

Guidelines for Formulating Mobile Phases for Various Reversed Phase HPLC Columns

Different manufacturing processes are used to produce packings for octadecylsilyl HPLC columns. The silica base surface area of these columns also differs, according to base coverage with the reversed phase. These factors dictate that different brands of columns require different proportions of solvent and water in mobile phases used for the same analysis. These factors also cause variation in the column-to-column performance among columns of any one brand.

Methanol-, acetonitrile-, and tetrahydrofuran-water mobile phases needed to elute benzene derivatives are compared for eight octadecylsilyl columns in this bulletin. Chromatographers can use these data as guidelines for formulating new mobile phases when they adapt their analyses to highly uniform SUPELCOSM LC-18 columns.

Key Words:

- Mobile phase ● octadecylsilyl ● ODS

Chromatographers often must spend time readjusting analytical conditions when they install a new octadecylsilyl (ODS) column in their HPLC system, even if the new column replaces one from the same manufacturer. The column-to-column performance of SUPELCOSM ODS columns is very uniform, but some analysts have hesitated to switch to these columns because they don't know how much time and effort are required to reformulate the analytical conditions. This bulletin explains why ODS columns perform differently, and provide time-saving guidelines for adapting analyses from other ODS packings to SUPELCOSM LC-18 packings.

Why Don't All ODS Columns Perform Identically?

The carbon content (phase loading) of an ODS packing affects the concentration of the organic component needed in an aqueous mobile phase to elute a specific analyte. Two brands of ODS columns require different mobile phases for the same analysis, primarily because they differ in the surface area of their silica base and to the extent the base is covered with the reversed phase. For example, a uniform brush monolayer of 3.4 $\mu\text{moles}/\text{m}^2$ of dimethyloctadecylsilane on SUPELCOSM LC-Si silica (170 m^2/g) and on Spherosil[®] XOA 600 silica (549-660 m^2/g) would produce almost a three-fold difference in the carbon content of the packings (approximately 12% and 34%, respectively). Packings with a small surface area and low degree of carbon coverage (Spherisorb ODS), a small surface area and high carbon coverage (SUPELCOSM LC-18), and other combinations are available. The carbon content of some common ODS packings ranges from approximately 7% to 20% (Table 1).

Differences in the column-to-column performance of a particular brand of ODS packing result from inconsistencies in applying the phase to the silica base. The use of di- or trichloroalkyl silanes will result in polymerization unless water is completely removed from the base silica and the reaction media. Such coverage cannot be exactly reproduced, and batch-to-batch variability in packing performance is the result. If the coverage of the silica surface is not complete, and a capping reaction is not included in the manufacturing process, the resulting HPLC packing material will have accessible surface silanol groups. Separations on a column filled with such a packing will occur through a mixture of normal phase and reversed phase processes. In contrast, monochlorodimethylalkyl silanes are used to manufacture SUPELCOSM LC-1, LC-8, LC-18, and LC-CN packings. This material does not polymerize, and it produces a uniform brush monolayer covering on the silica base. Trimethylchlorosilane is used in a final, capping step for SUPELCOSM packings. The product is a chemically uniform HPLC packing which is very reproducible from batch to batch.

Differences in the surface area and reversed phase coverage of commercial ODS columns have two consequences. First, because most ODS packings exhibit batch-to-batch variability, the mobile phase used for a particular analysis often must be adjusted when a new column is used. Second, the chromatographer who is transferring a separation from one ODS packing to another usually must adjust the mobile phase by trial and error. When an investigator using a column with mixed chromatographic properties changes to a true reversed phase column (such as SUPELCOSM LC-18), it may be difficult or impossible to formulate a mobile phase that provides exactly the same separations for all possible samples as the original column and mobile phase. The effort required to establish slightly different, but equal, true reversed phase separations will be rewarded with far greater column-to-column reproducibility.

