Scientific paper

Influence of Temperature on Retention Parameter of Bile Acids in Normal Phase Thin- Layer Chromatography: the Role of Steroid Skeleton

Mihalj Poša,* Ana Sebenji and Jovana Trifunović

Department of Pharmacy, Faculty of Medicine, University of Novi Sad, Hajduk Veljkova 3, 21000 Novi Sad, Serbia

** Corresponding author: E-mail: mihaljp@uns.ac.rs (M.P.)*
Tel: + 381 24 422 760; Fax: +381 24 422 760

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Abstract

In this paper, bile acids' retention parameter, R_M, obtained in normal phase thin-layer chromatography is determined as a function of the temperature (293–323 K). Analyzed bile acids belong to congeneric group with two oxygen atoms (OH or oxo groups) on the steroid core and congeneric group with three oxygen atoms. For molecules of both congeneric groups it is found that there is a linear relationship between R_M and temperature, therefore R_M decreases with the increase of temperature. In certain congeneric groups, parameters of linear function ($R_M - T$) can be linked to the structural characteristics of bile acids, firstly with their spatial orientation (steric position in relation to the steroid mean plane) of the steroid oxygen atom (OH or oxo groups). Absolute values of slope Θ of linear function ($R_M - T$) increase if steroid oxygen atoms are not in the polar plane, since then the possibility of forming hydrogen bonds with stationary phase decreases. Besides that, absolute value of the parameter Θ describes degree of hydrogen bond forming between bile acids and polar stationary phase in each congeneric group as well as hydrophobicity of the steroid skeleton.

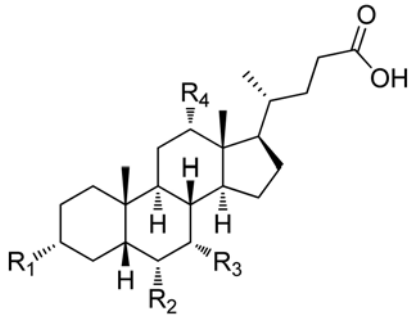
Keywords: Bile acids, bile acids' oxo derivatives, steroid skeleton, normal phase thin- layer chromatography, temperature dependence

1. Introduction

Bile acids are steroid amphiphilic compounds with hydrophobic-convex and hydrophilic-concave side of cyclopentanoperhydrophenanthrene ring.[1] Bile acids are (mainly) synthesised in the liver of vertebrates. Their main physiological role is in lipid digestion (emulgation and micellar transport from lumen to the bowel wall i.e. in homeostasis of cholesterol – nuclear modulator).[2,3] In certain concentrations (critical micellar concentration) bile acid salts form micelles. That is the basis for some of their pharmaceutical applications (emulgation and solubilisation of hydrophobic drugs).[1,4,5] Bile acid salts, especially their oxo derivatives, show promotoric action in transport of some drugs through lipid barriers (for example blood-brain barrier).[6–8] Interaction of bile acids with biological membranes (responsible for their promotoric and toxic activity) as well as self-association ability (micelle formation) is determined by their hydrophobicity.[1,9] Lately, oxo derivatives of bile acid salts have been intensively studied in biopharmaceutical experiments because of their lower membranotoxicity (membranolytic activity).[10,11]

In QSA(P)R research of bile acids, *in silico* descriptors or chromatographic values (retention parameters) obtained in reversed phase chromatography (RP-HPLC and RP-TLC) are used as independent variables.[12–14] QSA(P)R analysis have the aim to derive mathematical models to predict pharmacological features and self-association parameters, as parameters of hydrophobicity. However, huge number of *in silico* descriptors don't explain correctly or do not distinguish bile acids' oxo derivatives constitutional isomers.[4] Thus, finding chromatographic parameters that describe bile acids' hydrophobicity is of the great interest.

Thin-layer chromatography is widely applied for lipid analysis. From the aspect of green chemistry thin-layer chromatography has the advantage over liquid chromatography in terms of reduced consumption of organic solvents.[15,16]



