	66.0	3.1	6.1	63.8	5.3	5.8
Sulfamethazine	90.1	5.8	8.1	92.4	4.8	5.2	95.6	1.8	4.8	87.9	6.2	7.0	90.9	6.1	6.9	85.7	2.4	3.0
Sulfamethoxy-pyrid	95.6	1.8	3.4	97.8	1.3	3.9	97.4	2.8	6.1	70.9	2.1	4.2	75.9	3.8	4.7	72.5	4.0	4.9
Sulfamethoxazole	90.1	4.1	4.8	93.8	5.2	7.1	95.2	3.1	5.3	87.4	3.9	5.5	92.0	4.6	6.8	87.0	2.8	3.9
Risperidone*	93.7	2.5	6.4	99.8	3.5	4.4	100.0	2.9	4.7	90.1	8.1	8.9	99.8	9.1	12.0	99.2	6.2	7.0
Venlafaxine	100.9	5.8	6.1	99.7	7.1	9.1	101.0	2.8	9.0	112.0	3.8	6.2	115.0	6.0	6.1	119.0	12.1	13.0
Sulfaquinoxaline	75.6	3.9	5.4	80.7	2.3	5.1	83.4	1.4	4.5	69.8	4.5	6.1	73.1	4.2	6.3	70.1	3.4	5.0
Oxolinic acid	90.4	3.5	5.2	92.1	1.2	3.3	95.1	3.5	5.7	82.1	2.7	3.7	85.2	3.3	5.1	83.8	4.8	6.6
Paroxetine*	77.5	2.1	4.0	80.4	2.8	4.6	82.3	3.9	5.0	61.6	1.6	3.3	65.3	5.1	7.1	64.4	2.0	4.1
Cyclobenzaprine*	89.4	3.2	4.8	93.4	3.1	4.4	94.9	2.1	3.1	85.0	1.8	5.0	96.0	3.6	4.0	92.0	1.1	3.9
Erythromycin-H2o	79.8	2.4	3.4	82.2	5.1	6.0	83.4	2.6	5.1	72.1	4.1	4.9	77.1	6.4	7.5	72.9	3.2	4.4
Amitriptyline*	93.2	5.8	6.3	95.1	2.9	3.1	98.4	3.8	4.9	83.0	1.9	2.9	104.0	2.4	3.3	100.0	4.1	5.2

Fluoxetine*	92.8	3.4	4.1	94.3	3.1	3.8	99.3	4.0	5.8	104.0	2.6	3.8	97.4	2.9	3.1	93.9	3.5	3.9
Carbamazepine*	94.4	2.5	3.0	98.7	1.5	2.9	100.0	1.6	2.6	88.3	1.2	4.1	99.0	2.2	3.5	106.0	0.8	1.5
Clomipramine*	80.1	6.7	7.1	89.9	4.7	5.3	93.7	2.1	5.5	76.0	4.1	6.8	84.0	3.3	4.9	69.7	5.1	6.8
Acesulfame	59.9	6.7	7.2	60.1	1.8	2.4	59.3	4.2	7.6	54.3	5.0	8.2	56.9	1.8	2.7	55.8	7.5	9.3
Saccharin	93.7	3.5	3.9	99.4	6.3	6.7	98.7	2.7	4.5	90.1	1.1	4.3	93.8	6.3	7.9	92.2	3.5	5.8
Sucralose	98.4	1.1	2.5	99.2	1.9	3.0	99.3	3.6	5.1	98.2	2.1	5.2	110.0	1.9	3.8	95.4	5.0	6.3
Aspartame	92.1	2.8	4.0	97.4	8.2	9.2	97.1	4.7	6.2	89.9	6.6	7.7	105.0	8.2	10.0	99.2	4.5	8.0
Florfenicol	94.6	5.8	6.3	99.9	2.5	6.2	100.0	3.9	6.9	87.2	7.0	8.9	89.5	2.5	4.4	90.5	10.0	13.3
Salicylic acid	84.3	13.5	15.9	88.7	8.9	10.8	89.4	5.8	8.8	70.6	19.9	21.0	82.2	8.9	11.2	79.3	5.8	7.4
Indomethacin	79.4	8.6	11.0	83.6	2.2	3.1	86.1	3.9	7.1	71.2	8.1	13.0	73.0	2.2	3.9	69.7	4.5	5.2
Diclofenac	89.1	9.7	11.8	93.1	10.8	13.4	98.0	6.4	8.6	103.0	9.5	18.2	97.0	10.8	11.0	114.0	7.0	7.3
Gemfibrozil	62.0	8.5	10.1	65.7	12.9	14.6	67.1	13.9	17.0	53.9	10.4	14.4	59.7	12.9	13.3	55.5	11.0	14.2
Mefenamic Acid	58.4	16.3	13.2	62.1	10.2	13.1	69.7	8.1	9.4	55.0	13.3	16.3	59.2	10.2	14.8	56.9	2.2	5.1
Triclosan	60.1	5.4	2.8	61.3	9.8	10.3	62.9	7.4	8.6	56.8	7.1	7.6	58.8	9.8	11.2	57.6	3.1	5.9
Tolfenamic Acid	81.0	8.7	9.4	84.1	9.9	11.1	80.2	8.3	9.3	76.3	14.4	15.8	82.4	9.9	12.5	77.9	8.9	16.6

IS*: (a) olanzapine d3; (b) carbamazepine d10; (c) fluoxetine d5; (d) amitriptyline d6

Table 7.3. Parameters indicating the performance of SPE. Method detection and quantification limits (MDL, MQL), linearity(R^2) and matrix effect in all matrices studied

COMPOUND	R^2	MDL ng/L	MQL ng/L	ME %	R^2	MDL ng/L	MQL ng/L	ME %
	TAP WATER				WWTP EFFLUENT WATER			
Sulfacetamide	0.9992	3.6	11.0	-8.8	0.9973	10.5	33.3	-8.3
Sulfadiazine	0.9986	1.8	5.2	3.2	0.9967	7.4	23.5	8.6
Olanzapine	0.998	0.5	2.0	17.6	0.9961	2.7	5.5	22.7
Sulfathiazole	0.9989	1.2	3.5	-1.1	0.9970	6.9	19.4	-3.9
Sulfapyridine	0.9994	1.2	2.9	-7.8	0.9975	4.8	14.7	-44.7
Trimethoprim	0.9997	0.4	1.0	5.5	0.9978	1.1	4.0	-3.0
Sulfamethizole	0.9998	1.0	3.2	3.0	0.9959	2.4	8.1	-30.3
Sulfamethazine	0.9998	0.4	1.3	4.0	0.9949	1.9	4.8	-5.7
Sulfamethoxy-pyrid	0.9997	0.5	1.4	3.2	0.9928	1.7	5.4	-19.3
Sulfamethoxazole	0.9997	0.2	0.5	3.5	0.9978	0.9	3.0	-20.6
Risperidone	0.9983	0.6	1.8	-1.6	0.9924	1.4	4.2	-1.6
Venlafaxine	0.9999	0.1	0.3	-7.4	0.9980	0.5	1.1	7.5
Sulfaquinoxaline	1.0000	0.4	1.5	-7.8	0.9991	1.5	3.8	-30.6
Oxolinic acid	1.0000	0.2	0.6	6.3	0.9981	0.7	2.1	-37.2
Paroxetine	0.9990	1.0	2.6	4.6	0.9971	3.2	10.1	-34.4
Cyclobenzaprine	0.9996	0.1	0.4	-8.5	0.9977	0.4	1.2	-19.7
Erythromycin-H2o	0.9994	1.0	2.8	4.7	0.9945	2.7	8.4	-0.2
Amitriptyline	0.9995	0.4	0.9	-7.7	0.9936	0.9	2.3	0.4
Fluoxetine	0.9995	0.4	1.3	3.8	0.9976	1.2	3.9	-15.4
Carbamazepine	0.9999	0.1	0.3	-1.4	0.9993	0.3	1.0	0.4
Clomipramine	0.9991	0.6	1.5	-14.5	0.9972	1.3	3.9	-25.7
Acesulfame	0.9998	4.8	15.5	-4.1	0.9991	15.3	52.0	8.1
Saccharine	0.9999	3.5	10.6	9.1	0.9984	10.7	38.0	-30.1
Sucralose	0.9998	8.7	25.5	6.4	0.9973	28.7	101.0	-35.7
Aspartame	0.9993	12.5	38.7	9.3	0.9964	34.7	98.0	-36.7
Florfenicol	0.9997	0.2	0.6	20.0	0.9944	0.8	2.9	27.0
Salicylic acid	0.9991	3.8	10.8	7.2	0.9913	15.2	44.8	23.5
Indomethacin	0.9994	1.4	4.9	-4.4	0.9977	8.1	23.8	-42.7
Diclofenac	0.9996	3.1	10.4	12.9	0.9933	12.8	43.9	26.7
Gemfibrozil	0.9998	1.1	3.2	18.5	0.9922	10.4	31.3	39.5
Mefenamic Acid	0.9995	1.0	2.6	-13.1	0.9986	7.9	23.3	-25.6
Triclosan	0.9988	0.5	1.6	-15.0	0.9928	2.3	8.0	-26.4
Tolfenamic Acid	0.9993	0.3	1.0	11.3	0.9969	4.0	11.4	27.5

CHAPTER 8: OCCURRENCE OF TARGET ECS IN WWTPS-MONITORING STUDY

8.1 Sampling Area- Collection of Samples

8.1.1 WWTP of Amaliada city

Amaliada is a city located in the West part of Peloponnese in Greece. It is the capital of Municipality of Ilida and is situated in the middle of the fertile plain of Prefecture of Ilia. The western limits of the municipality are the shores of the Ionian Sea while the eastern ones are the lush hills, where concludes the massif of the central Peloponnese. It covers 401,9 km^2 and its population is about 32.000 habitants according to census of 2011. It regards a region with intense agricultural and veterinary activities. The WWTP of Amaliada city receives combined urban (domestic and stormwaters), hospital wastewaters as well as industrial wastewaters and discharges its effluents in the Ionian Sea.

Figure 8.1. Sampling site of WWTP of Amaliada city in the map of Greece and the corresponding unit

The WWTP of Amaliada initially performs preliminary treatment which is consisted of a screen and a grit-removal tank. The biological treatment consists of a conventional activated sludge system (recycling of ca. 80 % of the sludge) with an anaerobic step with phosphorus removal, followed by two aerobic and anoxic tanks where the water is alternated through them and nitrification, denitrification take place respectively. The next step is the secondary sedimentation and finally the disinfection with NaClO, which was executed before the effluent is released in the final recipient of Ionian Sea.

8.1.2 WWTP of Ioannina city

Ioannina is the capital and largest city of regional unit of Epirus, an administrative region in north-western Greece. According to the 2011 census, the city population was 65,574, while the municipality had 112,486 inhabitants. It lies at an elevation of approximately 500 meters (1,640 feet) above sea level, on the western shore of lake Pamvotis. The WWTP of Ioannina city receives combined urban (domestic and stormwaters), hospital wastewaters as well as industrial wastewaters, and discharges its effluents in Kalamas River.

Figure 8.2. Sampling site of WWTP of Ioannina city in the map of Greece and the corresponding unit

The municipal plant of Ioannina city is connected to a sewage system servicing all the municipal area. This system, as many others in Greece with old infrastructure, still have “combined” sewers that collect both domestic sewage and stormwaters, often deliver sewage volumes during rainstorm events that exceed the treatment capacity of the municipal WWTP. This system can therefore have numerous direct discharges of untreated effluents (Fig. 8.3). Primary treatment, known as mechanical treatment, consists of a screen, an aerated grit-removal tank, and a primary sedimentation tank. The next step is the biological treatment that separates and breaks down organic contaminants, with the aid of microorganisms. After the primary treatment, the effluent is directed to the activated sludge system for the removal of phosphorus, denitrification, and nitrification. The activated sludge process is used involving recycling of 60% of the sludge giving an average solid retention time (SRT) of 11 days. The hydraulic retention time (HRT) of the WWTP, calculated from the flow and the volume of the treatment tanks, varies between 1.52 and 4.05 h depending on seasonal variation and precipitation. The biological procedure consists of an anaerobic step followed by an anoxic and a larger aerobic decomposition step. Phosphorus removal is achieved first with the biological anaerobic step and then with a chemical treatment. In the chemical removal of phosphorus, a simultaneous precipitation takes place with the addition of $FeCl_2$ (500–600 L/day) after the biological step. Then the water is passed through the secondary sedimentation and as a final cleaning step the water is passed through a sand filter and is disinfected before it reaches the recipient of Kalamas River [662].

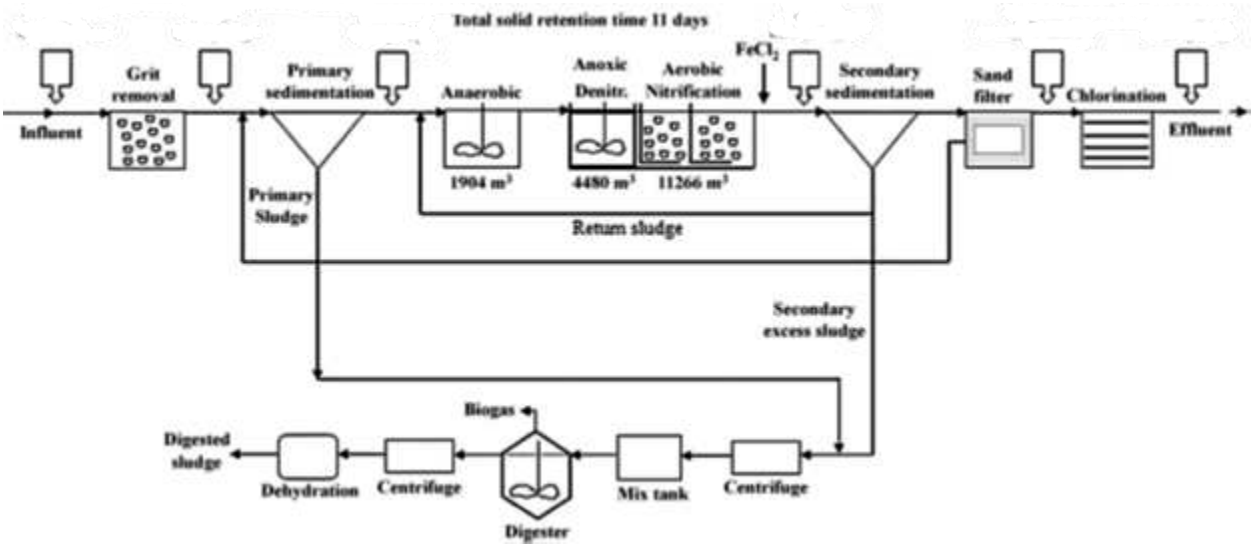


Figure 8.3. Scheme of the municipal WWTP of Ioannina city [662]

8.1.3 WWTP of University Hospital of Ioannina

The University hospital of Ioannina city has a capacity of 800 beds and provides a broad range of clinical services and medical specialties, since it is also a center of research. It serves a population of approximately 130,000 inhabitants since it is a reference hospital for Epirus region and suburbs. Wastewater effluents of the hospital are discharged into public sewer network, being co-treated with domestic wastewaters in municipal WWTPs. The hospital plant applies a pretreatment (grit-removal), a flow equilibration tank, and a biological secondary treatment concluding with disinfection with the addition of NaClO (15% solution), [662] (Fig. 8.4). The hydraulic retention time (HRT) of the WWTP is 6 h while the solid retention time (SRT) is 1.5 day.

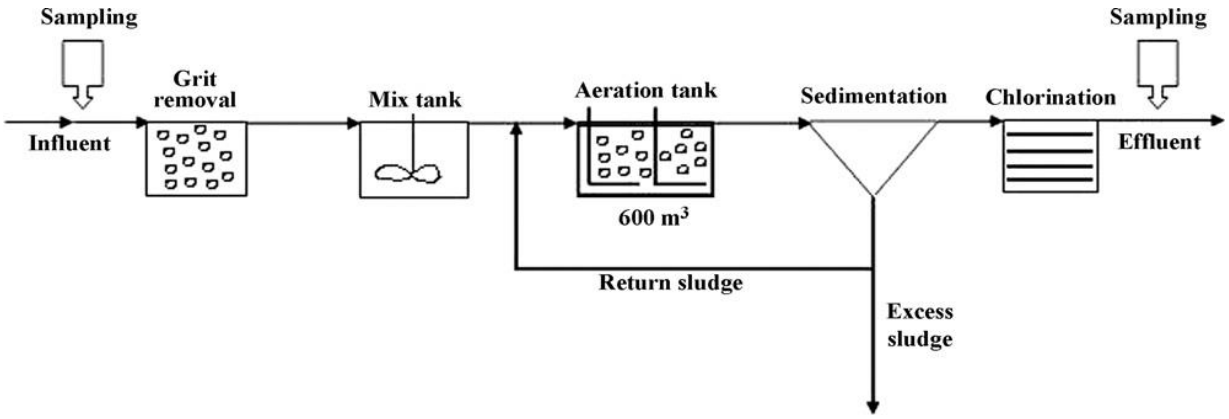


Figure 8.4. Scheme of the municipal WWTP of University Hospital of Ioannina city [662]

Hospital WWTP is the smaller unit from all the units investigated in this study, as it receives lower loads than the other bigger municipal WWTPs. However, this fact does not indicate that the hospital WWTP is an efficient unit since even though lower loads reach the unit, their variety and complexity makes them a major source of contamination that substantially contributes to the total wastewater loads of the municipal WWTP. For example a big number of undesirable organic xenobiotic compounds such as pharmaceutical residues, radionuclides, antibiotic resistant bacteria, solvents and disinfectants as well as laboratories and surgeries materials are included in the burden of the hospital wastewaters, making the loads that result into the urban network even more complex [136,138,685]. Table 8.1 shows the technical characteristics of the investigated WWTPs. Main differences among them refer to their water treatment capacity, the hydraulic retention times, and solid retention times.

Table 8.1. Characteristics of WWTP studied

WWTP	Population served	Average Flow (m^3/d)	Treated Wastewater	SRT (d)	HRT (h)	Primary treatment	Secondary treatment	Final recipient
Amaliada	32000	3371	Urban and industrial	28	20	Grit Removal	Activated Sludge	Ionian Sea
Ioannina	130000	25276	Urban and industrial	1.5	6	Grit Removal-Primary settling	Activated Sludge	Kalamas River
University Hospital	800	550	Hospital	11	1.5-4	Grit Removal	Activated Sludge	Urban network

8.2 Collection of Samples

A monitoring program was conducted embracing a total of twelve sampling periods (monthly) covering the four seasons over the period of one year. Sampling campaigns were performed from March 2019 until February of 2020. Thirty-six (36) in total wastewater samples, one for each month (n=12), were collected during the sampling period from WWTP of Amaliada (WWTP-AMA) and Ioannina city (WWTP-IOA), as well as from University hospital of Ioannina (WWTP-HOS).

The collection of the samples was performed according to EPA guidelines for wastewater sampling [686]. Composite samples were collected over time, manually, by continuous sampling to obtain a representative sample with average wastewater characteristics during the sampling day. A time composite sample consists of equal volume discrete sample aliquots collected at constant time intervals into one container. Specifically, in each sampling site the procedure adopted was as follows: samples were collected every 120 min, from 8:00 to 16:00 h, and then combined to provide a final representative composite sample. Final volumes of 1 L wastewater effluents were collected at the final stage of the plant after the secondary treatment and disinfection. One aliquot of sample was used for BOD and COD analysis and measuring basic physicochemical parameters while the other fraction was used for the analysis of target compounds. All samples were collected in amber glass bottles pre-rinsed with deionized water. Up on their arrival in the laboratory, were filtered with 1 μm glass fiber filters (GF/B, Whatman, UK) to eliminate the particulate matter. Afterwards, the samples were stored in the dark at 4 °C for analysis within 48 h. Otherwise, the samples were preserved at-20^{o}C for post laboratory processing. The wastewater characterization parameters measured during the monitoring period for all WWTPs are depicted in Table 8.2. BOD_5 values ranged from 1.0-9.6 mg/L

and 0.7-14.9 mg/L, for WWTP-IOA and WWTP-HOS, while for WWTP-AMA was up to 19 mg/L. COD values in WWTP-IOA and WWTP-HOS effluents were from 19.1-59.5 mg/L and 1.2-109.5 mg/L, respectively and 22.3-59.9 mg/L for WWTP-AMA effluents. Slightly higher BOD_5 and COD values of WWTP-HOS effluents, are observed in WWTP-HOS effluents. The physicochemical parameters usually accessed for hospital effluents include higher organic and inorganic loadings in terms of BOD, COD, conductivity, in comparison with domestic sewage [687]. The ranges of physicochemical parameters values in WWTP-HOS are within those reported for hospital effluents collected in different countries over a 20-year span [685]. A notable differentiation in physicochemical characteristics of the three types of effluents, occurs for values of total Nitrogen. Specifically, the effluent of WWTP-AMA presents higher mean concentration of total N, i.e. 7.6 mg/L, compared to WWTP-IOA and WWTP-HOS with corresponding values of 3.6 mg/L and 3.9 mg/L. A possible explanation is related to agricultural activities, which are the main financial source of Amaliada city. Through the runoff process from fertilized croplands, high nitrogen loads discharge into drains and flow to sewer system. In addition, functional parameters like nitrification, denitrification during treatment processes may differ. Concentration of total Phosphate followed similar trend in all WWTPs (P<1.2 mg/L)

.

Table 8.2. Physicochemical characteristics of effluent wastewater of each sampling site at each sampling month

Parameters	WWTP-AMALIADA-EFFLUENT											
	Mar-19	**Apr-19**	**May-19**	**Jun-19**	**Jul-19**	**Aug-19**	**Sep-19**	**Oct-19**	**Nov-19**	**Dec-19**	**Jan-20**	**Feb-20**
Conductivity at 25°C (μS/cm)	191.2	158.8	151.2	149.2	282.3	137.6	223.3	115.6	167.2	192.6	152.9	133.9
Temperature (°C)	17.8	18.2	22.8	24.9	27.1	28.1	24.4	22.9	23.1	21.6	17.8	17.5
pH	7.1	6.8	6.5	7.1	6.9	7.2	7.0	6.6	6.9	6.8	5.7	6.3
COD (mg/L)	56.6	34.0	36.2	31.7	29.5	24.6	42.7	37.1	34.6	59.9	24.9	22.3
BOD_5 (mg/L)	13.3	12.21	11.4	11.7	13.4	19.0	11.0	10.5	8.9	7.1	12.5	10.8
Total P (mg/L)	0.9	0.8	1.1	1.2	1.2	0.9	0.9	0.9	0.7	0.8	0.8	1.1
Total N (mg/L)	8.5	8.2	7.8	7.6	8.5	7.1	6.7	7.5	7.6	6.7	7.9	7.8
Parameters	**WWTP-IOANNINA-EFFLUENT**											
	Mar-19	**Apr-19**	**May-19**	**Jun-19**	**Jul-19**	**Aug-19**	**Sep-19**	**Oct-19**	**Nov-19**	**Dec-19**	**Jan-20**	**Feb-20**
Conductivity at 25°C (μS/cm)	242.2	208.5	237.2	308.2	332.9	299.5	242.4	214.9	207.7	172.0	217.2	211.7
Temperature (°C)	16.5	17.1	19.2	20.5	21.3	21.8	20.5	20.1	17.1	13.8	15.7	16.9
pH	6.8	7.1	6.9	7.5	7.2	6.9	7.3	7.7	7.2	6.7	6.8	6.9
COD (mg/L)	43.5	50.2	29.3	48.3	59.5	48.8	45.6	44.3	37.5	44.7	19.1	19.4
BOD_5 (mg/L)	6.2	4.5	7.6	8.1	8.0	9.6	3.3	2.0	1.0	1.4	6.2	6.7
Total P (mg/L)	0.5	0.7	0.9	0.7	0.8	0.9	0.6	0.7	0.9	0.9	0.9	0.6
Total N (mg/L)	4.7	4.9	4.4	2.6	2.0	2.1	2.6	2.7	2.3	4.0	5.9	4.8
Parameters	**WWTP-UNIVERITY HOSPITAL OF IOANNINA- EFLUENT**											
	Mar-19	**Apr-19**	**May-19**	**Jun-19**	**Jul-19**	**Aug-19**	**Sep-19**	**Oct-19**	**Nov-19**	**Dec-19**	**Jan-20**	**Feb-20**
Conductivity at 25°C(μS/cm)	382.9	423.3	352.5	384.2	394.8	402.8	364.2	332.8	485.3	345.9	447.6	523.8
Temperature (°C)	16.9	17.5	18.5	21.8	22.4	21.2	20.9	19.8	17.8	15.2	16.4	16.7

Table 8.2. Physicochemical characteristics of effluent wastewater of each sampling site at each sampling month, *(continued)*												
pH	6.5	6.8	6.1	6.2	6.5	6.6	6.7	7.1	7.3	6.6	6.9	6.2
COD (mg/L)	30.1	12.8	1.2	11.2	18.1	15.9.	89.3	14.2	9.5	23.9	109.5	29.9
BOD_5 (mg/L)	14.9	3.9	0.7	8.1	9.2	9.6	4.9	8.2	8.3	7.7	4.9	7.3
Total P (mg/L)	0.3	0.2	0.5	0.4	0.4	0.6	0.5	0.4	0.5	0.3	0.3	0.2
Total N (mg/L)	5.1	5.6	5.1	2.3	4.3	4.8	2.3	3.7	4.1	3.9	2.8	2.6

8.3 Occurrence of target ECs in WWTPs- 1-year monitoring study

The target analytes were investigated attempting to evaluate their occurrence and fate in a small and bigger municipal wastewater treatment plant of Amaliada and Ioannina (WWTP-AMA,WWTP-IOA) respectively, as well as in the University Hospital of Ioannina city (WWTP-HOS). The total assessment of ECs in wastewater effluents was performed with the multi-residue method of SPE described in Section 2.8. The results of three sampling campaigns in a period of year showed an occurrence of ECs in a range of 0–100%, depending on the compound, the sampling station and the month of collection, whereas the concentrations ranged from very few to some thousands of ng/L; therefore, all the results are expressed in ng/L units. The individual concentrations of ECs detected in effluent wastewater samples, collected during 1-year monitoring, at three WWTPs, are summarized in Tables 8.3, 8.4 and 8.5.

Table 8.3. Concentrations (ng/L) of target ECs in the WWTP of Ioannina city for each sampling month

WWTP Ioannina City-Concentrations (ng/L)												
Compounds	**Mar-19**	**Apr-19**	**May-19**	**Jun-19**	**Jul-19**	**Aug-19**	**Sept-19**	**Oct-19**	**Nov-19**	**Dec-19**	**Jan-20**	**Feb-20**
Artificial Sweeteners												
Acesulfame	10324.6	8542.1	12652.6	11834.1	13569.0	12331.3	9564.8	10234.2	7567.6	6543.9	7023.1	6054.8
Aspartame	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Saccharin	<MQL	<MQL	40.8	64.9	<MQL	52.8	<MQL	<MQL	<MQL	<MQL	<MQL	n.d
Sucralose	1246.1	1949.15	464.2	2463.4	3214.1	1098.3	3137.4	4075.25	1523.6	642.85	647.8	992.6
Antibiotics												
Erythromycin-H2O	<MQL	n.d	n.d	n.d	n.d	n.d	n.d	n.d	<MQL	<MQL	n.d	n.d
Florfenicol	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Oxolici acid	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Sulfacetamide	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Sulfadiazine	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Sulfamethazine	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Sulfamethizole	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Sulfamethoxazole	189.6	<MQL	n.d	n.d	n.d	35.9	21.4	5.8	40.2	29.7	81.2	23.4
Sulfamethoxy-pyrid	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Sulfapyridine	n.d	n.d	72.4	<MQL	n.d	n.d	<MQL	n.d	n.d	n.d	n.d	n.d
Sulfaquinoxaline	12.4	n.d	52.8	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Sulfathiazole	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Trimethoprim	n.d	53.4	42.9	32.5	<MQL	<MQL	<MQL	70.1	65.1	96.8	73.6	82.5
NAIDS-Analgesics												
Diclofenac	152.9	302.4	252.6	689.4	221.6	158.4	518.5	112.9	245.6	85.8	610.1	105.9
Indomethacin	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Mefenamic Acid	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	<MQL

Table 8.3. Concentrations (ng/L) of target ECs in the WWTP of Ioannina city for each sampling month, *(continued)*

Salicylic acid	723.6	881.8	630.2	1958.8	1632.6	1458.3	2321.8	2413.6	1856.0	2913.5	932.4	851.0
Tolfenamic Acid	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Disinfectants												
Triclosan	115.4	321.8	52.9	132.5	258.4	199.6	142.8	84.2	196.8	200.2	99.6	105.8
Antidepressants-Psychiatrics												
Amitriptyline*	15.5	42.6	23.5	10.9	23.1	28.6	45.2	72.1	5.5	23.4	38.6	<MQL
Carbamazepine*	133.2	301.9	98.9	120.8	132.4	117.6	95.8	82.1	77.6	211.3	172.6	98.9
Clomipramine*	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Cyclobenzaprine*	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Fluoxetine*	32.8	50.9	62.3	<MQL	<MQL	28.3	89.8	33.4	61.0	23.6	<MQL	<MQL
Olanzapine*	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Paroxetine*	<MQL	n.d	n.d	n.d	n.d	n.d	<MQL	<MQL	n.d	n.d	n.d	n.d
Risperidone*	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Venlafaxine	145.2	181.8	220.5	99.8	110.2	188.9	223.4	133.5	320.1	282.6	210.8	301.3
Lipid Regulators												
Gemfibrozil	n.d	453.8	312.1	n.d	n.d	n.d	<MQL	<MQL	<MQL	223.5	214.6	<MQL

<MQL= Below Method Quantification Limit, n.d = not detected

Table 8.4. Concentrations (ng/L) of target ECs in the WWTP of Amaliada city for each sampling month

WWTP-Amaliada city -Concentrations (ng/L)												
	Mar-19	**Apr-19**	**May-19**	**Jun-19**	**Jul-19**	**Aug-19**	**Sept-19**	**Oct-19**	**Nov-19**	**Dec-19**	**Jan-20**	**Feb-20**
Artificial Sweeteners												
Acesulfame	1001.2	428.6	1569.8	783.4	3935.8	4561.2	2564.8	842.5	1002.3	538.3	1421.3	987.9
Aspartame	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Saccharin	n.d	n.d	n.d	n.d	n.d	<MQL	n.d	n.d	n.d	n.d	n.d	n.d
Sucralose	384.6	198.6	286.9	732.4	918.6	1221.5	896.4	556.9	313.8	185.1	190.8	283.6
Antibiotics												
Erythromycin-H2o	11.8	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	<MQL	<MQL	<MQL
Florfenicol	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Oxolici acid	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Sulfacetamide	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Sulfadiazine	n.d	n.d	n.d	n.d	n.d	<MQL	<MQL	n.d	n.d	n.d	n.d	n.d
Sulfamethazine	<MQL	<MQL	n.d	n.d	n.d	<MQL	<MQL	n.d	5.6	n.d	n.d	n.d
Sulfamethizole	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Sulfamethoxazole	10.2	7.3	5.5	<MQL	<MQL	<MQL	n.d	n.d	23.4	279.7	381.2	323.4
Sulfamethoxy-pyrid	n.d	n.d	n.d	n.d	n.d	<MQL	n.d	n.d	n.d	n.d	n.d	n.d
Sulfapyridine	18.8	n.d	23.6	n.d	<MQL	<MQL	n.d	n.d	n.d	n.d	n.d	n.d
Sulfaquinoxaline	5.6	<MQL	7.2	<MQL	<MQL	4.9	10.0	12.3	8.2	6.6	<MQL	<MQL
Sulfathiazole	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Trimethoprim	25.9	14.2	<MQL	<MQL	<MQL	<MQL	<MQL	5.5	42.9	49.5	77.2	33.4
NAIDS-Analgesics												
Diclofenac	90.4	50.1	63.0	55.0	49.2	83.1	69.3	132.6	158.9	174.6	121.8	71.5
Indomethacin	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Mefenamic Acid	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	<MQL	<MQL	n.d	n.d

Table 8.4. Concentrations (ng/L) of target ECs in the WWTP of Amaliada city for each sampling month, *(continued)*

Salicylic acid	153.4	482.6	1801.8	1545.9	2632.6	984.3	1704.3	1623.6	436.5	1582.6	392.3	521.6
Tolfenamic Acid	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Disinfectants												
Triclosan	23.1	15.4	12.0	53.6	153.6	100.8	76.4	30.2	22.8	12.8	61.0	35.9
Antidepressants-Psychiatrics												
Amitriptyline*	15.3	4.8	12.6	3.5	7.8	9.6	5.7	14.6	3.0	20.1	13.5	10.0
Carbamazepine*	23.5	18.6	5.9	15.1	8.4	3.3	2.5	<MQL	<MQL	<MQL	n.d	n.d
Clomipramine*	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Cyclobenzaprine*	n.d	n.d	n.d	n.d	n.d	n.d	n.d	13.3	n.d	n.d	n.d	n.d
Fluoxetine*	5.5	<MQL	n.d	n.d	<MQL	n.d	23.2	<MQL	<MQL	n.d	n.d	n.d
Olanzapine*	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	<MQL	n.d	n.d	n.d
Paroxetine*	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Risperidone*	<MQL	<MQL	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Venlafaxine	45.6	86.3	52.1	14.8	n.d	32.1	28.9	n.d	47.9	69.8	n.d	n.d
Lipid Regulators												
Gemfibrozil	<MQL	73.9	51.3	n.d	<MQL	n.d	n.d	n.d	65.8	<MQL	<MQL	n.d

<MQL= Below Method Quantification Limit, n.d = not detected

Table 8.5. Concentrations (ng/L) of target ECs in the WWTP of University Hospital of Ioannina for each sampling month

WWTP-University Hospital of Ioannina -Concentrations (ng/L)												
	Mar-19	**Apr-19**	**May-19**	**Jun-19**	**Jul-19**	**Aug-19**	**Sept-19**	**Oct-19**	**Nov-19**	**Dec-19**	**Jan-20**	**Feb-20**
Artificial Sweeteners												
Acesulfame	157.6	60.8	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	<MQL	<MQL
Aspartame	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Saccharin	<MQL	<MQL	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Sucralose	<MQL	n.d	<MQL	n.d	n.d	n.d	n.d	<MQL	n.d	n.d	<MQL	n.d
Antibiotics												
Erythromycin-H2o	14.9	25.3	4.2	n.d	n.d	4.2	n.d	4.2	123.1	118.5	85.3	95.1
Florfenicol	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Oxolici acid	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Sulfacetamide	189.5	<MQL	<MQL	<MQL	52.6	n.d	n.d	n.d	44.8	<MQL	<MQL	<MQL
Sulfadiazine	<MQL	52.8	<MQL	n.d	n.d	n.d	<MQL	59.3	<MQL	168.6	499.2	36.7
Sulfamethazine	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Sulfamethizole	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Sulfamethoxazole	487.5	254.3	154.3	123.4	220.8	569.8	95.4	192.8	704.9	223.8	1578.0	446.7
Sulfamethoxy-pyrid	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Sulfapyridine	109.3	n.d	n.d	n.d	123.4	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Sulfaquinoxaline	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Sulfathiazole	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Trimethoprim	12.9	66.8	59.1	45.9	14.8	32	177.3	170.1	365.6	273.5	480.4	165.9
NAIDS-Analgesics												
Diclofenac	156.9	n.d	n.d	n.d	n.d	n.d	319.4	154.3	<MQL	<MQL	<MQL	<MQL
Indomethacin	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Mefenamic Acid	<MQL	n.d	n.d	n.d	n.d	n.d	n.d	<MQL	n.d	32.9	<MQL	<MQL

Table 8.5. Concentrations (ng/L) of target ECs in the WWTP of University Hospital of Ioannina for each sampling month, *(continued)*												
Salicylic acid	451.8	217.6	115.9	123.8	158.9	294.8	334.8	358.6	512.6	624.1	394.6	223.4
Tolfenamic Acid	n.d	n.d	n.d	n.d	n.d	n.d	<MQL	<MQL	<MQL	n.d	n.d	n.d
Disinfectants												
Triclosan	110.9	512.8	843.6	119.6	102.3	254.9	423.1	781.4	355.1	418.2	328.4	159.6
Antidepressants-Psychiatrics												
Amitriptyline*	25.8	13.3	n.d	n.d	72.8	89.9	27.6	30.8	56.2	52.5	31.0	n.d
Carbamazepine*	478.0	500.9	379.8	228.0	504.3	852.6	195.1	251.8	220.0	124.8	141.5	166.3
Clomipramine*	<MQL	<MQL	n.d	n.d	<MQL	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Cyclobenzaprine*	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Fluoxetine*	23.1	162.1	99.5	n.d	n.d	228.2	16.2	40.8	70.1	50.9	21.2	<MQL
Olanzapine*	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Paroxetine*	40.4	<MQL	<MQL	n.d	n.d	n.d	35.1	n.d	n.d	n.d	<MQL	n.d
Risperidone*	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Venlafaxine	165.2	190.1	n.d	284.9	648.5	592.1	796.9	832.4	919.8	863.3	492.1	58.2
Lipid Regulators												
Gemfibrozil	n.d	n.d	218.1	<MQL	n.d	n.d	<MQL	259.8	<MQL	<MQL	<MQL	<MQL

<MQL= Below Method Quantification Limit, n.d = not detected

Overall, considering all sampling campaigns, 28 different ECs out of the 33 monitored compounds, were detected in at least one effluent wastewater of all WWTPs even at concentrations <MQL, but only 17 were present in all of them. Additional 19 compounds out of the 33 investigated, were detected in at least two of the three sampling campaigns, and nine (9) in only one campaign. Specifically, 23 compounds were detected in WWTP of Amaliada city, whereas 18 and 22 compounds were detected in Ioannina and University hospital of Ioannina, respectively. Five (5) compounds aspartame, florfenicol, oxolinic acid, sulfathiazole, indomethacin, were not detected at any sample. We estimate that these compounds were either used in lower amounts than the rest and/or were degraded during their transport through the sewage channels [551]. It is noteworthy that salicylic acid, triclosan, trimethoprim, venlafaxine, carbamazepine, amitriptyline were the most ubiquitous compounds since they were detected in >90% of the water samples. The most abundant compounds in terms of mean concentrations were acesulfame (4862.5 ng/L), sucralose (993.8 ng/L) and salicylic acid (981.8 ng/L) throughout the three monitoring campaigns. Sulfaquinoxaline (9.3 ng/L), paroxetine (13.2 ng/L) and cyclobenzaprine (13.3 ng/L) exhibited the lowest concentration levels during the monitoring period.

Concerning therapeutic groups, the frequency of detection throughout all sampling sites followed the order psychiatrics/antidepressants, antibiotics, NSAIDs-analgesics, artificial sweeteners, disinfectants, and lipid regulators (Fig. 8.5).

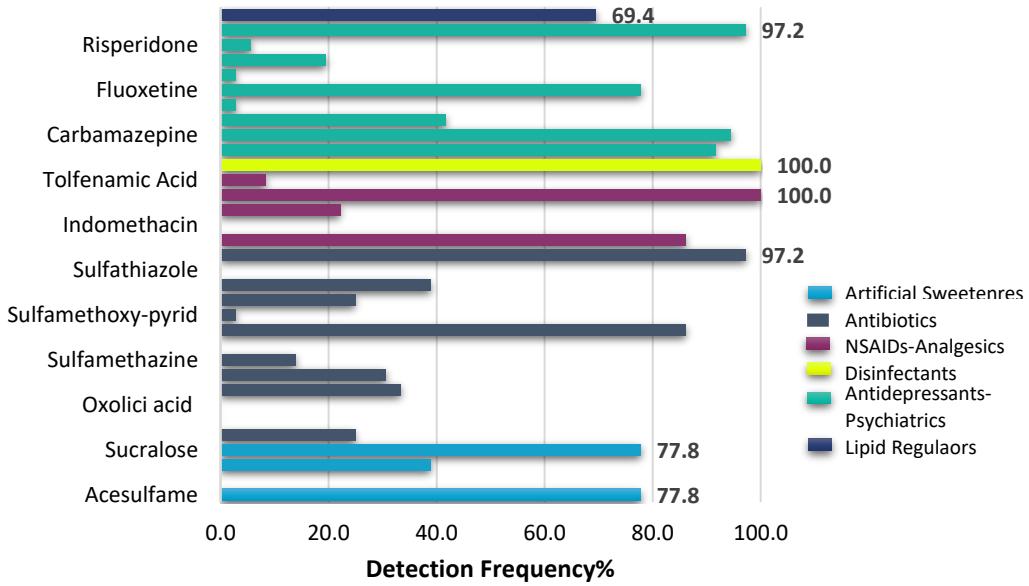


Figure 8.5. Detection Frequency (%) of ECs in total analyzed samples (n=36) of all WWTPs

Among psychiatrics the most frequent compound was venlafaxine, whereas among antibiotics and NSAIDs it was trimethoprim and salicylic acid, respectively. Among artificial sweeteners, acesulfame and sucralose provided the most positive findings equally. Finally, triclosan and gemfibrozil were the only representative compounds of their family groups (Fig. 8.5). Comparing the frequency of investigated compounds regarding their therapeutic group all over the WWTPs investigated, a similar trend is noticed for three therapeutic groups of NSAIDs, disinfectants and lipid regulators. A differentiation among the three WWTPs is denoted concerning the frequency of antibiotics and artificial sweeteners. In Amaliada and Ioannina city the percentage of positive findings of artificial sweeteners in the corresponding WWTPs, is significantly higher compared to WWTP of University Hospital (Fig. 8.7a, b). This was expected since the water that discharges in municipal effluents derives from numerous anthropogenic activities that include a variety of household products. Sweeteners have a widespread use in the human diet, to produce sugar-free low-calorie products, and exhibit countless applications in processed food items and beverages. Use of artificial sweeteners is even reported in drugs, sanitary products and also animal feed with many possible combinations [97,100,291,688,689]. For this purpose, the observed persistence of artificial sweeteners such as acesulfame and sucralose during municipal wastewater treatment resulted to their identification as useful tracers of wastewater input to environmental waters [96,109,112,690–693]. On the other hand, hospital WWTPs are differentiated from municipal plants since they are a source of restricted classes of contaminants. Due to their specific nature, it is expected that hospital effluents present a mixture of compounds, including antibiotics, NSAIDs, diagnostic agents, disinfectants, resulting from diagnostic, laboratory and research activities and principally from medicine excretion from patients [136]. On account to that, the maximum detection frequency of antibiotic class occurred in hospital WWTP simultaneously with the minimum detection frequency of artificial sweeteners of all sampling sites (Fig. 8.7c).

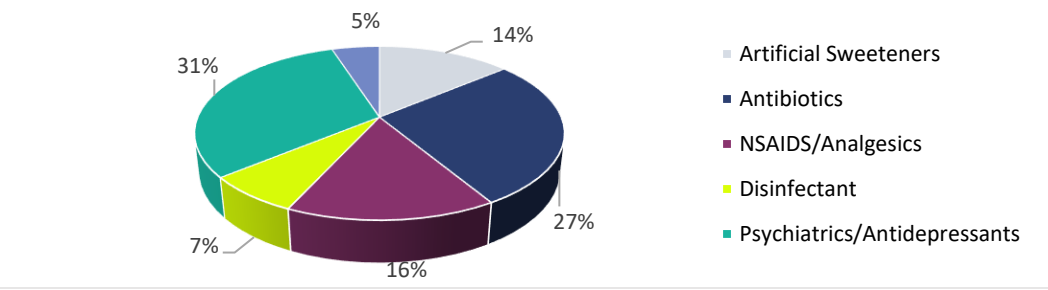


Figure 8.6. Frequency of detection of each therapeutic group throughout all sampling campaigns

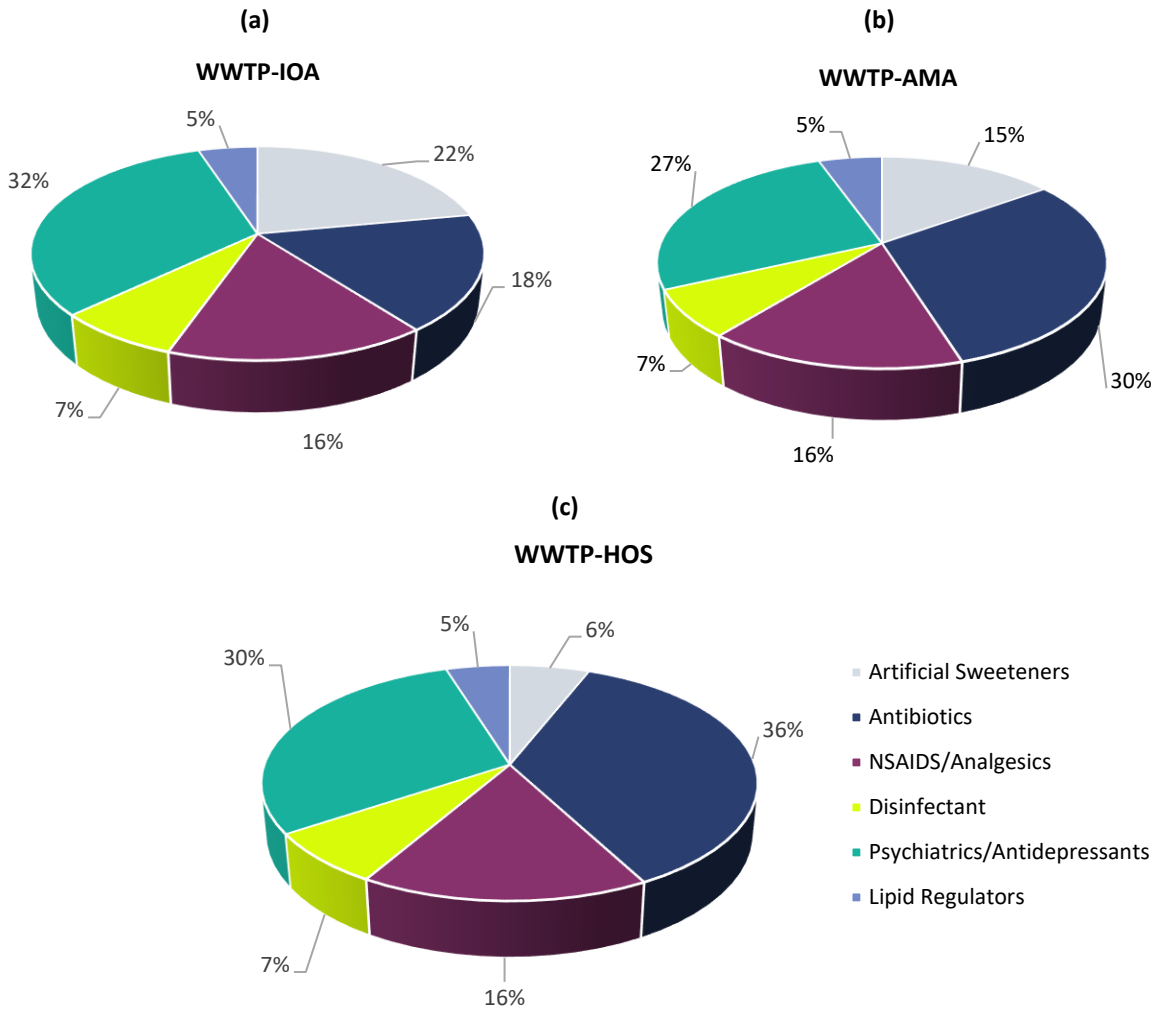


Figure 8.7. Frequency of detection of each therapeutic group per sampling site (a) WWTP-IOA, (b) WWTP-AMA, and (c) WWTP-HOS

Occurrence of target compounds in terms of concentration levels, follows a rather broad range of variability depending on the sampling station, the class of contaminants and the sampling month. Generally, the overall range of mean concentrations was from MQL levels to 4862.5 ng/L and the highest individual concentration was detected for acesulfame at WWTP-IOA (13569.0 ng/L), while the lowest for carbamazepine (2.5ng/L) at WWTP-AMA. The most abundant therapeutic group in terms of concentration level was artificial sweeteners with highest mean concentration for acesulfame at 4862.5 ng/L, followed by NSAIDS/analgesics with most predominant compound salicylic acid at 981.8 ng/L. The next higher concentration levels were observed for antidepressants/psychiatrics with maximum value of venlafaxine (278.7 ng/L), followed by antibiotics

with maximum mean concentration for sulfamethoxazole (235.7 ng/L). Finally, triclosan and gemfibrozil were the last compounds with decreasing concentrations levels representing the family groups of disinfectants and lipid regulators, respectively.

More specifically, municipal WWTPs of Ioannina and Amaliada city presented higher cumulative levels of concentrations of the total target compounds, compared to the hospital unit, since average flow rates per day differ as well. The compound detected with highest mean concentration in WWTP-IOA and WWTP-AMA was acesulfame at 9686.8 ng/L and 1636.4 ng/L, respectively. In WWTP-HOS the highest mean concentration was observed for venlafaxine (531.2 ng/L). High concentrations of acesulfame and sucralose provide a great contribution for the class of artificial sweeteners in the total concentration levels of the municipal plants (WWTP-IOA, WWTP-AMA). On the other hand, in hospital WWTP the therapeutic group that contributes more in the total level of concentrations is antibiotics group followed by antidepressants/Psychiatric drugs, NSAIDs since the hospital holds a Department of Psychiatry demonstrating that hospital effluents are an important source of input of these families of pharmaceuticals into WWTP (Fig. 8.8). The evaluation of individual contribution of each target compound to the load of ECs into the wastewater discharges during the sampling periods is depicted in Fig. 8.9.

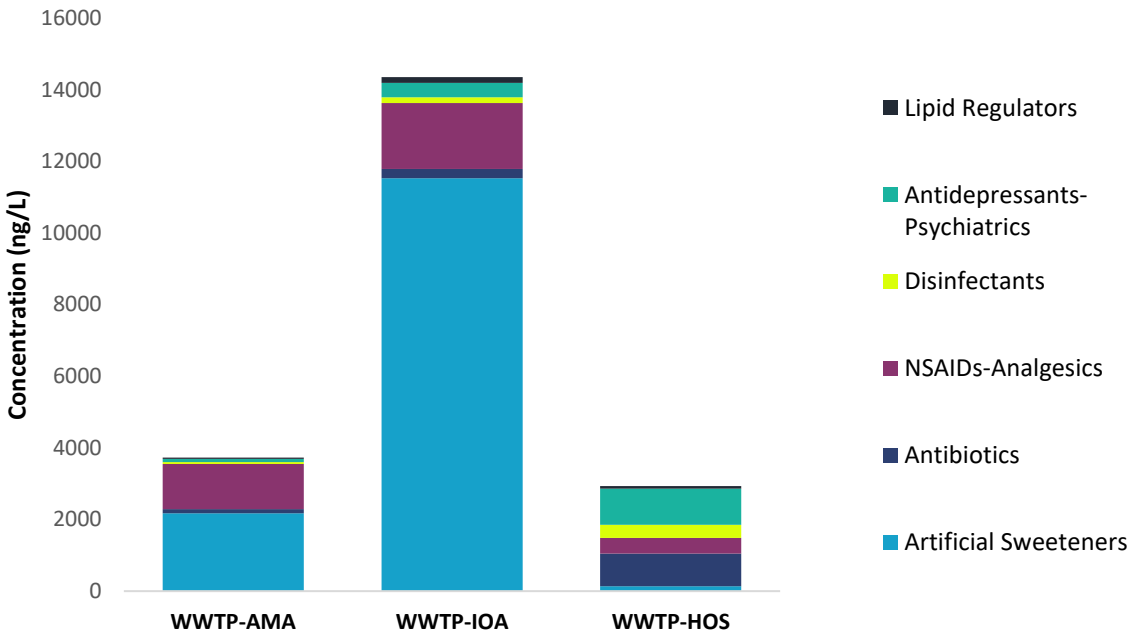


Figure 8.8. ECs cumulative concentrations measured in each WWTP, distinguishing between therapeutic groups

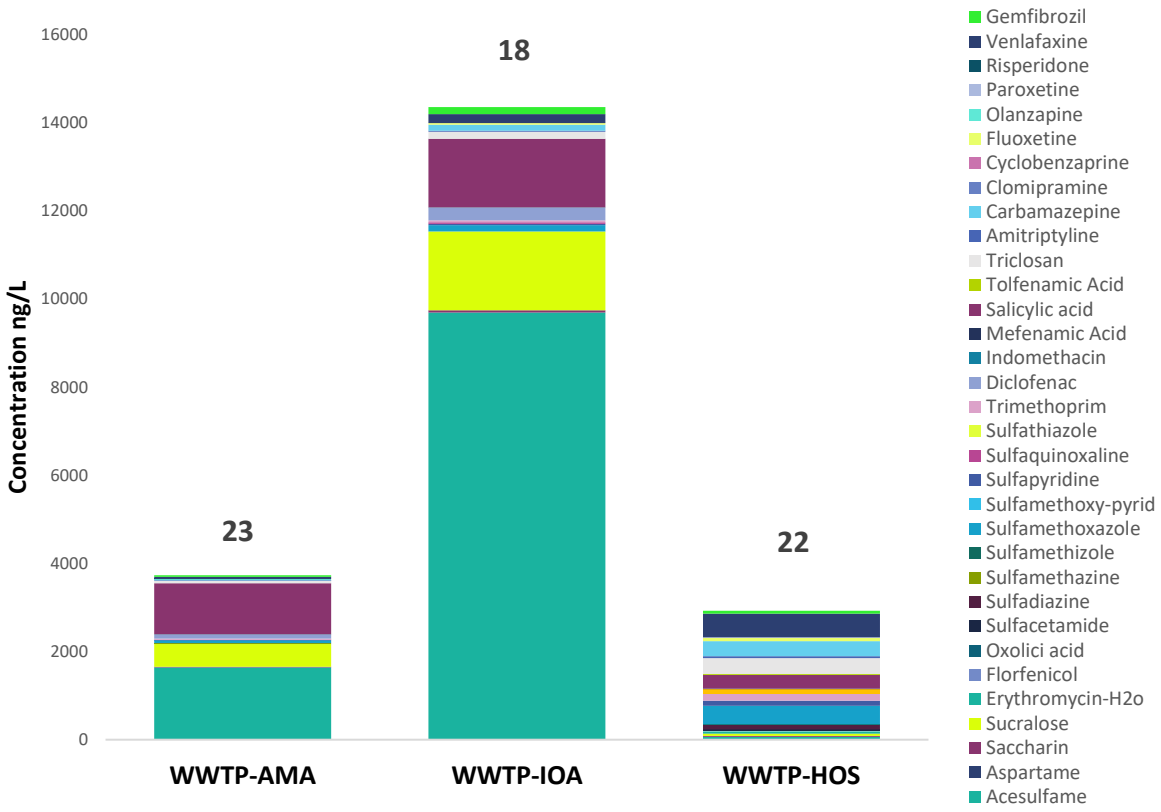


Figure 8.9. Cumulative levels of ECs displayed as a stacked bar plot at the different sampling stations. Numbers above bars depict the number of analytes found in each station

Variations in ECs concentrations between the sampling effluents of the three WWTPs were observed, primarily because of the nature of WWTPs since a hospital WWTP is included in our study, which differentiates to urban WWTPs. Moreover, comparing the two municipal WWTPs of Amaliada and Ioannina, possible different consumption patterns may occur between the two investigated regions, resulting in different inlet concentrations. Finally another reason that sustains the differences in concentration ranges among the three WWTPs, may be the different removal efficiencies achieved in the two systems, whose values depend on design and operational factors, reactor configuration, SRT, HRT, temperature, etc [694].

ECs concentration ranges measured at each sampling location are further illustrated with boxplots for each individual compound detected (Fig. 8.10, 8.11, 8.12). Moreover, mean concentrations of ECs for the three investigated areas, minimum and maximum values as well as detection frequency (D.F%), are reported in the following tables (Tables 8.6, 8.7 ,8.8), presented by alphabetical order for

the different therapeutic classes. A table summarizing all concentration ranges throughout the three sampling campaigns is also given. (Table 8.9).

Results obtained by 1-year monitoring study throughout all sampling campaigns are discussed in detail in the following subsections and are compared with concentrations reported in other studies. Differences in the results observed among studies can be explained by differences in pharmaceutical or artificial sweeteners consumption among countries, prescription, sampling strategies and season, type and size of WWTP, wastewater flow rate, hospital size and type of medical cares as well as cultural and geographic factors.

Table 8.6. Frequencies of detection, ranges of concentrations (min-max) and corresponding mean values of ECs detected in WWTP of Ioannina city

WWTP- Ioannina city					
Compounds	**D.F % n=12**	**Min Conc. (ng/L)**	**Max Conc. (ng/L)**	**Mean Conc[a] (ng/L)**	**Mean Conc[b] (ng/L)**
Artificial sweeteners					
Acesulfame	100	6054.8	13569.0	9686.8	9686.8
Aspartame	0	n.d	n.d	n.d	n.d
Saccharin	92	n.d	64.9	<MQL	<MQL
Sucralose	100	464.2	4075.2	1787.9	1787.9
Antibiotics					
Erythromycin-H2o	25	n.d	<MQL	<MQL	<MQL
Florfenicol	0	n.d	n.d	n.d	n.d
Oxolici acid	0	n.d	n.d	n.d	n.d
Sulfacetamide	0	n.d	n.d	n.d	n.d
Sulfadiazine	0	n.d	n.d	n.d	n.d
Sulfamethazine	0	n.d	n.d	n.d	n.d
Sulfamethizole	0	n.d	n.d	n.d	n.d
Sulfamethoxazole	75	n.d	381.2	142.1	98.1
Sulfamethoxy-pyrid	0	n.d	n.d	n.d	n.d
Sulfapyridine	25	n.d	72.4	29.0	n.d
Sulfaquinoxaline	17	n.d	52.8	32.6	n.d

Table 8.6. Frequencies of detection, ranges of concentrations (min-max) and corresponding mean values of ECs detected in WWTP of Ioannina city, *(continued)*

Sulfathiazole	0	n.d	n.d	n.d	n.d
Trimethoprim	92	n.d	96.8	47.5	43.6
NSAIDS-Analgesics					
Diclofenac	100	85.8	689.4	288.0	288.0
Indomethacin	0	n.d	n.d	n.d	n.d
Mefenamic Acid	8	n.d	<MQL	<MQL	<MQL
Salicylic acid	100	630.2	2913.5	1547.8	1547.8
Tolfenamic Acid	0	n.d	n.d	n.d	n.d
Disinfectants					
Triclosan	100	52.9	321.8	159.2	159.2
Antidepressants-Psychiatrics					
Amitriptyline	100	<MQL	72.1	27.5	27.5
Carbamazepine	100	77.6	301.9	136.9	136.9
Clomipramine	100	n.d	n.d	n.d	n.d
Cyclobenzaprine	0	n.d	n.d	n.d	n.d
Fluoxetine	100	<MQL	89.8	32.5	32.5
Olanzapine	0	n.d	n.d	n.d	n.d
Paroxetine	25	n.d	<MQL	<MQL	<MQL
Risperidone	0	n.d	n.d	n.d	n.d
Venlafaxine	100	99.8	320.1	201.5	201.5
Lipid Regulators					
Gemfibrozil	67	n.d	453.8	158.3	105.6

P.F: Positive Findings, a: Mean concentration of detected ECs, b: Mean concentration considering not detected as zero (n. d=0)

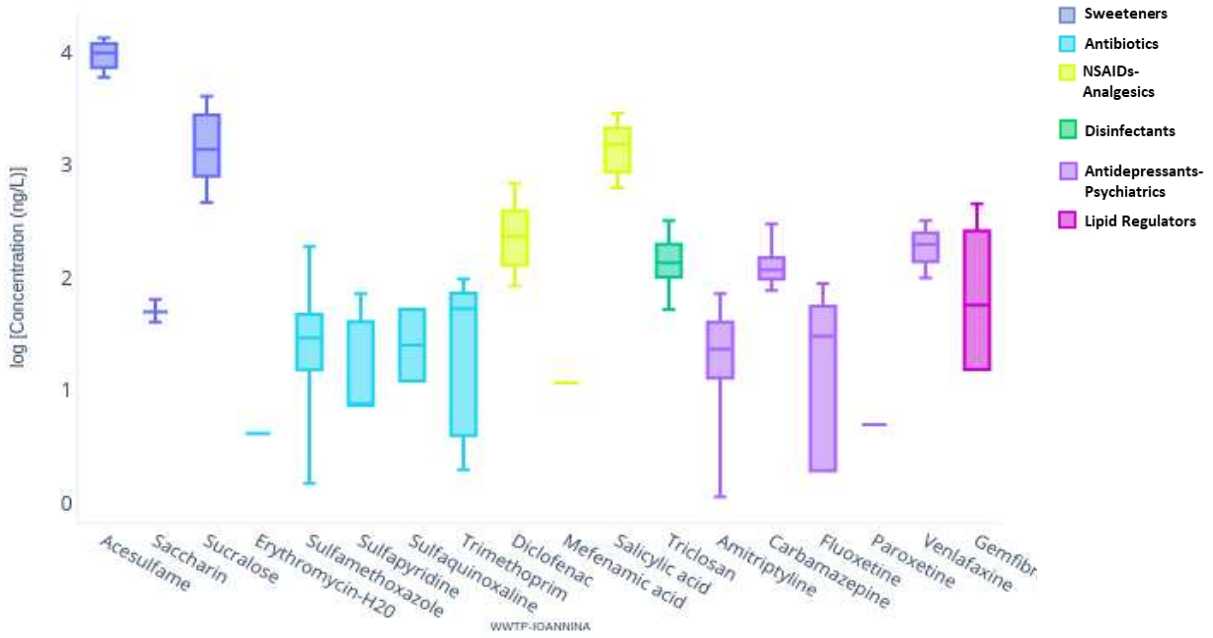


Figure 8.10. Box plots indicating the concentration range of individual ECs in the WWTP of Ioannina

Table 8.7. Frequencies of detection, ranges of concentrations (min-max) and corresponding mean values of ECs detected in WWTP of Amaliada city

WWTP- Amaliada city					
Compounds	**D.F % n=12**	**Min Conc. (ng/L)**	**Max Conc. (ng/L)**	**Mean Conc[a] (ng/L)**	**Mean Conc[b] (ng/L)**
Artificial Sweeteners					
Acesulfame	100	428.6	4561.2	1636.4	1636.4
Aspartame	0	n.d	n.d	n.d	n.d
Saccharin	8	n.d	<MQL	19.0	1.6
Sucralose	100	185.1	1221.5	514.1	514.1
Antibiotics					
Erythromycin-H2O	33	n.d	11.8	<MQL	<MQL
Florfenicol	0	n.d	n.d	n.d	n.d
Oxolici acid	0	n.d	n.d	n.d	n.d
Sulfacetamide	0	n.d	n.d	n.d	n.d
Sulfadiazine	17	n.d	<MQL	11.8	<MQL
Sulfamethazine	42	n.d	5.6	<MQL	<MQL

Table 8.7. Frequencies of detection, ranges of concentrations (min-max) and corresponding mean values of ECs detected in WWTP of Amaliada city, *(continued)*

Sulfamethizole	0	n.d	n.d	n.d	n.d
Sulfamethoxazole	83	<MQL	200.4	57.8	48.1
Sulfamethoxy-pyrid	8	n.d	<MQL	2.7	0.2
Sulfapyridine	33	<MQL	23.6	14.3	4.8
Sulfaquinoxaline	100	<MQL	12.3	5.4	5.4
Sulfathiazole	0	n.d	n.d	n.d	0.0
Trimethoprim	100	<MQL	77.2	21.6	21.6
NSAIDs-Analgesics					
Diclofenac	100	49.2	174.6	93.3	93.3
Indomethacin	0	n.d	n.d	n.d	n.d
Mefenamic Acid	17	n.d	n.d	11.7	1.9
Salicylic acid	100	153.4	2632.6	1155.1	1155.1
Tolfenamic Acid	0	n.d	n.d	n.d	n.d
Disinfectants					
Triclosan	100	12	153.6	49.8	49.8
Antidepressants- Psychiatrics					
Amitriptyline	100	3.0	20.1	10.0	10.0
Carbamazepine	83	n.d	23.5	7.9	6.6
Clomipramine	0	n.d	n.d	n.d	n.d
Cyclobenzaprine	8	n.d	13.3	13.3	1.1
Fluoxetine	50	n.d	23.2	6.1	3.0
Olanzapine	8	n.d	<MQL	2.8	0.2
Paroxetine	0	n.d	n.d	n.d	n.d
Risperidone	17	n.d	<MQL	2.1	0.4
Venlafaxine	100	14.8	86.3	47.2	31.5
Lipid Regulators					
Gemfibrozil	75	n.d	73.9	36.2	21.1

P.F: Positive Findings, a: Mean concentration of detected ECs, b: Mean concentration considering not detected as zero (n.d=0)

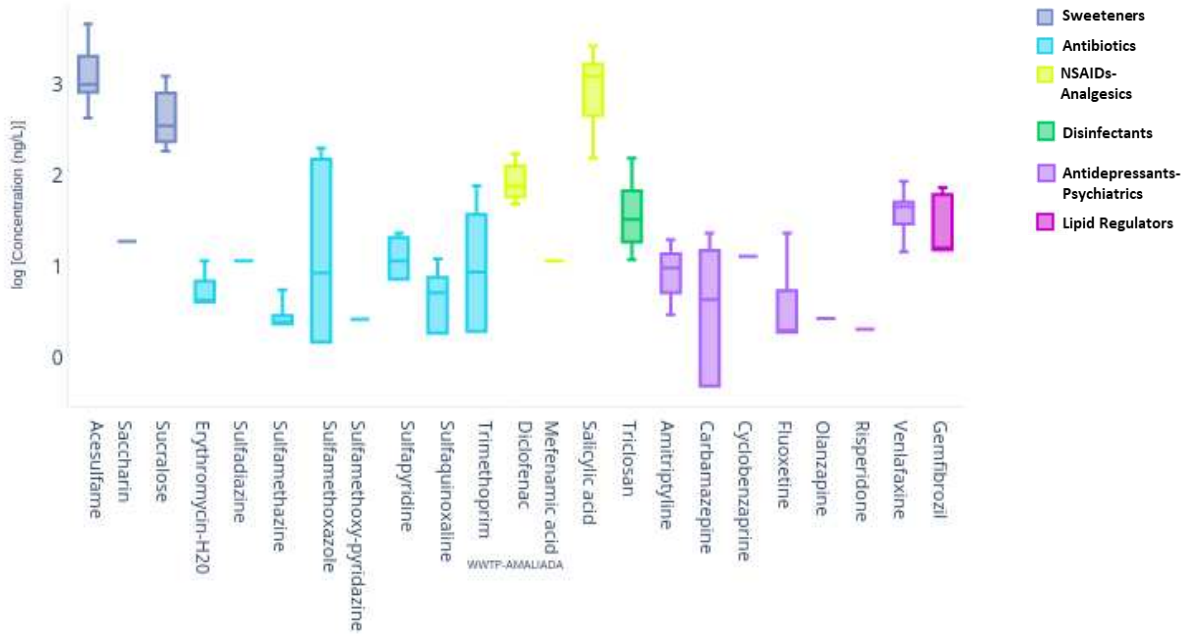


Figure 8.11. Box plots indicating the concentration range of individual ECs in the WWTP of Amaliada

Table 8.8. Frequencies of detection, ranges of concentrations (min-max) and corresponding mean values of ECs detected in WWTP of University Hospital of Ioannina

WWTP- University Hospital of Ioannina					
Compounds	**D.F % n=36**	**Min Conc. (ng/L)**	**Max Conc. (ng/L)**	**Mean Conc[a] (ng/L)**	**Mean Conc[b] (ng/L)**
Artificial Sweeteners					
Acesulfame	33	n.d	157.6	67.6	22.5
Aspartame	0	n.d	n.d	n.d	n.d
Saccharin	17	n.d	<MQL	19.0	3.2
Sucralose	33	n.d	<MQL	50.5	16.8
Antibiotics					
Erythromycin-H2O	75	n.d	123.1	52.8	39.6
Florfenicol	0	n.d	n.d	n.d	n.d
Oxolici acid	0	n.d	n.d	n.d	n.d
Sulfacetamide	75	n.d	189.5	43.7	32.8
Sulfadiazine	75	n.d	499.2	97.1	72.8
Sulfamethazine	0	n.d	n.d	n.d	n.d

Table 8.8. Frequencies of detection, ranges of concentrations (min-max) and corresponding mean values of ECs detected in WWTP of University Hospital of Ioannina, *(continued)*

Sulfamethizole	0	n.d	52.6	20.2	11.8
Sulfamethoxazole	100	95.4	1578.0	421.0	421.0
Sulfamethoxy-pyrid	0	n.d	n.d	n.d	n.d
Sulfapyridine	17	n.d	123.4	116.4	19.4
Sulfaquinoxaline	0	n.d	n.d	n.d	n.d
Sulfathiazole	0	n.d	n.d	n.d	n.d
Trimethoprim	100	n.d	480.4	155.4	155.4
NSAIDS-Analgesics					
Diclofenac	58	n.d	319.4	102.7	59.9
Indomethacin	0	n.d	n.d	n.d	n.d
Mefenamic Acid	42	n.d	32.9	15.9	6.6
Salicylic acid	100	115.9	624.1	317.6	317.6
Tolfenamic Acid	25	n.d	<MQL	5.7	1.4
Disinfectants					
Triclosan	100	102.3	843.6	367.5	367.5
Antidepressants-Psychiatrics					
Amitriptyline	75	n.d	89.9	44.4	33.3
Carbamazepine	100	124.8	852.6	336.9	336.9
Clomipramine	25	n.d	<MQL	2.0	0.5
Cyclobenzaprine	0	n.d	n.d	n.d	n.d
Fluoxetine	75	n.d	228.2	71.4	59.5
Olanzapine	0	n.d	n.d	n.d	n.d
Paroxetine	42	n.d	40.4	18.1	7.6
Risperidone	0	n.d	n.d	n.d	n.d
Venlafaxine	92	n.d	919.8	531.2	487.0
Lipid Regulators					
Gemfibrozil	67	n.d	259.8	71.5	47.7

P.F: Positive Findings, a: Mean concentration of detected ECs, b: Mean concentration considering not detected as zero (n.d=0)

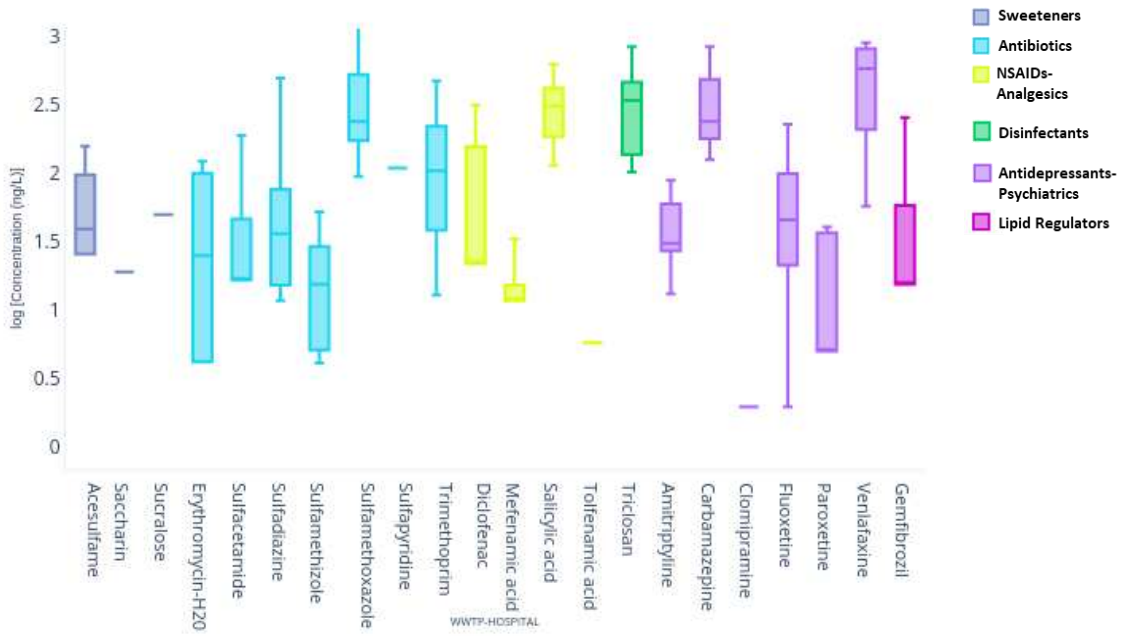


Figure 8.12. Box plots indicating the concentration range of individual ECs in the WWTP of University Hospital of Ioannina

Table 8.9. Frequencies of detection, ranges of concentrations (min-max) and corresponding mean values of ECs detected in all WWTPs investigated in this study

3 WWTPs					
Compounds	**P.F % n=36**	**Min Conc. (ng/L)**	**Max Conc. (ng/L)**	**Mean Conc[a] (ng/L)**	**Mean Conc[b] (ng/L)**
Artificial Sweeteners					
Acesulfame	78	n.d	13569	4862.5	3781.9
Aspartame	0	n.d	n.d	n.d	0.0
Saccharin	39	n.d	64.9	44.3	17.2
Sucralose	78	n.d	4075.25	993.8	772.9
Antibiotics					
Erythromycin-H2o	42	n.d	123.1	32.0	14.2
Florfenicol	0	n.d	n.d	n.d	0.0
Oxolici acid	0	n.d	n.d	n.d	0.0
Sulfacetamide	6	n.d	189.5	43.7	10.9
Sulfadiazine	17	n.d	499.2	81.5	24.9
Sulfamethazine	14	n.d	5.6	3.0	0.4

Table 8.9. Frequencies of detection, ranges of concentrations (min-max) and corresponding mean values of ECs detected in all WWTPs investigated in this study, *(continued)*

Sulfamethizole	0	n.d	n.d	20.2	3.9
Sulfamethoxazole	86	n.d	1578	235.7	203
Sulfamethoxy-pyrid	3	n.d	<MQL	2.7	0.1
Sulfapyridine	25	n.d	123.4	41.9	10.5
Sulfaquinoxaline	39	n.d	52.80	9.3	3.6
Sulfathiazole	0	n.d	n.d	n.d	0.0
Trimethoprim	94	n.d	480.4	72.7	70.7
NSAIDs-Analgesics					
Diclofenac	67	n.d	689.4	173.7	144.8
Indomethacin	0	n.d	n.d	n.d	0.0
Mefenamic Acid	22	n.d	32.9	122.1	30.5
Salicylic acid	100	115.9	2913.5	981.8	981.8
Tolfenamic Acid	8	n.d	<MQL	5.7	0.5
Disinfectants					
Triclosan	100	12	843.6	192.2	192.2
Antidepressants-Psychiatrics					
Amitriptyline	92	n.d	89.9	25.8	23.6
Carbamazepine	94	n.d	852.6	169.6	160.1
Clomipramine	42	n.d	<MQL	2.0	0.2
Cyclobenzaprine	3	n.d	13.3	13.3	0.4
Fluoxetine	75	n.d	228.2	40.7	31.7
Olanzapine	3	n.d	n.d	2.8	0.1
Paroxetine	22	n.d	40.4	13.2	2.9
Risperidone	6	n.d	<MQL	2.1	0.1
Venlafaxine	97	n.d	919.8	278.7	240.0
Lipid Regulators					
Gemfibrozil	69	n.d	453.8	91.0	58.1

n.d= non detected

8.3.1 Artificial Sweeteners

Artificial sweeteners were the most ubiquitous contaminants in terms not only of frequency of detection but also of concentration levels. First Acesulfame (ACE) followed by sucralose (SUC) were the compounds that contributed the most to the total concentrations of ECs measured. WWTP of Ioannina presented the highest concentrations of artificial sweeteners followed by WWTP of Amaliada whereas for reasons that are already mentioned, hospital WWTP had limited contribution to artificial sweetener contamination. Specifically, in WWTP of Ioannina the highest individual concentration of all monitoring study was observed for acesulfame (13.6 μg/L). The determined levels of acesulfame (4862.5 ng/L) and sucralose (993.8 ng/L) in wastewater samples, investigated in this study ,have similar orders of magnitude to those reported elsewhere in Greece [664] and other European countries [97,327,674,688]. The intense occurrence of these two artificial sweeteners, acesulfame and sucralose, is not limited in wastewater effluents since their widespread distribution in different aquatic environmental matrices has also been reported [93,97,695]. Artificial sweeteners are used worldwide as sugar substitutes in remarkable amounts in food, beverages, as well as in drugs and sanitary products, such as mouthwashes. They provide no, or negligible energy and thus, consist ingredients of dietary products [1]. The high environmental concentrations of acesulfame and sucralose combined with their persistence, high water solubility, made them ideal anthropogenic wastewater markers [93,111,112,690]. Using ACE as a marker, one can detect even wastewater-derived proportions of a water resource of less than 1 ‰ in this way [112]. The suitability of acesulfame and sucralose as environmental traces, apparently arises from its unequivocal production for consumption by human activity. These artificial sweeteners are excreted mostly unchanged from the human body, flow down the drain, and are discharged into the environment through WWTPs, if incomplete degradation and limited retention times are observed during wastewater treatment. It has been reported as the most resistant artificial sweetener to removal efficiency in WWTPs [111]. For instance, removal efficiencies for acesulfame and sucralose in WWTPs were reported to be less than 20% [111,112,664,691]. It is well recognized that WWTP removal efficiencies for any chemical are driven by two independent sets of variables. The first relates to the inherent physical–chemical properties of the compound and the second is associated with the specific operational parameters of the WWTP [696]. The chemical inherent properties determine to what extent the main processes, including biodegradation, hydrolysis, sorption to sludge solids, volatilization, and photodegradation, will occur and influence the rate of removal. The WWTP operating parameters that are critical for

understanding removal efficiencies, include sludge and hydraulic retention times, activated sludge concentrations and metabolic capabilities, pH, and operating temperature [133].

