Modeling of ibuprofen on reversed phase liquid chromatography: I. The effect of mobile phase composition

Tarab Ahmad [a*], Bobilla Srihari[a], Bakheet Alresheedi [a], Siddegowda Chandraju [b] Netkal Made Gowda [a]

[a] *Department of Chemistry,Western Illinois University, 324 Currens Hall, Macomb, IL 61455*
[b]*Department of Studies in Sugar Technology, Sir M.Vishweshwaraya Post-Graduate Center, University of Mysore, Tubinakere, Mandya 571402, Karnataka,India.*

Abstract

The present work investigates the modeling of ibuprofen on reversed phase liquid chromatography using a mobile phase containing aqueous solution of acetonitrile at pH 2.2. The adsorption isotherms of ibuprofen are determined by frontal analysis method and by the inverse method. It was found that the adsorption data fit the S-shaped model. All the parameters of the isotherm decrease by increasing the amount of acetonitrile in the mobile phase. There was a good agreement between the experimental and the calculated band profiles.

Keywords: adsorption isotherm, ibuprofen, effect of mobile phase, RPLC, C_{18}, retention mechanism.

1.0 Introduction

Many analytical or preparative separations are now performed by reversed phase liquid chromatography (RPLC). A better understanding of the retention mechanism of the RPLC is required in order to optimize the speed and cost of the separations or the production of the components of interest. There are many experimental parameters that control the retention of the compounds on RPLC like the temperature [Kim, 2004, Ahmad, 2007], pressure [Liu, 2003], mobile phase composition [Ahmad, 2006], the concentration and type of the salts added to the mobile phase and the nature of the buffer [Gritti, 2004] and the pH of the mobile phase [Gritti, 2009]. To predict the elution times or the shape of overloaded band profiles for single-component and multi-component systems and to study the retention mechanism, it is crucial to be able to determine the adsorption equilibria (single and competitive isotherms) of the compounds between the mobile and stationary phases. The determination of the equilibrium isotherms of the important component of the feed is critical to perform a computer assisted optimization of the separation by preparative liquid chromatography. This is very important step in simulated moving bed (SMB) separations because it will save time and cost for the separation process.

Ibuprofen [2-(3-isobutylphenyl)propanoic acid] is a non-steroidal anti-inflammatory drug widely used in the treatment of pain and inflammation in rheumatic disease and other musculoskeletal disorders. High performance liquid chromatography is the major technique used for the determination of ibuprofen in pharmaceutical preparations as well as in biological samples [Ghulam 2008]. RP-HPLC is by far the most widely used technique for the determination of ibuprofen and its impurities [USP convention, 2003]. Although most of these RPLC separations are based on silica based column, there are some reports in the literature in which Zirconia based columns are used for the determination of ibuprofen, its related compounds and its degradation products [Kalafut,2005; Kalafut, 2009].

The aim of this work is to model ibuprofen on reversed phase chromatography and to understand its retention mechanism using acetonitrile as a modifier. The parameters of the adsorption isotherms for ibuprofen will be determined by two methods; the frontal analysis method and by the inverse method which is a numerical method.

2.0 Theory

2.1. Determination of single-component isotherms by frontal analysis

Single-component adsorption isotherm data was acquired by frontal analysis because it is the most accurate method [Guiochon, 2006]. This method consists of successively replacing, at increasing concentrations the stream of mobile phase percolating through the column with streams of solutions of the studied compound, and of recording 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 breakthrough curve is eluted allows the calculation of the adsorbed amount, q*, of solute on the stationary phase at equilibrium with the mobile phase concentration, C.

This amount is best measured by integrating the breakthrough curve, using the equal area method [Guiochon, 2006]. The adsorbed amount is given by

$$q^* = \frac{c_0 F_v (t_{shock} - t_{ext} - t_o)}{\pi r^2 L - F_v t_0} \quad (1)$$

Where F_v is the mobile phase flow rate, t_{shock} the elution time of the front shock of the breakthrough curve, t_{ext} the extra-column hold-up time (measured from the elution time of the inflection time of the inflection point of the same breakthrough curve injected with no column), to the hold-up time, r_{in} the internal radius of the column tube, and L the length of the column in cm.

