CHROMATOGRAPHIC APPROACH IN THE ASSESSMENT OF LIPOPHILICITY OF CHLOROACETAMIDE DERIVATIVES

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Abstract: Chloroacetamides are widely used and highly effective herbicides. However, in addition to the positive aspects of application, their residues and metabolites remain in water and soil for a long time, which is why they represent a threat to the ecosystem. Lipophilicity, as the most important physico-chemical parameter closely related to bioavailability or potential biological activity of compounds for the newly synthesized chloroacetamide derivatives was determined computationally by using appropriate software packages, as well as experimentally, by using thin-layer chromatography on reversed phases, RPTLC C18/UV254s in a mixture of water and four organic modifier, separately. It was found that the chromatographic behavior of the studied derivatives of chloroacetamide is affected to a small extent by the applied organic modifier, and to a greater extent by the total number of carbon atoms in the structure of their molecules, as well as by the type of hydrocarbon substituents. Dependence between the chromatographic parameters and the software-derived values of the partition coefficient as a standard measure of lipophilicity, as well as the relevant parameters of acute ecotoxicity, was examined by using the linear regression analysis. The reliable application of chromatographic parameters in the assessment of lipophilicity and ecotoxicity of the tested chloroacetamide derivatives was confirmed by the obtained mathematical models.

Keywords: herbicides, chloroacetamides, chromatography, lipophilicity, acute ecotoxicity.

1. INTRODUCTION

Since the discovery of the herbicidal activity of chloroacetamide in 1952, these compounds, thanks to their efficiency, selectivity, and price, have remained widely used in agriculture until today [1]. These herbicides affect the disruption of lipid synthesis in plants, which inhibits their growth, and they are mainly used to control annual grassy weeds in the cultivation of soybeans, corn, and cotton [2]. Along with the constant increase in the demand for food, the use of herbicides has also increased, which in addition to positive also has negative aspects such as endangering the safety of the ecosystem [3]. Numerous studies have established that the residues of chloroacetamide herbicides show good solubility in water and a low rate of degradation with a half-life of over 800 days [2]. Bearing this in mind, as well as their continued use over the past 15 years, the fact that their residues and metabolites have often been detected in surface and groundwater, soil and crops is not surprising. The possibility of manifesting harmful effects on human health is one of the important tasks in the synthesis of new chloroacetamide herbicides, therefore great attention is paid to the study of the ecotoxicity of already known as well as new herbicides of this type [4].

In general, most of today's research is focused on the intensive study of new ways and methods of synthesis of various compounds that show pro-

nounced biological activity. A modern approach to the design of new biologically active compounds is unthinkable without establishing the relationship between the chemical structure of the molecule and its biological activity. To establish the aforementioned dependence, parameters called molecular descriptors are applied. The most applied molecular descriptor, which affects the biological activity of a compound, i.e. its absorption, distribution, metabolism, elimination, and toxicity, is lipophilicity [5]. The first systematic studies of lipophilicity were performed in 1964 with the assumption that the path of a substance through the body could correspond to its distribution in the n-octanol/water system (cell membrane simulation) [6,7]. As a standard, the lipophilicity of a substance is quantitatively expressed by the logarithmic value of the partition coefficient, log$P$ [6,8]. In addition to the above, parameters obtained by different chromatographic techniques are also used as alternative measures of lipophilicity [9-12]. The advantage of chromatographic methods in determining the lipophilicity of substances is reflected in simplicity, efficiency, the use of small sample masses, but also the similarity of intermolecular interactions that determine the behavior of the substance in the biological and chromatographic environment.

In this paper, the lipophilicity of newly synthesized chloroacetamide derivatives was determined experimentally, by using thin-layer chromatography on reversed phases in two different organic modifiers, and computationally, using appropriate software packages. The influence of the nature of the substituent and applied organic modifiers on the chromatographic behavior of the studied chloroacetamides was investigated. Also, the relationships between chromatographic parameters (R_M^0 and m), software obtained values of the partition coefficient as a standard measure of lipophilicity, and selected parameters of acute ecotoxicity were examined using the linear regression method.

2. EXPERIMENTAL

Chromatographic parameters of newly synthesized chloroacetamide derivatives were determined by applying reversed phases thin-layer chromatography (Table 1). Ethanol solutions of the investigated compounds in concentration of 2 mgcm-3 were applied on commercial chromatographic plates RPTLC C18/UV254s, (Macherey-Nagel, Germany).

