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Short communication

Simultaneous quantitative determination of thirteen urea pesticides at sub-ppb levels on a Zorbax SB-C_{18} column

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Abstract

A simple multi-residue method is described for simultaneously determining ten urea and three benzoylurea pesticides residues in drinking water with quantitation limits below the European regulatory limit of 0.1 μg/l. The residues were extracted from drinking water with dichloromethane and analysed by HPLC on a Zorbax 5 μm SB-C_{18} column with diode array detection (DAD) at 240 nm. Recoveries were determined by spiking drinking water with 13 pesticides (benzthiazuron, metoxuron, monuron, fluometuron, isoproturon, diuron, linuron, chloroxuron, chlorbromuron, diflubenzuron, neburon, triflumuron and flucycloxuron) at the 0.05–0.50 μg/l level.

Keywords: Urea; Benzoylurea; Pesticides

1. Introduction

The substituted urea herbicides are used for the control of many annual and perennial weeds, for brush control, and for weed control in irrigation and drainage ditches. Likewise the benzoylurea insecticides are widely used on a large number of crops.

Several of these compounds are highly persistent in the environment and misuse, carelessness, and leaching can contaminate surface waters used for crop irrigation or for a potable water supply. This can lead to damaged crops if water contaminated with pesticides is used for irrigation and increases the possibility of unacceptable residues in drinking water. It is thus necessary to have an analytical procedure that can estimate the level of contamination by these kinds of pesticides.

Many extraction methods are described either, liquid–liquid extraction (LLE) or solid-phase extraction (SPE).

LLE does not require special instrumentation and materials; these are the main reasons why it is employed in most methods for the determination of pesticides, including official ones [1–4], rather than much more recent methods which use SPE for pesticide analysis.

Hennion et al. [5] reported an on-line and off-line preconcentration technique to analyse phenylurea pesticides at the ppb level from 500 ml of water. Bagheri et al. [6] developed an on-line SPE system using a polystyrene–divinylbenzene copolymer cartridge; the liquid chromatography–thermospray mass spectrometry (LC–TSP-MS) procedure was in the 0.005–0.02 μg/l range for the analysis of phenylurea herbicides from surface and drinking water samples. Slobodnik et al. [7] described a fully automated column LC separation system using a styrene–divinylbenzene copolymer cartridge for the on-line trace enrichment system. The high-performance liq-

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uid chromatography–diode array detection (HPLC–DAD) determination limit was 0.1 μg/l for all tested compounds.

In the recent years, an on-line single short column was used as preconcentration and separation technique as described by Hogenboom et al. [8]. The detection system was between 0.002 and 0.75 μg/l by selected ion monitoring. In addition, several authors used immunoaffinity chromatography [9] or selective trace enrichment on immunosorbents to determine phenylurea herbicides [10,11].

Apart from the choice of the extraction method, the main object of this study was to investigate the Zorbax SB-C_{18} column performance for the separation of a certain number of urea and benzoyl urea pesticides. The results were encouraging yielding to the separation of 13 pesticides with quantitation limits below the European regulatory limit of 0.1 μg/l for each compound.

2. Experimental

2.1. Materials

Water and acetonitrile were of HPLC grade purchased from Carlo Erba (Milan, Italy). Dichloromethane (reagent grade) from Carlo Erba was re-distilled in glass. Orthophosphoric acid (85% analytical-reagent grade) was from BDH (Milan, Italy). An Anotop 10 filter 0.2 μm was from Merck (Darmstadt, Germany). Orion application solution, pH 4.01 buffer and pH 7.00 buffer were from Orion (Boston, MA, USA). Pesticide reference standards were from the collection in this laboratory. Stock solutions of individual pesticides were prepared in acetonitrile at 200 μg/ml. Internal standard (I.S.) solution of phenylsulphone was prepared in acetonitrile at 440 μg/ml. Mixed working solutions of the pesticides in acetonitrile at 1, 2, 5, 10 μg/ml were used for the construction of calibration curves and for the preparation of fortified drinking water samples.

2.2. Equipment

2.2.1. HPLC apparatus and chromatographic conditions

A Varian Model 9012Q pump (CA, USA), valve injector Reodyne (Cotati, CA, USA) equipped with a 10-μl loop and a Varian Model 9065 polychrom detector set to 240 nm was employed with a Varian Star 4.51 acquisition system. Chromatography was carried out on a Zorbax (DuPont) 5 μm SB-C_{18} column (250 mm×4.6 mm I.D.).

Acid water and acetonitrile were used as mobile phase. For this purpose orthophosphoric acid 85% was added to water to obtain a pH of 2.5, tested with Orion pH meter. The following gradient was employed: 10% acetonitrile from 0 to 5 min, 90% at 35 min. Flow-rate: 2 ml/min.

