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Dedicated to the memory of
Professor Candin Liteanu on his 100th anniversary

THE LIPOPHILICITY OF SOME DRUGS WITH TRICYCLIC STRUCTURE ESTIMATED BY THIN-LAYER CHROMATOGRAPHY AND COMPUTED BY VARIOUS METHODS

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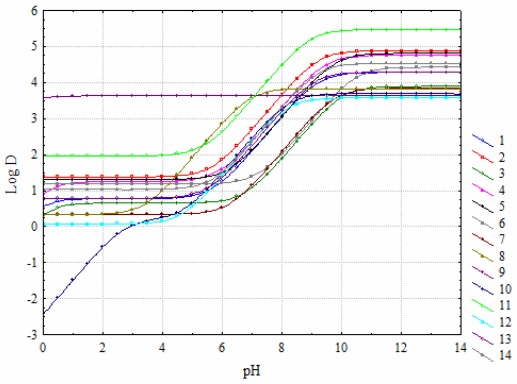
The lipophilicity of some drugs with tricyclic structure (antidepressants and phenothiazines) was investigated by thin-layer chromatography, on three stationary phases of RP-18, RP-8, and CN. The mobile phases were based on ethanol and different pH buffers (2, 4, 6, 7, 8, 10 and 12). The investigated lipophilicity indices were both experimentally (mR_M, R_{M0}, b, φ_0, $PC1/R_M$) and theoretically expressed (Log D and Log P). The effect of pH modification over the chromatographic behaviour has been evaluated. In order to find an objective manner of quantitative comparison of different chemically bonded stationary phases and evaluate the reliability of the obtained results some comparison procedures based on correlation diagrams and matrices were performed. The results indicated that all the stationary phases were useful for the lipophilicity investigation and that the chromatographic behaviour is highly dependent by the ionization state of the investigated compounds. This is also confirmed through the significant correlations obtained between pK_a values and experimental lipophilicity indices.



INTRODUCTION

Mental disorder is a psychological anomaly, most often reflected in behaviour, which is not considered part of normal development of a person. This may be associated with particular regions or functions of the brain or rest of the nervous system. The most serious mental illnesses, such as schizophrenia, bipolar disorder, major depression, and schizoaffective disorder, are often chronic and can cause serious disability. The cause of mental disorders is multiple and in many cases there is no single accepted or consistent cause currently established.[1]

Even if many of these disorders cannot be treated, they may be kept under control through adequate treatment. For example, the phenothiazines and trycyclic antidepressants (TCAs) are intensively used in treatment of schizophrenia and depression respectively. The TCAs action is based on raising the levels of neurotransmitters, serotonin and norepinephrine, in the brain by slowing/blocking the rate of reuptake or re-absorption by the presynaptic neuronal

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membrane. In addition, they can cause β-adrenergic down-regulation by blocking the postsynaptic receptors and inhibiting the reuptake of different neurotransmitters.[2, 3] Besides their beneficial activity, TCAs cause a number of side effects, amongst which can be mentioned the weight gain, dry mouth, constipation, drowsiness, dizziness, cardiotoxicity, poor dental health, etc.[4] On the other side, the phenothiazines are generally known as antipsychotic drugs, but they are exhibiting also anticancer, antiplasmid, antibacterial activities, antibiotic, reversal of multidrug resistance and potential treatment in Alzheimer's, Creutzfeldt-Jakob, and AIDS diseases.[5-8] However, they have also some side effects, such as urinary retention and urinary incontinence, allergies, dermatological problems, or even cardiac side effects.[9]

In spite of the complexity of the interaction of drugs and biological control systems, many drug responses correlate with simple physicochemical properties of the drug molecules concerned. The lipophilicity represents such a property and it is defined by IUPAC as being the affinity of a molecule or a moiety for a lipophilic environment. Lipophilicity is generally measured by evaluating the distribution behavior of compounds in biphasic systems, either liquid–liquid or solid–liquid.[10] Over the years, it has been generally accepted that the lipophilicity may be represented by the logarithm of the partition coefficient of the substance distribution between two immiscible phases.[11] Nowadays, the most common methods used for lipophilicity estimation are the chromatographic ones. They are versatile, flexible and from some points of view simulate biological conditions better, mainly because they involve a dynamic process of compound transfer between the used immiscible phases (stationary and mobile phase). In addition, these methods require only the determination of some retention parameters.[12, 13] The chromatographic approaches involved in the estimation of the lipophilic character are usually based on the reversed-phase partition mechanism, when the stationary phase is totally non-polar, such as RP-18 or RP-8, but since the biological environment is characterized by a high complexity described by various chemical entities, a pertinent analysis should be performed on stationary phases exhibiting different characteristics.[14-18] For example, more recently it was revealed that a CN modified stationary phase may often offer more descriptive lipophilicity indices, which can be considered a consequence of the multiple types of interactions that take place (both hydrophilic and lipophilic) during the separation process.[19, 20] The CN stationary phase, along with RP-18 and RP-8, has gained a strong position in lipophilicity determination and it should not be ignored in forthcoming studies.

