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Selection of stationary phases for the liquid chromatographic analysis of basic compounds using chemometric methods

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Abstract

The analysis of basic compounds by means of reversed-phase liquid chromatography is often hampered by poor peak shapes. In this paper chemometrical methods are used to select and reduce the number of test compounds and to detect differences in applicability of stationary phases designed for the analysis of basic drugs.

In the first part principal component analysis was applied to reduce the number of test compounds necessary to characterize a stationary phase. From a data set of the asymmetry values of 32 test compounds analyzed on six different LC columns, five representative compounds were selected. Subsequently, these five compounds were used for evaluation of commercially available columns.

For the column judgement the asymmetry of the test compounds, the efficiency and the short-term reproducibility of the capacity factor and the plate number, were taken into account. Graphical presentation using bar charts, multi-criteria decision making based on the Pareto optimality and bi-plots were used to distinguish between columns. First of all eight columns were compared at individual pH values of 3.0, 7.0 and 11.0. Finally, all results were combined and revealed that for our test compounds very good results were obtained at a pH of 11 using a column containing zirkonium oxide particles coated with polybutadiene (3MZ-18). At low pH values good results were obtained with a Supelcosil LC-ABZ and a Zorbax Rx-C_{18} column.

Overall it can be concluded that a chemometric approach is successfully applied for the development of a method for in-house column testing and evaluation dedicated to the Organon type of compounds. Other columns developed for the analysis of basic solutes can now be efficiently tested with the method described in this paper. Chemometric methods were useful to efficiently reduce the number of test compounds and for column evaluation. However, the final selection of a column also depends on the special requirements defined by the expert. The requirements, which are, for example, for routine quality control clearly different than for purity testing of new chemical entities in drug development, can be translated to weighing factors for the variables tested. For this the advice of the expert remains indispensable.

1. Introduction

In pharmaceutical analysis reversed-phase liquid chromatography (LC) is the most frequently used technique. It is used as a tool to quantify active ingredients in pharmaceutical formulations, to determine impurities, to investigate the stability of a product, etc. For the analysis of basic drugs, however, often asymmetric peaks are obtained due to ionic interactions of compounds with residual silanol groups of the stationary phase [1,2].

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To improve peak shape, optimization of both the mobile and stationary phase should be considered. In the literature, many suggestions have been given to optimize the mobile phase, e.g. by adding silanol blockers, using ion-pair reagents and by selecting an optimal pH [1,2]. Also the number of stationary phases specially designed for the analysis of basic compounds is exponentially increasing [1,3]. Because many possibilities are available, it is difficult to select the best system.

Recently, we reported about the analysis of 32 basic compounds using several columns with different eluents [1]. Relations between retention, asymmetry and basicity of the compounds were studied for a μBondapak C_{18} column. Together with data of the peak shape of the 32 test compounds on five other columns, a data set was obtained which contains a lot of information. However, to extract useful information from such a set is often problematic and the use of chemometric methods can be helpful.

Multivariate techniques, like principal component, cluster, correspondence factor and discriminant analysis can be used to analyse and reduce the number of variables of a data set [4–8]. In the literature, Musumarra et al. [6] reported results from principal component analysis (PCA) of chromatographic retention data of drugs for identification purposes. Delaney et al. [7] used PCA to select a set of test compounds in LC. Schmitz et al. [8] compared different multivariate techniques for the characterization of stationary phases in LC and to select test compounds.

From a data set of 32 compounds and different columns, a number of test compounds was selected using PCA. The test compounds were selected on basis of the asymmetry obtained on six stationary phases which are recommended for the analysis of basic compounds. Subsequently, the test compounds were used for testing of several other stationary phases. For selecting the optimal column not only the peak shapes are important, but also the efficiency and ruggedness. In these situations methods of multi-criteria decision making (MCDM) can be applied for column judgement [9–12]. Within this approach it is not necessary to make a priori decisions and experiments can be compared easily. Also bar charts and bi-plots can be helpful tools to characterize stationary phases [13]. For the column judgement presented in this paper the obtained asymmetry values, the plate height, and the repeatability of the capacity factor and plate height are used.

2. Experimental

2.1. Apparatus

The HPLC experiments were carried out using an HP1090M liquid chromatograph equipped with an HP1040M diode-array detector (Hewlett-Packard, Amstelveen, Netherlands). HPLC chromatograms were collected on a HP 79994A HPLC Workstation.

The software packages Unscrambler 5.03, Camo (Trondheim, Norway) and Microsoft Excel 4.0 (Redmond, WA, USA) were used to analyse the data.

2.2. Chemicals

All basic drugs were synthesized by Organon (Oss, Netherlands).

As organic modifiers methanol (MeOH) and acetonitrile (ACN) were used. Methanol was freshly distilled before use. Analytical-grade acetonitrile was obtained from J.T. Baker (Deventer, Netherlands).

For the preparation of the buffers disodium hydrogenphosphate, sodium dihydrogenphosphate and boric acid, supplied by J.T. Baker were used. To obtain 25 m*M* buffers, adequate amounts were dissolved in water of Milli-Q quality. Sodium hydroxide and concentrated orthophosphoric acid were obtained from Merck (Darmstadt, Germany) and were added to the buffers until the desired pH value was reached.

The amount of basic drugs injected was 2 μg and was achieved by injecting 2 μl from a 1 mg/ml solution in methanol.

The stationary phases used in this study were obtained from the suppliers as pre-packed columns (Table 1).

Table 1
Overview of the stationary phases used for selection of the test compounds (A) and for the evaluation of the column performance (B)

	Column	Abbreviation	Manufacturer	Dimensions (length × I.D., mm)	Particle size (μm)
A	μBondapak C_{18}	BON	Waters–Millipore	300 × 3.9	10
	NovaPak C_{18}	NOV	Waters–Millipore	300 × 3.9	4
	Kromasil KR100-5-C_{18}	KRO	Eka Nobel	250 × 4.6	5
	Exsil 100 ODS-B	EXS	Exmere	250 × 4.6	5
	Suplex pKb-100	PKB	Supelco	250 × 4.6	5
	Zorbax Rx-C_{18}	ZRX	Rockland Technologies	250 × 4.6	5
B	Zorbax Rx-C_{18}	ZRX	Rockland Technologies	250 × 4.6	5
	Hypersil BDS-C_{18}	BDS	Shandon Scientific	150 × 4.6	5
	Chromspher B	CHB	Chrompack	250 × 4.6	5
	Supelcosil LC-ABZ	ABZ	Supelco	150 × 4.6	5
	Polyspher RP-18	POL	Merck	150 × 4.6	10
	Asahipak ODP-50	ASA	Asahi Chemical Co.	125 × 4.0	5
	Aluspher RP-Select B	ALU	Merck	125 × 4.0	5
	3M-Z18	3MZ	Cohesive Technologies	150 × 4.6	6

2.3. Experimental set-up

The experiments carried out to characterize the columns were performed in strict order. Initially, for each column data were collected in duplicate at the highest pH to be tested. Subsequently, this was done for the lower pH. All experiments were repeated on a subsequent day.

