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Monitoring of new silica-based reversed-phase stationary phases for the liquid chromatographic analysis of basic pharmaceuticals using principal components analysis

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Abstract

In this study, fourteen commercially available reversed-phase stationary phases were compared for the high performance liquid chromatographic analysis of basic pharmaceutical compounds. The influence of silanol blocking compounds on retention and peak shape was investigated using phosphate buffers at pH values of 3 and 7. Principal components analysis was used to analyse the data set, enabling an evaluation of the various stationary phases. The commercially available stationary phases showed distinct differences in their suitability for the analysis of basic compounds, whereas addition of N,N-dimethyloctylamine to mobile phase buffer at pH 3 caused an improvement in the peak shapes.

Keywords: Principal components analysis; Stationary phases, LC; Basic drugs

1. Introduction

In the pharmaceutical industry, many compounds of interest contain basic nitrogen atoms, e.g., CNS (central nerve system) drugs, cardiovascular drugs, etc. To analyse these pharmaceutically relevant compounds, reversed-phase high-performance liquid chromatography (LC) using stationary phases based on silica modified with octadecyl (C_{18}) or octyl (C_8) chains is, in many cases, the preferred method. The LC analysis of pharmaceutical compounds is promoted by efficient separations and symmetrical peaks, which enable the quantification of analytes at low concentration levels. The LC analysis of basic compounds, however, can be problematic. Due to

ionic interactions of residual silanols on the silica material with basic compounds, an ion-exchange retention mechanism can occur in conjunction with the reversed-phase retention mechanism. This mixed retention mechanism will depend on the character of the LC column used and will result in varying peak shapes. Also, changes in separation and peak shape can occur as the stationary phase ages [1–5].

Today, many stationary phases that were specially designed for the analysis of basic compounds are available [6]. For many basic compounds, however, asymmetric peaks are still obtained using these stationary phases [7]. Therefore, testing of stationary phases before applying them in development or quality control applications is highly beneficial [8].

Testing of silica-based reversed-phase stationary phases can be carried out using numerous test

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procedures. In the literature, examples can be found where test compounds were selected to reveal differences between stationary phases such as silanol activity, hydrophobicity and metal activity [9]. A high amount and a high acidity of residual silanols present on the stationary phase's silica caused asymmetric peaks, whereas the presence of metals in the silica influenced the acidity of the residual silanols [1]. Recently, a selection of five basic compounds that were used to discern stationary phases suitable for the LC analysis of basic compounds was reported, which represented a set of 32 pharmaceutically relevant compounds [7]. The set of five compounds was selected from a data set containing the asymmetry values of the 32 compounds analysed on six different LC columns, using principal components analysis (PCA). The selection was based on the mutual distance between the compounds in the score plots [7]. In this study, the five selected compounds were used to evaluate the applicability of commercially available stationary phases for the analysis of pharmaceutically relevant basic compounds.

Since the number of stationary phases is very large and still increasing, classification of stationary phases into groups with similar characteristics can be helpful when selecting a suitable stationary phase. This can be obtained by chromatographic characterisation followed by chemometric evaluation. Schmitz et al. [9] and Walczak et al. [10] presented differences between stationary phases using correspondence factor analysis (CFA), by which similarities in the structure of the objects (stationary phases) and the variables were presented simultaneously. PCA was used by Delany et al. [11] to discern stationary phases, whereas Hamoir et al. [12] used spectral mapping analysis (SMA) to study sixteen stationary phases. CFA, PCA and SMA are closely related chemometric analysis tools.

In this paper, the comparison of fourteen silica-based reversed-phase stationary phases for the analysis of pharmaceutically relevant basic compounds is reported using PCA as a chemometric evaluation tool. In the study, the influence of the addition of the silanol-blocking compounds triethylamine and *N,N*-dimethyloctyl amine to the mobile phase, consisting of phosphate buffers at pH values of 3 and 7 with MeOH, on retention and peak shape was determined.

PCA was used to present the objects (stationary phases) in score plots and the variables (asymmetry, retention, plate height) in loading plots.

2. Experimental

2.1. HPLC apparatus

The HPLC experiments were carried out using a HP 1090 M liquid chromatograph equipped with a HP 1040 M diode array detector (Hewlett-Packard, Amstelveen, Netherlands). HPLC chromatograms were collected using a HP 79994A HPLC Workstation or a HPLC 3^D Chemstation.

2.2. Chemicals

The basic compounds were synthesised by the Department of Medicinal Chemistry, N.V. Organon (Oss, Netherlands). The structures are shown in Fig. 1.

Methanol (MeOH), supplied by J.T. Baker (Deventer, Netherlands), was used as the organic modifier. For the preparation of the buffers, disodium hydrogen phosphate (Na_2HPO_4) and sodium dihydrogen phosphate (NaH_2PO_4) (J.T. Baker) were used. The aqueous buffers were prepared using Milli

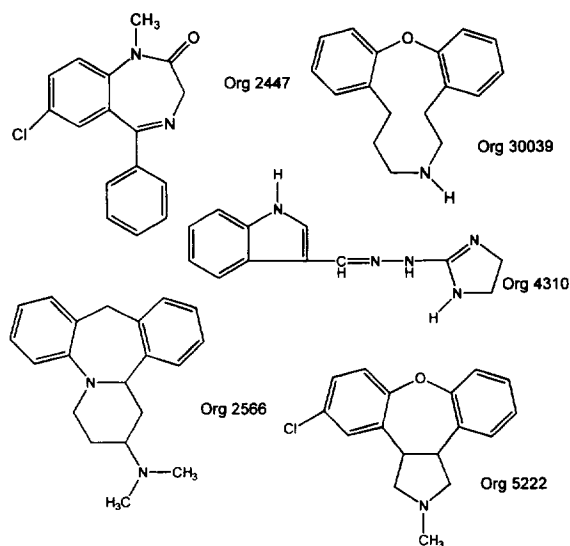


Fig. 1. Molecular structures of the test compounds.

