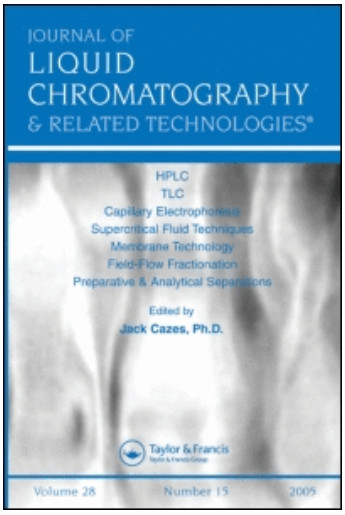

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Separation of Selected Bile Acids by TLC. IX. Separation on Silica Gel 60 and on Silica Gel 60F_{254} Aluminum Plates Impregnated with Cu(II), Ni(II), Fe(II), and Mn(II) Cations

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Abstract: Seven 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 separated using adsorption TLC on aluminum plates precoated with silica gel 60 and on aluminum plates precoated with silica gel 60F_{254}. The plates were impregnated with 1%, 2.5%, and 5% aqueous solutions of the following salts: $CuSO_4$, $MnSO_4$, $NiSO_4$, and $FeSO_4$. The mixtures of *n*–hexane–ethyl–acetate–acetic acid in the volume compositions: 22:20:5 and 25:20:2 (v/v/v) for both aluminum plates, 22:22:5 (v/v/v) only for #1.05554 plates and 25:20:5 (v/v/v) for #1.05553 plates were used as mobile phases. These mobile phases were not effective for the separation of bile acids on non impregnated silica gel 60 and silica 60F_{254} aluminum plates at 18°C. The plates impregnated with the salts whose application resulted in $\Delta R_F \geq 0.05$ and $R_S > 1$ for all neighboring pairs of examined bile acids were considered the most effective for bile acids separation. It was observed that impregnation of silica gel 60 and silica 60F_{254} aluminum plates with aqueous solutions of $CuSO_4$, $MnSO_4$, $NiSO_4$, and $FeSO_4$ improved the separation of GC/GDC and C/GLC, which separated poorly on aluminum plates precoated with non impregnated silica gel 60 and 60F_{254}. Moreover, it was stated that the use of a mobile phase in volume composition 25:20:2 (v/v/v) and aluminum plates precoated with silica gel 60F_{254} impregnated with a 5% aqueous solution of $CuSO_4$ made the separation of all neighboring pairs

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of investigated bile acids possible, in comparison with their separation on non impregnated aluminum plates.

Keywords: Bile acids, Adsorption TLC, Impregnation with metal cations, Silica gel 60 aluminum plates, Silica gel $60F_{254}$ aluminum plates

INTRODUCTION

Silica gel is the most important adsorbent used for TLC separations. Because of its attractive adsorptive properties, such as: large active surface, large pore volume, and possibilities of regeneration, silica gel is widely used as stationary phase for TLC.[1] Silica gel was used for identification and separation of different steroids, especially bile acids presented in biological samples. Modified silica gel (using impregnation with silver nitrate (V) and paraffin) was useful for identification and separation of difficult-to-separate bile acids.[2]

Our previous investigations of bile acids focused on determining the influence of non impregnated and impregnated TLC plates precoated with silica gel 60 and silica gel $60F_{254}$ and mobile phases, as well as chromatogram development temperature, on separation of bile acids with the use of adsorption TLC.[3–9] These investigations indicated that, on the non-impregnated aluminum plates precoated with silica gel 60 and silica gel $60F_{254}$ at 18°C (#1.05553 and #1.05554), the biggest problem was to separate GC from GDC and C from GLC.[5] The optimal separation of examined bile acids at 18°C on aluminum plates precoated with silica gel 60 (#1.05553) was obtained using *n*–hexane–ethyl acetate–acetic acid in volume compositions: 22:21:5, 22:22:5, 25:20:8, and 20:20:5 (v/v/v).[5] In the case of aluminum plates precoated with silica gel $60F_{254}$ (#1.05554), the optimal separation of all studied bile acids at 18°C was observed by using a mobile phase in volume compositions: 22:21:5 and 25:20:8 (v/v/v).[5] Moreover, it was stated that application of $DiolF_{254}$ plates facilitated the separation of examined bile acids.[6] It was proved that the temperature of 40°C improves the separation of GC from GDC performed on aluminum silica gel plates (#1.05553 and #1.05554).[7] The complete separation of all studied bile acids was obtained on aluminum plates precoated with silica gel 60 (#1.05553) with the use of a 2D technique.[8] It was stated that impregnation of silica gel $60F_{254}$ glass plates (#1.05715) with aqueous solutions of $CuSO_4$, $MnSO_4$, $NiSO_4$, and $FeSO_4$ facilitated the separation of GC from GDC and also C from GLC, in comparison with separation on non-impregnated silica gel $60F_{254}$ glass plates at 18°C.[9]

