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A comparison of silica and alumina columns for high-performance liquid chromatographic separations of basic drugs and plasma constituents following on-line solid-phase extraction with column switching*

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Abstract: A comparison has been made of a silica and an alumina column with respect to retention behaviour in high-performance liquid chromatography of pure drug solutions and plasma profiles following on-line solid-phase extraction. Using methanol–ammonium nitrate buffer (80:20, v/v) as eluent, it was found that these systems lend themselves well to the column switching technique. Increasing the pH of the mobile phase produced similar shifts in retention for basic drugs on both columns, but fewer plasma interferents are retained on the alumina column as compared to the silica column under the same operating conditions.

Keywords: *HPLC; silica and alumina columns; column switching; on-line plasma extraction.*

Introduction

Reversed-phase chromatography, where the eluent is more polar than the solid support, has become the method of choice for a wide range of analytical applications, particularly in the field of biopharmaceutical analysis. It is now possible to produce a diverse array of bonded phase materials which can be tailored to meet a variety of analytical needs. In spite, however, of the advanced technology in this area, it is possible only to achieve some 50% coverage of the hydroxyl groups on the silica surface [1, 2]. Solute molecules containing an amine functionality interact electrostatically with these residual silanol moieties, which are acidic in nature [3], and are therefore ionized at neutral or basic pH. These ionic interactions between the residual acidic sites and oppositely charged protonated bases give rise to poor chromatographic efficiency, long retention times, and badly tailing peaks for basic compounds, (including drugs of therapeutic and forensic interest) on reversed-phase columns [4, 5].

Much attention has been paid to circumventing the problem of unreacted silanol groups through ion suppression [6], paired ion techniques [6, 7], or by the use of end-

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capping [8]. Bidlingmeyer *et al.* have demonstrated that it is not specifically the presence of residual silanol groups which produce poor retention characteristics for basic compounds, but their inaccessibility (resulting in poor mass transfer rates) following reaction with the alkylsilane used to modify the silica surface [9].

By employing an unmodified silica column, as first reported by Jane in 1975, [10] efficient separations have been obtained using buffered aqueous, methanol-rich eluents in a chromatographic mode than has since been described as "pseudo reversed-phase" [11]. The retention mechanisms involved in these polar separations are complex and multifunctional, but are known to consist, at least in part, of ion-exchange interactions [3, 9, 11, 12].

A problem with the use of bonded-, and to a greater extent unmodified silica materials [13], is their tendency to dissolve in aqueous media at high pH, particularly in solutions containing sodium salts. This problem may be largely overcome by the use of eluents containing a high proportion of methanol, where ammonium salts are the source of hydroxide ions [13, 14], and by locating a column between the pump and injector in order to pre-saturate the mobile phase with silica [14].

An interesting development, however, is the employment of an alumina column rather than a silica column as a cation-exchanger in this type of chromatography. Alumina has the advantage of being more stable than silica over a wider pH range (up to pH 12) [15], although silica has a much higher specific surface area — 500 $m^2 g^{-1}$ for neutral silica as compared to 70 $m^2 g^{-1}$ for basic alumina. A higher surface area means greater capacity for silica, and this results in higher capacity ratios and more predictable retention behaviour [16]. The separation of bases at high pH proceeds in much the same way on the two materials, though unlike silica, alumina is amphoteric, and thus has the ability to behave as a cation- or anion-exchanger depending on the pH of the surrounding medium.

The pH at which alumina is neutral and bears no charge is the zero point charge (ZPC). The ZPC depends on the method of production of the alumina and the nature of the buffer ion in the surrounding medium [17].

It has previously been shown that this type of chromatography using methanol-rich eluents could readily be coupled to on-line extraction techniques using a single- [12], or dual-pump column switching assembly [18, 19]. In the former case, the concentration (extraction) column was mounted in place of the injector loop, and clean-up of plasma samples was effected manually by injecting water and a series of weak methanolic solutions onto the concentration column. In the latter case, the concentration column is mounted independent of the injector and, a second pump is used to deliver the washing eluent. Switching between the two columns is achieved using a six-port valve, and in this way, the concentration column may be re-equilibrated while separation of the previous sample is completed on the analytical column.

