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Interpretive strategy for optimization of surfactant and alcohol concentration in micellar liquid chromatography

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Abstract

An interpretive procedure for optimization of the retention of the solutes in a mixture, eluted with mobile phases containing a surfactant and an alcohol in micellar liquid chromatography (MLC), is proposed. Three optimization criteria were used: positional resolution, valley-to-peak ratio and overlapping. Retention data from several phenols, aromatic compounds and catecholamines were used to test the procedure. The positional criterion, together with the retention model given by an equation of the type $1/k' = c_0 + c_1\mu + c_2\varphi + c_3\mu\varphi$, led to a reliable optimum resolution using the retention data for a few mobile phases. However, the resolution criteria that take into account both the position and the peak shape are preferable in MLC, where the chromatographic peaks are asymmetric and have a low efficiency.

1. Introduction

The chromatographer is concerned with the achievement of the optimum mobile phase that permits the separation of the compounds in a mixture in the minimum time. This task may be complex when two or more variables are involved in the optimization process. The optimization may be sequential or interpretive. In a sequential strategy, the retention of the different solutes is not known a priori and each set of mobile phases is designed by taking into account the retention observed with previous eluents. In contrast, in an interpretive strategy, the experiments are designed before the optimization process. This strategy may be much more efficient and reliable, but the retention behaviour of each component in the mixture should be known, e.g., described by a mathematical equation. A sequential strategy is inadequate when several local optima exist (as occurs in chromatography), and may not give the best optimum.

In micellar liquid chromatography (MLC), the addition of a short-chain alcohol to the mobile phase, containing a surfactant above the critical micellar concentration, is usual. The alcohol improves the efficiency and increases the elution strength. In a previous paper, a model to describe the retention behaviour of solutes in any mobile phase containing a surfactant and an alcohol was proposed, which made use of the elution data in five mobile phases containing different amounts of surfactant and alcohol [1].

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A function of the type

$$\frac{1}{k'} = c_0 + c_1\mu + c_2\varphi + c_3\mu\varphi \quad (1)$$

where k' is the capacity factor and μ and φ are surfactant and alcohol concentration, respectively, proved to be satisfactory for different solutes.

In this work, an interpretive procedure for optimization of the retention of the solutes in a mixture, eluted with mobile phases of surfactant and alcohol, is proposed. Three optimization criteria were used: positional resolution, valley-to-peak ratio and overlapping. The optimization process consisted of (i) achievement of the retention equations for each solute to be separated, (ii) a search of the optimum mobile phase with the aid of contour maps of the global functions of resolution, (iii) simulation of the chromatogram for the optimum mobile phase and (iv) a search of a new optimum when the selected optimum is not satisfactory.

2. Experimental

2.1. Reagents

Sodium dodecyl sulphate (SDS) (99%) was obtained from Merck (Darmstadt, Germany) and propanol (analytical-reagent grade) from Panreac (Barcelona, Spain). The mobile phases were vacuum filtered through 0.47-μ nylon membranes from Micron-Scharlau (Barcelona, Spain).

Stock standard solutions of 2×10^{-3} M catecholamines were prepared in 0.1 M acetic acid from Probus (Barcelona, Spain): L-adrenaline (biochemical), DL-noradrenaline (pure), dopamine hydrochloride (very pure) and adrenalone hydrochloride (pure) from Fluka (Buchs, Switzerland), and isoprenaline, kindly donated by Boehringer-Ingelheim (Barcelona, Spain). The pH of the mobile phases was adjusted with 0.01 M sodium dihydrogenphosphate (Probus). Nanopure deionized water (Barnstead Sybron, Boston, MA, USA) was used throughout.

2.2. Apparatus

An HP 1050 chromatograph (Hewlett-Packard, Palo Alto, CA, USA) with a UV–visible detector (absorbance was measured at 280 nm) and an HP 3396A integrator were used. Data were acquired by means of a PC and Peak-96 software from Hewlett-Packard (Avondale, PA, USA). The sample was injected through a Rheodyne (Cotati, CA, USA) valve with a 20-μl loop. A Spherisorb octadecylsilane ODS-2 (5 μm) analytical column (12 cm × 4.6 mm I.D.) and a precolumn, placed before the injector, of identical characteristics (3.5 cm × 4.6 mm I.D.) from Scharlau were used. The mobile phase flow-rate was 1 ml min^{-1}. The dead volume was determined by injection of water.

The group of programs MICHROM for data treatment, written in QuickBasic 4.5 for IBM PC or compatible computers, was developed [2]. ASCII data files generated by the Peak-96 software can be treated directly with MICHROM. MICHROM allows data smoothing, measurement of chromatographic peak properties (e.g., efficiency, N, asymmetry factor, B/A, capacity factor, k', peak area) (see Fig. 1 for the meaning of B/A), experimental design, modelling of the retention of the solutes, selection of optimization criteria, drawing of the global response surface,

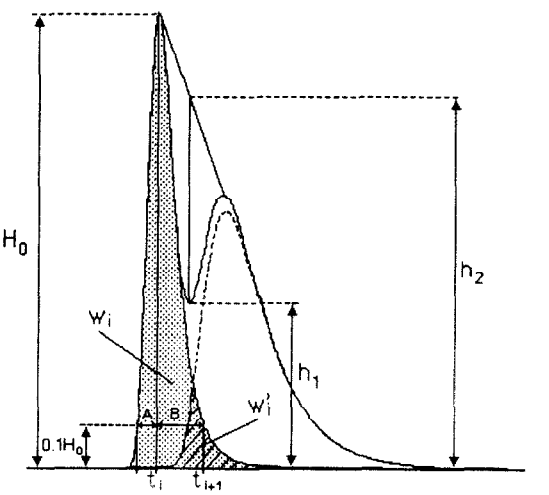

Fig. 1. Measurement of chromatographic peak properties. See text for meaning.

search and accurate definition of each resolution maximum by application of a simplex method of restricted evolution [3] and chromatogram simulation.

2.3. Optimization criteria

Three optimization criteria were used:

(i) Separation factor [4]:

$$S_{i,i+1} = \frac{t_{i+1} - t_i}{t_{i+1} + t_i} = \frac{k'_{i+1} - k'_i}{k'_{i+1} + k'_i + 2} \quad (2)$$

where t_i and t_{i+1} are the retention times of the solutes i and $i+1$.

(ii) Valley-to-peak ratio:

$$P_{i,i+1} = 1 - h_1/h_2 \quad (3)$$

where h_1 is the height of the valley and h_2 is an interpolated height between two adjacent peaks, measured at the abscissa of the valley (see Fig. 1).

