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Description of the retention behaviour in micellar liquid chromatography as a function of pH, surfactant and modifier concentration

J.R. Torres-Lapasió, J.J. Baeza-Baeza, M.C. García-Alvarez-Coque*

Departamento de Química Analítica, Facultad de Química, Universitat de Valencia, 46100 Burjassot, Valencia, Spain

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Abstract

Micellar liquid chromatography permits the elution of solutes of diverse polarity. One of the most outstanding advantages of the technique is its capability of predicting the retention with high accuracy, as a function of different experimental variables. The separation of a group of compounds is usually optimized by varying the concentrations of surfactant and modifier in the mobile phase. The pH is, however, for many solutes, a variable that should be considered in the description of their elution behaviour. A global model that takes into account, simultaneously, the concentrations of surfactant and modifier, and the pH as chromatographic variables, is proposed for ionizable solutes. The mean relative errors obtained in the prediction of the retention of seven compounds eluted with 81 mobile phases of sodium dodecyl sulphate (SDS) and 1-propanol, using equations fitted with the experimental data measured in 9 to 12 mobile phases was, usually, lower than 6%. The ranges of the variables considered were 0.05–0.15 *M* SDS, 0–0.08 (v/v) 1-propanol and pH 3–7.

Keywords: Retention behaviour; Surfactants; Modifiers; Mobile phase composition; Micelles; Sodium dodecyl sulfate; Propanol

1. Introduction

Many interesting examples have appeared in the literature on the use of a secondary chemical equilibrium involving micelles in reversed-phase liquid chromatography [1–7]. Selection of the optimal mobile phase pH is often extremely important owing to the side protonation reactions of many solutes. In fact, the manipulation of the pH can lead to the resolution of complex mixtures [8,9]. In the optimization of the separation of weak organic acids and bases with micellar mobile phases, it is usual to fix the pH and only optimize the concentration of surfactant and modifier. The best pH for the separation is selected after examining the retention in a reduced number of mobile phases, at two or three pH values. However, to achieve the full separation potential, the pH should be simultaneously optimized with the concentrations of surfactant and modifier, since the protonation constants suffer shifts depending on the composition of the micellar mobile phase. This is caused by the different partitioning of the acidic and basic species of a solute in the micellar pseudophase.

*Corresponding author.

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It has been demonstrated that, in micellar media containing a constant concentration of modifier at a given pH, the capacity factor, k', is related to the concentration of surfactant forming micelles, [M], through a very simple equation [10–12]:

$$k' = \frac{K^{\varphi}_{AS}}{1 + K^{\varphi}_{AM}[M]} \quad (1)$$

$K^{\varphi}_{AS} = \phi P^{\varphi}_{AS}$ being the phase ratio multiplied by the partition coefficient between stationary phase and water, and K^{φ}_{AM} the solute–micelle association constant. The superscript φ makes reference to the conditional character of these constants with respect to the concentration of modifier.

The equation describing the retention is more complex when the influence of surfactant and modifier are simultaneously considered, in a hybrid micellar mobile phase [13,14]. Assuming that the relative change in the concentration of solute in bulk water and micelles is proportional to the concentration of modifier, the constants K^{φ}_{AS} and K^{φ}_{AM} will be given by:

$$K^{\varphi}_{AS} = \frac{AS}{A + \Delta A} = K_{AS}\frac{1}{1 + K_{AD}\varphi} \quad (2)$$

$$K^{\varphi}_{AM} = \frac{AM + \Delta AM}{M(A + \Delta A)} = K_{AM}\frac{1 + K_{MD}\varphi}{1 + K_{AD}\varphi} \quad (3)$$

where φ is the concentration of modifier, A and AM are the concentrations of free solute in bulk water and solute associated to the micelle in a pure micellar solution (without modifier), and ΔA and ΔAM are the changes in the concentrations produced by the modifier. The constants K_{MD} and K_{AD} measure the relative variation in the concentration of solute in bulk water and micelle, respectively, in the presence of modifier, taking the pure micellar solution as reference. Thus, the equation of retention will be:

$$k' = \frac{K_{AS}\dfrac{1}{1 + K_{AD}\varphi}}{1 + K_{AM}\dfrac{1 + K_{MD}\varphi}{1 + K_{AD}\varphi}[M]} \quad (4)$$

This model is valid for polar or moderately hydrophobic solutes. For highly hydrophobic solutes, the change in the concentration of solute associated to the stationary phase, due to the presence of the modifier, should also be considered [14]. Eq. (4) can be modified to take this effect into account by substitution of $(1 + K_{SD}\varphi)/(1 + K_{AD}\varphi)$ by the $1/(1 + K_{AD}\varphi)$ factor of K_{AS}. This behaviour has been checked with mobile phases containing the surfactants sodium dodecyl sulphate (SDS) and cetyltrimethylammonium bromide (CTAB), and the modifiers propanol, butanol, pentanol and acetonitrile [6,15–17].

Arunyanart and Cline-Love [18], and Rodgers et al. [19,20], proposed a mathematical model to describe the retention of solutes as a function of the concentration of surfactant and pH. Strasters et al. [8] also considered the concentration of modifier, but proposed an approximate model to predict the retention: the method of triangles adapted to three dimensions, and linear log k' vs. pH, surfactant and modifier functions. In this strategy, the procedure begins with a design of fifteen points located in a three-dimensional space, which is divided in 24 tetrahedra. Therefore, 24 different equations of retention should be fitted. The retention in other mobile phases is calculated by a simple linear interpolation inside each tetrahedron. When the experimental and predicted retention data coincide, a confirmation of the assumed linearity is obtained. However, when strong deviations of the linear model are observed, subsequent measurements should be used to refine the prediction by a further subdivision of the parameter space into smaller tetrahedra. This method needs narrow variable ranges to avoid deviations from linearity, and may require a large number of measurements.

The range of pH values examined by Strasters et al. [8], for the prediction of the retention of several amino acids and peptides, was intentionally reduced to pH 2.5–3.5 to prevent deviations from linearity in their retention behaviour. For larger ranges, a sigmoidal retention vs. pH curve was observed. This is, obviously, a great limitation of the procedure. Also, the retention behaviour of solutes should be preferably described by a single equation, valid for the whole variable space.

In this work, a mathematical model is reported which extends the description made with Eq. (4), to take into account the influence of pH on retention. The model was applied to the prediction of the retention behaviour of solutes inside the working

range of a C_{18} column (pH 3–7), and for the concentration ranges of SDS and propanol, 0.05–0.15 *M*, and 0–0.08 (v/v), respectively. The model has proved to be adequate in the whole variable space.

