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Peak half-width plots to study the effect of organic solvents on the peak performance of basic drugs in micellar liquid chromatography

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ABSTRACT

The addition of the anionic surfactant sodium dodecyl sulphate (SDS) to hydro-organic mixtures of methanol, ethanol, propanol or acetonitrile with water yielded enhanced peak shape (i.e. increased efficiencies and symmetrical peaks) for a group of basic drugs (β-blockers) chromatographed with a Kromasil C18 column. The effect can be explained by the thin layer of surfactant associated to the hydrocarbon chain on the stationary phase in the presence of the organic solvents, which covers the free silanols on the siliceous support avoiding their interaction with the cationic basic drugs. These instead interact with the anionic head of the surfactant increasing their retention and allowing a more facile mass transfer. The peak shape behaviour with the four organic solvents (methanol, ethanol, propanol and acetonitrile) was checked in the presence and absence of SDS. The changes in peak broadening rate and symmetry inside the chromatographic column were assessed through the construction of peak half-width plots (linear relationships between the left and right half-widths at 10% peak height versus the retention time). The examination of the behaviour for a wide range of compositions indicated that the effect of acetonitrile in the presence of SDS is different from ethanol and propanol, which behave similarly. Acetonitrile seems to be superior to the alcohols in terms of peak shape, which can be interpreted by the larger reduction in the adsorbed surfactant layer on the C18 column. However, the decreased efficiencies observed at increasing surfactant concentration in the mobile phase should be explained by the reduction in retention times, more than by a change in the stationary phase nature.

1. Introduction

The incorporation of a low concentration of an additive into a conventional mobile phase in reversed-phase liquid chromatography (RPLC) can alter the stationary phase surface and the partition characteristics of the analytes [1,2]. The magnitude of the effect can be modulated by varying both the type and concentration of the additive. One interesting example is the addition of a surfactant above the critical micellar concentration (CMC). Owing to the existence of micelles (aggregates of surfactant molecules with the non-polar hydrocarbon chain oriented towards their core, and the neutral or ionic head towards their surface), the technique has been called micellar liquid chromatography (MLC) [3–5]. However, the surfactant also adsorbs on the stationary phase surface, giving rise to an open micelle-like structure, which is the main responsible of the observed behaviour.

Particularly attractive is the use of ionic surfactants for the RPLC analysis of compounds bearing an opposite charge, due to the formation of ion-pairs between compounds and surfactant molecules. Such is the case of positively charged basic drugs chromatographed with a mobile phase containing the anionic surfactant sodium dodecyl sulphate (SDS). Nuclear magnetic resonance (NMR) studies have revealed that the long hydrophobic chain of SDS is inserted in the bonded organic layer with the sulphate group protruding outside [6]. This makes the stationary phase negatively charged. Attraction to the modified SDS stationary phase affects the distribution equilibria of the cationic drugs, increasing their retention. Therefore, solutions containing only surfactant may be too weak to provide convenient retention times. Hence, the need of an organic solvent in the mobile phase, whose concentration should be tuned together with that of the surfactant, in order to achieve adequate retention and selectivity.

Concomitantly with the retention behaviour, the mass transfer kinetics on the modified stationary phase is affected. The MLC literature contains numerous comments on the reduced efficiency for compounds of different nature eluted with mobile phases containing exclusively a surfactant (either ionic or non-ionic) [3,7–9]. This has been explained by the thick SDS layer on the station-

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ary phase, which gives rise to an increased carbon loading [9–12]. The surfactant coverage produces also a significant decrease in the pore volume, dramatically reducing the active surface area [11]. Alcohol addition and temperature raise have been given as solutions to decrease the amount of adsorbed surfactant, and consequently improve the observed efficiency in MLC [3,7,9]. However, the efficiency can remain below that achieved with conventional hydro-organic mobile phases, particularly for neutral and anionic analytes.

On the other hand, the analysis by conventional RPLC of many drugs of interest containing basic nitrogens is problematic due to the severely low efficiencies and tailed peaks [13]. Peak asymmetry (and low efficiencies) is caused mostly by ionic interaction of the positively charged drugs with the free silanols of the packing. This can be at least partially avoided by lowering the pH of the mobile phase to suppress silanol ionization. A variety of base-deactivated packings from several manufacturers are also becoming widespread. However, the use of conventional RPLC packings is still common, and the problematic interactions of basic compounds with the siliceous supports are usually solved by the addition of amine modifiers to the mobile phase. These additives associate with silanol sites, blocking ion-exchange processes [14].

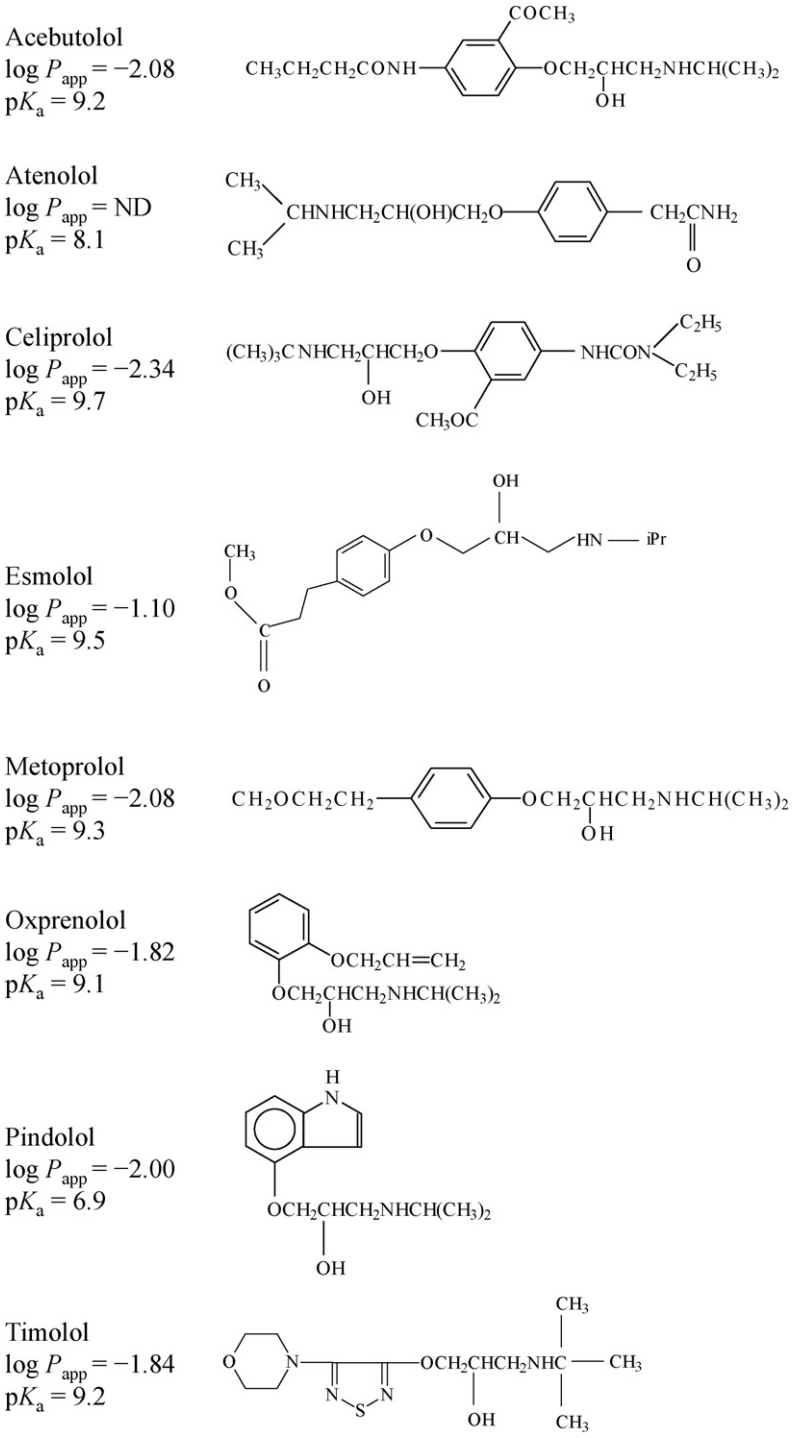

Fig. 1. Structure, logarithm of the apparent octanol–water partition coefficient at pH 3 ($\log P_{app}$), and acidity constant (pK_a) for the probe compounds [26].

