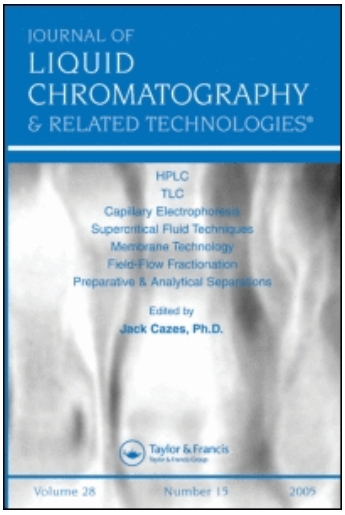

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Separation of Selected Bile Acids by TLC. VII. Separation by Reversed Partition HPTLC

A. Pyka[a]; M. Dołowy[a]
[a] Department of Analytical Chemistry, Faculty of Pharmacy, Silesian Academy of Medicine, Sosnowiec, Poland

To cite this Article Pyka, A. and Dołowy, M.(2005) 'Separation of Selected Bile Acids by TLC. VII. Separation by Reversed Partition HPTLC', Journal of Liquid Chromatography & Related Technologies, 28: 10, 1573 — 1581
To link to this Article: DOI: 10.1081/JLC-200058365
URL: http://dx.doi.org/10.1081/JLC-200058365

Journal of Liquid Chromatography & Related Technologies®, 28: 1573–1581, 2005

ISSN 1082-6076 print/1520-572X online
DOI: 10.1081/JLC-200058365

Separation of Selected Bile Acids by TLC. VII. Separation by Reversed Partition HPTLC

A. Pyka and M. Dołowy
Department of Analytical Chemistry, Faculty of Pharmacy, Silesian Academy of Medicine, Sosnowiec, Poland

Abstract: Selected bile acids: cholic acid (C), glycocholic acid (GC), glycodeoxycholic acid (GDC), chenodeoxycholic acid (CDC), deoxycholic acid (DC), lithocholic acid (LC), and glycolithocholic acid (GLC) were investigated with the use of reversed phase high performance thin-layer chromatography on RP18W (E. Merck, #1.14296), RP18 (E. Merck, #1.05914), RP2 (E. Merck, #1.13726), and CNF_{254} (E. Merck, #1.12571) plates using methanol–water, organic mixture (acetonitrile–methanol 50:50, v/v)–water, acetone–water, dioxane–water, and acetonitrile–phosphate buffer (pH 4.60) as mobile phases, in different volume compositions.

The obtained separations were carried out on the basis of separation factors values ΔR_F and R_S. None of the applied chromatographic conditions enabled completion of the separation of all examined bile acids. Five neighboring bile acids, i.e., LC/DC, CDC/GLC, GLC/C, C/GDC, GDC/GC, were separated only when CNF_{254} plates and the mobile phase acetone–water, 50:50; v/v were used. The biggest problem was to separate DC from CDC. These bile acids were separated only on RP2 plates by using methanol–water, 65:35, v/v as a mobile phase.

Keywords: Bile acids, RP-HPTLC, RP18W, RP18, RP2, CNF_{254}, Separation parameters

INTRODUCTION

TLC has been widely used as a method of bile acids separation because it is easy to operate, inexpensive, and it can be performed directly on biological fluids without prior sample purification.[1] One of the problems caused by applying

Address correspondence to A. Pyka, Department of Analytical Chemistry, Faculty of Pharmacy, Silesian Academy of Medicine, Jagiellońska 4, 41-200 Sosnowiec, Poland. E-mail: alinapyka@wp.pl

TLC to bile acids separations is the choice of their optimal separation conditions, i.e., selecting a proper adsorbent, composition of a mobile phase, temperature of developing chromatograms, and pH of eluent or adding modifiers to both the mobile phase and the standard mixture of separated bile acids.

In TLC, silica gel 60[2–4] or octadecyl–silica (RP18)[5–8] are used as stationary phases for bile acids separation. Besides RP18, cyano and polyacrylonitrile plates are effective for investigating selected physicochemical properties, including the lipophilicity of bile acids, when cholic acid and its derivatives are used as samples.[1] The proper choice of a mobile phase is essential to obtain the most efficient separation of bile acids. In partition TLC, the most frequently applied mobile phases are: methanol–water and methanol-water-acetic acid.[1]

The separation of chenodeoxycholic acid (CDC) and deoxycholic acid (DC), and their taurine and glycine conjugates, is one of the problems encountered during bile acids separation by TLC.[1]

Both, pH of eluent and the temperature of chromatograms development also influence the bile acids separation by TLC. The influence of temperature on efficiency of bile acids separations with the use of adsorption TLC, has been rarely described in literature.[9,10] Zarzycki et al. obtained the separation of DC from CDC at 50°C.

