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# Repeatability and reproducibility of retention data and band profiles on reversed-phase liquid chromatography columns IV. Results obtained with Luna $C_{18}$ (2) columns

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**Abstract**

The reproducibility of retention data and band profile characteristics was investigated for a series of columns packed with Luna $C_{18}$ (2), a silica-based, reversed-phase adsorbent. High precision data were obtained and statistically compared among five columns from the same batch (column-to-column reproducibility) and nine columns from as many different batches (batch-to-batch reproducibility). These data were acquired under five different sets of chromatographic conditions, for a group of 30 neutral, acidic, and basic compounds selected as probes following an experimental protocol previously described. This work is part of a study on the precision of chromatographic analyses. Its purpose is to illustrate the contribution of the stationary phase reproducibility to this precision. 

*Keywords:* Band profiles; Luna $C_{18}$ (2); Stationary phases, LC; Retention data

## 1. Introduction

Liquid chromatography is the most successful method for the quantitative analysis of organics in complex mixture. Until recently, however, there was only very limited data on the degree of reproducibility with which chromatographic data may be measured. For regulatory purposes or merely for benchmark reasons, it is important that analysts have a good idea of the level of performance they can rely on. This report is the continuation of a detailed investigation of the precision of chromatographic measurements. It addresses the short- and long-term repeatability, the column-to-column and batch-to-batch reproducibility of commercial reversed-phase packing materials. It uses an unmodified commercial instrument but follows a rigorous protocol [1]. The purpose of this work is to document the present state of the art in column manufacturing, using a few selected examples. In previous papers, we discussed results obtained with columns of two brands of monomeric chemically bonded $C_{18}$ silica, Waters Symmetry $C_{18}$ [2] and Kromasil $C_{18}$ [3]. In this study, we examine an another brand, Luna $C_{18}$ (2) from Phenomenex (Torrance, CA, USA). It is a relatively new product and little is known regarding its preparation. We will show that highly precise results allow the acquisition of useful, new information regarding its nature in particular and, more generally, the present state of the art of the RPLC column industry and its current trends.

In our experimental protocol [1], we described in detail the chromatographic tests used in this study and justified the selection of the test compounds and

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the choice of the experimental conditions. To be useful, a protocol for the quantitation of the precision of experimental data must be clearly defined and followed strictly, as we did in previous studies [2,3] and here. Data acquired in the process may also be used for other legitimate purposes, however. The data acquired are useful for the characterization of the packing materials studied. The *hydrophobicity* of these adsorbents and their *selectivity for neutral compounds* (especially the *methylene selectivity*) are well studied. They are more or less understood parameters of stationary phase characterization. This does not hold true for the *silanol activity*. The term itself is not well defined (which is why we avoid its use). At least five different methods are used to express this property: (1) the retention of selected basic compounds [4–6]; (2) the relative retention of selected pairs of a basic compound and a neutral one [7–10]; (3) the asymmetry of the peaks of selected basic compounds [4–6]; (4) the combination of retention, asymmetry and peak efficiency of selected basic compounds [11–14]; and (5) the pH dependence of the retention factors of selected basic compounds (or rather the deviation from the sigmoidal curve theoretically predicted in the case of pure hydrophobic interaction) [15]. The use of the second or third moment of the peak were also suggested.

All these parameters have advantages and disadvantages. For example, the relative retention of two compounds can be measured with a high accuracy but it is not necessarily sensitive enough to small changes of the properties of the underlying silica surface; furthermore, changes in this property depend much on the very compounds selected and on the experimental conditions. Parameters derived from the band profile may be very sensitive to changes of the stationary phase properties but they are also, unfortunately, strongly influenced by small changes in the experimental conditions. So, they cannot be measured with the same high accuracy as thermodynamic properties. To compare or combine parameters derived from retention data and parameters derived from band profiles requires a serious knowledge of the reproducibility of these values. Finally, the lack of data useful to understand the nature of the results obtained (e.g., the individual p$K_a$ values and the relative concentrations of the different types of silanols) further complicate the proper interpretation of the properties measured. The measurement of the 'silanol activity' of the products studied was not our primary goal. However, our results can support future studies by providing estimates of the probable precision of the data measured by others.

Another important issue was raised recently by several authors [10,13,16]. It deals with the choice of a buffered or a nonbuffered mobile phase as the best eluent for stationary phase characterization with basic compounds. Neue et al. [9] and McCalley [17] stated that tests carried out with nonbuffered mobile phases are not reproducible. Claessens et al. [16] carried out the Engelhardt test [4] both in buffered and nonbuffered mobile phases and compared the column ranking, showing quite significant differences. However, these authors did not give any information on the reproducibility of their data. In our hands, experimental values of the retention parameters are as reproducible in nonbuffered as in buffered mobile phases, as illustrated by the low RSD values obtained for the long-term repeatability [2,3][1]. Admittedly, this result is achieved because sufficient time is allowed for column equilibration to take place [1]. Moreover these tests use relatively weak bases which are not supposed to show strong interaction with the stationary phases used in our studies. The same reproducibility is not always achieved for band profile characteristics.

These issues will be addressed again in this work. Yet, the main goal was to investigate the reproducibility of the retention and band profile data obtained with the Luna material.

## 2. Experimental

The experimental conditions were described in detail and discussed earlier [1]. We merely summarize below the essential points of the protocol and discuss the minor changes required for its application to a new packing material.

[1]The RSDs observed in batch-to-batch reproducibility tend to be higher in nonbuffered than in buffered tests. This does not mean that the nonbuffered tests are poorly reproducible but rather that they are more sensitive to fluctuations of the surface chemistry.

### 2.1. Instrument

The experimental 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 these units were controlled by a dedicated computer (equipped with a Pentium processor and 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). The data are regularly 'burnt' into a CD-ROM for archiving and authentication purposes. They are also uploaded to a computer for further evaluation.

The instrument tests corresponding to the operational qualification and performance verification procedures for the HP 1100 Series HPLC modules [18] were performed weekly and after each maintenance of the equipment.

### 2.2. Conditions

The column temperature was maintained at 25.0°C by the instrument controller. Systematic measurements of the temperature with an independent thermometer, as previously described [1], confirm the stability of this parameter. The mobile phases (see composition later) were obtained by instructing the solvent delivery system to pump and mix the two required streams (pure water or buffer and pure methanol) in the proper ratio, using the binary pump. The total flow-rate was scaled up compared to the original protocol (see discussion in Ref. [3]) to adjust for the difference in column diameter. It was 1.39 ml/min in all tests. The columns were equilibrated with the required mobile phase for 5 h before the first sample injection.

The injection volume was 13.9 μl. Each sample was injected in five replicates. The changes in eluent composition at column outlet were detected with the UV detector at 220, 230, 254, 270 and 290 nm. The 254-nm signal was used for the data interpretation.

### 2.3. Stationary phase

The experimental results reported in this work were acquired with 14 columns (150×4.6 mm) packed with Luna $C_{18}$ (2), an RPLC packing material from Phenomenex. This packing material is based on a porous silica which has an exceptionally high surface concentration of silanol groups, 10.8 μmol/$m^2$ [19]. The silica surface is covered with a $C_{18}$ layer obtained with a proprietary bonding process. The stationary phase type (i.e., monomeric or polymeric) is not available from the company. All the batches studied were also end-capped. The particles are spherical with an average size of 5 μm. The main characteristics of the bare silica and the packing material are summarized in Tables 1 and 2. The values listed there were measured and supplied by the manufacturer.

We used five columns packed with material from the same batch and nine columns packed with material from nine different batches. The columns were packed by the manufacturer and used as received. The nine batches of packing material were based on four different batches of silica (see Table 2).

Table 1
Physicochemical properties of the nine batches of stationary phase (Luna $C_{18}$ (2)) supplied by the manufacturer (Phenomenex)

| Silica batch no. | Particle size (μm) | Particle size distribution (90:10% ratio) | Pore size (Å) | Pore volume (ml/g) | Surface area ($m^2$/g) | Metal content (ppm) |
|---|---|---|---|---|---|---|
| 1 | 5.05 | 1.93 | 102 | 1.05 | 411 | 31.4 |
| 2 | 5.10 | 1.85 | 108 | 1.01 | 374 | 31.2 |
| 3 | 4.94 | 1.98 | 108 | 1.04 | 386 | 29.9 |
| 4 | 5.01 | 2.05 | 100 | 1.04 | 418 | 32.1 |
| Mean | 5.065 | 1.92 | 104.5 | 1.035 | 397.3 | 31.4 |
| RSD (%) | 0.86 | 4.92 | 3.95 | 1.67 | 5.21 | 1.49 |

Table 2
Physicochemical properties of the nine batches of stationary phase (Luna $C_{18}$ (2)) supplied by the manufacturer (Phenomenex)

| Luna (2) $C_{18}$ batch no. | Corresponding silica batch no. | Total carbon (%) | Surface coverage[a] ($\mu mol/m^2$) |
|---|---|---|---|
| 5291-1 | 1 | 17.51 | 2.94 |
| 5291-2 | 1 | 18.32 | 3.51 |
| 5291-3 | 1 | 17.85 | 3.38 |
| 5291-4 | 2 | 17.43 | 3.27 |
| 5291-5 | 2 | 17.30 | 3.50 |
| 5291-6 | 2 | 17.28 | 3.30 |
| 5291-7 | 3 | 17.28 | 3.30 |
| 5291-8 | 4 | 18.22 | 3.33 |
| 5291-9 | 4 | 18.20 | 3.33 |
| | Mean | 17.71 | 3.32 |
| | RSD (%0 | 2.48 | 5.00 |

[a] Calculated based on the total carbon by a proprietary formula.

### 2.4. Samples and chemicals

The qualitative and quantitative compositions of the five test mixtures used are given below. Because the column dimensions and the average surface area were different from those of the first brand studied [1,2], the flow-rate, the sample concentration, and the sample load were scaled up compared to those described in the protocol [1] and used with the first brand studied (Symmetry $C_{18}$, Waters, Milford, MA, USA) [2]. The detailed explanation of the scale-up calculations were described earlier [3].

Sample 1: thiourea (14.1 mg/l), phenol (141.4 mg/l), 1-chloro-4-nitrobenzene (23.6 mg/l), toluene (614.9 mg/l), ethylbenzene (510.7 mg/l), propylbenzene (510.7 mg/l), butylbenzene (1216 mg/l), *o*-terphenyl (51.8 mg/l), triphenylene (14.1 mg/l) in methanol–water (80:20).

Sample 2: thiourea (14.1 mg/l), aniline (96.2 mg/l), phenol (141.4 mg/l), *o*-toluidine (94.0 mg/l), *p*-toluidine (23.6 mg/l), *m*-toluidine (69.9 mg/l), *N*,*N*-dimethylaniline (45.0 mg/l), ethylbenzoate (616.1 mg/l), toluene (1025 mg/l), ethylbenzene (1021 mg/l), in methanol–water (55:45).

Sample 3a: thiourea (14.1 mg/l), theobromine (21.2 mg/l), theophylline (35.4 mg/l), caffeine (37.7 mg/l), phenol (188.5 mg/l), 2,3-dihydroxynaphthalene (235.6 mg/l) in methanol–water (30:70). Sample 3b: thiourea (14.1 mg/l), pyridine (115.8 mg/l), 2,2-dipyridyl (188.4 mg/l) in methanol–water (30:70).

Sample 4: thiourea (14.1 mg/l), propranolol (471.2 mg/l), butylparaben (23.6 mg/l), dipropylphthalate (400.5 mg/l), acenaphthene (235.6 mg/l), amitriptyline (117.8 mg/l), in methanol–water buffer (65:35) (20 m*M*) with potassium phosphate, monobasic/dibasic at pH 7.00.

Sample 5a: thiourea (14.1 mg/l), benzylamine load 1 (235.6 mg/l), benzyl alcohol (706.8 mg/l), benzoic acid (235.6 mg/l) in methanol–water buffer (30:70) with phosphoric acid–potassium monophosphate buffer (20 m*M*) at pH 2.70. Sample 5b: procainamide (14.1 mg/l), benzylamine load 2 (117.8 mg/l), phenol (188.4 mg/l), in methanol–water buffer (30:70) with phosphoric acid–potassium monophosphate buffer (20 m*M*) at pH 2.70.

The tests were carried out in the order listed. The chemicals were obtained from Fluka, Sigma–Aldrich (Milwaukee, WI, USA), except *o*-toluidine, benzylamine, methanol, and water, which were from Fisher Scientific (Pittsburgh, PA, USA). The chemicals used in the work described here were recently acquired and not leftovers from a previous study. They were used as received. As shown by Neue et al. [9] in the validation of their own test method which uses the same mixture and the same experimental conditions as those of test 4 in our protocol, the retention properties of basic compounds are very sensitive to small changes in the pH of the buffer. In order to avoid possible errors caused by fluctuations of the buffer composition due to the lack of reproducibility of the buffer preparation, the same buffer solution was used for all the columns tested, for each test. For the same reason, the flasks containing the two mobile phase components were constantly sparged with a helium stream in order to avoid the dissolution of carbon dioxide from the laboratory.

Because of the coelution of amylbenzene and *o*-terphenyl under the experimental conditions in test 1, amylbenzene was removed from the test mixture and propylbenzene was used instead. Because of the coelution of caffeine and pyridine under the experimental conditions of test 3 and because a possible tailing of 2,2-dipyridyl could affect the peak shape of the 2,3-dihydroxynaphthalene which is

eluted immediately after, this test mixture was divided into two (see above, tests 3a and 3b). Because of the coelution of dipropylphthalate and naphthalene in test 4, naphthalene was removed from the test. Because of the accidental coelution of phenol and benzyl alcohol under the experimental conditions of test 5, this test mixture was also divided into two (see above, tests 5a and 5b). In test 5b benzylamine was used again but the loaded amount was 50% of the loaded amount defined in the original protocol and used in test 5a. This allowed for a test of a possible overloading of the column with the higher amount.

### 2.5. Presentation of the data

For the sake of clarity, the terms used in this paper are now defined and explained. The *short-term repeatability* is the relative standard deviation (RSD) of the results of five consecutive runs carried out with one column over a period of a few hours. Short-term repeatability data of retention times, retention factors and selectivity factors measured on columns of another brand were already published and discussed [1–3]. The values obtained in this study closely match those previously published. The *long-term repeatability* is the RSD obtained by repeating the series of five consecutive analyses of the test mixture on the same column after the measurements had been completed on all the columns tested (a total of 14). This interval was typically 10 days.

