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Journal of Chromatography A, 1085 (2005) 199–206
JOURNAL OF CHROMATOGRAPHY A
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Determination of triazines and dealkylated and hydroxylated metabolites in river water using a propazine-imprinted polymer

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Received 16 September 2004; received in revised form 23 May 2005; accepted 27 May 2005
Available online 21 June 2005

Abstract

A molecularly imprinted polymer (MIP) obtained by precipitation polymerisation using propazine as template has been employed as sorbent for the solid phase extraction of triazines and some of their hydroxylated and dealkylated metabolites from river water. Three configurations were studied: (a) use of the propazine-MIP as a selective sorbent for the extraction of triazines directly from water; (b) use of mixtures of LiChrolut EN (a polymeric sorbent of styrene divinylbenzene) and propazine-MIP as sorbent, and (c) use of propazine-MIP as a clean-up sorbent for organic extracts obtained in a prior SPE procedure with LiChrolut EN. The former two configurations imply that the analytes pass through the propazine-MIP in aqueous medium, whereas in the latter case the analytes percolate through the propazine-MIP in an organic medium coming from the previous SPE step. Different types of water were tested to assess matrix effects. The analytical characteristics of the three configurations were evaluated.

Keywords: Molecularly imprinted polymers; Analytical method; Triazines; Hydroxylated and dealkylated metabolites

1. Introduction

Triazine herbicides are widely used for weed control in several crops. Their prolonged use involves the risk of their retention in crops and soils, from which in turn, due to washing and leaching processes, these substances pass to surface and ground waters. Among other compounds, the technical health legislation [1] for drinking water includes herbicides, metabolites and degradation products, such that it is necessary to develop methods of analysis that include these compounds [2].

Molecularly imprinted polymers are extensively cross-linked polymers containing specific recognition sites with a predetermined selectivity for analytes of interest. Many authors have synthesised different MIPs, mainly in bulk, although more recently other methods for obtaining them, such as precipitation polymerisation [3–5], dispersion polymerisation [6] or suspension polymerisation [7,8] have been used.

The application of MIPs for clean-up after non-selective extraction has been proposed by several authors [9,10], and also their use as a stationary phase in LC or as selective sorbents for SPE. Since the first application described by Sellergren [11] in 1994, relating to an MIP used for the extraction of pentanamide from human urine, various reports have appeared showing – qualitatively – the use of MIPs in the analysis of samples such as water [12], urine [11], juice [13], human plasma [14,15], biological matrices [16], solid sample extracts [17]. However, little attention has been paid to quantitative determinations based on the use of MIPs as sorbents for the preconcentration of triazines [17,18].

MIPs for the selective extraction of triazines have been proposed, most of them aimed at the retention of chloro- and methylthio-triazines using MIPs obtained by bulk polymerisation. Although in some cases dealkylated chloro-metabolites [4,17,18] were also included, little attention has been directed at studying the behaviour of the

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doi:10.1016/j.chroma.2005.05.084

hydroxy-metabolites [12,13]. We have developed an MIP with methacrylic acid as functional monomer and propazine as template [19]. This propazine-MIP was prepared by precipitation polymerisation; it exhibits highly selective binding for triazines and dealkylated and hydroxylated metabolites, and can be used to retain these analytes from organic and aqueous media. However, different types of behaviour were observed as a function of the sample medium and of the type of analyte being studied. For both media it was observed that no specific recognition of non-related herbicides occurs.

Here we study the possibilities and the analytical characteristics of using a propazine-MIP as a sample treatment step for the quantitative determination of triazines and related metabolites in river water. The analytical data were evaluated using the propazine-MIP in three configurations: (a) as a single sorbent for selective SPE of triazines from water, (b) as sorbent mixed with a commercial polymeric sorbent, and (c) as a clean-up sorbent for organic extracts obtained in a prior SPE procedure. The analytes included in this study were: chloro-triazines, methylthio-triazines, hydroxy-metabolites, dealkylated chloro-metabolites, and dealkylated hydroxy-metabolites. To our knowledge, this is the first paper in which dealkylated hydroxy-metabolites are also included in the development of a MIP-based method for the determination of structurally related triazines in aqueous samples.

2. Experimental

2.1. Chemicals

The herbicides and the metabolites were obtained from Dr. Ehrenstorfer (Augsburg, Germany) and were used without further purification. The herbicides studied are indicated in Table 1. Stock solutions of each herbicide and metabolite were prepared in acetonitrile at 500 μg mL^{-1} except in the case of the hydroxy metabolites, which were prepared in acetonitrile–0.1 M hydrochloric acid (80:20, v/v) at a concentration of 200 μg mL^{-1}.