In previous work, we reported increased efficiencies and symmetrical peaks for a group of basic drugs (β-blockers) separated with aqueous mobile phases containing SDS and acetonitrile [15,16]. Although the SDS layer on the stationary phase is thinner in the presence of acetonitrile, the silanols are apparently still covered by the surfactant monomers, which change the stationary phase behaviour. SDS-mediated RPLC with acetonitrile as co-modifier seems to be superior to conventional RPLC in the separation of β-blockers. The combination of improved peak shape (i.e. peak sharpness and less tailing), larger selectivity, and smaller range in retention among compounds of extreme polarity leads to the logical observation that more β-blockers can be resolved in one run using isocratic elution [17–19].

Following a previous study on the separation of β-blockers with SDS and acetonitrile [19], our purpose was to compare the peak shape behaviour obtained using acetonitrile with that for other organic solvents (methanol, ethanol and propanol) used as co-modifiers in mobile phases containing SDS. With this aim, we have considered the external peak broadening contribution to the global variance, which becomes less significant as the retention time increases. The different modifiers provide different elution strengths, and consequently, the apparent deterioration in peak shape for compounds eluting at shorter retention times should be taken into account. The usefulness of peak half-width plots versus retention times for this kind of study is demonstrated.

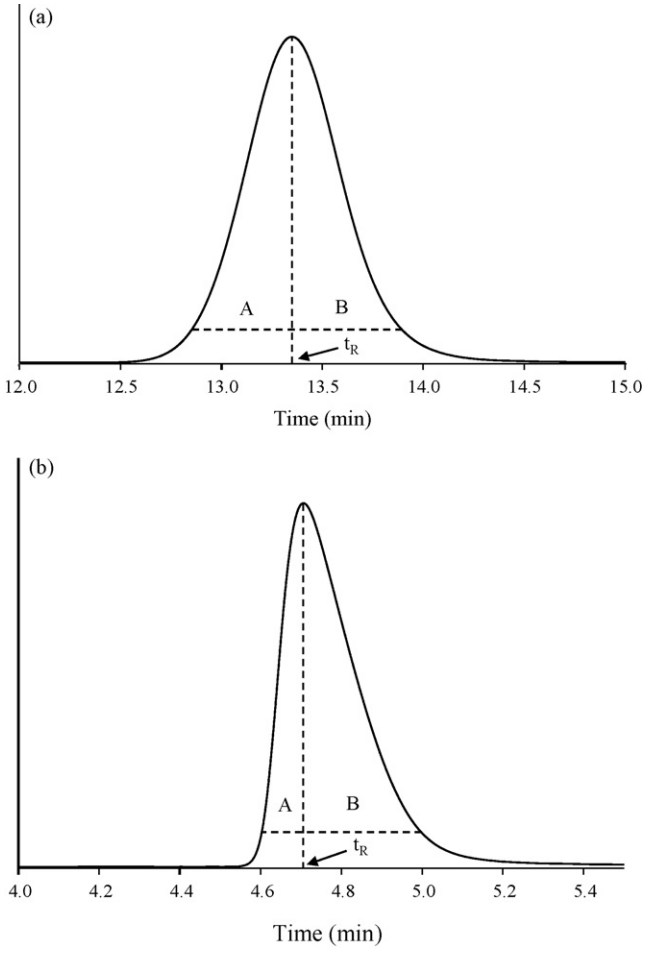

Fig. 2. Chromatographic peaks depicting the retention time, and left and right peak half-widths at 10% peak height for oxprenolol eluted with: (a) 0.15 M SDS/15% propanol, and (b) 15% propanol.

2. Experimental

2.1. Reagents

Eight β-blockers were used as probe compounds (Fig. 1): atenolol, pindolol, timolol (Sigma, St. Louis, MO, USA), esmolol (Du Pont-De Nemours, Le Grand Saconnex, Switzerland), acebutolol (Italfarmaco, Alcobendas, Madrid, Spain), celiprolol (Rhône-Poulenc Rorer, Alcorcón, Madrid), metoprolol and oxprenolol (Ciba-Geigy, Barcelona, Spain). The drugs were dissolved in a small amount of organic solvent and diluted with water. The concentration of the injected solutions was 20 μg/ml.

Hydro-organic mobile phases were prepared in the absence and presence of sodium dodecyl sulphate (99% purity, Merck, Darmstadt, Germany). The organic solvents were: methanol, ethanol, propanol and acetonitrile (Scharlau, Barcelona). 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. Drug solutions and mobile phases were filtered through 0.45 μm nylon membranes (Micron Separations, Westboro, MA, USA).

2.2. Apparatus

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. A Kromasil C18 column with the following characteristics: 125 mm × 4.6 mm i.d., 5 μm particle size, and 110 Å pore diameter (Análisis Vínicos, Ciudad Real, Spain) was used. A 12.5 mm C18 guard column was connected to the analytical column. The flow-rate was 1 ml/min and the temperature, 25 °C. Duplicate injections were made using an injection volume of 20 μl. In the presence of surfactant, the mean dead time measured as the first perturbation of the baseline ranged between 1.07 and 1.20 min, and without surfactant, between 1.25 and 1.40 min. The shorter dead times in the presence of surfactant can at least be partially explained by the decreased pore volume.

Data acquisition was carried out with an HPChemStation (Agilent, B.02.01), and mathematical treatment was performed in Excel (Microsoft Office 2003, Redmond, WA, USA). The MICHROM software [20] was used to measure the retention times, and peak half-widths.

3. Theory

The efficiency (number of chromatographic plates) is the most conventional parameter used to describe peak performance in chromatography. It is defined as follows [21,22]:

$$N = \left(\frac{t_R}{\sigma}\right)^2 \quad (1)$$

where t_R is the retention time and σ the standard deviation in time units for a chromatographic peak eluted in the isocratic mode. Since most peaks are non-Gaussian, the most appropriate way to evaluate the efficiency is the moment method [9,22], which gives a good approximation of the peak standard deviation:

$$N = \frac{M_1^2}{M_2} \quad (2)$$

where M_1 accounts for the retention time, and M_2 for the peak variance. Owing to the need of digital curve fitting to get the moments and inaccuracies related to the noise background at the peak front and tail, other approaches based on the exponentially modified Gaussian model and measurement of the widths above the baseline have been proposed, from which the most generally accepted

