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Development and optimization of a method for the determination of simazine, atrazine and propazine using solid-phase extraction and HPLC/GC

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A solid-phase extraction (SPE) method, coupled with HPLC/DAD and GC/FID analysis has been developed for the simultaneous determination of simazine, atrazine and propazine in water samples. The compounds of interest were enriched on Envi-carb SPE tubes. The recoveries for simazine, atrazine and propazine from spiked Nanopure water were 101 5.6 %, 99 4.9 % and 96 5.7 %, respectively. The detection limits were 4.00, 8.00 and 10.00 ng absolute sample mass in the column for simazine, atrazine and propazine, respectively. Standard curve r^2 values of 0.9828–0.9988 for the analyzed compounds were consistently obtained.

Keywords: determination, solid-phase extraction, HPLC/GC, atrazine, simazine, propazine.

INTRODUCTION

The separation, identification and determination of pesticide residues in different water samples is necessary for solving various environmental problems.[1] This is an analytical problem of increasing importance. Different techniques have been applied for the determination of pesticides. The accuracy and precision of the analyses are dependent on both sample preparation and instrumental performance. The analysis is carried out using gas chromatography (GC) or liquid chromatography (LC).[2,3] These chromatographic techniques require efficient isolation and concentration procedures, such as solid-phase extraction procedure.[4–8]

Triazines are widely used as herbicides in agriculture. These herbicides have been reported as contaminants in both surface and ground water.[9,10] The methods actually used for the determination of trace amounts of triazines include gas chromatography[11,12] and high performance liquid chromatography (HPLC).[13,14] Solid-phase extraction has been successfully applied for the extraction of triazines.[15,16]

In this paper, the results of the separation, identification and quantitative determination of simazine, atrazine and propazine herbicides by HPLC/DAD and GC/FID after an SPE procedure are presented.

EXPERIMENTAL

HPLC Analysis

Acetonitrile (HPLC grade) was from Sigma-Aldrich (Germany). Methanol and water (for liquid chromatography) were from Merck (Germany). The compounds analyzed are listed in Table I. The general structure of triazines is shown in Fig. 1. All triazine standards (purity 99 %) were from Supelco.

Concentrated stock solutions of the analyzed triazines (1000 ppm) were prepared by dissolving 10 mg of the respective triazine in 10 ml of methanol. Stock solutions were used to prepare standard mixtures with different traizaine concentrations. These standard mixtures were prepared in methanol/water (50/50 v/v). For solid-phase extraction, recovery studies and calibration curves, commercial water (for liquid chromatography) was spiked with the standard mixtures containing all the triazines of interest. Seven standard mixtures with individual herbicide concentrations of 1.0, 2.0, 4.0, 5.0, 8.0, 10.0 and 12.0 g/l in methanol/water (50/50 v/v) were prepared for external calibration. All solutions were stored at 4 ºC. Prior to analysis, the samples were allowed to attain room temperature.

HPLC analysis was performed using a Varian HPLC system equipped with a ternary gradient pump (9012), loop (Rheodine) and a polychrome diode array detector (Varian 9065). The sample volume injected into the HPLC system was 20 l. An analytical column Lichrosorb RP 18, 200 4.6 mm, 5 m (Hewlett-Packard) was used for separation of the triazines.

TABLE I. Structures of the investigated triazines

	Substituent in position (Fig. 1)		
Compound	R_1	R_2	R_3
Simazine	Cl	$NH–C_2H_5$	$NH–C_2H_5$
Atrazine	Cl	$NH–C_2H_5$	$NH–CH(CH_3)_2$
Propazine	Cl	$NH–CH(CH_3)_2$	$NH–CH(CH_3)_2$

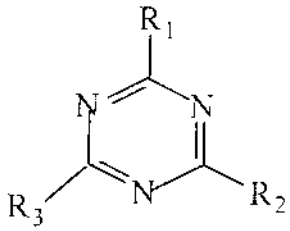

Fig. 1. Schematic structure of triazines. (Substituents R_1, R_2 and R_3 are listed in Table I).

GC Analysis

Sample eluates were anlyzed using a gas chromatograph Model 5890 Series II Plus (Hewlett-Packard) equipped with an automated injector 7673 (Hewlett-Packard) and a flame ionization detector. Samples (1 l injected volume) were injected in the splitless mode into the gas chromatograph. The injector temperature was 250 ºC. The herbicides were separated on a HP-5 capillary col-

umn, 30 m 0.53 mm (ID), with a film thickness of 1.5 m (Hewlett-Packard). The carrier gas was nitrogen with a constant flow-rate of 1 ml/min. The detector temperature was 320 ºC.

