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Accuracy and precision of adsorption isotherm parameters measured by dynamic HPLC methods

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Abstract

The fluctuations of the column temperature, the composition and the flow rate of the mobile phase affect the accuracy and precision of the adsorption isotherm parameters measured by dynamic HPLC methods. Experimental data were acquired by frontal analysis (FA) for phenol in equilibrium between C_{18}-bonded Symmetry and a methanol:water mixture (20:80, v/v), at 303 K and a flow rate of 1 mL/min. The fluctuations of the experimental parameters were 0.1 K for the temperature, 0.1% for the mobile phase composition and 0.001 mL/min for the flow rate. The best isotherm model was shown to be the tri-Langmuir isotherm. Random errors were calculated and shown to agree with experimental results. Overloaded band profiles of phenol were acquired at low (sample size, 100 μL, concentration 3 g/L) and high (same sample size, concentration 60 g/L) loadings, at seven temperatures (298, 300, 302, 303, 304, 306, and 308 K), for seven mobile phase compositions (methanol 16, 18, 19, 20, 21, 22, and 24%), and with seven mobile phase flow rates (0.95, 0.97, 0.99, 1.00, 1.01, 1.03, and 1.05 mL/min), always keeping two experimental parameters at the values selected for the FA runs. Assuming that the isotherm model stays the same, the inverse method (IM) was used to derive the isotherm parameters in each case. Temperature affects the equilibrium constants according to Van't Hoff law. A temperature change of 1 K around 303 K causes a relative variation of 1.5% of the high-energy adsorption constant b_3 and of 0.6% of the saturation capacity q_3. The isotherm parameters are very sensitive to the mobile phase composition, especially the highest energy mode. Both adsorption constants b_2 and b_3 follow the linear strength solvent model (LSSM). A methanol volume fraction change of 1% causes a relative decrease of 3.2 and 5.0% of b_2 and b_3, respectively and a 2% decrease of the saturation capacity q_3. Finally, flow rate changes affect only the saturation capacities. A flow rate change of 1% causes a 2% change in the saturation capacity parameters.

Keywords: Adsorption equilibrium; Frontal analysis; Mobile phase composition; Accuracy; Precision; Adsorption isotherms; Phenol

1. Introduction

The experimental determination of adsorption isotherm is fundamental in order to predict overloaded band profiles in HPLC. Much time and chemicals can then be spared in the process of optimizing the experimental conditions in order to achieve the highest production rate of a given separation process by replacing actual trials and errors with the calculation of overloaded band profiles [1]. Knowing with accuracy the thermodynamics of the chromatographic system is the crucial information required by the programs used in computer-assisted optimization, provided that the column efficiency is high enough.

The acquisition of isotherm data may be done by static methods, which are poorly accurate, by dynamic chromatographic methods, or by numerical methods [1]. All have their advantages and limits in terms of rapidity, consumption of chemicals, and accuracy. The accuracy of the isotherm data were already assessed by investigating the column-to-column reproducibility of isotherms for different lots of packed Kromasil columns [2,3] and monolithic Chromolith columns [4]. For a single column and when an accurate measurement of the isotherm becomes the main request, the best method used for isotherm determination is frontal analysis chromatography. This method consists in recording breakthrough curves at increasing plateau concentrations and measuring the mass of analyte adsorbed at

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equilibrium by applying the principle of mass conservation. This method is long and tedious as it requires the acquisition of an important number of data points. The acquisition of a complete isotherm containing between twenty and thirty data points usually takes about 12 h when the retention time under analytical conditions is about 10 min and the flow rate 1 mL/min. During this long period of time, some set-up conditions may not remain constant (e.g., the temperature of the chromatographic system, the average column pressure, the mobile phase composition, or the flow rate delivered by the pumps). All these experimental parameters must be controlled accurately in order to record consistent adsorption data from the first breakthrough curve to the last, no matter the time delay that separates them. However, there is yet no discussion in the literature devoted to the effect of uncontrolled experimental parameters on the accuracy of isotherm determination. There is no information on the extent to which slight modifications of these parameters may affect individually the isotherm parameters whose values are critical for further investigations of a separation process.

The aim of this work is to assess quantitatively the precision of isotherm data by measuring the influence of the fluctuations of the experimental parameters, the temperature, the mobile phase composition, and the mobile phase flow rate, on the isotherm parameters obtained by the dynamic methods of determination of isotherm data. By contrast to what some authors did before, the errors on these parameters were not generated using a simulation program or any theoretical model. They were determined directly by measuring the effect of small changes while the parameters were tightly controlled, the temperature by a thermostat (accuracy 0.1 K), the mobile phase composition (obtained by mixing two streams of constant flow rates, accuracy on the volume fraction of methanol 0.1%) and the flow rate delivery (accuracy 1 μL/min). The elementary error steps for the temperature, the mobile phase composition and the flow rate delivery were fixed at 1 K, 1% and 10 μL/min, respectively. The interval was 10 K, 8% and 100 μL/min large and centered at 303 K, 20% and 1 mL/min for the temperature, the mobile phase composition, and the flow rate delivery, respectively. The best isotherm parameters for each parameter combination were estimated by using the inverse method (IM) of isotherm determination. The model of isotherm chosen in the inverse method procedure was determined from frontal analysis (FA) data measured at the central values. Finally, the sensitivities of the isotherm parameters to fluctuations of the three experimental parameters was obtained by interpolation.

1.1. Determination of the adsorption isotherm data by frontal analysis (FA)

Frontal analysis [1,5,6] was used to measure the single-component adsorption isotherm data used in this work. The mobile phase composition is selected so that the retention of the probe is sufficiently large to allow accurate measurements of retention data. The derivation of the amount of the studied compound adsorbed on the column at equilibrium with a solution of known concentration is explained in details elsewhere [7].

1.2. Sources of error in isotherm determination by FA

Fluctuations of the experimental parameters during a series of measurements causes fluctuations in the coordinates of the data points, hence errors in the isotherm parameters. There are several important sources of error when adsorption data are measured with a dynamic method such as the FA method. The distribution of the analyte between the stationary and the mobile phases depends on the stationary phase selected (e.g., silica structure, nature of the bonded material, surface density of the bonded layers, etc.), the mobile phase composition (e.g., percentage of organic modifier in an aqueous solution), the temperature and, possibly the flow velocity. The latter may influence data in two different ways. First, a systematic error can be made if the isotherm data are measured before equilibrium is reached. The use of the integration of the breakthrough curve to determine the amount of solute adsorbed should eliminate this source of error. Also, flow rate fluctuations are associated with fluctuations of the pressure gradient along the column. Although this effect is usually minor, there are cases in which the pressure dependence of the isotherm is sufficient to cause additional errors.

The errors caused by fluctuations of the experimental parameters can be assessed from classical models of the relationship between retention and the experimental parameters investigated. For instance, it is well known that the equilibrium constant is related to the temperature through Van't Hoff equation. The relative error on the equilibrium constant b caused by small fluctuations of the temperature of amplitude ΔT can be written:

$$\frac{\Delta b}{b} = \frac{\epsilon}{RT^2}\Delta T \tag{1}$$

where ϵ is the adsorption energy at temperature T. The linear strength solvent model (LSSM) gives a good approximate description of the relationship between the equilibrium constant and the organic modifier content, φ, of the aqueous mobile phase in RPLC. The relative error made on the equilibrium constant for a small variation of the mobile phase composition, $\Delta\varphi$, is then:

$$\frac{\Delta b}{b} = S\Delta\varphi \tag{2}$$

where S is the slope of the empirical $\ln b$ versus φ plot.

