Reproducibility of low and high concentration data in reversed-phase liquid chromatography II. Overloaded band profiles on Chromolith-C$_{18}$

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Abstract

Single-component adsorption isotherm data were acquired by frontal analysis (FA) for six low molecular weight compounds (phenol, aniline, caffeine, *o*-toluidine, *p*-toluidine and propylbenzoate) on one Chromolith-C$_{18}$ column (#30, Merck, Darmstadt, Germany), using different methanol:water solutions (composition between 60/40 and 15/85 v/v, depending on the solute) as the mobile phase. These data were modeled for best agreement between the experimental data points and the adsorption isotherm model. The adsorption-energy distributions were also derived and used for the selection of the best isotherm model. Widely different models were obtained for these six compounds, four being convex upward (i.e., Langmuirian) and two having at least one inflection point. Overloaded band profiles corresponding to two different sample sizes (a low and a high loading factor) were recorded on six monolithic columns (#30–35) belonging to the same manufactured lot. These experimental band profiles were compared to the profiles calculated from the isotherm measured by FA on the first column, using the equilibrium-dispersive (ED) model of chromatography. For four of the six columns (#30, #32, #33, and #35), the reproducibility was better than 5 and 2.5% for the low and the high concentration profiles, respectively. On the other two columns (#31 and #34), the bands showed significant and systematic retention time shifts for all six compounds (with nearly identical band shapes), the relative adsorption being between 6 and 15% stronger on column #31 or between 2 and 7% lower on column #34. These differences seem to be correlated with the differences in the total porosities of these columns, which differ by 3% from columns #31 to #34, the higher porosity column giving the stronger adsorption.
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1. Introduction

The rapid development of the applications of preparative liquid chromatography in the pharmaceutical industry has lead to the recent renewal of interest in the fundamentals of nonlinear chromatography [1–3]. It has now become possible to calculate the design and operating conditions of a separation that render optimum the value of any objective function, e.g. for maximum production rate, for minimum solvent consumption, or for the optimum value of any combination of the production rate and the recovery yield [1,4]. However, this computer-assisted method of optimization re-quires a prior accurate understanding of the thermodynamics and the kinetics of the chromatographic process involved in the separation studied. The former is characterized by the competitive isotherms of the feed components, the latter by the rate coefficients of the various steps involved in the mass transfer across the column [1–3].

It was demonstrated that thermodynamics controls band profiles, particularly at high concentrations and when the mass transfer kinetics is not very slow [1]. Accordingly, it determines the maximum recovery yield and production rate that any industrial unit can achieve. For obvious economic reasons, preparative chromatography is carried out at high concentrations, the injected sample often being at a concentration close to that of the saturated solution. Under such conditions, the equilibrium isotherms between the two phases of the chromatographic system are rarely linear.

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The stronger the nonlinear behavior of the isotherm at the maximum band concentration, the more unsymmetrical the band profile, the lower the resolution of this band and its neighbors, hence the lower the recovery yield and the production rate [1,4]. The mass transfer kinetics affects the precise shape of the band profiles, dispersing to some extent the profiles predicted by thermodynamics alone. Accordingly, it has an impact on the band resolution that increases with decreasing column efficiency [1].

Thus, it is of paramount importance to determine accurately the competitive isotherms of the feed components if one wants to use computer-assisted optimization of the experimental conditions of a separation. This task alone proves to be an important project for any laboratory. It may require several weeks to acquire the experimental competitive isotherm data of a binary mixture. Fortunately, it has been shown that, in many instances, competitive isotherms can be derived from the single-component isotherms of the compounds involved [1,5,6]. This latter task is far easier than the former. Numerous methods are available for the acquisition of equilibrium isotherm data and for the derivation of the best equation of these single-component isotherms. The methods currently accepted as practical and reliable are frontal analysis (FA) [1,6–8], elution by characteristic point (ECP) [1,9,10], pulse perturbations [1,11,12], and numerical inverse [13] methods. Each one of these methods has its own advantages and drawbacks which must be taken into account in any specific case in order to minimize measurement errors and costs [1]. In this work, we use the FA method, which is the most accurate but also the slowest and the most costly. In this context, the accuracy on the measurements of single-component isotherm data and the reproducibility of these data become important issues. Adsorption phenomena are sensitive to several physico-chemical properties of the stationary phase. In RPLC, the most important ones are the specific surface area of the adsorbent, the bonding density and/or the carbon content, the density of residual silanols, and the surface concentration of the metal impurities. These primary properties are characterized by chromatographic parameters such as the column hydrophobic selectivity, the methylene selectivity, the silanol activity, that all have an impact on the retention factor of analytes, on the degree of asymmetry of its peak, and on the column efficiency. These properties vary significantly from column brand to column brand. Their reproducibility from batch to batch and, within one batch, from column to column has been assessed by Kele and Guiochon under linear conditions for four different brands of packed C_{18}-silica columns [14–17] and for a lot of monolithic columns [18]. They reported RSDs of the column to column fluctuations of the retention factors below 2–3% for most solutes and repeatabilities close to 0.1%.

We recently reported the repeatability and reproducibility of high concentration data on ten columns packed with Kromasil-C_{18} [19]. The column-to-column and the batch-to-batch reproducibilities of the band profiles obtained was better than 4%, except for one column. Our low concentration data agree with those obtained under linear conditions by Kele [15]. These results illustrate the progress made in the control of the manufacturing of large batches of packing materials, involving the preparation of the bare silica, the C_{18} bonding, and the endcapping reaction. Although it had not been documented earlier, this result was suspected by most specialists. It is particularly important in simulated moving bed chromatography, an industrial process that uses series of at least 4 and up to 16 columns whose adsorption properties must be as close as possible to avoid losses of production rate and/or purity [20–22]. Because Kele and Guiochon [14–17] obtained results that were highly consistent with the different brands of materials that they had studied, a systematic investigation of the reproducibility of high concentration data does not seem required. However, a reproducibility study of the monolithic columns is needed, even though they are not yet available in diameters consistent with their use in preparative HPLC. Each monolithic column is prepared in its own and unique glass cylinder [23], independently of the other rods, so there is only one column per batch. Furthermore, some specific treatments differentiate the monolithic columns from the conventional silica packing materials. For instance, the cylindrical rod of silica gel must be dried. The rate of this step determines to a large extent the final structure of the rod (e.g. the average macropore size, the radial heterogeneity of the rod, the average size of the silica skeleton ribs). This step of the fabrication process could be more difficult to control than the other ones and could affect the degree of reproducibility of the column characteristics, particularly the total porosity.

Thus, a study of the reproducibility of the chromatographic properties of monolithic columns under linear and nonlinear conditions would be useful. Kele and Guiochon already measured the repeatability (on one column) and the reproducibility (on six columns) of data measured under linear conditions on Chromolith Performance columns (Merck, Darmstadt, Germany) [18]. They reported that these columns exhibited a high degree of repeatability of the retention times or retention factors of 30 compounds of widely different polarity. However, they did not measure the Henry constant (i.e., the initial slope of the adsorption isotherm) that characterizes the liquid–solid equilibrium, hence the chemistry of the adsorbent surface, independently of the influence of the total porosity that may vary from column to column. The goal of this work was to determine the reproducibility of the adsorption isotherm data measured for different compounds, using different chromatographic systems, on a series of six monolithic columns of the same lot. This was done by measuring the reproducibility of the Henry constant of six compounds and that of their overloaded band profiles at a low and a high column loading. To save time and significant amounts of solvent solutions and sample compounds, the FA measurements were performed on one column only. The experimental adsorption data acquired were modeled and the best isotherm model derived. For each compound, this model was used to calculate overloaded band profiles. These

profiles were compared to those recorded on the different columns. The six compounds studied and the three mobile phase compositions used were selected so as to elicit most diverse isotherms and band profiles. The degree of agreement between the calculated and the experimental profiles in each case illustrates the reproducibilities of the adsorption data that can be achieved with the actual technology of preparation of monolithic silica material.

2. Theory

2.1. Determination of single-component isotherms by frontal analysis

Among the various chromatographic methods available to determine single-component isotherms, frontal analysis is the most accurate [1–4,6,7]. It consists in the step-wise replacement of the stream of mobile phase percolating through the column with streams of solutions of the studied compound of increasing concentrations and in the recording of the breakthrough curves at the column outlet. Mass conservation of the solute between the times when the new solution enters the column and when the plateau concentration is reached allows the calculation of the adsorbed amount, q^*, of the solute in the stationary phase at equilibrium at the corresponding mobile phase concentration, C. This amount is best measured by integrating the breakthrough curve (equal area method) [24]. The adsorbed amount q^* is given by:

$$q^* = \frac{C(V_{eq} - V_0)}{V_a} \tag{1}$$

where V_{eq} and V_0 are the elution volume of the equivalent area and the hold-up volume, respectively, and V_a is the volume of stationary phase. This relationship applies to all breakthrough curves recorded. This method was used for the acquisition of all the experimental isotherm data measured on the monolith column #30.

2.2. Models of single-component isotherm

Two general types of adsorption isotherms were used in this work. On the one hand, the adsorption of phenol and caffeine was best described by a strict convex upward or multiLangmuirian isotherm model. This mirrored the surface heterogeneity of the monolithic stationary phase under the experimental conditions used (i.e., with the mobile phase composition selected). A tri- and a quadri-Langmuir isotherms were found to best model the adsorption data of phenol and caffeine, respectively (see later). These isotherm equations are written:

$$q^* = \sum_{i=1}^{i=3,4} q_{s,i} \frac{b_i C}{1 + b_i C} \tag{2}$$

where b_i is the adsorption constant and $q_{s,i}$ the saturation capacity of the sites i. Accordingly, the adsorption affinity distribution is the sum of three or four δ-functions, each of them related to the adsorption energy, $\epsilon_{a,i}$, corresponding to b_i by [25]:

$$b_i = b_0 \exp\left(\frac{\epsilon_{a,i}}{RT}\right) \tag{3}$$

where $\epsilon_{a,i}$ is the adsorption energy on the sites i and b_0 is a preexponential factor that is usually assumed to be the same, whatever the type of adsorption sites i [25].

On the other hand, the adsorption of aniline, o-toluidine, p-toluidine and propylbenzoate were best described by a polynomial isotherm of the fifth order, exhibiting an inflection point. Beyond the second or third order, there are no obvious physical interpretations of the parameters of the adsorption isotherms of these compounds on the monolithic stationary phase. The isotherm equation is given by [6]:

$$q^* = q_s \frac{a_1 C + 2a_2 C^2 + 3a_3 C^3 + 4a_4 C^4 + 5a_5 C^5}{1 + a_1 C + a_2 C^2 + a_3 C^3 + a_4 C^4 + a_5 C^5} \tag{4}$$

where $5q_s$ is the saturation capacity of the adsorbent and the coefficients a_1, a_2, a_3, a_4 and a_5 are theoretically related to the partition functions for an individual adsorbed molecule on a hypothetical ith ($1 \le i \le 5$) monomolecular layer.

In the next section, we describe the method used to calculate the affinity energy distribution in the case of strictly convex downward isotherms.

2.3. Calculation of the adsorption energy distributions

Actual surfaces are neither homogeneous nor paved with homogeneous tiles, as was assumed so far. These surfaces are characterized by an adsorption energy distribution (AED) that may have several more or less well-resolved modes, each mode having a finite width. The experimental isotherm on such a heterogeneous surface is the sum of the isotherms on each one of the types of sites covering the surface. There are different possible mathematical approaches to calculate the affinity energy distribution or distribution of the adsorption energy constants on the surface [25–28]. In this work, we derive it from the raw adsorption data by using the expectation-maximization method [28], a method previously described in detail [19]. This method assumes that the adsorption isotherm on each homogeneous type of sites is a Langmuir or a Jovanovic isotherm. Accordingly, the method affords the energy distribution on heterogeneous adsorbents but only if the adsorbate–adsorbate interactions are negligible.

2.4. Modeling of high-performance liquid chromatography

The overloaded band profiles were calculated using the best model of the isotherm of the compound stud-

ied and the equilibrium-dispersive model of chromatography [1,6,29]. The ED model assumes instantaneous equilibrium between the mobile and the stationary phase and a finite column efficiency originating from an apparent axial dispersion coefficient, D_a, that accounts for the dispersive phenomena (molecular and eddy diffusion) and for the non-equilibrium effects that take place in a chromatographic column. The axial dispersion coefficient is related to the experimental parameters through the following equation:

$$D_a = \frac{uL}{2N} \tag{5}$$

where u is the mobile phase linear velocity, L the column length, and N the number of theoretical plates or apparent efficiency of the column. In the ED model, the mass balance equation for a single component is written:

$$\frac{\partial C}{\partial t} + u\frac{\partial C}{\partial z} + F\frac{\partial q^*}{\partial t} = D_a\frac{\partial^2 C}{\partial z^2} \tag{6}$$

where q^* and C are the stationary and the mobile phase concentrations of the adsorbate, respectively, t is the time, z the distance along the column, and $F = (1 - \epsilon_t)/\epsilon_t$ is the local phase ratio, with ϵ_t the total column porosity at time t and distance z. If ϵ_t is assumed to be constant so is F. q^* is related to C through the isotherm equation, $q^* = f(C)$.

2.4.1. Numerical solutions of the ED model

The mass balance equation was integrated numerically using a computer program based on an implementation of the method of orthogonal collocation on finite elements (OCFE) [30–32]. The set of discretized ordinary differential equations was solved with the Adams-Moulton method, implemented in the VODE procedure [33]. The relative and absolute errors of the numerical calculations were 10^{-6} and 10^{-8}, respectively.

2.4.2. Initial and boundary conditions for the ED model

At $t = 0$, the concentration of the solute in the column is uniformly equal to zero, and the stationary phase is in equilibrium with the mobile phase components (methanol and water in this work). The boundary conditions used are the classical Danckwerts-type boundary conditions [1,34] at the inlet and outlet of the column. In all the calculations, the inlet profiles were assimilated to rectangular profiles.

3. Experimental

3.1. Chemicals

The mobile phase used in this work, whether for the determination of the adsorption isotherm data or for the recording of large or small size band profiles, was a mixture of methanol and water (15:85 v/v for phenol, caffeine and aniline; 20/80v/v for o-toluidine and p-toluidine and 60/40 v/v for propylbenzoate). Both solvents were of HPLC grade and were purchased from Fisher Scientific (Fair Lawn, NJ, USA). The mobile phase was filtered before use on a surfactant-free cellulose acetate filter membrane, 0.2 μm pore size (Suwannee, GA, USA). Thiourea was chosen to measure the column hold-up volume at the different methanol contents in the mobile phase. Thiourea, phenol, caffeine, aniline, o-toluidine, p-toluidine and propylbenzoate were obtained from Aldrich (Milwaukee, WI, USA).

3.2. Materials

The lot of six new 100 mm × 4.6 mm Chromolith columns (serial UM20622, numbers 30–35) used was generously offered by Merck (Darmstadt, Germany, EU). These columns are C_{18}-bonded, endcapped, porous silica. The main characteristics of the bare porous silica and of the packing material used are summarized in Table 1, according to the manufacturer. The total porosities of these columns were derived from the retention times of two consecutive injections of thiourea at variable methanol contents after the column was equilibrated during 1 h at the given mobile phase composition. Results are gathered in Table 2.

