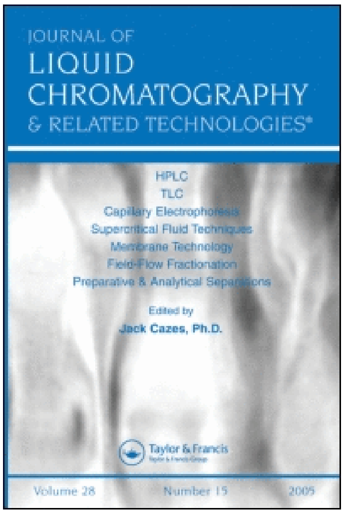

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Effect of Alkyl Chain Length of Bonded Silica Phases on Separation, Resolution and Efficiency in High Performance Liquid Chromatography

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EFFECT OF ALKYL CHAIN LENGTH OF BONDED SILICA PHASES ON SEPARATION, RESOLUTION AND EFFICIENCY IN HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A comparative study of alkyl bonded phases was carried out under optimum solvent conditions for each phase. Three columns, RP-2, RP-8 and RP-18, 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.

The results indicate that each of the three factors, i.e. solvent, solute and bonded alkyl chain length, play an important role, with the solvent being the most significant. When tetrahydrofuran-water was used as the mobile phase, the ratio of THF/H_2O did not vary by much when an RP-2, RP-8 or RP-18 column was used to separate naphthalene from biphenyl, dimethylphthalate from diethylphthalate or anthraquinone from methyl anthraquinone and ethyl anthraquinone. When acetonitrile-water and methanol-water were used the ratio of organic modifier to water changed so as to accomodate the hydrophobic properties of the columns. The efficiency of the columns, expressed as theoretical plates per meter (TPM) was highest when acetonitrile-water was used as the mobile phase. Although there were variations in TPM and resolution from column to column, the three columns gave good separation of the components of the three groups of compounds.

INTRODUCTION

Modification of the adsorbent is an important aspect of liquid chromatography which enables the chromatographer to achieve separations that are not otherwise possible. Reverse-phase (RP) adsorbents for both high performance liquid chromatography (HPLC) and thin-layer chromatography are both good examples of this. In RP today chemically bonded phases are used in which an organic moiety is bonded to silica. Such moieties may include an alkyl chain varying in length from C_2 to C_{22}, a phenyl or other organic groups (cyano, amine, etc.). Currently, the most popular RP is the C_{18} bonded phase, although other phases are commercially available.

Majors and Hopper (1) and Korpi and Janicki (2) have studied the effect of alkyl chain length using C_6, C_8 and C_{18} packing materials. The results indicated an increase in selectivity and longer retention times with increase in chain length, when the columns were compared using the same mobile phase.

Hemetsberger et al (3) studied the effect of chain length (C_8-C_{22}) of bonded organic phases. They reported that selectivity depended on the chain length of the bonded phase and the molecular structure of the solute. They also found that the utilization of long chain phases made it possible to reduce the water content of the water:methanol mobile phase, which increases efficiency and the loading capacity. Hemetsberger et al (4,5) studied the behavior, and effect of structure, of bonded phases. The effect of ligand structure and geometry on selectivity in RP was also investigated (6). Kikta and Grushka (7) studied the retention behavior on alkyl bonded phases as a function of chain length, surface coverage, solute type, mobile phase composition and temperature. Conditions during the experiments were kept constant. Colin and Guiochon (8) compared some packings for RPHPLC. In this study the alkyl chain lengths were between C_6 and C_{22}. Also, the experimental conditions, and especially the mobile phase composition, were kept the same. No attempt was made to optimize separation by changing the ratios of the binary mobile phase to fit the hydrophobicity of the bonded phase. As a result, the shorter length alkyl chain columns generally gave the worst resolution and efficiency.

The objectives of the present study are (a) to compare the separations achieved on commercially available alkyl bonded phases C_2, C_8 and C_{18}, (referred to as RP-2, RP-8 and RP-18, respectively), and (b) to compare the efficiency of each column under its optimum mobile phase conditions. Three different mobile phases (acetonitrile/water, methanol/water, and tetrahydrofuran/water) and three groups of solutes [anthraquinone (A), 1-methyl anthraquinone (MA) and 1-ethyl anthraquinone (EA); naphthalene (N) and biphenyl (BP); and dimethylphthalate (MP) and diethylphthalate (EP)] with varying chemical properties were used.

