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Journal of Chromatography A, 671 (1994) 151–157

JOURNAL OF CHROMATOGRAPHY A

On-line preconcentration and ion chromatography of triazine compounds

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Abstract

An ion chromatographic method was developed for the determination of triazine herbicides in environmental samples. Considering the polar nature of triazines, a cation-exchange column with a multi-mode pellicular packing was used. Optimization of the eluent (organic modifier concentration and ionic strength) is described with reference to the separation, on-line clean-up and preconcentration procedure. Several enrichment materials were evaluated and detection limits below 100 ng/l were achieved with a reversed-phase silica. The determination of triazines in tap water and river water samples was performed with UV detection (220 or 263 nm).

1. Introduction

Triazine herbicides are widely used in agriculture and their determination is of great importance in environmental studies and water control. The residual amount of these compounds in rivers and ground waters is normally very low so that samples require preconcentration and clean-up procedures.

The first triazine preconcentration techniques were based on liquid–liquid extraction but owing to their high cost and long analysis times they were replaced by solid-phase extraction. The solid phases used for enrichment of triazines include cation-exchange resins, adsorbent copolymers, porous octadecylsilica and graphitized carbon black [1–4]. As natural samples usually require a matrix-removal step, double-trap systems have been developed [4]. They consist of two successive solid-phase extractions, which allow analytes to be retained and the concentration of interferents to be reduced as a final result. Such methods are usually off-line and only a few studies have integrated on-line preconcentration and determination in the same apparatus [5,6].

The methods actually used for the determination of trace amounts of triazines include gas chromatography [3,7] and high-performance liquid chromatography (HPLC) [8–13]. LC methods are to be preferred for polar or thermolabile analytes [8]. Liquid–liquid partition chromatography represents the most popular HPLC technique for triazine determinations with a variety of detection methods, including mass spectrometry [14]. A reversed-phase ion interaction study for the separation of triazines has recently been reported [15], but ion chromatographic (IC) separation is not usually considered in triazine determinations.

In this work, an IC separation method was developed and coupled with an on-line (single or

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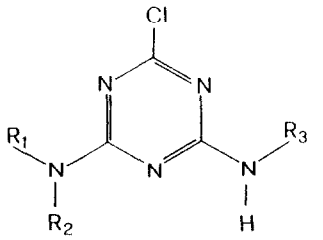
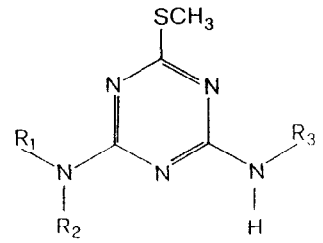
0021-9673/94/$07.00
SSDI 0021-9673(93)E1197-8



Names	R_1	R_2	R_3	pK_a
Simazine	H	Et	Et	1.7
Atrazine	H	Et	i-Pr	1.7
Propazine	H	i-Pr	i-Pr	1.7
Terbutylazine	H	Et	t-Bu	2.0
Cyanazine	H	Et	C-C=N CH3	1.0



Names	R_1	R_2	R_3	pK_a
Ametryne	H	Et	i-Pr	4.1
Prometryne	H	i-Pr	i-Pr	4.1
Terbutryne	H	Et	t-Bu	4.3

Fig. 1. Structures and pK_a values of Cl- and S-triazines. Et = Ethyl; i-Pr = isopropyl; t-But = *tert.*-butyl.

double) trap system in order to determine trace amounts of triazine herbicides in natural waters. The analytes investigated included eight 1,3,5-triazines, containing either chlorine or sulphur (Fig. 1).

2. Experimental

2.1. Reagents

Acetonitrile (chromatographic grade), phosphoric acid, sodium hydroxide and sodium chloride were of analytical-reagent grade from Merck. All solutions were prepared with high-purity water obtained with a Milli-Q System (Millipore, Bedford, MA, USA).

Reference standards for triazines were obtained from Riedel-de Haën (Seelze, Germany) (98.0–99.9% purity).

Stock standard solutions of triazines (200 mg/l) were prepared in acetonitrile and stored in the dark at 4°C. Working standard solutions were obtained daily by successive dilutions with water of the stock standard solutions.

The eluents, filtered and degassed under vacuum, were acetonitrile–water mixtures containing sodium phosphate buffer (1.0 m*M* H_3PO_4 + NaOH up to pH 4.5).