For a better understanding of the behavior of the biologically active compounds, the lipophilic character should be estimated. However, it should be taken into consideration that in some cases the experimental conditions, such as the pH of mobile phase may play a crucial role in chromatographic retention of analytes with acid/base properties, mainly because it can affect the ionization degree of the compounds. In fact, slight variation in the mobile phase pH when it is close to pKa value of the analytes, may cause notable changes in the retention values.[21, 22] This may be also the situation of TCAs and phenothiazines, which are exhibiting ionic properties. In this context, the purpose of this work is to evaluate their lipophilicity on different stationary phases (exploited under the reversed-phase separation mechanism) using mobile phases of different pH values. One of the major goals is also to illustrate the similarity and differences existing between the investigated compounds, by using advanced chemometric methods, such as principal component analysis (PCA).

THEORY

Chromatographic estimation of lipophilicity

The chromatographic retention obtained for a given system is a direct consequence of the compound structure, and implicitly of their lipophilic character. The retention parameters resulting from a thin-layer chromatographic (TLC) process may be also associated to the lipophilic character of the analytes, and further extrapolated to biological effects. The most influent lipophilicity descriptor obtained by TLC has been purposed by Soczewiński–Wachtmeister[23] and it is expressed through the following equation:

$$R_M = R_{M0} + bC \quad (1)$$

where R_{M0} represents the intercept of the regression equation and it is associated with the R_M value of an analyte for a hypothetical mobile phase containing 100% water. The regression slope (b) is directly related to the specific surface area of the stationary phase, while C represents the volume fraction of the organic solvent in the mobile phase. The slope is considered an alternative descriptor of lipophilicity. The R_{M0} is widely accepted as the most powerful lipophilicity descriptor, despite the lack of physical meaning, as its value is more or less different from those experimentally determined through direct methods, when applicable. Moreover, it is fairly dependent by the organic modifier being used.[24] The R_M may be computed as depicted in Eq. (2):[25]

$$R_M = \log\left(\frac{1}{R_F} - 1\right) \quad (2)$$

The list of descriptors has been completed during the last few years with the arithmetical mean of retention parameters (mean of k, logk, R_F or R_M), which may be appreciated as very powerful lipophilicity descriptors[26, 27] and by the new insights offered by PCA, which has the ability to provide highly descriptive lipophilicity indices and new insights in the chromatographic mechanisms. PCA is also known as eigenvector analysis, eigenvector decomposition or Karhunen–Loéve expansion. PCA practically transforms the original data matrix ($X_{n\times m}$) into a product of two matrices, one of which contains the information about the objects ($S_{n\times m}$) and the other about the variables ($V_{m\times m}$). The S matrix contains the scores of *n* objects on *m* principal components (the scores are the projection of the objects on principal components). The V matrix is a square matrix and contains the loadings of the original variables on the principal components (the loadings are the weights of the original variables in each principal component). Loadings and respectively scores plots are very useful as a display tool for examining the relationships between characteristics and between compounds, looking for trends, grouping or outliers.[18, 26]

EXPERIMENTAL

Materials

Ethanol used as an organic modifier in the mobile phase was of analytical grade (Chemical Company, Iaşi, Roumania). The distilled water was produced in the laboratory by means of a Multilab GFL-2008 instrument and used during experiments. Standardized buffer solutions of pH 2, 4, 6, 7, 8, 10 and 12 were purchased from Sigma Aldrich. The tricyclic compounds used in this study, obtained from commercial sources (Fluka, Sigma-Aldrich, Redox, Bucharest, Roumania), were of analytical grade. The studied compounds were as follows: **1** imipramine hydrochloride; **2** clomipramine hydrochloride; **3** desipramine hydrochloride; **4** trimipramine maleate salt; **5** amitriptyline hydrochloride; **6** nortriptyline hydrochloride; **7** doxepin hydrochloride; **8** mianserin hydrochloride; **9** phenothiazine; **10** perphenazine; **11** thioridazine hydrochloride; **12** mesoridazine benzenesulfonate; **13** promethazine hydrochloride; and **14** chlorpromazine hydrochloride. Stock solutions, having a concentration of 1 mg mL^{-1} were obtained from each of the tested compounds through direct dissolution in ethanol. All the used stationary phases were Merck products (Darmstadt, Germany). The chemically bonded plates (10 × 10 cm) were by RP-18, RP-8 and the CN-modified plates that are based on a silica gel 60 modified with cyanopropyl. The spots were applied with a semiautomatic Linomat V system (Camag, Switzerland), equipped with a 100 μL syringe. After separation all spots were visualized under UV light at 245 nm, by using a UV lamp.