Table 1
Stationary phases used in this study

Stationary phase	Abbreviation used in this study	Manufacturer	Dimension length × I.D. (mm)	Particle size (μm)
Hypersil BDS	BDSSH	Shandon	150 × 4.6	5
Hypersil BDS	BDSHP	Shandon	125 × 3.0	3
Inertsil 5 ODS-2	INCH	GL Sciences	150 × 4.6	5
Inertsil ODS-80Å	INGL	GL Sciences	150 × 4.6	5
Supelcosil LC-ABZ	ABZ	Supelco	150 × 4.6	5
Supelcosil LC-ABZ+Plus	ABZ ⁺	Supelco	150 × 4.6	5
Zorbax SB-C ₁₈	ZSB	Rockland Technologies	150 × 4.6	5
Zorbax Rx-C ₁₈	ZRX	Rockland Technologies	250 × 4.6	5
Chromspher B	CHB	Chrompack	250 × 4.6	5
Purospher RP-18	PUR	Merck	250 × 4.0	5
Symmetry C ₁₈	SYM	Waters	150 × 3.9	5
Prodigy 5 ODS-2	PROD	Phenomenex	150 × 4.6	5
YMC Basic	YMC	YMC	150 × 4.6	5
Nucleosil 100-5C ₁₈ AB	NUCAB	Machery Nagel	250 × 4.0	5

Q filtered water (Millipore, Bedford, MA, USA). Triethylamine (TEA, sequanal grade) was obtained from Pierce (Rockford, IL, USA) and N,N-dimethyloctylamine (DMOA, 95%) was obtained from Aldrich (Gillingham, UK). To obtain 0.1% (v/v) solutions of the silanol blockers, 1 ml of TEA or DMOA was added to 1 l of the buffer solution, before mixing with MeOH. Concentrated phosphoric acid (H₃PO₄) was obtained from Merck (Darmstadt, Germany) and was added to the buffers, after addition of TEA or DMOA and before mixing with MeOH, to adjust the pH of the mobile phase.

The amount injected was 2 μg and this was achieved by injecting 2 μl from a 1 mg/ml solution of the basic analytes in MeOH. The stationary phases studied are shown in Table 1.

2.3. Experimental set-up

In this study, mobile phases with pH values of 3 and 7 were used. The stationary phases were tested without addition of a silanol blocker, and with addition of 0.1% TEA and 0.1% DMOA to the buffer solution in the mobile phase.

The MeOH–buffer ratio was adjusted to ensure that $k' > 1$ without the addition of the silanol blockers. After addition of the blocker to the mobile phase buffer, the MeOH–buffer ratio remained unchanged. The sequence of experiments for each stationary phase was performed as shown in Table 2. All experiments were performed in duplicate.

The Chromspher B (CHB) and Inertsil ODS-80 Å (INGL) stationary phases were not tested at a buffer

Table 2
Sequence of experiments

Sequence number	Mobile phase
1	MeOH–25 mM Na ₂ HPO ₄ pH 7
2	MeOH–25 mM NaH ₂ PO ₄ pH 3
3	MeOH–25 mM Na ₂ HPO ₄ pH 7+0.1% (v/v) TEA
4	MeOH–25 mM NaH ₂ PO ₄ pH 3+0.1% (v/v) TEA
5	MeOH–25 mM Na ₂ HPO ₄ pH 7+0.1% (v/v) DMOA
6	MeOH–25 mM NaH ₂ PO ₄ pH 3+0.1% (v/v) DMOA

pH of 7. Due to the apolar character of these stationary phases, the test compounds did not elute using 80% (v/v) methanol in the mobile phase.

2.4. Peak performance calculations

The asymmetry factor (A_s) was calculated for the compounds Org 4310, Org 5222, Org 2566 and Org 30039 at 10% of the peak height and expressed as the ratio of the width at the rear and at the front of the peak. To describe the retention of the test compounds, the k' values of Org 4310, Org 5222, Org 2566 and Org 30039 were used. The first deviation of the baseline was used to determine the dead volume of the LC column. For calculation of the plate height (HETP) of the LC column, the second moment of the Org 2447 peak was used [13].

2.5. Principal components analysis

PCA was performed using the software package Unscrambler 5.03 (Camo AS, Trondheim, Norway), installed on a personal computer with a 486/66 MHz Intel processor. The Unscrambler results were transferred to Lotus 123 3.0 software (Lotus Development, USA), to enable graphical presentation.

3. Results and discussion

3.1. Selection of variables

The first consideration for stationary phase evaluation was the selection of variables known to describe the LC performance, e.g., peak shape and plate height. In Tables 3 and 4, the data obtained for each stationary phase listed in Table 1 are shown, when performing the sequence of experiments shown in Table 2. During the experiments, it was observed that the LC analysis of compound Org 2447 always revealed symmetrical peaks (asymmetry factor ≤ 1.2). Therefore, this compound was used to calculate the efficiency (HETP) of the stationary phases. Together with the individual asymmetry values of Org 4310, Org 30039, Org 2566 and Org 5222, the HETP values were used in PCA evaluation. As can be seen in Tables 3 and 4, for an individual stationary phase and compound, a reduction of k' also resulted in reduction of A_s . Since the amount of modifier