In our previous papers,[11–16] we presented the data which allowed estimating the usefulness of adsorption TLC for bile acids separation. There we examined the following mobile phases: n-heptane-ethyl acetate acetic acid and n-hexane-ethyl acetate-acetic acid in different volume compositions, which were used for bile acids separation, i.e., C, GC, GLC, DC, CDC, GDC, and LC, at 18°C and 40°C, on various plates precoated with silica gel, mixture of silica gel and Kieselguhr, and also on silica gel modified by CN and Diol groups. The estimation of separation was carried out on the basis of the separation factors values: ΔR_F and R_S. When $\Delta R_F \geq 0.05$ and $R_S > 1$ were obtained for each pair of neighboring bile acids, the used mobile phases were considered the most useful. We also described the chromatographic conditions of the two-dimensional technique for bile acids separation.[12] The similarity analysis was applied to compare the separations of studied bile acids.[14]

The aim of this work was to apply RP-HPTLC to study the separation of a/m bile acids.

EXPERIMENTAL

Chemicals

The following components of a mobile phase: methanol (Merck, Germany; pure p. a), acetonitrile (Merck, Germany pure p. a), acetone (POCh, Gliwice, Poland; pure p. a), dioxane (POCh, Gliwice, Poland; pure p. a), ethanol (POCh, Gliwice, Poland; pure p. a), and distilled water (Department of

Analytical Chemistry, Faculty of Pharmacy, Sosnowiec, Poland) were used for TLC analysis. The commercial samples of C, DC, CDC, LC, GLC, GDC, and GC (St. Louis, Sigma Company, USA) were used as test solutes. Methanol (POCh, Gliwice, Poland; pure p. a.) was used for the preparation of bile acids solutions. Sulfuric acid 95% (Chempur, Piekary Śląskie, Poland) and phosphomolibdic acid (POCh, Gliwice, Poland) were used to prepare a visualizing reagent.

Sample Preparation

The methanolic solutions of the above mentioned bile acids in concentration 50 mg/10 mL of each acid were prepared.

Reversed-Phase High Performance Thin-Layer Chromatography

Thin-layer chromatography was done on RP-HPTLC RP18W, (E. Merck, #1.14296), RP2 (E. Merck, #1.13726), CNF_{254} (E. Merck, #1.12571), and RP18 (E. Merck, #1.05914) glass plates. Solutions of examined bile acids were spotted on chromatographic plates in quantities of 5 μg of each bile acid in 1 μL of methanol. The chromatograms were developed by using the mixture of organic modifier (methanol, dioxane, acetonitrile, acetone)–water in the following volume compositions: methanol–water, the content of acetone in mobile phase was gradually varied by 5% [%, v/v] from 35–80 [%, v/v]; organic mixture (methanol–acetonitrile, 50:50, v/v)-water, the content of organic mixture in mobile phase was gradually varied by 5% [%, v/v] from 30–100 [%, v/v]; acetone–water, the content of acetone in mobile phase was gradually varied by 5% [%, v/v] from 35–80 [%, v/v]; dioxane–water, the content of acetone in mobile phase was gradually varied by 5% [%, v/v] from 30–90 [%, v/v]; the mixture of acetonitrile–phosphate buffer (pH 4.60) with acetonitrile content 30–80 [%, v/v] was used as a mobile phase.

A mobile phase (50 mL) was placed into a classical chamber. The chamber was saturated with solvent for 20 minutes. The development distance was 8.5 cm. After development and drying the plates, the spots were visualized by spraying the plates with a 10% water solution of H_2SO_4, or by dipping them in a 10% ethanol solution of phosphomolibdic acid and then heating for 20 minutes at 120°C. The chromatograms were run in triplicate.

