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Simple, rapid solid-phase extraction procedure for the determination of ultra-trace levels of pyrethroids in ground and sea water by liquid chromatography/electrospray ionization mass spectroscopy

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A method based on liquid chromatography/mass spectroscopy with electrospray ionization in positive mode (LC/ESI-MS) to determine trace levels of pyrethroids in environmental water samples has been developed. The chromatographic and the MS parameters were optimized to obtain the best sensitivity and selectivity for all pesticides. Solid-phase extraction (SPE) using C_{18} cartridges was applied for preconcentration of pesticide trace levels (ng/L) in both ground and sea water samples. The preconcentration step was carried out with 800 mL of water sample modified with 200 mL of MeOH to improve the recovery percentages in the SPE procedure. The SPE-LC/ESI-MS methodology was applied to determine pyrethroids in ground and sea water samples spiked at ng/L concentration levels. Recoveries obtained in ground water were satisfactory (between 72 and 110%). However, an enhancement of the signals of all pesticides in the sea water was found due to the negative effect of the salt in the ionization source. To eliminate this effect a simple cleanup step of the SPE cartridge using 200 mL of Milli-Q water was performed. The cleanup removed the matrix effect completely from the marine samples. Thus, the recovery percentages ranged from 80 to 115%. The method was applied to determine ng/L of pyrethroids in both ground and marine water samples with precision values lower than 10%. Copyright © 2006 John Wiley & Sons, Ltd.

Pyrethroids are manufactured chemicals which are very similar in structure to pyrethrins, but are often more toxic to insects, as well as to mammals, and last longer in the environment. Many pyrethroids have been linked to the disruption of the endocrine system, which can adversely affect reproduction, interfere with the immune system and increase changes in breast cancer.[1]

Pyrethroids are currently used in agricultural activities and they constitute the major alternative to the acutely toxic organophosphates.[2] Even though they are eventually degraded by microorganisms in soil and water, and can also be degraded by sunlight on the surface of water, soil or plants, some of the more recent pyrethroids can persist in the environment for a few months before they are degraded.

Since synthetic pyrethroids are among the most toxic pesticides for aquatic invertebrates,[3] they are usually not sprayed onto water, but they can enter lakes, ponds, rivers and streams from rainfall or by runoff from agricultural fields.

To protect aquatic life, countries such as the UK have proposed environmental quality standards (EQSs) for some pyrethroids, considering as potential effects on interest features of European marine sites: (i) acute toxic effects on invertebrates, in particular crustaceans, and fish at concentrations above EQS values of 0.001 μg/L for cyfluthrin and 0.01 μg/L for permethrin in the water column. An EQS for cypermethrin is under development; (ii) accumulation in sediments where concerns exist about the effects of sediment-dwelling organisms; and (iii) identification of permethrin as an endocrine-disrupting substance.[3]

Thus the residue analysis of pyrethoids is of importance in environmental sciences. Almost all analytical methods which have been developed to determine pyrethroids are based on the use of chromatographic techniques,[4–17] mainly gas chromatography (GC), but over recent years liquid chromatography (LC) techniques have seen an accelerated application for the determination of pyrethroid residues in different fields,[12,15–17] due to some advantages. For example, LC does not require an extensive cleanup of the real samples because of the strong retention of these compounds in the reversed-phase column, while polar interferences contained in the matrix samples are only slightly retained. In addition, LC coupled to an MS detector offers a powerful tool for the determination and confirmation of these compounds in complex matrices such as environmental water samples. However, the relatively low sensitivity of LC/MS makes the detection of pesticide residues difficult in these matrices, at the very low levels at which these contaminants are usually

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present in the environment. In order to comply with the above-mentioned tolerance levels for water samples, it is necessary to introduce efficient preconcentration and cleanup procedures before the chromatographic stage. At present, solid-phase extraction (SPE) is the most useful method for the enrichment of pesticides in water, because it combines the advantages of convenience, low cost and minimal consumption of organic solvents.

Few methods have been devoted to determine pyrethroid residues in water samples.[18–23] In this sense, van der Hoff *et al.* proposed different methods with this aim. The first of them involves liquid–liquid extraction (LLE) of aqueous samples, followed by automated cleanup over silica SPE cartridges and large volume GC introduction.[18] The second consists of a fully automated method including the LLE extraction step in the chromatographic system.[19] Finally, these same authors[20] have proposed a method for the determination of pyrethroids at a low ng/L level using an automated SPE method combined with large-volume GC injection.

On the other hand, Martínez Vidal and co-workers[21] determined several pyrethroids by a GC/MS multiresidue method using C_{18} as sorbent in an off-line SPE step. Recently,[22] a GC/MS multiresidue method for determining 31 endocrine-disrupting pesticides, among which some pyrethroids appear, has been applied in environmental matrices, such as water and surface sediment samples. Finally, in a previous investigation[23] we performed an on-line SPE method coupled to LC/LC methodology with fluorescence detection to determine pyrethroids in surface water.