Generally, silica-based columns are claimed to be only stable from pH values of 2 to 8. In order to avoid to operate the silica-based columns to close to the operating boundaries, they were tested at pH values of 3 and 7 using methanol as modifier. The non-silica-based columns were tested, using acetonitrile as modifier, at pH values of 7 and 11, and for the Asahipak ODP-50 and the 3MZ-18 column also at pH 3.

The modifier–buffer ratio was adjusted to ensure k' values larger than 1.

2.4. Calculations

The asymmetry factor (A_s) was calculated at 10% of the peak height and expressed as the ratio of the width of the rear and the front side of the peak. For the calculation of the plate height (HETP) the second moment of the peak was used [14].

For the stability of the column the repeatability of the plate height (RH) and the capacity factor (Rk') were determined. This was done by repeating the analyses on the next day. The difference between two days divided by the highest value (in most cases the first day) times 100% is reported.

3. Results and discussion

3.1. Selection of test compounds

Until now, the applicability of stationary phases for the analysis of basic compounds was tested using 32 compounds [1]. However, analysing 32 compounds each time is rather time consuming. The use of a representative set of test compounds, extracted from the 32 compounds, will result in a reduction of experimental work. This extraction, using the asymmetry data obtained for the test compounds on six different columns, was achieved using PCA. The information obtained with the reduced number of test compounds should be comparable with the information obtained with 32 compounds.

The data matrix used is shown in Table 2. For the missing values the particular column averages were used. The data were collected using methanol–10 m*M* phosphate buffer pH 7.4 as eluent [1]. The asymmetry factors for the BON column were calculated at a capacity factor 5.0. On the other columns the asymmetry values were taken at capacity factors varying from 2 to 8 with an average value of about 5.

In Fig. 1 the results of the PCA analysis of the six variables (LC columns) and the 32 objects (test compounds) are shown. Before performing PCA the data were autoscaled. With the first two principal components (PCs) 79% of the variance was described, while using the first three PCs 86% of the variance could be described. In Fig. 1A and B the score and the loading plot of the first and second PC, respectively, are given. In the loading plot can be seen that for the six different columns almost the same value is obtained for the first PC. A better distinction between the columns is obtained by plotting the second against the third PC (Fig. 1D). From this plot can be seen that the BON column and the NOV column behave very similar, which is not unexpected as they are produced by the same manufacturer. Also with the KRO column comparable results are obtained. In Fig. 1C the score plot is given for the second and the third PC.

Looking at the score plots, the compounds

Table 2
Asymmetry factors for 32 compounds (objects) and 6 LC colums (variables)

Objects	BON	NOV	ZRX	KRO	PKB	EXS
1	3.600	5.740	2.760	2.630	1.300	2.020
2	5.600	10.440	4.820	4.190	3.860	2.400
3	1.700	1.570	1.570	1.300	1.280	1.470
4	2.300	Missing	Missing	1.440	1.330	Missing
5	1.100	1.050	1.240	1.200	1.430	1.930
6	4.600	6.920	Missing	Missing	1.730	7.490
7	1.900	2.390	1.640	1.840	1.870	2.340
8	2.300	3.220	3.300	2.380	1.400	2.140
9	1.100	1.210	1.490	1.170	1.170	1.460
10	1.000	1.150	1.220	1.120	1.170	1.060
11	1.100	1.190	Missing	1.170	1.190	0.990
12	0.900	1.070	1.210	1.190	1.230	1.080
13	2.800	3.070	2.200	2.320	1.480	2.950
14	2.200	2.350	1.500	2.160	1.290	1.690
15	1.100	1.240	1.230	Missing	1.210	1.230
16	2.600	5.410	1.960	4.080	2.070	3.100
17	2.300	1.890	1.540	1.700	1.500	2.500
18	5.200	5.510	2.770	5.320	1.850	6.570
19	9.100	14.440	2.940	6.110	2.170	6.430
20	3.600	5.810	1.920	Missing	Missing	4.240
21	3.600	1.590	1.550	1.370	1.380	2.520
22	2.400	2.350	1.910	1.080	Missing	3.280
23	2.300	1.560	1.630	1.340	1.460	2.600
24	1.900	1.230	1.510	1.190	1.250	1.540
25	2.300	2.130	1.790	Missing	Missing	2.830
26	4.800	8.020	2.290	4.190	Missing	8.290
27	1.700	1.370	1.530	1.290	1.720	2.240
28	2.900	3.420	1.990	3.310	1.190	2.130
29	2.400	1.950	1.880	1.700	2.550	3.500
30	5.700	6.990	3.110	4.890	3.550	6.510
31	4.000	6.050	2.470	3.020	2.350	4.740
32	3.770	4.190	4.160	4.510	1.560	2.990