In this work, the impregnation of TLC aluminum plates precoated with silica gel 60 (#1.05553) and silica gel $60F_{254}$ (#1.05554) with 1%, 2.5%, and 5% aqueous solutions of $CuSO_4$, $MnSO_4$, $NiSO_4$, and $FeSO_4$ was applied. *n*–Hexane–ethyl acetate–acetic acid was used as a mobile phase

only in the volume compositions which were not optimal for the separation of bile acids on non impregnated silica gel at 18°C. It was observed that impregnation of TLC plates (#1.05553 and #1.05554) with a/m salts improves the separation of GDC from GC and C from GLC in comparison to their separation on non impregnated plates.

EXPERIMENTAL

Chemicals

The following components of mobile phase *n*-hexane (Merck, Germany), ethyl acetate (POCh, Gliwice, Poland), acetic acid 99.5% (POCh, Gliwice, Poland), and distilled water (Department of Analytical Chemistry, Faculty of Pharmacy, Sosnowiec, Poland) 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. Methanol (POCh, Gliwice, Poland; pure p. a.) was used for the preparation of bile acid solutions. Sulfuric acid, 95% (Chempur, Piekary Śląskie, Poland) was used to prepare a visualizing reagent. The salts of appropriate metals, i.e., $MnSO_4 \cdot 7\ H_2O$, $FeSO_4 \cdot 7\ H_2O$, $CuSO_4 \cdot 5\ H_2O$, and $NiSO_4 \cdot 7\ H_2O$ (POCh, Gliwice, Poland; pure p. a.) were used to prepare impregnation solutions.

Sample Preparation

The methanolic solutions of above-mentioned bile acids, at a concentration 50 mg/10 mL of each acid were prepared.

Impregnation of Applied Stationary Phase with Cu(II), Ni(II), Fe(II), and Mn(II) Cations

Aluminum plates precoated with silica gel 60 (#1.05553) and silica gel 60 F_{254} (#1.05554) were dipped for 30 sec. in aqueous solutions of the following salts: $CuSO_4$, $MnSO_4$, $NiSO_4$ and $FeSO_4$ in concentrations: 1%, 2.5%, and 5%. After impregnation, the plates were dried at room temperature (18°C) for 24 h. The modified plates were applied to separate the examined bile acids using adsorption thin layer chromatography.

Thin Layer Chromatography

Adsorption TLC was performed on 20 × 20 cm aluminum plates precoated with silica gel 60 and silica gel $60F_{254}$ (E. Merck, #1.05553 and

Table 1. The R_F values and separation factors (ΔR_F and R_S) of studied bile acids separated on aluminum plates precoated with silica gel $60F_{254}$ (#1.05554) with *n*–hexane–ethyl acetate–acetic acid as a mobile phase in different volume compositions (v/v/v), which were not optimal for bile acids separation on non impregnated silica gel $60F_{254}$ at 18°C[4,5]

