Journal of Pharmaceutical & Biomedical Analysis
Vol. 10, No. 2/3, pp. 167–179, 1992
Printed in Great Britain

0731–7085/92 $5.00 + 0.00

Fundamental studies in reversed-phase liquid–solid extraction of basic drugs; I: ionic interactions

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Abstract: Seven basic solutes with known and controlled pK_a (7.93–9.5) and log P (0.23–6.63) values have been used as test probes to study the mechanisms involved in liquid–solid extraction with C2 and C18 bonded silica phases. A limited comparison has also been made with underivatized silica and CN phases.

In addition to the reversed-phase mechanism, cation-exchange was shown to play a very significant role in the retention process. Various cations both organic and inorganic were assessed for their elution strength, and the ordering was similar to that for classical ion-exchange chromatography. Control of selectivity in the elution process can be achieved by varying the concentration of cation or methanol in the eluent. The C2 cartridge in combination with an aqueous ammonium acetate–methanol eluent proved to be the most versatile in that all compounds, irrespective of pK_a or log P could be recovered in high yield. The optimal eluent in terms of selectivity with respect to related compounds could be predicted from the solute log P.

Blocking of silanols by pre-conditioning the cartridges with cations prior to sample applications was also studied. The order of cation strengths although somewhat variable was similar to that established at the elution stage. To achieve quantitative elution with methanol or aqueous methanol solutions however, high concentrations of inorganic cations, equivalent to 1 ml of a 1 M solution were required to pre-condition a 100 mg cartridge.

Keywords: *Basic drugs; liquid–solid extraction; solid-phase extraction; cation exchange; reversed-phase; retention mechanism; extraction selectivity; physico-chemical parameters; silanols; conditioning.*

Introduction

Over the past 10 years, solid-phase extraction procedures have become well established in the field of drug analysis. Any new user, however, turning to the literature for guidance on the development of solid-phase extraction procedures, would find no logic or systematic guidance on the development of such methods. He or she would also be most confused by the great dissimilarity in reported methods for compounds of the same chemical class, e.g. the beta-blockers [1–8], and even for the same compound [2, 3, 8]. For example, the beta-blocker atenolol, has been assayed by a number of workers using solid-phase extraction. Although these workers used the same silica cyano-propyl (CN) bonded phase, they all used very different application, wash and elution stages. In two reports [2, 3] plasma was applied directly, in the third [8] however, plasma was first deproteinized. The wash solvents varied between water [8], water and acetonitrile [3] and acetone [2]. The elution solvents however showed the greatest diversity. Musch *et al.* [8], used 0.1% propylamine in methanol (1 ml), Verghese *et al.* [2], used a methanolic solution of acetic acid (10 mM) and triethylamine (50 mM) (3 × 0.2 ml), and Harrison *et al.* [3] used a complex mixture of NaH_2PO_4 (50 mM)–acetonitrile (70:30, v/v) containing triethylamine (4 mM) adjusted to pH 4 with H_3PO_4 (2 × 0.25 ml). The diverse conditions used for elution in particular, involving both acidic and basic eluents, would appear highly confusing to say the least. Except in the case of Musch *et al.* [8] where the procedure was part of a general approach to drug extraction [9], there was little rationalization or discussion on the choice of the conditions used. To facilitate more rapid and efficient development of solid-phase extraction procedures, fundamental studies, examining the underlying mechanisms of retention are obviously required.

A study of the literature shows that most workers use reversed-phase type cartridges for drug extraction, particularly C18. It would appear, however, that hydrophobic interaction is not the only retention mechanism operating. Work from this laboratory [5, 10] has suggested that cation-exchange may have an important role to play even when nominally

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reversed-phase cartridges such as C18 are used. A number of authors [5, 11, 12] make mention of cation-exchange along with other mechanisms of retention on reversed-phase cartridges although these effects have never been clearly demonstrated nor rationalized in terms of the behaviour of the solutes studied.

The purpose of this and subsequent publications is to show clearly how these secondary mechanisms operate in the extraction of basic drugs with reversed-phase type cartridges. From a knowledge of the underlying mechanisms more rapid development of selective drug isolation procedures based on sound scientific principles should then be possible.

In this present publication, studies have concentrated on the ionic-interactions that are believed to occur when reversed-phase type cartridges are used for the isolation of basic drugs. Rather than considering these interactions to be troublesome and attempting to block them as is generally the case in reversed-phase high-performance liquid chromatography (HPLC), this study shows how they can be exploited and used to control the selectivity of the extraction process.

There are over 20 different extraction cartridge types available with different bonded groups, although most of the published work has been carried out using C18 and to a lesser extent CN and C2 phases. This study has concentrated initially on these three sorbents and the more polar Si (silica) phase. In the case of Si, the high concentration of unbonded silanols would be expected to lead to a pronounced cation-exchange effect.

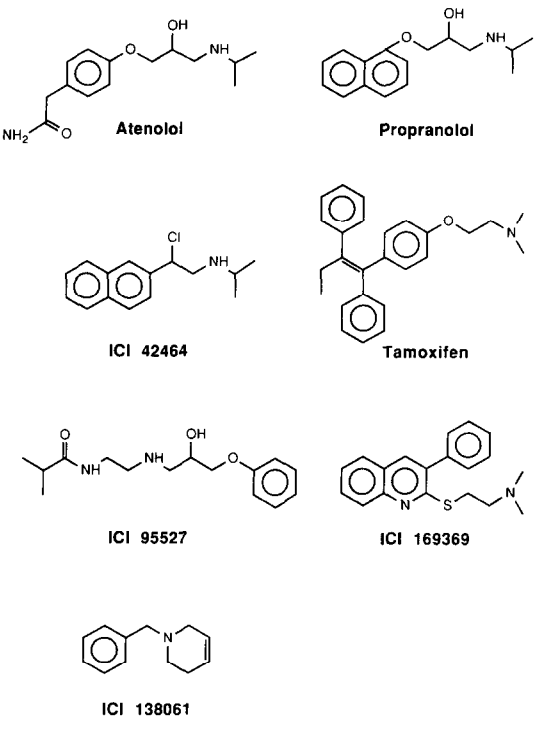

Figure 1
Structures of the seven radiolabelled solutes studied.

