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Characterisation of reversed-phase liquid chromatography stationary phases for the analysis of basic pharmaceuticals: eluent properties and comparison of empirical test methods

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Abstract

The reversed-phase liquid chromatographic analysis of basic pharmaceuticals can be problematic. Both the properties of the eluent and the stationary phase can influence the chromatographic performance. Therefore selection of suitable experimental conditions for the analysis of basic compounds can be difficult. This paper shows that the organic modifier and the nature of the buffer influence the eluent properties. Moreover, the nature and amount of modifier also influence the basicity of the analytes. Investigations showed that the nature of the buffer can have a significant influence on retention and peak shape of basic compounds. Test procedures using basic analytes as test probes provided relevant information with respect to selecting columns to analyse basic pharmaceutical compounds. Test procedures using compounds like aniline, phenol and benzene were found to be less suitable. © 2001 Published by Elsevier Science B.V.

Keywords: Stationary phases, LC; Mobile phase composition; Column selection; Basic drugs

1. Introduction

Reversed phase high performance liquid chromatography (RPLC) using silica-based C_{18} or C_8 stationary phases is used in the pharmaceutical industry for quality control and biomedical analysis. Generally the RPLC analysis is promoted by symmetrical peaks which, compared to asymmetrical peaks, will result in lower limits of detection and quantitation, improved resolution and less dispersion between the calculated quantitative results because of improved determination of the area of the chromatographic peak [1,2].

Due to ionic interactions of basic pharmaceutical compounds with residual silanols and other active sites of the reversed-phase stationary phase, ionic

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interactions occur together with the hydrophobic interaction. These can result in asymmetrical peaks and irreproducible retention. The ionic interactions comprise a number of stationary phase–solute interactions such as ion–ion (ion-exchange), ion–dipole, dipole–dipole (e.g. hydrogen bonding), dipole–induced dipole and induced dipole–induced dipole (London forces) [3,4]. Developments in manufacturing of stationary phases have led to the existence of a large number of different phases. For the analysis of basic compounds these phases can, due to the different manufacturing procedures and silicas used, result in large differences between peak shape and selectivity. The development of stationary phases suitable to analyse basic substances is concentrated on minimising the interaction between residual silanols responsible for asymmetrical peaks, with the basic analyte. Examples of such phases are phases utilising high purity silica [5–7], end-capping procedures [8–11], polymer encapsulation [12–15], embedded polar groups [16,17], bidentate phases [7,18] and phases from which the silica backbone is modified by introducing organic functional groups [19,20].

The selection of a suitable stationary phase is an important parameter in the development of LC methods. Characterisation of stationary phases using generally accepted test procedures would make the choice of a suitable column for a dedicated application easier. As recently discussed, column evaluation tests can be divided into empirical tests, thermodynamically-based tests and tests based on a retention model [21]. Empirical tests have in common that the information is obtained by using test compounds and eluents chosen according to a certain line of thought. Determination of silanol interactions is commonly based on relative retention of compounds of which the retention is mainly expected to be caused by hydrophobic and silanol interaction, and compounds from which the retention is based on hydrophobic interactions [22,23]. Tests based on retention models are e.g. the test of Galushko [24–26] and linear solvent retention relationship studies [27–30], whereas thermodynamically-based tests use van het Hoff plots [31–33]. Several of these tests were recently compared with respect to determining ionic and hydrophobic properties. The hydrophobic properties determined with the various tests were in good agreement, but the tests determined different ionic properties and other polar activities [21].

Next to the nature of the stationary phase, an important parameter in RPLC is the nature of the mobile phase. Some column characterisation tests are performed using buffered eluents whereas others are performed using non-buffered eluents. Both buffering and not buffering of the mobile phase shows advantages and disadvantages. Since buffers can mask silanol interactions, testing in unbuffered systems can be advantageous for silanol activity determination [23]. However, a test generally accepted for column characterisation should be rugged and reproduced in many different labs worldwide. This can only be achieved by using a stable well-defined eluent, which can be obtained using buffered solvents. It was recently shown that buffering of the eluent is mandatory for reproducible test results [34,35]. Another aspect is the nature of the buffer. In the majority of tests developed, phosphate buffers are used to control the pH of the eluent [34–39]. However, since buffer ions can interact with residual silanols and with basic analytes [40,41], it is obvious that the choice of the buffer can influence the results of the column tests. As part of testing various mobile phase compositions, also the applicability of volatile buffers was investigated. Volatile buffers present an alternative to the involatile phosphate buffers to enhance the compatibility with MS.

In the present study several column test procedures are compared. Most test procedures are developed for general column characterisation purposes and the majority of these tests mainly determine two stationary phase characteristics, i.e. hydrophobic and ionic properties. In this study especially the applicability of stationary phases in the analysis of basic pharmaceuticals is investigated. The tests were performed to determine ionic properties of the stationary phases, which are most important for the analysis of basic compounds. The results of the column characterisation using the tests of Engelhardt [23], Tanaka [22], Galushko [24–26] and McCalley [36–38] were related to the chromatographic data obtained by analysing a selection of seven basic pharmaceutical drugs from NV Organon. The influence of various buffers on the analysis of these basic compounds and on the results of column characterisation tests was included.

2. Experimental

2.1. Apparatus

The determinations of eluent pH were carried out using a Methrom 713 pH meter (Metrohm, Herisau, Switzerland) and a combined glass electrode (Hamilton, Bonaduz, Switzerland). The pH meter was calibrated using buffers pH 4.00, 7.00 and 9.00. The titrations of the eluent were performed using a Metrohm 670 Titriprocessor.

The HPLC experiments were performed using a HP1100 liquid chromatograph consisting of a quaternary pump, solvent degasser, autosampler, column oven and diode array detector (Agilent Technologies, Amstelveen, The Netherlands). LC–UV chromatograms were collected using a HPLC 3^D Chemstation (Agilent Technologies).