Chromatography

Spots of 2 μL were applied on the chromatographic plates on a 1.5 cm distance from the bottom and the margin of the plates, starting from left to right, with 0.7 cm space between two consecutive spots. The elution distance was 8 cm. The thin layer chromatographic separation was performed in an ascendant chromatographic chamber, priory saturated with the mobile phase for 15 min. The plates were developed at room temperature, in duplicate, and a small difference between runs has been observed. The mobile phases containing different mixtures of ethanol and standardized buffer solutions were optimized for each stationary phase type, in order to observe a significant migration of the compounds while the elution step was changed. The investigated compounds are having basic properties and that makes them difficult analytes in chromatographic analysis, mainly because of strong interactions between their cationic forms and surface-active groups. These interactions cause widening of spots, poor separation efficiency and spot asymmetry.[28] In our case, when the compounds were in single ionization state (either ionic or molecular; pH = 2, 4, 8, 10), the retention was increasing directly proportional with the organic modifier fraction in the mobile phase and decreasing when the compounds states were in more than one ionization state (pH = 6 and 7).

For each stationary phase, five steps were performed, at different fractions of ethanol, between 70 and 90%. The increment was changed with 5% per step in each case. The spots were observed as dark spots on the stationary plate background. The R_M values were calculated from retardation coefficients R_F, according to Eq. 2. The chromatographic lipophilicity indices accepted in this experiment are R_{M0}, mR_M and $PC1/R_M$. R_{M0} has been computed by means of Eq. (1), the mR_M represents the arithmetical mean of R_M values obtained with five mobile phases, while the $PC1/R_M$ are the scores corresponding to the first principal component obtained by applying PCA on the covariance matrix (14 cases × 5 variables) resulted from the obtained R_M values.

Computed lipophilicity indices

The log*P* values were estimated by means of various computer software or Internet available modules that apply different algorithms based on structural, atomistic, topological, electro-topological or other considerations, on a drawn chemical structure.[29] Some of the most common software for log*P* estimation are ChemOffice 8.0 (www.cambridgesoft.com), Alchemy 2000 (www.tripos.com) and Dragon Plus 5.4 (www.talete.mi.it). In addition, ALOGPS 2.1 (www.vcclab.org) and Marvin Sketch 5.5.01 (http://intro.bio.umb.edu/111-112/OLLM/ 111F98/newclogp.html) internet modules are also contribute by various log*P* values. In this experiment, prior to the computation, the chemical structures were pre-optimized and the resulting geometries were loaded by the above presented software in order to calculate the lipophilicity descriptors. For the investigated drugs, the Chem Office 8.0 had offered two indices (log(*p*)C – Crippen's method, log(*p*)V – Viswanadhan's method), Dragon Plus gave two values (MLOGP – Moriguchi's method, ALOGP – Ghose–Crippen's method), ALOGPS 2.1 allowed computation of seven values (ALOGPs, AC log*P*, miLog*P*, KOWWIN, XLOGP2 and XLOGP3), and finally Marvin Sketch allowed the computation of one descriptor (CLOGP). Since the experiments were carried out with mobile phases of different pH, the correlation of the chromatographic lipophilicity indices with a computed log D seems appropriate. The computation of log D and pK_a values has been made by means of the Marvin Sketch 5.5.01 Internet module. Moreover, the log *D* computing algorithm takes into account the contribution of pK_a, by means of some correction indices. All computed lipophilicity indices are enlisted in Table 1. All the experimental lipophilicity descriptors and graphs are computed through the Statistica 8.0 program (www.statsoft.com).

RESULTS AND DISCUSSION

The TCAs and phenothiazines are compounds that may be found in different ionization states, as a consequence of the environment pH. The highest ionization level may be observed in strong acidic environment, while the basic one favors the molecular state. This behavior may be directly observed for all compounds in the variance of the log D values while the pH is modified, excepting of ***9***, which is stable in all pH media (Fig. 1).

