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# Performance of short-chain alcohols versus acetonitrile in the surfactant-mediated reversed-phase liquid chromatographic separation of β-blockers

M.J. Ruiz-Ángel[a,*], J.R. Torres-Lapasió[a], S. Carda-Broch[b], M.C. García-Álvarez-Coque[a]

[a] Departament de Química Analítica, Universitat de València, c/Dr. Moliner 50, Burjassot, Spain
[b] Departament de Química Física i Analítica, Universitat Jaume I, Cra. Borriol s/n, Castelló, Spain

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ABSTRACT

Organic solvents are traditionally added to micellar mobile phases to achieve adequate retention times and peak profiles, in a chromatographic mode which has been called micellar liquid chromatography (MLC). The organic solvent content is limited to preserve the formation of micelles. However, at increasing organic solvent contents, the transition to a situation where micelles do not exist is gradual. Also, there is no reason to neglect the potentiality of mobile phases containing only surfactant monomers instead of micelles (high submicellar chromatography, HSC). This is demonstrated here for the analysis of β-blockers. The performance of four organic solvents (methanol, ethanol, 1-propanol, and acetonitrile) was compared in mobile phases containing the anionic surfactant sodium dodecyl sulphate in the MLC and HSC modes. The association of the organic solvent molecules with micelles gives rise to a significant loss in the elution strength of the organic solvent; whereas upon disruption of micelles, it tends to that observed in the hydro-organic mode. The elution behaviour of the β-blockers was modelled to predict the retention times. This allowed the detailed exploration of the selectivity and resolution of the chromatographic systems in relatively wide ranges of concentration of surfactant and organic solvent. The best performance in terms of resolution and analysis time was achieved using HSC with acetonitrile, being able to base-line resolve a mixture of eight β-blockers. Ethanol also provided a good separation performance, significantly improved with respect to methanol and 1-propanol. In contrast, the hydro-organic mode using acetonitrile or any of the short-chain alcohols could not succeed with the separation of the β-blockers, owing to the poorer selectivity and wider peaks.



## 1. Introduction

The addition of a surfactant above the critical micellar concentration (CMC) in water to a reversed-phase liquid chromatographic (RPLC) system gives rise to significant modifications in its performance (i.e. retention, selectivity and peak shape), especially for charged solutes eluted with mobile phases containing an ionic surfactant bearing an opposite charge. This mode has been called micellar liquid chromatography (MLC) [1]. The presence of surfactant in the mobile phase allows the use of organic solvents scarcely miscible with water, reaching concentrations which are useful in RPLC. In spite of the wide range of compatible solvents, only three are routinely used to develop analytical methods: 1-propanol, 1-butanol and 1-pentanol, being the former the most common [1,2]. Surprisingly, there are only few reports in MLC using acetonitrile [3–7], which is the solvent of choice in hydro-organic RPLC, and to our knowledge there is only one reported analytical method using ethanol [8], in spite that this solvent is attracting considerable attention in recent time owing to its low toxicity (green chemistry).

In the field of MLC, a particularly interesting case is the separation of basic drugs, such as β-blockers and tricyclic antidepressants, using an alkyl-bonded phase and mobile phases containing sodium dodecyl sulphate (SDS) [9–13]. In such systems, the surfactant monomers cover the stationary phase, their hydrophobic tail associated to the alkyl-chains bonded to the silica support, and the polar head group, to which basic drugs are strongly attracted, oriented away from the surface [14]. This is revealed by the higher retention and improved peak profile of the basic drugs (i.e. narrower and more symmetric peaks), with regard to conventional hydro-organic RPLC [13,15]. The excess surfactant is dissolved in the mobile phase as monomers, associated in small clusters or forming micelles. These entities and the organic solvent molecules are responsible of the elution of the drugs. Different solutes experience the inter-

* Corresponding author. Tel.: +34 963544005.
*E-mail address:* Maria.J.Ruiz@uv.es (M.J. Ruiz-Ángel).

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actions with the components of the stationary and mobile phases in different degree, giving rise to diverse selectivity.

There is an extensive literature on the use of mobile phases containing micelles [1,2,16]. Only a few references deal with the use of pure micellar mobile phases, owing to the high retention and poor efficiency for most solutes. An organic solvent is usually needed to achieve adequate retention times and peak profiles. There is also an extensive discussion on the association of solutes with micelles. However, there is little information about the effect of micelles or surfactant monomers on the elution behaviour of organic solvents used as modifiers. Short-chain alcohols (i.e. methanol to propanol) have a small penetration capability into SDS micelles. The binding constants (mole fraction ratio of alcohol between bulk solvent and micellar pseudo-phase) are: 0.4, 1.1, and 3.5 for methanol, ethanol, and 1-propanol, respectively, at 25 °C [17,18]. These values correlate with the logarithm of the octanol–water partition coefficient of the solvents ($\log P_{o/w}$ = 0.18, 0.48, 2.2, respectively [19]). Log $P_{o/w}$ for acetonitrile is similar to that for ethanol (0.46). However, the effect of acetonitrile on the CMC of SDS is similar to that of methanol (i.e. the CMC increases at increasing concentration of the organic solvent), and opposite to the effect of ethanol and 1-propanol (i.e. the CMC decreases) [20].

Beyond a certain concentration of organic solvent, micelles disaggregate (with SDS: 40, 30, 22 and 30% (v/v) for methanol, ethanol, 1-propanol and acetonitrile, respectively [12,21]). However, there is no sudden breakdown of micelles when the concentration of organic solvent is increased, but a progressive reduction in the aggregation number [22]. It is, thus, not surprising that some authors supposedly working with MLC were not aware of the non-existence of micelles in the mobile phase [23–30]. More recently, a chromatographic mode containing a relatively large amount of surfactant (above its CMC in water) together with an amount of organic solvent that prevents the formation of micelles has been described, which has been called "high submicellar chromatography" (HSC) [12,13,31,32].

In previous work, the chromatographic behaviour of several β-blockers was examined using a Kromasil C18 column and mobile phases containing SDS and acetonitrile [12,13]. Depending on the concentration of both modifiers, different separation environments with particular behaviours were yielded:

(i) Hydro-organic (conventional RPLC in the absence of SDS).
(ii) Low submicellar, with concentrations of SDS and acetonitrile below the CMC in water and above 30%, respectively, which corresponds to ion-pair chromatography (IPC).
(iii) Micellar (MLC), with stable micelles in the hydro-organic medium.
(iv) High submicellar (HSC), with high concentrations of both surfactant and organic solvent (above the CMC in water and above 30%, respectively), where no micelles exist in the mobile phase.

Among the four modes, HSC was the most promising, as it allowed full resolution of the β-blockers in practical times.