The *column-to-column reproducibility* is the RSD of the 25 injections (five consecutive injections on each column) made on the five columns packed with packing material coming from the same batch. The

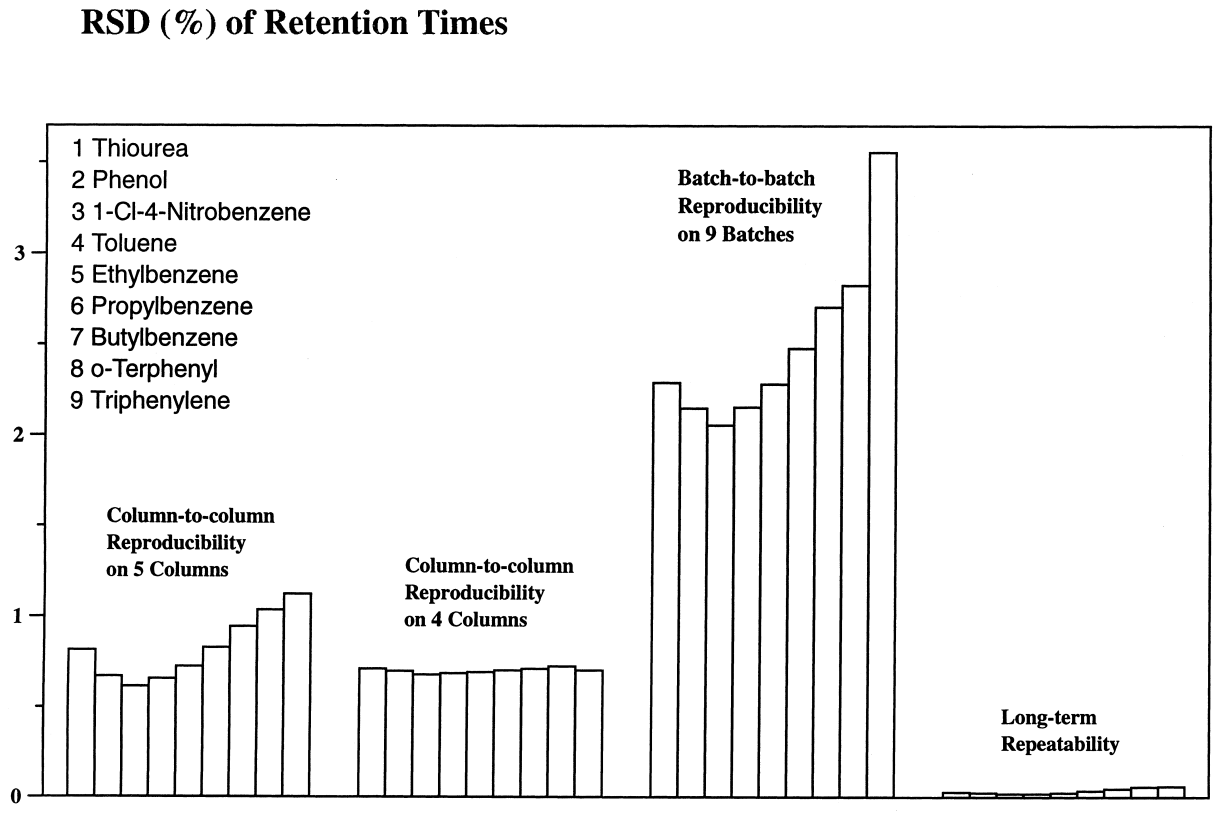



Fig. 1. Reproducibility of the retention time measured in the first test. (1) Thiourea, (2) phenol, (3) 1-chloro-4-nitrobenzene, (4) toluene, (5) ethylbenzene, (6) propylbenzene, (7) butylbenzene, (8) *o*-terphenyl, (9) triphenylene. Mobile phase, methanol–water (80:20) at 1.39 ml/min.

*batch-to-batch reproducibility* is the RSD of the 45 injections made on nine columns packed with material from the nine different batches of the reversed-phase packing material.

## 3. Results and discussion

### *3.1. Absolute retention data*

The long-term repeatability, the column-to-column and the batch-to-batch reproducibilities of the retention times are plotted in Figs. 1–5 for the five different tests (data pertaining to tests which were split into two parts are pooled together). The column-to-column reproducibility is plotted both for five columns (assigned as column-to-column reproducibility on five columns) and also for four columns only (assigned as column-to-column reproducibility on four columns). In this last case, the excluded data were those measured on a column which was packed 3 months later than the other four. Note, however, that all the chromatographic tests were carried out consecutively on the five columns, after delivery of this last column. The retention time RSD on the four columns is between 0 and 50% lower than on the five columns, which indicates a high degree of reproducibility of the packing process and the column hardware.

The RSD of the retention times measured on the nine columns packed with nine different batches vary between 1.6% (pyridine in test 3, Fig. 3) and 3.8% (benzylamine in test 5, Fig. 5). Among the neutral and acidic test solutes, triphenylene had the highest retention time RSD (3.6%). The highest value among basic compounds was measured for benzylamine (3.8%). The RSDs of the retention times of basic compounds are not significantly higher than those of neutral compounds. A comparison of the long-term repeatability of the retention times of basic compounds measured in buffered (tests 4 and 5) and nonbuffered (tests 1–3) solutions proves that the

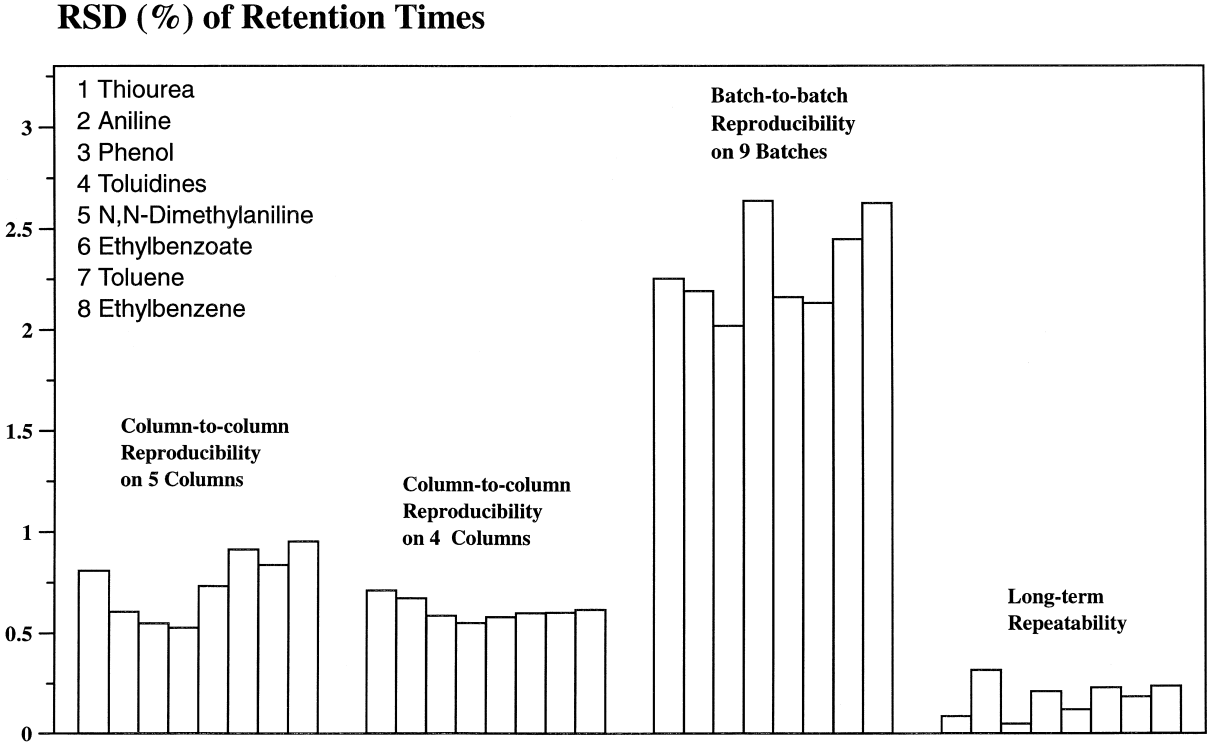



Fig. 2. Reproducibility of the retention time measured in the second test. (1) Thiourea, (2) aniline, (3) phenol, (4) toluidines, (5) *N,N*-dimethylaniline, (6) ethylbenzoate, (7) toluene, (8) ethylbenzene. Mobile phase, methanol–water (55:45) at 1.39 ml/min.

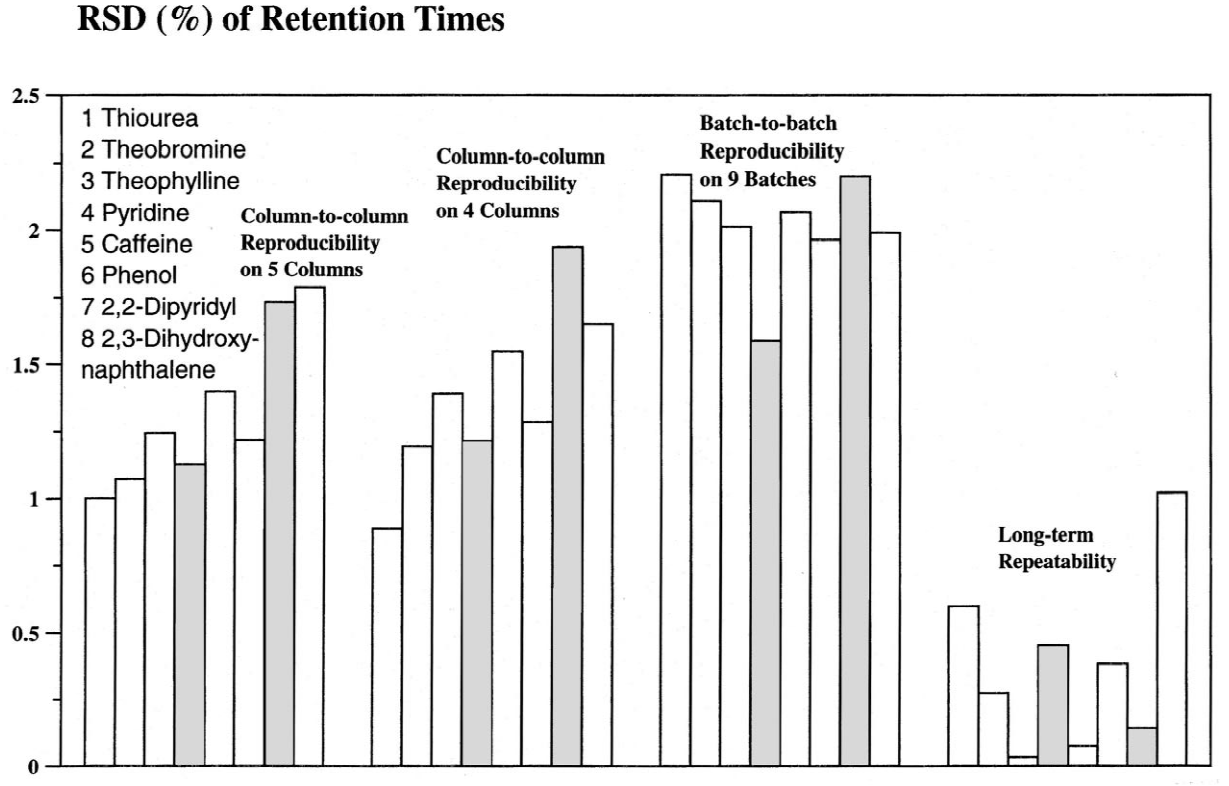



Fig. 3. Reproducibility of the retention time measured in the third test. (1) Thiourea, (2) theobromine, (3) theophylline, (4) caffeine, (5) pyridine, (6) phenol, (7) 2,2-dipyridyl, (8) 2,3-dihydroxynaphthalene. Mobile phase, methanol–water (30:70) at 1.39 ml/min.

tests carried out in nonbuffered solutions are as reproducible as those in buffered ones. This observation is in agreement with our previous findings on two other brands of columns [2,3].

A comparison of the data from tests 5a and 5b shows that the 50% decrease of loaded amount of benzylamine did not affect the column-to-column RSD for the five columns nor the batch-to-batch RSD. The average retention time on the five columns from the same batch was 1.634 min for the larger sample and 1.640 min for the lower one. The corresponding values on the nine batches were 1.616 and 1.620 min. These differences are not significant and no overloading effect was observed, at least in terms of retention.

The retention time RSD of the toluidine isomers was based on the retention time of the maximum of the composite peak. With most batches, there are no indications of even a small degree of resolution of these isomers. On two out of the nine batches, however, we observed a shoulder on the peak, indicating a partial separation of the isomers.