Experimental

Materials and equipment

Bond-Elut cartridges, 100 mg size (Si, CN, C2 and C18) and a Vac-Elut manifold were obtained from Jones Chromatography (Hengoed, UK). Methanol and acetonitrile were HPLC grade from Fisons (Loughborough, UK) tetrabutylammonium dihydrogen phosphate (97%) was from Aldrich (Gillingham, UK) and all other chemicals were Analar grade from BDH (Liverpool, UK).

The solutes studied (Fig. 1) were obtained from the Radiochemistry Laboratory at ICI Pharmaceuticals. The compounds were all radiolabelled with ^{14}C and the specific activity ranged from 1.21 to 27.8 μCi mg^{-1}. The radiochemical purity was typically >98%. The selection of these compounds was governed in part by their availability in radiolabelled form. However, they represent a diverse set of basic solutes with a relatively wide range of lipophilicity (measured by log P, the octanol–water partition coefficient) and basicity (pK_a). Physico-chemical data for these compounds, which were determined using standard techniques are presented in Table 1. Scintillation counting was carried out using a Canberra Packard 1900CA TRICARB counter with in-built quench correction. The scintillation fluid was Ready Protein Plus from Beckman.

Methods

All the drugs studied were diluted for use and applied in water or methanol–water

Table 1
Physico-chemical data for the seven solutes studied

Compound	pK_a	log P
Tamoxifen	8.57	6.63
ICI 169369	8.23	4.50
ICI 42464	8.25	3.85
Propranolol	9.42	3.56
ICI 95527	7.93	1.07
ICI 138061	8.7	1.66
Atenolol	9.5	0.23

(10:90, v/v) at a concentration of 0.9–2.0 μg ml^{-1}, equivalent to a radioactive concentration, ranging from 6×10^3 to 50×10^3 decay per minute (dpm) per 1 ml. All cartridges were conditioned with methanol (1 ml) and water (1 ml) before use. The vacuum to each cartridge was individually controlled with a small stopcock to prevent the cartridge drying prior to sample application. A vacuum pressure of 34 kPa (equivalent to a flow rate of approximately 10 ml min^{-1} for methanol) was used throughout.

A typical experiment involved application of the drug solution (1 ml) to a Bond-Elut cartridge and then sequential elution with a range of reagent solutions (1 ml). These solutions were mixtures of either methanol or acetonitrile and water, with or without the addition of various inorganic and organic salts. The application volume along with the elution solvents were collected into polypropylene vials, scintillation fluid added, and the radioactivity eluted at the various stages determined. The data was then processed by plotting the cumulative percentage of compound eluted against the concentration of the active species in the eluent. From these elution profiles, an EC_{50} or EC_{90} was determined. These are the concentrations of the active species, either a cation or organic modifier, giving 50 or 90% cumulative elution, respectively. All experiments were carried out in duplicate and results presented are mean data. EC_{90} and EC_{50} values were obtained by fitting a curve through the experimental data using a four parameter logistic routine.

Results and Discussion

Demonstration of a cation-exchange mechanism

The evidence that a cation-exchange mechanism operates on reversed-phase extraction cartridges is mainly indirect. It is based primarily on the fact that once a basic compound has been isolated on a cartridge (C2 or C18) the cartridge can be repeatedly washed with pure methanol or acetronitrile, i.e. strongly eluotropic solvents, without eluting the basic solute. This data is in stark contrast to what is expected from experience with reversed-phase HPLC, where the use of pure organic solvents would result in rapid elution with minimal or zero retention. As an initial part of this investigation the experiment described above was repeated using the two basic beta-blockers propranolol and atenolol, which have pK_a values of approximately 9.5.

The two compounds were applied to C2 and C18 cartridges and sequentially eluted with aliquots of methanol in water (1 ml), where the concentration of methanol ranged from 0 to 100%, in 20% increments. The cumulative percentage of compound eluted was very low for all four combinations studied, less than 7% over the six wash solvents used. The worst case, involving the most polar compound (atenolol) and the most polar stationary phase (C2) where the reversed-phase interaction would be at its weakest, actually showed the lowest elution (0.27%). In further experiments involving more extreme conditions, atenolol and propranolol were eluted from C2 cartridges with six 1 ml aliquots of methanol. In this case, the cumulative elution was also minimal, never exceeding 13% overall.

The above data suggests that the basic solutes are being retained by a mechanism other than, or in addition to, the hydrophobic interaction. If this mechanism is cation-exchange, then inclusion of a suitable counter-ion (M^+) in the methanol–water eluent should bring about elution. Also, it may be expected that cations such as Cu^{2+} which have a greater affinity for the ion-exchange matrix would result in more facile elution than cations such as Li^+, which shows less affinity for ion-exchange matrices [13].

To investigate this hypothesis, propranolol was eluted from four different cartridges (C2, C18, CN and Si) using methanol–water (50:50, v/v) containing a range of salts covering the concentration range 10^{-5} to 10^{-1} molar. The inorganic cations, which were chosen to cover a range of elution strength were; H^+, Li^+, Na^+, K^+, NH_4^+, Cu^{2+} and Pb^{2+}. The organic cations tetrabutylammonium (TBA^+) and triethylammonium$^+$ (TEA^+) which are commonly used as HPLC and solid-phase extraction eluent constituents were also evaluated.

