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Solid-phase extraction followed by high-performance liquid chromatographic analysis for monitoring herbicides in drinking water

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ABSTRACT

A multiresidue analytical method based on C_{18} solid-phase extraction and one-run HPLC determination has been developed for the analysis of eleven acidic, neutral and weak basic herbicides in drinking water. A 1-l sample of water was preconcentrated by passage through a 500-mg C_{18} solid phase extraction column. The retained compounds were eluted from the column with 1 ml of methanol. After concentration of the extract the pesticides were separated and quantified by reversed-phase HPLC with UV detection. Bentazone, 2,4-D, MCPA, fluazifop-acid, metoxuron, monolinuron, metobromuron, diuron, linuron, atrazine and simazine were determined simultaneously in a single run on a C_{18} HPLC column. Reanalyses of the sample extracts on a second cyano column were used to confirm the identity of the neutral and basic compounds. The limit of determination, defined as four times the baseline noise, varied between 0.01 µg/l and 0.1 µg/l depending on the compound, the detection sensitivity of the instrument and the type of HPLC column used.

INTRODUCTION

The high standards for drinking water purity laid down by the European Community, maximum admisible concentrations (MACs) of 0.1 µg/l for any individual pesticide, require the development of suitable analytical methods with high sensitivity, selectivity, accuracy and reliability. Recent publications on this subject show that there is a tendency to use sophisticated techniques such as gas chromatography (GC) with mass spectrometry (MS) [1,2], liquid chromatography with particle beam mass spectrometry [3], HPLC with diode-array detection [4,5], and HPLC with column switching [6] for determining pesticide residues in water.

Pesticide monitoring of waters is also possible with rapid and simple methods that use less sophisticated instruments, and which still provide reliable identification of analytes. Reversed-phase HPLC is widely used in analyses of pesticides with high polarity, low volatility and thermal instability [4, 7–9].

Solid-phase extraction (SPE) has recently been accepted as a powerful tool for extraction of water samples prior to analysis [10]. Compared with traditional methods such as liquid–liquid extraction, SPE reduces sample handling, labour and solvent consumption. The most popular sorbent for SPE of pesticides from water is octadecyl (C_{18}) bonded silica [1,2,5]. Graphitized carbon black cartridge extraction of pesticides from water has also been reported [8,11,12].

A simple, rapid and reliable multiresidue method has been developed for the analysis of eleven herbicides in drinking water. It includes herbicides that cannot be analysed directly by GC owing to poor volatility, polarity or thermal instability. The selected compounds are widely used in agriculture and are known to be potential pollutants of natural waters. Triazine herbicides, though they can be determined by GC without preliminary derivatization, are also included because they (especially atrazine) are some of most common water pollutants. The method allows the simultaneous determi-

nation of acidic, neutral and weak basic compounds using C_{18} SPE and one-run reversed-phase HPLC determination. Positive peak identification of basic and neutral compounds has been achieved by means of an alternative HPLC column with different polarity.

EXPERIMENTAL

Chemicals and reagents

All reagents and solvents were of reagent grade. Methanol distilled in glass and bidistilled water were used for HPLC. Octadecyl C_{18} SPE packing, Supel-clean LC-18, was obtained from Supelco. Individual standard stock solutions, 1 mg/ml in methanol, were prepared from analytical-purity standards. Composite working standard solutions were prepared by mixing appropriate known volumes of each standard stock solution and diluting to 100 ml with HPLC mobile phase.

Apparatus

A Pye Unicam liquid chromatograph was equipped with a PU 4010 pump, a PU 4020 variable-wave-

length UV detector and a Rheodyne Model 7125 injector with a 20-μl loop. A cartridge column, RP-18 Spheri 5 μm (100 mm × 4.6 mm I.D.) (Pye Unicam), and a LiChrosorb-CN 5 μm column (250 mm × 4.6 mm I.D.) (Merck), were used for the determination of the compounds. A guard cartridge, RP-18 5 μm (40 mm × 4.6 mm I.D.), was used with the RP-18 column.

