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ARTICLES

A Distribution Chromatography Method for the Identification of Pesticides in Their Mixtures and Agricultural Products

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Abstract—The distribution of 166 pesticides of various classes (amides, dinitroanilines, pyrethroids, thiocarbamates, triazines, etc.) was studied at 20 ± 1°C in multiple extraction systems. The distribution constants (*P*) of pesticides between hexane and a polar phase are calculated. Based on the distribution constants of pesticides, a possibility of using distribution chromatography for their identification was considered. It is demonstrated on an example of pesticides with similar retention times that the hexane–water extraction system is most selective and universal for the identification of most compounds by gas and liquid chromatography. Using this system, logarithms of distribution constants of the substances under consideration are maximally differentiated and ranged from –1.32 to 8.0. Such a range makes it possible, with an acceptable volume ratio of the hexane and water phases (up to 1 : 500), to achieve a significant decrease in the peak area of the pesticide in hexane when it is washed with water in accordance with its *P* value up to $\log P = 3.0$. In the case of more hydrophobic pesticides, extraction systems of hexane–ethylene glycol, hexane–acetonitrile, and hexane–mixtures of acetonitrile with water and ethylene glycol can be used.

Keywords: identification of pesticides, pesticide distribution constants, distribution chromatography method, agricultural products, gas and liquid chromatography

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Chromatographic methods are used not only for the separation of substances but also for their identification by comparing the retention parameters of the components of a test mixture with the retention parameters of individual substances [1–3]. It should be noted that, despite significant advances in chromatographic equipment, the possibilities of separating and identifying substances by this method are limited mainly by the efficiency of chromatographic columns. In some cases, to improve the efficiency of the chromatographic system, it is advisable to use two-dimensional chromatography, based on a combination of chromatographic systems with various stationary or mobile phases [4]. To confirm the presence of a component in the test sample, columns with phases different in nature are sometimes used in chromatography [4].

The creation of highly selective mass spectrometric (MS) detectors significantly expanded the capabilities of chromatographic analysis by solving the problem of identifying compounds and lowering their detection limits. The application of such systems, in turn, led to the emergence of a multitude of procedures for the simultaneous determination of several dozens to several hundred substances in different matrices [5–9]. The high cost of chromatographic devices with mass spectrometric detectors limits their widespread use.

A rather simple method of liquid extraction seems capable of increasing the reliability of chromatographic analysis and confirming the identification of components [1–3, 9–13]. In particular, the distribution constants (*P*) of substances can be used along with the chromatographic parameters of retention for their identification. The procedure includes the distribution of a test mixture in a particular extraction system and a comparison of the ratio of substance concentrations in the equilibrium phases with their distribution constants [1–3]. This approach was used to identify substances of a rather simple molecular structure in mixtures with a limited number of components [2, 3], which does not offer the evaluation of the full the possibilities of the method known as a distribution chromatography method [1–3].

One of promising directions of using distribution constants for the identification of substances is the chromatographic analysis of such complex matrices as agricultural and food products for the concentration of residual amounts of pesticides. This direction seems promising given the successful use of distribution constants and distribution ratios of pesticides for the

development of sample preparation procedures for their subsequent determination in various objects [9–13].

The goal of the present work was to assess a possibility of using a distribution ratios chromatography method for identifying some pesticides in agricultural products, including the cases of their joint presence.

EXPERIMENTAL

Reagents. We used standard samples of pesticides with the concentration of active substances 98.0–99.9% (Dr. Ehrenstorfer, Germany); acetonitrile for HPLC–MS, 99.9% (Scharlau, Spain); methanol for HPLC–MS, 99.9% (Scharlau, Spain); *n*-hexane for gas chromatography (GC), 96% (Scharlau, Spain); acetone for GC, 99.8% (Scharlau, Spain); ethylene glycol, analytical grade (Merck, Germany); formic acid for HPLC–MS, 98.3% (Merck, Germany); and ammonium formate for HPLC–MS, 100.0% (Merck, Germany). Deionized water was prepared using a Milli-Q integral system for water purification (Millipore, United States). Nitrogen, 99.996% (Linde, Hungary), was used to evaporate solutions. Helium, 99.9999% (Linde, Hungary), was used as a carrier gas for gas chromatography.

Apparatus. *The GC–MS/MS analysis* was performed using a Varian CP3800 gas chromatograph equipped with a CombiPAL robotic system, a Best injector with a programmable evaporator temperature, and a 320-MS TQ triple quadrupole mass spectrometer (Varian, United States). Substances were separated in a capillary column, 30 m in length and 0.25 mm in internal diameter, with a layer of VF-1-MS stationary phase 0.25 μm in thickness (Agilent, United States).

The HPLC–MS/MS analysis was performed using a Varian ProStar liquid chromatograph equipped with an autosampler and a 320-MS TQ triple quadrupole mass spectrometer (Varian, United States). The substances were separated in a Restek Ultra C18 column, 100 mm in length and 2.1 mm in internal diameter, packed with a stationary phase with the particle size of 3 μm coupled with a similar 10-mm precolumn (Restek, United States).

MS Workstation software (Varian, United States) was used to control instruments and for data processing and quantitative calculations.

Conditions of chromatographic separation and detection. For GC–MS/MS analysis, 4 mL of sample was injected into the evaporator at a rate of 1 μL/s in the solvent-vent mode for 0.5 min after the sample injection, at an evaporator temperature of 80°C and a flow split ratio of 1/40. The split valve was then closed, and the temperature of the evaporator was raised at a rate of 200 deg/min to 300°C. The total transfer time was 4.0 min. The split valve was then opened (1/40).

