Determination of triazines and dinitroanilines in waters by high-performance liquid chromatography after solid-phase extraction

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ABSTRACT

A rapid and selective method for the simultaneous determination of triazines and dinitroanilines in real water matrices is suggested based on a preliminary adsorption on an RP-18 cartridge, an elution step using acetonitrile and HPLC separation with a Lichrosorb RP-Select B column and UV detection. The washing step cartridge is critical for triazines: terbutryn is eluted with quantitative recovery only after washing with an NH_3 solution. The degree of enrichment of the compounds studied has been determined: triazine recoveries are quantitative, while dinitroaniline recoveries are between 66% and 78% at the lowest fortification level. The detection limits for the ten herbicides are in the range 0.03–0.1 μg/l. The analysis time is 2 h.

INTRODUCTION

In the Polyspecialist Prevention Centre, we work to ensure that environmental laws are respected, to detect pollution and alterations of soil, water, food and air, and to survey quality trends and risk factors. Especially in emergency situations, it is not enough to prove analytically respect of the environmental law, it is necessary to have analytical results in real time. For this it is necessary to detect the origins, to define the consequences and to dictate the particular prevention steps.

In northern Italy environmental contamination from herbicides used in agriculture, particularly involving water matrices, is common.

The quantities reaching water by leaching will depend upon the herbicide, rainfall and soil type. Very important also is the question of accidental contamination of water supplies by a concentrate or a diluted spray. The Council of the European Community in 1980 adopted a directive [1] concerning standards for water intended for human consumption: the permitted level of any individual pesticide is 0.1 μg/l, and the total permitted concentration in water supplies is 0.5 μg/l.

Accordingly, the demands on analysis are very high, since relevant individual substances must be included, and the levels set by legislation necessitate an analytical method with high sensitivity. The traditional methods for determination of herbicides in water by HPLC or GC apply to compounds of the same structural class [2–10].

Therefore we developed an HPLC method that follows a preconcentration step, optimized for routine work and applied to ten herbicides in two different classes, triazines and dinitro-

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TABLE I

HERBICIDES STUDIED AND TOXICITY LEVELS [12]

Common name	Toxicity LD$_{50}$ for rats (mg technical grade/kg)
Triazines	
Atrazine	1869–3080
Metamitron	3343
Metribuzin	2200–2345
Propazine	>7700
Simazine	>5000
Terbuthylazine	2000
Terbutryn	2000
Dinitroanilines	
Ethalfluralin	>10 000
Pendimethalin	1050–1750
Trifluralin	>10 000

anilines. The ten herbicides studied and their toxicological data are reported in Table I. Although the use of atrazine in agriculture has been banned in Italy since 1991 [11], this compound is included in the list because its residues are still detected in the environment.

EXPERIMENTAL

Apparatus

A chromatograph equipped with a Model 501 solvent-delivery system and a Model 680 automated gradient controller was used, fitted with a Model 994 photodiode array detector (Waters, Division of Millipore, Milford, MA, USA) and a Rheodyne rotary injection valve (20-μl loop). The data-processing system was a Waters 746 Data Module. The column used was a LiChrosorb RP-Select B (5 μm), 250 × 4 mm I.D. (E. Merck, Darmstadt, Germany). Herbicides were extracted from water with a Vac-Elut vacuum system (Analitichem International, Harbor City, CA, USA).

Chemicals and materials

HPLC-grade acetonitrile (Carlo Erba, Milan, Italy), Milli-Q high-grade water (Millipore system) and 25% ammonia solution (E. Merck) were used. Herbicide standards were purchased from Labor Dr. Ehrenstorfer, Augsburg, Ger-

many (metamitron, simazine, propazine, terbutryn, metribuzin, pendimethalin, terbuthylazine, ethalfluralin and trifluralin) and from Riedel De Haen Seelze, Hannover, Germany (atrazine). Bakerbond solid-phase extraction columns (3 ml size with sorbent octadecyl, weight of 500 mg; J.T. Baker, Phillipsburg, USA) were used in the preconcentration step. Sample filtration was obtained using a 0.45-μm HA filter (Millipore).