EXPERIMENTAL

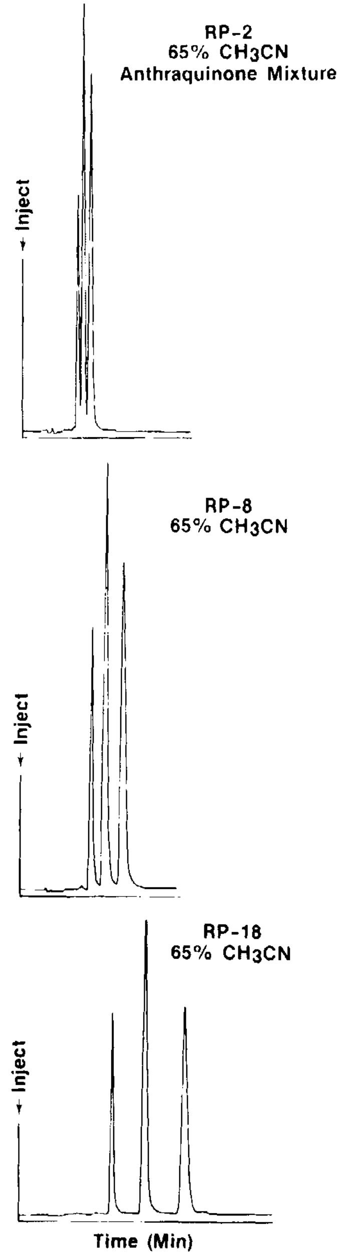
Materials: All solvents were glass distilled (Burdick and Jackson). The chemicals were analytical grade (Aldrich Chemical Co.) and used without further purification.

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, a Rheodyne injector, and a strip-chart recorder operated at 0.2 in/min was used.

The columns were all 250 mm X 4.6 mm pre packed with 10 μm particle size materials (Merck). The reverse phase materials were RP-2, RP-8 and RP-18 10 μl sample solution were injected. Experiments were run at room temperature using a mobile phase flow rate of 1.2 ml/min. Retention times, peak widths at half height($W_{1/2}$), theoretical plates/meter (TPM) and resolution (R) were determined by 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).

RESULTS AND DISCUSSION

Since RP-2, RP-8 and RP-18 columns have different hydrophobic properties it seemed logical to optimize the mobile phase ratio and composition for each column and solute mixture separately. It was felt that a comparison of the three columns under the same mobile phase conditions may, or may not, be the correct approach depending on the solute tested and the organic modifier used. Figure (1) shows the separation of anthraquinone, 1-methyl anthraquinone and 1-ethyl anthraquinone (referred to as the quinone mixture) using the mobile