Other herbicides studied were: *chlorsulfuron* (Cls) 1-(2-clorophenylsulfonyl)-3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)urea; *isoproturon* (Ipn) 3-(4-isopropylphenyl)-1,1-dimethylurea; *linuron* (Lin) 3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea and *lenacil* (Len) 3-cyclohexyl-1,5,6,7-tetrahydrocyclopentapyrimidine-2,4(3H)-dione.

Methacrylic acid (MAA), ethylene dimethacrylate (EGDMA) and 2,2′-Azobis(2-methyl-propionitrile) (AIBN) were obtained from Acros Organics (Geel, Belgium). Toluene, dichloromethane, acetonitrile (ACN) and methanol (MeOH) were of HPLC grade (Merck) and were used as received. Ultra-high quality (UHQ) water was obtained with an Elgastat UHQ water purification system.

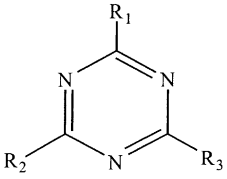
LiChrolut EN cartridges (Merck) containing 200 mg of a styrene-divinylbenzene polymer were used for the preconcentration of the analytes from river water.

All other chemicals were of analytical reagent grade.

2.2. Synthesis of the imprinted polymer

The procedure to obtain the MIP by precipitation was as follows: the template, propazine (1 mmol) and the functional monomer, MAA (4 mmol), were added to a glass tube and after 5 min EGDMA (20 mmol), toluene (30 mL) and AIBN (0.2 mmol) were added. The solution was degassed with nitrogen for 5 min and the tubes were closed and sealed under this atmosphere. Microspheres were obtained by precipitation polymerisation in a water bath at 60 °C for 10 h with stirring at 350 rpm. The resulting microspheres were washed twice with acetone to remove the fine particles. The template was removed by Soxhlet extraction with methanol:acetic acid (9:1, v/v) until no template was found in the washing solution.

Table 1
General structure of the triazine herbicides and metabolites studied



Compounds	R_1	R_2	R_3
Deisopropylhydroxyatrazine (DIHA)	—OH	—NH_2	—NH—CH_2—CH_3
Deethylhydroxyatrazine (DEHA)	—OH	—NH—$CH(CH_3)_2$	—NH_2
Deisopropylatrazine (DIA)	—Cl	—NH_2	—NH—CH_2—CH_3
Deethylatrazine (DEA)	—Cl	—NH—$CH(CH_3)_2$	—NH_2
Hydroxyterbutylazine (HT)	—OH	—NH—$C(CH_3)_3$	—NH—CH_2—CH_3
Simazine (Smz)	—Cl	—NH—CH_2—CH_3	—NH—CH_2—CH_3
Atrazine (Atz)	—Cl	—NH—$CH(CH_3)_2$	—NH—CH_2—CH_3
Propazine (Ppz)	—Cl	—NH—$CH(CH_3)_2$	—NH—$CH(CH_3)_2$
Terbutylazine (Tbz)	—Cl	—NH—$C(CH_3)_3$	—NH—CH_2—CH_3
Prometryn (Pmn)	—SCH_3	—NH—$CH(CH_3)_2$	—NH—$CH(CH_3)_2$
Terbutryn (Tbn)	—SCH_3	—NH—$C(CH_3)_3$	—NH—CH_2—CH_3

Non-imprinted polymers (NIP) were prepared in the same way but without the addition of the template to the polymerisation mixture.

2.3. Preconcentration of triazines from river water

2.3.1. SPE with propazine-MIP

As sorbent for SPE, we used 200 mg of the propazine-MIP packed in an empty extraction cartridge. The cartridge was conditioned with 10 mL of dichloromethane, 10 mL of acetonitrile/acetic acid (9:1, v/v) and 10 mL of water. The sample of water (100 mL) was passed through the cartridge by a peristaltic pump at a flow rate of 6.5 mL min^{-1}. Once analyte retention had been completed, the cartridges were dried for 30 min under a vacuum of −15 mm Hg. Dichloromethane (5 mL) was used as washing solvent. The analytes were eluted with 10 mL of acetonitrile/acetic acid (9:1, v/v). The eluate was brought to dryness and the dry residue was reconstituted with 500 μL of a mixture of water/acetonitrile (9:1, v/v).

