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HPLC of basic drugs and quaternary ammonium compounds on microparticulate strong cation-exchange materials using methanolic or aqueous methanol eluents containing an ionic modifier

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Abstract

Sulphopropyl (SCX)-modified silica HPLC columns used with methanolic or aqueous methanol eluents of appropriate pH and ionic strength can give good retention and peak shape for quaternary ammonium compounds and basic drugs. In the system studied, eluent pH influenced retention via protonation of basic analytes, the p*K*a indicating the pH where retention began to decrease at constant ionic strength. At constant eluent pH retention was inversely proportional to ionic strength for protonated bases and quaternary ammonium compounds. However, this effect was less marked at pH 8.3 as compared to results obtained under acidic conditions. Except for codeine, morphine and lignocaine, the addition of water had no major effects on retention or selectivity at concentrations up to 30% (v/v) at pH 6.7. However, and in contrast to behaviour on unmodified silica, the addition of up to 5% (v/v) water under strongly acidic conditions caused a doubling of retention for most analytes studied.

SCX-modified silica columns can be used in the HPLC of a range of basic drugs, including many compounds which are poorly retained on unmodified silica using methanol-rich eluents. The underlying retention mechanism appears to be ion exchange with the SCX moieties, although ionized surface silanols may also contribute to retention at higher eluent pH values. Applications of SCX columns in the HPLC of basic drugs include the analysis of antimalarials such as chloroquine, desethylchloroquine, hydroxychloroquine and quinine, benzodiazepines such as clonazepam, bronchodilators such as salbutamol and terbutaline, cardioactive drugs such as flecainide and lignocaine, and tricyclic antidepressants such as amitriptyline, dothiepin, and imipramine, and their N-demethyl, hydroxyl and sulphoxide metabolites.

1. Introduction

Chemically modified (bonded phase) silica–aqueous methanol or acetonitrile eluent ('reversed-phase') systems have been widely used in the HPLC of basic drugs. However, in addition to buffer salts, various additives (pairing- or counter-ions, such as alkylsulphonates, alk-

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ylamines, or quaternary ammonium compounds) are often needed to give efficient performance [1]. Moreover, the effect of altering the eluent water content on retention may not be predictable in terms of interaction with the bonded phase. This is probably because ionic interactions between the (protonated) analyte and (ionized) surface silanols are as strong or stronger than hydrophobic interactions with the stationary phase [2]. Similar considerations apply when using chemically modified silica solid-phase extraction columns [3–5].

In the HPLC of basic drugs similar retention and selectivity (and generally better peak shapes) to those obtained on bonded phase packings can often be obtained on unmodified silica using 'reversed-phase' eluents, i.e. aqueous methanol or acetonitrile eluents of an appropriate pH and ionic strength [6–12]. Ion exchange with surface silanols is thought to be the predominant retention mechanism, eluent pH influencing retention via (i) the ionization of surface silanols and (ii) the protonation of basic analytes. Although the fact that acidic or neutral compounds and indeed some basic drugs are either not retained or very poorly retained excludes interference from such sources, the analysis of compounds in these latter categories is not possible using unmodified silica unless very high eluent water contents are employed. However, if this is attempted very poor efficiencies are attained [13].

When analyzing basic drugs by reversed-phase HPLC, evaporation of solvent extracts and reconstitution in an aqueous medium is often required. The use of nonaqueous ionic eluents was originally investigated with the aim of analyzing solvent extracts directly [14]. It was found that efficient performance could be obtained for many basic drugs on unmodified silica using 100% methanol containing perchloric acid (0.01 or 0.02% v/v, approximately 1 or 2 mmol/l) as eluent. An ammonium perchlorate-modified (10 mmol/l, apparent pH 6.7) 100% methanol eluent balances retention, peak shape and electrochemical oxidation response for many analytes [15,16]. Perchloric acid, ammonium perchlorate, and some sulphates and sulphonates are useful ionic modifiers since they have UV cutoffs below 210 nm, and are adequately soluble and appear to be highly dissociated in methanolic solution [14].

Spherisorb S5W silica (5 μm average particle size) has been used extensively with nonaqueous ionic eluents, although similar results may be obtained on other silicas [15,17]. Microparticulate sulphopropyl- (SCX) or sulphophenylpropyl-modified silicas also give good retention of many basic drugs with nonaqueous ionic eluents. The use of two such materials, Zorbax 300 SCX (Du Pont) and Spherisorb S5SCX (Phase Separations), has been described [12,17–19]. The aims of the present paper are (i) to present data to aid the use of microparticulate SCX columns in the HPLC of basic drugs and quaternary ammonium compounds, (ii) to compare some results obtained using the sulphopropyl-modified material to those obtained on unmodified silica and on phenylpropyl-modified silica, and (iii) to discuss the mechanism whereby retention of basic drugs is achieved on unmodified silica and on SCX-modified silica.

2. Experimental

2.1. Materials and reagents

Methanol and acetonitrile (HPLC grade) were from Rathburn (Walkerburn, UK), perchloric acid (60% w/v) and sodium hydroxide (both analytical reagent grade) from BDH (Poole, UK), and ammonium perchlorate from Aldrich (Gillingham, UK). Water was deionised (Elgastat Option 3). Pure drugs used as test compounds were obtained from a variety of manufacturers. Drug nomenclature follows that of Reynolds [20]; pK_a values were obtained from this same source.

2.2. High-performance liquid chromatography

A constant-flow reciprocating pump (Shimadzu, Model LC-9A) was used with a syringe-loading injection valve (Rheodyne, Model 7125). Column effluents were monitored by UV absorption (Applied Chromatography Systems, Model 750/11/AZ) or fluorescence

(Applied Biosystems Model 980, glass photomultiplier tube). The columns used were (i) stainless-steel packed with Spherisorb S5SCX (sulphopropyl-modified silica), S5P (phenylpropyl-modified silica), or Spherisorb S5W (unmodified silica) (all from Phase Separations, Deeside, UK), or (ii) PEEK (polyether ether ketone) (Jour No-Met) packed with Spherisorb S5SCX (Hichrom, Reading, UK). Spherisorb S5W is a spherical packing [size of particles: 90% in the range 5 ± 2 μm (area distribution), surface area: 220 m^2/g, mean pore diameter: 8 nm (range 5.4–11 nm)]. Spherisorb S5P and S5SCX are prepared from S5W (maximum carbon loadings 3 and 1.2% w/w, respectively). S5SCX has a maximum ion-exchange capacity of 6 μmol/g (6 μEquiv/g).

The eluents were solutions of perchloric acid or ammonium perchlorate of appropriate pH and ionic strength. Eluent pH values were measured without correction using a standard glass electrode (Jenway 3020) calibrated against aqueous buffers. Experiments designed to study the effects of eluent pH, ionic strength and water content on retention and peak shape of the test compounds (Fig. 1) were performed at ambient temperature (normally 22°C) and at a flow rate of 2.0 ml/min. Effluent pH was measured to ensure column equilibration was complete before each experiment. Analyte retention times were measured using a Hewlett-Packard Model 3392A recording integrator. Retention factors (k, also known as mass distribution ratio, capacity factor, capacity ratio, k') were calculated using the formula $k = (t_R - t_0)/t_0$ where t_R is the retention time of the analyte and t_0 is the retention time of the non-retained peak (taken as the first deviation of the baseline following the injection of 5 μl acetone).

3. Results and discussion

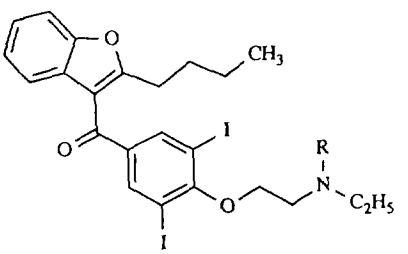
3.1. HPLC of basic drugs and quaternary ammonium compounds on Spherisorb S5SCX

Effect of pH on retention at constant ionic strength: methanol eluent

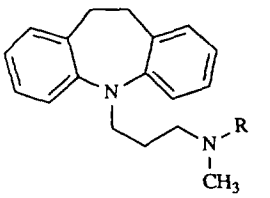
When studying the effect of pH on retention at constant ionic strength on unmodified silica, Flanagan and Jane [15] used sodium perchlorate (10 mmol/l) to provide the eluent ionic strength. However, this salt has no buffering capacity. The silica column itself provided buffering at acidic–neutral pH values, but it was difficult to obtain stable readings at pH values > 7. To obviate this problem, ammonium perchlorate (50 mmol/l) was used to provide the eluent ionic strength in this study, pH adjustment being by adding either perchloric acid or sodium hydroxide (both 50 mmol/l in methanol). Unfortunately, it was not possible to obtain stable pH values between 1 and 5 using ammonium perchlorate and 8.7 was the highest pH which could be attained.

