Simultaneous Determination of Sixteen Phenylurea Herbicides in Water by High Performance Liquid Chromatography and Solid Phase Extraction

Fangshi LI[1,2], Dieter MARTENS[2], Antonius KETTRUP[2]

(*1. Department of Applied Chemistry, Nanjing University of Technology, Nanjing 210009, China;*
2. Institute of Ecological Chemistry, GSF-National Research Centre for Environment and Health, Ingolstaedter Landstrasse 1, 85764 Neuherberg, Germany)

Abstract: A high performance liquid chromatography (HPLC) procedure for simultaneous determination of sixteen phenylurea herbicides in water is described. A Lichrospher 100 RP-18e column and spectrophotometric detection at 240 nm were used. Adequate retention was achieved with a mobile phase of acetonitrile/water solution with gradient elution. The HPLC analysis time was less than 20 min. The herbicides were isolated from water samples by using a single solid phase extraction procedure with C_{18} solid-phase columns. An enrichment factor of 1 000 was achieved. Recoveries were in the range between 87.8% and 103.7%. The detection limits of the whole procedure achieved were less than one-tenth of the maximum limit permitted by the European Community standard for drinking water.

Key words: high performance liquid chromatography; solid phase extraction; phenylurea herbicide; water

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1 Introduction

Phenylureas are selective herbicides widely used for agricultural purpose both in Europe and in China. The leaking of these substances from the soil into local ground water is a common phenomenon. Under environmental conditions phenylureas can persist at the mg/L level in ground water for a number of days or weeks depending on the temperature and pH[1]. These substances are highly toxic for mammalians. If such ground water is to be used as the source of drinking water, it is necessary to screen them. The European Union gives 0.1 μg/L as the admissible concentrations of any individual herbicide in drinking water[2]. Different analytical procedures for determining phenylurea herbicides in aqueous samples have been proposed, mostly gas chromatography (GC)[3] and liquid chromatography (LC)[4,5]. Because most of these compounds are thermally labile, thermal degradation products are often detected by GC instead of the molecular herbicides. In LC, the pesticides in question can be analyzed without the risk of thermal degradation, however, due to the low resolution of LC compared to GC, complete separation of all solutes is often difficult to achieve[6].

This paper describes a simple, sensitive, and rapid procedure for simultaneously determining residues of sixteen phenylureas in drinking water after preconcentration of the samples on C_{18} solid-phase column. The compounds were separated on a reversed-phase column by using a solvent gradient. The results obtained indicate that the proposed method is well suitable for monitoring phenylureas in compliance with the European Community standard for drinking water.

2 Experimental

2.1 Apparatus

Perkin Elmer 200 LC pump, sample processor ISS 200, diode array detector 235C and Turbochrom 4 acquisition system were used. Uniflows Degasys DG-1310, Mistral Thermal and Lichrospher 100 RP-18e

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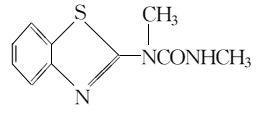
Biography: Fangshi LI (1956-), male, professor, Ph. D. of chemistry, Tel/Fax: (025)3240556, E-mail: njfangli@jlonline.com.

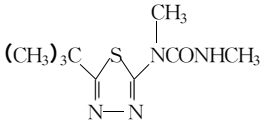
column (5 μm, 250 mm × 4 mm i.d.) with precolumn (4 mm × 4 mm i.d.) were employed. Solid phase extraction (SPE) vacuum station Vac Elut 20 and Chromabond HP-P/3 mL/200 mg solid phase extraction cartridges were adopted. A mobile phase of acetonitrile/water solution was used. The gradient was: 30% acetonitrile reached 50% at 10 min after the injection and then reached 100% at 20 min. The column was thermostated at 20 ℃. Flow rate was 1 mL/min. Injection volume was 10 μL. Detection was at 240 nm.

2.2 Reagents and standards

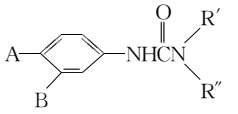
Acetonitrile (HPLC grade) and phenylurea herbicide standards (94%-99% purity) were from Riedel-de-Haen (Seelze, Germany). Milli-Q water (180 kΩ·m) was used throughout. Stock solutions of individual herbicides were prepared with methanol at 1.0 g/L level and stored at 4 ℃. Dilute solutions for analysis were prepared daily with Milli-Q water. Fig.1-a, b, c shows the names and structures of the phenylurea herbicides studied. The groups of A, B, R′ and R″ in Fig.1-c were listed in Table 1.

