# On the use of ionic liquids as mobile phase additives in high-performance liquid chromatography. A review

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## Abstract

The popularity of ionic liquids (ILs) has grown during the last decades in several analytical separation techniques. Consequently, the number of reports devoted to the applications of ILs is still increasing. This review is focused on the use of ILs (mainly imidazolium-based associated to chloride and tetrafluoroborate) as mobile phase additives in high-performance liquid chromatography (HPLC). In this approach, ILs just function as salts, but keep several kinds of intermolecular interactions, which are useful for chromatographic separations. Both cation and anion can be adsorbed on the stationary phase, creating a bilayer. This gives rise to hydrophobic, electrostatic and other specific interactions with the stationary phase and solutes, which modify the retention behaviour and peak shape. This review updates the advances in this field, with emphasis on topics not always deeply considered in the literature, such as the mechanisms of retention, the estimation of the suppressing potency of silanols, modelling and optimisation of the chromatographic performance, and the comparison with other additives traditionally used to avoid the silanol problem.

*Keywords:* High-performance liquid chromatography; Ionic liquids; Additives; Mechanisms of retention; Silanol suppression; Optimisation

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Abbreviations: $C_1C_1IM$: 1,3-dimethylimidazolium; $C_2C_1IM$: 1-ethyl-3-methylimidazolium; $C_4C_1IM$: 1-butyl-3-methylimidazolium; $C_6C_1IM$: 1-hexyl-3-methylimidazolium; $C_8C_1IM$: 1-octyl-3-methylimidazolium; $C_{10}C_1IM$: 1-decyl-3-methylimidazolium; $C_{12}C_1IM$: 1-dodecyl-3-methylimidazolium; $C_2PY$: ethyl-pyridinium; $C_4PY$: butyl-pyridinium; $C_4C_1PY$: N-butyl-3-methylpyridinium; TEA: triethylamine; DMOA: dimethyloctylamine.

## 1. Introduction

The term ionic liquid concerns, in principle, inorganic and organic molten salts, composed entirely of ions, with an exactly equal number of positive and negative charges. The melting point of salts containing exclusively inorganic ions, such as NaCl and $LiClO_4$, is above or well above 400K (temperatures at which they are called molten salts). In contrast, the crystal packing of salts formed by large dissymmetrical nitrogen or phosphorous heterocyclic rings (imidazolium, pyridinium or pyrrolidinium), or quaternary ammonium or phosphonium cations, all attaching different alkyl chains, combined with a variety of inorganic ($Br^-$, $Cl^-$, $NO_3^-$, $ClO_4^-$, $AlCl_4^-$, $BF_4^-$, or $PF_6^-$), or organic (triflate, dicyanamide, acetate, trifluoroacetate, and trifluoromethylsulphate) anions, is much less stable. This results in a significant decrease of the melting temperature. Depending on the kind of cation and the length of the alkyl chain, the resulting melting point can be above or below room temperature (298K). In this case, the salts are called room-temperature ionic liquids (RTILs). A more general term including those reagents with melting points above or below 298K is ionic liquids (ILs) [1–3].

Besides the attractive feature of the low melting point of ILs that has given rise to a new class of solvents with a non-molecular nature and low vapour pressure at room temperature, high thermal stability and wide temperature range in the liquid state, the large variety of possible combinations of cations and anions (and consequently, new liquid reagents), with physico-chemical properties strongly depending on both the nature and size of the cation and

anion should be highlighted. Depending on its nature, the resulting IL may have hydrophobic or hydrophilic properties, higher or lower viscosity and miscibility with water or organic solvents, and specific electrochemical properties, among other that will be commented below [4–7]. This easy adjustability has gained for this class of solvents the term “designer” or “tailor-made” solvents. More than two thousand ILs are known today, but it has been estimated that up to $10^{18}$ combinations are possible [4]. This makes this class of substances one of the biggest in chemical chest of substances.

However, it should be commented that the claim for ILs of been “green solvents” (mainly due to their low volatility) is a bit controversial [8,9]. Not all ILs are safe and non-toxic, especially during their synthesis. Also, there are several reports dealing on the bioactivity of the anion/cation IL combinations, their carcinogenicity, cytotoxicity, genotoxicity and teratological effects [10]. But, indeed, there is great interest in synthesizing really “green ILs”.

Besides the success of ILs in the fields of organic synthesis and catalysis, these reagents have enjoyed high applicability in a variety of analytical techniques, due to several reasons [4,6,11–16]:

(i) Their electrical conductivity, which allow their use as electrolytes in capillary electrophoresis (CE) and electrochemical techniques.

(ii) Their coating properties related to their viscosity and surface tension, which make them appropriate for gas chromatography (GC).

(iii) Their solubility in a variety of solvents (aqueous and non-aqueous), and their solvating properties, which are important to stabilise, pre-concentrate, or extract analytes.

(iv) The electrostatic interactions associated with the cation and anion moieties comprised within the ILs, which affect the selectivity.

(v) The possibility of imparting specific selectivity by the incorporation of different substituents in the ILs, from polar groups (to promote dipolar interactions with polar

analytes) to long aliphatic alkyl chains (to enhance their ability to undergo dispersion interactions with non-polar analytes), and the capability of chiral behaviour, which allows the analysis of isomers.

All these features and others are making ILs an indispensable alternative to traditional organic solvents (which often possess high toxicity and volatility) in many sample preparation (extraction, microextraction and supported membranes), detection (electrochemical sensing and mass spectrometry, MS), and separation (capillary electrophoresis, and gas and liquid chromatography) techniques. This can be easily checked by the exponential increase in the number of publications in these fields in the last 5–10 years.

This review will focus on the use of ILs as mobile phase additives in high-performance liquid chromatography (HPLC). It should be first said that since the first reports on this particular application, several authors have summarised the published advances in several reviews, together with other analytical techniques [5,7,11,12,17,18]. Reviews have been also published on the use of ILs as mobile phase additives or immobilised on HPLC stationary phases, the former approach being found the most convenient and popular [19–25]. The HPLC analysis of ILs has also called some attention [26–30]. Based on our own laboratory experience in the use of ILs as mobile phase additives in HPLC and the study of the published reports, we present and discuss below the most recent knowledge on this topic.

Before starting, it should be noted that ILs used as mobile phase additives in HPLC (also in CE running buffers and as complexing agents for MS detection of anions, or those immobilised in solid-phase microextraction fibers and HPLC stationary phases) are merely starting materials and do not remain liquids. Thus, as additives in HPLC, ILs just function as salts and some of their special physical properties as solvents, such as their low volatility are no longer important [6].

## 2. A historical overview on the idea of using ILs in HPLC

It is first convenient to describe some facts that are important in the development of the idea of using ILs as mobile phase modifiers and/or additives in HPLC. Wilkes and Zaworotko were the first to report the synthesis of air and water stable ILs with imidazolium or pyridinium cations associated to acetate or $BF_4^-$ [31]. This was the starting point for the current research on the synthesis of different types of ILs useful in HPLC. However, the first attempts to use ILs in HPLC were not very promising. The main problem in considering them as possible mobile phase modifiers is their high viscosity, at least one order of magnitude higher than that of methanol or acetonitrile. In 1986, Poole et al. proved first the use of ILs in HPLC, using a microcolumn and a series of alkylammonium-based ILs with nitrate and thiocyanate anions (with the lowest viscosity), mixed with reversed-phase liquid chromatography (RPLC) solvents (water, methanol, acetonitrile, tetrahydrofuran and dichloromethane) [32,33]. The concentration of these ILs was maintained in the 0.5–1.5% v/v range to avoid a significant increase in the viscosity of the mobile phase, and the flow rate was at 0.05 mL $min^{-1}$, which resulted in acceptable back-pressure. The authors evaluated the solvent properties of the ILs and found that the selectivity was controlled by proton acceptor-donor and dispersive interactions that depended on the size of the IL cation and the nature of the IL anion [33]. They also found that ILs containing nitrate as anion act more like water, while ILs associated to thiocyanate act more like organic solvents.

ILs, such as ethylammonium nitrate (with similar elution strength as tetrahydrofuran) and acetate (similar to methanol) in the 20–80% v/v range (in water) [34–37], and 5% v/v methylammonium formate [38], were later tried as RPLC mobile phases, without better results. Besides the high viscosity, other disadvantages were found in the use of ILs as modifiers in HPLC, including the interference in UV detection since many ILs possess UV chromophores, the corrosion of the metallic parts of the HPLC system after extensive use, and

the high cost of ILs. Other issues were the poor baseline stability, the weaker elution strength compared to common organic solvents, the rapid deterioration of the silica-based packing, and the poor efficiencies. It was evident that ILs have little or no interest and no potential in replacing methanol or acetonitrile in routine HPLC.

More recently, in the search for efficient suppressors of free silanols, He et al. turned their attention to the use of imidazolium-based ILs associated to $BF_4^-$, in small amount (approximately, 0.5% v/v), as mobile phase additives in HPLC [39,40]. These ILs are water-stable compounds soluble in typical RPLC solvents and exhibit marked ability to participate in Coulombic interactions. The authors found that ILs effectively shield residual silanols and improve the peak profiles, and explained with large detail the reason of the observed decrease in peak tailing, reduction of band broadening and analysis time, with resolution enhancements, as due to the coating of the alkyl-bonded surface of $C_{18}$ stationary phases with the IL cation to form a weak bilayer that competes with the solute molecules.

Not less significant are two reports by Kaliszan et al., describing ILs ($C_1C_1IM{\cdot}BF_4$, $C_2C_1IM{\cdot}BF_4$, and $C_6C_1IM{\cdot}BF_4$) as additives in the mobile phase to suppress the free silanol effects on the retention of basic compounds in thin layer chromatography (TLC) and HPLC [41,42]. The first report focused mainly on the analysis of basic compounds by TLC, using either bare silica or $C_{18}$ plates, and acetonitrile as solvent modifier. The analytes were not eluted even with pure acetonitrile, while ILs-modified stationary phases decreased the retention in both normal and reversed-phase TLC, more effectively than alkylamines such as TEA, DMOA, and ammonia. This, together with the detailed study on the dual character of ILs in HPLC performed by two authors of this work, 10 years ago [43] (described in Section 4.2) triggered the interest of ILs as mobile phase additives in HPLC. Table 1 collects the reported procedures up-to-date on the use of ILs as mobile phase additives in HPLC. As observed, in most cases the analyses were carried out under isocratic conditions.

## 3. The silanol problem in RPLC

RPLC analysis is based mainly on the hydrophobic interaction of solutes with the alkyl-bonded layer of the stationary phase, together with the solving power of the organic solvent. However, the stationary phase support can add another mechanism to the retention. Thus, it is not possible to functionalise all silanols on the silica support used in most RPLC columns, making some silanols accessible to the solutes and mobile phase components [75,76]. Residual silanols are weakly to strongly ionised within the working pH range of typical RPLC columns ($2.5 < pH < 7.5$). This gives rise to a negatively charged stationary phase, which can act as a weak cation-exchanger that increases the retention of protonated basic compounds, which are positively charged [75–79]. Also, the chromatographic peaks of these compounds are broad and tailing, behaviour that has been explained by the slow kinetics of the sorption-desorption equilibria of cationic solutes with silanols. The poor peak profiles affect considerably peak resolution in both TLC and HPLC. The problem is of considerable importance, since a significant fraction of the drugs used in modern therapy and a large number of compounds of biomedical, biological and environmental significance have a basic character, being protonated at the usual working pH in RPLC. The problem concerns even the most modern highly purified silica supports [80]. Although residual silanols are often shielded from solutes by the steric hindrance of the bonded ligands, even a small amount of silanols present in the stationary phase can cause decreased retention, peak broadening and asymmetry for basic compounds.

The solution to the silanol problem could be working with stationary phases non-based on silica, but still silica-based columns are the most common in the laboratories. Frequently, the separations are performed with an acidic mobile phase at pH close to 3 to decrease silanol dissociation, but in these conditions silanol activity is still important [75,79]. Another practical and highly extended practice is the addition of ionic (cationic or anionic) additives

[81]. These can interact with the stationary phase through two main mechanisms, which can take place simultaneously: (i) direct neutralisation of the anionic silanol sites (only for cationic additives), which blocks ion-exchange processes with solutes, and (ii) hydrophobic interaction of the cation or anion in the additive with the alkyl-bonded stationary phase, which creates a charged bilayer that prevents the penetration of the basic compounds to reach the silanol sites. All these interactions give rise to changes in the chromatographic behaviour, related to the retention time and peak profile, as will be described in the following sections for ILs.

