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Retention studies on mixed-mode columns in high-performance liquid chromatography☆

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Abstract

The retention properties of a column prepared by mixing together strong cation exchange (SCX) and reversed-phase (C_{18}) packing materials were investigated using a range of test solutes. The column was found to exhibit chromatographic properties characteristic of both phases. The effects of changes in eluent composition, buffer ion, ionic strength and pH on the capacity factors of different compounds were determined. The dual nature of the retention mechanism allowed the retention of ionisable molecules to be adjusted by altering the composition of the aqueous component of the mobile phase while those of compounds uncharged over the pH range investigated remained unaffected. Results were compared with those obtained on a C_{18} column and it was found that the acidic and weakly basic compounds had higher capacity factors on this column whereas strongly basic compounds had higher capacity factors on the mixed-mode column.

1. Introduction

The term liquid chromatography encompasses a battery of separation techniques where a liquid mobile phase is the single common feature. A number of distinct separation modes are employed in liquid chromatography, but reversed-phase chromatography (RPLC) which consists of a non-polar bonded stationary phase in combination with a polar hydro-organic mobile phase is extensively, indeed almost exclusively, used in the majority of applications. The popularity of reversed-phase chromatography may be explained by its unmatched simplicity, wide applicability, and particularly by its versatility. Secondary chemical equilibria using ion suppression, ion pair formation, metal complexation and micelle formation may be exploited to effect changes in retention additional to those brought about by changing the ratio of the aqueous and organic components in the mobile phase. The exploitation of such secondary equilibria is commonly used to facilitate the separation of analytes of differing pK_a values.

While reversed-phase separation of charged analytes may be accomplished by ion pairing techniques, in recent years such separations are usually carried out on highly efficient HPLC phases especially designed to act as ion exchangers. Thus cations are separated on phases

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modified with a sulphonate group with mobile cationic counter ions, whereas anions are separated on phases modified with a quaternary ammonium group. Ions are retained by displacement of counter ions from the fixed charged sites and analyte binding may be modulated by the inclusion of competing ions in a buffered aqueous mobile phase.

For organic compounds which contain ionisable groups, the separation method of choice has traditionally been reversed-phase chromatography employing a buffered mobile phase to control the degree of ionisation and hence the retention of compounds on the hydrophobic stationary phase. However, the separation of basic analytes using reversed-phase high-performance liquid chromatography has often been problematic due to unwanted, or, more specifically, to uncontrolled secondary interactions with residual charged silanol groups on the silica surface. A number of approaches have been adopted to overcome this problem, such as ion suppression [1], paired ion chromatography [1,2] or by the addition of a deactivating agent such as an aliphatic amine to the eluent [3,4].

In an alternative approach, the ion-exchange properties of silica have actually been exploited by using an unmodified silica column in conjunction with an eluent containing a high percentage of organic modifier [5]. For example, methanol–ammonium nitrate eluents have been used in the separation of basic drugs including narcotics, analgesics and amphetamine-related compounds [6–8]. Such systems have been shown to offer superior retention characteristics for charged amines over conventional reversed-phase separations.

In this work we were interested in investigating the retention properties of columns containing equal proportions (w/w) of ion-exchange and reversed-phase materials, since the combined separation modes would increase the number of parameters that could be varied to optimise retention and resolution and thus permit the separation of both charged and uncharged compounds simultaneously. This report describes our experiences with a mixed-mode column containing both C_{18} and a strong cation-exchange material (SCX). The chromatographic characteristics of a number of drug compounds, including benzodiazepines, tricyclic antidepressants and barbiturates were investigated utilising typical "reversed-phase" mobile phases at various pHs, with different buffer ions, buffer ion concentrations and various percentages of organic component. Using the same series of mobile phases, the chromatographic characteristics of these compounds were determined on a single-mode C_{18} column for a comparison purposes.

2. Experimental

2.1. Materials

HPLC grade methanol and acetonitrile were obtained from Labscan Analytical Sciences, (Dublin, Ireland). AnalaR grade sodium hydroxide, phosphoric acid and acetic acid were supplied by BDH (Poole, UK). Analytical grade disodium hydrogen phosphate, sodium acetate, ammonium acetate and dipotassium hydrogen phosphate were used as buffer salts and were obtained from Merck (Darmstadt, Germany). Deionised water was obtained from an Elgastat spectrum water purification unit.