(MeOH) remained unchanged during the experiments, the reduction in peak asymmetry and retention was evidently caused by the addition of TEA and DMOA to the mobile phase buffer. Clearly, as shown in Table 3, the silanol-blocking compounds suppressed the interaction between residual silanols and basic compounds, resulting in improved peak symmetry and a decrease in retention times. The relation between retention and peak shape was also discussed in previous studies [14,15]. The stationary phases presented in this study (Table 1), however, showed a different chromatographic behaviour; variations in asymmetry and in retention after addition of silanol-blocking compounds to the mobile phase buffer. The deviating chromatographic behaviour is specific for a given stationary phase, e.g., the influence of silanol-blocking compounds in the mobile phase buffer on peak asymmetry is negligible for the Purospher RP-18 (PUR) column in Table 3 compared to the Inertsil 5 ODS-2 (INCH; and other) columns. To include these stationary phase characteristics in PCA, the k' values of the compounds, both with and without TEA and DMOA in the mobile phase buffer, were used. Since the data differed in scale, they were autoscaled prior to PCA.

In this study, various stationary phases that can be used for the HPLC analysis of pharmaceutical basic compounds are compared. Stationary phases showing comparable characteristics will cluster in the PCA score plots, whereas both score- and loading plots can show relationships between the stationary phases and the variables (retention, peak shape, plate height). First, stationary phases were compared, independently of the pH of the mobile phase buffer, using the combined data obtained with phosphate buffers with pH values of 3 and 7. Secondly, the stationary phases were compared using phosphate buffer with a pH value of 3 and 7, separately.

3.2. Stationary phase monitoring; phosphate buffers pH 3 and pH 7

To evaluate the stationary phases, the data obtained (Tables 3 and 4) were analysed using PCA. With PCA, a set of new variables (principal components, PC) are defined instead of using the original variables (retention, asymmetry, plate height). To find the PC of a data matrix, X , the first step is to

Table 3
Data obtained when testing the stationary phases using phosphate buffer, pH 3

Column	Asymmetry										Plate height (μm)											
	Org 4310		Org 30039		Org 2566		Org 5222		Org 2447		Org 4310		Org 30039		Org 2566		Org 5222		Org 2447			
	NS	TEA	DMOA	NS	TEA	DMOA	NS	TEA	DMOA	NS	TEA	DMOA	NS	TEA	DMOA	NS	TEA	DMOA	NS	TEA	DMOA	
CHB	3.7	2.9	2.5	6.0	2.7	2.4	6.5	3.6	2.8	8.0	3.3	2.7	32.7	39.7	39.5							
PUR	1.7	1.9	1.5	1.6	1.6	1.4	2.0	1.9	1.6	2.0	2.1	1.5	20.4	20.5	19.8							
SYM	3.1	2.5	1.5	3.0	2.4	1.2	2.6	2.2	1.4	2.5	2.1	1.4	18.3	19.7	19.2							
ABZ+	2.2	2.0	1.3	2.3	2.0	1.0	1.9	1.7	1.3	2.0	1.8	1.2	15.3	15.2	15.5							
ABZ	2.1	2.1	1.9	1.9	1.8	1.6	2.0	2.1	1.8	1.9	1.9	1.8	21.6	17.1	17.8							
YMC	3.4	2.7	1.3	3.4	2.9	1.2	2.8	2.2	m	2.7	2.2	1.2	13.1	13.0	13.2							
BDSHP	6.0	4.5	2.5	6.2	4.7	1.6	8.6	6.4	2.2	7.0	5.1	2.0	16.0	17.3	14.8							
BDSSH	12.1	2.0	1.4	8.9	2.5	1.2	10.2	3.5	1.5	12.6	2.5	1.3	20.9	19.5	21.4							
INCH	17.9	5.1	1.4	10.7	3.8	1.2	14.9	4.8	1.6	14.6	4.8	1.5	15.1	15.9	15.1							
INGL	3.3	2.8	1.6	4.3	2.7	1.7	3.8	2.6	1.6	3.7	2.5	1.6	23.9	29.7	26.9							
ZSB	4.7	3.2	1.5	3.5	2.4	1.3	4.4	2.9	1.4	3.6	2.4	1.2	15.2	16.1	15.7							
ZRX	3.9	2.9	1.8	3.9	2.6	1.6	4.1	2.8	1.5	4.1	2.7	1.3	18.0	24.7	24.9							
PROD	1.5	1.3	1.2	1.5	1.4	1.1	1.2	1.3	1.2	1.4	1.4	1.2	14.7	14.1	16.3							
NUCAB	2.9	2.5	1.6	2.0	1.8	1.2	1.9	1.7	1.1	2.8	2.2	1.3	19.4	19.3	19.3							