2.2. Chemicals

The basic compounds of the Organon compound mixture were obtained from NV Organon (Oss, The Netherlands). In Fig. 1 the structures and the related pK_a values are shown. The compounds for the Engelhardt, Galushko, Tanaka and McCalley tests were obtained from various manufacturers and are of p.a. quality. The McCalley test compounds and related pK_a values are shown in Fig. 2. As organic modifier methanol (MeOH), supplied by J.T. Baker (Deventer, The Netherlands), was used. The buffers were prepared from disodium hydrogen phosphate, sodium dihydrogen phosphate, ammonium phosphate, acetic acid (99–100%), orthophosphoric acid (85%) and citric acid monohydrate supplied by J.T. Baker. Ammonium citrate was purchased from Aldrich (Milwaukee, WI) and ammonium acetate from Janssen Chimica (Geel, Belgium). Aqueous (25 m*M*) solutions of acetic acid, ammonium acetate, citric acid, ammonium citrate, ammonia, ammonium monohydrogen phosphate, ammonium dihydrogen phosphate, sodium dihydrogen phosphate and sodium monohydrogen phosphate were prepared.

2.3. Determination of buffer pH and analyte pK_a

The buffers were prepared by titration of an electrolyte solution with an equimolar electrolyte solution with different pH, until the desired pH value was reached. For example, 25 m*M* sodium phosphate buffer pH 7 was obtained by titration of 25 m*M*

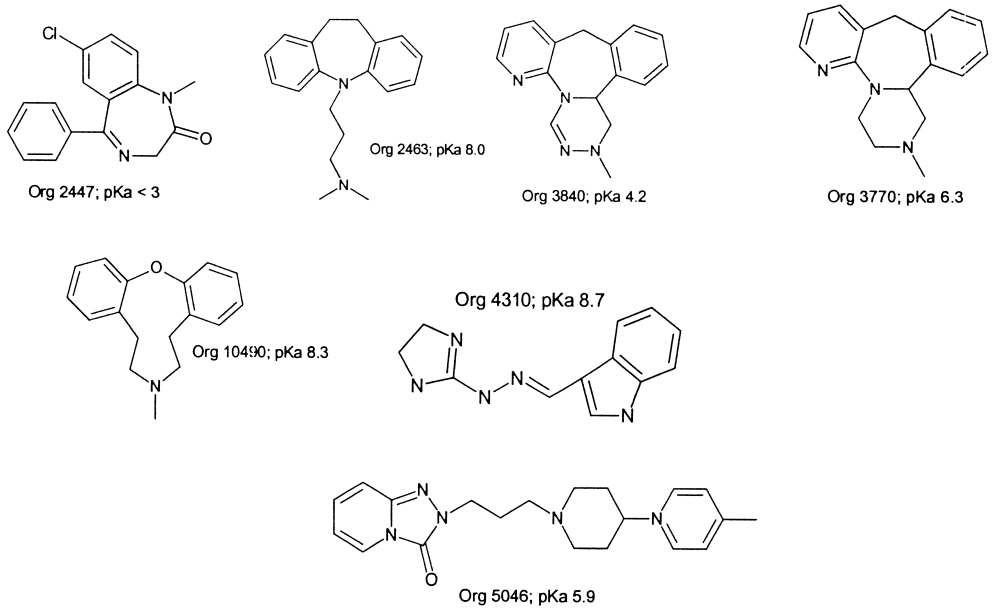

Fig. 1. Structures of the Organon compounds used in this study. The pK_a values were measured in MeOH + H_2O/60 + 40% v/v.

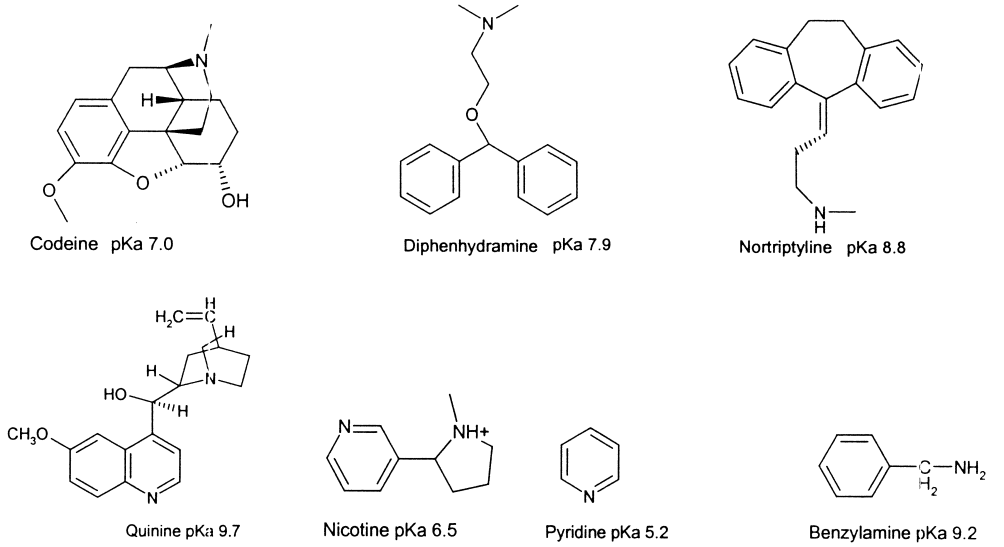

Fig. 2. Structures of the McCalley test compounds used in this study. The pK_a values were measured in water.

sodium monohydrogen phosphate with 25 m*M* sodium dihydrogen phosphate until pH 7.

To determine $^{s}_{w}pK_a$ values, solutions of mirtazipine in water, and in modifier+water mixtures of 10+90, 30+70 and 50+50% v/v were titrated with 0.03 *M* HCl. As $^{s}_{w}pK_a$ the $^{s}_{w}$pH value at half the equivalent point of the titration curve was taken.

2.4. Column tests

To avoid overloading of the stationary phases by basic compounds, as recently discussed [38], for the McCalley and the Organon test compounds amounts of 0.1 μg of each test compound were injected onto the column. For the Galushko test 2 μl of a solution of 0.03 mg uracil, 0.21 mg aniline, 1.78 mg phenol, 8 mg benzene and 8 mg toluene in 5 ml methanol were injected. For the other tests the amounts as described in the literature were injected onto the column [22,23]. The flow-rate was set to 1.0 ml/min. The stationary phases studied are shown in Table 1. The analytical conditions are given in Table 2.

Firstly, the columns were tested for ionic properties using the test procedures as summarised in Table 2. Secondly, to study the influence of the nature of buffers on the test results, the water or buffer moiety of the eluents of the original tests was replaced by 25 m*M* solutions of ammonium citrate, ammonium acetate, ammonium phosphate and sodium phosphate pH 3 and 7.

Table 1
Investigated stationary phases; dimensions are 150×4.6 mm I.D.

