This article was downloaded by:
On: *25 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK

Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Hydrophobicity Parameter (log K_{ow}) Estimation for Some Phenolic Compounds of Pharmaceutical Interest from Retention Studies with Mobile Phase Composition in Reversed-Phase Liquid Chromatography

Toma Galaon[a]; Andrei Medvedovici[ab]; Victor David[b]
[a] LaborMed Pharma S.A., Splaiul Independentei, Bucharest, Romania [b] Faculty of Chemistry, Department of Analytical Chemistry, University of Bucharest, Bucharest, Romania

To cite this Article Galaon, Toma , Medvedovici, Andrei and David, Victor(2008) 'Hydrophobicity Parameter (log K_{ow}) Estimation for Some Phenolic Compounds of Pharmaceutical Interest from Retention Studies with Mobile Phase Composition in Reversed-Phase Liquid Chromatography', Separation Science and Technology, 43: 1, 147 — 163
To link to this Article: DOI: 10.1080/01496390701764858
URL: http://dx.doi.org/10.1080/01496390701764858

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Separation Science and Technology, 43: 147–163, 2008
Copyright © Taylor & Francis Group, LLC
ISSN 0149-6395 print/1520-5754 online
DOI: 10.1080/01496390701764858

Downloaded At: 09:20 25 January 2011

Hydrophobicity Parameter (log K_{ow}) Estimation for Some Phenolic Compounds of Pharmaceutical Interest from Retention Studies with Mobile Phase Composition in Reversed-Phase Liquid Chromatography

Toma Galaon,[1] Andrei Medvedovici,[1,2] and Victor David[2]

[1]LaborMed Pharma S.A., Splaiul Independentei, Bucharest, Romania
[2]Faculty of Chemistry, Department of Analytical Chemistry, University of Bucharest, Bucharest, Romania

Abstract: Chromatographic retention of some phenolic compounds (impurities of related pharmaceutical active substances) was studied for different organic modifier contents in mobile phase. Their capacity factors k′ versus organic modifier content (acetonitrile, or methanol) in mobile phase were measured and the corresponding dependences were studied by linear and polynomial regressions and then they were extrapolated to a mobile phase with no content in organic modifier in order to estimate their octanol/water partition coefficient (log K_{ow}). The values of log K_{ow} from retention data were compared to known shake-flask experimental values and theoretical values predicted by fragment methodology. Linear dependences for methanol and second-degree polynomial dependences for acetonitrile between organic modifier volume percentage and log k′ provided good estimation of log K_{ow}. The chromatographic method can be considered as a method of choice for estimating hydrophobicity parameter for different solutes. The linear dependence between extrapolated values of log k′ for 0% organic modifier and log K_{ow} estimated by means of fragment methodology for

Received 8 May 2007, Accepted 24 July 2007
Address correspondence to Victor David, Faculty of Chemistry, Department of Analytical Chemistry, University of Bucharest, Sos. Panduri, no. 90, 050663, Bucharest-5, Romania. E-mail: vict_david@yahoo.com

the model compounds can be used to predict chromatographic retention of other similar compounds.

Keywords: Octanol-water partition coefficient, extrapolation, retention models, reversed-phase liquid chromatography, pharmaceuticals, linear versus polynomial regressions

INTRODUCTION

Prediction of solute hydrophobicity parameter (partition constant between octanol and water, denoted in this paper by log K_{ow}) by extrapolation to pure aqueous mobile phases (log k'_{ow}) in RP-HPLC has become increasingly important because of their application in quantitative structure–retention relationship studies, e.g. (1–6). In case of solutes containing different dissociable groups, the hydrophobicity index is replaced by log D_{ow} (distribution coefficient) (7–10). However, D_{ow} becomes practically K_{ow} when pH is much lower than pK_a of the acid-like solute.

The possibility of estimating solute hydrophobicity from RP-LC studies is a consequence of the similarity between aqueous mobile/stationary (C18) phase partition and the classical hydrophilic–hydrophobic system employing water and octanol (11–13). Correct hydrophobicity measurements using RP-LC should be performed with mobile phase consisting entirely of aqueous component, but this situation is seldom used owing to several impediments:

1) weak elutropic power of such a mobile phase leading to strong solute retention in the stationary phase (high retention time, broad peaks);
2) collapse of the hydrocarbonaceous stationary phase (14, 15).

This is the reason why the hydrophobicity measurements are performed using a mobile phase containing both aqueous and organic solvent and then extrapolating the capacity factor k' to 100% water, followed by a predetermined mathematical function allowing the determination of capacity factor for this composition.

The accuracy of this method can be verified from other experimental data, such as from flask-shake experiments. The lack of such data can be substituted by data predicted by theoretic approaches, such as, for instance, the fragment methodology that is frequently used in estimating the hydrophobicity parameter (16–18). Thus, this theoretic method becomes very useful in predicting molecular hydrophobicity, which is very important for method development in RP-LC. A field of interest could be, for instance, the RP-LC assay of impurities in pharmaceutical active

Downloaded At: 09:20 25 January 2011

substances, which are generally less affordable in finding out their octanol/water partition constant.