(iii) Overlapping of two adjacent peaks:

$$O_{i,i+1} = 1 - W'_i/W_i \quad (4)$$

where W_i is the total area of a given peak and W'_i the area overlapped by other peaks (see Fig. 1).

The three functions may vary from 0 to 1. Proximity to unity indicates a better separation. The normalized products of these functions were used to describe the overall separation of the peaks in the chromatogram:

$$r = \prod_{i=1}^{n-1} \frac{X_{i,i+1}}{\left(\sum \frac{X_{i,i+1}}{n-1}\right)^{n-1}} \quad (5)$$

where $X_{i,i+1}$ may be $S_{i,i+1}$, $P_{i,i+1}$ or $O_{i,i+1}$. The function of resolution, r, is maximized to obtain the optimum mobile phase. Contour maps were used to find the position of the maxima and the shape of the response surface. A higher precision in the determination of the optimum mobile phase was achieved by applying the modified simplex method [3] in the final step of the optimization process, when a local optimum has been selected. This was especially useful when the optimum was located on a plateau in the contour map.

2.4. Simulation of the chromatograms

The position of the chromatographic peaks in any mobile phase was obtained from Eq. 1, fitted with the retention data of a few experimental mobile phases (usually five). To improve the accuracy in the prediction of the retention, the capacity factors used in the fitting process were calculated with the void volumes obtained for each mobile phase. However, since it was checked that the void volumes for different mobile phases were similar, the chromatograms were simulated assuming a mean value of void volume.

The efficiencies, measured as the number of theoretical plates, N, and the asymmetry factors, B/A, for the experimental mobile phases were measured. The values of N and B/A used to simulate and predict the peak profile were interpolated by fitting to a plane the values of these parameters for the three experimental mobile phases closer to the simulated mobile phase. For the examples taken from the literature [5,6], the values of N and B/A for several mobile phases were not available, and therefore a constant value of these parameters was assumed. In all instances the areas of the chromatographic peaks were normalized for the simulation.

The simulated chromatographic peaks were drawn using an asymmetric Gaussian function, where the standard deviation depended on the efficiency and the asymmetry factor:

$$h = H_0 \exp\left[-0.5\left(\frac{x - k'}{\sigma(N, B/A)}\right)^2\right] \quad (6)$$

where H_0, the height of the chromatographic peak of a given solute, is a function of $B + A$ since the areas of the peaks are normalized. The peaks were split into two parts of variable standard deviation. The width of the Gaussian curve varied asymptotically between $(B + A)/2$ and A or B, for the leading or tailing edge of the peak, respectively. The values of A and B were calculated from the efficiency and asymmetry

factor calculated from the equation of Foley and Dorsey [7]:

$$N = \frac{41.7[t_R/(A+B)]^2}{(B/A)+1.25} \quad (7)$$

2.5. Test compounds

The procedure was checked using the chromatographic data for several groups of compounds: a mixture of catecholamines (adrenaline, noradrenaline, adrenalone, dopamine and isoprenaline) at pH 6.8 (Table 1) and 3.5 (Table 2), and a mixture of aromatic compounds (anisole, benzene, naphthalene, 1-naphthalenemethanol, phenol and toluene) at pH 6.8, eluted with SDS–1-propanol micellar mobile phases as reported in Ref. [5], and a mixture of phenols (4-benzamidephenol, 4-*tert.*-butylphenol, 4-fluorophenol, 4-hydroxyacetophenone, 4-hydroxybenzaldehyde, 4-hydroxybenzyl alcohol, 4-hydroxybenzophenone, 4-hydroxybenzyl cyanide, 4-hydroxydiphenylmethane, 4-hydroxyphenemethyl alcohol, 4-hydroxypropiophenone, 4-isopropylphenol, 4-methylphenol, 4-nitrophenol and phenol) at pH 7 with cetyltrimethyl-

Table 1
Capacity factors, efficiencies and asymmetry factors in several mobile phases of SDS (μ) and propanol (φ) at pH 6.8

Catecholamine	Mobile phase composition														
	Component	Concentration													
	SDS (M)	0.035			0.035			0.035			0.052			0.052		
	Propanol (v/v)	0.000			0.050			0.100			0.015			0.085		
		k'	N	B/A	k'	N	B/A	k'	N	B/A	k'	N	B/A	k'	N	B/A
Noradrenaline		20.3	3000	1.7	11.7	280	6.7	8.5	34	9.1	10.5	950	3.4	6.2	23	12.7
Adrenaline		26.5	2010	2.1	11.9	240	7.5	8.2	31	11.5	11.6	830	3.6	6.2	20	12.9
Adrenalone		40.6	180	6.2	22.5	10	7.5	13.0	150	8.4	18.6	23	10.4	10.0	7	12.5
Dopamine		51.4	3600	1.3	20.6	1710	2.6	12.5	950	3.9	20.9	2090	1.9	9.7	400	5.2
Isoprenaline		53.1	1600	2.1	19.0	320	6.9	11.4	86	11.7	20.2	460	4.3	8.8	39	13.0
	SDS (M)	0.092			0.092			0.092			0.133			0.133		
	Propanol (v/v)	0.000			0.050			0.100			0.015			0.085		
		k'	N	B/A	k'	N	B/A	k'	N	B/A	k'	N	B/A	k'	N	B/A
Noradrenaline		6.9	2530	1.4	4.3	120	6.5	3.1	33	10.0	3.8	840	2.2	2.2	27	8.9
Adrenaline		8.4	1600	1.6	4.3	60	7.9	3.1	12	12.5	4.1	680	2.4	2.2	24	8.9
Adrenalone		12.7	110	7.2	7.4	6	10.3	5.9	3	8.0	6.4	20	8.5	4.0	1	12.5
Dopamine		15.9	2360	1.4	7.1	830	3.0	4.8	76	6.8	7.1	1700	1.6	3.4	220	4.9
Isoprenaline		16.6	1100	2.0	6.8	38	9.5	4.4	12	12.9	7.0	380	2.8	3.2	12	11.2
	SDS (M)	0.150			0.150			0.150								
	Propanol (v/v)	0.000			0.050			0.100								
		k'	N	B/A	k'	N	B/A	k'	N	B/A						
Noradrenaline		4.0	1340	1.7	2.6	380	3.2	1.9	10	11.9						
Adrenaline		5.0	1200	1.7	2.6	280	3.5	1.9	5	13.5						
Adrenalone		7.7	130	4.8	3.9	8	11.5	3.1	1	17.4						
Dopamine		9.3	1830	1.5	4.1	1180	1.9	2.9	17	9.4						
Isoprenaline		9.8	830	2.0	3.8	240	3.6	2.6	11	12.1						

