Green Chemistry

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/greenchem

Reversed-phase Liquid Chromatography with Mixed Micellar Mobile Phases of Brij-35 and Sodium Dodecyl Sulphate: A Method for the Analysis of Basic Compounds

M.J. Ruiz-Angel, E. Peris-García and M.C. García-Alvarez-Coque*

Departament de Química Analítica, Universitat de València, c/Dr. Moliner 50, Burjassot (Spain)

E-mail: celia.garcia@uv.es; Tel. +34 96354 4005

Micellar liquid chromatography (MLC) is a reversed-phase liquid chromatographic (RPLC) mode, which uses a surfactant as modifier, with significant changes in retention and selectivity with regard to the classical RPLC mode that employs mixtures of water and organic solvent. The anionic sodium dodecyl sulphate (SDS) is the most usual surfactant in MLC, but it also requires the addition of an organic solvent to decrease the retention times and increase the efficiency. Particularly, positively charged basic compounds are strongly retained by the stationary phase modified by adsorption of SDS monomers and require the addition of a strong solvent, such as propanol or pentanol. The non-ionic surfactant Brij-35 is much less common in MLC, but has the interesting feature of reducing the stationary phase polarity which remains neutral. This decreases the retention significantly and can eliminate the need of organic solvent, giving rise to successful “green” RPLC procedures. However, the retention of polar compounds may be too short if these do not exhibit specific interactions with the non-ionic surfactant. In this work, MLC with Brij-35 and mixtures of Brij-35 and SDS without organic solvent is investigated for the analysis of basic compounds. The research has been carried out with tricyclic antidepressants (TCAs) and β-blockers, which are compounds of pharmaceutical interest with different polarity. The chromatographic performance in the mixed micellar system is examined in terms of retention behaviour and peak profiles, and compared with the performance achieved with MLC systems containing a single surfactant. In the mixed micellar system, the analysis of β-blockers of diverse polarity is carried out with good resolution and adequate analysis time. For TCAs, mobile phases with only Brij-35 are preferable.

Keywords: Micellar liquid chromatography; Brij-35; Sodium dodecyl sulphate; Mixed micellar system; Basic compounds

Introduction

The idea of adding a surfactant to the mobile phase in reversed-phase liquid chromatography (RPLC) is a practice that has been explored over the three last decades, with significantly different results in the analysis of compounds of diverse nature with respect to those obtained in classical RPLC that employs mixtures of water and organic solvent.[1–3] Surfactant monomers are adsorbed on the alkyl-bonded chains of the stationary phase (usually C8 or C18) through hydrophobic interactions, modifying its nature. This creates a neutral or charged double layer (depending on the nature of the adsorbed surfactant), which interacts with solutes. For stationary phases modified with a charged surfactant, a dynamic ion-exchanger is yielded. Moreover, above the critical micelle concentration, surfactant monomers in the mobile phase aggregate to form small clusters or micelles that also interact with solutes. The formation of micelles has given rise to the most accepted name for this chromatographic mode: micellar liquid chromatography (MLC). However, the main changes in the observed chromatographic performance are due to the adsorption of surfactant monomers on the stationary phase. An attractive feature of MLC is the significant reduction in the amount of organic solvent with respect to the classical RPLC. Another fascinating feature is the capability of micelles of some surfactants to solubilize proteins that has been effectively exploited for the direct injection of untreated biological fluids onto RPLC columns, avoiding previous extraction steps with organic solvents.[4,5] For this reason, MLC is considered a "green" RPLC mode.[6]

Although several surfactants of diverse nature can be used in MLC, the anionic sodium dodecyl sulphate (SDS) has been selected in most reports.[1,2] The frequent use of SDS has somehow relegated the research on the potential of other surfactants as modifiers, such as the non-ionic surfactants. One of such surfactants is polyoxyethylene(23)lauryl ether ($(C_2H_4O)_{23}C_{12}H_{25}OH$), commercially known as Brij-35, which has been explored by a few

authors as an alternative to SDS with satisfactory results.[7–17] Brij-35 has been also reported as an ideal modifier in quantitative structure-activity relationship studies (QSARs) in RPLC, due to its capability to mimic biopartitioning processes.[18,19]

When RPLC columns are used with mixtures of water and organic solvent, solute retention is mainly based on the hydrophobic interactions with the alkyl-bonded layer of the stationary phase, together with the solving power of the organic solvent in the mobile phase. When cationic compounds are analysed, additional ion-exchange interaction with residual anionic silanols on the silica packing are established. These interactions are also characterised by slow kinetics, which results in broad and skewed peaks.[20,21] Mobile phases containing SDS have demonstrated to minimise the interaction of cationic solutes with the residual silanols: the long hydrophobic chain of SDS monomers covers the stationary phase with the sulphate group oriented outside, resulting in a negatively charged stationary phase.[22] This enhances remarkably the efficiency and peak symmetry of basic compounds, such as tricyclic antidepressants (TCAs) and β-blockers.

However, due to the attraction of the cationic basic compounds to the anionic SDS modified stationary phase their retention increases significantly. This forces the addition of a relatively high amount of acetonitrile or propanol to elute most β-blockers,[23,24] and pentanol is required to elute TCAs.[25] If Brij-35 is used instead of SDS, its monomers are adsorbed on the stationary phase with the hydrophilic polar end of the molecule oriented away from the surface. This increases the polarity of the stationary phase without providing a net charge, which allows compounds of low or intermediate polarity be eluted without the addition of organic solvent.[26,27] However, polar compounds as most β-blockers, which do not establish specific interactions with Brij-35, are not retained.

In this work, it is shown that a solution for the described limitations of mobile phases containing a single surfactant (Brij-35 or SDS), in the RPLC analysis of β-blockers, is the use

of mobile phases that include both surfactants, so that the favourable characteristics of each surfactant are combined. These mixed systems have been investigated along the last decades outside the field of chromatography.[28] Thus, it is known that when an anionic surfactant (such as SDS) and a non-ionic surfactant (such as Brij-35) are mixed in aqueous solution, their tails establish hydrophobic interactions, and their head groups ion-dipole and hydrophilic interactions, giving rise to the formation of mixed micelles. Systems containing mixed surfactants have been scarcely used in MLC,[29–32] being the combination of Brij-35 and SDS the most common. The mixed systems may result in improvements in the chromatographic performance with respect to the use of mobile phases containing a single surfactant.

The capability of mobile phases containing exclusively Brij-35 or the combination of Brij-35 and SDS to elute basic compounds, specifically TCAs and β-blockers, is here studied. The results are analysed in terms of retention, peak profiles, selectivity and resolution. Since there is no organic solvent in the mobile phase, the greenness of the method is increased with respect to classical RPLC or MLC with hybrid mobile phases of SDS and organic solvent. Another important advantage is the biodegradable character of the reagents used in the mobile phase: SDS is a fatty alcohol sulphate that is aerobically degraded,[33] and Brij-35 is a derivative of fatty alcohol ethoxylate, developed as an eco-friendly alternative to alkyl phenol ethoxylates.[34] It is shown how their combined use gives rise to a successful “green” RPLC separation of β-blockers.