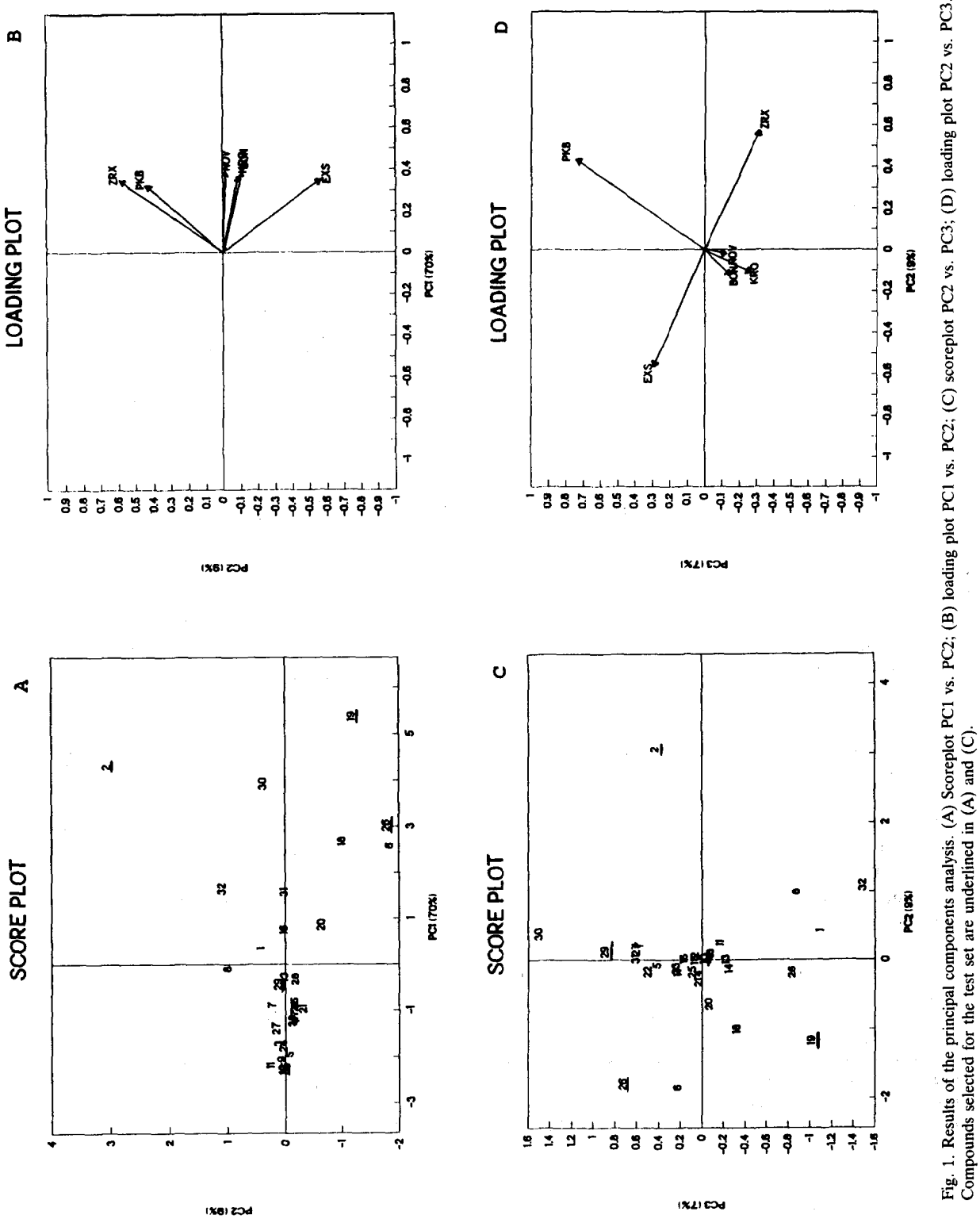

Fig. 1. Results of the principal components analysis. (A) Scoreplot PC1 vs. PC2; (B) loading plot PC1 vs. PC2; (C) scoreplot PC2 vs. PC3; (D) loading plot PC2 vs. PC3. Compounds selected for the test set are underlined in (A) and (C).

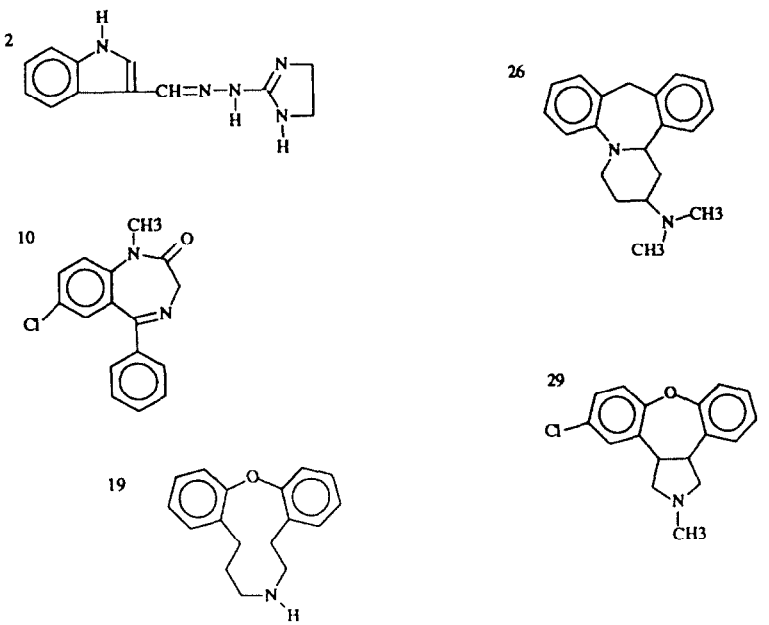

Fig. 2. Structures of the compounds selected with PCA.

positioned to the left in Fig. 1A and in the middle of Fig. 1C always gave symmetrical peaks. Compound 10 was selected as a "neutral" test compound, giving symmetrical peaks on all six columns tested. This compound is used to calculate the efficiency of the columns. The first PC seems to correspond with a general asymmetry effect; with increasing value for the PC the average asymmetry for the compounds is increasing. Other test compounds were selected from Fig. 1A and C on basis of their position somewhere on the edge of the cluster in order to obtain maximum discrimination. Compounds 2, 19, 26 and 29 were selected. Looking at Fig. 1C, compounds 30 and 32 would also have been good choices. The selection of the test compounds on basis of PCA fitted very well with the compounds which would have been selected by the analytical expert based on the polarity, the pK_a values and the peak shapes of the compounds [1]. However, other factors involved can easily be overlooked by the expert or can be difficult to interpret. In Fig. 2 the structures of the selected compounds are shown.

