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Determination of herbicides and metabolites by solid-phase extraction and liquid chromatography Evaluation of pollution due to herbicides in surface and groundwaters ☆

Rita Carabias-Martínez[a,*], Encarnación Rodríguez-Gonzalo[a], Eliseo Herrero-Hernández[a], Francisco Javier Sánchez-San Román[b], M. Guadalupe Prado Flores[a,1]

[a]*Departamento de Química Analítica, Nutrición y Bromatología, Facultad de Química, Universidad de Salamanca, Plaza de la Merced s/n, 37008 Salamanca, Spain*

[b]*Departamento de Geología, Facultad de Geología, Universidad de Salamanca, 37008 Salamanca, Spain*

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Abstract

A procedure based on solid-phase extraction (SPE) has been developed for the simultaneous preconcentration of three widely used herbicides and seven of their most common degradation products. The compounds studied were atrazine and its metabolites, desethylatrazine, desethyldesisopropylatrazine (DEDIA), 2-hydroxyatrazine, desethyl-2-hydroxyatrazine and desisopropyl-2-hydroxyatrazine (DIHA), terbutryne and its metabolite 2-hydroxyterbutylazine, and chlorotoluron and its metabolite 3-chloro-4-methylphenylurea. A HPLC system with diode array detection was used for the separation, identification and quantification of all these analytes. In the SPE preconcentration step, different types of sorbent were studied: C_{18} on silica and polymeric sorbents (Oasis and LiChrolut EN), the best results being obtained with the styrene–divinylbenzene cartridge and when the elution was performed with methanol and ethyl acetate. The detection limits obtained were between 0.1 $\mu g\ l^{-1}$ for DIHA and DEDIA and 0.02 $\mu g\ l^{-1}$ for the other analytes. The method used permitted the determination of these herbicides in drinking water at the concentration levels demanded by current legislation. The proposed method was used to evaluate the presence and evolution with time of these herbicides and their degradation products in samples of surface and ground waters from agricultural zones of the provinces of Salamanca and Zamora (basins of the Rivers Guareña and Almar), Spain.

Keywords: Water analysis; Environmental analysis; Pesticides

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E-mail address: rcm@gugu.usal.es (R. Carabias-Martínez).

*Corresponding author. Tel.: +34-923-294-483; fax: +34-923-294-574.

[1]Permanent address: Departamento de Producción Agrícola y Animal, División de Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana-Xochimilco, 04960 Ciudad de México, Mexico.

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1. Introduction

Herbicides represent 50% of the demand for agricultural chemicals and, within them, the triazine derivatives and those of the phenyl ureas are the ones most commonly used in Europe to combat weeds. These herbicides are widely used as selective pre- and post-emergence herbicides for the control of broadleaf and grassy weeds in many agricultural crops. Accordingly, their prolonged use may lead to pollution of surface and ground waters by the herbicides themselves and their metabolites [1]. In previous work [2,3] evaluating these herbicides in an agricultural zone close to the city of Salamanca (Spain), we observed that surface and ground water samples contained levels of atrazine, terbutryne and chlorotoluron above 0.1 $\mu g\ l^{-1}$.

In water and soil, parent s-triazine herbicides are subjected to various biotic and abiotic degradation processes such as photolysis, oxidation, hydrolysis and biodegradation, leading to dealkylation of the amine groups, dechlorination, hydroxylation, deamination, etc. [4,5]. The main degradation products in water are the dealkylated chloro metabolites: desethyldesisopropylatrazine (DEDIA) and, mainly, desethylatrazine (DEA) [6,7]. In soils, the main metabolites found are 2-hydroxyatrazine (HA), 2-hydroxyterbutylazine (HT), desethylhydroxyatrazine (DEHA), and desisopropylhydroxyatrazine (DIHA). It has also been shown that the hydroxylated degradation products (HA, HT, DEHA and DIHA) are more persistent in soils than DEA since they are adsorbed by the organic matter of the soil. Despite its low solubility in water, HA may be present in ground water owing to a leaching process [8,9].

In the case of the herbicide chlorotoluron, the main reported metabolites are *N*-(3-chloro-4-methylphenyl)urea (CMPU) and 3-chloro-4-methylaniline [9].