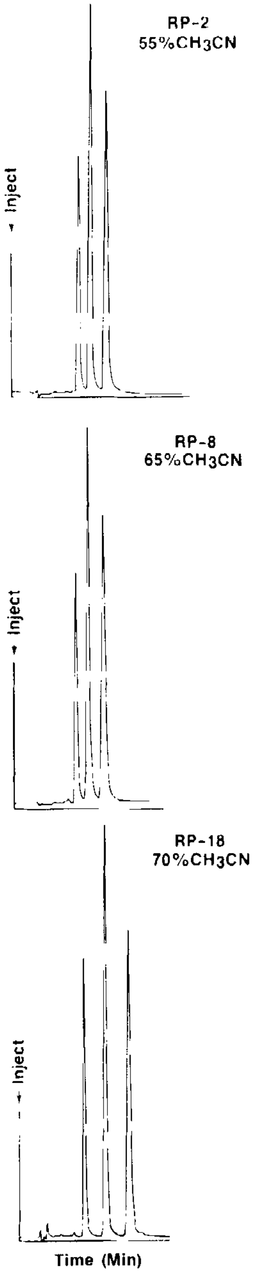


Comparison of Three Reversed Phase Columns Using the Same Solvent System

FIGURE 1

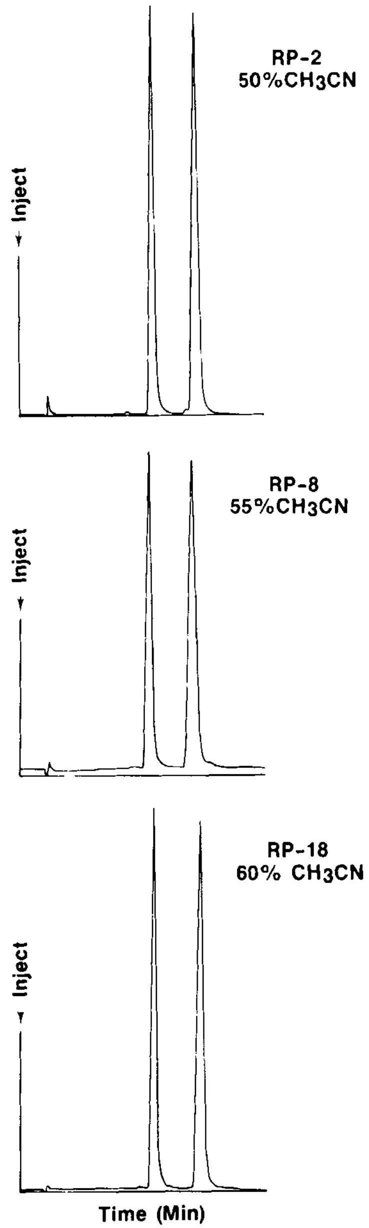
phase 65% acetonitrile:water. As expected, the resolution of the quinone mixture on each column is different, because the experimental conditions did not take into consideration the lower retaining power of the shorter chain length columns (RP-2 vs RP-8 vs RP-18). As a result, the best resolution was obtained with the RP-18 column. On the other hand, when the binary mobile phase ratios were altered for each column to accomodate the hydrophobic properties of each, resolution of the quinone mixture was comparable with RP-2, RP-8 and RP-18 (Figure 2). The same was true, when optimum binary solvent mixtures were used for the separation of naphthalene and biphenyl on the three reverse phase columns (Figure 3). An optimum binary mixture is that mobile phase which gives baseline separation in the minimum amount of time. Comparable results were obtained for dimethyl and diethyl phthalates.

Table 1 compares the retention time, width of peak at half height, theoretical plates per meter and resolution of the quinone mixture on RP-2, RP-8 and RP-18 using the solvent system 65% acetonitrile:water. The table clearly shows that the shorter the alkyl chain of the bonded phase the worse the resolution. When the mobile phase ratios were adjusted for each column (Table 2) better resolution of the quinones was obtained, but the RP-18 column still gave the best resolution if the retention times on each column were comparable. Table 2 also shows that when acetonitrile:water was used as the mobile phase the percentage of acetonitrile dropped from 70% (RP-18) to 55% (RP-2) in order to obtain comparable retention times. The same was true when methanol:water was used, Table 3. Also note that the resolution between methyl- and ethyl anthraquinones is better with RP-2 than with the other two columns. When tetrahydrofuran (THF):water was used as the mobile phase, the THF percentage variation for the separation of the quinone mixture using RP-2, RP-8 and RP-18 was only ±4% giving comparable resolution Table 4. The same trend of mobile phase composition was observed for the resolution of the naphthalene and biphenyl mixture using the three solvent systems, Tables 5-7. Again, it was found that the variation in mobile phase composition was smallest when THF: water was used.



Separation of Anthraquinone, 1-Methylanthraquinone and 1-Ethylanthraquinone at Optimum Solvent Mixture for Each Column

FIGURE 2



Separation of Naphthalene and Biphenyl at Optimum Solvent Mixture for Each Column

FIGURE 3

TABLE 1

COMPARATIVE SEPARATION OF THE QUINONE MIXTURE ON RP-2, RP-8 AND RP-18 USING 65% ACETONITRILE/WATER

COLUMN	MOBILE PHASE	COMPOUND	R_t	$W_{1/2}$	TPM	R
RP-2	65% CH_3CN	AQ	4.61	0.206	11112	-
		MAQ	5.08	0.225	11249	2.19
		EAQ	5.72	0.245	12021	2.70
RP-8	65% CH_3CN	AQ	5.98	0.282	9926	-
		MAQ	7.14	0.332	10209	3.76
		EAQ	8.65	0.397	10529	4.15
RP-18	65% CH_3CN	AQ	7.18	0.292	15756	-
		MAQ	10.65	0.386	16786	8.34
		EAQ	14.08	0.502	17402	7.71

TABLE 2

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

COLUMN	MOBILE PHASE	COMPOUND	R_t	$W_{1/2}$	TPM	R
RP-2	55% CH_3CN	AQ	6.47	0.266	13063	-
		MAQ	7.56	0.304	13630	3.79
		EAQ	9.14	0.355	14639	4.79
RP-8	65% CH_3CN	AQ	5.98	0.282	9926	-
		MAQ	7.14	0.332	10209	3.76
		EAQ	8.65	0.397	10529	4.15
RP-18	70% CH_3CN	AQ	6.36	0.233	16417	-
		MAQ	8.41	0.293	18170	7.76
		EAQ	10.71	0.377	17896	6.85

