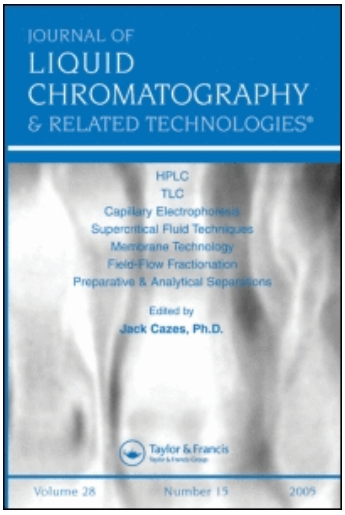

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J. M. Sanchis-Mallols[a]; S. Sagrado[a]; M. J. Medina-Hernández[a]; R. M. Villanueva Camañas[a]; E. Bonet-Domingo[b]

[a] Departamento de Quimica Analítica, Facultad de Farmacia Universidad de Valencia. C/Vicente A, València, Spain [b] General de Análisis Materiales y Servicios (GAMASER), S.L. València, Spain

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DETERMINATION OF PHENYLUREA HERBICIDES IN DRINKING WATERS BY HPLC AND SOLID PHASE EXTRACTION

J. M. Sanchis-Mallols,[1] S. Sagrado,[1] M. J. Medina-Hernández,[1,*]
R. M. Villanueva Camañas,[1] E. Bonet-Domingo[2]

[1] Departamento de Química Analítica.
Facultad de Farmacia
Universidad de Valencia.
C/ Vicente A. Estellés s/n
E-46100 Burjassot, València (Spain)

[2] General de Análisis Materiales y Servicios (GAMASER) S.L.
València (Spain)

ABSTRACT

An HPLC procedure for determining phenylurea herbicides in waters is described. A LichroSpher RP select B octadecyl-silane analytical column and spectrophotometric detection at 247 nm were used. Adequate retention was achieved with a mobile phase containing ACN/H_2O 35/55 (v/v) and 10^{-2} M phosphate (pH = 7). 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 333 is achieved. The coefficients of variation of the method were lower than 8% at 3 $\mu g\ L^{-1}$ herbicides concentration level. Recoveries ranged between 93 and 105%. The results obtained indicate that the proposed method is well suitable for monitoring phenylureas in compliance with the European Community standard for drinking water.

INTRODUCTION

Substituted phenylureas are selective herbicides used extensively in agriculture. The leaking of these substances from the soil into local ground water is a common phenomenon. Phenylureas can persist under environmental conditions at the mg L^{-1} level in the aquatic environment for a number of days or weeks depending on temperature and pH.[1]

These substances are highly toxic for mammalian, therefore, if such ground waters are to be used as sources of drinking water, it is necessary to screen them. The high standards for drinking water purity laid down by the European Union give 0.1 μg L^{-1} as the admissible concentrations of any individual herbicide.

Different analytical procedures for determining phenylurea herbicides in aqueous samples have been proposed, mostly gas chromatography[2,3] and liquid chromatography.[4-17] However, the polar nature of such compounds, their termolability and low vapor pressure, make difficult the direct analysis by gas chromatography; consequently, prior to chromatography, steps such as hydrolysis and/or derivatization are required.[2,3]

High-performance liquid chromatography (HPLC) often allows the development of sensitive analytical procedures for the determination of organic compounds in water without derivatization.

In the literature, both -normal and reversed-phase- separations with UV at 240-250 nm,[4-11] electrochemical,[12-14] or mass spectrometry detection[15-17] have been reported.

Reversed-phase separations of phenylurea herbicides usually requires the use of complicated gradient concentrations of the organic solvents in order to achieve enough resolution and adequate time of analysis.

This paper describes a new simple, sensitive, and rapid procedure for determining residues of twelve phenylureas in drinking waters after preconcentration of the samples on C_{18} solid-phase columns. The compounds were separated on a reversed-phase column by using a flow rate 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.