The objective of the present study was to compare a silica and an alumina column in regard to retention characteristics of pure drug solutions and plasma profiles following extraction employing the column switching technique.

Experimental

Reagents and solvents

The drugs were a gift from the Institute of Clinical Pharmacology, Dublin, Ireland. Ammonium nitrate (reagent grade) was obtained from BDH Chemicals, Poole, UK, and

analytical grade ammonia solution (25%) from Riedel de Haen, Seelze, Hannover, FRG. HPLC grade methanol was obtained from Labscan Analytical Sciences, Dublin, Ireland. Deionized water was produced by passing distilled water through a Millipore Milli-Q water purification system. Dried human plasma from the Blood Transfusion Board, Dublin was dissolved in deionized water, and used within seven days of reconstitution.

Drug standards

Stock solutions equivalent to 1 mg ml^{-1} of the drugs in methanol were prepared. Working standards were made to 50–10,000 ng ml^{-1}, depending on the detector response of the drug.

Plasma standards

Aliquots of blank plasma were spiked with stock solutions to produce the required concentrations. These plasma solutions were then diluted [1 + 1] with deionized water, and 500 μl were injected through a loop onto a pre-concentration column for on-line solid-phase extraction prior to analysis.

Instrumentation and operating conditions

The columns used were a LiChrosorb silica 60, 5 μm column (250 × 4.6 mm ID), and a Techsphere alumina 5 μm column (250 × 4.6 mm ID), both supplied by HPLC Technology, Macclesfield, UK. Stock solutions of ammonia and ammonium nitrate (both 1 M) were mixed to produce the required pH. The resulting solutions were diluted with deionized water to produce the desired ionic strength. Mobile phases were made by mixing the aqueous component with methanol to produce solutions containing 80% organic phase. The mobile phase was passed through a 0.45 μm filter and delivered by a Waters (Milford, MA, USA) Model 501 HPLC pump. The drugs were detected by UV absorption at 254 nm using an Applied Chromatography Systems (Macclesfield, UK), Model 750/11 fixed wavelength detector, with a sensitivity setting of 0.02 AUFS. The resulting chromatograms were recorded with a Linseis (Selb, FRG) recorder at a chart speed of 200 mm h^{-1}.

For direct injection, 20 μl aliquots of drug solutions were introduced into the chromatographic system. For the purposes of column switching, a second Waters model 501 HPLC pump and the concentration column were connected to the analytical assembly via a Rheodyne (Cotati, CA, USA) 7000 six-port, two-position switching valve. The 10 × 1.5 mm ID concentration columns were dry-packed with Corasil (Waters Associates) RP-18 material. The second pump eluent was deionized water, filtered and degassed prior to use.

Column switching procedure

The instrument arrangement used in this part of the study is shown in Fig. 1. The spiked plasma sample is introduced via the injector port, and swept onto the concentration column by water from pump A. The drug compounds are selectively retained by the packing material in the column while the endogenous plasma components are eluted to waste. In the meantime, the mobile phase eluent is being passed by pump B through the analytical column, which is thus maintained in a state of constant equilibration. Upon switching the valve the mobile phase flow is diverted in a

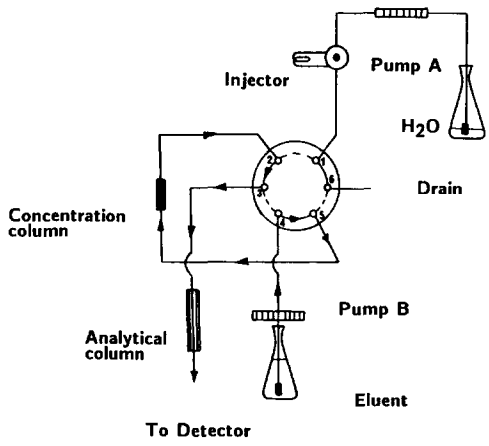

Figure 1
Column switching arrangement.

backflush mode via the concentration column where it desorbs the drugs and sweeps them onto the analytical column for separation.

