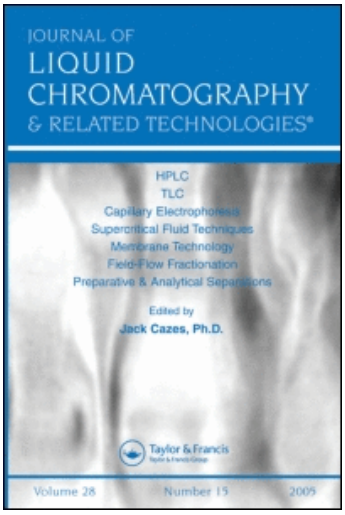


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Evaluation of Alkyl Bonded Silica and Solvent Phase Modifiers for the Efficient Elution of Basic Drugs on HPLC

Dennis W. Hill[a]
[a] Microchemistry Laboratory University of Connecticut Storrs, Connecticut

To cite this Article Hill, Dennis W.(1990) 'Evaluation of Alkyl Bonded Silica and Solvent Phase Modifiers for the Efficient Elution of Basic Drugs on HPLC', Journal of Liquid Chromatography & Related Technologies, 13: 16, 3147 — 3175
To link to this Article: DOI: 10.1080/01483919008049094
URL: http://dx.doi.org/10.1080/01483919008049094

JOURNAL OF LIQUID CHROMATOGRAPHY, 13(16), 3147-3175 (1990)

EVALUATION OF ALKYL BONDED SILICA AND SOLVENT PHASE MODIFIERS FOR THE EFFICIENT ELUTION OF BASIC DRUGS ON HPLC

DENNIS W. HILL
Microchemistry Laboratory
University of Connecticut
Storrs, Connecticut 06269-4193

ABSTRACT

Nine representative drugs were used to evaluate the effects of alkyl bonded stationary phases containing type A and type B silica and the effects of an amine modifier on the efficiency of high performance liquid chromatographic elution of basic and acidic drugs. The theoretical plate count and asymmetry factor of the eluted peaks were compared to that of acetophenone as a reference to the maximal efficiency of each system evaluated. Zorbax^R C_8 was used as the stationary phase prepared from type A silica and Zorbax RX^R was used as the stationary phase prepared from the type B silica. The theoretical plate count and asymmetry factor of acetophenone was observed to be the same on both columns when analyzed in an acidic aqueous/acetonitrile mobile phase. An improvement in the efficiency and peak shape of the amine containing compounds was observed using the Zorbax RX^R stationary phase as compared to the efficiency and peak shape of these compounds on the Zorbax^R C_8 stationary phase. Interestingly, the acidic compounds salicylic acid and mefenamic acid showed better peak shapes on the Zorbax RX^R column than on the Zorbax C_8. For all drugs studied the theoretical plate count and asymmetry factor was better on both the Zorbax^R C_8 and the Zorbax RX^R stationary phases when the amine modifier triethylamine was used in the mobile phase. Except for salicylic acid, the theoretical plate count and asymmetry factor for each drug was similar on the Zorbax^R C_8 and the Zorbax RX^R columns when the amine modifier

triethylamine was incorporated into the mobile phase. Analysis of this group of drugs in a solvent programmed systems showed similar peak symmetry as those observed in the isocratic systems of the same stationary and mobile phase conditions.

INTRODUCTION

Many of the drugs of interest in the regulatory analysis of biological samples have basic functional groups. Organic bases analyzed on alkyl bonded silica high performance liquid chromatographic (HPLC) columns generally elute in broad asymmetric bands (1, 2, 3, 4, 5, 6, 7). The degree of peak asymmetry and band dispersion varies between stationary phase materials produced by different manufacturers (1). These differences may reflect variations in the type of silica and/or the differences in the chemistry of alkylation of silica silanol groups that are used between manufacturers. It is commonly speculated that the poor chromatographic properties of basic compounds analyzed on these reversed phase columns is due to hydrogen bonding of the ionized basic functional groups of the solute to certain nonbonded silanol groups on the silica based stationary phase. The overall retention of basic compounds at a mobile phase pH that results in ionization of the solute may be due to a combination of solvophobic and ionic mechanisms. A variation in the quantitative contribution of these two mechanisms between alkyl bonded silica columns may result in the different retention characteristics of ionized basic compounds analyzed on columns of similar carbon load.

Kohler and Kirkland (2) observed that certain commercially prepared alkyl bonded silica materials that exhibited low base adsorptivity (type B) were prepared from silica polymers that had a higher concentration and a more homogeneous distribution of silanol groups than silica polymers that were used to prepare certain commercial alkyl bonded phase silica materials that exhibited high base adsorptivity (type A). Following alkylation of type A and type B silica materials, associated and non-associated silanol groups were present on the bonded phase silica product. A correlation was observed between low base adsorptivity of an alkyl bonded silica phase and a high

concentration of associated silanol groups over isolated silanol groups. It was suggested that homogeneous distribution of silanol groups favored association of silanol functional groups which reduced the interaction of these groups with ionized basic solutes.

Stadalius et. al. (3) suggested that the adsorptive interaction of ionized basic solutes with a silica based alkyl bonded phase was due to an ion-exchange mechanism. Based on this assumption, conditions were suggested to improve HPLC peak symmetry of basic compounds on alkyl-bonded silica columns. One approach was to eliminate the solute-silica phase ion-exchange mechanism by: 1) reducing the number of acidic silanol groups on the silica stationary phase, 2) suppressing the ionization of the acidic sites on the silica phase by using a low mobile phase pH, and 3) reducing the interaction of the ionized solute with the ionized silanol group by including an inorganic or organic cation in the mobile phase at a concentration that would effectively displace the solute from the silica phase silanol. It was additionally suggested that one might prevent solute saturation of the ion-exchange sites on the silica phase by applying a smaller quantity of the solute to the column.

Kohler and Kirkland (2) have produced a silica polymer with a homogeneous distribution of silanol groups. The alkyl bonded phases prepared from this material have shown reduced adsorptivity of ionized organic base compounds when used as the stationary phase in reverse phase HPLC. This type of alkyl bonded phase was made available in a commercial product (Zorbax RX^R) produced by DuPont. A comparison of the acidity of 19 commercially available alkyl bonded phases (2) indicated that, of this group, Zorbax RX^R (octyl) was the least acidic and Zorbax^R (octyl) was the second most acidic.

A number of studies have shown varying degrees of success in the use of primary (5, 6), secondary (6), tertiary (1, 3, 4, 5, 6, 7) and quaternary (4, 5, 7) organic amine modifiers in the mobile phase to improve the symmetry of basic solutes in reversed phase HPLC analysis. Of the amine modifiers used, triethylamine (TEA) and N, N-dimethyl, n-octylamine (DMOA) appeared to be effective in enhancing peak symmetry and efficiency of many basic solutes.