2.2. Apparatus

An LC 5000 liquid chromatograph (Varian, Walnut Creek, CA, USA) equipped with a Rheodyne injector and a UV 100 spectrophotometric detector was used. A 100-μl loop was used throughout. Chromatograms and data were registered with a Vista 401 data system. A second Rheodyne injection valve was used as a switching valve in the double-trap preconcentration system. Samples were preconcentrated with a DQP-1 pump (Dionex, Sunnyvale, CA, USA).

2.3. Procedure

The separation column was a Dionex OmniPac PCX 500 (250 × 4 mm I.D.). The preconcentration columns investigated for on-line optimization were OmniPac PCX 500 Guard (50 × 4 mm I.D.) (Dionex) and silica-based microcolumns (4 × 4 mm I.D.) LiChrospher 100 RP-18 (5 μm), LiChrospher 100 RP-8 (5 μm), LiChrospher 100 Si 60 (5 μm), LiChrospher 100 CN (5 μm) and LiChrospher 100 DIOL (5 μm) (all from Merck). Other preconcentration columns were obtained by packing a Merck cartridge holder (20 × 3.5 mm I.D.) with 0.16 g of Supelclean Envi-18 (40–60 μm) or with Supelclean Envi-Carb (Supelco, Bellefonte, PA, USA).

Preconcentration flow-rates were 4.0 ml/min for the silica-based microcolumns and 2.0 ml/min for the polymer-based cation-exchange col-

umn. After the sample loading (100.0 ml), the preconcentration column was rinsed with 10.0 ml of high-purity water. The Supelclean Envi-18 stationary phase was activated before use by washing with 10 ml of hexane–diethyl ether (50:50, v/v) followed by 6 ml of methanol and rinsing with 6 ml of water.

After optimization, the eluent composition was acetonitrile–buffer (70:30, v/v), the buffer being 1.0 m*M* phosphate (pH 4.5) containing 30 m*M* NaCl; the flow-rate was 0.7 ml/min.

Unless stated otherwise, UV detection was performed at 220 nm.

3. Results and discussion

3.1. Ion chromatographic separation

The analytes, according to their pK_a values (Fig. 1), may be in cationic form at appropriate pHs. The cation-exchange column chosen for the determination of triazines utilizes a multi-mode pellicular packing to combine an ion-exchange and a reversed-phase mechanism. Several parameters, *i.e.*, pH, dielectric constant, ionic strength and organic modifier concentration, affect the separation of triazines and were considered in eluent optimization. An acidic pH was chosen in order to take advantage of the ion-exchange mechanism.

Chromatographic retention times (t_R) were the means of triplicate determinations and the dead time (t_0) was evaluated by injection of water (water dip), taken as an unretained peak.

As expected, the k' values decrease sharply with increase in acetonitrile concentration, indicating a substantial contribution of liquid–liquid partitioning to the retention mechanism. The additional contribution of an ion-exchange mechanism allows greater flexibility than that obtained with reversed-phase columns during the optimization of the separation procedure. In this instance, in comparison with previous HPLC results [15], the IC separation shows an inversion for the retention order of ametryne and terbutylazine which can be related to their pK_a values. Fig. 2 shows the variation of k' for

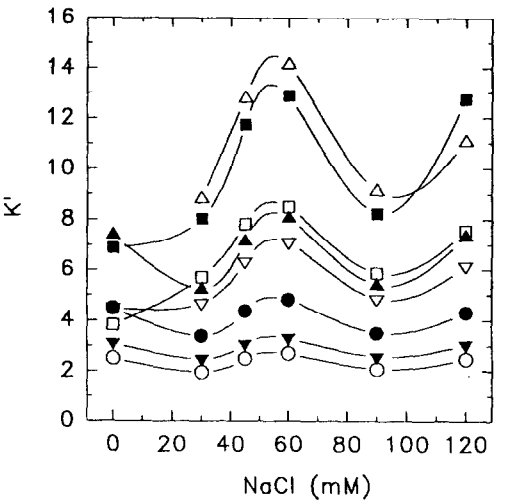


Fig. 2. Effect of ionic strength on capacity factors (k') of triazines. ○ = Cyanazine; ▼ = simazine; ● = atrazine; ▽ = propazine; □ = ametryne; ▲ = terbutylazine; ■ = prometryne; △ = terbutryne. Chromatographic conditions: mobile phase, acetonitrile–buffer (60:40, v/v) containing NaCl as shown (buffer composition: 1.0 m*M* H_3PO_4 and NaOH up to pH 4.50); flow-rate, 0.7 ml/min; samples 100 μl; each species 6.0 mg/l.

