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Method Article

# Simple analytical methodology based on solid phase extraction for monitoring pesticide residues in natural waters

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A B S T R A C T

Pesticide contamination of natural waters due to agricultural activities has been a widely publicized topic over the past 30 years and will continue to be a problem in the future. The determination of pesticide residues in water samples is necessary for solving various environmental problems. The aim of this work was to develop an efficient method on the basis of solid phase extraction (SPE) technique for the determination of 34 multiclass pesticides in natural waters. SPE using C18 extraction disks followed by gas chromatography (GC-MS) and liquid chromatography (LC-MS) were used for the determination of various pesticides residues in environmental waters. The developed SPE method provided good repeatability and reproducibility range, high extraction efficiency and low LODs. The performance results confirm the usefulness of the proposed methodology for the analysis multiclass pesticides in natural waters.

The key benefits of this methodology are:

- It possesses the advantages of SPE (fast, simple, highly sensitive) and could be potentially extended to other classes of pesticides.
- It can be used as a useful tool for monitoring purposes on natural waters.
- The validated methodology meets regulatory requirements established by the EU [1] and other authorities of developed countries [2].



A R T I C L E I N F O

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**Specifications Table**

| | |
|---|---|
| Subject Area | • *Environmental Science* |
| More specific subject area: | *Describe narrower subject area* |
| Method name: | Solid phase extraction of pesticides coupled to GC/MS and LC/MS |
| Name and reference of original method | |
| Resource availability | |

## Method details

*Reagents and standards*

Pesticides, namely acetamiprid, acetochlor, atrazine, azinphos-ethyl, buprofezin, chloropyriphos-methyl, dimethenamid-P, disulfoton, eptc, endosulfan-alpha, endosulfan-beta, endosulfan-sulfate, ethoprophos, fenpyroximate, fluometuron, iprodione, methoxyfenozide, metolachlor, molinate, myclobutanil, pendimethaline, pirimiphos-methyl, propachlor, quinalphos, quizalofop-ethyl, spirodiclofen, tebufenozide, tebufenpyrad, terbuthylazin, tetramethrin, triadimefon, triadimenol-A, triadimenol, trifluralin were obtained from Sigma-Aldrich, (St. Louis, Missouri, USA), and were of high purity grade (>96%) with exception of ethoprophos 93.1%. Acetone and methanol, was supplied by Carlo Erba (Milan Italy), methanol (LC-MS grade), water (LC-MS grade) and dichloromethane were purchased by Fisher Scientific (Leicestershire, UK), and ethyl acetate was supplied by Pestiscan (Labscan, Ltd., Dublin, Ireland). All solvents and reagents were analytical grade. An individual stock solution of each compound was prepared (2000 mg $L^{-1}$) in methanol, and two standard mixture solutions one for LC-ESI-MS and one for GC-MS of all target analytes were prepared in the same solvent at a concentration of 50 mg $L^{-1}$ and stored in the dark at −20 °C. Calibration solutions were prepared in LC water by appropriate dilution of the above standard solutions. C18 disks were purchased by Agilent Technologies (Santa Clara, California, USA) and sodium sulfate by Merck KGaA (Darmstadt, Germany). Glass fibre filters (1 μm were purchased from Whatman (United Kingdom).

*Sampling*

Details of the water sampling campaign have already been described elsewhere and are not the topic of this work [3]. Nevertheless concerning the sampling details, water samples from Louros river (N.W. Greece) were collected into amber glass bottles (volume 2.5 L) from each sampling station in the mid-depth of the water column. All samples were collected with the aid of Niskin sampler. After being filled with water, the bottles were sealed with screw caps and lined with aluminum foil. The bottles were placed in a portable cooler filled with ice for the transportation in the laboratory on the same day, where the samples were extracted within 48 h.

*Solid phase extraction (SPE) procedure*

1. Vacuum-filter the water sample through 1 μm glass fibre filters GF/B (Whatman, UK) prior to analysis, in order to remove the suspended solid matter, avoiding in this way potential interferences during the analysis.
2. Precondition the C18 disks with 10 mL of acetone, followed by 10 mL of ethyl acetate, 10 mL of methanol and 10 mL of deionised water.
3. Before the disk becomes dry, pass the water samples (230 mL) through the SPE disks, at a flow rate of approximately 10 mL/min, using a vacuum manifold that maintains a constant pressure differential between the inlet and the outlet of the disk.
4. Once the total sample is percolated, rinse the disks with 2 × 5 mL of deionised water.
5. Dry the disks under vacuum for 10 min to remove residual water.
6. Elute the analytes with 9 mL of ethyl acetate / dichloromethane (85:15), drop-by-drop, at flow rate of 1 mL/min
7. Dry the final extract over anhydrous sodium sulfate.