## 4. Mechanisms of retention

### *4.1. Types of interactions*

ILs at small concentration does not change the mobile phase viscosity drastically and can be applied in HPLC conditions. Also, they keep several kinds of intermolecular interactions, which are useful for chromatographic separations. In aqueous mobile phases, often the properties of the cations are taken as the properties of the ILs themselves, but it should not be forgotten that ILs are made of a cation and an anion. In most cases, they are constituted of a large imidazolium (or pyridinium) cation, associated with a relatively large anion as $BF_4^-$, $PF_6^-$, $Cl^-$ or $Br^-$ (Table 1). Therefore, ILs have a dual nature, which means that both species may contribute to the chromatographic behaviour of analytes [43].

When an IL is added to the flowing mobile phase, the chromatographic system is dynamically modified. This means that the IL will interact uniformly with the components in the mobile phase and with the surface of the stationary phase, forming a pseudo-stationary phase [39]. This changes the properties of the chromatographic system, leading to completely different mechanisms of retention. In fact, ILs play a multiplicity of roles (Fig. 1). Both cation and anion can be adsorbed on the stationary phase, which creates a bilayer, positively or

negatively charged depending on the relative strength of the adsorption of cation and anion, respectively. The IL cation can interact through specific electrostatic interactions with the silanols on the alkyl-bonded silica surface, competing with the polar group of basic solutes. At the same time, different alkyl groups of the heterocyclic ring or quaternary cation in the IL can interact with the non-polar alkyl groups of the stationary phase through hydrophobic and other unspecific interactions [5,17,19,23,39,82].

In addition to the interactions that exist in conventional organic solvents (hydrogen bonding, dipole-dipole and van der Waals interactions), ILs also can establish ionic interactions (electrostatic attraction or repulsion) with analytes. Therefore, they cannot be adequately characterised on the basis of polarity or any single parameter. ILs are complex entities compared with the relatively simple solvents used in most chromatographic processes, capable of undergoing multiple solvation interactions to separate polar molecules. These interactions make them also highly miscible with polar solvents. At the same time, the presence of the alkyl chain on the cation determines the solubility of the ILs in less polar solvents. Hydrogen bonding is also thought to exist between the oxygen or halide atoms on the anion and the hydrogen atoms on the imidazolium or pyridinium ring of the cation in ILs [22,82].

The adsorption of ILs also affects the retention of non-polar analytes, since the alkyl-bonded groups of the stationary phase are covered by the additive decreasing the possibility of dispersion interactions. Although the carbon content of the stationary phase is increased, no deleterious effect has been observed on the peak profiles, as happens with surfactants [83]. Besides this, specific solute-salt interactions can take place in the mobile phase, where ILs can act as ion-pairing reagents. All these interactions increase the complexity of the chromatographic system (and consequently, the mechanisms of retention), with regard to other common additives. The retention mechanisms are thus mixed and involve

hydrophobic partitioning, ion-exchange and ion-pairing. The extension of these interactions depends on the selected IL (e.g., the length of the alkyl group on the imidazolium ring and its counterion), and the IL concentration. One can conclude that adding an IL to a mobile phase produces chromatographic systems applicable for basic, acidic and neutral analytes.

*4.2. Anion and cation adsorption on the stationary phase*

As indicated, both cation and anion in ILs are able to interact with the stationary phase, affecting the chromatographic behaviour. Consequently, the adsorption capability of both ions has been studied in detail [43]. It was demonstrated that ions can adsorb on hydrophobic stationary phases in amounts depending on the Hofsmeister series:

For cations:

$Al^{3+} > Ca^{2+} > Mg^{2+} > H^{+} > Na^{+} > K^{+} > Rb^{+} > Cs^{+} > NH_4^{+} > N(CH_3)_4^{+} > NH(CH_3CH_2)_3^{+}$

and for anions:

$PF_6^{-} > SCN^{-} > ClO_4^{-} \sim BF_4^{-} > NO_3^{-} > I^{-} > Br^{-} > Cl^{-} > F^{-} > H_2PO_4^{-} > SO_4^{2-} > \text{citrate}(^{3-})$

This ranking was first proposed in 1888 and was related to a special physico-chemical property of the ions called “lyotropy”, a notion close to “hydrophobicity”. An ion is said to be lyotropic if it can stabilise the quaternary structure of proteins. An opposite correlation was found between lyotropy for anions and cations with their degree of strong hydration. Strongly hydrated ions are highly lyotropic cations ($Al^{3+}$, $Ca^{2+}$, or $Mg^{2+}$) but weakly lyotropic anions ($F^{-}$, $H_2PO_4^{-}$, $SO_4^{2-}$). These are called kosmotrope ions. On the contrary, weakly hydrated ions are highly lyotropic anions ($PF_6^{-}$, $SCN^{-}$, $ClO_4^{-}$ or $NO_3^{-}$) and weakly lyotropic cations ($Rb^{+}$, $Cs^{+}$, $NH_4^{+}$, or $N(CH_3)_4^{+}$). In this case, they are called chaotropic ions. It is noteworthy that most ILs used as additives in RPLC mobile phases are made by a large imidazolium cation associated with a relatively large anion. Both ions are weakly hydrated, being thus chaotropic ions. The chaotropic character of the anion may introduce ion-pairing with cationic solutes, as

well as adsorption on the stationary phase. The hydrophobicity of the cation may further induce stationary phase adsorption [43,84].

Adsorption of ions having different lyotropy was extensively studied by Gritti and Guiochon [85–90]. Later, nine alkylmethyl-imidazolium ILs with different alkyl chain length associated to $Cl^-$, $BF_4^-$ or $PF_6^-$ anions were used as additives in acetonitrile-water mixtures, and the amount of ions adsorbed on a Kromasil $C_{18}$ column was determined using a frontal analysis method [43]. In order to isolate the anion adsorption, sodium salts of $Cl^-$, $BF_4^-$ or $PF_6^-$ were also used. It was checked that $Cl^-$ showed a weak affinity for the stationary phase, whereas the anion $BF_4^-$ was moderately adsorbed on the column when compared with $PF_6^-$ (according to the Hofsmeister series): 15 versus 32 µmol adsorbed on the 15 cm Kromasil $C_{18}$ column, using a mobile phase containing 30% v/v acetonitrile and 0.05 M $NaBF_4$ or $NaPF_6$, respectively. The adsorbed amount of the IL cations was shown to increase linearly with their concentration in the mobile phase, and with the hydrophobicity of the side alkyl chain of the imidazolium cation. Thus, on the Kromasil $C_{18}$ column, using mobile phases of 30% v/v acetonitrile containing 0.05 M $C_2C_1IM{\cdot}PF_6$, $C_4C_1IM{\cdot}PF_6$ or $C_6C_1IM{\cdot}PF_6$, ~50, 125 and 380 µmol was adsorbed, respectively.

### *4.3. Linear solvation free energy relationship studies*

The Abraham solvation parameter model or linear solvation free energy relationship (LSER) has been widely used for characterizing ILs based on their multiple solvation interactions [2,29,91–94]. This model has also been shown useful for understanding the solute-IL interactions and retention behaviour in HPLC. The LSER model relates the retention of analytes on a stationary phase (log *k*) to a linear relationship of fundamental solute descriptors (the Abraham's descriptors) [95,96], associated to system constants represented by lowercase letters: *a*, *b*, *e* or *r*, *s* and *v*. The model was first outlined as:

$$\log k = \log k_0 + r R_2 + s \pi_2^* + a \sum \alpha_2^{\mathrm{H}} + b \sum \beta_2^{\mathrm{H}} + v V_2 \quad (1)$$

More recently, it has adopted the following expression:

$$\log k = \log k_0 + e E + s S + a A + b B + v V \quad (2)$$

The equations represent the contribution of specific intermolecular interactions to the overall retention factor: $R_2$ or $E$ represent the excess molar refraction, which reflects polarizability contributions from π- and n-electrons; $\pi_2^*$ or $S$ are the solute dipolarity/polarizability; $a \sum \alpha_2^{\mathrm{H}}$ or $A$, and $b \sum \beta_2^{\mathrm{H}}$ or $B$, are the overall solute hydrogen-bond acidity and basicity, respectively; and $V_2$ or $V$ are the McGowan's characteristic volume. The system constants are obtained for a particular chromatographic configuration (mobile phase/stationary phase) by making a regression analysis using a set of compounds with known descriptors, which have been reported for more than 4000 compounds [97]. This shows the huge experimental work needed to make LSER studies. Each different chromatographic condition needs its own multiple linear regression analysis of retention data, for a training set of usually neutral compounds, although the approach has been also extended to include ionic interactions. The system constants or coefficients describe the difference in the associated interaction with the mobile and stationary phases. The sign and the magnitude of the coefficients indicate whether the stationary or the mobile phase displays the greater interaction. If the defined property is stronger in the solvated stationary phase than in the mobile phase, the corresponding system coefficient has a positive sign, contributing to solute retention. The first four terms are the polar contributions to retention, and the last term represents the hydrophobic contribution.

The LSER model has been used in several reports to explain and predict the retention of probe compounds using immobilised ILs on porous silica particles [98–102]. There is also one report where this approach has been used to evaluate the capability of ILs as additives in

HPLC, using mobile phases of acetonitrile-water containing $C_6C_1IM{\cdot}BF_4$ and $C_8C_1IM{\cdot}BF_4$ [70]. The study has the problem that it was carried out with only nine aromatic probe compounds, which compromises its reliability. However, using the obtained LSER models, it was possible to predict retention factors with high correlation coefficients ($r^2 > 0.99$ for $C_8C_1IM{\cdot}BF_4$ and $r^2 = 0.96$ for $C_6C_1IM{\cdot}BF_4$). This allowed approximately reproducing the experimental log *k* values for the solutes studied in the different mobile phases. The most relevant result was that the excess molar refraction and hydrogen-bond basicity exhibited a dominant effect role on the solute/IL interaction. Also, the effect with $C_8C_1IM{\cdot}BF_4$ was greater.

## 5. Chromatographic behaviour in the presence of ionic liquids

### *5.1. Changes in retention behaviour*

An active research is found in many reports on the role of ILs to modify the retention of different types of solutes, mainly of cationic nature [39,49,57–59,61,62]. In general, these studies are conceived using an experimental design based on the use of alkylimidazolium ILs associated to different anions, which are added at increasing concentrations into aqueous mobile phases or mobile phases that contain a fixed concentration of organic solvent. Most published reports on the use of ILs as mobile phase additives do not especially emphasise on changes in solute retention. The results are considered more relevant with regard to peak shape improvement, although this improvement is closely related with changes in the retention factors. Since alkylimidazolium ILs associated to moderate or weakly chaotropic anions ($Cl^-$, $Br^-$, $BF_4^-$) are usually selected, faster separations are mostly observed for all kind of solutes, at increasing concentrations of ILs.

Several reports that examined in detail the use of alkylimidazolium ILs with side alkyl chains of different size and associated to anions of different chaotropicity, to elute a set of

β-blockers [58–61], are especially illustrative of the changes in solute retention that can be expected and will be described here. In a Kromasil $C_{18}$ column, the addition to an acetonitrile-water mixture of 0.01 M $C_2C_1IM^+$, $C_4C_1IM^+$ and $C_6C_1IM^+$ associated to $Cl^-$ yielded a larger effect in the reduction of solute retention, in the presence of the imidazolium cations with the longer side chain, when compared against a similar aqueous-organic mobile phase in the absence of additive [61]. At increasing concentration of the ILs (0.01–0.04 M), the changes in the retention of β-blockers were minimal for $C_2C_1IM{\cdot}Cl$, whereas for $C_4C_1IM{\cdot}Cl$ and $C_6C_1IM{\cdot}Cl$, the retention decreased significantly, especially for the latter. This behaviour can be exclusively attributed to the cation, since the affinity of $Cl^-$ for the stationary phase is low (see Section 4.2) and will not be (or only scarcely) involved in the chromatographic separation. Fig. 2a shows these trends for oxprenolol.

The use of $BF_4^-$ or $PF_6^-$ instead of $Cl^-$, both with a moderate or significant affinity for the stationary phase, respectively, changed notably the chromatographic behaviour [58,59,61]. In the presence of $C_2C_1IM{\cdot}BF_4$ and imidazolium cations associated to $PF_6^-$ (Fig. 2c and d), the retention of the β-blockers increased with respect to the aqueous-organic mobile phase in the absence of additive. At higher concentrations of the ILs, the retention reached a maximum and finally it is expected to decrease, although this was only experimentally observed for $C_4C_1IM{\cdot}PF_6$ at the assayed concentrations. Initially in these cases, the interaction of the cationic solutes with the amount of $BF_4^-$ or $PF_6^-$ adsorbed on the stationary phase appears to be more important than the interaction with the alkylimidazolium cations. After reaching a maximum, the repulsion from the positively charged stationary phase by adsorption of the cation, or ion-pairing with the anion dissolved in the mobile phase prevails, decreasing the retention. This effect is stronger for $C_2C_1IM{\cdot}PF_6$ and $C_4C_1IM{\cdot}PF_6$, and is not observed for $C_4C_1IM{\cdot}BF_4$ and $C_6C_1IM{\cdot}BF_4$, which led to shorter retention times at increasing concentrations in the mobile phase.

