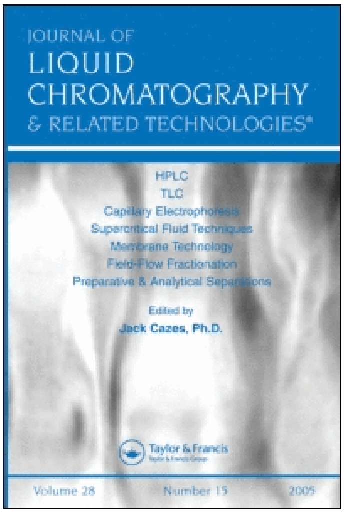

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SEPARATION OF SELECTED PESTICIDES BY AN HPLC TECHNIQUE. I

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ABSTRACT

Two groups of the selected pesticides were investigated: 1st group—monolinuron (1), chlorotoluron (2), diuron (3), isoproturon (4), linuron (5); and 2nd group—dimefuron (6), diflubenzuron (7), teflubenzuron (8), and lufenuron (9). The retention times (t_R), the peak resolutions (R_S), and the separation factors (α) indicate that the mobile phases containing 55% and 85% methanol in water are the best for separations of first and second groups of investigated pesticides, respectively.

Key Words: Urea pesticides; RP-HPLC

INTRODUCTION

At present the urea derivatives are universally applied herbicides.[1] The solubility of urea in water is almost equal to 100%. The solubility of the urea

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compounds in water and in fats depends on the type of the substitutents introduced instead of hydrogen atoms, which are on nitrogen groups.[2] The modifications in basic structure of compounds induce the changes of their biological activities.[3,4] Moreover, the urea compounds are stored in thumbs as dangerous wastes.[5] Some of these substances show a large durability in soil, because they are resistant to the microorganisms. The urea pesticides were determined in water,[6–10] fruits,[11–15] and meat.[16] The processes of biodegradation, as well as the products of transformation of herbicides, were studied.[17–21]

The aim of our study was to work out the optimum conditions of the separation of selected biologically active substances of urea derivatives, which were investigated by high performance liquid chromatography (HPLC). The physical characteristics of studied urea pesticides are presented in Table 1.

EXPERIMENTAL

Chemicals

The components of mobile phases: methanol (POCH, Poland) and water (Millipore, France) were for HPLC analysis. The commercial samples of monolinuron, chlortoluron, diuron, isoproturon, linuron, diflubenzuron (Institute of Organic Industry, Poland), dimefuron (Riedel-de Haën, Germany), tefluberlnzuron (Labor Dr. Ehrenstorfier, Germany), and lufenuron (Ciba Geigy, Switzerland) were used as test solutes.

Sample Preparation

Studied urea pesticides were divided into two groups, applying the following criteria:

(a) the molar mass: first group of compounds possessing the molecular mass form 206.28 to 249.09 g/mol; second group of compounds possessing the molecular mass form 310.68 to 511.15 g/mol.
(b) first group of compounds—pesticides including exclusively one benzene ring; second group of pesticides including two benzene rings or one benzene ring and one heterocyclic ring.

Other physical data, presented in Table 1, confirm the division of the investigated pesticides. Monolinuron (1), chlorotoluron (2), diuron (3), isoproturon (4), and linuron (5) were the first group; however, dimefuron (6), diflubenzuron (7), teflubenzuron (8), and lufenuron (9) were the second group of

