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MOBILE PHASE OPTIMIZATION FOR THE SEPARATION OF SOME TRIAZINE SAMPLES USING RP/HPLC

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Introduction

Triazine herbicides are widely used to control weeds in food crops. These toxic chemicals generally degrade slowly, so they can persist in fields for a year or more after application. Therefore, for consumer protection, residual triazines in food plants and ground water must be accurately monitored. For that, analytical methods are more than need.

Gas chromatography (GC) is a common method for the determination of pesticides and its non-polar degradation products with high sensitivity and good separation efficiency[1,2]. A disadvantage of GC is that it is limited to volatile chlorotriazines. The hydroxy derivatives cannot be analyzed without derivatization.

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[3]. Conventional detection in HPLC is usually achieved with UV or diode-array detection (DAD). UV detection often provides adequate sensitivity. In combination with a diode-array detector, an unambiguous identification in environmental samples is sometimes possible for certain compound classes[4-6].

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

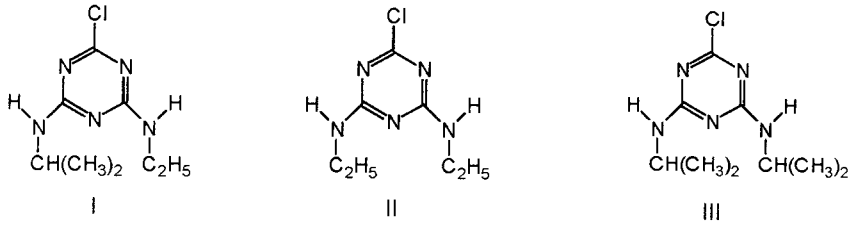

Figure 1. Structures of simazine (I), atrazine (II) and propazine (III).

Experimental

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. For separation was used an analytical column Hypersil ODS, 100×2.1 mm, 3 µm (Hewlett-Packard). Detection was carried out at a wavelength of 220 nm, where the compounds have an absorption maximum.

Methanol and water (for liquid chromatography) were from Merck (Germany). All triazine standards (purity 99%) were from Supelco. Concentrated stock solutions of 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 triazine concentrations. These standard mixtures were prepared in methanol/water (50/50 V/V). Seven mixtures with individual herbicide concentrations of 1.0, 2.0, 4.0, 5.0, 8.0, 10.0 and 12.0 µg/L were prepared

* Editorial note: Recognized by Greece as FYROM.

for external calibration. All solutions were stored at 4°C. Prior to analysis, the samples were allowed to attain room temperature.

Results and Discussion

The analytes investigated included three 1,3,5-triazines, containing chlorine (Fig. 1).

To separate and determine a mixture of triazines containing simazine, atrazine and propazine, an intense study was made to optimize the chromatographic conditions, emphasizing the composition of the mobile phase and flow rate. So, for best separation and symmetrical peak shape, 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. After consideration of both analysis time and resolution, the optimum mobile phase to carry out the separation of mixture of these herbicides was found to be methanol/water, 50:50, V/V at a flow rate 0.5 mL/min.

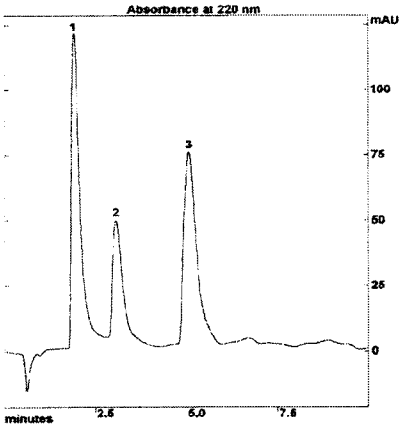

Figure 2. HPLC/DAD separation for simazine (I), atrazine (II) and propazine (III). For all other conditions, see text.

The chromatogram of the separation to the components of interest is shown in Fig.2. The absorbance was measured continuously in the range 190-360 nm using diode array detector. The peaks were quantified at a wavelength of 220 nm, were the compounds have an absorption maximum. The retention times for simazine, atrazine and propazine are 1.94, 3.08 and 5.08 min, respectively.

The values calculated for the retention factors (k), separation factors (α) and resolution (R) between adjacent peaks[7] are given in Table 1.

Table 1. Retention factors (k), separation factors (α) and resolution (R) for investigated pesticides.

Compound	t_R (min)	k	α	R
Simazine	1.94	1.90	1.89	4.58
Atrazine	3.08	3.61	1.83	6.25
Propazine	5.08	6.61		
Acetonitrile*	0.67			

*solution for determination dead time

In addition of this work, the sensitivity of the method was determined (Table 2.) The limit of detection (LOD) was calculated as three times the ratio between the SD and the slope of the low concentration curve (LOD = 3·SD/slope) and the limit of quantification (LOQ) as ten times the same ratio (LOQ = 10S·D/slope. Under these chromatographic conditions, simazine show the best sensitivity.

Table 2. Limit of detection (LOD) and limit of quantification (LOQ) for investigated pesticides.

Compound	Simazine	Atrazine	Propazine
LOD / ng	12.3	16.4	14.5
LOQ / ng	41.0	54.6	48.3

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 25-200 ng. Calibration equations are reported in Table 3. The correlation coefficients are all satisfactory (R^2>0.99).

Table 3. Statistical data of calibration curves for investigated pesticides.

Compound	Regression equation*	RSD (%)	R^2
Simazine	$y = 2.5313 \cdot 10^3 x$	7.445	0.9983
Atrazine	$y = 2.6681 \cdot 10^3 x$	4.796	0.9962
Propazine	$y = 1.6756 \cdot 10^3 x$	6.331	0.9973

* y - peak area, x - mass of pesticide

Whereas the reproducibility during one-day experiments is often excellent to satisfactory, it is often poorer if determined over longer periods. Typical reproducibilities for several pesticides from standard injections are presented in Table 4. The RSD of retention time and peak area for different sample amounts range from 2.66 to 7.62 %.

Table 4. Intra day precision of retention time and peak area for investigated pesticides.

simazine (100 ng, n = 7)			
t_R (min)	Mean = 2.17	SD = 0.064	RSD = 2.95 %
Area	Mean = 200112	SD = 15239.23	RSD = 7.62 %
atrazine (80 ng, n = 7)			
t_R (min)	Mean = 3.43	SD = 0.09	RSD = 2.66 %
Area	Mean = 177679.7	SD = 12126.1	RSD = 6.82 %
propazine (85 ng, n = 7)			
t_R (min)	Mean = 5.73	SD = 0.17	RSD = 3.04 %
Area	Mean = 154524.9	SD = 10764.2	RSD = 6.97 %

Conclusion

This study shows the possibility of separation and simultaneous determination a mixture of triazines containing simazine, atrazine and propazine by the reverse phase HPLC/DAD. For separation was used an analytical column Hypersil ODS, 100×2.1 mm, □□m, mobile phase methanol/ water (50:50), at a flow rate 0.5 mL/min. Detection was carried out at 220 nm. Hence, the developed method is simple, fast and precise enough for routine analysis of investigated pesticides in the different matrix, food and water.

References

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