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Comparison of the chromatography of octadecyl silane bonded silica and polybutadiene-coated zirconia phases based on a diverse set of cationic drugs

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Abstract

In this study, we compare the separation of basic drugs on several octadecyl silane bonded silica (ODS) phases and a polybutadiene-coated zirconia (PBD–ZrO_2) phase. The retention characteristics were investigated in detail using a variety of cationic drugs as probe solutes. The ODS phases were selected to cover a relatively wide range in silanol activity and were studied with ammonium phosphate eluents at pH 3.0 and 6.0. Compared to any of the ODS phases, the PBD–ZrO_2 phase showed very significant differences in selectivities towards these drugs. Due to the presence of both reversed-phase and ion-exchange interactions between the stationary phase and the basic analyte on ODS and PBD–ZrO_2, mixed-mode retention takes place to some extent on both types of phases. However, very large differences in the *relative* contributions from ion-exchange and reversed-phase interactions on the two types of phases led to quite different selectivities. When phosphate is present in the eluent and adsorbs on the surface, the PBD–ZrO_2 phase takes on a high negative charge over a wide pH range due to phosphate adsorption on its surface. On ODS phases, ion-exchange interactions result from the interactions between protonated basic compounds and ionized residual silanol groups. Since the pH of the eluent influences the charge state of the silanol groups, the ion-exchange interactions vary in strength depending on pH. At pH 6.0, the ion-exchange interactions are strong. However, at pH 3.0 the ion-exchange interactions on ODS are significantly smaller because the silanol groups are less dissociated at the lower pH. Thus, not only are the selectivities of the ODS and PBD–ZrO_2 phases different but quite different trends in retention are observed on these two types of phases as the pH of the eluent is varied. More importantly, by using the large set of ''real'' basic analytes we show the extreme complexity of the chromatographic processes on the reversed stationary phases. Both the test condition and solute property influence the column performance. Therefore, use of only one or two probe solutes is not sufficient for column ranking.

Keywords: Stationary phases, LC; Silanol activity; Column characterization; Octadecyl silane bonded silica phase; Polybutadiene-coated zirconia phase; Cationic drugs

1. Introduction

Silica-based stationary phases remain, after several decades of development, the first choice for most RPLC separations [1,2]. Despite its numerous posi-

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tive features [3,4], silica has several limitations. It has low thermal and pH stability [5,6], and the silanol activity of the specific phase must be considered when a silica-based column is used for the separation of basic (cationic) analytes [7]. The silanol activity of a silica-based column originates in the accessible residual silanol groups present on the surface even after it is chemically modified [8]. Under certain conditions, negatively charged silanol groups can strongly interact with positively charged basic solutes [8]. The detailed mechanism of silanol interactions is far from clear. However, ion-exchange is recognized as a major contribution to silanol interactions [7]:

$$(A^+)_m + (SiO^-X^+)_s \rightleftarrows (X^+)_m + (SiO^-A^+)_s \qquad (1)$$

where A^+ represents a protonated basic analyte, and X^+ stands for the counter-ion that is associated with the ionized silanol groups. The subscripts m and s denote the mobile and stationary phases, respectively.

Silanol activity can have deleterious effects on the separation of ionizable solutes, specifically on the peak asymmetry and width. Because elimination of all silanol groups from silica's surface by the bonding reaction is impossible due to steric factors [9], a wide array of methods to minimize the number and effects of the residual silanols have been investigated. Differences in silanol activity between different stationary phases continue to complicate chromatography and remain a significant problem with the application of silica-based stationary phases [10]. However, residual silanol activity can also be quite beneficial due to its effect, sometimes profound, on chromatographic selectivity, especially for analytes with similar hydrophobicity but significant differences in basicity or accessibility of the charge. It is often the case that only one particular type of reversed-phase material works for the separation of a given mixture due to the seemingly unique selectivity originating in the silanol interactions on that specific phase. Thus, the ion-exchange characteristics of a stationary phase are very important in selecting a specific column for a specific application.

Numerous tests with various types of probe solutes have been reported for the characterization of interactions on silica phases [11–13]. A considerable number of studies in classifying phases according to their silanol activity have been performed. For example, in one compilation of ODS stationary phases [14], 60 phases were ranked according to their efficiency toward basic compounds. However, column evaluation is not straightforward due to the many factors affecting the chromatography. No universally acceptable simple test for the characterization of all columns has been devised. A complete comparison is not available due to the diversity in test methods and the effect of the experimental conditions on the outcome. Furthermore, the simple molecules (e.g. pyridine, toluidines, and aniline) frequently used for column characterization are not able to reflect the very complex set of interactions that are commonly found in most pharmaceutical mixtures. Therefore, simple probes can only provide information concerning the major differences among columns. Silanol activity ''ranking'' of columns depends not only on the test conditions but also on the specific solutes [15]. General conclusions cannot be made using simple probes. Studies based on compounds with different structures and basicities are still necessary and important to achieve a better understanding and application of silica-based columns in the separation of basic drugs. McCalley has made many contributions based on the study of cationic analytes on silica-based reversed-phases [15–23]. He systematically studied the influence of analyte stereochemistry and basicity, solvent modifier type and solvent strength, sample mass, temperature, and flow-rate on the chromatography of basic analytes. Both low pH and intermediate pH conditions were used in his studies [15,19]. Based on McCalley's studies, the use of buffered eluents appears to be essential to probe silanol interactions when strong basic analytes are used due to the variable ionization effects [22]. Only major differences between columns can be quantified with unbuffered eluents. He found that compounds with high pK_as could give greater peak asymmetry simply because of the increased ion-exchange interactions with the ionized silanol groups. According to McCalley, tests based on solutes of different structures at both high and low pH conditions are important for column evaluation. He also pointed out that column evaluation based on two or three probes is insufficient. It is clear from McCalley's studies that the use of test probes similar to real analytes is essential.

Zirconia-based phases are relatively recent intro-

ductions into HPLC and they offer some advantages relative to silica-based phases. Although they have not been studied as extensively as silica-based phases, zirconia-based phases have gained more and more attention due to several unique characteristics [24–26]. The most useful features are their high thermal and chemical stability over a wide range in temperature (up to 200 °C) and pH (1 to 14) [27–29]. The major complexity in using zirconia-based phases is their extremely different surface chemistry relative to the nearly ubiquitous silica phases [27]. The presence of coordinatively unsaturated strong, hard Lewis acid zirconium (IV) sites on the surface of zirconia allows Lewis base functional groups (e.g. $R–SO_3^-$, $R–PO_3^-$, $R–COO^-$) of both analytes and eluent components to interact with the surface. Usually, these interactions are very strong and frequently cause poor peak shape and large peak width due to the slow dissociation kinetics of analytes that interact strongly with the surface. Complete coverage of the surface to eliminate these interactions is difficult. For example, only about 70% of zirconia's surface can be covered even with a very high loading of a polybutadiene (PBD) coating [30].

In an earlier study, we showed that we could dynamically modify PBD–ZrO_2 phases by adding Lewis bases to the eluent [31]. When Lewis base buffers (e.g. PO_3^-, F^-, $R–COO^-$) are used in the eluent, the adsorbed anions generate a negatively charged surface. Also, at high pH surface hydroxyl groups (i.e. Zr–OH group) can dissociate to give negatively charged sites. The PBD coating provides the reversed-phase component. Consequently, when eluents containing Lewis base additives are used, PBD–ZrO_2 phases acquire both reversed-phase and cation-exchange characteristics which can be used to control the selectivity of cationic analytes. The adsorbed Lewis base anions and the ionized zirconol groups serve as the cation-exchange sites by the following processes:

$$(Zr–L^-:X^+)_s + (A^+)_m \rightleftharpoons (Zr–L^-:A^+)_s + (X^+)_m \quad (2)$$

$$(Zr–O^-:X^+)_s + (A^+)_m \rightleftharpoons (Zr–O^-:A^+)_s + (X^+)_m \quad (3)$$

where A^+, X^+, $Zr–O^-$, and L^- represent the positively charged basic analyte, the counter-ion, the dissociated zirconol group, and the adsorbed Lewis base anion, respectively. Thus, basic compounds can undergo reversed-phase and ion-exchange mixed-mode retention on the PBD–ZrO_2 phase.

It is clear from the above discussion that basic compounds can undergo mixed-mode retention on both alkyl silane bonded silica and PBD–ZrO_2 phases. The retention mechanisms are similar in that the two major contributions are reversed-phase and ion-exchange interactions. In this study, we want to determine how different the ODS and PBD–ZrO_2 stationary phases are in terms of selectivity and column efficiency for the separation of basic analytes. Of special interest is the column selectivity stemming from different stationary phase materials. We expect that this comparison will provide useful information for column selection for the separation of basic compounds.