Probably, in part, because there has been few systematic determinations of the dependence of the saturation capacities of adsorbents on the experimental conditions, there are yet no general laws describing the variation of the saturation capacity of an adsorbent with either the column temperature or the mobile phase composition (it should be independent

of the flow rate but not necessarily of the pressure). Experimental data are still required in each particular case and empirical conclusions will be presented later.

There are a few other sources of errors, arising not from the limited stability of the experimental parameters but from the measurement errors made on the intermediate data needed to calculate the isotherm data points, the data used in FA to calculate the mass of adsorbed component and its concentration in the adsorbed phase. These parameters are the mobile phase flow rate, F_v, the column tube volume, V_c (e.g. the column diameter ϕ and its length, L), the column hold-up time, t_0 (or the total column porosity $\epsilon_t = F_v t_0 / V_c$) and the extra-column time, t_e, or transit time between the pump mixer and the column inlet, and between the column outlet and the detector cell. In the case of a strictly convex upward isotherm, when the mass transfer kinetics are fast, the front of the breakthrough curve is a thin shock layer which is nearly symmetrical. Then, the general expression for calculating the mass adsorbed per unit of adsorbent volume is given by:

$$q^* = \frac{F_v(t_{shock} - t_0 - t_e)C}{V_c - F_v t_0} \quad (3)$$

where t_{shock} is the elution time of the front shock of the breakthrough curve and C the plateau concentration at equilibrium with which q^* is calculated.

Differentiation of Eq. (3) gives the error propagation coefficient of any parameter. For instance, the impact of the measurement error made on the flow rate on the concentration q^* is:

$$\frac{\Delta q^*}{q^*} = \frac{1}{1 - \epsilon_t} \frac{\Delta F_v}{F_v} \quad (4)$$

Note that, in practice, it is far more accurate to derive q^* from the integral of the breakthrough curve. The effect of the mass transfer kinetics on the isotherm data point is drastically reduced, provided the breakthrough curve can be registered until the outlet concentration of the eluent is equal to its inlet concentration, C. Then, the isotherm data points are independent of the flow rate as they should be.

Similar equations can be derived for the other parameters V_c, t_0, and t_e but this does not concern the experiments performed in this work.

1.3. Model of isotherm

Previous studies have shown that the isotherm model that best accounts for the adsorption behavior of phenol on C_{18}-bonded adsorbents such as the one used in this work is a tri-Langmuir isotherm [8–10]. The existence of three different types of sites was attributed to the coexistence on the surface of sites differently buried inside the bonded alkyl layer and, so, partaking differently in adsorption and partition in the complex structure of C_{18}-bonded chains. The tri-Langmuir model assumes that the surface consists in three different patches of sites, each homogeneous and acting independently from the other ones, and on each of which a different Langmuir model applies. So, the isotherm equation is:

$$q^* = q_{s,1} \frac{b_1 C}{1 + b_1 C} + q_{s,2} \frac{b_2 C}{1 + b_2 C} + q_{s,3} \frac{b_3 C}{1 + b_3 C} \quad (5)$$

where $q_{s,1}$, $q_{s,2}$, $q_{s,3}$, b_1, b_2 and b_3 are the monolayer saturation capacities and the low-concentration equilibrium constants for sites 1, 2, and 3, respectively.

The equilibrium constants b_1, b_2, and b_3 are associated with the adsorption energies $\epsilon_{a,1}$, $\epsilon_{a,2}$ and $\epsilon_{a,3}$, through the following equation [11]:

$$b_i = b_0 e^{\epsilon_{a,i}/RT} \quad (6)$$

where $\epsilon_{a,i}$ is the energy of adsorption, R is the universal gas constant, T is the absolute temperature and b_0 is a pre-exponential factor that could be derived from the molecular partition functions in both the bulk and the adsorbed phases. b_0 is often considered to be independent of the adsorption energies, $\epsilon_{a,i}$ [11]. Another treatment of the adsorption equilibrium data can be used to supply the affinity energy distribution [11]. It is important to note here that it is not possible to derive accurate estimates of the absolute values of the adsorption energies without making an assumption regarding b_0. However, it is possible to determine the difference between two adsorption energies (i.e., the difference between the energies of two modes, see later, Fig. 2) directly from the distribution of adsorption constants which is afforded by the method used here and described elsewhere [8–10].

1.4. The inverse method of isotherm determination

This method consists in adjusting the coefficients of an isotherm model in order to minimize the differences between a recorded experimental band profile and the profiles calculated with the equilibrium-dispersive model of chromatography (see next section) and the isotherm model selected. The main advantage of this method of isotherm determination is that it requires the measurement of only one or, at most, a few overloaded band profiles [12–15]. Accordingly, the method is fast and requires only relatively small amounts of solvent and sample. This method was described and discussed previously [16]. It gives results that are in excellent agreement with those of FA [15].

1.5. Modeling of band profiles in HPLC

The equilibrium-dispersive model (ED) of chromatography was used to calculate the overloaded band profiles of phenol needed for the IM procedure [1,17,18]. This model assumes instantaneous equilibrium between the mobile and the stationary phases 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 tri-Langmuir model was used in this case since it had been shown previously to account for the adsorption data of phenol in the system studied.

The axial dispersion coefficient is related to the column efficiency by:

$$D_a = \frac{uL}{2N} \quad (7)$$

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 measured under linear conditions, i.e., with a small sample size, so that the product $b_i C_M$, where b_i is the largest equilibrium constant of the tri-Langmuir model (Eq. (5)) and C_M the maximum concentration of the elution band, be significant compared to 1, i.e., of the order of at least 0.4 [1].

1.5.1. Initial and boundary conditions for the ED model

At $t = 0$, the concentrations of the solute and the adsorbate in the column are uniformly equal to zero (except in staircase FA), and the stationary phase is in equilibrium with a stream of the pure mobile phase. The boundary conditions used are the classical Danckwerts-type boundary conditions [1,19] at the inlet and outlet of the column.

1.6. Numerical solutions of the ED model

The ED model was solved using the Rouchon program based on the finite difference method [1,20–22].

2. Experimental

2.1. Chemicals

The mobile phases used in this work were aqueous solutions of methanol with concentrations between 16 and 24% (v/v). Both water and methanol were of HPLC grade, purchased from Fisher Scientific (Fair Lawn, NJ, USA). Prior to their use, the solvents were filtered on an SFCA filter membrane, 0.2 μm pore size (Suwannee, GA, USA). Thiourea was chosen to measure the column hold-up volume. Phenol was the only solute used. Thiourea was obtained from Aldrich (Milwaukee, WI, USA).

