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Retention reproducibility of basic drugs in high-performance liquid chromatography on a silica column with a methanol–high-pH buffer eluent

Effect of operating conditions on separations using an organic buffer

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ABSTRACT

Basic drugs can be separated by high-performance liquid chromatography on silica using a methanol–aqueous pH-10 buffer eluent prepared from 3-(cyclohexylamino)-1-propanesulphonic acid and sodium 3-(cyclohexylamino)-2-hydroxy-1-propanesulphonate. The buffer could be reproducibly prepared. The effects of small changes in the buffer and eluent composition and in the operating temperature on the relative retentions of the different groups of drugs were determined.

INTRODUCTION

Basic drugs often cause problems in high-performance liquid chromatography (HPLC) because of interactions with the stationary phase. In reversed-phase HPLC poor peak shapes are often seen unless a deactivating agent, such as an aliphatic amine, is added to the eluent[1,2]. An alternative approach has been to use silica as an ion-exchange material with eluents containing high proportions of methanol and either perchloric acid[3,4] or high-pH buffers[5–9].

Jane[5] reported that retention was primarily controlled by analyte pK_a and stereochemistry. Subsequently, Bidlingmeyer *et al.*[10] suggested that the silica column also showed marked hydrophobicity and that the order of retention of related compounds often resembled that on reversed-phase HPLC. They attributed this effect

to the presence of siloxane groupings. To test these findings Law[11] examined 69 monobasic aryl alkylamines using an aqueous methanol eluent at pH 9.1. He showed that there was a linear relationship between the retention times of the amines and the reciprocal of the ionic strength and concluded that cation exchange was the predominant mechanism of retention and the separation was primarily controlled by eluent pH. Although there were deviations from the expected linearity these were small and the proposed hydrophobic mechanism was ruled out. Non-polar compounds were effectively unretained. There was only a fair correlation between pK_a and capacity factors. The sizes of the substituents were important but this was difficult to rationalise. Law and Chan[12] have also confirmed the long-term stability of silica columns towards mixed aqueous–organic eluents at high pH.

Lingeman *et al.*[13] have shown that the retention of amines is predominantly controlled by the pH of the eluent. They also found that as the proportion of modifier increased the retention of the amines initially decreased and then increased again at high proportions. This effect was attributed to the solvation of organic competing ions.

In recent studies Cox and Stout[14] have looked in detail at the retention of ionic compounds on silica using "pseudo-reversed-phase" conditions. They used a limited set of test compounds and concentrated on the pH ranges 2.1–7.0 and 15–75% methanol. They also observed a minimum capacity factor at approximately 50% organic modifier and found a linear relationship between reciprocal ionic strength and capacity factors. However, the curve showed a positive intercept suggesting that a second retention mechanism was effective in addition to the ion-exchange mode. This extra effect depended on the method of preparation of the silica.

Schmid and Wolf[15] examined a group of tricyclic antidepressants at pH 7.9 and also suggested that some hydrophobic interaction was present. They noted that at low buffer concentration the systems could be unstable. They found that as the pH increased from 4 to 10 the retentions of primary and secondary amines were affected more than tertiary amines. Above pH 10.0 the capacity factors of all three groups of amines decreased and this was attributed to a reduction in the degree of protonation of the bases. They claimed that there was little difference in selectivity between different brands of silica but the chromatograms in the paper showed significant changes.

Over the past few years, work in our laboratories on the development of robust and reliable methods for the analysis of basic drugs on silica columns by HPLC has examined the use of a methanol–aqueous ammonia–ammonium nitrate eluent[6–9]. The reproducibility of the experimental conditions[6] and the stationary phase[8] has been determined and the conclusions have been tested in national[7] and international collaborative studies[9]. Within a single laboratory good reproducibility could be obtained under controlled conditions[8], but in interlaboratory studies[7,9] the variations were much larger and it appeared that the method was very sensitive to changes in the operating conditions. Two areas of particular concern were the column temperature and the differences in concentrations of the ammonia stock solutions used to prepare the buffer. Although the pH appeared to remain unaltered, significant changes in the ionic strength would result from changes in the ammonia concentration[8,9]. It was concluded that this eluent lacked the required reproducibility for the analysis of basic drugs and it was therefore decided to examine alternative buffer systems, preferably those which could be made up by weight from single-component bases.

In an initial study, ethylenediamine was examined[16] and found to be better than ammonia. However, solid buffer components were still considered preferable. The present paper describes separations carried out using buffers prepared from non-volatile organic sulphonic acid amine buffer components. The effects of changes in the operating conditions on the selectivity and retentions were examined, and the robustness of the method with respect to the buffer composition was studied.