2.2. Fitting of the isotherm data to isotherm models

2.2.1. Fisher parameter

Non-linear regression of the experimental data to adsorption isotherm models was performed using the origin lab software. The experimental data were fitted to each isotherm model with weighing ($1/Y^2$) to put an even emphasis on each data point during the fitting process. The best isotherm parameters were selected by minimizing the residual sum of squares (RSS) for each isotherm model. The different adsorption isotherm models were compared using the Fisher test (equation below). The best model selected for the experimental data is the one, which gives the highest value of the Fisher parameter, F_p [Quinones, 1998].

$$F_p = \frac{(n-p)\Sigma_{i=1}^{n}\left(q_{ex,i} - \overline{q_{ex}}\right)^2}{(n-1)\Sigma_{i=1}^{n}\left(q_{ex,i} - q_{theor,i}\right)^2} \quad (2)$$

Where $\overline{q_{ex}}$ is the mean value of the experimental data $q_{ex,i}$, p the number of adjusted parameters of the model, and n is the number of data points acquired by FA. $q_{theor,i}$ the estimate given by the isotherm model for the solid phase concentration of the adsorbate in equilibrium with the mobile phase concentration $C_{i,}$. A higher value of F_p suggests a better fit to the experimental data. F_p increases when (n-p) increases (more experimental data points is available), $\Sigma\left(q_{ex,i} - q_{theor,i}\right)^2$ decreases with increasing goodness of the fit and $\Sigma\left(q_{ex,i} - \overline{q_{ex}}\right)^2$ increases with increasing width of the data range. The Fisher parameter assumes that the residual are normally distributed. To determine if a model provides data that are statistically more accurate than the other one, the ratio of the two Fisher parameters corresponding to the data obtained with this method is calculated and compared to critical F-ratios.

2.3. Models of isotherm

2.3.1. The Langmuir model

The adsorption isotherm models that are used to fit the experimental isotherm data were the Langmuir, the bi-Langmuir, and the S-shaped, and bi-Moreau and Moreau isotherm models. In spite of its semi-empirical nature, the Langmuir model is most frequently used in general studies of liquid–solid chromatographic equilibria [Guiochon, 2006]

$$q^* = q_s \cdot \frac{bC}{1+bC} \quad (3)$$

In this model, q_s is the monolayer saturation capacity of the adsorbent, and b is the equilibrium constant of adsorption. This model assumes that the surface of the adsorbent is homogeneous, that adsorption is located, and that there are no adsorbate-adsorbate interactions. The equilibrium constant b is given by the following equation [Guiochon, 2006]:

$$b = b_o e^{\frac{\varepsilon_a}{RT}} \quad (4)$$

,where ε_a is the energy of adsorptions, R is the universal ideal gas constant, T the absolute temperature and b_0 is a pre-exponential factor that could be derived from the molecular partition function in both the bulk and the adsorbed phases.

Consistent with the basic assumption of this model, the affinity energy distribution (AED), F (ε), corresponding to the Langmuir isotherm is a Dirac function:

$$F(\epsilon) = \delta(\varepsilon - \varepsilon_a) \qquad (5)$$

The surface is homogeneous, hence has a unimodal energy distribution with a mode width equal to 0.

2.3.2. The bi-Langmuir model

The bi-Langmuir isotherm [Graham, 1953] is the simplest isotherm model for nonhomogenous surfaces. The equation of this model for a pure compound uses the sum of two Langmuir isotherms terms;

$$q^* = q_{s,1} \cdot \frac{b_1 C}{1 + b_1 C} + q_{s,2} \cdot \frac{b_2 C}{1 + b_2 C}$$

This model assumes that the adsorbent surface is heterogeneous and is covered with a quilt of two different and independent surfaces with two saturation capacities, $q_{s,1}$ and $q_{s,2}$, corresponding to each one of the two type the adsorption of sites. The two equilibrium constants b_1 and b_2 are associated with the adsorption energies $\varepsilon_{a,1}$ and $\varepsilon_{a,2}$ and the AED becomes:

$$F(E) = \frac{q_{s,1}}{q_s}\delta(\varepsilon - \varepsilon_{a,1}) + \frac{q_{s,2}}{q_s}\delta(\varepsilon - \varepsilon_{a,2}) \qquad (7)$$

2.3.3. The Moreau model

The Moreau adsorption isotherm model is the simplest extension of the Langmuir adsorption isotherm model in the case of a homogeneous adsorbent on a surface where significant adsorbate–adsorbate interactions take place [Guiochon, 2006]. The bi-Moreau adsorption isotherm is the corresponding extension of the bi-Langmuir model to a surface covered with two types of sites, on which adsorbate–adsorbate interactions take place , following Moreau model behavior.

2.3.4. The bi-Moreau model

The bi-Moreau model is written:

$$q^* = q_{S,1} \frac{b_1 C + I_1 b_1^2 C^2}{1 + 2b_1 C + I_1 b_1^2 C^2} + q_{s,2} \frac{b_2 C + I_2 b_2^2 C^2}{1 + 2b_2 C + I_2 b_2^2 C^2} \qquad (8)$$

where q* and C are the equilibrium concentration of the compound considered in the adsorbed and the liquid phase respectively, and $q_{s,1}$, $q_{s,2}$, b_1, b_2, I_1, and I_2 are the monolayer saturation capacities, the equilibrium constants, and the adsorbate-adsorbate interaction parameters on the sites of types 1 and 2, respectively [Moreau, 1991].