MATERIALS AND METHODS

Apparatus

A Hewlett-Packard HP 1050 chromatograph with a isocratic pump, a UV-visible detector, and an HP 3396A integrator was used (Palo Alto, CA, USA). Data acquisition was made with the Peak-96 software from Hewlett-Packard (Avondale, PA, USA). The solutions were injected into the chromatograph through a Rheodyne valve (Cotati, CA, USA) with a 20 μL loop. A LichroSpher RP select B octadecyl-silane column (5 μm, 250 x 4 mm) and a guard column of similar characteristics (30 x 4 mm) (Scharlau, Barcelona, Spain) were used. The mobile phase flow rate varied between 1 and 1.5 mL min^{-1} in linear gradient for 50 min and it remained at 1.5 mL min^{-1} from now on. The detection was performed in UV at 245 nm. All the assays were carried out at room temperature.

A solid phase extraction vacuum station Vac Elut 20 (Varian Sample Preparation products, Harbor City, CA, USA) was used.

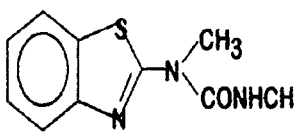
Reagents and Standards

Mobile phases were prepared by mixing 0.01 M phosphate buffer solution (pH 7) and acetonitrile, ACN, (analytical reagent grade, Scharlau, Barcelona, Spain) to obtain the working concentration. A ratio 65/35 (phosphate buffer/acetonitrile, v/v) was recommended. The phosphate buffer was prepared with disodium hydrogen phosphate and phosphoric acid (analytical reagent, Panreac, Barcelona, Spain).

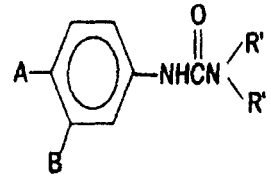
Stock standard solutions of the herbicides fenuron (FE), metoxuron (MX), monuron (MO), chlortoluron (CL), fluometuron (FL), metabenzthiazuron (MZ), isoproturon (IP), diuron (DI), chloroxuron (CX), chlorbromuron (CB), buturon (BU), and neburon (NB) (99.3 %, Dr Ehrenstorfer GmbH, Augsburg, Germany) were prepared in acetonitrile (10 mg L^{-1}) and stored at -18°C in the dark. Working solutions were prepared in acetonitrile by dilution of the stock standard solutions. Table 1 shows the structure and the logP values of the phenylurea herbicides studied. The logP values for the compounds were calculated using the ACD-logP software.[18] Bond Elut C18 3CC/500MG solid phase extraction columns (Varian Sample Preparation products, Harbor City, CA, USA) were used.

Table 1

Structure of the Phenylureas Studies



Metabenzthiazuron



General structure of phenylureas

Herbicide	Symbol	A	B	R'	R"	log P
Fenuron	FE	H	H	CH_3	CH_3	0.98
Metoxuron	MX	OCH_3	Cl	CH_3	CH_3	1.92
Monuron	MO	Cl	H	CH_3	CH_3	1.89
Chlortoluron	CL	CH_3	Cl	CH_3	CH_3	2.46
Fluometuron	FL	H	CF_3	CH_3	CH_3	2.36
Isoproturon	IP	$(CH_3)_2CH$	H	CH_3	CH_3	2.32
Diuron	DI	Cl	Cl	CH_3	CH_3	2.78
Chloroxuron	CX	4-Cl-C_6H_4O	H	CH_3	CH_3	3.84
Chlorbromuron	CB	Br	Cl	CH_3	OCH_3	3.37
Buturon	BU	Cl	H	CH_3	$CH(CH_3)C{=}CH$	2.61
Neburon	NB	Cl	Cl	C_4H_9	CH_3	4.38

Barnstead E-pure, deionized water (Sybron, Boston, MA, USA) was used throughout. The mobile phase and the solutions injected into the chromatograph were vacuum-filtered through 0.45 μm and 0.22 μm Nylon membranes, respectively (Micron Separations, Westboro, MA, USA).