The aim of this paper is to study the retention of some phenolic compounds, which are encountered as impurities in different pharmaceutical active substances. Different mathematical models were applied to extrapolate these data to mobile phase containing only water. These extrapolated values were compared to theoretical values predicted by fragment methodology. In order to check the accuracy of these results two solutes with known shake-flask experimental log K_{ow} values were used.

EXPERIMENTAL

Instrumentation

All experiments were performed with an Agilent 1100 liquid chromatograph, equipped with degasser, binary pump, autosampler, column thermostat, and diode-array detector (Agilent Technologies, Waldbronn, Germany). The chromatographic system was operationally qualified before this study. Chromatographic data were acquired by means of Chemstation software rev. B.01.03 (Agilent Technologies).

Downloaded At: 09:20 25 January 2011

Chromatographic Conditions

A chromatographic column containing octadecyl-silicagel stationary phase, Purospher Star RP-18e (125 mm × 4.0 mm, 5 μm particles) from Merck, was used for the entire study. Column temperature was set up to 25°C.

Elution was performed in isocratic mode at a fixed flow-rate of 1 mL/min; the mobile phase consisted of variable ratios between aqueous phosphate buffer (0.1% H_3PO_4 brought to pH 2.5 with 2.5 M NaOH solution) and organic modifier (ACN or MeOH). The range of the organic modifier percentage was indicated for each of the investigated solutes in the tables containing experimental data.

Detection was achieved at 280 and 240 nm, according to the spectral properties of the studied compounds. UV spectral data was also acquired for checking the chromatographic peaks.

The time retention values for each studied solutes were averaged between three consecutive chromatographic measurements. Column void time (t_0) was calculated from retention time of uracil.

Chemicals

All solvents were HPLC-grade from Merck (Darmstadt, Germany). Water for chromatography (minimum resistivity −18 MΩ and maximum total

organic content −30 ppb) was produced in-house by means of a TKA Lab HP 6UV/UF instrument and used during experiments.

Phenol and salicylic acid were purchased from Riedel-de-Haën, metoprolol impurity B from Recordati SA, betaxolol impurity D from Sifavitor SPA, while fenofibrate impurity A, amiodarone impurity D, and amiodarone impurity E were purchased from Council of Europe. Standard solutions containing 50 μg/mL uracil and one or two maximum solutes, each at a concentration level of 100 μg/mL in methanol were used in this study. Injection volume of 2 μL of these solutions was applied.

RESULTS AND DISCUSSION

The present paper studies the RP-LC retention behavior of several model compounds, which are structurally related from the following points of view: they have a hydrophobic character that covers a large domain of values; the presence of phenolic polar groups in their molecular structure; six of them are phenolic moiety impurities of important active substances, such as metoprolol, betaxolol, fenofibrate, amiodarone, and acetylsalycilic acid. These impurities are officially assayed in the specified drugs following the requirements of European Pharmacopoeia. The structures of the investigated impurities are given in Fig. 1. Their inferior homologue–phenol–was also taken into consideration in the present study as an "internal standard" in order to check the accuracy of the determinations.

The adsorption model frequently used to describe the retention mechanism in RP-LC is based on the interaction between the solute A and the hydrocarbonaceous chain L from the stationary phase according to the equilibrium, e.g. (19–21):

$$L + A \underset{\leftarrow}{\overset{\rightarrow}{}} L \cdot A \tag{1}$$

This equilibrium is characterized by equilibrium constant denoted by K_{LA}, which can be written in the following two ratios:

$$K_{LA} = \frac{[LA]_s}{[L]_s \cdot [A]_m} = \frac{[A]_s}{[L]_s \cdot [A]_m} \tag{2}$$

This model basically supposes that one ligand interacts with one solute molecule, and therefore the concentration of $L \cdot A$ is equal to the concentration of A found in stationary phase, $[A]_s$. On the other hand, it must be taken into account that the concentration of L is much larger that the concentration of A, which is a sum of its concentration in stationary phase, $[A]_s$ and in the mobile phase, $[A]_m$, i.e. $[L] >> [A]_s + [A]_m$. This assumption may be used in modifying the Eq. (2) in order to derive the partition constant of solute A between mobile and stationary phase ($K_{p,A}$):