Stationary phase	Manufacturer	Abbreviation	Bonding characteristics
Symmetry shield C_{18}	Waters	SYSH	Embedded polar group
Symmetry C_{18}	Waters	SYMM	High purity silica
Zorbax SB-C_{18}	Agilent technologies	ZOSB	Sterically protected
Zorbax extend-C_{18}	Agilent technologies	ZOBI	Bidentate bonded alkyl chains
Alltima C_{18}	Alltech	ALLT	Polymeric bonded phase, endcapped
Luna C_{18}	Phenomenex	LUNA	High purity silica
Discovery C_{18}	Supelco	DISC	High purity silica

Table 2
Column tests used in this study

Test	Test compounds	Calculation	Experimental conditions	Reference
Tanaka	Caffeine, benzylamine, phenol	Hydrogen bonding: $k_{caffeine}/k_{phenol}$ Ion exchange capacity: $k_{benzylamine}/k_{phenol}$	Methanol + water/55 + 45% (v/v), column temperature 40°C Methanol + 25 m*M* sodium phosphate pH 3/55 + 45% (v/v) Methanol + 25 m*M* sodium phosphate pH 7/55 + 45% (v/v)	[22]
Engelhardt	Aniline, phenol, 4-ethylaniline	Silanol activity: $k_{aniline}/k_{phenol}$ $Tf_{4\text{-ethylaniline}}$	Methanol + water/55 + 45% (v/v), column temperature 40°C	[23]
Galushko	Aniline, phenol, benzene, toluene	NH-interaction and polarity calculated using Chromlife software	Methanol + water/60 + 40% (v/v), column temperature 30°C	[24–26]
McCalley	Pyridine, quinine, benzylamine, diphenhydramine, codeine, nortriptyline, nicotine	Tailing and retention of the test compounds	Methanol + 25 m*M* sodium phosphate pH 3: for pyridine nicotine, codeine, quinine and benzylamine ratio 30 + 70% (v/v); for diphenhydramine and nortriptyline ratio 55 + 45% (v/v) Methanol + 25 m*M* sodium phosphate pH 7 for all compounds ratio 65 + 35% (v/v)	[36–38]
Organon	Org 2447, Org 4310, Org 3840, Org 10490, Org 2463, Org 3770, Org 5046	Tailing and retention of the test compounds	Methanol + 25 m*M* sodium phosphate pH 3/50 + 50% (v/v) Methanol + 25 m*M* sodium phosphate pH 7/50 + 50% (v/v)	[39]

2.5. Calculations

The column characteristics were calculated according to the literature [22–26,36–39] and as shown in Table 2. The results of the Galushko test were calculated using the software program Chromlife (Merck, Darmstadt, Germany). The USP tailing factors were calculated at 5% of the peak height using the HPLC^{3D} Chemstation software:

$$Tf = \frac{w_{0.05}}{2w_{a,0.05}} \quad (1)$$

where $w_{0.05}$ is the width of the peak at 5% of the peak height and $w_{a,0.05}$ is the width of the front side of the peak at 5% of the peak height. Correlation coefficients between the test variables were calculated using Microsoft Excel 5.0 software.

3. Results and discussion

3.1. Eluent composition

In the analysis of substances that are ionisable, the pH of the eluent will affect the degree of ionisation of the basic analyte. Moreover, the pH of the eluent will also control the degree of ionisation of residual silanols present on the surface of the stationary phase. Therefore, the pH of the eluent will influence the ionic interactions between the basic analyte and the stationary phase. It is obvious that eluent pH is a very important parameter in the RPLC analysis of basic analytes and can influence the results obtained with the various test procedures applied in this study.

In LC it is common practice to measure the pH of the mobile phase buffer before mixing with the organic modifier. However, it is also recognised that the pH changes after addition of organic solvents [34,42]. For organic modifier/buffer mixtures the pH can be calculated using measurements with conventional pH electrodes:

$$^{s}_{s}\mathrm{pH} = {}^{s}_{w}\mathrm{pH} - \delta \quad (2)$$

where $^{s}_{s}$pH is the pH value in an aqueous–organic system, $^{s}_{w}$pH is the measured (apparent) value in an aqueous–organic system and δ is a correction factor for the liquid junction between the electrode and the eluent [43]. Values of δ have been published for several organic modifier–aqueous systems. The correction factor (δ) is small (~0.2) for amounts of methanol up to 80% and acetonitrile up to 50% [44–47].

In Fig. 3 the relation between the pH of the pure buffer solution and the pH of the methanol + 25 m*M* sodium phosphate buffers/50 + 50% v/v solutions from pH 3 up to pH 7 is shown. As can be seen, addition of methanol to the sodium phosphate buffers

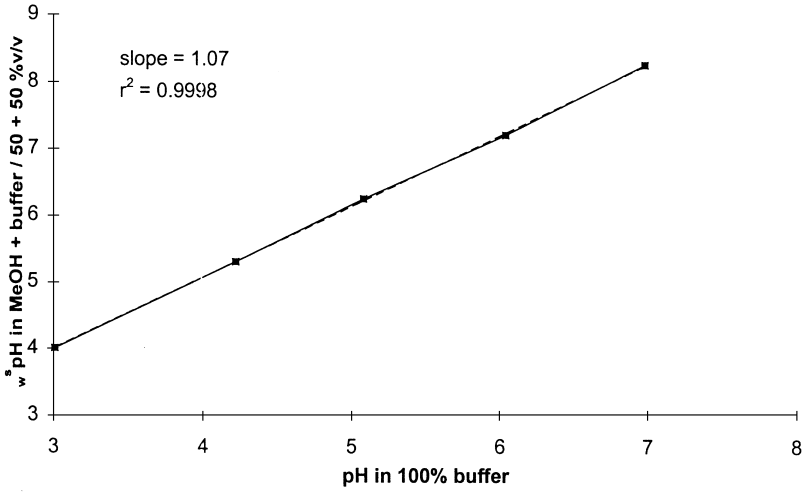

Fig. 3. Relation between buffer pH and ${}^{s}_{w}$pH of the eluent. The buffers used are for 25 m*M* sodium phosphate and the solvents used are MeOH + buffer/50 + 50% v/v.

resulted in an increased eluent pH and, in the pH range studied, the relation between the buffer pH and the pH of the methanol + 25 m*M* sodium phosphate buffers/50 + 50% v/v solutions is linear ($r^2 = 0.9998$). Comparable data were obtained using ammonium acetate, ammonium citrate and ammonium phosphate buffers. Differences were found between the slopes of the curves. For sodium phosphate, the slope of the curve is 1.07, meaning that the pH shift depends slightly on the buffer pH. For ammonium acetate the slope was 0.96, for ammonium citrate 1.06 and for ammonium phosphate 1.01, respectively. The discussed data were obtained using methanol. Barbosa et al. [42] also showed that the nature of the modifier influences the eluent pH. Different eluent pH values were obtained mixing buffers with methanol, acetonitrile and tetrahydrofuran.

