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Repeatability and reproducibility of high-concentration data in reversed-phase liquid chromatography III. Isotherm reproducibility on Kromasil C_{18}

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Abstract

Single component equilibrium isotherms of six compounds (aniline, caffeine, ethylbenzene, phenol, propranolol, and theophylline) were determined by the inverse method on 10 Kromasil-C_{18} columns, using water–methanol solutions as the mobile phase. This method offers an economic and fast isotherm determination by means of the overloaded band profiles of the compounds. Five out of the ten columns used in this test come from the same batch whilst the other five columns represent five additional batches. Statistical evaluation was used to assess the reproducibility of the isotherm parameters. We found that the column-to-column reproducibility of the isotherm parameters is of the same magnitude as the batch-to-batch reproducibility (with the exception of one outlier column). In most of the cases, the reproducibilities of the saturation capacities and that of the retention factors are excellent, they are typically between 1.2 and 3%, and very often below 2%. Within the limits of the experimental precision, these results agree with those obtained earlier, using a conventional method of isotherm determination.

Keywords: Repeatability; Reproducibility; Adsorption isotherms; Aniline; Caffeine; Ethylbenzene; Phenol; Propranolol; Theophylline

1. Introduction

The reproducibility of reversed-phase packing materials has drawn considerable attention recently. In a most thorough, rigorous series of studies, Kele and Guiochon determined the repeatability and reproducibility of the most important retention and efficiency parameters for thirty compounds on four commercial silica-based C_{18} stationary phases [1–5] and on a monolithic reversed phase [6]. They observed that nowadays the reproducibility of silica-based C_{18} packing materials is quite remarkable. For instance, the relative standard deviation (R.S.D.) of the retention factors is usually of the order of 0.15–1.5% for column-to-column and 1.3–3.0% for batch-to-batch reproducibility, depending mostly on the basicity of the solute considered. Investigations of the production and testing of reversed-phase packing materials confirm that manufacturers have been providing stationary phases with stable physical chemical and chromatographic properties [7,8].

The most important parameters that affect the reproducibility of retention data were identified by Felinger et al. [9]. They found that the reproducibility of the retention times are mostly determined by the fluctuation of the total column volume (i.e., essentially of its inner diameter), whereas the phase ratio has the most important influence on the reproducibility of the retention factors. Most of the data available regarding the reproducibility of chromatographic data are determined with analytical-size columns, under linear conditions. These data, however, cannot necessarily be extrapolated to overloaded conditions, where the nonlinear behavior of the chromatographic process controls the migration of the solute bands and the evolution of their profiles. Furthermore, in preparative chromatography, large diameter columns are often used and their dynamics can be profoundly different from that of analytical-scale columns. Recently, Laub studied the column-to-column reproducibil-

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ity of the retention time and of the efficiency parameters on reversed-phase and chiral stationery phases packed into axial compression columns with inner diameter of 25 mm or 50 mm [10]. A reproducibility better than 2% for retention times, and better than 5% for column efficiency was achieved.

In a previous study, the adsorption isotherm of six low molecular weight test compounds were measured by frontal analysis (FA) on one Kromasil-C_{15} column [11]. Overloaded band profiles of the test compounds were determined on nine additional columns and were compared to the band profiles calculated using the isotherm parameters determined on the first column. The columns were evaluated after the differences between the positions and the shapes of the experimental and the calculated band profiles.

In this study, we determine the equilibrium isotherm of each test compound on every column and calculate the reproducibility of each isotherm parameter. With the experimental set-up used in [11], this would have required the determination of 70 equilibrium isotherms, a task that would have lasted more than half-a-year. This task can be efficiently achieved via the inverse method of isotherm determination. The inverse method was developed recently [12–14]. It derives the adsorption isotherm from a single overloaded band profile. The inverse method can be used to determine the single component isotherm of a pure compound [15] or the competitive isotherms of mixtures [16,17]. The equilibrium isotherms are determined by numerically integrating a proper model of nonlinear chromatography and by tuning the values of the isotherm parameters to minimize the difference between the calculated and the measured band profiles.

2. Theory

2.1. The inverse method

The inverse method of isotherm determination estimates the parameters of an a priori selected isotherm model from the profiles of overloaded elution bands. Overloaded band profiles are calculated with a properly chosen model of nonlinear chromatography, then the measured and the calculated band profiles are compared by evaluating the following objective function:

$$\min \sum_i (C_i^{\mathrm{sim}} - C_i^{\mathrm{meas}})^2 \tag{1}$$

where C_i^{sim} and C_i^{meas} are the calculated and the measured concentrations at point i. At the end of each loop, the isotherm parameters are changed to minimize the objective function, using an optimization routine.

The equilibrium-dispersive model of chromatography can be employed for the modeling of many nonlinear separations [18], particularly when low molecular weight compounds of moderate polarity are used. In this model, we assume constant equilibrium between the stationary and the mobile phases and use an apparent dispersion term to account for the band broadening effects of both axial dispersion and the finite rate of the mass transfer kinetics. The following mass balance equation is written for the solute:

$$\frac{\partial C}{\partial t} + F\frac{\partial q}{\partial t} + u\frac{\partial C}{\partial z} = D_{\mathrm{a}}\frac{\partial^2 C}{z^2} \tag{2}$$

where C and q are the concentrations of the solute in the mobile and the stationary phases, respectively, z the length, t the time, u the superficial linear velocity of the mobile phase, and F is the phase ratio, with $F = (1-\varepsilon_t)/\varepsilon_t$, where ε is the total porosity of the column. The total porosity of the column is defined as the ratio of the mobile phase volume to the total column volume. D_{a} is the apparent dispersion coefficient that can be calculated from the number of theoretical plates (N) determined by an analytical injection:

$$D_{\mathrm{a}} = \frac{uL}{2N} \tag{3}$$

where L is the column length.

Because methanol is very weakly adsorbed (the retention factor of methanol in pure water is of the order of unity) on the columns, it is legitimate to consider the mobile phase as if it were a pure compound, i.e., not to use a mass balance for this organic modifier (at the concentrations used here, the saturated monolayer is formed), and to consider the equilibrium isotherms of the solutes as those of single components not as binary isotherms [18].

The initial condition $C(z, 0) = 0$ states that at $t = 0$ the column is equilibrated with the pure mobile phase. The boundary condition is given by the inlet concentration profile. The true inlet profile can be determined by eliminating the column from the instrument and performing a large-volume injection. Ideally, the concentration profile is a rectangular impulse but in reality it is smoothed by dispersion effects that occur in the extra-column volumes of the instrument.

The mass balance equation with the proper isotherm equation is to be integrated numerically to obtain the concentration profiles at the column outlet.

2.2. Models of single component isotherms

The isotherm models used in this study are the ones that were selected in a previous study [11], on the basis of the adsorption energy distributions determined by the expectation maximization method from frontal analysis data.

2.2.1. Langmuir isotherm

The Langmuir isotherm is the most commonly used isotherm to model adsorption. It assumes a homogeneous surface characterized by a single value of the adsorption energy.

$$q = \frac{q_{\mathrm{s}} bC}{1 + bC} \tag{4}$$

where q_s is the saturation capacity and b is the equilibrium constant.

2.2.2. Jovanović isotherm

Whereas most isotherm models assume a local Langmuirian behavior, the Jovanović isotherm is different [19]. The Jovanović isotherm is a local isotherm, in the form of $q = 1 - e^{-bC}$ and the global isotherm for a homogeneous surface is written as:

$$q = q_s(1 - e^{-bC}) \tag{5}$$

where q_s is the saturation capacity and b is the equilibrium constant.

2.2.3. BiLangmuir isotherm

The biLanguir isotherm is an extension of the Langmuir equation to a two-site heterogeneous surface. Two different types of adsorption sites are assumed to be present on the surface of the adsorbent. Either adsorption site is characterized by a unique adsorption energy.

$$q = \frac{q_{s,1}b_1C}{1 + b_1C} + \frac{q_{s,2}b_2C}{1 + b_2C} \tag{6}$$

where the saturation capacity of the analyte of site i is and its $q_{s,i}$ equilibrium constant is b_i.

2.2.4. Tóth isotherm

The Tóth isotherm is another extension of the Langmuir isotherm toward heterogeneous surfaces, but with a unimodal energy distribution.

$$q = q_s\frac{bC}{[1 + (bC)^\nu]^{1/\nu}} \tag{7}$$

where q_s is the saturation capacity, b the equilibrium constant, and ν is the heterogeneity parameter. When $\nu = 1$, the Tóth isotherm reduces to the Langmuir isotherm. The Tóth equation is widely used to characterize adsorption on heterogeneous surfaces [20].

2.2.5. Quadratic isotherm

Statistical thermodynamical considerations lead to an isotherm equation which is the ratio of two polynomials of the same degree [21]. The quadratic isotherm is obtained with second-order polynomials.

$$q = q_s\frac{b_1C + 2b_2C^2}{1 + b_1C + b_2C^2} \tag{8}$$

The saturation capacity is $2q_s$, b_1 and b_2 are the equilibrium constants. When the numerical coefficients meet certain condition, this isotherm has an inflection point. Note that the biLangmuir isotherm is also a quadratic isotherm (with a denominator that has two negative roots).

2.2.6. The extended liquid–solid BET isotherm

The classical multilayer adsorption isotherm of Brunauer, Emmett, and Teller was extended to liquid solid adsorption [22]. It is given by the following expression:

$$q = q_s\frac{b_sC}{(1 - b_LC)(1 - b_LC + b_sC)} \tag{9}$$

where q_s is the monolayer saturation capacity, b_s the equilibrium constant between the analyte and the surface and b_L is the adsorption equilibrium constant of the analyte in the adsorbed layers.

3. Experimental

A Hewlett-Packard 1090 liquid chromatography system (Agilent Technologies, Palo Alto, CA, USA), equipped with a multisolvent delivery system, an automatic injector with a 22 μl sample loop, a column thermostat, a diode array detector, and a computer data station, was used for all experiments.

