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Determination of imidacloprid, cypermethrin and chlorpyrifos ethyl in water samples using high-performance liquid chromatography

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A high-performance liquid chromatography method is developed and validated for determination of imidacloprid, cypermethrin and chlorpyrifos ethyl in water samples. Separation is performed with a chromatographic column C18 (Microsorb-MV, 100-5, C18, 150 × 4.6 mm) using a mobile phase consisting of methanol/water (70:30, v/v) at a flow rate of 0.5 mL min^{-1} and UV detection at 205 nm. The method exhibits good linearity in the range of 1-1000 μg L^{-1} for the analyzed pesticides. The percentage recovery of the method at three concentration levels (10, 100, and 1000 μg L^{-1}) is within 98.6 to 101.6% for the three pesticides. The limit of quantification is low (0.51, 1.20 and 1.50 μg L^{-1} for imidacloprid, cypermethrin and chlorpyrifos ethyl, respectively) which enables their determination in water samples at low concentration levels. A stability test of imidacloprid, cypermethrin and chlorpyrifos ethyl was also performed to predict their stability in surface water under variation of ambient temperature.

Keywords: Pesticides, Water samples, Stability test, HPLC-DAD.

INTRODUCTION

Pesticides are useful in agricultural practice and their application increases the agricultural outputs since they protect crops from pests' infestations. In spite of their importance in agriculture, the use of some pesticides poses a significant risk to non-target populations especially wildlife and aquatic organisms [1]. The health of aquatic organisms and humans may be impaired by pesticides residues in surface water above certain limits [2]. Pesticide residues reach the water body through direct runoff, leaching, equipment washing, etc. Several factors such as soil characteristics, topography, weather, agricultural practices, chemical and environmental properties can affect the transportation of pesticides from agricultural fields to surface waters [3, 4].

Imidacloprid belongs to the neonicotinoid insecticide class. It acts as an antagonist by binding to nicotinic acetylcholine receptors and provides excellent control of a wide range of chewing and sucking pests, such as aphids, jassids and termites [5]. It has very high toxicity and stability in the soil for several months and can easily reach ground water [6].

Cypermethrin is an insecticide used for control of insects in and around residential areas. It belongs to the group of pyrethroids and is a non-systemic insecticide with contact and stomach action. Cypermethrin acts on the nervous system of the insect [7] and consist of eight optical isomers [8]. Among the eight isomers, only two pairs of diastereomers of cypermethrin (cisB and transB) possess biological activity against pests and insects [9-13].

Chlorpyrifos ethyl is one of the organophosphate class pesticides commonly used in small and large agricultural areas. High acute toxicity of chlorpyrifos ethyl poses a potential risk to human and aquatic organisms. Several studies have reported that chlorpyrifos ethyl residues pose a significant risk to children, pregnant women and infants, and this demands continuous monitoring in aquatic ecosystems [14, 15].

Normally these pesticides are present in water in low concentrations, therefore a method with low limit of detection (LOD) and limit of quantification (LOQ) is required to detect the compounds. Several methods have been reported for the separate determination of imidacloprid, cypermethrin and chlorpyrifos ethyl in different environmental matrices by high-performance liquid chromatography (HPLC) with UV-detection [5, 16-23], but in many cases, due to their widespread use, it is necessary to determine the three pesticides in one run. No method has been developed to analyze them simultaneously in the same sample.

The aim of the present paper is to develop a rapid and simple reversed-phase (RP) HPLC method with UV detection for the determination of imidacloprid, cypermethrin and chlorpyrifos ethyl

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after liquid-liquid extraction (LLE) of the pesticides from the water samples. The potential application of the method is in the assessment of the environmental behavior of the pesticides in surface waters.

EXPERIMENTAL

Chemicals

HPLC-grade solvents and reagents and double deionized water (MilliQ) were used throughout the analysis. Methanol, acetonitrile, dichloromethane (99.5%) were from Merck (Darmstadt, Germany). Imidacloprid (1-(6-chloro-3-pyridinylmethyl)-N-nitroimidazolidin-2-ylideneamine) – 99.9%, cypermethrin ((RS)-α-cyano-3-phenoxybenzyl (1RS,3RS;1RS,3SR)-3-(2,2-dichlorovinyl) 2,2 dimethylcyclopropane- carboxylate) – 99.9%, and chlorpyrifos ethyl (O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphate) – 99.9% were purchased from Sigma-Aldrich.