R_1 = OH; R_2 = R_3 = R_4 = H	3α-hydroxy-5β-cholanoic acid (lithocholic a.); **L**
R_1 = R_4 = OH; R_2 = R_3 = H	3α,12α-dihydroxy-5β-cholanoic acid (deoxycholic a.); **DC**
R_1 = =O; R_4 = OH; R_2 = R_3 = H	12α-hydroxy-3-oxo-5β-cholanoic acid; **3-DD**
R_1 = OH; R_4 = =O; R_2 = R_3 = H	3α-hydroxy-12-oxo-5β-cholanoic acid (12-oxolithocholic a.); **12-OL**
R_1 = R_4 = =O; R_2 = R_4 = H	3,12-dioxo-5β-cholanoic acid; **3,12-DD**
R_1 = R_3 = OH; R_2 = R_4 = H	3α,7α-dihydroxy-5β-cholanoic acid (chenodeoxycholic a.); **CD**
R_1 = =O; R_3 = OH; R_2 = R_4 = H	7α-hydroxy-3-oxo-5β-cholanoic acid; **3-DCD**
R_1 = OH; R_3 = =O; R_2 = R_4 = H	3α-hydroxy-7-oxo-5β-cholanoic acid (7-oxolithocholic a.); **7-OL**
R_1 = R_3 = =O; R_2 = R_4 = H	3,7-dioxo-5β-cholanoic acid; **3,7-DCD**
R_1 = R_2 = OH; R_3 = R_4 = H	3α,6α-dihydroxy-5β-cholanoic acid (hyodeoxycholic acid); **HD**
R_1 = R_3 = R_4 = OH; R_2 = H	3α,7α,12α-trihydroxy-5β-cholanoic acid (cholic a.); **C**
R_1 = R_4 = OH; R_3 = =O; R_2 = H	3α,12α-dihydroxy-7-oxo-5β cholanoic acid (7-oxodeoxycholic a.); **7-ODC**
R_1 = R_3 = OH; R_4 = =O; R_2 = H	3α,7α-dihydoxy-12-oxo-5β-cholanoic acid (12-oxochenodeoxycholic a.); **12-OCD**
R_1 = R_3 = =O; R_4 = OH; R_2 = H	12α-hydroxy-3,7-dioxo-5β-cholanoic acid; **3,7-DOC**
R_1 = R_4 = =O; R_3 = OH; R_2 = H	7α-hydroxy-3,12-dioxo-5β-cholanoic acid; **3,12-DOC**
R_1 = OH; R_3 = R_4 = =O; R_2 = H	3α-hydroxy-7,12-dioxo-5β-cholanoic acid; **7,12-DOC**
R_1 = R_4 = R_3 = =O; R_2 = H	3,7,12-trioxo-5β-cholanoic acid; **3,7,12-TOC**

Fig. 1. Studied bile acids.

The aim of the paper is examination of the influence of temperature in normal phase thin-layer chromatography of bile acids in order to obtain parameters (independent variables) of hydrophobicity and capacity of hydrogen bonding in QSA(P)R model. Two congeneric groups are investigated: the first one (**I**), that contains bile acids with two oxygen atoms (in OH or oxo group) and the second one (**II**), with three oxygen atoms bonded to steroid skeleton rings (**Fig. 1**). For illustration of temperature dependence monohydroxylitocholic acid is examined as well. In this paper the simplest, underivatized silica gel is chosen as a stationary phase, which is ecologically most favorable (sometimes toxic materials shouldn't be used for derivatization). Working hypothesis was that information about bile acids' hydrophobicity can be obtained at different temperatures on the underivatized silica gel.

2. Materials and Methods

2. 1. Bile Acids

Bile acids: cholic, deoxycholic, chenodeoxycholic, litocholic and hiodeoxycholic are obtained from Sigma Aldrich, 98%. Each bile acid was recrystallized from the mixture of methanol: water = 3 : 2 three times and once from the ethanol. For synthesis of oxo derivatives cholic, deoxycholic and chenodeoxycholic acid were used. Procedures of synthesis were described in earlier papers.[4–10] Each solution was of HPLC purity.

2. 2. Chromatographic Experiment

In the experiment, dichloromethane – acetone (5: 3) mixture was used as mobile phase, and silica gel (Merck, Germany) was the stationary phase. The investigated compounds were separately dissolved in dichloromethane (1 mg/mL) and 2 mL of each solution was spotted on the plate. Chromatographic chamber was placed in the water bath with thermostat (Ministat 650), and water bath was placed in the air bath with thermostat (Iskra). Temperature was determined in the vapour phase (Te1) of the chromatographic chamber, and difference between temperatures in air bath and vapour phase was less than ±0.1 °C. Chromatographic plate (S) with sample was placed in the manipulator holder (M) placed in the air bath. After 10 min of tempering chromatographic plate was placed in the chromatographic chamber (Fig. 2). Temperature was changed during the experiment in the interval from 293 to 323 K in steps of 5 K. Above 323 K, derivatives with two oxo groups and trioxo derivative have $R_f \approx 1$. After being developed, dried plates were sprayed with manganese chloride in sulphuric acid solution, and placed in the oven at 100–120 °C. In each case sharp spots without tailing tendency were obtained. This visualization method per-

mitted the identification of compounds at 254 nm as yellow–brown spots. For each spot, R_f value was determined and retention parameter was calculated R_M:

$$R_M = \log\left(\frac{1}{R_f} - 1\right). \quad (1)$$

For each bile acid, R_M value as a function of temperature was calculated:

$$R_M = R_{M(T=273K)} + \Theta T \,. \quad (2)$$

droxyl groups are placed on the α side of the molecule in a so-called polar plane (i.e. all three OH groups are in the same plane, which favours spatial orientation of hydroxyl groups during the binding to the stationary phase).[10] Bile acids bind to the silanol stationary phase with hydrogen bonds via steroid OH groups.[18] If there are more OH groups in the polar plane connected to the steroid skeleton, equilibrium of bile acids' binding is shifted towards the adsorption process (Fig. 3), i.e. the standard free enthalpy of adsorption on silanol stationary phase $\Delta_{ad}G$ is more negative. Standard free enthalpy is linked to the adsorption constant by the following equation:

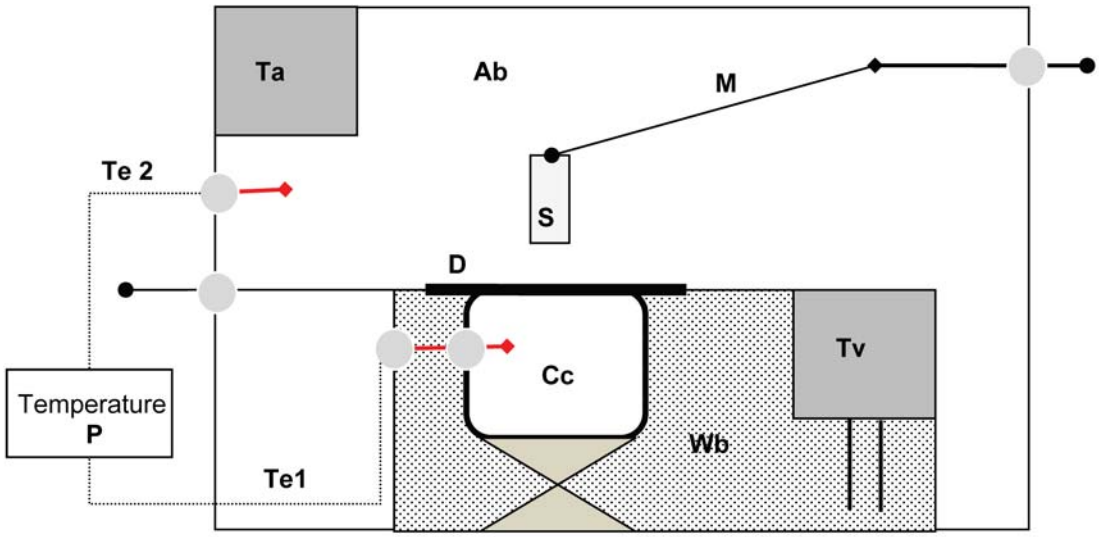


Fig. 2. Apparatus used to determine influence of temperature in normal phase chromatography: Wb = water bath, Ab = air bath, Tv = water bath thermostat, Ta = air bath thermostat, Te1 = thermo-element that measures the temperature in the chromatographic chamber, Te2 = thermo-element that measures the temperature in the air chamber, P = processor, Cc = chromatographic chamber, S = stationary phase (plate), D = chromatographic chamber cover, M = manipulator.

3. Results and Discussion

Zarzycki et al. used RP-18W HPTLC technique for separation of cholesterol and bile acids (with OH group in steroid core). They varied methanol–water rate and temperature was up to 60 °C.[17] However, in this paper normal phase TLC was used. In order to obtain different R_f values (especially above 35 °C) of analysed dioxo and trioxo bile acid derivatives (bile acid with lower amphilicity on steroid core)[10], it was necessary that polarity of stationary and mobile phase differ as much as possible. Thus, mobile phase containing dichloromethan : acetone = 5:3 was used.

For each bile acid there is a linear relationship obtained between retention parameter R_M and temperature T in the temperature interval from 293 K to 323 K (Table 1), whereby R_M decreases with the increase of temperature.

As it can be seen from Fig. 3, cholic acid (C) contains three OH groups in the steroid skeleton. These hy-

$$\Delta_{ad}G^\circ = -RT\ln K_{ad} \quad (\text{Eq} \quad (3)$$

while there is a direct relationship between chromatographic parameter R_M and $\ln K_{ad}$ (if R_M is higher, R_f is smaller, which means that mobility of the analyzed molecule in the stationary phase is lower as well, and value of K_{ad} i.e. ln K_{ad} is higher). Equilibrium is, to a greater extent, moved to the adsorption process $R_M = b \ln K_{ad}$. Thus, the equation (Eq. 3) is transformed into:

$$\Delta_{ad}G^\circ = -RTbR_M \text{ or } \frac{\Delta_{ad}G^\circ}{T} = -RbR_M \quad (4)$$

By differentiating the above equation for temperature T and using Gibbs-Helmholn's equation $(\partial(\Delta G / T) / \partial T) = -\Delta H / T^2$ the next equation follows:

$$Rb\left(\frac{\partial R_M}{\partial T}\right)_p = Rb\Theta = \frac{\Delta_{ad}H^\circ}{T^2} \quad (5)$$