The extraction and preconcentration of atrazine, terbutryne and chlorotoluron by solid-phase extraction (SPE) has been successfully achieved by different authors using C_{18} sorbents [10,11]. However, it has been shown that this sorbent is not very suitable for the extraction and preconcentration of the degradation products of triazines [12,13]. For these metabolites, other sorbents have been proposed, such as graphitised carbon [14,15], strong cation-exchange (SCX) propylbenzenesulfonic acid cartridges [16], strong anion-exchange (SAX) disks based on a styrene–divinylbenzene matrix combined with C_{18} disks [17], sorbents containing mixtures of styrene–divinylbenzene and metacrylate macroporous resins [18] and polymeric materials of styrene–divinylbenzene [11,19–22]. In comparison with silica-based sorbents, most polymeric ones have a hydrophobic structure and a different particle size and degree of crosslinking. Hennion and Pichon [23] have shown that they have a good capacity for preconcentrating polar analytes.

In the present work, we developed a method employing SPE for the simultaneous preconcentration of three widely used herbicides and seven of their most common degradation products. The compounds studied were as follows: atrazine and its metabolites DEA, DEDIA, HA, DEHA and DIHA; terbutryne and its metabolite HT, and chlorotoluron and its metabolite CMPU. A high-performance liquid chromatography (HPLC) system with diode array detection (DAD) was used for the separation, quantification and identification of these analytes.

In the preconcentration step, a comparative study was made of the efficiency of C_{18} and polymeric sorbents. Within the latter, sorbents studied were Oasis HLB, a co-polymer of poly(divinylbenzene–co-*N*-vinylpyrrolidone) which exhibits both hydrophilic and lipophilic retention characteristics, and LiChrolut EN polymeric cartridge, a hydrophobic polymer of styrene–divinylbenzene. Choice of the latter was based on the fact that styrene–divinylbenzene polymers display a very large accessible surface area (1200 $m^2\ g^{-1}$) [24].

Finally, the method was applied to the determination of these herbicides and their metabolites in surface and ground waters in an agricultural zone in the provinces of Salamanca and Zamora (Spain) and to study the evolution over time of the pollution levels. The analyses, conducted from October 2000 to January 2001, pointed to the presence of atrazine, desethylatrazine and chlorotoluron at levels between approximately 0.03 and 1.5 $\mu g\ l^{-1}$.

2. Experimental

2.1. Chemicals

The herbicides were obtained from Riedel-de

Haën (Seelze-Hannover, Germany) and were used without further purification (minimum percent purity greater than 98%). The herbicides studied were as follows: chlorotoluron (Clt), 3-(3-chloro-*p*-tolyl)-1,1-dimethylurea; atrazine (At), 6-chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine and terbutryne (Tb), N^2-*tert*.-butyl-N^4-ethyl-6-methylthio-1,3,5-triazine-2,4-diamine. Stock solutions of each herbicide were prepared in methanol at 200 μg ml^{-1}.

The metabolites were obtained from Dr. Ehrenstorfer (Augsburg, Germany) and were as follows: DIHA, 2-hydroxy-*N*-ethyl-1,3,5-triazine-4,6-diamine; DEDIA, 6-chloro-1,3,5-triazine-2,4-diamine; DEHA, 2-hydroxy-*N*-isopropyl-1,3,5-triazine-4,6-diamine; HA, 2-hydroxy-N^4-ethyl-N^6-isopropyl-1,3,5-triazine-4,6-diamine; DEA, 6-chloro-*N*-isopropyl-1,3,5-triazine-2,4-diamine; HT, N^2-*tert*.-butyl-N^4-ethyl-6-hydroxy-1,3,5-triazine-2,4-diamine; and CMPU, *N*-(3-chloro-4-methylphenyl)urea. Stock solutions of DEA and CMPU were prepared in acetonitrile at 200 μg ml^{-1} and solutions of the rest of the metabolites were prepared in acetonitrile–0.1 *M* hydrochloric acid (80:20, v/v), at a concentration of 200 μg ml^{-1}.

Different types of sorbents were used to accomplish SPE: polymeric cartridges (Oasis HLB and Oasis MCX, Waters, and LiChrolut EN, Merck), and silica-based bonded C_{18} cartridges (Sep-Pak Plus, Waters).

The organic solvents, acetonitrile (ACN), methanol (MeOH) and ethyl acetate (AcOEt) were of HPLC grade (Merck) and were used as received. Ultra-high-quality water was obtained with a Elgastat UHQ water purification system.

All chemicals used for the preparation of the buffers were of analytical-reagent grade.

2.2. Apparatus and chromatographic conditions

HPLC was performed on a HP 1100 Series chromatograph from Hewlett-Packard (HP; Waldbronn, Germany), equipped with two pumps, a membrane degasser, an autosampler and a diode-array detector. The system was controlled by a HP ChemStation, which also performed data collection from the diode array detector and quantitative measurements. The analytical column used was a 250× 4.0 mm I.D. Spherisorb S5 ODS2 packed with 5 μm particles (Waters, Milford, MA, USA). The diode array detector was set at 210, 220, 230 and 245 nm. The spectra were recorded in the 190–400 nm range.

