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Comparison of high-performance liquid chromatographic methods for the analysis of basic drugs

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ABSTRACT

Problems that are often encountered in the high-performance liquid chromatographic analysis of basic compounds are severe peak asymmetry and low separation efficiency. In attempting to solve these problems, one can become confused by the variety of suggestions given by the specialists and by the numerous stationary phases available. In this work, the analysis of basic drugs was studied from two directions. In both approaches a set of 32 basic drugs was used, differing in basicity, polarity and number and type of nitrogen atoms. In the first approach the effect of mobile phase additives and buffers on the performance of a single column was determined. It was found that tertiary and quaternary amines can be applied successfully as silanol blockers. The latter proved to be aggressive towards silica-based stationary phases. Addition of triethylamine showed a remarkable improvement in peak shape in different columns. Other aspects, such as pK_a, retention and amount injected, were systematically studied. In the second approach, eight different columns, specially recommended for the chromatography of basic drugs, were evaluated. The chromatographic results showed great variability. As far as peak shape as a function of pH is concerned, an electrostatically shielded stationary phase was most promising for the analysis of basic compounds. This column can even be used without buffers, which can be an advantage in liquid chromatography–mass spectrometry coupling. Because some results were inconsistent with published results, a third approach was to study three columns in more detail.

INTRODUCTION

Peak asymmetry, which is often observed in the chromatography of basic drugs, is an important performance characteristic for a given stationary phase. In routine analysis, system suitability criteria are set for the maximum allowable asymmetry, expressed as asymmetry factor. For quantitative analysis an asymmetry factor of less than 1.5 is preferred. It is generally accepted that the severe peak asymmetry of basic drugs in reversed-phase chromatography is caused by ionic interaction of the charged solutes with free silanol groups of the packing. Despite these negative effects, reversed-phase high-performance liquid chromatography (RP-HPLC) is still the most widely used method because of the fast equilibration time, the selectivity characteristics and the retention reproducibility. As can be seen in Fig. 1, we are in fact in a bad situation. The

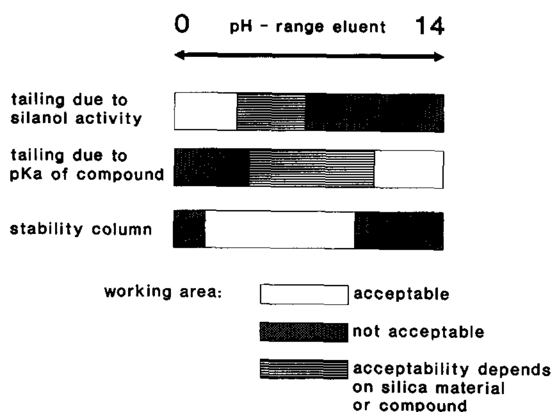

Fig. 1. Qualitative illustration of the working area for the analysis of basic compounds on silica-based reversed-phase materials.

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requirements for a good chromatographic performance with strongly basic compounds conflict with the demands for high stability of the column. Good performance requires a high pH to avoid ionization of the basic solute or a low pH to suppress the formation of free silanol groups.

Over the years, numerous suggestions have been put forward to improve this situation. Special attention has been given (i) to reducing the number of free silanols (end-capping), (ii) to diminishing the effect of free silanols by choosing the correct mobile phase pH and reducing the acidity of the silica and (iii) to eliminating silanol effects by adding inorganic and organic salts. The use of blocking agents such as tertiary and quaternary amines proved to be a useful approach [1,2].

Alternative solutions have been proposed by workers who used bare silica, dynamically modified silica or alumina [3,4]. These methods, however, did not gain widespread application. In RP-HPLC, the most obvious solution is the choice of a suitable column. Stadalius *et al.* [1] pointed to the existence of "acidic" and "basic" columns. The latter clearly are the best for this particular task. Over more than a decade our approach has been to improve the mobile phase composition and to test new stationary phases. As a result of this, μBondapak C_{18} and Nova-Pak C_{18} columns have been used more or less as a standard for the analysis of basic drugs. However, research on LC packings is continuing and recently resulted in the introduction of new columns specially designed for the chromatography of basic drugs [5].

In this paper, we present data on the influence of the mobile phase composition (pH, buffers, additives) on peak performance and on the stability of μBondapak C_{18}. We also compared the chromatographic performance of special column materials. For both, a set of 32 basic drugs was used. These drugs differ widely in pK_a, polarity and number and structural type of the nitrogen atoms.

EXPERIMENTAL

Chemicals

All basic drugs were obtained from Organon International (Oss, Netherlands). Methanol was freshly distilled and water was obtained from a Milli-Q quality purification system (Millipore). Tetramethylammonium hydroxide (TMAH) was obtained from Southwestern Analytical Chemicals (Austin, TX, USA). Disodium hydrogenphosphate (Na_2HPO_4), sodium dihydrogenphosphate (NaH_2PO_4) and 25% ammonia solution were supplied by J. T. Baker (Deventer, Netherlands), acetonitrile (CH_3CN) and concentrated phosphoric acid (H_3PO_4) by Merck (Darmstadt, Germany), sodium 1-hexanesulphonate by Eastman Kodak (Rochester, NY, USA) and triethylamine (TEA, Sequanal grade) by Pierce (Rockford, IL, USA).

The pK_a values of the basic drugs were determined in 60% methanol at 37°C because of the low solubility in water. As a rule these pK_a values are *ca.* 0.5–1 unit lower than in water.

Apparatus

The experiments were carried out on an HP 1090M liquid chromatograph equipped with an HP 1040M diode-array detector. Data were collected on an HP 79994A HPLC workstation (Hewlett-Packard, Amstelveen, Netherlands).

Experimental set-up

The experiments can be divided into three parts. In the first part of the study we used a μBondapak C_{18} column and chromatographed a set of 32 basic drugs with different mobile phases, as explained in Fig. 2A. The influence of the mobile phase parameters on the asymmetry factor was studied quantitatively, and more qualitatively on plate number. We also checked their influence on the stability of the stationary phase.

In the second part, recent developments in column technology were explored. For this we used different stationary phases, specially recommended by the manufacturers for the chromatography of basic drugs. With a constant mobile phase composition we studied the chromatographic performance of these columns with our set of basic drugs. In Fig. 2B an overview is shown of these experiments. The results will be described in a qualitative way.