The purpose of this study was to develop a reversed phase HPLC system using a mechanically efficient alkyl bonded silica phase column to elute basic drugs of a wide range of polarities in one system. This was to be accomplished by utilizing a solvent programmed mobile phase in which both the strong and weak solvent differed substantially in polarity. It was desirable to use a diode array detector to collect ultraviolet spectra of the eluting compounds for qualitative analysis, which required that the strong and weak solvent be reasonably transparent to ultraviolet radiation between 200 and 400 nm.

EXPERIMENTAL SECTION

Materials and Reagents

Source of Materials. Acetonitrile (Merck Ominisolv^R, AX0124-1) and o-phosphoric acid (Fisher A260-500) were obtained as HPLC grade materials. Triethylamine (Eastman, 616) was reagent grade quality. Reagent grade water was generated in a Millipore Milli-RO4/Milli Q^R reverse osmosis/ion-exchange/charcoal water purification system. Zorbax^R (880952.706) and Zorbax RX^R (880967.901) 250 mm x 4.6 mm,id HPLC columns were obtained from Mod-Mac Analytical Inc.

Standard Drug Solutions. Table I lists the drugs used in this study. Acetophenone was obtained from Aldrich Chemical Co., Ethylmorphine and Morphine were obtained from Alltech Applied Science and the remainder from Sigma Chemical Co. Individual aqueous solutions of each were prepared at a concentration of 500 µg/mL. Dissolution of some basic compounds (desipramine, imipramine and morphine) was aided by the addition of 50% H_2SO_4 to a final concentration of 1.25% H_2SO_4. Dissolution of the acid compounds (mefenamic acid, phenylbutazone and salicylic acid) was aided by the addition of saturated sodium borate to a final concentration of 10% saturated sodium borate.

A standard solution containing each of the ten drugs at a concentration of 500 µg/mL was prepared by dissolving 5.0 mg of each drug in 5.0 mL of methanol. One drop of concentrated NaOH was added to aid in the dissolution

of phenylbutazone and mefenamic acid and the total was diluted to 10.0 mL with reagent grade water.

Purification of Triethylamine. Neutral alumina (Merck, 1077) was placed in a beaker and washed 3 times with 2 bed volumes of pentane (Burdick & Jackson), 3 times with 2 bed volumes of methylene chloride (Burdick & Jackson) and 3 times with 2 bed volumes of methanol (Merck Ominisolv^R, MX0488-1). The solvent was allowed to evaporate from the alumina under a fume hood overnight. The alumina was then heated at 130° for 2 hrs and stored in a glass bottle. Preparative liquid chromatographic columns were prepared by dry packing a 29 cm x 2.2 cm, id glass chromatographic column with a 14 cm bed of the washed alumina. One head volume of methanol was allowed to pass through the column. Triethylamine was applied to the column and allow to elute through the column. When an amine odor was detected in the effluent, an additional twenty mL of solvent was allowed to pass through the column and was discarded. The next 20 mL of the triethylamine was collected and used immediately in the preparations of the HPLC solvents. Following the purification of 20 mL of triethylamine, the remaining material on the column was drained and discarded. The column was immediately washed with one head volume of methanol and stored for future use with approximately 2 mL of methanol above the alumina. An alumina column prepared and used in this manner has been used 5 times to purify triethylamine before preparation of a new column was necessary.

HPLC Solvent System 1. A 1.0% (v/v) solution of o-phosphoric acid was prepared by diluting 1.0 mL of concentrated o-phosphoric acid to 1 L with reagent grade water. Solvent 1A (0.1% H_3PO_4) was prepared by diluting 100.0 mL of the 1.0% o-phosphoric acid solution to 1 L with reagent grade water. Solvent 1B (0.1% H_3PO_4, 20% H_2O in CH_3CN) was prepared by diluting 100.0 mL of the 1.0% o-phosphoric acid solution and 100.0 mL of reagent grade water to 1 L with acetonitrile

HPLC Solvent System 2. Solvent 2A (0.15 M H_3PO_4, 0.05 M triethylamine) was prepared by diluting 10.0 mL of concentrated o-phosphoric acid and 7.0 mL of purified triethylamine to 1 L with reagent grade water. Solvent 2B (0.15 M H_3PO_4, 0.05 M triethylamine, 20% H_2O in CH_3CN) was

Table I. Model Drugs

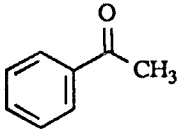
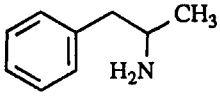
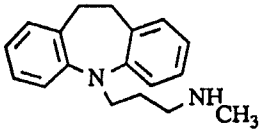
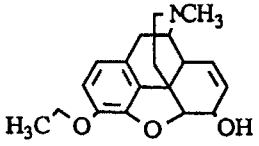
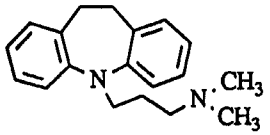
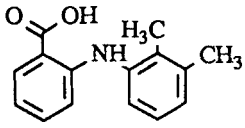
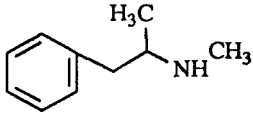
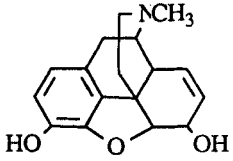
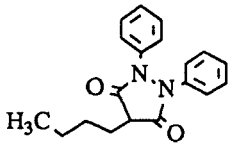
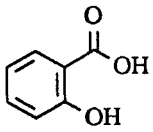
COMPOUND	STRUCTURE	ACTIVE HYDROGEN
Acetophenone		None
Amphetamine		1° Amine
Desipramine		2° & 3° Amine
Ethylmorphine		3° Amine & AA
Imipramine		2(3° Amines)

Table I. Model Drugs (cont.)

COMPOUND	STRUCTURE	ACTIVE HYDROGEN
Mefenamic Acid		2° Amine & CA
Methamphetamine		2° Amine
Morphine		3° Amine, AA, P
Phenylbutazone		Pyrazolidine
Salicylic Acid		P & CA

AA- Aliphatic Alcohol
CA- Carboxylic Acid
P- Phenol

prepared by diluting 10.0 mL of phosphoric acid, 7.0 mL of purified triethylamine and 200.0 mL of reagent grade water to 1 L with acetonitrile.

HPLC Systems

The HPLC system utilized in this study consisted of a Water's 6000A solvent delivery system (pump A) delivering solvent A, and a Water's Model 590 solvent delivery system (pump B) delivering solvent B to either a Zorbax^R C_8 or Zorbax RX^R reverse phase column. The solvent ratio was controlled by a Water's Model 720 system controller. Five μL sample injections were made using a Water's Model 710B autoinjector. The absorbance of the effluent from the column was monitored with a Hewlett-Packard 1040 diode array detector at a wavelength of 210 nm.