2.2. Test solutions

Phenolphthalein and phenol were supplied by Sigma Chemical Co. (Poole, UK). The remaining compounds were obtained from commercial suppliers and were of pharmaceutical quality. Stock solutions were prepared by dissolving the appropriate amount of analyte in methanol (100%) to give a concentration equivalent to 1 mg/ml. The solutions were then diluted to 100 μg/ml with deionised water and stored at 4°C. These stock solutions were freshly prepared on a weekly basis. Daily working solutions were prepared by diluting the stock solutions to 1–10 μg/ml depending on the extinction coefficient of the compounds at the detection wavelength.

2.3. HPLC eluents

The organic component used was either acetonitrile or methanol; the buffer salts were sodium acetate, ammonium acetate, disodium hydrogen phosphate and dipotassium hydrogen phosphate. The pH was adjusted as required with 10% phosphoric acid, 10% glacial acetic acid or 0.1 *M* sodium hydroxide.

2.4. HPLC separations

The HPLC system consisted of a Waters Associates (Milford, MA, USA) dual-piston chromatographic pump (Model 510) fitted with a Rheodyne (Cotati, CA, USA) injection port with a 20-μl injection loop. Detection was achieved with a Waters Model 486 spectrophotometric detector set at 254 nm. The sensitivity was 0.05 AUFS and data was manipulated using an integrator (Waters 746 Data Module). The column under evaluation was a Hypersil (250 × 4.6 mm I.D.) SCX/C_{18} column (Shandon Scientific, Cheshire, UK). The column contained equal quantities of 5 μm C_{18} and 5 μm sulphonate-modified silica. The column used for the comparison study was a Hypersil (250 × 4.6 mm I.D., 5 μm) single-mode column containing the same batch of C_{18} silica. All separations were carried out at ambient temperature.

2.5. Calculations

The capacity factors (k') of the components were calculated from the equation

$$k' = (t_r - t_0)/t_0$$

where t_r is the retention time of the analyte and t_0 is the retention time of the first peak following injection of the corresponding organic modifier in the eluent.

3. Results and discussion

Multidimensional chromatography, which makes uses of coupled column technology to link two separate chromatographic modes is now well known and examples of this approach can be found in Refs. [9,10]. However, this requires the use of switching valves, two or more pumps and two separate HPLC columns, and if the two mobile phases are incompatible, a purge and dry sequence. A single column containing a mixture of phases has formerly been evaluated for the separation of acidic compounds and their application to the simultaneous separation of both ionised/ionisable and non-ionic compounds was demonstrated [11–13]. Although the secondary cation-exchange interactions on nominally reversed-phase materials is well documented, no work on the retention properties of a column containing fixed and controlled amounts of both reversed-phase and strong cation-exchange materials has been published. The aim of this study was to investigate how capacity factors of a series of test compounds were affected by alterations in mobile phase composition which would be expected to affect both ion-exchange and hydrophobic interactions.

Table 1 lists the compounds used in the study along with their systematic names in order to give some indication of their structures and, where available, their pK_a's to aid in the rationalisation of their retention properties. The compounds investigated were mostly of pharmaceutical or medical interest and included strongly basic compounds such as tricylic antidepressants (TCAs) and β-blockers, weakly basic compounds such as the benzodiazepines, compounds that are essentially acidic in character such as the barbiturates, and furosemide, a diuretic drug that contains a carboxylic acid group with a pK_a of 3.9.

3.1. Effect of eluent pH on K'

The aqueous component of the mobile phase used in this part of the study consisted of a 0.025 *M* sodium phosphate buffer adjusted to pH 3, 4, 5, 6 or 7 and mixed in a 50:50 (v/v) ratio with acetonitrile. The filtered degassed eluents were allowed to equilibrate on the column overnight to ensure that the desired on-column pH conditions were obtained.