Results and Discussion

It is known that anions tend to absorb onto alumina causing a negative shift in the zero point charge (ZPC), and that the magnitude of this shift depends on the nature of the anion present in the surrounding medium. Other workers have demonstrated that in presence of acetate ions the ZPC occurs at *ca* pH 6.5, and in the presence of citrate ions, it is as low as 3.5 [17]. As ammonium nitrate was the salt used in the present study, the ZPC of alumina in the presence of nitrate anions was estimated by measuring the retention of both a positively and a negatively charged solute over the pH range 5.5–10.0. The mobile phase used was a wholly aqueous mixture of ammonium nitrate and ammonia where the cation (NH_4^+) concentration was kept constant. The negatively charged benzoate ion (pK_a = 4.2) was strongly retained below a pH of about 7.0, and unretained at higher values, while the opposite was true for the positively charged tyramine (pK_a = 10.2) (Fig. 2). These findings indicate that the ZPC of alumina in the presence of nitrate ions occurs at pH 7.3 (±0.3), and that below this value it behaves as an anion exchanger, and above this value as a cation exchanger.

The capacity ratios and pK_a values for some of the basic drugs investigated on silica and alumina columns using mobile phases where the aqueous component was buffered to pH 7.0 and 9.5 are shown in Table 1. As may be seen, there is, as the pH is increased, a general increase in retention for the more basic amines while retention is seen to decrease for the compounds of lower pK_a values. Except for a few exceptions, this trend is common to both columns. These findings may be explained in terms of the degree of column and solute ionization. As the pH of the surrounding medium is increased, the anionic character of the stationary support is enhanced due to increasing dissociation of ionic moieties on the column surface. For the more basic compounds, the greater negative charge facilitates more solute–column interaction, and thus, these compounds are retained longer. On the other hand, the less basic drugs elute earlier because they rapidly become less ionized as their pK_a values are exceeded with increasing pH. Furthermore, common to both silica and alumina is a pronounced retention shift for the

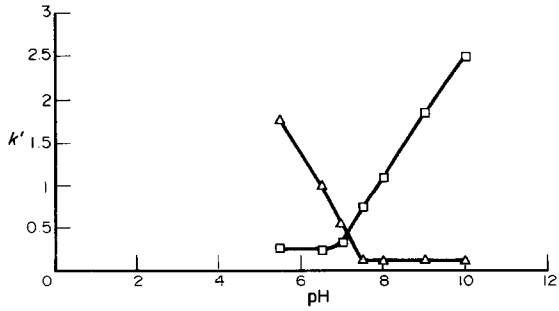

Figure 2
Estimation of the zero-point charge of the column using nitrate ions in the mobile phase. Mobile phase: 0.1 M ammonium nitrate–ammonia. Key: □ Tyramine; △ benzoic acid.

Table 1
Effect of pH on capacity factor for some basic drugs

Drug	pK_a	Silica		Alumina	
		pH 7.0	pH 9.5	pH 7.0	pH 9.5
Amitriptyline	9.4	3.0	5.8	1.4	4.1
Atenolol	9.6	1.6	5.9	1.1	1.8
Acetopromazine	NA	2.5	5.6	2.6	3.0
Chlorpromazine	9.3	3.7	5.1	3.4	3.0
Diltiazem	NA	0.7	1.0	1.6	0.4
Desipramine	10.2	1.6	9.8	3.2	8.0
Fluphenazine	8.1	1.4	1.1	3.2	0.8
Imipramine	9.5	3.6	6.6	4.4	5.4
Lignocaine	7.9	0.7	0.4	0.8	0.2
Mefloquine	NA	3.5	7.7	4.8	6.4
Mepivacaine	7.7	0.9	0.4	0.8	0.2
Nortriptyline	10.0	1.9	8.3	3.2	7.6
Perphenazine	7.8	1.5	1.1	3.6	1.2
Phenylpropanolamine	9.5	1.0	3.6	1.6	5.8
Pindolol	9.7	1.0	3.6	1.1	1.6
Propranolol	9.5	0.8	4.0	1.4	2.4
Protriptyline	10.0	1.6	8.9	2.6	6.7
Trimethoprim	7.2	0.6	0.4	2.0	0.5
Verapamil	NA	1.6	1.4	1.0	1.5