However, the determination of pesticides in sea water is very difficult to carry out because the high salinity of these samples reduces the ionization efficiency of the analytes and causes a matrix effect with MS detection.[24,25] In addition, in sea water, concentrations of organic contaminants are low and the preconcentration of a large volume is necessary, so that the concentration of co-extracted substances increases, which can interfere with the ionization processes. Different strategies are established to eliminate this problem: matrix-matched calibration, standard addition and isotope dilution, and even though these three options compensate the matrix effect, none one of them eliminate it and, for this reason, important efforts are usually devoted to successfully reducing the matrix effect.

In this paper, the determination of pyrethroids at ultra-trace levels in ground and sea water has been accomplished by LC/MS with an electrospray ionization (ESI) source, with previous enrichment by SPE. In both cases, the SPE step involved the addition of MeOH as an organic modifier to water samples and the use of C_{18} cartridges.

In sea water samples, a reduction in the ESI was observed, which was eliminated by an additional washing step of the SPE cartridges after retention of the analytes on the stationary phase.

EXPERIMENTAL

Chemicals and standards

Analytical standards (pestanal quality) of fenpropathrin (FENP), λ-cyhalothrin (λ-CYH), deltamethrin (DELT), fenvalerate (FENV), permethrin (PERM), τ-fluvalinate (τ-FLUV), and bifenthrin (BIFE) were obtained from Riedel-de Haën (Seelze, Germany).

Acetonitrile (ACN), methanol (MeOH), and n-hexane (HPLC-grade) were obtained from Merck (Darmstadt, Germany). Ammonium formate and formic acid were purchased from Fluka (Buchs, Switzerland). LC-grade water was obtained by purifying demineralized water in a Milli-Q system (Millipore, Bedford, MA, USA).

Mobile phases were filtered through a 0.45 μm cellulose acetate (water or aqueous mixtures) or Teflon (organic solvents), both obtained from Millipore.

SPE cartridges of Plus C_{18} (3 mL) containing 360 mg of strongly hydrophobic silica-based phase (particle size 55–105 μm) obtained from Waters (Milford, MA, USA) were used for the preconcentration of pesticides in water samples.

Individual stock solutions of seven pyrethroids were prepared in n-hexane at concentrations of 200 μg/mL and were protected from light and stored at 4°C. These solutions were stable for a period of at least 3 moths.

A working standard solution of 1 μg/mL of all pyrethroids was prepared daily by evaporating to dryness aliquots of the standard solutions in n-hexane under a gentle N_2 stream and redissolving in ACN.

Calibration standard solutions of the analytes were prepared in ACN/water (30:70 v/v) and were filtered through Millipore membrane Teflon filters (0.45 μm particle size) before injection into the chromatographic system.

Instrumentation

LC separation was carried out with a Hewlett Packard (H-P) series 1100 system (Hewlett-Packard, Wilmington, DE, USA) with an H-P Chem Station for MS control and spectral processing. The LC system consisted of a model G 1311 gradient pump and a Rheodyne six-port injection valve (model 7725i) with a 20 μL loop. The analytical separation of pyrethroids was performed with a 250 mm × 4.6 mm i.d. Waters Symmetry C_{18} column (5 μm particle size).

An H-P G 1948 A Platform benchtop single quadrupole mass spectrometer with an ESI interface was used to detect and quantify the target compounds in the LC column effluent.

A Büchi Vac V-500 (Switzerland) vacuum system, connected to an extraction manifold from Waters (Milford, MA, USA), was used for SPE preconcentration of water samples.

LC/MS operating conditions

The separation of seven pyrethroids was carried out with a solvent gradient consisting of solvent A (ACN) and solvent B (ammonium formate 50 mM, 5% of ACN, acidified at pH 3.5 by adding formic acid) into a Symmetry C_{18} column. The gradient program was as follows: initially 3 min with 70% A, 17 min linear gradient to 80% A, 10 min linear gradient to 100% A, 3 min at 100% A and finally the mobile phase was returned to the initial conditions in 4 min for subsequent analysis. The mobile phase was adjusted to a flow rate of 1 mL/min. The temperature of the column was set at 25°C and the injection volume was 20 μL. The seven pyrethroids

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were completely separated in the analytical column under the established gradient program in 29 min.

The MS detector was used in positive ion mode with a capillary voltage of 3000 V. The desolvation was optimized in order to obtain the highest analytical response for the pyrethroids. The source temperature of ESI desolvation was selected at 325°C and the fragment ions were generated using highly pure nitrogen as drying gas at a flow rate of 9 L/min and nebulizing gas at a pressure of 40 psig. LC/MS chromatograms were obtained by operating in the time-scheduled one selected ion monitoring (SIM) acquisition mode.

SPE procedure

MeOH (200 mL) was added to water samples (800 mL) and the mixtures were then percolated through the SPE cartridge at a flow rate of 10 mL/min in the extraction manifold system at a pressure of 500 psig for the vacuum system. Previously, the SPE cartridge was preconditioned with 5 mL of MeOH, 5 mL of n-hexane, 5 mL of MeOH and finally 5 mL of Milli-Q water.

