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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

Changes in selectivity with the age of the stationary phase

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ABSTRACT

The long-term reproducibility of the separation of basic drugs by high-performance liquid chromatography using a silica column with a methanol–high-pH eluent was examined. The relative capacity factors of specific tertiary amines changed systematically compared with the other drugs on storage of the columns for 1 year. The changes were enhanced on storing the column in mobile phase and were accelerated by prolonged washing with water. A comparison of freshly packed columns suggested that similar changes also occurred with the dry silica on storage. These effects may be a contributor to previously observed batch-to-batch differences in the silica.

INTRODUCTION

The analysis of basic analytes by high-performance liquid chromatography (HPLC) often causes considerable problems because of interactions between the analytes and free silanol groups on the surface of the silica stationary phase [1]. A number of approaches have been used to overcome this problem in reversed-phase chromatography, including the use of deactivating agents such as alkylamines in the mobile phase [1] and highly coated silica stationary phases [2,3]. An alternative technique has been to use silica as an ion-exchange medium [4] with mobile phases containing high proportions of methanol and high-pH buffers. Most of these studies have used eluents consisting of 90:10 methanol–ammonia/ammonium nitrate (pH 9.2) buffers [5].

However, in previous work in these laboratories it was found that although reproducible results could be obtained under closely controlled conditions on a single column over a limited time period within one laboratory [6], considerable variation was found in the relative retentions of a number of the test analytes on different batches and brands of silica [7]. In addition, in collaborative trials large variations in absolute and relative retentions between laboratories were obtained for columns prepared from a single common batch of silica [8,9]. It

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was considered that these differences might be due to variations in the ionic strength of the mobile phase caused by differences in the concentration of the ammonia solutions used to prepare the buffer [9].

In order to develop a more robust method, alternative high-pH buffers were examined. A mixture of 3-(cyclohexylamino)-1-propanesulphonic acid (CAPS) and sodium 3-(cyclohexylamino)-2-hydroxy-1-propanesulphonate (CAPSO-Na), which could be prepared by weight, was selected as this would avoid the problems due to the volatility of the ammonia buffer [10]. This new method showed good robustness and reproducibility but differences were again noted between different batches of the same brand of silica.

This paper extends this work and reports a study of changes in the separation of basic analytes with the age of the silica before packing, changes in the selectivity of columns on storage, the effect of different storage conditions and of accelerated ageing by washing the columns with water. These studies were not possible previously as the limited long-term reproducibility of the ammonia-based mobile phase would have masked any changes. In early work, Wheals [11] found that a silica column was stable towards dissolution for a long period provided that the mobile phase contained a high percentage of methanol and ammonia was used as the source of the high pH. Subsequently, Law [12] noted an overall decrease in retention times with time for the separation of a set of 69 related monofunctional arylalkylamines. Because of widespread scepticism about the stability of the silica when used with these high-pH eluents, Law and Chan [13] carried out an extended study and reported that a single column could be used for over 9 months for a range of antimalarial drugs. However, the selectivity of the column changed with time and this was attributed to irreversible adsorption on the column which might have been prevented by using a guard column.

Few previous studies have examined the long-term stability on silica based column materials. Claessens *et al.* [14] studied the ageing processes of alkyl-bonded phases and attributed many of the changes to alterations in the underlying silica matrix, in particular the hydrolysis of siloxane groups to silanols. More recently, Hetem *et al.* [2] used retention and NMR studies to examine the changes in the underlying silica of three bonded-phase materials with different ageing conditions. Although Cox and Stout [15] compared the effects on the retention of bases on silica from different sources and suggested that a slow equilibrium existed between siloxanes and silanols groups, they did not report any changes in properties with time.

EXPERIMENTAL

Chemicals

Sample and eluent components were as reported previously [10]. The eluent buffer was prepared by mixing CAPS (0.8852 g) and CAPSO-Na (1.0372 g) in water and making up to 50 ml.

The columns were all packed using Spherisorb S5W (batch F5493/1 or batch 2752; Phase Separations, Deeside, UK). The former batch had been subdivided into 10-g sub-batches, which were stored separately.

