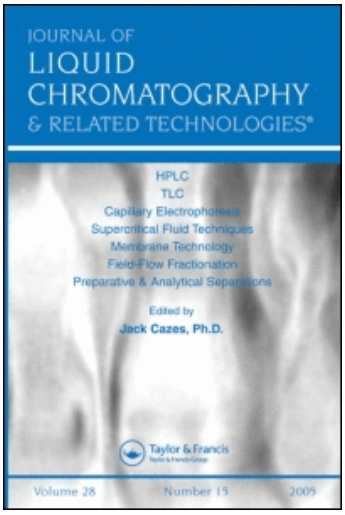

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SEPARATION OF TRIAZINE HERBICIDES BY ION-INTERACTION HPLC AND APPLICATION TO SURFACE WATERS

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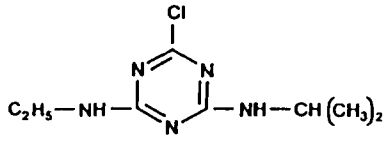
ABSTRACT

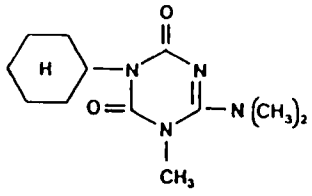
An ion-interaction reverse phase chromatographic method is presented for the separation and determination of atrazine, hexazinone, prometryn, propazine, simazine, terbuthylazine. The separation is carried out in isocratic conditions on a octadecyl silica stationary phase, using an hydroorganic solution (acetonitrile/water 45/55) of 5.0 mmol/L octylamine o-phosphate at pH 6.4 as mobile phase, with spectrophotometric detection at 230 nm. The detection limit (around 1.0 μg/L) reached without derivatization makes the method suitable for the analysis of surface waters without preconcentration. Application to river and lagoon water samples are reported.

INTRODUCTION

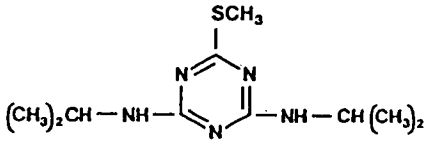
Triazine herbicides, widely used as pre-emergence weed-killers, are characterized by a very high chemical stability, a low water solubility, a low volatility and a high trend to be absorbed in organic and clayey colloids. All these properties lead to a consequent long persistence in the environment.



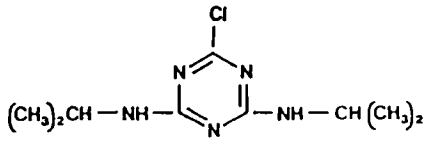
atrazine



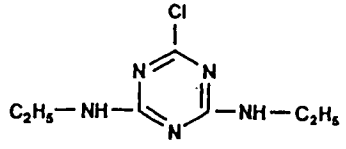
hexazinone



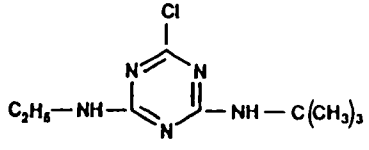
prometryn



propazine



simazine



terbuthylazine

Figure 1. Chemical structures of the analytes.

Their toxicity, expressed as oral LD50 for rats, ranges from 182 (cyanazine) to more than 5000 mg/Kg (simazine, propazine, anilazine)[1,2].

As concerns structure, the triazine derivatives generally used as weed-killers are symmetric triazines in which two carbon atoms of the aromatic ring are substituted with two alkylamine- groups and the third by a chloro-, a methoxy- or an azido- group. About twenty five triazine herbicides are used, being atrazine and simazine the most widely employed[2].

Preconcentration methods are often necessary before the analytical determination. They make use of supercritical fluid extraction[3-5]; off-line[6-10] and on-line[11-14] solid-phase extraction with various sorbents; liquid-liquid extraction[15-16]; microwave techniques[17], size-exclusion chromatography[18].

The chromatographic techniques are the most widely used for triazine herbicide analysis: gas chromatography with thermoionic[19], atomic emission[20], selective N and P[5, 6, 18, 21-23], mass spectrometry[6, 13, 22, 24] detection systems and HPLC analysis with UV detection[5, 7-10, 2, 16, 18, 25]. Examples are also reported[26] of gas-liquid chromatography, of HPLC thermospray MS[11] and MS-MS[27] methods.

In this paper an ion-interaction chromatographic method which permits detection limit of around 1.0 μg/L without preconcentration is presented. The method is applied in the separation of terbuthylazine, prometryn, atrazine, hexazinone, simazine and propazine (chemical structures reported in Figure 1).