With the exception of TBA^+, which was in the dihydrogen ortho phosphate form, all the other compounds were the acetate salts. Acetate was selected as the counter anion since it has poor ion-pairing ability and hence the data were less likely to be confounded by other retention mechanisms. Also, acetate salts possess reasonable solubility in the hydro-organic eluents necessary to overcome the hydrophobic interaction. The pH of these

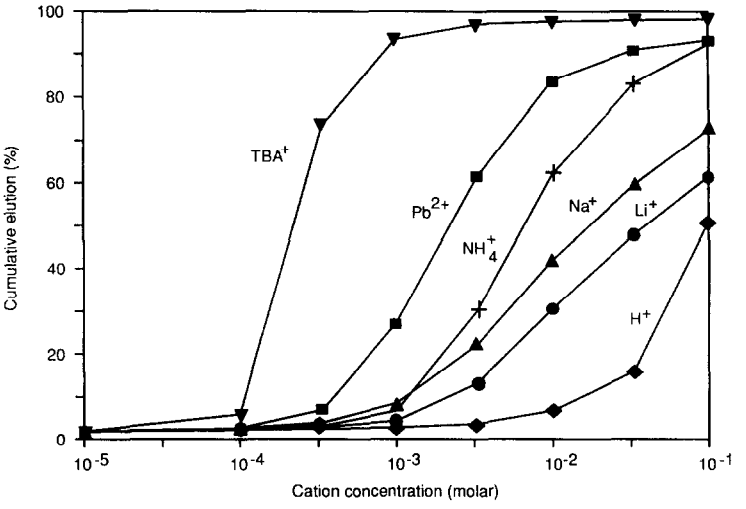

Figure 2
Cumulative elution profiles for propranolol eluted from C18 cartridges with a range of salts in 50% methanol.

solutions varied from 3.26 for acetic acid to 8.12 for sodium acetate with a mean pH of 6.23. A selection of elution profiles for propranolol on a C18 cartridge are shown in Fig. 2. As the concentration of any of the salt solutions is increased, the elution of the basic solute propranolol also increases. Furthermore, the ordering of the elution power of the inorganic cations is in general agreement with data quoted for classical ion-exchange chromatography [13] and is also similar to that quoted by Analytichem [14] for use with their sulphonic acid based cation-exchange Bond-Eluts.

The data for propranolol, where the greatest selectively difference between cations was observed are shown in Table 2. The major differences between this and published data are the reversal of position of Li^+ and H^+. This may be a function of the very low pH of the proton source, acetic acid, in comparison to the other solutions used, although some authorities, consider the strength of H^+ to be poorly defined [15]. The other difference is the reversal in the order of NH_4^+ and K^+, although again this position is less well defined with one source stating that these ions are of equivalent strength [16]. These data therefore are highly supportive of the view that a cation-exchange mechanism is operating.

Table 2
Cation-exchange selectivity or eluting power of various inorganic and organic cations measured in terms of their EC_{50} values

Cation	$EC_{50} \times 10^{-3}$ (molar)*
TBA^+	0.24
Pb^{2+}	2.24
TEA^+	2.64
Cu^{2+}	3.51
K^+	5.18
NH_4^+	6.74
Na^+	16.7
Li^+	35.7
H^+	93.5

Data for propranolol eluted from C18 cartridge with methanol–water (50:50, v/v) solutions.
*EC_{50} is the concentration of cation giving 50% elution of propranolol under the conditions described.

The ordering of cation-exchange strength or selectivity quoted in the literature (e.g. refs 13–15) only ever includes inorganic ions. In this present case, involving a mixed retention mechanism we have been able to show that the organic cations tetrabutylammonium$^+$ and triethylammonium$^+$ have eluting power comparable to, or greater than the classically considered strong inorganic ions such as Pb^{2+}.

The ordering of elution power was the same on all four cartridges, however, the differences were most pronounced on C18 with EC_{50} values ranging from 2.4×10^{-4} M to 9.35×10^{-2} M. On C2 cartridges the range was reduced to just over one order of magnitude. Most surprising however, the range of elution power on Si and CN was very much reduced with only the CN cartridge showing any difference in eluting strength for the cations studied, and then only 2.8-fold between the strongest and the weakest.

The above experiment was repeated using the more polar compound atenolol, where a reduced lipophilic interaction in comparison with propanolol would be expected to take place. In general, the trends observed previously were also seen with this compound. The selectivity difference or range of cation eluting strength also decreased across the series C18 > C2 > CN > Si. The poor selectivity with respect to the cations on the CN and Si cartridges was also reflected in the lack of selectivity between the two solutes. On the C18 cartridge the EC_{50} for atenolol and propanolol with a given cation differed by over 2 orders of magnitude, but on CN this was much reduced and on silica there was virtually no difference at all between atenolol and propanolol. The reason for this is unclear but it may be due to the large lipophilicity difference between atenolol and propranolol (approximately 3.3 log *P* units) which is emphasized on the C2 and C18 cartridges. The absence or reduced hydrophobic interaction on the polar CN or Si cartridges results in a loss of selectivity between compounds of differing lipophilicity.

As the main objective of this study was to maximize the degree of control and in particular the ion-exchange properties of the cartridges, C2 and C18, which appeared to offer the greatest selectivity, were selected for subsequent work.

Irreproducibility

Batch to batch irreproducibility of solid-phase extraction cartridges is well known amongst bio-analysts and has also been reported in the literature [17]. We selected four batches of C18 Bond-Eluts, which were tested with propranolol using ammonium acetate in methanol–water (50:50, v/v) as the elution reagent. There was a small but significant difference in the retentivity of the four batches studied with the EC_{50} for propanolol ranging from 2.5×10^{-3} M to 6.3×10^{-3} M. The reproducibility within a given batch (72212) however, was found to be excellent, with the relative standard deviation in the percentage eluted across the elution profile being <2% ($n = 7$). It is strongly recommended therefore that for large blocks of work, a single batch of cartridges is used. Also, the sensitivity of these cartridges to the effect of cations should be assessed before use.

