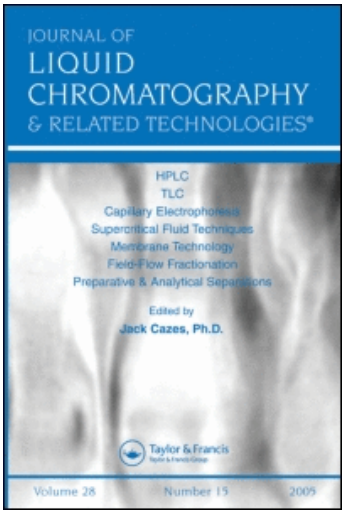

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SEPARATIONS IN THIN LAYER AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY USING ALKYL SILICA GEL BONDED PHASES[1]

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ABSTRACT

Separations in thin layer (TLC) and high performance liquid chromatography (HPLC) using alkyl bonded phases were carried out under optimum solvent conditions for each of three phases, RP-2, RP-8 and RP-18. The phases were tested for their efficiency and resolving power using three groups of compounds in three binary organic-water mobile phases. The organic solvents were acetonitrile, methanol and tetrahydrofuran, which are widely used as solvent modifiers in reversed phase liquid chromatography.

The results showed that, unlike HPLC, TLC using RP-18 plates was the most, and RP-2 plates the least, efficient. A naphthalene and biphenyl mixture which was resolved by HPLC using any of the three solvents and columns was not resolved by TLC using any plate or solvent combination, unless the plate was prewashed with an organic modifier. The addition of NaCl (1-2% wt/vol) to the solvent for TLC speeded development unless an alcohol was used, but did not greatly affect the separation.

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INTRODUCTION

In previous work (1) we described the affect of using normal phase thin layer chromatography (TLC) conditions (silica gel) in normal phase high performance liquid chromatography (HPLC). Good agreement between results with TLC and HPLC was obtained. We recommended that, in a preliminary test for a mobile phase for HPLC, TLC be used, due to its low cost, speed and simplicity. Okumura (2) found good correlation between TLC and HPLC separation of cephalosporin antibiotics on dimethylsilyl silica gel. TLC was also used (3) to develop optimum condition for use in preparative column chromatography.

This study compares the separations in reversed phase HPLC and TLC with three different solute mixtures developed in three different mobile phases using three different silica gel alkyl bonded stationary phases (RP-2, RP-8 and RP-18). Commercially available precoated TLC plates and packed columns were used.

EXPERIMENTAL

Materials: Tetrahydrofuran, methanol and acetonitrile were glass distilled (Burdick and Jackson). Anthraquinone (AQ), 1-methylanthraquinone (MAQ), 1-ethylanthraquinone (EAQ), naphthalene (N), biphenyl (B), dimethylphthalate (MP) and diethylphthalate (EP) were analytical grade (Aldrich Chemical Co.).

Apparatus: A modular HPLC system consisting of Laboratory Data Control (LDC) constametric I and II pumps attached to an LDC Gradient Master, a Chromatronix dual-channel UV absorbance detector (254 and 280 nm), a Rheodyne injector, and a strip-chart recorder operated at 0.2 in/min was used.

The columns were 250 mm x 4.6 mm prepacked with 10 µm particle size materials (Merck). The reversed phase materials were RP-2, RP-8 and RP-18. Sample solutions (10µl) were injected. Experiments were run at room temperature using a mobile phase flow rate of 1.2 ml/min. Retention times and separation factors (α) were determined with a 3352A Laboratory Data System (Hewlett-Packard) linked

through a Hewlett-Packard 1865 A/D converter to the UV detector output of the liquid chromatograph. The output from the data system was recorded on a 9866A thermal line printer (Hewlett-Packard). Precoated RP-2, RP-8 and RP-18 TLC plates (Whatman, Inc.) were used without pretreatment. Micropipettes were used to spot the sample solution, after which plates were developed in standard rectangular TLC tanks. After development, plates were dried and spots observed under short wave (254 nm) UV light.

Procedure: Optimum HPLC mobile phases, which were previously found (4), were used to test the RP-2, RP-8 and RP-18 TLC plates. hR_f is defined as R_f X 100.