a 

Metabenzthiazuron (MT)

b 

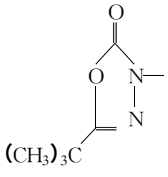
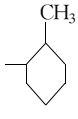
Tebuthiuron (TE)

c 

General structure of phenylurea

Fig.1 Structures of the phenylurea herbicides studied

Table 1 Groups of A, B, R′ and R″ in Fig.1-c

Herbicide	Symbol	A	B	R′	R″
Fenuron	FE	H	H	CH_3	CH_3
Metoxuron	MX	OCH_3	Cl	CH_3	CH_3
Thidiazuron	TH	H	H	H	
Monuron	MO	Cl	H	CH_3	CH_3
Chlortoluron	CL	CH_3	Cl	CH_3	CH_3
Fluometuron	FL	H	CF_3	CH_3	CH_3
Isoproturon	IP	$(CH_3)_2CH$	H	CH_3	CH_3
Diuron	DI	Cl	Cl	CH_3	CH_3
Metobromuron	MT	Br	H	CH_3	OCH_3
Dimefuron	DM		Cl	CH_3	CH_3
Siduron	SD	H	H	H	
Linuron	LI	Cl	Cl	CH_3	OCH_3
Chlorbromuron	CB	Br	Cl	CH_3	OCH_3
Neburon	NB	Cl	Cl	C_4H_9	CH_3

2.3 Solid phase extraction procedure

Two column volumes of methanol and then one column volume of distilled water were used for the column conditioning. One liter of water sample was aspirated through the column. The column was dried in a stream of nitrogen. Elution was carried out with 3 times of 1 mL methanol/acetonitrile (1:1, *V*/*V*) or methanol/acetone (3:2, *V*/*V*) and then the eluate was evaporated with nitrogen to 1 mL or diluted to the volume required.

3 Results and discussion

Lichrospher 100 RP-18e column showed good performance in separating the 16 phenylurea herbicides.

Reproducible elution with symmetrical peaks was obtained. Fig.2-a shows the chromatogram of a 10 μL standard solution containing 10 mg/L of each herbicides with the exception of MT (6.0 mg/L). No overlapping between the analyte peaks was observed under the set instrumental conditions. SD showed a double peak chromatographic pattern due to the presence of two isomers. The total analysis time was less than 20 min. Detector response was directly proportional to the standard concentration (10 μL sample injection). The calibration curves deduced from five tests were linear in the tested range from 0.1 mg/L to 10 mg/L. The coefficients of regression (r^2) were higher than 0.99 for all the pesticides. The limits of detection (LOD, 3 σ) for all the pesticides without the SPE preconcentration were 0.008 3 mg/L-0.023 4 mg/L (Table 2).

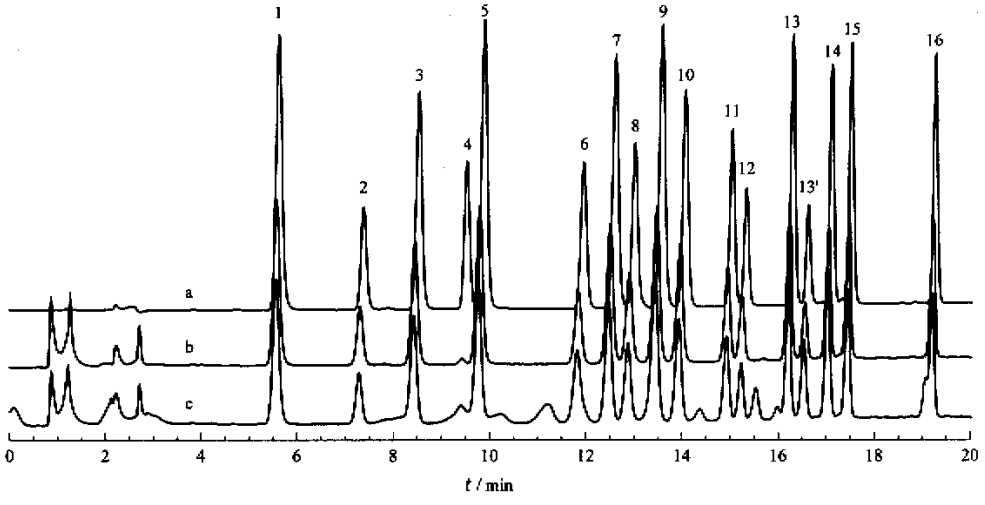


Fig.2 Chromatograms of the phenylurea herbicides

Flow rate: 1 mL/min. Detection wavelength: 240 nm.

a. standards (10 mg/L each, except 6.0 mg/L of MT); b, c. solid phase extract of the spiked water (5 μg/L each, except 3.0 μg/L of MT), eluted with methanol/acetonitrile (1:1, *V*/*V*) for b or with methanol/acetone (3:2, *V*/*V*) for c.