Retention (k')	Org 30039										Org 2566										Org 5222									
	NS		TEA		DMOA		MeOH (%)		NS		TEA		DMOA		MeOH (%)		NS		TEA		DMOA		MeOH (%)							
CHB	1.2	1.0	0.9	7.0	3.1	1.8	1.6	70	4.0	3.0	2.5	70	5.7	2.5	2.2	70	5.7	2.5	2.2	70	5.7	2.5	2.2							
PUR	2.6	3.1	1.7	20	2.4	2.9	2.4	35	6.0	6.5	5.7	35	5.1	6.0	5.1	35	5.1	6.0	5.1	35	5.1	6.0	5.1							
SYM	4.2	3.7	0.9	25	2.4	2.7	1.7	45	4.7	5.2	4.2	45	4.8	4.4	3.1	45	4.8	4.4	3.1	45	4.8	4.4	3.1							
ABZ+	1.3	1.3	0.6	30	1.6	1.7	1.1	40	3.3	3.4	2.4	40	3.0	3.1	2.2	40	3.0	3.1	2.2	40	3.0	3.1	2.2							
ABZ	2.2	1.9	1.3	30	2.3	2.4	1.8	40	4.5	4.5	3.5	40	4.2	4.2	3.3	40	4.2	4.2	3.3	40	4.2	4.2	3.3							
YMC	5.6	4.9	0.9	20	3.7	3.6	3.5	40	6.7	6.4	m	40	6.2	5.9	3.3	40	6.2	5.9	3.3	40	6.2	5.9	3.3							
BDSHP	4.3	3.5	1.3	25	3.6	3.2	1.7	45	6.4	5.6	3.0	45	5.7	5.1	2.8	45	5.7	5.1	2.8	45	5.7	5.1	2.8							
BDSSH	5.8	4.0	0.8	25	2.0	1.2	0.8	55	4.0	2.0	1.4	55	3.3	1.8	1.3	55	3.3	1.8	1.3	55	3.3	1.8	1.3							
INCH	2.8	2.8	0.7	30	1.0	0.8	0.6	60	1.8	1.4	1.0	60	1.6	1.4	0.9	60	1.6	1.4	0.9	60	1.6	1.4	0.9							
INGL	3.1	4.8	1.0	25	2.5	2.2	1.1	50	4.0	3.7	1.9	50	3.8	3.7	1.9	50	3.8	3.7	1.9	50	3.8	3.7	1.9							
ZSB	5.1	5.0	1.0	25	3.9	3.9	2.0	45	6.3	6.3	3.3	45	6.0	6.2	3.3	45	6.0	6.2	3.3	45	6.0	6.2	3.3							
ZRX	4.3	3.4	0.7	25	3.1	2.5	1.3	45	5.7	4.4	2.5	45	5.1	4.2	2.4	45	5.1	4.2	2.4	45	5.1	4.2	2.4							
PROD	3.2	3.1	0.9	30	2.4	2.5	1.1	50	4.2	4.4	2.9	50	3.7	4.0	2.7	50	3.7	4.0	2.7	50	3.7	4.0	2.7							
NUCAB	3.6	3.4	0.6	25	3.1	3.3	1.5	45	5.6	5.7	2.8	45	4.8	4.8	2.6	45	4.8	4.8	2.6	45	4.8	4.8	2.6							

NS: no silanol-blocking compound added to the mobile phase.

TEA: triethylamine added to the mobile phase.

DMOA: N,N-dimethyloctylamine added to the mobile phase.

m: no data.

Table 4
Data obtained when testing the stationary phases using phosphate buffer pH 7

Column	Asymmetry												Plate height (μm)		
	Org 30039				Org 2566				Org 5222				Org 2447		
	NS	TEA	DMOA	NS	TEA	DMOA	NS	TEA	DMOA	NS	TEA	DMOA	NS	TEA	DMOA
PUR	4.1	5.1	3.8	5.8	6.4	6.1	4.8	4.8	4.8	2.6	2.0	2.4	1.9	18.9	19.0
SYM	2.4	2.0	1.4	2.5	2.4	2.0	1.6	1.5	1.5	1.2	1.2	1.2	1.1	20.0	18.8
ABZ+	4.4	5.1	4.2	1.6	1.6	1.5	1.5	1.5	1.4	m	m	1.2	1.1	13.8	14.9
ABZ	5.5	5.2	3.9	2.1	2.3	2.0	2.0	3.0	1.7	1.3	1.3	1.7	1.4	21.1	19.2
YMC	3.3	2.6	1.2	4.3	2.8	1.5	3.3	2.1	1.0	1.3	1.2	1.2	1.0	13.0	13.1
BDSHP	5.3	4.8	2.4	15.1	5.2	2.8	9.1	4.0	1.2	4.4	4.4	2.0	1.2	18.2	16.1
BDSSH	7.3	3.6	1.6	9.7	4.1	2.7	11.7	2.6	1.2	3.6	3.6	1.3	1.0	20.2	23.2
INCH	21.2	2.7	2.7	12.3	7.3	6.2	4.3	3.3	1.9	1.9	1.8	1.8	1.4	15.3	15.9
ZSB	4.6	3.3	2.1	5.0	3.1	3.2	2.4	1.6	1.2	1.2	1.1	1.1	1.1	15.2	16.1
ZRX	3.3	m	2.0	6.1	5.2	3.7	5.3	2.4	1.8	2.9	1.3	1.3	1.1	17.1	24.6
PROD	1.7	1.4	1.2	3.5	2.1	2.1	1.9	1.4	1.2	1.3	1.2	1.2	1.2	14.6	13.3
NUCAB	4.2	2.7	1.7	10.4	3.7	3.9	6.0	1.6	1.4	4.3	1.5	1.5	1.2	19.4	19.8