Pair of acids	*n*–Hexane–ethyl acetate–acetic acid (v/v/v)								
	22:20:5			22:22:5			25:20:2		
	R_F	ΔR_F	R_S	R_F	ΔR_F	R_S	R_F	ΔR_F	R_S
GC/GDC	0.01/0.05	0.04	1.00	0.01/0.05	0.04	1.00	0.01/0.01	0.00	0.00
GDC/C	0.05/0.15	0.10	2.24	0.05/0.14	0.09	1.85	0.01/0.06	0.05	1.20
C/GLC	0.15/0.23	0.07	1.55	0.14/0.22	0.08	1.85	0.06/0.09	0.03	0.76
GLC/CDC	0.23/0.49	0.26	4.93	0.22/0.48	0.26	5.07	0.09/0.26	0.17	4.00
CDC/DC	0.49/0.56	0.07	1.33	0.48/0.54	0.06	1.06	0.26/0.32	0.06	1.07
DC/LC	0.56/0.95	0.39	8.62	0.54/0.89	0.35	6.47	0.32/0.78	0.46	11.73

#1.05554) and impregnated with the respective cations: Cu(II), Ni(II), Fe(II), and Mn(II). Before use, the plates were activated at 120°C for 30 min. Micropipettes (5 μL, Camag, Switzerland) were used to apply the standard solutions onto the plates. Solutions of the standard acids were spotted onto a chromatographic plate in quantities 15 μg of each standard acid in 3 μL methanol. The chromatograms were developed at room temperature in a 20 cm × 20 cm classical chamber (Camag, Switzerland) using *n*-hexane-ethyl acetate-acetic acid in the following volume compositions: 22:20:5, 22:22:5, 25:20:2, and 25:20:5 as mobile phases. Fifty mL of mobile phases were used in all cases. 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 10% solution of sulfuric acid in water as visualizing reagent. The spots were developed by heating the sprayed plates at 120°C for 20 min. The values of ΔR_F and R_S for the studied bile acids were calculated according to the formulae which were presented in our previous papers.[3,5] The calculated parameters are the mean values of five analyses.

Table 2. Description of separation effects for the respective pairs of studied bile acids on aluminum plates precoated with silica gel $60F_{254}$ (#1.05554) impregnated with aqueous solutions of inorganic salts developed by using *n*-hexane–ethyl acetate–acetic acid as a mobile phase at 18°C

The volume composition of mobile phase (v/v/v)	Pair of acids					
	GC/GDC	GDC/C	C/GLC	GLC/CDC	CDC/DC	DC/LC
25:20:2	5% $CuSO_4$[a]	1% $MnSO_4$	W[b]	W	1% $CuSO_4$ 2.5% $NiSO_4$	W
22:22:5	W except of 1% $NiSO_4$	W except of 2.5% $NiSO_4$	W	W except of 5% $CuSO_4$	5% $CuSO_4$ 1% $MnSO_4$ 2.5% $MnSO_4$ 1% $NiSO_4$ 2.5% $NiSO_4$ 1% $FeSO_4$	W
22:20:5	W except of 1% $MnSO_4$	W	W	W	W except of 1% $MnSO_4$ 2.5% $FeSO_4$	W

[a] Successful separation of the pair of neighboring bile acids on silica gel impregnated with a/m salts solution.

[b]W-successful separation of the pair of neighboring bile acids on silica gel impregnated with all applied salt solutions.

RESULTS AND DISCUSSION

In this study, the impregnation of TLC aluminum plates precoated with silica gel 60 (#1.05553) and silica gel $60F_{254}$ (#1.05554) with 1%, 2.5%, and 5% aqueous solutions of $CuSO_4$, $MnSO_4$, $NiSO_4$, and $FeSO_4$ was applied. *n*–hexane–ethyl acetate–acetic acid was used as a mobile phase only in the volume compositions which were not optimal for the separation of bile acids on non-impregnated aluminum plates precoated with silica gel at 18°C.[5]

According to previous investigations, it was proven that the non impregnated aluminum plates precoated with silica gel $60F_{254}$ (#1.05554) didn't separate GC from GDC when *n*–hexane–ethyl acetate-acetic acid in the volume compositions: 25:20:2, 22:22:5, and 22:20:5 was used as a mobile phase (Table 1).

