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Water pollution

DETERMINATION OF SOME ORGANOPHOSPHORUS INSECTICIDES AND UREA HERBICIDES FROM WATER BY HPLC

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Abstract. The aim of this study was to develop a method for the simultaneous analysis in water samples of some organophosphorus pesticides (dichlorvos, fenitrothion, coumaphos, phoxim) and phenyl urea pesticides (monolinuron, linuron) included in the EU list of dangerous substances. For this purposes, a solid phase extraction (SPE) technique coupled to high performance liquid chromatography (HPLC) with multi-waved detection (MWD) was studied and optimised. The linearity domain, as well as the limits of detection were determined for each compounds. The limits of detection varied between 0.05 and 0.1 μg/l. The method was applied to the determination of these pesticides in surface water samples. Recoveries ranged between 74.3 and 115.3% and relative standard deviation values between 6.7 and 13.2%.

Keywords: organophosphorus insecticides, phenyl urea herbicides, HPLC, analysis, water.

AIMS AND BACKGROUND

Determination of organophosphorus and phenyl urea pesticides found in water has become in recent years an important issue due to the widespread and indiscriminate use of these compounds in agricultural field. The study of mixture of these compounds is of general interest because they appear generally together in well-known commercial formulation[1–3]. For pesticides, efficient separations can be realised by gas chromatography (GC) with nitrogen-phosphorus[4–6], flame photometric[7], flame thermoionic[8] or mass spectrometric[9–11] detection. However, numerous pesticides residues are not amenable to GC separation because of their thermal lability and/or polarity; thermal degradation products are often detected instead of the molecular pesticides[12]. To overcome these problems, derivatisations procedures are developed. However, the added sample treatment is often time-consuming and generally leads to some interferences[13]. A better choice for multi-residues monitoring of pesticides in all kinds of environmental samples is high-performance liquid chromatography (HPLC) due to its versatility and capability to separate between them or from matrix components[14–19].

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The objective of this work was to develop an analytical method, based on solid-phase extraction-liquid chromatography (SPE-HPLC-MWD) for the simultaneous determination, in a single run, of 6 selected urea and organophosphorus pesticides in water and to apply this method for the investigation of their occurrence in the Danube river. The target analytes selected for the present work are included in the list of dangerous substances in the field of water[20]. Additionally, the EEC Directive 98/83/CE on water quality for human consumption set limit values at 0.1 μg/l for each individual pesticide and 0.5 μg/l for total pesticides. For surface water used to produce drinking water, these values are 2 μg/l for each individual substance and 5 μg/l for total pesticides[21].

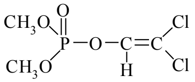
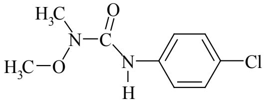
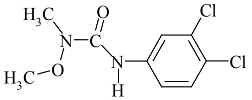
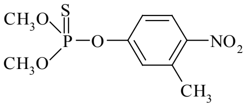
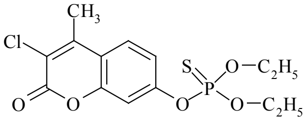
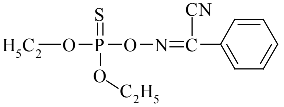
EXPERIMENTAL

Reagents and chemicals. High purity (95.4–99.9%) standards of the 6 selected pesticides monolinuron, linuron, dichlorvos, coumaphos, fenitrothion and phoxim were purchased from Riedel de Haën (Seelze, Germany). Structures, IUPAC name and CAS number are shown in Table 1. All these compounds were used as they were received. Stock standard solutions for each of the analytes were prepared in methanol at 0.5 mg/ml and stored in the dark at 4°C. A stock mixed standard solution of 5.0 μg/ml was prepared weekly. A set of 10 calibration standard solutions of the mixtures of all compounds at concentrations ranging between 0.1 and 4.0 μg/ml were prepared by appropriate dilution of the stock mixed standard solution in methanol. The standard mixtures were used also as spiking solutions in the recovery studies. Spiked water samples for the extraction procedure study were prepared by adding an appropriate amount of a working standard solution to 1000 ml of river water. The spiked water samples were stirred and left to stand for 10 min to stabilise. The river water samples used in the matrix effect study were obtained at two sampling points, situated in the Danube delta biosphere: Uzlina and Murighiol.

HPLC-grade solvents methanol, acetonitrile and water were supplied by Merck (Darmstadt, Germany). All the solvents and samples were filtered through 0.45-μm membrane filter prior to use (Agilent Technologies, USA).

