

Re-evaluation of strong cation-exchange high-performance liquid chromatography for the analysis of basic drugs

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Abstract

Strong cation-exchange (SCX) HPLC has been systematically evaluated for the chromatography of basic compounds using an eluent of 80% methanol containing ammonium formate (0.02 *M*) with the pH adjusted to 2.45 with trifluoroacetic acid. Contrary to common perception SCX-HPLC is capable of high efficiencies (>60 000 plates/m) and good peak symmetries (A_s typically <1.2). Retention shows the expected dependency on the reciprocal of the eluent ammonium concentration and eluent pH. Other mechanisms of retention have also been demonstrated including partitioning and hydrogen bond interactions. Control of the separation selectivity is possible through systematic adjustment of the eluent parameters such as ionic strength, pH and methanol concentration. SCX-HPLC is shown to be a powerful separation procedure for the analysis of basic compounds.

Keywords: Ion-exchange chromatography; Mobile-phase composition; Drugs, basic; β -Blockers

1. Introduction

Strong cation-exchange (SCX) bonded phases for high-performance liquid chromatography were introduced and evaluated for the analysis of drugs in the 1970s [1,2]. Apart from a series of reports by Piotrovskii and co-workers [3–7] in the 1980s, SCX has received scant attention or systematic study for the analysis of basic drugs [8–10]. This may be attributed to the fact that ion-exchange chromatography is still considered to be a low-performance technique even when used in the high-performance mode [11]. This can be traced to the fact that classically, ion-exchange chromatography has been

carried out using large particle size organic resins (typically styrene divinyl benzene) and highly aqueous eluents which resulted in limited chromatographic efficiency.

In the authors' opinion this view of ion-exchange-HPLC is unfounded. For a number of years now silica has been widely used as a cation-exchanger giving excellent efficiencies ($2 < h < 4$) and near perfect symmetries for a wide range of basic solutes [12]. The only serious disadvantage with this approach is the low pK_a of the silica silanols and the high eluent pH (typically 9.1) which makes it unsuited to the analysis of weak bases. Recognising this limitation we decided to re-evaluate commercially available SCX-HPLC phases for the analysis of weakly basic drugs. Initial results were encouraging

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[13] and we present here a systematic evaluation of SCX-HPLC. A similar study [10] was reported whilst this manuscript was in preparation and whilst overall the views are similar there are significant differences in some of the specific findings.

2. Experimental

2.1. Equipment

Chromatography was carried out using a Milton Roy constametric 3000 pump, a Perkin-Elmer ISS200 autosampler and an LDC Spectromonitor III UV detector set at 270 nm. Results were recorded using a Hewlett-Packard 3392A, a Perkin-Elmer 1020 integrator or a chart recorder.

A Beckman 3500 digital pH meter fitted with a CMAWL combined pH glass/reference cell (Russell pH Ltd, Fife, UK) was calibrated with aqueous buffers and used to determine eluent pH.

HPLC columns were stainless steel (100×4.6 mm I.D.) containing Spherisorb 5SCX (Phase Separations, Deeside, UK) packed by Hichrom (Reading, UK) or Phase Separations. The stationary phase is a bonded propyl sulphonic acid.

2.2. Materials

The following chemicals were used: ammonium formate, Analar grade (BDH, Liverpool, UK); trifluoroacetic acid (Fluka, Gillingham, UK); methanol and acetic acid (both HPLC grade from Fisons, Loughborough, UK). Solutes were a selection of substituted anilines and pyridines, phenyl-alkyl amines, beta-blockers or basic drug compounds. These were obtained from the Zeneca compound collection or from a number of commercial sources.

2.3. Methods

Eluents were prepared from mixtures of methanol and water containing various proportions of acids and ammonium formate. All separations were carried out at a flow-rate of 1 ml/min at ambient temperature. Solutes were dissolved in methanol at ca. 1 mg/ml and 1 to 10 μ l injected onto the column.

Diphenylamine being a sterically hindered weak base was selected as the t_0 marker.

3. Results and discussion

3.1. Selection of chromatographic conditions

Our intention was to develop an HPLC system which was compatible with LC-MS and which could be used for preparative chromatography. These two criteria therefore necessitated the use of volatile mobile-phase additives and accordingly ammonium salts of acetate and formate were investigated along with acetic, formic and trifluoroacetic acid for adjustment of the eluent pH.

For the purpose of this work we have defined weak bases as having pK_a values between 3 and 7. To ensure full ionisation of all the solutes studied the eluent pH needed to be <3.