The Zorbax^R C_8 column was used in HPLC systems I and II and the Zorbax RX^R columns were used in HPLC systems III and IV. The mobile phases for HPLC systems I and III utilized solvents 1A and 1B; whereas, solvents 2A and 2B were used to generate the mobile phases in HPLC systems II and IV.

The solvent composition for the isocratic analysis of each compound was adjusted to give a capacity factor (k') of approximately 3 to 4. Because of the strong retention of imipramine and desipramine in system I a larger k' value was used for the evaluation of these two compounds in each system.

The nominal solvent flow rate was set at 2.0 mL/min. The actual flow rate of each pump using the solvent conditions of HPLC system I was measured as the time required to deliver 2.0 mL of the appropriate solvent. Under these conditions, pump A had a flow rate of 1.88 mL/min when delivering solvent 1A and pump B had a flow rate of 1.93 mL/min when delivering solvent 1B. When pump A and B were used in concert to generate an isocratic solvent composition, the flow rate of the system was calculated as a linear interpolation of the fractional contribution of the two pumps.

The void volume of the two columns was determined for each system by injecting 5 μL of formic acid at 100% delivery of solvent A and then at 100% delivery of solvent B. The void volume of the column was calculated as the average elution volume of the formic acid in the two solvent systems.

For solvent programmed analysis 5 μL of the drug standard mixture was injected on the respective HPLC systems (ISP, IISP, IIISP and IVSP) at a solvent composition of 100% A. A solvent gradient of 0 to 100% B in 30 min was generated at a flow rate of 2.0 mL/min.

Calculations

The retention volume of the compounds in the respective HPLC systems were calculated as the product of the flow rate and the retention time of the peak. The capacity factor (k') was then calculated from the void volume (V_0) and the retention volume (V_x) by the equation (8):

$$k' = (V_x - V_0)/V_0.$$

The theoretical plate count ($N_{5\sigma}$) of a peak was calculated from the retention time (t_R) and the peak width ($w_{4.4}$) at 4.4% of the peak height. These data were determined from the raw data by a computer program and the theoretical plates calculated by the equation (8):

$$N_{5\sigma} = 25(t_r/w_{4.4})^2.$$

The symmetry factor was calculated by a stock program in the Hewlett-Packard Diode Array applications program. The reciprocal of this value was taken as the asymmetry factor. Peak height sensitivity (S) was calculated for each drug as the ratio of the peak height response (H) to the peak area response (A):

$$S = H/A.$$

Analysis of Efficiency and Peak Shape

Under the appropriate isocratic solvent conditions, 5 μL of the 500 μg/mL solution of each model drug was analyzed in triplicate on HPLC systems I, II, III, and IV. The capacity factor, peak asymmetry factor, and

theoretical plate count were calculated for each chromatographic peak using the appropriate equation. The value of each of these parameters for each drug in each HPLC system was taken as the average of the three determinations.

In each of the solvent programmed systems (ISP, IISP, IIISP and IVSP) 5 μL of the 500 μg/mL mixture of each model drug was analyzed in triplicate. The peak asymmetry factors and peak height sensitivities were calculated for each chromatographic peak as the average for the three determinations.

RESULTS AND DISCUSSION

The mobile phase composition and the resultant capacity factors for each compound analyzed in each HPLC system evaluated in this study are listed in Table II. Except for imipramine and desipramine the capacity factors obtained between compounds are within 2.88 to 4.29. For the analysis of an individual compound between HPLC systems the range of capacity factor values is less than 0.55. The capacity factor range for desipramine and imipramine between HPLC systems is less than 1.3.

The average and standard deviation of triplicate analyses of the efficiency of elution, measured as the average theoretical plate count, and the peak shape, measured as the asymmetry factor, are listed in Tables III and IV, respectively, for each model compound analyzed in each HPLC system. To demonstrate the change in peak shape for representative model compounds in the evaluated HPLC systems, comparative chromatograms of morphine, desipramine, salicylic acid and acetophenone are diagrammed in Figures 1, 2 and 3.

To evaluate the peak shapes of the model compounds as they elute in a solvent programmed system, the asymmetry factor of each compound is compared in the solvent programmed system with the asymmetry factor in the isocratic system of the same solvent and column parameters (Table V). The effect of solvent and column type on the peak height sensitivity is compared for each compound analyzed in the solvent programmed system in Figure 4. A comparison of the solvent programmed chromtogram of the model

Table II. Solvent Composition and Capacity Factors

	%B[a]				k'[b]			
COMPOUND	[c]I	II	III	IV	I	II	III	IV
Acetophenone	47	47	45	46	3.25	3.22	3.33	3.26
Amphetamine	16	11	6	8	3.56	3.39	3.28	3.59
Desipramine	100	38	29	35	5.09	5.21	5.68	4.51
Ethylmorphine	21	15	11	14	4.29	3.66	4.05	3.95
Imipramine	100	38	29	34	6.05	5.91	6.31	5.78
Mefenamic Acid	76	76	72	72	3.41	3.25	3.46	3.26
Methamphetamine	21	13	7	10	3.90	3.51	3.95	3.75
Morphine	8	2	2	2	2.95	3.11	3.39	3.37
Phenylbutazone	74	74	71	71	3.49	3.44	3.49	3.42
Salicylic Acid	41	41	42	40	3.21	2.88	3.31	3.21

[a]Percent Composition of Solvent B in the Isocratic Mobile Phase
[b]Capacity Factor (Average of 3 Determinations)
[c]HPLC System as Described in Text

Table III. Theoretical Plate Count ($N_{5\sigma}$)

	[a]SYS I		SYS II		SYS III		SYS IV	
COMPOUND	N[b]	%CV[c]	N	%CV	N	%CV	N	%CV
Acetophenone	18560	4.2	18012	5.1	18655	1.8	17906	0.8
Amphetamine	767	8.1	10694	1.2	3239	1.9	10400	0.9
Desipramine	1039	8.5	12205	1.6	4810	3.2	10750	1.8
Ethylmorphine	728	4.9	10570	1.8	4875	2.0	9340	0.9
Imipramine	1080	13.1	12364	0.3	5029	1.8	10810	6.5
Mefenamic Acid	4496	1.1	10126	0.9	14444	0.4	14154	3.9
Methamphetamine	580	6.6	10549	0.8	2842	2.7	10063	1.2
Morphine	998	1.8	8144	4.1	2520	3.4	6995	6.2
Phenylbutazone	16052	2.5	15576	1.0	15448	0.6	15034	2.8
Salicylic Acid	499	1.1	2247	3.0	6813	2.7	12894	0.5