Additionally, in the case of mobile phase n-hexane-ethyl acetate-acetic acid in volume composition 25:20:2 didn't separate C from GLC ($\Delta R_{F(C/GLC)} = 0.03$ and $R_{S(C/GLC)} = 0.76$) (Table 1). The impregnation of plates with aqueous solutions of $CuSO_4$, $MnSO_4$, $NiSO_4$, and $FeSO_4$ didn't allow separation of all pairs of examined bile acids. Application of aluminum plates precoated with silica gel $60F_{254}$ (#1.05554) and impregnated with salts of inorganic acids (1%, 2.5%, and 5% $CuSO_4$; 1%, 2.5%, and 5% $MnSO_4$; 1%, 2.5%, and 5% $NiSO_4$, and 1%, 2.5%, and 5% $FeSO_4$ facilitated, especially, the separation of the following pairs of bile acids, i.e., GC/GDC and C/GLC separated using

Table 3. The selected values[a] of separation factors ΔR_F and R_S of bile acids examined on aluminum plates precoated with silica gel $60F_{254}$ (#1.05554) impregnated with salts of inorganic acids developed by using *n*–hexane–ethyl acetate–acetic acid as a mo mobile phase in volume composition 22:22:5 at 18°C

Pair of acids	*n*–Hexane–ethyl acetate–acetic acid (v/v/v)					
	22:22:5					
	1%$MnSO_4$		2.5%$MnSO_4$		1%$FeSO_4$	
	ΔR_F	R_S	ΔR_F	R_S	ΔR_F	R_S
GC/GDC	0.08	1.77	0.07	1.60	0.06	1.64
GDC/C	0.15	2.62	0.13	2.18	0.08	1.57
C/GLC	0.19	3.71	0.27	4.88	0.19	3.38
GLC/CDC	0.24	4.06	0.16	2.71	0.21	3.29
CDC/DC	0.08	1.15	0.13	2.22	0.06	1.06
DC/LC	0.19	3.79	0.20	5.18	0.35	7.54

[a]Table presents only the values of optimal separation of studied bile acids for given impregnated concentrations.

Table 4. The selected values[a] of separation factors ΔR_F and R_S of bile acids examined on glass plates precoated with silica gel $60F_{254}$ (#1.05554) impregnated with salts of inorganic acids developed by using *n*–hexane–ethyl acetate–acetic acid as a mobile phase in volume composition 22:20:5 at 18°C

	n–Hexane–ethyl acetate–acetic acid (v/v/v)																			
	22:20:5																			
	1%$CuSO_4$		2.5%$CuSO_4$		5%$CuSO_4$		2.5%$MnSO_4$		5%$MnSO_4$		1%$NiSO_4$		2.5%$NiSO_4$		1%$FeSO_4$		5%$FeSO_4$		5%$NiSO_4$	
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	ΔR_F	R_S	ΔR_F	R_S	ΔR_F	R_S	ΔR_F	R_S
GC/GDC	0.10	2.00	0.12	2.29	0.19	4.33	0.08	1.04	0.12	2.00	0.19	2.29	0.08	4.33	0.12	2.41	0.09	1.85	0.13	2.92
GDC/C	0.12	2.00	0.13	2.25	0.10	1.93	0.09	2.06	0.13	2.00	0.10	2.25	0.09	1.93	0.12	1.89	0.10	1.62	0.12	2.00
C/GLC	0.22	4.69	0.21	3.73	0.21	4.07	0.22	4.01	0.21	4.69	0.21	3.73	0.22	4.07	0.24	3.50	0.29	4.44	0.26	4.56
GLC/CDC	0.21	4.64	0.16	2.87	0.28	5.69	0.18	2.22	0.16	4.64	0.28	2.87	0.18	5.69	0.21	3.26	0.17	2.47	0.20	3.80
CDC/DC	0.10	1.06	0.07	1.19	0.07	1.24	0.09	2.21	0.07	1.06	0.07	1.19	0.09	1.24	0.10	1.65	0.07	1.18	0.08	1.22
DC/LC	0.23	5.89	0.24	5.50	0.10	2.40	0.26	5.00	0.24	5.89	0.10	5.50	0.26	2.40	0.16	4.18	0.24	6.70	0.17	4.50

[a]In table presents only the values of optimal separation of the studied bile acids for given impregnated concentrations.