$$K_{LA} \cdot [L]_s = K_{p,A} = \frac{V_m}{V_s} \cdot k'_A \tag{3}$$

Downloaded At: 09:20 25 January 2011

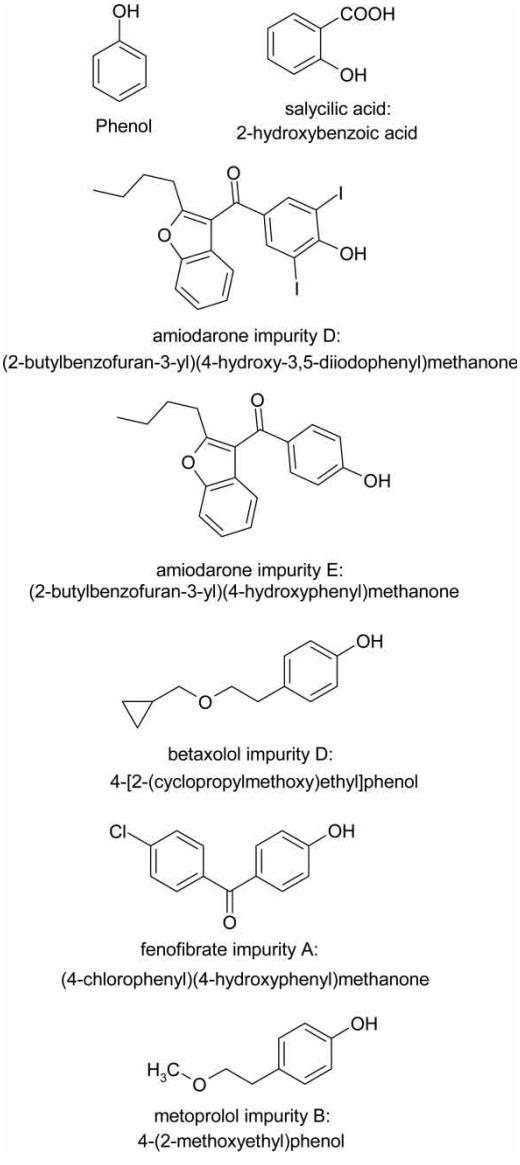

Figure 1. Molecular structure and chemical identification of studied phenolic compounds.

where V_m and V_s are the volume of mobile phase, and stationary phase, respectively, and k'_A represents the measured capacity factor for the solute A. This dependence can be studied for different mobile phase composition, dependable on the organic modifier content denoted by C_m (expressed as volume fraction in mobile phase). The octanol-water partition constant,

Downloaded At: 09:20 25 January 2011

K_{ow}, is related to the extrapolated value of $K_{p,A}$ for a mobile phase consisting of only aqueous component, denoted by $K_{p,A}^{C_m \to 0}$, which at its turn is dependent on the extrapolated value of capacity factor, denoted by $k'^{C_m \to 0}_A$. In conclusion, $K_{p,A}^{C_m \to 0}$ is proportional to partition constant of solute A between octanol and water, i.e. $K_{ow}(A)$. Such dependence can be written in a simplified form given by the proportional constant δ:

$$K_{p,A}^{C_m \to 0} = \delta \cdot K_{ow}(A) = \frac{V_m}{V_s} \cdot k_A'^{C_m \to 0} \tag{4}$$

According to this simplified dependence the ratio of the extrapolated values of capacity factors of two solutes A and B will give the ratio of their hydrophobicity indexes:

$$\frac{K_{ow}(A)}{K_{ow}(B)} = \frac{k_A'^{C_{m \to 0}}}{k_B'^{C_{m \to 0}}} \tag{5}$$

For a series of n solutes one of them can be considered as an "internal standard" with the condition that its K_{ow} value must be accurately known.

On the other hand, in RP-LC the dependence of k' on C_m is most often described by the following equations (22–26):

$$\log k' = b \cdot C_m + c \tag{6}$$

$$\log k' = a' \cdot C_m^2 + b' \cdot C_m + c' \tag{7}$$

where a log is taken as ten-base logarithm, and a, b, c, and b′, c′, respectively, are regression parameters for the two above dependences. The linear dependence in Eq. (6) is based on experimental data that showed linear correlation but for a limited range of mobile phase composition. Investigation of the entire domain of mobile phase composition ($C_m \in [0, 1]$) proves that most of the solutes present non-linear behavior at both ends of the range. Practically, the model based on linearity is kept approximately for C_m situated within 0.1–0.2 units of volume fraction in mobile phase. When linear behavior is disobeyed, then Eq. (7) can be successfully applied (24). Non-linear behavior can be observed mainly when $C_m \to 0$. It is assumed that this deviation from linearity (curvature towards higher retention) is due to modification in stationary phase solvation (27). Consequently, extrapolations based on linear relationships of type (6) should be more affected by errors than corresponding polynomial Eq. (7).

Different organic modifiers (acetonitrile or methanol), several different mobile phase compositions and a single C18 column were used to measure retention parameters (t_R, k' and then $\log k'$); experimental retention data were fitted to the organic modifier content. Extrapolations of the linear/polynomial dependences to the theoretical case when mobile phase was composed entirely of aqueous solvent ($C_m = 0$) were achieved in order to estimate the

Downloaded At: 09:20 25 January 2011

Table 1. Experimental log k′ obtained for different mobile phase compositions for less hydrophobic studied solutes (from 50:50 to 30:70–ACN: aqueous solvent ratio) at 25°C and pH = 2.5

C_m–ACN fraction	Phenol	Salicylic acid	Metoprolol impurity B	Betaxolol impurity D	Fenofibrate impurity A
0.500	0.133	0.121	0.106	0.471	0.687
0.475	0.177	0.172	0.151	0.541	0.772
0.450	0.225	0.230	0.205	0.619	0.877
0.425	0.280	0.296	0.265	0.710	0.995
0.400	0.327	0.353	0.315	0.787	1.096
0.375	0.428	0.386	0.384	0.894	1.236
0.350	0.438	0.490	0.442	0.980	1.353
0.300	0.561	0.653	0.591	1.206	1.656

Downloaded At: 09:20 25 January 2011

value of log K_{ow}. For more accurate hydrophobicity estimation, dissociation of the phenolic group in the solute molecule in the mobile phase was prevented by using an aqueous component of mobile phase with pH = 2.5. The weak acidic character of the studied compounds and the low pH of aqueous component in the mobile phase will eliminate the necessity to correct the extrapolated value of log K_{ow} for solute dissociation in the aqueous mobile phase (28). The experimental retention data obtained for the two organic modifiers in mobile phase for all studied analytes are presented in Tables 1–4.