triazines as a function of ionic strength. Sodium chloride was chosen as an ionic strength modifier taking into account its low absorbance at the detection wavelength. The complex chromatographic trends that the triazines show, are due to the different effects of ionic strength on the various species. The capacity factors of the more polar S-triazines (ametryne, prometryne and terbutryne) are affected by both a stronger ion-exchange competition and liquid–liquid partition equilibria. The two effects, concomitant to the ionic strength improvements, are opposite and tend to give lower and higher k' values, respectively. The retention of Cl-triazines is less influenced by ionic strength than S-triazines and shows, for low ionic strength values, opposite behaviour with respect to the S-triazines. The optimized eluent composition allows, without preconcentration, detection limits below 80 μg/l to be achieved (Table 1) with UV detection (220 nm). The values were decreased (1000-fold) to the ng/l level after the optimization of the clean-up and preconcentration procedure (see below). The linear calibration ranges for the triazines

Table 1
Detection limits and linear ranges ($r > 0.995$), without preconcentration, for triazines

Analyte	Detection limit (μg/l)	Linear range (mg/l)
Cyanazine	10	0.5–10
Simazine	20	0.5–6
Atrazine	20	0.5–10
Propazine	30	0.3–6
Terbutylazine	20	0.5–6
Ametryne	20	0.5–10
Prometryne	30	1–10
Terbutryne	80	1–10

studied are summarized in Table 1. Fig. 3 shows a typical chromatogram for all the investigated triazines, obtained with the stated eluent composition.

3.2. On-line preconcentration with single trap

For lowering the detection limits of triazines through an enrichment step prior to the chromatographic separation, an on-line preconcentration procedure was developed. For this purpose the 100-μl injection loop was replaced with a microcolumn and the enrichment efficiency was evaluated for different stationary phases. The efficiency represents the ability of the column to retain and to release quantitatively the analytes in both the loading and release steps. Moreover, the microcolumn should allow adequate matrix removal. The preconcentration recovery for the eight triazines was evaluated by comparing the peak areas obtained by direct injection (100-μl loop) of samples (2.0 mg/l for Cl-triazines and 5.0 mg/l for S-triazines) with those obtained by loading the preconcentration column (100.0-ml mixtures, 2.0 μg/l for Cl-triazines and 5.0 μg/l for S-triazines).

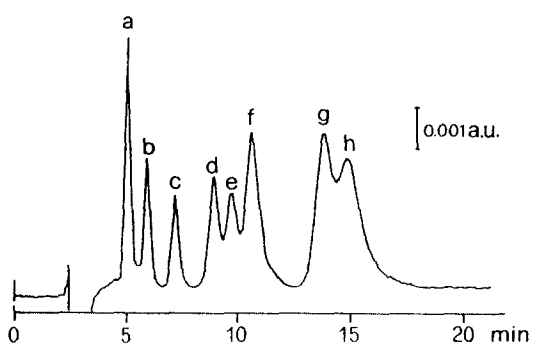


Fig. 3. Chromatogram of (a) cyanazine (100 μg/l), (b) simazine (100 μg/l), (c) atrazine (100 μg/l), (d) propazine (200 μg/l), (e) terbutylazine (100 μg/l), (f) ametryne (100 μg/l), (g) prometryne (200 μg/l) and (h) terbutryne (400 μg/l). Chromatographic conditions: mobile phase, acetonitrile–buffer (70:30, v/v) (buffer composition: 1.0 m*M* H_3PO_4 and NaOH up to pH 4.50, containing 30 m*M* NaCl); flow-rate, 0.7 ml/min; sample, 100 μl.

Several stationary phases and packings (listed under Experimental) were tested. Only Omnipac PCX 500 and Supelclean Envi-18 gave satisfactory results (see Table 2).

As the analytes show both a hydrophobic and an ionic retention mechanism, a multi-mode pellicular cation-exchange PCX guard column was used for preconcentration. The column packing is the same as in the separation column. It requires a low flow-rate in sample loading (2.0 ml/min) because of its high back-pressure due to the polymeric resin. Recoveries ranged between 25 and 86% and sub-μg/l detection limits (Table 2) were obtained. Acidified samples (pH 1.0) were also processed to enhance the ion-exchange contribution to the retention of cationic species but under these conditions hydrogen ion competition did not allow the yields to be improved.