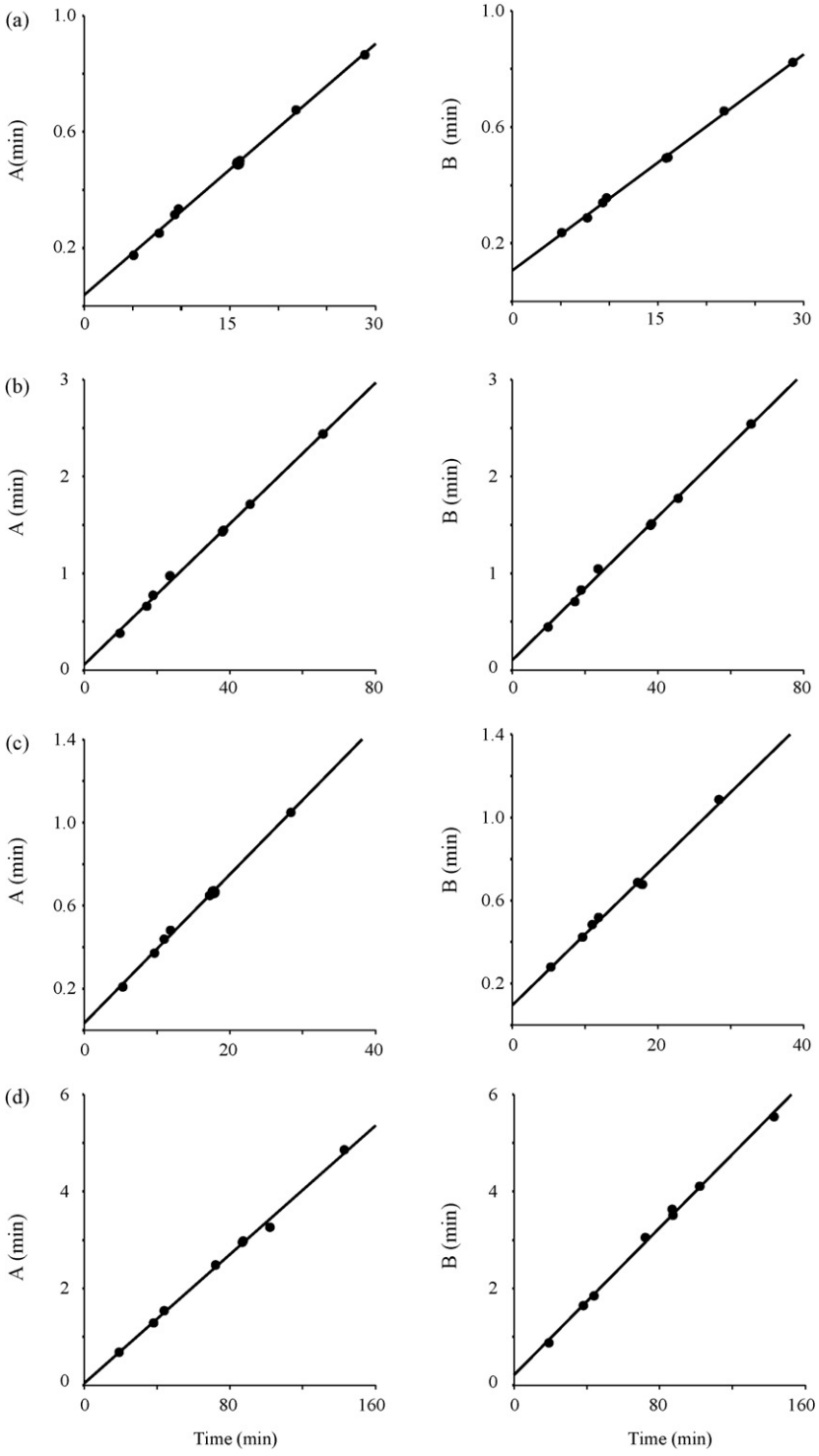

Fig. 3. Peak half-width plots for the set of eight β-blockers corresponding to mobile phases containing 0.075 M SDS and (a) 60% methanol, (b) 15% ethanol, (c) 15% propanol, and (d) 15% acetonitrile.

is the Foley and Dorsey approach [23]:

$$N = \frac{41.7\left(t_R/w_{0.1}\right)^2}{(B/A)_{0.1}^2 + 1.25} \quad (3)$$

where the width ($w_{0.1}$), and the left and right half-widths ($A_{0.1}$ and $B_{0.1}$, respectively) are measured at 10% peak height, being

$$w_{0.1} = A_{0.1} + B_{0.1} \quad (4)$$

To illustrate the meaning of the parameters in Eq. (3), Fig. 2 depicts two chromatographic peaks, where the retention times and half-widths at 10% peak height are shown. The peaks exhibit different asymmetry factors (B/A), and were selected from those obtained for this work.

Eq. (3) allows the calculation of the efficiency for single peaks, obtained at specific mobile phase compositions, provided the information about the retention time and peak half-widths is available. In some cases, however, it is convenient to have an estimation of

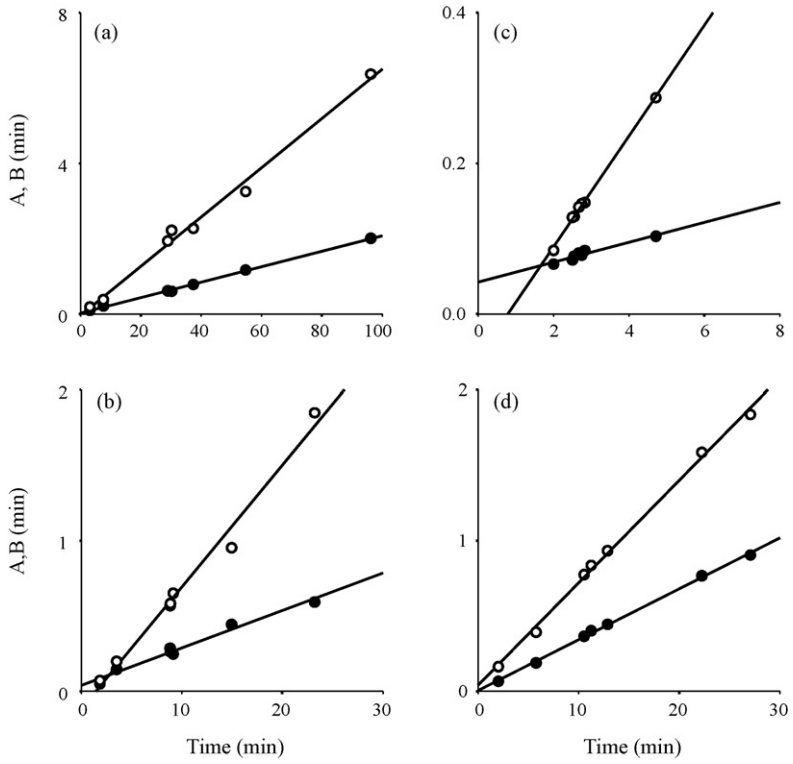

Fig. 4. Peak half-width plots for the set of eight β-blockers corresponding to hydro-organic mobile phases containing: (a) 20% methanol, (b) 15% ethanol, (c) 15% propanol, and (d) 15% acetonitrile. Half-widths: *A* (●) and *B* (○).

the efficiency at a particular retention time, from which no experimental data are available. This is the case when chromatographic columns or solvents should be compared, or peak resolution optimized. For this purpose, a model that relates the peak half-widths with the retention time should be useful.

In previous work [24,25], we observed an approximately linear correlation between the two peak half-widths and the retention time (indeed parabolic, but this is only evident for wide ranges of retention times):

$$A = m_A t_R + A_0 \quad (5)$$

$$B = m_B t_R + B_0 \quad (6)$$

where m_A and m_B are the slopes of the linear correlations, and A_0 and B_0 the corresponding intercepts. These equations allow the prediction of the peak half-widths and provide parameters useful to characterize the column performance: the sum of slopes ($m_A + m_B$) represents the peak broadening rate, and the ratio m_B/m_A the peak asymmetry, both yielded inside the column.

4. Results and discussion

4.1. Experimental designs

In surfactant-mediated mobile phases, the surfactant modifies the stationary phase by coating it totally or partially. The coverage depends on the surfactant/organic solvent ratio. Basic compounds have demonstrated to be useful to test this coverage, since the free silanols that interact with them are masked, and mass transfer with the surfactant seems to be more facile [16]. In order to test this behaviour, we used eight β-blockers of different polarity as probe compounds (Fig. 1).

The discussion in this work is based on the measurement of the peak half-widths at 10% peak height and retention times for the set of β-blockers eluted with a large number of mobile phases containing SDS and organic solvent, which were compared with mobile phases in the absence of the surfactant (hydro-organic mixtures). In both chromatographic modes, the behaviour of four organic solvents was examined: acetonitrile and the alcohols methanol, ethanol and propanol.

Table 1
Parameters of the fitted peak half-width plots (Eqs. (5) and (6)) and determination coefficient (r^2) corresponding to Figs. 3 and 4.