Table 1
Compounds used in the study and their pK_a values

Compound	pK_a	Systematic name
Amitriptyline	9.4	3-(10,11-Dihydro-5H-dibenzo[a,d] cyclo--hept-5-ylidene)-propyldimethylamine
Clomipramine	n/a	3-Chloro-10,11-dihydro-N,N-di-methyl--5H-dibenz[b,f]azepine-5-propanamine
Desmethyl-clomipramine	n/a	3-Chloro-10,11-dihydro-N,N-di-methyl--5H-dibenz[b,f]azepine-propyl--(methyl)amine
Desipramine	10.2	3-(10,11-Dihydro-5H-dibenz[b,f]azepin--5-yl)propyl-(methyl)amine
Imipramine	9.5	3-(10,11-Dihydro-5H-dibenz[b,f]azepin-5-yl) -NN-dimethyl-propylamine
Pindolol	8.8	1-(indol-4-yloxy)-3-isopropylamino propan-2-ol
Propranolol	9.5	N-(2-Hydroxy-3-naphth-1-tloxypropyl) -N-isopropyl-ammonium chloride
Norephedrine	n/a	Ethylamine-1-phenyl propan-1-ol
N-Methyl ephedrine	9.6	2-(Dimethyl-amino)-1-phenyl propan-1-ol
Terbutaline	8.7, 10.0, 11.0	(2-tert.-Butylamino-1-[3,5-dihydroxy-phenyl]ethanol)
Xylazine	n/a	(2-[2,6-Dimethylphenylamino]-4) 5,6-dihydrothiazine
Clonazepam	1.5, 10.5	5-(*o*-Chlorophenyl)-1,3-dihydro-7--nitro-2H-1,4-benzodiazepin-2-one
Diazepam	3.3	7-Chloro-1,3-dihydro-1-methyl-5-phenyl -2H-1,4-benzodiazepin-2-one
Flurazepam	1.9,8.2	7-Chloro-1-(2-diethylaminoethy)-5-(2-fluorophenyl)-1,3-Dihydro-2H-1,4-benzodiazepin-2-one
Nitrazepam	3.1, 10.8	1,3-dihydro-7-nitro-5-phenyl-2H-1,4--benzodiazepin-2-one
Barbitone	8.0	5,5-Diethylbarbituric acid
Butobarbitone	8.0	5-*n*-Butyl-5-ethylbarbituric acid
Phenobarbitone	7.3, 11.8	5-Ethyl-5-phenylbarbituric acid
Quinalbarbitone	7.9	5-Allyl-5 (1 methylbutyl)-barbituric acid
Benzoic acid	4.2	Benzoic acid
Phenol	9.98	Phenyl hydroxide
Phenolphthalein	9.7	3,3-Bis(4-hydroxyphenyl)phthalide
Resorcinol	9.5,10.1	1,3-Dihydroxybenzoic acid
Salicylic acid	3.0	2-Hydroxybenzoic acid
Furosemide	3.9	(4-Chloro-N-furfuryl-5-sulfamoylanthranilic acid)

n/a = not available.

Variation of the eluent pH alters the degree of ionisation of ionisable compounds, resulting in different proportions of neutral and ionised forms. As only the unionised form of the compounds will partition into the hydrophobic portion of the stationary phase, and the ionised form of the bases will interact with the sulphonate portion and the residual silanol groups, then it is to be expected that small changes in the pH of the eluent will elicit major changes in retention times.

Strong bases

Changes in the pH of the eluent were observed to cause marked changes in the capacity factors of the strongly basic TCAs and β-block-

ers: the retention of these compounds decreased between pH 3 and pH 5 and then rose sharply between pH 6 and pH 7 [Table 2(A)]. In reversed-phase chromatography, the retention of basic compounds is strongly influenced by secondary equilibria between the oppositely charged amines and residual silanol groups on the stationary phase. These secondary equilibria will also be expected to contribute to the observed retention patterns on the mixed-mode phase, though their significance cannot be evaluated in terms of the primary ion-exchange interactions with the SCX component in the column. It is possible that the decrease in retention observed between pH 3 and pH 5 is due to the decreasing degree of ionisation as the pH approaches the pK_a of the drugs. However, as all these amines are still 99% ionised at pH 7, the contribution from this source is probably not significant. It is more likely that since the fixed-charge moiety is ionised over the entire pH range, the slight decrease in retention results from the increased molar concentration of competing cation (sodium) with increasing pH. Between pH 6 and pH 7, ionisation of the silanol moieties becomes significant and the secondary silanophilic interaction could account for the dramatic increase in retention at this point.