The increased retention shown by basic compounds in the presence of $C_2C_1IM{\cdot}BF_4$, $C_2C_1IM{\cdot}PF_6$ and $C_4C_1IM{\cdot}PF_6$ is not attractive in principle. For this reason, an exhaustive study on the retention of β-blockers eluted with a set of six different $C_{18}$ columns using ILs as additives in the mobile phase, considered only $C_6C_1IM{\cdot}BF_4$ and $C_4C_1IM{\cdot}BF_4$ [59]. The selected columns contained different amounts of silanols: Zorbax SB-$C_{18}$, X-Terra MS $C_{18}$, Kromasil, Nucleosil (type B columns, with less silanol groups), and Lichrospher and Spherisorb (type A columns, with more silanols). The retention behaviour of β-blockers with $C_4C_1IM{\cdot}BF_4$ and $C_6C_1IM{\cdot}BF_4$ differed, depending on the considered column. In the presence of both ILs the retention decreased, except for Zorbax, X-Terra and Kromasil in the presence of $C_4C_1IM{\cdot}BF_4$, where the changes in retention were small and followed different trends. This suggests that the adsorption strengths of the cation and the anion in the IL were similar. Surprisingly, Spherisorb reached the shortest retention times, being the effect more pronounced for the most strongly adsorbed $C_6C_1IM^+$. The results indicated that in terms of retention, the different performance obtained for the type-A silica packings in the absence of additive is minimised by the addition of any of the two ILs associated to $BF_4^-$, whereas for the type-B stationary phases, the effect on retention is less significant.

The studies carried out on the ILs retention by other authors, from which those in Refs. [39,49,62] should be highlighted, yielded, in principle, similar results to those with β-blockers using alkylimidazoilum ILs with chains of different lengths, different associated anions, and different types of columns [57–61], but they are not so comprehensive. With the β-blockers, both the retention behaviour, and as commented below, the peak profile (both width and asymmetry) were studied in detail, which is not the case for other reports.

*5.2. Effect of pH*

The pH is an experimental factor with a large influence on retention and selectivity for ionisable compounds. At fixed modifier and additive contents, the retention in RPLC is a weighted mean of the behaviour of the basic and acidic species [103,104]:

$$k = k_A \delta_A + k_{HA} \delta_{HA} = k_A \frac{1}{1 + K h} + k_{HA} \frac{K h}{1 + K h} = \frac{k_A + k_{HA} K h}{1 + K h} \quad (3)$$

where $k$ is the measured retention factor, $k_A$ and $k_{HA}$ are the retention factors for the basic and acidic species, respectively, $\delta_A$ and $\delta_{HA}$ are their molar fractions, and $h$ is the molar proton concentration. For simplicity, the acid-base equilibrium is here described by the apparent protonation constant, which is the reciprocal of the acidity constant ($K = K_a^{-1}$). The protonation of an acidic compound is produced along at least two pH units (within log $K \pm 1$). Since the retention for each species ($k_A$ and $k_{HA}$) is different, a sudden change in solute retention will happen at pH values close to p$K_a$ = log $K$. The protonation constant is affected by all interactions of both the acidic and basic species with the components of the mobile and stationary phases, as those that take place with ILs.

Only few authors have checked the effect of pH on retention, in the presence of ILs [21,47, 50,57,62,105], since there is a preference to fix the pH at values close to 3 to avoid the silanol problem. Silanols are weakly acidic, with average protonation constants ranging from ca. log $K$ = 4.5 to ca. 7, depending on the type of silica [106]. Thus, the ionisation degree of silanols changes with the mobile phase pH, inducing changes in the silanol-cationic solute interaction, but at pH = 3 the interactions are highly reduced.

In general, the addition of ILs with a poorly adsorbed anion decreases the retention of basic compounds, independently of the pH value, but an increase in the pH may increase the retention due to a decrease in the solute ionisation (the charged species is less retained). Depending on the log $K$ value, changes in retention are or not observed. Thus, for

*N*-ethylaniline with log $K$ < 7, eluted in the presence of $C_4C_1IM{\cdot}BF_4$, the retention factors were observed to increase at increasing pH ($k$ = 4.92, 11.09 and 16.56, at pH 3, 4 and 5, respectively) [47]. In contrast, for β-blockers with log $K$ = 9–10, the retention did not change in the usual 2.5 < pH < 7.5 range, since the solutes remain in their protonated form [57].

On the other hand, ILs have been indicated to exhibit minimal influence on the pH of the mobile phase when used as additives [13]. However, in a study performed with $C_2C_1IM{\cdot}BF_4$ and $C_4C_1IM{\cdot}BF_4$, the mobile phase pH was found to slightly increase, while for mobile phases containing the methylsulphates of the same IL cations, the pH was decreased, at increasing IL concentration [50].

*5.3. Enhancement of peak profiles*

Authors working with ILs have described enhancements in the peak profiles by the addition of ILs to the mobile phase in HPLC. In some reports included in Table 1, several chromatograms are shown that allow the comparison of the changes in the peak performance upon addition of several ILs to an aqueous or aqueous-organic mobile phase. It is also usual to report values of efficiencies and peak asymmetries to appraise the enhancements. However, to obtain a clear conclusion these parameters should be measured at similar retention times, which is not often the case. For a fair comparison of peak profiles, a global overview of the changes that occur in the width and asymmetry of chromatographic peaks using different experimental conditions is needed. This is possible simply by plotting the left ($A$) and right ($B$) half-widths of chromatographic peaks, at a given height, versus the retention time ($t_R$) (Fig. 3) [107,108]. This approach is particularly useful to evaluate the suppression of the silanol problem [58,59,80,107].

The plots are built more conveniently with the half-widths measured at 10% peak height (instead of the most usual 50% peak height), where the values are not affected by the baseline

noise, and there is a larger sensitivity to the peak skewness. The half-widths can be usually measured with sufficiently high accuracy using appropriate tools, based on the interpolation of the signals, or on the non-linear fitting of the chromatographic peaks to appropriate models [109,110]. A software tool that was commercialised in 2000 can assist in this measurement [111,112]. Only for low efficiencies and highly asymmetrical peaks, the measurement of peak half-widths may be problematic. However, even in this case, the provided information is useful.

The construction of half-width plots needs data from peaks obtained at several retention times with a given column. These data can correspond to a single compound eluted at varying mobile phase composition, or to several compounds eluted at the same composition, with the condition that the interaction kinetics with the chromatographic column is the same for all compounds and mobile phases. This fact is revealed by the degree of alignment of the half-widths versus the retention times [113]. The latter approach is preferable when the column is modified with additives, since the column nature can change significantly by changing the concentration of the additive (and consequently, the kinetics). However, in some instances, adequate plots can be obtained even using the data from several solutes eluted at different compositions (e.g., different concentrations of ILs). A particular good set of probe compounds to perform this characterisation is constituted by commercially available β-blockers, such as atenolol, pindolol, timolol, esmolol, acebutolol, celiprolol, metoprolol and oxprenolol.

The coincidence of slopes for the plots built for the right and left half-widths indicates that the peaks of compounds eluting at different retention times will be symmetrical. Usually, peaks are tailing (the slope of the right half-width is larger) and the peak asymmetry is indicated by the angle between both plots. The plots give a direct reading of the half-widths

for peaks at different retention times. These values can be used to calculate the peak efficiencies and asymmetries for particular compounds and conditions, if desired.

Compared with the complexity in the retention behaviour of basic compounds observed for ILs with different combinations of cation and anion, the results obtained with regard to the peak profiles go in the same direction: significant enhancements are always achieved. We show here only a few illustrative examples, but similar results were achieved in most reports given in Table 1. Fig. 3 shows half-width plots obtained in the absence and presence of ILs. Without additive, the peaks were broad and rather skewed. The peak profile was significantly enhanced (the peaks were narrower and almost symmetrical) upon the addition of an IL, with more evident changes in the right half-width. Peak profile enhancements are observed independently of the relative adsorption of the cation and anion. However, $C_6C_1IM{\cdot}BF_4$ or $C_6C_1IM{\cdot}Cl$, for which the cation is more strongly adsorbed, both showed the largest enhancement. Fig. 4 shows another interesting example, where the effect of an increasing concentration of $C_4C_1IM{\cdot}BF_4$ to an aqueous mobile phase on the peak profiles for several ephedrines is shown.

## 6. Estimation of the suppressing potency of silanols

### *6.1. Measurements based on the changes in retention*

In the absence of additive, the retention of a protonated basic compound in a silica-based column is given by a two-site retention model:

$$k_0 = k_1 + k_2 \qquad (4)$$

where $k_0$ is the retention factor, and $k_1$ and $k_2$ are the hydrophobic and silanophilic contributions to the retention. Masking the silanols will reduce the retention. If the basic compounds do not experience any other additional electrostatic interaction, the decreased retention will indicate undeniably the suppressing potency of the silanol effect. Based on this

assumption, Horváth et al. proposed a mathematical model to evaluate the silanol suppressing potency of an amine by measuring the retention for basic compounds at varying concentration of the amine [114,115].

When a cationic additive (the assumed silanol suppressor) is added to the mobile phase, a secondary equilibrium is established:

$$A + Silanol \leftrightarrows ASilanol \quad (5)$$

The retention factor will decrease according to:

$$k = k_1 + \frac{k_2}{1 + K_A[A]} \quad (6)$$

where [*A*] is the additive molar concentration, and $K_A$ the binding constant of the additive-silanol complex, which is assumed to evaluate the ability to suppress the silanol effect. If Eqs. (4) and (6) are subtracted, the silanophilic contribution to retention is isolated:

$$k_0 - k = k_2 - \frac{k_2}{1 + K_A[A]} = \frac{k_2 K_A[A]}{1 + K_A[A]} \quad (7)$$

resulting in:

$$\frac{[A]}{k_0 - k} = \frac{1}{k_2 K_A} + \frac{[A]}{k_2} \quad (8)$$

which is known as the Horváth equation. If this equation holds, plots of [*A*]/($k_0 - k$) versus [*A*] will yield a straight line with the reciprocal of the silanophilic retention factor ($k_2$) as the slope. The slope to intercept ratio will indicate the binding constant $K_A$.

Eq. (8) has been used more recently to measure $K_A$ for ILs added to the mobile phase [20, 58,59,61,65,67]. The question is: are the assumptions of the Horváth equation fulfilled? The most extensive study was performed by Kaliszan et al., who evaluated $K_A$ for 15 imidazolium-based ILs with different alkyl chains and anions (chloride, bromide, tosylate, tetrafluoroborate, methylsulphate, ethylsulphate, and octylsulphate), using a non-endcapped LiChrospher RP-18 column to increase the silanol problem [67]. In all cases, good

correlations ($r^2 > 0.999$) were obtained. The authors emphasised that the screening effect was achieved at low concentration of the additives. The $K_A$ constant was observed to increase with increasing length of the alkyl chain at position C-1 of the imidazolium ring, with the greatest effect observed for the most hydrophobic $C_8C_1IM^+$, stabilised by interaction of its $C_8$ chain with the $C_{18}$ chains in the stationary phase. It was also highlighted that $Cl^-$ and $Br^-$ yielded higher $K_A$ values than $BF_4^-$. The authors used also Eq. (8) to calculate the additive concentration needed to reduce the retention to a given extent (90%).

Other authors have also put attention in the estimation of the suppression potency of silanols, using retention data. However, the results have not been so satisfactory. Thus, in the separation of six amines using ILs as additives and two stationary phases with moderate silanol activity (Nova-Pak $C_{18}$ and TSK-Gel ODS-80TM) [65], the authors described some unexpected behaviour, and considered that the most important conclusion with Eq. (8) was that it can be used as a useful guide in the selection of adequate concentrations of ILs to reduce the retention to a pre-fixed value. More conclusive were a series of studies carried out with a set of β-blockers of different polarity (already commented), eluted with acetonitrile-water mixtures in the presence of a variety of ILs with alkyl chains of different length and $Cl^-$, $BF_4^-$ and $PF_6^-$ as anions, and using six $C_{18}$ stationary phases with different silanol activity (Zorbax SB-$C_{18}$, X-Terra MS $C_{18}$, Kromasil, Lichrospher, Nucleosil, and Spherisorb) [58,59,61]. Some results are compared in Table 2. It was found that:

(i) A certain scattering is obtained in the calculation of the silanol binding constant $K_A$ with Eq. (8), for different compounds. Thus, for a Kromasil column: $K_A$ = 36.5±7.4 for $C_4C_1IM{\cdot}BF_4$ and 436±90 for $C_6C_1IM{\cdot}BF_4$, but the scattering was much larger for Nucleosil and Lichrospher. However, since $K_A$ is the binding constant of the silanol-additive complex, it should be a single value. Although some inaccuracy in the measurement of the column hold-up time and the retention times (especially for the

least retained compounds) may be partially the reason, the scattering should be the result of particular interactions of each compound with the stationary and mobile phases.

