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Chemical Industry & Chemical Engineering Quarterly 19 (1) 1–6 (2013) CI&CEQ

VESNA DESPOTOVIĆ[1]
NEMANJA TRIŠOVIĆ[2]
ANAMARIJA MANDIĆ[3]
GORDANA UŠĆUMLIĆ[2]
TATJANA ĐAKOVIĆ-SEKULIĆ[1]

[1]University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Novi Sad, Serbia
[2]University of Belgrade, Faculty of Technology and Metallurgy, Belgrade, Serbia
[3]University of Novi Sad, Institute for Food Technology, Novi Sad, Serbia

SCIENTIFIC PAPER

UDC 66:547.783:543.544.5

DOI 10.2298/CICEQ120124037D

LIPOPHILICITY ASSESSMENT OF SOME 5,5-DISUBSTITUTED HYDANTOINS BY THE MEANS OF REVERSED PHASE LIQUID CHROMATOGRAPHY

The retention of some 5,5-disubstituted hydantoins was investigated by reversed phase high performance thin-layer chromatography (RP HPTLC) and reversed phase high-pressure liquid chromatography (RP HPLC). The mobile phases were mixtures of methanol-water and acetonitrile-water in various volume fractions. In order to explore and visualize similarities and differences among the investigated compounds and chromatographic system, Principal Component Analysis (PCA) was used. Results show that the experimental lipophilicity indices estimated from retention data ($R_{M,W}$, log k_w) and PC1 are directly correlated with logP values at a high significant statistical level.

Keywords: $R_{M,W}$, log k_w, PC1, log P, lipophilicity.

Hydantoins (2,4-imidazolidinediones) are important anticonvulsant drugs [1,2]. Besides anticonvulsant, various additional activities of hydantoin derivatives were reported in the literature, such as antiarrhythmic [3,4], antimicrobial, antifungal [5] or anticancer activity [6]. Since 1938, when Merrit and Putman found that 5,5-diphenylhydantoin showed anticonvulsant properties [5], hydantoins were a focus of research. Investigations have shown that the anticonvulsant action of hydantoins is a result of targeting the trans-membrane sodium channels in neurons and the reproduction of the normal ion potential. Since membrane interactions are related to transport phenomena through the blood-brain barrier on the way to the receptor binding site, the activity depends in great extent on lipophilicity [7,8]. It has been shown that optimal lipophilicity for penetration through the blood-brain barrier for anticonvulsant drugs is about log $P = 2$ [9]. However, the real importance of the lipophilicity has been pointed out within the QSAR (Quantitative Structure-Activity Relationship), QSRR (Quantitative Structure-Retention Relationship) and QSPR (Quantitative Structure-Property Relationship). QSAR tends to create a realistic image over the capacity of a compound to produce an impact over a biological system while QSRR and QSPR highlight the correlation between structure and the retention of compounds or their properties.

Lipophilicity represents the tendency of a compound to distribute between an immiscible organic, non-polar solvent (usually *n*-octanol), and water. It is expressed as logarithm of the partition coefficient (log *P*) [10]. Numerous methods for determination of log *P* are available today [11]. Besides the traditional experimental shake flask method, a number of software and internet modules for calculation log *P* are available. Moreover, the shake flask method for log *P* determination, especially in the case of bioactive compounds, is very often replaced by alternative chromatographic methods [12-17] since the partition (*i.e.*, lipophilicity) of a compound between aqueous and or-

Correspondening author: V. Despotović, University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Trg Dositeja Obradovica 3, 21000 Novi Sad, Republic of Serbia.
E-mail: vesna.despotovic@dh.uns.ac.rs
Paper received: 24 January, 2012
Paper revised: 19 April, 2012
Paper accepted: 20 April, 2012

ganic phase determines both its permeation through biological membranes and retention in RPLC. For this reason, RPLC has received considerable attention in predicting the pharmacological and pharmacokinetic properties of drugs in the early stages of the drug discovery phase.

The aim of this work was to analyze and compare the experimental lipophilicity estimated by different chromatographic retention indices ($R_{M,W}$, log k_w,) with calculated log *P* values for 5,5-disubstituted hydantoin derivatives. The investigated compounds are derivatives of known active drugs, and hence potentially active [18,19]. In order to explore and visualize similarities and differences among the compounds and the chromatographic systems principal, component analysis was used.