The obtained experimental data (retention factor logarithm) were plotted against volume percentage of the organic modifier (acetonitrile or methanol) in the mobile phase, denoted previously by C_m. Linear and second-degree

Table 2. Experimental log k′ obtained for different mobile phase compositions for more hydrophobic studied solutes (from 90:10 to 40:60–ACN: aqueous solvent ratio) at 25°C and pH = 2.5

C_m–ACN fraction	Amiodarone impurity D	Amiodarone impurity E
0.90	0.477	0.041
0.85	0.653	0.157
0.80	0.822	0.279
0.75	1.000	0.413
0.70	1.180	0.554
0.65	1.371	0.706
0.60	1.584	0.875
0.55	1.820	1.063
0.50	2.096	1.283
0.40	—	1.844

Table 3. Experimental log k′ obtained for different mobile phase compositions for less hydrophobic studied solutes (from 60:40 to 37.5:62.5–MeOH: aqueous solvent ratio) at 25°C and pH = 2.5

C_m–MeOH fraction	Phenol	Salicylic acid	Metoprolol impurity B	Betaxolol impurity D	Fenofibrate impurity A
0.600	−0.029	0.191	0.022	0.535	0.904
0.550	0.104	0.346	0.171	0.738	1.150
0.525	0.165	0.428	0.242	0.834	1.266
0.500	0.226	0.508	0.317	0.937	1.393
0.450	0.352	0.671	0.475	1.149	1.652
0.425	0.412	0.754	0.555	1.256	1.785
0.400	0.479	0.838	0.642	1.372	1.927
0.375	0.541	0.919	0.722	1.482	2.066

Downloaded At: 09:20 25 January 2011

polynomial dependences were applied to fit the experimental data to organic modifier content in mobile phase Eqs. (6) and (7). The regression parameters (intercept, slope, and correlation coefficient) for both types of dependences using acetonitrile or methanol as organic modifiers are given in Tables 5 and 6.

Extrapolation in the linear or polynomial regressions to C_m=0 allowed the determination of log $k'^{C_m \to 0}$ for all investigated compounds. Afterwards, the log $k'^{C_m \to 0}$ values were corrected with column phase ratio in order to obtain log K_{ow}. Their values for different types of regressions are given in Table 7. The ratio V_m/V_s for Purospher STAR RP-18e column (dimensions: 125 mm length and 4.0 mm i.d.) was estimated from retention data (using an average dead-time from experiments, t_0) and column constructive dimensions to be approximately equal to 1.18. However, there is still a serious debate on the significance and computation of these parameters (29). Moreover, the

Table 4. Experimental log k′ obtained for different mobile phase compositions for more hydrophobic studied solutes (from 95:5 to 55:45–MeOH: aqueous solvent ratio) at 25°C and pH = 2.5

C_m–MeOH fraction	Amiodarone impurity D	Amiodarone impurity E
0.95	0.234	−0.186
0.90	0.521	0.055
0.85	0.844	0.302
0.80	1.176	0.559
0.75	1.514	0.821
0.70	1.847	1.101
0.65	—	1.399
0.60	—	1.719
0.55	—	2.051

Downloaded At: 09:20 25 January 2011

Table 5. Linear Eq. (6) and polynomial Eq. (7) regression parameters obtained for log k′–organic modifier percentage (acetonitrile) dependence

	Linear correlation			Polynomial correlation			
Compound	y-intercept (c)	Slope (b)	r^2	y-intercept (c′)	b′	a′	r^2
Phenol	1.192	−0.0214	0.9968	1.507	−0.0376	$2.03 \cdot 10^{-4}$	0.9999
Salicylic acid	1.971	−0.0402	0.9743	2.236	−0.0688	$5.34 \cdot 10^{-4}$	0.9999
Metoprolol impurity B	1.293	−0.0241	0.9941	1.751	−0.0474	$2.91 \cdot 10^{-4}$	0.9996
Betaxolol impurity D	2.270	−0.0365	0.9939	2.978	−0.0726	$4.49 \cdot 10^{-4}$	0.9996
Fenofibrate impurity A	3.056	−0.0482	0.9924	4.105	−0.1017	$6.66 \cdot 10^{-4}$	0.9996
Amiodarone impurity D	3.992	−0.0396	0.9935	5.262	−0.0771	$2.68 \cdot 10^{-4}$	0.9993
Amiodarone impurity E	3.028	−0.0344	0.9722	4.590	−0.0847	$3.82 \cdot 10^{-4}$	0.9985

Downloaded At: 09:20 25 January 2011

Table 6. Linear Eq. (6) and polynomial Eq. (7) regression parameters obtained log k′–organic modifier percentage (methanol) dependence