Fortification of the water

Stock standard solutions (*ca.* 100 mg/l each) were prepared in acetonitrile and stored at 4°C. Working standard solutions were obtained by dilution with acetonitrile, so that 0.5 ml of solution added to a 250-ml sample of water gave the required concentration of herbicides.

Extraction procedure

Extraction of triazines and dinitroanilines was carried out using the following procedure. An RP-18 cartridge was activated with two column volumes of acetonitrile followed by two column volumes of water. The sample (250 ml), after filtration through a 0.45-μm HA filter (Millipore), was added (using a reservoir) and allowed to percolate slowly, at 5 ml/min flow-rate (Vac-Elut system). The sorbent was washed using two column volumes of water to remove salt residues and 2 × 0.5 ml of 0.25% NH$_3$ solution. The cartridge was air dried under vacuum for 15 min and then herbicides were eluted with 2 × 1 ml of acetonitrile. The extract was completely evaporated with a gentle stream of nitrogen. The residue was recovered with 0.5 ml of acetonitrile.

Chromatographic determination

A LiChrosorb RP-Select B (5 μm) column (250 × 4 mm I.D.) was used for analysing the samples. The mobile phase was acetonitrile-water: 4 min at 40% acetonitrile, from 40% to 75% in 26 min, convex gradient curve, then 5 min in isocratic conditions, at 1 ml/min flow-rate.

The amount injected was 20 μl and the working wavelength 222 nm with a bandwidth of 3 nm.

Ten compounds were identified and quantified

by comparing their retention values, spectral data and integrated peak areas with those of known external standards.

RESULTS AND DISCUSSION

Triazine enrichment in the preconcentration procedure is critical. The pH conditions during the washing step, following the sample adsorption, dramatically affect the recovery of terbutryn. For instance, the recovery after washing with neutral water is *ca.* 5%, whereas it becomes approximately quantitative using an ammonia solution.

In contrast, the recovery was very low and poorly reproducible in the acidic region. Other studies [2] have shown that this low recovery can be ascribed to the conversion of triazines to other compounds under highly acidic conditions. Terbutryn is eluted only after a washing step with a NH_3 solution, probably owing to a secondary cation exchange of the reversed-phase column. Experiments at different pH values showed that the ideal washing pH is *ca.* 11. At pH >11 the recovery of metamitron decreases dramatically because of its instability in high alkaline conditions [12].

The percentage recoveries for the ten herbicides studied are reported in Table II. The average is based on five values.

Under the adopted experimental conditions the recovery of dinitroanilines is lower than that of triazines. Preliminary results [4] showed that

TABLE II

MEAN RECOVERIES (%) AND STANDARD DEVIATION OF HERBICIDES IN MILLI-Q WATER

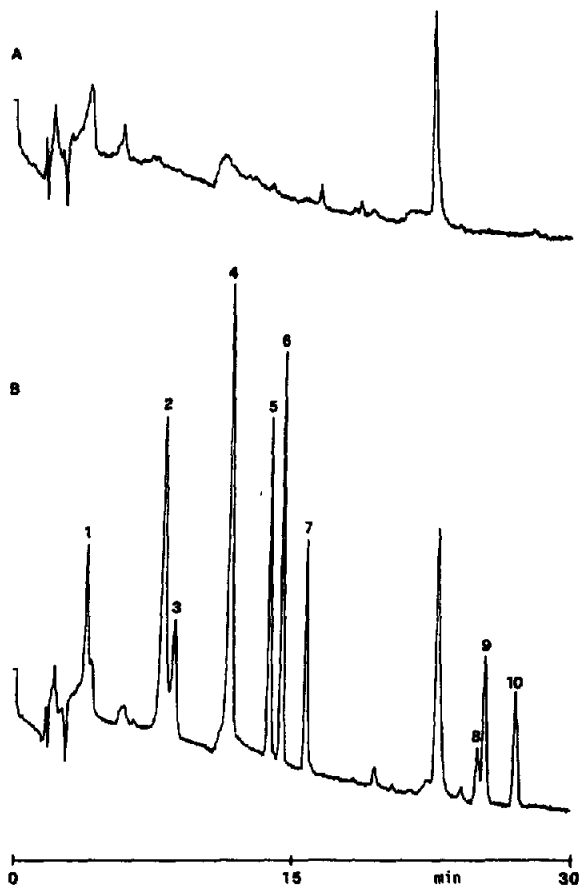| Compound	Fortification level (μg/l)	Mean recovery (%)	±S.D. ($n = 5$)
Metamitron	3.0	89.7	10.0
	0.6	77.0	7.0
Simazine	0.84	95.1	5.7
	0.16	90.6	2.0
Metribuzin	2.6	97.6	4.9
	0.5	95.7	3.3
Atrazine	1.6	98.9	1.5
	0.3	95.6	5.7
Propazine	0.8	97.5	2.9
	0.16	94.0	5.4
Terbuthylazine	1.3	98.9	3.0
	0.26	98.5	1.6
Terbutryn	0.8	94.8	8.1
	0.16	98.4	4.6
Ethalfluralin	2.6	54.6	5.4
	0.5	77.5	6.5
Pendimethalin	2.9	54.7	3.4
	0.58	65.9	3.9
Trifluralin	4.16	50.7	4.6
	0.8	67.0	7.6

