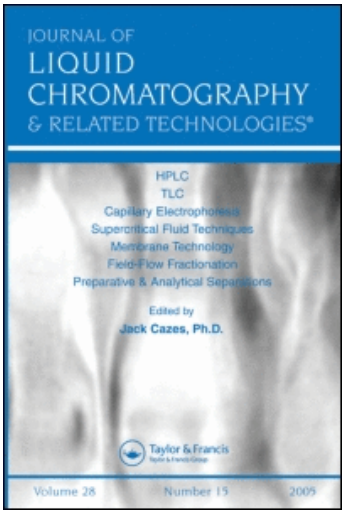

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Chromatographic Separation of Some Coumarins and Flavonoids on Diol-Modified Silica Gel Phase

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CHROMATOGRAPHIC SEPARATION OF SOME COUMARINS AND FLAVONOIDS ON DIOL-MODIFIED SILICA GEL PHASE

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ABSTRACT

The chromatography of polar compounds, such as coumarins and flavonoids, has been investigated on diol-bonded silica gel layers using various organic modifiers (2-propanol (2PrOH), ethyl acetate (EtOAc), methyl ethyl ketone (MeEtCO), 2-propyl ether $(2Pr)_2O$, 1,4-dioxane (DX) and tetrahydrofuran (THF)) in n–heptane as the mobile phase. It was found that diol phases are very suitable for separation of the above-mentioned compounds in normal phase thin-layer chromatography. The selectivity of the systems, the adsorptive properties of the diol–modified phases, and the influence of the organic modifiers on retention are discussed. Some results, obtained on diol-bonded silica gel, are compared to underivatized silica gel as R_M diol vs. R_M silica relationship.

INTRODUCTION

In recent years, high performance liquid chromatography (HPLC) has become very important as an analytical technique well established in the

Table 1

The Compounds Studied

No.	Popular Name	Systematic Name
1	Coumarin	
2	4-Hydroxycoumarin	
3	Umbelliferon	7-Hydroxycoumarin
4	4-Methylesculetin	4-Methyl-6,7-dihydroxycoumarin
5	Isopimpinellin	5,8-Dimethoxypsoralen
6	Esculin	6,7-Dihydroxycoumarin-6-glucoside
7	Flavone	
8	α-Naphthoflavone	
9	Kaempferol	3,4',5,7-Tetrahydroxyflavone
10	Quercetin	3,3',4',5,7-Pentahydroxyflavone
11	Isoquercitrin	Quercetin-3-O-glucoside
12	Robinetin	3,3',4',5',7-Pentahydroxyflavone
13	Robinin	Kaempferol-3-O-robinoside-7-O-rhamnoside
14	Myricetin	3,3',4',5,5',7-Hexahydroxyflavone
15	Luteolin-7-O-glucoside	3',4',5,7-Tetrahydroxyflavone-7-O-glucoside
16	Rutin	3,3',4',5,7-Pentahydroxyflavone-3-O-rhamnoglucoside
17	Hesperetin	3',5,7-Trihydroxy-4'-methoxyflavanone
18	Hesperidin	Hesperetin-7-O-rhamnoglucoside
19	Naringin	4',5,7-Trihydroxyflavanone-7-O-rhamnoglucoside
20	Pelargonidin chloride	3,4',5,7-Tetrahydroxyflavylium chloride
21	Malvin chloride	3,4',5,7-Tetrahydroxy-3',5'-dimethoxyflavylium-3,5-diglucoside

separation of coumarins and flavonoids.[1-8] At present, after the introduction of HPTLC plates with various chemically bonded stationary phases (aminopropyl, phenyl, alkyl, cyanopropyl, diol, and others), they are applied even to difficult separations of closely related compounds in mixtures. The most frequent silica and silanized silica gel plates were used,[9-15] but the polar bonded phases with amino[16] or diol functional groups were used considerably less. Despite many publications on TLC of coumarins and flavonoids, no publication on the separation of investigated compounds on diol phases could be found. These very polar compounds are strongly retained on silica gel plates, but diol phases which behave like a deactivated silica gel with a similar retention mechanism should be more suitable for chromatographic analysis.[17,19]

In this paper, the retention of coumarins and flavonoids on HPTLC-Diol plates, in normal-phase systems, was investigated. The experimental data obtained on diol-phases was compared to the data obtained with bare silica gel phases.

