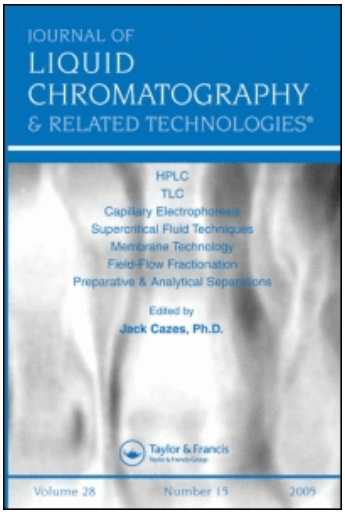

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Use of Structural Descriptors to QSRR Analysis of Selected Bile Acids Separated by NP-TLC

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Abstract: The selected bile acids such as: cholic acid (C), glycocholic acid (GC), glycolithocholic acid (GLC), deoxycholic acid (DC), chenodeoxycholic acid (CDC), glycodeoxycholic acid (GDC), lithocholic acid (LC), were separated by using normal phase thin-layer chromatography (NP-TLC) on glass plates precoated with silica gel 60 with concentrating zone (E. Merck, #1.11845) and *n*-hexane-ethyl acetate–methanol-acetic acid in volume composition 20:20:5:2 as the mobile phase. The selected topological indexes based on connectivity (M^{ν}, ${}^{0}\chi^{\nu}$, ${}^{1}\chi^{\nu}$, ${}^{2}\chi^{\nu}$, and χ^{ν}_{012}), on distance matrix (W, A, ${}^{0}B$, ${}^{1}B$, and C), and selected electrotopological states ($SdO_{(acid)}$, $SsOH_{(acid)}$, $SsOH_{(aliph)}$, $SdO_{(amide)}$, and SsNH) were calculated for investigated bile acids. The most accurate prediction of the R_M values of the investigated bile acids was achieved by using a monoparametric equation employing the topological index M^{ν}.

Keywords: Bile Acids, Densitometry, NP-TLC, QSRR, Structural descriptors

INTRODUCTION

Bile acids fulfil many important functions, e.g., they facilitate digestion and absorption of fat, vitamins soluble in fat (A, E, D, and K), and cholesterol. Separation and quantification of bile acids (free, glycine, and taurine conjugated acids) from biological materials are very important

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diagnostic indicators of liver and gastrointestinal diseases in humans.[1] Because of their structural similarities, separation of bile acids and their metabolites is difficult.[1–6] Many visualizing reagents were described for the visualization of bile acids, namely: anisaldehyde-sulphuric acid reagent,[7] a solution of antimony (III) chloride (Carr Price reagent) in chloroform (1:5, w/v),[7] a manganese (II) chloride-sulphuric acid reagent,[7] or a water solution of sulphuric acid.[8] Bile acids can also be detected by dipping plates into phosphomolybdic acid in ethanol and then heating for 10 min at 105–120°C[7] or by spraying the plates with a 10% solution of phosphomolybdic acid in methanol and then heating for 20 min at 50–80°C.[9] Chromatographic bands of bile acids on the densitogram after the use of spray solution of phosphomolybdic acid in methanol were irregular.[9] Therefore, this way of the detection of bile acids can not be recommended. Regular chromatographic bands of bile acids on the densitogram were obtained after the use of dipping water solution of sulphuric acid.[8]

Earlier, selected structural descriptors were used in QSAR and QSPR analysis of selected bile acids. Correlation between selected physicochemical properties; i.e., molar mass (M_w), molar refraction (R_m), molar volume (V_m), parachor (P), refraction index (I_r), density (d), lipophilicity parameter (R_{MW} and φ_0), and obtained structural descriptions, was found.[10] Different possibilities of application of the structural descriptors to calculate certain physicochemical data of examined bile acids (QSPR), depending on examined physicochemical properties, were found. The structural descriptions are not useful for calculating the density (d) of the examined bile acids. Substance density, especially the densities of liquids, not only changes with mass and structure; but, to a large extent, also depends on molecular interaction.