Given the above constraints, the results obtained using the Spherisorb S5SCX column (Fig. 2) were similar to those obtained using unmodified silica [15] except that (i) retention on the SCX column was much greater for a given eluent ionic strength, and (ii) the effect of changes in pH on retention under strongly acidic conditions were much less marked on SCX than on silica. In addition, methdilazine gave a very poor peak shape under strongly acidic conditions on SCX but not on silica. The reason for this finding is not clear. Other phenothiazines tested, for example chlorpromazine and thioridazine, also gave very poor peak shapes at pH 0 on SCX.

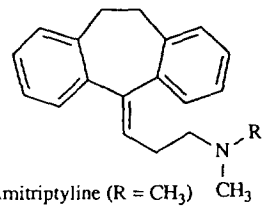
Thus, methdilazine excepted, the retention of all the test compounds increased slightly as the pH increased from 0 to 5 (Fig. 2). This may have been due to ionization of residual silanols on the SCX material rather than increased ionization of the very strongly acidic sulphopropyl moieties. At eluent pH values > 5 the N,N-diethylamines amiodarone and lignocaine and the alicyclic N-methylamines codeine and morphine showed marked decreases in retention. Desethylamiodarone and the N,N-dimethylamines (pK_a values < 9.5) also showed decreased retention at pH 7 and above, although this was less marked than with lignocaine and amiodarone. In contrast, the retention of emepronium and the primary and secondary aliphatic amines studied (pK_a values generally 9.7 or greater) was maintained up to pH 8. Emepronium then showed increased retention on going to pH 8.7, possibly reflecting



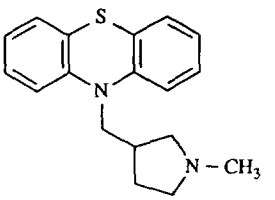
Amiodarone ($R = C_2H_5$)
Desethylamiodarone ($R = H$)



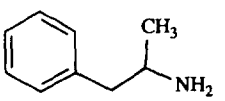
Imipramine ($R = CH_3$)
Desipramine ($R = H$)



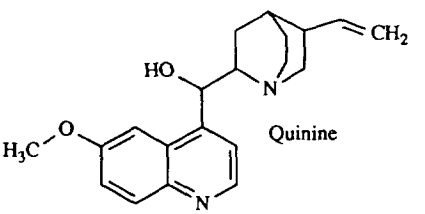
Amitriptyline ($R = CH_3$)
Nortriptyline ($R = H$)



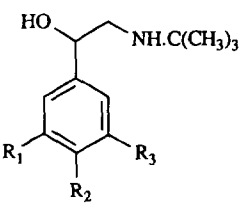
Methdilazine



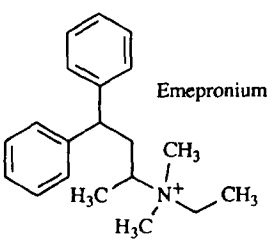
Amphetamine



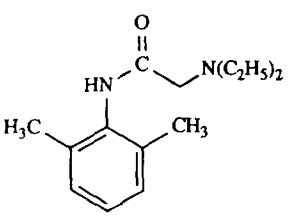
Quinine



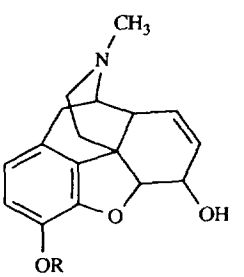
Terbutaline ($R_1 = OH$, $R_2 = H$, $R_3 = OH$)
Salbutamol ($R_1 = H$, $R_2 = OH$, $R_3 = CH_2OH$)



Emepronium



Lignocaine



Codeine ($R = CH_3$)
Morphine ($R = H$)

Fig. 1. Test compounds. Amiodarone (pK_a 5.6); desethylamiodarone (DEA, no published pK_a); amitriptyline (pK_a 9.4); nortriptyline (pK_a 9.7); imipramine (pK_a 9.5); desipramine (pK_a 10.2); methdilazine (pK_a 7.5); amphetamine (pK_a 9.9); quinine (pK_a 4.1, (amine) 8.5); terbutaline [pK_a 8.7, (amine) 10.0, 11.0]; salbutamol [pK_a 9.3, (amine) 10.3]; lignocaine (lidocaine, pK_a 7.9); emepronium; codeine (pK_a 8.2); morphine [pK_a (amine) 8.0, 9.9].

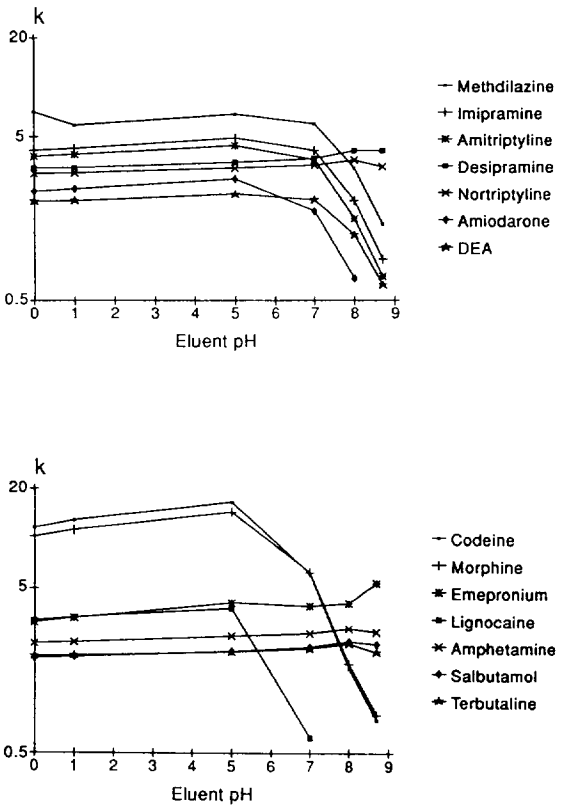


Fig. 2. Effect of eluent pH on the retention (k) of some test compounds at constant ionic strength. Column: 100 × 4.6 mm I.D. stainless steel packed with Spherisorb S5SCX. Eluent: methanol containing ammonium perchlorate (50 mmol/l) adjusted to the appropriate pH by adding either perchloric acid or sodium hydroxide (both 50 mmol/l in methanol). Flow rate: 2.0 ml/min. Detection: UV, 254 nm. Injections: 20–100 μl each analyte (10 mg/l) in methanol.

increased ionization of residual surface silanols, but the remaining compounds showed decreased retention. By analogy with the results obtained using unmodified silica [15], the retention of the basic drugs would be expected to continue to decrease at higher eluent pH values although the retention of the quaternary ammonium should be maintained.

Effect of pH on peak shape at constant retention: methanol eluent

The test compounds (amphetamine, nortriptyline, amitriptyline, imipramine, methdilazine, morphine, emepronium and quinine) and eluents [methanolic perchloric acid (pH < 0) and methanolic ammonium perchlorate (pH 6.7 and pH 8.3)] used were those studied by Flanagan and Jane [15] on unmodified silica. Eluent ionic strengths were adjusted to give similar retention times at each pH to facilitate comparison of peak shapes.

The elution sequence of the test compounds at pH < 0 and at pH 6.7 (Fig. 3) was similar to that obtained on unmodified silica [15]. For example, the sequence amphetamine < nortriptyline < amitriptyline < imipramine < methdilazine was the same on both columns. The efficiency and shape of the amphetamine, nortriptyline, amitriptyline, imipramine and methdilazine peaks were similar at each pH on both columns except that methdilazine gave a badly tailing peak on SCX at pH < 0. The emepronium peak was similar (very slight tail) at each pH (compare Ref. 15, Fig. 5). Quinine, which gave a badly tailing peak on unmodified silica at pH < 0, was not eluted at this pH on the SCX column. At higher pH values quinine gave a good peak shape on silica [15] and on SCX (Fig. 3). Finally, and in marked contrast to the results obtained with unmodified silica, morphine gave a symmetric although relatively broad peak at pH < 0 but broad, tailing peaks at pH 6.7 and at pH 8.3.

Effect of ionic strength on retention at constant pH: methanol eluent

In HPLC, plots of retention against the reciprocal of eluent ionic strength may give information on the retention mechanism. A straight line plot is expected if an ion-exchange mechanism is operating, the intercept on the y axis (infinite ionic strength) indicating the contribution of retention mechanism(s) other than ion exchange to retention [21].