The mobile phase consisted of an acetonitrile (solvent A)–0.005 *M* phosphate buffer at pH 7.2 (solvent B) linear gradient, from 2 to 90% of solvent A in 60 min. Flow-rate was 1 ml min^{-1} and the volume injected was 100 μl. The analytical column was thermostatted at 25 °C.

Preconcentration of water samples was performed with LiChrolut EN polymeric cartridges (200 mg). Samples were pumped through the cartridge using a Gilson Minipuls 2 HP 4 peristaltic pump at a maximum flow-rate of 8.0 ml min^{-1}.

2.3. SPE procedure

The SPE cartridges were conditioned with 5 ml of methanol, then, 5 ml of ethyl acetate and, finally, 5 ml of water.

The passage of the samples (volume 250 ml) through the cartridges was carried out at a flow-rate of 7 ml min^{-1} by means of a peristaltic pump. Once the retention step had been completed, the cartridges were dried for 15 min under a vacuum of 15 mmHg (1 mmHg=133.322 Pa). The components retained were eluted with 5 ml of methanol and 5 ml of ethyl acetate. The organic phase thus obtained was evaporated to complete dryness in a rotary evaporator (Büchi, Flawil, Switzerland) at a temperature of 45–50 °C. The dry residue was dissolved in 500 μl of an acetonitrile–0.005 *M* phosphate buffer, pH 7.2 (50:50, v/v) solution.

2.4. Analysis of herbicides and metabolites in waters

Samples were taken from three rivers and six wells from an agricultural zone between the River Almar and the River Guareña in the provinces of Salamanca and Zamora (Spain). The sampling zone has been described in a previous work [2].

Six samplings were carried out between 28th October 2000 and 11th January 2001 (sampling 1: 28 October 2000; sampling 2: 10 November 2000; sampling 3: 25 November 2000; sampling 4: 1 December 2000; sampling 5: 15 December 2000, sampling 6: 11 January 2001).

Samples were collected in 1-l glass bottles. They were brought to the laboratory the same day of

sampling and were stored at 4 °C in the dark until SPE, which was carried out in 4 days or less after sampling. All samples were filtered through 0.45-μm pore-size cellulosic membrane filters from Osmonics (Kent, WA, USA).

2.5. Identification and quantification

Analyte identification was accomplished on the basis of the retention times of the analytes and by comparison between the UV spectrum of the reference compound in the library and the UV spectrum of the detected peak in the sample. A match equal or higher than 990 was fixed to confirm identification between both spectra for all herbicides. Quantification was performed by external calibration. Sample analyses were run in duplicate and, in most, relative standard deviations (RSDs) of less than 10% were achieved.

3. Results and discussion

3.1. Characteristics of the method

In order to determine the most appropriate conditions to carry out chromatographic separation of the herbicides and their metabolites, a linear acetonitrile–0.005 *M* phosphate buffer gradient (from 2 to 90% acetonitrile in 60 min) was chosen. Under these conditions, a good separation of the 10 analytes was achieved in an acceptable time (about 45 min). However, when the analytes were injected dissolved in pure acetonitrile, a deficient peak morphology was observed, especially in the case of the more polar metabolites, chromatographic peaks with frontal asymmetry being observed. This asymmetry was reduced when the composition of the sample injected was modified by the addition of water; thus, injection of the analytes dissolved in acetonitrile–water (50:50, v/v) allowed us to obtain better defined chromatographic peaks (Fig. 1).

Linear calibration graphs were found between peak areas and analyte concentration in the whole range studied (0.25–6 mg l^{-1}). The detection limits of the method, calculated as the ratio between three times the noise and the calibration slope, ranged between 60 and 6 μg l^{-1}, for DIHA and At, respectively. The determination of lower concentrations required the setting up of a previous preconcentration step.

3.2. Solid-phase extraction and recovery studies

With a view to obtaining a more sensitive method for the quantification of the herbicides and their metabolites, a study was performed using SPE with different sorbents as a preconcentration step prior to chromatographic determination. To do so, a 100-ml sample of deionised water spiked with the 10 analytes at a concentration of 20 μg l^{-1} was used to evaluate the capacity of different sorbents (C_{18}, Oasis HLB cartridges) to retain the compounds. Methanol, acetonitrile and ethyl acetate were used to elute the analytes from the cartridges. Recoveries are shown in Table 1.

