Application of solid-phase microextraction for determination of pyrethroids in groundwater using liquid chromatography with post-column photochemically induced fluorimetry derivatization and fluorescence detection

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Abstract

Solid-phase microextraction (SPME) is a rapid and simple analytical technique which uses coated fused-silica fibers to extract analytes from aqueous samples. This study develops a method of SPME analysis for seven pyrethroids, including fenpropathrin, α-cyhalothrin, deltamethrin, fenvalerate, permethrin, γ-fluvalinate and bifenthrin in groundwater samples using high performance liquid chromatography combined with post-column photochemically induced fluorimetry derivatization and fluorescence detection (SPME–LC-PIF-FD). To perform the SPME, a 60 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber was used for the extraction of the pesticides from groundwater samples. The main factors affecting the SPME process, such as extraction time, stirring rate, extraction temperature, pH and the desorption process were studied. The use of photochemically induced fluorescence for detection improved sensitivity and selectivity. The limits of quantification (LOQs) obtained in the matrix, with respect to EURACHEM Guidance, varied between 0.03 and 0.075 μg L⁻¹. Relative recoveries ranged from 92 to 109% and relative standard deviations values ranged from 2 to 9%.

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Keywords: Solid-phase microextraction; Pyrethroids; Photochemically induced fluorescence; Liquid chromatography; Groundwater

1. Introduction

Pyrethroid insecticides are currently used in agricultural practices and they constitute the major alternative to the acutely toxic organophosphates [1]. However, many pyrethroids have been linked to the disruption of the endocrine system, which can adversely affect reproduction, interfere with the immune system and increase the chances of breast cancer [2]. Whilst the development of the synthetic pyrethroids was heralded as providing selective toxicity to insects, they are extremely toxic to aquatic organisms, including fish such as bluegill and lake trout, with LC₅₀ values of less than 1.0 parts per billion (www.beyondpesticides.org, 16/05/2005). The nonlethal effects of pyrethroids on fish include damage to the gills and behavioral changes [3].

Few methods have been reported to determine pyrethroids in water, probably due to the fact that these compounds are strongly adsorbed into the soil. Even so, they can enter surface, ground and drinking water from rainfall or runoff from agricultural land [4]. Therefore, the residue analysis of pyrethroids is of importance in environmental science. The low concentrations of pyrethroids usually present in water make the development of sensitive trace analysis methods necessary. Conventional pesticide residue analysis of pyrethroids in environmental samples is often laborious, since it involves manual samples extraction, as well as several concentration steps. The most difficult and time-consuming step is the extraction of the target analytes from the matrix, using LLE and SPE [5–10].

Solid-phase microextraction (SPME), introduced by Pawliszyn’s group in 1990 is an excellent alternative technique. SPME is significantly more rapid and simpler than LLE and SPE. The requirement for solvents has been eliminated and only a small volume of sample is required. SPME also

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incorporates sample extraction, along with concentration and sample introduction, all in a single procedure [11,12].

SPME was successfully coupled to LC by Chen and Pawliszyn in 1995, so as to analyze a wide variety of compounds [13]. Several applications of SPME–high performance liquid chromatography (HPLC) have been found in the literature, such as polyaromatic hydrocarbons, alkyl-phenol ethoxylate, surfactants, proteins, pesticides, corticosteroids, etc. [13,14].

Pyrethroids are usually determined by GC with electron capture (ECD) [3,15] or mass spectrometry (MS) detection [16]. However, in GC methods, there are usually relatively high retention times. The applications of HPLC to pyrethroid residue analysis are limited. Some applications include multiresidue methods for the analysis of a variety of agrochemicals, including many pyrethroids, in fruits and vegetables [17,18] and some applications are used on isolated pyrethroids [19,20] using UV detection. Certain HPLC applications used on in different matrices, by means of fluorescence detectors [21,22] or a chemiluminescence detector [23], have recently been developed and reported by our group. However, in the above procedures, samples were extracted by LLE and further clean up steps, using SPE, were necessary.