2.3.2. SPE with a mixture of LiChrolut EN and propazine-MIP

The same procedure as in Section 2.3.1 was employed, but using as sorbent 200 mg of propazine-MIP mixed with 200 mg of LiChrolut EN, packed in an empty extraction cartridge.

2.3.3. SPE with LiChrolut EN as sorbent and clean-up step with propazine-MIP

Preconcentration was accomplished by passing 250 mL of water through a LiChrolut EN cartridge, following the procedure described previously [2], and the dry residue was dissolved in 2 mL of toluene. This solution was passed through 200 mg of the polymer imprinted with propazine packed in an empty extraction cartridge. The propazine-MIP cartridge had previously been conditioned with 10 mL of acetonitrile/acetic acid (9:1, v/v), 10 mL of dichloromethane, and 10 mL of toluene. To eliminate non-specific interactions, cartridges were washed with 5 mL of dichloromethane and the analytes were eluted with 10 mL of acetonitrile/acetic acid (9:1, v/v). The eluate was evaporated to dryness and the dry residues were reconstructed in 500 μL of water/acetonitrile (9:1, v/v).

2.4. LC conditions

LC-DAD UV was performed on a HP 1100 Series chromatograph from Hewlett Packard (Waldbronn, Germany) equipped with a quaternary pump, a membrane degasser, an autosampler and a diode-array UV detector (DAD UV system). The system was controlled by an HP ChemStation, which also performed data collection from the diode array detector and quantitation. The analytical column was a 250 mm × 40 mm I.D. Spherisorb S5 ODS2 packed with 5 μm particles (Waters, Milford, MA, USA). The DAD UV detector was set at 210 and 245 nm. Spectra were recorded in the 190–400 nm range.

A gradient acetonitrile (solvent A) and 5 mM phosphate buffer at pH 7.2 (solvent B) was used: from 5 to 45% of solvent A in 15 min, from 45 to 50% of solvent A in 15 min and returned to initial conditions in 2 min, with 3 min for equilibrating the column. The flow rate was 1 mL min^{-1} and the volume injected was 100 μL. The analytical column was thermostatted at 25 °C.

3. Results and discussion

The retention of triazines and related metabolites by propazine-MIP has been studied previously [19]. It was observed that the use of the propazine-MIP for SPE of these analytes depends on the type of analyte and on the medium in which the sample is dissolved. Thus, in organic medium, the chloro- and methylthio-triazines, together with the dealkylated chloro-metabolites, could be preconcentrated efficiently. However, hydroxy-metabolites and dealkylated hydroxy-metabolites were not retained on the MIP. By contrast, in aqueous medium it was possible to use the propazine-MIP to extract chloro-triazines, the dealkylated chloro-metabolites and the hydroxy- and dealkylated hydroxy-metabolites, but the methylthio-triazines were not retained on the MIP.

3.1. SPE with propazine-MIP

First, the effect of the volume of water to be preconcentrated on the recoveries was studied. The improvement in sensitivity based on an increase in the volume of sample to be preconcentrated has some limitations, such as the fact that the loading capacity of the cartridge might be surpassed or that, for highly polar analytes, the breakthrough phenomenon may occur.

The preconcentration from sample volumes of 2–100 mL of UHQ water containing the same total amount of herbicides (200 ng each) was studied. Fig. 1 shows that there was almost no change in recoveries, except for the highly polar dealkylated hydroxy-metabolites DIHA and DEHA. For all other analytes, the recoveries remained more or less constant. In later studies it was decided to use a sample volume of 100 mL.

3.1.1. Influence of concentration

To study the influence of concentration, 100 mL of UHQ water spiked with fifteen compounds (six triazines, five metabolites, and four non-triazine herbicides) was preconcentrated. A concentration range of 0.5–100 μg L^{-1} (preconcentrated amount, 50 ng–10 μg for each compound) was assayed. The absence of the peaks corresponding to the non-triazine herbicides shows that the propazine-MIP is selective for triazine and related metabolites.

A linear relationship up to a concentration of 4 μg L^{-1} was observed for all triazines. However, with the chloro-triazines, a plateau was reached for concentrations above 15 μg L^{-1},