Procedure

Aqueous samples were fortified with known volumes of standard solutions. After adjusting the pH to 2 and adjusting the ionic strength by addition of 2.5 M sulphuric acid and 10 g of sodium chloride, the samples were mixed well and forced to percolate through the SPE column under vacuum at a rate of *ca.* 10 ml/min. The SPE column was prepared by placing in a 5 mm I.D. glass tube a plug of quartz wool, 0.5 g of C_{18} packing material and then another plug of quartz wool to prevent clogging or crushing of the particles. The column was conditioned with 10 ml of methanol and equilibrated with 10 ml of distilled water. Just after the sample was passed through the column, it was washed with 5 ml of

TABLE I

ACCURACY AND PRECISION OF THE METHOD AT DIFFERENT LEVELS OF FORTIFICATION AND TWO SAMPLE VOLUMES

No.	Compound	Recovery (% ± S.D., n = 5)				
		1 l		0.5 l		1 l
		5 × LOD[a]	20 × LOD[b]	5 × LOD[a]	20 × LOD[b]	0.1 μg/l
1	Bentazone	39.2 ± 6.3	52.7 ± 4.8	75.8 ± 4.6	77.3 ± 9.0	86.7 ± 0.3[c]
2	2,4-D	72.3 ± 3.8	75.6 ± 6.2	90.9 ± 7.1	102.8 ± 6.5	77.1 ± 4.9
3	MCPA	80.1 ± 4.2	90.3 ± 5.6	92.3 ± 9.9	98.0 ± 8.4	91.9 ± 8.1
4	Metoxuron	83.5 ± 7.4	93.6 ± 4.8	95.8 ± 9.9	92.6 ± 5.8	84.6 ± 8.8
5	Fluazifop-acid	90.7 ± 5.2	96.7 ± 4.1	91.3 ± 8.1	95.8 ± 5.2	85.3 ± 5.2
6	Simazine	77.3 ± 7.9	89.9 ± 5.1	94.1 ± 10.1	98.3 ± 4.2	[d]
7	Atrazine	82.7 ± 5.2	97.5 ± 2.9	91.8 ± 3.9	94.4 ± 3.1	[d]
8	Monolinuron	84.9 ± 11.6	91.7 ± 6.9	104.6 ± 10.4	97.3 ± 6.1	92.3 ± 6.7
9	Metobromuron	86.7 ± 10.8	96.4 ± 8.3	100.6 ± 10.6	99.5 ± 4.4	80.5 ± 7.3[e]
10	Diuron	87.7 ± 11.9	95.3 ± 9.8	89.1 ± 7.4	96.1 ± 3.5	82.4 ± 9.5[e]
11	Linuron	86.5 ± 13.9	91.5 ± 10.1	82.1 ± 9.5	94.7 ± 7.3	79.2 ± 5.1[e]

[a] Fortification level five times limit of determination (μg/l).
[b] Fortification level twenty times limit of determination (μg/l).
[c] Sample volume 0.5 l.
[d] Equal to (5 × LOD) μg/l.
[e] Determinations on a CN column.

distilled water, the eluate discarded and the sorbent bed dried under vacuum for 5 min. Analytes were eluted with 1 ml of methanol. The solvent was evaporated to dryness under a stream of air. The residue was dissolved first in 0.25 ml of methanol and then in an equal volume of 0.1 M acetic acid–sodium acetate buffer (pH 3.8) to a final sample volume of 0.5 ml.

For the separation and quantification of acidic, neutral and basic compounds on the RP-18 cartridge column the composition of the mobile phase was 50% methanol and 50% 0.1 M acetic acid–sodium acetate buffer (pH 3.8). The flow-rate was 1 ml/min and UV detection at 230 nm was used. The second HPLC column containing CN packing was used for the determination of urea and triazine herbicides with a mobile phase of methanol–water (2:8, v/v) at a flow-rate of 1 ml/min and the same UV wavelength.

The concentration of the herbicides in water samples was calculated by measuring the peak heights and comparing them with those obtained with standard solutions.