	A_0	m_A	r^2	B_0	m_B	r^2
Micellar mobile phases						
0.075 M SDS/60% methanol	0.076	0.029	0.9974	0.108	0.027	0.9990
0.075 M SDS/15% ethanol	0.059	0.036	0.9980	0.105	0.037	0.9976
0.075 M SDS/15% propanol	0.033	0.036	0.9977	0.096	0.034	0.9942
0.075 M SDS/15% acetonitrile	0.040	0.033	0.9967	0.211	0.038	0.9976
Hydro-organic mobile phases						
20% methanol	0.023	0.020	0.9986	–0.037	0.065	0.9919
15% ethanol	0.039	0.025	0.9777	–0.117	0.081	0.9846
15% propanol	0.041	0.013	0.9506	–0.059	0.074	0.9984
15% acetonitrile	0.006	0.034	0.9985	0.041	0.068	0.9970

For the surfactant-mediated mobile phases, the concentration of surfactant allowed the formation of micelles (micellar conditions), but in some cases a large amount of organic solvent was added, which prevented the aggregation of the surfactant monomers (submicellar conditions) [16]. In the case of methanol, the added percentage was necessarily high due to the extremely low elution strength of this solvent in the presence of surfactant. The number of mobile phases in the experimental design, and the concentration range for the surfactant and organic solvent were the following: methanol (5, 0.075–0.15 M/50–60%, v/v), ethanol (11, 0.075–0.15 M/5–40%), propanol (19, 0.020–0.15 M/5–35%), and acetonitrile (17, 0.075–0.15 M/5–50%). In the absence of surfactant, four mobile phases were enough to describe the behaviour. In this case, the composition ranges were: methanol (20–50%), ethanol (10–25%), propanol (5–15%), and acetonitrile (15–30%).

As commented, the stationary phase modified by the anionic SDS attracts the positively charged β-blockers, increasing their retention with respect to the unmodified stationary phase (i.e. in the absence of surfactant). For this reason, the surfactant-mediated modes allowed wider concentration ranges for the organic solvents, except for methanol. It should be also noted that the exploration of the chromatographic behaviour is more complex (i.e. a larger number of experiments is required) for the mobile phases containing two modifiers (surfactant and organic solvent), where the number of interactions is larger.

4.2. Peak half-width plots

In previous reports, we built a few peak half-width plots (linear plots representing the left and half-width values of chromatographic peaks versus the retention time) for a set of β-blockers eluted from a monolithic column at several mobile phase compositions and flow-rates [27], and from several chromatographic columns (microparticulate and monolithic) [28] at fixed flow-rate, using in both cases hydro-organic mobile phases of acetonitrile (without other additives). The usefulness of the peak half-width plots to assess peak performance was demonstrated. In this work, we apply these plots to compare the effect of several organic solvents, and the difference in peak performance between mobile phases prepared in the presence and absence of SDS. The study has involved the construction of a large number of peak half-width plots (one plot for each mobile phase composition, 52 and 16 in the presence and absence of surfactant, respectively), which allowed to assess their suitability.

Figs. 3 and 4 depict (as representative) the peak half-width plots for the set of eight β-blockers eluted with different mobile phase compositions in the presence of surfactant (0.075 M SDS/15% organic solvent, except for methanol which was 60%), and without surfactant (15% organic solvent, except for methanol which was 20%). All half-width plots drawn for other mobile phase compositions in this work showed similar trends (i.e. data points aligned depicting a straight-line). For clarity, in the presence of surfactant, the plots for the left and right half-widths (Fig. 3) were drawn separately due to the similar slopes (the peaks were nearly symmetrical, in contrast with the peaks in the hydro-organic mode for which the asymmetry was significant, see Fig. 2).

The model parameters and determination coefficient (r^2) corresponding to the plots in Figs. 3 and 4 (Eqs. (5) and (6)) are given in Table 1. As observed, the linear fittings for the mobile phases in the presence of surfactant are highly satisfactory ($r^2 > 0.99$). The points appear somewhat more disperse without surfactant ($r^2 > 0.96$). Also, in the hydro-organic mode, the outliers were more frequent. However, the fittings can be still considered as satisfactory. It should be noted that the peaks are broader and more asymmetrical in the hydro-organic mode. Therefore, the reason of the poorer quality of the fittings in this mode could be the larger difficulty in measuring the peak half-widths, but we cannot discard that in specific cases, particular interactions between a given solute and the stationary phase make the behaviour depart from the fitted trend.

Fig. 5 shows the accuracy in the prediction of peak half-widths using the linear plots for the left and right half-widths (Eqs. (5) and (6)), considering all peaks obtained with the whole set of mobile phases in the experimental designs, for ethanol (Fig. 5a and b) and acetonitrile (Fig. 5c and d), in the presence (Fig. 5a and c) and absence (Fig. 5b and d) of SDS. The agreement between the experimental and predicted values is highly satisfactory.

The information gathered for the set of β-blockers with all mobile phases for each particular organic solvent, in the presence and absence of surfactant, is depicted altogether in the plots shown in Figs. 6 and 7, respectively. Owing to the attraction of the basic drugs to the anionic surfactant-modified stationary phase, the maximal retention times in the presence of surfactant were larger with respect to the hydro-organic mode. For comparison purposes, the plots for the different organic solvents show the same ranges in half-widths and retention time, but it should be noted that the data at longer retention times exhibited the same trend.

In the presence of surfactant, the correlation for the plot obtained with all mobile phases containing methanol (Fig. 6a) was excellent ($r^2 > 0.99$), and similar to that achieved for the plots for each particular mobile phase, as that depicted in Fig. 3a. This reveals that the stationary phase is scarcely modified in the studied range (50–60% methanol). We would like to indicate that we were not able to increase the methanol content in the mobile phase above 60% due to the high back-pressure, and that the retention behaviour pointed out that in these conditions the stationary phase was still covered with surfactant. Without surfactant, all β-blockers in the set eluted close to the dead time with a hydro-organic mobile phase containing 50% methanol.

Ethanol and propanol (Fig. 6b and c) showed a larger scattering ($r^2 > 0.97$–0.98), whereas the plots for acetonitrile (Fig. 6d) were rather poor ($r^2 > 0.89$–0.91). The plots for acetonitrile after removing the mobile phases above 25% or 40% acetonitrile (submicellar mode) were similar to that shown in Fig. 6d. In order to support the following comments, the quality of the fittings in Fig. 6b–d should be compared with those in Fig. 3b–d. The observed behaviour (data scattering) should be related to the changes in the stationary phase nature with the mobile phase composition. The column is modified at different surfactant/organic solvent ratios, especially for acetonitrile. In contrast, the quality of the plots in Fig. 7 for the whole set of hydro-organic mobile phases is similar to that obtained for each particular mobile phase (see also Fig. 4). This indicates that, at least for the studied composition range, the changes in the stationary phase were less significant. We should remind that concomitantly with the modification of the stationary phase, the SDS micelle in the mobile phase experiences changes in the presence of organic solvent, and is destroyed above a given concentration of this modifier [29,30].

4.3. Effect of different organic modifiers on the peak half-widths

As commented, the most relevant difference between the chromatographic modes in the absence and presence of surfactant, related to peak performance, is the thickness of the surfactant layer associated to the alkyl-bonded chains, and the presence of free silanols on the siliceous support. In hydro-organic RPLC, the stationary phase is scarcely modified when the organic solvent content is changed, at least in narrow composition ranges [1,31]. Meanwhile, SDS molecules associate to the C18 chains and, above the CMC, the surfactant coverage reaches saturation as indicated by the adsorption isotherms (these show that above the CMC the

amount of adsorbed surfactant is constant or the adsorption rate is small) [32,33]. Upon addition of an organic solvent, the coverage is affected, since the solvent dissolves the adsorbed surfactant at least partially. Consequently, a change in the surfactant-to-organic solvent ratio modifies the stationary phase nature.

In this work, we show that the peak half-width plots of β-blockers are useful to study such changes. As explained above, the sum and ratio of the slopes for the half-width plots ($(m_A + m_B)$ and m_B/m_A) give information about the peak broadening rate and asymmetry inside the column, respectively. It should be also reminded that due to the extra-column effects, the observed efficiency decreases with the retention time.

The comparison of the peak half-width plot profiles at diverse mobile phase compositions is, however, problematic, owing to the different retention time windows. At increasing concentration of surfactant and organic solvent, the retention times are shortened.