In case of ionisable compounds the lipophilicity values may be strongly affected by the pH of the mobile phase. In our particular case this influence has been strongly observed during the development process on all type of stationary phases. Analyzing Table 2, there can be observed that at pH values where the compounds were in singular state of ionization (2, 4, 8, 10), the R_{M0} values are much higher than those obtained at pH values when the compounds are in all ionization states (6, 7). This may be corroborated with different behavior associated to each state of ionization. In consequence, a mixture of different ions will lead to atypical values. In addition, this may be physically observed through spots enlargement, tailing effect, week correlation between retention parameter and mobile phase composition. Moreover, it could be remarked that at pH = 12, the R_{M0} values are also questionable. This is associated with the sensitivity of silica stationary phases at strongly basic pHs. In this case the silica gel layer is degrading and the results may not be considered as viable. According to the computed lipophilicity indices, the most lipophilic compound considered in this study were ***11***, while all other compounds had comparative lipophilcity values. Anyway, this is also a consequence of different chromatographic behavior exercised by ***9***, in comparison with all other compounds, thereby it can be accepted that ***11*** is the most lipophilic compound investigated in this study. At opposite pole may be found compound ***12***. The lipophilicity of the two investigated groups is generally at same level.

The lipophilicity of the investigated compounds is varying according to structural particularities. However, since each group is dedicated to cure a particular medical condition, appear the question about, which one of them is more appropriate to be used? A pertinent suggestion can be made by applying PCA on the matrix formed by the R_M values obtained for different mobile phases at given pH and stationary phase. This analytical tool is appropriate to be used since the first two PCs are retaining almost entire information stocked in the initial matrix (RP-18: $99.41_{(pH = 6)} \leq PC1 + PC2 \leq 99.92_{(pH = 7)}$; RP-8: $99.42_{(pH = 6)} \leq PC1 + PC2 \leq 99.84_{(pH = 2)}$; CN = $99.37_{(pH = 6)} \leq PC1 + PC2 \leq 99.91_{(pH = 10)}$). The inconsistences existed at pH = 6, caused by the presence of all ionization species can be underlined once again by PCs values, since the information retained by the first two PCs was always the lowest. This situation was constant independently by the nature of the stationary phase. By investigating the charts of the scores of the first two principal components (PCs) obtained by applying principal components analysis (PCA) on the matrices formed by the R_M values obtained on each stationary type at certain pH values (examples of PC1-PC2 charts are presented in Fig. 2 a-c) may be observed that compounds ***9*** and ***10*** are exhibiting the most different behavior amongst all others. The other phenothiazines are more similar from structural point of view, which is why they are forming linear clusters in the PC1-PC2 charts. Moreover, this behavior was observed on all stationary phases at all pHs.

Table 1

The computed lipophilicity indices and pK_a values

Cpd	pKa	Log D (pH = 2)	Log D (pH = 4)	Log D (pH = 6)	Log D (pH = 7)	Log D (pH = 8)	Log D (pH = 10)	Log D (pH = 12)	CLOGP	ALOGPs	AC logP	miLogP	KOWWIN	XLOGP2	XLOGP3	log P^C	log P^V	MLOGP	ALOGP
1	9.20	0.77	0.79	1.26	2.10	3.06	4.22	4.28	4.28	4.53	4.47	4.16	5.01	4.03	4.80	4.32	4.01	3.88	4.39
2	9.20	1.38	1.39	1.86	2.70	3.66	4.82	4.88	4.88	5.04	5.08	4.82	5.65	4.65	5.19	4.87	4.52	4.37	5.05
3	10.02	0.65	0.65	0.72	1.08	1.90	3.59	3.89	3.90	4.02	4.25	3.92	4.80	3.79	4.90	3.94	3.64	3.64	3.85
4	9.42	1.25	1.26	1.60	2.37	3.32	4.66	4.76	4.76	4.67	4.81	4.64	5.43	4.33	2.24	4.80	4.51	4.10	4.84
5	9.76	1.31	1.31	1.50	2.12	3.05	4.61	4.81	4.81	5.10	4.22	4.19	4.95	4.93	5.04	4.63	4.64	4.76	4.77
6	10.47	1.18	1.18	1.21	1.39	2.02	3.83	4.41	4.43	4.65	4.00	3.94	4.74	4.69	4.51	4.25	4.27	4.53	4.24
7	9.76	0.34	0.34	0.53	1.15	2.08	3.64	3.84	3.84	4.08	3.24	3.86	3.99	3.99	4.29	3.72	3.68	3.53	3.91
8	6.92	0.35	1.01	2.86	3.57	3.80	3.83	3.83	3.83	3.52	3.25	3.62	3.35	3.42	3.38	3.85	3.99	3.38	3.71
9	11.48	3.63	3.63	3.63	3.63	3.63	3.63	3.63	3.63	4.19	3.62	4.01	3.43	3.79	2.25	3.34	3.52	2.86	3.67
10	8.21	-0.57	0.25	1.50	2.46	3.27	3.69	3.69	3.69	4.15	3.66	4.29	3.42	3.86	2.24	3.48	3.68	2.63	4.16
11	8.93	1.97	1.98	2.64	3.55	4.49	5.43	5.47	5.47	5.93	5.00	5.68	6.06	5.94	3.91	4.84	4.69	4.06	5.56
12	8.18	0.07	0.15	1.40	2.36	3.17	3.57	3.57	3.57	3.83	4.07	4.09	3.89	3.91	2.52	3.06	3.18	3.14	4.45
13	9.05	0.79	0.80	1.37	2.25	3.21	4.24	4.29	4.29	4.52	3.46	4.44	4.09	4.40	2.57	3.90	4.16	3.26	4.39
14	9.20	1.03	1.04	1.51	2.36	3.31	4.47	4.53	4.54	5.18	4.14	5.03	4.81	4.92	3.12	4.24	4.32	3.77	4.74

