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Repeatability and reproducibility of retention data and band profiles on reversed-phase liquid chromatographic columns II. Results obtained with Symmetry C_{18} columns

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Abstract

The long-term repeatability, the column-to-column and the lot-to-lot reproducibilities of the retention and profile of the peaks obtained with 15 different Symmetry C_{18} columns were investigated. This packing material is an octadecyl bonded silica for RP-LC. Data characterizing the retention, the steric selectivity, the hydrogen bonding capacity, the hydrophobic interaction selectivity, the column efficiency, and the peak asymmetry are reported for a group of 30 neutral, acidic and basic compounds selected as probes. These data were acquired under five different sets of chromatographic conditions, using an HP 1100 liquid chromatograph and following an experimental protocol previously described. Statistical comparison of five different columns of one batch and of columns representative of 10 different batches are based on the high precision data obtained. For example, for 28 of the 30 components, the R.S.D.s of the retention factors measured on 10 columns from as many different batches were better than 2%. The R.S.D.s of the separation factors for 27 of these 30 components were below 1.4%. Such a level of column reproducibility is most impressive.

Keywords: Retention data; Band profiles; Stationary phases, LC; Symmetry C_{18} columns

1. Introduction

In a companion paper [1], we described in detail an experimental protocol for the systematic investigation of the repeatability and reproducibility of the quantitative chromatographic data obtained with commercial columns. This protocol was applied to the study of columns of Symmetry C_{18} (Waters, Milford, MA, USA) and the results are reported here. The aim of this work is to provide independent data regarding the performance currently available from commercial columns. An experimental investigation such as this one is necessarily limited in scope. Although our protocol includes 30 different compounds to be analyzed in five different groups, using different mobile phases, it cannot include all the compounds that most analysts would like to see. When judging the suitability of a given column to a certain application, one must take into account not only the data presented here but also the specific properties of the column, such as the balance between the silanophilic interactions and the hydrophobic selectivity of all the columns available.

There is a wide variety of reversed-phase liquid

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chromatography (RP-LC) columns available on the market today and this is certainly one of the important reasons for the great popularity of this mode of chromatographic separations. This flexibility is an enormous advantage and it should not be reduced by attempts at normalizing, standardizing, referencing or otherwise regulating a dynamic and vibrant field. If the level of reproducibility achieved by a few commercial products, a level which was unknown at the beginning of this work, proves sufficient, there would be no need to develop new reference materials, especially if they are not able to demonstrate a markedly better level of performance. On the other hand, there is certainly a need for some classification of the available columns or packing materials. Another of our goals in providing these reproducibility data is to contribute to a better understanding of the possibilities of RP-LC analyses by allowing comparisons based on data of known precision, hence more meaningful.

2. Experimental

The reader is referred to the companion paper [1] for a detailed discussion of the experimental protocol. Only a summary description of the essential points and the required information regarding the columns used in this work and their characteristics are presented here.

2.1. Instrument

The data were acquired using a Hewlett-Packard (Palo Alto, CA, USA) HP 1100 liquid chromatograph including a binary solvent delivery system, an autosampler, a diode array UV detector, a column thermostat and a data station. All of these units were controlled by a dedicated computer (Pentium processor, operating under Windows 95). Automatic data acquisition and the determination of most parameters were performed using the standard features of this instrument (ChemStation Software, Rev. A. 05.03).

Because it was essential to maintain a high degree of accuracy, the periodic tests of this instrument suggested by the manufacturer were performed weekly. These tests correspond to the operational qualification and performance verification procedures for the HP 1100 Series HPLC modules [2]. They were described in detail in the companion paper [1].

2.2. Stationary phase

The experimental results reported in this work were acquired with 15 columns (150×3.9 mm) packed with Symmetry C_{18} (5 μm), a packing material from Waters (Milford, MA, USA). Symmetry C_{18} is a porous silica, chemically bonded with octadecylsilane. The main characteristics of the initial silica and the packing material batches are summarized in Table 1. Five columns were packed with the packing material from the same batch (referred to as ''1 batch, 5 columns''). Ten columns were packed with 10 different batches of packing material (referred to as ''10 batches''). The 10 batches of packing material are derived from eight different batches of silica, one of which had been bonded three times (Table 1). The columns were packed by the manufacturer and used as received.

The study involved first the determination of the repeatability of the data acquired with one column during a few hours [1]; second, the long term repeatability of the data acquired with one column over a period of 10 days; third the column-to-column within one batch reproducibility of the data acquired with the five columns packed with the same batch of packing material and fourth, the batch-to-batch reproducibility of the data acquired with the 10 columns packed with 10 different batches of packing material. Data regarding the short-term (over a few hours) repeatability were previously published [1]. The column used for this preliminary work belongs to the same batch as the five columns of one batch used in the present study.

No attempts were made at aging the columns. On the contrary, the data reported correspond to virgin columns, the aging corresponding only to the possible effects of the samples described below and of the volume of mobile phase percolated during the test described later. For any given column, the tests lasted a maximum of a few days (see later).

2.3. Samples and chemicals

Fresh solutions of the samples required for each of

Table 1
Physico–chemical properties of the 10 batches of stationary phase (Symmetry C_{18}) supplied by the manufacturer (Waters)

Silica batch No.	Particle size (μm)	Particle size distribution (90:10, ratio)	Pore size (Å)	Surface area (m^2/g)	% Porosity	Na, Al, Fe content (ppm)	Particle shape
31 A	5.00	1.42	91	344	65	<1; <1; 1.5	Spherical
32 A	5.07	1.44	89	343	65	1.5; 1.0; 1.6	‘
33 A	4.96	1.47	91	340	65	<1; <1; <1	‘
34 A	5.09	1.47	90	337	65	1; <1; <1	‘
35 A	4.95	1.47	93	332	66	<1; <1; 3.4	‘
36 A	4.99	1.45	93	337	66	2.3; 1.4; 8.7	‘
38 A	5.02	1.45	92	335	65	1.2; <1; 3.5	‘
40 A	4.99	1.44	91	340	65	<1; <1; <1	‘
Mean	5.02	1.45	90.8	339.4	65.2	N/A	
R.S.D. (%)	1.01	1.15	1.71	1.19	0.65	N/A	

Symmetry C_{18} batch No.	Corresponding silica batch No.	Total carbon (%)	Mean surface coverage ($\mu mol/m^2$)
124	31 A	19.81	3.18
125	32 A	20.03	3.19
126	32 A	19.82	3.21
127	33 A	19.66	3.16
128	34 A	19.47	3.16
129	35 A	19.37	3.21
130	36 A	19.57	3.20
131	32 A	19.83	3.24
132	38 A	19.48	3.22
133	40 A	19.22	3.13
All batches of Symmetry C_{18} were end-capped	Mean	19.63	3.19
	R.S.D. (%)	1.27	1.03

the five tests carried out were prepared weekly by dilution of the selected chemicals in the corresponding mobile phase, using filtered solvents and carefully cleaned glassware. They were conserved in a refrigerator between use. Sample volumes of 10 μl were injected successively, using the autosampler. The qualitative and quantitative compositions of the five test mixtures were described in the companion paper. The names of the compounds are found in the figure captions and the tables. Typical chromatograms of these mixtures were also given in the companion paper [1] and need not be reproduced here.

2.4. Presentation of the data

Unless otherwise mentioned, all the data measured or calculated and reported in this work are the average of five consecutive measurements carried out under identical conditions. Figs. 1–5 illustrate the repeatability and reproducibility of the retention times of the probe compounds in the five different tests. The data characterizing the short-term repeatability are the average values of the parameters measured for five consecutive injections carried out into one column over a period of a few hours. They were already published for this brand of columns [1]. Only the long-term repeatability data are given here, for the sake of reference. The data characterizing the long-term repeatability of the measurements were obtained by repeating the series of five consecutive analyses of the test mixture on the same column after the measurements had been performed on all the columns tested (a total of 15). This interval was typically 10 days.

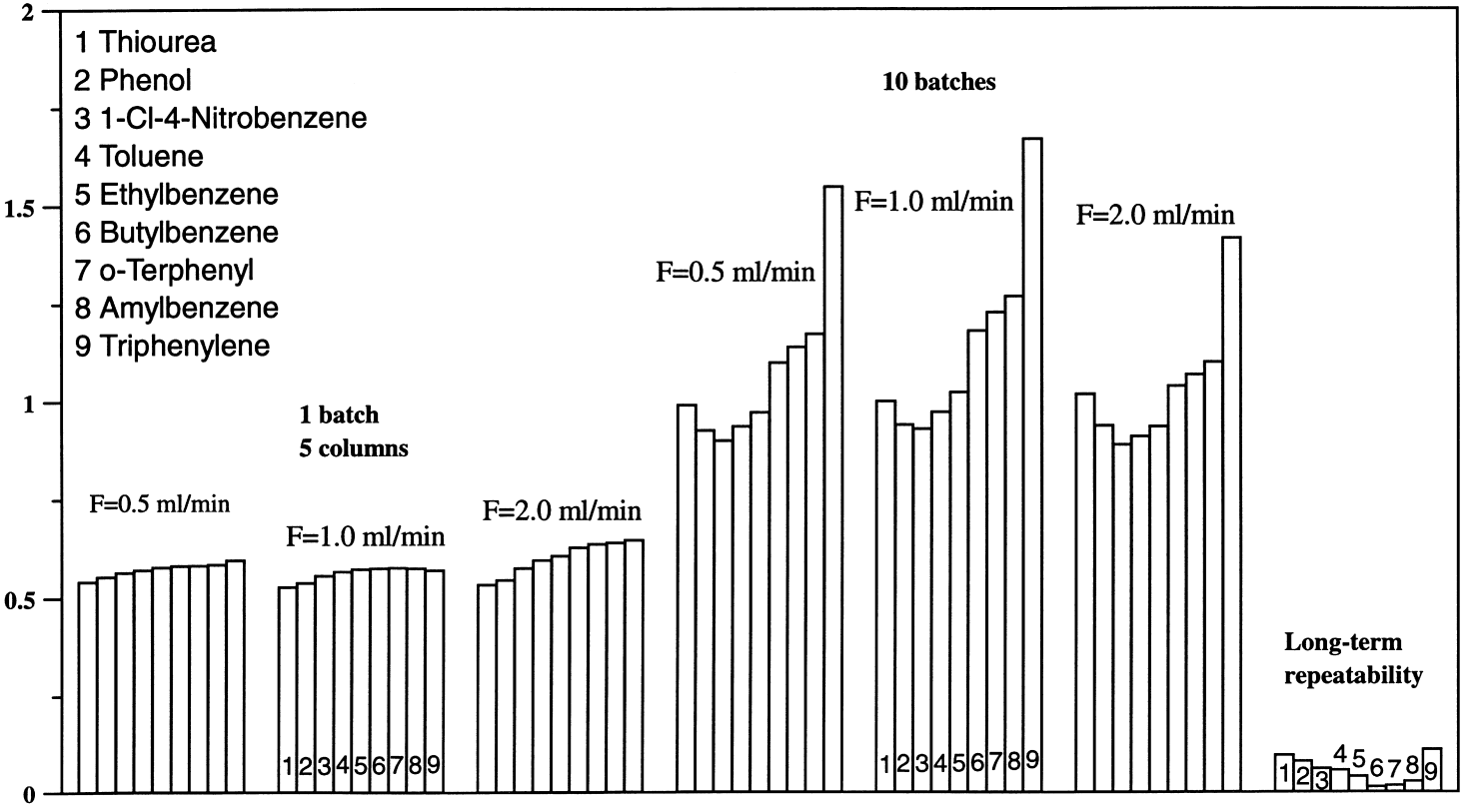

Fig. 1. Reproducibility of the retention time measured in the first test. Mobile phase, methanol–water (80:20) at 1 ml/min. $T=25°C$, detection wavelength, 254 nm. Each bar represents the R.S.D. observed for one given compound (identified by its number, top left). For the data set ''1 batch, 5 columns'', a bar represents the R.S.D. of 25 injections made on five columns, packed with packing material from the same batch. For the data set ''10 batches'', a bar represents the R.S.D. of 50 injections made on 10 columns, packed with 10 different batches of packing material. For the data set ''long-term repeatability'', a bar represents the R.S.D. of 10 injections made on one column over a 10-day period.

