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Use of solid-phase extraction and high-performance liquid chromatography for the determination of triazine residues in water: validation of the method

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Abstract

A method for determination of some triazine residues in water has been developed. The method involves concentration with C_{18} solid-phase extraction cartridges followed by high-performance liquid chromatographic analysis using a C_{18} column with UV detection at 230 nm, a mobile phase of methanol–water (60:40, v/v) at pH 4.6 (phosphoric acid) and a flow-rate of 0.8 ml/min. After optimization of the extraction and separation conditions, the method was validated. The method can be used for determination of atrazine, simazine, cyanazine and ametryn in water, within the international limits of 0.1 μg/l. © 2000 Published by Elsevier Science B.V. All rights reserved.

Keywords: Validation; Water analysis; Triazines; Pesticides

1. Introduction

Herbicides are used in agriculture to eliminate weeds that would otherwise compete with the desired crop [1]. The triazine herbicides form a wide group of substances used for pre- and post-emergence weed control. The use of triazines started in 1952 when Geigy (Basel, Switzerland) synthesized and investigated the use of triazine derivatives as possible herbicides [1,2]. The first triazine herbicide, chlorazine, was introduced in 1954, simazine was introduced in 1955, and others followed [1]. Today, more than 30% of all agricultural herbicides are triazines [2].

The more important triazines are the symmetric triazines (*s*-triazines) that have a six-membered heterocycle with symmetrically located nitrogen atoms and substitution in positions 2, 4 and 6 (Fig. 1). The stability of *s*-triazines is lower than that of benzene because the perfectly delocalized π-bond system is disturbed by the introduction of nitrogen atoms in the ring at positions 1, 3 and 5, with a subsequent increase in the electron density at these positions and a corresponding decrease in the electron density at positions 2, 4 and 6. Thus, nucleophilic substitution in the latter positions is facilitated [2]. *s*-Triazine names and their principal properties are primarily determined by the substituent in position 2, this is most often –Cl (the commercial name ending in -azine), $-SCH_3$ (-tryn) and $-OCH_3$ (-ton). The properties of the chloro derivatives present significant differences, compared to those of the other two groups, while the properties of methylthio- and methoxy-derivatives are quite similar. Positions 4 and 6 are usually occupied by substituted amino groups and exert substantially

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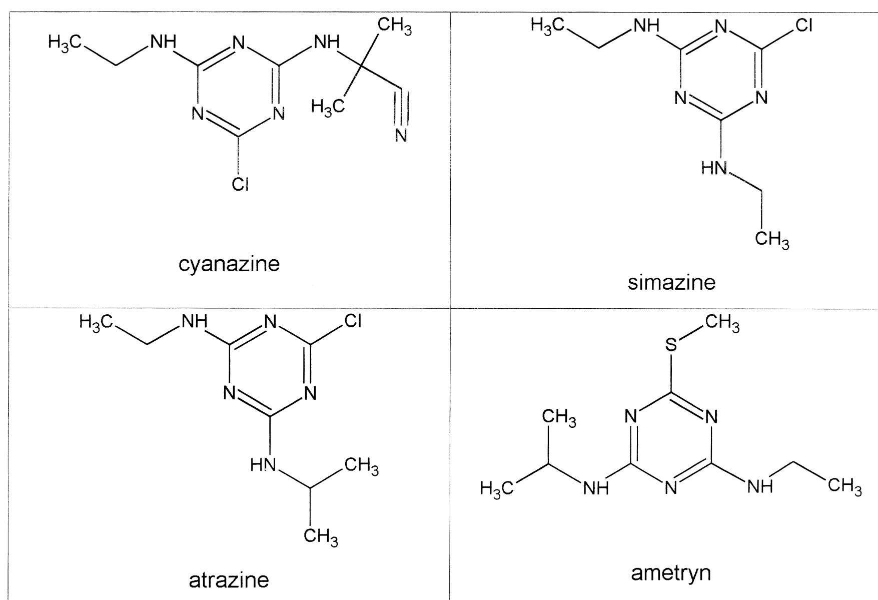

Fig. 1. Structure of triazine herbicides used in this work [28–30].

smaller effects on the properties. Thus, the water solubility of each triazine compound is dependent on the substituent in the 2-position and varies over the range 5–750 mg/l [1,2]. *s*-Triazine derivatives are among the most important selective herbicides. They, and their degradation products, are very toxic and highly resistant and survive many years in the soil [3], water, plants and animals [2]. Atrazine has been classified as a possible human carcinogen and has been banned in European countries [4]. Thus, the determination of triazines is very important for environmental control [4]. Because of this, the European Union established a maximum admissible concentration of 0.1 μg/l for individual pesticides in drinking water [4–10]. The US Environmental Protection Agency (EPA) considers the toxicity of the pesticides and has established different limits for each one [11]. These rigorous standards for drinking water purity require the availability of suitable analytical methods with high sensitivity, selectivity, accuracy and precision.