EXPERIMENTAL

Chemicals

Sodium 3-(cyclohexylamino)-2-hydroxy-1-propanesulphonate (CAPSO-Na), and 3-(cyclohexylamino)-1-propanesulphonic acid (CAPS) were obtained from Sigma (Poole, U.K.). Sodium nitrate was analytical-reagent grade and methanol was HPLC grade, both from FSA Laboratory Supplies (Loughborough, U.K.). Water was reagent grade, purified on site using a Millipore Liquipure water purification system. The drug samples were obtained from the reference collection of the Central Research and Support Establishment of the Home Office Forensic Science Service.

Buffer solution

The buffer solution was prepared by mixing CAPS (0.8852 g) and CAPSO-Na (1.0372 g) in water and making up to 50 ml.

HPLC separations

The HPLC separations were performed using a Pye Unicam 4010 pump and a Pye Unicam 4020 UV detector set at 254 nm. The eluent consisted of methanol–buffer (90:10, v/v) and was pumped at 2 ml min^{-1}. The samples (1 μl) were injected using a 7125 Rheodyne valve, fitted with a 20-μl loop, onto a Shandon column (25 cm $\times$ 5 mm I.D.) packed with Spherisorb S5W (5 μm, batches 5123 and 5493/1; Phase Separations, Queensferry, U.K.). The temperature of the analytical column was maintained at 30°C using a circulating water bath. The system was fitted with a pre-column (3 cm $\times$ 5 mm I.D.) filled with open-column-grade silica sieved to 60 mesh. The retention times were determined using a Hewlett-Packard 3390 integrator or a Shimadzu Chromatopac C-R3A integrator.

Test solutions of basic drugs

A set of nine solutions of basic drugs, which have been described previously[9], was used in the study. With the second column, solutions G, H and I were replaced by solutions K, L, M and N below.

Compositions in mg ml^{-1} in ethanol–water (90:10,v/v):

(K) Papaverine, 0.036– dipipanone hydrochloride, 0.82– methdilazine hydrochloride, 0.07– protriptyline hydrochloride, 0.24.

(L) Procaine hydrochloride, 0.044– promazine, 0.04– ethoheptazine citrate, 7.32– protriptyline hydrochloride, 0.40.

(M) Codeine phosphate, 3.20– L-phenylephrine hydrochloride, 1.05– protriptyline hydrochloride, 0.22.

(N) Nortriptyline hydrochloride (used as a secondary standard), 0.16– strychnine hydrochloride, 0.13.

CALCULATIONS

The retention times (t_R) were measured in duplicate and the capacity factors (k') were calculated as $k' = (t_R - t_0)/t_0$, where t_0 is the retention time of methanolic sodium nitrate (solution I). Relative capacity factors were calculated as k'/k'_p where k'_p is the capacity factor for protriptyline present as an internal standard. Solution N contained nortriptyline as a secondary standard whose k' value from solution C was used to determine the relative k' of strychnine.

RESULTS AND DISCUSSION

Because it was difficult to control the concentration of ammonia stock solutions used in the buffer, it was considered necessary to devise an alternative method for the analysis of basic drugs on silica. In developing the new method, the following desirable properties were identified based on experience gained from the use of the ammonia eluent. The buffer solution must be easy to prepare reproducibly and should not contain any volatile components. It should have a similar pH to that used in the ammonia system (pH 10.1)[7] but the eluting power of the eluent should be weaker than that of the ammonia eluent so that weakly retained compounds can be resolved from the solvent front. It should also extend the overall retention times and thus increase the discrimination capacity. However, the retention of the longest retained compounds should not be excessive and unduly extend the analysis time.

Trials using buffers based on the liquid base ethylenediamine[16] and on ethanolamine hydrochloride (unpublished) gave acceptable results but failed to meet all of the criteria. So the present study concentrated on potential solid organic buffer components of high pK_a including 2-(N-cyclohexylamino)ethanesulphonic acid (CHES, $pK_a = 9.3$), CAPSO-Na ($pK_a = 9.6$) and CAPS ($pK_a = 10.4$). A range of buffers of different composition and pH were prepared and examined. Buffers prepared from ethanolamine hydrochloride and CHES with a lower pH were found to be unsuitable because they gave poor peak shapes and long retention times.

Combinations of CAPS and CAPSO-Na gave buffer solutions of high pH, in the region of 9.6–10.4. In initial studies using a simplified test set of drug compounds (from ref. 8) a buffer containing the two compounds in a 1:1 molar ratio, 0.1 *M* for each component, gave reasonable retention times (protriptyline 10.6 min), longer than those on the ammonia system, but the efficiencies of some compounds were very low and their peak shapes where poor. On increasing the concentration of CAPSO-Na in the aqueous buffer to 0.2 *M*, giving a 2:1 molar ratio of CAPSO-Na/CAPS, much better results were obtained, although phenylephrine still exhibited low efficiency.