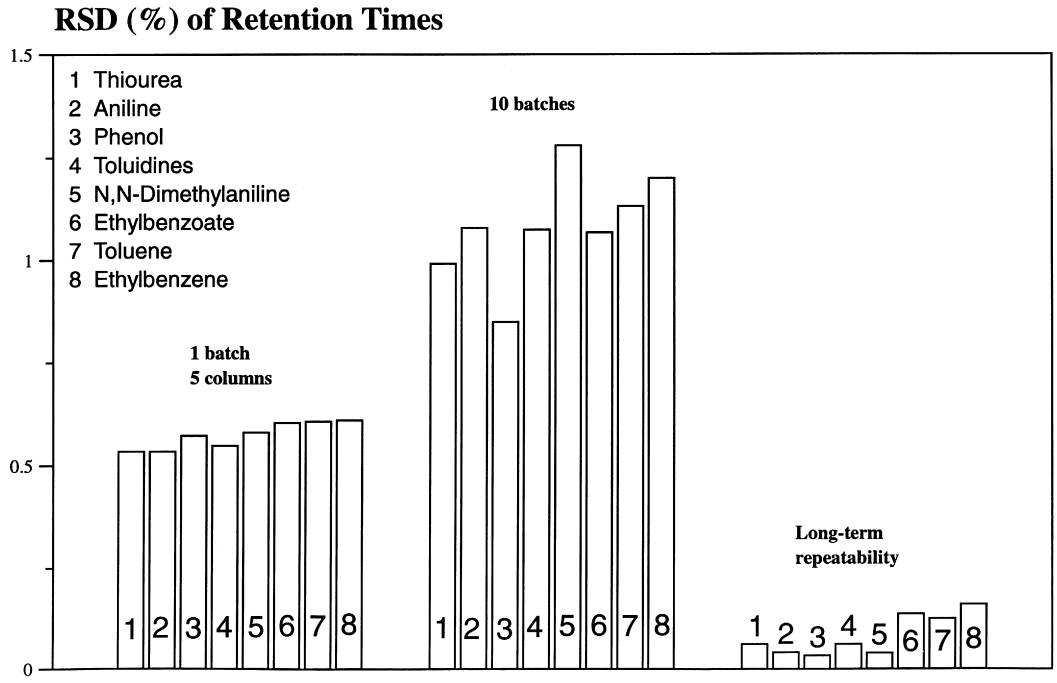

Fig. 2. Reproducibility of the retention time measured in the second test. Mobile phase, methanol–water (55:45) at 1 ml/min. $T=25°C$, detection wavelength, 254 nm. Same data presentation as in Fig. 1.

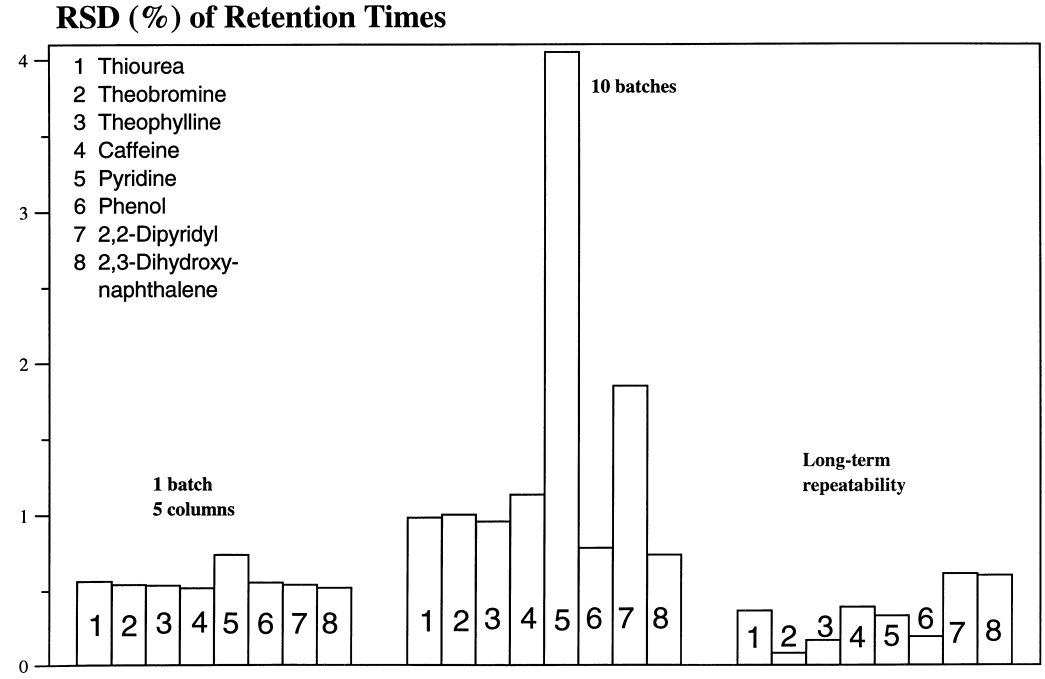

Fig. 3. Reproducibility of the retention time measured in the third test. Mobile phase, methanol–water (30:70) at 1 ml/min. $T=25°C$, detection wavelength, 254 nm. Same data presentation as in Fig. 1.

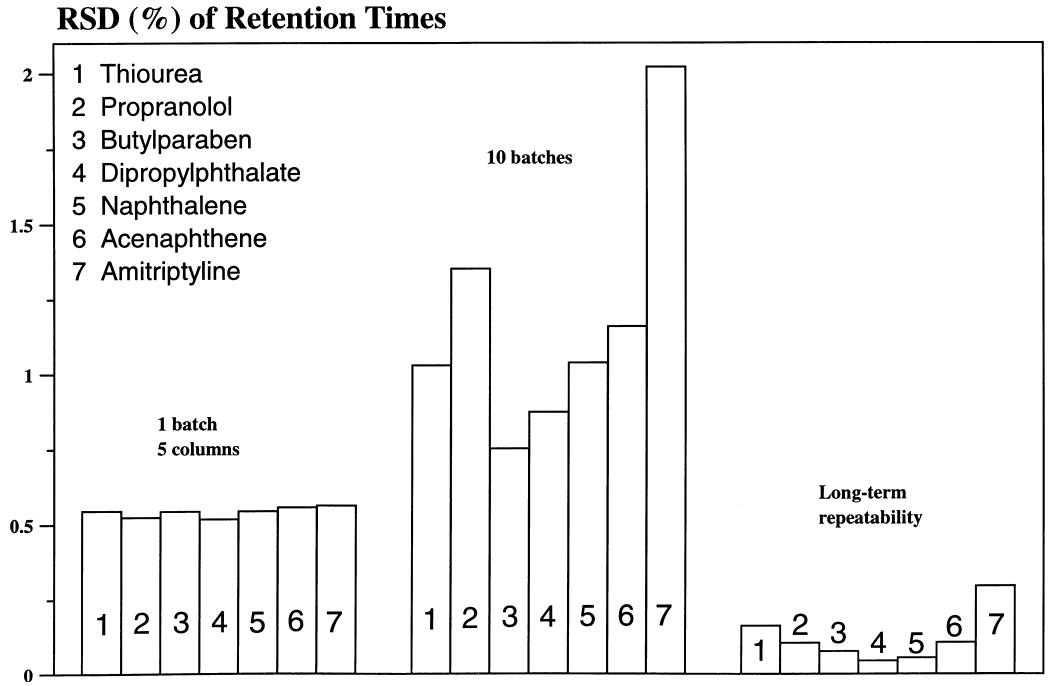

Fig. 4. Reproducibility of the retention time measured in the fourth test. Mobile phase, methanol–water (65:35) buffer with potassium phosphate, monobasic/dibasic at pH 7.00, $T=25^{\circ}C$, detection wavelength, 254 nm. Same data presentation as in Fig. 1.

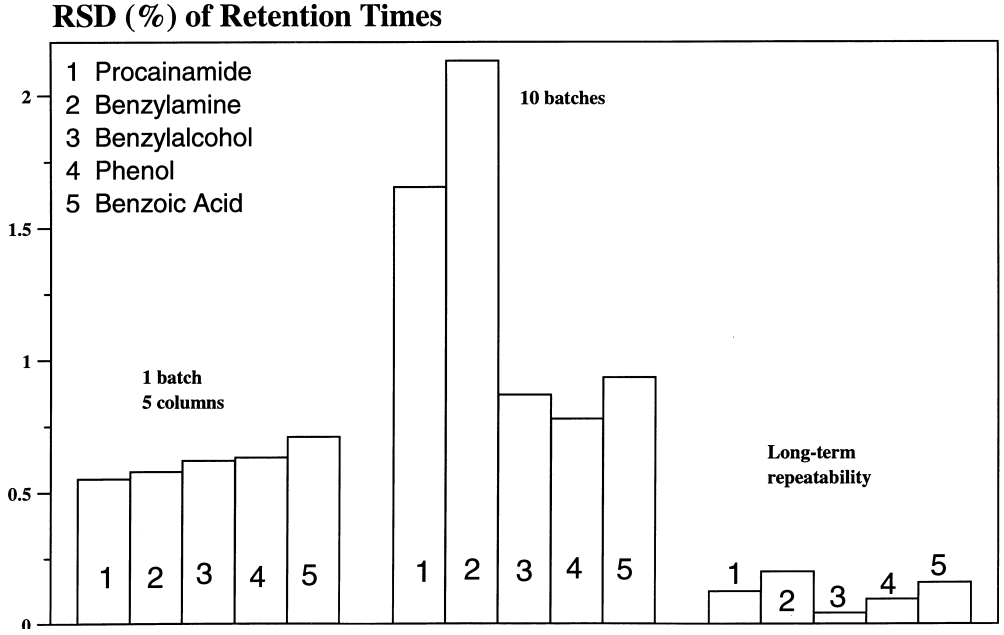

Fig. 5. Reproducibility of the retention time measured in the fifth test. Mobile phase, methanol–water (30:70) buffer with phosphoric acid/potassium monophosphate buffer at pH 2.70, $T=25^{\circ}C$, detection wavelength, 254 nm. Same data presentation as in Fig. 1.

The data characterizing the column-to-column reproducibility within one batch are the standard deviations of the whole set of data obtained on the five columns packed with the packing material from the same batch. The data characterizing the batch-to-batch reproducibility are the standard deviations of the whole set of data obtained on the 10 columns packed with packing material from 10 different batches. For each chromatographic column, these data are the average of five consecutive measurements.

3. Results and discussion

Data on the long-term repeatability and on the column-to-column within one batch and the batch-to-batch reproducibility of the retention times of the different components of the five test mixtures are summarized in Figs. 1–5. The column-to-column within one batch and the batch-to-batch reproducibility data of the retention factors are shown in Figs. 6–10 and quantified in Tables 2–6. The same reproducibility data for the hydrophobic selectivity, the steric selectivity, and the separation factors of the basic test compounds are given in Figs. 11–13, respectively. The data regarding the reproducibility of the separation factors are reported in Tables 7–9. The short-term and long-term repeatability and the column-to-column within one batch and the batch-to-batch reproducibility data of the column efficiency for the different components of the five test mixtures are summarized by the data in Figs. 14–18. The column-to-column within one batch and the batch-to-batch reproducibility data of the tailing factors are summarized in Table 10.