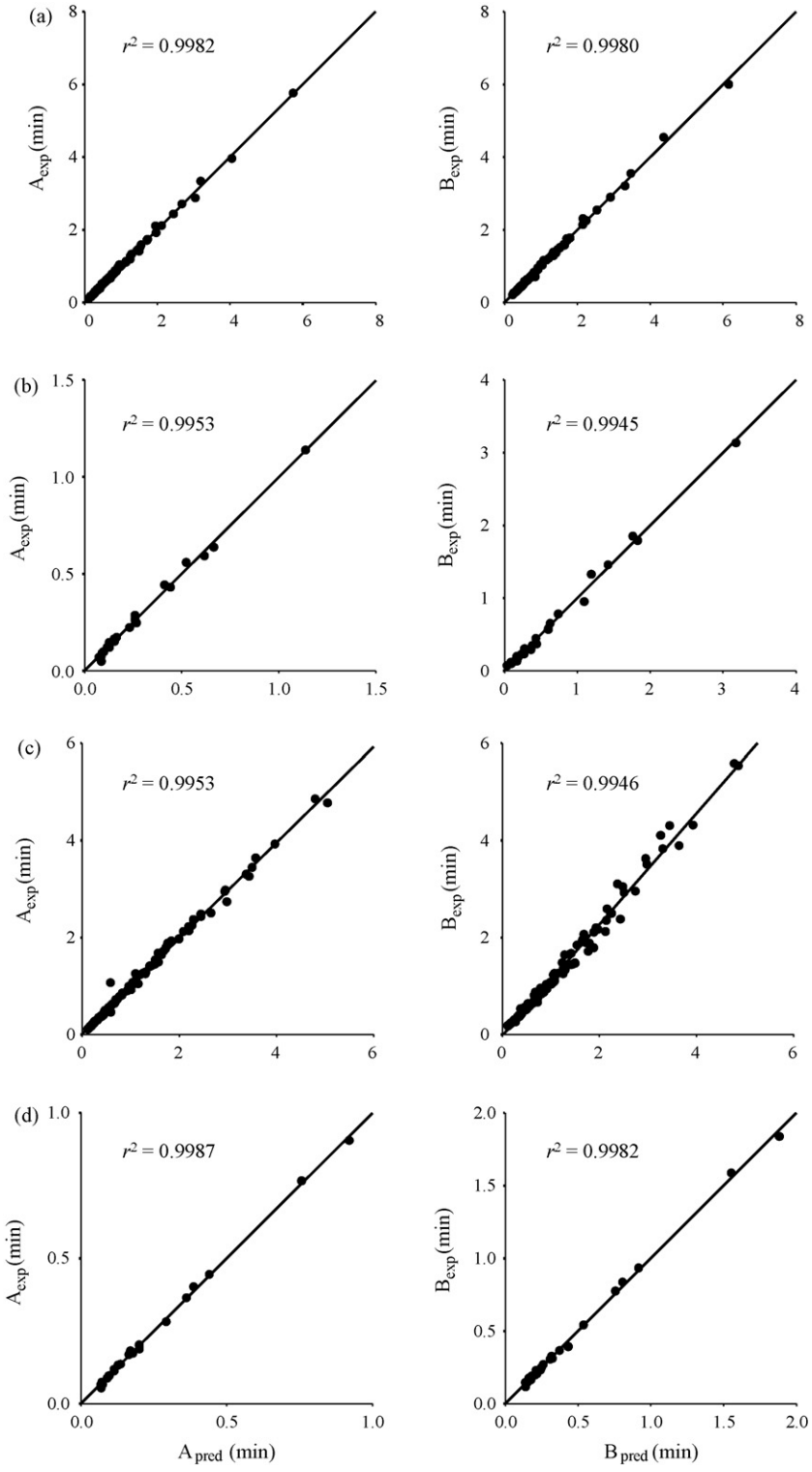

Fig. 5. Accuracy in the predictions of the left and right peak half-widths for the set of β–blockers according to Eqs. (5) and (6), for micellar (a and c), and hydro-organic (b and d) mobile phases, containing ethanol (a and b) and acetonitrile (c and d). The whole set of mobile phases in the experimental design was considered.

Also, the effect of each organic solvent on retention (the elution strength) is different, and is modified by the presence of surfactant (see the retention time axis in the plots in Figs. 3 and 4). For this reason, from the peak half-width plots fitted with the experimental data (A, B and t_R) we obtained theoretical plots, where the retention time axis was extended between 2 and 20 min for the micellar/submicellar (Fig. 8) and hydro-organic (Fig. 9) modes, using propanol and acetonitrile as organic solvents. The larger concentrations required for acetonitrile with respect to propanol are due to the smaller elution strength of the former.

From the plots, some observations arise:

(i) The peaks of the β-blockers in the presence of surfactant are nearly symmetrical: the lines for A and B are close to each other. Meanwhile, in the hydro-organic mode, peak deformation is significant (the lines diverge).

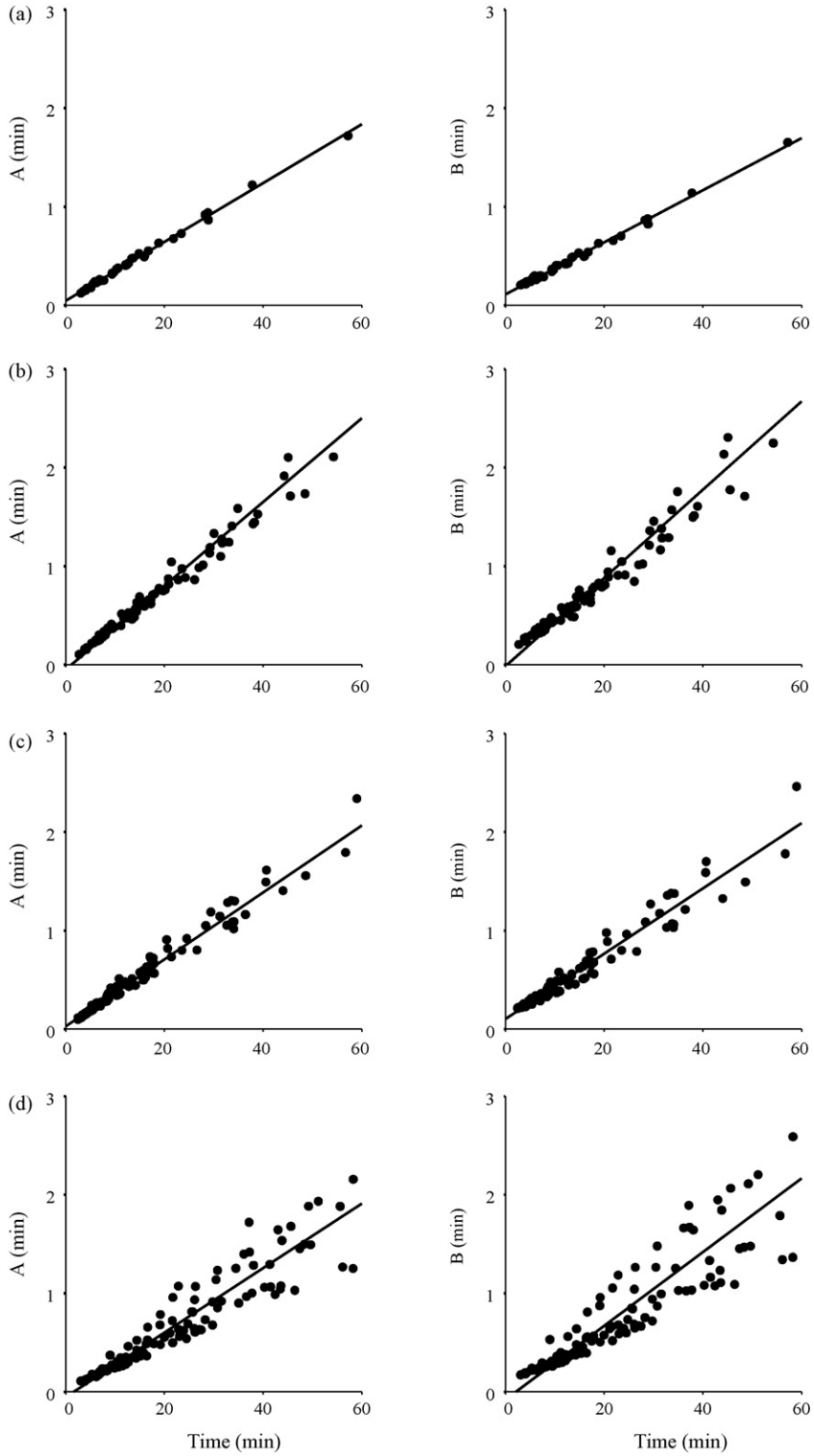

Fig. 6. Peak half-width plots for the set of β-blockers using all mobile phases in the experimental design containing SDS and: (a) methanol, (b) ethanol, (c) propranol, and (d) acetonitrile. Only the data for peaks eluting up to 60 min are shown.