We also investigated the silanol interactions on silica phases. Since ODS phases are the most common silica stationary phases, ODS columns were used in this study. Selected basic drugs were used to explore the efficiency of columns with different silanol activity to provide some insights for proper column selection. Our purpose is not to find a procedure to universally characterize different columns, but rather to use practical application to obtain information that is not available from simple probes.

2. Experimental

2.1. Instruments

All chromatographic work was performed on a Hewlett-Packard 1090 chromatography system, equipped with a binary pump, helium sparger, autosampler, thermostatted-column compartment, diode array UV detector and a computer-based ChemStation software (ChemStation for LC 3D, Rev. A.08.03 [847], Agilent Technologies, Hewlett-Packard, Wilmington, DE, USA). The retention time, plate count ((retention time$\times$2.35/(half height peak width))^2), and asymmetry factor were reported by ChemStation.

2.2. Analytical columns

All ODS columns used in this study were

15 cm×0.46 cm I.D. (particle size 5 μm). The Zorbax columns were donations from Agilent Technologies. The ACE column was a gift from MacMod Analytical (Chadds Ford, PA, USA). The Alltima and Inertsil columns were obtained from Alltech Associates (Deerfield, IL, USA).

PBD–ZrO_2 particles (batch No. 24-124, particle size 4.1 μm) used in this work were obtained from ZirChrom Separations (Anoka, MN, USA). A 5 cm× 0.46 cm I.D. column was packed by the downward slurry method at 5000 p.s.i.

Table 1 summarizes the physical characteristics of the eight columns used in this study.

2.3. Reagents

All chemicals used in this study were reagent grade or better. HPLC-grade methanol (MeOH) was purchased from Pharmco (Brookfield, CT, USA). HPLC water was obtained from a Barnsted Nanopure deionizing system (Dubuque, IA, USA) run through an ''organic-free'' cartridge followed by a 0.2 μm particle filter. The water was boiled to remove carbon dioxide. Solvents were filtered through a 0.45 μm filter (Lida Manufacturing, Kenosha, WI, USA) before use. The ammonium phosphate (monobasic) was purchased from Fisher Chemicals (Fair Lawn, NJ, USA). The antihistamine and antidepressant drugs were obtained from Theta (Newtown Square, PA, USA). The other chemicals were obtained from Aldrich (Milwaukee, WI, USA).

2.4. Chromatographic conditions

Chromatography was performed at a flow-rate of 1 ml/min with UV detection at 254 nm. The injection volume was 1 μl. Analyte concentrations were adjusted to avoid overload of the columns. The experimental temperature was controlled at 35 °C with a precision of ±0.2 °C, unless specified otherwise. The dead times of the ODS columns were determined by injecting uracil, and acetone was used as the dead time marker for the PBD–ZrO_2 column. The eluents were prepared by first adjusting the aqueous monobasic ammonium phosphate (prepared with HPLC water) with phosphoric acid or ammonium hydroxide to the desired pH (measured before the addition of the organic modifier), then filtering the buffer through a 0.45 μm membrane filter prior to use, and finally mixing the aqueous buffer with pre-filtered methanol.

3. Results and discussions

Many approaches have been used to evaluate reversed-phase columns. Simple molecules, such as toluene, ethylbenzene, aniline, and pyridine, are commonly used as probe solutes [11,12]. However, ''real'' analytes such as drugs generally have much more complex structures. For example, more than 80% of all drugs have one or more basic groups [4]. In this work, a set of seven judiciously selected ODS phases were first evaluated using simple basic and

Table 1
Characteristics of the stationary phases[a]

Column	Designation	Surface area (m^2/g)	Pore size (Å)	Carbon content (% w/w)	Endcapped
ACE	ACE	300	100	15.5	Yes
Zorbax Eclipse	EC	186	80	10	Yes
Inertsil ODS-3	INER	436	95	14.7	Yes
Zorbax Extend	EX	179	80	10	Yes
Zorbax SB	SB	180	80	10	No
Alltima	ALLT	350	100	16	Yes
Zorbax RX	RX	172	80	10	No
PBD–ZrO_2	PBD	11.2[b]	500	2.5	No

[a] Data provided by the manufacturers unless noted otherwise.
[b] Data obtained by BET.

neutral probes to define their overall performance. Then, a set of basic drugs, predominantly antihistamines and antidepressants, were used to further probe their chromatographic performance.

3.1. Overview of column performance

To achieve a more general understanding of silanol interactions, ODS phases covering a wide range in silanol activity were selected. We specifically picked columns according to the silanol activity ranking in Ref. [14]. Columns with very low, low, and moderate silanol activity were used. To obtain reasonable column efficiency and ensure that all analytes would elute under the same test conditions, columns with very high silanol activity were avoided. Initially, the silanol activity and hydrophobicity of each column were characterized by amitriptyline, pyridine, and acenaphthene [14,32].

As can be seen in Table 2, the retention factor (k') of amitriptyline (pK_a 9.4) which is fully protonated under the test condition varies considerably from column to column. The trend in k' for pyridine from column to column is slightly different from that of amitriptyline. However, since pyridine (pK_a 5.17) is predominantly uncharged under the test condition (pH 6.0), amitriptyline is a better probe of silanol activity. Neue and co-workers prefer to use the retention factor ratio of a basic compound to a neutral compound as the probe parameter of silanol activity [33,34]. From Table 2, we see that, among the ODS phases, k'_{am}/k'_{ac} increases twofold from the Extend (0.47) to the SB (0.99) column. This indicates that these columns differ considerably in their silanol activities. Table 2 also compares the plate count and asymmetry factor of amitriptyline for the different phases. Again, these data can be used to rank columns for their silanol activity [14,22]. Among the ODS phases, the ACE column has the best peak shape based on both the plate count and asymmetry factor for amitriptyline. On the other hand, based on this table the Alltima and RX columns have relatively high silanol activities and low column efficiencies.

An interesting result is that the three methods for phase ''ranking'' give different results. Clearly, different orders of silanol activity can be obtained depending on the use of relative retention factor, plate count, and asymmetry factor even with the *same* probe solute. We also point out that based on

Table 2
Stationary phase comparison based on ion-exchange and reversed-phase interactions[a]

Column[b]	k'_{am}[c]	k'_{py}[d]	k'_{ac}[e]	N_{am}[f]	Rank 1[g]	As_{am}[h]	Rank 2[i]	k'_{am}/k'_{ac}[j]	Rank 3[k]
PBD	5.16	0.01	0.88	52 600	1	0.90	3	5.89	8
ACE	1.90	0.16	3.76	50 700	2	0.99	1	0.51	2
EC	2.32	0.17	4.21	49 600	3	0.81	4	0.55	3
INER	3.02	0.25	5.46	37 500	4	0.95	2	0.55	4
EX	2.07	0.16	4.38	35 600	5	0.67	5	0.47	1
SB	3.20	0.25	3.25	34 300	6	0.60	6	0.99	7
ALLT	4.51	0.31	5.26	17 800	7	0.34	7	0.86	6
RX	2.97	0.25	4.09	11 000	8	0.31	8	0.73	5

[a] Test condition: MeOH–25 m*M* ammonium phosphate buffer (80:20, v/v, pH 6.0); temperature, ambient (about 25 °C).
[b] Columns are listed in descending order of the plate count (N) for amitriptyline.
[c] Retention factor (k') for amitriptyline.
[d] k' for pyridine.
[e] k' for acenaphthene.
[f] N (per metre) for amitriptyline.
[g] Column ranking based on N for amitriptyline.
[h] Asymmetry factor (As, greater than 1, fronting; less than 1, tailing) for amitriptyline.
[i] Column ranking based on As for amitriptyline.
[j] Retention factor ratio for amitriptyline relative to acenaphthene.
[k] Column ranking based on retention factor ratio for amitriptyline relative to acenaphthene.

the plate count for amitriptyline, the PBD–ZrO_2 column ranks first but based on the ratio of k' for amitriptyline relative to acenaphthene it ranks last. This clearly points towards the very different chemical properties of this material compared to the seven ODS phases studied. Table 2 also shows that differences in hydrophobicity for the ODS columns are not as large as are the differences in silanol activity since the range in the retention of acenaphthene for different columns is not large (5.46/3.25 = 1.68). However, what we have obtained here is just a rough picture of the ion-exchange interactions of these phases since this property, in our opinion, cannot be fully defined by use of only one or two probes [35–37].

3.2. Phase comparison based on selectivity and retention of antihistamine and antidepressant analytes at pH 6.0

Tables 3 and 4 show a comparison of the different phases based on the retention and selectivity at pH 6.0 for some antihistamines and antidepressants (see Figs. 1 and 2 for structures). The antihistamines exhibit a common elution sequence for the seven ODS phases. For the antidepressants, there are some minor changes in elution order for the ODS phases. However, the PBD–ZrO_2 phase shows significant differences in elution order relative to the ODS phases for *both* sets of probes.

