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Fundamental studies in reversed-phase liquid–solid extraction of basic drugs; II: hydrogen bonding effects

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Abstract: Anomalies in the elution order of the β-blockers atenolol and propranolol on reversed-phase liquid–solid extraction cartridges have been studied. The hydrogen bond acceptor properties of the polar ring substituent in atenolol was found to result in greater retention than would be expected from the reversed-phase or cation-exchange mechanisms alone. This hydrogen bonding interaction could be attenuated by inclusion of a strong hydrogen bond acceptor in the eluent or more successfully by increasing the eluent water content. The conclusions from this work have been used to explain previously reported anomalies in the solid-phase extraction of polar basic drugs.

Keywords: *Basic drugs; polar drugs; hydrogen bonding; liquid–solid extraction; solid-phase extraction; retention mechanism; extraction selectivity.*

Introduction

The use of reversed-phase extraction cartridges for the isolation of basic drugs is a complex process involving several retention mechanisms in addition to the obvious hydrophobic interaction. In Part I of this paper [1] data was presented which clearly showed evidence for an ion-exchange mechanism involving the protonated basic solutes and the ionized unbonded silanols on the silica surface. Further examination of the data indicated that other retention mechanisms were operating, giving rise to anomalies in elution order. A study of the literature also indicated unusual behaviour of certain solutes on solid-phase extraction, particularly the poor recovery of the polar drugs atenolol [2], sotalol [3] and labetalol [2, 3]. Taken together these data suggested some form of polar, hydrogen bonding type interaction between H-bond acceptor groups on the solute and unionized residual silanols.

These observations lead us to investigate more fully the possibility of a significant H-bonding effect in the isolation of basic drugs by solid-phase extraction with apolar sorbents. This work should add to our related studies [1, 4] in giving a better understanding of the mechanisms involved in solid-phase extraction processes. These data can then be used to aid the more rational and systematic development of drug isolation procedures.

Experimental

Materials and equipment

The equipment and reagents were as described in Part I [1]. Several additional reagents were used, which were obtained from a variety of sources. These were either of Analar grade or the purest grade available. ICI 40979 radiolabelled with ^{14}C to a specific activity of 1.21 μCi mg^{-1} and a radiochemical purity of >98% was obtained from the Radiochemistry Unit, ICI Pharmaceuticals. Carbon-14 radiolabelled phenol, specific activity 655 μCi mg^{-1} and radiochemical purity >98% was a gift from ICI Chemicals and Polymers Ltd, Billingham, UK.

Methods

The experimental procedures were essentially as described previously [1]. These involved application of an aqueous drug solution (1 ml) to a Bond-Elut cartridge (100 mg size C2 or C18) and then sequential elution with a series of reagent solutions (1 ml). These solutions were mixtures of either methanol or acetonitrile, and water, with the addition of ammonium acetate and/or H-bond acceptors. The application and elution volumes were collected and radioactivity determined. Cumulative elution profiles were then generated as described in Part I [1].

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Results and Discussion

Evidence for a hydrogen bonding interaction

The original indication that mechanisms other than reversed-phase and ion-exchange were operating came from an analysis of data for the basic beta-blockers propranolol and atenolol. The results for these two compounds eluted with increasing concentrations of ammonium acetate in 50% (v/v) aqueous methanol, from C2 and C18 cartridges, are shown in Fig. 1(a) and (b), respectively.

The data for propranolol (Fig. 1a) is as expected in that the more lipophilic C18 stationary phase results in greater retention than the more polar C2 phase. In contrast however, the order of elution for atenolol is in fact reversed, with the apolar C18 cartridge appearing the least retentive. Comparing results for a given cartridge type shows atenolol to elute much more easily than propranolol from C18. This can be rationalized by the fact that propranolol is highly lipophilic with a log P of 3.56 whereas atenolol is very much more polar with a log P of only 0.23. This difference in lipophilicity would be unaffected by pH since both compounds have very similar pK_a values of around 9.5. In contrast, on the C2 cartridge, atenolol appears to be more highly retained than would be explained by reversed-phase or ionic interactions alone.