EXPERIMENTAL

Chemicals and Reagents

Ultrapure water from Millipore MilliQ system was used for the preparation of the solutions. Sodium nitrate was analytical grade Merck reagent. Prometryn, propazine, simazine, terbuthylazine, hexazinone, atrazine were analytical grade LabService Analytica reagents. Octylamine, ortho-phosphoric acid and HPLC grade acetonitrile were Fluka chemicals.

Apparatus

The chromatographic analyses were performed with a Merck-Hitachi Lichrograph chromatograph Model L- 6200 equipped with a two channel D-2500 chromato-integrator interfaced with a UV-Vis detector L-4200 and with a conductivity detector with temperature control L-3720 from the same firm.

Absorbance measurements were performed with a Hitachi model 150-20 spectrophotometer.

For pH measurement a Metrohom 654 pH-meter provided with a

combined glass-calomel electrode was employed.

Chromatographic Conditions

A Spherisorb Phase Separation S5 ODS-2 (5 μm, 250 x 4.6 mm) cartridge column was used as the stationary phases together with a guard pre-column Merck Lichrospher RP-18 (5 μm).

In the optimization study the mobile phases were prepared at different concentrations of octylammonium o-phosphate and acetonitrile and brought to pH 6.4 with o-phosphoric acid. The pH value so obtained in the hydroorganic solution is known as operational pH. The optimal conditions are: 5.0 mmol/L octylammonium phosphate in acetonitrile/water 45/55.

The chromatographic system was conditioned by eluting the mobile phase until a stable baseline signal was obtained; a minimum of 1 hour was necessary at flow rate of 1.0 mL/min. After the use, the column was washed by flowing a 50/50 v/v water/acetonitrile mixture (1.0 mL/min for one hour), and then acetonitrile (0.7 mL/min for 20 min).

Our results fit the model[28, 29] according to which the interaction reagent contained in the mobile phase is bound onto the surface of the stationary phase through adsorption and electrostatic forces, giving rise to an electrical double layer. The interaction properties of the original reverse phase packing material are therefore modified. The new surface is able to simultaneously retain anions and cations[30-32].

Preparation of Herbicide Standard Solutions

Standard solutions, 10.00 mg/L were prepared in acetonitrile for successive dilutions of 400.00 mg/L acetonitrile solutions and preserved in brown bottles at 4°C for a maximum time of a month; the work solutions were prepared in acetonitrile/water (30/70) just before the injection in the HPLC system.

Sampling and Storage of Surface Waters

Surface water samples were collected in 1 L pyrex glass with wide neck and PP-screw-capwal bottles, previously washed with 0.2 mol/L

hydrochloric acid and repeatedly rinsed with ultrapure water. During the sampling, bottles were rinsed twice with the sample water, then filled and tightly capped. The entire sample was immediately filtered in the laboratory through Millipore 0.45 μm filters and stored at 4°C. All the analyses were performed within three days.

RESULTS

Table 1 shows for the triazine derivatives here investigated, the absorptivity values evaluated at 230 nm (which wavelenght offers the best average sensitivity in the separation) and the absorptivities at the wavelenght of the maximum absorbance. In order to obtain the best resolution and sensitivity in the lowest total analysis time, the experimental conditions of organic modifier, ion-interaction reagent concentration and flow-rate were optimized.

Table 1

Absorptivity Values at the Wavelenghts of Maximum Absorbance and at 230 nm.

	λ_{max}	ε_{max} (L mol^{-1} cm^{-1})	ε_{230nm} (L mol^{-1} cm^{-1})
atrazine	220	$(3.72\pm0.05)10^4$	$(2.26\pm0.04)10^4$
hexazinone	244	$(1.40\pm0.03)10^4$	$(8.98\pm0.11)10^3$
propazine	220	$(3.11\pm0.03)10^4$	$(1.72\pm0.04)10^4$
prometryn	220	$(4.26\pm0.05)10^4$	$(3.60\pm0.04)10^4$
simazine	220	$(3.24\pm0.05)10^4$	$(1.71\pm0.02)10^4$
terbuthylazin	222	$(3.93\pm0.06)10^4$	$(2.51\pm0.04)10^4$

Figure 2 shows, as an example, the separation of a mixture containing hexazinone, simazine, atrazine, propazine, terbuthylazine, prometryn, at concentration of 6.0 μg/L each, obtained in the optimized conditions, namely: octylammonium o-phosphate 5.0 mmol/L in acetonitrile/water (45/55), flow rate: 0.7 mL/min.