The eluent pH values employed in the present study were again those used by Flanagan and Jane [15] with unmodified silica, i.e. <0, 6.7 and 8.3. The k values measured on SCX at different eluent ionic strengths are presented in Table 1. Representative data are plotted (k vs. 1/ionic strength) in Fig. 4; some data points have been omitted at higher ionic strengths to clarify presentation. Straight line plots with intercepts

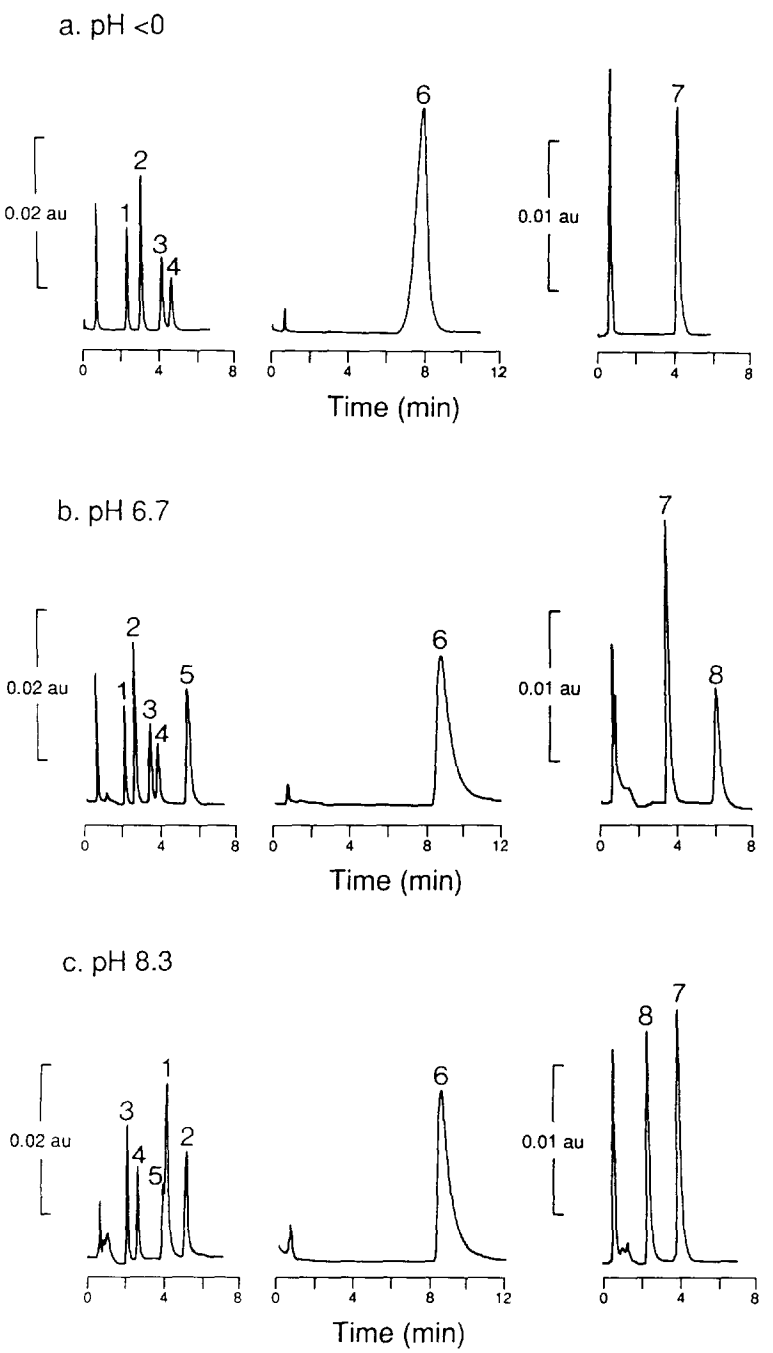


Fig. 3. Chromatography of some test compounds: SCX column. Eluents: (a) pH < 0. Methanolic perchloric acid (25 mmol/l). (b) pH 6.7. Methanolic ammonium perchlorate (40 mmol/l). (c) pH 8.3. Methanolic ammonium perchlorate (20 mmol/l) (3 mmol/l morphine; 40 mmol/l emepronium–quinine). See legend to Fig. 2 for other chromatographic conditions. Injections: 10 μl (100 μl emepronium, quinine) 10 mg/l each analyte (100 mg/l emepronium, 0.5 g/l amphetamine, 1 g/l morphine). Peaks: 1 = amphetamine, 2 = nortriptyline, 3 = amitriptyline, 4 = imipramine, 5 = methdilazine, 6 = morphine, 7 = emepronium, 8 = quinine.

Table 1
Effect of eluent ionic strength on the retention (k) of some test compounds on SCX column

Compound	Ionic strength (mmol/l)								
	10	20	40	50	80	100	160	200	400
Eluent: methanol containing perchloric acid, pH < 0									
Amiodarone	12.2	6.5	3.4		1.8		1.0		
Amitriptyline	20.8	11.1	5.8		3.1		1.6		
Amphetamine	9.5	5.1	2.8		1.6		0.9		
Codeine	58.5	30.7	16.3		8.6		4.3		
Desethylamiodarone	9.4	5.0	2.6		1.4		0.8		
Desipramine	16.8	8.9	4.8		2.6		1.4		
Emepronium	22.6	11.7	6.3		3.3		1.7		
Imipramine	25.9	13.5	7.1		3.8		1.9		
Lignocaine	16.4	8.5	4.6		2.5		1.3		
Methdilazine	37.5	20.8	12.2		7.0		3.2		
Morphine	50.0	26.3	14.0		7.4		3.8		
Nortriptyline	13.8	7.3	3.9		2.2		1.2		
Salbutamol	6.8	3.7	2.1		1.2		0.7		
Terbutaline	6.7	3.6	2.0		1.2		0.7		
Eluent: methanol containing ammonium perchlorate, pH 6.7									
Amiodarone	11.2			2.2		1.0		0.5	0.3
Amitriptyline	22.1			4.1		1.8		0.9	0.5
Amphetamine	12.0			2.3		1.2		0.6	0.4
Codeine	61.2			11.6		5.1		2.8	1.3
Desethylamiodarone	10.4			2.0		0.9		0.5	0.3
Desipramine	17.8			3.9		1.6		0.8	0.4
Emepronium	23.5			4.5		1.8		0.9	0.4
Imipramine	24.8			4.7		2.1		1.0	0.5
Lignocaine	4.6			1.3		0.6		0.4	0.3
Methdilazine	39.8			6.8		3.0		1.4	0.7
Morphine	57.2			11.0		5.0		2.6	1.3
Nortriptyline	15.8			2.9		1.4		0.7	0.4
Salbutamol	8.6			1.8		1.0		0.6	0.3
Terbutaline	9.2			1.9		1.1		0.6	0.4
Eluent: methanol containing ammonium perchlorate, pH 8.3									
Amiodarone	1.2			0.3		0.3		0.2	0.1
Amitriptyline	4.2			1.1		0.7		0.6	0.4
Amphetamine	13.2			2.7		1.3		0.7	0.4
Codeine	3.6			1.2		0.9		0.9	0.7
Desethylamiodarone	3.5			0.9		0.6		0.3	0.2
Desipramine	20.0			3.9		1.9		1.0	0.6
Emepronium	31.3			4.6		2.0		1.0	0.5
Imipramine	5.5			1.4		0.9		0.6	0.5
Lignocaine	0.2			0.1		0.1		0.1	0.1
Methdilazine	9.1			2.2		1.4		1.0	0.6
Morphine	3.9			1.3		1.0		0.9	0.7
Nortriptyline	16.6			3.2		1.7		0.8	0.5
Salbutamol	10.5			2.2		1.2		0.7	0.5
Terbutaline	9.6			2.0		1.1		0.6	0.4

See legends to Figs. 2 and 4 for details of chromatographic conditions.