2. Experimental

2.1. Reagents

The probe compounds were seven TCAs (doxepin, amitriptyline, clomipramine, imipramine, maprotiline, nortriptyline, and trimipramine) and six β-blockers (alprenolol, atenolol, celiprolol, metoprolol, oxprenolol, and propranolol), all from Sigma (St. Louis, MO, USA). All these compounds are basic (pK_a = 9–10), which means that at the working pH of the mobile phase (~3) they are positively charged. Most experiments were carried out with the seven TCAs and the two most hydropobic β-blockers (propranolol and alprenolol), all of them sufficiently retained with Brij-35. As will be commented below, atenolol, celiprolol, metoprolol and oxprenolol eluted close to the dead time with Brij-35.

Stock solutions of 100 μg/mL of the drugs were prepared in a small amount of ethanol with the aid of an Elmas 15h ultrasonic bath from Elmasonic (Singen, Germany), and diluted with water. These solutions were stable during at least two months at 4^{o}C and were diluted before injection with an aqueous solution of 0.02 M Brij-35 (Fluka, Buchs, Switzerland) up to a final concentration of 20 μg/mL. Uracil (Acros Organics, Geel, Belgium) was used as dead time marker.

Mobile phases containing Brij-35 or a mixture of Brij-35 and SDS (99% purity, Merck, Darmstad, Germany) were prepared at different concentrations, buffered at pH ~3 with 0.01 M sodium dihydrogen phosphate (Panreac, Barcelona, Spain) and HCl, to reduce the amount of free silanols in the column. The solutions of the probe compounds and mobile phases were filtered through 0.45 μm Nylon membranes (Micron Separations, Westboro, MA, USA). Nanopure water (Barnstead, Sybron, Boston, MA, USA) was used throughout.

2.2. Chromatographic system and column

An Agilent chromatograph (Waldbronn, Germany), equipped with a quaternary pump (Series 1260), an autosampler (Series 1200), a thermostated column compartment (Series 1100) set at 25ºC, a diode array detector, and an HPChemStation (Agilent, B.02.01) for data acquisition, was used. TCAs and β-blockers were monitored at 254 and 225 nm, respectively.

The chromatographic column was a Zorbax Eclipse C18 (Agilent) with the following characteristics: 150 mm × 4.6 mm i.d., 5 μm particle size, 10% carbon load, 180 m^2/g surface area, and 80 Å pore size, which was connected to a similar 30 mm pre-column for protection. The flow-rate was 1 mL/min. Duplicate injections were made using an injection volume of 20 μL. The mobile phases were recycled between runs and also during the analysis (as long as a small number of injections was made) to reduce the consumption of reagents. This increases the sustainability of the procedure. The chromatographic system was periodically rinsed with water and methanol (around 20 mL) to remove the surfactant from the stationary phase.

2.3. Experimental design

Based on previous experience,[24–26] two mobile phases containing either 0.02 M Brij-35 or 0.15 M SDS were selected as references. SDS was added to the 0.02 M Brij-35 solution at the following concentrations: 0.02, 0.04, 0.08, 0.12, and 0.15 M. Similarly, Brij-35 was added to the 0.15 M SDS solution at the concentrations: 0.01, 0.02, 0.03, 0.04, and 0.05 M (the latter concentration being close to the solubility of Brij-35 in water). The minimal and maximal concentrations of the surfactants in the mobile phase were selected to achieve enough retention for the most polar compounds, and not excessive retention for the most apolar.

3. Results and discussion

3.1. Retention capability of the mixed Brij-35/SDS micellar systems

The modified stationary phase coated by polyoxyethylene chains of Brij-35 is significantly more polar than the original C18 bonded phase. This reduces the retention times of the analysed compounds, if no specific interactions with the adsorbed surfactant are established, such as hydrogen-bonding between the hydroxyl groups in the surfactant and phenolic compounds.[27] The micellised surfactant in the mobile phase also changes the elution strength and selectivity (relative retention). Micelles formed by Brij-35 contain a dodecyl apolar core (similarly to SDS) and a relatively polar surface formed by oxyethylene chains, which interact with the solutes in the mobile phase.

Surfactant monomers of SDS and Brij-35 compete for adsorption sites on the stationary phase. The long hydrophobic chain of SDS monomers is inserted into the alkyl-bonded layer (similarly to Brij-35), with the sulphate group oriented outside (Fig. 1). Therefore, in the mixed system, the modified stationary phase will have a negative charge, although with smaller density than in a system exclusively modified with SDS. Different studies have also demonstrated that Brij-35 and SDS form mixed micelles in the mobile phase, with a common core involving their hydrophobic chains.[35] Therefore, mixed micellar systems should provide different chromatographic behaviour with respect to the single systems.

Fig. 2a shows the changes in retention for the whole set of TCAs and the two most apolar β-blockers eluted with a mobile phase containing 0.02 M Brij-35 and increasing concentrations of SDS in the 0.02–0.15 M range. As observed, the trends are similar for TCAs and β-blockers. It can be observed that the retention factors increased dramatically with the first addition of SDS. This is mainly due to the strong electrostatic attraction of the basic compounds (positively charged) to the anionic SDS monomers adsorbed on the stationary

phase. Further addition of SDS reduces the retention, due to the increase in micelle concentration which attracts the cationic solutes towards the mobile phase.

Fig. 2b depicts the changes in retention by adding increasing concentration of Brij-35 into a 0.15 M SDS mobile phase. The retention of TCAs and β-blockers in the absence of Brij-35 was excessively large (often above 80 min) and could not be measured. However, the addition of a small amount of Brij-35 (0.01 M) decreased the retention factors to practical analysis times. Successive additions of the non-ionic surfactant gradually reduced the retention, although in a smaller extent than the addition of SDS to a mobile phase containing a fixed amount of Brij-35.

When TCAs and β-blockers are eluted with SDS mobile phases, the addition of a relatively high amount of organic solvent (such as acetonitrile, propanol, butanol or pentanol), or the use of a column with a shorter alkyl-bonded chain (e.g., a C8 column) is required to decrease the retention times to practical values (Fig. 3a, and Fig. 4b and c).[23–26] Thus, it was checked that using a C18 column, the retention times of propranolol and alprenolol (not shown) were above 120 and 30 min with SDS mobile phases in the presence of 10 and 45% acetonitrile, respectively.

The retention times were smaller with mobile phases containing exclusively Brij-35. The apolar TCAs (with octanol-water partition coefficients, log $P_{o/w}$, ranging between 3.9 and 5.3)[36] eluted at practical retention times in these conditions (Fig. 3b). However, the retention of most β-blockers (with log $P_{o/w}$ between 0.25 and 3.4)[36] was excessively low. Thus, for example, the retention times for oxprenolol and propranolol (log $P_{o/w}$ = 2.4 and 3.4, respectively) with a mobile phase containing a small concentration of Brij-35 (0.01 M) were 2.7 and 11.8 min, respectively, and other more polar β-blockers eluted close to the dead time. Also, the retention of the most retained β-blockers decreased significantly with 0.02 M Brij-35.

The retention capability of the C18 stationary phase simultaneously modified with both Brij-35 and SDS, towards basic compounds (such as TCAs and β-blockers), is larger compared to a stationary phase exclusively modified with Brij-35, and significantly smaller with regard to a stationary phase exclusively modified with SDS. The increased retention with the mixed Brij-35/SDS system is not advantageous for TCAs (compare Figs. 3b and c), but for β-blockers, it allows modulating the retention to practical values (Fig. 4d), without the requirement of adding an organic solvent.

