Determination of Triazines in Water Samples by High-Performance Liquid Chromatography with Diode-Array Detection

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Abstract

Triazines are widely used herbicides that can be detected in the environment at trace level. A preconcentration step is necessary to determinate them before analysis. In this study, carbonaceous and polymeric adsorbents are compared with C_{18} for the solid-phase extraction of simazine, atrazine, and propazine in water samples in order to quantitate their levels by high-performance liquid chromatography using photodiode-array detection.

Introduction

The use of pesticides in agriculture has increased over the last several years. Such different products can be damaging to the human health and environment. The presence of pesticides in the environment has forced official international institutions to establish maximum concentration levels allowed in drinking water and foods (1). One of the most frequently employed herbicides is triazine atrazine (2). Most triazines are derived from *s*-triazine, a six-atom heterocyclic compound characterized by the presence of nitrogen atoms symmetrically arranged in the 1, 3, and 5 positions and various substituents in the 2, 4, and 6 positions. The persistence of triazines and their degradation products in soil, water, plant matter, and animals can be significant (3).

The 98/83/CE Directive (4), relating to the quality of waters for human consumption, establishes 0.1 µg/L as the highest concentration allowed for individual plaguicides and 0.5 µg/L for the total of plaguicides. These levels are not detected by the usual analytical techniques, thus the combination of analytical methods and preconcentration procedures that will achieve the enrichment of the analyte necessary for their determination in the environment.

The enrichment of trace triazine can be attained by liquid–liquid or liquid–solid procedures. Liquid–liquid extraction (LLE) has been the most employed procedure, but today, solid-phase extraction (SPE) is being applied as an alternative to LLE in many official methods of analysis established by the regulatory agencies from the U.S. and Europe. SPE has some advantages over LLE, such as the availability of different solid sorbents and the need for smaller volumes of organic solvents. However, the highly polar water-soluble degradation products are more difficult to extract in many organic solvents (5).

High-performance liquid chromatography (HPLC) has been used these last few years because of its ability to analyze both polar and nonpolar thermodegradable compounds without the need for a derivatization step (5). Triazines are easily determined by liquid chromatography (LC) using a diode-array–UV detector because of their strong absorbance at the 220-nm line, which gives detection limits almost equivalent to those obtained by gas chromatography (GC)–nitrogen–phosphorus detection, the analytical method initially used (6).

In this study, the determination of simazine, atrazine, and propazine (Figure 1) in water samples are concentrated by an SPE

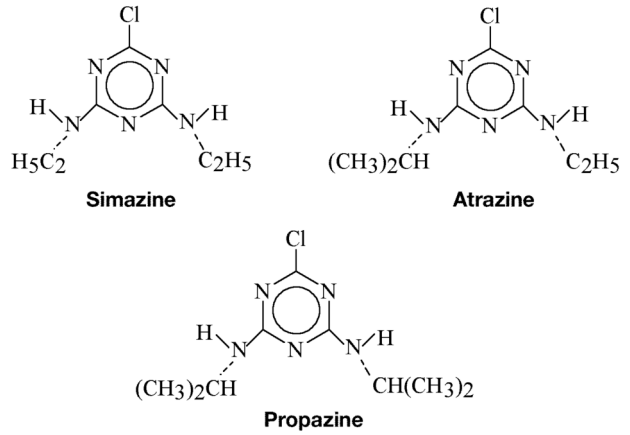

Figure 1. Chlorotriazine structures.

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step, offline, and followed by subsequent chromatographic analysis. V. Pichon in 1998 (7) recognized three kinds of sorbents for SPE. *N*-alkyl silica sorbents have been the universal extraction sorbents, being particularly appropriate for polar compounds in having log K_{ow} < 2.5–3. Additional sorbents showing a high capability to extract polar analytes are highly cross-linked styrene-divinylbenzenes (SDB) as well as carbon-based sorbents. The effectiveness of three kinds of sorbents in the SPE step has been studied and a reversed-phase HPLC with a diode-array detection method has been developed.