Table 1. General Physical Data of Investigated Urea Pesticides

Pesticide (Code)	Molar Mass [g/mol][a]	Molar Refractivity [cm^3][a]	Molar Volume [cm^3][a]	Polarizability × 10^{-24} [cm^3][a]
		Group I		
Monolinuron (1) $C_9H_{11}ClN_2O_2$	214.65	55.51 ± 0.3	164.5 ± 3.0	22.00 ± 0.5
Chlorotoluron (2) $C_{10}H_{13}ClN_2O$	212.68	58.58 ± 0.3	174.5 ± 3.0	23.23 ± 0.5
Diuron (3) $C_9H_{10}Cl_2N_2O$	233.09	58.66 ± 0.3	170.1 ± 3.0	23.25 ± 0.5
Isoproturon (4) $C_{12}H_{18}N_2O$	206.28	63.06 ± 0.3	196.3 ± 3.0	25.00 ± 0.5
Linuron (5) $C_9H_{10}C_{l2}N_2O_2$	249.09	60.41 ± 0.3	176.5 ± 3.0	23.95 ± 0.5
		Group II		
Dimefuron (6) $C_{15}H_{19}ClN_4O_3$	338.79	87.89 ± 0.5	259.4 ± 7.0	34.84 ± 0.5
Diflubenzuron (7) $C_{14}H_9ClF_2N_2O_2$	310.68	73.96 ± 0.3	211.1 ± 3.0	29.32 ± 0.5
Teflubenzuron (8) $C_{14}H_6Cl_2F_4N_2O_2$	381.11	78.85 ± 0.3	231.4 ± 3.0	31.25 ± 0.5
Lufenuron (9) $C_{17}H_8Cl_2F_8N_2O_3$	511.15	95.68 ± 0.3	313.2 ± 3.0	37.93 ± 0.5

[a] Data calculated by ChemSketch 4.5 program.

investigated pesticides. The methanolic solutions of above-mentioned two groups of compounds about concentration 0.1 mg/mL of each pesticide were prepared. The purity of the studied standard pesticides samples were at least 97.6%.

Reverse Phase High Performance Liquid Chromatography

The chromatographic investigations were conducted using the liquid chromatograph obtained from Gynkotek (Germany). Dionex Softron GmbH certified the chromatograph. The conditions of applied HPLC were the following: pump—P 580 LPG, detector UVD 340 S, column—Econosphere C18 5µ (250 mm × 4.6 mm), injection volume—20 mL, eluent flow—1 mL/min. The detection of the first group of pesticides investigated was conducted at 248 nm; however, the second group was done at $\lambda = 260$ nm. *n*-Hexane was selected for the determination of the retention time of an unretained compound.

Isocratic elution was applied using, as mobile phase, a mixture of methanol and water, in which the proportional content of methanol was comprised from 45% to 80% for the first group, as well as from 65% to 90% for the second group of pesticides.

Logarithm of the Retention Factor[22]

The logarithm of the retention factor ($\log k$) was calculated using the formula:

$$\log k = \log \frac{t'_R}{t_M} = \log \frac{t_R - t_M}{t_M} \tag{1}$$

where t_R is the retention time of investigated compound, t'_R is adjusted retention time of investigated compound; whereas t_M is retention time of an unretained compound (*n*-hexane).

Separation Factor α[22]

The separation factor (α) was calculated using the equation:

$$\alpha = \frac{k_2}{k_1} \tag{2}$$

where k_2 and k_1 are retention factors of two adjacent peaks ($k_2 > k_1$).

Peak Resolution R_S[22]

The peak resolution (R_S) was calculated using the formula:

$$R_S = \frac{2(t_{R2} - t_{R1})}{W_{b1} + W_{b2}} \tag{3}$$

where t_{R2} and t_{R1} are retention times of two adjacent peaks on chromatogram ($t_{R2} > t_{R1}$), whereas w_{b1} and w_{b2} are peaks-width at base.