3.2. Solute-stationary phase and solute-mobile phase interactions

In the early development of MLC, a three-phase model (stationary phase, water and micelle) was proposed to understand the mechanism of retention. This model gave rise to equations that describe the changes in solute retention at increasing concentration of the modifiers (surfactant and organic solvent).[37,38] The approach is valid for both ionic and non-ionic surfactants and considers two association equilibria between solute and stationary phase, and solute and micelle. The equation proposed by Arunyanart and Cline-Love is particularly useful. The following chemical equilibria are considered:[38]

$$A + S \leftrightarrows AS \quad (1)$$

$$A + M \leftrightarrows AM \quad (2)$$

which describe the association of a solute (A) in bulk water with the stationary phase binding sites (S), and with the surfactant monomers in the micelles dissolved in the mobile phase (M). The equilibria in Eqs. (1) and (2) are described by the association constants K_{WS} and K_{AM}, respectively. The retention factor, k, can be expressed by:

$$k = \phi \frac{[AS]}{[A]+[AM]} = \frac{\phi K_{WS}[S]}{1+K_{AM}[M]} = \frac{K_{AS}}{1+K_{AM}[M]} \quad (3)$$

where ϕ is the phase ratio (ratio between the stationary phase and mobile phase volumes), [*AS*] and [*AM*] are the solute concentrations associated to the stationary phase and mobile phase, respectively, [*S*] is the concentration of active sites on the stationary phase, and [*M*] the molar concentration of surfactant monomers in the mobile phase. Since [*S*] is constant (or practically constant), and assuming the column is saturated with surfactant, the product $\phi K_{WS}[S]$ is also constant (K_{AS}). Eq. (3) can be rewritten as:

$$\frac{1}{k} = \frac{1}{K_{AS}} + \frac{K_{AM}}{K_{AS}}[M] \quad (4)$$

which describes a $1/k$ versus surfactant concentration linear plot. The extrapolation of the linear segments give a measurement of the strength of the interaction between the solute and stationary phase (K_{AS}), expressed as the inverse of the intercept. The slope combined with the value of K_{AS} indicates the interaction between the solute and mobile phase (K_{AM}).

To our knowledge, Eq. (4) has not been applied to measure the strength of the interaction of solutes with stationary phases modified by the simultaneous adsorption of two surfactants in the presence of mixed micelles. Both Brij-35 and SDS in the mixed micellar system experience similar equilibria to those described by Eqs. (1) and (2). This allows the fitting to Eq. (4) of the data obtained at increasing concentration of SDS, in the presence of fixed Brij-35, and similarly, at increasing concentration of Brij-35 in the presence of fixed SDS. The estimated association constants K_{AS} and K_{AM} are given in Table 1. For comparative purposes, the values obtained with the micellar system containing only Brij-35 are included. Owing to the strong solute-stationary phase interaction between TCAs and β-blockers with the sulphate group of SDS, which yield extremely long retention times, the estimation of these constants was not possible for purely micellar mobile phases of this surfactant. However, based on previous work, it is known that the intercept in Eq. (4) is practically null for the studied solutes eluted exclusively with SDS, indicating very high K_{AS} and K_{AM} values.[23,24]

As observed in Table 1, the set of runs where SDS was increased and Brij-35 was fixed yielded stronger solute-stationary phase interactions, whereas the runs where Brij-35 was increased with fixed SDS provided solute affinity to the stationary phase similar or smaller than that observed with the only presence of Brij-35. Thus, in a mixed Brij-35/SDS system, the interaction between the basic solutes and each surfactant in the modified stationary phase was different (stronger with SDS). Finally, the solute-micelle association constants (K_{AM}) in the mixed micellar systems were significantly smaller. This suggests that the affinity of the basic solutes to the mixed micelles is smaller, giving rise to a decreased elution strength.

3.3. Peak profiles in the mixed Brij-35/SDS micellar systems

The graphical representation of the left (A) and right (B) half-widths, measured at 10% peak height, versus the retention time, allows an overview of the changes that occur in the width and asymmetry of the chromatographic peaks obtained with a given column. Measurement at 10% peak height allows the characterisation of the asymmetry without being affected by the baseline noise of chromatograms. The validity of these plots to compare the behaviour of different families of compounds, using different types of columns and mobile phases, has been demonstrated in previous work.[26,39–42] The construction of half-width plots is very simple, being represented by the following equations:

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

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

where m_A and m_B are the slopes of the linear correlations for the left and right half-widths, respectively, and A_0 and B_0 the corresponding intercepts representing the extra-column contribution to the peak broadening. Eqs. (5) and (6) allow for the prediction of the peak half-widths for compounds eluted at different retention times, and the calculation of the apparent efficiencies associated to each compound. These parameters are also useful to

characterise chromatographic columns. The sum of m_A and m_B represents the broadening rate of chromatographic peaks inside the column, and its ratio (m_B/m_A) indicates the peak asymmetry at high retention times. The study of the effect of the surfactant mediated systems on the peak profiles was performed based on the construction of plots at each mobile phase composition, using the half-widths for several probe compounds eluted at that condition.

Fig. 5 shows the half-width plots for the TCAs and β-blockers eluted with the Brij-35 and/or Brij-35/SDS systems. The slopes of the linear segments for the left (m_A) and right (m_B) half-widths, and its sum and ratio for the assayed mobile phases are given in Table 2. Fig. 5a depicts the half-width plots for a mobile phase containing only 0.02 M Brij-35. The correlations were satisfactory for both half-widths. The larger slope for the right half-width indicates an appreciably peak tailing. Fig. 5b and c shows the half-width plots obtained for a mixed Brij-35/SDS system. The coincidence of the slopes of the linear segments for both half-widths ($m_B/m_A \approx 1.0$, which means highly symmetrical peaks) is remarkable (compare with Fig. 5a). This indicates that SDS is able to protect the silanol groups in the column, hindering the access of the basic compounds. Although the peak asymmetry with Brij-35 ($m_B/m_A = 2.33$) is significantly larger with respect to the mixed Brij-35/SDS systems, it should be noted that when the basic compounds are eluted from C18 columns with aqueous-organic mobile phases, the peak asymmetry may be even larger ($m_B/m_A = 3.60$, see also Fig. 4a).[42]

The silanol masking capability of SDS has been extensively demonstrated using hybrid mobile phases of SDS and organic solvent.[39–42] As noted, the effect is similar for the mixed Brij-35/SDS system. However, m_A+m_B values are appreciably larger (i.e., the peaks are broader) with respect to the mobile phases containing only Brij-35, probably due to the larger carbon contents when both surfactants are adsorbed.

3.4. Selectivity and resolution

In order to explore the selectivity achieved with the mixed micellar systems, the retention factors obtained for the TCAs, propranolol and alprenolol with a mobile phase containing only Brij-35 were correlated with those using mobile phases containing both Brij-35 and SDS (Fig. 6a). The retention factors for different mixed micellar mobile phases were also correlated (Fig. 6b and c). The observed changes in relative retention can be explained by the changes in the stationary phase nature and elution strength with the mobile phase composition. Besides the significant changes in absolute retention in the presence and absence of SDS, and with changes in the concentration of both surfactants, the three plots show differences in selectivity. Similar results were obtained at other concentrations. We should here recall that more polar β-blockers elute close to the dead time with mobile phases containing only Brij-35.