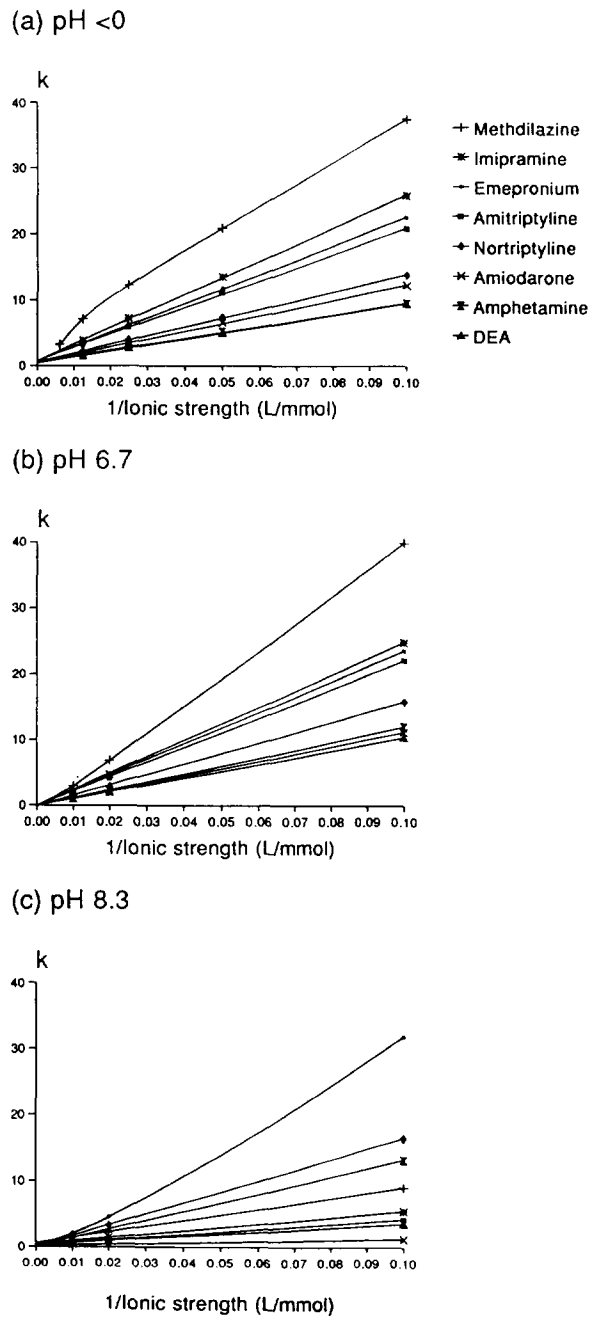


Fig. 4. Effect of eluent ionic strength on the retention (k) of some test compounds. Column: 100 × 4.6 mm I.D. Spherisorb S5SCX. Eluent: methanol containing (a) perchloric acid, (b) ammonium perchlorate adjusted to pH 6.7 by adding methanolic sodium hydroxide, (c) ammonium perchlorate adjusted to pH 8.3 by adding methanolic sodium hydroxide. See Fig. 2 for other experimental details.

passing close to the origin (intercept of approximately 0.4 in the case of the pH < 0 plots) were obtained at all three pH values except for methdilazine (pH < 0, very poor peak shape, convex curve at higher ionic strengths; pH 6.7, slight concave curve at higher ionic strengths) and emepronium (pH 8.3, concave curve). Codeine and morphine (results not shown) gave straight line plots with small intercepts on the y axis except at pH < 0 (intercepts approximately 1.4).

In order to facilitate comparison with the results obtained on unmodified silica (Spherisorb S5W, Ref. 15), analogous plots (k vs. 1/ionic strength) are given in Fig. 5. Some data points have again been omitted at higher ionic strengths to clarify presentation. At pH 0 all of the test compounds gave approximately straight line plots and all of the plots passed through the origin except those given by emepronium and methdilazine (intercepts at $k = 0.3$). At pH 6.7 all the plots again approximated to straight lines. Methdilazine gave the biggest intercept on the y axis (0.4) and emepronium one of the smallest (0.1). At pH 8.3, however, all the plots were convex curves, that given by emepronium having the biggest slope and passing closest to the origin. The intercepts given by the other plots ranged up to 0.8 in the case of methdilazine. Codeine and morphine behaved similarly giving intercepts of approximately 0.5 (results not shown).

Effect of eluent water content on retention: constant pH and ionic strength

(*i*) *Initial pH* < 0

All of the test compounds showed marked increases in retention on raising the eluent water content from 0 to 5% (v/v) on the SCX column under strongly acidic conditions (Fig. 6). The retention of codeine and morphine decreased thereafter up to 50% water. The retention of the remaining compounds stayed relatively constant except that amiodarone and desethylamiodarone showed proportionately greater increases in retention at eluent water contents of 30% (v/v) and above. Flanagan et al. [17] reported only very slight increases in retention up to 1% water

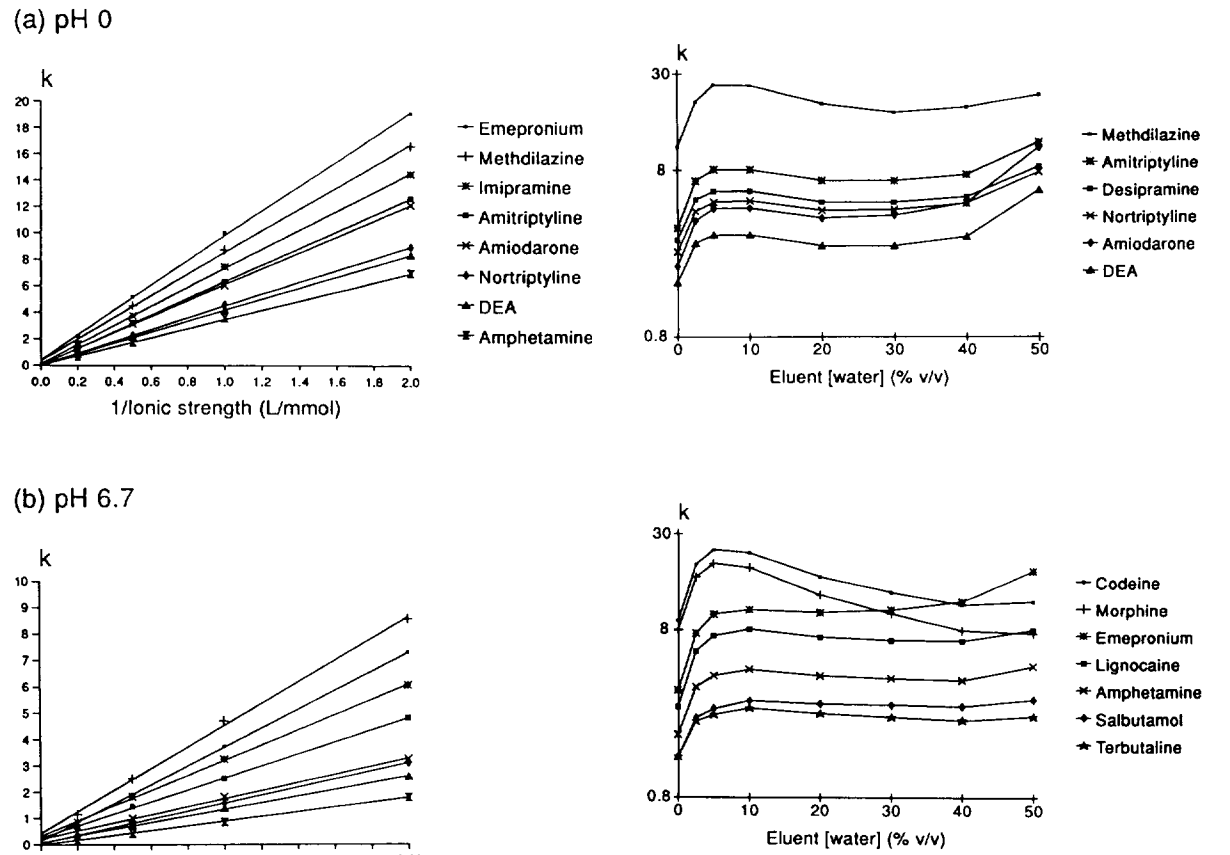


Fig. 5. Effect of eluent ionic strength on the retention (k) of some test compounds. Column: 250 × 4.9 mm I.D. Spherisorb S5W silica. Eluent: methanol containing perchloric acid or ammonium perchlorate at an appropriate pH and ionic strength. See Flanagan and Jane [15] for other experimental details.

Fig. 6. Effect of eluent water content on the retention (k) of some test compounds: SCX column. Eluent: methanol or methanol–water containing perchloric acid [50 mmol/l (0.5% v/v)]. See legend to Fig. 2 for other chromatographic conditions.

(perchloric acid eluent) when studying strong bases and emepronium on unmodified silica. Subsequently there was little change in retention up to 10% water. The reason for this difference in behaviour between the SCX column and unmodified silica is not clear.

