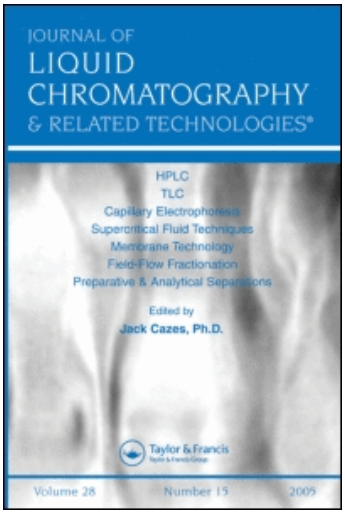

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PEAK IDENTIFICATION OF THE CONJUGATED BILE ACIDS IN REVERSED-PHASE LIQUID CHROMATOGRAPHY BY USING THE CONJUGATION SELECTIVITY

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ABSTRACT

It has been found for the first time that the selectivity between five glycine and taurine conjugated bile acids remains constant despite a considerable variation in retention values for each pair of the compounds in reversed-phase liquid chromatography (RPLC). The dependence of parameter S on $\log k'_w$ in retention equation $\log k' = \log k'_w - S\varphi$ results in parallel lines for glycine and taurine conjugates. The selectivity between glycine and taurine conjugates has been utilized for the identification of the conjugated bile acids in RPLC.

INTRODUCTION

Bile acids occur naturally in conjugation with glycine(G) or taurine(T). The determination of glycine and taurine conjugates in biological fluids is very important for studying bile acids metabolism in heptatobiliary and other diseases. Good resolution has been obtained by using reversed－phase liquid chromatography(RPLC) or ion－pair RPLC(IP－RPLC) (1－8), but there has been little studies on the retention behaviour of the conjugated bile acids in RPLC.

The aim of this work has been to develop a method for the identification of the conjugated bile acids based on the rule of the selectivity between glycine and taurine conjugates in RPLC.

It has been observed for the first time that the selectivity between five glycine and taurine conjugates is a constant despite the variation in retention time for each pair of the compounds.

The dependence of parameter S on log k'w in retention eguation log k' = log k'$_w$ －Sφ has been found to be parallel lines for glycine and taurine conjugates in RPLC. The conjugation selectivity has been shown to be a promising method for the identification of the conjugated bile acids in routine analysis.

EXPERIMENTAL

The experimental results utilized in this work were taken from papers by Scalia(2), Tietz et al. (3), Zhang (4), Zhou (5), Reid et al. (6) and Wildgrube et al. (7) which give an exact description of analytical conditions employed. The conjugated bile acid standards were taurocholic acid (TC), taurochenodeoxycholic acid (TCDC), taurodeoxycholic acid (TDC), tauroursodeoxycholic acid (TUDC), taurolithocholic acid (TLC), glycocholic acid (GC), glycochenodeoxycholic acid (GCDC), glycodeoxycholic acid (GDC), glycoursodeoxycholic acid (GUDC), glycolithocholic acid (GLC).

RESULTS AND DISCUSSION

In RPLC, the retention is usually adjusted by the concentration of mobile phase, approximately linear relationship has been widely used to describe the effect of mobile phase concentration on retention as shown as follows(9 −11):

$$\log k' = \log k'_w - S\varphi \quad (1)$$

Where k' is the capacity factor, φ is the volume fraction of organic modifier in binary mobile phase. Parameter S reflects the difference in solvation interaction between solute−weak solvent and solute−strong solvent and is constant for a specific solute even when column system with different C_{18} packings are used(11). log k'$_w$ refers to the logarithm of extrapolated value of k' in pure water. log k'$_w$ mainly describes the difference in hydrophobic interaction between solute−water and solute−bonded phases.

For structural related compounds, a linear relationship between parameter S and log k'$_w$ has been strictly observed(10−11), as shown as follows:

$$S = L_1 * \log k'_w + L_2 \quad (2)$$

Where L_1, L_2 are constants.

The structure of glycine and taurine conjugates lies in the difference in the glycine and taurine groups.

The dependence of parameter S on log k'$_w$ results in parallel lines for glycine and taurine conjugates in RPLC, as is demonstrated by Figure 1.

Therefore we can obtain:

For glycine conjugate:

$$S^G = L_1(\log k'_w)^G + L'_2 \quad (3)$$

For taurine conjugates:

$$S^T = L_1(\log k'_w)^T + L''_2 \quad (4)$$

Where S^G, S^T are S−values for glycine and taurine conjugates respectively, $(\log k'_w)^G$ and $(\log k'_w)^T$ are log k'$_w$−values for glycine and taurine conjugates respectively. L'_2 and L''_2 are constants.