The main objective of modifying the stationary and mobile phases is the selectivity improvement. This depends on the relative interactions of solutes with both phases. In this work, the potentiality of MLC and HSC with SDS for the separation of β-blockers using the Kromasil C18 column, and methanol, ethanol and 1-propanol as modifiers, was examined and compared with acetonitrile. The performance of the conventional hydro-organic mode with each solvent was also checked. The exploration of the selectivity and resolution was performed based on a detailed description of the elution behaviour.

## 2. Experimental

### 2.1. Reagents

A set of eight β-blockers was used: acebutolol, atenolol, metoprolol, pindolol, timolol (Sigma, St. Louis, MO, USA), celiprolol (Rhône-Poulenc Rorer, Alcorcón, Spain), esmolol (Du Pont-De Nemours, Le Grand Saconnex, Switzerland), and oxprenolol (Ciba-Geigy, Barcelona, Spain). The drugs were dissolved in a small amount of the organic solvent used as modifier in the mobile phase, and diluted with water. The concentration of the injected solutions was 20 μg/mL.

The mobile phases contained methanol, ethanol, 1-propanol or acetonitrile (Scharlab, Barcelona). Sodium dodecyl sulphate (99% purity, Merck, Darmstadt, Germany) was added to switch the system to the assayed surfactant-mediated modes (MLC and HSC). The mobile phases were buffered at pH 3 with 0.01 M citric acid monohydrate and sodium hydroxide (Panreac, Barcelona). Nanopure water (Barnstead, Sybron, Boston, MA, USA) was used throughout. The drug solutions and mobile phases were filtered through 0.45 μm nylon membranes (Micron Separations, Westboro, MA, USA).

### 2.2. Apparatus and column

The liquid chromatograph (Agilent, Waldbronn, Germany) was equipped with an isocratic pump (Series 1200), an autosampler and a UV–visible detector (Series 1100) set at 225 nm. Data acquisition was carried out with an HPChemStation (Agilent, B.02.01), and the mathematical treatment performed in MATLAB 6.5 (The Mathworks, Natick, MA, USA). The operating pump pressure was below 400 bar.

A Kromasil C18 column (Análisis Vínicos, Ciudad Real, Spain) was used, with the following characteristics: 150 mm × 4.6 mm i.d., 5 μm particle size, 19% carbon load, 320 $m^2$/g surface area, and 110 Å pore diameter. The column was connected to a similar 30 mm guard column. The flow-rate was set at 1 mL/min. Duplicate injections were made using an injection volume of 20 μL.

### 2.3. Experimental designs

The minimal concentrations of the alcohols in the mobile phase were selected to attain a measurable retention for the most retained solutes. In spite of the high retention achieved for <10% (v/v) acetonitrile in the presence of surfactant, mobile phases containing 5–10% were also tested to assure micellar conditions. On the other hand, the maximal concentration of organic solvent was limited by the system pressure (case of alcohols), or the reduction of the retention to excessively small values.

In the surfactant-mediated modes, a two-factor space of concentrations of SDS and organic solvent was investigated. The weak elution strength of methanol, ethanol and acetonitrile forced the use of concentrations of SDS ≥ 0.075 M. A high volume fraction of methanol was needed, owing to its extremely low elution strength in the presence of surfactant. The concentrations of the surfactant and organic solvent in the experimental designs were the following (SDS molar concentration/% (v/v) organic solvent are given): methanol (0.075 M/50 and 60%; 0.1125 M/55%; 0.15 M/50 and 60%), ethanol (0.075 M/5, 15, 25 and 40%; 0.1125 M/10, 20 and 30%; and 0.15 M/5, 15, 25 and 40%), 1-propanol (0.04 M/5, 15, 25 and 35%; 0.075 M/5, 15, 25 and 35%; 0.1125 M/10, 20 and 30%; and 0.15 M/5, 15, 25 and 35%), and acetonitrile (0.075 M/5, 15, 20, 30, 40 and 50%; 0.1125 M/10, 17.5, 25, 35 and 45%; and 0.15 M/5, 15, 20, 30, 40 and 50%).

In the hydro-organic mode, experimental designs consisting in four mobile phases were used to study the elution behaviour.

The organic solvent contents in the aqueous mobile phases were: methanol (20, 30, 40 and 50%), ethanol (10, 15, 20 and 25%), 1-propanol (5, 7.5, 10 and 15%), and acetonitrile (15, 20, 25 and 30%).

## 3. Results and discussion

Several authors have carried out comparison studies on the performance of different organic solvents added to micellar mobile phases [33–37]. Most reports deal exclusively with the changes in the chromatographic behaviour in the micellar mode; they do not include a comparison with conventional hydro-organic RPLC. Also, the behaviour using selected mobile phases is often discussed, and trial-and-error optimization strategies applied.

Along the last decade, we have carried out in our laboratories a systematic research on the application of MLC to the analysis of drugs [2,38]. With optimization purposes, we have developed an interpretive strategy based on the reliable description of the retention behaviour of the analytes using mathematical models [39–41]. This strategy has the advantage of allowing a comprehensive examination of the changes in the chromatograms of individual solutes, or mixtures of two or more solutes. This facilitates the selection of the optimal mobile phase for a given separation, offering the maximal resolution, or at least, satisfactory resolution in an adequate analysis time, or with a smaller amount of modifier in the mobile phase.

In order to compare the performance of the short-chain alcohols methanol, ethanol and 1-propanol versus acetonitrile, for the separation of a set of eight β-blockers in the surfactant-mediated and hydro-organic modes, we first built several models to enable the prediction of retention times. These models facilitated the detailed exploration of the selectivity and resolution of the chromatographic systems. Our final aim was finding out the potentiality of each modifier for the separation of β-blockers.

### 3.1. Elution behaviour

The development of a model aimed to describe the elution behaviour of a solute in a surfactant-mediated system requires the measurement of the retention times, using a set of mobile phases containing variable concentrations of surfactant and organic solvent. We obtained this information for eight β-blockers eluted with SDS and the alcohols methanol, ethanol and 1-propanol, or acetonitrile (see Section 2.3). Concentrations of SDS below 0.04 M were avoided, since surfactant desorption from the stationary phase was significant at moderate organic solvent contents. However, among the alcohols, only 1-propanol allowed the inspection of a wide range of experimental conditions. The feasible experimental domain was narrower for ethanol and methanol, especially for the latter, owing to the smaller elution strength giving rise to excessive retention. We were also especially interested on 1-propanol, since this is the most usual organic solvent in MLC to elute moderately polar compounds.

#### 3.1.1. Changes in elution strength

The elution strength (traditionally measured as the slope of the plot of the logarithm of the retention factor versus the concentration of modifier) changes with the concentration of the modifier, except in relatively narrow concentration ranges, which holds for the organic solvent (in the absence and presence of a surfactant) and the surfactant itself. The elution strength in a ternary system (as is the case of the surfactant-mediated modes) depends also on the concentration of the accompanying modifier. We have also observed that in the analysis of β-blockers, the elution strength for the surfactant (SDS) is significantly larger than that of the short-chain alcohols and acetonitrile.