(ii) Even considering the particular contributions for each compound to the retention, the mean $K_A$ values indicated the greater ability to decrease the retention for ILs with a longer alkyl chain and bearing the same anion (e.g., $C_6C_1IM \cdot BF_4$ versus $C_4C_1IM \cdot BF_4$, or $C_6C_1IM \cdot Cl$ versus $C_4C_1IM \cdot Cl$).

(iii) The ability to decrease the retention depends on the associated anion, being larger as the anion adsorption is poorer. Thus, the most significant decrease in retention (and consequently, larger $K_A$ value) is observed when the IL cations are associated to chloride, the least chaotropic anion.

(iv) Eq. (8) cannot be applied with ILs where the adsorption of the anion is significant. In some cases, the anion adsorbs so strongly with respect to the cation that the retention of the positively charged basic compounds is higher with respect to the absence of the IL ($k > k_0$) (Fig. 2c,d), over a wide range of practical concentrations.

(v) $K_A$ silanol binding constants are obtained by comparison of the retention with mobile phases containing the same amount of organic solvent in the presence and absence of additive. As a consequence, they are relative values: the larger the amount of residual silanols, the larger the constant (case of Nucleosil, Lichrospher and Spherisorb). Therefore, $K_A$ depends on the stationary phase. This may be at least one of the reasons of the different values reported by different authors for the same additive.

(vi) $K_A$ cannot be evaluated for stationary phases that contain a small amount of silanols (such as type B silica-based Zorbax and X-Terra), owing to the small extension of the adsorption of the cation.

(vii) When the IL is added in excess, the amount of additive in the mobile phase also modulates the retention due to ion-pairing with charged solutes.

Therefore, the use of $K_A$ as an estimation of the suppressing potency is arguable. This comment can be extended even to additives where the associated anion is weakly adsorbed on the stationary phase as amines, or additives with cations of relatively small size as $C_2C_1IM^+$, for which the changes in retention do not reveal specifically the suppression of the silanol effect (which in fact is observed by the improvement in peak profiles). What is then measuring $K_A$? It can be only affirmed that in most cases, it is just a parameter that measures the capability of the additives to reduce the retention of the cationic solutes, as a result of antagonist interactions.

*6.2. Measurements based on the changes in peak profile*

As commented, the slow interaction kinetics of cationic basic compounds with anionic residual silanols has been given as the reason of the observed poor peak profiles (i.e., broad and asymmetrical peaks). Thus, blocking the accessibility of the cationic solutes to the silanols by the addition of ionic reagents in the mobile phase yields thinner and better shaped peaks. Several authors have confirmed the suppression of the silanophilic interaction by ILs, which should coat the stationary phase surface forming a layer associated to the bonded alkyl chains. For some reagents, a direct interaction with the free silanol groups on the silica-based supports may exist. All these processes are accompanied by a change in the retention mechanisms, which is especially complex for ILs, since both ions may contribute to the retention of analytes and can affect it with positive or negative changes.

In view of this behaviour, it can be concluded that the estimation of the silanol suppressing potency for ILs through the changes in retention (when possible) can be misleading. Eq. (8) can only strictly be applied to ILs where the anion is very weakly

adsorbed (i.e., no other secondary equilibria modify the retention of the basic compounds). In other cases (with partial or high anion adsorption), the observed retention should be interpreted by considering the dual nature of ILs: the blocking of the silanols by the IL cation, which decreases the retention, and the attraction of the positively charged basic compounds to the adsorbed IL anion, which increases the retention. However, other electrostatic interactions with the ions adsorbed on the stationary phase (even the IL cation), or dissolved in the mobile phase, also cooperate to the changes in retention.

Thus, comments like “In the light of conducted experiments, it is obvious that the cation of IL is responsible for the silanol screening effect, whereas the retention factor primarily depends on the properties of its anion” [20] are not describing the true situation. The changes in retention respond to several interactions, which may be more or less related to the efficient protection of silanols. Therefore, the Horváth equation (Eq. (8)) does not seem to be the best way to estimate the silanol suppressing potency with ILs and other additives. Meanwhile, the enhancement in peak profile itself should offer a reliable estimation of the suppression potency of residual silanols (i.e., the reduction of the accessibility to silanols), independently of the type of interaction. For this purpose, the half-width plots described in Section 5.2 are useful. In many instances, the plots can be approximated to straight-lines [107,108]:

$$A = m_A t_R + A_0 \quad (9)$$

$$B = m_B t_R + B_0 \quad (10)$$

where $m_A$ and $m_B$ are the slopes of the linear correlations, and $A_0$ and $B_0$ the intercepts, which are associated to the extra-column contributions to the peak broadening. Eqs. (9) and (10) allow the prediction of the peak half-widths at any retention time, and provide parameters useful to characterise the column performance. The sum of the slopes ($m_A + m_B$) indicates the peak broadening rate inside the column (increasing rate of peak width with the retention time), and the ratio of slopes ($m_B / m_A$) the expected peak skewness for retained compounds

(for which the extra-column contribution is non-significant). The peaks are symmetrical for $m_A = m_B$. A smaller peak broadening rate implies narrower peaks at similar times, provided that the width is the same close to the dead time. The information in Table 2 is given as an example of the evaluation of the silanol suppression by ILs using these parameters.

Therefore, the multiple interactions that ILs undergo inside the chromatographic system suggest that the suppressing potency should be measured based on the peak profiles, instead of the changes in retention.

## 7. Special ionic liquids

### *7.1. Ionic liquids capable of forming micelles*

Approximately 50 ILs with surfactant properties (the so-called IL-based surfactants) have been synthesised. Most of these ILs present melting points above 100ºC. IL-based surfactants have found application in HPLC, among other analytical fields [116]. They have been shown to improve the performance obtained with conventional cationic surfactants. Thus, IL-based surfactants seem to be promising reagents and have called some attention. These mainly comprise two classes of compounds, which are monocationic and dicationic (or Gemini). The monocationic ILs consist of imidazolium, pyridinium or pyrrolidinium with alkyl substituents of several lengths (attached at positions one and three for imidazolium, at the nitrogen of pyridinium, and at positions one and/or two of pyrrolidinium). The dicationic ILs are comprised of two imidazolium (or pyridinium) cations connected by an alkyl linkage chain (called the spacer), where the position three of imidazolium or the nitrogen position of pyridinium are substituted with alkyl chains of varying lengths. The properties of a new family of tricationics have been recently reported [117].

The first reports on the amphiphilic association of ILs in water were carried out in 2004 by two independent research groups [118,119]. The authors observed that at a relatively low

concentration, IL-based surfactants aggregate forming micelles (Fig. 5). The critical micelle concentration (CMC) in water is approximately 0.04 M for $C_{10}C_1IM{\cdot}Br$, 0.1 M for $C_8C_1IM{\cdot}Cl$ and $C_8C_1IM{\cdot}I$, and 0.8 M for $C_4C_1IM{\cdot}BF_4$. More recently, the influence of a variety of organic solvents on the micellisation process has been examined [116]. As a general trend, the increase in the alkyl chain length within the IL-based surfactant is accompanied by an increase in the micelle aggregation number and a decrease in the CMC values. Thus, for instance, the following trends in CMC are observed: $C_{16}C_1IM{\cdot}Br$ « $C_6C_1IM{\cdot}Br$, $C_{12}C_{12}IM{\cdot}Br$ « $C_{12}C_1IM{\cdot}Br$, and for the dicationic $C_{12}IM\text{-}C_4IM\text{-}C_{12}{\cdot}Br_2$ « $C_8IM\text{-}C_4IM\text{-}C_8{\cdot}Br_2$. Gemini dicationic IL-based surfactants present much smaller CMC values than their monocationic analogs.

Since the 80s, an HPLC mode has been developed that uses surfactants above the CMC in the aqueous mobile phase, which has been called micellar liquid chromatography (MLC) [120,121]. To be considered a good candidate for MLC, a surfactant should possess a low CMC which is a requirement fulfilled by many IL-based surfactants. Thus, these ILs can be appropriate to increase the number of possible surfactants to be used in MLC. However, only few reports have appeared up-to-date describing this possibility [51,122,123]. The IL-based surfactants used in these reports were $C_{16}C_4IM{\cdot}Br$ (with benzene as probe compound and different stationary phases) [123], $C_{12}C_1IM{\cdot}Br$ and $C_{14}C_1IM{\cdot}Br$ (to analyse inorganic anions) [122], and $C_8C_1IM{\cdot}BF_4$ (to determine new analgesic active urea derivatives) [51]. The performance of these ILs in MLC was found comparable to that of cetyltrimethylammonium chloride ($C_{12}TAB{\cdot}Cl$).

*7.2. Ionic liquids that allow the separation of isomers*

Some ILs may be especially suitable for the separation of positional and optical isomers, although still little work has been published in this regard. Already in 2007 a report was

published on the successful separation of three positional isomers (*o*-, *m*- and *p*-amino benzoic acid), with four different ILs ($C_2C_1IM{\cdot}BF_4$, $C_4C_1IM{\cdot}BF_4$, $C_2C_1IM{\cdot}CH_3SO_4$ and $C_8C_1IM{\cdot}CH_3SO_4$) as additives in RPLC [50]. The authors commented that "conventional analysis in RPLC of the isomers was an intractable problem": without ILs the peaks of the *m*- and *p*-isomers were almost completely overlapped, whereas after the addition of the ILs, the isomers were partially or completely resolved. It was observed that the anion also had an influence on the elution order. Thus, $C_2C_1IM{\cdot}CH_3SO_4$ was superior to $C_2C_1IM{\cdot}BF_4$ in the separation of the amino benzoic acid isomers.

Different chiral ILs can be fabricated by varying the cation, the anion or both cation and anion in the same IL [7]. However, although the synthesis of surface-bonded IL stationary phases with chiral properties for HPLC is an active field [24], the search for chiral ILs as additives in HPLC is still under development. The reference work in this field is a chapter by the Warner's group [124]. Yuan *et al*. proposed, for the first time, the chiral IL (R)-*N*,*N*,*N*-trimethyl-2-aminobutanol-bis(trifluoromethanesulfon)imidate as a chiral selector in HPLC to separate eight racemates, and obtained a better resolution than without the chiral IL [125]. Both achiral and chiral ILs were employed as additives for the separation of ofloxacin enantiomers by ligand-exchange HPLC [54]. The chiral amino acid IL $C_4C_1IM{\cdot}$L-leucine demonstrated be also superior compared to the achiral ILs to separate enantiomers of mandelic acid and derivatives [66**]**. These reports indicated that the use of chiral ILs could soon become very attractive as new chiral selectors in HPLC.

## 8. Modelling and optimisation of the chromatographic performance

### *8.1. Measurement of lipophilicity*

ILs added to the mobile phase have been demonstrated to provide reliable parameters to measure the lipophilicity for basic compounds, owing to the suppression of the silanophilic

interaction which improves the linearity of log $k$ versus the volume fraction of organic modifier ($\varphi$) in the mobile phase [20,23,41]:

$$\log k = \log k_w - S\varphi \quad (11)$$

where log $k_w$ is the retention factor extrapolated to pure water, and $S$ the slope of the regression straight-line. The correlation between the chromatographically determined parameters (log $k_w$ and $S$) versus the reference lipophilicity parameter octanol-water partition coefficient (log $P_{o/w}$) is statistically significant mainly for a congeneric group of neutral solutes. The presence of ILs in the mobile phase (such as $C_2C_1IM{\cdot}BF_4$) improved this correlation for basic compounds [41]. According to the authors, the elimination of the uncontrollable attractive interactions of basic compounds with free silanols seems to be more effective than that provided by typical amines used as silanol suppressors.

The approach was also applied to measure the lipophilicity of several newly synthesised derivatives of urea, active on the central nervous system, using mixtures of methanol or acetonitrile and water with addition of imidazolium-based ILs ($C_4C_1IM{\cdot}Cl$, $C_4C_1IM{\cdot}BF_4$, $C_4C_1IM{\cdot}PF_6$ and $C_8C_1IM{\cdot}BF_4$) [51]. The correlation of log $k_w$ with a computational lipophilicity scale was highly improved by the addition of ILs to the mobile phase, with the effectiveness improving according to $C_8C_1IM{\cdot}BF_4$ < $C_4C_1IM{\cdot}PF_6$ < $C_4C_1IM{\cdot}Cl$. The existence of an interaction between the cation of the imidazolium-based IL and neutral solutes, affecting the chromatographic behaviour was demonstrated.