The values of R_F, ΔR_F, and R_S for studied bile acids were calculated according to the formulas which were presented in our previous papers.[11–16]

RESULTS AND DISCUSSION

The studied bile acids were separated on the following chromatographic plates: RP18W (#1.14296), RP2 (#1.13726), CNF_{254} (#1.12571), using

methanol–water, dioxane–water, acetone–water, organic mixture (methanol-acetonitrile; 50:50)–water as mobile phases, as well as on RP18 W (#1.14296), and RP18 (#1.05914) using acetonitrile–phosphate buffer (V) in different volume compositions as a mobile phase. The total number of 161 combinations, which included the changes of mobile and stationary phases, were examined. The organic mixture–water was chosen as a mobile phase, because on the a/m plates the R_F values of examined bile acids were relatively low values, i.e., from 0.05 to 0.20, when pure acetonitrile was used as a mobile phase. The dependence between R_F values and the respective component of a mobile phase showed that the lines which represented C and GDC, as well as DC, CDC, and GLC ran near each other. Thus, it can be concluded that C and GDC, as well as DC, CDC, and GLC separate poorly under the applied chromatographic conditions.

The values of separation factors: ΔR_F and R_S for the bile acids separated on RP18W, RP18, RP2, and CNF_{254} plates, developed using selected mobile phases in different volume compositions (optimal conditions for separation), are listed in Tables 1–3, respectively. None of the applied chromatographic conditions facilitated the separation of all neighboring pairs of bile acids. The complete separation ($\Delta R_F \geq 0.05$ and $R_S > 1$) was obtained for four or five pairs of the examined acids, i.e., LC/DC, DC/GLC, C/GDC, and GDC/GC on RP18W plates, using organic mixture–water 60:40 (v/v) as a mobile phase; LC/GLC, GLC/DC, CDC/C, GDC/GC on RP18W plates, using acetonitrile–phosphate buffer (V), 50:50 (v/v) as a mobile phase; LC/DC, CDC/GLC, GLC/C, GDC/GC on RP18 plates, using acetonitrile–phosphate buffer (V), 80:20 (v/v) as a mobile phase, LC/DC, CDC/C, C/GDC, GDC/GC on RP2 plates, using methanol–water, 70:30 (v/v) as a mobile phase; LC/CDC, DC/GLC, GLC/GDC, and C/GC on RP2 plates, using organic mixture–water, 65:35 (v/v) as a mobile phase; LC/CDC, GLC/C, C/GDC, GDC/GLC on RP2 plates, using dioxane–water, 65:35 (v/v) as a mobile phase; LC/DC, CDC/GLC, GLC/C, and GDC/GC on CNF_{254} plates, using methanol–water, 60:40 (v/v) as a mobile phase; LC/DC, CDC/GLC, C/GDC, GDC/GC on CNF_{254} plates, using methanol–water, 55:45 (v/v) organic mixture–water, 40:60 (v/v) and acetone–water, 55:45 (v/v) as mobile phases; LC/DC, GLC/C, C/GDC, and GDC/GC on CNF_{254} plates, using organic mixture–water, 50:50 (v/v) as a mobile phase; LC/DC, CDC/GLC, GLC/C, C/GDC, and GDC/GC on CNF_{254} plates, using acetone–water, 50:50 (v/v) as a mobile phase. Thus, the selection of experimental conditions ought to depend on the type of bile acids which are separated.

The presented comparisons lead to the conclusion that the best separation of examined bile acids was obtained on CNF_{254} plates and with the use of acetone–water in volume composition 50:50 (v/v) as a mobile phase. Five of six studied neighboring pairs of bile acids can be separated using the a/m chromatographic conditions. Figure 1 presents the model comparison of R_S values for the studied bile acids separated on CNF_{254} plates, using

Table 1. The values[a] of separation factors ΔR_F and R_S of examined bile acids separated on RP18 and R18W plates by using selected mobile phases in different volume compositions

Pair of acids	Mobile phase (v/v) Organic mixture–water (60:40)	
	ΔR_F	R_S
R18W		
LC/DC	0.06	1.25
DC/CDC	0.01	0.12
CDC/GLC	0.06	1.25
GLC/C	0.04	0.75
C/GDC	0.05	1.12
GDC/GC	0.14	2.88
	Acetonitrile–phosphate buffer (V) (50:50)	
	ΔR_F	R_S
LC/GLC	0.06	2.00
GLC/DC	0.04	1.40
DC/CDC	0.01	0.36
CDC/C	0.10	3.00
C/GDC	0.00	0.00
GDC/GC	0.08	2.89
	Acetonitrile–phosphate buffer (V) (80:20)	
	ΔR_F	R_S
RP18		
LC/DC	0.20	2.67
DC/CDC	0.01	0.15
CDC/GLC	0.09	1.09
GLC/C	0.10	1.27
C/GDC	0.02	0.26
GDC/GC	0.09	1.53

[a]In table are presented the data only for optimal separations for studied bile acids.

