Acta Poloniae Pharmaceutica – Drug Research, Vol. 72 No. 2 pp. 235–244, 2015 ISSN 0001-6837
Polish Pharmaceutical Society

LIPOPHILICITY ASSESSMENT OF SPIRONOLACTONE BY MEANS OF REVERSED PHASE LIQUID CHROMATOGRAPHY AND BY NEWLY DEVELOPED CALCULATION PROCEDURES

MAŁGORZATA DOŁOWY* and ALINA PYKA

Medical University of Silesia in Katowice, School of Pharmacy and the Division of Laboratory Medicine, Department of General and Analytical Chemistry, Institute of Analytical Chemistry, 4 Jagiellońska St., 41-200 Sosnowiec, Poland

Abstract: The parameters of lipophilicity of spironolactone (a single member of steroids group), which is widely applied as diuretic and antihypertensive agent, were experimentally determined by reversed-phase TLC and HPLC methods as well as calculated using different computer programs and also by a novel mode based on topological indices. Various stationary phases, such as RP-18WF_{254}, RP-2F_{254}, RP-18F_{254} and also different binary solvent systems composed of organic modifier (e.g., methanol, dioxane, acetone) and water were used as mobile phases in order to predict the following chromatographic parameters: R_{MW} and $logk_w$, respectively. LogP of examined spironolactone calculated with respective theoretical procedures: AlogPs, $logP_{KOWWIN}$, xlogP2, xlogP3, AClogP, AlogP, MlogP and also $logP_{average}$ were obtained from online package software. The partition coefficients expressed as $logP_1$, $logP_2$ and $logP_3$ were calculated by means of the formulae based on the numerical values of the following topological indices: , ^0B, ^1B, W, $^0\chi^v$ and I_B, which was novelty of this study. A good agreement between logP calculated by new method and experimentally estimated lipophilicity parameters (by chromatography and by shake flask method) was found. The results confirmed applicability of the topological indices for calculating lipophilicity of spironolactone as alternative procedure to the experimental and other computed logP values.

Keywords: lipophilicity, logP, $logk_w$, R_{MW}, spironolactone, topological indices, RP-TLC, RP-HPLC

For many years, increasing development of new biologically active compounds for application in medicine as potential drugs is observed. The pharmacokinetic profile of newly discovered drugs depends on various factors. Among different physicochemical properties that has significant impact on drug behavior in biological system is lipophilicity (hydrophobicity). This property plays decisive role in drug design, especially in the prediction of transport of biomolecule trough cell membranes in biological system. The most common lipophilicty measure is logP (logarithm of partition coefficient) determined by different separation methods including chromatography. The traditional method which has been widely used for the determination of lipophilicty (logP) of organic compounds in *n*-octanol - water system is the shake flask technique (1). As it is well known, this method is rather time consuming and allows to determine logP in limited range from -3.0 to +3.0, therefore in order to eliminate this limitation, the chromatographic methods can be utilized. Among numerous chromatographic approaches like reversed phase thin layer chromatography (RP-TLC) or reversed phase high performance liquid chromatography (RP-HPLC), which can be currently performed in lipophilicity investigations with the use of modern mobile and stationary phases, such as immobilized articificial membranes (IAM), an alternative technique to those may be micellar liquid chromatography (MLC) in TLC and HPLC systems. Electrophoretic methods are suitable for the estimation of the lipophilicity of various biomolecules in the wide range of logP.

The most commonly used chromatographic lipophilicity descriptors are: R_{MW} – in thin layer chromatography and also $logk_w$ in column chromatography. Analogously to both, the micellar $logk_m$ parameter can be evaluated as lipophilicity descriptor. Many researches were applying RP-TLC, RP-HPLC and also MLC in lipophilicity study of novel drugs with very different structures and functionalities like, for example: some oxicams from a group of nons-

* Corresponding author: e-mail: mdolowy@sum.edu.pl

teroidal anti-inflammatory drugs, anitiproliferative 8,10-substituted quinobenzothiazines, selected phenylthioamides and 1,2,4-triazoles with antifungal activity, biologically active imidazolinum based ionic liquids, some β-blockers drugs, and also γ-butyrolactone derivatives with anticonvulsant and analgesic activity (2-15). The predicted lipophilicity parameters were found to be significantly correlated with the activities of these compounds.

Despite of the widely applied experimental techniques, such as described chromatographic methods, lipophilicity of biologically active compounds could be determined by the use of computational methods (1, 16, 17). Computed methods of prediction of logP from compound structure are still in development and show different power of calculation of this descriptor (16). From this fact arises conclusion that in order to obtain reliable lipophilicity parameter, the computed logP should be compared with those which have been obtained experimentally.

In the past decade, the electrotopological state indices and also topological indices are becoming increasingly popular for modeling of lipophilicity of different organic compounds (18-21). The current literature review demonstrates the topological approach to estimating lipophilicity of 223 heterogeneous organic compounds (21). Moreover, it was found that topological distance indices are useful descriptors for correlating a variety of biological properties (e.g., pharmacological activity) of chemical compounds in QSAR studies (22-25). For example, using the well known in literature distance-based topological indices, a QSAR analysis on the antibacterial activity of some sulfa drugs was carried out (23). In another work, similar QSAR studies on a series of imidazole derivatives as novel ORL1 receptor antagonist with the use of number of structural descriptors including topological index (Balaban Index) was performed (25).