The nine batches of reversed-phase packing material are based on four batches of silica (see Table 2). In order to investigate the possible effect of the underlying silica on the retention properties of the packing material, we calculated separately the RSD data for the batches of packing material derived from the same silica batch. These data are plotted in Fig. 6. This figure demonstrates that the underlying silica has no or almost no effect on the retention properties of the different batches since the RSDs of the batches of packing material based on the same batch of silica are practically the same as those for batches of packing materials based on different batches of silica. Moreover, the RSD of the retention times do not correlate with those of the surface carbon content or the surface coverage. For example, the two batches of Luna $C_{18}$ (2) packing which are based on the same batch of silica and have the same carbon

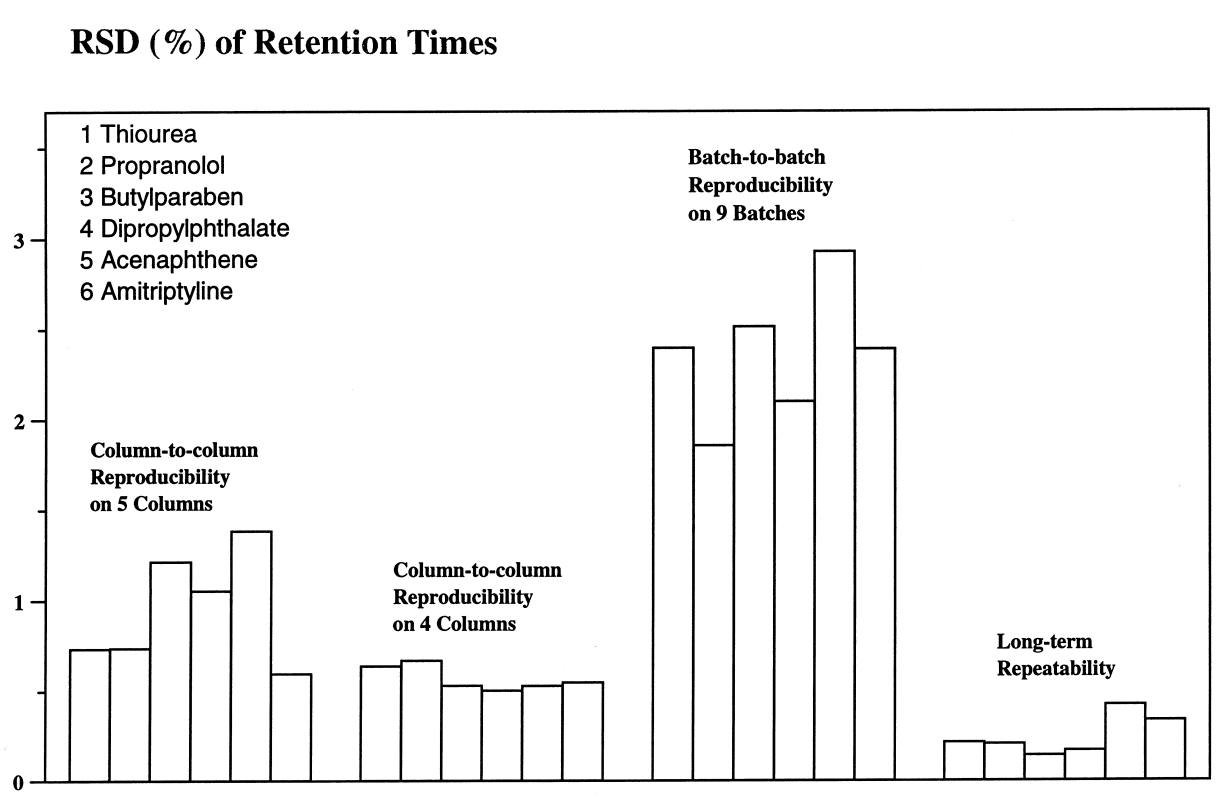



Fig. 4. Reproducibility of the retention time measured in the fourth test. (1) Thiourea, (2) propranolol, (3) butylparaben, (4) dipropylphthalate, (5) acenaphthene, (7) amitriptyline. Mobile phase, methanol–water buffer (65:35) with potassium phosphate, monobasic/dibasic at pH 7.00 at 1.39 ml/min.

content and surface coverage (batches 8 and 9, Table 2) give the same retention time RSD as the first three batches (batches 1–3, Table 2) for which the carbon content has a 2.3% and the surface coverage a 9.2% RSD

This result is quite different from those previously observed and showing a strong effect of the underlying silica on the retention times measured for Kromasil $C_{18}$ [3]. Although this difference could be related to the nature of the surface of the packing materials, other interpretations are possible. This issue will be addressed in a forthcoming publication.

### *3.2. Retention and separation factors*

Tables 3–7 report the RSDs of the retention factors derived from the measurements of the retention times of the different probe compounds made on either the five columns of one batch or the four columns of this batch, after exclusion of the data measured on the column packed 3 months later than the others. They also include the RSDs of these retention factors on the nine columns of different batches. The retention factors, calculated as the average of the results of five injections made on each column, are plotted in Figs. 7–11 versus the column rank. The RSDs for the column-to-column reproducibility of the retention factors are around 1% with a few exceptions. Propranolol in test 4 gives an unexpectedly low value (0.2%), difficult to explain. The highest value (3.2%) was measured for benzylamine in test 5. A 50% decrease of the amount loaded did not change the RSD (test 5b). The RSDs of the retention factors measured on the set of four columns are 3–5 times lower than those of the

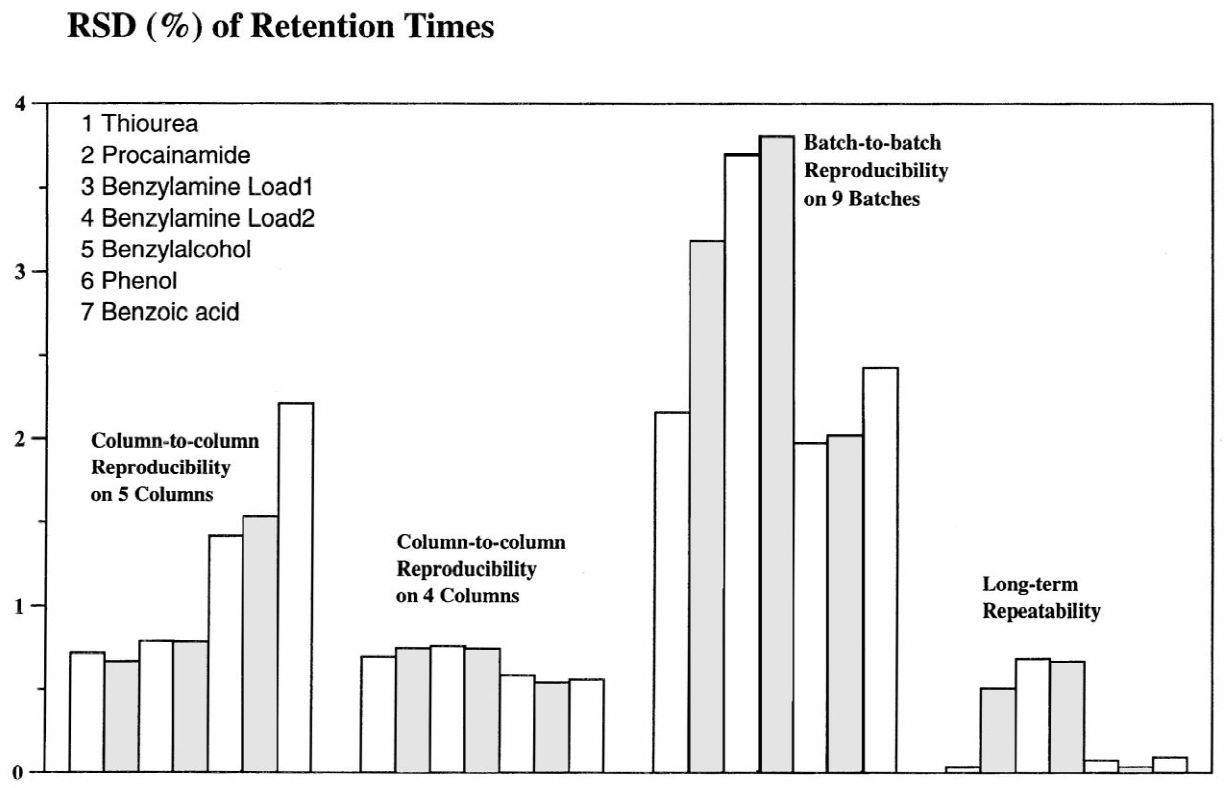



Fig. 5. Reproducibility of the retention time measured in the fifth test. (1) Thiourea, (2) procainamide, (3) benzylamine load 1, (4) benzylamine load 2, (5) benzyl alcohol, (6) phenol, (7) benzoic acid. Mobile phase, methanol–water buffer (30:70) with phosphoric acid/potassium monophosphate buffer at pH 2.70 at 1.39 ml/min.

factors measured on the set of five columns, except for the compounds in test 3. The batch-to-batch reproducibilities of the retention factors are 2–3 times larger than the column-to-column reproducibilities.

Table 8 lists the average relative retention data (i.e., separation factors, $\alpha$) for pairs of successively eluted peaks, for all the tests carried out, and their RSDs. In general, the average relative retention values of these arbitrarily selected compound pairs are close to those measured on the monomeric-type stationary phases previously studied [1–3], with a few exception. The most notable of these exceptions are triphenylene/*o*-terphenyl, amitriptyline/acenaphthene, and benzyl alcohol/benzylamine. In the first test, the RSDs are all below 0.1% for the set of five columns packed with the same packing material. The highest RSD on the five column set were obtained for the pairs butylparaben/propranolol (2.0%, test 4), dihydroxynaphthalene/dipyridyl (1.4%, test 3) and benzyl alcohol/benzylamine (1.4%, test 5). The basic/neutral compound pairs have RSDs which are 2–10 times higher than the neutral/neutral pairs.

For the set of nine columns from different batches, the RSDs of the relative retentions vary from 0.14% (ethylbenzene/toluene) to 1.40% (acenaphthene/dipropylphthalate) for the neutral/neutral pairs. The highest RSD for batch-to-batch reproducibility of the relative retention was measured for the pair benzyl alcohol/benzylamine in test 5 (9.8%). These RSDs are 2–30 times higher than on the set of five columns from the same batch, with the exception of the pairs dipyridyl/phenol and dihydroxynaphthalene/dipyridyl for which the RSDs were lower on the nine batches than on the five columns. Out of a total of 27 separation factors calculated in Table 8, 13 have

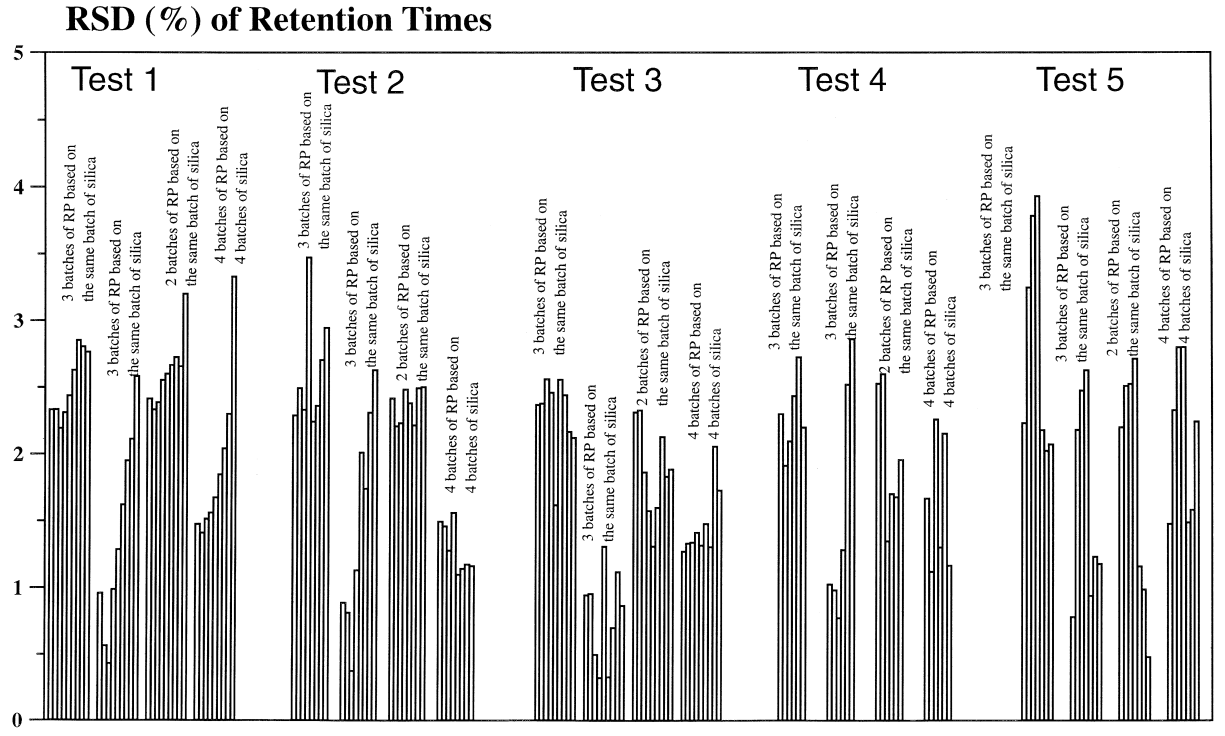



Fig. 6. The effect of underlying silica on the retention time. RSDs on the retention times of the components of all the test mixtures plotted for the reversed-phase (RP) batches based on the same batch of silica and for the RP batches based on different batches of silica.

batch-to-batch reproducibilities within 1.0 and 22% are better than 2.0%.

As in earlier studies [1–3], we calculated the values of different separation factors suggested by various authors [4,7–10,20–23] for the characterization of the surface properties of different brands of RPLC packing materials. These values are discussed later under the headings hydrophobic selectivity, steric selectivity, and separation factors of basic compounds.

