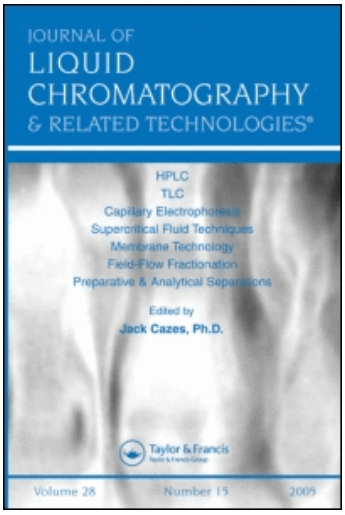

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The Effects of Type B Silica and Triethylamine on the Retention of Drugs in Silica Based Reverse Phase High Performance Chromatography

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THE EFFECTS OF TYPE B SILICA AND TRIETHYLAMINE ON THE RETENTION OF DRUGS IN SILICA BASED REVERSE PHASE HIGH PERFORMANCE CHROMATOGRAPHY

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ABSTRACT

The effect of type B silica and the presence of triethylamine (TEA) in an acidic mobile phase on the retention time of drugs containing neutral, acidic and basic functional groups was investigated. At the pH of the mobile phase (2.2) used in the study, none of the test drugs that contained acidic functional groups were ionized, whereas most of the drugs containing basic functional groups were ionized. The retention time of the non-ionized compounds showed little change when analyzed on reverse phase columns prepared from type A (Zorbax C_8) or type B silica (Zorbax RX). Without TEA in the mobile phase the retention times of the ionized basic compounds were different on the two types of phases. When TEA was used in the mobile phase, the retention times of the ionized bases were similar on the two phases. On Zorbax RX the between column standard deviation of the retention times of all the test drugs on four different batches of the column material, with and without TEA in the mobile phase, was <0.21 min. When TEA was used in the mobile phase, the between column standard deviation of the ionized bases was significantly ($p<0.05$) less than that of the non-ionized drugs. The data suggested that the best system for obtaining reliable retention data for reverse phase HPLC analysis of drug mixtures containing ionized basic compounds was a reverse phase column prepared from type B silica and the use of an acidic mobile phase containing TEA.

INTRODUCTION

High Performance Liquid Chromatography (HPLC) has increasingly been the chromatographic method of choice for the screening of biological fluids for drugs (1, 2, 3) because of its ease of use and greater resolution than thin layer chromatography. The problems of reproducing retention characteristics of drugs on reverse phase HPLC systems have been investigated over the past few years (4, 5, 6, 7, 8, 9). It has been demonstrated (10, 11, 12) that a good reverse phase system for the efficient elution of drugs utilizes an acidic mobile phase (to suppress the ionization of acidic drugs and acidic silanol groups on the stationary phase) containing an amine modifier (to displace the interaction of ionized basic drugs with non-reacted stationary phase silanol groups). Because the hydrophobic character of reverse phase systems has varied between columns, the absolute retention time of drugs has not been reproducible (4, 9, 13, 14); however, if the system has been reduced to one retention mechanism (i.e., solvophobic), then the retention of drugs might be expected to be more consistent. Previous work (4) has indicated that the use of amine modifiers in the mobile phase appeares to reduce this variability. Recently workers have evaluated the ability to reproduce retention indices of drugs in reverse phase systems using reverse phase columns from different manufactures (1, 15, 16). Variability of these parameters was still observed, and these investigators have suggested the use of type compounds to correct deviant retention behavior.

Recent work in our laboratory confirmed the ability of a low pH mobile phase containing triethylamine (TEA) to decrease band dispersion in ionized basic drugs (11). The mobile phase was capable of eluting these compounds with the same efficiency on a reverse phase column prepared from type B (low concentration of acidic silanol groups) and type A (high concentration of acidic

silanol groups) silica. The present study investigates the effects of type B silica and the presence of an amine modifier on the reproducibility of drug retention times.

EXPERIMENTAL

Materials and Reagents

Source of Material. Acetonitrile (Merck Ominisolv®, AX0142-1), methanol (Merck Ominisolv®, MX0488-1) 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® reverse osmosis/ion-exchange/charcoal water purification system. Zorbax C_8 (880952.706) and Zorbax RX (880967.901) 250 mm x 4.6 mm (id) HPLC columns were obtained from Mac-Mod Analytical Inc.

Standard Drug and Reference Solutions. A column test solution (LC2) of model drugs (morphine, amphetamine, methamphetamine, ethylmorphine, salicylic acid, desipramine, imipramine, phenylbutazone and mefenamic acid), was used to daily check column efficiency. This solution was prepared at 0.50 mg/mL of each compound as described previously (11). Acetophenone was prepared at 0.50 mg/mL in reagent grade water or methanol. Test mixtures of the drugs listed in Table 1 (LCA, LCB and LCC, respectively) were prepared in the indicated solvent at 0.50 mg/mL concentrations. Before HPLC analysis, a test drug solution was mixed with an equal volume of acetophenone solution prepared from the same solvent.

Purification of Triethylamine. Neutral alumina (Merck, 1077) and activated charcoal (Norit® A, Fisher, C176-500) were placed into separate beakers and each was washed 3 times with 2 bed volumes of pentane (Burdick & Jackson, 312-4), 3 times with 2 bed volumes of methylene chloride (Burdick & Jackson,

Table 1. List of Test Compound (500 µg/mL each) Mixtures Used in Present Study.