**Table 1**
Instrumental parameters for target pesticides using GC-MS in SIM mode.

| Pesticide | Time window (min) | Retention Time (min) | Molecular weight (g mol -1) | Quantification and identification ions (m/z) | | |
|---|---|---|---|---|---|---|
| Eptc | 5.00–21.00 | 16.20 | 189.3 | 128 | 132 | 189 |
| Molinate | 5.00–21.00 | 20.82 | 187.3 | 126 | 187 | 127 |
| Propachlor | 21.00–25.00 | 22.05 | 211.6 | 120 | 176 | 175 |
| Ethoprophos | 21.00–25.00 | 22.93 | 242.3 | 158 | 127 | 139 |
| Trifluralin | 21.00–25.00 | 23.47 | 335.2 | 264 | 306 | 248 |
| Atrazine | 25.00–29.00 | 25.74 | 215.6 | 200 | 215 | 201 |
| Terbuthylazin | 25.00–29.00 | 26.13 | 229.7 | 214 | 173 | 216 |
| Disulfoton | 25.00–29.00 | 26.8 | 274.4 | 129 | 186 | 153 |
| Dimethenamid-p | 25.00–29.00 | 27.97 | 275.8 | 154 | 203 | 230 |
| Acetochlor | 25.00–29.00 | 28.12 | 322.5 | 146 | 174 | 223 |
| Chloropyrifos-methyl | 25.00–29.00 | 28.08 | 269.7 | 286 | 288 | 125 |
| Pirimiphos-methyl | 29.00–32.00 | 29.29 | 305.3 | 276 | 290 | 305 |
| Metolachlor | 29.00–32.00 | 29.93 | 283.8 | 162 | 238 | 240 |
| Pendimethaline | 29.00–32.00 | 31.61 | 281.3 | 251 | 191 | 161 |
| Quinalphos | 32.00–35.00 | 32.68 | 298.3 | 157 | 156 | 146 |
| Triadimenol A | 32.00–35.00 | 32.96 | 295.7 | 168 | 111 | 128 |
| Endosulfan-$\alpha$ | 32.00–35.00 | 34.13 | 406.9 | 195 | 241 | 170 |
| Myclobutanil | 35.00–42.00 | 36.49 | 406.9 | 179 | 150 | 181 |
| Endosulfan-$\beta$ | 35.00–42.00 | 38.47 | 422.9 | 197 | 231 | 195 |
| Endosulfan-sulfate | 35.00-42.00 | 41.62 | 288.7 | 229 | 271 | 241 |
| Azinphos-ethyl | 42.00–47.00 | 45.69 | 345.3 | 160 | 104 | 105 |
| Quizalofop-ethyl | 47.00–60.00 | 48.67 | 372.8 | 299 | 372 | 243 |

8. Evaporate the methanol extracts to dryness under a gentle stream of nitrogen.
9. Reconstitute in 0.1 mL methanol and store at −20 °C until chromatographic analysis

*GC/MS conditions*

Analyses were performed using a Trace GC Ultra instrument (Thermo Scientific, Austin, Texas, USA) coupled to an ISQ mass spectrometer controlled by a computer running X-Calibur software. The separation was performed using a DB-5-MS column with a film thickness of 0.25 µm (30 mm x 0.25 mm i.d., Thermo Fisher Scientific, Austin, Texas, USA). Helium (purity > 99.999 vol) was used as the carrier gas at a flow rate of 1 mL min−1. The GC oven temperature program was as follows: initial temperature of 55 °C, 5 °C $min^{-1}$ to 200 °C, 1 °C $min^{-1}$ to 210 °C (held for 2 min), and finally 20 °C $min^{-1}$ to 270 °C (held for 16 min). The injector was set at 220 °C in the splitless mode. The temperatures of the ion source and the interface were set at 240 °C and 290 °C, respectively. The mass spectrometer was operated in the electron ionization mode at ionization energy of 70 eV. In the selected-ion monitoring (SIM) acquisition mode, the target ions were monitored at different time windows defined by the corresponding retention times. The quality criteria adopted for the retention times of the analytes as well as the relative intensities of the selected ions were within the tolerances established by the 2002/657/EC directive concerning the performance of analytical methods and the interpretation of results [4]. The retention times as well as the identification and quantification ions selected for the target compounds are shown in Table 1.