Column	Retention (k')														
	Org 30039				Org 2566				Org 5222						
	NS	TEA	DMOA	NS	TEA	DMOA	NS	TEA	DMOA	NS	TEA	DMOA	NS	TEA	DMOA
PUR	3.3	4.2	3.1	40	8.0	7.5	9.0	80	80	9.0	8.5	8.3	80	7.0	6.8
SYM	6.3	5.3	4.4	25	2.3	2.8	2.8	75	75	5.8	5.6	5.6	75	5.7	5.7
ABZ+	1.4	1.4	1.2	50	2.0	1.8	1.9	70	70	3.9	3.9	3.9	70	m	4.2
ABZ	5.3	3.2	2.7	50	4.2	2.9	2.9	70	70	6.3	5.3	5.0	70	5.6	5.1
YMC	3.1	2.8	2.2	25	1.8	1.7	1.6	65	65	4.6	4.4	4.0	65	4.8	4.4
BDSHP	2.4	2.7	2.2	30	3.0	3.1	2.9	70	70	5.4	6.4	6.0	70	5.3	6.0
BDSSH	3.6	3.1	2.3	30	2.2	2.2	2.0	80	80	2.7	2.7	2.6	80	2.4	2.4
INCH	3.2	2.8	2.8	35	3.2	3.7	3.5	80	80	4.1	5.0	4.2	80	3.8	3.9
ZSB	5.3	4.4	3.0	35	5.9	5.1	5.3	80	80	5.8	5.6	5.5	80	4.9	4.8
ZRX	3.1	m	2.6	35	7.6	10.2	6.8	80	80	5.4	7.3	4.8	80	4.1	3.9
PROD	3.4	3.2	2.7	30	1.9	1.8	1.9	80	80	3.4	3.3	3.2	80	3.3	3.2
NUCAB	3.5	3.3	2.7	25	1.5	0.9	1.0	80	80	1.8	1.7	1.7	80	1.9	1.8

NS: no silanol-blocking compound added to the mobile phase.

TEA: triethylamine added to the mobile phase.

DMOA: N,N-dimethyloctylamine added to the mobile phase.

m: no data.

look for a vector t_1 (score) = Xp_1 that is a linear function of X with maximum variance, where p_1 (loading) is a vector of m constants $p_{11}, p_{12}, \dots, p_{1m}$ and length, l . The second step is to look for a linear function Xp_2 , orthogonal to Xp_1 , which has maximum variance, etc. Summarising for the data in Table 3, a 14×27 data matrix (14 stationary phases \times 27 variables) can be written as: $X = t_1 p_1^T + t_2 p_2^T + \dots$. Since the first few PC contain most of the data variability, a graphical presentation of the data is possible, i.e., the scores reflect the objects (stationary phases) and the loadings reflect the variables (retention, peak shape, plate height) [16].

In Fig. 2, the score and loading plots are shown using the data in Tables 3 and 4. The first three PC explained 64% of the variance present in the dataset. To evaluate the stationary phases qualitatively, this was found to be sufficient since conclusions drawn from the score and loading plots could additionally be verified using the data in Tables 3 and 4. As can be seen in Tables 3 and 4, the Prodigy 5 ODS-2 (PROD) stationary phase showed a lower asymmetry and plate height compared to the other stationary phases. These low A_s and low HETP are favourable for the separation of basic compounds. In the PC1–PC2 score plot, the PROD stationary phase was situated in the lower right quadrant. The vectors for A_s and HETP in the corresponding loading plot pointed in the opposite direction, indicating low A_s and low HETP for the PROD stationary phase. The stationary phases situated close to the PROD stationary phase (the lower left and lower right quadrants) in the score plots also showed low asymmetry and low plate height, e.g., the Supelcosil LC-ABZ+Plus (ABZ+) and Nucleosil 100-5C₁₈ AB (NUCAB) stationary phases using phosphate buffer, pH 3. Using phosphate buffer, pH 7, the YMC Basic (YMC) and Symmetry C₁₈ (SYM) stationary phases showed low asymmetry and low plate height. Stationary phases situated apart from the PROD stationary phase in the score plots were less suitable for the LC analysis of basic pharmaceutical compounds, as used in this study (Fig. 1). Apparently, not all stationary phases developed for the separation of basic compounds are suitable for analysing all types of basic pharmaceuticals. As can be seen in the literature, other types of basic compounds showed different peak symmetries. For instance, the use of

the INCH stationary phase, showing asymmetric peaks when used for the test compounds, had an acceptable low asymmetry factor in another study [17]. Nevertheless, it should be kept in mind that the results presented in this study were valid for a rather limited set of 32 basic compounds, represented by the five compounds shown in Fig. 1.

For several stationary phases, like PROD, YMC and Zorbax SB-C₁₈ (ZSB), the peak shapes obtained when using phosphate buffer, pH 3, were similar to the peak shapes obtained when using phosphate buffer, pH 7, as shown by the vectors in the PC1–PC2 and PC1–PC3 score plots. Other stationary phases showed a different pattern. For the PUR stationary phase, a difference could be observed depending on whether the pH value of the buffer used was 3 or 7. Using buffer with a pH of 3, relatively symmetrical peaks ($1.4 \leq A_s \leq 2.1$) were obtained for the basic compounds, whereas using a buffer of pH 7, asymmetrical peak shapes ($1.9 \leq A_s \leq 6.4$) were observed. Generally, for the selected compounds, the use of phosphate buffer, pH 3, yielded better peak symmetry than the use of phosphate buffer, pH 7. Furthermore, differences in peak shape were observed for apparently similar packing materials, e.g., INCH and INGL, both of which were manufactured from Inertsil silica material, and Hypersil BDS (BDSSH) and Hypersil BDS (BDSHP), both of which were prepared using Hypersil BDS stationary phase.

3.3. Stationary phase monitoring; phosphate buffers pH 3 and 7, separately

In Fig. 3, the score and loading plots of the first three PC are shown using the data in Table 3 (phosphate buffer, pH 3), explaining 76% of the variance. In Fig. 4, the score and loading plots of the first three PC are shown using the data in Table 4 (phosphate buffer, pH 7), explaining 70% of the variance.