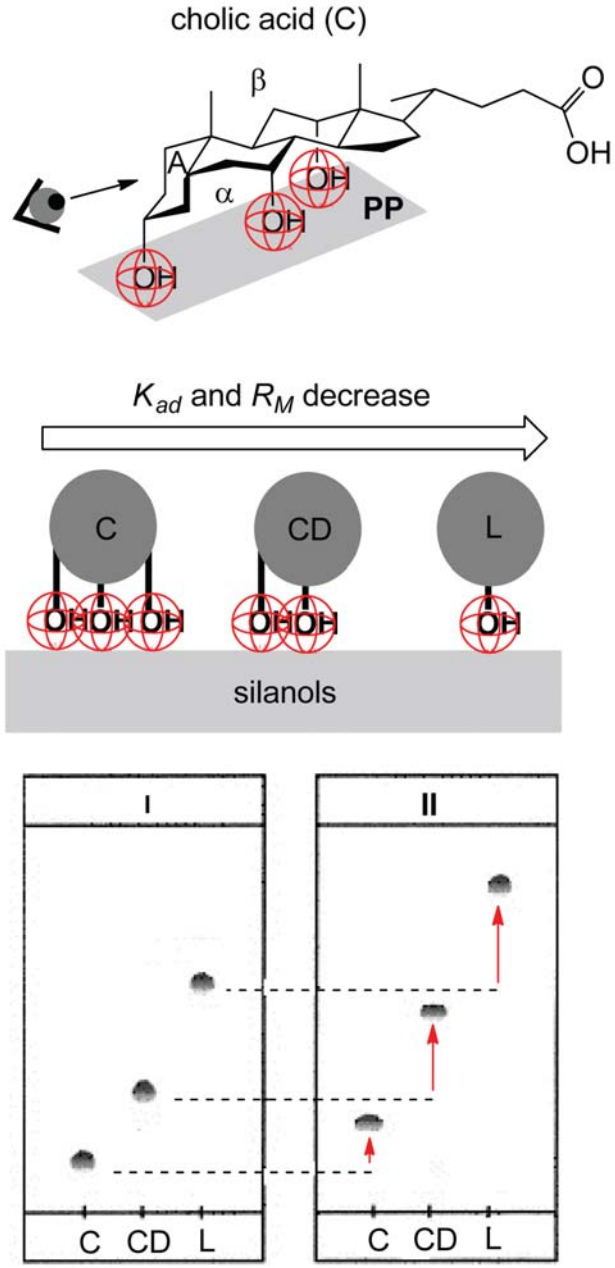


Fig. 3. Polar plane (PP) and bonding of bile acids to silanol stationary phase (view in the A ring direction).

Table 1. Parameters of the equation $R_M = R_{M(T=273K)} + \Theta T$ and critical micellar concentrations (CMC).[10]

Bile acids	$-\Theta$ [1/°C]	$R_{M(T=273K)}$	R^2	CMC [mM]
L	0.0199	0.288	0.9859	nd
I congeneric group				
DC	0.0136	0.849	0.9978	3
3-DD	0.0182	0.340	0.9825	17
12-OL	0.0163	0.387	0.9875	21
3,12-DD	0.0197	0.285	0.9941	51
CD	0.0132	0.861	0.9793	5
3-DCD	0.0170	0.352	0.9887	20
7-OL	0.0153	0.382	0.9994	24
3,7-DCD	0.0189	0.297	0.9959	53
HD	0.0148	0.390	0.9897	12
II congeneric group				
C	0.009	1.238	0.9932	8
7-ODC	0.0126	0.856	0.9964	55
12-OCD	0.0123	0.869	0.9889	58
3,7-DOC	0.0160	0.374	0.9983	94
3,12-DOC	0.0155	0.402	0.9892	98
7,12-DOC	0.0144	0.421	0.9921	108
3,7,12-TOC	0.0189	0.319	0.9887	130

nd = not defined (does not form micelles)

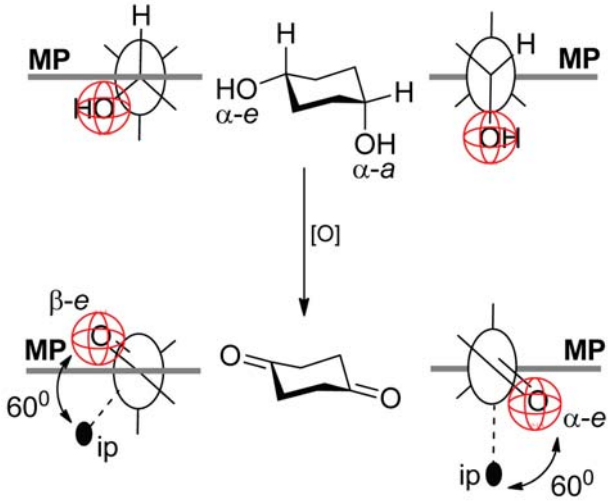


Fig. 4. The change of the oxygen atom position after oxidation of (steroid) OH groups bound to cyclohexane (ip = initial position, MP= mean plane).