The main goal in a chromatographic separation is to achieve the resolution of all peaks. In order to observe the resolution capability of the column simultaneously modified with Brij-35 and SDS, mixtures of the two sets of probe compounds (TCAs and β-blockers) were eluted with mixed micellar Brij-35/SDS mobile phases. Fig. 3c shows a chromatogram corresponding to the separation of several TCAs. As observed, for these compounds, mixed Brij-35/SDS mobile phases do not offer any advantage with respect to the use of mobile phases containing Brij-35: in the presence of SDS the peaks are significantly broader and show longer retention. The TCAs remain unresolved in MLC, either with SDS/pentanol (the retention times with a less polar solvent are too high), and with Brij-35 or Brij-35/SDS without organic solvent. However, samples containing the individual TCAs can be analysed with good results using a green RPLC method with Brij-35 in the absence of organic solvent in sufficiently small analysis times. This procedure has been demonstrated to be competitive against classical RPLC with an optimised mobile phase (32% acetonitrile).[26]

In contrast, the mixed Brij-35/SDS system is revealed as promising to succeed in the separation of mixtures of β-blockers, with a favourable effect on retention and resolution. The most polar β-blockers (such as atenolol, celiprolol, metoprolol, oxprenolol, with log $P_{o/w}$ values between 0.25 and 2.0), which are not sufficiently retained with mobile phases containing only Brij-35, and are excessively retained with mobile phases with only SDS, are eluted at practical retention times with the mixed Brij-35/SDS system. Fig. 4d depicts the chromatogram for a mixture of six β-blockers, using an isocratic mobile phase containing 0.02 M Brij-35 and 0.15 M SDS. The mixed micellar mobile phase was able to separate the set of β-blockers with an analysis time below 35 min in the absence of organic solvent. A smaller analysis time will be obtained by optimising the mobile phase composition, which will depend on the particular analysed β-blocker or set of β-blockers. For comparison purposes, Fig. 4a shows the chromatogram of the most polar β-blockers studied in this work, obtained in 15% acetonitrile. The retention of alprenolol and propranolol was above 60 min in these conditions.

The repeatability of the retention time, and the peak efficiency and area, performing ten-fold injections, are indicated in Table 3 for the six β-blockers at three concentrations. The results show that the analysis can be carried out successfully with a mobile phase only composed by water and two detergents at room temperature.

4. Conclusions

More than two-thirds of the reported applications in MLC employ the anionic surfactant SDS, with a special relevance in the pharmaceutical field. The references on the analytical use of Brij-35 in MLC are few, except in the field of QSAR studies. Although procedures using the Brij-35/SDS mixture are found in the MLC literature for several types of compounds, there are no previous descriptions on its application to basic compounds. Also, detailed

comparisons between the mixed micellar systems and those using a single surfactant (as shown in this work) have not been carried out.

This work shows that the separation of basic compounds of diverse polarity, with Brij-35/SDS mobile phases, yields retention times and peak profiles that are dominated by the strong association of the cationic solutes with the adsorbed SDS on the stationary phase. However, the simultaneous adsorption of Brij-35 confers the stationary phase higher polarity that decreases the retention times, which are significantly shorter than those obtained with mobile phases containing only SDS. This avoids the addition of organic solvent.

The preference for the mixed Brij-35/SDS system against the single Brij-35 system depends on the polarity of the basic compounds. Thus, aqueous mobile phases containing only Brij-35 are preferable to analyse apolar basic compounds (as TCAs). Meanwhile, the retention of polar and moderately polar basic compounds (as β-blockers), which is too short with mobile phases containing only Brij-35, can be modulated to practical values by the addition of SDS to the mobile phase containing Brij-35, and may yield successful resolution. Therefore, the described methods with Brij-35 in the absence or presence of SDS can be the basis of successful "green" chromatographic analyses of basic compounds. The studies in this work should be used as a guideline to develop the analytical procedures.

Acknowledgements

This work was supported by Project CTQ2013–42558-P (Ministry of Economy and Competitiveness, Spain), and FEDER funds.

References

1 A. Berthod and M.C. García-Alvarez-Coque, *Micellar Liquid Chromatography*, Marcel Dekker, New York, 2000.

2 M.J. Ruiz-Angel, M.C. García-Alvarez-Coque and A. Berthod, *Sep. Purif. Rev.*, 2009, **38**, 45–96.

3 M.J. Ruiz-Angel, S. Carda-Broch, J.R. Torres-Lapasió and M.C. García-Alvarez-Coque, *J. Chromatogr. A*, 2009, **1216**, 1798–1814.

4 E. Bonet-Domingo, M.J. Medina-Hernández and M.C. García-Alvarez-Coque, *Quím. Anal.*, 1993, **12**, 167–172.

5 M.C. García-Alvarez-Coque and S. Carda-Broch, *J. Chromatogr. B*, 1999, **736**, 1–18.

6 Li Zhu, Li Ding, Qianli Zhang, Lin Wang, Fei Tang, Qian Liu and Shouzhuo Yao, *Green Chemistry*, 2009, **11**, 132–137.

7 M.F. Borgerding and W.L. Hinze, *Anal. Chem.*, 1985, **57**, 2183–2190.

8 M.F. Borgerding, F.H. Quina, W.L. Hinze, J. Bowermaster and H.M. McNair, *Anal. Chem.*, 1988, **60**, 2520–2527.

9 M.F. Borgerding, W.L. Hinze, L.D. Stafford, G.W. Fulp Jr. and W.C. Hamlin Jr., *Anal. Chem.*, 1989, **61**, 1353–1358.

10 L.J. Cline-Love and J.J. Fett, *J. Pharm. Biomed. Anal.*, 1991, **9**, 323–333.

11 M.L. Marina, O. Jiménez, M.A. García and S. Vera, *Microchem. J.*, 1996, **53**, 215–224.

12 P. Menéndez-Fraga, A. Blanco-González, A. Sanz-Medel and J.B. Cannata-Andia, *Talanta*, 1997, **45**, 25–33.

13 A. Berthod, S. Tomer and J.G. Dorsey, *Talanta*, 2001, **55**, 69–83.

14 M. Gil-Agustí, L. Alvarez-Rodríguez, L. Monferrer-Pons, D. Bose, A. Durgbanshi and J. Esteve-Romero, *Anal. Lett.*, 2002, **35**, 1721–1734.

15 N. Memon, M.I. Bhanger and M.Y. Khuhawer, *J. Sep. Sci.*, 2005, **28**, 635–638.

16 W. Thogchai and B. Liawruangrath, *Int. J. Cosmetic Sci.*, 2013, **35**, 257–263.

17 Y.M. Dong, N. Li, Q. An and N.W. Lu, *J. Liq. Chromatogr., Rel. Technol.*, 2015, **38**, 251–258.

18 J.M. Sanchis-Mallols, R.M. Villanueva-Camañas, S. Sagrado and M.J. Medina-Hernández, *Chromatographia*, 1997, **46**, 605–612.

19 C.R. Yin, L.Y. Ma, J.G. Huang, L. Xu, Z.G. Shi, *Anal. Chim. Acta*, 2013, **804**, 321–327.

20 J. Nawrocki, *J. Chromatogr. A*, 1997, **779**, 29–71.

21 S. Bocian, B. Buszewski, *J. Sep. Sci.*, 2012, **35**, 1191–1200.

22 B.K. Lavine, S. Hendayan, W.T. Cooper and Y. He, *ACS Symp. Ser.*, 2000, **740**, 290–313.

23 M.J. Ruiz-Angel, J.R. Torres-Lapasió, M.C. García-Alvarez-Coque and S. Carda-Broch, *Anal. Chem.*, 2008, **80**, 9705–9713.