In general, fluorimetry is more sensitive than other detection systems, such as classical UV absorption, and less expensive than MS detection. Additionally, fluorescence detectors are very selective, overcoming matrix interference. Sensitivity can be increased by using derivatization photo-reactions [24]. It was recently demonstrated that UV irradiation yields photochemical reactions in several classes of pesticides, the photochemistry of which has been reviewed by Crosby et al. [25] and Marcheterre et al. [26] and more recently by Coly and Aaron [24].

Pyrethroid insecticides have no native fluorescence, however some pyrethroid insecticides can be derivatized into strong fluorescent photoproducts by exposure to UV irradiation [27]. An interesting property of photochemical reactions is that they can be easily implemented as a detection system within a continuous system. Thus, post-column photochemically induced fluorimetry (PIF) is coupled to LC with fluorescence detectors (FD). This method has several advantages compared to other fluorogenic derivatization techniques, including simplicity, shorter analysis time, and enhanced sensitivity and selectivity. In addition, neither post-column pumps nor other devices are required.

In this work we have developed a method for the analysis of seven pyrethroids: fenpropathrin, λ-cyhalothrin, deltamethrin, fenvalerate, permethrin, α-fluvalinate and bifenthrin in ground-water using a sample preparation step of SPME coupled to HPLC, combined with photochemically induced fluorimetry and fluorescence detection (SPME–HPLC-PIF-FD) for the analysis of these pesticides. In the present work, the use of SPME-PIF-FD offers a much more powerful technique for the qualitative and quantitative analysis of the target compounds compared to the conventional LLE/SPE-GC-ECD or MS methods, since SPME combined with the sensitivity and selectivity of PIF-FD yield low cost, a reduction in time, the elimination of solvent requirements and the need for only a small volume of sample.

2. Experimental

2.1. Chemical and solvents

Analytical standards (Pestanal quality) of fenpropathrin (FP), lambda-cyhalothrin (λ-CH), deltamethrin (DL), fenvalerate (FV), permethrin (PM), tau-fluvalinate (τ-FL) and bifenthrin (BF) were obtained from Ridel-de Haën (Sellez, Germany).

The HPLC-grade acetonitrile (ACN) and methanol (MeOH) were obtained from Merck (Darmstadt, Germany); n-hexane and anhydrous sodium sulphate for pesticide residue analysis were obtained from Panreac (Barcelona, Spain).

Ultrapure water, obtained from a Milli-Q water purification system Millipore (Bedford, MA, USA) was used. Phosphate buffer (0.1 M) was prepared by dissolving the appropriate amount of dipotassium hydrogen phosphate in Milli-Q water and adding the appropriate amount of 0.1 M H3PO4.

The mobile phase was filtered through a 0.45 μm cellulose acetate (water) or PTFE (ACN) and degassed with helium prior to and during use.

2.2. Apparatus and materials

The SPME fiber assembly and SPME–HPLC interface were purchased from Supelco (Bellefonte, PA, USA). The SPME–HPLC interface consists of a six-port injection valve and desorption chamber (chamber volume 60 μL) which replaces the injection loop of a six-port injection system. The SPME fibers (for HPLC use): polydimethylsiloxane (PDMS 100 μm); polydimethylsiloxane/divinylbenzene (PDMS/DVB 60 μm) and carbowax/templated resin (CW/TPR 50 μm) were purchased from Supelco (Bellefonte, PA, USA).

The HPLC, gradient Model 600E LC pump and a Model 474 variable-wavelength scanning fluorescence detector were from Waters (Milford, MA, USA). Liquid chromatography separations were performed with a 250 mm × 4.6 mm ID column packed with 3.5 μm Symmetry C18 (Waters).

The photochemical reaction was carried out in a post-column photochemical reactor (Softron GmbH, Gynkotek HPLC, Germany) fitted with a knotted open tube reactor coil (5 m × 1.66 mm OD and 0.3 mm ID) PTFE and a 4-W Xenon lamp. Recording of chromatograms and quantitative measurements of peak areas were performed with Millennium32 Software from Waters (Milford, MA, USA). A schematic diagram of the system is shown in Fig. 1.