*LC/MS conditions*

Analysis was carried out using a SPD 20A UV–Vis detector coupled in series with the LC–MS 2010EV mass selective detector, equipped with an atmospheric pressure ionization source electrospray (ESI) interface. The chromatographic column used for analyte separation was a C18, 150 × 4.6 mm with 5 µm particle size (Restek, USA). Injection volume was set at 20 $\mu$L. The samples were analyzed using the ESI interface in positive (PI) ionization mode. For the analysis a gradient elution was performed by a binary gradient composed of solvent A (methanol with 0.1% formic acid) and solvent

**Table 2**
Instrumental parameters for target pesticides using LC-ESI-MS in SIM mode.

| Pesticide | Polarity (ESI) | Retetion Time (min) | Molecular weight (g mol$^{-1}$) | Quantification and identification ions (m/z) | | |
|---|---|---|---|---|---|---|
| Acetamiprid | + | 14.967 | 222.6 | 245 | 277 | 223 |
| Buprofezin | + | 20.96 | 305.4 | 233 | 287 | 273 |
| Fenpyroximate | + | 24.383 | 421.4 | 391 | 313 | 423 |
| Fluometuron | + | 24.783 | 232.2 | 294 | 316 | 348 |
| Iprodione | + | 25.258 | 330.1 | 296 | 318 | |
| Methoxyfenozide | + | 26.217 | 368.4 | 297 | 375 | 407 |
| Spirodiclofen | + | 29.517 | 411.3 | 354 | 386 | 395 |
| Tetramethrin | + | 29.575 | 331.4 | 306 | | |
| Triadimefon | + | 29.583 | 293.8 | 334 | 356 | 388 |
| Tebufenozide | + | 31.208 | 352.4 | 465 | 313 | 433 |
| Tebufenpyrad | + | 31.83 | 333.8 | 422 | 444 | 476 |
| Triadimenol | + | 37.596 | 259.7 | 394 | | |

B (water LC-MS) according to the following program: Initial conditions 90% B, decreased to 40% in 15 min, decreased to 10% in 5 min, returns to the initial conditions after 2 min and re-equilibration time was set at 3 min. The total run analysis lasted 40 min, returns to the initial conditions after 2 min and re-equilibration time was set at 3 min. The total run analysis lasted 50 min. Column temperature was set at 40 °C and the flow rate was 0.5 mL min$^{-1}$. The drying gas was operated at flow 10 L min$^{-1}$ at 200 °C. The nebulizing pressure was 100 psi, capillary voltage was 4500 V and the fragmentation voltage was set at 5 V. For each compound the precursor molecular ion, [M+H]+ for positive ESI, and at least one confirming ion in the selected-ion monitoring (SIM) mode were acquired. The most abundant ion was used for quantification, except tetramethrin and triadimenol (Table 2). Tetramethrin and triadimenol did not show any precursor molecular ion response, so the most intense signal was at m/z 306 and 394 respectively. The identification of target compounds was performed by matching the retention time (within 2.5%) and mass spectrum with standards.

*Validation of the analytical methodology*

To ensure that the optimized procedure was suitable for the application in routine analysis, the basic analytical performance parameters such as selectivity, linearity and linear range, limits of detection and quantification, precision, accuracy, trueness (recovery), stability, as well as robustness were determined and assessed.

*Selectivity*

Water sample used for optimization purposes was free of pesticides contamination collected from the springs of Louros River (Epirus, NW Greece). For selectivity, which is essentially a qualitative assessment, analyses of matrix-blank samples (samples of ultrapure and river water) were performed to test interferences using the proposed extraction procedure and chromatographic and spectroscopic conditions. The results were compared to those obtained with an aqueous solution of the analytes at concentrations near the limit of quantification. No significant interference has been detected in the retention time of the compounds. Absence of peaks for any organic compound indicated good selectivity of the analytical method [5].