Table 2
Mean efficiencies (Eq. (3)) and time ranges (min) for the β-blockers eluted with mobile phases containing SDS.

Compound	SDS–methanol[a]	SDS–ethanol[b]	SDS–propanol[b]	SDS–acetonitrile[b]
Acebutolol	2700 ± 900 (4.2–13.6)	2100 ± 300 (9.2–30.0)	2100 ± 300 (5.9–76.8)	3300 ± 1500 (16.5–51.2)
Atenolol	2000 ± 700 (3.0–6.6)	2100 ± 400 (5.6–16.8)	1600 ± 500 (3.2–36.4)	3100 ± 1400 (7.4–26.2)
Celiprolol	2700 ± 900 (4.2–14.8)	2100 ± 300 (12.7–45.1)	2000 ± 300 (6.1–96.8)	3300 ± 1500 (25.5–84.3)
Esmolol	3900 ± 1000 (6.8–28.8)	2500 ± 400 (19.5–73.5)	2600 ± 300 (8.4–143.9)	3500 ± 1700 (29.8–115.2)
Metoprolol	4000 ± 900 (7.1–28.2)	2700 ± 300 (20.2–70.0)	2700 ± 300 (8.7–147.6)	3700 ± 1900 (31.6–110.3)
Oxprenolol	4800 ± 800 (12.1–57.2)	2700 ± 400 (33.0–131.4)	2900 ± 200 (13.4–125.9)	3600 ± 1600 (48.4–193.9)
Pindolol	2800 ± 1000 (3.9–12.1)	2400 ± 300 (9.4–29.2)	2200 ± 400 (5.3–68.3)	3400 ± 1400 (14.3–45.7)
Timolol	4200 ± 800 (9.4–37.8)	2600 ± 400 (22.8–92.9)	2800 ± 400 (8.7–54.9)	3100 ± 1100 (34.5–143.8)

[a] All mobile phases in the experimental design were considered (50–60% methanol).
[b] The mean values correspond to the mobile phases in the experimental design containing 5–20% organic solvent (i.e. micellar mode).

(ii) Peak broadening with retention time is also significantly smaller (the slopes of the lines are smaller) in the presence of surfactant with respect to the hydro-organic mode.
(iii) In the hydro-organic mode, *B* is usually significantly larger than *A*, which indicates that the peaks are tailing. The asymmetry factor *B*/*A* progressively decreases with retention time, but at sufficiently long times, a constant value will be reached.
(iv) In the presence of surfactant, the lines are almost parallel, or diverge or converge only slightly. In the case of convergence (Fig. 8b–d, g and h), the plots predict the change from tailing to fronting peaks above a certain retention time (see Fig. 8h). However, the retention times of the probe compounds did not surpass the crossing point in such plots (the existence of such point could not be confirmed for propanol and acetonitrile).

The behaviours commented above have been also observed for other mobile phase compositions, and for methanol and ethanol. Although not shown, the plots obtained for methanol usually converged, and in this case, the experimental data confirmed the existence of a crossing point, above which the peaks were fronting.

Fig. 10 depicts the change in the peak broadening rate and asymmetry with the organic solvent content for mobile phases containing 0.075 M (left) or 0.15 M (right) SDS, and ethanol (top), propanol (middle) and acetonitrile (bottom). Larger concentrations of the organic solvents could not be assayed due to the short retention (the compounds eluted close to the dead time). As observed, the behaviours are similar at both surfactant concentrations for the four organic solvents.

The slope ratios (m_B/m_A) were mostly close to one for the hybrid micellar mobile phases containing SDS and organic solvent. However, they tended to give values <1, which means that the peaks of highly retained compounds will be fronting. The experimental peaks obtained for this work were, however, nearly symmetrical or slightly tailing (except for some peaks eluted with SDS/methanol mobile phases, which were fronting). This can be interpreted as the compensation between the behaviour inside the column (slightly fronting) and the tailing effect of the extra-column components.

In the absence of surfactant, the asymmetry ratio m_B/m_A was rather high for the four organic solvents (usually in the range 2.5–5). Methanol provided the smallest change in peak asymmetry with organic solvent addition, whereas the largest change corresponded

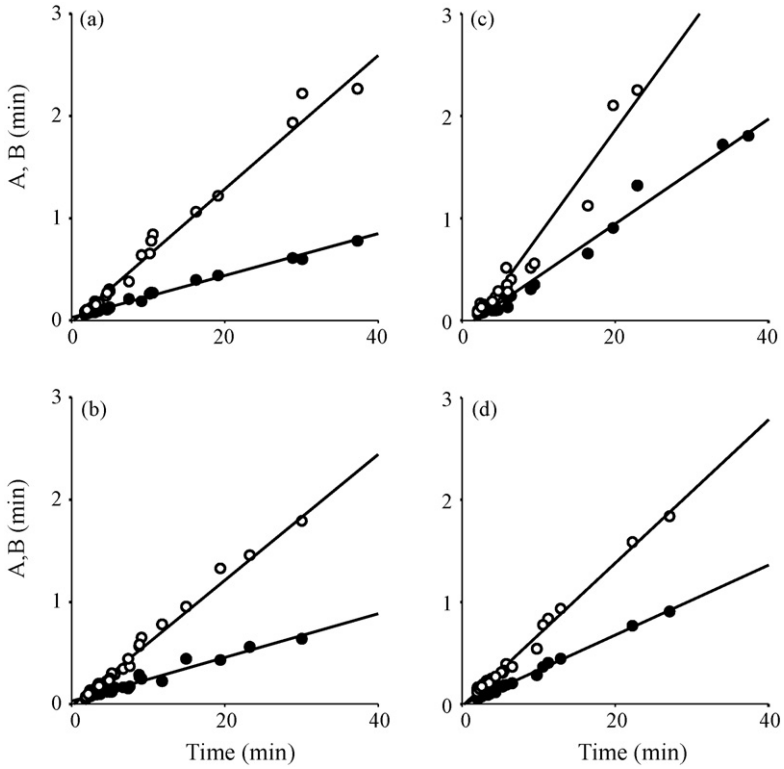

Fig. 7. Peak half-width plots for the set of β-blockers using all hydro-organic mobile phases in the experimental design for: (a) methanol, (b) ethanol, (c) propranol, and (d) acetonitrile. Half-widths: *A* (●) and *B* (○). Only the data for peaks eluting up to 40 min are shown.