The Symmetry columns cannot separate the three toluidine isomers in test mixture 2. A broad triplet is obtained instead. In the following, we consider this multiplet as just another peak. The determination of the reproducibility of the characteristics of this peak constitutes a particularly harsh test of the reproducibility of the columns studied. Relatively minor variations in the relative retention of the toluidine

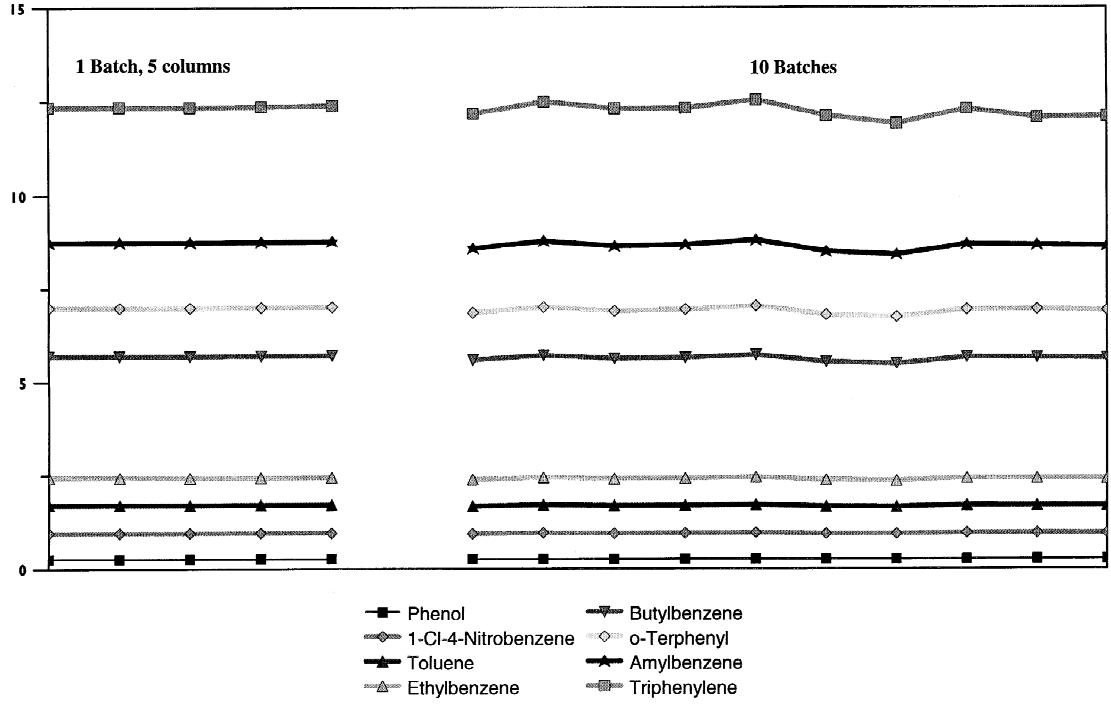

Fig. 6. Retention factors of the components of the first test mixture. Each data point represents the average of five consecutive injections carried out on a column.

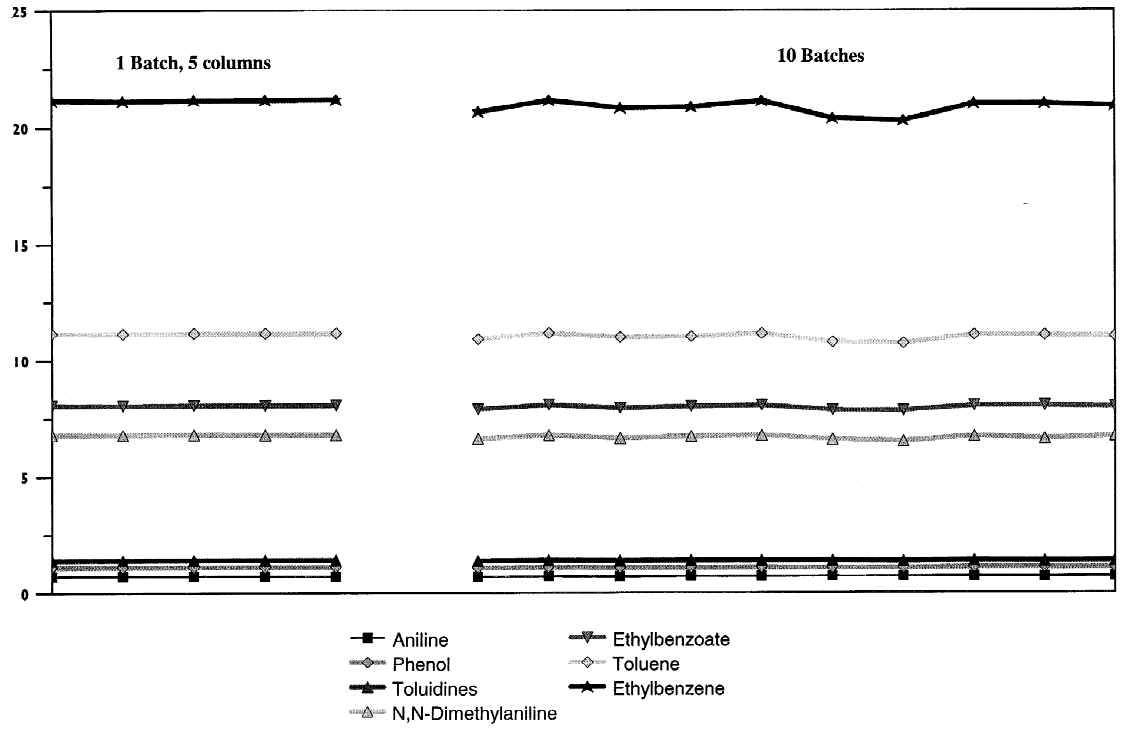

Fig. 7. Retention factors of the components of the second test mixture. Same data presentation as in Fig. 6.

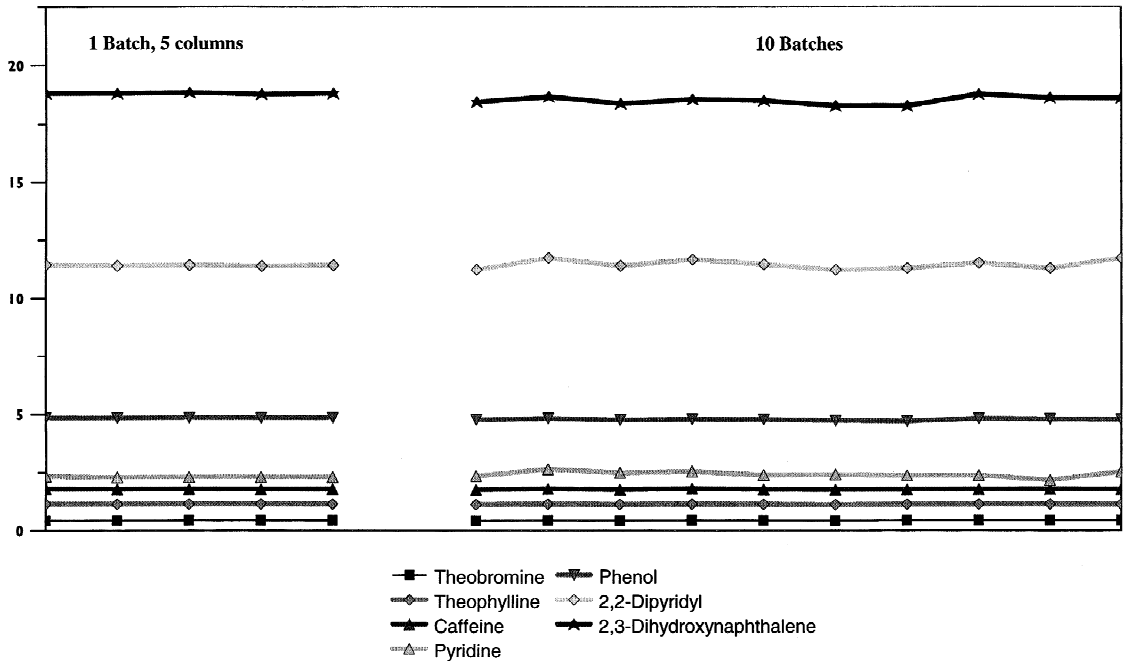

Fig. 8. Retention factors of the components of the third test mixture. Same data presentation as in Fig. 6.

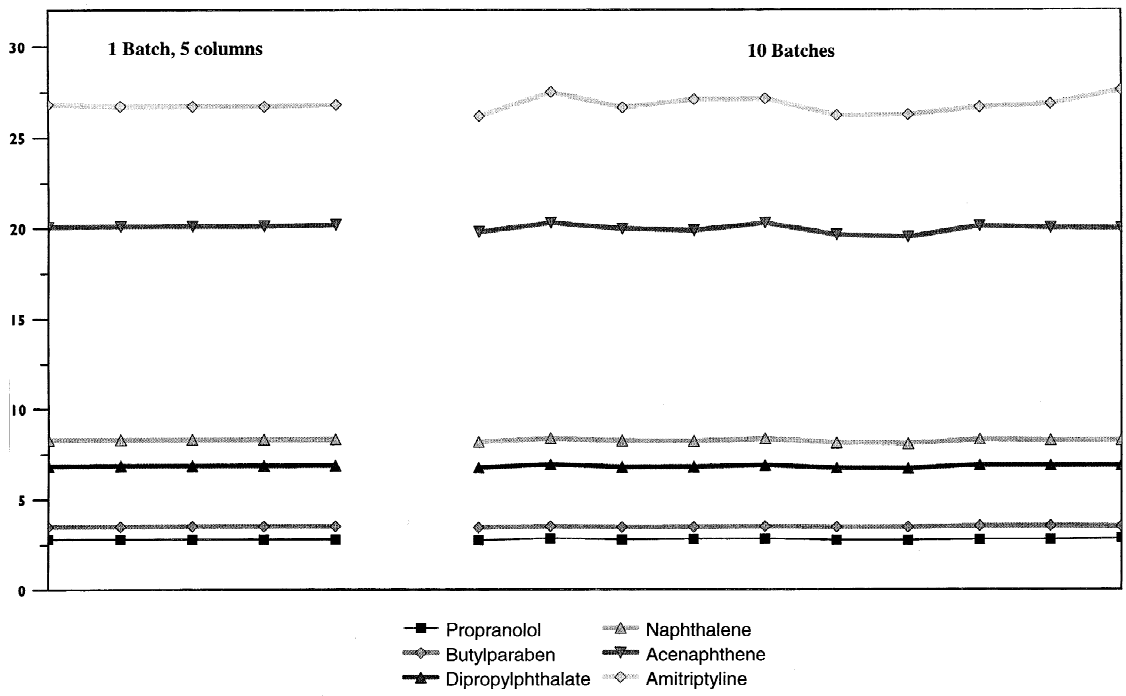

Fig. 9. Retention factors of the components of the fourth test mixture. Same data presentation as in Fig. 6.

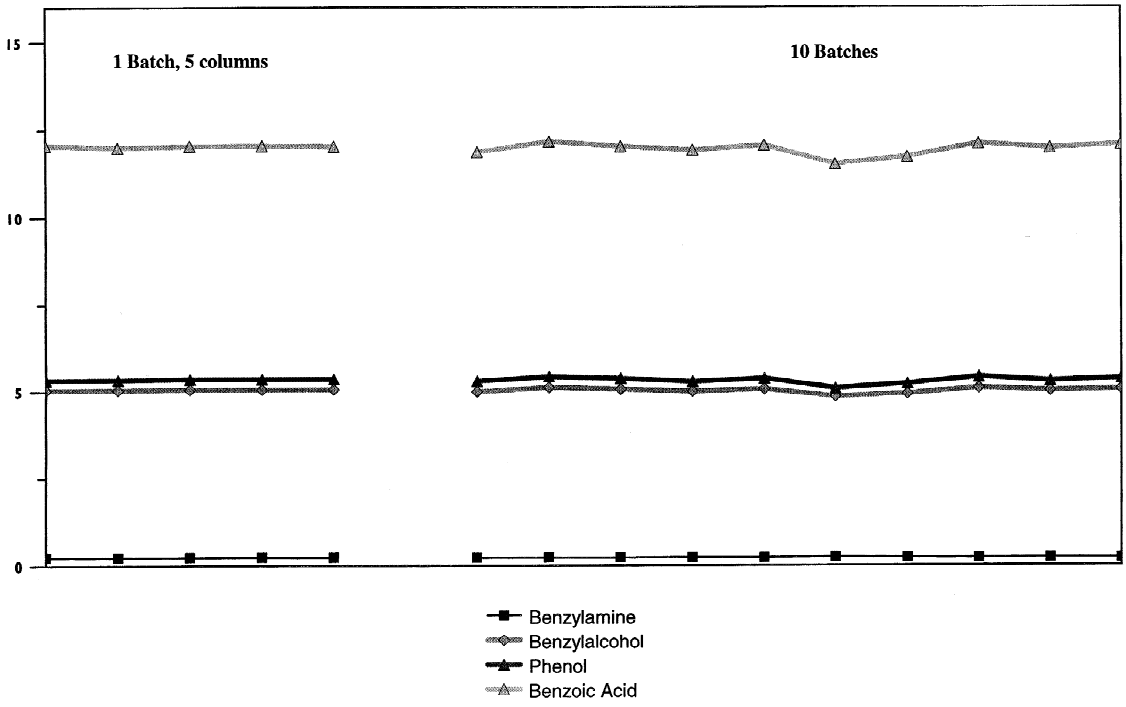

Fig. 10. Retention factors of the components of the fifth test mixture. Same data presentation as in Fig. 6.