Group A (Water)	Group B (Methanol)	Group C (Methanol)
Acetaminophen	Aprobarbital	Avermectin Bla
N- Acetylprocainamide	Brucine	Biphenyl
m- Aminobenzamide	Butabarbital	Butylparaben
Ethylmorphine	Butethal	Chloroxylenol
β- Hydroxyethyltheophylline	Chlordiazepoxide	Danazol
5- Hydroxyquinoline	Chlorpromazine	Danthron
Methylaminomethylbenzyl alcohol	Clenbuterol	Diflunisal
Nicotine	Cortisone	Estrone
p- Phenetidine	Doxapram	Flunitrazepam
Phentermine	Fluoxymesterone	Lormetazepam
Phenylpropanolamine	Indole 3-Carboxyaldehyde	Mefenamic acid
Sulfanilamide	2- Naphthoxyacetic acid	Methyltestosterone
Tranylcypromine	Oxazepam	Progesterone
Tripelennamine	Pyrithyldione	Testosterone
	Sulfamethazine	Testosterone propionate
	Vincamine	

300-4) and 3 times with 2 bed volumes of methanol (Merck Ominisolv®, MX0488-1). The solvent was allowed to evaporate under a fume hood overnight. The alumina and activated charcoal were then heated at 130° for 2 hrs and stored in a glass bottle. (*Care should be taken to insure that most of the solvent has evaporated to prevent explosion prior to drying these materials in the oven.*) Liquid chromatographic columns were prepared by dry-packing 29 cm x 2.2 cm, id glass chromatographic columns with a 14 cm bed of the washed alumina. The column was solvent-initialized by allowing one head volume of methanol to pass through. For a newly prepared column, one head volume of reagent grade TEA was allowed to flow through to clear TEA soluble contaminates that may be present. After the clearance of another head volume of methanol the column was ready for use. A 60 mL bed volume of cleaned activated charcoal was placed in a 150 mL graduated beaker. Reagent grade TEA was added to the beaker to the 140 mL mark. The slurry was mixed using a magnetic stirring bar for 30 min. After the charcoal had settled to the bottom of the beaker, the TEA supernatant

phase was applied to the column and allowed to elute. When an amine odor was detected in the effluent, an additional twenty mL of solvent were allowed to pass through the column and were then discarded. The next 20 mL of TEA were collected and used immediately in the preparations of the HPLC solvents. Following the purification of 20 mL of TEA, 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 TEA before preparation of a new column was necessary.

HPLC Solvent System 1. Solvent 1A (0.1% H_3PO_4) was prepared by diluting 1.0 mL of concentrated o-phosphoric acid to 1 L with reagent grade water. Solvent 1B (0.1% H_3PO_4, 20% H_2O in CH_3CN was prepared by adding 200.0 mL of reagent grade water and 1.0 mL of concentrated o-phosphoric acid to a 1 L volumetric flask and diluting to volume with acetonitrile.

HPLC Solvent System 2. Solvent 2A (0.15 M H_3PO_4, 0.05 M TEA) was prepared by diluting 10.0 mL of concentrated o-phosphoric acid and 7.0 mL of purified TEA to 1 L with reagent grade water. Solvent 2B (0.15 M H_3PO_4, 0.05 M TEA, 20% H_2O in CH_3CN) was prepared by diluting 10.0 mL of o-phosphoric acid, 7.0 mL of purified TEA and 200.0 mL of reagent grade water to 1 L with acetonitrile.

HPLC Systems

A Hewlett-Packard HP1090 HPLC system interfaced to a Hewlett-Packard 1040 diode array detector was used in this study. The column temperature was maintained at 30° C and the mobile phase was delivered to the column at a flow rate of 2.0 mL/min and consisted of a 2.20 min initial isocratic hold at 0% B

followed by a 30 min linear gradient to 100% B. The dwell time for this system at 2.0 mL/min was determined to be 0.47 min so that the actual isocratic hold time was 2.67 min.

The effluent from the column was monitored at 210 nm and UV spectral data was collected from 200 to 402 nm at 640 ms intervals. The identity of compounds that were analyzed as a mixture was determined by computer identification of the UV spectral profile of the compounds in a UV library data base (17).

Determination of Drug HPLC Retention

Comparison of Drug Retention Data on Columns of Different Batch Lots and Different Silica Type. Four Zorbax RX (1,1-diisopropyl-1-octylsilicone bonded to type B silica) columns, each from a different batch preparation of the bonded silica, and a Zorbax C_8 (1,1-dimethyl-1-octylsilicone bonded to type A silica) were used in these experiments. Five μL of each of the drug mixtures listed in Table 1 (LCA, LCB, LCC) were analyzed in triplicate on each of the columns. The mixtures were analyzed using the described solvent gradient program with solvent 1A and 2B and then with solvent 2A and 2B. In order to minimize the effects of differences in solvent preparation, solvents 1 and 2 were prepared in sufficient volume to perform the analysis on each column without preparing another batch of solvent.

Between Day Variation of Drug Retention Data. During the routine periodic analysis of extracts of biological fluids on the solvent gradient HPLC system using solvents 2A and 2B, 5 μL of 250 μg/mL or 20 μL of 100 μg/mL of the column test drug mixture (LC2) was analyzed to monitor the efficiency of the column. During a nine month period, 30 samples of this drug mixture were

analyzed. The deviation of the retention time of each compound was determined as the mean and standard deviation of these data.