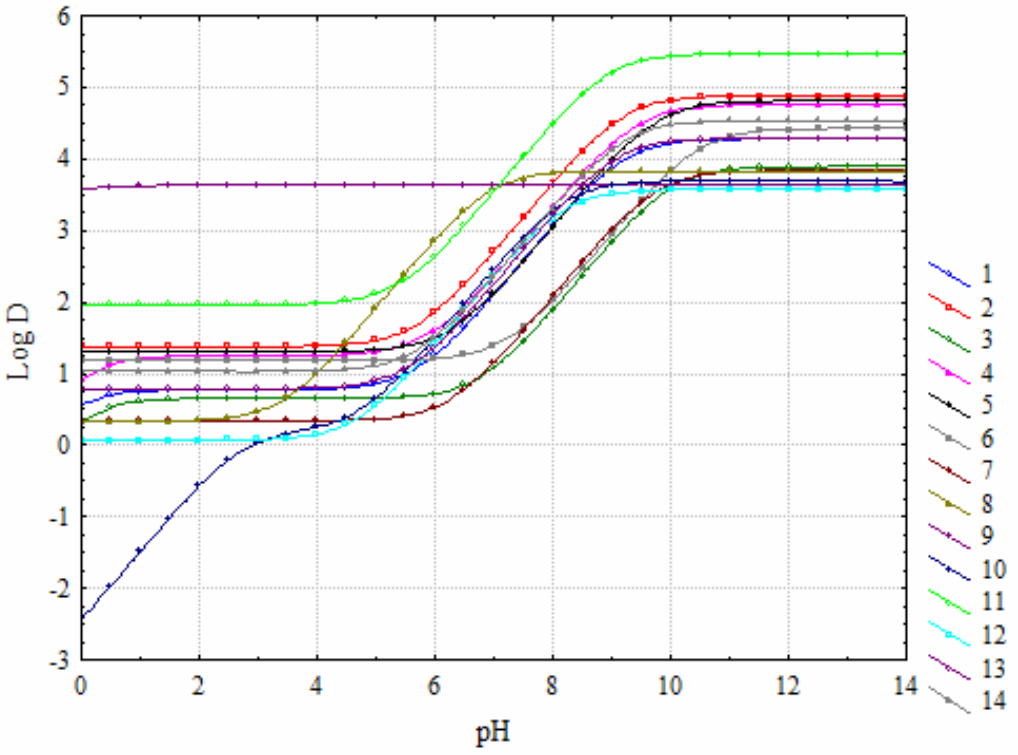
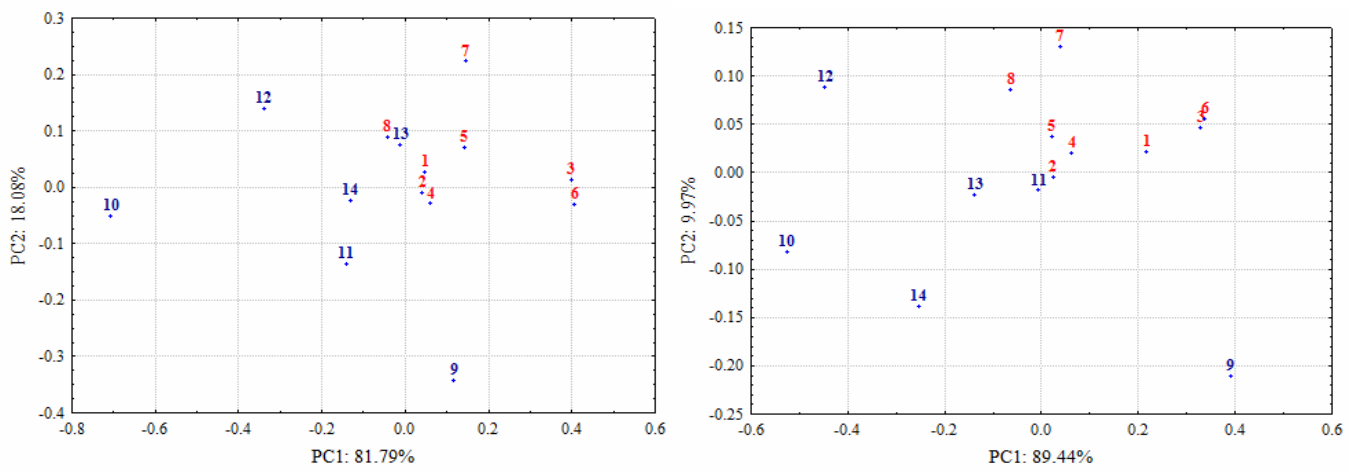