The advantage of buffering the mobile phase is maintaining a constant pH during the RPLC analysis, which for ionisable compounds is important to obtain reproducible and robust analyses. A stable pH is obtained when the applied pH is in the buffering range of the buffer. In Fig. 4 the influence of the nature of modifiers commonly used in LC, e.g. methanol, acetonitrile and tetrahydrofuran, on the buffering range of the eluent containing sodium phosphate buffer is shown. As can be seen the nature of the modifier did not significantly influence the buffering range of the eluent. Detailed studies about the influence of the nature of modifier and buffer on eluent pH were reported previously [46,47].

Besides the pH of the eluent, the organic modifier will also influence the pK_a of the basic analyte. Comparable to the pH of the eluent, the pK_a values of the analytes are often measured in pure water. In Fig. 5 the influence of the nature and concentration of organic modifier on the pK_a of a basic pharmaceutical, i.e. mirtazipine, is shown. Since the correction factor δ up to 50% of modifier is small [44–47] the determined ${}^{s}_{w}$pH values are approximately equal to ${}^{s}_{s}$pH values. Hence, the ${}^{s}_{w}$pK_a values can be used to discuss differences and trends in pK_a values of compounds upon addition of organic modifier. As can be expected, the pK_a of the basic substance is influenced: increasing the modifier concentration lowers the pK_a value of the basic analyte. The effect was similar for acetonitrile and methanol, however, the effect was significantly different for tetrahydrofuran. The latter can be explained by comparing the normalised hydrogen bond acceptability (β) of the solvents used. A higher hydrogen bond acceptability of the solvent will lower the pK_a value of the basic analyte. For MeOH and ACN β is 0.29 and 0.25, whereas for THF β is 0.49, respectively [48]. Therefore, the pK_as for mirtazipine with MeOH and ACN are comparable, and for THF is significantly lower.

It is clear that the influence of modifier on eluent

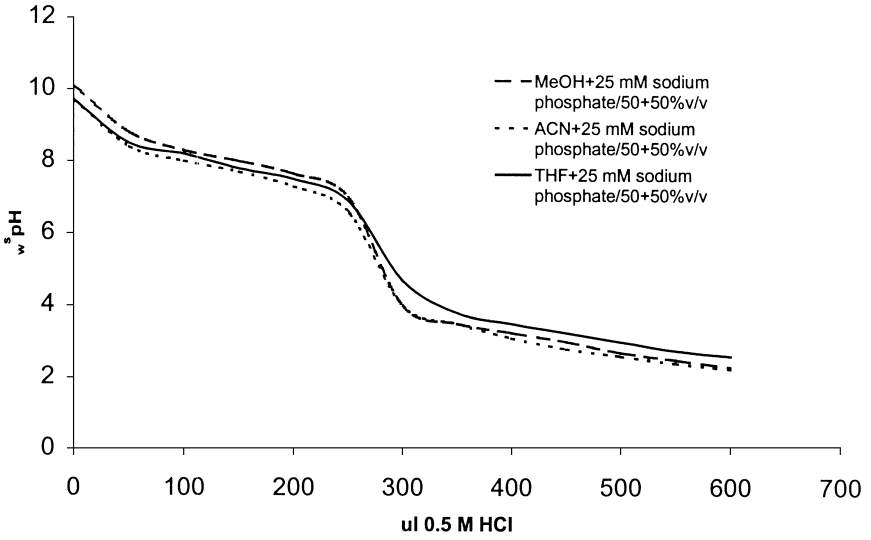

Fig. 4. Influence of the nature of the modifier on the buffering range of the eluent.

pH and analyte pK_a should be taken into account when performing and discussing LC analysis of basic analytes. As an example the shift in retention for propranolol as recently described by Neue et al. [34,35] can be taken. The shift in retention for propranolol was not expected from the pH of the buffer and pK_a of the analyte. Both the pH and pK_a values were measured in pure water. In this study it is shown that in the eluent containing organic modifier the pK_a of the analyte will be lower, whereas the pH of the eluent will be higher. As a result, the analyte might be analysed as a deprotonated compound, explaining the retention shift. Moreover, the possible influence of the stationary phase on the pH of the eluent inside the column should also be considered as well as the influence of

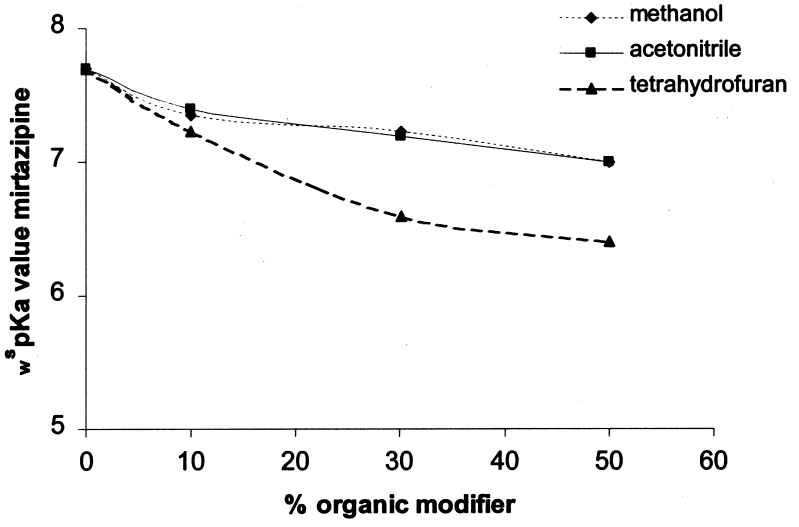

Fig. 5. Influence of the nature and concentration of the modifier on $^{s}_{w}$pK_a value of mirtazipine.

the organic modifier on the pK_a of the residual silanol groups of the stationary phase [49].