where $\Delta_{ad}H$ is the change of standard enthalpy of the adsorption process. As binding on the stationary phase by hydrogen bonds is an exothermic process ($\Delta_{ad}H < 0$),[19] the value of the slope Θ in the equation of R_M as function of T is negative, which is consistent with experimental data (Table 1). Constant value of the slope Θ in the investigated temperature interval means that rising of $\Delta_{ad}H$, caused by higher temperature values in the quotient $\Delta_{ad}H°/T^2$, is compensated with the rising of T^2.

In the congeneric group **I** absolute value of Θ increases in the next series: CD≈D<HD≈7-OL<12-OL<3-DD<3,7-DCD<3,12-DD. This can be explained by shifting of the oxygen atoms (OH or oxo) connected to the steroid skeleton toward steroid cyclohexane mean plane (MP). This results in a reduction of bile acids (steroids) biplanarity (amphiphilicity) and respectively hinders the formation of hydrogen bonds with silanol stationary phase (due to steric reasons). Namely, after oxidation of α axial (α-*a*) or α equatorial (α-*e*) bile acids' OH groups, oxo group is obtained whose oxygen atom is switched for 60° (compared to the starting position of the oxygen atom of the OH group in the Newmanns' projection formula) to-

ward β side of the steroid skeleton (to mean plane MP) i.e. it is taken out from PP (Fig. 4). If a bile acid contains more OH groups dislocated from PP (if more OH groups are substituted with oxo groups), i.e. moved toward β side of the steroid skeleton, bile acid is poorly adsorbed on the polar stationary phase – it is less strongly bounded. Thus, rising temperature to a greater extent causes the lowering of the retention parameter R_M (higher absolute value of Θ) i.e. lower amount of heat is needed for desorption. For monooxo derivatives of bile acids from the congeneric group **I** (7-OL<12-OL<3-DD) both derivatives: 7-OL and 12-OL contain OH group on a C3 position of the steroid skeleton, therefore the difference in the values of Θ is a result of different environment of the oxo group (to some extent oxo group can form hydrogen bonds with silanol stationary phase). If oxo group is placed in a position C12, this group is spatially screened with C17 side chain (intramolecular hydrogen bond with carboxyl group of the side chain), which is not the case with C7 oxo group[1,4]. Thus, the derivative 7-OL is to a higher extent bound to the stationary phase than the derivative 12-OL. For the derivative 3-DD, which is C3 oxo derivative, interaction is possible between C12 α-axial OH group and carboxyl group of the side chain, i.e. as the same OH group in synclinal positions has three methine groups (C9, C14, C17) additional decrease in hydrogen bonding with stationary phase occurs. In contrast, C3 α-equatorial OH group of 7-OL and 12-OL derivatives doesn't have groups in synclinal positions and spatially it can form hydrogen bonds in the easiest way. Explanation for different values of Θ for dioxo derivatives (3,7-DCD<3,12-DD) also originates from the intra-molecular forming of hydrogen bonds between C12 oxo group and carboxyl group of the side chain.

Hyodeoxycholic acid's (HD) Θ value doesn't statistically differ from the value for 7-oxolitocholic acid (7-OL) (Table 1). Namely, HD possesses an α equatorial (α-*e*) OH group in the B ring of the steroid skeleton whose spatial orientation is similar to the C7 oxo group of 7-OL derivative (in a proper Newmann's projection formula oxygen atoms of both groups are shifted for 60 in order to α axial position of the oxygen atom of the OH group, i.e. they are moved from the PP, Fig 4. and Fig 5. **I**).

However, for the analyzed bile acids with C3 α equatorial OH group, steric position of this group does differ from the steric position of α equatorial OH groups of the steroid skeleton B and C ring (if bile acid has an α equatorial OH group in B and C ring, then oxygen from this group has the same spatial position as the oxygen of the oxo group – it is moved toward the steroid skeleton mean plane (Fig. 4)). If the isolated ring A (cyclohexane ring) is represented in the way that axis (*ax*) (axis parallel with axial bonds) is parallel with the same axis (*ax*) of the B ring of the steroid skeleton, then C3 and C6 α equatorial OH groups in a proper Newmanns' projection formula have the same stereo-chemical positions. However, for the examined bile acids (most of them are natural bile acids) A and B rings are cis connected (A/B-cis), which means that an isolated A ring (Fig 5. II) in a steroid skeleton is rotated for 60 and Newmann's projection is rotated as well. C3 α equatorial OH group of the A ring of the