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

| | RSD (%) of $k$ | | |
|---|---|---|---|
| | Column-to-column reproducibility on | | Batch-to-batch reproducibility on nine batches |
| | Five columns | Four columns | |
| Phenol | 1.115 | 0.235 | 1.628 |
| 1-Chloro-4-nitrobenzene | 1.239 | 0.244 | 2.171 |
| Toluene | 1.204 | 0.231 | 2.764 |
| Ethylbenzene | 1.266 | 0.236 | 2.893 |
| Propylbenzene | 1.344 | 0.245 | 3.057 |
| Butylbenzene | 1.430 | 0.253 | 3.233 |
| *o*-Terphenyl | 1.509 | 0.267 | 3.245 |
| Triphenylene | 1.611 | 0.270 | 3.826 |

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

| | RSD (%) of $k$ | | |
|---|---|---|---|
| | Column-to-column reproducibility on | | Batch-to-batch reproducibility on nine batches |
| | Five columns | Four columns | |
| Aniline | 0.953 | 0.320 | 2.302 |
| Phenol | 1.271 | 0.454 | 1.346 |
| Toluidines | 1.227 | 0.518 | 2.221 |
| *N*,*N*-Dimethylaniline | 1.244 | 0.500 | 2.424 |
| Ethylbenzoate | 1.475 | 0.599 | 2.053 |
| Toluene | 1.327 | 0.520 | 2.797 |
| Ethylbenzene | 1.431 | 0.581 | 2.990 |

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

| | RSD (%) of $k$ | | |
|---|---|---|---|
| | Column-to-column reproducibility on | | Batch-to-batch reproducibility on nine batches |
| | Five columns | Four columns | |
| Theobromine | 1.515 | 0.891 | 1.822 |
| Theophylline | 1.510 | 0.925 | 1.899 |
| Pyridine | 0.720 | 0.715 | 2.886 |
| Caffeine | 1.525 | 1.014 | 1.758 |
| Phenol | 1.283 | 0.492 | 1.559 |
| 2,2-Dipyridyl | 1.247 | 1.319 | 2.214 |

### *3.3. Hydrophobic selectivity*

Three estimates of the hydrophobic selectivity were derived from the retention data measured in two different tests. The methylene selectivity, $\alpha$(CH2), was calculated as the ratio of the retention factors of the three following pairs of compounds, butylbenzene/propylbenzene (test 1, Fig. 12a), ethylbenzene/toluene (tests 1 and 2, Fig. 12b,c, respectively) and propylbenzene/ethylbenzene (test 1, Fig. 12d). These figures report both the values obtained (as the average derived from five injections on each

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

| | RSD (%) of $k$ | | |
|---|---|---|---|
| | Column-to-column reproducibility on | | Batch-to-batch reproducibility on nine batches |
| | Five columns | Four columns | |
| Propranolol | 0.209 | 0.229 | 2.128 |
| Butylparaben | 2.022 | 0.550 | 2.016 |
| Dipropylphthalate | 1.629 | 0.408 | 1.990 |
| Acenaphthene | 1.894 | 0.435 | 3.192 |
| Amitriptyline | 0.834 | 0.121 | 2.904 |

Table 7
Reproducibility of the retention factors of the components of the fifth test mixture

| | RSD (%) of $k$ | | |
|---|---|---|---|
| | Column-to-column reproducibility on | | Batch-to-batch reproducibility on nine batches |
| | Five columns | Four columns | |
| Procainamide | 15.505 | 4.378 | |
| Benzylamine load 1 | 3.224 | 0.705 | 9.444 |
| Benzylamine load 2 | 3.233 | 0.501 | 9.846 |
| Benzyl alcohol | 2.005 | 0.303 | 1.643 |
| Phenol | 2.155 | 0.315 | 1.792 |
| Benzoic acid | 2.721 | 0.371 | 2.088 |

column) and their RSDs. The long-term repeatability values were of the same order as those measured earlier [2,3] on other brands of RPLC columns, which is a reflection of the constancy of the equipment performance. The repeatability of the hydrophobic selectivity is usually controlled by the instrument stability [1].

The relative retentions measured for the four columns packed at the same time exhibit very small fluctuations. The value measured on the fifth column

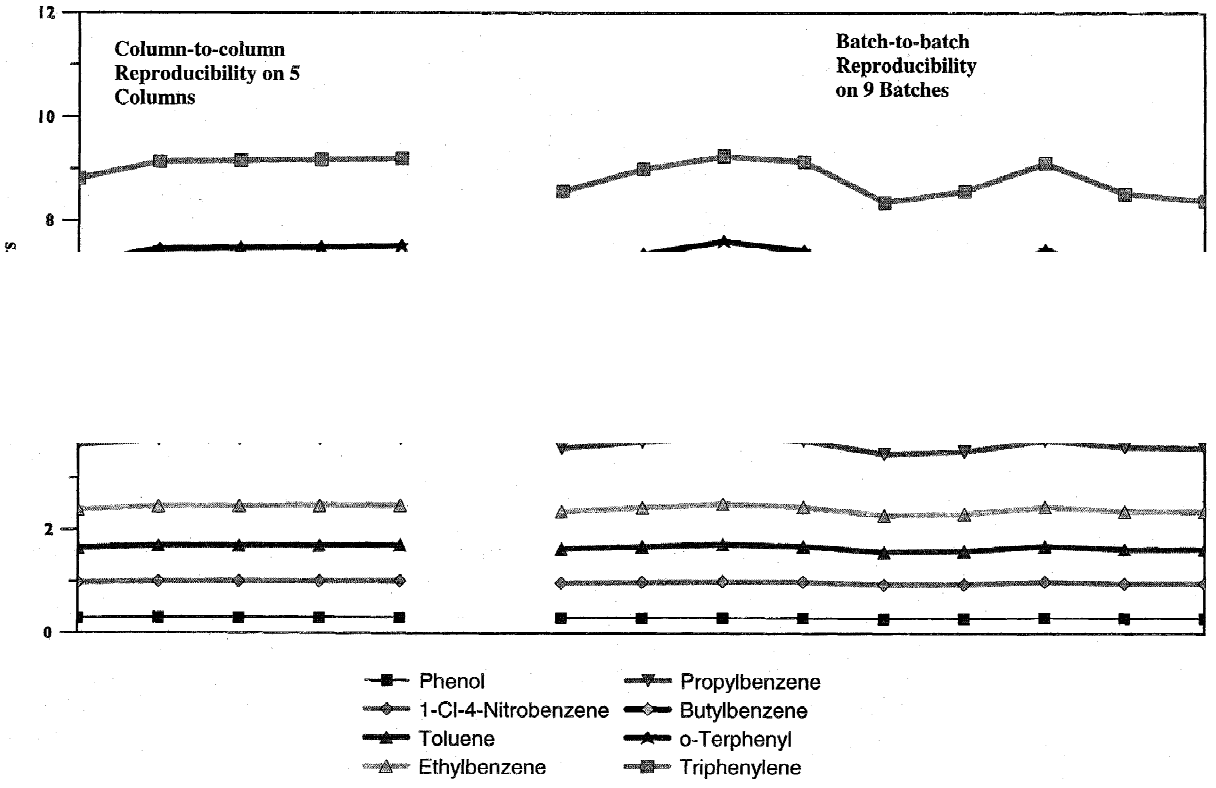



Fig. 7. Retention factors of the components of the first test mixture. Each data point represents the average of five consecutive injections carried out on a column.

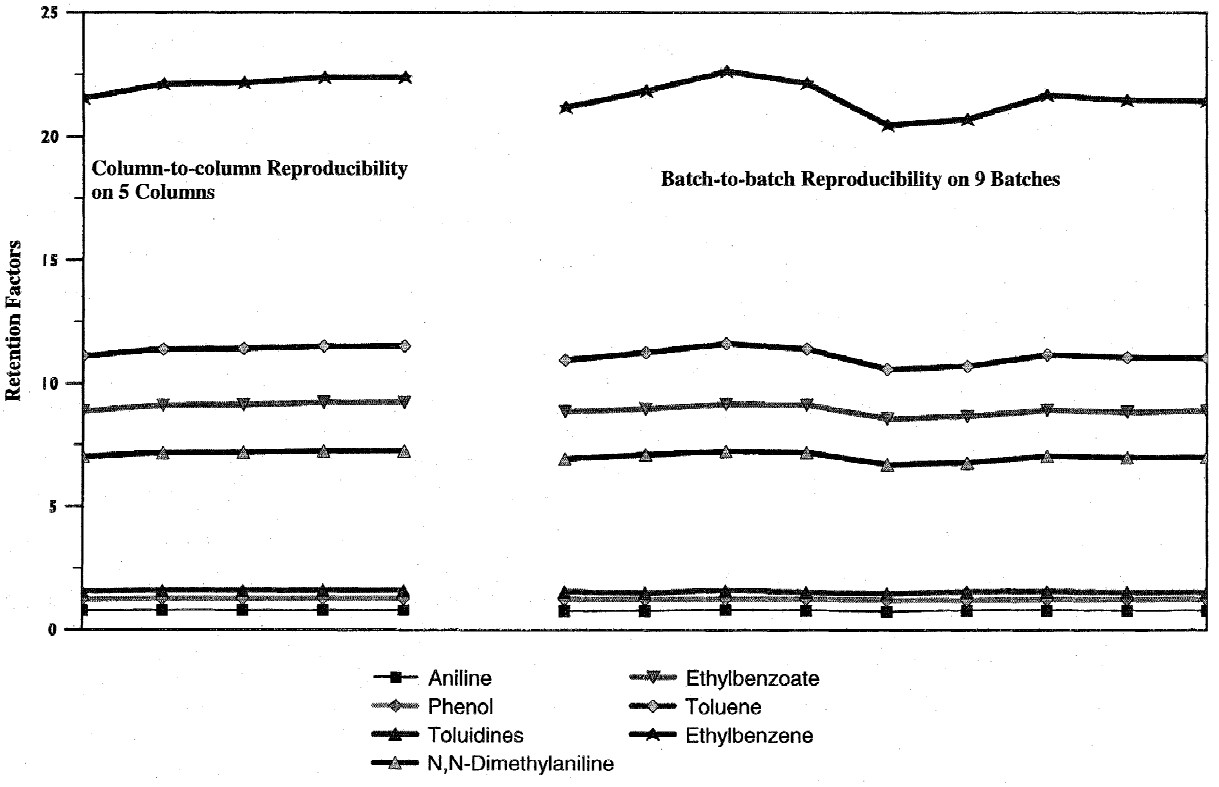



Fig. 8. Retention factors of the components of the second test mixture. Same data presentation as in Fig. 7.

(first data value on the plots), which was packed later, is slightly smaller for all pairs. The difference is small but still significant. The batch-to-batch reproducibility was twice higher than the column-to-column reproducibility on five columns (Fig. 12a–d). The correlations between the values derived under different test conditions are satisfactory.

The data corresponding to the batches of RP packing materials made with the same batch of silica are marked on Fig. 12a–d with circles, squares and dashed circles (see also Table 2). The differences observed are significant, albeit very small. They are not easy to explain. There is no correlation between the values measured or their RSDs and the fact that the underlying silica comes or not from the same batch. For certain batches made with the same batch of silica, the relative retentions are very close. For other sets, the fluctuations are much higher. The relative retention observed do not correlate with the total carbon or the surface coverage of the corresponding batches.

Finally, note that the differences between the relative retentions measured on the different batches are very small. They should not concern the analyst. They may be of interest for the surface chemist who might try to relate them to other properties of the packing materials.

### *3.4. Steric selectivity*

The steric selectivity is characterized by the separation factor of triphenylene and *o*-terphenyl, two polynuclear aromatics which have a similar polarity but different shapes [7]. All the values of the steric selectivity measured are plotted in Fig. 13. Each set of five successive data points correspond to one column. The two sets of data obtained for the determination of the long-term repeatability are

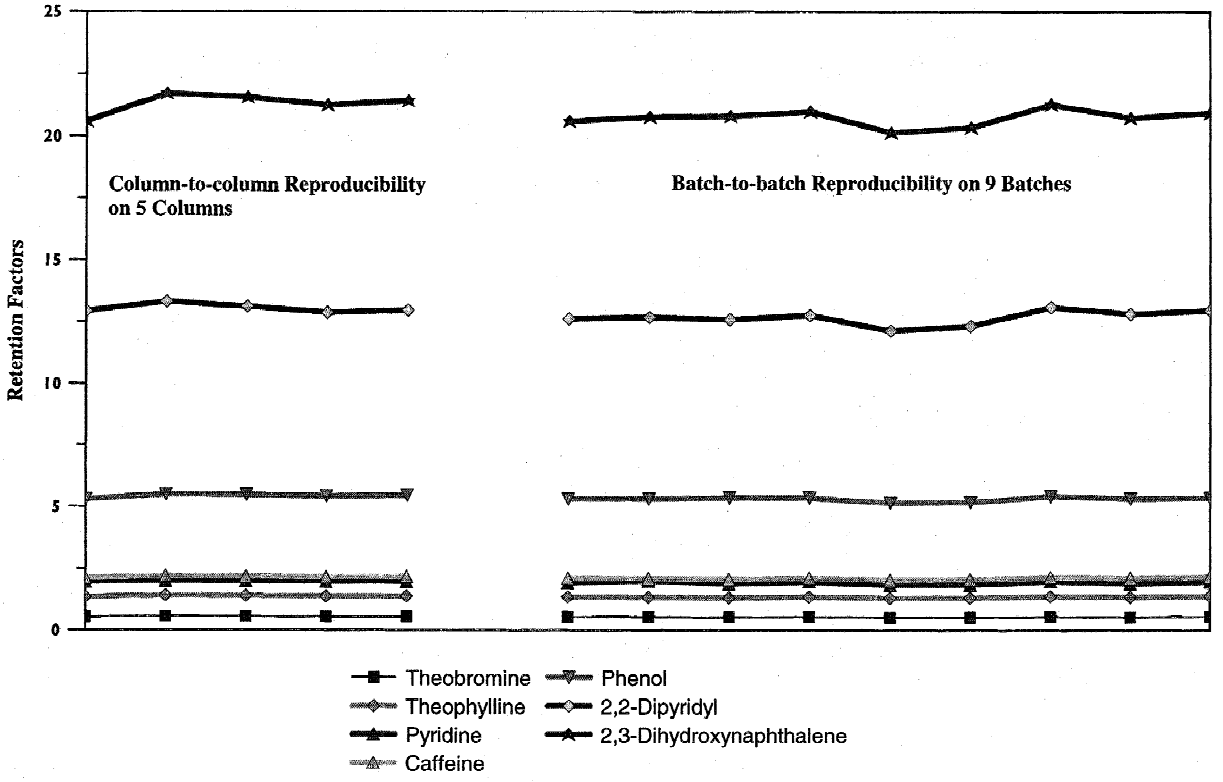



Fig. 9. Retention factors of the components of the third test mixture. Same data presentation as in Fig. 7.

marked with rectangles showing the initial results and those obtained 10 days later on the same column. The data corresponding to columns packed with RP batches based on the same batch of silica are grouped together as sets.

The average value obtained for the triphenylene/*o*-terphenyl relative retention is 1.22. The monomeric-type stationary phases previously studied are based on narrow-pore silicas (Waters Symmetry $C_{18}$ [2], Kromasil $C_{18}$ [3], and Hypersil HyPURITY Elite $C_{18}$). The average values found for this same parameter were 1.77, 1.72, and 1.76, respectively. On six batches of a polymeric-type stationary phase (Vydac RP $C_{18}$), which is based on a wide-pore silica, we found an average value of 3.15 [24]. It was shown [25] that the two most commonly used steric selectivity parameters (i.e., the planar recognition test described by Kimata et al. [7] which uses *o*-terphenyl/triphenylene and the shape selectivity test of Sander and Wise [26] which uses phenanthrophenanthrene, benzo[*a*]pyrene and tetrabenzonaphthalene) allow the classification of stationary phases as 'monomer', 'intermediate' or 'polymer' phases, depending on the numerical values obtained. The two tests do not necessarily give the same classification [25], but both give valuable information on the surface density of the bonded groups, the bonded alkyl chain lengths and, if values obtained on the same base silica are compared with the steric selectivity, on the functionality of the silylating reagent [7]. The values of the steric selectivity of the monomeric-type $C_{18}$ stationary phases found in previous work and in the literature are consistent. They were measured on phases which have a surface coverage close to that of the material studied here. The difference between them and the value obtained for Luna $C_{18}$ (2) demonstrate that this last brand does not belong to the same group. However, it is not a polymeric stationary phase either.