2.2.2. pH meter

An Orion Research Model 701/digital pH meter (Boston, MA, USA) and an Orion 8104 Ross combinator pH electrode glass body with rugged bulb were used.

2.3. Calibration

Stock solutions of individual pesticides in acetonitrile at 200 μg/ml were prepared. Phenylsulphone as I.S. at 440 μg/ml parent concentration was used.

A series of calibration solutions for HPLC–UV analysis at 1, 2, 5, 10 μg/ml of each pesticide and at a constant concentration of 11 μg/ml for the I.S. were prepared by appropriate mixing of the stock solutions.

2.4. Extraction procedure

Volumes of 100 μl of the calibration solutions (1, 2, 5, 10 μg/ml) were added to the drinking water samples resulting in spiked levels of 0.05 μg/l, 0.1 μg/l, 0.25 μg/l and 0.5 μg/l, respectively. The samples were extracted in separatory funnel using three portions of 100 ml of dichloromethane for 10 min under mechanical shaking.

The organic phase was collected and concentrated to a small volume using a rotary evaporator (40°C; reduced pressure). The organic phase was then quantitatively transferred to a 5-ml tube, concentrated to dryness and dissolved in 1 ml acetonitrile.

The acetonitrile phase was passed through an Anotop 10 filter (0.2 μm).

Finally, the filtered extract was concentrated to dryness under a gentle stream of nitrogen, dissolved in 100 μl acetonitrile and analysed by HPLC.

3. Results and discussion

Although the traditional LLE requires the handling of a large volume of chlorinated solvents and tends to be difficult to automate, the nature of the environmental sample can determine the choice between LLE and SPE methods. The analysis of wastewater and surface water requires the treatment of the total sample, including pesticides adsorbed on fine materials. LLE should be then preferred since the total sample can be handled without filtration.

Zorbax SB-C_{18} is a base-deactivated microparticulate C_{18} bonded silica used for reversed-phase HPLC. This column showed good performance in separating the 13 pesticides and the I.S. considered. Reproducible elution with symmetrical peaks was obtained acidifying the water phase to pH 2.5. Column bleeding also was very low under the gradient conditions increasing the signal-to-noise ratio and allowing a good performance in terms of sensitivity.

The minimum amount injected was ca. 10 ng for each compound, comparable with the amount injected after the extraction procedure of a drinking water sample spiked at 0.05 μg/l.