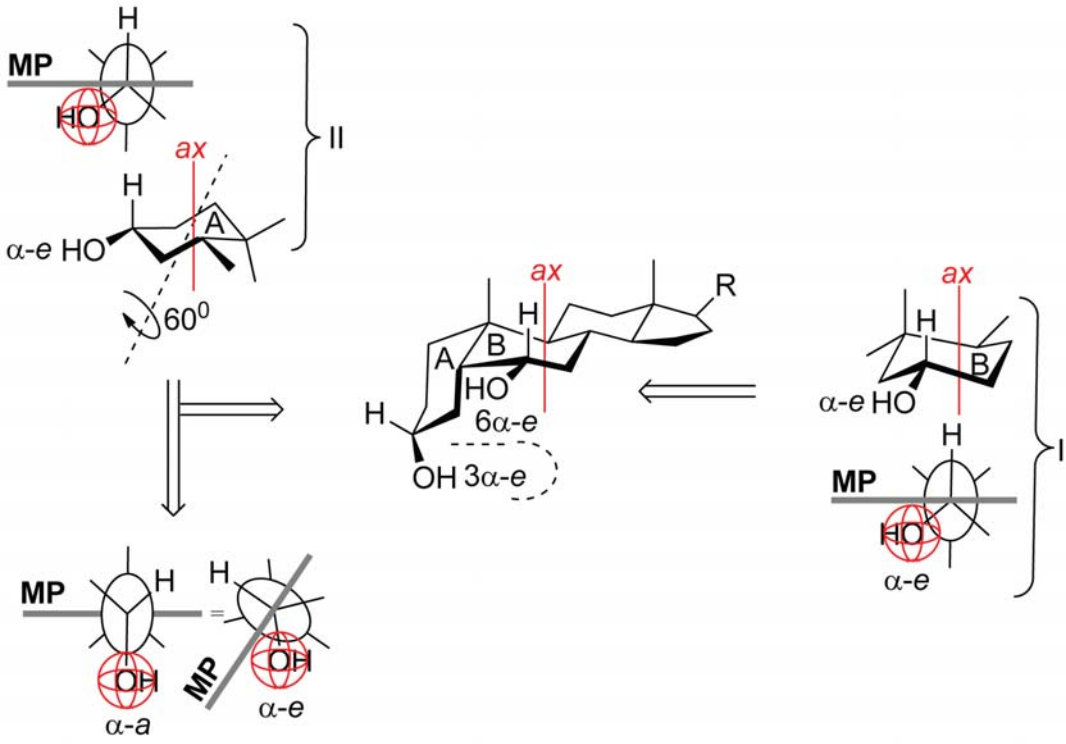


Fig. 5. Spatial orientation of equatorial OH groups in hyodeoxycholic acid (MP = mean plane).

steroid skeleton has the same spatial arrangement as it is axially OH group, i.e. although C3 is equatorial, it is placed in the planar plane (PP) with α axial OH groups of the B and C ring (for alo series of bile acids (A/B-trans) C3 α equatorial OH group has the same orientation as equatorial OH groups – it is of the B and C rings – it is moved from PP).

In Table 1 parameter $R_{M(T=273K)}$ represents ($R_M = \Theta T + R_{M(T=273K)}$) extrapolated value R_M for temperature 273 K. There is a reverse proportion between the absolute value of Θ and $R_{M(T=273K)}$. Bile acids with higher absolute value of Θ are weakly bound to polar stationary phase and they move faster on stationary phase i.e. have the lower value of R_M. In congeneric group **I** values of R_M decrease in the next series: CD≈D>HD≈7-OL≈12-OL>3-DD>3, 7-DCD>3,12-DD. This is in accordance with the structure of bile acids.

In congeneric group **II** absolute value of parameter Θ grows in the next series (Table 1): C<12-OCD≈7-ODC<7,12-DOC<3,12-DOC<3,7<DOC<3,7,12-TOC, i.e. values of $R_{M(T=273K)}$ grow in a reverse series. Changes of the parameter Θ, $R_{M(T=273K)}$ in congeneric group **II** of bile acids are in accordance with the regiochemical and sterochemical characteristics of OH and oxo groups of the steroid skeleton explained for the congeneric group **I**.