The main goal of this experiment was to apply reversed phase TLC and HPLC to indirectly determine lipophilicity descriptor (R_{MW} and $logk_w$) of spironolactone, a single member of steroids, which has been widely applied in medicine as antidiuretic agent. The second stage was determination of other lipophilicity parameters by computational methods: AlogPs, $logP_{KOWWIN}$, xlogP2, xlogP3, AClogP, AlogP, MlogP, $logP_{average}$ and also by the newly developed procedures based on topological indices: $logP_1$, $logP_2$ and $logP_3$. The third stage was comparison and assessment of all obtained results.

The present work is a part of our extensive study on the use of the two experimental methods like TLC and HPLC and also selected theoretical procedures based on compound structure to estimate the lipophilic properties of pharmaceutically important steroids with different biological activity (26-34). In our previous investigations, various steroid compounds belonging to conjugated and unconjugated bile acids and also some steroid anabolics were investigated for lipophilic properties using RP-TLC, RP-HPTLC and also some theoretical methods. In the present research, the applicability of both techniques, including the newly developed calculating procedure based on topological indices as alternative to reference shake flask method, in studying lipophilicity of spironolactone was estimated.

EXPERIMENTAL

Reagents and materials

For the preparation of mobile phases, methanol, acetone and dioxane of HPLC grade POCh (Gliwice, Poland) and distilled water for HPLC (E. Merck, Darmstadt, Germany) in RP-TLC, RP-HPTLC and also RP-HPLC analyses were used. The standard of spironolactone (97%, No. Catalog. S3378-1G) was procured from Sigma-Aldrich (St. Louis, MO, USA).

Preparation of standard solution

Standard solution of tested compound for RP-TLC and RP-HPTLC analysis was prepared by dissolving 10 mg of accurately weighted amount of this substance in 10 mL of methanol. Thus, final concentration of analyte was 1 mg/mL. For the purpose of RP-HPLC analysis, methanol solution of spironolactone at concentration of 3 mmol/L was utilized.

Chromatographic investigations

RP-TLC and RP-HPTLC analysis

Lipophilicity of spironolactone was evaluated by thin-layer chromatography on various stationary phases, such as 6 cm × 10 cm aluminum RP-TLC plates (RP-18F_{254}, Art. 1.05559), glass RP-HPTLC plates: RP-18WF_{254} (Art. 1.13124) and also RP-2F_{254} (Art 1.13726) manufactured by E. Merck (Darmstadt, Germany). Three microliters of examined solution was spotted onto the chromatographic plates (1 cm distance from the bottom) in each case. The chromatograms were developed with the use of mobile phases consisting of organic modifier (e.g., methanol, acetone or dioxane) - water in different volume compositions. The content of methanol, acetone and also dioxane in applied mobile phases were gradually varied by 5% (v/v) in the range from 50 to 90% (v/v).

Fifty milliliters of mobile phase was used in all cases. The chromatograms were developed at 18 ± 2°C in a 10 cm × 20 cm chromatographic chamber (Camag, Switzerland) which has been previously saturated with solvent vapors during 30 min. The development distance was 8 cm. After developing, the chromatographic plates were dried at 18 ± 2°C using a fume cupboard. Spectrodensitometric scanning was done using a Camag TLC Scanner 3 (Muttenz, Switzerland) which was controlled by WinCATS 1.4.2 software. All spectrodensitometric measurements were conducted in reflectance absorbance mode at wavelength of 238 nm. This wavelength was an optimum for examined spironolactone, and hence, it was selected for densitometric analysis. The source of radiation was a deuterium lamp. The scanning speed was 20 nm/s and the data resolution was 100 µm/step. The slit dimension was kept at 10.0 mm × 0.40 mm, Macro. Each analysis was repeated three times. Mean R_F value was used to calculate R_M.

Reversed-phase high performance liquid chromatography (RP-HPLC)

The compound was examined using a chromatograph HPLC Hewlett Packard 1050 (Canada) with the UV detector. The chromatographic conditions of applied HPLC method were as follows: the column C-18 (Eurospher 100-5) of the size 250 × 4 mm, packing of a 5 µm diameter, additionally equipped with precolumn (Knauer, Germany). The injection volume was 10 µL, the eluent flow was 1 mL/min. The detection of spironolactone was conducted at 238 nm. The isocratic elution of separated compound was carried out by the use of mobile phases: methanol – water and also dioxane – water. The content of methanol and dioxane in mobile phase was gradually varied by 5% (v/v) in the range from 55-95% (v/v). The t_R values are mean value from three independent analyses.

Lipophilicity parameters

Chromatographic parameter of lipophilicity (R_{MW})

For subsequent calculations, mean R_F values obtained under applied chromatographic conditions (various mobile phases and stationary phases) were converted to retention parameter R_M according to the expression:

$$R_m = \log(\frac{1}{R_F} - 1) \quad [1]$$

Linear correlation between R_M and volume fraction of organic modifier in mobile phase (φ) permits the extrapolation of obtained R_M values to the zero concentration of organic modifier (methanol, acetone or dioxane) in accordance with Soczewiński-Wachtmeister equation [2] and estimate relative retention parameter R_{MW} (1):

$$R_M = R_{MW} - S \times \varphi \quad [2]$$

where: R_M is the R_M value of spironolactone, R_{MW} is the R_M value extrapolated to zero concentration of organic modifier in mobile phase, S is the slope of the regression plot, φ is the volume fraction of organic modifier in mobile phase used (e.g., methanol, acetone, dioxane).