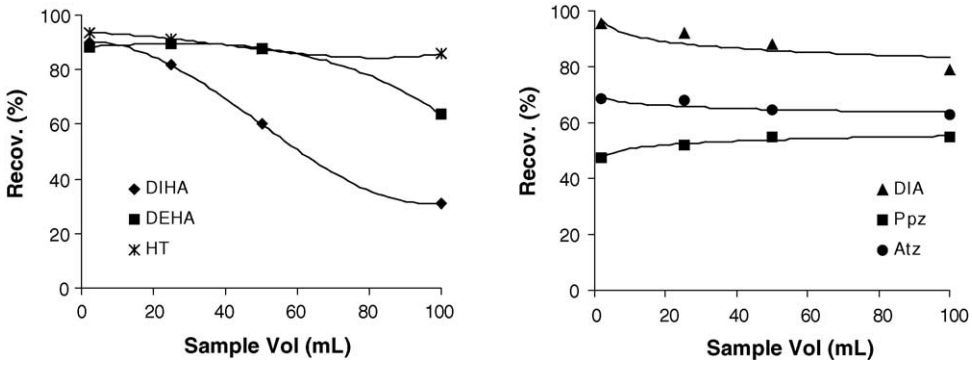


Fig. 1. Influence of the sample volume on recovery. Sorbent: 200 mg of propazine-MIP.

indicating that the binding cavities were saturated. However, for the dealkylated and hydroxylated metabolites, no such plateau was reached. Nevertheless, the slopes corresponding to this latter section of the plot were different, being greater for the dealkylated (DEA and DIA) than for the hydroxylated (DEHA and DIHA) metabolites (Fig. 2a).

The different behaviour shown by the chloro-triazines and the dealkylated chloro-triazines (Fig. 2b) is due to the fact that the MIP has a heterogeneous surface with cavities of different sizes and energies. DEA and DIA which are smaller than the chloro-triazines may be retained more easily in the cavities, while the larger analytes, such as propazine, have more difficulty in accessing. These findings suggest that the linear range is associated with the sites with the largest pores, which are the first to be occupied. As pore size decreases, only the smaller metabolites are able to enter.

A similar study was conducted, percolating a volume of 100 mL of UHQ water spiked only with atrazine and DEA at concentrations of 0.5–40 $\mu g\,L^{-1}$. In this case, linearity, corresponding to extraction with constant recovery, was found up to concentrations of 40 $\mu g\,L^{-1}$. This increase in the range of linearity when the number of competing analytes decreases is consistent with the above indicated mechanism.

Upon calculating the capacity of the propazine-MIP, a value of 8 μg (40 $\mu g\,L^{-1}$) was obtained; this was maintained despite the volume of sample preconcentrated, as long as this was lower than 100 mL. These findings differ from those reported by other authors [13] who used polymers imprinted by bulk polymerisation, for which capacity is strongly affected by the volume of sample percolating through the MIP. Such differences must be related to the greater homogeneity of the distribution of active sites exhibited by polymers obtained by precipitation.

From these studies it may be inferred that the range of linearity is a function of the concentrations of the analytes to be preconcentrated. Hence, in order to ensure that quantification will be carried out in the linear zone it is necessary to work with concentrations lower than 4 $\mu g\,L^{-1}$.

3.1.2. Analytical data

Table 2 shows the analytical characteristics for concentrations below 4 $\mu g\,L^{-1}$. The precision of the method is similar to that obtained with conventional sorbents and ranges from 3% for DIA and DEA to 17% for hydroxyterbutylazine and propazine. The limits of detection (LODs) determined for a signal-to-noise ratio of 3, were 0.1–0.2 $\mu g\,L^{-1}$. They allow the quantification of triazines in water at the levels demanded by current legislation.

3.1.3. Matrix effects

Three types of water—UHQ, tap, and river water—were used to study matrix effects. A volume of 100 mL of each type

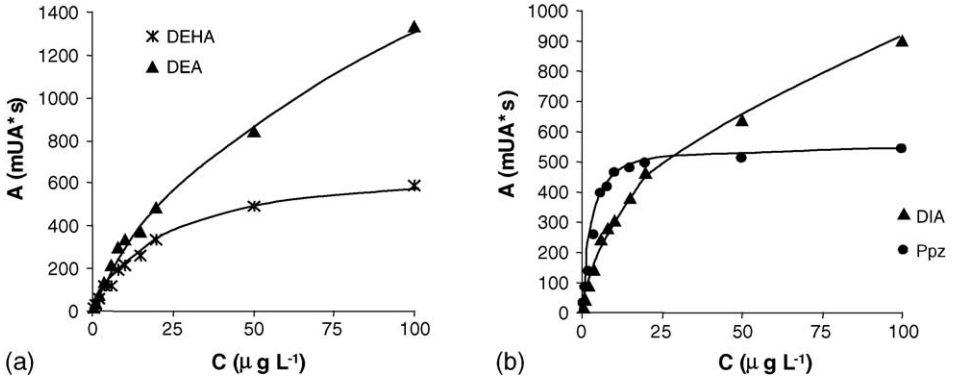


Fig. 2. Variation of peak area with the concentration of the analytes. (a) Dealkylated chloro-metabolites (DEA) and dealkylated hydroxy-metabolites (DEHA), (b) chloro-triazines (Ppz) and dealkylated chloro-metabolites (DIA). Sample: 100 mL of UHQ water spiked with all the analytes studied.