To reveal the effects of pH, Figs. 3 and 4 are illustrative. In Fig. 3, it can be seen that the loadings of the asymmetry values of the four compounds obtained, without addition of silanol-blocking compounds to the mobile phase, show clusters. This is also observed for the loadings of the asymmetry values after addition of TEA, and for the loadings of

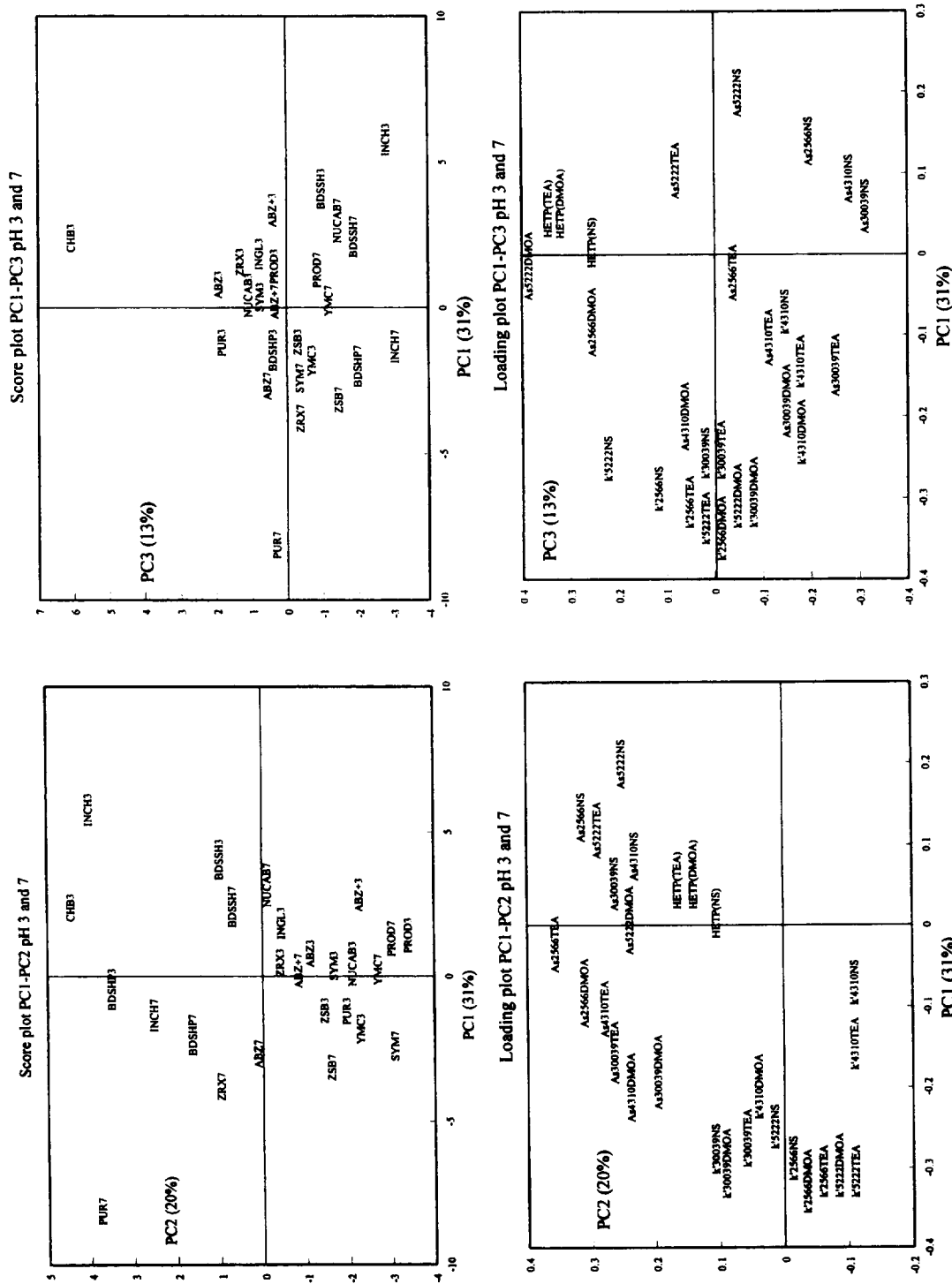


Fig. 2. Score and loading plots using the first three principal components (PC) and the data obtained using phosphate buffers, pH 3 and pH 7 (Tables 3 and 4). The numbers behind the column abbreviations (Table 1) in the score plots are the pH values used. The abbreviations in the loading plots are related to the following: As2566DMOA means the loading of the asymmetry value (*A_s*) of Org 2566 (2566) after addition of DMOA.