In order to show column effects and differences between columns as well as possible, we performed comparative experiments under critical conditions. Therefore, we chose pH values of 3.5 and 7.4 and plain buffer without additives, as the addition of silanol blockers can mask differences.

For this study a set of 32 basic drugs was selected

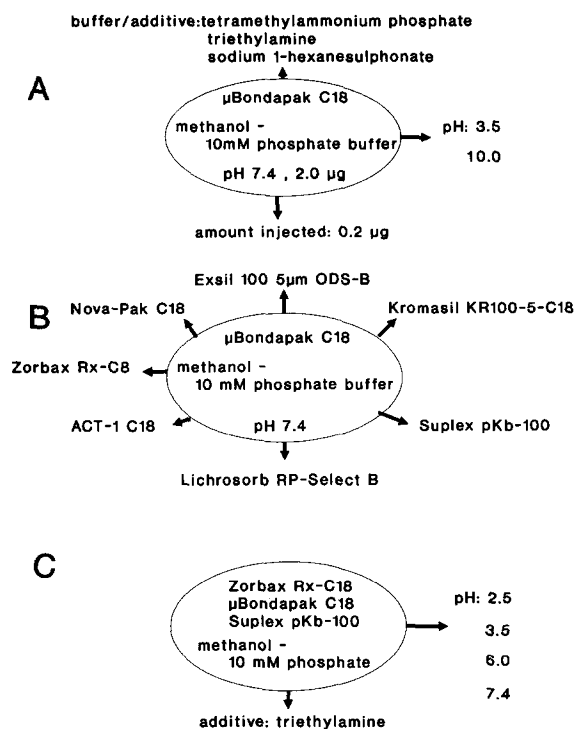

Fig. 2. Overview of the experiments in this study.

and a representative group of them is shown in Table I. The selection of 32 compounds was based on the following characteristics: basicity, with pK_a values ranging from 3 to 9, polarity, with retention indices [6] between 400 and 1600, number of nitrogen atoms, from 1 to 5, and different structural type of nitrogen atom.

In the third part the effects of pH and the addition of triethylamine were studied for seven "difficult" compounds on three selected columns (see Fig. 2C).

Chromatographic conditions

All bonded-phase materials were obtained from the manufacturers as prepacked columns. An overview of the stationary phases is given in Table II. The mobile phases used in this study are shown in Table III.

The pH of the buffers was measured before mixing with the organic modifier. They were prepared by dissolving 3.58 g (10 m*M*) of disodium hydrogenphosphate or 9.05 g (50 m*M*) of tetramethylammonium hydroxide in 1 l of water. Occasionally 2 ml (15 m*M*) of triethylamine was added to the phosphate solution. Concentrated phosphoric acid or 0.5 *M* sodium hydroxide was finally added until the desired pH was reached.

The amount of basic drug injected was 2.0 µl of a 1 mg/ml solution in methanol, *i.e.*, 2 µg injected, unless indicated otherwise. After 10–20 injections of the basic drugs, a test solution was injected to check the column performance for changes in silanol activity, hydrophobicity and metal activity. The test solution consisted of a mixture of acetylacetone, aniline, phenol, benzene and anthracene. Methanol–water (60:40, v/v) was used as mobile phase for the test solution. The column temperature was 40°C and UV detection was carried out at 210 nm. Triethylamine should be of a high-purity grade to prevent a high UV offset. For basic drugs the flow-rate was set at 1.0 ml/min and for the test solution at 1.5 ml/min.

Calculations

The asymmetry factors were calculated at 10% of the peak height using the ratio of the width of the rear and front sides of the peak [7]. Because most peaks do not have an ideal Gaussian shape, we used the second moment of the peak for the calculation of the plate numbers [8].

RESULTS AND DISCUSSION

Varying the mobile phase composition using a µBondapak C_{18} column

The aim of this study was to find the best mobile phase conditions for the analysis of basic compounds. In the first set of experiments we chromatographed all basic drugs on a µBondapak column, using the starting conditions given in Fig. 2A [methanol–10 m*M* phosphate (pH 7.4), 2 µg injected]. Clearly, as can be seen in Fig. 3, there is a strong correlation between the pK_a value of the compound and the asymmetry factor. Generally, peak tailing increases with increasing pK_a values. These results have been confirmed by others [1]. Peak tailing is also a function of the capacity factor, as illustrated in Fig. 4 for a number of basic drugs. Therefore, the data given in Fig. 3 were normalized for a k' value of 5.

TABLE I

COMPOUNDS USED WITH THEIR pK_a VALUES AND RETENTION INDICES (I)

Asymmetry factors (A_s) were obtained under the conditions described in Fig. 3.

No.	Structure	pK_a	I	A_s	No.	Structure	pK_a	I	A_s
1		<3	790	1.0	**7**		7.9	1360	4.8
2		5.0	460	1.7	**8**		8.0	1310	4.0
3		6.5	1120	2.6	**9**		7.7	1310	5.7
4		8.2	1380	5.2	**10**		8.7	690	5.6
5		8.3	1160	3.6	**11**		7.7 (not included in the test set)		
6		8.7	1550	9.1					

TABLE II

STATIONARY PHASES USED

No.	Column	Manufacturer	Dimensions [length × I.D. (mm)]	Particle size (μm)
1	μBondapak C_{18}	Waters–Millipore	300 × 3.9	10
2	Novapak C_{18}	Waters–Millipore	300 × 3.9	4
3	Zorbax Rx-C_8	Rockland Technologies	250 × 4.6	5
4	Zorbax Rx-C_{18}	Rockland Technologies	250 × 4.6	5
5	LiChrosorb RP-Select B	Merck	250 × 4.0	5
6	Exsil 100 5 μm ODS-B	Exmere	250 × 4.6	5
7	Kromasil KR100-5-C_{18}	Eka Nobel	250 × 4.6	5
8	Suplex pKb-100	Supelco	250 × 4.6	5
9	ACT-1 C_{18}	Interaction	150 × 4.6	10

For compounds having $pK_a < 6$ we did not observe any problems. Peaks were symmetric with asymmetry factors <1.5 and plate numbers were about 4000–6000. Asymmetric peaks were obtained for compounds with $pK_a > 6$. These compounds are partly protonated and hence more strongly bound to the acidic silanol groups:

$$XH^+ + SiO^-Na^+ \rightleftharpoons SiO^-XH^+ + Na^+ \quad (1)$$

There are indications that the pK_a value is not the only parameter that influences peak tailing. It is thought that structural factors also play a role. For example, substances having comparable pK_a values show widely differing asymmetry factors. Generally it is observed that the flexibility of the protonated N atom and hence the possibility of interacting with silanol sites plays a predominant role. For instance, when we compare substances **6**, **4** and **5** (see Table I) the decrease in the flexibility in this order of the (protonated) N atom is significant. The asymmetry factor decreased likewise. Data on the flexibility of protonated and unprotonated N atoms were taken from NMR measurements.