Interplay of ionic and hydrophobic mechanisms

To give a clear understanding of the balance of the retention mechanisms involved, and how this related to the physico-chemical properties of the solutes, the range of compounds studied was enlarged to include all those shown in Fig. 1.

On the basis of the work carried out above, ammonium acetate was selected as the ionic reagent of choice for further work. This selection was based on the fact that ammonium acetate was a middle-ranking ion in terms of elution strength. It is widely used as a constituent of HPLC eluents and is unlikely to interfere with analysis, also, it is volatile, thus allowing preparative applications of solid-phase extraction, e.g. metabolite isolation. Fifty per cent aqueous methanol, being of intermediate polarity, was used as the solvent to overcome the hydrophobic interactions.

The compounds shown in Fig. 1 were applied to C2 and C18 cartridges and eluted with 50% methanol containing a range of ammonium acetate concentrations. The data for C2 and C18 cartridges are shown in Figs 3 and 4, respectively. These data show that the ammonium acetate in combination with 50% methanol solution was of insufficient eluotropic strength to give quantitative elution for all of the compounds. In particular, the more lipophilic solutes, ICI 169369 and tamoxifen, especially on the more lipophilic C18 cartridge, were highly retained suggesting that 50% methanol was insufficient to overcome the hydrophobic interaction with these compounds. This problem however, was resolved by increasing the methanol concentration in the eluent to 90% as shown for the C2 cartridge (Fig. 5). As expected, near quantitative elution for ICI 169,369 and tamoxifen was obtained, and the curves for all the other compounds shifted to the left also. A similar effect was also seen on the C18 cartridge, although even with 0.1 M ammonium acetate complete elution of tamoxifen was only just achieved. These data suggest that the relatively low concentration of ammonium is sufficient to overcome the ionic interaction and that final elution is brought about by the methanol overcoming the lipophilic interaction. A control experiment involving the application of atenolol to the C2 cartridge and washing with nine 1 ml aliquots of 50, 90 or 100% methanol was carried out. Under these extreme conditions, i.e. the most polar compound and the

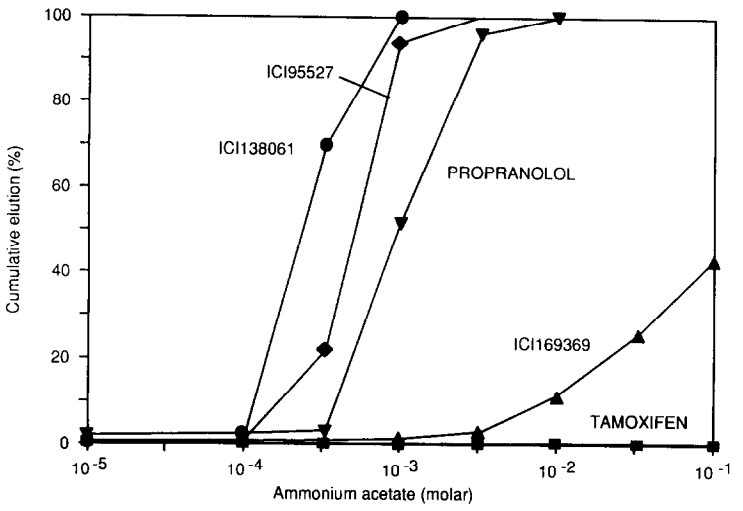

Figure 3
Cumulative elution profiles for a range of basic solutes eluted from a C2 cartridge with ammonium acetate in 50% methanol.

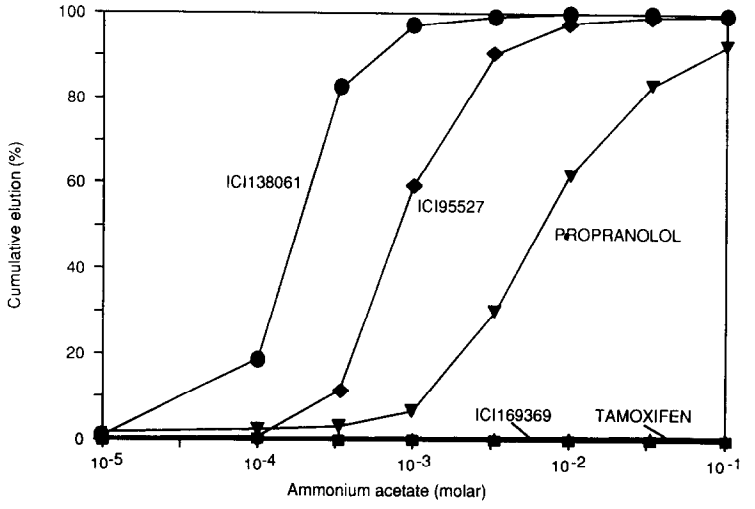

Figure 4
Cumulative elution profiles for a range of basic solutes eluted from a C18 cartridge with ammonium acetate in 50% methanol.

most polar stationary phase, a maximum of 20% elution was observed, showing the necessity of the ammonium acetate for quantitative recovery.

The above experiments suggest that the C2 cartridge is to be preferred to the more widely used C18 since it is capable of giving good recovery for drugs with a wide range of lipophilicity. In contrast, when used with very liphophilic drugs, e.g. tamoxifen, total elution from C18 may be difficult.

Prediction of elution conditions

The preceding work has shown how the ionic and reversed-phase mechanisms can be manipulated to allow fine tuning of the extraction procedure. To broaden the applicability of this approach and to facilitate rapid method development, further experiments were carried out with the aim of predicting the necessary elution conditions from the physicochemical properties of the solutes under investigation.