Data Evaluation

pK_a Values for Test Compounds. The pK_a values of the test compounds were obtained from the literature (18, 19, 20) where available. The pK_a values for compounds for which a specific experimental value could not be found was estimated by comparison to compounds with similar functional groups for which the pK_a values were known. The pH of the mobile phases used in this study were measured to be 2.2. The ionization state of a compound was determined to be ionized as a base (IB) if the compound contained one or more basic functional groups that had a pK_a value greater than 3.2 and partially ionized as a base (PB) if the compound contained a basic functional group that had a pK_a value between 1.2 and 3.2. A compound which had no basic functional group with a pK_a value greater than 1.2 and no acid functional group with a pK_a value less than 3.2 was considered to be in an non-ionized (NI) state in the mobile phases. A compound with an acid functional group with a pK_a value of 3.2 or less would have been considered either an ionized or partially ionized acid, however none of the compounds used in this study had an acid functional group that met this condition. Assigned ionization states for each compound used in this study are listed in Table 2 and 3.

Statistics. For comparison of data between groups of compounds in a similar ionization state, the ionized bases and the partially ionized bases were grouped together. The similarity of the variance between two test groups of data was tested by the F-test. The average difference in the retention times obtained for ionized bases and non-ionized compounds with and without TEA in the mobile phase were compared by the two tailed student t-test for equal or unequal

Table 2. Retention Time of Test Compounds on Zorbax C_8 with and without TEA in the Mobile Phase.

Cpd #	Compound	Ion[a]	Retention Time (min)[b]	
			No TEA	TEA
1	Acetaminophen	NI	8.306	8.271
2	Acetophenone	NI	17.226	17.350
3	N- Acetylprocainamide	IB	13.552	8.625
4	*m*- Aminobenazmide	IB	3.859	1.730
5	Aprobarbital	NI	14.749	14.877
6	Avermection B1a	NI	30.970	30.937
7	Biphenyl	NI	27.371	27.478
8	Brucine	IB	18.661	11.651
9	Butabarbital	NI	15.840	15.951
10	Butethal	NI	16.326	16.450
11	Butylparaben	NI	22.566	22.676
12	Chlordiazepoxide	IB	20.719	13.995
13	Chloroxylenol	NI	22.409	22.503
15	Clenbuterol	IB	19.248	12.461
16	Cortisone	NI	16.945	17.002
17	Danazol	IB	26.420	26.522
18	Danthron	NI	25.241	25.394
19	Diflunisal	NI	23.745	23.675
20	Doxapram	IB	24.743	14.913
21	Estrone	NI	22.409	22.503
23	Flunitrazepam	PB	20.905	20.897
24	Fluoxymesterone	NI	18.888	18.917
25	β- Hydroxyethyltheophylline	IB	9.268	9.253
27	Indole-3-carboxylaldehyde	IB	14.819	14.804
28	Lormetazepam	PB	21.396	21.429
29	Mefenamic acid	IB	26.541	26.569
30	Methylaminomethylbenzyl alcohol	IB	9.671	5.408
31	Methyltestosterone	NI	22.735	22.803
32	2- Naphthoxyacetic acid	NI	19.816	19.805
34	Oxazepam	PB	18.879	18.697
35	*p*- Phenetidine	IB	11.409	7.037
36	Phentermine	IB	14.976	10.097
37	Phenylpropanolamine	IB	10.065	6.363
38	Progesterone	NI	26.297	26.409
39	Pyrithyldione	NI	12.459	12.522
40	Sulfamethazine	IB	11.357	10.423
41	Sulfanilamide	IB	4.096	3.039
42	Testosterone	NI	21.728	21.799
43	Testosterone propionate	NI	30.058	30.149
46	Vincamine	IB	23.694	14.561

[a]Ionization State of Compound at pH 2.2 (IB: ionized base, PB: partially ionized base, NI: non-ionized).

[b]Average Retention Time of Triplicate Analyses.

Table 3. Retention Time and Between Column Standard Deviation of Retention Time of Test Compounds on Zorbax RX.

Cpd #	Compound	Ion[a]	Retention Time (min)[b]		Standard Deviation[c]	
			No TEA	TEA	No TEA	TEA
1	Acetaminophen	NI	8.350	8.341	0.075	0.057
2	Acetophenone	NI	16.484	16.625	0.106	0.099
3	N- Acetylprocainamide	IB	8.469	8.353	0.079	0.049
4	*m*- Aminobenzamide	IB	2.131	1.656	0.038	0.010
5	Aprobarbital	NI	14.181	14.316	0.074	0.067
6	Avermectin B1a	NI	29.744	29.779	0.072	0.045
7	Biphenyl	NI	25.942	26.098	0.102	0.100
8	Brucine	IB	10.828	11.176	0.091	0.065
9	Butabarbital	NI	15.194	15.336	0.069	0.066
10	Butethal	NI	15.673	15.826	0.069	0.067
11	Butylparaben	NI	21.753	21.885	0.076	0.079
12	Chlordiazepoxide	IB	12.769	13.149	0.124	0.057
13	Chloroxylenol	NI	21.292	21.400	0.088	0.091
14	Chlorpromazine	IB	17.795	18.875	0.209	0.099
15	Clenbuterol	IB	11.560	11.823	0.129	0.058
16	Cortisone	NI	17.399	17.496	0.043	0.044
17	Danazol	IB	26.485	26.596	0.072	0.068
18	Danthron	NI	24.319	24.500	0.094	0.097
19	Diflunisal	NI	23.479	23.243	0.152	0.089
20	Doxapram	IB	13.479	13.993	0.139	0.063
21	Estrone	NI	22.545	22.678	0.066	0.070
22	Ethylmorphine	IB	10.019	10.194	0.100	0.059
23	Flunitrazepam	PB	20.428	20.585	0.061	0.064
24	Fluoxymesterone	NI	19.495	19.561	0.044	0.037
25	β- Hydroxyethyltheophylline	IB	9.371	9.406	0.049	0.044
26	5- Hydroxyquinoline	IB	6.649	5.946	0.072	0.058
27	Indole-3-carboxyaldehyde	IB	14.827	14.857	0.089	0.086
28	Lormetazepam	PB	20.628	20.723	0.055	0.052
29	Mefenamic acid	IB	25.522	25.611	0.081	0.077
30	Methylaminomethylbenzyl alcohol	IB	5.786	3.878	0.094	0.076
31	Methyltestosterone	NI	23.960	24.062	0.060	0.058
32	2- Naphthoxyacetic acid	NI	19.306	19.253	0.102	0.087
33	Nicotine	IB	2.298	1.307	0.152	0.006
34	Oxazepam	PB	18.022	18.098	0.049	0.046
35	*p*- Phenetidine	IB	7.011	5.928	0.093	0.063
36	Phentermine	IB	9.420	9.269	0.117	0.054
37	Phenylpropanolamine	IB	6.327	4.875	0.088	0.103
38	Progesterone	NI	27.481	27.646	0.094	0.097
39	Pyrithyldione	NI	12.079	12.163	0.062	0.053
40	Sulfamethazine	IB	11.027	10.534	0.042	0.023
41	Sulfanilamide	IB	3.721	2.983	0.062	0.026
42	Testosterone	NI	22.833	22.941	0.057	0.057
43	Testosterone proprionate	NI	30.563	30.716	0.122	0.120
44	Tranylcypromine	IB	7.968	7.035	0.109	0.087
45	Tripelennamine	IB	8.777	7.297	0.126	0.035
46	Vincamine	IB	13.175	13.806	0.145	0.079