The column-to-column reproducibility of the steric

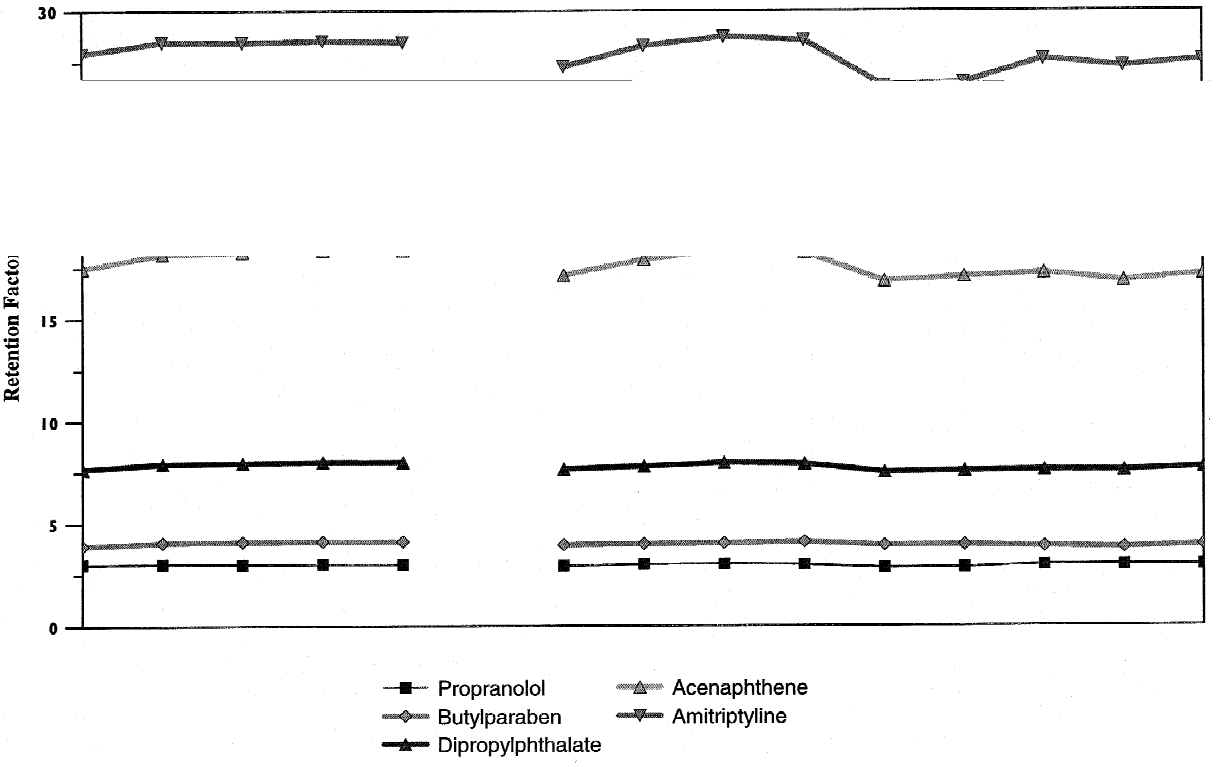



Fig. 10. Retention factors of the components of the fourth test mixture. Same data presentation as in Fig. 7.

selectivity for the five columns have a RSD similar to that observed for the hydrophobic selectivity (RSD = 0.108%, Fig. 13). However, the batch-to-batch reproducibility (RSD = 1.15%) is 5–6 times higher than for the hydrophobic selectivity. The differences observed between the different batches cannot be correlated with their carbon content nor with their surface coverage. Fig. 13 shows that the RSDs obtained for the batches of packing material made from the same batch of silica are approximately half the RSD obtained for the whole set of data.

### *3.5. Separation factors of the basic compounds*

The purpose of the last four tests of the series was to assess the interactions of basic compounds with the stationary phase surface under different experimental conditions (i.e., with a nonbuffered mobile phase, with buffered mobile phases at high and low pH), and using test compounds having different molecular size and p$K_a$ values. As discussed in an earlier publication [3], the expected type of interaction contributing most to the retention should be different under the different conditions selected. As a consequence, we expect to obtain different results from the different tests. However, the purpose of the study, the determination of the precision of the measurements of column characteristics with modern RPLC columns, brought some constraints. Because of the large number of factors which can affect the separation of basic compounds, studies using our protocol are too limited in scope and cannot address all problems related to the separation of basic compounds.

The behavior of the columns studied toward basic compounds was characterized by the separation factors of pairs of one of the test basic compounds and a neutral, nonpolar compound. Fig. 14a–f illustrates the results obtained, showing the separation factors of the following pairs: aniline and toluene (Fig. 14a) and *N*,*N*-dimethylaniline and toluene (Fig. 14b) — both from test 2 — amitriptyline and

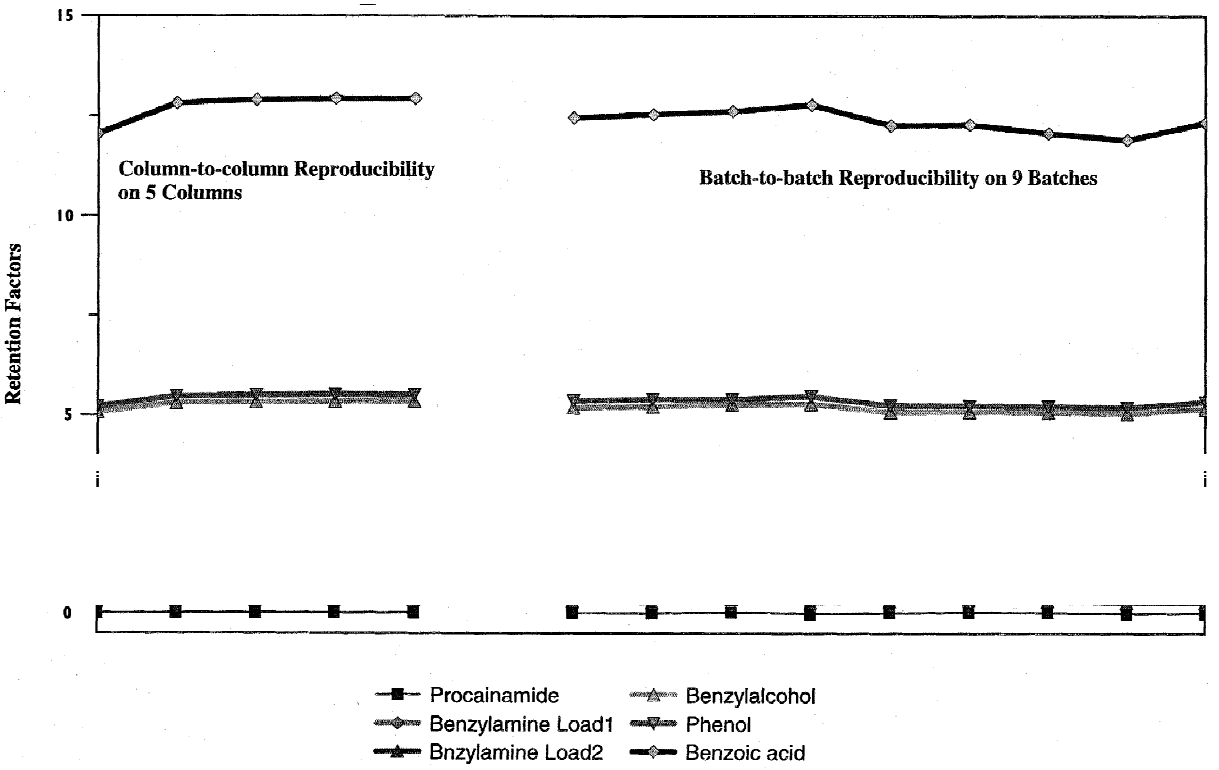



Fig. 11. Retention factors of the components of the fifth test mixture. Same data presentation as in Fig. 7.

acenaphthene (Fig. 14c) and propranolol and acenaphthene (Fig. 14d) — both from test 4 — benzylamine and benzyl alcohol (Fig. 14e) — from test 5 — and pyridine and phenol (Fig. 14f) — from test 3. Both the values (given as the average of the results of five successive injections on each column) and their RSDs are plotted in the figures. The bars marked with asterisks indicate the two sets of results obtained in the determination of the long-term repeatability.

The column-to-column reproducibility measured for five columns varies between 0.09% (*N*,*N*-dimethylaniline/toluene in test 2) and 1.94% (propranolol/acenaphthene in test 4). In all cases, except for benzylamine/benzyl alcohol in test 5, the column packed later (first bar in the set) gives a higher value than the four columns packed on the same day. Exclusion of the values obtained with this column improves the column-to-column reproducibility. For example the RSD of the relative retention measured on five columns drops from 1.11 to 0.45% for the pair amitriptyline/acenaphthene, from 1.94 to 0.74% for the pair propranolol/acenaphthene and from 1.33 to 0.46% for the pair benzylamine/benzyl alcohol when the RSD is calculated for the four columns packed the same day.

The RSDs for the batch-to-batch reproducibility vary between 0.46% (*N*,*N*-dimethylaniline/toluene in test 2) and 10.0% (benzylamine/benzyl alcohol in test 5). Those for the pairs amitriptyline/acenaphthene and propranolol/acenaphthene in test 4 are slightly higher than those measured for the column-to-column reproducibility with five columns. The difference between the results given by the fifth column and by the other four is more pronounced under this test condition than in the other cases discussed before. This may be because at pH 7.0 the strong bases are partly protonated, partly nonionized and the ions may interact with the dissociated surface silanol groups, through strong ion-exchange interactions. The density and dissociation constant of these silanols might be the less reproducible characteristics

Table 8
Reproducibility of the relative retention data of the components of the five test mixtures

| | One batch, five columns | | Nine batches | |
|---|---|---|---|---|
| | Average value of relative retentions | RSD (%) of relative retentions | Average value of relative retentions | RSD (%) of relative retentions |
| *Test 1 (MeOH–water, 8:2)* | | | | |
| Cl-nitrobenzene/phenol | 3.310 | 0.089 | 3.298 | 0.681 |
| Toluene/Cl-nitrobenzene | 1.687 | 0.039 | 1.687 | 0.759 |
| Ethylbenzene/toluene | 1.450 | 0.065 | 1.449 | 0.148 |
| Propylbenzene/ethylbenzene | 1.525 | 0.080 | 1.523 | 0.181 |
| Butylbenzene/propylbenzene | 1.539 | 0.088 | 1.537 | 0.189 |
| *o*-Terphenyl/butylbenzene | 1.290 | 0.082 | 1.287 | 0.246 |
| Triphenylene/*o*-terphenyl | 1.225 | 0.108 | 1.215 | 1.149 |
| *Test 2 (MeOH–water, 55:45)* | | | | |
| Phenol/aniline | 1.571 | 0.357 | 1.558 | 1.928 |
| Toluidines/phenol | 1.262 | 0.096 | 1.251 | 1.990 |
| Dimethylaniline/toluidines | 4.525 | 0.070 | 4.544 | 2.376 |
| Ethylbenzoate/dimethylaniline | 1.268 | 0.236 | 1.270 | 0.479 |
| Toluene/ethylbenzoate | 1.250 | 0.152 | 1.247 | 0.859 |
| Ethylbenzene/toluene | 1.941 | 0.108 | 1.939 | 0.216 |
| *Test 3 (MeOH–water, 3:7)* | | | | |
| Theophylline/theobromine | 2.469 | 0.137 | 2.473 | 0.381 |
| Pyridine/theophylline | 1.435 | 1.004 | 1.432 | 1.950 |
| Caffeine/pyridine | 1.092 | 0.988 | 1.094 | 2.023 |
| Phenol/caffeine | 2.506 | 0.471 | 2.515 | 1.186 |
| 2,2-Dipyridyl/phenol | 2.398 | 1.126 | 2.384 | 1.000 |
| Dihydroxynaphth./dipyridyl | 1.634 | 1.375 | 1.637 | 0.918 |
| *Test 4 (MeOH–pH 7.0 buffer, 65:35)* | | | | |
| Butylparaben/propranolol | 1.349 | 2.032 | 1.341 | 2.548 |
| Dipropylphth./butylparaben | 1.943 | 0.409 | 1.942 | 1.329 |
| Acenaphthene/dipropylphth. | 2.292 | 0.283 | 2.263 | 1.402 |
| Amitriptyline/acenaphthene | 1.565 | 1.114 | 1.582 | 1.404 |
| *Test 5 (MeOH–pH 2.7 buffer, 3:7)* | | | | |
| Benzyl alcohol/benzylamine L1 | 16.634 | 1.365 | 17.152 | 9.394 |
| Benzyl alcohol/benzylamine L2 | 16.372 | 1.348 | 16.948 | 9.788 |
| Phenol/benzyl alcohol | 1.032 | 0.166 | 1.032 | 0.401 |
| Benzoic acid/phenol | 2.340 | 0.587 | 2.327 | 0.653 |

of RPLC adsorbents. Be like as it may, this higher 'noise' observed on the five columns prevents from drawing reliable conclusions on the values measured for the nine batches.

As was stated earlier for the hydrophobic selectivity, the differences reported in this subsection are generally small and of little concern for the analytical chemist. They are of greater interest for the surface chemist trying to understand the nature of the surface of RPLC materials and their origin.