2. Theory

2.1. Influence of pH and concentration of surfactant on the retention in micellar liquid chromatography

The retention in a reversed-phase system of solutes that exhibit an acid–base behaviour, depends on the pH of the mobile phase. For a monoprotic system showing a protonation equilibrium in water:

$$A + H \rightleftarrows HA \quad K_H = \frac{[HA]}{[A]h} \tag{5}$$

where h is the proton concentration and K_H is the protonation constant, the capacity factor will be given as follows:

$$k' = \phi \frac{[AS] + [HAS]}{[A] + [HA] + [AM] + [HAM]} \tag{6}$$

[AS] and [A] being the concentrations of the non-protonated species present in the stationary phase and bulk water, respectively, and [HAS] and [HA] the concentrations of the respective acidic species. Finally, [AM] and [HAM] refer to the concentrations of the basic and acidic species associated to the micelle, respectively. Inside the column and with mobile phases without an organic modifier, several partitioning equilibria will take place:

(i) Association of the basic species to the micelle:

$$A + M \rightleftarrows AM \quad K_{AM} = \frac{[AM]}{[A][M]} \tag{7}$$

(ii) Association of the basic species to the stationary phase:

$$A \rightleftarrows AS \quad P_{AS} = \frac{[AS]}{[A]} \tag{8}$$

(iii) Association of the acidic species to the micelle:

$$HA + M \rightleftarrows HAM \quad K_{HAM} = \frac{[HAM]}{[HA][M]} \tag{9}$$

(iv) Association of the acidic species to the stationary phase:

$$HA \rightleftarrows HAS \quad P_{HAS} = \frac{[HAS]}{[HA]} \tag{10}$$

By substitution of Eqs. (5,7–10) in Eq. (6), and making $K_{AS} = \phi P_{AS}$ and $K_{HAS} = \phi P_{HAS}$, the following is obtained:

$$k' = \frac{K_{AS} + K_{HAS}K_H h}{1 + K_H h + K_{AM}[M] + K_{HAM}K_H h[M]} \tag{11}$$

Rewriting this expression, an equation similar to Eq. (1) is obtained:

$$k' = \frac{\dfrac{K_{AS} + K_{HAS}K_H h}{1 + K_H h}}{1 + \dfrac{K_{AM} + K_{HAM}K_H h}{1 + K_H h}[M]} = \frac{K^H_{AS}}{1 + K^H_{AM}[M]} \tag{12}$$

where K^H_{AS} and K^H_{AM} are conditional constants with respect to proton concentration, that show a sigmoidal dependence with pH. Eq. (11) may also be rewritten as follows:

$$k' = \frac{\dfrac{K_{AS}}{1 + K_{AM}[M]} + \dfrac{K_{HAS}}{1 + K_{HAM}[M]} \dfrac{1 + K_{HAM}[M]}{1 + K_{AM}[M]} K_H h}{1 + \dfrac{1 + K_{HAM}[M]}{1 + K_{AM}[M]} K_H h} \tag{13}$$

A similar equation was reported previously [18,19]. Eq. (13) indicates that the retention varies with pH, following a sigmoidal behaviour between the retention of the acidic species and the retention of the basic species. Eq. (13) is also valid for mobile phases having the same concentration of organic modifier, and may easily be extended to solutes exhibiting several protonation equilibria in the pH working range.

2.2. Simultaneous influence of pH, surfactant and modifier on the retention

Most micellar liquid-chromatographic analytical procedures reported in the literature make use of hybrid micellar eluents. The modifier shortens the elution times of the solutes, especially important for the most non-polar solutes, and often improves the shape of the chromatographic peaks. The effect of the modifier on the description of the retention can be considered by substitution of Eqs. (2,3), and other similar for the protonated species, in Eq. (13). In this way, the following is obtained:

$$k' = \frac{\dfrac{\dfrac{K_{AS}}{1+K_{AD}\varphi}}{1+K_{AM}\dfrac{1+K_{MD}\varphi}{1+K_{AD}\varphi}[M]} + \dfrac{\dfrac{K_{HAS}}{1+K_{HAD}\varphi}}{1+K_{HAM}\dfrac{1+K_{HMD}\varphi}{1+K_{HAD}\varphi}[M]}\kappa K_H h}{1+\kappa K_H h} \tag{14}$$

where:

$$\kappa = \frac{1+K_{HAM}\dfrac{1+K_{HMD}\varphi}{1+K_{HAD}\varphi}[M]}{1+K_{AM}\dfrac{1+K_{MD}\varphi}{1+K_{AD}\varphi}[M]}$$

Eq. (14) may be rewritten as:

$$k' = \frac{k'_A + k'_{HA}K_H^{M\varphi}h}{1+K_H^{M\varphi}h} \tag{15}$$

where k'_A and k'_{HA} are the capacity factors of the basic and acidic species, respectively, and $K_H^{M\varphi}$ is the conditional protonation constant that depends on the concentration of surfactant and modifier, and on the association capability of both acid–base species with the micelle.

Eq. (14) contains nine constants (K_{AS}, K_{AM}, K_{AD}, K_{MD}, K_{HAS}, K_{HAM}, K_{HAD}, K_{HMD} and K_H), and describes the change in the capacity factors of acid–base solutes at any concentration of surfactant, modifier and pH, in the mobile phase. The modification of the protonation constant, K_H, in a water-modifier bulk solvent with the concentration of modifier, has not been considered, because this would complicate excessively the model by the introduction of a new constant. It was checked that the inclusion of this constant did not improve significantly the description of the retention, since the large number of parameters of the model provides a high flexibility to the fitting, absorbing the deviations produced by this simplification.

The mean relative errors in the predicted capacity factors were calculated as:

$$\epsilon_r = \frac{\sum_{i=1}^{n}|k'_{exp} - k'_{calc}|}{\sum_{i=1}^{n}k'_{exp}} \times 100 \tag{16}$$

since this expression avoids achievement of relative errors dependent on the capacity factors, owing to the wide range of k' values.

3. Experimental

3.1. Apparatus

A Hewlett–Packard HP 1050 (Palo Alto, CA, USA) liquid chromatograph with an isocratic pump, and automatic injector, a UV–visible detector and an HP 3396A integrator were used. The injection volume was 20 μl, and the detection was performed at 254 nm. The mobile phase flow-rate was 1 ml min^{-1}. The dead volume in each one of the 81 mobile phases was determined by injection of water, taking the first deviation from the base-line. A Spherisorb ODS-2 column (5 μm particle size, 125 mm×4.6 mm I.D.) and precolumn (35 mm×4.6 mm I.D.) from Scharlau (Barcelona, Spain) were used. The mobile phase and the solutions to be injected were vacuum filtered through 0.45-μm and 0.22-μm nylon membranes, respectively (Micron Separations, Westboro, MA, USA).