The mobile phase used in this study was a mixture of HPLC-grade methanol and water, both purchased from Fisher Scientific (Fair Lawn, NJ, USA). Uracil, aniline, phenol, caffeine, theophylline, propranolol, ethylbenzene, acetic acid and sodium acetate were obtained from Aldrich (Milwaukee, WI, USA). The composition of the mobile phase was adjusted for each solute studied, in order to provide a convenient retention.

Ten manufacturer-packed Kromasil-C_{18} columns (Eka nobel, Bohus, Sweden) were used in this study. These are C_{18}–silica bonded, endcapped columns. The same columns were used by Kele and Guiochon [3], under linear conditions, to study reproducibility and repeatability. The physical properties of these columns are summarized in Tables 1 and 2 of [11]. The repeatability of the overloaded elution profiles obtained on one given column was already assessed and so was the reproducibility of the elution profiles on the set of columns studied here [11]. The reproducibility of the retention data of thirty different compounds on these columns was also investigated in detail [3]. In view of these results, the repeatability of the data obtained on each column was not studied here, only the column-to-column reproducibility which can only be less good.

4. Results and discussion

4.1. Inlet profile

The inlet profile constitutes the boundary condition of the differential mass balance equation, thus it should be accurately modeled. Ideally, the shape of the inlet profile should be a rectangle but, in practice, significant deviations from this profile are almost always observed [17]. In the sample loop and in the capillaries connecting the injection port and the column, dispersion takes place, enhanced by the consequences of the Hagen–Poiseuille velocity profile in a tube.

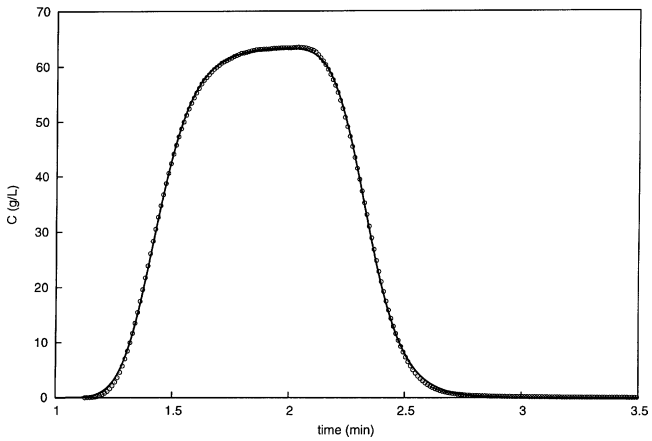

Fig. 1. Plot of the inlet concentration profile for $t_p = 0.9$ mm injection of aniline.

Thus, the inlet concentration profile is not a pulse with sharp boundaries.

The exponentially modified Gaussian (EMG) function is often used in chromatography, to model asymmetrical peaks [23]. Its application, however, is usually not related to the physical origin of the model. It is the convolution of a Gaussian peak—describing the band broadening in the connecting tubes—and an exponential decay function—modeling mixer-type extra volumes. Thus, the EMG model is a proper choice to model the overall effect of different extra-column band broadening effects. In case of a large-volume injection, the true inlet concentration can be modeled by the convolution of the EMG function and a rectangular pulse. The resulting profile is [17]:

$$C(t) = \frac{1}{2a}\left\{\mathrm{erfc}\frac{m-t}{\sqrt{2\sigma}} - \mathrm{erfc}\frac{t_p+m-t}{\sqrt{2\sigma}} + \exp\left(\frac{\sigma^2}{2\tau^2}+\frac{m-t}{t}\right) \times \left[e^{t_p/\tau}\mathrm{erfc}\left(\frac{\sigma}{\sqrt{2\tau}}+\frac{t_p+m-t}{\sqrt{2\sigma}}\right) - \mathrm{erfc}\left(\frac{\sigma}{\sqrt{2\tau}}+\frac{m-t}{\sqrt{2\sigma}}\right)\right]\right\} \quad (10)$$

where m is the residence time in the connecting tube, σ the Gaussian band width and τ the time constant of the mixer-type extra-column volume and t_p is the injection time.

The measured inlet concentration profile for the injection of aniline is reported in Fig. 1 (symbols). The fitted model described in Eq. (10) (solid lines) follows remarkably well the measured concentration profile. The inlet profile has been recorded for every analyte and the parameters of Eq. (10) were determined by nonlinear curve fitting. The parameters are summarized in Table 1 for all the analytes. We can see that the numerical values vary slightly between the different compounds.

Table 1
The parameters of Eq. (10) describing the injection profile

	t_p (min)	m (min)	σ (min)	τ (min)
Aniline	0.9038	1.349	0.0946	0.0968
Caffeine	0.9046	1.336	0.1105	0.1282
Ethylbenzene	0.9004	1.368	0.0820	0.0924
Phenol	0.9040	1.350	0.0927	0.0905
Propranolol[a]	0.8984	1.343	0.1083	0.1130
Propranolol[b]	0.9085	1.325	0.1143	0.1287
Theophylline	0.8838	1.356	0.1012	0.1034

[a] Without buffer.
[b] With acetate buffer.

4.2. Reproducibility of the isotherm parameters

The choice of a proper equilibrium isotherm model for each analyte was facilitated by the use of the adsorption energy distributions previously calculated from the isotherm data determined on column I [11]. In this study, we applied the same isotherm models in most calculations. However, to assess the effect that the choice of an incorrect isotherm model itself could have on the reproducibility of the parameters, we used additional isotherm models for two of the compounds studied. Figs. 2–8 compare the high-concentration band profiles recorded and those calculated using the best isotherm parameters supplied by the inverse method.

4.2.1. Caffeine

The adsorption behavior of caffeine on the Kromasil-C_{18} columns was modeled with a biLangmuir isotherm. The experimental band profiles and the calculated ones obtained with the best-fit isotherm parameters are plotted in Fig. 2 for the 10 columns used in this study. The chromatograms plotted in Fig. 2 demonstrate that the agreement between the experimental and the calculated bands is remarkably good. This confirms that the combination of the equilibrium-dispersive model and the biLangmuir isotherm can be considered as providing an accurate model of chromatography for the nonlinear process considered.

The numerical results characterizing the reproducibility of the biLangmuir isotherm parameters are summarized in Table 2. For the statistical analysis of the data, we chose two tools of multivariate statistics: principal component analysis (PCA) and cluster analysis (CA). The results of the multivariate statistical analysis are plotted in Figs. 9 (PCA) and 10 (CA). In the statistical analysis of the data of the caffeine isotherm, the four parameters of the biLangmuir isotherm ($q_{s,1}$, $q_{s,2}$, b_1, b_2) were used. The average values of these parameters and their relative standard deviation are reported in the first four columns of Table 2.

Both PCA and CA were carried out on a data set organized in a 10×4 matrix (four isotherm parameters determined on 10 chromatographic columns). The PCA revealed that the first two factors explain 99% of the variance within the data set (see Fig. 9). The positions of the 10 columns in the space of the two factors show two outliers: columns IX and

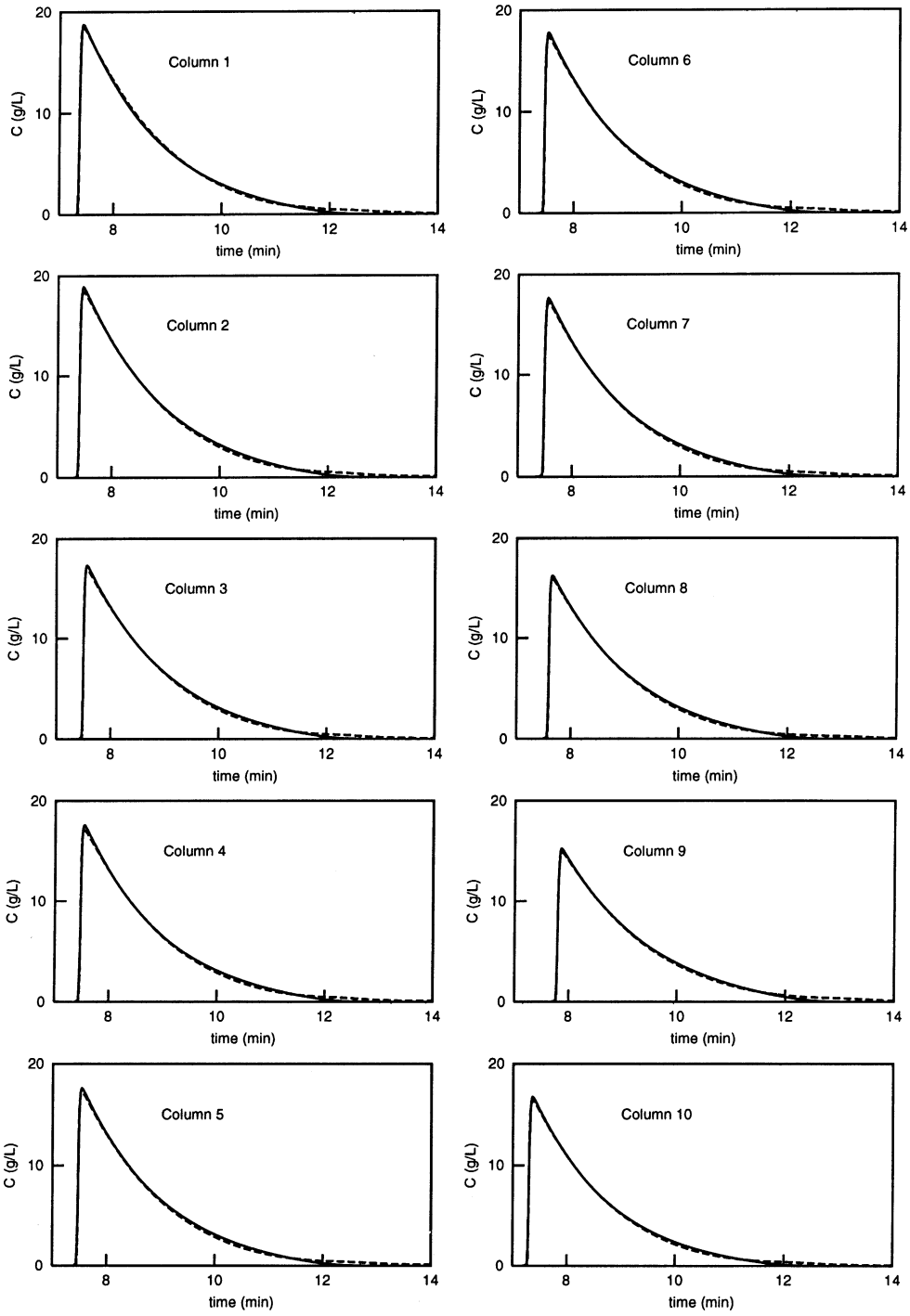

Fig. 2. Comparison between the experimental (dashed lines) and calculated (solid line) band profiles of caffeine.