Quantification of reversed-phase effects

In these experiments the seven compounds previously studied were applied to C2 and C18 cartridges and sequentially eluted with meth-

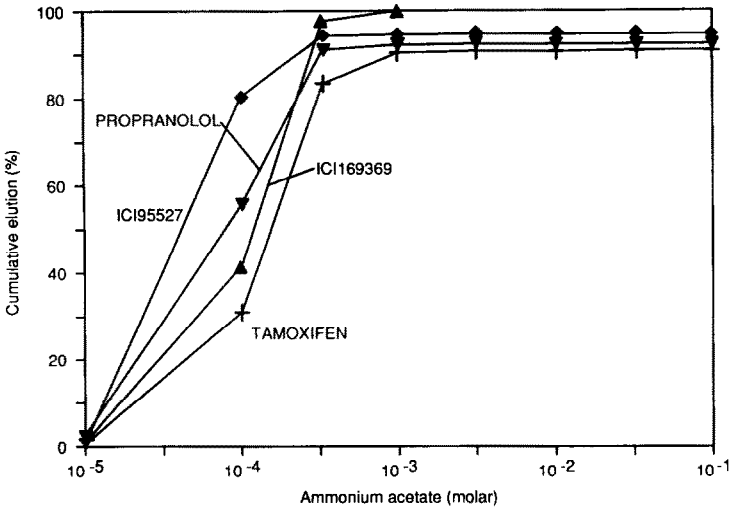

Figure 5
Cumulative elution profiles for a range of basic solutes eluted from a C2 cartridge with ammonium acetate in 90% methanol.

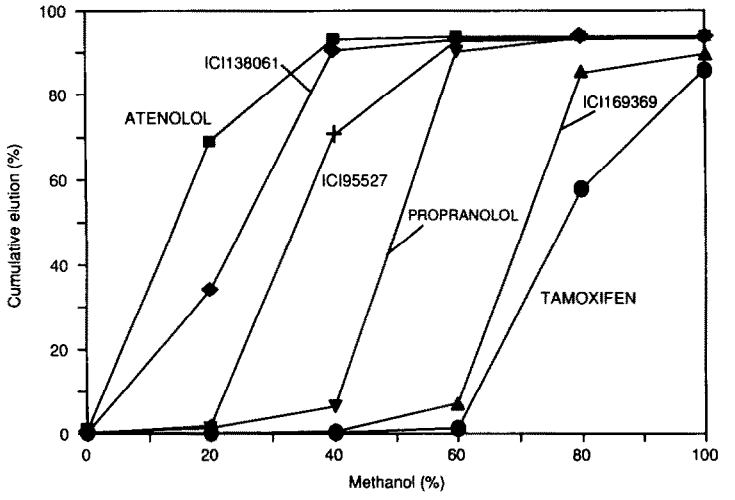

Figure 6
Cumulative elution profiles for a range of basic solutes eluted from C2 cartridges with methanol–water solutions containing ammonium acetate (0.003 M).

anol solutions (1 ml), ranging from 0 to 100% in 20% increments. The solutions contained ammonium acetate at either 0.003 or 0.1 M. The results were as expected for all four combinations studied in that the more lipophilic compounds required a greater percentage of methanol to achieve quantitative elution. However, as observed previously, recovery from the C18 cartridge was relatively poor for the lipophilic compounds even when using high methanol (100%) and high ammonium acetate (0.1 M) concentrations. A series of typical profiles for C2 with 0.003 M ammonium acetate are shown in Fig. 6. To quantify the effects observed, the concentration of methanol giving 50 or 90% elution (i.e. EC_{50} or EC_{90}) was determined and this was correlated with the solute log *P*. Generally, the best correlation was observed with EC_{90} values. The correlation with EC_{50} was poorer, probably because of the rapidly changing nature of the elution profile at this point.

The best linear correlation was obtained for the C2/0.1 M combination, with the equation

of the best fit line shown below (equation 1); the figures in parenthesis are the standard deviations of the slope and intercept.

$$\text{Methanol } EC_{90} = 10.2(\pm 1.5)\log P + 16.9(\pm 8.2) \quad (1)$$

$$r = 0.9494, \qquad n = 7, \qquad P < 0.01,$$

where r is the correlation coefficient, n is the number of data points and P is the significance level. This was also the only example where all compounds eluted totally and hence this did not involve extrapolation of the elution profiles to obtain EC_{90} data. This shows that there is a very good correlation between the percentage of methanol required for elution and the log P of the various compounds, with the latter parameter actually covering a very wide range, over 6 log P units. The methanol–aqueous ammonium acetate eluents used had pH values of approximately 6.7 as did the application and wash solvents. Under these conditions, all the solutes would be at least 95% ionized. It would be expected therefore that use of log D (the distribution coefficient) as opposed to log P, would provide a better correlation. The former parameter (log D) takes into account the degree of ionization and its effect on lipophilicity as opposed to log P which is the lipophilicity of the unionized molecule. Surprisingly however, the correlation was not improved ($r = 0.9390$) by the use of log D calculated for a pH of 6.7.

The data for the other combinations i.e. C2/0.003 M, and C18/0.1 M did not give such a good correlation, with r typically 0.925. This may in part be due to the fact that data for the lipophilic compounds, ICI 169369 and tamoxifen, which were not fully eluted, were obtained by extrapolation of the profiles.

The ability to predict the optimal conditions for elution of a compound from a solid-phase extraction cartridge (albeit from a given batch) could be of enormous benefit in certain areas of drug analysis such as the early stages of pharmaceuticals development and toxicological screening. In this type of work where it is common to analyse groups of compounds differing only in lipophilicity, it should be a simple matter to establish an EC_{90}–log P relationship and thus minimize the development time of optimized extraction methods.

Quantification of cation-exchange effects

It has been shown previously that in the chromatography of bases on silica where a cation-exchange mechanism operates, that retention is dependent on the solute pK_a [18]. It would be expected therefore that on C2 or C18 cartridges, providing there was a sufficiently high methanol concentration to overcome the reversed phase-effect, that cation elution EC_{50} values would be dependent on solute pK_a. Compounds with a low pK_a would be eluted by solutions with a low cation concentration whilst those with a high pK_a would require a higher ionic concentration to give similar elution.