acetone–water in volume composition 55:45 and 50:50 as a mobile phase. The biggest problem was to separate DC from CDC. These bile acids were separated only on RP2 plates ($\Delta R_{F(DC/CDC)} = 0.07$, $R_{S(DC/CDC)} = 1.26$), which were developed using methanol–water, 65:35 (v/v) as a mobile

Table 2. The values[a] of separation factors ΔR_P and R_S of examined bile acids separated on RP2 plates by using selected mobile phases in different volume compositions

	Mobile phase (v/v) Methanol–water (70:30)	
Pair of acids	ΔR_F	R_S
LC/DC	0.14	2.29
DC/GLC	0.01	0.14
GLC/CDC	0.02	0.29
CDC/C	0.10	1.60
C/GDC	0.07	1.20
GDC/GC	0.07	1.20
	Methanol–water (65:35)	
	ΔR_F	R_S
LC/DC	0.04	0.60
DC/CDC	0.07	1.26
CDC/GLC	0.09	1.45
GLC/GDC	0.03	0.36
GDC/C	0.01	0.08
C/GC	0.15	2.08
	Organic mixture–water (65:35)	
	ΔR_F	R_S
LC/CDC	0.09	1.60
CDC/DC	0.02	0.36
DC/GLC	0.06	1.11
GLC/GDC	0.08	1.47
GDC/C	0.01	0.10
C/GC	0.15	2.08
	Dioxane–water (65:35)	
	ΔR_F	R_S
LC/CDC	0.11	2.53
CDC/DC	0.04	0.86
DC/GLC	0.01	0.25
GLC/C	0.08	1.62
C/GDC	0.06	1.83
GDC/GLC	0.06	1.54

[a]In the table are presented the data only for optimal separations for studied bile acids.

Table 3. The values[a] of separation factors ΔR_F and R_S of examined bile acids separated on CNF_{254} plates by using selected mobile phases in different volume compositions

	Mobile phase (v/v)											
	Methanol–water				Organic mixture–water				Acetone–water			
	60:40		55:45		50:50		40:60		55:45		50:50	
Pair of acids	ΔR_F	R_S	ΔR_F	R_S	ΔR_F	R_S	ΔR_F	R_S	ΔR_F	R_S	ΔR_F	R_S
LC/DC	0.15	2.78	0.12	2.62	0.13	2.44	0.09	2.00	0.14	2.53	0.10	1.38
DC/CDC	0.00	0.00	0.02	0.29	0.02	0.29	0.03	0.59	0.05	0.73	0.04	0.46
CDC/GLC	0.10	2.25	0.10	1.70	0.05	0.80	0.10	2.13	0.08	1.40	0.08	1.56
GLC/C	0.06	1.11	0.05	0.76	0.07	1.60	0.01	0.22	0.02	0.36	0.08	1.04
C/GDC	0.04	0.63	0.06	1.05	0.10	1.68	0.13	2.00	0.11	1.80	0.12	1.38
GDC/GC	0.14	2.53	0.15	2.78	0.11	2.11	0.15	2.89	0.09	1.88	0.09	1.23

[a]In table are presented the data only for optimal separations for studied bile acids.

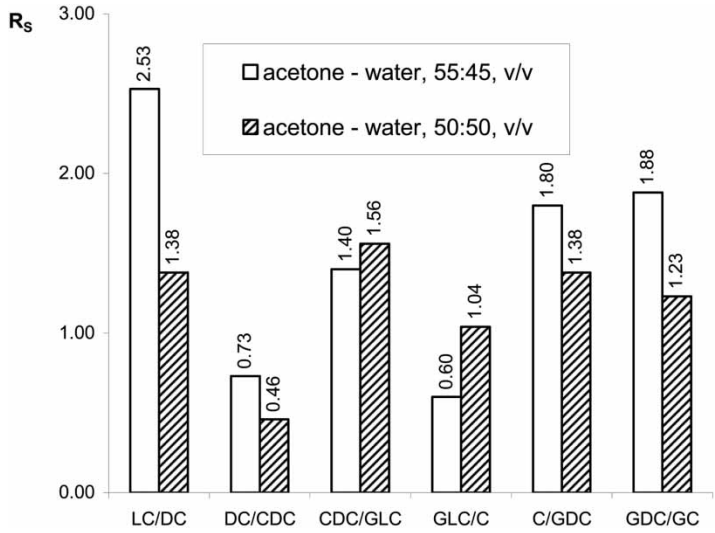

Figure 1. Comparison of R_S values for neighboring pairs of studied bile acids separated on CNF_{254} (#1.12571) plates by using mobile phase acetone-water in the following volume compositions: 55:45 and 50:50.

phase. However, under these conditions only two pairs of bile acids, i.e., CDC/GLC and C/GC, separate.