Table 2
Recoveries and detection limits for triazines (three replicates)

Analyte	Recovery (%)		Detection limit (μg/l)	
	a	b	a	b
Cyanazine	30	93 ± 5	0.3	0.04
Simazine	56	97 ± 3	0.3	0.03
Atrazine	83	98 ± 1	0.4	0.04
Propazine	86	96 ± 2	0.8	0.08
Terbutylazine	25	99 ± 3	0.8	0.08
Ametryne	38	102 ± 7	1	0.07
Prometryne	80	104 ± 4	1	0.07
Terbutryne	34	104 ± 5	2	0.07

On-line preconcentration with (a) OmniPac PCX 500 Guard and (b) Supelclean Envi-18; samples, 100.0 ml.

The silica-based preconcentration columns may be used with a high sample flow-rate in the loading step and with a high back-pressure during elution. Therefore, in order to reduce the loading duration, a 4.0 ml/min loading flow-rate was maintained for such microcolumns without too high a back-pressure. Among the silica-based packings, only LiChrospher 100 RP-18 and Supelclean Envi-18 gave significant recoveries. LiChrospher 100 RP-18 gave a low recovery (10%) for all the analytes and this value did not change significantly on varying the sample pH. Supelclean Envi-18 gave quantitative recoveries for S-triazines and 93–99% for Cl-triazines (Table 2). The detection limits were 30–80 μg/l (signal-to-noise ratio = 3). The two hydrophobic silica materials differ with Supelclean Envi-18 having a higher surface area, and this explains the higher recoveries reached with this material.

Such an optimized on-line preconcentration method with a single trap was applied to a real sample (Turin tap water). A 100.0-ml sample was analysed but matrix interferences were too high at the maximum absorption wavelength of triazines (220 nm). The second absorbance maximum of triazines at 263 nm, which is characterized by lower intensity, was selected for detection, being free from interferences. Nevertheless, higher detection limits were obtained and all the triazines were detected at sub-μg/l levels. Fig. 4 shows the chromatogram obtained for a tap water sample analysed without or with addition of spikes (simazine, ametryne and terbutryne, 500 ng/l each).

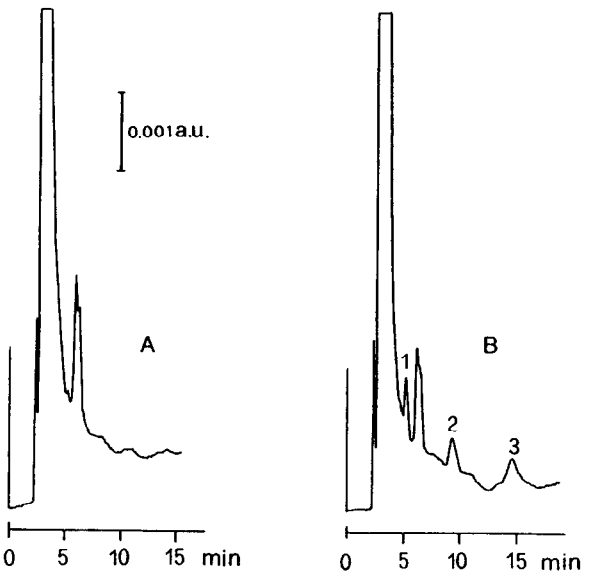


Fig. 4. Analysis of tap water with single-trap preconcentration and IC separation. (A) 100.0-ml samples as such and (B) spiked with (1) cyanazine, (2) propazine and (3) prometryne, 500 ng/l of each species. Chromatographic conditions: mobile phase, acetonitrile–buffer (70:30, v/v) (buffer composition: 1.0 m*M* H_3PO_4 and NaOH up to pH 4.50, containing 30 m*M* NaCl); eluent flow-rate, 0.7 ml/min; sample loading flow-rate, 4.0 ml/min; detection at 263 nm.

3.3. On-line preconcentration with double trap

In order to achieve good detection limits also in the presence of interferents usually found in natural waters, *e.g.*, river waters, a double-trap preconcentration system was developed. The system (Fig. 5) utilizes the previous column (Supelclean Envi-18) coupled with a LiChrospher 100 RP-18 microcolumn which is characterized by a high retention ability for lipophilic compounds and shows a very low (<10%) retention for triazines.