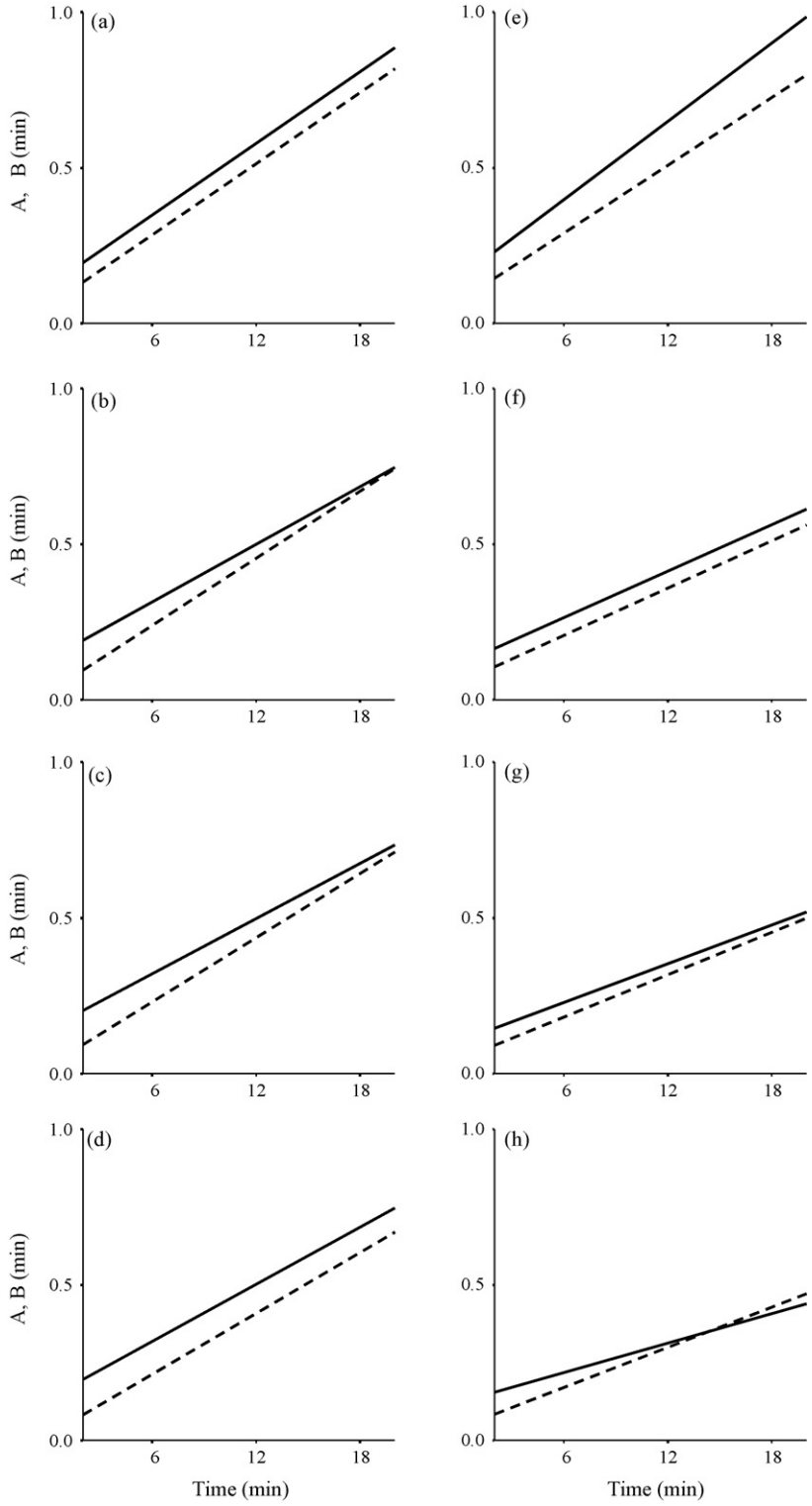

Fig. 8. Change in the peak half-width plots for the set of β-blockers using 0.15 M SDS mobile phases containing propanol: (a) 5%, (b) 15%, (c) 25%, and (d) 35%, and acetonitrile: (e) 15%, (f) 30%, (g) 40%, and (h) 50%. Half-widths: *A* (- -) and *B* (—). The fitted lines are depicted.

to propanol. For ethanol and propanol, the asymmetry increased with the organic solvent content, while it decreased for acetonitrile.

Upon addition of the organic solvent to a pure micellar mobile phase, peak broadening is decreased. Although we could not measure the peak half-widths for the β-blockers in the absence of organic solvent (pure micellar mobile phases) due to the excessive retention, peak broadening ($m_A + m_B$) in these conditions should be significantly above 10%. Apparently, the initial decrease in peak broadening by addition of propanol is somewhat larger than for ethanol (observe the first point at 5% organic solvent in the plots of Fig. 10, top and middle). However, peak broadening tended to stabilize at high solvent content (30–45%) for ethanol and especially for propanol. The behaviour for acetonitrile is different: although initially the reduction in peak broadening is similar to that of propanol,

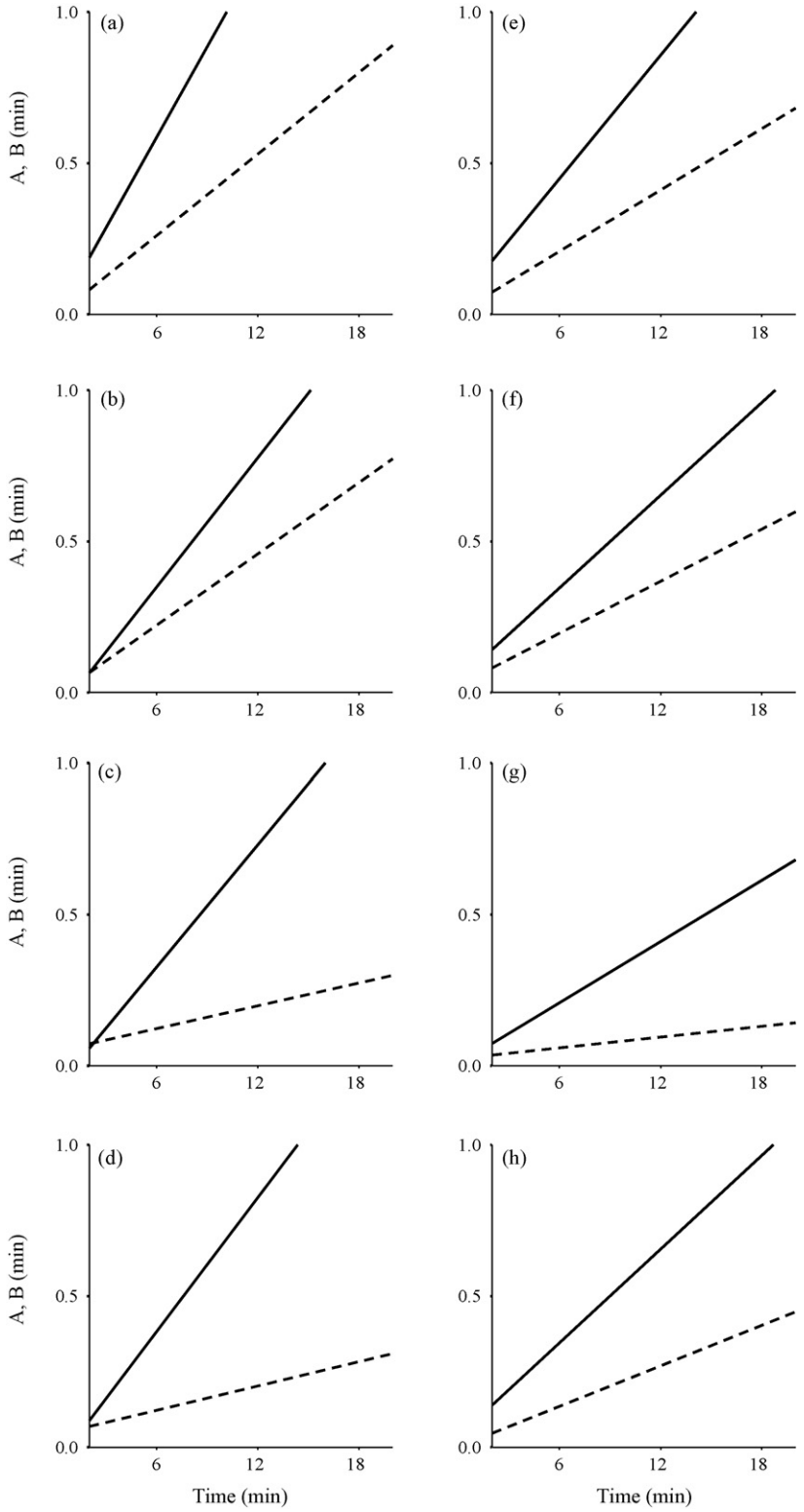

Fig. 9. Change in the peak half-width plots for the set of β-blockers using hydro-organic mobile phases containing propanol: (a) 5%, (b) 7.5%, (c) 10%, and (d) 15%, and acetonitrile: (e) 15%, (f) 20%, (g) 25%, and (h) 30%. Half-widths: *A* (- -) and *B* (—). The fitted lines are depicted.

smaller ($m_A + m_B$) values are achieved at the highest organic solvent concentrations (4% against 6% for ethanol and propanol). The peaks become thus narrower with acetonitrile.

Only two mobile phases were assayed for methanol at each surfactant concentration (0.075 and 0.15 M SDS/50 and 60% methanol, not shown). Peak broadening was similar in all cases, with ($m_A + m_B$) ≈ 5.5%, which is similar to the values reached for 40–45% ethanol and propanol.