NA = Not available in standard reference texts.
Mobile phase: (0.05 M ammonium nitrate–ammonia)–methanol (20:80, v/v).

strongly basic (i.e. pK_a = 10.0 or greater) tricyclic drugs, protriptyline and desipramine, whereas this effect is less manifest for compounds of lower basicity. This correlation between retention shift and pK_a is, however, not absolute, as evidenced by the fact that pindolol, which has a pK_a of 9.7, is less affected by increased eluent pH than amitriptyline which has a pK_a of 9.4. It is believed that factors other than simple ion exchange govern retention in these systems; in fact, it is known that the siloxane bridges on silica are hydrophobic, and there is evidence to suggest that hydrophobic interactions involving solutes and these siloxane bridges play a significant, if lesser, role in controlling retention [9].

Of particular interest in this study was the coupling of column switching technology to these systems in order to facilitate on-line solid-phase extractions of plasma followed by

chromatography on bare silica and alumina. The objective was to compare the two columns in terms of plasma profiles using methanol-rich eluents containing ammonium nitrate, and to determine whether drug-free plasma profiles were as profoundly affected by the pH shift as the pure drug solutions.

The instrument arrangement used in this part of the study is shown in Fig. 1, and its operation described in the Experimental section. The 10 × 1.5 mm ID concentration columns were dry-packed in-house with Corasil RP-18 material. We have previously found [18], that octadecyl material retained less plasma components during the wash phase than octyl(C_8) material, a finding which may be attributed to the more hydrophobic packing having less affinity for the relatively polar endogenous plasma constituents. Water had been established as a suitable solvent for the wash phase, and based on previous work in this area [20, 21], a wash time of 1 min at 1.5 ml min^{-1} was deemed a suitable regimen for the removal of plasma while retaining the compounds of interest. Sample chromatograms generated from this study are presented in Fig. 3. The results demonstrate that, under the same mobile phase conditions, there are generally less interferences originating in the plasma matrix seen on the alumina column than on the silica column. At the higher eluent pH, all interfering peaks have eluted after about 3.6 min from the alumina column, whereas there are later eluting peaks seen on the silica column under the same conditions. When the mobile phase pH is lowered to 7.0, two small peaks appear later in the chromatogram on alumina, though the major plasma constituents continue to elute early. There are no differences between the drug-free plasma profiles on silica at the two pH values, though the baseline is marginally more stable at the higher pH.

