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SEPARATION OF SIMAZINE, ATRAZINE AND PROPAZINE USING REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract

The aim of this work is to evaluate a HPLC method for simultaneous determination of these herbicides: simazine, atrazine and propazine.

The optimum mobile phase was found to be methanol/water, 70/30, V/V at a flow rate 1.0 mL/min. The linearity of the method, the sensitivity of the method (LOD and LOQ) and intra day precision of the retention times and peak areas were determined.

HPLC analyses were performed by HPLC system (Varian) equipped with ternary gradient pump (9012), loop (Rheodine), polychrome diode array detector (Varian 9065) and analytical column Lichrosorb RP 18, 200 x 4.6 mm, 5 μm (Hewlett-Packard).

Introduction

Triazines are still widely used as herbicides. Atrazine is one of the most used pesticides worldwide[1,2]. During and after the herbicide application onto farming land, triazines may be transported to the ground and surface water and into the atmosphere. Therefore, residual triazines in food plants and ground water must be accurately monitored. For that, analytical methods are more than need.

The majority of the analytical methods published to date report the determination of triazines by gas chromatography (GC) as a common method for the determination of pesticides [3.4]. A disadvantage of GC is that it is limited to volatile chlorotriazines. However, in order to determine total residues of these herbicides in different matrix, the polar degradation products have to be included. High-performance liquid chromatography (HPLC) is directly applicable to triazines and their degradation products [5,6].

This paper describes the optimization of HPLC conditions for separation of herbicides simazine, atrazine and propazine.

Experimental

All triazine standards (purity 99 %) were from Supelco. Methanol (for liquid chromatography) was from Merck (Germany). Concentrated stock solutions 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 triazine concentrations.

HPLC analysis was performed by HPLC system (Varian) equipped with ternary gradient pump (9012), loop (Rheodine) and polychrome diode array detector (Varian

9065). The sample volume injected into HPLC system was 20 µL. Analytical column used for separation was Lichrosorb RP 18, 200 x 4.6 mm, 5 µm (Hewlett-Packard), mobile phase methanol/water, 70/30, V/V at a flow rate 1.0 mL/min. Detection was carried out at a wavelength of 220 nm, where the compounds have an absorption maximum.

Results and Discussion

The compounds analyzed are listed in Table 1. Structure of triazines is given in Figure 1.

Table 1. Substitutents in the structure of investigated triazines

Compound	R_1	R_2	R_3
Simazine	Cl	NH-C_2H_5	NH-C_2H_5
Atrazine	Cl	NH-CH$(CH_3)_2$	NH-C_2H_5
Propazine	Cl	NH-CH$(CH_3)_2$	NH-CH$(CH_3)_2$

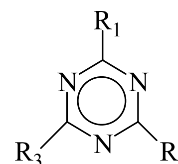

Figure 1. Structure of triazines

To separate a mixture of triazines an intense study was made to optimize the chromatographic conditions. For best separation several isocratic and gradient methods of methanol/water (10 – 90 % methanol) and several flow rate of mobile phase (0.5 – 1.5 mL/min) were evaluated. The optimum mobile phase was found to be methanol/water, 70/30, V/V at a flow rate 1.0 mL/min.

The chromatogram obtained under these conditions is shown in Fig.2.

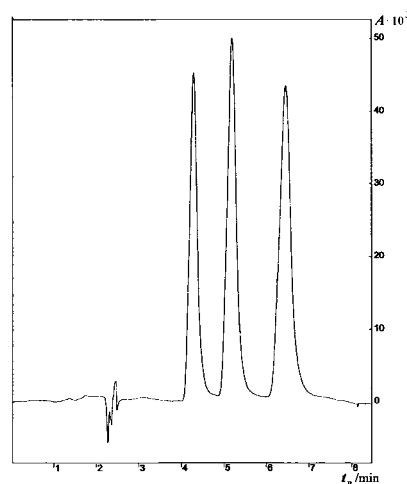

Figure 2. HPLC/DAD separation for simazine (4.32 min) , atrazine (5.24 min) and propazine (6.53 min). For all other conditions, see text.

The peaks were quantified at a wavelength of 220 nm, were the compounds have an absorption maximum.

The values calculated for the retention factors (*k*), separation factors (α) and resolution (*R*) between adjacent peaks are given in Table 2.

Table 2. Retention factors (*k*), separation factors (α) and resolution (*R*) for investigated pesticides

Compound	t_R/min	*k*	*α*	*R*
Simazine	4.32	0.73	1.50	1.98
Atrazine	5.24	1.10	1.47	2.25
Propazine	6.53	1.62	-	-

Calibration plots for seven different concentration levels encompassing the range of interest were drawn for simazine, atrazine and propazine. They were linear over the range of 20 - 167 ng. The correlation coefficients are all satisfactory ($R^2 > 0.99$).

In addition of this work, the sensitivity of the method was determined. Under these chromatographic conditions, the best sensitivity was obtained for propazine (limit of detection 1.1 ng and limit of quantification 3.2 ng).

Typical reproducibilities for investigated pesticides from standard injections were measured. The peak area data , with a relative standard deviation (RSD) of less than 8.64 %, and the retention time data with a relative standard deviation of less than 0.46 %, are, by themselves, very acceptable.

Conclusion

The described method allows simultaneous separation of cited pesticides easily and rapidly. It is sensitive and precise analytical method. The advantage of this method is also the possibility of measuring peak purity and confirming the identity of the pesticide by UV spectra. This method can be used for determination of these pesticides in water samples.

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