

Article

Retention Mechanisms of Basic Compounds in Liquid Chromatography with Sodium Dodecyl Sulfate and 1-Hexyl-3-Methylimidazolium Chloride as Mobile Phase Reagents in Two C18 Columns

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Abstract: Reversed-phase liquid chromatography (RPLC) relies on a non-polar stationary phase and a more polar hydro-organic mobile phase, where compound retention is primarily governed by hydrophobicity, with more hydrophobic compounds being retained longer. The introduction of secondary equilibria in the chromatographic system through additives, such as anionic surfactants and ionic liquids (ILs), was proposed to mitigate ionic interactions between positively charged analytes and the anionic free silanol groups in non-encapped stationary phases, thereby preventing increased retention and peak tailing. Additionally, the combined hydrophobic and ionic interactions between cationic analytes and the ions in these additives was demonstrated to create mixed retention mechanisms that influence retention and selectivity. In this regard, this study investigates aqueous chromatographic systems incorporating both the anionic surfactant sodium dodecyl sulfate (SDS) and the IL 1-hexyl-3-methylimidazolium chloride as mobile phase reagents. This combination of reagents modulates the retention, eliminating the need for organic solvents and resulting in highly sustainable HPLC procedures. The chromatographic behavior was assessed using two different C18 columns (Zorbax Eclipse and XTerra-MS). The strength of solute interactions was estimated by calculating equilibrium parameters and the contributions of hydrophobic and ionic interactions through simple mathematical models. Focusing on the retention of six basic drugs (β -adrenoceptor antagonists), the study highlighted the significant role of ionic interactions. The results demonstrate the feasibility of using aqueous systems combining SDS and an IL for the efficient separation of moderately polar basic compounds without the use of organic solvents.

Keywords: liquid chromatography; sodium dodecyl sulfate; ionic liquids; mobile phase without organic solvents; β -adrenoceptor antagonists; retention mechanism



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1. Introduction

The combination of an ionic liquid (IL) and an ionic surfactant as reagents for the mobile phase in liquid chromatography was first investigated in the context of microemulsion liquid chromatography (MELC) with sodium dodecyl sulfate (SDS) and 1-butanol [1,2]. In these reports, the focus was on analyzing phenolic and basic compounds, using various alkyl-methyl imidazolium ILs. The ILs examined included 1-ethyl, 1-butyl, 1-hexyl, and 1-octyl-3-methyl imidazolium cations ($[C_2C_1IM]^+$, $[C_4C_1IM]^+$, $[C_6C_1IM]^+$, and $[C_8C_1IM]^+$), paired with the anions chloride (Cl^-), tetrafluoroborate (BF_4^-), hexafluorophosphate (PF_6^-), and bis(trifluoromethylsulfonyl)imide. In the MELC systems, these ILs functioned as oils, while SDS was used as a surfactant and 1-butanol was used as a co-surfactant.

Peng et al. conducted a pioneering study on the analysis of a set of phenolic compounds, identifying $[C_6C_1IM][PF_6]$ as the optimal IL due to its favorable retention times and selectivity [1]. A subsequent study on the analysis of basic compounds in MELC

revealed that adding an IL to the mobile phase minimized the need for 1-butanol in the microemulsion, which could be eliminated without significantly altering the chromatographic behavior [2]. It was also found that aqueous mobile phases containing only SDS and IL provided satisfactory separations. This result led to a new aqueous liquid chromatographic method capable of achieving practical retention times and high resolution for analyzing mixtures of basic compounds, without the need of an organic solvent in the mobile phase. However, environmental considerations regarding the anion in the IL are still important. ILs paired with fluoride anions are less sustainable than those paired with chloride anions. Therefore, ILs with chloride anions are preferable for environmentally friendly liquid chromatographic applications.

Aqueous liquid chromatography with surfactant and 1-alkyl-3-methylimidazolium ILs integrates features of both micellar liquid chromatography (MLC) and RPLC, with ILs serving as additives in the mobile phase. Similarly to MLC, the surfactant is added above its critical micelle concentration (CMC), allowing the surfactant monomers to organize into micelles [3,4]. Simultaneously, the IL is incorporated into the mobile phase in relatively small amounts, acting like a dissociated salt that increases the ionic strength of the mobile phase [5–9]. It is noteworthy that some ILs, known as surface-active ILs (SAILs), composed of 1-alkyl-3-methylimidazoliums with long alkyl chains, can form organized structures similar to typical surfactants [10,11].

In MLC, it has been demonstrated that surfactant monomers adsorbed onto the alkyl-bonded chains create a modified stationary phase that exhibits a net charge with ionic surfactants [3,4]. Similarly, 1-alkyl-3-methylimidazolium cations and their associated anions can adsorb onto the alkyl-bonded stationary phase, forming a charged bilayer depending on their intrinsic properties [12]. When SDS and 1-alkyl-3-methylimidazolium ILs are combined in the mobile phase, there is a competition between the surfactant monomers and the 1-alkyl-3-methylimidazolium cations and anions for adsorption sites on the stationary phase, as suggested by recent studies [13,14]. Furthermore, the presence of the charged bilayer composed of both additives inhibits the activity of residual silanols. This inhibition is particularly relevant for the analysis of basic compounds using non-encapped stationary phases as it significantly improves peak profiles [15,16].

It is important to note that using only surfactant in MLC or only IL in RPLC, with mobile phases lacking any organic solvent, leads to excessive retention and broad peaks for low-polarity compounds. To mitigate this, the addition of an organic solvent (such as acetonitrile or short-chain alcohols like propanol, butanol, and pentanol) to the mobile phase becomes necessary to reduce retention and enhance efficiency [17]. Although the amount of the organic solvent used in these mobile phases is significantly smaller compared to conventional RPLC, its use still poses potential health hazards, generates toxic wastes that require treatment, and increases overall analysis costs [18]. Additionally, in MLC, the concentration of the organic solvent must be carefully controlled to prevent micelle breakdown [15].

The wide array of interactions available to solutes in a chromatographic system incorporating SDS and alkyl-imidazolium ILs effectively fine-tunes separation selectivity [13]. By precisely controlling the concentrations of SDS and IL in the aqueous mobile phase, retention behavior can be conveniently modulated without the need for adding an organic solvent. However, it should be taken into account that anions associated with the ILs, which have an affinity for the alkyl-bonded stationary phase, can significantly affect the retention of positively charged basic compounds [14,19]. Indeed, the retention mechanisms occurring within the column create a complex scenario within the chromatographic system.

The present study aims to shed light on the behavior of aqueous liquid chromatographic systems composed of a combination of SDS and 1-alkyl-3-methylimidazolium ILs without the use of organic solvents. Specifically, it focuses on elucidating the interactions within two distinct alkyl-bonded C18 columns: Zorbax Eclipse and XTerra-MS. To achieve this, solute–stationary and solute–mobile phase equilibrium parameters, as well as the contribution of hydrophobic and ionic interactions, were estimated using two simple math-

emational models. SDS and 1-hexyl-3-methylimidazolium chloride were selected as the mobile phase reagents. Chloride was chosen because it shows minimal adsorption on the stationary phase, ensuring that any observed impact of retention is solely attributable to the cationic component. The results were analyzed in terms of retention times and resolution for chromatograms of mixtures containing basic β -adrenoceptor antagonists, which are commercially available drugs used in the treatment of various cardiac diseases [20].