In the presence of SDS, the organic solvent molecules can be found free in the bulk mobile phase, associated to micelles or to surfactant monomers. The association with the organized structure (the micelle) in the mobile phase is stronger, giving rise to a loss in elution strength for the organic solvent. In previous work carried out with β-blockers, a drastic increase in the elution strength of acetonitrile (at constant surfactant concentration) was observed at increasing organic solvent contents, with a transition region in the range 20–30% acetonitrile [12,13]. In this region, two effects are happening that affect the retention: the micelles disaggregate and the organic solvent desorbs significantly the surfactant monomers covering the stationary phase. The disruption of the surfactant organized structures is translated into an increase in the elution strength of the organic solvent, which becomes similar to that observed in an acetonitrile–water mobile phase.

In this work, the retention of the β-blockers in mobile phases comprising the micellar and high submicellar regions was measured for ethanol and 1-propanol, but the transition region (around 20–25% for ethanol and 15–20% for 1-propanol) was far less evident in comparison to acetonitrile (i.e. the change in elution strength was more gradual). On the other hand, the small elution strength of methanol in the presence of surfactant forced the use of mobile phases containing a large amount of this solvent, where micelles cannot be formed (i.e. HSC mode). All the observed effects should be gathered by the models describing the retention behaviour.

#### 3.1.2. Retention models

In hydro-organic RPLC, the elution behaviour is classically modelled using a quadratic relationship between the logarithm of the retention factor, $k$, and the volume fraction of organic solvent in the aqueous–organic mobile phase, $\varphi$:

$$\log k = \log\frac{t_R - t_0}{t_0} = c_0 + c_1\varphi + c_{11}\varphi^2 \quad (1)$$

where $t_R$ and $t_0$ are the retention time and dead time, respectively, and $c_0$, $c_1$ and $c_{11}$, regression coefficients with characteristic values for a given solute and column/solvent system.

For MLC and HSC, mechanistic models have been developed [13], which can be rewritten as quadratic polinomia. For MLC:

$$\frac{1}{k} = \frac{1}{K_{AS}}(1 + K_{AD}\varphi) + \frac{K_{AM}^{MLC}}{K_{AS}}(1 + K_{MD}\varphi)[M]$$
$$= c_0 + c_1\varphi + c_2[M] + c_{12}\varphi[M] \quad (2)$$

and for HSC:

$$\frac{1}{k} = \frac{1}{K_{AS}}(1 + K_{AD}\varphi) + \frac{K_{\varphi}}{K_{AS}}(1 + K_{AD}\varphi)\varphi^2 + \frac{K_{AM}^{HSC}}{K_{AS}}(1 + K_{MD}\varphi)[S]$$
$$= c_0 + c_1\varphi + c_{11}\varphi^2 + c_{111}\varphi^3 + c_2[S] + c_{12}\varphi[S] \quad (3)$$

which can be simplified to:

$$\frac{1}{k} = \frac{1}{K_{AS}}(1 + K_{AD}\varphi) + \frac{K_{\varphi}}{K_{AS}}\varphi^2 + \frac{K_{AM}^{HSC}}{K_{AS}}(1 + K_{MD}\varphi)[S]$$
$$= c_0 + c_1\varphi + c_{11}\varphi^2 + c_2[S] + c_{12}\varphi[S] \quad (4)$$

where $[M]$ is the concentration of surfactant monomers involved in micelle formation and $[S]$ the concentration of surfactant monomers in the submicellar mode (where no micelles are formed); $K_{AS}$ describes the partition of the solute between bulk water and the surfactant-modified stationary phase, and $K_{AM}^{MLC}$ and $K_{AM}^{HSC}$ the partition between bulk water and the surfactant monomers forming micelles (MLC) or the free monomers (HSC); $K_{AD}$ and $K_{MD}$ are constants that account for the displacement of the partitioning equilibria produced by the addition of the organic solvent. Finally, the ratio $K_{\varphi}$ to $K_{AS}$ is a regression coefficient similar to

**Table 1**
Correlation between predicted and experimental retention factors for the eight β-blockers eluted in the surfactant-mediated modes with SDS.

| Organic solvent | SDS range (M) | Solvent range (%, v/v) | Retention model | Correlation equation | $R$ | $n$[a] |
|---|---|---|---|---|---|---|
| Micellar mode | | | | | | |
| Acetonitrile | 0.075–0.15 | 5–15 | Eq. (2) | $k_{pred} = 0.11 + 0.9985k_{exp}$ | 0.99993 | 40 |
| Methanol | Non-available[b] | | | | | |
| Ethanol | 0.075–0.15 | 5–15 | Eq. (2) | $k_{pred} = 0.03 + 0.9994k_{exp}$ | 0.99998 | 40 |
| 1-Propanol | 0.04–0.15 | 5–15 | Eq. (2) | $k_{pred} = 0.05 + 0.9999k_{exp}$ | 0.99997 | 56 |
| High submicellar mode | | | | | | |
| Acetonitrile | 0.075–0.15 | 30–50 | Eq. (4) | $k_{pred} = 0.04 + 0.999k_{exp}$ | 0.9998 | 64 |
| Methanol | 0.075–0.15 | 50–60 | Eq. (2) | $k_{pred} = 0.08 + 0.9964k_{exp}$ | 0.9994 | 40 |
| Ethanol | 0.075–0.15 | 20–40 | Eq. (4) | $k_{pred} = 0.04 + 0.9983k_{exp}$ | 0.9998 | 47 |
| 1-Propanol | 0.04–0.15 | 20–35 | Eq. (4) | $k_{pred} = 0.012 + 0.998k_{exp}$ | 0.9997 | 64 |

[a] Number of points.
[b] Data for methanol in the micellar mode were not available due to the high retention.

$c_{11}$ in Eq. (1). The quadratic and cubic terms in $\varphi$ for the HSC models account for the larger role of the organic solvent in the mobile phase [12,15].

