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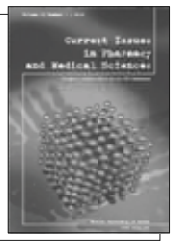

Lipophilicity study of ursodeoxycholic acid

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ABSTRACT

In this work, the researchers determined the chromatographic parameter of the lipophilicity (R_{MW}) of ursodeoxycholic acid under various chromatographic conditions, and using the RP-TLC and RP-HPTLC methods. This chromatographic analysis was performed on different glass and aluminum plates (RP-18F_{254}, RP-18WF_{254} and on RP-2F_{254}), with the use of a mixture of various organic modifiers (methanol, acetone or dioxane) and water, in respective volume compositions. The results of these chromatographic investigations (R_{MW} values) were compared with the partition coefficient (logP) obtained by way of the use of several theoretical methods (expressed as: AlogPs, $logP_{KOWWIN}$, xlogP2, xlogP3, milogP, AlogP, and MlogP) and with the experimental logP ($logP_{exp}$), respectively. The similarity between the chromatographically determined and computational calculated parameters of lipophilicity shows the possibility of applying both the chromatographic and theoretical methods to predict the lipophilicity value of ursodeoxycholic acid. The further direction of this study will be towards the application of the obtained lipophilicity parameters of examined bile acid in SAR studies (structureactivity relationships).

Keywords: Lipophilicity, Ursodeoxycholic acid, RP-TLC, RP-HPTLC, Bile acids, Cluster analysis

INTRODUCTION

Ursodeoxycholic acid (3,7-dihydroxycholan-24-oic acid, UDC) is a secondary bile acid formed in the intestines, and pharmaceutical formulations of ursodoxycholic acid are used to treat cholestatic liver diseases by preventing and dissolving gallstones [12]. To understand the mechanism of the pharmacological action of ursodeoxycholic acid in the biological system, knowledge of its respective physicochemical properties, e.g. its lipophilicity value, is necessary. This property is essential for the transport process of organic compounds in biological systems, and it correlates well with the pharmacological action of bioactive compounds.

Lipophilicity is expressed in many ways [5]. The most popular of these is by way of its "partition coefficient" (P) or its logarithm (logP). Partition coefficient and logP characterize the tendency of a respective compound to self-partition between two phases: polar and non-polar. The partition coefficient of any compound is usually determined by the classical "shake-flask" method, with the use of n-octanol and water. In practice, the traditional, but time consuming "shake-flask" method is substituted by chromatography, by way of a reversed–phase system such as thin-layer chromatography (RP-TLC and RP-HPTLC), and also by high performance liquid chromatography (RP-HPLC).

Many papers describe the application of chromatographically obtained lipophilicity parameters: R_{MW} (determined by RP-TLC and RP-HPTLC) or logk (determined by RP-HPLC), to predict the lipophilic character in a lot of organic compounds with respective biological activities. Among these: anticonvulsant, anticancer, antimycotic, antibacterial and others [1-2, 7-11].

The work described in this paper is a continuation of previous studies of lipophilicity investigations in a group of bile acids. Our previously presented results indicate the usefulness of RP-TLC, RP-HPTLC, as well as the usefulness of selected computational programs to determine the lipophilicity of particular bile acids (such as cholic, deoxycholic, chenodeoxycholic and lithocholic) and their conjugates with glycine and taurine, respectively [3, 13-15]. Of all the bile acids, only two free bile acids are pharmaceutically important. These are dehydrocholic acid and ursodeoxycholic acid. The lipophilicity study of dehydrocholic acid was performed in a previous work [4].

In this paper, the lipophilicity of ursodeoxycholic acid (UDC) was determined. To estimate the lipophilic character of UDC, thin-layer chromatography in a reversed phase system (RP-TLC and RP-HPTLC) was applied. The chromatographic parameter of lipophilicity (R_{MW}) was determined under the following conditions: on different chromatographic plates type: RP-18F_{254}, RP-18WF_{254}, RP-2F_{254},, and with the use of a mixture of organic modifier (methanol, acetone, dioxane) and water in various volume compositions. To visualize the spots of ursodeoxycholic acid, a water solution of sulfuric acid was used. The chromatographically determined lipophilicity parameter (R_{MW}) was compared with its corresponding experimental partition coefficient ($logP_{exp}$).

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This was determined using the classical shake-flask method and with partition coefficients calculated by means of computational methods such as AlogPs, AlogP, milogP, MlogP, $logP_{KOWWIN}$, XlogP2 and XlogP3.

