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Chromatographic Separation of Pesticides from Various Chemical Classes

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Abstract

Features of chromatographic separation of pesticides from various chemical classes by the reversed-phase (RP) high-performance liquid chromatography (HPLC) method under isocratic mode of elution are shown. The most complete separation of the test substances is achieved using a lead electrolyte containing acetonitrile and 0.01 M ortho-phosphoric acid in a ratio of 3:2 (v/v), a flow rate of 0.5 mL min^{-1}, a column thermostat temperature of 40°C, a detection wavelength of 230 nm, and the volume of the injected sample of 10 µL. The separation time is 35 min. Under these conditions, the values of the chromatographic parameters, in particular, peak resolution (Rs), capacitance factor (k'), asymmetry factor (As), and selectivity coefficient (α), have optimal limits.

***Keywords:** Pesticides; HPLC; Chromatographic parameters; Eluent; Schatz hydrophobicity parameter*

Introduction

At the current stage of development of agricultural production, the range of chemical and biological methods of plant protections is constantly changing. In particular, drugs causing remote environmental consequences are excluded, and a list of useful tools is supplemented by effective compounds with new mechanisms of action in safer preparative forms. In recent years, highly toxic and persistent drugs (mercury-containing, chloroorganic, many organophosphorus, etc.) have been excluded from the list of pesticides used in agriculture [1-5].

Pesticides of the 21st century include sulfonylurea preparations and heterocyclic compounds of various series, in particular, pyridine herbicides, pyrimidine insecticides and fungicides, herbicides based on aryloxyphenoxypropionic acid derivatives, imidazolinone herbicides, insecticides of the phenylpyrazole and neonicotinoide classes, triazole and imidazolinone

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fungicides. The number of works on the synthesis of these compounds is continuously growing, and they successfully compete in efficiency with previously used pesticides [6].

Along with the use of individual pesticides, their various mixtures are often used in agriculture. Combined pesticides allow simultaneously to destroying weeds, pests and diseases, and are an important reserve for increasing the biological and economic efficiency of chemical methods of plant protection. It is known that the use of combinations of small doses of two or more pesticides can provide the same biological efficacy and duration of action as treatment with a large dose of a more toxic preparation. However, the expansion of simultaneous use of pesticides of various classes leads to a noticeable contamination of soil, surface waters, ground waters and drinking water as well as agricultural products [7,8].

The complexity and diversity of the composition of pesticide preparations, the large number of interfering substances make the control over the pollution of waters by pesticides a very complex analytical task. In addition, the composition of pesticides used varies significantly over time [9]. The search for optimal methods for the analysis of pesticides is one of the most important problems in environmental analytical chemistry. For analytical control of residual amounts of pesticides in agricultural and environmental facilities, methods of chromatography-mass spectrometry and gas chromatography, electrochemical methods, polarography, and enzyme immunoassay are widely used. The method of high-performance liquid chromatography (HPLC) has been used most in routine analyzes; however, the use of toxic substances as solvents and expensive reagents necessitates the selection of appropriate eluents for chromatographic separation and detection [10].

The purpose of this work was to develop optimal conditions for simultaneous chromatographic separation of active substances of pesticides of various chemical classes [11].

Experimental

Chromatographic studies were performed on a liquid chromatograph from Applied Biosystems (USA) equipped with a spectrophotometric detector Applied Biosystems Kratos 757 with a deuterium lamp. The operating range is from 190 nm to 360 nm, and the maximum sensitivity is 0.005 AUFS. Isocratic elution of the mobile phase was carried out through a Reprosil-PUR ODS column (4 mm × 150 mm size and 5.0 μm grain size). For the preparation of the mobile phase, acetonitrile purchased from Cryochrome (Russia), bidistilled water and ortho-phosphoric acid were used (FIG. 1). A degasser DG-18 was used to remove air bubbles in the mobile phase. Standard samples of pesticides (>98%) were taken as subjects of study: imazapyr (BASF, Germany), imidacloprid (BASF, Germany), imazethapyr (BASF, Germany), tsiprosulfamid (Bayer CropScience, Germany), metribuzin (Bayer CropScience, Germany), phenmedipham (Bayer CropScience, Germany), flumioxazine (Sumitomo Chemical Co., Japan), quizalofop-P-ethyl (Bayer CropScience, Germany), ethofumesate (Bayer CropScience, Germany), iprodion (BASF, Germany), flufenacet (Bayer Crop-Science, Germany), flubendiamide (Bayer CropScience, Germany), famoxadone (DuPont de Nemours International, Switzerland), pencikuron (Bayer CropScience, Germany), diflufenican (Bayer CropScience, Germany). Standard solutions of pesticides with a concentration of 100 μg mL^{-1} were prepared from dry samples using acetonitrile as the solvent. Calibration solutions of pesticides were stored in the working chamber of the refrigerator at a temperature of +3°C to -5°C in hermetically sealed glass containers for not more than 3 months. Before use, the solutions were kept at room temperature for at least 20 min. Working solutions of a mixture of