Instrumentation

The chromatographic analysis was performed using a HPLC Varian Pro Star (Mulgrave, Australia) system equipped with a quaternary gradient pump and a ternary solvent delivery system, an injection valve with a 20 μL sample loop and a diode-array detector (DAD) detector. The separation was performed with a chromatographic column C18 (Microsorb-MV, 100-5, C18, 150 × 4.6 mm) purchased from Varian (Netherland, Europe). Ultrapure water, purified by a Milli-Q water purification system (Millipore purification system Synergy, France), was used throughout the experiments.

The temperature variation during the stability test of the analyzed pesticides was achieved in a climate chamber Model HPP 108 (Memmert GmbH, Germany).

Preparation of standard solutions

The stock standard solution of each pesticide (10 mg L^{-1}) was prepared in HPLC-grade methanol in amber reagent bottles and kept in the refrigerator at +4°C. Calibration standard solutions in the following concentrations: 10.0, 100.0, 300.0, 500.0, 700.0, and 1000.0 μg L^{-1} of each pesticide were then prepared from the stock solution by appropriate dilution with the mobile phase and were used for instrument calibration.

Extraction procedure

Three parallel water samples of 100 mL volume containing spiked 100 μg L^{-1} of imidacloprid, cypermethrin and chlorpyrifos ethyl each were extracted three times with dichloromethane. The volumes of organic solvent were 3 × 60 mL. The organic extracts were then collected and concentrated by rotary vacuum evaporation until few drops of the solution were left. Then, 1.0 mL of methanol was added and the final sample was analyzed by HPLC-DAD.

Accuracy and precision of the method

The percent recovery of the studied pesticides was estimated by spiking deionized water with three concentrations (10.0, 100.0 and 1000.0 μg L^{-1}) of each pesticide. The spiked samples were extracted according to the extraction procedure described in the section above and analyzed by HPLC-DAD.

The precision of the method was evaluated by the relative standard deviation (RSD, %) of the areas of six replicate injections of each pesticide at the three concentrations (10.0, 100.0 and 1000.0 μg L^{-1}).

Limit of detection (LOD) and limit of quantification (LOQ)

LOD of the three pesticides was calculated by preparing spiked solutions of imidacloprid, cypermethrin and chlorpyrifos ethyl at low concentrations that were expected to produce a response 3-10 times baseline noise. LOQ was determined in the same manner and selected as the concentration of the pesticide that gives an S/N ratio of 10-20 [16].

Stability test

Two series of three parallel river water samples each free from detectable amounts of imidacloprid, cypermethrin and chlorpyrifos ethyl were spiked with 0.5 mg L^{-1} imidacloprid, 0.5 mg L^{-1} cypermethrin and 0.5 mg L^{-1} chlorpyrifos ethyl.

The first series was stored at room temperature (22±1°C) for six months. The second series was placed in a climate chamber with temperature variation (every 12 hours the temperature was changed, so that it approached the average daily 22±1°C and nightly temperatures 4±0.5°C) for six months.

RESULTS AND DISCUSSION

Determination of λ max of the pesticides

The correct choice of the wavelength is an important step to ensure the highest possible sensitivity of the analysis. The absorption maxima of imidacloprid are reported to be 270 nm [17] and 220 nm (±2 nm) [16], of cypermethrin are 235 nm [21] and 220 nm [20] and chlorpyrifos ethyl at 230 nm (±1 nm) [23]. In this work, the absorption spectra of the three pesticides in the entire UV range from 190 nm to 360 nm range were investigated. The obtained spectra showed that the most intensive absorption peaks were at 202 nm, followed by 205 nm and 212 nm for cypermethrin, 205 nm for chlorphyrifos ethyl, followed by 202 nm and 230 nm, and 270 nm for imidacloprid, followed by 212 nm and 205 nm. In order to analyze the three pesticides in one run, the selected working wavelength was 205 nm as a reasonable compromise. To avoid potential interferences at 205 nm LLE was applied to isolate the pesticides, as described in the experimental part.

Method development (Optimization of peak separation)

The commonly used mobile phases for the separate elution of imidacloprid, cypermethrin and chlorpyrifos ethyl with a C18 chromatographic column are acetonitrile, methanol and water. A mobile phase of acetonitrile/water (80:20 v/v) was used to elute imidacloprid by Al-Rimawi *et al.* [16] who determined a mixture of pesticides in surface water. Kumar *et al.* [22] used the same acetonitrile/water ratio of the eluent for the determination of cypermethrin. Successful elution of chlorpyrifos ethyl was achieved using ratios 90:10 v/v and 75:25 v/v acetonitrile/1mM PO_4 [24].