I, can be written as

$$I = e^{\varepsilon AA/RT} \qquad (9)$$

where ε_{AA} is the interaction energy between two molecules of A adsorbed on close adsorption sites. Note that the bi-Moreau model morphs into the bi-Langmuir model when $I_1 = I_2 = 0$, since the equation above is reduced to that of the bi-Langmuir model [Moreau, 1991]. The equilibrium constants b_1 and b_2 are associated with the adsorbtion energy $\varepsilon_{a,1}$ and $\varepsilon_{a,2}$, respectively, through the following classical equation.

2.3.5. The quadratic model

The quadratic isotherm equation is the first equation to which the experimental data should be fitted when an inflection point is observed. Statistical thermodynamic considerations lead to an adsorption equation which is the ratio of two polynomials of the same degree [Svoboda, 1990]

$$q^* = q_S \frac{b_1 C + 2b_2 C^2 + \ldots + n b_n C^n}{1 + b_1 C + b_2 C^2 + \ldots + b_n C^n} \qquad (10)$$

The Langmuir adsorption isotherm, obtained with a first-degree polynomial (n=1), is the first such model. The quadratic adsorption isotherm, obtained with a second-order polynomial, is the second one:

$$q^* = q_S \frac{(b_1 + 2b_2 C)C}{1 + b_1 C + b_2 C^2} \qquad (11)$$

The saturation capacity is q_s; b_1 and b_2 are the equilibrium constants. When the numerical coefficients meet certain conditions, this adsorption isotherm has an inflection point. The quadratic equation may be used also when more than one inflection point are observed.

2.4. The inverse method of adsorption isotherm determination:

The adsorption isotherm model uses, and consists of adjusting the coefficients of a preselected adsorption isotherm model in order to minimize the difference between one or several experimental band profiles and the calculated profiles using the equilibrium dispersive (ED) model [Felinger, 2003] . The main advantage of this method of adsorption isotherm determination is that it requires the measurement of one or a few overloaded band profiles only [Felinger, 2003]. The measurements are fast and require very small amounts of chemicals. In practice, band profiles are recorded, the recorded profile (detector response) is converted into a concentration profile through the use of a calibration curve, and the coefficients of a selected adsorption isotherm model are adjusted to minimize the sum:

$$\sum_i (C_i^{sim} - C_i^{meas})^2 \qquad (12)$$

The equilibrium-dispersive model of chromatography is used to calculate the band profiles [Felinger, 2003]. It is particularly suitable for low molecular weight compounds of moderate polarity. In this model, we assume constant equilibrium between the stationary and the mobile phases and use an apparent dispersion term to account for the band broadening effects of both axial dispersion and the finite rate of the mass transfer kinetics. The following mass balance equation is written for the solute [Guiochon, 2006]:

$$\frac{\delta C}{\delta t} + F\frac{\delta C}{\delta t} + u\frac{\delta C}{\delta Z} = D_a\frac{\delta^2 C}{\delta Z^2} \qquad (13)$$

where C and q are the concentrations of the solute in the mobile and the stationary phases, respectively, z the length, t the time, u the superficial linear velocity of the mobile phase, and F the phase ratio of the column (with $F = (1-\varepsilon)/\varepsilon$ and ε the total column porosity). Da is the apparent dispersion coefficient that can be calculated from the column efficiency or number of theoretical plates N:

$$D_a \frac{uL}{2N} \qquad (14)$$

where L is the column length, and N, the number of theoretical plates of the column, is measured under Linear A condition, which is with a small sample size. This method requires recording few experimental data followed by computer optimization to find the adsorption isotherms. The advantages of this method are that it saves time and it reduces the consumption of solvents and chemicals.

The initial conditions states that at t = 0, the concentrations of the solute in the mobile and in the adsorbed phase in the column are uniformly equal to zero. 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 at the inlet and the outlet of the column. The ED model was solved using a computer program based on an implementation of the method of orthogonal collocation on finite elements (OCFE). The set of discredited ordinary differential equations was solved with the Adams–Moulton method, implemented in the VODE procedure [Guiochon, 2006]. The relative and absolute errors of the numerical calculations were 1×10^{-6} and 1×10^{-8}, respectively [*Rouchon, 1985*].

3.0 Experimental

3.1. Chemicals

Water, acetonitrile and phosphoric acid (85%), were all HPLC grade, and were purchased from Fisher Scientific (Fair Law, NJ, and USA). The solutes used were ibuprofen and thiourea both purchased from Aldrich (Milwaukee, WI, USA).

3.2. Chromatographic column

The column that is used for this work is Alltech Altima C_{18} with dimensions of 250 mm ×4.6 mm with 5 µm particle size.

3.3. Origin lab software

Origin 7.5 SR6, Origin Lab Corporation, One round house plaza, Northampton, MA 01060 USA, 1991-2006.

3.4. Apparatus

Shimadzu liquid chromatograph, model 20A, equipped with auto sampler (SIL 20A / 20 AC), UV-VIS detector (SPD-20A / SPD-20AV), online degasser (DGU-20 A3 / DGU 20 A5) and system Controller (BM-20 A / 20 A Lite). The extra-column volumes are 0.08 and 0.09 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. All measurements were carried out at a constant temperature of 22 °C, fixed by the laboratory air conditioner. The daily variation of the ambient temperature never exceeded 1 °C.