With C_{18} cartridges, recovery values in the 9–20% range were obtained for the metabolites more polar than HA. These values were almost independent of

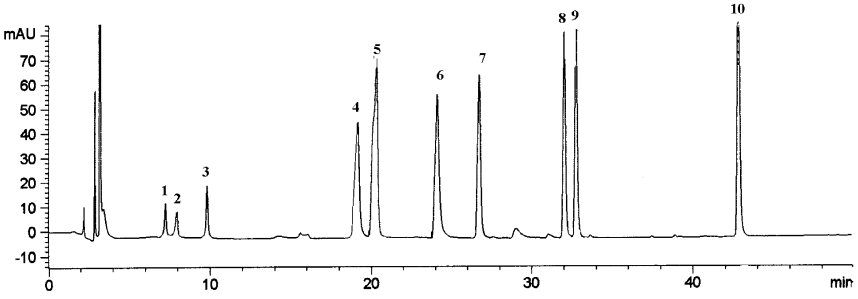

Fig. 1. Chromatogram of a standard solution of the three herbicides and the seven degradation products at 2 mg l^{-1}. Mobile phase: acetonitrile–0.005 *M* phosphate buffer (pH 7.2). Linear gradient, from 2 to 90% of acetonitrile in 60 min. UV detection at 220 nm. Peaks: (1) DIHA; (2) DEDIA; (3) DEHA; (4) HA; (5) DEA; (6) HT; (7) CPMU; (8) Clt; (9) At; (10) Tb.

Table 1
Recoveries obtained after solid-phase extraction with C_{18} and Oasis HLB cartridges

Herbicide	Recovery (%)					
	C_{18}			Oasis HLB		
	ACN	MeOH	AcOEt	ACN	MeOH	AcOEt
DIHA	10	9	9	0	0	0
DEDIA	10	11	13	10	13	12
DEHA	22	19	19	15	18	16
HA	30	78	90	60	54	55
DEA	94	86	121	84	102	111
HT	85	92	119	96	110	113
CMPU	77	90	107	77	99	94
Clt	108	83	108	103	102	104
At	97	81	103	99	95	102
Tb	99	67	81	85	83	93

Sample: 100 ml of water spiked with 20 μg l^{-1} of each herbicide.

the eluent used (Table 1). Similar values were obtained when the Oasis HLB sorbent was used. The recovery values for the more polar metabolites were found not to be a function of the eluent and it was observed that the most polar metabolite, DIHA, was not retained by this sorbent while for DEHA recovery values close to 16% were obtained (Table 1).

A second approach using C_{18} sorbent was evaluated, carrying out preconcentration in the presence of salts. The addition of salts to the samples to be preconcentrated can often improve recovery when conventional extraction methods are used. Fig. 2 shows the recovery obtained when modifying the percentage of NaCl added to the samples. On increasing the percentage of salt, an initial increase in recovery was obtained for the metabolites DIHA, DEDIA and DEHA, whereas for the parent herbicides and CMPU a decrease in recovery was observed. This behaviour can be explained by considering two simultaneously occurring processes. Initially, analyte recovery is enhanced due to salting out because the water molecules form hydration spheres around the ionic salt molecules and reduce the water available to dissolve the analyte. Also, the analytes may interact with the salts, thus reducing their capacity to be retained in the sorbent [25,26]. Since in this case the analytes were of very different polarities, the presence of salts did not allow recovery values over 70% to be obtained for any of them.

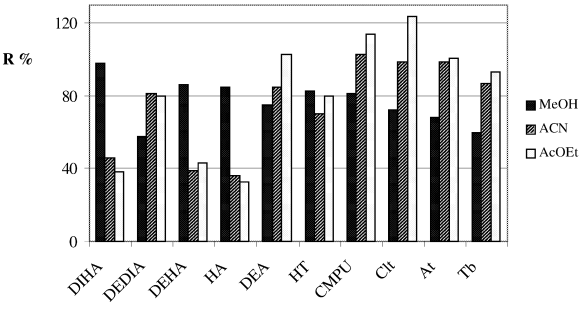

Fig. 3. Effect of the eluent on recovery (R). Sample: 100 ml of ultrapure water spiked at 20 μg l^{-1}; sorbent: LiChrolut EN.

