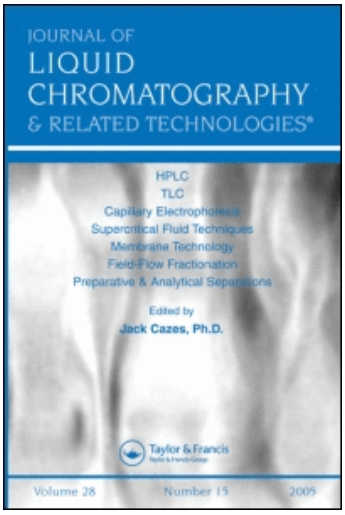

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Comparative Study of the Lipophilicity of Selected Tauro-Conjugates Bile Acids Determined with the Use of RPTLC and RPHPTLC Methods

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Abstract: Lipophilicity of selected tauro-conjugates bile acids such as: taurocholic acid (TC), taurodeoxycholic acid (TDC), taurochenodeoxycholic acid (TCDC), and taurolithocholic acid (TLC), by means of RPTLC and RPHPTLC methods was found. Reversed–phase thin layer chromatography was performed on $RP18F_{254}$, $RP18WF_{254}$, and on $RP2F_{254}$ chromatographic plates with the use of a mixture of an organic modifier (methanol, acetone, dioxane)–water in different volume compositions. The obtained chromatographic parameters of lipophilicity (R_{MW} and φ_0) indicates that regardless of applied chromatographic conditions, the lipophilicity of four examined tauro-conjugates bile acids should decrease in the following order: TLC > TCDC ≅ TDC > TC. A good correspondence between experimentally determined lipophilic parameters and the theoretical value of lipophilicity ($\log P_{virtual}$) for investigated bile acids was observed. The cluster analysis (CA) of lipophilic parameters indicates a large similarity of lipophilicity for tauro-conjugates bile acids. All examined bile acids form exactly one cluster in almost all chromatographic systems used.

Keywords: Bile acids, Lipophilicity, log P, RPTLC

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INTRODUCTION

Lipophilicity is one of the parameters which influence the transport of bioactive compounds through lipid membranes in the human body. The measurement of the lipophilicity is partition coefficient ($\log P$). Experimentally, $\log P$ is determined with the use of different analytical methods like the shake flask method or with the use of chromatographic techniques, such as RPTLC, RPHPTLC, or RPHPLC.[1] The theoretical values of partition coefficients for respective compounds can be obtained by means of commercial computer programs based on calculations related to chemical structure of studied substances.[2]

Nowadays, description of the relationships between $\log P$ values determined with the use of various methods and respective structural descriptors for compounds investigated is the objective of SAR (Structure–Activity–Relationship) studies.

Previous investigations referred to SAR-studies of selected free and glyco-conjugates bile acids.[3–6] The aim of this work was to determine the lipophilicity of tauro-conjugates bile acids with the use of RPTLC and RPHPTLC techniques. Cluster Analysis (CA) was used for estimation of similarity between lipophilicity of four examined bile acids. The chromatographic lipophilicity parameters (R_{MW}, φ_0) obtained for four examined bile acids in various chromatographic systems were compared with computational calculated $\log P$ ($\log P_{virtual}$). Virtual $\log P$ is suitable for prediction of lipophilicity for compounds which exist in stereoisomeric conformation (3D structure) like tauro-conjugates bile acids.

EXPERIMENTAL

Chemicals

The following components of mobile phase: methanol (Merck, Germany), acetone (POCh, Gliwice), dioxane (POCh, Gliwice), and distillated water (Department of Analytical Chemistry, Faculty of Pharmacy, Sosnowiec, Poland) were used for RPTLC and RPHPTLC analysis. All chemicals were analytical grade.

The commercial samples in the form of sodium salts such as: taurocholic acid (TC) No. 345909-26-4, taurodeoxycholic acid (TDC) No. 207737-97-1, taurochenodeoxycholic acid (TCDC) No. 6009-98-9, and taurolithocholic acid (TLC) No. 6042-32-6 (Sigma–Aldrich) were used as test solutes. Ethanol 95% (POCh, Gliwice, Poland) and phosphomolibdic acid (POCh, Gliwice, Poland) were applied to prepare a visualizing reagent.

Preparation of Sample Solutions

The methanolic solutions of the above mentioned bile acids were prepared in 5 mg/mL concentration for each bile acid.