In MLC, hyperbolic relationships have been demonstrated to be more appropriate than the logarithmic ones for alcohols [42]. We checked, therefore, also the performance of a hyperbolic model for conventional hydro-organic RPLC:

$$\frac{1}{k} = c_0 + c_1\varphi + c_{11}\varphi^2 \quad (5)$$

*3.1.3. Accuracy of the retention models*

The performance of the models given in Eqs. (1)–(5), fitted with the retention data of each β-blocker eluted with the mobile phases described in Section 2.3, was measured using the correlation coefficient ($R$), and the mean relative prediction error calculated as:

$$\text{RE} = \frac{\sum_{i=1}^{n} |k_{exp} - k_{pred}|}{n\bar{k}_{exp}} \times 100 \quad (6)$$

where $k_{exp}$ and $k_{pred}$ are the experimental and predicted retention factors, respectively, for each individual compound eluted with

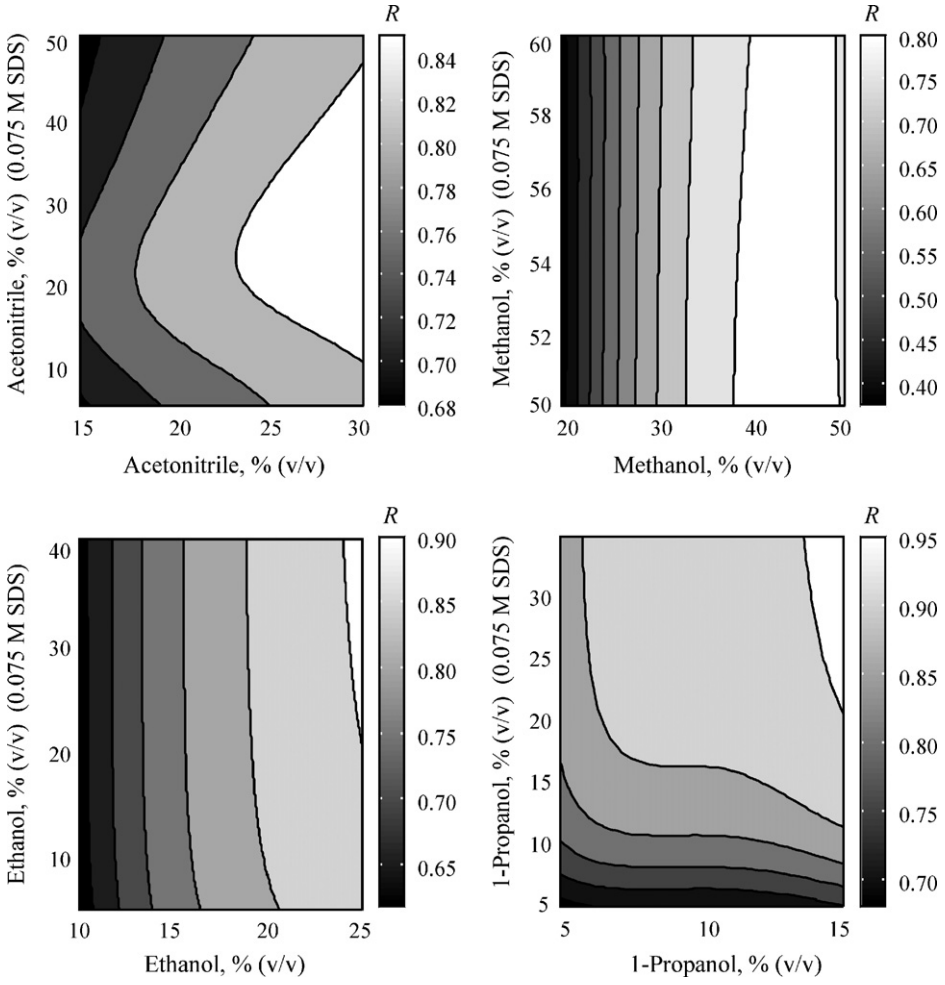


**Fig. 1.** Similarities in selectivity between the surfactant-mediated and hydro-organic modes, expressed as the $R$ coefficient for the correlation of the retention times of the eight β-blockers separated in both modes with the Kromasil C18 column, at varying mobile phase composition. The concentration of SDS in the surfactant-mediated modes was fixed at 0.075 M in all cases.

each mobile phase, and $\bar{k}_{exp}$ is the mean experimental retention factor considering all compounds and mobile phases.

For acetonitrile, the retention in MLC (<15% acetonitrile), HSC (>30%), and the transition region (15–30%) was modelled using three different equations: Eq. (2) for MLC, and Eq. (3) or (4) for both HSC and the transition region. The predictions were highly satisfactory. For MLC, $n = 40$, $R = 0.9999$ and RE = 0.51%; and for HSC, $n = 64$, $R = 0.9998$ and RE = 1.08% for the quadratic model (without significant improvement with the cubic model: $R = 0.99992$ and RE = 0.78%). However, when the whole search space is considered

