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Lipophilicity of Selected Bile Acids as Determined by TLC. I

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ABSTRACT

The selected bile acids were chromatographed on RP-HPTLC plates using methanol–water in different volume proportions as mobile phases. The linear relationships were found between R_M values of bile acids and the volume fraction of methanol in the mobile phase. The retention parameters R_{MW} were extrapolated to zero methanol content and were compared with measured ($\log P_{\text{exp}}$), as well as calculated partition coefficients ($\text{IAlog } P$ and $\log P_{\text{Kowin}}$). It was stated that the investigated bile acids might be considered as compounds belonging to the same group. The most significant correlation was found between the R_{MW} and $\log P_{\text{Kowin}}$ values. The results indicate that chromatographic R_{MW} values may be used as a measure of lipophilicity of the investigated bile acids.

Key Words: Lipophilicity; Bile acids; RP-HPTLC method.

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INTRODUCTION

The terms lipophilicity and the less frequently encountered analogue hydrophobicity are used to indicate the physical property of the molecule, which governs its partitioning into the nonaqueous partner of an immiscible or partially immiscible solvent pair.^[1] Lipophilicity of a substance is one parameter, which influences biological activity. The importance of the use of partition coefficients in quantitative structure, i.e., activity relationships (QSAR) is well established.^[2-20] The measurement of partition coefficients by equilibration methods is frequently made difficult or even impossible by the impurity or instability of compounds, by a strong preference of the compounds for one of the two phases of the system, or by the formation of stable emulsions after shaking.^[3] The compatibility of experimental and calculative approaches to quantify organic compound lipophilicity remain a focus of scientific interest.^[2,14,15,19]

Reversed-phase thin-layer chromatography (RP-TLC) is an alternative method for lipophilicity estimations.^[2-20]

The aim of the present study was to find a linear correlation between R_M values obtained for some bile acids by RP-HPTLC and the volume fraction of methanol in the mobile phase. In addition, the retention parameter R_{MW} extrapolated to pure water was compared with measured, as well as calculated partition coefficients.

EXPERIMENTAL

Chemicals

The components of mobile phases: methanol (POCh, Gliwice, Poland) and distilled water were used for RP-TLC analysis. The commercial samples of cholic acid (C), chenodeoxycholic acid (CDC), deoxycholic acid (DC), lithocholic acid (LC), glycocholic acid (GC), glycodeoxycholic acid (GDC), and glycolithocholic acid (GLC) (St. Louis, Sigma Chemical Company, USA) were used as test solutes. Methanol (POCh, Gliwice, Poland; pure p.a.) was used to prepare the solutions of bile acids. Sulfuric acid (Chempur, Piekary Śląskie, Poland) was used to prepare the visualizing reagent.

Sample Preparation

The methanolic solutions of the above-mentioned bile acids at about a concentration 5 mg/mL of each acid were prepared.





Partition Thin-Layer Chromatography

Thin-layer chromatography was done on RP-TLC glass plates 10×20 cm RP-18 WF (Merck, Darmstadt, No. 1.14296). The mixtures of methanol–water were used as the mobile phases. The methanol content was varied by 5% volumes from 60% to 100%. Fifty milliliter of the mobile phase was used in each case.

Micropipetes (5 μ L, Camag) were used to apply the standard solutions to the plates. Solutions of the standard acids were spotted on a chromatographic plate in quantities 15 μ g of each standard in 3 μ L of methanol. The chromatograms were developed at room temperature (20°C) in a 20×20 cm standard chamber (Camag, Switzerland). The development distance was 8.5 cm.

The plates were dried at room temperature (20°C) using a fume cupboard. The investigated bile acids were evaluated on plates using a 10% solution of sulfuric acid in water as the visualizing reagent. The spots were developed by heating the sprayed plates at 120°C for 20 min. The chromatograms were run in triplicate. The R_F values were the mean values that were used for calculation of R_M parameters according to the expression $R_M = \log[(1/R_F) - 1]$.

The R_M values were extrapolated to the zero methanol concentration (R_{MW}) using the expression^[16,20]

$$R_M = R_{MW} + a\phi \quad (1)$$

where ϕ is the volume fraction of methanol (in v/v%,) in the mobile phase and slope a is the change in R_M value due to the 1% increase of methanol content in the mobile phase (associated with the specific hydrophobic surface area).

The relationship between R_{MW} values and slopes a was given by the equation:^[20]

$$R_{MW} = A + Ba \quad (2)$$

Theoretical Partition Coefficients

The lipophilicity of the investigated compounds, expressed by partition coefficient ($\log P$) was also calculated theoretically ($|\log P$, $\log P_{Kowin}$) using the internet databases.^[21,22]





RESULTS AND DISCUSSION

Regular retention behavior was observed for each solute on RP-18W layers. The R_M values of the investigated acids were decreasing with the increasing fraction volume of methanol in the mobile phase (Fig. 1).