Liquid-chromatographic separation. Liquid-chromatographic separation of the pesticides was investigated by using an Agilent 1100 (Agilent Technologies, USA) system equipped with a degasser, quaternary pump, autosampler, column thermostat and multiple wavelength detector (MWD). The separations were performed on a Zorbax Eclipse XDB-C18 analytical column (150 mm length, 4.6 mm i.d.; 5 μm particle size) packed with a reversed phase featured by dimethyl-*n*-octadecylsilane bonded to silica-organic support, double end-capped, acquired from Agilent Technologies. The HPLC separations were carried out at 25°C with a flow-rate of 1 ml/min under isocratic conditions. The injection volume was 10 μl and the elution of the compounds into the chromatographic column was performed with acetonitrile–water (65:35, v/v).

Table 1. Identification data on the studied pesticides

Commercial name	IUPAC name and CAS number	Structure
Dichlorvos	1,1-dichloro-2-dimethoxyphosphoryloxyethene (000062-73-7)	
Monolinuron	3-(4-chlorophenyl)-1-methoxy-1-methylurea (1746-81-2)	
Linuron	3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea (000330-55-2)	
Fenitrothion	Dimethoxy-(3-methyl-4-nitro-phenoxy)-sulfanylidene-phosphorane (000122-14-5)	
Coumaphos	3-chloro-7-diethoxyphosphinothioyloxy-4-methyl-chromen-2-one (000056-72-4)	
Phoxim	2(E)-2-diethoxyphosphinothioyloxyimino-2-phenyl-acetonitrile (014816-18-3)	

System control and data acquisition were achieved by means of a computer equipped with an Agilent ChemStation program. The chromatographic system was operationally qualified before performing this study.

Multiple wavelength detection. Under the above described chromatographic conditions, all the analytes were simultaneously determined by using a multiple wavelength detector (MWD). In order to attain maximum sensitivity, the quantitative measurements are made at 220 nm for dichlorvos and at 254 nm – for the rest of analytes.

SPE procedure. Disposable vacuum manifold system was obtained from Vacuubrand GmbH (Wertheim, Germany) and solid-phase extraction cartridges from Macherey-Nagel (Duren, Germany). The SPE cartridges (Chromabond C18 Hydra, 6 ml, 2000 mg) were conditioned with 3 × 3 ml methanol and then with 3 × 3 ml

HPLC-grade water, taking care not to let the cartridge dry out. The passage of the samples (volume 1000 ml) through the cartridges was carried out at a flow-rate of 10 ml/min by means of a vacuum pump. After the retention step the cartridges were cleaned with 10 ml of HPLC-grade water and were dried under a gentle stream of air (45 min, room temperature). The retained compounds were eluted with 10 ml methanol. The obtained solutions were evaporated to dryness by mean of a rotary evaporator at 35°C. The dry residue was dissolved in 1 ml acetonitrile.

RESULTS AND DISCUSSION

OPTIMISATION OF SEPARATION CONDITIONS IN HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

The first aim was the development of a sensitive, selective and precise method for the simultaneous determination of the 6 selected pesticides in water samples. The UV absorption spectra measured from 190 to 400 nm has shown that all the compounds provide distinguishable absorption spectra within the range from 200 to 290 nm. In order to select the monitoring wavelength for the selected pesticides and also to attain low limits of detection, the chromatogram for a standard mixture has been simultaneously recorded at 4 wavelengths: 218, 220, 254 and 280 nm. The simultaneous detection at 220 nm, the most sensitive wavelength corresponding to maximum absorption for dichlorvos, and 254 nm – for the rest of analytes, allows the increase of the sensitivity.

For a optimal simultaneous elution of the analytes, the mobile phase composition was established as result of the experiments regarding the analyte retention dependences on organic modifier concentration using an octadecyl silica stationary phase. The dead time of the column was determined by injection of uracil-containing solution. The mobile phase with the composition of acetonitrile: acetonitrile: water = 65:35 (v/v) as isocratic elution separates successfully the chromatographic signals with a baseline resolution of all the constituents within 10 min. Using the above-mentioned chromatographic conditions clear chromatograms were obtained as is shown in Fig. 1.

Retention times of the selected pesticides were inversely proportional to their polarity and their solubility in water. The shortest retention time was for dichlorvos which solubility is 18 g/l; water solubilities of monolinuron, linuron, fenitrothion, coumaphos and phoxim are 735, 63.8, 14, 1.5 and 1.5 mg/l, respectively.

VALIDATION OF THE METHOD

Linearity and detection limits. The linearity of this method for analysing standard solution has been investigated over the range of 0.1 to 4.0 μg/ml. Each calibration level was analysed in duplicate. Regression analysis was used to construct calibration curves for each compound. All the analytes were found to be linear in the tested range with correlation coefficients (R^2) from 0.9971 to 0.9998.