The initial experiments in the current study were performed using several mobile phase compositions. As a first step isocratic elution with a mobile phase of acetonitrile/water (80:20 v/v) at a flow rate of 0.5 mL min^{-1} was performed. The results demonstrated that the peak's retention times were very close to each other, thus although the mobile phase consisting of acetonitrile/water (80:20 v/v respectively) is suitable to determine the pesticides separately, it was not suitable for separation of the mixture.

In order to reduce the time for analysis, gradient elution for the separation of the analyzed compounds was used with methanol as an organic eluent. Many authors applied it as a third component in the already used mobile phase [20, 21]. A successful separation of the analyzed pesticides was achieved with a mobile phase containing methanol:water (70% methanol and 30% water, v/v) with isocratic elution at a flow rate of 0.5 mL min^{-1}. The resulting chromatogram of imidacloprid, cypermethrin and chlorpyrifos ethyl separation is presented in Figure. 1. Cypermethrin had two peaks due to cis- and trans- forms.

The obtained retention times t_r (min) of the analyzed pesticides were as follows: for imidacloprid - t_r=3.95 min, for cypermethrin - t_{r1}= 11.87 min; t_{r2}= 14.20 min and for chlorpyrifos ethyl - t_r= 17.80 min.

Method validation

Linearity and range. To evaluate the linearity of the method, different calibration standards of the pesticides were analyzed by HPLC-DAD and the responses were recorded. The dependence concentration *versus* peak response and the respective correlation coefficient are presented in Figure 3. A plot of the peak areas of the pesticides *versus* concentration (in μg L^{-1}) was found to be linear in the range of 10-1000 μg L^{-1} for all analyzed pesticides with a correlation coefficient (R^2) greater than 0.999. This result indicates that the studied pesticides can be determined in surface water samples in a wide concentration range.

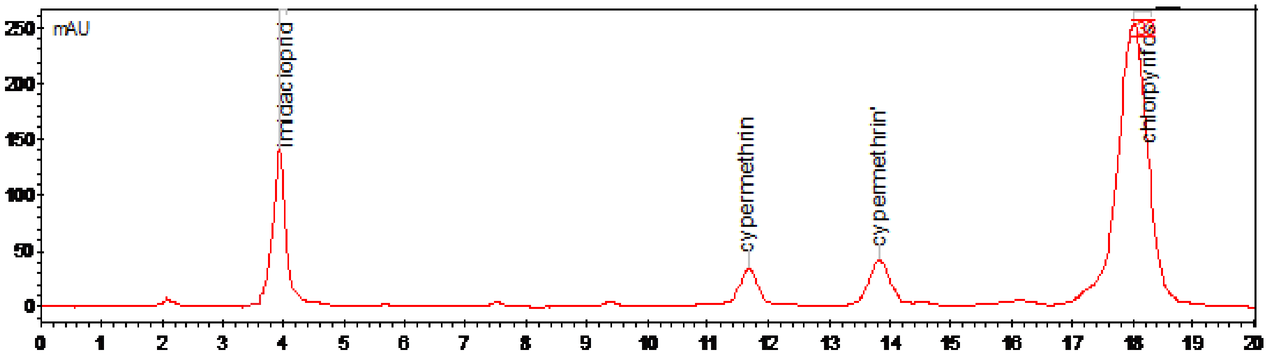

Fig. 1. Chromatogram of the separation of imidacloprid, cypermethrin and chlorpyrifos ethyl with mobile phase methanol/water (70:30 v/v).