EXPERIMENTAL

Thin-layer chromatography was performed with 10 x 10 cm glass HPTLC-DIOL F_{254} precoated plates (E. Merck, Darmstadt, Germany) in a sandwich chamber with an eluent distributor. Samples (2 μL) of solution (0.2% w/v) from the solutes in methanol were spotted and developed to a distance of 8 cm. The location of the spots was determined under UV light (λ = 254 nm). The results (an average of three measurements, differing by no more than 0.02 R_F units) and chromatographic conditions used are given either in the figures or in the text. The temperature was maintained at 20±1°C. All reagents were of analytical reagent grade from E. Merck. The investigated coumarins and flavonoids are listed in Table 1.

RESULTS

Plots showing the effect of organic modifier concentration on the retention of a series of coumarins and flavonoids on HPTLC-DIOL plates as adsorbent are presented in Figures 1-3. In most instances, the linear relationship R_M = f(log C%) was obtained in accordance with the equation $R_M = R^0_M - m \log C\%$. The experimental data obtained for dioxane and 2-propanol are presented in Figs. 1 and 2. The lines sometimes intersect, giving different selectivities relative to the concentration of the stronger component in the mobile phase. In Fig. 1A and 1B, the results obtained for dioxane in n-heptane as the mobile phase are presented. The very high slopes obtained on the diol-silica phase indicate a multipoint adsorption, as well as solvation in the mobile phase. An increased number of the active sites (e.g.OH groups) in the solute molecule causes a considerably stronger adsorption (lower R_F values) and also increased intercept (R^0_M) values. The glucosides, as very polar compounds, have a lower R_F value on the diol-phase than do their aglycones. Methylation of the hydroxyl group decreases the retention and, also, the slopes and intercepts are lower. Generally, the order of R_F values is in accordance with their hydrophobicity $ROCH_3$ >ROH > $R(OH)_2$ > RO-sugar.[12] Malvine chloride, hesperetin and isopimpinellin exhibit lower slope and intercept values according to their hydrophobic methoxyl groups; they have higher R_F values. Coumarins and flavonoids possessing hydroxyl groups have the possibility of forming hydrogen bonds with alcoholic hydroxyl groups on the diol-adsorbent.