Although SCX columns are often run in purely aqueous eluents (the test chromatogram supplied with the column involves an eluent of aqueous ammonium phosphate) we decided to investigate mixed methanol–aqueous eluents. Initial experiments involving a range of test compounds showed a significant increase in column efficiency as the proportion of methanol in the eluent was increased (Fig. 1). The efficiency reached a plateau at around

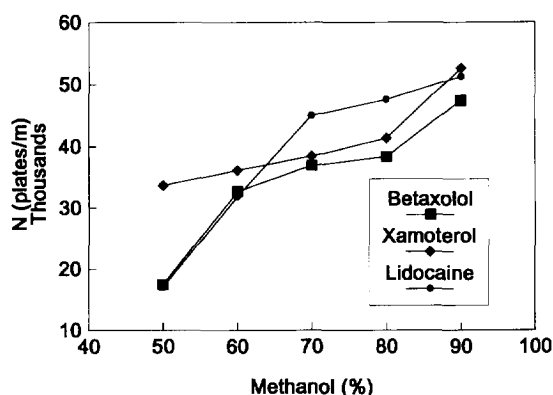


Fig. 1. Variation in the efficiency for three solutes on an SCX column with changing mobile-phase methanol content over the range 50–90%. Column, Spherisorb 5 μ m SCX (100×4.6 mm I.D.); eluent, methanol–water (80:20) containing ammonium formate (0.02 M) and TFA (2.3 ml/l).

80 to 90% methanol. We have attributed this effect to the changing viscosity of the eluents which decreases between 40% and 100% methanol [14]. The high efficiencies observed under optimum conditions (Fig. 1 and see below) shows that SCX columns are capable of very high performance.

Having defined the mobile-phase organic modifier concentration we set about optimising the eluent pH. Acetic acid was rapidly eliminated from the investigation. Even when the proportion of acid in the eluent was as high as 15%, the pH could only be reduced to 3.4. Further experiments showed TFA to be preferable to formic acid in giving lower pHs for a given volume of acid. TFA also gave lower UV absorbance at short wavelengths than formic acid (between 1.6 and 4 times).

Methanol–water–TFA (800:200:2.3, v/v) containing 0.02 M ammonium formate was eventually selected as a general purpose eluent and this was used as the basis for further study.

3.2. Mobile-phase ionic concentration

The effect of variations in the mobile-phase ammonium formate concentration on the retention of a range of test solutes is shown in Fig. 2. Five concentrations of ammonium formate were used, two either side of the recommended concentration. Reducing the concentration of the competing ammonium ion clearly increases retention as expected.

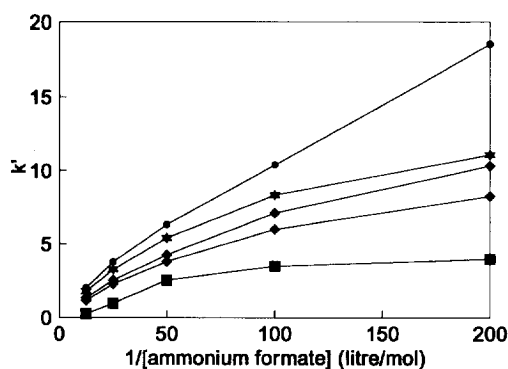


Fig. 2. Variation in the capacity factor with mobile-phase ammonium formate concentration for a range of simple solutes. Column, Spherisorb 5 μm SCX (100 \times 4.6 mm I.D.); eluents, methanol–water (80:20) containing ammonium formate and TFA (2.3 ml/l).

Contrary to theory the relationship between $\log k'$ and the reciprocal of the ionic concentration is non-linear. This suggests the presence of retention mechanisms in addition to the cation-exchange process. It is clear however that retention can be predictably controlled by varying the concentration of the mobile-phase ionic component.

The results obtained here are in marked contrast to the work of Croes et al. [10] who obtained linear relationships for most compounds using methanol and methanol–water as the eluent. These differences may be attributable to the different t_0 markers used or the different ionic components in the eluent. For example, Croes et al. used perchlorate in their eluent which is known to form ion pairs.

3.3. Effect of eluent pH

The effect of eluent pH on solute retention was studied by varying the amount of TFA added to the eluent; from 1 ml to 5 ml acid per litre giving three final measured pHs of 4.7, 2.45 (the recommended pH) and 2.0. The test solutes studied included weak bases such as anilines and pyridines with $\text{p}K_a$ values around 4 to 6 as well as several phenyl-alkyl amines and beta-blockers ($\text{p}K_a$ around 9). Within their chemical classes these compounds generally showed the same trend and some typical data for a selection of these are presented in Fig. 3. The data for the weak bases, compounds 1–4, show maximum retention at the intermediate pH of 2.45. The increase in retention in moving from pH 4.7 to 2.45 can be explained by an increase in the ionisation of the weakly basic solutes. As the pH is reduced further, from 2.45 to 2.0, there is little further ionisation of the amines. At this lower pH however, the ionisation of the stationary-phase sulphonic acid groups appears to be suppressed. Thus in moving from a pH of 2.45 to 2.0 the overall effect is dominated by the loss in ion-exchange capacity and a reduction in retention ensues.