Data acquisition was made through the PEAK-96 software from Hewlett–Packard (Avondale, PA, USA), and data treatment was performed with MICHROM, a package of programs developed in our laboratory [21]. MICHROM takes part in all the stages of the analytical process. It allows the determination of the dead time, smoothing of chromatograms, measurement of peak parameters and fitting of

Table 1
Capacity factors in several mobile phases of SDS and propanol at increasing pH

Compound	SDS (*M*)	0.05	0.05	0.05	0.10	0.10	0.10	0.15	0.15	0.15
	Propanol (v/v)	0.00	0.04	0.08	0.00	0.04	0.08	0.00	0.04	0.08
	pH									
Benzocaine	3.0	77.3	32.0	18.4	35.4	16.1	9.11	23.3	9.61	6.77
	3.5	64.8	24.5	14.2	31.6	13.2	7.55	21.4	8.40	5.30
	4.0	51.5	17.5	10.1	26.0	9.76	5.84	16.5	6.45	4.27
	4.5	40.0	13.5	8.09	21.2	7.80	5.06	13.7	5.35	3.94
	5.0	34.8	11.9	7.86	18.9	7.18	4.87	12.5	5.07	3.72
	5.5	33.1	11.4	7.70	18.0	6.97	4.79	11.7	5.01	3.73
	6.0	32.6	11.3	7.57	17.8	6.96	4.75	11.6	4.98	3.63
	6.5	32.5	11.1	7.53	17.6	6.93	4.75	11.6	4.96	3.61
	7.0	31.2	11.1	7.26	17.5	6.92	4.75	11.6	4.96	3.61
Bumetanide	3.0	30.4	14.6	10.9	14.5	7.82	5.86	10.1	5.11	3.97
	3.5	30.3	14.6	10.7	14.3	7.79	5.68	10.1	5.30	3.90
	4.0	30.3	14.6	10.2	14.2	7.77	5.47	10.0	5.03	3.87
	4.5	28.9	12.6	8.15	13.5	7.26	4.87	9.76	4.81	3.69
	5.0	25.5	9.70	5.76	12.3	5.99	3.63	8.99	3.95	2.94
	5.5	18.8	6.16	3.47	9.86	4.25	2.37	7.26	2.88	1.93
	6.0	10.3	3.11	2.00	6.39	2.37	1.51	5.00	1.88	1.24
	6.5	5.14	1.96	1.36	3.52	1.58	1.11	2.99	1.26	0.84
	7.0	3.28	1.54	1.17	2.26	1.26	0.92	1.86	1.02	0.72
Ethacrynic acid	3.0	45.0	20.2	14.2	22.8	10.9	7.46	15.2	7.17	5.38
	3.5	40.5	17.7	11.7	21.6	10.2	6.49	14.8	6.73	4.88
	4.0	32.0	13.3	7.83	18.2	8.45	4.77	12.7	5.39	3.79
	4.5	21.2	8.09	4.81	13.0	5.27	3.14	8.71	3.58	2.68
	5.0	11.9	4.48	3.03	7.59	3.39	2.23	5.15	2.43	1.75
	5.5	7.41	3.20	2.31	4.60	2.53	1.76	3.62	1.95	1.38
	6.0	5.59	2.76	2.11	3.53	2.22	1.58	2.91	1.69	1.25
	6.5	4.60	2.64	2.00	3.13	2.11	1.55	2.67	1.62	1.26
	7.0	4.39	2.66	1.98	3.13	2.05	1.51	2.57	1.61	1.27
Furosemide	3.0	15.9	8.09	5.64	7.94	4.74	3.31	5.75	3.24	2.47
	3.5	15.5	7.73	5.18	7.86	4.61	3.16	5.63	3.15	2.39
	4.0	14.1	6.88	4.06	7.44	4.18	2.70	5.37	2.85	2.17
	4.5	10.9	4.56	2.61	6.19	3.15	1.95	4.72	2.32	1.71
	5.0	6.27	2.35	1.49	4.25	1.83	1.22	3.32	1.48	1.14
	5.5	3.25	1.27	0.91	2.43	1.20	0.81	2.16	1.01	0.76
	6.0	1.69	0.82	0.63	1.43	0.80	0.61	1.36	0.73	0.59
	6.5	1.15	0.69	0.56	1.08	0.67	0.53	0.97	0.64	0.51
	7.0	0.96	0.64	0.53	0.91	0.62	0.51	0.87	0.61	0.48
Sulfanilamide	3.0	4.10	2.07	1.20	2.53	1.41	1.03	1.88	1.08	0.93
	3.5	2.32	1.28	0.85	1.64	0.98	0.76	1.38	0.82	0.71
	4.0	1.59	0.86	0.66	1.24	0.76	0.63	1.11	0.69	0.59
	4.5	1.26	0.74	0.61	1.10	0.69	0.59	1.03	0.66	0.55
	5.0	1.17	0.70	0.59	1.04	0.66	0.57	0.98	0.64	0.53
	5.5	1.15	0.68	0.58	1.02	0.65	0.56	0.95	0.63	0.53
	6.0	1.14	0.68	0.58	1.02	0.65	0.56	0.95	0.63	0.53
	6.5	1.13	0.68	0.58	1.01	0.65	0.56	0.95	0.63	0.52
	7.0	1.13	0.68	0.58	1.01	0.65	0.56	0.95	0.63	0.52

Table 1. Continued

Compound	SDS (*M*)	0.05	0.05	0.05	0.10	0.10	0.10	0.15	0.15	0.15
	Propanol (v/v)	0.00	0.04	0.08	0.00	0.04	0.08	0.00	0.04	0.08
	pH									
Tyrosine	3.0	19.5	9.37	4.46	9.47	5.39	2.96	6.77	3.39	2.41
	3.5	14.6	5.50	2.66	7.23	3.36	1.75	5.88	2.16	1.32
	4.0	8.07	2.64	1.51	4.61	1.79	0.97	3.43	1.28	0.69
	4.5	3.83	1.45	1.10	2.49	1.00	0.67	1.81	0.72	0.48
	5.0	1.67	1.07	0.88	1.32	0.72	0.57	0.98	0.45	0.40
	5.5	1.04	0.84	0.81	0.88	0.62	0.53	0.70	0.49	0.36
	6.0	0.88	0.81	0.77	0.73	0.58	0.52	0.56	0.42	0.34
	6.5	0.85	0.79	0.77	0.68	0.58	0.51	0.54	0.44	0.36
	7.0	0.84	0.77	0.76	0.68	0.57	0.50	0.52	0.42	0.35
Xipamide	3.0	36.9	16.4	11.6	18.4	9.12	6.40	12.6	6.14	4.59
	3.5	36.8	16.6	11.6	18.4	9.19	6.29	12.6	6.13	4.60
	4.0	36.5	16.4	11.2	18.4	9.13	6.21	12.5	6.10	4.59
	4.5	36.0	15.6	10.2	17.9	9.00	5.95	12.4	5.97	4.56
	5.0	34.1	13.6	8.30	17.1	8.53	5.11	12.2	5.60	4.14
	5.5	28.3	9.32	5.45	15.6	6.81	3.71	11.0	4.69	3.27
	6.0	18.2	4.89	2.76	11.6	4.22	2.21	9.08	3.23	1.94
	6.5	9.15	2.35	1.38	6.70	2.20	1.13	5.88	1.86	1.10
	7.0	4.17	1.28	0.78	3.22	1.23	0.74	3.01	1.03	0.64