pesticides were prepared by diluting standard solutions of individual pesticides with acetonitrile immediately before use. Data processing was performed using the MultiChrom v.1.5 software (Ampersand Ltd., USA). The optimal conditions for separation of the components of the separated mixture were determined by the achievement of the following values: the peak resolution Rs ≥ 1.0, the capacitance factor 0.5 ≤ k' ≤ 20, the asymmetry factor 0.7 ≤ As ≤ 1.5 and the selectivity coefficient α ≥ 1.1.

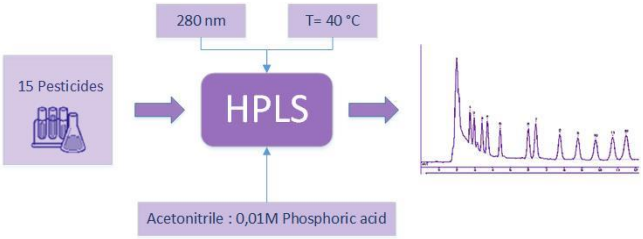

FIG. 1. **Acetonitrile: 0. 01 M phosphoric acid.**

Results and Discussion

When choosing the conditions for chromatographic separation of the mixture of the active substances of pesticides studied, the physical and chemical properties of the separated compounds were taken into account (TABLE 1). All substances are thermally unstable, low molecular weight aromatic carbocyclic and heterocyclic compounds containing both electron-withdrawing and electron-donating groups as substituents. To assess the degree of hydrophilicity and hydrophobicity of the compounds, the Schatz hydrophobicity parameter (H) and the octanol water partition coefficient (log P) were used, which were calculated by the formulas:

$$H = n_h - 4\sqrt{n_f} \qquad (1)$$

Where, n_h is the number of elementary hydrophobic fragments in the molecule, and n_f is the number of polar groups.

$$K_{ow} = \frac{C_0}{C_w} \qquad (2)$$

Where, C_o is the concentration of the test substance in *n*-octanol saturated with water, and C_w is the concentration of the test substance in water saturated with *n*-octanol.

TABLE 1. **Brief physicochemical characteristic of the active substances of pesticides.**

Active substance	**Class**	**Molar mass**	***H***	$\log K_{ow}$
Imazapyr	Imidazolinones	261.3	5	0.11
Imidacloprid	Neonicotinoids	255.7	0.6	0.57
Imazethapyr	Imidazolinones	289.3	5	1.49
Tsiprosulfamid	Methoxybenzamide derivatives	374.0	8	0,8
Metribuzin	Triazinones	214.3	0.1	1.65
Phenmedipham	Carbamates	300.3	6	3.59
Flumioxazine	Phenylphthalimides	354.3	10	2.55
Quizalofop-P-ethyl	Aryloxyphenoxypropionates	372.8	9	4.61
Ethofumesate	Benzofuranylalkanesulfonates	286.3	5	2.7
Iprodion	Dicarboxamides	330.2	5	3.1
Flufenacet	Oxyacetanilides	363.3	10	3.2
Flubendiamide	Benzenediracarboxamides	682.4	15	4.2
Famoxadone	Oxazolidinediones	374.4	12	4.8
Pencikuron	Urea derivatives	328.8	13	4.68
Diflufenican	Carboxamides	394.3	16	4.2

All investigated active substances of pesticides belong to low (H=0-4) and medium hydrophobic (H=4-20) substances, which are respectively partially soluble in water and are readily soluble in polar solvents. Given these characteristics, the most effective is the use of a reversed-phase (RP) version of liquid chromatography. A chromatographic column containing a silica-based sorbent chemically modified with alkylsilanes Cl-Si $(CH_3)_2$-R was used as a stationary phase, where R is an alkyl chain with eighteen carbon atoms or an octadecylsilated silica gel. A mixture of acetonitrile with a 0.01 M solution of ortho-phosphoric acid in various ratios (4:1, 7:3, 3:2, 1:1) was used as a mobile phase. Taking into account that the investigated active substances of pesticides are thermally unstable, the temperature of the column thermostat did not exceed 40°C. According to the methodological instructions for determining the residual quantities of individual pesticides, the detector wavelength of 230 nm was chosen. At the given wavelength, the compounds under study give the maximum response. Chromatographic mobility of the investigated active substances of pesticides was studied and the retention time for different composition of the eluent was determined. The results of chromatography in systems with different reagent contents, as an average of five parallel determinations, are presented in TABLE 2.