24 M.J. Ruiz-Angel, S. Carda-Broch and M.C. García-Alvarez-Coque, *J. Chromatogr. A*, 2010, **1217**, 7082–7089.

25 M.J. Ruiz-Angel, S. Carda-Broch, E.F. Simó-Alfonso and M.C. García-Alvarez-Coque, *J. Pharm. Biomed. Anal.*, 2003, **32**, 71–84.

26 J.J. Fernández-Navarro, M.J. Ruiz-Angel and M.C. García-Alvarez-Coque, *J. Sep. Sci.*, 2012, **35**, 1303–1309.

27 J.J. Baeza-Baeza, Y. Dávila, J.J. Fernández-Navarro and M.C. García-Alvarez-Coque, *Anal. Bioanal. Chem.*, 2012, **404**, 2973–2984.

28 K. Ogino, H. Uchiyama and M. Abe, *Mixed Surfactant Systems*, Marcel Dekker, New York, 1993.

29 X. Li and J.S. Fritz, *Anal. Chem.*, 1996, **68**, 4481–4488.

30 P. Ebrahimi and M.R. Hadjmohammadi, *Anal. Bioanal. Chem.*, 2006, **384**, 851–858.

31 J. Sun, J. Mao, X. Liu, Y. Wang, Y. Sun and Z. He, *J. Sep. Sci.*, 2009, **32**, 2043–2050.

32 R. Nakao and C. Haldin, *J. Chromatogr. A*, 2013, **1281**, 54–59.

33 O.R.T. Thomas and G.F. White, *Biotechnol. Appl. Biochem.*, 1989, **11**, 318–327.

34 M.T. Scott and M.N. Jones, *Biochim. Biophys. Acta.*, 2000, **1508**, 235–251.

429 35 H. Gao, S. Zhao, S. Mao, H. Yuan, J. Yu, L. Shen and Y. Du, *J. Coll. Int. Sci.*, 2002,
430 **249**, 200–208.
431 36 C.J. Drayton (Ed.), *Comprehensive Medicine Chemistry*, Vol. 6, Pergamon Press,
432 Oxford, 1990.
433 37 D.W. Armstrong and F. Nome, *Anal. Chem.*, 1981, **53**, 1662–1666.
434 38 M. Arunyanart and L.J. Cline-Love, *Anal. Chem.*, 1984, **56**, 1557–1561.
435 39 M.J. Ruiz-Angel, S. Carda-Broch and M.C. García-Alvarez-Coque, *J. Chromatogr. A*,
436 2010, **1217**, 1786–1798.
437 40 J.J. Fernández-Navarro, M.C. García-Alvarez-Coque and M.J. Ruiz-Angel,
438 *J. Chromatogr. A*, 2011, **1218**, 398–407.
439 41 S. Pous-Torres, M.J. Ruiz-Angel, J.R. Torres-Lapasió and M.C. García-Alvarez-Coque,
440 *J. Sep. Sci.*, 2009, **32**, 2841–2853.
441 42 M.J. Ruiz-Angel, S. Pous-Torres, S. Carda-Broch and M.C. García-Alvarez-Coque,
442 *J. Chromatogr. A*, 2014, **1344**, 76–82.
443

444 **FIGURE CAPTIONS**

445 **Fig. 1.** Simplified scheme of the environment of cationic solutes in a C18 stationary phase, in
446 the presence of Brij-35 and SDS.

447 **Fig. 2.** Effect of the addition of increasing concentrations of surfactant on the retention of
448 TCAs and β-blockers in a mobile phase containing a fixed concentration of a second
449 surfactant: (a) 0.02 M Brij-35 and increasing concentrations of SDS, and (b) 0.15 M SDS and
450 increasing concentrations of Brij-35. Compound identity: (+) alprenolol, (◊) propanolol,
451 (□) amitriptyline, (■) clomipramine, (△) doxepin, (▲) imipramine, (×) maprotiline,
452 (◆) nortriptyline, and (▶) trimipramine.

453 **Fig. 3.** Chromatograms for a mixture of TCAs eluted with: (a) 0.10 M SDS and 3.4% v/v
454 pentanol (Eclipse XDB C8 column), (b) 0.02 M Brij-35 (Zorbax Eclipse C18), and (c) mixed
455 micellar system composed of 0.02 M Brij-35 and 0.15 M SDS (Zorbax Eclipse C18).
456 Compound identity: (1) doxepin, (2) imipramine, (3) amitriptyline, (4) trimipramine,
457 (5) nortriptyline, and (6) clomipramine.

458 **Fig. 4.** Chromatograms for a mixture of β-blockers, eluted with: (a) 15% v/v acetonitrile
459 (Kromasil C18), (b) 0.1125 M SDS and 10% v/v acetonitrile (Kromasil C18), (c) 0.1125 M
460 SDS and 45% v/v acetonitrile (Kromasil C18), and (d) mixed micellar system composed of
461 0.02 M Brij-35 and 0.15 M SDS (Zorbax Eclipse C18). Compound identity: (1) atenolol,
462 (2) celiprolol, (3) metoprolol, (4) oxprenolol, (5) propranolol, and (6) alprenolol.

463 **Fig. 5.** Half-width plots for mobile phases containing: (a) 0.02 M Brij-35, (b,c) 0.02 M
464 Brij-35/0.15 M SDS. Left (*A*, ○) and right (*B*, ●) half-widths. Compounds: (a,b) TCAs,
465 propranolol and alprenolol, and (c) atenolol, celiprolol, metoprolol and oxprenolol.

466 **Fig. 6.** Comparison of the selectivity of chromatographic systems containing only Brij-35,
467 and both Brij-35 and SDS (retention factors are plotted). The data correspond to the seven
468 TCAs, propranolol and alprenolol.

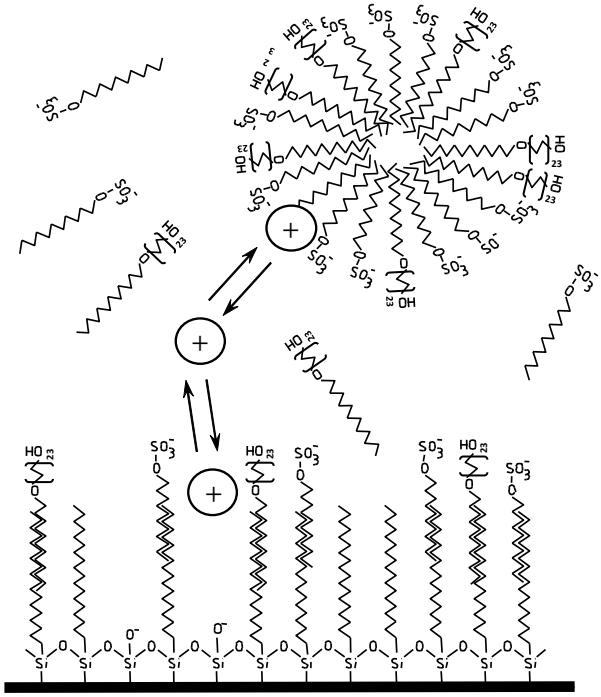
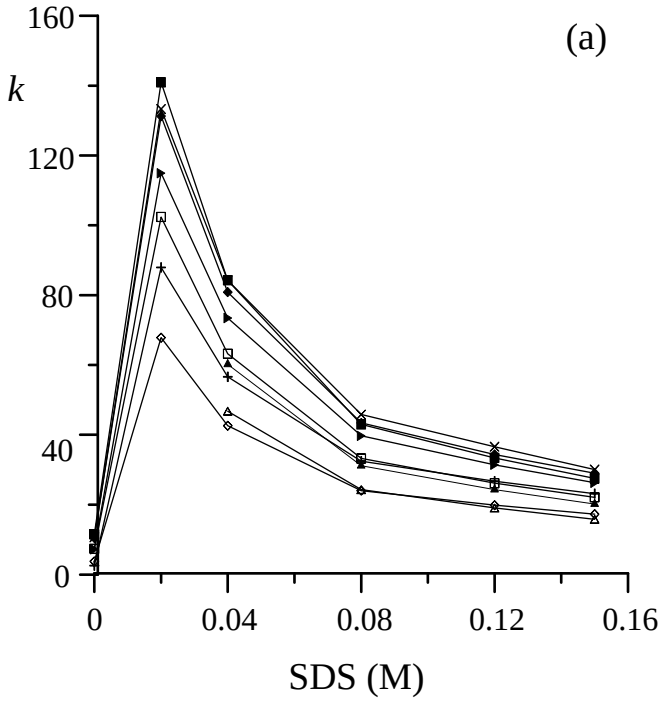