How Mobile Phases Used with Different ODS Columns Were Compared

The capacity factor, k' , \uparrow expresses the ratio of distribution of a compound between the stationary phase and the mobile phase in an HPLC column. The k' for a compound will vary, depending on the characteristics of the stationary phase from which the compound is eluted, and on the concentration of the organic component in the mobile phase. If k' is held constant when a compound is eluted from two or more columns, the mobile phases needed for constant analysis times can be compared. If several compounds with different characteristics are eluted at the same k' from two or more columns, then generalizations may be made about the mobile phases needed with each column.

\uparrow The k' value is obtained from the following formula: $k' = t_r - t_0 / t_0$, where t_0 is the retention time of an unretained peak and t_r is the retention time of a retained peak.

For practical purposes, compounds of interest usually are eluted within the k' range of 1 to 10. In the following comparison of SUPELCOSIL LC-18 and seven ODS packings from other manufacturers, we eluted each of six standards from each packing with a series of organic solvent:water mixtures (i.e., 30, 40, 50, and 60% organic solvent). The organic component:water mixture at which the k' equaled an arbitrarily chosen value of 3 was determined for each standard, with each packing, using three different organic solvents.

Single columns of SUPELCOSIL LC-18, Spherisorb ODS, Chromosorb® LC-7, Nucleosil® C18, LiChrosorb® RP-18, and Zorbax® BP-ODS were packed in Supelco laboratories from materials available commercially. Single Partisil 5 ODS and μ Bondapak® C18 columns were obtained from their manufacturers. These materials were assumed to be representative of the eight chosen packings. Several important characteristics of each packing were determined under standardized conditions and are presented in Table 1. These data indicate that the eight ODS packings can be expected to differ in performance.

Benzene (C_6H_6), phenol (C_6H_5OH), acetophenone ($C_6H_5COCH_3$), nitrobenzene ($C_6H_5NO_2$), methyl benzoate ($C_6H_5CO_2CH_3$), and toluene ($C_6H_5CH_3$), were eluted individually from each of the columns. These compounds were used as the analytical standards because each has a distinctive functional group and is easily detected by UV at 254nm. Methanol:water, acetonitrile:water, and tetrahydrofuran:water mobile phases were selected because they are commonly used mixtures. Mobile phases were generated using a Spectra-Physics 8000 liquid chromatograph, and the reproducibility of k' values was verified.

How Different Are the Mobile Phases Needed with Different ODS Columns?

When methanol:water and acetonitrile:water mobile phases were used, the benzene derivatives eluted from the ODS packings in similar patterns. Although there were exceptions among a few of the compounds, progressively more methanol or acetonitrile in water generally was required to elute the analytes from Spherisorb, Chromosorb, μ Bondapak, SUPELCOSIL, LiChrosorb, Partisil, Nucleosil, and Zorbax BP-ODS, respectively. ■

The percentages of methanol or acetonitrile in water needed to elute each of the six benzene derivatives from each of the ODS

packings, at a k' of 3.0, are shown in Figures A1 and A2, respectively. Some of the benzene derivatives were eluted from two or more of the packings with very similar mobile phases. In extreme cases, however, differences of 15-17% in the concentration of methanol or acetonitrile in water were required to elute a derivative from two packings. For example, little or no change in mobile phase composition would be required to transfer an analysis for toluene from μ Bondapak or Chromosorb to SUPELCOSIL LC-18 (Figures A1 and A2). The organic component needed to transfer such an analysis from Spherisorb to SUPELCOSIL, however, would increase by 5-7%. From Zorbax BP-ODS to SUPELCOSIL, it would decrease by approximately 10%.

When tetrahydrofuran:water mobile phases were used and the packings were ranked by the amount of organic component required to elute the derivatives, a different sequence occurred (Figure A3). The maximum difference in the tetrahydrofuran concentration needed to elute a specific derivative from two packings was 9-10%.