Parameters (Θ and $R_{M(T=273K)}$) of the linear equation for the relationship between the retention parameter and the temperature in both congeneric groups adequately describe the influence of the steroid core on binding to the silanol stationary phase. This parameters can be used as independent variables in describing bile acids binding for biomolecules (enzymes) if this interaction occurs via hydrogen bonds. However, prominent bile acids' features, as micelle formation,[20,21] interaction with cell membrane, binding to albumins, etc. are mainly determined by their hydrophobic surface.[1]

In order to check if information obtained from the temperature dependence of R_M on silanol stationary phase is valid for describing bile acids' hydrophobicity, linear regression between parameter Θ and critical micellar concentration (CMC) is applied. If dependence between binding of bile acids to polar stationary phase with hydrogen bonds (absolute value of parameter Θ) and their hydrophobicity is observed (in bile acids if the CMC value is higher, observed bile acids' β side of the steroid skeleton is less hydrophobic i.e. they have a lower tendency for self-association)[4,20] then in congeneric group with constant number of O atoms (OH or oxo group), there is a reverse proportion, or in other words, the absolute values of parameters Θ and CMC are directly proportional (Fig 6. and Fig 7). Namely, if bile acid has more O atoms moved from PP (higher absolute value of parameter Θ) that at the same time means that these oxygen atoms (α equatorial OH or oxo group) are shifted in the direction of steroid skeleton mean plane. It means that hydrophobic surface of the β side decreases, i.e. tendency for self association is lower – and CMC value rises.

After linear regression is performed on congeneric group **I** (Fig 6. 1), bile acids 3-DD and 3-DCD appear as outliers. Derivative 3-DCD has lower mobility on polar stationary phase than derivative 3-DD, i.e. 3-DCD derivative is strongly bonded to silanol stationary phase since it has C7 α axial OH group, while derivative 3-DD has C12 α axial OH group that can form intra-molecular hydrogen bond with carboxyl group of the side chain. Thus, 3-DD is weakly bound to the silanol stationary phase and has higher absolute value of the parameter Θ than derivative 3-DCD (according to that, 3-DD should have the higher CMC value). However, during the primary micelle forming process side chain spatially shields C12 α axial OH group,[1] and because of that, derivative 3-DD is a bit more hydrophobic than derivative 3-DCD, i.e. derivative 3-DD has the lower critical micellar concentration.

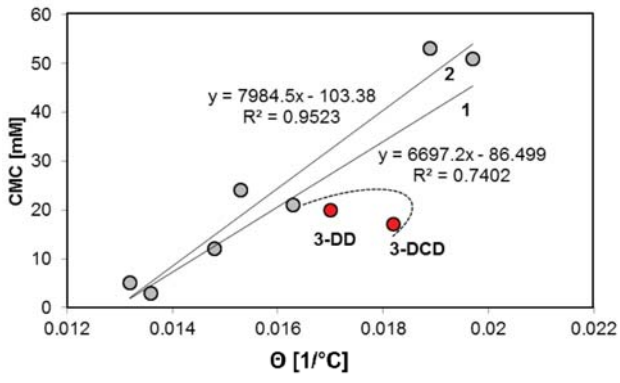


Fig. 6. Critical micellar concentration (CMC) as a function of the parameter Θ in congeneric group **I**: in linear regression **1** each bile acid from congeneric group **I** is included, while in linear regression **2** derivatives 3-DD i 3-DCD are omitted.

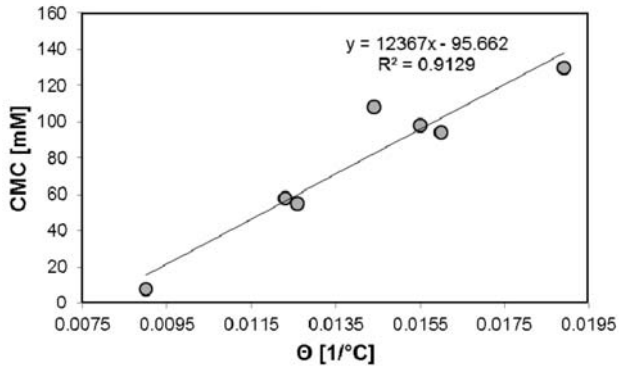


Fig. 7. Critical micellar concentration (CMC) as function of the parameter Θ in congeneric group **II.**

If bile acids of both congeneric groups are analysed at the same time, it can be concluded that there is no correlation between parameters Θ and CMC. Considering deoxycholic acid (from congeneric group **I**) and cholic acid (from congeneric group **II**), according to

parameters Θ, and $R_{M(T=273K)}$ deoxycholic acid has features of mono-oxo derivatives from congeneric group **II,** while mono-oxo derivatives of the congeneric group **I** possess properties of di-oxo derivatives of the second congeneric group (Table 1). In contrast, in reversed phase chromatography there is a good correlation between retention parameters and bile acids' hydrophobicity even if bile acids from both congeneric groups are analyzed.[10,22] However, correlation between parameters Θ and CMC inside certain congeneric groups indicates that parameter Θ can be used for deriving descriptor that describes bile acids' behaviour for the whole analyzed set (congeneric groups **I+II**). In order to extract information about structure of bile acids from some of the congeneric groups, vector of variable $|\Theta|$ (absolute value of the Θ) is centred $|\Theta|^c$. Reciprocal value of R_{M0} (parameter from reversed-phase TLC experiment)[10] for hyodeoxycholic acid (HD) is added to each value $|\Theta|^c$ of bile acids' of the first congeneric group, while reciprocal value of R_{M0} for cholic acid (C)[10] is added to $|\Theta|^c$ values of bile acids of the second congeneric group. Reciprocal values of R_{M0} are selected for HD and C because according to their R_{M0} values difference between cluster of congeneric group **I** and cluster of congeneric group **II** (in reversed phase TLC)[10] is the smallest (single linkage methods)[23]. In this way descriptor D is derived, describing difference between bile acids inside certain congeneric group (information from absolute values of Θ) and difference between congeneric groups as a unity (information from R_{M0}) (Table 2). According to linear regression between *D* values (bile acids from congeneric groups **I** and **II** at the same time) and CMC, correlation exists (Fig 8. **1**) in which cholic acid appears as outlier. After omitting