Table 2
Effect of eluent pH on capacity factors; C_{18}/SCX column

Compound	Capacity factor				
	pH 3	pH 4	pH 5	pH 6	pH 7
(A)					
Amitriptyline	3.79	3.61	2.83	4.46	7.47
Clomipramine	3.99	3.75	3.59	4.61	8.59
Desmethyl-clomipramine	3.45	3.26	3.02	3.77	4.69
Desipramine	3.28	2.95	3.03	3.59	4.34
Imipramine	3.37	3.36	3.21	4.31	6.31
Pindolol	2.35	2.18	2.13	2.74	2.88
Propranolol	2.81	2.56	2.54	3.14	3.40
Norephedrine	2.72	2.47	2.35	2.92	2.89
N-Methyl ephedrine	4.11	3.93	4.33	5.21	5.86
Terbutaline	3.39	3.01	3.50	3.62	3.65
Xylazine	4.53	4.25	4.17	5.56	4.67
(B)					
Clonazepam	1.35	1.13	1.03	1.24	1.20
Diazepam	2.32	2.19	1.98	2.32	2.37
Flurazepam	4.59	4.15	4.23	4.66	4.83
Nitrazepam	1.28	1.06	0.94	1.05	1.11
(C)					
Barbitone	0.44	0.48	0.39	0.41	0.46
Butobarbitone	0.85	0.80	0.72	0.82	0.78
Phenobarbitone	0.77	0.73	0.67	0.96	0.79
Quinalbarbitone	1.17	1.08	0.90	1.07	1.20
Benzoic acid	0.69	0.62	0.45	0.52	0.37
Phenolphthalein	0.96	0.96	0.87	1.08	1.03
Phenol	0.79	0.83	0.78	0.85	0.86
Resorcinol	0.38	0.45	0.48	0.59	0.47
Salicylic acid	0.50	0.36	0.39	0.61	0.36
Furosemide	0.42	0.52	0.34	0.49	0.52

Eluent: Acetonitrile–disodium hydrogen phosphate 0.025 *M* (50:50, v/v).

The benzodiazepines (nitrazepam, clonazepam, diazepam, flurazepam) are all weakly basic with pK_a values of less than 3.5. Hence, the retention times of these compounds are determined principally by their interaction with the C_{18} component over most of the pH range investigated. However, as shown by the data in Table 2(B), they showed the same retention patterns with changing pH as the strong bases. This would suggest that their retention is governed by ion exchange which becomes progressively weaker up to pH 5 and which is then taken over by hydrophobic interaction mechanisms at the higher pHs.

Phenobarbitone, barbitone, quinalbarbitone, butobarbitone, phenolphthalein and phenol are all weakly acidic with pK_a values between 7.2 and 11.8. These compounds only interact with the hydrophobic component in the column and then only when unionised. It was found, as expected that their retention times remained unchanged over the pH range examined [Table 2(C)]. Similar results were obtained for benzoic acid, salicylic acid, resorcinol and furosemide.

The weakly basic and acidic analytes all have very low k' values over the pH range suggesting that either there is insufficient C_{18} material present to give them reasonable capacity factors or that these compounds, even when unionised have low affinities for very hydrophobic octadecyl carbon.

3.2. Ionic strength

To study the effect of ionic strength, a mobile phase was prepared which consisted of either 0.01, 0.025 or 0.05 *M* disodium hydrogen phosphate (pH 5) mixed in equal proportions with acetonitrile. The filtered degassed mobile phase was allowed to equilibrate on-column for at least 2 h.