3.3. Apparatus

The breakthrough curves and the overloaded band profiles of all compounds were acquired using a Hewlett-Packard (Palo Alto, CA, USA) HP 1090 liquid chromatograph. This instrument includes a multi-solvent delivery system (three tank, volume 1 l each), an auto-sampler with a 250 μl sample loop, a column thermostat, a diode-array UV-detector, and a data station. Compressed nitrogen and helium bottles (National Welders, Charlotte, NC, USA) are connected to the instrument to allow the continuous operations of the pump, the auto-sampler, and the solvent sparging. The extra-column volumes are 0.068 and 0.90 ml, as measured from the auto-sampler and from the pump system, respectively, to the column inlet. All the retention data were

Table 1
Physico-chemical properties of the six monolithic Chromolith-C_{18} columns (from the manufacturer Merck)

Batch no. UM20622					
Macropore size (μm)	Mesopore size (Å)	Surface area (m²/g)	Total metal content (ppm)	Total carbon (mass%)	Surface coverage (μmol/m²)
1.95	130	314	<5	18.0	3.60

Table 2
Total porosities[a] of the six monolithic Chromolith-C_{18} columns (Merck) measured by the injection time of thiourea at a flow rate of 1 ml/min for the six compounds studied

| Column | Mobile phase (methanol/water, v/v) | | | | | |
| | 15/85 | | | 20/80 | | 60/40 |
	Aniline	phenol	Caffeine	o-Toluidine	p-Toluidine	Propylbenzoate
30 (I)	0.8671	0.8665	0.8677	0.8629	0.8647	0.8418
31 (II)	0.8821	0.8839	0.8845	0.8767	0.8779	0.8532
32 (III)	0.8677	0.8689	0.8695	0.8623	0.8629	0.8382
33 (IV)	0.8689	0.8695	0.8707	0.8629	0.8647	0.8406
34 (V)	0.8593	0.8599	0.8611	0.8538	0.8544	0.8304
35 (VI)	0.8701	0.8713	0.8731	0.8659	0.8659	0.8424

The extra-column volume was $0.058\,cm^3$. The volume of the column tubes is $1.662\,cm^3$ ($100\,mm \times 4.6\,mm$).

[a] The column porosities are calculated from the column-tube volume (inner diameter and length) supplied by the manufacturer.

corrected for this contribution. The flow-rate accuracy was controlled by pumping the pure mobile phase at 23 °C and 1 ml/min during 50 min, from each pump head, successively, into a volumetric glass of 50 ml. The relative error was less than 0.4%, so that we can estimate the long-term accuracy of the flow-rate at 4 µl/min at flow rates around 1 ml/min. All measurements were carried out at a constant temperature of 23 °C, fixed by the laboratory air-conditioner. The daily variation of the ambient temperature never exceeded ±1 °C.

3.4. Frontal analysis isotherm measurements on Chromolith #30

The mobile phase composition at which the FA measurements were performed on the reference Chromolith Performance column #30 was chosen on the basis of the results of measurements of the retention factor at infinite dilution. In order to make accurate measurements of adsorption isotherm data, the retention factor, k' should be neither too high (which limits the number of data points that can be acquired in a reasonable period) nor too low (which would cause a decrease in the accuracy of the adsorption data). Values of k' between 2 and 6 are ideal to achieve a satisfactory isotherm determination. Prior to any isotherm measurement, the solubilities at 23 °C (the temperature at which the isotherm data were acquired) of all the compounds in their respective mobile phase (see Section 3.1) were determined approximately by the stepwise addition of 0.5 ml of the pure mobile phase into a volume of 25 ml of a saturated solution containing a small amount of undissolved compound, until complete dissolution. Accordingly, the maximum concentrations (in the corresponding mobile phase) used in the FA measurements were 40, 75, 27, 15, 15 and 13.5 g/l for aniline, phenol, caffeine, o-toluidine, p-toluidine and propylbenzoate, respectively. Two master sample solutions were prepared, with concentrations of 10 and 100% of these maximum concentrations, respectively. Accordingly, two consecutive sequences of FA measurements were carried out with these two solutions (see procedure below), giving an accurate isotherm determination at both low and high concentrations. Twenty-six experimental adsorption data points were recorded for each compound. One pump of the HPLC instrument was used to deliver a stream of the pure mobile phase, the second pump a stream of the pure master sample solution. The concentration of the studied compound in the stream percolating through the column is determined by the concentration of the master sample solution and by the ratio of the flow rates delivered by the two pumps. The breakthrough curves are recorded successively at a flow rate of 1 cm^3/min, with a sufficiently long time interval between each breakthrough curve to allow for the reequilibration of the column with the pure mobile phase. The injection time of each new solution was taken long enough (typically between 5 and 10 min) to reach a stable plateau at the column outlet. The overloaded profiles needed for the validation of the fitted isotherms (column #30) were recorded at the time when the frontal analysis experiments were carried out. An isotherm was accepted only if it accurately predicts the band profiles at both low and high column loadings (see later, Section 4.1). To avoid recording any UV-absorbance signal larger than 1500 mAU and the associated too large signal noise, the detection of the breakthrough curves and the overload band profiles of aniline, phenol, caffeine, o-toluidine, p-toluidine and propylbenzoate were carried out at 330, 291, 307, 310, 310 and 289 nm, respectively. The detector responses for the samples were calibrated accordingly.

4. Measurements of overloaded profiles on Chromolith columns #31 to #35

A reserve of 2.5 l of the pure mobile phase (a mixture of methanol and water at a concentration depending on the sample, see Section 3.1) was prepared manually before performing FA measurements on Chromolith Performance column #30. The mixing of methanol and water being exothermic, it takes at least 2 h for its temperature to stabilize at room temperature (23 °C). This amount of mobile phase was used, first, for the recording of the breakthrough curves and of the two overloaded profiles on column #30, then for the recording of the same two overloaded profiles on each other column, #31 to #35. Using the same mobile phase for all columns was of paramount importance in order to reduce the unavoidable experimenter errors when attempting manu-

ally to prepare another mobile phase mixture. Columns #30 to #35 were used in this order for the study of all compounds.

For each column, the following sequence was followed. First, the column was equilibrated with the pure mobile phase for at least 120 min. If, after this time, the UV detector signal was still drifting or had a background noise of more than 0.2 mAU, an additional equilibration time of 30 min was applied, until full equilibration was considered to have been reached. At the end, the state of equilibrium was checked by determining the stability of the hold-up column volume, measured every 5 min during half an hour. Second, the column hold-up volume and the sample retention time at infinite dilution were measured by performing two successive 2 μl injections, one of a uracil and the second of a sample solutions, both at concentrations of about 0.1 gl (concentration for which the isotherms are all linear) of the respective compound in the corresponding mobile phase. It was observed that the total porosity of each column decreases steadily with decreasing water content of the mobile phase (Table 2), by approximately 3% when the composition varies from 15:85 to 60:40 methanol/water, v/v.

Finally, the small and the large overloaded profiles (see earlier) were successively recorded. The first one corresponds to a low column loading, with an injection that lasted for 60 s of a solution at a concentration of 5% of the 100% master sample solution. The second one corresponding to a high column loading, with an injection that lasted for 60 s of a solution at a concentration of 80% of the same master sample solution.

4.1. Comparison between calculated and experimental band profiles

4.1.1. Parameters used to calculate the band profiles

In this work, the calculations of all band profiles were done by using the equilibrium dispersive model of chromatography and the numerical method of orthogonal collocation on finite elements (OCFE). The input parameters needed to run these calculations are: the mobile phase superficial velocity, the column length, the column total porosity, its number of theoretical plates, the injected concentration of the rectangular injection profile (or the boundary condition at column inlet) and the isotherm parameters.

The flow rate was set at 1 ml/min for all the measurements. Accordingly, the superficial velocity was 0.1003 cm/s for the six Chromolith Performance columns (assuming that all have the same 4.6 mm inner diameter). The column length is the same for all columns ($L = 10$ cm). The total porosities of the six columns are listed in Table 2, for all the mobile phase compositions used. The total porosity does not need to be a constant parameter in the calculations because the local concentration of the sample in the adsorbed phase does not depend on the local column void volume nor on the time spent by the solute in the mobile phase. Adsorption and retention depend on the chemical nature of the bare silica (e.g., mesopore structure, density and strength of the active sites, metal impurities) and on the modifications of the surface chemistry resulting from the C_{18} bonding (e.g., carbon content, C_{18} chain density, endcapping). Those are all surface properties. Then, all the band profiles were calculated according to the measured values of the column total porosity (Table 2). For each compound, the number of theoretical plates was kept constant for all six columns. The common efficiency was the one that gives the same maximum concentration for the calculated and the experimental band profiles recorded on the reference column #30. Finally, the isotherm parameters used in all calculations were those derived from the FA measurements made on the reference Chromolith Performance column #30 (see parameters in Table 3).

Table 3
Best adsorption isotherm parameters obtained by fitting the experimental FA data of the six adsorption systems on the Chromolith-C_{18} column 30 (I)

	Quadri-Langmuir							
	$q_{s,1}$ (g/L)	b_1 (L/g)	$q_{s,2}$ (g/L)	b_2 (L/g)	$q_{s,3}$ (L/g)	b_3 (L/g)	$q_{s,4}$	b_2
Caffeine	219.03	0.030715	37.63	0.17605	25.76	0.65377	0.57	7.51574
	Fifth-order polynomial							
	q_s (g/L)	a_1 (L/g)	a_2 (L^2/g^2)	a_3 (L^3/g^3)	a_4 (L^4/g^4)	a_5 (L^5/g^5)		
Aniline	154.42	0.11513	0.005383	7.22e−5	−3.873e−6	6.944e−8		
o-Toluidine	67.04	0.50616	0.08759	0.01937	−0.00056	8e−5		
p-Toluidine	74.21	0.52839	0.12275	0.02060	0.00036	5e−5		
Propylbenzoate	260.29	0.08544	0.0021	0.0002307	−1.711e−5	1.08e−6		
	Tri-Langmuir							
	$q_{s,1}$ (g/L)	b_1 (L/g)	$q_{s,2}$ (g/L)	b_2 (L/g)	$q_{s,3}$ (g/L)	b_3 (L/g)		
Phenol	236.79	0.009495	109.66	0.13345	28.65	0.40212		

The best isotherm parameters are obtained by regression analysis on three different models of isotherm (tri-Langmuir, quadri-Langmuir, and the fifth-order statistical polynomial isotherms).

4.1.2. Definitions

As will become apparent later, the shape of the calculated overloaded band profiles agrees excellently with those of the experimental profiles. For each compound, at both loading factors, the use of the same value of the column efficiency results in an excellent description of the experimental band profiles recorded on all the columns. Actually, this means that, in all cases, provided that we use a constant dispersive correction, the presence and the locations of all the diffuse and the self-sharpening fronts observed in all the experimental bands are well accounted for by an adsorption behavior following the one described by the selected isotherm model.

The only significant differences observed between the calculated and the experimental profiles were small shifts in the time positions of these fronts. These changes are due to small numerical differences in the values of the isotherm parameters, particularly their initial slopes. These differences characterize the reproducibility of the packing material studied.

In this work, we focus our attention on the relative positions of the shock layers in the calculated and the experimental profiles. All the low-loading band profiles have a front shock, whatever the compound analyzed. This is in agreement with all the isotherms being convex upward at low concentrations (see later, in Figs. 3–8, the negative
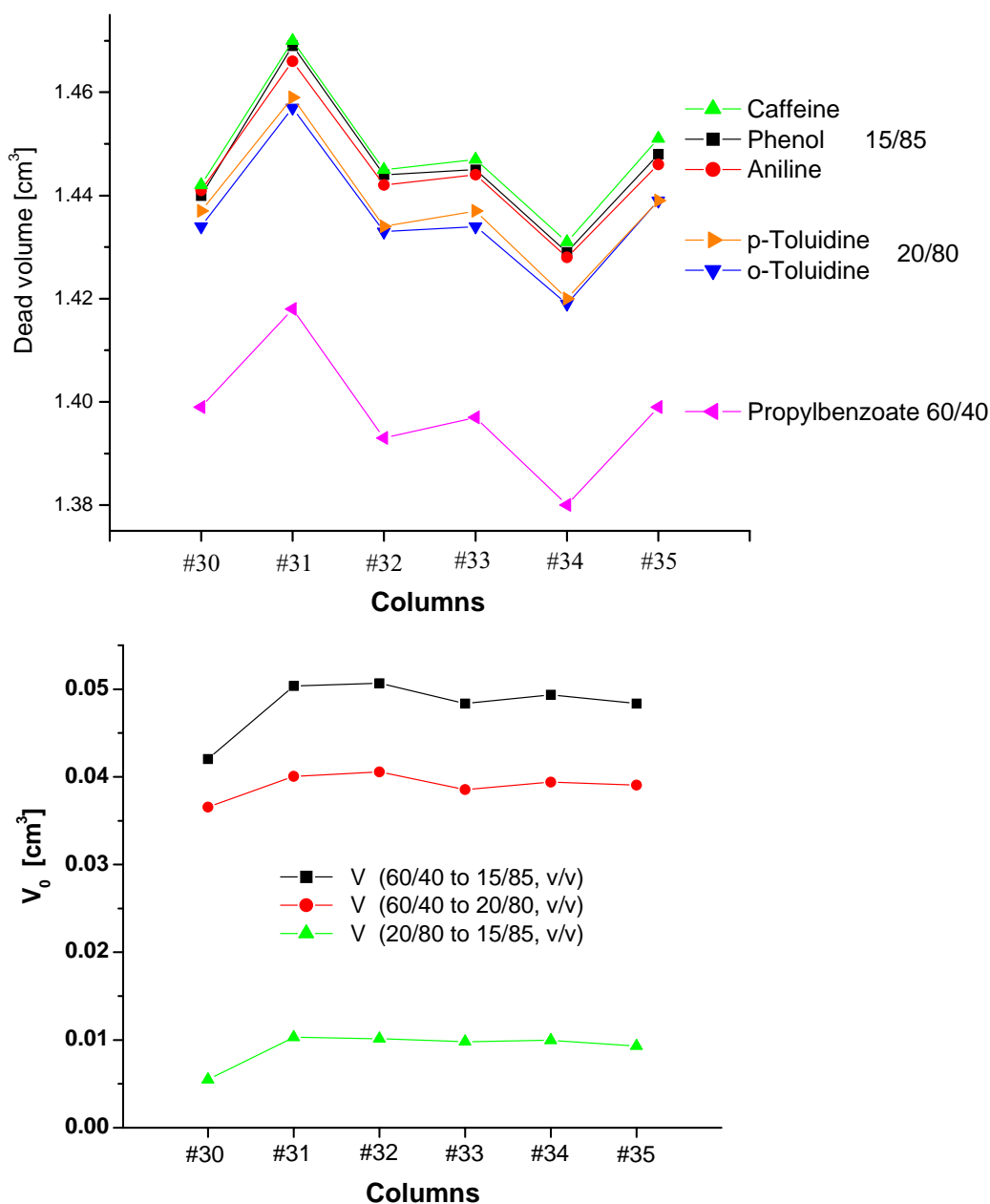
Fig. 1. (A) Hold-up volumes of the six Chromolith columns for the different chromatographic systems used in this study. (B) Increase of the total hold-up volume of the six columns when the methanol content decreases from 60 to 20, 60 to 15 and 20 to 15% in volume. Note the same parallel between the three curves and the difference on column #30.

value of the initial slope of the q^*/C plot). The position of the front parts of these bands will be analyzed. The same treatment will be applied to the high-loading band profiles, except for that of propylbenzoate, whose isotherm has an inflection point in the middle of the concentration range and whose band has two shocks, one in the front and one in the rear part of the band.

Accordingly, the retention time of the point at half-height of the peak defines the position of the whole band for all overloaded band profiles. To compare the shape of the different profiles, we define for each column, X, the relative difference E between the calculated and the experimental profiles as:

$$E(X) = \frac{t_{shock}^{Calc} - t_{shock}^{Exp}}{t_{shock}^{Exp}} \quad (7)$$

Of course, $E(X)$, has no fundamental meaning. It is merely a useful tool to compare simply the relative strength of the adsorption of the different compounds on the six different columns (the reference being the strength measured on column #30). For instance, $E(\#33) \geq 0$ would indicate that the adsorption on column #33 is weaker than on column #30.