Fig. 3 shows the recoveries obtained when using 200 mg of LiChrolut EN as sorbent. With this sorbent, using methanol as eluent, recoveries ranging between 98%, for DIHA, and 78%, for HA, were obtained. However, for the herbicides atrazine, terbutryne and chlorotoluron the most suitable eluents proved to be acetonitrile and ethyl acetate. To be able to elute the metabolites and herbicides, two series of experiments were carried out. In one,

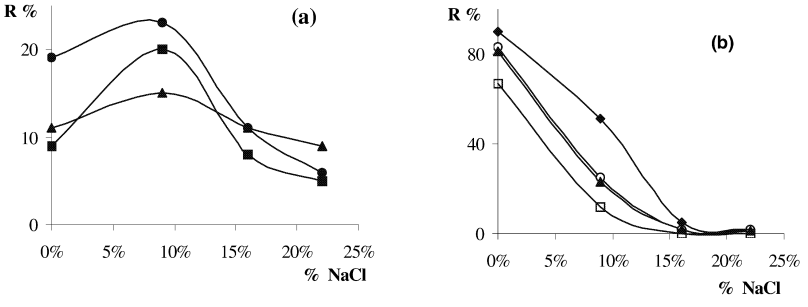

Fig. 2. Effect of sample salt concentration on recovery (R). Sample: 100 ml of ultrapure water spiked at 20 μg l^{-1}; sorbent: C_{18}; eluent: methanol. (a) (■) DIHA; (▲) DEDIA; (●) DEHA; (b) (♦) CMPU; (○) Clt; (▲) At; (□) Tb.

Table 2
Recoveries obtained after solid-phase extraction with 200 mg of LiChrolut EN cartridges

	Recovery±SD[a] (%)	
	(a)	(b)
DIHA	91±4	86±12
DEDIA	69±12	68±9
DEHA	91±4	103±8
HA	96±7	92±12
DEA	106±9	99±8
HT	88±13	103±14
CMPU	102±3	109±9
Clt	107±10	103±11
At	98±5	95±9
Tb	82±4	91±6

Sample: 100 ml of water spiked with (a) 20 μg l^{-1} of each herbicide, (b) 0.5 μg l^{-1} of each herbicide.
[a] $n=4$.

methanol and acetonitrile were used and in the other methanol and ethyl acetate were used as eluents of the sorbents. The results obtained were very similar in both cases. Table 2 shows the recovery values at two different concentration levels—0.5 and 20 μg l^{-1}—together with the confidence intervals obtained with methanol and ethyl acetate, ranging between 68% for DEDIA and 109% for CMPU. Additionally, these values were not significantly modified when preconcentration was accomplished with 400 mg of LiChrolut EN.

To determine the recovery values in natural water, two samples were selected—one of river water and the other of ground water—that were not contaminated with the pesticides under study. After spiking them with 20 μg l^{-1} of the herbicides, it was observed that the recoveries were significantly equal to those obtained when distilled water was used as matrix.

Another way in which the detection limit can be decreased consists of increasing the volume of water to be preconcentrated. However, in the case of highly polar analytes, breakthrough may occur when the volume to be preconcentrated is increased [27]. Fig. 4 shows that the metabolites DIHA, DEDIA, DEHA, HA and DEA display the breakthrough phenomenon, this being most marked for DEDIA. Nevertheless, for CMPU and the parent herbicides, recovery was independent of the volume to be preconcentrated. A volume of 250 ml was selected as a compromise between the most satisfactory recovery values and the lowest detection limits that it was possible to obtain.

3.3. Analytical data for SPE preconcentration

As sorbent to conduct preconcentration, 200 mg of LiChrolut EN was chosen. Calibration graphs were obtained by preconcentrating a volume of 250 ml (preconcentration factor of 500). Linear relationships were found between peak areas and metabolites and herbicide concentrations in the 0.1–6 μg l^{-1} range. The detection limits, calculated as the ratio between three times the noise and the calibration slope, ranged between 0.1 μg l^{-1} for DIHA and DEDIA, and 0.02 μg l^{-1} for the rest of the analytes (Table 3). This method permits therefore the detection of these herbicides in drinking water at the concentration

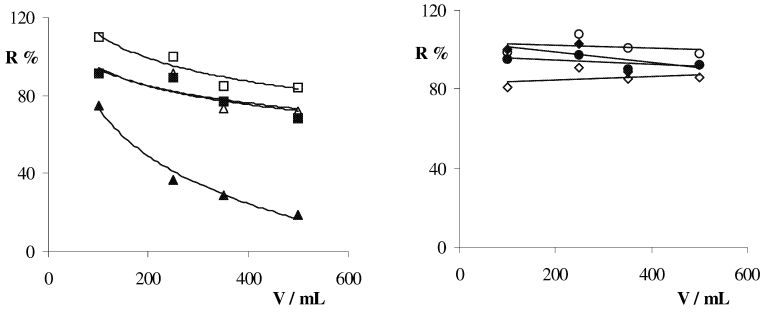

Fig. 4. Influence of the sample volume on recovery (R). Sorbent: LiChrolut EN. (■) DIHA; (▲) DEDIA; (□) DEA; (△) HT; (♦) CMPU; (○) Clt; (●) At; (◇) Tb.