The conjugation selectivity, $\alpha_{G/T}$, is defined as the ratio of capacity factor of

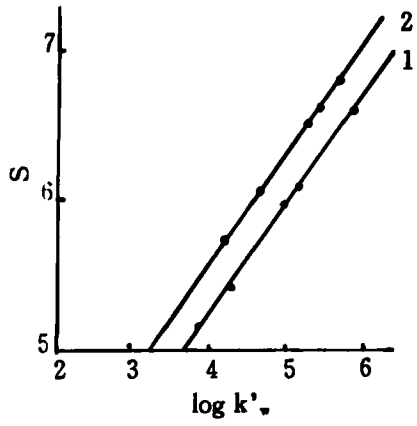

Figure 1.
Dependence of parameter S on log k'_w for glycine and taurine conjugates in methanol — water eluent. Parameters S and log k'_w were recalculated from ref. 5,
1: glycine conjugates;
2: taurine conjugates.

glycine conjugate, k'(G), to that of taurine conjugate, k'(T), as is shown in the following equation:

$$\alpha_{G/T} = k'(G)/k'(T) \tag{5}$$

Therefore we obtain:

$$\log \alpha_{G/T} = (1 - L_1\varphi)\triangle \log k'_w - (L'_2 - L'_1)\varphi \tag{6}$$

As the difference in parameter log k'_w between glycine and taurine conjugates ($\triangle$log k'_w) approaches a constant, therefore the conjugation selectivity between five glycine and taurine conjugates remains a constant despite a considerable variation in retention values for each pair of the compounds. Tables 1—2 summarized the retention behaviour and the selectivity factors between glycine and taurine conjugates on different RP packing materials. There is no serious change in the conjugation selectivity for five glycine and taurine conjugate pairs with a change of the retention values. These data basically agree with our theoretical assumption.

The conjugation selectivity can be widely used in the practical identification of the conjugated bile acids in RPLC. Based on the retention of taurine bile acids, the peaks of glycine conjugates can be identified by using the conjugation selectivity. The examples of using the conjugation selectivity for peak

Table 1

Peak identification results of the conjugated bile acids based on the retention of taurine bile acids by using conjugation selectivity on different packing materials in RPLC

Date are recalculated from ref. 2

Bile acid	1[a]			2[b]			3[c]		
	k' exp.	α	k' pre.	k' exp.	α	k' pre.	k' exp.	α	k' pre.
GUDC	3.15	1.30	3.15	2.00	1.43	2.00	2.20	1.55	2.20
TUDC	2.42			1.40			1.42		
GC	5.00	1.30	4.99	3.93	1.44	3.89	2.94	1.50	3.04
TC	3.84			2.72			1.96		
GCDC	10.20	1.30	10.20	8.11	1.45	7.98	6.23	1.57	6.15
TCDC	7.86			5.58			3.97		
GDC	11.78	1.32	11.65	9.68	1.47	9.40	7.13	1.59	6.95
TDC	8.96			6.57			4.49		
GLC	23.18	1.31	22.98	18.67	1.47	18.15	15.50	1.63	14.71
TLC	17.68			12.69			9.49		

a: μ—Bondapak C_{18}, methanol: 0.02M phosphate buffer pH=4.2(60:30).

b: Supelcosil LC 18—DB, methanol: acetate buffer pH=4.2(60:30).

c: Lichrospher CH8, eluent is the same as b.

Table 2

The results of peak identification of glycine bile acids by using conjugation selectivity based on the retention behaviour of taurine conjugates in RPLC

Bile acid	1[a]			2[b]		
	k' exp.	α	k' pre.	k' exp.	α	k' pre.
GUDC	3.06	1.46	3.06	2.02	1.62	2.02
TUDC	2.10			1.25		
GC	4.98	1.45	5.02	3.21	1.59	3.27
TC	3.44			2.02		
GCDC	10.80	1.47	10.70	6.13	1.63	6.09
TCDC	7.33			3.76		
GDC	12.68	1.49	12.47	7.07	1.66	6.89
TDC	8.54			4.25		
GLC	26.03	1.47	25.78	13.04	1.67	12.67
TLC	17.66			7.82		

a: μ-Bondapak C_{18}, methanol: 0.1 M KH_2PO_4 pH = 4.6 (60:40), unretained time was measured on the chromatogram, data are recalculated from ref. 3.

b: μ-Bondapak C_{18}, acetonitrile: methanol: 0.03 M phosphate buffer pH = 3.40 (10:60:30) (V/V/V), data are recalculated from ref. 8.

Table 3

The comparison of experimental k' and predicted ones by using the conjugation selectivity based on the retention behaviour of taurine conjugates in RPLC and IP−RPLC

Bile acid	1[a]			2[b]		
	k' exp.	α	k' pre.	k' exp.	α	k' pre.
TUDC	1.28					
GUDC	1.80	1.41	1.80			
TC	2.06			0.53	1.40	
GC	2.84	1.38	2.90	0.38		0.38
TCDC	3.86			1.30	1.40	
GCDC	5.26	1.36	5.44	0.93		0.94
TDC	4.60			1.62	1.39	
GDC	6.36	1.38	6.49	1.17		1.16
TLC	8.20			4.52	1.42	
GLC	11.60	1.41	11.56	3.18		3.23

a: Radial−Pak C_{18}, methanol: water (75:25) containing 2.5% (V/V) acetic acid and adjusted to pH = 5.25 with 10 M sodium hydroxide, 2.0 ml/min, retention time was measured on chromatogram, from ref. 6.

b: Ultrasphere IP−C_{18}, 50 ml acetonitrile in 45 ml water and 0.5M tetrabutylammonium phosphate, data are recalculated from ref. 7.

identification of glycine conjugates based on retention behaviour of taurine conjugates can be seen in Tables 1—2.

The conjugation selectivity can also be applied to the ion pair reversed phase LC (IP—RPLC) system which is summarized in Table 3, but the introduction of ion pair reagent to the binary mobile phase leads to the reversal of elution order for glycine and taurine conjugated bile acids, it is mainly due to the fact that sulphonic acid is much stronger acid than carboxylic acid. Therefore different retention mechanism in RPLC and IP—RPLC has been observed.

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