MATERIALS AND REAGENTS

Chemicals:

Components of mobile phase: methanol, acetone and dioxane, were from POCh (Gliwice, Poland). Distilled water was acquired from the Department of Analytical Chemistry, Faculty of Pharmacy (Sosnowiec, Poland). All chemicals were analytical grade. A commercial sample of ursodeoxycholic acid min. 99% (Sigma – Aldrich, St. Louis, MO, USA) was applied as test solute. Sulfuric acid 95% (POCh, Gliwice, Poland) was used to prepare a visualizing reagent.

METHODS

RP-TLC and RP-HPTLC Analysis:

Chromatographic analysis was performed on RP-TLC aluminum plates 4cm×10cm: RP-$18F_{254}$ (E. Merck, Germany, Art. 1.05559) and also on RP-HPTLC glass plates 4cm×10cm: RP-$18WF_{254}$ (E. Merck, Art. 1.13124) and RP-$2F_{254}$ (E. Merck, Germany, Art. 1.13726). 3 μ L of solution of ursodeoxycholic acid in methanol at concentration of 5 mg/ml was then spotted on the chromatographic plates. Next, the chromatograms were developed using a mixture of organic modifier and water as a mobile phase, mixed in various volume compositions.

The content of organic modifier (methanol, acetone and dioxane) in mobile phase was gradually varied by 5% [v/v], from 50–90% [v/v]. 50 mL of mobile phase was used in all cases. The chromatograms were developed at a temperature of 18°C, in a chromatographic chamber (20cm×20cm, Camag, Switzerland), using the particular mobile phase. The development distance was 8 cm.

After developing, the chromatographic plates were dried at room temperature, using a fume cupboard. Next, the spots were visualized by spraying them with 10% water solution of sulfuric acid and then heating the plates for 15 minutes at 120 C. On the basis of the obtained chromatograms, the R_F value of ursodeoxycholic acid for all applied chromatographic conditions, was calculated. The R_F values were then averaged from three separate analyses.

Calculation of the chromatographic lipophilicity parameter (R_{MW}):

The R_F values determined under applied chromatographic conditions were converted into the R_M values according to the equation [5, 13]:

$$R_M = \log(1/R_F - 1) \quad (1)$$

Next, the results of R_M values were extrapolated to the zero concentration of organic modifier (methanol, acetone and dioxane) in mobile phase, by use of Soczewiński – Wachtmeister equation [2, 9]:

$$R_M = R_{MW} - S \cdot \varphi \quad (2)$$

where: S – is the slope of the regression plot, and φ – is the volume fraction of organic modifier in mobile phase used.

Finally, equation No. 2 enables determination the normalized chromatographic parameter of lipophilicity (R_{MW}).

Determination of the theoretical partition coefficient (logP):

To determine the theoretical values of the partition coefficient (logP) of the examined bile acid (which are expressed as: AlogPs, $logP_{KOWWIN}$, xlogP2, xlogP3, milogP, AlogP and MlogP), the selected algorithms for determining the partition coefficient, based on the structure of the investigated compound, were used. The on-line available database: Virtual Computational Chemistry Laboratory, was helpful in obtaining the above–mentioned theoretical partition coefficients and also the experimental value of logP ($logP_{exp}$) for ursodeoxycholic acid (which was determined using shake-flask method [6]).

Linear regression and cluster Analysis:

Linear regression and cluster analysis of the obtained results were done with the use of the computer program *Statistica 8.0*.

RESULTS AND DISCUSSION

The R_F values obtained for ursodeoxycholic acid by means of the RP-TLC and RP-HPTLC methods, using different chromatographic plates (RP-$18F_{254}$, RP-$18WF_{254}$, RP-$2F_{254}$), and evaluated with methanol-water, acetone- water and dioxane-water, respectively, as the mobile phases, were converted into the chromatographic lipophilicity parameter R_M. The linear relationships between the R_M values and the volume composition of the organic modifier in the mobile phase (φ) (methanol, acetone or dioxane, respectively), allowed an extrapolation procedure. The extrapolated value of R_M to the zero content of the organic modifier in the mobile phase (R_{MW}) is the measure of the lipophilicity of the examined bile acid. Fig. 1–3 reveals the linear dependencies type $R_M = f(\varphi)$ that were obtained on the chromatographic plates: RP-$18F_{254}$, RP-$18WF_{254}$, RP-$2F_{254}$ using the respective mobile phase:

The figures presented above indicate that the R_M values decreased linearly with increasing methanol, acetone and dioxane content in the mobile phase, used independently of the applied mobile phase and the kind of chromatographic plate used.