Gas chromatography (GC) and high-performance liquid chromatography (HPLC) are good options for monitoring triazines in water [4,10,12,13]. A disadvantage of GC is its limitation to volatile *s*-triazines; the hydroxy derivatives cannot be analyzed directly by GC [3]. HPLC is favored over GC for acidic pesticides, with high polarities, low volatilities and thermal instabilities, because GC can only be used following a prior derivatization step [13–16]. In addition, as triazines strongly absorb in the UV region 210–240 nm, they make excellent compounds for UV detection in liquid chromatography [1,13]. Diode-array detection (DAD) can be used to improve identification [13]. Thus, reversed-phase HPLC, with UV or DAD, is widely used for the analysis of triazines [17,18]. A mass spectrometric (MS) detector is also a powerful tool for confirmation of pesticides traces in water, including the triazines herbicides [19,20].

Because of the rigorous limits for water purity, methods for extraction and preconcentration of the pesticides present in water have become necessary. For these purposes, solid-phase extraction (SPE) is replacing traditional methods such as liquid–liquid extraction (LLE) [8], and has been widely used for extraction of water samples prior to analysis. SPE reduces sample handling, labor and solvent consumption [8–10,12,13,17]. The most popular SPE sorbent for pesticides in water is octadecyl (C_{18}) bonded silica.

In the present work, a simple and efficient method

for the determination of triazines in water was developed. After the method was developed, method validation [21–24] was applied, determining precision (repeatability), accuracy, recovery, detection and quantification limits, and linearity. The method was applied to some samples.

2. Experimental

2.1. Chemicals and reagents

Standards were obtained from: Novartis (atrazine, 97.7%; simazine, 98.3%; ametryn, 96.8%), and Cyanamid (cyanazine, 98.0%). The methanol (Ominosolv, Merck) was chromatographic grade. Sodium chloride (Mallinkrodt) and phosphoric acid (Synth) were analytical reagent grade. Water was purified with a Millipore Milli-Q Plus system.

The extraction cartridges were Envi C_{18}, Supelclean (Supelco), packed with 500 mg silica–octadecyl C_{18}.

2.2. Instrumentation

Chromatography was performed with a modular HPLC system equipped with a Rheodyne 7725i injector with a 10-μl loop, a Waters 510 pump, a UV–Vis absorbance detector (Waters Model 486) coupled to a CHROM PERFECT for Windows, version 3.03, program in a microcomputer, for acquisition and treatment of data. The column (150×3.9 mm I.D.) and guard column (20×3.9 mm I.D.) were Waters Nova-Pak C_{18}, 4 μm.

The pH of the mobile phase was determined using a Digimed, model DM21, pH meter, with glass and thermal compensation electrodes. The spectra of atrazine, simazine, cyanazine and ametryn in mobile phase were taken using a Waters liquid chromatograph, with a 10-μl loop, diode array detector (model 996) and MILLENNIUM system of data acquisition.

2.3. Procedure

The mobile phase was prepared volumetrically from individually measured aliquots of methanol and water. The pH was then adjusted to 4.6 with phosphoric acid. The mobile phase flow-rate was 0.8 ml/min and detection was performed at 230 nm. The column dead time, t_M, was determined using methanol as the unretained compound. All measurements were carried out at ambient temperature.

Stock solutions for calibration were prepared in methanol at 0.1 g/l. The samples were diluted in mobile phase and stored in the refrigerator ($T=4°C$).