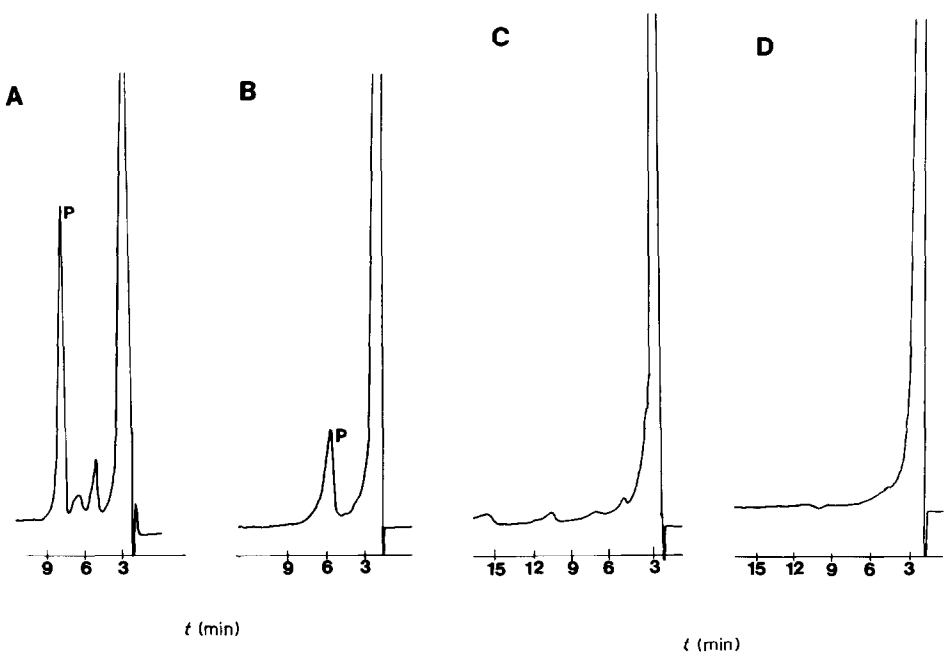

Figure 3
Blank plasma chromatograms on silica or alumina following solid-phase extraction. A: Silica, pH 7.0; B: silica, pH 9.5; mobile phase: (0.05 M ammonium nitrate–ammonia)–methanol (20:80, v/v); C: alumina, pH 7.0; D: alumina, pH 9.5; mobile phase: (0.05 M ammonium nitrate–ammonia)–methanol (20:80, v/v).

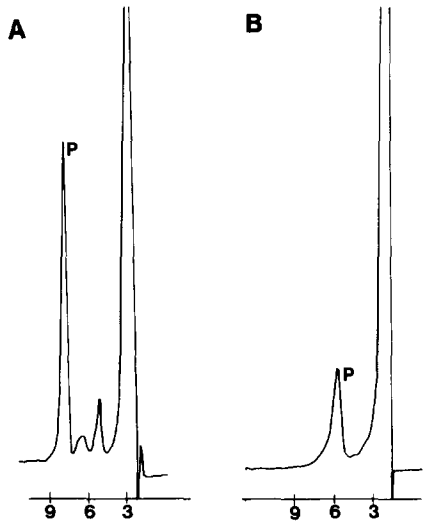

Figure 4
Spiked plasma chromatograms on silica and alumina following solid-phase extraction. A: Propranolol (P) on silica; B: propranolol on alumina; mobile phase (0.05 M ammonium nitrate–ammonia pH 9.5)–methanol (20:80, v/v).

The chromatograms of plasma samples spiked with propranolol (250 ng ml^{-1}), and subjected to the same pre-treatment and analytical procedures as the drug-free aliquots described above, are shown in Fig. 4. They show that it is possible to effect a good separation of a drug from plasma components on both columns by judicious choice of pH of the aqueous component of the mobile phase. This is presumably because plasma components are not sufficiently ionized under the employed experimental conditions to partake in ionic interactions with the alumina or silica columns. On reversed-phase systems, where solute–column interactions are hydrophobic rather than ionic in nature, plasma interferents frequently pose a problem to the analyst engaged in biopharmaceutical studies. An important example is caffeine, which can interfere with drug analysis in reversed-phase systems, but because it does not ionize, it will not interact with either alumina or silica by the principal mechanism of retention, i.e. ion-exchange, and as a result would elute too early to interfere with any of the compounds of interest.

Conclusions

It has been shown in this and other studies that silica and alumina columns with methanol-rich buffered aqueous eluents can be applied to the separation of basic compounds of medicinal interest. In this study, the effect of increasing eluent pH from 7.0 to 9.5 was seen to produce the same effect for most drugs on both columns, and that where there was an increase in capacity factor, it was most manifest on the silica column. Both columns were coupled to a column-switching assembly in order to facilitate on-line plasma extraction. The results show that fewer plasma interferents appear on the alumina column, especially at the higher pH. Evidence suggests that within both systems there is wide scope to manipulate the mobile phase pH, which is a major factor controlling retention, with little danger of introducing unwanted peaks from the plasma matrix.

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