TABLE III

MOBILE PHASES USED WITH THE VARIOUS COLUMNS

Mobile phase	Columns used
CH_3OH–50 m*M* TMAH, pH 7.4	1
CH_3OH–10 m*M* NaH_2PO_4, pH 2.5	1,4,8
CH_3OH–10 m*M* NaH_2PO_4, pH 3.5	1,4,8
CH_3OH–10 m*M* Na_2HPO_4, pH 6.0	1,4,8
CH_3OH–10 m*M* Na_2HPO_4, pH 7.4	1,2,3,4,5,6,7,8
CH_3OH–10 m*M* Na_2HPO_4, pH 10.0	1
CH_3OH–10 m*M* NaH_2PO_4 + 15 m*M* TEA, pH 2.5	1,4,8
CH_3OH–10 m*M* NaH_2PO_4 + 15 m*M* TEA, pH 3.5	1,4,8
CH_3OH–10 m*M* Na_2HPO_4 + 15 m*M* TEA, pH 6.0	1,4,8
CH_3OH–10 m*M* Na_2HPO_4 + 4 m*M* TEA, pH 7.4	1
CH_3OH–10 m*M* Na_2HPO_4 + 15 m*M* TEA, pH 7.4	1,4,8
CH_3OH–10 m*M* Na_2HPO_4 + 10 m*M* IPR, pH 3.5	1
CH_3OH–H_2O	6,8
CH_3CN–1% NH_3, pH 11.2	9

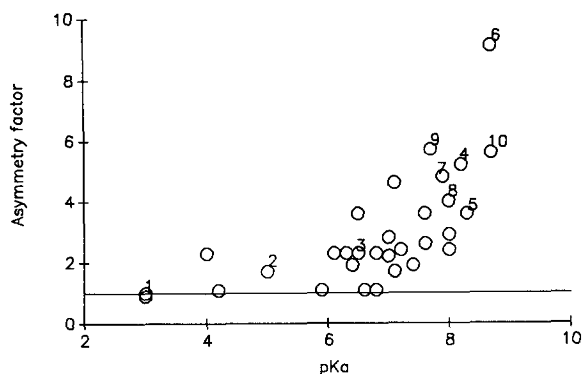

Fig. 3. Scatter plot of pK_a *versus* peak asymmetry, normalized for $k' = 5$. The numbers in the plot correspond to compounds in Table I. For HPLC conditions, see starting conditions in Fig. 2A.

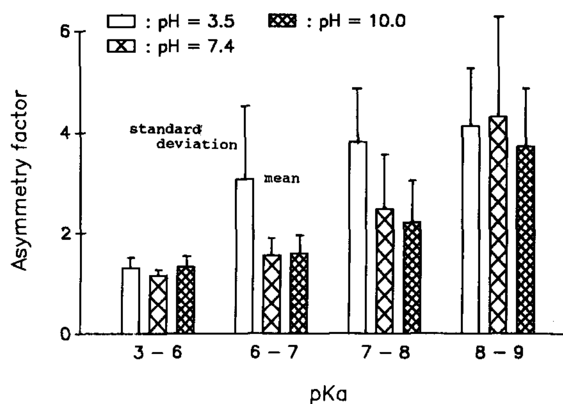

Fig. 5. Influence of the buffer pH on peak asymmetry. Methanol was used as modifier and 2.0 μg were injected. For HPLC conditions, see Fig. 2A. The bars represent the mean and standard deviation (as shown).

Influence of the pH of the buffer

The influence of the pH of the buffer was tested with 10 m*M* sodium phosphate at different pH values in the range 3.5–10. Lowering the pH to 3.5 will result in less dissociation of the silanol groups, whereas at pH 10.0 none of the compounds is protonated. In the literature a pH of less than 3.5 is often recommended to suppress silanol activity as effectively as possible. On purpose we chose a pH of 3.5, which in our view was sufficiently critical to show differences between columns. The effects of lower pH will be discussed later.

As is shown in Fig. 5, the overall results on a μBondapak column were in favour of the higher pH values, especially for substances with pK_a = 6–8. The effects on peak asymmetry are probably not statistically different for compounds with pK_a 3–6 and 8–9. At pH 7.4 most of the test compounds are not protonated and the effect of dissociated silanols is therefore small. Strongly basic compounds (pK_a > 8) will be retained by the mechanism shown in eqn. 1. An effect that is often overlooked is that the pH of a methanol–buffer mixture differs significantly from that of the starting buffer. For instance, 10 m*M* phosphate buffer (pH 7.4) shows a virtual pH of 8.6 in a 1:1 mixture with methanol. These results conflict with the general opinion that a low pH is the best condition for the chromatography of basic compounds. In order to support our conclusions, we present additional data on this pH effect for three different columns in the last section of this paper.

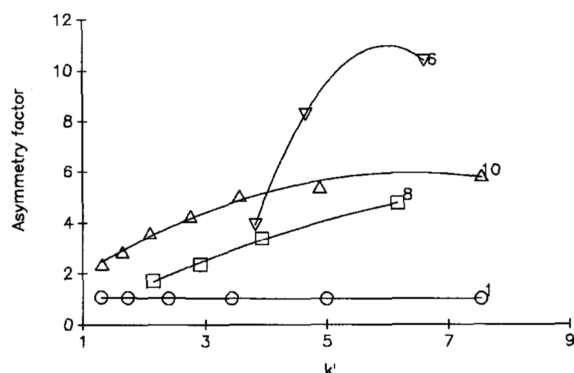

Fig. 4. Relationship between asymmetry factor and k' for substances covering the pK_a range <3–8.7.