In methanol:water or acetonitrile:water mobile phases, phenol eluted from each ODS packing with the lowest concentration of organic component, followed (with a few exceptions) by acetophenone, nitrobenzene, methyl benzoate, benzene, and toluene, in that order (Figures A1 and A2). The order in which these compounds eluted with tetrahydrofuran:water mobile was different (Figure A3). In tetrahydrofuran:water mobile phases, methyl benzoate eluted from each packing at a lower percentage of organic component than did nitrobenzene. Acetophenone generally eluted at a lower tetrahydrofuran concentration than did phenol. Additional data (not shown) indicate that reversal of the acetophenone-phenol elution order could be obtained with Zorbax BP-ODS or with several of the other packings.

The mean difference in the methanol-, acetonitrile- or tetrahydrofuran-water mobile phases used with SUPELCOSIL LC-18 and any other ODS packing in this comparison can be estimated from Figures A1, A2, or A3, respectively. This mean difference is approximately the extent of the mobile phase adjustment needed to adapt an analysis from another packing to SUPELCOSIL LC packing.

The Importance of Determining Component Separations

Three factors are important to a chromatographer comparing different ODS columns: the mobile phase composition needed

Table 1. Characteristics of Different Manufacturers' ODS Packings

Column*	Carbon Content (%)	Theoretical PPlates/ meter (103)	Asymmetry at 10% of peak Height	Back Pressure (psig)	Particle Diameter
SUPELCOSIL LC-18	10.76	73	1.03	950	5 μ m (spherical)
Spherisorb ODS	7.33	71	1.33	1290	5 μ m (spherical)
Chromosorb LC-7	12.90	39	1.12	1500	5 μ m (irregular)
Nucleosil C18	15.28	80	1.29	1800	5 μ m (spherical)
LiChrosorb RP-18	20.13	54	1.18	1830	5 μ m (irregular)
Zorbax BP-ODS++	13.86	63	1.16	850	7-8 μ m (spherical)
Partisil 5 ODS	10	45	1.51	2710	5 μ m (irregular)
μ Bondapak C18	10	10	1.46	1690	10 μ m (irregular)

+ Column dimensions: Partisil 250 x 3.9mm, μ Bondapak 300 x 4.6mm, all others 150 x 4.6mm. Column characteristics were determined under the following conditions: Mobile Phase: Methanol:water, 66:34 (v/v), Flow Rate: 1mL/min.

++ Theoretical plates/meter and asymmetry values for the first column filled by Supelco with Zorbax BP-ODS packing were 52 x 103 and 1.47 respectively. Typical production values for a 150mm x 4.6mm column are given in the table.

■ This order was obtained from a rank-order analysis.

Figure A. Organic Solvent Concentrations Needed to Elute Benzene Derivatives at a k' of 3 from Various ODS Packings