5. Results and discussion

5.1. Reproducibility of the hold-up volumes of the six Chromolith Performance columns

Fig. 1A illustrates the batch-to-batch reproducibility of the column hold-up volumes of the six Chromolith Perfor-

mance columns and its dependence on the nature of the chromatographic system selected (i.e., the mobile phase composition). Thiourea was used to measure the column hold-up volume before the injection of the six analytes chosen in this study, i.e., phenol, caffeine, aniline, *o*-toluidine, *p*-toluidine and propylbenzoate. From one mobile phase composition to another, the variation of the total porosity is the same for all the columns. The porosity increases by 3% when the methanol concentration decreases from 60 to 15% (v/v). The relative standard deviation (RSD) of the hold-up volumes of the six columns is about 0.88% at constant mobile phase composition. This fluctuation is not negligible. There is, for instance, a difference of more than 3% between the total porosities of columns #31 and #34. Note, however, that the RSD of the hold-up volumes of four columns (#30, #32, #33 and #35) is only 0.25 % (corresponding to differences in the porosity values of less than 1%). This suggests that the reproducibility of only four of the six Chromolith columns is truly satisfactory.

Fig. 1B shows the variation, ΔV_0, of the hold-up volume with the mobile phase composition. This change is explained by the unfolding of the C_{18} bonded alkyl chains, which swell when increases the methanol content of the mobile phase in contact with the solid surface. So, the change in column hold-up volume is actually the mechanical consequence of a change in the volume of the stationary phase, i.e., in the volume occupied by the bonded chains in contact with the mobile phase. Three variations of the void volume are plotted in the figure, from a mobile phase composition of 15/85 to 60/40, 20/80 to 60/40 and 15/85
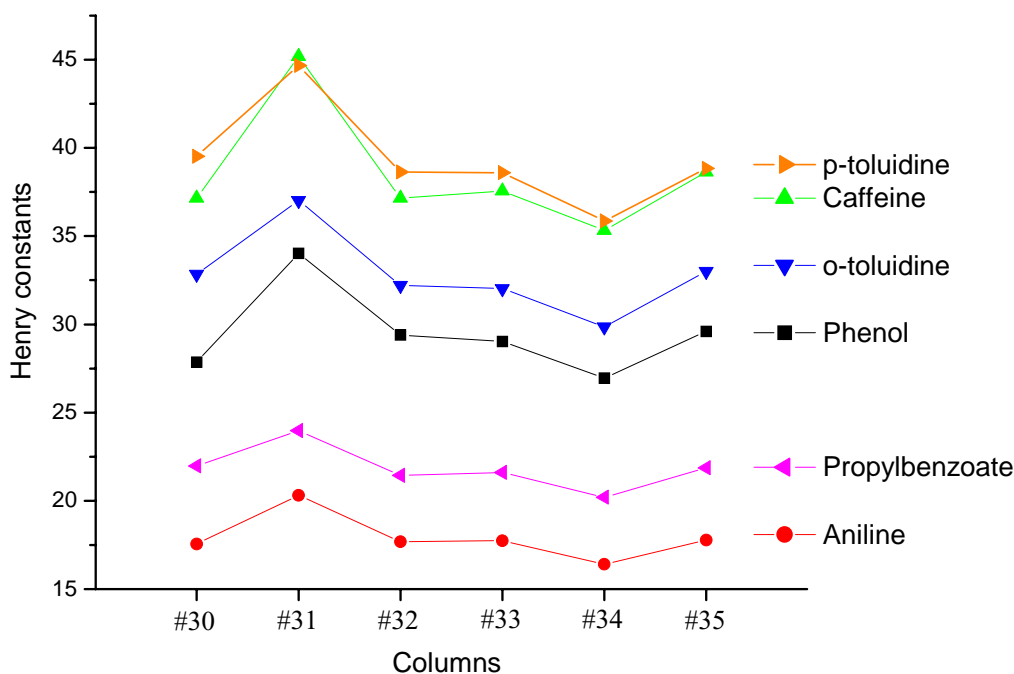

Fig. 2. Henry constants H of the six solutes measured on the lot of six monolithic columns. Note the systematic higher and lower Henry constant measured on columns #31 and #34, respectively.

to 20/80 (v/v). We observed that all the columns exhibit the same behavior, except column #30 for which the volume difference is systematically smaller. The number of accessible C_{18}-bonded chains is probably lower on this column.

To summarize, the analysis of the retention times of thiourea on the six Chromolith Performance columns shows some obvious and systematic differences between these columns. These differences are due to differences in their total porosity and possibly in the amount of C_{18} chains bonded onto the silica. However, the origin of these differences is not clear. It may stem out from any or all the steps involved in the process of preparation of the C_{18}-Chromolith columns developed by Merck, on which we have limited information. These steps include the sol–gel reaction in the mold, the chemical treatment of the silica rods formed, the drying process (that may generate radial heterogeneity), and the final C_{18} deriva-

tization and endcapping steps. In addition, it is not impossible that small, scattered empty volumes form at the walls or around the frits. Similar, important differences between the total porosities of packed columns of different diameters have been already reported by Heuer et al. [35].

5.2. Reproducibility of the Henry constants of the six Chromolith Perfomance columns

Fig. 2 shows the Henry constants, H, of the compounds studied on the six Chromolith Performance columns. These constants are calculated as follows :

$$H = a = \frac{t_R - t_0}{Ft_0} = \frac{k'}{F} \tag{8}$$

where t_R and t_0 are the retention time of an analytical injection and the hold-up column time, F is the column
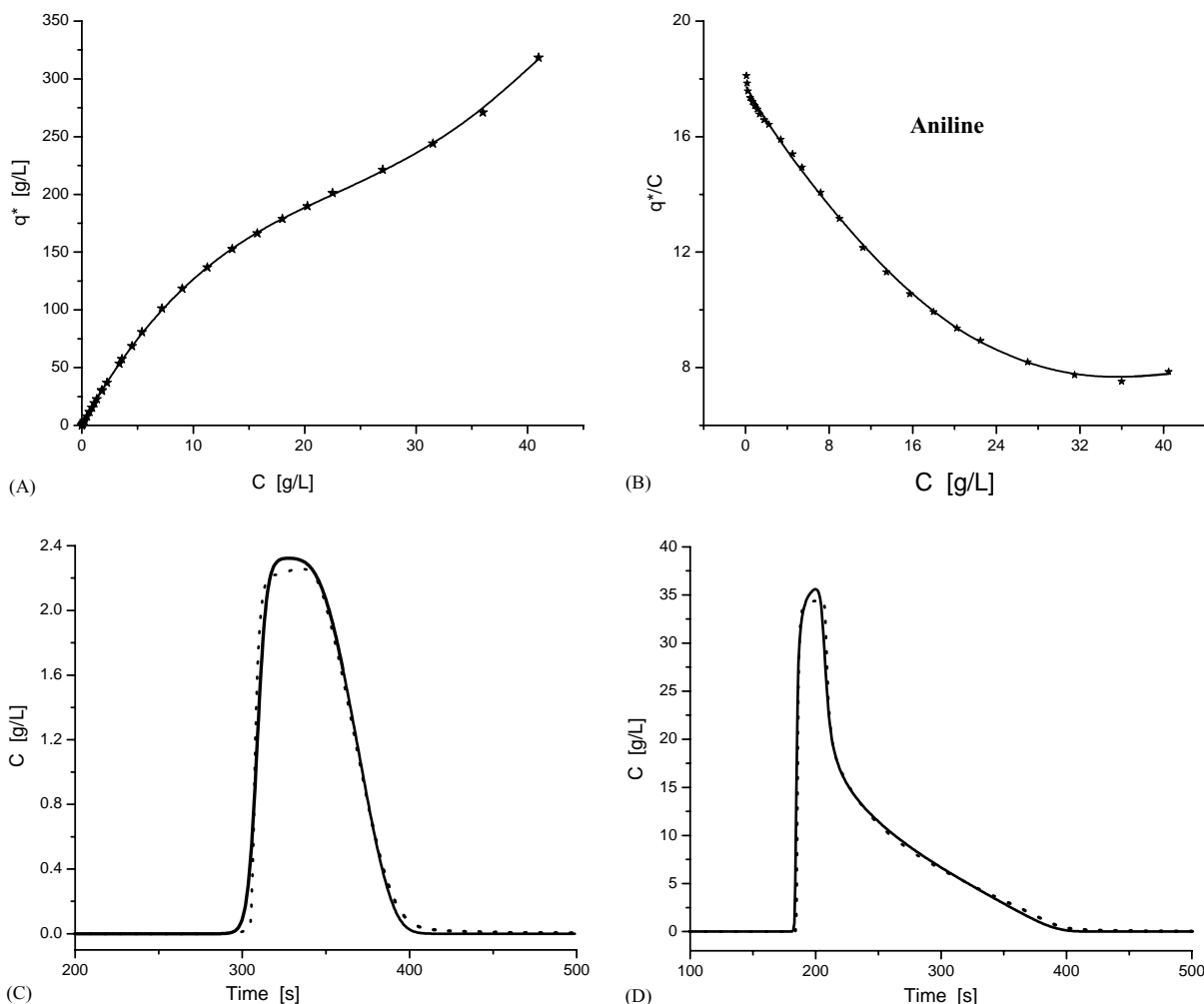
Fig. 3. (A) Experimental isotherm (stars) and best fifth-order polynomial isotherm (solid line) of aniline on the Chromolith column #30. (B) Experimental isotherm chord q^*/C (stars) and the same best 5th order polynomial isotherm as in (A) (solid line). (C) Comparison between calculated (solid line) and experimental band profile (dotted line) measured for a low column loading. (D) Comparison between calculated (solid line) and experimental band profile (dotted line) measured for a high column loading.

phase ratio. H allows a comparison of the distribution constants of the solutes between the stationary and the mobile phases, eliminating the spurious influence of the differences in the total porosity between the columns that we would observe if we compare retention volumes or retention factors. The batch-to-batch RSD of H for the set of columns #30, #32, #33 and #35 varies between 1.3 and 3.4%, depending on the compound considered. The RSD for the whole lot is far larger and varies between 5.6 and 8.3%. This suggests significant discrepancies in the strength of adsorption of the different compounds on the columns #31 and #34 and, accordingly, important differences in the surface chemistry of the C_{18}-bonded stationary phases. Column number #31 adsorbs all compounds more strongly, and column #34 less strongly, than the four other columns (see Fig. 2, the maximum and minimum of each curve). The parallelism between the curves in Fig. 2 comforts this result and demonstrates that it is independent of the solute injected, but utterly related to the character-

istics of the column surface. These significant variations of the initial slopes of the adsorption isotherms on the six Chromolith columns suggest a certain lack of reproducibility of the surface properties of the stationary phase, including the surface heterogeneity and the C_{18} chain density.

5.3. Measurement of the adsorption isotherms on reference Chromolith Performance column #30

Column #30 was arbitrarily chosen to serve as the reference in the comparison between the profiles calculated and those recorded on all the columns of the lot. FA experiments were carried out on this one column to measure the adsorption isotherm data of the compounds studied. For each compound, Figs. 3A–8A show the isotherm data (symbols) and the best isotherm models (solid line). Figs. 3B–8B show the plots of the isotherm chords, $q^*/C = f(C)$, versus C for those compounds
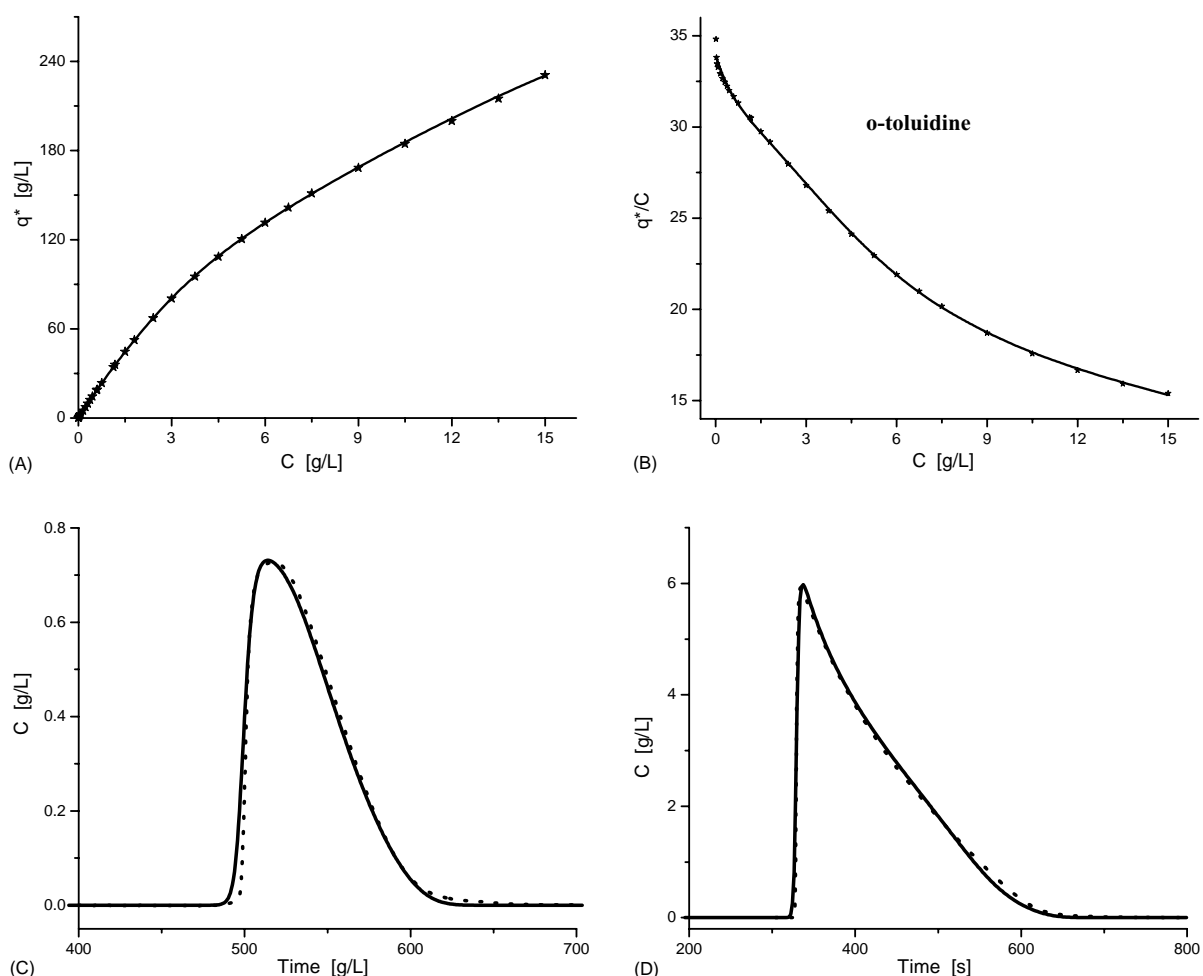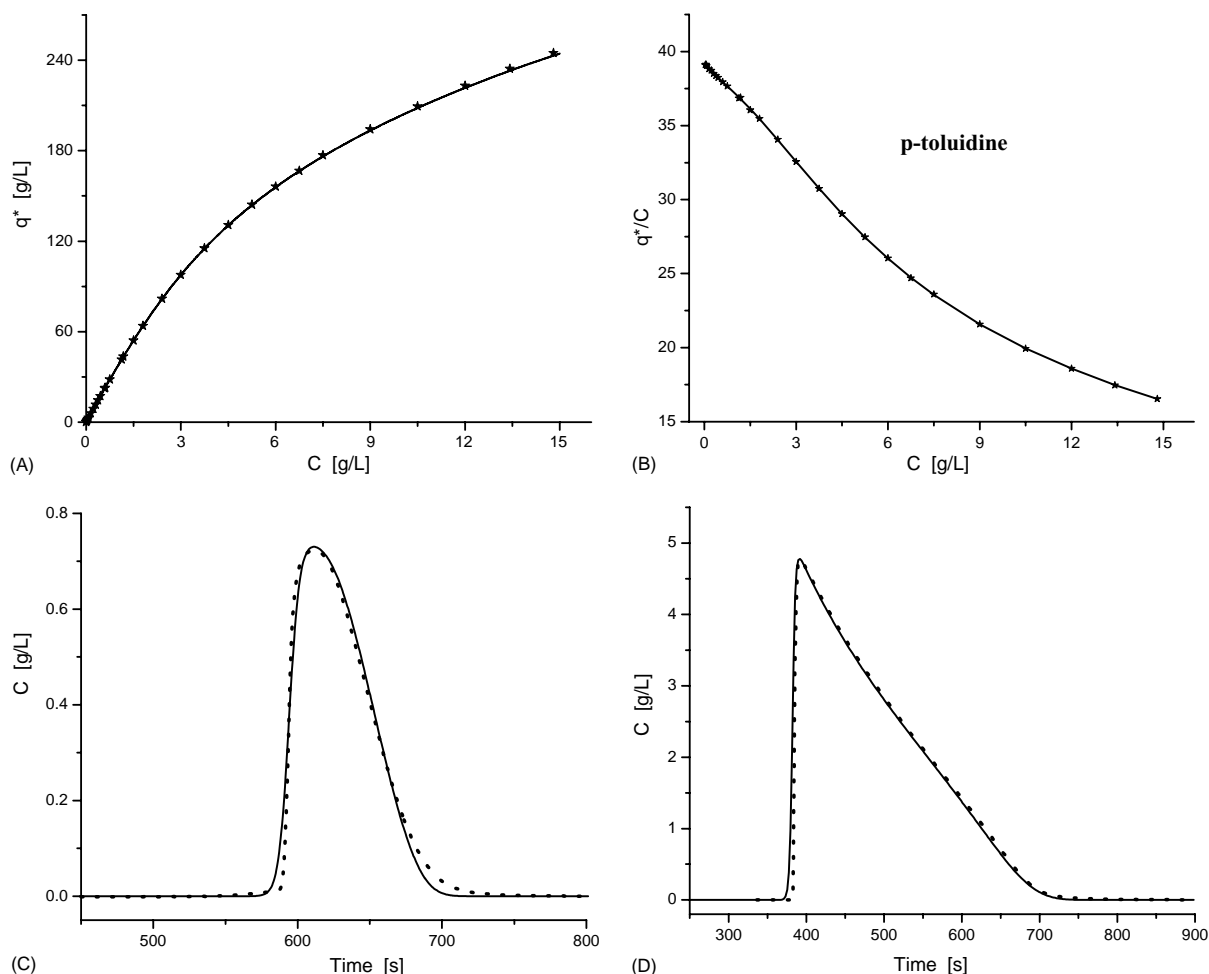