In an attempt to quantify the ionic interaction the seven compounds were applied to a C2 cartridge, but in this instance they were eluted sequentially, in an initial experiment with 90% methanol in water containing an increasing concentration of ammonium acetate from 10^{-5} to 10^{-1} M, in concentration increments of one order of magnitude or less. Ninety per cent methanol was chosen for this experiment since other studies (data not shown) indicated that 50% methanol, even with a high concentration (0.1 M) of ammonium acetate, failed to elute the lipophilic compounds. Furthermore, to be able to observe a clear correlation with pK_a it was felt that a high methanol concentration was necessary to overcome the reversed-phase effects. Under these conditions however, the correlation between cation EC_{50} or EC_{90} values and pK_a was very poor or non-existent ($r < 0.12$). Surprisingly also, further analysis of the data showed a very good correlation between the cation EC_{50} values and solute log P, with $r = 0.9526$. The two compounds which showed the greatest deviation, atenolol and ICI 95527 we believe to be anomalous because of a significant hydrogen bonding type interaction, this phenomenon has been studied in more detail and is reported in Part II of this paper [19].

The lack of a correlation with pK_a in this experiment showed retention was still being dominated by reversed-phase effects. To overcome this, the experiment was repeated using ammonium acetate with pure methanol as the solvent. The data from this experiment, treated as previously gave a small improvement in the correlation of EC_{50} with pK_a and a small drop in the correlation with log P, suggesting superficially that the use of the higher methanol concentration had overcome, in part, the reversed-phase interaction. In

comparison to the experiment with 90% methanol however, the range of EC_{50} values in this experiment were much reduced, ranging from only 1.1×10^{-4} M to 1.7×10^{-4} M. The change in the correlation coefficients were therefore considered artifactual.

It is difficult to rationalize the reason for the lack of correlation with pK_a. A possible explanation is that even when using pure methanol as solvent the reversed-phase effects still dominate. The possibility of repeating the experiment with a more highly eluotropic solvent, e.g. ethanol or propanol, was discounted, because of the very narrow range of EC_{50} values expected, and also because these solvents could possibly suppress the ionization of the ammonium acetate, thus reducing the likelihood of an ionic interaction. It was also possible that at high concentration, the ionic constituent of the eluent was actually salting out the solute from the mobile phase thus increasing retention in opposition to the cation-exchange effect. As all cations, even the weakest Li^+, appeared to show some eluting power, it was not possible to carry out an experiment at constant ionic strength, with a view to studying this phenomenon.

Although we have been unable to demonstrate a correlation between ionic strength and solute pK_a, the ionic interaction appears to be a dominating force in terms of the elution of basic solutes even with pK_a values as low as 7.93.

Use of acetonitrile as eluent

The foregoing work was carried out with methanol as the organic component of the eluent. In practice, much solid-phase extraction work is carried out using acetonitrile as an eluent or wash solvent and it was considered of interest to evaluate this solvent in the present circumstances. In reversed-phase HPLC at least, acetonitrile is considered a stronger, more highly eluotropic solvent than methanol. All things being equal therefore, a change from methanol to acetonitrile would be expected to result in a shift of the elution profiles to the left, i.e. more facile elution.

Limited experiments were carried out using propranolol and atenolol with C2 and C18 cartridges. The cations chosen for these experiments were Li^+, NH_4^+ and TBA^+, i.e. ions covering the range of cation elution strength. The elution profiles produced in this work were, in general, similar to those generated using methanol as the solvent. With the exception of propranolol on C18 however, all the curves showed a shift to the right, implying that acetonitrile is less eluotropic than methanol. This surprising result is unlikely to be due to differences in the degrees of ionization of the salts in the two different hydro-organic mixtures, since Marko *et al.* [20] and Musch and Massart [9] have reported similar findings using unadulterated methanol and acetonitrile as eluents in solid-phase extraction. It is possible that the difference may be due to a hydrogen bonding type interaction which is mediated to a greater extent by methanol with its donor and acceptor properties in contrast to acetonitrile which only has hydrogen bond acceptor character.

A further possible explanation may be that the physico-chemical processes in HPLC, which is a continuous equilibrium process, are actually different to those in solid-phase extraction, which when used in the so called 'digital mode', involves discontinuous equilibria. Also, the manufacturing processes used for the production of the two types of silica are quite different, and this may lead to significantly different trace impurities and surface characteristics.

Overall, the results of these experiments suggest that there is little to choose between methanol and acetonitrile as eluting solvents. The slightly greater eluting strength of methanol, at least under the conditions used here, and the greater solubility of ammonium salts in methanol solution [21] make this the preferred choice as an eluting solvent. The use of acetonitrile as a wash solvent for the removal of non-cationic materials would also appear to be indicated.

Pre-conditioning of cartridges with ionic reagents

In the work described so far, the aim has been to utilize the ion-exchange properties of the silanols, rather than suppress them as has been attempted by some workers in solid phase extraction [9, 22], and as is widely practiced in HPLC. In certain reports where pre-conditioning with buffers has been employed, the exact make-up of the buffer, particularly with respect to the cationic component, has not been specified (e.g. refs 20, 23 and 24). In view of the data presented above, which showed very significant differences in the cation exchange strength, particularly between Na^+ and K^+,

which are common buffer constituents, a number of preliminary experiments were carried out.

As 0.1 M concentrations of cations in 50% methanol were found to be successful in terms of elution, these conditions were used for the initial pre-conditioning experiments. The exact procedure (for C2 and C18 cartridges) involved conditioning with methanol (1 ml) followed by the cation solution (1 ml, 0.1 M) in 50% methanol and finally water (1 ml). The test compounds, propranolol and atenolol in water (1 ml) were then applied to the cartridge. The application volume was collected and the cartridge washed with water (1 ml), 50% methanol (1 ml), and then methanol (2 × 1 ml). The radioactivity in the application and the various washes volumes was then determined and cumulative elution profiles generated.