Chromatographic parameter of lipophilicity ($logk_w$)

The logarithm of retention factor logk of examined spironolactone obtained under applied solvent systems was calculated from retention time (t_R) determined by means of RP-HPLC method according to the formula:

$$\log k = \log \frac{t_R - t_M}{t_M} \quad [3]$$

where: t_R and t_M – is the retention time [min] of spironolactone and also dead-time, respectively.

For each mobile phase, the logk walue was determined and then the extrapolation of obtained logk to zero content of organic modifier (methanol and dioxane) in mobile phase: methanol - water and dioxane -to water accordance with Snyder-Soczewiński equation allowed obtain the $logk_w$ (1):

$$\log k = \log k_w - S \times \varphi \quad [4]$$

Table 1. Partition coefficient (logP) obtained by means of different theoretical methods and by use of shake flask method in *n*-octanol - water system ($logP_{exp}$).

Partition coefficient	
Taken from online software package	
$logP_{exp}$	2.78
AlogPs	3.10
AClogP	2.98
AlogP	3.59
MlogP	3.77
$logP_{KOWWIN}$	2.88
xlogP2	3.41
xlogP3	2.93
$logP_{average}$	3.24 (± 0.36)
Calculated on the basis of topological indices	
$logP_1$	2.73
$logP_2$	3.24
$logP_3$	3.00

where: logk is the logk value of spironolactone, $logk_w$ is the logk value of spironolactone extrapolated to zero concentration of organic modifier in mobile phase, S is the slope of the regression plot, φ is the volume fraction of organic modifier in applied mobile phase.

Calculations of partition coefficients by computational methods

Theoretical partition coefficients of spironolactone, such as AlogPs, $logP_{KOWWIN}$, xlogP2, xlogP3, AClogP, AlogP, MlogP and also average value of logP, which have been predicted on the basis of chemical structure of investigated compound by means of various computational procedures, were obtained from drugbank and also from another database available *via* online at VCCLAB.org website (36, 37). All theoretically determined partition coefficients and also the *n*-octanol partition coefficient ($logP_{exp}$) predicted by the use of classical shake flask method taken from VCCLAB.org website are presented in Table 1.

Newly developed method of calculation of logP

In order to calculate the logP value the selected topological indices based on adjacency matrix: Gutman (M^v and M), Randic ($^0\chi^v$, $^0\chi$ and $^1\chi$) and also based on distance matrix: Pyka (^0B, ^1B), Wiener (W), and Balaban (I_B) were calculated. The numerical values of calculated topological indices are listed in Table 2. The method of calculation of these indices have been described elsewhere (38-41). Topological indices based on distance matrix were calculated by building a distance matrix and by determining its elements by means of values given by Barysz et al. (42).

Table 2. Numerical values of the selected topological indices calculated for examined spironolactone.

The topological indices based on:	
Adjacency matrix	
M	216.000
$^0\chi$	10.286
$^1\chi$	12.384
M^v	340.440
$^0\chi^v$	18.608
Distance matrix	
W	1678
^0B	2.7288
^1B	0.3079
I_B	1.6936

The proposed new methods of calculation of logP value denoted as $logP_1$, $logP_2$ and $logP_3$ for examined compound based on its topological indices are characterized by the following formulae: [5], [6] and [7]

$$logP_1 = {}^0B \quad [5]$$

$$logP_2 = \frac{W}{M^v} - I_B \quad [6]$$

$$logP_3 = {}^0\chi^v \cdot {}^1B - {}^0B \quad [7]$$

where: , ^0B, ^1B, W, $^0\chi^v$ and I_B are topological indices.

Regression and cluster analysis

Regression and cluster analysis of obtained results were performed with the use of computer software STATISTICA 10.0.

RESULTS AND DISCUSSION

This work is a part of our previous study on lipophilicity determination of biologically active steroids. Recently, we have estimated the applicability of reversed phase thin-layer chromatography (RP-TLC and RP-HPTLC) as well as computational methods to describe the lipophilicity of selected bile acids, some steroid anabolics, plant sterols (26-34) and non-steroidal compounds namely salicylic and acetylsalicylic acids (35). Numerous chromatographic systems were applied in order to determine lipophilicity descriptor (R_{MW}) for examined steroids which have shown various pharmacological action. Our investigations confirmed that the experimentally determined by thin-layer chromatography lipophilicity parameter (R_{MW}) and some computed logP (calculated by use of appropriate programs) may be used as alternative to *n*-octanol - water partition coefficient in describing lipophilic properties of steroids. Besides obtaining reliable lipophilicity descriptor of previously tested steroid compounds (e.g., bile acids), the advantage of the proposed TLC method was a possibility of examination of several discussed steroids such as bile acids in parallel stage (on the same chromatographic plate). According to our knowledge, until today, there is no paper containing a comparative study of the chromatographically (by TLC and HPLC) and also computed determined lipophilicity descriptors of spironolactone.