The carrier gas flow rate was kept constant at 1.2 mL/min. The substances were separated in a mode of raising gradient of the column thermostat temperature: 0 min, 50°C; 1.5 min, 50°C; 5.83 min, 180°C; 25.83 min, 280°C; and 26.5 min, 280°C. The temperature of the transfer line to the mass detector was maintained at 290°C. Ionization was performed by electron impact (70 eV) at a source temperature of 200°C. The data were obtained in the selected reaction monitoring mode after the solvent delay from 4.7 to 26.5 min.

In HPLC–MS/MS analysis, an aqueous solution of formic acid (0.1 vol %) and ammonium formate (1 mM) was used as eluent A, and methanol was used as eluent B. The substances were separated in a gradient elution mode: 0 min, 10% B; 14 min, 100% B; 36 min, 100% B; 37 min, 10% B; and 43 min, 10% B. The eluent flow rate was maintained constant at 0.25 mL/min. The eluent from the column was fed to the mass detector without splitting the flow. The injected sample volume was 10 μL. The chromatographic column temperature was 40°C.

Electrospray ionization was used with the following parameters: the ion-spray potential was 5000 V, the nebulizer gas (nitrogen) pressure was 4.08 atm, the pressure of the drying gas (nitrogen) was 1.36 atm, and the collision gas (argon) pressure was 2.43×10^{-6} atm.

The data were obtained in the selected reaction monitoring mode with recording positive and negative ions. The delay time before the detector is turned on was 1.5 min.

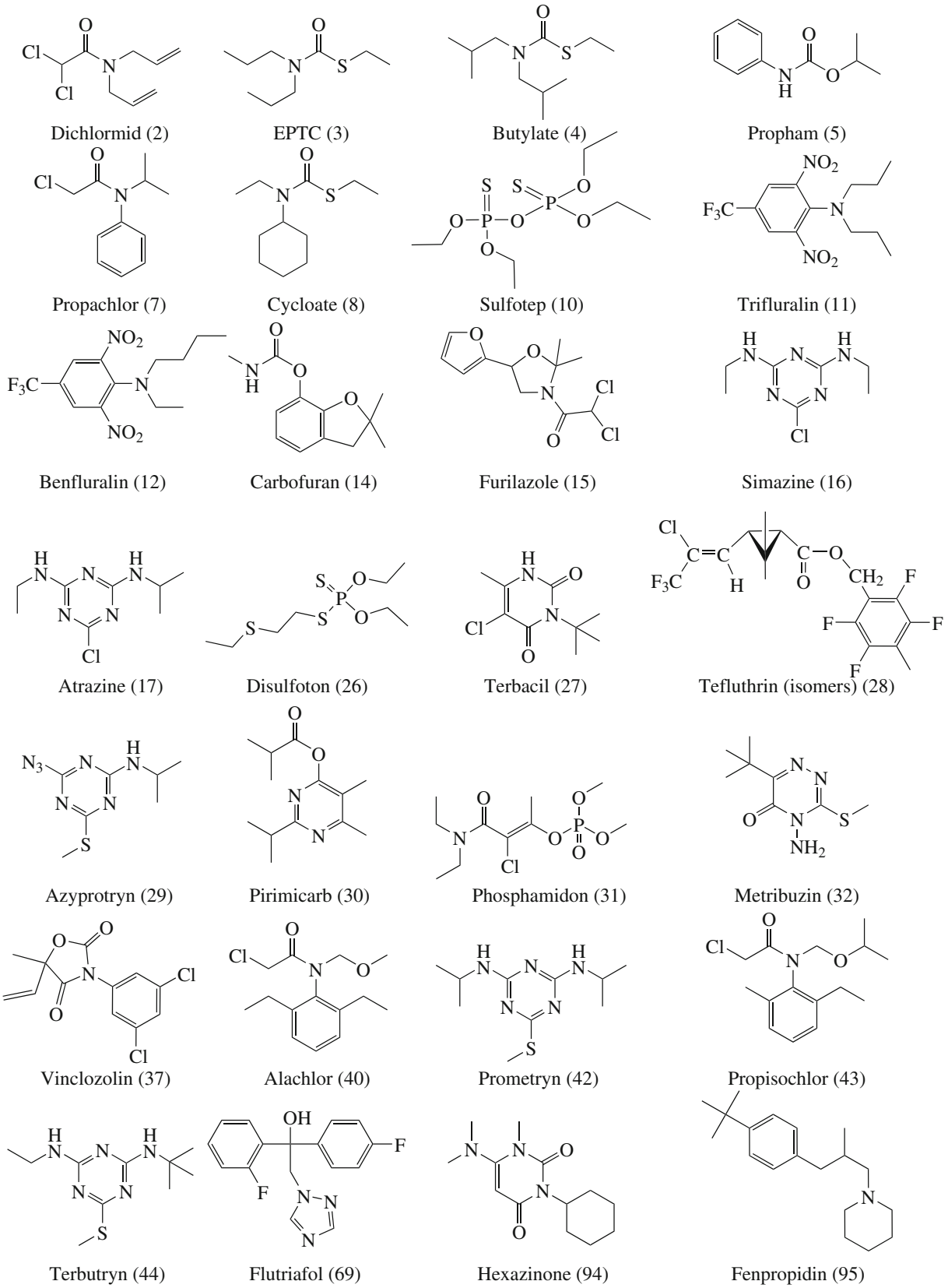
Determination of distribution constants. The distribution constants of pesticides in the extraction systems under study were determined at 20 ± 1°C. The distribution constants of pesticides between hexane and water in the range from 0.25 to 200 were calculated by a decrease in the concentration of the pesticides from the hexane phase at a volume ratio of hexane–water of 1 : 100. At $P > 200$, the distribution constants were calculated by re-extraction of the equilibrium polar phase with hexane, followed by the analysis of the hexane extract [12].