Sample Preparation

SPE columns were conditioned by washing with 4 mL of MeOH/ACN 70/30 (v/v) mixture and 6 mL of deionized water. 500 mL of water sample were forced through the C_{18} SPE column using vacuum at a flow rate of 9 mL min^{-1}. Phenylurea herbicides were eluted using 1.5 mL of pure acetonitrile.

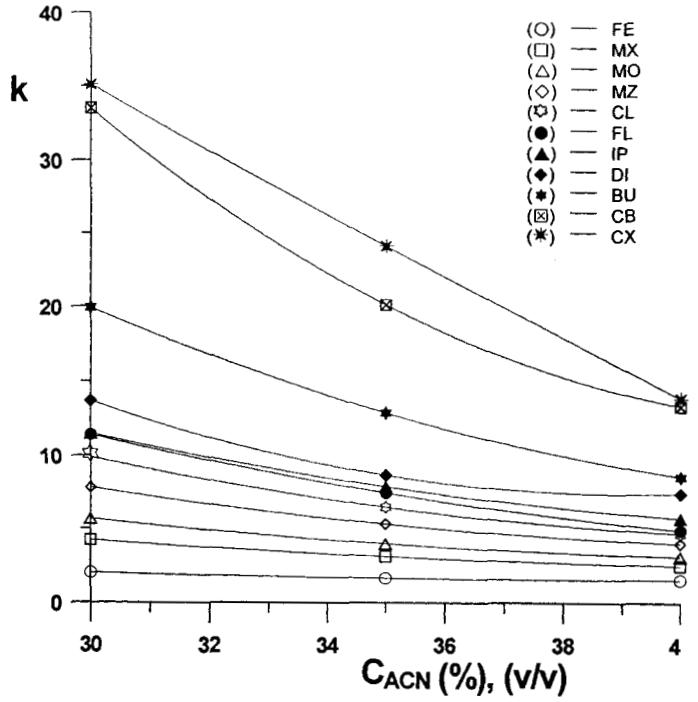


Figure 1. Effect of the acetonitrile concentration in the mobile phase on the retention of the phenylurea herbicides. 0.01 M phosphate buffer at pH 7 was used throughout.

RESULTS

Chromatographic Conditions

A study to select the adequate composition of the mobile phase (pH, and acetonitrile concentration) was performed. Figure 1 shows the effect of the acetonitrile mobile phase concentration on the retention of compounds. As can be observed, for the highly hydrophobic compounds studied (CX,. CB and NB), large changes in the retention were obtained upon increasing the acetonitrile concentration in the mobile phase, while for the slightly hydrophobic compounds (FE and MX) the retention was scarcely modified. This behaviour indicates that the eluent strength of the acetonitrile increases as the hydrophobicity of the compounds increases.