	Linear correlation			Polynomial correlation			
Compound	y-intercept (c)	Slope (b)	r^2	y-intercept (c′)	b′	a′	r^2
Phenol	1.484	−0.0252	0.9999	1.446	−0.0236	$-1.65\cdot10^{-5}$	0.9999
Salicylic acid	2.135	−0.0325	0.9999	2.257	−0.0376	$5.30\cdot10^{-5}$	0.9999
Metoprolol impurity B	1.886	−0.0312	0.9993	2.173	−0.0434	$1.25\cdot10^{-4}$	0.9999
Betaxolol impurity D	3.051	−0.0421	0.9994	3.396	−0.0567	$1.51\cdot10^{-4}$	0.9999
Fenofibrate impurity A	3.986	−0.0516	0.9991	4.517	−0.0740	$2.31\cdot10^{-4}$	0.9999
Amiodarone impurity D	6.386	−0.0650	0.9994	7.757	−0.0986	$2.04\cdot10^{-5}$	0.9999
Amiodarone impurity E	5.037	−0.0556	0.9968	6.547	−0.0971	$2.76\cdot10^{-4}$	0.9999

Downloaded At: 09:20 25 January 2011

Table 7. Comparison between extrapolated and theoretical log K_{ow} (using acetonitrile or methanol as organic modifier in mobile phase composition)

	Acetonitrile				Theoretic (fragment methodology)	Methanol			
Compound	From linear eq	Error[a] (%)	From polynom eq	Error[a] (%)		From linear eq	Error[a] (%)	From polynom eq	Error[a] (%)
Phenol	1.26	16.5	1.53	−1.3	1.51	1.56	−3.3	1.52	−0.6
Salicylic acid	1.50	33.0	1.97	12.0	2.24	2.21	1.3	2.33	−4.0
Metoprolol impurity B	1.37	23.4	1.82	−1.6	1.79	1.96	−9.4	2.25	−25.6
Betaxolol impurity D	2.34	23.7	3.05	0.6	3.07	3.12	−1.6	3.47	−13.0
Fenofibrate impurity A	3.13	5.4	4.18	−26.2	3.31	4.06	−22.6	4.59	−38.6
Amiodarone impurity D	4.06	46.3	5.33	29.5	7.57	6.46	14.6	7.83	−3.4
Amiodarone impurity E	3.10	40.7	4.66	10.8	5.23	5.11	2.2	6.62	−26.5

[a]Computed as (theoretic-experimental)*100/theoretic.

accuracy of t_0 (determined by means of the peak of uracil) may strongly influence the estimation of V_m and k' (30).

Experimental log K_{ow} values from shake-flask experiments are available only for phenol (1.46) and salycilic acid (2.26). In order to check the accuracy of log K_{ow} values estimated from these retention studies we used a theoretical approach, namely the fragment methodology (17), based on the following equation that has been used frequently in the literature for calculating the theoretical octanol/water partition coefficient, denoted by log $K_{ow}^{theoretic}$ (31):

$$\log K_{ow}^{theoretic} = \sum_{i=1}^{n} n_i \cdot \log K_{ow}^{(i)} + \sum_{j=1}^{m} F_j + \zeta \quad (8)$$

where: n_i represents the number of fragments of the same type i, having log $K_{ow}^{(i)}$; F_j–the factor correction applied for different groups, and ζ-an empirical equation constant (0.229). This possibility has been already applied to different isomeric compounds in RP-TLC (32).

Analyzing individual values for log K_{ow} obtained from extrapolation for all 7 model compounds (Table 7) and their comparison with theoretical values derived by fragment methodology, several considerations can be emphasized:

a) Extrapolated and theoretical values of the hydrophobicity index (log K_{ow}) are of the same order of magnitude.
b) Comparing the relative errors with respect to theoretical values when using linear or polynomial equations for acetonitrile, it can be observed that extrapolation in polynomial regressions leads to a better concordance with the expected values; on the contrary, for methanol, extrapolation in linear regressions leads to smaller deviation from theory. A same conclusion has been emphasized by other authors after a RP-LC study on a set of several selected solutes and C18 column, when the linear model was more suitable for methanol as organic modifier and quadratic model for acetonitrile (33).
c) A better accuracy for log $K_{o,w}$ value determination was obtained with linear log k'–C_m plots for methanol and polynomial plots in the case of acetonitrile; thus, linear model for methanol generates 0.1–0.2 units deviation with respect to theoretical log K_{ow} for 5 compounds from 7 studied compounds, while for acetonitrile as organic modifier, the polynomial regression gives a maximum 0.6 units of deviation from theoretical log K_{ow} for the same 5 compounds.
d) The largest relative errors are observed for amiodarone impurity D and amiodarone impurity E (Table 7), which are the most hydrophobic compounds of the investigated series; thus, their retention was measured in highly organic mobile phases (C_m between 0.40 and 0.95) leading to large errors in the extrapolation to 0% modifier. In reverse it can be observed that the measuring retention in highly aqueous mobile phases results in much smaller deviations from theoretical values; this