Therefore, the present lipophilicity study is a continuation of those earlier reported, in which comparison of different lipophilicity descriptors determined by use of chromatographic methods: RP-TLC, RP-HPTLC, RP-HPLC and also those calculated including the newly developed based on

numerical values of topological indices for spironolactone was performed. Application of the numerical values of selected topological indices, such as M^v, ^0B, ^1B, W, $^0\chi^v$ and I_B to calculate the partition coefficients: $logP_1$, $logP_2$ and $logP_3$ of tested spironolactone was the novelty of this study.

In order to determine and then to estimate the compatibility of the chromatographic lipophilicity parameter (R_{MW}) determined by reversed phase TLC with those calculated by means of RP-HPLC and also obtained by use of other procedures, e.g., theoretical and with *n*-octanol - water partition coefficient $logP_{exp}$ (obtained by shake flask method), regardless of the applied chromatographic systems, extrapolation of R_M values to zero content of organic modifier φ (methanol, acetone, dioxane) in mobile phase according to Eq. 2 was done. Parameters of linear relationships between R_M and φ such as *r* - correlation coefficient, *s* - standard error, p - significance level and *F* value of Fischer test are listed in Table 3. Analysis of correlation coefficients in Table 3 (*r* above 0.9) indicates that strong correlations were obtained for all modifiers in the range of 55-90%. Thus, all discussed linear dependences may be satisfactory applied to determine relative lipophilicity parameter R_{MW} for tested compound. The results of R_{MW} (± SD) obtained under 9 chromatographic systems: on different chromatographic plates and by various mobile phases are presented in Table 3. From the data presented there, it could be concluded that obtained R_{MW} values are placed in the range of: 2.513 - 3.476. In order to estimate the impact of organic modifier of all applied (methanol, acetone and dioxane) on chromatographic retention of tested compound, the R_{MW} values determined using RP-TLC and RP-HPTLC plates and by use of these three mobile phase systems were compared using cluster analysis (single-bond method, *Euclidean-distance*) in Figure 1. Dendrogram (see Fig. 1) indicates a big similarity between R_{MW} values determined on all applied chromatographic plates used in this experiment which have been developed with the use of dioxane - water ($R_{MW(d)}$) and also acetone - water ($R_{MW(a)}$) as the mobile phases. This fact could be explained by similar behavior of spironolactone developed in both solvent systems. Thus, it

Table 3. Parameters of Eq. [2] (RP-TLC and RP-HPTLC) and Eq. [4] (RP-HPLC) calculated for tested compound.

Parameters of linear correlations $R_M=R_{MW}-S\times\varphi^*$						
Stationary phase type	R_{MW}(±SD)	S(±SD)	r	s	F	n
methanol – water (v/v)						
Silica gel RP-18WF_{254}	3.476 (± 0.229)	4.593 (± 0.322)	0.983	0.124	203.8	9
Silica gel RP-18F_{254}	3.474 (± 0.243)	4.264 (± 0.319)	0.986	0.080	178.4	7
Silica gel RP-2F_{254}	2.942 (± 0.080)	4.283 (± 0.113)	0.998	0.044	1442.1	9
acetone – water (v/v)						
Silica gel RP-18WF_{254}	2.564 (± 0.182)	3.756 (± 0.261)	0.988	0.092	206.9	7
Silica gel RP-18F_{254}	3.035 (± 0.190)	4.064 (± 0.272)	0.989	0.095	223.8	7
Silica gel RP-2F_{254}	2.513 (± 0.220)	3.804 (± 0.316)	0.983	0.011	145.2	7
dioxane – water (v/v)						
Silica gel RP-18WF_{254}	2.527 (± 0.162)	3.931 (± 0.247)	0.988	0.106	254.2	7
Silica gel RP-18F_{254}	2.855 (± 0.103)	4.238 (± 0.155)	0.997	0.066	746,0	8
Silica gel RP-2F_{254}	2.778 (± 0.133)	4.464 (± 0.203)	0.994	0.087	481.8	8
Parameters of linear correlations (± SD) $logk = logk_w - S \times \varphi^*$						
Stationary phase type	$logk_w$	S	r	s	F	n
methanol – water (v/v)						
Silica gel RP-18	3.144 (± 0.030)	3.921 (± 0.040)	0.999	0.020	8112.0	9
dioxane – water (v/v)						
Silica gel RP-18	1.952 (± 0.124)	3.193 (± 0.163)	0.994	0.053	382.9	6

Notes: *n*-number of points used to derive the particular regressions; *r*-correlation coefficient; *s*- standard error; *F*-value of Fischer test; * for all equations the significance level $p < 0.001$.

could be suggested that acetone may be applied alternatively to dioxane as mobile phase component in lipophilicity study of examined compound.