Repeating the experiment with acetonitrile (50% v/v) as the organic modifier produced a shift to the right for all curves with the exception of that for propranolol on the C18 cartridge. The anomalously high retention of

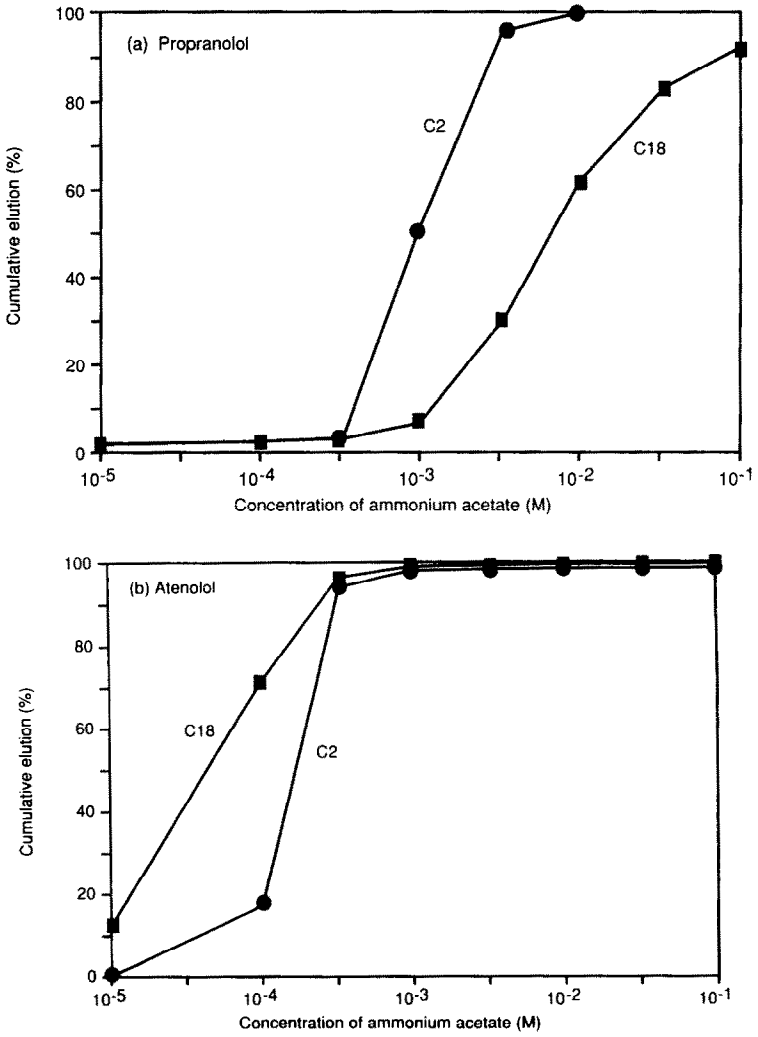

Figure 1
Cumulative elution profiles for (a) propranolol and (b) atenolol eluted from a C2 (●) and C18 (■) Bond-Elut cartridge with 1 ml aliquots of 50% methanol containing increasing concentrations of ammonium acetate.

atenolol on the C2 cartridge, however, was still apparent.

The major difference in structure between the two beta-blockers, and the reason for the great difference in lipophilicity (3.3 log P units) is the presence of the very polar amide function in atenolol. The polarity of functional groups in terms of their hydrogen bond donor and acceptor ability has been recently quantified by Abraham *et al.* [5]. On the basis of their data the phenylacetamide function of atenolol has a high acceptor value, log K_β = 3.0, on a scale of −0.4 to 4.2.

Since this is the major difference between propranolol and atenolol we theorized that the anomaly in retention was due to H-bonding between the amide carbonyl group of atenolol and exposed residual unbonded and unionized silanols on the surface of the C2 phase.

It would be expected that the C2 phase would show a greater potential for interactions involving unbonded silanols since the smaller C2 moiety in comparison to C18, would allow greater access to the silanols.

It has been shown previously [6] that H-bonding interactions in reversed-phase high-performance liquid chromatography (HPLC) are controlled to some degree by the polarity of the solvent medium. In highly polar, aqueous rich solvents hydrogen bonding between the solute and stationary phase is minimal, due to masking of the silanols by water. However, in organic rich solvents, H-bonding can become significant resulting in the frequently observed parabolic relationships in HPLC between log k' and the eluent mole fraction of organic modifier.