TABLE 3

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

COLUMN	MOBILE PHASE	COMPOUND	R_t	$W_{1/2}$	TPM	R
RP-2	60% MeOH	AQ	8.22	0.401	9273	-
		MAQ	10.77	0.502	10176	5.64
		EAQ	14.56	0.650	11121	6.57
RP-8	64% MeOH	AQ	8.76	0.427	9335	-
		MAQ	11.81	0.555	10013	6.21
		EAQ	15.18	0.692	10675	5.4
RP-18	76% MeOH	AQ	8.42	0.385	10571	-
		MAQ	11.94	0.532	11162	7.66
		EAQ	15.24	0.674	11305	5.45

TABLE 4

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

COLUMN	MOBILE PHASE	COMPOUND	R_t	$W_{1/2}$	TPM	R
RP-2	44% THF	AQ	10.36	0.458	11481	-
		MAQ	12.38	0.532	11985	4.1
		EAQ	16.36	0.659	13632	6.66
RP-8	40% THF	AQ	11.15	0.554	9303	-
		MAQ	13.86	0.654	9966	4.51
		EAQ	19.06	0.877	10460	6.78
RP-18	44% THF	AQ	9.64	0.436	10815	-
		MAQ	12.10	0.534	11346	5.05
		EAQ	16.23	0.698	11978	6.70

TABLE 5

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

COLUMN	MOBILE PHASE	COMPOUND	R_t	$W_{1/2}$	TPM	R
RP-2	50% CH_3CN	N	10.43	0.374	17364	-
		BP	13.9	0.491	17961	8.07
RP-8	55% CH_3CN	N	10.04	0.387	14866	-
		BP	13.41	0.511	15245	7.49
RP-18	60% CH_3CN	N	10.58	0.349	20307	-
		BP	14.33	0.471	20489	9.14

TABLE 6

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

COLUMN	MOBILE PHASE	COMPOUND	R_t	$W_{1/2}$	TPM	R
RP-2	64% MeOH	N	6.25	0.326	8109	-
		BP	8.21	0.401	9268	5.37
RP-8	75% MeOH	N	6.13	0.315	8403	-
		BP	7.86	0.383	9314	4.93
RP-18	80% MeOH	N	6.43	0.261	13375	-
		BP	8.22	0.329	13795	6.07

TABLE 7

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

COLUMN	MOBILE PHASE	COMPOUND	R_t	$W_{1/2}$	TPM	R
PR-2	44% THF	N	14.03	0.560	13902	-
		BP	19.32	0.750	14703	8.07
RP-8	44% THF	N	11.17	0.500	11056	-
		BP	14.92	0.661	11289	7.83
RP-18	44% THF	N	13.88	0.567	13180	-
		BP	19.03	0.770	13512	7.69

The separation of dimethyl and diethyl phthalates on RP-2, RP-8 and RP-18 using acetonitrile:water, methanol:water and THF:water was also achieved, Tables 8-10. A comparison of Tables 2-10 indicates that when THF is used as the modifier of the mobile phase there are two results: (a) the percentage of THF is lower than any of the other two organic components of the mobile phase; and (b) the percentage of composition of THF in the mobile phase does not change appreciably (∓4%) from column to column (RP-2, RP-8 or RP-18), or from one sample mixture to the other, quinones, phthalates or naphthalene and biphenyl (see Tables 4, 7 and 10).

It is also interesting to note that, in the case of the quinone mixture, with acetonitrile as the mobile phase, the highest TPM and lowest $W_{1/2}$ are obtained, while methanol and THF give lower TPM and higher $W_{1/2}$ values, Tables 2-4. The same is true in the case of naphthalene and biphenyl; however TPM, $W_{1/2}$ and R values for phthalates when THF was used were comparable for both RP-2 and RP-18, Table 7. Again when the phthalate mixture was analyzed

TABLE 8

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

COLUMN	MOBILE PHASE	COMPOUND	R_t	$W_{1/2}$	TPM	R
RP-2	50% CH_3CN	MP	5.56	0.235	12306	-
		EP	8.08	0.317	14398	9.1
RP-8	55% CH_3CN	MP	4.83	0.218	10885	-
		EP	7.12	0.291	13240	8.96
RP-18	60% CH_3CN	MP	4.17	0.178	12131	-
		EP	6.12	0.239	14424	9.29