Acesulfame has the lowest logP (-0.552) among all studied compounds after aspartame, with a high-water solubility of 237 g/L at 20 °C. These two characteristics along with the very low estimated vapor pressure ($9.03X10^{-6}$ mm Hg at 25 °C) indicate that acesulfame will not volatize appreciably during wastewater operations, will remain in the aqueous phase and will not bioconcentrate or bioaccumulate in aquatic organisms. Furthermore, the measured Kd values of ACE ranged from 10.1 to 43.7 L/kg [697], indicate a low sorption of this compound to solids (i.e., sludge), which would suggest a low potential for exposure in soil and sediment compartments.

Generally, the Kd values <500 indicate a strong potential to migrate with water rather than attach to soil or sludge particles [698]. In addition, the pKa of ACE is 2.0 which suggests that it will exist almost completely in the anion form in the environment. Anions commonly do not absorb tightly to soils with high organic carbon and clay when compared to their corresponding neutral form of compounds. Confirming these conclusions, ACE has been detected in sewage sludge samples at low mean concentrations ranging from 32 to 163 ng/g, with low removal rate of 16% to 21% from nitrifying activated sludge. A suggestion for this removal of ACE was attributed to biodegradation and the presence of autotrophic and heterotrophic microorganisms in the nitrifying activated sludge as well as potential induction of nonspecific oxidative enzymes rather than any abiotic factors [699].

Furthermore, direct photolysis by sunlight is not very possible to occur since ACE does not absorb well at wavelengths >290 nm (the cutoff of solar irradiation) at neutral pH [698]. Although there may be some indirect photolysis of ACE in surface waters exposed to sunlight, the extent to which it might be photodegraded in a typical WWTP would be limited to only the exposed top layers of wastewater treatment effluents and the typical high turbidity of those wastewaters can be assumed to block most sunlight [106]. Given the above discussion of the insignificance of abiotic factors influencing the removal of ACE-K in a typical activated sludge treatment plant, we can conclude that removal of ACE from WWTPs is difficult, but when it is observed, the rate of removal is likely associated with one form of biodegradation or another.

Sucralose (SUC), the second most abundant artificial sweetener throughout the one-year monitoring survey presented highest mean concentration value of 993.8 ng/L, with the highest mean value observed at WWTP-IOA (1787.9 ng/L) and then at WWTP-AMA (514.1 ng/L). The maximum individual concentration was 4075.3 ng/L in WWTP-IOA. Sucralose provides almost five-fold lower

concentration than acesulfame, a concentration ratio that comes in agreement with European studies [674], [96] unlike with studies conducted in United States that showed the prevalence of sucralose instead of acesulfame. This may be may be anticipated due to much longer period of availability of SUC (U.S. introduction in 1988 versus 2004 in Europe) and different regional dietary habits [96].

Taking under consideration the similar physicochemical properties of ACE, SUC presents eliminated removal efficiency from wastewater treatment plants, only < 20%, in many countries studied. Sucralose was barely removed from WWTPs in Switzerland (−5%) [112], China (17.7%) [691], Germany (20%) [96] and Sweden(<10%) [700]. In addition, in Greece the study of Kokotou et al. [664] about the occurrence of artificial sweeteners in WWTP of Athens reported negligible removal of SUC from WWTP.

Moreover, the resistance of SUC to degradation through natural and water treatment processes is another parameter that enables its persistence in the wastewater samples. Sucralose degradation through wastewater treatment facilities has also been demonstrated to be minimal for measurements through full-scale facilities and laboratory-scale aerobic biodegradation reactors [96,111,298,700]. Finally, SUC is not liable for microbial degradation in the last part of disinfection processes due to the presence of chlorine atoms [701,702]. Given its lack of any electron rich sites for oxidation and its already trichlorinated structure, the resistance of SUC to chlorination is expected [690].

Saccharin (SAC) is another popular artificial sweetener due to its low price, and is widely consumed by humans as a calorie-free artificial sweetener in more than 90 countries [703]. However, concentration levels of this sweetener in wastewater samples varied significantly compared to other sweeteners: approximately 100 times lower, with a total frequency of detection at 38%. The mean concentration of saccharin from all wastewater samples was 44.3ng/L, with higher occurrence observed at WWTP of Ioannina (mean concentration of 51.1 ng/L). The determined levels of sucralose in the other two WWTPs were in both cases below quantification limit (<MQL). The concentration levels measured in this study were comparable to other studies for effluent wastewaters (mean concentration of 64ng/L) [664]. These findings are in contrast to other European reports, eg. Buerge et al. [112] reported concentrations for saccharin ranged from <MDL to 3.200 ng/L [112], while Scheurer et al. [111] and Ordoñez et al. [327] reported ranges of saccharin between MDL-400ng/L [96] and 7100-9100 ng/L, respectively for effluent wastewaters. In any case, in all the above studies

the levels of saccharin compared to other sweeteners are significantly lower despite the wide applications of saccharin not only in food industry but also to nickel-plating, personal care and pharmaceutical products, antiseptics, preservatives, antistatic agents, feeds, etc. [112,701,704–706]. An explanation for this observation could be the efficient removal of saccharin from WWTPs. Studies about occurrence of saccharin in influent and effluent water conclude to high rates of elimination from waste water treatment processes (>90%) [96,97,664] WWTPs therefore play a vital role in preventing and controlling the ecological environmental risk induced by saccharin.

Finally, aspartame (ASP) was the only artificial sweetener with no detection at any water sample of the total sampling campaigns. These results are in accordance to other studies of aspartame in Greece [664] and Spain [688], in contrast with non-European studies that highlight the presence of aspartame not only in effluent water but also in groundwater and drinking water [691,692,707] Unlike with the other sweeteners that have been excreted unchanged by the kidney, aspartame is almost completely metabolized in the human body [291], [708] and possibly excreted as a conjugate of glucuronide or sulfate. In this way, heterocyclic saccharin can undergo ring opening and be transformed into other products that reach the sewage treatment plants. Excretion after human consumption is undoubtedly a major source of artificial sweeteners in the environment, but it is surely not the only one. For instance, in the European Union, SAC is authorized for use as an additive in animal feed for piglets, pigs, bovines and calves, and it is also the major degradation product of certain sulfonylurea herbicide [100,101,709]. In the case that the parent molecule of aspartame is finally discharged into wastewater facilities, ASP can lose its acetyl or amine group under different pH conditions during wastewater treatment [701]. ASP, in its dry form, is relatively stable, but below pH 3 it is unstable and is hydrolyzed to aspartylphenylalanine. Above pH 6, it is transformed to 5-benzyl-3,6-dioxo-2-piperazine acetic acid [93,710].

8.3.2 NSAIDs-Analgesics

The average concentration of total NSAIDs throughout the three sampling campaigns, followed the order WWTP of Ioannina (1847.5 ng/L), WWTP of Amaliada (1260 ng/L) and University hospital of Ioannina (441.8ng/L). In the first two sampling campaigns, NSAIDs were the second most abundant compounds after artificial sweeteners, whereas in hospital, the therapeutic groups which were more dominant were differed. Among NSAIDs, salicylic acid (SA) was the most frequent and abundant compound in all sampling stations with concentration ranges varying from 630-2913.5 ng/L in WWTP-

IOA, 153-263.2.6 ng/L in WWTP-AMA and 115.9-624.1 ng/L in WWTP-HOS. The highest mean concentration of salicylic acid was exhibited in Ioannina, (1547.8 ng/L) along with the maximum individual concentration of 2913.5 ng/L. The mean concentration in WWTP-AMA and WWTP-HOS was 1260 ng/L and 441.8 ng/L, respectively. The concentrations of SA measured in this study are significantly higher compared to the effluents of WWTPs investigated in other countries where SA varied from 110-130ng/L in Italy [32], 100-109ng/L in Portugal [133], MQL-497 ng/L in UK [711] and MQL-437ng/L in Greece [712]. For instance, remarkable differences were reported compared to previous study in 2016 for salicylic acid in Greece including the WWTP system of Ioannina city. Some possible explanations are that the occurrence of SA along the years is continually increasing and previous studies developed, had higher method quantification limits (210 ng/L). High concentrations of salicylic acid can be explained by the large amounts of this pharmaceutical dispensed in Greece from WWTP despite the satisfactory removals during treatment process (~70%) [260,712]. SA is the major metabolite of acetylsalicylic acid (aspirin®), one of the most popular and a first-line analgesic in Greece, which is freely available over-the counter. Aspirin, the parent compound of salicylic acid, is susceptible to direct photolysis by sunlight, and both are hydrolyzed, with half-lives ranging from 1.2 h to 12.5 days [713,714].

Concentration levels of SA found in municipal WWTPs of Ioannina and Amaliada (1847.5 and 1260.0 ng/L respectively) were significantly different with the corresponding of the University hospital (441.8ng/L), announcing the limited use of SA as a component of aspirin or other anti-inflammatory medicines. However, the multiplex urban activities include additional possible sources of salicylic acid, increasing its occurrence in the corresponding urban sewage systems. Salicylic acid is widely used as component of food preservatives and flavoring agents as well as antiseptics. Furthermore, a common application in cosmetic industry is its use in skin care products as keratolytic protector for treating acne, dermatitis, etc. It is also used in anti-dandruff shampoos and relative cosmetic products. Salicylic acid is typically found at concentrations of 2-3 percent in household products and occurs naturally in the environment [715,716].

Another compound that has been of interest and has been detected in most samples (86% D.F) was diclofenac. Diclofenac was detected in all samples of WWTP-IOA and WWTP-AMA whereas its presence in WWTP-HOS was less frequent (58%). Higher concentrations of diclofenac were detected in WWTP-IOA with mean concentration of 288.0 ng/L, while the corresponding mean concentrations for WWTP-AMA and WWTP-HOS were 93.3 ng/L and 102.7 ng/L, respectively. Taking into

consideration its low detection frequency in hospital WWTP, the concentration was significantly high. Similar results have been also reported to other studies carried out for hospital effluents [56,662].

Diclofenac is used either as a tablet or as a body cream; thus, it can be released in the WWTPs via human excretion or via body cleaning or washing of clothes. Removal rates of diclofenac in WWTPs vary and several studies by various authors have shown contradictory results (0–90%), [260,712], [717,718]. Diclofenac is one of the ECs that is included in the first watch list, to gather monitoring data for the purpose of facilitating the determination of appropriate measures to address the risk posed. The persistence of diclofenac and the possible inherent effects in the environment suggested its possible inclusion along with 17-α- ethynyl estradiol and β-oestradiol, in the Directive 2013/ 39/EU as priority substances [719]. However, is not yet regulated and may enter the dynamic list of priority substances in future updates.

As for the other NSAIDs regards, Mefenamic acid (MA) and tolfenamic acid (TA), their detected concentrations were only at levels <MQL, while their frequency of detection was 22% and 8.3%, respectively. Mefenamic acid was detected occasionally in all sampling sites, whereas tolfenamic acid was detected only in hospital WWTP. Other studies confirm the low occurrence of these NSAIDs in [260,712] excreted unchanged in only 8.8%, therefore, low concentrations might enter the influents. The fact that MA and TA did not exceed the quantification levels in any sample, may be attributed mainly to the decreased prescription of these NSAIDs compared to other ones, which not only are prescribed but also are consumed widely over the counter. Finally, indomethacin was not detected in any sample analyzed throughout all sampling campaigns. Absence of indomethacin also occurs in other studies [662,720,721].

8.3.3 Antibiotics

Overuse and misuse of antibiotics can promote the development of antibiotic-resistant bacteria. Antibiotics have attracted increasing concern due to their high human and veterinary use. Greece remains in the first place in the consumption of antibiotics all these years according to antimicrobial consumption database (ESAC-Net) [260].

The highest concentration of antibiotics was observed in WWTP-HOS (906.4 ng/L), while the cumulative concentration of antibiotics in WWTP-IOA and WWTP-AMA was 161.0 ng/L and 61.6 ng/L, respectively. As expected, the average concentration of total antibiotics in hospital wastewater was significantly higher than those reported in effluent from urban WWTP. This is in agreement with other

studies focused on comparisons between hospital wastewater and urban effluents [56,139,140]. Among antibiotics the most ubiquitous in terms of detection frequency was sulfamethoxazole (SMX), (86.8%). The presence of sulfamethoxazole occurred in all sampling sites, with the highest mean concentration among all antibiotics at 235.7ng/L, while in Amaliada and hospital were 18.4ng/L and 421.0 ng/L, respectively. The intense occurrence of sulfamethoxazole in monitoring studies of WWTPs is highlighted by other studies too [663,712,722–725]. SMX is one of the most broad-spectrum antibiotics consumed worldwide. Because of its low-price, stable properties, it has been extensively applied for human health and veterinary around the global world. Its concentration showed discrepancies, reaching a maximum concentration of 1578.0 ng/L in WWTP-HOS where it was detected in 100% of the samples. SMX belongs in sulfonamide group of antibiotics (SAs), a particular therapeutic group in terms of physicochemical properties. SAs are very hydrophilic (logKow<1) and have a low potential to volatize, denoting its persistence in aqueous phase during treatment processes [726,727]. They are not easy to degrade, thus lower removal efficiencies for these analytes have been associated (below 50%) [728,729].

Moreover, another finding which can explain its high occurrence in effluent waters is the transformation product of SMX during excretion in urine. 40% of a sulfamethoxazole (SMZ) is metabolized into N4-acetyl-sulfamethoxazole (ASMZ) which according to evidence is transformed back to the parent compound during wastewater treatment [730].

Along with SMX, the other sulfonamides investigated in this study were also prevalent in overall monitoring survey. Mean concentrations of sulfadiazine, sulfacetamide, sulfapyridine, sulfamethizole and sulfaquinoxaline were 81.5 ng/L, 43.7 ng/L, 41.9 ng/L, 20.2 ng/L, 9.3 ng/L, respectively. On the other hand, sulfamethazine and sulfamethoxy-pyridazine were detected in concentrations <MQL, whereas sulfathiazole was not detected in any effluent sample. These results coincide with other studies which refer low average concentrations of the specific antibiotics [663,731].

Significant differences in sulfonamides concentrations between sampling campaigns were observed, which is not surprising since different usage of antibiotics is expected in the WWTPs along the days [732]. In fact, significant differences in antibiotic concentrations were observed within the same day [733].

Specifically, sulfapyridine, was detected in all sampling sites with concentration levels ranged from n.d-72.4 ng/L in WWTP-IOA, <MQL-23.6 ng/L in WWTP-AMA and n.d-123.4 ng/L in WWTP-HOS. Sulfadiazine was found in quantified concentrations in hospital WWTP , with a range of n.d-499.2

ng/L, whereas in Amaliada city was detected below MQLs. Sulfadiazine was not detected in WWTP-IOA. Sulfaquinoxaline was detected only in the two sampling sites of WWTP-IOA and WWTP-AMA varied from n.d to 52.8 ng/L and <MQL to 12.3ng/L, respectively. Sulfamethazine and sulfamethoxy-pyridazine was presented only in WWTP of Amaliada in non-quantified levels of concentrations (<MQL). Sulfacetamide and sulfamethizole were found only in WWTP-HOS with ranges from n.d to 189.5 ng/L and n.d to 52.6 ng/L. Only three SAs where detected in WWTP-IOA (sulfamethoxazole, sulfaquinoxaline and sulfapyridine), five in hospital and six in Amaliada. Actually in WWTP of Amaliada the exclusive presence of sulfamethazine and sulfamethoxy-pyridazine, compounds with main use as animal drugs, indicates a possible contribution of nonurban inputs such as runoff from animal feeding operations or agricultural fields nearby in which biosolids have been applied. Amaliada is a city, belonging to the region of Ileias, which is characterized by intense agricultural activities. As a matter of fact, according to Hellenic Statistical Authority (ELSTAT), irrigated areas of crops in Amaliada city are amounted to 443,367 stremmas in 2018 compared to Ioannina sector of total irrigated area of 58,551 stremmas [734]. On the other hand, drug animals and antibiotics with exclusive veterinary use such as sulfamethazine, sulfamethoxy-pyridazine, sulfaquinoxaline were not present in WWTP of hospital, a reasonable result, since we expected pharmaceuticals of human consumption generated by human medicinal care, analysis and research activities.

Another compound strongly associated with sulfonamides and especially sulfamethoxazole, is trimethoprim antibiotic. Trimethoprim (TMP), has been mainly consumed along with sulfamethoxazole (SMX) in one medicine, namely co-trimoxazole, since 1968 [735] in a 5:1 ratio (SMX:TMP), allowing synergistic effects between the two compounds [736]. In the present study TMP was the second in order most ubiquitous and abundant antibiotic in all effluent samples, with detection frequency of 97.2%. Several studies report the detection of TMP in lower concentrations compared to SMX [604,663,712,720,722–725,737–740]. The mean concentration of TMP was 72.7 ng/L, with maximum occurrence in WWTP-HOS ranged from n.d to 480 ng/L, while it was the first in rank antibiotic detected in WWTP-AMA, with mean concentration value of 21.6 ng/L. TMP was also presented with high prevalence in WWTP-IOA with concentration range of n.d-96.8 ng/L.

Currently, the global use of TMP and SMX in association (i.e. labelled J01E according to WHO) is the fourth highest after penicillins, macrolides and fluoroquinolones [741]. This antimicrobial association is considered as an essential medicine by the World Health Organization (WHO) [742] due to its use in the treatment of various infections such as pneumonia and urinary tract infection [183,743]. High

consumption of TMP along with low average removal efficiencies confirm its high persistence in WWTPs. Trimethoprim has a high potential to resist biodegradation and according to studies exhibits low removal efficiencies of 55 % [744], or 35 -70% [745].

Regarding erythromycin, is the only representative antibiotic of macrolides in this study, which is also included in the first Watch List, highlighting the need of monitoring data [120]. Erythromycin is easily degraded in the aquatic environment and converted into anhydro erythromycin (ERY-H2O), therefore, in many studies [549,744,746–750], including this, is always detected as its dominant form of this metabolite [549,550]. Considering all the monitoring survey, ERY-H_2O was detected at mean concentration of 32.0 ng/L. Its presence in WWTP-IOA and WWTP-AMA was below quantification limits, and only in WWTP-HOS was found in quantified levels at mean concentration of 52.8 ng/L. Its maximum concentration was occurred in WWTP-HOS too with individual value of 123.1 ng/L. Low concentrations of erythromycin were also denoted in other studies concerning domestic effluents in Greece [260,712]

Finally, antibiotics that did not detected at any effluent sample were oxolinic acid, florfenicol and sulfathiazole. Similar results were obtained from Dasenaki et al. [663] and Dilanka et al. [751] concerning sulfathiazole and florfenicol, although the first study confirms the presence of oxolinic in effluent waters at mean concentration of 23.5 ng/L. On the other hand several studies contradict this result by denoting the low occurrence or absence of oxolinic acid in effluent wastewater systems [717,722,732,752,753]. The high removal efficiency of up to 100 percent of florfenicol was likely to play an important role in the absence of the specific antibiotic from effluent samples [754]. Sulfamethazine and sulfamethoxy-pyridazine were found occasionally in concentrations below quantification limits.

8.3.4 Antidepressants and Psychiatrics

Prescription of psychiatric drugs, along with the consumption of antidepressant drugs has been increased on average in OECD countries in the period between 2000 and 2012 [755]. After their intake, psychiatrics undergo metabolism in the human body and are excreted in urine and faeces either unchanged or in form of biologically active metabolites. However, these metabolites may deconjugate back to the parent compound when reaching WWTPs [712]. Therefore, their detection in WWTP discharges, is gaining interest [30,215,216,756].

Their ubiquitous occurrence in the investigated WWTPs in this study (31% detection frequency) indicates either large consumption or removal inefficiencies and poor degradation rates. High consumption of psychiatric drugs has been mentioned as a possible socioeconomic impact of the financial European crisis which increased the insecurity and might lead to various neurological disorders [215–217]. Especially Greece is among the most affected countries by the severe economic crisis plaguing Europe. In fact, an extensive study conducted by Thomaidis et al. [217] in Athens confirms this assume since, high increase in the use of antipsychotics (35-fold) and antidepressants (11-fold) between 2010 and 2014 has been denoted.

Psychiatric compounds contributed about 1300 ng/L of mean concentration level and ranked third in order after artificial sweeteners and NSAIDs. All psychiatrics were detected at least in one sample, presenting the most positive findings among the other therapeutic groups. Venlafaxine exhibited the highest mean concentration of 278.7 ng/L followed by carbamazepine with 169.6 ng/L and fluoxetine with 40.7 ng/L. Venlafaxine, carbamazepine, fluoxetine, amitriptyline were detected in all sampling sites, whereas cyclobenzaprine, olanzapine and risperidone were occasionally detected only in WWTP of Amaliada. Paroxetine was found in WWTP-IOA and WWTP-HOS, while clomipramine was detected only in WWTP-HOS. Considering the total 1-year monitoring survey the concentration levels of psychiatrics were ranged from <MQL to 919.8 ng/L. Mean concentrations of olanzapine, risperidone and clomipramine did not exceed the method quantification limits.

The highest average concentration of psychiatrics was observed at WWTP of University Hospital (1004.1 ng/L) followed by WWTP of Ioannina and WWTP of Amaliada with average concentrations of 403.5 ng/L and 89.3 ng/L, respectively. This result was expected since the University hospital holds a psychiatry clinic, contributing to high percentages of psychiatric drugs in the effluents of the specific plant. In contrast with the psychiatric drug's concentration levels, the number of compounds detected in each sampling station followed the order WWTP-AMA (7 compounds), WWTP-HOS (6 compounds) and WWTP-IOA (5 compounds). The rank order of mean concentrations of the investigated psychiatrics in WWTP-HOS was : Venlafaxine(531.2 ng/L), carbamazepine(336.9 ng/L), fluoxetine (71.4 ng/L), amitriptyline(44.4 ng/L), paroxetine (18.1 ng/L), clomipramine(<MQL). Similar trend of occurrence was observed in WWTP-IOA, whereas WWTP-AMA followed a different pattern. Specifically, in WWTP-AMA the highest mean concentration was similarly presented for venlafaxine, whereas the order of occurrence in this site changed to cyclobenzaprine, amitriptyline, carbamazepine, fluoxetine, olanzapine, and risperidone. The regional difference in the compositions

of investigated psychiatrics in wastewaters is a reflection of variable prescription and consumption patterns [757].

Venlafaxine is a clinical commonly prescribed antidepressant drug belonging in the category of serotonin noradrenergic reuptake inhibitors (SNaRIs). As mentioned before, presented the highest concentration levels in total the monitoring survey. More specifically it was the first compound in rank of the psychiatrics group in every sampling station with detection frequency 100% in WWTP - IOA and WWTP-AMA. The detection of venlafaxine in WWTP-HOS was less frequent (92%), nevertheless in this sampling site presented its highest mean concentration (531.2 ng/L), along with its maximum detection value of 919.8 ng/L. The corresponding concentration levels of venlafaxine in WWTPs of Ioannina and Amaliada presented some discrepancies with ranges from 99.8 to 320.1 ng/L and 14.8 to 86.3 ng/L, respectively. Higher concentrations of venlafaxine among other psychotropic drugs were also reported in other studies [723], [745]. Low removal rates (RR) of venlafaxine or even negative ones (RR = −31.1%) that have been reported from other studies, [758],[759] enable its occurrence in WWTPs. These compounds are excreted in conjugated form as glucuronide after human metabolization [30].

Other psychiatric drug that merits the attention was carbamazepine (CBZ). In the present study, carbamazepine was present at 94.4% of the samples analyzed, with mean concentration of 169.6 ng/L. It was present with absolute detection frequency of 100% in WWTP-IOA and WWTP-HOS, whereas in WWTP-AMA was found with a frequency of 83%. Its highest mean concentrations were observed in WWTP-HOS (336.9 ng/L), along with its maximum individual concentration value of 852.6 ng/L. The corresponding mean concentration levels of carbamazepine in WWTP-IOA and WWTP-AMA were 136.9ng/L and 7.9 ng/L, respectively. The concentration range of occurrence of CBZ in WWTP of Amaliada was significantly lower compared to other WWTPs of the study, probably due to different temperature and operational conditions of the WWTPs during the sampling period, or because of different consumption patterns that may occur.

According to other studies, CBZ is also one of the most frequently detected drugs in municipal wastewater treatment plants with high occurrence too. Recently its concentrations in municipal wastewater has been reported from a few tens to several thousands of ng/L, [59,215,721,758,760], while in hospital wastewater the corresponding concentration range was up to several μg/[140,724,761,762]. Carbamazepine is extensively metabolized in humans and only 10% is excreted unchanged [713,763,764]. Therefore, its persistence is mainly attributed to poor removal efficiencies,

(typically less than 10%), from conventional wastewater treatment processes [765–767]. Furthermore, excretion of glucuronides is a typical reason of occurrence for this compound, which may act as a reservoir from which a later yield of the parent substance can occur [30]. The persistence of CBZ in conventional WWTPs followed by its widespread occurrence in water bodies, triggered the establishment of water quality standards for regular monitoring [10,142,768]. In this context, carbamazepine has been proposed as an anthropogenic marker of sewage contamination in freshwater bodies [769,770]

Fluoxetine is another antidepressant drug found at lower concentrations and a detection frequency of 77.8 % in all sampling stations. It is presented in all WWTPs, with highest frequency in WWTP-IOA followed by WWTP-HOS, whereas in WWTP-AMA was determined in half of the samples investigated. Its highest mean concentration occurs in WWTP-HOS (71.4 ng/L), along with the maximum individual detection of 228.2ng/L and the lowest in WWTP-AMA (6.1 ng/L). Fluoxetine, with less than 10% excreted unchanged, is mainly excreted as norfluoxetine and according to literature was found in lower frequencies in WWTPs [216,604] or in some studies it was detected only in influent wastewaters [216,759]. Fluoxetine, according to literature has mean removal efficiencies oscillating between 80.37 and 100%, enabling its lower occurrence in aquatic environment [216]. With regards to the occurrence of fluoxetine in hospital WWTPs, its concentration range in effluent waters of WWTP-HOS is similar to the corresponding range of a previous study performed for the same system [146].

Amitriptyline was ubiquitous in WWTP-IOA and WWTP-AMA, whereas in WWTP-HOS a detection frequency of 75 % was observed. Nevertheless, the highest mean concentration of 44.4 ng/L occurred in WWTP-HOS while the lowest was observed in WWTP-AMA (10.0 ng/L), although it ranked third in the specific sampling site. Similar mean concentration levels were obtained for hospital wastewater compared to the most recent investigation for the same system by Kosma et. al 2020 [146]. Regarding domestic effluents, amitriptyline was found in lower concentration levels ranged from 1.57 to 4.27ng/L [759], in a previous study, while other findings report no detection at any effluent sample [771]. There are studies as well in Germany and China with mean concentrations reported of 51.7 ng/L [622] and 1.2 ng/L [772] respectively.

Paroxetine was found in lower frequencies and even lower concentrations than the other psychiatric drugs. Specifically, considering all the monitoring study, the detection frequency of paroxetine was only 13.2 %, while in WWTP-AMA was not detected at any sample. In WWTP-IOA was detected with

a frequency of 25.0% at non quantified levels of concentrations. The highest frequency was occurred in WWTP-HOS (42.0%) along with the highest mean concentration of 18.1 ng/L, and the maximum individual value of 40.4 ng/L. Paroxetine according to literature presents efficient elimination rates >80% from WWTPs [216], enabling our results of low occurrence in the present study. In a previous research about psychiatric drugs conducted in Ioannina city, paroxetine did not exceed quantification limits [30].

Clomipramine remained undetected in WWTP-IOA and WWTP-AMA, whereas it was present sporadically (25% frequency) in WWTP-HOS in non-quantified concentration levels (<MQL). Cyclobenzaprine was detected only in WWTP-AMA in significant concentration levels (from n.d to 13.3 ng/L), since it was the second in order, psychiatric drug detected in the specific sampling campaign. It is noteworthy to mention that despite the low frequency of detection (8.0%) in WWTP-AMA, the contribution of cyclobenzaprine by means of concentration abundance was rather significant. Generally in other studies cyclobenzaprine is presented as a compound with no occurrence data, however its monitoring study is very important since it was flagged a decade ago, as compound of potentially high environmental relevance [773] and it is estimated to be persistent and/or bio-accumulative [774].

Risperidone, another drug included in the wide therapeutic class of psychiatrics, is a second-generation atypical antipsychotic and was also investigated in this study. It remained undetected in WWTP-IOA and WWTP-HOS, whereas it was detected sparsely in concentrations of <MQL in WWTP-AMA. The evidence that risperidone is readily metabolized to 9-hydroxy risperidone may be an explanation to the fact that it is not found in the expected concentrations, since it is the highest consumed pharmaceutical treatment of schizophrenia in the Greek market [713].

Finally, olanzapine, was detected only in WWTP-AMA with a low frequency of 8.0% and concentration range of n.d to <MQL. These results are in accordance with studies exhibited in Santorini in Greece and a specific area of Valencia in Spain [215,717]. On the contrary, a study in Portugal focused on hospital contribution to effluent wastewaters, reported detection of olanzapine in effluents at a concentration range of 15.0-36.1 ng/L [56].

8.3.5 Disinfectants & Lipid Regulators

Triclosan is the only representative compound of the class of disinfectants. It was ubiquitous in all sampling campaigns with absolute detection frequency of 100%. Its mean concentration throughout

the 1-year monitoring was 192.2 ng/L and its contribution as contaminant was significant, taking into consideration that was the only compound investigated from the group of disinfectants. Its highest mean concentration was observed in WWTP-HOS, ranked third in abundance, with a value of 367.5 ng/L, along with its maximum absolute concentration of 843.6ng/L. Next, the occurrence of triclosan followed the decreasing order of WWTP-IOA and WWTP-AMA with mean concentrations of 159.2 ng/L and 49.8 ng/L, respectively. Ranges of triclosan in the domestic effluents of the study were oscillated from 52.9 ng/L to 321.8 ng/L and 12.0 ng/L to 153.6 ng/L in WWTP-IOA and WWTP-AMA, respectively. Comparing these results with other monitoring studies, variabilities in concentration ranges occur. For example, Papageorgiou et al. 2016 [260], reported no detection of triclosan in municipal WWTP of Volos, a city in Central Greece, while a few years later in 2019, triclosan was presented in the same city with mean concentration of 27.6 ng/L [775], indicating a possible increase of the antimicrobial agent along the years. The trend of low occurrence of triclosan was also reported by Kosma et al., 2014, in a study of several WWTPs in Greece, including Ioannina city and University Hospital of Ioannina. In this study, triclosan was found in concentrations below method quantification limit, with only exceptions the WWTPs of Ioannina Hospital and the municipal plant of Veroia, with mean concentration of 133.6 ng/L and 139.2 ng/L, respectively [712]. In other European studies the occurrence of triclosan in effluent waters, ranges from 0.01 to 6.6 μg/L [32], while a wide European monitoring survey reports mean concentration of triclosan in level of 150 ng/L [674].

In our study triclosan is detected in rather high concentrations, especially in Ioannina Hospital. Its use as antiseptic agent in hospitals, as well as main ingredient in medical supplies such as surgical scrubs, hand soaps, gloves, surgical instruments, toilet detergents etc., is quite common [776]. Nevertheless, high concentrations were also observed in municipal effluents , since triclosan is commonly found in the ingredient list of several household and personal care products (PCPs), exploded onto the market because of its broad-spectrum antimicrobial activity [776]. Triclosan is commonly used in PCPs as synthetic preservative in the manufacture of toothpastes, footwear, shampoos, deodorants, skin creams, while recently it is being incorporated into plastic products from children's toys or sport equipment, kitchen utensils such as cutting boards or furniture [694,712,713,777]. Triclosan is efficiently eliminated from WWTPs (>65 %), [32,674,778] about ~ 50% will be settled in sludge due to its high octanol/water partition coefficient (log Kow = 4.76), rendering it labile to biodegradation and adsorption in the suspended matter [779]. Consequently, in spite of the high removal rates of

triclosan, it remains ubiquitous and is continuously released not only in WWTPs but in freshwater bodies too [713,714,780].

As regards the therapeutic group of lipid regulators, gemfibrozil was the representative compound in our study. It was found in all WWTPs with a frequency of 69.0% and a total mean concentration of 91.0 ng/L. The highest mean concentration was detected in WWTP-IOA, whereas the lowest in WWTP- AMA. Gemfibrozil was more frequent in WWTP-AMA (75.0%) despite its low occurrence compared to the other WWTPs, with a concentration range from n.d to 73.9ng/L, while in WWTP-IOA and WWTP-HOS presented the same frequency of 66.7 %. The maximum individual concentration of gemfibrozil was observed in WWTP-IOA (453.8 ng/L), while in some sampling sites no detection was occurred. These findings are controversial to a study in Greece about municipal effluents [260] where no detection of Gemfibrozil is reported, but are in line about the presence, with another study in Greece [712]. Kosma et al. [712].refers to higher concentrations of Gemfibrozil in several Greek cities, with average concentrations of 215-347.1 ng/L. Meanwhile similar trend of results is presented in the European wide monitoring study, which reports mean concentration of gemfibrozil about 138 ng/L and detection frequency of 60% [674]. Finally, a recent study assessed exclusively for the effluents of Ioannina University hospital refers to average concentration of 33.1 ng/L, which is confirmed by our study [146]. The rather high occurrence of gemfibrozil in terms of detection frequency as well as of concentration levels, advocates the fact that gemfibrozil belongs to the class of antihyperlipidaemics, which comprises the highest sales in the worldwide pharmaceutical market. For this reason gemfibrozil has been classified as high priority pharmaceutical for the water cycle by the Global Water Research Coalition [468]. Although it is mainly found in the aqueous phase, its possible sorption to organic carbon enriched matrices, sediments, and biota cannot be excluded owing to its log Kow value (4.77) [781–784]. In fact, the European Medicines Agency Guidelines, attract the concern to drug substances with a logKow > 4.5 which are possible candidate contaminants for persistence, bioaccumulation and toxicity [785].

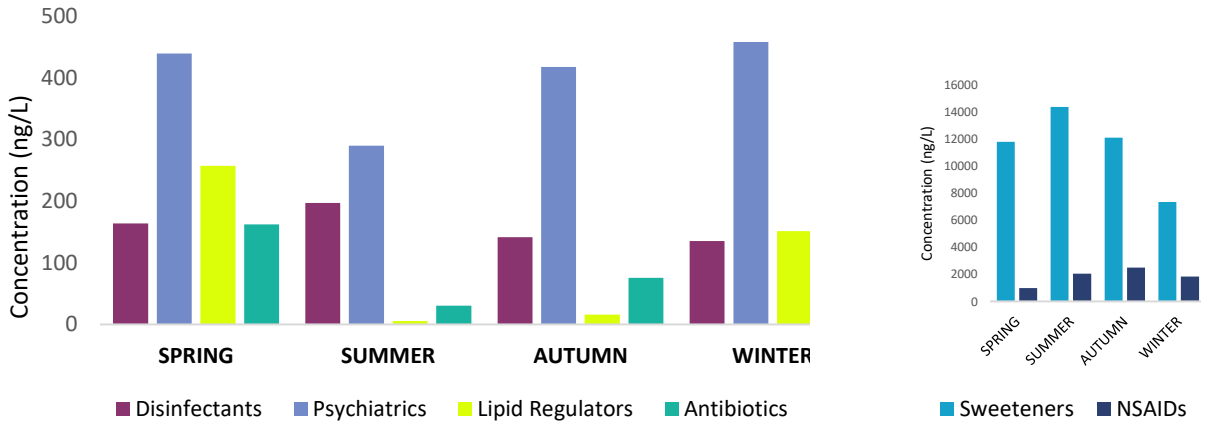
8.4 Seasonal and temporal variation

Recently, various studies have reported that concentrations of artificial sweeteners and pharmaceutical compounds, especially the ones belonging in Personal Care Products are subjected to significant seasonal variations in the wastewater effluents [702,786–789]. Generally, seasonal variations are attributed to consumption patterns of artificial sweeteners or pharmaceuticals

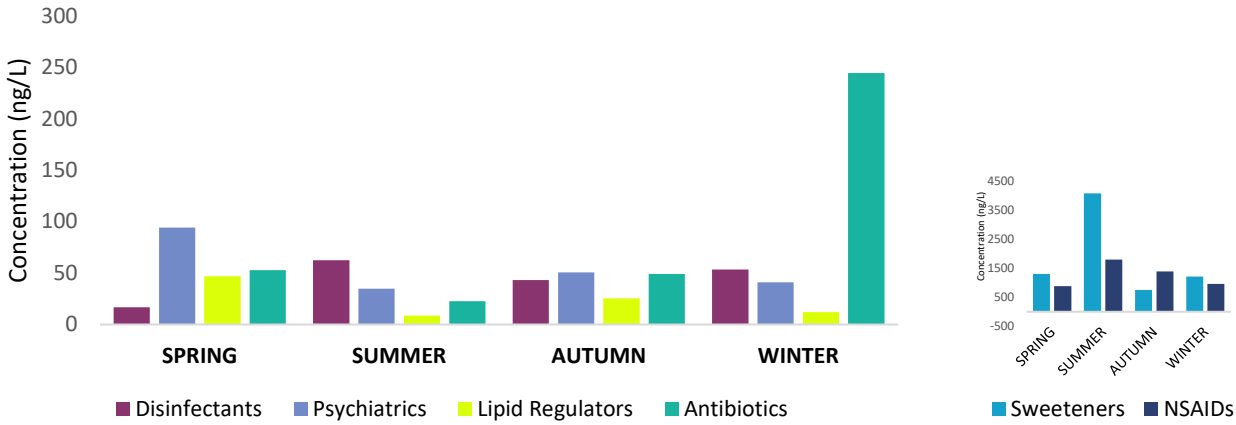
depending also on their family group they belong [712]. Additionally, it may be associated to the amount of precipitation during the sampling period or to socio-geographical differences as well (e.g. tourism during Summer, some important events in the investigated area etc.) [790]. Furthermore, dilution factors due to the increased rainfall in winter period might lead in low concentrations of the target ECs. For instance, it has been reported that generally lowest effluent loadings were observed for some pharmaceuticals in winter period in three WWTPs in Dublin region [786]. Finally, photo degradation or biodegradation and sorption that occur in WWTPs, are supposed to be key processes for the removal efficiency of the target contaminants. These processes are associated with environmental elements such as solar irradiation, precipitation, and temperature. For example, for some compounds, sorption increases with decreased temperature while biodegradation decreases in lower temperatures [786]. Finally, the physicochemical characteristics of each target compound plays as well an important role, since are involved in the above removal processes [260,791].

Therefore, our findings were compared in terms of seasonal fluctuations in the observed concentrations of ECs in analyzed water samples. Four periods in accordance with the seasons can be distinguished: winter (December–February), spring (March–May), summer (June–August), and autumn (September–November). The following figures (Fig. 8.13) illustrate the average concentrations of the ECs during the four seasons of the year, for each WWTP, categorized in the corresponding family groups. In addition, Fig. 8.14-Fig.8.16 provide information about the individual concentration of target ECs monthly, for each WWTP investigated.

(a) WWTP-IOA



(b) WWTP-AMA



(c) WWTP-HOS

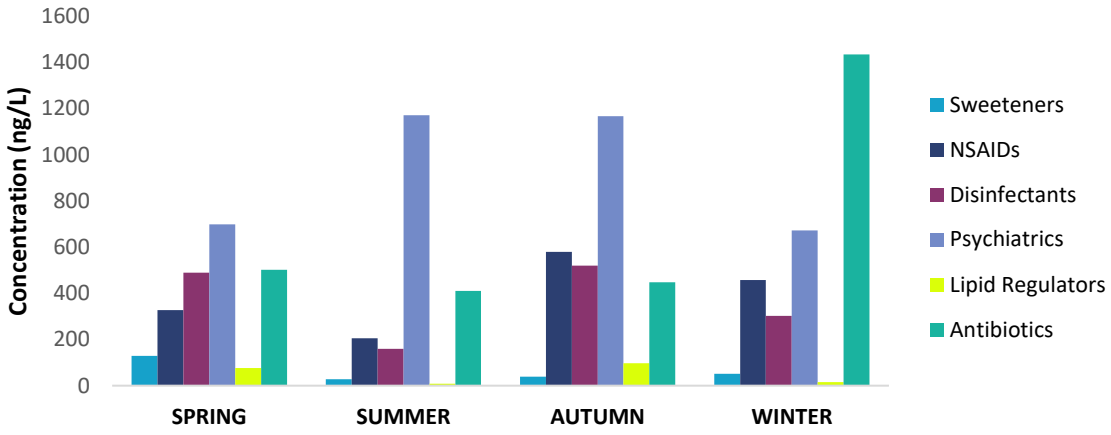
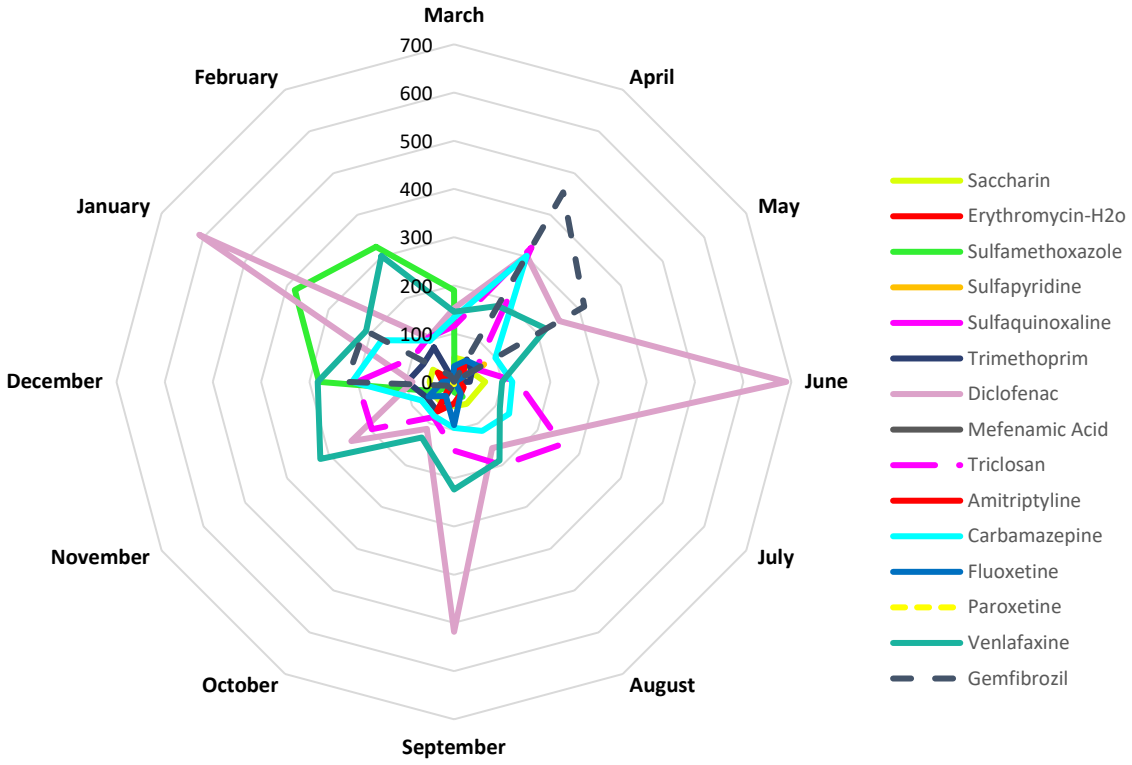


Figure 8.13. Seasonal average concentrations of the diverse groups of ECs in effluent water of **(a)** WWTP-IOA, **(b)** WWTP-AMA and **(c)** WWTP-HOS



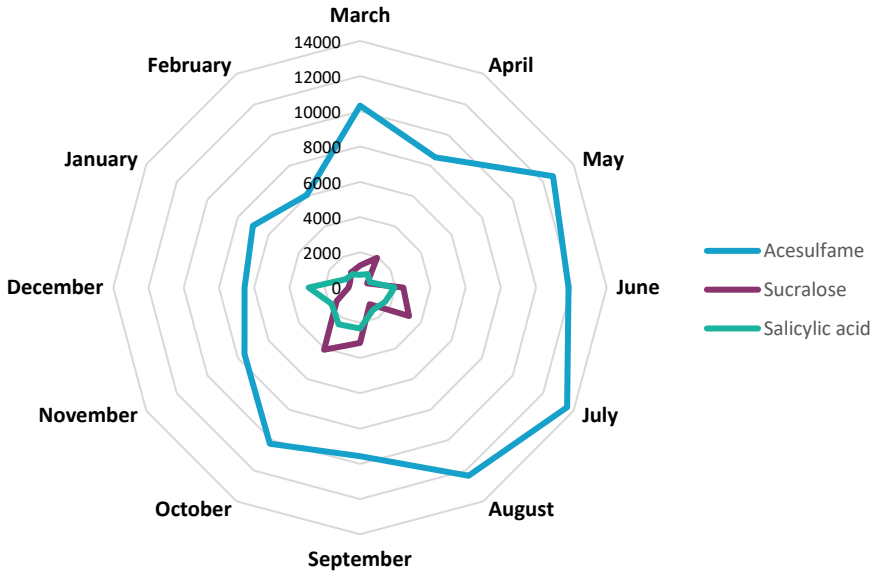


Figure 8.14. Concentrations (ng/L) of ECs in WWTP of **Ioannina** effluent during one-year period

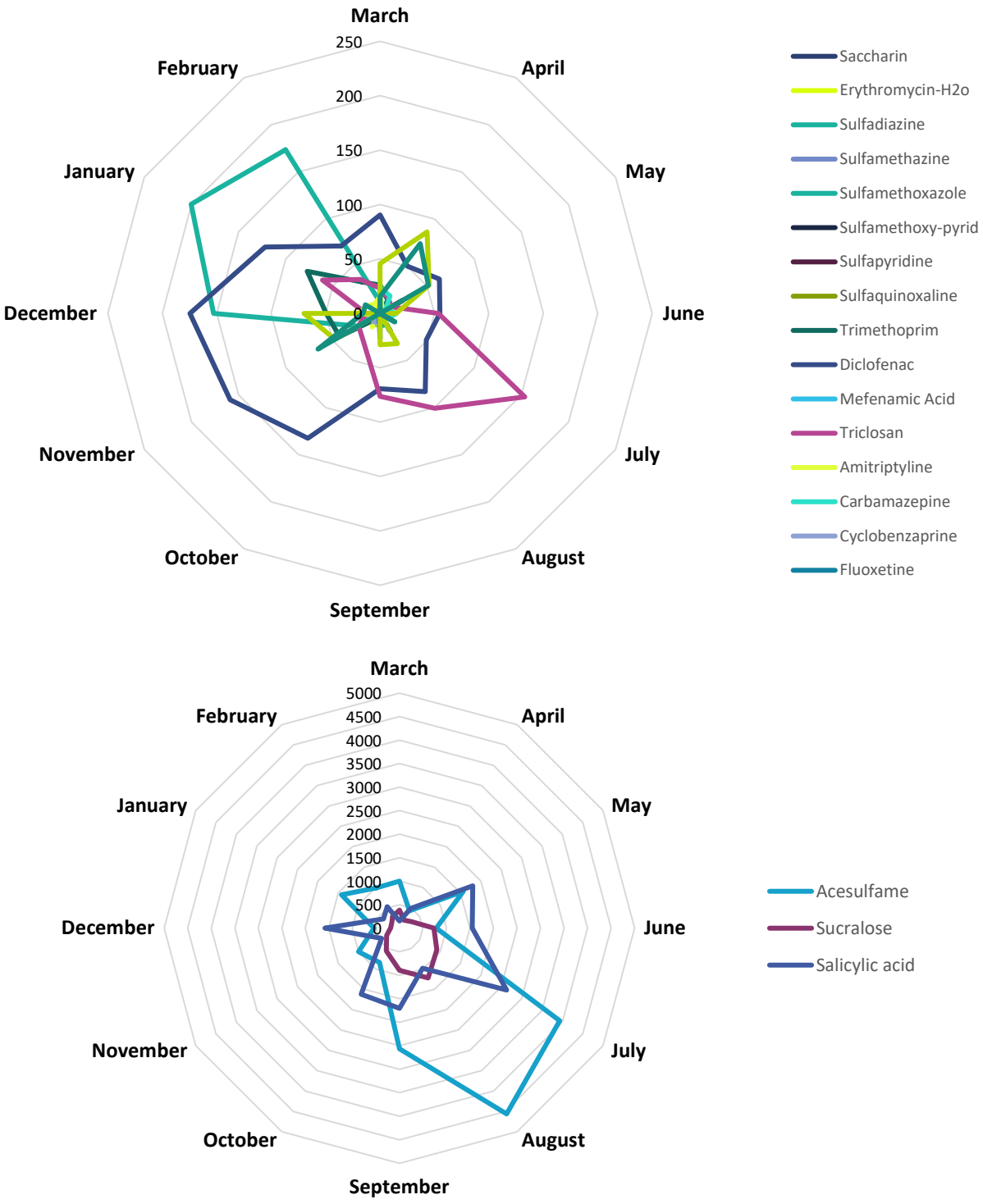


Figure 8.15. Concentrations (ng/L) of ECs in WWTP of **Amaliada** effluent during one-year period

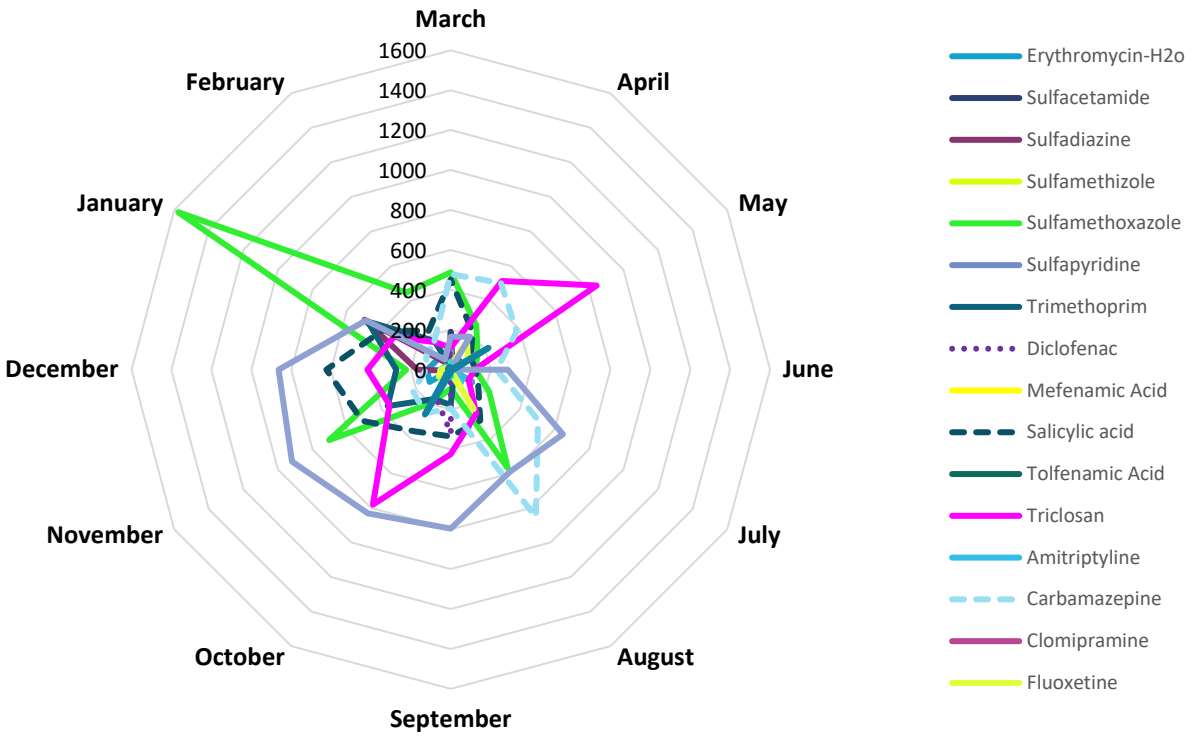


Figure 8.16. Concentrations (ng/L) of ECs in WWTP of **University Hospital** effluent during one-year period

A visually discernible trend shows that the concentration levels of antibiotics are higher in winter compared to other seasons with a significant difference from 3-fold to almost 10-fold. This trend occurs all sampling stations of WWTP-IOA, WWTP-AMA and WWTP-HOS. These findings are consistent with the expected usage patterns for human antibiotic use, for the reason that they are used for flu or other respiratory infections which mainly occur in cold months of the year [792].Other studies also reported similar results regarding the occurrence of antibiotics in winter months (December–February) [30,146,792,793]. Another reason for the higher concentrations in winter is the fact that antibiotics might be bio- and photo-degraded in higher rates during summer months [794].

In details, the highest concentrations of sulfamethoxazole (SMX), the most wide-spread antibiotic of the study, was found in winter months in all sampling stations. In WWTP-IOA the highest concentration was detected in December, at the value of 381.2ng/L, while in WWTP-AMA and WWTP-HOS the maximum concentrations were detected in January with corresponding values of 200.4 and 1578.0 ng/L respectively. Lower concentrations of SMX were observed in spring at WWTP-IOA and WWTP-AMA, while in autumn was sporadically detected in both WWTPs. On the other hand,

SMX was not detected at any sample in WWTP-IOA in summer period, while for the same period in WWTP-AMA was detected only below quantification levels. Concerning WWTP hospital, the presence of SMX in winter period had a distinct difference compared to the other months. Nevertheless, high concentrations of SMX occurred also the other seasons of the year and found in significant amounts even in summer period. A possible explanation to these findings is, that antibiotics in hospital facilities are used throughout the total months of year, for the therapy of a wide range of diseases such as : ear infections, urinary tract infections, bronchitis, pneumonia etc.

Trimethoprim, (TMP) was also detected with high occurrence in winter period and for instance in December and January presented its highest value at WWTP-IOA (96.8 ng/L) and WWTP-AMA,(77.2ng/L), WWTP-HOS (480.4ng /L), respectively. On the other hand, lower occurrence of TMP were observed in warmer months. TMP was not detected at any sample at WWTP-IOA in July and August, while at WWTP-AMA, was detected only in non- quantified levels, the summer period. In WWTP-HOS, TMP has presented some variations in concentration ranges throughout the seasons, following although the same temporal pattern, of sulfamethoxazole, implying in some way their associated use. The presence of sulfapyridine, sulfadiazine, sulfaquinoxaline, sulfamethoxy-pyridazine, erythromycin-H_2O, is characterized by temporal and spatial discrepancies, therefore is difficult to conclude to a seasonal pattern for these compounds. However, it is noteworthy to mention the ubiquitous detection of sulfacetamide and sulfamethizole in all winter months in WWTP-HOS, along with the much lower concentrations levels or non-detection in summer, similarly to sulfadiazine which presented maximum concentrations in January (499.2 ng/L) and December (168 ng/L), while in summer was not detected at all. Another highlight may be the presence of sulfaquinoxaline and sulfamethazine in WWTP-AMA, the warmer months of the year and especially the summer when they presented higher concentration levels than in winter. A possible explanation for this observation may lie to the use and application of the specific antibiotics exclusively in veterinary medicine. Veterinary antibiotics usually are applied in summer [795], compared to human antibiotics which are used mostly in winter [260]. Summer is the period of high disease incidence, in animal farms hence, the higher use of feed incorporated with antibiotics can lead to the higher concentrations of these compounds. The recent findings of Im et al., [791] as regards the tempo-spatial distribution of sulfonamide antibiotics supports this assumption. Im et al. reported notably higher concentration of sulfonamides in summer and spring than in winter [791]. The seasonal variations of antibiotics are presented in Figure 8.17.

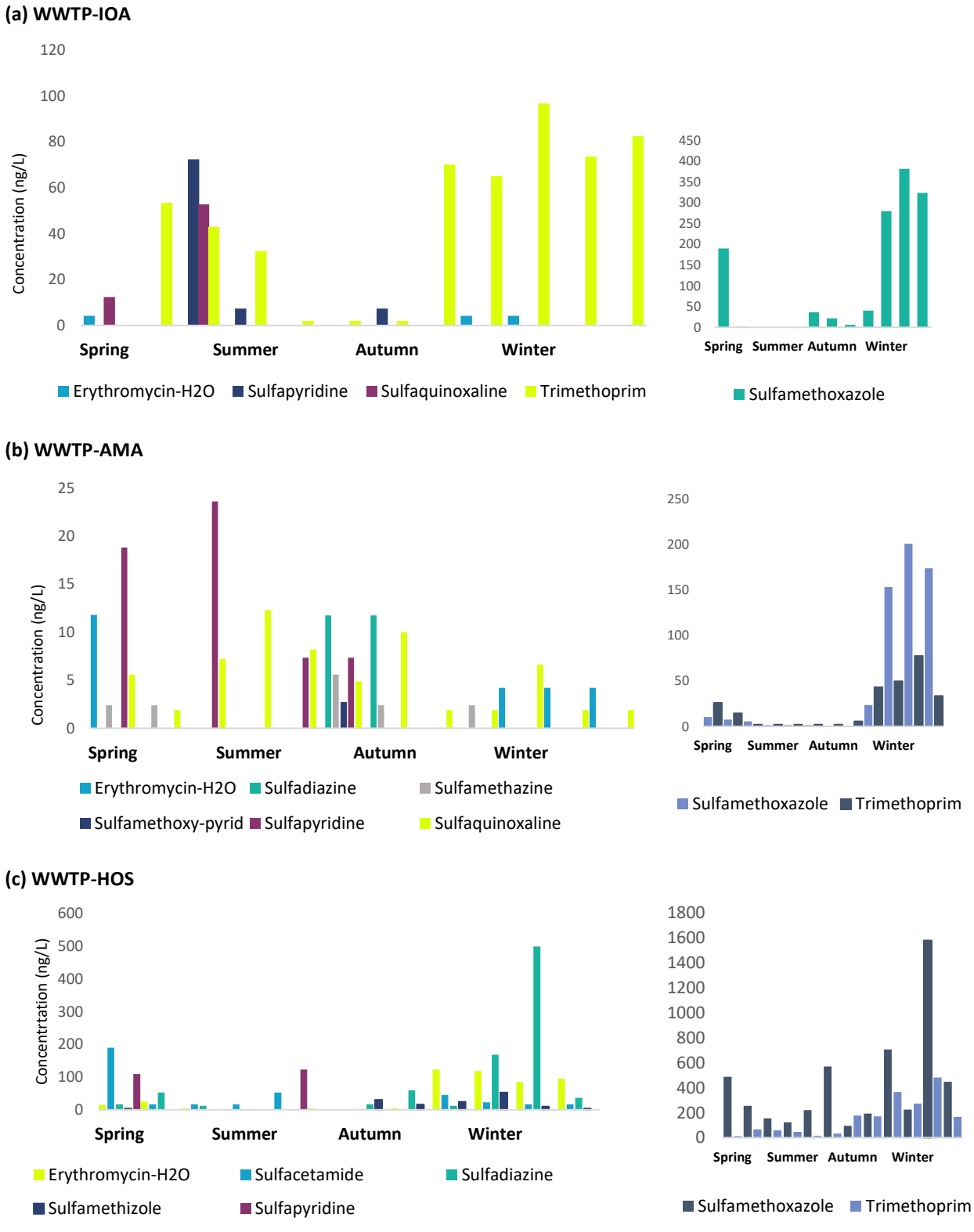


Figure 8.17. Seasonal concentration levels of Antibiotics in **(a)** WWTP-IOA, **(b)** WWTP-AMA, **(c)** WWTP-HOS

Artificial Sweeteners, tend to follow a seasonal pattern, since in the sampling sites where the occurrence is intense (WWTP-IOA, WWTO-AMA), the higher concentrations are accumulated in summer period, whereas the lowest in colder months, in winter and autumn, respectively. The total amount of sweeteners in summer is controversial to the general suggestion which implies that in higher temperatures, biodegradation and photodegradation by solar irradiation is enabled. A possible explanation is the higher consumption of products containing artificial sweeteners during the summer months. Cold beverages, soft drinks, powdered drink mixes, ice-creams etc. are consumed in higher rates in summer months than in winter or autumn. Acesulfame's concentration levels are the maximum during July and August in both domestic WWTPs of Ioannina and Amaliada, with corresponding values of 13569 ng/L, 12331 ng/L and 3935.8 ng/L, 4561.2 ng/L, respectively. The rather higher persistence of ACE in Amaliada city in August compared to other months, may attributed to the increased population of the city during that period. Amaliada, is a seaside city and a summer holiday destination of interest. Tourism, after agriculture, is an important economic sector of the city, at the same time though, has a negative impact regarding the contamination of natural ecosystems [796]. Increased intake of products containing sweeteners, consumption of pharmaceuticals, and loads of personal care products in WWTPs may conclude to insufficient removals and eventually to higher discharges in freshwater bodies. These tourism-generated negative effects may occur seasonally. Seasonal changes induced by anthropogenic factors were also observed earlier in other studies [797,798].

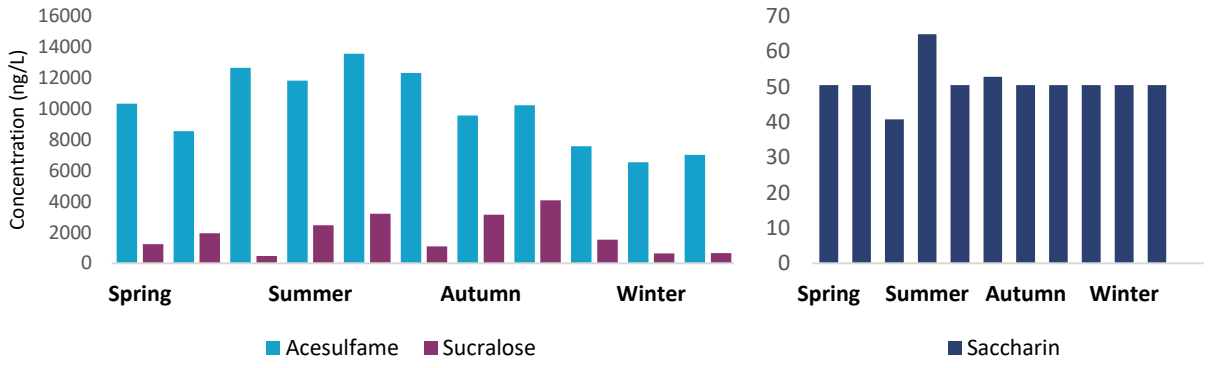
The high occurrence of ACE in summer at WWTP-IOA and WWTP-AMA is followed by lower concentrations in autumn and spring with descending order, while in winter is denoted the lowest level of concentration (~ 6540 and 982 ng/L). In WWT-IOA, the concentration ranges of ACE were oscillated from 8542.1 to 12652 ng/L and 7567.6 ng/L to 10234.2 in spring and autumn, respectively. Similar concentrations of ACE were observed in Amaliada effluents during winter and spring (~1000.0 ng/L) and are differentiated significantly from the corresponding levels of summer (~3093ng/L). Alike to ACE, sucralose is detected in high levels in summer and autumn in both domestic effluents. In WWTP-IOA, the highest concentration is observed in October (4075.2 ng/L), while in WWTP-AMA the highest is observed in August (1221.5 ng/L). In both cases the lowest concentrations are denoted in winter period followed by spring months.

Saccharin was the artificial sweetener with a negligible occurrence in effluent of the three WWTPs investigated. Nevertheless, its occasional presence seems to be occurred in summer months in both cases, in June and August, even in <MQL concentrations.

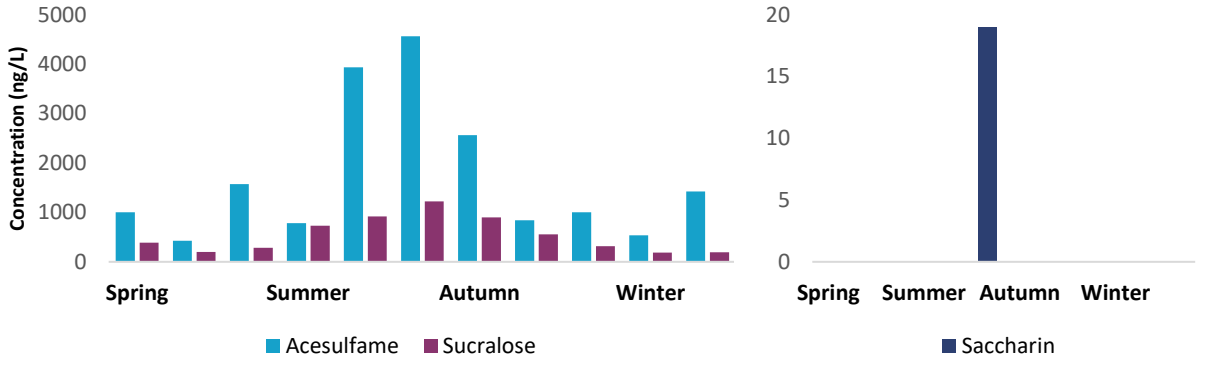
As regards WWTP-HOS, was not a representative sampling point to investigate the seasonality of artificial sweeteners, since it was the WWTP with the less occurrence of these compounds.

To conclude the persistence of artificial sweeteners is gathered in warmer months and especially in summer period indicating not only high usage patterns during summer but also a severe lack of efficiency in current domestic wastewater treatment plants. Photodegradation processes are insufficient for acesulfame and sucralose even in high temperatures and intense and prolonged solar irradiation. This fact is highlighted by numerous studies focusing on the removal of artificial sweeteners [702], [799–801]. Seasonal concentrations levels of antibiotics per sampling campaign is presented in Fig.8.17.

(a) WWTP-IOA



(b) WWTP-AMA



(c) WWTP-HOS

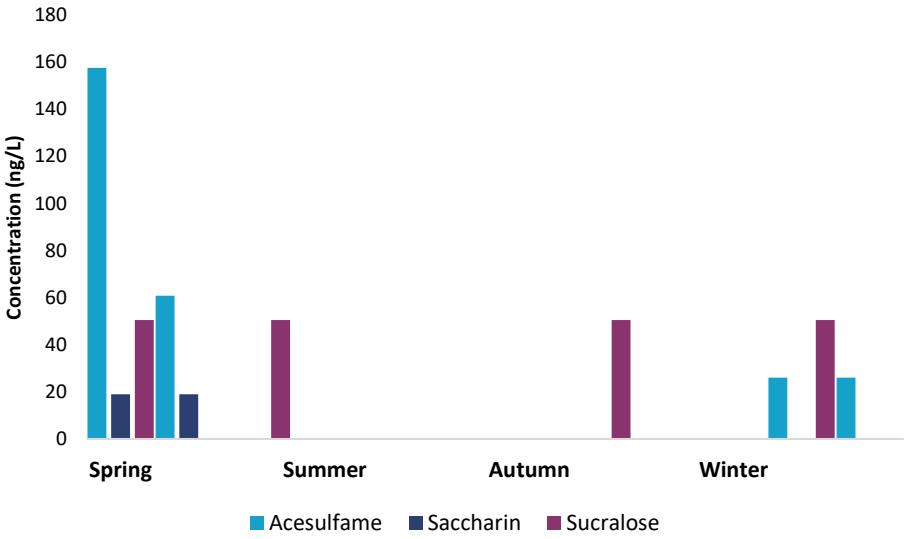


Figure 8.17. Seasonal concentration levels of Artificial Sweeteners in **(a)** WWTP-IOA, **(b)** WWTP-AMA, **(c)** WWTP-HOS

NSAIDs-Analgesics, present higher concentrations in Autumn and lowest in Spring in WWTP-IOA and WWTP-HOS, indicating a similar usage pattern since the effluents of hospital water discharge in the municipal plant of Ioannina. Salicylic acid is the compound that contributes the most in this therapeutic group and as mentioned in previous sections, constitutes the main metabolite of aspirin, an over the counter drug, very popular in Greece and can derive from many sources since is used as additive in food products, in cosmetics, in toothpastes and other personal care products. Therefore, the occurrence of salicylic acid by means of usage pattern, is expected all over the year. However, considering its main use as anti-inflammatory drug, is consumed also to cure inflammation caused by influenza, which occurs more frequently during colder months [802]. In fact, in WWTP-IOA and WWTP-HOS, the highest concentration of Salicylic acid occurs in December with concentration values of 2913.5 ng/L and 624.1 ng/L, respectively. Unlike these findings, in WWTP-AMA, the highest concentration of salicylic acid was detected in summer and especially in July at value of 2632.6 ng/L. This controversial to our expectation result may be attributed, to the seasonal increase in the population of Amaliada city during summer. The use of this drug may have increased as the number of residents in the city increased. Simultaneously, the decrease of salicylic acid in winter suggests a possible dilution effect due to rainfalls in winter months [260].