2.3. Preparation of standard and spiked samples

Individual analytical standard solutions of pesticides (400 mg L⁻¹) were prepared by carefully weighing and dissolving the corresponding compounds in n-hexane. Furthermore, the standard solutions were protected from light and stored at 4 °C in a refrigerator. Under such conditions, they were stable for at least 3 months.

Working solutions were prepared daily by evaporating to dryness aliquots of the standard solutions in n-hexane under a gentle N2 stream and redissolving in acetonitrile:water 25:75 (v/v),
Fig. 1. Schematic diagram of the instrumental system.

The volume of each aliquot and the final volume of the working solution were set according to the concentration needed.

2.4. SPME procedure

The PDMS/DVB fiber was conditioned before the initial application, in accordance with the instructions provided by the supplier. The fiber to be used was conditioned in the interface with the mobile phase each day before use, until it was free of contaminants. The SPME process consists of 4 mL vials filled with 3 mL aqueous samples of standard solutions or real samples containing the mixture of pesticides and corresponding amount of acetonitrile (25%, v/v). The extraction was carried out through the vials sealed with hole caps and PTFE septa, which were pre-punctured to permit the passage of the fiber. The depth of immersion was kept constant. The sample solution is buffered to pH 3, using the phosphate buffer, and stirred with a stirring bar controlled at 1100 rpm by a magnetic stirrer. The temperature of the solution was kept at 65 ± 2 °C. The sample was extracted by direct immersion for a period of 30 min. After the sample extraction, the SPME fiber was introduced into the desorption chamber full of mobile phase. Inside the chamber, the fiber was soaked for 7 min before loading the analytes into the column, carried out by switching the valve to the injection position. The mobile phase circulated via the chamber containing the fiber for 5 min, this being the desorption time. The valve was then switched to load position, allowing the conventional LC analysis in the HPLC system. To minimize the possibility of any analyte carry-over or contaminant between extraction runs, the fiber was cleaned by flushing with several portions of acetonitrile (three portions of a minimum 1 mL) followed by flushing with several portions of water (three portions of a minimum 1 mL). In this way, it was made ready for further extraction.

2.5. HPLC procedure

The SPME extracted samples were chromatographed by a programmed gradient with ACN:water as mobile phase for 34 min at a constant flow rate of 1 mL min⁻¹. The solvent program was as follows: initially 18 min isocratic with ACN:water 80:20 (v/v); then 12 min linear gradient to ACN:water 100:0 (v/v) followed by an additional period of 4 min to the initial conditions; finally 5 min under the initial conditions allowing sufficient time before a subsequent analysis run. The programmed excitation and emission wavelengths of the fluorescence detector, λex and λem, were 283 and 330 nm, respectively.

3. Results and discussions

3.1. HPLC analysis

After the extraction step with SPME, the analytes were desorbed inside the interface chamber and transferred to the HPLC column for separation and analysis. To do this, the composition and flow rate of mobile phase were optimized. A series of aqueous ACN and MeOH gradient elution programs were evaluated
in order to obtain maximum responses and selectivity yet with minimum broadening on the chromatograms. The highest fluorescence responses and the best separation in the shortest time were achieved using an ACN:water gradient program described previously in the HPLC procedure section.

3.2. Fiber selection

SPME is performed using a fused-silica fiber which is coated with a thin layer of polymeric stationary phase. The principle behind SPME is the partition of analytes between the sample matrix and the extraction medium [11]. In order to compare the effect of the fiber coating for the extraction of selected pyrethroids, several commercially available polymeric coatings such as polydimethylsiloxane (PDMS 100 μm), polydimethylsiloxane/divinylbenzene (PDMS/DVB 60 μm), and carbowax/templated resin (CW/TPR 50 μm) were evaluated under the optimum and sub-optimum conditions mentioned in Fig. 2. From the results shown in Fig. 2, the PDMS/DVB was shown to be the most appropriate fiber coating for the analysis of a standard aqueous solution of 100 μL−1 of each pesticide. The PDMS/DVB has shown the highest sensitivity to: -CH; DL; FV; PM; -FL and BF. However, for fenpropathrin, the highest sensitivity was shown using CW/TPR. Therefore, PDMS/DVB was selected for the SPME extraction steps because of its greater sensitivity. This choice is consistent with the observations from other works on pyrethroid analysis [28,29].