Table 2
Reproducibility of the retention factors of the components of the first test mixture

	R.S.D. (%) of k 1 batch, 5 columns $F=0.5$ ml/min	R.S.D. (%) of k 1 batch, 5 columns $F=1.0$ ml/min	R.S.D. (%) of k 1 batch, 5 columns $F=2.0$ ml/min	R.S.D. (%) of k 10 batches $F=0.5$ ml/min	R.S.D. (%) of k 10 batches $F=1.0$ ml/min	R.S.D. (%) of k 10 batches $F=2.0$ ml/min
Phenol	0.192	0.125	0.183	0.707	0.720	0.738
1-Cl-4-Nitrobenzene	0.153	0.139	0.181	0.951	0.969	0.967
Toluene	0.153	0.136	0.191	1.110	1.137	1.145
Ethylbenzene	0.150	0.140	0.212	1.140	1.170	1.177
Butylbenzne	0.154	0.144	0.237	1.219	1.264	1.258
o-Terphenyl	0.169	0.150	0.252	1.217	1.267	1.264
Amylbenzene	0.159	0.147	0.247	1.260	1.318	1.302
Triphenylene	0.144	0.148	0.244	1.420	1.530	1.342

Each value in the table represents the R.S.D. obserevd for one given compound. For the data set ''1 batch, 5 columns'', a value represents the R.S.D. of 25 injections made on five columns, packed with packing material from the same batch. For the data set ''10 batches'', a value represents the R.S.D. of 50 injections made on 10 columns, packed with 10 different batches of packing material.

Table 3
Reproducibility of the retention factors of the components of the second test mixture

	R.S.D. (%) of k 1 batch, 5 columns	R.S.D. (%) of k 10 batches
Aniline	0.168	0.933
Phenol	0.120	0.754
Toluidines	0.083	0.866
N,*N*-Dimethylaniline	0.080	1.230
Ethylbenzoate	0.114	1.114
Toluene	0.115	1.294
Ethylbenzene	0.113	1.364

Same data presentation as in Table 2.

isomers may cause a larger change in the retention time of the triplet and multiply to modify importantly its profile. This observation explains the results obtained with toluidines.

Table 4
Reproducibility of the retention factors of the components of the third test mixture

	R.S.D. (%) of k 1 batch, 5 columns	R.S.D. (%) of k 10 batches
Theobromine	0.262	0.841
Theophylline	0.227	0.793
Caffeine	0.283	0.867
Pyridine	0.560	5.105
Phenol	0.122	0.755
2,2-Dipyridyl	0.194	1.702
2,3-Dihydroxynaphthalene	0.141	0.872

Same data presentation as in Table 2.

Table 5
Reproducibility of the retention factors of the components of the fourth test mixture

	R.S.D. (%) of k 1 batch, 5 columns	R.S.D. (%) of k 10 batches
Propranolol	0.160	1.330
Butylparaben	0.282	0.828
Dipropylphthalate	0.252	1.043
Naphthalene	0.244	1.146
Acenaphthene	0.269	1.247
Amitriptyline	0.228	1.857

Same data presentation as in Table 2.

3.1. Absolute retention data

Long-term repeatability of the retention times of the neutral and acidic compounds (Figs. 1–5) is characterized by a relative standard deviation (R.S.D.) lower than 0.2% for all the five tests. This

Table 6
Reproducibility of the retention factors of the components of the fifth test mixture

	R.S.D. (%) of k 1 batch, 5 columns	R.S.D. (%) of k 10 batches
Benzylamine	0.264	2.804
Benzyl alcohol	0.180	1.528
Phenol	0.242	1.835
Benzoic acid	0.206	1.572

Same data presentation as in Table 2.

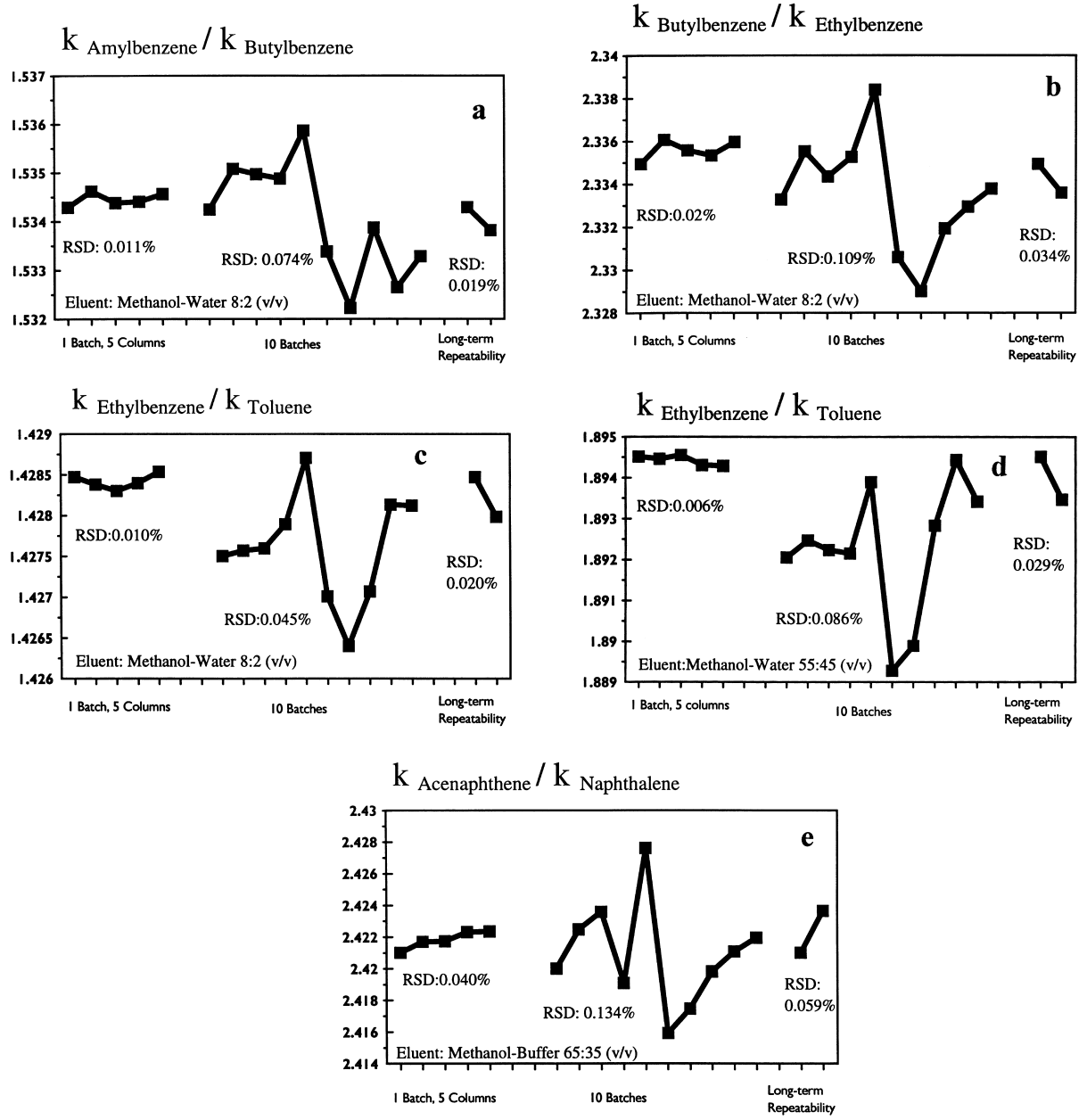

Fig. 11. Reproducibility of the hydrophobic selectivity. Same data presentation as in Fig. 6. In addition, for the data set ''long-term repeatability'', the first dat point represents the average of five consecutive injections carried out on a column, and the second data point represents the average of five consecutive injections repeated on the same column over a 10-day period. (a) Amylbenzene/butylbenzene (test 1). (b) Butylbenzene/ethylbenzene (test 1). (c) Ethylbenzene/toluene (test 1). (d) Ethylbenzene/toluene (test 2). (e) Acenaphthene/naphthalene (test 4).

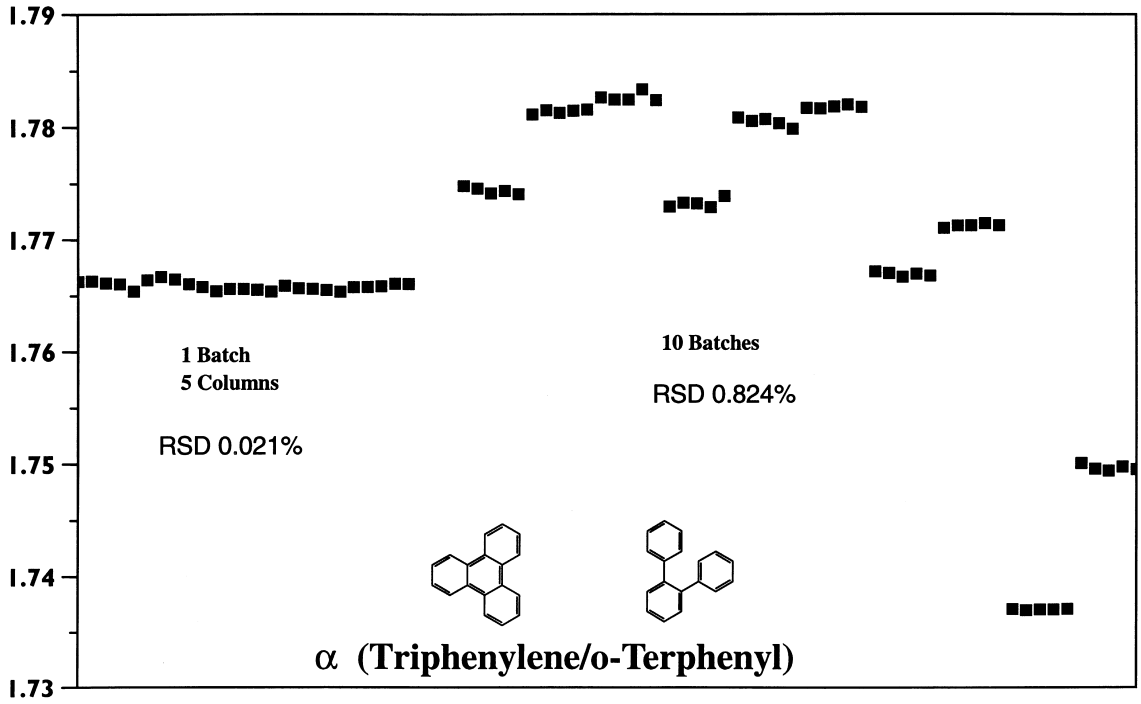

Fig. 12. Reproducibility of the steric selectivity. For the data set ''1 batch, 5 columns'', the R.S.D. value represents the R.S.D. of 25 injections made on five columns, packed with packing material from the same batch. For the data set ''10 batches'', the R.S.D. value represents the R.S.D. of 50 injections made on 10 columns, packed with 10 different batches of packing material. Each data point represents a steric selectivity value calculated based on the results of one injection on a column.

high measurement precision allows a meaningful comparison of the five columns packed from the same batch of packing material. The R.S.D. of the retention times measured on these columns was typically 0.6%. Because the five columns were packed with the same stationary phase, there should not have been such a comparatively large difference between the results afforded by each column. The larger value of the R.S.D. compared with that of the long-term repeatability (ca. 0.1%) can be explained by the fluctuations in the column size, causing slight differences in column volumes, hence in the retention volumes measured at constant mobile phase flow-rate. This fluctuation is indeed of the order of 0.5%, as derived from the reproducibility of the retention volume of thiourea (unretained). A correction for this effect is easy, it suffices to calculate the retention factors (see later).

Note that phenol was used in four of the five test mixtures. As shown by the data in Figs. 1–5, the R.S.D. values observed are practically the same in all the tests, regardless of the eluent composition.