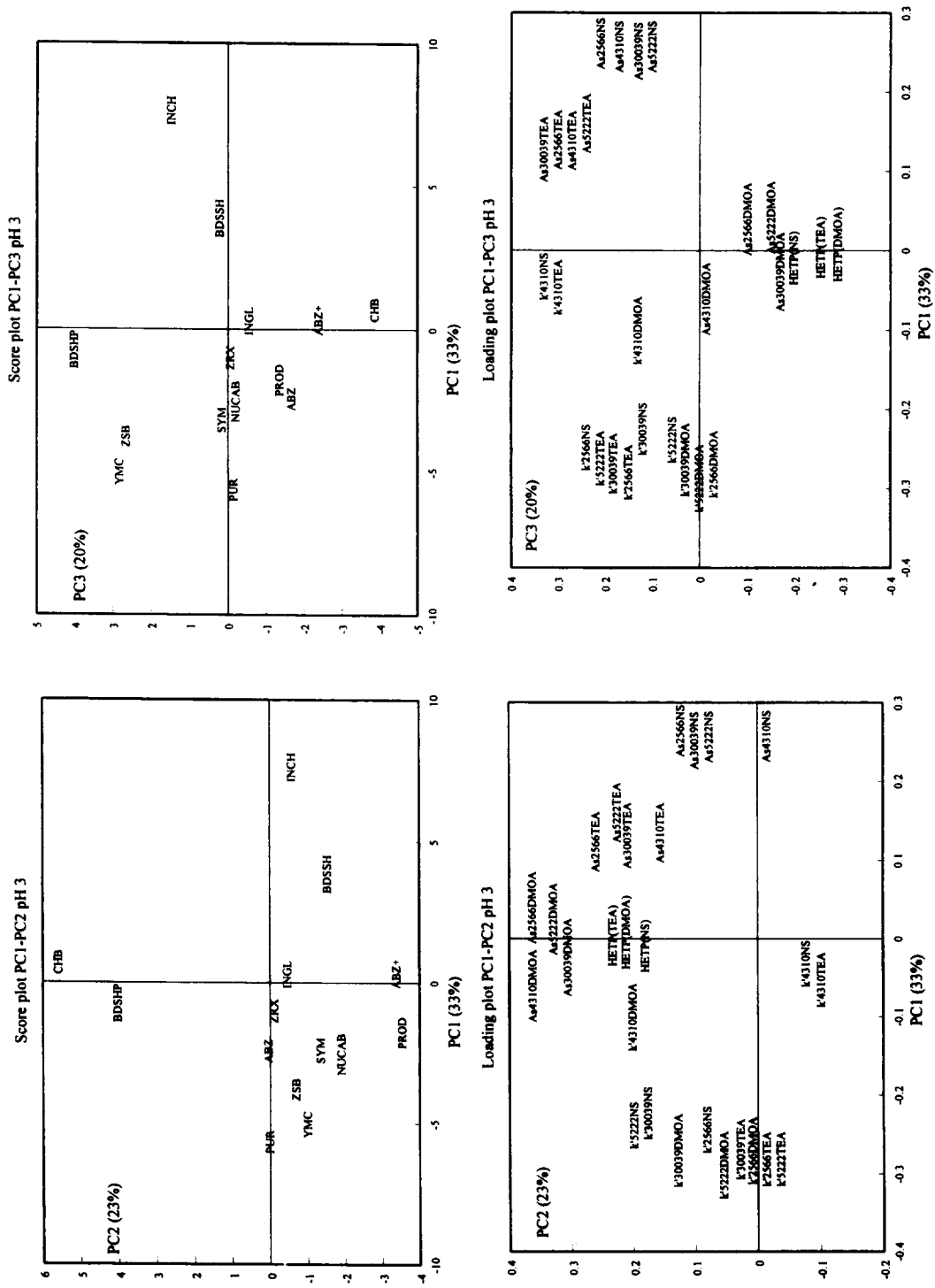


Fig. 3. Score and loading plots using the first three principal components and the data obtained using phosphate buffer, pH 3 (Table 3). For the column abbreviations, see Table 1. The abbreviations in the loading plots are as explained in Fig. 2.