3.3. Desorption mode

Analytes can be desorbed using either a dynamic mode or a static mode. In the dynamic mode, the analytes are desorbed on-line by a stream of the HPLC mobile phase. In the static mode, the valve of the SPME assembly is switched so that the desorption chamber is off-line, the solvent is introduced and the analytes are desorbed from the fiber before the solvent passes on to the column. All of the analytes showed higher desorption efficiency in the static desorption mode than in the dynamic mode. Better resolution between the peaks of deltamethrin and fenvalerate (Fig. 3) was also achieved. The static mode was used for desorption in this study.

3.4. Soaking period

In the soaking period, the fiber remains inside the desorption chamber before it is flushed with the desorption solvent. All analytes showed an increase in the extraction efficiencies, when the soaking time was increased up to 9 min. However, the maximum desorption for FP was reached at 7 min. Seven minutes were selected as the soaking time for further experiments.

3.5. Desorption period

The desorption period refers to the period of time which the fiber is washed by the desorption solvent (the mobile phase in this case) in the desorption chamber. The desorption period was evaluated up to 7 min. The maximum desorption efficiency was obtained at 5 min for all analytes. A 5 min time period was used as the desorption time since good extraction and resolution were achieved.

3.6. Soaking solvent

Soaking solvent refers to the solvent which is used for desorping the analytes from the fiber inside the chamber during the period of soaking. Different mixtures of acetonitrile–water solvent were evaluated. The best results were obtained using ACN:water 80:20 (v/v) since better resolution and extraction were achieved. However, increasing the ACN concentration by more than 80% led to peak interferences. Therefore, the mobile phase was used as a soaking solvent.

3.7. Extraction time profile

The extraction time for the analysis of seven pyrethroids was evaluated. The experimental results of the extraction time profile are presented in Fig. 4. According to these results, the extraction
efficiency improved as the time of extraction increased for all of the pesticides. However, the equilibrium was not reached even with an 80 min extraction time under fixed sub-optimal conditions. In order to avoid too long extraction time for the analysis of the target pyrethroids, and given that SPME is not an exhaustive extraction but an equilibrium process, a period of 30 min was selected and used for further experiments as the extraction time. It was a compromise between sensitivity and time of analysis. The application of SPME under non-equilibrium conditions has been used for the analysis of several organic compounds and pesticides in aqueous matrices with satisfactory results [30–34].

3.8. Effect of stirring rate

The rate at which the extraction process reaches an equilibrium state depends primarily upon the transfer in the aqueous phase [33,35], which is improved by stirring. The effect of the stirring rate for the seven pyrethroid analytes was evaluated by analyzing samples at different stirring rates. The results of the stirring effect are shown in Fig. 5. From the results obtained, it can be stated that an increase in extraction efficiencies was achieved when the stirring rate was increased for all of the analytes. However, the amount of extracted analytes either decreased or did not significantly alter after increasing the stirring rate to over 1100 rpm. This is due to stirring bar vibration at higher stirring rates. Therefore, the optimum selected stirring rate was 1100 rpm, which was used for further experiments.

3.9. Effect of the salt additives

The addition of salt (NaCl or Na2SO4) often improves recovery when conventional extraction methods are used [36]. In this study, the influence of a Na2SO4 additive was evaluated. Different concentrations of Na2SO4 (up to 15%, w/v) were used, analyzing a solution mixture of 100 μg L−1 for FP, λ-CH, DL, FV, PM and τ-FL, and 75 μg L−1 for BF. The addition of salt led to a decrease in extraction efficiencies for all analytes. Fig. 6 shows the results obtained when using different concentrations of Na2SO4. Better extraction levels of pyrethroids were found when the extraction was performed without addition of salt during the extraction process. The addition of salt may lead to a decreased extraction when the compounds solubility does not change as has been observed for pyrethroids and other groups of pesticides in other works [37,38]. This relates to the dependence of partition coefficients on the activities rather than to the concentration of analytes in a solution [39].