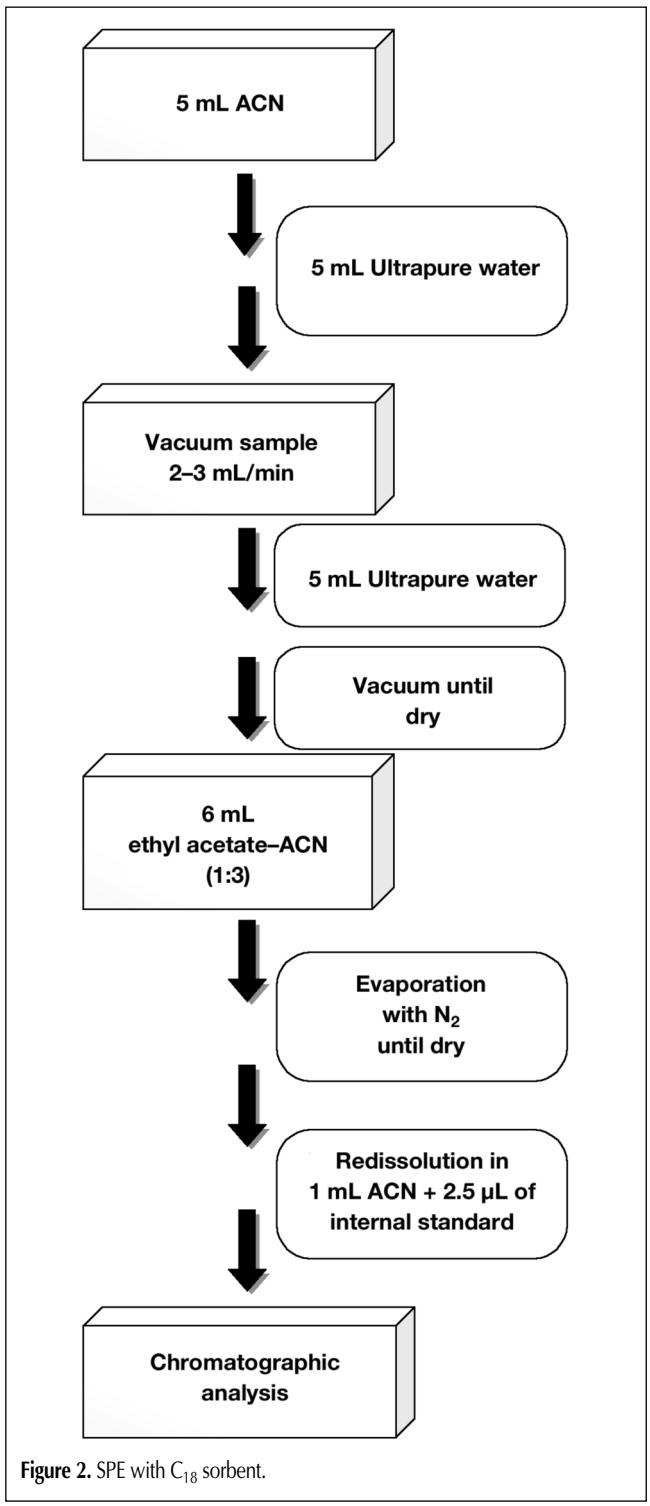

Figure 2. SPE with C_{18} sorbent.

Experimental

Materials and reagents

Acetonitrile (ACN) (HPLC grade) was obtained from Panreac Química S.A. (Barcelona, Spain). Potassium dihydrogen phosphate (KH_2PO_4) was from Merck (Darmstadt, Germany). Toluene for analysis was from Vorquímica S.L. (Vigo, Spain). Helium (C_{50})