Table 2
Analytical data for the extraction of triazines from water by SPE with propazine-MIP as sorbent

Analyte	Intercept (mAU)	Slope (mAU/μg L^{-1})	R^2	RSD[a] (%)	LOD (μg L^{-1})
DIHA	26 ± 15	15 ± 3	0.993	11	0.2
DEHA	0 ± 6	58 ± 3	0.999	7	0.1
DIA	1 ± 40	78 ± 12	0.991	3	0.1
DEA	4 ± 21	70 ± 6	0.997	3	0.1
HT	−4 ± 95	271 ± 28	0.996	17	0.1
Simazine	−30 ± 72	140 ± 21	0.991	10	0.1
Atrazine	−12 ± 27	96 ± 8	0.997	13	0.1
Propazine	16 ± 45	127 ± 13	0.996	17	0.1
Terbutylazine	−6 ± 36	67 ± 11	0.990	10	0.1

[a] Concentration, 4 μg L^{-1}; $n = 6$.

of sample was spiked with the triazines, the dealkylated and hydroxylated metabolites, and four non-triazine herbicides at a level of 2 μg L^{-1} each. The recoveries are shown in Fig. 3. Chlorsulfuron, lenacil, isoproturon and linuron were not detected in any samples, confirming the selectivity of the MIP towards triazine herbicides and related compounds (Fig. 4a). Also, the recoveries for the chloro-triazines, hydroxyterbutylazine (HT) and the dealkylated chloro-metabolites (DIA and DEA) were essentially the same in all water samples tested. However, with the dealkylated hydroxy-metabolites, DEHA and DIHA, the recoveries from tap and river water (both with a hardness of 2 °f, *French degrees*) were lower than for UHQ water.

To confirm the existence of this matrix effect, 100 mL of river water (with an initial hardness of 2 °f), to which a high concentration of calcium (final hardness, 40 °f) had been added, were passed through the MIP. DEHA and DIHA were not retained on the sorbent, whereas the other analytes were removed in the washing process. Under these conditions, the protons of the carboxylic acids of methacrylic acid in the polymeric matrix are exchanged for calcium ions, preventing the formation of hydrogen bridges with the analytes [13]. In order to eliminate this effect, a washing step was implemented using 1 mL of 0.1 M HCl to regenerate the surface of the MIP and replace the calcium ions by protons. When the washing step was carried out after the MIP drying step, no analyte recognition was observed. However, when the HCl washing step was performed prior to the drying step, the same

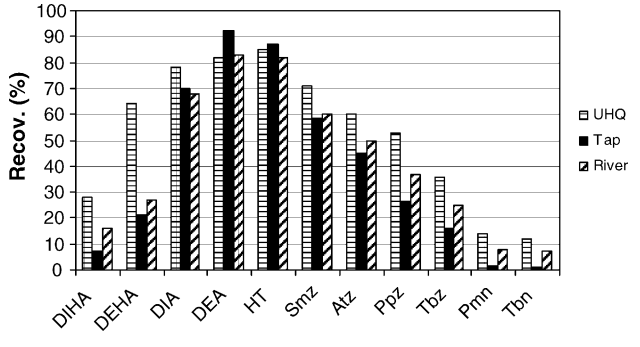


Fig. 3. Recoveries (%) for different type of water samples. Sorbent: 200 mg of propazine-MIP. Sample: 100 mL of water spiked with 2 μg L^{-1} of each analyte.

recoveries were obtained as upon analysing river water with a hardness of 2 °f. DIHA and DEHA were not retained on the MIP in any case.

3.2. SPE with a mixture of propazine-MIP and LiChrolut EN

To develop a quantitative SPE method for the preconcentration of the dealkylated hydroxy-metabolites, DIHA and DEHA, together with the other triazines, a sorbent containing a mixture of MIP and LiChrolut EN was prepared. Fig. 4b shows that by using this mixed sorbent chlorsulfuron, lenacil, isoproturon and linuron, as well as the methylthio-triazines, were eliminated in the washing step. DIHA and DEHA were now retained on LiChrolut EN, with recoveries ranging from 67% for DIHA in river water to 89% for DEHA in UHQ water (Table 3).