Fig. 1 – Log D variance as a function of the pH modification.

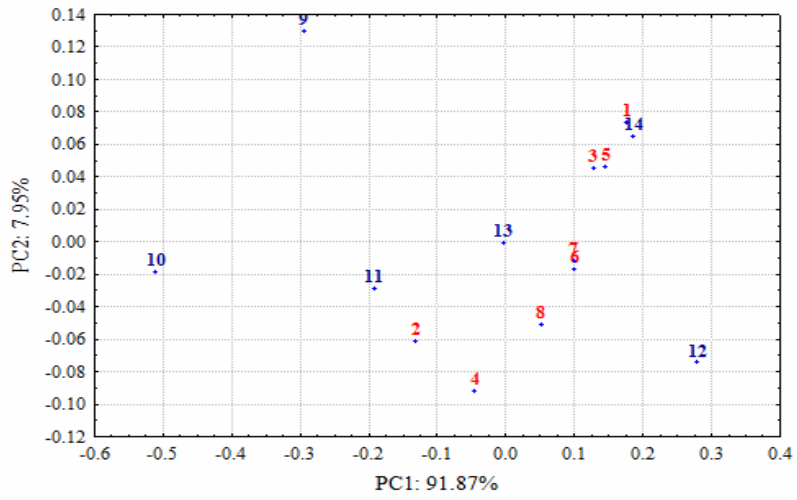
Table 2

The chromatographic lipophilicity indices

Cpd.	R_{M0}																				
	RP-18							RP-8							CN						
	pH = 2	pH = 4	pH = 6	pH = 7	pH = 8	pH = 10	pH = 12	pH = 2	pH = 4	pH = 6	pH = 7	pH = 8	pH = 10	pH = 12	pH = 2	pH = 4	pH = 6	pH = 7	pH = 8	pH = 10	pH = 12
1	0.34	0.29	-1.24	-0.84	0.66	0.34	-0.55	0.72	0.62	-1.47	-0.22	1.06	1.03	-0.72	0.57	0.71	-1.55	-0.81	0.71	0.86	-0.79
2	0.98	0.59	-0.86	-0.98	0.88	0.52	-0.71	1.02	0.01	-0.96	-0.14	1.40	0.89	-0.35	0.19	0.41	-0.93	-0.50	0.74	1.15	0.02
3	0.33	0.58	-0.40	-0.78	1.00	0.72	-0.76	0.94	0.36	0.16	-0.51	1.41	1.09	-0.03	0.85	0.61	-0.81	-0.96	0.50	0.83	-0.35
4	0.85	0.49	-1.08	-0.67	0.81	0.63	-0.81	0.61	0.34	-1.20	-0.73	1.16	0.83	-0.08	0.88	0.03	-1.06	-0.75	0.13	0.92	-0.56
5	0.56	0.05	-1.01	-1.00	0.29	0.21	-1.33	0.42	0.43	-0.94	-0.48	1.03	0.68	-0.65	0.64	0.58	-1.13	-0.82	0.21	0.88	-0.76
6	0.71	0.91	-0.55	-0.80	0.88	0.95	-1.15	1.08	0.72	-0.66	-0.31	1.25	1.03	-1.07	0.91	0.24	-0.71	-0.68	0.37	1.08	-0.71
7	-0.04	-0.51	-1.53	-1.10	0.01	-0.55	-1.70	0.41	0.27	-1.01	-0.82	0.64	0.24	0.10	0.10	0.29	-1.29	-0.95	-0.44	0.55	-1.16
8	0.17	-0.33	-1.49	-0.78	0.15	0.99	-1.00	-0.02	-0.01	-1.47	-0.79	0.66	0.31	0.74	0.41	0.12	-0.99	-1.02	0.38	0.51	-0.70
9	2.53	2.10	2.32	2.28	2.21	2.25	2.13	1.39	1.56	2.32	-3.40	2.29	2.37	3.28	2.42	1.98	2.05	1.85	1.92	2.48	2.11
10	0.41	-0.02	-1.69	-0.69	0.77	0.05	-0.28	0.60	-0.30	-1.66	-0.84	0.75	0.49	-1.18	0.68	1.44	-0.69	-1.24	0.97	-0.92	-0.68
11	1.15	0.66	-0.43	0.16	1.53	0.99	0.07	0.39	0.84	-0.75	-0.26	1.49	0.92	1.29	1.05	0.73	-0.46	-0.65	1.03	1.12	-1.05
12	-0.53	-0.94	-2.11	-1.23	-0.44	-0.57	-1.24	-1.05	-0.60	-1.49	-1.12	0.38	-0.25	1.14	-0.76	-0.49	-1.76	-1.53	1.26	0.16	-1.29
13	0.49	0.05	-1.23	-1.15	0.49	0.05	-0.86	-0.56	0.30	-0.71	-0.56	0.91	0.75	-0.29	0.07	0.56	-0.82	-0.96	-1.04	0.62	-1.07
14	0.70	0.12	-1.08	-0.18	1.12	0.43	-0.55	-0.03	0.48	-0.76	0.09	1.27	1.15	1.97	-0.02	0.62	-0.68	-0.42	0.74	0.88	-0.24