[a]HPLC System as Described in Text using Solvent system Listed in Table III

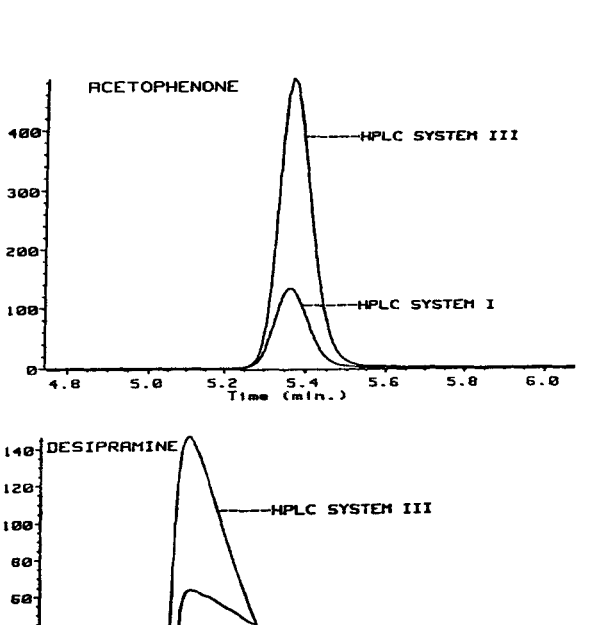
[b]Theoretical Plate Count (Average of 3 Determinations)

[c]Percent Coefficient of Variation of 3 Theoretical Plate Count Determinations

Table IV. Asymmetry Factor

	[a]SYS I		SYS II		SYS
COMPOUND	ASYM[b]	%CV[c]	ASYM	%CV	ASY
Acetophenone	1.17	0.9	1.16	1.0	1.1
Amphetamine	5.64	3.5	1.95	0.7	3.9
Desipramine	9.39	9.4	1.95	0.9	3.4
Ethylmorphine	5.32	2.1	1.44	0.0	3.2
Imipramine	9.48	16.7	1.94	1.2	3.2
Mefenamic Acid	3.27	1.5	1.76	0.6	1.3
Methamphetamine	5.51	4.5	2.00	1.0	4.1
Morphine	5.39	0.7	1.26	0.9	3.4
Phenylbutazone	1.13	0.9	1.13	0.5	1.1
Salicylic Acid	7.10	5.8	4.10	1.2	3.0

[a]HPLC System as Described in Text using Solvent Sy
III
[b]Asymmetry Factor (Average of 3 determinations)
[c]Percent Coefficient of Variation in Asymmetry Fac



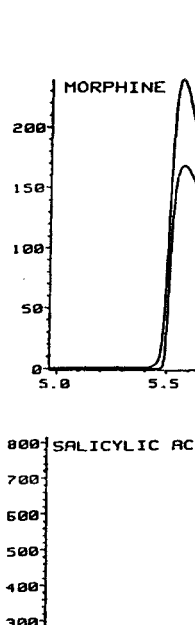


Figure 1. Comparison of the chromatographic peak shape
drugs analyzed in a phosphoric acid/aqueous/acetonitrile
system on a Zorbax C_8^R (HPLC SYSTEM I) and Zorba
SYSTEM III) column. The retention time scale is that of s
chromatogram of system I was reconstructed so as to alig
peaks in the two systems. (See Table I for isocratic solvent co

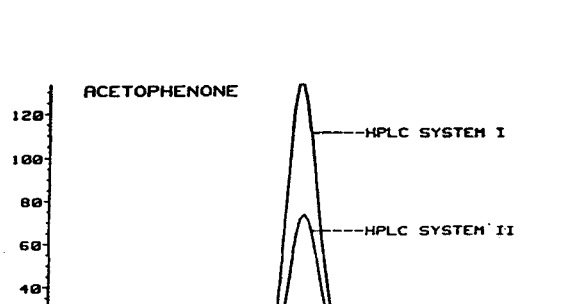
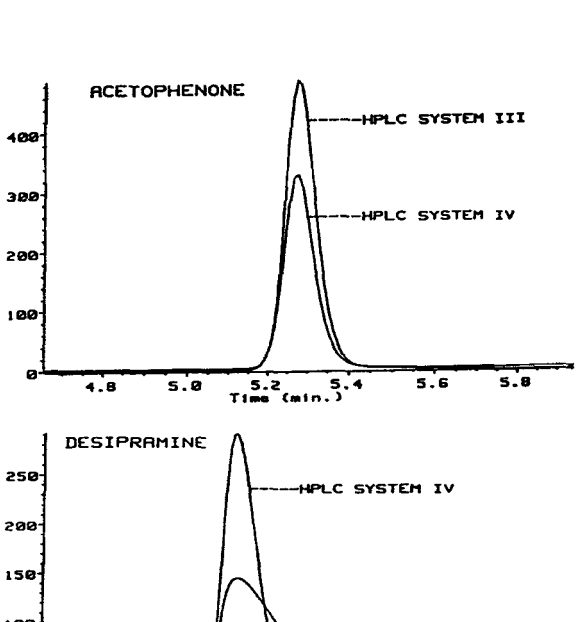


Figure 2. Comparison of the chromatographic peak shape drugs analyzed in a phosphoric acid/aqueous/acetonitrile system (HPLC SYSTEM I) and a acid/triethylamine/aqueous/acetonitrile system (HPLC SY Zorbax C_8^R column. The retention time scale is that of s chromatogram of system I was reconstructed so as to alig peaks in the two systems. (See Table I for isocratic solvent co



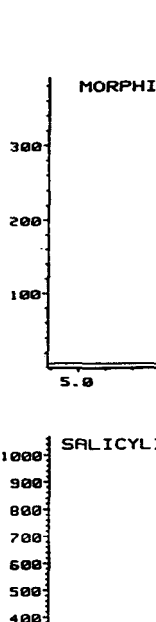


Figure 3. Comparison of the chromatographic peak shape
drugs analyzed in a phosphoric acid/aqueous/acetonitrile
system (HPLC SYSTEM III) and a
acid/triethylamine/aqueous/acetonitrile system (HPLC SYS
Zorbax RX^R column. The retention time scale is that of sy
chromatogram of system III was reconstructed so as to alig
peaks in the two systems. (See Table I for isocratic solvent co

Table V. Comparison of Asymmetry Factors of Compounds Eluted in the Isocratic and the Solvent Programmed HPLC Systems

COMPOUND	HPLC SYSTEMS							
	I	SPI	II	SPII	III	SPIII	IV	SPIV
Morphine	5.39	4.29	1.26	1.32	3.45	2.59	1.53	1.29
Amphetamine	5.64	6.67	1.95	2.06	3.92	3.53	1.90	1.81
Methamphetamine	5.51	6.53	2.00	2.11	4.14	3.83	2.00	1.78
Ethylmorphine	5.32	5.57	1.44	1.49	3.20	2.54	1.62	1.34
Salicylic Acid	7.10	2.91	4.10	1.85	3.05	3.53	1.81	1.74
Acetophenone	1.17	1.06	1.16	1.08	1.17	1.10	1.17	1.09
Desipramine	9.39	7.91	1.95	2.17	3.44	2.70	1.89	1.43
Imipramine	9.48	NC	1.94	2.44	3.23	3.10	1.96	1.50
Phenylbutazone	1.13	NC	1.13	1.08	1.16	1.14	1.15	1.12
Mefenamic Acid	3.27	NC	1.76	1.27	1.30	1.26	1.25	1.17

NC - Asymmetry Factor not calculated because of incomplete resolution from neighboring peak.