2. Materials and Methods

2.1. Reagents and Mobile Phases

The following β -adrenoceptor antagonists were selected as probe compounds for this research (organized by their log $P_{o/w}$ values in Table A1): atenolol, carteolol, acebutolol, metoprolol, oxprenolol, and propranolol (all sourced from Sigma, St. Louis, MA, USA). Initially, the solid drugs were dissolved in a small volume of acetonitrile from VWR International (Rosny-sous-Bois, France), and were then diluted with water to prepare stock solutions of approximately $500 \mu\text{g mL}^{-1}$. These solutions remained stable for at least two months when stored at 4°C . Prior to injection into the chromatograph, the stock solutions were further diluted with water to achieve a final concentration of around $40 \mu\text{g mL}^{-1}$. Additionally, a mixed solution of all six β -adrenoceptor antagonists was prepared from the stock solutions, resulting in final concentrations of $80 \mu\text{g mL}^{-1}$ for atenolol, $10 \mu\text{g mL}^{-1}$ for carteolol and acebutolol, and $150 \mu\text{g mL}^{-1}$ for metoprolol, oxprenolol, and propranolol. These concentrations were selected to ensure sufficiently large signals for each drug within the chromatographic mixture, facilitating accurate UV detection.

Aqueous mobile phases were prepared using SDS from Merck (99% purity, Darmstadt, Germany) and 1-hexyl-3-methylimidazolium chloride ($[\text{C}_6\text{C}_1\text{IM}][\text{Cl}]$) from Sigma. SDS concentrations ranged from 0.05 to 0.20 M (0.05, 0.10, 0.15, and 0.20 M), while $[\text{C}_6\text{C}_1\text{IM}][\text{Cl}]$ concentrations varied from 0.005 to 0.03 M (0.005, 0.01, 0.02, and 0.03 M). Aqueous mobile phases containing only SDS were also prepared in concentrations ranging from 0.10 to 0.25 M (0.10, 0.15, 0.20, and 0.25 M), and containing only $[\text{C}_6\text{C}_1\text{IM}][\text{Cl}]$ in concentrations ranging from 0.02 to 0.05 M (0.02, 0.03, 0.04, and 0.05 M). In all cases, no organic solvents were added. During experimentation, mobile phases were systematically run from lower to higher concentrations of SDS and $[\text{C}_6\text{C}_1\text{IM}][\text{Cl}]$. The concentration ranges were selected to ensure adequate retention for highly polar β -adrenoceptor antagonists, while avoiding excessive retention for the more hydrophobic ones.

To minimize silanol ionization, the mobile phases were buffered to a pH of approximately 3.0 (within the range of 2.9 to 3.2), using 0.01 M sodium dihydrogen phosphate from Fluka (Buchs, Switzerland) and hydrochloric acid from Scharlau (Barcelona, Spain). Given the basic nature of the β -adrenoceptor antagonists ($\text{pK}_a \geq 9$), these compounds were fully protonated within the acidic pH environment of the mobile phases.

The drug working solutions and mobile phases were filtered using $0.45 \mu\text{m}$ Nylon membranes from Micron Separations (Westboro, MA, USA) and then degassed in an Elmasonic S15 H ultrasonic bath from Elma (Singen, Germany). Throughout the process, nanopure water obtained from an Adrona system (Riga, Latvia) was used consistently.

2.2. Chromatographic Instrumentation and Columns

An Agilent chromatograph (Waldbronn, Germany) was employed for the study. The system comprised an isocratic pump (Series 1200), an automatic injector (Series 1260 Infinity II), a thermostatted column compartment (Series 1290 Infinity II), and a diode array detector (Series 1100). The β -adrenoceptor antagonists were monitored at a wavelength of 254 nm. Retention data were collected at a constant temperature of 25°C with a steady flow rate of 1 mL min^{-1} under isocratic conditions. Duplicate injections of $20 \mu\text{L}$ were performed.

Chromatographic control was managed via OpenLAB CDS LC Chemstation (Agilent C.02.01). Peak parameter measurements, such as retention times and baseline corrections in the chromatograms, were conducted using CHRODATA software (first version, FUSCHROM group, Burjassot Valencia, Spain) (still in development). This updated version

of the MICHROM software, written in QBasic [21,22], enables precise data analysis and interpretation and operates within GNU Octave (version 6.3.0). Data treatment, including retention factor calculations and regression analysis, was carried out using Excel (Microsoft Office 2010, Redmond, WA, USA).

Analytical separations were performed using the following two C18 columns, both measuring 150 mm × 4.6 mm i.d. and with a particle size of 5 µm: the Zorbax Eclipse XDB column from Agilent and the XTerra-MS column from Waters (Milford, MA, USA). The Zorbax column features a mean pore diameter of 80 Å, a surface area of 180 m²/g, and a total carbon content of 10% by weight, while the XTerra-MS column has a mean pore diameter of 125 Å, a surface area of 175 m²/g, and a total carbon content of 16% by weight. Zorbax Eclipse XDB columns employ extra-dense bonding of organo-silane ligands and double endcapping to protect the ultra-pure (Type B) silica support from dissolution in the mobile phases of intermediate and high pH levels. XTerra-MS employs hybrid particle technology, where one in every three silanols is substituted with a methyl group. This general-purpose reversed-phase C18 column, designed to be compatible with mass spectrometry applications, offers superior pH stability compared to traditional silica-based columns. While both stationary phases are designed to be inert, the deactivation of the silica surface is not entirely complete. To protect the analytical column from the mobile phase and enhance the durability and efficiency of the chromatographic system, a 30 mm guard column packed with C18 particles was placed before the main column. The dead times were determined from the initial baseline perturbations in the chromatograms, measuring 1.04 min for the Zorbax column and 0.93 min for the XTerra column.

The mobile phases were efficiently recycled both between runs and throughout the analysis, thanks to the absence of organic solvents in the mobile phases. This sustainable approach minimized reagent consumption and waste production. Regular rinsing cycles were performed on the chromatographic system, typically using 10 volumes of a methanol–water solution (5:95 v/v), followed by approximately 30 mL of pure methanol [23]. These rinsing steps effectively removed any residual surfactant and IL from the stationary phase. During weekends or periods of inactivity, the columns were stored in methanol to maintain their integrity and cleanliness.

3. Results and Discussion

3.1. Retention Behaviour of β-Adrenoceptor Antagonists with Mobile Phases Containing Ionic Additives

In previous work [14], we reported the behavior observed for the β-adrenoceptor antagonists with mobile phases containing ionic additives using the XTerra column. For this reason, we only show the below figures with the detailed results for the Zorbax column. The results for the XTerra column can be found in the Supplementary Material (Figures S1 and S2).

3.1.1. Effect on Retention of Mobile Phases Containing Only SDS or [C₆C₁IM][Cl]

Figure 1a illustrates the effect of varying concentrations of the anionic surfactant SDS on the retention of β-adrenoceptor antagonists using a Zorbax column. The aqueous mobile phases contained only SDS with concentrations increasing from 0.1 to 0.25 M (0.1, 0.15, 0.2, and 0.25 M), all well above the surfactant CMC in aqueous medium (0.008 M). At these concentrations, surfactant monomers are organized into micelles in the mobile phase and the column is maximally saturated with surfactant monomers [4]. Retention times were notably high at an SDS concentration of 0.1 M, particularly for the more hydrophobic β-adrenoceptor antagonists (oxprenolol and propranolol), with retention times exceeding 90 min. This high retention at lower SDS concentrations is attributed to the strong interaction between the positively charged basic compounds formed at acidic pH and the anionic SDS monomers adsorbed onto the stationary phase. At higher surfactant concentrations, the elution strength was increased due to the greater abundance of SDS micelles in the mobile phase. The XTerra column exhibited a similar retention pattern to the

Zorbax column, with only minor differences (see Supplementary Material). Specifically, the XTerra column resulted in lower retention times for the more hydrophobic β -adrenoceptor antagonists, while comparable retention times were found for the more polar ones. For example, using a mobile phase with 0.15 M SDS, the retention factors (k) for β -adrenoceptor antagonists on the XTerra column were as follows: acebutolol, 13.47; atenolol, 7.41; carteolol, 11.34; metoprolol, 28.05; oxprenolol, 44.93; and propranolol, 62.53. These values can be compared with those presented in Figure 1a for the Zorbax column, using the same SDS concentration.