To test whether this phenomenon was operating here, the above experiment involving atenolol was repeated using 10 and 20% methanol as solvent, with the same ammonium acetate concentration as previous. With the reduced methanol concentrations, the elution curves shifted to the right, i.e. elution was more difficult, in accordance with the reduced reversed-phase eluotropic properties of the eluents (Fig. 2). More interestingly, however, was the change in relative positions of the curves with decreasing eluent methanol concentration. With 10% methanol (Fig. 2, broken line) a reversal of elution order occurred, so that atenolol now eluted from C2 more easily than it eluted from C18. These results and the previous work of Bij *et al.* [6] are indirect evidence for the presence of a significant H-bonding interaction on reversed-phase extraction cartridges.

Experiments to overcome the hydrogen bonding interaction

In Part I of this paper [1] the ionic interaction was overcome by inclusion of a competitive cation in the eluent. This type of approach was adopted here and the elution experiments with propranolol and atenolol were repeated with the addition to the methanol–water–ammonium acetate eluent of reagents with known H-bonding acceptor ability. These re-

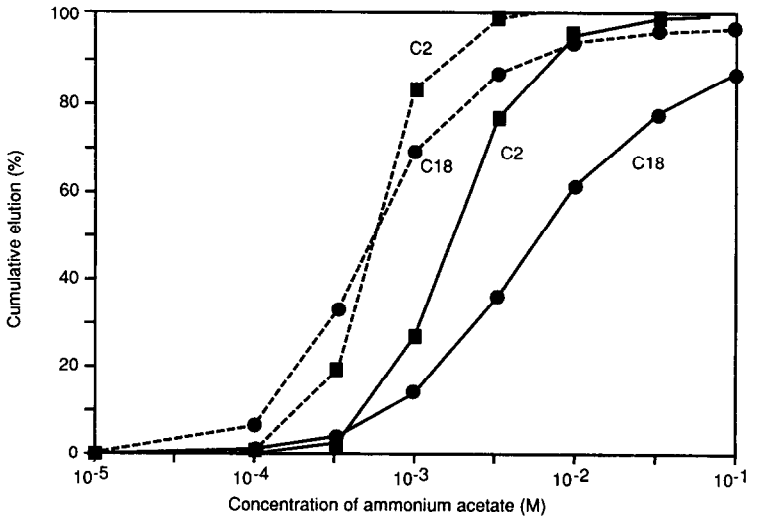

Figure 2
Cumulative elution profiles for atenolol eluted from C2 (■) and C18 (●) cartridges with 1 ml aliquots of 10% methanol (broken line) and 20% methanol (solid line) containing increasing concentrations of ammonium acetate.

agents included compounds which were strong acceptors, e.g. triphenylphosphine oxide (log K_β = 3.9), *N*,*N*′-dimethyl urea (3.2), dimethyl sulphoxide (3.0) as well as compounds with weaker acceptor ability, e.g. dichloromethane (−0.2), phenol (0.2) and acetone (1.6). All compounds were used at a concentration of 1% (m/v for solids and v/v for liquids) giving a typical molar concentration of 0.014 M. The results from these experiments were compared with a control which involved elution by 50% methanol containing ammonium acetate alone, as shown in Fig. 1. In general the use of the powerful H-bond acceptors resulted in a shift of the elution profiles for atenolol to the left, i.e. elution was apparently easier. In contrast and as expected propranolol, which does not possess the same H-bond donor ability as atenolol, showed very little or no change in the shape or position of its elution profiles.

Although consistent with an H-bonding interaction some of the data was anomalous. For example, the strongest H-bond acceptor triphenylphosphine oxide gave only a small effect with atenolol, and propranolol actually moved to the right indicating increased retention. Trifluoroacetone (log K_β = 0) [7] produced a very pronounced shift to the left in the profiles of both propranolol and atenolol, indicating more facile elution.