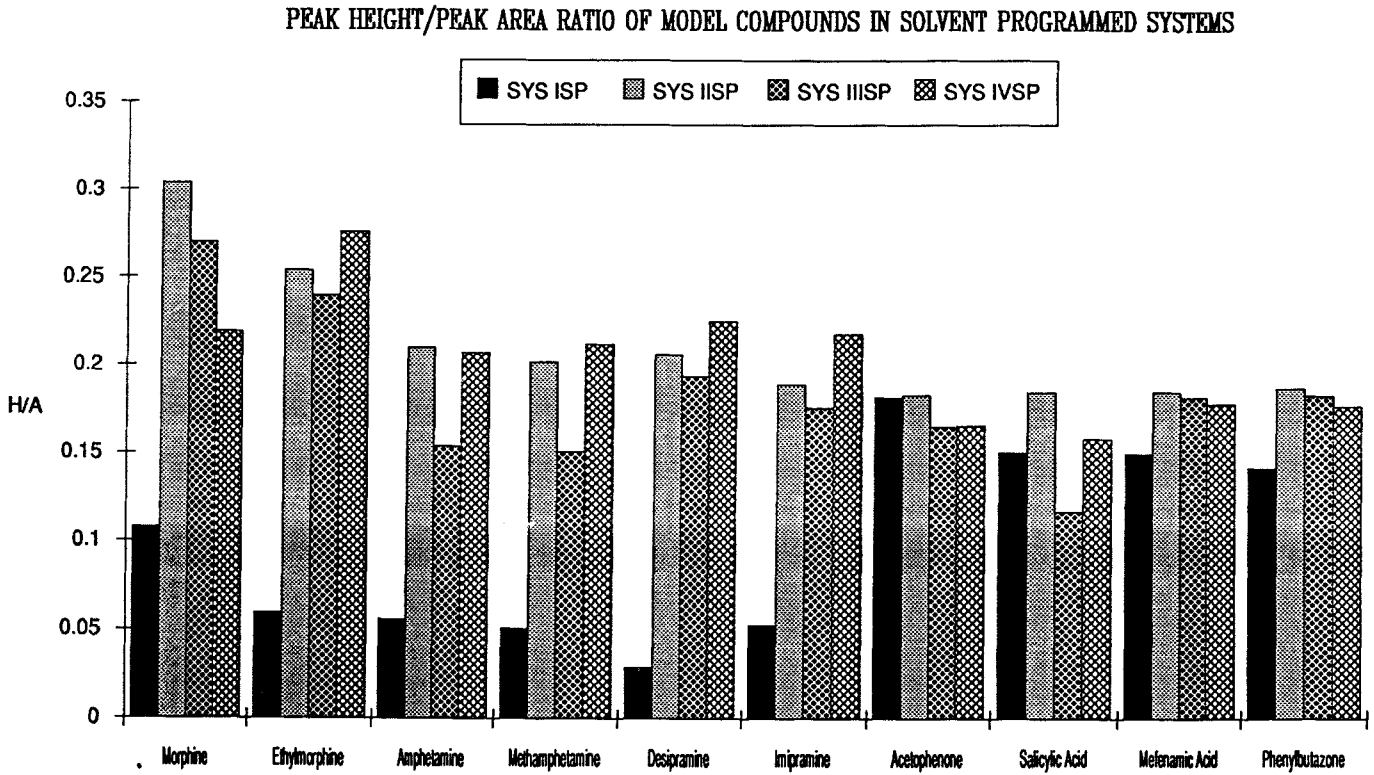


Figure 4. Comparison of peak height sensitivity (Peak Height(H)/Peak Area(A)) for model compounds analyzed on solvent programed systems SPI, SPII, SPIII and SPIV. (See Experimental Section for the conditions of the HPLC systems.)

compounds analyzed on the Zorbax^R C_8 column without triethylamine in the mobile phase is made to the chromatogram of the compounds eluted on the Zorbax RX^R column without triethylamine in the mobile phase (Figure 5) and the chromatogram of the compounds eluted on the Zorbax^R C_8 column with triethylamine in the mobile phase (Figure 6).

It has been demonstrated that a homogeneous distribution of isolated silanol groups on an alkyl bonded silica HPLC stationary phase favors ionic association between these groups and renders them less reactive with ionized basic solute molecules in a chromatographic analysis (2). The Zorbax RX^R packing material used in this study consisted of an isopropyl octyl ether linkage to the silanol groups of a silica polymer that was treated to generate a homogeneous distribution of surface silanol groups. Low adsorptivity of ionized organic bases on this phase may have been due to the fact that 1) most of the non-alkyl bonded silanol groups were close enough to each other to form an interatomic association and, thus, were unavailable for ionic interaction with ionized base solutes and 2) the bulky isopropyl group provided steric hindrance to the interaction of ionized solute molecules with the non-alkyl bonded silanol groups of the stationary phase. Zorbax^R C_8 reverse phase material was prepared from a silica polymer that had a heterogeneous distribution of isolated silanol groups and showed high adsorption of ionized organic bases.

In the present study, all of the basic solutes (amphetamine, desipramine, ethylmorphine, imipramine, methamphetamine and morphine) that were evaluated in the acid mobile phase without an amine modifier showed improvement in efficiency and peak shape on the Zorbax RX^R column (system III) as compared to the elution of these compounds on the Zorbax^R C_8 (system I) column. Salicylic acid and mefenamic acid showed poor peak symmetry and efficiency on the Zorbax C_8 column when analyzed in the mobile phase without the amine modifier. These compounds showed improved elution characteristic on the Zorbax RX column in the same mobile phase. Both of these compounds had a carboxylic functional group and mefenamic acid also contained a secondary amine in its structure. It was possible that the poor efficiency of salicylic acid (a metal chealator) was due