In summary, from the results discussed above it is clear that control and proper usage of the pH of the eluent is important in the analysis of basic analytes. The amount and nature of the organic modifier influence pH of the eluent, pK_a of the basic substance and pK_a of the silanol groups of the stationary phase. Increasing the amount of modifier will increase the pH of the eluent, whereas the pK_a value of the basic pharmaceuticals will decrease. This means that in e.g. gradient analysis, besides a change in modifier concentration, also a change in eluent pH and pK_a of the analytes occurs.

3.2. Comparison of various test procedures

The applicability of the testing procedures described in Table 2 to characterise stationary phases for the analysis of basic pharmaceuticals is investigated by testing the stationary phases shown in Table 1. Comparing the test procedures in Table 2, differences are observed between the test compounds as well as the eluent conditions used. Analytes like aniline and phenol are frequently used both in empirical (Engelhardt) and model-based (Galushko) tests [23–26], whereas Tanaka used caffeine, benzylamine and phenol [22]. McCalley developed a test focused on testing columns for the analysis of basic substances using compounds generally available and structurally comparable to the compounds of interest, i.e. basic analytes [36–38]. One of the McCalley test compounds is amphetamine, which belongs to the class of controlled drugs. For safety reasons these types of compounds are not generally available and can therefore not be included in a test applied worldwide. Therefore, in the McCalley test quinine, nicotine, pyridine, benzylamine, diphenhydramine, codeine and nortriptyline were used as test compounds (Fig. 2). For comparison and as a reference to daily practice, also seven basic Organon drugs differing in pK_a range from <3 to 8.7 were studied (Fig. 1). For the Organon and McCalley tests the average *Tf* values obtained for the test compounds were used. The Engelhardt and Galushko tests are as prescribed not buffered, as well as the hydrogen bonding test procedure of the Tanaka test. With the McCalley test, the ion-exchange capacity of the Tanaka tests and the Organon test phosphate buffers are used to control the ionisation and retention of the test analytes. Retention (k) and peak tailing (Tf) data are used in the various tests. Since *Tf*, compared to k, is more sensitive to extra-column effects and/or column ageing, the neutral compound in the Organon test mix (Org 2447, p$K_a<3$) was used to control these effects. It was observed that with all eluents and all columns used the shape of the Org 2447 peak was symmetrical: $0.95 < Tf < 1.15$ and therefore it was concluded that the columns studied were in acceptable condition when used.

In the first approach a qualitative comparison of the tests was performed by ranking the columns (see Table 3) based on the parameters calculated as described in Table 2. A lower ranking number represents a better suitability of the column to analyse basic pharmaceuticals. As can be seen, no uniform ranking of the columns is obtained using the five tests. For the LUNA column, however, the McCalley and Organon data were in agreement, i.e. symmetrical peak shapes were obtained using the LUNA column. Using the tests of Engelhardt, Tanaka and Galushko however, the SYSH column generally showed the best characteristics and on the basis of these tests the LUNA column would not be the column of choice. The other columns were ranked differently using the various tests. It should be noted, however, that the differences between the ranking in some cases were relatively small. As shown in Table 3 testing must be performed at more than one eluent pH. For the McCalley and Organon compounds, the DISC column showed good performance when used with buffer pH 7, but when used with buffer pH 3 asymmetrical peaks were obtained.

As recently discussed by Kele and Guiochon, the interaction and/or the accessibility of the silica surface most probably depends on the molecular structure of the analyte, as illustrated by different trends for aniline and *N*,*N*-dimethylaniline [49]. As the structural diversity between the Engelhardt, Tanaka and Galushko test compounds on one side, and the McCalley and Organon test compounds on the other side are relatively large (e.g. aniline vs. diphenhydramine, benzylamine vs. Org 2463), determination of stationary phase characteristics using the various test analytes can result in different test

Table 3
Ranking of stationary phases using the tests as described in Table 2. The lower the ranking number, the more suitable the column for analysing basic substances

Test	SYSH	SYMM	ZOSB	ZOBI	ALLT	LUNA	DISC
Engelhardt silanol activity	1	4	6	4	7	2	2
Engelhardt *Tf* 4-ethylaniline	4	1	3	5	7	1	5
Tanaka hydrogen bonding	1	2	7	2	6	5	4
Tanaka ion-exchange capacity pH 3	1	2	7	4	5	3	6
Tanaka ion-exchange capacity pH 7	1	2	7	2	6	5	4
Galushko NH interaction	1	4	6	5	7	2	3
McCalley pH 3[a]	1	5	1	1	6	1	7
McCalley pH 7[a]	2	2	5	6	7	1	2
Organon pH 3[a]	1	1	5	4	7	1	6
Organon pH 7[a]	3	5	4	6	6	1	2

[a] Based on average *Tf* values of the test compounds.

results depending on the test analytes used. In comparison, the data of the McCalley test are more in agreement with the data of the Organon test compounds. As is shown taking both buffer pH 3 and pH 7 into account, both tests classify the LUNA column as most suitable, the SYSH column shows to be a good alternative, whereas the ALLT column is less suitable, for the analysis of basic solutes with regard to peak shape. Moreover, both the McCalley and Organon tests classify the DISC column as second best when used with buffer pH 7, but less useful when used with buffer pH 3. The ALLT column being the highest ranked column of the seven columns studied is similarly ranked by the Engelhardt and Galushko test results. Therefore, it was concluded that differences between generations of columns can be determined using tests like the Engelhardt test. However, often small differences in separation performance exist between the modern phases of today. It was concluded that these small differences can best be detected using basic compounds as test analytes. Using the McCalley test compounds the SYMM, ZOSB and ZOBI were also ranked differently, compared to the ranking obtained using the Organon compounds. This is also due to the differences between the McCalley and Organon test compounds. When comparing the ranking of the columns using test compounds with comparable molecular structures i.e. diphenhydramine and Org 2463 (both compounds have a tertiary amine situated at the end of an aliphatic chain) a comparable ranking of the columns was obtained (data not shown). To illustrate the importance of a proper column selection for LC method development, in Fig. 6 the LC–UV analysis of a candidate drug