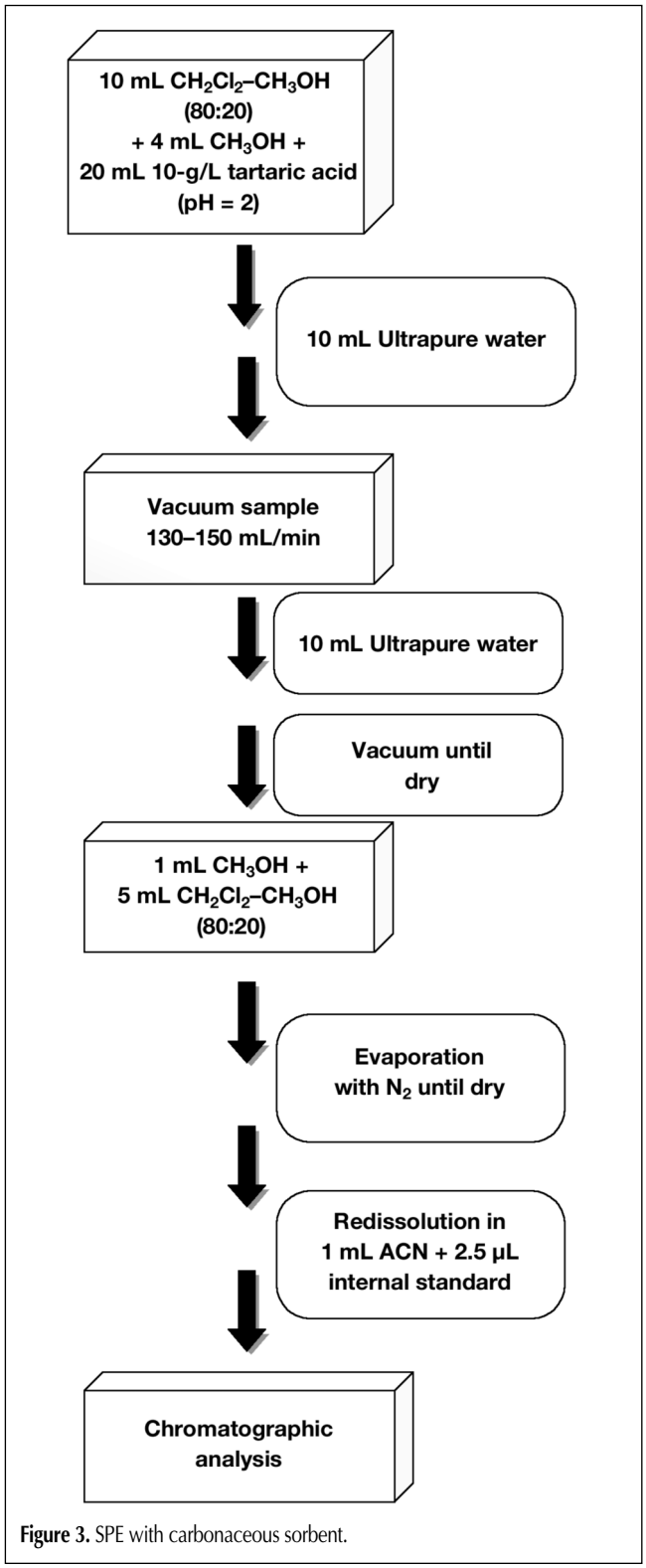

Figure 3. SPE with carbonaceous sorbent.

was from Carburos Metálicos (Barcelona, Spain) and was used for degassing the mobile phases.

C_{18} columns (900-mg w/2 frits) and Carbograph columns (carbonaceous sorbent, 12 cc, 1-g w/2 frits) were obtained from Lida Manufacturing Corp. (Kenosha, WI). Polymeric Resins HR-P (highly porous sorbent resin on the basis of polystyrene– divinylbenzene, 6 mL, 1 Kg Chromabond) was from Macherely-Nagel (Postfach, Düren, Germany). ACN used for UV–infrared (IR)–HPLC–HPLC prep-PAR instrumental analysis, dichloromethane used to stabilize with (~20 mg/L amylene for LC), and ethyl acetate used for UV–IR–HPLC–HPLC prep-PAR instrumental analysis were obtained from Panreac Química S.A. Methanol for LC was obtained from Merck. Tartaric acid crystallized pure was obtained from Probus-Merck (Darmstadt, Germany), and N_2 (C_{55}) was from Carburos Metálicos.