X.[2] The retention times of the shock in Fig. 2 also suggest that columns IX and X differ from the rest of the set. The variation along the axis of the first factor is much larger than that along the second one (86% versus 13%). Thus, column X is much more different from columns I to VIII than column IX. The results of the cluster analysis show a similar conclusion: columns I–VIII form a homogeneous group from which column IX differs somewhat, the distance of column X from all other columns being by far the largest.

As columns I–V were packed with stationary phase from the same batch, we would expect that columns I–V in Figs. 9 and 10 form a subset, but it is not so. Neither PCA nor

[2] Note that, consistent with the terminology used in previous reports [3,11], we identify the columns in the text with roman numerals. This, however, would cause confusion in Figs. 9–16 because symbols often overlap. Therefore, the columns are identified with the corresponding Arabic numerals in the figures.

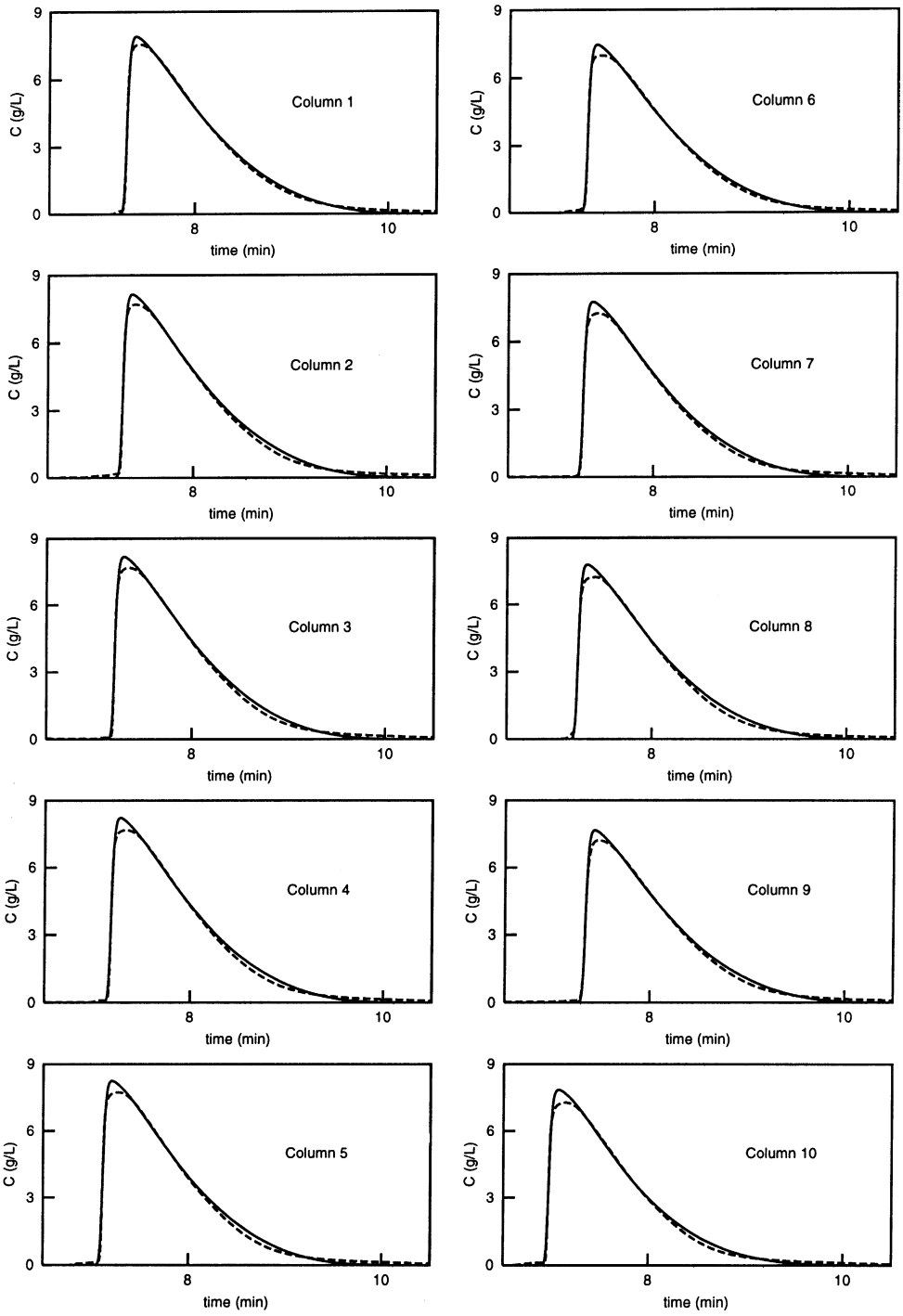

Fig. 3. Comparison between the experimental (dashed lines) and calculated (solid line) band profiles of theophylline.

CA show that the column-to-column reproducibility is better than the batch-to-batch reproducibility when the two outlier columns are not considered.

In Table 2, we report the R.S.D. of the isotherm parameters for different subsets of the original data. Besides the four isotherm parameters, the R.S.D. of some additional terms are also calculated. The a terms ($a_i = q_{s,i}, b_i$) can be considered as the contribution of the individual sites to the retention factor under analytical conditions. The R.S.D. of the total saturation capacity ($q_{s,1} + q_{s,2}$) is also calculated.

The R.S.D. of the isotherm parameters is not better for columns I–V than for columns I–VIII. It is remarkable, however, that the batch-to-batch reproducibility of the isotherm parameters is excellent; for most of the parameters, the R.S.D. is between 1 and 2%. The only exception is the saturation capacity of the less abundant site. Note, however, that these stronger sites account for only about 6% of the total

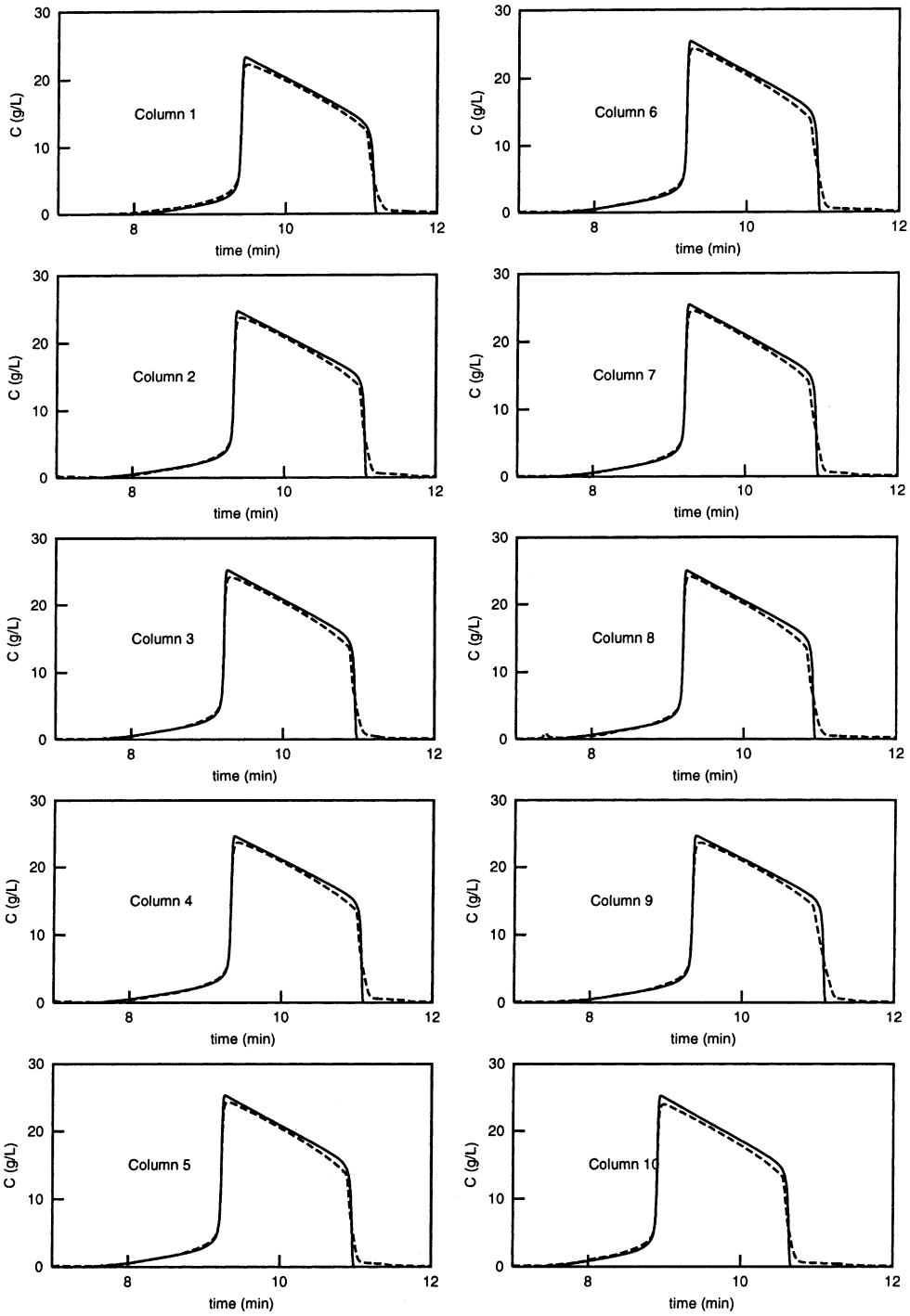

Fig. 4. Comparison between the experimental (dashed lines) and calculated (solid line) band profiles of propranolol with no buffer in the mobile phase.

saturation capacity of the stationary phase, so they cannot be characterized as precisely as the other parameters. A comparison with the frontal analysis data determined for column I (reported in the first line of Table 2) show that there is a significant difference between the $q_{s,2}$, values determined by FA and those derived by the inverse method. Again, this is due to the fact that the less abundant sites represent only a small fraction of the total sites.