(a) (b)



(c)

Fig. 2 – The lipophilicity charts corresponding to R_M values estimated on different stationary phase, with mobile phases of different pH values: (a) RP-18, pH=10, (b) RP-8, pH=10, (c) CN, pH=4.

An interesting particularity may be observed on the RP-18 charts, where the group of TCAs are distinguished in the left side of the charts at acidic conditions, while when increasing the pH, the position of TCAs group is moving on the right side. This may be correlated with a high sensitivity of these compounds at pH value. This can be observed also on RP-8 stationary phases, excepting on pH = 4. On the other side, it is well known that the CN stationary phases are inducing a mixt retention mechanism, and this may be observed also on the PC1-PC2 charts. The TCAs are generally inducing a very similar behavior and they may be distinguished as linear cluster or as a compact group. However, there may be distinguished that compounds ***3*** and ***6*** are always a bit outside of the group, and they are also very closely positioned to each other. All these observations are indicating that the pH values are uniformly influencing the investigated compounds, but it doesn't mean that the determined lipophilicity is valuable at all pHs. This has been proven by direct correlation of R_{M0} values with values determined through computational method (Table 3). The correlation coefficients are reaching values of 0.90 in case of log P^V vs. $R_{M0;\ pH\ =\ 2,\ RP\text{-}18}$. The highest correlations were obtained for the R_{M0} values obtained on RP-18, when the mobile phase had the pH value of 2. In this case the compounds were totally ionized. However, in case of RP-8 and CN, the highest correlations were obtained when the mobile phase had pH of 8, and the compounds were in their unionized form. As can be concluded for a pertinent determination of lipophilicity of ionizable compounds it is necessary to know the appropriate pH that must be chosen when setting up the working conditions.