The use of a buffer of pH 10.0 led to a small improvement only for very basic substances. This high pH is in fact unrealistic and was only incorporated to see whether unexpected effects would arise. Summarizing, we recommend a pH of *ca.* 7.

Influence of the type of buffer

To determine the influence of the type of buffer, we compared 50 m*M* tetramethylammonium phosphate and 10 m*M* sodium phosphate buffers. A low concentration of sodium phosphate was necessary because of the low solubility in eluents with a high methanol concentration. In our laboratory tetra-

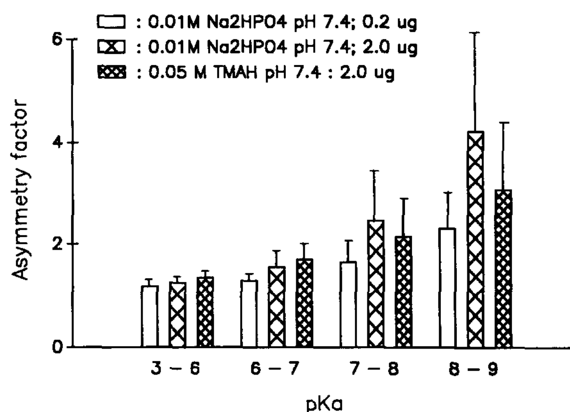

Fig. 6. Influence of the type of buffer and the amount injected on peak asymmetry. Methanol was used as modifier. For HPLC conditions, see Fig. 2A, using tetramethylammonium phosphate (TMAH) buffer.

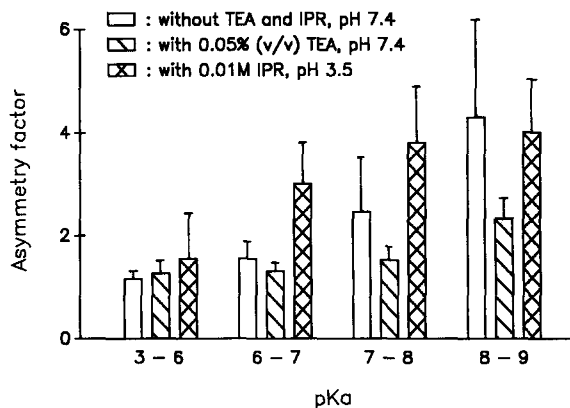

Fig. 7. Influence of triethylamine (TEA) and sodium 1-hexanesulphonate (IPR) on peak asymmetry. Methanol was used as modifier and 2.0 μg were injected. For HPLC conditions, see Fig. 2A.

methylammonium phosphate buffer is being used as a standard for the HPLC of basic drugs. The results obtained with the two buffers are summarized in Fig. 6. For compounds with $pK_a < 8$ almost no difference in peak shape was noticed. As far as plate numbers are concerned the use of sodium phosphate resulted in a slight increase. For compounds with $pK_a > 8$ tetramethylammonium phosphate showed better results (although not statistically significant), but the asymmetry factors were still > 2. Overall it can be concluded that tetramethylammonium phosphate can be used successfully to improve peak shapes by effectively blocking silanol activity. It should be added, however, that the tetramethylammonium ion is aggressive.

Influence of the type of additive

To determine the influence of a silanol blocking agent, triethylamine was used with 10 m*M* sodium phosphate buffer (pH 7.4). The results were compared with those without triethylamine and are shown in Fig. 7 and Table V. Triethylamine (TEA) is a very basic compound ($pK_a \approx 11$) which will also interact with silanol groups and so compete with the basic drugs. Dimethyloctylamine (DMOA) has been successfully used by others [1] for the same purpose. A comparison between TEA and DMOA is now under study. As can be seen in Fig. 7, the addition of triethylamine resulted in a clear improvement in peak shape. Particularly for compounds with $pK_a > 6$ the tailing clearly decreased, resulting in plate numbers of 3000–6000 with acceptable symmetry. However, several basic drugs still have unacceptable peak shapes. Comparing Figs. 6 and 7 it can be concluded that the effect of TEA is better than that of tetramethylammonium. Whether the addition of TEA is to be preferred at high (*ca.* 7) or low (*ca.* 3) pH will be discussed later.

Sodium 1-hexanesulphonate was also tested as an ion-pair reagent (IPR). For this the basic drugs have to be protonated and 10 m*M* sodium phosphate (pH 3.5) was used as a buffer. Comparing these results with those obtained with 10 m*M* sodium phosphate buffer (pH 3.5), the addition of IPR did not result in a clear improvement of peak symmetry. Compared with sodium phosphate (pH 7.4), the peak symmetry is even worse, except for compounds with pK_a = 8–9, where addition of IPR seems to result in less tailing.

Influence of the amount injected

For the determination of the influence of the amount of basic drug injected on to the column, several experiments were compared. Amounts of 0.2 and 2.0 μg were injected and 50 m*M* tetramethylammonium phosphate (pH 7.4) and 10 m*M* sodium phosphate (pH 3.5 and 7.4) were used.

Decreasing the amount injected can dramatically improve the peak symmetry (Fig. 6). Although Fig. 6 shows only data at pH 7.4, the effect is also seen at

pH 3.5, for both sodium phosphate and tetramethylammonium phosphate. Most asymmetry factors were < 2 when 0.2 μg was injected. For very tailing compounds the asymmetry factor was even reduced by a factor of 2 for small amounts injected. Especially for substances with low UV absorbance (see compound **11**, Table I) the large amounts injected are the main reason for peak tailing. When the amount of sample is reduced, the saturation of the acidic sites of the residual silanol groups will be avoided.

Influence of mobile phase composition on column stability

The analysis of basic compounds with RP-HPLC is often used in quality control and stability studies. This means that many samples have to be analysed and that mobile phase volumes of the order of 1000 ml and more are pumped over the column. Therefore, it is important to use a mobile phase–stationary phase combination that is not destructive.

During the experiments, as shown in Fig. 2A, the μBondapak column was tested for changes in silanol activity, hydrophobicity and metal activity. To check the silanol activity a mixture of aniline and phenol was injected. For well deactivated stationary phases aniline elutes before phenol [9,10]. To check the hydrophobicity a mixture of anthracene and benzene was injected. A change in the ratio of the retention times indicates a change in hydrophobicity [9]. Acetylacetone was used to determine the metal activity [9]. It can form a complex with metal ions, resulting in a broad and tailing peak that is retained. In Fig. 8 a chromatogram of the test solution is shown.