Table 2
Capacity factors, efficiencies and asymmetry factors in several mobile phases of SDS (μ) and propanol (φ) at pH 3.5

Catecholamine	Mobile phase composition														
	Component	Concentration													
	SDS (M)	0.05			0.15			0.05			0.05			0.15		
	Propanol (v/v)	0.00			0.00			0.05			0.10			0.10		
		k'	N	B/A	k'	N	B/A	k'	N	B/A	k'	N	B/A	k'	N	B/A
Noradrenaline		12.1	3620	1.2	4.4	1970	1.3	5.5	3990	1.2	3.8	3780	1.0	1.6	1890	1.3
Adrenaline		15.6	3180	1.2	5.5	1680	1.2	5.9	3370	1.2	3.7	2950	1.5	1.7	1990	1.4
Adrenalone		24.3	2950	1.2	8.3	1710	1.3	9.6	2870	1.4	6.2	3260	1.3	2.5	1560	1.5
Dopamine		28.1	3460	1.3	9.5	2350	1.1	9.9	3850	1.2	6.6	4000	1.4	2.5	2240	1.2
Isoprenaline		29.0	2700	1.3	9.7	1650	1.1	9.1	2890	1.2	5.4	3100	1.2	2.6	1750	1.7

ammonium bromide (CTAB)–2-propanol mobile phases as reported in Ref. [6].

3. Results and discussion

3.1. Equations of retention

In previous work [1], several equations and about 100 experimental designs were studied to describe the retention behaviour in MLC, when hybrid mobile phases containing an alcohol are used. The models were evaluated with the retention data for several compounds (catecholamines, phenols, amino acids and different aromatic compounds). Among all the equations and designs, Eq. 1 gave the most reliable results. For an experimental design of five mobile phases (design 6 in Fig. 2), the global mean relative error in the prediction of the retention of five catecholamines and thirteen mobile phases at pH 6.8 was 3.5% [1].

However, in an attempt to reduce the error in the prediction of the retention behaviour of solutes, some modifications of Eq. 1 were checked. The retention data of the catecholamines for thirteen mobile phases at pH 6.8 were again used. Initially, linear regression was applied to fit the data, and the equation parameters were further refined using non-linear regression (Powell method [8]). More than 100 experimental designs were considered. The distribution of the experimental data for some of these designs is shown in Fig. 2 and the equations studied are indicated in Table 3, together with the global mean relative errors obtained in the prediction of the retention.

The inclusion of an interaction term in the equations was needed to obtain an adequate description of the retention behaviour of the solutes in MLC with hybrid mobile phases (see Eq. e in Table 3). The results confirmed that Eq. 1 (Eq. a in Table 3) gave the best description. The addition of a new term ($\mu\sqrt{\varphi}$ or $\varphi\sqrt{\mu}$) improved the accuracy of the prediction for some experimental designs, and Eq. g, which also contained a $\varphi\sqrt{\mu}$ term, was frequently good. Although an experimental design of four points (such as design 1) was enough to achieve the fitting parameters of the equation, design 6 is

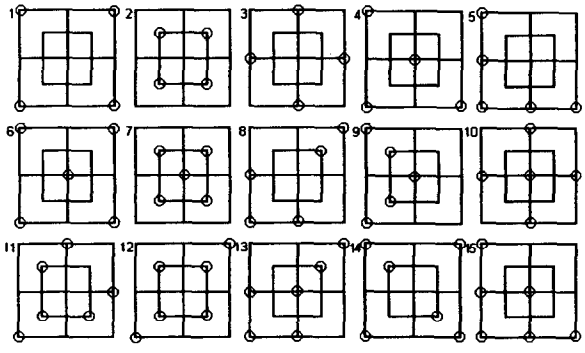

Fig. 2. Experimental designs used to check the retention equations in Table 3.

Table 3
Description of the retention behaviour and global mean errors obtained with the five catecholamines and thirteen mobile phases at pH 6.8

Equation	Relative error (%) ($n = 65$)[a]														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
(a) $1/k' = c_0 + c_1\mu + c_2\varphi + c_3\mu\varphi$	3.4	4.9	100	3.3	250	3.5	4.9	5.7	5.3	207	5.9	4.1	5.2	3.6	3.4
(b) $1/k' = c_0 + c_1\mu + c_2\varphi + c_3\mu\varphi + c_4\mu\sqrt{\varphi}$	–[b]	–[b]	–[b]	–[b]	111	2.8	9.8	12.8	5.5	260	7.7	7.3	3.5	2.4	4.4
(c) $1/k' = c_0 + c_1\mu + c_2\varphi + c_3\mu\varphi + c_4\varphi\sqrt{\mu}$	–[b]	–[b]	–[b]	–[b]	380	3.4	5.2	6.2	5.7	70.2	5.2	23.9	4.5	3.6	111
(d) $1/k' = c_0 + c_1\mu + c_2\varphi + c_3\mu\varphi + c_4\sqrt{\mu\varphi}$	–[b]	–[b]	–[b]	–[b]	559	2.9	8.8	42.9	4.8	153	4.8	4.2	3.7	3.4	3.8
(e) $1/k' = c_0 + c_1\mu + c_2\varphi$	727	70.2	70.9	23.1	31.6	185	44.3	90.4	15.4	69.4	17.8	27.3	121	135	21.8
(f) $1/k' = c_0 + c_1\mu + c_2\varphi + c_3\mu\sqrt{\varphi}$	7.3	117	41.7	7.2	43.2	9.2	656	10.5	17.1	66.1	16.5	26.2	10.1	9.4	7.3
(g) $1/k' = c_0 + c_1\mu + c_2\varphi + c_3\varphi\sqrt{\mu}$	4.1	7.3	52.6	4.5	1250	4.2	7.2	5.5	7.9	111	8.5	6.5	5.6	4.7	4.6
(h) $1/k' = c_0 + c_1\mu + c_2\varphi + c_3\sqrt{\mu\varphi}$	14.4	88.2	291	11.7	33.9	73.5	121	116	15.2	216	37.9	56.4	26.8	54.9	12.1

[a] Numbers correspond to the experimental designs in Fig. 2.
[b] No results could be obtained.