The distribution constants of pesticides in the remaining extraction systems were determined as the ratio of the equilibrium concentrations in the hexane and polar phases, respectively. The relative standard deviations of the calculated distribution constants P did not exceed 10%, as in the case of extraction of other organic substances [12].

The concentrations of substances were determined either directly in solutions or after preliminary transfer (blowing the solvent in a nitrogen stream and dissolving the dry residue or re-extraction) into a suitable solvent (hexane or acetone for the gas chromatograph and acetonitrile for the liquid chromatograph).

RESULTS AND DISCUSSION

The retention times of some pesticides when determined by gas and liquid chromatography and their distribution constants in various extraction systems are presented in Tables 1 and 2. The chemical structures of some of the pesticides in question are shown in Scheme 1.



Scheme 1. Chemical structures of some of the examined pesticides.

The data of Tables 1 and 2 show that the distribution constants of compounds with similar retention times in different extraction systems often differ significantly. For example, in the GC analysis, the differ-

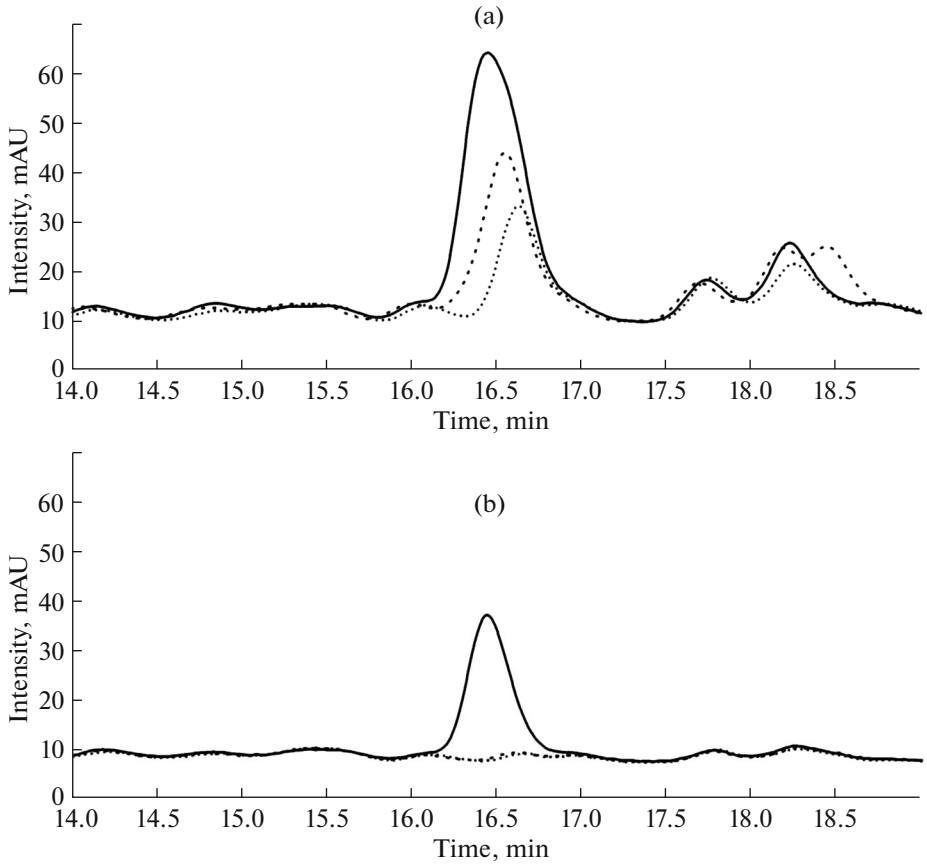


Fig. 1. Chromatograms (λ = 250 nm) of the acetonitrile extracts of control samples of barley grain (dotted line) without and (solid line) with addition of 0.200 mg/kg of halauxifen-methyl and (dashed line) the test barley grain sample after its treatment with halauxifen-methyl (a) without washing and (b) after washing with the same volume of hexane.

ence between the retention times of terbacil (27) and tefluthrin (28) is only 0.01 min (~1 s), and their distribution constants in the hexane–water system differ by five orders of magnitude. In the HPLC analysis, the difference between the retention times of haloxyfop (131) and cyprodinil (132) is only 0.06 min (~4 s), and the distribution constants in the hexane–water system differ by four orders of magnitude.

The principle of using the distribution constants of substances for their identification in chromatographic analysis consists in comparing the experimental value of the distribution constant of a component, the retention time of which is close to the expected substance, with the distribution constant of the proposed substance.

Of the wide variety of existing extraction systems, hexane–water and hexane–acetonitrile systems, most widely used in the analytical chemistry of pesticides, have been selected to illustrate their applicability for the identification of substances [5–10]. We also examined the systems of hexane–ethylene glycol, hexane–20% solution of water in acetonitrile, and hexane–20% ethylene glycol in acetonitrile.

It is known that the hexane–water system most strongly differentiates the distribution constants of substances due to the most pronounced hydrophobic and hydration effects of water and the least solvation properties of hexane [12].