In further investigations, to estimate utility of high-performance RP-HPLC to predict the lipophilicity parameter of spironolactone expressed as $logk_w$, the logk values obtained on the basis of t_R values (Eq. 3) were extrapolated to zero content of organic modifier (methanol or dioxane) in applied mobile phases: methanol - water and dioxane – water, respectively, in accordance with Eq. 4. Exemplary TLC and HPLC chromatograms of spironolactone obtained with methanol - water systems are shown in Figure 2 A and B, respectively. Parameters of linear correlations between logk and j, such as *r*-correlation coefficient, *s*-standard error, p-significance level and *F* value of Fischer test are presented in Table 3. Satisfactory results of *r* values which range from 0.994 to 0.999 show that the obtained linear correlations between logk and φ allow to determine relative lipophilicity descriptor expressed in HPLC as $logk_w$. It can be observed that $logk_w$ predicted by use of dioxane - water ($logk_{w(d)}$) is visibly lower compared to those predicted with the use of methanol - water ($logk_{w(m)}$). Obtained $logk_w$ was 1.952 in the case of used dioxane as organic modifier of mobile phase and 3.144 for methanol, respectively (Table 3). We have disqualified in HPLC lipophilicity measurements the third of applied organic modifier - acetone, due to observed inacceptable results of t_R caused by irregular noise baseline on chromatograms of spironolactone detected using mobile phase acetone - water. This fact confirmed the suggestion performed by Komsta et al. (16) that of several modifiers, methanol and dioxane are the best in lipophilicity determination by use of TLC and HPLC methods.

Partition coefficients logP calculated by means of software packages available online (at the Virtual Computational Chemistry Laboratory) and also experimental logP (determined by shake flask method) summarized in Table 1 indicate certain discrepancies among themselves. Generally, computed logP which has been predicted by various algorithms is placed in the range: from 2.88 ($logP_{KOWWIN}$) to 3.77 (MlogP). Thus, average value of partition coefficient calculated on the basis of all computed logP is 3.24 (± 0.36). As it can be seen, this value is relatively higher in relation to *n*-octanol - water partition coefficient ($logP_{exp}$) obtained from available software. Among all computationally calculated logP the most similar to $logP_{exp}$ is $logP_{KOWWIN}$ and $xlogP_3$. In addition to this, the greatest similarity to average logP indicates AlogPs and also xlogP2. These results indicate that in order to apply the computed logP as a measure of lipophilicity of examined compound, critical review of all available logP for spironolactone should be done.

Analysis of partition coefficients obtained by the use of proposed procedure based on topological indices (Table 1) demonstrates that the first method of calculations (based on topological index ^0B, Eq.

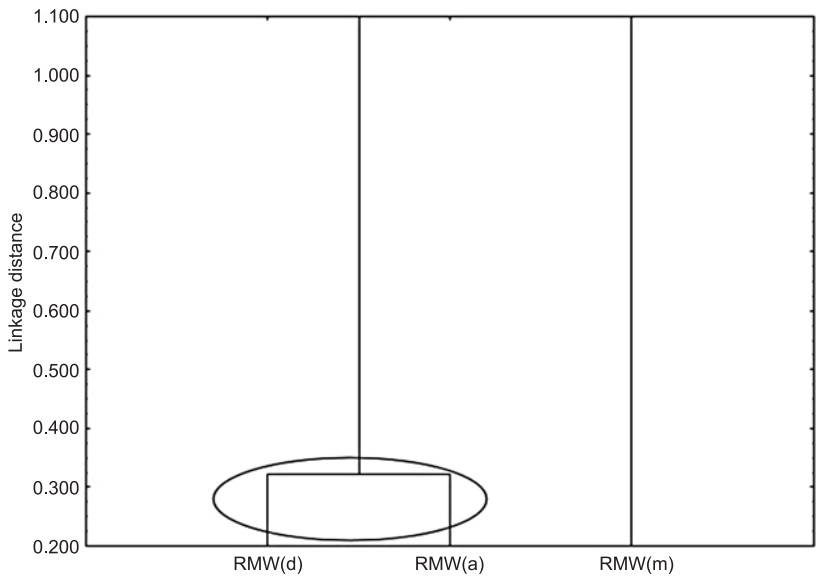

Figure 1. Cluster analysis of lipophilicity descriptors (R_{MW}) determined for spironolactone by means of RP-TLC and RP-HPTLC and binary solvent systems: methanol – water (m); acetone – water (a) and dioxane – water (d) as mobile phases