The reproducibility of the retention parameters of the compounds studied here was determined by Kele and Guiochon on the same Kromasil-C_{18} columns [3] under strictly linear conditions. They found that the R.S.D. of the retention factors are typically 0.54–0.69% for the column-to-column reproducibility and 2.2–3.1% for the batch-to-batch reproducibility. The retention factors are proportional to the value of the a terms of the isotherms. For a biLangmuir isotherm,

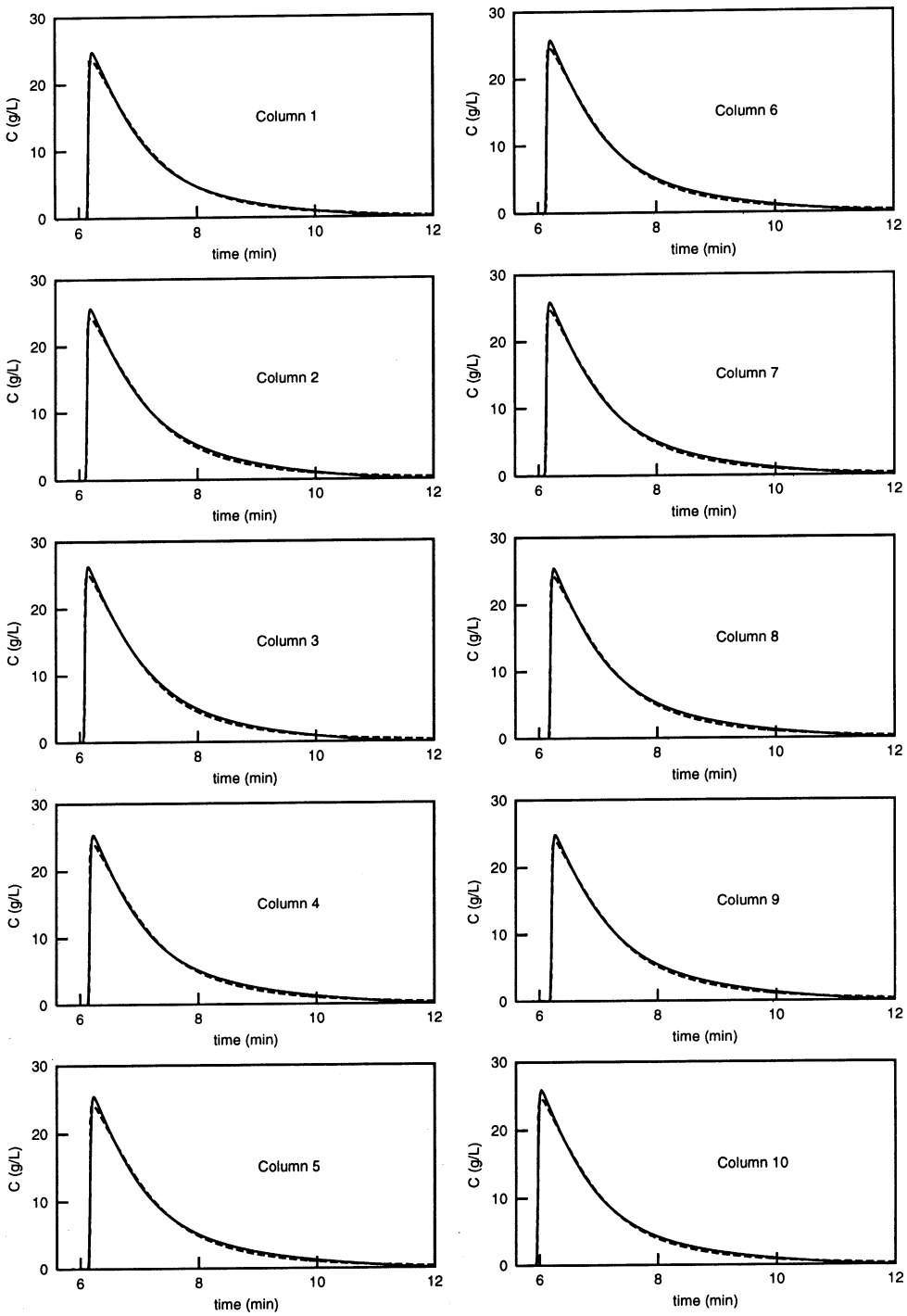

Fig. 5. Comparison between the experimental (dashed lines) and calculated (solid line) band profiles of propranolol with acetate buffer in the mobile phase.

we have $a = a_1 + a_2$ and $k' = Fa$, where F is the phase ratio. The variation of the $a_1 + a_2$ term determined here under nonlinear conditions shows a 1.12% column-to-column reproducibility and a 2.99% batch-to-batch reproducibility. The reproducibility of the total saturation capacity is 1.24% (column-to-column) and 5.44% (batch-to batch).

Although the experimental technique and the numerical procedure used to obtain the parameters in nonlinear chromatography are both far more complex than in linear chromatography, the reproducibility of the retention parameters is only 30–60% worse under overloaded conditions than under analytical ones which is quite an impressive result on the part of the manufacturer.

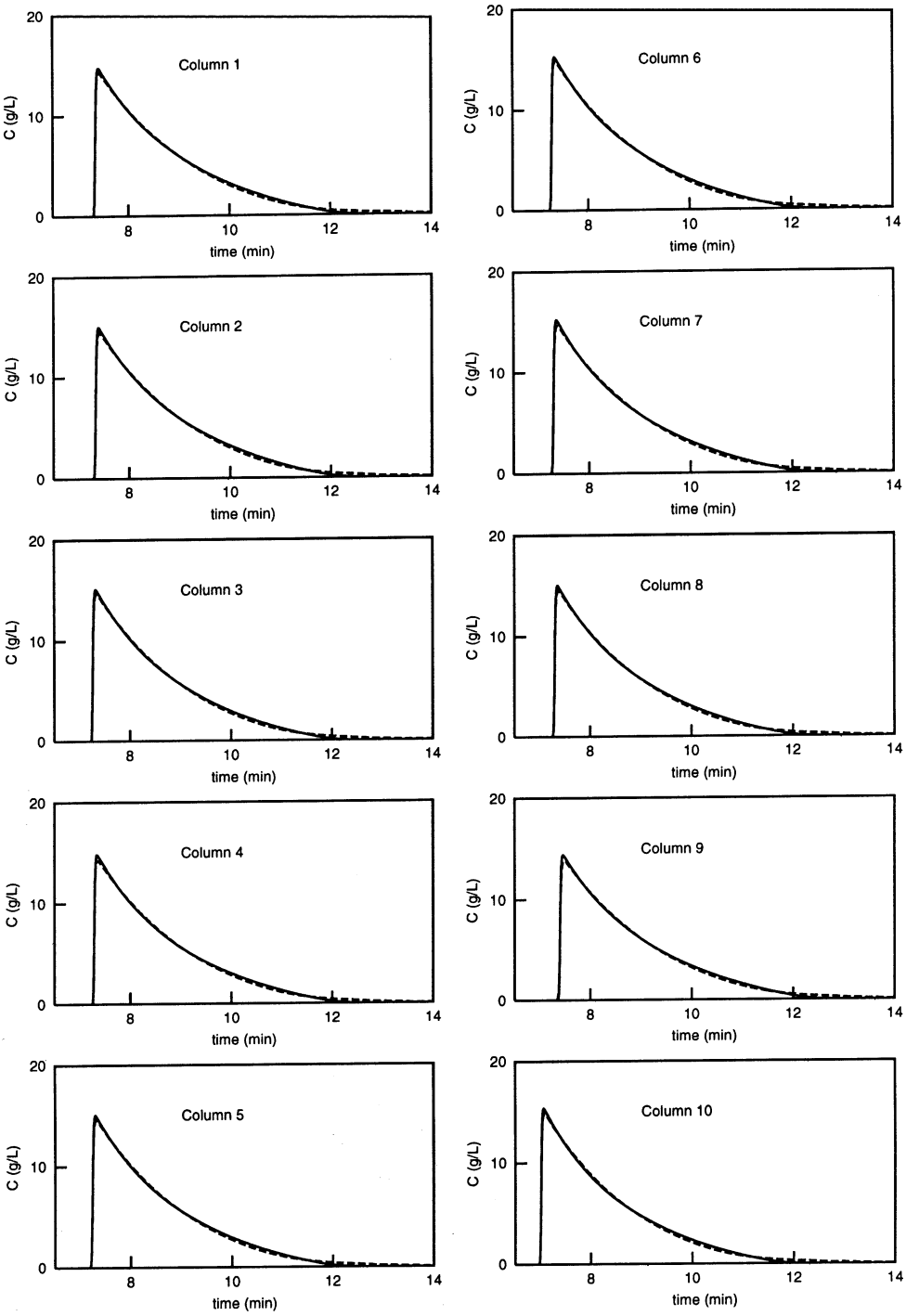

Fig. 6. Comparison between the experimental (dashed lines) and calculated (solid line) band profiles of phenol.

4.2.2. Theophylline

The adsorption of theophylline was modeled with the Tóth isotherm, because a broad unimodal energy distribution was calculated from the frontal analysis isotherm data [11]. The experimental band profiles and those calculated with the best-fit isotherm parameters are plotted in Fig. 3 for the 10 columns used in this study. As in the case of caffeine, we see that the retention times of the front shock layer of the bands on columns IX and X are different from those recorded for the rest of the columns. The PCA of the isotherm parameters (see the upper part of Fig. 11), however, indicates that only column X is an outlier. The retention time difference of the shock layer on column IX compared to those on columns I–VIII can most probably be attributed to the difference between the column total porosities. Since CA does not give significant extra information over PCA, only the results of PCA will be discussed in the following.