3.10. Effect of organic solvent

The effect of the addition of an organic solvent was carried out by preparing a series of samples, each containing different concentrations of acetonitrile ranging from 0 to 30% (v/v). Extraction was applied to these samples using the previously selected conditions. The effect of ACN on the adsorption of analytes is shown in Fig. 7. The extraction efficiencies of pyrethroids improved as a result of increasing the ACN percentage up to 30% for the pesticides λ-CH, DL, FV, PM, τ-FL and BF. For FP, the increase continued up to equilibrium at 20%. The amount of ACN was added so as to have 25% ACN (v/v) for SPME samples, thus providing an enhancement in the extraction levels. The
enhancement of extraction efficiency, using amounts of ACN, 
is due to a reduction in the interaction between the analytes and  
the walls of the container as well as because of a reduction in  
the polarity of the bulk.

3.11. pH effect

The influence of pH on the extraction efficiencies was inves-
tigated in order to find a pH value at which the extraction of the  
pyrethroids was enhanced. When varying the pH value from 3 to  
10, using buffered samples (the recommended range given in the  
instructions provided by the fiber supplier), a significant effect  
was observed on both the extraction level and peak resolution.  
The maximum extraction levels were obtained when the sample  
pH was adjusted to 3 and the lowest extraction levels were at  
pH 10 (Fig. 8). In addition, working at higher than pH 7 led to  
broadening of peaks, as well as peak interference between the  
peaks of DL and FV. This interference was maximized at pH  
10. In order to have better extraction levels and good resolution,  
the sample was adjusted to pH 3 in all the SPME extractions of  
groundwater samples.

3.12. Effect of extraction temperature

The effect of temperature on extraction is influenced by two  
factors: kinetic and thermodynamic. With the kinetic factor,  
higher temperature increases the diffusion rate of the analytes,  
thus the extraction efficiencies may increase at higher temper-

tature. Thermodynamically, because adsorption is generally an  
exothermic process, the amount of analytes adsorbed decreases  
with an increase in temperature. The two effects compete against  
each other and different analytes are affected in different ways  
[40]. Fig. 9 shows the extraction temperature profile for the  
seven pyrethroids in this study. From the results obtained for  
the temperature effect, kinetics played more of a role for the  
pesticides λ-CH, DL, FV, PM, τ-FL and BF. An increase in tem-
perature led to an increase in extraction levels. On the other  
hand, in the case of FP, no significant effect was observed  
when changing the temperature between 25 and 75 °C. As a  
compromise, 65 °C was selected as the extraction tempera-

ture.

3.13. Effect of carry-over

With SPME techniques, a significant amount of the analytes  
often remain adsorbed on the fiber after the desorption step. To  
evaluate the effect of carry-over, a blank desorption experiment  
was performed after extraction of the sample, spiked with seven  
pyrethroid pesticides at a concentration of 50 µg L⁻¹. For the  
pesticides tested, the carry-over values ranged between 0 and  
1.3%, being 0.8, 0.4, 1.3, 0.8, 0.7, 0.5 and 0.0% for FP, λ-CH,  
DL, FV, PM, τ-FL and BF, respectively. This carry-over was  
eliminated by washing the fiber with portions of acetonitrile  
(three portions of a minimum 1 mL) followed by portions of  
water (three portions of a minimum 1 mL).

3.14. Validation

The proposed analytical method was validated using the  
above optimum conditions. The limits of detection, limits of

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Table 1: Analytical figures of merit obtained using Milli-Q water