Table 1. *Structures of the tested newly synthesized chloroacetamide derivatives*

derivative	structure
1. N-ethyl-N-n-butyl-chloroacetamide	
2. N-ethyl-N-phenyl-chloroacetamide	
3. N-ethyl-N-n-pentyl-chloroacetamide	
4. N-methyl-N-n-hexyl-chloroacetamide	
5. N-ethyl-N-n-hexyl-chloroacetamide	

Chromatograms were developed in mixtures of water and 1-propanol (J.T. Backer, Deventer, The Netherlands), 2-propanol ((J.T. Backer, Deventer, The Netherlands), tetrahydrofuran (Sigma Aldrich, France) and acetonitrile (Sigma Aldrich, France), separately. The chromatograms are developed for about 20 minutes at room temperature, using a one-dimensional ascending technique, without prior saturation of the atmosphere of the chromatographic chamber with organic modifier vapors, whose volume fraction varied in the range φ= 0.36–0.52. After development, the

chromatographic plates were dried at room temperature, and the studied compounds were identified under UV light with a wavelength of λ = 254 nm, whereby dark spots appeared on the fluorescent basis.

Three chromatograms were developed for each applied modifier, and for each investigated derivative, the average R_f values were calculated, which were further used to calculate R_M values [13]. The linear dependence of the R_M value and the volume fraction of the organic modifier, φ, gave as a section the chromatographic retention constant R_M^0, while the slope of the line gave the value of the parameter m [14] (equation 1):

$$R_M = R_M^0 + m\phi \qquad (1)$$

Calculation of log$P$ partition coefficient values and ecotoxicity parameters was performed using online programs: Chemdraw Ultra 12.0, Chemaxon, Molinspiration, PreADMET and Simulation plus [15-19]. The experimental results were processed using the computer program Origin 6.1. The structures of the tested compounds are shown in Table 1.

3. RESULTS AND DISCUSSION

3.1. Determination of the lipophilicity of chloroacetamide derivatives

In the first phase, the lipophilicity of the studied chloroacetamides was determined computationally. Table 2 shows the software-obtained values of the partition coefficient log$P$, as a standard measure of the lipophilicity of the examined chloroacetamides. The data in Table 2 show that the values of the log$P$ partition coefficient differ for the same compound. The reason for this is different mathematical approaches used to obtain the log$P$ values.

Regardless of the calculation procedure, the lowest log$P$ values were obtained for the derivative 1, which can be explained by the smallest total number of C atoms in its structure among the studied derivatives, as well as the shortest alkyl substituents. In contrast, the highest log$P$ values were obtained for the derivative 5, which, unlike the derivative 2 (with the same number of C atoms), has a long alkyl substituent, and consequently, a more expressed hydrophobic nature. In the second phase, the lipophilicity of the studied derivatives of chloroacetamides was determined experimentally using thin-layer chromatography. The values of chromatographic parameters R_M^0 and m for the examined derivatives are shown in Table 3 and Table 4.

Comparing the R_M^0 values of different derivatives in the same modifier, it was observed that R_M^0 values increase with the overall number of C atoms in their structure, as well as with the increasing hydrophobic nature of the whole molecule. The lowest values of the chromatographic retention constant, R_M^0, in all applied organic modifiers were obtained for the derivative 1, while the highest were obtained for the derivative 5.

Table 2. logP values of studied derivatives of chloroacetamides

derivative	logP_{cd}	Clog$P$	milog$P$	logP_{ms}	Alog$P$	AlogP_2	AlogP_{98}
1.	1.620	1.927	2.080	1.600	2.002	4.009	1.874
2.	2.040	2.249	2.340	2.080	2.250	5.063	2.278
3.	2.040	2.456	2.590	2.000	2.458	6.044	2.264
4.	2.120	2.456	2.720	2.050	2.566	6.583	2.264
5.	2.450	2.985	3.090	2.390	2.915	8.495	2.654

Table 3. Values of chromatographic parameters of investigated chloroacetamides in protic modifiers

derivative	1-propanol			2-propanol		
	R_M^0	m	r	R_M^0	m	r
1.	1.794	-3.144	0.997	1.659	-2.518	0.995
2.	2.040	-3.394	0.998	1.859	-2.760	0.994
3.	1.968	-3.313	0.996	1.815	-2.715	0.999
4.	2.086	-3.454	0.999	1.949	-2.865	0.998
5.	2.220	-3.560	0.995	2.123	-3.069	0.996