skewed peaks. Tools for the experimental design, optimization of the mobile phase composition to resolve a mixture of analytes, and simulation of chromatograms in several experimental conditions, are implemented. Routines for the graphical representation of chromatograms, resolution surfaces, contour maps, management of data series, optimization and regression analysis, are also included.

3.2. Reagents

The following compounds were used: benzocaine (Fluka, Buchs, Switzerland), bumetanide (Boehringer Ingelheim, Barcelona, Spain), ethacrynic acid (Merck, Sharp and Dohme, Madrid, Spain), furosemide (Lasa, Barcelona), sulfanilamide (Sigma, St. Louis, MO, USA), tyrosine (Scharlau), and xipamide (Lacer, Barcelona). The diuretics benzocaine, bumetanide, ethacrynic acid, furosemide, and xipamide, were kindly donated by the Spanish pharmaceutical industries. The micellar mobile phases were prepared with sodium dodecyl sulphate (Merck, Darmstadt, Germany) and 1-propanol (Panreac, Barcelona, Spain). The solutes were eluted at different pH values, which were adjusted with 0.01 *M* citrate buffer, after the addition of the alcohol. The buffer was prepared with citric acid monohydrate and sodium hydroxide (Panreac).

The capacity factors of the seven compounds, eluted with mobile phases containing varying concentrations of SDS and propanol, at the pH values: 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, and 7.0, are shown in Table 1. The number of mobile phases studied was 81. The spatial distribution of the mobile phases is shown in Fig. 1.

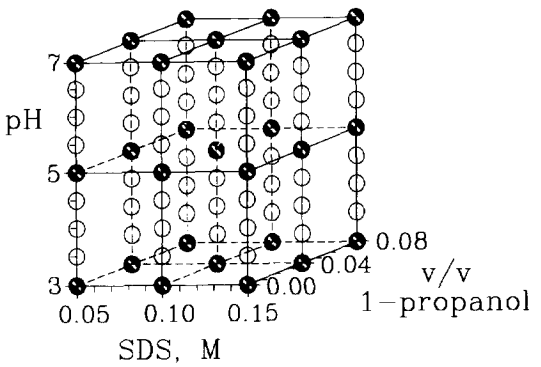

Fig. 1. Spatial distribution of the mobile phases used in this work.

4. Results and discussion

The models proposed above were validated with the retention data of seven solutes that present an acid–base behaviour in the 3–7 pH range. The protonation constants of the solutes (log K_H) in water are: benzocaine, 2.5; bumetanide, 10.0 and 5.2; ethacrynic acid, 3.5; furosemide, 3.9; sulfanilamide, 10.4 and 2.3; tyrosine, 9.2 and 2.2, and xipamide, 10.0 and 4.8 [22,23]. For the diprotic solutes, only the protonation equilibrium in acid medium was considered.

Fig. 2 shows the modification of the capacity factors with pH for several compounds, in eluents containing 0.05 *M* SDS/0.08 (v/v) propanol and 0.15 *M* SDS without propanol. The same sigmoidal behaviour was observed for mobile phases with other concentrations of SDS and propanol. The capacity factors of the basic and acidic species, and the conditional protonation constants for the seven compounds studied, for diverse concentrations of surfactant and modifier, are indicated in Table 2. The parameters were obtained by fitting to Eq. (15) the experimental data obtained in mobile phases of SDS and propanol, at increasing pH (nine values). The concentration of micelles was calculated by subtraction of the critical micellar concentration (cmc) in micellar solutions without alcohol ($8.15 \cdot 10^{-3}$ *M*). In

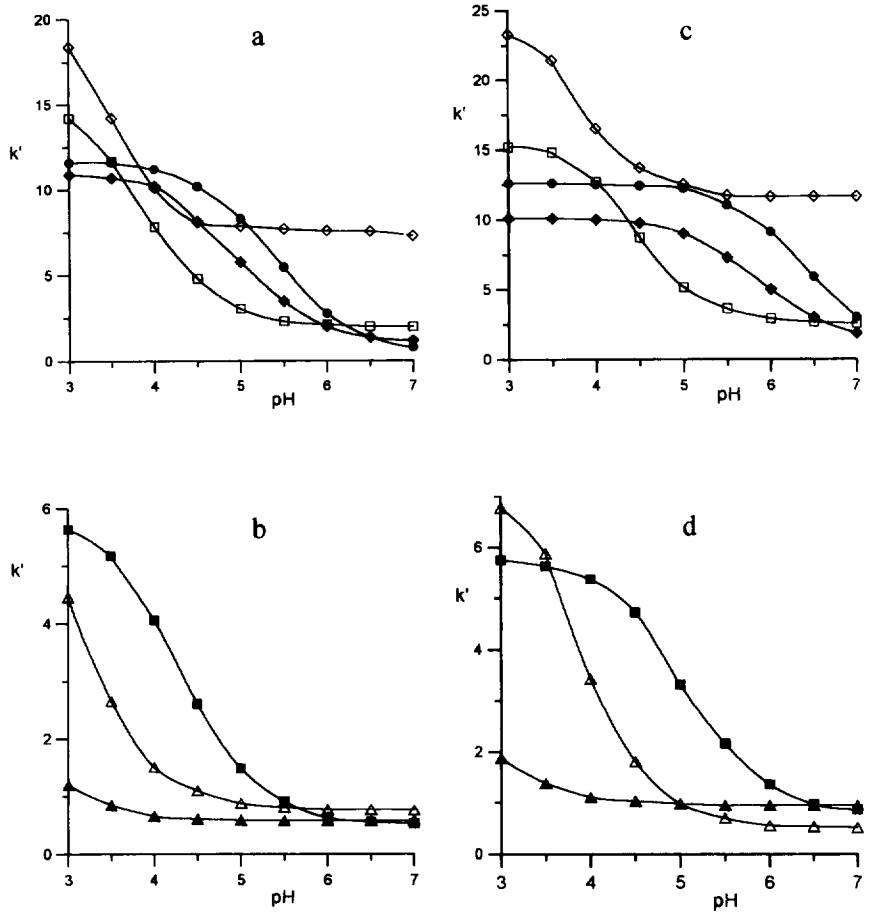

Fig. 2. Modification of the capacity factors with pH for: benzocaine (◇), ethacrynic acid (□), xipamide (●), bumetanide (♦), furosemide (■), tyrosine (△) and sulfanilamide (▲), for 0.05 *M* SDS/0.08 (v/v) propanol (a,b), and 0.15 *M* SDS (c,d) mobile phases.