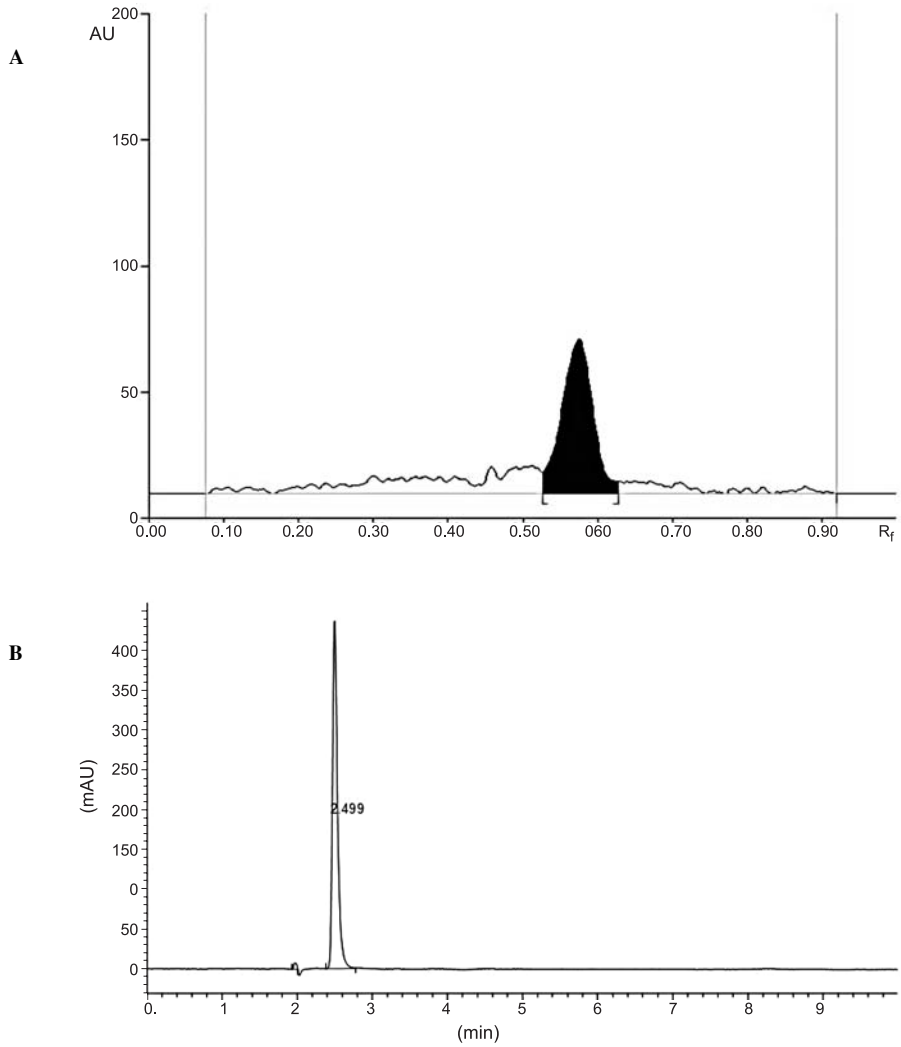

Figure 2. Exemplary TLC chromatogram of examined spironolactone obtained on chromatographic plates RP-18F_{254} using methanol – water: 90 : 10 (v/v) (**A**); example of HPLC chromatogram of spironolactone investigated on column RP18 by the use of methanol - water in volume composition: 90 : 10 (v/v) (**B**)

5) gives the result of lipophilicity descriptor (designated as $logP_1$) very similar to $logP_{exp}$. Other partition coefficients calculated by means of various topological indices according to Eq. 6 and Eq. 7 presented in Table 1 are $logP_2$ and $logP_3$ which show higher similarity to $logP_{average}$. It could be suggested that topological indices may be useful in predication of lipophilic properties of steroid compounds like, for example, spironolactone.

As it was accurately emphasized in introduction part of this work, in order to estimate which of the theoretically determined (by different calculations procedures) partition coefficient may be a reliable measure of lipophilicity of examined biomolecule there is a need to compare all calculated logP values with those obtained by appropriate experimental method. Therefore, the third stage of this study was the comparison and assessment of all obtained results. Compared experimental and calculated lipophilicity descriptors for spironolactone are presented in Figure 3.

As results from Figure 3, of all chromatographically determined lipophilicity descriptors (R_{MW}) the biggest similarity to $logP_{exp}$ shows R_{MW} obtained on glass RP-HPTLC plates RP-18F_{254} and RP-2F_{254} developed with mobile phase: dioxane - water: $RMWRP18_{(d)}$ and also $RMWRP2_{(d)}$. Among computed partition coefficients, these which are comparable to those are $logP_{KOWWIN}$ and $xlogP_3$. The results of RP-HPLC analysis indicate that the chro-

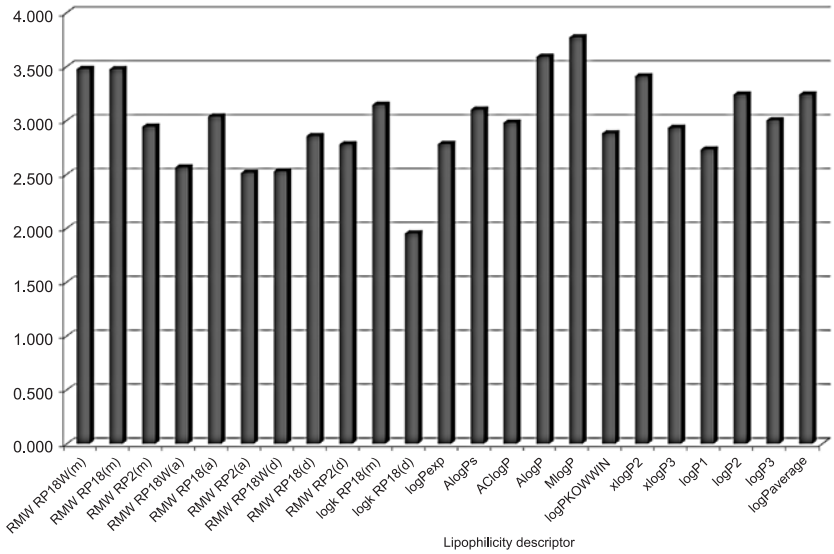

Figure 3. Comparison of experimental and calculated lipophilicity descriptors for spironolactone determined using various methods: (m) methanol - water; (a) acetone - water and (d) dioxane - water,; $logP_{exp}$ – the experimental partition coefficient determined by shake flask method; $logP_1$, $logP_2$ and $logP_3$ – partition coefficients calculated on the basis of topological indices