Although supportive of the H-bond interaction these data were not equivocal. Apart from having H-bond acceptor properties of varying degrees a number of the reagents used were also quite lipophilic, for example trifluoroacetone and triphenylphosphine oxide have log *P* values of 0.29 and 2.90, respectively, in comparison to methanol which has a log *P* of only −0.77. We felt therefore, that these reagents may have been affecting the reversed-phase interaction and possibly through some unknown mechanism affecting the ion-exchange interaction also.

In an attempt to demonstrate more clearly that H-bonding was taking place experiments were carried out using an additional solute; ICI 40979 (Fig. 3). This compound had no basic function, hence no ionic-interaction was possible, however, it possessed a strong H-bond acceptor in the form of the amide (log K_β = 3.0) similar to that for atenolol. The absence of an ionic interaction with this compound was first confirmed by the following experiments. ICI 40979 was eluted from C2 and C18 cartridges using solutions of increasing methanol concentrations with and without the addition of ammonium acetate (6×10^{-3} M). The cumulative elution profiles so generated were the same irrespective of whether ammonium acetate was included in the eluent or not, indicating absence of any ionic interaction.

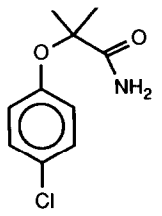

Figure 3
Structure of ICI 40979.

From the results of the above experiment, the concentration of methanol was determined which just failed to bring about elution. This was 20% for the C2 and 40% for the C18 cartridge. With these concentrations of methanol, we postulated that the reversed-phase interaction should be at or near a minimum and the H-bonding interaction, if present, should be at its most significant.

Elution profiles for ICI 40979 were then generated using the methanol solutions defined above, both with and without the addition of increasing concentrations of dimethylsulphoxide (DMSO) as an H-bond competitor. DMSO was chosen since it possessed a relatively high log K_β = 3.0, but was relatively polar with a log *P* of only −1.38, thus the potential for a reversed-type effect was reduced.

The elution profiles generated using the C2 and C18 cartridges are shown in Fig. 4. These clearly show the positive effect of an increasing concentration of the strong H-bond acceptor DMSO. Despite the relatively high concentration used however, the overall effect of DMSO was relatively poor. In comparison to the control, the addition of DMSO only increased elution by 2.5× in the case of the C2 cartridge. It is possible that the effect of the DMSO was attenuated somewhat by H-bonding to water, which has acceptor properties in the bulk state which are much greater than any of the reagents studied here. This would also explain why a reversal of the elution order for atenolol on C2 and C18 could be achieved by increasing the eluent water content, but no effect could be achieved with 10% DMSO in the eluent (data not shown). One surprising observation from Fig. 4 is the

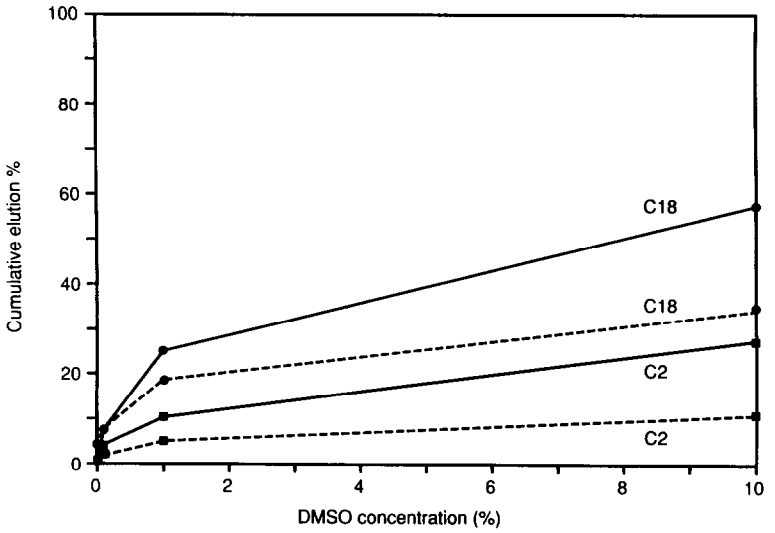

Figure 4
Cumulative elution profiles for ICI 40979 eluted from C2 and C18 cartridges with 1 ml aliquots 20 and 40% methanol, respectively. Solid line with, and broken line without increasing concentrations of DMSO.