Table 2
Estimated capacity factors of the acidic and basic species and conditional protonation constants, in hybrid mobile phases of SDS and propanol at increasing pH, using Eq. (15)

Compound	SDS (*M*)	Propanol (v/v)	k'_{HA}	k'_{A}	log $K_H^{M\varphi}$	ϵ_r	r
Benzocaine	0.05	0.00	85.2	32.3	3.73	0.6	0.9997
	0.05	0.04	39.0	11.1	3.47	0.2	1.0000
	0.05	0.08	23.8	7.42	3.32	1.6	0.9988
	0.10	0.00	38.0	17.6	3.84	0.1	1.0000
	0.10	0.04	19.1	6.85	3.51	1.0	0.9994
	0.10	0.08	11.0	4.71	3.39	0.9	0.9990
	0.15	0.00	25.5	11.5	3.80	1.2	0.9981
	0.15	0.04	10.9	4.90	3.60	1.4	0.9981
	0.15	0.08	8.97	3.65	3.15	0.7	0.9993
Bumetanide	0.05	0.00	30.7	1.80	5.64	1.0	0.9998
	0.05	0.04	14.9	1.28	5.22	1.6	0.9994
	0.05	0.08	11.0	1.16	4.94	1.6	0.9995
	0.10	0.00	14.4	1.59	5.76	0.9	0.9997
	0.10	0.04	7.92	1.04	5.42	1.3	0.9996
	0.10	0.08	5.84	0.91	5.11	1.0	0.9998
	0.15	0.00	10.1	1.36	5.84	0.6	0.9998
	0.15	0.04	5.22	0.95	5.41	1.6	0.9991
	0.15	0.08	4.01	0.63	5.33	1.3	0.9993
Ethacrynic acid	0.05	0.00	46.6	4.65	4.30	1.8	0.9997
	0.05	0.04	21.5	2.51	4.11	1.0	0.9999
	0.05	0.08	15.8	2.01	3.88	0.8	0.9999
	0.10	0.00	23.5	2.96	4.47	0.6	0.9999
	0.10	0.04	11.5	2.00	4.27	1.9	0.9992
	0.10	0.08	8.03	1.54	4.01	0.8	0.9999
	0.15	0.00	15.9	2.49	4.45	2.0	0.9993
	0.15	0.04	7.59	1.60	4.22	1.3	0.9996
	0.15	0.08	5.67	1.22	4.15	1.3	0.9997
Furosemide	0.05	0.00	16.3	0.86	4.76	0.8	0.9999
	0.05	0.04	8.44	0.56	4.52	2.3	0.9993
	0.05	0.08	5.89	0.55	4.30	1.2	0.9999
	0.10	0.00	8.09	0.85	4.95	0.6	0.9999
	0.10	0.04	4.88	0.61	4.65	1.5	0.9995
	0.10	0.08	3.40	0.52	4.50	0.8	0.9999
	0.15	0.00	5.79	0.84	5.03	1.1	0.9997
	0.15	0.04	3.29	0.60	4.72	0.9	0.9997
	0.15	0.08	2.51	0.48	4.68	0.4	1.0000
Sulfanilamide	0.05	0.00	10.2	1.14	2.68	0.5	0.9999
	0.05	0.04	4.39	0.67	2.78	0.6	0.9998
	0.05	0.08	2.28	0.58	2.75	0.4	0.9997
	0.10	0.00	5.34	1.01	2.73	0.2	1.0000
	0.10	0.04	2.55	0.65	2.83	0.2	1.0000
	0.10	0.08	1.80	0.56	2.78	0.4	0.9998
	0.15	0.00	2.96	0.96	2.93	0.5	0.9997
	0.15	0.04	1.82	0.63	2.78	0.3	0.9998
	0.15	0.08	1.47	0.53	2.88	0.3	0.9998

Table 2. Continued

Compound	SDS (*M*)	Propanol (v/v)	k'_{HA}	k'_{A}	log $K_H^{M\varphi}$	ϵ_r	r
Tyrosine	0.05	0.00	23.3	0.75	3.69	1.3	0.9999
	0.05	0.04	14.8	0.79	3.20	1.0	0.9999
	0.05	0.08	7.33	0.78	3.11	1.2	0.9998
	0.10	0.00	10.8	0.70	3.80	2.0	0.9997
	0.10	0.04	7.80	0.57	3.30	0.3	1.0000
	0.10	0.08	5.02	0.51	3.08	0.7	0.9999
	0.15	0.00	7.79	0.50	3.86	3.5	0.9986
	0.15	0.04	4.65	0.42	3.36	2.7	0.9993
	0.15	0.08	4.75	0.35	2.94	1.0	0.9999
Xipamide	0.05	0.00	37.1	0.88	5.98	0.8	0.9998
	0.05	0.04	16.7	0.61	5.58	1.1	0.9997
	0.05	0.08	11.6	0.58	5.38	1.0	0.9998
	0.10	0.00	18.4	0.82	6.20	0.6	0.9998
	0.10	0.04	9.28	0.51	5.89	1.5	0.9992
	0.10	0.08	6.37	0.53	5.58	0.7	0.9999
	0.15	0.00	12.6	0.78	6.37	0.5	0.9998
	0.15	0.04	6.15	0.59	5.96	0.3	1.0000
	0.15	0.08	4.67	0.37	5.80	1.5	0.9993

previous work, it was checked that the subtraction of this value of cmc decreases the uncertainty in the regression process [14].

The correlation coefficients in Table 2 indicate the reliability of the proposed model. It may be observed that the modifier decreases the conditional protonation constant, log $K_H^{M\varphi}$, whereas this constant slightly increases with the concentration of surfactant (see also Figs. 3 and 4). The shift in the conditional protonation constants upon variation of the modifier concentration is caused by both the modification of the thermodynamic constants in the water–modifier bulk solvent, and the displacement of the acid–base equilibria, because of the modification of the interaction of the solute with the micelles, in the presence of the modifier.

