P2-33

DEVELOPMENT AND OPTIMIZATION FOR SIMULTANEOUS HPLC SEPARATION OF TERBUTHYLAZINE, PHOSMET, NAPROPAMIDE AND FOLPET

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Introduction

The monitoring of pesticide residues in food and water became a priority in pesticides research and health care. It presents a real interest for the protection of environment and for the evalution of food quality.

High-performance liquid chromatographic (HPLC) methods for pesticide analysis in food occasionally used fluorescence, electron-capture and electrochemical detection method[1-3] that present low selectivity. On the other hand, diode array UV-Vis and MS detectors[4,5] were frequently used and provided a selective detecton.

Terbuthylazine, phosmet, napropamide and folpet are the active compounds in some pesticide formulations which are used on farming land - vineyards, apple and pear trees in Macedonia.

The purpose of the present method was to develop a rapid, specific and sensitive method for separation and routine analysis of above-mentioned pesticides.

Experimental

HPLC analysis were performed with a Varian LC System Workstation including a ternary gradient pump (9012), loop (Rheodyne) and a Model 9065 Polychrom diode array detector. For separation was used an analytical column Hypersil ODS, 100×2.1 mm, 3 μm (Hewlett-Packard).

HPLC-grade acetonitrile was from Sigma-Aldrich (Germany). LC-quality water was prepared by distiling deionized water in a glass apparatus. All solvents and solutions for HPLC analysis were vacuum degassed by sonication before use. Pesticide standards were from BASF (Germany).

Stock solution (1.0 mg/mL) were prepared by dissolving the appropriate amount of the standard pesticude in methanol and stored frozen. Working solution (5 μg/mL) were prepared by diluting aliquotes of the spock solutions with a mixture of acetonitrile/water (50:50) and stored at 4°C.

The sample volume injected into HPLC system was 20 μL.

Results and Discussion

The investigation pesticides were terbuthylazine, phosmet, napropamide and folpet, which are herbicide, insecticide, herbicide and fungicide, respectively. Their chemical structures are given on Fig. 1.

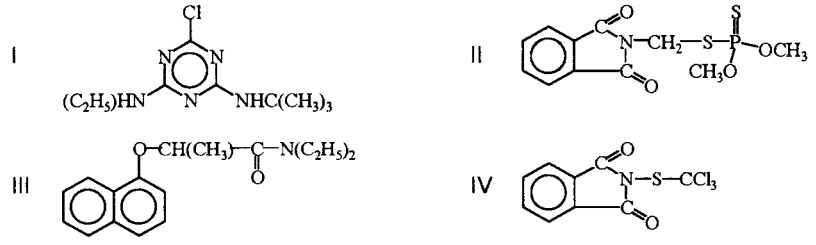

Figure 1. Chemical structures of terbuthylazine (I), phosmet (II), napropamide (III) and folpet (IV).

* Editorial note: Recognized by Greece as FYROM.

Detection was carried out at a wavelength of 220 nm, which is the best for simultaneous determination of all mentioned pesticides.

The chromatogram (Fig.2) shows the separation of investigation pesticides.