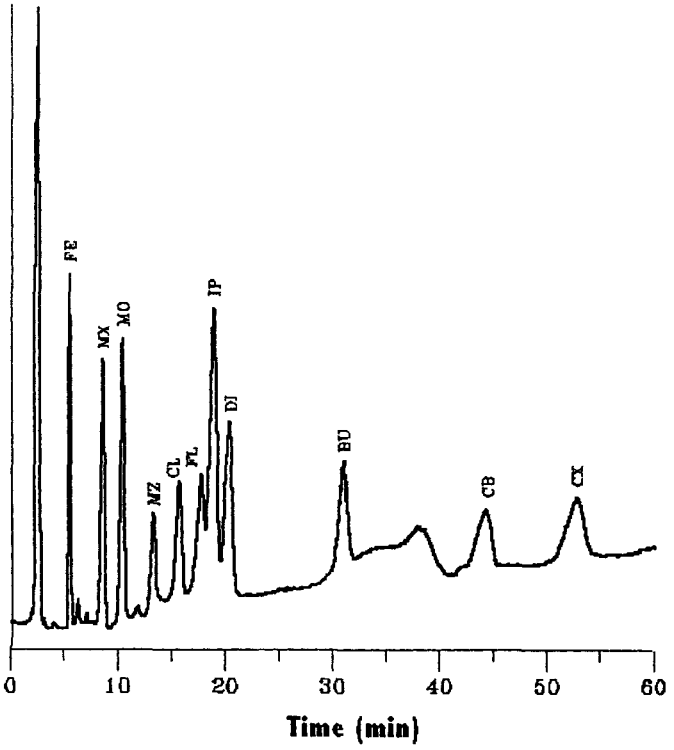


Figure 2. Chromatogram corresponding to a solution containing 1.25 mg L^{-1} of each phenylurea herbicides. Mobile phase: ACN/phosphate buffer 35/65 (v/v), pH 7. Flow rate 1 mL min^{-1}. Wavelength 245 nm.

No significative changes in the retention factors of compounds were observed as the mobile phase pH was modified in the range 3-7 at a fixed acetonitrile concentration. On the other hand, larger retention factors of compounds were obtained when unbuffered mobile phases were used. This phenomenon was attributed to the influence of the ionic strength on the retention. A mobile phase composition of ACN / phosphate buffer (pH 7) 35/65 (v/v) was selected. Higher concentrations of acetonitrile produced important overlapping in the peaks of the phenylureas. Figure 2 shows the chromatogram corresponding to a solution containing 1.25 mg L^{-1} of each compound using this mobile phase. As can be observed, the separation of the peaks of the phenylureas was adequate for this mobile phase, but the retention times for the most hydrophobic herbicides were too high (44, 53 and >90 min

Table 2

Regression Statistics for the Calibration Curves of the Phenylureas Studied

	Area = $aC_{ppm} + b$				
Compound	**$a \pm ts_a$**	**$b \pm ts_b$**	**r^2**	**F**	**SE**
FE	53.9 ± 1.1	-0.5 ± 0.4	0.99990	26415	0.10
MO	72 ± 3	-1.3 ± 0.9	0.9996	4967	0.3
MZ	23 ± 4	0 ± 1	0.993	405	0.4
FL	41 ± 4	0 ± 1	0.998	1224	0.4
DI	60 ± 8	1 ± 3	0.9994	540	0.8
CB	50 ± 19	-2 ± 5	0.984	123	1.0
CX	50 ± 8	-1 ± 3	0.991	346	0.8
NB	28 ± 6	0 ± 2	0.990	228	0.6
MX	56 ± 4	-0.4 ± 1.2	0.9990	2626	0.3
CL	51 ± 6	0.8 ± 1.9	0.996	833	0.6
IP	54 ± 12	-1 ± 4	0.990	215	1.2
BU	53 ± 5	-0.9 ± 1.5	0.998	1432	0.5

for CB, CX and NB, respectively). In order to reduce the time of analysis, maintaining an adequate resolution, a flow rate gradient was assayed. The mobile phase flow rate varied between 1 and 1.5 mL min^{-1} in linear gradient for 50 min; it remained unchanged from then on. The retention times for the most hydrophobic compounds, CB, CX and NB were, in these conditions 41, 47, and 78 min, respectively.

Analytical Data

The calibration curves of each compound were obtained by triplicate injections of standard solutions containing different concentrations of the analytes in the 0.1-0.5 mg L^{-1} range. Peak area was used as dependent variable. Table 2 shows regression statistics for the calibration curves of each compound.

Except for CB, linear relationships were obtained in the working interval. For this compound the linear range was 0.1-0.4 mg L^{-1}. In all cases, the calibration curves showed adequate regression coefficients and significant levels. In addition, for all compounds the intercept values were significantly equal to zero (95% probability level).