The results of the fitting to Eq. (4) of the k'_A and k'_{HA} values shown in Table 2 are given in Table 3. This mathematical treatment is equivalent to the fitting of the experimental data to Eq. (14) in the pH regions where the basic and acidic species predominate. The poor results obtained with the protonated tyrosine are due to the limited extension of the protonation in the studied pH range (see Fig. 2). A poor fitting was also usually achieved for the k'_A values, due to the scarce retention of the basic species, which makes the fitting very sensitive to the experimental error.

The retention data at pH 3.0 and 7.0, and diverse concentrations of surfactant and modifier (nine values), for each solute, were also fitted to Eq. (4), giving the physicochemical constants for the acidic species (K_{HAS}, K_{HAM}, K_{HAD}, and K_{HMD}), and basic species (K_{AS}, K_{AM}, K_{AD}, and K_{MD}), shown in Table 4. At pH 3.0, an acceptable agreement was observed between these values and those given in Table 3, except for benzocaine, sulfanilamide and tyrosine, owing to their incomplete protonation (Fig. 2). The high retention of benzocaine in acid medium produces inaccurate extrapolations at concentrations of surfactant close to the cmc, that lead to abnormally high constants. The fitting, however, is good and allows an excellent prediction of the retention behaviour in the studied range. At pH 7.0, the agreement between the results in Tables 3 and 4 was satisfactory, considering the difficulty in the fitting of low capacity factors. The worst values corresponded to bumetanide and xipamide, whose basic species did not predominate completely at pH$\leq$7.

The 81 experimental data obtained for each solute, at several concentrations of surfactant and modifier and varying pH, were all fitted to Eq. (14) by using the non-linear method of Powell [24]. The results are given in Table 5. The fitting errors and correlation coefficients indicate that the proposed model adequately describes the experimental behaviour.

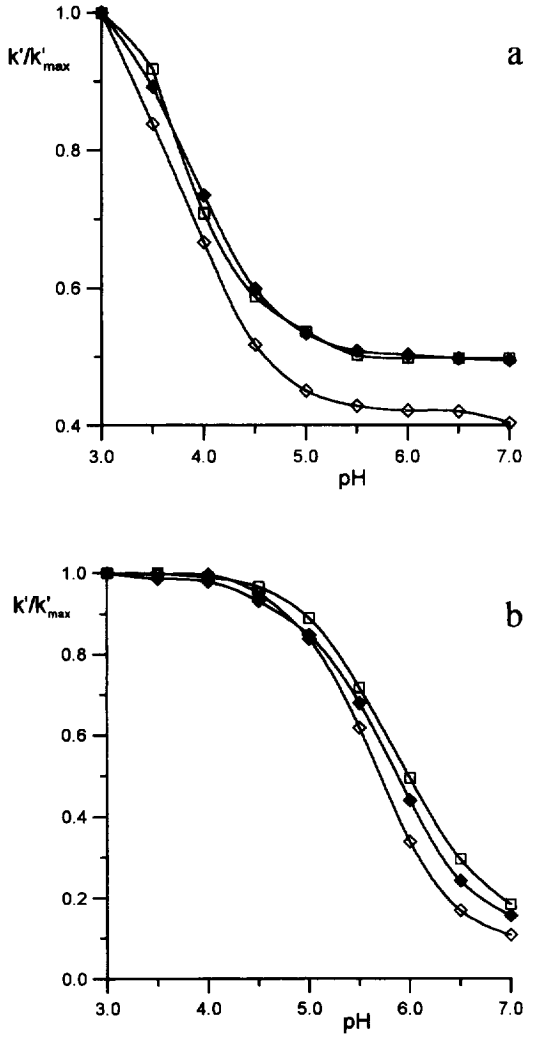

Fig. 3. Normalized capacity factors as a function of pH for the following mobile phases: 0.05 *M* (◇), 0.10 *M* (♦) and 0.15 *M* (□) SDS, without propanol, for (a) benzocaine and (b) bumetanide.

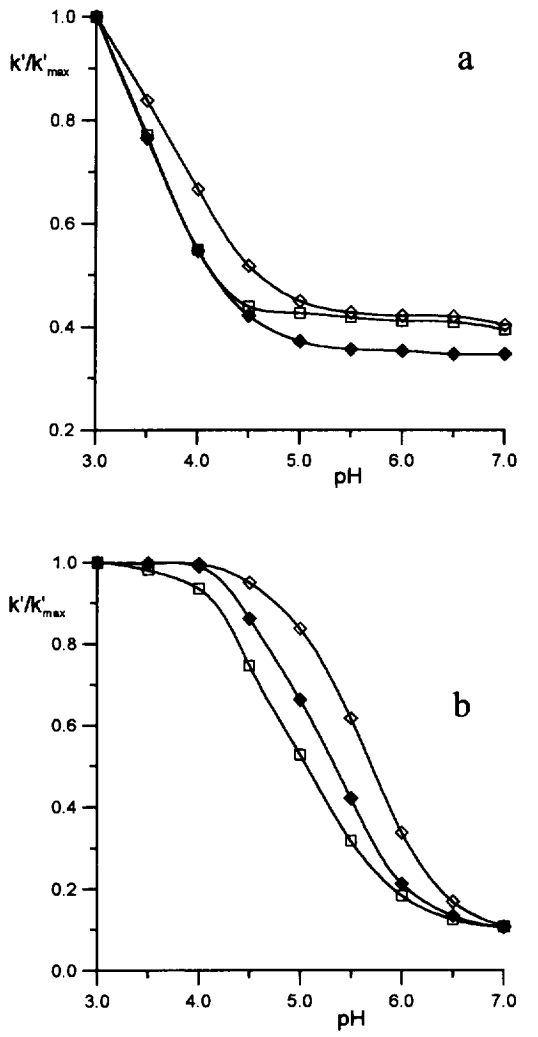

Fig. 4. Normalized capacity factors as a function of pH for the following mobile phases: 0 (◇), 0.04 (♦), and 0.08 (□) (v/v) propanol, containing 0.05 *M* SDS, for (a) benzocaine and (b) bumetanide.

Also, the values of log K_H agreed acceptably with the data found in the literature, although the effect of the modifier on the aqueous acid–base equilibria was not considered.