Fig. 3 compares the selectivity of each column based on all 17 basic drugs. Among all the solutes, the retention factors of perphenazine on each column have the smallest relative standard deviation. As it becomes clear later (see below), the pH of the eluent has a very small effect on the retention of buclizine. We specifically use perphenazine and buclizine as the reference solutes to compare the selectivity difference among the stationary phases. We note that in Fig. 3 PBD–ZrO_2 phase is dramatically different from all ODS phases. Difference in retention order between different ODS phases is much less than for PBD–ZrO_2 phase regardless of the solute used for normalization.

According to Horvath et al. [38], plots of log k' under one set of chromatographic conditions versus log k' under other chromatographic conditions, which are called κ–κ plots, can be used to assess the

Table 3
Stationary phase comparison based on retention factors and selectivities of the antihistamines at pH 6.0[a]

Solute[b]/column[c]	ACE	EC	INER	EX	SB	ALLT	RX	PBD
1. Thenyldiamine	1.09	1.20	2.29	1.09	1.85	2.58	1.77	2.88
2. Methapyrilene	1.17	1.31	2.47	1.19	1.88	2.59	1.77	2.74
3. Pyrilamine	1.31	1.47	3.07	1.29	2.27	3.13	2.01	3.11
4. Tripelennamine	1.45	1.62	3.17	1.45	2.5	3.48	2.36	3.18
5. Brompheniramine	1.84	1.94	4.08	1.76	3.33	5.23	3.77	6.04
6. Triprolidine	2.13	2.38	4.96	2.08	4.31	5.42	3.82	3.42
Column[c]	ACE	EC	INER	EX	SB	ALLT	RX	PBD
$\alpha_{2/1}$[d]	1.07	1.09	1.08	1.09	1.02	1.00	1.00	0.95
$\alpha_{3/2}$	1.12	1.12	1.24	1.08	1.21	1.21	1.14	1.14
$\alpha_{4/3}$	1.11	1.10	1.03	1.12	1.10	1.11	1.17	1.02
$\alpha_{5/4}$	1.27	1.20	1.29	1.21	1.33	1.50	1.60	1.90
$\alpha_{6/5}$	1.16	1.23	1.22	1.18	1.29	1.04	1.01	0.57
Med.[e]	1.12	1.12	1.22	1.12	1.21	1.11	1.14	1.14
Max.[e]	1.27	1.23	1.29	1.21	1.33	1.50	1.60	1.90
Min.[e]	1.07	1.09	1.03	1.08	1.02	1.00	1.00	1.02

[a] Test condition: MeOH–25 m*M* ammonium phosphate buffer (60:40, v/v, pH 6.0), see Experimental section for other conditions.
[b] The solutes are listed in ascending order of the k' values on the ACE column.
[c] ODS columns (left to right) are ordered according to the *N* values for amitriptyline (see Table 2).
[d] Selectivity $\alpha_{i/j}$ calculated as k_i/k_j.
[e] Median, maximum, and minimum of selectivity on each column. All α values less than 1.0 were inverted to $1/\alpha$ for calculations of median, maximum, and minimum.

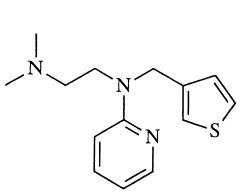
Table 4
Stationary phase comparison based on retention factors and selectivities of the antidepressants at pH 6.0[a]

Solute[b]/column	ACE	EC	INER	EX	SB	ALLT	RX	PBD
1. Chlordiazepoxide	0.53	0.57	0.80	0.51	0.59	0.77	0.45	0.12
2. Desipramine	0.87	0.91	1.36	0.61	2.06	2.26	1.28	13.69
3. Nortriptyline	1.06	1.16	1.71	0.81	2.32	2.80	1.56	13.96
4. Doxepin	1.65	1.96	2.84	1.73	2.66	4.60	2.43	4.62
5. Thiothixene	2.28	2.73	3.68	2.38	3.70	5.96	2.72	3.53
6. Imipramine	2.40	2.93	4.21	2.51	4.12	6.90	3.76	7.22
7. Amitriptyline	3.15	3.91	5.05	3.33	5.41	8.87	4.73	6.60
8. Buclizine	3.39	3.91	5.56	3.91	3.70	5.18	3.40	0.61
9. Hydroxyzine	3.39	3.95	5.64	3.93	3.70	5.25	3.40	1.40
10. Thioridazine	4.53	5.79	7.99	4.92	7.43	17.77	7.97	13.81
11. Perphenazine	5.37	6.35	8.43	5.78	6.47	8.87	5.30	7.40
Column[c]	ACE	EC	INER	EX	SB	ALLT	RX	PBD
$\alpha_{2/1}$[d]	1.64	1.60	1.70	1.20	3.49	2.94	2.84	114
$\alpha_{3/2}$	1.22	1.27	1.26	1.33	1.13	1.24	1.22	1.02
$\alpha_{4/3}$	1.56	1.69	1.66	2.14	1.15	1.64	1.56	0.33
$\alpha_{5/4}$	1.38	1.39	1.30	1.38	1.39	1.30	1.12	0.76
$\alpha_{6/5}$	1.05	1.07	1.14	1.05	1.11	1.16	1.38	2.05
$\alpha_{7/6}$	1.31	1.33	1.20	1.33	1.31	1.29	1.26	0.91
$\alpha_{8/7}$	1.08	1.00	1.10	1.17	0.68	0.58	0.72	0.09
$\alpha_{9/8}$	1.00	1.01	1.01	1.01	1.00	1.01	1.00	2.30
$\alpha_{10/9}$	1.34	1.47	1.42	1.25	2.01	3.38	2.34	9.86
$\alpha_{11/10}$	1.19	1.10	1.06	1.17	0.87	0.50	0.66	0.54
Med.[e]	1.27	1.30	1.23	1.22	1.23	1.47	1.39	2.17
Max.[e]	1.64	1.69	1.70	2.14	3.49	3.38	2.84	114
Min.[e]	1.00	1.00	1.01	1.01	1.00	1.01	1.00	1.02

[a] Test condition: MeOH–25 m*M* ammonium phosphate buffer (72:28, v/v, pH 6.0), see Experimental section for other conditions.
[b] The solutes are listed in ascending order of the k' values on the ACE column.
[c] ODS columns (left to right) are ordered according to the *N* values for amitriptyline (see Table 2).
[d] Selectivity $\alpha_{i/j}$ calculated as k_i/k_j.
[e] Median, maximum, and minimum of selectivity. All α values less than 1.0 were inverted to $1/\alpha$ for calculation of median, maximum, and minimum.

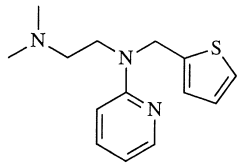
energetic difference in retention under the different conditions. A strong linear correlation (R^2 close to 1) in a κ–κ plot implies that both conditions are governed by the same retention mechanism. A scattered correlation (R^2 substantially less than 1) means that different retention mechanisms present under the two conditions. Zhao and Carr [39] have discussed the chemical meaning and practical implications of κ–κ plots in detail. They concluded that the correlation or lack of correlation of such plots can be used as a criterion for differences in chromatographic selectivity. This approach has been used to evaluate column selectivity [39–41]. The standard deviation of a κ–κ plot can also be used, as a larger standard deviation implies a bigger difference in selectivity.

Fig. 4 shows two κ–κ plots obtained in this work. The correlation coefficients of all κ–κ plots are summarized in Tables 5 and 6. The selectivity comparison based on standard deviations of the κ–κ plots are shown in Fig. 5. For both the antihistamines and antidepressants, the selectivity differences among the ODS phases are not very large ($0.998 \geq R^2 \geq 0.774$) compared to that of the PBD–ZrO_2 versus any of the ODS phases ($0.589 \geq R^2 \geq 0.035$). Fig. 5 clearly shows that the PBD–ZrO_2 differs significantly from any ODS phase, as reflected in the very weak correlations and large standard deviations in the κ–κ plots for any of the ODS phases and the PBD–ZrO_2 phase. The selectivity differences between ODS phases and the PBD–ZrO_2 phase are very striking, especially for the antidepressants. For



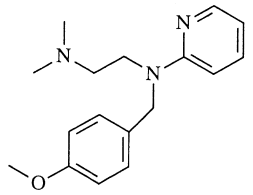
Thenyldiamine

(3.9, 8.9)



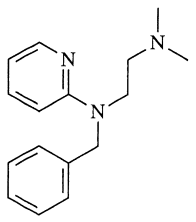
Methapyrilene

(3.7, 8.9)



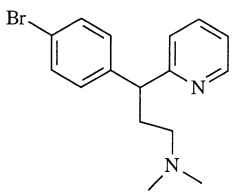
Pyrilamine

(4.0, 8.9)



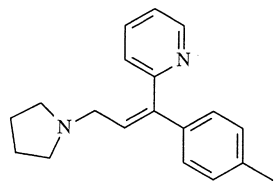
Tripelennamine

(4.2, 8.7)



Brompheniramine

(3.6, 9.8)



Triprolidine

(6.6)

Fig. 1. Structures and pK_as of the antihistamines.

example, examination of Table 4 indicates that the separation of desipramine and nortriptyline on the PBD–ZrO_2 is almost impossible at pH 6.0 ($\alpha = 1.02$), but quite easy ($\alpha > 1.1$) on any of the ODS phases. In stark contrast, the separation of hydroxyzine and buclizine is all but impossible ($\alpha \approx 1.0$) on any of the ODS phases but quite easy ($\alpha = 2.3$) on the PBD–ZrO_2.