The correlation matrix of the variables (columns) is given in Table 3. In this table again the high correlation between the BON and the NOV column can be noticed. For the other columns probably different mechanisms play a role which influence the (a)symmetry. These different elu-

Table 3
Correlation matrix of the variables (columns) calculated from Table 2

	BON	NOV	ZRX	KRO	PKB	EXS
BON	1.000	0.935	0.678	0.877	0.636	0.771
NOV		1.000	0.696	0.871	0.674	0.704
ZRX			1.000	0.720	0.658	0.387
KRO				1.000	0.608	0.755
PKB					1.000	0.524
EXS						1.000

tion mechanisms can be a result of the different ways residual silanols are shielded. For instance, with the ZRX stationary phase highly pure silica was used, whereas residual silanols of the PKB stationary phase are shielded electrostatically.

3.2. Testing of HPLC columns specially designed for the analysis of basic compounds

In a previous paper [1] eight HPLC columns were compared in a more qualitative way. From these results it was concluded that the PKB column was the most promising column. Later on an improved version of this column appeared on the market, viz. ABZ. Also with the ZRX column reasonable results were obtained. Therefore, these two columns and six other LC columns were further tested using the five compounds selected in the first part of this study.

The silica-based columns used in the second part of this study (Table 1B), differed in the manner the residual silanols are shielded. As mentioned before, very pure silica was used in the preparation of the ZRX column. Residual silanols in the ABZ column are shielded by electrostatic repulsion. The stationary phase used for the CHB column are polymer-coated silica particles whereas for the BDS column silica with a homogeneous surface is used which is end-capped after bonding the C_{18} phase.

For the 3MZ and the ALU stationary phase, polybutadiene was coated on particles of zirkonium oxide and aluminium oxide, respectively. The ASA column consisted of macroporous particles of polyvinylalcohol-based polymer, in which reversed-phase chains were introduced by binding stearic ester chains through an ester bond. The POL column consisted of particles of polystyrene–divinylbenzene polymer with C_{18} chains.

Besides the asymmetry factor an important parameter for the comparison of columns is the efficiency. For example for purity analysis of unknown compounds, i.e. new chemical entities in drug development, a high separation efficiency is necessary in an acceptable time. Low efficiency and high asymmetry will lead to poor purity analyses. High asymmetry will also hamper a correct integration. To make it more complicated, columns with low efficiency will mask the factors which contribute to the tailing. For mutual optimization MCDM is therefore necessary.

The primary goal of the column selection is to select the best column which can be broadly applied. Therefore the average asymmetry of the test compounds was used and not the single values. For the calculation of the efficiency of the column the results of test compound 10 were used. In order to compare the efficiency of the columns and to compensate for differences in the column length the plate height was calculated.

Table 4
Chromatographic data obtained at pH 7

Column	Asymmetry (A_s)					Capacity factor (k')					Average				
	2	10	19	26	29	2	10	19	26	29	A_s	k'	HETP (μm)	RH (%)	Rk' (%)
ZRX	3.8	1.1	6.8	5.9	3.1	3.4	4.9	7.6	5.2	4.0	4.1	5.0	17.3	1.7	10.3
BDS	7.1	0.9	8.3	7.4	2.5	3.5	6.1	2.3	2.9	2.6	5.2	3.5	19.9	2.5	3.3
CHB	4.7	1.1	7.5	6.5	3.9	5.0	2.6	24.4	16.4	8.6	4.7	11.4	31.4	1.0	1.9
ABZ	5.8	0.9	2.1	2.2	1.3	5.2	7.8	4.1	6.4	5.7	2.5	5.8	20.7	4.1	3.9
ASA	6.1	1.7	4.6	4.5	5.0	2.0	5.1	2.8	2.0	2.9	4.4	2.9	64.3	11.4	2.0
ALU	1.3	0.6	1.6	1.7	1.4	12.9	5.0	5.5	6.2	6.5	1.3	7.2	74.8	14.1	6.1
POL	3.3	1.1	1.8	1.3	1.2	0.5	5.6	1.2	3.2	8.0	1.8	3.7	298.3	69.3	9.0
BMZ	1.6	1.0	1.7	1.4	1.2.	4.5	2.6	3.2	3.1	3.1	1.4	3.3	25.2	8.9	3.9

Another factor which is important for a good column performance is the reproducibility of analyses. In this study the capacity factors and the plate heights were measured on two subsequent days. In this way the short-term reproducibility or repeatability is measured. Testing the ruggedness of a column requires more extensive testing which should be done after the initial selection described in this report. The relative difference between two days is reported for compound 10. The obtained data must be seen as qualitative data in comparison with the more precise data obtained for the asymmetry and the plate height. The repeatability of the capacity factor is an indication of the stability of the stationary phase, while the repeatability of the plate height is an indication of the stability of the packing material in combination with the stationary phase.

3.3. Testing of columns at pH 7

The results are shown in Table 4. The first question was whether k' values should be treated as a factor. In ref. [1] a correlation was found for k' and A_s. Because the k' values vary from column to column, for a honest comparison of the asymmetry factors the k' values should be included. However, in this case there was hardly any correlation between k' and A_s, as can be seen from the correlation matrix presented in Table 5. Because in the column evaluation only the asymmetry plays a role, the k' values were excluded.

In order to interpret the results, bar charts were made (see Fig. 3). For four of the columns recommended for basic solutes an average asymmetry for the test compounds of more than 4 was observed. Lowest asymmetry factors were obtained for the 3MZ, the ALU and the POL column. From these three columns only the 3MZ column showed a reasonable low plate height. Poor efficiency was observed for the POL column. However, the repeatability of the 3MZ column is moderate in comparison with for example the CHB column. A qualitative interpretation of the bar charts is given in Table 6. In this table the repeatability of the capacity factor and of the plate height are combined. It can be concluded that the best results are obtained with the 3MZ column, although there are still doubts about the ruggedness of the column.

MCDM using the Pareto optimal (PO) points [15] is another method to interpret the results. A PO plot is the best possible combination of two criteria. Applying four factors, in principle 6 plots of 2 criteria can be made. The most important one is the asymmetry vs. plate height. As the asymmetry is the starting point for the optimization it was decided to add the PO plots for asymmetry vs. repeatability of the plate height, and asymmetry vs. the repeatability of the capacity factor. When three or more criteria are involved a stacked PO plot can be made. A stacked PO plot is a stack of scatter plots of the individual criteria. The individual plots are placed on top of each other. Points which are exactly vertical to each other (over the different plots) belong to the same stationary phase [15].