Starting at 80 ºC, the column was heated at 30 ºC/min to 178 ºC. This temperature was maintained for 4 min before the column was further heated at 2 ºC/min to 205 ºC. After 2 min at this temperature heating was continued at 30 ºC/min to 290 ºC. The held at the final temperature was 1 min.

Extraction procedure

The extraction of the determined triazines was performed using ENVI-carb tubes, 3 ml, 250 mg sorbent (Supelco). For the SPE procedure, the concentrations of the fortified commercial water solutions were 2.0, 4.0 and 10.0 g/l. The exact volume of 1000 ml of spiked water solution was used for the enrichment procedure. For each concentration level four samples ($n = 4$) were prepared. The SPE cartridge was conditioned by passing 5 ml CH_2Cl_2/CH_3OH (80/20 v/v), 1 ml CH_3OH and 10 ml 2 % CH_3COOH. Then, the fortified water sample was passed through the cartridge. Subsequently, the cartridge was dried for 3–5 minutes with air. The retained compounds were eluted with 1 ml CH_3OH and 2 3.5 ml CH_2Cl_2/CH_3OH (80/20 v/v). The eluate was evaporated to dryness, the residue was collected by 3 500 l CH_3OH and transferred into a vial for analysis. Each sample in the set was sequentially prepared in the same way.

RESULTS AND DISCUSSION

The sample eluates were analyzed by both the HPLC/DAD and GC/FID methods. Several HPLC columns were tested to find the one which gave the best separation. It was found that the column Lichrosorb RP 18, 200 4.6 mm, 5 m (Hewlett-Packard) gave the best results for the analysis of the standard solutions as well as the artificial samples.

Several isocratic and gradient elutions, with two mobile phases: methanol/water and acetonitrile/water, were used for the separation of the compounds of interest. The best separation with the most symmetrical peaks were obtained using the mobile phase acetonitrile/water (70/30 v/v) under isocratic conditions, so this mobile phase was used for further investigations.

Several mobile phase flow rates (0.5 – 1.5 ml/min) were evaluated. The best separation was achieved using a flow rate of 1.0 ml/min.

The chromatogram of the separation to the compounds of interest under the above-mentioned HPLC conditions is shown in Fig. 2. The retention times for simazine, atrazine and propazine are 3.41, 3.91 and 4.56 min, respectively.

GC/FID separation of the same compounds is shown in Fig. 3. The retention times for simazine, atrazine and propazine are 23.66, 23.88 and 24.03 min, respectively.

The absorbance was measured continuously in the range 190–360 nm using a diode array detector. The peaks were quantified at a wavelength of 220 nm, where the compounds have an absorption maximum.

The calibration was carried out by injecting standard solutions onto the HPLC column. The concentrations of the chlorotriazines in the samples were calculated by comparing the individual peak areas with an external calibration. For positive compound assignment, besides the retention times, the UV spectra were compared with a spectra library.

The r^2 values obtained from the respective calibration curves were 0.9893, 0.9828, 0.9929 for HPLC/DAD determination and 0.9988, 0.9982, 0.9971 for GC/FID

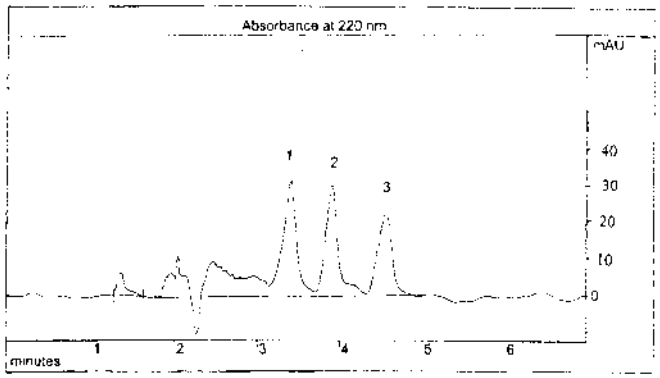

Fig. 2. HPLC/DAD separation of simazine (1), atrazine (2) and propazine (3).