Peak identification: 1. FE; 2. TE; 3. MX; 4. TH; 5. MO; 6. MT; 7. CL; 8. FL; 9. IP; 10. DI; 11. MT; 12. DM; 13/13′. SD; 14. LI; 15. CB; 16. NB.

Table 2 Results of HPLC for herbicides in standard solution (0.1 mg/L-10 mg/L) and SPE-HPLC for spiked water (0.02 μg/L-0.20 μg/L)

Herbicide	t_R (min)	HPLC		SPE-HPLC			
		r^2	LOD (μg/L)	r^2	LOD (ng/L)	recovery[1] (%)	recovery[2] (%)
FE	5.61	0.9970	8.7	0.9989	3.36	102.7	92.2
TE	7.38	0.9957	23.4	0.9911	4.35	102.4	87.3
MX	8.52	0.9995	11.0	0.9911	5.46	103.7	98.3
TH	9.52	0.9947	16.2	-	-	-	-
MO	9.88	0.9944	8.3	0.9985	4.10	99.2	85.1
MT	11.94	0.9927	16.4	0.9965	3.80	87.8	87.7
CL	12.60	0.9926	9.4	0.9956	6.69	98.7	83.5
FL	13.01	0.9928	14.6	0.9843	4.48	98.4	83.8
IP	13.57	0.9927	8.5	0.9943	6.37	98.6	89.7
DI	14.06	0.9928	11.0	0.9958	6.09	98.4	83.1
MT	15.03	0.9990	8.1	0.9936	5.77	101.8	96.3
DM	15.33	0.9944	20.4	0.9912	7.01	97.6	84.3
SD	16.29/16.61	0.9941	8.8	0.9912	6.30	102.1	87.5
LI	17.10	0.9945	10.1	0.9969	3.10	99.4	101.3
CB	17.51	0.9947	9.2	0.9955	4.18	99.5	85.4
NB	19.25	0.9946	9.6	0.9948	3.59	98.9	87.5

1) Elution with methanol/acetonitrile (1:1, *V*/*V*); 2) elution with methanol/acetone (3:2, *V*/*V*).

In considering the LOD, the analytes should be extracted from a relatively large volume of sample water (1 L) and eluted with a small volume of eluent (1.0 mL). Under this condition, an enrichment factor of 1 000 was achieved, assuring the procedure suitable for determining the phenylurea herbicides in drinking water at a level lower than the admissible concentrations of the European Union. The analytical recovery from spiked tap water was high for all the components except TH, ranging 87.8%-103.7% by elution with methanol/acetonitrile (1:1, *V*/*V*) or 83.1%-101.3% by elution with methanol/acetone (3:2, *V*/*V*) (see Table 2). The reason that TH could not be extracted is under investigation. It may degrade during the extraction. The blank did not show any interference with the analyte peaks (Fig. 2-b, Fig. 2-c). As a result, the use of an internal standard was not taken into consideration. The elution with methanol/acetonitrile was preferred to that with methanol/acetone because the former gave a better base line.

The calibration curves of the phenylurea herbicides in the spiked water (0.02 μg/L, 0.05 μg/L, 0.08 μg/L, 0.11 μg/L, 0.14 μg/L, 0.17 μg/L and 0.20 μg/L) after SPE eluted with methanol/acetonitrile were linear (for the r^2 of them see Table 2). The determination limits were less than one-tenth of the maximum limit (0.1 μg/L) permitted by the European Union.

The results shown above indicate that the proposed method could be considered sufficiently sensitive and reliable for the routine analysis of phenylurea herbicides in drinking water.

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固相萃取-高效液相色谱法同时测定水中的 16 种苯脲除草剂

李方实[1,2], Dieter MARTENS[2], Antonius KETTRUP[2]

(1. 南京工业大学应用化学系, 江苏 南京 210009; 2. Institute of Ecological Chemistry, GSF-National Research Centre for Environment and Health, Ingolstaedter Landstrasse 1, 85764 Neuherberg, Germany)

摘要 建立了固相萃取-高效液相色谱(SPE-HPLC)同时测定水中 16 种苯脲除草剂的方法。HPLC 采用 Lichrospher 100 RP-18e 柱,紫外检测波长为 240 nm,流动相为乙腈水溶液,流速为 1 mL/min,采用梯度洗脱方式。HPLC 分析时间少于 20 min。水中的除草剂用 C_{18} 柱固相萃取富集 1 000 倍。在优化的条件下,各成分的添加回收率为 87.8%～103.7%。此方法的检测限低于欧盟允许的水中除草剂含量上限的 1/10。

关键词 高效液相色谱 固相萃取 苯脲除草剂 水