3.5. Mobile phase preparation

3.5.1. Mobile phases

Mobile phases containing aqueous solutions of acetonitrile (40, 50, or 60%) were prepared by mixing 400, 500, and 600 mL of acetonitrile respectively with an appropriate amount of hplc water. The pH = 2.2 was obtained by adding phosphoric acid dropwise to the solution.

4.0 Results and Discussion

4.1. The effect of mobile phase composition at pH 2.2 on the adsorption behavior of ibuprofen

4.1.1. Modeling of ibuprofen on Altima C_{18}

Fig.1A-C shows the adsorption data of ibuprofen from the mobile phases containing 40%, 50% and 60% acetonitrile (ACN) derived from single component frontal analysis (symbols), the best bi-Langmuir isotherm (filled squares), the best S-shaped to the third degree, (filled triangles) and the bi-Moreau model (filled circles). Also the figure shows the best fits to Langmuir, Moreau and the S-shaped of the second order (empty squares, circles and triangles respectively). While the main figures shows all the data, the insets show only the data at low concentration (C< 0.1 g/L). The agreement between the experimental data and the bi-Langmuir, bi-Moreau and S-shaped of the 3rd degree models are slightly better than the unimodels (Langmuir and Moreau) and the S-shaped 2nd order especially in the lower concentration range. A similar conclusion can also be derived from the Fisher's test of the calculation results (see Table 1) and from the scatchard plot shown in Fig. 1D-F.

The scatchard plots q/C versus C for ibuprofen for the three mobile phases used (40, 50 and 60% ACN) are shown in Fig. 1D-F. These graphs are convex downward which confirms that the simple Langmuir model is inadequate to model the data since these plots are not the straight lines corresponding to Langmuirian behavior. This indicates that the surface of adsorption is not homogenous for ibuprofen and it would be better modeled if we assume that it consists of patches of at least two different types of sites. Therefore, we tried to fit the adsorption data for all mobile phases initially to the bi-Langmuir model.

The fitting was successful for all three isotherms. However because the scatchard plots of ibuprofen using the 50% and 60% ACN mobile phases indicate the presence of at least one inflection point in the isotherms at low concentration we tried to fit the adsorption data of ibuprofen for all mobile phases used to other models like the quadratic (S-shaped) model and the bi-Moreau model. For our surprise, the ibuprofen adsorption data fit very easily to all of these models. Therefore, we calculated the Fisher parameter for all the models Langmuir, bi-Langmiur, Moreau, bi-Moreau, and the S-shaped (2nd and 3rd degree) for the three mobile phases used (40, 50 and 60% ACN) to determine which model is better.

The best values of the Fisher parameters obtained for the six isotherm models and for the three mobile phases used to elute ibuprofen are reported in Table 1. The value of the Fisher parameter characterizes the quality of the fit of the data to the corresponding model; the larger the value, the better the fit. For the mobile phase containing 40%ACN, the Fisher parameter values for the bi-Langmuir, bi-Moreau and S-shaped of the 3rd degree are: 7549, 7678 and 7727 respectively. The values of the Fisher parameter for the S-shaped 2nd order, the Moreau and the Langmuir models are less than 3000 and 3 times less than the bi-modals and the S-shaped of the 3rd degree. Table 1 show that there is a significant difference between the Langmuir, Moreau, S-shaped (2nd) models and the bi-Langmuir, bi-Moreau and the S-shaped 3rd. Since the scatchard plot supports only a bi-Langmuir model when the mobile phase is 40% acetronitrile, the other models will be excluded. In addition, all the overloaded band profiles of ibuprofen exhibit langmuirian shapes in which there is a sharp front and diffuse tail. Furthermore, the validity of the bi-Langmuir model is further supported by the agreement between the calculated band profiles using the inverse method (IM) and the experimental band profiles in Fig. 3

For the second mobile phase 50% ACN, the Fisher parameter values are: 5181, 4811, 5468, 6207, 5466 and 6829 for the Langmuir, bi-Langmuir, Moreau, bi-Moreau, S-shaped 2nd and 3rd order respectively. For the third mobile phase used (60% ACN), the Fisher parameter values are: 2985, 2986, 3069, 2777, 2181, 3069 for the same models mentioned above respectively. The highest Fisher parameter for these two mobile phases was also for the S-shaped 3rd model, but there was no significant difference between this model and the other models. Since the scatchrad plots doesn't support unimodals , the Langmuir and the Moreau modules can be excluded. Also because there was more than one inflection point in each of the scatchard plots of ibuprofen for the 50 and 60% ACN mobile phases the bi-Langmuir model can be excluded. On the other hand, it was difficult to decide which one of the two models; the bi-Moreau or the S-shaped (3rd) accounts better for the adsorption of ibuprofen on the C_{18} stationary phase since the respective Fisher test values is less than the required threshold of 2.0 ($F_{model1}/ F_{model2} = F_{cal} < F_{table}$).