The aim of this work was the use of selected structural descriptors in QSRR analysis of selected bile acids after their separation by NP-TLC. The selected bile acids: cholic acid (C), glycocholic acid (GC), glycolithocholic acid (GLC), deoxycholic acid (DC), chenodeoxycholic acid (CDC), glycodeoxycholic acid (GDC), and lithocholic acid (LC) were a subject of investigations.

EXPERIMENTAL

Chemicals and Sample Preparation

The following components of the mobile phase *n*-hexane (Merck, Germany), ethyl acetate (POCh, Gliwice, Poland), acetic acid 99.5% (POCh, Gliwice, Poland), and methanol (Merck, Germany) were used for the adsorption TLC analysis. The commercial samples of C, DC,

CDC, LC, GLC, GDC, and GC (St. Louis, Sigma Company, USA) were used as test solutes. Ethanol (96%, POCh, Gliwice, Poland; pure p. a.) was used for the preparation of bile acids solution. Sulphuric acid, 95% (Chempur, Poland) was used to prepare the visualizing reagent. The ethanolic solution of the above mentioned bile acids in concentration 0.7 mg/1 mL of each acid was prepared.

Thin Layer Chromatography

Adsorption TLC was performed on 20×20 cm glass plates precoated with silica gel 60 with concentrating zone (E. Merck, #1.11845). The plates were prewashed with methanol and dried for 24 h at room temperature ($18 \pm 1°C$). Before use the plates were activated at 120°C for 30 min. A micropipette (Camag, Switzerland) was used to apply the standard solution to the plates. Solution of the standard acids was spotted on a chromatographic plate in the quantity 0.7 μg of each standard in 1 μL ethanol. The chromatograms were developed at room temperature ($18 \pm 1°C$) in a classical chamber (Camag, Switzerland) using *n*-hexane-ethyl acetate-methanol – acetic acid in volume composition 20:20:5:2. The development distance was 14 cm. The plates were dried at room temperature using a fume cupboard.

The investigated bile acids were evaluated on the plates using the solution of sulphuric acid in methanol in volume composition 1:19. The plate was immersed in a dipping solution of sulphuric acid for 15 s. It was then heated to 90°C for 20 min.

Densitometric Analysis

Densitometric scanning was then performed with a Camag Scanner TLC 3 operated in absorbance mode and controlled by winCATS 1.4.1 software. Densitometric scanning was then performed at multi wavelength in the range of 380 to 460 nm, at a change of wavelength at every step 20 nm. The radiation sources were a deuterium lamp emitting a continuous UV spectrum between 190 and 450 nm and a wolfram lamp emitting a spectrum between of 370 to 800 nm. The slit dimensions were 12.00×0.90 mm, macro; the optimized optical system was light; the scanning speed was 20 mm s^{-1}; the data resolution was 100 μm $step^{-1}$; the measurement type was remission; and the measurement mode was absorption; the optical filter was second order. Each track was scanned three times and baseline correction (lowest slope) was used.

Structural Descriptors

Selected topological indexes based on adjacency matrix: Gutman (M^{ν}),[11–14] Randić ($^{0}\chi^{\nu}$, $^{1}\chi^{\nu}$, and $^{2}\chi^{\nu}$),[11,12] Pyka (χ^{ν}_{012}),[13,14] and also based on distance matrix: Wiener (W),[12,15] and Pyka (A, ^{0}B, ^{1}B, and C)[13,14] were calculated. Pyka and Wiener indices were calculated by building a distance matrix and determining its elements by means of values given by Barysz et al.[16] The electrotopological states:[17] $SdO_{(acid)}$, $SsOH_{(acid)}$, $SsOH_{(aliph)}$, $SdO_{(amide)}$, and SsNH were obtained from Internet data base.[18] The methods of calculation of structural descriptors have been described elsewhere.[11,12,17]