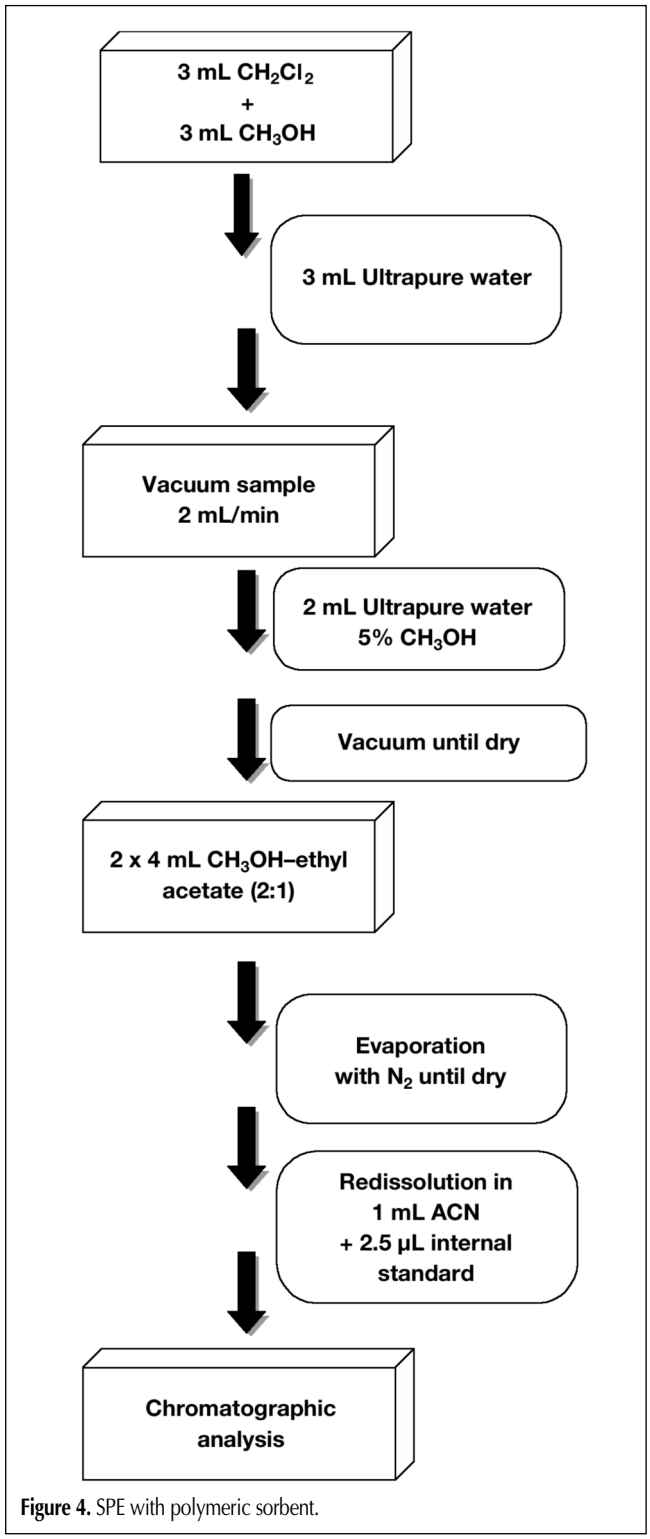

Figure 4. SPE with polymeric sorbent.

Instrumentation

Chromatography was performed with a modular HPLC system (Waters Corporation, SA, Milford, MA) equipped with a Waters 600 quaternary pump for HPLC, a Waters 600 solvents distribution unit, a Waters U6K injector, a Waters 996 diode-array detector, and a Millennium system Version 2.15 chromatography manager for acquisition and treatment of data. Column Symmetry C_{18} (5 µm, 150 mm) and precolumn Symmetry C_{18} were from Waters Corporation, SA. A Crison pH-meter and a Sartorius $\pm 1 \times 10^{-4}$ analytical balance were used.

Chromatographic determination

The chromatographic conditions used were as follows. The mobile phase was water–phosphate buffer (1µM, pH 6–7, 55:45). The flow rate was 1.5 mL/min, the injection volume 70 µL, and the internal standard toluene. Chromatograms were obtained at the 220-nm line and, when necessary, at the 260-nm line.

A 80-mg/L stock solution was obtained by dissolving 4.0 ± 0.1 mg of each triazine standard (Supelco, Bellefonte, PA) in 50 mL of ACN. A 2M HCl sample was added in order to improve the dissolution of the triazines in ACN (8).

The working standards were prepared from the 80-mg/L stock solution by dilution with ACN in 10-mL flasks. Ten microliters of 0.001% (v/v) toluene were added as the internal standard. Aqueous samples were spiked by adding known volumes of the triazine standards, resulting in 2- and 20-µg/L levels.

All of the solutions were maintained at 4°C in glass flasks protected from light (9).