[a]Ionization State of Compound at pH 2.2 (IB: ionized base, PB: partially ionized base, NI: non-ionized).
[b]Average Retention Time of Triplicate Analyses on 4 Different Batch Columns.
[c]Standard Deviation of Retention Times Between 4 Different Batch Columns.

variance depending on the results of the F-test. The average between column retention time standard deviations between the ionized bases and non-ionized compounds with and without TEA in the mobile phase were compared by a single factor analysis of variance with a Newman-Keul conversion. For each statistical comparison the null hypothesis was rejected for $P<0.05$.

RESULTS

Table 2 lists the retention time of the test compounds on one Zorbax C_8 column with and without TEA in the mobile phase. (Chloropromazine, ethylmorphine, 5-hydroxyquinoline, nicotine, tranylcypromine and tripelennamine were not resolved in the test mixtures when analyzed on Zorbax C_8 without TEA in the mobile phase. The retention times for these compounds were therefore not available for use in evaluations involving the Zorbax C_8 column.) The difference in the retention time between the two mobile phase systems is illustrated in Figure 1. This graphic representation suggest that the presence of TEA in the mobile phase effects the ionized basic compounds more than the non-ionized compounds. The average absolute difference in retention time between the use and non use of TEA in the mobile phase for the ionized/partially ionized bases was 3.306 ± 3.304 min and ranged from 0.008 to 9.830 min. For the non-ionized compounds the average change in retention time was 0.084 ± 0.039 min and ranged from 0.010 to 0.153 min. Statistical comparison of the absolute retention time difference of the ionized/partially ionized bases to that of the non-ionized compounds showed a significantly ($P<0.01$) greater change in retention time for the ionized/partially ionized bases.

The retention times and between column standard deviations for the test compounds analyzed on four different lot batches of Zorbax RX columns with and without TEA in the mobile phase are listed in Table 3. Figure 2 illustrates

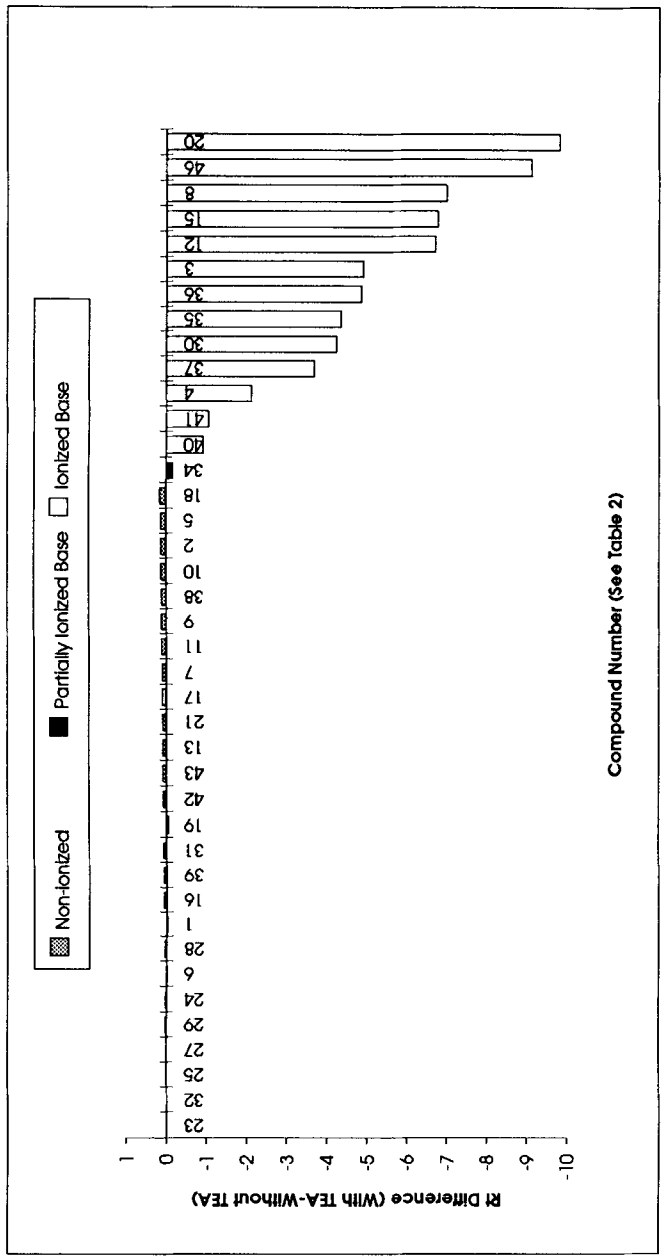

Figure 1. Retention time difference of compounds analyzed on Zorbax C8 column with and without TEA in the mobile phase.