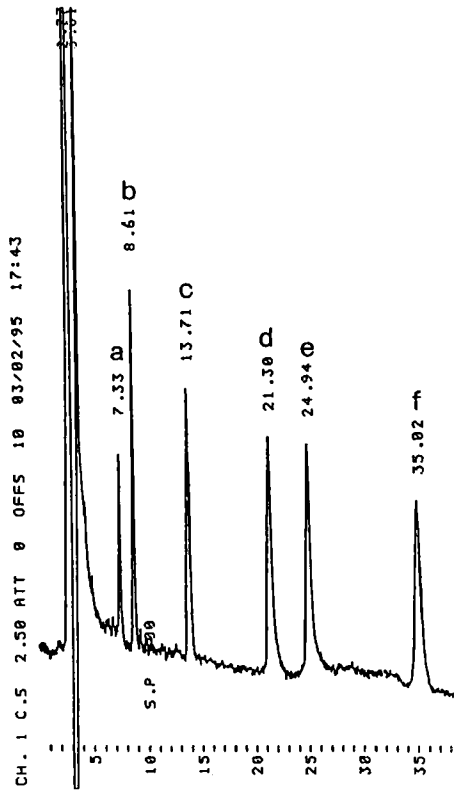


Figure 2. Chromatogram of the standard mixture. a: hexazinone, b: simazine, c: atrazine, d: propazine, e: terbuthylazine, f: prometryn, 6.0 μg/L each. Stationary phase: Phase Separation Spherisorb 5S ODS-2, 250 x 4.6 mm (5 μm) cartridge-type column, mobile phase: 5.0 mmol/L octylammonium o-phosphate in acetonitrile/water (45/55), pH 6.4; flow rate 0.7 mL/min. Spectrophotometric detection at 230 nm.

Calibration Curves

Under the optimized conditions the calibration curves reporting the peak areas (as evaluated by the integrator) vs standard concentrations were built. Standard concentrations range between 5.0 and 40 μg/L for all the pesticides.

Table 2

Slopes (m) of the Regression Equation y= mx and Correlation Coefficients (r^2) of the Plot Analyte Peak Area (y) vs Analyte Standard Concentration (x).

Analyte	m	r^2
atrazine	$(8.15\pm0.05)\ 10^2$	0.9996
hexazinone	$(2.50\pm0.04)\ 10^2$	0.9979
propazine	$(9.72\pm0.13)\ 10^2$	0.9992
prometryn	$(1.47\pm0.01)\ 10^3$	0.9997
simazine	$(8.97\pm0.06)\ 10^2$	0.9996
terbuthylazine	$(1.41\pm0.01)\ 10^3$	0.9998

In all cases linear plots were obtained: Table 2 reports the regression equation and the correlation factors and Table 3 reports the detection limits (*d.l.*), calculated through the sensitivity *S* (expressed as the peak area given by the integrator for 1 μg/L solution) and the evaluation in the chromatogram of a peak area (*a*) corresponding to an average signal to noise ratio = 3 (*d.l.* = *a*/*S* (μg/L)). Detection limits resulted of 3.0 μg/L for hexazinone and equal or lower than 1.0 μg/L for the other herbicides.

Application of the Method to Surface Water Samples

Figures 3-5 (A) show the chromatograms recorded under the optimized conditions for some samples of surface waters and namely samples of Po and Dora Riparia rivers, which flow near Turin and collect effluents from agricultural zones, and a sample of Venice lagoon water, collected near the outlet of the Dese River. No herbicide, at least at the detectable level, seems to be present in all the samples studied.

Figure 3-5 (B) show the chromatograms of the same samples added with a mixture of hexazinone, simazine, atrazine, propazine, terbuthylazine, prometryn, at concentration of 8.0 (Figure 4) and 10.0 μg/L (Figures 3 and 5) each. The recovery yields, evaluatedc through standard addition method are