Table 5
Correlation matrix at pH 7 calculated from Table 4

	A_s	k'	Rk'	HETP	RH
A_s	1.00	0.14	−0.34	−0.40	−0.46
k'		1.00	−0.21	−0.21	−0.29
Rk'			1.00	0.46	0.48
HETP				1.00	0.99
RH					1.00

Table 6
Qualitative interpretation of the results of Fig. 3

Column	Asymmetry	Efficiency	Repeatability
ZRX	−	+	0
BDS	−	+	+
CHB	−	+	+
ABZ	0	+	+
ASA	−	0	0
ALU	+	0	0
POL	+	−	−
3MZ	+	+	0

+ = Good; 0 = moderate; − = poor.

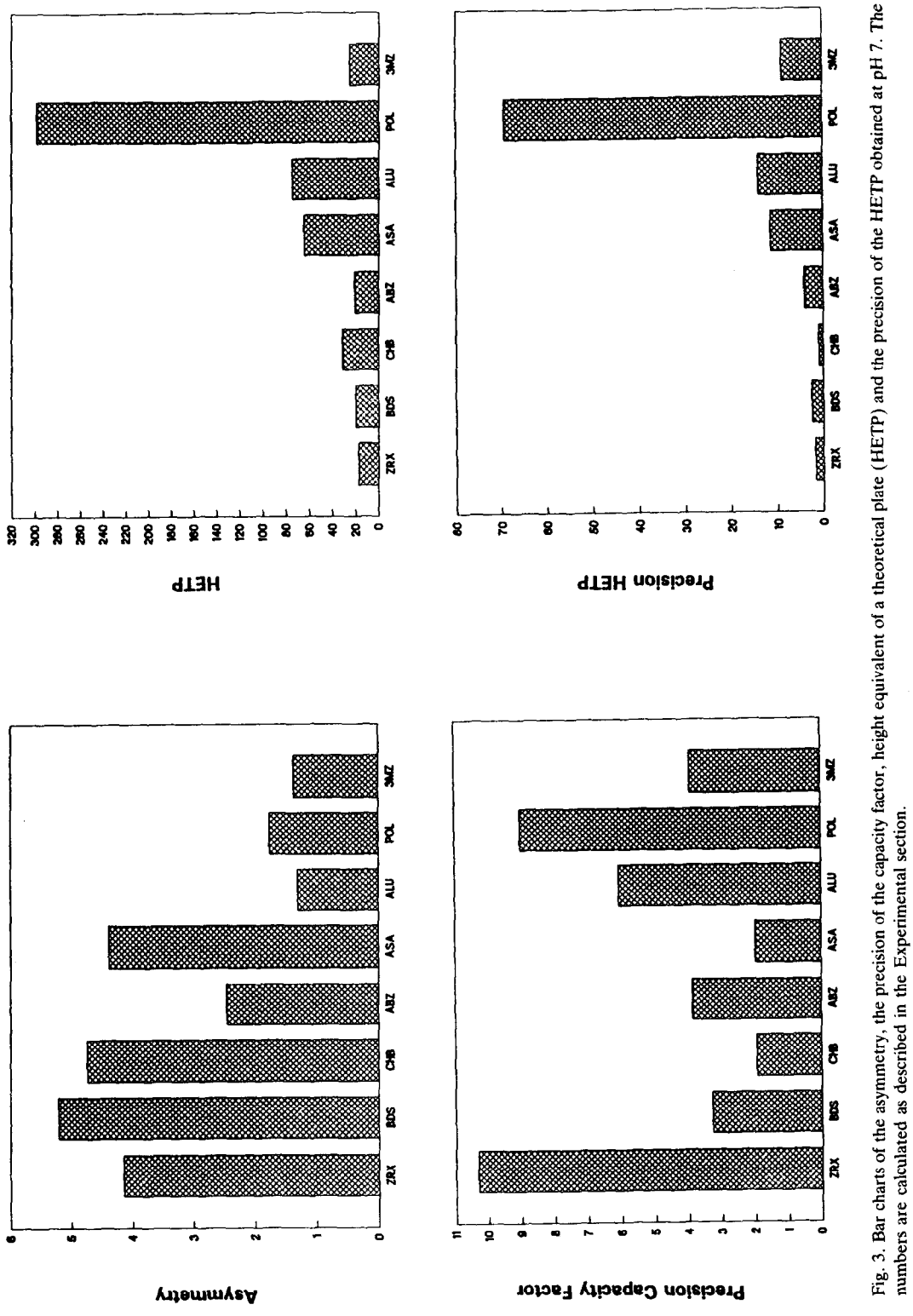

Fig. 3. Bar charts of the asymmetry, the precision of the capacity factor, height equivalent of a theoretical plate (HETP) and the precision of the HETP obtained at pH 7. The numbers are calculated as described in the Experimental section.