When tetramethylammonium phosphate and sodium phosphate (pH 7.4) were compared it was seen that the former is more aggressive and strips off the stationary phase. As shown in Figs. 9 and 10, the change in silanol activity and hydrophobicity is less pronounced with sodium phosphate. These conclusions were confirmed by Wherli *et al.* [11], who also found that quaternary ammonium compounds are aggressive towards silica-based stationary phases. Adding triethylamine or sodium 1-hexanesulphonate to the mobile phase did not clearly influence the stability of the column.

As far as the metal activity is concerned, when tetramethylammonium phosphate was used the peak shape of acetylacetone was sharp and eluted after $k' = 1$ at the beginning of the experiment. The tailing steadily increased and the retention decreased to $k' = 0.1$ after 1000 ml mobile phase had been pumped over the column. Subsequently the peak symmetry and retention time remained stable. An increase in tailing indicates an increase in metal

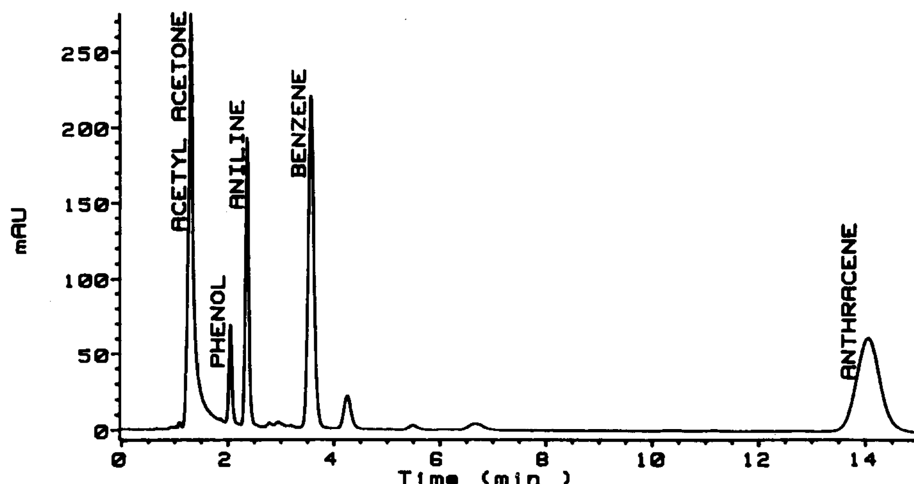

Fig. 8. Chromatogram of the test compounds on a μBondapak C_{18} column (300 × 3.9 mm I.D.). Methanol–water (60:40, v/v) was used as eluent.

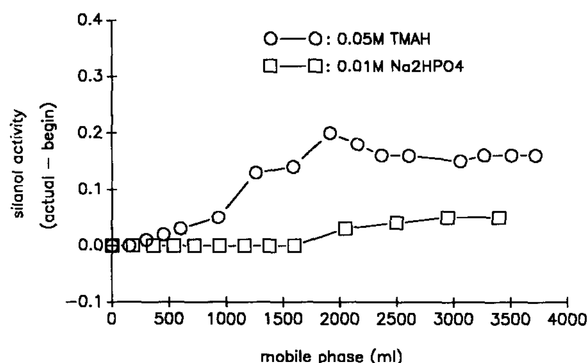

Fig. 9. Influence of the type of buffer and the volume pumped over the column on silanol activity (expressed as the difference between the actual and the starting values). For HPLC conditions, see Figs. 2A and 5.

activity. The decrease in retention indicated a decrease in metal activity or could be a result of reduced reversed phase activity due to a loss of bonded phase. When phosphate was used the peak shape was asymmetric but stable. The retention time decreased from $k' = 0.4$ to 0.1 after 3400 ml of mobile phase had been pumped over the column.

Another parameter that can influence the stability of the column is the pH of the mobile phase. In Fig. 11 the influence of the pH on silanol activity is shown. It was found that the use of high pH resulted in a rapid increase in silanol activity and a decrease in hydrophobicity. This high pH will strip off the stationary phase. The use of pH 3.5 showed no change in silanol activity and hydrophobicity, whereas for pH 7.4 a slight change after about 1500 ml was noticed.

Varying the stationary phase

Stationary phases specially developed for the analysis of basic compounds were investigated and the results were compared with those with the μBondapak column, using methanol–10 mM sodium phosphate buffer (pH of 7.4) as the mobile phase. In order to ensure a fair appraisal, the columns were compared in the same k' range. The results are described in a qualitative way.

Nova-Pak C_{18}. The Nova-Pak C_{18} column showed more tailing for most compounds with a $pK_a > 7$. For compounds with $pK_a < 7$ there is less difference. Most compounds have asymmetry factors of $\leqslant 2$. Owing to the smaller particle size of the Nova-Pak phase, for symmetrical peaks it gives about twice as many plates as μBondpak C_{18} under comparable conditions.

Zorbax Rx-C_8, Kromasil KR-5-C_{18} and LiChrosorb RP-Select B. These three columns are offered as stationary phases specially deactivated for basic compounds. They did not show a clear improvement in peak symmetry compared with a μBondapak C_{18} column. For nine compounds with $pK_a > 7$ the Kromasil column even showed asymmetry factors of $\geqslant 3$. However, for symmetrical peaks the plate number was over 10 000.

Exsil 100 5 μm ODS-B. The Exsil phase is also specially deactivated for basic compounds and, ac-

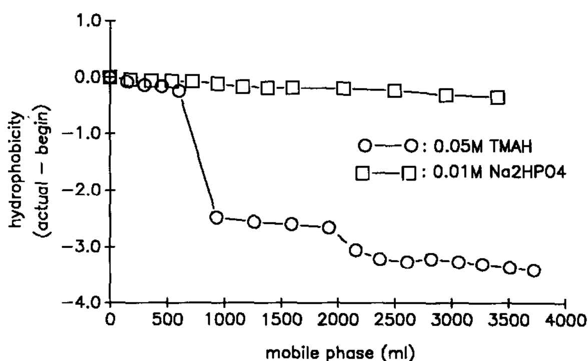

Fig. 10. Influence of the type of buffer and the volume pumped over the column on hydrophobicity (expressed as the difference between the actual and the starting values). For HPLC conditions, see Figs. 2A and 5.