Since the results for these eluents seemed to be promising, a further set of experiments was performed in which an extended test set of drug solutions[9] was used. Six separations were carried out using buffers varying in composition from 1:2 to 4:1 CAPSO-Na/CAPS and with different overall ionic strengths. The eluents giving the best results were those prepared from a buffer with a calculated pH about 10.0 and ionic strength 0.075–0.080 *M*. If the pH was lower (1:2, pH 9.79) the later peaks were too highly retained (*e.g.* protriptyline, 15.6 min; strychnine, 16.70 min) whilst buffers of higher pH (4:1, pH 10.43) caused more rapid elution (protriptyline, 7.75 min) and thus reduced the resolution of the earlier eluting drugs.

TABLE I

CAPACITY FACTORS AND RELATIVE CAPACITY FACTORS USING THE CAPS/CAPSO-Na ELUENT AND THE AMMONIUM NITRATE ELUENT IN THE CHROMATOGRAPHY OF BASIC DRUGS ON A SILICA COLUMN

Conditions: column, Spherisorb S5W (batch 5123); eluent methanol–aqueous CAPS/CAPSO-Na buffer (each component 0.08 *M*) (90:10, v/v); temperature 30°C.

Compound	*Ionisation constant*[a]	*Capacity factor*		*Relative capacity factor (× 100)*[b]	
		CAPS/CAPSO-Na	*Ammonia*[c]	*CAPS/CAPSO-Na*	*Ammonia*[c]
Nitrazepam	3.2,10.8	0.22	0.02	2.7	1.3
Diazepam	3.3	0.25	0.02	3.1	1.3
Papaverine	6.4	0.31	0.06	3.9	2.6
Caffeine	14.0	0.41	0.10	5.1	5.0
Dextropropoxyphene	6.3	0.56	0.09	7.0	4.5
Cocaine	8.6	0.71	0.11	8.9	6.0
Procaine	9.0	0.81	0.17	10.2	8.8
Amitriptyline	9.4	1.44	0.39	18.0	19.9
Chlorpromazine	9.3	1.53	0.44	19.9	22.4
Propranolol	9.5	1.66	0.44	20.8	22.5
Imipramine	9.5	2.13	0.60	26.7	31.1
Dipipanone	8.5	2.32	0.45	29.0	22.9
Promazine	9.4	2.56	0.75	32.0	38.5
Phentermine	10.1	2.60	0.61	32.5	31.4
Codeine	8.2	2.64	0.91	33.0	46.6
Morphine	8.0,9.9	2.69	0.96	33.7	49.7
Amphetamine	9.9	2.72	0.69	34.1	35.6
Phenylephrine	8.9,10.1	3.51	1.24	43.9	63.8
Pholcodine	8.0,9.3	3.53	1.23	44.2	63.4
Prolintane	9.7[d]	3.89	0.93	48.6	47.7
Ethoheptazine	8.5	4.03	1.19	50.0	61.1
Nortriptyline	9.7	4.32	1.19	54.1	60.9
Methdilazine	7.5	4.36	1.32	54.6	67.9
Ephedrine	9.6	4.62	1.35	57.8	69.5
Pipazethate	n/a	4.64	1.07	58.1	54.9
Methylamphetamine	10.1	5.61	1.54	70.2	79.1
Protriptyline[e]	10.0	8.00	1.94	100.0	100.0
Strychnine	2.3,8.0	8.75	2.71	109.3	139.5

[a] From ref. 17 (n/a = not available).
[b] Relative capacity factors relative to protriptyline.
[c] Data taken from ref. 7.
[d] pK_a unpublished value from Boehringer Ingelheim.
[e] Based on test solution H. pK_a from ref. 15.

From these conclusions a buffer of pH 10.0 containing the two compounds in a 1:1 molar ratio at 0.08 *M* for each component was chosen for a more detailed study (Table I) as it gave better efficiencies than a more concentrated 1:1 molar buffer (0.1 *M* for each component) (*e.g* ephedrine, plate number $N = 3497$ compared to $N = 2573$ and prolintane, 3718 compared to 2957). The eluent had a good UV range, with an absorbance < 1 at 215 nm and the retentions of the drugs ranged from 1.60 to 13.06 min.