Table 3

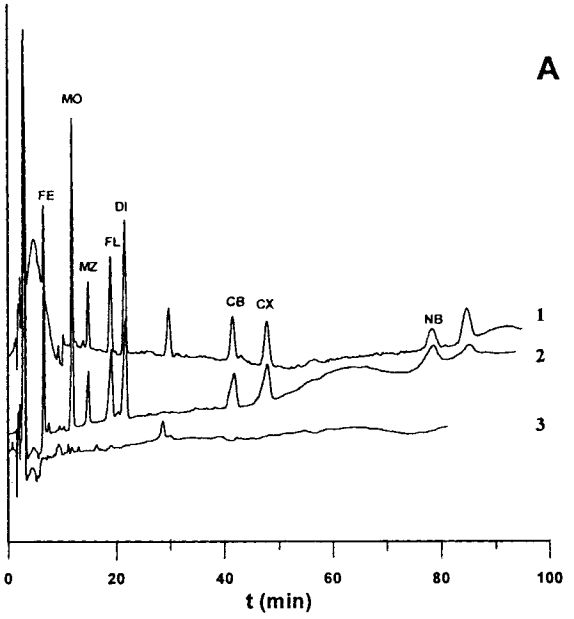
Repeatability and Limits of Detection of the Phenylureas

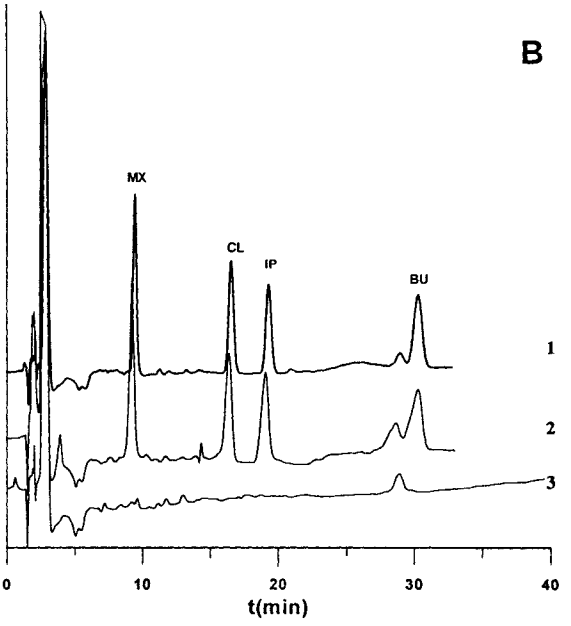
Compound	Repeatability C.V. (%), n - 5	LOD ($\mu g l^{-1}$)
FE	1.8	5.4
MO	1.1	3.8
MZ	2.8	12.4
FL	1.5	5.3
DI	1.4	5.2
CB	3.1	6.6
CX	1.7	4.5
NB	6.7	43.6
MX	4.6	13.8
CL	4.7	14.0
IP	3.5	9.7
BU	5.3	15.6

The repeatability was evaluated from series of five injections of standard solutions of phenylurea herbicides in concentration 0.1 mg L^{-1}. Table 3 shows the coefficient of variation values (CV) found for each compound. As can be observed, the CV values were ranged between 1 to 5.4% except for NB, the most retained compound, which showed a relative standard deviation value of 12.6%.

Limits of detection (LODs) were calculated from the standard deviation corresponding to five-fold injections of 100 $\mu g\ L^{-1}$ solutions of each herbicide (3σ criterium). The LODs values for each herbicide are shown in Table 3 and they were ranged between 3.8 and 15.6 $\mu g\ L^{-1}$, except for NB, which showed a limit of detection of 44 $\mu g\ L^{-1}$. These values indicate that a preliminary preconcentration step is necessary in order to achieve sufficiently high enrichment factors, which enable phenylurea herbicides to be monitored in drinking waters samples at 0.1 $\mu g\ L^{-1}$ level.