SPE conditions

Three kinds of sorbents were employed: C_{18} (Junker-Bucheit et al. [10], Martin-Esteban et al. [11], and Ferrer et al. [8]); polymeric (Junker-Bucheit et al. [10], Aguilar et al. [12], and Lacorte et al. [13]); and carbonaceous sorbents (Berg et al. [14], Pichon et al. [15], and Di Corcia et al. [16]). The different steps of the process (passage of the sample through the sorbent, washing, and elution) were optimized. In order to improve the elution step and obtain the smallest volume of eluent, two kinds of experiments were performed. In the early investigations, mixtures of the elution solvents at different percentages were used to optimize their polarity. Then, the solvent volume was studied in order to settle the smallest solvent volume to desorb analytes completely. The optimized concentration step in the solid phase for each kind of sorbent is shown in Figures 2, 3, and 4.

Sample preparation

Samples were collected according to ISO 5667-3:1994 (17) using dark glass bottles previously washed with water and detergent, rinsed with distilled water, dried for 2 h at 105°C in a heater, and finally rinsed with ACN (the solvent used in the extraction step). According to Martín-Esteban et al. (1), the pH of the sample

must be adjusted to pH 5–9, then it must be stored at 4°C, extracted before 7 days, and the extract analyzed before 40 days. The pH of the samples was determined showing that the values for all of them were approximately 7–7.5. Samples were preserved in the refrigerator and filtered by using 0.45-µm Millipore (Bedford, MA) filters prior to the preconcentration step. According to Sabik et al. (2), the previous filtration of the sample does not affect the determination of triazines.

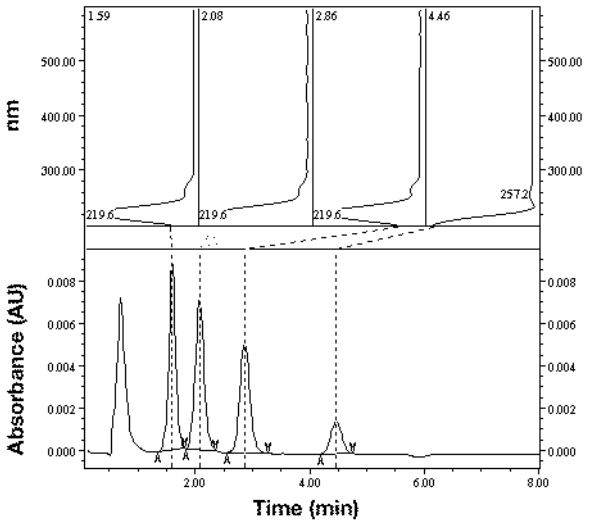

Figure 5. Chromatogram obtained with 0.2 mg/L dissolution and an injection volume of 70 µL. The mobile phase was 55:45 ACN–water–phosphate buffer (1 × 10–6M) with a 1.5-mL/min flow rate. The stationary phase was Symmetry C18 (5 µm, 150 mm, 220 nm).

Table I. Repeatability of the Chromatographic Method*

Concentration RSD (%)	1 mg/L		0.1 mg/L	
	$\%t_R$†	$\%A/A_{IS}$	$\%t_R$	$\%A/A_{IS}$
Simazine	0.24	5.7	0.22	15
Atrazine	0.18	5.6	0.33	11
Propazine	0.25	5.8	0.18	16

* $n = 10$.
† t_R, retention time.

Results and Discussion

Chromatographic method

The chromatogram of Figure 5 (carried out at a 1-mL/min flow rate and with an injection volume of 50 µL) shows that the elution order for the studied triazines is simazine (k' = 1.17), atrazine (k' = 1.72), and propazine (k' = 2.59). Noble (18) gives log K_{ow} = 1.51–2.26, 2.0–2.5, and 2.91–3.02 for simazine, atrazine, and propazine, respectively. This shows that the apolar character of the triazines increases with the length of the alkylic chain, which agrees with the elution order observed. The spectra corresponding with each peak showed a first maximum at 220 nm and a second one of less intensity at 260 nm. Therefore, the individual triazines must be distinguished by their retention times. The presence of a peak coeluting with the atrazine peak and interfering with its response was observed when standard solutions of small concentrations were injected. This peak seems to be a result of some impurity present in the ACN. Thus, the evaluation of the response of the atrazine must be made at the 260-nm line in order to avoid their interference.