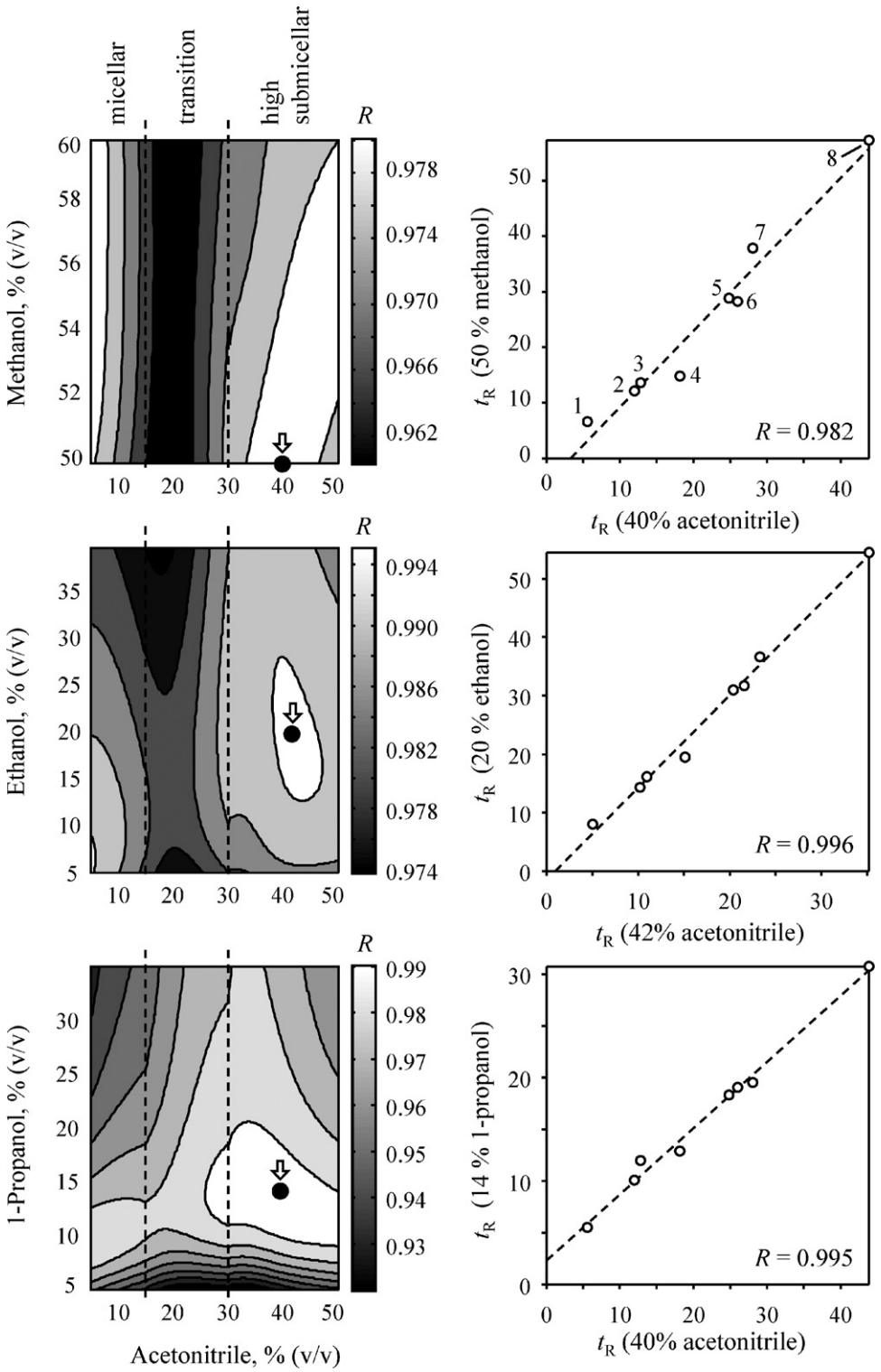


**Fig. 2.** Left: Similarities in selectivity between the short-chain alcohols and acetonitrile in the surfactant-mediated modes, expressed as the *R* coefficient for the correlation of the retention times of the eight β-blockers separated with the Kromasil C18 column, at varying mobile phase composition. The concentration of SDS was fixed at 0.075 M in all cases. The predictions for the micellar region were made with Eq. (2), and those for the transition and high submicellar regions with Eq. (4), except for methanol for which Eq. (2) was used. Right: Correlation plots corresponding to the marked points in the regions of maximal selectivity. Compounds: (1) atenolol, (2) pindolol, (3) acebutolol, (4) celiprolol, (5) esmolol, (6) metoprolol, (7) timolol, and (8) oxprenolol. The order of elution was the same for the four solvents, except for esmolol and metoprolol, which reversed their elution for methanol.

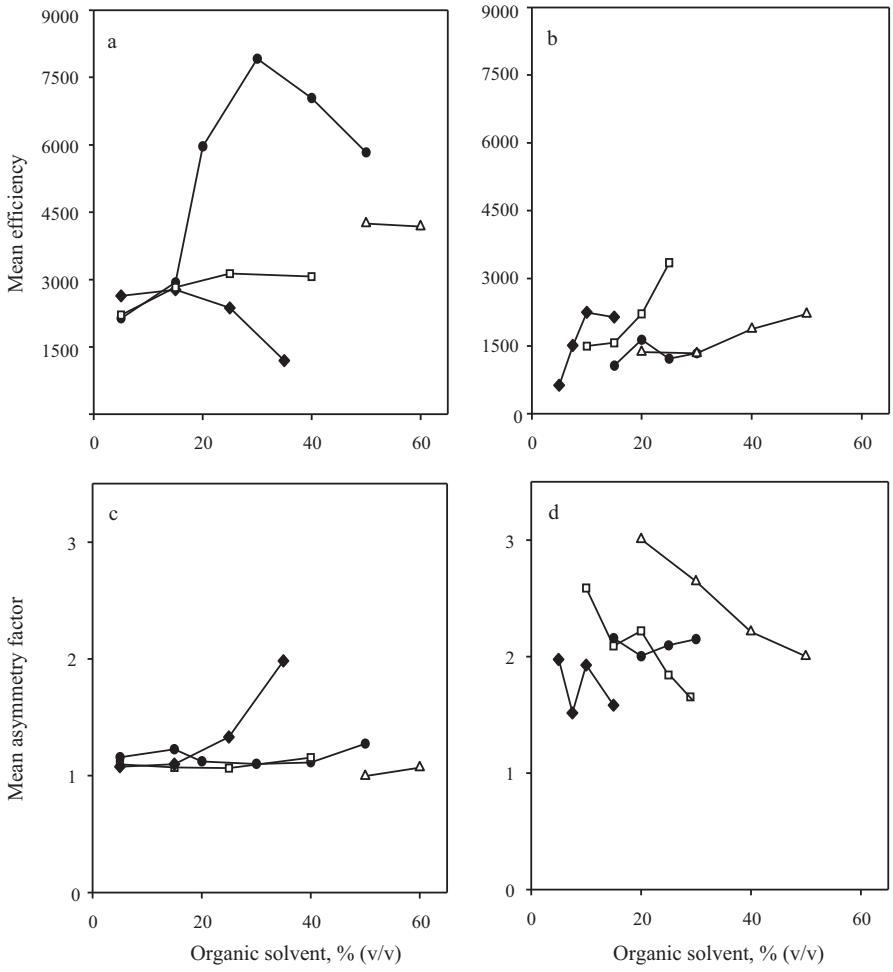


**Fig. 3.** Mean values of efficiency (a and b) and asymmetry factor (c and d) considering the eight β-blockers, eluted in the surfactant-mediated modes (a and c) and hydro-organic mode (b and d). Organic solvents: acetonitrile (●), methanol (△), ethanol (□), and 1-propanol (♦). The efficiencies were calculated according to Foley and Dorsey [48], and the asymmetry factors as the right to left half-widths ratio at 10% peak height. The concentration of SDS in the surfactant-mediated modes was 0.075 M.

(e.g. 5–50% acetonitrile in this work), the use of specific models for different regions of the factor space complicates the search of the optimal experimental conditions. For this reason, we sought also a model that fitted satisfactorily the elution behaviour in the whole domain (5–50%). The best description was achieved with the cubic equation (Eq. (3)), with $n = 136$, $R = 0.9992$ and RE = 2.8%, against $R = 0.988$ and RE = 8.7% for the quadratic model (Eq. (2)). As expected, the quality of the predictions was enhanced when the domain was divided in regions described by specific models.

With methanol, only the HSC separation could be examined, owing to the high retention in MLC, but for ethanol and 1-propanol, both MLC and HSC mobile phases were assayed. As commented, for methanol, we assayed only five mobile phases covering a relatively narrow factor space. In this case, we preferred to model the retention with Eq. (2), which allowed one degree of freedom ($n = 40$, $R = 0.9994$ and RE = 1.85%). For ethanol in the MLC mode: $n = 40$, $R = 0.9999$ and RE = 0.25%; and in HSC, $n = 47$, $R = 0.9998$ and RE = 0.91% for the quadratic model. For 1-propanol in the MLC mode: $n = 56$, $R = 0.9999$ and RE = 0.48%; and in HSC, $n = 64$, $R = 0.9997$ and RE = 1.07% for the quadratic model. The excellent descriptions obtained for acetonitrile and the alcohols can be also appraised in Table 1.

For ethanol and 1-propanol, the use of a single model to predict the retention factors in the whole range of organic solvent concentrations was also considered. For these solvents, the transition between MLC and HSC was rather smooth, but again the cubic model offered the best accuracy (for ethanol: $n = 87$, $R = 0.9998$ and RE = 1.33%, against $R = 0.9990$ and RE = 2.9% for the quadratic model; and for 1-propanol: $n = 120$, $R = 0.99994$ and RE = 1.10%, against $R = 0.9997$ and RE = 2.25%).