Tables 3 and 4 also demonstrate that even the relatively small differences in selectivities among the ODS phases will have very significant effects on the separation of basic drugs. The variation in selectivity for each solute pair from phase to phase will have very important and practical chromatographic consequences. Selectivities greater than 1.1 correspond to quite easy separations while selectivities less than 1.02–1.03 can be extremely difficult and require an excessive number of plates. Even a cursory inspection of Tables 3 and 4 indicates many instances where a separation is nearly impossible on one phase and easy on another. There is no pattern of selectivity that would indicate that one silica-based phase offers globally or even commonly superior performance to another phase in terms of this critical parameter. The observation is hardly surprising but clearly rationalizes the reason for the existence of nearly 400 different commercial ODS phases [42].

Since basic solutes undergo mixed-mode retention on both PBD–ZrO_2 and ODS phases, it is reasonable to postulate that the differences in retention factor and selectivity arise from the different relative contributions of ion-exchange and reversed-phase interactions to the overall retention on each type of phase. We know that for ‘‘pure’’ ion-exchange

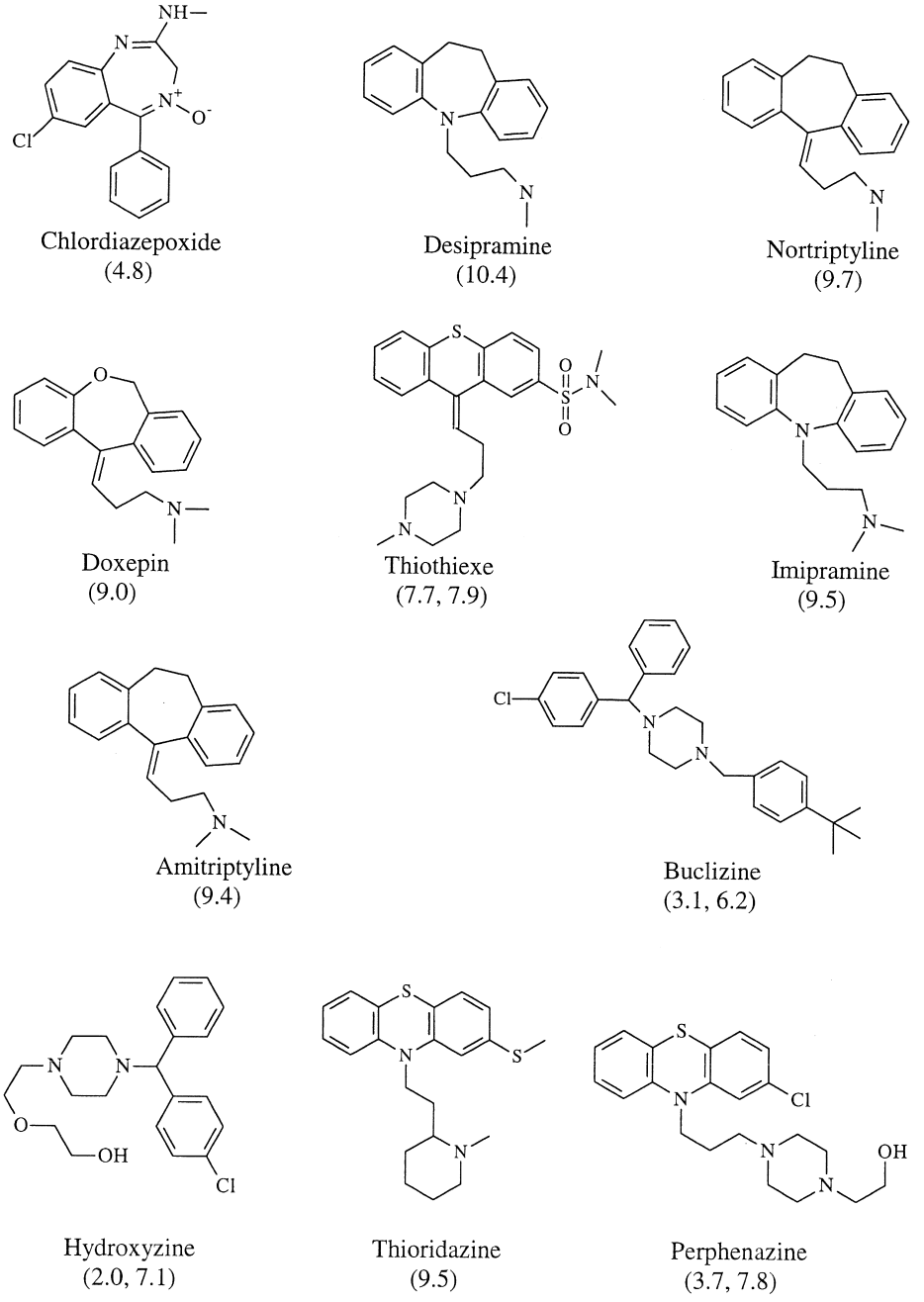


Fig. 2. Structures and pK_as of the antidepressants.

chromatography, the following equation, based on a stoichiometric displacement model, can be used to relate k' to the counter-ion concentration in the eluent [43,44]:

$$\log k' = -s \log [C] + \text{constant}$$

where s is a constant that depends on the charge of the displacer (counter-ion) relative to the analyte, [C]

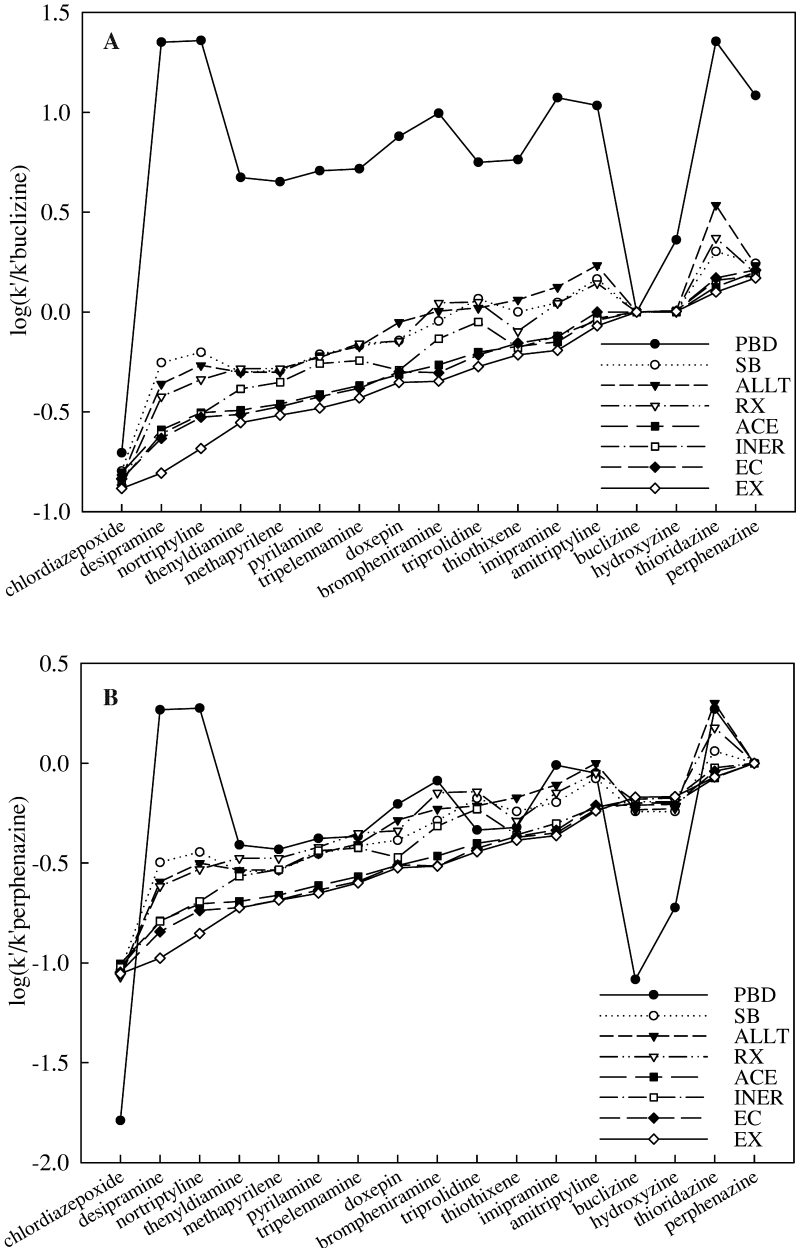


Fig. 3. Stationary phase comparison based on normalized retention factors relative to buclizine (A) and perphenazine (B). Solutes are ordered in ascending order of the normalized retention factors on the ACE column (data based on Tables 3 and 4).