Fig. 4. (A) Experimental isotherm (stars) and best fifth-order polynomial isotherm (solid line) of o-toluidine on the Chromolith column #30. (B) Experimental isotherm chord q^*/C (stars) and the same best fifth-order polynomial isotherm as in (A) (solid line). (C) Comparison between calculated (solid line) and experimental band profile (dotted line) measured for a low column loading. (D) Comparison between calculated (solid line) and experimental band profile (dotted line) measured for a high column loading.

Fig. 5. (A) Experimental isotherm (stars) and best fifth-order polynomial isotherm (solid line) of *p*-toluidine on the Chromolith column #30. (B) Experimental isotherm chord q^*/C (stars) and the same best fifth-order polynomial isotherm as in (A) (solid line). (C) Comparison between calculated (solid line) and experimental band profile (dotted line) measured for a low column loading. (D) Comparison between calculated (solid line) and experimental band profile (dotted line) measured for a high column loading.

that have a complex isotherm with at least one inflection point (i.e., aniline, *o*-toluidine, *p*-toluidine and propylbenzoate) and the best affinity energy distribution function for those compounds that have of strictly convex-upward isotherm (i.e., phenol and caffeine). Finally, Figs. 3C and D, and 8C and D compare the experimental overloaded band profiles (dotted lines) and the corresponding calculated profiles (solid lines) at low and high loadings. Table 3 summarizes the numerical values of the best isotherm parameters.

5.3.1. Adsorption of aniline, o-toluidine, p-toluidine and propylbenzoate: the fifth polynomial isotherm model

The adsorption data of aniline, *o*-toluidine, *p*-toluidine and propylbenzoate were difficult to interpret because each one of them exhibits at least one inflection point. Numerous attempts were carried out to fitting the adsorption data to simpler isotherm models that might have provided that kind of isotherm shape, like the B.E.T. and

the quadratic models which successfully accounted for the adsorption data of low molecular mass compounds in RPLC [19,36]. Except for the adsorption of propylbenzoate, these efforts failed, however, because neither the fit never converged nor the model could describe accurately the adsorption data observed at low and at high concentrations (Statisctic Fisher number *F* below 1000). Furthermore, the positions and the shapes of the calculated band profiles were not consistent with those of the experimental band profiles. The B.E.T. adsorption isotherm could have been selected to describe the adsorption of propylbenzoate, only, ($F = 15,000$) as reported in precedent reports [36,37] but its prediction of band profile was less accurate than the one using a high-order polynomial isotherm.

A more systematic approach was then adopted. The isotherm data were fitted to a series of polynomial isotherm models [6], the order of which was increased stepwise until excellent agreement between experimental data and best
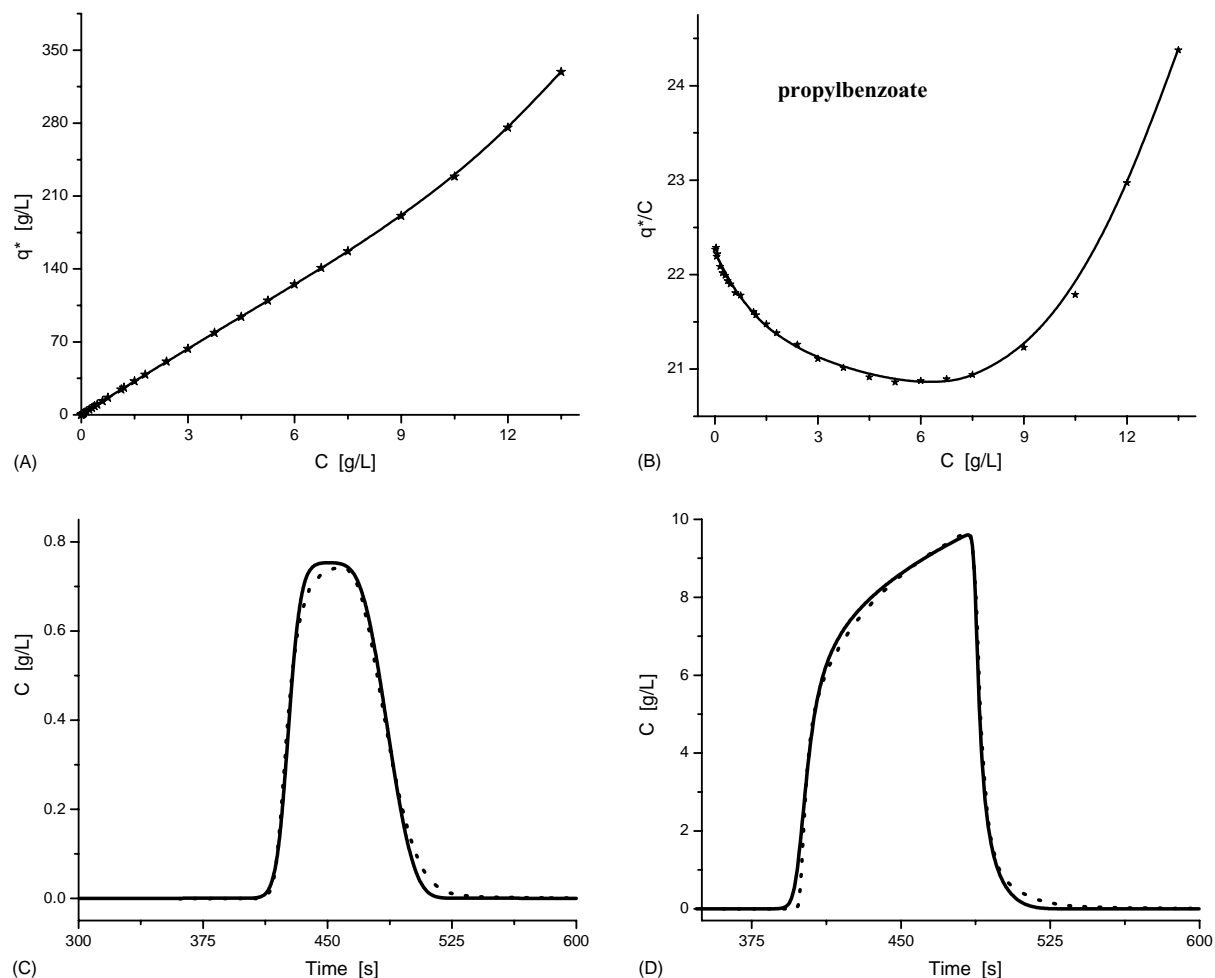
Fig. 6. (A) Experimental isotherm (stars) and best fifth-order polynomial isotherm (solid line) of propylbenzoate on the Chromolith column #30. (B) Experimental isotherm chord q^*/C (stars) and the same best fifth-order polynomial isotherm as in (A) (solid line). (C) Comparison between calculated (solid line) and experimental band profile (dotted line) measured for a low column loading. (D) Comparison between calculated (solid line) and experimental band profile (dotted line) measured for a high column loading.

fitted model was achieved ($F \geq 10,000$). The minimum polynomial order required to achieve such an agreement was five for all four compounds. At this stage, it would be uncertain and certainly incautious to attribute any physical meaning to the parameters of the adsorption isotherms of these four compounds. Their process of adsorption is far more complex than could have been expected with these simple, low molecular mass molecules. This complexity may in part arise from the high accuracy of the data measured that requires more parameters for an accurate fit, especially at low concentration. Whatever it may be, this isotherm model provides an excellent agreement between the experimental and the calculated experimental isotherm data and overloaded band profiles, at low and at high column loading. It is intriguing to observe that, in a similar study, the adsorption isotherm of aniline on C_{18}-Kromasil was found to be best modeled by a Jovanovic isotherm [19], a convex-upward isotherm model that is usually associated with a homogeneous surface.

This suggests a serious difference between the chemistry of the octadecyl-bonded silica material in the Kromasil particles and in the silica monolith studied here. To conclude, the fifth-order polynomial isotherm model gives an accurate prediction of the overloaded band profiles of aniline, o-toluidine, p-toluidine and propylbenzoate recorded on column #30. Despite its lack of physical meaning, it satisfies our goal of comparing the overloaded band profiles measured on the five other Chromolith Performance columns.

5.3.2. Adsorption of phenol and caffeine: the tri- and quadri-Langmuir isotherm models

By contrast with what was observed for the other four compounds, the adsorption isotherm data of caffeine and phenol were described by a convex-upward isotherm with no inflection point. In both cases, the plot of the isotherm chord versus the mobile phase concentration is a monotonically decreasing function, whose convex-
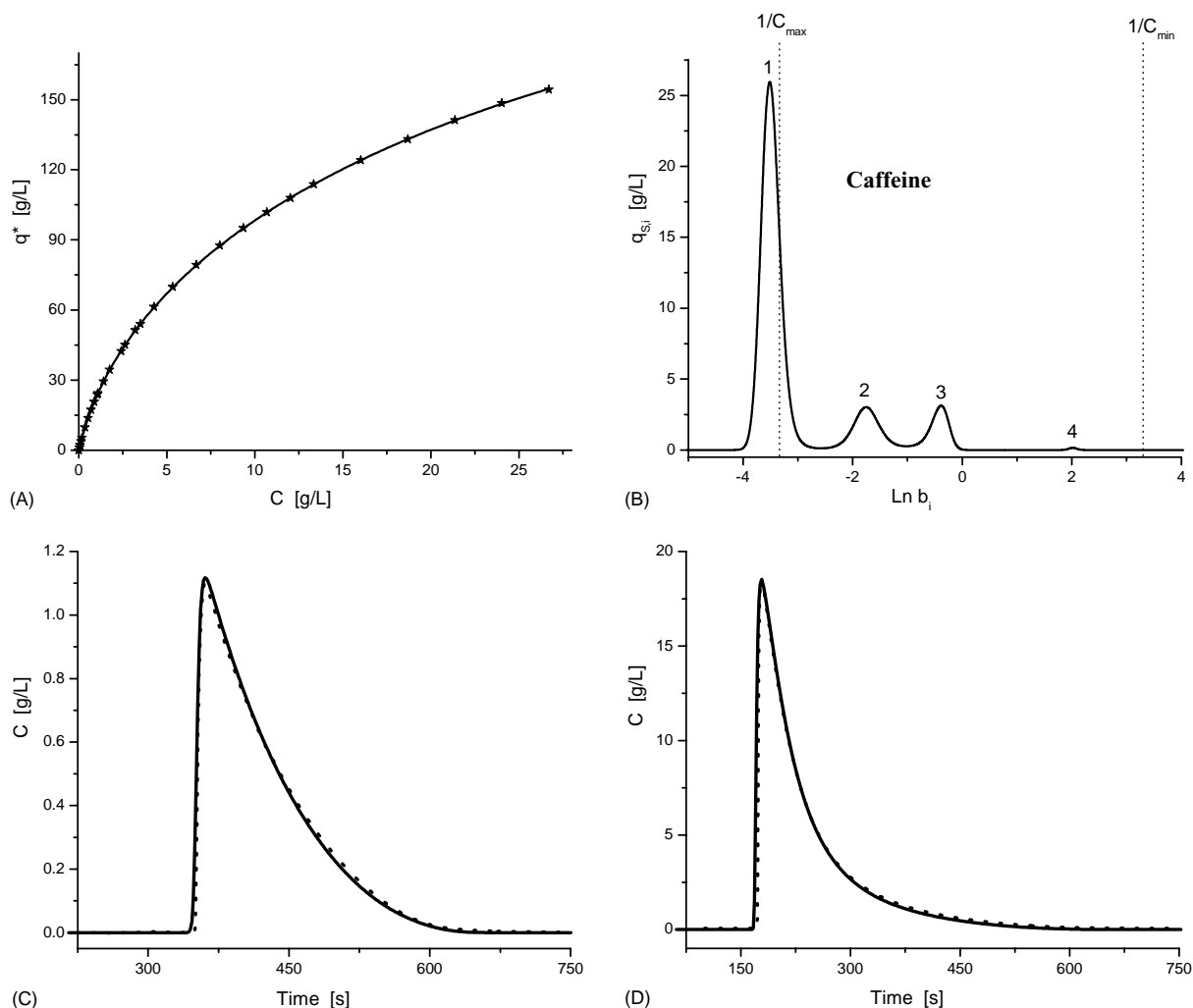
Fig. 7. (A) Experimental isotherm (stars) and best quadri-Langmuir isotherm (solid line) of caffeine on the Chromolith column #30. (B) Quadrimodal adsorption energy distribution calculated from the raw adsorption data by the expectation-maximization method. Local Langmuir isotherm. (C) Comparison between calculated (solid line) and experimental band profile (dotted line) measured for a low column loading. (D) Comparison between calculated (solid line) and experimental band profile (dotted line) measured for a high column loading.

ity remains constant (it is convex downward). The first attempts at accounting for the experimental data with a simple Langmuir or bi-Langmuir model were unsuccessful, probably for the same reasons as in the precedent section ($F \leq 10{,}000$). The affinity energy distribution was then calculated from the raw adsorption data, in order to obtain complementary information on the unusual adsorption behavior of these two compounds on the monolithic column. The AEDs converged to a tri- and quadrimodal energy distribution for phenol and caffeine, respectively (see Figs. 7 and 8). Accordingly, the best parameters of the isotherms of phenol and caffeine were fitted to a sum of three (six parameters) and four (eight parameters) Langmuir isotherms, respectively. Fisher values greater than 100,000 were found in both cases. An excellent consistency was found between the numerical values of the parameters obtained by the AED analysis

and those derived from the direct isotherm fit of the data. The best parameters of the AED calculation were selected and they are listed in Table 3. The validity of the tri- and the quadri-Langmuir model in the case of caffeine and phenol, respectively, was confirmed by the excellent agreement that is illustrated in Figs. 7 and 8 between the experimental and the calculated band profiles of these two compounds.