Table 5. The R_F values and separation factors (ΔR_F and R_S) of studied bile acids separated on aluminum plates precoated with silica gel 60 (#1.05553) with *n*–hexane–ethyl acetate–acetic acid as a mobile phase in different volume compositions (v/v/v), which were not optimal for bile acids separation on non impregnated silica gel 60 at 18°C[4,5]

Pair of acids	*n*–Hexane–ethyl acetate–aceticacid (v/v/v)								
	22:20:5			25:20:2			25:20:5		
	R_F	ΔR_F	R_S	R_F	ΔR_F	R_S	R_F	ΔR_F	R_S
GC/GDC	0.01/0.05	0.04	1.00	0.01/0.01	0.00	0.00	0.01/0.06	0.05	1.62
GDC/C	0.05/0.14	0.09	2.38	0.01/0.05	0.04	1.10	0.06/0.20	0.14	4.00
C/GLC	0.14/0.21	0.07	1.48	0.05/0.07	0.02	0.64	0.20/0.25	0.05	0.96
GLC/CDC	0.21/0.46	0.25	4.24	0.07/0.22	0.15	3.44	0.25/0.58	0.33	7.00
CDC/DC	0.46/0.53	0.07	1.14	0.22/0.26	0.04	0.77	0.58/0.67	0.09	1.47
DC/LC	0.53/0.89	0.36	6.67	0.26/0.70	0.44	9.76	0.67/0.98	0.31	6.92

Table 6. Description of separation effects for the respective pairs of studied bile acids on aluminum plates precoated with silica gel 60 (#1.05553) impregnated with aqueous solutions of inorganic salts developed by using *n*–hexane–ethyl acetate–acetic acid as a mobile phase at 18°C

The volume composition of mobile phase (v/v/v)	Pair of acids					
	GC/GDC	GDC/C	C/GLC	GLC/CDC	CDC/DC	DC/LC
25:20:2	5% $CuSO_4$[a]	2.5% $CuSO_4$ 5% $CuSO_4$ 1% $NiSO_4$	W[b]	W	5% $CuSO_4$ 2.5% $FeSO_4$ 5% $FeSO_4$	W
25:20:5	W	W	W	W	1% $CuSO_4$ 1% $MnSO_4$ 2.5% $MnSO_4$ 1% $NiSO_4$ 2.5% $NiSO_4$ 1% $FeSO_4$ 5% $FeSO_4$	W
22:20:5	W	W	W	W except of 5% $CuSO_4$	W except of 2.5% $CuSO_4$ 5% $MnSO_4$ 5% $NiSO_4$ 2.5% $FeSO_4$	W except of 5% $CuSO_4$

[a]Successful separation of the pair of neighboring bile acids on silica gel impregnated with a/m salts solution.

[b]W-successful separation of the pair of neighboring bile acids on silica gel impregnated with all applied salt solutions.

n–hexane–ethyl acetate–acetic acid in volume compositions 22:22:5 and 22:20:5 (Table 2).

All pairs of studied bile acids were separated on aluminum plates precoated with silica gel $60F_{254}$ (#1.05554) impregnated with the following aqueous solutions of inorganic salts:

- 1% $MnSO_4$, 2.5% $MnSO_4$, and 1% $FeSO_4$ with a mobile phase in volume composition 22:22:5 (v/v/v) (Table 3);
- 1%, 2.5%, and 5% $CuSO_4$, 2.5% and 5% $MnSO_4$, 1%, 2.5%, and 5% $NiSO_4$ and 1% and 5% $FeSO_4$ with a mobile phase in volume composition 22:20:5 (v/v/v) (Table 4).

On the non impregnated aluminum plates precoated with silica gel 60 (#1.05553) and developed using mobile phase: *n*–hexane–ethyl acetate–acetic

acid in volume composition 25:20:2 (v/v/v) didn't separate four pairs of neighboring bile acids, i.e., GC/GDC, C/GLC, GDC/C, and CDC/DC (Table 5).