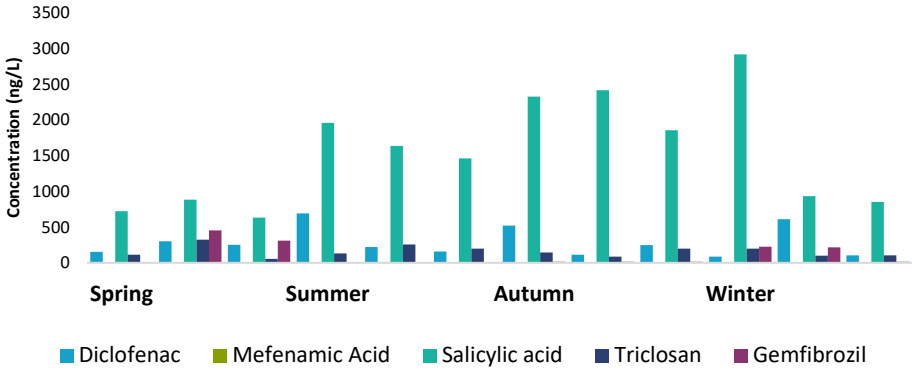
Diclofenac on the other hand, seems not to follow a seasonal pattern. Its occurrence all over the seasons is denoted with several discrepancies in concentration levels. For instance, in WWTP-IOA, average concentrations of diclofenac throughout the year were ranged from 236.0 ng/L to 365.5 ng/L with some peaks of concentrations in several months of each season. In WWTP-AMA, similar average concentrations of 67.8 and 62.4 ng/L were observed during spring and summer, while a double-fold increase is detected in autumn and winter with corresponding concentrations of 120.3 ng/L and 122.6 ng/L, respectively. In WWTP-HOS, diclofenac was occasionally found throughout the seasons with highest concentrations occurring in autumn with maximum individual in September (319.4 ng/L).

As for the other two NSAIDS-analgesics concerns, Mefenamic acid and tolfenamic acid, their detected concentrations in the monitoring study were mainly at levels below quantification limits (<MQL).

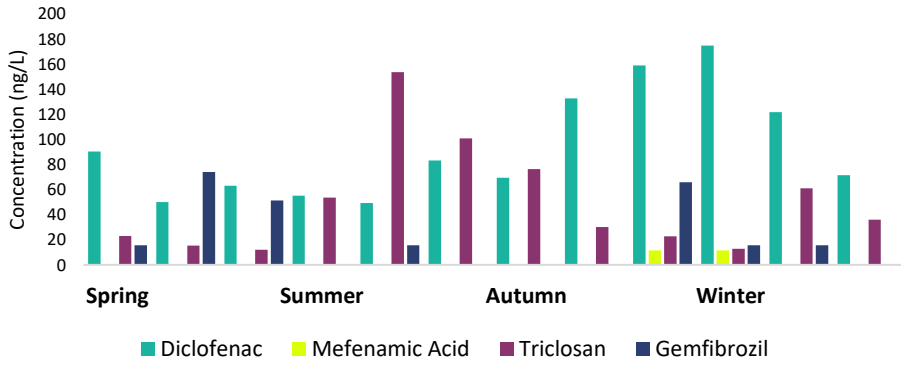
Triclosan throughout the seasons, showed no significant variances in some sampling sites. Specifically, in WWTP-IOA, the average concentrations were from 135.2ng/L in winter to 196.8 in summer, while in WWTP-HOS was 302.1 ng/L to 489.1 ng/L from winter to spring, with lower average presented in summer 158.9 ng/L. On the other hand, in WWTP-AMA, a significant increase of triclosan was observed during summer months, since the average concentration that period was 102.7ng/L, compared to other seasons that occurred in range of 16.8 -43.5ng/L. Urban wastewaters are the discharge of a wide range of applications of triclosan as an important ingredient in personal care products. Furthermore, the use of personal care products such as soaps, deodorants, sunscreens etc. are increased during summer period [803]. This fact, associated with a possible inefficient removal from WWTP, justifies the high occurrence of triclosan in Amaliada during summer, with maximum concentration in July (153.6ng/L).

Concerning lipid regulators i.e. gemfibrozil, it was not possible to result in a seasonal pattern about its concentration levels. According to studies, lipid regulators are generally daily administered drugs in clinical practice, and thus, no variation is expected in their consumption per season [260]. The temporal and spatial variances of gemfibrozil during this study may occur due to geographical consumption patterns, or efficient eliminations during treatment processes depending also on climate changes. For example, a noteworthy observation for gemfibrozil is that in all sampling stations it presented its lowest occurrence , even at <MQL levels, during summer, implying successful removals since biodegradation or photodegradation processes are enhanced in higher temperatures and solar irradiation. Figure 8.18 represents the seasonal concentration levels of representative compounds from three therapeutic groups of NSAIDs, disinfectants and lipid regulators.

(a) WWTP-IOA



(b) WWTP-AMA



(c) WWTP-HOS

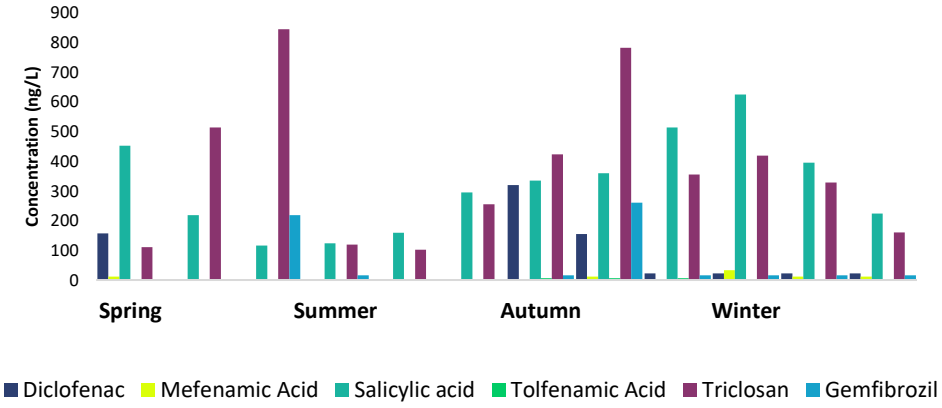


Figure 8.18. Seasonal concentration levels of NSAIDs-Analgesics in **(a)** WWTP-IOA, **(b)** WWTP-AMA, **(c)** WWTP-HOS

Antidepressants-Psychiatrics (PSAs) presented several variations in their concentration levels, in each sampling site, indicating different seasonal trend per sampling campaign. The mean concentrations

of antidepressants-psychiatrics in WWTP-AMA are by far the lowest compared to WWTP-IOA and WWTP-HOS, reflecting probably to a different consumption pattern. In Amaliada city, the average concentration of PSAs from summer to winter are quite similar, with average concentration range of 34.9 to 50.7ng/L with a high peak in spring (94.1 ng/L), contributed mainly from venlafaxine and carbamazepine which is supposed to be recalcitrant in all seasons [713]. On the other hand, in Ioannina city the concentration effluents during spring, autumn and winter are approximately the same (417.0-457.7ng/L), whereas in summer a significant decrease occurs (289.8ng/L). This may depend on several parameters, such as geographical region, socio-economic factors, biodegradation processes, and climate conditions. For example according to meteorogical data, the characteristics of the weather such as the total rainfall, cloud cover, sunlight hours, vary from city to city since Ioannina is located in north western part of Greece, while Amaliada in southern part. As a matter of fact the percentage of cloud cover in Ioannina was almost 40%, during the survey, whereas in Amaliada approximately 25% [804]. In addition, the total amount of rainfall in Ioannina (1756.3 mm of rain), as illustrated in Fig.8.19 is significantly higher than the corresponding of Amaliada (718.8mm of rain) [804].

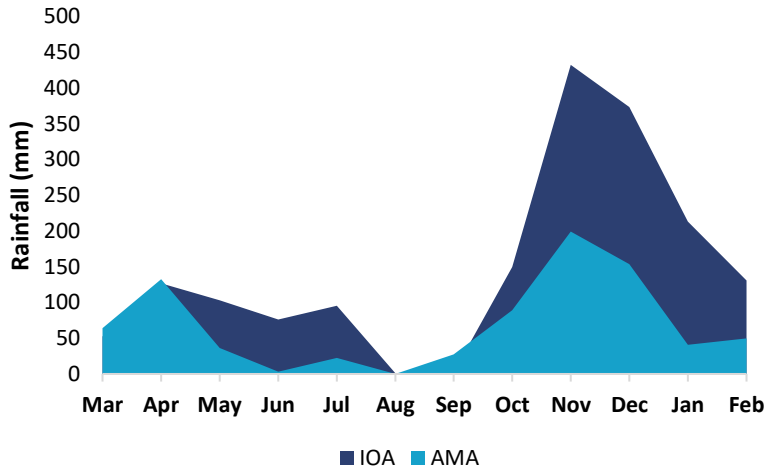


Figure 8.19. Amount of rain (mm) during the period of the monitoring study (March '19-February '20) in Ioannina and Amaliada city [804].

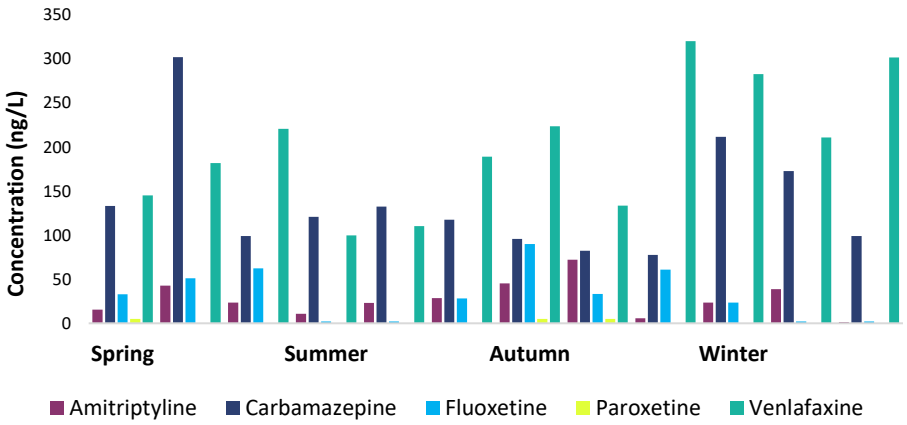
According to psychiatric studies, meteorological conditions and climate have been long suspected to play a role in the onset and development of mental and psychiatric disorders like depression [805–808]. Interestingly, a recent study from Spain using local objective climate data found that those living

in areas with the highest temperatures, least rain and longest duration of sunshine were less likely to become depressed [806], [807]. For instance, Seasonal Affective disorder (SAD) is a combination of biologic and mood disorders characterized by recurrent episodes of major depression occurring with a seasonal pattern [809], [810]. The symptoms occur in a nearly yearly pattern in autumn/winter and disappear completely in spring/summer[792],[811]. Pharmacotherapy with antidepressants is usually an option for an appropriate treatment [216], [809]. The following findings in association with meteorological data reported, during the 1-year monitoring, seem to sustain this evidence. Amitriptyline, venlafaxine, fluoxetine are common antidepressant drugs and are detected in Ioannina city in colder months of the year. Venlafaxine presented highest mean concentration (264.9 ng/L) during winter, while fluoxetine and amitriptyline were found in their highest rates during autumn at average concentrations of 123.6 and 61.4 ng/L, respectively. A remarkable observation was that all these antidepressant drugs were decreased significantly in summer period, with lowest mean concentrations of 133.0 ng/L, 20.9 ng/L, 9.2 ng/L for venlafaxine, amitriptyline, fluoxetine, respectively. Similar results observed for amitriptyline in WWTP-AMA, with highest concentration in winter and especially in December at 20.1 ng/L, while fluoxetine was detected in concentrations close to MQL or <MQL. Low concentrations of carbamazepine and occasional detection was occurred in the same sampling point. On the other hand, carbamazepine was frequent in WWTP-IOA throughout the year with consistent presence across the seasons. Specifically, the mean concentrations detected in three of the four seasons, spring, summer, winter, were oscillated in similar range 123.6 ng/L - 160.9 ng/L, with slight decrease in autumn (~95.0 ng/L). This makes sense, since carbamazepine is an antiepileptic drug mainly used for treatment of chronic disease (certain types of seizures, trigeminal neuralgia, episodes of mania and depression), [812] which usually occurs all over the year [45]. Considering the usage of carbamazepine as well as its low removal efficiency by WWTPs, its persistence is not expected to follow a seasonal pattern. Contrary to this, and to the results of the other WWTPs, concentrations of carbamazepine detected in WWTP-HOS, showed several seasonal variations. The mean concentrations of carbamazepine in hospital effluents were higher in summer at 528.3 ng/L and especially in August (853.6 ng/L), and decreased in the following order: spring at 452.9 ng/L, autumn at 222.3 ng/L, and winter at 144.2 ng/L. The low occurrence in winter may attributed to increased rainfall which reduces relatively constant loads of contaminants due to dilution effects [787], [813]. Concentration variances in hospital effluents across the four seasons were observed for venlafaxine and amitriptyline too. Highest concentrations of venlafaxine occurred

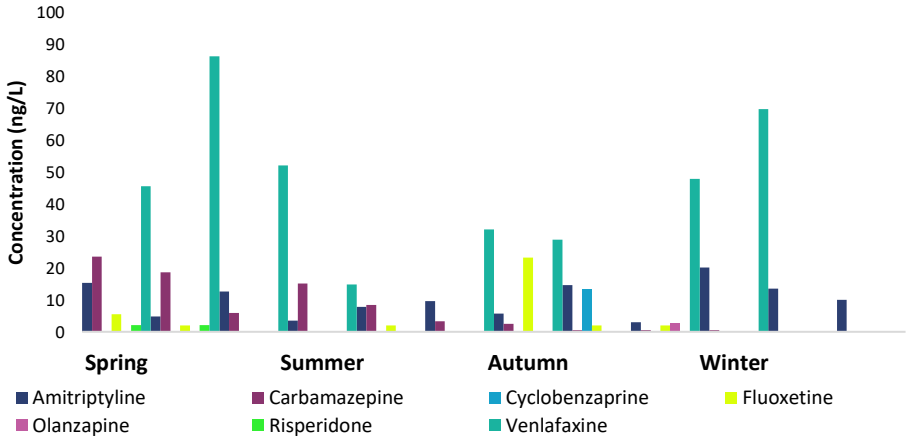
during colder months in autumn and winter at 849.7ng/L and 471.2 ng/L, respectively, whereas the lowest was in spring 118.4 ng/L. Amitriptyline was detected in hospital effluents with higher concentration during summer at 54.2 ng/L, remained at similar levels during autumn (38.2 ng/L) and winter 27.8 ng/L, and presented its lowest abundance in spring (13.0 ng/L). The prominent level of variances in hospital wastewater is mainly attributed to consumption usage in psychiatric clinic, depending on the applied techniques and therapies per patient case during the month of the sampling campaign. Finally, the amount of precipitation during the sampling period, may also contribute to variations [790].

The rest of the psychiatrics were detected with less frequency or in concentrations close to MQLs and <MQLs. Seasonal concentration levels of antidepressants-psychiatrics are illustrated in Fig.8.20.

(a) WWTP-IOA



(b) WWTP-AMA



(c) WWTP-HOS

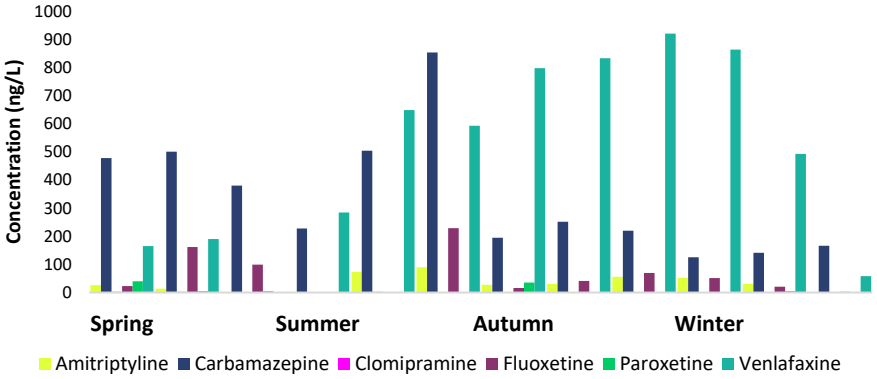
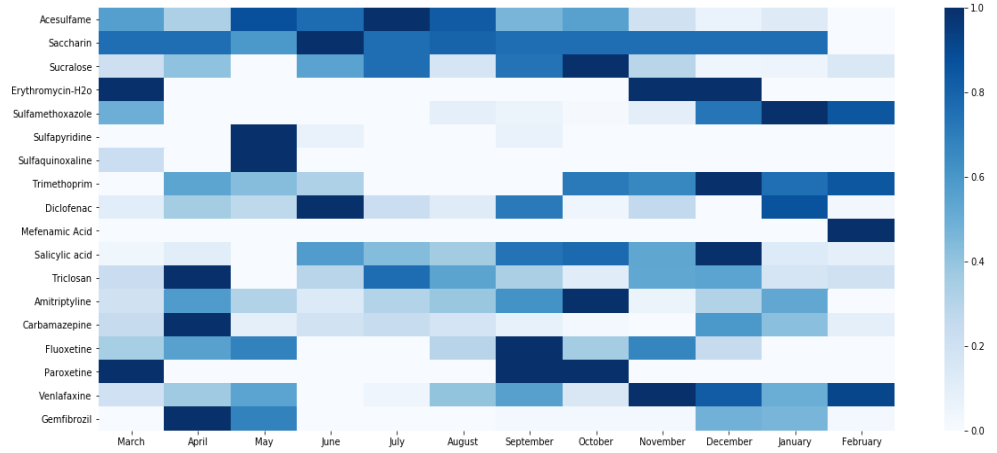


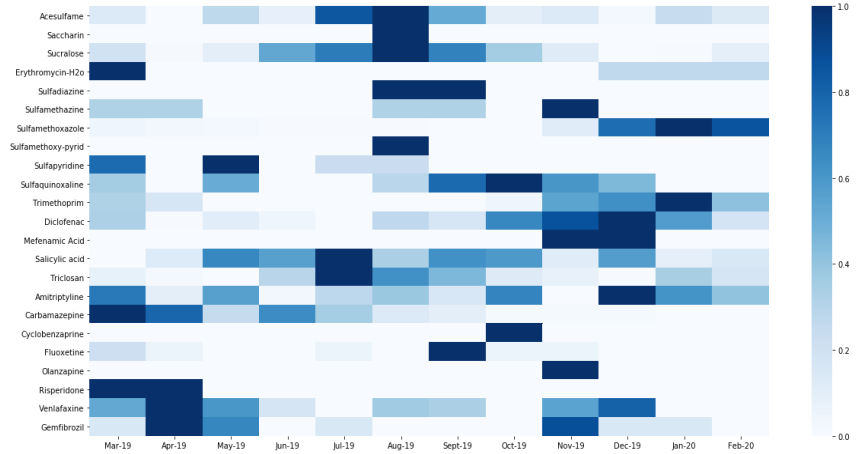
Figure 8.20. Seasonal concentration levels of Antidepressants-Psychiatrics in **(a)** WWTP-IOA, **(b)** WWTP-AMA, **(c)** WWTP-HOS

The detailed temporal (monthly) variations of individual target ECs for each sampling site of (a) WWTP-IOA, (b) WWTP-AMA and (c) WWTP-HOS are illustrated in Figure 8.21 with the aid of heatmaps. Concentration heat map (Fig.8.21) visualizes all the above discussion for each sampling point. For example, the higher concentrations variation (dark blue color) of antibiotics is mainly located in months December, January and February, while the high occurrence of sweeteners mainly contributed from acesulfame and sucralose is located in August, July, June. On the other hand, some compounds belonging in psychiatrics, NSAIDs, are depicted as clear blue color-cells in the heat map, indicating rather lower concentrations in summer, taking into consideration that exceptional weather conditions contributed to their removal processes. Compounds like sulfapyridine, sulfaquinoxaline in WWTP-IOA, olanzapine, cyclobenzaprine, risperidone and mefenamic acid, although their low concentrations and the sporadic occurrence are illustrated with intense change of color in the heatmaps, since the normalization of concentration values was exhibited by row.

(a) WWTP-IOA



(b) WWTP-AMA



(c) WWTP-HOS

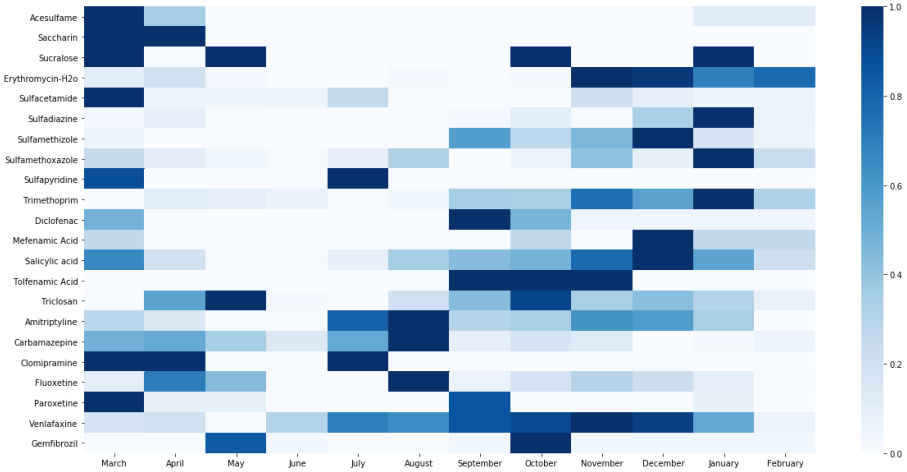


Figure 8.21. Heat map of ECs detected during the 1-year monitoring in (a) WWTP-IOA, (b) WWTP-AMA, (c) WWTP-HOS The color of each cell represents the concentration intensity (abundance of each analyte) (ng/L). Concentrations of individual compound were normalized (by row). The color gradient represents the relative concentration from the lowest (0.0) to highest (1.0).

8.5 Identification in real samples with Orbitrap-MS

Finally, the identification of detected target compounds was employed with data-dependent MS/MS by using the predominant advantage of Orbitrap mass spectrometry, of high mass accuracy both for precursor ion and fragment ion as well. The main process was based to the criteria for both screening and confirmatory analytical methods for pharmaceutical residues according to the identification points proposed by EU Commission Decision 2002/657/EC with combination of FDA guidelines that takes full advantage of the capabilities of modern HRMS instruments [814], [576] and also the last update exploring the means of identification of small molecules [576]. An example of identification with MS/MS fragmentation is illustrated in Figure 8.22 for carbamazepine compound detected in August at WWTP of Amaliada. The full scan MS spectrum of the chromatographic peak detected at 4.57 min (on the top of Figure 8.22), showed an abundant signal at m/z 237.1023 which corresponds within 0.255 ppm to the theoretical exact mass of carbamazepine. Additional MS/MS data shows two intense fragment ions which correspond to protonated molecules of $C_{14}H_{12}N^+$ (194.096 m/z) and $C_{14}H_{10}N^+$ (192.0810 m/z) with mass errors in relation to exact mass below 5 ppm in both cases. High mass accuracy results for precursor ion as well as for its fragments confirm the presence of carbamazepine in real effluent water

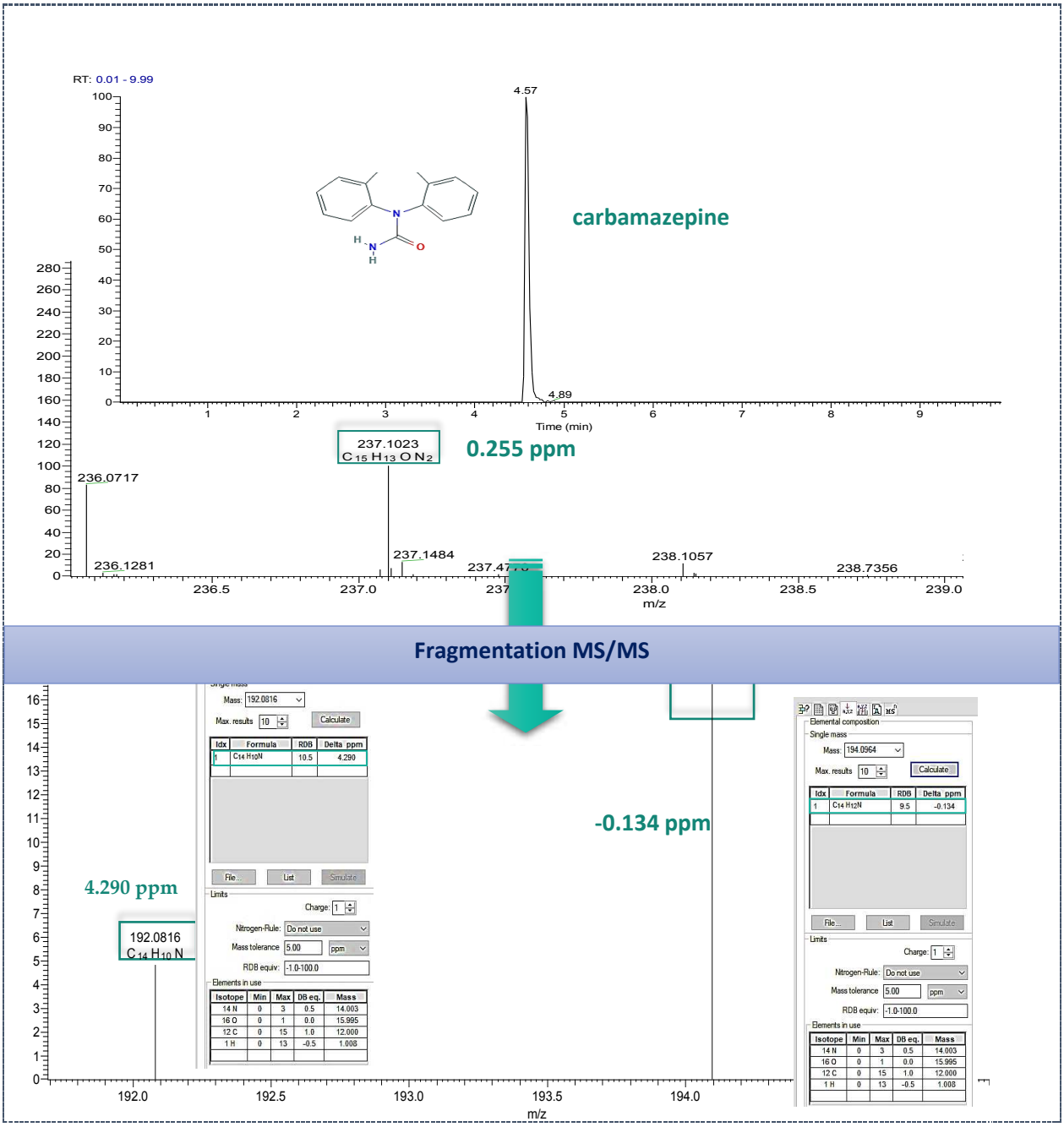


Figure 8.22. Identification of carbamazepine in effluent wastewater by performing UHPLC–LTQ Orbitrap tandem mass spectrometry fragmentation

CHAPTER 9: CONCLUSIONS

Conclusions

A considerable number of emerging contaminants (ECs) resulting mainly from anthropogenic activities, is present in the aquatic environment. These contaminants are not commonly monitored but have the potential to enter the environment and cause adverse ecological and human health effects. The increasing use of chemicals and resulting chemicalization of the environment is a global environmental problem that poses new challenges for analytical laboratories. This doctorate research tried to respond to those challenges by addressing to novel analytical strategies in terms of sample preparation and analytical techniques for their accurate determination. Based on our knowledge for the transport of these contaminants in the aqueous environment, wastewater treatment plants consisted the main route of their release. On account to that a wide range of emerging contaminants including pharmaceutical active compounds, artificial sweeteners and personal care products was selected considering consumption patterns, prescription rates as well as detection frequency. A monitoring survey in selected WWTPs addresses the objective of providing relevant and representative data on the organic contamination of ECs and also attempts to investigate the influence of some anthropogenic practices.

Specifically, in this research a sensitive chromatographic technique using a UHPLC-LTQ-Orbitrap HRMS was developed and optimized for the simultaneous analysis of 33 emerging contaminants belonging to the class of pharmaceutical active compounds and to artificial sweeteners. Chromatographic conditions and parameters affecting signal response, resolution, mass accuracy was evaluated in positive and negative ionization mode to obtain the most suitable performance characteristics of UHPLC-LTQ-Orbitrap. Mass errors below 5 ppm were performed in all the assayed compounds while low IQLs up to 0.06 μg/L were accomplished.

Magnetic nanoparticles were successfully synthesized and characterized with XRD, SEM, FT-IR providing the desired morphological and structural characteristics for their applications in Magnetic Solid Phase Extraction. Magnetic reduced graphene oxide (mrGO) MNPs and C18 silica-based MNPs consisted the sorbents for the magnetic solid phase extraction of 19 and 16 emerging

contaminants respectively. Optimization of parameters affecting the extraction processes such as the pH, the amount of the MNPs, desorption solvent and time were assessed for both magnetic materials. The optimized MSPEs were individually validated for the analysis of wastewater effluent sample . The analytical characteristics were excellent including linearity with coefficients of determination (R^2) greater than 0.99, high repeatability and reproducibility and recoveries ranged from 58.4% to 102.6% for both MSPEs .MSPEs employed with C18 and mrGO MNPs provided high sensitivity with minimum MDLs and MQLs up to 0.6 and 1.8ng/L, respectively. In addition, the method was also validated for tap water and displayed excellent analytical characteristics as well, suggesting the magnetic solid-phase extraction for further application in other environmental matrices with similar physicochemical characteristics. Additional evidence that advocates to this perspective is the findings of the matrix effect evaluation. Medium and low matrix effects in general, indicate the suitability of the sorbent materials C18 and mrGO MNPs in the sample pretreatment and the efficiency of the method to eliminate sample matrix of complex samples like wastewater.

The extraction capabilities of silica and graphene based MNPs employed in MSPE in combination with UHPLC-LTQ-Orbitrap MS provided a fast (15-20min extraction) sensitive, effective and green, compared with previous analytical methods with excessive use of organic solvents. For this reason, this doctoral research proposes the MSPE as sample pretreatment technique, for the enhancement of polar and non-polar emerging contaminants lying to its simplicity, flexibility, non-time consuming. Multiple steps including centrifugation and/or filtration are eliminated since the separation of the materials with analytes adsorbed on the surface is easily achieved by using an external magnetic field. Finally, the advantage of reusability of MNPs employed in MSPE, minimizes the cost of analysis making the MSPE not only appealing for laboratory use but also in line with environmental demands.

Despite the ‘’greener’’ approach of MSPE in analytical methods, remains a method that does not coincide precisely with the Principles of Green Analytical Chemistry. Even though irone oxides, the core of magnetic nanoparticles are not toxic, still modification with some functional groups can introduce the use of toxic reagents (such as hydrazine). For this reason, a new generation technique that could effectively address most of the shortcomings already mentioned, was developed.

A novel FPSE protocol has been developed and optimized to follow the green analytical chemistry demands for the extraction and determination of 21 selected emerging contaminants in

wastewater and tap water. FPSE was combined with UHPLC-MS-Orbitrap to take advantage the utilities of the new sample pretreatment for the enrichment of the analytes of interest and the powerful mass analyzer of Orbitrap. This is the first time (according to our knowledge) that a new generation technique is combined with the advanced technology of Orbitrap MS. Fabric medium consisted of a fiber glass substrate and a sol-gel coating of PEG, was synthesized, characterized, and employed as an FPSE device. Parameters affecting extraction efficiency and desorption performance such as sample volume and pH, extraction time, ionic strength as well as elution solvent and desorption volume were optimized. Under optimized conditions validation assays were followed for two aqueous matrices, tap water and effluent wastewater. FPSE exhibited excellent analytical characteristics in terms of linearity, intra-day, and inter-day precision for both tap water and effluent. It is noteworthy to mention that the recoveries of the method expressing the accuracy were remarkably high (>83%) for all the compounds. MDLs and MQLs were satisfactory, taking into consideration the low preconcentration factor of FPSE, with maxim values up to 105.9 ng/L and 317.8 ng/L for tap water, respectively, while in effluent water the corresponding MDLs and MQLs ranged from 3.1-149.4 ng/L and 9.3-447.7 ng/L. As regards the capability of the FPSE to eliminate interferences from the matrix, medium matrix effects occurred for effluent water for most of the analytes with only two compounds displaying signal enhancement. Taking into consideration the complexity of wastewater and the employment of ESI ionization in the applied analytical technique, FPSE managed to reduce matrix interferences. To emphasize this accomplishment, matrix effects have been reduced without involving additional washing steps in the procedure and with acidic sample loadings. All these achievements render the FPSE as a useful sample preparation technology, which can satisfy the demands of modern analytical laboratories thanks to its numerous positive attributes: (a) simplicity, minimum consumption of solvents, low cost; (b) flexibility in the selection of organic solvents that can be used as eluent (c) minimization of sample preparation steps, reducing the time of sample pretreatment and the potential sources of errors; (d) a variety of effective sol–gel coatings can be employed as sorbent; (e) high chemical resistance of the FPSE media thanks to a strong chemical bonding between the sorbent phase and the substrate.

The high selectivity of FPSE is one of the main advantages of the method, nevertheless at the same time this technique introduces a limitation on the number of analytes during multi-residues determination methods. This results in a restricted use for monitoring surveys where many contaminants with different physicochemical properties has to be detected. This shortcoming along with the relatively low preconcentration factor requires the employment of a reference

method such as Solid-Phase Extraction (SPE) for the investigation of the occurrence of the target ECs in the aqueous environment.

The developed multiresidue method, based on SPE followed by UHPL-LTQ-Orbitrap MS analysis, allows simultaneous extraction and pre-concentration of 33 emerging contaminants including pharmaceutical active compounds and artificial sweeteners with a variety of structures and physicochemical properties, allowing satisfactory recoveries above 55%. Although there is evidence of matrix effect in most of the compounds, the range of signal suppression in wastewater samples did not exceed the accepted level indicating the efficiency of Solid Phase Extraction as pretreatment technique. The SPE-UHPLC-Orbitrap-MS methodology presented high repeatability and reproducibility with excellent linearity. High preconcentration factor along with calibration with internal standard for a group of contaminants provided low detection and quantification limits up to 0.3 ng/L and 1.0 ng/L despite the low recoveries for specific compounds. Simultaneous analysis of multi-class compounds with quite different physicochemical characteristics often imposes compromises between the performance parameters, in case of sample pretreatment (cartridge selection, elution solvent, pH) or analytical part (chromatographic condition and MS detection). Despite the drawbacks of SPE including : time-consuming, multi-step technique, higher volumes of solvents compared to MSPE and FPSE, higher cost (one-use cartridge) etc., high loading of samples etc., remains the mainstream technique for the multi-residue determination methods. High enrichment factors, elimination of matrix effects and generic cartridges offered the desired analytical performance for trace analysis even at low concentrations(ng/L), that is particularly required in environmental analysis. For this reason, in consistence with other relative studies, SPE was the technique of choice for the conduction of one-year monitoring study in municipal and hospital WWTPs.

Moreover, an one-year monitoring survey was carried out in a small and bigger municipal wastewater treatment plant of Amaliada and Ioannina (WWTP-AMA,WWTP-IOA) respectively, as well as in the University Hospital of Ioannina city (WWTP-HOS). A detailed investigation about the concentration occurrence, the frequency detection, the temporal and seasonal variations was overviewed and compared with relevant studies.

Consumption patterns and explanations about the occurrence of emerging contaminants were estimated.

Overall, considering all sampling campaigns, 28 different ECs out of the 33 monitored compounds, were detected in at least one effluent wastewater of all WWTPs even at concentrations <MQL,

but only 17 were present in all of them. It is noteworthy that salicylic acid, triclosan, trimethoprim, venlafaxine, carbamazepine, amitriptyline were the most ubiquitous compounds since they were detected in >90% of the water samples. The most abundant compounds in terms of mean concentrations were acesulfame (4862.5 ng/L), sucralose (993.8 ng/L) and salicylic acid (981.8 ng/L) throughout the three monitoring campaigns. Concerning therapeutic groups, the frequency of detection throughout all sampling sites followed the order psychiatrics/antidepressants, antibiotics, NSAIDs-analgesics, artificial sweeteners, disinfectants, and lipid regulators.

Comparing the frequency of investigated compounds regarding their therapeutic group all over the WWTPs investigated, a similar trend is noticed for three therapeutic groups of NSAIDs, disinfectants and lipid regulators. A differentiation among the three WWTPs is denoted concerning the frequency of antibiotics and artificial sweeteners. In Amaliada and Ioannina city the percentage of positive findings of artificial sweeteners in the corresponding WWTPs, is significantly higher compared to WWTP of University Hospital. On the other hand, hospital WWTPs are differentiated from municipal plants with the maximum detection frequency of antibiotic class occurred in hospital WWTP simultaneously with the minimum detection frequency of artificial sweeteners of all sampling sites.

Occurrence of target compounds in terms of concentration levels, follows a rather broad range of variability depending on the sampling station, the class of contaminants and the sampling month. Generally, the overall range of mean concentrations was from MQL levels to 4862.5 ng/L and the highest individual concentration was detected for acesulfame at WWTP-IOA (13569.0 ng/L), while the lowest for carbamazepine (2.5ng/L) at WWTP-AMA.

The most abundant therapeutic group in terms of concentration level was artificial sweeteners with highest mean concentration for acesulfame at 4862.5 ng/L following by NSAIDs and antipsychotic drugs. Generally, variations in ECs concentrations between the sampling effluents of the three WWTPs were observed. Comparing the two municipal WWTPs of Amaliada and Ioannina, possible different consumption patterns may occur between the two investigated regions, resulting in different inlet concentrations. Differences in the results can be explained by differences in pharmaceutical or artificial sweeteners consumption among different area, prescription, sampling strategies and season, type and size of WWTP, wastewater flow rate, hospital size and type of medical cares as well as cultural and geographic factors.

Our findings were compared in terms of seasonal fluctuations in the observed concentrations of ECs in analyzed water samples A visually discernible trend shows that the concentration levels of antibiotics are higher in winter compared to other seasons with a significant difference from 3-

fold to almost 10-fold. This trend occurs all sampling stations. Moreover, artificial sweeteners, display higher concentrations in summer period, whereas the lowest in colder months, in winter and autumn, respectively. Generally. the temporal and seasonal fluctuation depends on several parameters, such as geographical region, socio-economic factors, biodegradation processes, and climate conditions.

Finally, the identification of detected target compounds was employed with data-dependent MS/MS by using the predominant advantage of Orbitrap mass spectrometry, of high mass accuracy both for precursor ion and fragment ion as well. High mass accuracy results of <5ppm were exhibited for both precursor ion and its fragments as well, confirming the presence of target analytes in real effluent water.

Bibliography

1. United States Geological Survey - USGS Contaminants of Emerging Concern in the Environment Available online: https://toxics.usgs.gov/investigations/cec/index.php (accessed on Oct 17, 2020).
2. USEPA Emerging contaminants and federal facility contaminants of concern Available online: https://www.epa.gov/fedfac/emerging-contaminants-and-federal-facility-contaminants-concern (accessed on Oct 17, 2020).
3. CRC CARE *Development of guidance for contaminants of emerging concern*; 2014; ISBN 0008-543X (Print)n0008-543x.
4. NORMAN Emerging substances | NORMAN Available online: https://www.norman-network.net/?q=node/19 (accessed on Oct 17, 2020).
5. Lord, E.M. *DoD Instruction 4715.18 Emerging Chemicals (ECs) of Environmental Concern*;
6. British Geological Survey (BGS) Science Briefing: Emerging Contaminants in Groundwater. Available online: https://www2.bgs.ac.uk/groundwater/quality/emerging_contaminants.html (accessed on Oct 17, 2020).
7. Hernández-Maldonado, A.J.; Blaney, L. *Contaminants of emerging concern in water and wastewater: Advanced treatment processes*; 2019; ISBN 9780128135617.
8. Kostianoy, E.O.H.D.B.A.; Fabian, D.B.P.; Frank, H.F.H.; Hites, J.P.G.R.A.; Mackay, M.A.K.K.D.; Paasivirta, A.H.N.J.; Safe, H.P.S.H.; Wangersky, P.J. *The Handbook of Environmental Chemistry Part J Advisory Board* .; 2008; Vol. 5; ISBN 9783540002789.
9. Stuart, M.E., Manamsa K., Talbot, J.C., Crane, E.J. *Emerging contaminants in groundwater*; 2011;
10. Lapworth, D.J.; Baran, N.; Stuart, M.E.; Ward, R.S. Emerging organic contaminants in groundwater: A review of sources, fate and occurrence. *Environ. Pollut.* 2012, *163*, 287–303.
11. Bueno, M.J.M.; Gomez, M.J.; Herrera, S.; Hernando, M.D.; Agüera, A.; Fernández-Alba, A.R. Occurrence and persistence of organic emerging contaminants and priority pollutants in five sewage treatment plants of Spain: Two years pilot survey monitoring. *Environ. Pollut.* **2012**, *164*, 267–273, doi:10.1016/j.envpol.2012.01.038.
12. Murray, K.E.; Thomas, S.M.; Bodour, A.A. Prioritizing research for trace pollutants and emerging contaminants in the freshwater environment. *Environ. Pollut.* 2010, *158*, 3462–3471.
13. Tankiewicz, M.; Fenik, J.; Biziuk, M. Determination of organophosphorus and organonitrogen pesticides in water samples. *TrAC Trends Anal. Chem.* **2010**, *29*, 1050–1063, doi:10.1016/J.TRAC.2010.05.008.
14. Resources, W. Contaminants of Emerging Concern in Water and Wastewater. *Contam. Emerg. Concern Water Wastewater* **2020**, doi:10.1016/c2016-0-05074-x.
15. US EPA, O. Endocrine Disruption.
16. Milmo, S. *Pharmaceuticals in the environment*; 2018; Vol. 42; ISBN 9783540746638.
17. Rivera-Utrilla, J.; Sánchez-Polo, M.; Ferro-García, M.Á.; Prados-Joya, G.; Ocampo-Pérez, R. Pharmaceuticals as emerging contaminants and their removal from water. A review. *Chemosphere* 2013, *93*, 1268–1287.
18. Calvo-flores, F.G. Therapeutic Classes of PCs in the Environment. *Emerg. Pollut.* **2017**, 103–166, doi:10.1002/9783527691203.ch5.
19. Calvo-flores, F.G. Overview of Pharmaceutical Products as Emerging Pollutants. *Emerg. Pollut.* **2017**, 57–101, doi:10.1002/9783527691203.ch4.
20. Patel, M.; Kumar, R.; Kishor, K.; Mlsna, T.; Pittman, C.U.; Mohan, D. Pharmaceuticals of emerging concern in aquatic systems: Chemistry, occurrence, effects, and removal methods. *Chem. Rev.* **2019**, *119*, 3510–3673, doi:10.1021/acs.chemrev.8b00299.
21. Gogoi, A.; Mazumder, P.; Tyagi, V.K.; Tushara Chaminda, G.G.; An, A.K.; Kumar, M. Occurrence and fate of emerging contaminants in water environment: A review. *Groundw. Sustain. Dev.* 2018, *6*, 169–180.
22. World Health Organization *Promoting Rational Use of Medicines*; 2006; Vol. 183;.
23. Lei, M.; Zhang, L.; Lei, J.; Zong, L.; Li, J.; Wu, Z.; Wang, Z. Overview of emerging contaminants and associated human health effects. *Biomed Res. Int.* **2015**, *2015*, doi:10.1155/2015/404796.
24. Data, K. The Pharmaceutical Industry in Figures The Pharmaceutical Industry : A Key Asset to Scientific and Medical Progress. *Eur. Fed. Pharm. Ind. Assoc.* **2016**.
25. IOBE *The Pharmaceutical Market in Greece. Facts and Figures 2019*; 2019;
26. ATCC WHOCC - ATC/DDD Index Available online: https://www.whocc.no/atc_ddd_index/ (accessed on Oct 31, 2020).
27. ESAC-Net Survillance data Data source overview of antimicrobial consumption Data source overview of antimicrobial consumption. **2016**, *12*, 1–4.

28. Pérez, S.; Barceló, D. Application of advanced MS techniques to analysis and identification of human and microbial metabolites of pharmaceuticals in the aquatic environment. *TrAC - Trends Anal. Chem.* **2007**, *26*, 494–514, doi:10.1016/j.trac.2007.05.004.
29. Webb, S.F. *A Data-based Perspective on the Environmental Risk Assessment of Human Pharmaceuticals I — Collation of Available Ecotoxicity Data*; 2004; ISBN 9783662092613.
30. Kosma, C.I.; Nannou, C.I.; Boti, V.I.; Albanis, T.A. Psychiatrics and selected metabolites in hospital and urban wastewaters: Occurrence, removal, mass loading, seasonal influence and risk assessment. *Sci. Total Environ.* **2019**, *659*, 1473–1483, doi:10.1016/j.scitotenv.2018.12.421.
31. García-Galán, M.J.; Díaz-Cruz, S.; Barceló, D. Multiresidue trace analysis of sulfonamide antibiotics and their metabolites in soils and sewage sludge by pressurized liquid extraction followed by liquid chromatography-electrospray-quadrupole linear ion trap mass spectrometry. *J. Chromatogr. A* **2013**, *1275*, 32–40, doi:10.1016/j.chroma.2012.12.004.
32. Verlicchi, P.; Al Aukidy, M.; Zambello, E. Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment-A review. *Sci. Total Environ.* 2012, *429*, 123–155.
33. Farré, M. la; Pérez, S.; Kantiani, L.; Barceló, D. Fate and toxicity of emerging pollutants, their metabolites and transformation products in the aquatic environment. *TrAC - Trends Anal. Chem.* **2008**, *27*, 991–1007, doi:10.1016/j.trac.2008.09.010.
34. Council, N.R. *A Framework to Guide Selection of Chemical Alternatives*; National Academies Press, 2014;
35. Arnold, K.E.; Brown, A.R.; Brown, A.R.; Ankley, G.T.; Sumpter, J.P. Medicating the environment: Assessing risks of pharmaceuticals to wildlife and ecosystems. *Philos. Trans. R. Soc. B Biol. Sci.* **2014**, *369*, doi:10.1098/rstb.2013.0569.
36. Brooks, B.W.; Berninger, J.P.; Ramirez, A.J.; Huggett, D.B. Perspectives on Human Pharmaceuticals in the Environment. In; Brooks, B.W., Huggett, D.B., Eds.; Emerging Topics in Ecotoxicology; Springer New York: New York, NY, 2012; Vol. 4, pp. 1–16 ISBN 978-1-4614-3419-1.
37. Kümmerer, K. The presence of pharmaceuticals in the environment due to human use - present knowledge and future challenges. *J. Environ. Manage.* 2009, *90*, 2354–2366.
38. Kodešová, R.; Grabic, R.; Kočárek, M.; Klement, A.; Golovko, O.; Fér, M.; Nikodem, A.; Jakšík, O. Pharmaceuticals' sorptions relative to properties of thirteen different soils. *Sci. Total Environ.* **2015**, *511*, 435–443, doi:10.1016/j.scitotenv.2014.12.088.
39. Palacios-Rosas, E.; Castro-Pastrana, L.I. Pharmaceuticals Reaching the Environment: Concepts, Evidence, and Concerns. In *Handbook of Environmental Chemistry*; Springer Verlag, 2019; Vol. 66, pp. 21–41.
40. Newman, M.C. *Fundamentals of Ecotoxicology*; CRC Press, 2014;
41. Larsson, D.G.J.; de Pedro, C.; Paxeus, N. Effluent from drug manufactures contains extremely high levels of pharmaceuticals. *J. Hazard. Mater.* **2007**, *148*, 751–755, doi:10.1016/j.jhazmat.2007.07.008.
42. Cardoso, O.; Porcher, J.M.; Sanchez, W. Factory-discharged pharmaceuticals could be a relevant source of aquatic environment contamination: Review of evidence and need for knowledge. *Chemosphere* **2014**, *115*, 20–30, doi:10.1016/j.chemosphere.2014.02.004.
43. Larsson, D.G.J. Pollution from drug manufacturing: Review and perspectives. *Philos. Trans. R. Soc. B Biol. Sci.* 2014, *369*.
44. Verlicchi, P.; Galletti, A.; Petrovic, M.; Barceló, D. Micro-pollutants in Hospital Effluent: Their Fate, Risk and Treatment Options. *Handb. Environ. Chem.* **2012**, *20*, 139–171, doi:10.1007/698_2011_134.
45. Moreno-González, R.; Rodriguez-Mozaz, S.; Gros, M.; Barceló, D.; León, V.M. Seasonal distribution of pharmaceuticals in marine water and sediment from a mediterranean coastal lagoon (SE Spain). *Environ. Res.* **2015**, *138*, 326–344, doi:10.1016/j.envres.2015.02.016.
46. H.Bell, C.; Gentile, M.; Kalve, E.; Ross, I.; Horst, J.; Suthersan, S. *Emerging Contaminants Handbook*; Taylor & Francis Group: Boca Raton, 2019; ISBN 9781138062948.
47. Tong, A.Y.C.; Peake, B.M.; Braund, R. Disposal practices for unused medications around the world. *Environ. Int.* 2011, *37*, 292–298.
48. European Comission *Directive 2004/27/EC on the Community code relating to medicinal products for human use*; 2004; Vol. 2003;.
49. Makki, M.; Hassali, M.A.; Awaisu, A.; Hashmi, F. The Prevalence of Unused Medications in Homes. *Pharmacy* **2019**, *7*, 61, doi:10.3390/pharmacy7020061.
50. Bound, J.P.; Voulvoulis, N. Household disposal of pharmaceuticals as a pathway for aquatic contamination in the United Kingdom. *Environ. Health Perspect.* **2005**, *113*, 1705–1711, doi:10.1289/ehp.8315.
51. Braund, R.; Peake, B.M.; Shieffelbien, L. Disposal practices for unused medications in New Zealand. *Environ. Int.* **2009**, *35*, 952–

955, doi:10.1016/j.envint.2009.04.003.

52. Albaroodi, K.A.I. Pharmacists' Knowledge Regarding Drug Disposal in Karbala. *Pharmacy* **2019**, *7*, 57, doi:10.3390/pharmacy7020057.
53. Alvarez-Cohen, L.; Sedlak, D.L. Emerging contaminants in water. *Environ. Eng. Sci.* **2003**, *20*, 387–388, doi:10.1089/109287503768335887.
54. Chonova, T.; Keck, F.; Labanowski, J.; Montuelle, B.; Rimet, F.; Bouchez, A. Separate treatment of hospital and urban wastewaters: A real scale comparison of effluents and their effect on microbial communities. *Sci. Total Environ.* **2016**, *542*, 965–975, doi:10.1016/j.scitotenv.2015.10.161.
55. Mullot, J.U.; Karolak, S.; Fontova, A.; Levi, Y. Modeling of hospital wastewater pollution by pharmaceuticals: First results of Mediflux study carried out in three French hospitals. *Water Sci. Technol.* **2010**, *62*, 2912–2919, doi:10.2166/wst.2010.986.
56. Santos, L.H.M.L.M.; Gros, M.; Rodriguez-Mozaz, S.; Delerue-Matos, C.; Pena, A.; Barceló, D.; Montenegro, M.C.B.S.M. Contribution of hospital effluents to the load of pharmaceuticals in urban wastewaters: Identification of ecologically relevant pharmaceuticals. *Sci. Total Environ.* **2013**, *461–462*, 302–316, doi:10.1016/j.scitotenv.2013.04.077.
57. Glassmeyer, S.T.; Hinchey, E.K.; Boehme, S.E.; Daughton, C.G.; Ruhoy, I.S.; Conerly, O.; Daniels, R.L.; Lauer, L.; McCarthy, M.; Nettesheim, T.G.; et al. Disposal practices for unwanted residential medications in the United States. *Environ. Int.* **2009**, *35*, 566–572, doi:10.1016/j.envint.2008.10.007.
58. OECD *Pharmaceutical Residues in Freshwater Hazards and Policy Responses*; 2019;
59. Rivera-Jaimes, J.A.; Postigo, C.; Melgoza-Alemán, R.M.; Aceña, J.; Barceló, D.; López de Alda, M. Study of pharmaceuticals in surface and wastewater from Cuernavaca, Morelos, Mexico: Occurrence and environmental risk assessment. *Sci. Total Environ.* **2018**, *613–614*, 1263–1274, doi:10.1016/j.scitotenv.2017.09.134.
60. Furuhagen, S.; Liewenborg, B.; Breitholtz, M.; Gorokhova, E. Feeding activity and xenobiotics modulate oxidative status in daphnia magna: Implications for ecotoxicological testing. *Environ. Sci. Technol.* **2014**, *48*, 12886–12892, doi:10.1021/es5044722.
61. Silva, B.; Neves, I.C. *SPRINGER BRIEFS IN MOLECULAR SCIENCE Psychiatric Pharmaceuticals as Emerging Contaminants in Wastewater*; ISBN 9783319204925.
62. Elizalde-Velázquez, A.; Martínez-Rodríguez, H.; Galar-Martínez, M.; Dublán-García, O.; Islas-Flores, H.; Rodríguez-Flores, J.; Castañeda-Peñalvo, G.; Lizcano-Sanz, I.; Gómez-Oliván, L.M. Effect of amoxicillin exposure on brain, gill, liver, and kidney of common carp (Cyprinus carpio): The role of amoxicilloic acid. *Environ. Toxicol.* **2017**, *32*, 1102–1120, doi:10.1002/tox.22307.
63. Schoenborn, A.; Kunz, P.; Koster, M. Estrogenic activity in drainage water: a field study on a Swiss cattle pasture. *Environ. Sci. Eur.* **2015**, *27*, 1–15, doi:10.1186/s12302-015-0047-4.
64. Besse, J.-P.; Garric, J. Progestagens for human use, exposure and hazard assessment for the aquatic environment. *Environ. Pollut.* **2009**, *157*, 3485–3494, doi:10.1016/j.envpol.2009.06.012.
65. Sanjuan-Reyes, N.; Gómez-Oliván, L.M.; Galar-Martínez, M.; Vieyra-Reyes, P.; García-Medina, S.; Islas-Flores, H.; Neri-Cruz, N. Effluent from an NSAID-manufacturing plant in Mexico induces oxidative stress on Cyprinus Carpio. *Water. Air. Soil Pollut.* **2013**, *224*, 1–14, doi:10.1007/s11270-013-1689-8.
66. Islas-Flores, H.; Gómez-Oliván, L.M.; Galar-Martínez, M.; Colín-Cruz, A.; Neri-Cruz, N.; García-Medina, S. Diclofenac-induced oxidative stress in brain, liver, gill and blood of common carp (Cyprinus carpio). *Ecotoxicol. Environ. Saf.* **2013**, *92*, 32–38, doi:10.1016/j.ecoenv.2013.01.025.
67. JD, C.-V.; H, I.-F.; N, S.-R.; EI, M.-C.; M, G.-M.; S, G.-M.; A, E.-V.; O, D.-G.; LM, G.-O. Comparative study of diclofenac-induced embryotoxicity and teratogenesis in Xenopus laevis and Lithobates catesbeianus, using the frog embryo teratogenesis assay: Xenopus (FETAX). *Sci. Total Environ.* **2017**, *574*, doi:10.1016/J.SCITOTENV.2016.09.095.
68. Cuthbert, R.J.; Taggart, M.A.; Prakash, V.; Chakraborty, S.S.; Deori, P.; Galligan, T.; Kulkarni, M.; Ranade, S.; Saini, M.; Sharma, A.K.; et al. Avian scavengers and the threat from veterinary pharmaceuticals. *Philos. Trans. R. Soc. B Biol. Sci.* **2014**, *369*, 20130574, doi:10.1098/rstb.2013.0574.
69. Hutchinson, T.H.; Madden, J.C.; Naidoo, V.; Walker, C.H. Comparative metabolism as a key driver of wildlife species sensitivity to human and veterinary pharmaceuticals. *Philos. Trans. R. Soc. B Biol. Sci.* 2014, *369*.
70. Harding, J. Medicare Part D enrollees' use of out-of-plan discounted generic drugs, revisited. *J. Am. Geriatr. Soc.* 2013, *61*, 309–310.

71. Montesinos, M.L. Roles for DSCAM and DSCAML1 in central nervous system development and disease. *Adv. Neurobiol.* 2014, *8*, 249–270.
72. Gómez-Oliván, L.M. *Ecopharmacovigilance Multidisciplinary Approaches to Environmental Safety of Medicines*; 2017; ISBN 9783319734750.
73. (Who), W.H.O. *Pharmaceuticals in Drinking-water Public Health and Environment Water, Sanitation, Hygiene and Health*;
74. Development, O. of R.& Active Pharmaceutical Ingredients and Aquatic Organisms.
75. Brooks, B.W.; Chambliss, C.K.; Stanley, J.K.; Ramirez, A.; Banks, K.E.; Johnson, R.D.; Lewis, R.J. Determination of select antidepressants in fish from an effluent-dominated stream. *Environ. Toxicol. Chem.* **2005**, *24*, 464–469, doi:10.1897/04-081R.1.
76. Chu, S.; Metcalfe, C.D. Analysis of paroxetine, fluoxetine and norfluoxetine in fish tissues using pressurized liquid extraction, mixed mode solid phase extraction cleanup and liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A* **2007**, *1163*, 112–118, doi:10.1016/j.chroma.2007.06.014.
77. Muir, D.; Simmons, D.; Wang, X.; Peart, T.; Villella, M.; Miller, J.; Sherry, J. Bioaccumulation of pharmaceuticals and personal care product chemicals in fish exposed to wastewater effluent in an urban wetland. *Sci. Rep.* **2017**, *7*, doi:10.1038/s41598-017-15462-x.
78. Sabri, N.A.; van Holst, S.; Schmitt, H.; van der Zaan, B.M.; Gerritsen, H.W.; Rijnaarts, H.H.M.; Langenhoff, A.A.M. Fate of antibiotics and antibiotic resistance genes during conventional and additional treatment technologies in wastewater treatment plants. *Sci. Total Environ.* **2020**, *741*, 140199, doi:10.1016/j.scitotenv.2020.140199.
79. Andersson, D.I.; Hughes, D. Evolution of antibiotic resistance at non-lethal drug concentrations. *Drug Resist. Updat.* **2012**, *15*, 162–172, doi:10.1016/j.drup.2012.03.005.
80. Manaia, C.M.; Vaz-Moreira, I.; Nunes, O.C. Antibiotic Resistance in Waste Water and Surface Water and Human Health Implications. *Handb. Environ. Chem.* **2012**, *20*, 173–212, doi:10.1007/698_2011_118.
81. Kraemer, S.A.; Ramachandran, A.; Perron, G.G. Antibiotic pollution in the environment: From microbial ecology to public policy. *Microorganisms* 2019, *7*.
82. Karkman, A.; Do, T.T.; Walsh, F.; Virta, M.P.J. Antibiotic-Resistance Genes in Waste Water. *Trends Microbiol.* 2018, *26*, 220–228.
83. Pazda, M.; Kumirska, J.; Stepnowski, P.; Mulkiewicz, E. Antibiotic resistance genes identified in wastewater treatment plant systems – A review. *Sci. Total Environ.* 2019, *697*.
84. Davies, J. Origins and evolution of antibiotic resistance. *Microbiologia* 1996, *12*, 9–16.
85. Ecdc *SURVEILLANCE REPORT. Surveillance of antimicrobial resistance in Europe 2018*; 2018;
86. Peñalva, G.; Högberg, L.D.; Weist, K.; Vlahović-Palčevski, V.; Heuer, O.; Monnet, D.L.; Vandael, E.; Ivanov, I.N.; Payerl-Pal, M.; Skjold Selle Pedersen, K.; et al. Decreasing and stabilising trends of antimicrobial consumption and resistance in Escherichia coli and Klebsiella pneumoniae in segmented regression analysis, European Union/European Economic Area, 2001 to 2018. *Eurosurveillance* **2019**, *24*, doi:10.2807/1560-7917.ES.2019.24.46.1900656.
87. Frieden, T. Antibiotic resistance threats in the United States. *Centers Dis. Control Prev.* **2013**, 114.
88. Davies, S.C.; Fowler, T.; Watson, J.; Livermore, D.M.; Walker, D. Annual report of the chief medical officer: Infection and the rise of antimicrobial resistance. *Lancet* 2013, *381*, 1606–1609.
89. Smith, J.M.; Feil, E.J.; Smith, N.H. Population structure and evolutionary dynamics of pathogenic bacteria. *BioEssays* 2000, *22*, 1115–1122.
90. ECDC ECDC/EMEA JOINT TECHNICAL REPORT The bacterial challenge: time to react Available online: https://www.ecdc.europa.eu/en/publications-data/ecdcemea-joint-technical-report-bacterial-challenge-time-react (accessed on Nov 4, 2020).
91. Chattopadhyay, S.; Raychaudhuri, U.; Chakraborty, R. Artificial sweeteners - A review. *J. Food Sci. Technol.* **2014**, *51*, 611–621, doi:10.1007/s13197-011-0571-1.
92. Ebrahimzadeh, V.; Ardalan, M.R.; Mahdavi, A.M.; Gorbani, A. A review of the health hazards of artificial sweeteners: Are they safe? *Prog. Nutr.* **2018**, *20*, 36–43, doi:10.23751/pn.v20i2-S.5901.
93. Kokotou, M.G.; Asimakopoulos, A.G.; Thomaidis, N.S. Artificial sweeteners as emerging pollutants in the environment: Analytical methodologies and environmental impact. *Anal. Methods* **2012**, *4*, 3057–3070, doi:10.1039/c2ay05950a.
94. Xie, L.; Jiang, R.; Zhu, F.; Liu, H.; Ouyang, G. Application of functionalized magnetic nanoparticles in sample preparation. *Anal.*

Bioanal. Chem. **2014**, *406*, 377–399, doi:10.1007/s00216-013-7302-6.

95. European Union 2008 Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. *Off. J. Eur. Union* **2008**, *51*, 16–33.
96. Scheurer, M.; Brauch, H.J.; Lange, F.T. Analysis and occurrence of seven artificial sweeteners in German waste water and surface water and in soil aquifer treatment (SAT). *Anal. Bioanal. Chem.* **2009**, *394*, 1585–1594, doi:10.1007/s00216-009-2881-y.
97. Lange, F.T.; Scheurer, M.; Brauch, H.J. Artificial sweeteners-A recently recognized class of emerging environmental contaminants: A review. *Anal. Bioanal. Chem.* 2012, *403*, 2503–2518.
98. Calvo-flores, F.G. Lifestyle Products as Emerging Pollutants. In *Emerging Pollutants*; 2017; pp. 233–264.
99. Petrovic, M. Methodological challenges of multi-residue analysis of pharmaceuticals in environmental samples. *Trends Environ. Anal. Chem.* 2014, *1*, e25–e33.
100. Buerge, I.J.; Keller, M.; Buser, H.R.; Müller, M.D.; Poiger, T. Saccharin and other artificial sweeteners in soils: Estimated inputs from agriculture and households, degradation, and leaching to groundwater. *Environ. Sci. Technol.* **2011**, *45*, 615–621, doi:10.1021/es1031272.
101. *Metabolic Pathways of Agrochemicals*; Royal Society of Chemistry, 2007;
102. Von Holst, C.; Bellorini, S.; Chedin, M.; D'arco, G.; De Smet, M.; Drooghmans, M.; Fernandez Orozco, R.; Gonzalez De La Huebra, M.J.; Kaklamanos, G.; Keltti, J.; et al. *Activity Report 2013 2 0 1 4*; 2014;
103. Houtman, C.J. Emerging contaminants in surface waters and their relevance for the production of drinking water in Europe. *J. Integr. Environ. Sci.* **2010**, *7*, 271–295, doi:10.1080/1943815X.2010.511648.
104. Perkola, N.; Sainio, P. Quantification of four artificial sweeteners in Finnish surface waters with isotope-dilution mass spectrometry. *Environ. Pollut.* **2014**, *184*, 391–396, doi:10.1016/j.envpol.2013.09.017.
105. Klára Kobetičová, Klára Anna Mocová, Lucie Mrhálková, Z.F.; Kočí, and V. Artificial Sweeteners and the Environment. *Czech J. Food Sci.* **2016**, *34*, 149–153, doi:10.1021/acs.est.6b00706.
106. Belton, K.; Schaefer, E.; Guiney, P.D. A Review of the Environmental Fate and Effects of Acesulfame-Potassium. *Integr. Environ. Assess. Manag.* **2020**, *16*, 421–437, doi:10.1002/ieam.4248.
107. Subedi, B.; Lee, S.; Moon, H.B.; Kannan, K. Emission of artificial sweeteners, select pharmaceuticals, and personal care products through sewage sludge from wastewater treatment plants in Korea. *Environ. Int.* **2014**, *68*, 33–40, doi:10.1016/j.envint.2014.03.006.
108. Rahn, A.; Yaylayan, V.A. Thermal degradation of sucralose and its potential in generating chloropropanols in the presence of glycerol. *Food Chem.* **2010**, *118*, 56–61, doi:10.1016/j.foodchem.2009.04.133.
109. Oppenheimer, J.; Eaton, A.; Badruzzaman, M.; Haghani, A.W.; Jacangelo, J.G. Occurrence and suitability of sucralose as an indicator compound of wastewater loading to surface waters in urbanized regions. *Water Res.* **2011**, *45*, 4019–4027, doi:10.1016/j.watres.2011.05.014.
110. Van Stempvoort, D.R.; Roy, J.W.; Grabuski, J.; Brown, S.J.; Bickerton, G.; Sverko, E. An artificial sweetener and pharmaceutical compounds as co-tracers of urban wastewater in groundwater. *Sci. Total Environ.* **2013**, *461–462*, 348–3359, doi:10.1016/j.scitotenv.2013.05.001.
111. Scheurer, M.; Storck, F.R.; Graf, C.; Brauch, H.J.; Ruck, W.; Lev, O.; Lange, F.T. Correlation of six anthropogenic markers in wastewater, surface water, bank filtrate, and soil aquifer treatment. *J. Environ. Monit.* **2011**, *13*, 966–973, doi:10.1039/c0em00701c.
112. Buerge, I.J.; Buser, H.R.; Kahle, M.; Müller, M.D.; Poiger, T. Ubiquitous occurrence of the artificial sweetener acesulfame in the aquatic environment: An ideal chemical marker of domestic wastewater in groundwater. *Environ. Sci. Technol.* **2009**, *43*, 4381–4385, doi:10.1021/es900126x.
113. Nakada, N.; Kiri, K.; Shinohara, H.; Harada, A.; Kuroda, K.; Takizawa, S.; Takada, H. Evaluation of pharmaceuticals and personal care products as water-soluble molecular markers of sewage. *Environ. Sci. Technol.* **2008**, *42*, 6347–6353, doi:10.1021/es7030856.
114. Buerge, I.J.; Kahle, M.; Buser, H.R.; Müller, M.D.; Poiger, T. Nicotine derivatives in wastewater and surface waters: Application as chemical markers for domestic wastewater. *Environ. Sci. Technol.* **2008**, *42*, 6354–6360, doi:10.1021/es800455q.
115. Buerge, I.J.; Poiger, T.; Müller, M.D.; Buser, H.R. Caffeine, an anthropogenic marker for wastewater contamination of surface waters. *Environ. Sci. Technol.* **2003**, *37*, 691–700, doi:10.1021/es020125z.

116. Clara, M.; Strenn, B.; Kreuzinger, N. Carbamazepine as a possible anthropogenic marker in the aquatic environment: Investigations on the behaviour of Carbamazepine in wastewater treatment and during groundwater infiltration. *Water Res.* **2004**, *38*, 947–954, doi:10.1016/j.watres.2003.10.058.

117. The European Parlament and the Council of the European Union Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. *Off. J. Eur. Parliam.* **2000**, *L327*, 1–82, doi:10.1039/ap9842100196.

118. European Comission Directive 2008/105/EC of the European Parliament and the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC. *Off. J. Eur. Union* **2008**.

119. European Comission *Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 - Amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy*; 2013; Vol. L 226;

120. European Comission Commission Implementing Decision (EU) 2015/495 of 20 March 2015 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council. *Off. J. Eur. Union* **2015**, *L78/40*, 20–30, doi:http://eur-lex.europa.eu/pri/en/oj/dat/2003/l_285/l_28520031101en00330037.pdf.

121. European Commission Commission Implementing Decision (EU) 2018/840 of 5 June 2018 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council and repealing Comm. *Off. J. Eur. Parliam.* **2018**, *141*, 9–12.

122. Official Journal of the European Communities Directive 91/271/EEC concerning urban waste wate treatment. *Off. J. Eur. Communities* 1991, *21*, 196.

123. European Comission Directive 2001/82/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to veterinary medicinal products. *Off. J. Eur. Communities* **2001**.

124. European Commission *Directive 2001/83/EC of The European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use*; 2001;

125. US EPA, O. 2012 Recreational Water Quality Criteria.

126. De Voogt, P.; Janex-Habibi, M.L.; Sacher, F.; Puijker, L.; Mons, M. Development of a common priority list of pharmaceuticals relevant for the water cycle. *Water Sci. Technol.* **2009**, *59*, 39–46, doi:10.2166/wst.2009.764.

127. Richardson, S.D.; Kimura, S.Y. Water Analysis: Emerging Contaminants and Current Issues. *Anal. Chem.* **2016**, *88*, 546–582, doi:10.1021/acs.analchem.5b04493.

128. World Health Organization World Health Organization - Food additives Available online: https://www.who.int/news-room/fact-sheets/detail/food-additives (accessed on Nov 5, 2020).

129. Amchova, P.; Kotolova, H.; Ruda-Kucerova, J. Health safety issues of synthetic food colorants. *Regul. Toxicol. Pharmacol.* **2015**, *73*, 914–922, doi:10.1016/j.yrtph.2015.09.026.

130. Cheeseman, M. *Global Regulation of Food Additives*; 2014;

131. Paíga, P.; Lolić, A.; Hellebuyck, F.; Santos, L.H.M.L.M.; Correia, M.; Delerue-Matos, C. Development of a SPE-UHPLC-MS/MS methodology for the determination of non-steroidal anti-inflammatory and analgesic pharmaceuticals in seawater. *J. Pharm. Biomed. Anal.* **2015**, *106*, 61–70, doi:10.1016/j.jpba.2014.06.017.

132. Edokpayi, J.N.; Odiyo, J.O.; Durowoju, O.S. Impact of Wastewater on Surface Water Quality in Developing Countries: A Case Study of South Africa. In *Water Quality*; InTech, 2017.

133. Paíga, P.; Correia, M.; Fernandes, M.J.; Silva, A.; Carvalho, M.; Vieira, J.; Jorge, S.; Silva, J.G.; Freire, C.; Delerue-Matos, C. Assessment of 83 pharmaceuticals in WWTP influent and effluent samples by UHPLC-MS/MS: Hourly variation. *Sci. Total Environ.* **2019**, *648*, 582–600, doi:10.1016/j.scitotenv.2018.08.129.

134. Radjenovic, J.; Petrovic, M.; Barceló, D. Analysis of pharmaceuticals in wastewater and removal using a membrane bioreactor. In Proceedings of the Analytical and Bioanalytical Chemistry; 2007; Vol. 387, pp. 1365–1377.

135. Townsend, R. Mass spectrometric investigation of pharmaceuticals in environmental matrices : homogenate analysis, Swansea, 2019.

136. Verlicchi, P.; Galletti, A.; Petrovic, M.; BarcelÓ, D. Hospital effluents as a source of emerging pollutants: An overview of

micropollutants and sustainable treatment options. *J. Hydrol.* 2010, *389*, 416–428.