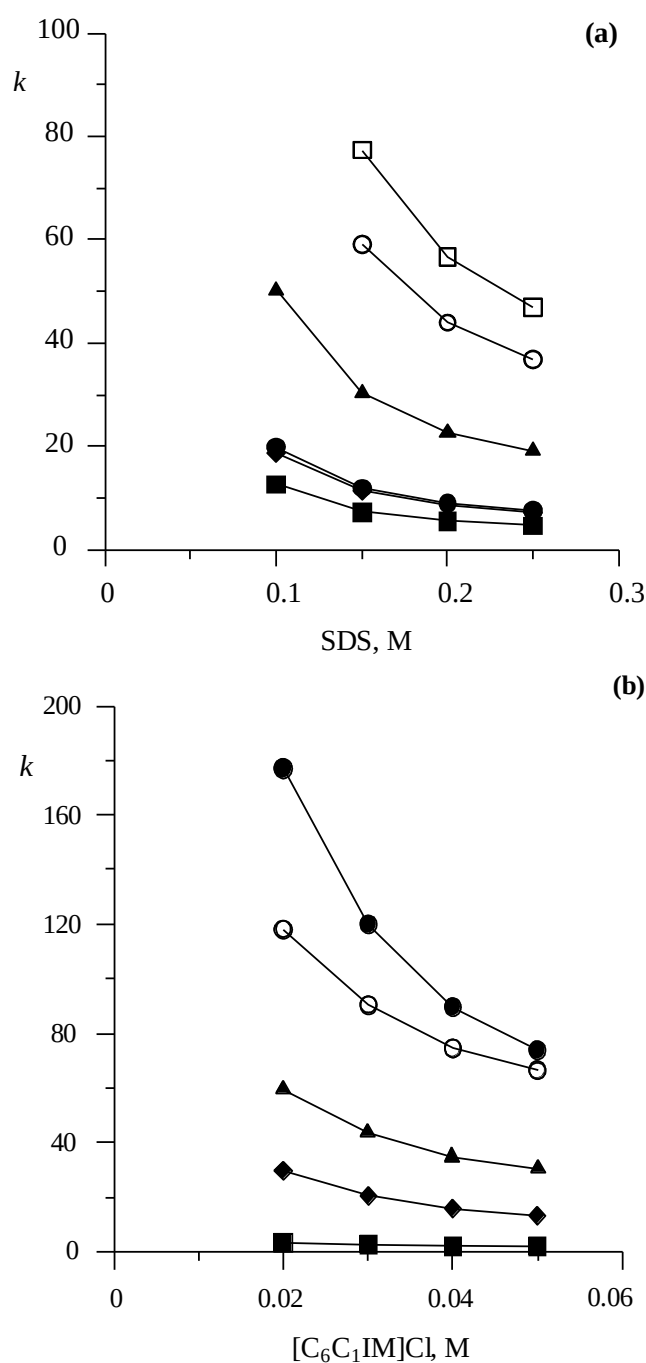


Figure 1. Retention behavior of the β -adrenoceptor antagonists propranolol (□), oxprenolol (○), metoprolol (▲), carteolol (◆), atenolol (■), and acebutolol (●) for aqueous mobile phases containing additives at increasing concentrations: (a) SDS and (b) $[C_6C_1IM][Cl]$. The analyses were conducted using a Zorbax column.

Figure 1b illustrates the effect of increasing concentrations of $[C_6C_1IM][Cl]$ (0.02, 0.03, 0.04, and 0.05 M), using the Zorbax column with mobile phases containing only the IL with no organic solvent or surfactant. A similar trend was observed with the XTerra column (see Supplementary Material). It is important to note that the chloride anion exhibits minimal adsorption on alkyl-bonded stationary phases, as previously reported [12]. Consequently, the observed retention changes highlight the impact of the IL cation on chromatographic behavior. The variations in retention shown in Figure 1b are attributed to the increasing adsorption of the $[C_6C_1IM]^+$ cation onto the C18 stationary phase with higher IL concentrations. This adsorption results in the significant repulsion of positively charged solutes, leading to decreased retention.

It is notable that acebutolol exhibits enhanced retention in the presence of $[C_6C_1IM][Cl]$ (see Figure 1b), considering its $\log P_{o/w}$ value (see Table A1). This behavior, also observed with an XTerra C18 column [13], can be attributed to the incomplete coating of the alkyl-bonded stationary phase by adsorbed imidazolium cations from ILs. This allows hydrophobic interactions between the solutes and C18 chains, as well as with silanols [24]. The acebutolol chemical structure significantly influences these interactions. This compound contains a ketone and an amide group, allowing its oxygen atoms to form hydrogen bonds with protonated silanols. These interactions are particularly pronounced in the absence of both organic solvent and SDS, the latter being able to extensively cover (i.e., mask) residual silanols on the stationary phase. The retention factor is also affected by the intrinsic properties of the C18 stationary phases. The XTerra column features endcapping with methyl groups substituted for one out of every three silanols, a modification not present in the Zorbax column. The resulting effect from the distinct column properties is that acebutolol is the most retained compound on the XTerra column [14], whereas it ranks second in retention on the Zorbax column, following propranolol (not shown in the figure).

3.1.2. Effect on Retention of Mobile Phases Containing Both SDS and $[C_6C_1IM][Cl]$

Figure 2 illustrates the changes in retention on the Zorbax column under varying SDS and $[C_6C_1IM][Cl]$ concentrations. Figure 2a,b show the retention at fixed $[C_6C_1IM][Cl]$ concentrations (0.01 and 0.03 M) with increasing SDS, while Figure 2c,d display the retention at fixed SDS concentrations (0.05 M and 0.20 M) with increasing $[C_6C_1IM][Cl]$. The addition of progressively higher concentrations of SDS and $[C_6C_1IM][Cl]$ to the mobile phase generally leads to the decreased retention of the β -adrenoceptor antagonists, consistent with the trend observed in Figure 1. An exception is observed in Figure 2c (obtained at 0.05 M SDS), where IL concentrations above 0.01 M result in a perceptible increase in retention for acebutolol and carteolol. The XTerra column exhibited similar retention patterns and times under identical conditions (see Figure S2 in the Supplementary Material).

The reduction in retention with increasing concentrations of SDS and $[C_6C_1IM][Cl]$ can be attributed to two main factors: the increased presence of micelles in the mobile phase and the repulsion exerted by the $[C_6C_1IM]^+$ cation on the positively charged molecules of the β -adrenoceptor antagonists. The anionic surfactant (SDS) and the IL cation ($[C_6C_1IM]^+$) compete for adsorption sites on the stationary phase. As the IL/SDS ratio in the mobile phase increases, the repulsive effect of the $[C_6C_1IM]^+$ cation on the β -adrenoceptor antagonists becomes more pronounced, thus influencing retention and elution strength.