2.2. Columns

The column used in this study (Symmetry-C_{18}) was given by the manufacturer (Waters, Milford, MA, USA). It is 1 of the lot of 15 columns previously used to test the column-to-column and batch-to-batch reproducibility under linear conditions [23]. The tube dimension is 150 × 3.9 mm. The main characteristics of the packing material are summarized in Table 1. The hold-up volume of this column was derived from the elution volume of two consecutive thiourea injections (1.077 mL). The column porosity remained constant at 0.601, whatever the temperature and the mobile phase composition used in this study.

Table 1
Physico-chemical properties of the C_{18}-bonded packed Symmetry column (150 mm × 3.9 mm)

Particle shape	Spherical
Particle size (μm)	5
Pore size[a] (Å)	86
Pore volume[a] (mL/g)	0.90
Surface area[a] (m^2/g)	346
Total carbon (%)	19.6
Surface coverage (μmol/m^2)	3.18
Endcapping	Yes
Total column porosity	0.6010[b]

[a] Data for the packings before derivatization.
[b] Data from thiourea injections in a methanol/water mobile phase (20/80, v/v).

2.3. Apparatus

The perturbation signals and the overloaded band profiles were acquired using a Hewlett-Packard (Palo Alto, CA, USA) HP 1100 liquid chromatograph. This instrument includes a multi-solvent delivery system (volume of each tank, 1 L), an auto-sampler with a 100 μL sample loop, a UV-vis detector, a column thermostat and a data station. The extra-column volumes are 0.10 and 0.50 mL, as measured from the auto-sampler and from the pump system, respectively, to the column inlet. All the retention data were corrected for these contributions. 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 0.1%, so we estimate the long-term accuracy of the flow rate at 1 μL/min at flow rates around 1 mL/min. The temperature was controlled by the thermostat at ± 0.1 K.

2.4. Measurements of overloaded band profiles of phenol

The measurements of overloaded band profiles of phenol were made using the auto-sampler syringe (maximum volume 100 μL). Hundred microliters samples of solutions at 3 and 60 g/L were injected to record low and relatively high overloaded band profiles, respectively. These profiles were recorded at 290 nm. The UV signal was transformed into a concentration profile by using the calibration curve obtained from the plateau concentrations measured during FA at the same wavelength of 290 nm. Segments of the elution profiles having between 500 and 1000 data points were used to perform the IM calculations and derive the isotherm parameters.

3. Results and discussion

3.1. Adsorption of phenol on Symmetry-C_{18}

The main source of systematic errors introduced by the inverse method of isotherm determination arises from the important contribution that non-equilibrium phenomena, axial dispersion and mass transfer kinetics between the stationary and the mobile phases, may have on the exact shape of the overloaded band profiles. Accordingly, the use of an incorrect model of chromatography in the calculation program of the band profiles could lead to important errors in the estimation of the thermodynamic parameters. Unless previous experience with the particular problem investigated informs otherwise, it is cautious to acquire clearly correct equilibrium isotherm data using an accurate method like FA, and to model these data before performing isotherm determinations by the IM method. Then, this method can be used for the systematic, rapid determination of isotherms in a wide range of experimental conditions.

In this study, we measured the influence on the isotherm parameters of the temperature, between 298 and 308 K, the methanol concentration in the mobile phase, between 16 and 24%, and the flow rate, between 0.95 and 1.05 mL/min. The equilibrium adsorption data of phenol on C_{18}-bonded Kromasil were measured by FA at the center of this three-dimensional space (i.e., at 303 K, 20% methanol, and 1.00 mL/min). Fig. 1 shows the isotherm data (q^* versus C), the Scatchard plot representation (q^*/C versus q^*), and the adsorption energy distribution (AED) derived from these experimental data. The AED is clearly trimodal, but the first mode is still very broad after one hundred millions iterations. There are two reasons that explain why so many iterations were required to obtain the distribution showed in Fig. 1: first, the maximum concentration applied in FA analysis did not exceed 120 g/L, a value for which the amount adsorbed is less than half the total saturation capacity of the column. The low energy sites are certainly not sufficiently populated ($b_1 C_{max}$ is only 0.252, see Table 2) to permit the accurate determination of the first mode by the EM method. Secondly, the distance between the three modes is rather small, which makes their complete resolution hard

Table 2
Best isotherm parameters of the tri-Langmuir model estimated by the inverse method (IM) for isotherm determination

Parameters	FA	IM[a] (low loading)	IM[b] (high loading)
$q_{s,1}$ (g/L)	278.0		
b_1 (L/g)	0.0021		
$q_{s,2}$ (g/L)	73.2		70.0
b_2 (L/g)	0.0321		0.0319
$q_{s,3}$ (g/L)	44.8	46.4	45.4
b_3 (L/g)	0.2003	0.1891	0.1960

Optimization made on band profiles recorded after the injection of 100 mL of a 3 and 60 g/L solution of phenol.

[a] The best efficiency in the ED model was fixed at 4500.

[b] The best efficiency in the ED model was fixed at 1300.

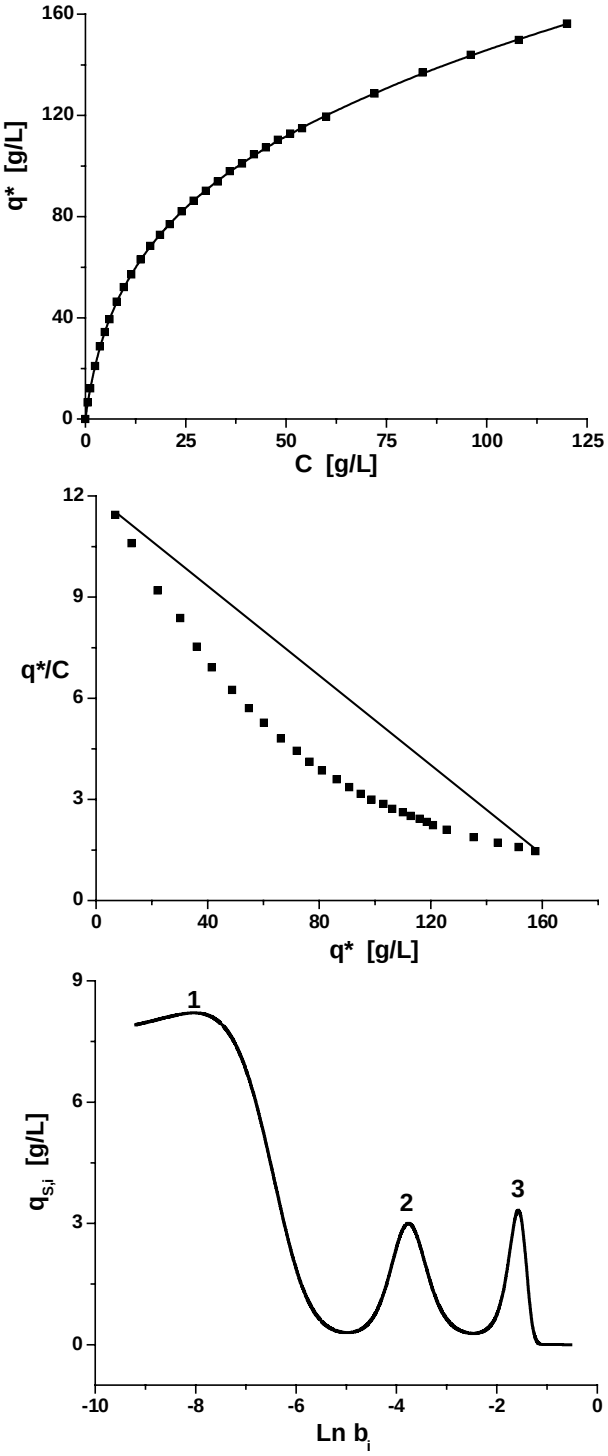

Fig. 1. (Top) Frontal analysis isotherm data points (squares) of phenol on the C_{18}-bonded Symmetry stationary phase with a mixture of methanol and water (20:80, v/v) as the mobile phase. $T = 303$ K, flow rate 1 ml/min. The solid line is the best fitting using a Tri-langmuir isotherm model. (Middle) Scatchard plot representation of the adsorption data shown in the top figure. Note the non-linearity as well as the convex downward shape of the plot suggesting an heterogeneous adsorption model. (Bottom) Calculation of the AED from the raw adsorption data presented in the top figure. One hundred millions iterations. Note the existence of three modes.