The SPE cartridge was dried for 30 min under vacuum and then the retained analytes were eluted with 7 mL of n-hexane. After evaporation of the n-hexane extract, the residue was redissolved in 0.5 mL of ACN/water (70:30) v/v and finally 20 μL were injected into the LC/MS system under the optimized conditions.

For the analysis of sea water, an additional washing step was performed, after percolating the water samples, by passing 200 mL of Milli-Q water to eliminate the salts retained in the SPE cartridge. Finally, the analytes were eluted from the cartridge and injected into the LC/MS system as described above.

RESULTS AND DISCUSSION

Optimization of LC/MS parameters

The optimization of the MS conditions was carried out by direct injection of a standard solution containing 10 mg/L of each pyrethroid prepared in ACN/water (50:50 v/v). For every pesticide the MS signal was optimized in the positive and negative ionization mode. The optimal fragmentation occurred in the positive ionization mode with cone voltages ranging between 60 and 85 V for all compounds. On the other hand, the addition of buffer solutions was evaluated in agreement with the literature data.[26] The results obtained showed that the MS signals increased when ammonium formate/formic acid was added to standard solutions for all target analytes. Thus, a concentration of 50 mM ammonium formate acidified at pH 3.5 by adding formic acid was selected in order to increase the sensitivity of the pyrethroid signals. Figure 1 shows two mass spectra of a standard of λ-CHY in ACN/ammonium formate/formic acid (50:50 v/v) and in ACN/water (50:50 v/v). As can be seen the signals obtained using buffer solution are more than 5-fold higher than those obtained using Milli-Q water.

The cone voltage was optimized between 60 and 120 V for each pesticide in order to obtain the most intense ion (Fig. 2). Table 1 shows the ions chosen for quantification of

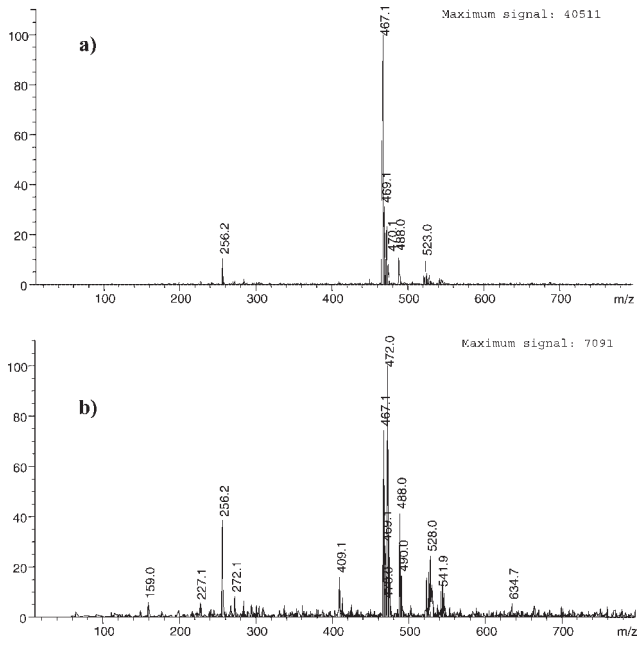

Figure 1. ESI-MS spectra of λ-CHY obtained using as solvent: (a) ACN/ammonium formate/formic acid (50:50 v/v) and (b) ACN/water (50:50 v/v).

pyrethoids and the optimum voltage used. The higher signals (abundance of 100%) were obtained with the pseudomolecular ion $[M+H]^+$ for fenpropathrin (*m/z* 350) and with the adduct ions $[M+NH_4]^+$ for λ-cyhalothrin, deltamethrin and fenvalerate (*m/z* 467, 523 and 437, respectively). Finally, permethrin, τ-fluvalinate and bifenthrin yielded fragment ions corresponding to $[CH_2–C_6H_4–O–C_6H_5]^+$ (*m/z* 183), $[CNCH–C_6H_4–O–C_6H_5]^+$ (*m/z* 208) and $[CH_2–C_6H_3CH_3–C_6H_5]^+$ (*m/z* 181), respectively. Following the SANCO guidance,[27] a second confirmation ion (the second most intense) for each pyrethoid (Table 1) was selected to improve selectivity of the MS method.

The LC method was optimized to achieve the best separation and the optimum peak shape for the seven pyrethroids. In order to evaluate the influence of mobile-phase composition on the ionization efficiency, different gradient programs using aqueous binary mixtures of ammonium formate/formic acid with organic solvent (MeOH or ACN) as mobile phase were checked. The best separation and highest MS signals were obtained with the ACN gradient program described in the 'LC/MS operating conditions' section. The flow rate of the LC mobile phase was 1 mL/min and the injection volume was 20 μL. Finally, SIM mode was selected for quantification of pyrethroids using the peak area of quantification ions of each compound which appear in Table 1.

Figure 3 shows a LC/ESI-MS SIM chromatogram of the seven pyrethroids under the optimized conditions. As can be seen, one chromatographic peak was obtained for each pesticide, except permethrin, which yields two chromatographic peaks (5a and 5b) corresponding to its two isomers, according to the solid standard used.