HPLC separations

The equipment and samples were as described previously [10]. The test drugs were injected as mixtures of 2–5 components and were eluted using methanol–buffer (90:10, v/v) at 30°C. Protriptyline was used as an internal standard. The separations were carried out in triplicate and the mean retention times were used to calculate the relative capacity factors compared with protriptyline.

Between separations the columns were stored at room temperature after washing with either methanol or fresh mobile phase. Each column was equilibrated with fresh eluent overnight prior to being tested.

RESULTS AND DISCUSSION

One-year storage trials

Three columns were prepared from a single sub-batch of Spherisorb S5W (batch F5493/1: 10.A, 10.B and 10.C). The columns were tested immediately after packing using a set of 27 test compounds and the CAPS-CAPSO-Na mobile phase and then after *ca.* 1, 3, 6 and 12 months. In order to determine whether the storage conditions influenced the stability of the silica after each test, columns 10.A and 10.B were washed with methanol prior to storage and column 10.C was washed with fresh eluent.

Results are not given for column 10.B after 6 months because equipment problems caused poor reproducibility. It was also necessary to re-pack the top of this column with fresh silica after 1 year before any meaningful chromatographic results could be recorded. Particular problems were experienced on all three columns with methylamphetamine, the peak shape of which deteriorated in this study compared with earlier work [10]. Reasonable peak shapes could only be obtained using a tenfold diluted test solution. Frequently the methylamphetamine peak was distorted and reliable capacity factors could not be obtained. This problem is the subject of further study.

The capacity factors (k') for the test compounds decreased on all three columns over the period of the study, *e.g.*, for protriptyline on column 10.A, initially $k' = 10.40$, which decreased after 1 year to $k' = 8.04$. This agrees with the study by Law [12], who also found a decrease in retention over a prolonged period of elution. Initially the relative capacity factors of the test compounds compared with protriptyline on each of the three columns were almost identical (Tables I–III). The only differences in elution order were for the closely eluted compounds nortriptyline and prolintane, which were reversed on column 10.A compared with the other two columns, and pholcodine and phenylephrine, which were reversed on column 10.C. However, the variations in the relative capacity factors of these compounds were small and were similar to the variations found earlier for repeated separations on a single column [10].

Over the period of the study, the relative capacity factors for most of the analytes showed only small variations (less than 2 units). However, a number of compounds showed greater changes and their relative capacity factors altered steadily over the year (Table IV and Fig. 1 for column 10.A). For the two columns stored in methanol, the relative capacity factors for morphine, codeine, phenylephrine, pholcodine and methdilazine all increased on average by between 2 and 4 units. In contrast, decreased relative capacity factors were observed for dipipanone (−10 units), prolintane (−6 units) and pipazethate (−4 units). Larger differences were observed for the first group of compounds on column 10.C, which had been stored filled with the high-pH eluent, with many of the compounds changing by 4 to 7 units. Significant changes were also found on this column for ethoheptazine (3 units) and ephedrine (4 units). The effects on dipipanone (−11 units) and prolintane (−6 units) were similar to the changes on storage in methanol. However, pipazethate showed a small increase in its relative capacity factor on this column rather than a decrease. Propanolol, whose capacity factor changed during the study by Law and Chan [13], was unchanged in this study.

The three compounds which suffered the largest effects, dipipanone, prolintane and pipazethate,

TABLE I

CHANGES IN RELATIVE CAPACITY FACTORS WITH AGE OF SILICA COLUMN 10.A (BATCH F5493/1) STORED IN METHANOL

Relative capacity factors: rel. $k' \times 100$ (relative to protriptyline).