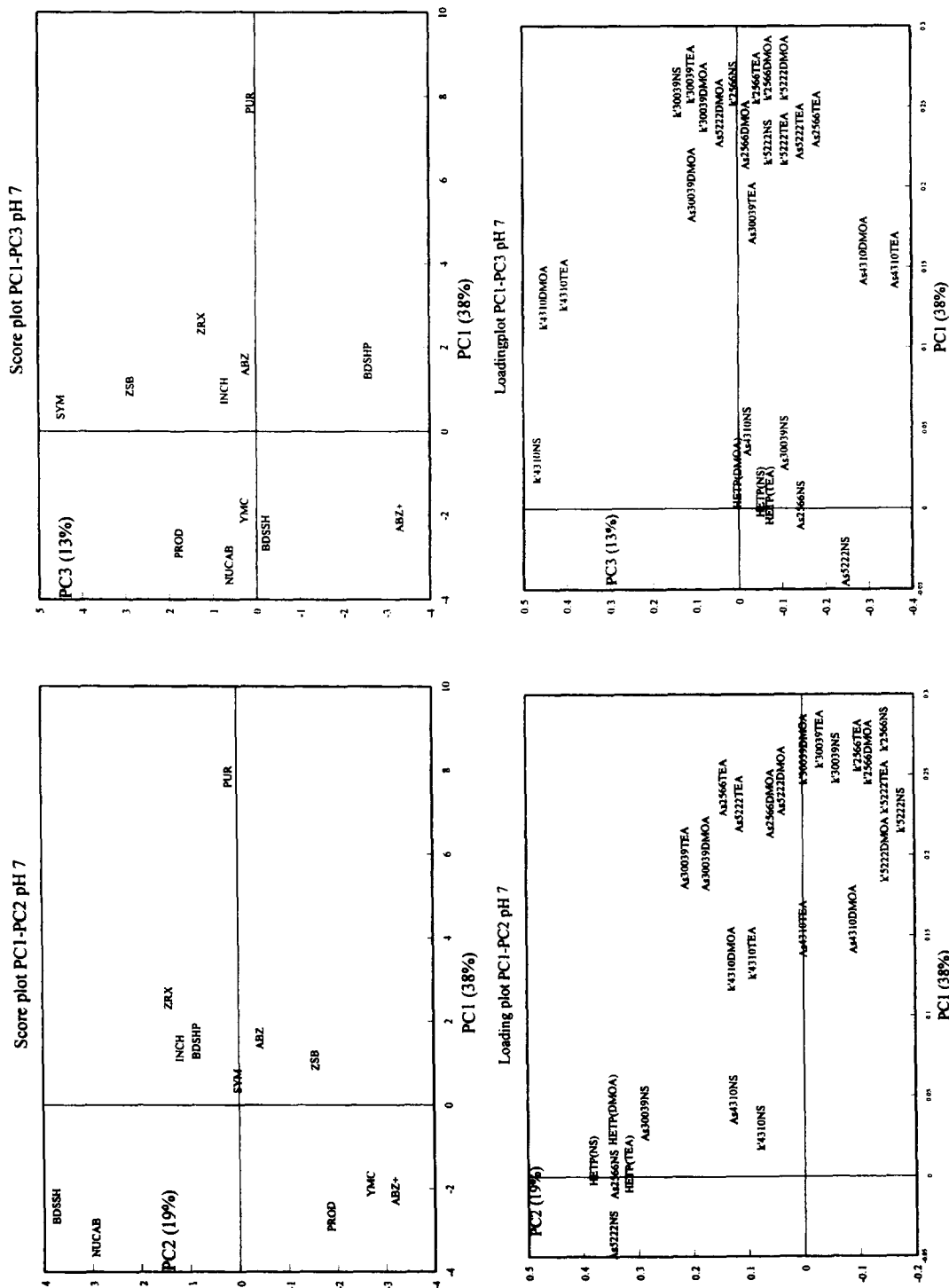


Fig. 4. Score and loading plots using the first three principal components and the data obtained using phosphate buffer, pH 7 (Table 4). For the column abbreviations, see Table 1. The abbreviations in the loading plots are as explained in Fig. 2.

the asymmetry values after addition of DMOA to the mobile phase. Hence, the four test compounds showed similar chromatographic characteristics, i.e., peak shape. Therefore, it can be concluded that the use of one of the test compounds, instead of four, would yield similar information about the performance of the LC stationary phases when phosphate buffer, pH 3, was used in the mobile phase.

A loading plot reveals information about the relationship between variables, since each loading vector describes a different source of variation in the data. The correlation between variables is expressed by the angle between the various variable vectors in the loading plot, i.e., a small angle between the variable vectors means a high correlation between the variables. Low correlations between k' -values (retention) and asymmetry values (peak shape) were observed in the loading plots. In particular, this was observed for the data when DMOA was added to the phosphate buffer, pH 3, expressed by the orthogonality of the retention and asymmetry vectors in the loading plots, meaning that no correlation exists between A_s and k' . Clearly, the addition of DMOA to the mobile phase yielded symmetrical peaks for the basic compounds. Since interactions between the basic solutes and residual silanols yield asymmetrical peaks, symmetrical peaks obtained after the addition of DMOA to phosphate buffer, pH 3, indicates that blocking of residual silanols has occurred.

Contradictory to the results in Fig. 3, the loading vectors for the asymmetry values on addition of TEA and DMOA to phosphate buffer, pH 7, shows no clusters in the PC1–PC2 and the PC1–PC 3 loading plots (see Fig. 4). However, without the addition of silanol-blocking compounds, in the PC1–PC2 loading plot, the loadings for the asymmetry values of Org 5222, Org 2566 and Org 30039 are clustered and in the PC1–PC3 loading plot, Org 4310 was also included in the cluster. Obviously, the effect of adding silanol-blocking compounds to mobile phase buffer, pH 7, is different for the test compounds. As can be seen in Table 4, after addition of TEA to phosphate buffer, pH 7, the peak symmetry improved. After addition of DMOA to the phosphate buffer, pH 7, symmetric peaks were obtained for Org 5222 and Org 2566, whereas for Org 4310 and Org 30039, the enhancement of the peak symmetry was less significant.

As was shown in the loading plots in Figs. 3 and 4, the loadings for the k' -values of Org 4310 differed from the loadings for the k' -values of Org 2566, Org 5222 and Org 30039. The loadings for retention without the addition of silanol-blocking compounds (and after addition of TEA and DMOA) were highly correlated for Org 2566, Org 5222 and Org 30039 using phosphate buffers, pH 3 and 7, meaning that, for these compounds, the influence of silanol-blocking compounds on retention was similar. The loadings for retention of Org 4310 were not included in these clusters, indicating that the effect on retention compared to Org 2566, Org 30039 and Org 5222 for TEA and DMOA, was not similar.

Besides the influence on the symmetry and k' -value of the chromatographic peak, some practical considerations of using silanol-blocking compounds should be noted. Stabilisation times were substantially longer (approx. 1 h) compared to eluents without silanol-blocking compounds. Also, the removal of the silanol-blocking compounds from the stationary phase by rinsing procedures with methanol–water is difficult and will take some time (approx. 1 h). Additionally, the removal of DMOA from the stationary phase will take approximately twice the time required to remove TEA from the stationary phase.

4. Conclusions

The monitoring of stationary phases for the LC analysis of basic compounds was performed by analysing the chromatographic data (retention, peak symmetry and plate height) of five basic test compounds by PCA. PCA was found to be useful for discerning stationary phases. Explanation of 64% of the variation present in the data set was found to be acceptable for graphical presentation and qualitative discernment of stationary phases. Stationary phases with similar chromatographic behaviour (retention, peak shape, plate height) clustered in the score plots. In the loading plots, the relationships between the variables were depicted.

It was found that the observed peak symmetries of the test compounds obtained with some stationary phases when using phosphate buffer, pH 3, were different from those obtained using phosphate buffer,

pH 7. Other stationary phases showed similar peak symmetries when using phosphate buffer pH 3 and pH 7.

The use of silanol-blocking compounds, such as triethylamine (TEA) and N,N-dimethyloctylamine (DMOA), resulted in an improvement in the shape of the chromatographic peak. In particular, after addition of DMOA to mobile phase buffer, pH 3, symmetrical peaks were obtained using the stationary phases presented in this study.

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