Figure 1



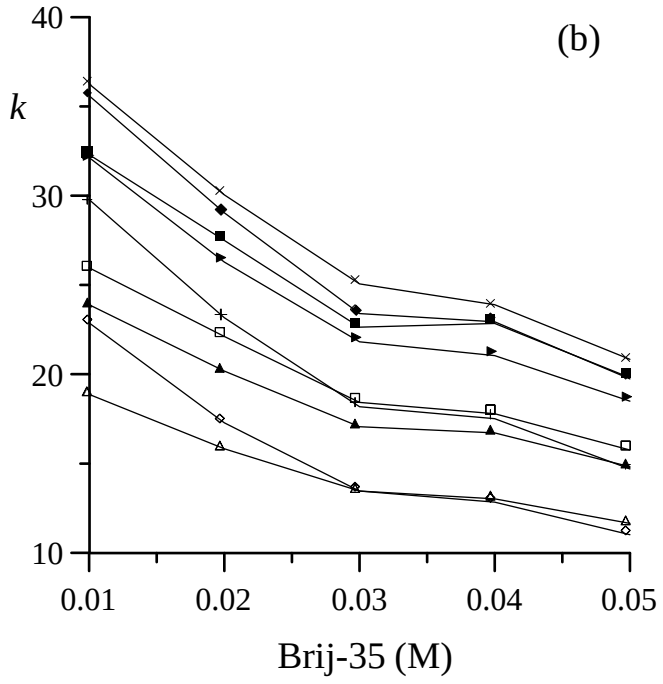


Figure 2

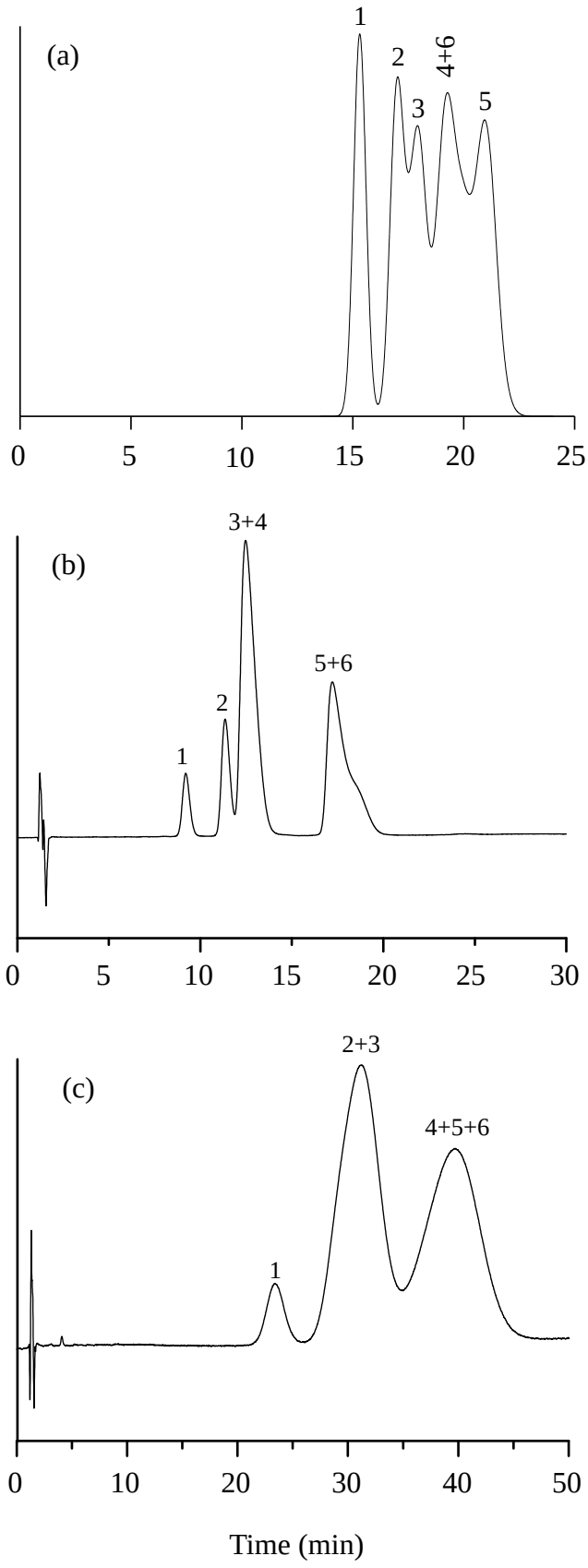


Figure 3

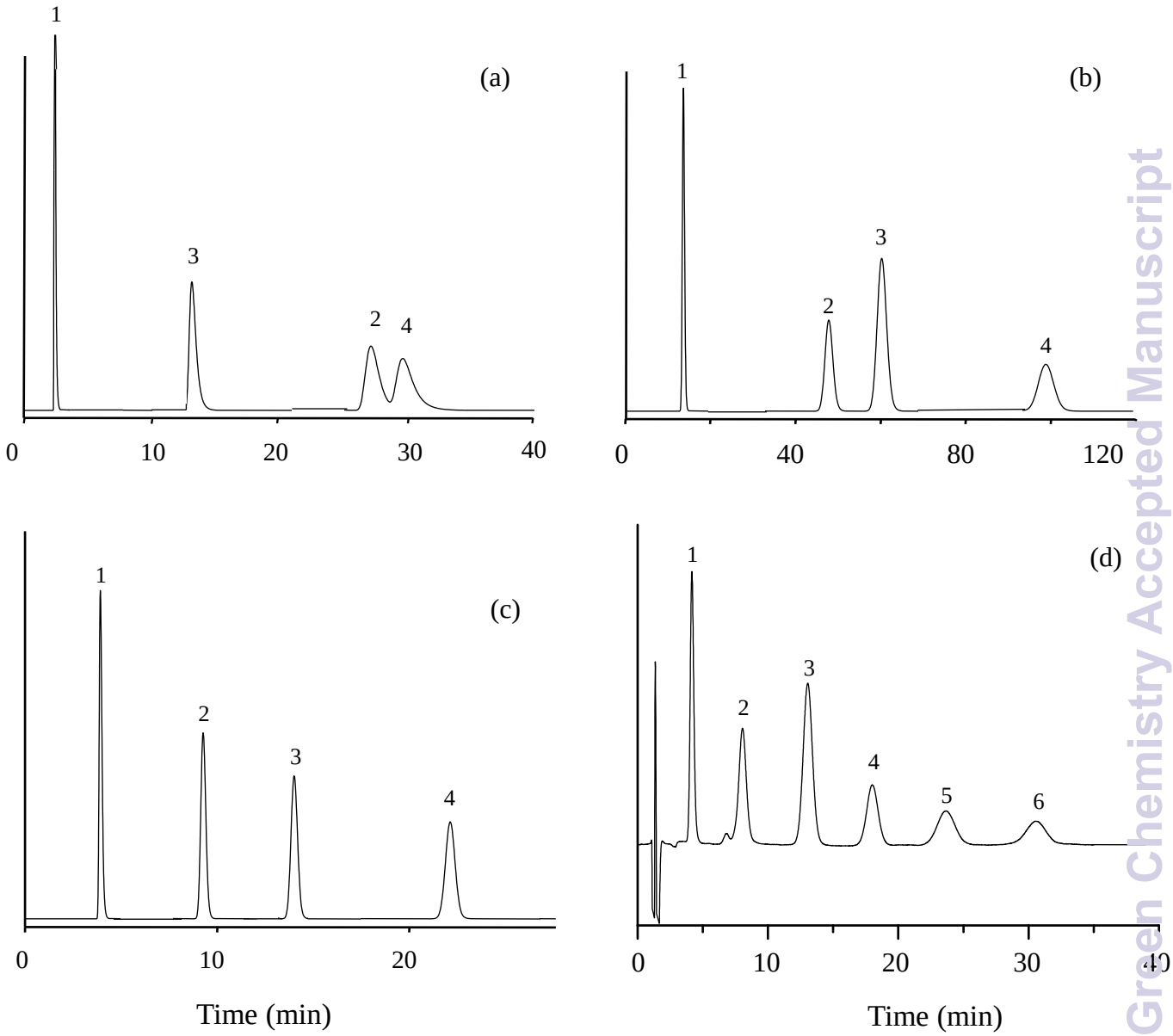