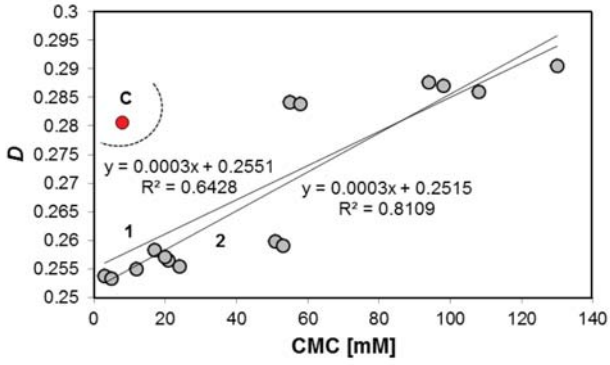


Fig. 8. Descriptor *D* as function of CMC if bile acids from both congeneric groups are analysed with **1** and without cholic acid **2**.

cholic acid, this correlation is improved (Fig 8. **2**). Similar influence of cholic acid is discovered during modeling of the partition coefficient (log*P*) of bile acids.[12] Such behaviour of cholic acid can be explained with cooperative effect of three α OH groups in hydrogen binding process.

Table 2. Values of the centered parameter Θ and descriptor *D*.

Bile acids	$\|\Theta\|^c$	*D*
DC	–0.0027	0.2537
3-DD	0.0019	0.2583
12-OL	0	0.2564
3,12-DD	0.0034	0.2598
CD	–0.0031	0.2533
3-DCD	0.0007	0.2571
7-OL	–0.001	0.2554
3,7-DCD	0.0026	0.259
HD	–0.0015	0.2549
C	–0.0051	0.2806
7-ODC	–0.0015	0.2842
12-OCD	–0.0018	0.2839
3,7-DOC	0.002	0.2877
3,12-DOC	0.0014	0.2871
7,12-DOC	0.0003	0.286
3,7,12-TOC	0.0048	0.2905

4. Conclusion

From the bile acids investigated by normal phase thin-layer chromatography, it can be concluded that linear function between the retention parameter R_M and temperature (293–323 K) exists. Parameters of linear function, Θ and $R_{M(T=273K)}$ adequately reflect structural differences of the bile acids' steroid skeleton and beside hydrogen bonding capacity, they can be connected to the hydrophobicity of the β side of the steroid skeleton within certain congeneric groups. If the whole group of studied molecules is analysed, descriptor can be derived that describes hydrophobic properties of bile acids but it is necessary to take into account differences between chromatographic parameter in reversed phase chromatography between clusters **I** and **II** of congeneric groups (actually, difference between any two bile acids from congeneric groups **I** and **II** is enough).

5. Acknowledgments

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Povzetek

V tem članku smo določili retencijski parameter R_M za žolčne kisline pri normalno-fazni tankoplastni kromatografiji kot funkcijo temperature (293 – 323 K). Analizirane žolčne kisline spadajo v istovrstno skupino z dvema kisikovima atomoma (OH ali okso skupine) na steroidnem jedru in v istovrstno skupino s tremi kisikovimi atomi. Za molekule iz obeh istovrstnih skupin smo ugotovili, da obstaja linearna zveza med R_M in temperaturo, tako da se R_M zmanjšuje z naraščajočo temperaturo. V posamezni istovrstni skupini je parametre linearne funkcije ($R_M - T$) možno razložiti s strukturnimi karakteristikami žolčnih kislin, predvsem s prostorsko orientacijo (prostorski položaj glede na srednjo ravnino steroida) steroidnega kisikovega atoma (OH ali okso skupine). Absolutne vrednosti naklona Θ linearne funkcije ($R_M - T$) naraščajo, če steroidni kisikovi atomi niso v polarni ravnini, saj se s tem zmanjša možnost tvorbe vodikovih vezi s stacionarno fazo. Poleg tega absolutna vrednost parametra Θ opisuje stopnjo tvorbe vodikovih vezi med žolčnimi kislinami in polarno stacionarno fazo v vsaki od istovrstnih skupin, kakor tudi hidrofobnost steroidnega ogrodja.