It was found that the R_M values obtained from 60% to 100% content of methanol in the mobile phase for LC, DC, and CDC and from 65% to 100% content of methanol for the C, and from 70% to 100% content of methanol for the GLC, GC, and GDC have to be considered to extrapolate of R_M values to

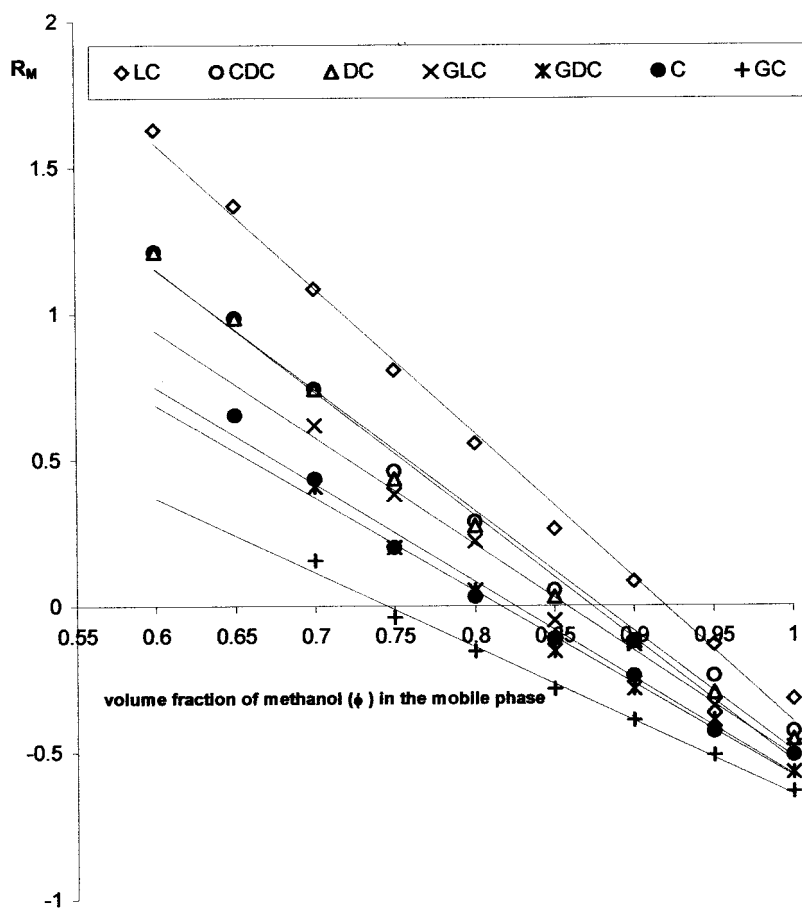


Figure 1. Relationship between R_M values and the volume fraction of methanol (ϕ) in the methanol-water mobile phase.





pure water in methanol–water mobile phase. Contents of the water in the mobile phase higher than 30% caused the tailing of spots of the conjugated acids GC, GDC, and GLC on the chromatograms.

The lines of the dependence of the R_M values vs. the volume fraction of methanol (ϕ) in the mobile phase (Fig. 1) for the C and GDC, as well as DC and CDC, were running next to each other. It follows that the C and GDC, as well as DC and CDC, ought to have similar hydrophobic properties. The R_M obtained for the all investigated acids were extrapolated to pure water (to 0%, v/v of methanol in mobile phase) according to Eq. (1). Seven correlation Eqs. (2)–(8) were obtained (Table 1). The high correlation coefficients (r), the values of the Fisher test (F), the significance levels (p), and small values of the standard errors of the estimates (s) indicated that all the equations obtained were highly significant. The intercepts in these equations are the R_{MW} values. The parameters of lipophilicity (R_{MW}) for investigated bile acids are between $1.888(\pm 0.080)$ and $4.552(\pm 0.109)$. Lithocholic acid has the highest and GC the lowest lipophilicity. Cheno-deoxycholic acid and DC have similar R_{MW} values: $3.626(\pm 0.126)$ and $3.683(\pm 0.127)$, respectively. Similarly, the GDC and C have similar R_{MW} values: $2.591(\pm 0.106)$ and $2.736(\pm 0.136)$, respectively. Thus, the R_{MW} values for both of the pairs of acids are included in limits of their errors. The R_{MW} values indicate that lipophilicity of the investigated bile acids should decrease in the order: $LC > DC \approx CDC > GLC > C \approx GDC > GC$.