Compound	Rel. $k' \times 100$				
	Time from packing (days)				
	0	34	97	197	370
Nitrazepam	2.0	2.2	2.3	2.4	2.1
Diazepam	2.5	2.8	2.8	2.9	2.6
Papaverine	3.3	3.5	3.9	3.7	3.5
Caffeine	3.9	4.0	4.2	4.3	4.4
Dextropropoxyphene	6.9	6.8	7.0	7.2	6.6
Cocaine	8.2	8.0	8.2	8.5	8.2
Procaine	8.9	8.9	9.2	9.5	9.2
Amitriptyline	16.3	16.4	17.0	17.1	16.9
Chlorpromazine	17.2	17.4	17.8	18.0	18.1
Propranolol	20.2	20.0	20.2	20.6	19.9
Imipramine	25.1	25.0	25.6	25.7	25.4
Morphine	27.8	27.5	28.3	28.7	30.7
Codeine	27.9	27.8	28.3	29.0	30.6
Promazine	29.4	29.5	30.0	30.6	30.7
Phentermine	32.5	31.7	31.9	32.1	31.4
Amphetamine	32.8	31.9	32.5	32.7	32.9
Phenylephrine	38.0	37.4	37.6	38.4	40.7
Pholcodine	38.4	36.8	38.0	38.9	42.2
Dipipanone	40.7	37.5	36.7	36.3	30.9
Ethoheptazine	45.6	45.0	45.7	46.7	47.0
Methdilazine	48.5	48.6	49.7	50.6	51.4
Nortriptyline	53.1	52.8	53.0	53.5	53.1
Prolintane	53.5	50.4	52.0	51.8	47.4
Ephedrine	54.2	53.2	53.6	54.3	55.3
Pipazethate	61.6	58.9	59.0	58.5	57.3
Methylamphetamine	71.4	70.8	N.R.[a]	71.6	N.R.[a]
Protriptyline	100.0	100.0	100.0	100.0	100.0
Strychnine	107.0	104.4	104.4	106.0	107.6

[a] Not reported because of distorted peaks.

TABLE II

CHANGES IN RELATIVE CAPACITY FACTORS WITH AGE OF SILICA COLUMN 10.B (BATCH F5493/1) STORED IN METHANOL

Rel. $k' \times 100$ as in Table I.

Compound	Rel. $k' \times 100$			
	Time from packing (days)			
	0	29	92	365
Nitrazepam	2.2	2.2	2.2	2.1
Diazepam	2.6	2.7	2.7	2.6
Papaverine	3.5	3.5	3.6	3.7
Caffeine	4.1	3.9	4.2	4.4
Dextropropoxyphene	7.0	6.9	6.9	6.5
Cocaine	8.1	7.8	8.1	8.2
Procaine	8.9	8.8	9.1	9.1
Amitriptyline	16.2	16.2	16.6	16.7
Chlorpromazine	17.2	17.4	17.6	18.1
Propranolol	20.0	19.8	20.0	19.7
Imipramine	25.0	24.9	25.2	25.2
Morphine	27.9	27.2	28.0	31.4
Codeine	28.1	27.7	28.4	31.0
Promazine	29.3	29.2	29.8	30.9
Phentermine	31.9	31.7	31.6	31.1
Amphetamine	32.6	32.1	32.2	33.1
Phenylephrine	38.5	37.1	37.7	41.4
Pholcodine	38.6	37.3	37.9	42.2
Dipipanone	39.2	37.6	35.9	28.2
Ethoheptazine	45.1	44.6	45.4	46.7
Methdilazine	48.5	48.7	49.3	51.4
Nortriptyline	52.5	52.8	52.7	53.3
Prolintane	51.9	50.2	51.4	44.9
Ephedrine	53.6	53.2	53.6	55.7
Pipazethate	59.9	58.5	58.8	55.6
Methylamphetamine	68.3	N.R.[a]	67.8	N.R.[a]
Protriptyline	100.0	100.0	100.0	100.0
Strychnine	106.3	105.2	104.1	105.7

[a] Not reported because of distorted peaks.

have been found previously to be particularly sensitive to small changes in the elution conditions [10]. Differences in the relative retentions of these compounds were also a major factor in discriminating different batches of silica, both with this eluent [10] and with the ammonia-buffered eluent [7]. The changes for pipazethate in those studies were smaller than for the other two compounds. Phenylephrine had also been identified as a compound whose retention was sensitive to the separation conditions and, as in this study, its relative retention always changed in the opposite direction to dipipanone but to a lesser extent.