RESULTS AND DISCUSSION

The separation of bile acids: cholic acid (C), glycocholic acid (GC), glycolithocholic acid (GLC), deoxycholic acid (DC), chenodeoxycholic acid (CDC), glycodeoxycholic acid (GDC), and lithocholic acid (LC) on silica gel 60 with concentrating zone and by use of *n*-hexane-ethyl acetate-methanol – acetic acid in volume composition 20:20:5:2 enables optimum separation of the investigated bile acids. Absorption maximum of investigated bile acids after separation on silica gel and after application of sulphuric acid as visualizing occur at 458, 393, 397, 457, 379, 386, and 380 nm, respectively for GC, GDC, GLC, C, CDC, DC, and LC.[8] Therefore, densitometric scanning was then performed at multi wavelength in the range of 380 to 460 nm, at a change of wavelength at every step 20 nm. The densitogram of investigated bile acids at different wavelengths (380, 400, 420, 440, and 460 nm) is presented in Figure 1. The chromatograms were done in triplicate and each track was scanned three times; and the mean of R_F values were calculated. Next, the R_F values were recalculated on R_M values.

One of the current tendencies in chemical investigations is the prediction of physicochemical and biological properties of chemical compounds from their structural parameters. The fundamental finding of these investigations is the fact that the structure of a molecule determines it properties. Only quantum mechanics completely describes the structure of a molecule, characterizing its geometrical and electron structure. The structural descriptors are the simplest way of a structural description of a molecule. The applications of the structural descriptors in investigations of quantitative structure retention relationships (QSRR) for organic compounds have been described in the scientific literature. In this connection, the selected topological indexes and electrotopological states were used to show the dependence among their numerical values and the R_M values of the investigated bile acids by using n-hexane-ethyl acetate-methanol – acetic acid in

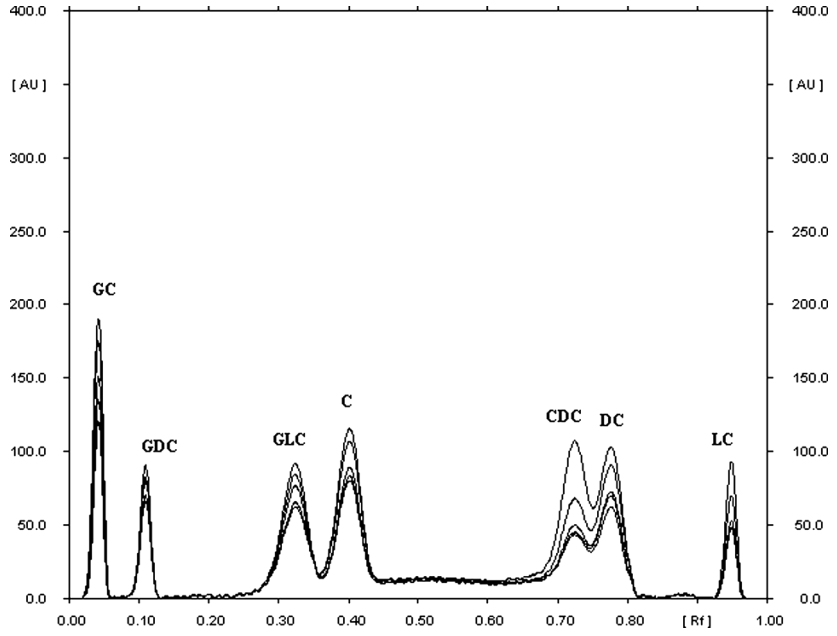

Figure 1. The densitograms of bile acids investigated (GC – glycocholic acid, GDC – glycodeoxycholic acid, GLC – glycolithocholic acid, C – cholic acid, CDC – chenodeoxycholic acid, DC – deoxycholic acid, LC – lithocholic acid) at wavelengths 380, 400, 420, 440, and 460 nm after their separation using a *n*-hexane – ethyl acetate – methanol – acetic acid, 20:20:5:2 (v/v/v/v) as mobile phase and after application of sulphuric acid as visualizing reagent.