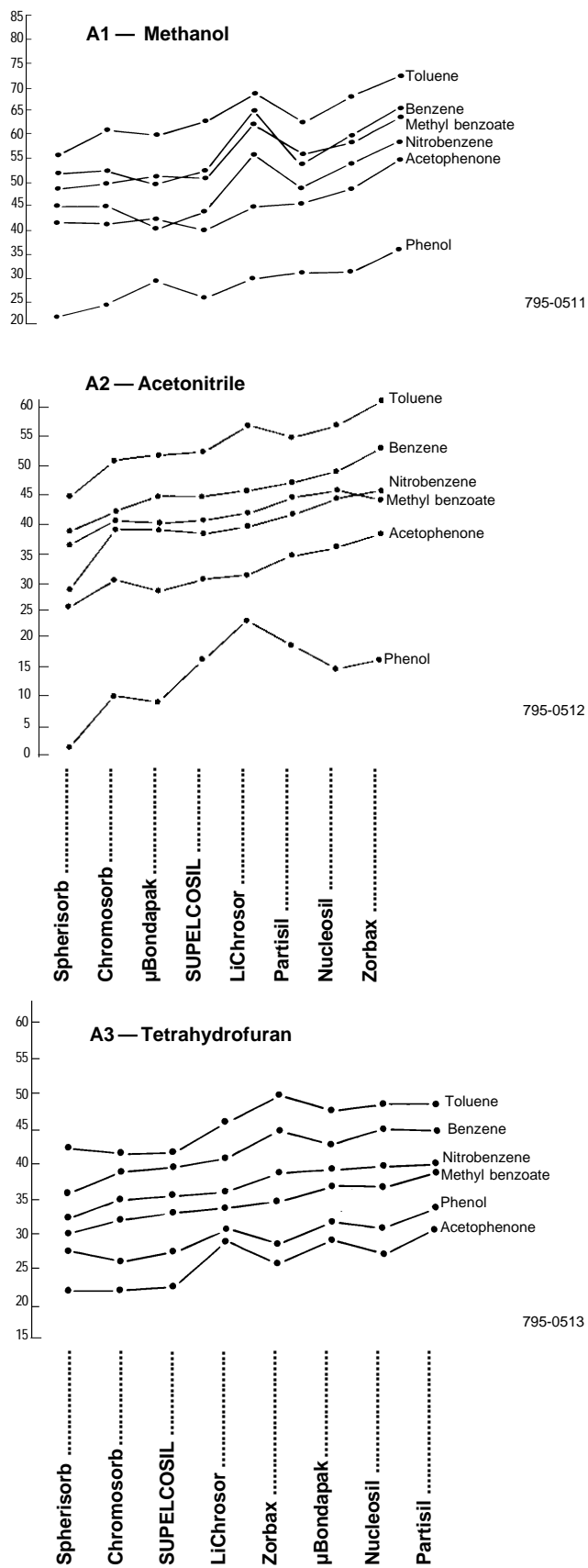
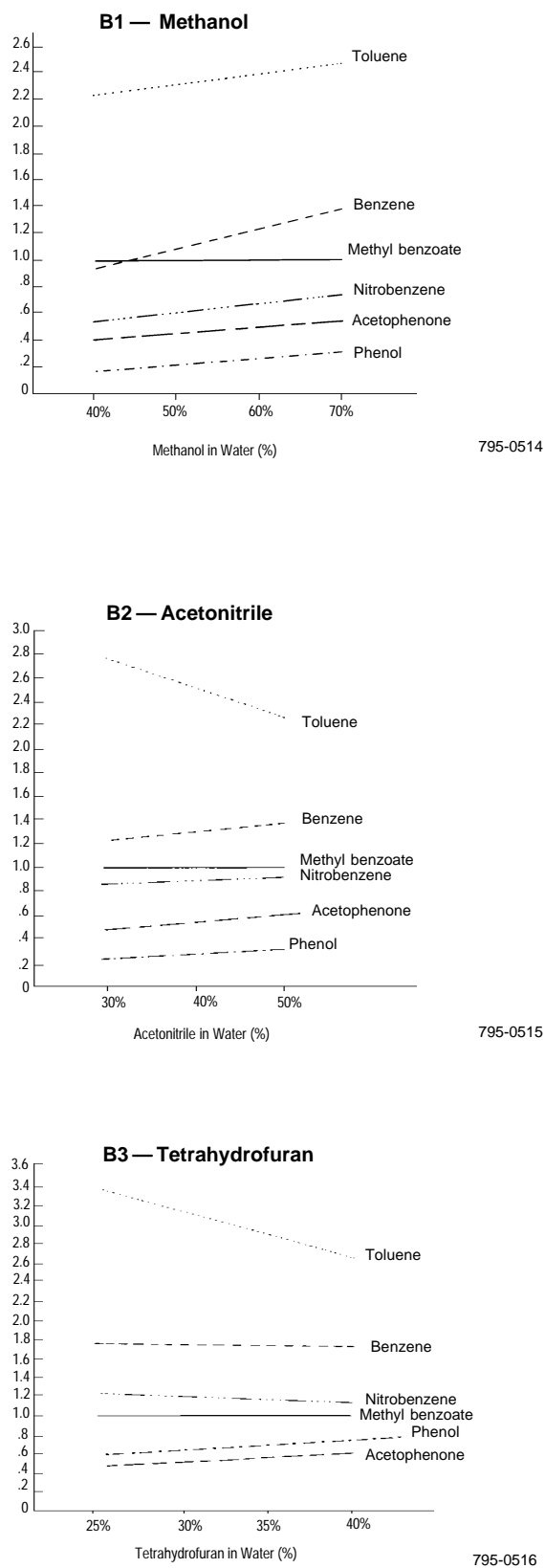