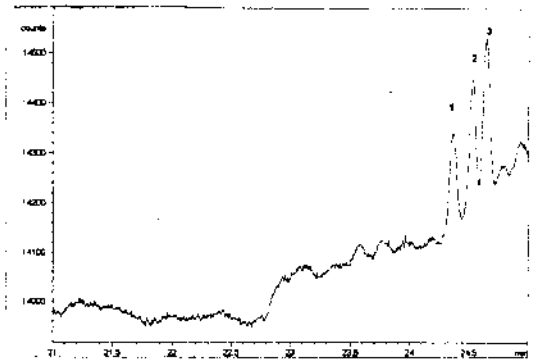

Fig. 3. GC/FID separation of simazine (1), atrazine (2) and propazine (3).

determination for simazine, atrazine and propazine, respectively. It is important to maintain high r^2 values to have good control over the lower range in the standard curve.

The detection limits were 4.00, 8.00 and 10.00 ng absolute sample mass of the herbicide in the column, which corresponds to 0.3, 0.6 and 0.75 g/l for simazine, atrazine and propazine, respectively.

The results for the solid-phase extraction procedure, obtained by both HPLC/DAD and GC/FID methods, are given in Table II.

The analyses by the GC/FID method were used as an independent check of the accuracy of the HPLC method. In general, the results obtained using the GC/FID method agree within 2 to 9 % of the results obtained using the HPLC method.

The two methods were compared by studying test samples containing different concentrations of analyte and analysing the difference between each pair of results using the *t*-test.[17] The obtained results are given in Table III where:

x_1, x_2 – experimental means; n_2, n_2 – numbers of replicate analyses; s_1, s_2 – sample standard deviations and s - mean standard deviation.

The critical value of t is 2.45 ($P = 0.05$) and since the calculated values of t (Table III) are less than this, the null hypothesis is retained, *i.e.*, the methods do not give signifi-

TABLE II. Recoveries and relative standard deviations of the solid-phase extraction in water samples

Compound	Conc. ppb	Recovery (%)(*RSD*)	
		HPLC/DAD	GC/FID
Simazine	2	98.50 (5.2)	93.90 (7.1)
	4	105.00 (1.6)	100.40 (5.7)
	10	104.70 (6.5)	106.20 (8.3)
Atrazine	2	93.50 (4.9)	89.40 (5.5)
	4	104.50 (2.5)	99.80 (7.2)
	10	105.30 (4.7)	103.70 (4.9)
Propazine	2	85.00 (3.9)	90.50 (4.1)
	4	103.50 (7.4)	97.30 (8.6)
	10	100.50 (3.8)	99.40 (5.8)

TABLE III. *t* - Test comparison of the two methods for the determination of herbicides in water samples

Compound	Simazine	Atrazine	Propazine
HPLC/DAD			
x_1/ppm	1.31	1.25	1.13
s_1 ($n_1 = 4$)	0.052	0.049	0.039
GC/FID			
x_2/ppm	1.25	1.19	1.21
s_2 ($n_2 = 4$)	0.071	0.055	0.041
s	0.062	0.052	0.040
t (exp)	1.36	1.15	2.11
t (tab)	2.45	2.45	2.45

cantly different values for the mean concentration of the determined pesticides. Hence, the two chromatographic methods, HPLC/DAD and GC/FID, give statistically similar results and are proposed for the determination of simazine, atrazine and propazine in water samples. The HPLC/DAD method has the advantage that the identification of the pesticides based on the retention time is confirmed by the UV spectrum.

ИЗВОД

РАЗВОЈ И ОПТИМИЗАЦИЈА КОД ОДРЕЂИВАЊА СИМАЗИНА, АТРАЗИНА И ПРОПАЗИНА КОРИСТЕЋИ ЧВРСТО ФАЗНУ ЕКСТРАКЦИЈУ И HPLC/GC

ВЕРА ТРАЈКОВСКА, СИМКА ПЕТРОВСКА-ЈОВАНОВИЋ и МИРКО ЦВЕТКОВСКИ*

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Развијен је метод за симултано одређивање симазина, атразина и пропазина у воденим растворима, користећи чврсто фазну екстракцију (SPE) у спрези са HPLC/DAD и GC/FID. За изоловање и концентровање једињења која су од интереса, примењени су Envi-carb носачи за чврсто фазну екстракцију. При томе, добијени приноси су: 101 5,6 %, 99 4,9 % и 96 5,7 %, за симазин, атразин и пропазин, за сваког посебно из спајковане дестиловане воде. Границе детекције су 4,00, 8,00 и 10,00 ng апсолутних маса које су унесене у колону за сва три испитивана једињења. Из одговарајуће калибрационе криве добијене су r^2 вредности у следећим границама 0,9828–0.9988.

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