### *8.2. Modelling of the retention behaviour*

As described above, ILs give rise to secondary equilibria in both stationary and mobile phases. Solutes interact with the IL cation (and in many instances, with the IL anion) that coat the stationary phase, by means of hydrophobic and electrostatic interactions. They also may interact with the excess IL in the mobile phase, forming ion-pairs, and in some instances, get

associated with IL-based surfactant micelles. The mechanisms of retention of solutes in the presence of such variety of equilibria has been described in other fields, in the presence and absence of aggregate entities in the mobile phase. The proposal of such mechanisms (i.e., retention models) has been very useful to increase the knowledge on the chromatographic systems and also with prediction purposes [126]. It is not surprising that these models were adapted to HPLC with ILs [58,59], as described below.

The models assume the existence of three phases (stationary phase, water/organic solvent and individual IL molecule or micelle). Following the Arunyanart and Cline-Love approach [127], two chemical equilibria are established:

$$A + S \leftrightarrows AS \quad (12)$$

$$A + IL \leftrightarrows AIL \quad (13)$$

which describe the association of the solute in bulk water (A) with the stationary phase binding sites (S, which are modified with the IL), or with an IL molecule dissolved in the mobile phase (as individual entity or as a part of micelles). These equilibria can be expressed by the stability constants $K_{WS}$ and $K_{AIL}$, respectively. Accordingly, at fixed organic solvent content, the retention factor will be expressed by:

$$k = \phi \frac{[AS]}{[A]+[AIL]} = \frac{\phi K_{WS}[S]}{1+K_{AIL}[IL]} \quad (14)$$

where $\phi$ is the phase ratio (the ratio between the volume of active stationary phase surface and the column dead volume) and [*IL*] the molar concentration of IL molecules. Considering [*S*] is constant (or practically constant), and rearranging Eq. (14):

$$\frac{1}{k} = \frac{1}{K_{AS}} + \frac{K_{AIL}}{K_{AS}}[IL] = c_0 + c_1[IL] \quad (15)$$

where $K_{AS} = \phi K_{WS}[S]$. The linear plots of the inverse of the retention factor (1/*k*) versus the IL concentration evidence the stationary phase saturation by the IL molecules. The extrapolation of the linear segments gives a measurement of the strength of the interaction

between the solute and stationary phase, expressed as the inverse of the intercept. In a study carried out with β-blockers eluted with acetonitrile-water mobile phases in the presence of several ILs associated to $BF_4^-$ and $PF_6^-$, the strongest interaction corresponded to $C_4C_1IM{\cdot}PF_6$ (suggesting a higher affinity of the β-blockers to the stationary phase modified with this IL, favoured by the larger adsorption of $PF_6^-$ on the stationary phase [43]), and the lowest interaction to $C_6C_1IM{\cdot}BF_4$ (which revealed a low or moderate adsorption for $BF_4^-$ and the greater interaction of $C_6C_1IM^+$ with the alkyl-bonded stationary phase with respect to $C_4C_1IM^+$ that repelled β-blockers).

The linear Eq. (15), with the IL concentration as unique factor, describes the retention at fixed organic solvent content or in aqueous medium. However, often, appropriate resolution needs the control of the concentration of both organic solvent and IL in the aqueous-organic mobile phase. In this case, the retention has been checked to be accurately described using polynomial models with two variables [60]:

$$\log k = c_0 + c_1 \varphi + c_2 [IL] + c_{12} \varphi [IL] \quad (16)$$

However, modelling the retention in HPLC using ILs as additives is still an open field.

*8.3. Optimisation of chromatographic performance*

ILs have demonstrated great potential in HPLC. Excellent separations have been reported for a number of analytes in short times [19,22,39,46,60,62,72]. However, the large variety of ILs in the market and their wide range of behaviours do not facilitate the selection of the best. This selection is easier with purely aqueous mobile phases (with only one factor: the IL concentration), for which trial-and-error strategies may work. Nevertheless, in most cases, the elution strength should be increased or modulated using aqueous-organic mobile phases to which the IL is added. In general, the selectivity and resolution of analytes depend on the concentration and type of IL and organic solvent used. The search of an IL reducing

sufficiently the retention is usually the primary aim. However, with some ILs better resolution may be achieved at the expense of longer analysis time [19].

With mobile phases that contain both organic solvent and IL, the best conditions can only be reliably searched using an interpretive optimisation [104,128]. This should indicate the best resolution in the minimal analysis time. In one of the first reports on the use of ILs as additives in HPLC, we can read: “Such systems should allow for a reliable prediction of retention as a function of eluent composition and hence for a rational optimisation of separation conditions.” [41]. A decade later still the consequences of the changes in retention and peak profile on the resolution, with added ILs, have not received enough attention. In the published reports, only a reduced number of mobile phases with a fixed organic solvent content and a narrow concentration range of IL are usually examined.

In order to appraise the performance or convenience of ILs in HPLC, more comprehensive information is needed, provided by a wider range of chromatographic conditions. Such comprehensive optimisation is only shown in one report, where several β-blockers were separated with three different $C_{18}$ stationary phases [60]. The study shows that the extremely poor chromatographic performance using acetonitrile-water mixtures in the absence of additive is highly improved in the presence of $C_6C_1IM{\cdot}BF_4$, yielding shorter analysis times, smaller consumption of acetonitrile, significantly narrower and more symmetrical peaks, and the most important, higher resolution (Fig. 6). In that work, the retention times were predicted through Eq. (16), and the peak profiles from the fitted half-width plots (Eqs. (9) and (10)). In all cases, the agreement between predicted and experimental chromatograms was highly satisfactory. Although it was expected that the IL covered the three studied stationary phases in a significant extent, these kept particular behaviours with respect to the analysis times, peak profile and selectivity (and consequently, different resolution). The resolution improvement was especially striking for the Spherisorb

5 μm column (type-A column), which yielded the poorest results in the absence of additive (Fig. 6c and d). This cannot be explained only by the changes in the selectivity upon addition of $C_6C_1IM{\cdot}BF_4$, but also (and especially) because of the significant enhancement in peak profiles. Also, in contrast to the absence of IL, the three assayed columns exhibited robust optima (not affected by small changes in mobile phase composition), in wide regions of the experimental domain.

*8.4. Selection of ionic liquids*

In most published reports on the use of ILs as mobile phase additives in HPLC, alkyl-imidazolium ILs associated to $BF_4^-$ and $Cl^-$ have been used (Table 1). As described in previous sections, the most universal effect of ILs (at least for basic compounds), is the peak profile enhancement (i.e., narrower and almost symmetrical peaks), due to the deactivation of the residual silanols on the support. This effect is related to the association of the IL cation to the stationary phase, and in a lesser extent, of the IL anion, through several mechanisms not completely understood. Concomitantly, such adsorption gives rise to a change in the forces that maintain solutes associated to the stationary phase, inducing increased or decreased retention [43] (see also Fig. 2). Finally, the particular changes in the retention of the analytes in a mixture may affect significantly the selectivity, and this together with the improved peak profiles will have a profound effect on the resolution. In all cases, ILs will exert considerable impact on the analysis times.

A wise choice of the appropriate combination of the chaotropicity of the IL anion with the hydrophobicity of the IL cation allows playing with solute selectivity and analysis time [43]. ILs yielding decreased retention are usually preferred, since this allows shorter analysis times and the reduction of the consumption of organic solvent. This is possible when the anion show a relatively weaker adsorption than the cation. In this regard, several authors have

pointed out $C_4C_1IM{\cdot}BF_4$, and especially, $C_6C_1IM{\cdot}BF_4$ or $C_6C_1IM{\cdot}Cl$ as the best additives [39,54,60,61,63,64].

However, we cannot be so categoric in indicating the “most suitable” IL. The relative adsorption of the anion and the cation of an IL can be used to adjust the selectivity, as well as to enhance the chromatographic resolution by improving the peak profiles of cationic solutes. If the solutes elute too rapidly, an IL with a lyotropic anion, such as $PF_6^-$, $BF_4^-$, or $ClO_4^-$, can be used with a short alkyl chain imidazolium cation such as $C_4C_1IM^+$ or $C_2C_1IM^+$ to increase the retention factors. If the compounds are highly retained, a long alkyl chain cation such as $C_6C_1IM^+$ can be used associated with an anion with a low lyotropy, such as $Cl^-$. If there is no problem with the retention, $C_6C_1IM{\cdot}BF_4$ or $C_6C_1IM{\cdot}Cl$ are recommended as a first choice. Of course, other usual experimental parameters, such as organic modifier content, pH, and temperature, should also be adjusted. Also, there are other considerations in the selection of an appropriate IL, as the following [43]:

(i) $BF_4^-$ should be preferred to $PF_6^-$ as the IL anion, since $PF_6^-$ can be slowly hydrolysed in water releasing HF, which could damage the silica support.

(ii) $ClO_4^-$ with similar adsorption as $BF_4^-$ give rise to ILs not commonly found in the market, due to an intrinsic explosive hazard associated to alkyl-imidazolium cations.

(iii) The best efficiency enhancer cation seems to be $C_6C_1IM^+$, since poor solubility does not favor the longer $C_8C_1IM^+$.

*8.5. Detection techniques*

Common ILs formed by an alkyl-imidazolium cation and an inorganic anion absorb UV light strongly in the 200–280 nm range [50]. This may add noise or a background signal to HPLC with UV detection. In a study performed with 15 ILs [67], ILs showed absorbance maxima at about 210–215 nm in water (except for two cases, $C_2C_1IM^+$ associated to tosylate

and $C_4C_1PY{\cdot}Cl$). This absorbance may make detection problematic, giving rise to a signal depression for some analytes. Fortunately, detection is possible at 254 nm with the most common ILs used in HPLC. However, alkylimidazolium-based ILs not transparent at common working wavelengths will provide unacceptable absorbance and cannot be used with UV detection [129,130]. Care should also be taken with the use of such ILs as extractants, as they can interfere in the further HPLC analysis. Such shortcoming of ILs as additives interfering during the detection has been mitigated by immobilizing them on the surface of silica [24].

Based on their UV absorption, imidazolium-based ILs have been applied as background reagents for the HPLC determination of morpholinium cations using UV indirect detection [68]. Morpholinium cations lack a UV absorption group in their molecular structures, while imidazolium ILs as mobile phase additives of aqueous-organic mixtures cannot only act as background UV absorbents, but also improve their separation. $C_2C_1IM{\cdot}BF_4$ was used in 20% v/v methanol-water as mobile phase. This IL shows maximal absorption at 200 nm. However, in order to avoid the interference of other UV absorbents as methanol, detection at 210 nm was chosen. The chromatographic baseline noise was smaller with respect to that offered by $C_4C_1IM{\cdot}BF_4$ or $C_5C_1IM{\cdot}BF_4$.

Most reported applications of ILs as mobile phase additives in HPLC have been related to UV or diode array detection (Table 1). Few reports make use of fluorescence detection. It should be noted that besides its inherent sensitivity, fluorescence detection can eliminate one of the limitations in the application of ILs in HPLC, associated to the fact that several ILs are not UV transparent at wavelengths where analytes are detected. In one of such applications, the beneficial effect of $C_4C_1IM{\cdot}BF_4$ as mobile phase additive with a Nova-Pak® $C_{18}$ stationary phase was shown for the determination of heterocyclic aromatic amines in commercial meat extracts [64,65].

Electrochemical (EC) detection has also been assayed with good results in HPLC using ILs as mobile phase additives [63,71]. This is based on the electrochemical response of the compounds at the operating potential, and requires the presence of an ionic mobile phase in order to have an adequate electrolyte solution. Therefore, the implementation of EC detection needs the evaluation of the influence of the organic solvent content and IL on the relationship between the applied potential and the EC signal intensity. Besides the improved efficiencies and resolution, and the smaller analysis times obtained by the addition of ILs ($C_4C_1IM{\cdot}BF_4$, $C_6C_1IM{\cdot}BF_4$, and $C_8C_1IM{\cdot}BF_4$) to the mobile phase, instead of the common ammonium acetate, the main improvement was the remarkable decrease (about 300%) in the background current, which guaranteed less noisy chromatograms. The same conclusion was obtained with mobile phases free of organic solvent.

A particular application for the speciation of inorganic and organic selenium in biological samples was developed using ILs as mobile phase additives combined with inductively coupled plasma-mass spectrometry (ICP-MS) [74]. The advantages with respect to other RPLC-ICP-MS methods for selenium speciation comprised the ability to regulate the retention time of the target selenium species by selecting the suitable IL and its concentration, together with other advantages like simplicity, rapidness and low injection volume.