Table 3
Analytical characteristics of the method after SPE with LiChrolut EN

Herbicide	Intercept	Slope	r^2	RSD[a] (%)	D.L.[b] ($\mu g\ l^{-1}$)
DIHA	0.3±0.9	19±1	0.998	15	0.09
DEDIA	0.3±0.5	10±1	0.966	12.3	0.10
DEHA	−4±6	52±2	0.997	8.7	0.04
HA	1±34	276±14	0.997	17.7	0.02
DEA	−16±41	392±17	0.997	10.8	0.02
HT	4±29	229±12	0.996	13.7	0.02
CMPU	−6±41	247±17	0.993	11.1	0.02
Clt	6±30	227±12	0.995	13.2	0.02
At	5±21	338±9	0.999	9.8	0.02
Tb	58±23	291±9	0.998	7.6	0.01

[a] RSD=Relative standard deviation for a concentration of 0.5 $\mu g\ l^{-1}$ (n=10).
[b] D.L.=Detection limit for a signal-to-noise ratio of 3.

levels demanded by current legislation [28]. The relative standard deviations obtained from 10 replicate analyses at a concentration level of about 0.5 $\mu g\ l^{-1}$ ranged between 7.6 and 17.7% (Table 3).

3.4. Analysis of herbicides and metabolites in ground waters

For the analysis, six wells designated P-3, P-6, P-8, P-9, P-9a and P-22 were chosen. In P-3, P-6, P-8 and P-9a none of the herbicides studied was detected at concentrations above the detection limit, except for the single case of well P-8, in which atrazine ($0.02\pm0.08\ \mu g\ l^{-1}$) was found in the second sampling, and for well P-9a, in which chlorotoluron ($0.06\pm0.2\ \mu g\ l^{-1}$) was found in the fifth sampling.

Fig. 5a shows the chromatogram obtained on analysing the sample from well P-22 corresponding to the first sampling. In that sample, the contents of atrazine and DEA found were 1.67 ± 0.08 and $1.3\pm0.1\ \mu g\ l^{-1}$, respectively.

To verify the results obtained, this same sample was analysed using the Oasis MCX sorbent, following the procedure recommended by the manufacturer [29]. This sorbent contains cation-exchange sulfonic acid groups on the surface of the Oasis HLB. Fig. 5b shows the chromatogram obtained when this sorbent was used. The values found using this procedure were 1.6 ± 0.2 and $1.3\pm0.1\ \mu g\ l^{-1}$ for atrazine and DEA, respectively.

When the SPE step was carried out using Oasis MCX cartridges, a much cleaner chromatogram was obtained than when the LiChrolut EN sorbent was employed. However, the procedure proposed for preconcentration with Oasis MCX can only be applied to the preconcentration of basic compounds and is not suitable for multiresidue analysis. With these sorbents, it was seen that 42 and 47% of chlorotoluron and its metabolite, respectively, were eluted in the washing step with methanol.

The concentrations found for atrazine and DEA using the Oasis MCX sorbent were consistent with those obtained when using LiChrolut EN. This points to the reliability of the procedure proposed in this work.

Regarding the samples from the wells, herbicide levels above the detection limit were found in P-9 and P-22. In P-22, atrazine and DEA were detected: the presence of DEA seems to be justified owing to its high solubility in water. These findings are in agreement with those reported by other authors [21,30,31], who only detected this degradation product of atrazine in ground waters. However, it should be noted that the values found in the present study are threefold higher than those reported in [30] and [31] and 10-fold higher than those described by Loos and Niessner [21].

Table 4 shows the evolution of the atrazine and DEA content in P-22 and the evolution of the chlorotoluron content in P-9. The concentrations of atrazine and DEA decrease, tending progressively to become equal up to the fifth sampling, after which this trend changes and an increase occurs in the concentration of both, although atrazine does so more markedly. In the case of chlorotoluron, its concentration remained almost constant up to the last