The statistic parameters of linear equations type $R_M = R_{MW} - S \cdot \varphi$ obtained for all the applied chromatographic conditions such as correlation coefficient (r), standard error of estimation (s) and value of Fisher test (F) are listed in Table 1.

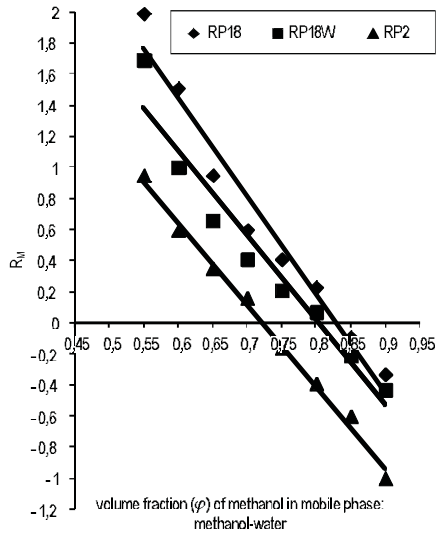

Fig. 1. Dependence R_M value of ursodeoxycholic acid on methanol content (φ) in mobile phase: methanol-water obtained on chromatographic plates: RP-18F_{254}, RP-18WF_{254} and RP-2F_{254}

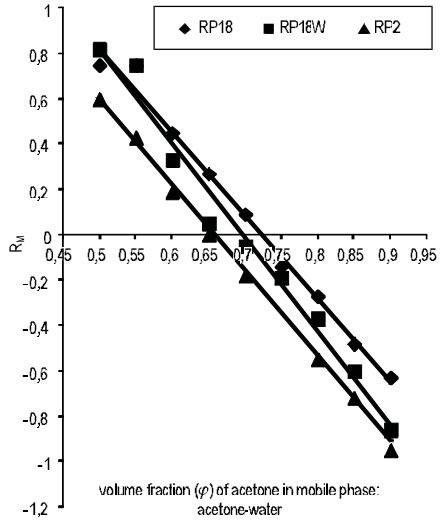

Fig. 2. Dependence R_M value of ursodeoxycholic acid on acetone content (φ) in mobile phase: acetone-water obtained on chromatographic plates: RP-18F_{254}, RP-18WF_{254} and RP-2F_{254}

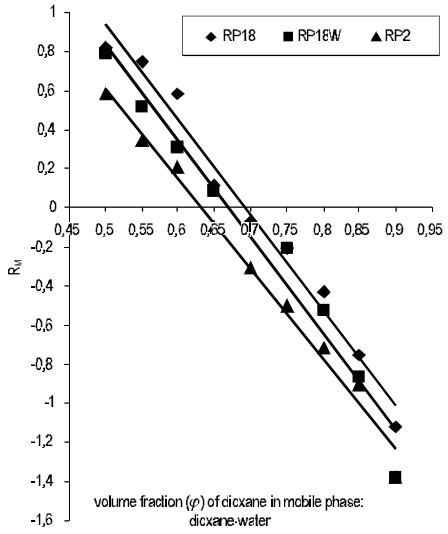

Fig. 3. Dependence R_M value of ursodeoxycholic acid on dioxane content (φ) in mobile phase: dioxane-water obtained on chromatographic plates: RP-18F_{254}, RP-18WF_{254} and RP-2F_{254}

Because the p (significance level) value is less than 0.01 for all the linear dependencies presented in Table 1, there are a statistically significant relationships between R_M and φ. These relationships can be used to predict the chromatographic parameter of lipophilicity (R_{MW}) for the examined ursodeoxycholic acid under all applied chromatographic conditions. The values of the correlation coefficient (r) of the presented dependencies are high, and show strong relationships between variables. The standard error of estimation (s) is also small (Table 1). The R_{MW} values presented in Table 1 show that the R_{MW} values determined using acetone-water exist in the range: 2.489–2.882, and for dioxane-water: 2.924–3.375. In regard to the mixture of methanol-water, a higher value of R_{MW} is observed: 3.819–5.249.