Other double-trap on-line enrichment procedures have been described [5,6]. Both methods use a combination of reversed-phase and ion-exchange traps, whereas in this work two reversed-phase adsorbents were used: the first retains all lipophilic compounds while the second is a specially treated reversed-phase material for trapping triazine derivatives.

Fig. 5 shows the loading and eluting procedures. When the two injection valves are in the load position (Fig. 5a) the sample passes through both columns. The LiChrospher 100 RP-18 microcolumn retains the organic liphophilic substances while allowing triazines to reach the second Supelclean Envi-18 microcolumn, where they are preconcentrated. The system is then rinsed with 10 ml of high-purity water, both Rheodyne values are switched to the injection position (Fig. 5b) and only the triazine analytes are injected towards the separation column by exclusion of the LiChrospher column. The latter

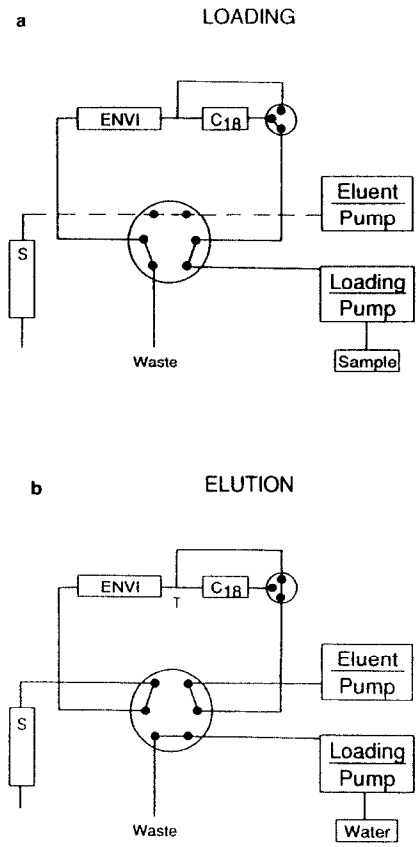


Fig. 5. Block diagram of the double-trap preconcentration apparatus in (a) the loading mode and (b) the elution mode.

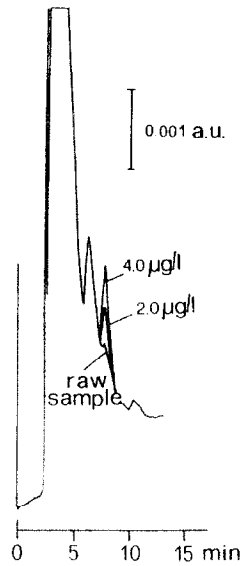


Fig. 6. Analysis of tap water with double-trap preconcentration and IC separation. 100.0-ml samples as such and spiked with atrazine (2.0 and 4.0 μg/l). Chromatographic conditions: mobile phase, acetonitrile–buffer (70:30, v/v) (buffer composition: 1.0 m*M* H_3PO_4 and NaOH up to pH 4.50, containing 30 m*M* NaCl); eluent flow-rate, 0.7 ml/min; sample loading flow-rate, 4.0 ml/min; detection at 263 nm.

microcolumn is cleaned by passing the eluent for 2 min before the next analysis.

The on-line double-trap system was applied to river water analysis (Po river, Turin). Fig. 6 shows the chromatogram obtained by loading 100.0-ml samples injected as such or after addition of spikes (2.0 and 4.0 μg/l of atrazine). Samples were filtered (0.45 μm) before the analysis.

4. Conclusions

The developed IC procedure is suitable for triazine detection and determination at levels down to 10 μg/l. By coupling on-line clean-up and preconcentration procedures the method permits the trace analysis of river water samples, also at a less sensitive wavelength for UV detection, as removal of spectral interferences from sample impurities can be achieved with the treatment. However, it should be noted that the occurrence of "matrix" peaks in real samples with a heavy matrix could affect the detection limits reported in Table 1. The use of gradient elution might improve the chromatographic resolution of the present procedure (shown in Fig. 3), especially for those samples with the concomitant presence of all triazines.

5. Acknowledgements

Financial support from the Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST, Rome) and from the Italian National Research Council (CNR, Rome) is gratefully acknowledged.

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