(ii) pH 6.7

In contrast to the results obtained under strongly acidic conditions on the SCX column, the retention of all test compounds generally decreased slightly on going from 0 to 10% water at pH 6.7 and increased thereafter at higher eluent water contents (Fig. 7). Amiodarone and desethylamiodarone again showed proportionately greater increases at eluent water con-

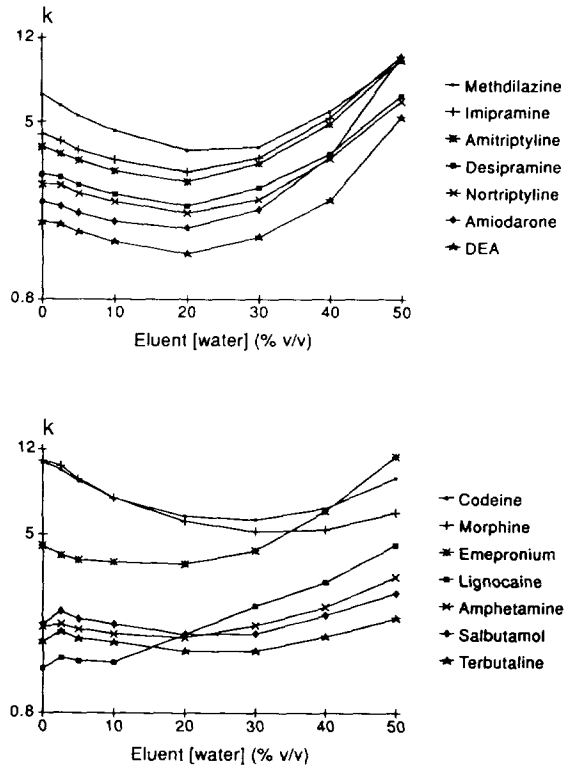


Fig. 7. Effect of eluent water content on the retention (k) of some test compounds: SCX column. Eluent: methanol or methanol–water containing ammonium perchlorate (50 mmol/l) adjusted to pH 6.7 with sodium hydroxide (50 mmol/l). See legend to Fig. 2 for other chromatographic conditions.

tents above 30% (v/v). However, lignocaine showed marked increases in retention with increasing water content from 10% (v/v). The retention of codeine and morphine decreased up to 30% water and thereafter showed relatively small increases up to 50% water. Similar results were obtained on unmodified silica [15], although lignocaine, codeine and morphine were not studied.

The behaviour of amiodarone, desethylamiodarone and lignocaine in the presence of water (Figs. 6 and 7) deserves further comment. In summary, amiodarone and desethylamiodarone showed similar retention characteristics to each other (and to the other compounds studied except at higher eluent water contents) under strongly acidic conditions, at pH 5.4 (results not presented graphically), and at pH 6.7. Lignocaine, however, behaved similarly to the other compounds studied (codeine/morphine excepted) under strongly acidic conditions and at pH 5.4, but showed marked increases in retention with increasing water content from 10% (v/v) at pH 6.7 (Fig. 7). The reason for these differences in chromatographic behaviour is not clear but could be related to the fact that both amiodarone and lignocaine are N,N-diethylamines which have relatively low pK_a values compared to the N,N-dimethylamines studied (see Fig. 1).

We have not studied systematically the effect of eluent water content on retention at eluent pH values above 6.7. However, addition of water (1.5% v/v) to a methanolic ammonium perchlorate eluent was effective in resolving chloroquine and monodesethylchloroquine at pH 8.0 on an SCX column [19].

Effect of ionic strength on retention at pH 6.7: methanol–water eluent

In order to investigate the possible contribution of the propyl moiety of the sulphopropyl-modified silica to retention in the presence of water, the effect of changes in ionic strength on retention using methanol–water (7 + 3), pH 6.7 as eluent was studied. Unmodified silica (S5W) and phenylpropyl-modified silica (S5P) were studied under these same conditions in order to provide comparative data. The results are presented in Fig. 8; some data points have again been omitted at higher ionic strengths in order to clarify presentation. Amphetamine was not studied in detail on the S5W and S5P columns since elution was so rapid on the 100 mm columns used that k values could not be measured accurately at higher ionic strengths. On the SCX column amphetamine co-eluted with amiodarone at all the ionic strengths studied.

The results obtained on the SCX column were similar to those obtained in the absence of water (Fig. 4) save that methdilazine gave a linear plot. All the plots had an intercept on the y axis of approximately 0.4. Other compounds studied on SCX behaved similarly except that the codeine and morphine plots had intercepts on the y axis

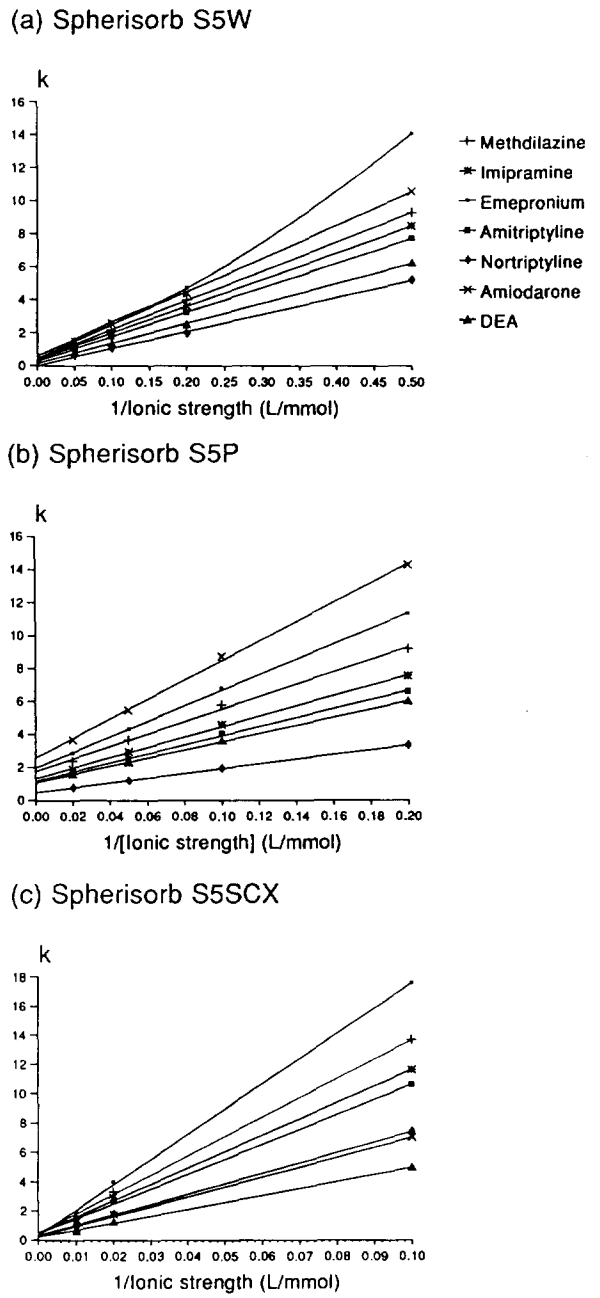


Fig. 8. Effect of eluent ionic strength on the retention (k) of some test compounds at pH 6.7 in the presence of water. Columns: 100 × 4.6 mm I.D. stainless steel packed with (a) Spherisorb S5W silica, (b) Spherisorb S5P phenylpropyl-modified silica, and (c) Spherisorb S5SCX sulphopropyl-modified silica. Eluent: methanol–water (70:30) containing ammonium perchlorate adjusted to pH 6.7 with sodium hydroxide (50 mmol/l) in methanol–water (70:30). See legend to Fig. 2 for other chromatographic conditions.

of 0.7 (results not shown). The results obtained on unmodified silica were also very similar to those obtained using methanol alone (Fig. 5) except that the plot given by emepronium, which was linear in the absence of water, was a concave curve. Codeine and morphine behaved similarly to the other basic drugs studied and gave linear plots (intercepts on the y axis of 0.7 and 0.9, respectively; results not shown).

Spherisorb S5P gave interesting results in that, although all the plots were linear, none passed through the origin, intercepts on the y axis of between 0.4 (nortriptyline) and 2.4 (amiodarone) being obtained. The codeine and morphine plots were also linear and had intercepts on the y axis of 1.6 and 1.2, respectively (results not shown). Further points of interest were (i) that amiodarone was strongly retained in comparison to results on unmodified silica and SCX, and (ii) that the ionic strength needed in order to promote elution at a given retention time lay in general between that needed on unmodified silica (lowest) and on SCX (highest). Presumably this is because a trifunctional silylating reagent was used in preparing the S5P material and this served to add additional silanols (ion-exchange sites) as well as the phenylpropyl moiety (S5SCX is prepared using a monofunctional silylating reagent [information from Phase Separations]). However, the presence of water is also important since under nonaqueous conditions at pH 6.7 retention of nortriptyline, amitriptyline, imipramine and methdilazine on S5P was only half that on S5W at a given ionic strength, selectivity being unaffected [12].