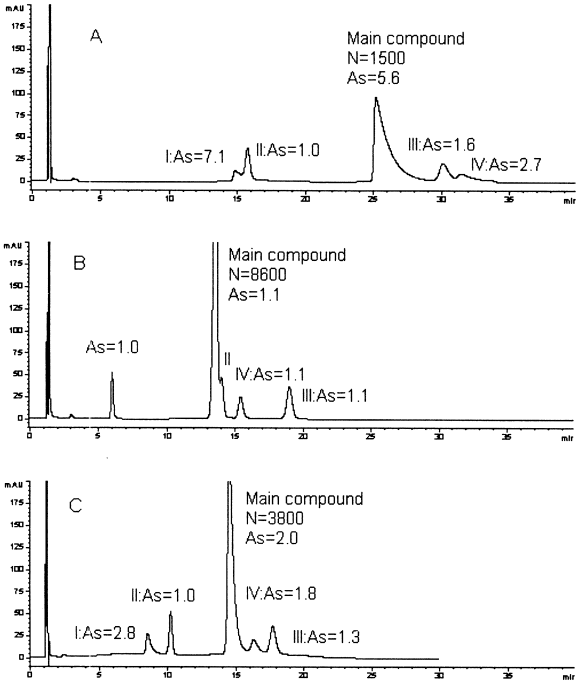

Fig. 6. Comparison of Alltima C_{18} (A), Luna C_{18} (B) and Zorbax SB-C_{18} (C) columns. The eluent used was MeOH+25 m*M* NH_4Ac pH 7/50+50% v/v at a flow-rate of 1.0 ml/min and the columns were thermostated at 40°C. The main compound was spiked with 10% of related substances.

currently under development and spiked with related substances is shown, using the ALLT, LUNA and ZOSB columns. In a second approach the influence of the nature of the buffer and mobile phase pH on the test results was studied. Brereton and McCalley already demonstrated that the nature of the modifier influenced the applicability of modern packing materials for the analysis of basic analytes [50]. In this study we investigated the influence of the nature of the buffer by replacing the water or buffer moiety of the test eluents in Table 2 by 25 m*M* ammonium citrate, ammonium acetate, ammonium phosphate and sodium phosphate buffers pH 7 and 3. Using buffers pH 7, the influence of the nature of the buffers on ranking of the columns was small. Using buffers pH 3, the nature of the buffers showed a larger influence on column ranking (data not shown). The McCalley and Organon results were based on average peak tailing data. However, we noticed that the influence of eluent composition on chromatographic performance of individual basic compounds was large. Therefore, the influence of the nature of the buffer on peak shape and retention of basic compounds will be discussed in the next part in more detail.

3.3. Influence of the nature of buffer on peak tailing and retention of basic analytes

To study the influence of the nature of the buffer on retention and peak tailing using the McCalley and Organon test compounds, correlations for retention and peak tailing values obtained with the seven columns were determined. Low correlation between the measured variables with the different buffers indicates a significant effect of the nature of the buffer on retention or peak shape.

In several cases for peak tailing a significant influence of the nature of the buffer was observed. As an example, correlations between the peak tailing data for the McCalley analytes are shown in Table 4. Using buffers pH 7, for pyridine, nicotine, benzylamine and nortriptyline besides the stationary phase, the nature of the buffer also affected the shape of the peak. For codeine, quinine and diphenhydramine the peak shape is hardly affected by the nature of the buffers. The peak shape of the latter compounds was affected by the nature of the stationary phase only. Generally when using buffers pH 3, the correlation for peak tailing between the buffers was low. Except when comparing the peak shapes obtained when using ammonium phosphate and sodium phosphate buffers e.g. for codeine, quinine, benzylamine, diphenhydramine and nortriptyline the correlation was ≥ 0.9 meaning that the effect of the cation on peak shape for these compounds was small. In Fig. 7 for diphenhydramine, examples of a high (ammonium phosphate versus sodium phosphate) and a low (ammonium phosphate versus ammonium acetate) correlation between peak tailing obtained with the seven columns is shown. For the Organon analytes the correlation between the peak tailing data usually was low. Only when analysing Org 10490 using buffers pH 3 the type of buffer hardly influenced the peak shape, i.e. correlation ≥ 0.9.

For some McCalley compounds correlation coefficients between the retention factors, obtained with the four buffers at pH 7 were ~ 0.85, but > 0.95 for the other compounds. This showed that the retention behaviour of the compounds when using the buffers at pH 7 with the seven columns is comparable: the nature of the buffer at buffer pH 7 did not affect the selectivity of the McCalley test compounds using the columns shown in Table 1. With buffers pH 3, however, an effect of the nature of the buffer was observed. Using ammonium acetate low correlation for retention with the other buffers were obtained with pyridine, nicotine, codeine and benzylamine (correlation ≤ 0.7), meaning the selectivity for the compounds with the seven columns used with ammonium acetate buffer pH 3 is different. Comparison of ammonium phosphate and sodium phosphate buffers pH 3 showed a good correlation (≥ 0.9) for retention, i.e. the type of cation of the buffer (sodium vs. ammonium) had no significant effect on the retention. For the Organon compounds, comparable phenomena were observed. Using buffers pH 7 comparable selectivity is obtained with the columns in Table 1. When using buffers pH 3, also for the Organon compounds a low correlation is obtained when comparing ammonium acetate with the other buffers.

From these data it is clear that the mechanism of ionic interactions between the stationary phase and the basic analytes is very complex. As discussed by

Table 4
Correlation matrix for *Tf* for the McCalley compounds using buffers pH 3 and pH 7

pH 3	AmAc	AmCit	AmPho	NaPho	pH 7	AmAc	AmCit	AmPho	NaPho
Pyridine									
AmAc	1	−0.50	0.56	0.43	AmAc	1	−0.50	−0.21	−0.14
AmCit		1	−0.60	0.13	AmCit		1	0.64	−0.16
AmPho			1	−0.17	AmPho			1	0.33
NaPho				1	NaPho				1
Nicotine									
AmAc	1	−0.25	−0.33	0.56	AmAc	1	0.77	0.72	0.65
AmCit		1	−0.51	−0.80	AmCit		1	0.91	0.21
AmPho			1	0.40	AmPho			1	0.06
NaPho				1	NaPho				1
Codeine									
AmAc	1	−0.30	−0.13	−0.05	AmAc	1	0.90	0.94	0.76
AmCit		1	0.71	0.72	AmCit		1	0.97	0.89
AmPho			1	0.99	AmPho			1	0.97
NaPho				1	NaPho				1
Quinine									
AmAc	1	0.46	0.07	0.18	AmAc	1	0.98	0.99	0.96
AmCit		1	0.89	0.95	AmCit		1	0.99	0.97
AmPho			1	0.96	AmPho			1	0.98
NaPho				1	NaPho				1
Benzylamine									
AmAc	1	−0.04	−0.05	0.10	AmAc	1	0.81	0.94	0.17
AmCit		1	0.96	0.98	AmCit		1	0.81	0.32
AmPho			1	0.93	AmPho			1	0.25
NaPho				1	NaPho				1
Diphenhydramine									
AmAc	1	0.80	0.11	0.13	AmAc	1	0.99	0.93	0.96
AmCit		1	0.52	0.53	AmCit		1	0.92	0.94
AmPho			1	0.99	AmPho			1	0.80
NaPho				1	NaPho				1
Nortriptyline									
AmAc	1	0.65	−0.02	0.01	AmAc	1	0.80	0.40	0.92
AmCit		1	0.54	0.55	AmCit		1	0.86	0.89
AmPho			1	0.99	AmPho			1	0.55
NaPho				1	NaPho				1