Fig. 1. Chromatography of triazine and dinitroaniline herbicides in surface water. Mobile phase: acetonitrile–water (convex gradient programme: 4 min, 40% acetonitrile; from 40% acetonitrile to 75% in 26 min). Flow-rate: 1 ml/min. Detection: UV at 222 nm. (A) Control, (B) sample fortified with: (1) metamitron 3.0 μg/l; (2) simazine 0.84 μg/l; (3) metribuzin 2.6 μg/l; (4) atrazine 1.6 μg/l; (5) propazine 0.8 μg/l; (6) terbuthylazine 1.3 μg/l; (7) terbutryn 0.8 μg/l; (8) ethalfluralin 2.6 μg/l; (9) pendimethalin 2.9 μg/l; (10) trifluralin 4.16 μg/l.

adoption of diethyl ether in the elution step improves dinitroaniline recovery, however the effect of this eluent on triazine recovery needs further verification.

The HPLC separation of herbicides in a surface water sample is shown in Fig. 1. There are no significant interferences due to the matrix. To optimize the separation between pendimethalin and ethalfluralin a gradient programme including a convex curve was used. A linear curve gradient does not allow satisfactory separation. Quantitative determinations were carried out using the external standard method.

The UV absorbances of solutions were measured at 222 nm as a compromise between the highest wavelength absorptions for triazines and dinitroanilines. The detector response is linear in the tested range (metamitron, 1.5–15.0 ng; terbutryn, 0.4–8.0 ng; pendimethalin, 1.5–15.0 ng; trifluralin 2.0–20.0 ng). The linear regression coefficients square root are between $r = 0.9947$ for pendimethalin and $r = 0.9989$ for terbutryn.

The detection limits for each compound are reported in Table III based on a signal-to-noise ratio of ≥ 2. After the preconcentration step, the herbicide enrichment factor is ca. 500. Consequently, under these conditions the detection limits are from 0.03 $\mu g/l$ for propazine and terbutryn to 0.14 $\mu g/l$ for trifluralin.

Work is in progress to improve the sensitivity of the analytical method: a larger volume of injected sample in HPLC analysis can be used. Other elution solvents can be tested to obtain a quantitative recovery for dinitroanilines, but it is necessary to evaluate the influence on triazine recoveries.

TABLE III

RETENTION TIME AND LIMITS OF DETECTION

UV detection at 222 nm.

Compound	Retention time (min)	Limit ($\mu g/l$)
Metamitron	3.9	0.1
Simazine	8.1	0.03
Metribuzin	8.7	0.1
Atrazine	11.7	0.05
Propazine	13.8	0.03
Terbuthylazine	14.5	0.05
Terbutryn	15.7	0.03
Ethalfluralin	24.9	0.09
Pendimethalin	25.3	0.1
Trifluralin	26.9	0.14

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