Based on the assumption that chromatographic analysis can reliably fix the change in the peak area by at least 1/3 and the convenience of the volume ratio of hexane and water from 50 : 1 to 1 : 500 for practical use, the hexane–water system can be successfully used for the identification of pesticides with log P from –2.0 to 3.0. One hundred and ten (110) of the 166 pesticides studied fall in this range (Tables 1 and 2). In the gas chromatographic analysis, the distribution constants of substances can be calculated by a decrease in their peak areas in hexane after washing with water, and in liquid chromatography, by a decrease in the corresponding peaks in the water after washing with hexane.

If two analytes are characterized by close retention parameters under chromatographic conditions, it is advisable to select such a volume ratio of hexane and aqueous phases that satisfies the conditions of the optimal extraction separation of substances [9, 12]. When the peak area of the analyte with a higher the

Table 1. Retention times (t_R), their standard deviations (s), and logarithms of the distribution constants of substances determined by gas chromatography ($n = 5$)

No.	Substance	t_R, min	s	log P in hexane–polar phase systems				
				water	ACN	20% of water in ACN	EG	20% of EG in ACN
1	Dichlobenil	5.62	0.02	2.25	–0.74	1.48	0.10	–0.42
2	Dichlormid	5.68	0.01	1.18	–0.87	–0.11	–0.28	–1.00
3	EPTC	5.69	0.01	2.98	0.21	–	1.46	1.09
4	Butylate	6.08	0.01	3.21	0.43	–	1.92	1.24
5	Propham	6.23	0.02	1.65	–0.91	–0.58	–0.45	–1.33
6	Molinate	6.58	0.01	2.36	–0.02	0.96	0.84	0.11
7	Propachlor	6.95	0.01	1.19	–0.85	–0.87	–0.47	–1.11
8	Cycloate	7.10	0.01	3.32	0.24	1.15	1.42	0.33
9	Chlorpropham	7.23	0.02	2.40	–0.84	–0.73	–0.40	–1.20
10	Sulfotep	7.27	0.01	3.58	–0.76	–0.05	1.41	–0.69
11	Trifluralin	7.35	0.01	4.61	–0.44	0.56	2.09	–0.20
12	Benfluralin	7.37	0.01	4.49	–0.42	0.56	2.05	–0.21
13	Atraton	7.62	0.02	0.21	–1.02	–1.59	–	–1.65
14	Carbofuran	7.63	0.02	–0.28	–1.72	–2.07	–1.25	–2.12
15	Furilazole	7.70	0.01	1.25	–	–1.39	–0.42	–1.75
16	Simazine	7.72	0.04	–0.17	–	–1.87	–1.38	–2.05
17	Atrazine	7.77	0.01	0.52	–1.07	–1.41	–1.38	–1.72
18	Clomazone	7.79	0.01	1.83	–0.81	–0.83	0.00	–1.05
19	Propazine	7.82	0.01	1.28	–0.86	–1.06	–0.83	–1.32
20	Dioxathion	7.83	0.01	3.50	–1.09	–0.66	1.12	–1.07
21	Lindane	7.90	0.01	3.17	–0.62	–0.13	0.27	–0.71
22	Terbuthylazine	7.96	0.01	1.70	–0.87	–0.97	–0.80	–1.29
23	Quintozen	8.00	0.01	4.17	0.04	1.00	1.53	0.18
24	Profluralin	8.06	0.01	>3.7	–0.51	0.49	1.93	–0.43
25	Pyrimethanil	8.15	0.03	2.04	–0.64	–0.59	–0.33	–1.04
26	Disulfoton	8.16	0.01	3.64	–0.45	0.23	1.51	–0.38
27	Terbacil	8.28	0.02	–0.41	–1.52	–2.19	–1.70	–2.19
28	Tefluthrin	8.29	0.01	4.75	–0.08	0.90	2.54	0.19
29	Azyprotryn	8.31	0.04	2.00	–0.64	–0.54	–0.19	–0.90
30	Pirimicarb	8.41	0.01	1.70	–0.86	–1.13	–0.27	–1.06
31	Phosphamidon	8.59	0.02	–0.82	–2.05	–3.07	–1.83	–2.78
32	Metribuzin	8.69	0.02	–0.10	–1.46	–1.96	–1.41	–1.85
33	Dimethachlor	8.69	0.01	1.05	–1.04	–1.23	–0.25	–1.31
34	Dimethenamid	8.72	0.01	1.74	–0.85	–0.85	0.04	–1.10
35	Acetochlor	8.81	0.02	2.49	–0.74	–0.59	0.47	–0.88
36	Tolclofos-methyl	8.86	0.01	3.83	–0.70	–0.11	0.94	–0.60
37	Vinclozolin	8.87	0.01	3.17	–0.99	–0.71	0.67	–1.16
38	Spiroxamine I	8.96	0.01	2.99	–0.71	–0.17	–1.45	–1.19
39	Ametryn	8.97	0.02	1.56	–0.86	–0.91	–1.06	–1.36
40	Alachlor	8.97	0.01	2.56	–0.73	–0.59	0.47	–0.83
41	Metalaxyl	9.00	0.01	0.34	–1.26	–1.72	–0.69	–1.62

Table 1. (Contd.)