It is to be expected that ionic strength will exert a considerable influence on the retention times of the cationic analytes since there are both primary (sulphonate) and secondary (silanophilic) cationic exchange mechanisms occurring. The data in Fig. 1 demonstrate that while the capacity factors for furosemide, diazepam and

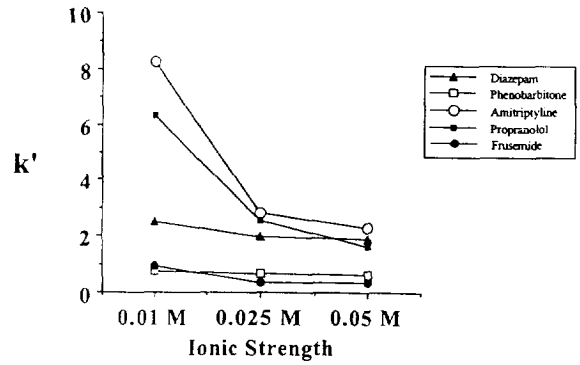

Fig. 1. Effect of ionic strength on k': C_{18}/SCX column. Mobile phase: acetonitrile–disodium hydrogen phosphate, pH 5 (50:50, v/v).

phenobarbitone are not significantly affected by ionic strength, the capacity factors of propranolol and amitriptyline are dramatically reduced as the ionic strength is increased. Hence, the concentration of competing cation in the mobile phase influences retention in a characteristic ion-exchange manner. Furthermore, compounds with different pK_a values (showing different degrees of ionisation) are susceptible to different extents, though all the strongly basic compounds showed similar effects; decreasing k' values as the ionic strength is increased. Chromatograms demonstrating the effect of ionic strength on strong and weakly basic analytes are presented in Fig. 2.

3.3. Type of buffer cation

To investigate the effect of buffer cation, the mobile phase consisted of either sodium, potassium or ammonium phosphate, each at both 0.025 *M* and 0.05 *M* (pH 5) mixed in equal proportions with acetonitrile. As expected, increasing the ionic strength of the buffer cation resulted in a decrease in k' values for compounds that are ionised at pH 5 regardless of the type of buffer cation. But at any given ionic strength, varying retention values were obtained depending on the affinity of the cations for the fixed anionic sites. The data in Fig. 3 demonstrate that the type of buffer cation strongly influences the capacity factors of the strongly basic analytes in a