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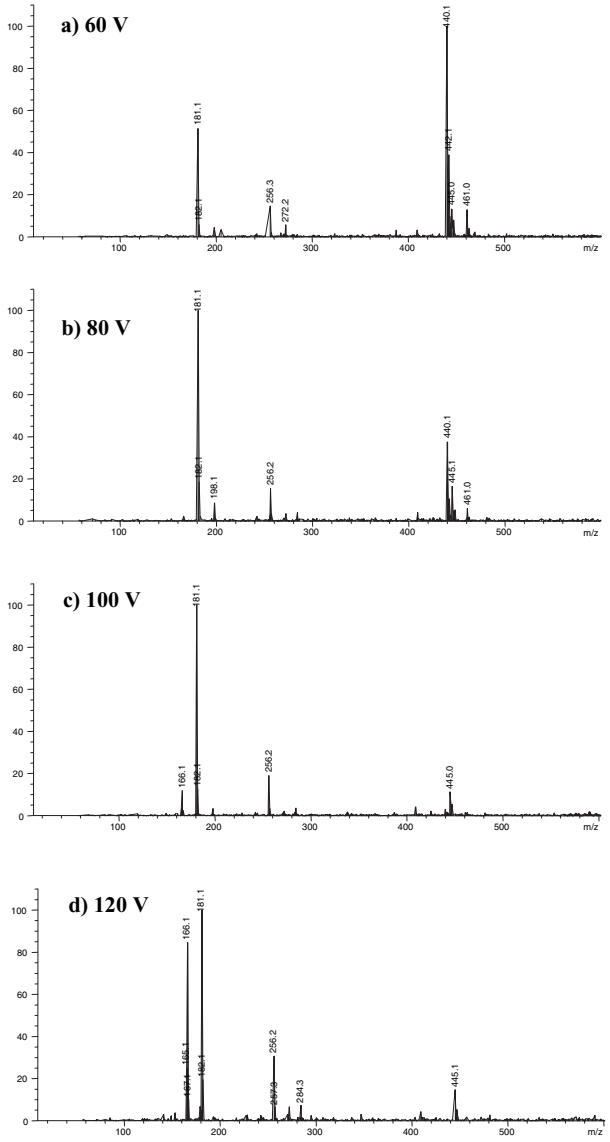

Figure 2. ESI-MS spectra of BIFE obtained at different cone voltages: (a) 60 V, (b) 80 V, (c) 100 V, and (d) 120 V.

SPE procedure

Initial SPE experiments were carried out with 250 mL of Milli-Q water spiked at 0.1 μg/L of each pyrethroid by adding 25 μL of a standard solution of 10 μg/L prepared in ACN. The spiked samples were stirred for 5 min and then were allowed to stand for 30 min before SPE preconcentration. For the extraction of pyrethroids from the water samples, C_{18} SPE cartridges were selected according to other authors.[20] The spiked water samples were passed through the SPE cartridge previously conditioned as described above at a flow rate of 10 mL/min.

In addition, n-hexane was tested as solvent for the elution of retained compounds and 7 mL of this solvent was selected as minimum volume for the elution of the pesticides from the SPE cartridges. The elution with additional volumes of organic solvent did not provide better recoveries.

Results achieved using this SPE method (Table 2) showed low recoveries (between 56.8 and 75.1%) for the pesticides, in agreement with other authors.[20] The lowest recoveries may be due to the fact that the large volume of water removes the activating organic solvent from the C_{18} sorbent and the penetration of the compounds between the octadecyl chains is hampered.

On the one hand, a previous work[28] has demonstrated that the addition of organic solvent (MeOH or ACN) to water samples increases the retention of the compounds on the SPE cartridge. Therefore, in order to evaluate the effect of organic modifier in the extraction procedure, different percentages of MeOH were added to the water samples spiked with all pyrethroids and it was found that the presence of organic modifier increased the recovery percentages for all of them. As can be seen in Table 2, maximum recoveries (between 94.1 and 108.2%) were achieved with a percentage of 20% MeOH as organic modifier with the lower dilution of the water sample. No significant differences[29] for pesticide recoveries of pyrethroids were found when the percentage of MeOH in the water sample was increased over 20% (p-value <5%). So this percentage was selected to retain the pesticides in the SPE cartridge.

On the other hand, the addition of higher organic modifier content increases the eluotropic strength of the sample, so the analytes can be eluted from the SPE cartridge simultaneously with the water sample. Therefore, the breakthrough volume was studied in order to establish the maximum volume of water sample that can be passed through the SPE cartridge. Different Milli-Q water sample volumes ranging between 250 and 1000 mL (containing 20% of MeOH) and spiked with the same amounts of pesticides (5 ng) were passed through the C_{18} cartridge under the conditions specified in the Experimental section. No breakthrough on the C_{18} cartridge was observed for all pesticides, even for the volume of 1000 mL of water sample modified with 20% of MeOH. Thus, this volume was selected to achieve lower concentration levels of pesticides in the water samples in a reasonable analysis time (12 water samples were preconcentrated in about 3 h).