The R.S.D. of the retention times measured on the columns packed with 10 different batches of the same brand vary between 0.7% (butylparaben in test 4, Fig. 4) and 2.1% (benzylamine in test 5, Fig. 5), with only one exception, the value of 4.05% measured for pyridine in test 3. This high R.S.D. value is essentially caused by a retention of pyridine which is significantly lower on one of the 10 batches than on all the others. After excluding from the calculation the data measured on this batch, the R.S.D. of the retention times of pyridine drops to 3.0%, still the largest value observed. Surprisingly, most of the other basic compounds show no anomalous retention behavior on this one batch, except for the toluidines which are partially separated on this one column. This fact, however, does not affect the R.S.D. of the retention time of the toluidines because their mean retention time (Fig. 2) and mean retention factor

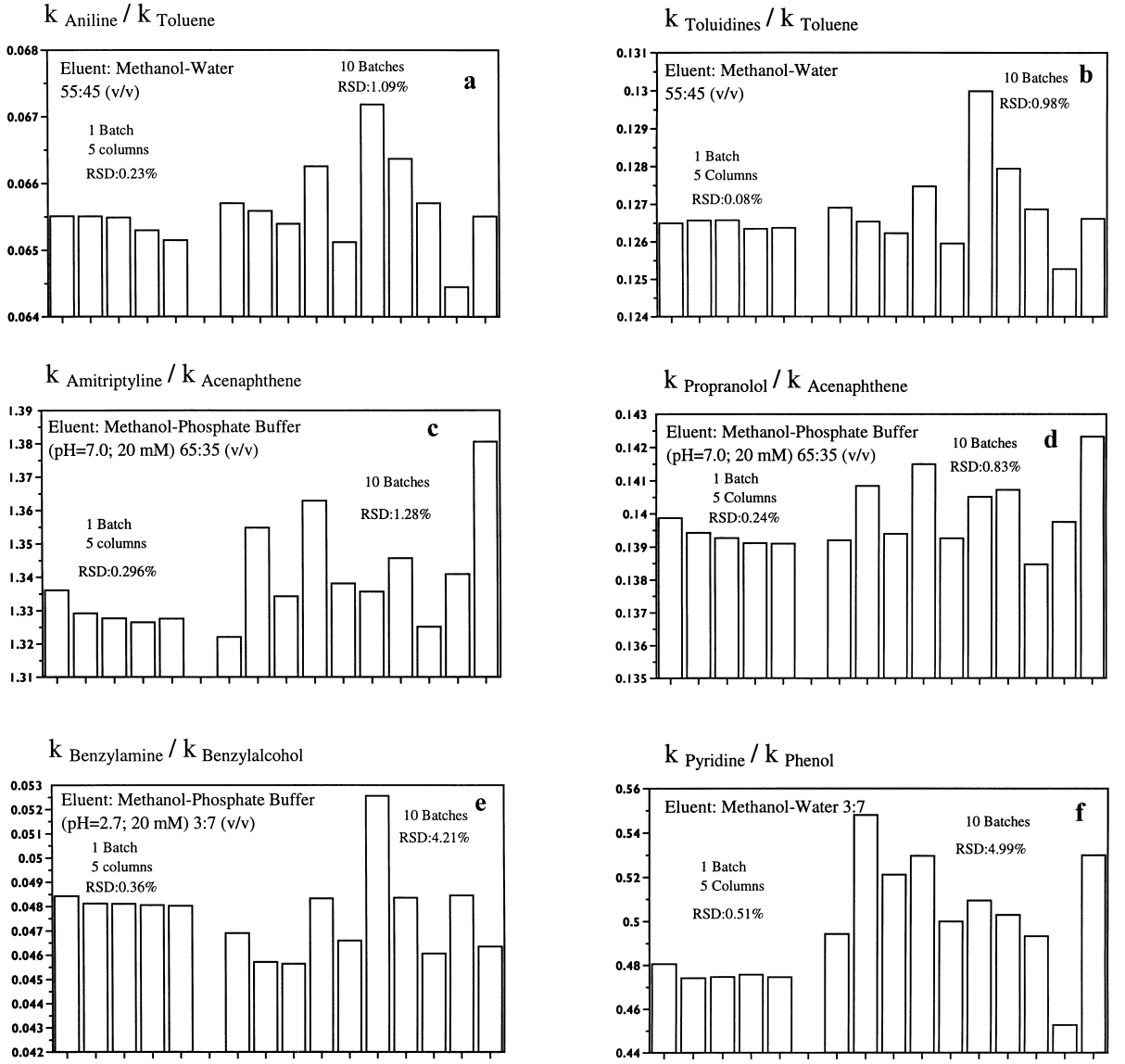

Fig. 13. Reproducibility of the separation factors of basic compounds. (a). Aniline/toluene (test 2). (b) Toluidines/toluene (test 2). (c) Amitryptiline/acenaphthene (test 4). (d) Propranolol/acenaphthene (test 4). (e) Benzylamine/benzylalcohol (test 5). (f) Pyridine/phenol (test 3). Same data presentation as in Fig. 6.

(Fig. 7) do not change. A serious peak distortion is observed on the chromatograms, however. The separation factors of the three isomers are too close to unity for any separation to be noticeable and the three peaks are integrated together. They are sufficiently different from 1, however, for slight batch-to-batch fluctuations of these factors to significantly affect the peak profile. The anomalous behavior of this particular batch could not be traced to a contamination of the column.

Table 7
Column-to-column reproducibility of the relative retention data of the compounds of the first test mixture

Relative retention	Cl-Nitrobenzene/ phenol	Toluene/ nitrobenzene	Ethylbenzene/ toluene	Butylbenzene/ ethylbenzene	*o*-Terphenyl/ butylbenzene	Amylbenzene/ *o*-terphenyl	Triphenylene/ amylbenzene
Column 1	3.5866	1.7876	1.4288	2.3351	1.2272	1.2503	1.4127
	3.5868	1.7874	1.4283	2.3352	1.2272	1.2504	1.4127
	3.5864	1.7874	1.4284	2.3351	1.2273	1.2502	1.4127
	3.5868	1.7875	1.4284	2.3352	1.2273	1.2502	1.4126
	3.5876	1.7875	1.4285	2.3341	1.2270	1.2501	1.4122
Column 2	3.5851	1.7879	1.4283	2.3362	1.2274	1.2503	1.4128
	3.5850	1.7878	1.4283	2.3361	1.2274	1.2505	1.4128
	3.5857	1.7877	1.4284	2.3361	1.2275	1.2502	1.4129
	3.5858	1.7875	1.4284	2.3359	1.2274	1.2503	1.4126
	3.5868	1.7874	1.4285	2.3362	1.2274	1.2502	1.4124
Column 3	3.5863	1.7877	1.4284	2.3359	1.2273	1.2503	1.4120
	3.5860	1.7879	1.4283	2.3356	1.2272	1.2503	1.4122
	3.5852	1.7879	1.4283	2.3355	1.2272	1.2503	1.4122
	3.5859	1.7879	1.4283	2.3357	1.2272	1.2502	1.4122
	3.5861	1.7877	1.4283	2.3353	1.2283	1.2501	1.4121
Column 4	3.5858	1.7881	1.4283	2.3356	1.2273	1.2502	1.4125
	3.5858	1.7878	1.4283	2.3355	1.2274	1.2501	1.4124
	3.5861	1.7876	1.4283	2.3353	1.2273	1.2502	1.4123
	3.5868	1.7878	1.4286	2.3351	1.2274	1.2501	1.4123
	3.5873	1.7874	1.4285	2.3352	1.2274	1.2502	1.4121
Column 5	3.5874	1.7876	1.4286	2.3359	1.2275	1.2501	1.4125
	3.5867	1.7876	1.4285	2.3361	1.2275	1.2501	1.4125
	3.5873	1.7873	1.4285	2.3359	1.2275	1.2502	1.4125
	3.5877	1.7875	1.4285	2.3360	1.2275	1.2503	1.4126
	3.5878	1.7875	1.4286	2.3360	1.2274	1.2503	1.4126
Average	3.5864	1.7876	1.4284	2.3356	1.2273	1.2502	1.4125
R.S.D. (%)	0.0227	0.0113	0.0098	0.0208	0.0100	0.0077	0.0173

The data were obtained on five columns packed with packing material from the same batch.

3.2. Retention and separation factors

The R.S.D.s of the retention factors derived for the measurements made on the five columns of one batch and the 10 different batches are listed in Tables 2–6 and illustrated in Figs. 6–10. All the column-to-column reproducibilities within a batch are below 0.26%, in all the tests (except pyridine). They are even lower than 0.16% in tests 1 and 2. The batch-to-batch reproducibilities of the retention factors are four- to 10-times larger. All, except pyridine and benzylamine, are below 2%.

Quite expectedly, the batch-to-batch reproducibility of the separation factors is better than that of the retention factors in most cases. In Table 7, we list the relative retention data of the first test on the five columns packed from the same batch. The same data are given in Table 8 for the 10 batches. The pairs of successively eluted peaks were used for these calculations. The R.S.D. values are below 0.023% for the five columns of one batch and below 0.74% for the 10 columns of different batches.

In Table 9, the average relative retention data of the pairs of successively eluted peaks are listed for all the tests carried out, together with their R.S.D.s. The highest R.S.D. value obtained was that measured in the third test for the pair phenol/pyridine (5.2% R.S.D.) on the 10 batches. The same value was

Table 8
Batch-to-batch reproducibility of the relative retention data of the compounds of the first test mixture

Relative retention	Cl-Nitrobenzene/ phenol	Toluene/ nitrobenzene	Ethylbenzene/ toluene	Butylbenzene/ ethylbenzene	*o*-Terphenyl/ butylbenzene	Amylbenzene/ *o*-terphenyl	Triphenylene/ amylbenzene
Batch 1	3.5834	1.7850	1.4274	2.3330	1.2262	1.2512	1.4185
	3.5834	1.7851	1.4274	2.3334	1.2264	1.2512	1.4183
	3.5837	1.7851	1.4275	2.3333	1.2263	1.2511	1.4181
	3.5845	1.7852	1.4276	2.3332	1.2263	1.2511	1.4183
	3.5843	1.7849	1.4276	2.3336	1.2264	1.2510	1.4181
Batch 2	3.6016	1.7862	1.4277	2.3359	1.2262	1.2518	1.4228
	3.6011	1.7861	1.4275	2.3351	1.2263	1.2519	1.4230
	3.6011	1.7864	1.4276	2.3355	1.2262	1.2519	1.4229
	3.5999	1.7863	1.4275	2.3356	1.2262	1.2519	1.4230
	3.5998	1.7864	1.4275	2.3356	1.2263	1.2518	1.4232
Batch 3	3.5972	1.7864	1.4275	2.3344	1.2260	1.2518	1.4240
	3.5976	1.7865	1.4277	2.3346	1.2262	1.2518	1.4239
	3.5976	1.7864	1.4275	2.3343	1.2261	1.2519	1.4238
	3.5975	1.7862	1.4276	2.3342	1.2261	1.2522	1.4242
	3.5978	1.7864	1.4276	2.3343	1.2261	1.2519	1.4238
Batch 4	3.5975	1.7804	1.4280	2.3352	1.2288	1.2489	1.4196
	3.5975	1.7802	1.4279	2.3352	1.2288	1.2491	1.4197
	3.5981	1.7803	1.4279	2.3354	1.2288	1.2490	1.4197
	3.5974	1.7802	1.4279	2.3353	1.2288	1.2489	1.4196
	3.5972	1.7804	1.4278	2.3353	1.2290	1.2493	1.4199
Batch 5	3.6071	1.7875	1.4286	2.3385	1.2287	1.2501	1.4246
	3.6076	1.7876	1.4287	2.3384	1.2287	1.2500	1.4245
	3.6077	1.7875	1.4287	2.3386	1.2288	1.2500	1.4246
	3.6078	1.7874	1.4288	2.3384	1.2287	1.2499	1.4244
	3.6073	1.7863	1.4287	2.3381	1.2286	1.2499	1.4240
Batch 6	3.5706	1.7756	1.4271	2.3306	1.2275	1.2492	1.4263
	3.5699	0.7755	1.4270	2.3306	1.2275	1.2492	1.4263
	3.5708	1.7757	1.4270	2.3307	1.2276	1.2491	1.4265
	3.5703	1.7756	1.4271	2.3306	1.2274	1.2492	1.4265
	3.5707	1.7753	1.4269	2.3305	1.2275	1.2492	1.4262
Batch 7	3.5716	1.7742	1.4261	2.3289	1.2277	1.2474	1.4167
	3.5730	1.7748	1.4265	2.3289	1.2283	1.2476	1.4164
	3.5729	1.7749	1.4265	2.3290	1.2280	1.2475	1.4162
	3.5730	1.7749	1.4264	2.3290	1.2283	1.2477	1.4162
	3.5727	1.7748	1.4265	2.3292	1.2283	1.2476	1.4161
Batch 8	3.5883	1.7811	1.4270	2.3317	1.2261	1.2508	1.4159
	3.5867	1.7808	1.4271	2.3319	1.2263	1.2510	1.4159
	3.5883	1.7809	1.4271	2.3324	1.2261	1.2509	1.4160
	3.5872	1.7811	1.4271	2.3320	1.2261	1.2510	1.4161
	3.5891	1.7813	1.4271	2.3318	1.2262	1.2510	1.4159
Batch 9	3.5863	1.7822	1.4282	2.3331	1.2292	1.2468	1.3932
	3.5863	1.7825	1.4283	2.3330	1.2293	1.2468	1.3931
	3.5864	1.7825	1.4282	2.3326	1.2293	1.2468	1.393
	3.5860	1.7825	1.4281	2.3330	1.2292	1.2468	1.3932
	3.5870	1.7825	1.4279	2.3331	1.2292	1.2470	1.3931