137. Orias, F.; Perrodin, Y. Characterisation of the ecotoxicity of hospital effluents: A review. *Sci. Total Environ.* 2013, *454–455*, 250–276.
138. Chonova, T.; Labanowski, J.; Bouchez, A. Contribution of hospital effluents to the load of micropollutants in WWTP influents. In *Handbook of Environmental Chemistry*; Springer Verlag, 2018; Vol. 60, pp. 135–152.
139. Carraro, E.; Bonetta, S.; Bertino, C.; Lorenzi, E.; Bonetta, S.; Gilli, G. Hospital effluents management: Chemical, physical, microbiological risks and legislation in different countries. *J. Environ. Manage.* 2016, *168*, 185–199.
140. Verlicchi, P.; Al Aukidy, M.; Galletti, A.; Petrovic, M.; Barceló, D. Hospital effluent: Investigation of the concentrations and distribution of pharmaceuticals and environmental risk assessment. *Sci. Total Environ.* **2012**, *430*, 109–118, doi:10.1016/j.scitotenv.2012.04.055.
141. Verlicchi, P.; Aukidy, M. Al; Chalabi, S. Al *Hospital Wastewaters Characteristics, Management, Treatment and Environmental Risks*; 2018; ISBN 9783319621777.
142. Vieno, N.; Tuhkanen, T.; Kronberg, L. Elimination of pharmaceuticals in sewage treatment plants in Finland. *Water Res.* **2007**, *41*, 1001–1012, doi:10.1016/j.watres.2006.12.017.
143. Pauwels, B.; Noppe, ; H; De Brabander, ; H; Verstraete, W. Comparison of Steroid Hormone Concentrations in Domestic and Hospital Wastewater Treatment Plants., doi:10.1061/ASCE0733-93722008134:11933.
144. Yves Chartier, Jorge Emmanuel, Ute Pieper, Annette Prüss, Philip Rushbrook, Ruth Stringer, William Townend, S.W. and R.Z. WHO | Safe management of wastes from health-care activities. *WHO* **2019**.
145. Ebrahimi, S.M.; Reyhani, R.D.; Asghari-JafarAbadi, M.; Fathifar, Z. Diversity of antibiotics in hospital and municipal wastewaters and receiving water bodies and removal efficiency by treatment processes: A systematic review protocol. *Environ. Evid.* 2020, *9*, 19.
146. Kosma, C.I.; Kapsi, M.G.; Konstas, P.G.; Trantopoulos, E.P.; Boti, V.I.; Konstantinou, I.K.; Albanis, T.A. Assessment of multiclass pharmaceutical active compounds (PhACs) in hospital WWTP influent and effluent samples by UHPLC-Orbitrap MS : Temporal variation , removals and environmental risk assessment. *Environ. Res.* **2020**, *191*, 110152, doi:10.1016/j.envres.2020.110152.
147. Galán, M.J.G.; Díaz-Cruz, M.S.; Barceló, D. Sulfonamide Antibiotics in Natural and Treated Waters: Environmental and Human Health Risks. *Handb. Environ. Chem.* **2012**, *20*, 71–92, doi:10.1007/698_2011_129.
148. Pan, X.; Qiang, Z.; Ben, W.; Chen, M. Residual veterinary antibiotics in swine manure from concentrated animal feeding operations in Shandong Province, China. *Chemosphere* **2011**, *84*, 695–700, doi:10.1016/j.chemosphere.2011.03.022.
149. Ibrahim, M.; Ahmad, F.; Yaqub, B.; Ramzan, A.; Imran, A.; Afzaal, M.; Mirza, S.A.; Mazhar, I.; Younus, M.; Akram, Q.; et al. Current trends of antimicrobials used in food animals and aquaculture. In *Antibiotics and Antimicrobial Resistance Genes in the Environment*; Elsevier, 2020; pp. 39–69.
150. Lavanya, R. *Sulphonamides: A Pharmaceutical Review*; Online, 2017; Vol. 6;.
151. Maren, T.H. Relations Between Structure and Biological Activity of Sulfonamides. *Annu. Rev. Pharmacol. Toxicol.* **1976**, *16*, 309–327, doi:10.1146/annurev.pa.16.040176.001521.
152. Carta, F.; Scozzafava, A.; Supuran, C.T. Sulfonamides: A patent review (2008 2012). *Expert Opin. Ther. Pat.* 2012, *22*, 747–758.
153. Scozzafava, A.; Owa, T.; Mastrolorenzo, A.; Supuran, C. Anticancer and Antiviral Sulfonamides. *Curr. Med. Chem.* **2005**, *10*, 925–953, doi:10.2174/0929867033457647.
154. Jabusch, T.W.; Tjeerdema, R.S. Chemistry and fate of triazolopyrimidine sulfonamide herbicides. *Rev. Environ. Contam. Toxicol.* **2008**, *193*, 31–52, doi:10.1007/978-0-387-73163-6_2.
155. Chohan, Z.H.; Rauf, A.; Naseer, M.M.; Somra, M.A.; Supuran, C.T. Antibacterial, antifungal and cytotoxic properties of some sulfonamide-derived chromones. *J. Enzyme Inhib. Med. Chem.* **2006**, *21*, 173–177, doi:10.1080/14756360500533059.
156. Baran, W.; Adamek, E.; Ziemiańska, J.; Sobczak, A. Effects of the presence of sulfonamides in the environment and their influence on human health. *J. Hazard. Mater.* 2011, *196*, 1–15.
157. Summers, W.A. Antagonism of Sulfonamide Inhibition by Para-aminobenzoic Acid and Folic Acid in Toxoplasma Infected Mice. *Exp. Biol. Med.* **1947**, *66*, 509–511, doi:10.3181/00379727-66-16138.
158. Ahmad, I.; Ahmad, T.; Usmanghani, K. Sulfacetamide. *Anal. Profiles Drug Subst. Excipients* **1994**, *23*, 471–509, doi:10.1016/S0099-5428(08)60610-3.

159. Del Rosso, J.Q. The use of sodium sulfacetamide 10%-sulfur 5% emollient foam in the treatment of acne vulgaris. *J. Clin. Aesthet. Dermatol.* **2009**, *2*, 26–9.
160. Klaron Side Effects: Common, Severe, Long Term - Drugs.com Available online: https://www.drugs.com/sfx/klaron-side-effects.html (accessed on Oct 19, 2020).
161. Supuran, C.T. Special issue: Sulfonamides. *Molecules* 2017, *22*.
162. Sakharkar, M.K.; Rajamanickam, K.; Chandra, R.; Khan, H.A.; Alhomida, A.S.; Yang, J. Identification of novel drug targets in bovine respiratory disease: An essential step in applying biotechnologic techniques to develop more effective therapeutic treatments. *Drug Des. Devel. Ther.* **2018**, *12*, 1135–1146, doi:10.2147/DDDT.S163476.
163. Branion, H.D. The Merck Veterinary Manual. *Poult. Sci.* **1963**, *42*, 552–553, doi:10.3382/ps.0420552.
164. Sulfathiazole | DrugBank Online Available online: https://go.drugbank.com/drugs/DB06147 (accessed on Oct 23, 2020).
165. Ji, K.; Choi, K.; Lee, S.; Park, S.; Khim, J.S.; Jo, E.H.; Choi, K.; Zhang, X.; Giesy, J.P. Effects of sulfathiazole, oxytetracycline and chlortetracycline on steroidogenesis in the human adrenocarcinoma (H295R) cell line and freshwater fish Oryzias latipes. *J. Hazard. Mater.* **2010**, *182*, 494–502, doi:10.1016/j.jhazmat.2010.06.059.
166. Sulfapyridine | C11H11N3O2S - PubChem Available online: https://pubchem.ncbi.nlm.nih.gov/compound/Sulfapyridine#section=Fire-Hazards (accessed on Oct 23, 2020).
167. Pratt, J.; Hutchinson, J.; Klein Stevens, C.L. Sulfapyridine (polymorph III), sulfapyridine dioxane solvate, sulfapyridine tetra-hydro-furan solvate and sulfapyri-dine piperi-dine solvate, all at 173 K. *Acta Crystallogr. Sect. C Cryst. Struct. Commun.* **2011**, *67*, doi:10.1107/S0108270111041825.
168. Sulfapyridine | DrugBank Online Available online: https://go.drugbank.com/drugs/DB00891 (accessed on Oct 23, 2020).
169. Mabeck, C.E.; Vejlsgaard, R. Treatment of urinary tract infections in general practice with sulfamethizole, trimethoprim or co-trimazine (sulphadiazine-trimethoprim). *J. Antimicrob. Chemother.* **1980**, *6*, 701–708, doi:10.1093/jac/6.6.701.
170. Sulfamethizole | DrugBank Online.
171. Jørgensen, G. Effects of drugs on the liver. A review illustrated with 3 clinical cases. *Ugeskr. Laeger* **1994**, *156*, 155–161.
172. Sulfamethazine | DrugBank Online Available online: https://go.drugbank.com/drugs/DB01582 (accessed on Oct 23, 2020).
173. Friaza, V.; Morilla, R.; Respaldiza, N.; de la Horra, C.; Calderón, E.J. Pneumocystis jiroveci dihydropteroate synthase gene mutations among colonized individuals and Pneumocystis pneumonia patients from Spain. *Postgrad. Med.* **2010**, *122*, 24–28, doi:10.3810/pgm.2010.11.2219.
174. Thijssen, H.H.W. A simplified radioassay method for dihydropteroate synthetase activity in Escherichia coli and its application for an inhibition study of p-aminobenzoic acid derivatives. *Anal. Biochem.* **1973**, *53*, 579–585, doi:10.1016/0003-2697(73)90109-7.
175. Hong, Y.L.; Hossler, P.A.; Calhoun, D.H.; Meshnick, S.R. Inhibition of recombinant Pneumocystis carinii dihydropteroate synthetase by sulfa drugs. *Antimicrob. Agents Chemother.* **1995**, *39*, 1756–1763, doi:10.1128/AAC.39.8.1756.
176. WHO | JECFA Available online: https://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=3194 (accessed on Oct 23, 2020).
177. Sulfamethoxypyridazine | DrugBank Online Available online: https://go.drugbank.com/drugs/DB13773 (accessed on Oct 23, 2020).
178. Maichuk Yu., F.; Pozdnyakov, V.I.; Kuryazov, I.K.; Moldaver, B.L.; Rusak, A. V. Sodium sulfapyridazine as new bases prolonging its effect in treatment of bacterial conjunctivitis. *Antibiot. i Khimioterapiya* **1991**, *36*, 26–28.
179. Mehra, K.S.; Agrawal, N. Role of tromaril - A new anti-inflammatory drug in eye diseases. *Ann. Ophthalmol.* **1984**, *16*, 132–133.
180. Novelli, A.; Rosi, E. Pharmacological properties of oral antibiotics for the treatment of uncomplicated urinary tract infections. *J. Chemother.* **2017**, *29*, 10–18, doi:10.1080/1120009X.2017.1380357.
181. van der Ven, A.; Vree, T.; van Ewijk-Beneken Kolmer, E.; Koopmans, P.; van der Meer, J. Urinary recovery and kinetics of sulphamethoxazole and its metabolites in HIV-seropositive patients and healthy volunteers after a single oral dose of sulphamethoxazole. *Br. J. Clin. Pharmacol.* **1995**, *39*, 621–625, doi:10.1111/j.1365-2125.1995.tb05721.x.
182. Wright, S.W.; Wrenn, K.D.; Haynes, M. Lou Trimethoprim-sulfamethoxazole resistance among urinary coliform isolates. *J. Gen. Intern. Med.* **1999**, *14*, 606–609, doi:10.1046/j.1525-1497.1999.10128.x.
183. Guneysel, O.; Onur, O.; Erdede, M.; Denizbasi, A. Trimethoprim/Sulfamethoxazole Resistance in Urinary Tract Infections. *J. Emerg. Med.* **2009**, *36*, 338–341, doi:10.1016/j.jemermed.2007.08.068.

184. Chio, L.C.; Bolyard, L.A.; Nasr, M.; Queener, S.F. Identification of a class of sulfonamides highly active against dihydropteroate synthase from Toxoplasma gondii, Pneumocystis carinii, and Mycobacterium avium. *Antimicrob. Agents Chemother.* **1996**, *40*, 727–733, doi:10.1128/aac.40.3.727.

185. Aspen Pharmacare Canada Inc. *Health Canada Product Monograph: Septra (sulfamethoxazole/trimethoprim) for intravenous injection*; 2018;

186. Ribera, E.; Pou, L.; Fernandez-Sola, A.; Campos, F.; Lopez, R.M.; Ocaña, I.; Ruiz, I.; Pahissa, A. Rifampin reduces concentrations of trimethoprim and sulfamethoxazole in serum in human immunodeficiency virus-infected patients. *Antimicrob. Agents Chemother.* **2001**, *45*, 3238–3241, doi:10.1128/AAC.45.11.3238-3241.2001.

187. Heimpel, H.; Raghavachar, A. Hematological side effects of co-trimoxazole. *Infection* **1987**, *15*, S248–S253, doi:10.1007/BF01643198.

188. Ho, J.M.W.; Juurlink, D.N. Considerations when prescribing trimethoprim-sulfamethoxazole. *CMAJ* 2011, *183*, 1851–1858.

189. Harr, T.; French, L.E. Toxic epidermal necrolysis and Stevens-Johnson syndrome. *Orphanet J. Rare Dis.* 2010, *5*, 39.

190. Parajuli, P.; Ibrahim, A.M.; Siddiqui, H.H.; Lara Garcia, O.E.; Regmi, M.R. Trimethoprim-sulfamethoxazole Induced Pancytopenia: A Common Occurrence but A Rare Diagnosis. *Cureus* **2019**, *11*, doi:10.7759/cureus.5071.

191. FDA *FDA Approved Drug Products: Bactrim (sulfamethoxazole/trimethoprim) oral tablets*;

192. Sulfamethoxazole | DrugBank Online Available online: https://go.drugbank.com/drugs/DB01015 (accessed on Oct 23, 2020).

193. Preusch, P.C.; Hazelett, S.E.; Lemasters, K.K. Sulfaquinoxaline inhibition of vitamin K epoxide and quinone reductase. *Arch. Biochem. Biophys.* **1989**, *269*, 18–24, doi:10.1016/0003-9861(89)90082-9.

194. Righter, H.F.; Worthington, J.M.; Zimmerman, H.E.; Mercer, H.D. Tissue-residue depletion of sulfaquinoxaline in poultry. *Am. J. Vet. Res.* **1970**, *31*, 1051–1054.

195. FDA *FDA Approved Drug Products: Trimethoprim oral tablets*;

196. Rich, J.D.; Sullivan, T.; Greineder, D.; Kazanjian, P.H. Trimethoprim/sulfamethoxazole incremental dose regimen in human immunedeficiency virus-infected persons. *Ann. Allergy, Asthma Immunol.* **1997**, *79*, 409–414, doi:10.1016/S1081-1206(10)63034-7.

197. Trimethoprim | C14H18N4O3 - PubChem Available online: https://pubchem.ncbi.nlm.nih.gov/compound/Trimethoprim (accessed on Oct 25, 2020).

198. Pham, T.D.M.; Ziora, Z.M.; Blaskovich, M.A.T. Quinolone antibiotics. *Medchemcomm* 2019, *10*, 1719–1739.

199. Adjei, M.D.; Deck, J.; Heinze, T.M.; Freeman, J.P.; Williams, A.J.; Sutherland, J.B. Identification of metabolites produced from N-phenylpiperazine by Mycobacterium spp. *J. Ind. Microbiol. Biotechnol.* **2007**, *34*, 219–224, doi:10.1007/s10295-006-0189-x.

200. Anderson, V.; Osheroff, N. Type II Topoisomerases as Targets for Quinolone Antibacterials Turning Dr. Jekyll into Mr. Hyde. *Curr. Pharm. Des.* **2005**, *7*, 337–353, doi:10.2174/1381612013398013.

201. Sárközy, G. Quinolones: A class of antimicrobial agents. *Vet. Med. (Praha).* **2001**, *46*, 257–274, doi:10.17221/7883-vetmed.

202. Vázquez, M.M.P.; Vázquez, P.P.; Galera, M.M.; García, M.D.G. Determination of eight fluoroquinolones in groundwater samples with ultrasound-assisted ionic liquid dispersive liquid–liquid microextraction prior to high-performance liquid chromatography and fluorescence detection. *Anal. Chim. Acta* **2012**, *748*, 20–27, doi:10.1016/J.ACA.2012.08.042.

203. Soni, K. Fluoroquinolones: Chemistry & Action-A Review. *Indo Glob. J. Pharm. Sci.* **2012**, *2*, 43–53.

204. Touraki, M.; Ladoukakis, M.; Prokopiou, C. High-performance liquid chromatographic determination of oxolinic acid and flumequine in the live fish feed Artemia. *J. Chromatogr. B Biomed. Sci. Appl.* **2001**, *751*, 247–256, doi:10.1016/S0378-4347(00)00474-6.

205. Pouliquen, H.; Morvan, M.L. Determination of residues of oxolinic acid and flumequine in freeze-dried salmon muscle and skin by HPLC with fluorescence detection. *Food Addit. Contam.* **2002**, *19*, 223–231, doi:10.1080/02652030110072731.

206. Varaldo, P.E. The 'borderline methicillin-susceptible'staphylococcus aureus. *J. Antimicrob. Chemother.* **1993**, *31*, 1–4, doi:10.1093/jac/31.1.1.

207. Kanoh, S.; Rubin, B.K. Mechanisms of action and clinical application of macrolides as immunomodulatory medications. *Clin. Microbiol. Rev.* 2010, *23*, 590–615.

208. Alvarez-Elcoro, S.; Enzler, M.J. The macrolides: Erythromycin, clarithromycin, and azithromycin. In Proceedings of the Mayo Clinic Proceedings; Elsevier Ltd, 1999; Vol. 74, pp. 613–634.

209. Farzam, K.; Nessel, T.A.; Quick, J. *Erythromycin*; StatPearls Publishing, 2020;

210. Shaeer, K.M.; Chahine, E.B.; Varghese Gupta, S.; Cho, J.C. Macrolide Allergic Reactions. *Pharm. (Basel, Switzerland)* **2019**, *7*, 135, doi:10.3390/pharmacy7030135.

211. Carter, B.L.; Woodhead, J.C.; Cole, K.J.; Milavetz, G. Gastrointestinal side effects with erythromycin preparations. *Drug Intell. Clin. Pharm.* **1987**, *21*, 734–738, doi:10.1177/106002808702100914.

212. Voigt, A.M.; Zacharias, N.; Timm, C.; Wasser, F.; Sib, E.; Skutlarek, D.; Parcina, M.; Schmithausen, R.M.; Schwartz, T.; Hembach, N.; et al. Association between antibiotic residues, antibiotic resistant bacteria and antibiotic resistance genes in anthropogenic wastewater – An evaluation of clinical influences. *Chemosphere* **2020**, *241*, 125032, doi:10.1016/j.chemosphere.2019.125032.

213. Hoyer, D.; Zorrilla, E.P.; Cottone, P.; Parylak, S.; Morelli, M.; Simola, N.; Simola, N.; Morelli, M.; Lane, J.D.; Morgan, M.M.; et al. Classification of Psychoactive Drugs. In *Encyclopedia of Psychopharmacology*; Springer Berlin Heidelberg: Berlin, Heidelberg, 2010; pp. 293–302.

214. Mars, B.; Heron, J.; Kessler, D.; Davies, N.M.; Martin, R.M.; Thomas, K.H.; Gunnell, D. Influences on antidepressant prescribing trends in the UK: 1995–2011. *Soc. Psychiatry Psychiatr. Epidemiol.* **2017**, *52*, 193–200, doi:10.1007/s00127-016-1306-4.

215. Borova, V.L.; Maragou, N.C.; Gago-Ferrero, P.; Pistos, C.; Thomaidis, N.S. Highly sensitive determination of 68 psychoactive pharmaceuticals, illicit drugs, and related human metabolites in wastewater by liquid chromatography-tandem mass spectrometry. *Anal. Bioanal. Chem.* **2014**, *406*, 4273–4285, doi:10.1007/s00216-014-7819-3.

216. Silva, L.J.G.; Pereira, A.M.P.T.; Meisel, L.M.; Lino, C.M.; Pena, A. A one-year follow-up analysis of antidepressants in Portuguese wastewaters: Occurrence and fate, seasonal influence, and risk assessment. *Sci. Total Environ.* **2014**, *490*, 279–287, doi:10.1016/j.scitotenv.2014.04.131.

217. Thomaidis, N.S.; Gago-Ferrero, P.; Ort, C.; Maragou, N.C.; Alygizakis, N.A.; Borova, V.L.; Dasenaki, M.E. Reflection of Socioeconomic Changes in Wastewater: Licit and Illicit Drug Use Patterns. *Environ. Sci. Technol.* **2016**, *50*, 10065–10072, doi:10.1021/acs.est.6b02417.

218. OCDE *Health at a Glance 2019*; Health at a Glance; OECD, 2019;

219. Puri, B.; Treasaden, I. *Textbook of Psychiatry 3rd Edition*; Churchill Livingstone 2011, 2011; ISBN 978-0-7020-3157-1.

220. Khetan, S.K.; Collins, T.J. Human pharmaceuticals in the aquatic environment: A challenge to green chemisty. *Chem. Rev.* **2007**, *107*, 2319–2364, doi:10.1021/cr020441w.

221. Maher, A.R.; Theodore, G. Summary of the comparative effectiveness review on off-label use of atypical antipsychotics. *J. Manag. Care Pharm.* 2012, *18*.

222. FDA FDA Approved Drug Products: Zyprexa (olanzapine) tablets and powder. *Metab. Clin. Exp.* **2008**.

223. Olanzapine - PubMed Available online: https://pubmed.ncbi.nlm.nih.gov/30422498/ (accessed on Oct 25, 2020).

224. Yang, T.; Liu, Q.; Lu, M.; Ma, L.; Zhou, Y.; Cui, Y. Efficacy of olanzapine for the prophylaxis of chemotherapy-induced nausea and vomiting: a meta-analysis. *Br. J. Clin. Pharmacol.* **2017**, *83*, 1369–1379, doi:10.1111/bcp.13242.

225. Kemp, D.E.; Canan, F.; Goldstein, B.I.; McIntyre, R.S. Long-acting risperidone: A review of its role in the treatment of bipolar disorder. *Adv. Ther.* 2009, *26*, 588–599.

226. Kennedy, S.H.; Lam, R.W.; McIntyre, R.S.; Tourjman, S.V.; Bhat, V.; Blier, P.; Hasnain, M.; Jollant, F.; Levitt, A.J.; MacQueen, G.M.; et al. Canadian Network for Mood and Anxiety Treatments (CANMAT) 2016 clinical guidelines for the management of adults with major depressive disorder: Section 3. Pharmacological Treatments. *Can. J. Psychiatry* 2016, *61*, 540–560.

227. Risperidone: Uses, Dosage, Side Effects - Drugs.com Available online: https://www.drugs.com/risperidone.html (accessed on Oct 25, 2020).

228. Corena-McLeod, M. Comparative pharmacology of risperidone and paliperidone. *Drugs R D* 2015, *15*, 163–174.

229. Shelton, R.C. Serotonin and Norepinephrine Reuptake Inhibitors. In *Handbook of Experimental Pharmacology*; Springer New York LLC, 2019; Vol. 250, pp. 145–180.

230. Gallagher, H.C.; Gallagher, R.M.; Butler, M.; Buggy, D.J.; Henman, M.C. Venlafaxine for neuropathic pain in adults. *Cochrane Database Syst. Rev.* 2015, *2017*.

231. Handley, A.P.; Williams, M. The efficacy and tolerability of SSRI/SNRIs in the treatment of vasomotor symptoms in menopausal women: A systematic review. *J. Am. Assoc. Nurse Pract.* 2015, *27*, 54–61.

232. Venlafaxine Uses, Dosage & Side Effects - Drugs.com Available online: https://www.drugs.com/venlafaxine.html (accessed on Oct

25, 2020).

233. Venlafaxine | DrugBank Online Available online: https://go.drugbank.com/drugs/DB00285 (accessed on Oct 25, 2020).

234. Nevels, R.M.; Gontkovsky, S.T.; Williams, B.E. Paroxetine-The Antidepressant from Hell? Probably Not, But Caution Required. *Psychopharmacol. Bull.* **2016**, *46*, 77–104.

235. Paroxetine Uses, Dosage & Side Effects - Drugs.com Available online: https://www.drugs.com/paroxetine.html (accessed on Oct 25, 2020).

236. Zegarra, M. Cyclobenzaprine. In *The Essence of Analgesia and Analgesics*; Cambridge University Press, 2010; pp. 370–372 ISBN 9780511841378.

237. Calandre, E.P.; Rico-Villademoros, F.; Slim, M. An update on pharmacotherapy for the treatment of fibromyalgia. *Expert Opin. Pharmacother.* 2015, *16*, 1347–1368.

238. Amitriptyline: Uses, Dosage, Side Effects, Warnings Available online: https://www.drugs.com/amitriptyline.html (accessed on Oct 25, 2020).

239. Bryson, H.M.; Wilde, M.I. Amitriptyline. A review of its pharmacological properties and therapeutic use in chronic pain states. *Drugs and Aging* 1996, *8*, 459–476.

240. Lawson, K. A brief review of the pharmacology of amitriptyline and clinical outcomes in treating fibromyalgia. *Biomedicines* 2017, *5*.

241. Guaiana, G.; Barbui, C.; Hotopf, M. Amitriptyline versus other types of pharmacotherapy for depression. In *The Cochrane Database of Systematic Reviews*; John Wiley & Sons, Ltd, 2003.

242. Hisaoka, K.; Tsuchioka, M.; Yano, R.; Maeda, N.; Kajitani, N.; Morioka, N.; Nakata, Y.; Takebayashi, M. Tricyclic antidepressant amitriptyline activates fibroblast growth factor receptor signaling in glial cells: Involvement in glial cell line-derived neurotrophic factor production. *J. Biol. Chem.* **2011**, *286*, 21118–21128, doi:10.1074/jbc.M111.224683.

243. USP, A.H.T. Product monograph. *Toxicology* **2010**, 1–55.

244. Fluoxetine: Drug Uses, Dosage & Side Effects - Drugs.com Available online: https://www.drugs.com/fluoxetine.html (accessed on Oct 25, 2020).

245. Fluoxetine: MedlinePlus Drug Information Available online: https://medlineplus.gov/druginfo/meds/a689006.html (accessed on Oct 25, 2020).

246. Fluoxetine | DrugBank Online Available online: https://go.drugbank.com/drugs/DB00472 (accessed on Oct 25, 2020).

247. Chen, C.H.; Lin, S.K. Carbamazepine treatment of bipolar disorder: A retrospective evaluation of naturalistic long-term outcomes. *BMC Psychiatry* **2012**, *12*, doi:10.1186/1471-244X-12-47.

248. Barrons, R.; Roberts, N. The role of carbamazepine and oxcarbazepine in alcohol withdrawal syndrome. *J. Clin. Pharm. Ther.* 2010, *35*, 153–167.

249. Clomipramine | C19H23ClN2 - PubChem Available online: https://pubchem.ncbi.nlm.nih.gov/compound/Clomipramine (accessed on Oct 25, 2020).

250. Balant-Gorgia, A.E.; Gex-Fabry, M.; Balant, L.P. Clinical Pharmacokinetics of Clomipramine. *Clin. Pharmacokinet.* 1991, *20*, 447–462.

251. Carbamazepine | DrugBank Online Available online: https://go.drugbank.com/drugs/DB00564 (accessed on Oct 25, 2020).

252. Toušová, Z.; Vrana, B.; Smutná, M.; Novák, J.; Klučárová, V.; Grabic, R.; Slobodník, J.; Giesy, J.P.; Hilscherová, K. Analytical and bioanalytical assessments of organic micropollutants in the Bosna River using a combination of passive sampling, bioassays and multi-residue analysis. *Sci. Total Environ.* **2019**, *650*, 1599–1612, doi:10.1016/j.scitotenv.2018.08.336.

253. González-Alonso, S.; Merino, L.M.; Esteban, S.; López de Alda, M.; Barceló, D.; Durán, J.J.; López-Martínez, J.; Aceña, J.; Pérez, S.; Mastroianni, N.; et al. Occurrence of pharmaceutical, recreational and psychotropic drug residues in surface water on the northern Antarctic Peninsula region. *Environ. Pollut.* **2017**, *229*, 241–254, doi:10.1016/j.envpol.2017.05.060.

254. Gumbi, B.P.; Moodley, B.; Birungi, G.; Ndungu, P.G. Detection and quantification of acidic drug residues in South African surface water using gas chromatography-mass spectrometry. *Chemosphere* **2017**, *168*, 1042–1050, doi:10.1016/j.chemosphere.2016.10.105.

255. Wong, R.S.Y. Role of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) in Cancer Prevention and Cancer Promotion. *Adv. Pharmacol. Sci.* 2019, *2019*.

256. Tyumina, E.A.; Bazhutin, G.A.; Cartagena Gómez, A. d. P.; Ivshina, I.B. Nonsteroidal Anti-inflammatory Drugs as Emerging Contaminants. *Microbiol. (Russian Fed.* **2020**, *89*, 148–163, doi:10.1134/S0026261720020125.

257. Cleuvers, M. Mixture toxicity of the anti-inflammatory drugs diclofenac, ibuprofen, naproxen, and acetylsalicylic acid. *Ecotoxicol. Environ. Saf.* **2004**, *59*, 309–315, doi:10.1016/S0147-6513(03)00141-6.

258. Vieno, N.; Sillanpää, M. Fate of diclofenac in municipal wastewater treatment plant - A review. *Environ. Int.* 2014, *69*, 28–39.

259. Paíga, P.; Santos, L.H.M.L.M.; Delerue-Matos, C. Development of a multi-residue method for the determination of human and veterinary pharmaceuticals and some of their metabolites in aqueous environmental matrices by SPE-UHPLC–MS/MS. *J. Pharm. Biomed. Anal.* **2017**, *135*, 75–86, doi:10.1016/j.jpba.2016.12.013.

260. Papageorgiou, M.; Kosma, C.; Lambropoulou, D. Seasonal occurrence, removal, mass loading and environmental risk assessment of 55 pharmaceuticals and personal care products in a municipal wastewater treatment plant in Central Greece. *Sci. Total Environ.* **2016**, *543*, 547–569, doi:10.1016/j.scitotenv.2015.11.047.

261. Botero-Coy, A.M.; Martínez-Pachón, D.; Boix, C.; Rincón, R.J.; Castillo, N.; Arias-Marín, L.P.; Manrique-Losada, L.; Torres-Palma, R.; Moncayo-Lasso, A.; Hernández, F. An investigation into the occurrence and removal of pharmaceuticals in Colombian wastewater. *Sci. Total Environ.* **2018**, *642*, 842–853, doi:10.1016/j.scitotenv.2018.06.088.

262. Bashaar, M.; Thawani, V.; Hassali, M.A.; Saleem, F. Disposal practices of unused and expired pharmaceuticals among general public in Kabul. *BMC Public Health* **2017**, *17*, 45, doi:10.1186/s12889-016-3975-z.

263. Fatta-Kassinos, D.; Meric, S.; Nikolaou, A. Pharmaceutical residues in environmental waters and wastewater: Current state of knowledge and future research. *Anal. Bioanal. Chem.* **2011**, *399*, 251–275, doi:10.1007/s00216-010-4300-9.

264. Salicylic acid | DrugBank Online Available online: https://go.drugbank.com/drugs/DB00936 (accessed on Oct 25, 2020).

265. Indomethacin | DrugBank Online Available online: https://go.drugbank.com/drugs/DB00328 (accessed on Oct 25, 2020).

266. Nalamachu, S.; Wortmann, R. Role of indomethacin in acute pain and inflammation management: A review of the literature. *Postgrad. Med.* **2014**, *126*, 92–97, doi:10.3810/pgm.2014.07.2787.

267. Chen, L.; Yang, G.; Grosser, T. Prostanoids and inflammatory pain. In Proceedings of the Prostaglandins and Other Lipid Mediators; 2013; Vol. 104–105, pp. 58–66.

268. Davies, N.M.; Andersen, K.E. Clinical pharmacokinetics of diclofenac. Therapeutic insights and pitfalls. *Clin. Pharmacokinet.* 1997, *33*, 184–213.

269. Gan, T.J. Diclofenac: An update on its mechanism of action and safety profile. *Curr. Med. Res. Opin.* 2010, *26*, 1715–1731.

270. Diclofenac | DrugBank Online Available online: https://go.drugbank.com/drugs/DB00586 (accessed on Oct 25, 2020).

271. Mefenamic acid for pain and inflammation - Ponstan | Patient Available online: https://patient.info/medicine/mefenamic-acid-for-pain-and-inflammation-ponstan (accessed on Oct 25, 2020).

272. Mefenamic Acid: MedlinePlus Drug Information Available online: https://medlineplus.gov/druginfo/meds/a681028.html (accessed on Oct 25, 2020).

273. Mefenamic acid | DrugBank Online Available online: https://go.drugbank.com/drugs/DB00784 (accessed on Oct 25, 2020).

274. Tolfenamic acid | C14H12ClNO2 - PubChem Available online: https://pubchem.ncbi.nlm.nih.gov/compound/Tolfenamic-acid (accessed on Oct 25, 2020).

275. Tolfenamic acid | DrugBank Online Available online: https://go.drugbank.com/drugs/DB09216 (accessed on Oct 25, 2020).

276. Davison, J.; Maillard, J. *Opinion on triclosan - Antimicrobial resistance*; 2010; ISBN 9789279124846.

277. Dhillon, G.S.; Kaur, S.; Pulicharla, R.; Brar, S.K.; Cledón, M.; Verma, M.; Surampalli, R.Y. Triclosan: Current status, occurrence, environmental risks and bioaccumulation potential. *Int. J. Environ. Res. Public Health* 2015, *12*, 5657–5684.

278. Fang, J.L.; Stingley, R.L.; Beland, F.A.; Harrouk, W.; Lumpkins, D.L.; Howard, P. Occurrence, efficacy, metabolism, and toxicity of triclosan. *J. Environ. Sci. Heal. - Part C Environ. Carcinog. Ecotoxicol. Rev.* 2010, *28*, 147–171.

279. Rodricks, J. V.; Swenberg, J.A.; Borzelleca, J.F.; Maronpot, R.R.; Shipp, A.M. Triclosan: A critical review of the experimental data and development of margins of safety for consumer products. *Crit. Rev. Toxicol.* 2010, *40*, 422–484.

280. Yang, J.; Dong, H.; Hammock, B.D. Profiling the regulatory lipids: Another systemic way to unveil the biological mystery. *Curr. Opin. Lipidol.* **2011**, *22*, 197–203, doi:10.1097/MOL.0b013e3283468c10.

281. Pahan, K. Lipid-lowering drugs. *Cell. Mol. Life Sci.* 2006, *63*, 1165–1178.

282. Henriques, J.F.; Almeida, A.R.; Andrade, T.; Koba, O.; Golovko, O.; Soares, A.M.V.M.; Oliveira, M.; Domingues, I. Effects of the lipid

regulator drug gemfibrozil: A toxicological and behavioral perspective. *Aquat. Toxicol.* **2016**, *170*, 355–364, doi:10.1016/j.aquatox.2015.09.017.

283. FDA Foods and Veterinary Medicine Program, C. *LOPID -Gemfibrozil information product*;
284. Yılmaz, S.; Uçar, A. A review of the genotoxic and carcinogenic effects of aspartame: does it safe or not? *Cytotechnology* 2014, *66*, 875–881.
285. Marinovich, M.; Galli, C.L.; Bosetti, C.; Gallus, S.; La Vecchia, C. Aspartame, low-calorie sweeteners and disease: Regulatory safety and epidemiological issues. *Food Chem. Toxicol.* 2013, *60*, 109–115.
286. Shankar, P.; Ahuja, S.; Sriram, K. Non-nutritive sweeteners: Review and update. *Nutrition* 2013, *29*, 1293–1299.
287. Iyyaswamy, A.; Rathinasamy, S. Effect of chronic exposure to aspartame on oxidative stress in brain discrete regions of albino rats. *J. Biosci.* **2012**, *37*, 679–688, doi:10.1007/s12038-012-9236-0.
288. Adaramoye, O.A.; Akanni, O.O. Effects of long-term administration of aspartame on biochemical indices, lipid profile and redox status of cellular system of male rats. *J. Basic Clin. Physiol. Pharmacol.* **2016**, *27*, 29–37, doi:10.1515/jbcpp-2014-0130.
289. Tsakiris, S.; Giannoulia-Karantana, A.; Simintzi, I.; Schulpis, K.H. The effect of aspartame metabolites on human erythrocyte membrane acetylcholinesterase activity. *Pharmacol. Res.* **2006**, *53*, 1–5, doi:10.1016/j.phrs.2005.07.006.
290. Simintzi, I.; Schulpis, K.H.; Angelogianni, P.; Liapi, C.; Tsakiris, S. The effect of aspartame on acetylcholinesterase activity in hippocampal homogenates of suckling rats. *Pharmacol. Res.* **2007**, *56*, 155–159, doi:10.1016/j.phrs.2007.05.003.
291. Soffritti, M.; Belpoggi, F.; Degli Esposti, D.; Lambertini, L.; Tibaldi, E.; Rigano, A. First experimental demonstration of the multipotential carcinogenic effects of aspartame administered in the feed to Sprague-Dawley rats. *Environ. Health Perspect.* **2006**, *114*, 379–385, doi:10.1289/ehp.8711.
292. Soffritti, M.; Belpoggi, F.; Tibaldi, E.; Esposti, D.D.; Lauriola, M. Life-span exposure to low doses of aspartame beginning during prenatal life increases cancer effects in rats. *Environ. Health Perspect.* **2007**, *115*, 1293–1297, doi:10.1289/ehp.10271.
293. Kroger, M.; Meister, K.; Kava, R. Low-calorie sweeteners and other sugar substitutes: A review of the safety issues. *Compr. Rev. Food Sci. Food Saf.* 2006, *5*, 35–47.
294. FDA Additional Information about High-Intensity Sweeteners Permitted for Use in Food in the United States Available online: https://www.fda.gov/food/food-additives-petitions/additional-information-about-high-intensity-sweeteners-permitted-use-food-united-states (accessed on Oct 28, 2020).
295. *EUROPEAN COMMISSION DIRECTORATE-GENERAL III INDUSTRY Industrial affairs III: Consumer goods industries Foodstuffs-Legislation and scientific and technical aspects ANNEX III TO DOCUMENT III/5157/97 SCIENTIFIC COMMITTEE FOR FOOD CS/ADD/EDUL/148-FINAL*; 1997;
296. Schiffman, S.S.; Rother, K.I. Sucralose, a synthetic organochlorine sweetener: Overview of biological issues. *J. Toxicol. Environ. Heal. - Part B Crit. Rev.* **2013**, *16*, 399–451, doi:10.1080/10937404.2013.842523.
297. Sharma, V.K.; Oturan, M.; Kim, H. Oxidation of artificial sweetener sucralose by advanced oxidation processes: A review. *Environ. Sci. Pollut. Res.* 2014, *21*, 8525–8533.
298. Torres, C.I.; Ramakrishna, S.; Chiu, C.A.; Nelson, K.G.; Westerhoff, P.; Krajmalnik-Brown, R. Fate of sucralose during wastewater treatment. *Environ. Eng. Sci.* **2011**, *28*, 325–331, doi:10.1089/ees.2010.0227.
299. Li, X.; Du, Z.; Huang, X.; Yuan, W.; Ying, H. Solubility of sucralose in different solvents from (283.15 to 333.15) K. *J. Chem. Eng. Data* **2010**, *55*, 2600–2602, doi:10.1021/je9008427.
300. Loney, G.C.; Torregrossa, A.M.; Carballo, C.; Eckel, L.A. Preference for sucralose predicts behavioral responses to sweet and bittersweet tastants. *Chem. Senses* **2012**, *37*, 445–453, doi:10.1093/chemse/bjr126.
301. Roberts, A.; Renwick, A.G.; Sims, J.; Snodin, D.J. Sucralose metabolism and pharmacokinetics in man. *Food Chem. Toxicol.* **2000**, *38*, 31–41, doi:10.1016/s0278-6915(00)00026-0.
302. Płotka-Wasylka, J.; Szczepańska, N.; de la Guardia, M.; Namieśnik, J. Miniaturized solid-phase extraction techniques. *TrAC - Trends Anal. Chem.* 2015, *73*, 19–38.
303. Locatelli, M.; Tartaglia, A.; Piccolantonio, S.; Di Iorio, L.A.; Sperandio, E.; Ulusoy, H.I.; Furton, K.G.; Kabir, A. Innovative Configurations of Sample Preparation Techniques Applied in Bioanalytical Chemistry: A Review. *Curr. Anal. Chem.* **2019**, *15*, 731–744, doi:10.2174/1573411015666190301145042.
304. Pérez-Fernández, V.; Mainero Rocca, L.; Tomai, P.; Fanali, S.; Gentili, A. Recent advancements and future trends in environmental

analysis: Sample preparation, liquid chromatography and mass spectrometry. *Anal. Chim. Acta* **2017**, *983*, 9–41, doi:10.1016/j.aca.2017.06.029.

305. Azzouz, A.; Kailasa, S.K.; Lee, S.S.; J. Rascón, A.; Ballesteros, E.; Zhang, M.; Kim, K.H. Review of nanomaterials as sorbents in solid-phase extraction for environmental samples. *TrAC - Trends Anal. Chem.* 2018, *108*, 347–369.
306. Calderilla, C.; Maya, F.; Leal, L.O.; Cerdà, V. Recent advances in flow-based automated solid-phase extraction. *TrAC - Trends Anal. Chem.* 2018, *108*, 370–380.
307. Andrade-Eiroa, A.; Canle, M.; Leroy-Cancellieri, V.; Cerdà, V. Solid-phase extraction of organic compounds: A critical review (Part I). *TrAC - Trends Anal. Chem.* 2016, *80*, 641–654.
308. Maciel, E.V.S.; de Toffoli, A.L.; Neto, E.S.; Nazario, C.E.D.; Lanças, F.M. New materials in sample preparation: Recent advances and future trends. *TrAC - Trends Anal. Chem.* **2019**, *119*, 115633, doi:10.1016/j.trac.2019.115633.
309. Zhang, H.; Zhu, P. Solid phase extraction. *Fenxi Huaxue* **2000**, *28*, 1178–1180, doi:10.1002/9780470682494.ch3.
310. Telepchak, M.J.; August, T.F.; Chaney, G. *Forensic and Clinical Applications of Solid Phase Extraction*; Humana Press, 2004;
311. Walker, V.; Mills, G.A. Solid-phase extraction in clinical biochemistry. *Ann. Clin. Biochem.* 2002, *39*, 464–477.
312. Zygler, A.; Wasik, A.; Kot-Wasik, A.; Namieśnik, J. Determination of nine high-intensity sweeteners in various foods by high-performance liquid chromatography with mass spectrometric detection. *Anal. Bioanal. Chem.* **2011**, *400*, 2159–2172, doi:10.1007/s00216-011-4937-z.
313. Feng, J.; Wang, L.; Dai, I.; Harmon, T.; Bernert, J.T. Simultaneous determination of multiple drugs of abuse and relevant metabolites in urine by LC-MS-MS. *J. Anal. Toxicol.* **2007**, *31*, 359–368, doi:10.1093/jat/31.7.359.
314. Chang, S.H.; Lai, Y.H.; Huang, C.N.; Peng, G.J.; Liao, C.D.; Kao, Y.M.; Tseng, S.H.; Wang, D.Y. Multi-residue analysis using liquid chromatography tandem mass spectrometry for detection of 20 coccidiostats in poultry, livestock, and aquatic tissues. *J. Food Drug Anal.* **2019**, *27*, 703–716, doi:10.1016/j.jfda.2019.02.004.
315. Rodriguez-Mozaz, S.; Lopez de Alda, M.J.; Barceló, D. Advantages and limitations of on-line solid phase extraction coupled to liquid chromatography-mass spectrometry technologies versus biosensors for monitoring of emerging contaminants in water. *J. Chromatogr. A* 2007, *1152*, 97–115.
316. Adelhelm, C.; Niessner, R.; Pöschl, U.; Letzel, T. Analysis of large oxygenated and nitrated polycyclic aromatic hydrocarbons formed under simulated diesel engine exhaust conditions (by compound fingerprints with SPE/LC-API-MS). *Anal. Bioanal. Chem.* **2008**, *391*, 2599–2608, doi:10.1007/s00216-008-2175-9.
317. Carlson, J.C.; Challis, J.K.; Hanson, M.L.; Wong, C.S. Stability of pharmaceuticals and other polar organic compounds stored on polar organic chemical integrative samplers and solid-phase extraction cartridges. *Environ. Toxicol. Chem.* **2013**, *32*, 337–344, doi:10.1002/etc.2076.
318. Packer, N.H.; Lawson, M.A.; Jardine, D.R.; Redmond, J.W. A general approach to desalting oligosaccharides released from glycoproteins. *Glycoconj. J.* **1998**, *15*, 737–747, doi:10.1023/A:1006983125913.
319. Van Heukelem, L.; Thomas, C.S. Computer-assisted high-performance liquid chromatography method development with applications to the isolation and analysis of phytoplankton pigments. *J. Chromatogr. A* **2001**, *910*, 31–49, doi:10.1016/S0378-4347(00)00603-4.
320. Epa Method 1694 : Pharmaceuticals and Personal Care Products in Water , Soil , Sediment , and Biosolids by HPLC / MS / MS. *EPA Method* **2007**, 77.
321. Płotka-Wasylka, J.; Szczepańska, N.; de la Guardia, M.; Namieśnik, J. Modern trends in solid phase extraction: New sorbent media. *TrAC - Trends Anal. Chem.* **2016**, *77*, 23–43, doi:10.1016/j.trac.2015.10.010.
322. Erger, C.; Schmidt, T.C. *Disk-based solid-phase extraction analysis of organic substances in water*; Elsevier B.V., 2014; Vol. 61, pp. 74–82;.
323. Huck, C.W.; Bonn, G.K. Recent developments in polymer-based sorbents for solid-phase extraction. *J. Chromatogr. A* 2000, *885*, 51–72.
324. Fontanals, N.; Marcé, R.M.; Borrull, F. Materials for solid-phase extraction of organic compounds. *Separations* **2019**, *6*, doi:10.3390/separations6040056.
325. Postigo, C.; Lopez De Alda, M.J.; Barceló, D. Fully automated determination in the low nanogram per liter level of different classes of drugs of abuse in sewage water by on-line solid-phase extraction-liquid chromatography-electrospray-tandem mass

spectrometry. *Anal. Chem.* **2008**, *80*, 3123–3134, doi:10.1021/ac702060j.

326. Babić, S.; Mutavdžić Pavlović, D.; Ašperger, D.; Periša, M.; Zrnčić, M.; Horvat, A.J.M.; Kaštelan-Macan, M. Determination of multi-class pharmaceuticals in wastewater by liquid chromatography-tandem mass spectrometry (LC-MS-MS). In Proceedings of the Analytical and Bioanalytical Chemistry; Anal Bioanal Chem, 2010; Vol. 398, pp. 1185–1194.
327. Ordóñez, E.Y.; Quintana, J.B.; Rodil, R.; Cela, R. Determination of artificial sweeteners in water samples by solid-phase extraction and liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A* **2012**, *1256*, 197–205, doi:10.1016/j.chroma.2012.07.073.
328. Azzouz, A.; Rascón, A.J.; Ballesteros, E. Simultaneous determination of parabens, alkylphenols, phenylphenols, bisphenol A and triclosan in human urine, blood and breast milk by continuous solid-phase extraction and gas chromatography-mass spectrometry. *J. Pharm. Biomed. Anal.* **2016**, *119*, 16–26, doi:10.1016/j.jpba.2015.11.024.
329. Roy, K.S.; Purohit, A.K.; Chandra, B.; Goud, D.R.; Pardasani, D.; Dubey, D.K. Polymeric Sorbent with Controlled Surface Polarity: An Alternate for Solid-Phase Extraction of Nerve Agents and Their Markers from Organic Matrix. *Anal. Chem.* **2018**, *90*, 7025–7032, doi:10.1021/acs.analchem.8b01428.
330. Fontanals, N.; Marcé, R.M.; Cormack, P.A.G.; Sherrington, D.C.; Borrull, F. Monodisperse, hypercrosslinked polymer microspheres as tailor-made sorbents for highly efficient solid-phase extractions of polar pollutants from water samples. *J. Chromatogr. A* **2008**, *1191*, 118–124, doi:10.1016/j.chroma.2007.11.061.
331. Bratkowska, D.; Fontanals, N.; Borrull, F.; Cormack, P.A.G.; Sherrington, D.C.; Marcé, R.M. Hydrophilic hypercrosslinked polymeric sorbents for the solid-phase extraction of polar contaminants from water. *J. Chromatogr. A* **2010**, *1217*, 3238–3243, doi:10.1016/j.chroma.2009.08.091.
332. Castiglioni, S.; Bagnati, R.; Melis, M.; Panawennage, D.; Chiarelli, P.; Fanelli, R.; Zuccato, E. Identification of cocaine and its metabolites in urban wastewater and comparison with the human excretion profile in urine. *Water Res.* **2011**, *45*, 5141–5150, doi:10.1016/j.watres.2011.07.017.
333. Tomková, J.; Ondra, P.; Válka, I. Simultaneous determination of mushroom toxins α-amanitin, β-amanitin and muscarine in human urine by solid-phase extraction and ultra-high-performance liquid chromatography coupled with ultra-high-resolution TOF mass spectrometry. *Forensic Sci. Int.* **2015**, *251*, 209–213, doi:10.1016/j.forsciint.2015.04.007.
334. Janda, J.; Nödler, K.; Brauch, H.J.; Zwiener, C.; Lange, F.T. Robust trace analysis of polar (C2-C8) perfluorinated carboxylic acids by liquid chromatography-tandem mass spectrometry: method development and application to surface water, groundwater and drinking water. *Environ. Sci. Pollut. Res.* **2019**, *26*, 7326–7336, doi:10.1007/s11356-018-1731-x.
335. Zhang, P.; Bui, A.; Rose, G.; Allinson, G. Mixed-mode solid-phase extraction coupled with liquid chromatography tandem mass spectrometry to determine phenoxy acid, sulfonylurea, triazine and other selected herbicides at nanogram per litre levels in environmental waters. *J. Chromatogr. A* **2014**, *1325*, 56–64, doi:10.1016/j.chroma.2013.12.021.
336. Huang, C.; Li, Y.; Yang, J.; Peng, J.; Tan, J.; Fan, Y.; Wang, L.; Chen, J. Hyperbranched mixed-mode anion-exchange polymeric sorbent for highly selective extraction of nine acidic non-steroidal anti-inflammatory drugs from human urine. *Talanta* **2018**, *190*, 15–22, doi:10.1016/j.talanta.2018.07.033.
337. Hu, K.; Zhao, G.; Liu, J.; Jia, L.; Xie, F.; Zhang, S.; Liu, H.; Liu, M. Simultaneous quantification of three alkylated-purine adducts in human urine using sulfonic acid poly(glycidyl methacrylate-divinylbenzene)-based microspheres as sorbent combined with LC-MS/MS. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2018**, *1081–1082*, 15–24, doi:10.1016/j.jchromb.2018.02.028.
338. Fontanals, N.; Cormack, P.A.G.; Sherrington, D.C. Hypercrosslinked polymer microspheres with weak anion-exchange character. Preparation of the microspheres and their applications in pH-tuneable, selective extractions of analytes from complex environmental samples. *J. Chromatogr. A* **2008**, *1215*, 21–29, doi:10.1016/j.chroma.2008.10.124.
339. Bratkowska, D.; Marcé, R.M.; Cormack, P.A.G.; Sherrington, D.C.; Borrull, F.; Fontanals, N. Synthesis and application of hypercrosslinked polymers with weak cation-exchange character for the selective extraction of basic pharmaceuticals from complex environmental water samples. *J. Chromatogr. A* **2010**, *1217*, 1575–1582, doi:10.1016/j.chroma.2010.01.037.
340. Republic, C.; Republic, C.; Šafaříková, M.; Šafařík, I. Magnetic solid-phase extraction. *J. Magn. Magn. Mater.* **1999**, *194*, 108–112, doi:10.1016/S0304-8853(98)00566-6.
341. Ibarra, I.S.; Rodriguez, J.A.; Galán-Vidal, C.A.; Cepeda, A.; Miranda, J.M. Magnetic solid phase extraction applied to food analysis. *J. Chem.* **2015**, *2015*, doi:10.1155/2015/919414.

342. Hagarová, I. Magnetic Solid Phase Extraction as a Promising Technique for Fast Separation of Metallic Nanoparticles and Their Ionic Species: A Review of Recent Advances. *J. Anal. Methods Chem.* 2020, *2020*.

343. Pyrzynska, K. Nanomaterials in speciation analysis of metals and metalloids. *Talanta* **2020**, *212*, 120784, doi:10.1016/j.talanta.2020.120784.

344. Huang, X.; Liu, Q.; Yao, S.; Jiang, G. Recent progress in the application of nanomaterials in the analysis of emerging chemical contaminants. *Anal. Methods* 2017, *9*, 2768–2783.

345. Spietelun, A.; Marcinkowski, Ł.; de la Guardia, M.; Namieśnik, J. Recent developments and future trends in solid phase microextraction techniques towards green analytical chemistry. *J. Chromatogr. A* 2013, *1321*, 1–13.

346. Herrero-Latorre, C.; Barciela-García, J.; García-Martín, S.; Peña-Crecente, R.M.; Otárola-Jiménez, J. Magnetic solid-phase extraction using carbon nanotubes as sorbents: A review. *Anal. Chim. Acta* **2015**, *892*, 10–26, doi:10.1016/j.aca.2015.07.046.

347. Ríos, A.; Zougagh, M.; Bouri, M. Magnetic (nano)materials as an useful tool for sample preparation in analytical methods. A review. *Anal. Methods* 2013, *5*, 4558–4573.

348. Synaridou, M.E.S.; Sakkas, V.A.; Stalikas, C.D.; Albanis, T.A. Evaluation of magnetic nanoparticles to serve as solid-phase extraction sorbents for the determination of endocrine disruptors in milk samples by gas chromatography mass spectrometry. *J. Chromatogr. A* **2014**, *1348*, 71–79, doi:10.1016/j.chroma.2014.04.092.

349. Dinali, L.A.F.; de Oliveira, H.L.; Teixeira, L.S.; da Silva, A.T.M.; D'Oliveira, K.A.; Cuin, A.; Borges, K.B. Efficient development of a magnetic molecularly imprinted polymer for selective determination of trimethoprim and sulfamethoxazole in milk. *Microchem. J.* **2020**, *154*, 104648, doi:10.1016/j.microc.2020.104648.

350. Wu, J.; Xiao, D.; Zhao, H.; He, H.; Peng, J.; Wang, C.; Zhang, C.; He, J. A nanocomposite consisting of graphene oxide and Fe3O4 magnetic nanoparticles for the extraction of flavonoids from tea, wine and urine samples. *Microchim. Acta* **2015**, *182*, 2299–2306, doi:10.1007/s00604-015-1575-8.

351. Lv, T.; Zhao, X.E.; Zhu, S.; Qu, F.; Song, C.; You, J.; Suo, Y. Determination of bisphenol A, 4-octylphenol, and 4-nonylphenol in soft drinks and dairy products by ultrasound-assisted dispersive liquid-liquid microextraction combined with derivatization and high-performance liquid chromatography with fluorescence detection. *J. Sep. Sci.* **2014**, *37*, 2757–2763, doi:10.1002/jssc.201400612.

352. Li, G.; Wen, A.; Liu, J.; Wu, D.; Wu, Y. Facile extraction and determination of organophosphorus pesticides in vegetables via magnetic functionalized covalent organic framework nanocomposites. *Food Chem.* **2021**, *337*, doi:10.1016/j.foodchem.2020.127974.

353. Safari, M.; Shahlaei, M.; Yamini, Y.; Shakorian, M.; Arkan, E. Magnetic framework composite as sorbent for magnetic solid phase extraction coupled with high performance liquid chromatography for simultaneous extraction and determination of tricyclic antidepressants. *Anal. Chim. Acta* **2018**, *1034*, 204–213, doi:10.1016/j.aca.2018.06.023.

354. Eskandari, H.; Naderi-Darehshori, A. Preparation of magnetite/poly(styrene-divinylbenzene) nanoparticles for selective enrichment-determination of fenitrothion in environmental and biological samples. *Anal. Chim. Acta* **2012**, *743*, 137–144, doi:10.1016/j.aca.2012.07.012.

355. Shan, S.; Ma, Y.; Sun, C.; Guo, X.; Zheng, H.; Xu, X.; Qin, L.; Hu, J. A novel magnetic solid-phase extraction method for detection of 14 heterocyclic aromatic amines by UPLC-MS/MS in meat products. *Food Chem.* **2021**, *337*, 127630, doi:10.1016/j.foodchem.2020.127630.

356. Asgharinezhad, A.A.; Ebrahimzadeh, H.; Mirbabaei, F.; Mollazadeh, N.; Shekari, N. Dispersive micro-solid-phase extraction of benzodiazepines from biological fluids based on polyaniline/magnetic nanoparticles composite. *Anal. Chim. Acta* **2014**, *844*, 80–89, doi:10.1016/j.aca.2014.06.007.

357. Zhang, M.; Lian, K.; Ai, L.; Kang, W.; Zhao, T. Simultaneous determination of 11 antiseptic ingredients in surface water based on polypyrrole decorated magnetic nanoparticles. *RSC Adv.* **2020**, *10*, 37473–37481, doi:10.1039/d0ra07064e.

358. Rodriguez, J.A.; Ibarra, I.S.; Miranda, J.M.; Barrado, E.; Santos, E.M. Magnetic solid phase extraction based on fullerene and activated carbon adsorbents for determination of azo dyes in water samples by capillary electrophoresis. *Anal. Methods* **2016**, *8*, 8466–8473, doi:10.1039/c6ay02631a.

359. Zhang, H.-F.; Shi, Y.-P. Preparation of Fe3O4 nanoparticle enclosure hydroxylated multi-walled carbon nanotubes for the determination of aconitines in human serum samples. *Anal. Chim. Acta* **2012**, *724*, 54–60, doi:10.1016/j.aca.2012.02.039.

360. Zhu, L.; Xu, H. Magnetic graphene oxide as adsorbent for the determination of polycyclic aromatic hydrocarbon metabolites in

human urine. *J. Sep. Sci.* **2014**, *37*, 2591–2598, doi:10.1002/jssc.201400363.

361. Cai, M.Q.; Su, J.; Hu, J.Q.; Wang, Q.; Dong, C.Y.; Pan, S.D.; Jin, M.C. Planar graphene oxide-based magnetic ionic liquid nanomaterial for extraction of chlorophenols from environmental water samples coupled with liquid chromatography–tandem mass spectrometry. *J. Chromatogr. A* **2016**, *1459*, 38–46, doi:10.1016/j.chroma.2016.06.086.
362. Liu, Q.; Shi, J.; Wang, T.; Guo, F.; Liu, L.; Jiang, G. Hemimicelles/admicelles supported on magnetic graphene sheets for enhanced magnetic solid-phase extraction. *J. Chromatogr. A* **2012**, *1257*, 1–8, doi:10.1016/j.chroma.2012.08.028.
363. Giakisikli, G.; Anthemidis, A.N. Magnetic materials as sorbents for metal/metalloid preconcentration and/or separation. A review. *Anal. Chim. Acta* **2013**, *789*, 1–16, doi:10.1016/j.aca.2013.04.021.
364. Li, X.S.; Zhu, G.T.; Luo, Y.B.; Yuan, B.F.; Feng, Y.Q. Synthesis and applications of functionalized magnetic materials in sample preparation. *TrAC - Trends Anal. Chem.* **2013**, *45*, 233–247, doi:10.1016/j.trac.2012.10.015.
365. Reddy, L.H.; Arias, J.L.; Nicolas, J.; Couvreur, P. Magnetic nanoparticles: Design and characterization, toxicity and biocompatibility, pharmaceutical and biomedical applications. *Chem. Rev.* **2012**, *112*, 5818–5878, doi:10.1021/cr300068p.
366. Massart, R. Preparation of Aqueous Magnetic Liquids in Alkaline and Acidic Media. *IEEE Trans. Magn.* 1981, *17*, 1247–1248.
367. Wu, W.; He, Q.; Jiang, C. Magnetic iron oxide nanoparticles: Synthesis and surface functionalization strategies. *Nanoscale Res. Lett.* **2008**, *3*, 397–415, doi:10.1007/s11671-008-9174-9.
368. Baresel, C.; Schaller, V.; Jonasson, C.; Johansson, C.; Bordes, R.; Chauhan, V.; Sugunan, A.; Sommertune, J.; Welling, S. Functionalized magnetic particles for water treatment. *Heliyon* **2019**, *5*, e02325, doi:10.1016/j.heliyon.2019.e02325.
369. Li, X.S.; Wu, J.H.; Zhao, Y.; Zhang, W.P.; Gao, Q.; Guo, L.; Yuan, B.F.; Feng, Y.Q. Preparation of magnetic polymer material with phosphate group and its application to the enrichment of phosphopeptides. *J. Chromatogr. A* **2011**, *1218*, 3845–3853, doi:10.1016/j.chroma.2011.04.044.
370. de Dios, A.S.; Díaz-García, M.E. Multifunctional nanoparticles: Analytical prospects. *Anal. Chim. Acta* **2010**, *666*, 1–22, doi:10.1016/j.aca.2010.03.038.
371. Alejandro-Arellano, M.; Ung, T.; Blanco, Á.; Mulvaney, P.; Liz-Marzán, L.M. Silica-coated metals and semiconductors. Stabilization and nanostructuring. In Proceedings of the Pure and Applied Chemistry; Walter de Gruyter GmbH, 2000; Vol. 72, pp. 257–267.
372. Wang, L.; Zhao, W.; Tan, W. Bioconjugated silica nanoparticles: Development and applications. *Nano Res.* **2008**, *1*, 99–115, doi:10.1007/s12274-008-8018-3.
373. Chen, H.; Deng, C.; Zhang, X. Synthesis of Fe304@Si02@PMMA core-shell-shell magnetic microspheres for highly efficient enrichment of peptides and proteins for MALDI-ToF MS analysis. *Angew. Chemie - Int. Ed.* **2010**, *49*, 607–611, doi:10.1002/anie.200904885.
374. Arkles, B. Tailoring Surfaces with Silanes. *Chemtech* **1977**, *7*, 766, doi:10.1002/9781118217689.ch5.
375. Deng, Y.; Qi, D.; Deng, C.; Zhang, X.; Zhao, D. Superparamagnetic high-magnetization microspheres with an Fe 3O4@SiO2 core and perpendicularly aligned mesoporous SiO2 shell for removal of microcystins. *J. Am. Chem. Soc.* **2008**, *130*, 28–29, doi:10.1021/ja0777584.
376. Zhu, G.-T.; He, X.-M.; Li, X.-S.; Wang, S.-T.; Luo, Y.-B.; Yuan, B.-F.; Feng, Y.-Q. Preparation of mesoporous silica embedded pipette tips for rapid enrichment of endogenous peptides. *J. Chromatogr. A* **2013**, *1316*, 23–28, doi:10.1016/j.chroma.2013.09.068.
377. Li, Y.; Wang, W.H.; Yang, S.D.; Li, B.J.; Feng, C.; Shi, Z.J. Oxidative dimerization of N-protected and free indole derivatives toward 3,3'-biindoles via Pd-catalyzed direct C-H transformations. *Chem. Commun.* **2010**, *46*, 4553–4555, doi:10.1039/c0cc00486c.
378. Park, S.K.; Kim, K. Do; Kim, H.T. Preparation of silica nanoparticles: Determination of the optimal synthesis conditions for small and uniform particles. *Colloids Surfaces A Physicochem. Eng. Asp.* **2002**, *197*, 7–17, doi:10.1016/S0927-7757(01)00683-5.
379. Zhang, X.; Niu, H.; Pan, Y.; Shi, Y.; Cai, Y. Chitosan-coated octadecyl-functionalized magnetite nanoparticles: Preparation and application in extraction of trace pollutants from environmental water samples. *Anal. Chem.* **2010**, *82*, 2363–2371, doi:10.1021/ac902589t.
380. Caon, N.B.; Cardoso, C.D.S.; Faita, F.L.; Vitali, L.; Parize, A.L. Magnetic solid-phase extraction of triclosan from water using n-octadecyl modified silica-coated magnetic nanoparticles. *J. Environ. Chem. Eng.* **2020**, *8*, doi:10.1016/j.jece.2020.104003.
381. Ma, J.; Yan, F.; Chen, F.; Jiang, L.; Li, J.; Chen, L. C18-functionalized magnetic silica nanoparticles for solid phase extraction of microcystin-LR in reservoir water samples followed by HPLC-DAD determination. *J. Liq. Chromatogr. Relat. Technol.* **2015**, *38*, 655–661, doi:10.1080/10826076.2014.936611.

382. Moreno, V.; Zougagh, M.; Ríos, Á. Hybrid nanoparticles based on magnetic multiwalled carbon nanotube-nanoC18SiO2 composites for solid phase extraction of mycotoxins prior to their determination by LC-MS. *Microchim. Acta* **2016**, *183*, 871–880, doi:10.1007/s00604-015-1722-2.

383. Cai, P.; Xiong, X.; Li, D.; Zhou, Y.; Xiong, C. Magnetic solid-phase extraction coupled with UHPLC-MS/MS for four antidepressants and one metabolite in clinical plasma and urine samples. *Bioanalysis* **2020**, *12*, 35–52, doi:10.4155/bio-2019-0171.

384. Wang, X.; Deng, C. Preparation of C18-functionalized magnetic polydopamine microspheres for the enrichment and analysis of alkylphenols in water samples. *Talanta* **2016**, *148*, 387–392, doi:10.1016/j.talanta.2015.11.008.

385. Srivastava, N.; Kumari, S.; Nair, K.; Alam, S.; Raza, S.K. Determination of organophosphorous pesticides in environmental water samples using surface-engineered C18 functionalized silica-coated core-shell magnetic nanoparticles-based extraction coupled with GC-MS/MS analysis. *J. AOAC Int.* **2017**, *100*, 804–809, doi:10.5740/jaoacint.16-0312.

386. Kuráň, P.; Pilnaj, D.; Ciencialová, L.; Pšenička, M. Preparation of Magnetic Sorbent with Surface Modified by C18 for Removal of Selected Organic Pollutants from Aqueous Samples. In Proceedings of the IOP Conference Series: Earth and Environmental Science; Institute of Physics Publishing, 2017; Vol. 95.

387. Qiao, Z.; Perestrelo, R.; Reyes-Gallardo, E.M.; Lucena, R.; Cárdenas, S.; Rodrigues, J.; Câmara, J.S. Octadecyl functionalized core–shell magnetic silica nanoparticle as a powerful nanocomposite sorbent to extract urinary volatile organic metabolites. *J. Chromatogr. A* **2015**, *1393*, 18–25, doi:10.1016/j.chroma.2015.03.026.

388. Guo, B.; Ji, S.; Zhang, F.; Yang, B.; Gu, J.; Liang, X. Preparation of C18-functionalized Fe3O4@SiO2 core-shell magnetic nanoparticles for extraction and determination of phthalic acid esters in Chinese herb preparations. *J. Pharm. Biomed. Anal.* **2014**, *100*, 365–368, doi:10.1016/j.jpba.2014.08.027.

389. Xiong, X.; Li, D.; Du, Z.; Xiong, C.; Jiang, H. Magnetic solid-phase extraction modified Quick, Easy, Cheap, Effective, Rugged and Safe method combined with pre-column derivatization and ultra-high performance liquid chromatography-tandem mass spectrometry for determination of estrogens and estrogen mimics in pork and chicken samples. *J. Chromatogr. A* **2020**, *1622*, 461137, doi:10.1016/j.chroma.2020.461137.

390. Maddah, B.; Sabouri, A.; Hasanzadeh, M. Magnetic Solid-Phase Extraction of Oxadiazon and Profenofos from Environmental Water Using Magnetite Fe3O4@SiO2–C18 Nanoparticles. *J. Polym. Environ.* **2017**, *25*, 770–780, doi:10.1007/s10924-016-0859-3.

391. Wen, Y.; Chen, L.; Li, J.; Liu, D.; Chen, L. Recent advances in solid-phase sorbents for sample preparation prior to chromatographic analysis. *TrAC - Trends Anal. Chem.* 2014, *59*, 26–41.

392. Grajek, H.; Jonik, J.; Witkiewicz, Z.; Wawer, T.; Purchała, M. Applications of Graphene and Its Derivatives in Chemical Analysis. *Crit. Rev. Anal. Chem.* **2020**, *50*, 445–471, doi:10.1080/10408347.2019.1653165.

393. Hou, X.; Tang, S.; Wang, J. Recent advances and applications of graphene-based extraction materials in food safety. *TrAC - Trends Anal. Chem.* 2019, *119*, 115603.

394. Li, G.; Deng, R.; Peng, G.; Yang, C.; He, Q.; Lu, Y.; Shi, H. Magnetic solid-phase extraction for the analysis of bisphenol A, naproxen and triclosan in wastewater samples. *Water Sci. Technol.* **2018**, *77*, 2220–2227, doi:10.2166/wst.2018.137.

395. Bitas, D.; Kabir, A.; Locatelli, M.; Samanidou, V. Food sample preparation for the determination of sulfonamides by high-performance liquid chromatography: State-of-the-Art. *Separations* **2018**, *5*, doi:10.3390/separations5020031.

396. Wang, J.; Xu, J.; Ji, X.; Wu, H.; Yang, H.; Zhang, H.; Zhang, X.; Li, Z.; Ni, X.; Qian, M. Determination of veterinary drug/pesticide residues in livestock and poultry excrement using selective accelerated solvent extraction and magnetic material purification combined with ultra-high-performance liquid chromatography–tandem mass spectrometry. *J. Chromatogr. A* **2020**, *1617*, 460808, doi:10.1016/j.chroma.2019.460808.

397. Marcano, D.C.; Kosynkin, D. V.; Berlin, J.M.; Sinitskii, A.; Sun, Z.; Slesarev, A.; Alemany, L.B.; Lu, W.; Tour, J.M. Improved synthesis of graphene oxide. *ACS Nano* **2010**, *4*, 4806–4814, doi:10.1021/nn1006368.

398. Babaei, A.; Zeeb, M.; Es-haghi, A. Magnetic dispersive solid-phase extraction based on graphene oxide/Fe $_3$ O $_4$ @polythionine nanocomposite followed by atomic absorption spectrometry for zinc monitoring in water, flour, celery and egg. *J. Sci. Food Agric.* **2018**, *98*, 3571–3579, doi:10.1002/jsfa.8873.

399. Wen, Y.; Niu, Z.; Ma, Y.; Ma, J.; Chen, L. Graphene oxide-based microspheres for the dispersive solid-phase extraction of non-steroidal estrogens from water samples. *J. Chromatogr. A* **2014**, *1368*, 18–25, doi:10.1016/j.chroma.2014.09.049.

400. Singh, R.K.; Kumar, R.; Singh, D.P. Graphene oxide: Strategies for synthesis, reduction and frontier applications. *RSC Adv.* 2016, *6*,

64993–65011.

401. Smith, A.T.; LaChance, A.M.; Zeng, S.; Liu, B.; Sun, L. Synthesis, properties, and applications of graphene oxide/reduced graphene oxide and their nanocomposites. *Nano Mater. Sci.* **2019**, *1*, 31–47, doi:10.1016/j.nanoms.2019.02.004.
402. AlKinani, A.; Eftekhari, M.; Gheibi, M. Ligandless dispersive solid phase extraction of cobalt ion using magnetic graphene oxide as an adsorbent followed by its determination with electrothermal atomic absorption spectrometry. *Int. J. Environ. Anal. Chem.* **2019**, doi:10.1080/03067319.2019.1659254.
403. Chandra, V.; Park, J.; Chun, Y.; Lee, J.W.; Hwang, I.C.; Kim, K.S. Water-dispersible magnetite-reduced graphene oxide composites for arsenic removal. *ACS Nano* **2010**, *4*, 3979–3986, doi:10.1021/nn1008897.
404. Ai, L.; Zhang, C.; Chen, Z. Removal of methylene blue from aqueous solution by a solvothermal-synthesized graphene/magnetite composite. *J. Hazard. Mater.* **2011**, *192*, 1515–1524, doi:10.1016/j.jhazmat.2011.06.068.
405. Calza, P.; Hadjicostas, C.; Sakkas, V.A.; Sarro, M.; Minero, C.; Medana, C.; Albanis, T.A. Photocatalytic transformation of the antipsychotic drug risperidone in aqueous media on reduced graphene oxide-TiO2 composites. *Appl. Catal. B Environ.* **2016**, *183*, 96–106, doi:10.1016/j.apcatb.2015.10.010.
406. Dhara, K.; Debiprosad, R.M. Review on nanomaterials-enabled electrochemical sensors for ascorbic acid detection. *Anal. Biochem.* **2019**, *586*, 113415, doi:10.1016/j.ab.2019.113415.
407. Rowley-Neale, S.J.; Randviir, E.P.; Abo Dena, A.S.; Banks, C.E. An overview of recent applications of reduced graphene oxide as a basis of electroanalytical sensing platforms. *Appl. Mater. Today* 2018, *10*, 218–226.
408. Mohan, V.B.; Lau, K. tak; Hui, D.; Bhattacharyya, D. Graphene-based materials and their composites: A review on production, applications and product limitations. *Compos. Part B Eng.* 2018, *142*, 200–220.
409. Dreyer, D.R.; Park, S.; Bielawski, C.W.; Ruoff, R.S. The chemistry of graphene oxide. *Chem. Soc. Rev.* 2010, *39*, 228–240.
410. Singh, V.; Joung, D.; Zhai, L.; Das, S.; Khondaker, S.I.; Seal, S. Graphene based materials: Past, present and future. *Prog. Mater. Sci.* 2011, *56*, 1178–1271.
411. Hummers, W.S.; Offeman, R.E. Preparation of Graphitic Oxide. *J. Am. Chem. Soc.* **1958**, *80*, 1339, doi:10.1021/ja01539a017.
412. Fang, M.; Wang, K.; Lu, H.; Yang, Y.; Nutt, S. Covalent polymer functionalization of graphene nanosheets and mechanical properties of composites. *J. Mater. Chem.* **2009**, *19*, 7098–7105, doi:10.1039/b908220d.
413. Rosca, I.D.; Watari, F.; Uo, M.; Akasaka, T. Oxidation of multiwalled carbon nanotubes by nitric acid. *Carbon N. Y.* **2005**, *43*, 3124–3131, doi:10.1016/j.carbon.2005.06.019.
414. Kovtyukhova, N.I. Layer-by-layer assembly of ultrathin composite films from micron-sized graphite oxide sheets and polycations. *Chem. Mater.* **1999**, *11*, 771–778, doi:10.1021/cm981085u.
415. Qi, T.; Huang, C.; Yan, S.; Li, X.-J.; Pan, S.-Y. Synthesis, characterization and adsorption properties of magnetite/reduced graphene oxide nanocomposites. *Talanta* **2015**, *144*, 1116–1124, doi:10.1016/J.TALANTA.2015.07.089.
416. Reis, E.O.; Foureaux, A.F.S.; Rodrigues, J.S.; Moreira, V.R.; Lebron, Y.A.R.; Santos, L.V.S.; Amaral, M.C.S.; Lange, L.C. Occurrence, removal and seasonal variation of pharmaceuticals in Brasilian drinking water treatment plants. *Environ. Pollut.* **2019**, *250*, 773–781, doi:10.1016/j.envpol.2019.04.102.
417. Chua, C.K.; Pumera, M. Chemical reduction of graphene oxide: A synthetic chemistry viewpoint. *Chem. Soc. Rev.* 2014, *43*, 291–312.
418. Kotov, N.A.; Dékány, I.; Fendler, J.H. Ultrathin graphite oxide-polyelectrolyte composites prepared by self-assembly: Transition between conductive and non-conductive states. *Adv. Mater.* **1996**, *8*, 637–641, doi:10.1002/adma.19960080806.
419. Stankovich, S.; Dikin, D.A.; Piner, R.D.; Kohlhaas, K.A.; Kleinhammes, A.; Jia, Y.; Wu, Y.; Nguyen, S.B.T.; Ruoff, R.S. Synthesis of graphene-based nanosheets via chemical reduction of exfoliated graphite oxide. *Carbon N. Y.* **2007**, *45*, 1558–1565, doi:10.1016/j.carbon.2007.02.034.
420. Stankovich, S.; Piner, R.D.; Chen, X.; Wu, N.; Nguyen, S.T.; Ruoff, R.S. Stable aqueous dispersions of graphitic nanoplatelets via the reduction of exfoliated graphite oxide in the presence of poly(sodium 4-styrenesulfonate). *J. Mater. Chem.* **2006**, *16*, 155–158, doi:10.1039/b512799h.
421. Zalan, Z.; Lazar, L.; Fulop, F. Chemistry of Hydrazinoalcohols and their Heterocyclic Derivatives. Part 1. Synthesis of Hydrazinoalcohols. *Curr. Org. Chem.* **2005**, *9*, 357–376, doi:10.2174/1385272053174949.
422. Pei, S.; Cheng, H.M. The reduction of graphene oxide. *Carbon N. Y.* **2012**, *50*, 3210–3228, doi:10.1016/j.carbon.2011.11.010.