However there are two reasons which made the S-shaped model better for fitting the ibuprofen adsorption data, the first is that the adsorption data fits very quickly and easily to the S-shaped model. Second, the goodness of this fit was validated by using the inverse method of determining the isotherm. There was an excellent agreement between the experimental and the calculated band profiles. Therefore, the s-shaped is the best model for fitting the adsorption data of ibuprofen using 50 and 60 % acetonitrile in the mobile phase. This model is suitable when there is an anti-Langmuirian behavior or when the isotherm is concave upward at low concentration (S-shaped). Usually, this behavior results when the amount adsorbed at equilibrium increases more rapidly than the concentration in the mobile phase. Such an effect results usually from strong adsorbate-adsorbate interactions. Based on the assumptions that there is more than the one adsorption site and that there is adsorbate-adsorbate interactions, we can assume that ibuprofen is adsorbed from the alkyl group side on the alkyl chains of the C_{18} surface with the carboxylic groups projected away from the C_{18} surface. While, this constitutes the majority of the adsorption sites, the other sites on the stationary phase might be polar and their numbers are less. Probably these later sites are occupied by the ibuprofen molecule form the propanoic acid side.

The adsorbed ibuprofen molecules and the acetonitrile form more than one layer on the C_{18} surface and the ibuprofen molecules interact with each other. The interaction between the ibuprofen molecules is enhanced by increasing the acetonitrile in the mobile phase because the solubility of ibuprofen increases with increasing acetonitrile. There are some reports in the literature [5] which support our assumption that there are adsorbate-adsorbate interaction between the neutral analytes adsorbed on the different layers of acetonitrile which are adsorbed on the surface of the C_{18} stationary phase [5]. The same study showed that the shapes of the adsorption isotherms of neutral compounds like phenol and caffeine on C_{18} stationary phase using acetonitrile-water is different from adsorption isotherms using methanol-water.

With methanol water mobile phases the adsorption isotherms are concave upward and they were best modeled by a bi-Langmuir or tri-Langmuir models. On the other hand, the adsorption isotherms of the same compounds using acetonitrile-water mobile phases are concave upward at low concentration and downward at high concentration and they have inflection points. This behavior was explained based on the assumption of the accumulation of the analyte in an adsorbed multilayer system of acetonitrile on the bonded alkyl chains. The formation of up to four layers of acetonitrile was confirmed by the excess adsorption isotherm data measured for acetonitrile on the C_{18} stationary phase [47]. Since ibuprofen (pKa ~4.5) is neutral at the pH used for this study (pH =2.20) it is expected that it behaves like phenol and caffeine on RPLC using acetonitrile water mobile phases.

4.1.4. Parameters of the isotherm

Table 2 shows the best fit of the adsorption data acquired by the frontal analysis (FA) for the three mobile phases used to the six models above. The table shows that the all the parameters of the adsorption isotherm exhibit the same trend with increasing the amount of the acetonitrile in the mobile phase in terms of the saturation capacities and the equilibrium constants. For example the saturation capacities for ibuprofen using the bi-Langmuir model are: 475.3, 223.9, and 183.2 and the corresponding equilibrium constants are: 0.0359, 0.0340 and 0.02217 for the mobile phases 40, 50 and 60 % acetonitrile respectively. Therefore, the saturation capacities and the equilibrium constants decrease by increasing the amount of acetonitrile in the mobile phase. Similarly, in the case of S-shaped isotherm (n=3), the saturation capacities and the adsorption constants for ibuprofen decrease by increasing the amount of acetonitrile in the mobile phase.

For example: q_{s1}: 76.51, 38.44 and 36.8 , b_1: 0.3402, 0.2266 and 0.09969, b_2: 0.0197 , 0.0149 and 0.0045 and b_3: .00306, 0.00177 and 0.0002 for the mobile phases with 40%, 50% and 60% ACN respectively. Fig. 3A-C show an overlay of the experimental band profile with the calculated band profiles obtained using the inverse method of determining the isotherm. Table 2 shows the parameters of the adsorption isotherms obtained by using the inverse method (IM).

5.0 Conclusions

This work provides new insights on the retention mechanism of ibuprofen on reversed phase liquid chromatography using acetonitrile as a modifier. Simple unimodals like the Langmuir model is not suitable to model the adsorption of ibuprofen on the C_{18} surface. The bi-Langmuir is the best model that fits the adsorption data of ibuprofen on C_{18} stationary phase using 40% acetonitrile as a modifier in the mobile phase, while the S-shaped model is the best model that fits the adsorption data when the mobile phase contains 50% and 60% acetonitrile. These models are validated by the inverse method (IM). There is a good agreement between the experimental and the calculated band profiles. All the models that have been used in this study indicate that the parameters of the adsorption isotherm; the saturation capacities and the equilibrium constants of ibuprofen decrease with increasing the amount of acetonitrile in the mobile phase.