Nawrocki [4], ionic interactions comprise of a number of stationary phase–solute interactions such as ion–ion (ion-exchange) ion–dipole, dipole–dipole (e.g. hydrogen bonding), dipole–induced dipole and induced dipole–induced dipole. Moreover, the influence of counterions of different lipophilicity, size and flexibility on ion-pair partition was recently studied for some drugs [51–53]. The size and flexibility of both analyte and counterion were essential in the interaction between these, whereas the lipophilicity of the analytes could be influenced by the size of the counterions. LoBrutto et al. showed that retention of bases depends on the nature of buffer ions and its concentration in the eluent [40,41]. The effects can be attributed to the ionic interactions of protonated analytes with oppositely charged buffer ions, which results in the formation of ion pairs or the disruption of the analyte solvation. It

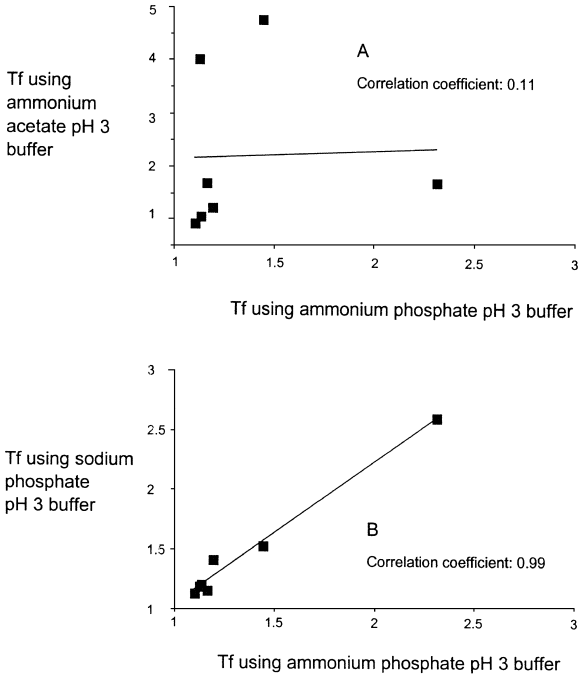

Fig. 7. Example of low (A) and high (B) correlation with calculated regression line. The tailing factor values (*Tf*) were obtained for diphenhydramine using the seven columns in Table 1. The mobile phases used were: methanol+25 m*M* ammonium phosphate pH 3/55+45% (v/v), methanol+25 m*M* sodium phosphate pH 3/55+45% (v/v) and methanol+25 m*M* ammonium acetate pH 3/55+45% (v/v).

is obvious that various factors affect the interaction between analyte and residual silanols. It depends on the molecular structure of the analyte, the nature of the stationary phase and on the properties of the eluent used.

In summary, it is shown that characterisation of stationary phases for the analysis of basic pharmaceuticals depends on the type of test analyte and on the properties of the eluent. Although the nature of the buffer showed a small influence on ranking of the columns, the influence of the nature of the buffer on the peak shape for individual basic compounds was obvious. From the literature it is clear that stationary phases are usually characterised using phosphate buffer. This study showed that testing under different eluent conditions provides additional information. For example, testing of columns using ammonium acetate buffer gives valuable information for the selection of columns suitable for use in LC–MS analysis, which nowadays is routinely applied in many laboratories.

4. Conclusions

As known from the literature and also shown in this paper, the amount and nature of the modifier influence the pH of the eluent. Furthermore, the addition of methanol, acetonitrile and tetrahydrofuran to 25 m*M* sodium phosphate buffer did not significantly influence the buffering range of the eluent. The amount and nature of the modifier also influence the pK_a of basic analytes. For example, for methanol and acetonitrile upon addition of these modifiers the pK_a shift was ~0.7 for mirtazipine. For tetrahydrofuran however, for the same amount of modifier the change of the pK_a value of this compound decreased with more than 1 pK_a unit. It is obvious that the influence of the nature and amount of modifier on eluent pH and analyte pK_a must be taken into account when developing methods for the analysis of ionisable compounds.

Comparing the classification of seven RPLC stationary phases of the latest generation with respect to their applicability for the analysis of basic pharmaceuticals, the Engelhardt, Tanaka and Galushko tests resulted in mutual different rankings.

These three latter tests were also in disagreement with classification of the RPLC columns using basic compounds from the McCalley and Organon tests. In general, comparing the result of the McCalley test with the data of the Organon compounds, the ranking of the investigated RPLC columns was comparable.

The mutual replacement of 25 m*M* ammonium acetate, ammonium citrate, ammonium phosphate and sodium phosphate aqueous buffers pH 3 and 7 in the various eluents did not result in a comparable ranking of the investigated columns. From the investigated tests only results of the McCalley test could predict suitability of columns for the analysis of the Organon basic compounds. For individual basic compounds a significant influence of the nature of the buffer on peak shape was observed in several cases. In addition, the results of this study revealed the necessity of column testing at different pH-values and various types of buffering salts. The data in this paper show that for selection of a suitable column for

method development analysts should first define the eluent conditions. For instance, if MS detection will be used, select volatile eluent constituents. The next step is to characterise a number of relevant columns with a number of suitable test analytes using a number of applicable eluent conditions. The results allow the selection of suitable mobile and stationary phase conditions useful in method development for a specific application.