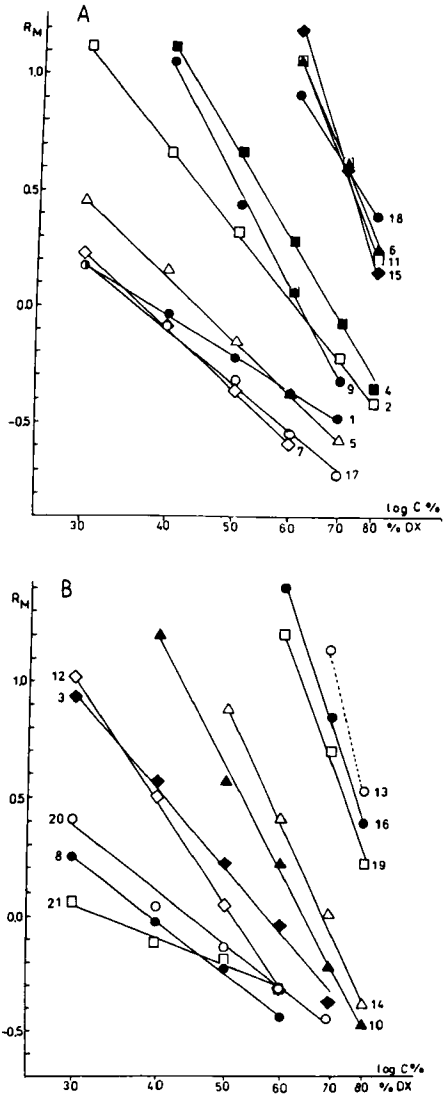

Figure 1. Dependence of coumarins and flavonoids R_M values on log C (% v/v) for HPTLC on diol-modified silica with dioxane - n-heptane as mobile phase. Compound identities are as in Table 1.

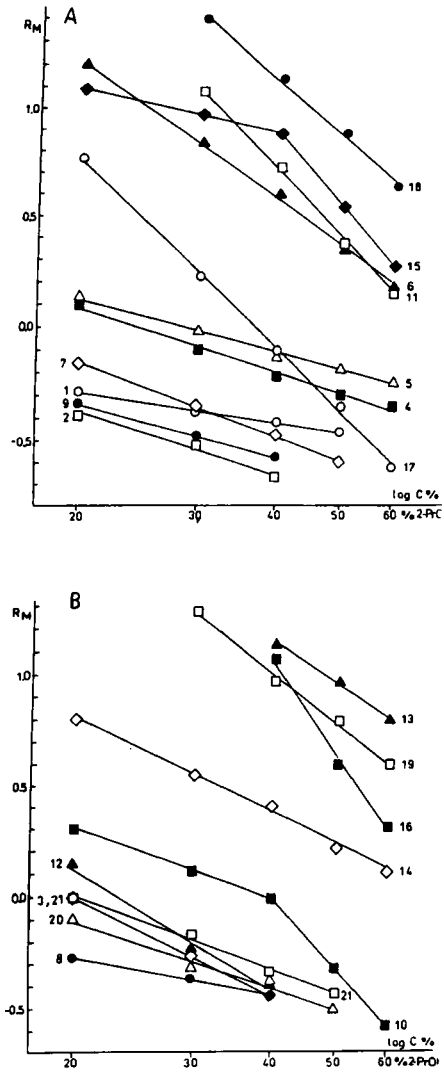

Figure 2. Dependence of coumarins and flavonoids R_M values on log C (% v/v) for HPTLC on diol-modified silica with 2-propanol - n-heptane as mobile phase. Compound identities are as in Table 1.