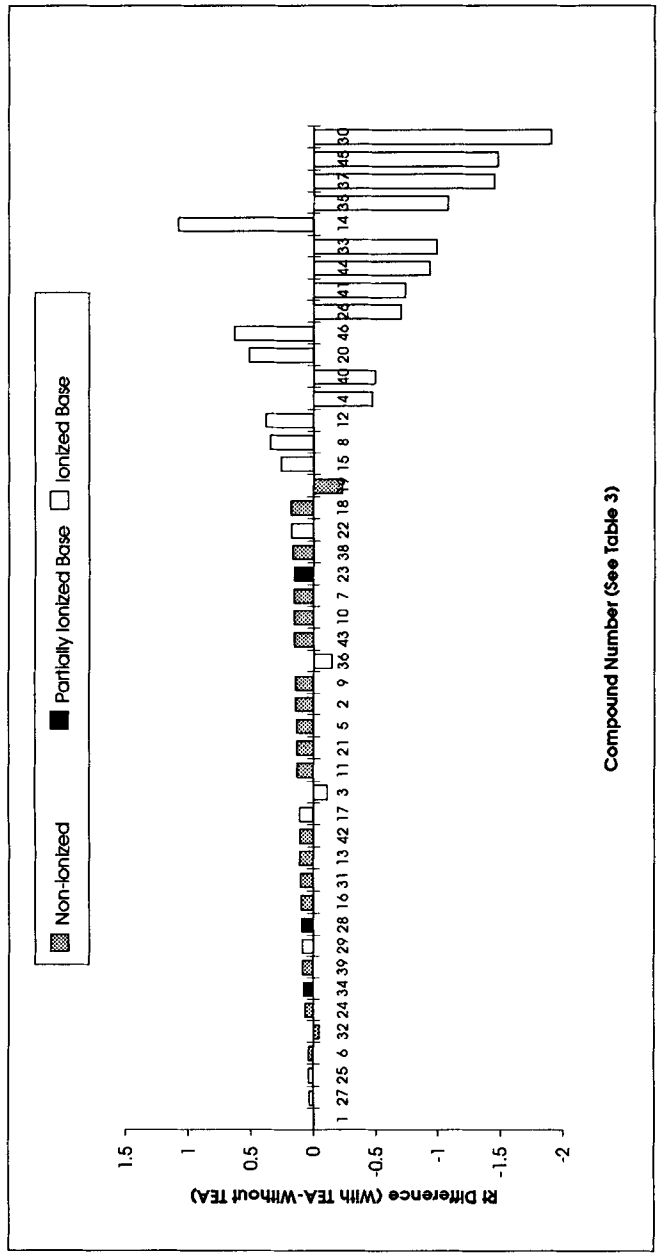

Figure 2. Retention time difference of compounds analyzed on Zorbax RX columns with and without TEA in the mobile phase.

the difference in the retention time between the two mobile phase systems on the Zorbax RX columns. As with the Zorbax C_8 column, the presence of TEA in the mobile phase appears to have a greater effect on the ionized basic compounds. The average absolute difference in retention time between the two mobile phases for the ionized/partially ionized bases was 0.558 ± 0.517 min and ranged from 0.030 to 1.908 min. The change in the retention times for the non-ionized compounds on the Zorbax RX column averaged 0.119 +/- 0.053 min and ranged from 0.008 to 0.236 min. The change in retention of the ionized/partially ionized bases was significantly ($P<0.01$) greater than the change for the non-ionized compounds.

The average effect of the use of TEA in the mobile phase on the retention times of ionized/partially ionized bases was significantly greater ($P<0.05$) on the Zorbax C_8 column than on the Zorbax RX column (3.306 min compared to 0.558 min). The average change in the retention time between the two mobile phases for the non-ionized compounds was less ($P<0.05$) on the Zorbax C_8 than on the Zorbax RX column (0.084 min compared to 0.119 min).

The between column variability, measured as the standard deviation of the retention times between the four different lot batches of Zorbax RX columns, is listed in Table 2 with and without TEA in the mobile phase for each of the test compounds. The difference in the retention time between column standard deviations with and without TEA in the mobile phase is illustrated in Figure 3. The average standard deviation and range of standard deviation for the ionized/partially ionized bases and the non-ionized compounds with and without TEA in the mobile phase is shown in Table 4. There was no significant ($P>0.05$) difference in the standard deviation of ionized/partially ionized bases and non-ionized compounds when TEA was not used in the mobile phase. When TEA was used in the mobile phase, the standard deviation of the non-ionized