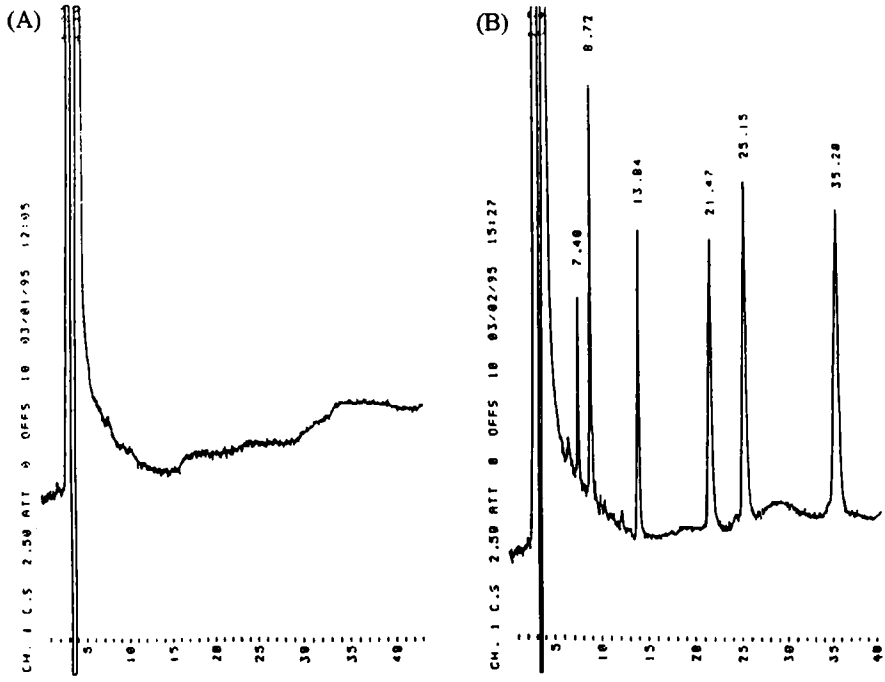


Figure 3. Chromatogram of a native (A) and spiked (B) sample of Po river water with 10.0 μg/L of hexazinone, simazine, atrazine, propazine, terbuthylazine and prometryn. Experimental conditions as in Figure 2.

always greater than 96% for all the herbicides, so thus showing a negligible matrix interference.

The results indicate that the method proposed can be successfully applied without preconcentration treatment in the analysis of surface waters, where the generally reported maximum concentration of herbicides is 30 μg/L[33]. The method proposed here does not require any pretreatment or derivatization process, except for filtration through a 0.22 μm membrane.

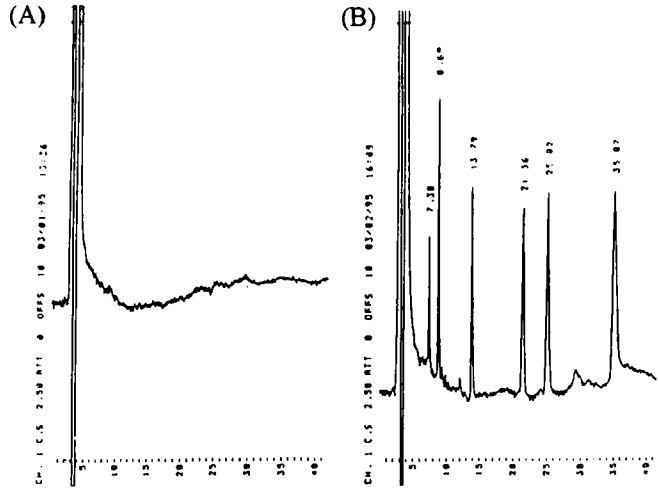


Figure 4. Chromatogram of a native (A) and spiked (B) sample of Dora Riparia river water with 8.0 μg/L of hexazinone, simazine, atrazine, propazine, terbuthylazine and prometryn. Experimental conditions as in Figure 2.

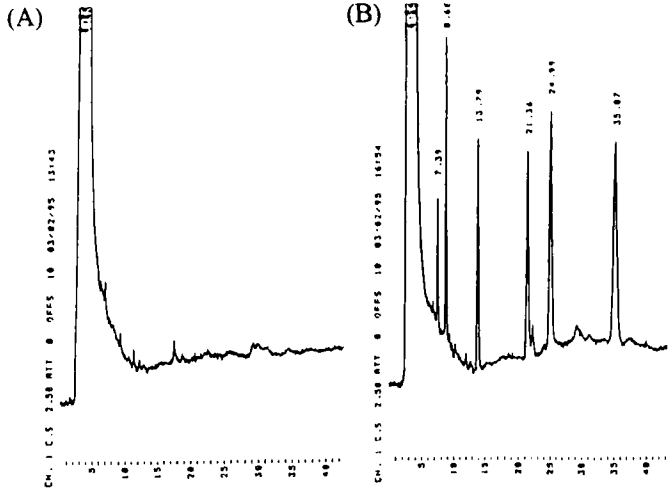


Figure 5. Chromatogram of a native (A) and spiked (B) sample of Venice lagoon water with 10.0 μg/L of hexazinone, simazine, atrazine, propazine, terbuthylazine and prometryn. Experimental conditions as in Figure 2.

Table 3

Detection Limits (μg/L) of the Analytes Investigated in the Optimized Chromatographic Conditions*

Analyte	Detection Limit (μg/L)
atrazine	1.0
hexazinone	3.0
propazine	0.8
prometryn	0.8
simazine	1.0
terbuthylazine	0.9

* Stationary phase: Phase Separation Spherisorb 5S 250 x 4.6 mm (5μm) cartridge column; mobile phase: octylamine 5.0 mmol/L in water/acetonitrile solution (55/45) brought to pH 6.4 with o-phosphoric acid, flow-rate 0.7 mL/min; spectrophotometric detection at 230 nm.

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