Table 3

The correlation matrix of the chromatographic and computed lipophilicity indices

Lipophilicity index	Stationary phase	pH	pKa	Log D (pH = 2)	Log D (pH = 4)	Log D (pH = 6)	Log D (pH = 7)	Log D (pH = 8)	Log D (pH = 10)	Log D (pH = 12)	CLOGP	ALOGPs	AC logP	miLogP	KOWWIN	XLOGP2	XLOGP3	log P^C	log P^V	MLOGP	ALOGP
R_{M0}	RP-18	2	0.33	**0.76**	**0.85**	0.37	0.29	0.38	**0.81**	**0.87**	**0.87**	**0.81**	0.62	**0.70**	**0.74**	**0.72**	0.21	**0.88**	**0.90**	0.57	0.67
		4	0.56	0.66	0.68	0.07	-0.07	-0.02	0.50	0.66	0.66	0.59	0.60	0.40	**0.71**	0.50	0.41	**0.75**	0.66	0.60	0.39
		6	0.61	**0.76**	**0.73**	0.05	-0.12	-0.08	0.49	0.67	0.68	0.60	0.53	0.38	**0.73**	0.58	0.56	**0.72**	0.63	0.66	0.36
		7	-0.03	0.46	0.61	0.48	0.45	0.48	0.59	0.57	0.57	0.63	0.42	**0.72**	0.48	0.62	-0.06	0.46	0.48	0.14	0.51
		8	0.34	0.57	0.65	0.21	0.15	0.23	0.60	0.66	0.66	**0.70**	0.58	0.68	0.66	0.61	0.18	0.65	0.61	0.32	0.50
		10	0.04	0.56	**0.74**	0.55	0.35	0.26	0.42	0.52	0.52	0.34	0.39	0.25	0.45	0.31	0.25	0.61	0.59	0.45	0.20
		12	-0.21	0.23	0.40	0.47	0.53	0.60	0.52	0.42	0.42	0.49	0.51	**0.70**	0.39	0.40	-0.17	0.33	0.31	-0.13	0.50
	RP-8	2	0.54	0.31	0.35	-0.16	-0.29	-0.29	0.17	0.33	0.33	0.26	0.39	0.01	0.46	0.13	0.61	0.57	0.39	0.51	0.04
		4	0.60	**0.74**	0.66	-0.01	-0.13	-0.07	0.51	0.66	0.67	0.64	0.31	0.35	0.63	0.61	0.46	0.68	0.66	0.63	0.32
		6	0.65	0.46	0.28	-0.33	-0.46	-0.42	0.10	0.26	0.27	0.28	0.18	0.13	0.40	0.31	0.44	0.27	0.20	0.36	0.03
		7	0.41	0.66	0.62	0.10	0.05	0.14	0.58	0.65	0.66	**0.72**	0.50	0.54	0.65	0.61	0.49	0.68	0.66	0.57	0.47
		8	0.55	**0.77**	**0.74**	0.10	-0.01	0.08	0.63	**0.75**	**0.76**	**0.72**	**0.71**	0.58	**0.84**	0.63	0.47	**0.80**	0.69	0.63	0.53
		10	0.56	0.61	0.58	-0.04	-0.14	-0.06	0.47	0.59	0.60	0.59	0.49	0.41	0.65	0.46	0.40	0.70	0.63	0.52	0.32
		12	-0.34	0.19	0.16	0.36	0.41	0.41	0.23	0.12	0.12	0.17	0.07	0.42	0.09	0.25	-0.26	-0.06	-0.03	-0.14	0.26
	CN	2	0.34	0.40	0.55	0.16	0.00	-0.02	0.33	0.46	0.46	0.35	0.32	0.16	0.43	0.30	0.29	0.58	0.52	0.40	0.14
		4	0.08	-0.09	0.07	-0.02	0.02	0.09	0.17	0.15	0.15	0.31	0.01	0.27	0.04	0.19	0.09	0.16	0.21	-0.16	0.07
		6	0.14	0.35	0.52	0.31	0.20	0.20	0.38	0.45	0.45	0.48	0.12	0.48	0.25	0.49	-0.03	0.38	0.51	0.11	0.25
		7	0.46	**0.78**	**0.75**	0.15	0.06	0.14	0.67	**0.76**	**0.77**	**0.72**	0.50	0.54	**0.72**	0.62	0.44	**0.84**	**0.82**	**0.70**	0.50
		8	-0.28	-0.05	0.06	0.32	0.34	0.33	0.10	0.04	0.04	0.14	0.48	0.27	0.19	0.12	0.03	-0.03	-0.14	-0.03	0.29
		10	0.51	**0.89**	**0.72**	0.09	-0.04	0.01	0.56	0.69	0.69	0.54	0.52	0.30	**0.76**	0.52	0.58	**0.74**	0.63	**0.83**	0.43
		12	0.18	0.24	0.31	0.07	0.00	0.01	0.22	0.26	0.26	0.21	0.43	0.18	0.36	0.04	0.31	0.48	0.38	0.34	0.12

CONCLUSIONS

The lipophilicity of the most important TCAs and phenothiazines has been investigated on three reverse stationary phases, i.e. RP-18, RP-8, CN, using ethanol–buffer mixtures as mobile phases. The mobile phases pH had different pH values; i.e. 2, 4, 6, 7, 8, 10, and 12. It has been observed that the chromatographic behaviour is influenced by the pH, which is inducing variability in the ionization state of molecules. The investigated compounds do not present high variations from the lipophilicity point of view, however compound ***11*** can be distinguished as the most lipophilic, while ***12*** can be placed at the opposite pole. The pH induced behaviour could be investigated at a larger scale by studying the PCA charts. They were highly suggestive, indicating the (dis-)similarities existed between investigated compounds. Furthermore, the correlation matrix proved that in case of ionisable compounds is important to select the best combination of pH and mobile phase to obtain the most conclusive results.

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