The application of mobile phase in volume composition 25:20:2 (v/v/v) and silica gel 60 (#1.05553) aluminum plates, which were impregnated with 5% $CuSO_4$ allowed a separation of all studied bile acids (Table 6). Comparing ΔR_F and R_S values of bile acids separated on non impregnated and impregnated with 5% $CuSO_4$ aluminum plates (#1.05553) with the use of *n*–hexane–ethyl acetate-acetic acid in volume composition 25:20:2 (v/v/v) as mobile phase presents Fig. 1.

On the non impregnated silica gel 60 plates (#1.05553) with the use of mobile phase in volume composition 25:20:5 (v/v/v) C didn't separate from GLC and GC from GDC when a mobile phase 22:20:5 (v/v/v) was used. It was observed that application of aluminum plates precoated with silica gel 60 (#1.05553) and impregnated with respective aqueous solution of inorganic salts: $CuSO_4$, $MnSO_4$, $NiSO_4$, and $FeSO_4$ allows complete separation of GC from GDC, GDC from C and C from GLC developed with a mobile phase in volume compositions: 22:20:5 and 25:20:5 (v/v/v) (Tables 6 and 7). However, both pairs of neighboring bile acids GLC/CDC and DC/LC separated on all impregnated plates except for 5% $CuSO_4$ (Table 6).

CDC separates from DC on aluminum plates impregnated with selected solutions of examined salts.

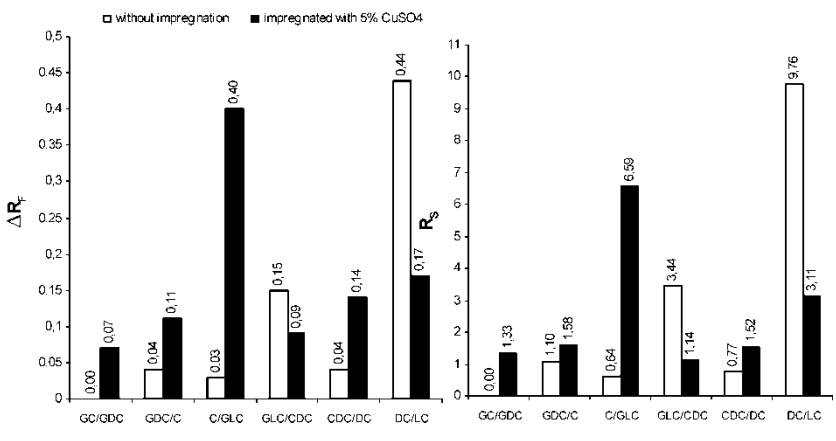

Figure 1. Comparison of the ΔR_F and R_S values for studied bile acids separated on aluminum plates precoated with non impregnated and impregnated silica gel 60 (#1.05553) with 5% aqueous solutions of $CuSO_4$ and developed by using *n*–hexane–ethyl acetate–acetic acid in a volume composition 25:20:2 (v/v/v) as a mobile phase.

Table 7. The selected values[a] of separation factors ΔR_F and R_S of bile acids examined on aluminum plates precoated with silica gel $60F_{254}$ (#1.05553) impregnated with salts of inorganic acids developed by using *n*–hexane–ethyl acetate–acetic acid as a mobile phase in volume compositions 22:20:5 and 25:20:2 at 18°C

	n–Hexane–ethyl acetate–acetic acid (v/v/v)															
	22:20:5								25:20:2							
	1%$CuSO_4$		1%$MnSO_4$		2.5%$MnSO_4$		1%$NiSO_4$		2.5%$NiSO_4$		1%$FeSO_4$		5%$FeSO_4$		5%$CuSO_4$	
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	ΔR_F	R_S	ΔR_F	R_S
GC/GDC	0.13	2.40	0.10	2.67	0.08	1.69	0.07	1.67	0.09	1.87	0.09	1.93	0.13	2.52	0.07	1.33
GDC/C	0.11	1.75	0.18	3.75	0.10	1.76	0.09	1.85	0.13	3.08	0.12	2.18	0.11	1.87	0.11	1.58
C/GLC	0.26	4.24	0.22	4.38	0.19	3.50	0.19	4.73	0.21	4.71	0.22	4.40	0.34	5.22	0.40	6.59
GLC/CDC	0.23	3.37	0.26	5.19	0.17	2.94	0.23	4.92	0.19	3.16	0.15	2.93	0.19	3.06	0.09	1.14
CDC/DC	0.08	1.05	0.07	1.20	0.08	1.16	0.08	1.29	0.08	1.26	0.10	1.56	0.07	1.33	0.14	1.52
DC/LC	0.17	3.83	0.14	2.71	0.28	5.12	0.31	7.25	0.22	4.53	0.24	5.38	0.13	3.78	0.17	3.11

[a]Table presents only the values of optimal separation of studied bile acids for given impregnated concentrations.