Aqueous samples (250 ml) were fortified by addition of measured volumes of the stock solutions of the triazines, resulting in two levels of fortification, 0.1 and 1.0 μg/l. After adjusting the pH to <2, by addition of phosphoric acid, to increase the herbicide retention, and the ionic strength with 5 g of sodium chloride, the samples were mixed well and percolated through the SPE column under vacuum at a rate of 3 ml/min. Before sample application, the SPE column was conditioned with 10 ml of methanol and equilibrated with 10 ml of Milli-Q water. After the sample had passed through the column, the column was washed with 5 ml of Milli-Q water, the eluate discarded, and the sorbent bed dried under vacuum for 5 min. The analyte was then eluted with 1 ml of methanol. The solvent was evaporated to dryness under a stream of nitrogen and the residue was dissolved in 0.5 ml of mobile phase.

3. Results and discussion

Fig. 2 presents the UV absorption spectra for cyanazine, simazine, atrazine and ametryn. The spectra show that the maximum UV absorptions are: 220 nm (cyanazine), 222 nm (simazine), 222 nm (atrazine) and 222 nm (ametryn). Thus, 230 nm was chosen as a good wavelength for simultaneous analysis of these triazines, using methanol–water as a mobile phase.

Fig. 3 shows the separation of the triazines. The elution order of cyanazine, simazine, atrazine and ametryn can be explained by the structures of these triazines. The basicity of these herbicides increases with the substituents: $-Cl < -SCH_3 < -OCH_3$. Substitutions on positions 4 and 6 affect the basicity less, although this increases with the increase in the number of H substituents on the amino group and with the length of alkyl chain [2]. The inductive effect (−I) is strongest for $-Cl^-$, the substituent in position 2 on cyanazine, simazine and atrazine, in

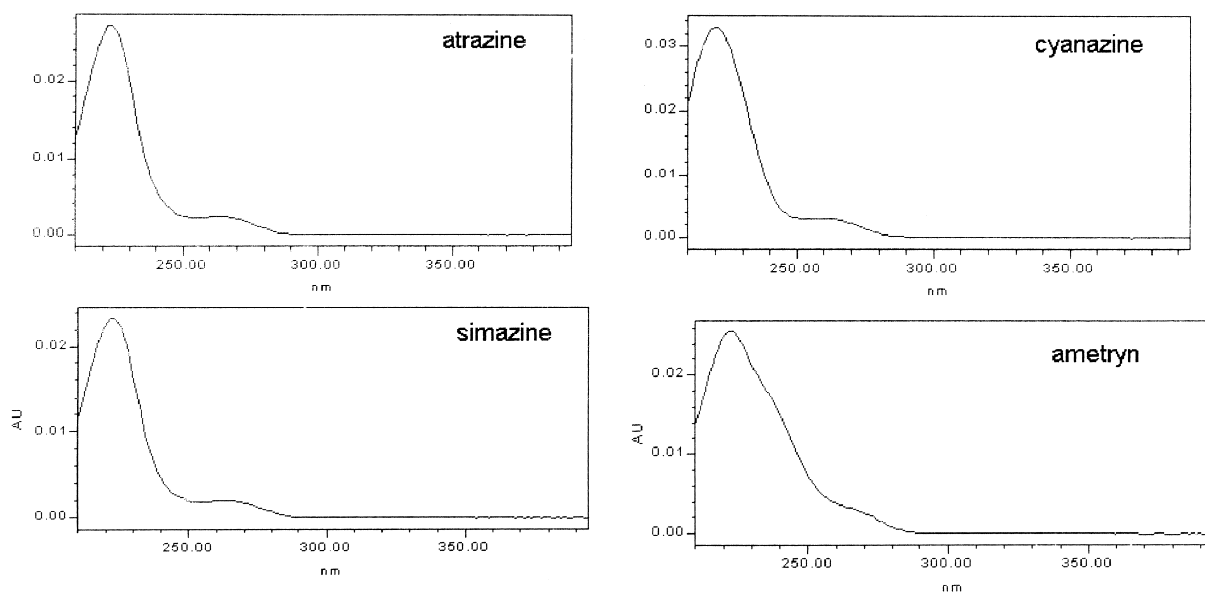

Fig. 2. UV DAD absorption spectrum for triazines in methanol–water (60:40, v/v).

relation the $-SCH_3$ group of ametryn and causes an increase in charge density in the triazine ring, in particular in the N atoms at the 1 and 3 positions, resulting in a more polar structure with lower affinity to the stationary phase. Thus, the Cl-triazines [cyanazine ($k=1.4$), simazine ($k=2.0$) and atrazine