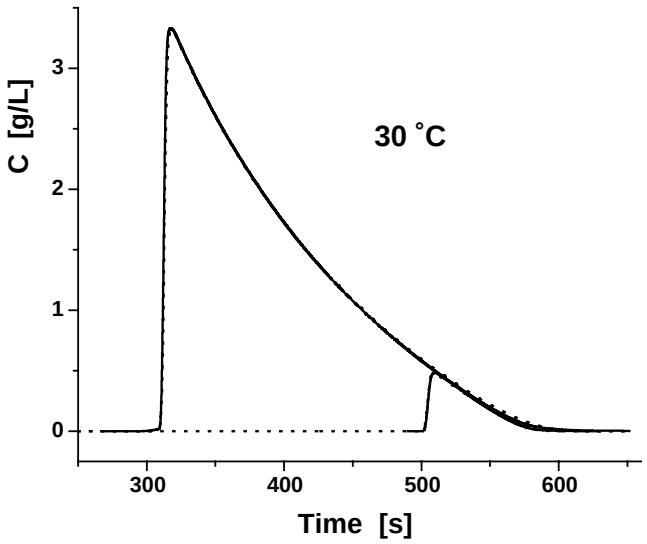

Fig. 2. Comparison between simulated (solid lines) and experimental (dotted lines) band profiles of phenol (two loadings, one low and high corresponding to the injection of 100 μL of a 3 and 60 g/L solution, respectively). Same experimental conditions as in Fig. 1. The constant efficiency in the ED model was fixed at 4500 and 1300 for the low and high loading, respectively. Note the excellent agreement between the two band profiles in each case.

to reach even with a large number of iterations. However, a multi-langmuirian isotherm behavior is supported by the convex downward shape of the Scatchard plot [9]. This plot is a straight line for a Langmuir isotherm. In the present case, the best isotherm model accounting for the adsorption data is the tri-Langmuir model. The differences between the three adsorption energies were calculated from the FA data and their best model. They are 6.9 kJ/mol ($\epsilon_{a,2} - \epsilon_{a,1}$) and 4.6 kJ/mol ($\epsilon_{a,3} - \epsilon_{a,2}$), respectively.

Two overloaded band profiles were recorded in order to validate the IM results. Fig. 2 shows the excellent fit between the experimental band profiles and those calculated using the tri-Langmuir isotherm. The column efficiency is clearly concentration dependent since, in order for the profiles of the calculated shock layers to match exactly the experimental profiles, the best efficiencies were 4500 and 1300 theoretical plates for the low and the high loading profiles, respectively. Despite this difference, the two profiles exhibit a common rear part which guarantees a good assessment by the IM method of the thermodynamic information available in the band profile. The isotherm parameters derived by IM are compared to those derived from the fitting of the FA data in Table 2.

The concentrations at the apex of the two profiles are 0.5 and 3.5 g/L for the low and the high loadings, respectively. The corresponding values of the products $b_i C_M$ are approximately 0.001, 0.007, and 0.2 at the apex of the low loading profile, 0.01, 0.1, and 0.7 at that of the high loading profile. Not surprisingly, it was impossible to obtained a reasonable estimate of the parameters of the first two energy modes with the low loading profile. Only the parameters $q_{s,3}$ and b_3 of the third mode could be accurately assessed. Similarly, only the parameters of the second and third modes could be derived from the high loading band profile but no accurate estimate of the parameters of the first mode could be achieved. These limitations are in large part due to the important broadening of the band during its propagation along the column, a broadening that is due to both thermodynamic and non-equilibrium phenomena, dilutes rapidly the injected pulse, prevents the migration of the high concentrations along the column to take place over a long distance, and the lowest adsorption energies significantly to contribute to the elution profile. A simple solution would consist in injecting a larger volume of solution. Unfortunately, the HP 1100 injection device cannot deliver larger samples. Although such a sample injection could be made with the solvent delivery system, it would cause an important axial dispersion of the sample along the extra-column connecting tubes. Although IM could be performed with a boundary condition that reflects this dispersion, the results lose in precision due to the limited reproducibility of the injection profile.

In spite of these experimental limitations, a very good agreement is obtained between the parameters of the second and third Langmuir modes derived from FA and IM. Because the apex concentration of the band profiles is always less than 4 g/L, the IM calculations were carried out assuming that the parameters of the first mode were constant and equal to those derived by FA ($q_{s,1} = 278$ g/L, $b_1 = 0.0021$ L/g). The contributions of the first, the second and the third modes to the overall Henry constant account approximately for 5, 20 and 75% of the total constant, H, respectively, with:

$$H = q_{s,1}b_1 + q_{s,2}b_2 + q_{s,3}b_3 = 0.584 + 2.350 + 8.973 = 11.907 \quad (8)$$

Accordingly, all the calculations of band profiles were performed with constant values of $q_{s,1}$ and b_1.

Note that the use of a multi-Langmuir isotherm model makes sense only if the different modes are operating in widely different concentration ranges. This is consistent with well separated, distant energy modes in the AED. This requires that the experimental data be acquired in a sufficiently wide range of solute concentrations, so that a sufficient number of data points are found in concentration ranges within which the contribution of each term varies significantly, those of the other modes remaining practically constant. The experimental difficulties encountered are often connected to the limited solubility of the compounds studied.

The comparison between the FA and IM methods of isotherm determination shows a good agreement between their results (Table 2). The parameters of the third and second modes determined by IM agree with the FA parameters within 5%. Accordingly, the IM method can now be applied directly to all experimental band profiles recorded under different temperatures, mobile phase compositions, and flow rates. These results will be used to investigate the influence of these parameters on the values obtained for the thermodynamic parameters.