No.	Substance	t_R, min	s	log *P* in hexane–polar phase systems				
				water	ACN	20% of water in ACN	EG	20% of EG in ACN
42	Prometryn	9.02	0.01	2.30	–0.67	–0.65	–0.74	–1.04
43	Propisochlor	9.06	0.01	2.86	–0.62	–0.34	0.75	–0.70
44	Terbutryn	9.27	0.01	2.60	–0.62	–0.67	–0.65	–0.98
45	Pirimiphos-methyl	9.29	0.01	3.78	–0.54	0.00	1.41	–0.40
46	Dichlofluanid	9.40	0.01	3.18	–1.21	–0.92	0.22	–1.29
47	Spiroxamine II	9.44	0.01	2.94	–0.63	–0.14	–1.46	–1.29
48	Metolachlor	9.65	0.01	2.49	–0.73	–0.59	0.41	–0.84
49	Isocarbophos	9.78	0.01	1.09	–1.75	–2.04	–1.05	–2.19
50	Triadimefon	9.82	0.02	2.23	–1.15	–1.32	–0.36	–1.59
51	Flurochloridon	9.82	0.04	2.13	–1.56	–1.79	–0.62	–1.93
52	Tetraconazole	9.85	0.01	1.91	–1.71	–2.01	–1.18	–2.19
53	Fenson	9.86	0.03	3.26	–1.19	–0.89	0.10	–1.40
54	Isofenphos-methyl	10.16	0.01	3.36	–0.90	–0.66	0.67	–1.01
55	Metazachlor	10.26	0.02	0.98	–1.36	–1.55	–0.71	–1.68
56	Pendimethalin	10.35	0.01	4.43	–0.45	0.27	1.64	–0.24
57	Penconazole	10.40	0.02	2.53	–0.92	–1.06	–0.64	–1.34
58	Tolylfluanid	10.41	0.01	3.58	–1.03	–0.75	0.48	–1.19
59	Chlorfenvinphos	10.47	0.01	2.89	–1.01	–0.98	0.02	–1.19
60	Phenthoate	10.50	0.01	3.72	–1.03	–0.69	0.79	–1.09
61	Fluopyram	10.57	0.03	1.85	–1.47	–1.67	–0.65	–1.83
62	Pethoxamid	10.71	0.01	2.25	–0.92	–0.97	0.22	–1.13
63	Fipronil	10.75	0.01	1.41	–2.03	–2.14	–1.87	–2.39
64	Procymidone	10.77	0.02	2.53	–1.04	–0.82	0.21	–1.25
65	Triadimenol	10.87	0.06	0.64	–1.27	–1.79	–	–
66	Chinomethionate	10.95	0.04	5.07	–0.35	0.14	1.03	–0.49
67	Bromophos-ethyl	11.01	0.01	>3.7	–0.11	0.69	1.93	0.06
68	Mepanipyrim	11.21	0.03	2.68	–1.13	–1.05	–0.39	–1.44
69	Flutriafol	11.25	0.02	0.26	–1.67	–1.81	–1.53	–1.82
70	Chlorfenson	11.34	0.04	4.10	–0.98	–0.64	0.43	–1.12
71	Napropamid	11.40	0.01	2.44	–0.93	–0.98	–0.02	–1.27
72	Hexaconazole	11.52	0.01	2.47	–0.81	–1.08	–0.75	–1.26
73	Profenofos	11.61	0.02	3.81	–0.55	–0.29	0.81	–0.72
74	Myclobutanil	11.81	0.01	1.04	–1.65	–2.04	–1.32	–2.08
75	Kresoxim-methyl	12.00	0.01	3.26	–1.16	–1.05	0.71	–1.30
76	Oxyfluorfen	12.02	0.01	4.5*	–1.15	–0.59	0.95	–1.21
77	Bupirimate	12.03	0.01	2.93	–	–1.13	–0.09	–1.32
78	Isoxadifen-ethyl	13.28	0.01	3.55	–1.19	–0.99	0.49	–1.29
79	Cyanofenphos	13.46	0.02	3.75	–1.20	–0.99	0.34	–1.40
80	Benalaxyl	13.46	0.01	2.84	–1.01	–1.01	0.17	–1.19
81	Propiconazole	13.84	0.10	2.50	–0.96	–0.99	–0.59	–
82	Nuarimol	14.16	0.03	1.42	–1.20	–1.59	–1.33	–1.83
83	Tebuconazole	14.21	0.03	1.57	–1.16	–1.54	–1.33	–1.71
84	Propargite	14.33	0.01	–	–0.80	–0.24	1.56	–0.82
85	Piperonyl butoxide	14.53	0.02	3.79	–0.54	–0.19	1.63	–0.52

Table 1. (Contd.)

No.	Substance	t_R, min	s	log P in hexane–polar phase systems				
				water	ACN	20% of water in ACN	EG	20% of EG in ACN
86	Dimoxystrobin	15.16	0.01	2.24	−1.40	−1.60	−0.41	−1.83
87	Phenkapton	15.43	0.01	>3.9	−0.49	0.21	1.54	−0.39
88	Fenpropathrin	15.55	0.01	4.8*	−0.70	−0.01	1.71	−0.63
89	Bifenox	15.76	0.16	–	−1.42	−1.15	0.45	–
90	Lambda-cyhalothrin	17.08	0.04	8.0*	−0.96	−0.27	1.80	−0.94
91	Fenarimol	17.11	0.17	2.13	−0.98	−1.39	−1.08	−1.62
92	Dialifos	17.31	0.05	4.00	−1.21	−0.98	0.71	−1.40
93	Acrinathrin	17.64	0.19	5.9*	−1.09	−0.34	2.27	−1.09

* The values of the distribution constants are taken from [9].
Abbreviations: ACN, acetonitrile, and EG, ethylene glycol.

distribution constants in hexane decreases by 1/3, and with a smaller constant, by 2/3, the separation factor is $P_1/P_2 = 4$ ($\Delta\log P = 0.6$), which enables the reliable differentiation of the substances.