References

Ahmad, T. and G. Guiochon (2007). Numerical determination of the adsorption isotherm of tryptophan at different temperatures and mobile phase compositions, J. Chromatogr. A, 1142, 148-163.

Ahmad, T. and G. Guiochon (2006).Effect of the mobile phase composition on the adsorption behavior of tryptophan in reversed-phase liquid chromatography, J. Chromatogr. A, 2006, 1114, 111–122.

A. Felinger, A., A. Cavazzini and G. Guiochon (2003). Determination of the single component and competitive adsorption isotherms of the 1-indanol enantiomers by the inverse method. J. Chromatogr. A. 986 issue 2, 207-225.

Gritti, F. and G. Guiochon (2009). Band profiles of reacting acido-basic compounds with water–methanol eluents at different pHs and ionic strengths in reversed-phase liquid chromatography, J. Chromatogr. A, 12163175–3184.

Gritti, F. and G. Guiochon (2005). Critical contribution of nonlinear chromatography to the understanding of retention mechanism in reversed-phase liquid chromatography, J. Chromatography A, 1099, 1–42.

Ghulam A. Shabir, Abdul Hamid. And Shafique A. Arain (2008). J. Liq. Chromatogr. & Rel. Technonol. 31, 497-508.

Graham, D. (1953). The characterization of physical adsorption: the equilibrium function and standard free energy of adsorption. J. Phys. Chem. 57, 665-669.

Guiochon, G. A. Felinger, A.M. Katti and D. Shirazi (2006). Fundamentals of Preparative and Nonlinear Chromatography, second ed., Elsevier, Amsterdam.

Kalafut, P., J. Sochor, J. Klimeš and J. Dohnal (2005). Use of the zirconia-based stationary phase for separation of ibuprofen and its impurities. J. Pharm. Biomed . Anal 38, 4, 609-618.

Kalafut, P.,, R. Kučera, J. Klimeš and J. Sochor (2009). An innovative approach to the analysis of 3-[4-(2-methylpropyl)phenyl]propanoic acid as an impurity of ibuprofen on a carbon-coated zirconia stationary phase. J. Pharm Biomed anal, 49, 5, 1150-1156.

Kim, H., F. Gritti and G. Guiochon (2004). Effect of the temperature on the isotherm parameters of phenol in reversed-phase liquid chromatography, J. Chromatogr. A, 1049, 25-36.

Proceeding of the united states pharmacopeia XXVI, The United States Pharmacopoeia Convention, Rockville, USA, 2003.

Moreau, M. P. Valentin, C. Vidal-Madjar, B. Lin and G. Guiochon (1991). Adsorption isotherm model for multicomponenet adsorbate adsorpbate interactions. J. Colloid. Interface Sci., 141, 127 D 136.

Rouchon, P., M. Schoenauer, P. Valentin, C. Vidal-Madjar and G. Guiochon (1985). Theory of the propagation of finite concentration bands in gas chromatography. A numerical solution of the ideal problem. J. Phys. Chem., 89(10), 2076-2082.

Quiñones, I. and G. Guiochon (1998). Extension of a Jovanoic-Freundlich isotherm model to multicomponent adsorption on heterogenous surface. J. Chromatogr. A, 796, 1,13, 15-40.

Svoboda, V. (1990). Multi-component elution overload chromatography of compounds with S-shaped isotherms : A theoretical study. J. Chromatogr. A, 518, 77-97.

Table1. Fisher parameters table

% Acetonitrile	Isotherm model					
	Langmuir	Bilangmuir	Moreau	bi-Moreau	S-shaped 2nd	S-shaped 3rd
	1832	7549	2038	7678	2036	7727
40	5181	4811	5468	6207	5466	6829
50	2985	2986	3069	2777	2181	3069
60						

Table 2. The best fit of the isotherm parameters to different models using the FA and the IM of ibuprofen using mobile phases containing different amounts of acetonitrile