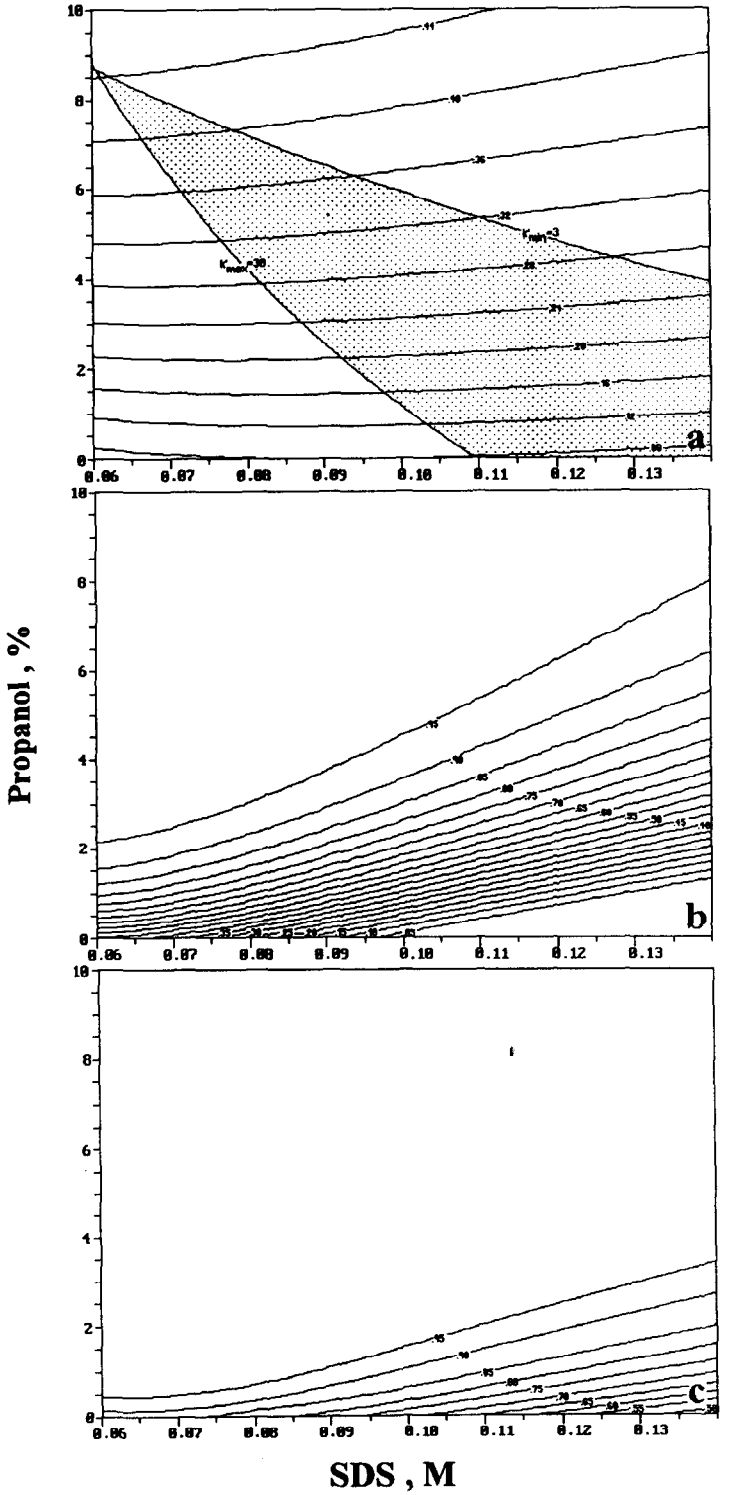

Fig. 3. Contour maps of global resolution for the mixture of six aromatic compounds, and SDS–propanol mobile phases (optima indicated): (a) positional criterion (0.06 M–10%); (b) valley-to-peak ratio criterion (0.06 M–10%); (c) overlapping criterion (0.06 M–10%).

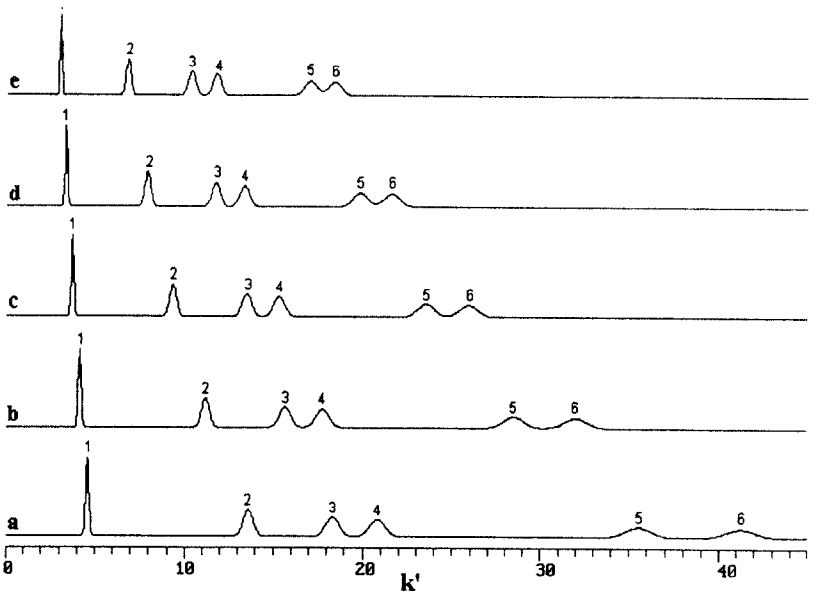

Fig. 4. Chromatograms for the mixture of aromatic compounds for mobile phases with a global positional resolution of $r(S) = 0.25$, containing the following SDS–propanol composition: (a) 0.06 M–3.23%; (b) 0.08 M–3.20%; (c) 0.10 M–3.34%; (d) 0.12 M–3.56%; (e) 0.14 M–3.83%. Peaks: 1 = phenol; 2 = 1-naphthalenemethanol; 3 = anisole; 4 = benzene; 5 = toluene; 6 = naphthalene.

recommended as it allows checking of the accuracy of the fitting. Design 2 is similar to design 1, but it considers the prediction of the retention for mobile phases with concentrations outside the concentration range used for the prediction. Designs 14 and 15, with six mobile phases, are also good. When available, design 6 was always used in this work for the optimization process. If two new phases are added in the middle of two adjacent sides of the design square, design 1 will be produced with a more restricted concentration range. Thus, with only two new phases added to design 6 in the region where the optimum appears, the precision of the predictions will be increased.

3.2. *Positional criterion vs. positional–shape criteria*

The positional criterion, together with the retention model given by Eq. (1), leads to a reliable optimum resolution using the retention data for a few mobile phases. However, as the shape and width of the chromatographic peaks are not considered, achievement of an unacceptable optimum with a high positional resolution may be possible, with peaks largely overlapped.