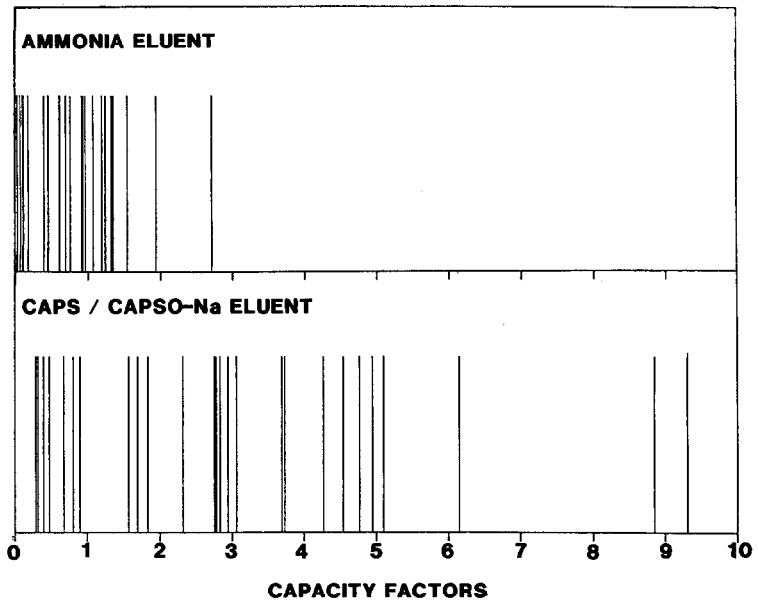

Fig. 1. Comparison of capacity factors using the ammonia–ammonium nitrate buffer (upper) and the CAPS/CAPSO-Na buffer (lower) showing the improvement in resolution and discriminating power with the latter eluent.

Comparison of the results for this eluent and the previous ammonia system showed that the present eluent gave an increase in retention time for all the drug compounds. This greater separation of the basic drugs (Fig. 1) would improve the resolution and thus enable better discrimination between similarly retained compounds, aiding more positive identification. The capacity factors and relative retentions with the two eluents differed significantly particularly for moderately retained compounds (relative capacity factors of 30–50) (Table I).

The changes in relative retentions caused some compounds to be eluted relatively more rapidly in the new system including imipramine, 26.7 (ammonia system, 31.1); promazine, 32.0 (38.5); codeine, 33.0 (46.6); morphine, 33.7 (49.7); phenylephrine 43.9 (63.8); pholcodine 44.2 (63.4); ethoheptazine, 50.0 (61.1); and strychnine, 109.3 (139.5). Other basic drugs were relatively more highly retained, including cocaine, 8.9 (6.0); dipipanone, 29.0 (22.9); prolintane, 48.6 (47.7); and pipazethate, 58.1 (54.9). These changes reflect those caused by decreasing the ionic strength of the buffer in the ammonia system[7], when the retentions of the last four compounds all increased whereas the earlier compounds decreased. There is no correlation with the pK_a of the analytes; dipipanone and ethoheptazine, both pK_a 8.5 behaving in a markedly different manner.

These results therefore contrast with the earlier studies[11,14,15], where, except at very low ionic strengths, there was generally no change in the relative order of retention with the strength of the buffer. However, a wider range of structural types is being examined in this study.