*Linearity and linear range*

To investigate the linearity of the method, extractions of seven spiked water samples were used. The correlation coefficients ranged between 0.991 and 0.999 (Table 3), which is an evidence for a very good linearity. The 'on-line linearity (LOL)', expressed as

$$LOL(\%) = 100 - R.S.D.(b)$$

**Table 3**
Quality factors for the analytical performance of SPE.

| Compound | $R^2$ | LOD ($\mu g\ L^{-1}$) | LOQ ($\mu g\ L^{-1}$) | Linear range ($\mu g\ L^{-1}$) | LOL% |
|---|---|---|---|---|---|
| Eptc | 0.999 | 0.012 | 0.040 | 0.050–0.750 | 97.1 |
| Molinate | 0.995 | 0.014 | 0.046 | 0.050–0.750 | 97.6 |
| Propachlor | 0.998 | 0.009 | 0.030 | 0.050–0.750 | 97.1 |
| Ethoprophos | 0.996 | 0.010 | 0.033 | 0.050–0.750 | 97.9 |
| Trifluralin | 0.998 | 0.006 | 0.020 | 0.025–0.500 | 97.8 |
| Atrazine | 0.999 | 0.010 | 0.033 | 0.050–0.750 | 97.7 |
| Terbuthylazin | 0.998 | 0.008 | 0.026 | 0.050–0.750 | 98.4 |
| Disulfoton | 0.998 | 0.010 | 0.033 | 0.050–0.750 | 97.9 |
| Dimethenamid-P | 0.999 | 0.005 | 0.017 | 0.025–0.500 | 97.4 |
| Chloropyrifos-methyl | 0.996 | 0.008 | 0.026 | 0.050–0.750 | 97.2 |
| Acetochlor | 0.998 | 0.008 | 0.026 | 0.050–0.750 | 98.1 |
| Pirimiphos-methyl | 0.999 | 0.006 | 0.020 | 0.025–0.500 | 98.3 |
| Metolachlor | 0.998 | 0.010 | 0.033 | 0.050–0.750 | 98.1 |
| Pendimethaline | 0.997 | 0.008 | 0.026 | 0.050–0.750 | 97.2 |
| Quinalphos | 0.994 | 0.012 | 0.040 | 0.050–0.750 | 97.5 |
| Triadimenol | 0.995 | 0.015 | 0.050 | 0.050–0.750 | 97.2 |
| Endosulfan-$\alpha$ | 0.999 | 0.008 | 0.026 | 0.050–0.750 | 98.2 |
| Endosulfan-$\beta$ | 0.991 | 0.006 | 0.020 | 0.025–0.500 | 97.2 |
| Endosulfan-sulfate | 0.999 | 0.010 | 0.033 | 0.050–0.750 | 98.5 |
| Myclobytanil | 0.993 | 0.013 | 0.043 | 0.050–0.750 | 98.1 |
| Azinphos-ethyl | 0.997 | 0.005 | 0.017 | 0.025–0.500 | 98.1 |
| Quizalofop-ethyl | 0.993 | 0.006 | 0.020 | 0.025–0.500 | 98.1 |
| Acetamiprid | 0.999 | 0.020 | 0.066 | 0.075–1.000 | 97.6 |
| Buprofezin | 0.999 | 0.015 | 0.050 | 0.075–1.000 | 98.1 |
| Fenpyroximate | 0.991 | 0.028 | 0.092 | 0.100–1.000 | 97.2 |
| Fluometuron | 0.999 | 0.018 | 0.059 | 0.075–1.000 | 97.6 |
| Iprodione | 0.997 | 0.022 | 0.073 | 0.075–1.000 | 98.3 |
| Methoxyfenozide | 0.999 | 0.024 | 0.079 | 0.100–1.000 | 98.1 |
| Spirodiclofen | 0.999 | 0.022 | 0.073 | 0.075–1.000 | 98.5 |
| Tetramethrin | 0.998 | 0.017 | 0.056 | 0.075–1.000 | 97.9 |
| Triadimefon | 0.998 | 0.030 | 0.099 | 0.100–1.000 | 98.6 |
| Tebufenozide | 0.995 | 0.018 | 0.059 | 0.075–1.000 | 97.4 |
| Tebufenpyrad | 0.999 | 0.024 | 0.079 | 0.100–1.000 | 97.9 |
| Triadimenol | 0.994 | 0.030 | 0.099 | 0.100–1.000 | 97.2 |

in which R.S.D.(b) is the relative standard deviation of the slope (expressed as a percentage), with values higher than 97.1% (Table 3), also corroborated that the method was linear within the entire range of concentration investigated. The linear range for each compound is listed in Table 3.

*Limits of detection and quantification*

The LODs and LOQs were calculated by signal-to-noise (S/N) ratio for each pesticide obtained from the blank water matrix. Three times S/N value was used for LOD determination and ten times for LOQ determination [6]. Limits of detection and quantification were in the range 5–99 ng $L^{-1}$ (Table 3) for both GC/MS and LC/MS analysis.