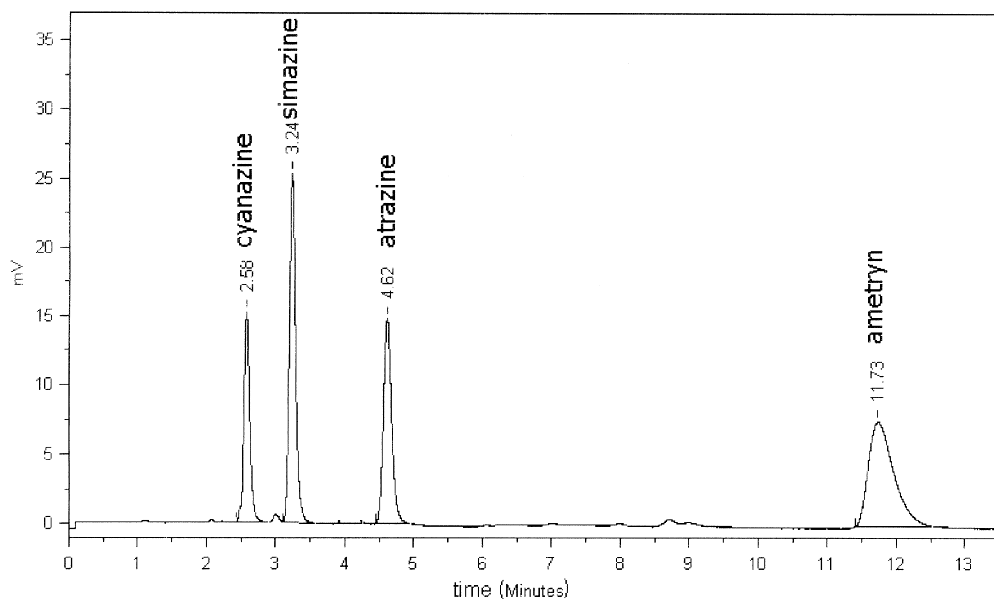

Fig. 3. Chromatogram showing triazines separation. Mobile phase: methanol–water (60:40, v/v), pH 4.6; flow-rate=0.8 ml/min; $\lambda=230$ nm; injection volume=10 μl.

($k=3.2$)] have shorter retention times and consequently smaller retention factors, k, than S-triazines [ametryn ($k=9.7$)] [2,18].

Comparing different compounds with the same substituent in the 2 position, the value of k can be explained considering the substituent groups in positions 4 and 6. Thus, the Cl-triazine k values have the order: cyanazine<simazine<atrazine. Cyanazine has the lowest retention because the $-NHC(CH_3)_2CN$ substituent group is more polar because of the –CN [18].

The values of pK_a (0.63 for cyanazine, 1.62 for simazine, 1.70 for atrazine and 4.10 for ametryn) show that a significant protonation occurs at pH<2 for Cl-triazines, and at pH<4 for S-triazines. Thus, for the pH 4.6 mobile phase used, significant protonation should not occur for any of these triazines. However, the pH value influences others parameters, such as resolution. This fact is also confirmed by the asymmetry values, which are higher for S-triazines in acidic mobile phases [17].

After defining the analytical conditions, tests were made on the recovery of triazines with C_{18} SPE extraction cartridges.

The effect of sample volume on SPE recovery is of crucial importance for samples of environmental interest. A sample volume higher than 200 ml is necessary in order to determine low levels of pollutants. The maximum sample volume from which 100% recovery can be achieved, above which the solute of interest begins to elute from the cartridge is called the breakthrough volume and it is determined by the retention factor of the solute in the sample solvent, that is, the sample solvent strength. For a reversed-phase sorbent, the breakthrough volume is a function of the hydrophobicity of the solute and the mass of sorbent used [25]. In the present work, the sample volume was 250 ml because this permitted determination of low levels of herbicides without loss due to breakthrough for cartridges of 500 mg of sorbent.

The recoveries using SPE and subsequent quantification were subjected to method validation. The definitions of each validation parameter are presented in detail in an earlier paper on the RP-HPLC quantification of bentazon [26].

The linear regression equation ($y=a+bx$) parameters for triazine calibration are presented in Table 1. Throughout the analytical curve, obtained over three orders of magnitude of concentration, linearities were evaluated by means of the ratio between signal (S) and concentration (Q), defined by $(S/Q_i)=(S_i-a)/Q_i$, where the ratio signal/concentration for the ith point of the analytical curve, $(S/Q)_i$, is calculated from the corresponding measured signal S_i, at the corresponding concentration Q_i and slope of the analytical curve (b).