Table 1. Optimized ESI-MS parameters

Pesticide	Cone voltage (V)	Molecular weight (g/moL)	Quantification ion (m/z)	Second confirmation ion (m/z)
FENP	60	349.4	350	125
λ-CYH	80	449.9	467	488
DELT	80	505.2	523	544
FENV	80	419.9	437	458
PERM	85	391.3	183	355
τ-FLUV	85	502.9	208	503
BIFE	80	422.9	181	440

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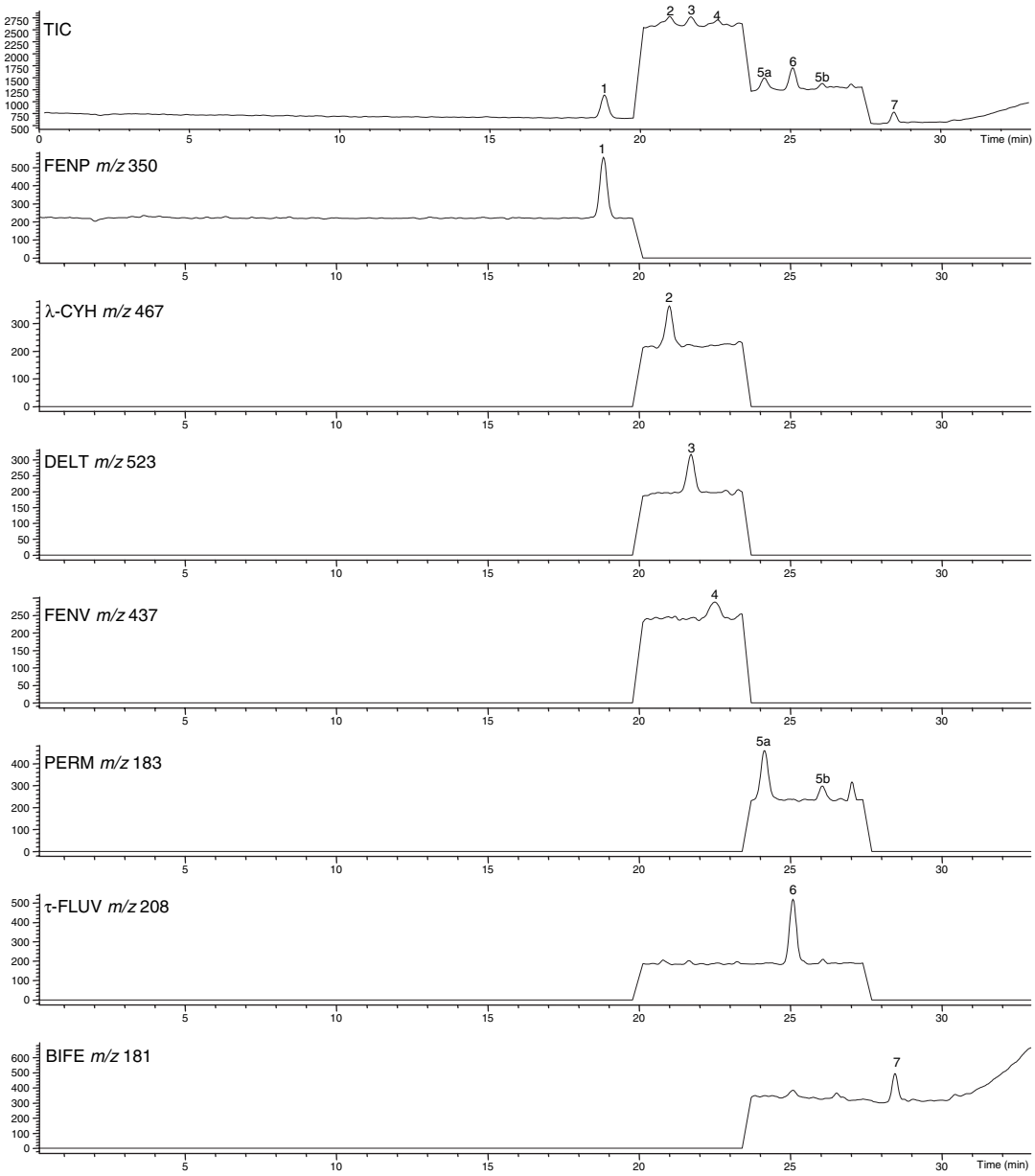

Figure 3. LC-ESI-MS SIM and TIC chromatograms corresponding to 25 ng/mL of a standard of: (1) FENP, (2) λ-CYH, (3) DELT, (4) FENV, (5a) and (5b) PERM isomers, (6) τ-FLUV, and (7) BIFE.