RPTLC and RPHPTLC Analysis

Thin layer chromatography was performed on RPTLC aluminum plates 6 cm × 10 cm $RP18F_{254}$ (E. Merck, Art. 1.05559) and also on RPHPTLC glass plates 6 cm × 10 cm such as: $RP18WF_{254}$ (E. Merck, Art. 1.13124) and $RP2\,F_{254}$ (E. Merck, Art 1.13726). Solutions of four studied bile acids were spotted on chromatographic plates in quantities of 5 μg in 1 μL of methanol.

The chromatograms were developed with the use of organic modifier (methanol, acetone and dioxane)–water in the following volume compositions:

- methanol–water, the content of methanol in mobile phase was gradually varied by 5% [v/v] from 50–80% [v/v].
- acetone–water, the content of acetone in mobile phase was gradually varied by 5% [v/v] from 40–80% [v/v].
- dioxane–water, the content of acetone in mobile phase was gradually varied by 5% [v/v] from 40–80% [v/v].

The chromatograms were developed at room temperature in a 20 cm × 20 cm horizontal chamber (Camag, Switzerland) using respective mobile phase. The development distance was 9 cm. Mobile phases of 50 mL were used in all cases. Next, the plates were dried at room temperature using a fume cupboard. The spots were visualized by dipping the plates in 10% ethanol solution of phosphomolibdic acid and next heating them for 20 minutes at 120°C.

The chromatograms were run in triplicate.

Lipophilicity Parameters

Chromatographic Parameter of Lipophilicity R_{MW}

The lipophilicity parameter R_{MW} can be determined by RPTLC and RPHPTLC methods on the basis of retention data like R_M values. The experimentally determined R_{MW} values related to lipophilicity. R_M values predicted for each mobile phase: methanol–water, acetone–water, and dioxane–water by means of different chromatographic plates were

extrapolated to the zero concentration of respective organic modifier in mobile phase according to equation:[7]

$$R_M = R_{MW} - S \times \varphi \quad (1)$$

where: R_M is the R_M value of examined bile acid, R_{MW} is the R_M value of bile acid for analyte 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.

Parameter of Lipophilicity φ_0

In each case, when a high linear correlation between R_{MW} and S is observed, parameter φ_0 can be calculated according to equation:[7]

$$\varphi_0 = \frac{R_{MW}}{S} \quad (2)$$

We have previously stated that, φ_0 can be used as the relative measure of lipophilicity for bioactive compounds.[3–6]

Theoretical Partition Coefficient

A lot of computational programs allow predicting the partition coefficient expressed as: Alog Ps, IAlog P, clog P, xlog P.[2] For four examined tauro-conjugates bile acids the $\log P_{virtual}$ was calculated with the use of the Vega ZZ program, which includes the stereoisomeric conformation of bioactive compounds.[8]

Chemometric Analysis of Data

Regression analysis between experimental and theoretical values of lipophilicity obtained for four examined bile acids was made by means of program STATISTICA 7.1. To estimate the similarity between experimentally determined lipophilicity (R_{MW} and φ_0) under different chromatographic conditions and also theoretical value predicted as $\log P_{virtual}$, the CA (Cluster Analysis) was used, which was available in program STATISTICA 7.1.

RESULTS AND DISCUSSION

The purpose of this work was to determine the lipophilicity of tauro-conjugates bile acids such as: taurocholic acid (TC), taurodeoxycholic

acid (TDC), taurochenodeoxycholic acid (TCDC), and taurolithocholic acid (TLC) with the use of reversed–phase thin layer chromatography (RPTLC and RPHPTLC). The R_M values obtained on $RP18F_{254}$, $RP18WF_{254}$, and $RP2F_{254}$ chromatographic plates developed by means of different mobile phases: methanol–water, acetone–water, and dioxane–water in various volume compositions, were extrapolated to zero content of organic modifier in mobile phase. Tables 1–3 present the statistical parameters of linear regressions between R_M values and the content of organic modifier in mobile phase type: $R_M = R_{MW}\text{-}S \times \varphi$, such as: correlation coefficient (r), standard error (s), value of Fisher test (F), significance level (p), and number of points used to derive the particular regression (n). The statistical parameters presented in Tables 1-3 indicate that all obtained linear equations were highly significant ($0.9906 < r < 0.9992$) under applied chromatographic conditions.