Table 4. *Values of chromatographic parameters of investigated chloroacetamides in aprotic modifiers*

derivative	tetrahydrofuran			acetonitrile		
	R_M^0	m	r	R_M^0	m	r
1.	1.803	-3.898	0.993	0.963	-1.993	0.999
2.	1.964	-4.049	0.998	1.203	-2.215	0.998
3.	1.911	-3.990	0.996	1.147	-2.143	0.999
4.	1.997	-4.089	0.999	1.248	-2.289	0.994
5.	2.084	-4.138	0.997	1.349	-2.373	0.995

It is also visible that the values of the chromatographic retention constant R_M^0 for the same derivative are little different in the applied modifiers, indicating that it is not independent of the nature of the applied organic modifier. In protic modifiers, an increase in the R_M^0 values with an increase in the polarity of the modifier was observed, while in aprotic modifiers, the opposite phenomenon was observed, i.e. a decrease in the R_M^0 value with an increase in their polarity. The highest R_M^0 values were obtained in the least polar tetrahydrofuran, while the lowest were in the most polar acetonitrile.

In addition to the mentioned observations, it was noticed that for all examined derivatives in all applied modifiers, the slope value (m) follows the same trend of change as the intercept value (R_M^0). Assuming that the chromatographic parameters R_M^0 and m depend on the same physicochemical properties, they are correlated with each other by using linear regression analysis. The equations of the ob-

Table 5. *Equations of the dependencies $R_M^0 – m$ for the examined derivatives in used modifiers*

modifier	equation	r	sd	p
1-propanol	$R_M^0 = -1.352 – 1.000m$	0.998	0.012	$1.335 \cdot 10^{-4}$
2-propanol	$R_M^0 = -0.475 – 0.846m$	0.999	0.005	$< 1 \cdot 10^{-4}$
tetrahydrofuran	$R_M^0 = -2.544 – 1.115m$	0.993	0.014	$7.111 \cdot 10^{-4}$
acetonitrile	$R_M^0 = -0.975 – 0.979m$	0.993	0.020	$7.184 \cdot 10^{-4}$

Table 6. *Correlation matrix of the dependency between $R_M^0 – logP$ and $m – logP$*

	$logP_{cd}$	ClogP	milogP	$logP_{ms}$	AlogP	$AlogP_2$	$AlogP_{98}$
1-propanol							
R_M^0	0.981	0.916	0.912	0.978	0.918	0.912	0.975
m	0.968	0.894	0.899	0.964	0.905	0.896	0.941
2-propanol							
R_M^0	0.977	0.947	0.948	0.957	0.953	0.954	0.956
m	0.981	0.951	0.951	0.961	0.957	0.956	0.961
tetrahydrofuran							
R_M^0	0.975	0.909	0.907	0.972	0.914	0.908	0.954
m	0.949	0.864	0.876	0.946	0.882	0.872	0.916
acetonitrile							
R_M^0	0.979	0.906	0.906	0.977	0.912	0.900	0.956
m	0.960	0.888	0.902	0.951	0.906	0.897	0.927

tained linear dependencies and their regression coefficients are shown in Table 5.

The validity of the established $R_\mathrm{M}^0 - m$ dependencies is indicated by the high values of the regression coefficient, which confirmed that the mentioned chromatographic parameters depend on the same physicochemical properties (Table 5).

The potential application of chromatographic parameters as reliable alternative measures of the lipophilicity of newly synthesized chloroacetamides, was investigated by their correlation with computationally obtained $\log P$ values by using linear regression analysis (Table 6).

Based on the results shown in Table 6, it can be seen that among the values of the partition coefficient, the best agreement with R_M^0 and m values was obtained in the case of $\log P_\mathrm{cd}$, while the weakest agreement was observed with $A\log P_2$ values. High values of the regression coefficient, r, confirm that the obtained linear dependencies are valid, indicating that chromatographic parameters can be reliably applied in assessing the lipophilicity of the studied chloroacetamide derivatives.

3.2. The correlation of chromatographic parameters with ecotoxicity parameters

The preliminary ecotoxicity testing of chloroacetamide derivatives included the calculation of their effective concentrations as a measure of acute toxicity for selected test organisms, EC_{50} mgkg^{-1} (Table 7).