RESULTS AND DISCUSSION

The retention time values (t_R) were introduced for the first and second groups of investigated pesticides, as well as *n*-hexane as an unretained compound in methanol + water as mobile phase in various volume fractions. However, the dependencies of the logarithm of the retention factors on the composition of mobile phase are presented in Figs. 1 and 2 for the first and second groups of investigated urea pesticides, respectively. The course of these dependencies indicates that the best separation of the first group was obtained

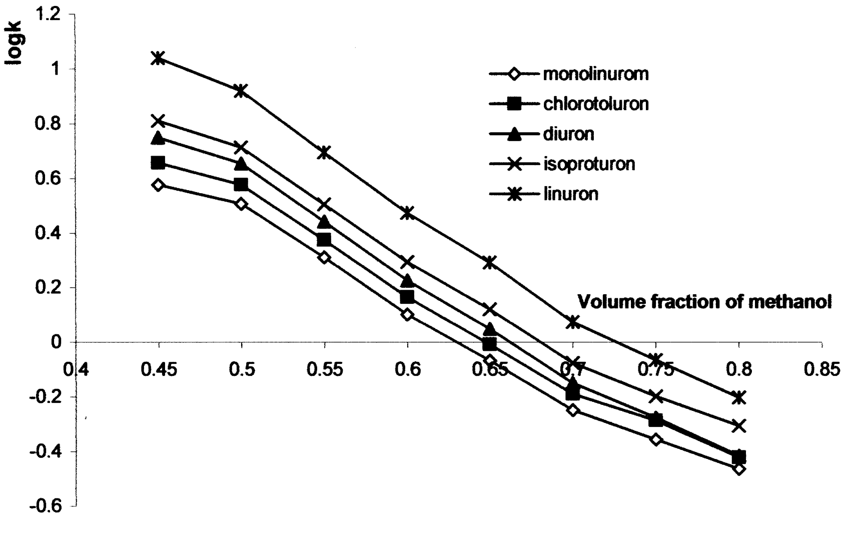

Figure 1. The dependence between $\log k$ and volume fraction of methanol in water for the first group of investigated pesticides.

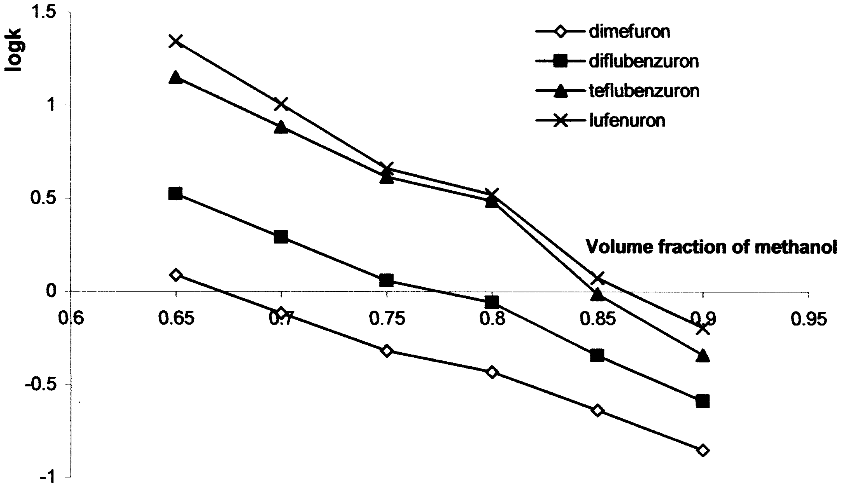

Figure 2. The dependence between log k and volume fraction of methanol in water for the second group of investigated pesticides.

using the mobile phases from 0.45 to 0.60 of volume fraction of methanol in water. However, for the second group of pesticides, the best separations were obtained using the mobile phases 0.65, 0.70, 0.85, as well as 0.90 of volume fraction of methanol in water. The peak resolutions (R_S), as well as the separation factors (α), of investigated pesticides with methanol + water in different volume fractions as mobile phase were presented in Table 3. The highest value of peak resolutions (R_S), the separation factors (α), as well as the largest differences in retention times, were obtained when applying the eluents of about 45% of content of methanol in water for the first group of studied pesticides, and about 65% of methanol in water for the second group of pesticides (see Table 3). However, from a practical point of view the retention time in liquid chromatography is the most important. The retention times should be comparatively short, giving, simultaneously, the total separation of individual components of the studied mixture. The retention times (t_R) of studied substances, the peak resolutions (R_S), and the separation factors (α) obtained for the studied pesticides, indicate that the optimum mobile phase for separation of selected pesticides was with the mobile phase containing about 55% of methanol in water for first group, and about 85% content of methanol in water for the second group of pesticides (see Tables 2 and 3). The chromatograms obtained using optimum conditions of separation for the first and second groups of studied pesticides are presented in Figs. 3 and 4, respectively.