Although the R_{MW} values show that, the higher lipophilicity of tauro–conjugates bile acids has taurolithocholic acid (TLC). Both, taurodeoxycholic acid (TDC) and taurochenodeoxycholic acid (TCDC) have similar lipophilicity, which is lower than the lipophilicity of TLC.

Table 1. Parameters of linear correlations (±SD) between R_M values of tauro-conjugates bile acids obtained on $RP18\,F_{254}$ plates (Art. 1.05559) and content of organic modifier (methanol, acetone or dioxane) in mobile phase according to Eq. $R_M = R_{MW} - S \times \varphi$[a]

Acid	R_{MW}	S	r	s	F	n	Eq. no.
Methanol-water							
TC	2.6116 (±0.1747)	3.7465 (±0.2545)	0.9931	0.053	216.67	5	3
TDC	2.8205 (±0.0763)	3.7571 (±0.1158)	0.9981	0.031	1052.67	6	4
TCDC	2.7669 (±0.0299)	3.7286 (±0.0454)	0.9997	0.012	6755.8	6	5
TLC	3.1717 (±0.1048)	4.0000 (±0.1590)	0.9968	0.042	632.47	6	6
Acetone-water							
TC	1.0853 (±0.0847)	2.4612 (±0.1342)	0.9911	0.047	336.08	6	7
TDC	1.5412 (±0.0515)	2.8100 (±0.0816)	0.9975	0.029	1184.44	7	8
TCDC	1.5794 (±0.0922)	2.9283 (±0.1460)	0.9926	0.052	402.29	6	9
TLC	1.8035 (±0.0682)	2.9794 (±0.1080)	0.9961	0.038	760.31	6	10
Dioxane-water							
TC	1.0397 (±0.0704)	2.3829 (±0.1166)	0.9941	0.034	417.25	6	11
TDC	1.3830 (±0.0488)	2.5584 (±0.0773)	0.9973	0.027	1095.06	6	12
TCDC	1.3767 (±0.0467)	2.5724 (±0.0740)	0.9975	0.026	1209.62	5	13
TLC	1.7219 (±0.0693)	2.9323 (±0.1098)	0.9958	0.039	713.44	6	14

Note. *n*-number of points used to derive the particular regressions; *r*-correlation coefficient; *s*-standard error; *F*-value of Fisher test.

[a]For all equations the significance level $p < 0.001$.

Table 2. Parameters of linear correlations (±SD) between R_M values of tauro-conjugates bile acids obtained on $RP18WF_{254}$ plates (Art. 1.13124) and content of organic modifier (methanol, acetone or dioxane) in mobile phase according to Eq. $R_M = R_{MW} - S \times \varphi^a$

Acid	R_{MW}	S	r	s	F	n	Eq. no.
Methanol-water							
TC	1.8113 (±0.1066)	3.0406 (±0.1630)	0.9943	0.042	348.14	6	15
TDC	2.2174 (±0.1119)	3.2626 (±0.1793)	0.9925	0.062	330.99	7	16
TCDC	2.1883 (±0.1333)	3.2126 (±0.2122)	0.9914	0.073	229.14	6	17
TLC	2.7085 (±0.1503)	3.8174 (±0.2392)	0.9922	0.083	254.65	6	18
Acetone-water							
TC	0.8702 (±0.0763)	2.3514 (±0.1214)	0.9934	0.043	375.06	7	19
TDC	1.3323 (±0.0966)	2.7188 (±0.1531)	0.9906	0.054	315.80	8	20
TCDC	1.2997 (±0.0841)	2.6642 (±0.1333)	0.9926	0.047	399.65	8	21
TLC	1.8329 (±0.0537)	3.3286 (±0.0877)	0.9983	0.028	1441.30	7	22
Dioxane-water							
TC	1.0272 (±0.0573)	2.4846 (±0.0945)	0.9971	0.030	690.78	7	23
TDC	1.3659 (±0.0771)	2.6822 (±0.1221)	0.9938	0.030	482.39	8	24
TCDC	1.3678 (±0.0909)	2.6976 (±0.1465)	0.9927	0.050	339.05	7	25
TLC	1.7073 (±0.1221)	3.0475 (±0.2086)	0.9908	0.057	213.47	6	26

Note. *n*-number of points used to derive the particular regressions; *r*-correlation coefficient; *s*-standard error; *F*-value of Fisher test.
[a]For all equations the significance level $p < 0.0001$.