The parameters of the model should be obtained using an experimental design with data allocated in the most significant regions of the variable space, i.e., the experimental data should belong to the regions where the acidic or basic species predominate, but also some data should be taken in an intermediate pH region to achieve information on the protonation constant. A partial fitting of the data can be made to obtain initial values that facilitate the rapid and reliable convergence towards the correct solution, and avoid the achievement of local minima. Thus, the capacity factors in four mobile phases at sufficiently acid medium could be used to calculate the four constants of the protonated species (Eq. (4)). The parameters of the non-protonated species could be obtained similarly at pH 7, whereas the estimation

Table 3
Fitting to Eq. (4) of the capacity factors given in Table 2

Compound	Acidic species					
	K_{HAS}	K_{HAM}	K_{HAD}	K_{HMD}	ϵ_r	r
Benzocaine	[a]	[a]	[a]	26.3	2.7	0.9993
Bumetanide	330	234	99.4	16.7	2.9	0.9988
Ethacrynic acid	247	103	40.6	23.7	2.1	0.9991
Furosemide	82.7	97.8	62.9	12.7	1.5	0.9996
Sulfanilamide	86.9	178	244	9.39	4.9	0.9966
Tyrosine	346	327	216	5.65	7.8	0.987
Xipamide	228	123	76.3	19.8	1.7	0.9996
	Basic species					
	K_{AS}	K_{AM}	K_{AD}	K_{MD}	ϵ_r	r
Benzocaine	119	64.2	102	24.7	2.5	0.9994
Bumetanide	2.02	3.28	4.51	31.0	3.9	0.989
Ethacrynic acid	7.62	15.8	27.4	4.15	3.5	0.9946
Furosemide	0.844	0.051	8.19	157	3.5	0.975
Sulfanilamide	1.21	2.05	15.0	−3.36	4.0	0.988
Tyrosine	0.946	5.25	−4.85	21.0	3.4	0.984
Xipamide	0.881	0.864	5.18	62.0	6.4	0.952

[a] Very high values of the parameters were obtained because of an inaccurate extrapolation.

of the protonation constant requires additional mobile phases at intermediate pH values. With the initial parameters, the global non-linear fitting of the complete set of experimental data can be made.

The retention equation should be fitted using the data obtained in a reduced number of mobile phases to facilitate the experimental work. Eq. (14) requires the use of experimental data from at least nine mobile phases, but extra data can be used to improve the reliability of the predictions. The quality of the

Table 4
Fitting to Eq. (4) of the experimental data taken at pH 3.0 and 7.0

Compound	Acidic species (pH 3.0)					
	K_{HAS}	K_{HAM}	K_{HAD}	K_{HMD}	ϵ_r	r
Benzocaine	4011	1216	405	29.8	2.0	0.9996
Bumetanide	264	184	81.7	17.4	3.0	0.9987
Ethacrynic acid	249	108	53.7	23.8	1.7	0.9995
Furosemide	75.9	90.4	64.3	12.6	1.7	0.9994
Sulfanilamide	8.42	25.2	49.8	5.32	3.0	0.9977
Tyrosine	120	123	113	16.2	5.3	0.9952
Xipamide	212	114	71.6	20.4	2.0	0.9994
	Basic species (pH 7.0)					
	K_{AS}	K_{AM}	K_{AD}	K_{MD}	ϵ_r	r
Benzocaine	116	62.4	103	23.8	2.1	0.9996
Bumetanide	4.89	12.1	32.1	10.6	2.7	0.9978
Ethacrynic acid	6.32	10.7	18.2	9.46	1.4	0.9991
Furosemide	0.995	1.02	11.1	4.90	1.7	0.9963
Sulfanilamide	1.21	2.09	14.8	−2.39	3.9	0.990
Tyrosine	1.09	7.12	−2.67	14.9	2.1	0.9962
Xipamide	4.99	5.13	61.3	17.1	3.2	0.9980

Table 5
Fitting to Eq. (14) of the experimental data obtained in 81 mobile phases of SDS and propanol at increasing pH

Compound	K_{AS}	K_{AM}	K_{AD}	K_{MD}	K_{HAS}	K_{HAM}	K_{HAD}	K_{HMD}	log K_H	ϵ_r	r
Benzocaine	120	65.2	94.0	27.3	3916	1080	157	28.7	2.64	2.3	0.9995
Bumetanide	2.89	10.9	14.6	4.09	413	297	135	15.9	4.66	2.8	0.9992
Ethacrynic acid	7.04	13.2	17.2	15.3	285	121	65.2	21.1	3.70	2.7	0.9995
Furosemide	1.06	3.27	11.0	2.57	89.6	107	69.4	12.5	4.06	2.6	0.9995
Sulfanilamide	1.27	3.09	16.2	−6.37	92.0	260	47.8	13.6	1.85	4.2	0.9955
Tyrosine	1.22	12.7	−7.37	9.90	267	251	68.9	8.06	2.81	5.1	0.9985
Xipamide	0.957	1.54	9.66	30.0	267	147	104	17.8	5.13	2.2	0.9996

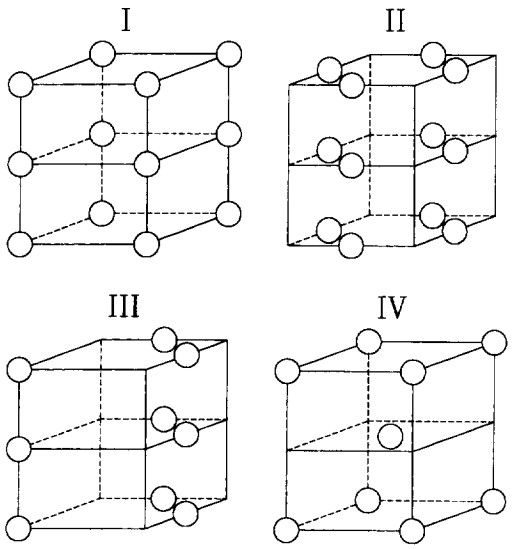

Fig. 5. Experimental designs.

predictions performed with several experimental designs was studied. Fig. 5 shows four of the designs considered, which were selected according to the quality of the information given by the experimental data, and using our previous experience [16]. The equations fitted according to these designs were used to predict the retention in the experimental mobile phases not employed in the fitting (see Table 1). The mean relative errors and correlation coefficients obtained are given in Table 6. The errors corresponding to the prediction of the retention of each one of the 81 mobile phases, using the remaining 80 experimental data in the fitting process, are indicated in the same table. In this case, the mean relative errors were only slightly larger than the errors obtained in the global fitting of the 81 mobile phases (Table 5), and can be considered as the best results attainable in the prediction of the retention.