RESULTS AND DISCUSSION

The separation of AQ, MAQ and EAQ, N and B, and MP and EP by reversed phase HPLC and TLC (RP-2, RP-8 and RP-18) using three aqueous mobile phases with different organic modifiers, acetonitrile (CH_3CN), methanol (MeOH) and tetrahydrofuran (THF), is given in tables 1-9. The results show that the three groups of compounds were separated by HPLC using any combination of stationary or mobile phase. This was not the case when TLC plates were used. Tables 1-3 show that good separation of the MP, and EP was achieved except in 50% CH_3CN using RP-2 plates (Table 3). The same was true for the anthraquinone mixture in 55% CH_3CN again using RP-2 plates (Table 6), while in HPLC, good separation was achieved. The separation factors (α) obtained for both HPLC and TLC (Tables 1-6) are comparable which indicates that both the mobile and stationary phases give good resolution of the component's mixtures.

No separation of the N-B mixture was obtained using RP-2, RP-8 or RP-18 TLC plates with any of the three mobile phases (Tables 7-9), although these compounds gave base line separations by HPLC using the same type of bonded phases and the same solvent mixtures.

We do not know why this occurred with only N-B mixture. It is perhaps due to the fact that HPLC columns are continuously washed by the mobile phase

TABLE 1

COMPARATIVE HPLC AND TLC SEPARATION OF DIMETHYL AND DIETHYLPHTHALATES ON RP-2, RP-8 AND RP-18 USING METHANOL/WATER

R.Phase	Mobile Phase	Compound	R_t	α_{HPLC}	hR_f	α_{TLC}
RP-2	56% MeOH	MP	5.99	-	54.2	-
		EP	8.73	1.56	43.3	1.25
RP-8	56% MeOH	MP	4.82	-	23.8	-
		EP	7.98	1.66	9.6	2.48
RP-18	64% MeOH	MP	5.03	-	43.8	-
		EP	9.39	1.87	25.8	1.70

TABLE 2

COMPARATIVE HPLC AND TLC SEPARATION OF DIMETHYL AND DIETHYLPHTHALATES ON RP-2, RP-8 AND RP-18 USING TETRADYROFURAN/WATER

R.Phase	Mobile Phase	Compound	R_t	α_{HPLC}	hR_f	α_{TLC}
RP-2	48% THF	MP	4.64	-	33.8	-
		EP	6.57	1.42	25.4	1.33
RP-8	44% THF	MP	4.43	-	32.1	-
		EP	6.35	1.43	21.3	1.51
RP-18	48% THF	MP	3.82	-	30.8	-
		EP	5.29	1.38	20.8	1.48

TABLE 3

COMPARATIVE HPLC AND TLC SEPARATION OF THE QUINONE MIXTURE ON RP-2, RP-8 AND RP-18 USING ACETONITRILE/WATER

R.Phase	Mobile Phase	Compound	R_t	α_{HPLC}	hR_f*	α_{TLC}
RP-2	55% CH_3CN	AQ	6.47	-	74.6	-
		MAQ	7.56	1.17	74.6	1.0
		EAQ	9.14	1.21	74.6	1.0
RP-8	65% CH_3CN	AQ	5.98	-	35.8	-
		MAQ	7.14	1.21	30.0	1.19
		EAQ	8.65	1.21	24.2	1.24
RP-18	70% CH_3CN	AQ	6.36	-	42.1	-
		MAQ	8.41	1.32	34.2	1.23
		EAQ	10.71	1.27	32.1	1.07

and equilibrated before use, and the TLC plates are not so treated. The sample solution is spotted on a dry plate which has not been washed or predeveloped in the mobile phase. However, when the plate was washed with the organic modifier, dried, and then spotted with the sample solution, the results were much better. For example, when the AQ, MAQ and EAQ mixture was spotted on an RP-2 plate which was not pre-washed one spot (hR_f 74.6) was observed for the three compounds (Table 6), but when a prewashed plate was used, separation was achieved (hR_f 48, 44 and 41). The same was true for MP and EP using unwashed RP-2 plates. In this case one spot at hR_f 95.4 (Table 3) was observed. However, when a prewashed plate with CH_3CN was used the two components were resolv-