The last investigated bile acid, taurocholic acid (TC), shows the lowest lipophilicity regardless of applied chromatographic conditions. To compare the experimental R_{MW} values with theoretical $\log P_{virtual}$, the cluster analysis was made (CA). The CA analysis of all R_{MW} values indicates that the highest similarity was observed between $\log P_{virtual}$ and R_{MW} obtained on all applied chromatographic plates ($RP18F_{254}$, $RP18WF_{254}$, and $RP2F_{254}$) developed by means of methanol as mobile phase. Under these conditions, the R_{MW} values and $\log P_{virtual}$ make exactly one cluster (see Figure 1).

The good linear correlation between R_{MW} values calculated according to Equation (1) and the slope (S) of obtained linear curves allowed determining the φ_0 values in accordance with Equation (2). Table 4 presents the φ_0 values of examined bile acids for 9 chromatographic conditions.

Based on the φ_0 values of four examined tauro-conjugates bile acids, it can be observed that the lipophilicity of all bile acids decreases in the same order according to R_{MW} values: $TLC > TCDC \cong TDC > TC$ (Table 5).

Table 3. Parameters of linear correlations (±SD) between R_M values of tauro-conjugates bile acids obtained on RP2 F_{254}plates (Art. 1.13726) and content of organic modifier (methanol, acetone or dioxane) in mobile phases according to Eq. $R_M = R_{MW} - S \times \varphi$[a]

Acid	R_{MW}	S	r	s	F	n	Eq. no.
Methanol-water							
TC	1.7526 (±0.0990)	3.5706 (±0.1635)	0.9958	0.052	477.09	6	27
TDC	2.4620 (±0.1304)	4.3780 (±0.2151)	0.9952	0.069	414.08	6	28
TCDC	2.1785 (±0.0860)	3.8700 (±0.1539)	0.9976	0.034	631.94	5	29
TLC	2.6636 (±0.0840)	4.3078 (±0.1387)	0.9979	0.044	964.84	5	30
Acetone-water							
TC	0.2985 (±0.0524)	1.8077 (±0.0809)	0.9960	0.027	498.44	6	31
TDC	0.8940 (±0.0504)	2.4640 (±0.0847)	0.9976	0.024	846.06	6	32
TCDC	0.8595 (±0.0269)	2.4862 (±0.0428)	0.9992	0.015	3366.10	7	33
TLC	1.3403 (±0.0396)	3.0061 (±0.0630)	0.9989	0.022	2279.82	7	34
Dioxane-water							
TC	0.9367 (±0.0580)	2.7540 (±0.0911)	0.9967	0.032	913.89	8	35
TDC	1.3695 (±0.0467)	3,1325 (±0.0733)	0.9984	0.026	1827.76	8	36
TCDC	1.3738 (±0.0363)	3.1672 (±0.0578)	0.9992	0.020	3002.82	7	37
TLC	1.6666 (±0.1062)	3.4129 (±0.1793)	0.9945	0.048	362.11	6	38

Note. *n*-number of points used to derive the particular regressions; *r*-correlation coefficient; *s*-standard error; *F*-value of Fisher test.
[a]For all equations the significance level $p < 0.0001$.

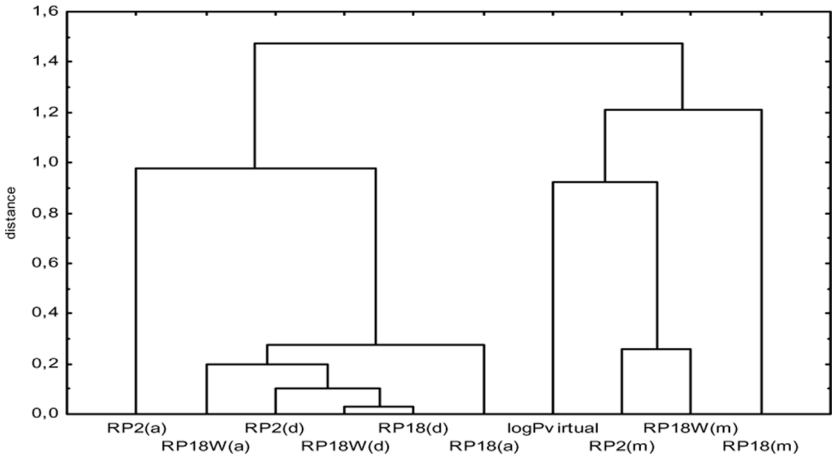

Figure 1. Cluster analysis (CA) of $\log P_{virtual}$ and R_{MW} values of tauro-conjugates bile acids determined on $RP18F_{254}$, $RP18WF_{254}$ and $RP2F_{254}$ chromatographic plates developed with an mixture of organic modifier (methanol, acetone or dioxane)-water in different volume compositions.