	Models		Parameters					
			q_{s1}	b_1	q_{s2}	B_2		
40% ACN	Langmuir	FA	322.2	0.06939				
		IM	242.4	0.09325				
	bi-Langmuir	FA	475.3	0.0359	0.7846	12.0228		
		IM	311.0	0.06372	6.113	0.4946		
			$q_{s,1}$	b_1	I_1	$q_{s,2}$	b_2	I_2
	Moreau	FA	416.9	0.0542	0.602			
		IM	252.3	0.05931	3.046			
	Bi-Moreau	FA	313.8	0.05910	1.686	8.607	0.8144	2.526
		IM	324.4	0.06977	0.6273	0.1656	40.986	19.945
			$q_{s,1}$	b_1	b_2	b_3		
	S-shaped2	FA	214.8	0.1055	0.00154			
		IM	170.3	0.1346	0.00222			
	S-shaped3	FA	76.51	0.3402	0.0197	0.00306		
		IM	161.7	0.1427	0.00141	0.00157		
50% ACN			$q_{s,1}$	b_1	$q_{s,2}$	b_2		
	Langmuir	FA	246.3	0.03408				
		IM	140.3	0.05542				
	bi-Langmuir	FA	223.9	0.03408	0.03407	22.30671		
		IM	148.1	0.05117	0.4133	0.734		
			$q_{s,1}$	b_1	I_1	$q_{s,2}$	b_2	I_2
	Moreau	FA	184.1	0.04455	1.49236			
		IM	193.5	0.04027	0.5709			
	bi-Moreau	FA	135.2	0.04408	3.66145	0.29479	9.95909	0.8475
		IM	134.6	0.05696	0.7802	12.51	0.01262	29.14
			$q_{s,1}$	b_1	b_2	b_3		
	S-shaped2	FA	92.41	0.08878	0.00292			
		IM	107.7	0.07237	0.0005457			
	S-shaped3	FA	38.44	0.22636	0.01499	0.00177		
		IM	71.12	0.01104	0.002426	0.0002501		
60% ACN			$q_{s,1}$	b_1	$q_{s,2}$	b_2		
	Langmuir	FA	183.3	0.02215				
		IM	107.7	0.029				
	bi-langmuir	FA	183.2	0.02217	-18.0605	-0.00008		
			$q_{s,1}$	b_1	I_1	$q_{s,2}$	b_2	I_2
	Moreau	FA	109.5	0.03597	1.77841			
		IM	79.84	0.03921	1.492			
	bi-Moreau	FA	95.71	0.03911	2.1691	2.50181	0.29728	0.00799
		IM	**68.29**	0.03853	2.179	2.483	0.2022	0.06689
			$q_{s,1}$	b_1	b_2	b_2		
	S-shaped2	FA	54.21	0.07254	0.00237			
		IM	29.36	0.09389	0.00665			
	S-shaped3	FA	36.80	0.09969	0.00665	0.00002		
		IM	35.78	0.0831	0.002704	0.00006809		