The other compounds, whose relative capacity factors increased, had not been identified in the previous work [6,10] as compounds that were sensitive to separation conditions. In contrast, strychnine, whose relative capacity factor remained virtually constant in this study, had been found to be very sensitive to variations in the ammonia eluent [6,7]. This compound might be particularly sensitive to changes in the ionic strength of the eluent as its relative capacity factor decreased markedly on changing from the ammonia-based eluent to the weaker CAPS–CAPSO-Na eluent [10].

TABLE III

CHANGES IN RELATIVE CAPACITY FACTORS WITH AGE OF SILICA COLUMN 10.C (BATCH F5493/1) STORED IN MOBILE PHASE

Rel. $k' \times 100$ as in Table I.

Compound	Rel. $k' \times 100$				
	Time from packing (days)				
	0	33	96	198	369
Nitrazepam	2.1	2.2	2.3	2.4	2.2
Diazepam	2.8	2.5	2.7	2.8	2.7
Papaverine	3.4	3.5	3.5	3.8	3.5
Caffeine	3.9	4.2	4.3	4.4	4.5
Dextropropoxyphene	6.8	6.8	6.8	6.8	6.2
Cocaine	8.0	8.4	8.5	8.6	8.8
Procaine	8.8	9.1	9.4	9.7	9.6
Amitriptyline	16.1	16.4	16.8	17.1	17.0
Chlorpromazine	17.1	17.6	17.9	18.2	18.3
Propranolol	20.0	20.2	20.2	20.3	20.0
Imipramine	24.1	25.4	25.1	25.4	25.3
Morphine	27.2	29.8	30.3	31.3	33.0
Codeine	27.9	29.7	30.4	31.3	32.7
Promazine	29.1	30.1	30.6	31.1	31.2
Phentermine	32.0	32.0	31.6	31.6	31.3
Amphetamine	32.1	32.6	32.7	32.7	33.6
Phenylephrine	38.3	39.4	39.9	40.7	43.3
Pholcodine	37.7	40.2	40.6	41.6	44.6
Dipipanone	39.1	35.2	33.5	32.0	27.7
Ethoheptazine	44.9	46.7	47.2	47.9	47.9
Methdilazine	47.9	50.0	51.1	52.1	52.7
Nortriptyline	52.5	53.0	53.1	53.1	53.4
Prolintane	52.2	49.6	50.6	49.9	46.4
Ephedrine	53.6	55.5	55.2	55.9	57.3
Pipazethate	59.5	60.0	60.1	59.0	60.9
Methylamphetamine	68.1	N.R.[a]	70.2	70.7	N.R.[a]
Protriptyline	100.0	100.0	100.0	100.0	100.0
Strychnine	107.1	108.7	108.6	109.7	108.7

[a] Not reported because of distorted peaks.

TABLE IV

MAJOR CHANGES IN RELATIVE CAPACITY FACTORS ON STORAGE OF PACKED COLUMNS

Compound	Changes in relative capacity factors over 1 year	
	Storage solvent	
	Methanol[a]	Eluent[b]
Morphine	3.2	5.8
Codeine	2.8	4.8
Phenylephrine	2.8	5.0
Pholcodine	3.7	6.9
Dipipanone	−10.4	−11.4
Ethoheptazine	1.5	3.0
Methdilazine	2.9	4.8
Prolintane	−6.5	−5.8
Ephedrine	1.6	3.7
Pipazethate	−4.3	1.4

[a] Mean value from columns 10.A and 10.B
[b] Column 10.C.

Effect of water on the ageing of the columns

It was suspected that the changes in the relative retentions on storage could be due to the effects of water, either from the mobile phase or from the atmosphere, on the silica surface. After the initial storage trial had been completed, the columns (10.A, 10.B and 10.C) were therefore each washed with 1200–1300 ml of HPLC-grade water without a guard column in an attempt to produce "accelerated ageing" of the silica. This treatment also had the effect of partially restoring the poor peak shapes observed with methylamphetamine. The columns were then re-tested.