Figure 4

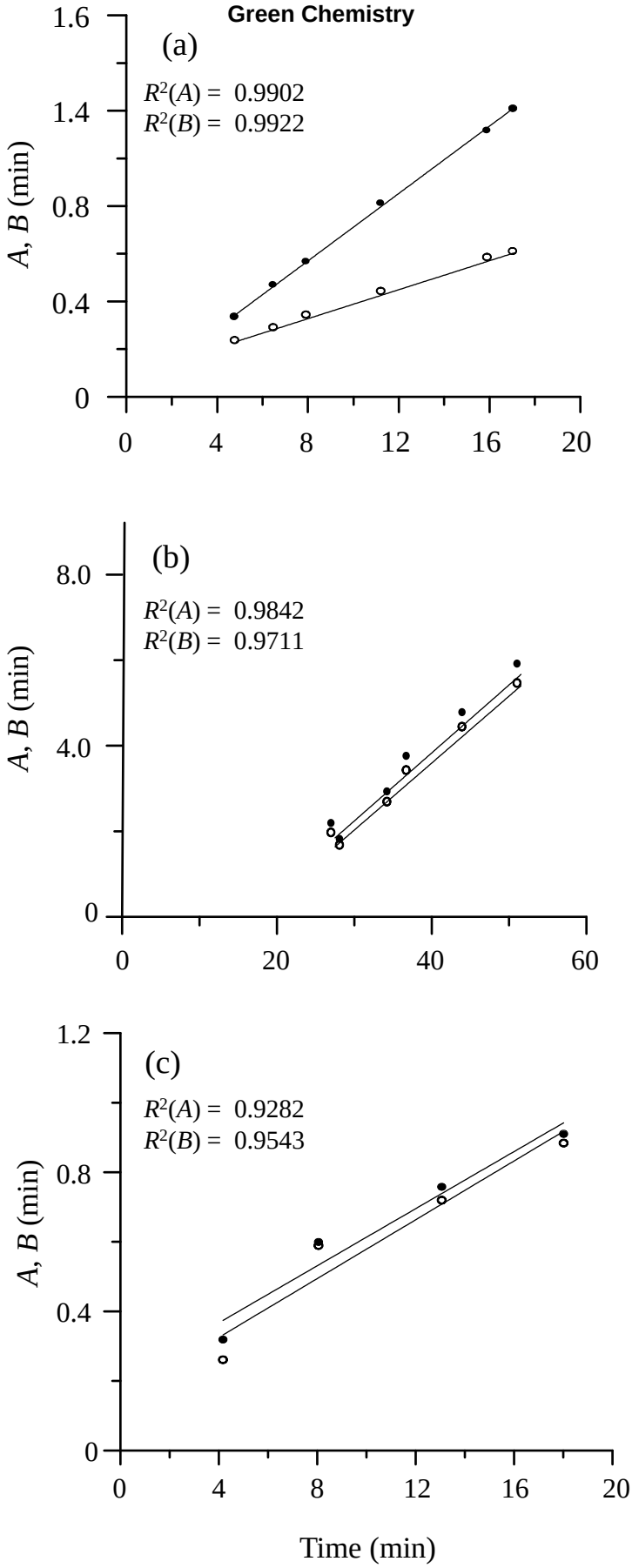
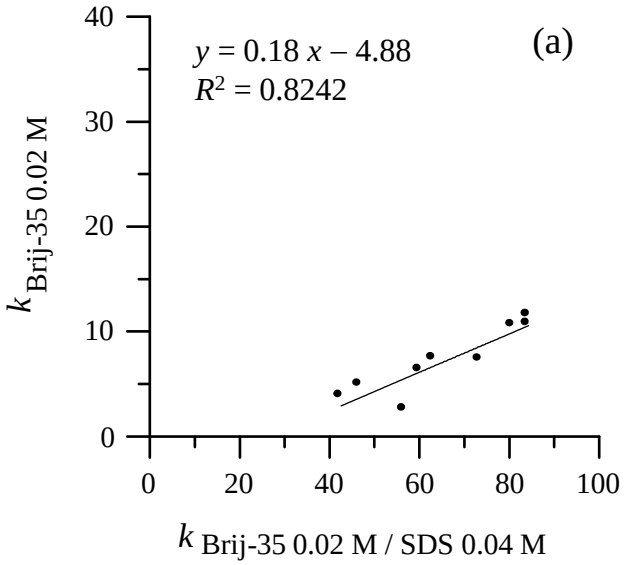
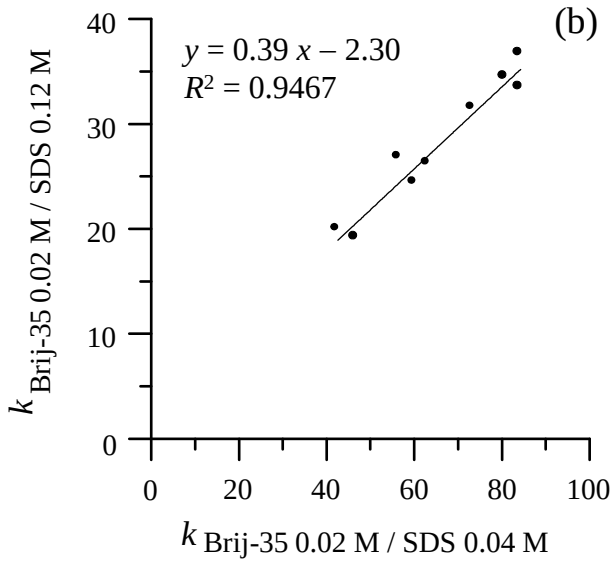