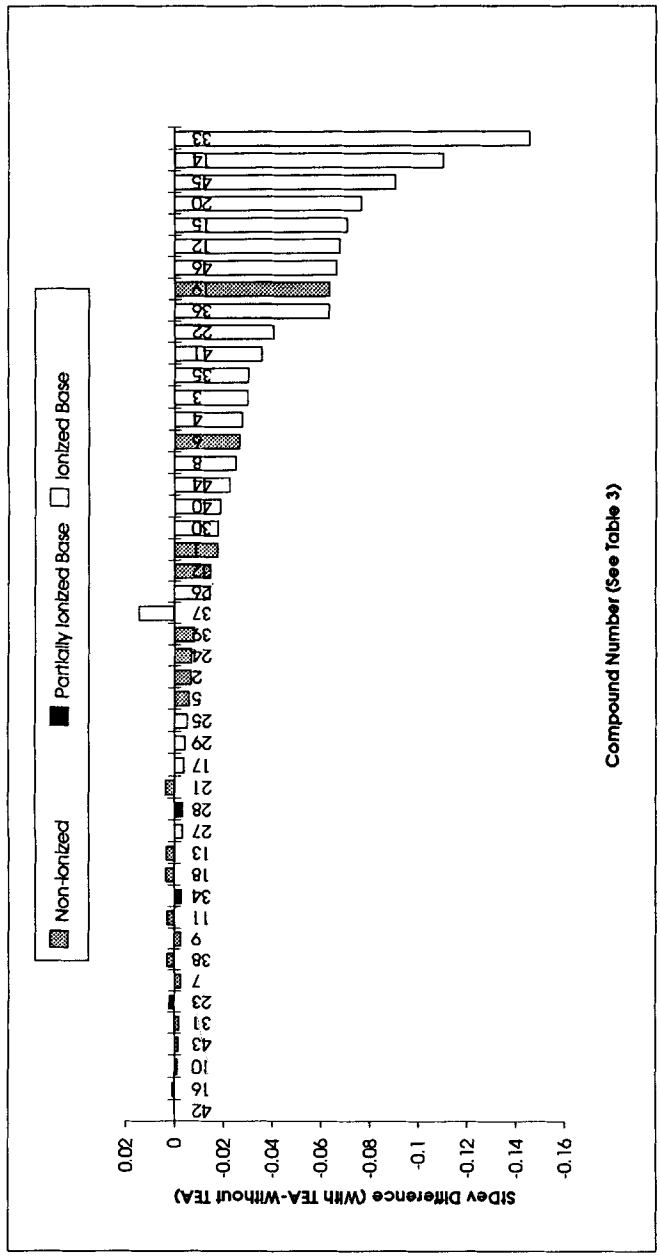

Figure 3. Between column retention time standard deviation difference of compounds analyzed on Zorbax RX with and without TEA in the mobile phase.

Table 4. Average and Range of Retention Time Standard Deviation of Test Compounds on Zorbax RX With and Without TEA in the Mobile Phase.

Ion[a]	No TEA[b]			TEA[c]		
	Av	Low	High	Av	Low	High
IB	0.095	0.038	0.209	0.058	0.006	0.103
NI	0.081	0.043	0.152	0.074	0.037	0.120

[a]IB: ionized and partially ionized bases; NI: non-ionized compounds.
[b]Standard deviation of retention time on Zorbax RX without TEA in the mobile phase.
[c]Standard deviation of retention time on Zorbax RX with TEA in the mobile phase.

compounds did not change ($P>0.05$); however, the average standard deviation of the ionized/partially ionized compounds was significantly ($P<0.05$) lower than the non-ionized compounds.

Figure 4 shows the relationship of the retention time of the test compounds on Zorbax C_8 and Zorbax RX without TEA in the mobile phase. The linear regression analysis for all compounds gave a slope of 1.00 and a low correlation coefficient (0.8174). The linear regression analysis for the non-ionized compounds indicated a slope of 1.00 and a good correlation coefficient of 0.9805; however the linear regression analysis of the ionized/partially ionized bases gave a poor correlation coefficient (0.7273) and slope of 0.83 which was significantly lower ($P<0.05$) than the slope of the non-ionized compounds. The relationship of the retention time of the test compounds on the two column types when TEA was used in the mobile phase is shown in Figure 5. The linear regression analysis of all the data gave a slope of 1.01 and a good correlation coefficient of 0.9901. The linear regression analysis of the non-ionized compounds gave a slope of 1.00 which was the same ($P>0.05$) as the 1.02 slope obtained for the ionized/partially ionized bases. The y-intercept of the two equations were not significantly ($P>.05$) different.