TABLE 4

COMPARATIVE HPLC AND TLC SEPARATION OF THE QUINONE MIXTURE ON ON RP-2, RP-8 AND RP-18 USING METHANOL/WATER

R.Phase	Mobile Phase	Compound	R_t	α_{HPLC}	hR_f	α_{TLC}
RP-2	60% MeOH	AQ	8.22	-	43.5	-
		MAQ	10.77	1.31	37.5	1.16
		EAQ	14.56	1.35	32.7	1.15
RP-8	64% MeOH	AQ	8.76	-	12.5	-
		MAQ	11.81	1.35	7.5	1.67
		EAQ	15.18	1.29	4.2	1.79
RP-18	76% MeOH	AQ	8.42	-	24.5	-
		MAQ	11.94	1.42	17.9	1.37
		EAQ	15.24	1.28	13.3	1.35

ed (hR_f 53 and 46). This also occurred with the other mixture, but the spots were irregular in shape.

Prewashing also affected the migration distance of the solute (R_f value). It was less on a washed plate, which indicates an increased interaction between the solute and the solid phase.

Another difference between TLC and HPLC which may affect α was that in TLC the plates were developed for 12 cm while in HPLC a column 25 cm long was used. Also, the adsorbent layer in TLC is 0.25 mm while the columns internal diameter was 4.6 mm.

One of the disadvantages of TLC, which is not encountered in HPLC, is that high water content mobile phases (above 25%) lead to (a) collapse of the plate

adsorbent and (b) long development time due to increased viscosity of the mobile phase. It has been suggested (4,5) that the addition of sodium chloride (1-2% wt/v) to the mobile phase would eliminate these problems. We studied the effect of adding 1% NaCl on time of development, separation and R_f values. Solvent compositions selected were such that the mobile phase can be used with or without the addition of salt. The results indicate (Table 10) that (a) development time decreases with the addition of 1% NaCl except when an alcohol is the organic modifer (methanol or 2-ethoxyethanol); (b) the resolutions are better the longer the development time; (c) R_f values vary with each organic modifier but, in general, R_f values are higher when salt is used in the mobile

TABLE 5

COMPARATIVE HPLC AND TLC SEPARATION OF THE QUINONE MIXTURE ON RP-2, RP-8 AND RP-18 USING TETRAHYDROFURAN/WATER

R.Phase	Mobile Phase	Compound	R_t	α_{HPLC}	hR_f	α_{TLC}
RP-2	44% THF	AQ	10.36	-	21.7	-
		MAQ	12.38	1.19	19.2	1.13
		EAQ	16.36	1.32	15.8	1.22
RP-8	40% THF	AQ	11.15	-	16.7	-
		MAQ	1.386	1.24	13.8	1.21
		EAQ	19.06	1.37	10.4	1.33
RP-18	44% THF	AQ	9.64	-	15.4	-
		MAQ	12.10	1.26	12.1	1.27
		EAQ	16.23	1.34	8.3	1.46

TABLE 6

COMPARATIVE HPLC AND TLC SEPARATION OF NAPHTHALENE AND BIPHENYL ON RP-2, RP-8 AND RP-18 USING ACETONITRILE/WATER

R.Phase	Mobile Phase	Compound	R_t	α_{HPLC}	hR_f	α_{TLC}
RP-2	50% CH_3CN	N	10.42	-	95.8	-
		BP	13.9	1.33	95.8	1.0
RP-8	55% CH_3CN	N	10.04	-	21.7	-
		BP	13.41	1.34	21.7	1.0
RP-18	60% CH_3CN	N	10.58	-	16.5	-
		BP	14.33	1.35	16.5	1.0

TABLE 7

COMPARATIVE HPLC AND TLC SEPARATION OF NAPHTHALENE AND BIPHENYL ON RP-2, RP-8 AND RP-18 USING METHANOL/WATER

R.Phase	Mobile Phase	Compound	R_t	α_{HPLC}	hR_f	α_{TLC}
RP-2	64% MeOH	N	6.25	-	38.8	-
		BP	8.21	1.31	38.8	1.0
RP-8	75% MeOH	N	6.13	-	21.3	-
		BP	7.86	1.28	21.3	1.0
RP-18	80% MeOH	N	6.43	-	31.2	-
		BP	8.22	1.28	22.7	1.37