(*Continued overleaf*)

Table 8. Continued

Relative retention	Cl-Nitrobenzene/ phenol	Toluene/ nitrobenzene	Ethylbenzene/ toluene	Butylbenzene/ ethylbenzene	*o*-Terphenyl/ butylbenzene	Amylbenzene/ *o*-terphenyl	Triphenylene/ amylbenzene
Batch 10	3.5826	1.7889	1.4282	2.3339	1.2269	1.2496	1.4005
	3.5834	1.7881	1.4282	2.3338	1.2272	1.2495	1.4003
	3.5849	1.7881	1.4280	2.3334	1.2272	1.2495	1.4001
	3.5852	1.7882	1.4282	2.3340	1.2271	1.2496	1.4003
	3.5858	1.7881	1.4280	2.3340	1.2272	1.2494	1.4003
Average	3.5889	1.7827	1.4276	2.3335	1.2274	1.2498	1.4161
R.S.D. (%)	0.3206	0.2593	0.0448	0.1093	0.0962	0.1321	0.7410

The data were obtained on 10 columns packed with packing material from 10 different batches.

Table 9
Reproducibility of the relative retention data of the components of the five test mixtures

	1 batch, 5 columns		10 batches	
	Average value of relative retentions	R.S.D. (%) of relative retentions	Average value of relative retentions	R.S.D. (%) of relative retentions
Test 1 (MeOH–water, 8:2)				
Cl-Nitrobenzne/phenol	3.5864	0.0227	3.5889	0.3206
Toluene/Cl-nitrobenzene	1.7876	0.0113	1.7827	0.2593
Ethylbenzene/toluene	1.4284	0.0098	1.4276	0.0448
Butylbenzene/ethylbenzene	2.3356	0.0208	2.3335	0.1093
o-Terphenyl/butylbenzene	1.2273	0.0100	1.2274	0.0962
Amylbenzene/*o*-terphenyl	1.2502	0.0077	1.2498	0.1321
Triphenylene/amylbenzene	1.4125	0.0173	1.4161	0.7410
Test 2 (MeOH–water, 55:45)				
Phenol/aniline	1.5217	0.2435	1.5197	0.7193
Toluidine/phenol	1.2710	0.0859	1.2713	0.5599
Dimethylaniline/toluidine	4.8118	0.0385	4.7793	0.6790
Ethylbenzoate/dimethylaniline	1.1867	0.0678	1.1957	0.5743
Toluene/ethylbenzoate	1.3847	0.0178	1.3782	0.3141
Ethylbenzene/toulene	1.8944	0.0093	1.8923	0.0839
Test 3 (MeOH–water, 3:7)				
Theophylline/theobromine	2.5662	0.1149	2.5656	0.3929
Caffeine/theophylline	1.5688	0.0917	1.5720	0.3491
Pyridine/caffeine	1.2914	0.4332	1.3645	4.8961
Phenol/pyridine	2.1009	0.5061	1.9729	5.1672
2,2-Dipyridyl/phenol	2.3538	0.1036	2.3990	1.3621
Dihydroxynaphthalene/dipyridyl	1.6476	0.1311	1.6154	1.3130
Test 4 (MeOH–pH 7.0 buffer, 65:35)				
Butylparaben/propranol	1.2539	0.2537	1.2496	1.0581
Dipropylphth./butylparaben	1.9517	0.0635	1.9519	0.4780
Naphthalene/dipropylphth.	1.2108	0.0444	1.2081	0.3283
Acenaphthene/naphthalene	2.4218	0.0403	2.4209	0.1344
Amitriptyline/acenaphthene	1.3294	0.2961	1.3440	1.2811
Test 5 (MeOH–pH 2.7 buffer, 3:7)				
Benzyl alcohol/benzylamine	20.7667	0.3548	21.0868	3.9810
Phenol/benzyl alcohol	1.0581	0.0732	1.0582	0.3625
Benzoic acid/phenol	2.2508	0.2629	2.2487	0.4117

For the data set ''1 batch, 5 columns'', a value represents the R.S.D. of 25 injections made on five columns, packed with packing material from the same batch. For the data set ''10 batches'', a value represents the R.S.D. of 50 injections made on 10 columns, packed with 10 different batches of packing material.

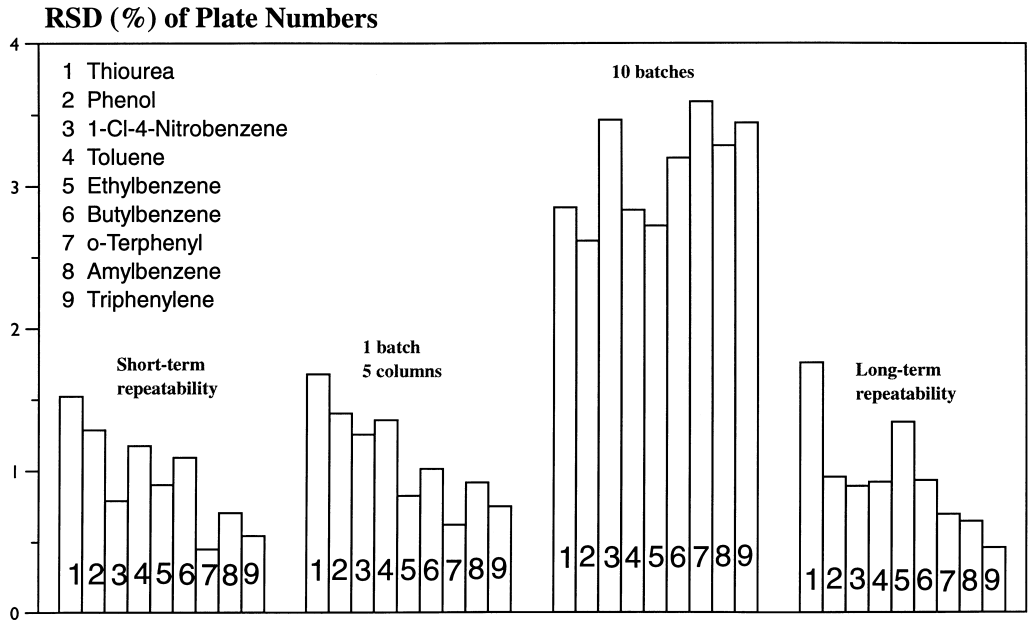

Fig. 14. Column efficiency for the components of the first test mixture. R.S.D. of the number of theoretical plates. Same data presentation as in Fig. 1. In addition for the term ''short-term repeatability'' a bar represents the R.S.D. of five consecutive injections carried out on one column over a period of a few hours.

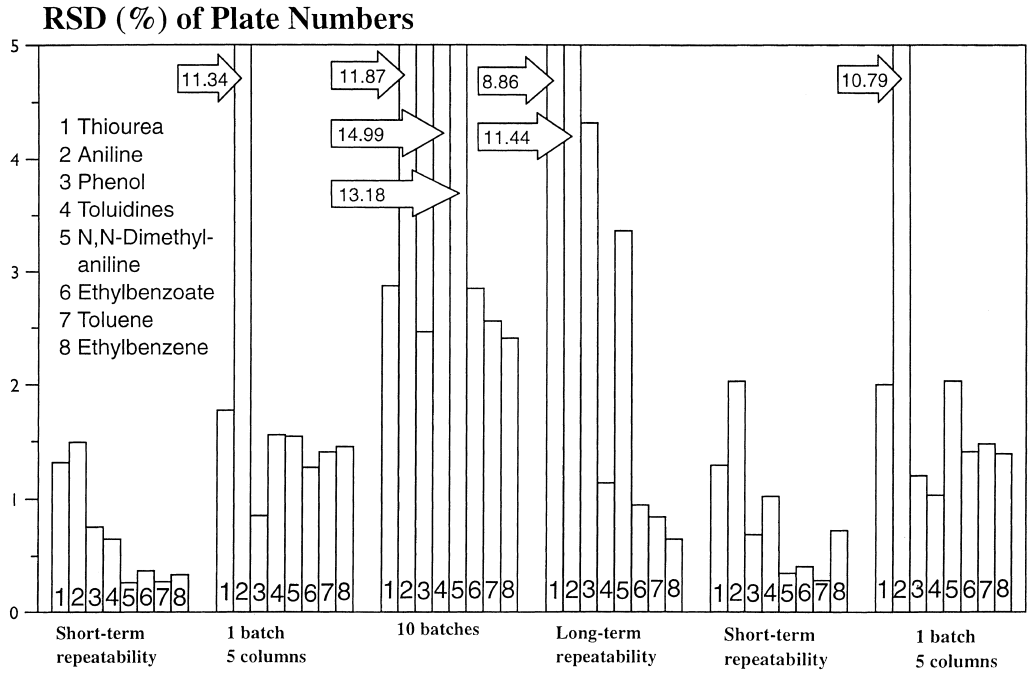

Fig. 15. Column efficiency for the components of the second test mixture. Same data presentation as in Fig. 14.

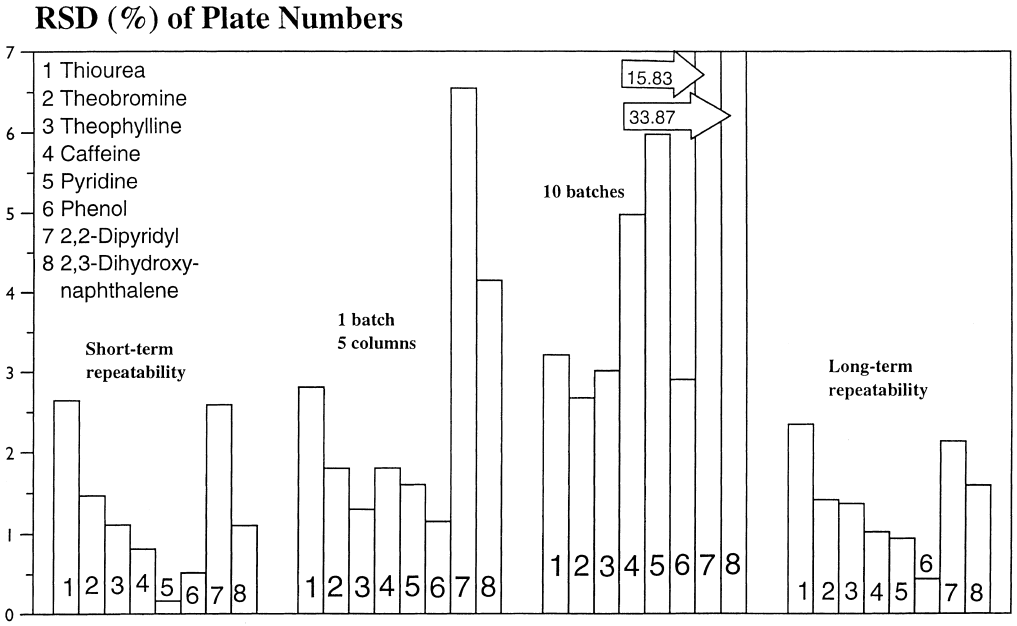

Fig. 16. Column efficiency for the components of the third test mixture. Same data presentation as in Fig. 14.