For comparison purposes, models describing the retention in the hydro-organic mode were also obtained. We assayed both logarithmic and hyperbolic relationships (Eqs. (1) and (5)). For acetonitrile, methanol and ethanol, the predictions were similar for the logarithmic and hyperbolic models, with RE < 1.0%. However, for 1-propanol, these were improved with the hyperbolic model: $R = 0.999990$ and RE = 0.43%, against $R = 0.9993$ and RE = 3.8% for the logarithmic.

### 3.2. Comparison of the selectivity in the different systems

The resolution capability of a chromatographic system depends on its selectivity and peak performance (i.e. peak broadening and skewness). The selectivity is traditionally measured through the ratio of the retention factors (relative retention) for the peaks of two compounds, which is called the "selectivity factor". This pairwise comparison is usually extended to two or more peaks [43]. The selectivity factor is also applied to column characterization, using selected pairs of probe compounds, eluted under specific conditions. These compounds are assumed to measure different properties, such as column hydrophobicity, silanol activity, steric hindrance, hydrogen bonding capacity and ion-exchange capability, for which several test compounds have been proposed [44].

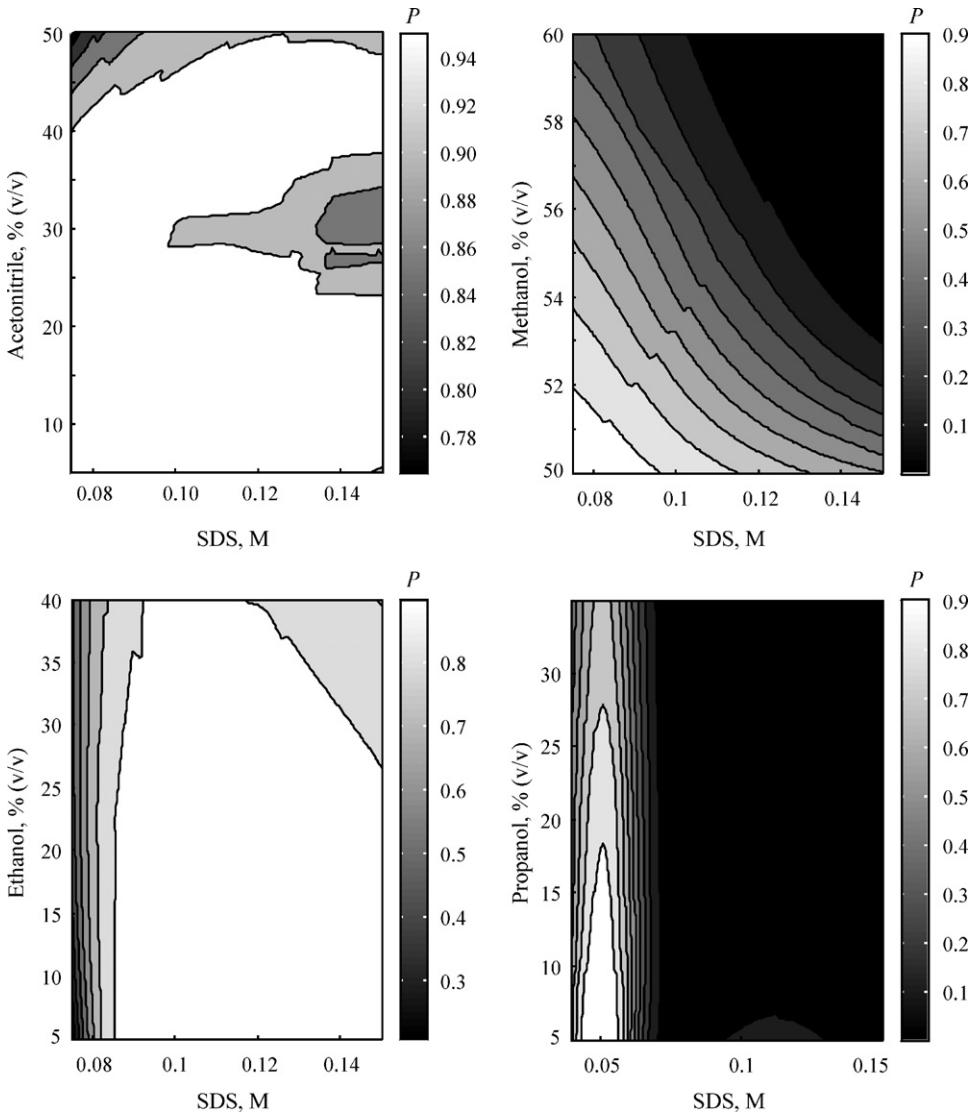


**Fig. 4.** Contour maps showing the peak purities in a two-factor space of concentrations of surfactant and organic solvent, for a set of seven β-blockers (without esmolol), eluted in the surfactant-mediated modes.

However, although the conclusions about the hydrophobicity generally agree between the tests, those for other properties differ. Also, it should be noted that the selectivity changes with mobile phase composition. It is, thus, possible that two columns or systems show similar for a given composition region and differ extremely for another [45].

The addition of a surfactant to an RPLC system gives rise to a fundamental change in the stationary phase nature that affects its selectivity. Also, in the surfactant-mediated systems, different organic solvents may give rise to different behaviours. In order to obtain a comprehensive description of the selectivity, we decided to compare the relative retention for all probe compounds in each system (MLC, HSC or hydro-organic) considering the incidental variations with mobile phase composition. Note that this study comprises the information given by selected pairs of compounds. For this purpose, the retention times of the set of β-blockers, separated with two different modes or solvents, were regressed each other at varying mobile phase composition. The retention times in each case were predicted using the models described in Section 3.1.2 and the dead time values, for a number of mobile phase compositions distributed in the solvent content range. The correlation coefficient ($R$) was used as a descriptor of the similarity between the peak distribution (selectivity) of the systems. Such approach was previously applied to the comparison of different columns using acetonitrile–water mixtures [45].

Fig. 1 depicts contour maps showing the $R$ coefficients for the correlations between the retention times for the surfactant-mediated and hydro-organic modes, for each organic solvent. For the surfactant-mediated modes, the concentration of SDS was arbitrarily fixed at 0.075 M. As observed, the addition of surfactant gives rise to important changes in the selectivity. However, the similarities increase at increasing concentration of the organic solvent, especially for ethanol and 1-propanol, with maximal $R$-values of 0.90 and 0.95.

The similarities among the four organic solvents in the surfactant-mediated systems were also checked. The contour maps in Fig. 2 compare each alcohol with acetonitrile. The diagrams were obtained using the best retention models for acetonitrile in each region (MLC, transition and HSC, which are marked). In each case, a narrow region of high similarity ($R > 0.975$) between systems is achieved (the plots at the right of each figure depict the best correlations, corresponding to the marked points). Similar diagrams were built to compare the alcohols. These revealed that methanol and ethanol were similar in selectivity, in a wide composition range.