Figure B. Selectivity of Benzene Derivatives (Relative to Methyl Benzoate) at Various Mobile Phase Compositions*



*For a SUPELCOSIL LC-18 column.

elute analytes in reasonable time, the separation among analytes, and the elution order of analytes. The data in Figure A illustrate only one aspect of selecting a useful mobile phase (i.e., analysis time). Increasing the percentage of the organic constituent in the mobile phase reduces analysis time, but it also reduces resolution. Therefore, rapid, high quality separations usually are best achieved with the column that requires the least organic constituent to elute a compound in a given period of time.

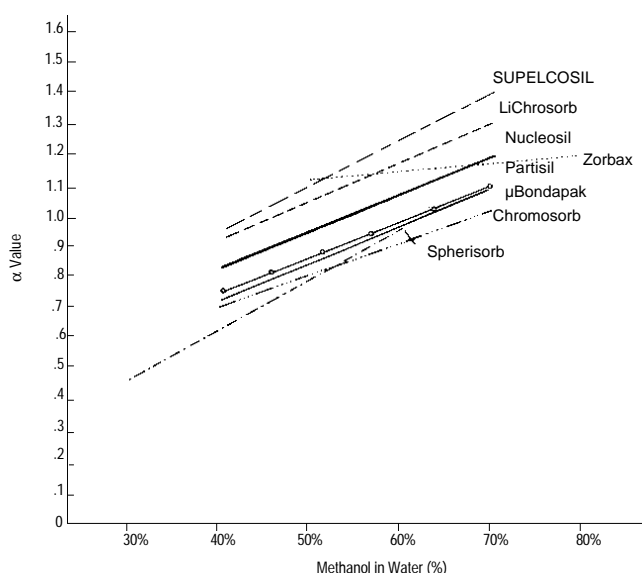
The separation of analytes is conveniently discussed in terms of the selectivity factor, α , between a specific reference analyte and each of the other components of the sample. In the mixture of benzene derivatives used for these examples, methyl benzoate was considered the reference analyte, and $\alpha = k'_x/k'$ methyl benzoate, where k'_x represents the k' of any other derivative. The α values for these derivatives on a SUPEL COSIL LC-18 column are plotted against the percentage of methanol in water (Figure B1), acetonitrile in water (Figure B2), and tetrahydrofuran in water (Figure B3). In general, the α values shift toward 1.0 (no separation) as the percentage of the organic constituent is progressively increased. For instance, in Figure B1 we can see that at about 43% methanol the separation factor for benzene and methyl benzoate is equal to 1. Thus, no separation is obtained. The intersection between benzene and methyl benzoate (Figure B1) should also alert the analyst to the possibility that reversals in peak order can accompany changes in mobile phase composition. Only intersections occurring at mobile phase compositions that produce reasonable analysis times, however, are of concern.

The α values for each analyte are relatively similar for all eight ODS packings with each mobile phase mixture used (Table 2).

Figures A1, A2, and A3 provide first approximations of the mobile phase modifications necessary when converting analyses from other ODS packings to SUPEL COSIL LC-18 packings. However, reversals in the elution order of sample components can occur when the mobile phase composition is changed (especially if the packing has accessible surface silanol groups). To determine if this will happen with a specific analysis compare, on the original column, selectivity factors and elution order of a polar and nonpo-

lar component of the mixture you wish to separate on SUPEL COSIL LC-18 column. This comparison will indicate if the sample components will elute in a different order with the mobile phase intended for use with the SUPEL COSIL column. As an example, a values for a polar (methyl benzoate) and a nonpolar (benzene) compound used in this study are plotted in Figure C for each of the eight ODS packings. Figure C shows that, in general, crossover points ($\alpha = 1.0$) in the elution order of benzene and methyl benzoate occur at 40-65% methanol with these ODS columns. Thus, you might separate benzene and methyl benzoate on a Nucleosil column with a methanol:water mixture of 55:45. If you now want to separate these compounds on a SUPEL COSIL LC-18 column, and determine (from Figure A1) that a 50:50