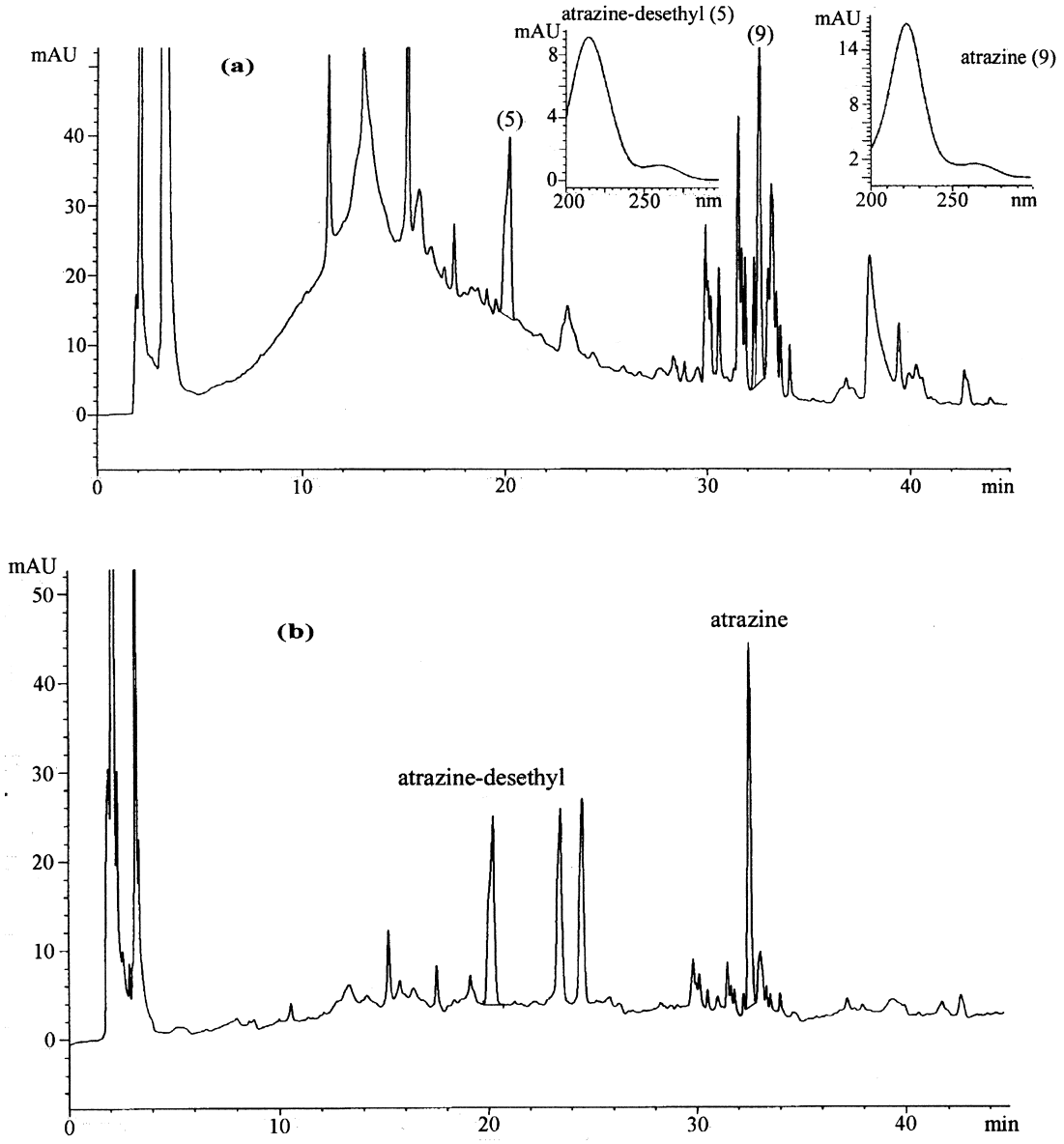

Fig. 5. Chromatograms obtained after solid-phase extraction with: (a) LiChrolut EN and (b) Oasis MCX of a ground water sample. Experimental conditions as in Fig. 1.

samplings, in which an increase was seen.

3.5. Analysis of herbicides and metabolites in river waters

The results obtained in the samples from the three rivers analysed—R-2b and R-5 from the basin of the River Almar and R-8 from that of the River Guareña—are shown in Table 4. The table reflects mainly the presence, at the highest concentration, of chlorotoluron, although the presence of atrazine was also detected in samples R-2b and R-5, in the first samplings; DEA was detected in the first sampling of R-5.