Table 4. Parameters of linear correlations (±S.D.) between R_{MW} values and slope for tauro–conjugates bile acids obtained in various chromatographic systems and determined according to equation: $R_{MW} = a \times S + b$

	Statistical parameters of equation: $R_{MW} = a \times S + b$						
R_{MW}	a	b	r	F	S	p	Eq. no
$RP18F_{254}$							
$R_{MW(m)}$	1.7062 (±0.4886)	−3.6547 (±1.8616)	0.9269	12.19	0.109	0.073	39
$R_{MW(a)}$	1.2581 (±0.2025)	−2.0137 (±0.5675)	0.9750	38.58	0.082	0.025	40
$R_{MW(d)}$	1.1751 (±0.1976)	−1.6883 (±0.5176)	0.9728	35.35	0.079	0.027	41
$RP18WF_{254}$							
$R_{MW(m)}$	1.0604 (±0.1890)	−1.3034 (±0.6326)	0.9696	31.46	0.110	0.030	42
$R_{MW(a)}$	0.9512 (±0.1090)	−1.2969 (±0.3040)	0.9871	76.11	0.077	0.013	43
$R_{MW(d)}$	1.1650 (±0.1582)	−1.8109 (±0.4327)	0.9820	54.24	0.064	0.018	44
$RP2F_{254}$							
$R_{MW(m)}$	0.9853 (±0.2287)	−1.7082 (±0.9250)	0.9501	18.56	0.151	0.050	45
$R_{MW(a)}$	0.8683 (±0.0317)	−1.2715 (±0.0785)	0.9987	751.78	0.027	0.001	46
$R_{MW(d)}$	1.1040 (±0.0365)	−2.1040 (±0.1141)	0.9989	914.06	0.017	0.001	47

Note. *r*–correlation coefficient; *s*-standard error; *F*-value of Fisher test; *p*-significance level –methanol-water; *a*–acetone—water; *d*-dioxane-water.

Cluster analysis of φ_0 values found in 9 chromatographic systems shows the highest similarity between experimentally determined φ_0 by means of RPTLC and RPHPTLC plates and mixture of methanol–water, acetone–water, and dioxane–water used as mobile phases (Figure 2). All φ_0 make one cluster.

Linear correlations between $\log P_{virtual}$ and experimentally determined lipophilic parameter (R_{MW}) in all applied chromatographic systems was observed. The good correlation between R_{MW} values and the theoretical parameter of lipophilicity ($\log P_{virtual}$) for examined tauro–conjugates bile acids in the form of respective linear equations allow predicting experimental R_{MW} values on all applied chromatographic plates and mobile phases used. The statistical parameters of linear equations type: $R_{MW} = \log P_{virtual} \times a + b$ are given below:

$$R_{MWRP18(m)} = \log P_{virtual} \times 0.2562\ (\pm 0.0432) + 2.2654\ (\pm 0.1031),$$
$$r = 0.9727,\ s = 0.255,\ F = 35.09,\ p = 0.027,\ n = 4 \quad (48)$$
$$R_{MWRP18(a)} = \log P_{virtual} \times 0.3245\ (\pm 0.0595) + 0.7711\ (\pm 0.1419),$$
$$r = 0.9680,\ s = 0.093,\ F = 29.72,\ p = 0.032,\ n = 4 \quad (49)$$
$$R_{MWRP18(d)} = \log P_{virtual} \times 0.3099\ (\pm 0.0048) + 0.6818\ (\pm 0.0115),$$
$$r = 0.9998,\ s = 0.008,\ F = 4113.37,\ p = 0.000,\ n = 4 \quad (50)$$

Table 5. The values of lipophilicity parameters φ_0 obtained for investigated tauro-conjugates bile acids on various RPTLC and RPHPTLC plates ($RP18F_{254}$, $RP18WF_{254}$, $RP2F_{254}$) with the use of different mobile phases according to Eq. (2)