423. Fernández-Merino, M.J.; Guardia, L.; Paredes, J.I.; Villar-Rodil, S.; Solís-Fernández, P.; Martínez-Alonso, A.; Tascón, J.M.D. Vitamin C is an ideal substitute for hydrazine in the reduction of graphene oxide suspensions. *J. Phys. Chem. C* **2010**, *114*, 6426–6432, doi:10.1021/jp100603h.

424. Maciel, E.V.S.; Mejía-Carmona, K.; Jordan-Sinisterra, M.; da Silva, L.F.; Vargas Medina, D.A.; Lanças, F.M. The Current Role of Graphene-Based Nanomaterials in the Sample Preparation Arena. *Front. Chem.* 2020, *8*, 664.

425. Manousi, N.; Rosenberg, E.; Deliyanni, E.; Zachariadis, G.A.; Samanidou, V. Magnetic solid-phase extraction of organic compounds based on graphene oxide nanocomposites. *Molecules* **2020**, *25*, doi:10.3390/molecules25051148.

426. Taghvimi, A.; Hamishehkar, H.; Ebrahimi, M. Magnetic nano graphene oxide as solid phase extraction adsorbent coupled with liquid chromatography to determine pseudoephedrine in urine samples. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2016**, *1009–1010*, 66–72, doi:10.1016/j.jchromb.2015.12.005.

427. Lu, Q.; Guo, H.; Zhang, Y.; Tang, X.; Lei, W.; Qi, R.; Chu, J.; Li, D.; Zhao, Q. Graphene oxide-Fe3O4 nanocomposite magnetic solid phase extraction followed by UHPLC-MS/MS for highly sensitive determination of eight psychoactive drugs in urine samples. *Talanta* **2020**, *206*, 120212, doi:10.1016/j.talanta.2019.120212.

428. Zeng, S.; Gan, N.; Weideman-Mera, R.; Cao, Y.; Li, T.; Sang, W. Enrichment of polychlorinated biphenyl 28 from aqueous solutions using Fe3O4 grafted graphene oxide. *Chem. Eng. J.* **2013**, *218*, 108–115, doi:10.1016/j.cej.2012.12.030.

429. Zhang, X.; Ma, X.; Li, X.; Li, C.; Wang, R.; Chen, M. Development of Ultra-sensitive Method for Determination of Trace Atrazine Herbicide in Environmental Water Using Magnetic Graphene Oxide-Based Solid-Phase Extraction Coupled with Dispersive Liquid-Liquid Microextraction Prior to Gas Chromatography-Mass Spectrometry. *Water. Air. Soil Pollut.* **2018**, *229*, 1–11, doi:10.1007/s11270-018-3930-y.

430. Shi, P.; Ye, N. Magnetite-graphene oxide composites as a magnetic solid-phase extraction adsorbent for the determination of trace sulfonamides in water samples. *Anal. Methods* **2014**, *6*, 9725–9730, doi:10.1039/c4ay02027h.

431. Li, N.; Chen, J.; Shi, Y.P. Magnetic reduced graphene oxide functionalized with β-cyclodextrin as magnetic solid-phase extraction adsorbents for the determination of phytohormones in tomatoes coupled with high performance liquid chromatography. *J. Chromatogr. A* **2016**, *1441*, 24–33, doi:10.1016/j.chroma.2016.02.077.

432. Kazemi, E.; Dadfarnia, S.; Haji Shabani, A.M.; Abbasi, A.; Rashidian Vaziri, M.R.; Behjat, A. Iron oxide functionalized graphene oxide as an efficient sorbent for dispersive micro-solid phase extraction of sulfadiazine followed by spectrophotometric and mode-mismatched thermal lens spectrometric determination. *Talanta* **2016**, *147*, 561–568, doi:10.1016/j.talanta.2015.10.033.

433. Wang, Y.; Liu, L.; Xiao, C.; Chen, L.; Yang, P.; Liu, Q.; Wang, J.; Liu, X. Rapid Determination of Trace Sulfonamides in Milk by Graphene Oxide-Based Magnetic Solid Phase Extraction Coupled with HPLC–MS/MS. *Food Anal. Methods* **2016**, *9*, 2521–2530, doi:10.1007/s12161-016-0433-6.

434. Xiao, R.; Zhang, X.; Zhang, X.; Niu, J.; Lu, M.; Liu, X.; Cai, Z. Analysis of flavors and fragrances by HPLC with Fe3O4@GO magnetic nanocomposite as the adsorbent. *Talanta* **2017**, *166*, 262–267, doi:10.1016/j.talanta.2017.01.065.

435. Feng, Y.; Hu, X.; Zhao, F.; Zeng, B. Fe 3 O 4 /reduced graphene oxide-carbon nanotubes composite for the magnetic solid-phase extraction and HPLC determination of sulfonamides in milk. *J. Sep. Sci.* **2019**, *42*, 1058–1066, doi:10.1002/jssc.201801177.

436. Fatta-Kassinos, D.; Nikolaou, A.; Ioannou-Ttofa, L. *Advances in analytical methods for the determination of pharmaceutical residues in waters and wastewaters*; Second Edi.; Elsevier, 2019; Vol. 1; ISBN 9780444639523.

437. Baltussen, E.; Sandra, P.; David, F.; Cramers, C. Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples: Theory and principles. *J. Microcolumn Sep.* **1999**, *11*, 737–747, doi:10.1002/(SICI)1520-667X(1999)11:10<737::AID-MCS7>3.0.CO;2-4.

438. Bruheim, I.; Liu, X.; Pawliszyn, J. Thin-film microextraction. *Anal. Chem.* **2003**, *75*, 1002–1010, doi:10.1021/ac026162q.

439. Kabir, A.; Furton, K.G. Fabric Phase Sorptive Extraction (FPSE) 2016.

440. Kabir, A.; Furton, K.G. *Fabric phase sorptive extraction: A new genration, green sample preparation approach*; Elsevier Inc., 2019; ISBN 9780128169063.

441. Kabir, A.; Furton, K.G.; Malik, A. Innovations in sol-gel microextraction phases for solvent-free sample preparation in analytical chemistry. *TrAC - Trends Anal. Chem.* 2013, *45*, 197–218.

442. Kumar, R.; Gaurav; Heena; Malik, A.K.; Kabir, A.; Furton, K.G. Efficient analysis of selected estrogens using fabric phase sorptive extraction and high performance liquid chromatography-fluorescence detection. *J. Chromatogr. A* **2014**, *1359*, 16–25,

doi:10.1016/j.chroma.2014.07.013.

443. Racamonde, I.; Rodil, R.; Quintana, J.B.; Sieira, B.J.; Kabir, A.; Furton, K.G.; Cela, R. Fabric phase sorptive extraction: A new sorptive microextraction technique for the determination of non-steroidal anti-inflammatory drugs from environmental water samples. *Anal. Chim. Acta* **2015**, *865*, 22–30, doi:10.1016/j.aca.2015.01.036.

444. Segro, S.S.; Tran, M.; Kesani, S.; Alhendal, A.; Turner, E.B.; Malik, A. Sol-gel microextraction phases for sample preconcentration in chromatographic analysis. *J. Sep. Sci.* 2010, *33*, 3075–3096.

445. Kazantzi, V.; Anthemidis, A. Fabric sol–gel phase sorptive extraction technique: A review. *Separations* **2017**, *4*, doi:10.3390/separations4020020.

446. Kabir, A.; Mesa, R.; Jurmain, J.; Furton, K.G. Fabric phase sorptive extraction explained. *Separations* **2017**, *4*, doi:10.3390/separations4020021.

447. Zilfidou, E.; Kabir, A.; Furton, K.G.; Samanidou, V. Fabric phase sorptive extraction: Current state of the art and future perspectives. *Separations* **2018**, *5*, 1–23, doi:10.3390/separations5030040.

448. Furton, K.G.; Kabir, A. Novel Sol-Gel Sorbents in Sorptive Microextraction Analytical Microextraction Techniques. *Anal. Microextraction Tech.* **2016**, 28–69.

449. Lakade, S.S.; Borrull, F.; Furton, K.G.; Kabir, A.; Fontanals, N.; Marcé, R.M. Comparative study of different fabric phase sorptive extraction sorbents to determine emerging contaminants from environmental water using liquid chromatography-tandem mass spectrometry. *Talanta* **2015**, *144*, 1342–1351, doi:10.1016/j.talanta.2015.08.009.

450. Kalaboka, M.; Chrimatopoulos, C.; Jim, C.; Boti, V. Exploring the Efficiency of UHPLC-Orbitrap MS for the Determination of 20 Pharmaceuticals and Acesulfame K in Hospital and Urban Wastewaters with the Aid of FPSE. *Separations* **2020**.

451. Karageorgou, E.; Manousi, N.; Samanidou, V.; Kabir, A.; Furton, K.G. Fabric phase sorptive extraction for the fast isolation of sulfonamides residues from raw milk followed by high performance liquid chromatography with ultraviolet detection. *Food Chem.* **2016**, *196*, 428–436, doi:10.1016/j.foodchem.2015.09.060.

452. Kabir, A.; Furton, K.G.; Tinari, N.; Grossi, L.; Innosa, D.; Macerola, D.; Tartaglia, A.; Di Donato, V.; D'Ovidio, C.; Locatelli, M. Fabric phase sorptive extraction-high performance liquid chromatography-photo diode array detection method for simultaneous monitoring of three inflammatory bowel disease treatment drugs in whole blood, plasma and urine. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2018**, *1084*, 53–63, doi:10.1016/j.jchromb.2018.03.028.

453. Locatelli, M.; Kabir, A.; Innosa, D.; Lopatriello, T.; Furton, K.G. *A fabric phase sorptive extraction-High performance liquid chromatography-Photo diode array detection method for the determination of twelve azole antimicrobial drug residues in human plasma and urine*; Elsevier B.V., 2017; Vol. 1040, pp. 192–198;.

454. Kaur, R.; Heena; Kaur, R.; Grover, A.; Rani, S.; Malik, A.K.; Kabir, A.; Furton, K.G. Trace determination of parabens in cosmetics and personal care products using fabric-phase sorptive extraction and high-performance liquid chromatography with UV detection. *J. Sep. Sci.* **2020**, *43*, 2626–2635, doi:10.1002/jssc.201900978.

455. Ulusoy, H.İ.; Köseoğlu, K.; Kabir, A.; Ulusoy, S.; Locatelli, M. Fabric phase sorptive extraction followed by HPLC-PDA detection for the monitoring of pirimicarb and fenitrothion pesticide residues. *Microchim. Acta* **2020**, *187*, 1–11, doi:10.1007/s00604-020-04306-7.

456. Celeiro, M.; Vazquez, L.; Nurerk, P.; Kabir, A.; Furton, K.G.; Dagnac, T.; Llompart, M. Fabric phase sorptive extraction for the determination of 17 multiclass fungicides in environmental water by gas chromatography-tandem mass spectrometry. *J. Sep. Sci.* **2020**, *43*, 1817–1829, doi:10.1002/jssc.201901232.

457. Jiménez-Holgado, C.; Chrimatopoulos, C.; Stathopoulos, V.; Sakkas, V. Investigating the utility of fabric phase sorptive extraction and HPLC-UV-Vis-DAD to determine antidepressant drugs in environmental aqueous samples. *Separations* **2020**, *7*, 1–14, doi:10.3390/separations7030039.

458. Tartaglia, A.; Kabir, A.; D'Ambrosio, F.; Ramundo, P.; Ulusoy, S.; Ulusoy, H.I.; Merone, G.M.; Savini, F.; D'Ovidio, C.; De Grazia, U.; et al. Fast off-line FPSE-HPLC-PDA determination of six NSAIDs in saliva samples. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2020**, *1144*, 122082, doi:10.1016/j.jchromb.2020.122082.

459. Ruan, X.; Xing, L.; Peng, J.; Li, S.; Song, Y.; Sun, Q. A simplified fabric phase sorptive extraction method for the determination of amphetamine drugs in water samples using liquid chromatography-mass spectrometry. *RSC Adv.* **2020**, *10*, 10854–10866, doi:10.1039/c9ra10138a.

460. Montesdeoca-Esponda, S.; Sosa-Ferrera, Z.; Kabir, A.; Furton, K.G.; Santana-Rodríguez, J.J. Fabric phase sorptive extraction followed by UHPLC-MS/MS for the analysis of benzotriazole UV stabilizers in sewage samples. *Anal. Bioanal. Chem.* **2015**, *407*, 8137–8150, doi:10.1007/s00216-015-8990-x.

461. García-Guerra, R.B.; Montesdeoca-Esponda, S.; Sosa-Ferrera, Z.; Kabir, A.; Furton, K.G.; Santana-Rodríguez, J.J. Rapid monitoring of residual UV-stabilizers in seawater samples from beaches using fabric phase sorptive extraction and UHPLC-MS/MS. *Chemosphere* **2016**, *164*, 201–207, doi:10.1016/j.chemosphere.2016.08.102.

462. Guedes-Alonso, R.; Ciofi, L.; Sosa-Ferrera, Z.; Santana-Rodríguez, J.J.J.J.; Del Bubba, M.; Kabir, A.; Furton, K.G.K.G. Determination of androgens and progestogens in environmental and biological samples using fabric phase sorptive extraction coupled to ultra-high performance liquid chromatography tandem mass spectrometry. *J. Chromatogr. A* **2016**, *1437*, 116–126, doi:10.1016/j.chroma.2016.01.077.

463. Rigkos, G.; Alampanos, V.; Kabir, A.; Furton, K.G.; Roje, Ž.; Vrček, I.V.; Panderi, I.; Samanidou, V. An improved fabric-phase sorptive extraction protocol for the determination of seven parabens in human urine by HPLC–DAD. *Biomed. Chromatogr.* **2020**, doi:10.1002/bmc.4974.

464. Zilfidou, E.; Kabir, A.; Furton, K.G.; Samanidou, V. An improved fabric phase sorptive extraction method for the determination of five selected antidepressant drug residues in human blood serum prior to high performance liquid chromatography with diode array detection. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2019**, *1125*, 121720, doi:10.1016/j.jchromb.2019.121720.

465. Alampanos, V.; Kabir, A.; Furton, K.G.; Samanidou, V.; Papadoyannis, I. Fabric phase sorptive extraction for simultaneous observation of four penicillin antibiotics from human blood serum prior to high performance liquid chromatography and photo-diode array detection. *Microchem. J.* **2019**, *149*, 103964, doi:10.1016/j.microc.2019.103964.

466. Kaur, R.; Kaur, R.; Rani, S.; Malik, A.; Kabir, A.; Furton, K.; Samanidou, V. Rapid Monitoring of Organochlorine Pesticide Residues in Various Fruit Juices and Water Samples Using Fabric Phase Sorptive Extraction and Gas Chromatography-Mass Spectrometry. *Molecules* **2019**, *24*, 1013, doi:10.3390/molecules24061013.

467. Pérez-Mayán, L.; Rodríguez, I.; Ramil, M.; Kabir, A.; Furton, K.G.; Cela, R. Fabric phase sorptive extraction followed by ultra-performance liquid chromatography-tandem mass spectrometry for the determination of fungicides and insecticides in wine. *J. Chromatogr. A* **2019**, *1584*, 13–23, doi:10.1016/j.chroma.2018.11.025.

468. Santana-Viera, S.; Guedes-Alonso, R.; Sosa-Ferrera, Z.; Santana-Rodríguez, J.J.; Kabir, A.; Furton, K.G. Optimization and application of fabric phase sorptive extraction coupled to ultra-high performance liquid chromatography tandem mass spectrometry for the determination of cytostatic drug residues in environmental waters. *J. Chromatogr. A* **2017**, *1529*, 39–49, doi:10.1016/j.chroma.2017.10.070.

469. Mesa, R.; Kabir, A.; Samanidou, V.; Furton, K.G. Simultaneous determination of selected estrogenic endocrine disrupting chemicals and bisphenol A residues in whole milk using fabric phase sorptive extraction coupled to HPLC-UV detection and LC-MS/MS. *J. Sep. Sci.* **2019**, *42*, 598–608, doi:10.1002/jssc.201800901.

470. Calvo-flores, F.G. Detection and Analysis of Chemical Pollutants. *Emerg. Pollut.* **2017**, 43–55, doi:10.1002/9783527691203.ch3.

471. Meyer, V.R. *CHROMATOGRAPHY | principles*; Elsevier Inc., 2019; ISBN 9780081019832.

472. Hage, D.S. *Chromatography*; 2018; ISBN 9780128160633.

473. Mattarozzi, M.; Careri, M. *Liquid Chromatography/Mass Spectrometry in Environmental Analysis*; 2015; ISBN 9780470027318.

474. Čelić, M.; Jaén-Gil, A.; Briceño-Guevara, S.; Rodríguez-Mozaz, S.; Gros, M.; Petrović, M. Extended suspect screening to identify contaminants of emerging concern in riverine and coastal ecosystems and assessment of environmental risks. *J. Hazard. Mater.* **2021**, *404*, doi:10.1016/j.jhazmat.2020.124102.

475. Meng, Y.; Liu, W.; Liu, X.; Zhang, J.; Peng, M.; Zhang, T. A review on analytical methods for pharmaceutical and personal care products and their transformation products. *J. Environ. Sci. (China)* 2021, *101*, 260–281.

476. Chang, W.C.W.; Wu, H.Y.; Yeh, Y.; Liao, P.C. Untargeted foodomics strategy using high-resolution mass spectrometry reveals potential indicators for fish freshness. *Anal. Chim. Acta* **2020**, *1127*, 98–105, doi:10.1016/j.aca.2020.06.016.

477. Cambrai, A.; Marchioni, E.; Julien-David, D.; Marcic, C. Discrimination of Cocoa Bean Origin by Chocolate Polyphenol Chromatographic Analysis and Chemometrics. *Food Anal. Methods* **2017**, *10*, 1991–2000, doi:10.1007/s12161-016-0744-7.

478. Shi, T.; Song, E.; Nie, S.; Rodland, K.D.; Liu, T.; Qian, W.J.; Smith, R.D. Advances in targeted proteomics and applications to biomedical research. *Proteomics* 2016, *16*, 2160–2182.

479. Sathe, G.; Na, C.H.; Renuse, S.; Madugundu, A.K.; Albert, M.; Moghekar, A.; Pandey, A. Quantitative Proteomic Profiling of Cerebrospinal Fluid to Identify Candidate Biomarkers for Alzheimer's Disease. *Proteomics - Clin. Appl.* **2019**, *13*, doi:10.1002/prca.201800105.

480. Mahmud, I.; Garrett, T.J. Mass Spectrometry Techniques in Emerging Pathogens Studies: COVID-19 Perspectives. *J. Am. Soc. Mass Spectrom.* 2020, *31*, 2013–2024.

481. Akçan, R.; Taştekin, B.; Yildirim, M.Ş.; Aydogan, H.C.; Sağlam, N. Omics era in forensic medicine: Towards a new age. *Turkish J. Med. Sci.* 2020, *50*, 1480–1490.

482. Xiao, Y.; Deng, J.; Yao, Y.; Fang, L.; Yang, Y.; Luan, T. Recent advances of ambient mass spectrometry imaging for biological tissues: A review. *Anal. Chim. Acta* 2020, *1117*, 74–88.

483. Thevis, M.; Kuuranne, T.; Geyer, H. *Annual banned-substance review: Analytical approaches in human sports drug testing*; John Wiley and Sons Ltd, 2018; Vol. 10, pp. 9–27;.

484. Awad, H.; Khamis, M.M.; El-Aneed, A. Mass spectrometry, review of the basics: Ionization. *Appl. Spectrosc. Rev.* **2015**, *50*, 158–175, doi:10.1080/05704928.2014.954046.

485. Hopfgartner, G. Theory and Instrumentation of Mass Spectrometry. *Mass Spectrom. Drug Metab. Dispos. Basic Princ. Appl.* **2011**, 255–290, doi:10.1002/9780470929278.ch8.

486. Hoffmann, E.; Stroobant, V. *Mass spectrometry Mass spectrometry*; 2016; Vol. 1040; ISBN 9783642107092.

487. Zhang, Y.V.; Wei, B.; Zhu, Y.; Zhang, Y.; Bluth, M.H. Liquid Chromatography–Tandem Mass Spectrometry: An Emerging Technology in the Toxicology Laboratory. *Clin. Lab. Med.* **2016**, *36*, 635–661, doi:10.1016/j.cll.2016.07.001.

488. Interfaces for LCMS : SHIMADZU (Shimadzu Corporation) Available online: https://www.shimadzu.com/an/service-support/technical-support/analysis-basics/fundamental/interfaces_for_lcms.html#section1 (accessed on Nov 16, 2020).

489. Naegele, E. Making your LC Method Compatible with Mass Spectrometry Technical Overview Author. *Tec. Overveiw* **2011**, 1–20.

490. SIUZDAK, G. An introduction to mass spectrometry ionization: An excerpt from The Expanding Role of Mass Spectrometry in Biotechnology, 2nd ed.; MCC Press: San Diego, 2005. *J. Assoc. Lab. Autom.* **2004**, *9*, 50–63, doi:10.1016/j.jala.2004.01.004.

491. Lifshitz, C.; Märk, T.D. Mass spectrometry, ionization theory. *Encycl. Spectrosc. Spectrom.* **2016**, 748–757, doi:10.1016/B978-0-12-803224-4.00184-9.

492. Mass analyzers - Mass analyzers - Chromedia.

493. Xian, F.; Hendrickson, C.L.; Marshall, A.G. High-resolution Mass spectrometry. *Anal. Chem.* **2012**, *84*, 708–719, doi:doi.org/10.1021/ac203191t.

494. Capability, Q.; Testing, F.S.; Contaminants, E.; Discovery, P.; Notes, A. Orbitrap quantitation : lab of the future Close your uncertainty gap with the selectivity and con dence of HRAM.

495. Strupat, K.; Scheibner, O.; Bromirski, M. Orbitrap Mass Spectrometry – Definitions , Opportunities , and Advantages. *Thermo Sci. Tech. Note 64287* **2013**.

496. Crawford Mass Spectrometry Fundamental LC-MS Orbitrap Mass Analyzers. *Crawford Sci.* **2011**.

497. Kaufmann, A.; Dvorak, V.; Crüzer, C.; Butcher, P.; Maden, K.; Walker, S.; Widmer, M.; Schürmann, A. Study of High-Resolution Mass Spectrometry Technology as a Replacement for Tandem Mass Spectrometry in the Field of Quantitative Pesticide Residue Analysis. *J. AOAC Int.* **2012**, *95*, 528–548, doi:10.5740/jaoacint.11-074.

498. Perry, R.H.; Cooks, R.G.; Noll, R.J. Orbitrap mass spectrometry: Instrumentation, ion motion and applications. *Mass Spectrom. Rev.* **2008**, *27*, 661–699, doi:10.1002/mas.20186.

499. Hu, Q.; Noll, R.J.; Li, H.; Makarov, A.; Hardman, M.; Cooks, R.G. The Orbitrap: A new mass spectrometer. *J. Mass Spectrom.* **2005**, *40*, 430–443, doi:10.1002/jms.856.

500. Makarov, A. Orbitrap Mass Spectrometry: Ultra- high Resolution for Every Lab Symposium: New Alternatives in High- Resolution Mass Spectrometry. **2012**.

501. Zubarev, R.A.; Makarov, A. Orbitrap mass spectrometry. *Anal. Chem.* **2013**, *85*, 5288–5296, doi:10.1021/ac4001223.

502. Hecht, E.S.; Scigelova, M.; Eliuk, S.; Makarov, A. *Fundamentals and Advances of Orbitrap Mass Spectrometry*; 2019; ISBN 9780470027318.

503. Bhattacharyya, D. LC-MS innovations : review of the technology and its advancements Triple quadrupole (QqQ) and Orbitrap-based mass spectrometry technology — choose the right tools for the right trade. *Thermo Fish. Sci. Boston, MA* **2018**.

504. Donna LTQ Orbitrap XL混合线性离子阱质谱仪对绿茶和红茶提取物的代谢组学分析. **中国食品 2012**, 82–85.

505. Domon, B.; Aebersold, R. Mass spectrometry and protein analysis. *Science (80-.).* **2006**, *312*, 212–217, doi:10.1126/science.1124619.

506. Thermo Scientific TSQ Series Manual. **2010**.

507. Kappler, L.; Lehmann, R. Mass-spectrometric multi-omics linked to function – State-of-the-art investigations of mitochondria in systems medicine. *TrAC - Trends Anal. Chem.* 2019, *119*, 115635.

508. Girolamo, F.; Lante, I.; Muraca, M.; Putignani, L. The Role of Mass Spectrometry in the "Omics" Era. *Curr. Org. Chem.* **2013**, *17*, 2891–2905, doi:10.2174/13852728178881311181627 25.

509. Stewart-Miller, H.; Massey, R. The mass spectrometry innovations simplifying drug discovery workflows. *Drug Discov. World* **2018**, *19*, 34–39.

510. Beccaria, M.; Cabooter, D. Current developments in LC-MS for pharmaceutical analysis. *Analyst* **2020**, *145*, 1129–1157, doi:10.1039/c9an02145k.

511. Siddiqui, M.R.; AlOthman, Z.A.; Rahman, N. Analytical Techniques in Pharmaceutical Analysis: A Review. *Arab. J. Chem.* **2017**, S1409–S1421.

512. Fernandes, C.L.; Carvalho, D.O.; Guido, L.F. Determination of acrylamide in biscuits by high-resolution orbitrap mass spectrometry: A novel application. *Foods* **2019**, *8*, doi:10.3390/foods8120597.

513. Alcántara-Durán, J.; Moreno-González, D.; Gilbert-López, B.; Molina-Díaz, A.; García-Reyes, J.F. Matrix-effect free multi-residue analysis of veterinary drugs in food samples of animal origin by nanoflow liquid chromatography high resolution mass spectrometry. *Food Chem.* **2018**, *245*, 29–38, doi:10.1016/j.foodchem.2017.10.083.

514. Chen, Q.; Pan, X.D.; Huang, B.F.; Han, J.L. Quantification of 16 β-lactams in chicken muscle by QuEChERS extraction and UPLC-Q-Orbitrap-MS with parallel reaction monitoring. *J. Pharm. Biomed. Anal.* **2017**, *145*, 525–530, doi:10.1016/j.jpba.2017.07.019.

515. Hong, Y.H.; Xu, X.L.; Li, W.Q.; Xu, B.Z.; Wu, H.Q.; Cheng, Y.; Chen, H.; Zhang, B.S.; Zhang, F. A high-accuracy screening method of 44 cephalosporins in meat using liquid chromatography quadrupole-orbitrap hybrid mass spectrometry. *Anal. Methods* **2017**, *9*, 6534–6548, doi:10.1039/c7ay01903c.

516. Wang, J.; Chow, W.; Chang, J.; Wong, J.W. Ultrahigh-performance liquid chromatography electrospray ionization Q-orbitrap mass spectrometry for the analysis of 451 pesticide residues in fruits and vegetables: Method development and validation. *J. Agric. Food Chem.* **2014**, *62*, 10375–10391, doi:10.1021/jf503778c.

517. Gardana, C.; Simonetti, P. Determination of steviol glycosides in commercial extracts of Stevia rebaudiana and sweeteners by ultra-high performance liquid chromatography Orbitrap mass spectrometry. *J. Chromatogr. A* **2018**, *1578*, 8–14, doi:10.1016/j.chroma.2018.09.057.

518. Liang, M.; Xian, Y.; Wang, B.; Hou, X.; Wang, L.; Guo, X.; Wu, Y.; Dong, H. High throughput analysis of 21 perfluorinated compounds in drinking water, tap water, river water and plant effluent from southern China by supramolecular solvents-based microextraction coupled with HPLC-Orbitrap HRMS. *Environ. Pollut.* **2020**, 114389, doi:10.1016/j.envpol.2020.114389.

519. Liu, S.; Junaid, M.; Zhong, W.; Zhu, Y.; Xu, N. A sensitive method for simultaneous determination of 12 classes of per- and polyfluoroalkyl substances (PFASs) in groundwater by ultrahigh performance liquid chromatography coupled with quadrupole orbitrap high resolution mass spectrometry. *Chemosphere* **2020**, *251*, 126327, doi:10.1016/j.chemosphere.2020.126327.

520. Nannou, C.I.; Boti, V.I.; Albanis, T.A. Trace analysis of pesticide residues in sediments using liquid chromatography–high-resolution Orbitrap mass spectrometry. *Anal. Bioanal. Chem.* **2018**, *410*, 1977–1989, doi:10.1007/s00216-018-0864-6.

521. Roy-Lachapelle, A.; Vo Duy, S.; Munoz, G.; Dinh, Q.T.; Bahl, E.; Simon, D.F.; Sauvé, S. Analysis of multiclass cyanotoxins (microcystins, anabaenopeptins, cylindrospermopsin and anatoxins) in lake waters using on-line SPE liquid chromatography high-resolution Orbitrap mass spectrometry. *Anal. Methods* **2019**, *11*, 5289–5300, doi:10.1039/c9ay01132c.

522. Ccanccapa-Cartagena, A.; Pico, Y.; Ortiz, X.; Reiner, E.J. Suspect, non-target and target screening of emerging pollutants using data independent acquisition: Assessment of a Mediterranean River basin. *Sci. Total Environ.* **2019**, *687*, 355–368, doi:10.1016/j.scitotenv.2019.06.057.

523. Guo, C.; Li, K.; Xing, S.; Sun, H.; Shi, F.; Zhang, G.; Sun, H. Application of quadrupole-Orbitrap high-resolution mass spectrometry in rapid screening and identification of synthetic dyes in herbal medicines. *Eur. J. Mass Spectrom.* **2019**, *25*, 419–427, doi:10.1177/1469066719829872.

524. Li, J.; Jiang, Y.; Li, D. Determination of imidacloprid and its relevant metabolites in tomato using modified QuEChERS combined with ultrahigh-pressure liquid chromatography/Orbitrap tandem mass spectrometry. *J. Sci. Food Agric.* **2019**, *99*, 5211–5218, doi:10.1002/jsfa.9769.

525. Helfer, A.G.; Michely, J.A.; Weber, A.A.; Meyer, M.R.; Maurer, H.H. Liquid chromatography-high resolution-tandem mass spectrometry using Orbitrap technology for comprehensive screening to detect drugs and their metabolites in blood plasma. *Anal. Chim. Acta* **2017**, *965*, 83–95, doi:10.1016/j.aca.2017.03.002.

526. Peters, R.J.B.; Stolker, A.A.M.; Mol, J.G.J.; Lommen, A.; Lyris, E.; Angelis, Y.; Vonaparti, A.; Stamou, M.; Georgakopoulos, C.; Nielen, M.W.F. Screening in veterinary drug analysis and sports doping control based on full-scan, accurate-mass spectrometry. *TrAC - Trends Anal. Chem.* 2010, *29*, 1250–1268.

527. Jagerdeo, E.; Schaff, J.E. Rapid screening for drugs of abuse in biological fluids by ultra high performance liquid chromatography/Orbitrap mass spectrometry. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2016**, *1027*, 11–18, doi:10.1016/j.jchromb.2016.05.010.

528. Miyaguchi, H.; Inoue, H. Determination of amphetamine-type stimulants, cocaine and ketamine in human hair by liquid chromatography/linear ion trap-Orbitrap hybrid mass spectrometry. *Analyst* **2011**, *136*, 3503–3511, doi:10.1039/c0an00850h.

529. Scarpone, R.; Rosato, R.; Chiumiento, F.; Cipolletti, C.; Sergi, M.; Compagnone, D. Preliminary Study to Develop an Alternative Method for the Non-targeted Determination of Xenobiotics in Food by Means of Ultra High Performance Liquid Chromatography Coupled to High Resolution and Accuracy Mass Spectrometry. *Food Anal. Methods* **2020**, *13*, 1099–1110, doi:10.1007/s12161-020-01727-1.

530. Blaga, G.V.; Chițescu, C.L.; Lisă, E.L.; Dumitru, C.; Vizireanu, C.; Borda, D. Antifungal residues analysis in various Romanian honey samples analysis by high resolution mass spectrometry. *J. Environ. Sci. Heal. - Part B Pestic. Food Contam. Agric. Wastes* **2020**, *55*, 484–494, doi:10.1080/03601234.2020.1724016.

531. Rusko, J.; Pugajeva, I.; Perkons, I.; Reinholds, I.; Bartkiene, E.; Bartkevics, V. Development of a rapid method for the determination of phenolic antioxidants in dark chocolate using ultra performance liquid chromatography coupled to orbitrap mass spectrometry. *J. Chromatogr. Sci.* **2019**, *57*, 434–442, doi:10.1093/chromsci/bmz013.

532. Saito-Shida, S.; Hamasaka, T.; Nemoto, S.; Akiyama, H. Multiresidue determination of pesticides in tea by liquid chromatography-high-resolution mass spectrometry: Comparison between Orbitrap and time-of-flight mass analyzers. *Food Chem.* **2018**, *256*, 140–148, doi:10.1016/j.foodchem.2018.02.123.

533. Li, H.; Shen, H.; Fang, J.; Zhang, J.; Ding, X. Rapid determination of 66 antibiotics in cosmetics using ultra high performance liquid chromatography-linear ion trap/Orbitrap mass spectrometry. *Chinese J. Chromatogr. (Se Pu)* **2018**, *36*, 643–650, doi:10.3724/SP.J.1123.2018.01041.

534. Lee, H.J.; Ryu, H.D.; Lim, D.Y.; Chung, E.G.; Kim, K.; Lee, J.K. Characteristics of veterinary antibiotics in intensive livestock farming watersheds with different liquid manure application programs using UHPLC-q-orbitrap HRMS combined with on-line SPE. *Sci. Total Environ.* **2020**, *749*, doi:10.1016/j.scitotenv.2020.142375.

535. Wang, S.Y.; Fodjo, E.K.; Kong, C.; Yu, H.J. Multi-residue screening of pesticides in aquaculture waters through ultra-high-performance liquid chromatograph-Q/orbitrap mass spectrometry. *Water (Switzerland)* **2020**, *12*, doi:10.3390/W12051238.

536. Moreno-González, D.; Cutillas, V.; Hernando, M.D.; Alcántara-Durán, J.; García-Reyes, J.F.; Molina-Díaz, A. Quantitative determination of pesticide residues in specific parts of bee specimens by nanoflow liquid chromatography high resolution mass spectrometry. *Sci. Total Environ.* **2020**, *715*, doi:10.1016/j.scitotenv.2020.137005.

537. Gray, A.D.; Todd, D.; Hershey, A.E. The seasonal distribution and concentration of antibiotics in rural streams and drinking wells in the piedmont of North Carolina. *Sci. Total Environ.* **2020**, *710*, doi:10.1016/j.scitotenv.2019.136286.

538. Plassmann, M.M.; Schmidt, M.; Brack, W.; Krauss, M. Detecting a wide range of environmental contaminants in human blood samples—combining QuEChERS with LC-MS and GC-MS methods. *Anal. Bioanal. Chem.* **2015**, *407*, doi:10.1007/s00216-015-8857-1.

539. Abou-Elwafa Abdallah, M.; Nguyen, K.H.; Ebele, A.J.; Atia, N.N.; Ali, H.R.H.; Harrad, S. A single run, rapid polarity switching method for determination of 30 pharmaceuticals and personal care products in waste water using Q-Exactive Orbitrap high resolution accurate mass spectrometry. *J. Chromatogr. A* **2019**, *1588*, 68–76, doi:10.1016/j.chroma.2018.12.033.

540. Nannou, C.I.; Boti, V.I.; Albanis, T.A. A modified QuEChERS approach for the analysis of pharmaceuticals in sediments by LC-

Orbitrap HRMS. *Anal. Bioanal. Chem.* **2019**, *411*, 1383–1396, doi:10.1007/s00216-018-01570-8.

541. Beck, O.; Rylski, A.; Stephanson, N.N. Application of liquid chromatography combined with high resolution mass spectrometry for urine drug testing. In *Critical Issues in Alcohol and Drugs of Abuse Testing*; Elsevier, 2019; pp. 321–332 ISBN 9780128156070.
542. Althakafy, J.T.; Kulsing, C.; Grace, M.R.; Marriott, P.J. Determination of selected emerging contaminants in freshwater invertebrates using a universal extraction technique and liquid chromatography accurate mass spectrometry. *J. Sep. Sci.* **2018**, *41*, 3706–3715, doi:10.1002/jssc.201800507.
543. Ahad, J.M.E.; Pakdel, H.; Lavoie, D.; Lefebvre, R.; Peru, K.M.; Headley, J. V. Naphthenic acids in groundwater overlying undeveloped shale gas and tight oil reservoirs. *Chemosphere* **2018**, *191*, 664–672, doi:10.1016/j.chemosphere.2017.10.015.
544. Asghar, M.A.; Zhu, Q.; Sun, S.; Peng, Y.; Shuai, Q. Suspect screening and target quantification of human pharmaceutical residues in the surface water of Wuhan, China, using UHPLC-Q-Orbitrap HRMS. *Sci. Total Environ.* **2018**, *635*, 828–837, doi:10.1016/j.scitotenv.2018.04.179.
545. Huysman, S.; Van Meulebroek, L.; Vanryckeghem, F.; Van Langenhove, H.; Demeestere, K.; Vanhaecke, L. Development and validation of an ultra-high performance liquid chromatographic high resolution Q-Orbitrap mass spectrometric method for the simultaneous determination of steroidal endocrine disrupting compounds in aquatic matrices. *Anal. Chim. Acta* **2017**, *984*, 140–150, doi:10.1016/j.aca.2017.07.001.
546. Prosen, H.; Fontanals, N.; Borrull, F.; Marcé, R.M. Determination of seven drugs of abuse and their metabolites in surface and wastewater using solid-phase extraction coupled to liquid chromatography with high-resolution mass spectrometry. *J. Sep. Sci.* **2017**, *40*, 3621–3631, doi:10.1002/jssc.201700287.
547. Alves, A.; Giovanoulis, G.; Nilsson, U.; Erratico, C.; Lucattini, L.; Haug, L.S.; Jacobs, G.; De Wit, C.A.; Leonards, P.E.G.; Covaci, A.; et al. Case Study on Screening Emerging Pollutants in Urine and Nails. *Environ. Sci. Technol.* **2017**, *51*, 4046–4053, doi:10.1021/acs.est.6b05661.
548. Guidi, L.R.; Santos, F.A.; Ribeiro, A.C.S.R.; Fernandes, C.; Silva, L.H.M.; Gloria, M.B.A. A simple, fast and sensitive screening LC-ESI-MS/MS method for antibiotics in fish. *Talanta* **2017**, *163*, 85–93, doi:10.1016/j.talanta.2016.10.089.
549. McArdell, C.S.; Molnar, E.; Suter, M.J.F.; Giger, W. Occurrence and Fate of Macrolide Antibiotics in Wastewater Treatment Plants and in the Glatt Valley Watershed, Switzerland. *Environ. Sci. Technol.* **2003**, *37*, 5479–5486, doi:10.1021/es034368i.
550. Fohner, A.E.; Sparreboom, A.; Altman, R.B.; Klein, T.E. PharmGKB summary. *Pharmacogenet. Genomics* **2017**, *27*, 164–167, doi:10.1097/FPC.0000000000000270.
551. Afsa, S.; Hamden, K.; Lara Martin, P.A.; Mansour, H. Ben Occurrence of 40 pharmaceutically active compounds in hospital and urban wastewaters and their contribution to Mahdia coastal seawater contamination. *Environ. Sci. Pollut. Res.* **2020**, *27*, 1941–1955, doi:10.1007/s11356-019-06866-5.
552. Hillebrand, O.; Musallam, S.; Scherer, L.; Nödler, K.; Licha, T. The challenge of sample-stabilisation in the era of multi-residue analytical methods: A practical guideline for the stabilisation of 46 organic micropollutants in aqueous samples. *Sci. Total Environ.* **2013**, *454–455*, 289–298, doi:10.1016/j.scitotenv.2013.03.028.
553. Vanderford, B.J.; Mawhinney, D.B.; Trenholm, R.A.; Zeigler-Holady, J.C.; Snyder, S.A. Assessment of sample preservation techniques for pharmaceuticals, personal care products, and steroids in surface and drinking water. *Anal. Bioanal. Chem.* **2011**, *399*, 2227–2234, doi:10.1007/s00216-010-4608-5.
554. Baker, D.R.; Kasprzyk-Hordern, B. Critical evaluation of methodology commonly used in sample collection, storage and preparation for the analysis of pharmaceuticals and illicit drugs in surface water and wastewater by solid phase extraction and liquid chromatography-mass spectrometry. *J. Chromatogr. A* **2011**, *1218*, 8036–8059, doi:10.1016/j.chroma.2011.09.012.
555. Schubert, B.; Heininger, P.; Keller, M.; Claus, E.; Ricking, M. Monitoring of contaminants in suspended particulate matter as an alternative to sediments. *TrAC - Trends Anal. Chem.* 2012, *36*, 58–70.
556. Merck Analytical Quality Assurance , Standard for COD. **2012**, *49*, 1–2.
557. Merck-Appication Photometric determination using the PMB method after decomposition with the thermoreactor. **2016**, *49*, 2–4.
558. Chemicals, M. Phosphate Cell Test. **2011**, *49*, 64271.
559. US EPA, O. *Total Nitrogen*;
560. WTW Nitrogen Cell Test. **2018**, *49*, 64271.

561. WTW-Application Report *Respirometric BOD5 determination of domestic waste water*; 2010;

562. WTW-Application Report Respirometric BOD 5 determination of waste water polluted with organic or inorganic toxins or inhibitors. **2010**, 1–8.

563. Fan, W.; Lai, Q.; Zhang, Q.; Wang, Y. Nanocomposites of TiO2 and reduced graphene oxide as efficient photocatalysts for hydrogen evolution. *J. Phys. Chem. C* **2011**, *115*, 10694–10701, doi:10.1021/jp2008804.

564. Chatzimitakos, T.G.; Karali, K.K.; Stalikas, C.D. Magnetic graphene oxide as a convenient nanosorbent to streamline matrix solid-phase dispersion towards the extraction of pesticides from vegetables and their determination by GC–MS. *Microchem. J.* **2019**, *151*, 104247, doi:10.1016/j.microc.2019.104247.

565. Maidatsi, K. V.; Chatzimitakos, T.G.; Sakkas, V.A.; Stalikas, C.D. Octyl-modified magnetic graphene as a sorbent for the extraction and simultaneous determination of fragrance allergens, musks, and phthalates in aqueous samples by gas chromatography with mass spectrometry. *J. Sep. Sci.* **2015**, *38*, 3758–3765, doi:10.1002/jssc.201500578.

566. Jiang, C.; Sun, Y.; Yu, X.; Gao, Y.; Zhang, L.; Wang, Y.; Zhang, H.; Song, D. Application of C18-functional magnetic nanoparticles for extraction of aromatic amines from human urine. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2014**, *947–948*, 49–56, doi:10.1016/j.jchromb.2013.12.008.

567. Zhang, Y.; Kuang, M.; Zhang, L.; Yang, P.; Lu, H. An accessible protocol for solid-phase extraction of N-linked glycopeptides through reductive amination by amine-functionalized magnetic nanoparticles. *Anal. Chem.* **2013**, *85*, 5535–5541, doi:10.1021/ac400733y.

568. Smith, G.; Wermuth, U.D.; Healy, P.C. Hydrogen bonding in proton-transfer compounds of 5-sulfosalicylic acid with ortho-substituted monocyclic heteroaromatic Lewis bases. *J. Chem. Crystallogr.* **2006**, *36*, 841–849, doi:10.1007/s10870-006-9122-4.

569. Tahmasebi, E.; Yamini, Y.; Moradi, M.; Esrafili, A. Polythiophene-coated Fe3O4 superparamagnetic nanocomposite: Synthesis and application as a new sorbent for solid-phase extraction. *Anal. Chim. Acta* **2013**, *770*, 68–74.

570. Maddah, B.; Shamsi, J. Extraction and preconcentration of trace amounts of diazinon and fenitrothion from environmental water by magnetite octadecylsilane nanoparticles. *J. Chromatogr. A* **2012**, *1256*, 40–45, doi:10.1016/j.chroma.2012.07.085.

571. Kabir, A.; Furton, K.G. Fabric Phase Sorptive Extractor (FPSE). *U.S. Pat. US 20140274660A1* 2014, *18*, 1–24.

572. Celeiro, M.; Acerbi, R.; Kabir, A.; Furton, K.G.; Llompart, M. Development of an analytical methodology based on fabric phase sorptive extraction followed by gas chromatography-tandem mass spectrometry to determine UV filters in environmental and recreational waters. *Anal. Chim. Acta X* **2020**, *4*, doi:10.1016/j.acax.2019.100038.

573. Jiménez-Holgado, C.; Chrimatopoulos, C.; Stathopoulos, V.; Sakkas, V. Investigating the Utility of Fabric Phase Sorptive Extraction and HPLC-UV-Vis/DAD to Determine Antidepressant Drugs in Environmental Aqueous Samples. *Separations* **2020**, *7*, 39, doi:10.3390/separations7030039.

574. Samanidou, V.; Galanopoulos, L.D.; Kabir, A.; Furton, K.G. Fast extraction of amphenicols residues from raw milk using novel fabric phase sorptive extraction followed by high-performance liquid chromatography-diode array detection. *Anal. Chim. Acta* **2015**, *855*, 41–50, doi:10.1016/j.aca.2014.11.036.

575. Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. *Off. J. Eur. Communities* **2002**, *L221*, 8–36.

576. FDA, F. and V.M.P. Acceptance Criteria for Confirmation of Identity of Chemical Residues using Exact Mass Data within the Office of Foods and Veterinary Medicine. **2015**, 1–16.

577. Schymanski, E.L.; Jeon, J.; Gulde, R.; Fenner, K.; Ruff, M.; Singer, H.P.; Hollender, J. Identifying small molecules via high resolution mass spectrometry: Communicating confidence. *Environ. Sci. Technol.* **2014**, *48*, 2097–2098, doi:10.1021/es5002105.

578. *2002/657/EC: Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (Text with EEA relevance) (notified under document number C(2002) 3044) - Publica*;

579. Bipm *International vocabulary of metrology-Basic and general concepts and associated terms (VIM) 3rd edition 2008 version with minor corrections Vocabulaire international de métrologie-Concepts fondamentaux et généraux et termes associés (VIM) 3 e édition*; 2012;

580. Eurachem *The Fitness for Purpose of Analytical Methods A Laboratory Guide to Method Validation and Related Topics The Fitness for Purpose of Analytical Methods A Laboratory Guide to Method Validation and Related Topics Second Edition 2014 i Eurachem Guide The Fitn*; 2014; ISBN 9789187461590.

581. ISO 5725-1:1994 Accuracy (trueness and precision) of measurement methods and results - Part 1: General principles and definitions. *Measurement* 1994, *ISO 5725-1*.

582. Gosetti, F.; Mazzucco, E.; Zampieri, D.; Gennaro, M.C. Signal suppression/enhancement in high-performance liquid chromatography tandem mass spectrometry. *J. Chromatogr. A* 2010, *1217*, 3929–3937.

583. Luigi Silvestro, T.S. and R.S. Matrix Effects in Mass Spectrometry Combined with Separation Methods — Comparison HPLC, GC and Discussion on Methods to Control these Effects. *Intech* **2012**, 13, doi:10.1016/j.colsurfa.2011.12.014.

584. Zielinski, T.; Dragan-górska, A. *Insights onEnvironmentalChanges*; 2014; ISBN 9783319036830.

585. Annesley, T.M. Ion suppression in mass spectrometry. *Clin. Chem.* 2003, *49*, 1041–1044.

586. Furey, A.; Moriarty, M.; Bane, V.; Kinsella, B.; Lehane, M. Ion suppression; A critical review on causes, evaluation, prevention and applications. *Talanta* 2013, *115*, 104–122.

587. Panuwet, P.; Hunter, R.E.; D'Souza, P.E.; Chen, X.; Radford, S.A.; Cohen, J.R.; Marder, M.E.; Kartavenka, K.; Ryan, P.B.; Barr, D.B. Biological Matrix Effects in Quantitative Tandem Mass Spectrometry-Based Analytical Methods: Advancing Biomonitoring. *Crit. Rev. Anal. Chem.* 2016, *46*, 93–105.

588. Ghosh, C.; Shinde, C.P.; Chakraborty, B.S. Influence of ionization source design on matrix effects during LC-ESI-MS/MS analysis. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2012**, *893–894*, 193–200, doi:10.1016/j.jchromb.2012.03.012.

589. Ikonoraou, M.G.; Blades, A.T.; Kebarle, P. Investigations of the Electrospray Interface for Liquid Chromatography/Mass Spectrometry. *Anal. Chem.* **1990**, *62*, 957–967, doi:10.1021/ac00208a012.

590. Cech, N.B.; Enke, C.G. Practical implications of some recent studies in electrospray ionization fundamentals. *Mass Spectrom. Rev.* **2001**, *20*, 362–387, doi:10.1002/mas.10008.

591. Trufelli, H.; Palma, P.; Famiglini, G.; Cappiello, A. An overview of matrix effects in liquid chromatography-mass spectrometry. *Mass Spectrom. Rev.* 2011, *30*, 491–509.

592. Hall, T.G.; Smukste, I.; R., K.; Wang, Y.; McKearn, D.; E., R. Identifying and Overcoming Matrix Effects in Drug Discovery and Development. In *Tandem Mass Spectrometry - Applications and Principles*; InTech, 2012.

593. Mei, H.; Hsieh, Y.; Nardo, C.; Xu, X.; Wang, S.; Ng, K.; Korfmacher, W.A. Investigation of matrix effects in bioanalytical high-performance liquid chromatography/tandem mass spectrometric assays: Application to drug discovery. *Rapid Commun. Mass Spectrom.* **2003**, *17*, 97–103, doi:10.1002/rcm.876.

594. Matuszewski, B.K. Standard line slopes as a measure of a relative matrix effect in quantitative HPLC-MS bioanalysis. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2006**, *830*, 293–300, doi:10.1016/j.jchromb.2005.11.009.

595. Dams, R.; Benijts, T.; Günther, W.; Lambert, W.; De Leenheer, A. Influence of the eluent composition on the ionization efficiency for morphine of pneumatically assisted electrospray, atmospheric-pressure chemical ionization and sonic spray. *Rapid Commun. Mass Spectrom.* **2002**, *16*, 1072–1077, doi:10.1002/rcm.683.

596. Holčapek, M.; Volná, K.; Jandera, P.; Kolářová, L.; Lemr, K.; Exner, M.; Církva, A. Effects of ion-pairing reagents on the electrospray signal suppression of sulphonated dyes and intermediates. *J. Mass Spectrom.* **2004**, *39*, 43–50, doi:10.1002/jms.551.

597. Gustavsson, S.Å.; Samskog, J.; Markides, K.E.; Långström, B. Studies of signal suppression in liquid chromatography-electrospray ionization mass spectrometry using volatile ion-pairing reagents. *J. Chromatogr. A* **2001**, *937*, 41–47, doi:10.1016/S0021-9673(01)01328-0.

598. Mallet, C.R.; Lu, Z.; Mazzeo, J.R. A study of ion suppression effects in electrospray ionization from mobile phase additives and solid-phase extracts. *Rapid Commun. Mass Spectrom.* **2004**, *18*, 49–58, doi:10.1002/rcm.1276.

599. Blomberg, L.G. Two new techniques for sample preparation in bioanalysis: Microextraction in packed sorbent (MEPS) and use of a bonded monolith as sorbent for sample preparation in polypropylene tips for 96-well plates. *Anal. Bioanal. Chem.* 2009, *393*, 797–807.

600. Cappiello, A.; Famiglini, G.; Pierini, E.; Palma, P.; Trufelli, H. Advanced liquid chromatography-mass spectrometry interface based on electron ionization. *Anal. Chem.* **2007**, *79*, 5364–5372, doi:10.1021/ac070468l.

601. Bonfiglio, R.; King, R.C.; Olah, T. V.; Merkle, K. The effects of sample preparation methods on the variability of the electrospray ionization response for model drug compounds. *Rapid Commun. Mass Spectrom.* **1999**, *13*, 1175–1185, doi:10.1002/(SICI)1097-0231(19990630)13:12<1175::AID-RCM639>3.0.CO;2-0.

602. Yaroshenko, D. V.; Kartsova, L.A. Matrix effect and methods for its elimination in bioanalytical methods using chromatography-

mass spectrometry. *J. Anal. Chem.* 2014, *69*, 311–317.

603. King, R.; Bonfiglio, R.; Fernandez-Metzler, C.; Miller-Stein, C.; Olah, T. Mechanistic investigation of ionization suppression in electrospray ionization. *J. Am. Soc. Mass Spectrom.* **2000**, *11*, 942–950, doi:10.1016/S1044-0305(00)00163-X.
604. Gros, M.; Rodríguez-Mozaz, S.; Barceló, D. Fast and comprehensive multi-residue analysis of a broad range of human and veterinary pharmaceuticals and some of their metabolites in surface and treated waters by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem. *J. Chromatogr. A* **2012**, *1248*, 104–121, doi:10.1016/j.chroma.2012.05.084.
605. Huerta, B.; Rodriguez-Mozaz, S.; Nannou, C.; Nakis, L.; Ruhí, A.; Acuña, V.; Sabater, S.; Barcelo, D. Determination of a broad spectrum of pharmaceuticals and endocrine disruptors in biofilm from a waste water treatment plant-impacted river. *Sci. Total Environ.* **2016**, *540*, 241–249, doi:10.1016/j.scitotenv.2015.05.049.
606. Economou, A.; Botitsi, H.; Antoniou, S.; Tsipi, D. Determination of multi-class pesticides in wines by solid-phase extraction and liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A* **2009**, *1216*, 5856–5867, doi:10.1016/j.chroma.2009.06.031.
607. Arias, J.L. de O.; Rombaldi, C.; Caldas, S.S.; Primel, E.G. Alternative sorbents for the dispersive solid-phase extraction step in quick, easy, cheap, effective, rugged and safe method for extraction of pesticides from rice paddy soils with determination by liquid chromatography tandem mass spectrometry. *J. Chromatogr. A* **2014**, *1360*, 66–75, doi:10.1016/j.chroma.2014.07.082.
608. MARCHI, I.; RUDAZ, S.; SELMAN, M.; VEUTHEY, J. Evaluation of the influence of protein precipitation prior to on-line SPE–LC–API/MS procedures using multivariate data analysis☆. *J. Chromatogr. B* **2007**, *845*, 244–252, doi:10.1016/j.jchromb.2006.08.045.
609. Chernetsova, E.S.; Koryakova, A.G. High-performance liquid chromatography coupled to mass spectrometry for studying new pharmaceutical entities. *J. Anal. Chem.* 2010, *65*, 1436–1445.
610. Hsieh, Y.; Merkle, K.; Wang, G.; Brisson, J.M.; Korfmacher, W.A. High-performance liquid chromatography-atmospheric pressure photoionization/tandem mass spectrometric analysis for small molecules in plasma. *Anal. Chem.* **2003**, *75*, 3122–3127, doi:10.1021/ac0300082.
611. Dams, R.; Huestis, M.A.; Lambert, W.E.; Murphy, C.M. Matrix effect in bio-analysis of illicit drugs with LC-MS/MS: Influence of ionization type, sample preparation, and biofluid. *J. Am. Soc. Mass Spectrom.* **2003**, *14*, 1290–1294, doi:10.1016/S1044-0305(03)00574-9.
612. Liang, H.R.; Foltz, R.L.; Meng, M.; Bennett, P. Ionization enhancement in atmospheric pressure chemical ionization and suppression in electrospray ionization between target drugs and stable-isotope-labeled internal standards in quantitative liquid chromatography/tandem mass spectrometry. In Proceedings of the Rapid Communications in Mass Spectrometry; John Wiley and Sons Ltd, 2003; Vol. 17, pp. 2815–2821.
613. Van Eeckhaut, A.; Lanckmans, K.; Sarre, S.; Smolders, I.; Michotte, Y. Validation of bioanalytical LC-MS/MS assays: Evaluation of matrix effects. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* 2009, *877*, 2198–2207.
614. Kamel, A.; Jeanville, P.; Colizza, K.; J-Rivera, L.E. Mechanism of [M + H]+ Formation in Atmospheric Pressure Photoionization Mass Spectrometry: Identification of Propionitrile in Acetonitrile with High Mass Accuracy Measurement and Tandem Mass Spectrometry and Evidence for Its Involvement in the Protonation Phenomenon. *J. Am. Soc. Mass Spectrom.* **2008**, *19*, 1579–1589, doi:10.1016/j.jasms.2008.06.027.
615. Tiller, P.R.; Romanyshyn, L.A. Implications of matrix effects in ultra-fast gradient or fast isocratic liquid chromatography with mass spectrometry in drug discovery. *Rapid Commun. Mass Spectrom.* **2002**, *16*, 92–98, doi:10.1002/rcm.544.
616. Müller, C.; Schäfer, P.; Störtzel, M.; Vogt, S.; Weinmann, W. Ion suppression effects in liquid chromatography-electrospray-ionisation transport-region collision induced dissociation mass spectrometry with different serum extraction methods for systematic toxicological analysis with mass spectra libraries. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2002**, *773*, 47–52, doi:10.1016/S1570-0232(02)00142-3.
617. Gianotti, V.; Chiuminatto, U.; Mazzucco, E.; Gosetti, F.; Bottaro, M.; Frascarolo, P.; Gennaro, M.C. A new hydrophilic interaction liquid chromatography tandem mass spectrometry method for the simultaneous determination of seven biogenic amines in cheese. *J. Chromatogr. A* **2008**, *1185*, 296–300, doi:10.1016/j.chroma.2008.02.038.
618. Pascoe, R.; Foley, J.P.; Gusev, A.I. Reduction in matrix-related signal suppression effects in electrospray ionization mass spectrometry using on-line two-dimensional liquid chromatography. *Anal. Chem.* **2001**, *73*, 6014–6023, doi:10.1021/ac0106694.
619. Choi, B.K.; Hercules, D.M.; Gusev, A.I. Effect of liquid chromatography separation of complex matrices on liquid chromatography-

tandem mass spectrometry signal suppression. *J. Chromatogr. A* **2001**, *907*, 337–342, doi:10.1016/S0021-9673(00)01052-9.

620. Gros, M.; Petrović, M.; Barceló, D. Development of a multi-residue analytical methodology based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) for screening and trace level determination of pharmaceuticals in surface and wastewaters. *Talanta* **2006**, *70*, 678–690, doi:10.1016/j.talanta.2006.05.024.
621. Steiner, D.; Krska, R.; Malachová, A.; Taschl, I.; Sulyok, M. Evaluation of Matrix Effects and Extraction Efficiencies of LC-MS/MS Methods as the Essential Part for Proper Validation of Multiclass Contaminants in Complex Feed. *J. Agric. Food Chem.* **2020**, *68*, 3868–3880, doi:10.1021/acs.jafc.9b07706.
622. Gurke, R.; Rossmann, J.; Schubert, S.; Sandmann, T.; Rößler, M.; Oertel, R.; Fauler, J. Development of a SPE-HPLC-MS/MS method for the determination of most prescribed pharmaceuticals and related metabolites in urban sewage samples. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2015**, *990*, 23–30, doi:10.1016/j.jchromb.2015.03.008.
623. Loos, G.; Van Schepdael, A.; Cabooter, D. Quantitative mass spectrometry methods for pharmaceutical analysis. *Philos. Trans. R. Soc. A Math. Phys. Eng. Sci.* **2016**, *374*, doi:10.1098/rsta.2015.0366.
624. Hopper, J.T.S.; Sokratous, K.; Oldham, N.J. Charge state and adduct reduction in electrospray ionization-mass spectrometry using solvent vapor exposure. *Anal. Biochem.* **2012**, *421*, 788–790, doi:10.1016/j.ab.2011.10.034.
625. Shou, W.Z.; Jiang, X.; Beato, B.D.; Naidong, W. A highly automated 96-well solid phase extraction and liquid chromatography/tandem mass spectrometry method for the determination of fentanyl in human plasma. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 466–476, doi:10.1002/rcm.255.
626. Naidong, W.; Chen, Y.L.; Shou, W.; Jiang, X. Importance of injection solution composition for LC-MS-MS methods. *J. Pharm. Biomed. Anal.* **2001**, doi:10.1016/S0731-7085(01)00439-3.
627. Makarov, A.; Denisov, E.; Lange, O.; Horning, S. Dynamic Range of Mass Accuracy in LTQ Orbitrap Hybrid Mass Spectrometer. *J. Am. Soc. Mass Spectrom.* **2006**, *17*, 977–982, doi:10.1016/j.jasms.2006.03.006.
628. Roiffé, R.R.; Ribeiro, W.D.; Sardela, V.F.; de la Cruz, M.N.S.; de Souza, K.R.; Pereira, H.M.G.; Aquino Neto, F.R. Development of a sensitive and fast method for detection of catecholamines and metabolites by HRMS. *Microchem. J.* **2019**, *150*, doi:10.1016/j.microc.2019.104173.
629. Kalli, A.; Smith, G.T.; Sweredoski, M.J.; Hess, S. Evaluation and optimization of mass spectrometric mode: Focus on LTQ-Orbitrap Mass analyzers. *J. Proteome Res.* **2014**, *12*, 3071–3086, doi:10.1021/pr3011588.Evaluation.
630. Rusko, J.; Jansons, M.; Pugajeva, I.; Zacs, D.; Bartkevics, V. Development and optimization of confirmatory liquid chromatography—Orbitrap mass spectrometry method for the determination of 17 anticoccidials in poultry and eggs. *J. Pharm. Biomed. Anal.* **2019**, *164*, 402–412, doi:10.1016/j.jpba.2018.10.056.
631. Pugajeva, I.; Rusko, J.; Perkons, I.; Lundanes, E.; Bartkevics, V. Determination of pharmaceutical residues in wastewater using high performance liquid chromatography coupled to quadrupole-Orbitrap mass spectrometry. *J. Pharm. Biomed. Anal.* **2017**, doi:10.1016/j.jpba.2016.11.008.
632. Agüera, A.; Martínez-Piernas, A.B.; Campos-Mañas, M.C. Analytical Strategies Used in HRMS. In *Applications in High Resolution Mass Spectrometry: Food Safety and Pesticide Residue Analysis*; Elsevier Inc., 2017; pp. 59–82 ISBN 9780128096482.
633. Paíga, P.; Silva, L.M.S.; Delerue-Matos, C. Optimization of the Ion Source-Mass Spectrometry Parameters in Non-Steroidal Anti-Inflammatory and Analgesic Pharmaceuticals Analysis by a Design of Experiments Approach. *J. Am. Soc. Mass Spectrom.*, doi:10.1007/s13361-016-1459-0.
634. Lee, J.H.; Park, H.N.; Kim, N.S.; Park, S.; Lee, Y.M.; Kang, H. Development of a specific fragmentation pattern-based quadrupole-Orbitrap™ mass spectrometry method to screen drugs in illicit products. *Sci. Justice* **2020**, *60*, 86–94, doi:10.1016/j.scijus.2019.08.001.
635. Guo, L.; Ye, P.; Wang, J.; Fu, F.; Wu, Z. Three-dimensional Fe3O4-graphene macroscopic composites for arsenic and arsenate removal. *J. Hazard. Mater.* **2015**, *298*, 28–35, doi:10.1016/j.jhazmat.2015.05.011.
636. Feng, L.; Cao, M.; Ma, X.; Zhu, Y.; Hu, C. Superparamagnetic high-surface-area Fe 3O 4 nanoparticles as adsorbents for arsenic removal. *J. Hazard. Mater.* **2012**, *217–218*, 439–446, doi:10.1016/j.jhazmat.2012.03.073.
637. Thi, N.; Hoan, V.; Thu, A.; Duc, H. Van; Cuong, N.D.; Khieu, D.Q.; Vo, V.; Vuong, N.T.; Vn, H. Fe3O4/Reduced Graphene Oxide Nanocomposite: Synthesis and Its Application for Toxic Metal Ion Removal. *J. Chem.* **2016**, *2016*, 1–10, doi:10.1155/2016/2418172.
638. Rai, A.K.; Gim, J.; Thi, T.V.; Ahn, D.; Cho, S.J.; Kim, J. High Rate capability and long cycle stability of Co3O 4/cofe2O4 nanocomposite

as an anode material for high-performance secondary lithium ion batteries. *J. Phys. Chem. C* **2014**, *118*, 11234–11243, doi:10.1021/jp502004c.

639. Chatzimarkou, A.; Stalikas, C. Adsorptive Removal of Estriol from Water Using Graphene-Based Materials and Their Magnetite Composites: Heterogeneous Fenton-Like Non-toxic Degradation on Magnetite/Graphene Oxide. *Int. J. Environ. Res.* **2020**, *14*, 269–287, doi:10.1007/s41742-020-00255-4.
640. Kyzas, G.Z.; Travlou, N.A.; Kalogirou, O.; Deliyanni, E.A. Magnetic graphene oxide: Effect of preparation route on reactive black 5 adsorption. *Materials (Basel).* **2013**, *6*, 1360–1376, doi:10.3390/ma6041360.
641. Sheshmani, S.; Falahat, B.; Nikmaram, F.R. Preparation of magnetic graphene oxide-ferrite nanocomposites for oxidative decomposition of Remazol Black B. *Int. J. Biol. Macromol.* **2017**, *97*, 671–678, doi:10.1016/j.ijbiomac.2017.01.041.
642. Silva, V.A.J.; Andrade, P.L.; Silva, M.P.C.; Bustamante, A.D.; De Los Santos Valladares, L.; Albino Aguiar, J. Synthesis and characterization of Fe3O4 nanoparticles coated with fucan polysaccharides. *J. Magn. Magn. Mater.* **2013**, *343*, 138–143, doi:10.1016/j.jmmm.2013.04.062.
643. Liu, Y.X.; Jian, G.Q.; He, X.W.; Chen, L.X.; Zhang, Y.K. Preparation and application of core-shell structural carbon nanotubes-molecularly imprinted composite material for determination of nafcillin in egg samples. *Fenxi Huaxue/ Chinese J. Anal. Chem.* **2013**, *41*, 161–166, doi:10.1016/S1872-2040(13)60626-X.
644. Mhatre, A.M.; Chappa, S.; Ojha, S.; Pandey, A.K. Functionalized glass fiber membrane for extraction of iodine species. *Sep. Sci. Technol.* **2019**, *54*, 1469–1477, doi:10.1080/01496395.2018.1520729.
645. Ochoa, M.; Durães, L.; Matos Beja, A.; Portugal, A. Study of the suitability of silica based xerogels synthesized using ethyltrimethoxysilane and/or methyltrimethoxysilane precursors for aerospace applications., doi:10.1007/s10971-011-2604-7.
646. Shameli, K.; Ahmad, M. Bin; Jazayeri, S.D.; Sedaghat, S.; Shabanzadeh, P.; Jahangirian, H.; Mahdavi, M.; Abdollahi, Y. Synthesis and characterization of polyethylene glycol mediated silver nanoparticles by the green method. *Int. J. Mol. Sci.* **2012**, *13*, 6639–6650, doi:10.3390/ijms13066639.
647. Park, J.Y.; Daksha, P.; Lee, G.H.; Woo, S.; Chang, Y. Highly water-dispersible PEG surface modified ultra small superparamagnetic iron oxide nanoparticles useful for target-specific biomedical applications. *Nanotechnology* **2008**, *19*, doi:10.1088/0957-4484/19/36/365603.
648. Montarsolo, A.; Periolatto, M.; Zerbola, M.; Mossotti, R.; Ferrero, F. Hydrophobic sol-gel finishing for textiles: improvement by plasma pre-treatment. *Text. Res. J. 83*, 1190–1200.
649. Módolo, M.L.; Valandro, S.R.; Pessoa, C.A.; Fujiwara, S.T. Carbon ceramic electrodes obtained by basic catalysis of sol-gel process. *Electrochim. Acta* **2013**, *112*, 783–790, doi:10.1016/j.electacta.2013.09.028.
650. Montesdeoca-Esponda, S.; Sosa-Ferrera, Z.; Kabir, A.; Furton, K.G.; Santana-Rodríguez, J.J. Fabric phase sorptive extraction followed by UHPLC-MS/MS for the analysis of benzotriazole UV stabilizers in sewage samples. *Anal. Bioanal. Chem.* **2015**, *407*, 8137–8150, doi:10.1007/s00216-015-8990-x.
651. Liu, Q.; Shi, J.; Zeng, L.; Wang, T.; Cai, Y.; Jiang, G. Evaluation of graphene as an advantageous adsorbent for solid-phase extraction with chlorophenols as model analytes. *J. Chromatogr. A* **2011**, *1218*, 197–204, doi:10.1016/j.chroma.2010.11.022.
652. Chatzimitakos, T.; Samanidou, V.; Stalikas, C.D. Graphene-functionalized melamine sponges for microextraction of sulfonamides from food and environmental samples. *J. Chromatogr. A* **2017**, *1522*, 1–8, doi:10.1016/J.CHROMA.2017.09.043.
653. Chen, H.; Gao, B.; Li, H. Removal of sulfamethoxazole and ciprofloxacin from aqueous solutions by graphene oxide. *J. Hazard. Mater.* **2015**, *282*, 201–207, doi:10.1016/j.jhazmat.2014.03.063.
654. Al-Behadili, M.B.H.; Shah-Hosseini, A.; Mohebinia, A.; Eftekhari, M. Polythiophene-coated cerium oxide nanocomposite for efficient solid-phase extraction of trace levels of Zn2+ followed by flame atomic absorption spectrometry. *Polym. Bull.* **2020**, *77*, 323–337, doi:10.1007/s00289-019-02748-5.
655. Kazemi, E.; Dadfarnia, S.; Haji Shabani, A.M. Dispersive solid phase microextraction with magnetic graphene oxide as the sorbent for separation and preconcentration of ultra-trace amounts of gold ions. *Talanta* **2015**, *141*, 273–278, doi:10.1016/j.talanta.2015.04.024.
656. Sun, J.; Liang, Q.; Han, Q.; Zhang, X.; Ding, M. One-step synthesis of magnetic graphene oxide nanocomposite and its application in magnetic solid phase extraction of heavy metal ions from biological samples. *Talanta* **2015**, *132*, 557–563, doi:10.1016/j.talanta.2014.09.043.

657. Liu, F.F.; Zhao, J.; Wang, S.; Xing, B. Adsorption of sulfonamides on reduced graphene oxides as affected by pH and dissolved organic matter. *Environ. Pollut.* **2016**, doi:10.1016/j.envpol.2015.11.053.

658. Chatzimitakos, T.G.; Stalikas, C.D. Melamine sponge decorated with copper sheets as a material with outstanding properties for microextraction of sulfonamides prior to their determination by high-performance liquid chromatography. *J. Chromatogr. A* **2018**, *1554*, 28–36, doi:10.1016/J.CHROMA.2018.04.015.

659. Chatzimitakos, T.; Binellas, C.; Maidatsi, K.; Stalikas, C. Magnetic ionic liquid in stirring-assisted drop-breakup microextraction: Proof-of-concept extraction of phenolic endocrine disrupters and acidic pharmaceuticals. *Anal. Chim. Acta* **2016**, *910*, 53–59, doi:10.1016/j.aca.2016.01.015.

660. Wu, L.; Liu, L.; Gao, B.; Muñoz-Carpena, R.; Zhang, M.; Chen, H.; Zhou, Z.; Wang, H. Aggregation kinetics of graphene oxides in aqueous solutions: Experiments, mechanisms, and modeling. *Langmuir* **2013**, doi:10.1021/la404134x.

661. Chowdhury, I.; Duch, M.C.; Mansukhani, N.D.; Hersam, M.C.; Bouchard, D. Colloidal properties and stability of graphene oxide nanomaterials in the aquatic environment. *Environ. Sci. Technol.* **2013**, doi:10.1021/es400483k.

662. Kosma, C.I.; Lambropoulou, D.A.; Albanis, T.A. Occurrence and removal of PPCPs in municipal and hospital wastewaters in Greece. *J. Hazard. Mater.* **2010**, *179*, 804–817, doi:10.1016/j.jhazmat.2010.03.075.

663. Dasenaki, M.E.; Thomaidis, N.S. Multianalyte method for the determination of pharmaceuticals in wastewater samples using solid-phase extraction and liquid chromatography-tandem mass spectrometry. *Anal. Bioanal. Chem.* **2015**, *407*, 4229–4245, doi:10.1007/s00216-015-8654-x.

664. Kokotou, M.G.; Thomaidis, N.S. Determination of eight artificial sweeteners in wastewater by hydrophilic interaction liquid chromatography-tandem mass spectrometry. *Anal. Methods* **2013**, *5*, 3825–3833, doi:10.1039/c3ay40599k.