The R-2b samples initially contained no chlorotoluron, a maximum being observed in sampling 3,

Table 4
Results obtained for the analysis of herbicides in river and ground waters

Sample	Herbicide	Sampling					
		1	2	3	4	5	6
River water[a]							
R-2b	At	0.17±0.08	–	–	–	–	–
	Clt	–	–	1.3±0.2	0.9±0.2	0.5±0.2	0.6±0.2
R-5	DEA	0.3±0.1	–	–	–	–	–
	At	0.12±0.08	0.15±0.08	–	–	–	–
	Clt	–	–	–	0.03±0.2	0.6±0.2	1.3±0.2
R-8	Clt	–	0.3±0.2	0.5±0.2	0.5±0.2	0.5±0.2	1.9±0.2
Ground water[b]							
P-9	Clt	0.5±0.2	0.5±0.2	0.5±0.2	0.4±0.2	0.4±0.2	0.7±0.2
P-22	DEA	1.3±0.1	1.0±0.1	0.8±0.1	0.7±0.1	0.7±0.1	1.0±0.1
	At	1.67±0.08	1.22±0.08	1.03±0.08	0.76±0.08	0.76±0.08	1.39±0.08

Number of samples analysed was nine in each sampling: (a) 18 for river water samples; (b) 36 for ground water samples. Number of replicates=2, each replicate injected twice.

then decreasing to sampling 6. The behaviour of the R-5 samples was slightly different. Initially, no chlorotoluron was detected up to sampling 3, after which its concentrations increased. The samples taken at R-8 showed a similar type of behaviour to that corresponding to R-5.

Fig. 6 shows the chromatogram obtained on analysing the sample from river R-5 corresponding to the fifth sampling. In this sample, the content of chlorotoluron found was 0.6±0.2 μg l^{-1}.

4. Conclusions

An LC–DAD method, after SPE, is proposed for the separation and quantification of atrazine and its metabolites DIHA, DEDIA, DEHA, DEA and HA, of terbutryne and its metabolite HT, and of chlorotoluron and its metabolite CMPU. The results indicate that the polymeric LiChrolut EN sorbents can be satisfactorily used for the simultaneous preconcentration of analytes of different polarities, the parent

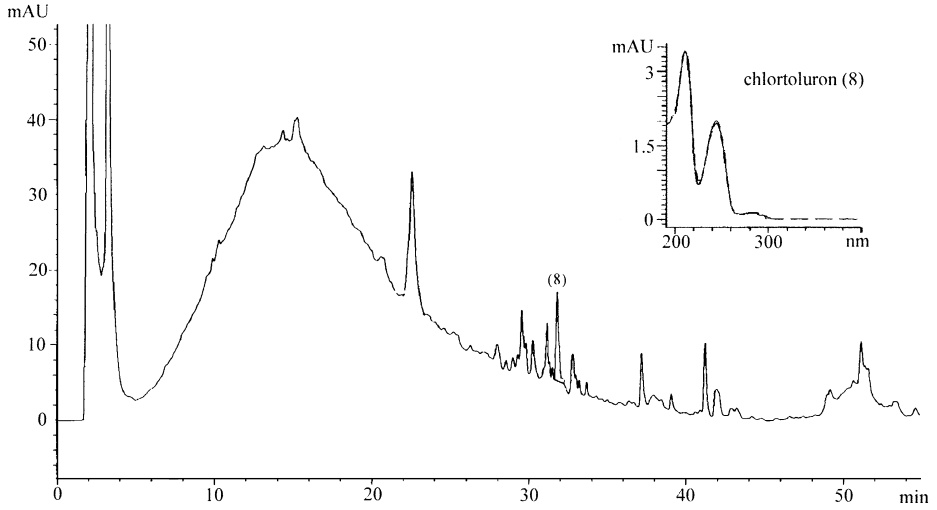

Fig. 6. Chromatogram obtained after solid-phase extraction with LiChrolut EN of a river water sample. Experimental conditions as in Fig. 1.

herbicides and their metabolites, as long as the maximum volume to be preconcentrated is of the order of 250 ml. The proposed method permitted the determination of these herbicides at concentration levels of 0.1 $\mu g\ l^{-1}$, as demanded by current legislation for individual pesticides in drinking waters.

The described method has been used to evaluate pollution of three rivers in the basins of the rivers Almar and Guareña (provinces of Salamanca and Zamora, Spain). The herbicides found were chlorotoluron, atrazine and, exceptionally, DEA. The evolution with time of the content of these herbicides shows that in surface waters pollution is a function of time and is related to the application and use of these herbicides.

Of the six wells analysed, pollution due to herbicides was only observed in two. In one of them, atrazine and DEA were found and chlorotoluron in another. It should be noted that the contents of these herbicides and of DEA in ground waters are almost independent of the time of analysis, indicating that pollution in ground waters is persistent and seems to be related to the frequency of application, soil permeability, the frequency of rainfall, and the recharge rate of the aquifer.

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