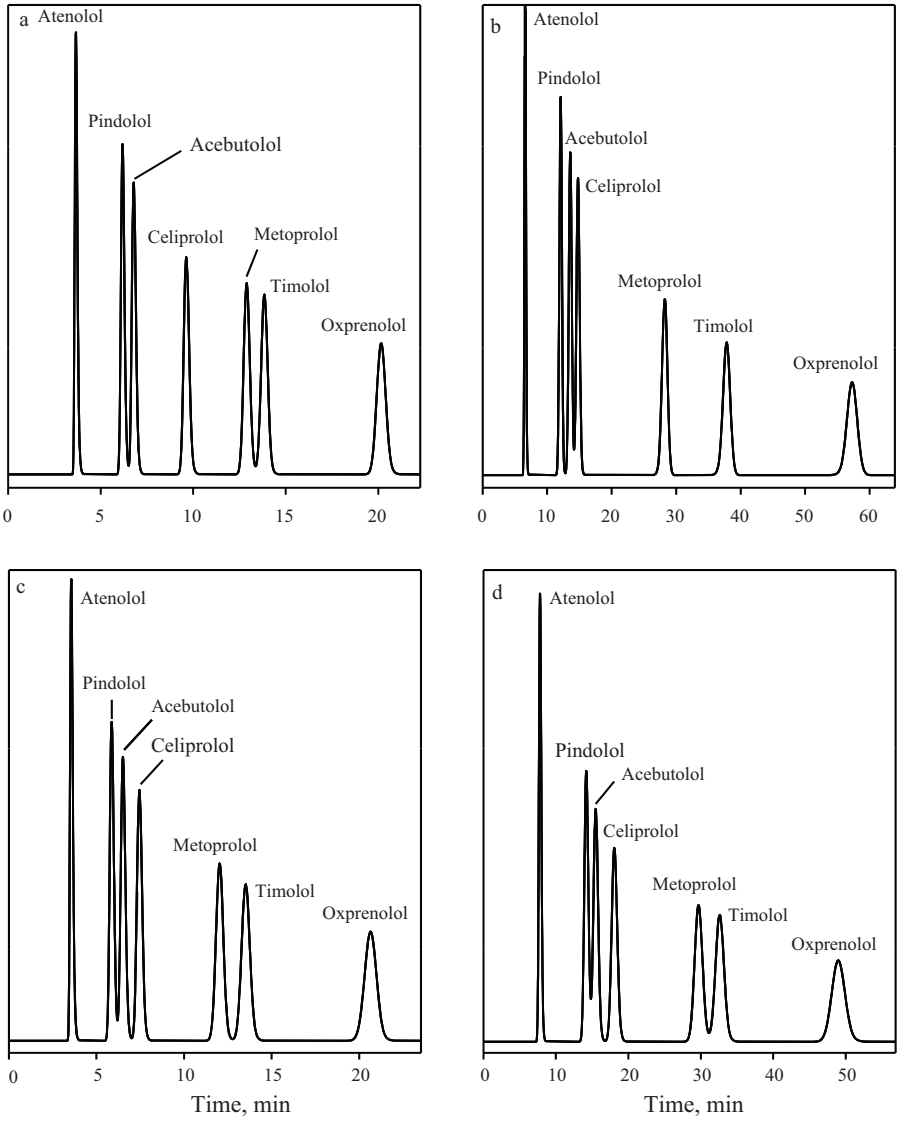


**Fig. 5.** Selected chromatograms for a mixture of seven β-blockers, eluted with: (a) 0.15 M SDS/42.7% acetonitrile ($P = 0.976$), (b) 0.075 M SDS/50.0% methanol ($P = 0.972$), (c) 0.10 M SDS/40.0% ethanol ($P = 0.972$), and (d) 0.078 M/7.8% 1-propanol ($P = 0.921$).

### 3.3. Changes in peak width and asymmetry

In hydro-organic RPLC, protonated basic drugs interact with free silanols on the stationary phase in a slow process, which implies tailing peaks and low efficiencies affecting the chromatographic resolution [46]. SDS is an effective silanol-blocking agent for the analysis of β-blockers [47], with implications in the resolution. Fig. 3 shows the changes in the mean efficiency and asymmetry factor for the set of eight β-blockers in the surfactant-mediated and hydro-organic modes, using acetonitrile and the three alcohols as modifiers. In all cases, the presence of SDS in the mobile phase yielded a significant improved peak shape (i.e. larger efficiencies and smaller asymmetry factors). Also, in the surfactant-mediated modes, the peaks were enhanced at increasing solvent content, up to reach a maximal value of efficiency, which is more evident for acetonitrile and 1-propanol. Note that the maximum is found in the region where micelles are being disrupted (∼30% acetonitrile and ∼20% 1-propanol). At this concentration, surfactant desorption from the stationary phase should be significant. The best peaks were obtained in the SDS/acetonitrile systems. Note that the peaks for methanol correspond only to the HSC mode, where no micelles are formed (as commented, the retention in the MLC mode was excessive to be measured). However, it seems that methanol yields also better efficiencies than ethanol and 1-propanol.

### 3.4. Resolution performance

The reduction of the information related to the chromatographic resolution was performed using the peak purity concept (peak area fraction free of interferences), which is a normalized measurement ranging from zero for full overlapping to one for full resolution [39,40]. The global resolution ($P$) was assessed as the product of elementary peak purities ($p_i$). The measurement of peak purities was proposed in the 80s [49], but its use was not possible until the development of fast computers, and more practical and reliable peak models. The most important features of the peak purity concept are its straightforward meaning, and the capability of evaluating the separation degree of a given peak, without linking it to the identity of the interferences or being affected by changes in the elution order. It also gives information about the expectancies of resolution (i.e. limiting resolutions) for a single compound, a group of compounds, or for the whole mixture [50].