Table 1. Results of the linear correlations between R_{MW} values and type: $R_M = R_{MW} - S \cdot \varphi$ obtained with the use of various chromatographic conditions

Chromatographic plates	R_{MW}	S	r	s	F	n
methanol – water (v/v)						
RP-18WF_{254}	4.371	5.443	0.972	0.174	102.4	7
RP-18F_{254}	5.249	6.331	0.981	0.164	157.2	7
RP-2F_{254}	3.819	5.283	0.997	0.052	1075.0	7
acetone – water (v/v)						
RP-18WF_{254}	2.882	4.137	0.990	0.087	339.0	8
RP-18F_{254}	2.668	3.687	0.995	0.054	705.5	8
RP-2F_{254}	2.489	3.773	0.993	0.064	521.8	8
dioxane – water (v/v)						
RP-18WF_{254}	3.298	4.927	0.984	0.131	210.8	8
RP-18$F25_4$	3.375	4.870	0.990	0.099	364.7	8
RP-2F_{254}	2.924	4.617	0.993	0.078	528.9	8

Notes: r – correlation coefficient, *s* – standard error of estimation, *F* – value of Fisher test, *n* – number of points used to derive the particular regressions

To compare the R_{MW} values obtained on all types of chromatographic plates (RP-18F_{254}, RP-18WF_{254}, RP-2F_{254}), using the varied mobile phases: methanol-water (m), acetone–water (a) and dioxane–water (d) (R_{MW}(m), R_{MW}(a) and R_{MW}(d), respectively), cluster analysis was used (Fig. 4):

A dendrogram of similarity of R_{MW} values, shows the greatest similarity between R_{MW} values obtained on all ap-

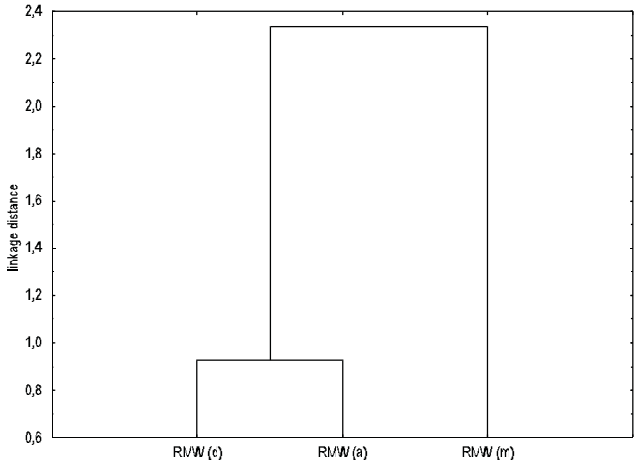

Fig. 4. Dendrogram of similarity of chromatographically determined lipophilicity parameters (R_{MW}) of ursodeoxycholic acid

Notes: RMW(m) – R_{MW} value obtained with the use of methanol-water as a mobile phase, RMW(a) – R_{MW} value obtained with the use of acetone-water as a mobile phase, RMW(d) – R_{MW} value obtained with the use of dioxane-water as a mobile phase

plied chromatographic plates developed with the use of dioxane-water ($R_{MW(d)}$) and acetone-water ($R_{MW(a)}$) as the mobile phases, respectively. A large similarity in R_{MW} values obtained with the use of acetone-water and dioxane-water as the mobile phases, confirm the fact that the both solvents have a similar elution force in the RP system. This is higher than for methanol [10].

The lipophilicity excess of ursodeoxycholic acid observed in regard to the methanol-water mixture (R_{MW} = 3.819–5.249), in comparison to the R_{MW} values obtained using acetone-water and dioxane-water as the mobile phases on different chromatographic plates (RP-18F_{254}, RP-18WF_{254} and also on RP-2F_{254}), show that the big influence for ursodeoxycholic acid retention in the RP system is engendered by a strong hydrogen bond which is formed between the molecule of ursodeoxycholic acid and the methanol used as a mobile phase in this part of the experiment.

Further analysis of R_{MW} values obtained on different types of chromatographic plates: RP-18F_{254}, RP-18WF_{254}, RP-2F_{254}, using acetone-water and dioxane-water as the mobile phases, indicate the minor influence of the kind of stationary phase used in these conditions, on R_{MW} values of the investigated bile acid. As can be seen in Table 1, the R_{MW} values obtained in these chromatographic conditions are very similar.

In regard to the methanol-water mixture used as a mobile phase and the above-mentioned chromatographic plates, a noticeable difference between all obtained R_{MW} values is observed. This situation confirms the effect of specific interactions between the surface of the adsorbent methanol used as a mobile phase and the analyzed bile acid.

Besides the chromatographic parameters of lipophilicity (R_{MW}), the experimental and theoretical partition coefficient (logP) for the investigated ursodeoxycholic acid was determined. In Table 2, the value of $logP_{exp}$ and the theoretical values of partition coefficient expressed as AlogPs, $logP_{KOWWIN}$, xlogP2, xlogP3, milogP, AlogP and MlogP, were performed.