Figure C. Selectivity Factors (α) for Benzene vs. Methyl Benzoate on ODS Columns



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Table 2. Selectivity Factors for Benzene Derivatives (Relative to Methyl Benzoate) on Eight ODS Columns

Mobile Phase (%) Methanol in Water	C ₆ H ₅ OH [▲]		C ₆ H ₅ COCH ₃		C ₆ H ₅ NO ₂		C ₆ H ₆		C ₆ H ₅ CH ₃	
	α Range	Average	α Range	Average	α Range	Average	α Range	Average	α Range	Average
40	0.15-0.18	0.17	0.38-0.50	0.43	0.48-0.60	0.54	0.66-0.90	0.76	1.49-2.17	1.74
50	0.19-0.23	0.22	0.45-0.51	0.49	0.57-0.71	0.63	0.74-1.10	0.94	1.45-2.40	1.98
60	0.23-0.28	0.26	0.50-0.64	0.55	0.67-0.79	0.73	0.97-1.28	1.10	1.71-2.56	2.07
70	0.26-0.31	0.29	0.50-0.60	0.56	0.70-0.85	0.76	1.02-1.33	1.13	1.67-2.40	1.93
% Acetonitrile in Water										
30	0.20-0.26	0.22	0.48-0.58	0.51	0.83-0.91	0.87	1.00-1.33	1.12	1.80-2.72	2.30
40	0.20-0.29	0.27	0.55-0.63	0.57	0.84-0.96	0.91	1.11-1.33	1.21	1.86-2.50	2.18
50	0.23-0.33	0.30	0.59-0.68	0.62	0.84-0.95	0.91	1.09-1.33	1.21	1.61-2.24	1.95
60	0.22-0.39	0.32	0.63-0.70	0.66	0.81-0.94	0.87	1.13-1.30	1.21	1.67-2.13	1.84
70	0.26-0.41	0.33	0.65-0.73	0.69	0.76-0.91	0.84	1.16-1.23	1.20	1.66-1.90	1.78
% Tetrahydrofuran in Water										
25	0.60-0.66	0.63	0.47-0.50	0.49	1.21-1.26	1.24	1.47-1.77	1.60	2.75-3.48	3.04
30	0.64-0.69	0.67	0.49-0.53	0.51	1.20-1.28	1.25	1.62-1.83	1.70	2.68-3.35	2.97
35	0.56-0.71	0.67	0.52-0.62	0.56	1.17-1.25	1.21	1.55-1.94	1.70	2.30-3.19	2.68
40	0.64-0.72	0.68	0.59-0.68	0.61	1.15-1.26	1.19	1.52-2.08	1.72	2.12-3.21	2.54
45	0.62-0.72	0.68	0.60-0.65	0.63	1.12-1.16	1.14	1.56-1.95	1.72	2.19-2.86	2.44
50	0.60-0.73	0.67	0.63-0.68	0.66	1.10-1.13	1.11	1.58-1.89	1.69	2.08-2.64	2.30

▲ Zorbax BP-ODS packing is not included in the data for Phenol (C₆H₅OH) with methanol:water mobile phases.

methanol:water mixture will do so, you may find the compounds elute in reversed order. If reversals are not acceptable, it may be necessary to modify the mobile phase to be used with the SUPELCO column.