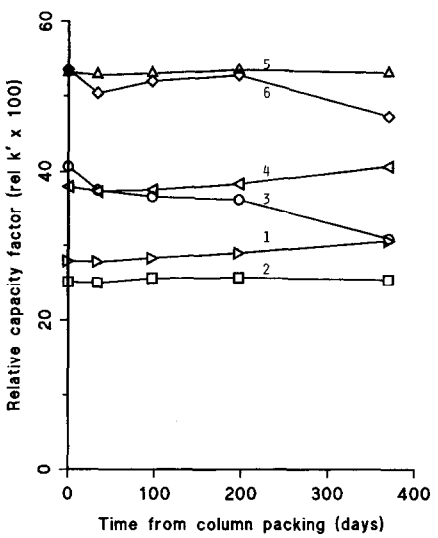

Fig. 1. Changes in relative capacity factors with time for selected analytes on column 10.A batch F5493/1. For conditions, see text. Compounds: 1 = codeine; 2 = imipramine; 3 = dipipanone; 4 = phenylephrine; 5 = nortriptyline; 6 = prolintane.

The capacity factors of all the analytes decreased as a result of washing, *e.g.*, protriptyline from $k' = 8.04$ to 7.59 on column 10.A. Changes in the relative capacity factors were also observed (for selected examples, see Table V). On all three columns the tertiary amines dipipanone, prolintane and pipazethate showed further reductions in their relative capacity factors compared with the earlier study. The relative capacity factor of pipazethate on column 10.C now matched the value on the other two columns, rather than its much higher relative capacity factor after 1 year in the time trial. In addition, the relative capacity factor of strychnine decreased markedly, which had not been observed previously with these columns. Phenylephrine showed much larger increases in relative capacity factors on each column than in the time study. Ephedrine again showed only small effects. In all instances the increased relative retention for pholcodine over the 1-year study was reversed, the final results being only marginally different from the data recorded on the columns when new (Tables I–III).

Storage changes in dry silica

Because of concern that some changes in the nature of the surface of the silica could be occurring during storage of the stationary phase as a dry powder before packing, the retentions of the drug compounds were compared on four columns, which had been packed over an 18-month period using silica from the same original batch (batch F5493/1). Two of these columns were packed from the same sub-batch (columns 4.A and 4.B) and the others were from different sub-batches. Each column was packed, equilibrated with eluent and then tested within 5 days. In the packed column ageing study, the three columns 10.A, 10.B and 10.C had been packed within a few days so would have had the same age from the manufacture of the silica.

The capacity factors measured on the four col-

TABLE V

EFFECT OF A WATER WASH ON RELATIVE CAPACITY FACTORS OF SELECTED TEST COMPOUNDS

Rel. $k' \times 100$ as in Table I.

Compound	Rel. $k' \times 100$					
	Column 10.A		Column 10.B		Column 10.C	
	Before	After	Before	After	Before	After
Dipipanone	30.9	25.6	28.2	25.3	27.7	24.7
Phentermine	31.4	31.1	31.1	31.3	31.3	30.9
Phenylephrine	40.7	47.8	41.5	49.7	43.3	51.5
Pholcodine	42.2	37.6	42.2	39.5	44.6	37.6
Amphetamine	32.9	34.0	33.1	34.3	33.6	34.1
Prolintane	47.4	38.7	44.9	39.8	46.4	38.1
Ephedrine	55.3	56.3	55.7	57.9	57.3	55.2
Pipazethate	57.3	50.7	55.6	52.3	60.9	49.5
Strychnine	107.6	97.0	105.7	100.2	108.7	96.4

umns decreased with increasing age of the silica before packing (*e.g.*, protriptyline changed from $k' = 10.40$ on column 10.A packed 31 months after manufacture to $k' = 7.84$ on column 9.A packed after 48 months). Differences were also observed in the relative capacity factors of many of the compounds (Table VI). The effects were less than for the columns which had been stored in solvent and were not as systematically related to the age of the silica. The most marked effects in the relative capacity factors were again for prolintane, pipazethate and dipipanone, which all decreased. In contrast to the storage study, the relative capacity factor of strychnine also decreased. Morphine, ephedrine and phenylephrine showed small increases in relative retentions with increasing age of the silica.