The development of the new eluent was carried out on Spherisorb S5W (batch

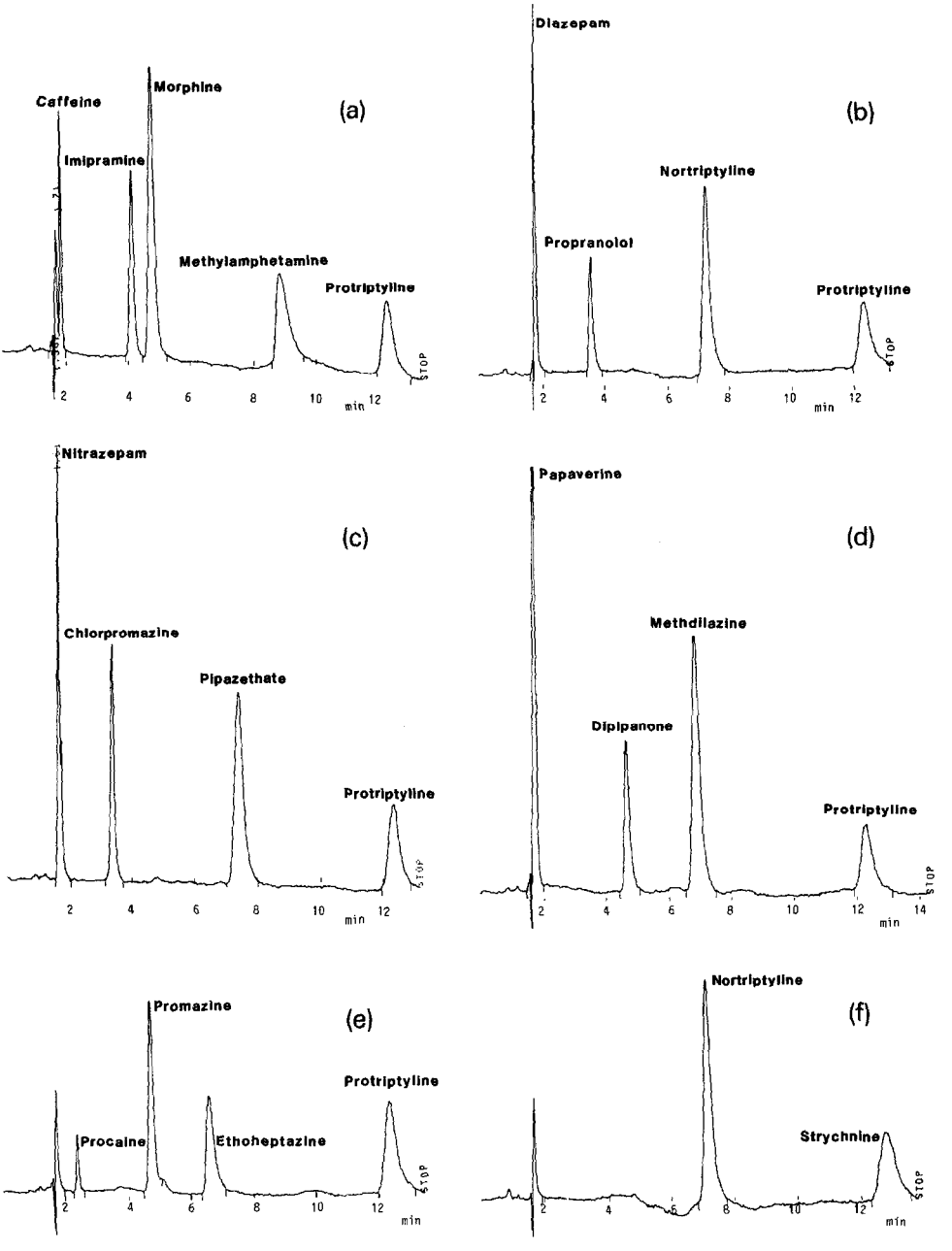

Fig. 2. Examples of separations of basic drugs on silica using the CAPS/CAPSO-Na eluent. Conditions: column: 25 cm × 5 mm I.D. packed with Spherisorb S5W (batch 5493/1); eluent: methanol–aqueous CAPS/CAPSO-Na buffer (90:10, v/v); buffer composition: each component 0.08 *M*; flow-rate = 2 ml/min; temperature: 30°C; detection wavelength = 254 nm. (a) Solution A; (b) solution C; (c) solution E; (d) solution K; (e) solution L; (f) solution N.

5123) but when the method was transferred to a new column containing a different batch of Spherisorb S5W (batch 5493/1) significantly different results were obtained and some of the components of the mixtures were unresolved. Strychnine was now unresolved from the internal standard protriptyline, whilst codeine and dipipanone in solution G co-eluted. Consequently the test mixtures G, H and I were replaced by mixtures K, L, M and N with nortriptyline as a secondary standard for strychnine in solution N (see Experimental). Good separations were now observed for all the test compounds, and examples are shown in Fig. 2.

TABLE II

REPRODUCIBILITY OF THE CAPACITY FACTORS AND RELATIVE CAPACITY FACTORS FOR THE SEPARATION OF BASIC DRUGS ON A SILICA COLUMN

Five repeated separations; column Spherisorb S5W (batch 5493/1); eluent, methanol–aqueous CAPS/CAPSO-Na buffer (each component 0.08 *M* (90:10, v/v); temperature 30°C.