Figure 5





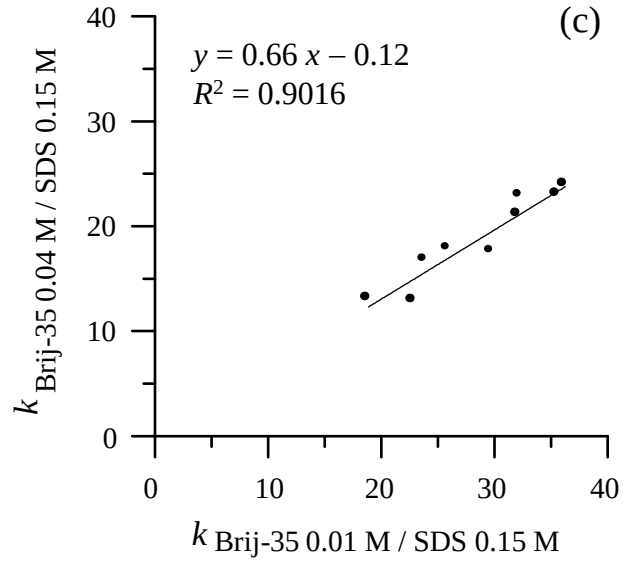


Figure 6

Table 1. Solute-stationary phase (K_{AS}) and solute-mobile phase (K_{AM}) association constants for the studied basic compounds eluted with mobile phases containing Brij-35 or mixtures of Brij-35 and SDS.

	Brij-35[a]		Brij-35 0.02 M / SDS[b]		SDS 0.15 M / Brij-35[a]	
Compound	K_{AS}	K_{AM}	K_{AS}	K_{AM}	K_{AS}	K_{AM}
Alprenolol	–	–	138.9	34.0	39.1	32.4
Propanolol	–	–	108.7	36.1	30.0	34.3
Amitryptiline	35.7	148.9	185.2	50.7	30.7	18.7
Clomipramine	98.0	306.5	303.0	68.2	37.7	17.5
Doxepin	14.7	74.8	161.3	61.5	21.8	17.3
Imipramine	21.9	91.8	212.8	64.0	27.6	17.0
Maprotiline	62.9	204.6	232.6	45.7	43.7	21.6
Nortryptiline	57.5	188.9	227.3	46.9	43.7	23.7
Trimipramine	36.4	151.4	200.0	45.1	38.3	21.2

[a] Increasing concentration of Brij-35 from 0.01 to 0.05 M.

[b] Increasing concentration of SDS from 0.02 to 0.15 M.

Table 2. Half-width plots parameters for TCAs and β-blockers eluted with different micellar mobile phases: slopes for the left (m_A) and right (m_B) half-width plot, sum of slopes and slopes ratio.

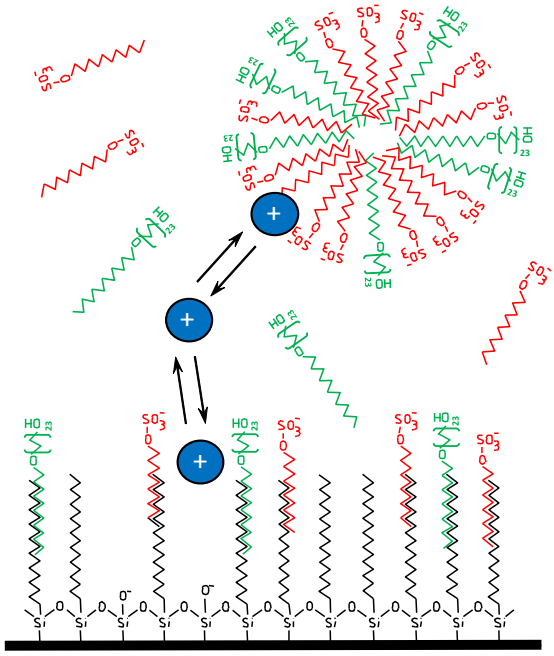
Mobile phase	m_A	m_B	m_A+m_B	m_B/m_A
Brij-35 0.02 M[a]	0.030	0.071	0.101	2.33
Brij-35 0.02 M / SDS 0.02 M[a]	0.083	0.082	0.165	0.99
Brij-35 0.02 M / SDS 0.04 M[a]	0.098	0.100	0.198	1.02
Brij-35 0.02 M / SDS 0.08 M[a]	0.141	0.150	0.291	1.06
Brij-35 0.02 M / SDS 0.12 M[a]	0.157	0.159	0.316	1.01
Brij-35 0.02 M / SDS 0.15 M[a]	0.123	0.119	0.242	0.96
Brij-35 0.01 M / SDS 0.15 M[a]	0.207	0.213	0.420	1.03
Brij-35 0.02 M / SDS 0.15 M[a]	0.123	0.119	0.242	0.96
Brij-35 0.03 M / SDS 0.15 M[a]	0.141	0.152	0.293	1.13
Brij-35 0.04 M / SDS 0.15 M[a]	0.160	0.180	0. 340	1.07
Brij-35 0.05 M / SDS 0.15 M[a]	0.127	0.139	0.266	1.10
Brij-35 0.02 M / SDS 0.15 M[b]	0.0423	0.0410	0.0833	0.97

[a] TCAs, alprenolol and propranolol.

[b] Atenolol, celiprolol, metoprolol and oxprenolol.

Table 3. Repeatability in retention times, area and efficiency at three different concentrations of β-blockers.

	2 μg/mL			7 μg/mL			14 μg/mL		
Compound	t_R (min)	Area	*N*	t_R (min)	Area	*N*	t_R (min)	Area	*N*
Atenolol	4.13 ± 0.01	1.45 ± 0.02	940 ± 30	4.14 ± 0.01	6.08 ± 0.02	880 ± 14	4.16 ± 0.01	11.19 ± 0.02	870 ± 12
Celiprolol	7.95 ± 0.02	1.57 ± 0.05	910 ± 60	8.00 ± 0.02	4.96 ± 0.02	865 ± 8	8.05 ± 0.01	9.41 ± 0.06	840 ± 12
Metoprolol	12.86 ± 0.02	2.92 ± 0.05	1450 ± 50	12.95 ± 0.04	9.31 ± 0.07	1400 ± 27	13.06 ± 0.02	17.30 ± 0.07	1400 ± 9
Oxprenolol	17.71 ± 0.05	1.37 ± 0.07	1820 ± 150	17.86 ± 0.07	4.19 ± 0.03	1900 ± 38	18.01 ± 0.04	7.84 ± 0.07	1840 ± 36
Propranolol	23.28 ± 0.12	0.89 ± 0.16	1130 ± 320	23.42 ± 0.09	3.89 ± 0.09	1300 ± 76	23.66 ± 0.07	6.97 ± 0.09	1290 ± 45
Alprenolol	30.04 ± 0.14	0.95 ± 0.17	1560 ± 470	30.26 ± 0.11	3.04 ± 0.10	1700 ± 160	30.60 ± 0.08	5.54 ± 0.22	1700 ± 83



Mixed micellar systems of Brij-35 and sodium dodecyl sulphate without organic solvent allow the analysis of polar and moderately polar basic compounds, giving rise to a type of more sustainable RPLC.