These values can be considered satisfactory, since the achieved LOQ levels were suitable for surveillance of pesticides maximum permitted value of 0.1 $\mu g\ L^{-1}$ established by the European Union for water intended for human consumption [1].

*Accuracy and Precision*

The accuracy of the method (also termed as trueness) was evaluated at three levels of concentration including: LOQ of each compound, 5xLOQ and 10xLOQ, representing low, medium and high concentration levels within the linear dynamic range. As reported in Table 4, all compounds showed good accuracy ranging between 63.26% and 104.99% and were within the acceptable range of recovery (50–120%) in accordance with the European guidelines [7].

**Table 4**
Data concerning accuracy and precision of the method.

| Compound | Recovery % | | | $RSD_r$% | $R.S.D._R$% | CV Horwitz equation |
|---|---|---|---|---|---|---|
| | Concentration level LOQ | Concentration level 5x LOQ | Concentration level 10x LOQ | | | |
| Eptc | 95.71 | 100.53 | 87.06 | 3.4 | 4.4 | 6.5 |
| Molinate | 98.92 | 92.60 | 84.30 | 4.7 | 2.6 | 6.4 |
| Propachlor | 77.53 | 63.26 | 75.13 | 3.1 | 4.6 | 6.8 |
| Ethoprophos | 75.33 | 100.37 | 84.67 | 2.8 | 4.8 | 6.7 |
| Trifluralin | 67.32 | 89.80 | 94.70 | 4.1 | 2.3 | 7.2 |
| Atrazine | 100.70 | 97.45 | 94.99 | 3.2 | 2.5 | 6.7 |
| Terbuthylazin | 98.60 | 75.17 | 80.70 | 1.6 | 3.8 | 6.9 |
| Disulfoton | 91.03 | 97.27 | 89.55 | 4.5 | 2.4 | 6.7 |
| Dimethenamid-P | 104.99 | 88.95 | 92.27 | 3.1 | 4.9 | 7.4 |
| Chloropyrifos-methyl | 98.32 | 99.84 | 96.86 | 2.8 | 3.2 | 6.9 |
| Acetochlor | 96.18 | 102.02 | 93.63 | 4.7 | 3.9 | 6.9 |
| Pirimiphos-methyl | 97.62 | 89.02 | 90.50 | 1.7 | 3.5 | 7.2 |
| Metolachlor | 97.43 | 95.73 | 93.56 | 3.1 | 4.9 | 6.7 |
| Pendimethaline | 96.33 | 108.46 | 95.89 | 4.8 | 3.1 | 6.9 |
| Quinalphos | 99.27 | 76.16 | 71.55 | 4.9 | 3.8 | 6.5 |
| Triadimenol | 91.14 | 81.23 | 90.96 | 4.1 | 3.3 | 6.3 |
| Endosulfan-$\alpha$ | 95.20 | 70.91 | 80.62 | 3.8 | 4.7 | 6.9 |
| Endosulfan-$\beta$ | 97.60 | 83.58 | 95.08 | 2.8 | 4.1 | 7.2 |
| Endosulfan-sulfate | 98.16 | 90.76 | 100.02 | 3.5 | 3.7 | 6.7 |
| Myclobytanil | 83.49 | 92.50 | 78.23 | 4.6 | 3.4 | 6.4 |
| Azinphos-ethyl | 83.74 | 80.00 | 88.00 | 3.9 | 4.6 | 7.4 |
| Quizalofop-ethyl | 99.00 | 76.83 | 72.43 | 4.6 | 3.7 | 7.2 |
| Acetamiprid | 93.91 | 94.33 | 96.10 | 4.0 | 4.4 | 6.0 |
| Buprofezin | 95.19 | 100.42 | 93.79 | 3.5 | 4.8 | 6.3 |
| Fenpyroximate | 93.59 | 92.69 | 91.69 | 4.8 | 4.6 | 5.7 |
| Fluometuron | 88.39 | 92.09 | 97.84 | 3.4 | 3.7 | 6.1 |
| Iprodione | 100.63 | 93.92 | 95.51 | 4.9 | 4.9 | 5.9 |
| Methoxyfenozide | 82.84 | 94.24 | 94.55 | 4.8 | 3.1 | 5.9 |
| Spirodiclofen | 101.94 | 93.46 | 87.08 | 4.6 | 3.6 | 5.9 |
| Tetramethrin | 93.97 | 92.24 | 90.20 | 3.8 | 4.1 | 6.2 |
| Triadimefon | 97.54 | 103.88 | 92.54 | 4.6 | 4.1 | 5.7 |
| Tebufenozide | 97.20 | 95.75 | 95.77 | 3.1 | 4.0 | 6.1 |
| Tebufenpyrad | 97.86 | 88.20 | 100.16 | 3.3 | 4.7 | 5.9 |
| Triadimenol | 98.43 | 92.97 | 94.93 | 4.8 | 4.4 | 5.7 |