This result is surprising because, in earlier reports, we showed that the experimental adsorption data acquired for phenol on a number of RPLC columns was best accounted for by the bi-Langmuir isotherm model. This result was first demonstrated for columns packed with particles of C_{18}-Kromasil, in aqueous solutions of methanol, with methanol concentrations between 30 and 60% [38,39]. It was later extended to the concentration range extending down to 0% methanol [40,41] on the same C_{18}-Kromasil. It
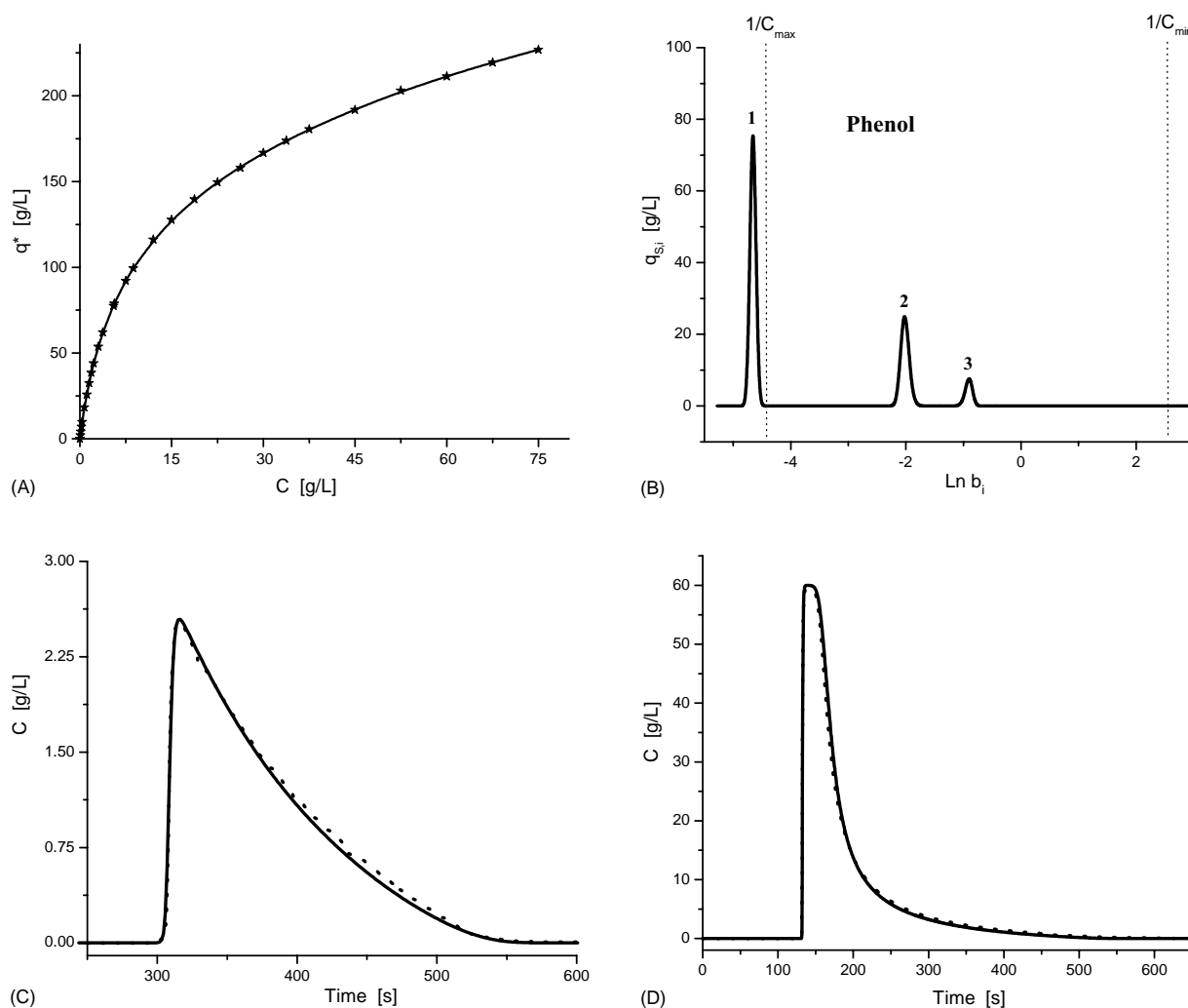
Fig. 8. (A) Experimental isotherm (stars) and best tri-Langmuir isotherm (solid line) of phenol on the Chromolith column #30. (B) Trimodal adsorption energy distribution calculated from the raw adsorption data by the expectation-maximization method. Local Langmuir isotherm. (C) Comparison between calculated (solid line) and experimental band profile (dotted line) measured for a low column loading. (D) Comparison between calculated (solid line) and experimental band profile (dotted line) measured for a high column loading.

also applies to columns packed with Waters Symmetry-C_{18}, Phenomenex Luna-C_{18}, and Hypersil-$_{18}$ particles with mobile phases containing 30% methanol [42]. This latter report discussed the adsorption energy distributions on these packing materials and showed that this distribution was bimodal on the materials just described. This result was also extended to caffeine [38,42] for which these packing materials exhibit also a bimodal adsorption energy distribution. This observation applied also to one of the monolithic column [42] previously used by Kele and Guiochon [18], although, admittedly, the mobile phase composition in this last case was slightly different from the one used here (30% methanol instead of 15% in this work). The validity of the bi-Langmuir model, on the columns and under the experimental conditions reported in these previous publications, was confirmed by the excellent agreement between the experimental band profiles recorded

and those calculated from this isotherm model. Obviously, there are significant differences between the chemistry of the surface of the different adsorbents named here. In Sections 5.3.1 and 5.3.2 we have determined and validated the isotherm models of adsorption for the six compounds investigated on the reference column #30. These models account very well for the recorded band profiles at both low and high concentrations. The experimental and calculated profiles nearly overlay in all cases. The adsorption isotherms and their best parameters can now be used to predict the band profiles measured on the five other monolithic columns (#31 to #35). Any differences between the calculated and the experimental profiles will be characterized by the relative error E defined earlier (Eq. (14)). The value of this empirical parameter will show the degree of reproducibility of the adsorption properties of these columns.

5.4. Prediction of band profiles from isotherm data measured on Chromolith column #30 and comparison with the experimental band profiles measured on five distinct columns

Figs. 9–14 compare the experimental band profiles recorded for each of the six compounds studied, on each of the six Chromolith Performance columns and the corresponding calculated profiles. These profiles were calculated using: (1) the isotherm model derived from the FA measurements made on column #30; and (2) the characteristics (and particularly the phase ratio) of the individual column considered (see Section 4.1.1). Two different sample sizes were used (see end of Section 4). For a given compound
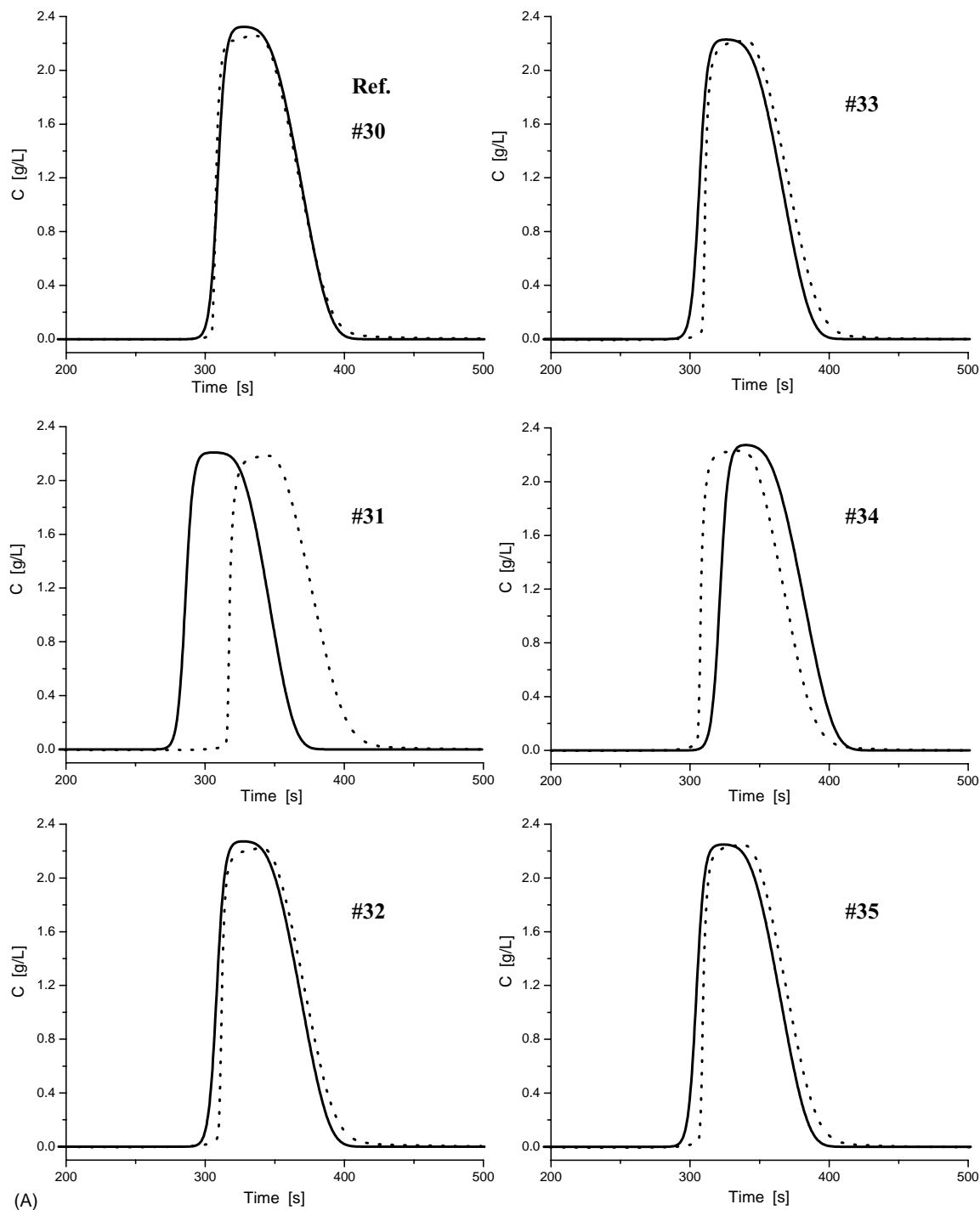
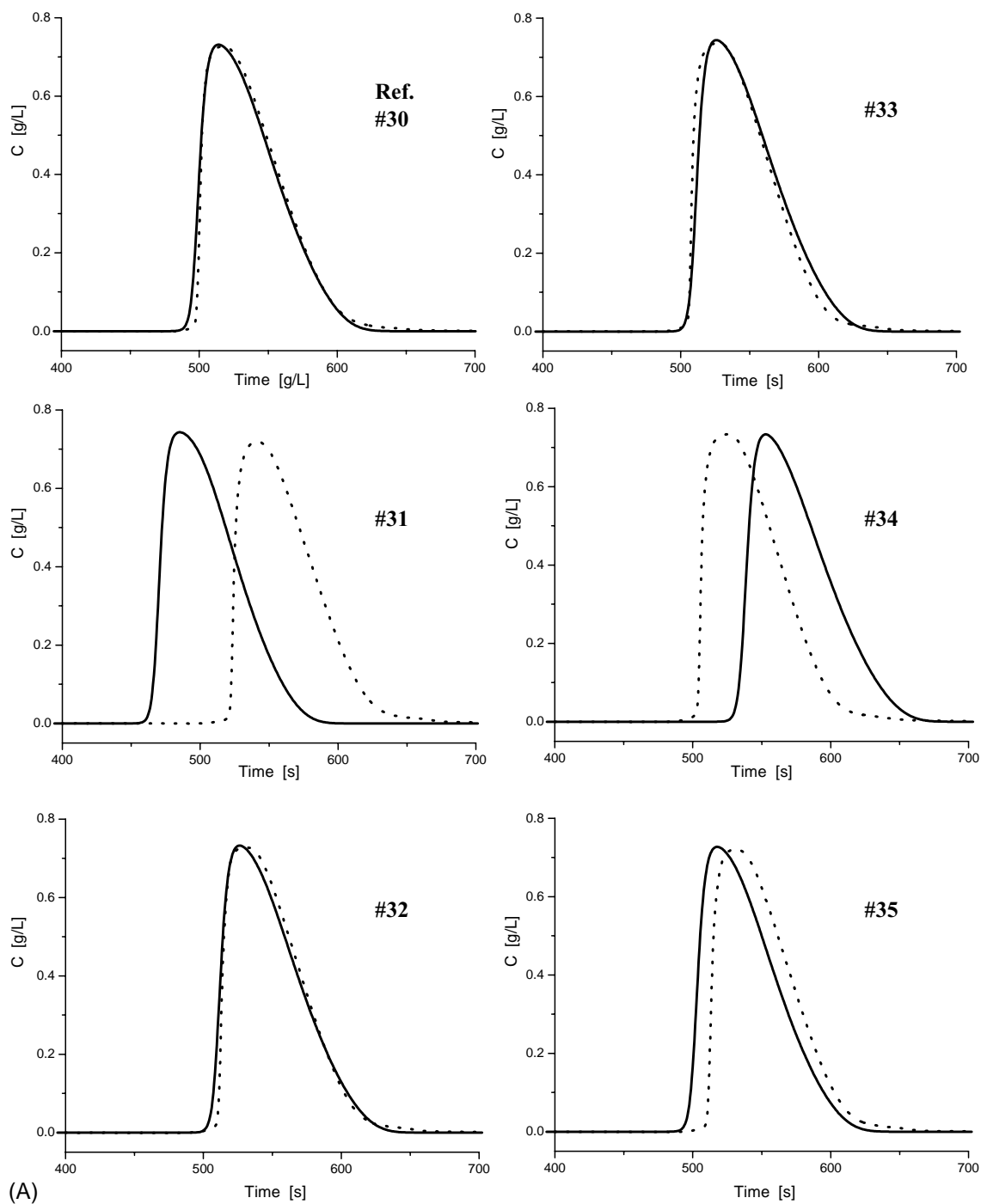
Fig. 9. Comparison between the experimental overloaded (dotted line) and simulated (solid line) band profiles of aniline on the six Chromolith columns. (A) Low loading. (B) High loading.
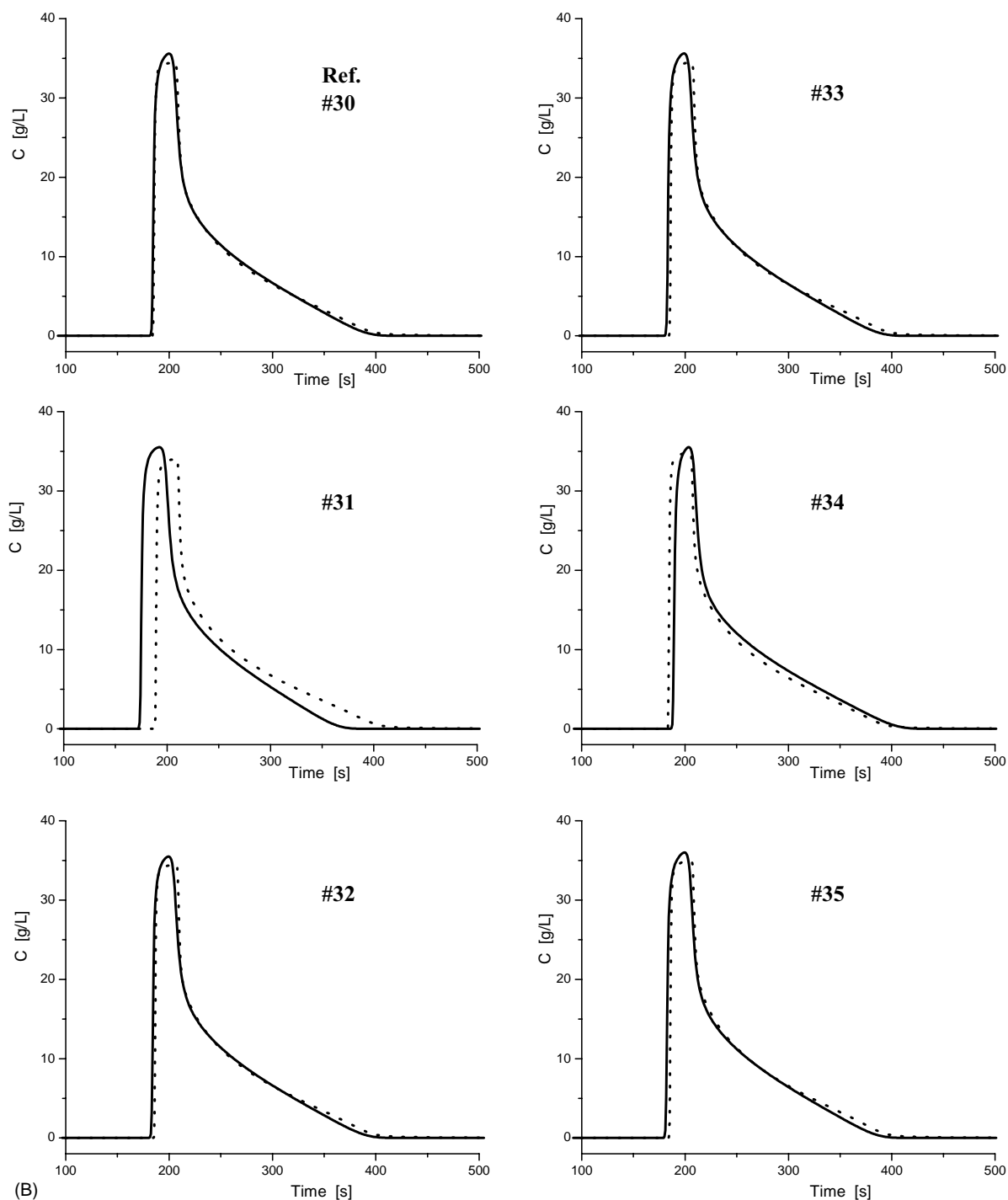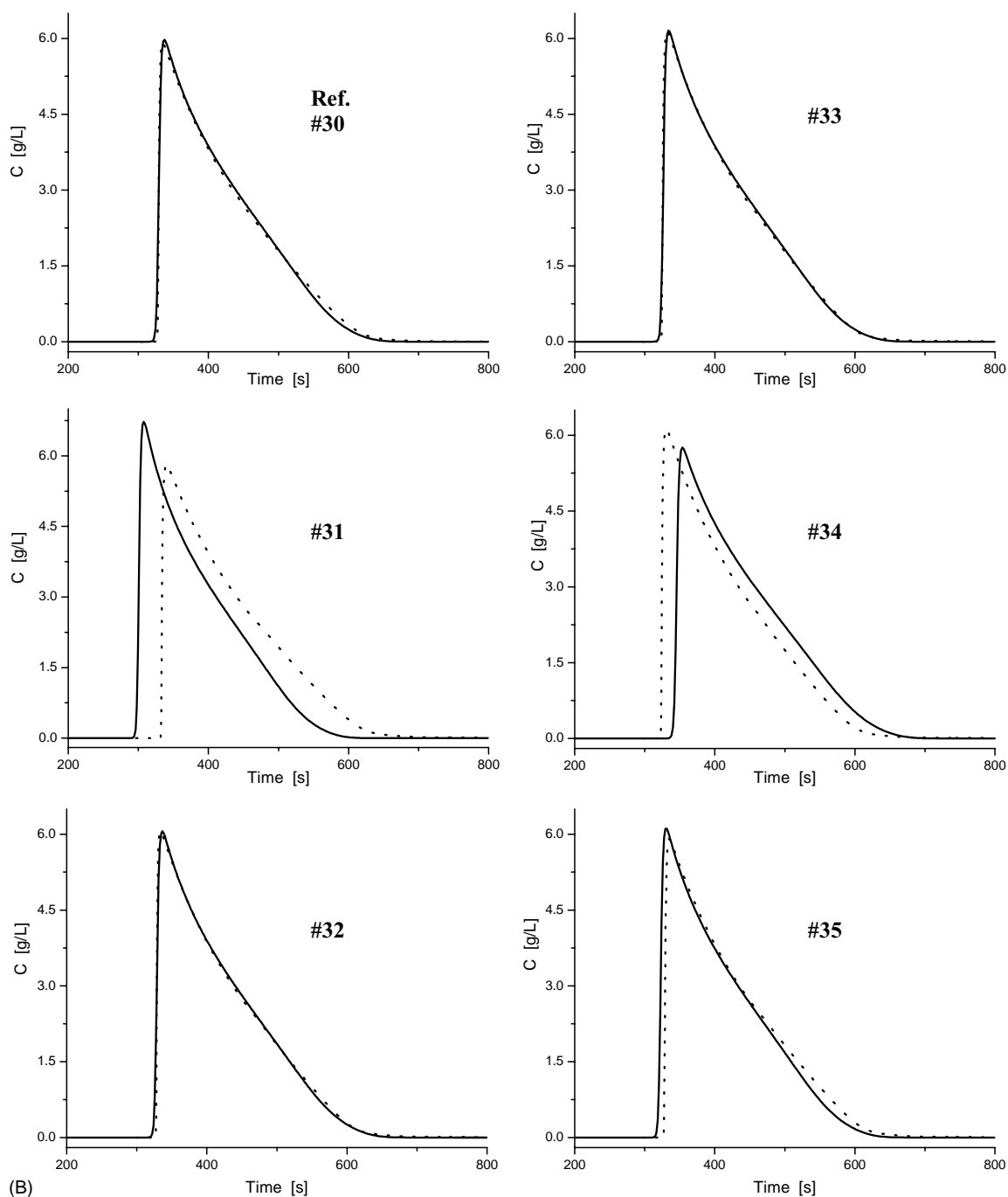
Fig. 9. (*Continued*).