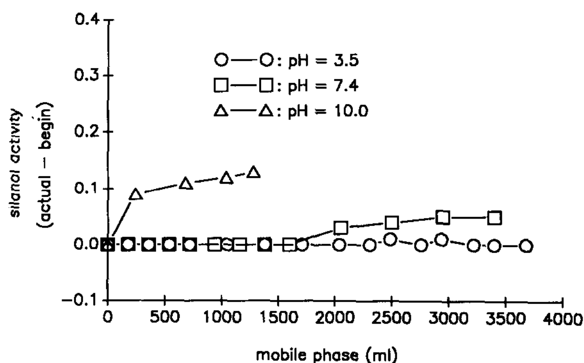

Fig. 11. Influence of the pH of sodium phosphate buffer and volume pumped over the column on silanol activity (expressed as the difference between the actual and the starting values). For HPLC conditions, see Figs. 2A and 6.

cording to the manufacturer, basic compounds can be eluted even without buffer. For the compounds in our test series this was correct. All compounds, however, had extremely asymmetric peaks with asymmetry factors of ≥ 6. The use of 10 m*M* sodium phosphate (pH 7.4) instead of water did not reduce the peak asymmetry significantly.

Suplex pKb-100. The Suplex column is a silica-based C_{18} stationary phase in which residual silanols are electrostatically shielded. First this column was tested with methanol–10 m*M* sodium phosphate (pH 7.4) as eluent. As can be seen in Fig. 12, good peak shapes were obtained. Only six compounds had an asymmetry factor larger than 2 and plate numbers were in the range 3000–11 000. Compared with μBondapak, this stationary phase is more suitable for the analysis of basic compounds.

The column was also tested without buffer using a methanol–water mobile phase, which can be advantageous when the system is coupled to a mass spectrometer. In that event the column should be "conditioned" by washing with 10 m*M* buffer. Under these conditions good peak shapes were also obtained. Only one compound showed an asymmetry factor > 2. Plate numbers were also in the range 3000–11 000. However, five compounds with pK_a > 8 showed fronting peaks. This phenomenon

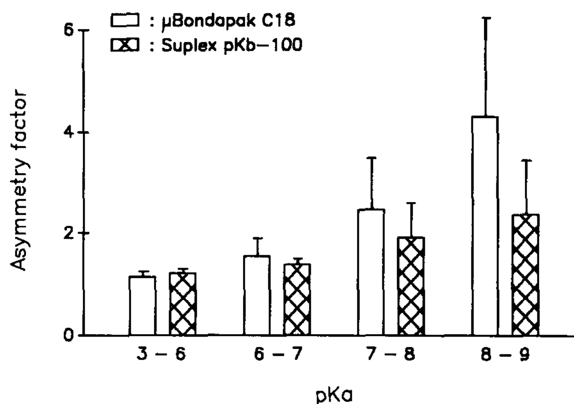

Fig. 12. Comparison between μBondapak C_{18} and Suplex pKb-100. Methanol–10 m*M* sodium phosphate (pH 7.4) was used as eluent and 2.0 μg were injected.

was not observed using a phosphate buffer. Of course, for a rugged method involving ionic and ionizable compounds, the mobile phase should always be carefully buffered. In Fig. 13 chromatograms of a compound with pK_a = 8.7 with and without buffer are shown.

In our experience, it was found that for this column the equilibration time is longer than usual. Reproducibility proved to be good in comparison with

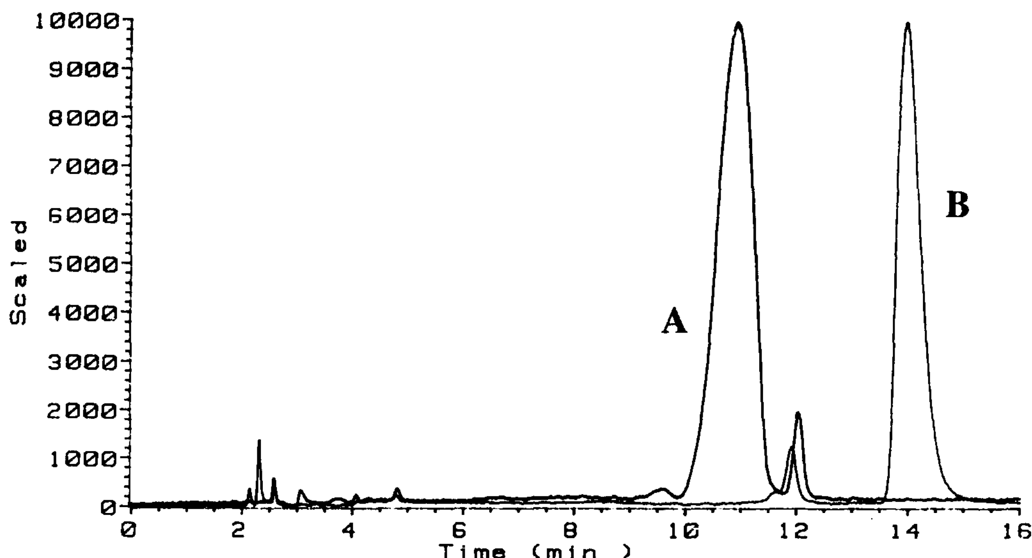

Fig. 13. Chromatograms of a basic compound (pK_a = 8.7) (A) without and (B) with 10 m*M* sodium phosphate (pH 7.4) on the Suplex pKb-100 column.

TABLE IV

COMPARISON OF THE STATIONARY PHASES

Column	Peak shape[a]	Plate number[a]	Stability[a]	Remarks
μBondapak C_{18}	0	0	0	
Nova-Pak C_{18}	−	+	0	
Kromasil KR100-5-C_{18}	0	+	0	
Zorbax Rx-C_8	0	+	0	
Lichrosorb RP-Select B	0	+	0	
Exsil 100 5 μm ODS-B	− −	−	0	Can be used without buffer
Suplex pKb-100	+	+	0	Can be used without buffer
ACT-1 C_{18}	0	− −	+	Very pH stable

[a] − −, very bad; −, bad; 0, acceptable; +, good.

conventional columns. For better stability acetonitrile should be used instead of methanol.