Finally, it should be noted that ILs complicate the use of mass spectrometric detection. The non-volatility of ILs will be responsible for IL condensation and pollution in the electron-spray or atmospheric pressure-ionisation sources.

## 9. Are ionic liquids really superior to amines?

ILs have been considered as alternative additives to amines in RPLC, in order to enhance the peak performance in the analysis of basic compounds, using conventional stationary phases. However, we should note that in most reports, the performance of ILs has been

compared only with TEA [39,45,52,56–58,65,67], which is the common silanol suppressing agent. In all cases, at least one of the assayed ILs resulted in much better performance. However, TEA is not the best amine to be used as silanol suppressor. Therefore, a fair comparison was not being carried out.

To check this, a detailed study was recently reported that compares the performance of imidazolium-based ILs with varying alkyl-chain lengths attached to the C1 position, with the performance of TEA and DMOA, using several β-blockers as basic probe compounds, acetonitrile-water mixtures and a Kromasil $C_{18}$ column [61]. The results for both retention and peak profiles are shown in Figs. 2 and 3. As observed, the retention behaviour and peak profiles for $C_2C_1IM{\cdot}Cl$ and TEA, on the one hand, and $C_6C_1IM{\cdot}Cl$ and DMOA, on the other, are similar. For $C_2C_1IM{\cdot}Cl$ and TEA, the changes in retention at increasing concentration of additive were rather small with no clear trend. Meanwhile, the addition of a relatively small amount of $C_6C_1IM{\cdot}Cl$ and DMOA to the mobile phase yielded a significant decrease in the retention. Certainly, the trends will change with the type of stationary phase, but still the effect on the retention of those reagents with stronger adsorption on the bonded phase will be larger. These results show that the reduction of retention depends on the size of the cation in the additive, rather than on its nature. Meanwhile, the four reagents produce a significant enhancement of the peak profiles, although peak broadening is somewhat smaller for $C_6C_1IM{\cdot}Cl$, and the peaks are more symmetrical for DMOA. The least symmetrical peaks corresponded to TEA and $C_2C_1IM{\cdot}Cl$, which are adsorbed in lesser amount.

Therefore, the comments in the literature about the better performance of ILs with respect to amines are not accurate. If an IL and an amine of similar adsorption are compared, the performance is similar. In our opinion, this topic deserves a more extensive study. In fact, various amine compounds have worked well in the improvement of peak profile for basic compounds for many years, and some are compatible with MS, opposed to ILs. The appeal of

ILs should be emphasised in other terms. ILs do not change radically the pH of the mobile phase as found for amines, and seem to be less environmentally harmful than volatile amines. ILs do not appear either to damage the silica-based columns as indicated for amines, although some precautions should be taken, especially when working with ILs associated to $PF_6^-$. Thus, it has been recommended that each time these mobile phases are used, the HPLC system should be rinsed overnight with 30% v/v acetonitrile at low flow-rate (0.1 mL $min^{-1}$) [43]. No unusual column aging was observed after one year of experiments with two Kromasil $C_{18}$ columns. Finally, the main attractive of ILs is their great variety, with the possibility to be task-specific for basic compounds and other analytes.

## 10. Some final comments on the future perspective of ILs as mobile phase additives in HPLC

In spite of the publication of several successful examples of application of ILs as mobile phase additives in HPLC, the approach is not widely used so far, although the number of reports is notably increasing. The mechanisms of retention in the presence of ILs are complex, involving hydrophobic interactions, ion-exchange and ion-pairing with the IL cation, and in many cases also with the IL anion. Even the interaction between the matrix of a real sample and the IL may affect the separation [22]. Therefore, it may be affirmed that the related knowledge is still in an early stage. On the other hand, the great variety of known ILs and the synthesis of new ones (compared to other common additives as the amines or conventional surfactants used in MLC) has been considered as “a powerful stimulus for further studies” [19]. However, these numerous ILs may complicate finding the proper one to separate the analytes of interest in a sample. Consequently, a more structured research is needed in this field that offers clear guidelines for analysts.

Most work with ILs in HPLC deals with the separation of basic compounds, trying to eliminate the silanol problem with conventional (5 μm particles, 3 to 4.6 mm i.d.) silica-based columns, and improve the resolution. However, there are other fields of interest to exploit, as the synthesis of more task-specific ILs to improve complex separations of target compounds with different properties, or the separation of optical enantiomers. Undoubtedly, the use of ILs opens new opportunities to solve difficult separations of different analytes. There is also interest in the development of MLC with IL-based surfactants, or in general, in a "greener HPLC" with the reduction of the organic solvent content, or the use of aqueous solutions containing a single IL [39] (see Fig. 4), or a combination of ILs with complementary behaviour. In this regard, an interesting procedure has been recently developed for the separation of selenium species using a mobile phase composed of 0.4% $C_4C_1IM{\cdot}Cl$ and 0.4% $C_4C_1IM{\cdot}BF_4$ in water [74]. Here it is worth to indicate a comment that appeared in the first report on the use of ILs as mobile phase additives in HPLC: "To find a solvent which has a benign effect on the environment and can be used in HPLC efficiently and with good separation for polar to non-polar compounds is one of the greatest challenges for research workers in this field" [39].

However, as is the case for amines, with ILs there has been a big concern on the possible damage of chromatographic columns. This is the reason of the frequent comments in the published reports using ILs in HPLC pointing out that "no stationary phase damage has been observed with the use of ILs, in contrast to what has been observed with other reagents as phosphate or amines" [20,53,54,67]. In spite of this, other comments made by several researchers should be indicated: "It should be noted that toxicity and long-term stability of ILs can vary widely and must be taken into consideration when choosing an IL. Some ILs are relatively impure, and precautions should be taken since impurities could affect both the properties of the IL and the application in which it is used" [6] (see similar comments in Refs.

[7,23]); and “although ILs exhibit negligible vapour pressure at room temperature, which results in virtually no air pollution, improper disposal of these compounds may adversely affect the ecosystem. Therefore, it is important to continue investigating and monitoring the toxicity of ILs and their effects on the surrounding environment” [13]. Finally, not less important is the economy of use of ILs. Their cost is still too high. Thus, ILs have great potential in future HPLC analysis, but this field needs more research to be understood and still several problems must be solved.

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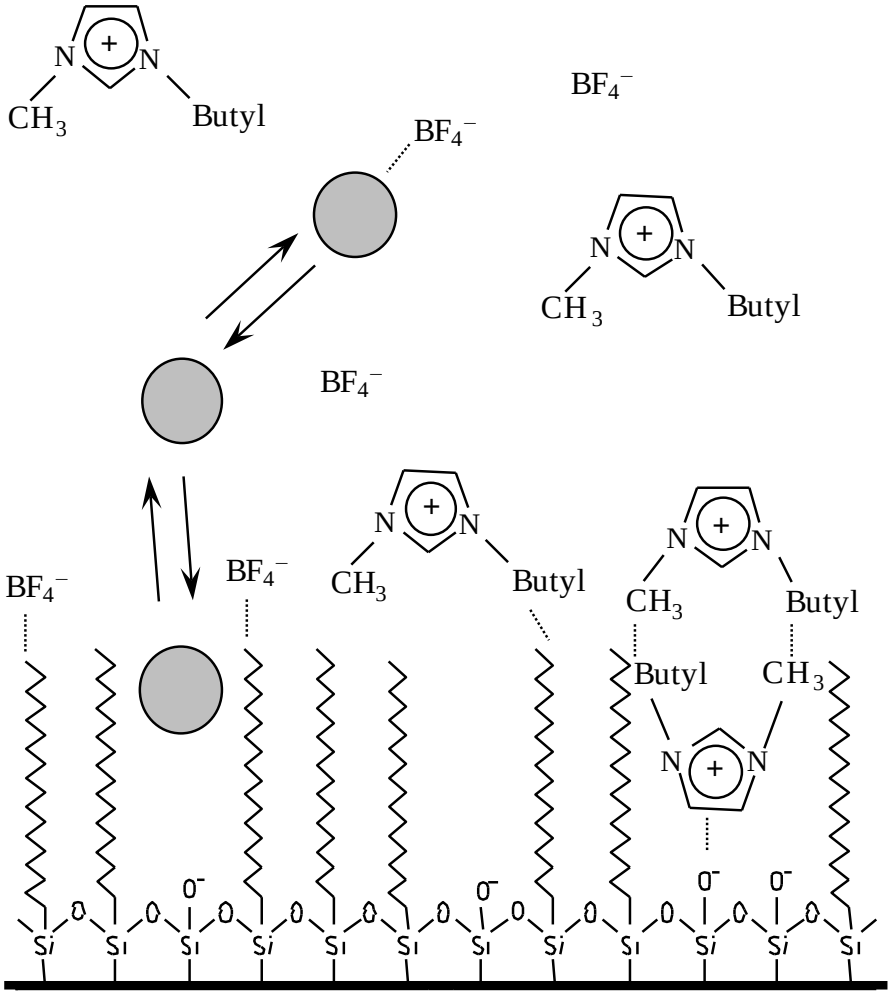


**Fig. 1**. Simplified scheme of positively charged solute environment in a $C_{18}$ stationary phase in the presence of $C_4C_1IM \cdot BF_4$.

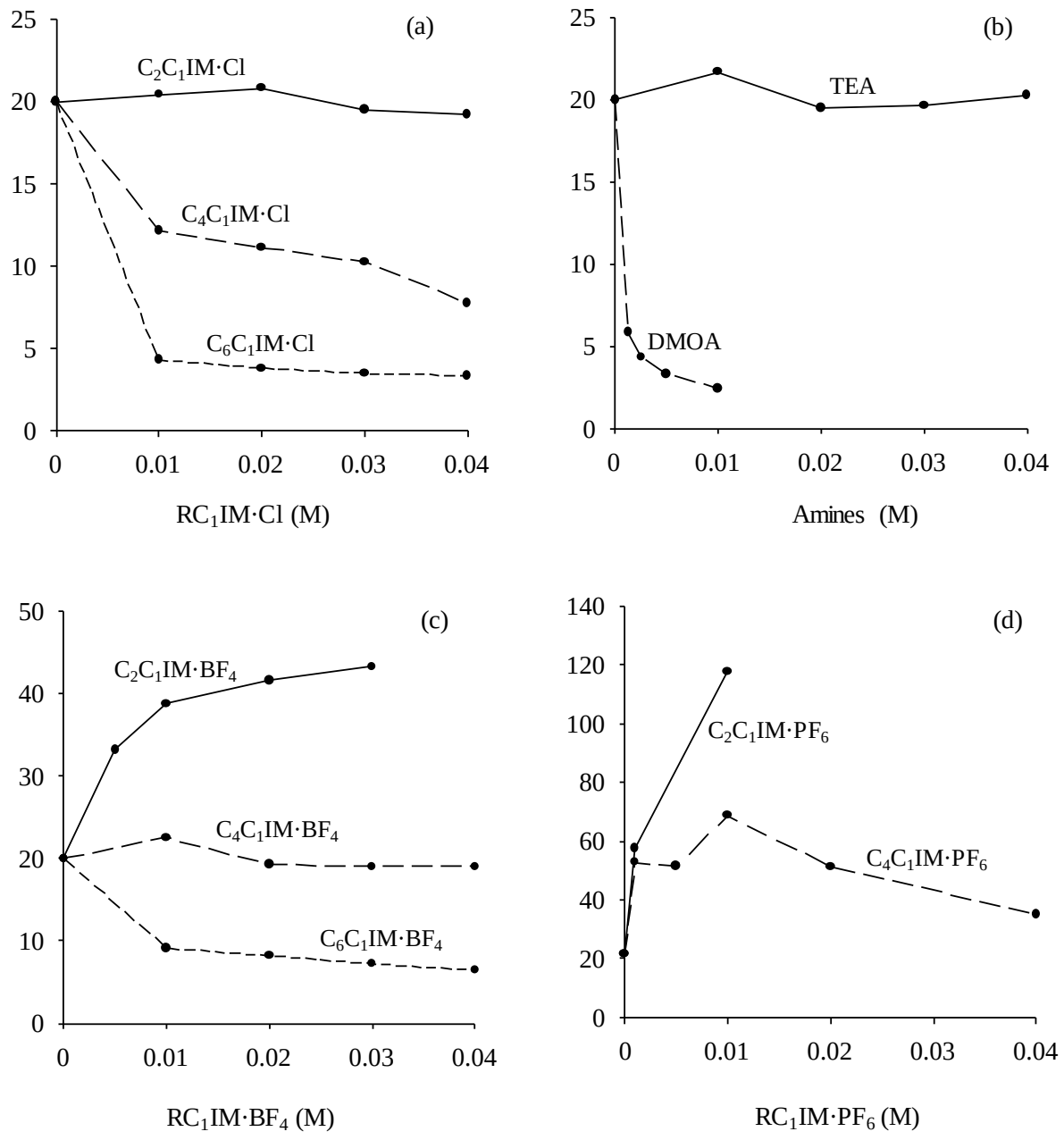


**Fig. 2.** Retention of oxprenolol in aqueous mobile phases containing 15% v/v acetonitrile and increasing concentrations of different alkylimidazolium ILs (a,c,d) and amines (b). Data taken from Refs. [58,61].