Many pairs of substances with close retention times satisfy the conditions $-2.0 < \log P_{1,2} < 3.0$ and $\Delta\log P \geq 0.6$. In the GC analysis, examples are dichlobenil (1) and dichlormid (2), dichlormid (2) and EPTC (3), propham (5) and molinate (6), molinate (6) and propachlor (7), carbofuran (14) and furilazole (15), simazine (16) and atrazine (17), pirimicarb (30) and phosphamidon (31), metribuzin (32) and dimethachlor (33), ametryn (39) and alachlor (40), metalaxyl (41) and prometryn (42), mepanipyrim (68) and flutriafol (69), etc. (Table 1). In the HPLC analysis, examples of such pairs of substances are hexazinone (94) and fenpropidin (95), thiodicarb (96) and carbaryl (97), isoxaflutole (102) and bromoxynil (103), etc. (Table 2).

A significant number of pesticides (Tables 1 and 2) are highly hydrophobic substances: in the hexane–water system, the value of $\log P$ for them are >3.0. Consequently, the possibilities of this system in identifying substances are limited by the need to use small volume ratios of the hexane and aqueous phases.

When passing from the hexane–water system to the hexane–acetonitrile system, the logarithms of the distribution constants of the vast majority of known pesticides, including those considered in this paper, sharply decrease and acquire negative values. This change ensures the use of the hexane–acetonitrile system for identifying highly hydrophobic substances. In comparison with the hexane–water system, a decrease in the peak area of the substance from hexane can be detected at a much higher ratio of hexane to acetonitrile volumes, which in turn enables hexane to be washed with acetonitrile directly in the vial for chromatography and opens up possibilities for the automation of the method.

The examples of pairs of substances with similar retention times in GC analysis, satisfying the conditions $-2.0 < \log P_{1,2} < 3.0$ and $\Delta\log P \geq 0.6$ in the hexane–acetonitrile system, are dichlormid (2) and EPTC (3), butylate (4) and propham (5), propachlor (7) and cycloate (8), cycloate (8) and sulfotep (10), lindane (21) and quintozen (23), and many others (Table 1). Wherein, it is not possible to identify the pesticides unambiguously from the latter two pairs of substances by washing hexane with water. Similarly, the washing of acetonitrile with hexane can be used to identify substances in HPLC analysis (Table 2).

There are many pesticides among those listed in Tables 1 and 2 that simultaneously have not only close retention times but also close values of $\log P$ in the hexane–acetonitrile system, while in the hexane–water system, they are characterized by $\log P > 3.0$ or $\Delta\log P < 0.6$. These include sulfotep (10) and trifluralin (11), dioxation (20) and lindane (21), disulfoton (26) and tefluthrin (28), alachlor (40) and prometryn (42), propisochlor (43) and terbutryn (54), napropamide (71) and hexaconazole (72), malathion (109) and fluopicolide (111), fluquinconazole (116) and flufenacet (119), rotenone (124) and picoxystrobin (125), and others. This fact greatly complicates the use of hexane–water and hexane–acetonitrile systems to identify such compounds.

This problem can be solved using the hexane–ethylene glycol extraction system, which is characterized by intermediate values of increment for the methylene group of the logarithm of distribution constant I_{CH_2} [10, 11] and $\log P$ of pesticides in comparison with the systems considered above. In the hexane–ethylene glycol system, many of the studied pesticides with close retention times are characterized by significantly

Table 2. Retention times (t_R), their standard deviations (s), and logarithms of the distribution constants of substances determined by liquid chromatography ($n = 5$)