matographic parameter of lipophilicity predicted by this technique in methanol - water system and denoted as $logkRP18_{(m)}$ is relatively higher in relation to $logP_{exp}$ but correlates well with the computed logP, AlogPs and also with average value of logP ($logP_{average}$). The second parameter estimated by means of RP-HPLC and dioxane as organic modifier of mobile phase – $logkRP18_{(d)}$ demonstrates much lower value (about 2) in comparison with other lipophilicity parameters. Thus, no significant relation between this parameter and also others obtained in this study was observed. It confirms previous suggestion that dioxane gives better results of lipophilicity measurements conducted by TLC than by HPLC. The last group of estimated lipophilicity parameters of examined spironolactone are those, which have been calculated on the basis of the numerical values of selected topological indices based on adjacency and on distance matrix, respectively: M^v, ^0B, ^1B, W, $^0\chi^v$ and I_B according to the proposed formulae (Eq. 5-7). These partition coefficients: $logP_1$, $logP_2$ and $logP_3$ are placed in the range of: 2.73-3.24. Among them, $logP_1$ based on topological index ^0B is in good agreement with $logP_{exp}$ and also with chromatographically predicted lipophilicity parameter R_{MW} in dioxane - water system on RP-$2F_{254}$ plates (RMWRP2(d)). Good correlation could be observed also between $logP_1$ and the following computationally determined partition coefficients: AClogP, $logP_{KOWWIN}$ and $xlogP_3$.

Next partition coefficient determined by newly developed procedure based on topological indices: W, M^v, and I_B described by Eq. 6 enabled calculate $logP_2$ which shows the biggest similarity to chromatographic parameter of lipophilicity $logk_w$ determined by use of methanol - water (logkRP18(m)) and also with computationally determined $logP_{average}$. The third developed partition coefficient ($logP_3$) indicates the biggest similarity to the R_{MW} obtained on RP-$2F_{254}$ plates developed with mobile phase: methanol - water and also on silica gel RP-$18F_{254}$ using acetone - water as the mobile phase.

Finally, it can be concluded, that the results of lipophilicity parameters of spironolactone obtained by the use of TLC and HPLC indicate that liquid chromatography can play important role as an experimental method in lipophilicity study of certain steroids like spironolactone because is accurate, not expensive and does not require a large amount of compound in comparison with classical shake flask method. Additionally, it has been stated that the best (optimal) chromatographic conditions which allowed obtain the lipophilicity results (expressed as R_{MW} and $logk_w$) similar to those determined by the use of reference shake flask method are: dioxane – water and silica gel RP-$2F_{254}$ and RP-$18F_{254}$ in the case of TLC. In the case of HPLC a mixture of methanol - water (as mobile phase) and column RP18 (as the stationary phase) are optimal in lipophilicity study of spironolactone.

Further investigations will be continued. The predicted by different theoretical methods and also chromatographically determined lipophilicity descriptors of spironolactone will be applied not for description of its lipophilicity only but also to estimate the efficiency and applicability of newly developed logP calculation models based on topological indices to evaluate the pharmacokinetic properties of tested spironolactone and its metabolite like canrenone in future QSAR study.

CONCLUSIONS

From the analysis of obtained data, it can be concluded that:

- liquid chromatography in reversed-phase system, such as RP-TLC, RP-HPTLC and also RP-HPLC can be an alternative method to traditional shake flask procedure for studying lipophilicity of spironolactone;
- R_{MW} and $logk_w$ parameters can be used as an estimation of the lipophilicity of spironolactone;
- partition coefficients logP calculated according to molecular structure of tested compound by use of online available package software, such as AlogPs, $logP_{KOWWIN}$, xlogP2, xlogP3, AClogP, AlogP and MlogP demonstrate certain discrepancies which could be explained by differences in accuracy of these calculations;
- newly developed logP calculation models based on topological indices are suitable for predication of partition coefficients of investigated spironolactone denoted as $logP_1$, $logP_2$ and $logP_3$, respectively;
- among performed theoretical lipophilicity parameters, those which are comparable with *n*-octanol - water partition coefficient ($logP_{exp}$) determined by shake flask method are: computed $logP_{KOWWIN}$, xlogP3 and also the newly developed $logP_1$;
- of all chromatographic lipophilicity descriptors: R_{MW} and $logk_w$ those which correlate well with $logP_{exp}$ are R_{MW} values determined by use of dioxane - water system and silica gel (RP-$2F_{254}$ and RP-$18F_{254}$);
- obtained lipophilicity parameters including chromatographic results, such as R_{MW} and $logk_w$ can be used in future QSAR study of spironolactone and its metabolite like canrenone.

Acknowledgment

This work was financed by Medical University of Silesia as part of statutory research project in 2014 year, No. KNW-1-006/N/4/0.