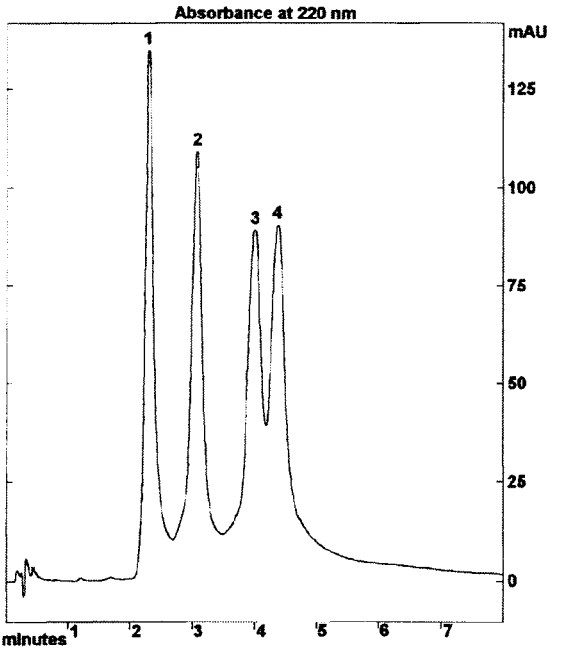

Figure 2. HPLC/DAD separation for terbuthylazine (1), phosmet (2), napropamide (3) and folpet (4). For all other conditions, see text.

The best separation and symmetrical peak shape was performed using isocratic elution at a flow rate of 0.75 mL/min with a mobile phase acetonitrile / water, 28/72 V/V. Under these chromatographic conditions, the retention times were 2.31, 3,08, 4,01 and 4,39 min for terbuthylazine, phosmet, napropamide and folpet, respectively.

The values calculated for the retention factors (k), separation factors (α) and resolution (R) between adjacent peaks give high efficiency of separation (Table 1).

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

Compound	t_R (min)	k	α	R
Terbuthylazine	2.31	5.35	1.40	4.81
Phosmet	3.08	7.49	1.34	4.10
Napropamide	4.01	10.05	1.10	1.37
Folpet	4.39	11.08		
Methanol*	0.36			

* solution for determination dead time

For each compound the limit of detection and quantification (LOD and LOQ) was determined[6]. The obtained results are listed in Table 2. It can see that napropamide show the best sensitivity.

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

Compound	Terbuthylazine	Phosmet	Napropamide	Folpet
LOD / ng	19.3	18.0	17.9	18.7
LOQ / ng	64.4	60.0	59.6	62.2

Statistical date, i.e. regression equation, relative standard deviation (RSD) and the value of the multiplete correlation coefficients (R^2) are listed in Table 3. The date shows very good linearity for all compounds in the investigation area.

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

Compound	Regression equation*	RSD (%)	R^2
Terbuthylazine	$y = 9.2245 \cdot 10^2 x$	6.734	0.9956
Phosmet	$y = 1.1173 \cdot 10^3 x$	4.000	0.9981
Napropamide	$y = 1.1688 \cdot 10^3 x$	5.278	0.9959
Folpet	$y = 1.1543 \cdot 10^3 x$	4.173	0.9967

y - peak area
x - mass of pesticide

The results obtained for intra day precision[7,8] of retention time and peak area for terbuthylazine, phosmet, napropamide and folpet, are listed in Table 4. A very good repeatability of the retention time for all compounds was achieved, but the repeatability of the peak area was some worse.

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

Terbuthylazine (180 ng, n = 7)			
t_R (min)	Mean = 2.35	SD = 0.042	RSD = 1.77 %
Area	Mean = 183156.4	SD = 11475.95	RSD = 6.26 %
Phosmet (170 ng, n = 7)			
t_R (min)	Mean = 3.11	SD = 0.06	RSD = 1.93 %
Area	Mean = 209390.7	SD = 9885.9	RSD = 4.72 %
Napropamide (165 ng, n = 7)			
t_R (min)	Mean = 4.04	SD = 0.08	RSD = 2.16 %
Area	Mean = 196936.7	SD = 9805.9	RSD = 4.98 %
Folpet (165 ng, n = 7)			
t_R (min)	Mean = 4.37	SD = 0.09	RSD = 2.21 %
Area	Mean = 241052.7	SD = 6050.3	RSD = 2.51 %

Conclusion

HPLC is a sensitive, specific and versatile technique for the determination of pesticides in aqueous environmental samples. A reversed-phase isocratic separation was developed which permits the determination of terbuthylazine, phosmet, napropamide and folpet. The reproducibilites, linearities and instrumental detection limits obtained are adequate for environmental monitoring of a broad range of investigated pesticides.

Acknowledgments

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