ACT-1 C_{18}. This column is based on a C_{18}-polymer stationary phase. The advantage over silica-based columns is that there are no ionic or ionizable species present. Further, these columns are very stable and a pH range of 0–14 can be used. Our results with this column show acceptable tailing, of the order of 1.5–3. However, a great disadvantage is the low plate numbers obtained. They are of the order of 50–250, which is less than 10% of the plate numbers obtained with a μBondapak C_{18} column.

The results obtained with the different stationary phases are summarized in Table IV, with the advantages and/or disadvantages of each column.

Comparison of μBondapak C_{18}, Zorbax Rx-C_{18} and Suplex pKb-100

The experiments in the first section under Results and Discussion strongly suggest that at pH 3.5, where the compounds are protonated, the dissociated silanols are still fully active. Even at pH 2.5, a pH recommended by most manufacturers, the peak shape improved only marginally (see Table V). Plain buffers are therefore unable to mask effectively silanol effects.

A further illustration, also using a Zorbax Rx-C_{18} column, is given in Fig. 14. At a buffer pH of 7.4 the substances are not protonated and a high portion of methanol is needed to elute them from the column. With a buffer of pH 6.0 the compounds

TABLE V

COMPARISON OF THREE COLUMNS TESTED UNDER LOW pH CONDITIONS

Column	Eluent[a]	Compound	pK_a	pH 3.5		pH 2.5		pH 2.5 + 15 mM TEA	
				k'	A_s	k'	A_s	k'	A_s
Zorbax Rx-C_{18}	A	**7**	7.9	3.2	5.1	3.1	4.3	3.1	2.1
		6	8.7	2.1	4.1	1.7	4.3	1.8	2.5
		9	7.7	2.6	4.5	2.1	4.0	2.2	2.0
		8	8.0	4.4	5.1	4.1	4.5	4.3	2.2
μBondapak C_{18}	A	**7**	7.9	3.3	8.6	3.3	7.3	3.2	1.7
		6	8.7	2.2	7.4	2.3	5.6	2.2	2.1
		9	7.7	2.4	7.7	2.5	5.8	2.4	1.6
		8	8.0	4.6	9.7	4.6	7.6	4.5	1.7
Suplex pKb-100	B	**7**	7.9	4.5	1.9	4.8	1.9	4.1	1.7
		6	8.7	1.9	1.6	2.0	1.5	1.8	1.7
		9	7.7	2.9	1.7	2.9	1.6	2.7	1.5
		8	8.0	6.2	2.0	6.4	1.9	5.4	1.7

[a] A = methanol–10 mM sodium dihydrogenphosphate (50:50); B = methanol–10 mM sodium dihydrogenphosphate (25:75).

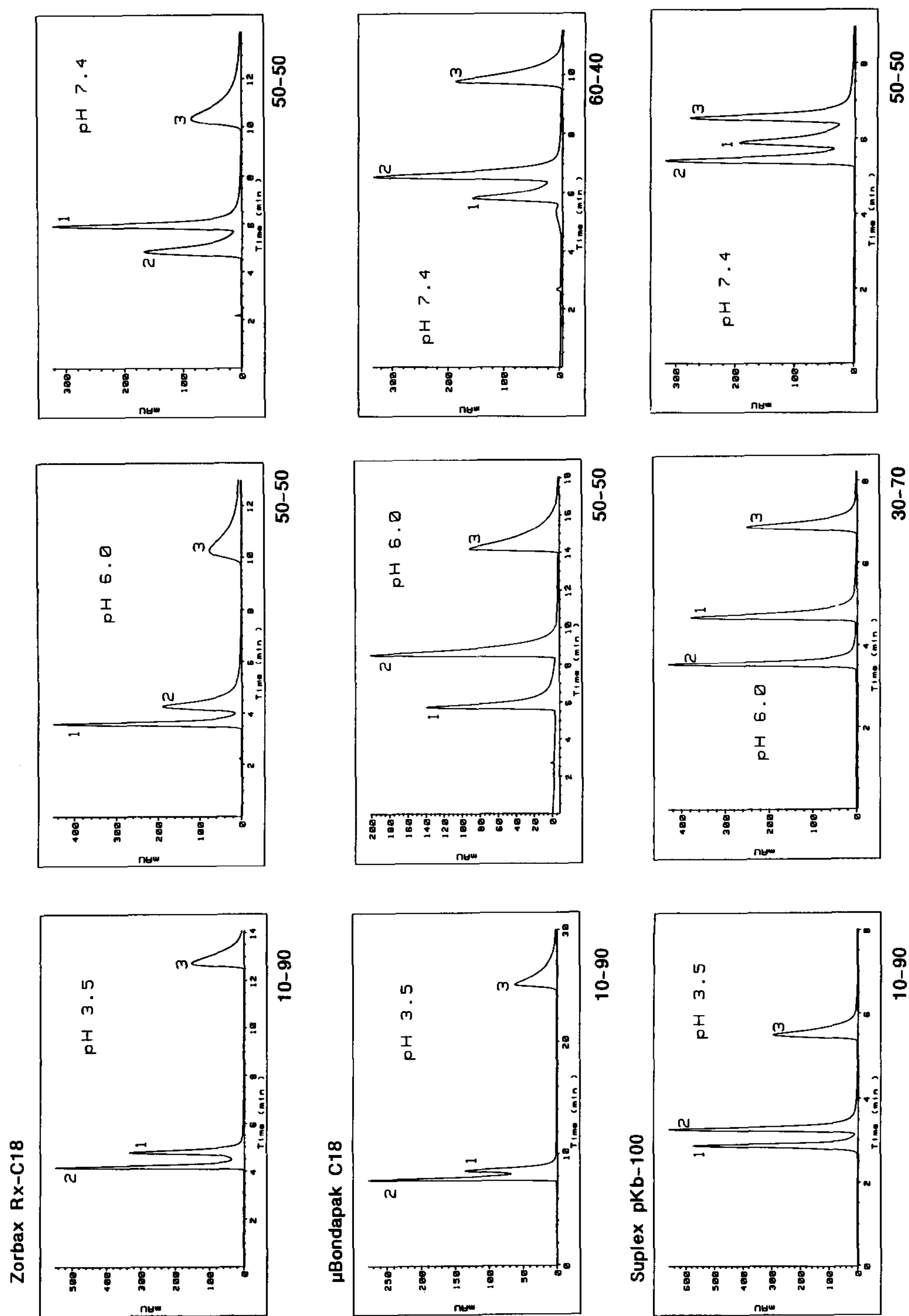

Fig. 14. Comparison of Zorbax Rx-C_{18}, μBondapak C_{18} and Supex pKb-100 at pH 7.4, 6.0 and 3.5. Solutes: 1 = oxymorphone, pK_a = 7.65; 2 = morphine, pK_a = 7.08; 3 = codeine, pK_a = 7.00. The methanol–10 mM phosphate compositions are given beneath the plots.