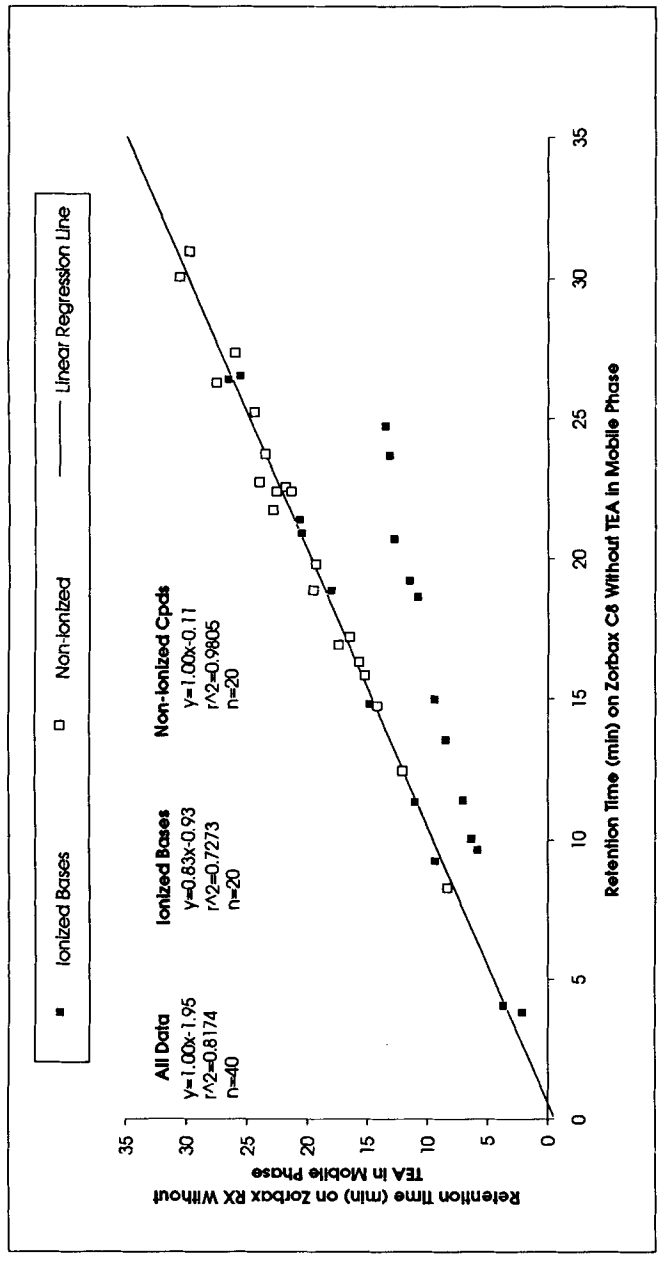

Figure 4. Comparison of retention time of test compounds on Zorbax RX and Zorbax C8 without TEA in the mobile phase.

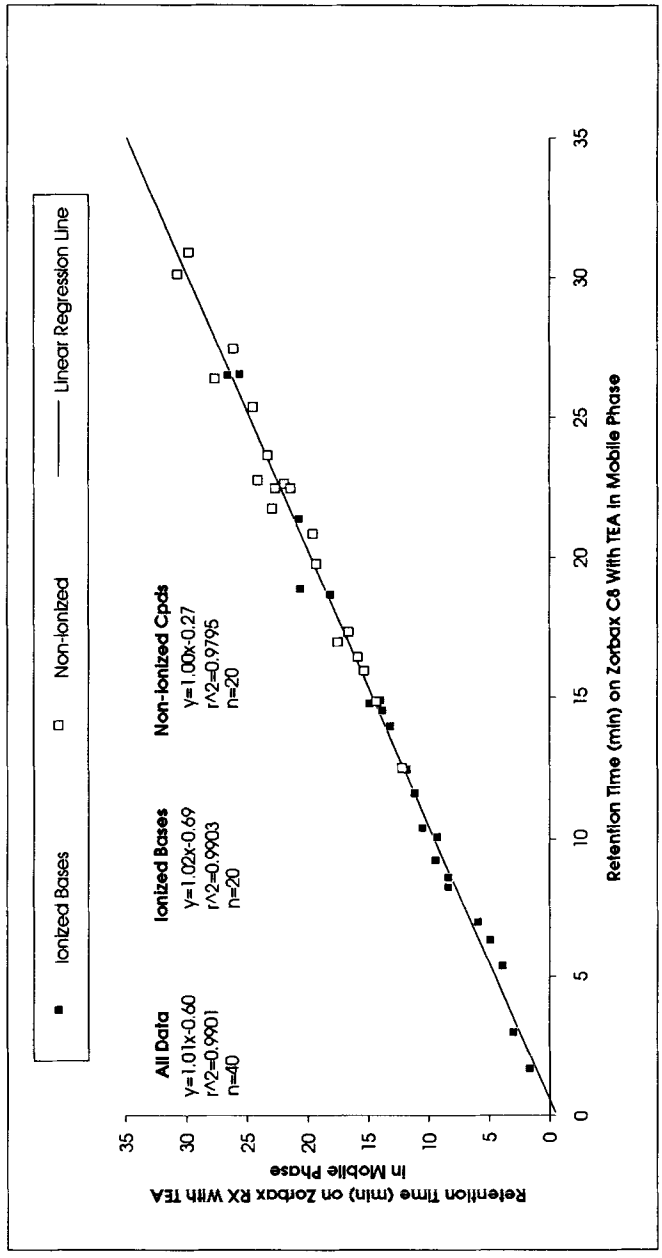

Figure 5. Comparison of retention time of test compounds on Zorbax RX and Zorbax C8 with TEA in the mobile phase.

Table 5. Reproducibility of Retention Time of HPLC Test Drugs on One Zorbax RX Column from 12/7/90 to 9/23/91 (n=30)*.

Compound	Retention Time (min) Mean	StDev	%CV
Morphine	5.995	0.092	1.53
Amphetamine	7.756	0.077	1.00
Methamphetamine	8.696	0.081	0.93
Ethylmorphine	10.282	0.096	0.93
Salicylic Acid	15.600	0.123	0.79
Acetophenone	16.874	0.091	0.54
Desipramine	17.574	0.181	1.03
Imipramine	18.004	0.236	1.31
Phenylbutazone	25.660	0.103	0.40
Mefenamic Acid	25.825	0.117	0.45

***Analysis on HP1090 HPLC System; 0% (2.2 min isocratic) to 100% 2B/2A in 30 min at 2.0 mL/min.**

The reproducibility of the retention time of selected ionized bases (morphine, amphetamine, methamphetamine, ethylmorphine, desipramine, imipramine and mefenamic acid) and non-ionized compounds (salicylic acid, acetophenone, and phenylbutazone) over a 10 month period on one column is given in Table 5. The standard deviation in the retention time of the 30 analyses ranged from 0.077 min to 0.236 min with a percent coefficient of variation of 1.5 % or less.