EXPERIMENTAL

Investigated compounds

The compounds investigated, listed in Table 1, were synthesized as described elsewhere [20,21]. For chromatographic analysis each substance was separately dissolved in ethanol (Zorka Pharma, Šabac, Serbia). The concentrations of the solutions were 1 and 0.1 mg mL^{-1} for HPTLC and HPLC, respectively.

Reversed phase liquid chromatography

Two chromatographic techniques of reversed phase liquid chromatography were applied: HPLC and HPTLC. Experimental conditions for both were described previously [22,23]. In addition to the HPLC methanol-water mobile phase, one more aqueous mobile phase was used: acetonitrile-water with volume fraction of acetonitrile 30-60% (v/v).

Log *P* calculations

The log *P* values of investigated compounds **1**-**15** were calculated by means of different programs available online (http://www.vcclab.org and http://www.chemsilico.com). Because all log *P* represent the same distribution coefficient in one and the same distribution system, all of them should be intercorrelated. However, the correlation matrix between different calculated log *P* values shows that it is not so (Table 2). The cross-correlation coefficient ranges between 0.879 (programs AC log *P vs.* COSMOF) and 0.996 (*ABI*log *P vs. m*log *P*). This was no surprise, since different algorithms were used for calculation for log *P* values of the same compound.

Principal Component Analysis (PCA)

PCA has been performed on the retention data matrix (R_M and log *k* values) using a computer program Past, ver. 2.05, (http://folk.uio.no/ohammer/past) and covariance matrix.

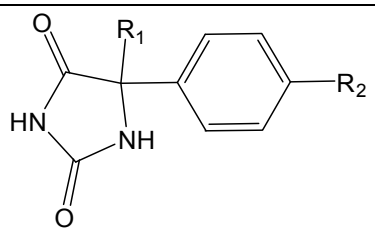
RESULTS AND DISCUSSION

In order to understand factors that affect to the activity, and hence to the retention, QSRR of chromatographic data was considered.

Principal Component Analysis of retention data

Principal Component Analysis, PCA, is a mathematical procedure that reduces complex multivariable data sets to more simple sets [24-27]. PCA combines original retention data into new variables (so-called principal components, PC) and gives both coordinate of scores of the studied compounds and the loadings of variables (retention obtained in particular mobile phases). PCs are formed in such way that the first principal component (PC1) covers as much of the va-

Table 1. The structure of the compounds investigated



I Series			II Series			III Series		
Compound	R_1	R_2	Compound	R_1	R_2	Compound	R_1	R_2
1	CH_3	OH	**6**	C_2H_5	OH	**11**	CH_3	H
2	CH_3	CH_3	**7**	C_2H_5	CH_3	**12**	C_2H_5	H
3	CH_3	OCH_3	**8**	C_2H_5	OCH_3	**13**	nC_3H_7	H
4	CH_3	Cl	**9**	C_2H_5	Cl	**14**	nC_4H_9	H
5	CH_3	Br	**10**	C_2H_5	Br	**15**	C_6H_5	H

Table 2. Correlation matrix between different calculated log P

	AlogP$_s$	CosmoF	Kowin	XlogP	milogP	AClogP	AB/logP	CslogP
*A*log *P*$_s$	1.000	0.956	0.957	0.961	0.978	0.944	0.977	0.942
CosmoF		1.000	0.982	0.960	0.971	0.879	0.978	0.892
*K*owwin			1.000	0.979	0.986	0.893	0.988	0.894
*X*log *P*				1.000	0.982	0.939	0.985	0.922
*mi*log *P*					1.000	0.945	0.996	0.924
*AC*log *P*						1.000	0.941	0.957
AB/log *P*							1.000	0.929
*CS*log *P*								1.000

riation within the data set as possible. The second principal component (PC2) describes the maximum amount of residual variation after PC1 has been taken into consideration, and so on. Using only a limited number of PCs the dimensionality of the data, space is reduced, simplifying further analysis.

Results of Principal component analysis are summarized in Table 3. In HPTLC, two or three principal components, for methanol and acetonitrile, respectively, as mobile phase modifiers are sufficient to describe most of the variation in the retention data. For HPLC, only one principal component (PC1) is sufficient to describe more than 99% of the variation in the retention.

Plotting scores in the space described by PC1 and PC2 (Figure 1) are very useful as a display tool for examining the relationships between the compounds and looking for trends, groupings, or outliers.