apparent H-bond interaction on the C18 phase, where the access to the silanols may have been expected to be limited. This may in part, be due to the fact that elution from a C18 cartridge requires a higher methanol concentration, than does C2, and as noted above, H-bond interactions become more significant as the eluent organic modifier concentration is increased. Another possible reason may be due to a reversed-phase effect of DMSO. Although this H-bond competitor was chosen to minimize potential reversed-phase effects this type of interaction could not be totally ruled out. To confirm the H-bond theory and eliminate the possibility of a reversed-phase interaction a final experiment was carried out using ^{14}C-phenol in place of ICI 40979. Phenol, which has very poor acceptor properties (log K_β = 0.2), would be expected to be sensitive to the reversed-phase properties of DMSO but not the H-bond acceptor character.

Phenol was therefore applied to C2 and C18 cartridges, but because of the relatively low log P of the compound (1.46) but particularly because of the lack of secondary interactions, significant retention could only be obtained on the C18 phase. Following application to a C18 cartridge, phenol was then eluted with 5% (v/v) methanol both with and without added DMSO. The cumulative elution profiles were seen to be identical, thus showing DMSO to be free of significant reversed-phase eluotropic properties. These data therefore, prove that the interaction seen with atenolol and ICI 40979 is due to the H-bonding acceptor properties of these solutes.

Rationalization of other data

The observations reported here, we believe, also explain some unexpected results in other published work on solid-phase extraction involving polar solutes and reversed-phase cartridges. Musch *et al.* [3] experienced problems with low recovery of sotalol using a CN phase. Sotalol, a beta-blocker, has a methylsulphonamide substituent with moderate H-bond acceptor properties (log K_β = 1.4) attached to the aromatic ring. Both Musch *et al.* [3] and Leloux *et al.* [2] experienced problems with low recovery of labetalol using CN and C2 phases, respectively. Labetalol, like atenolol, which also showed poor recovery in the hands of Leloux *et al.* [2], has an amide with a high log K_β.

The drug amiloride was also shown by Musch and Massart [8] to have anomalously high retention on a CN phase which was inconsistent with its lipophilicity, assessed in their work by carbon number. The H-bonding ability of amiloride cannot be assessed exactly, due to the complex nature of the molecule and the potential for interaction between the various functional groups. The molecule however does possess two pyrazine nitrogens (log K_β = 1.4) and a carbonyl with a log K_β of 0–2 depending on the state of ionization of the guanidino function. It would appear therefore, that amiloride could also

undergo a H-bonding interaction. In these four cited examples [2, 3, 8] it is probable that the additional H-bonding interaction results in greater retention on the cartridge than would be predicted from lipophilicity alone. It is this H-bonding therefore which is responsible for the low recovery or high retention observed.

Conclusions

This work indicates that in addition to the obvious reversed-phase and cation-exchange mechanisms reported in Part I of this paper, H-bonding plays an important role in the isolation of basic drugs using nominally reversed-phase cartridges.

The evidence suggests that the more exposed surface of the C2, as opposed to a C18 phase will give rise to a greater interaction. This interaction however, cannot be manipulated as easily as the ionic or reversed-phase effects. Addition of a strong H-bond acceptor, e.g. DMSO to the eluent produces only small changes in elution behaviour, although some flexibility can be achieved by the variation of the aqueous–organic eluent ratio. The H-bonding interaction can be enhanced when elution solvents high in methanol or acetonitrile are used, and minimized when water rich eluents are employed. This control of the interaction could be used profitably to manipulate the selectivity of extraction procedures, providing one or other of the compounds of interest had H-bond acceptor properties. The main use of these data would appear to be the prediction of those compounds which will show non-ideal elution behaviour, and a rationalization of unexpected selectivity differences.

Drugs or other solutes containing strong hydrogen bond acceptors such as amide or sulphoxide groups would be particularly susceptible to this type of interaction, which would be manifested in unexpectedly high retention. An indication of which drugs may be susceptible to such effects can be obtained by consulting published data on functional group H-bonding acceptor values [5].

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