The positional and positional–shape criteria (valley-to-peak ratio and overlapping) give similar results when the compounds to be separated show symmetrical peaks of high efficiency. Fig. 3 shows contour maps of positional resolution, valley-to-peak ratio and overlapping for diverse aromatic compounds. Assuming an efficiency of $N = 2500$ and an asymmetry factor of $B/A = 1$ for any mobile phase, the solutes appeared well resolved along almost all the composition range studied, since the mean width of the peaks was small compared with the mean separation of adjacent peaks.

With the positional criterion (Fig. 3a), the maximum global resolution was obtained for a 0.06 M SDS–10% propanol mobile phase [$r(S) = 0.476$]. Fig. 4 shows several simulated chromatograms for the separation of the aromatic compounds for mobile phases with a global positional resolution of $r(S) = 0.25$. Although the value of global positional resolution was

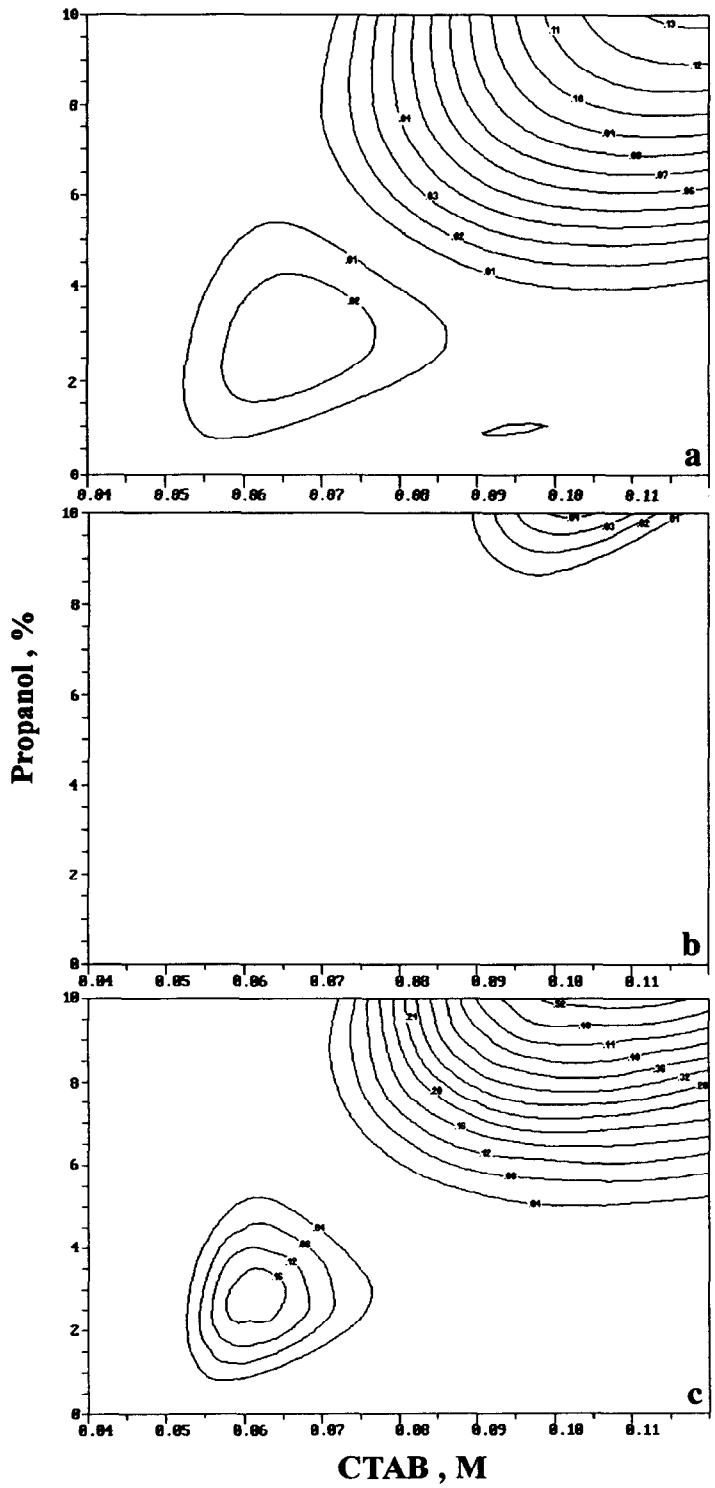

Fig. 5. Contour maps of global resolution for the mixture of fifteen phenols, and CTAB–2-propanol mobile phases (optima indicated): (a) positional criterion (0.12 M–10%); (b) valley-to-peak ratio criterion (0.102 M–10%); (c) overlapping criterion (0.107 M–10%).

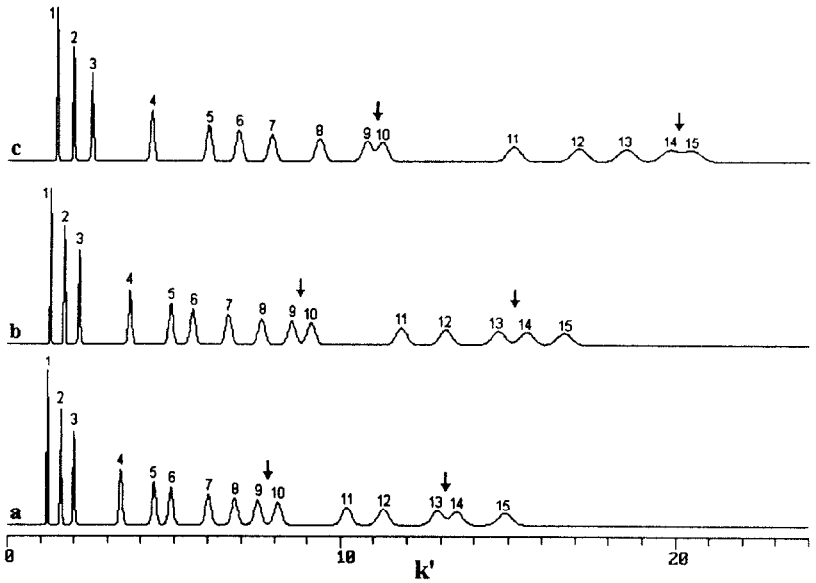

Fig. 6. Chromatograms for the mixture of phenols in mobile phases containing 10% 2-propanol and CTAB, with CTAB concentrations of (a) 0.12 *M*, (b) 0.10 *M* and (c) 0.08 *M*. Peaks: 1 = 4-benzamidephenol; 2 = 4-hydroxybenzyl alcohol; 3 = 4-hydroxyphenemethyl alcohol; 4 = 4-hydroxybenzyl cyanide; 5 = 4-hydroxyacetophenone; 6 = 4-hydroxybenzaldehyde; 7 = phenol; 8 = 4-fluorophenol; 9 = 4-hydroxypropiophenone; 10 = 4-methylphenol; 11 = 4-nitrophenol; 12 = 4-hydroxybenzophenone; 13 = 4-isopropylphenol; 14 = 4-hydroxydiphenylmethane; 15 = 4-*tert.*-butylphenol.

relatively low, a good separation was achieved for all these mobile phases, but that giving the shorter retention times is preferable. It may also be interesting to limit the parameter space by establishing maximum and minimum capacity factors. The lines corresponding to $k'_{max} = 30$ and $k'_{min} = 3$ have been drawn in Fig. 3a.