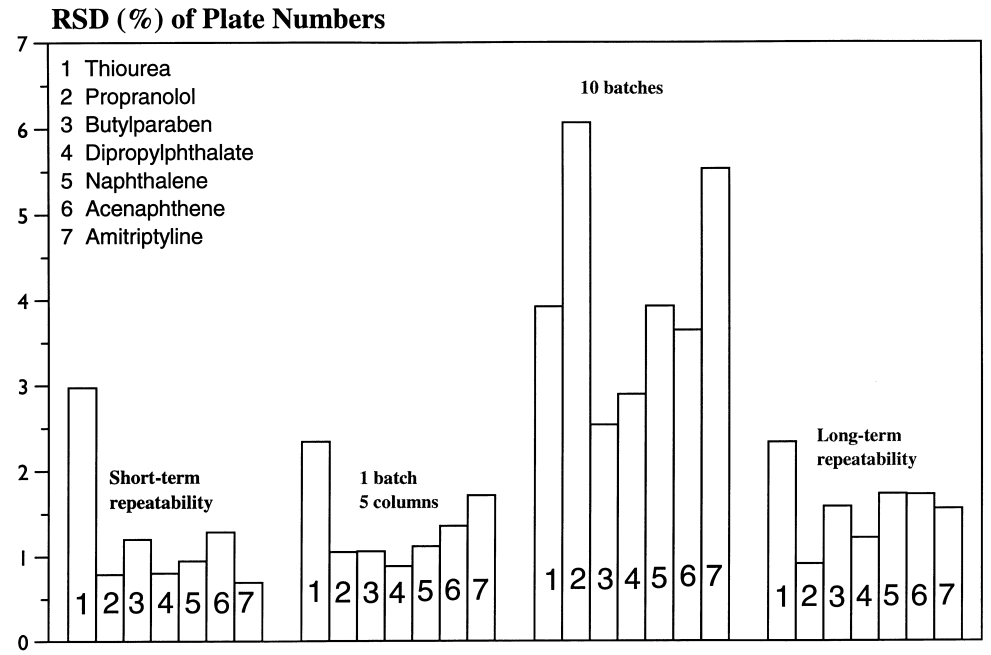

Fig. 17. Column efficiency for the components of the fourth test mixture. Same data presentation as in Fig. 14.

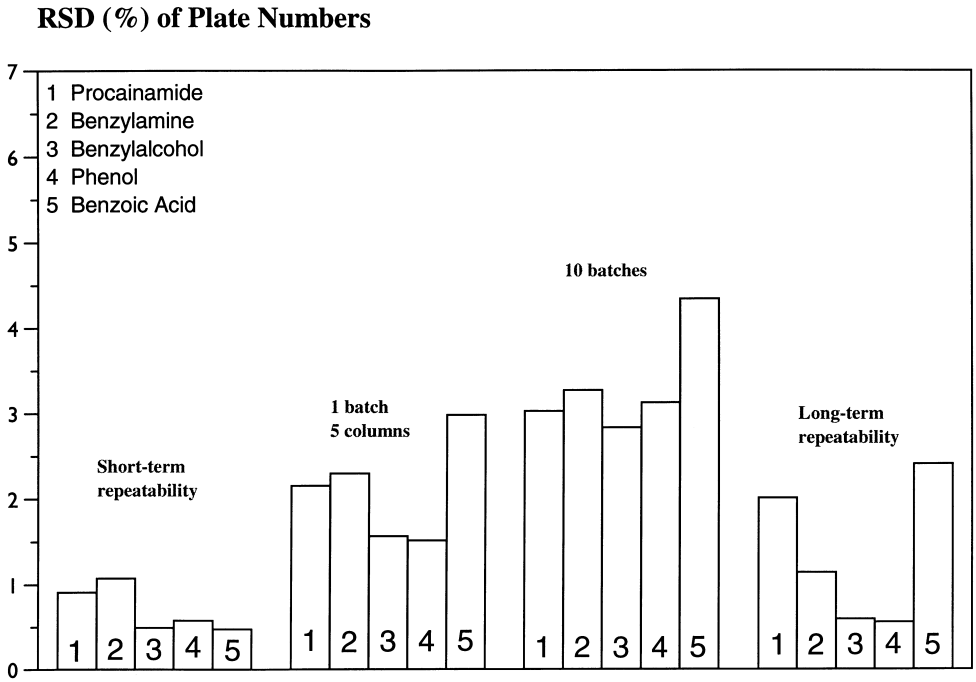

Fig. 18. Column efficiency for the components of the fifth test mixture. Same data presentation as in Fig. 14.

measured on the five columns from the same batch, which indicates that the accuracy of the measurement is the lowest in this case. Out of a total of 27 separation factors calculated in Table 9, 15 have batch-to-batch reproducibilities better than 0.5%, 20 better than 1%, and 24 better than 2%. Two of the three larger than 2% involve pyridine, the last one benzylamine.

The separation factors discussed above have no real physical sense in terms of retention mechanisms and cannot be used to characterize columns because the selection, hence the elution orders of the components of the test mixtures are arbitrary, so we also calculated the values of the separation factors which have been suggested by different authors for the characterization and comparison of columns of different brands. These values are now discussed.

3.3. Hydrophobic selectivity

Estimates for the hydrophobic selectivity of the different batches could be derived from retention data measured in two different tests. First, we calculated $\alpha(CH_2)$ as the ratio of the retention factors of the three following pairs of compounds, amylbenzene/butylbenzene (test 1), butylbenzene/ethylbenzene (test 1) and ethylbenzene/toluene (tests 1 and 2). The $\alpha(CH_2)$ values obtained are a good measure of the degree of surface coverage by the bonded octadecyl groups [3]. Second, from the data measured in the fourth test, we derived the acenaphthene/naphthalene separation factor (Fig. 11e). The reproducibility of all these data was extremely high. The column-to-column and the batch-to-batch reproducibilities were of the order of 0.02 and 0.1%, respectively (Fig. 11a Fig. 11b Fig. 11c Fig. 11d Fig. 11e).

Because of the high precision of these measurements, it is possible to observe small but possibly significant differences between the data obtained with the different batches. The R.S.D. for five columns of the same batch is between four and 15-times smaller than for the 10 columns of different batches. The different values obtained for the five tests are plotted in Fig. 11a–e. Obviously, although similar, the trends exhibited by the four plots are different. For example, batches for which the separation factor α for butylbenzene/ethylbenzene (Fig.

Table 10
Tailing factor of the different compounds studied

	1 batch, 5 columns		10 batches	
	Average value of tailing factors	R.S.D. (%) of tailing factors	Average value of tailing factors	R.S.D. (%) of tailing factors
(a) Column-to-column reproducibility (within one batch)				
Test 1 (MeOH–water, 8:2)				
Thiourea	1.313	0.908	1.252	1.561
Phenol	1.222	0.702	1.261	1.841
1-Cl-4-Nitrobenzene	1.136	0.554	1.202	4.115
Toluene	1.091	0.728	1.138	2.799
Ethylbenzene	1.056	0.710	1.100	2.815
Butylbenzene	1.021	0.650	1.056	2.937
o-Terphenyl	1.022	0.607	1.058	2.706
Amylbenzene	1.014	0.800	1.046	2.876
Triphenylene	1.027	0.603	1.062	3.010
Test 2 (MeOH–water, 55:45)				
Thiourea	1.316	0.636	1.376	1.592
Aniline	1.085	1.579	1.145	5.847
Phenol	1.128	0.758	1.181	2.272
Toluidines	0.954	1.824	0.935	8.519
N,N-Dimethylaniline	0.943	0.781	0.961	7.205
Ethylbenzoate	1.064	1.102	1.107	3.198
Toulene	1.017	1.029	1.042	2.763
Ethylbenzene	0.983	0.951	1.001	2.859
Test 3 (MeOH–water, 3:7)				
Thiourea	1.287	1.425	1.327	1.945
Theobromine	1.186	0.908	1.253	1.976
Theophylline	1.113	1.072	1.169	2.377
Caffeine	1.155	1.129	1.241	3.612
Pyridine	1.718	1.121	1.974	12.452
Phenol	1.098	0.977	1.136	2.383
2,2-Dipyridyl	3.262	16.201	6.037	34.723
Batch-to-batch reproducibility (10 different batches)				
Test 4 (MeOH–pH 7.0 buffer, 65:35)				
Thiourea	1.315	0.711	1.369	1.681
Propranol	1.345	0.506	1.557	9.691
Butylparaben	1.071	0.789	1.115	2.440
Dipropylphthalate	1.076	0.854	1.110	2.572
Naphthalene	1.071	1.084	1.107	3.533
Acenaphthene	1.044	1.276	1.075	3.106
Amitriptyline	1.898	0.961	1.959	4.206
Test 5 (MeOH–pH 2.7 buffer, 3:7)				
Procainamide	1.326	0.797	1.371	1.948
Benzylamine	1.446	0.606	1.485	2.923
Benzyl alcohol	1.044	1.042	1.073	4.223
Phenol	1.093	1.409	1.120	4.125
Benzoic acid	1.423	2.529	1.657	6.667

Average values and their reproducibility. Same data presentation as in Table 9.

11b) are different have similar values for the separation factor of ethylbenzene and toluene (Fig. 11c Fig. 11d), and conversely. Even the data in Fig. 11c Fig. 11d, which correspond to the ethylbenzene/toluene separation factor under slightly different conditions, do not correlate well.

We can conclude, that, at least in this range of differences, with R.S.D. of the hydrophobic selectivity values below 0.13%, the differences between batches remain observable but are difficult to correlate with any actual physical property of the stationary phase, so one could question the practical importance of such small differences. At this level of precision, these differences are of no concern to the analyst, if they may be of interest to the physical chemist. It is worth noting, however, that recent independent data [4] question the actual physical significance of many parameters derived for the purpose of comparing columns of different brands. It was shown that parameters having a similar physico–chemical basis correlate poorly, even between brands which exhibit important differences of their main physico–chemical properties.

3.4. Steric selectivity

According to Sander and Wise [5,6] the steric selectivity measured with selected polyaromatic hydrocarbons having different molecular shape depends on the phase type (monomeric versus polymeric) and, through this, on the nature of the silylating reagent (mono-, di-, trifunctional), so, on the average distance between alkyl chains. In our study, the steric selectivity can be characterized only by the separation factor of triphenylene and *o*-terphenyl [3]. The values of this separation factor derived from the retention factors measured for the different columns studied are reported in Fig. 12. The values of the steric selectivity obtained for the different columns of the same batch are highly reproducible (R.S.D.$\simeq$ 0.02%).

The values of the steric selectivity obtained for the different batches exhibit a much lower degree of reproducibility (R.S.D.=0.82), in large part because of differences observed between the (quite similar) values obtained for most columns and those measured for the last two batches. This was an unexpected result, since one would expect the same values of the steric selectivity for the different batches. Again, the high accuracy of the measurements allows the observation of minor differences of properties between the different batches, but the explanation of these results is not so straightforward as it was in previous studies in which different types or brands of stationary phases were compared [5,6].

3.5. Separation factors of basic compounds

Because basic compounds have always been more difficult to analyze by RP-LC on alkyl bonded silica, careful attention is paid to them in all studies on the characterization of these stationary phases. The main factors which affect the separation of basic compounds originate from both the stationary and the mobile phase. The stationary phase factors are the concentration of the silanol groups on the surface, the relative concentration of the different silanol types, the concentration of metal impurities and the accessibility of the corresponding sites. The mobile phase factors are the pK_a of the solute, the pH of the mobile phase, the buffer concentration (nature and concentration of the counter ions), possible steric effects (affecting the penetration of the analyte toward the silica surface), and the nature of the organic solvent modifier. Finally, the nature of the solute in general (because overloading effects vary from solute to solute and some compounds may show unexpected results) is also important. Note that the pK_a of a compound in an organic solvent–water mixture decreases with increasing organic solvent content and that the pH of the mobile phase depends on the nature and the concentration of the organic modifier in an unbuffered solution. These two effects complicate considerably the interpretation of the experimental results. The large number of these factors makes it insufficient to characterize the ability of a stationary phase to separate basic compounds with only a few solutes. In our study we used nine different basic compounds under four test conditions. To illustrate our results, we show in Fig. 13a–f the results obtained for the separation factors of aniline and toluene (Fig. 13a), and of the toluidines and toluene (Fig. 13b), both from test 2, of amitryptiline and acenaphthene (Fig. 13c) and of propranolol and acenaphthene (Fig. 13d), both from

test 4, of benzylamine and benzyl alcohol (Fig. 13e), from test 5, and of pyridine and phenol (Fig. 13f), from test 3.