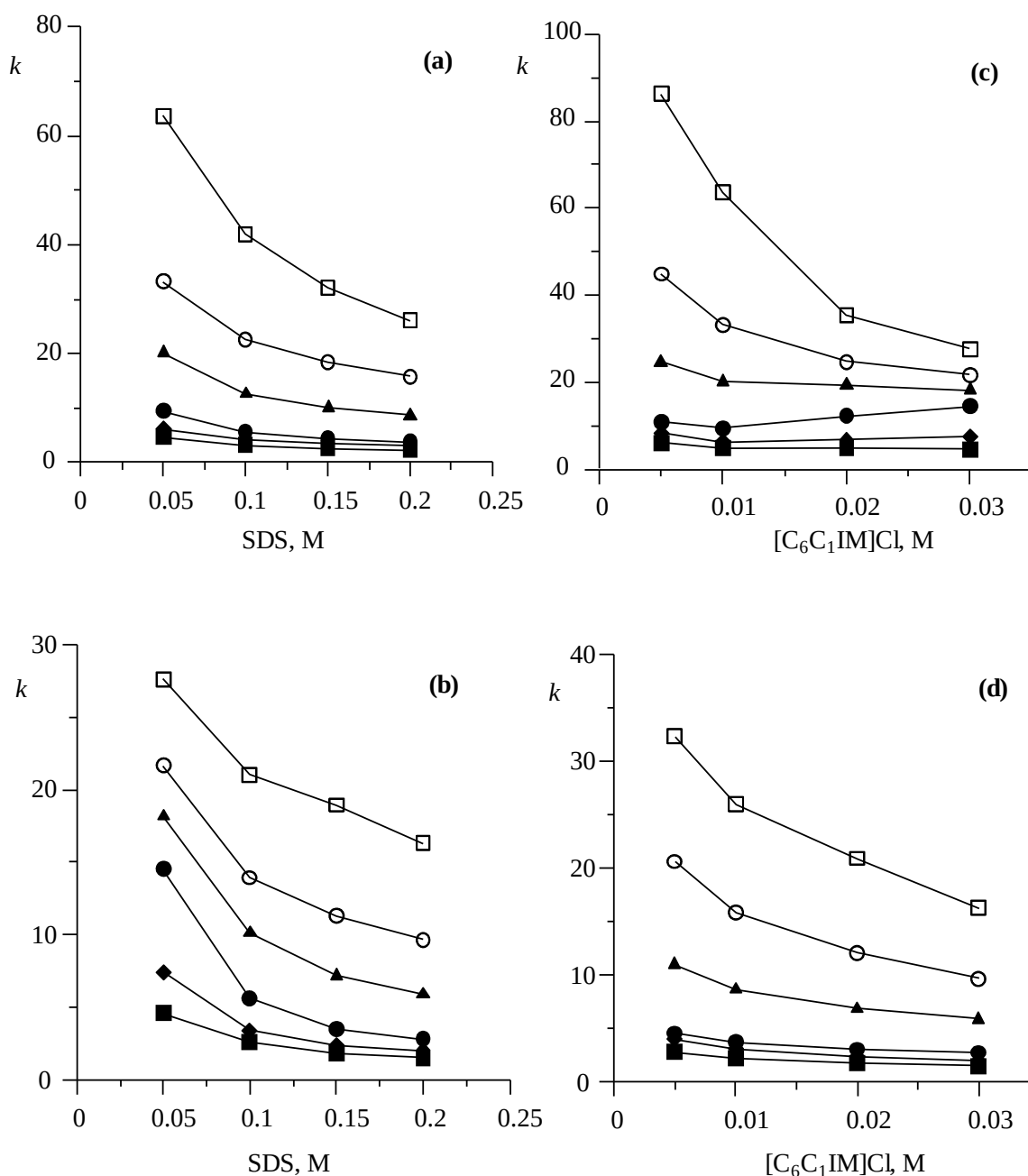


Figure 2. Retention behavior of the β -adrenoceptor antagonists, using aqueous mobile phases containing $[C_6C_1IM][Cl]$ at concentrations of (a) 0.01 M and (b) 0.03 M, with increasing SDS, and containing SDS at concentrations of (c) 0.05 M and (d) 0.20 M, with increasing $[C_6C_1IM][Cl]$. A Zorbax column was used for the analysis. For compound identities, refer to Figure 1.

3.2. Solute–Stationary Phase and Solute–Mobile Phase Interactions

The strength of interactions between solutes and either the stationary phase or mobile phase can be estimated by calculating the equilibrium constants that describe the solute partitioning between these phases during chromatographic separation. This can be achieved using the mathematical model proposed by Arunyanart and Cline-Love in the early years of MLC to elucidate potential retention mechanisms [25]. This model suggests three pseudo-phases in the separation environment: the modified stationary phase, the phase formed by water and the organic solvent, and a third phase (a pseudo-phase) formed by the surfactant micelles [26]. The equations describing solute retention are based on the following two chemical equilibria:



These equilibria involve the association of the solute in bulk water (A) with interaction sites of the stationary phase (S) or with micelles in the mobile phase (M). The constants K_{WS} and K_{AM} associated with Equations (1) and (2), respectively, describe the shift of these equilibria. Based on this, the retention factor can be expressed as follows:

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

where ϕ is the phase ratio (the ratio between the active surface volume of the stationary phase and the dead volume of the column), $[M]$ is the molar concentration of the surfactant monomers forming micelles, and $[S]$ (which is constant or nearly constant above the CMC) is the amount of modified stationary phase binding sites. Equation (3) can be rewritten as follows:

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

where K_{AS} and K_{AM} are known as the solute–stationary phase and solute–micelle association constants, respectively. Equation (4) has been successfully applied in MLC [25,27] and also in RPLC using aqueous–organic mobile phases containing an IL or an amine as additives [28]. Its application in a micellar medium with SDS in the presence of an IL is novel, but the chromatographic separation environment created by both additives conforms to the three-phase model.

Equation (4) can, in principle, be applied in two ways: by keeping the IL concentration constant while varying the SDS concentration or by keeping the SDS concentration constant while varying the IL concentration. However, the change in retention for the more polar β -adrenoceptor antagonists as the IL concentration increases (see Figure 2c, where retention factors decline until reaching 0.01 M IL and then increase) indicates that the $1/k$ relationship versus the $[C_6C_1IM][Cl]$ concentration is not always linear for these solutes and conditions, especially at lower surfactant concentrations. For this reason, we adjusted Equation (4) only for varying SDS concentrations while keeping the IL concentration constant. Figure 3 illustrates some of the linear regressions obtained with both columns. The linear behavior is consistent for all solutes and both columns. However, at the lower IL concentration, the linearity is lost for most polar compounds (carteolol, atenolol, and acebutolol) due to the competition of the IL with the surfactant for adsorption.

Tables 1 and 2 present the K_{AS} and K_{AM} values determined for mobile phases containing SDS and $[C_6C_1IM][Cl]$, along with their corresponding precision, for the Zorbax and XTerra columns, respectively. It is important to note that the estimation of K_{AS} for β -adrenoceptor antagonists eluted solely with SDS was not possible as the y -intercept for Equation (4) was zero for both columns. This indicates a strong solute–stationary phase interaction between the protonated β -adrenoceptor antagonists and the sulfate group of the surfactant adsorbed onto the stationary phase.