TABLE 2. **Retention times of active substances of pesticides with different composition of the mobile phase (n=5, P=0.95).**

Eluting system / Active substance	Retention time, min			
	CH_3CN/ 0.01 M H_3PO_4 (4:1)	CH_3CN/ 0.01 M H_3PO_4 (7:3)	CH_3CN/ 0.01 M H_3PO_4 (3:2)	CH_3CN/ 0.01 M H_3PO_4 (1:1)
Imazapyr	-	-	2.735	6.861
Imidacloprid	-	-	2.964	6.681
Imazethapyr	-	2.951	3.403	9.312
Tsiprosulfamid	3.234	3.032	3.705	12.012
Metribuzin	-	4.311	4.420	13.375
Phenmedipham	3.876	5.304	5.957	24.645
Flumioxazine	4.458	6.982	6.368	28.234
Quizalofop-P-ethyl	5.653	7.321	7.699	32.912
Ethofumesate	6.234	7.512	8.714	38.042
Iprodion	7.231	8.612	9.702	43.612
Flufenacet	8.342	9.432	10.663	45.453
Flubendiamide	9.054	10.234	11.425	45.567
Famoxadone	11.321	12.241	15.008	46.921
Pencikuron	14.765	15.442	18.245	49.925
Diflufenican	15.076	17.453	21.243	-

As seen from the TABLE 1, the active substances of pesticides with a low Schatz parameter and octanol water partition coefficient are not detected with the eluent containing 80% acetonitrile and 20% 0.01 M ortho-phosphoric acid solution. Imazapyr, imidacloprid, imazethapyr and metribuzin are sorbed irreversibly, i.e. the system has insufficient eluting power with respect to these substances. A decrease in acetonitrile in the mobile phase up to 70% leads to a similar pattern for imazapyr and imidacloprid, while the other active substances of pesticides are separated. With the composition of the mobile phase of 60% acetonitrile and 40% 0.01 M ortho-phosphoric acid solution, all the active substances of the pesticides are separated, and the chromatograms are characterized by fairly narrow peaks. It should be noted that with a further decrease in the volume fraction of acetonitrile in the mobile phase, the time for the release of active substances of pesticides is significantly increased, and for some substances, such as diflufenican, the retention time is not determined.

As a result, the complete separation of the test substances was achieved under the following conditions: mobile phase (acetonitrile and 0.01 M ortho-phosphoric acid in a ratio of 3:2 by volume) in isocratic mode; flow rate of 0.5 mL min^{-1}, the temperature of the column thermostat of 40°C, the detection wavelength of 230 nm, the sample volume of 10 μL, the analysis duration of 35 min. FIG. 2 shows a chromatogram of a mixture of standard substances obtained under these conditions.

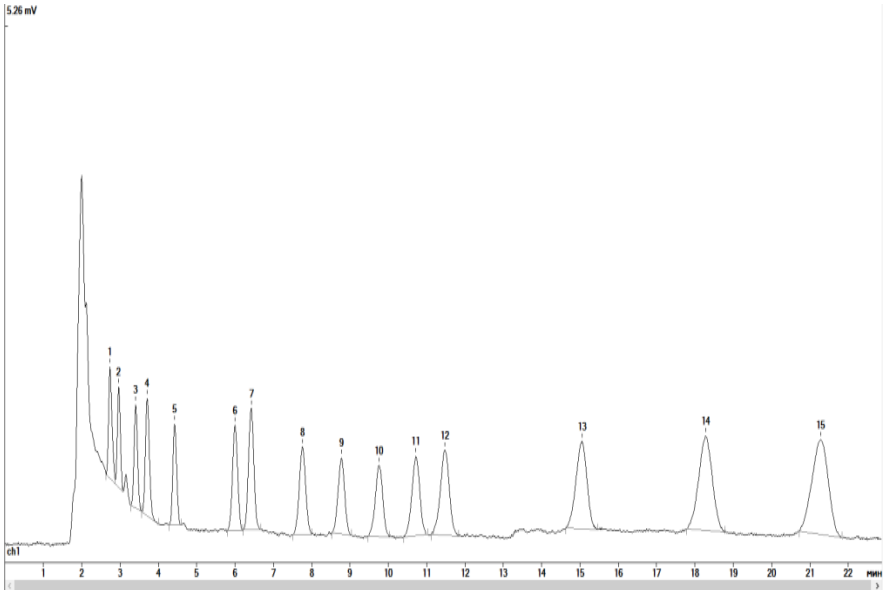

FIG. 2. **Chromatogram of a standard mixture of pesticides: 1-imazapyr, 2-imidacloprid, 3-imazethapyr, 4-tsiprosulfamid, 5-metribuzin, 6-phenmedipham, 7-flumioxazine, 8-quizalofop-P-ethyl, 9-ethofumesate, 10-iprodion, 11-flufenacet, 12-flubendiamide, 13-famoxadone, 14-pencikuron, 15-diflufenican.**

When choosing the optimal conditions for separation and identification of substances in the mixture, the dependence between the composition of the mobile phase and the chromatographic parameters (R_s-peak resolution, α-selectivity coefficient, A_s-asymmetry factor, k'-capacitance factor, N-number of theoretical plates). The results of calculation of chromatographic characteristics are presented in TABLE 3.