3.2. Sensitivity of the isotherm parameters to temperature fluctuations

In a series of experiments, we recorded fourteen overloaded band profiles (low and high loadings) at seven different temperatures (298, 300, 302, 303, 304, 306, and 308 K). Given the level of control of temperature afforded by the thermostat, these temperature increments are large enough to lead to the acquisition of significantly different band profiles. Then, an accurate interpolation of the local variation of the isotherm parameters permits the calculation of the effect of infinitesimal changes in the column temperature. Fig. 3A shows the fourteen corresponding chromatograms, at low (left) and high (right) loadings. The calculations showed that

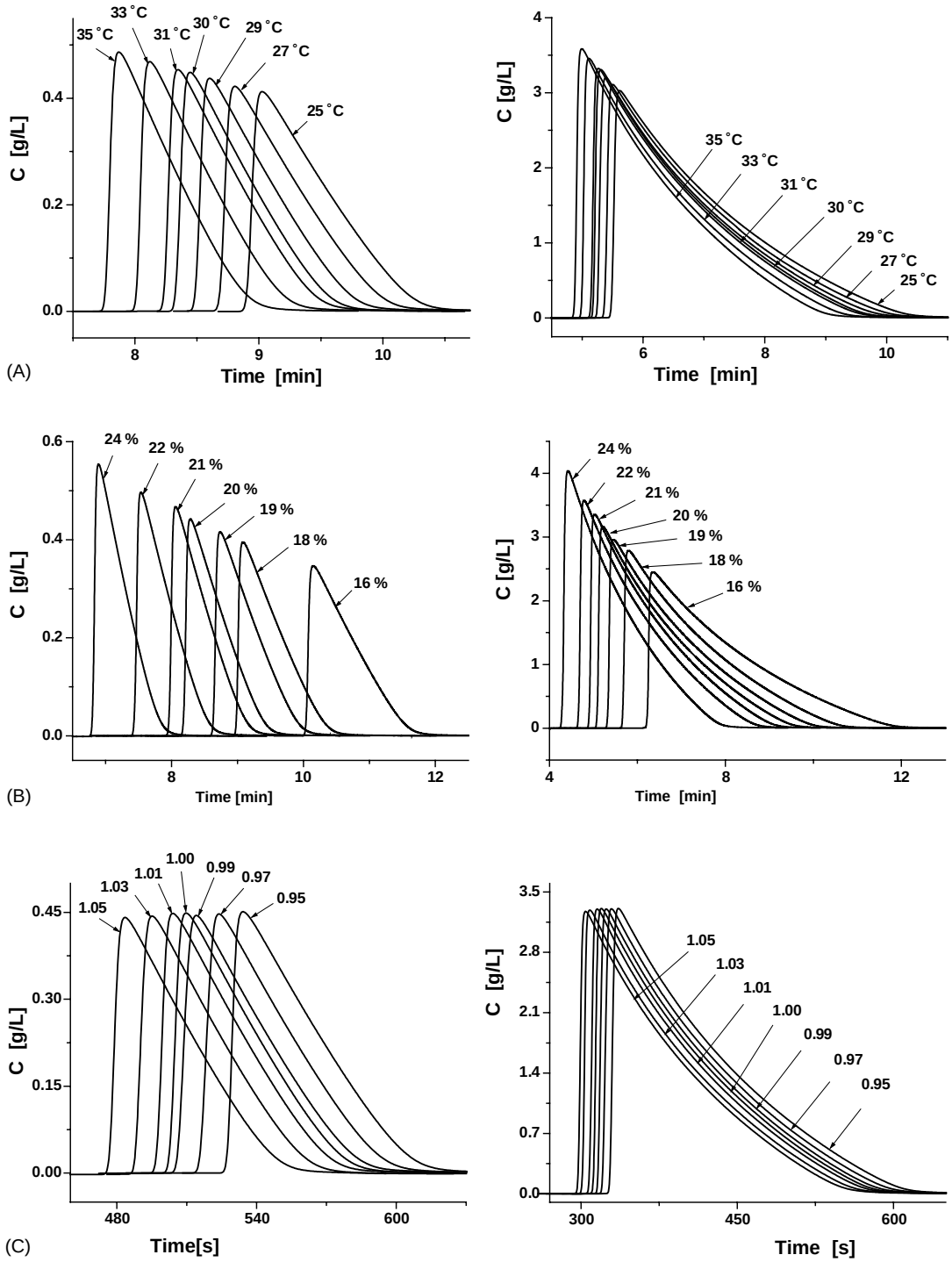

Fig. 3. Overloaded band profiles of phenol recorded at low (left) and high (right) column loadings on the C_{18}-Symmetry column for seven different temperatures (A, mobile phase composition 20:80, v/v, flow rate 1 mL/min), seven different mobile compositions (B, temperature 303 K, flow rate 1 mL/min) and seven different mobile phase flow rates (C, temperature 303 K, mobile phase composition 20:80, v/v).

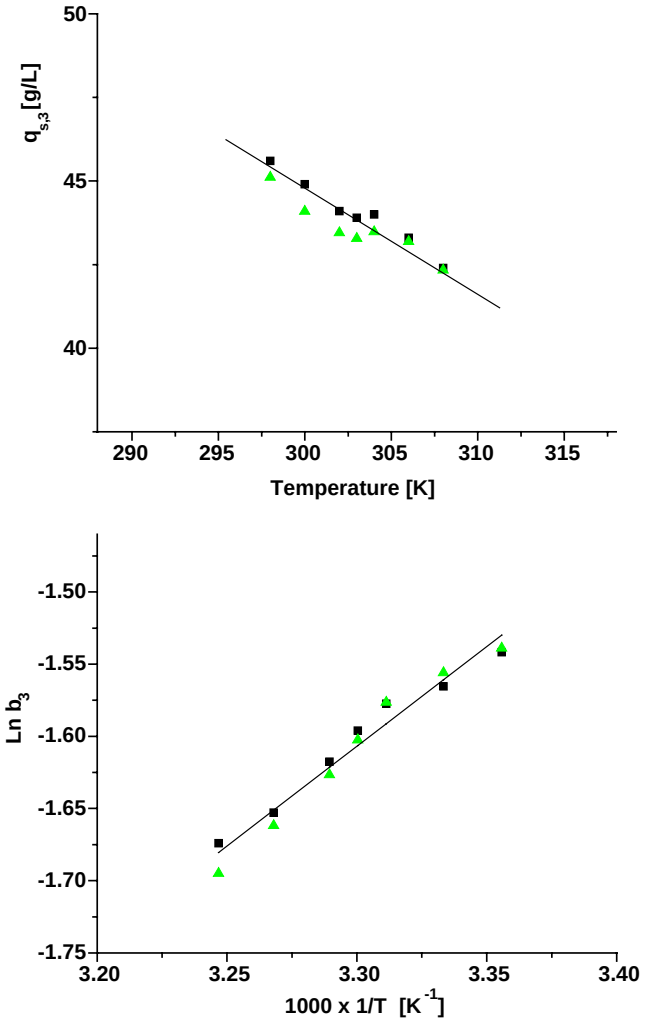

Fig. 4. Evolution of the best estimated isotherm parameters from the IM (squares: high loading bands; triangles: low loading bands) as a function of the temperature. (Top) Saturation capacity of the highest energy mode. (Bottom) Associated equilibrium constant. Note that the saturation capacity decrease with the temperature and that the equilibrium constant follows well the classical Van't Hoff law.

all these experimental band profiles can be accounted for by a mere change of the parameters of the third mode. Allowing the adjustment of the parameters of the second energy mode does not give satisfactory results as the numerical problem is indeterminate. The values obtained for this second mode are non reproducible and the improvement is not statistically significant. The evolution of the best values of the parameters $q_{s,3}$ and $\ln b_3$ as a function of the temperature and the reciprocal temperature, respectively, are shown in Fig. 4.