3.2. Use of Spherisorb S5SCX in the HPLC of basic drugs

Lignocaine (Fig. 1) is poorly retained on unmodified silica columns under acidic or neutral conditions [14,16]. However, the use of an SCX-modified silica column gives good retention and forms the basis of a reliable assay [18]. Salbutamol and terbutaline (Fig. 1) are also relatively poorly retained on unmodified silica and an SCX-modified column used with methanol–acetonitrile–water (40:40:20) containing per-

chloric acid (20 mmol/l) has proved valuable in the analysis of these compounds in plasma extracts. Addition of water to the eluent under strongly acidic conditions served to enhance retention and improve the separation attained (compare Fig. 6), whilst acetonitrile was added to the eluent to reduce the column back-pressure [22].

The measurement of the antiarrhythmic flecainide is sometimes requested in the presence of the lipophilic β-adrenoceptor blocker propranolol. Although these compounds can be analyzed independently using an unmodified silica column [17], they are difficult to separate. However, this analysis is easy using an SCX column under strongly acidic conditions (Fig. 9). As with the salbutamol–terbutaline assay discussed above, water and acetonitrile were added to the eluent to improve the separation and lower the back-pressure, respectively. An SCX-modified column has also been used in the assay of chloroquine, desethylchloroquine, hydroxychloroquine and quinine in plasma [19]. Here a methanolic ammonium perchlorate (66 mmol/l, pH 8.0) eluent was used. This gave good peak shapes for all the compounds of interest but the addition of water (1.2% v/v) was required in order to separate chloroquine and desethylchloroquine effectively (Fig. 10). Further increases in the eluent water content had little effect on the separation.

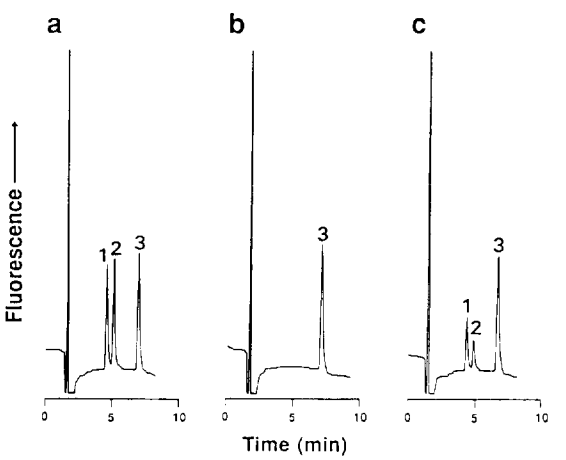


Fig. 9. Analysis of flecainide in the presence of propranolol in plasma–serum. Column: 150 × 4.6 mm I.D. Spherisorb S5SCX. Eluent: Methanol–acetonitrile–water (2:2:1) containing perchloric acid (25 mmol/l). Flow rate: 2.0 ml/min. Detection: Fluorescence: excitation 215 nm, no emission filter. Injection: 100 μl sample extracts. Chromatograms: (a) Standard solution prepared in newborn calf serum containing flecainide (0.50 mg/l) and propranolol (0.10 mg/l). (b) Drug-free human plasma. (c) Plasma from a patient treated with flecainide and propranolol (plasma concentrations 0.26 and 0.03 mg/l, respectively). Peaks: 1 = flecainide, 2 = propranolol, 3 = benzimidazole (internal standard).

A consistent but unexplained finding is the very poor peak shape given by phenothiazines such as methdilazine and chlorpromazine under strongly acidic conditions on the SCX column. Clearly a higher eluent pH is needed to assay such compounds satisfactorily on SCX (see Fig. 3), although the corollary is that interference from phenothiazines themselves in assays carried under strongly acidic conditions will be minimal. Strong acid conditions are sometimes mandatory if an ion-exchange mechanism is to be exploited, as in the analysis of very weak bases such as benzodiazepines. The separation of some benzodiazepines and of dothiepin and some metabolites is illustrated in Fig. 11. In conjunction with appropriate sample preparation procedures, this system can be used to assay (i) benzodiazepines such as clonazepam, and (ii) tricyclic antidepressants such as amitriptyline, dothiepin, or imipramine and their N-demethyl, hydroxyl and sulphoxide metabolites.

3.3. General discussion

The factors influencing the retention of basic drugs and quaternary ammonium compounds on unmodified silica and on SCX-modified silica using 100% methanol or aqueous methanol eluents containing an ionic modifier clearly have many similarities. It is appropriate therefore to review previous work on the HPLC of basic drugs on unmodified silica prior to discussing general aspects of the use of SCX-modified packings.

HPLC of basic drugs on unmodified silica

Silica column–nonaqueous ionic eluent systems are useful in the HPLC of basic drugs in biological extracts since only protonated bases

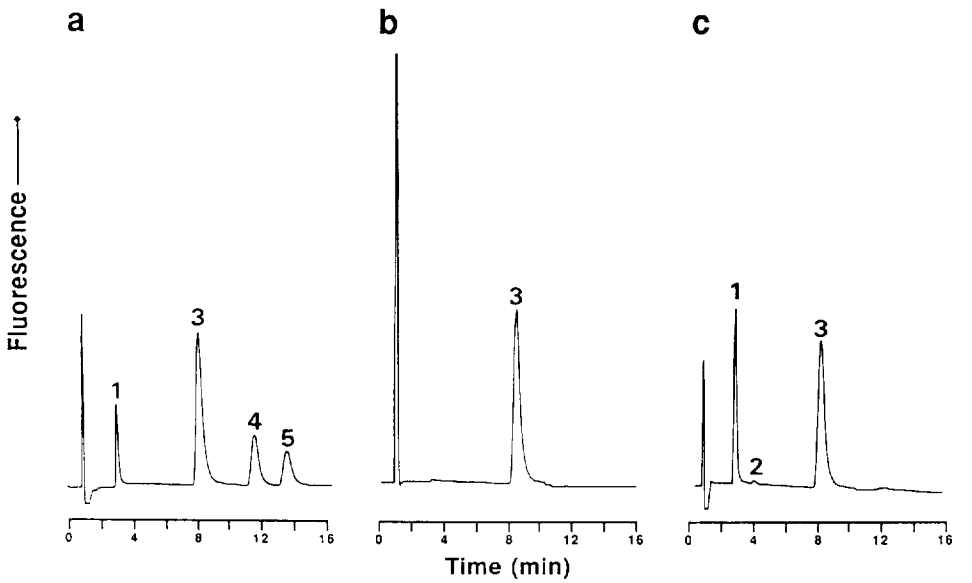


Fig. 10. Analysis of chloroquine and quinine in plasma or serum [19]. Column: 150 × 5 mm I.D. Spherisorb S5SCX. Eluent: Methanol–water (98.5:1.5) containing ammonium perchlorate (80 mmol/l), adjusted to pH 8.0 with 50 mmol/l methanolic sodium hydroxide (final eluent ammonium perchlorate concentration 66 mmol/l). Flow rate: 1.5 ml/min. Detection: Fluorescence: excitation 215 nm, no emission filter. Injection: 100 μl sample extracts. Chromatograms: (a) Standard solution prepared in newborn calf serum containing chloroquine (0.50 mg/l), monodesethylchloroquine (0.25 mg/l) and quinine (1.00 mg/l). (b) Drug-free human plasma. (c) Plasma from a patient treated with quinine (plasma concentration 2.2 mg/l). Peaks: 1 = quinine, 2 = hydroquinine, 3 = hydroxychloroquine (internal standard), 4 = monodesethylchloroquine, 5 = chloroquine.

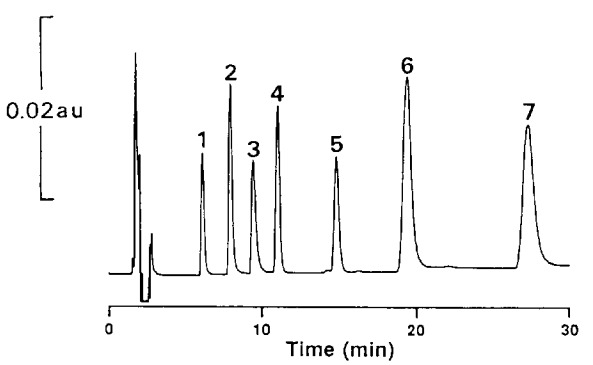


Fig. 11. Analysis of some benzodiazepines, tricyclic antidepressants and metabolites. Column: 250 × 4.6 mm I.D. PEEK packed with Spherisorb S5SCX. Eluent: methanol–water–perchloric acid (60%) (97.5:1.75:0.75). Flow rate: 1.0 ml/min. Detection: UV, 220 nm. Injection: 30 μl methanolic solution containing (1) clonazepam, (2) nordiazepam (both 0.7 mg/l), (3) diazepam, (4) nordothiepin, (5) dothiepin, (6) nordothiepin sulphoxide (all 2.1 mg/l), and (7) dothiepin sulphoxide (4.3 mg/l).

and quaternary ammonium compounds are retained. In addition, N-dealkylated, phenolic, and other metabolites such as sulphoxides are often resolved from the parent compound [14,23]. However, one experimental finding which has not been explained is that the peak shape of some analytes, most notably alkaloids such as morphine, quinine, and strychnine, is influenced by eluent pH, higher pH values usually giving better peaks.