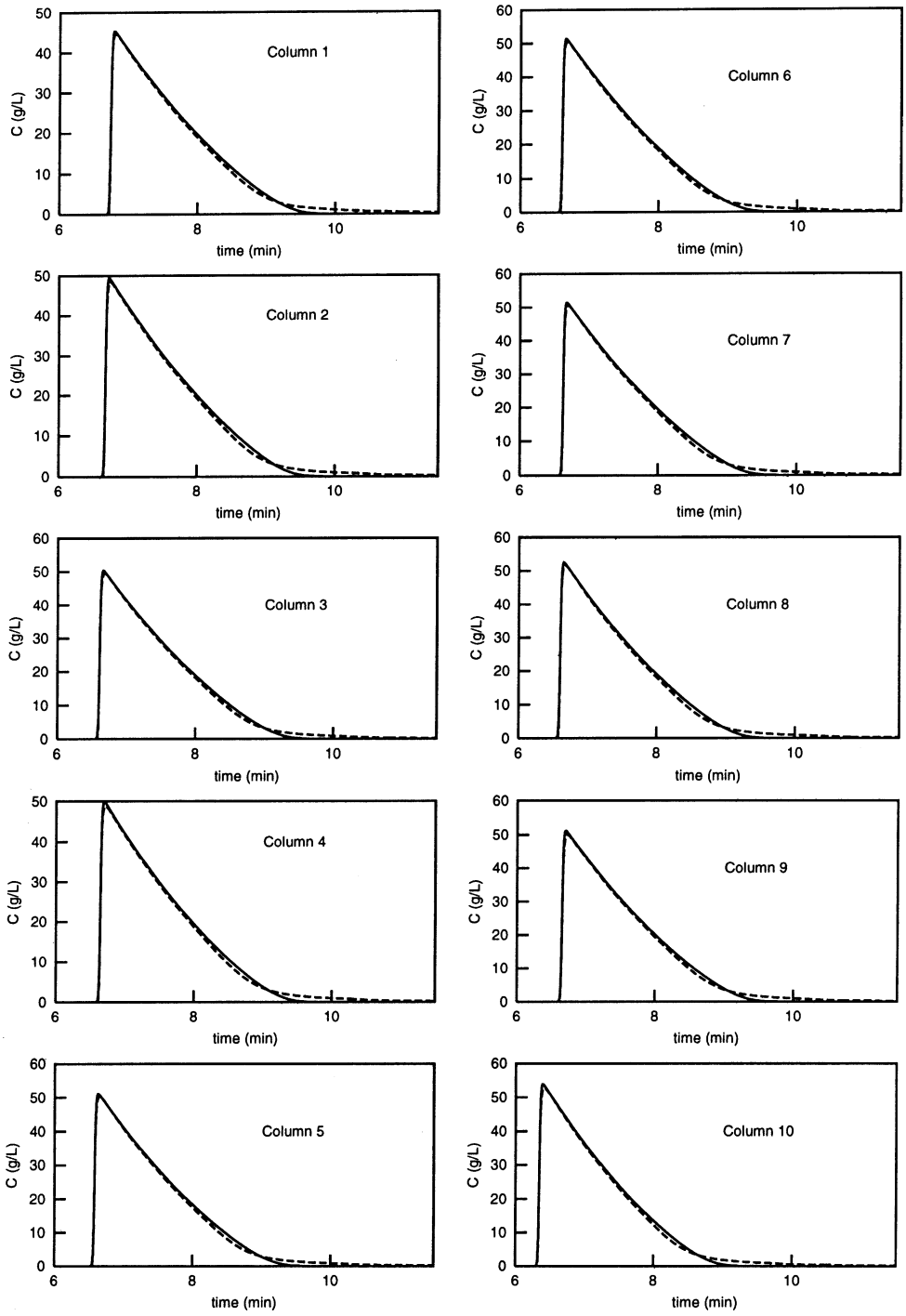

Fig. 7. Comparison between the experimental (dashed lines) and calculated (solid line) band profiles of aniline.

The numerical results of the reproducibility of the Tóth isotherm of theophylline are summarized in Table 3. We can observe again that, after the elimination of the data from column X, the column-to-column reproducibility is not better than the batch-to-batch reproducibility. We can also see in Table 3 that there is a major systematic difference between the parameters of the Tóth isotherm determined by FA or derived by the inverse method. We note, however, that when the isotherm determined by FA was used to calculate the band profiles, the curvatures of the diffuse rears of the experimental and the calculated band profiles differed slightly (see Fig. 13 in [11]). These two observations are probably related.

Since the difference in the shapes of the calculated and experimental band profiles of theophylline was larger than that observed for the caffeine band and since it is inconvenient to fit experimental data to the Tóth isotherm, due to the presence of a variable in the exponent of the model equa-

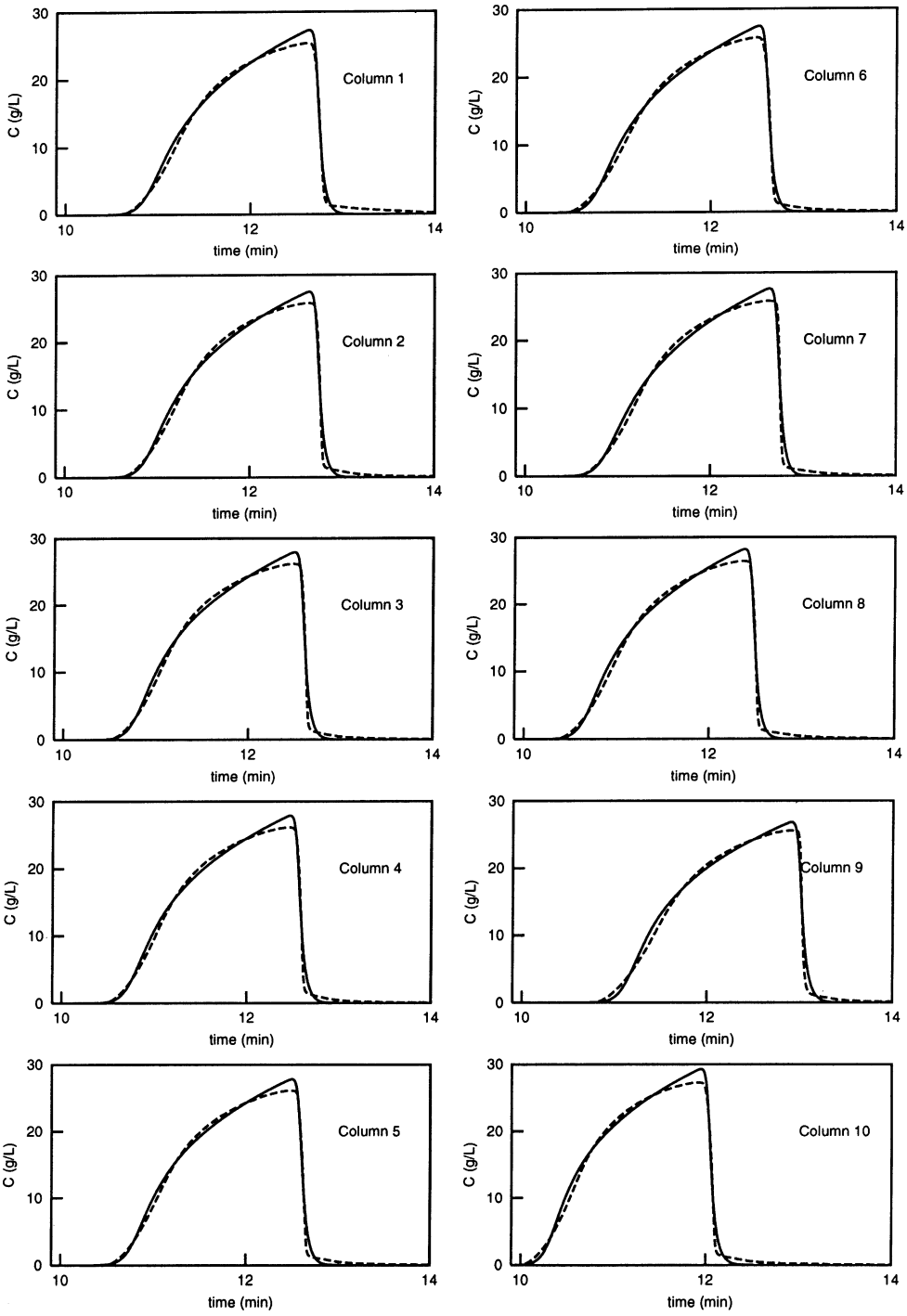

Fig. 8. Comparison between the experimental (dashed lines) and calculated (solid line) band profiles of ethylbenzene.

tion, we fitted the biLangmuir isotherm to the theophylline bands, too. The band profiles calculated with the biLangmuir isotherm fit better the experimental peaks than the ones obtained with the Tóth isotherm, though the peaks calculated with the two isotherms run closer to each other than either of them to the experimental profile.