Compound	*Capacity factor*			*Relative capacity factor (× 100)*[a]		
	Mean	*S.D.*	*C.V.*	*Mean*	*S.D.*	*C.V.*
Nitrazepam	0.29	0.00	–	3.3	0.1	3.0
Diazepam	0.32	0.01	3.1	3.7	0.1	2.7
Papaverine	0.40	0.01	2.5	4.5	0.1	2.7
Caffeine	0.48	0.01	2.1	5.5	0.2	3.6
Dextropropoxyphene	0.68	0.02	3.0	7.7	0.0	–
Cocaine	0.81	0.03	3.7	9.2	0.1	1.1
Procaine	0.90	0.03	3.3	10.1	0.1	1.0
Amitriptyline	1.57	0.05	3.2	17.8	0.2	1.1
Chlorpromazine	1.67	0.05	3.0	18.9	0.2	1.1
Propranolol	1.83	0.06	3.3	20.7	0.1	0.5
Imipramine	2.32	0.07	3.0	26.3	0.2	0.8
Codeine	2.75	0.10	3.6	31.1	0.2	0.6
Promazine	2.77	0.10	3.6	31.3	0.2	0.6
Dipipanone	2.78	0.14	5.0	31.4	0.5	1.6
Morphine	2.83	0.09	3.2	32.1	0.3	0.9
Phentermine	2.94	0.11	3.8	33.3	0.2	0.6
Amphetamine	3.06	0.12	3.9	34.6	0.1	0.3
Phenylephrine	3.69	0.14	3.8	41.7	0.2	0.5
Pholcodine	3.73	0.15	4.0	42.2	0.2	0.5
Ethoheptazine	4.27	0.17	4.0	48.2	0.2	0.4
Prolintane	4.27	0.18	4.2	48.4	0.3	0.6
Methdilazine	4.54	0.17	3.7	51.3	0.3	0.6
Nortriptyline	4.77	0.17	3.6	54.0	0.3	0.6
Pipazethate	4.94	0.22	4.5	55.8	0.4	0.7
Ephedrine	5.10	0.19	3.7	57.7	0.2	0.4
Methylamphetamine	6.15	0.24	3.9	69.6	0.2	0.3
Protriptyline[b]	8.85	0.38	4.3	100.0	–	–
Strychnine	9.31	0.35	3.8	105.2	0.7	0.7

[a] Relative capacity factors relative to protriptyline.
[b] Based on test solution L.

Effect of changes in the operating conditions

The robustness and reproducibility of the method was determined by varying the experimental parameters temperature, flow-rate, injection volume, and buffer composition. These included five runs using the selected standard conditions to monitor the reproducibility over a period of time (Table II). This included two eluents prepared from one batch of buffer solution, and three eluents prepared from a second batch of buffer.

The coefficient of variation (C.V.) in capacity factors was about 4% and, except for the rapidly eluting compounds, the variation in relative capacity factors was much lower (Table II) although dipipanone stood out as being poorer than other compounds with similar retentions. In previous studies with the ammonia eluent this compound was particularly sensitive to changes in experimental conditions[7]. The variations in retention were much smaller than the difference between the results on the two columns and suggested that batch-to-batch variations in the silica cause significant effects on retention in a similar manner to the differences observed with the ammonia[8,9] and diamine eluents[16].

In this series of separations the retention times and capacity factors showed a consistent downward drift with each subsequent analysis, although the relative capacity factors remained consistent. Inspection of the column at the end of the series of experiments revealed a 1-mm void at the top, indicating that the analytical column was slowly dissolving or being etched by the eluent. It is possible that the drift in retention was related to the dissolution of the silica during the study. A silica pre-column was being used between the pump and the injector to extend the column lifetime and its presence would appear to be essential, despite the study by Law and Chan[12] which found dissolution to be negligible.

To investigate the effect of small pH changes in the buffer on the separation of the drugs, buffers of pH 9.7 and 10.3, with ionic strengths equal to that of the standard buffer (0.080 *M*), were tested. For all of the analytes the retention times decreased on going from low to higher pH, which is probably caused by a reduction in the degree of protonation of the bases as observed earlier by Schmid and Wolf[15]. However, some of the bases were affected more than the others but for most of the drugs, the relative capacity factors also decreased as the pH was increased (Fig. 3). Particularly large decreases were observed for dipipanone (36.05 to 28.13), prolintane (52.41 to 45.25), pipazethate (61.64 to 51.43) and methylamphetamine (74.53 to 67.30). However, the pK_a values of these compounds are similar to those of many of the other drugs (Table I). The steric environment of the basic groups appears to be an important factor as the first three of these compounds all contain a cyclic tertiary amine with a substituted N-alkyl side chain. As noted earlier these three compounds also showed particular sensitivity to changes in separation conditions. In contrast, tertiary amines containing only N-methyl substituents, such as methdilazine and cocaine, showed much smaller effects relative to protriptyline which is also an N-methyl compound. In his study Law[11] had found that size of alkyl substituents had a marked effect. The introduction of N-methyl groups caused positive retention changes whereas larger alkyl substituents had a negative effect on retention. These effects may suggest that the larger substituents on a cyclic amine may limit the interaction of the basic group to a particular type of silanol site on the silica surface whose ionisation changes to a different extent than the other silanol groups with changes in eluent pH.