TABLE 3. **Parameters of chromatographic separation of active substances of pesticides.**

Active substance	R_s	α	A_s	k'	N
Imazapyr			1.68	0.57	4174
Imidacloprid	1.48	1.3	1.11	0.59	6513
Imazethapyr	2.79	1.5	1.20	0.71	6619
Tsiprosulfamid	1.71	1.2	1.20	0.86	6034
Metribuzin	3.82	1.4	1.14	1.22	8811
Phenmedipham	7.15	1.6	1.01	1.99	9436
Flumioxazine	1.65	1.1	1.04	2.19	9691
Quizalofop-P-ethyl	4.73	1.3	0.99	2.86	9595
Ethofumesate	3.20	1.2	0.93	3.37	10932
Iprodion	2.89	1.1	0.91	3.85	11471
Flufenacet	2.60	1.1	0.90	4.35	11879
Flubendiamide	1.87	1.1	0.91	4.71	10614
Famoxadone	7.34	1.4	0.85	6.52	11671
Pencikuron	5.30	1.2	0.95	8.16	11256
Diflufenican	3.99	1.2	0.83	9.64	10289

Under these chromatographic conditions, the peak resolution (Rs) values were in the range from 1.48 to 7.34, indicating a fairly complete separation of the two adjacent components. The values of the capacitance factor (k') for the pesticide active substances studied were from 0.37 to 9.64, which characterizes their retention in the column as optimal. If the selectivity coefficient (α) is ≥ 1.1, the separation is considered complete. In our case, α was in the range from 1.1 to 1.6, which indicates

the correct choice of the sorbent in the column and solvent in the mobile phase. The values of the asymmetry factor (As) calculated for the peaks of the investigated compounds were in the range from 0.83 to 1.68 that testifies about the absence of significant ion-exchange interactions. The number of theoretical plates (N) under these chromatographic conditions was in the range from 4174 to 11879, which indicates a large number of steady-state equilibria and high efficiency of the chromatographic column. In the selected optimal chromatographic conditions, calibration characteristics were obtained. The graphs for each active substance of pesticides have a linear relationship with the correlation coefficients R2 ≥ 0.9978 (TABLE 4).

TABLE 4**. Analytical characteristics of the procedure for determining the active substances of pesticides (n=5, P=0.95).**

Active substance	Retention time, min	Linearity range, μg mL^{-1}	Equation of calibration graph	Correlation coefficient
Imazapyr	2.70	0.03 – 4	y=0.022100x	0.9978
Imidacloprid	2.93	0.08 – 10	y=0.081246x	0.9986
Imazethapyr	3.36	0.04 – 5	y=0.039133x	0.9993
Tsiprosulfamid	3.66	0.05 – 6	y=0.036583x	0.9996
Metribuzin	4.37	0.02 – 3	y=0.020694x	0.9996
Phenmedipham	5.89	0.02 – 3	y=0.017073x	0.9997
Flumioxazine	6.31	0.02 – 2	y=0.009263x	0.9998
Quizalofop-P-ethyl	7.64	0.05 – 6	y=0.031069x	0.9998
Ethofumesate	8.66	0.08 – 10	y=0.058973x	0.9997
Iprodion	9.65	0.06 – 7	y=0.041369x	0.9996
Flufenacet	10.61	0.08 – 10	y=0.049935x	0.9997
Flubendiamide	11.37	0.08 – 10	y=0.036661x	0.9997
Famoxadone	14.96	0.05 – 6	y=0.018735x	0.9998
Pencikuron	18.22	0.06 – 8	y=0.017986x	0.9996
Diflufenican	21.23	0.08 – 10	y=0.018329x	0.9996

Conclusion

In conclusion, based on the results of present study, the composition of the mobile phase was selected and optimum elution conditions were found for identification of active substances of pesticides of various chemical classes: imazapyr, imidacloprid, imazethapyr, tsiprosulfamid, metribuzin, phenmedipham, flumioxazine, quizalofop-P-ethyl, ethofumesate, iprodion, flufenacet**,** flubendiamide, famoxadone, pencikuron, diflufenican, The obtained data indicate that this method ensures complete separation and identification of the investigated pesticides that are simultaneously in the mixture. The developed method of separation of a mixture of pesticides from different classes allows us to proceed to an analysis of the pollution of natural objects with pesticides, which is the subject of our next study.

Conflicts of Interest

No potential conflict of interest was reported by the authors.

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