Table 2. Retention Time Values (t_R)[min] of Investigated Pesticides and *n*-Hexane Using Methanol + Water in Different Volume Fraction as Mobile Phases

	Volume Fraction of Methanol in Water									
	0.45	0.50	***0.55***	0.60	0.65	0.70	0.75	0.80	***0.85***	0.90
Pesticide	t_R [min][a]									
				Group I						
Monolinuron (1)	22.067	16.187	***10.924***	7.722	6.147	4.984	4.435	4.057	—	—
Chlorotoluron (2)	25.595	18.338	***12.065***	8.391	6.559	5.249	4.671	4.161	—	—
Diuron (3)	30.520	21.201	***13.481***	9.140	7.005	5.440	4.709	4.178	—	—
Isoproturon (4)	34.253	23.742	***15.017***	10.091	7.679	5.863	5.031	4.512	—	—
Linuron (5)	55.311	35.867	***21.327***	13.527	9.784	6.970	5.730	4.918	—	—
				Group II						
Dimefuron (6)	—	—	—	—	7.384	5.625	4.557	4.138	***3.643***	3.339
Diflubenzuron (7)	—	—	—	—	14.398	9.413	6.594	5.654	***4.305***	3.682
Teflubenzuron (8)	—	—	—	—	50.048	27.582	15.747	12.263	***5.834***	4.265
Lufenuron (9)	—	—	—	—	76.272	35.650	17.184	13.032	***6.476***	4.811
n-Hexane	4.619	3.848	3.584	3.406	3.308	3.185	3.077	3.017	2.956	2.926

[a] Average of five measurements.

Table 3. Peak Resolutions (R_S) and Separation Factors (α) of Investigated Pesticides Using Methanol + Water in Different Volume Fractions as Mobile Phases

	Volume Fraction of Methanol in Water									
R_S α	0.45	0.50	***0.55***	0.60	0.65	0.70	0.75	0.80	***0.85***	0.90
					Group I					
$R_{S1\text{-}2}$	**2.542**	2.094	***1.663***	1.388	1.014	0.486	0.254	0.183	—	—
$R_{S2\text{-}3}$	**2.803**	2.433	***1.838***	1.298	0.962	0.284	0.028	0.025	—	—
$R_{S3\text{-}4}$	**1.771**	1.929	***1.792***	1.808	1.452	0.842	0.439	0.746	—	—
$R_{S4\text{-}5}$	**7.671**	7.158	***5.802***	4.999	4.252	2.602	2.167	1.379	—	—
					Group II					
$R_{S6\text{-}7}$	—	—	—	—	**9.694**	7.622	5.325	5.012	***2.566***	1.508
$R_{S7\text{-}8}$	—	—	—	—	**17.399**	15.378	11.833	10.951	***4.524***	2.277
$R_{S8\text{-}9}$	—	—	—	—	**6.594**	3.935	1.241	0.894	***1.525***	1.854
					Group I					
$\alpha_{1\text{-}2}$	**1.202**	1.174	***1.155***	1.155	1.145	1.147	1.174	1.100	—	—
$\alpha_{2\text{-}3}$	**1.235**	1.198	***1.167***	1.150	1.137	1.092	1.024	1.015	—	—
$\alpha_{3\text{-}4}$	**1.144**	1.146	***1.155***	1.166	1.182	1.188	1.197	1.288	—	—
$\alpha_{4\text{-}5}$	**1.711**	1.609	***1.552***	1.514	1.482	1.413	1.358	1.272	—	—
					Group II					
$\alpha_{6\text{-}7}$	—	—	—	—	**2.721**	2.552	2.376	2.352	***1.968***	1.830
$\alpha_{7\text{-}8}$	—	—	—	—	**4.215**	3.917	3.602	3.506	***2.136***	1.771
$\alpha_{8\text{-}9}$	—	—	—	—	**1.561**	1.331	1.113	1.083	***1.223***	1.408