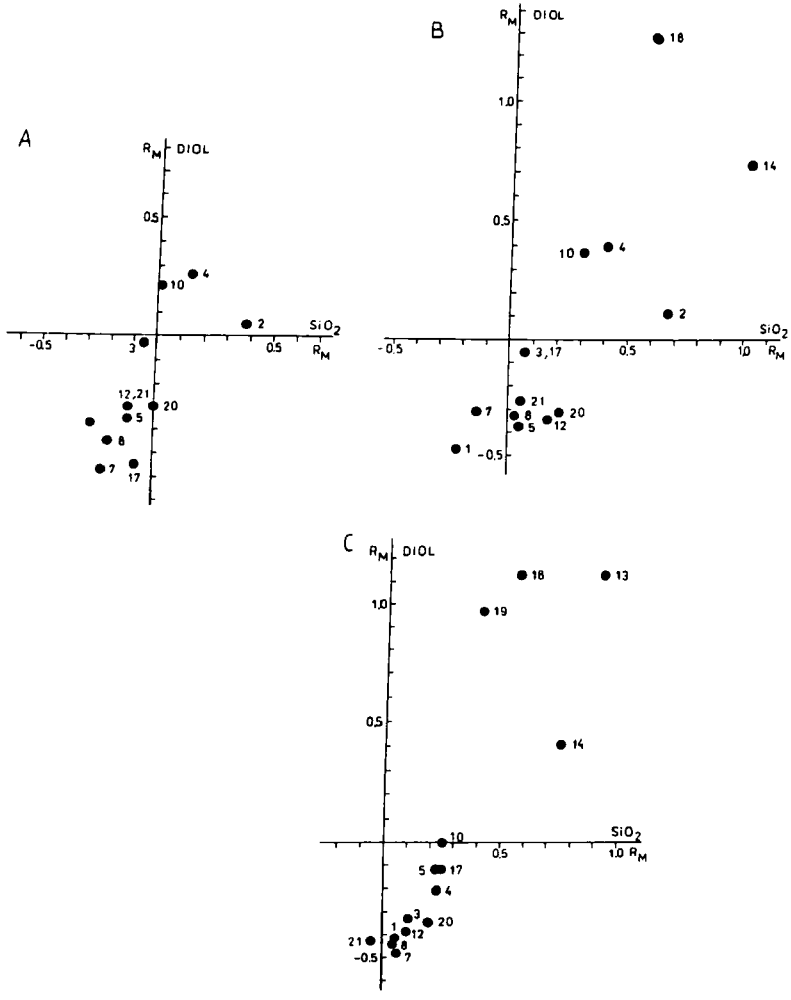

Figure 3. Correlation between R_M values of coumarins and flavonoids obtained on diol and silica layers. Mobile phases: 3A, 60:40 (v/v) dioxane - n-heptane for diol and 80:20 (v/v) dioxane - n-heptane for silica; 3B, 60:40 (v/v) ethyl-acetate - n-heptane for diol and 80:20 (v/v) ethyl-acetate - n-heptane for silica; 3C, 40:60 (v/v) 2-propanol for both adsorbents. For identification of solutes, see Table 1.

Table 2

R_M(HPTLC$_{DIOL}$) and ΔR_M Values Relative to Coumarin*

	40% 2-PrOH + Heptane		60% EtOAc + Heptane		60% MeEtCO + Heptane		50% THF + Heptane		60% DX + Heptane		80% $(2\text{-Pr})_2O$ + Heptane	
No.	R_M	ΔR_M	R_M	ΔR_M	R_M	ΔR_M	R_M	ΔR_M	R_M	ΔR_M	R_M	ΔR_M
1	-0.44	---	-0.51	---	-0.43	---	-0.40	---	0.37	---	0.08	---
2	-0.67	-0.23	0.10	0.61	0.00	0.43	-0.12	0.28	0.04	0.41	0.73	0.65
3	-0.33	0.11	-0.04	0.47	-0.04	0.39	-0.13	0.27	-0.03	0.34	0.55	0.47
4	-0.21	0.23	0.40	0.91	0.31	0.74	0.06	0.46	0.27	0.64	1.07	0.99
5	-0.13	0.31	-0.38	0.13	-0.31	0.12	0.29	0.11	-0.37	0.00	0.37	0.29
6	0.60	-0.16	---	---	1.20	1.63	1.08	1.48	1.08	1.45	---	---
7	-0.52	-0.08	-0.31	0.20	-0.21	0.22	-0.40	0.00	-0.57	-0.20	0.51	0.43
8	-0.47	-0.03	-0.34	0.17	-0.23	0.20	-0.38	0.02	-0.47	-0.10	0.48	0.40
9	-0.58	-0.14	-0.31	0.20	0.06	0.49	-0.15	0.25	0.06	0.43	0.41	0.33
10	0.00	0.44	0.37	0.88	0.41	0.84	0.06	0.46	0.22	0.59	1.14	1.06
11	0.73	1.17	---	---	---	---	1.08	1.48	1.08	1.45	---	---
12	-0.40	0.04	-0.23	0.28	-0.25	0.18	-0.34	0.06	-0.32	0.05	0.36	0.28
13	1.13	1.57	---	---	---	---	---	---	---	---	---	---
14	0.41	0.85	0.73	1.24	0.84	1.27	0.17	0.57	0.40	0.77	1.38	1.30
15	0.88	1.32	---	---	---	---	1.20	1.60	1.20	1.57	---	---
16	1.06	1.50	---	---	---	---	---	---	---	---	---	---
17	-0.11	0.33	-0.05	0.46	0.06	0.49	-0.18	0.22	-0.53	-0.16	0.88	0.80
18	---	---	---	---	---	---	1.13	1.53	0.93	1.30	---	---
19	0.98	1.42	---	---	---	---	---	---	1.20	1.57	---	---
20	-0.36	0.08	-0.34	0.17	-0.25	0.18	-0.29	0.11	-0.32	0.05	0.43	0.35
21	-0.43	0.01	-0.28	0.23	-0.08	0.35	-0.37	0.03	-0.32	0.05	0.22	0.14