The elution order of the β-blockers in the surfactant-mediated modes was the same for the four organic solvents, except for

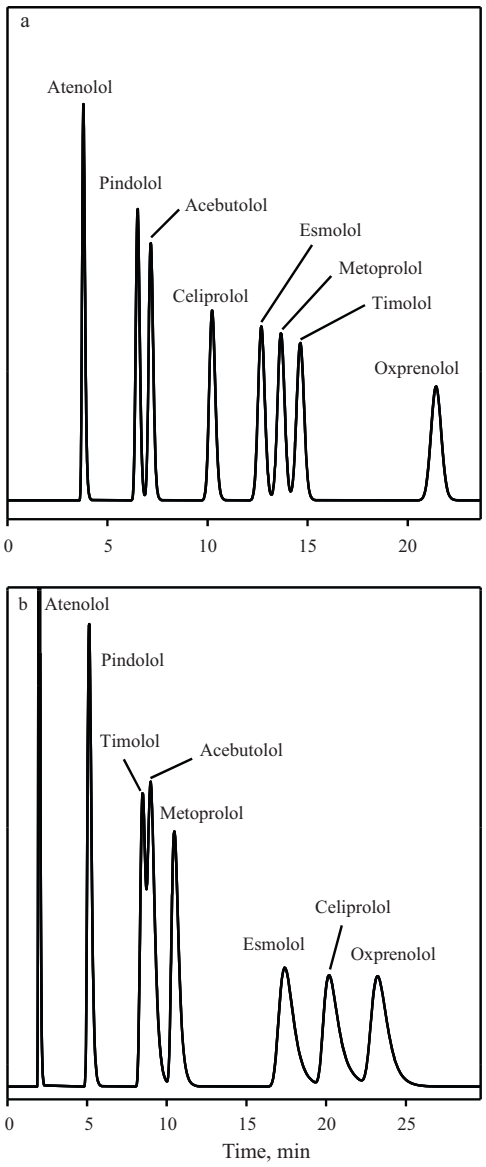


**Fig. 6.** Optimal chromatograms for a mixture of the eight β-blockers, eluted with: (a) 0.15 M SDS/41.1% acetonitrile ($P$ = 0.965), and (b) 16.35% acetonitrile ($P$ = 0.586).

esmolol and metoprolol which constitutes the critical pair in the mixture (these compounds eluted closely and reversed their retention at certain compositions). The global resolution was thus affected by the overlapping of a single pair. Therefore, in order to appraise the resolution behaviour of the different β-blockers, we obtained first the peak purity contour maps for a mixture of seven β-blockers (without esmolol, which belonged to the critical pair) (Fig. 4), and considered further the overlapping of the critical pair. As observed, for acetonitrile and ethanol, there is a wide composition range where satisfactory resolution is achieved. Instead, the region of acceptable resolution for methanol and 1-propanol is rather narrow. The optima were found at 0.0915 M SDS/14% acetonitrile ($P$ = 0.99999), 0.075 M SDS/50% methanol ($P$ = 0.972), 0.075 M SDS/25% ethanol ($P$ = 0.996), and 0.04 M SDS/7.7% 1-propanol ($P$ = 0.995). At these compositions, the analysis time was however too long, but it could be decreased to acceptable values maintaining good resolution, by increasing the concentration of organic solvent (and eventually, SDS). Fig. 5 shows the chromatograms for four selected conditions, which corresponded to the HSC mode for acetonitrile, methanol and ethanol, and MLC for 1-propanol. The analysis time could not be further decreased for methanol and 1-propanol, owing to the overlapping of the peaks of pindolol, acebutolol and celiproplol.

The limiting peak purities for esmolol and metoprolol (the critical pair) were $p_{lim}$ = 0.998 and 0.990 for acetonitrile, 0.649 and 0.649 for methanol, 0.791 and 0.787 for ethanol, and 0.751 and 0.652 for 1-propanol, respectively. This means that only acetonitrile was able to resolve the critical pair. Acetonitrile also succeeded in the resolution of the mixture of the eight β-blockers, with optimal peak purity at 0.124 M SDS/39.2% acetonitrile ($P$ = 0.977), and an analysis time of 30 min, which can be decreased to 21 min with still good resolution at 0.15 M SDS/41.1% acetonitrile (Fig. 6a).

The most outstanding differences between the surfactant-mediated and hydro-organic modes are the different elution order (see Fig. 6 for acetonitrile), the multiple peak reversals in the hydro-organic mode, and the improved efficiencies in the presence of surfactant. For the hydro-organic mode, the critical peaks corresponded to acebutolol and timolol for acetonitrile, metoprolol and timolol for methanol and ethanol, and acebutolol, metoprolol and timolol for 1-propanol; meanwhile, esmolol was well resolved in a wide range of compositions for all solvents.

In all cases, the resolution was poorer in the hydro-organic mode, but again acetonitrile and ethanol offered the best performance. For 1-propanol and methanol, several peaks overlapped in the optimal separations, at relatively low concentrations of the organic solvents (20% for methanol and 5.5% for 1-propanol), with long analysis times (50–100 min). Thus, these solvents cannot be recommended in the analysis of the mixture of β-blockers with or without surfactant in the mobile phase, using the Kromasil C18 column. The analysis times for acetonitrile and ethanol were appreciably shorter in the hydro-organic mode. With these solvents, it was possible to resolve a mixture of seven β-blockers (without timolol) in 22 min using 16.65% acetonitrile ($P$ = 0.97), and in 10 min using 21.7% ethanol ($P$ = 0.998). However, only the SDS/acetonitrile RPLC system could resolve the mixture of eight β-blockers (Fig. 6).

## 4. Conclusions

Analysts working with mobile phases containing a surfactant at relatively high concentration (i.e. above the CMC in water) have been concerned with the preservation of micelles. However, as commented, there is no sudden transition between MLC and HSC. Also, some authors using high organic solvent concentrations claimed to be working in MLC conditions, without being aware that no micelles were formed. However, in fact, there is no reason to neglect the potentiality of mobile phases containing surfactant monomers instead of micelles. This has been demonstrated for the analysis of β-blockers.

Another common topic in the MLC literature is the role of micelles in the chromatographic behaviour. Certainly, micelles increase the solubility of analytes, and contribute to their desorption from the stationary phase, with an elution strength often larger than that of the organic solvent. Thus, the organic solvent is seen as a secondary modifier, which can affect the micelle nature and displace the analyte partition equilibrium towards the bulk mobile phase. However, in fact, the role of the organic solvent is similar to that in an aqueous–organic mixture. The loss of protagonism of the organic solvent in a surfactant-mediated mobile phase can be explained by its association with the micelles or surfactant monomers, which decreases its capability to interact with analytes. Since the stabilization with an organized structure (as the micelles) is stronger, the disruption of micelles at high concentration of the organic solvent gives rise to a significant increase in elution strength, which cannot solely be explained by the increase in the concentration of the organic solvent. In these conditions, the

elution strength of the organic solvent tends to that in a hydro-organic medium (in the absence of surfactant).

Among the RPLC systems investigated in this work to analyse a mixture of β-blockers, using four organic solvents (acetonitrile, methanol, ethanol and 1-propanol), the best in terms of resolution and analysis time was HSC with acetonitrile. The anionic surfactant adsorbed on the stationary phase increases the retention and improves the peak properties. This extends the separation space, giving rise to high resolution in wide concentration ranges of both surfactant and organic solvent. Acetonitrile consumption is relatively high, but its evaporation is decreased in the presence of surfactant, which facilitates mobile phase recycling. On the other hand, the hydro-organic mode did not succeed with the separation of the eight β-blockers.

Ethanol, which has not attracted attention in the MLC literature, showed better performance than methanol and 1-propanol. Ethanol and acetonitrile have the same polarity, and give rise to similar elution strength, with some differences in the selectivity and wider peaks for ethanol (see Fig. 5).

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