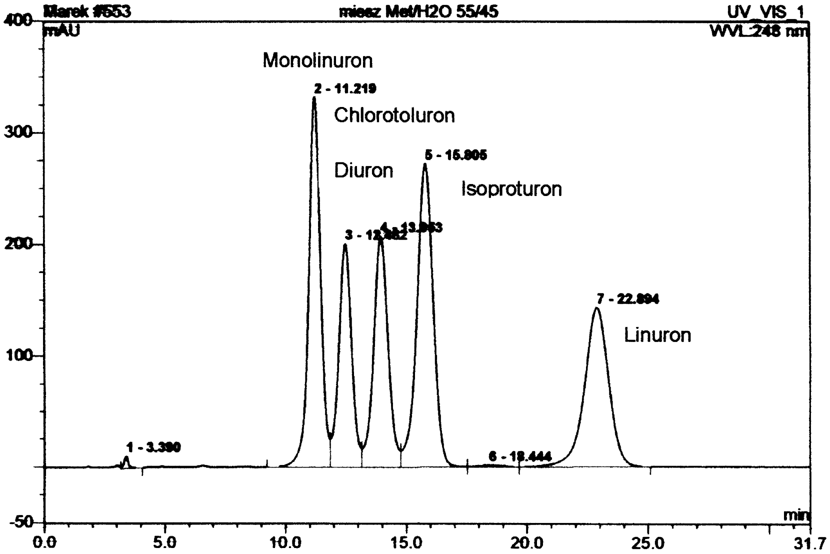

Figure 3. Chromatogram with methanol + water (55 : 45, v/v) as mobile phase for the first group of investigated pesticides.

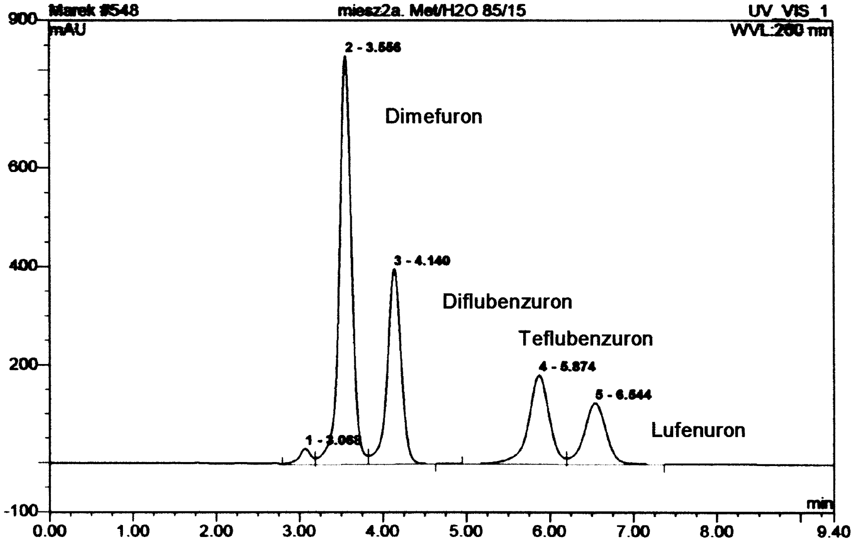

Figure 4. Chromatogram with methanol + water (85 : 15, v/v) as mobile phase for the second group of investigated pesticides.

Further investigations will concern the estimation of hydrophobic proprieties, as well as the determination of the above-mentioned substances in the environment.

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