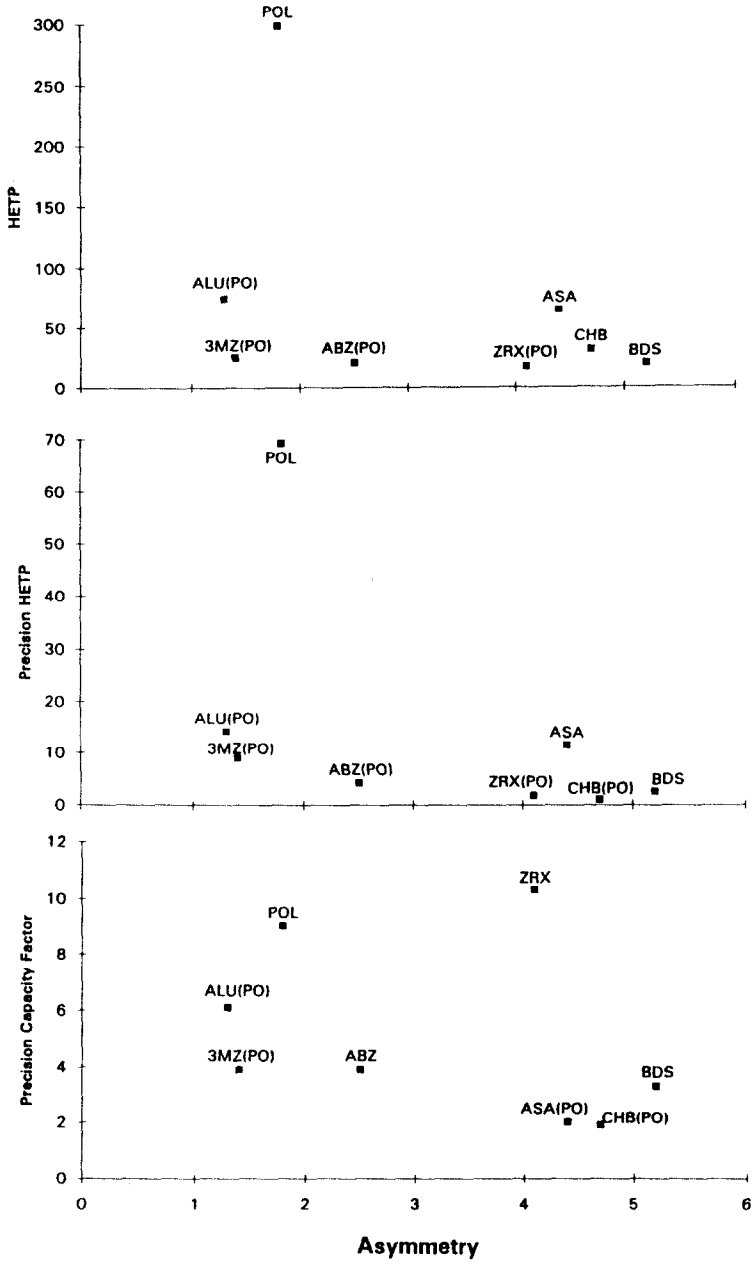

Fig. 4. Stacked PO plot of the asymmetry vs. HETP, precision of the HETP and precision of the capacity factor, obtained at pH 7. The PO points are indicated between brackets (PO) in the plot.

The stacked PO plot is shown in Fig. 4 in which the PO points are indicated. Very good asymmetry values are obtained for the ALU and the 3MZ. Comparing the efficiency, the 3MZ is clearly better. With respect to this aspect the ZRX and the ABZ are even better. However,

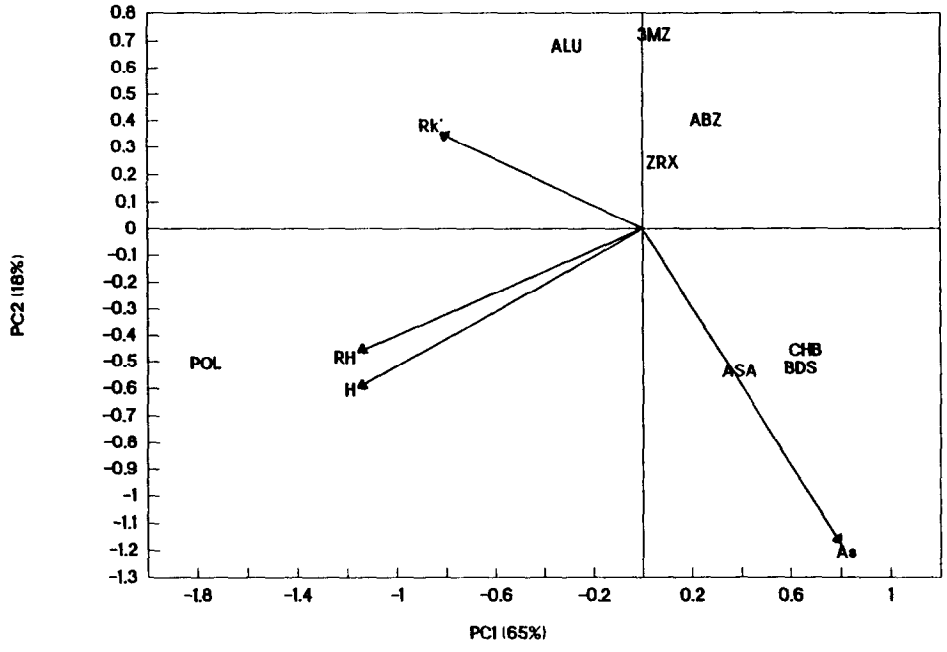

Fig. 5. Bi-plot obtained at pH 7. The data used for the bi-plot are the average values given in Table 4.

the increased asymmetry is likely to be more substantial than the gain in efficiency. Therefore, the 3MZ is to be preferred.

Still it is interesting to see whether other chemometrical methods can improve the column evaluation. In Fig. 5 a bi-plot is given based on the information presented in Table 4. As pretreatment the data were autoscaled. The first two PCs explain 83% of the variance. As can be seen the demands on "asymmetry" and "repeatability

Table 7
Chromatographic data obtained at pH 3, 7 and 11

Column	pH 3				pH 7				pH 11			
	A_s^a	HETP (μM)	RH (%)	Rk' (%)	A_s^a	HETP (μM)	RH (%)	Rk' (%)	A_s^a	HETP (μM)	RH (%)	Rk' (%)
ZRX	3.3	17.5	5.4	4.7	4.1	17.3	1.7	10.3				
BDS	8.8	20.5	4.4	0.0	5.2	19.9	2.5	3.3				
CHB	5.1	32.6	0.5	4.1	4.7	31.4	1.0	1.9				
ABZ	1.8	21.0	5.7	4.0	2.5	20.7	4.1	3.9				
ASA	3.2	62.0	27.1	6.3	4.4	64.3	11.4	2.0	2.2	64.0	14.9	3.6
ALU					1.3	74.8	14.1	6.1	0.7	73.8	10.6	25.6
POL					1.8	298.3	69.3	9.0	1.4	323.6	89.3	6.2
3MZ	1.6	26.5	13.8	6.8	1.4	25.2	8.9	3.9	1.0	24.7	4.9	7.7

[a]Average values of compounds 2, 10, 19, 26 and 29.

Table 8
Pareto optimal (PO) points at pH 3, 7 and 11

Column	PO points at pH 3			PO points at pH 7			PO points at pH 11			PO points all pH values		
Column	A_s-HETP	A_s-RH	A_s-Rk'	A_s-HETP	A_s-RH	A_s-Rk'	A_s-HETP	A_s-RH	A_s-Rk'	A_s-HETP	A_s-RH	A_s-Rk'
ZRX	**	*		**	*		–	–	–	**	*	
BDS			*				–	–	–			*
CHB		*			*	*	–	–	–		*	
ABZ	**	*	*	**	*		–	–	–	**	*	
ASA						*			*			
ALU	–	–	–	**	*	*	**	*	*	**	*	*
POL	–	–	–						*			
3MZ	**	*	*	**	*	*	**	*	*	**	*	*

– = Not tested. **PO points are considered to be more important than *PO points.