is the concentration of the counter-ion in the eluent. For a pure ion-exchange process, s equals the charge ratio of the analyte ion to the counter-ion. However, for a typical reversed-phase column, there are significant deviations between s and the related charge ratio. According to our recent work, a comparison of the intercept and the slope of a plot of k' versus $1/[C]$ can be used as a criterion of the relative contribution of ion-exchange to the total retention [45]. Such data for several drugs on both ODS and

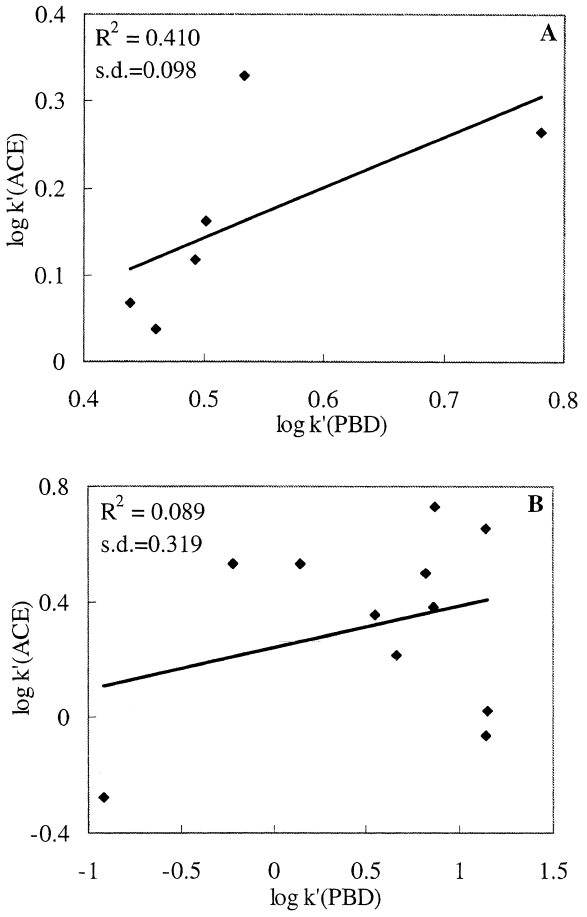


Fig. 4. κ–κ plots of the ACE and PBD columns for the antihistamines (A) (data based on Table 3) and the antidepressants (B) (data based on Table 4) at pH 6.0.

PBD–ZrO_2 (see Table 7, experimental data adapted from Ref. [46]) show that the counter-ion concentration has a much larger effect on retention on the PBD–ZrO_2 phase than that on the ODS phase at neutral pH (i.e. pH 7.0). These results indicate that a very different relative contribution of ion-exchange to reversed-phase interactions exists on the ODS and PBD–ZrO_2 as shown by the percentage of the ion-exchange contribution to the overall retention.

On the ODS phases, the reversed-phase contribution is dominant, and ion-exchange plays a minor role (see Table 7). The situation on the PBD–ZrO_2 is exactly opposite. This was confirmed by examining the retention factors of amitriptyline and nortriptyline (see Table 4). The only difference between these two probe solutes is the additional methyl group of amitriptyline (see Fig. 2). On ODS phases, nortriptyline elutes before amitriptyline, indicating that the reversed-phase interaction is the major contributor. However, on the PBD–ZrO_2 phase, nortriptyline is much more retained than is amitriptyline. This is consistent with the fact of the dominance of ion-exchange over reversed-phase interactions on the PBD–ZrO_2 phase. It is the difference in the *relative contribution* of ion-exchange and reversed-phase interactions that causes the different column selectivities between the ODS and PBD–ZrO_2 phases. A more detailed description and comparison of the retention mechanism on PBD–ZrO_2 and ODS phases can be found in Ref. [45].

3.3. Phase comparison based on selectivity and retention of antihistamine and antidepressant analytes at pH 3.0

pH 3.0 is often used for the separation of basic analytes on ODS phases because of the improvement in peak shape and suppression of silanol interactions compared to high pH [19]. However, it has been

Table 5
Correlation coefficients (R^2) of κ–κ plots of the antihistamines at pH 6.0[a]

R^2	EX	EC	RX	INER	SB	ALLT	PBD
ACE	0.997	0.992	0.957	0.985	0.989	0.972	0.410
EX		0.996	0.938	0.979	0.986	0.952	0.365
EC			0.915	0.988	0.988	0.937	0.328
RX				0.916	0.937	0.993	0.589
INER					0.983	0.949	0.369
SB						0.955	0.355
ALLT							0.565

[a] Data based on Table 3.

Table 6
Correlation coefficients (R^2) of κ–κ plots of the antidepressants at pH 6.0[a]

R^2	EX	EC	RX	INER	SB	ALLT	PBD
ACE	0.982	0.998	0.907	0.997	0.861	0.840	0.089
EX		0.985	0.852	0.980	0.774	0.785	0.035
EC			0.919	0.998	0.866	0.861	0.092
RX				0.926	0.970	0.980	0.275
INER					0.871	0.863	0.099
SB						0.956	0.382
ALLT							0.325

[a] Data based on Table 4.

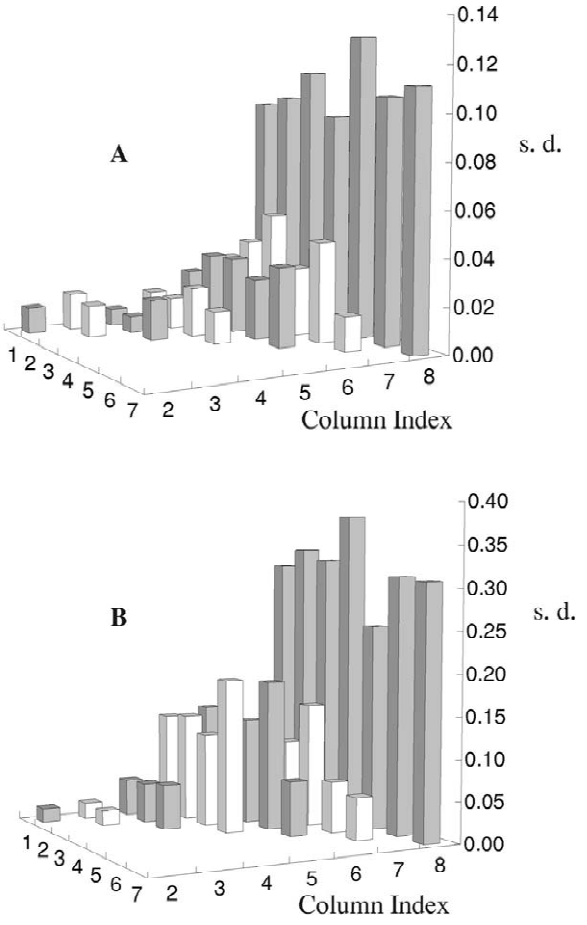


Fig. 5. Three-dimensional plots of standard deviations of the κ–κ plots for the antihistamines (A) (data based on Table 3) and the antidepressants (B) (data based on Table 4) at pH 6.0. (Note: the vertical scales differ in A and B.) Column index: 1, ACE; 2, EC; 3, INER; 4, EX; 5, SB; 6, ALLT; 7, RX; 8, PBD.

suggested that the optimum pH may depend on the individual analyte [15,19,22]. McCalley has compared the chromatographic performance of different columns at pH 7.0 and 3.0 using both simple molecules and basic drugs. He pointed out that column performance is pH dependent; therefore, testing at the pH of intended use is advisable. The most troublesome probes at pH 7.0 may not be the most difficult at pH 3.0 [19]. He found that the average asymmetry factor improved considerably upon decreasing the pH from 7.0 to 3.0. Furthermore, he found that intercolumn differences were smaller at pH 3.0 than 7.0. Column performance is more solute-dependent at pH 7.0 than at 3.0.