From Figure 1 it is evident that the compounds are grouped more or less in agreement with their chemical structure. PCA of HPTLC data shows that compounds from Series III (**11**-**14**) are distinguished from other substances according to PC2 with both acetonitrile and methanol modifiers. In all systems, compounds **1** and **6** (with R_2=OH), with the most polar substituent, have the most negative PC1 values forming one separated cluster. This cluster is more evident in the case of HPLC comparing to HPTLC, particularly in the methanol.

Although the PC's are abstract, PC1 is often well correlated with lipophilicity, molecular size, or steric factors, whereas PC2 seems more strongly correlated with dipole-dipole interactions and electronic factors [28-31]. For this reason, the logical step was to investigate correlation with lipophilicity.

Correlation with log *P*

The retention of hydanthoin derivatives varied in accordance with the methanol or acetonitrile content in the mobile phase; expressed as: $\log k = \log k_w + S\varphi$, *i.e.*, $R_M = R_{M,W} + S\varphi$, where the slope (S) is negative in case of reversed phase chromatography. Intercepts $\log k_w$ and $R_{M,W}$ are the most often used chromatographic lipophilicity parameters, given in Table 4.

The intercept is often used as a chromatographic parameter in QSAR correlations, too. As a partition parameter, it should be in agreement with log *P*. Table 5 lists correlations of log *P* vs. $R_{M,W}$ and log *P* vs. $\log k_w$ as well as correlation with PC1 (additional lipophilicity indices).

The correlation matrix from Table 5 shows relatively good correlation of retention indices ($\log k_w$ and $R_{M,W}$) with log *P*. This demonstrates that chromatographic systems used are good models to present the physiological conditions, since the chromatographic retention data are result of a dynamic distribution process very similar to the dynamic process of diffusion through the cell membrane [15]. For this reason, the $\log k_w$ and $R_{M,W}$ are considered as a lipophilicity measure. Better correlation with log *P* was obtained in HPLC mode compared to HPTLC.

Table 3. Eigenvalues of the covariance matrix and variance corresponding to the HPTLC and HPLC

PC	RP HPTLC				RP HPLC			
	CH_3OH		ACN		CH_3OH		ACN	
	Eigenvalue	Variance	Eigenvalue	Variance	Eigenvalue	Variance	Eigenvalue	Variance
1	0.631	96.963	0.291	79.774	0.165	99.758	0.102	99.136
2	0.019	2.919	0.063	17.364	3.3E-4	0.199	7.4E-4	0.714
3	0.001	0.087	0.008	2.061	3.3E-5	0.019	8.3E-5	0.081
4	0.001	0.019	0.002	0.555	2.9E-5	0.017	4.9E-5	0.047
5	6.7E-5	0.010	0.001	0.246	1.1E-5	0.006	2.3E-5	0.022