and a given column loading, the column efficiency used in the calculation was that found to be best for column #30, i.e., the one that gives the same height for the calculated band and for the recorded profile (see in Figs. 3–8). So, it was assumed that the column efficiency was the same for all the columns. The rationale for this assumption is that: (1) small changes of the column efficiency do not affect much

the band profiles; and (2) that the columns have very close properties. The experimental profiles in Figs. 9–14 confirm that, indeed, all the columns have nearly the same efficiency for a given compound. Thus, the minor variations in the column efficiency have no significant effect on the value of E. The main information E contains is the thermodynamic one.

Table 4
Relative error E expressed in percentage between the simulated (using the isotherm parameters measured on column #30) and experimental (measured for columns #30 to #35) at low column loading for each compound

|	Phenol	Aniline	Caffeine	o-Tolouidine	p-Tolouidine	Propylbenzoate	Average
#30	+0.14	+0.38	−0.22	−0.24	−0.02	+0.18	+0.04
#31	−14.76	−10.07	−12.96	−10.33	−10.94	−6.41	−10.91
#32	−4.8	−1.25	−4.60	−0.31	+0.03	+2.28	−1.44
#33	−4.14	−1.45	−1.98	+0.64	−0.20	+1.77	−0.89
#34	+1.79	+4.40	+3.57	+6.46	+6.18	+7.39	+4.97
#35	−5.59	−1.63	−3.93	−2.03	−1.15	+0.65	−2.23

Fig. 10. Comparison between the experimental overloaded (dotted line) and simulated (solid line) band profiles of o-toluidine on the six Chromolith columns. (A) Low loading. (B) High loading.
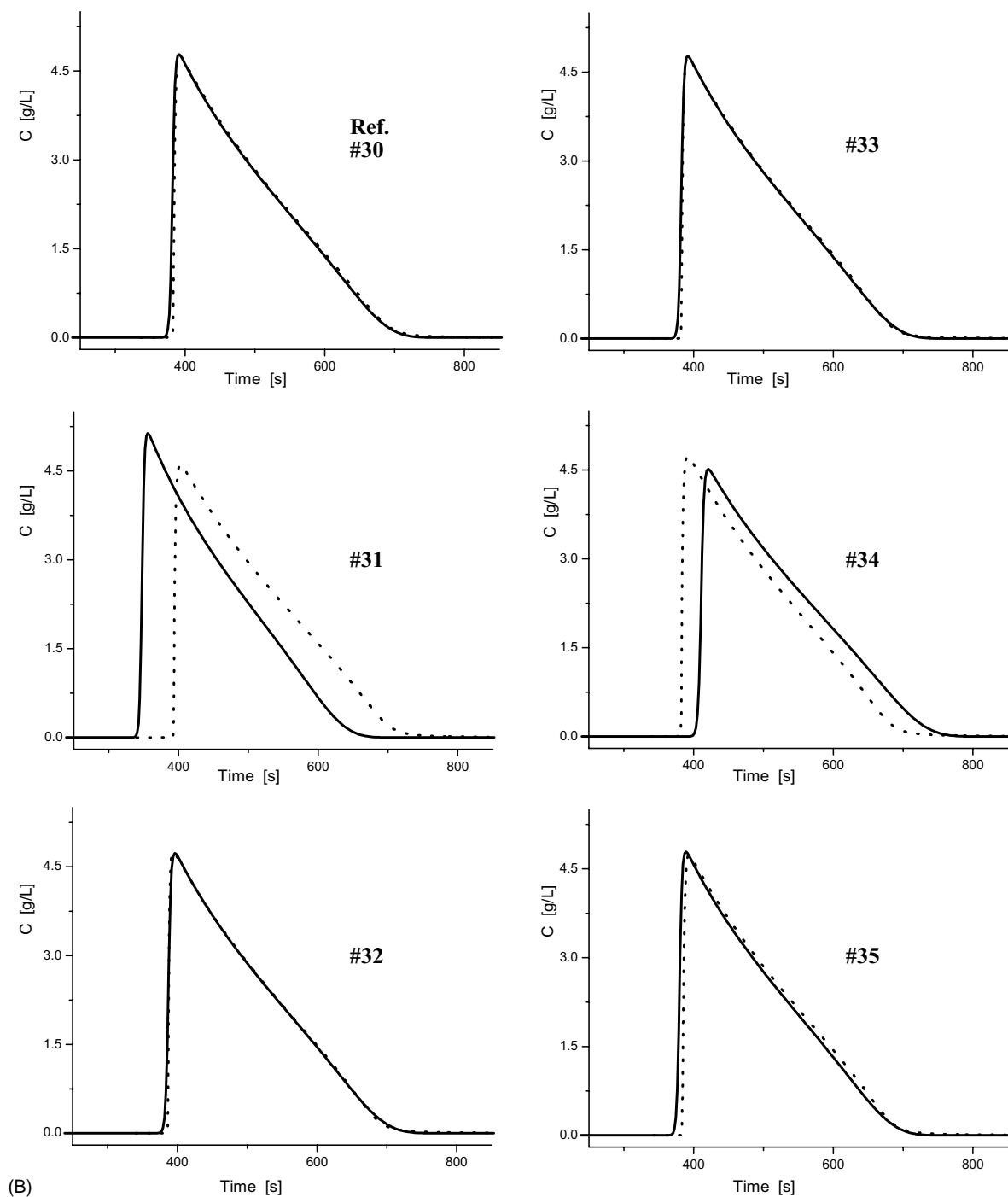
Fig. 10. (*Continued*).

Table 5
Relative error E expressed in percentage between the simulated (using the isotherm parameters measured on column #30) and experimental (measured for columns #30 to #35) at high column loading for each compound

	Phenol	Aniline	Caffeine	*o*-Tolouidine	*p*-Tolouidine	Propylbenzoate	Average
#30	+0.10	−0.40	−1.60	−0.03	−0.60	−0.06	−0.43
#31	−5.53	−7.50	−7.46	−10.01	−11.88	−4.92	−7.88
#32	−1.25	−1.21	−2.46	−0.10	−0.28	+2.22	−0.51
#33	−1.00	−1.42	−0.91	+0.17	−0.38	+1.75	−0.30
#34	+1.63	+2.83	+2.67	+6.27	+7.12	+6.44	+3.31
#35	−1.67	−1.60	−2.36	−1.84	−1.54	+0.73	−1.38

5.4.1. Profiles at low loading factor

Figs. 9A–14A show the low concentration profiles recorded and calculated for the six compounds on the six Chromolith Performance columns. Except for column #31 and #34, there is a very good agreement between these two profiles. The values of the relative error, E, between the retention times of the front shock layer of