volume composition 20:20:5:2 as mobile phase. R_M values and numerical values only of those structural descriptors, which were useful in correlation analysis for calculation of the R_M values of the investigated bile acids, are presented in Table 1. Those topological indexes can be also used for the prediction of relative situation of the bile acids investigated on a thin-layer chromatogram.

The best relationships for chromatographic parameter R_M were obtained using topological indexes W, A, M^{ν}, ${}^{0}\chi^{\nu}$, and χ^{ν}_{012}. These regression equations have good linearity, usually with $r > 0.90$. For example:

$$R_M = -3.077(\pm 0.604) + 0.0013(\pm 0.0002) \times W$$
$$n = 7;\ r = 0.921;\ s = 0.353,\quad F = 27;\ p < 0.005 \tag{1}$$

$$R_M = -5.609(\pm 0.746) + 0.0042(\pm 0.0008) \times A$$
$$n = 7;\ r = 0.912;\ s = 0.372,\quad F = 24;\ p < 0.005 \tag{2}$$

Table 1. R_M values and the selected topological indexes for investigated bile acids

Symbol of bile acid	Retention parameter R_M	Topological indexes				
		W	A	M^{ν}	${}^{0}\chi^{\nu}$	χ^{ν}_{012}
GC	1.195	3080.69	1100.80	376	20.3726	14.7912
GDC	0.826	2897.28	1051.02	346	20.0551	14.6920
GLC	0.288	2738.61	1008.43	316	19.7376	14.4784
C	0.140	2000.75	761.39	304	18.2572	13.5232
CDC	−0.432	1873.88	725.60	274	17.9398	13.3774
DC	−0.550	1864.88	722.25	274	17.9398	13.3552
LC	−1.195	1743.50	687.40	244	17.6223	13.2831

$$R_M = -12.448(\pm 2.253) + 0.662(\pm 0.119) \times {}^{0}\chi \quad n = 7;\ r = 0.928;\ s = 0.339,\quad F = 30;\ p < 0.005 \tag{3}$$

$$R_M = -15.090(\pm 3.236) + 1.086(\pm 0.232) \times \chi_{012} \quad n = 7;\ r = 0.902;\ s = 0.391,\quad F = 21;\ p < 0.01 \tag{4}$$

$$R_M = -5.457(\pm 0.304) + 0.018(\pm 0.001) \times M^{\nu} \quad n = 7;\ r = 0.993;\ s = 0.110,\quad F = 332;\ p < 0.0001 \tag{5}$$

This experiment indicates that the topological index M^{ν} is connected in a special way with the chromatographic parameter R_M of investigated bile acids.

One test of a predictive equation like Equation (5) is how well it predicts values of a compound not included in the training set. One compound, GLC, was removed from the training set, and a monoparametric equation was recalculated as:

$$R_M = -5.450(\pm 0.333) + 0.018(\pm 0.001) \times M^{\nu} \quad n = 6;\ r = 0.993;\ s = 0.121,\quad F = 273;\ p < 0.0005 \tag{6}$$

The R_M value for GLC was predicted from Equation (6) and is equal to 0.231, whereas the experimental value was 0.288.

From data presented in this work, it is apparent that the topological M^{ν} describes additional important elements of the chemical structure of the bile acids not given by other structural descriptors. The Gutman index (M^{ν}) has previously been used to calculate the dissociation constants of phenol derivatives,[19] the molar volume of saturated fatty acids,[20] the logk values of anilides separated by RP-HPLC,[21] retention times of PAH separated by HPLC,[22] and lipophilicity of selected pesticides.[22,23]

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