Table 2. Log P value of ursodeoxycholic acid obtained using computational methods [6]

Partition coefficient	The value of partition coefficient
$logP_{exp}$	3.00
AlogPs	3.01 (+0.01)
milogP	4.25 (+1.25)
AlogP	3.99 (+0.99)
MlogP	4.07 (+1.07)
$logP_{KOWWIN}$	5.06 (+2.06)
XlogP2	4.91 (+1.91)
XlogP3	4.92 (+1.92)

Among the above presented theoretical logP values, the most similar are AlogP and MlogP and the pair of partition coefficients xlogP2 and xlogP3. The highest value of all theoretical partition coefficients, 5.06, is shown by $logP_{KOWWIN}$. AlogPs is similar to the experimental partition coefficient ($logP_{exp}$) which was determined using the classical shake-flask method.

Finally, the results of the chromatographic lipophilicity parameter (R_{MW}) from RP-TLC and RP-HPTLC analysis were compared with those determined using the computational programs: AlogPs, $logP_{KOWWIN}$, xlogP2, xlogP3, milogP, AlogP, MlogP, and with the experimental logP ($logP_{exp}$). This is shown in Fig. 5.

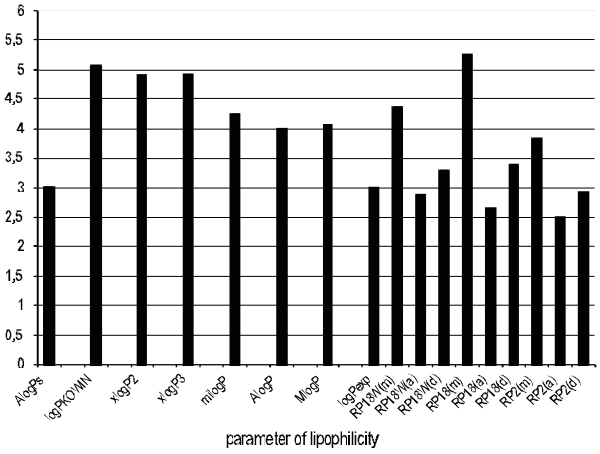

Fig. 5. Comparison of the chromatographic and theoretical values of lipophilicity parameters of ursodeoxycholic acid

Notes: RP18, RP18W, RP2 – the R_{MW} values obtained on the chromatographic plates: RP-18F_{254}, RP-18WF_{254} and RP-2F_{254} developed using mobile phase: methanol-water (m), acetone-water (a) and dioxane-water (d)

The data performed in Fig. 5 indicate, that of all the chromatographic lipophilicity parameters, the greatest similarity to the $logP_{exp}$ are the R_{MW} values obtained on RP-18WF_{254} plates using acetone-water ($RP18W_{(a)}$) and the R_{MW} obtained on RP-2F_{254} plates developed with mobile phase dioxane-water ($RP2_{(d)}$). However, a large similarity between the R_{MW} values determined on RP-18WF_{254} and RP-18F_{254} plates developed using dioxane-water ($RP18W_{(d)}$ and $RP18_{(d)}$), was also found. A similar value was seen between milogP and R_{MW} obtained on RP-18WF_{254} using the methanol-water mixture ($RP18W_{(m)}$). However, the highest chromatographically determined value of R_{MW}, using methanol-water and the plates RP-18F_{254} ($RP18_{(m)}$), is similar to the theoretical partition coefficient expressed as a $logP_{KOWWIN}$.

CONCLUSIONS

Results from both RP-TLC and RP-HPLC analysis show a linear dependence between the chromatographically obtained R_M of the examined ursodeoxycholic acid and the content of the organic modifier in the mobile phase φ. The equations of linear correlations between R_M and φ type: $R_M = R_{MW} - S \cdot \varphi$ obtained for all chromatographic conditions, allowed the researchers to calculate the normalized lipophilicity parameter R_{MW} of ursodeoxycholic acid.

A comparison of the chromatographic lipophilicity parameters with the partition coefficient (log P) that was determined using different computational methods: AlogPs, $logP_{KOWWIN}$, xlogP2, xlogP3, milogP, AlogP, MlogP, and also with the experimental value of logP ($logP_{exp}$), shows similarity between the respective R_{MW} and selected logP values. This fact indicates the possibility of applying both

chromatographic (RP-TLC, RP-HPTLC) and theoretical methods (commercial computer programs) to predict the lipophilicity of ursodeoxycholic acid.

The further direction of this study will be towards the application of obtained lipophilicity values of examined bile acid in SAR studies (structure-activity relationships).

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