the calculated and experimental profiles are reported in Table 4. The calculation of E is based on the position of the front of the profiles, measured at half-height. Fig. 15 shows that E ranges between -5 and $+2\%$ among the four columns #30, #32, #33 and #35, only. However, it is as large as -15 and $+8\%$ for columns #31 and #34, respectively. The curves corresponding to the six com-
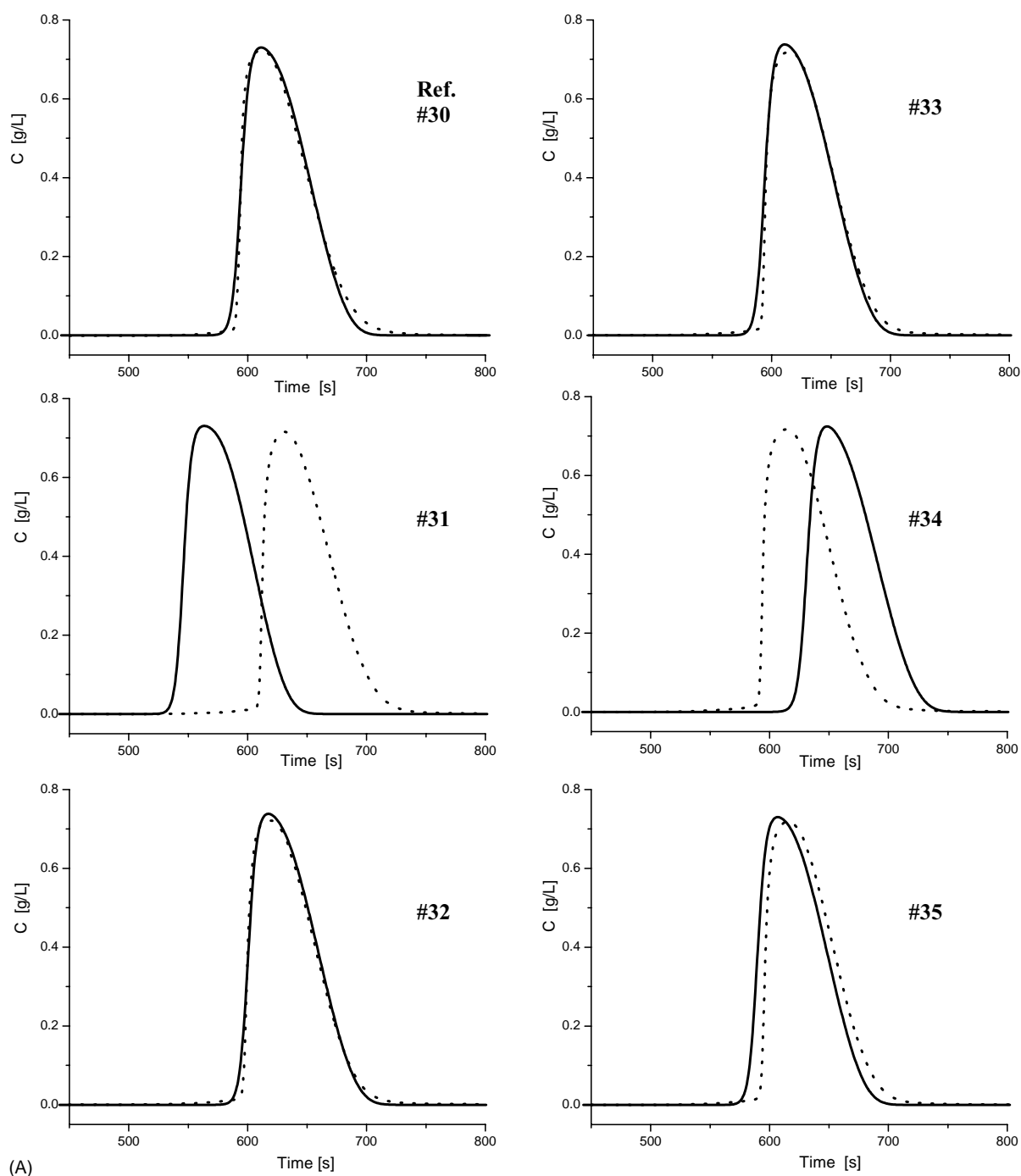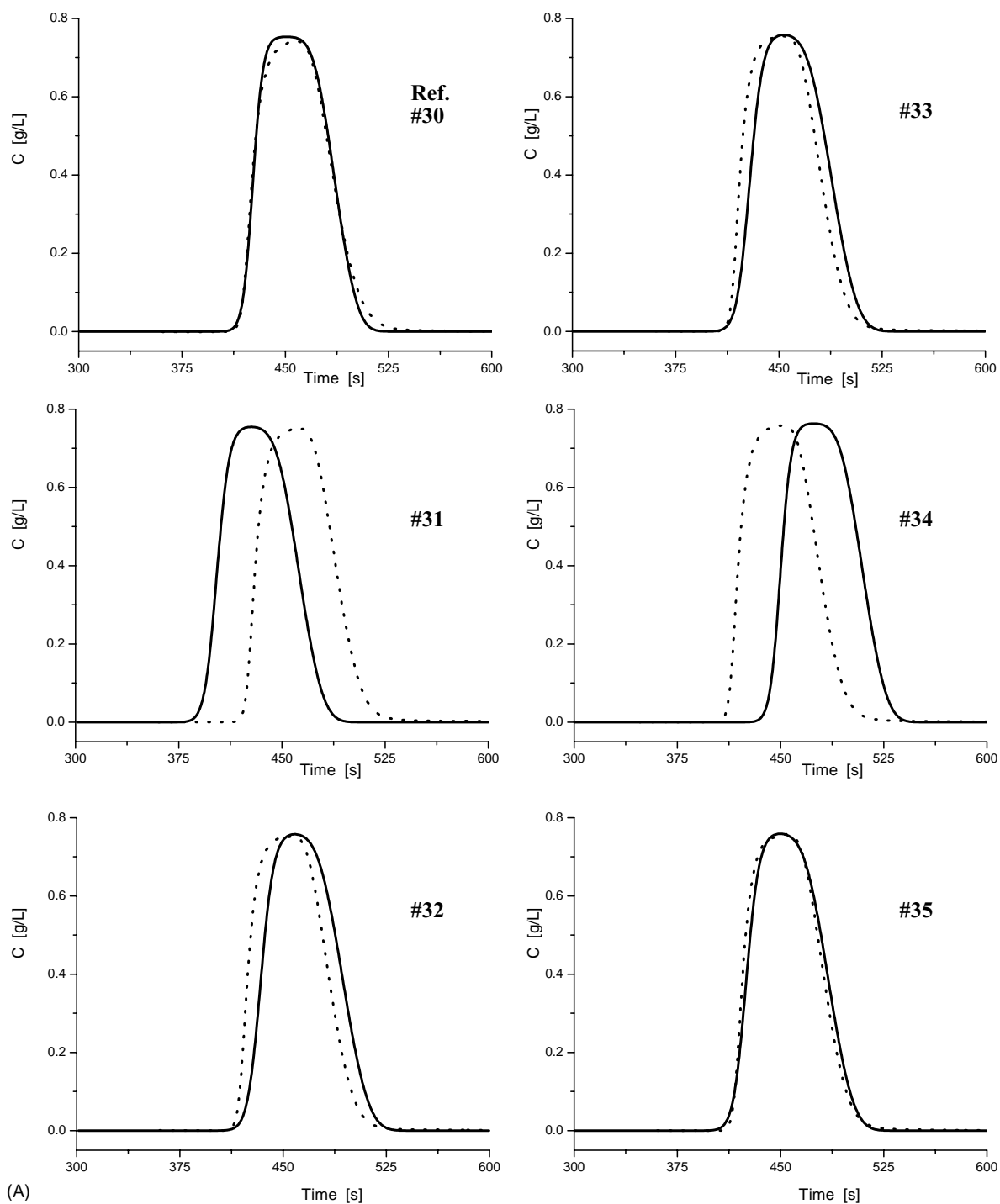

Fig. 11. Comparison between the experimental overloaded (dotted line) and simulated (solid line) band profiles of *p*-toluidine on the six Chromolith columns. (A) Low loading. (B) High loading.

Fig. 11. (*Continued*).

pounds are parallel. This mirrors the fact that the order of increasing adsorption is the same, independently of the compound chosen. The average value of E allows a classification of the columns by increasing order of the adsorption strength of their surface. This order is : #31 $\leq$ #35 $\leq$ # $\leq$ #33 $\leq$ #30 $\leq$ #34. It was unexpected to observe that the column with the highest porosity pected to observe that the column with the highest porosity

(#31) is also the one containing the strongest adsorbent and that vice versa, the column with the lowest porosity is the one exhibiting the weakest adsorption strength (#34). The differences observed earlier regarding the column hold-up volume seem to mirror the obvious discrepancies between the adsorption behavior of the columns studied.
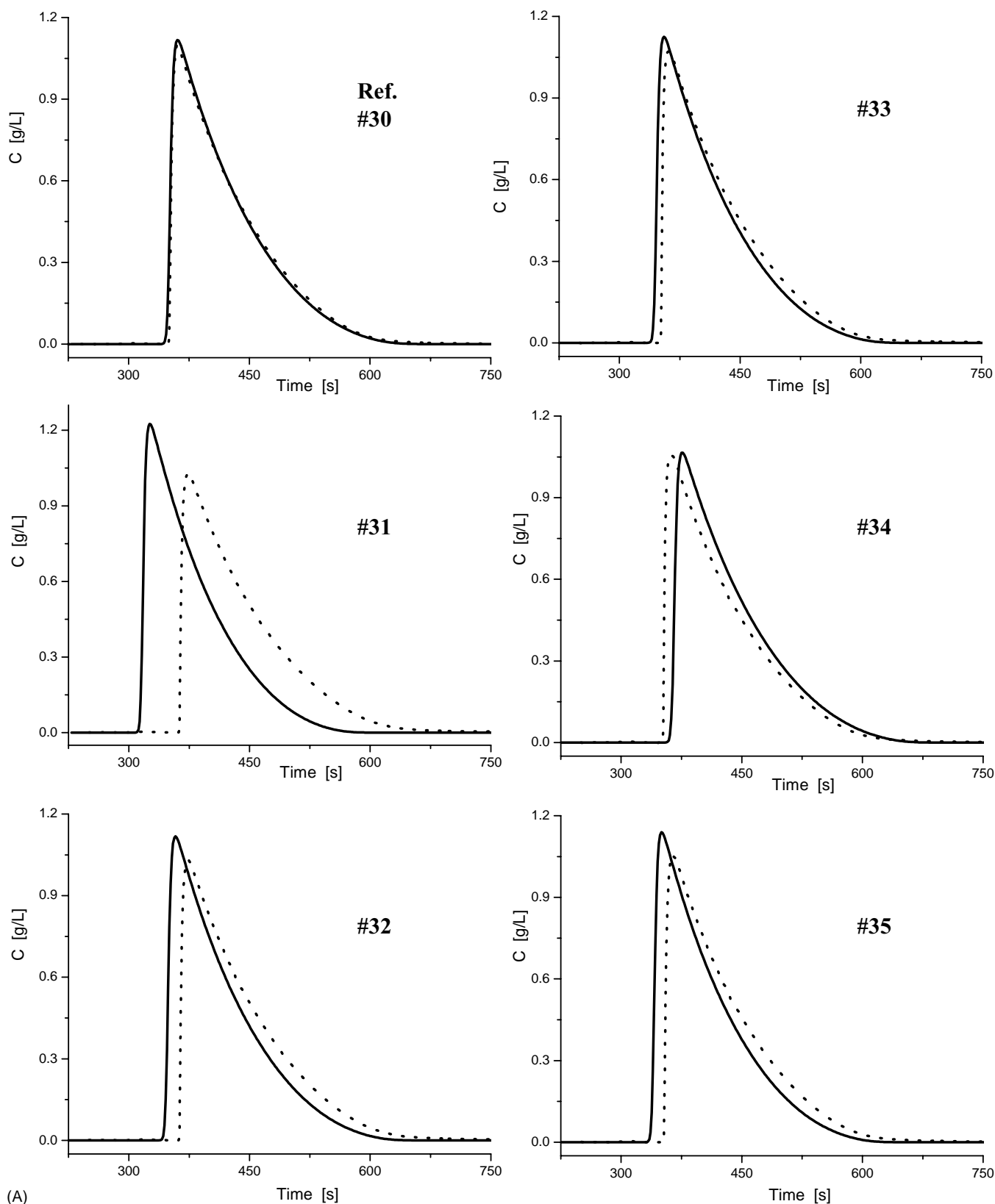
Fig. 12. Comparison between the experimental overloaded (dotted line) and simulated (solid line) band profiles of propylbenzoate on the six Chromolith columns. (A) Low loading. (B) High loading.

5.4.2. Profiles at high loading factors

The calculated and experimental band profiles corresponding to a high column loading factor (Table 5), 16 times larger than in the previous section, are shown in Figs. 9B–14B. Again, columns #31 and #34 are clearly different from the other four columns of the lot studied. A slightly better agreement is observed between the ex-

perimental and the calculated profiles obtained for high loading factors than was obtained between the similar profiles at low loading factors. E varies between -2 and $+2\%$, only, for all the compounds on the four closest columns. It is -12 and $+7\%$ for columns #31 and #34. Fig. 16 shows the same order of relative adsorption strength for the six columns as did Fig. 15, with a minor
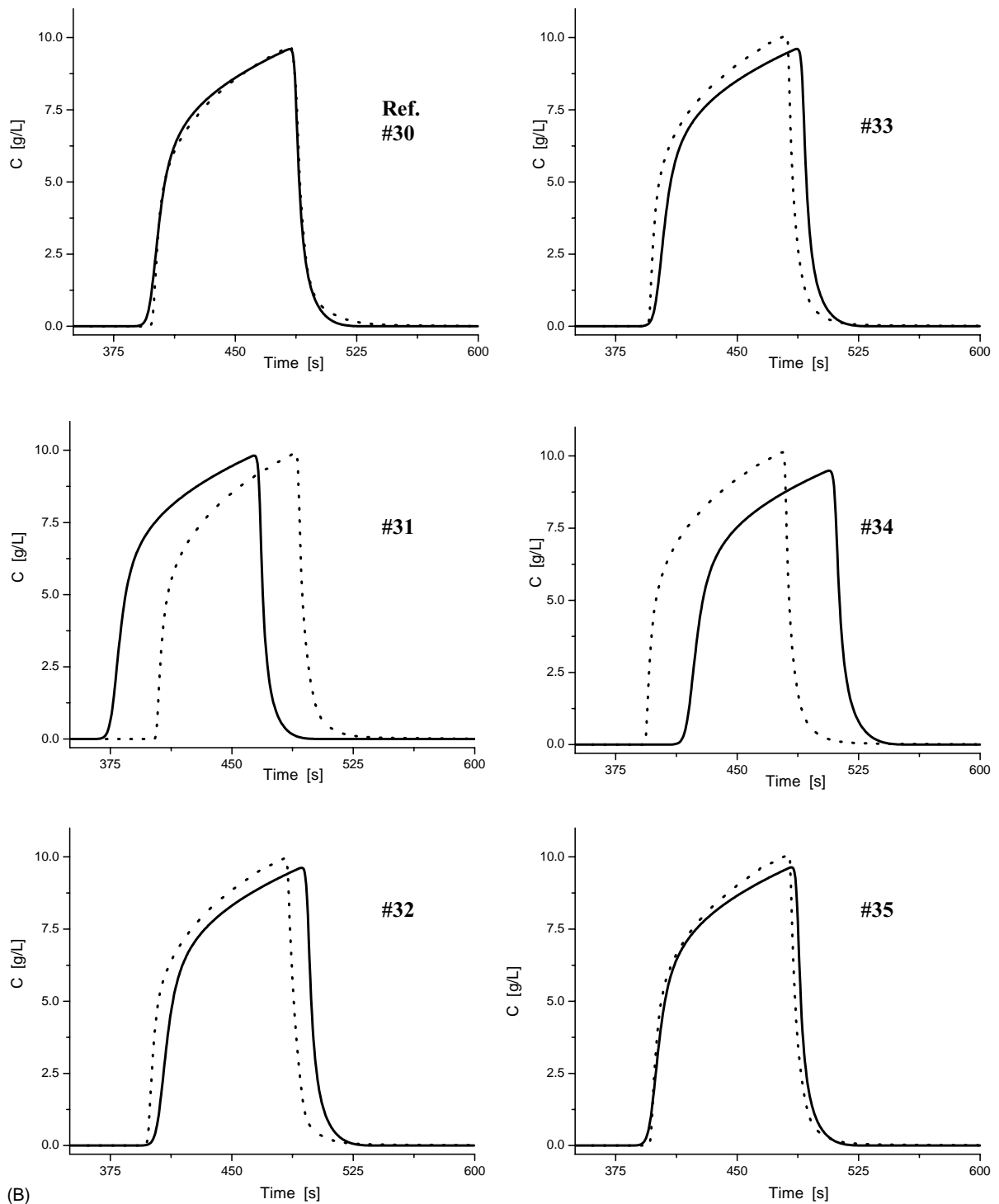
Fig. 12. (*Continued*).

difference, the inversion of columns #30 and #33 in the ranking.

To summarize, columns #30 (the reference colume), #32 and #33 have almost exactly the same properties and can be considered as made of the same material (the average value of $|E|$ is always less than 1.5%). Column #35 has a slighly higher adsorption strength than those three columns (with an average value of E of about −2%). Column #31 has a much stronger adsorption strength that all the others (with an average of E of about −10%) while column #34 has the lowest adsorption strength (the average value of E is about +4%).