It has been shown in the literature that between the intercept and slope of Eq. (1) usually the linear correlation is established.^[15,20] If linearity exists, the compounds form a congeneric class. The linear relationship was found for the R_{MW} and slopes a values (Fig. 2) with a correlation coefficient of -0.9996 . This result proves that the investigated bile acids may be considered as compounds belonging to the same group under the circumstances described.

Therefore, that bile acid forms a congeneric class, and it is possible to correlate R_{MW} with $\log P$ values. The comparison of chromatographic R_{MW} values vs. experimental $\log P$ data available in the literature^[21] are presented in Fig. 3. The correlation ($r = 0.9318$) shown in Fig. 3 proves that R_{MW} values of bile acids are a reliable alternative for partition coefficient between *n*-octanol and water.

Relationships between R_{MW} values and calculated $\log P$ values, applying two different calculation softwares, IALog P and $\log P_{Kowin}$, are presented in Figs. 4 and 5, respectively.

It can be seen that there was usually a correct linear correlation between R_{MW} values and $\log P$ values calculated using a commercial program. Comparing two calculation procedures, $\log P_{Kowin}$ is more appropriate for chromatographic R_{MW} ($r = 0.9578$) than Interactive Analysis IALog P ($r = 0.9256$).





Table 1. Parameters of linear correlation between R_M values of bile acids and methanol content in the methanol–water mobile phase (according to Eq. (1): $R_M = R_{MW} + a\phi$).

| Acid | Code | Range of the volume fraction of methanol (ϕ) | Parameters of equation: $R_M = R_{MW} + a\phi$ | | | Statistical data ^a | | | Eq. no | | |
|------------------|------|---|--|----------------------------|-----------------------|-------------------------------|-----|---------|--------|---------|-----|
| | | | R_{MW} | Standard error of R_{MW} | Standard error of a | r | s | F | | | |
| Lithocholic | LC | 0.60–1.00 | 4.552 | 0.109 | -4.948 | 0.134 | 9 | -0.9974 | 0.052 | 1353.56 | (2) |
| Deoxycholic | DC | 0.60–1.00 | 3.683 | 0.127 | -4.215 | 0.156 | 9 | -0.9952 | 0.060 | 726.19 | (3) |
| Chenodeoxycholic | CDC | 0.60–1.00 | 3.626 | 0.126 | -4.124 | 0.156 | 9 | -0.9950 | 0.060 | 700.11 | (4) |
| Glycolithocholic | GLC | 0.70–1.00 | 3.129 | 0.144 | -3.646 | 0.168 | 7 | -0.9947 | 0.044 | 470.72 | (5) |
| Cholic | C | 0.65–1.00 | 2.736 | 0.136 | -3.315 | 0.163 | 8 | -0.9928 | 0.053 | 413.06 | (6) |
| Glycodeoxycholic | GDC | 0.70–1.00 | 2.591 | 0.106 | -3.173 | 0.124 | 7 | -0.9962 | 0.032 | 659.66 | (7) |
| Glycocholic | GC | 0.70–1.00 | 1.888 | 0.080 | -2.534 | 0.094 | 7 | -0.9966 | 0.025 | 7373.72 | (8) |

Note: n , number of points used to derive the particular regression Eq. (2); r , correlation coefficient; s , standard error of the estimate; F , value of the Fisher test.

^aFor all equations the significance levels $p < 0.001$.



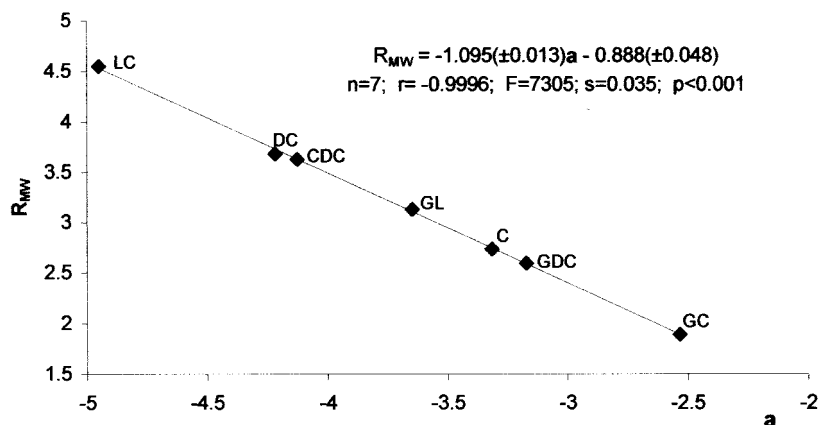


Figure 2. Relationship between the R_{MW} values and slope a for methanol-water system based on the data presented in Table 1.