Table 8. The selected values[a] of separation factors ΔR_F and R_S of bile acids examined on glass plates precoated with silica gel $60F_{254}$ (# 1.05553) impregnated with salts of inorganic acids developed by using *n*–hexane–ethyl acetate–acetic acid as a mobile phase in volume composition 25:20:5 at 18°C 87

	n–Hexane–ethyl acetate–acetic acid (v/v/v)													
	25:20:5													
	1%$CuSO_4$		1%$MnSO_4$		2.5%$MnSO_4$		1%$NiSO_4$		2.5%$NiSO_4$		1%$FeSO_4$		5%$FeSO_4$	
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	ΔR_F	R_S
GC/GDC	0.05	1.04	0.09	1.87	0.07	1.46	0.08	1.64	0.07	1.54	0.09	1.93	0.07	1.46
GDC/C	0.10	1.87	0.21	3.26	0.07	1.43	0.14	2.06	0.14	2.44	0.10	1.87	0.07	1.36
C/GLC	0.19	4.00	0.25	3.90	0.19	3.50	0.18	3.12	0.13	2.45	0.33	5.62	0.20	3.86
GLC/CDC	0.12	2.46	0.24	4.50	0.18	2.63	0.28	4.81	0.19	3.48	0.16	2.50	0.15	2.67
CDC/DC	0.10	1.47	0.07	1.31	0.08	1.16	0.09	1.20	0.06	1.06	0.09	1.42	0.06	1.20
DC/LC	0.30	4.94	0.11	2.05	0.30	6.54	0.20	5.16	0.25	5.07	0.19	4.25	0.33	6.85

[a]Table presents only the values of optimal separation of studied bile acids for given impregnated concentrations.

All pairs of studied bile acids were separated on plates impregnated with the following aqueous solutions of inorganic salts (Tables 7 and 8):

- 5% $CuSO_4$ with mobile phase 25:20:2 (v/v/v);
- 1% $CuSO_4$, 1% and 2.5% $MnSO_4$, 1% and 2.5% $NiSO_4$, 1% and 5% $FeSO_4$ with mobile phase 25:20:5 (v/v/v);
- 1% $CuSO_4$, 1% and 2.5 % $MnSO_4$, 1% and 2.5% $NiSO_4$, 1% and 5% $FeSO_4$ with mobile phase 22:20:5 (v/v/v).

CONCLUSIONS

In the present study, the impregnation of TLC aluminum plates precoated with silica gel 60 (#1.05553) and with silica gel $60F_{254}$ (#1.05554) for separation of selected bile acids was applied. The mixture of *n*-hexane-ethyl acetate-acetic acid was used as a mobile phase only in the volume compositions which were not optimal for the separation of bile acids on non impregnated aluminum plates at 18°C. The aluminum plates were impregnated with 1%, 2.5%, and 5% aqueous solutions of the following salts: $CuSO_4$, $MnSO_4$, $NiSO_4$, and $FeSO_4$. It was observed that impregnation of silica gel 60 (#1.05553) and silica $60F_{254}$ with aqueous solution of $CuSO_4$, $MnSO_4$, $NiSO_4$, and $FeSO_4$ improved the separation of GC/GDC and C/GLC, which separated poorly on aluminum plates precoated with non impregnated silica gel 60 and $60F_{254}$. The obtained results indicate that the mobile phase *n*–hexane–ethyl acetate–acetic acid in volume composition 25:20:2 (v/v/v) allowed to separate on impregnated with 5% $CuSO_4$ aluminum plates (#1.05553) all pairs of studied bile acids. It can be concluded that the appropriate modification of TLC plates can improve the separation of bile acids.

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