Typical elution profiles for atenolol and propranolol on C2 cartridges are shown in Fig. 7(a) and (b), respectively. In contrast to cartridge conditioned only with methanol and water, conditioning with a salt solution allows elution of propranolol or atenolol with aqueous methanol or pure methanol. In general the ordering of the cations strength was similar to that the previously established, i.e. strong ions such as TBA^+ give more facile elution. The weak ion, H^+, gave minimal elution and was comparable to a control where the cationic conditioning stage was omitted. As expected, the more polar atenolol was more easily eluted, and the overall picture was very similar for the C18 cartridge. Although the

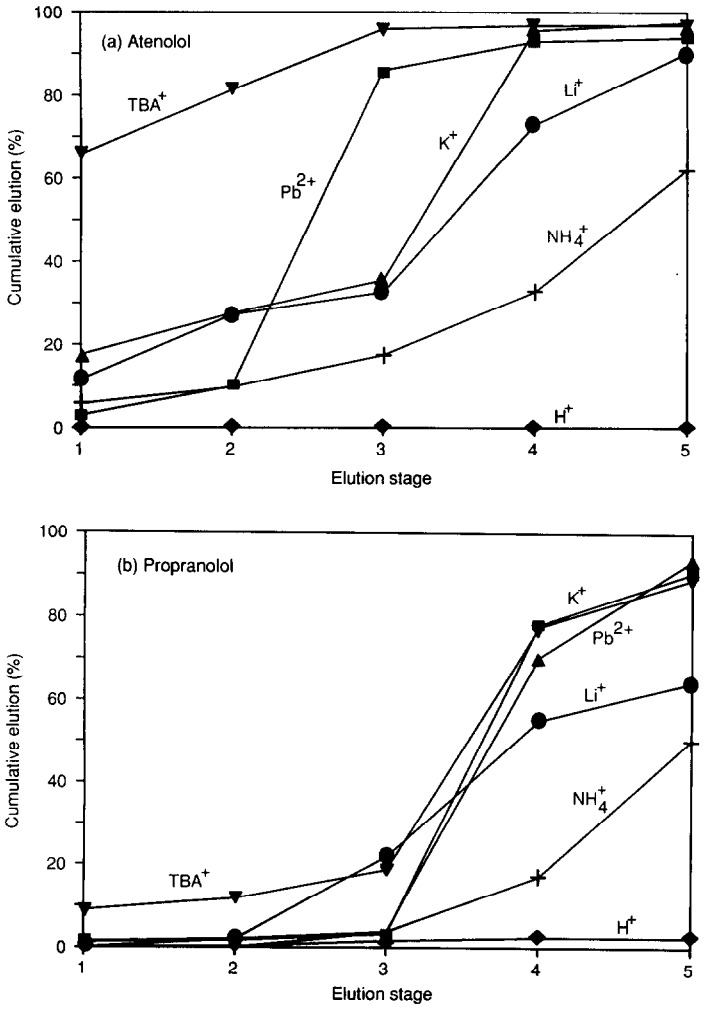

Figure 7
Cumulative elution profiles for (a) atenolol and (b) propranolol eluted from a C2 cartridge which had been pre-conditioned with a range of cations. Stage 1, application; 2, water wash; 3, 50% methanol wash; 4, first methanol wash; and 5, second methanol wash.

ordering of strength at the extremes was similar to the elution data (Table 2) the strengths of the intermediate ions were difficult to determine exactly due to the extreme non-parallel nature of the elution profiles. In general however the order, with one exception, was similar to that seen previously. The major difference was the relative position of NH_4^+, which under these conditions appeared weaker than the three alkaline earth metals which had relative strengths $K^+ > Na^+ > Li^+$.

A further interesting feature was the relatively low recoveries for propranolol (irrespective of cartridge) despite the two methanol washes. This suggested that blocking of the ionized silanols was not fully effective under the conditions used. The number of moles of silanols on the surface of the silica was calculated assuming a concentration of 7 μmol m^{-2}, a surface area of 600m^2 g^{-1} and 50% coverage of bonded phase, a typical figure for a bonded silica. This gave a value of 490 μmol of unbonded silanols for a 100 mg cartridge. As a 1 ml aliquot of a 0.1 M solution of the cationic reagent contains only 100 μmol of M^+, it would appear that there was insufficient cations to block every silanol present, assuming they were all ionized.

A second experiment was therefore carried out using three concentrations (0.01, 0.1 and 1.0 M) of two cations (Na^+ and NH_4^+) in the conditioning solution. The data for atenolol on a C2 cartridge (Fig. 8) shows more facile elution with increasing cation concentration. This supports the view that a cation mass of >490 μmole (i.e. 1 ml of 1 M solution) is necessary to block the ionized silanols on the cartridge and allow the stationary phase to act in a purely reversed-phase manner.