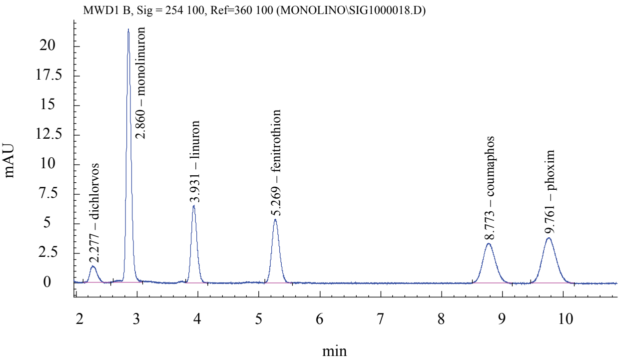


Fig. 1. Chromatogram of a standard mixture solution of dichlorvos, monolinuron, linuron, fenitrothion, coumaphos and phoxim (their concentrations in the sample solution were: 5.16 µg/ml monolinuron, 3.56 µg/ml linuron and 4.00 µg/ml – for the rest of analytes)

The calculation of the detection limits (LODs) was based on the lowest detectable peak for any single analyte that gave a signal to noise ratio (S/N) of 3. The obtained LODs were lower than the maximum concentration acceptable for individual pesticides in drinking water according to current legislation.

Statistical evaluation data of the linear calibration function and the method detection limits (LODs) are presented in Table 2.

Table 2. Retention time of analytes, linear regression analysis, method correlation coefficients (R^2), limits of detection (LODs) obtained in the chromatographic conditions of the method

Analyte	Ret. time (min)	Dynamic range (µg/l)	Regression equation	R^2	LOD (µg/l)
Dichlorvos	2.214	0.2–4.0	y=2.3837x + 0.2701	0.9971	0.10
Monolinuron	2.776	0.1–4.0	y=21.8421x+ 1.2186	0.9996	0.05
Linuron	3.803	0.1–4.0	y=12.3781x + 0.3933	0.9990	0.10
Fenitrothion	5.053	0.1–4.0	y=11.2578x + 0.1877	0.9998	0.05
Coumaphos	8.773	0.1–4.0	y=11.3534x + 0.2918	0.9996	0.07
Phoxim	9.761	0.1–4.0	y=15.2044x + 0.6402	0.9997	0.10

Recovery and precision. Recovery studies were performed in order to evaluate the performance of the developed methodology for the extraction of the target analytes from water samples. Different extraction solvents were used and it was found that methanol gave the best results for all compounds. Five replicate 1000 ml deionised water samples were each spiked to obtain the final concentration 1 µg/l of each compound. Recovery in spiked deionised water samples varied between 78.2 and 102.7% with relative standard deviations (RSDs) of 7.7 to 9.6% (Fig. 2). These values are acceptable for the concentration level used.

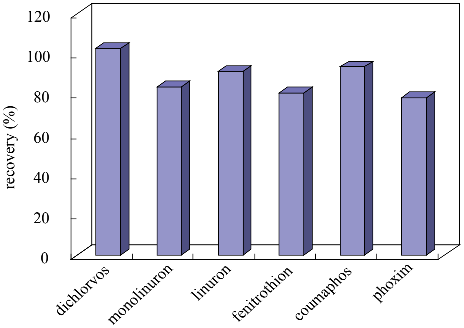


Fig. 2. Recoveries obtained for the analysis of pesticides in deionised spiked water using the SPE-HPLC-MWD method (average for 5 replicate)

Matrix effects study. The optimised method was further validated by investigating its reproducibility in real river samples to confirm its adequacy and feasibility to quantify these analytes in surface water samples.

The river samples used in this study present no analytes peaks or interferences in the chromatograms. As target analytes were not found in these river samples, in order to investigate the matrix effect on the method, triplicate aliquots of each samples were spiked with 2 levels of concentrations and further analysed with the developed method. The average concentrations obtained for these spiked samples with 0.8 μg/l correspond to mean recoveries ranging from 74.3 to 115.3% and the average concentrations obtained for the samples spiked with 3.0 μg/l correspond to mean recoveries ranging from 76.3 to 107.5%.

The precision of the three replicate river samples from the Danube delta for 0.8 μg/l, as indicated by RSD, ranged from 8.8 to 13.2% and for 3.0 μg/l ranged from 6.7 to 9.4%. The values obtained confirm the reliability of the proposed method.

CONCLUSIONS

A multi-residue method by SPE-HPLC with MWD detection has been developed for the analysis of some pesticides that are commonly found in water. The presented experimental results demonstrate that the method can be routinely applied for monitoring water quality in regard to these compounds, in the frame of the new EU regulation.

The analytical protocol developed in this study will be applied toward the quantification of these compounds in water from the Danube delta biosphere area.

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