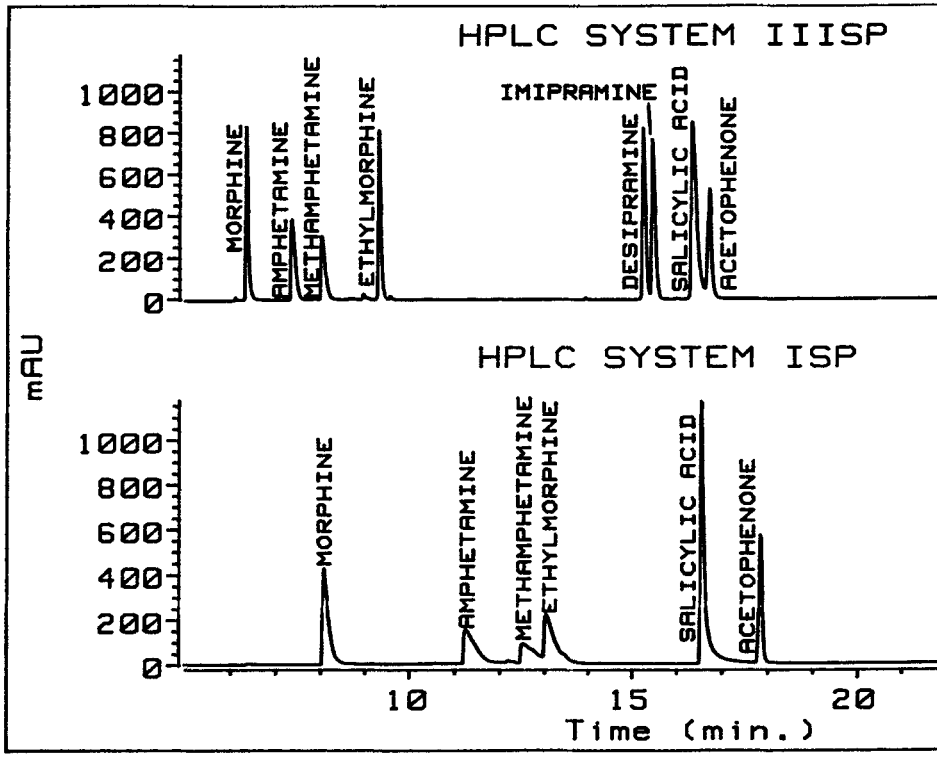


Figure 5. Chromatogram of the elution of model comp programmed HPLC systems IIISP and ISP. (See Experimen conditions of the HPLC systems.)

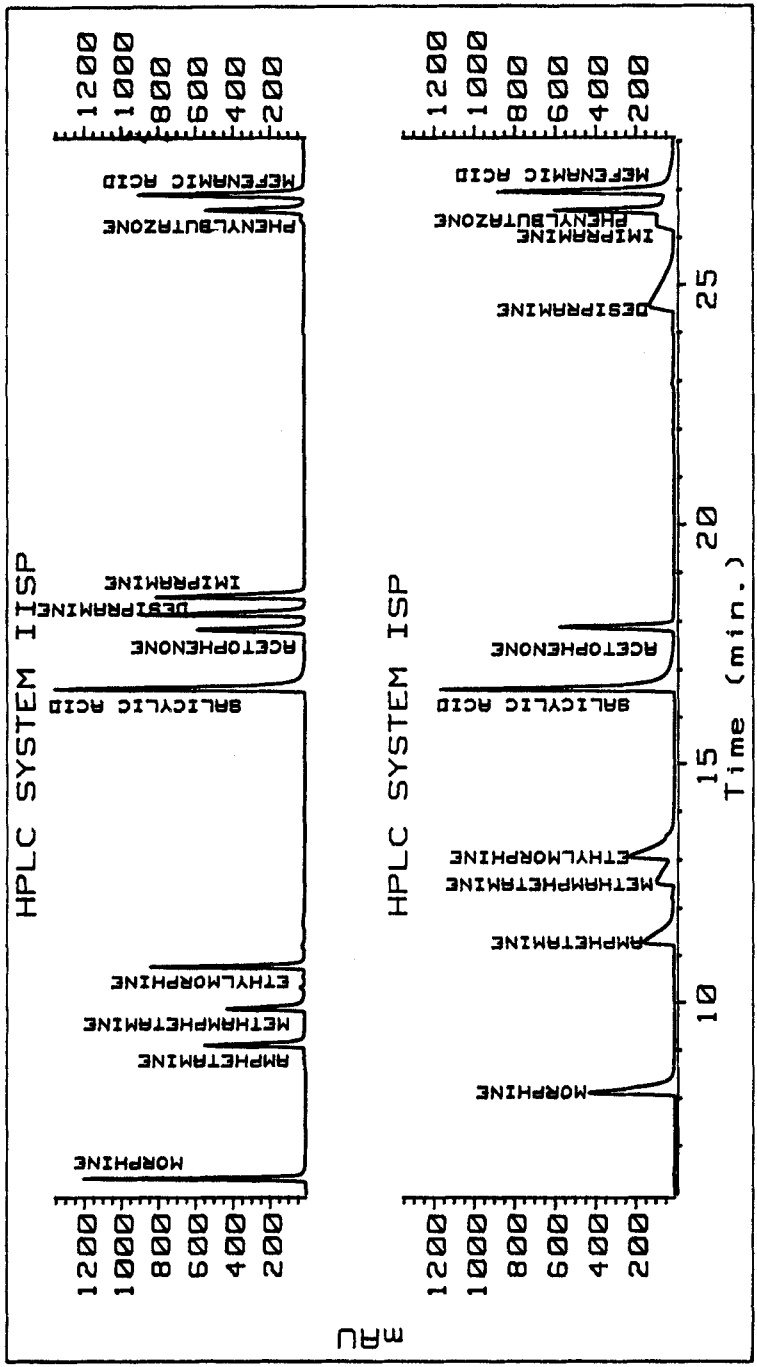


Figure 6. Chromatogram of the elution of model compounds in solvent programmed HPLC systems IISP and ISP. (See Experimental Section for the conditions of the HPLC systems.)

to the ionic interaction of this compound with metal ions on the silica of the Zorbax C_8 column material. A lower abundance of metal ions on the Zorbax RX^R column might account for the improvement in chromatographic efficiency on this material. However, at the low pH of the mobile phase (2.1), it would be expected that the ionization of salicylic acid (pK_a=3.5) would be suppressed and would therefore have minimal chealating interactions.

Acetophenone was expected to show good peak shape and efficiency in these reverse phase systems because its retention mechanism would be primarily due to a solvophobic mechanism. Therefore, the theoretical plate count and the asymmetry factor for acetophenone in each of the chromatographic systems served to monitor the mechanical efficiency of each column and was used as a reference for the maximum expected efficiency that could be obtained in the individual HPLC systems by a purely hydrophobic compound. In the HPLC systems using an acidic mobile phase without an amine modifier, acetophenone and the weakly acidic (pKa=4.4) phenylbutazone each had similar efficiency and peak symmetry on the Zorbax^R C_8 and the Zorbax RX^R columns. The data for these compounds suggested that a plate count of 15,000 to 18,000 and an asymmetry factor of 1.13 to 1.17 might be expected in these systems for compounds of similar molecular volume that were retained primarily by a solvophobic mechanism.