665. ChemAxon ChemAxon - Software Solutions and Services for Chemistry and Biology Available online: https://chemaxon.com/ (accessed on Aug 3, 2020).

666. Carpinteiro, I.; Ramil, M.; Rodríguez, I.; Nogueira, J.M.F. Combining stir-bar sorptive extraction and large volume injection-gas chromatography-mass spectrometry for the determination of benzotriazole UV stabilizers in wastewater matrices. *J. Sep. Sci.* **2012**, *35*, 459–467, doi:10.1002/jssc.201100448.

667. Serôdio, P.; Nogueira, J.M.F. Multi-residue screening of endocrine disrupters chemicals in water samples by stir bar sorptive extraction-liquid desorption-capillary gas chromatography-mass spectrometry detection. *Anal. Chim. Acta* **2004**, *517*, 21–32, doi:10.1016/j.aca.2004.04.045.

668. Prieto, A.; Basauri, O.; Rodil, R.; Usobiaga, A.; Fernández, L.A.; Etxebarria, N.; Zuloaga, O. Stir-bar sorptive extraction: A view on method optimisation, novel applications, limitations and potential solutions. *J. Chromatogr. A* 2010, *1217*, 2642–2666.

669. Samanidou, V.; Michaelidou, K.; Kabir, A.; Furton, K.G. Fabric phase sorptive extraction of selected penicillin antibiotic residues from intact milk followed by high performance liquid chromatography with diode array detection. *Food Chem.* **2017**, *224*, 131–138, doi:10.1016/j.foodchem.2016.12.024.

670. Čizmić, M.; Babić, S.; Kaštelan-Macan, M. Multi-class determination of pharmaceuticals in wastewaters by solid-phase extraction and liquid chromatography tandem mass spectrometry with matrix effect study. *Environ. Sci. Pollut. Res.* **2017**, *24*, 20521–20539, doi:10.1007/s11356-017-9660-7.

671. Semreen, M.H.; Shanableh, A.; Semerjian, L.; Alniss, H.; Mousa, M.; Bai, X.; Acharya, K. Simultaneous Determination of Pharmaceuticals by Solid-phase Extraction and Liquid Chromatography-Tandem Mass Spectrometry: A case study from sharjah sewage treatment plant. *Molecules* **2019**, *24*, 1–16, doi:10.3390/molecules24030633.

672. Seifrtová, M.; Pena, A.; Lino, C.M.; Solich, P. Determination of fluoroquinolone antibiotics in hospital and municipal wastewaters in Coimbra by liquid chromatography with a monolithic column and fluorescence detection. *Anal. Bioanal. Chem.* **2008**, *391*, 799–805, doi:10.1007/s00216-008-2020-1.

673. *Oasis Sample Extraction Products Purity by SPE*;

674. Loos, R.; Carvalho, R.; António, D.C.; Comero, S.; Locoro, G.; Tavazzi, S.; Paracchini, B.; Ghiani, M.; Lettieri, T.; Blaha, L.; et al. EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents. *Water Res.* **2013**, *47*, 6475–6487, doi:10.1016/j.watres.2013.08.024.

675. Kim, C.; Ryu, H.D.; Chung, E.G.; Kim, Y.; Lee, J. kwan A review of analytical procedures for the simultaneous determination of medically important veterinary antibiotics in environmental water: Sample preparation, liquid chromatography, and mass

spectrometry. *J. Environ. Manage.* 2018, *217*, 629–645.

676. Díaz-Cruz, M.S.; Barceló, D. Determination of antimicrobial residues and metabolites in the aquatic environment by liquid chromatography tandem mass spectrometry. *Anal. Bioanal. Chem.* 2006, *386*, 973–985.
677. Gros, M.; Petrović, M.; Barceló, D. Tracing pharmaceutical residues of different therapeutic classes in environmental waters by using liquid chromatography/quadrupole-linear ion trap mass spectrometry and automated library searching. *Anal. Chem.* **2009**, *81*, 898–912, doi:10.1021/ac801358e.
678. Gómez, M.J.; Petrović, M.; Fernández-Alba, A.R.; Barceló, D. Determination of pharmaceuticals of various therapeutic classes by solid-phase extraction and liquid chromatography-tandem mass spectrometry analysis in hospital effluent wastewaters. *J. Chromatogr. A* **2006**, *1114*, 224–233, doi:10.1016/j.chroma.2006.02.038.
679. Steen, R.J.C.A.; Hogenboom, A.C.; Leonards, P.E.G.; Peerboom, R.A.L.; Cofino, W.P.; Brinkman, U.A.T. Ultra-trace-level determination of polar pesticides and their transformation products in surface and estuarine water samples using column liquid chromatography-electrospray tandem mass spectrometry. *J. Chromatogr. A* **1999**, *857*, 157–166, doi:10.1016/S0021-9673(99)00772-4.
680. Schmitt-Kopplin, P.; Burhenne, J.; Freitag, D.; Spiteller, M.; Kettrup, A. Development of capillary electrophoresis methods for the analysis of fluoroquinolones and application to the study of the influence of humic substances on their photodegradation in aqueous phase. *J. Chromatogr. A* **1999**, *837*, 253–265, doi:10.1016/S0021-9673(99)00079-5.
681. Zhou, W.; Yang, S.; Wang, P.G. Matrix effects and application of matrix effect factor. *Bioanalysis* 2017, *9*, 1839–1844.
682. Wu, D.; Sui, Q.; Yu, X.; Zhao, W.; Li, Q.; Fatta-Kassinos, D.; Lyu, S. Identification of indicator PPCPs in landfill leachates and livestock wastewaters using multi-residue analysis of 70 PPCPs: Analytical method development and application in Yangtze River Delta, China. *Sci. Total Environ.* **2021**, *753*, 141653, doi:10.1016/j.scitotenv.2020.141653.
683. Pan, X.; Qiang, Z.; Ben, W.; Chen, M. Simultaneous determination of three classes of antibiotics in the suspended solids of swine wastewater by ultrasonic extraction, solid-phase extraction and liquid chromatography-mass spectrometry. *J. Environ. Sci.* **2011**, *23*, 1729–1737, doi:10.1016/S1001-0742(10)60590-6.
684. Ben, W.; Qiang, Z.; Adams, C.; Zhang, H.; Chen, L. Simultaneous determination of sulfonamides, tetracyclines and tiamulin in swine wastewater by solid-phase extraction and liquid chromatography-mass spectrometry. *J. Chromatogr. A* **2008**, *1202*, 173–180, doi:10.1016/j.chroma.2008.07.014.
685. Verlicchi, P. *Hospital wastewaters : The handbook of environmental chemistry*; 2018; Vol. 60; ISBN 978-3-319-62177-7.
686. Us-epa; Region; Sesd; Athens; Ga *Wastewater Sampling*;
687. Khan, N.A.; Ahmed, S.; Farooqi, I.H.; Ali, I.; Vambol, V.; Changani, F.; Yousefi, M.; Vambol, S.; Khan, S.U.; Khan, A.H. Occurrence, sources and conventional treatment techniques for various antibiotics present in hospital wastewaters: A critical review. *TrAC - Trends Anal. Chem.* 2020, *129*, 115921.
688. Arbeláez, P.; Borrull, F.; Pocurull, E.; Marcé, R.M. Determination of high-intensity sweeteners in river water and wastewater by solid-phase extraction and liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A* **2015**, *1393*, 106–114, doi:10.1016/j.chroma.2015.03.035.
689. Sylvetsky, A.C.; Rother, K.I. Trends in the consumption of low-calorie sweeteners. *Physiol. Behav.* **2016**, *164*, 446–450, doi:10.1016/j.physbeh.2016.03.030.
690. Soh, L.; Connors, K.A.; Brooks, B.W.; Zimmerman, J. Fate of sucralose through environmental and water treatment processes and impact on plant indicator species. *Environ. Sci. Technol.* **2011**, *45*, 1363–1369, doi:10.1021/es102719d.
691. Gan, Z.; Sun, H.; Feng, B.; Wang, R.; Zhang, Y. Occurrence of seven artificial sweeteners in the aquatic environment and precipitation of Tianjin, China. *Water Res.* **2013**, *47*, 4928–4937, doi:10.1016/j.watres.2013.05.038.
692. Subedi, B.; Kannan, K. Fate of artificial sweeteners in wastewater treatment plants in New York State, U.S.A. *Environ. Sci. Technol.* **2014**, *48*, 13668–13674, doi:10.1021/es504769c.
693. Van Stempvoort, D.R.; Brown, S.J.; Spoelstra, J.; Garda, D.; Robertson, W.D.; Smyth, S.A. Variable persistence of artificial sweeteners during wastewater treatment: Implications for future use as tracers. *Water Res.* **2020**, *184*, doi:10.1016/j.watres.2020.116124.
694. Al Aukidy, M.; Verlicchi, P.; Jelic, A.; Petrovic, M.; Barcelò, D. Monitoring release of pharmaceutical compounds: Occurrence and environmental risk assessment of two WWTP effluents and their receiving bodies in the Po Valley, Italy. *Sci. Total Environ.* **2012**,

438, 15–25, doi:10.1016/j.scitotenv.2012.08.061.

695. Tran, N.H.; Hu, J.; Ong, S.L. Simultaneous determination of PPCPs, EDCs, and artificial sweeteners in environmental water samples using a single-step SPE coupled with HPLC-MS/MS and isotope dilution. *Talanta* **2013**, *113*, 82–92, doi:10.1016/j.talanta.2013.03.072.
696. Douziech, M.; Conesa, I.R.; Benítez-López, A.; Franco, A.; Huijbregts, M.; Van Zelm, R. Quantifying variability in removal efficiencies of chemicals in activated sludge wastewater treatment plants-a meta-analytical approach. In Proceedings of the Environmental Science: Processes and Impacts; Royal Society of Chemistry, 2018; Vol. 20, pp. 171–182.
697. Tran, N.H.; Gan, J.; Nguyen, V.T.; Chen, H.; You, L.; Duarah, A.; Zhang, L.; Gin, K.Y.H. Sorption and biodegradation of artificial sweeteners in activated sludge processes. *Bioresour. Technol.* **2015**, *197*, 329–338, doi:10.1016/j.biortech.2015.08.083.
698. Perkola, N.; Vaalgamaa, S.; Jernberg, J.; Vähätalo, A. V. Degradation of artificial sweeteners via direct and indirect photochemical reactions. *Environ. Sci. Pollut. Res.* **2016**, *23*, 13288–13297, doi:10.1007/s11356-016-6489-4.
699. Tran, N.H.; Nguyen, V.T.; Urase, T.; Ngo, H.H. Role of nitrification in the biodegradation of selected artificial sweetening agents in biological wastewater treatment process. *Bioresour. Technol.* **2014**, *161*, 40–46, doi:10.1016/j.biortech.2014.02.116.
700. Neset, T.S.S.; Singer, H.; Longrée, P.; Bader, H.P.; Scheidegger, R.; Wittmer, A.; Andersson, J.C.M. Understanding consumption-related sucralose emissions - A conceptual approach combining substance-flow analysis with sampling analysis. *Sci. Total Environ.* **2010**, *408*, 3261–3269, doi:10.1016/j.scitotenv.2010.04.003.
701. Ferrer, I.; Thurman, E.M. Analysis of sucralose and other sweeteners in water and beverage samples by liquid chromatography/time-of-flight mass spectrometry. *J. Chromatogr. A* **2010**, *1217*, 4127–4134, doi:10.1016/j.chroma.2010.02.020.
702. Sang, Z.; Jiang, Y.; Tsoi, Y.K.; Leung, K.S.Y. Evaluating the environmental impact of artificial sweeteners: A study of their distributions, photodegradation and toxicities. *Water Res.* **2014**, *52*, 260–274, doi:10.1016/j.watres.2013.11.002.
703. Pang, L.; Borthwick, A.G.L.; Chatzisymeon, E. Determination, occurrence, and treatment of saccharin in water: A review. *J. Clean. Prod.* 2020, *270*, 122337.
704. Rashidi, A.M.; Amadeh, A. The effect of saccharin addition and bath temperature on the grain size of nanocrystalline nickel coatings. *Surf. Coatings Technol.* **2009**, *204*, 353–358, doi:10.1016/j.surfcoat.2009.07.036.
705. Watanabe, E.; Nascimento, A.P.; Guerreiro-Tanomaru, J.M.; Razaboni, A.M.; de Andrade, D.; Tanomaru-Filho, M. Antiseptic mouthwashes: in vitro antibacterial activity. *Acta Odontol. Latinoam.* **2015**, *28*, 180–184, doi:10.1590/S1852-48342015000200014.
706. Spoelstra, J.; Roy, J.W.; Brown, S.J. Syringe-Tip Filters May Contain the Artificial Sweetener Saccharin. *Groundw. Monit. Remediat.* **2016**, *36*, 71–74, doi:10.1111/gwmr.12183.
707. Li, D.; O'Brien, J.W.; Tscharke, B.J.; Choi, P.M.; Zheng, Q.; Ahmed, F.; Thompson, J.; Li, J.; Mueller, J.F.; Sun, H.; et al. National wastewater reconnaissance of artificial sweetener consumption and emission in Australia. *Environ. Int.* **2020**, *143*, 105963, doi:10.1016/j.envint.2020.105963.
708. Soffritti, M.; Padovani, M.; Tibaldi, E.; Falcioni, L.; Manservisi, F.; Belpoggi, F. The carcinogenic effects of aspartame: The urgent need for regulatory re-evaluation. *Am. J. Ind. Med.* **2014**, *57*, 383–397, doi:10.1002/ajim.22296.
709. Ezerskis, Z.; Keltti, J.; Robouch, P. *EURL Evaluation Report on the Analytical Methods submitted in connection with the Application for the Authorisation of Feed Additives according to Regulation (EC) No 1831/2003 Active Substance(s): Report revised by: Date: EUROPEAN COMMISSION JOINT RESEARCH CENTRE Institute for Reference Materials and Measurements European Union Reference Laboratory for Feed Additives*; 2012;
710. Sardesai, V.M.; Waldshan, T.H. Natural and synthetic intense sweeteners. *J. Nutr. Biochem.* 1991, *2*, 236–244.
711. Kasprzyk-Hordern, B.; Dinsdale, R.M.; Guwy, A.J. The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. *Water Res.* **2009**, *43*, 363–380, doi:10.1016/j.watres.2008.10.047.
712. Kosma, C.I.; Lambropoulou, D.A.; Albanis, T.A. Investigation of PPCPs in wastewater treatment plants in Greece: Occurrence, removal and environmental risk assessment. *Sci. Total Environ.* **2014**, *466–467*, 421–438, doi:10.1016/j.scitotenv.2013.07.044.
713. Nannou, C.I.; Kosma, C.I.; Albanis, T.A. Occurrence of pharmaceuticals in surface waters: analytical method development and environmental risk assessment. *Int. J. Environ. Anal. Chem.* **2015**, *95*, 1242–1262, doi:10.1080/03067319.2015.1085520.
714. Stamatis, N.; Triantafyllidis, V.; Hela, D.; Konstantinou, I. Occurrence and distribution of selected pharmaceutical compounds on

sewage-impacted section of River Acheloos, Western Greece. *Int. J. Environ. Anal. Chem.* **2013**, *93*, 1602–1619, doi:10.1080/03067319.2013.814121.

715. Valanciene, E.; Jonuskiene, I.; Syrpas, M.; Augustiniene, E.; Matulis, P.; Simonavicius, A.; Malys, N. Advances and prospects of phenolic acids production, biorefinery and analysis. *Biomolecules* 2020, *10*, 1–41.
716. Verenitch, S.S.; Lowe, C.J.; Mazumder, A. Determination of acidic drugs and caffeine in municipal wastewaters and receiving waters by gas chromatography-ion trap tandem mass spectrometry. *J. Chromatogr. A* **2006**, *1116*, 193–203, doi:10.1016/j.chroma.2006.03.005.
717. Gracia-Lor, E.; Sancho, J. V.; Serrano, R.; Hernández, F. Occurrence and removal of pharmaceuticals in wastewater treatment plants at the Spanish Mediterranean area of Valencia. *Chemosphere* **2012**, *87*, 453–462, doi:10.1016/j.chemosphere.2011.12.025.
718. Aydin, S.; Aydin, M.E.; Ulvi, A. Monitoring the release of anti-inflammatory and analgesic pharmaceuticals in the receiving environment. *Environ. Sci. Pollut. Res.* **2019**, *26*, 36887–36902, doi:10.1007/s11356-019-06821-4.
719. The European Parlament and the Council of the European Union Directives of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. *Off. J. Eur. Union* **2013**, *2013*, 1–17, doi:http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:32013L0039.
720. Oliveira, T.S.; Murphy, M.; Mendola, N.; Wong, V.; Carlson, D.; Waring, L. Characterization of Pharmaceuticals and Personal Care products in hospital effluent and waste water influent/effluent by direct-injection LC-MS-MS. *Sci. Total Environ.* **2015**, *518–519*, 459–478, doi:10.1016/j.scitotenv.2015.02.104.
721. Couto, C.F.; Lange, L.C.; Amaral, M.C.S. Occurrence, fate and removal of pharmaceutically active compounds (PhACs) in water and wastewater treatment plants—A review. *J. Water Process Eng.* 2019, *32*, 100927.
722. Quoc Tuc, D.; Elodie, M.G.; Pierre, L.; Fabrice, A.; Marie-Jeanne, T.; Martine, B.; Joelle, E.; Marc, C. Fate of antibiotics from hospital and domestic sources in a sewage network. *Sci. Total Environ.* **2017**, *575*, 758–766, doi:10.1016/j.scitotenv.2016.09.118.
723. Yilmaz, G.; Kaya, Y.; Vergili, I.; Beril Gönder, Z.; Özhan, G.; Ozbek Celik, B.; Altinkum, S.M.; Bagdatli, Y.; Boergers, A.; Tuerk, J. Characterization and toxicity of hospital wastewaters in Turkey. *Environ. Monit. Assess.* **2017**, *189*, doi:10.1007/s10661-016-5732-2.
724. Serna-Galvis, E.A.; Silva-Agredo, J.; Botero-Coy, A.M.; Moncayo-Lasso, A.; Hernández, F.; Torres-Palma, R.A. Effective elimination of fifteen relevant pharmaceuticals in hospital wastewater from Colombia by combination of a biological system with a sonochemical process. *Sci. Total Environ.* **2019**, *670*, 623–632, doi:10.1016/j.scitotenv.2019.03.153.
725. Thai, P.K.; Ky, L.X.; Binh, V.N.; Nhung, P.H.; Nhan, P.T.; Hieu, N.Q.; Dang, N.T.T.; Tam, N.K.B.; Anh, N.T.K. Occurrence of antibiotic residues and antibiotic-resistant bacteria in effluents of pharmaceutical manufacturers and other sources around Hanoi, Vietnam. *Sci. Total Environ.* **2018**, *645*, 393–400, doi:10.1016/j.scitotenv.2018.07.126.
726. Wegst-Uhrich, S.R.; Navarro, D.A.G.; Zimmerman, L.; Aga, D.S. Assessing antibiotic sorption in soil: A literature review and new case studies on sulfonamides and macrolides. *Chem. Cent. J.* 2014, *8*.
727. Kolpin, D.W.; Furlong, E.T.; Meyer, M.T.; Thurman, E.M.; Zaugg, S.D.; Barber, L.B.; Buxton, H.T. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: A national reconnaissance. *Environ. Sci. Technol.* **2002**, *36*, 1202–1211, doi:10.1021/es011055j.
728. Marx, C.; Günther, N.; Schubert, S.; Oertel, R.; Ahnert, M.; Krebs, P.; Kuehn, V. Mass flow of antibiotics in a wastewater treatment plant focusing on removal variations due to operational parameters. *Sci. Total Environ.* **2015**, *538*, 779–788, doi:10.1016/j.scitotenv.2015.08.112.
729. Bengtsson-Palme, J.; Larsson, D.G.J. Concentrations of antibiotics predicted to select for resistant bacteria: Proposed limits for environmental regulation. *Environ. Int.* **2016**, *86*, 140–149, doi:10.1016/j.envint.2015.10.015.
730. Zhang, H.; Du, M.; Jiang, H.; Zhang, D.; Lin, L.; Ye, H.; Zhang, X. Occurrence, seasonal variation and removal efficiency of antibiotics and their metabolites in wastewater treatment plants, Jiulongjiang River Basin, South China. *Environ. Sci. Process. Impacts* **2015**, *17*, 225–234, doi:10.1039/c4em00457d.
731. García-Galán, M.J.; Díaz-Cruz, M.S.; Barceló, D. Occurrence of sulfonamide residues along the Ebro river basin. Removal in wastewater treatment plants and environmental impact assessment. *Environ. Int.* **2011**, *37*, 462–473, doi:10.1016/j.envint.2010.11.011.
732. Gros, M.; Rodríguez-Mozaz, S.; Barceló, D. Rapid analysis of multiclass antibiotic residues and some of their metabolites in hospital,

urban wastewater and river water by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry. *J. Chromatogr. A* **2013**, *1292*, 173–188, doi:10.1016/j.chroma.2012.12.072.

733. Lindberg, R.; Jarnheimer, P.Å.; Olsen, B.; Johansson, M.; Tysklind, M. Determination of antibiotic substances in hospital sewage water using solid phase extraction and liquid chromatography/mass spectrometry and group analogue internal standards. *Chemosphere* **2004**, *57*, 1479–1488, doi:10.1016/j.chemosphere.2004.09.015.
734. Statistics - ELSTAT Available online: https://www.statistics.gr/en/statistics/-/publication/SPG06/- (accessed on Sep 19, 2020).
735. Salter, A.J. Overview trimethoprim-sulfamethoxazole: An assessment of more than 12 years of use. *Rev. Infect. Dis.* **1982**, *4*, 196–236, doi:10.1093/clinids/4.2.196.
736. Hitchings, G.H. Mechanism of action of trimethoprim-sulfamethoxazole wellcome research laboratories, research triangle park, north carolina. *J. Infect. Dis.* **1973**, *128*, S433–S436, doi:10.1093/infdis/128.Supplement_3.S433.
737. Lien, L.T.Q.; Hoa, N.Q.; Chuc, N.T.K.; Thoa, N.T.M.; Phuc, H.D.; Diwan, V.; Dat, N.T.; Tamhankar, A.J.; Lundborg, C.S. Antibiotics in wastewater of a rural and an urban hospital before and after wastewater treatment, and the relationship with antibiotic use-a one year study from Vietnam. *Int. J. Environ. Res. Public Health* **2016**, *13*, 588, doi:10.3390/ijerph13060588.
738. González-Curbelo, M.Á.; Socas-Rodríguez, B.; Herrera-Herrera, A.V.; González-Sálamo, J.; Hernández-Borges, J.; Rodríguez-Delgado, M.Á. Evolution and applications of the QuEChERS method. *TrAC Trends Anal. Chem.* **2015**, *71*, 169–185, doi:10.1016/J.TRAC.2015.04.012.
739. Thiebault, T. Sulfamethoxazole/Trimethoprim ratio as a new marker in raw wastewaters: A critical review. *Sci. Total Environ.* 2020, *715*.
740. Rodriguez-Mozaz, S.; Chamorro, S.; Marti, E.; Huerta, B.; Gros, M.; Sànchez-Melsió, A.; Borrego, C.M.; Barceló, D.; Balcázar, J.L. Occurrence of antibiotics and antibiotic resistance genes in hospital and urban wastewaters and their impact on the receiving river. *Water Res.* **2015**, *69*, 234–242, doi:10.1016/j.watres.2014.11.021.
741. Van Boeckel, T.P.; Gandra, S.; Ashok, A.; Caudron, Q.; Grenfell, B.T.; Levin, S.A.; Laxminarayan, R. Global antibiotic consumption 2000 to 2010: An analysis of national pharmaceutical sales data. *Lancet Infect. Dis.* **2014**, *14*, 742–750, doi:10.1016/S1473-3099(14)70780-7.
742. World Health Organization; Organization, W.H. *Executive summary: the selection and use of essential medicines 2019: report of the 22nd WHO Expert Committee on the selection and use of essential medicines: WHO Headquarters, Geneva, 1-5 April 2019*; 2019;
743. Di Cocco, P.; Orlando, G.; Bonanni, L.; D'Angelo, M.; Clemente, K.; Greco, S.; Gravante, G.; Madeddu, F.; Scelzo, C.; Famulari, A.; et al. A Systematic Review of Two Different Trimetoprim-Sulfamethoxazole Regimens Used to Prevent Pneumocystis jirovecii and No Prophylaxis at All in Transplant Recipients: Appraising the Evidence. *Transplant. Proc.* **2009**, *41*, 1201–1203, doi:10.1016/j.transproceed.2009.03.004.
744. Wang, J.; Chu, L.; Wojnárovits, L.; Takács, E. Occurrence and fate of antibiotics, antibiotic resistant genes (ARGs) and antibiotic resistant bacteria (ARB) in municipal wastewater treatment plant: An overview. *Sci. Total Environ.* 2020, *744*, 140997.
745. Collado, N.; Rodriguez-Mozaz, S.; Gros, M.; Rubirola, A.; Barceló, D.; Comas, J.; Rodriguez-Roda, I.; Buttiglieri, G. Pharmaceuticals occurrence in a WWTP with significant industrial contribution and its input into the river system. *Environ. Pollut.* **2014**, *185*, 202–212, doi:10.1016/j.envpol.2013.10.040.
746. Tadeo, L. and liquid chromatography-tandem mass spectrometry. *J. Pharm. Biomed. Anal.* **2017**, doi:10.1016/j.jpba.2017.08.013.
747. Karthikeyan, K.G.; Meyer, M.T. Occurrence of antibiotics in wastewater treatment facilities in Wisconsin, USA. *Sci. Total Environ.* **2006**, *361*, 196–207, doi:10.1016/j.scitotenv.2005.06.030.
748. Pérez, R.A.; Albero, B.; Férriz, M.; Tadeo, J.L. Analysis of macrolide antibiotics in water by magnetic solid-phase extraction and liquid chromatography–tandem mass spectrometry. *J. Pharm. Biomed. Anal.* **2017**, *146*, 79–85, doi:10.1016/j.jpba.2017.08.013.
749. Abuin, S.; Codony, R.; Compañó, R.; Granados, M.; Prat, M.D. Analysis of macrolide antibiotics in river water by solid-phase extraction and liquid chromatography-mass spectrometry. *J. Chromatogr. A* **2006**, *1114*, 73–81, doi:10.1016/j.chroma.2006.02.032.
750. Yang, S.; Carlson, K.H. Solid-phase extraction-high-performance liquid chromatography-ion trap mass spectrometry for analysis of trace concentrations of macrolide antibiotics in natural and waste water matrices. *J. Chromatogr. A* **2004**, *1038*, 141–155, doi:10.1016/j.chroma.2004.02.084.

751. Samaraweera, D.N.D.; Liu, X.; Zhong, G.; Priyadarshana, T.; Naseem Malik, R.; Zhang, G.; Khorram, M.S.; Zhu, Z.; Peng, X. Antibiotics in two municipal sewage treatment plants in Sri Lanka: Occurrence, consumption and removal efficiency. *Emerg. Contam.* **2019**, *5*, 272–278, doi:10.1016/j.emcon.2019.08.001.

752. Mhuka, V.; Dube, S.; Nindi, M.M. Occurrence of pharmaceutical and personal care products (PPCPs) in wastewater and receiving waters in South Africa using LC-Orbitrap™ MS. *Emerg. Contam.* **2020**, *6*, 250–258, doi:10.1016/j.emcon.2020.07.002.

753. Manoli, K.; Morrison, L.M.; Sumarah, M.W.; Nakhla, G.; Ray, A.K.; Sharma, V.K. Pharmaceuticals and pesticides in secondary effluent wastewater: Identification and enhanced removal by acid-activated ferrate(VI). *Water Res.* **2019**, *148*, 272–280, doi:10.1016/j.watres.2018.10.056.

754. Ahmed, M.B.; Zhou, J.L.; Ngo, H.H.; Guo, W. Adsorptive removal of antibiotics from water and wastewater: Progress and challenges. *Sci. Total Environ.* **2015**, *532*, 112–126, doi:10.1016/j.scitotenv.2015.05.130.

755. Silva, L.J.G.; Pereira, A.M.P.T.; Meisel, L.M.; Lino, C.M.; Pena, A. Reviewing the serotonin reuptake inhibitors (SSRIs) footprint in the aquatic biota: Uptake, bioaccumulation and ecotoxicology. *Environ. Pollut.* 2015, *197*, 127–143.

756. Subedi, B.; Kannan, K. Occurrence and fate of select psychoactive pharmaceuticals and antihypertensives in two wastewater treatment plants in New York State, USA. *Sci. Total Environ.* **2015**, *514*, 273–280, doi:10.1016/j.scitotenv.2015.01.098.

757. Miao, X.S.; Bishay, F.; Chen, M.; Metcalfe, C.D. Occurrence of antimicrobials in the final effluents of wastewater treatment plants in Canada. *Environ. Sci. Technol.* **2004**, *38*, 3533–3541, doi:10.1021/es030653q.

758. Gurke, R.; Rößler, M.; Marx, C.; Diamond, S.; Schubert, S.; Oertel, R.; Fauler, J. Occurrence and removal of frequently prescribed pharmaceuticals and corresponding metabolites in wastewater of a sewage treatment plant. *Sci. Total Environ.* **2015**, *532*, 762–770, doi:10.1016/j.scitotenv.2015.06.067.

759. Ma, L. dan; Li, J.; Li, J. jun; Liu, M.; Yan, D. zhi; Shi, W. yan; Xu, G. Occurrence and source analysis of selected antidepressants and their metabolites in municipal wastewater and receiving surface water. *Environ. Sci. Process. Impacts* **2018**, *20*, 1020–1029, doi:10.1039/c8em00077h.

760. Subedi, B.; Balakrishna, K.; Sinha, R.K.; Yamashita, N.; Balasubramanian, V.G.; Kannan, K. Mass loading and removal of pharmaceuticals and personal care products, including psychoactive and illicit drugs and artificial sweeteners, in five sewage treatment plants in India. *J. Environ. Chem. Eng.* **2015**, *3*, 2882–2891, doi:10.1016/j.jece.2015.09.031.

761. Niemi, L.; Taggart, M.; Boyd, K.; Zhang, Z.; Gaffney, P.P.J.; Pfleger, S.; Gibb, S. Assessing hospital impact on pharmaceutical levels in a rural 'source-to-sink' water system. *Sci. Total Environ.* **2020**, *737*, doi:10.1016/j.scitotenv.2020.139618.

762. Ort, C.; Lawrence, M.G.; Reungoat, J.; Eaglesham, G.; Carter, S.; Keller, J. Determining the fraction of pharmaceutical residues in wastewater originating from a hospital. *Water Res.* **2010**, *44*, 605–615, doi:10.1016/j.watres.2009.08.002.

763. Kasprzyk-Hordern, B.; Dinsdale, R.M.; Guwy, A.J. Illicit drugs and pharmaceuticals in the environment - Forensic applications of environmental data. Part 1: Estimation of the usage of drugs in local communities. *Environ. Pollut.* **2009**, *157*, 1773–1777, doi:10.1016/j.envpol.2009.03.017.

764. Luo, Y.; Guo, W.; Ngo, H.H.; Nghiem, L.D.; Hai, F.I.; Zhang, J.; Liang, S.; Wang, X.C. A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Sci. Total Environ.* 2014, *473–474*, 619–641.

765. Wijekoon, K.C.; Hai, F.I.; Kang, J.; Price, W.E.; Guo, W.; Ngo, H.H.; Nghiem, L.D. The fate of pharmaceuticals, steroid hormones, phytoestrogens, UV-filters and pesticides during MBR treatment. *Bioresour. Technol.* **2013**, *144*, 247–254, doi:10.1016/j.biortech.2013.06.097.

766. Tixier, C.; Singer, H.P.; Oellers, S.; Müller, S.R. Occurrence and fate of carbamazepine, clofibric acid, diclofenac, ibuprofen, ketoprofen, and naproxen in surface waters. *Environ. Sci. Technol.* **2003**, *37*, 1061–1068, doi:10.1021/es025834r.

767. Ying, G.G.; Kookana, R.S.; Kolpin, D.W. Occurrence and removal of pharmaceutically active compounds in sewage treatment plants with different technologies. *J. Environ. Monit.* **2009**, *11*, 1498–1505, doi:10.1039/b904548a.

768. Brack, W.; Dulio, V.; Ågerstrand, M.; Allan, I.; Altenburger, R.; Brinkmann, M.; Bunke, D.; Burgess, R.M.; Cousins, I.; Escher, B.I.; et al. Towards the review of the European Union Water Framework management of chemical contamination in European surface water resources. *Sci. Total Environ.* 2017, *576*, 720–737.

769. Hai, F.I.; Yang, S.; Asif, M.B.; Sencadas, V.; Shawkat, S.; Sanderson-Smith, M.; Gorman, J.; Xu, Z.Q.; Yamamoto, K. Carbamazepine as a Possible Anthropogenic Marker in Water: Occurrences, Toxicological Effects, Regulations and Removal by Wastewater Treatment Technologies. *Water (Switzerland)* 2018, *10*, 107.

770. Kumar, A.; Batley, G.E.; Nidumolu, B.; Hutchinson, T.H. Derivation of water quality guidelines for priority pharmaceuticals. *Environ. Toxicol. Chem.* **2016**, *35*, 1815–1824, doi:10.1002/ETC.3336.

771. Pivetta, R.C.; Rodrigues-Silva, C.; Ribeiro, A.R.; Rath, S. Tracking the occurrence of psychotropic pharmaceuticals in Brazilian wastewater treatment plants and surface water, with assessment of environmental risks. *Sci. Total Environ.* **2020**, *727*, 138661, doi:10.1016/j.scitotenv.2020.138661.

772. Wu, M.; Xiang, J.; Que, C.; Chen, F.; Xu, G. Occurrence and fate of psychiatric pharmaceuticals in the urban water system of Shanghai, China. *Chemosphere* **2015**, *138*, 486–493, doi:10.1016/j.chemosphere.2015.07.002.

773. Daughton, C.C. Pharmaceuticals and personal care products in the environment: Overarching issues and overview. *ACS Symp. Ser.* **2001**, *791*, 2–38, doi:10.1021/bk-2001-0791.ch001.

774. Howard, P.H.; Muir, D.C.G. Identifying new persistent and bioaccumulative organics among chemicals in commerce. III: Byproducts, impurities, and transformation products. *Environ. Sci. Technol.* **2013**, *47*, 5259–5266, doi:10.1021/es4004075.

775. Papageorgiou, M.; Zioris, I.; Danis, T.; Bikiaris, D.; Lambropoulou, D. Comprehensive investigation of a wide range of pharmaceuticals and personal care products in urban and hospital wastewaters in Greece. *Sci. Total Environ.* **2019**, *694*, 133565, doi:10.1016/j.scitotenv.2019.07.371.

776. Kumari, R.; Sachan, S.G.; Sachan, A. Environmental and human exposure to antimicrobial agent triclosan: A review. In *In vitro Plant Breeding towards Novel Agronomic Traits: Biotic and Abiotic Stress Tolerance*; Springer Singapore, 2019; pp. 237–261 ISBN 9789813298248.

777. von der Ohe, P.C.; Schmitt-Jansen, M.; Slobodnik, J.; Brack, W. Triclosan-the forgotten priority substance? *Environ. Sci. Pollut. Res.* **2012**, *19*, 585–591, doi:10.1007/s11356-011-0580-7.

778. Heidler, J.; Halden, R.U. Meta-analysis of mass balances examining chemical fate during wastewater treatment. *Environ. Sci. Technol.* 2008, *42*, 6324–6332.

779. Mohan, S.; Balakrishnan, P. Triclosan in Treated Wastewater from a City Wastewater Treatment Plant and its Environmental Risk Assessment. *Water. Air. Soil Pollut.* **2019**, *230*, 1–13, doi:10.1007/s11270-019-4098-9.

780. Lalonde, B.; Garron, C.; Dove, A.; Struger, J.; Farmer, K.; Sekela, M.; Gledhill, M.; Backus, S. Investigation of Spatial Distributions and Temporal Trends of Triclosan in Canadian Surface Waters. *Arch. Environ. Contam. Toxicol.* **2019**, *76*, 231–245, doi:10.1007/s00244-018-0576-0.

781. FENT, K.; WESTON, A.; CAMINADA, D. Ecotoxicology of human pharmaceuticals. *Aquat. Toxicol.* **2006**, *76*, 122–159, doi:10.1016/j.aquatox.2005.09.009.

782. Nikolaou, A.; Meric, S.; Fatta, D. Occurrence patterns of pharmaceuticals in water and wastewater environments. In Proceedings of the Analytical and Bioanalytical Chemistry; 2007; Vol. 387, pp. 1225–1234.

783. Sanderson, H.; Johnson, D.J.; Wilson, C.J.; Brain, R.A.; Solomon, K.R. Probabilistic hazard assessment of environmentally occurring pharmaceuticals toxicity to fish, daphnids and algae by ECOSAR screening. *Toxicol. Lett.* **2003**, *144*, 383–395, doi:10.1016/S0378-4274(03)00257-1.

784. Mandaric, L.; Celic, M.; Marcé, R.; Petrovic, M. Introduction on emerging contaminants in rivers and their environmental risk. In *Handbook of Environmental Chemistry*; Springer Verlag, 2016; Vol. 46, pp. 3–25.

785. EMEA/CHMP—European Medicines Evaluation Agency/Committee for Medicinal Products for Human use Doc. Ref. EMEA/CHMP/SWP/4447/00, Guideline on the Environmental Assessment of Medicinal Products for Human Use. *Reproduction* **2005**.

786. Lacey, C.; Basha, S.; Morrissey, A.; Tobin, J.M. Occurrence of pharmaceutical compounds in wastewater process streams in Dublin, Ireland. *Environ. Monit. Assess.* **2012**, *184*, 1049–1062, doi:10.1007/s10661-011-2020-z.

787. Valcárcel, Y.; Alonso, S.G.; Rodríguez-Gil, J.L.; Castaño, A.; Montero, J.C.; Criado-Alvarez, J.J.; Mirón, I.J.; Catalá, M. Seasonal variation of pharmaceutically active compounds in surface (Tagus River) and tap water (Central Spain). *Environ. Sci. Pollut. Res.* **2013**, *20*, 1396–1412, doi:10.1007/s11356-012-1099-2.

788. Yu, Y.; Wu, L.; Chang, A.C. Seasonal variation of endocrine disrupting compounds, pharmaceuticals and personal care products in wastewater treatment plants. *Sci. Total Environ.* **2013**, *442*, 310–316, doi:10.1016/j.scitotenv.2012.10.001.

789. Pereira, A.M.P.T.; Silva, L.J.G.; Meisel, L.M.; Lino, C.M.; Pena, A. Environmental impact of pharmaceuticals from Portuguese wastewaters: Geographical and seasonal occurrence, removal and risk assessment. *Environ. Res.* **2015**, *136*, 108–119,

doi:10.1016/j.envres.2014.09.041.

790. Česen, M.; Heath, D.; Krivec, M.; Košmrlj, J.; Kosjek, T.; Heath, E. Seasonal and spatial variations in the occurrence, mass loadings and removal of compounds of emerging concern in the Slovene aqueous environment and environmental risk assessment. *Environ. Pollut.* **2018**, *242*, 143–154, doi:10.1016/j.envpol.2018.06.052.

791. Im, J.K.; Kim, S.H.; Noh, H.R.; Yu, S.J. Temporal-spatial variation and environmental risk assessment of pharmaceuticals in tributaries of the Han River watershed, South Korea. *Sci. Total Environ.* **2020**, *741*, 140486, doi:10.1016/j.scitotenv.2020.140486.

792. Golovko, O.; Kumar, V.; Fedorova, G.; Randak, T.; Grabic, R. Seasonal changes in antibiotics, antidepressants/psychiatric drugs, antihistamines and lipid regulators in a wastewater treatment plant. *Chemosphere* **2014**, *111*, 418–426, doi:10.1016/j.chemosphere.2014.03.132.

793. Kot-Wasik, A.; Jakimska, A.; Śliwka-Kaszyńska, M. Occurrence and seasonal variations of 25 pharmaceutical residues in wastewater and drinking water treatment plants. *Environ. Monit. Assess.* **2016**, *188*, 1–13, doi:10.1007/s10661-016-5637-0.

794. Pan, C.; Bao, Y.; Xu, B. Seasonal variation of antibiotics in surface water of Pudong New Area of Shanghai, China and the occurrence in typical wastewater sources. *Chemosphere* **2020**, *239*, 124816, doi:10.1016/j.chemosphere.2019.124816.

795. Lu, S.; Lin, C.; Lei, K.; Wang, B.; Xin, M.; Gu, X.; Cao, Y.; Liu, X.; Ouyang, W.; He, M. Occurrence, spatiotemporal variation, and ecological risk of antibiotics in the water of the semi-enclosed urbanized Jiaozhou Bay in eastern China. *Water Res.* **2020**, *184*, 116187, doi:10.1016/j.watres.2020.116187.

796. Maasz, G.; Mayer, M.; Zrinyi, Z.; Molnar, E.; Kuzma, M.; Fodor, I.; Pirger, Z.; Takács, P. Spatiotemporal variations of pharmacologically active compounds in surface waters of a summer holiday destination. *Sci. Total Environ.* **2019**, *677*, 545–555, doi:10.1016/j.scitotenv.2019.04.286.

797. Bojarczuk, A.; Jelonkiewicz, Ł.; Lenart-Boroń, A. The effect of anthropogenic and natural factors on the prevalence of physicochemical parameters of water and bacterial water quality indicators along the river Białka, southern Poland. *Environ. Sci. Pollut. Res.* **2018**, *25*, 10102–10114, doi:10.1007/s11356-018-1212-2.

798. Barakat, A.; El Baghdadi, M.; Rais, J.; Aghezzaf, B.; Slassi, M. Assessment of spatial and seasonal water quality variation of Oum Er Rbia River (Morocco) using multivariate statistical techniques. *Int. Soil Water Conserv. Res.* **2016**, *4*, 284–292, doi:10.1016/j.iswcr.2016.11.002.

799. Calza, P.; Sakkas, V.A.; Medana, C.; Vlachou, A.D.; Dal Bello, F.; Albanis, T.A. Chemometric assessment and investigation of mechanism involved in photo-Fenton and TiO2 photocatalytic degradation of the artificial sweetener sucralose in aqueous media. *Appl. Catal. B Environ.* **2013**, *129*, 71–79, doi:10.1016/j.apcatb.2012.08.043.

800. Castronovo, S.; Wick, A.; Scheurer, M.; Nödler, K.; Schulz, M.; Ternes, T.A. Biodegradation of the artificial sweetener acesulfame in biological wastewater treatment and sandfilters. *Water Res.* **2017**, *110*, 342–353, doi:10.1016/j.watres.2016.11.041.

801. Kahl, S.; Kleinsteuber, S.; Nivala, J.; Van Afferden, M.; Reemtsma, T. Emerging Biodegradation of the Previously Persistent Artificial Sweetener Acesulfame in Biological Wastewater Treatment. *Environ. Sci. Technol.* **2018**, *52*, 2717–2725, doi:10.1021/acs.est.7b05619.

802. Tang, Y.; Guo, L. lu; Hong, C. yang; Bing, Y. xin; Xu, Z. cheng Seasonal occurrence, removal and risk assessment of 10 pharmaceuticals in 2 sewage treatment plants of Guangdong, China. *Environ. Technol. (United Kingdom)* **2019**, *40*, 458–469, doi:10.1080/09593330.2017.1397758.

803. Üstün-Odabaşı, S.; Maryam, B.; Özdemir, N.; Büyükgüngör, H. Occurrence and seasonal variations of pharmaceuticals and personal care products in drinking water and wastewater treatment plants in Samsun, Turkey. *Environ. Earth Sci.* **2020**, *79*, 311, doi:10.1007/s12665-020-09047-7.

804. World Weather Online West Greece Weather Averages | Monthly Average High and Low Temperature | Average Precipitation and Rainfall days | World Weather Online Available online: https://www.worldweatheronline.com/amaliada-weather-averages/west-greece/gr.aspx (accessed on Sep 29, 2020).

805. Sarran, C.; Albers, C.; Sachon, P.; Meesters, Y. Meteorological analysis of symptom data for people with seasonal affective disorder. *Psychiatry Res.* **2017**, *257*, 501–505, doi:10.1016/j.psychres.2017.08.019.

806. Henríquez-Sánchez, P.; Doreste-Alonso, J.; Martínez-González, M.A.; Bes-Rastrollo, M.; Gea, A.; Sánchez-Villegas, A. Geographical and climatic factors and depression risk in the SUN project. *Eur. J. Public Health* **2014**, *24*, 626–631, doi:10.1093/eurpub/cku008.

807. O'Hare, C.; O'Sullivan, V.; Flood, S.; Kenny, R.A. Seasonal and meteorological associations with depressive symptoms in older

adults: A geo-epidemiological study. *J. Affect. Disord.* **2016**, *191*, 172–179, doi:10.1016/j.jad.2015.11.029.

808. Terman, M.; Terman, J.S. Light therapy for seasonal and nonseasonal depression: Efficacy, protocol, safety, and side effects. *CNS Spectr.* 2005, *10*, 647–663.

809. Lurie, S.J.; Gawinski, B.; Pierce, D.; Rousseau, S.J. Seasonal affective disorder. *Am. Fam. Physician* 2006, *74*, 1521–1524.

810. https://www.worldweatheronline.com/ Available online: https://books.google.gr/books?hl=el&lr=&id=-JivBAAAQBAJ&oi=fnd&pg=PT18&ots=cfNL26NKu9&sig=ZKqHnCm_fHWMoNe2Wp-OFZyO2C0&redir_esc=y#v=onepage&q&f=false (accessed on Sep 29, 2020).

811. Bai, X.; Lutz, A.; Carroll, R.; Keteles, K.; Dahlin, K.; Murphy, M.; Nguyen, D. Occurrence, distribution, and seasonality of emerging contaminants in urban watersheds. *Chemosphere* **2018**, *200*, 133–142, doi:10.1016/j.chemosphere.2018.02.106.

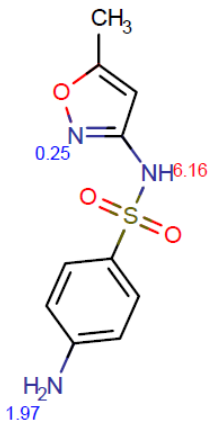
812. Mohapatra, D.P.; Brar, S.K.; Daghrir, R.; Tyagi, R.D.; Picard, P.; Surampalli, R.Y.; Drogui, P. Photocatalytic degradation of carbamazepine in wastewater by using a new class of whey-stabilized nanocrystalline TiO2 and ZnO. *Sci. Total Environ.* **2014**, *485–486*, 263–269, doi:10.1016/j.scitotenv.2014.03.089.

813. Brunsch, A.F.; ter Laak, T.L.; Rijnaarts, H.; Christoffels, E. Pharmaceutical concentration variability at sewage treatment plant outlets dominated by hydrology and other factors. *Environ. Pollut.* **2018**, *235*, 615–624, doi:10.1016/j.envpol.2017.12.116.

814. *Commission Decision of 12 August (2002/657/EC).*; 2002;

Appendix

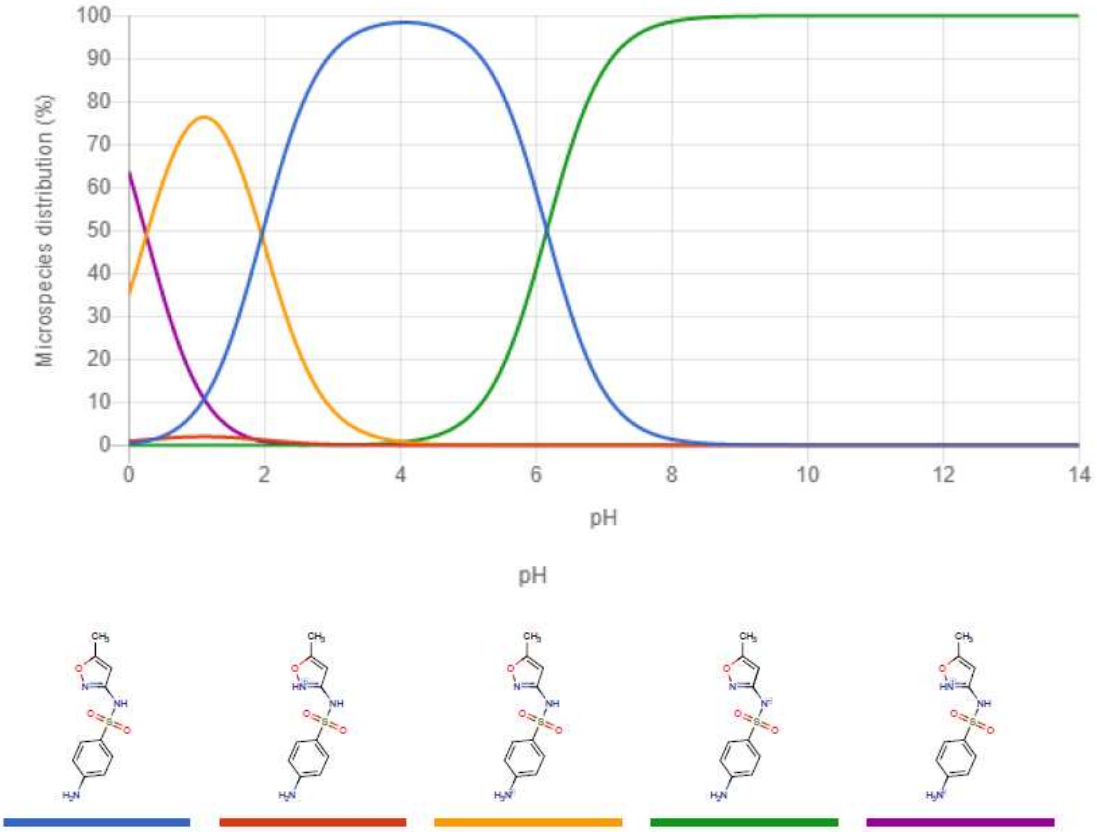
Sulfamethoxazole



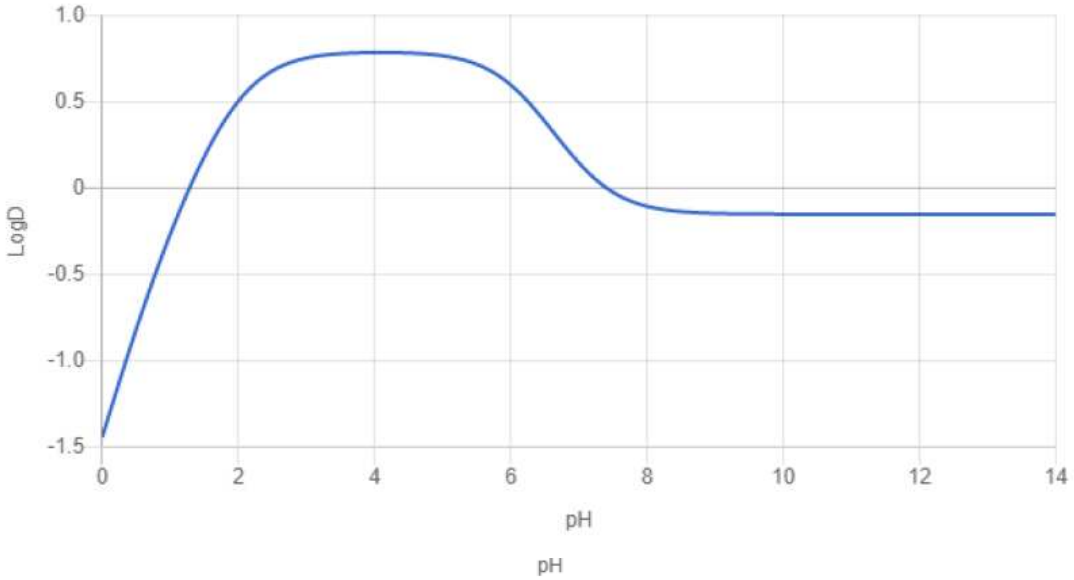
Pka

Strongest acidic pKa: 6.16

Strongest basic pKa: 1.97



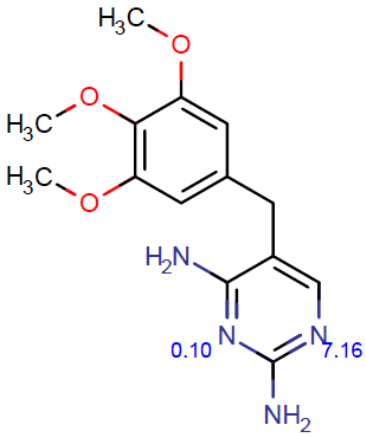
LogP and logD



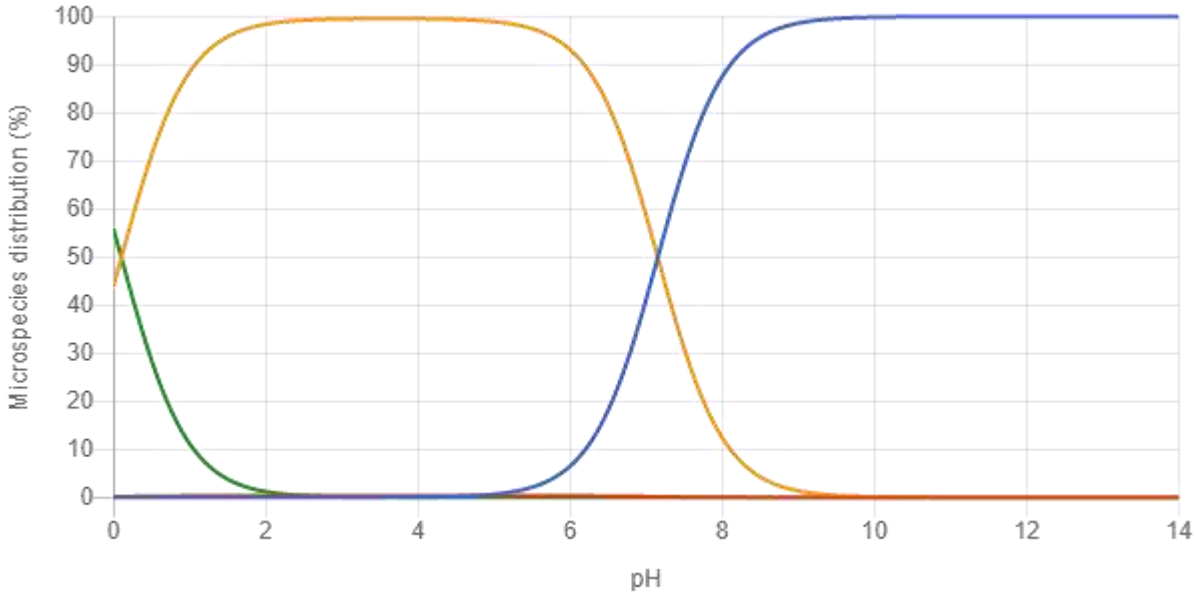
LogP: 0.791

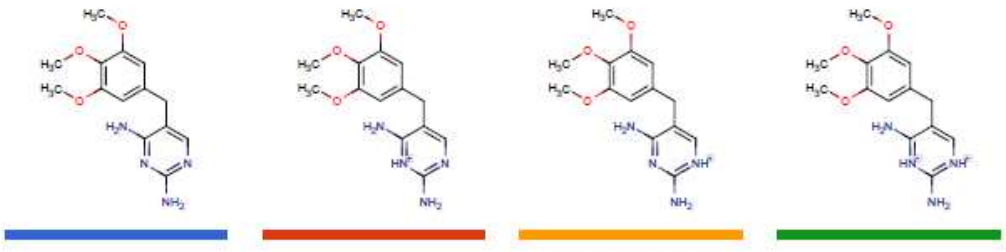
pH	logD
1.7	0.33
4.6	0.78
6.5	0.38
7.4	0.00
8.0	-0.11

Trimethoprim

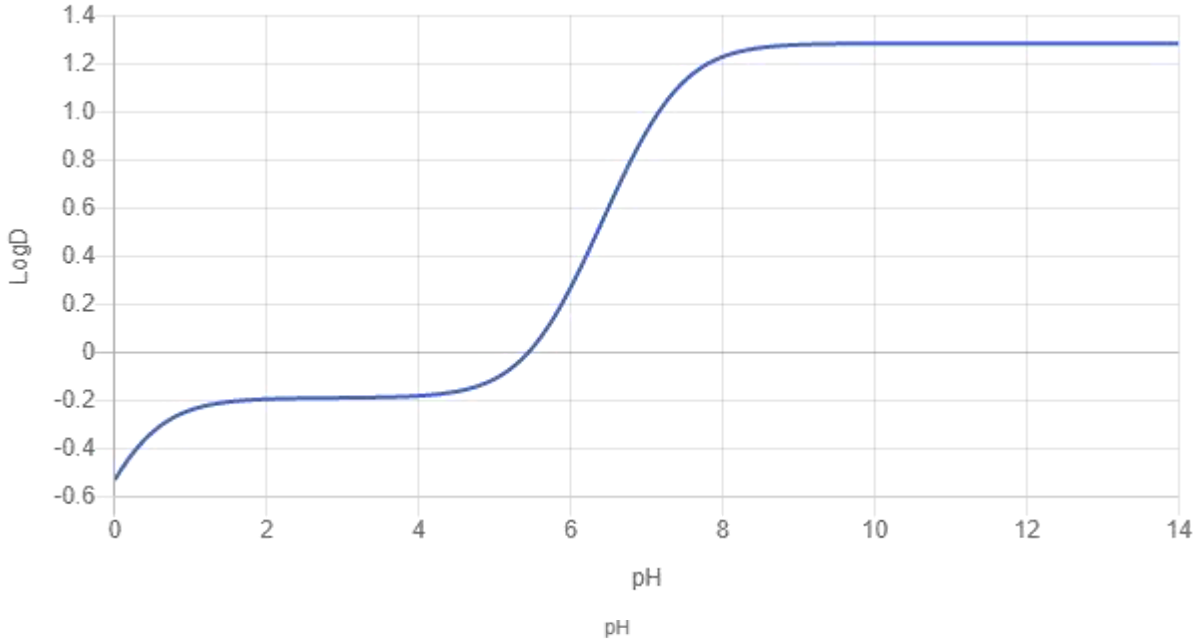


pKa





logP and logD

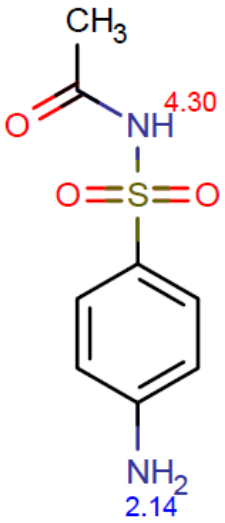


pH

LogP: 0.791

pH	logD
1.7	0.33
4.6	0.78
6.5	0.38
7.4	0.00
8.0	-0.11

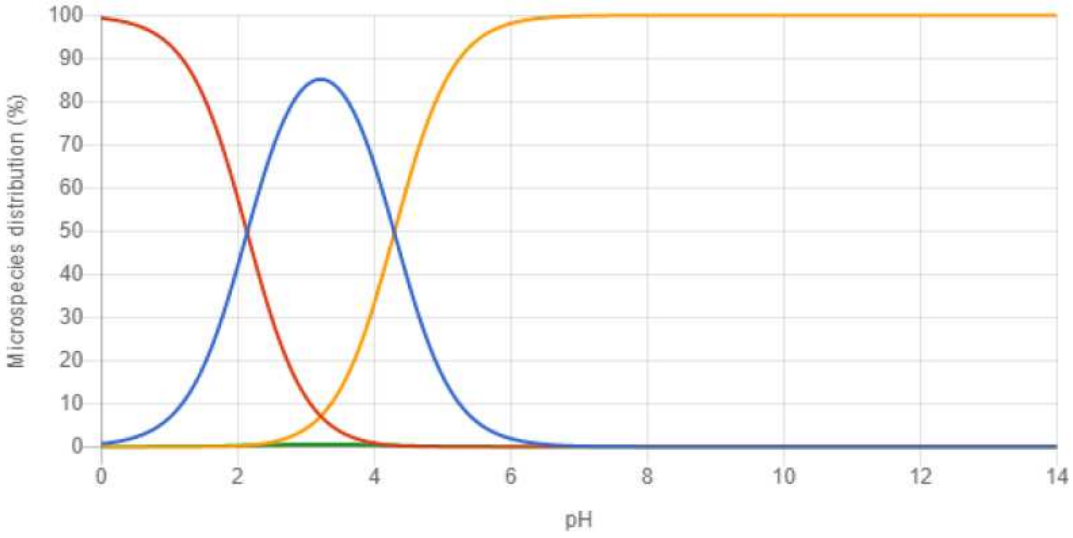
Sulfacetamide

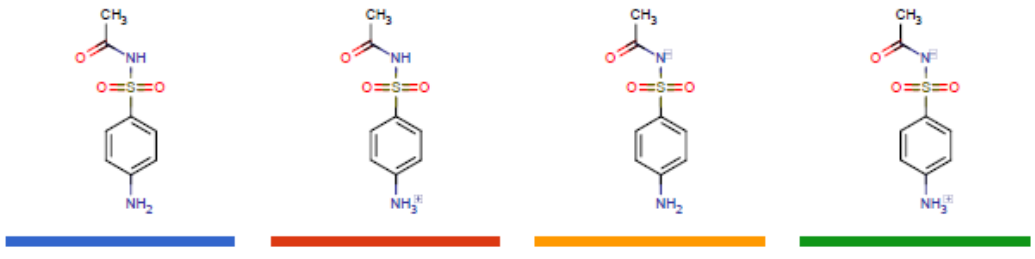


pKa

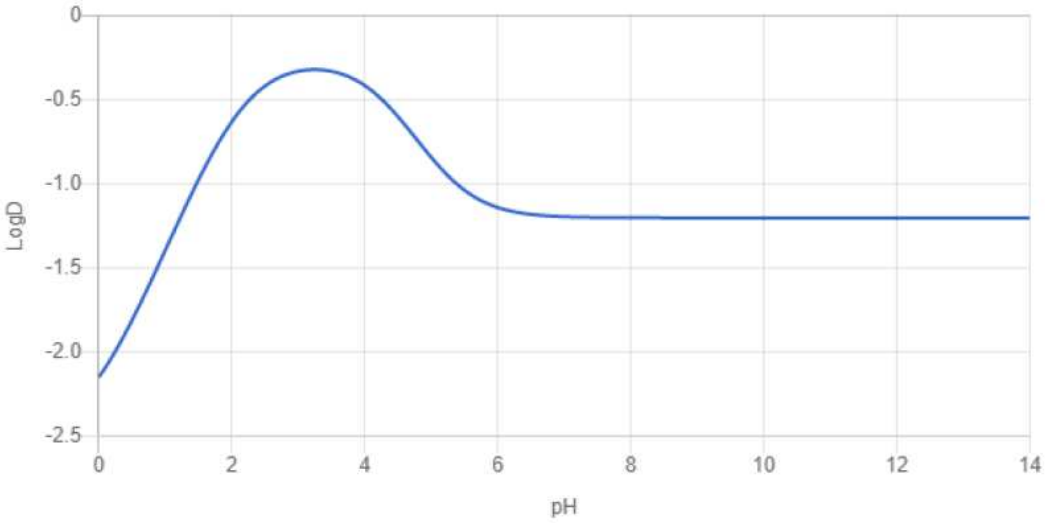
Strongest acidic pKa: 4.3

Strongest basic pKa: 2.14





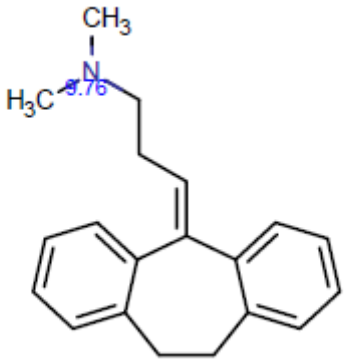
logP and logD



LogP: -0.261

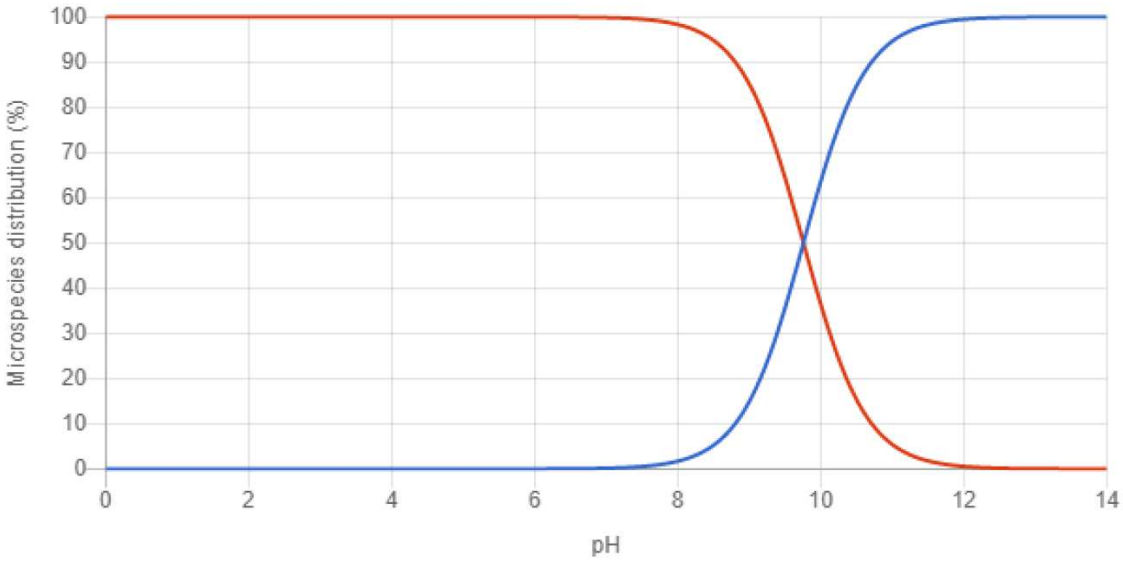
pH	logD
1.7	-0.83
4.6	-0.65
6.5	-1.18
7.4	-1.20
8.0	-1.20

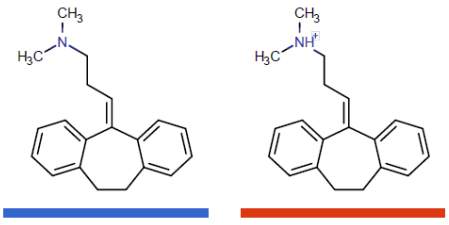
Amitriptyline



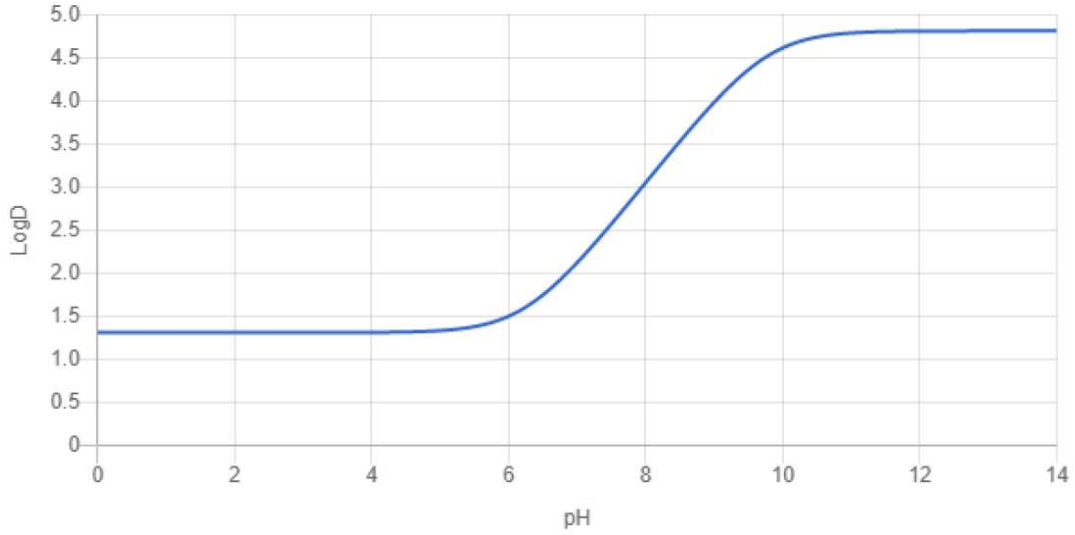
pKa

Strongest basic pKa: 9.76





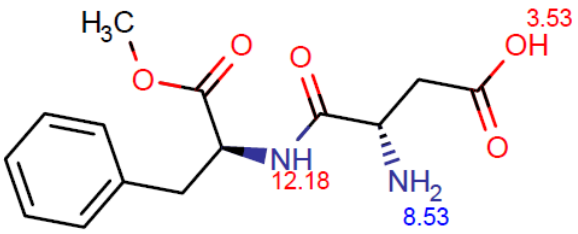
LogP and logD



LogP: 4.81

pH	logD
1.7	1.31
4.6	1.32
6.5	1.75
7.4	2.48
8.0	3.05

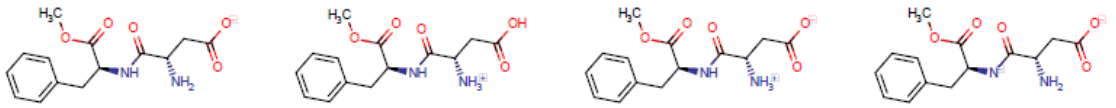
Aspartame



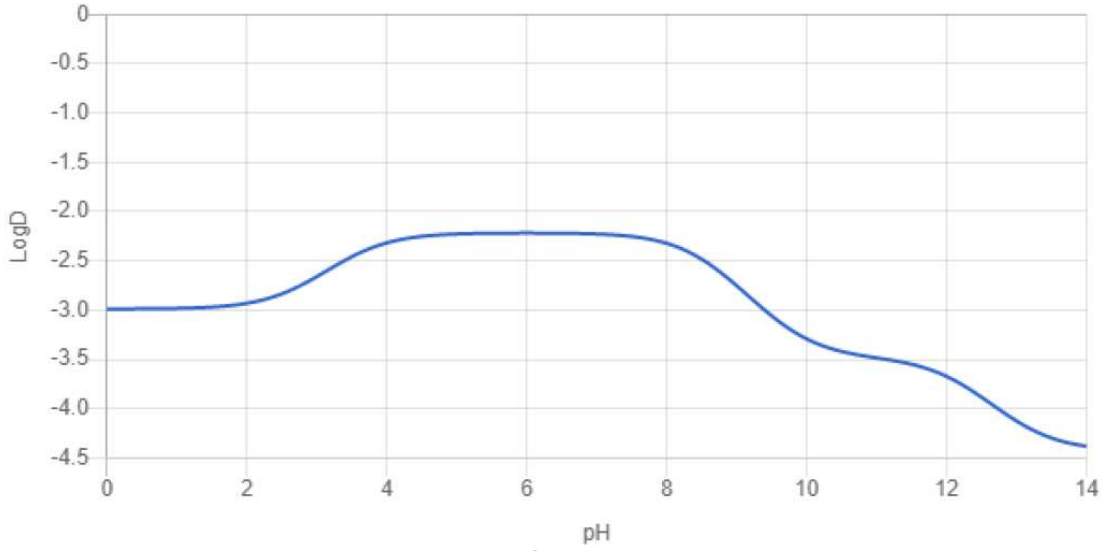
pKa

Strongest acidic pKa: 3.53

Strongest basic pKa: 8.53



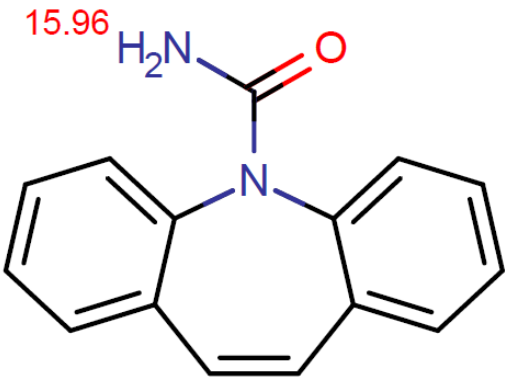
LogP and logD



LogP: -2.218

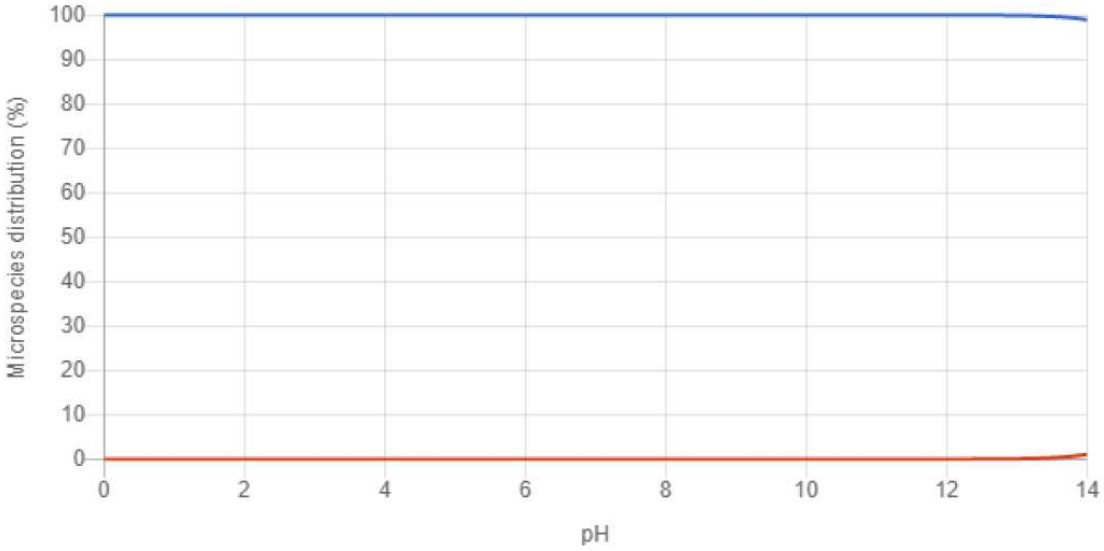
pH	logD
1.7	-2.96
4.6	-2.24
6.5	-2.22
7.4	-2.25
8.0	-2.32