The precision was measured as relative standard deviation to the recoveries values of 10xLOQ concentration level. Values of $RSD_r$% in within intra-day (n = 5) and $R.S.D._R$% for inter-day (n = 5) reproducibility conditions had to be lower than the value calculated according to Horwitz equation: $CV = 2^{(1-0.5 \times \log c)}$, where c is the concentration of analyte expressed as a decimal fraction [8]. According to Commission Decision 2002/657/EC, the coefficient of variance for the repeated analysis of fortified material under reproducible conditions should not exceed the level calculated by the Horwitz equation. As shown in Table 4 relative standard deviations were $\leq$ the acceptable Horwitz relative standard deviations and therefore indicated good precision.

*Stability of the analytes in matrix*

The stability of the analytes in the sample during storage was also evaluated. A set of nine fortified water samples was prepared on the same day. Three samples were immediately analyzed (time zero), three were stored for 24 h at 4 °C in the dark and subsequently analyzed, while the other three samples were stored for 48 h at 4 °C in the dark and analyzed. No significant deviation of the results indicated the good stability of the proposed extraction method.

*Robustness*

The robustness of an analytical procedure is the measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The robustness of the method, concerning the effect of the matrix that leads to false negative results, was determined by calculating the recovery values from the extraction and analysis of river, lake and sea water samples fortified with 10xLOQ $\mu$g/L of the target analytes. The calculated recovery values did not show any variation among the different water sample matrix, confirming the robustness of the proposed method.

*Application of the method to real samples*

The optimized method was applied in a case-control study carried out on Louros River (Epirus region, north-western Greece) for a period of one year [3]. 42% of the pesticides, belonging to different chemical classes, were detected. Their occurrence was observed throughout the whole survey period with the minimum detection of the winter months when dilution effects and degradation reduced concentrations. Seasonal variations of pesticide detection in Louros River water samples, corresponding to pesticide application periods, were observed. Pesticide detection tended to be more frequent and levels more elevated during the late spring and summer months. For most of the pesticides detected the decrease in rainfall in summer results in an increase in pesticide concentrations at this time of year, in addition to the fact that the summer period comes just after their application and most pesticides have soil half-lives of several weeks.

*Additional information*

Contamination of water resources by pesticides residues is one of the major challenges for the preservation and sustainability of the environment [9,10,11]. Their extensive use in world- wide agricultural practice in addition to industrial emission during their production has led to substantial occurrence of pesticide residues and their metabolites in water [12]. Pesticides are among the most dangerous environmental pollutants because of their stability, mobility and long-term effects on living organisms [13]. Thus, pesticide residue analysis in environmental samples has received increasing attention in the last few decades, resulting in many environmental monitoring programs for a broad range of pesticides [14]. There are many sources of pesticides discharge into the aquatic environment. They can come from both area sources (e.g., atmospheric precipitation or farmland) and point source. They can also be transported over long distances through the air [15]. Pesticides that are found in water samples, belong to different structural groups, such as organophosphates, pyrethrins, carbamates, organochlorine e.t.c. Exposure to such contaminated water is harmful to the health and the life of not just humans, but also living organisms. That is why pesticides levels in water must be monitored continuously, especially in sources of water.

The main objectives of this study were to demonstrate the applicability of the proposed method for rapid and accurate screening multiresidue pesticide analysis in environmental water samples. The developed SPE method provided good repeatability and reproducibility range, high extraction efficiency and allowed the determination of the selected pesticides at very low concentrations with LODs ranged from 0.005 to 0.024 $\mu$g/L, depending on the compound. The performance results confirm the usefulness of the proposed methodology for the analysis multiclass pesticides in natural waters. The optimized method was applied in a case-control study carried out on a Louros River (Epirus region, north-Western Greece) for a period of one year [3]. Twenty five pesticides residues belonging to different chemical classes were detected and the results confirmed the need of pesticides monitoring programs in natural waters.

The greatest advantage of the methodology relays in the analysis of different classes of pesticides (insecticides, fungicides and herbicides) at low levels and at the same time. The method is effective and rapid, and can be applied in routine analyses as an excellent tool for monitoring pesticide residues in various water samples such as river, lake, sea waters.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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