RESULTS AND DISCUSSION

In the published multiresidue methods for simultaneous analysis of acidic, neutral and weak basic pesticides in waters, after SPE they are separated by stepwise elution to fractions [2,12,13]. Determination of acidic compounds is carried out separately from neutral and basic compounds by HPLC, GC or another technique.

In the method developed, conditions for the simultaneous HPLC determination in one fraction of acidic, neutral and weak basic compounds after trace enrichment with C_{18} SPE were determined. Under these conditions, great losses of phenylureas and triazines were not observed, as was reported by Di Corcia and Marchetti [8,11] using 0.5-g C_{18} cartridges. As Table I shows, recoveries of all compounds are not less than 75%. The exception to this is bentazone, whose recovery is reduced to 39% with an increase in sample volume to 1 l and 2,4-D, whose recovery is reduced to 72%.

The influence of sample volume on recovery has been studied by analysis of five replicates of 0.5 l and 1 l of drinking water spiked with known quantities of herbicides corresponding to five times the limit of

determination. Table I shows that with a doubling of sample volume only bentazone and to some extent 2,4-D show loss of analyte.

It is known that HPLC methods using only one chromatographic column for identifying a large number of pesticides in waters have a high probability of false positives. Undoubtedly, mass spectrometry does provide the most definitive confirmation. Since sophisticated instruments such as liquid chromatographs coupled to mass selective detectors are not available to most routine analytical laborato-
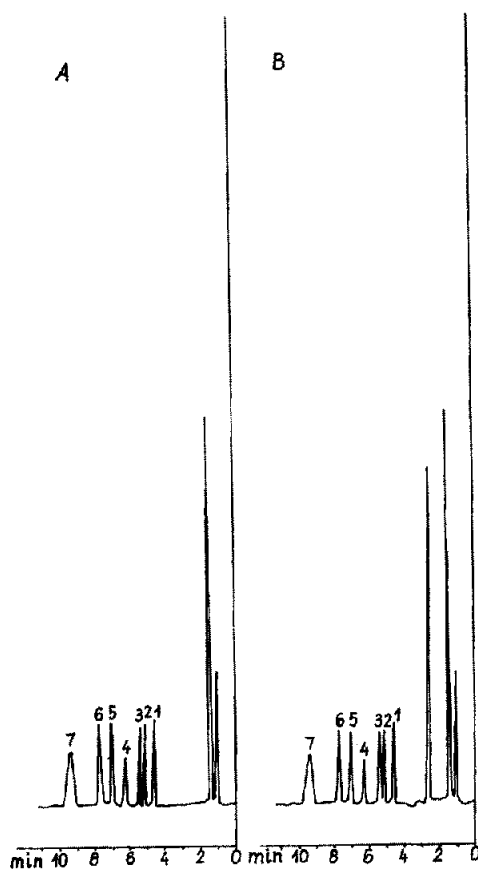

Fig. 1. HPLC chromatograms obtained by injecting (A) a mixed working standard solution of neutral and basic herbicides and (B) a tap water sample fortified with herbicides at the same concentrations. The column was a LiChrosorb-CN column and the chromatographic conditions were as described in the Experimental section. Attenuation 0.02 a.u.f.s. Peak numbering: 1 = simazine 1 ng; 2 = atrazine 1 ng; 3 = metoxuron 2 ng; 4 = monolinuron 2 ng; 5 = metobromuron 5 ng; 6 = diuron 5 ng; 7 = linuron 5 ng.

TABLE II

RETENTION TIMES AND LIMITS OF DETERMINATION ON TWO HPLC COLUMNS OF HERBICIDES ADDED TO 1 l OF TAP WATER SAMPLE

t_R = Retention time; LOD = limit of determination defined as four times baseline noise.