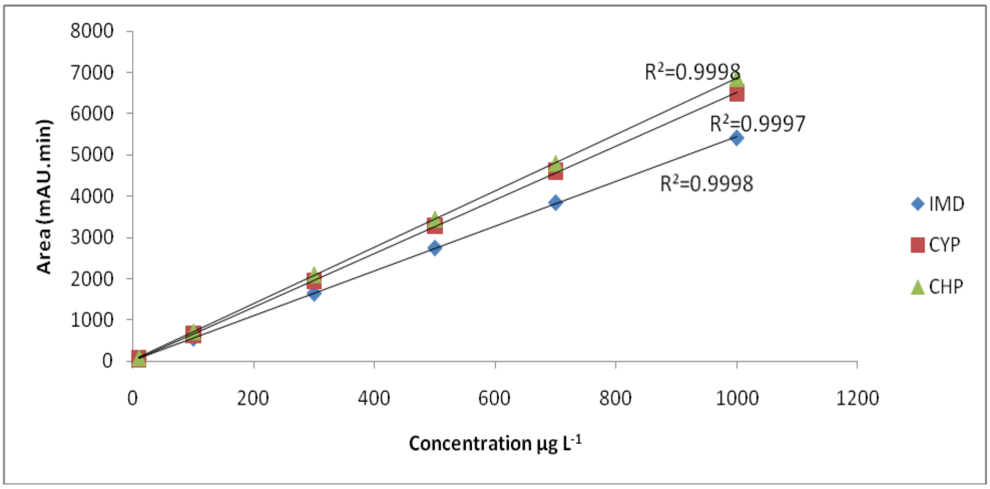

Fig. 2. Calibration curves of imadacloprid (IMD), cypermethrin (CYP) and chlorpyrifos ethyl (CHP).

Table 1. Recovery of imidacloprid, cypermethrin and chlorpyrifos ethyl at three concentration levels (10, 100, and 1000 µg L^{-1}).

	µg L^{-1}	Recovery (%)			Mean	SD*	RSD**
Imidacloprid	10.0	101.2	100.5	101.6	101.1	0.56	0.55
	100.0	98.9	99.5	100.4	99.6	0.75	0.76
	1000.0	99.3	99.5	98.6	99.1	0.47	0.48
Cypermethrin	10.0	100.5	101.0	101.3	100.9	0.40	0.40
	100.0	99.8	98.9	101.4	100.3	1.26	1.26
	1000.0	98.6	99.7	99.8	99.4	0.66	0.67
Chlorpyrifos ethyl	10.0	101.0	101.5	100.6	101.0	0.45	0.44
	100.0	100.2	99.8	99.0	99.7	0.61	0.61
	1000.0	99.4	100.4	99.6	99.8	0.52	0.53

*SD: standard deviation. **RSD (%): relative standard deviation.

Recovery. For the determination of the recovery of the investigated pesticides, the spiked samples were subjected to LLE, and analyzed by HPLC-DAD. The average recovery for each concentration was calculated by the ratio of the peak area of the pesticide in the spiked solution to the peak area of the standard solution with the same concentration. The results showed that the current method had good recovery (from 98.6% to 101.6%) for the three pesticides at the studied concentrations (10.0, 100.0, and 1000.0 µg L^{-1}) with a RSD better than 1.3% (see Table 1).

Precision. The precision of the current method for determination of the three pesticides was evaluated by calculating the RSD of the peak areas of six replicate injections of standard solutions with three concentrations (10.0, 100.0, and 1000.0 µg L^{-1}), and was found to be less than 5.0%.

Efficiency of the extraction procedures

The LOD of the three pesticides were found not low enough (180 µg L^{-1} for imidacloprid, 450 µg L^{-1} for cypermethrin and 800 µg L^{-1} for chlorpyrifos ethyl) to allow the detection and quantification of the pesticides in surface and ground water at low concentrations. This demanded a preliminary step for extraction and pre-concentration. LLE was performed with organic solvent - dichloromethane as described in the experimental part. The extraction efficiency was found to be 98.5%, 99.6% and 99.8% for imidacloprid, cypermethrin and chlorpyrifos ethyl, respectively.

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ of each compound were determined before and after LLE. After applying the extraction procedure, the samples were concentrated to the final volume of 1 mL and then HPLC analysis was performed. Table 2 presents the LOD and LOQ of the analyzed pesticides before and after LLE.

Table 2. LOD (μg L^{-1}) and LOQ (μg L^{-1}) of imidacloprid, cypermethrin and chlorpyrifos ethyl before and after the application of liquid-liquid extraction.

Pesticide	Before LLE		After LLE	
	LOD	LOQ	LOD	LOQ
Imidacloprid	180	560	0.17	0.51
Cypermethrin	450	1350	0.30	1.20
Chlorpyrifos ethyl	800	2800	0.40	1.50