Acid	$\varphi_{0RP18(m)}$	$\varphi_{0RP18(a)}$	$\varphi_{0RP18(d)}$	$\varphi_{0RP18W(m)}$	$\varphi_{0RP18W(a)}$	$\varphi_{0RP18W(d)}$	$\varphi_{0RP2(m)}$	$\varphi_{0RP2(a)}$	$\varphi_{0RP2(d)}$
TC	0.6971	0.4409	0.4363	0.5957	0.3701	0.4134	0.4908	0.1651	0.3401
TDC	0.7507	0.5485	0.5406	0.6796	0.4900	0.5092	0.5624	0.3628	0.4372
TCDC	0.7421	0.5394	0.5352	0.6812	0.4878	0.5070	0.5629	0.3457	0.4338
TLC	0.7929	0.6053	0.5872	0.7095	0.5506	0.5602	0.6183	0.4459	0.4883

Note. *m*-methanol-water; *a*-acetone–water; *d*-dioxane-water.

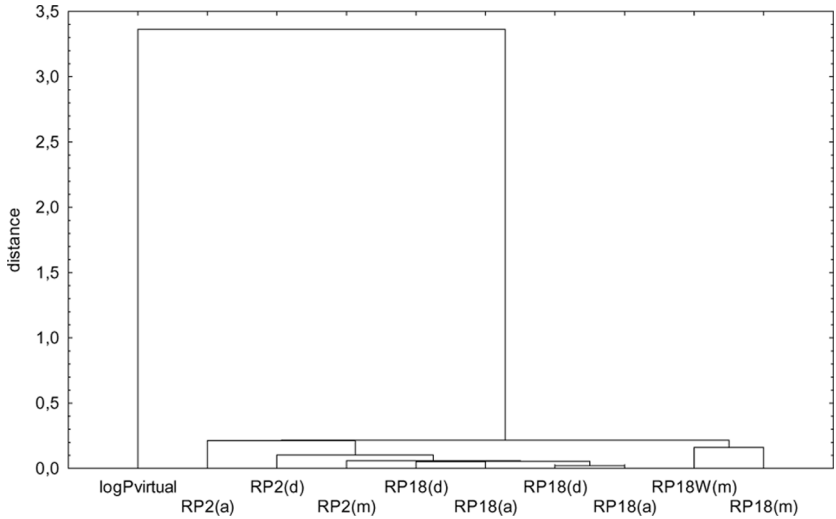

Figure 2. CA of $\log P_{virtual}$ and φ_0 values of tauro-conjugates bile acids calculated on the basis of R_{MW} values according to Eq. (2).

$$R_{MWRP18W(m)} = \log P_{virtual} \times 0.4086\ (\pm 0.0209) + 1.3107\ (\pm 0.0499),$$
$$r = 0.9974,\ s = 0.032,\ F = 381.12,\ p = 0.003,\ n = 4 \quad (51)$$
$$R_{MWRP18W(a)} = \log P_{virtual} \times 0.4381\ (\pm 0.0110) + 0.3465\ (\pm 0.0263),$$
$$r = 0.9994,\ s = 0.017,\ F = 1575.46,\ p = 0.001,\ n = 4 \quad (52)$$
$$R_{MWRP18W(d)} = \log P_{virtual} \times 0.3089\ (\pm 0.0070) + 0.6709\ (\pm 0.0168),$$
$$r = 0.9995,\ s = 0.011,\ F = 1924.58,\ p = 0.000 \quad (53)$$
$$R_{MWRP2(m)} = \log P_{virtual} \times 0.4158\ (\pm 0.1010) + 1.3273\ (\pm 0.2407),$$
$$r = 0.9458,\ s = 0.157,\ F = 16.96,\ p = 0.054,\ n = 4 \quad (54)$$
$$R_{MWRP2(a)} = \log P_{virtual} \times 0.4730\ (\pm 0.0321) - 0.2178\ (\pm 0.0766),$$
$$r = 0.9954,\ s = 0.050,\ F = 216.74,\ p = 0.005,\ n = 4 \quad (55)$$
$$R_{MWRP2(d)} = \log P_{virtual} \times 0.3307\ (\pm 0.0367) + 0.5914\ (\pm 0.0874),$$
$$r = 0.9879,\ s = 0.057,\ F = 81.32,\ p = 0.012,\ n = 4 \quad (56)$$