On the Zorbax^R C_8 column , using the mobile phase without the amine modifier, the plate count of the six basic organic compounds that were evaluated ranged from 580 to 1080. This was an average of 17,730 units less than the plate count for acetophenone in this system. On the Zorbax RX^R system, using the same mobile phase without the amine modifier, the plate count of the six basic organic compounds ranged from 2520 to 5029 with an average plate count 14,131 units less than the plate count for acetophenone in this system. Even though the basic compounds showed improved efficiency and peak shape on the Zorbax RX^R column, the efficiency was considerably less than the maximum efficiency suggested by the elution characteristics of acetophenone.

Stadalius et al. (3) have suggested several ways of suppressing the interaction of the stationary phase silanol groups with basic solute molecules.

The suppression of solute ionization by using a mobile phase with a high pH was impractical because of the rapid degradation of silica polymeric phases at these pH values. Reduction of the solute applied to the column to prevent saturation of the ion-exchange sites would greatly reduce the dynamic range of the analysis. Also, if retention was dependent on the solute concentration in the mobile phase, the qualitative use of the retention characteristics of a compound would be greatly compromised. Three other methods that were proposed dealt with reducing the number of reactive silanol groups on the stationary phase. In addition to reducing the number of free silanol groups on the stationary phase, it was suggested that the activity of the existing silanol groups could be reduced by lowering the pH of the mobile phase to suppress the ionization of the acidic silanols, and by using an organic or inorganic cation in the mobile phase to compete with and displace solute molecules from ionized stationary phase silanol groups. In the evaluation of the two columns without amine modifier, the pH of the mobile phase was 2.1. This satisfied the condition to suppress the ionization of all but the most acidic silanol groups.

For use in solvent programming over the desired polarity range, the cationic mobile phase modifier had to be soluble in the weak acidic-aqueous solvent and the stronger acidic-acetonitrile solvent so that a polar to hydrophobic solvent gradient could be generated without precipitating the cation. Preliminary studies with inorganic cations such as Na^+ and K^+ resulted in their precipitation in acetonitrile. Since a diode array detector was to be used to collect ultraviolet spectral profiles, the cation had to have low absorption in the 200 to 400 nm region of the electromagnetic spectrum. Several studies have demonstrated the usefulness of primary, secondary, tertiary and quaternary amines as mobile phase modifiers in suppressing the interaction of solute amines and stationary phase silanol groups (1,3,4,5,6,7). Of the amines studied, triethylamine (TEA) and N,N-dimethyl, n-octylamine (DMOA) appear to be the most efficient for this purpose. Preliminary studies in our laboratory, using the Zorbax C_8 column and morphine and amphetamine as the test compounds, indicated that TEA at a concentration of 0.05 M in the acidic mobile phase was more effective in enhancing efficiency

and peak symmetry than n-hexylamine, diethylamine or a mixture of the three amines at the same concentration. Other studies indicated that the use of 0.05 M DMOA in the weak acid-aqueous solvent resulted in the elution of morphine, amphetamine and methamphetamine in the void volume when this solvent was used as the mobile phase. Triethylamine at a concentration of 0.05 M was found to be sufficiently transparent to the electromagnetic spectrum between 190 and 400 nm and was soluble in the acid-aqueous and acid-acetonitrile (80%) mobile phase solvents. For these reasons triethylamine was chosen as the cationic modifier in the mobile phase.

On either the Zorbax^R C_8 or the Zorbax RX^R column, the use of TEA in the mobile phase greatly improved the efficiency and peak symmetry of the six basic compounds over the same chromatographic characteristics in the acid mobile phase without amine modifier. The plate count and asymmetry factor for acetophenone and phenylbutazone remained the same on the two columns whether or not the amine modifier was present in the mobile phase.

The use of the amine modifier in the mobile phase also improved the efficiency and peak shape of salicylic acid over that which occurred without the amine modifier. The efficiency of salicylic acid was better on the Zorbax RX^R column with the amine modifier in the mobile phase than on the Zorbax^R C_8 column with the amine modifier in the mobile phase. The use of the amine modifier in the mobile phase improved the elution characteristics of mefenamic acid on Zorbax C_8 but did not change these values on the Zorbax RX^R. The efficiency and peak symmetry of mefenamic acid on Zorbax C_8 with amine modifier in the mobile phase was slightly lower than these values on the Zorbax RX^R with or without the amine modifier in the mobile phase.

Bayer and Paulus (1) have reported that the capacity factor of disopyramid (tertiary, amide and pyridine functional groups) analyzed on five different commercial alkyl bonded silica stationary phases decreased with an increase in the concentration of TEA in the mobile phase. At 0.023 M TEA concentration in the mobile phase the k' of disopyramid on a Nucleosil^R C_{18} column had stabilized to further changes in the TEA concentration. On Hypersil^R ODS, Spherisorb^R ODS-2, LiChrospher^R CH-18 and Zorbax^R ODS columns a concentration of TEA greater than 0.038 M appeared to be required

to obtain the minimal k' value. According to the relative acidity rating of reversed-phase columns reported by Stadalius et al. (3) Nucleosil^R was one of the least acidic columns whereas the other commercial columns used in the Bayer and Paulus study were intermediate to more acidic. The 0.05 M TEA concentration required in the present study to maximize column efficiency on the Zorbax C_8 column was consistent with the reported TEA concentrations required to minimize k'. Since the Zorbax RX^R column was rated less acidic than the Nucleosil^R C_{18} column, it may be that a lower concentration of TEA on this column would be just as effective as the 0.05 M concentration.

Other investigators (4,5) have reported the use of 0.03 to 0.05 M DMOA in the mobile phase to effect the reversed phase elution of tricyclic amines, such as desipramine, with peak asymmetry factors of 1.1 to 2.4 depending on the brand of alkyl bonded silica stationary phase used. Similarly DMOA at a concentration of 0.05 M has been used to improve peak symmetry of 2-phenylethylamines (6) (asymmetry factors of 1.5 to 2.0) and at a concentration of 0.001 M to improve peak symmetry of phenoxypropanolamines (7) to asymmetry factors of 1.5 to 2.0.

Stadalius et al. (3) observed a plate count of 7280 for morphine on a Zorbax RX^R column using a mobile phase of 25 mM sodium phosphate buffer (pH 3.5) containing 7% CH_3CN, whereas in the present study a plate count of 2520 was obtained in a 0.1% H_3PO_4 (pH 2.1) mobile phase containing approximately 1.6% a CH_3CN. The capacity factor for the analysis in the 7% organic modified system appeared to be approximately 0.63. The low organic modifier concentration in the mobile phase in the present study was necessary to maintain the target k' value and gave a k' for morphine of 3.39. While adjusting the mobile phase composition to obtain the proper k' value for the morphine analysis, a mobile phase composition containing 8.8% CH_3CN gave a k' value of 2.09 and a plate count of 3250. It may be that the high aqueous content of the mobile phase was affecting the efficiency of morphine elution by other mechanisms. With 0.1% (w/v) TEA in the pH 3.5 mobile phase Stadalius et al. observed a plate count of 7000 for morphine. In the present study the use of 0.05 M TEA at pH 2.1 resulted in a similar plate count of 6995.