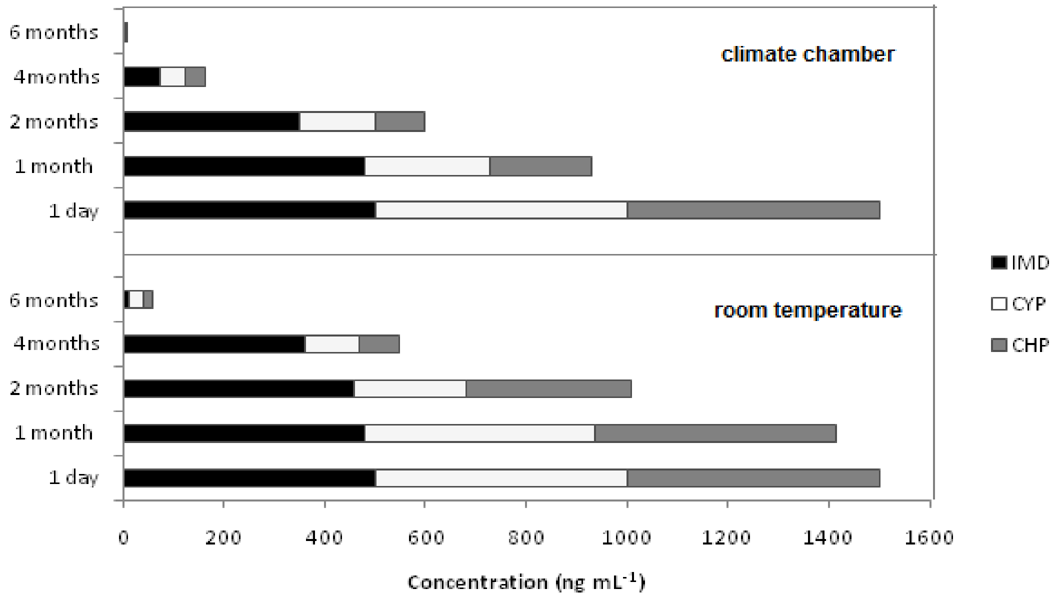

Fig. 3. Stability test of imidacloprid (IMD), cypermethrin (CYP) and chlorpyrifos ethyl (CHP), stored at room temperature (22±1 °C) and in a climate chamber simulating the average daily 22±1°C and nightly temperatures 4±0.5 °C).

The results showed that the imidacloprid had the lowest LOD and LOQ of the analyzed pesticides in water samples. The LODs of all three pesticides decrease between 1000-2000 times after applying the extraction and pre-concentration step which enables the detection and quantification of the pesticides in surface water at low (μg L^{-1}) concentration levels.

Stability test of the analyzed pesticides in a real water sample

The stability of the analyzed pesticides varies in the soil. Imidacloprid is stable for several months [6], cypermethrin more than 50 days [25], and the reported half-life for chlorpyrifos ethyl is in the range of 120-450 days [26]. The high stability of the pesticides enables their entry into surface and groundwater. However, their degradation to hydrolysis products in water depends on many factors - pH, temperature, dissolved oxygen, etc.

To investigate the stability of the analyzed pesticides a river water sample with pH = 6.58 was prepared in triplicate as described in the experimental part. The spiked samples were analyzed immediately after preparation and after periods of 1 month, 2 months, 4 months and 6 months. The results are shown on figure 3.

Imidacloprid was characterized by the highest pesticide stability. After the second month the amount of cypermethrin and chlorpyrifos ethyl detected at room temperature was about 50% of the introduced concentrations and below 30% in the sample with temperature variation. Significantly faster degradation of the analyzed pesticides was observed in the sample stored in the climate chamber with temperature variation. After the sixth month all analyzed pesticides were transformed to degradation products.

Application of the method to real water sample

The applicability of the proposed method was verified with river water samples collected from the basin of Struma river (South West Bulgaria) and analyzed for the three pesticides by the developed method. For comparison tap water samples from the town of Pernik (West Bulgaria) were analyzed. Results showed that only cypermethrin was detected in the river water samples at a concentration of 0.42 ± 0.06 μg L^{-1}. Imidacloprid and chlorpyrifos ethyl were below 0.17 μg L^{-1} and

0.40 μg L^{-1}, respectively. As expected, no signal of the studied pesticides was detected in the tap water samples.

CONCLUSION

This work shows the potential of HPLC-DAD for the determination of pesticides in surface water samples as a good alternative to gas chromatographic methods.

A simple, accurate, precise, and selective HPLC method has been developed and validated for determination of imidacloprid, cypermethrin and chlorpyrifos ethyl with minimal use of toxic organic solvents. The method is accurate within a wide dynamic range with a recovery from 98.6 to 101.6%. The mobile phase consisting of methanol and water provides short run time with good separation of the analytes. The chromatographic separation was achieved at ambient room temperature.

Low LOD and LOQ of the pesticides analyzed in this study enable their detection and quantification in river water at low concentrations.

The method can be applied for the determination of imidacloprid, cypermethrin and chlorpyrifos ethyl in real water samples, including groundwater and surface water. The results indicate that the proposed method is not time-consuming and does not need extensive clean-up sequence. The method can be recommended for routine analysis of imidacloprid, cypermethrin and chlorpyrifos ethyl in water sample analysis.

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