References

[1] U.D. Neue, D.J. Philips, T.H. Walter, M. Capparella, B. Alden, R.P. Fisk, LC–GC 12 (1994) 468.
[2] Y. Guillaume, C. Guinchard, J. Liquid Chromatogr. 17 (1994) 2809.
[3] M. Stadalius, J. Berus, L. Snyder, LC–GC 6 (1988) 494.
[4] J. Nawrocki, J. Chromatogr. A 779 (1997) 29.
[5] U. Nawrocki, Chromatographia 31 (1991) 193.
[6] B. Buszewski, M. Jezierska, M. Welniak, D. Berek, J. High Resol. Chromatogr. 21 (1998) 267.
[7] J.J. Kirkland, J.L. Glajch, R.D. Farlee, Anal. Chem. 61 (1989) 2.
[8] A. Pryde, J. Chromatogr. Sci. 12 (1974) 486.
[9] R.E. Majors, M.J. Hopper, J. Chromatogr. Sci. 12 (1974) 767.
[10] C.H. Lochmuller, D.B. Marshall, Anal. Chim. Acta 142 (1982) 63.
[11] J.J. Kirkland, J.W. Henderson, J.J. DeStefano, M.A. van Straten, H.A. Claessens, J. Chromatogr. A 762 (1997) 97.
[12] S. Kobayashi, I. Tanaka, O. Shirota, T. Kanda, Y. Ohtsu, J. Chromatogr. A 828 (1998) 75.
[13] Y. Ohtsu, H. Fukui, T. Kanda, K. Nakamura, M. Nakano, O. Nakata, Y. Fujiyama, Chromatographia 24 (1987) 380.
[14] G. Schomburg, J. Kohler, H. Figge, A. Deege, V. Bien-Vogelsang, Chromatographia 18 (1984) 265.
[15] M. Petro, D. Berek, Chromatographia 37 (1993) 549.
[16] U. Neue, HPLC Columns: Theory, Technology and Practice, Wiley–VCH, New York, 1997.
[17] T. Ascah, B. Feibush, J. Chromatogr. 506 (1990) 357.
[18] J.J. Kirkland, J.B. Adams, M.A. van Straten, H.A. Claessens, Anal. Chem. 70 (1998) 434.
[19] K.K. Unger, N. Becker, P. Roumeliotis, J. Chromatogr. 125 (1976) 115.
[20] T. Walter, B. Alden, E. Bouvier, R. Crowley, R. Fisk, C. Gendreau, Z. Jiang, J. O'Gara, D. Walsh, Lecture 73 presented at HPLC'99, Granada, June 1999, Spain.
[21] H.A. Claessens, M.A. van Straten, C.A. Cramers, M. Jezierska, B. Buszewski, J. Chromatogr. A 826 (1998) 135.
[22] K. Kimata, K. Iwaguchi, S. Onishi, K. Jinno, R. Eksteen, K. Hosoya, M. Araki, N. Tanaka, J. Chromatogr. Sci. 27 (1989) 721.
[23] H. Engelhardt, M. Arangio, T. Lobert, LC–GC Int. 10 (1997) 803.
[24] S.V. Galushko, J. Chromatogr. 552 (1991) 91.
[25] S.V. Galushko, Chromatographia 36 (1993) 93.
[26] S.V. Galushko, A.A. Kamenchuk, G.L. Pit, J. Chromatogr. A 660 (1994) 47.
[27] P.C. Sadek, P.W. Carr, R.M. Doherty, M.J. Kamlet, R.W. Taft, M.H. Abraham, Anal. Chem. 57 (1985) 2971.
[28] R. Kaliszan, Quantitative Structure–Chromatographic Retention Relationships, John Wiley, New York, 1987.
[29] A. Nasal, P. Haber, R. Kaliszan, E. Forgacs, T. Cserhati, M.W. Abraham, Chromatographia 43 (1996) 484.
[30] R. Kaliszan, M.A. van Straten, M. Markuszewski, C.A. Cramers, H.A. Claessens, J. Chromatogr. A 855 (1999) 455.
[31] L.C. Sander, L.R. Field, Anal. Chem. 52 (1980) 2009.
[32] L.A. Cole, J.G. Dorsey, Anal. Chem. 64 (1992) 1317.
[33] H.J.A. Philipsen, H.A. Claessens, H. Lind, B. Klumperman, A.L. German, J. Chromatogr. A 790 (1997) 101.
[34] U.D. Neue, E. Serowik, P. Iraneta, B.A. Alden, T.H. Walter, J. Chromatogr. A 849 (1999) 87.
[35] U.D. Neue, B.A. Alden, T.H. Walter, J. Chromatogr. A 849 (1999) 101.
[36] D.V. McCalley, J. Chromatogr. A. 738 (1996) 169.
[37] D.V. McCalley, J. Chromatogr. A 769 (1997) 169.
[38] D.V. McCalley, J. Chromatogr. A 828 (1998) 407.
[39] R.J.M. Vervoort, M.W.J. Derksen, A.J.J. Debets, J. Chromatogr. A 765 (1997) 157.
[40] R. LoBrutto, A. Jones, Y.V. Kazakevich, J. Chromatogr. A 913 (2001) 189.
[41] R. LoBrutto, A. Jones, Y.V. Kazakevich, H.M. McNair, J. Chromatogr. A 913 (2001) 173.
[42] J. Barbosa, I. Marques, D. Barron, V. Sanz-Nebot, TrAC 18 (1999) 543.
[43] D. Sykora, E. Tesarova, M. Popl, J. Chromatogr. A 758 (1997) 37.
[44] W.J. Gelsema, C.L. de Ligny, A.G. Remijnse, H.A. Blijlevens, Recueil 85 (1966) 647.
[45] R.G. Bates, Determination of pH, Theory and Practice, John Wiley, New York, 1973.
[46] I. Canals, J.A. Portal, E. Bosch, M. Roses, Anal. Chem. 72 (2000) 1802.
[47] S. Espinosa, E. Bosch, M. Roses, Anal. Chem. 72 (2000) 5193.
[48] L.R. Snyder, P.W. Carr, S.C. Rutan, J. Chromatogr. A 656 (1993) 537.
[49] M. Kele, G. Guiochon, J. Chromatogr. A. 855 (1999) 423.
[50] R.G. Brereton, D.V. McCalley, Analyst 123 (1998) 1175.
[51] K. Takacs-Novak, G. Szasz, Pharm. Res. 16 (1999) 1633.
[52] D. Quintanar-Geurrero, E. Allemann, H. Fessi, E. Doelker, Pharm. Res. 14 (1997) 119.
[53] J.D. Meyer, M.C. Manning, Pharm. Res. 15 (1998) 188.