Fig. 1 shows the chromatogram of an extract of a

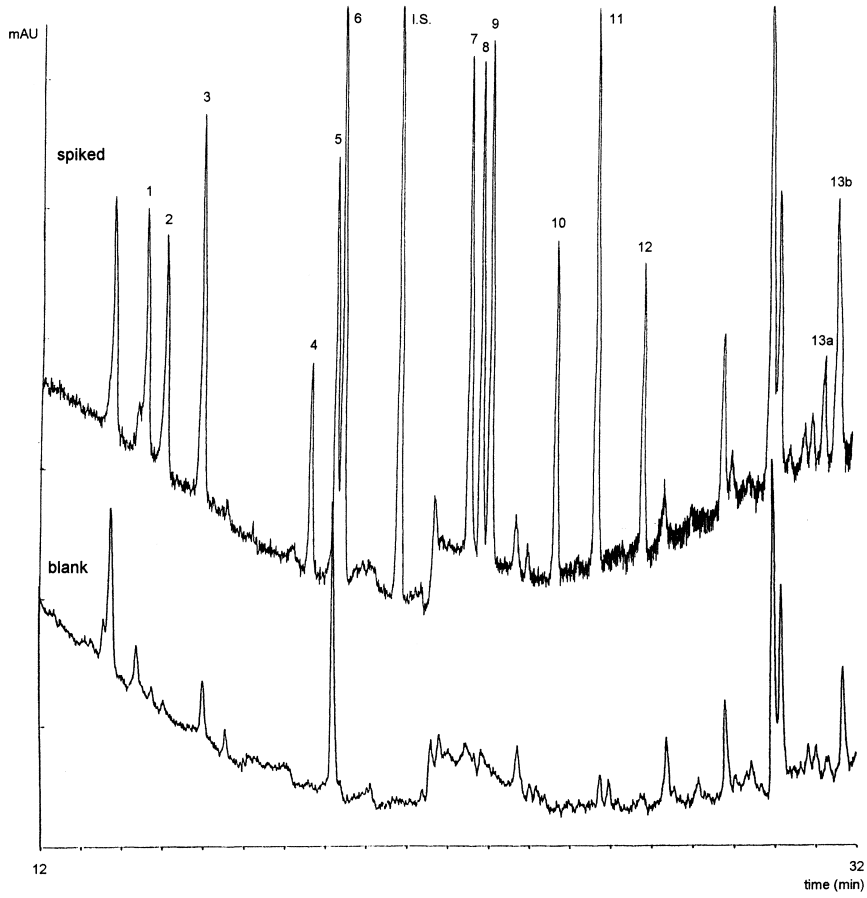

Fig. 1. Chromatogram of an extract of a drinking water sample spiked at 0.05 μg/l of each compound and 110 ng of I.S., and the blank trace on a Zorbax 5 μm SB-C_{18} column, 250 mm×4.6 mm I.D., with diode array detection at 240 nm. Flow: 2 ml/min. Peaks: 1=Benzthiazuron, 2=metoxuron, 3=monuron, 4=fluometuron, 5=isoproturon, 6=diuron, 7=linuron, 8=chloroxuron, 9=chlorbromuron, 10=diflubenzuron, 11=neburon, 12=triflumuron, 13a–b=flucycloxuron.

drinking water sample spiked at 0.05 μg/l with each pesticide, respectively and a blank trace of a water sample collected simultaneously to the spiked one.

No overlapping between the analytes peaks was observed under the set instrumental conditions.

Flucycloxuron showed a double-peak chromatographic pattern due to the presence of two optical isomers. Flucycloxuron was calculated on the basis of the sum of the areas of the two peaks.

Furthermore, the simple extraction procedure provided good recoveries and the blank did not show any interference with the analyte peaks with the exception of the isoproturon peak and the second peak of flucycloxuron. The quantitative calculations for isoproturon and flucycloxuron were performed by subtracting the blank contribution.

A multilevel internal standard calibration was performed. Four concentration levels to define a calibration curves were used; two replicates for each level were averaged.

Linear regression was used to calculate the curves. The coefficients of regression (r^2) were higher than 0.99 for all the pesticides as deduced from the four calibrations in the linear dynamic range considered.

The recoveries of the pesticides were performed with the internal standard method analysing four replicates at 0.1, 0.25 and 0.5 μg/l, respectively, and six replicates at 0.05 μg/l. The phenylsulphone recovery which was calculated with the external standard method, at a concentration of 0.55 μg/l (five replicates) was 98±9%.

The average recoveries varied from 69±6% to 127±16% as shown in Table 1. The detection limits of the 13 pesticides are also given in Table 1.

A commonly used definition of the limit of detection (LOD) is the ''analyte concentration giving a signal equal to the blank signal, y_B, plus three standard deviation of the blank S_B''. The y_B values were calculated by the average of 20 determinations of the blank signal before the appearance of the peaks.

The corresponding detection and quantitation limits expressed in concentration units were derived from the calibration equation for each compound. The detection limits varied from 0.001 μg/l to 0.007 μg/l. The quantitation limits (LOQs) (defined as 10×LOD) ranged between 0.01 μg/l and 0.07 μg/l.

Although the LOQs of diuron, diflubenzuron and triflumuron were a little higher than 0.05 μg/l, it has been deemed suitable calculate the LOQs of these three pesticides as described above.

Our final aim to develop a method suitable to determine pesticides at the 0.1 μg/l level on a Zorbax SB-C_{18} column was successful as the LOQs obtained in this study are well below this level.

This work indicates that the Zorbax SB-C_{18} col-

Table 1
Recoveries from fortified drinking water and limits of detection (LODs) of 13 pesticides

Pesticide	Average recovery (%) ±S.D.				LOD (μg/l)
	Fortification level				
	0.05 μg/l	0.1 μg/l	0.25 μg/l	0.5 μg/l	
Benzthiazuron	75±10	70±5	69±6	77±3	0.001
Metoxuron	94±2	94±5	94±3	95±6	0.003
Monuron	130±8	101±10	103±3	99±4	0.002
Fluometuron	93±2	98±3	98±6	97±3	0.005
Isoproturon	107±19	127±16	115±9	105±12	0.002
Diuron	91±2	96±1	97±3	95±3	0.007
Linuron	92±2	108±2	99±4	98±5	0.001
Chloroxuron	104±4	98±2	97±4	97±4	0.001
Chlorbromuron	100±2	99±1	98±4	97±4	0.001
Diflubenzuron	90±11	117±6	105±7	95±10	0.006
Neburon	104±2	103±4	98±3	98±5	0.004
Triflumuron	107±5	101±4	98±1	98±8	0.006
Flucycloxuron	102±16	105±3	106±2	99±9	0.005

umn shows a good performance in separating the 13 urea pesticides and, what is more, the low bleeding of the column allows one to determine the pesticides at sub-ppb levels.

In conclusion, the present procedure can be considered sufficiently sensitive and reliable for routine analysis of drinking water.

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