The above hypothesis was confirmed by analysis of a series of phenyl-alkyl amines which possessed a minimum $\text{p}K_a$ of 8.6 (1,2-diphenylethylamine), i.e. compounds 5–8 in Fig. 3. As 1,2-diphenylethylamine is a moderately strong base it is already >99.95% ionised at pH 4.7. Thus as the pH of the eluent decreases the retention of the phenyl-alkyl

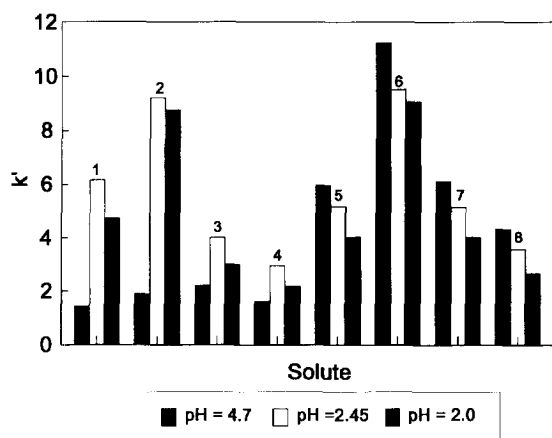


Fig. 3. Variation in capacity factor with eluent pH for a series of anilines (1–4) and phenyl-alkyl amines (5–8). 1=N-Methylaniline; 2=N,N-dimethylaniline; 3=4-methylaniline; 4=4-butylaniline; 5=2-phenethylamine; 6=N,N-dimethyl benzylamine; 7=N-isopropylbenzylamine; 8=amphetamine. Column, Spherisorb 5 μm SCX (100 \times 4.6 mm I.D.); eluent, methanol-water (80:20) containing ammonium formate (0.02 M) with the pH adjusted using TFA.

amines also decreases reflecting the suppression of the stationary-phase ionisation.

These data are difficult to compare with the findings of Croes et al. [10] since in the latter work the authors used sodium hydroxide solution to adjust the pH of some of the eluents. However not only will the pH be modified but there will also be a change in ionic strength which will confound the observed results.

3.4. The effect of solute pK_a

A series of basic solutes (9–21) with pK_a s distributed over the range 2.33 to 9.39 were chromatographed using the recommended mobile-phase with a pH of 2.45. A plot of $\log k'$ vs. pK_a is shown in Fig. 4, with retention increasing with increasing pK_a up to a limiting value, resulting in a plateau at $pK_a > \text{ca. } 4$. Increasing the mobile-phase pH to 4.7 reduces retention for most of the solutes (as expected) with the data shifting to the right and the plateau coming in at higher pK_a (≥ 6). Once again significant selectivity changes are evident as pH is varied. It can be seen that there is regular sigmoid relationship in the data which is essentially sigmoid in nature. It is also interesting to note that for compounds having k' of

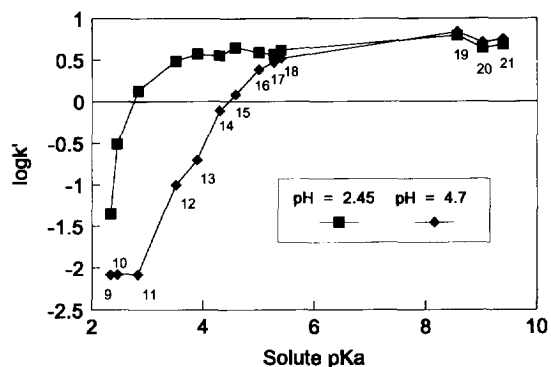


Fig. 4. Variation in $\log k'$ with solute pK_a for a series of anilines and benzylamines. Compound 9=4-nitroaniline; 10=3,5-dichloroaniline; 11=3,4-dichloroaniline; 12=3-chloroaniline; 13=4-chloroaniline; 14=3-ethoxyaniline; 15=aniline; 16=4-methylaniline; 17=4-ethoxyaniline; 18=4-methoxyaniline; 19=4-nitrobenzylamine; 20=3-iodobenzylamine; 21=benzylamine. Chromatographic conditions as for Fig. 1.

around 3 there are significant differences in retention (0.25 k' units) for compounds which only differ in pK_a by around 0.1.