TABLE 9

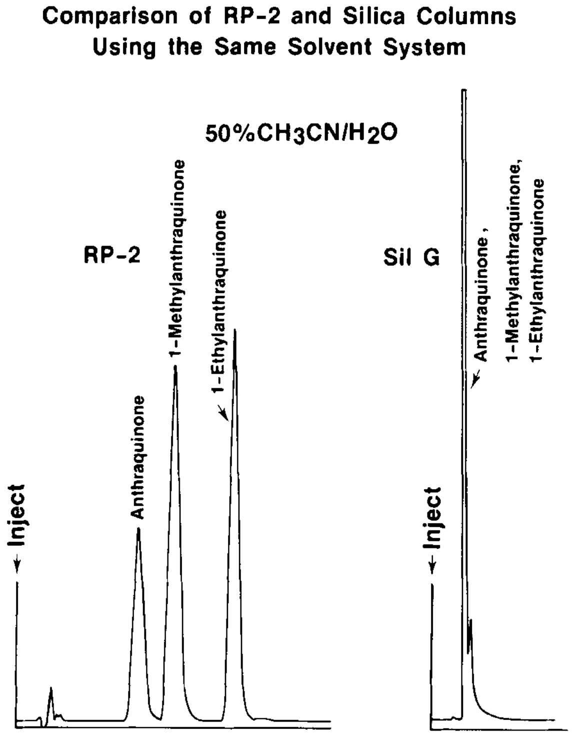
COMPARATIVE SEPARATION OF DIMETHYL AND DIETHYLPHTHALATES ON RP-2, RP-8 AND RP-18 USING METHANOL/WATER

COLUMN	MOBILE PHASE	COMPOUND	R_t	$W_{1/2}$	TPM	R
RP-2	56% MeOH	MP	5.59	0.293	8035	-
		EP	8.73	0.418	9671	8.82
RP-8	56% MeOH	MP	4.82	0.279	6624	-
		EP	7.98	0.402	8727	9.27
RP-18	64% MeOH	MP	5.03	0.253	8739	-
		EP	9.39	0.424	10835	12.84

TABLE 10

COMPARATIVE SEPARATION OF DIMETHYL AND DIETHYLPHTHALATES ON RP-2, RP-8 and RP-18 USING TETRADYDROFURAN/WATER

COLUMN	MOBILE PHASE	COMPOUND	R_t	$W_{1/2}$	TPM	R
RP-2	48% THF	MP	4.64	0.284	5909	-
		EP	6.57	0.346	7985	6.12
RP-8	44% THF	MP	4.43	0.272	5900	-
		EP	6.35	0.338	7800	6.26
RP-18	48% THF	MP	3.82	0.225	6406	-
		EP	5.29	0.276	8094	5.83



Comparison of RP-2 and Silica Columns Using the Same Solvent System

FIGURE 4

using the three columns and the three organic modifiers, the highest values for $W_{1/2}$ and TPM were obtained when acetonitrile was used, Table 8. Note that R, $W_{1/2}$ and TPM values are almost identical using RP-2 or RP-8, Table 8. In the case of THF, Table 10 the retention times are lower and that may account for low TPM values.

To test its reverse-phase properties, an RP-2 column was compared with a silica gel column. In Figure (4) the results obtained on both columns are shown. It is clear that RP-2 gives better resolution under the same experimental conditions. The order of elution of the quinones, phthalates, naphthalene and biphenyl on RP-2 was the same as when RP-8, and RP-18 columns were used. These results agree with those reported by Van Der Wal and Huber (9) for the separation of estrogen conjugates.

CONCLUSION

It is clear from the results that acetonitrile/water gives better TPM values for the compounds tested, and that 40-50% THF/water is an adequate mobile phase for the resolution of the three tested mixtures on either RP-2, RP-8 or RP-18 columns.

It was surprising that the RP-2 column gave such good results. It is also obvious, except in the case of THF, that the comparison of reverse phase columns of various alkyl chain length under the same mobile phase conditions, can result in erroneous chromatographic values, R, $W_{1/2}$ and TPM. In certain cases altering the ratios of the binary mobile phase can give base line separation of a mixture.

The results also show, that TPM is a function of the column, the solvent, and the sample used if other parameters are kept constant.

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