For the aromatic compounds, the application of the positional–shape criteria, valley-to-peak ratio (Fig. 3b) and overlapping (Fig. 3c) gave similar optima [0.06 *M* SDS–10% propanol, $r(P)$ and $r(O)$ being >0.999]. The contour maps for these criteria were different to that of the positional criterion. The positional resolution increased slowly. In contrast, the positional–shape resolution increased rapidly with increasing concentration of propanol for the lower concentrations and slowed above 4% propanol.

Fig. 5 shows the contour maps for the separation of fifteen phenols with mobile phases of CTAB and 2-propanol. An efficiency of $N = 2500$ was also considered. For the positional criterion, the optimum was found for a mobile phase of 0.12 *M* CTAB–10% 2-propanol (Fig. 5a), whereas for the valley-to-peak ratio criterion it was 0.102 *M* CTAB–10% 2-propanol [$r(P) = 0.047$] (Fig. 5b), and for the overlapping criterion it was 0.107 *M* CTAB–10% 2-propanol [$r(O) = 0.542$] (Fig. 5c). The simulated chromatograms for 10% 2-propanol and three concentrations of surfactant are given in Fig. 6. The disagreement between the positional and positional–shape criteria is due to the retention behaviour of peaks 13–15. As the concentration of surfactant decreased from 0.12 to 0.10 *M* (Fig. 6a and b), the overlapping of these peaks decreased, improving the valley-to-peak ratio and decreasing the overlapping to a lesser extent. However, the positional resolution became worse. A further decrease in the concentration of CTAB decreased both the positional and positional–shape resolution (see peaks 9–10 and 13–15).

The information given by the positional–shape criteria may be especially interesting when the chromatographic peaks are asymmetric, which is usual in MLC. Thus, poorly defined optima obtained with the positional criterion may be

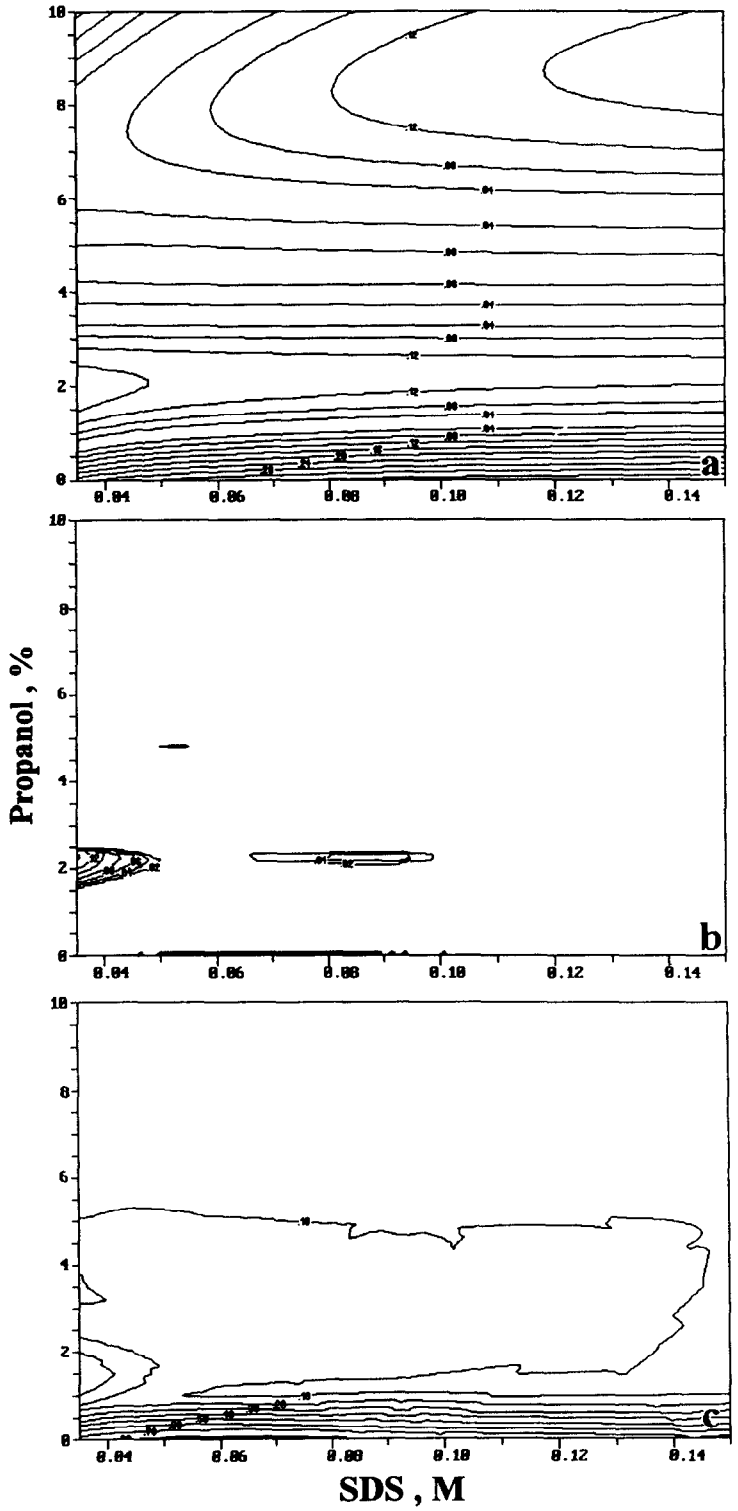

Fig. 7. Contour maps of global resolution for the mixture of five catecholamines, and SDS–propanol mobile phases at pH 6.8 (optima indicated): (a) positional criterion (0.035 to 0.15 *M*–0%); (b) valley-to-peak criterion (0.05 to 0.09 *M*–0% and 0.035 *M*–2.3%); (c) overlapping criterion (0.05 to 0.08 *M*–0%).