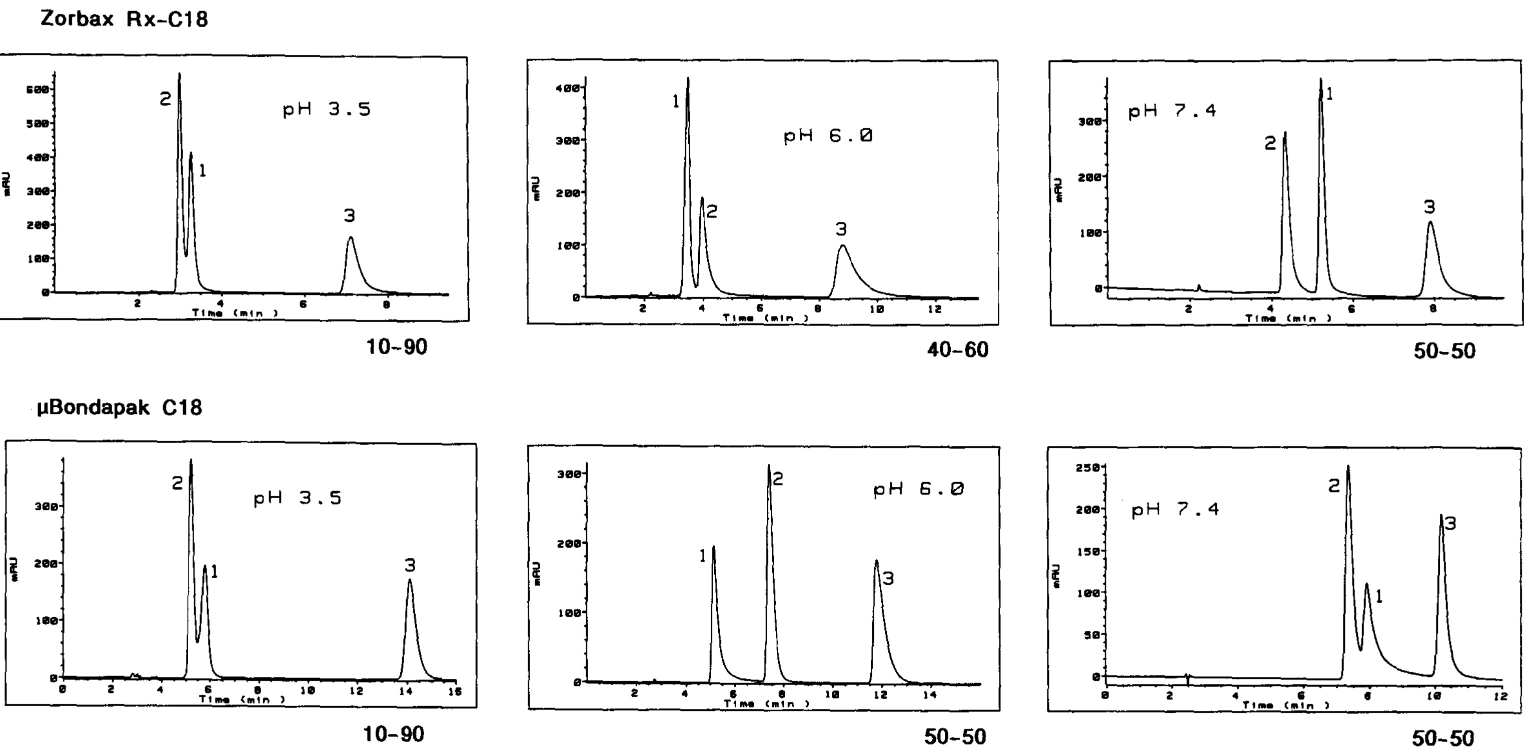

Fig. 15. Chromatograms of the separation of three morphines at pH 7.4, 6.0 and 3.5 after addition of 15 mM triethylamine to the mobile phase. The methanol–10 mM phosphate compositions are given beneath the plots.

are partly protonated (more polar, less methanol needed), which, especially for codeine, leads to increased tailing. At a pH of 3.5 the peaks are sharpened and silanol effects are suppressed. A remarkable effect is the inversion of morphine and oxymorphone as a result of the pH change.

For a set of four "difficult" compounds we also studied the effect of the addition of TEA at pH 2.5. As shown in Table V for μBondapak C_{18} and Zorbax Rx C_{18} dramatic improvements were obtained. We conclude from this critical evaluation that despite a low eluent pH and column deactivation, many active sites still exist that can only be masked by adding TEA.

A major conclusion for the chromatographer is that a low pH in the presence of TEA represents the best condition for the chromatography of basic compounds on conventional reversed-phase columns. This recommendation is again illustrated in Fig. 15. The morphines already shown in Fig. 14 were now eluted under the same conditions except for the addition of 15 m*M* TEA. Here also the results are significant.

For the Suplex column a different result was obtained. The special character of this stationary phase can also be deduced from the data in Table V. Clearly, the shielding is so effective that addition of TEA is no longer necessary. As a further illustration we refer to Fig. 14 for the separation of morphine, oxymorphone and codeine. The best condition proved to be pH 3.5, but without TEA.

CONCLUSIONS

The optimization of the peak shapes of basic compounds is difficult and many parameters can be varied. It was found that the peak tailing depends on the pK_a value and structural parameters of a compound. When tailing occurs the addition of silanol blocking reagents, such as triethylamine, was most effective in suppressing this effect. The addition of ion-pair reagents, however, did not improve the symmetry.

As far as the stationary phases are concerned, the use of an electrostatically shielded phase improved the peak shapes. This type of column showed acceptable tailing, even without the addition of additives that suppress silanol activity. With a polymer-based stationary phase reasonable peak shapes were obtained. However, owing to the low plate number, this column has a poor separation efficiency.

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