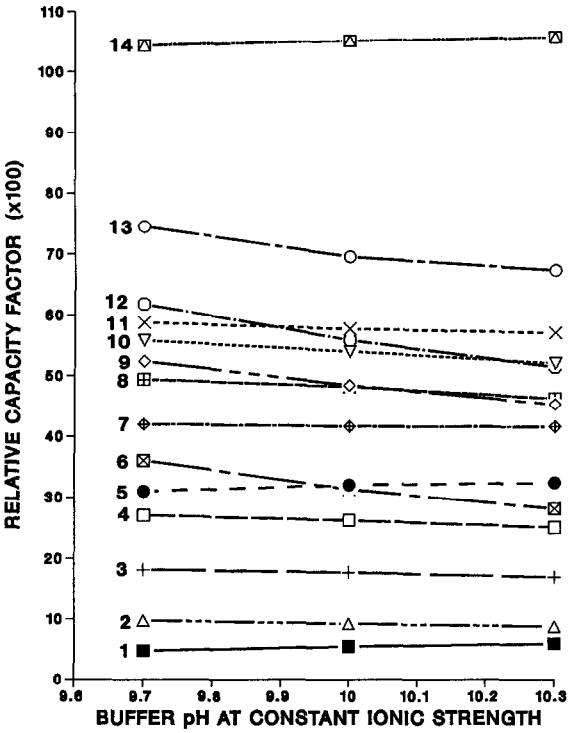

Fig. 3. Variation of relative capacity factors with pH. Conditions as in Fig. 2, but ratio of buffer components varied to give different buffer pH values at constant ionic strength. Compounds: 1 = caffeine; 2 = cocaine; 3 = amitriptyline; 4 = imipramine; 5 = morphine; 6 = dipipanone; 7 = phenylephrine; 8 = ethoheptazine; 9 = prolintane; 10 = nortriptyline; 11 = ephedrine; 12 = pipazethate; 13 = methylamphetamine; 14 = strychnine.

The relative retentions increased for a few compounds, including strychnine, codeine and morphine (31.03 to 32.40, pK_a 8.0 and 9.9). In the last case this might reflect the ionisation of the phenolic group to give a doubly charged species although phenylephrine which also contains a phenolic group changed very little. These relative changes were significant as a test of the robustness of the assay and emphasise the need for a constant buffer pH to obtain reproducible results. The lower pH also caused many of the compounds to elute with a lower efficiency but the higher pH reduced the efficiency of protriptyline. Clearly, although systematic changes with pH have been observed for small sample sets such as the tricyclic antidepressants[15] or aryl alkylamines[11], the resulting conclusions cannot be generalised to account for the relative changes observed in the present larger range of sample types.

The ionic strength of the present eluent was much lower than the previous ammonia eluent and this was a major factor leading to an increase in retention times. These changes agree with the predominant mode of retention being cation exchange. The effects of changes in the buffer concentration of $\pm$ 20% were examined by using buffers with ionic strengths of 0.096 and 0.064 *M* at a constant pH of 10.0. Most of the compounds showed a decrease in retention time on increasing the ionic strength, with

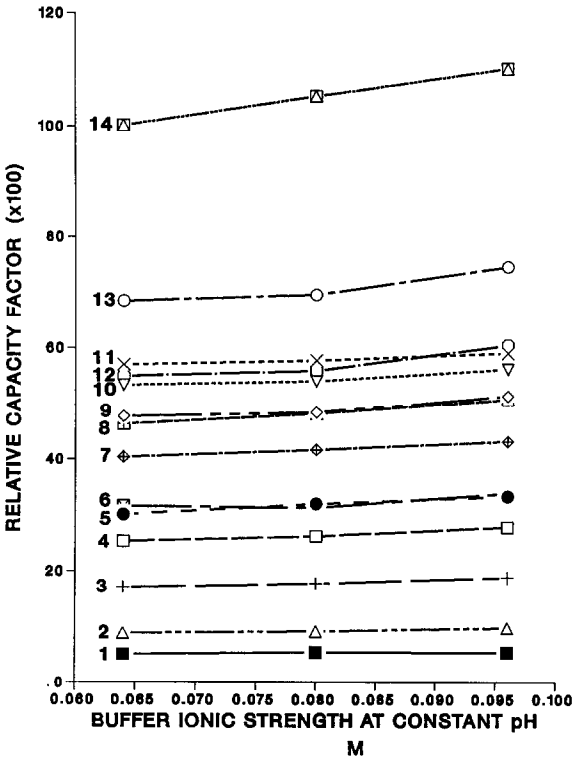

Fig. 4. Variation of relative capacity factors with ionic strength. Conditions as in Fig. 2, but ratio of buffer components varied to give different buffer ionic strengths at constant pH. Compounds as in Fig. 3.

protriptyline showing a quite significant change (13.4 min decreasing to 12.6 min) but strychnine (13.4 min to 13.7 min) and pipazethate (7.42 to 8.19 min) increased slightly. For most compounds the relative capacity factor increased slightly with some compounds showing a more marked effect (Fig. 4). The changes in the relative capacity factors were outside the experimental range for the repeated assays and suggest that small changes in the buffer could have a significant effect on an analysis. Again the compounds most affected were those which also markedly changed with pH.