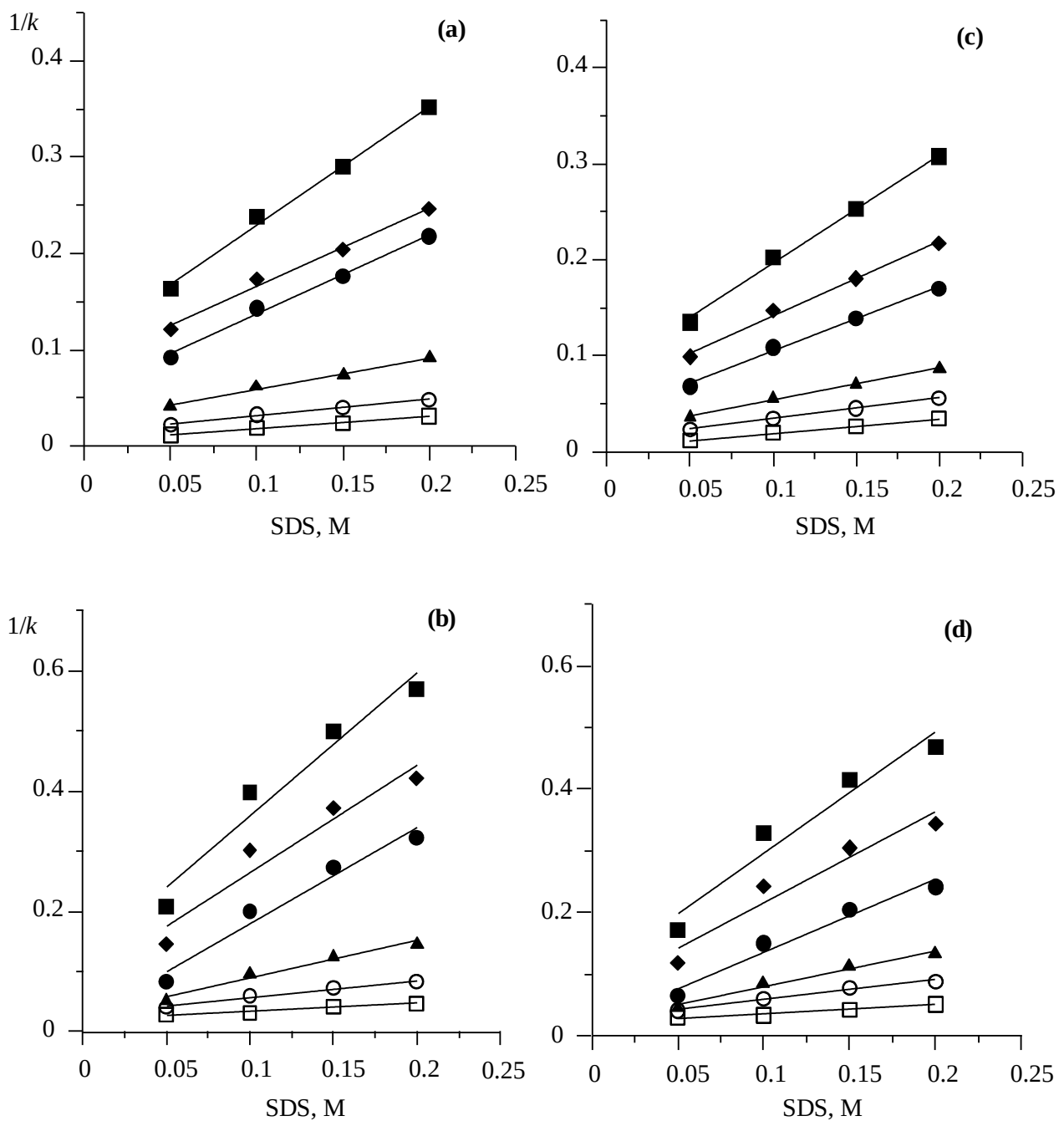


Figure 3. The representation of $1/k$ versus the concentration of SDS for the β -adrenoceptor antagonists, using aqueous mobile phases containing $[C_6C_1IM][Cl]$ at concentrations of 0.005 M (a,c), and 0.02 M (b,d), eluted from the Zorbax column (a,b) and the XTerra column (c,d). For compound identities, refer to Figure 1.

Table 1. Association constants between solutes and modified stationary phase (K_{AS}) and between solutes and surfactant micelles in the mobile phase (K_{AM}) for the Zorbax column in the presence of fixed concentrations of $[C_6C_1IM][Cl]$ and increasing concentrations of SDS.

Compound	0.005 M $[C_6C_1IM][Cl]$		0.01 M $[C_6C_1IM][Cl]$	
	K_{AS}	K_{AM}	K_{AS}	K_{AM}
Acebutolol	18.3 ± 2.3	15.1 ± 2.1	16 ± 4	17 ± 5
Atenolol	9.4 ± 0.7	11.6 ± 1.1	6.8 ± 0.9	10.6 ± 1.7
Carteolol	11.8 ± 1.0	9.6 ± 1.0	8.4 ± 1.2	8.8 ± 1.6
Metoprolol	39 ± 5	12.7 ± 1.7	32 ± 6	14 ± 3
Oxprenolol	69 ± 6	11.8 ± 1.3	49 ± 6	10.7 ± 1.5
Propranolol	186 ± 16	23.6 ± 2.1	119 ± 6	17.9 ± 1.0
	0.02 M $[C_6C_1IM][Cl]$		0.03 M $[C_6C_1IM][Cl]$	
	K_{AS}	K_{AM}	K_{AS}	K_{AM}
Acebutolol	50 ± 70	80 ± 110	—	—
Atenolol	8 ± 4	20 ± 9	13 ± 5	40 ± 15
Carteolol	12 ± 6	21 ± 12	30 ± 40	80 ± 100
Metoprolol	38 ± 14	23 ± 9	51 ± 15	39 ± 12
Oxprenolol	35 ± 4	10.0 ± 1.5	33 ± 5	12.5 ± 2.3
Propranolol	49 ± 4	6.5 ± 0.8	34 ± 3	5.5 ± 0.7

Table 2. Association constants between solutes and modified stationary phase (K_{AS}) and between solutes and surfactant micelles in the mobile phase (K_{AM}) for the XTerra column in the presence of fixed concentrations of $[C_6C_1IM][Cl]$ and increasing concentrations of SDS.

Compound	0.005 M $[C_6C_1IM][Cl]$		0.01 M $[C_6C_1IM][Cl]$	
	K_{AS}	K_{AM}	K_{AS}	K_{AM}
Acebutolol	27 ± 3	17.8 ± 2.4	23 ± 6	19 ± 5
Atenolol	12.1 ± 1.0	13.8 ± 1.3	8.4 ± 1.4	11.4 ± 2.2
Carteolol	15.6 ± 1.4	12.1 ± 1.2	11.4 ± 1.8	10.9 ± 2.0
Metoprolol	50 ± 5	17.1 ± 2.0	40 ± 8	17 ± 4
Oxprenolol	79 ± 6	17.3 ± 1.4	53 ± 7	14.1 ± 2.0
Propranolol	220 ± 40	34.0 ± 2.1	130 ± 170	23.1 ± 1.8
	0.02 M $[C_6C_1IM][Cl]$		0.03 M $[C_6C_1IM][Cl]$	
	K_{AS}	K_{AM}	K_{AS}	K_{AM}
Acebutolol	50 ± 60	60 ± 70	—	—
Atenolol	10 ± 5	19 ± 9	16 ± 6	39 ± 16
Carteolol	15 ± 8	22 ± 13	40 ± 40	80 ± 90
Metoprolol	46 ± 18	27 ± 11	70 ± 30	49 ± 18
Oxprenolol	37 ± 5	12 ± 2	35 ± 6	15 ± 3
Propranolol	50 ± 30	7.8 ± 1.0	40 ± 70	6.6 ± 0.9

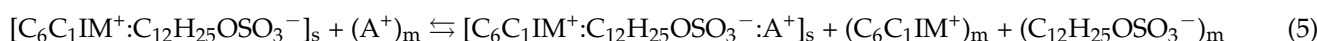
The data in Tables 1 and 2 reveal no significant differences between the two studied columns. The K_{AS} constants were generally higher than the K_{AM} constants, with some exceptions, suggesting a greater affinity of solutes for the stationary phases modified by SDS monomers and adsorbed IL cation. However, the K_{AS} values varied noticeably for the most polar β -adrenoceptor antagonists when the concentration of $[C_6C_1IM][Cl]$ reached 0.03 M. Conversely, for the most hydrophobic solutes (oxprenolol and propranolol), K_{AS} values decreased progressively with increasing IL concentrations. Regarding the K_{AM} constants, these increased significantly for the most polar β -adrenoceptor antagonists starting at 0.02 M $[C_6C_1IM][Cl]$ in both columns. For oxprenolol, the K_{AM} values did not vary appreciably across all tested IL concentrations, while for propranolol, the values decreased progressively with increasing IL concentrations.