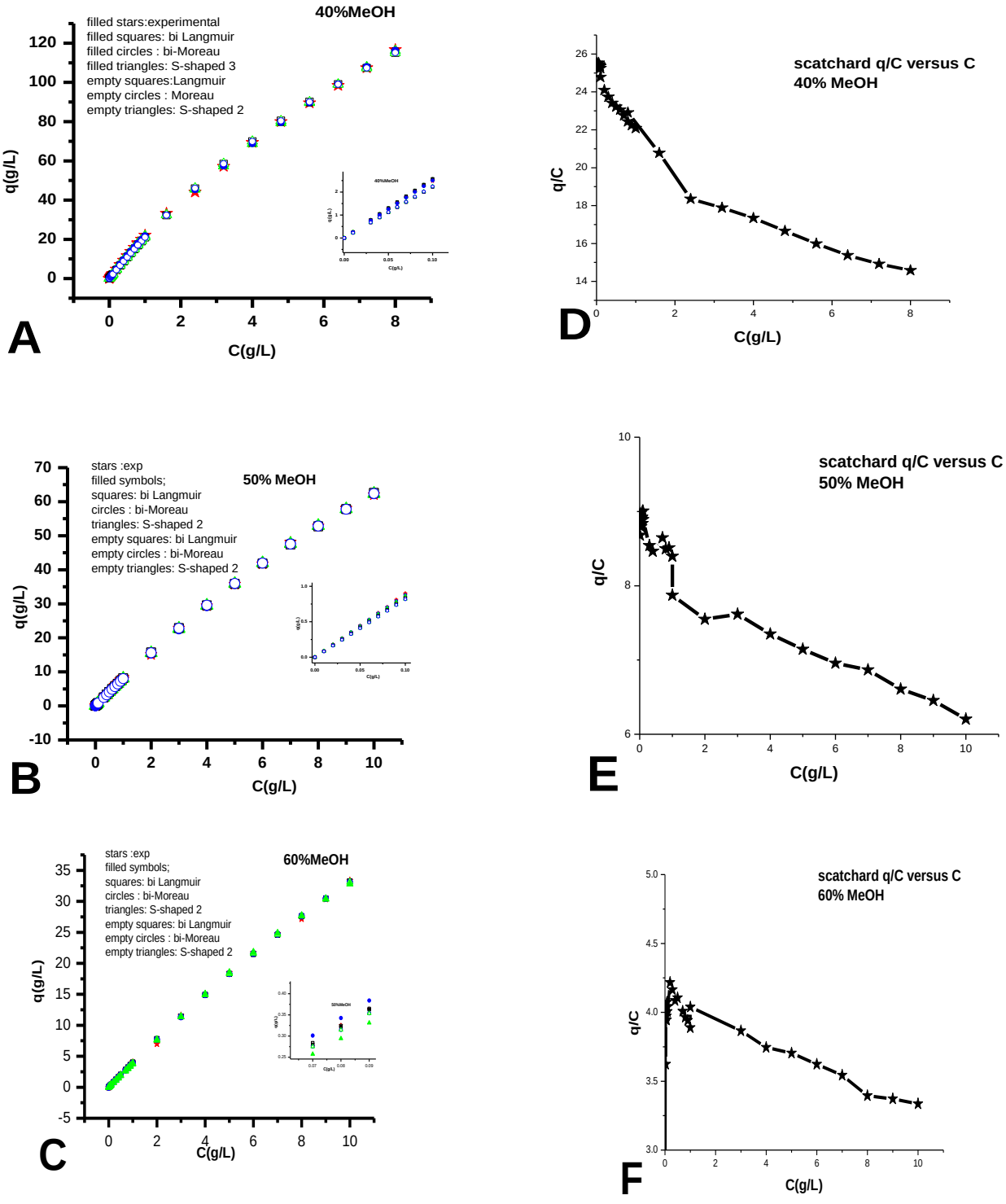

Fig.1. Right, the adsorption data of ibuprofen from the mobile phases containing aqueuous solutions that has (A) 40% (B) 50% and (C) 60% acetonitrile (ACN) and 1% phosphoric acid, at pH 2.2, derived from single component frontal analysis (filled stars), the best bi-Langmuir isotherm (filled squares), the best S-shaped to the third degree, (filled triangles) and the bi-Moreau model (filled circles), Langmuir(empty squares), Moreau (empty circles) and the S-shaped of the second order (empty triangles) respectively. Left (D-F)the corresponding scatchard plots. Column: C_{18} Alltech Altima, Flow rate 1.0 mL/min, wavelength 280 nm, and Temperature =296 K.

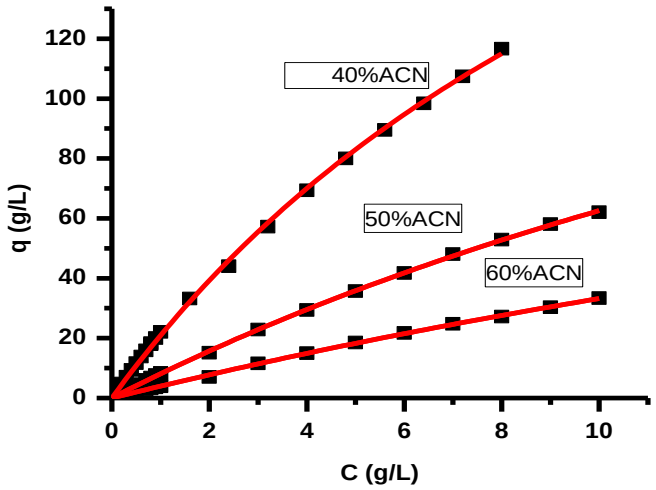

Fig. 2. An overlay of experimental isotherm data of ibuprofen using (0.1- 10.0) g/L ibuprofen solutions fitted to an S-shaped 3rd degree isotherm model. Same experimental conditions as in Fig. 1. Note that curvature of the isotherm decrease from low to high ACN concentrations.

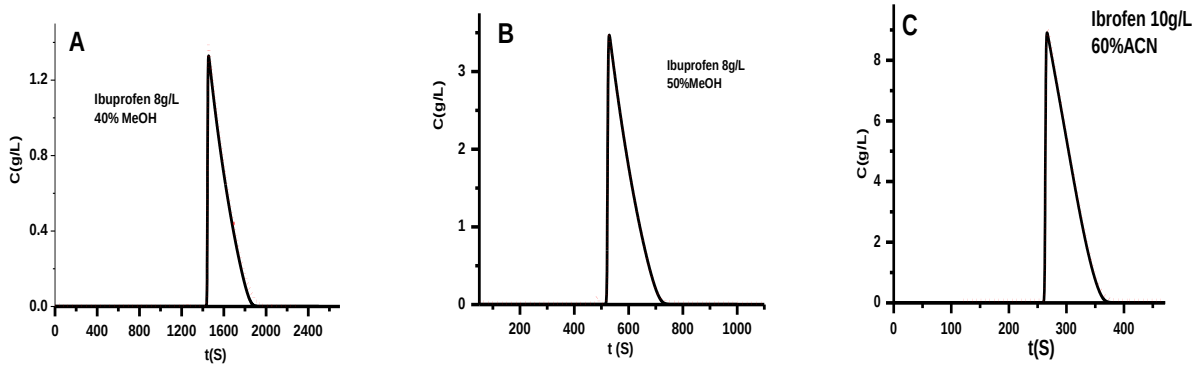

Fig. 3. (A-C). Experimental (dotted) and the calculated of profiles (solid lines) using the inverse method for ibuprofen. Same experimental conditions as in Fig. 1.