No.	Substance	t_R, min	s	log *P*				
				water	ACN	20% of water in ACN	EG	20% of EG in ACN
94	Hexazinone	6.71	0.02	–1.32	–1.97	–2.15	–1.91	–1.98
95	Fenpropidin	6.72	0.02	1.95	–0.46	–1.15	–1.48	–1.19
96	Thiodicarb	6.74	0.03	–1.17	–3.09	–0.86	–1.78	–2.79
97	Carbaryl	6.80	0.02	0.37	–1.75	–1.75	–1.65	–1.78
98	Carboxin	6.80	0.02	0.78	–1.48	–1.47	–1.15	–1.56
99	Fenpropimorph	6.86	0.02	3.16	0.55	0.42	–0.03	0.25
100	Fosthiazate	6.94	0.02	0.1	–1.67	–1.64	–1.22	–1.67
101	Fluometuron	6.97	0.02	–0.06	–1.75	–1.80	–	–1.75
102	Isoxaflutole	7.10	0.03	1.29	–3.13	–2.35	–0.62	–3.00
103	Bromoxynil	7.10	0.02	–0.42	–1.65	–1.88	–1.49	–1.92
104	Metobromuron	7.13	0.01	0.99	–1.28	–1.23	–0.31	–1.43
105	Isoprocarb	7.15	0.01	0.75	–1.33	–1.20	–0.42	–1.49
106	Lenacil	7.21	0.01	–0.50	–1.39	–1.87	–1.59	–1.93
107	Isoproturon	7.25	0.01	0.26	–1.31	–1.47	–1.19	–1.64
108	Dimethomorph	7.89	0.01	0.90	–1.55	–1.67	–0.88	–1.85
109	Malathion	7.90	0.01	2.35	–1.43	–0.85	0.95	–1.28
110	Paclobutrazol	7.91	0.01	1.02	–1.18	–1.21	–0.83	–1.43
111	Fluopicolide	7.91	0.01	2.16	–1.29	–0.91	–0.30	–1.40
112	Isoprothiolane	7.93	0.01	2.16	–0.85	–0.44	0.63	–0.79
113	Propyzamid	8.07	0.01	1.74	–1.20	–0.90	–0.17	–1.36
114	Cyproconazole	8.08	0.09	0.97	–1.19	–1.21	–0.89	–1.53
115	Pyrifenox	8.18	0.01	2.58	–0.46	–0.24	0.41	–0.64
116	Fluquinconazole	8.19	0.01	2.33	–1.34	–1.25	–0.21	–1.50
117	Iprovalicarb	8.21	0.01	0.79	–1.30	–1.35	–0.61	–1.68
118	Fenhexamid	8.25	0.01	1.23	–1.25	–1.62	–1.36	–1.95
119	Flufenacet	8.28	0.01	2.24	–1.17	–0.79	0.58	–1.28
120	Triticonazole	8.32	0.01	0.69	–1.33	–1.47	–1.19	–1.72
121	Cyazofamid	8.34	0.01	2.38	–1.83	–1.28	0.09	–1.82
122	Epoxiconazole	8.36	0.01	1.96	–1.49	–1.31	–0.47	–1.68
123	Bromuconazole	8.44	0.33	1.79	–1.15	–0.95	–0.52	–1.40
124	Rotenone	8.46	0.01	2.71	–1.74	–1.44	0.01	–1.62
125	Picoxystrobin	8.50	0.01	2.80	–1.46	–0.81	0.87	–1.33
126	Flufenzine	8.52	0.03	1.93	–1.30	–0.80	0.21	–1.14
127	Tebufenozide	8.52	0.01	1.56	–1.59	–1.55	–1.02	–1.77
128	Flusilazole	8.53	0.01	2.25	–1.37	–1.21	–0.39	–1.58
129	Prothioconazole-desthio	8.54	0.01	2.09	–0.72	–0.64	0.13	–0.95
130	Famoxadone	8.59	0.04	2.18	–1.27	–2.56	–1.17	–2.00
131	Haloxyfop	8.60	0.04	–1.09	–2.20	–1.80	–1.84	–2.64
132	Cyprodinil	8.66	0.01	2.92	–0.53	–0.11	0.56	–0.73
133	Zoxamide	8.81	0.04	1.78	–1.73	–1.52	–0.95	–1.30
134	Phoxim	8.82	0.04	3.58	–1.02	–0.69	0.79	–0.72

Table 2. (Contd.)

No.	Substance	t_R, min	s	log P				
				water	ACN	20% of water in ACN	EG	20% of EG in ACN
135	Metconazole	8.92	0.05	1.57	–1.17	–1.23	–1.13	–1.40
136	Metrafenone	9.03	0.04	2.28	–0.97	–0.44	0.46	–0.74
137	Pencycuron	9.04	0.05	2.29	–1.19	–1.00	–0.71	–1.25
138	Clofentezine	9.13	0.04	3.03	–1.04	–0.48	0.33	–0.83
139	Difenoconazole	9.15	0.05	1.93	–1.31	–1.18	–0.92	–1.42
140	Hexaflumuron	9.17	0.05	3.77	–2.16	–1.86	–1.29	–2.30
141	Diniconazole	9.19	0.04	1.56	–0.80	–0.77	–0.72	–0.83
142	Trifloxystrobin	9.21	0.05	3.61	–1.32	–0.72	0.87	–1.08
143	Novaluron	9.22	0.06	4.03	–2.14	–1.74	–0.86	–1.97
144	Diflufenican	9.29	0.05	3.31	–0.99	–0.45	0.80	–0.70
145	Triflumizole	9.37	0.05	2.77	–0.92	–0.78	–0.26	–1.11
146	Benfuracarb	9.56	0.05	3.59	–1.06	–0.47	1.95	–0.99
147	Metaflumizone	9.66	0.05	2.26	–2.59	–2.12	–1.62	–2.56
148	Furathiocarb	9.74	0.05	3.43	–0.82	–0.31	0.96	–0.63
149	Tebufenpyrad	9.87	0.04	2.06	–0.41	0.24	0.91	–0.35
150	Buprofezin	9.89	0.04	3.81	–0.35	0.15	0.92	–0.25
151	Lufenuron	9.91	0.05	3.99	–2.27	–0.74	–0.81	–2.01
152	Propaquizafop	9.93	0.05	2.13	–1.14	–0.76	0.53	–1.03
153	Fluazinam	9.99	0.05	3.97	–1.57	–0.51	0.37	–1.08
154	Teflubenzuron	10.12	0.05	3.00	–1.61	–1.27	–1.01	–1.68
155	Pyriproxifen	10.27	0.05	3.72	–0.51	0.11	1.19	–0.34
156	Flucythrinate	10.35	0.06	2.28	–0.48	–0.54	0.76	–1.32
157	Hexythiazox	10.36	0.05	3.76	–0.80	–0.06	0.99	–0.38
158	Quinoxyfen	10.51	0.05	3.37	–0.28	0.03	0.75	–0.28
159	Flufenoxuron	10.53	0.06	1.85	–1.98	–1.56	–0.52	–1.89
160	Triallat	10.63	0.04	4.13	0.09	0.61	1.59	0.27
161	Fenpyroximate	10.86	0.06	2.17	–0.65	–0.25	1.11	–0.59
162	Spirodiclofen	10.89	0.06	3.53	–0.68	0.07	1.19	–0.45
163	Proquinazid	11.35	0.05	3.44	–	0.52	1.26	0.09
164	Pyridaben	11.41	0.06	3.8	–0.49	0.10	1.12	–0.42
165	Fenazaquin	11.69	0.06	3.55	–0.17	0.24	1.11	–0.18
166	Meptyldinocap	11.73	0.06	2.05	–0.49	0.28	1.38	–0.26

different logarithms of the distribution constants, and their values often lie in the range from –1.5 to +1.5 and have a different sign. This system can thus be successfully used for the identification of substances, especially in those cases when hexane–water and hexane–acetonitrile systems are not applicable for these purposes.