TABLE 8

COMPARATIVE HPLC AND TLC SEPARATION OF NAPHTHALENE AND BIPHENYL ON RP-2, RP-8 AND RP-18 USING TETRAHYDROFURAN/WATER

R.Phase	Mobile Phase	Compound	R_t	α_{HPLC}	hR_f	α_{TLC}
PR-2	44% THF	N	14.03	-	11.3	-
		BP	19.32	1.38	11.3	1.0
PR-8	44% THF	N	11.17	-	7.5	-
		BP	14.92	1.34	7.5	1.0
PR-18	44% THF	N	13.88	-	7.5	-
		BP	19.03	1.37	7.5	1.0

TABLE 9

COMPARATIVE HPLC AND TLC SEPARATION OF DIMETHYL AND DIETHYLPHTHALATES ON RP-2, RP-8 AND RP-18 USING ACETONITRILE/WATER

R.Phase	Mobile Phase	Compound	R_t	α_{HPLC}	hR_f	α_{TLC}
RP-2	50% CH_3CN	MP	5.56	-	95.4	-
		EP	8.08	1.45	95.4	1.0
RP-8	55% CH_3CN	MP	4.83	-	64.6	-
		EP	7.12	1.47	48.3	1.34
RP-18	60% CH_3CN	MP	4.17	-	49.2	-
		EP	6.12	1.47	38.3	1.28

TABLE 10

EFFECT OF ADDITION OF NaCl TO THE MOBILE PHASE ON DEVELOPMENT TIME (DEVELOPMENT DISTANCE 12cm) AND SEPARATION IN REVERSED PHASE (RP-18) TLC

Solvent	Develop. Time (min)	Anthraquinones		Naphthalene + Biphenyl		Phthalate	
		hR_f	α	hR_f	α	hR_f	α
$THF:H_2O$ 70:30	123	53.8 50.0 50.0	1.08 1.00	50.8 50.8	1.00	65.8 58.3	1.13
$THF:H_2O$ 70:30 1% NaCl	57	78.3 75.8 75.8	1.03 1.00	72.9 72.9	1.00	81.7 79.2	1.03
$CH_3CN:H_2O$ 80:20	23	55.8 47.5 41.3	1.17 1.15	57.9 51.3	1.13	82.1 72.1	1.14
$CH_3CN:H_2O$ 80:20 1% NaCl	21	69.6 63.8 58.3	1.09 1.09	72.9 68.8	1.06	89.2 83.3	1.07
$MeOH:H_2O$ 80:20	43	25.8 19.2 19.2	1.34 1.00	29.2 21.3	1.37	58.8 46.7	1.26
$MeOH:H_2O$ 80:20 1% NaCl	57	32.9 25.4 20.0	1.30 1.27	37.5 28.3	1.33	66.7 55.4	1.20
2-EOH*:H_2O 80:20	126	62.9 57.5 53.3	1.09 1.08	60.4 55.4	1.09	47.5 47.5	1.0
2-EOH*:H_2O 80:20 1% NaCl	150	62.1 56.3 51.7	1.10 1.09	61.3 55.8	1.10	47.9 47.9	1.0

*2-EOH = 2-ethoxyethanol

phase. Also, the plate binder held up better when salt was added; and (d) α was not greatly affected.

CONCLUSION

The results of this study show that TLC and HPLC separations, using alkyl bonded silica gel phases, are, in most cases comparable, if experimental conditions are kept the same. Reversed-phase TLC on RP-18 plates is the most efficient system. No advantage was obtained with RP-2 plates which were found to be the least useful with the compounds studied. Addition of salt to the mobile phase in TLC speeded the analysis time (except when alcohol was used as the organic modifier), prevented the dislodgement of the bonded phase from the glass plate, and allowed the use of a higher percentage of water, which improved the selectivity of the mobile phase.

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