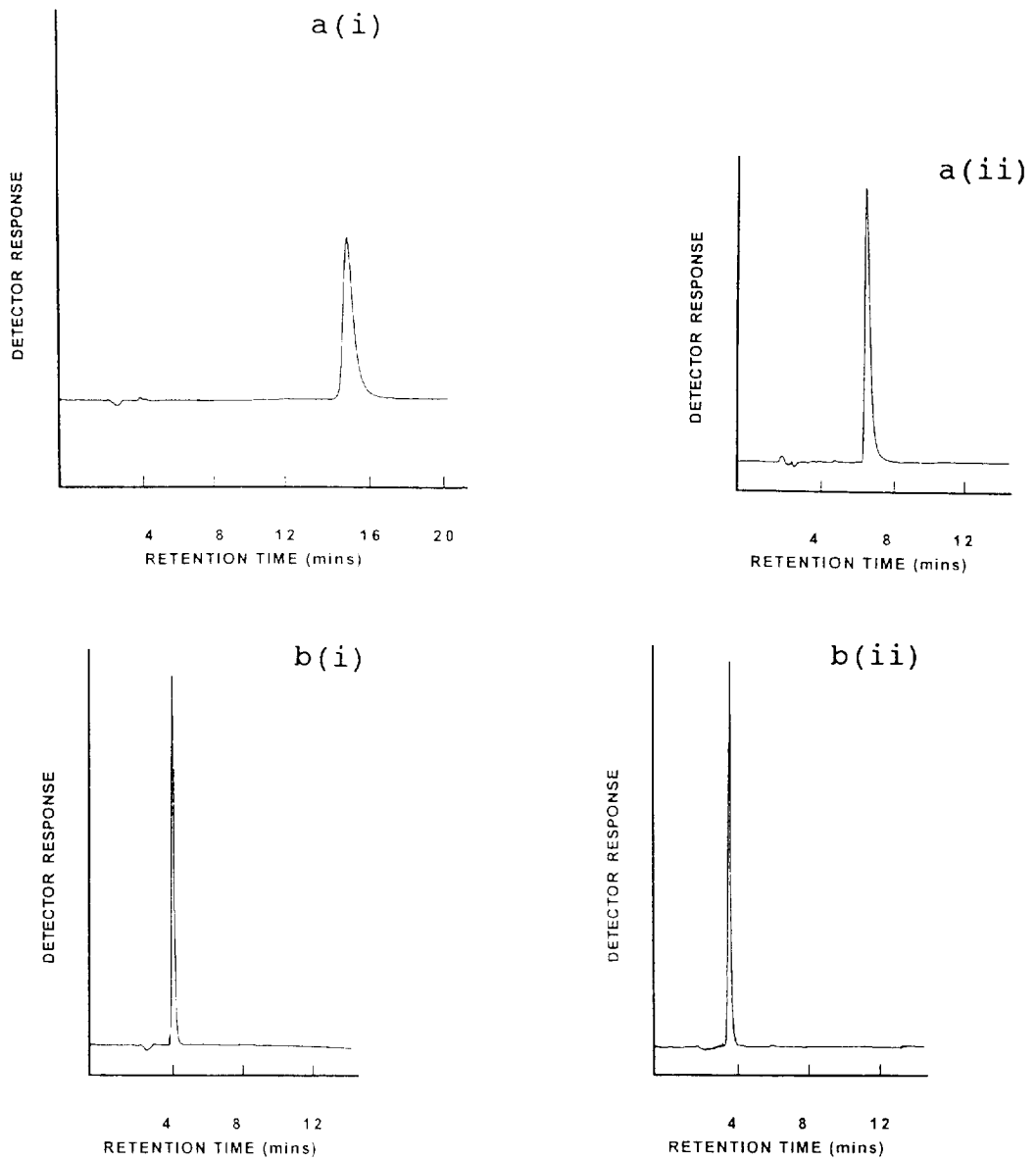

Fig. 2. (a) Effect of ionic strength on k' for clomipramine: C_{18}/SCX column. (i): Mobile phase, acetonitrile–disodium hydrogen phosphate 10 mM, pH 5 (50:50, v/v); (ii): mobile phase, acetonitrile–disodium hydrogen phosphate 50 m*M*, pH 5 (50:50, v/v). (b) Effect of ionic strength on k' for clonazepam: C_{18}/SCX column. (i): Mobile phase, acetonitrile–disodium hydrogen phosphate 10 m*M* pH 5 (50:50, v/v); (ii): mobile phase, acetonitrile–disodium hydrogen phosphate 50 m*M* pH 5 (50:50, v/v).

manner consistent with an ion-exchange mechanism, the order of competing ability being $Na^+ < NH_4^+ < K^+$. This finding is in accordance with the order of experimentally derived equilibrium constants for the exchange of cations for H^+ on sulphonated polystyrene resins [14]. Practically, this illustrates that shorter retention times may be obtained by switching from a sodium to potassium buffer without the need to increase the ionic strength.

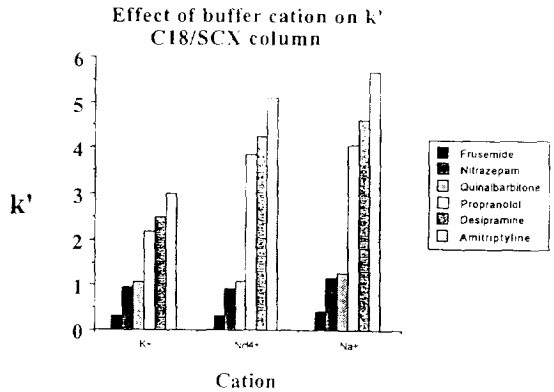

Fig. 3. Effect of type of buffer cation on k': C_{18}/SCX column. Mobile phase: 0.025 *M* phosphate buffer (pH 5)–acetonitrile (50:50, v/v).