The mean efficiencies for the different β-blockers studied in this work, eluted with micellar and hydro-organic mobile phases,

Table 3
Mean efficiencies (Eq. (3)) and time ranges (min) for the β-blockers eluted with hydro-organic mobile phases[a].

Compound	Methanol	Ethanol	Propanol	Acetonitrile
Acebutolol	1800 ± 400 (2.8–37.3)	1900 ± 500 (3.2–23.3)	1700 ± 900 (2.7–19.7)	1200 ± 200 (2.4–11.2)
Atenolol	2000 ± 400 (1.7–3.0)	2800 ± 900 (1.6–2.4)	1400 ± 500 (1.6–2.4)	700 ± 300 (1.6–2.0)
Celiprolol	1700 ± 500 (3.5–96.2)	1800 ± 600 (4.1–52.6)	1700 ± 800 (3.0–37.4)	1600 ± 400 (3.3–27.1)
Esmolol	1900 ± 500 (3.5–54.6)	2000 ± 600 (4.9–30.0)	1800 ± 900 (3.5–34.0)	1500 ± 300 (3.5–22.2)
Metoprolol	1300 ± 500 (3.0–30.1)	1400 ± 600 (3.7–20.8)	1400 ± 800 (3.0–25.7)	1400 ± 300 (2.8–12.9)
Oxprenolol	1000 ± 500 (4.7–69.8)	1200 ± 300 (7.4–23.2)	1200 ± 400 (5.4–49.9)	1400 ± 300 (4.4–29.6)
Pindolol	2240 ± 80 (2.0–7.5)	2300 ± 500 (2.2–5.6)	2700 ± 700 (2.0–5.7)	1300 ± 200 (2.3–5.8)
Timolol	1600 ± 600 (3.2–28.9)	1800 ± 600 (3.6–19.4)	1700 ± 900 (2.7–22.9)	1300 ± 300 (2.5–10.5)

[a] All mobile phases in the experimental designs were considered.

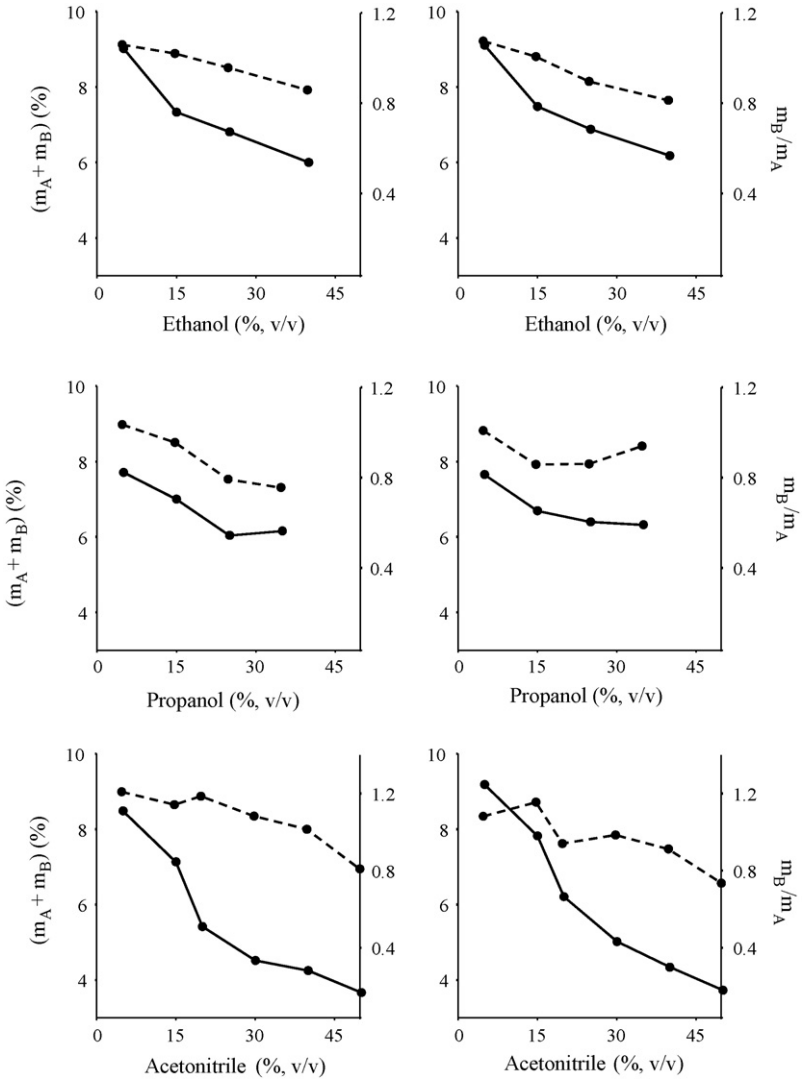

Fig. 10. Effect of the organic solvent content on the peak half-widths for the set of β-blockers in the presence of surfactant. SDS concentration: 0.075 M (left) and 0.15 M (right). The peak broadening rate ($m_A + m_B$, — and left axis) and the asymmetry factor (m_B/m_A, - - - and right axis) for highly retained compounds are depicted.

are given in Tables 2 and 3, respectively. The wide retention time ranges, which are different for each organic solvent make the comparison among them difficult. However, it is evident that the efficiencies in the presence of SDS are appreciably larger, corresponding the largest ones to acetonitrile. The range of efficiencies for this solvent is also wider. We would like to indicate that the efficiencies achieved for concentrations of acetonitrile above 20% (submicellar mode) can amount $N > 8000$ [16].

5. Conclusions

The peak shape and retention time data obtained for the β-blockers in a large number of mobile phases in the SDS micellar, submicellar and hydro-organic modes for four organic solvents, indicated that a linear behaviour can be assumed for the peak half-width versus retention time plots. The linear relationships between the left and right half-widths and the retention times provide useful

information about the peak broadening rate and asymmetry change with retention time inside a chromatographic column, which in the presence of surfactant is modified by the addition of organic solvent.

The peak shape parameters for the β-blockers depend on the presence of unprotected free silanols that can interact with the positively charged basic drugs, and on the thickness of the surfactant layer in the micellar and submicellar modes. The peak half-width plots can be considered as a simple tool to reveal the kinetics of the interactions of the basic drugs with the stationary phase. In the absence of surfactant, these compounds interact with the free silanols and give rise to broad and asymmetrical peaks. With the anionic surfactant covering the stationary phase, the peaks become nearly symmetrical and significantly narrower, which indicates that the interaction with the free silanols is negligible. The better peak performance at increasing organic solvent in the presence of surfactant should be explained by the thinner surfactant coverage.

The peak half-width plots allow an appropriate comparison of the behaviour of the different organic solvents. In the literature, this comparison is made based on the mean values of the efficiencies (or widths) and asymmetries for one or more compounds at different mobile phase compositions (as the values in Tables 2 and 3, which correspond to the experimental data obtained for this work). One main problem associated to the use of mean values is that different organic solvents give rise to different elution strengths, and consequently, the retention time ranges change for the same set of compounds. It should be pointed out that only the efficiencies for peaks eluting at similar retention time should be compared owing to the extra-column broadening. To our knowledge, this kind of comparison is rarely done.

The results shown in this work indicate that the effect of acetonitrile in the presence of SDS is different from ethanol and propanol, which behave similarly. Acetonitrile can give rise to significantly larger efficiencies, which can be interpreted by a larger reduction of the adsorbed surfactant layer on the stationary phase. It should be noted that the peak broadening rate in hydro-organic mobile phases containing acetonitrile is also smaller (7–8% against 8–15% for propanol), but still significantly larger than in the presence of surfactant.

Although a frequent comment in the MLC literature indicates that the efficiencies deteriorate at increasing surfactant concentration, we have not observed this trend, at least at the SDS concentrations used in this work. Seemingly, the observed decreased efficiencies in MLC could be explained by the reduction in retention times at increasing surfactant in the mobile phase, more than by a change in the stationary phase nature (i.e. thickness of the surfactant layer).

In this work, we have compared the chromatographic behaviour related to the peak shape. The elution strength and selectivity should be, however, also considered in order to have a complete description of the separation capability of a column/mobile phase system.

Acknowledgments

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