### *3.6. Column efficiency*

The RSDs of the peak efficiencies derived from the peak width measured at half-height are illustrated in Figs. 15–19. The *short-term repeatabilities* closely match those found in our previous studies [1–3]. The RSDs are all below 2%, except for thiourea in test 4. The *long-term repeatabilities* have stronger bias. In test 1 the values are practically the same as those for the short term repeatability. In test 2, all

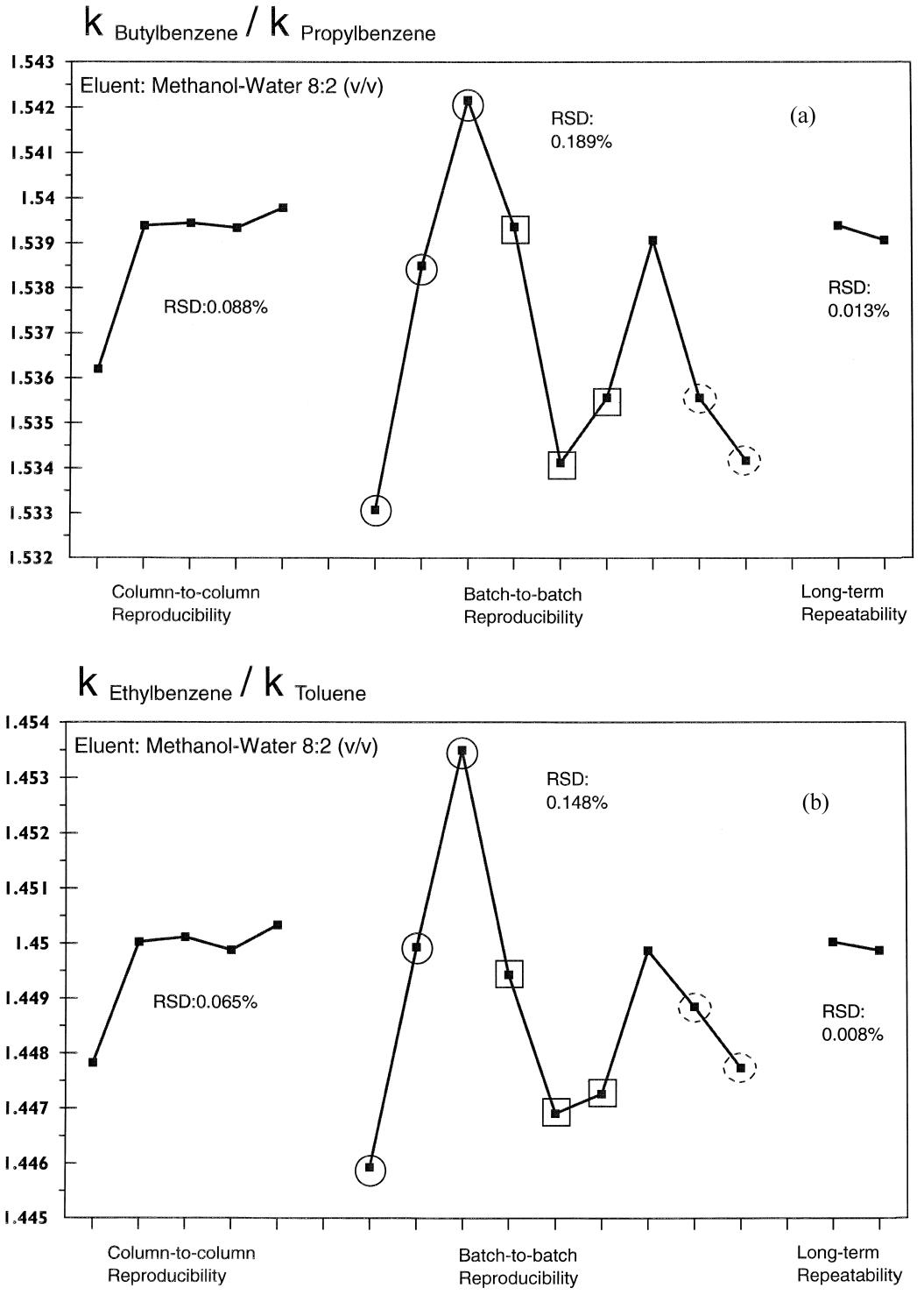
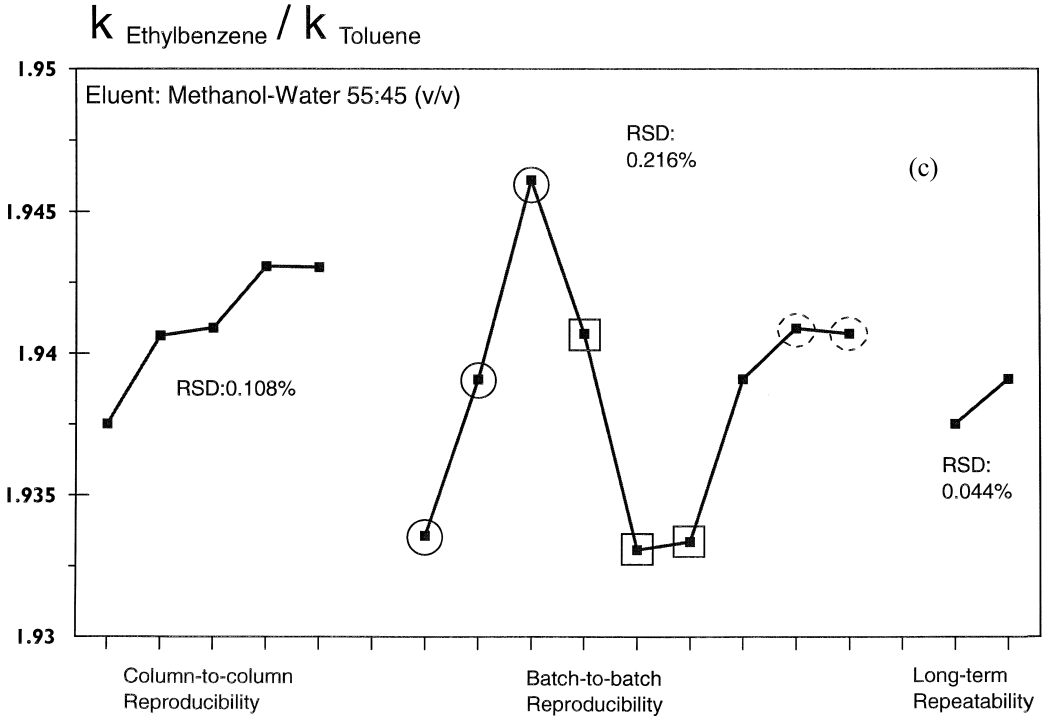



Fig. 12. Reproducibility of the hydrophobic selectivity. Each data point represents the average relative retention value calculated from the results of five consecutive injections carried out on a column. (a) Butylbenzene/propylbenzene (test 1); (b) ethylbenzene/toluene (test 1); (c) ethylbenzene/toluene (test 2); and (d) propylbenzene/ethylbenzene (test 1).



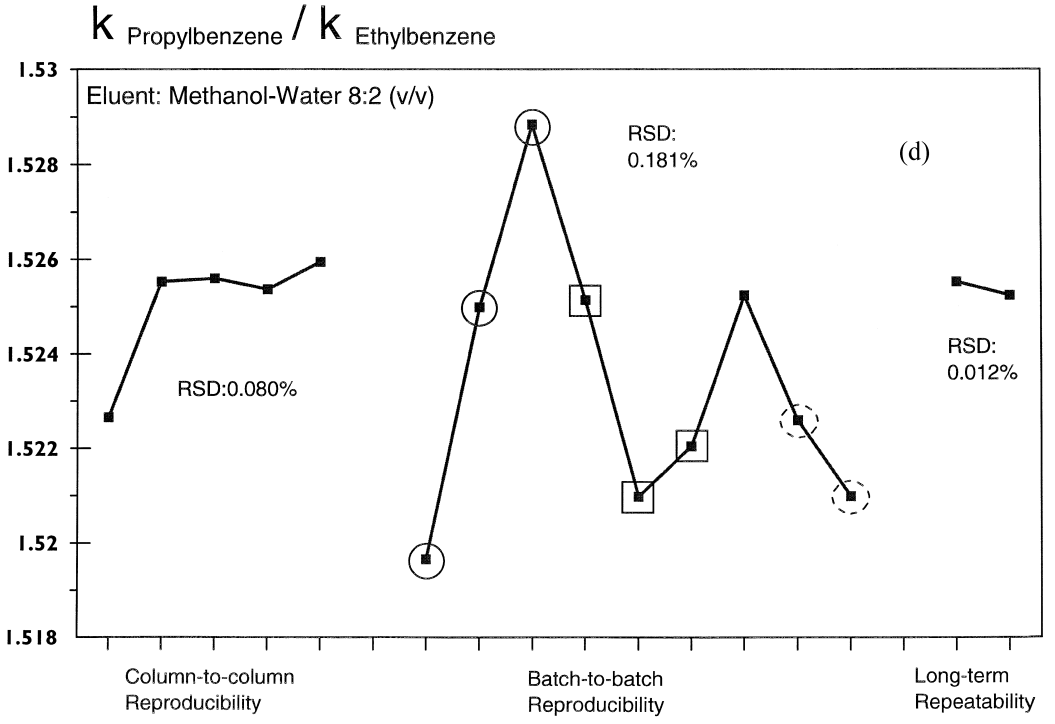




Fig. 12 (*continued*).

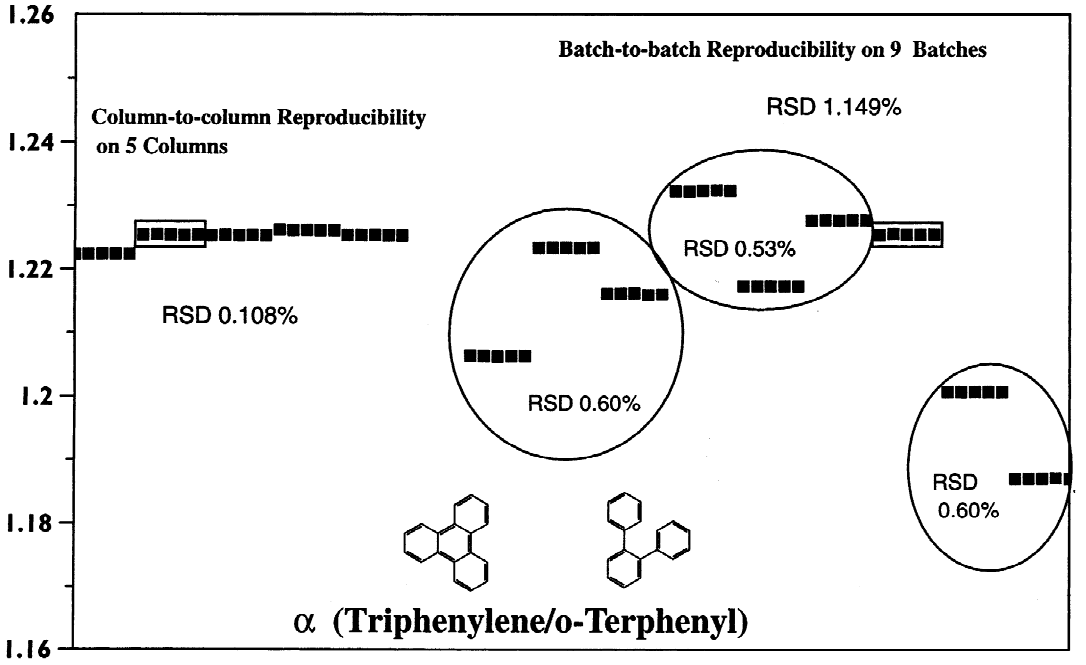



Fig. 13. Reproducibility of the steric selectivity.

RSDs are slightly higher in the long-term than in the short-term determinations, with the exception of aniline which gives a 10-times higher value for the long-term repeatability than for the short-term one. In test 3 two basic compounds, pyridine and 2,2-dipyridyl show, respectively, 6 and 10 times higher long-term repeatability RSDs than short-term ones, the other compounds giving just slightly higher values. In test 4, the long-term repeatability of the efficiency is high for thiourea and propranolol while the other compounds have a RSD of 2%. Finally, in test 5, the RSDs for the long-term repeatabilities are only slightly higher than for the short-term ones.

The RSDs of efficiency corresponding to the neutral compounds have practically the same value for the *column-to-column* and the *batch-to-batch reproducibility*, with the exception of acenaphthene in test 4, for which we observe a 4-times higher value on the nine batches than on the five columns. The batch-to-batch reproducibility of basic compounds is always higher than the column-to-column one, although the difference varies from compound to compound. Among basic compounds, aniline (test 2) has the highest batch-to-batch RSD for the efficiency (37%, almost 4 times higher than the column-to-column reproducibility). However, the RSD of the long-term repeatability is also high, possibly for the same reasons. The high value obtained for the toluidine isomers in test 2 (the column-to-column and the long-term reproducibilities are 3 and 4 times lower, respectively) originates from the fact that the test uses a mixture of the three isomers and that these isomers are partially separated on two batches of columns. Under the conditions used for test 2, the neutral forms of the bases predominate in the nonbuffered mobile phase. The separation of the toluidine isomers may be influenced by steric effects [27]. So, this observation is not necessarily a proof that strong interactions take place between these bases and the surface silanol groups. The RSD of the batch-to-batch reproducibility for pyridine (28.3%) is 4 times higher than that of the column-to-column reproducibility or the long-term repeatability of the same parameter.