As suggested in previous reports [8,10], the highest energy mode corresponds to the heterogeneity of the structure of the C_{18}-bonded alkyl chains between which the analyte can partition (in the adsorption field of the silica), rather than adsorb on the top of the C_{18} layer. It was reported that the equilibrium constant is higher when the solute follows such a partitioning mechanism. As the temperature increases, the structure of the C_{18} layer is expected to become more homogeneous and it is not surprising to observe that the saturation capacity (i.e., the number) of sites 3 decreases with increasing temperature. The rate of this decrease is:

$$\frac{\Delta q_{s,3}}{\Delta T} \simeq -0.26\,\text{g/L K} \tag{9}$$

A temperature change of 1 K causes a relative change of 0.6% for the saturation capacity $q_{s,3}$. This change is relatively small but still not negligible.

The plot of the logarithm of the adsorption constant b_3 versus the reciprocal temperature follows Van't Hoff law, e.g.

$$\frac{\Delta \ln b_3}{\Delta(1/T)} = \frac{\epsilon_{a,3}}{R} \tag{10}$$

From this numerical result ($\epsilon_{a,3}/R = 1.383\,\text{K}$), we can derive the absolute adsorption energy, $\epsilon_{a,3} = 11.5\,\text{kJ/mol}$, of the highest mode. This energy is exactly the sum of the two energy differences derived from the AED results in Fig. 1c (6.9 kJ/mol + 4.6 kJ/mol). This implies that the adsorption energy on the type 1 sites is practically negligible by comparison with the adsorption energies on type 2 and 3 sites. This observation validates the choice made earlier of keeping constant the parameters of the first mode during the calculation, since they account for only 5% of the overall Henry constant, H and since their temperature dependence is very small.

This result also demonstrates that the interactions between phenol and the stationary phase are likely to be due to weak hydrophobic interactions, with an energy of approximately 5 kJ/mol. By contrast, the interaction energies involved in hydrogen bond or dipole-dipole interactions are much higher ($\simeq$20 kJ/mol), so such interactions cannot be expected to take place between phenol and the stationary phase. Then, the best explanation for the retention mechanism observed is that phenol molecules may fit within the C_{18}-bonded layer and that the different adsorption sites correspond to different geometric structures of the hydrophobic cages formed inside this layer. These energy results are not consistent with phenol interacting with some free silanols trapped amidst the C_{18} chains. The tailing of phenol previously discussed [10] does not seem to be connected to a chemical heterogeneity of the surface of the stationary phase but rather to a structural heterogeneity of the C_{18}-bonded layer.

From Eq. (9), we can write the relative sensitivity of the adsorption constant b_3 to temperature fluctuations or error propagation coefficient for temperature:

$$\frac{\Delta \ln b_3}{\Delta T} = \frac{\epsilon_{a,3}}{RT^2} \simeq 0.015\,K^{-1} \tag{11a}$$

This means that at 303 K a temperature change of 1 K leads to a 1.5% change of the highest of the three equilibrium constants. Generalizing Eq. (10) to the first two modes, we can conclude that no significant change of the first equilibrium constant, b_1, can be observed (since $\epsilon_{a,1} \simeq 0$) and that a relative change of only 0.9% can be expected for the second constant, b_2.

3.3. Sensitivity of the isotherm parameters to fluctuations of the mobile phase composition

Band profiles were acquired using mobile phases with seven different volume fractions of methanol (16, 18, 19, 20, 21, 22, and 24%). The corresponding overloaded band profiles are shown in Fig. 3B, at low (left) and high (right) loading factors. By contrast to what happened in the case of the study of the effect of the temperature, it was not possible to describe well the experimental profiles at high loading factor with only two parameters ($q_{s,3}$ and b_3). The parameters of the second mode ($q_{s,2}$ and b_2) had also to be taken into account, requiring now that four parameters be determined in the IM procedure. Fig. 5 summarizes the influence