No.	Compound	t_R (min)		LOD (μg/l)	
		C_{18}	CN	C_{18}	CN
1	Bentazone	1.6	1.3	0.002	–
2	2,4-D	2.7	1.3	0.01	–
3	MCPA	3.5	1.4	0.01	–
4	Metoxuron	5.2	5.5	0.03	0.02
5	Fluazifop-acid	6.0	1.7	0.05	–
6	Simazine	7.6	4.8	0.02	0.01
7	Atrazine	11.8	5.2	0.02	0.01
8	Monolinuron	12.7	6.8	0.05	0.02
9	Metobromuron	14.6	7.6	0.1	0.03
10	Diuron	19.1	8.2	0.1	0.04
11	Linuron	30	10.4	0.15	0.05

ries, it is important to find more applicable methods for peak identity confirmation. The use of an alternative HPLC column of different polarity described in the method presented gives a reliable, cheap and accessible approach to validate pesticide identification.

Reanalysis of the sample extracts on a second cyano column was used. Cyano column was excellent for triazine and urea herbicides separation (Fig. 1). The elution order of the compounds on this column was different from on the C_{18} column (Table II). This fact was favourable to confirmation reliability. The retention of acidic compounds on the cyano column was very limited. Bentazone, 2,4-D and MCPA were not separated on that column (Table II). For that reason the alternative column could not be recommended for peak identification of acidic herbicides. The retention times of all triazine and urea (except metoxuron) herbicides were shorter on the cyano than on the C_{18} column. The time of
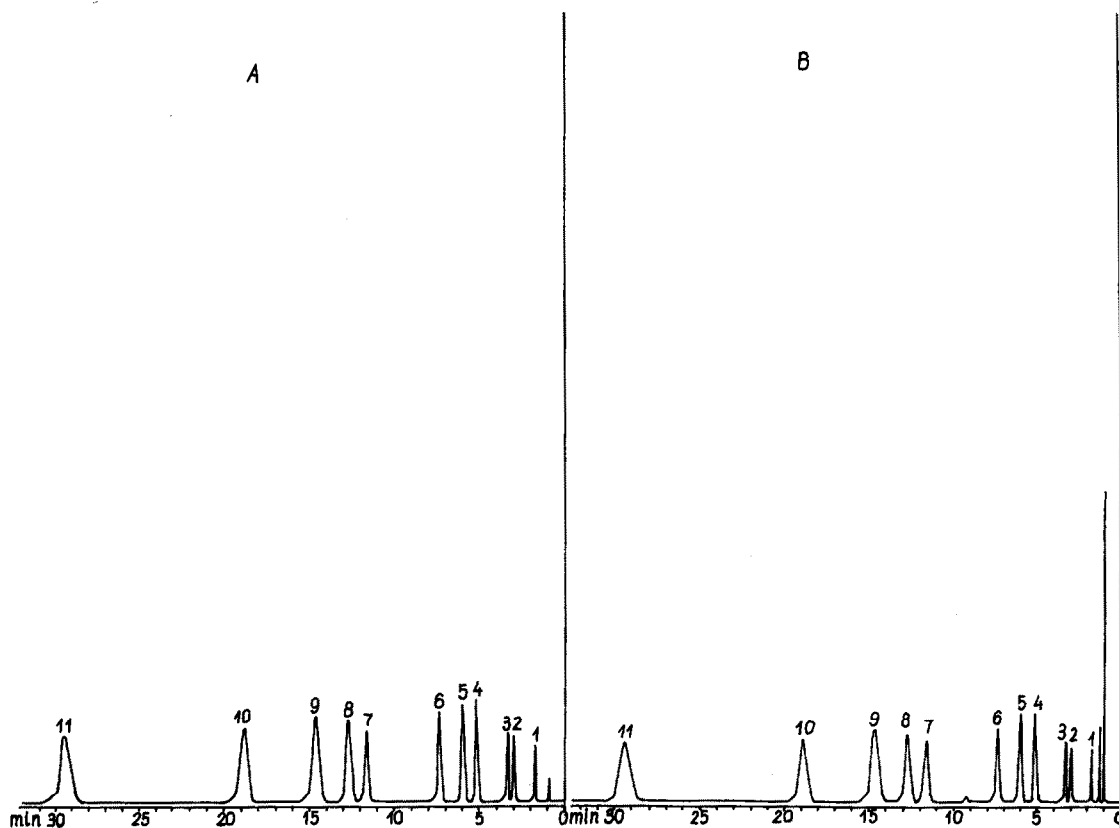