The retention times of the drugs decreased as the temperature increased from 20 to 40°C, but for most of the compounds the relative capacity factors increased with increasing temperature. The increases were proportionally more significant for the weakly retained compounds (relative $k' < 10.1$, see Fig. 5). For the rest of the compounds the changes were $\pm$ 4%. Exceptions to this trend were methylamphetamine and strychnine which both showed decreases in relative capacity factor with increasing temperature, whilst pipazethate, methdilazine and ephedrine did not exhibit any obvious trend. The relative capacity factors recorded at 20 and 40°C were outside the range of experimental error determined for the five standard assays. Thus to obtain reproducible results it is necessary to thermostat the column and to specify the temperature in any description of the method.

When the proportion of methanol in the eluent was changed from 90 to 88 or

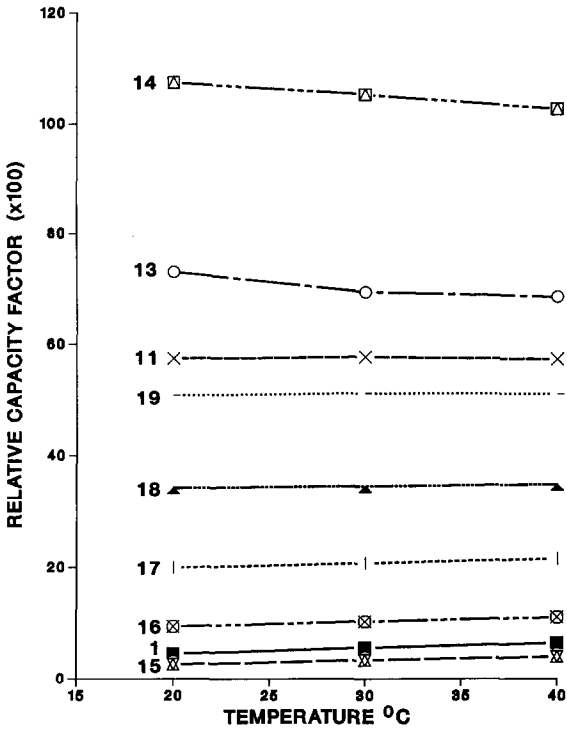

Fig. 5. Variation of relative capacity factors with temperature. Other conditions as in Fig. 2. Compounds as in Fig. 3, plus: 15 = nitrazepam; 16 = procaine; 17 = propranolol; 18 = amphetamine; 19 = methdilazine.

92%, variations in retention times and relative capacity factors were observed. Increasing methanol content caused an increase in the retention times but a very small change in the relative capacity factors for most compounds. Decreasing the methanol content had a more significant effect and the relative capacity factors were between 1.8 and 12% higher than the standard results. Thus care will be needed in the preparation of the eluent.

In a recent study Tanaka *et al.*[18] reported large changes in retention on changing the operating pressure in the column; these were ascribed to changes in the equilibria. An ion-exchange chromatographic separation might therefore be susceptible to similar effects, particularly if the drug is partially ionised. On changing the flow-rate from 2.0 ml min^{-1} to 1.0 ml min^{-1}, which changed the operating pressure from 145–148 bar to 76–78 bar, the capacity factors and relative capacity factors were unaffected.

In a case of a real-life sample submitted for analysis the concentration of the drug present in the solution will be unknown. Variations in relative capacity factors caused by changing the loading of the analyte on the column may therefore cause problems in identification. A sample of solution L was diluted to 20% of its original concentration and four replicate 1-μl injections were examined. The retention time of protriptyline was unaltered but the retentions and relative capacity factors of the test compounds were slightly increased. The changes were small and were less than two standard deviations (from Table II) for procaine and promazine and about three standard deviations for ethoheptazine.

CONCLUSIONS

This study has shown that the organic buffer salts CAPS and CAPSO-Na can be used to prepare reproducible buffer solutions for use in the analysis of basic drugs on silica. These eluents gave an increase in retention time and a better discrimination than the methanol–ammonium nitrate eluent.

The new method is susceptible to changes in the operating conditions and these parameters would need to be closely specified in the method protocol. Increases in the operating temperature or the ionic strength of the eluent, or a decrease in the proportion of the buffer caused the relative capacity factors to increase, whilst increasing the pH of the eluent or the proportion of methanol caused the relative capacity factors to decrease.

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