Figure 3. (right) Chromatograms corresponding to: 1.- mixtures A (upper part) and B (lower part) of phenylurea herbicides after preconcentration of solutions containing 3 (g l-1 of each compound; 2.- mixtures A (upper part) and B (lower part) of phenylurea herbicides solutions containing 1 mg l-1 of each compound; 3.- E-pure water after preconcentration step. See text for experimental conditions.





Sample Preconcentration

Owing the limits of detection obtained (Table 3), the analytes should be extracted from a relatively large volume of sample water (500 mL was assayed) and eluted with a small volume of eluent (1.5 mL was assayed). In these conditions, an enrichment factor of 333 is achieved, that assures the procedure is suitable for determining phenylurea herbicides in drinking water at lower concentration levels than the admissible concentrations by the European Union.

The ability of the Bond Elut C_{18} 3CC/500MG cartridges to retain quantitatively phenylureas was evaluated. To carry out these studies, two spiked E-pure water samples containing a concentration of 3 $\mu g\ L^{-1}$ of each herbicide were prepared. The sample A contained the herbicides FE, MO, MZ, FL, DI, CB, CX, and NB, and the sample B contained the herbicides MX, CL, IP, and BU. Phenylureas were extracted from 500 mL of the spiked water sample according to proposed procedure (experimental section). Acetonitrile and methanol was assayed as eluents. The use of methanol produced larger noise in the initial part of the chromatogram which made the determination of fenuron impossible. Phenylurea herbicides were adequately eluted using 1.5 mL of pure ACN and the extracts did not cause such problems.

The recovery achieved for each phenylurea herbicides after the preconcentration step was determined. The recovery values were obtained by comparing the peak areas corresponding to the extracts with those obtained by direct injection of standard solutions containing 1 mg L^{-1} of each herbicide (1 mg L^{-1} is the concentration of the herbicides in the eluates supposing a recovery equal to 100 %). Figure 3 shows the chromatograms corresponding to samples A and B of phenylurea herbicides after the preconcentration step together with the corresponding to the standard solutions containing 1 mg L^{-1} of each herbicide. The chromatogram corresponding to the blank of E-pure water after the preconcentration step is also included.

Table 4 shows the recovery values and the reproducibility (CV) obtained for each phenylurea herbicide corresponding to five independent analysis. The recovery values were ranged between 93 and 105%, except for BU and FE (150 and 32.6%, respectively). The high recovery value and low reproducibility obtained for BU could be due to the difficulty to determine the exact area of the BU peak since close to the BU peak another anomalous signal perturbations appeared. These perturbations may be due to change in the refractive index or to the presence of unidentified compounds which appear at similar retention times to BU. Fenuron, the least retained compound, showed a low recovery value due to its low affinity to the C_{18} stationary phase used in the SPE step.

Table 4

Recovery of Phenylureas from C_{18} Columns and Reproducibility of the Proposed Method

Compound	Recovery (%)	Reproducibility, C.V. (%), n=5
FE	32.6	3.1
MO	98	3.6
MZ	98	3.4
FL	99	5.6
DI	99	2.7
CB	93	4.7
CX	95	5.3
NB	99	5.4
MX	100	5.3
CL	103	6.6
IP	105	7.0
BU	150	7.7

The uncertainty of the method, including preconcentration and chromatographic analysis steps was evaluated. The method showed adequate reproducibility; the values of the coefficients of variation obtained were ranged between 2.6 to 7.7 % (Table 4). By comparing the variance values corresponding to the chromatographic analysis step (ranged between $4.0x10^{-6}$ and $1.8x10^{-3}$; average = $8.4x10^{-4}$) with the corresponding to the global procedure (ranged between $2.6x10^{-4}$ and $5.8x10^{-3}$, average = $21x10^{-4}$) it can be concluded that the uncertainty associated to the preconcentration step was, in general, the same order of that of the chromatographic analysis step. The results shown above indicate that the proposed method is adequate for determining phenylurea herbicides in drinking waters and well suitable for monitoring these compounds in compliance with the European Community standard for drinking water.

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