3.3. Estimation of Hydrophobic and Ionic Interactions

In RPLC with ionic additives, basic compounds experience chromatography via a mixed retention mechanism that simultaneously involves hydrophobic and ionic interactions as main contributions. To quantitatively describe this process, mathematical models have been proposed based on the assumption of solute interaction sites on the stationary phase. A model considering two interaction sites was initially proposed by Sokolowski and Wahlund [29] and was later refined by Horváth et al. [30,31]. This model postulates the existence of two distinct interaction sites on the alkyl-bonded stationary phases as follows:

- (i) The hydrocarbon chains of the stationary phase, which provide a site for hydrophobic interactions.
- (ii) The C18 chains modified through the adsorption of anionic SDS monomers and IL cations, enabling ionic interactions with the cationic solutes.

We have modified the original mathematical formulation of the interaction site model to include the presence of both the SDS anion and $[C_6C_1IM]^+$ cation. These ions are integrated into the stationary phase, where cationic solutes A^+ compete for binding:



The equilibrium constant for this process is as follows:

$$K_A = \frac{[A^+]_s [C_6C_1IM^+]_m [C_{12}H_{25}OSO_3^-]_m}{[C_6C_1IM^+:C_{12}H_{25}OSO_3^-]_s [A^+]_m} \quad (6)$$

where $[C_6C_1IM^+]_m$ and $[C_{12}H_{25}OSO_3^-]_m$ represent the concentration of the IL and surfactant in the mobile phase. This two-site interaction model elucidates the mixed-mode retention mechanism, offering a quantitative framework for predicting solute behavior in RPLC with ionic additives. The hydrophobic and ionic contributions to retention operate independently, allowing the retention factor to be expressed as the sum of these separate contributions as follows:

$$k = \phi_{RP} K_{RP} + \phi_{IEX} K_{IEX} = k_{RP} + k_{IEX} \quad (7)$$

where K_{RP} represents the distribution constant of the reversed-phase process and K_{IEX} denotes the distribution constant for the ionic process. Upon rearrangement, Equation (6) leads to

$$K_{IEX} = \frac{[A^+]_s}{[A^+]_m} = \frac{K_A [C_6C_1IM^+:C_{12}H_{25}OSO_3^-]_s}{[C_6C_1IM^+]_m [C_{12}H_{25}OSO_3^-]_m} \quad (8)$$

Therefore, the retention factor for the ionic interactions can be expressed as follows:

$$k_{IEX} = \phi_{IEX} K_{IEX} = \phi_{IEX} \frac{K_A [C_6C_1IM^+:C_{12}H_{25}OSO_3^-]_s}{[C_6C_1IM^+]_m [C_{12}H_{25}OSO_3^-]_m} \quad (9)$$

If the IL amount in the mobile phase is fixed, then

$$k_{IEX} = \frac{B_{IEX}}{[C_{12}H_{25}OSO_3^-]_m} \quad (10)$$

Equation (7) can then be rewritten as

$$k = k_{RP} + \frac{B_{IEX}}{[C_{12}H_{25}OSO_3^-]_m} \quad (11)$$

In logarithmic form,

$$\log k = \log(k_{RP} + \frac{B_{IEX}}{[C_{12}H_{25}OSO_3^-]_m}) \quad (12)$$

Given that k_{RP} remains relatively constant with varying additive concentrations, the first term inside the parentheses of Equation (12) stays nearly constant, while the second term varies inversely with the concentration of SDS in the mobile phase. Consequently, the slope of the relationship between $\log k$ and $\log [C_{12}H_{25}OSO_3^-]_m$ will depend on the dominant interaction. If k_{RP} is much greater than k_{IEX} , the slope predicted by Equation (12) will be close to zero. Conversely, if the ionic interaction dominates over the hydrophobic one, the model will yield a slope close to -1 .

Equation (12) was applied to mobile phases with fixed concentrations of $[C_6C_1IM][Cl]$ and varying concentrations of SDS. We should note that although the two-site model was originally developed for cationic additives, we have considered the concentration of SDS based on the principle that this anionic additive is able to modify hydrophobic and ionic interactions with the stationary phase similarly to cationic additives. Consequently, the correlation versus the logarithm of anionic additive concentration conforms the fundamental principle of the Horvath's model. Figure 4 illustrates the relationship between $\log k$ and the logarithm of the SDS concentration for β -adrenoceptor antagonists analyzed with mobile phases containing both additives. The slope values obtained are summarized in Tables 3 and 4 for the Zorbax and XTerra columns, respectively. These tables also include the results for mobile phases containing only SDS. In the absence of IL (micellar mobile phases containing only SDS), the slope values approached -1 for both columns. This indicates that the attraction of β -adrenoceptor antagonists to the adsorbed SDS monomers on the stationary phase and micelles in the mobile phase dominate the retention mechanism. Conversely, when both SDS and $[C_6C_1IM][Cl]$ were present, the slopes deviated significantly from -1 , with similar values for both columns, suggesting a mixed retention mechanism involving both hydrophobic and ionic interactions. However, as the concentration of $[C_6C_1IM][Cl]$ increased, the slopes progressively approached -1 for the more polar β -adrenoceptor antagonists (acebutolol, atenolol, carteolol, and metoprolol). This trend implies that the attraction of β -adrenoceptor antagonists to SDS became increasingly significant for these polar solutes.

Table 3. Slope (m) and determination coefficient for the $\log k$ vs. $\log [SDS]$ regression (Equation (9)) for the β -adrenoceptor antagonists analyzed with the Zorbax column in the presence of fixed concentrations of $[C_6C_1IM][Cl]$.

Compound	Without IL		0.005 M $[C_6C_1IM][Cl]$		0.01 M $[C_6C_1IM][Cl]$	
	m	R^2	m	R^2	m	R^2
Acebutolol	-1.07 ± 0.08	0.9899	-0.613 ± 0.022	0.9973	-0.67 ± 0.04	0.9942
Atenolol	-1.10 ± 0.09	0.9857	-0.545 ± 0.018	0.9978	-0.538 ± 0.013	0.9988
Carteolol	-1.04 ± 0.07	0.9919	-0.498 ± 0.023	0.9957	-0.497 ± 0.020	0.9968
Metoprolol	-1.07 ± 0.07	0.9905	-0.571 ± 0.023	0.9966	-0.61 ± 0.03	0.9956
Oxprenolol	–	–	-0.549 ± 0.023	0.9966	-0.536 ± 0.007	0.9996
Propranolol	–	–	-0.697 ± 0.024	0.9977	-0.641 ± 0.018	0.9984
	0.02 M $[C_6C_1IM][Cl]$		0.03 M $[C_6C_1IM][Cl]$			
	m	R^2	m	R^2		
Acebutolol	-1.00 ± 0.12	0.9727	-1.21 ± 0.08	0.9920		
Atenolol	-0.74 ± 0.08	0.9762	-0.83 ± 0.03	0.9975		
Carteolol	-0.77 ± 0.11	0.9623	-0.97 ± 0.07	0.9884		
Metoprolol	-0.75 ± 0.05	0.9897	-0.820 ± 0.019	0.9989		
Oxprenolol	-0.523 ± 0.011	0.9992	-0.583 ± 0.021	0.9975		
Propranolol	-0.36 ± 0.07	0.9321	-0.367 ± 0.024	0.9914		