Mixtures of polar solvents rather than individual solvents can be used to separate hydrophobic components more efficiently. It is known that the addition of water to acetonitrile leads to the growth of I_{CH_2} and, accordingly, log P of strongly hydrophobic pesticides. The values of log P of substances in the system of hexane–acetonitrile aqueous solutions usually lie in the range between the values of log P for the hexane–water and hexane–acetonitrile systems. Thus, such a ratio of water to acetonitrile can be selected using water–acetonitrile mixtures, at which the constant of one of the eluted components is higher than 1, and the constant of the other component is smaller than 1. This suggests

the use of the volume ratio of the hexane and water–acetonitrile phases close to 1, which is convenient from a practical point of view. Thus, the rate of decreasing of the peak area after washing hexane with a 20% solution of water in acetonitrile can be successfully used to confirm the presence of a sulfotep (10) or trifluralin (11), tefluthrin (28) or azyprotryn (29), etc. in the test hexane solution. The use of a hexane–20% solution of water in acetonitrile is sometimes more useful for the separation of pesticides than using other systems, for example, for azyprotryn (29) and pirimicarb (30), tolclofos-methyl (36) and vinclozolin (37), pyrifenox (115) and fluquinconazole (116), and iprovalicarb (117), and fenhexamid (118).

In some cases, effective separation and identification of substances is possible using a variety of extraction systems, for example, for terbuthylazine (22) and quintozen (23), disulfoton (26) and terbacil (27), metaflumizone (147) and furathiocarb (148), teflubenzuron (154) and pyriproxifen (155), and others.

The limitations of the use of extraction for the identification of substances in a chromatogram should also be noted. For example, structural isomers often have close retention times in the chromatogram and close distribution constants in different extraction systems. Trifluralin (11) and benfluralin (12), as well as prometryn (42) and terbutryn (44), can be examples.

On the other hand, the obtained distribution constants can be used to select the extraction system for both further purification of the samples and for the division of the analytes into groups, which will further exclude overlapping of peaks in the chromatogram and, accordingly, increase the reliability of the analysis as a whole.

For example, when analyzing the acetonitrile extracts of winter barley grain treated and not treated with halauxifen-methyl using HPLC with diode-array detection (λ = 250 nm), peaks at t_R = 16.5 min are recorded corresponding to the concentration of halauxifen-methyl of 0.13 mg/kg (Fig. 1a), which exceeds the maximum residue level (MRL) established in the countries of the European Union (0.02 mg/kg) [14].

Washing of barley grain extracts dissolved in a 30% aqueous solution of acetonitrile with the same volume of hexane leads to a 40-fold decrease in the intensity of the peaks with a retention time of 16.5 min in the control and test samples. The decrease of the peak area in the chromatogram of the control sample with the standard addition of halauxifen-methyl is approximately 2/3. At the same time, according to the distribution constant of halauxifen-methyl in the hexane–30% aqueous solution of acetonitrile system of 0.85 ($\log P = -0.07$), the decrease in the peak area should be approximately 46%. Thus, a single wash with hexane makes it possible to separate the matrix component, which prevents the determination of halauxifen-methyl.

The areas of peaks at t_R = 16.5 min in the chromatograms of the control and test barley grain samples, taking into account the correction for loss of halauxifen-methyl after washing with hexane, correspond to the herbicide concentration of 0.01 mg/kg ($0.13 \times 3/40$), which is twice smaller than the MRL (Fig. 1b). Thus, a single wash with hexane makes it possible to confirm or exclude the presence of an analyte in the test sample.

This approach concerns molecular extraction and is universal. When the substances exhibit acid–base properties, extraction with aqueous and nonaqueous solutions of acids and bases can be used for the identification of components [10, 11, 15]. The efficiency of the separation of substances when dissociative extraction is used is often much higher than the efficiency of molecular extraction.

The identification and separation of polar hydrophilic substances, for which the logarithm of the distribution constants in the hexane–water system is much less than zero, can be implemented using extraction systems with more active organic solvents. For example, the methylene chloride–water system is characterized with higher increments of the polar groups of the logarithm of the distribution constants and, accordingly, significantly higher distribution constants of substances [15, 16]. The possibilities of extraction systems can be extended to even more hydrophilic substances with the use of salting-out agents [17].

CONCLUSIONS

It is shown that the distribution constants of pesticides in various extraction systems can serve as a basis for the selection of suitable extraction conditions, under which chromatographic analysis of the initial and equilibrium phases significantly improves the reliability of the identification of substances with similar retention times, including in their joint presence. The hexane–water mixture is the most selective and universal extraction system for identifying pesticides in their determination by gas and liquid chromatography. Replacing water with acetonitrile enables the use of liquid extraction also to identify highly hydrophobic pesticides. For a more efficient separation of hydrophobic pesticides and their subsequent identification by chromatographic methods, extraction systems hexane–ethylene glycol and hexane–mixtures of acetonitrile with water and with ethylene glycol can be used.

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