Downloaded At: 09:20 25 January 2011

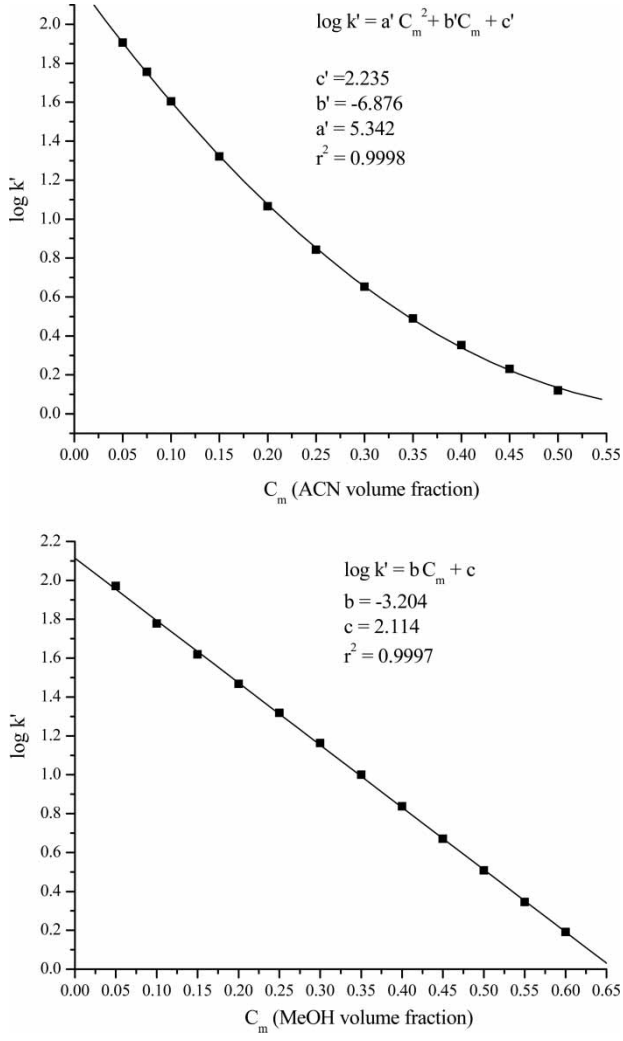

Figure 2. Extended dependences of log k′ of salicylic acid on organic modifier content in mobile phase (above for acetonitrile; below for methanol).

is the case for salicylic acid whose retention was measured again in a wider range of mobile phase composition than given in Tables 1 and 3 (ACN volume fraction between 0.05 and 0.50; MeOH volume fraction between 0.05 and 0.60). The two dependences obtained this time are given in Fig. 2. For methanol the accuracy was not improved owing to maintaining the linear tendency of the dependence log k′ on methanol concentration, while for acetonitrile the value of log K_{ow} was improved to 2.30 (error = 2.6%).

Downloaded At: 09:20 25 January 2011

e) However, the fragment methodology is not an absolute approach (34). It has limitations and therefore it can be used as a method of choice when shake-flask experimental values of log K_{ow} are not available.

All correlations between log K_{ow} values obtained by extrapolation in linear or polynomial plots and theoretical values of the same parameter lead to good fitting coefficients ($r^2 \geq 0.97$) regardless of the organic modifier employed in the mobile phase formation and the type of equation used (linear or polynomial). As a general trend it has been observed that log K_{ow} values derived for methanol studies were higher than those for acetonitrile studies. Moreover, the correlation coefficients obtained for methanol are somewhat higher than in the case of acetonitrile. The correlations between theoretical, experimental and extrapolated from retention values log K_{ow} are very good for phenol and salicylic acid. For this reason, the ratio given by the Eq. (5) was studied for all compounds from this work, using as an initial point

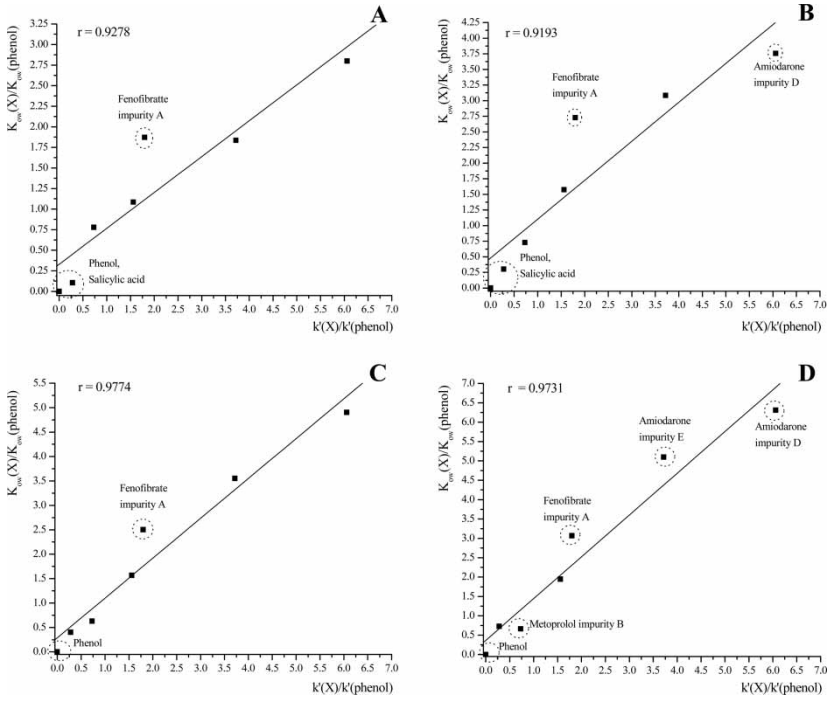

Figure 3. Correlations between theoretical values of log K_{ow} and extrapolated values of k′ for 0% organic modifier in mobile phase (as given by Eq. (5), for all seven analytes X, and the different variants applied in this study: A) linear regression for ACN; B) polynomial regression for ACN; C) linear regression for MeOH; D) polynomial regression for MeOH).