3.5. Effect of eluent organic modifier concentration

In the work reported so far the eluent methanol concentration has been maintained at a high concentration (80%) to ensure high chromatographic efficiency. When the mobile-phase methanol concentration was varied over a limited range (70–90%), there was some variation in solute retention and selectivity although there was no clear trend observable in the anilines, pyridines or phenyl-alkyl amines studied (data not shown). Some compounds showed an increase in retention with increasing methanol concentration whilst others showed a decrease. What was of interest however was the data for aniline and its 4-(*n*-alkyl) ring substituted analogues. These compounds showed decreasing retention with increasing substituent chain length (Fig. 5). With the exception of aniline (pK_a 4.58) the calculated pK_a s [15] for these compounds are very similar, ranging from 4.98 for the methyl substituted compound to 5.07 for the pentyl analogue. It is unlikely that the effect is related to pK_a since the difference is small and the most basic compound shows the least retention. The most likely explana-

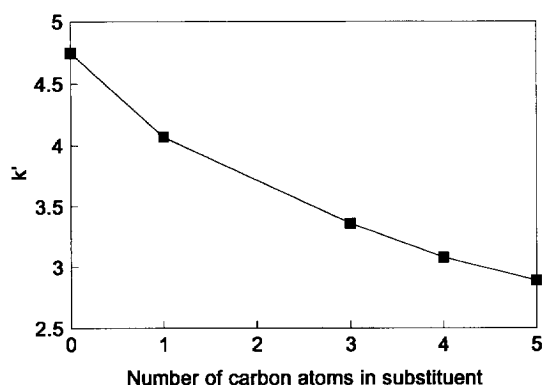


Fig. 5. Variation in capacity factor with increasing number of carbon atoms in the alkyl substituent of a series of 4-alkyl substituted anilines. Chromatographic conditions as for Fig. 1.

tion is that there is a secondary partitioning mechanism operating with the stationary phase more polar than the mobile phase. Thus the most lipophilic pentyl analogue shows the least retention.

In the light of previous findings employing a silica based ion-exchange system [16], we also investigated the possibility of a hydrogen bonding interaction. For this work we selected a set of beta-blocker compounds based on the structure shown in Fig. 6. These compounds had substituents in the 4-position of the aromatic ring. The substituents (Table 1), were chosen for their range of hydrogen bond acceptor properties ($\log K_{\beta}$) as defined by Abraham et al. [17].

The variation in retention with varying methanol concentration for the above set of compounds is shown in Fig. 7. The parent compound with no substituent in the ring showed very little change in retention as the methanol concentration was varied. Of the other four substituted compounds, the two with the highest $\log K_{\beta}$ values showed the greatest retention. Furthermore all four showed a decrease in retention with decreasing mobile-phase methanol

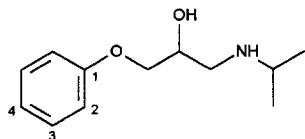


Fig. 6. The basic beta-blocker structure used for the study of hydrogen bonding interactions. The compounds were substituted at the 4-position.

Table 1
Beta-blocker substituents and their $\log K_{\beta}$ values

Compound no.	Substituent	$\log K_{\beta}$
22	-H	NA
23	-CH ₂ CONHCH ₃	3.0
24	-N(CH ₃)COCH ₃	2.8
25	-CN	1.0
26	-CH ₂ CH ₂ CN	1.2

NA=Not applicable.

concentration. This change can be understood by reference to previous work in RP-HPLC which showed that increasing organic modifier content of the eluent leads to increased secondary hydrogen bonding interactions [18]. We would suggest therefore that the results presented in Fig. 7 are in accord with previous findings [16,18] and are indicative of a hydrogen bond interaction between the acceptor group on the solute and the unbonded silanols or the sulphonic acid groups in the stationary phase.

Comparison with a similar report [10] is difficult since the compounds used in the present study were carefully controlled whereas those of Croes et al. [10] were drug compounds showing a high degree of physicochemical variability. However, despite this and the difference in eluent conditions both studies show a general reduction in retention as the mobile-