Hence silica stored as a dry powder appeared to undergo similar changes in chromatographic properties to silica stored as a packed column in solvent.

Comparison of batches of silica

To determine if these changes in relative capacity factor occurred with other batches of silica, columns were prepared from an older batch of Spherisorb S5W (batch 2752, manufactured about 34 months before batch F5493/1). An initial column (2752.A) was prepared, tested and then stored for 14 months in methanol before being re-tested. At that time a second column (2752.B) was packed from the same batch of silica and was tested to examine the effect of storage on the dry powder (Table VII). As expected from previous batch-to-batch comparisons, the retentions on these columns differed from those obtained with batch F5493/1.

The changes in the relative capacity factors with time on column 2752.A were generally smaller than for the F5493/1 batch of silica, but again dipipanone, prolintane and pipazethate showed marked decreases on storage of the column in solvent. In this instance strychnine was also affected. Phenylephrine showed a small increase but the changes for morphine and codeine were negligible.

The differences between the retentions on the two freshly packed columns, which would reflect the effects of the storage of the dry powder, were generally smaller, except for a marked change in strychnine (relative $k' = 95.2$ to 91.7).

It was felt that these changes in the properties of the column material might also have been responsible for some of the differences observed between batches of silica when they were examined using the ammonia buffer [8]. In that case the largest effects were found for the oldest batches and were particularly marked for strychnine.

Examination of the silica surface

To determine whether there had been any changes in the surface characteristics of the silica during the period of study, two silica samples (a dry sample of batch F5493/1 and a sample taken from column 4.A after storage for 18 months) were submitted for surface examination by ^{29}Si cross-polarization

TABLE VI

COMPARISON OF RELATIVE CAPACITY FACTORS ON NEW COLUMNS PACKED WITH SPHERISORB S5W (BATCH F5493/1) OVER AN 18-MONTH PERIOD

Each column was tested within 5 days of packing. Results for column 10.A from Table I. Rel. $k' \times 100$ as in Table I.

Compound	Rel. $k' \times 100$			
	Column (months from manufacture of silica)			
	4.A (30)	10.A (31)	4.B (46)	9.A (48)
Nitrazepam	2.3	2.0	2.1	2.1
Diazepam	3.1	2.5	2.5	2.6
Papaverine	3.6	3.3	3.3	3.3
Caffeine	4.2	3.9	4.0	4.3
Dextropropoxyphene	7.0	6.9	6.5	6.4
Cocaine	8.0	8.2	7.8	7.9
Procaine	8.8	8.9	8.4	8.5
Amitriptyline	16.2	16.3	16.0	16.3
Chlorpromazine	17.2	17.2	17.1	17.5
Propranolol	19.8	20.2	19.1	19.1
Imipramine	24.7	25.1	24.5	24.7
Morphine	27.8	27.8	28.7	29.6
Codeine	28.1	27.9	28.8	29.2
Promazine	29.0	29.4	29.1	29.7
Phentermine	31.8	32.5	31.5	31.3
Amphetamine	32.8	32.8	33.0	33.1
Phenylephrine	39.4	38.0	40.5	41.2
Pholcodine	38.0	38.4	38.9	38.5
Dipipanone	37.4	40.7	32.1	28.0
Ethoheptazine	43.9	45.6	43.9	44.1
Methdilazine	47.7	48.5	47.6	48.5
Nortriptyline	52.6	53.1	52.8	53.4
Prolintane	50.0	53.5	45.2	40.8
Ephedrine	53.4	54.2	54.4	55.0
Pipazethate	57.3	61.6	52.9	50.2
Methylamphetamine	63.9	71.4	64.1	64.5
Protriptyline	100.0	100.0	100.0	100.0
Strychnine	104.4	107.0	100.3	95.9

magic-angle spinning NMR spectroscopy (^{29}Si-CP-MAS-NMR). The spectra indicated that the ratio of the Q_3:Q_4 peaks (isolated silanols:siloxanes) changed from 2:3 for the dry silica to 3:3 for the sample removed from the column after treatment with eluent. However, the changes were subtle and not easily quantified. It appeared that hydrolysis of siloxanes to silanols had occurred on the silica surface.