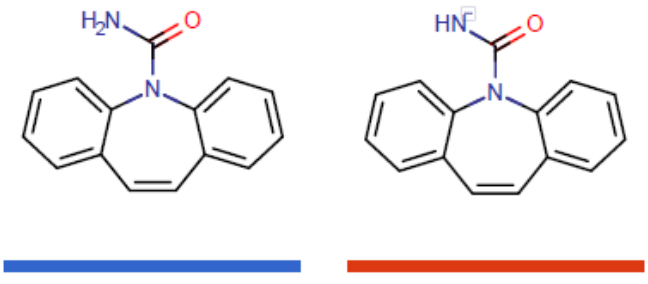
Carbamazepine



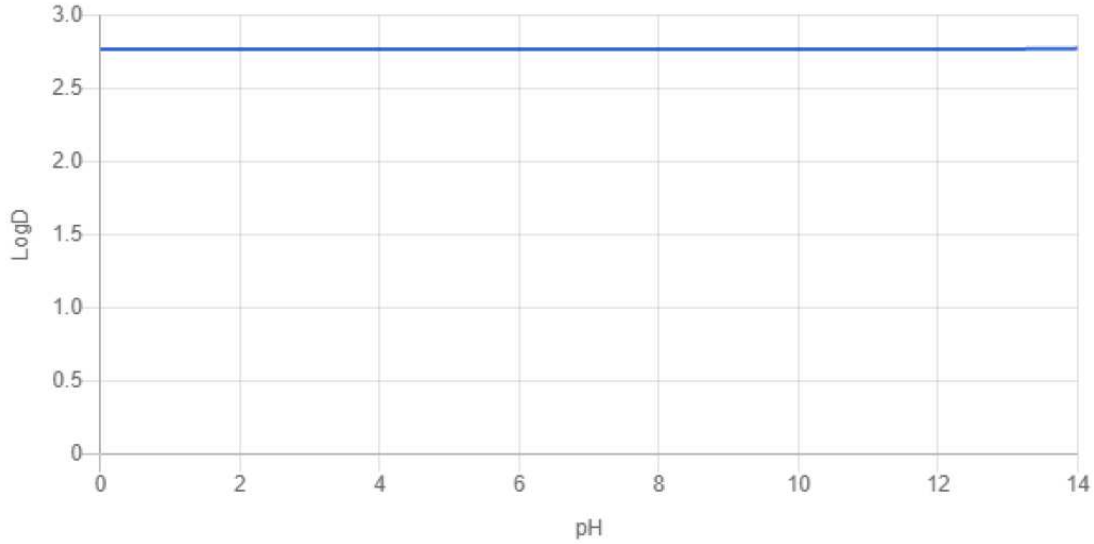
pKa

Strongest acidic pKa: 15.96





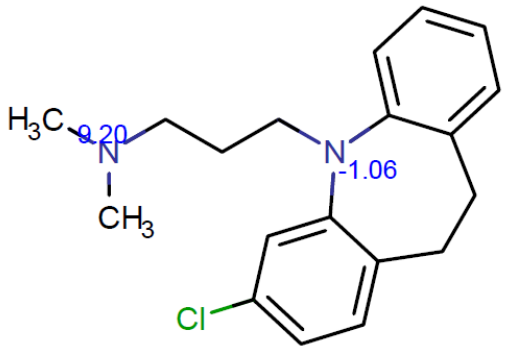
LogP and logD



LogP: 2.766

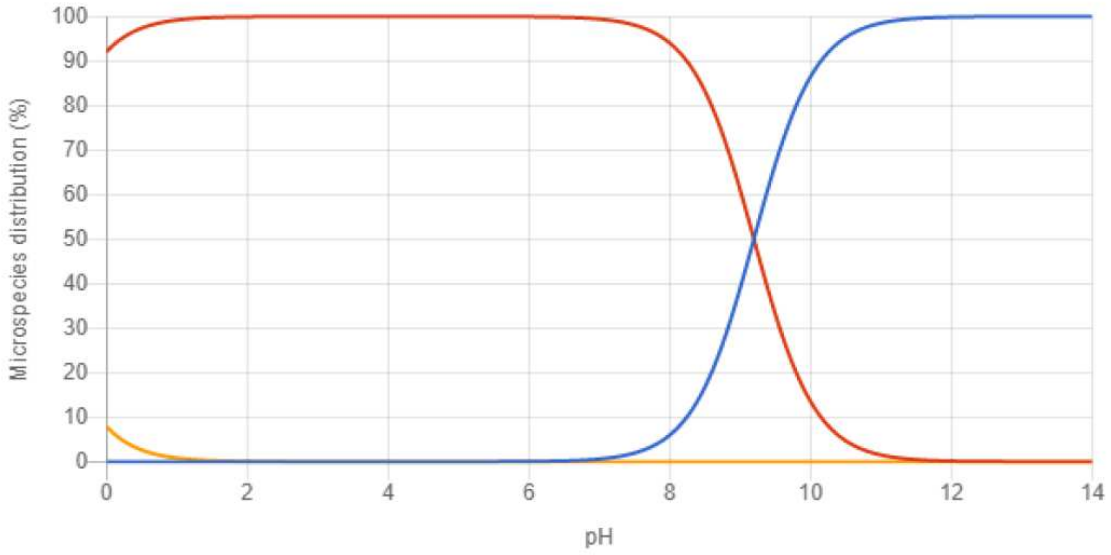
pH	logD
1.7	2.77
4.6	2.77
6.5	2.77
7.4	2.77
8.0	2.77

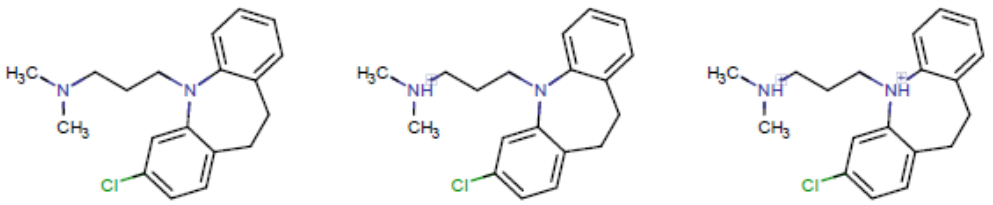
Clomipramine



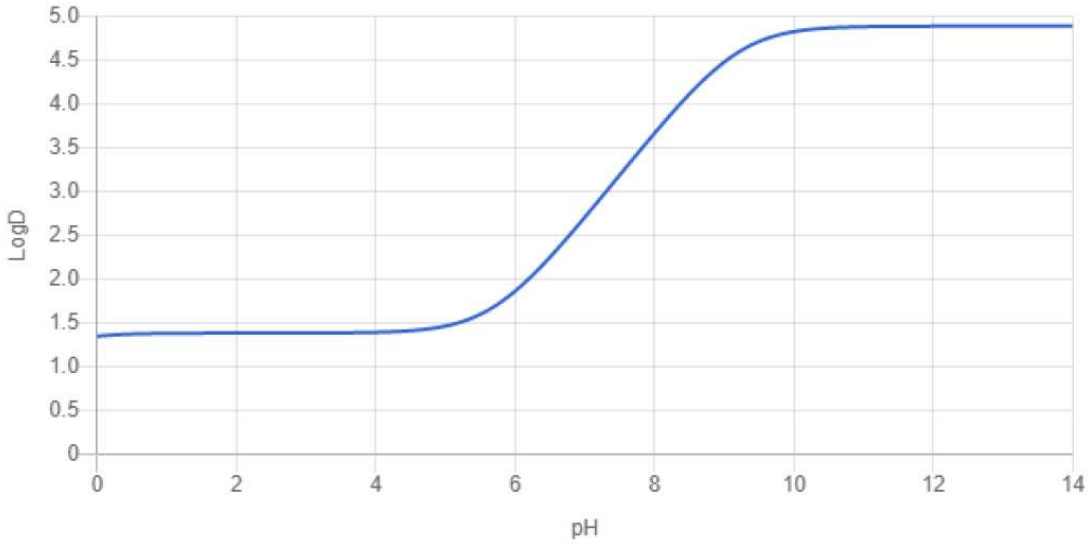
pKa

Strongest basic pKa: 9.2





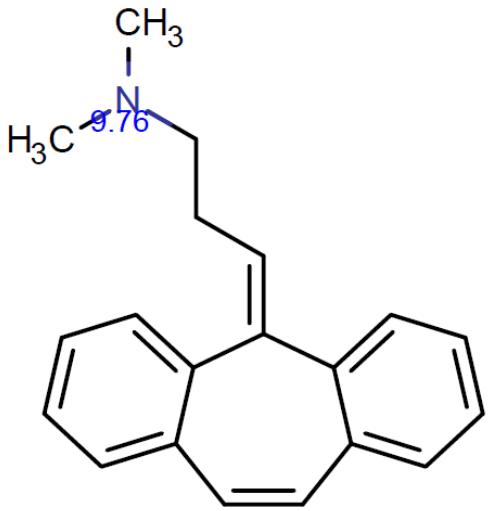
LogP and logD



LogP: 4.883

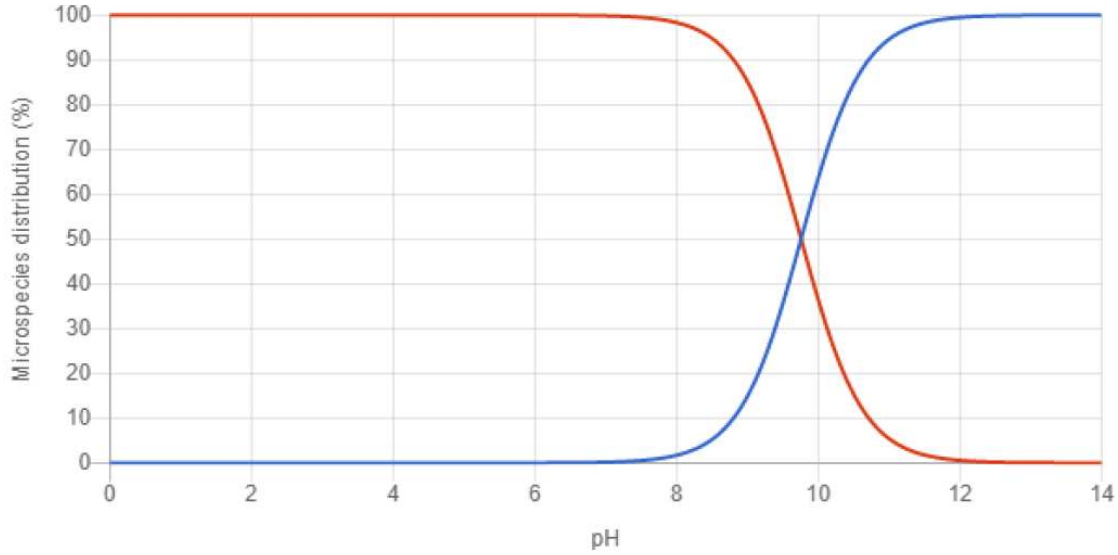
pH	logD
1.7	1.38
4.6	1.41
6.5	2.25
7.4	3.09
8.0	3.66

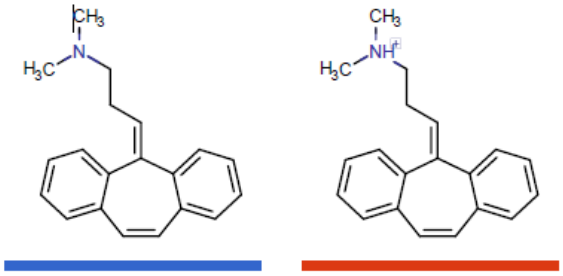
Cyclobenzaprine



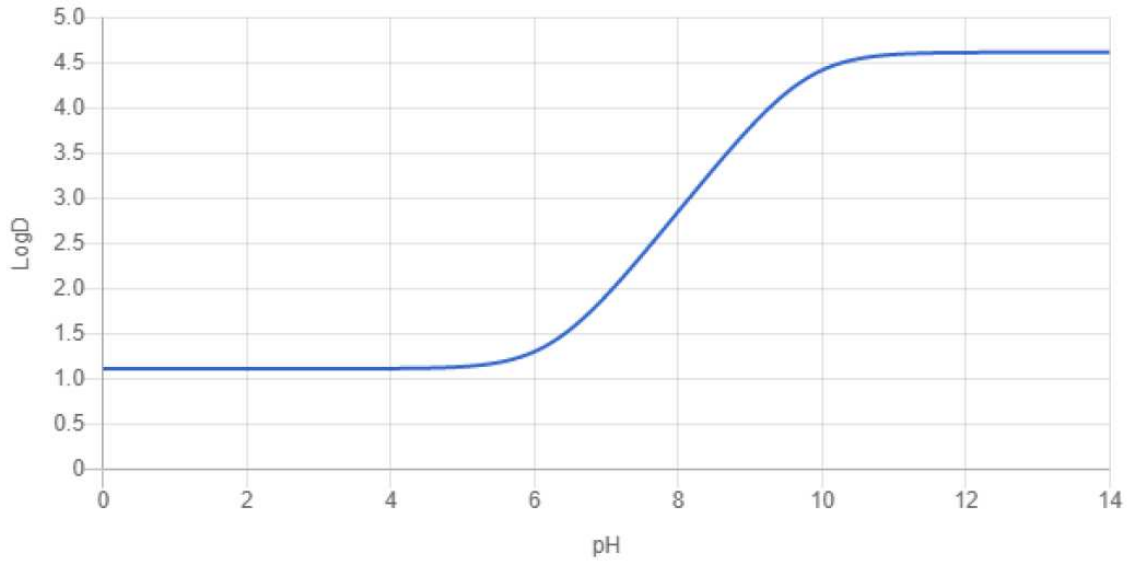
pKa

Strongest basic pKa: 9.76





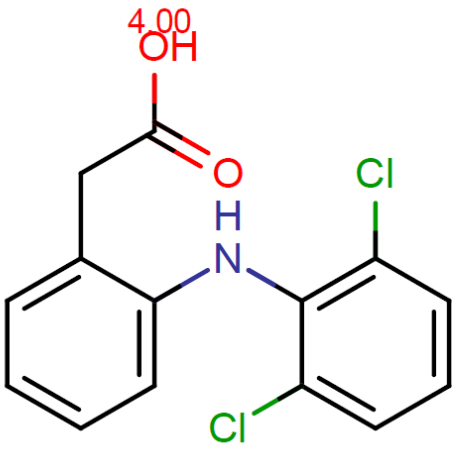
LogP and logD



LogP: 4.613

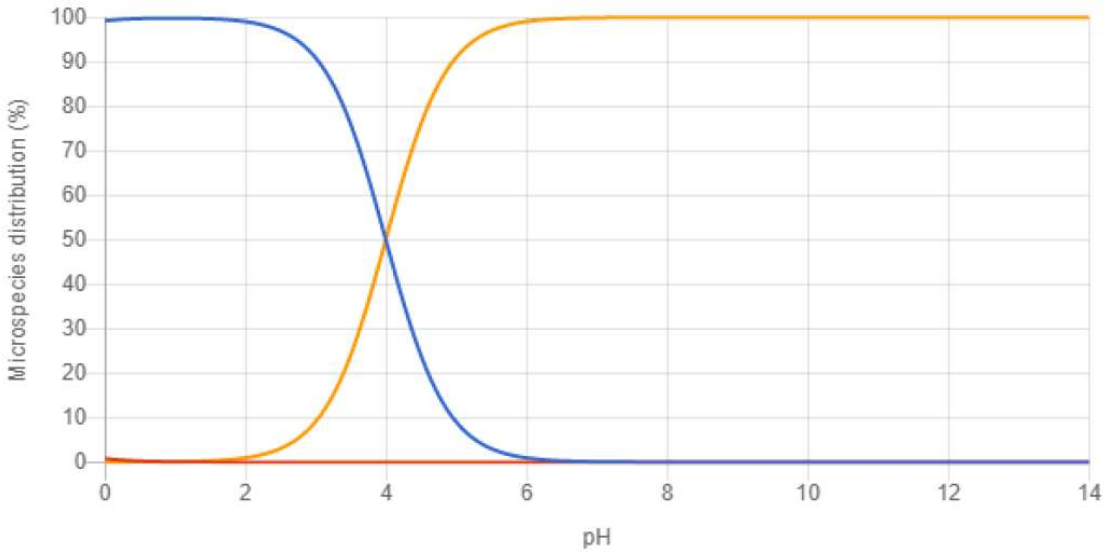
pH	logD
1.7	1.11
4.6	1.12
6.5	1.55
7.4	2.28
8.0	2.85

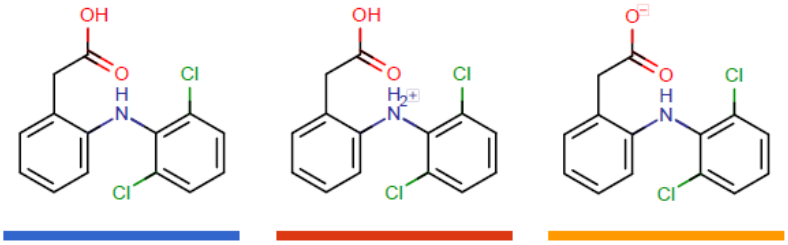
Diclofenac



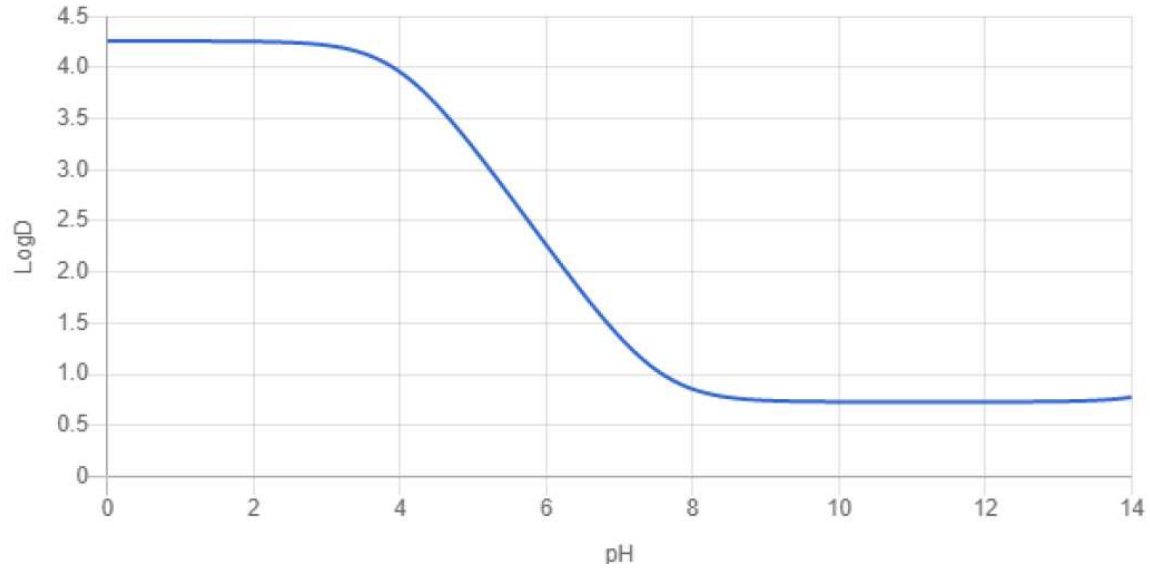
pKa

Strongest acidic pKa: 4





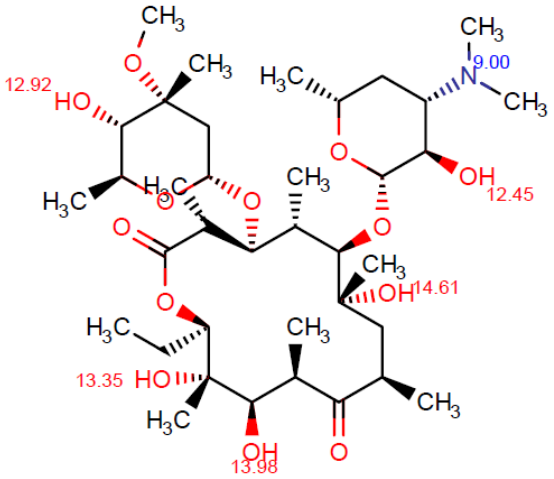
LogP and logD



LogP: 4.259

pH	logD
1.7	4.26
4.6	3.56
6.5	1.79
7.4	1.10
8.0	0.85

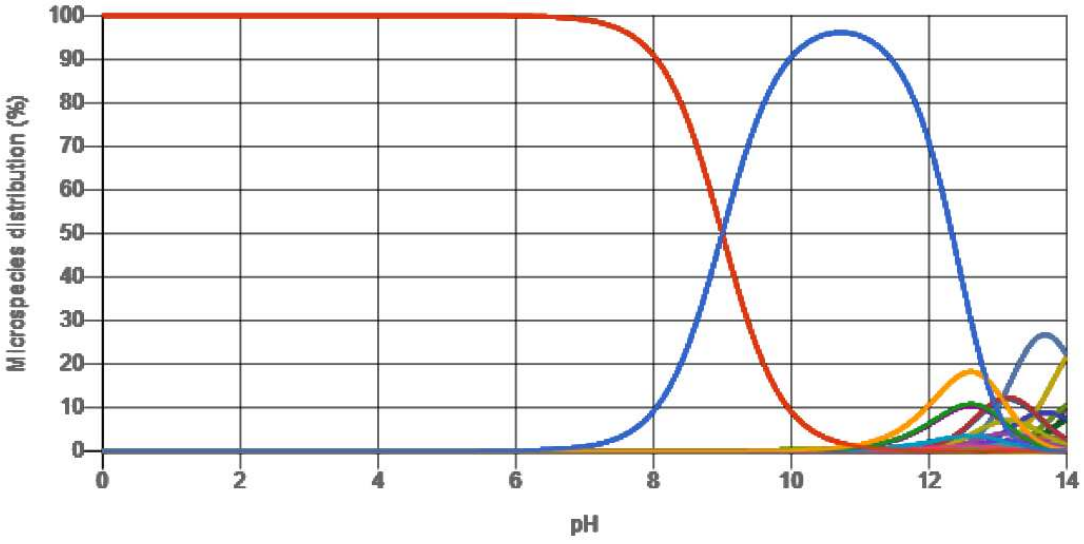
Erythromycin

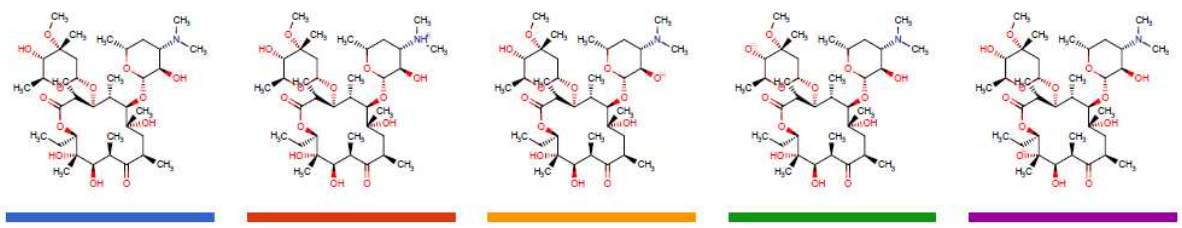


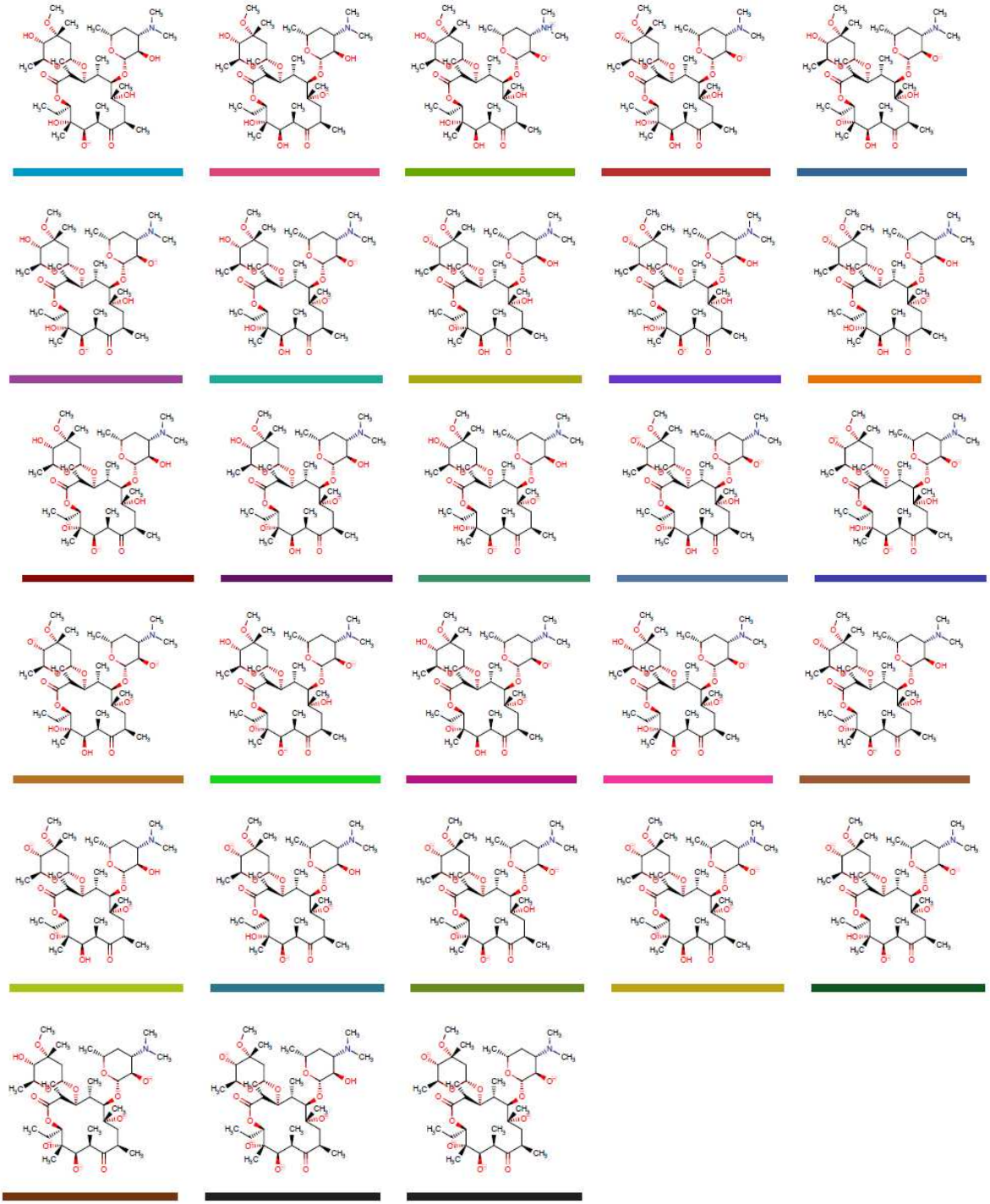
pKa

Strongest acidic pKa: 12.45

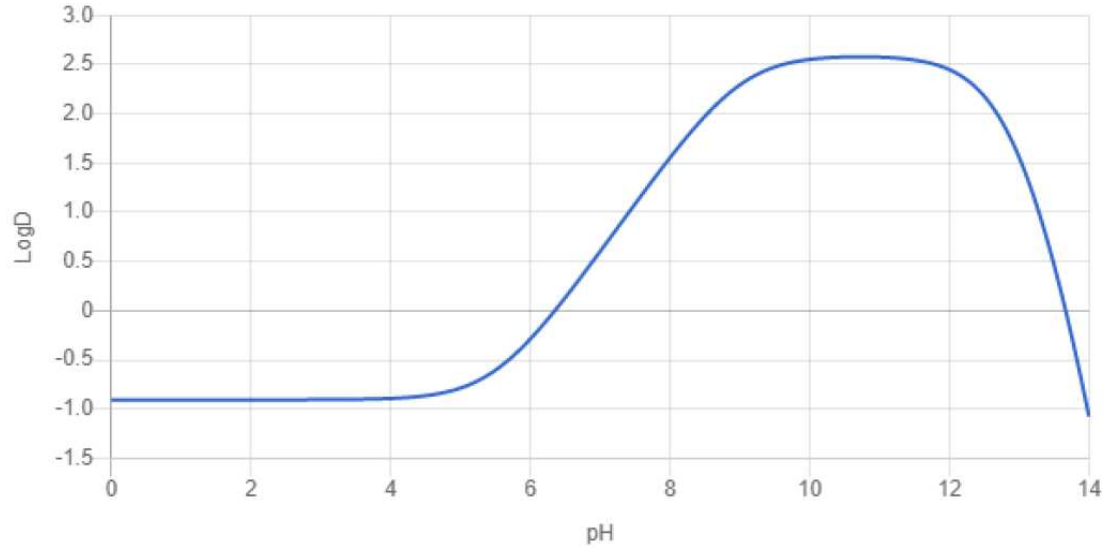
Strongest basic pKa: 9







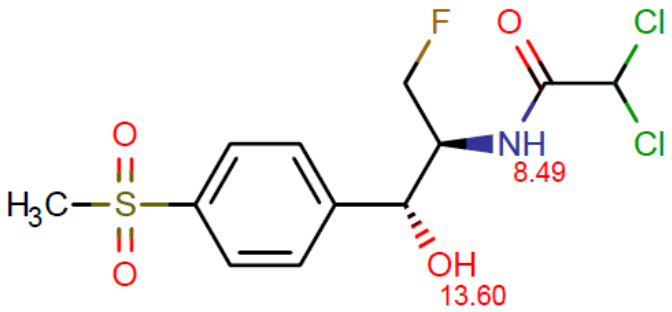
LogP nd logD



LogP: 2.596

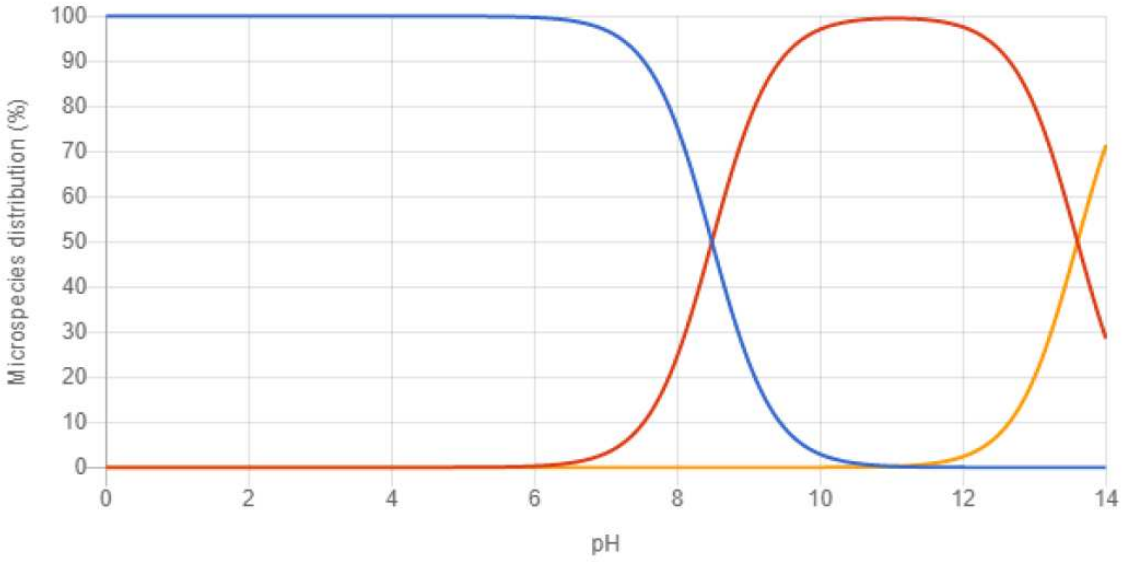
pH	logD
1.7	-0.90
4.6	-0.85
6.5	0.13
7.4	0.99
8.0	1.55

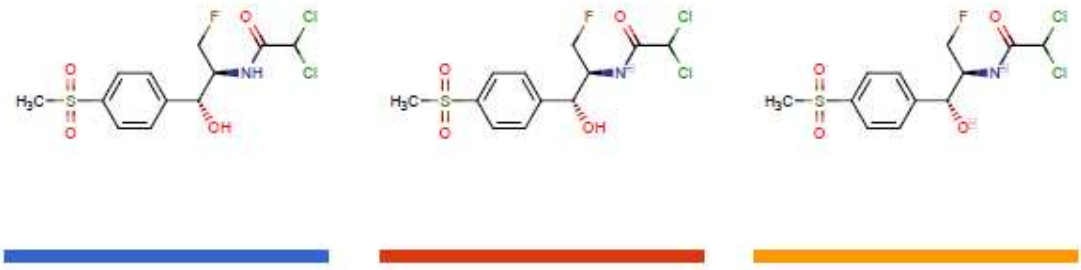
Florfenicol



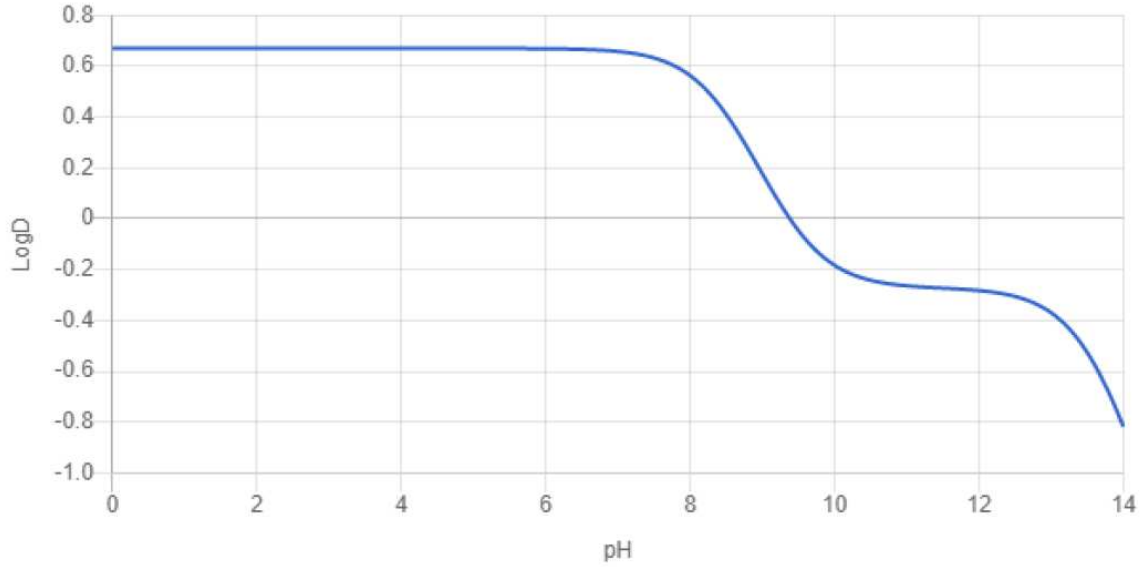
pKa

Strongest acidic pKa: 8.49





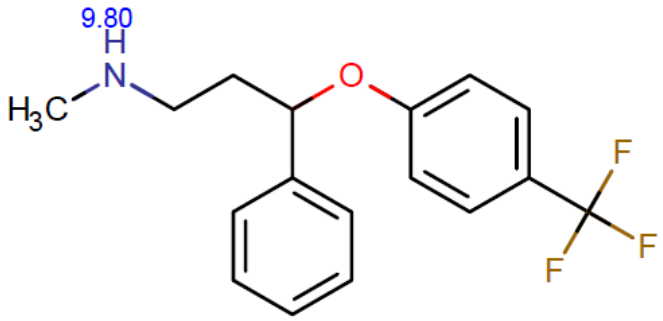
LogP and logD



LogP: 0.67

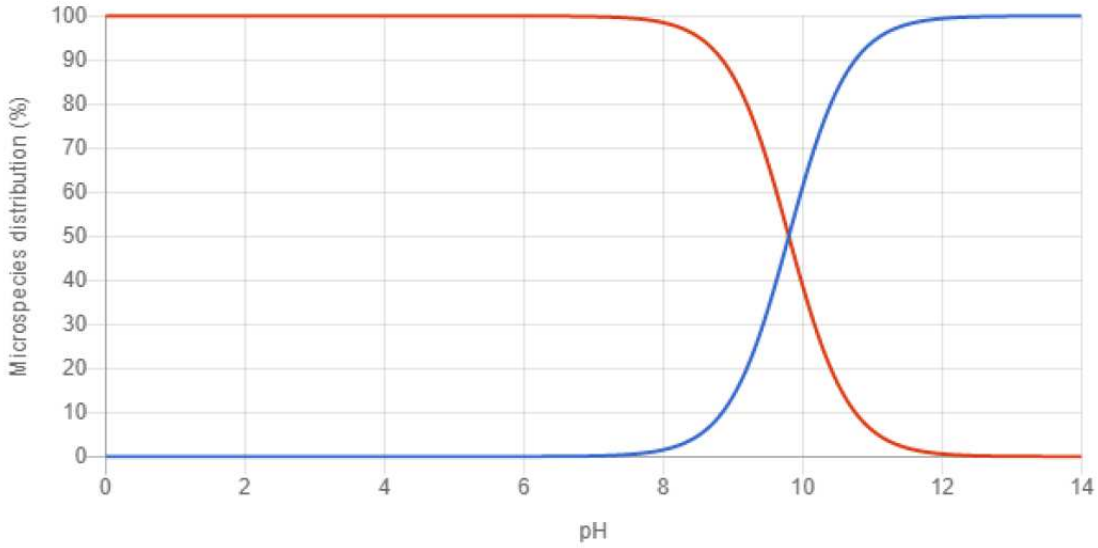
pH	logD
1.7	0.67
4.6	0.67
6.5	0.67
7.4	0.64
8.0	0.56

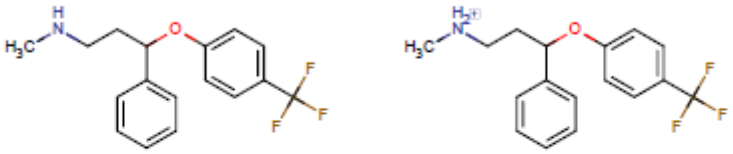
Fluoxetine



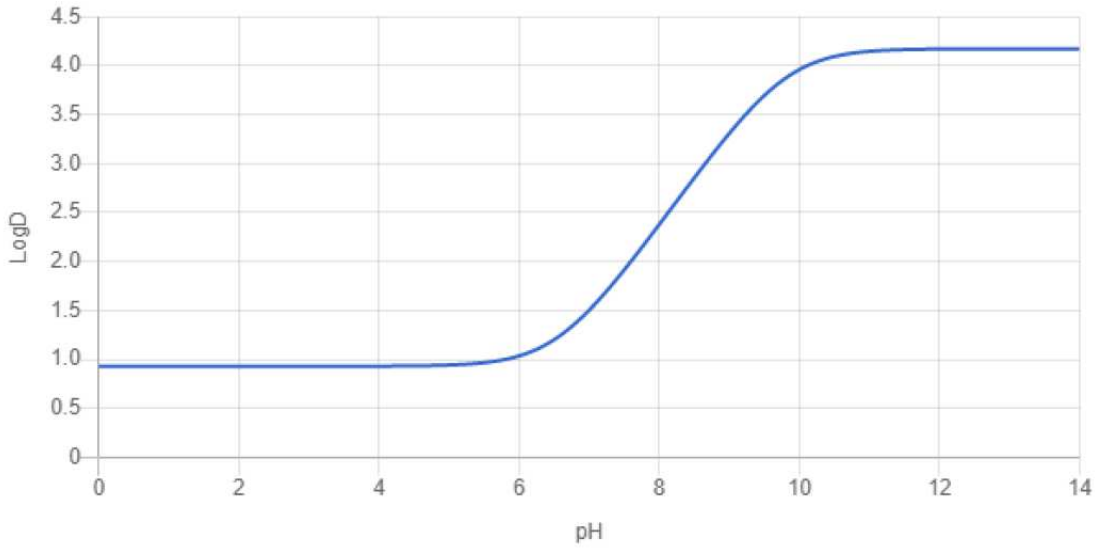
pKa

Strongest basic pKa: 9.8





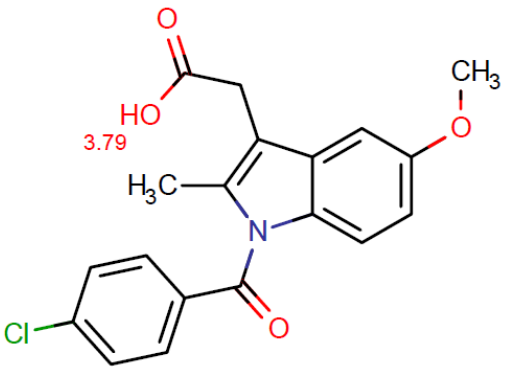
LogP and logD



LogP: 4.173

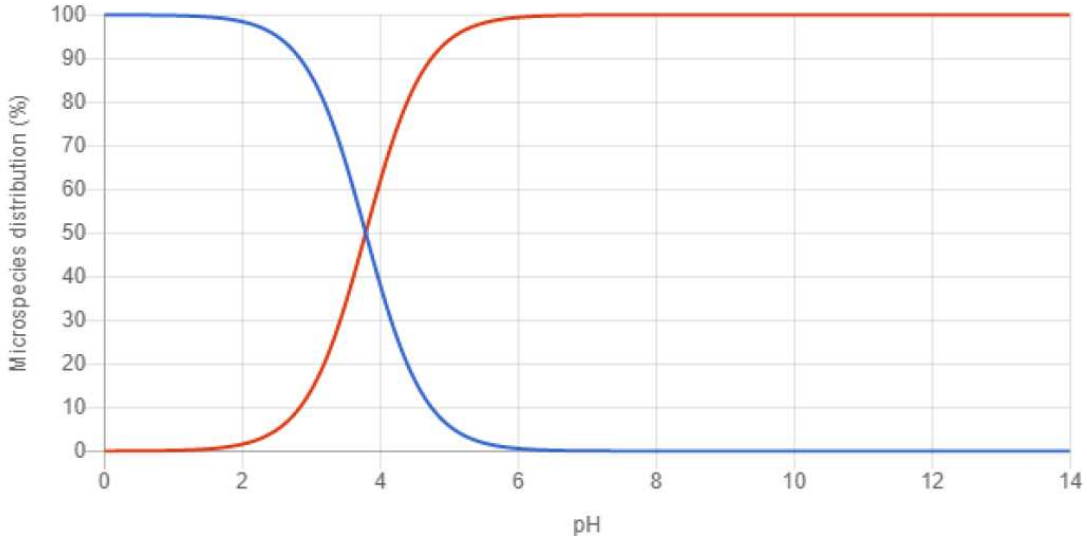
pH	logD
1.7	0.93
4.6	0.94
6.5	1.20
7.4	1.83
8.0	2.38

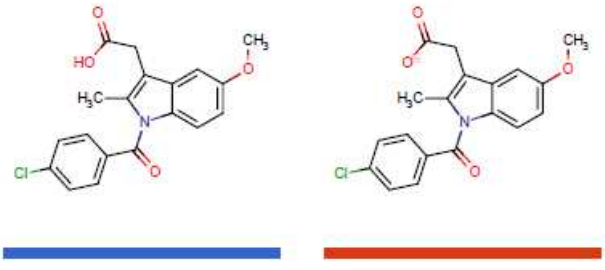
Indomethacin



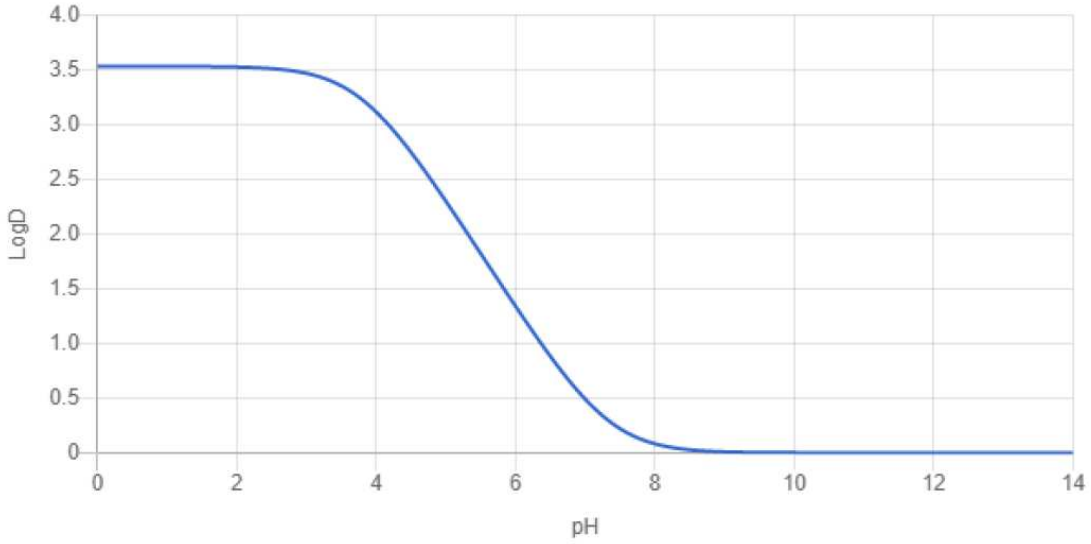
pKa

Strongest acidic pKa: 3.79





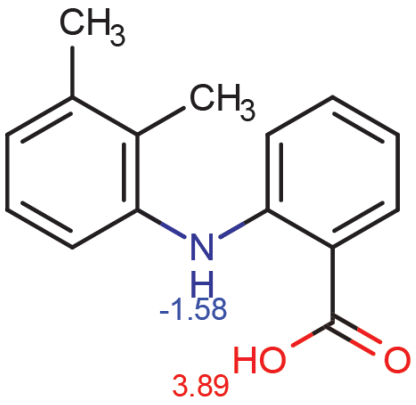
LogP and logD



LogP: 3.53

pH	logD
1.7	3.53
4.6	2.66
6.5	0.88
7.4	0.26
8.0	0.08

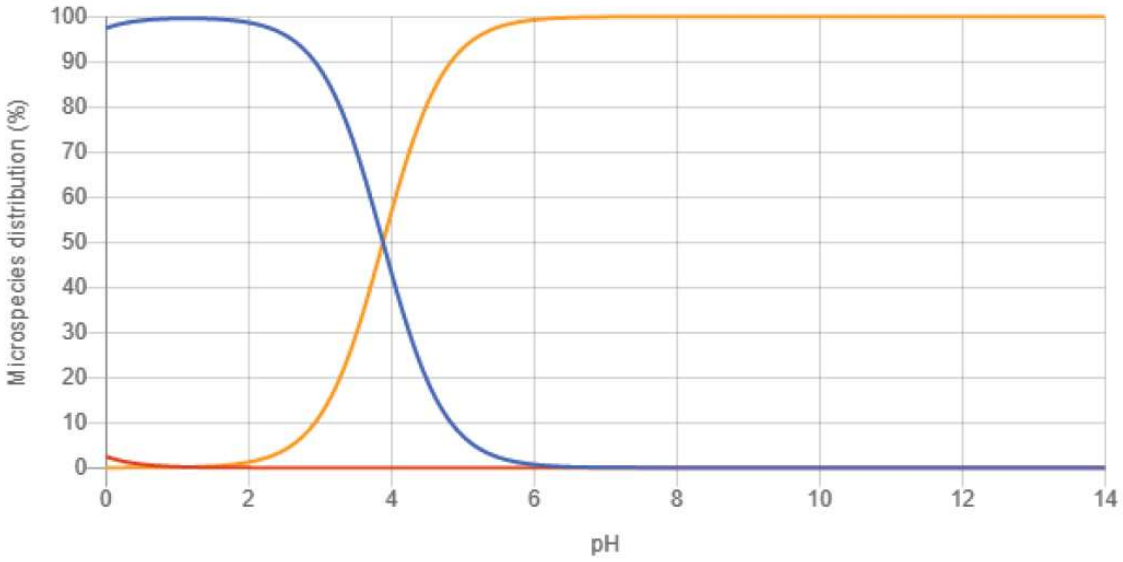
Mefenamic acid

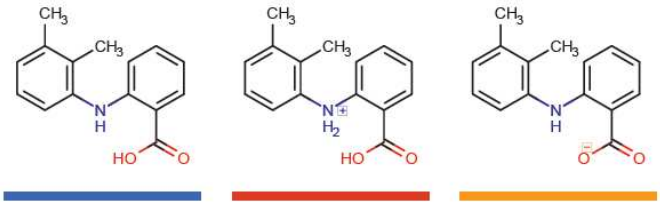


pKa

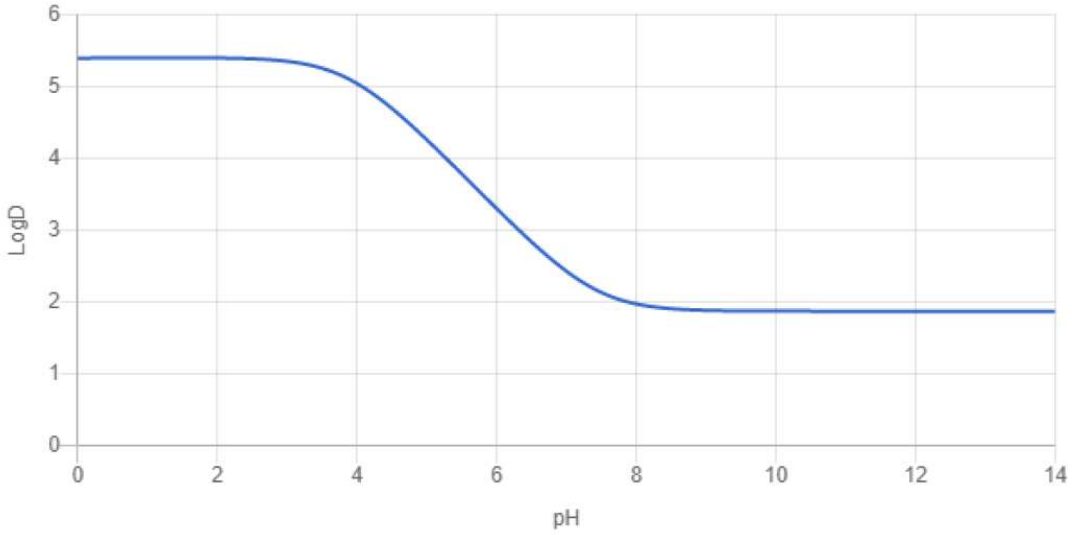
Strongest acidic pKa: 3.89

Strongest basic pKa: -1.58





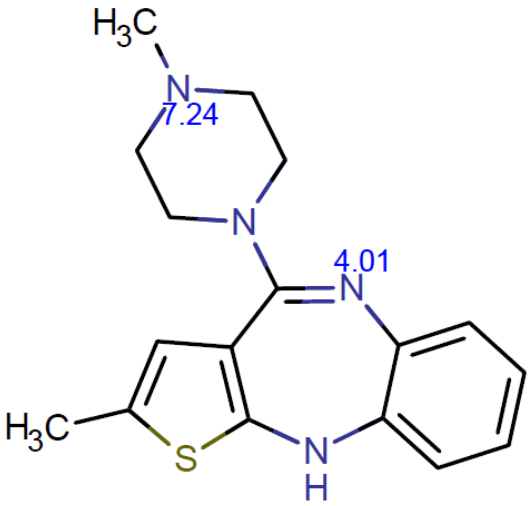
LogP and logD



LogP: 5.398

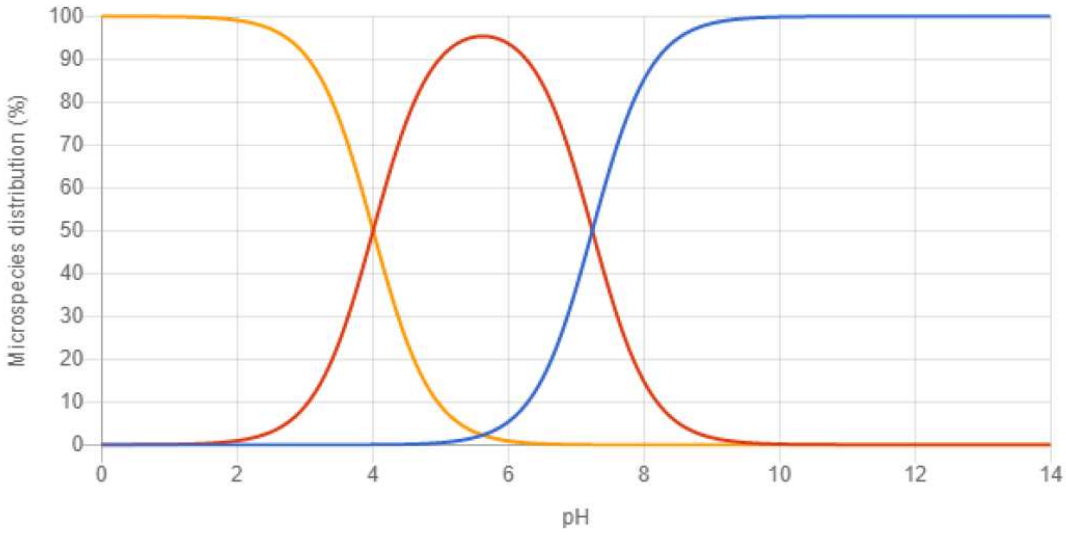
pH	logD
1.7	5.39
4.6	4.61
6.5	2.83
7.4	2.18
8.0	1.97

Olanzapine

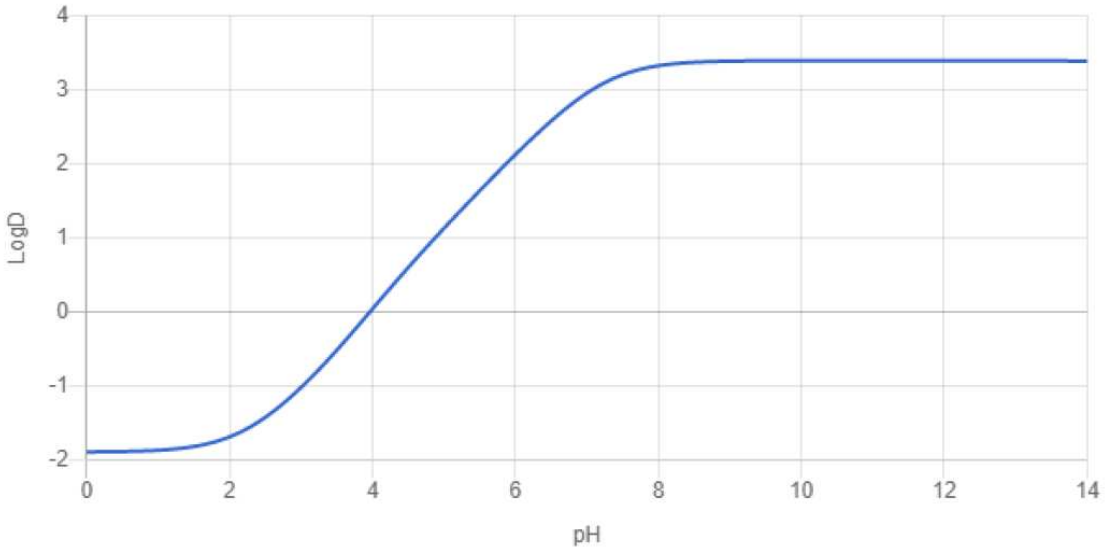


pKa

Strongest basic pKa: 7.24



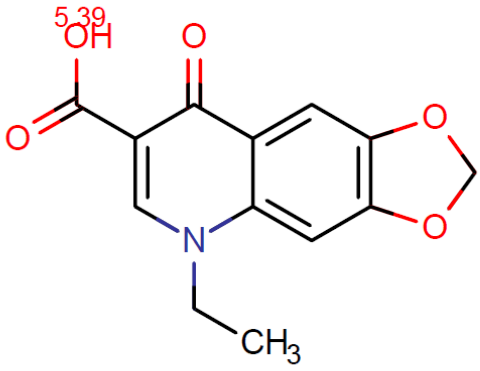
LogP and logD



LogP: 3.388

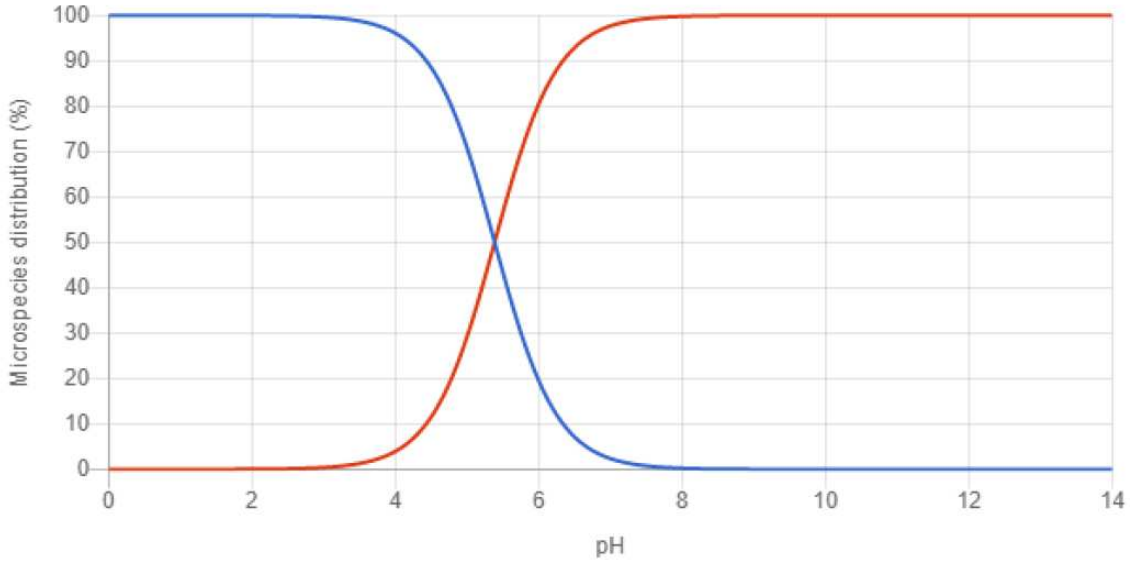
pH	logD
1.7	-1.78
4.6	0.70
6.5	2.57
7.4	3.16
8.0	3.32

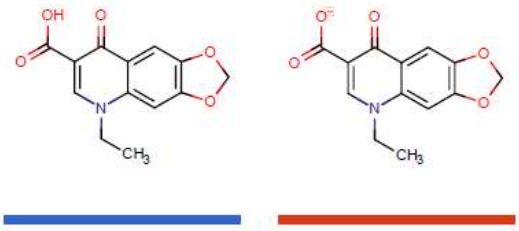
Oxolinic acid



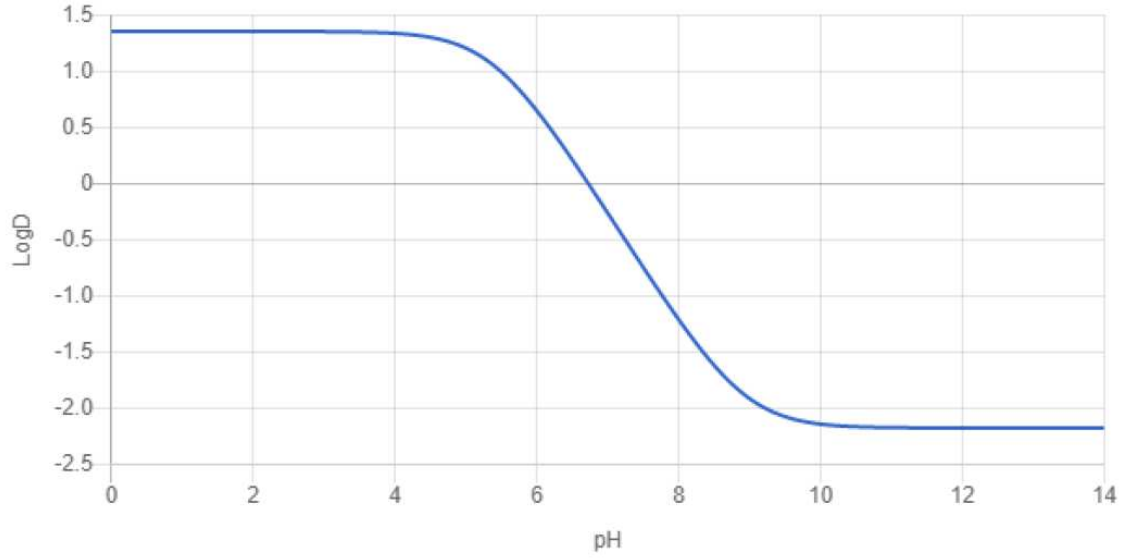
pKa

Strongest acidic pKa: 5.39





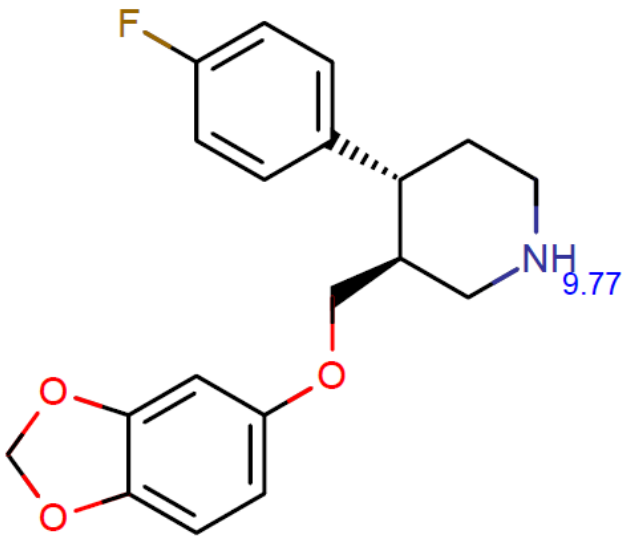
LogP and logD



LogP: 1.353

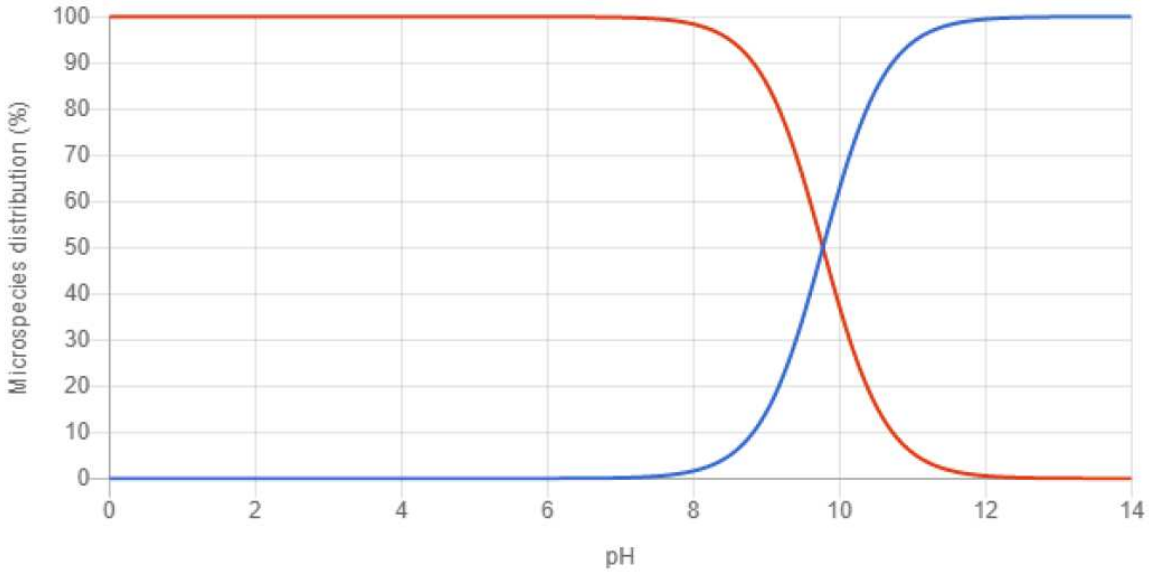
pH	logD
1.7	1.35
4.6	1.29
6.5	0.21
7.4	-0.65
8.0	-1.21

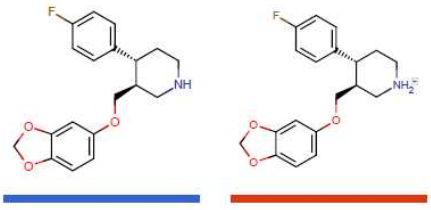
Paroxetine



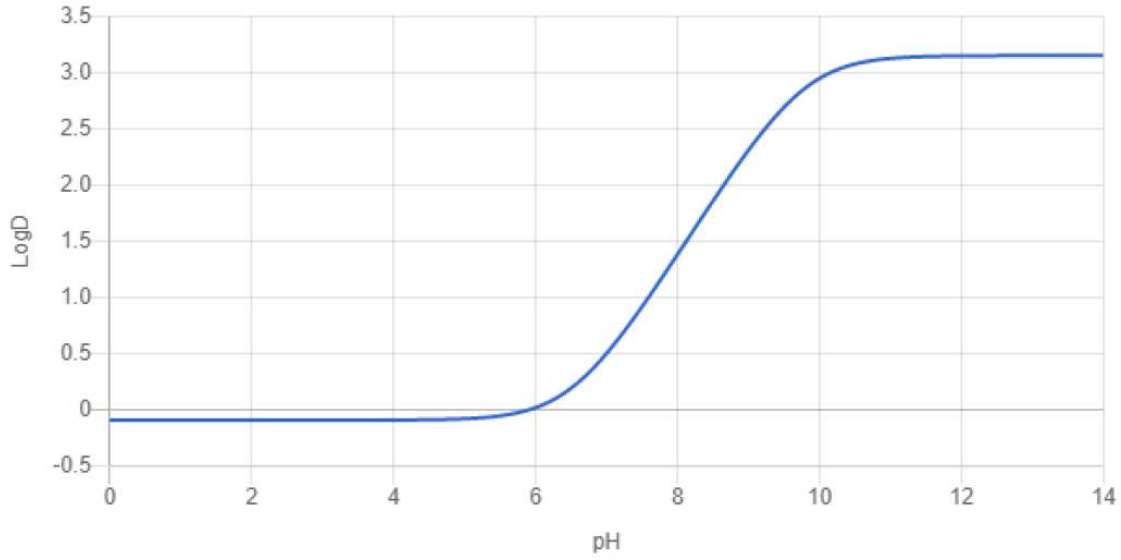
pKa

Strongest basic pKa: 9.77





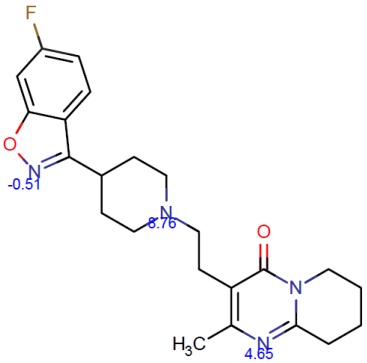
LogP and logD



LogP: 3.148

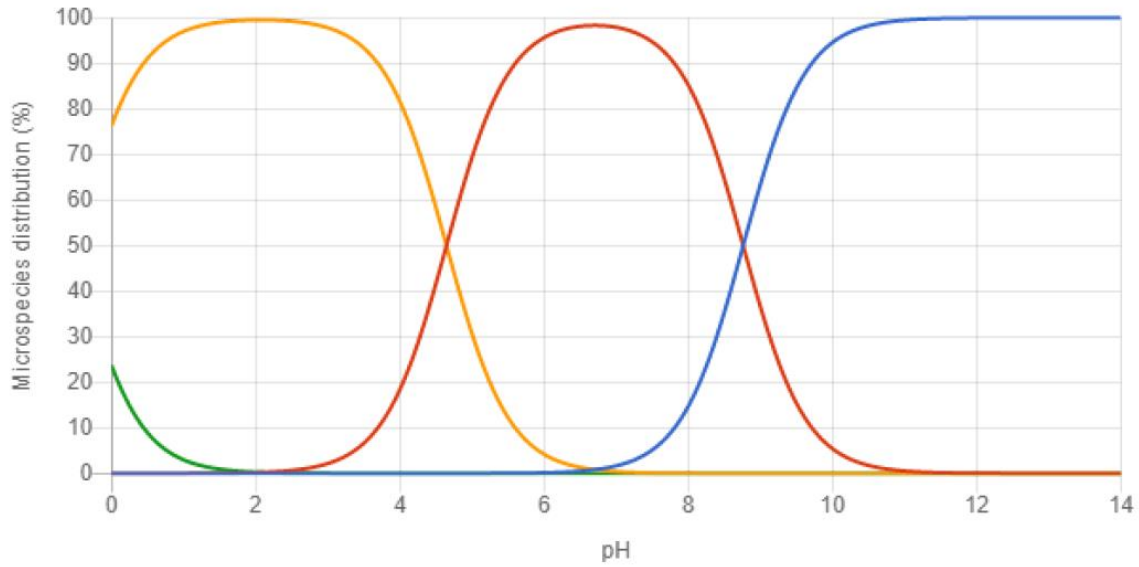
pH	logD
1.7	-0.09
4.6	-0.09
6.5	0.19
7.4	0.83
8.0	1.38

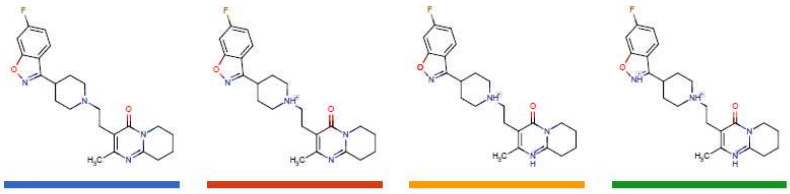
Risperidone



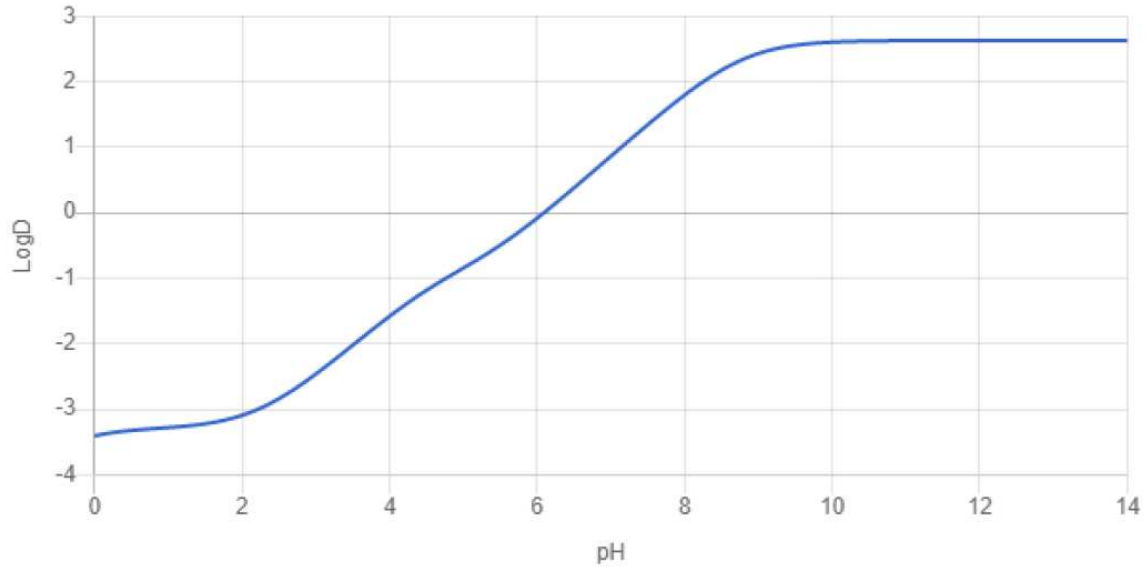
pKa

Strongest basic pKa: 8.76





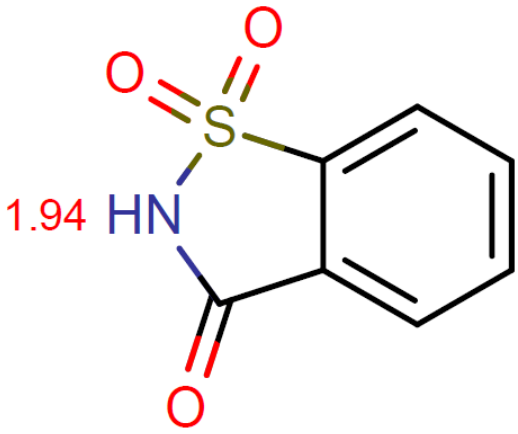
LogP and logD



LogP: 2.628

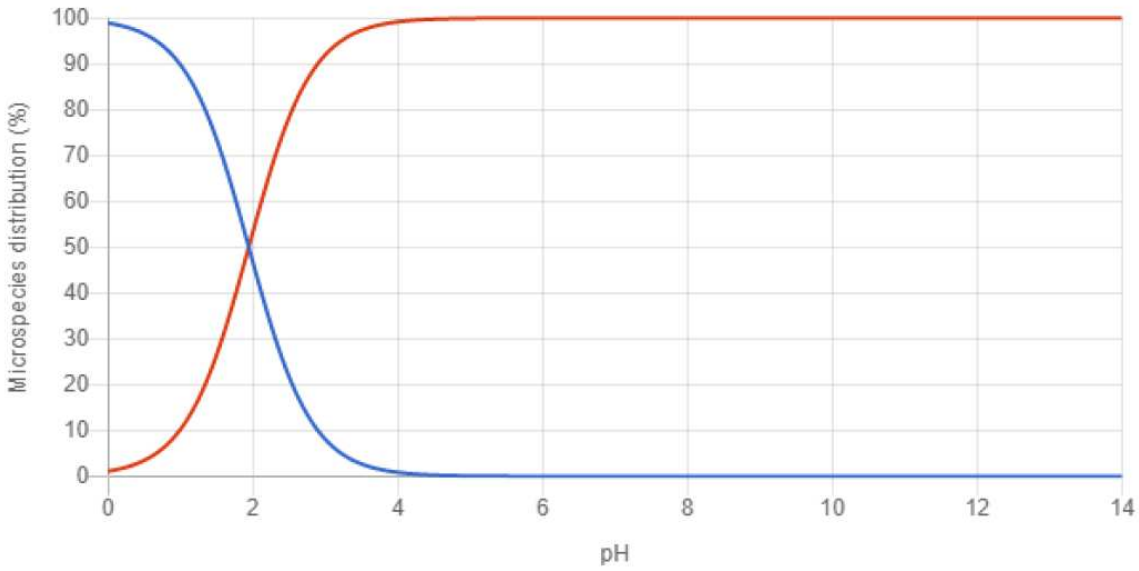
pH	logD
1.7	-3.18
4.6	-1.11
6.5	0.38
7.4	1.25
8.0	1.80