The retention of protonated bases and of quaternary ammonium compounds on unmodified silica using methanol or aqueous methanol eluents is thought to be mediated largely by cation exchange with surface silanols [7,15]. Eluent pH is thus a major influence on retention, ionization of surface silanols increasing at higher eluent pH values and thus giving increased retention for quaternary ammonium compounds and protonated bases. Reduced protonation of basic drugs at higher eluent pH values reduces retention, however, analyte pK_a

values indicating the eluent pH of maximum retention at constant ionic strength.

Ionic strength is also a major influence on retention, although the effect of ionic strength becomes less pronounced as the degree of protonation of basic analytes falls (see Fig. 5). Eluent water content had little effect on retention or selectivity for the fully protonated bases and the quaternary ammonium compound studied at concentrations up to 10% (v/v) at pH < 0 and up to 40% at pH 6.7 [15,17]. The use of more than 50% water enhanced retention markedly for most analytes at pH 6.7. However, except for amiodarone and desethylamiodarone, selectivity remained largely unaltered [7,15].

Smith, Gill and colleagues [24–28] have also used unmodified silica in the HPLC of a range of basic drugs with a methanol–water (9:1) eluent (pH in the range 9.2–10.1) based on that used by Jane [6] and have reported some variation in the retention of particular analytes. However, at alkaline pH values small alterations in eluent pH will alter the ionization of surface silanols and will also alter protonation of basic analytes. Changes in ionic strength may also alter selectivity at constant pH (Fig. 5). A complicating factor here is that ammonium hydroxide and ammonium salts of organic acids such as acetate used as modifiers in such systems may not be completely dissociated in methanol-rich media. Eluent water content and possible pH-induced breakdown of the silica matrix are further potential variables.

Much of the variation observed initially by Smith et al. was attributed to differences in the ammonium hydroxide solution used to prepare the buffer [25]. In subsequent work a non-volatile buffer was used but here certain compounds, notably dipipanone, pipazethate and prolintane (all of which contain an alicyclic tertiary amine moiety and are relatively weak bases; pK_a dipipanone, for example, 8.5), showed a consistent decrease in retention relative to protriptyline with increasing time since manufacture of the silica packing. These changes were attributed to increased hydroxylation of the silica surface arising from conversion of surface siloxanes to silanols [28]. Whilst this might be expected to give increased retention if ion exchange is the predominant retention mechanism, it may be that ion exchange is relatively unimportant for the compounds showing greatest variation since they are all relatively weak bases and thus will be present at pH 10.1 largely if not entirely in the unprotonated form. Even using methanolic ammonium perchlorate at pH 8.3 the plots of k vs. 1/ionic strength for basic drugs on silica were markedly different in slope to the plot given by the quaternary ammonium compound studied (Fig. 5).

Cox and Stout [21] have reported on a study of the retention mechanism of "a set of nitrogenous bases" on various silicas "over the entire range of concentration of organic solvent". Unfortunately, the only basic drug studied (morphine) is atypical as regards the HPLC of basic drugs on unmodified silica [14,15] and indeed on SCX-modified silica (this study), the quaternary ammonium compound studied (thiamine) is actually a thiazolium compound, whilst no methanol concentrations higher than 75% (v/v) were employed. A further complication is that sodium orthophosphate adjusted to an appropriate pH with orthophosphoric acid was used to provide the eluent ionic strength. This compound may not be fully dissociated at higher eluent methanol concentrations thus providing a possible explanation for their observation that apparent eluent pH increased with added methanol concentration from 4.6 (0% methanol) to 6.4 (75% methanol). Cox and Stout [21] interpreted this as showing that ionization of surface silanols would be increased at higher eluent methanol contents thereby explaining the increased retention of morphine and thiamine caused by simply adding methanol. In contrast, added water has no effect on apparent pH at eluent water contents from 0 to 60% (v/v) if ammonium perchlorate is used to provide the eluent ionic strength at pH 6.7 [15].

Retention mechanism(s) other than ion exchange with surface silanols must operate on unmodified silica using methanol or acetonitrile–water eluents at high eluent water contents (60% v/v or more). Adamovics [13], for example, has reported the analysis of acidic antibiotics on unmodified silica, whilst Flanagan [12] has shown

that a series of chlorophenoxy herbicides including 2,4-dichlorophenoxyacetic acid (pK_a 2.6) can be retained and have the same elution sequence on unmodified silica as on a number of bonded-phase materials at pH 3.5. Absolute retention could be increased by increasing the eluent water content, although efficiency decreased markedly with increasing retention. It is possible that hydrogen bonding with protonated silica silanols contributes to retention in such circumstances.

Use of SCX-modified silica

SCX-modified silica packings used with nonaqueous ionic eluents give good retention and peak shape for many basic drugs (Fig. 3). Ionization of the sulphopropyl moiety appears little influenced by eluent pH and residual silanols on the SCX material appeared to have little effect on the retention of the basic drugs studied (Fig. 2). As on unmodified silica, the retention of basic analytes decreases at higher eluent pH values, the pK_a indicating the eluent pH where retention begins to decrease at constant ionic strength. This property can be used to adjust selectivity for certain compounds. When using published pK_a values in this way, however, it must be remembered (i) that such measurements are performed in aqueous solution, and (ii) that there is always the possibility of error in published data. The published pK_a values for amiodarone (5.6) and methdilazine (7.5), for example, do seem rather low when compared to those for the structurally similar compounds lignocaine (7.9) and thioridazine (9.5).

At constant pH, retention on SCX columns is inversely proportional to ionic strength for fully protonated analytes and quaternary ammonium compounds (Table 1, Fig. 4). At pH 8.3 this effect is less marked for some of the weaker bases studied. Except for lignocaine, codeine and morphine, the addition of water had no major effect on retention or selectivity on SCX at concentrations below 30% (v/v) at pH 6.7 (Fig. 7). However, in contrast to behaviour with unmodified silica, the addition of up to 5% (v/v) water under strongly acidic conditions caused a doubling of retention for most analytes studied (Fig. 6). The reason for this is unclear. There was little further effect on going to 40% water.

Our approach to the HPLC of basic drugs on either unmodified or SCX-modified silica is to use acidic or neutral conditions if possible since the effect of changes in eluent pH and ionic strength (and even water content) are in general relatively predictable. The only reason for using high pH eluents in the HPLC of basic drugs on unmodified silica is in those instances when compounds (morphine, for example) give bad peak shapes at acid or neutral eluent pH values, as discussed above. If screening for a wide range of basic drugs is to be performed then Binder et al. [9] have shown that unmodified silica can be used with acetonitrile–aqueous potassium dihydrogen orthophosphate (6 mmol/l) containing tetramethylammonium hydroxide (5 mmol/l) and dimethyloctylamine (2 mmol/l), pH 6.50 (33:67) as eluent. Alternatively, if electrochemical oxidation detection is to be employed then the eluent described by Jane et al. [16] (10 mmol/l ammonium perchlorate in 100% methanol, pH 6.7) has the advantage of giving a relatively low background current at applied potentials up to +1.2 V vs. Ag/AgCl.

Practical advantages of nonaqueous ionic eluent systems are that methanol is much less viscous than water–methanol or water–acetonitrile mixtures and thus column back-pressures are low. Eluent degassing is not normally needed. In addition, use of methanol as the eluent solvent minimises the risks of silica dissolution. A further advantage of ionic eluent systems is that relatively large volume solvent extracts can be injected directly in a "non-eluting" (zero ionic strength) solvent with no loss of efficiency [14].

One problem with silica column–nonaqueous ionic eluent systems used under strongly acidic conditions (methanol–perchloric acid eluent) is that the ionic strength required to promote elution at a given retention time decreases with prolonged use of the column [14]. This could be due to, for example, dehydration of adjacent silanols to form a siloxane moiety or to irreversible binding of exogenous molecules at cation exchange sites. Although we have not studied

systematically the stability of SCX-modified columns used under strongly acidic conditions for the analysis of basic drugs, our experience is that such systems are probably more stable than those employing unmodified silica. This could be because the contribution of surface silanols to retention is relatively unimportant on the SCX materials and thus any tendency of adjacent silanols to lose water to form siloxane bridges, etc. will also be unimportant. Certainly hydrolysis of the sulphopropyl moieties under strongly acidic conditions does not seem to be important in routine use. A factor here may be the high organic content of the eluents used: the high methanol content of the methanol–water (9:1) pH 9.2–10.1 eluent used by Jane [6] and followers with unmodified silica is presumed to be an important factor in the stability of these systems.