River water was first analysed to check the absence of the herbicides under study. The water was then spiked in the 0.05–6 μg L^{-1} range. Preconcentration was carried out using a mixture of propazine-MIP and LiChrolut EN. The analytical characteristics of the method are shown in Table 4. The LODs

Table 3
Recoveries obtained using either propazine-MIP or propazine-MIP + LiChrolut EN as sorbents for SPE of aqueous samples (100 mL)

Analyte	Recovery (%)			
	UHQ water		River water	
	MIP	MIP + LiChr.	MIP	MIP + LiChr.
DIHA	31	78	7	67
DEHA	66	89	22	83
DIA	79	93	73	91
DEA	79	91	85	85
HT	85	92	83	90
Simazine	70	89	72	79
Atrazine	60	77	59	64
Propazine	51	56	38	45
Terbutylazine	38	40	30	28
Chlorsulfuron	–	–	–	–
Lenacil	–	–	–	–
Isoproturon	–	–	–	–
Linuron	–	–	–	–

(–) Not detected.

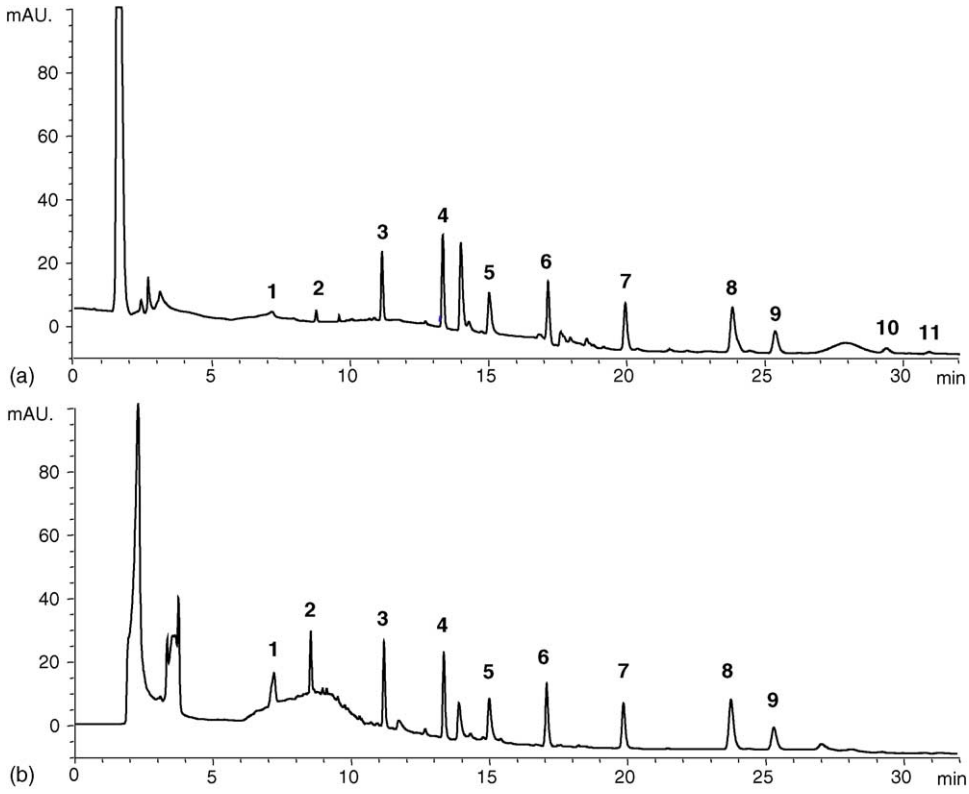


Fig. 4. Chromatograms of a river water sample spiked with 1.0 μg L^{-1} of triazine herbicides, dealkylated and hydroxylated metabolites and other non-related herbicides. (a) Preconcentration by SPE with propazine-MIP as sorbent; (b) Preconcentration by SPE with a mixture of propazine-MIP and LiChrolut EN as sorbent. Peaks: (1) DIHA; (2) DEHA; (3) DIA; (4) DEA; (5) HT; (6) simazine; (7) atrazine; (8) propazine; (9) terbutylazine; (10) prometryn and (11) terbutryn. Wavelength was set at 220 nm for triazines and metabolites, and at 245 nm for the other herbicides. LC conditions as described in Section 2.

ranged between 0.1 μg L^{-1} for propazine and 0.03 μg L^{-1} for DIA and DEA.