These changes would contrast with those obtained by Hetem *et al.* [2], who found that for octadecylsilyl-bonded silica, ageing treatment with a methanol–aqueous pH 8 mobile phase caused a decrease in the number of free silanol groups relative to siloxanes. It was proposed that siloxane bridge formation was occurring.

The three compounds, that showed consistent decreases in relative retentions with increasing silica age, dipipanone, pipazethate and prolintane, are all tertiary amines containing an alicyclic tertiary nitrogen atom to which the remainder of the molecule is linked, as an N-substituent on a pyrrolidine or

TABLE VII

COMPARISON OF RELATIVE CAPACITY FACTORS ON COLUMNS PACKED WITH SPHERISORB S5W (BATCH 2752)

Rel. $k' \times 100$ as in Table I.

Compound	Rel. $k' \times 100$		
	Column (days from start of set of experiments)		
	2752.A (0)	2752.A (440)	2752.B (packed 450)
Nitrazepam	2.6	2.2	2.3
Diazepam	3.0	2.7	2.8
Papaverine	3.9	3.4	3.5
Caffeine	4.9	4.7	4.6
Dextropropoxyphene	6.5	5.9	6.2
Cocaine	8.3	8.0	8.0
Procaine	9.3	8.8	8.7
Amitriptyline	17.4	17.2	17.2
Chlorpromazine	18.6	18.5	18.4
Propranolol	19.3	18.8	18.8
Dipipanone	24.0	20.5	22.7
Imipramine	25.7	25.2	25.4
Phentermine	31.2	30.3	30.4
Promazine	31.2	31.2	31.1
Codeine	31.3	31.8	30.5
Morphine	31.6	32.2	31.1
Amphetamine	33.1	33.4	33.0
Prolintane	39.6	36.1	37.2
Pholcodine	39.8	41.1	39.5
Phenylephrine	42.4	44.2	42.9
Ethoheptazine	46.5	46.0	45.2
Pipazethate	48.6	45.8	45.8
Methdilazine	51.8	52.2	51.1
Nortriptyline	53.8	54.0	54.0
Ephedrine	55.9	56.2	54.9
Methylamphetamine	66.3	65.6	64.6
Strychnine	95.2	92.3	91.7
Protriptyline	100.0	100.0	100.0

piperidine ring. The nitrogen atom is therefore sterically hindered compared with the other alicyclic tertiary amines in the test samples, such as cocaine and methdilazine, which contain less hindered N-methyl groups.

CONCLUSIONS

The rapid changes in the relative capacity factors of the basic drugs on treatment with water compared with storage in methanol or eluent and the slower changes on storage as a dry powder suggest that significant changes are occurring in the degree of hydroxylation of the silica surface. These were probably emphasized in this study, which used the silica as an ion-exchange medium, but from the work of Hetem *et al.* similar changes can also occur in the free silanols on bonded phases. In this study these changes caused differences in the overall retention properties of the silica. The effects on retention were selective and were probably dependent on the specific steric requirements of the interaction between the analyte and the silica surface, although clarification will require considerable further study. However, more than one type of change may be occurring because, although dipipanone and prolintane were always affected in a similar manner, the effect on the retention of strychnine was only observed on dry storage or on washing with water, but its retention was unaffected by storage in mobile phase.

These changes in the retention properties of silica with time or usage suggest that separations based on this mode of interaction might have inherent poor reproducibility over long periods. Irrespective of the consistency of the operating conditions, it will probably be difficult to replicate results in different laboratories unless all the analytes being considered interact with the column material in the same manner. Particular problems will be encountered if a wide range of structural types are being compared as in this study.

The differences in retention with the age of the silica following manufacture suggest that much of the batch-to-batch differences observed in earlier studies might have been due to an ageing process rather than inherent variations in the manufacturing or testing procedures. These changes in the silica surface with time may also be important in reversed-phase chromatography because of the frequently observed effects of interactions with residual silanols on bonded-phase columns, particularly during the separation of bases.

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