2,2-Dipyridyl has a basicity comparable with that of pyridine. The RSD for the batch-to-batch reproducibility of its efficiency is in between the values obtained for the five columns and for the long-term

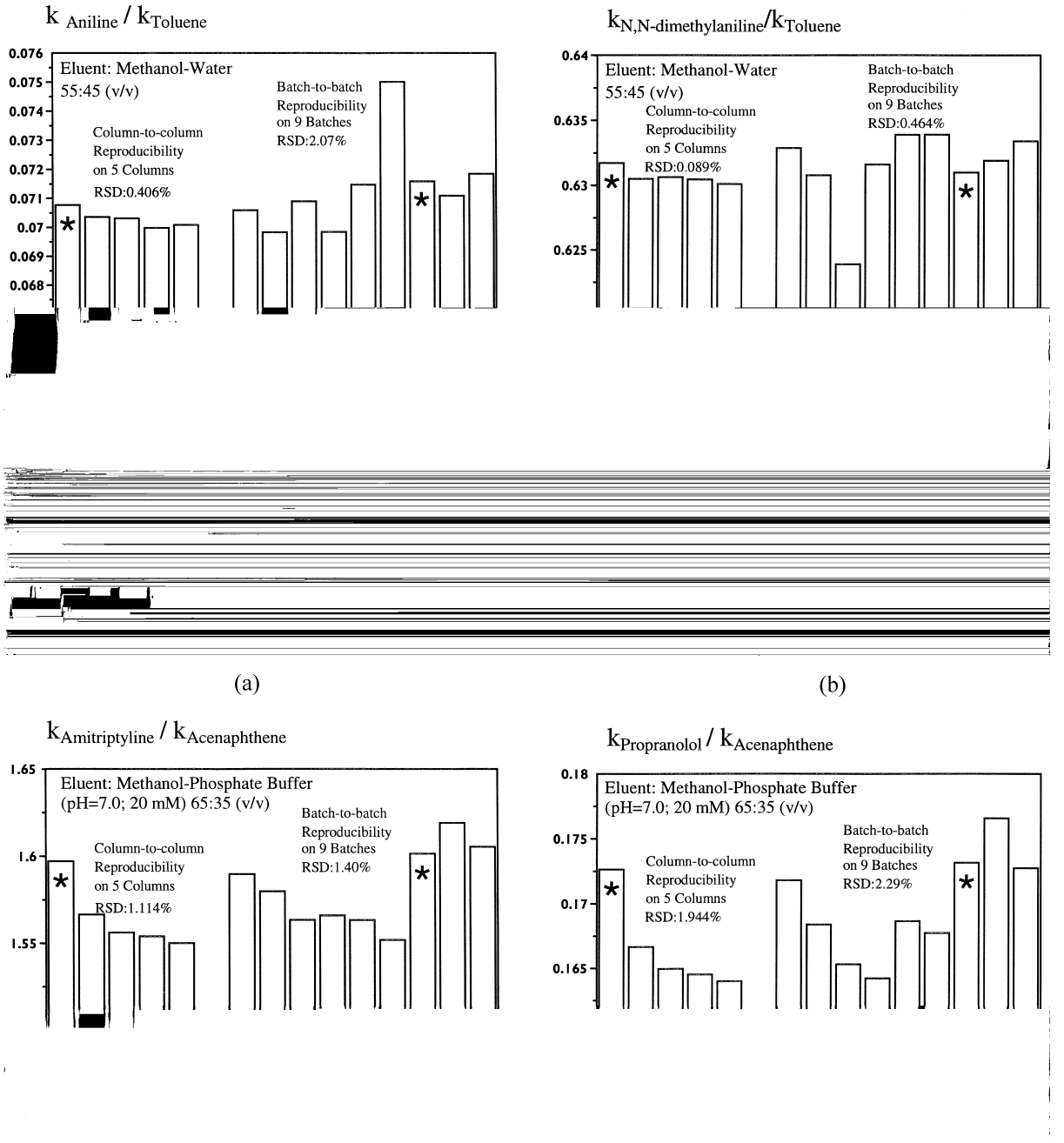



Fig. 14. Reproducibility of the separation factors of basic compounds. Each data point represents the average relative retention value calculated from the results of five consecutive injections carried out on a column. (a) Aniline/toluene (test 2); (b) *N,N*-dimethylaniline/toluene (test 2); (c) amitriptyline/acenaphthene (test 4); (d) propranolol/acenaphthene (test 4); (e) benzylamine/benzyl alcohol (test 5); and (f) pyridine/phenol (test 3).

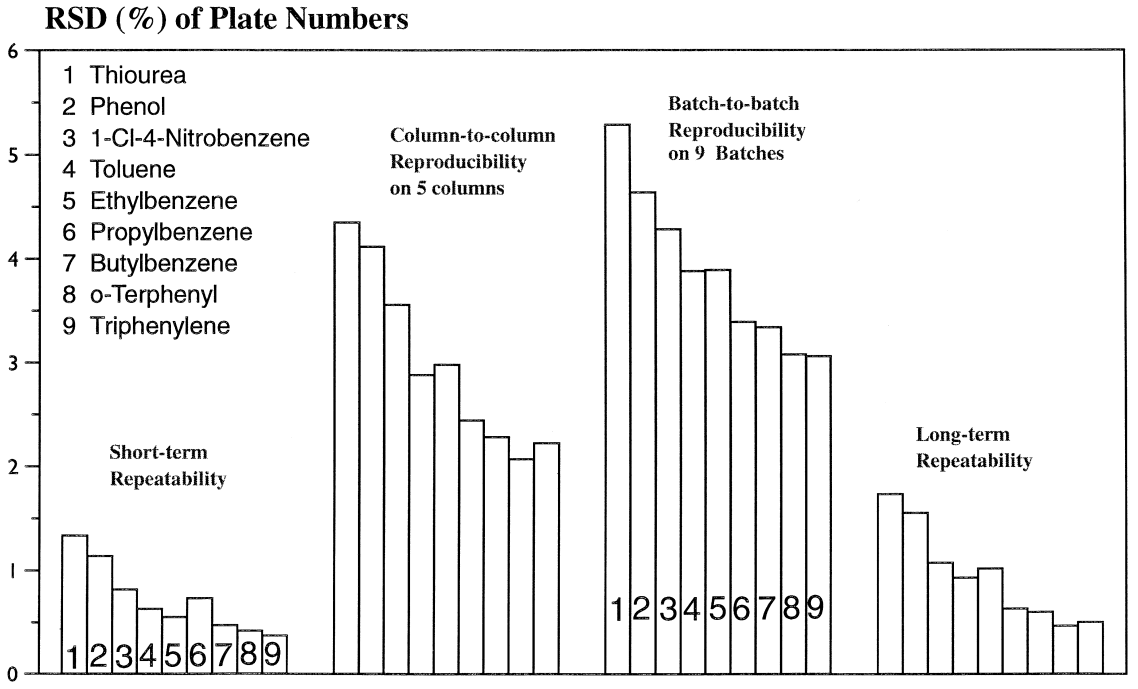



Fig. 15. Relative standard deviation of the number of theoretical plates for the components of the first test mixture.

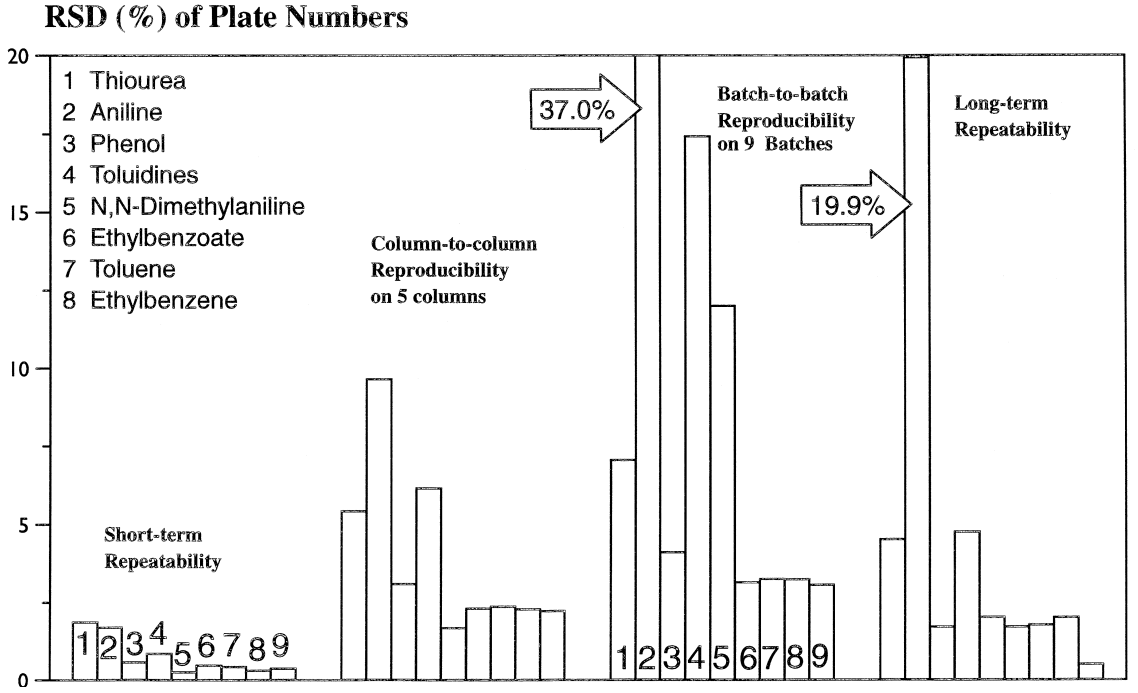



Fig. 16. Relative standard deviation of the number of theoretical plates for the components of the second test mixture.

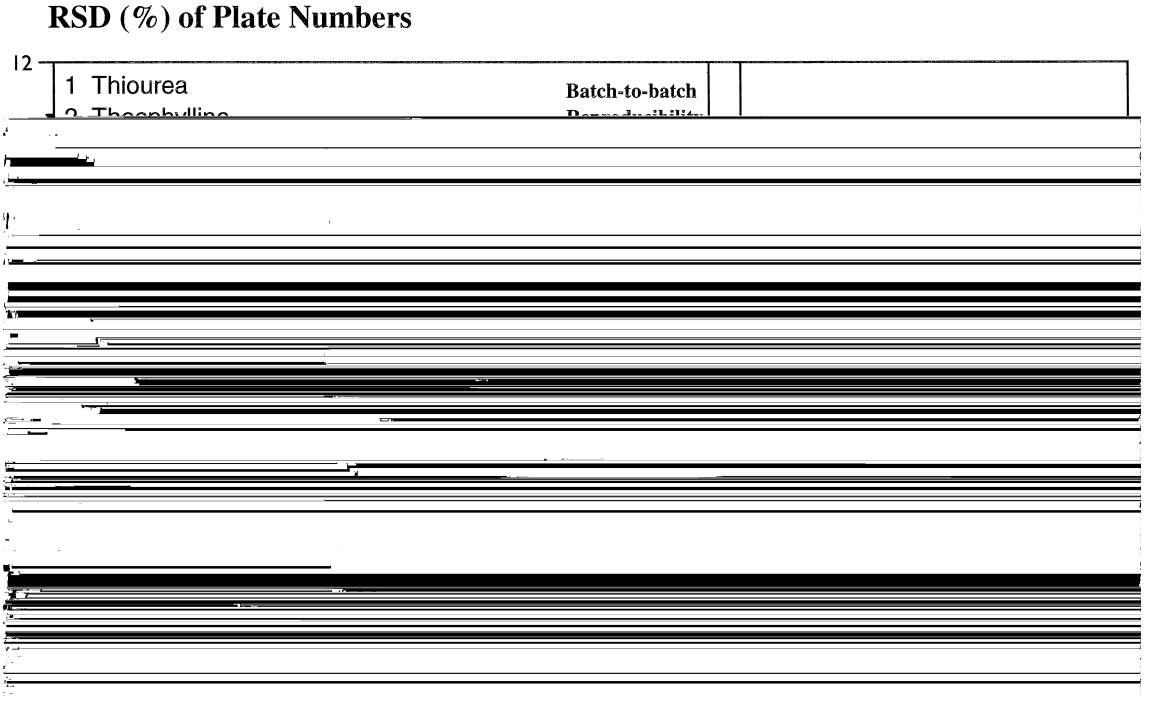



Fig. 17. Relative standard deviation of the number of theoretical plates for the components of the third test mixture.

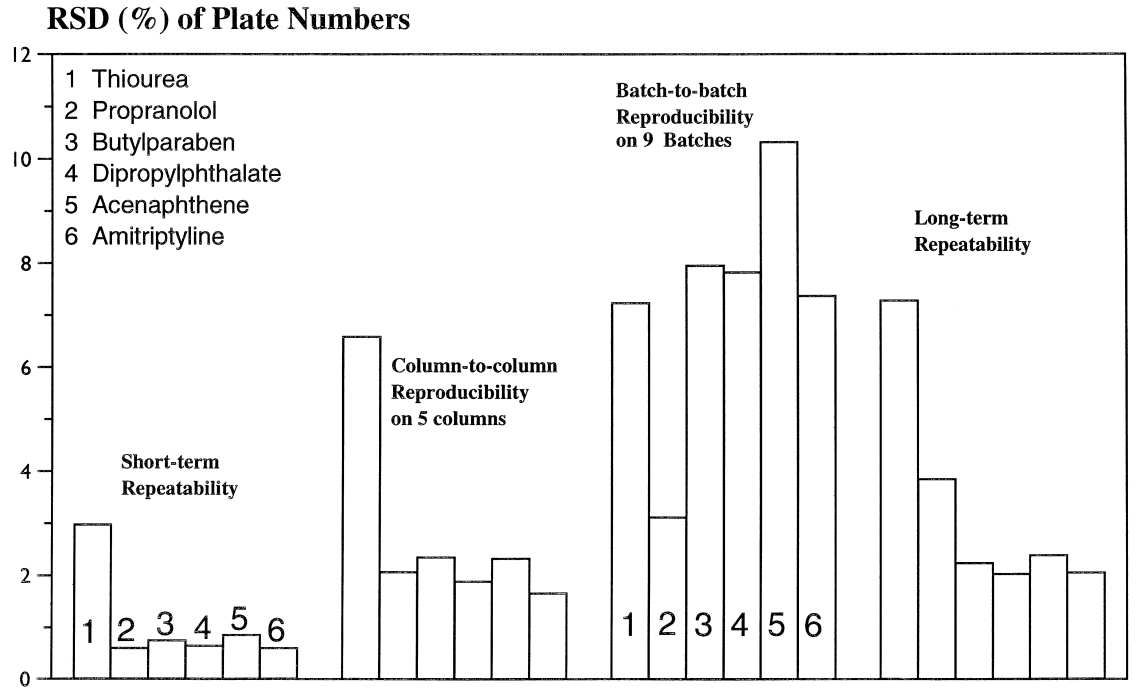



Fig. 18. Relative standard deviation of the number of theoretical plates for the components of the fourth test mixture.