The rationale behind this approach to enhance the chromatographic efficiency of the elution of basic compounds on alkyl-bonded silica polymers was to eliminate the ionic interaction between the stationary phase and the solute molecules. If an ion-exchange mechanism was the major cause of poor chromatographic efficiency of basic compounds, then the removal of this process should have resulted in maximum chromatographic efficiency of the elution of the basic test compounds. If we assumed that the molecular volume for the model compounds was similar to that of the reference compound, acetophenone, we would expect the theoretical plate count and asymmetry factors of these compounds to be similar to that of acetophenone. For the most efficient systems in this study these data for the basic compounds fall short of the maximum expected values, which suggests that either some ion-exchange sites on the stationary phase material were not inhibited or that some additional mechanisms were causing poor chromatographic efficiency.

The evidence in this study suggested that the best efficiency for the separation of basic drugs on an alkyl bonded stationary phase was obtained when an organic amine such a triethylamine was present in the mobile phase. Although the efficiency for the elution of these compounds in a system without triethylamine in the mobile phase was better on the phase prepared from type B silica, the use of triethylamine in the mobile phase resulted in a similar efficiency on both the type B silica and the type A silica alkyl bonded phases. Additionally, the Zorbax RX^R column material exhibited better efficiency for salicylic acid and mefenamic acid over the type A phase whether triethylamine was used in the mobile phase or not.

The use of the type B silica column material in combination with triethylamine in the mobile phase would appear to be the preferred system for the analysis of neutral, basic and acidic drugs in one HPLC system. To cover the broad polarity range of drugs required for general drug analysis, this system should be used in a solvent programmed mode. Table V list a comparison of the asymmetry factors of the model compounds in the isocratic systems and the solvent programmed systems. In each of the solvent/column systems studied, the peak asymmetry of acetophenone was slightly better in the solvent programmed systems. As observed for the isocratic analysis of

these compounds, the presence of triethylamine in the mobile phase improved the peak symmetry of the basic compounds on either the Zorbax^R C_8 or the Zorbax RX^R column. Also, an improvement in the peak symmetry of the basic compounds was observed on the Zorbax RX^{R}column over that of the Zorbax C_8 without triethylamine in the mobile phase. Similar improvement in the peak symmetry of salicylic acid and mefenamic acid occurred on both stationary phases when triethylamine was included in the mobile phase. Interestingly the improvement in peak symmetry that was observed in the isocratic system using a mobile phase without triethylamine when going from the Zorbax^R C_8 column to the Zorbax RX^R column was not observed in the solvent programmed analysis. In fact, in the solvent programmed analysis of salicylic acid the peak asymmetry factor on the Zorbax RX^R column was slightly higher.

With a few exceptions, the asymmetry factor of each compound in each solvent/column system was similar in the isocratic system and the solvent programmed system. Exceptions to this were the reduction of the asymmetry factor for desipramine and salicylic acid in the solvent programmed analysis on the Zorbax^R C_8 column without triethylamine in the mobile phase.

The reduction of peaking tailing results in an increase in the peak height response per amount of compound applied to the column. This leads to an increase in the sensitivity of the detection of the compound. Figure 4 compares the peak height sensitivity of each of the model compounds in each of the solvent programmed systems. It was observed that for all the compounds except acetophenone the poorest sensitivity occurred on the Zorbax^R C_8 column without triethylamine in the mobile phase. The highest sensitivity occurred for compounds analyzed on either the Zorbax^R C_8 or Zorbax RX^R column with triethylamine in the mobile phase. For mefenamic acid and phenylbutazone similar sensitivities were observed on the Zorbax RX^R column without triethylamine in the mobile phase as was observed for these compounds in the systems using Zorbax C_8 or Zorbax RX^R with triethylamine in the mobile phase. Solvent programmed chromatograms of the model compounds analyzed on Zorbax RX^R without triethylamine in the

mobile phase (Figure 4) and Zorbax^R C_8 with triethylamine in the mobile phase (Figure 5) compared to the chromatogram of these compounds analyzed on the Zorbax^R C_8 column without triethylamine in the mobile phase illustrate the increase of efficiency and resolution that was obtained by using either a Zorbax RX^R column or triethylamine in the mobile phase.

These data suggest that an efficient and sensitive elution of basic drugs can be accomplished in a solvent programmed system using an acidic mobile phase in which either triethylamine is incorporated into the mobile phase or a stationary phase prepared with type B silica is used. The combination of triethylamine in the acidic mobile phase and the use of a stationary phase similar to Zorbax RX^R appears to give the most efficient elution of both acidic and basic compounds in one system.

ACKNOWLEDGMENTS

The author thanks Robert T. Moody of Mac-Mod Analytical Inc. for the Zorbax RX^R column used in this study. The author also thanks Albert Kind and Karen Langner for technical assistance and aid in preparation of this manuscript.

This study is scientific contribution number 1327, Storrs Agricultural Experiment Station, The University of Connecticut, Storrs, Connecticut 06269.

LITERATURE CITED

(1) Bayer, E.; Paulus, A.,"Silanophilic Interactions in Reversed-Phase High-Performance Liquid Chromatography", *J. Chromatogr.* 400, 1., 1987.

(2) Kohler, J.; Kirkland J. J., "Improved Silica-Based Column Packings for High-Performance Liquid Chromatography", *J. Chromatogr.*, 385, 125, 1987.

(3) Stadalius, M. A.; Berus, J. S.; Snyder, L. R., "Reversed-phase HPLC of Basic Samples", *LC-GC*, 6(6), 495, 1988.

(4) Sokolowski, A.; Wahlund, K. G., "Peak Tailing and Retention Behaviour of Tricyclic Antidepressant Amines and Related Hydrophobic Ammonium Compounds in Reversed-Phase Ion-Paired Liquid CHromatography on Alkyl-Bonded Phases", *J. Chromatogr.*, 189, 299, 1980.

(5) Lagerstrom, P.; Marle, I.; Persson, B., "Solvent Extraction of Tricuyclic Amines from Blood Plasma and Liquid Chromatographic Determination", *J. Chromatogr.*, 273, 151, 1983.

(6) Gill, R.; Alexander, S. P.; Moffat, A. C., :Comparison of Amine Modifiers used to Reduce Peak Tailing of 2-Phenylethylamine Drugs in Reversed-Phase High-Performance Liquid Chromatography", *J. Chromatogr.*, 247, 39, 1982.

(7) Persson, B.; Jansson, S.; Johansson, M.; Lagerstrom, P., "Influence of Mobile Phase Additives in Liquid Chromatography of Phenoxypropanolamines", *J. Chromatogr.*, 316, 291, 1984.