Table 2. Recovery percentages on C_{18} cartridges with different percentages of MeOH added to the water

	Mean recovery (%)[a]				
	MeOH percentage (%)				
Pesticide	0	10	15	20	30
FENP	70.5 (5.4)	82.9 (4.8)	94.5 (4.7)	95.3 (4.2)	93.7 (5.0)
λ-CYH	60.4 (6.5)	80.4 (5.2)	91.8 (5.0)	108.2 (5.7)	108.5 (6.2)
DELT	75.1 (7.1)	87.1 (6.5)	104.4 (5.7)	107.2 (6.2)	107.1 (5.4)
FENV	68.7 (6.4)	73.7 (6.9)	95.1 (4.8)	105.1 (5.4)	99.0 (4.5)
PERM	70.5 (5.2)	83.3 (4.7)	99.0 (5.0)	105.0 (4.9)	107.8 (5.0)
τ-FLUV	56.8 (6.9)	72.0 (5.4)	85.2 (5.6)	95.6 (5.5)	107.2 (6.1)
BIFE	61.8 (7.3)	68.7 (6.8)	83.6 (5.9)	94.1 (4.8)	105.4 (5.2)

[a] RSD (%) in parentheses (three replicates).

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Table 3. Figures of merit for the determination of pyrethroids by the LC/ESI-MS method in pure solvent

Pesticide	Concentration range (μg/L)	R^2	Precision study RSD (%)	LOD[a] (μg/L)	LOQ[a] (μg/L)	LOQ[b] (μg/L)[c]
FENP	2.2–5000.0	0.9996	3.5	0.3	0.8	2.2 (6.2)
λ-CYH	2.3–5000.0	0.9999	4.0	0.5	1.3	2.3 (7.3)
DELT	2.6–5000.0	0.9999	3.8	0.3	0.8	2.6 (7.8)
FENV	2.8–5000.0	0.9990	2.5	0.5	1.3	2.8 (4.3)
PERM	2.5–5000.0	0.9996	4.2	0.3	0.8	2.5 (6.7)
τ-FLUV	1.6–5000.0	0.9999	4.5	0.5	1.3	1.6 (9.3)
BIFE	2.4–5000.0	0.9997	3.8	0.5	1.3	2.4 (5.8)

[a] IUPAC criterion.
[b] EURACHEM criterion.
[c] RSD (%) in parentheses.

Validation of the LC/MS method

The analytical figures of merit such as limits of detection (LODs), limits of quantification (LOQs), linear range and within-day precision for standards of pesticides in pure solvent, following the proposed method without the preconcentration step, are summarized in Table 3.

Limits of detection and quantification

LODs and LOQs in pure solvent were calculated statistically[30] and the latter were also calculated, according to the EURACHEM guidance,[31] for a relative standard deviation (RSD) equal to or less than 10% in our case (Table 3). LOQs ranged between 1.6 and 2.8 μg/L, according to this last criterion.

The method was found to be linear in the ranges between the LOQ calculated according to the EURACHEM criterion for each analyte and the upper limit, the concentration for which the signal deviates from the linearity by 3–5%,[29] with determination coefficients higher than 0.999 in all cases (Table 3).

Precision

The study of within-day precision, expressed as the RSD, was carried out by injecting 10 μg/L of each pesticide. The RSD (%) values (n = 6) obtained were lower than 4.5%.

Ruggedness

Ruggedness, describing the reproducibility of method results for identical samples under standard conditions, considering slight and sometimes unavoidable changes of system parameters, was tested. This parameter must be considered as at the beginning of the method validation[32] and it has been included in the normative[33] as another performance characteristic of the analytical method.

A Plackett Burman factorial design[34] was used for this test, seven different components or variables, as (−1,0,1) levels, being set as follows:

A: ESI spray voltage: (2995, 3000, 3005 V)
B: Cone voltages: (59, 60, 61 V) for FENP; (79, 80, 81 V) for λ-CYH, DELT, FENV and BIFE; and (84, 85, 86 V) for PERM and τ-FLUV.
C: Drying gas flow: (8.9, 9.0, 9.1 mL/min)
D: Nebulizing gas pressure: (39, 40, 41 psig)
E: Source temperature of ESI desolvation: (323, 325, 327°C)
F: HPLC mobile flow rate: (0.95, 1.00, 1.05 mL/min)
G: Sample solvent: (49:51, 50:50, 51:49 ACN/water v/v)

Each experimental sample belonging to the Plackett Burman design, containing 100 μg/L of each pyrethroid, was injected in triplicate and the influence of the target factors on the analytical response was evaluated by analysis of variance (ANOVA) for a confidence level of 95% ($\alpha = 0.05$). The *p*-values obtained for the different factors are detailed in Table 4, according to the compounds. A value of *p* lower than 0.05 means that the influence of the corresponding factor on the analytical response is significantly different from zero at the confidence level established. Thus, it can be stated that the analytical signal is hardly influenced by slight changes in