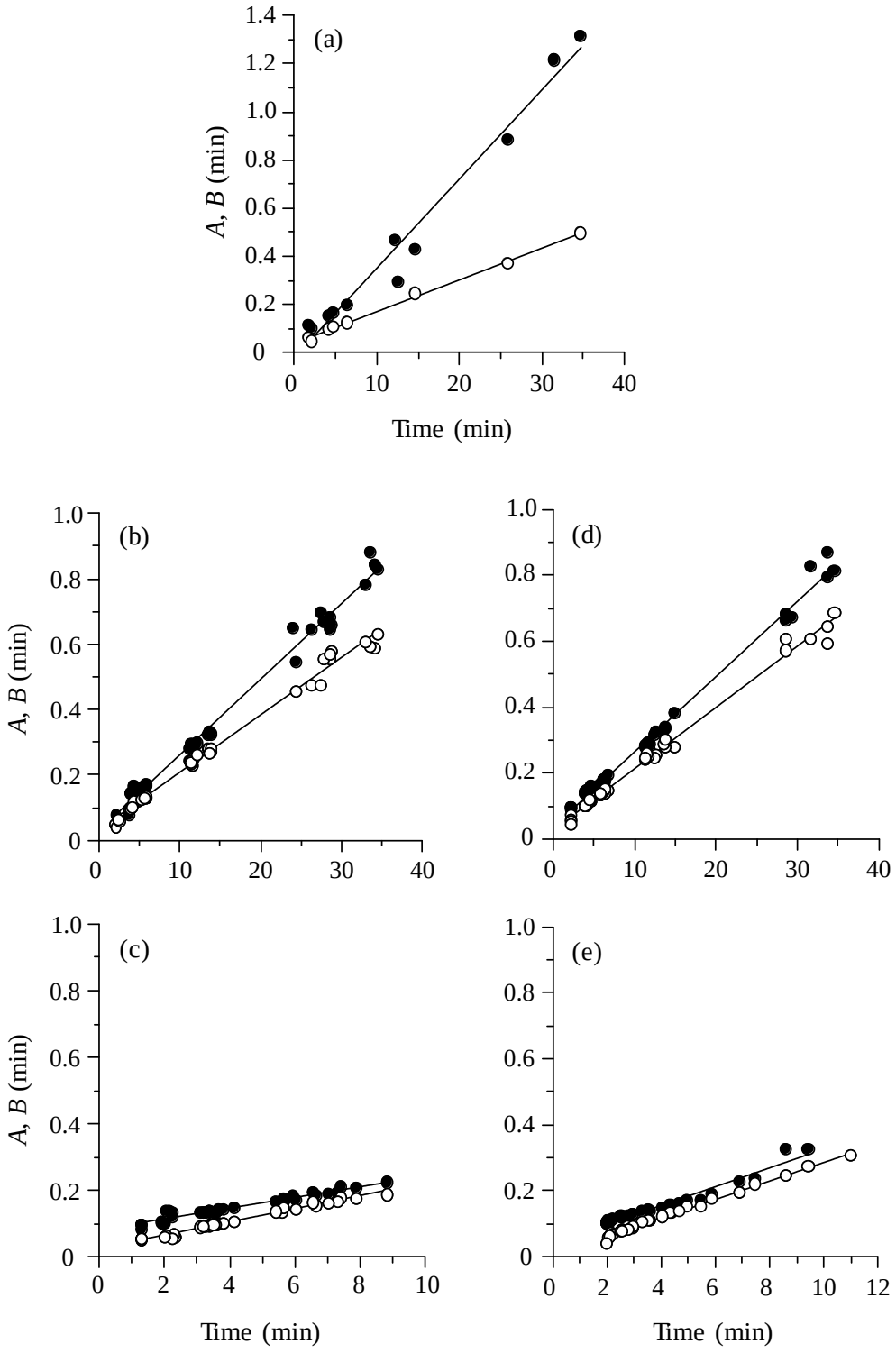


**Fig. 3.** Half-width plots (left, *A*) (○) and (right, *B*) (●), obtained for a set of 10 β-blockers including data obtained using four mobile phases at variable concentrations of ILs: (a) No additive, (b) $C_2C_1IM{\cdot}Cl$, (c) $C_6C_1IM{\cdot}Cl$, (d) TEA, and (e) DMOA. With permission from Ref. [61].

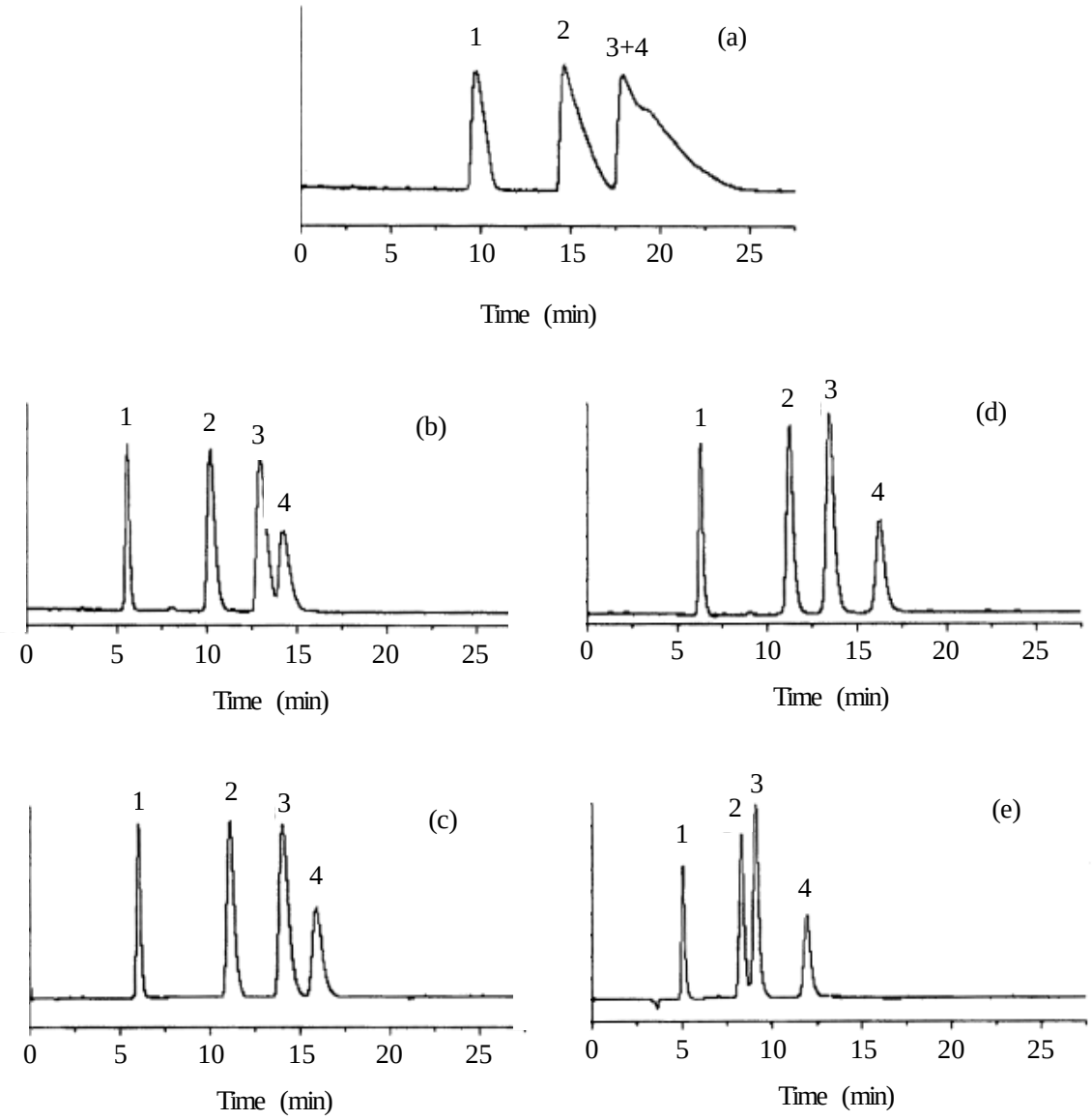


**Fig. 4.** Chromatograms of ephedrines obtained with a mobile phase in the absence (a) and presence (b–e) of $C_4C_1IM{\cdot}BF_4$ at different concentrations: (b) 2.6, (c) 5.2, (d) 20.8 and (e) 62.4 mM, using a Chromatorex ODS column (10 cm × 4.6 mm i.d.) and UV detection at 252 nm. Compounds: (1) norephedrine, (2) ephedrine, (3) pseudoephedrine and (4) methylephedrine. Data taken from Ref. [39].