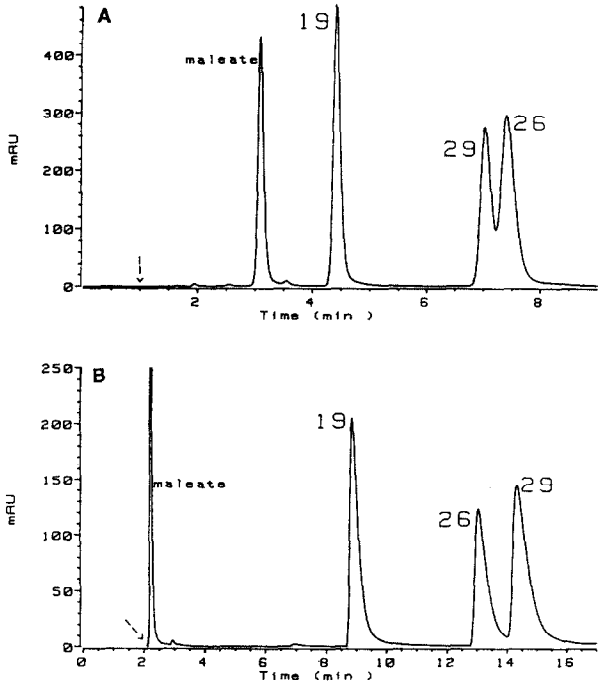

Fig. 6. LC–UV chromatograms of compounds 19, 26 and 29. Detection was done at UV 210 nm. The arrow in the chromatograms indicates the dead time. (A) A 2-μl volume from a 1 mg/ml solution was injected onto a Supelcosil LC-ABZ 150 × 4.6 mm I.D. column. The flow-rate was set to 1.0 ml/min and the eluent used was methanol–25 mM NaH_2PO_4 pH 3.0 (40:60, v/v). (B) A 2-μl volume from a 1 mg/ml solution was injected onto a Zorbax Rx-C18 250 × 4.6 mm I.D. column. The flow-rate was set to 1.0 ml/min and the eluent used was methanol–25 mM NaH_2PO_4 pH 3.0 (45:55, v/v).

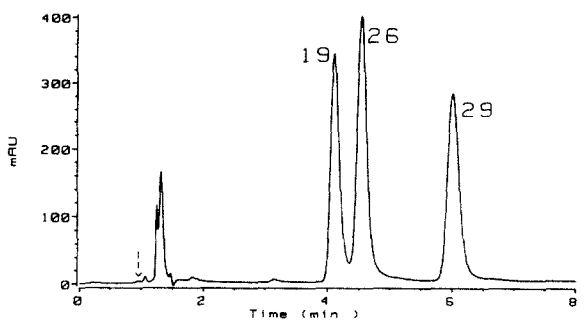

Fig. 7. LC–UV chromatogram of compounds 19, 26 and 29. A 2-μl volume from a 1 mg/ml solution was injected onto a 3MZ-18 150 × 4.6 mm I.D. column. The flow-rate was set to 1.0 ml/min and the eluent used was acetonitrile–25 mM borate pH 11.0 (45:55, v/v). Detection was done by UV at 210 nm. The arrow in the chromatogram indicates the dead time.

of the capacity factor" are contradictory to each other, while there is a high correlation between "plate height" and "repeatability of the plate height". For the optimal columns these chromatographic factors must be as low as possible, preferably in the opposite direction of the arrows in Fig. 5. The best columns are clustered together in the upper right quadrant of the figure. Also from this graph, taking the "asymmetry" and "plate height" as the most important factors, it can be concluded that the 3MZ is the most optimal column, although the differences with three other columns are small. An advantage of

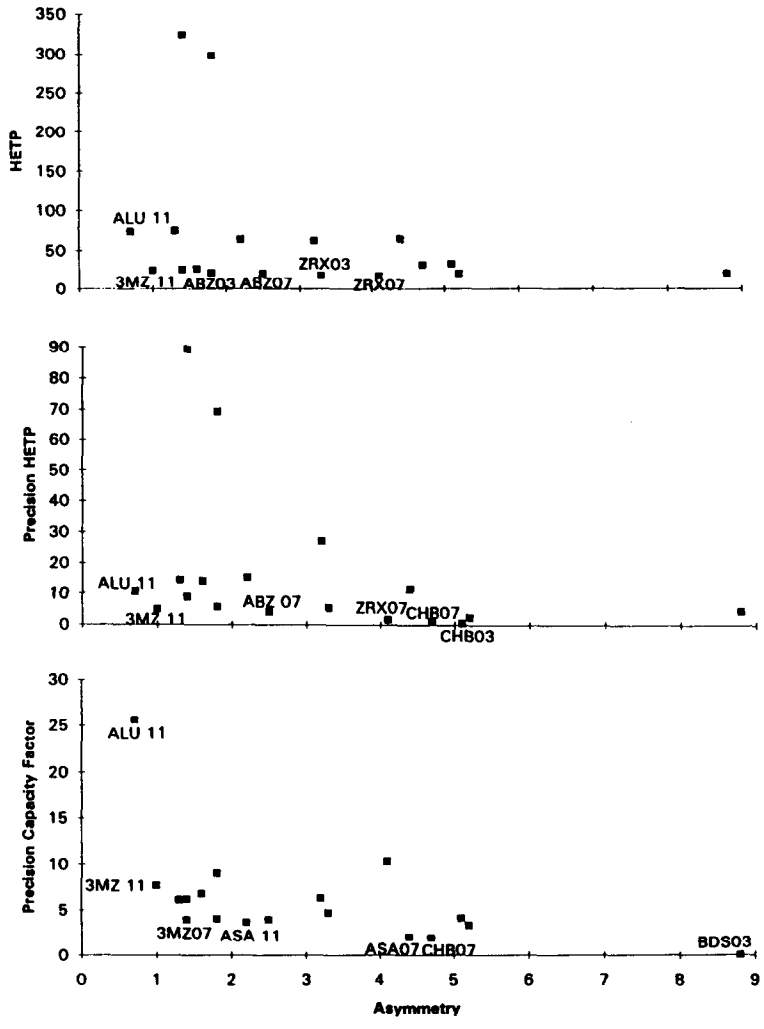

Fig. 8. Stacked PO plot of the asymmetry vs. HETP, precision of the HETP and precision of the capacity factor, obtained at pH values 3, 7 and 11. Only the PO points are indicated by giving the column abbreviation together with the pH used.

the bi-plot is that all information can be seen in one graph, while this is not the case for the bar charts and the MCDM plots. However, one has to realize that with the bi-plot information is lost, that the interpretation is sometimes difficult and that all factors are treated as being of the same importance.