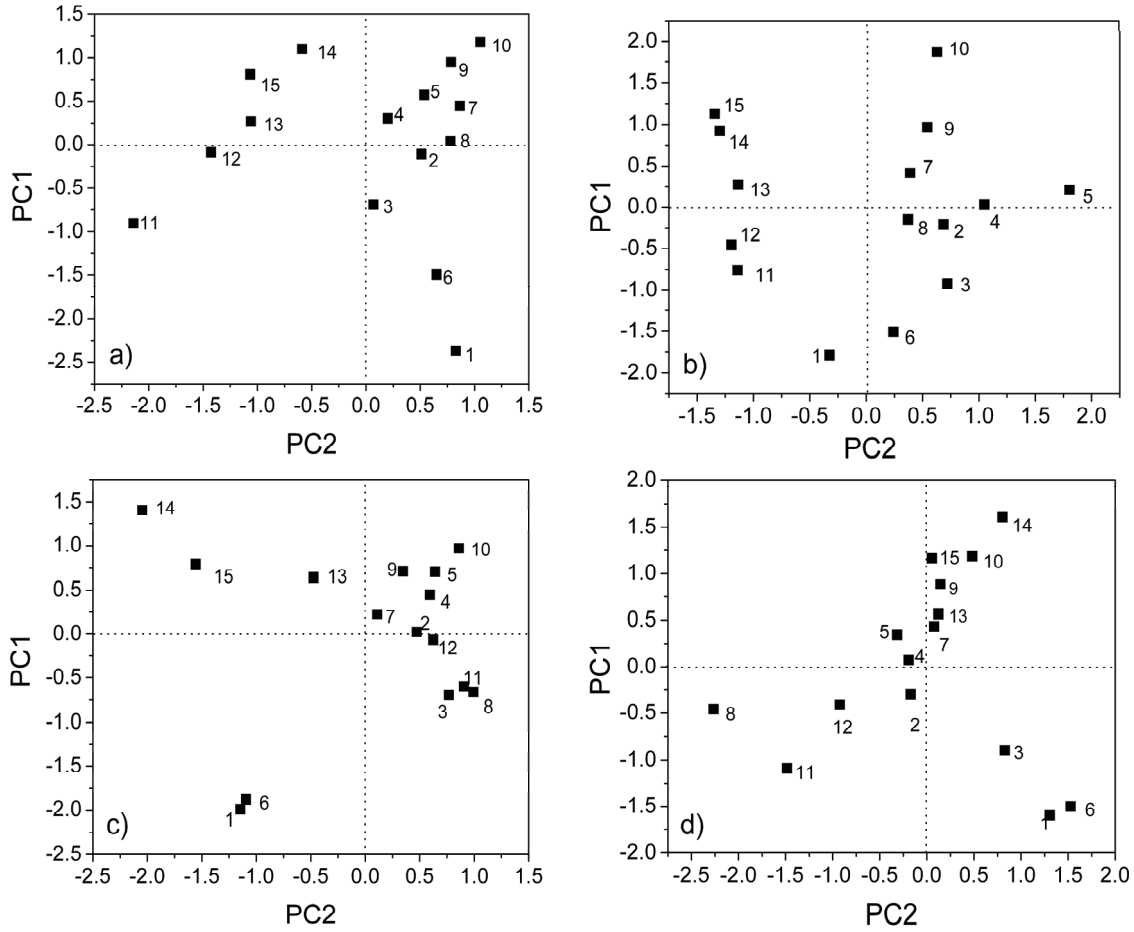


Figure 1. PC1-PC2 score plot of investigated compounds: a) methanol-water and b) acetonitrile-water in RP HPTLC mode; c) methanol-water and d) acetonitrile-water in RP HPLC mode.

In addition, log *P* values were compared with PC1 as well. It seems that PC1 (HPTLC) better expresses lipophilicity than $R_{M,W}$. In the case of HPLC, similar results were obtained for both PC1 and log k_w. This is in agreement with the observation that one PC is enough for HPLC. Comparing to HPTLC, HPLC

Table 4. Chromatographic lipophilicity parameters ($R_{M,W}$, log k_w) and PC1 for investigated compounds

Compound	RP HPTLC				RP HPLC			
	CH_3OH		ACN		CH_3OH		ACN	
	$R_{M,W}$	PC1	$R_{M,W}$	PC1	log k_w	PC1	log k_w	PC1
1	0.452	-2.371	0.101	-1.787	0.086	-1.995	0.074	-1.597
2	1.923	-0.109	1.183	-0.213	1.144	0.022	0.739	-0.301
3	1.701	-0.689	0.903	-0.921	0.701	-0.695	0.401	-0.894
4	2.318	0.303	1.396	0.025	1.380	0.449	0.904	0.073
5	2.412	0.572	1.789	0.205	1.523	0.704	1.027	0.3447
6	0.793	-1.494	0.542	-1.509	0.154	-1.883	0.088	-1.501
7	2.148	0.446	1.331	0.416	1.285	0.226	1.035	0.432
8	1.911	0.037	1.101	-0.151	0.701	-0.661	0.837	-0.457
9	2.609	0.948	1.613	0.964	1.550	0.708	1.231	0.881
10	2.655	1.177	2.166	1.869	1.668	0.968	1.335	1.184
11	2.529	-0.905	0.659	-0.758	0.748	-0.604	0.507	-1.086
12	2.804	-0.087	0.747	-0.456	1.082	-0.067	0.755	-0.412
13	2.897	0.264	1.138	0.267	1.569	0.638	1.088	0.5616
14	3.308	1.101	1.407	0.923	2.142	1.405	1.495	1.609
15	3.316	0.807	1.621	1.127	1.735	0.786	1.359	1.164