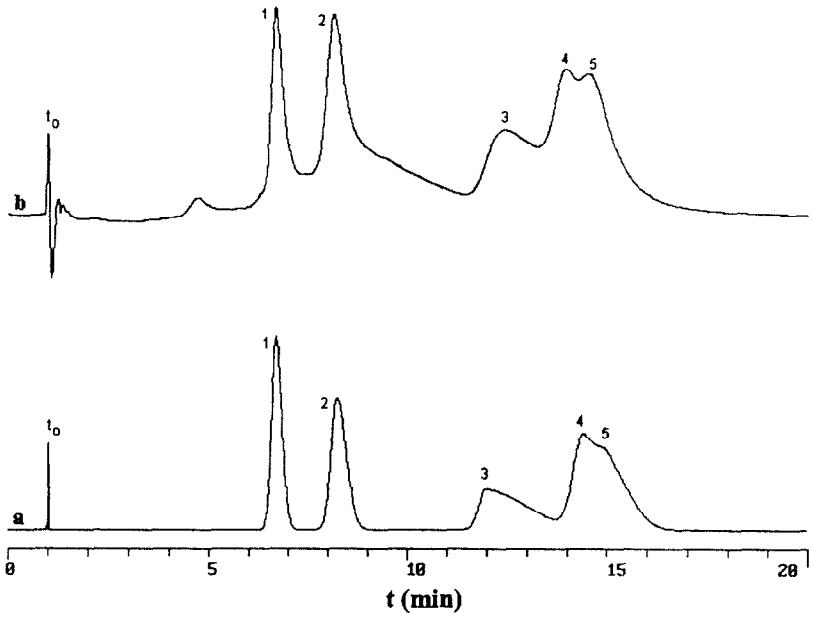

Fig. 8. (a) Simulated and (b) experimental chromatograms for the mixture of catecholamines in a mobile phase of 0.11 *M* SDS alone. Peaks: 1 = noradrenaline; 2 = adrenaline; 3 = adrenalone; 4 = dopamine; 5 = isoprenaline.

clearer when the shape of the peaks is considered. Besides, with the positional–shape criteria some characteristics of the contour maps may be magnified, such as the slope of the resolution function and the value of the maxima and minima, and some maxima may disappear. The comparison of the numerical values of the global resolution for the three criteria allows the evaluation of the quality of the optima. On the other hand, the chromatographic peaks will probably be asymmetric when marked differences are observed between the numerical values of the valley-to-peak ratio and overlapping criteria. The most satisfactory optima should be observed in the contour maps of the three criteria, and the global resolution for the valley-to-peak ratio and overlapping criteria should be both high.

The catecholamines showed very asymmetric peaks and low efficiencies when separated with a purely micellar eluent at pH 6.8. However, at pH < 4 the efficiencies increased and the asymmetry factors decreased. In contrast with the usual behaviour, the addition of short-chain alcohols (methanol, propanol and pentanol) to the micellar mobile phase at pH 6.8 resulted in extremely low efficiencies (see Table 1). The contour map of positional resolution for the five catecholamines eluted with SDS–propanol mobile phases at pH 6.8 is presented in Fig. 7a. Maximum resolution was observed for mobile phases without propanol containing 0.035–0.15 *M* SDS [$r(S) = 0.395$ for 0.15 *M* SDS]. The slope of the function at increasing concentrations of alcohol in this region was high, which is not detrimental for the preparation of the optimum mobile phase, as it does not contain an alcohol and the concentration of surfactant scarcely affected the resolution.

For the catecholamines at pH 6.8 and using the valley-to-peak ratio criterion, the optimum appeared in the 0.05–0.09 *M* SDS concentration range without alcohol [$r(P) = 0.14$], and for 0.035 *M* SDS–2.3% propanol [$r(P) = 0.144$]. Other secondary maxima disappeared (Fig. 7b). With the overlapping criterion the maximum of resolution was similar [0.05–0.08 *M* SDS without alcohol, $r(O) = 0.85$]. The maximum for 0.035 *M* SDS–1.5% propanol was less important (Fig. 7c).