Table 1
Analytical curve and linearity for triazines

Herbicides	Analytical curve[a]			Linearity (μg/l)
	a	b	r	
Atrazine	−468	110.7	0.99953	1120.0
Simazine	−1090	157.3	0.99981	1050.0
Cyanazine	−787	81.4	0.99898	1110.0
Ametryn	−3527	89.3	0.99948	1790.0

[a] $y=a+bx$, a=linear coefficient, b=angular coefficient, r=correlation coefficient.

In the absence of undetermined errors, i.e., with $r^2=1$, and within the linear range, it can be shown that $(S/Q)_i=b$ for all pairs of experimental values used to construct the curve. In the presence of undetermined errors ($r^2<1$), the real situation under experimental conditions, and within the linear range, $(S/Q)_i\approx b$. If $(S/Q)_i \ll b$ or $(S/Q)_i \gg b$, then it can be assumed to be out of the linear range. Points were considered to be in the linear range if their $(S/Q)_i$ values were in the interval $(1.00\pm0.05)b$, i.e., points whose signal:concentration ratios do not differ by more than 5% from the slope. This tolerance interval is based on IUPAC chromatography standards.

From Table 2, it is evident that the precision and accuracy are very good because both these measurements (~±4%) are well within the ±15% value admissible in literature at all concentrations [27].

The recovery tests were carried out on five replicates, permitting calculation of the relative estimated standard deviation (RSD). The recoveries were calculated using the equation [27]:

$$R=\frac{\text{mass of analyte after extraction}}{\text{mass of analyte added}}\cdot 100$$

The average results obtained for triazine re-

Table 2
Results for precision, accuracy and recovery for the SPE–HPLC determination of triazines[a]

Herbicides	Precision (%)	Accuracy (%)	Recovery (%)
Atrazine	2.3	0.3	76.0
Simazine	3.1	2.1	77.8
Cyanazine	1.5	−3.9	80.7
Ametryn	0.2	−2.2	97.4

[a] n = number of replicates = 5; level of fortification = 1 μg/l.

coveries (see Table 2) are very good, since a 70–110% recovery range is considered acceptable [27].

The limits of detection (LOD) and quantification (LOQ) were determined using a series of dilute triazine standard samples. From this series, a peak is selected whose height, h_s, is about 2–10 times larger than that of noise, h_n, ($C_s \approx 10$ μg/l). h_s is the height of the analyte peak measured from the average baseline level to the top of the peak, in mV, while h_n is measured at several points on the chromatogram within 10 peak widths of the t_R of analyte on a blank injection. The results of LOD and LOQ, before and after preconcentration, are presented in Table 3.

The method described here was applied to the analyses of tap water and of irrigation water. Some samples presented peaks with a retention time similar to the that of one of the herbicide standards (simazine). The results were confirmed by spectral comparison (DAD) and cochromatography. The level present was estimated as 0.1 μg/l (RSD = 0.4%).

4. Conclusion

The mobile phase, methanol–water (60:40, v/v), adjusted to pH 4.6 with phosphoric acid, is adequate for accurate analyses of cyanazine, simazine, atrazine and ametryn. The wavelength used (230 nm) permits good detection of the herbicide.

Table 3
Results for sensitivity (LOD and LOQ) of triazines

Herbicides	LOD (μg/l)	LOQ (μg/l)	LOD[a] (μg/l)	LOQ[a] (μg/l)
Cyanazine	4.9	14.8	0.0098	0.024
Simazine	6.2	18.6	0.012	0.037
Atrazine	9.3	27.9	0.018	0.055
Ametryn	16.7	50.1	0.034	0.10

[a] LOD and LOQ after 500-fold preconcentration; n = 5 for all measurements.

The results obtained for calibration, recovery, linearity, LOD, LOQ, precision and accuracy show that this is an efficient and simple method for the determination and quantification of these triazines in water samples. The total analysis time, including SPE and HPLC, is about 100 min.

Considering the 500-fold preconcentration step obtained with SPE, the effective LOD and LOQ values are adequate to determine triazines in water, at concentrations lower than 0.1 μg/l, satisfying the international limits.

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