In this work, we also studied the chromatography of the antihistamines and antidepressants at pH 3.0. Tables 8 and 9 give the retention data and the standard deviations of the κ–κ plots are shown in Fig. 6. Again, there are only weak correlations between the PBD–ZrO_2 and ODS phases, which can be explained by big differences in the relative contribution of ion-exchange interactions. The correlations among the κ–κ plots for the ODS phases at pH 3.0 are higher compared to the correlations at pH 6.0. This observation is consistent with McCalley's report that intercolumn differences are smaller at acidic pH compared to neutral pH. At pH 3.0, most, but we feel not all, of the silanol groups on ODS phases are protonated. Therefore, ion-exchange interactions between the basic compounds and silanol groups are significantly suppressed at pH 3.0. Thus selectivity differences resulting from differences in the silanol activity of the various phases are attenuated at pH 3.0. Most of these drugs are only

Table 7
Comparison of the relative contribution of ion-exchange interactions on ODS and PBD–ZrO_2 columns as a function of buffer concentration (data adapted from Ref. [46])[a]

Solute	k' [d]			Regression data		% IEX[h]		
	5[e]	25[e]	50[e]	Slope[f]	Intercept[g]	5[e]	25[e]	50[e]
ODS column[b]								
1. Pheniramine	0.97	0.66	0.53	2.3±0.4	0.53±0.05	46%	20%	1%
2. Chlorpheniramine	2.23	1.39	1.27	5.3±0.1	1.17±0.01	48%	16%	8%
3. Thenyldiamine	2.41	1.60	1.42	5.3±0.4	1.35±0.04	44%	16%	5%
4. Brompheniramine	2.56	1.66	1.42	6.1±0.6	1.35±0.07	47%	18%	5%
5. Cyclizine	10.10	7.87	6.91	16.3±3.2	6.9±0.4	32%	13%	0%
6. Pyrrobutamine	20.03	14.60	11.06	44±14	11±2	43%	22%	−3%
7. Chlorcyclizine	23.51	18.62	15.91	38±10	16±1	32%	14%	−1%
PBD–ZrO_2 column[c]								
1. Pheniramine	11.57	3.56	1.98	52±3	1.2±0.3	90%	66%	40%
2. Chlorpheniramine	24.33	7.70	4.54	107.6±5	2.9±0.6	88%	63%	37%
3. Thenyldiamine	15.61	5.22	3.13	68±4	2.1±0.4	86%	59%	32%
4. Brompheniramine	30.8	9.69	5.51	133±8	3.6±0.9	88%	63%	35%
5. Cyclizine	20.94	8.55	5.79	82±6	4.7±0.7	78%	45%	19%
6. Pyrrobutamine	86.38	38.54	21.95	335±50	20±6	77%	48%	9%
7. Chlorcyclizine	39.44	17.74	11.13	149±19	10±2	75%	44%	11%

[a] Test condition: acetonitrile–potassium phosphate buffer (40:60, v/v, pH 7.0); 30 °C; 1 ml/min.
[b] 5 cm×0.46 cm I.D. Zorbax Extend ODS column from Agilent, particle size 3.5 μm.
[c] 5 cm×0.46 cm I.D. PBD–ZrO_2 column from ZirChrom, particle size 3 μm.
[d] Retention factor under each condition.
[e] Buffer concentration in mM.
[f] Slope of k' versus 1/[buffer] with standard error.
[g] Intercept of k' versus 1/[buffer] with standard error.
[h] Relative contribution of ion-exchange interactions based on the two-site model [45]. The negative value is due to random experimental error.

weakly retained on the ODS phases at pH 3.0. However, on the PBD–ZrO_2, despite the partial protonation of the zirconol and adsorbed phosphate groups, there are still very substantial ion-exchange interactions due to the presence of the adsorbed Lewis bases (phosphate) and in some cases partial double protonation of the analytes. *It is important to note that on the PBD–ZrO_2 the retention factors of most drugs in this study at pH* 3.0 *are even greater than at pH* 6.0. Thus, significant retention and very different selectivity of basic drugs are observed on the PBD–ZrO_2 phase compared to the ODS phases.

Table 8
Stationary phase comparison based on k' of the antihistamines at pH 3.0[a]

Solute/column	ACE	EC	INER	EX	SB	ALLT	RX	PBD
1. Thenyldiamine	0.37	0.33	0.30	0.26	0.37	0.57	0.29	10.68
2. Methapyrilene	0.34	0.31	0.26	0.24	0.34	0.53	0.27	5.55
3. Pyrilamine	0.47	0.43	0.43	0.34	0.48	0.72	0.37	11.57
4. Tripelennamine	0.50	0.46	0.41	0.36	0.50	0.76	0.40	9.75
5. Brompheniramine	0.81	0.72	0.69	0.59	0.75	1.16	0.63	22.61
6. Triprolidine	0.65	0.60	0.58	0.47	0.69	0.98	0.51	9.48

[a] Test condition, same as Table 3 except pH. Solutes are ordered according to Table 3.

Table 9
Stationary phase comparison based on k' of the antidepressants at pH 3.0[a]

Solute/column	ACE	EC	INER	EX	SB	ALLT	RX	PBD
1. Chlordiazepoxide	0.54	0.51	0.79	0.49	0.57	0.78	0.47	0.13
2. Desipramine	0.72	0.75	0.60	0.55	0.74	1.02	0.58	14.24
3. Nortriptyline	0.81	0.72	0.68	0.61	0.82	1.12	0.64	13.67
4. Doxepin	0.33	0.34	0.26	0.24	0.33	0.50	0.27	7.00
5. Thiothixene	0.67	0.69	0.64	0.52	0.72	1.05	0.54	49.56
6. Imipramine	1.30	0.55	0.53	0.49	0.64	0.91	0.50	9.85
7. Amitriptyline	0.73	0.71	0.60	0.55	0.72	1.03	0.57	8.47
8. Buclizine	3.48	3.81	5.00	3.79	3.61	5.36	3.59	0.61
9. Hydroxyzine	3.49	4.35	5.69	3.80	3.61	5.35	3.58	4.25
10. Thioridazine	1.29	1.20	1.07	1.02	1.35	1.97	1.06	20.71
11. Perphenazine	1.54	1.55	1.47	1.21	1.53	2.17	1.26	NA[b]

[a] Test condition, same as Table 4 except pH. Solutes are ordered according to Table 4.
[b] No elution observed.

It is clear from Tables 8 and 9 that at pH 3.0, the PBD–ZrO_2 is a good alternative to ODS phases in that it can provide more retention, different band spacing, and unique selectivity. Furthermore, other studies show that at higher pH (pH>11), the retention of basic compounds decreases on PBD–ZrO_2 phase due to the deprotonation of the analytes and the subsequent decrease of ion-exchange interactions [31]. This is very different from the behavior of ODS phases [47] on which basic solutes usually have more retention at high pH (pH>10) due to the increase in reversed-phase interactions resulting from deprotonation of the analytes.

It is interesting to compare the retention factor ratio for each solute at the two pH conditions. We know that silanol interactions are weaker at pH 3.0 than at neutral pH. Upon comparing the retention of pH 3.0 and pH 6.0, we expect some changes in the performance of ODS phases. Tables 10 and 11 compare the retention factor ratios at pH 3.0 and 6.0. The medians of $k'_{\mathrm{pH\ 6.0}}/k'_{\mathrm{pH\ 3.0}}$ for each solute range from less than 1.0 to more than 6.0. The large range in the retention factor ratio clearly demonstrates the solute dependence of the overall chromatographic performance. Obviously, both column silanol activity and solute property influence retention.

Based on Table 10, we see that the retention factor ratios for the antihistamines are quite similar on each ODS column. The retention factors all increased three to four fold as the pH was increased from 3.0 to 6.0. However, for the antidepressants (see Table 11), while the ratios for some solutes show increases up to fivefold, a few actually have the same retention at pH 3.0 and 6.0. Examination of the structures of the basic drugs (see Figs. 1 and 2) indicates that both steric effects and pK_as of the solute have some influence on the observed retention factor ratios. It is interesting to note that the same classes of amines have similar ratios of $k'_{\mathrm{pH\ 6.0}}/k'_{\mathrm{pH\ 3.0}}$. For example, for cyclic amines (buclizine and hydroxyzine), the ratios are about 1; secondary amines (chlordiazepoxide, nortriptyline, and desipramine), the ratios are 1–2; and for the tertiary amines (the antihistamines and the other tertiary amines among the antidepressants), the ratios exceed 3. We can summarize the following effects that account for the increase in retention factor from pH 3.0 to 6.0.

1. Ionization of the silanol groups on the stationary phase at neutral pH results in an increase in ion-exchange interactions.
2. Partial deprotonation of the analyte at neutral pH leads to an increase in reversed-phase interactions.

We want to point out that the above two effects on the retention factors are very solute- and condition-dependent (e.g. the pK_a and steric effect of the solute, the pH of the eluent).