REFERENCES

1. Jóźwiak K., Szumiło H., Soczewiński E.: Wiad. Chem. 55, 1047 (2001).
2. Walerowicz T., Buszewski B.: Biomed. Chromatogr. 19, 725 (2005).
3. Giaginis C., Tsantili-Kakoulidou A.: J. Pharm. Sci. 97, 2984 (2008).
4. Starek M., Komsta Ł., Krzek J.: J. Pharm. Biomed. Anal. 85, 132 (2013).
5. Shweshein K.S.A.M., Andrić F., Radoičić A., Zlatar M., Gruden-Pavlović M., Tešić Ž., Milojković-Opsenica D.: Sci. World J. Article ID 862796 (2014) http://dx.doi.org/10.1155/2014/862796.
6. Jeleń M., Pluta K., Morak-Młodawska B.: J. Liq. Chromatogr. Rel. Technol. 37, 1373 (2014).
7. Pallicer J.M., Sales J., Rosés M., Ràfols C., Bosch E.: J. Chromatogr. A 1218, 6356 (2011).
8. Lu D., Chambers P., Wipf P., Xie X.Q., Englert D., Weber S.: J. Chromatogr. A 1258, 161 (2012).
9. Janicka M., Stępnik K., Pachuta-Stec A.: Chromatographia 75, 449 (2012).
10. Nasal A., Siluk D., Kaliszan R.: Curr. Med. Chem. 10, 381 (2003).
11. Poole S.K., Poole C.F.: J. Chromatogr. B 797, 3 (2003).
12. Welorowicz T., Buszewski B.: Biomed. Chromatogr. 19, 725 (2005).
13. Studzińska S., Stepnowski P., Buszewski B.: QSAR Comb. Sci. 26, 963 (2007).
14. Kostecka M.: Curr. Issues Pharm. Med. Sci. 64, 291 (2009).
15. Bajda M., Guła A., Więckowski K., Malawska B.: Electrophoresis 34, 3079 (2013).
16. Komsta Ł., Skibiński R., Berecka A., Gumieniczek A., Radkiewicz B., Radoń M.: J. Pharm. Biomed. Anal. 53, 911 (2010).
17. Stasiak J., Koba M., Bober L., Kawczak P., Bączek T.: Comb. Chem. High Throughput Screen. 16, 603 (2013).
18. Silva S.E., Zaramello L., Kuhnen C.A., da Silva Junkes B., Yunes R.A, Heinzen V.E.F.: Int. J. Mol. Sci. 12, 7250 (2011).
19. Agrawal V.K., Singh J., Khadikar P.V., Supuran C.T., J. Bioorg. Med Chem. Lett. 16, 2044 (2006).
20. Agrawal V.K., Bano S., Khadikar P.V.: Bioorg. Med. Chem. 11, 4039 (2005).
21. Agrawal V.K., Gupta M., Singh J., Khadikar P.V.: Bioorg. Med. Chem. 13, 2109 (2005).
22. Jaiswal M., Khadikar P.V., Supuran C.T.: J. Bioorg. Med. Chem. Lett. 14, 5661 (2004).

23. Mandloi D., Joshi S., Khadikar P.V., Khosla N.: J. Bioorg. Med. Chem. Lett. 15, 405 (2005).
24. Cash G.G., Anderson B., Mayo K., Bogaczyk S, Tunkel J.: Mutat. Res. 585, 170 (2005).
25. Srivastava A.K., Shukla N.: J. Saudi Chem. Soc. 17, 321 (2013).
26. Pyka A., Dołowy M.: Acta Pol. Pharm. Drug Res. 61, 407 (2004).
27. Pyka A., Dołowy M.: J. Liq. Chromatogr. & Rel. Technol. 26, 2741 (2003).
28. Pyka A., Dołowy M.: J. Liq. Chromatogr. Rel. Technol. 28, 297 (2005).
29. Pyka A., Dołowy M.: J. Liq. Chromatogr. Rel. Technol. 28, 1765 (2005).
30. Dołowy M.: J. Liq. Chromatogr. Rel. Technol. 32, 2281 (2009).
31. Dołowy M.: Curr. Issues Pharm. Med. Sci. 25, 29 (2012).
32. Dołowy M.: Farm. Pol. 65, 689 (2009).
33. Dołowy M.: Comparison of lipophilicity of the selected substances having anabolic activity, determined using TLC technique and by the means of calculations, in Chromatography in practice, Voelkel, A., Wasiak, W. Eds., p. 125, Technical University in Poznań, Poznań 2011.
34. Dołowy M.: Abstract book: 29th International Symposium on Chromatography ISC 2012, Toruń, 9-13. 2012, 155 (2012): [S1-P18].
35. Dołowy M., Pyka A.: J. Liq. Chromatogr. Rel. Technol. 38, 485 (2015).
36. Open Data Drug & Drug Target Database [Internet]. Available from: http://www.drug-bank.ca (accessed on October 08. 2014).
37. Tetko I.V., Tanchuk V.J. VCC – Lab, 2002, Interactive analysis logP prediction [Internet]. Available from: http://www.vcclab.org/lab/alog-ps (Accessed on October 08, 2014).
38. Devillers J., Balaban A.T.: Topological Indices and Related Descriptors in QSAR and QSPR, Gordon and Breach Science Publishers, The Netherlands 1999.
39. Pyka A.: J. Planar Chromatogr. – Modern TLC 4, 316 (1991).
40. Pyka A.: Wiad. Chem. 51, 784 (1997).
41. Pyka A. Wiad. Chem. 52, 727 (1998).
42. Barysz M., Jashari G., Lall R.S., Srivastava R.S., Trinajstić N.: Stud. Phys. Theor. Chem. 28, 222 (1983).

Received: 5. 12. 2014