Chromatographic parameters associated with the optimized conditions ($1 < k' < 5$, $\alpha < 1.14$) (19) show that they are suitable for the chromatographic analysis with regard to resolution and efficiency of the separation and commensurate with a short analysis time. In order to validate the chromatographic method, a study of the precision was carried out evaluating its repeatability and reproducibility. The evaluation of the repeatability of the chromatographic method was performed from the values

Table II. Parameters that Define the Graphic Chart

		Simazine (mg/L)	Atrazine (mg/L)	Propazine (mg/L)
Nominal level	Mean (A/A_{IS})	0.50	0.43	0.50
	SD*	0.10	0.09	0.08
Warning limits	Mean + 2SD	0.71	0.61	0.65
	Mean – 2SD	0.30	0.24	0.34
Control limits	Mean + 3SD	0.81	0.70	0.73
	Mean – 3SD	0.20	0.15	0.26

* SD, standard deviation.

Table III. Calibration Curve (10, 25, 50, 160, and 200 µg/L); Injection Volume (70 µL); and Flow Rate (1.5 mL/min) Used to Obtain the Calibration Graph by Linear Regression*

	a	b	r†	r^2	$S_{y/x}$‡	*x*LD§ (µg/L)	*x*LC** (µg/L)
Simazine	$-6.1 \times 10^{-2} \pm 2.8 \times 10^{-3}$	16 ± 0.91	0.9991	0.9983	0.0029	10	34
Atrazine	$2.2 \times 10^{-1} \pm 1.6 \times 10^{-3}$	7.4 ± 0.51	0.9988	0.9976	0.0303	12	41
Propazine	$-7.3 \times 10^{-3} \pm 2.1 \times 10^{-3}$	15 ± 0.68	0.9995	0.9989	0.0407	8.2	27

* $y = a + bx$; a = intercept; and b = slope.
† r, correlation coefficient.

obtained for 10 injections of a standard of the same solution using two different concentrations (1.0 and 0.1 mg/L) containing an internal standard. Table I shows a diminution in the variation coefficient between 76% and 85% for the 1.0-mg/L standard solution using toluene as the internal standard. For simazine and propazine, a diminution between 63% and 72% in the 0.1-mg/L standard solution was observed.

Peak-area reproducibility was evaluated by means of 10 injections of a 1.0-mg/L standard solution carried out on different dates over a month, giving the same relative standard deviation (RSD) of 17% for simazine, atrazine, and propazine. The values show the advantage of using an internal standard. In order to complete this reproducibility study, Shewmart graphics were obtained representing the variable of concern (A/A_{IS}) for each of the triazines versus time. Twenty determinations of the 1.0-mg/L standard solution (n = 3) were performed to define the middle value, the warning, and the action limits, taking into account the parameters that will affect the response of the instrument: different day, different operator, and different standard (two samples prepared with a month of difference were used) (Table II).

The calibration graph was obtained by linear regression (y = a + bx) for 10, 25, 50, 160, and 200 µg/L (Table III). The mean value of three measurements was calculated for each point. In order to improve the detection and quantitation limits, the initial conditions were changed (70-µL injection volume and 1.5-mL/min flow rate).

The values obtained for the chromatographic parameters and the precision of the chromatographic method were suitable. The detection and quantitation limits (8–12 µg/L and 27–41 µg/L, respectively) (Table III) were higher than those established by regulatory limits (0.1 µg/L), which confirms the need for a preconcentration step previous to the chromatographic analysis.

Solid-phase preconcentration

A study of the preconcentration for the three triazines was performed by evaluating the recovery for aqueous standard solutions. Table IV shows the results obtained for the preconcentration of 100 mL ultrapure water spiked with 2 µg/L each of the three triazines with the kinds of sorbents used.

The higher recovery percentages for the three triazines were obtained by using C_{18} or carbonaceous sorbent except for propazine, which showed a better recovery by using a polymeric sorbent. However, the smaller values of standard deviation and variation coefficient corresponding with the carbonaceous column were very similar for C_{18} and polymeric columns. The carbonaceous sorbent had also the advantage of a rapid analysis because it allows a high flow rate that cannot be achieved with the C_{18} or polymeric sorbents used (16).

Table IV. Recovery (%) of 100 mL of Ultrapure Water Spiked Until 2 µg/L of Simazine, Atrazine, and Propazine Results After SPE With C_{18}, Carbonaceous, and Polymeric Sorbents*

	C_{18}	Carbonaceous	Polymeric
Simazine	104 ± 7	99 ± 1	75 ± 8
Atrazine	104 ± 11	95 ± 6	87 ± 10
Propazine	105 ± 10	92 ± 3	100 ± 10

* n = 6.