DISCUSSION

Earlier studies (11) compared the chromatographic efficiencies of acidic and basic drugs on Zorbax RX and Zorbax C_8 columns using an acidic mobile phase with and without TEA. These data indicated that when using the mobile phase without TEA the chromatographic efficiency of basic drugs was better on the Zorbax RX than on the Zorbax C_8 column. However, when TEA was used in the mobile phase, the efficiency of basic drugs on both columns was similar and was enhanced over that obtained on either column when the mobile phase did not

contain TEA. The efficiency of the acidic drug salicylic acid and the zwitterionic drug mefenamic acid increased respectively in HPLC systems using Zorbax C_8 without TEA, Zorbax C_8 with TEA, Zorbax RX without TEA and Zorbax RX with TEA. The type of column or the presence or absence of TEA in the mobile phase did not appreciably effect the efficiency of the elution of the acidic drug phenylbutazone or the neutral compound acetophenone.

Similar enhancement of the chromatographic efficiency of basic drugs when TEA was used in the mobile phase was observed on Synchropak SCD (10), Superspher RP-18, Techsphere ODS-BDS, Nucleosil 100-5 C18, Encapharm RP18-TS, Lichrospher 60 RP-Selcet B, and Synchropak RP-SCD (12) columns. In one study (12) it was observed that on base deactivated reverse phase columns, five basic drugs were not eluted in an aqueous/acetonitrile mobile phase. However, the basic drugs were eluted in a phosphate buffer (pH-3.0)/acetonitrile mobile phase and the retention times were decreased when TEA was used in the buffered mobile phase.

The present study was designed to determine the effects of type B silica (represented by Zorbax RX) and type A silica (represented by Zorbax C_8) based reverse phase columns, and the presence of TEA in the mobile phase on the retention times of acidic, basic and neutral drugs. Although several drugs used in this study contained acidic functional groups, all of them had pK_a values that indicated that they were non-ionized at the pH of the mobile phase used. The drugs were therefore evaluated in groups as ionized/partially ionized bases and non-ionized compounds.

The effects of silica based stationary phases on the elution characteristics of ionized bases has been attributed to an ion exchange interaction of free ionized silanol groups on the stationary phase with ionized basic solute molecules (21).

Broad tailing peaks have been explained as being caused by the saturation of these sites, whereas shifts in retention time would be due to a mixture of ion exchange and reverse phase retention mechanisms. The variability of retention time between columns may be caused by the variability of the ratio of ion exchange surface area to reverse phase surface area between different batch preparations of stationary phases. In the present study the type B silica of Zorbax RX showed good retention time reproducibility (standard deviation <0.21 min) of basic and neutral compounds between four different batch columns. When TEA was not used in the mobile phase the between column retention time reproducibility was the same for the ionized/partially bases and the non-ionized compounds. The type B silica has a more homogenous distribution of silanol groups (21, 22) which results in most of the silanol groups interacting with near neighbor silanol groups (associated silanols) and thus unavailable for interaction with the ionized basic solute molecules. However, when TEA was used in the mobile phase, the between-column retention time reproducibility of the ionized basic compounds was further improved, while the retention time reproducibility of the non-ionized compounds remained the same as it was without TEA in the mobile phase. TEA may have associated with the remaining free unassociated silanol groups causing a decreased interaction with the ionized basic solutes.

Further evidence of TEA affecting the interaction of free silanol groups was observed in the retention time of ionized basic compounds on Zorbax RX and Zorbax C_8. On Zorbax C_8, TEA in the mobile phase resulted in a decrease in the retention time of most of the ionized bases. On Zorbax RX, TEA in the mobile phase caused a change in the retention time of the ionized basic compounds, but the direction of the change was not constant. If the presence of TEA decreased the interaction of the silanol groups with the ionized solutes, it would seem that a

decrease in the retention of non-ionized bases would result. The effect of TEA on the retention time of ionized bases on type B silica may be other than a simple blocking of silanol groups.

Whether or not TEA was used in the mobile phase, the retention time of non-ionized compounds was similar on the type A and the type B silica reverse phase columns. However, when TEA was not used in the mobile phase there was a significantly longer retention time of ionized basic compounds on the type A silica reverse phase column compared to the type B silica reverse phase column. When TEA was used in the mobile phase, the retention time of ionized basic compounds were similar on the type B and type A silica reverse phase columns. The greater change in retention times with and without TEA in the mobile phase for ionized/partially ionized bases on the Zorbax C8 column was expected due to the greater number of unassociated silanol groups on this phase relative to the Zorbax RX phase. The reason for the slightly greater change in the retention times with and without TEA in the mobile phase for the non-ionized compounds on the Zorbax RX was not clear.

Long term reproducibility of retention times of acidic, neutral and basic compounds on the Zorbax RX column with TEA in the mobile phase appears to be no greater than the variation observed between columns in the present study. The ionized bases, desipramine and imipramine showed the greatest deviation in retention time (0.181 min and 0.236 min, respectively). However, other ionized bases showed a standard deviation of less than 0.1 min. These data suggest a good long term consistence of drug retention times in this system.

CONCLUSION

The retention time of ionized bases and non-ionized compounds was reproducible between different batches of Zorbax RX columns with or without

TEA in the mobile phase. The reproducibility of ionized bases was somewhat better than that of unionzed compounds when TEA was used in the mobile phase. Although a poor correlation of retention times of ionized bases was observed between type A and type B silica reverse phase columns without TEA in the mobile phase, these retention times were similar when TEA was used in the mobile phase. A good correlation of retention times of non-ionized compounds on type A and type B silica reverse phase columns was obtained with or without TEA in the mobile phase. The data in the present study and a previous study on chromatographic efficiency (11) indicated that efficient and reproducible chromatography of ionized basic drugs was obtained by the use of a reverse phase column prepared from type B silica and a mobile phase containing an amine modifier such as triethylamine. Additionally, these data suggest that the use of an amine modifier in the mobile phase allows for correlative retention of basic compounds between columns of great difference in the acidity of the silica.

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