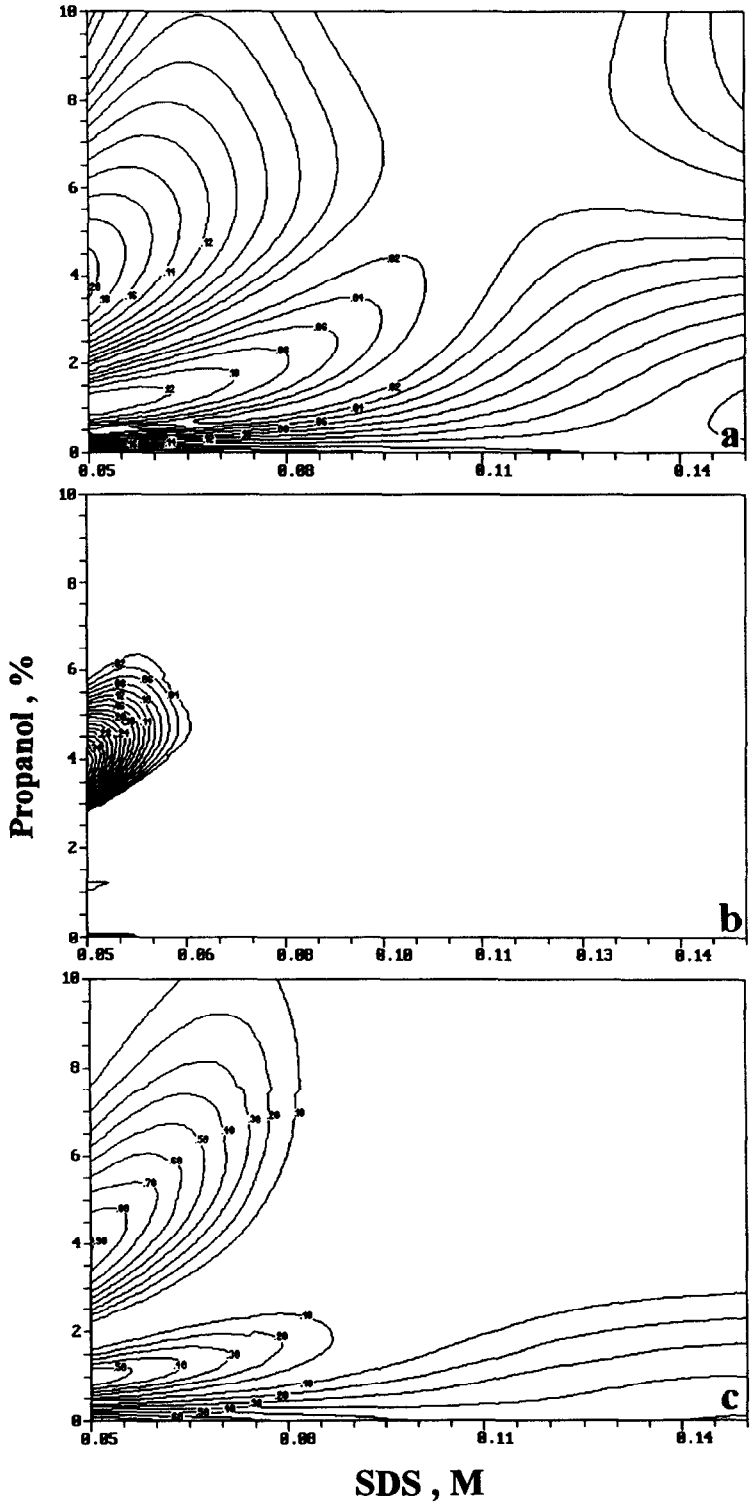

Fig. 9. Contour maps of global resolution for the mixture of five catecholamines, and SDS–propanol mobile phases at pH 3.5 (optima indicated): (a) positional criterion (0.05 *M*–0%, 0.05 *M*–1.1% and 0.05 *M*–4.2%); (b) valley-to-peak criterion (0.05 *M*–0%, 0.05 *M*–1.2% and 0.05 *M*–3.9%); (c) overlapping criterion (0.05 *M*–0%, 0.05 *M*–1.0% and 0.05 *M*–4.0%).

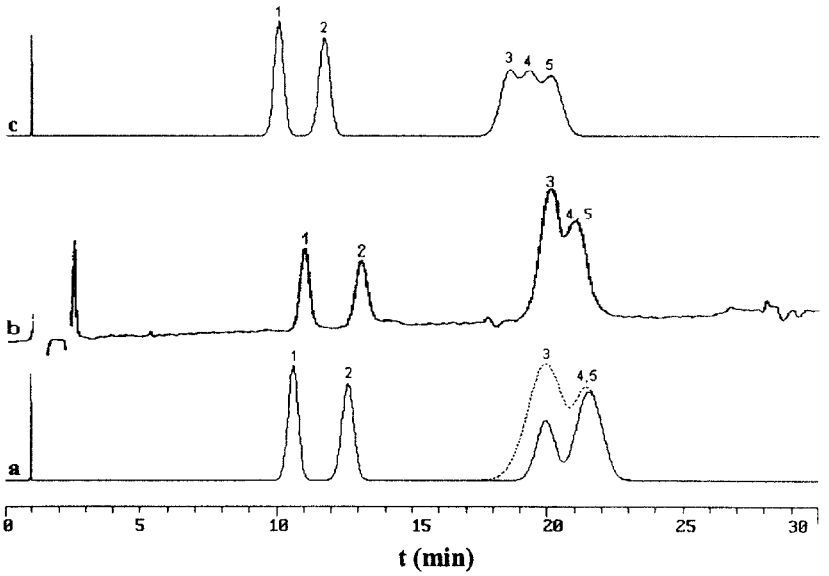

Fig. 10. (a) Simulated and (b) experimental chromatograms for the mixture of catecholamines in a 0.05 *M* SDS–0.8% propanol mobile phase at pH 3.5. (c) Simulated chromatogram for a 0.05 *M* SDS–1.1% propanol mobile phase at pH 3.5. Peaks: 1 = noradrenaline; 2 = adrenaline; 3 = adrenalone; 4 = dopamine; 5 = isoprenaline. Dashed lines in (a) correspond to a higher concentration of adrenalone.

Fig. 8 shows the simulated (a) and experimental (b) chromatograms for the separation of the five catecholamines with 0.11 *M* SDS mobile phases without alcohol at pH 6.8. Good agreement was observed between the experimental and simulated chromatograms, in spite of the low efficiencies. However, the separation of all the peaks in the chromatogram was not possible in any mobile phase at pH 6.8.

For the five catecholamines eluted with SDS–propanol at pH 3.5, several maxima of positional resolution were observed (Fig. 9a). The most interesting corresponded to 0.05 *M* SDS alone [$r(S) = 0.255$], 0.05 *M* SDS–1.1% propanol [$r(S) = 0.13$] and 0.05 *M* SDS–4.2% propanol [$r(S) = 0.21$]. These three maxima were also observed for the valley-to-peak ratio criterion for 0.05 *M* SDS alone [$r(P) = 0.168$], 0.05 *M* SDS–1.2% propanol [$r(P) = 0.04$] and 0.05 *M* SDS–3.9% propanol [$r(P) = 0.406$] (Fig. 9b) and for the overlapping criterion they were 0.05 *M* SDS alone [$r(O) = 0.80$], 0.05 *M* SDS–1.0% propanol [$r(O) = 0.55$] and 0.05 *M* SDS–4.0% propanol [$r(P) = 0.91$] (Fig. 9c). Fig. 10a and b show the simulated and experimental chromatograms for a 0.05 *M* SDS–0.8% propanol mobile phase. This mobile phase was close to a resolution maximum according to the positional and overlapping criteria, but it had a very low value of valley-to-peak resolution. A small increase in the concentration of propanol to 1.1% (Fig. 10c) led to the top of the local maximum of valley-to-peak resolution and, as observed, it gave the partial resolution of peaks 3–5.

4. Conclusions

Eq. 1 gave an adequate description of the retention behaviour of solutes in MLC with mixed mobile phases of SDS and propanol, which was useful for the determination of the optimum mobile phase in the separation of several mixtures of compounds. The use of a positional criterion of optimization may be acceptable for symmetrical peaks with high efficiencies. For asymmetric peaks, peaks with low efficiencies or mobile phases where the peaks are very close to each other, the use of positional–shape criteria may be advisable.

The combined use of the three criteria indicated in this work may give complementary information for selecting the optimum mobile phase. The positional criterion gives only a rough approximation of the region where the peaks will be separated, but it does not indicate how well separated they will be. The valley-to-peak ratio criterion gives information about the region where the peaks will be apparent. The overlapping criterion will indicate the region where the peaks will be better quantified, because a larger surface of each peak will be exposed.

As the application of the positional–shape criteria requires a good prediction of the position and shape of the chromatographic peaks (efficiency, asymmetry and retention), they are susceptible to error. However, these criteria are always preferable, even when asymmetric peaks are assumed to be symmetrical. In situations where the positional–shape criteria lead to large errors, the positional criterion also would give an unreliable prediction.

The selection of the optimum mobile phase should not only consider the value of the resolution function, but also the suitability of the preparation of the mobile phase. Thus, an optimum found in a region of large variations of the resolution function will not be adequate in practice, as the errors in the prediction of the retention and in the preparation of the mobile phase may lead to results different from those expected. For complex response surfaces showing several maxima and minima, additional experimental mobile phases should be prepared in the region where the optimum appears.

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