The reproducibility achieved for these five separation factors varies considerably from one case to the next as expected since the different tests represent different sets of experimental conditions, so different interactions take place. In test 2, the mobile phase is unbuffered and the pH value is around 7, so a majority of the silanol groups are dissociated. The pK_a values of the solutes are close to 4 (aniline 4.63, *o*-toluidine 4.44, *m*-toluidine 4.73, *p*-toluidine 5.08 in water), so these amines are unprotonated at the pH of the mobile phase. Little electrostatic interactions can take place. The aniline/toluene and the toluidine/toluene relative retention values show a fluctuation of 1% on the 10 batches while the R.S.D. of relative retention of two neutral compounds (e.g., ethylbenzene/toluene) was 0.08% under the same conditions, on the same columns.

In test 4 the mobile phase is buffered at pH 7.0, the solute compounds, amitriptyline and propranolol, have a pK_a value in water of 9.4 and 9.5, respectively. Under these conditions the silanol groups are dissociated and the amines are protonated. Strong ion-exchange interactions are expected between the amine and the silica surface. On the other hand, the potassium ions of the buffer solution can counter these interactions. The fluctuations of the separation factors of these two basic compounds relative to the neutral acenaphthene gives approximately the same value as in the second test, R.S.D.=0.83% for propranolol/acenaphthene and 1.28% for amitriptyline/acenaphthene on the 10 batches (Fig. 13 b Fig. 13c).

In tests 3 and 5 the methanol content of the mobile phase is 30%. In test 5 the mobile phase is buffered at pH 2.7, a pH which is believed to be low enough for the silanol groups to be fully protonated. The pK_a value of benzylamine is 9.3 in water making the amine fully protonated under these conditions. We observe that, in this case, the reproducibility of the retention factor of benzylamine/benzyl alcohol (Fig. 13e) is significantly poorer (4.21%) than the values measured in tests 2 and 4. The R.S.D. is also large (4.99%) for pyridine/phenol in test 3 (Fig. 13f) but this last result is consistent with the earlier observation that, of all the compounds tested, pyridine gave by far the worst reproducibility results for the retention time. Still, we must observe that an R.S.D. of 5% under the experimental conditions described, for the separation factor of an acid like phenol and a strongly basic and complexing reagent as pyridine is quite an impressive achievement in comparison with past performance [10].

3.6. Column permeability

The fluctuations of the column permeability from column to column result from the combined effects of the batch-to-batch fluctuations of the particle size, the particle size distribution and of the column-to-column fluctuations of the packing density and the column size (see earlier). To measure the fluctuations of these parameters between the columns of a given batch and those from different batches, we measured the pressure drop of these columns at three different flow-rates. For a 1.00 ml/min flow-rate, the largest difference between the back pressures of five columns packed with a packing material from the same batch was 1.7 bar (1.25% of the mean). This difference between the highest and the lowest pressure drops needed for the achievement of a 1 ml/min flow-rate through the 10 columns of different batches tested was 16.7 bar (13.9% of the mean). The average value of the inlet pressure recorded for each column at the three different flow-rates was plotted versus the flow-rate and a linear regression of the pressure/flow-rate data was calculated. The R.S.D. of the slopes (inversely proportional to the column permeability) was found to be 4.5% for the 10 columns of different batches, 0.5% for the five columns of one batch and only 0.1% for one column over several days[1]. This last value does include a certain contribution from the pressure gauge itself. Note that these R.S.D.s are consistent with the ranges of head pressures measured.

The fluctuations of the permeability measured for one column over several days reflect the error of measurement of the pressure and the fluctuations of the flow-rate. A R.S.D. of 0.1% for the combination of these contributions seems reasonable. The col-

[1]Note that the figures for the R.S.D.s are well compatible with those given earlier for the relative data spread, (maximum value − minimum value)/average value.

umn-to-column fluctuations of the permeability within a batch arise from the above sources, to which should be added the fluctuations of the column cross-section area and of the packing density. The fluctuations of the hold-up volumes of the five columns of the same batch have a R.S.D. of 0.5% (see Section 3.1). These fluctuations can be ascribed almost entirely to fluctuations of the column cross-section area. They suffice to explain the increase in the permeability R.S.D. to 0.5% in this case and the fluctuations of the packing density from column to column are probably small.

The R.S.D.s of the permeability of the columns belonging to 10 different batches arise from the same sources discussed above to which we should add the fluctuations of the particle size and the particle size distribution. The values supplied by the manufacturer are 1.01% and 1.15%, respectively, for these 10 batches (Table 1). The fluctuations of the hold-up volumes of the 10 columns have a R.S.D. of 1% (see Section 3.1). Again, these last fluctuations can be ascribed almost entirely to fluctuations of the column cross-section area. The combined influence of these known fluctuations on the R.S.D. of the column permeability amounts to approximately 2.4%. There is a significant disagreement with the larger experimental result (R.S.D.=4.5%) which is not easily explained. It could arise from the uncertainty in correlating permeability and particle size distribution, which would require more data on the latter than available, and to the lack of data regarding the external porosity of the bed.

3.7. Column efficiency

As expected, the column efficiencies which have always been known to be more difficult to measure with precision than retention data, do not exhibit the same high degree of reproducibility. The R.S.D.s on efficiency data are more than one order of magnitude larger than the R.S.D.s on the other parameters determined in this study. For the number of theoretical plates, the short-term and the long-term reproducibilities as well as the column-to-column repeatability within a batch of columns gave R.S.D. values which are all below 3% for the neutral compounds in all the tests performed (Figs. 14–18). The R.S.D.s of the short-term repeatability are in agreement with the values calculated by error propagation from the standard deviations of the measurements of the retention times and the peak widths at half height. Because of this relatively high ''noise'' in the determination of the column efficiency, there are no possibilities in discriminating between the five columns, should they differ slightly. The efficiency values of the neutral and polar compounds measured on the 10 batches are between 2.5 and 3.5%, in some cases barely higher than the long-term repeatability values.

The values for the basic compounds in the buffered test solutions are reasonably well reproducible (Figs. 17 and 18). The relative standard deviations of the data obtained for the five columns of the same batch are similar to those measured for the long-term repeatability of these parameters on one column. The R.S.D.s for the columns of different batches are definitely higher for propranolol (6.2% R.S.D.) and amitriptyline (5.8% R.S.D.) than they are for the five columns of the same batch. The values measured at pH=2.7 for procainamide and benzylamine are practically the same on the 10 batches and the five within a batch of columns (Fig. 18).

It is difficult to give a reasonable explanation of the high R.S.D. values observed with the compounds analyzed in the unbuffered solutions (Figs. 15 and 16). The R.S.D.s of the aniline efficiency are uniformly large, around 11% for the long-term repeatability, the column-to-column and the batch-to-batch reproducibilities. These values are too large to allow any conclusions. The column-to-column reproducibility experiments were repeated but the results remained the same. It seems clearly that something is not properly controlled in this experiment but we have not identified which factor yet. Note that the water used in the preparation of the mobile phase comes from a single manufacturer, from the same lot, but that we used different bottles in the different measurements. The other basic compounds in the second test mixture give a reasonably good repeatability value (~1% for the toluidines, 3.3% for *N*,*N*-dimethylaniline). This suggests that the high R.S.D. of the efficiencies measured on the different batches (~15% for the toluidines, 13% for *N*,*N*-dimethylaniline) might be due to actual differences between these batches. In the third test mixture (Fig. 16), however, five basic compounds were included.

The batch-to-batch R.S.D.s for theobromine and theophylline are only marginally higher than those for the column-to-column experiments. For caffeine and pyridine the R.S.D.s for batch-to-batch reproducibility are two- to three-times higher than for column-to-column or for long-term measurements. This set of results illustrates the difficulties that are encountered in high-performance liquid chromatography (HPLC) analysis of nitrogen compounds.

An explanation on the high values of the R.S.D. for the efficiency measured for 2,2-dipyridyl and 2,3-dihydroxynaphthalene on the 10 batches is not straightforward. First we note that the dipyridyl peak tails strongly on most columns, a phenomenon that might affect the performance of the dihydroxynaphthalene peak. For both compounds the efficiency derived from the first injection is different from the following four ones (five successive injections are always performed). For 2,2-dipyridyl the first injection always gives a higher efficiency than the other ones. For 2,3-dihydroxynaphthalene, it is the opposite, the first injection always gives a lower efficiency. This suggests that there is a small amount of sample which is strongly adsorbed, is slowly released, and is not entirely released before the second injection is carried out. A sort of steady-state equilibrium is reached, however, and the following injections are better reproducible. Finally, the peak of the last compound exhibits an important shoulder. The width of the peak at half-height, hence the efficiency measured for it are affected. The UV spectra recorded during elution of the shoulder peak are virtually the same as the spectra of the main peak. It is possible but improbable that the shoulder peak signals the elution of an isomer partly resolved from the main component or of a degradation product (the UV spectra are too close). The shoulder peak could rather originate from the interaction of the tailing of the previous 2,2-dipyridyl peak. This phenomenon deserves further investigation. It may suggest that there is too high a concentration of metal ions adsorbed on the stationary phase from the mobile phase in a steel column used with a steel liquid chromatograph for the test to be meaningful.

3.8. Peak asymmetry factor

The parameter measured in this study is the U.S.P. tailing factor. It is determined from the peak width at 5% of the peak height and defined as

$$T = \frac{a + b}{2a} \quad (1)$$

where a and b are the distances between the peak maximum and the ascending and descending fronts, also called the ascending and descending half peak width. The value obtained is lower than that of another widely used factor, the asymmetry factor (Af). The relationship between the two factors for an EMG peak can be expressed [11] as:

$$T = 0.6\mathrm{Af} + 0.4 \quad (2)$$

As was expected, the neutral compounds have a tailing factor (and also an asymmetry factor) which is practically equal 1.0, while the profiles of the basic compounds are asymmetrical and exhibit either leading peaks (toluidines and *N*,*N*-dimethylaniline) or tailing ones (pyridine, 2,2-dipyridyl, amitriptyline). The data are summarized in Table 10. The R.S.D. values of the tailing factor on the five columns of the same batch columns were in the range of 0.5 to 1.8%, except for 2,2-dipyridyl (16.2%) in test 3 and for benzoic acid (2.5%) in test 5. The values obtained were between 1.5 and 12.5% for the 10 columns studied in the batch-to-batch reproducibility, with the exception of 2,2-dipyridyl (34.7%). Given the extreme sensitivity of the tailing of the profiles of basic compounds to minor changes in the chemistry of the surface, this degree of reproducibility appears quite satisfactory.

4. Conclusion

Those who have worked more than 10 years in analytical chromatography will note that the improvement in column reproducibility reported here is remarkable. For a long time, porous silica had the reputation of being difficult to reproduce from batch to batch [7–9]. It is obvious that the manufacturer of Symmetry C_{18} made great progress in improving the reproducibility of its production of packing materials.

It seems to us that, for all practical purposes, the batch-to-batch reproducibility of the chromatograph-

ic properties of the C_{18} silica stationary phase studied should satisfy most analytical users. It is difficult to demand a better reproducibility of the columns when the R.S.D.s of most retention factors are in the 0.1% range for column-to-column and the 1% range for batch-to-batch reproducibilities. Obviously, this level of precision could not be obtained without the excellent control of the column temperature, the mobile phase flow-rate and the mobile phase composition obtained with the equipment selected. Achieving a better repeatability with a given column, on a routine basis, would probably require considerable improvements of the instrumentation itself. The next issue to address, however, is more probably the long-term stability of the retention data. This is an entirely different problem and a more difficult one to study because it is very specific of the user, as it depends largely on the nature of the samples analyzed, of their pretreatment, and of the composition of the mobile phase. Such an investigation was not within the scope of this study.

Obviously, the high precision of the analytical performance achieved with one commercially available packing material (Symmetry C_{18}) is insufficient to allow general conclusions regarding the performance of the whole industry. The systematic acquisition of similar data on a number of different, well-known brands is in progress. The data will be reported shortly and separately for each brand studied. Different column brands are prepared with different synthesis processes of porous silica and have different physico–chemical properties. The aim of our current study is not to compare the position of the different brands studied on charts comparing their retention properties (i.e., plots of hydrophobic selectivity versus silanophilic activity), as was done by several groups [3–6]. It is to estimate the error range of the points on such charts. Knowing the precision of these correlation plots will markedly improve their usefulness for the selection of the column needed to perform a separation.

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