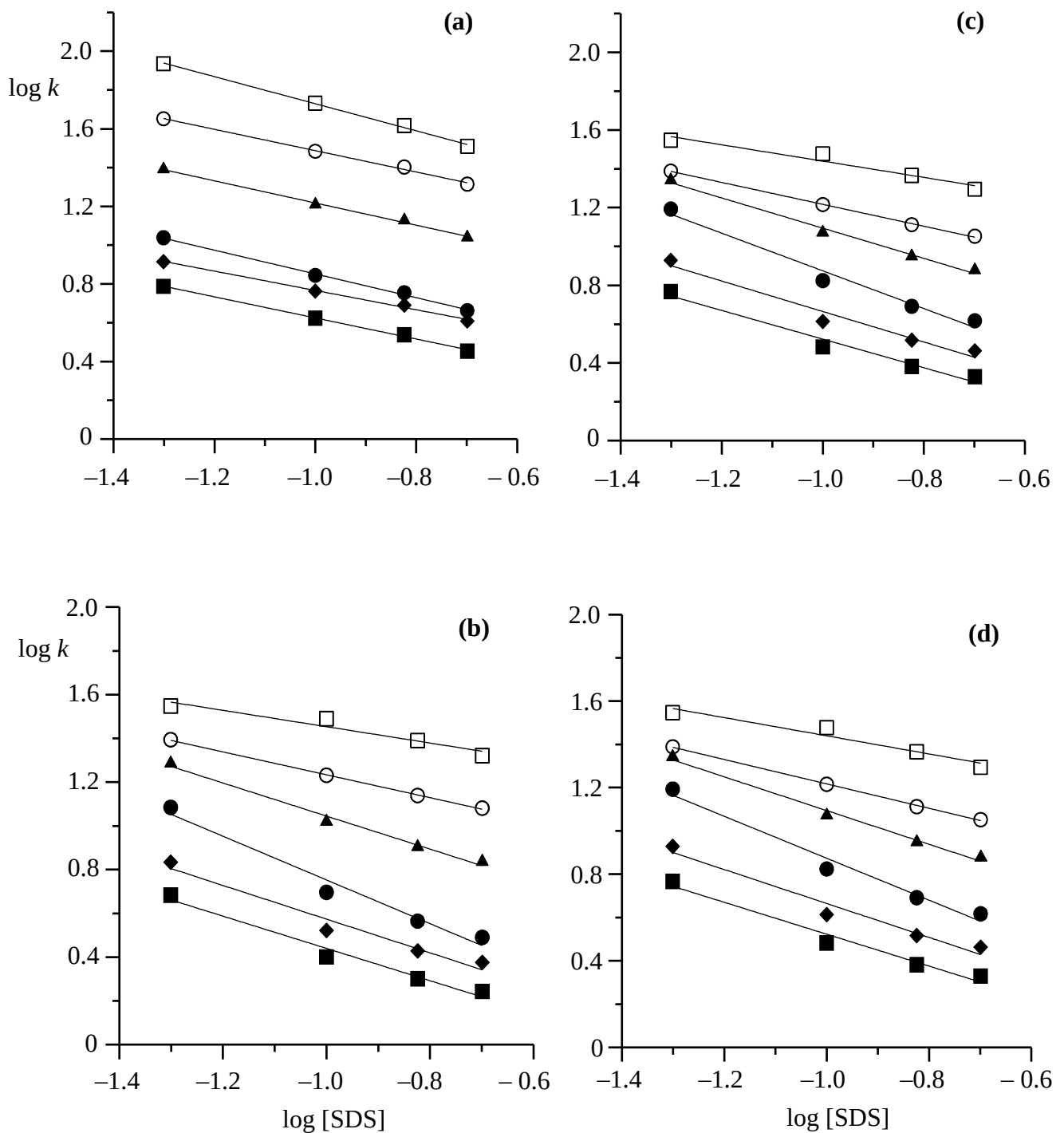


Figure 4. Representation of $\log k$ versus $\log [\text{SDS}]$ for the β -adrenoceptor antagonists, using aqueous mobile phases containing $[\text{C}_6\text{C}_1\text{IM}][\text{Cl}]$ at concentrations of 0.005 M (a,c) and 0.02 M (b,d), eluted from the Zorbax column (a,b) and XTerra column (c,d). For compound identities, refer to Figure 1.

Table 4. Slope (m) and determination coefficient for the log k vs. log [SDS] regression (Equation (9)) for the β -adrenoceptor antagonists obtained with the XTerra column in the presence of fixed concentrations of $[C_6C_1IM][Cl]$.

Compound	Without IL		0.005 M $[C_6C_1IM][Cl]$		0.01 M $[C_6C_1IM][Cl]$	
	m	R^2	m	R^2	m	R^2
Acebutolol	-1.14 ± 0.11	0.9824	-0.588 ± 0.012	0.9992	-0.562 ± 0.022	0.9971
Atenolol	-1.22 ± 0.13	0.9786	-0.558 ± 0.012	0.9990	-0.551 ± 0.020	0.9974
Carteolol	-1.16 ± 0.12	0.9793	-0.654 ± 0.011	0.9994	-0.70 ± 0.03	0.9956
Metoprolol	-1.23 ± 0.13	0.9771	-0.641 ± 0.012	0.9993	-0.659 ± 0.021	0.9979
Oxprenolol	-1.25 ± 0.13	0.9801	-0.636 ± 0.015	0.9989	-0.602 ± 0.006	0.9998
Propranolol	-1.29 ± 0.13	0.9807	-0.770 ± 0.014	0.9994	-0.695 ± 0.023	0.9979
	0.02 M $[C_6C_1IM][Cl]$		0.03 M $[C_6C_1IM][Cl]$			
	m	R^2	m	R^2		
Acebutolol	-0.74 ± 0.09	0.9736	-0.83 ± 0.03	0.9969		
Atenolol	-0.78 ± 0.11	0.9644	-0.96 ± 0.08	0.9879		
Carteolol	-0.97 ± 0.11	0.9768	-1.13 ± 0.06	0.9946		
Metoprolol	-0.78 ± 0.05	0.9916	-0.857 ± 0.024	0.9984		
Oxprenolol	-0.563 ± 0.012	0.9990	-0.619 ± 0.022	0.9974		
Propranolol	-0.419 ± 0.072	0.9440	-0.415 ± 0.022	0.9944		

3.4. Separation of β -Adrenoceptor Antagonists with Aqueous Mobile Phases in RPLC

Figure 5 depicts chromatograms for a mixture of six β -adrenoceptor antagonists analyzed using mobile phases buffered at pH 3.0, containing 0.15 M SDS and 0.02 M $[C_6C_1IM][Cl]$. The chromatographic separations were performed on both the Zorbax (Figure 5a) and XTerra (Figure 5b) columns. The β -adrenoceptor antagonists were injected at concentrations ranging from 10 to 150 $\mu\text{g mL}^{-1}$. Although 225 nm is typically optimal for the UV detection of the β -adrenoceptor antagonists, a detection wavelength of 254 nm was used to avoid interference from the IL absorption at the lower wavelength.

The chromatograms demonstrate that the combination of SDS and IL effectively separates the six basic cationic β -adrenoceptor antagonists on both columns, eliminating the need for an organic solvent. The peaks are well resolved and symmetric, with minimal overlap. The elution order of the β -adrenoceptor antagonists corresponds to their polarity, as indicated by their log $P_{o/w}$ values in Table A1. The XTerra column achieved slightly shorter analysis times compared to the Zorbax column. However, for the more polar β -adrenoceptor antagonists (atenolol, carteolol, and acebutolol), retention times were similar on both columns. Notably, the Zorbax column offered superior resolution for these compounds within the same elution window. It is important to highlight that aqueous mobile phases containing solely SDS or $[C_6C_1IM][Cl]$ were insufficient for analyzing the six β -adrenoceptor antagonists due to excessively long retention times. Therefore, incorporating $[C_6C_1IM][Cl]$ into the SDS micellar mobile phase is essential for achieving efficient separations and sufficiently short retention times without the need of organic solvents.