Table 4. Variation effects of the levels (-1, 0, 1) on the seven selected operating conditions on the analytical signal. *p*-values obtained by ANOVA

	p-value*							
Factor	FENP	λ-CYH	DELT	FENV	PERM[a]	PERM[b]	τ-FLUV	BIFE
Capillary voltage	0.0370*	0.0001*	0.0000*	0.0012*	0.0081*	0.0121*	0.0026*	0.8988
Cone voltage	0.0012*	0.6434	0.0011*	0.4157	0.0001*	0.0001*	0.0000*	0.5648
Drying gas	0.0009*	0.0672	0.0161*	0.0438*	0.0037*	0.0005*	0.0007*	0.0115*
Nebulizing gas pressure	0.7962	0.2174	0.2802	0.4006	0.2620	0.8281	0.5367	0.1957
Source temperature	0.5318	0.3127	0.0022*	0.2331	0.0453*	0.0076*	0.0086*	0.0906
HPLC flow rate	0.0666	0.0600	0.1423	0.0155*	0.0001	0.0001*	0.0009*	0.0219*
Sample solvent	0.3646	0.5832	0.4127	0.3805	0.2391	0.3800	0.9085	0.3205

* Significance level *p*-value <0.0500.

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those parameters which are key in the ionization step (i.e. capillary voltage, cone voltage and drying gas flow) for all pesticides except for BIF for which the signal was only influenced by the source temperature and the drying gas flow. Also, the analytical signal was modified by small changes in source temperature of ESI desolvation (DELT, PERM and τ-FLUV) and in the HPLC flow rate for the more retained compounds (FENV, PERM, τ-FLUV and BIFE).

Real samples and recovery studies

The developed LC/ESI-MS method was applied to the determination of selected pesticides in different types of water samples (ground and sea water). In these studies, extracts of blank water samples of both ground and sea water were used.

Recovery studies of the pyrethroids in real water samples (from ground and sea water) spiked at two levels of concentration (5 and 25 ng/L of all pyrethroids) were performed in order to assess the efficiency of the proposed SPE-LC/ESI-MS method. Table 5 shows the recovery data obtained in both kinds of samples. The results obtained in ground water samples were satisfactory, with recoveries ranging between 72 and 110% while in marine waters the recoveries were lower than 10%.

These low recoveries may be attributed to the effect of salinity which causes a suppression effect on the signal corresponding to pesticides in the sea water samples. The reduced ionization efficiency of the analytes in the presence of sample matrix constituents such as salts is a well-known phenomenon frequently observed in ESI sources[35] when determining traces of pesticides in environmental water samples. For this reason, efficient cleanup to eliminate matrix components is essential for quantitative and qualitative analysis.

Cleanup step to determine pyrethroids in sea water

In order to obtain good recoveries in the analysis of pyrethroids in the sea water samples a simple washing step of the cartridge with Milli-Q water after the retention of pesticides into the SPE cartridge was performed. A volume of 200 mL of Milli-Q water was selected to remove the retained salts when the pyrethroids remained in the cartridge.

After preconcentration of a sea water blank, absence of overlapping peaks in the LC/MS chromatogram at the same retention times as the target pesticides occurred, except for the second chromatographic peak of PERM (5b) for which an interference peak (I) was found at the same retention time (Fig. 4(a)) on the total ion chromatograms (TICs), i.e. when monitoring the selected ions (quantification and confirmation). Nevertheless, no interference peak appeared on the LC/MS chromatogram when the only quantification ion of PERM (*m*/*z*183) was monitored in the sea water blank (Fig. 4(b)). In this way PERM can be unequivocally detected and quantified by using both peaks when monitoring the most abundant ion (*m*/*z* 183). So, the MS detection provides an efficient and fast method to identify these pesticides in sea water samples.

To check the efficiency of the SPE cleanup for the determination of pyrethroids in sea water, the matrix effect was tested. This effect was evaluated by comparing the MS responses of known amounts of standard solutions of the pesticides with those measured in a blank water extract obtained after SPE and cleanup steps and spiked with the same amounts of the analytes. No matrix effect was found by comparing statistically[29] the slopes of calibration curves built with both solvent-based and matrix-matched standards. Therefore, calibration curves built with solvent were used for quantification of pesticides in real samples.

Finally, a recovery study in the sea water samples was carried out for all pyrethroids at two different concentration levels (5 and 25 ng/L), using the proposed SPE and cleanup method, yielding the results summarized in Table 5. It can be observed that with this additional washing with Milli-Q water the matrix interferences were eliminated and satisfactory recoveries for pyrethroids in sea water samples at ng/L (5 and 25 ng/L) were obtained (between 80 and 115%).

Limits of detection and quantification in real water samples

The LODs and LOQs were calculated in ground and sea water according to the two criteria[30,31] described above. Both parameters were determined using a blank extract of real water matrix as solvent. The obtained results (Table 6) were very similar in both water matrices and slightly higher than in pure solvent. LODs ranged between 0.2 and 0.7 ng/L and

Table 5. Recovery percentages on C_{18} cartridges for pyrethroids in ground and sea water

	Mean recoveries (%)[a]					
	Ground water		Sea water		Sea water[b]	
Pesticide	5 ng/L	25 ng/L	5 ng/L	25 ng/L	5 ng/L	25 ng/L
FENP	110.0	107.2	7.4	9.8	89.3	87.2
λ-CYH	82.8	88.8	8.2	8.4	89.3	100.5
DELT	90.9	94.3	7.9	9.0	86.9	86.9
FENV	81.0	97.1	9.2	10.2	104.4	105.9
PERM	91.9	98.3	7.6	8.8	93.3	115.6
τ-FLUV	71.8	73.4	3.2	4.5	80.0	85.6
BIFE	78.0	77.2	4.5	5.1	84.7	83.3

[a] n = 3;
[b] With additional washing step of the C_{18} cartridge.