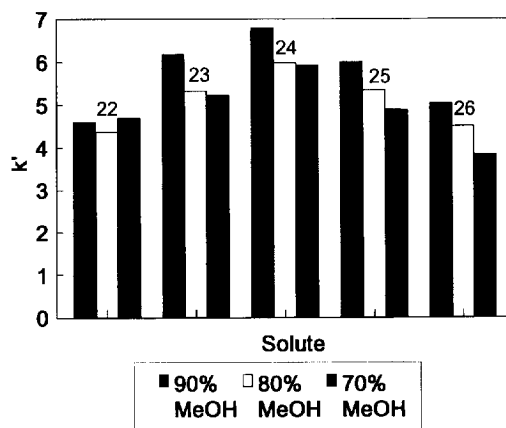


Fig. 7. Variation in retention for a range of beta-blockers with differing eluent methanol concentrations. Compound 22 is a control, the others have substituents with varying degrees of hydrogen bond acceptor ability at the 4-position in the ring (see Table 1). Column, Spherisorb 5 μ m SCX (100 \times 4.6 mm I.D.); eluent, mixtures of methanol and water containing ammonium formate (0.02 M) and TFA (2.3 ml/l).

phase methanol content is reduced from 90% to 70%.

3.6. Applicability

The HPLC system presented here has proved particularly useful in our laboratory since it is able to give good chromatographic performance for a wide range of basic compounds and their metabolites. Whilst many of these compounds are of a proprietary nature and cannot be reported, Fig. 8 shows a chromatogram for a selection of commonly occurring weakly basic drugs. The performance of the system in terms of efficiency and symmetry is shown by some typical performance data in Table 2. In general, efficiencies are high, often up to 70 000 plates/m. Peak symmetries are also generally good but more variable. Whilst many compounds give A_s values <1.2, compounds like diazepam are clearly an exception. The efficiency and symmetry data for three other columns were within $\pm 10\%$ of that shown in Table 2. We have however observed column-to-column variability which manifested itself in small selectivity differences. We believe that this variation in selectivity may be related to variability

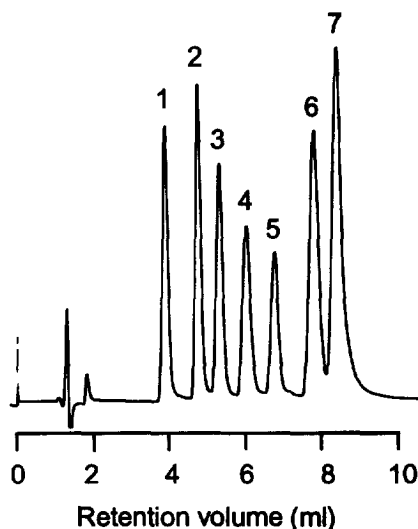


Fig. 8. Example chromatogram showing the quality of chromatography for a range of basic drugs. Peaks: 1=halofantrine, 2=minoxidil, 3=haloperidol, 4=reserpine, 5=cimetidine, 6=verapamil, 7=clomipramine. Chromatographic conditions as for Fig. 1.

Table 2

Typical performance data for the standard^a SCX chromatographic system

Compound	k'	N (plates/m)	A_s
Betaxolol	3.63	58 839	0.96
Clonidine	4.65	74 035	1.10
Xamoterol	5.79	58 602	1.05
Lidocaine	7.78	57 379	1.27
Diazepam	6.77	70 344	1.35

^a The column was a Spherisorb 5 μm SCX (100 \times 4.6 mm I.D.) and the eluent was methanol–water (80:20) containing ammonium formate (0.02 M) and TFA (2.3 ml/l). All values are the mean of two determinations.

seen with some of the simpler compounds studied. For example, aniline showed significant fronting ($A_s=0.75$) which was similar on four different columns. The efficiency however varied from 25 363 to 74 859 plates/m. This poor and variable chromatographic performance is probably due an impurity in the aniline sample used in the study. Although we tried where possible to use the best-grade materials available it is probable that this non-pharmaceutical grade chemical contains related compounds which have similar but variable chromatographic properties which results in the observed variations in performance.

4. Conclusions

The use of strong cation-exchange HPLC for the analysis of basic drugs has seen only infrequent use since its introduction in high-performance mode in the early 1970s. This is partially attributable to the widely held perception that ion-exchange is a low-performance technique. This view should be changed in the light of the results presented here and those from other workers [10]. The chromatographic performance with most basic compounds is excellent with efficiencies up to 70 000 plates/m.

The separation mechanism on SCX is undoubtedly complex, involving partitioning, and hydrogen bonding in addition to ion exchange. However, with some basic knowledge of the physico-chemical properties of the solutes under study, systematic control of retention and selectivity is possible.

Whilst this report was in preparation Croes et al.

published a study on the use of SCX-HPLC for the analysis of basic drugs [10]. Their work involved the use of predominantly methanolic eluents which may explain some differences in our respective findings. However the overall conclusion of our studies and that of Croes et al. is that SCX-HPLC is a powerful separation technique for the analysis of weak bases which is worthy of greater attention.

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