Downloaded At: 09:20 25 January 2011

$K_{ow}(A)/K_{ow}(B) = 1$ for the same tested compound, i.e. A = B = phenol. All four possibilities of correlating the theoretical value of log K_{ow} by fragment methodology and extrapolated values of the capacity factor for C_m=0 are shown in Fig. 3.

The linear regression applied to the dependence between the theoretical ratio $K_{ow}(X)/K_{ow}(phenol)$ and the experimental ratio $k'^{C_m\to 0}_{X}/k'^{C_m\to 0}_{phenol}$ (Fig. 3), for the four possibilities, showed good correlations of the two parameters confirming the validity of the theoretical models on which it was based. The most significant deviations were observed in all four cases for the compound named fenofibrate impurity A, which could be explained by highest structural difference among the lot of compounds studied in this work. In case of polynomial regression applied to the dependence of log k′ on C_m one impurity of amiodarone (containing two iodide atoms in molecule) exhibited also a large deviation to the above correlation. Moreover, the two phenolic compounds derived from amiodarone are very hydrophobic and their experimental study of the retention extended on a higher organic modifier concentration in mobile phase than for the other studied compounds. Therefore, the extrapolation of k′ to $C_m = 0$ could be less accurately for the two amiodarone related compounds than for the others.

Downloaded At: 09:20 25 January 2011

CONCLUSIONS

Hydrophobicity parameter (log K_{ow}) estimation can be investigated using linear/polynomial model equations relating chromatographic retention and organic modifier content in mobile phase; different organic modifiers and mobile phase composition ranges were used to establish these correlations. The determined functional dependences (log k′ − C_m) were extrapolated to 0% organic modifier, and the extrapolated values were compared to the values predicted by fragment methodology. Acceptable correlations were obtained by employing linear and second-order polynomial models: comparable extrapolated and theoretical log K_{ow} values and good correlation coefficients (between 0.92 and 0.97) have been achieved from these correlations.

ACKNOWLEDGMENTS

Two of the authors acknowledge partial support of this study from Romanian Agency MATNANTECH (grant CEEX 107/09.10.2006).

REFERENCES

1. Kaliszan, R. (1987) Quantitative structure—chromatographic retention relationships; Wiley: New York.

2. Hanai, T. (1991) Structure-retention correlation in liquid chromatography. *J. Chromatogr. A*, 550 (1): 313–324.
3. Forgacs, E., Kosa, A., Csiktusnadi, K., Cserhati, T., Kaliszan, R., and Nasal, H.A. (1998) Use of modified nonlinear mapping method in quantitative structure retention relationship study. *J. Liq. Chromatogr. Rel. Technol.*, 21 (6): 2523–2534.
4. Vonk, E.C., Lewandowska, K., Claessens, H.A., Kaliszan, R., and Cramers, C.A. (2003) Quantitative structure-retention relationships in reversed-phase liquid chromatography using several stationary and mobile phases. *J. Sep. Sci.*, 26 (9–10): 777–792.
5. David, V. and Medvedovici, A. (2007) Structure-retention correlation in liquid chromatography for pharmaceutical applications. *J. Liq. Chromatogr. Rel. Technol.*, 30 (5–7): 761–789.
6. Platts, J.A., Oldfiled, S.P., Reif, M.M., Palmussi, A., Gabano, E., and Osella, D. (2006) The RP-HPLC measurement and QSPR analysis of log Po/w values of several Pt (II) complexes. *J. Inorg. Biochem.*, 100 (7): 1199–1207.
7. Espinosa, S., Bosch, E., and Roses, M. (2000) Retention of ionizable compounds on HPLC. 5. pH scales and the retention of acids and bases with acetonitrile-water mobile phases. *Anal. Chem.*, 72 (21): 5193–5200.
8. Espinosa, S., Bosch, E., and Roses, M. (2002) Retention of ionizable compounds in high-performance liquid chromatography: IX. Modelling retention in reversed-phase liquid chromatography as a function of pH and solvent composition with acetonitrile–water mobile phases. *J. Chromatogr. A*, 947 (1): 47–58.
9. Kaliszan, R., Baczek, T., Bucinski, A., Buszewski, B., and Sztupecka, M. (2003) Prediction of gradient retention from the linear solvent strength (LSS) model, quantitative structure-retention relationships: (QSRR), and artificial neural networks (ANN). *J. Sep. Sci.*, 26 (3–4): 271–282.
10. Neue, U.D., Phoebe, C.H., Tran, K., Cheng, Y.F., and Lu, Z. (2001) Dependence of reversed-phase retention of ionizable analytes on pH, concentration of organic solvent and silanol activity. *J. Chromatogr. A*, 925 (1–2): 49–67.
11. Liu, X., Tanaka, H., Yamauchi, A., Testa, B., and Chuman, H. (2005) Determination of lipophilicity by reversed-phase high-performance liquid chromatography: influence of 1-octanol in the mobile phase. *J. Chromatogr. A*, 1091 (1–2): 51–59.
12. Poole, S.K. and Poole, C.F. (2003) Separation methods for estimating octanol–water partition coefficients. *J. Chromatogr. B*, 797 (1–2): 3–19.
13. Kepczynska, E., Bojarski, J., Haber, P., and Kaliszan, R. (2000) Retention of barbituric acid derivatives on immobilized artificial membrane stationary phase and its correlation with biological activity. *Biomed. Chromatogr.*, 14 (4): 256–260.
14. Engelhardt, H., Blay, C., and Saar, J. (2005) Reversed phase chromatography–the mystery of surface silanols. *Chromatographia*, 62 (13): s19–s29.
15. Gritti, F. and Guiochon, G. (2004) Physical origin of peak tailing on C_{18}-bonded silica in reversed-phase liquid chromatography. *J. Chromatogr. A.*, 1028 (1): 75–88.
16. Rekker, R.F. and Mannfold, R. (1992) Calculation of drug lipophilicity. The hydrophobic fragmental constant approach; VCH: Weinheim.
17. Meylan, W.M. and Howard, P.H. (1995) Atom/fragment contribution method for estimating octanol-water partition coefficients. *J. Pharm. Sci.*, 84 (1): 83–92.
18. Moldoveanu, S.C. and David, V. (2002) Sample Preparation in Chromatography. Elsevien: Amsterdam, p. 81.