The PCA data of this fit are plotted in the lower part of Fig. 11, the numerical reproducibility data are given in Table 4. A comparison of the two PCA calculations in Fig. 11 confirms that regardless of the chosen isotherm model, column X is an outlier and the rest of the nine columns form a largely scattered set in which it is impossible to localize the smaller subset of columns I–V. In both cases, the first factor explains 99% of the variation of the isotherm parameters, although the number of isotherm parameters in the Tóth and biLangmuir models are different (three and four). The reproducibility of the a parameter is around 3–4%, that of the saturation capacity is 4–7% de-

Table 2
Reproducibility of the biLangmuir isotherm parameters of caffeine

	$q_{s,1}$ (g/l)	b_1 (l/g)	$q_{s,2}$ (g/l)	b_2 (l/g)	a_1	a_2	$a_1 + a_2$	$q_{s,1} + q_{s,2}$ (g/l)
FA	171.3	0.01612	6.500	0.1903	2.761	1.237	3.998	177.8
I–X								
Mean	169.0	0.01506	11.59	0.1603	2.539	1.842	4.381	180.6
R.S.D. (%)	5.14	4.63	11.2	8.77	2.33	4.50	2.99	5.44
I–IX								
Mean	171.6	0.01487	11.95	0.1562	2.551	1.864	4.416	183.6
R.S.D. (%)	1.47	2.72	5.25	3.59	1.84	2.41	1.71	1.45
I–VIII								
Mean	171.9	0.01476	11.79	0.1579	2.537	1.860	4.398	183.7
R.S.D. (%)	1.45	1.65	3.38	1.47	0.874	2.50	1.29	1.53
I–V								
Mean	170.6	0.01488	11.64	0.1579	2.538	1.837	4.375	182.3
R.S.D. (%)	1.17	1.55	3.17	1.81	1.06	1.87	1.12	1.24

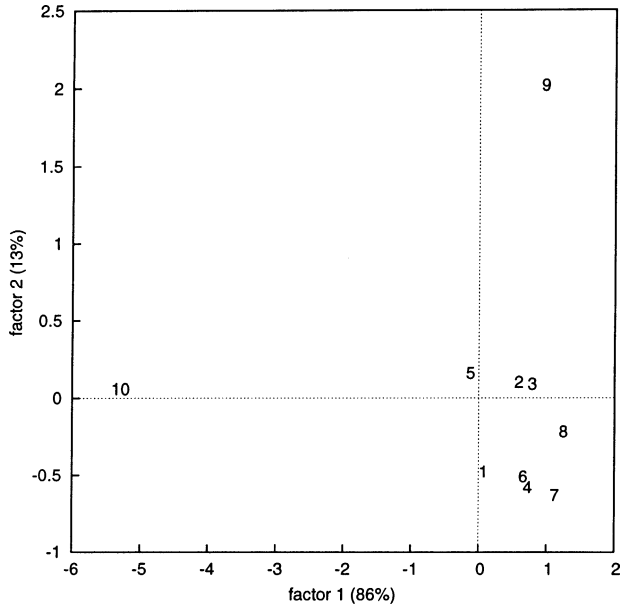

Fig. 9. Score plot of PCA performed on the biLangmuir isotherm parameters of caffeine.

Table 3
Reproducibility of the Tóth isotherm parameters of theophylline

	q_s (g/l)	b (l/g)	ν	a
FA	186.8	0.01367	0.8526	2.554
I–X				
Mean	370.3	0.008076	0.5669	2.914
R.S.D. (%)	19.5	14.6	5.61	2.88
I–IX				
Mean	348.2	0.008436	0.5766	2.932
R.S.D. (%)	5.83	3.82	1.62	2.25
I–V				
Mean	345.2	0.008448	0.5773	2.909
R.S.D. (%)	7.21	4.44	1.52	2.82

pending on the isotherm model and the subset of columns selected.

4.2.3. Propranolol with no buffer in the mobile phase

Under these experimental conditions, the band profiles of propranolol can be well modeled with a quadratic isotherm that is convex downward at low concentrations and becomes convex upward at high concentrations. The experimental band profiles and the bands calculated with the best-fit isotherm parameters are plotted in Fig. 4. The quality of the fit is very good. The results of PCA are plotted in Fig. 12. As we expect, column X is located again far from the rest of the columns, but it is very surprising that, this time, column I is as different from columns II to IX as column X. Although the band profiles plotted in Fig. 4 do not suggest that column I is markedly different from columns II to IX, the saturation capacity of column I is about 5% smaller and the equilibrium constants are about 10% larger than on columns II–IX. Still, the reproducibility of the $a = q_s b_1$ parameter is remarkable (see Table 5): the IISD is 1.72% for column-to-column reproducibility and it is 2.56% for batch-to batch reproducibility. These values for the reproducibility of the saturation capacity are 2.58 and 3.43%, respectively.

4.2.4. Propranolol with acetate buffer in the mobile phase

In the presence of an acetate buffer, the adsorption behavior of propranolol is strongly changed. It shows now a bimodal energy distribution, therefore the overloaded profiles were fitted by means of the biLangmuir model. The experimental and the calculated chromatograms in Fig. 5 show an excellent accuracy. The numerical data, summarized in Table 6, show some unexpected results. The R.S.D. of the isotherm parameters do not exhibit any of the trends found for the other compounds if we calculate them for the different subsets of columns. The elimination of column X resulted in an improved reproducibility for all the other

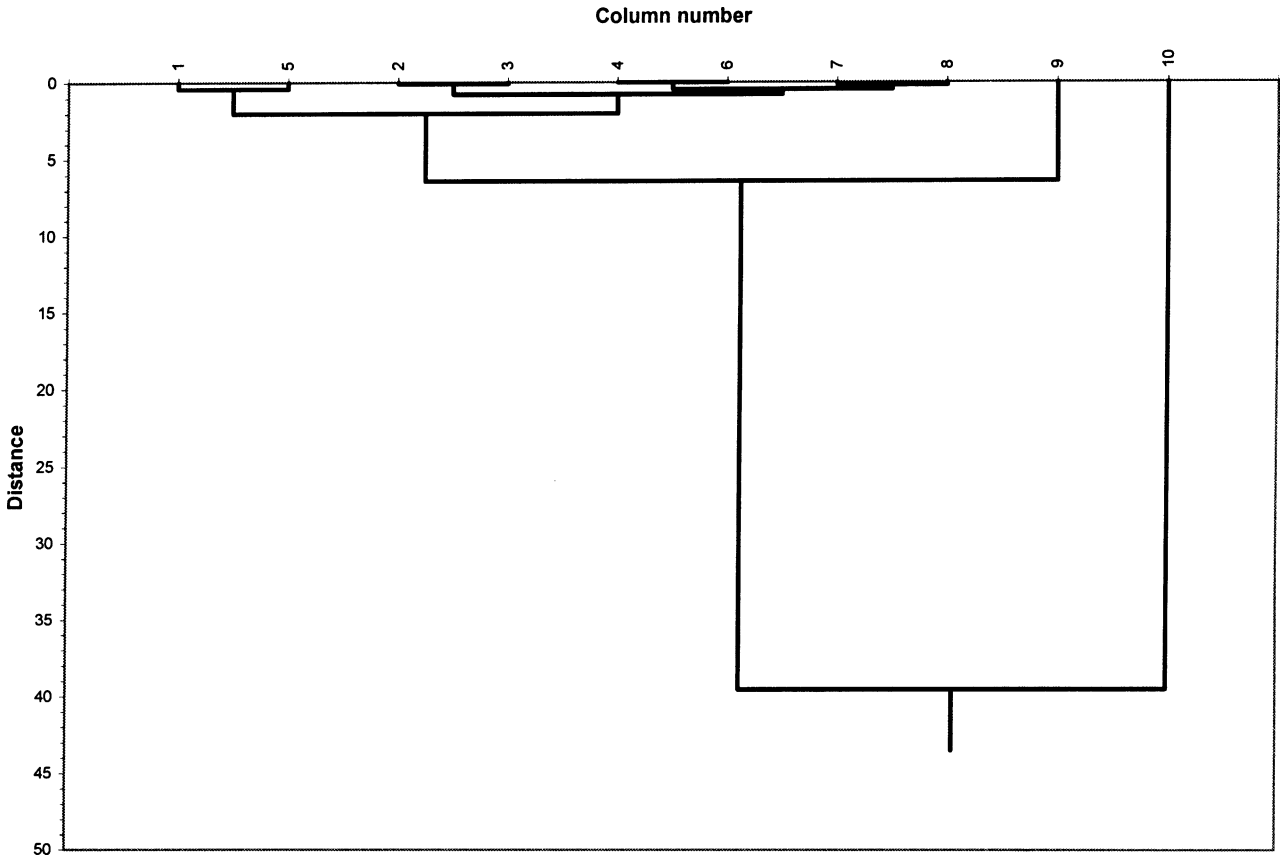

Fig. 10. Complete link dendrogram obtained by cluster analysis of the biLangmuir isotherm parameters of caffeine.

test compounds studied. This is not the case here. Furthermore, the column-to-column reproducibility of some isotherm parameters is worse than their batch-to-batch reproducibility. The scatter plot in Fig. 13 confirms these results. Columns I–V can be found randomly scattered in the whole factor space. No subset can be identified, with the sole exemption of the pair formed by columns IV and V.

4.2.5. Phenol

The adsorption behavior of phenol on Kromasil C_{18} was modeled with the biLangmuir isotherm. The chromatograms

Table 5
Reproducibility of the quadratic isotherm parameters of propranolol with no buffer in the mobile phase

	q_s (g/l)	b_1 (l/g)	b_2 (l/g)	a
FA	90.38	0.03005	0.00129	2.716
I–X				
Mean	92.68	0.02651	0.00129	2.455
R.S.D. (%)	3.43	3.29	6.68	2.56
I–IX				
Mean	93.50	0.02646	0.001296	2.473
R.S.D. (%)	2.09	3.44	6.91	1.35
I–V				
Mean	92.86	0.02674	0.001328	2.481
R.S.D. (%)	2.58	4.44	8.17	1.72

Table 4
Reproducibility of the biLangmuir isotherm parameters of theophylline

	$q_{s,1}$ (g/l)	b_1 (l/g)	$q_{s,2}$ (g/l)	b_2 (l/g)	a_1	a_2	$a_1 + a_2$	$q_{s,1} + q_{s,2}$ (g/l)
I–X								
Mean	0.4087	3.227	98.51	0.02469	1.294	2.429	3.723	98.92
R.S.D. (%)	18.1	15.5	5.62	4.06	11.5	4.30	5.54	5.65
I–IX								
Mean	0.4103	3.270	99.89	0.02463	1.313	2.458	3.771	100.3
R.S.D. (%)	19.1	15.6	3.62	4.26	11.0	2.14	3.89	3.68
I–V								
Mean	0.3980	3.307	99.41	0.02459	1.279	2.442	3.721	99.81
R.S.D. (%)	21.9	17.9	4.07	4.45	8.32	2.61	3.07	4.14