3.4. Testing of columns at pH 3, 7 and 11

An overview of the results at pH values of 3, 7 and 11 is given in Table 7. Again from the original data the correlation between the capacity factors and the asymmetries was so low that the capacity factor was excluded as a factor. From the correlation matrices at pH 3 and 11 again surprisingly high correlations of 0.82 and 1.00, respectively, were found between the plate height and the repeatability of the plate height, as was also observed in the previous section at a pH of 7 (see Table 5).

Because at pH of 3 and 11 less columns were used than at the pH of 7, the interpretation is

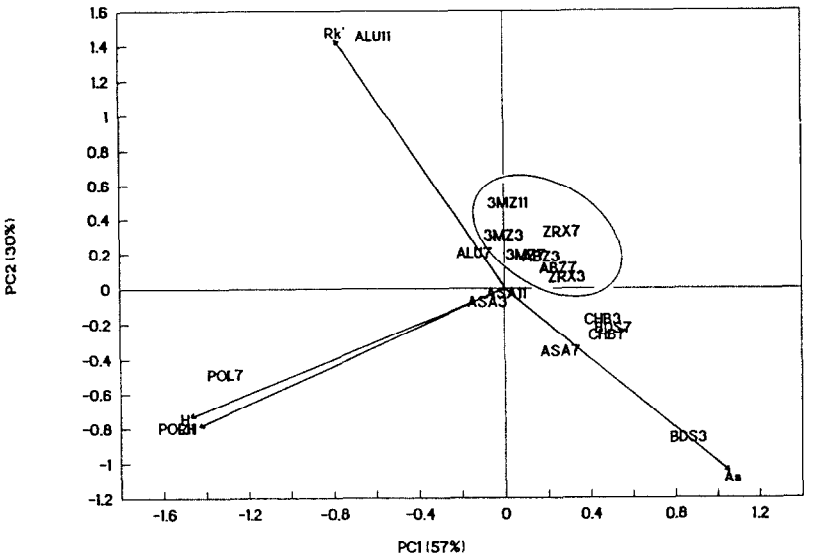

Fig. 9. Bi-plot of the data obtained at all pH values. The data used for the bi-plot are the values given in Table 7.

easier. The PO points were determined and are given in Table 8. At a pH of 3 the 3MZ column again shows the lowest tailing, although the difference with the ABZ column is very small. Even more, with the ABZ column a smaller plate height and a better repeatability is obtained. With the ZRX column a further (small) improvement in efficiency is obtained while the other factors are worse. In Fig. 6 chromatograms of compounds 19, 26 and 29 on the ABZ (Fig. 6A) and ZRX column (Fig. 6B) at pH 3 are shown. With the ABZ column the results at pH 3 are better than at pH 7.

At pH 11, both the ALU and 3MZ columns show good characteristics. However, with the ALU column fronting peaks were observed at this pH (Table 7). Also the plate height is in favour of the 3MZ column. Clearly, at pH 11 the 3MZ column is a good choice for analyzing basic solutes. This is confirmed by the repeatability data. In Fig. 7 a chromatogram of compounds 19, 26 and 29 on the 3MZ column at pH 11 is shown.

The ultimate goal of course is to select the best column operating at its optimal pH, although in practice there can be reasons to select on forehand a certain pH. In Table 8 the PO points at all pH values are given. The results are also presented graphically in Fig. 8. On basis of this MCDM plot the user can decide which column to use by weighing the importance of the different chromatographic factors. Overall the 3MZ column seems to give the best results of the columns tested. Looking at the individual data for this column (Table 7), operating at pH 11 is preferred at which very symmetrical peaks and acceptable plate heights are obtained. A bi-plot of the overall results is given in Fig. 9. For this the results of Table 7 are used and the data are autoscaled. The first two PCs explain 88% of the variance. A cluster of optimal columns is encircled. The 3MZ column shows better asymmetry values while the ZRX and ABZ columns show a better repeatability of the capacity factor. This latter factor, however, is only a qualitative factor while the asymmetry values are quite accurately determined. With respect to the efficiency and the repeatability of the efficiency the columns are equally good. It is interesting to see that at the different pH values for some columns, e.g. the 3MZ, ZRX and ABZ column, only small differences while for other columns, e.g. the ALU and BDS column, large differences were observed.

4. Conclusions

The selection of a set of test compounds out of a larger set with PCA was carried out. Using a test set of only five compounds, information about the applicability of stationary phases developed for the analysis of basic compounds was obtained. A complete column test requires only two LC analyses using two mobile phases for the analysis of 3 and 2 test compounds. Each analysis consists of a duplicate injection and the testing is repeated on the next day.

Using bar charts and several chemometrical techniques such as bi-plots and MCDM, differences between stationary phases in their applicability for the analysis of basic solutes were successfully made. The advantage of the MCDM plots is that the factors involved can be visually weighed in order to select a column. The bi-plots are more difficult to interpret. An interesting conclusion from the results is that the columns with a high efficiency generally show a good repeatability for the plate height.

A column consisting of zirkonium oxide particles coated with polybutadiene proved to be suitable at all pH values tested. The best results with this column were obtained at pH 11. However, one has to realize that at this moment too limited information is available on the ruggedness of the column. This requires specific testing on this aspect. Furthermore, the high price of this column is a limiting factor. At pH 3 a Supelcosil LC-ABZ and a Zorbax Rx-C_{18} column showed also promising results. Polymer-based columns showed inferior results because of the high plate heights obtained.

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