The same situation it can be observed in the case of relationships between φ_0 values and $\log P_{virtual}$. All obtained φ_0 values correlate well with $\log P_{virtual}$. The statistically significant correlations between φ_0 and $\log P_{virtual}$ in theform of linear plots type: $\varphi_0 = \log P_{virtual} \times a + b$ are presented below:

$$\varphi_{0RP18(m)} = \log P_{virtual} \times 0.0436\ (\pm 0.0023) + 0.6474\ (\pm 0.0055),$$
$$r = 0.9972,\ s = 0.004,\ F = 350.85,\ p = 0.003,\ n = 4 \quad (57)$$
$$\varphi_{0RP18(a)} = \log P_{virtual} \times 0.07445\ (\pm 0.0105) + 0.3655\ (\pm 0.0251),$$
$$r = 0.9806,\ s = 0.016,\ F = 50.04,\ p = 0.019,\ n = 4 \quad (58)$$

$$\varphi_{0RP18(d)} = \log P_{virtual} \times 0.0683\ (\pm 0.0128) + 0.3709\ (\pm 0.0304),$$
$$r = 0.9669,\ s = 0.020,\ F = 28.68,\ p = 0.033,\ n = 4\ (59)$$
$$\varphi_{0RP18W(m)} = \log P_{virtual} \times 0.0514\ (\pm 0.0134) + 0.5508\ (\pm 0.0318),$$
$$r = 0.9385,\ s = 0.021,\ F = 14.79,\ p = 0.062,\ n = 4\ (60)$$
$$\varphi_{0RP18W(a)} = \log P_{virtual} \times\ 0.0817\ (\pm 0.0140) + 0.2905\ (\pm 0.0133),$$
$$r = 0.9718,\ s = 0.022,\ F = 33.95,\ p = 0.028,\ n = 4\ (61)$$
$$\varphi_{0RP18W(d)} = \log P_{virtual} \times 0.0665\ (\pm 0.0105) + 0.3477\ (\pm 0.0251),$$
$$r = 0.9758,\ s = 0.016,\ F = 39.88,\ p = 0.024,\ n = 4\ (62)$$
$$\varphi_{0RP2(m)} = \log P_{virtual} \times 0.0578\ (\pm 0.0046) + 0.4283\ (\pm 0.011),$$
$$r = 0.9938,\ s = 0.007,\ F = 160.79,\ p = 0.006,\ n = 4\ (63)$$
$$\varphi_{0RP2(a)} = \log P_{virtual} \times 0.1272\ (\pm 0.0239) + 0.0432\ (\pm 0.0572),$$
$$r = 0.9662,\ s = 0.037,\ F = 28.14,\ p = 0.034,\ n = 4\ (64)$$
$$\varphi_{0RP2(d)} = \log P_{virtual} \times\ 0.0671\ (\pm 0.0105) + 0.2736\ (\pm 0.0251),$$
$$r = 0.9763,\ s = 0.016,\ F = 40.67,\ p = 0.024,\ n = 4\ (65)$$

Both types of linear regressions obtained between the theoretically found parameter of lipophilicity for stereoisomeric compounds $\log P_{virtual}$ and chromatographic parameters of lipophilicity (R_{MW}, φ_0) allow predicting the R_{MW} and $\varphi_{0,}$, respectively, for each applied chromatographic system.

CONCLUSIONS

The lipophilicity order of tauro-conjugates bile acids such as: TC, TDC, TCDC, and TLC, by means of 9 different chromatographic conditions was determined. The R_{MW} and φ_0 values indicate that the lipophilicity of bile acids examined should decrease in the following order: TLC > TCDC ≅ TDC > TC. On the basis of cluster analysis of experimentally determined R_{MW} values under 9 applied chromatographic conditions and also $\log P_{virtual}$ for bile acids examined, it can be concluded that, of all mobile phases used, only methanol-water mixture in different volume compositions is the optimal mobile phase, because it allowed obtaining the biggest similarity between R_{MW} values for TC, TDC, TCDC, and TLC and theoretical partition coefficient $\log P_{virtual}$. The R_{MW} values found with the use of the methanol-water mixture on all applied chromatographic plates for RPTLC and RPHPTLC ($RP18F_{254}$, $RP18WF_{254}$, and $RP2F_{254}$) and $\log P_{virtual}$ make exactly one group of similarity (one cluster). Comparison of φ_0 values for bile acids examined show that all bile acids make one cluster because their φ_0 values are similar. $LogP_{virtual}$ is similar to this one. Finally, it was stated that reversed–phase thin layer chromatography is useful for prediction of lipophilicity of tauro-conjugates bile acids.

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