Fig. 13. Comparison between the experimental overloaded (dotted line) and simulated (solid line) band profiles of caffeine on the six Chromolith columns. (A) Low loading. (B) High loading.
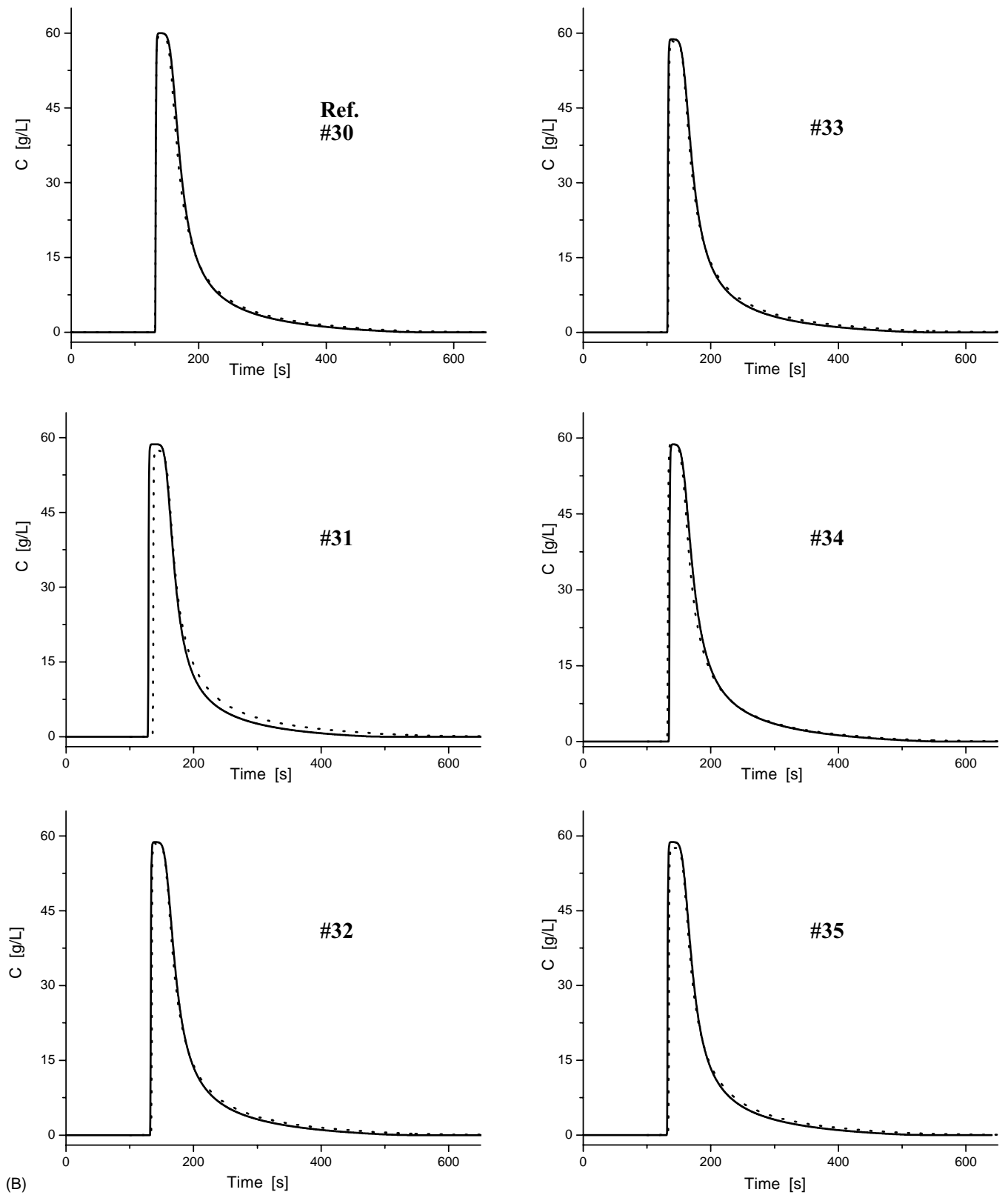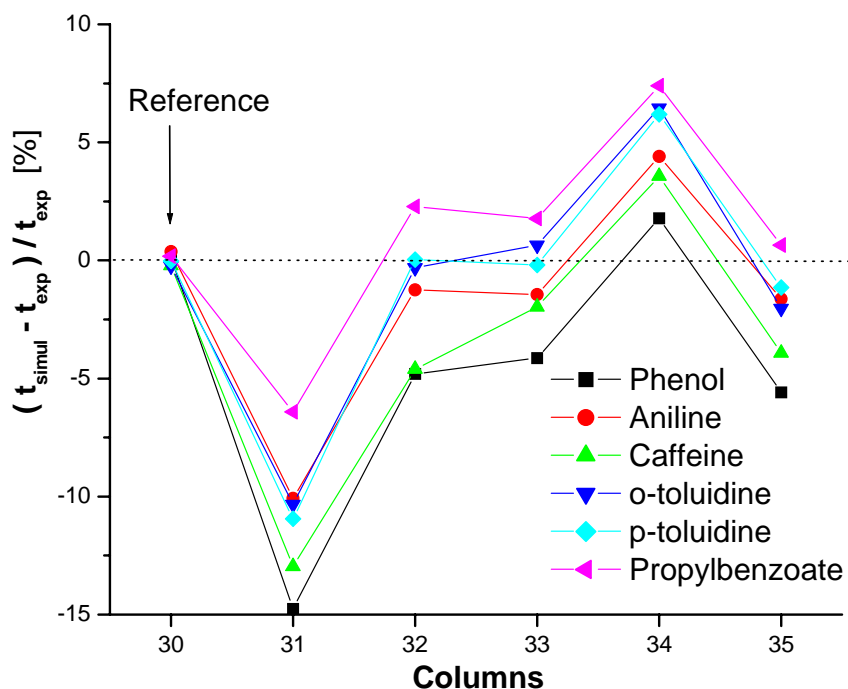
Fig. 13. (*Continued*).

(A)
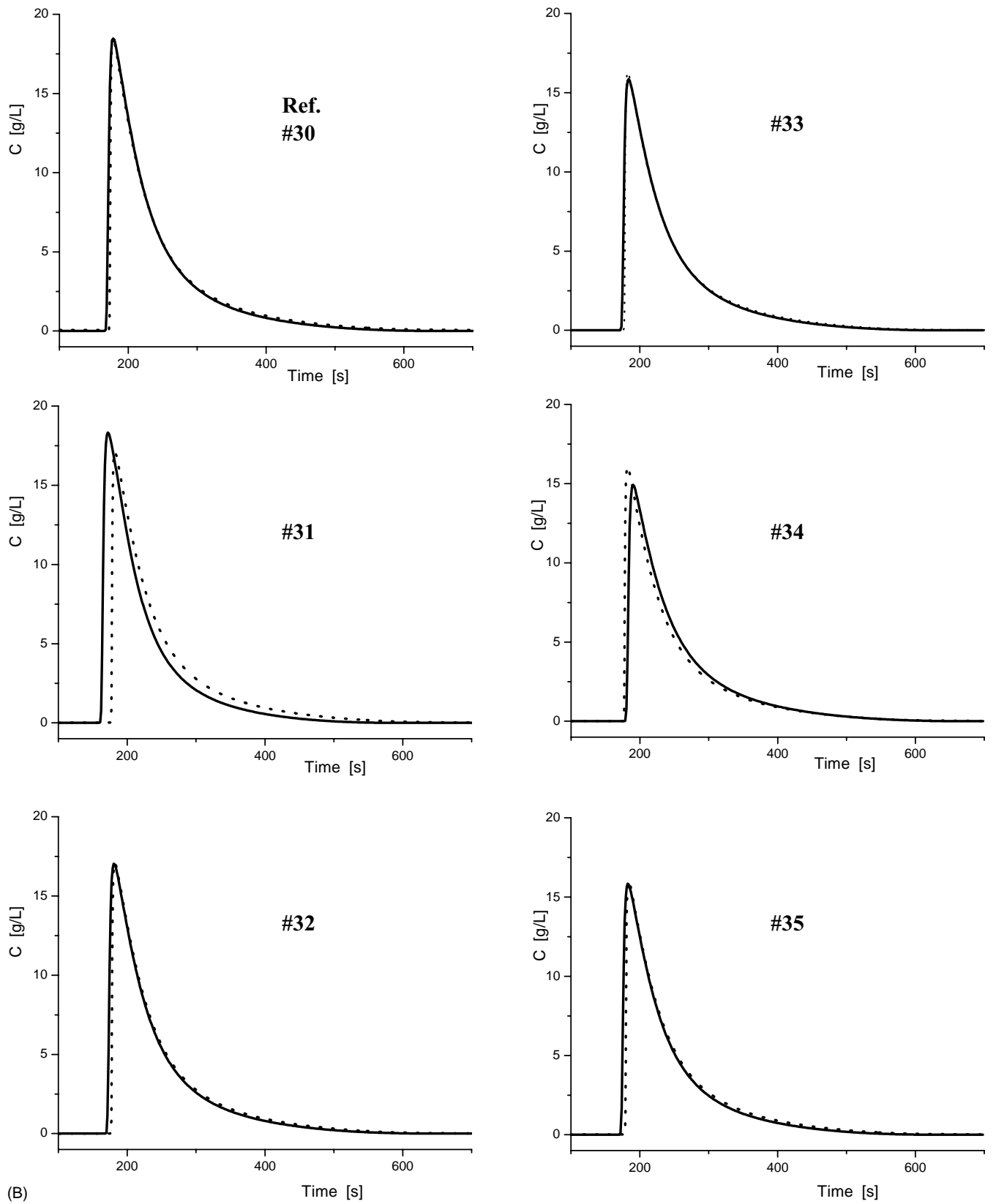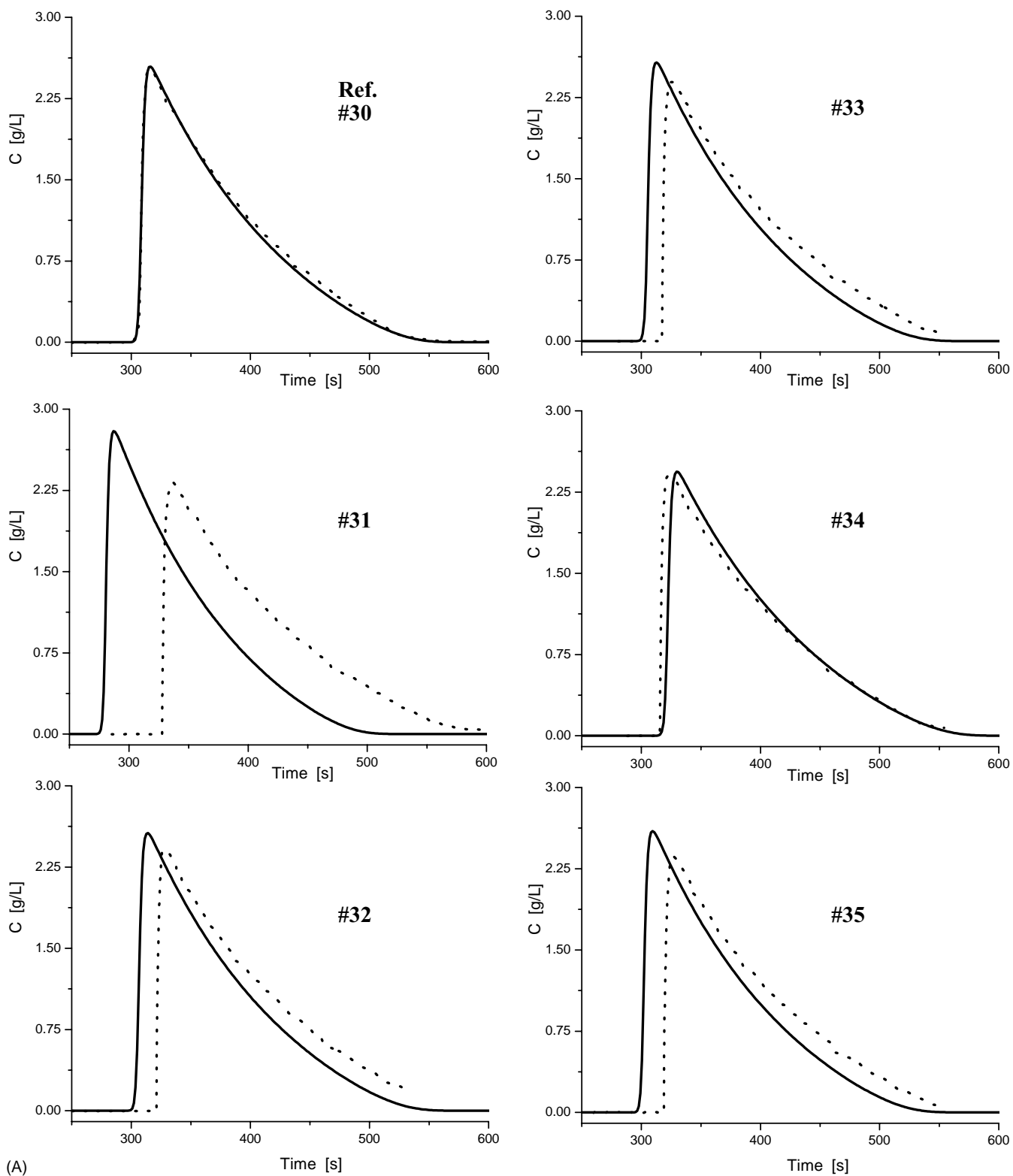
Fig. 14. Comparison between the experimental overloaded (dotted line) and simulated (solid line) band profiles of phenol on the six Chromolith columns. (A) Low loading. (B) High loading.

(B)

Fig. 14. (*Continued*).

Fig. 15. Relative error E between calculations and experiments at low column loading for all the chromatographic systems studied, calculated according to the points defined in Section 4.1.2. Note the parallel evolution of the curves and the significative difference between the groups of columns {#30, #32, #33, #35}, and {#31 and #34}.
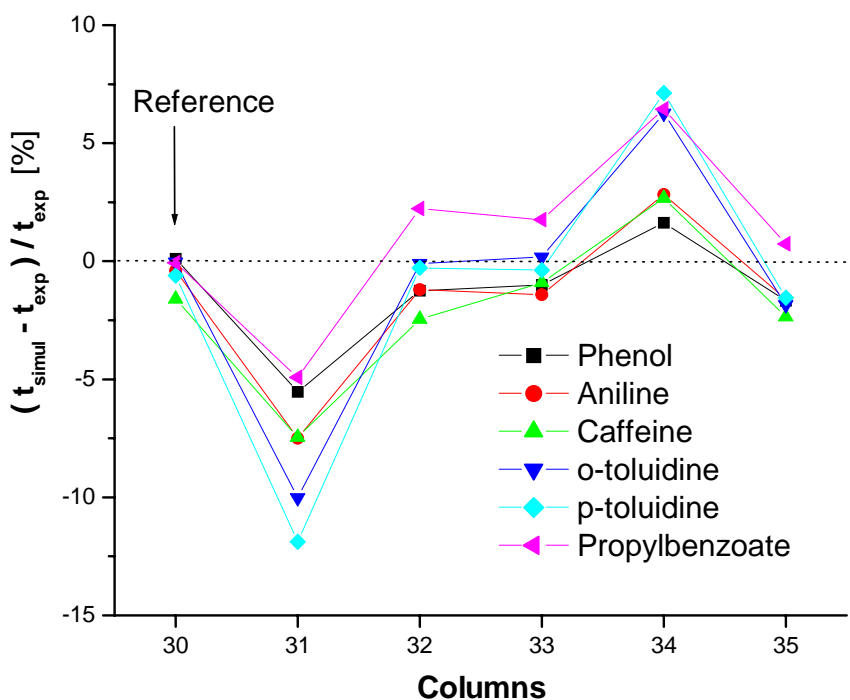

Fig. 16. Relative error E between calculations and experiments at high column loading for all the chromatographic systems studied, calculated according to the points defined in Section 4.1.2. Note, as in Fig. 15, the parallel evolution of the curves and the significative difference between the groups of columns {#30, #32, #33, #35}, {#31 and #34}.

6. Conclusion

Our results demonstrate the good reproducibility of the adsorption data measured on four columns out of a lot of six monolithic columns. The other two columns exhibit a significantly distinct adsorption behavior. The six compounds studied, aniline, phenol, caffeine, *o*-toluidine, *p*-toluidine and propylbenzoate, have markedly different molecular size, polarity and basicity. Unsurprisingly, different adsorption isotherms best model the adsorption data measured by frontal analysis. These isotherms are unusually complex, however. A tri- and a quadri-Langmuir isotherms described the adsorption behavior of phenol and caffeine (in methanol/water, 15/85, v/v), respectively, while on more traditional packing materials this behavior is best modeled by a bi-Langmuir isotherm. A fifth-order polynomial isotherm describes the adsorption behavior of aniline (15/85, v/v), *o*-toluidine, *p*-toluidine (20/80, v/v) and propylbenzoate (60/40, v/v) while the adsorption of aniline on a conventional packing material follows Jovanovic isotherm behavior. Whatever the compound considered, the shape of the band profiles obtained on the six monolithic columns are extremely similar at all loading factors studied. The only differences observed are minor shifts of the retention times axis for four out of the six columns, shifts lower than 5 and 2.5% at low and high loading factors, respectively. The band shapes recorded on the other two columns are also very similar, with larger retention times shifts, between 6 and 15%. The systematic procedure of band comparison used here is rapid and economical. It provides a simple but effective test of the batch-to-batch reproducibility of a large number of columns. Our results imply that the procedure currently followed to prepare monolithic columns is less reproducible than the one used to manufacture conventional packing materials [19]. However, the number of columns tested here is too small to derive general conclusions. This test should be repeated on a larger number of columns to become significant. Quantitative, reasonably accurate information regarding the reproducibility of the isotherm parameters for a series of monolithic columns can be derived from one or several high concentration elution band profiles. This more sophisticated and slower method requires the numerical calculation of the isotherms of the compounds of interest on each column by solving the inverse problem of chromatography and estimating the isotherm parameters from the overloaded profiles, assuming a suitable mathematical form for the isotherm model [13].

Acknowledgements

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