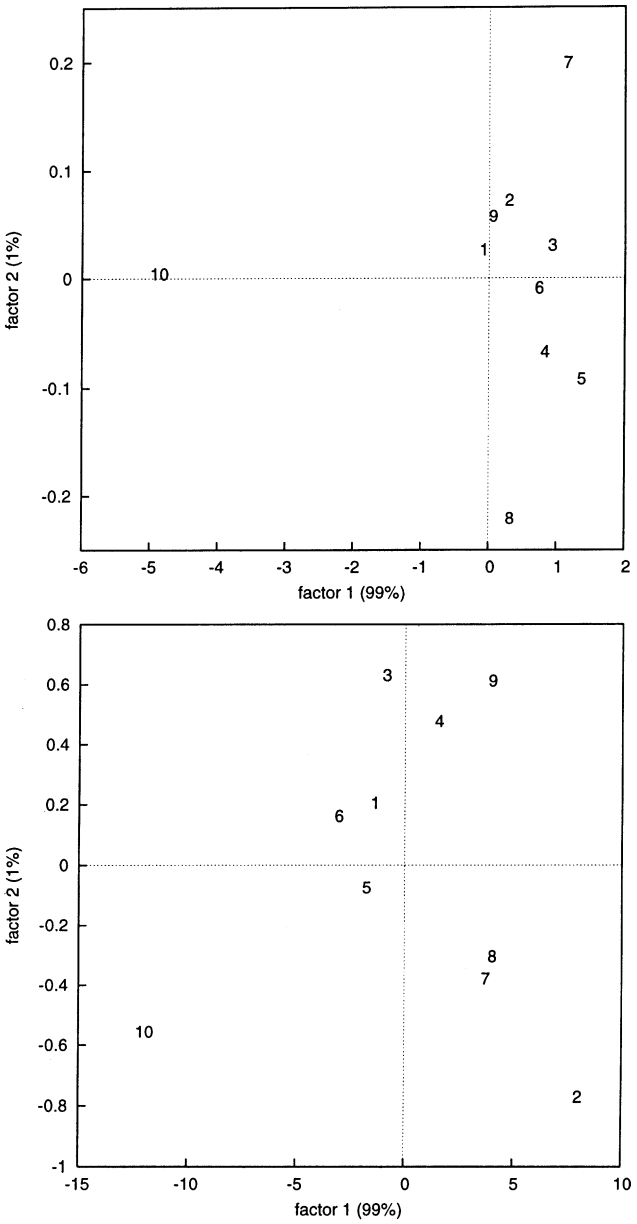

Fig. 11. Score plots of PCA performed on the Tóth and biLangmuir isotherm parameters of theophylline.

are plotted in Fig. 6. The agreement between the experimental and the calculated bands is excellent. The results of PCA (Fig. 14) show that the 10 columns are rather scattered in the space of the first two factors that explain 94% of the variation. The numerical results of the reproducibility of the isotherm parameters (see Table 7) give the impression that out of the several cases when the biLangmuir isotherm was used, the best reproducibility of every parameter is achieved with phenol. This is due to the fact that saturation capacities of the two sites are closer than in the case of caffeine, theophylline, or propranolol with acetate buffer. The biLangmuir isotherm of phenol shows that the saturation capacity of the weak sites is approximately four-fold

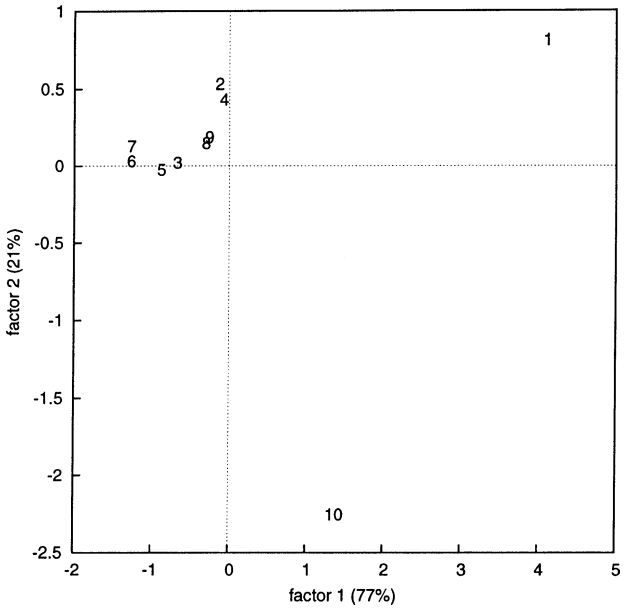

Fig. 12. Score plot of PCA performed on the quadratic isotherm parameters of propranolol with no buffer in the mobile phase.

larger than that of the strong sites. For the other analytes, this ratio is 15-fold or even 250-fold. Since, in the case of phenol, both sites have significant contribution to the overall adsorption isotherm, the individual saturation capacities and equilibrium constants can be estimated with a good precision. The column-to-column reproducibility of the *a* parameter is 1.25% and that of the saturation capacity is 2.37%. These values for the batch-to-batch reproducibility are 3.0 and 4.2%, respectively.

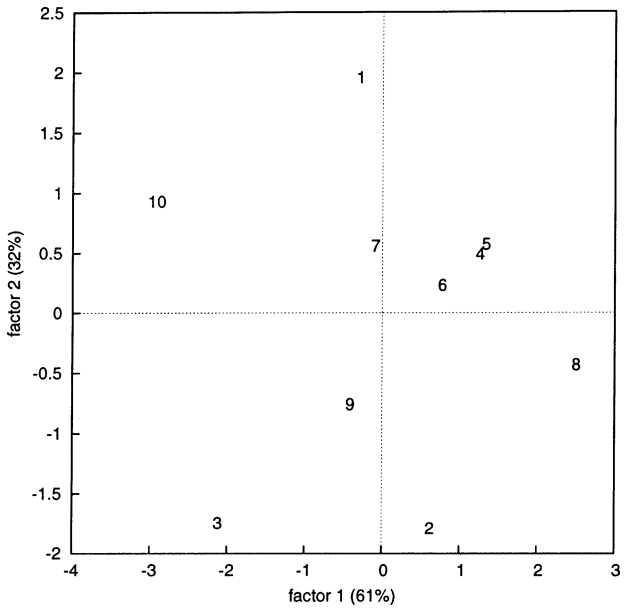

Fig. 13. Score plot of PCA performed on the biLangmuir isotherm parameters of propranolol with acetate buffer in the mobile phase.

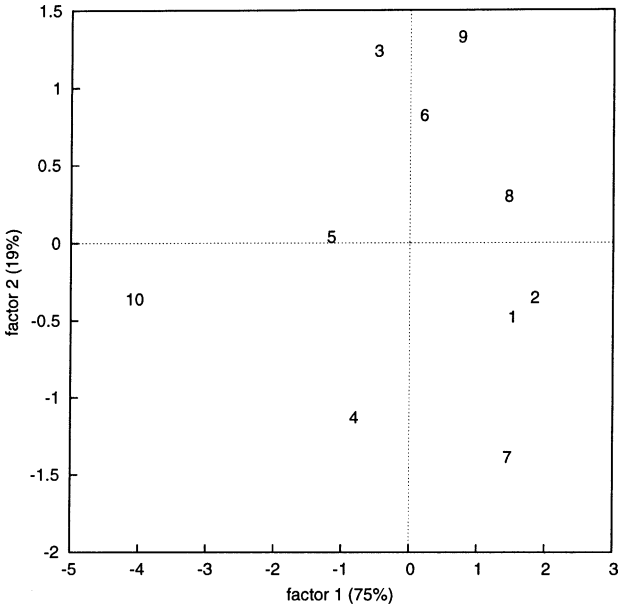

Fig. 14. Score plot of PCA performed on the biLangmuir isotherm parameters of phenol.

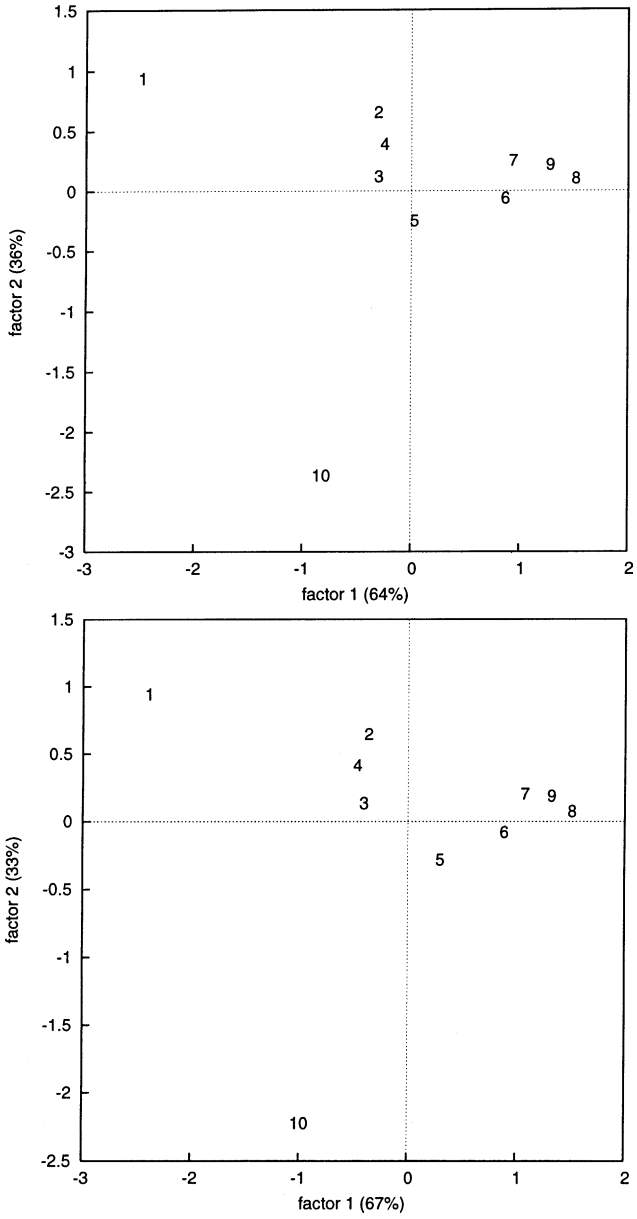

Fig. 15. Score plots of PCA performed on the Jovanović and Langmuir isotherm parameters of aniline.