Downloaded At: 09:20 25 January 2011

19. Horvath, Cs., Melander, W., and Molnar, I. (1977) Liquid chromatography of ionogenic substances with nonpolar stationary phases. *Anal. Chem.*, 49 (1): 142–153.
20. Dorsey, J.G. and Dill, K.A. (1989) The molecular mechanism of retention in reversed-phase liquid chromatography. *Chem. Rev.*, 89 (2): 331–36.
21. Miyabe, K. and Guiochon, G. (2003) Measurement of the parameters of the mass transfer kinetics in high performance liquid chromatography. *J. Sep. Sci.*, 26 (3–4): 155–173.
22. Hsieh, M-M. and Dorsey, J.G. (1993) Accurate determination of log k'_w in reversed-phase liquid chromatography. Implications for quantitative structure—retention relationships. *J. Chromatogr.*, 631 (1–2): 63–78.
23. Nikitas, P., Pappa-Louisi, A., and Agrafiotou, P. (2002) Effect of the organic modifier concentration on the retention in reversed-phase liquid chromatography: I. General semi-thermodynamic treatment for adsorption and partition mechanisms. *J. Chromatogr. A*, 946 (1–2): 9–32.
24. Nikitas, P., Pappa-Louisi, A., and Agrafiotou, P. (2002) Effect of the organic modifier concentration on the retention in reversed-phase liquid chromatography: II. Tests using various simplified models. *J. Chromatogr. A*, 946 (1–2): 33–45.
25. Neue, U.D. (2006) Nonlinear relationships in reversed phase chromatography. *Chromatographia*, 63 (13): s45–s53.
26. Torres-Lapasio, J.R., Garcia-Alvarez-Coque, M.C., Roses, M., and Bosch, E. (2002) Prediction of the retention in reversed-phase liquid chromatography using solute–mobile phase–stationary phase polarity parameters. *J. Chromatogr. A*, 955 (1): 19–34.
27. Scott, R.P. and Simpson, C.F. (1980) Solute-solvent interactions on the surface of reversed phases. I. Stationary phase interactions and their dependence on bonding characteristics. *J. Chromatogr.*, 197 (1): 11–20.
28. David, V., Albu, F., and Medvedovici, A. (2004) Structure-retention correlation of some oxicam drugs in reversed-phase liquid chromatography. *J. Liq. Chromatogr. Rel. Technol.*, 27 (6): 965–984.
29. Kazakevich, Y.V. (2006) High-performance liquid chromatography retention mechanisms and their mathematical descriptions. *J. Chromatogr. A*, 1126 (1–2): 232–243.
30. Rimmer, C.A., Simmons, C.R., and Dorsey, J.G. (2002) The measurement and meaning of void volumes in reversed-phase liquid chromatography. *J. Chromatogr. A*, 965 (1–2): 219–232.
31. http://www.syrres.com/esc/physdemo.htm (Syracuse Research Corporation, Online Interactive Physical Properties Database).
32. Stefaniak, M., Niestroj, A., Klupsch, J., Sliwiok, J., and Pyka, A. (2005) Use of RP-TLC to determine the log P values of isomers of organic compounds. *Chromatographia*, 62 (1–2): 87–89.
33. Bacsek, T., Markuszewski, M., Kaliszan, R., van Straten, M.A., and Claessens, H.A. (2000) Linear and quadratic relationships between retention and organic modifier content in eluent in reversed phase high-performance liquid chromatography: a systematic comparative statistical study. *J. High Res. Chromatogr.*, 23 (12): 667–676.
34. Mannhold, R., Rekker, R.F., Ter Laak, A.M., Sonntag, C., Dross, K., and Polymeropoulos, E.E. (1995) Comparative evaluation of the predictive power of calculation procedures for molecular lipophilicity. *J. Pharm. Sci.*, 84 (12): 1410–1419.

Downloaded At: 09:20 25 January 2011