As an application, two samples of river water were analysed. The samples were spiked at 0.5 and 1.0 μg L^{-1}, and quantification was performed by the standard addition method; satisfactory results were obtained. The RSDs obtained in triplicate analyses of each sample were always lower than 22%.

3.3. Use of propazine-MIP as clean-up sorbent

The configurations described in the previous sections imply that the sample passes through the MIP in aqueous solution. Under these conditions, methylthio-triazines are not specifically retained on the MIP [19]. The use of mixtures of LiChrolut EN and propazine-MIP does not permit their determination: although they are retained on LiChrolut EN, they are removed from the sorbent in the washing step. According, with a view to determining methylthio-triazines and chloro-triazines simultaneously, a third two-step configuration was tested. In the first step, the aqueous sample was preconcentrated on LiChrolut EN, and in the second one the organic extracts obtained were passed through propazine-MIP. Propazine-MIP now acts as a clean-up sorbent. The ability of the propazine-MIP to preconcentrate methylthio- and chloro-triazines simultaneously was evaluated.

Table 4
Analytical data for the extraction of triazines from river water by SPE with propazine-MIP + LiChrolut EN as sorbent

Analyte	Intercept (mAU)	Slope (mAU/μg L^{-1})	R^2	RSD[a] (%)	LOD (μg L^{-1})
DIHA	14 ± 19	88 ± 5	0.999	2	0.06
DEHA	−6 ± 31	124 ± 9	0.999	4	0.05
DIA	−4 ± 22	141 ± 6	0.999	16	0.03
DEA	8 ± 48	151 ± 14	0.998	3	0.03
HT	−9 ± 31	93 ± 9	0.997	11	0.09
Simazine	8 ± 10	140 ± 3	0.999	9	0.04
Atrazine	17 ± 39	128 ± 11	0.998	10	0.05
Propazine	6 ± 49	70 ± 13	0.990	20	0.10
Terbutylazine	−17 ± 76	53 ± 22	0.998	18	0.08

[a] Concentration, 1 μg L^{-1}; $n = 4$.

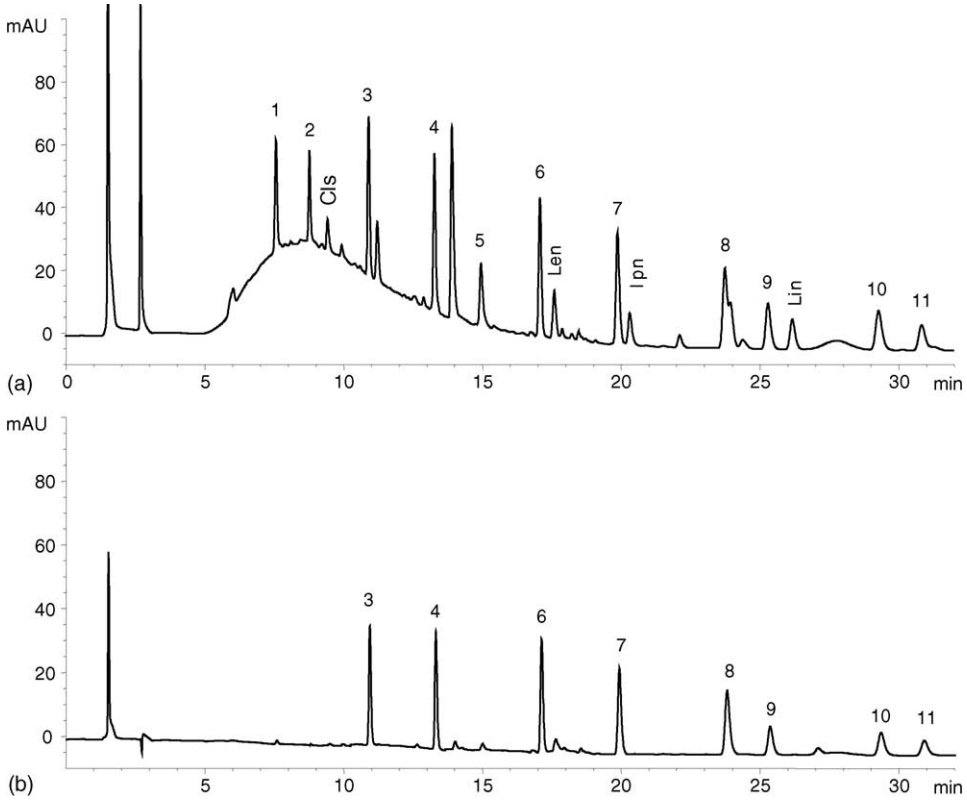


Fig. 5. Chromatograms of a river water sample spiked with 1.6 μg L^{-1} of triazine herbicides, dealkylated and hydroxylated metabolites and other non-related herbicides. (a) Preconcentration by SPE with LiChrolut EN cartridge; (b) Preconcentration by SPE with LiChrolut EN and a clean up step with the propazine-MIP. Conditions and peak numbers as in Fig. 4. Other peaks: (Cls) chlorsulfuron; (Len) lenacil; (Ipn) isoproturon; (Lin) linuron.