Table 7. EC_{50} values of the studied chloroacetamide derivatives for selected test organisms

derivative	*Algae*	*Daphnia*	*Medaka*	*Minnow*
1.	0.055	0.775	0.643	0.357
2.	0.093	0.438	0.241	0.154
3.	0.043	0.461	0.240	0.153
4.	0.040	0.344	0.138	0.126
5.	0.033	0.251	0.076	0.065

The data in Table 7 indicate that among all the examined chloroacetamides, the derivative 1, with the smallest number of C atoms, shows the lowest ecotoxicity on average, while the most lipophilic derivative 5 is also the most toxic. Additionally, all examined derivatives show the highest toxicity towards the *Algae* species, while they are least toxic to the *Daphnia* species.

By applying the linear regression method, the R_M^0 and m values of the investigated chloroacetamides were correlated with their EC_{50} values for different test organisms. The obtained results are shown in Table 8.

High values of the regression coefficient, r, given in Table 8, confirm the validity of the established linear dependencies.

4. CONCLUSION

Chloroacetamides have been widely used in agriculture as effective herbicides, but their residues and metabolites persist for a long time in water and

***Table 8.** Correlation matrix obtained from the linear correlation of EC_{50} values of the investigated chloroacetamide derivatives and their chromatographic parameters, R_M^0 and m*

	Algae	*Daphnia*	*Medaka*	*Minnow*
1-propanol				
R_M^0	0.983	0.975	0.942	0.949
m	0.977	0.978	0.947	0.948
2-propanol				
R_M^0	0.968	0.944	0.898	0.908
m	0.973	0.950	0.906	0.916
tetrahydrofuran				
R_M^0	0.977	0.970	0.934	0.941
m	0.963	0.971	0.940	0.935
acetonitrile				
R_M^0	0.991	0.989	0.966	0.970
m	0.969	0.975	0.944	0.940

soil, representing potential toxic agents for the ecosystem. Bearing in mind these facts, in this work, the retention behavior and then the lipophilicity of the newly synthesized chloroacetamide derivatives were studied by applying reversed phase thin-layer chromatography. It was found that the chromatographic behavior of the tested chloroacetamide derivatives was influenced to a small extent by the applied organic modifier, and to a greater extent by the total number of C atoms in the structure of their molecules, as well as the type of hydrocarbon substituents. It was also observed that an increase in the number of C atoms in the molecule leads to an increase in retention (lipophilicity). A linear dependence was observed between the chromatographic parameters (R_M^0 and m) and the mathematically calculated $\log P$ values of the tested chloroacetamide derivatives, that is, their EC_{50} values. All this indicated the reliability of applying reversed phase thin-layer chromatography for describing lipophilicity and acute ecotoxicity of newly synthesized chloroacetamide derivatives.

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ХРОМАТОГРАФСКИ ПРИСТУП У ПРОЦЕНИ ЛИПОФИЛНОСТИ ДЕРИВАТА ХЛОРОАЦЕТАМИДА

Сажетак: Хлороацетамиди припадају групи широко коришћених и високо ефикасних хербицида. Међутим, поред позитивних страна примене, њихове резидуе и метаболити се дуго задржавају у води и земљишту услед чега представљају опасност по екосистем. Липофилност, као најважнији физичко-хемијски параметар уско повезан са биорасположивошћу односно потенцијалном биолошком активношћу једињења је за новосинтетисане деривате хлороацетамида одређен рачунским путем применом одговарајућих софтвера, као и експерименталним путем, применом танкослојне хроматографије на обрнутим фазама, RPTLC C18/UV254s у смеши воде и четири органска модификатора, понаособ. Установљено је да на хроматографско понашање проучаваних деривата хлороацетамида у малој мери има примењени органски модификатор, а у већој мери укупан број угљеникових атома у структури њихових молекула, као и врста угљоводоничних супституената. Применом методе линеарне регресије је испитана зависност између хроматографских параметара и софтверски добијених вредности подеоног коефицијента као стандардног мерила липофилности, односно релевантних параметара акутне екотоксичности. Поуздана примена хроматографских параметара у процени липофилности и екотоксичности испитиваних деривата хлороацетамида је потврђена добијеним математичким моделима

Кључне ријечи: хербициди, хлороацетмиди, хроматографија, липофилност, акутна екотоксичност.