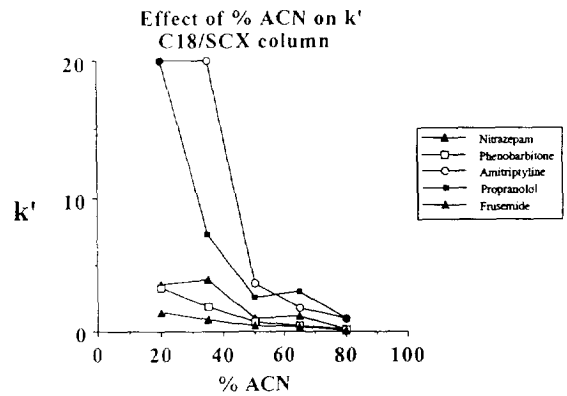

Fig. 4. Effect of % acetonitrile on k': C_{18}/SCX column. Mobile phase: 0.025 *M* disodium hydrogen phosphate (pH 5)–acetonitrile (50:50, v/v).

3.4. Organic component and percent organic component

Methanol and acetonitrile were compared by mixing either solvent with an equal volume of 0.025 *M* phosphate buffer, pH 5. The effect of changing the percentage organic component was investigated by adding acetonitrile to 0.025 *M* phosphate buffer, pH 5 in proportions ranging from 20% to 80% organic component.

Retention times were, on average, much shorter using acetonitrile than methanol in the mobile phase. Acetonitrile has virtually no effect on selectivity, probably indicating that it does not modify the type, but rather the intensity of interaction between solutes and stationary phase (it competes with the solutes for occupation of the C_{18} groups). In common with the usual observations made on reversed-phase materials for most analytes, high values of k' occurred with low proportions of acetonitirle and these values decreased as the amount of acetonitrile present increased. These observations, illustrated in Fig. 4, indicate that there is a hydrophobic component in the interaction mechanism for all compounds.

3.5. Comparison of C_{18} single mode with C_{18}/SCX

The same series of experiments were repeated on a nominally single-mode hydrophobic column, i.e. one containing only C_{18} material. Both columns were of the same dimensions and particle size and both were prepared form the same batch of silica base material. Therefore, any differences in retention properties could reasonably be attributed to the absence of the primary SCX mechanism on the single-mode column and the reduced volume of C_{18} sorbent in the mixed-mode column. The retention data for all the analytes as a function of pH are presented in Table 3 and a comparison of these data and those obtained on the mixed-mode column for selected analytes is shown in Fig. 5.

These data show that the strongly basic analytes have much lower retention indices on the single-mode column, an observation that can be explained in terms of the absence of the primary ion-exchange mechanism present in the mixed-mode column. Furthermore, their capacity factors are not strongly influenced by pH below about pH 6, but as on the mixed-mode column, their capacity factors increase significantly between pH 6 and pH 7, an effect that is consistent with the silanophilic interaction present in both columns and which becomes significant at around neutral and higher pHs. This demonstrates the added flexibility that the mixed-mode column has for the separation of strongly basic analytes both from similar compounds and from neutral and acidic analytes. The benzodiazepines have much lower capacity factors on the single-mode

Table 3
Effect of eluent pH on capacity factor; C_{18} column

Compound	Capacity factor				
	pH 3	pH 4	pH 5	pH 6	pH 7
Amitriptyline	2.01	2.13	2.27	2.90	6.75
Clomipramine	2.71	2.91	3.08	3.96	8.87
Desmethyl-clomipramine	2.39	2.53	2.64	3.05	3.86
Desipramine	1.56	1.63	1.73	1.95	2.51
Imipramine	1.78	2.01	1.97	2.31	4.93
Pindolol	0.52	0.54	0.53	0.60	0.94
Propranolol	0.83	0.90	0.96	1.07	1.61
Norephedrine	0.52	0.51	0.61	0.65	0.89
N-Methyl ephedrine	0.45	0.59	0.62	0.64	1.04
Terbutaline	0.46	0.51	0.56	0.70	0.71
Xylazine	0.67	0.73	0.80	0.95	1.92
Clonazepam	0.67	1.85	1.86	2.07	2.54
Diazepam	0.52	3.81	3.80	4.26	5.07
Flurazepam	1.00	1.16	1.18	1.82	4.97
Nitrazepam	0.17	1.70	1.70	1.88	2.36
Barbitone	0.59	0.66	0.67	0.73	1.01
Butobarbitone	1.17	1.26	1.26	1.15	1.75
Phenobarbitone	0.94	1.08	1.08	1.14	1.44
Phenolphthalein	1.49	1.60	1.61	1.75	2.34
Quinalbarbitone	1.67	1.99	2.00	2.13	2.71
Benzoic acid	0.83	0.48	0.40	0.48	0.79
Phenol	1.59	1.76	1.68	1.72	1.87
Resorcinol	0.56	0.62	0.62	0.70	1.05
Salicylic acid	0.83	0.55	0.43	0.51	0.87
Furosemide	1.24	0.86	0.51	0.51	0.91