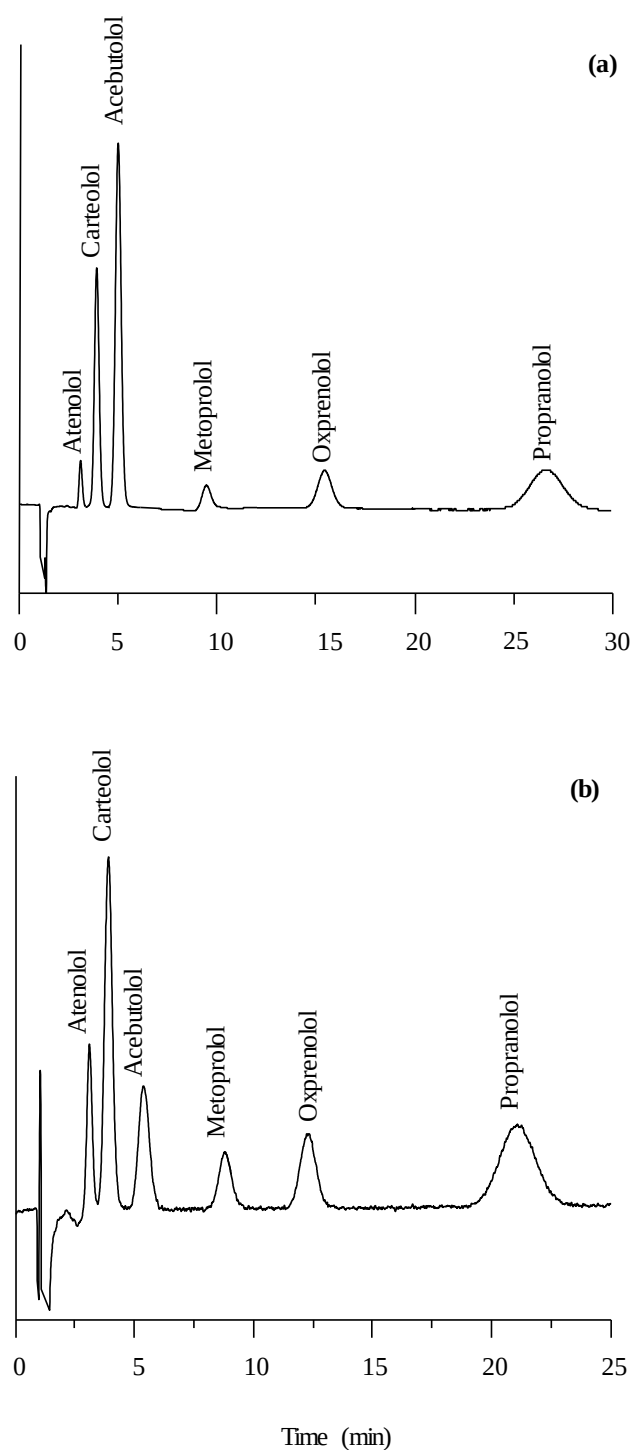


Figure 5. Chromatograms of a mixture of the six β -adrenoceptor antagonists analyzed with aqueous mobile phases containing 0.15 M SDS and 0.02 M $[\text{C}_6\text{C}_1\text{IM}][\text{Cl}]$ without an organic solvent, using the columns (a) Zorbax and (b) XTerra. Solute concentrations: atenolol— $80 \mu\text{g mL}^{-1}$; carteolol and acebutolol— $10 \mu\text{g mL}^{-1}$; and metoprolol, oxprenolol, and propranolol— $150 \mu\text{g mL}^{-1}$.

4. Conclusions

The study investigated the behavior of aqueous liquid chromatographic systems comprising the anionic surfactant SDS and the ionic liquid $[\text{C}_6\text{C}_1\text{IM}][\text{Cl}]$ in the absence of organic solvents to better understand the interactions of a series of positively charged basic β -adrenoceptor antagonists with varying polarities using two different C18 columns.

Simple mathematical models were employed to estimate equilibrium parameters and assess both hydrophobic and ionic contributions to retention.

The Arunyanart and Cline-Love mathematical model confirmed that solute interactions with the stationary phase and mobile phase in the SDS/IL system follow the classic three-phase model used in MLC when no IL is added. This model provided insights into the mixed retention mechanisms involved. Meanwhile, the two-site model validated the mixed retention mechanism when SDS and [C₆C₁IM][Cl] were combined. It showed that at higher concentrations of the IL, the attraction to SDS became increasingly significant, particularly for more polar solutes.

The findings revealed that using SDS above its CMC to form micelles, in conjunction with the IL [C₆C₁IM][Cl], effectively separates the β -adrenoceptor antagonists without the need for organic solvents. The use of [C₆C₁IM][Cl] at relatively low concentrations, combined with SDS, provides a good resolution of β -adrenoceptor antagonists within reasonable analysis times. This approach not only enhances the efficiency of the separation process but also contributes to a more environmentally sustainable analytical procedure by eliminating the need for organic solvents.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/separations11100300/s1>, Figure S1: Retention behavior of several β -adrenoceptor antagonists, eluted with mobile phases containing only SDS, or [C₆C₁IM][Cl], at increasing concentrations; Figure S2: Retention behavior of the β -adrenoceptor antagonists, using aqueous mobile phases containing [C₆C₁IM][Cl] and SDS.

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Appendix A

Table A1. Structures, dissociation constants (pK_a), and octanol–water partition coefficients ($\log P_{o/w}$) for the β -adrenoceptor antagonists studied, arranged from higher to lower polarity.

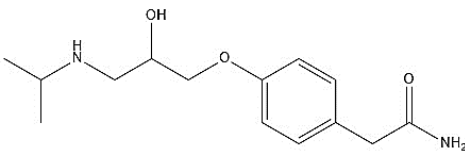
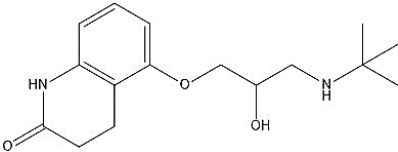
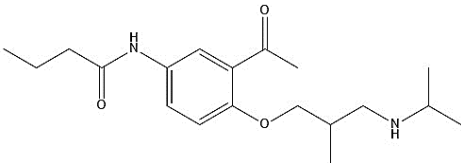
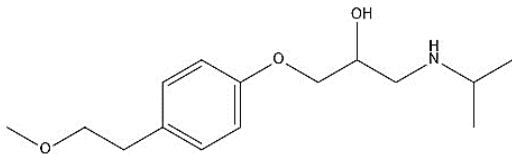
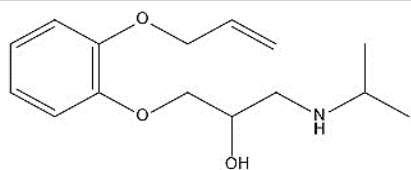
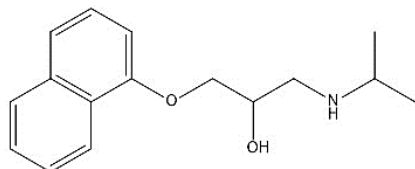
β -Adrenoceptor Antagonists	Structure	pK_a ^a	$\log P_{o/w}$ ^a
Atenolol		9.17	0.25
Carteolol		9.24	1.49

Table A1. Cont.

β -Adrenoceptor Antagonists	Structure	pK_a^a	$\log P_{o/w}^a$
Acebutolol		9.24	1.83
Metoprolol		9.31	1.90
Oxprenolol		9.08	2.30
Propranolol		9.25	3.41

^a From Ref. [32]

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