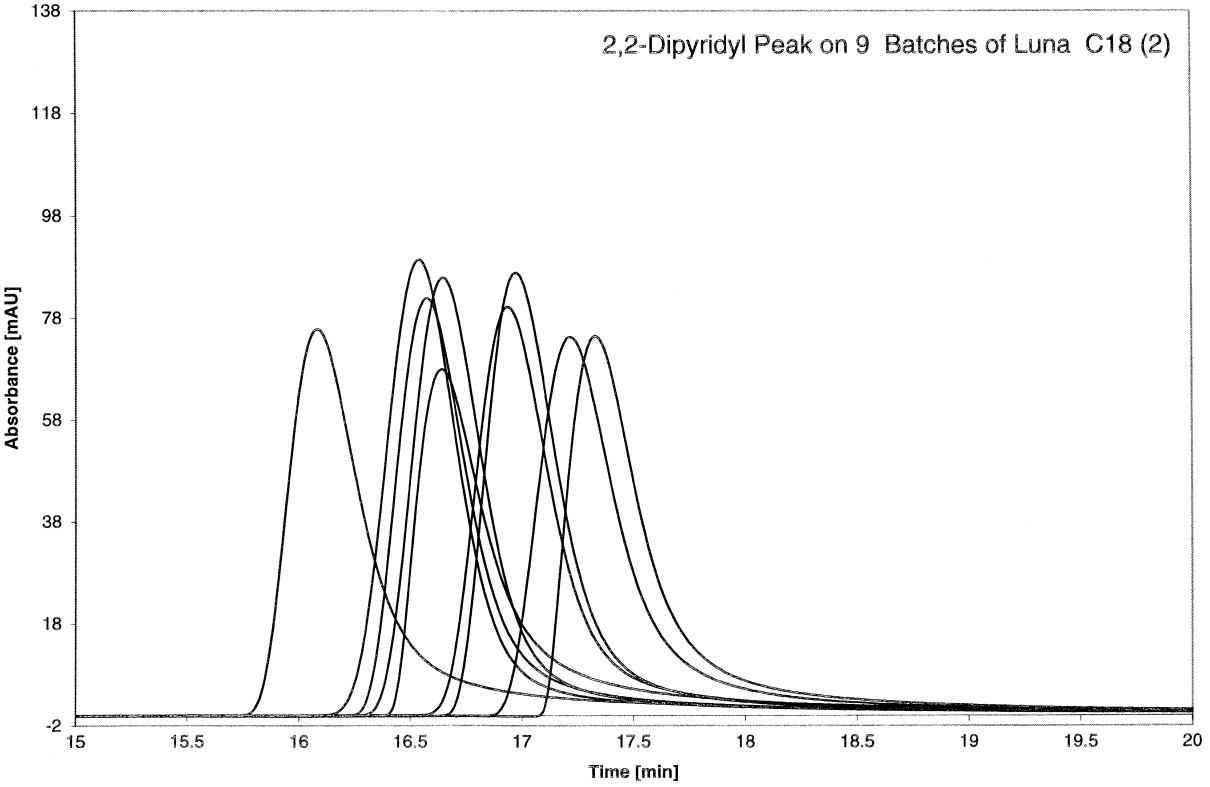



Fig. 21. 2,2-Dipyridyl peak on nine batches of Luna $C_{18}$ (2).

ing half peak-width. This is an empirical parameter, without physical meaning. Its only advantage is the ease of its derivation and the reasonable precision of its measurement. The average value on five columns packed with the same batch of packing material and the average value on nine batches together with their RSD are reported in Table 9. The absolute values reported here are not directly comparable with the values reported for the previously studied brands [2,3] because the column sizes and the peak efficiencies are different.

The neutral compounds have a tailing factor practically equal to 1, except those with low retention times, for which this factor is affected by extra-column effects. The column-to-column RSDs of the tailing factors of these compounds were below 3.5%, except for benzyl alcohol in test 5 (RSD= 5.4%). The batch-to-batch RSDs vary between 3.2% (ethylbenzene in test 2) and 8.8% (acenaphthene in test 4).

*N*,*N*-Dimethylaniline in test 2 and propranolol in test 4 gave leading peaks. The peak shape of this latter compound is also affected by extra-column effects which disperse and alleviate to a degree the leading of the peak (deconvolution of the extra-column effect would result in a lower tailing factor). *N*,*N*-Dimethylaniline gave also a leading peak on the stationary phases previously studied. The most probable cause of this effect on well packed columns, on which the neutral compounds elute with symmetrical peaks, is overloading with an analyte having an antilangmuirian isotherm [29]. The other basic compounds gave tailing peaks, the highest average value was measured for 2,2-dipyridyl (2.42) on the five columns and for pyridine (2.3) on the nine batches.

Only pyridine among the basic compounds has a tailing factor which shows important batch-to-batch fluctuations. The batch-to-batch RSD (11.7%) is almost 3 times higher than the column-to-column RSD and 4 times higher than the RSD obtained for

Table 9
Tailing factor of the different compounds studied: average values and their reproducibility

| | One batch, five columns | | Nine batches | |
|---|---|---|---|---|
| | Average value of tailing factors | RSD (%) of tailing factors | Average value of tailing factors | RSD (%) of tailing factors |
| *Test 1 (MeOH–water, 8:2)* | | | | |
| Thiourea | 1.400 | 3.178 | 1.400 | 3.178 |
| Phenol | 1.290 | 3.067 | 1.283 | 4.153 |
| 1-Chloro-4-nitrobenzene | 1.190 | 3.275 | 1.179 | 4.970 |
| Toluene | 1.153 | 3.383 | 1.137 | 5.199 |
| Ethylbenzene | 1.116 | 3.229 | 1.098 | 5.114 |
| Propylbenzene | 1.075 | 2.955 | 1.058 | 5.129 |
| Butylbenzene | 1.045 | 2.728 | 1.027 | 4.851 |
| *o*-Terphenyl | 1.063 | 2.380 | 1.050 | 4.832 |
| Triphenylene | 1.070 | 2.371 | 1.062 | 4.998 |
| *Test 2 (MeOH–water, 55:45)* | | | | |
| Thiourea | 1.303 | 2.105 | 1.308 | 3.073 |
| Aniline | 1.085 | 3.943 | 1.210 | 5.895 |
| Phenol | 1.170 | 3.497 | 1.151 | 4.374 |
| *N*,*N*-Dimethylaniline | 0.833 | 1.386 | 0.857 | 5.503 |
| Ethylbenzoate | 1.088 | 2.097 | 1.075 | 4.741 |
| Toluene | 1.032 | 1.957 | 1.017 | 4.060 |
| Ethylbenzene | 1.012 | 1.718 | 1.000 | 3.217 |
| *Test 3 (MeOH–water, 3:7)* | | | | |
| Thiourea | 1.300 | 2.525 | 1.309 | 3.221 |
| Theobromine | 1.176 | 2.471 | 1.171 | 3.195 |
| Theophylline | 1.120 | 2.573 | 1.101 | 2.641 |
| Pyridine | 2.002 | 4.147 | 2.295 | 11.720 |
| Caffeine | 1.117 | 2.218 | 1.103 | 4.075 |
| Phenol | 1.130 | 2.147 | 1.108 | 4.250 |
| 2,2-Dipyridyl | 2.424 | 24.263 | 2.214 | 24.446 |
| 2,3-Dihydroxynaphthalene | 1.548 | 11.215 | | |
| *Test 4 (MeOH–pH 7.0 buffer, 65:35)* | | | | |
| Thiourea | 1.283 | 4.328 | 1.287 | 5.132 |
| Propranolol | 0.939 | 2.943 | 0.951 | 6.886 |
| Butylparaben | 1.099 | 3.572 | 1.110 | 7.816 |
| Dipropylphthalate | 1.098 | 3.525 | 1.111 | 7.988 |
| Acenaphthene | 1.034 | 3.396 | 1.053 | 8.809 |
| Amitriptyline | 1.227 | 3.069 | 1.282 | 7.760 |
| *Test 5 (MeOH–pH 2.7 buffer, 3:7)* | | | | |
| Thiourea | 1.338 | 5.013 | 1.366 | 4.645 |
| Procainamide | 1.293 | 3.720 | 1.307 | 4.157 |
| Benzylamine (load 1) | 1.821 | 3.981 | 1.796 | 3.631 |
| Benzylamine (load 2) | 1.575 | 3.118 | 1.564 | 3.662 |
| Benzyl alcohol | 1.189 | 5.415 | 1.204 | 6.929 |
| Phenol | 1.159 | 5.652 | 1.163 | 6.971 |
| Benzoic acid | 1.144 | 5.106 | 1.160 | 6.832 |

the tailing factor of non-basic compounds in the same test. For all other basic compounds, either the batch-to-batch and the column-to-column RSDs are the same or their values are comparable with those obtained for non-basic compounds in the same test. The fact that the highest RSD was measured for

2,2-dipyridyl (both for the column-to-column and the batch-to-batch reproducibility) proves that it is not the accessability of the underlying silica surface which counts but the tailing type which differs for 2,2-dipyridyl peak on the monomeric packing materials previously studied and on the one studied here.

Finally, the average tailing factor measured for benzylamine is ~15% smaller with the lower than with the higher sample size on both the five column and the nine batch sets. The column-to-column RSD for five columns decreases slightly with decreasing loading. The batch-to-batch reproducibility is not affected by the sample size (RSD = 3.6% for both loadings).

## 4. Conclusion

The steric selectivity or relative retention of the pair triphenylene/*o*-terphenyl clearly indicates that Luna $C_{18}$ (2) does not belong to the classical family of monomeric stationary phases, i.e., phases prepared using monofunctional silylating reagents, which are believed to be the most reproducible packing materials. However, the reproducibility figures obtained on nine batches of this brand clearly indicate that a 'non-monomeric' stationary phase can give results of a precision comparable to those obtained with a monomeric phase, neglecting relatively minor fluctuations upward or downward. The RSDs for the batch-to-batch reproducibility of most retention factors are within a 2–3% range. Those for the batch-to-batch reproducibilities of the peak efficiencies and the tailing factors are below 10% for most compounds. This degree of reproducibility should satisfy most analysts. However, for a few compounds, we observed some serious reproducibility problems. This finding is consistent with our previous observation of similar problems on the Kromasil $C_{18}$ and Symmetry $C_{18}$ packings. The compounds involved are different for the different brands.

The results obtained with the Luna material confirm previous findings that the reproducibility of thermodynamic data is always much better than that of kinetic data. Moreover the RSDs of the relative retention of the neutral–neutral pairs are always much lower than those of basic–neutral or basic–basic pairs.

Unlike with Kromasil $C_{18}$, the possible differences between batches of the underlying silica of Luna $C_{18}$ (2) do not seem to influence the retention properties. The RSD figures obtained on different packing batches based on the same batch of silica are almost the same as those obtained for packing batches based on different silica batches.

Finally, a decrease of the sample amount does not affect the batch-to-batch reproducibility. The values of the peak efficiencies and their asymmetries change with the loading, as was expected, not the RSDs.

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## References

[1] M. Kele, G. Guiochon, J. Chromatogr. A 830 (1999) 41.
[2] M. Kele, G. Guiochon, J. Chromatogr. A 830 (1999) 55.
[3] M. Kele, G. Guiochon, J. Chromatogr. A 855 (1999) 423.
[4] H. Engelhardt, M. Jungheim, Chromatographia 29 (1990) 59.
[5] H. Engelhardt, H. Low, W. Gotzinger, J. Chromatogr. A 544 (1991) 371.
[6] H. Engelhardt, M. Arangio, T. Lobert, LC·GC 15 (1997) 856.
[7] K. Kimata, K. Iwaguchi, S. Onishi, K. Jinno, R. Eksteen, K. Hosaya, M. Araki, N. Tanaka, J. Chromatogr. Sci. 27 (1989) 721.
[8] U.D. Neue, in: HPLC Columns: Theory, Technology and Practice, Wiley–VCH, New York, 1997, p. 198.
[9] U.D. Neue, B.A. Alden, T.H. Walter, J. Chromatogr. A 849 (1999) 101.
[10] U.D. Neue, E. Serowik, P. Iraneta, B.A. Alden, T.H. Walter, J. Chromatogr.A 849 (1999) 87.
[11] R.J.M. Vervoort, M.W.J. Derksen, F.A. Maris, J. Chromatogr. A 678 (1994) 1.

[12] R.J.M. Vervoort, M.W.J. Derksen, A.J.J. Debets, J. Chromatogr. A 765 (1997) 157.
[13] D.V. McCalley, R.G. Brereton, J. Chromatogr. A 828 (1998) 407.
[14] R.G. Brereton, D.V. McCalley, Analyst 123 (1998) 1175.
[15] D. Sykora, E. Tesarova, M. Popl, J. Chromatogr. A. 758 (1997) 37.
[16] H.A. Claessens, M.A. van Straten, C.A. Cramers, M. Jezierska, B. Buszewski, J. Chromatogr. A 826 (1998) 135.
[17] D.V. McCalley, LC·GC 17 (1999) 440.
[18] Manual: Operational qualification/performance verification for complete HP 1100 series HPLC systems, Hewlett-Packard, 1997.
[19] Y. Bereznitski, M. Jaroniec, J. Chromatogr. A 828 (1998) 51.
[20] A.B. Scholten, J.W. de Haan, H.A. Claessens, L.J.M. van de Ven, C.A. Cramers, Anal. Chem. 64 (1994) 4085.
[21] A. Tchapla, H. Colin, G. Guiochon, Anal. Chem. 56 (1984) 621.
[22] A. Tchapla, S. Heron, H. Colin, G. Guiochon, Anal. Chem. 60 (1988) 1443.
[23] V. Bhagwat, Y. Bereznitski, B. Buszewski, M. Jaroniec, J. Liq. Chromatogr. 21 (1998) 923.
[24] M. Kele, G. Guiochon, in preparation.
[25] H. Engelhardt, M. Nikolov, M. Arangio, M. Scherer, Chromatographia 48 (1998) 183.
[26] L.C. Sander, S.A. Wise, Anal. Chem. 67 (1995) 3284.
[27] Q.-H. Wan, M.C. Davies, P.N. Shaw, D.A. Barrett, Anal. Chem. 68 (1996) 437.
[28] H. Engelhardt, T. Lobert, Anal. Chem. 71 (1999) 1885.
[29] G. Guiochon, S.G. Shirazi, A.M. Katti, Fundamentals of Preparative and Nonlinear Chromatography, Academic Press, Boston, MA, 1994.