<table>
<thead>
<tr>
<th>Compound</th>
<th>Linear range (µg L⁻¹)</th>
<th>Regression equation</th>
<th>$R^2$</th>
<th>LODa (µg L⁻¹)</th>
<th>LOQa (µg L⁻¹)</th>
<th>LOQb (µg L⁻¹)</th>
<th>Recovery% c (RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenpropathrin</td>
<td>0.025–125</td>
<td>(Y = 54769X + 94930)</td>
<td>0.9985</td>
<td>0.003</td>
<td>0.007</td>
<td>0.025</td>
<td>90 (9)</td>
</tr>
<tr>
<td>λ-Cyhalothrin</td>
<td>0.025–150</td>
<td>(Y = 38040X + 108294)</td>
<td>0.9994</td>
<td>0.001</td>
<td>0.002</td>
<td>0.025</td>
<td>88 (8)</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>0.05–150</td>
<td>(Y = 33788X + 149352)</td>
<td>0.9981</td>
<td>0.002</td>
<td>0.002</td>
<td>0.050</td>
<td>86 (6)</td>
</tr>
<tr>
<td>Fenvalerate</td>
<td>0.05–150</td>
<td>(Y = 33146X + 41122)</td>
<td>0.9977</td>
<td>0.006</td>
<td>0.020</td>
<td>0.050</td>
<td>94 (7)</td>
</tr>
<tr>
<td>Permethrin</td>
<td>0.05–150</td>
<td>(Y = 23054X + 43531)</td>
<td>0.9975</td>
<td>0.006</td>
<td>0.020</td>
<td>0.050</td>
<td>86 (9)</td>
</tr>
<tr>
<td>τ-Fluvalinate</td>
<td>0.05–150</td>
<td>(Y = 25236X + 7359)</td>
<td>0.9983</td>
<td>0.006</td>
<td>0.020</td>
<td>0.050</td>
<td>96 (8)</td>
</tr>
<tr>
<td>Bifenthrin</td>
<td>0.01–75</td>
<td>(Y = 95238X + 10382)</td>
<td>0.9986</td>
<td>0.001</td>
<td>0.004</td>
<td>0.010</td>
<td>101 (8)</td>
</tr>
</tbody>
</table>

a IUPAC criterion.

b EURACHEM criterion (RSD 10%).

c Spiked at level 50 µg L⁻¹ (bifenthrin 25 µg L⁻¹), n = 6.
quantification, precision (RSDs), linearity, and recovery, were all studied. The analytical figures of merit, obtained under the optimum conditions, are summarized in Table 1. Limits of detection (LODs) and limits of quantification (LOQs) were calculated statistically, according to IUPAC [41], as 3.84 and 10 times, respectively the standard deviation of the signal corresponding to 10 blank solutions divided by the slope of the calibration curve. The LOQs were also calculated, according to EURACHEM Guidance [42], as the lowest concentration of the analyte for which the relative standard deviation (RSD) of the signal is equal or less than a fixed percentage (10% in our case). LOD and LOQ results, in accordance with the two guidances, showed values of less than 0.1 µg L⁻¹ for all of the analytes. These values are in agreement with the recommended regulations for drinking and groundwater within the EU [43]. They are in the same order as others reported, using SPME-GC for the target compounds [6,38,44], and lower than those obtained by other SPME-GC methods [37,45,46]. In addition, no significant differences in the limits of detection and quantification were found between sample solutions prepared with Milli-Q water (Table 1) and sample solutions prepared with groundwater samples (Table 2).

The linear range was established for each pesticide, the lower limit being the LOQ calculated, according to the last criterion, and the upper limit of the concentration for which the signal deviated from linearity by 3–5% [47]. Calibration curves showed a good linear relationship ($R^2 > 0.998$) between 0.01 and 150 µg L⁻¹ (six standards covering the whole range were used and each point of the calibration graph was obtained in triplicate).

![Graph](image)

In order to establish the accuracy and precision of the total method, six replicates of groundwater samples were spiked and analyzed using the proposed method described. Mean recovery and RSDs percentages were evaluated at two concentration levels and they are shown in Table 3. In the results, good precision was obtained for all analytes (RSD% < 10%) and recoveries were good for all pesticides (between 92 and 109%), which are in the expected range.

Matrix effects have been investigated by performing calibrations on both groundwater matched and Milli-Q water-based standards at the same concentration [47] (data not shown). The results showed that no matrix effect was observed for any of the analytes.