Fig. 2. HPLC chromatograms obtained by injecting (A) a mixed working standard solution of the herbicides and (B) a tap water sample fortified with herbicides at the same concentrations. The column was a Spheri-5 RP-18 cartridge column and the chromatographic conditions were as described in the Experimental section. Attenuation 0.02 a.u.f.s. Peak numbering: 1 = bentazone 0.2 ng; 2 = 2,4-D 1 ng; 3 = MCPA 1 ng; 4 = simazine 2 ng; 5 = fluazifop-acid 5 ng; 6 = metoxuron 4 ng; 7 = atrazine 2 ng; 8 = monolinuron 4 ng; 9 = metobromuron 10 ng; 10 = diuron 10 ng; 11 = linuron 10 ng.

determination was considerably reduced and the sensitivity was higher. The cyano column is to be preferred to the C_{18} column in analyses of triazine and urea herbicides residues, especially at low concentrations (Figs. 1 and 2).

The accuracy and precision of the method have been evaluated at two fortification levels —five and twenty times the limits of determination (LOD) of the herbicides. Drinking water samples of 1 l were fortified with known quantities of the standard solutions and quantitative results were obtained (Table I). Since the LODs of the analytes varied to a large extent, recovery studies at a concentration of 0.1 μg/l were carried out for all herbicides to verify the suitability of the method for monitoring compliance with the European Community drinking water directive. The results shown in Table I prove that the method can be used to detect many of the herbicides at concentrations below the maximum admissible concentration for pesticides in drinking water. For metobromuron, diuron and linuron at this concentration, better results were obtained on the cyano column.

CONCLUSIONS

A simple multiresidue method has been developed for the analysis of herbicides belonging to different classes in drinking waters. Acidic, neutral and weak basic compounds are determined simultaneously in a single HPLC run, which saves apparatus time. Application of two HPLC columns for confirmation of positive identification of neutral and basic herbicides increases the reliability of determination, avoiding the use of expensive techniques.

The enrichment factor is about 2000. The sensitivity of the method is sufficient to achieve quantitative determination at or below 0.1 μg/l for each of the herbicides.

The method is also directly applicable to sample preparation automation. For its simplicity, reliability and usage of generally applied instrumentation this method is suitable for pesticide monitoring of waters.

REFERENCES

1 S. A. Schuette, R. Smith, L. Holden and J. Graham, *Anal. Chim. Acta*, 236 (1990) 141.
2 E. Boligo and N. C. Atreya, *Fresenius' Z. Anal. Chem.*, 339 (1991) 432.
3 In Suk Kim, F. Sasinos, R. Stephens, J. Wang and M. Broun, *Anal. Chem.*, 63 (1991) 819.
4 R. Reupert and E. Plöger, *Fresenius' Z. Anal. Chem.*, 331 (1988) 503.
5 R. Reupert and E. Plöger, *Vom Wasser*, 72 (1989) 211.
6 E. A. Hogendoorn, C. Goewie and P. van Zoonen, *Fresenius' Z. Anal. Chem.*, 339 (1991) 348.
7 P. Cabras, P. Diana, M. Meloni and F. Pirisi, *J. Chromatogr.*, 234 (1982) 249.
8 A. Di Corcia and M. Marchetti, *J. Chromatogr.*, 541 (1991) 365.
9 E. G. Cotterill, T. H. Byast, in J. F. Lawrence (Editor), *Liquid Chromatography Environmental Analysis*, Clifton, New York, 1984, p. 77.
10 M. J. M. Wells and J. L. Michael, *J. Chromatogr. Sci.*, 25 (1987) 345.
11 A. Di Corcia and M. Marahetti, *Anal. Chem.*, 63 (1991) 580.
12 A. Di Corcia and M. Marchetti, *Environ. Sci. Technol.*, 26 (1992) 66.
13 J. Legler, presented at the *7th International Congress of Pesticide Chemistry, Hamburg, August 5–10, 1990*, Vol. III, p. 217.