Comparing these results with that of other authors, it can be concluded that similar and even higher recoveries have been obtained. Sabik et al. (2) obtained a 51–84% recovery by using carbonaceous cartridges in the analysis of a sequence of pesticides including simazine, atrazine, and propazine. Nouri et al. obtained a 94–109% recovery by using SDB cartridges (2). Barceló et al. (20) obtained a 80–125% recovery by using C_{18} silica disks in the analysis of 22 pesticides including simazine and atrazine.

Sample analysis

The preconcentration and chromatographic analysis methods described were applied in the determination of the presence of simazine, atrazine, and propazine in the Grande of Xubia River (northwestern Spain). Five sampling points were established along the river. Samples were taken in July in duplicate with the purpose of making the analysis of each sampling point by using carbonaceous and C_{18} columns. None of the analyses showed the presence of the triazines studied (Figure 6). For C_{18} experiments, 500 mL of the sample was used and 1000 mL was used for the carbonaceous analysis. Mills et al. (21) showed that a 100% retention can be obtained by using C_{18} cartridges containing 360 mg of 40-µm bonded silica, with a breakthrough volume starting at 750, 1250, and 2500 mL, respectively, for pure water samples. In regards to carbonaceous sorbents, Di Corcia et al. (16) analyzed 2 L of water samples. Therefore, sample volumes of 500 and 1000 mL for C_{18} and carbonaceous columns will be below the breakthrough volume.

Conclusion

By introducing a solid-phase preconcentration step, the initial volume passes from 1 L for a carbonaceous sorbent to a final volume of 1 mL, obtaining an enrichment factor of 1000. Thus, with a 100% recovery, the detection and quantitation limits of the method (8–12 µg/L and 27–41 µg/L, respectively) will improve

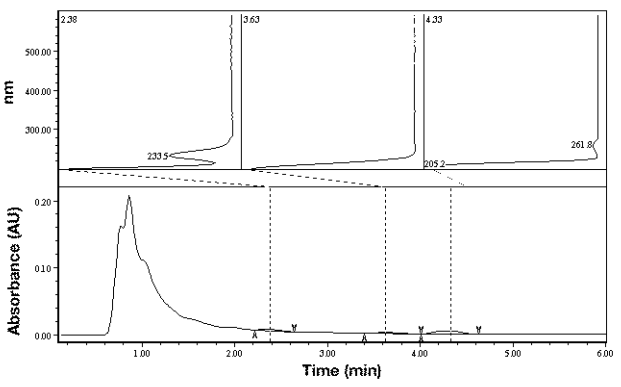

Figure 6. Chromatogram obtained after SPE with a carbonaceous sorbent of 1 L of the sample. The injection volume was 70 µL, and the mobile phase was 55:45 ACN–water–phosphate buffer (1 × 10^{-6}M). The flow rate was 1.5 mL/min, and the stationary phase was Symmetry C_{18} (5 µm, 150 mm, 220 nm).

between 0.008–0.012 µg/L and 0.027–0.041 µg/L, respectively. Because of this, the method could be used for the determination of samples with a concentration lower than the limit established by the legislation (0.1 µg/L for individual pesticides). Compared with other authors, it can be noted that the detection and quantitation limits are similar and even better than those obtained with other SPE–LC–UV detection analyses. Aguilar et al. (12) determined a sequence of pesticides including simazine and atrazine by SPE–LC–diode array coupled online, reaching a detection limit of 0.1 µg/L for both pesticides. Pinto et al. (22) determined simazine and atrazine by SPE–LC–UV with detection limits of 0.012 and 0.018 µg/L, respectively. The results obtained are even comparable with those obtained by GC. Choudhury et al. (2) determined 39 pesticides, including the studied chlorotriazines, by solid-phase microextraction–GC–MS obtaining detection limits ranging from 0.010 to 0.030 µg/L (2).

This method offers an important advantage with respect to multiresidual analysis such as its lower analysis time. For a 1-mL/min flow rate the chromatographic determinations take 8 min (and 6 min using a 1.5-mL/min flow rate). They contrast with their determination part of a multiresidual analysis, as for example in Junker-Buchleit et al. (10) in which propazine elutes at 43 min. Di Corcia et al. (16) reported atrazine elutes at 14.4 min. These results also show a faster elution than that indicated by Pinto et al. (22), who determined elution times for simazine and atrazine at 3.24 and 4.62 min, respectively.

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