Applying the Comparative Data to a Practical Analysis

The information obtained was subsequently used to determine the appropriate mobile phases for separating seven barbiturates on each of the ODS packings. On the SUPELCO LC-18 column, this separation requires a methanol:water mobile phase of approximately 50:50 (v/v). The mobile phase required for each of the other packings was easily determined, based on the average deviation from the SUPELCO column mobile phase composition (Figure A1). The resulting chromatograms are shown in Figures D1 to D8. In addition to demonstrating that analyses can be adapted from one ODS column to another, Figure D illustrates the high quality of the separation by the SUPELCO LC-18 column. The column-to-column reproducibility of SUPELCO LC-18 columns will amply repay for the experimentation required to make this transfer.

A Word of Warning

The single samples of ODS packings or columns used to obtain these data may not represent every lot of packing from each manufacturer. Therefore, the data provide only an approximation of the required change in mobile phase composition when switching from other manufacturers' ODS packings to SUPELCO packings. These data are provided solely as a guide to the analyst who wishes to use SUPELCO HPLC columns because of their high degree of uniformity.

Figure D. Mobile Phase Compositions Adjusted to Achieve Nearly Constant Barbiturate Analysis Times

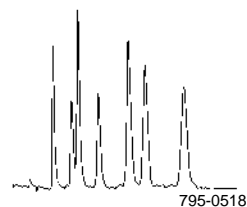


Figure D8
Zorbax BP-ODS, 150 x 4.6mm,
Mobile Phase: methanol:water,
53:47 (v/v), Flow Rate: 1 mL/min

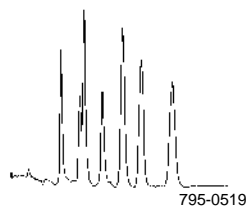


Figure D7
Nucleosil C-18, 150 x 4.6mm,
Mobile Phase: methanol:water,
53:47 (v/v), Flow Rate: 1 mL/min



Figure D6
Partisil PXS ODS, 250 x 4.6mm,
Mobile Phase: methanol:water,
53:47 (v/v), Flow Rate: 1.7 mL/min

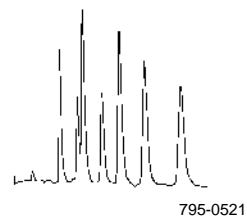


Figure D5
LiChrosorb RP-18, 150 x 4.6mm,
Mobile Phase: methanol:water,
52.3:47.7 (v/v), Flow Rate:
1 mL/min

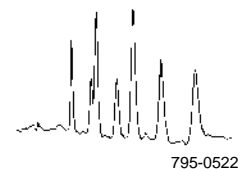


Figure D4
Mobile Phase: water: methanol,
μBondapak, C18, 300 x 3.9 mm,
51.5:48.5 (v/v), Flow Rate:
1.4 mL/min

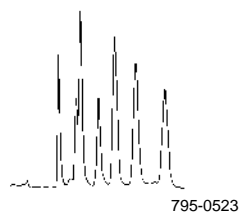


Figure D3
Chromosorb LC-7, 150 x 4.6 mm,
Mobile Phase: methanol:water,
50.5:49.5 (v/v), Flow Rate:
1 mL/min

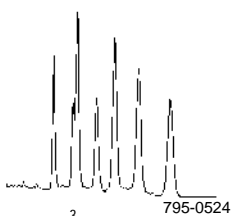


Figure D2
Spherisorb ODS, 150 x 4.6mm,
Mobile Phase: water:methanol,
54:46 (v/v), Flow Rate: 1 mL/min

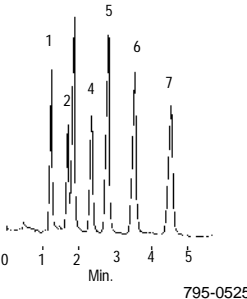


Figure D1
SUPELCO LC-18, 150 x
4.6mm, Mobile Phase: methanol:
water, 50.5:49.5 (v/v), Flow Rate:
1 mL/min

Detection: UV, 220nm
1cm = 1 min

Peak Order

1. Barbitol
2. Phenobarbital
3. Aprobarbital
4. Butethal
5. Mephobarbital
6. Amobarbital
7. Secobarbital

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