CONCLUSION

One hundred sixty one combinations, which included the changes of mobile and stationary phases, were examined using RP-HPTLC. The obtained results indicate that the applied technique, i.e., RP-HPTLC, did not facilitate the separation of all pairs of neighboring bile acids under the same chromatographic conditions. Five of the neighboring pairs of bile acids, i.e., LC/DC, CDC/GLC, GLC/C, C/GDC, and GDC/GC, can be separated only with the use of CNF_{254} plates and acetone–water, 50:50 (v/v) as a mobile phase. However, it was a problem to separate DC from CDC. These bile acids were separated only on RP2 plates developed using methanol–water, 65:35 (v/v) as a mobile phase.

REFERENCES

1. Scalia, S. Bile acid separation. J. Chromatogr. B. **1995**, *671*, 299–317.
2. Huang, C.T.L.; Nichols, B.L. New solvent systems for the separation of free and conjugated bile acids. III. Separation of free bile acids as a group. J. Chromatogr. **1975**, *109*, 427–431.

3. Ikawa, S.; Goto, M. Two-dimensional thin-layer chromatography of bile acids. J. Chromatogr. **1975**, *114*, 237–243.
4. Batta, A.K.; Salen, G.; Shefer, S. Thin-layer chromatography of conjugated bile acids. J. Chromatogr. **1979**, *168*, 557–561.
5. Touchstone, J.C.; Levitt, R.; Soloway, R.D.; Lewin, S.S. Separation of conjugated dihydroxy bile acids by thin-layer chromatography. J. Chromatogr. **1979**, *178*, 566–570.
6. Lepri, L.; Heilmer, D.; Desideri, P.G. Reversed-phase high performance thin-layer chromatography of free and conjugated bile acids. J. Chromatogr. **1984**, *88*, 461–468.
7. Iida, T.; Momose, T.; Shinohara, T.; Goto, J.; Nambara, T.; Chang, F.C. Separation of all bile acids stereoisomers by thin-layer and high performance liquid chromatography. J. Chromatogr. **1986**, *366*, 396–402.
8. Gaica, S.B.; Opsenica, D.M.; Šolaja; Tešić, Lj.Ž.; Milkovic-Opsenica, D.M. The retention behavior of some cholic acid derivatives and different adsorbents. J. Planar Chromatogr. **2002**, *15*, 299–305.
9. Yarabe, H.H.; Shamsi, S.A.; Warner, I.M. Capillary zone electrophoresis of bile acids indirect photometric detection. Anal. Chem. **1998**, *70*, 1412–1418.
10. Zarzycki, P.K.; Wierzbowska, M.; Lamparczyk, H. Retention and separation studies of cholesterol and bile acids using thermostated thin-layer chromatography. J. Chromatogr A. **1999**, *857*, 255–262.
11. Pyka, A.; Dołowy, M. Separation of selected bile acids by TLC. I. J. Liq. Chromatogr. & Rel. Technol. **2003**, *26* (7), 1095–1108.
12. Pyka, A.; Dołowy, M. Separation of selected bile acids by TLC II. One-dimensional and two-dimensional TLC. J. Liq. Chromatogr. & Rel. Technol. **2004**, *27* (13), 2031–2038.
13. Pyka, A.; Dołowy, M. Separation of selected bile acids by TLC III. Separation on various stationary phases. J. Liq. Chromatogr. & Rel. Technol. **2004**, *27* (16), 2613–2623.
14. Pyka, A.; Dołowy, M. Separation of selected bile acids by TLC IV. Comparison of separation of studied bile acids by the use of cluster analysis. J. Liq. Chromatogr. & Rel. Technol. **2004**, *27* (9), 2987–2995.
15. Pyka, A.; Dołowy, M.; Gurak, D. Separation of selected bile acids by TLC V. Influence of temperature on the separation. J. Liq. Chromatogr. & Rel. Technol. **2005**, *28* (4), 631–640.
16. Pyka, A.; Dołowy, M. Separation of selected bile acids by TLC. VI. Separation on cyano-and diol-modified silica layers. J. Liq. Chromatogr. & Rel. Technol. **2005**, *28* (9), 1383–1392.

Received December 30, 2004
Accepted February 2, 2005
Manuscript 6562