As expected, the designs with 9 points yielded

Table 6
Prediction errors for the retention of diverse solutes in mobile phases of SDS and propanol at increasing pH

Compound	Experimental designs[a]								Reference fitting[b]	
	I		II		III		IV			
	ϵ_r	r	ϵ_r	r	ϵ_r	r	ϵ_r	r	ϵ_r	r
Benzocaine	3.3	0.9989	6.3	0.9950	4.0	0.9971	6.1	0.9942	2.7	0.9993
Bumetanide	4.6	0.9978	5.0	0.9970	7.4	0.988	5.6	0.9958	3.2	0.9990
Ethacrynic acid	4.4	0.9984	4.1	0.9975	4.3	0.9981	6.8	0.9956	2.9	0.9994
Furosemide	3.7	0.9988	3.8	0.9984	5.3	0.9942	3.8	0.9981	2.8	0.9994
Sulfanilamide	5.9	0.980	9.6	0.964	5.7	0.982	10.9	0.932	4.8	0.9931
Tyrosine	11.3	0.986	7.7	0.9952	10.6	0.987	13.8	0.976	6.1	0.9981
Xipamide	4.4	0.9980	3.7	0.9986	5.0	0.9962	15.6	0.962	2.4	0.9994

[a] The roman numbers correspond to the experimental designs in Fig. 5.
[b] The data in 80 mobile phases were used to predict the retention in the 81st mobile phase. The process was repeated for each mobile phase.

errors somewhat larger than did the designs with 12 points, although design III gave fairly acceptable results. The smallest errors were achieved with design I, where the experimental data are located in the extreme of the variable space. In previous work, it was demonstrated that in a space of two variables (SDS and modifier), the best design contained four points in the corners of the variable space, although a fifth point was added to check the accuracy of the model [13,16].

5. Conclusions

The model given by Eq. (14) accurately describes the retention of solutes eluted in a C_{18} column with hybrid mobile phases of SDS and alcohol, at any pH. The errors obtained in the prediction of the retention of diverse solutes were lower than 6%, except for tyrosine, due to its peculiar behaviour. The predictive capability of micellar liquid chromatography (MLC) will allow the reliable and relatively rapid optimization of the composition of the mobile phase for the separation of a mixture of compounds, by using an interpretive method and a reduced number of mobile phases (at least nine).

The model gives a quantitative description of the equilibrium properties of the solutes in MLC. However, the accurate determination of the physicochemical parameters of the model requires the use of the cmc corresponding to each concentration of modifier. Also, experimental data taken in mobile phases with a concentration of surfactant close to the cmc are necessary to achieve an accurate extrapolation of the retention in non-micellar mobile phases, in order to obtain good values of the equilibrium constants of the solutes between the stationary phase and water. Errors in these constants will propagate to the calculation of other constants describing the retention.

The accurate prediction of the retention of each compound in a given mixture is more important with micellar mobile phases than with the conventional aqueous–organic eluents, owing to the usual low efficiencies in the former case. The many interactions that the solutes experience in a micellar chromatographic system enhances the differences among them. The possibility of using simultaneously the three most significant variables that affect the retention, e.g., surfactant and modifier concentrations, and pH, will improve the capability of resolution of complex mixtures of ionic and non-ionic compounds.

The number of reports on analytical applications in MLC have been increasing along this decade. The development of more applications needs that the resolution of complex mixtures be made and optimized in a short time, with a low waste of reagents. The results in this work may contribute for this purpose.

Acknowledgments

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References

[1] F.J. De Luccia, M. Arunyanart and L.J. Cline-Love, Anal. Chem., 57 (1985) 1564.
[2] M.F. Borgerding and W.L. Hinze, Anal. Chem., 57 (1985) 2183.
[3] M.G. Khaledi and J.G. Dorsey, Anal. Chem., 57 (1985) 2190.
[4] J. Haginaka, J. Wakai, H. Yasuda and T. Nakagawa, Anal. Chem., 59 (1987) 2732.
[5] M.G. Khaledi and E.D. Breyer, Anal. Chem., 61 (1989) 1040.
[6] E.F. Simó Alfonso, G. Ramis Ramos, M.C. García Alvarez-Coque and J.S. Esteve Romero, J. Chromatogr., 670 (1995) 183.
[7] I. Rapado Martínez, M.C. García Alvarez-Coque and R.M. Villanueva Camañas, The Analyst, 121 (1996) 1677.
[8] J.K. Strasters, S.T. Kim and M.G. Khaledi, J. Chromatogr., 586 (1991) 221.
[9] E. Bonet Domingo, J.R. Torres Lapasió, M.J. Medina Hernández and M.C. García Alvarez-Coque, Anal. Chim. Acta, 287 (1994) 201.
[10] D.W. Armstrong and F. Nome, Anal. Chem., 53 (1981) 1662.
[11] M. Arunyanart and L.J. Cline Love, Anal. Chem., 56 (1984) 1557.
[12] J.P. Foley, Anal. Chim. Acta, 231 (1990) 237.
[13] J.R. Torres Lapasió, R.M. Villanueva Camañas, J.M. Sanchis Mallols, M.J. Medina Hernández and M.C. García Alvarez-Coque, J. Chromatogr., 639 (1993) 87.
[14] M.C. García Alvarez-Coque, J.R. Torres Lapasió and J.J. Baeza Baeza, Anal. Chim. Acta, 324 (1996) 163.
[15] M.A. García, O. Jiménez and M.L. Marina, J. Chromatogr. A, 675 (1994) 1.

[16] J.R. Torres Lapasió, R.M. Villanueva Camañas, J.M. Sanchis Mallols, M.J. Medina Hernández and M.C. García Alvarez-Coque, J. Chromatogr. A, 677 (1994) 239.
[17] S. Torres Cartas, R.M. Villanueva Camañas and M.C. García Alvarez-Coque, Anal. Chim. Acta, in press.
[18] M. Arunyanart and L.J. Cline Love, Anal. Chem., 57 (1985) 2837.
[19] A.H. Rodgers, J.K. Strasters and M.G. Khaledi, J. Chromatogr., 636 (1993) 203.
[20] A.H. Rodgers and M.G. Khaledi, Anal. Chem., 66 (1994) 327.
[21] J.R. Torres Lapsió, M.C. García Alvarez-Coque and J.J. Baeza Baeza, Anal. Chim. Acta, in press.
[22] C. Hansch, The Rational Design, Mechanicistic Study and Therapeutic Application of Chemical Compounds, Vol. 6, Pergamon Press, Oxford, 1990.
[23] M. Windholz (Editor), The Merck Index, 10th ed., Merck, Rahway, NJ, 1983.
[24] S.S. Rao, Optimization: Theory and Applications, Wiley, New Delhi, 1985.