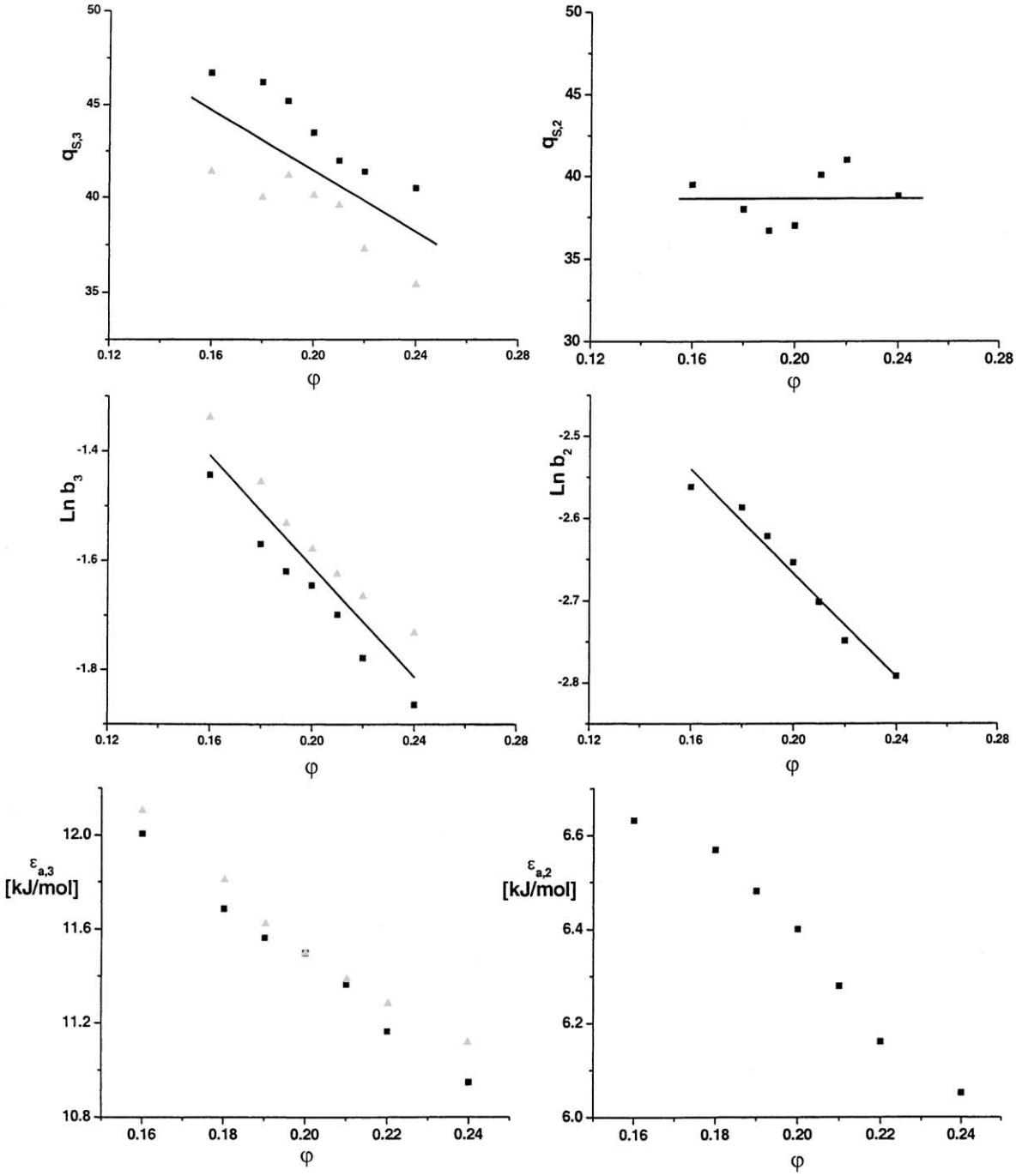

Fig. 5. Evolution of the best estimated isotherm parameters from the IM (squares: high loading bands; triangles: low loading bands) as a function of mobile phase composition in methanol φ. (Top) Saturation capacities of the third and second energy mode. (Middle) Associated equilibrium constants. Note the diminihing of the saturation capacity $q_{s,3}$ and the LSSM followed by the equilibrium constants. (Bottom) Adsorption energies of the third and second energy mode.

of the mobile phase composition on the two saturation capacities (top) and the two equilibrium constants (bottom). The values found for the two parameters of the third mode are fully consistent with those derived from the FA data. This is not so for those of the second mode ($q_{s,2} \simeq 38$ g/L instead of 73 g/l and $b_2 \simeq 0.070$ L/g instead of 0.032 L/g). Only the product $q_{s,2}b_2$ remains constant, which suggests that the high loading band profiles merely allow a good estimate of the product of these two parameters, not of their individual values. Better results could be obtained only if it was possible to acquire band profiles covering a wider range of concentrations. Nevertheless, the variations of the two equilibrium constants with the mobile phase composition are consistent with the linear strength solvent model and we obtain the following relationship between the equilibrium constants b_2 and b_3 and the volume fraction, φ, of the organic modifier in the aqueous mobile phase:

$$\ln b_i = \ln b_{0,i} - S_i\varphi \tag{11b}$$

where $b_{0,i}$ is the extrapolated equilibrium constant on sites i in pure water as the mobile phase. The corresponding numerical values of the parameters of b_i estimated by IM are $b_{0,2} = 0.13$ L/g, $S_2 = 3.16$, $b_{0,3} = 0.55$ L/g and $S_3 = 5.06$. When compared to data previously measured on C_{18}-Kromasil, these values make physical sense [24]. It was shown that the retention mechanism of phenol on this adsorbent was described by a two-sites adsorption model (i.e., a bi-Langmuir isotherm model) within a large range of methanol concentrations. In this case also, the high equilibrium constant followed the LSSM and the best values for $b_{0,2}$ and S_2 were 0.57 L/g and 4.558, respectively. The physical nature of the type 3 sites on C_{18}-Symmetry is almost certainly the same as that of type 2 sites on C_{18}-Kromasil, a conclusion confirmed by the variation of $q_{s,3}$ on Symmetry, which, like for Kromasil, decreases rapidly with increasing methanol content. The rate of decrease is:

$$\frac{\Delta q_{s,3}}{\Delta\varphi} \simeq -81.7\ g/L$$

The largest value of the number of sites 3 is reached for $\varphi = 0$ and it is 57.8 g/L. Accordingly, it can be extrapolated that the number of sites 3 becomes zero when $\varphi = 0.71$. On Kromasil the high energy sites of type 2 vanish when $\varphi = 0.65$. These critical values of the methanol:water mobile phase composition are very close, which confirms previous conclusions that a low methanol content of the mobile phase contributes to increase the column heterogeneity while a high methanol content renders the C_{18} layer more homogenous. We can also predict that if adsorption data were measured at methanol concentrations beyond 70%, they would lead to a unimodal AED, which is just what was observed previously with 4-*tert*-butylphenol [7,25–27]. Thus, all the results obtained by IM are consistent with former observations and are reinforced by them.

The parameter $q_{s,2}$ remains nearly constant. The error propagation coefficients for the two equilibrium constants, b_3 and b_2, are:

$$\frac{\Delta \ln b_3}{\Delta\varphi} \simeq 5.0$$

and

$$\frac{\Delta \ln b_2}{\Delta\varphi} \simeq 3.2$$

Thus, a 1% change of the volume fraction of methanol causes relative changes of 5.0, 3.2 and 2.0% for b_3, b_2 and $q_{s,3}$, respectively. The direct impact on the adsorption energies, $\epsilon_{a,3}$ and $\epsilon_{a,2}$, of the error made on the mobile phase composition can be assessed according to Eq. 6, assuming that b_0 is constant, knowing the adsorption energies derived from the Van't Hoff plot (adsorption energies for a mobile phase composition of 20%, 11.5 and 6.4 kJ/mol, respectively). The variation of the adsorption energies $\epsilon_{a,3}$ and $\epsilon_{a,2}$ are 0.13 and 0.08 kJ/mol for a change of 1% in the mobile phase composition by volume.

3.4. Sensitivity of the isotherm parameters to fluctuations of the mobile phase flow rate

The determination of equilibrium adsorption data by the FA method is usually done by applying the mass conservation and integrating the breakthrough curve. However, to calculate the amount adsorbed, it is critical to know accurately the mobile phase flow rate, or at least its integral over the duration of the breakthrough. It is also important to know the flow rate when using the IM method. Fourteen overloaded band profiles were recorded at seven different flow rates (0.95, 0.97, 0.99, 1.00, 1.01, 1.03, and 1.05 mL/min). Fig. 3C shows these band profiles, at low (left) and high (right) loading. These profiles were used for isotherm determination by the IM procedure. During the profile calculations with the ED model, the flow rate was kept constant at the central flow rate value, 1.00 mL/min. This procedure describes the situation in which the actual flow rate is either overestimated (0.95–0.99 mL/min) or underestimated (1.01–1.05 mL/min). As can be seen in Fig. 3C, the band profiles are merely translated, rightward to leftward, depending on whether the mobile phase flow rate is decreased or increased, respectively, while the band apex remains almost constant. This suggests that the mass transfer of phenol between the stationary and the mobile phase is little affected by the mobile phases flow rate in the range studied. Only $q_{s,3}$ and b_3 were estimated when using the IM method. The results are shown in Fig. 6.