A major consideration in HPLC is the hazard posed by the eluent. With SCX materials the hazard presented by use of relatively high perchlorate concentrations in methanolic or methanol–acetonitrile-rich media has to be balanced against the practical benefit obtained. The use of ammonium perchlorate at the concentrations discussed does not seem to cause excessive corrosion of stainless HPLC components. However, the perchloric acid-modified eluent used in the analysis of tricyclic antidepressants (Fig. 11), for example, is very corrosive and for this reason PEEK columns and fittings have been preferred. It should prove possible to use sulphuric acid, ammonium sulphate or even organic sulphonates such as camphorsulphonic acid as the ionic modifier [14] in order to obviate the requirements for perchlorates. Secondly, synthesis of SCX-modified materials with lower phase loadings should facilitate the use of eluents of lower ionic strength.

Observations on the mechanism of retention: ionic eluent systems

Although the underlying mechanism whereby separations are achieved either on unmodified silica or on SCX-modified silica packings using ionic eluent systems under acidic or neutral conditions appears to be ion exchange, the reason why separations are achieved remains obscure. The key analyte functionality is clearly the protonated basic group or quaternary ammonium moiety. The loss of an N-alkyl group from a tertiary or secondary amine (amitriptyline–nortriptyline, for example) is enough to give rise to a good separation under appropriate eluent conditions. If both analytes are fully protonated then the stronger base (nortriptyline, pK_a 9.7) is eluted before (has less affinity for the stationary phase than) amitriptyline (pK_a 9.4).

This apparent anomaly could be due to steric effects, the lack of one N-methyl group on nortriptyline as compared to amitriptyline permitting easier access of counter-ions (solvated H^+ or NH_4^+) to ionized silanols or SCX moieties and thus more rapid elution. Alternatively, the lack of the N-methyl group on nortriptyline could permit greater solvation of the analyte and thus reduce the affinity of the solvated complex for the stationary phase. At higher eluent pH values the net charge carried by amitriptyline, and thus ionic interaction with the stationary phase, is reduced hence permitting more rapid elution for a given ionic strength. In the case of the stronger base nortriptyline this process presumably begins at a higher eluent pH thus providing a possible explanation for the fact that nortriptyline elutes after (has greater affinity for the stationary phase) than amitriptyline at higher pH values.

Recently Law [11] has published a 'strategic' approach to the analysis of basic drugs by HPLC. However, the suggested 'tau' values (attempts to relate separation of parent compounds and metabolites to molecular structure) are only valid for the eluent used [methanol–aqueous ammonium acetate, pH 9.1 (9:1)] and are clearly subject to change with eluent pH, and also with ionic strength at alkaline pH (Figs. 4 and 5), and may be influenced by other factors such as eluent water content. Moreover, as noted above, Smith, Gill and colleagues [24–28] have reported variation in the k values obtained on such systems, especially with weaker bases.

In unmodified and SCX-modified silica HPLC systems eluent water may act to either (i) alter the affinity of the (solvated) analyte for the stationary phase or (ii) alter the affinity of the

(solvated) counter-ion for the stationary phase. Water could also influence the accessibility of the cation-exchange sites, as appears to happen on SCX under strongly acidic conditions (Fig. 6). Given that all the analytes studied (protonated or unprotonated) are very soluble in methanol it is unlikely that the solubility in the eluent contributes to retention except possibly when adding relatively large amounts of water (40% v/v or more). However, at higher concentrations water might act to hinder access of the counter-ion (increased solvation shell?) to the ionized silanol–SCX moiety thus giving increased analyte retention.

4. Conclusions

Microparticulate SCX-modified silica used with methanolic or aqueous methanol eluents containing an ionic modifier can be employed in the analysis of quaternary ammonium compounds and basic drugs, including compounds which are poorly retained on unmodified silica. The effects of changes in eluent pH and ionic strength on retention and selectivity are relatively predictable. The addition of water has little overall effect on retention or selectivity at concentrations up to 30% (v/v) at pH 6.7 except for certain compounds, notably lignocaine. However, addition of up to 5% (v/v) water under strongly acidic conditions caused a doubling of retention for most of the analytes studied. No SCX materials other than Spherisorb S5SCX have been evaluated systematically. However, use of materials with a lower SCX loading should permit lower eluent ionic strengths to be used. Past experience suggests that Zorbax 300 SCX (sulphophenylpropyl-modified silica) might be suitable.

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References

[1] R. Gill, S.P. Alexander and A.C. Moffat, *J. Chromatogr.*, 247 (1982) 39.
[2] D.C. Leach, M.A. Stadalius, J.S. Berus and L.R. Snyder, *LC·GC Internat.*, 1 (1988) 22.
[3] B. Law, S. Weir and N.A. Ward, *J. Pharmaceut. Biomed. Anal.*, 10 (1992) 167.
[4] B. Law and S. Weir, *J. Pharmaceut. Biomed. Anal.*, 10 (1992a) 181.
[5] B. Law and S. Weir, *J. Pharmaceut. Biomed. Anal.*, 10 (1992b) 487.
[6] I. Jane, *J. Chromatogr.*, 111 (1975) 227.
[7] B.A. Bidlingmeyer, J.K. Del Rios and J. Korpi, *Anal. Chem.*, 54 (1982) 442.
[8] H. Lingeman and W.J.M. Underberg, *Trends Anal. Chem.*, 7 (1988) 346.
[9] S.R. Binder, M. Regalia, M. Biaggi-McEachern and M. Mazhar, *J. Chromatogr.*, 473 (1989) 325.
[10] B. Law, *Trends Anal. Chem.*, 9 (1990) 31.
[11] B. Law, in E. Reid and I.D. Wilson (Editors), *Bioanalytical approaches for drugs, including antiasthmatics and metabolites*, Royal Society of Chemistry, Cambridge, 1992, p. 57.
[12] R.J. Flanagan, in R.B. Holman, A.J. Cross and M.H. Joseph (Editors), *High Performance Liquid Chromatography in Neuroscience Research*, Wiley, Chichester, 1993, p. 321.
[13] J.A. Adamovics, *J. Pharmaceut. Biomed. Anal.*, 5 (1987) 267.
[14] R.J. Flanagan, G.C.A. Storey, R.K. Bhamra and I. Jane, *J. Chromatogr.*, 247 (1982) 15.
[15] R.J. Flanagan and I. Jane, *J. Chromatogr.*, 323 (1985) 173.
[16] I. Jane, A. McKinnon and R.J. Flanagan, *J. Chromatogr.*, 323 (1985) 191.
[17] R.J. Flanagan, R.K. Bhamra, S. Walker, S.C. Monkman and D.W. Holt, *J. Liquid Chromatogr.*, 11 (1988) 1015.
[18] S.C. Monkman, S. Rosevear, R. Armstrong, R.J. Flanagan and D.W. Holt, *Biomed. Chromatogr.*, 3 (1989) 88.
[19] K. Croes, P.T. McCarthy and R.J. Flanagan, *J. Anal. Toxicol.*, 18 (1994) 255.
[20] J.E.F. Reynolds (Editor), *Martindale, The Extra Pharmacopoeia*, Edition 30, Pharmaceutical Press, London, 1993.
[21] G.B. Cox and R.W. Stout, *J. Chromatogr.*, 384 (1987) 315–336.

[22] P.T. McCarthy, S. Atwal, A.P. Sykes and J.G. Ayres, *Biomed. Chromatogr.*, 7 (1993) 25.
[23] J.R. Cashman and Z-C. Yang, *J. Chromatogr.*, 532 (1990) 405.
[24] R.M. Smith, T.G. Hurdley, J.P. Westlake, R. Gill and M.D. Osselton, *J. Chromatogr.*, 455 (1988) 77.
[25] R. Gill, M.D. Osselton and R.M. Smith, *J. Pharm. Biomed. Anal.*, 7 (1989) 447.
[26] R.M. Smith and J.O. Rabour, *J. Chromatogr.*, 464 (1989) 117.
[27] R.M. Smith, J.P. Westlake, R. Gill and M.D. Osselton, *J. Chromatogr.*, 514 (1990) 97.
[28] R.M. Smith, J.P. Westlake, R. Gill and M.D. Osselton, *J. Chromatogr.*, 592 (1992) 85.