When only the LiChrolut EN sorbent was used (Fig. 5a), the signal characteristic of the presence of humic acids was recorded, and chromatographic peaks for the fifteen analytes with which the water sample had been spiked were obtained. When the extract eluted from LiChrolut EN was evaporated, redissolved in 2 mL of toluene, and passed through the propazine-MIP, a cleaner chromatogram was obtained (Fig. 5b) and the peaks corresponding to the methylthio-triazines were observed, while no signals corresponding to DIHA, DEHA and HT were seen.

The recoveries and precision obtained upon analysing river water samples and a standard sample in toluene were compared using an unpaired two-tailed *t*-test. For a level of significance of 0.05, the results indicated that there was a significant difference in the recoveries obtained in both cases

Table 5
Recoveries obtained using a propazine-MIP as (A) clean-up sorbent for the extract of a river water sample, and (B) for direct preconcentration of a standard solution in toluene

Analyte	(A) MIP as clean-up sorbent[a]		(B) Standard solution in toluene[b]	
	Recovery (%)	RSD[c] (%)	Recovery (%)	RSD[d] (%)
DIA	64	7	96	9
DEA	62	9	93	8
Simazine	62	9	87	10
Atrazine	60	9	79	13
Propazine	67	10	83	9
Terbutylazine	51	10	77	10
Prometryn	54	11	89	7
Terbutryn	52	12	84	9

[a] River water (250 mL) spiked at 1.6 μg L^{-1} of each compound, preconcentrated through LiChrolut EN. Eluate evaporated and redissolved in 2 mL of toluene, then passed through propazine-MIP.
[b] Standard solution in toluene (2 mL) at 200 μg L^{-1} of each compound, directly passed through propazine-MIP.
[c] $n = 6$.
[d] $n = 4$.

(Table 5). Accordingly, it can be affirmed that there is a clear matrix effect attributable to other components of the matrix that are co-extracted on LiChrolut EN.

Comparison of the chromatogram in Fig. 5b with that obtained upon preconcentrating with mixtures of MIP and LiChrolut EN (Fig. 4b) shows that propazine-MIP is very effective as clean-up sorbent and allows the determination of the dealkylated metabolites and the chloro- and methylthio-triazines. However, it has the disadvantage that it is necessary to perform two steps: one involving preconcentration and the other cleaning. The use of mixtures of LiChrolut EN and MIP permits the preconcentration of chloro-triazines and dealkylated, hydroxylated and hydroxy-dealkylated metabolites in a single step. However, under these conditions it is not possible to determine methylthio-triazines.

4. Conclusions

A molecularly imprinted polymer obtained by precipitation using propazine as template can be used for the determination of triazine herbicides and related metabolites in river water in three different ways. Any of the three configurations allows the determination of chlorotriazines and dealkylated metabolites.

When propazine-MIP was used as sorbent, the sample volume to be preconcentrated was less than 100 mL. Matrix effects were only found for the hydroxylated metabolites DEHA and DIHA. When the samples of natural water had high hardness (around 40 °f) it was necessary to wash the MIP with HCl before the drying step.

To determine hydroxy dealkylated-metabolites it is necessary to use a sorbent that contains mixtures of MIP + LiChrolut EN. Under these conditions, the imprinted polymer provides selectivity and the LiChrolut EN allows the retention of DIHA and DEHA.

For the simultaneous determination of methylthio- and chloro-triazines in river water, it is necessary to perform a preconcentration on the LiChrolut EN sorbent, after a cleaning step using the imprinted polymer. In this configuration, propazine-MIP showed high selectivity towards chloro- and methylthio-triazines and dealkylated chloro-metabolites.

With all configurations studied the LODs were lower than 0.1 $\mu g\,L^{-1}$, the limit established by EU legislation as the maximum concentration allowed for individual pesticides in drinking water.

Acknowledgements

This work was supported by the MCYT-DGI (project BQU-2002-02314) and by the Consejería de Cultura y Turismo, Junta de Castilla y León, and the European Union (European Social Fund, Project SA044/02).

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