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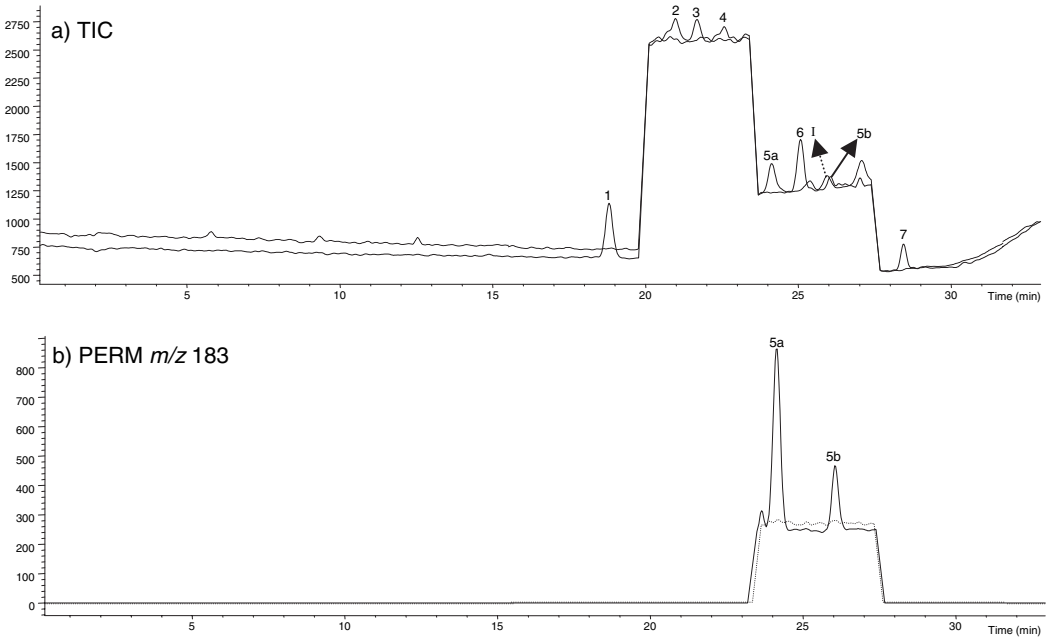

Figure 4. LC/ESI-MS chromatograms of a sea water blank (....) and a sea water blank spiked at a concentration level corresponding to the LOQ for each pyrethroid (—). The two water samples were monitored at: (a) TIC of selected ions for each compounds and (b) quantification ion for PERM (*m/z* 183). (1) FENP, (2) λ-CYH, (3) DELT, (4) FENV, (5a) and (5b) PERM isomers, (6) τ-FLUV, and (7) BIFE.

Table 6. LODs and LOQs for pyrethroids in ground and sea water

	Ground water			Sea water		
Pesticide	LOD[a] (ng/L)	LOQ[a] (ng/L)	LOQ[b] (ng/L)	LOD[a] (ng/L)	LOQ[a] (ng/L)	LOQ[b] (ng/L)
FENP	0.3	0.8	2.4	0.6	1.8	2.6
λ-CYH	0.4	1.1	2.2	0.5	1.5	2.5
DELT	0.2	0.7	2.4	0.3	0.8	2.7
FENV	0.4	1.3	2.1	0.5	1.4	2.6
PERM	0.4	1.1	2.3	0.7	2.1	3.0
τ-FLUV	0.5	1.4	2.2	0.5	1.4	2.4
BIFE	0.5	1.5	2.4	0.6	1.7	2.7

[a] IUPAC criterion;
[b] EURACHEM criterion (RSD lower than 10%).

LOQs between 2.1 and 3.0 ng/L. This fact proved that the SPE method proposed in this work is adequate for the determination of pyrethroids in complex matrices without the presence of background signal and interference peaks due to the matrix.

CONCLUSIONS

A rapid and simple LC/ESI-MS method combined with an off-line SPE preconcentration step has been developed for the determination of seven pyrethroids in surface and sea water samples. The addition of organic modifier to the water sample is crucial to improve the recoveries of pesticides in water samples.

Matrix effect in sea water due to the high salinity was eliminated using an additional washing of the cartridges with Milli-Q water, in a cost-free way. Satisfactory precision and recoveries were obtained ranging from 72 to 115% at trace levels in all cases, with a relative standard deviation (RSD) lower than 10%.

The appropriate selectivity and sensitivity of this methodology allows the identification and quantification of low levels of pyrethroids in environmental water using a single quadrupole like detection system.

The developed methodology allows the monitoring of these endocrine-disrupting pesticides in environmental water samples at concentration levels that can be toxic for aquatic organisms.

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