The work of Bosch and Roses [48,49] clearly shows that the solvent composition has a big effect on pK_as and perhaps more importantly on the

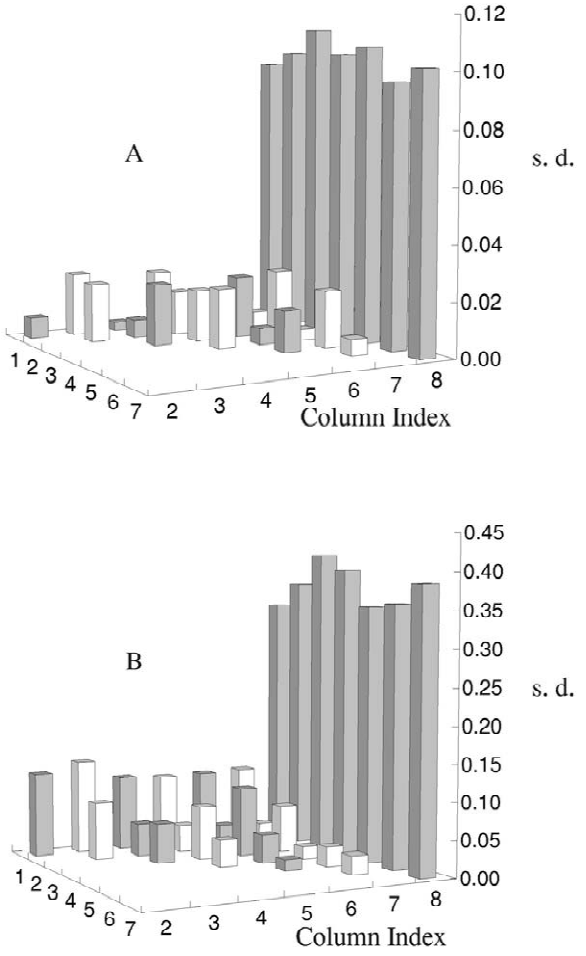


Fig. 6. Three-dimensional plots of standard deviations of the κ–κ plots for the antihistamines (A) (data based on Table 8) and the antidepressants (B) (data based on Table 9) at pH 3.0. (Note: the vertical scales differ in A and B.) Column index, same as Fig. 5.

effective hydrogen ion activity of the buffer. Because the organic modifier will affect the pK_a of the solute and the pH of the eluent, the above two effects create a very complex situation. To avoid any ambiguity in our understanding of the state of the protonation of the analytes in the work, we did proton NMR on a model compound (*p*-methylbenzylamine, pK_a 9.3) in the mobile phase used in the present study to measure the degree of protonation (data not shown here). We found that even at a pH of 6.0 (as established by the procedure used here), the analytes are more than 90% protonated. Therefore, for those amines whose pK_a is greater than 9.0, it is difficult to explain the large increase in k' upon changing the pH from 3.0 to 6.0. The degree of protonation of these strong bases changes only very slightly (<10%) from pH 3.0 to 6.0. In addition, studies of quaternary amines show that the ion-exchange interactions on ODS phase do not become much stronger upon changing the pH from 3.0 to 6.0 (data not shown here). On the other hand, the ''bulkiness'' of tertiary amines may explain their low retention at low pH.

In stark contrast to all the ODS phases, we see the opposite trend in retention on the PBD–ZrO_2 phase as pH is increased from 3.0 to 6.0. There is a significant decrease in k' for most solutes. We attribute this to the partial deprotonation of the dibasic amines which have their first pK_a between 3.0 and 6.0.

It is clear that the situation is very complex and more work is needed for further clarification. However, the above results definitely show that the relative column performance is not only eluent-dependent but also solute-dependent. Also, we must point out that at pH 3.0 the antihistamines are weakly retained on all the ODS phases and the reliability of the data could be significantly affected by small experimental errors. We must be very careful in using such retention data. Given the very low k' values of most drugs at pH 3.0 on the ODS phases, we will not interpret these results in detail.

3.4. Phase comparison based on efficiency and peak asymmetry

Both plate counts and asymmetry factors of basic analytes have been used to compare the silanol activity [14,19]. However, different results are often obtained using these two parameters. The amount of the analyte injected usually influences the plate count and asymmetry factor.

By comparing plate counts and asymmetry factors on the PBD–ZrO_2 and ODS phases, we can gain some insight as to the ''goodness'' of a column for a specific analyte. Tables 12 and 13 give the relevant

Table 10
Stationary phase comparison based on retention factor ratios at pH 6.0 and 3.0 for the antihistamines[a]

Solute[b]/column	ACE	EC	INER	EX	SB	ALLT	RX	PBD	Med.[c]	Max.[c]	Min.[c]
1. Thenyldiamine	2.95	3.64	7.63	4.19	5.00	4.53	6.10	0.27	4.36	7.63	0.27
2. Methapyrilene	3.44	4.23	9.50	4.96	5.53	4.89	6.56	0.49	4.92	9.50	0.49
3. Pyrilamine	2.79	3.42	7.14	3.79	4.73	4.35	5.43	0.27	4.07	7.14	0.27
4. Tripelennamine	2.90	3.52	7.73	4.03	5.00	4.58	5.90	0.33	4.30	7.73	0.33
5. Brompheniramine	2.27	2.69	5.91	2.98	4.44	4.51	5.98	0.27	3.71	5.98	0.27
5. Triprolidine	3.28	3.97	8.55	4.43	6.25	5.53	7.49	0.36	4.98	8.55	0.36
Med.[d]	2.92	3.58	7.68	4.11	5.00	4.55	6.04	0.30	–	–	–
Max.[d]	3.44	4.23	9.50	4.96	6.25	5.53	7.49	0.49	–	–	–
Min.[d]	2.27	2.69	5.91	2.98	4.44	4.35	5.43	0.27	–	–	–

[a] Data based on Tables 3 and 8. Retention factor ratios are calculated as $k'_{pH\ 6}/k'_{pH\ 3}$.
[b] Solutes are ordered according to Table 3.
[c] Median, maximum, and minimum of retention factor ratios of each solute.
[d] Median, maximum, and minimum of retention factor ratios of each column.

data. Since the retention factors of the analytes on the ODS phases are very small at pH 3.0, only the results at pH 6.0 are given. In those cases where exceptionally broad peaks were observed, especially in the case of the Alltima and RX columns, smaller amounts of sample were injected. The peak shape and width were not examined in detail as a function of the amount of the sample injected. However, we did observe some very confusing trends for a few solutes: the plate counts worsened, but the peak asymmetry improved when smaller amounts of sample were injected. McCalley reported a similar phenomenon at pH 7.0 [20,21]. For columns which generally gave good peak shapes (ACE, Eclipse, and Inertsil), the plate counts and asymmetry factors were more or less insensitive to the amount of sample injected and the results in these cases are reproducible. In those cases where the plate count

Table 11
Stationary phase comparison based on retention factor ratios at pH 6.0 and 3.0 for the antidepressants[a]

Solute[b]/column	ACE	EC	INER	EX	SB	ALLT	RX	PBD	Med.[c]	Max.[c]	Min.[c]
1. Chlordiazepoxide	0.98	1.12	1.01	1.04	1.04	0.99	0.96	0.92	1.00	1.12	0.92
2. Desipramine	1.21	1.21	2.27	1.11	2.78	2.22	2.21	0.96	1.71	2.78	0.96
3. Nortriptyline	1.31	1.61	2.51	1.33	2.83	2.50	2.44	1.02	2.02	2.83	1.02
4. Doxepin	5.00	5.76	10.92	7.21	8.06	9.20	9.00	0.66	7.63	10.92	0.66
5. Thiothixene	3.40	3.96	5.75	4.58	5.14	5.68	5.04	0.07	4.81	5.75	0.07
6. Imipramine	1.85	5.33	7.94	5.12	6.44	7.58	7.52	0.73	5.88	7.94	0.73
7. Amitriptyline	4.32	5.51	8.42	6.05	7.51	8.61	8.30	0.78	6.78	8.61	0.78
8. Buclizine	0.97	1.03	1.11	1.03	1.02	0.97	0.95	1.00	1.01	1.11	0.95
9. Hydroxyzine	0.97	0.91	0.99	1.03	1.02	0.98	0.95	0.33	0.98	1.03	0.33
10. Thioridazine	3.51	4.83	7.47	4.82	5.50	9.02	7.52	0.67	5.16	9.02	0.67
11. Perphenazine	3.49	4.10	5.73	4.78	4.23	4.09	4.21	NA	4.21	5.73	3.49
Med.[d]	1.85	3.96	5.73	4.58	4.23	4.09	4.21	0.76	–	–	–
Max.[d]	5.00	5.76	10.92	7.21	8.06	9.20	9.00	1.02	–	–	–
Min.[d]	0.97	0.91	0.99	1.03	1.02	0.97	0.95	0.07	–	–	–

[a] Data based on Tables 4 and 9. Retention factor ratios are calculated as $k'_{pH\ 6}/k'_{pH\ 3}$.
[b] Solutes are ordered according to Table 4.
[c] Median, maximum, and minimum of retention factor ratios of each solute.
[d] Median, maximum, and minimum of retention factor ratios of each column.