4.2.6. Aniline

Two isotherm equations were selected to model the adsorption of aniline: the Jovanović and the Langmuir equations. Both models assume homogeneous surfaces, they differ in their local isotherms. Furthermore, both models contain the same two parameters to fit, the saturation capacity and the equilibrium constant.

The experimental and the calculated chromatograms of the 10 columns are plotted in Fig. 7. In these figures, however, only the chromatograms calculated with the Jovanović isotherm are plotted. The chromatograms calculated with the Langmuir isotherms are indistinguishable from them at this scale, although the goodness-of-fit is slightly better with the Langmuir isotherm.

The results of PCA are plotted in Fig. 15. The upper part, which shows the results obtained with the Jovanović isotherm is almost completely identical to the scatter plot obtained with the Langmuir isotherm. The similarity of the results obtained with the two isotherm models is much more pronounced than in the case of theophylline. Remember, however, that the theophylline adsorption was modeled by two completely different isotherms: the Tóth and the biLangmuir models that have quite different affinity energy distributions, the former a broad unimodal, the latter a bimodal energy distribution with narrow modes. These isotherms also differ in the number of their parameters. It is interesting to observe that, besides column X, column I is again an outlier, regardless of which isotherm was used to model the adsorption of aniline. As Tables 8 and 9 show, the reproducibility values obtained with the two isotherms are very close to each other. The column-to-column reproducibility of the a parameter is about 2.3%, its batch-to-batch reproducibility is 4.3%, regardless of the isotherm chosen. If the outlier column I is eliminated from the calculation, the column-to-column reproducibility of the a parameter becomes 2.0%. The column-to-column reproducibility of the saturation capacity is about 1.7%, its batch-to-batch reproducibility is 3.7%. For columns II–V, the column-to-column reproducibility of the saturation capacity is an exceptionally good 0.6%.

4.2.7. Ethylbenzene

The BET isotherm was used to model the adsorption behavior of ethylbenzene. The chromatograms plotted in Fig. 8 show that the retention times on columns IX and X differ

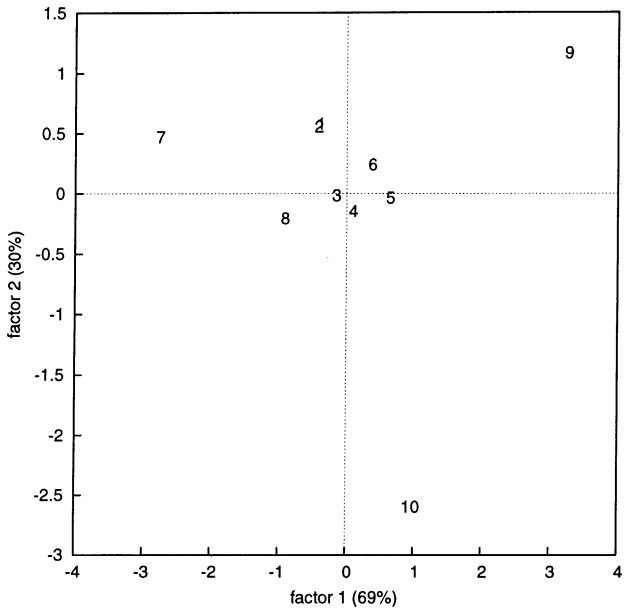

Fig. 16. Score plot of PCA performed on the BET isotherm parameters of ethylbenzene.

from those measured for the rest of the columns. The scatter plot in Fig. 16 confirms that these columns, together with column VII, differ from columns I to VI. The reproducibility of the isotherm parameters is remarkable (see Table 10).

Table 8
Reproducibility of the Jovanović isotherm parameters of aniline

	q_s (g/l)	b (l/g)	a
FA	183.5	0.0129	2.367
I–X			
Mean	166.5	0.01449	2.412
R.S.D. (%)	3.58	3.81	4.33
I–IX			
Mean	168.0	0.01455	2.442
R.S.D. (%)	2.39	3.78	1.84
I–V			
Mean	165.2	0.01489	2.458
R.S.D. (%)	1.79	3.51	2.27

The column-to-column reproducibility of the a parameter is 1.08%, its batch-to-batch reproducibility is 3.56%. The column-to-column reproducibility of the saturation capacity is 1.79%, its batch-to-batch reproducibility is 4.43%. The reproducibility of the equilibrium constants is also excellent. On the basis of the isotherm parameters of ethylbenzene, we are able to identify the subset of the five columns coming from the same batch. They are in the close center of the scatter plot, together with column VI that shows very similar properties.

Table 6
Reproducibility of the biLangmuir isotherm parameters of propranolol with acetate buffer in the mobile phase

	$q_{s,1}$ (g/l)	b_1 (l/g)	$q_{s,2}$ (g/l)	b_2 (l/g)	a_1	a_2	$a_1 + a_2$	$q_{s,1} + q_{s,2}$ (g/l)
FA	152.7	0.00975	7.641	0.1905	1.489	1.456	2.945	160.4
I–X								
Mean	125.0	0.01169	8.609	0.2538	1.458	2.179	3.637	133.6
R.S.D. (%)	5.27	5.38	8.10	6.43	2.82	6.95	3.97	5.26
I–IX								
Mean	126.5	0.01162	8.717	0.2509	1.468	2.184	3.652	135.2
R.S.D. (%)	3.75	5.39	7.39	5.70	1.94	7.32	3.98	3.73
I–V								
Mean	125.8	0.01166	8.550	0.2503	1.463	2.136	3.600	134.3
R.S.D. (%)	4.60	6.31	8.81	7.39	1.82	9.62	5.11	4.46

Table 7
Reproducibility of the biLangmuir isotherm parameters of phenol

	$q_{s,1}$ (g/l)	b_1 (l/g)	$q_{s,2}$ (g/l)	b_2 (l/g)	a_1	a_2	$a_1 + a_2$	$q_{s,1} + q_{s,2}$ (g/l)
FA	128.3	0.00993	38.70	0.06338	1.274	2.453	3.727	167.0
I–X								
Mean	124.0	0.01082	31.25	0.08642	1.342	2.697	4.039	155.3
R.S.D. (%)	4.16	2.03	5.37	2.59	2.94	3.35	3.01	4.19
I–IX								
Mean	125.4	0.01080	31.68	0.08594	1.354	2.721	4.075	157.1
R.S.D. (%)	2.26	1.97	3.27	2.03	0.946	1.96	1.18	2.02
I–V								
Mean	125.3	0.01082	31.26	0.0866	1.355	2.704	4.059	156.5
R.S.D. (%)	2.34	2.16	3.36	2.41	0.974	1.87	1.25	2.37

Table 9
Reproducibility of the Langmuir isotherm parameters of aniline

	q_s (g/l)	b (l/g)	a
I–X			
Mean	279.3	0.008865	2.475
R.S.D. (%)	3.76	3.91	4.30
I–IX			
Mean	281.9	0.008894	2.505
R.S.D. (%)	2.47	3.98	1.92
I–V			
Mean	276.9	0.009116	2.52
R.S.D. (%)	1.72	3.64	2.36

Table 10
Reproducibility of the BET isotherm parameter of ethylbenzene

	q_s	b_s	b_L	a
FA	170.0	0.02566	0.0109	4.285
I–X				
Mean	174.3	0.02351	0.01037	4.095
R.S.D. (%)	4.43	2.76	1.88	3.56
I–IX				
Mean	176.3	0.02348	0.01039	4.137
R.S.D. (%)	2.60	2.90	1.87	1.48
I–V				
Mean	175.9	0.02348	0.01037	4.129
R.S.D. (%)	1.79	0.823	0.337	1.08

5. Conclusions

We studied the reproducibility of the nonlinear isotherms of six test compounds, one of them in both buffered and nonbuffered mobile phases. These isotherm data were derived for 10 Kromasil-C_{18} columns, representing six batches of packing material. Since 5 out of the 10 columns were packed with stationary phase from the same batch, we could evaluate both the column-to-column and the batch-to-batch reproducibility of the data. We used quite diverse isotherm models to account for the nonlinear behavior of the adsorption of these solutes. In two instances, the results obtained by using two different isotherm models were processed. Thus, altogether, the results reported here are based on 70 isotherms determined experimentally. The inverse method offers a quick and economic alternative to FA for the determination of such a large number of isotherms with minimum sample and solvent use. The isotherm parameters were determined from a single overloaded band profile in each instance.

In agreement with our previous results, our results show that 1 column out of the 10 exhibits properties that are different from those of the other nine columns. In only one case (viz. the BET isotherm of ethylbenzene) could we show that the column-to-column reproducibility is much better than the batch-to-batch reproducibility. For the other test compounds the column-to-column and the batch-to-batch reproducibilities were rather similar. This result confirms that the production of the modern reversed-phase packing materials is well controlled, and that the different batches possess very similar physic chemical properties.

In most cases, the reproducibilities of both the saturation capacities and the retention factors (when we neglect the outlier column X) are between 1.2 and 3%, and very often below 2%. This is remarkable, considering the complexity of the nonlinear phenomena occurring the elution of a high-concentration sample, let alone the complexity of the manufacturing process of porous silica and its bonding. There are some isotherm parameters—for instance the saturation capacity or the equilibrium constant on a less abundant, higher energy site—that cannot be characterized with such a good reproducibility, but the contribution of these parameters to the overall isotherm is also small, thus the good reproducibility of the overall data is preserved.

These results agree well with the conclusions of the previous study where the isotherms of the test compound were determined on column I by frontal analysis and band profiles of the test compounds were measured on the other nine columns and were compared to the band profiles calculated using the isotherm parameters determined on the first column [11].

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