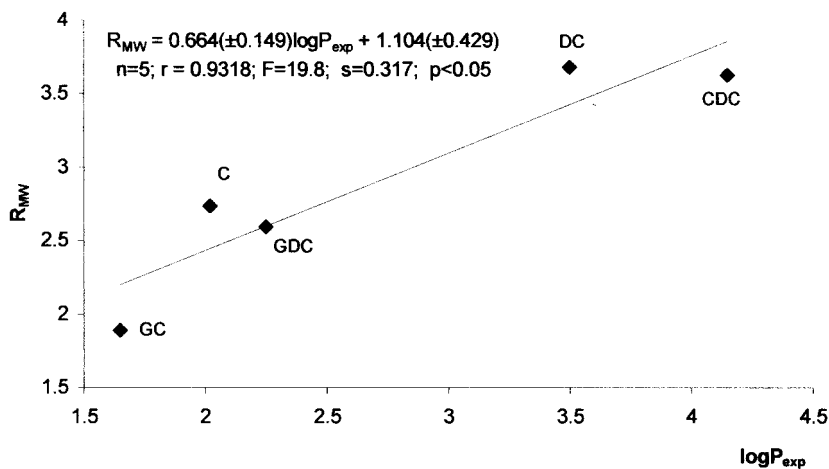


Figure 3. Chromatographic R_{MW} values vs. experimental $\log P$.

Further investigations are in progress and concern the use of other mobile phases to evaluate the lipophilicity of bile acids.

CONCLUSION

The linear relationships were found between R_M values of bile acids and the volume fraction of methanol in the mobile phase. The R_{MW} values indicate



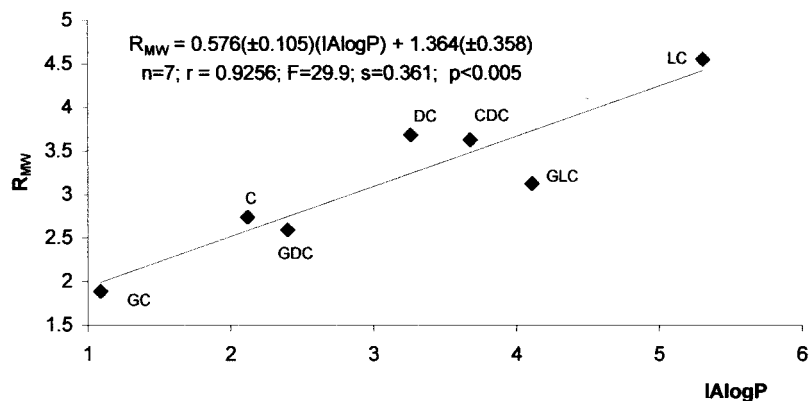


Figure 4. Correlation between R_{MW} and $I \log P$ calculated by use of the Interactive Analysis $\log P$ database.

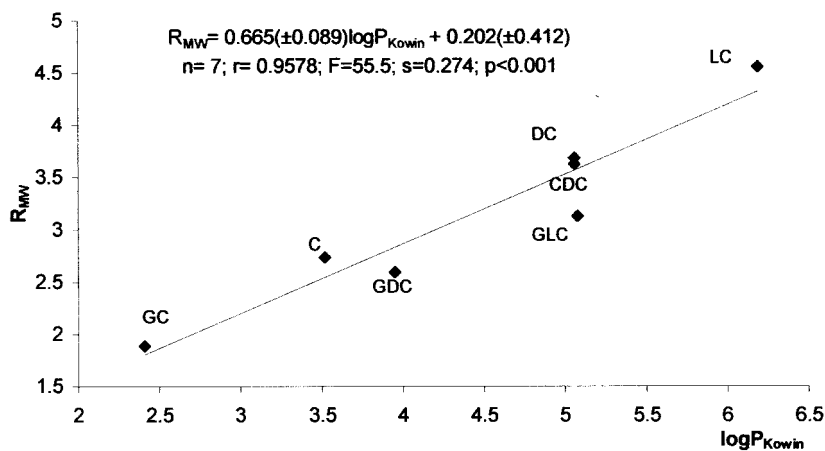


Figure 5. Correlation between R_{MW} and $\log P_{Kowin}$ calculated from the atom/fragmental contribution values.

that the bile acids investigated may be listed in order of decreasing lipophilicity as follows: $LC > DC \approx CDC > GLC > C \approx GDC > GC$. It was stated that the investigated bile acids might be considered as compounds belonging to the same group. The most significant correlation was found between R_{MW} values and $\log P_{Kowin}$ calculated from atom/fragmental contribution values.





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