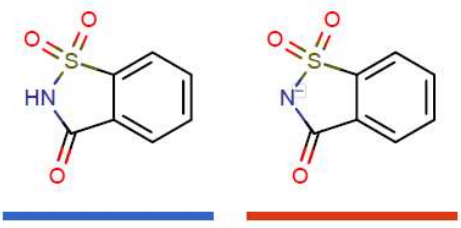
Saccharin



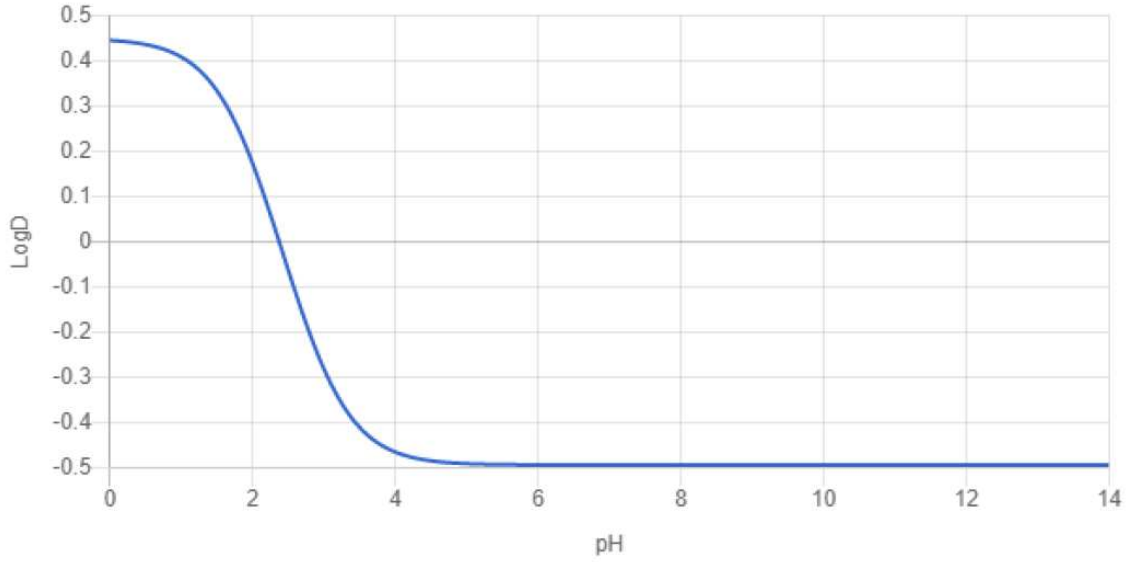
pKa

Strongest acidic pKa: 1.94





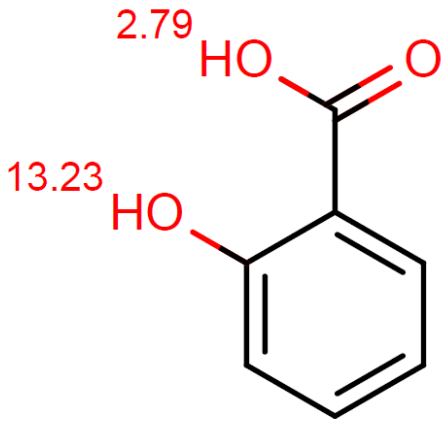
LogP and logD



LogP: 0.449

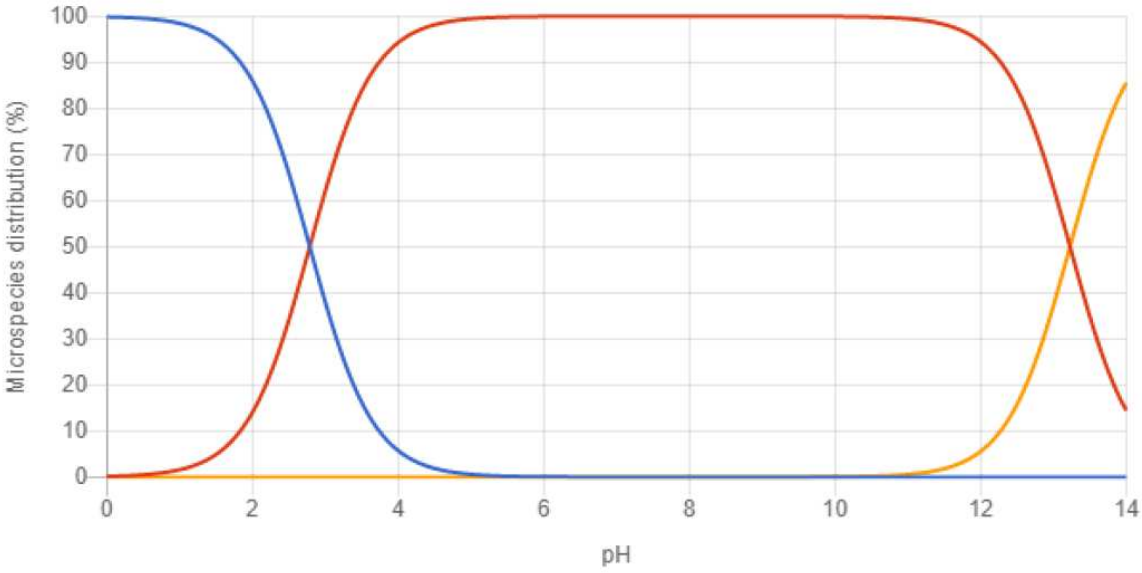
pH	logD
1.7	0.28
4.6	-0.49
6.5	-0.49
7.4	-0.49
8.0	-0.49

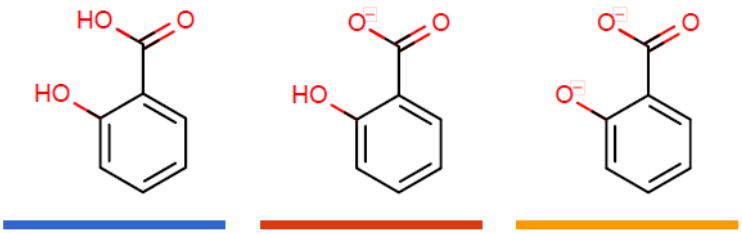
Salicylic acid



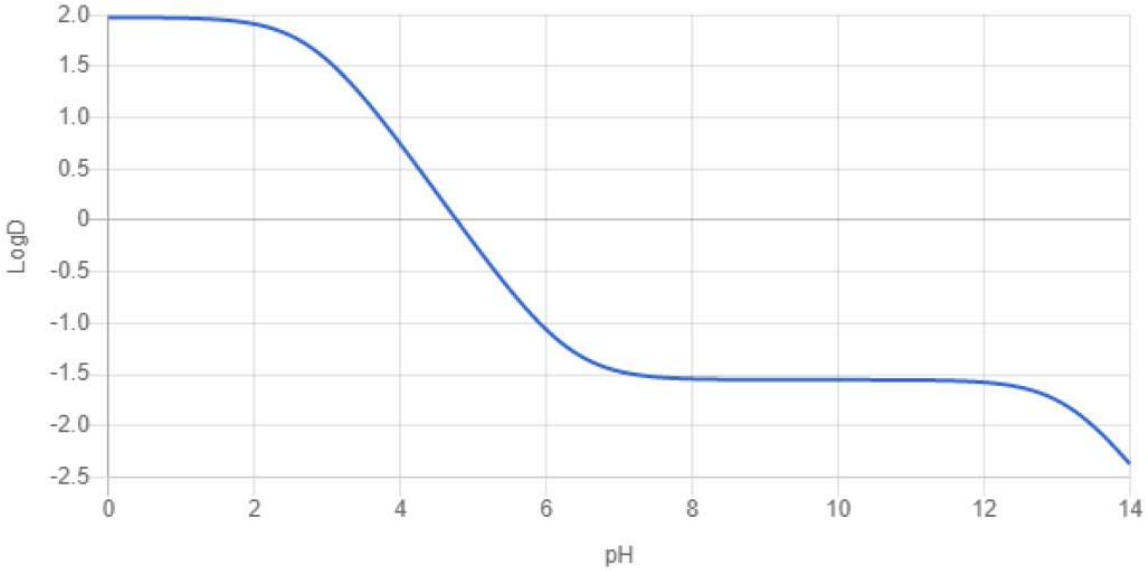
pKa

Strongest acidic pKa: 2.79





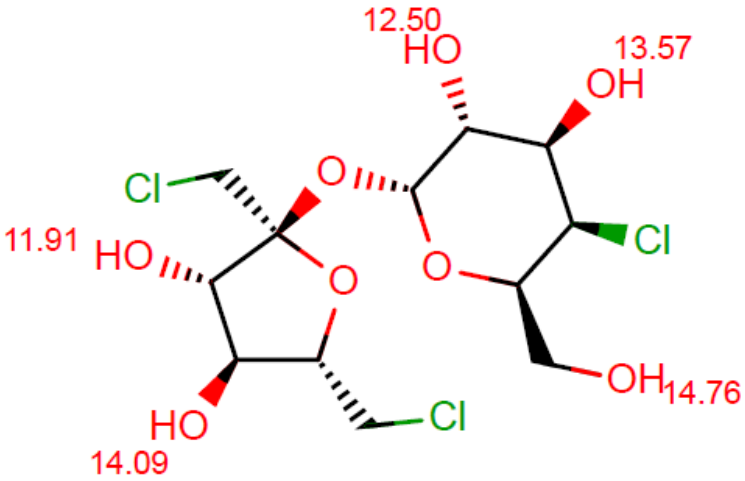
LogP and logD



LogP: 1.977

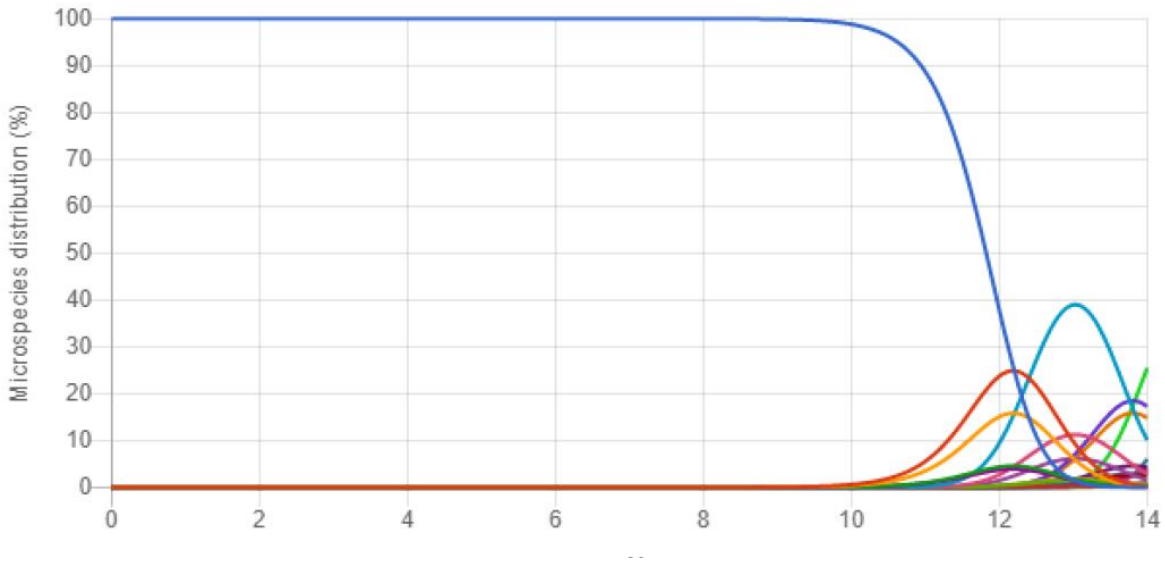
pH	logD
1.7	1.94
4.6	0.17
6.5	-1.33
7.4	-1.52
8.0	-1.54

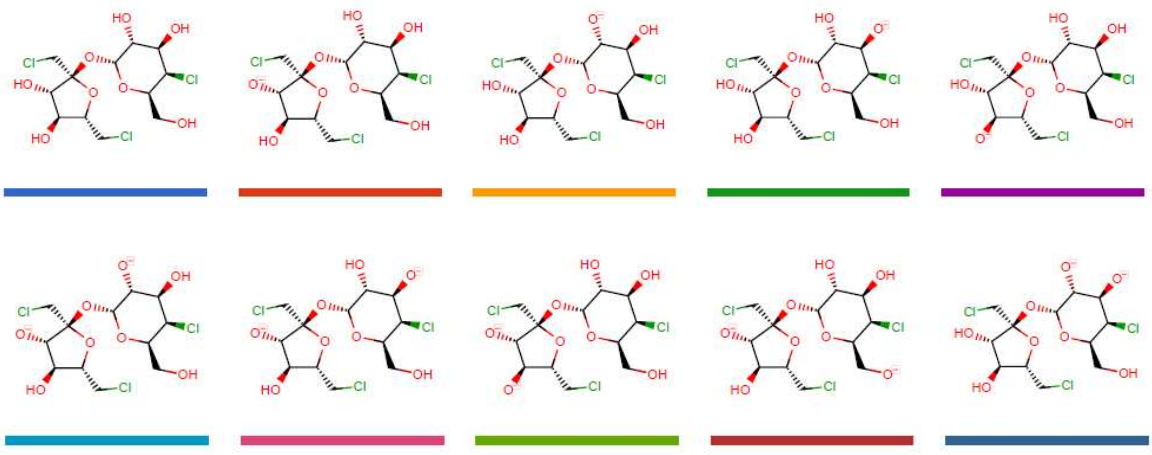
Sucralose

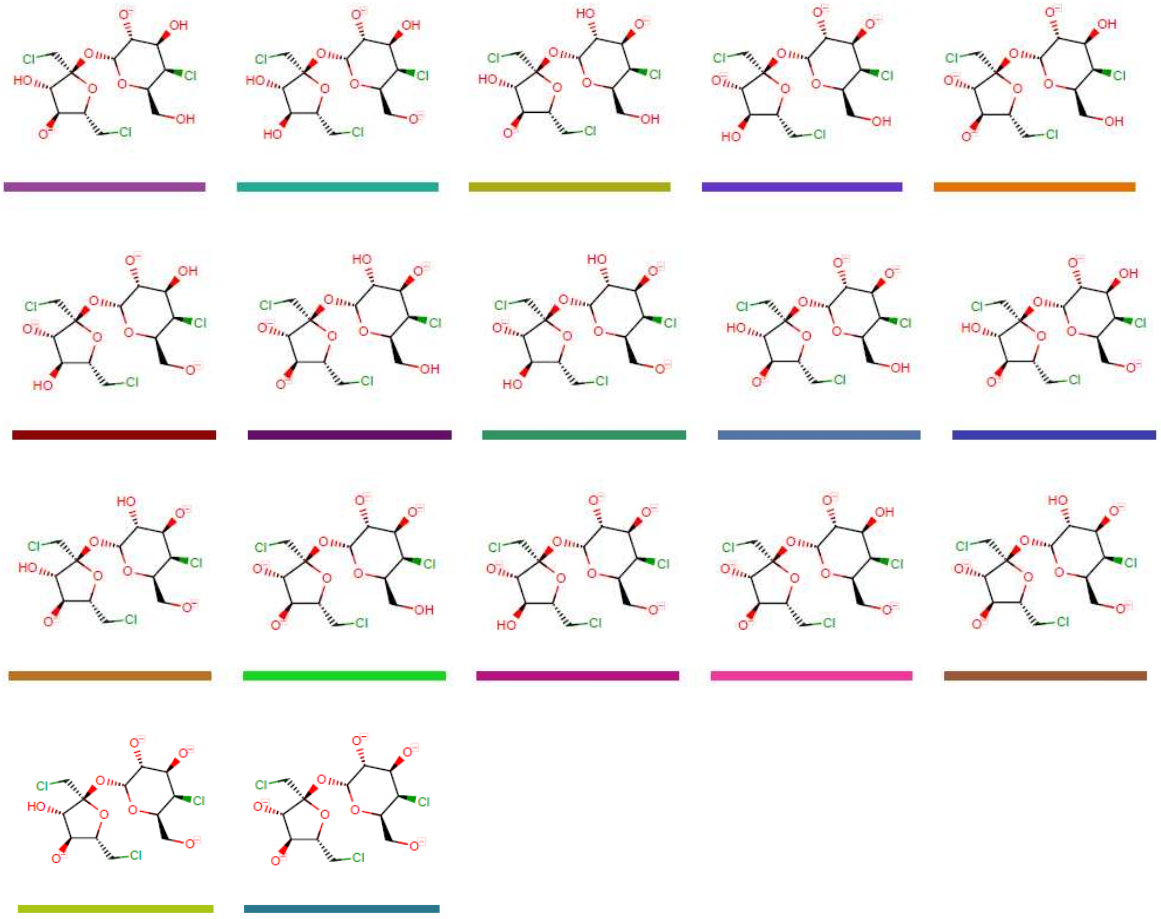


pKa

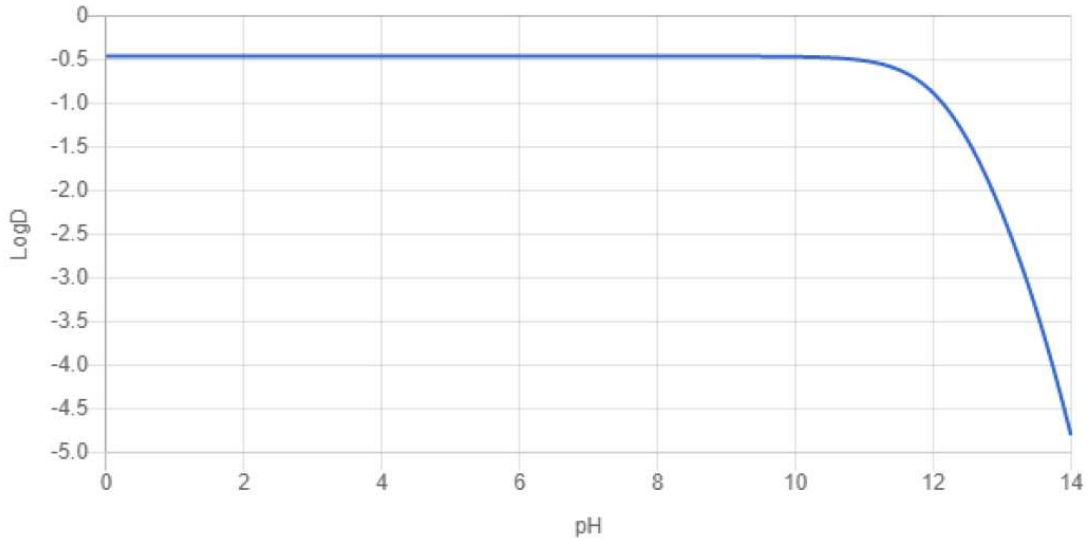
Strongest acidic pKa: 11.91







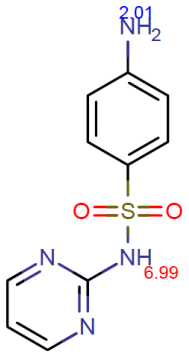
LogP and logD



LogP: -0.465

pH	logD
1.7	-0.47
4.6	-0.47
6.5	-0.47
7.4	-0.47
8.0	-0.47

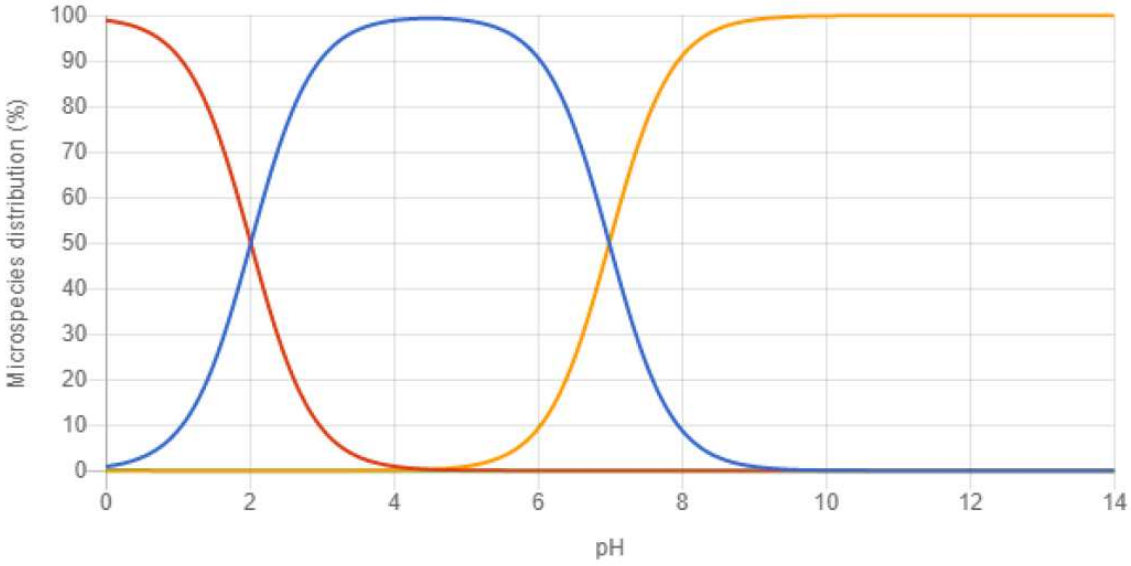
Sulfadiazine

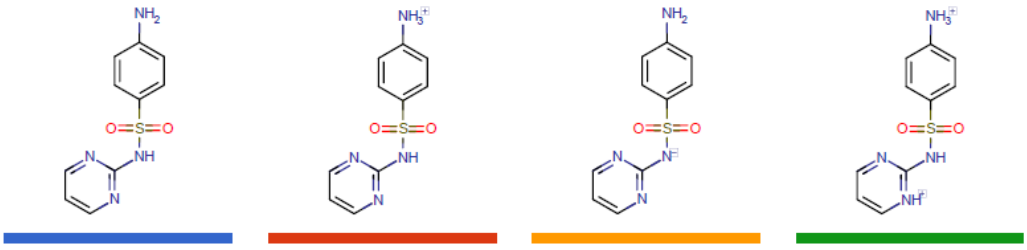


pKa

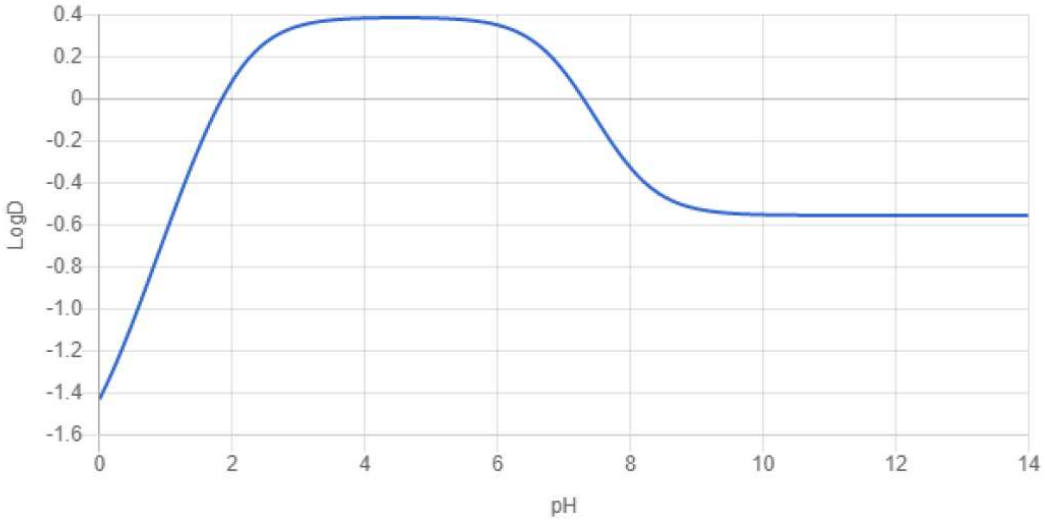
Strongest acidic pKa: 6.99

Strongest basic pKa: 2.01





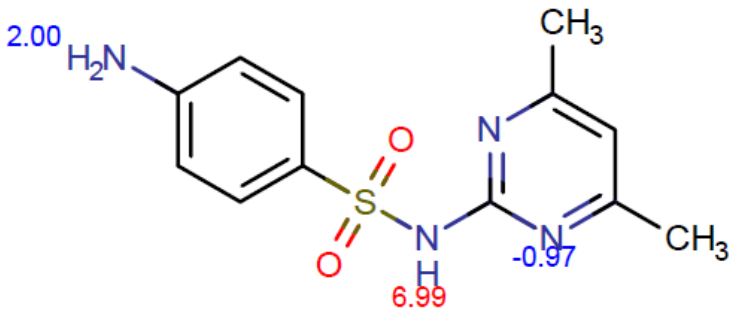
LogP and logD



LogP: 0.387

pH	logD
1.7	-0.09
4.6	0.38
6.5	0.28
7.4	-0.05
8.0	-0.33

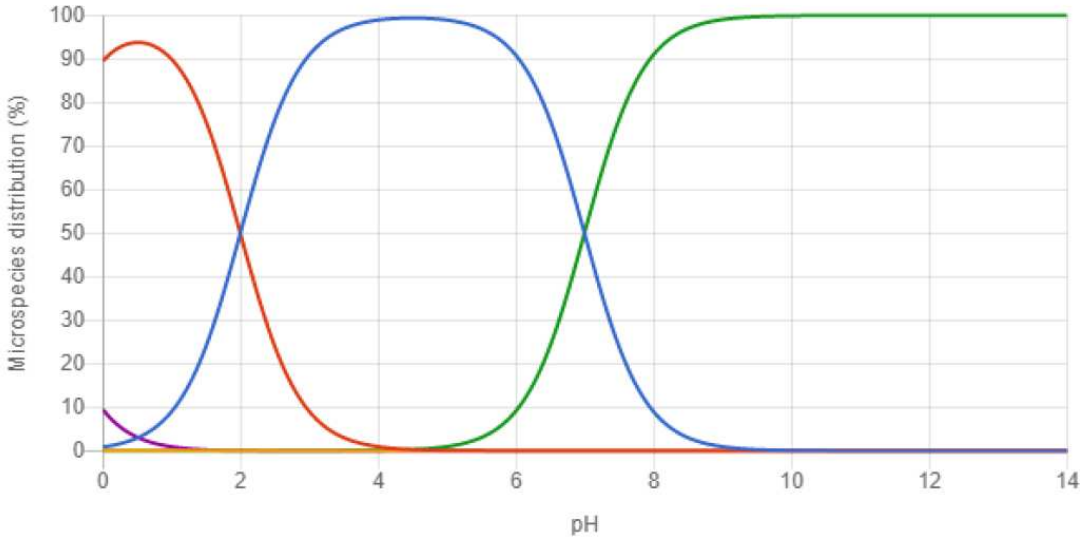
Sulphamethazine

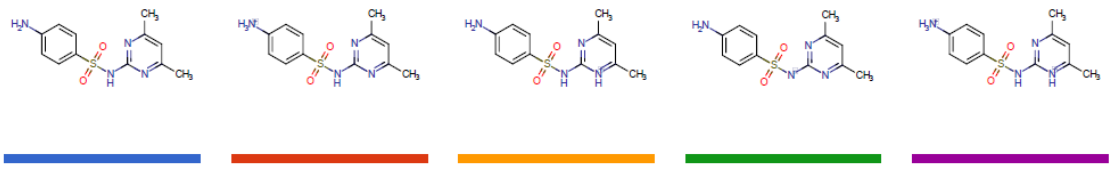


pKa

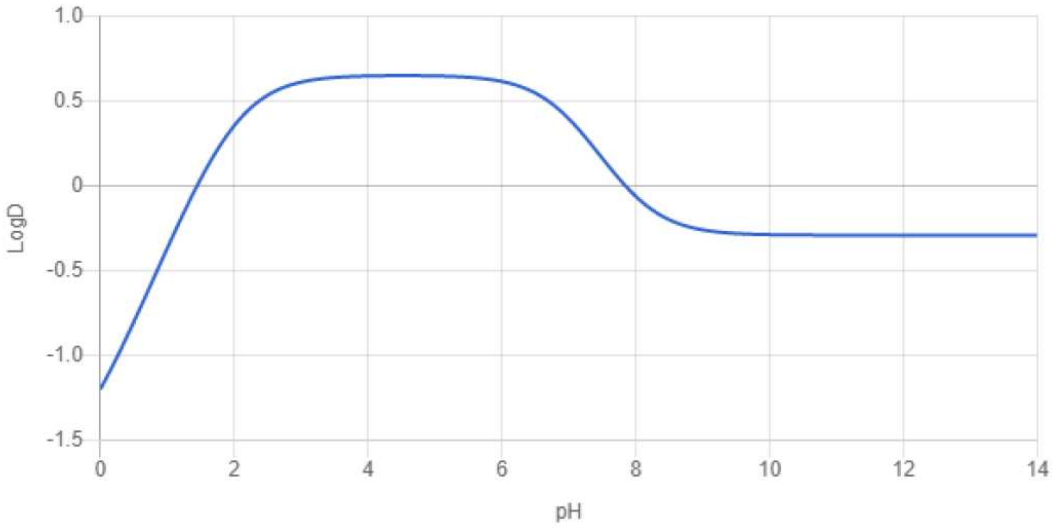
Strongest acidic pKa: 6.99

Strongest basic pKa: 2





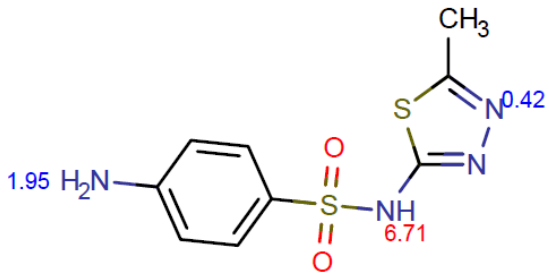
LogP and logD



LogP: 0.65

pH	logD
1.7	0.18
4.6	0.65
6.5	0.54
7.4	0.21
8.0	-0.06

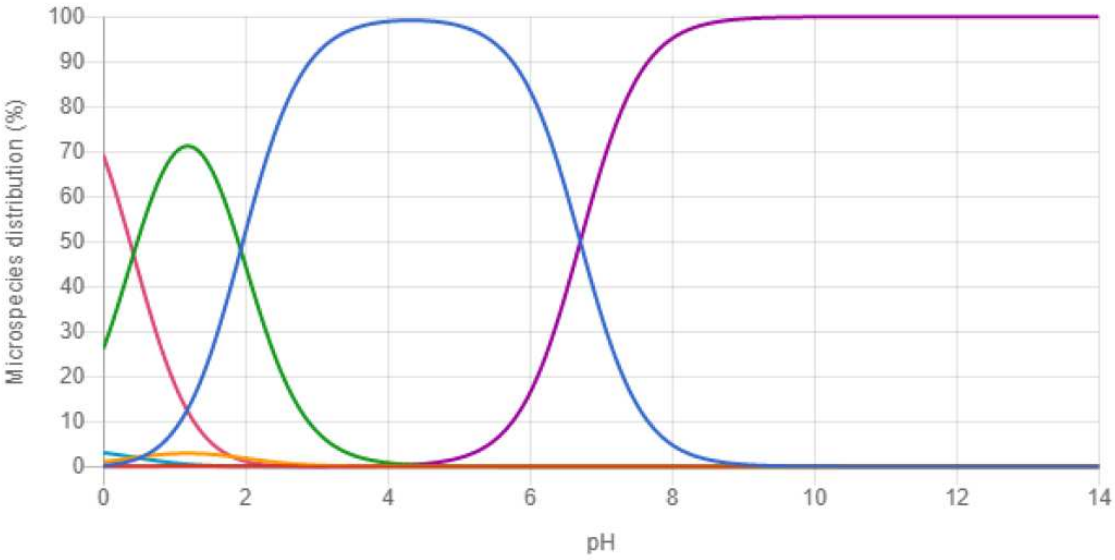
Sulfamethizole

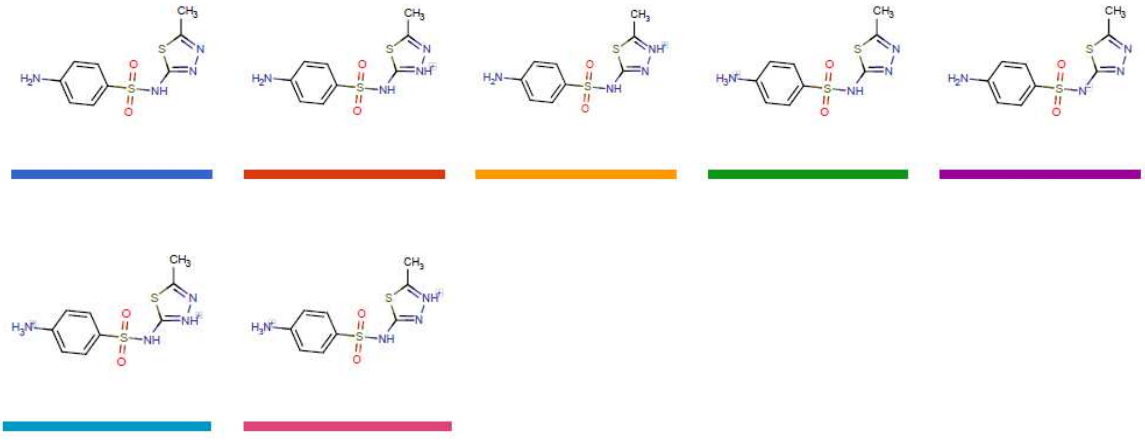


pKa

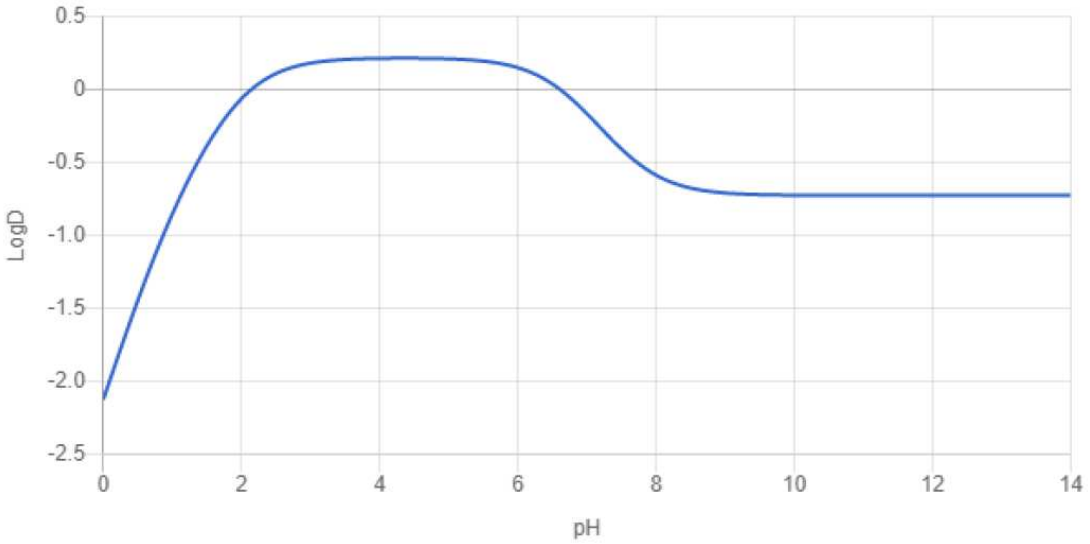
Strongest acidic pKa: 6.71

Strongest basic pKa: 1.95





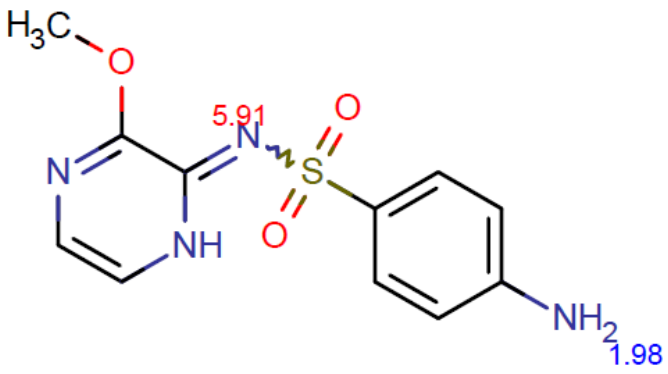
LogP and logD



LogP: 0.214

pH	logD
1.7	-0.24
4.6	0.21
6.5	0.03
7.4	-0.36
8.0	-0.59

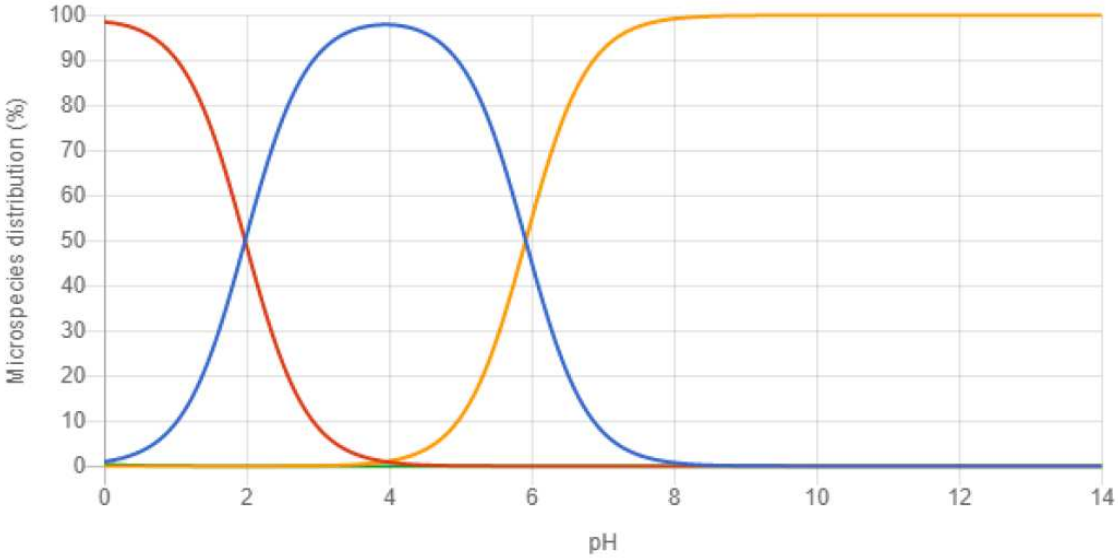
Sulfamethoxypyridazine

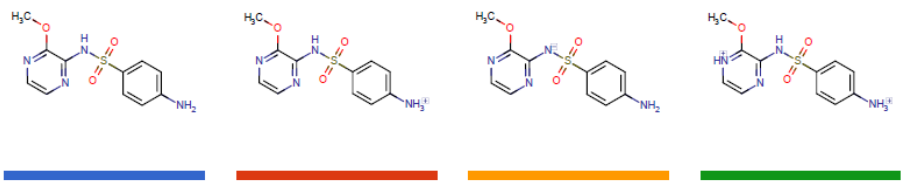


pKa

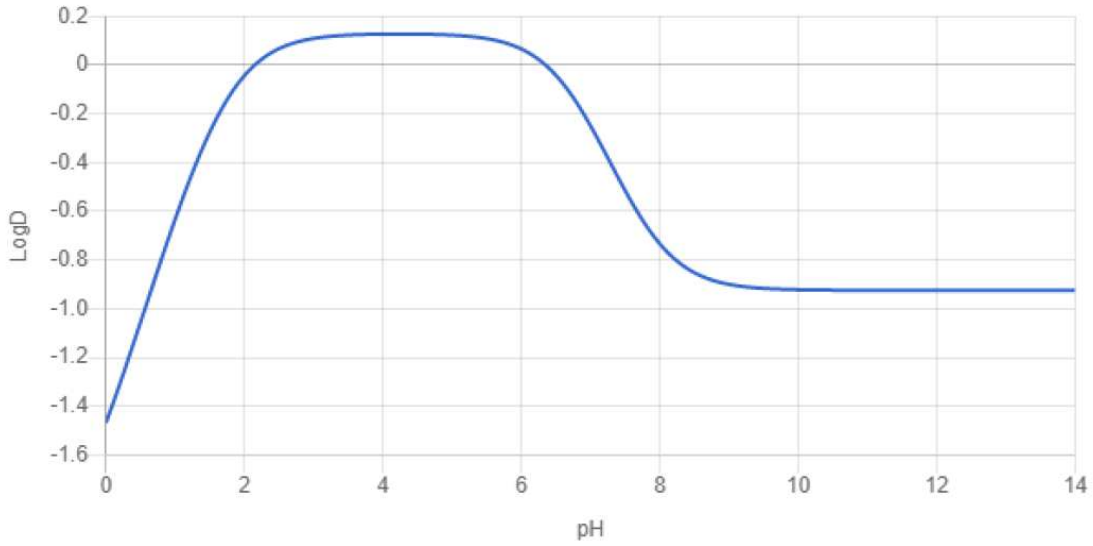
Strongest acidic pKa: 5.91

Strongest basic pKa: 1.98





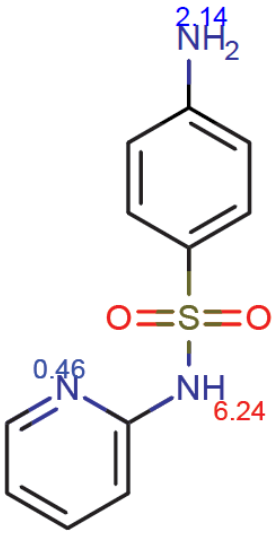
LogP and logD



LogP: 0.13

pH	logD
1.7	-0.17
4.6	0.13
6.5	-0.04
7.4	-0.46
8.0	-0.73

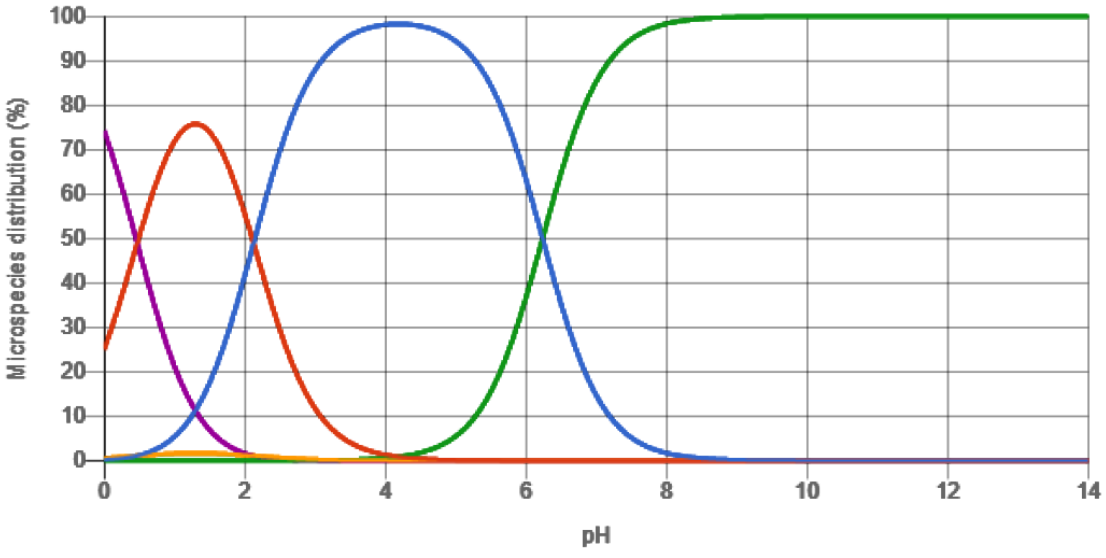
Sulfapyridine

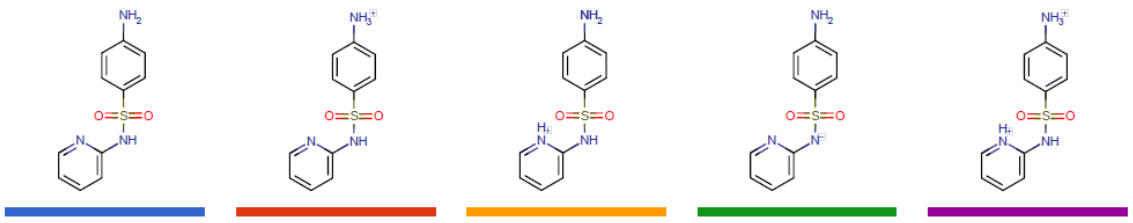


pKa

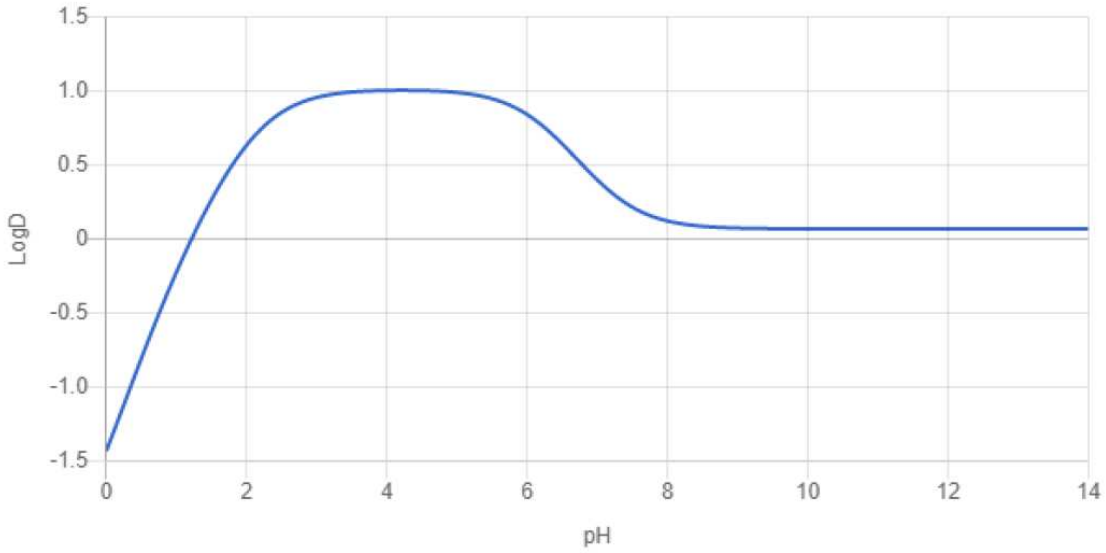
Strongest acidic pKa: 6.24

Strongest basic pKa: 2.14





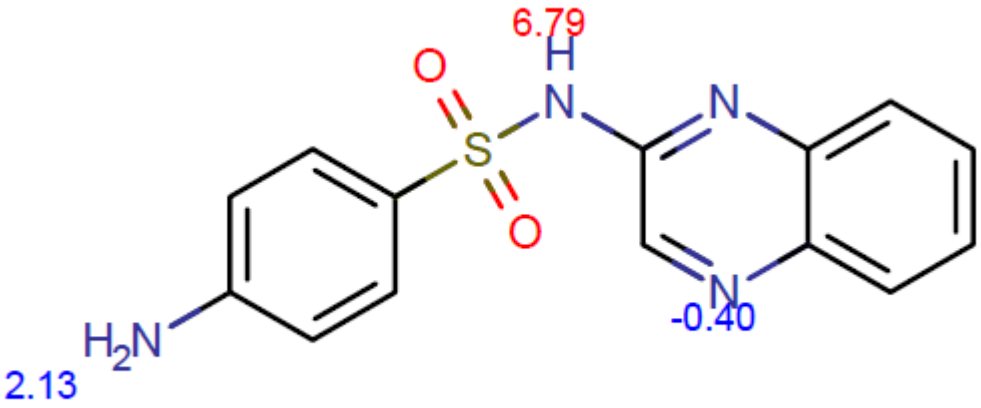
LogP and logD



LogP: 1.009

pH	logD
1.7	0.43
4.6	1.00
6.5	0.64
7.4	0.24
8.0	0.12

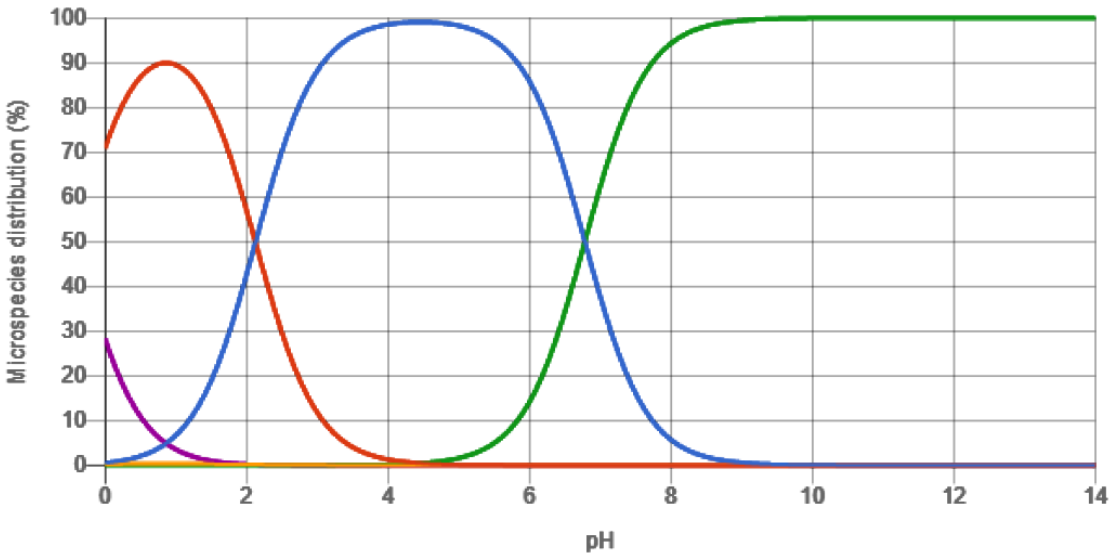
Sulfaquinoxaline

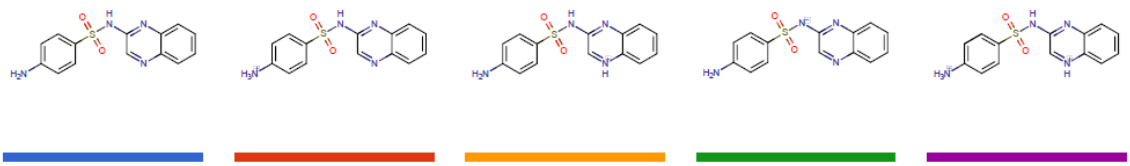


pKa

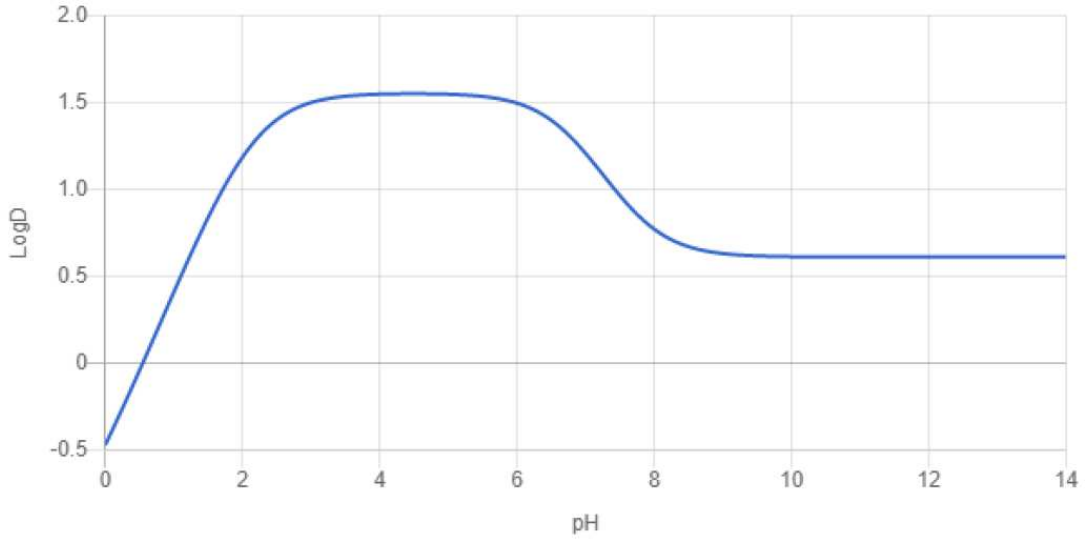
Strongest acidic pKa: 6.79

Strongest basic pKa: 2.13





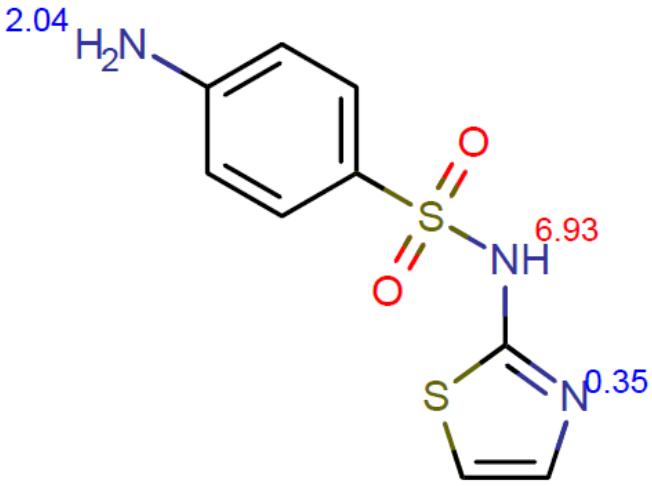
LogP and logD



LogP: 1.552

pH	logD
1.7	0.99
4.6	1.55
6.5	1.40
7.4	1.01
8.0	0.77

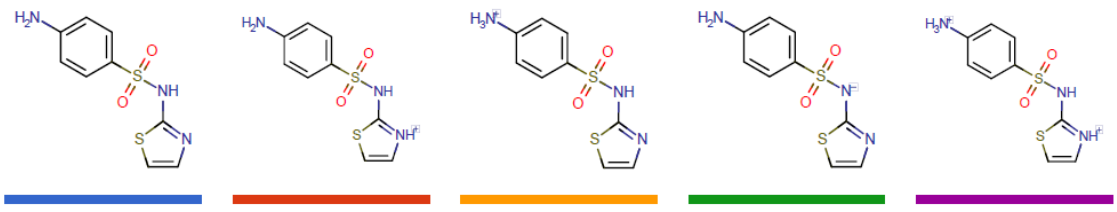
Sulfathiazole



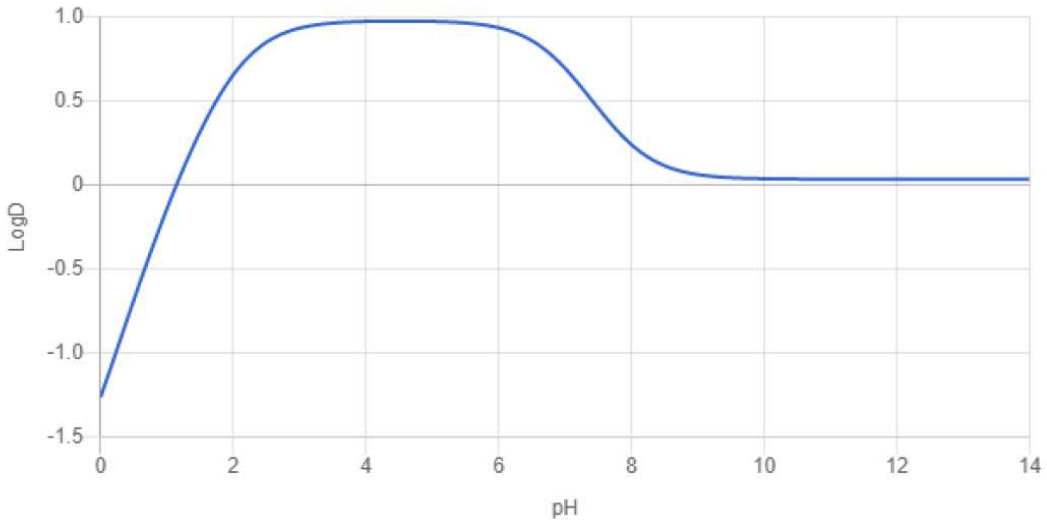
pKa

Strongest acidic pKa: 6.93

Strongest basic pKa: 2.04



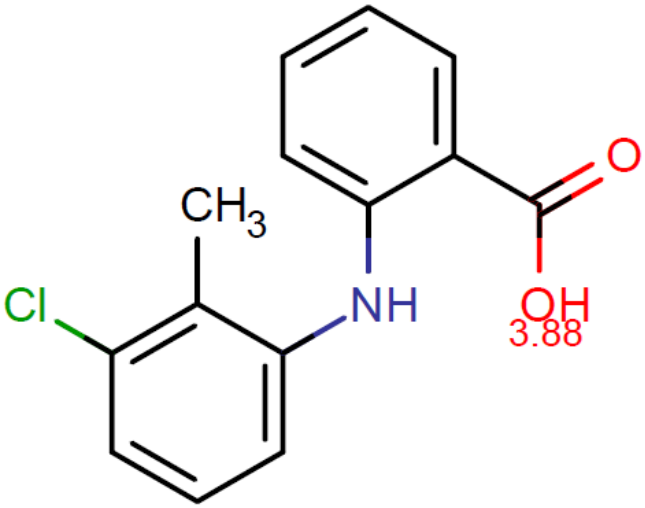
LogP and logD



LogP: 0.975

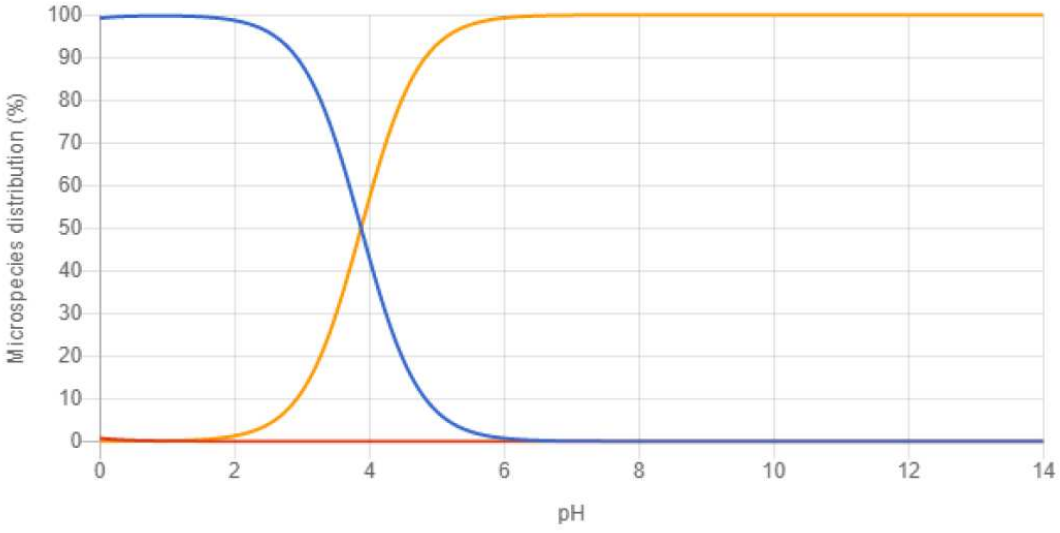
pH	logD
1.7	0.47
4.6	0.97
6.5	0.86
7.4	0.50
8.0	0.24

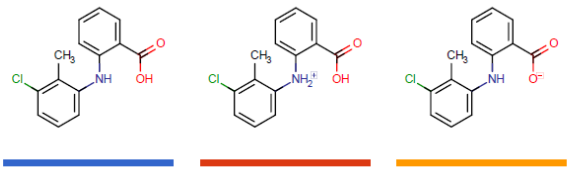
Tolfenamic acid



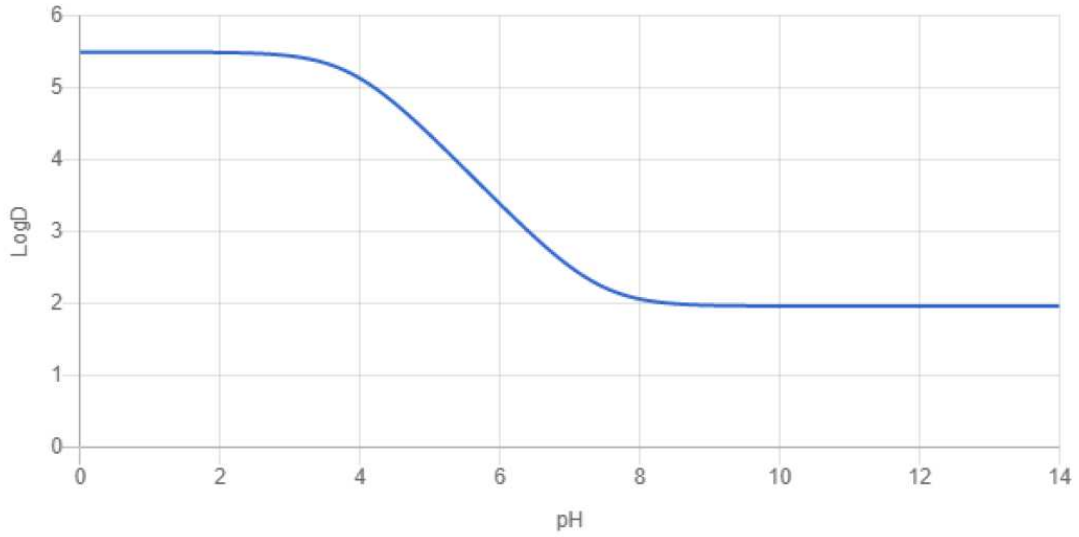
pKa

Strongest acidic pKa: 3.88





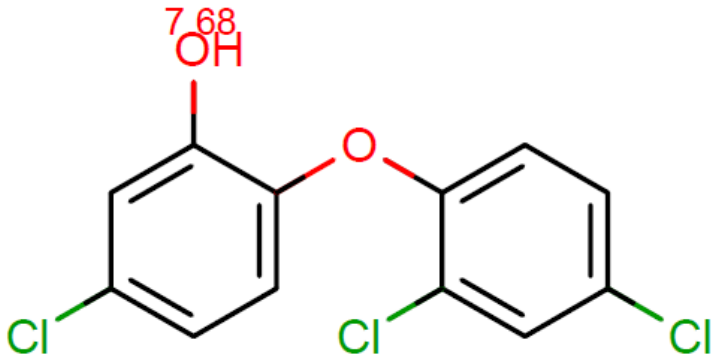
LogP and logD



LogP: 5.488

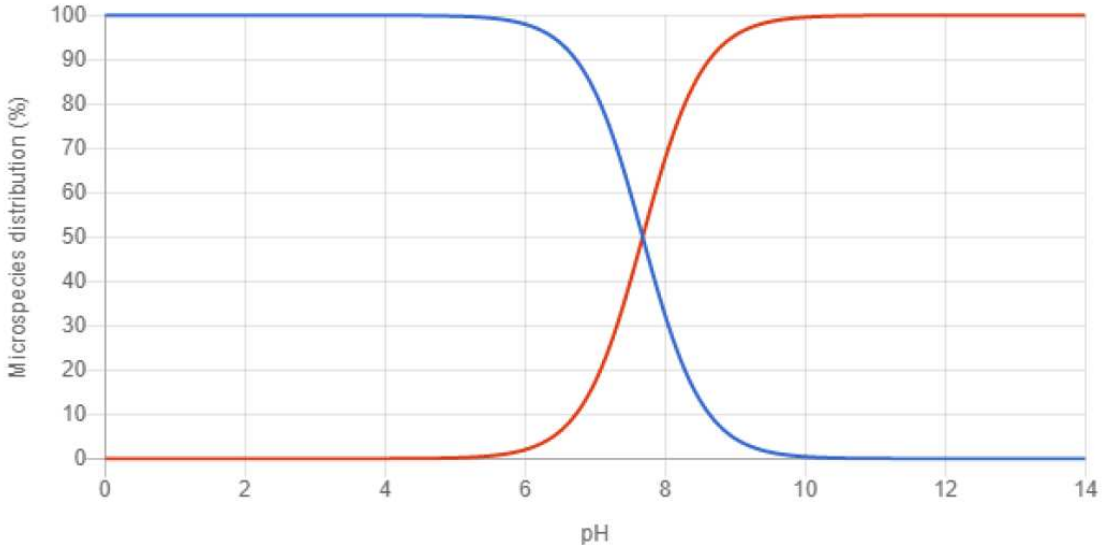
pH	logD
1.7	5.49
4.6	4.69
6.5	2.92
7.4	2.26
8.0	2.06

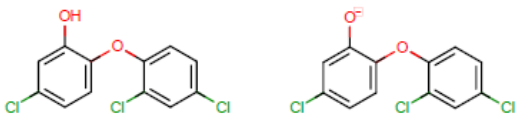
Triclosan



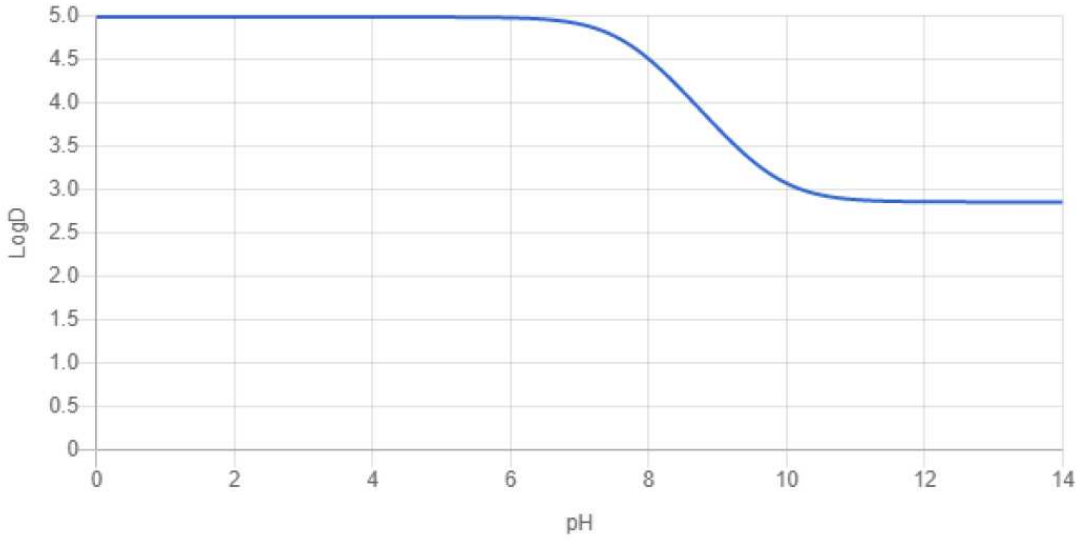
pKa

Strongest acidic pKa: 7.68





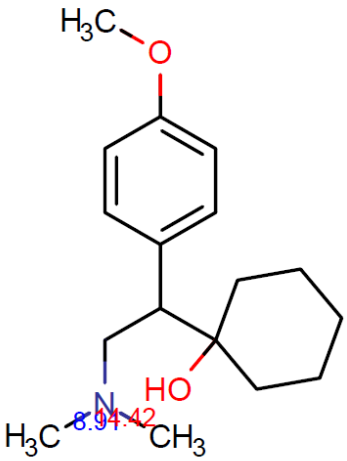
LogP and logD



LogP: 4.982

pH	logD
1.7	4.98
4.6	4.98
6.5	4.95
7.4	4.80
8.0	4.50

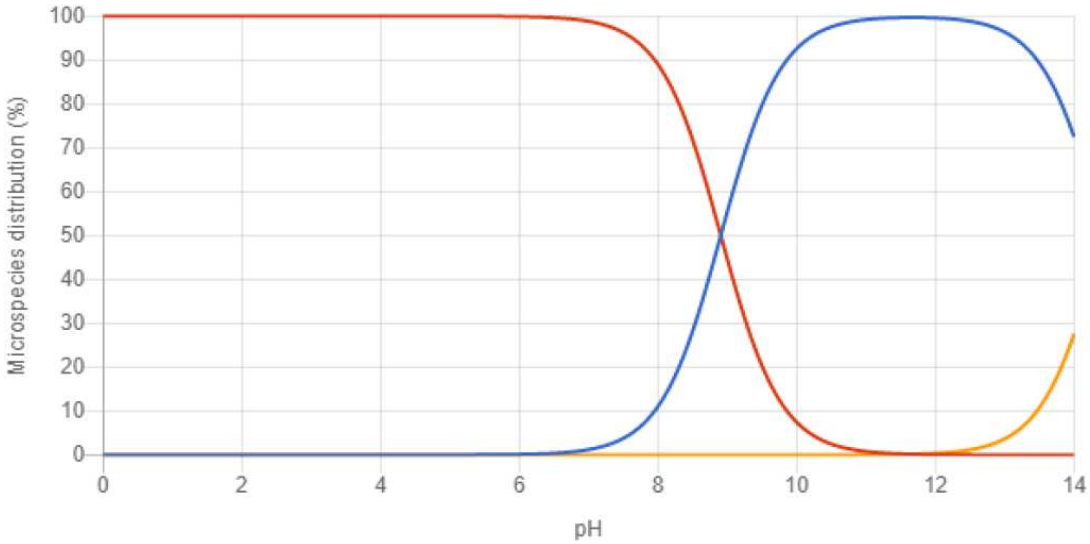
Venlafaxine

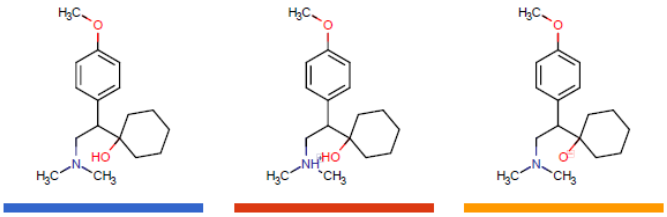


pKa

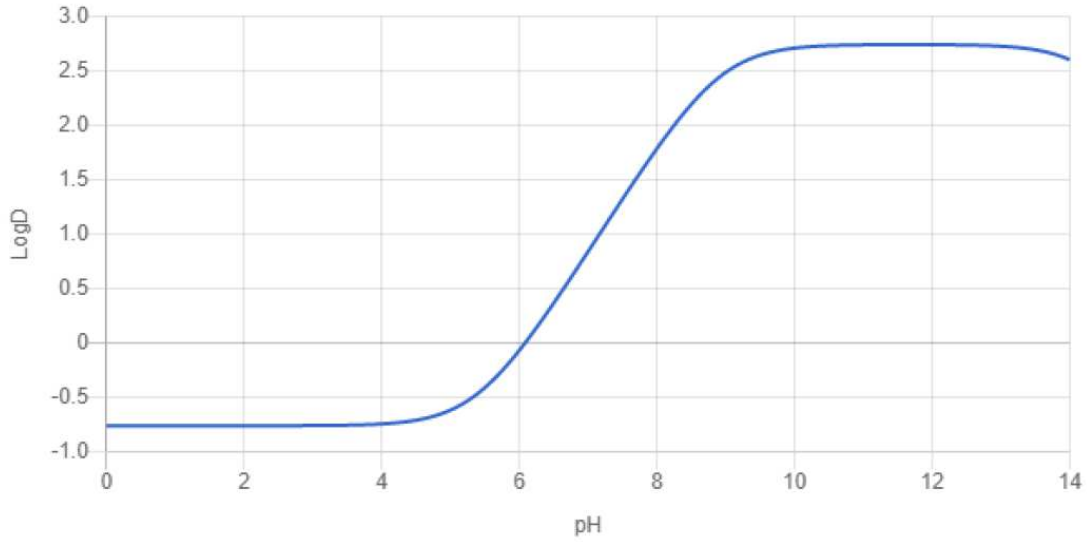
Strongest acidic pKa: 14.42

Strongest basic pKa: 8.91





LogP and logD



LogP: 2.739

pH	logD
1.7	-0.76
4.6	-0.70
6.5	0.36
7.4	1.22
8.0	1.78