Table 12
Stationary phase comparison based on plate counts[a]

Solute[b]/column	ACE	EC	INER	EX	SB	ALLT	RX	PBD
1. Chlordiazepoxide	61 900	68 100	48 300	57 000	64 000	57 600	62 000	39 600
2. Desipramine	50 700	45 750	24 600	29 400	38 700	12 800	10 300	54 600
3. Nortriptyline	50 600	44 700	23 600	26 400	27 000	10 400	13 300	56 600
4. Doxepin	49 200	48 700	36 400	33 200	33 300	19 500	13 900	36 300
5. Thiothixene	50 800	50 900	35 600	40 550	33 900	23 800	6800	37 200
6. Imipramine	56 000	55 500	39 400	35 900	38 600	18 000	11 600	51 300
7. Amitriptyline	57 400	55 300	41 600	38 200	27 900	13 300	9900	51 500
8. Buclizine	94 500	93 900	72 400	86 600	88 700	79 500	84 200	56 900
9. Hydroxyzine	64 200	60 700	70 200	51 000	86 300	85 000	52 200	43 600
10. Thioridazine	51 500	54 600	38 400	33 900	34 800	6900	4700	47 200
11. Perphenazine	64 900	62 700	46 300	51 200	52 400	39 600	37 300	15 200
12. Thenyldiamine	52 600	51 700	44 400	26 900	21 200	18 900	4500	56 300
13. Methapyrilene	56 400	56 400	44 100	32 800	29 000	40 800	3500	57 100
14. Pyrilamine	60 000	59 600	43 200	45 500	21 100	17 700	4200	52 500
15. Tripelennamine	62 300	61 300	45 800	45 000	19 600	15 500	4700	56 200
16. Brompheniramine	58 000	56 000	41 100	43 400	5800	53 000	830	48 800
17. Triprolidine	57 300	57 900	42 400	44 600	8200	10 740	1300	49 100
Med. N[c]	57 300	56 000	42 400	40 550	33 300	18 900	9900	51 300
Max. N[c]	94 500	93 900	72 400	86 600	88 700	85 000	84 200	57 100
Min. N[c]	49 200	44 700	23 600	26 400	5800	6900	830	15 200
n_{best}[d]	8	4	0	0	1	0	0	4
n_{worst}[e]	0	0	0	0	0	1	12	4
$n_{above\ median}$[f]	17	16	8	5	8	2	1	13

[a] N, per meter.
[b] Solute, from 1 to 11, antidepressants (see Table 4 for test condition); from 12 to 17, antihistamines (see Table 3 for test condition).
[c] Median, maximum, and minimum of N on each column.
[d] Number of solutes for which this column gave the best N.
[e] Number of solutes for which this column gave the worst N.
[f] Number of solutes for which this column gave N above the median N for that solute.

and asymmetry factor changed in the opposite directions as the amount of sample was varied, we attempted to strike a balance between the plate counts and asymmetry factors while still keeping an acceptable signal-to-noise ratio. Therefore, the values of the plate count and asymmetry factor are sensitive to the amount of the sample injected. We nonetheless believe that the overall trends in retention, plate count, and symmetry factor are reproducible. We ran several of the columns twice over a wide interval of time and the same trend was obtained upon each trial.

According to Tables 12 and 13, there is very large column to column variation in the plate count and asymmetry factor. No universal trend among the columns involved in the present study is observed. From the results, we see that every column has at least one plate count above the median for some specific analytes. When the columns are sorted according to the plate count and asymmetry factor, some interesting results were obtained. If the columns are ordered according to the number of solutes whose plate count is better than that of the median solute, we find the order:

$$\text{ACE} > \text{Eclipse} > \text{PBD–ZrO}_2 > \text{Inertsil} = \text{SB} > \text{Extend} > \text{Alitima} > \text{RX}.$$

When ranked by asymmetry factor, the following order is obtained:

$$\text{Eclipse} > \text{ACE} > \text{PBD–ZrO}_2 > \text{Inertsil} > \text{SB} = \text{Extend} > \text{Alltima} = \text{RX}.$$

In general, the ACE, Eclipse, and PBD–ZrO_2

Table 13
Stationary phase comparison based on asymmetry factors[a]

Solute[b]/column	ACE	EC	INER	EX	SB	ALLT	RX	PBD
1. Chlordiazepoxide	0.81	0.91	0.93	0.82	0.89	0.85	0.86	0.48
2. Desipramine	0.75	0.54	0.59	0.53	0.64	0.42	0.48	0.97
3. Nortriptyline	0.73	0.59	0.56	0.54	0.33	0.39	0.52	1.02
4. Doxepin	0.83	0.81	0.75	0.69	0.57	0.58	0.34	1.25
5. Thiothixene	0.73	0.83	0.84	0.80	0.56	0.60	0.37	0.98
6. Imipramine	0.65	0.54	0.70	0.52	0.53	0.54	0.46	1.02
7. Amitriptyline	0.77	0.72	0.84	0.69	0.46	0.30	0.29	0.94
8. Buclizine	0.92	1.02	0.91	0.92	1.00	0.97	0.98	0.79
9. Hydroxyzine	0.96	1.01	0.91	0.93	1.02	0.92	0.98	0.91
10. Thioridazine	0.68	0.64	0.95	0.59	0.59	0.37	0.30	0.95
11. Perphenazine	0.87	0.98	0.96	0.90	0.88	0.63	0.77	0.34
12. Thenyldiamine	0.68	0.64	0.97	0.49	0.39	0.27	0.33	0.95
13. Methapyrilene	0.75	0.70	0.79	0.53	0.51	0.30	0.37	0.99
14. Pyrilamine	0.99	1.01	0.76	0.78	0.46	0.38	0.47	0.93
15. Tripelennamine	1.00	0.97	0.76	0.69	0.45	0.32	0.44	1.01
16. Brompheniramine	0.89	1.04	0.53	0.66	0.31	0.31	0.43	0.95
17. Triprolidine	0.86	0.86	0.65	0.72	0.24	0.28	0.35	0.91
Med. (As-1)[c]	0.19	0.17	0.21	0.31	0.47	0.61	0.56	0.06
Max. (As-1)[c]	0.35	0.46	0.47	0.51	0.76	0.73	0.71	0.66
Min. (As-1)[c]	0.00	0.01	0.03	0.07	0.00	0.03	0.02	0.00
n_{best}[d]	3	4	3	0	1	0	0	7
n_{worst}[e]	0	0	1	0	3	6	5	4
$n_{above\ median}$[f]	14	16	12	5	5	3	3	13

[a] As (greater than 1, fronting; less than 1, tailing).
[b] Solute, same as Table 12.
[c] Median, maximum, and minimum of (As-1) on each column.
[d] Number of solutes for which this column gave the best As.
[e] Number of solutes for which this column gave the worst As.
[f] Number of solutes for which this column gave (As-1) below the median (As-1) for that solute.

columns are good; they have high column efficiency and better peak shapes for most solutes used in this study. On the other hand, the Alltima and RX columns have relatively low column efficiency for most solutes (i.e. the plate counts and asymmetry factors for most solutes were below the median values). Although there are some minor discrepancies in the above trends for the plate count and asymmetry factor, the overall picture is the same. We see that a column evaluation based on large number of solutes is more robust than one based on two or three probes. However, again, we want to point out that the above results only provide a rough picture of the column efficiency. Thus one must be very cautious in interpreting results based on a small number of probes. There is likely no optimum probe or small set of probes for completely characterizing silanophilic interactions.

4. Conclusion

Based on the above results, the following conclusions were obtained.

1. The PBD–ZrO_2 and ODS phases have quite different selectivities for basic compounds due to the very different relative contributions from the ion-exchange and reversed-phase interactions.
2. Ion-exchange interactions are dominant, perhaps preponderant, on the PBD–ZrO_2 phase; reversed-phase interactions are dominant on the ODS phases.
3. For the ODS phases, low pH (in this case, pH 3.0) is not necessarily the better condition for the separation of basic compounds due to the significant decrease in band spacing and lower retention compared to that at neutral pH (i.e. pH 6.0).
4. On all ODS phases tested, basic compounds have

much *less* retention at pH 3.0 than at pH 6.0. The reasons behind the large decrease in retention factors on the ODS phases at the lower pH are not entirely clear. Both protonation of ionized silanol groups and of the analyte are involved. In contrast, there is substantial retention on PBD–ZrO_2 phase at both pH 3.0 and 6.0, and generally in clear contrast to the silica-based phases, *more* retention at pH 3.0 than at 6.0.

5. PBD–ZrO_2 is frequently a good alternative to ODS phases for the separation of basic compounds in that it sometimes provides larger band spacing and usually quite different elution sequences, especially at low pH.
6. Column performance depends not only on the eluent but also on the individual solute. Both the pK_a and specifically steric effect of the solute affect the plate count and asymmetry.
7. Despite the much greater participation of ion-exchange interactions on PBD–ZrO_2 column, the plate count and peak shape for most basic molecules are quite acceptable and in many cases better than those observed with ODS columns which generally show much weaker ion-exchange interactions.
8. Caution must be taken in choosing a column for the separation of basic compounds. Generalized column ranking based on only one or two compounds is often an unreliable predictor for retention, efficiency, and asymmetry.

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