First, the best value of b_3 remains constant at about 0.20 L/g. It is independent of the possible error made on the flow rate. This result was expected since, with either loading factor, the rear part of the seven band profiles recorded are nearly parallel. The simple translation of the band causes a proportional shift of the saturation capacity, $q_{s,3}$, as shown in Fig. 6. The error measured is:

$$\frac{\Delta q_{s,3}}{\Delta F_v} \simeq -96.35\ \text{mg min mL}^{-2}$$

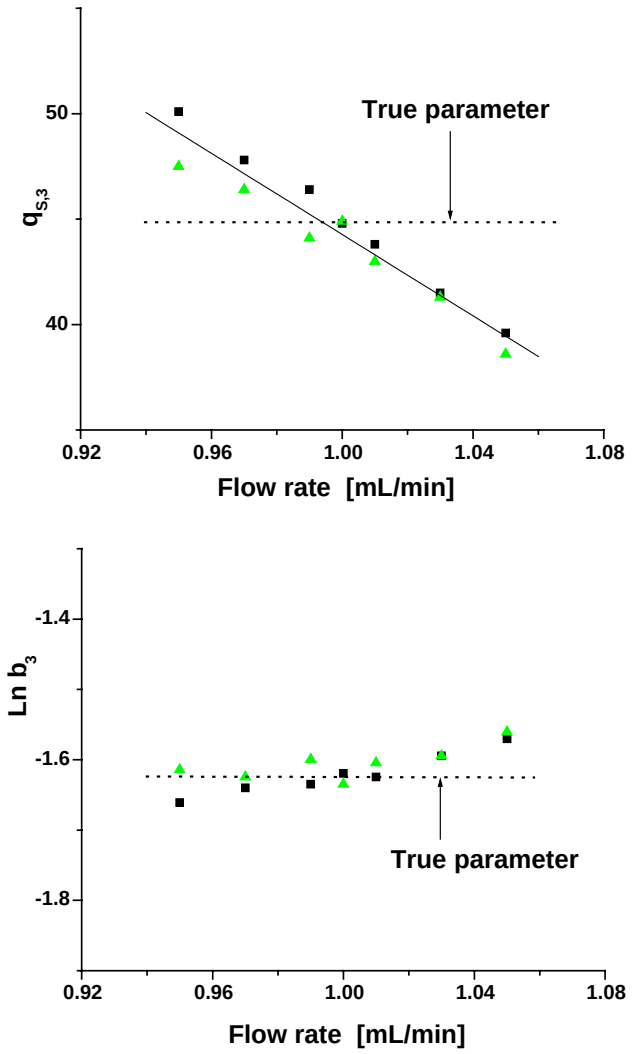

Fig. 6. Difference between the exact isotherm parameters measured at a flow rate of 1 mL/min and those estimated by the IM (squares: high loading bands; triangles: low loading bands) with experimental band profiles recorded at different flow rates. Note the strong impact on the determination of the saturation capacity.

In other words, underestimating or overestimating the actual flow rate by 0.01 mL/min causes a relative variation of 2.1% on the saturation capacity $q_{s,3}$. This result is consistent with Eq. (4). Since the equilibrium constant does not change and the column porosity being 0.601, we have:

$$\frac{\Delta q^*}{q^*} = \frac{\Delta(q_s b/1 + bC)}{q^*} = \frac{1}{q_s}\Delta q_s = \frac{1}{1-0.601} \times 0.01 \simeq 2.5\%$$

3.5. Comparison between the different error contributions

Table 3 compares the sensitivity of the saturation capacity $q_{s,3}$ and the equilibrium constants b_3 and b_2 to the fluctuations of the three main experimental parameters, the temperature, the mobile phase composition, and the mobile phase flow rate. According to the data in the table, the contribution of an error of 1 K on the temperature is equivalent to those of an error of 0.3% on the volume fraction of the organic modifier or to an error of 3 μL/min on the flow rate, regarding the errors made on the saturation capacity $q_{s,3}$. As for the equilibrium constants, an error of 1 K on the temperature is also equivalent to an error of 0.3% on the mobile phase composition. The high-energy isotherm parameters are more sensitive than the low-energy ones to errors made on the temperature and the mobile phase composition.

Table 3
Relative change (%) in the saturation capacity $q_{s,3}$ and equilibrium constants b_2 and b_3 when the temperature, the mobile phase composition and its flow rate are changed by 1 K, 1% and 0.01 mL/min

Parameters	$\Delta T = 1$ (K)	Δ MP[a] $= 1$ (%, v/v)	$\Delta F_v = 0.01$ (mL/min)
b_2 (L/g)	0.9	3.2	≃0
$q_{s,3}$ (g/L)	0.6	2.0	2.1
b_3 (L/g)	1.5	5.0	≃0

[a] MP: mobile phase composition.

4. Conclusion

Errors in the determination of the adsorption isotherms can arise at several different levels. Errors may be made in the measurement of adsorption isotherm data. They may be caused by fluctuations of the experimental conditions during the measurements. In this case, the data point measured is an average. However, since the dependence of the amount adsorbed at equilibrium with a given mobile phase concentration on the experimental condition is not a linear function of the temperature or mobile phase composition, this average does not correspond to the average temperature or the average mobile phase composition. Errors may arise also because the different data points are not measured exactly under the same experimental conditions or because they are referred to inexact values of these conditions. Finally, model errors stem from the use of improper isotherm model.

Combining the results of the FA and IM methods, we have shown that fluctuations of the temperature and mobile phase composition must be monitored rather carefully if data with a precision better than ca 1% must be obtained. A stability of the ambient temperature better than 1 K is not too difficult to achieve in an American laboratory. Yet, it is cautious to have an accurate thermometer in the laboratory and to check it several times daily. In most laboratories the world over, a column oven is necessary. The long term stability of the flow rates delivered by the pumping systems of modern HPLC instruments is sufficient to ensure a composition stability better than 0.1%. It is not always so to maintain to a negligible level the error contribution of flow rate fluctuations. This error contribution is surprisingly large.

Finally, errors made in the measurement of the column dimensions must be paid most careful attention. The prob-

lem is serious only if data are measured on one column, a microbore or an analytical column, to be used on another one, e.g., a preparative column. Then the dimensions of each column or rather, if possible, the amount of packing material that they contain must be known accurately. The case for the importance of this potential source of error has been made elsewhere [28].

Inaccuracy or fluctuations in the temperature and the mobile phase composition affect both the saturation capacity and the equilibrium constant of each isotherm contribution. Our results show that the saturation capacity of the high adsorption energy sites of phenol on C_{18}-bonded silicas used in RPLC decreases markedly with increasing temperature and with increasing concentration of the organic modifier in the aqueous mobile phase. This result is explained by the fact that both increases tend to make the stationary phase more homogeneous, as observed in a previous report [24]. A temperature change of 1 K and a variation of the methanol concentration of 0.3% cause each a change of 1.5% of the saturation capacity of phenol. As expected from the Van't Hoff equation and from the LSSM, respectively, the equilibrium constant decreases with increasing temperature and increasing organic modifier content of the mobile phase. The higher the adsorption energy, the higher the sensitivity of the equilibrium constant to the temperature and to the mobile phase composition. For an adsorption energy of 10 kJ/mol, which is within the range of energy corresponding to hydrophobic forces, a temperature variation of 1 K and a change of the methanol fraction of 1% lead to relative variations of 1.5 and 5% of the equilibrium constant. Although it is difficult to generalize at this stage and the numbers will be different in each particular case, it is probable that the trends will be the same in many other cases.

Despite the fact that two different values of the overall dispersion coefficient (i.e., the column efficiency) are needed to achieve a satisfactory agreement between the calculated and the experimental profiles obtained for the two different experimental column loadings used (because the overall mass tranfer is usually slower at high than at low concentrations), the isotherm parameters or at least the product $q_s \times b$ derived from the IM are almost identical. This result should not be generalized to systems for which the bands tail considerably at high loadings. For instance (results not shown), the band tailing of 4-*tert*-butylphenol on the same column at 15 °C is very sensitive to the initial concentration injected, which affects significantly the IM results.

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