### Table 2
Analytical figures of merit obtained using groundwater

<table>
<thead>
<tr>
<th>Compound</th>
<th>Linear range (µg L⁻¹)</th>
<th>Regression equation</th>
<th>$R^2$</th>
<th>LODa (µg L⁻¹)</th>
<th>LOQa (µg L⁻¹)</th>
<th>LOQb (µg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenpropathrin</td>
<td>0.05–125</td>
<td>Y = 52449X + 78594</td>
<td>0.9991</td>
<td>0.004</td>
<td>0.010</td>
<td>0.050</td>
</tr>
<tr>
<td>λ-Cyhalothrin</td>
<td>0.05–150</td>
<td>Y = 38718X + 122427</td>
<td>0.9997</td>
<td>0.004</td>
<td>0.010</td>
<td>0.050</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>0.05–150</td>
<td>Y = 34505X + 134623</td>
<td>0.9991</td>
<td>0.008</td>
<td>0.021</td>
<td>0.050</td>
</tr>
<tr>
<td>Fenvalerate</td>
<td>0.075–150</td>
<td>Y = 33228X + 57449</td>
<td>0.9986</td>
<td>0.007</td>
<td>0.018</td>
<td>0.075</td>
</tr>
<tr>
<td>Permethrin</td>
<td>0.075–150</td>
<td>Y = 23173X + 4384</td>
<td>0.9981</td>
<td>0.007</td>
<td>0.019</td>
<td>0.075</td>
</tr>
<tr>
<td>β-Fluvalinate</td>
<td>0.075–150</td>
<td>Y = 26631X + 42793</td>
<td>0.9985</td>
<td>0.009</td>
<td>0.022</td>
<td>0.075</td>
</tr>
<tr>
<td>Bifenthrin</td>
<td>0.03–75</td>
<td>Y = 98671X + 260947</td>
<td>0.9990</td>
<td>0.003</td>
<td>0.007</td>
<td>0.030</td>
</tr>
</tbody>
</table>

A total of five different groundwater samples from Almería (Spain) were analyzed by the proposed method, after pesticides were found in a monitoring program [48], but pyrethroid residues were not detected. The groundwater samples were collected from aquifers which constitute the drinking water resource for the rural and urban population. Fig. 10 shows two chromatograms corresponding to a real groundwater sample and a real groundwater sample spiked with 0.1 µg L⁻¹. It can be seen that no peaks appear at the retention times of the analytes, and

### Table 3
Recovery percentages and RSD (%) for the determination of seven pyrethroids in groundwater at two concentration levels (n = 6)

<table>
<thead>
<tr>
<th>Compound</th>
<th>a 10 µg L⁻¹</th>
<th>b 50 µg L⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean recovery (%)</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>Fenpropathrin</td>
<td>94</td>
<td>3</td>
</tr>
<tr>
<td>λ-Cyhalothrin</td>
<td>105</td>
<td>5</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>94</td>
<td>8</td>
</tr>
<tr>
<td>Fenvalerate</td>
<td>106</td>
<td>8</td>
</tr>
<tr>
<td>Permethrin</td>
<td>108</td>
<td>8</td>
</tr>
<tr>
<td>β-Fluvalinate</td>
<td>96</td>
<td>9</td>
</tr>
<tr>
<td>Bifenthrin</td>
<td>94</td>
<td>9</td>
</tr>
</tbody>
</table>

**Footnotes:**
- a IUPAC criterion.
- b EURACHEM criterion (RSD 10%).

3.15. Analysis of groundwater samples

![Graph](image)
therefore, the proposed method is suitable for the analysis of the seven pyrethroids in groundwater with detection limits meeting the levels assigned by the EU.

4. Conclusions

The combination of solid-phase microextraction and liquid chromatography coupled with photochemically induced fluorimetry derivatization and fluorescence detection (SPME-HPLC-PIF-FD) led to the development of a simple, efficient, selective and sensitive method for the determination of pyrethroid pesticides in groundwater samples. The proposed method showed no matrix effect when comparing the use of groundwater matched and Milli-Q water-based standards, making it possible to use Milli-Q water-based match standards for quantification. This method is less expensive than other conventional LLE/SPE-GC-ECD or MS methods, avoiding the use of extra amounts of organic solvents and substantially reducing sample manipulation.

References