Further experiments were carried out to study the effect of the cation conditioning solvent. In this work two cations (Na^+ and K^+) were prepared at the sub-optimal concentration of 0.1 M in either methanol, 50% methanol or water and the cartridges conditioned as described above. The results of this experiment using propranolol as test probe are shown in Fig. 9. It can be clearly seen that the nature of the conditioning solvent has a profound effect on the ability of the cation to block the silanol groups. To allow the stationary phase to act in a purely reversed-phase manner, the application of the silanol blocking agent (K^+ or Na^+ in this instance) in methanol as opposed to water, would appear to be necessary. This is in marked contrast to normal conditioning procedures (e.g. refs 20, 23 and 24) where, although the reason for conditioning with buffer is not stated, the conditioning step is obviously non-optimal in terms of blocking silanols and probably represents a poorly controlled variable in the analytical procedure. The reason for the effect of the solvent is unclear but it may be related to the solvation state in the different media. In contrast to the situation in water, in methanol the more lipophilic hydration sphere of the

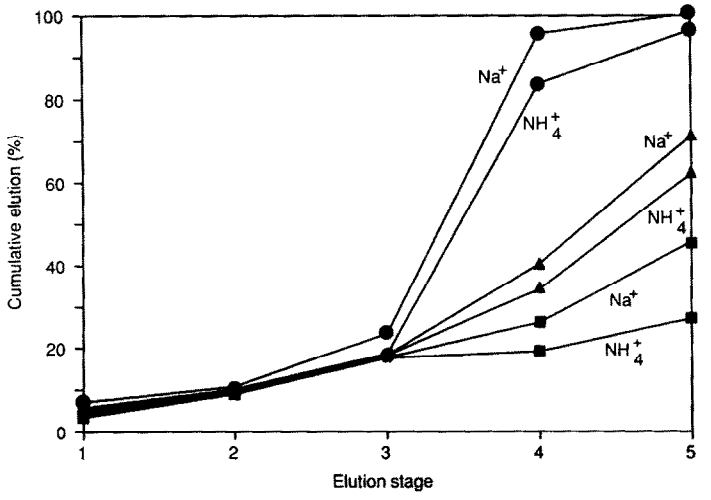

Figure 8
Cumulative elution profiles for atenolol eluted from a C2 cartridge which has been pre-conditioned with Na^+ or NH_4^+ in 50% methanol at three different concentrations: 0.01 M (■); 0.1 M (▲); and 1.0 M (●). Elution stages as Fig. 7.

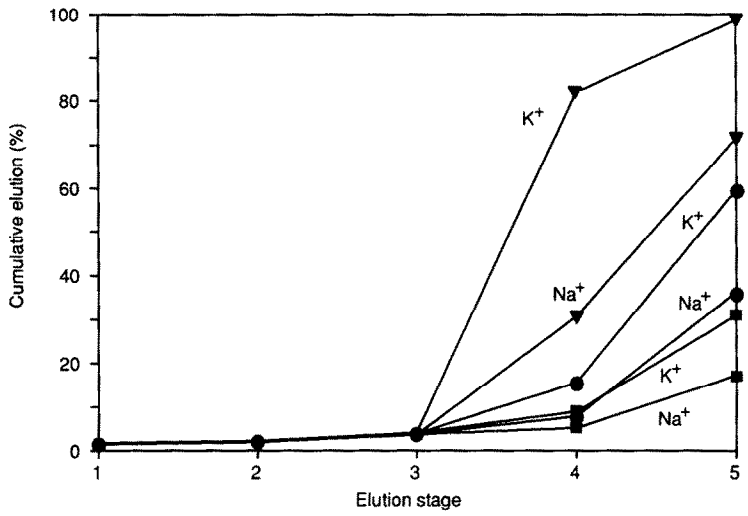

Figure 9
Cumulative elution profiles for propranolol eluted from a C2 cartridge which has been pre-conditioned with Na^+ or K^+ (0.1 M) in either methanol (▼); 50% methanol (●); or water (■). Elution stages as Fig. 7.

cation may allow greater penetration of the bonded phase and hence greater access to the silanols.

Conclusions

This work has clearly demonstrated that cation-exchange plays a very significnat role in the retention of basic compounds on reversed-phase extraction cartridges. For elution to take place it is necessary that both the reversed-phase and ionic interactions are overcome. This can be achieved through the use of aqueous methanol solvents containing a suitable competing cation. The ordering of the cation elution strength is comparable to that for classical ion-exchange chromatography. Furthermore, the organic cations TBA^+ and triethylammonium$^+$ have been shown to have eluotropic strengths comparable to strong inorganic ions such as Pb^{2+}.

Of the two reversed-phase cartridges studied, C2 is to be generally preferred to C18. On C18, the pronounced hydrophobic interaction with highly lipophilic compounds (log $P > 4$) can make elution difficult. In contrast to CN or Si cartridges, the combination of both the reversed-phase and cation-exchange mechanisms on the C2 (and C18) cartridge gives much greater control of selectivity. Although the use of CN cartridges may be of value in the development of general purpose methods [8, 9], for fine control of assay selectivity C2 and particularly C18 cartridges are preferred.

Using C2 with ammonium acetate in methanol–water, which is our system of choice, near quantitative elution could be obtained for all the solutes studied even over the wide log P range of approximately 6.5 units. Although the ionic effects are of major importance in governing retention, selectivity is best obtained by varying the eluent methanol–water ratio to suit the lipophilicity of the compound of interest. By reference to the solute log P it is also possible to predict, for a given batch of cartridges, the ammonium acetate concentration and the methanol–water ratio for optimal elution.

In contrast to reversed-phase HPLC, under the mixed mode conditions studied here, acetonitrile appears to be a weaker solvent than methanol. The former would appear to be more useful therefore as a wash solvent for the elimination of non-basic interferents.

Blocking of the silanols by pre-conditioning with cations was possible, the necessary conditions, however, involving high concentrations of salt solutions (1 M) and methanol as solvent instead of water, were different to those frequently employed in solid-phase extraction procedures. Apart from the low strength observed for the ammonium ion, the ordering of the cation strength was similar to that seen at the elution stage. These data suggest that more attention should be given to the conditioning stage of solid-phase extraction procedures. In particular, full details of all buffers used should be presented. Given the

differences in strength between sodium and potassium cations — common buffer constituents — it is less than satisfactory to merely describe a conditioning reagent as 'phosphate buffer'.

Overall the work described here should allow a more systematic approach to the development of solid-phase extraction procedures for basic compounds. The approach recommended can be adapted to give selective procedures for a single component or as a screening procedure for a group of compounds.

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[Received for review 24 June 1991; revised manuscript received 2 November 1991]