* Compound No. 1 in Table 1.

surface. Thus, the more OH groups in the molecule, the stronger binding with polar groups of the adsorbent and the lower the R_F values. Coumarins and flavonoids act as proton donors or proton acceptors towards the adsorbent-active sites that greatly influence retention and separation selectivity.

The results presented in the figures as plots R_M = f(log C%) clearly visualize the differences of the selectivity. 2-Propanol, with a higher eluent strength than dioxane, gave comparable R_F values at lower concentrations

(Figs. 2A and 2B). The elution order of the solutes changed, somewhat, but generally, the more polar compounds (similarly with dioxane as modifier) are more strongly retained. The dependence of retention and selectivity on the type of organic modifier in the mobile phase is shown in Table 2. Selectivities of modifiers are compared, and the best composition of the mobile phase to be chosen as complementary solvent systems for rechromatography or for two-dimensional development of the compounds not separated in one system. Some compounds had too low or too high R_F values (beyond the range 0.2 - 0.8 RF units). Robinine, luteolin 7-O-glucoside, rutin and pelargonidin, the more polar compounds, did not migrate from the origin, but α-naphthoflavone, a more hydrophobic compound, migrated at the solvent front. Diisopropyl ether is a more selective modifier of low eluent strength. An 80% concentration of 2-propanol in n-heptane was used to elute the majority of the investigated compounds in a reasonable range, in approximately the same order as was obtained for other systems used.

The selectivity is very different from one modifier to another. Figs. 3A, 3B, 3C show $R_{M(diol)}$ vs. $R_{M(silica)}$ relationships in dioxane, ethyl acetate and 2-propanol systems. The compounds are more strongly retained on silica than on the diol-phases (Fig. 3A). The selectivity on both types of adsorbent is very similar; for quercetin, 4-hydroxycoumarin, and 4-methylesculetin, a better selectivity on silica plates was observed and, for naringin, umbelliferon, malvin chloride, and hesperetin, on diol plates.

The second electron-donor solvent, ethyl acetate, was also compared on both adsorbents. For some compounds, the diol-phase is more selective; for other compounds, silica is more selective. In the 2-propanol - n-heptane systems (Fig. 3C), the solutes were adsorbed less strongly than in dioxane and ethyl acetate - n-heptane. 2-Propanol, a proton donor-acceptor solvent with the highest eluent strength, at 40% concentration in the mobile phase, gave comparable R_F values whereas, for dioxane and ethyl acetate, 60% concentrations were required. For many compounds (robinine, myricetin, quercetin, 4-methylesculetin and pelargonidin chloride), the diol phase was more selective than the silica gel phase; however, in both cases, hydroxyl groups were the active centers influencing the retention.

CONCLUSION

The diol-phases are very suitable for separation of coumarins and flavonoids. The investigated compounds are less strongly adsorbed on diol-modified than on underivatized silica gel. Differences in the selectivity of adsorbents and eluent systems can be utilized in the difficult separation of

closely related compounds and they offer the possibility of choosing suitable phase systems to identify the substances and they are useful in two-dimensional development.

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