Eluent: Acetonitrile–disodium hydrogen phosphate 0.025 *M* (50:50, v/v).

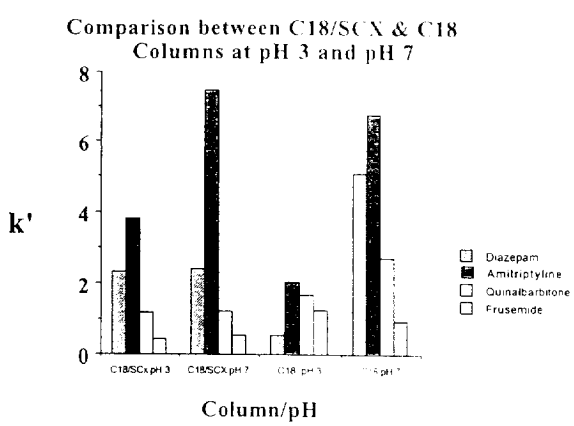

Fig. 5. Comparison between C_{18} SCX and C_{18} columns at pH 3 and pH 7. Mobile phase 0.025 *M* disodium hydrogen phosphate, (pH 3 or pH 7)–acetonitrile (50:50 v/v).

column at pH values where they are ionised (i.e. below pH 3) but exhibit a dramatic increase in retention as the pH is increased. That they have higher capacity factors at pH 4–7 on the single-mode column than on the mixed-mode column would suggest that the larger volume of C_{18} sorbent is a significant factor in their retention through hydrophobic mechanisms. This is supported by the fact that other test compounds which do not engage in ion-exchange interactions also have higher capacity factors at all pHs on the single-mode column.

The effect of eluent ionic strength (at pH 5; 50% acetonitrile) was also investigated on the single-mode column and it was found to exert a typical ion-exchange influence as a result of

secondary (silanophilic) interactions. As expected, the largest variations in capacity factors were observed for the most strongly basic analytes; analytes mostly unionised over the pH range were largely unaffected by changes in ionic strength. The other basic compounds also showed a reduction in capacity factor with increasing ionic strength, though in all cases, these changes were not nearly as large as those observed for the TCAs or indeed the same compounds on the SCX/C_{18} column.

4. Conclusion

The data obtained in this study indicates that ion exchange is a significant force in determining the retention indices of the more strongly basic analytes on the mixed-mode column. This is evidenced by the dramatic influence that pH, ionic strength and type of competing cation have on the capacity factors of these compounds. Acidic and neutral compounds were little influenced by pH changes, so the retention of strongly basic compounds could be altered independently of the acidic and neutral compounds. That the retention of all compounds on the mixed-mode column is also influenced by the type and percent organic modifier suggests that the hydrophobic interaction component is not inconsiderable and can also be used to tailor retention times. The route to devising a mobile phase to effect the separation of both strongly basic and acidic analytes would be to reduce the % acetonitrile to promote retention of the acidic or neutral compounds and to use a high ionic strength to effect elution of the strongly retained basic compounds in a reasonable time frame. The problem of solubility of phosphate salts in the organic component can be overcome by using acetate salts which are less insoluble in organic solvents. This approach has been used to design a mobile phase for the simultaneous separation of furosemide, pindolol and propranolol and will be presented in a future report. The weaker bases and acidic compounds generally have larger capacity factors on the single-mode column suggesting that this type of column would be more suitable for their analysis. However, if a less hydrophobic material (C_8, for example) was included with SCX in a mixed-mode column then the higher affinity which the more polar analytes would have for this sorbent would be expected to result in more favourable retention characteristics for such compounds in this environment.

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