Table 5. The correlation matrix calculated for various log P and $R_{M,W}$, log k_W or PC1 for investigated compounds

Parameter	RP HPTLC				RP HPLC			
	CH_3OH		ACN		CH_3OH		ACN	
	PC1	$R_{M,W}$	PC1	$R_{M,W}$	PC1	log k_w	PC1	log k_w
*A*log P_s	0.966	0.849	0.948	0.856	0.933	0.936	0.969	0.977
CosmoF	0.969	0.830	0.939	0.863	0.924	0.925	0.958	0.970
*K*owwin	0.966	0.804	0.956	0.883	0.929	0.937	0.979	0.973
*X*log *P*	0.917	0.830	0.928	0.833	0.925	0.954	0.984	0.966
*m*log *P*	0.955	0.819	0.943	0.851	0.927	0.942	0.982	0.975
*AC*log *P*	0.895	0.803	0.911	0.804	0.878	0.911	0.965	0.945
AB/log *P*	0.964	0.852	0.953	0.862	0.943	0.954	0.983	0.983
*CS*log *P*	0.937	0.7633	0.9465	0.9214	0.904	0.912	0.949	0.939

retention data better express the lipophilicity. HPLC mobile phase acetonitrile-water is the most suitable for lipophilicity characterization of the investigated compounds.

CONCLUSION

Principal component analysis of retention data shows that one principal component is sufficient for HPLC for both mobile phase used. In the case of HPTLC, two or three principal component are sufficient to explain correlation between compounds for both methanol and acetonitrile mobile phase modifiers, respectively. For this reason, the plot of PC1 vs. PC2 for HPTLC data better displays clustering of investigated compounds than HPLC. It is observed that in HPTLC systems Series III (compounds **11-15**) forms one cluster.

The most negative PC1 in all chromatographic systems is obtained for 1 and 6, both with R_2=OH.

Correlation with calculated log *P* shows that PC1 (HPTLC) better expresses lipophilicity than $R_{M,W}$. In the case of HPLC, similar results were obtained for both PC1 and log k_w. This is in agreement with the observation that one PC is enough for HPLC. The conclusion is that lipophilicity expression of investigated compound is better achieved by HPLC than HPTLC. The most suitable for lipophilicity characterization of investigated compounds is mobile phase acetonitrile-water in HPLC mode.

Acknowledgment

This work was performed within the framework of the research project: “Investigation of the Synthesis, Structure and Activity of Natural and Synthetic Organic Compounds” (grant number 172013) supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

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VESNA DESPOTOVIĆ[1]
NEMANJA TRIŠOVIĆ[2]
ANAMARIJA MANDIĆ[3]
GORDANA UŠĆUMLIĆ[2]
TATJANA ĐAKOVIĆ-SEKULIĆ[1]

[1]Univerzitet u Novom Sadu, Prirodno-matematički fakultet, Departman za hemiju, biohemiju i zaštitu životne sredine, Novi Sad, Srbija
[2]Univerzitet u Beogradu, Tehnološko-metalurški fakultet, Beograd, Srbija
[3]Univerzitet u Novom Sadu, Institut za prehrambene tehnologije, Novi Sad, Srbija

NAUČNI RAD

PROCENA LIPOFILNOSTI NEKIH 5,5-DISUPSTITUISANIH HIDANTOINA PRIMENOM TEČNE HROMATOGRAFIJE NA OBRNUTIM FAZAMA

Ispitano je retenciono ponašanje derivata 5,5-disupstituisanih hidantoina primenom dve tehnike tečne hromatografije na obrnutim fazama, i to hromatografije na tankom sloju velike moći razdvajanja (HPTLC) i tečne hromatografije pod visokim pritiskom (HPLC). Kao pokretne faze korišćene su smeše metanol-voda i acetonitril-voda u različitim zapreminskim odnosima. Da bi ispitali i vizuelno utvrdili sličnosti i razlike među ispitivanim supstancama u korišćenim hromatografskim sistemima, primenjena je analiza glavnih komponenti (PCA analiza). Rezultati su pokazali da između računskih logP vrednosti i hromatografskih parametara lipofilnostikoji su dobijeni ekstrapolacijom retencionih podataka ($R_{M,W}$, log k_w), kao i PC1 postoji značajna startistička povezanost.

Ključne reči: $R_{M,W}$, log k_w, PC1, log P, lipofilnost.