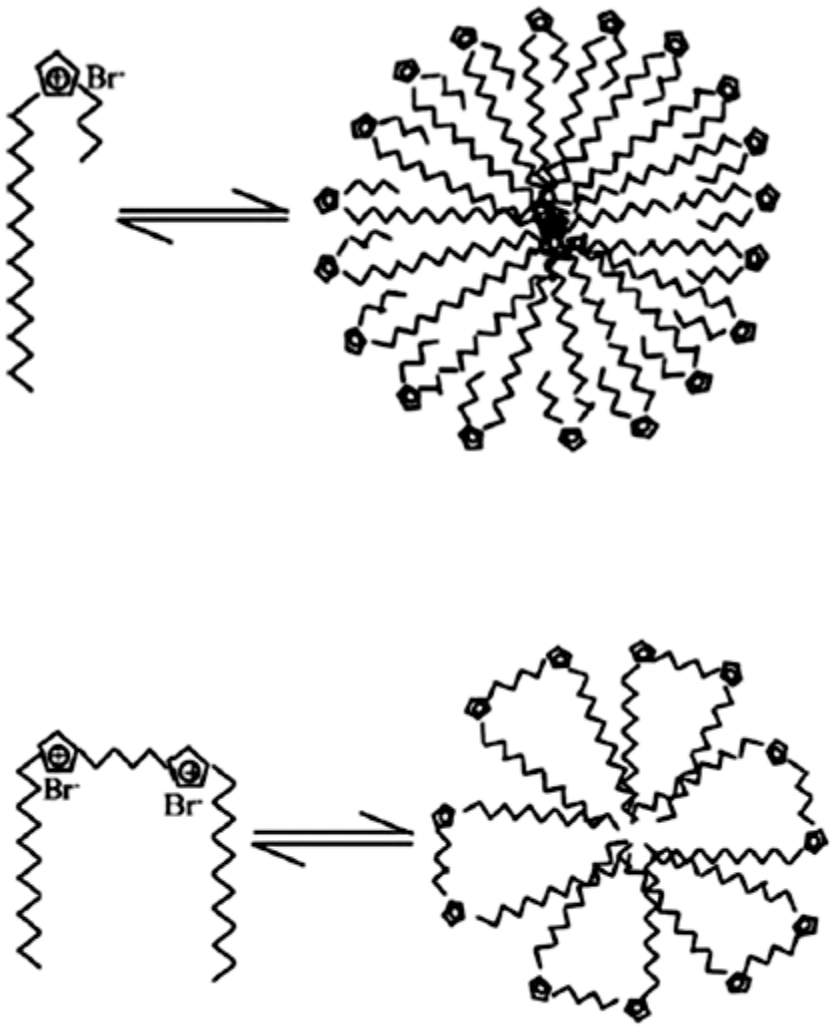


**Fig. 5.** Tentative scheme of a micellar two-dimensional shape IL-based surfactants. Adapted from Ref. [116].

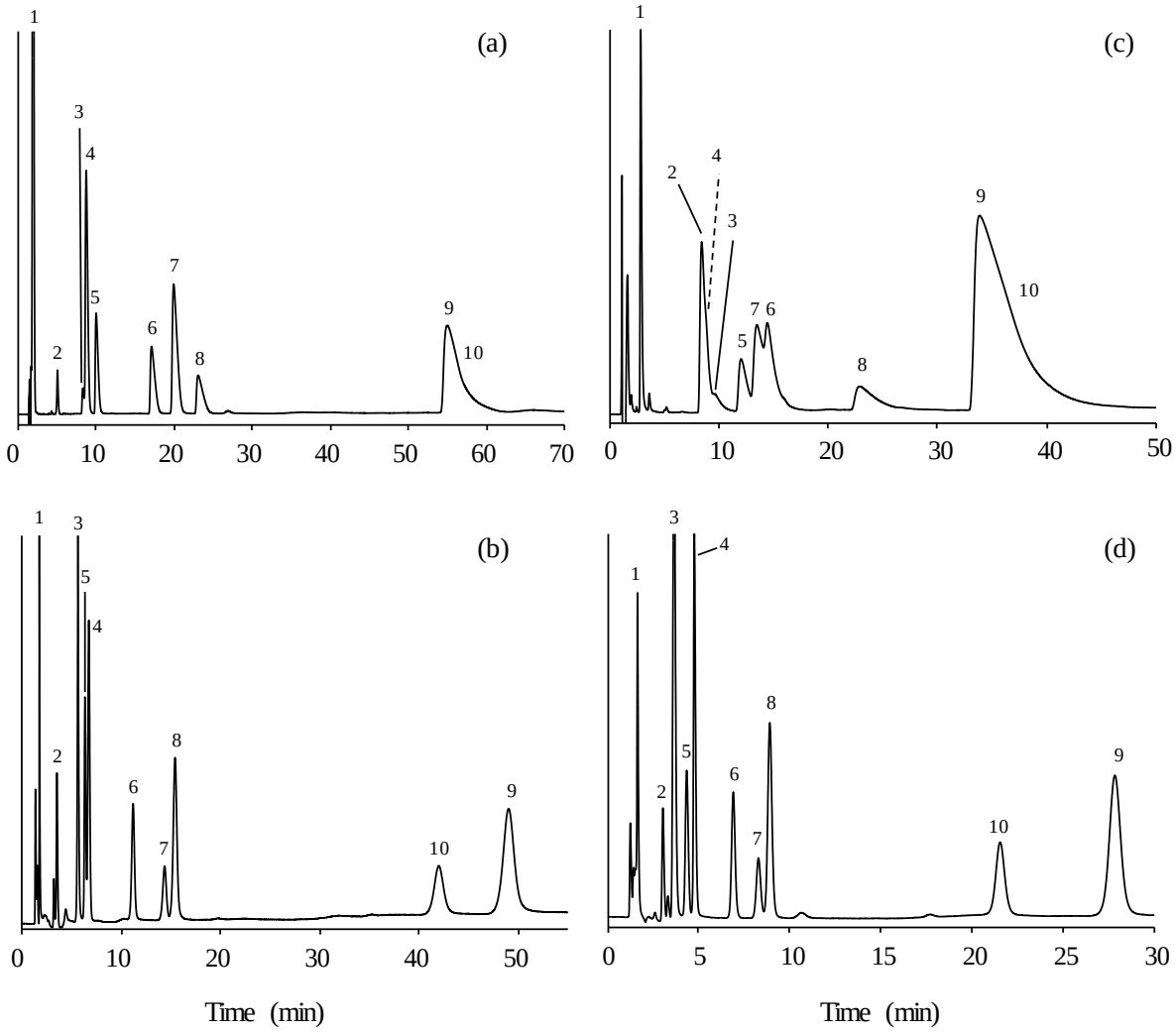


**Fig. 6.** Experimental chromatograms for the optimal conditions using Zorbax $C_{18}$ (a,b) and Spherisorb $C_{18}$ (c,d) (15 cm × 4.6 mm i.d., 5 μm columns), in the absence (a,c) and presence (b,d) of $C_6C_1IM{\cdot}BF_4$. Mobile phase: (a) 15.8% v/v acetonitrile, (b) 10.0% v/v acetonitrile and 0.0232 M $C_6C_1IM{\cdot}BF_4$, (c) 36.5% v/v acetonitrile, and (d) 10.5% v/v acetonitrile and 0.0418 M $C_6C_1IM{\cdot}BF_4$. UV detection at 254 nm. Data taken from Ref. [60]. Compounds: (1) atenolol, (2) pindolol, (3) timolol, (4) acebutolol, (5) metoprolol, (6) esmolol, (7) celiprolol, (8) oxprenolol, (9) propranolol, and (10) alprenolol.

**Table 1.** Experimental characteristics of RPLC procedures using ionic liquids as mobile phase additives.

| Compounds | Mobile phases with ionic liquids | Mobile phases with other additives | Stationary phase / pH / Temperature / Detection | Ref. |
|---|---|---|---|---|
| *Alkaloids*: allocryptopine, berberine, boldine, brucine, chelidoline, quinine, cinchonine, emetine, glaucine, codeine, laudanosine, matrine, noscapine, oxymatrine, papaverine, sophoramine, sophocarpine, sophoridine | 5–15% v/v acetonitrile-water and: 2% v/v $C_4C_1IM{\cdot}BF_4$, $C_8C_1IM{\cdot}BF_4$, $C_4C_1PY{\cdot}BF_4$, 1-butyl-2,3-dimethylimidazolium tetrafluoroborate 1-methyl-3-octyloxymethylimidazolium tetrafluoroborate, methyl-trioctylammonium trifluoroacetate | | XBridge $C_{18}$, XBridge Phenyl, Supelcosil C18, Supelcosil $C_{18}$ DB, Supelcosil LC-F / pH 3.5 / 22°C / UV 254 nm | [44] |
| | 10% v/v acetonitrile-water and: 2.6–83.4 mM $C_4C_1IM{\cdot}BF_4$, $C_6C_1IM{\cdot}BF_4$, $C_8C_1IM{\cdot}BF_4$, $C_{10}C_1IM{\cdot}BF_4$, $C_{12}C_1IM{\cdot}BF_4$ | 10% v/v acetonitrile-water and 3.6 mM TEA | $C_{18}$ / pH 3.4–6.4 / UV 220 nm | [45] |
| *Amines*: adrenaline, dopamine, noradrenaline, isoprotenerol, serotonine | 30% v/v acetonitrile-water and: 0–50 mM $C_2C_1IM{\cdot}PF_6$ | 30% v/v acetonitrile-water and 0–50 mM $NaPF_6$ | Discovery HS $C_{18}$ / pH 3 / 25°C / UV 210 and 280 nm | [46] |
| *Amines and phtalic acids*: benzidine, benzylamine, *N*,*N*'-dimethylaniline, *N*-ethylaniline, octopamine, *o*-, *m*-, *p*-phthalic acids, synephrine, tyramine | Buffered aqueous solution and: 0–60 mM $C_4C_1IM{\cdot}Br$, $C_2C_1IM{\cdot}BF_4$, $C_4C_1IM{\cdot}BF_4$, $C_6C_1IM{\cdot}BF_4$ | Buffered aqueous solution and: 0–50 mM tetrabutyl ammonium bromide | Chromatorex $C_{18}$ / pH 3 / UV 254 nm | [47] |
| | Buffered aqueous solution and: 0–100 mM $C_2C_1IM{\cdot}BF_4$, $C_4C_1IM{\cdot}BF_4$, $C_4C_1IM{\cdot}Cl$, $C_2C_1IM{\cdot}Br$, $C_2PY{\cdot}Br$, $C_4PY{\cdot}BF_4$ | | Spherigel $C_{18}$ / pH 2–7 / 5–50°C / UV 273 nm | [48] |
| *Aminoacids*: *N*-carbobenzyloxy-D-phenyl-alanine, D-tryptophan | 40–90% v/v acetonitrile-water and: 0–10 mM $C_4C_1IM{\cdot}BF_4$ | | $C_{18}$ / unbuffered / 20°C / UV 254 nm | [49] |
| *Aminobenzoic acids*: *o*-, *m*-, *p*-aminobenzoic acids | 25% v/v methanol-water and: 0–64 mM $C_2C_1IM{\cdot}BF_4$, $C_4C_1IM{\cdot}BF_4$, $C_2C_1IM{\cdot}CH_3SO_4$, $C_8C_1IM{\cdot}CH_3SO_4$ | | $C_{18}$ / pH 3–7 / UV 254 nm | [50] |
| *Analgesics*: 1-(1-arylimidazolidyn -2-ylidyn)-3-arylalkyl urea derivatives | 40–55% v/v acetonitrile-water or methanol-water and: 1–30 mM $C_4C_1IM{\cdot}Cl$, $C_4C_1IM{\cdot}BF_4$, $C_4C_1IM{\cdot}PF_6$, $C_8C_1IM{\cdot}BF_4$ | | Zorbax Extend-$C_{18}$ / pH 3 / 20°C / UV 220 or 265 nm | [51] |

**Table 1** (continued).

| Compounds | Mobile phases with ionic liquids | Mobile phases with other additives | Stationary phase / pH / Temperature / Detection | Ref. |
|---|---|---|---|---|
| *Antibiotics*: ampicilin, cefazolin, cefoxatime, ceftriaxone, cephradine, ciprofloxacin, danofloxacin, difloxacin, enrofloxacin, fleroxacin, lomefloxacin, ofloxacin (*R*) and (*S*), sarafloxacin | 10–20% v/v acetonitrile-water and: 0–10 mM $C_2C_1IM{\cdot}BF_4$, $C_4C_1IM{\cdot}BF_4$, $C_6C_1IM{\cdot}BF_4$, $C_8C_1IM{\cdot}BF_4$, Tetraethylammonium tetrafluoroborate | 10% v/v acetonitrile and 0.3% TEA or 100 mM formic acid. 13% v/v acetonitrile and ammonium acetate | Nova-Pak $C_{18}$ / pH 3 and 4 / fluorescence detection: excitation 280 nm, emission 450 nm | [52] |
| | 5–50% v/v acetonitrile-water and: 0.5–3 mM $C_4C_1IM{\cdot}BF_4$, $C_6C_1IM{\cdot}BF_4$, $C_8C_1IM{\cdot}BF_4$ | | $C_{18}$ / pH 3 / 25°C / UV 254 nm | [53] |
| | 12% v/v methanol-water and: 4 or 10 mM $C_2C_1IM{\cdot}Br$, $C_4C_1IM{\cdot}Br$, $C_4C_1IM{\cdot}Cl$, $C_4C_1IM{\cdot}BF_4$, $C_6C_1IM{\cdot}Br$, $C_8C_1IM{\cdot}Br$ | | $C_{18}$ / pH 3.0–5.2 / 25°–45°C / UV 293 nm | [54] |
| *Antidepressants:* amitryptiline, clomipramine, desipramine, fluoxetine imipramine, mianserine, nortryptiline, trazodone, trimipramine | 25/5/70 % v/v acetonitrile-methanol-water and: 0–20 mM $C_4C_1IM{\cdot}BF_4$, $C_4C_1IM{\cdot}PF_6$, $C_4C_1IM{\cdot}CF_3SO_3$ | | Eclipse X-DB-$C_8$ / pH 3 / UV 254 nm | [55] |
| *Antiretrovirals*: abacavir, didanosine, disoproxil fumarate, efavirenz, lamivudine, nevirapine, tenofovir, zidovudine | Methanol (Solvent B) and water (Solvent A) and: 0–10 mM $C_2C_1IM{\cdot}CH_3SO_4$, $C_4C_1IM{\cdot}Br$, $C_4C_1IM{\cdot}BF_4$, $C_4C_1IM{\cdot}CH_3(CH_2)_7SO_4$, $C_6C_1IM{\cdot}Cl$ Gradient program: Solvent B: 25% v/v (0 to 4 min), 25–70% v/v (4–6 min), 70–25% v/v (6–11 min), 25–20% v/v (11–12 min), 20–5% v/v (12–13 min), 5% v/v (13–17 min) | Methanol (Solvent B) and water (Solvent A) containing 10 mM TEA using the same gradient program | Chromolith Flash RP- $C_{18}$ / pH 4 / UV 254 nm | [56] |

**Table 1** (continued).

| Compounds | Mobile phases with ionic liquids | Mobile phases with other additives | Stationary phase / pH / Temperature / Detection | Ref. |
|---|---|---|---|---|
| *β-Blockers*: acebutolol, alprenolol, atenolol, celiprolol, esmolol, labetalol, metoprolol, nadolol, oxprenolol, pindolol, propranolol, timolol | 30% v/v acetonitrile-water and: 0–6 mM $C_4C_1IM{\cdot}BF_4$, $C_4C_1IM{\cdot}PF_6$, $C_8C_1IM{\cdot}BF_4$ | 30% v/v acetonitrile-water and 0–6 mM TEA | Kromasil $C_{18}$ / pH 3 / UV 254 nm | [57] |
| | 15% v/v acetonitrile-water and: 0–0.06 M $C_4C_1IM{\cdot}BF_4$, $C_6C_1IM{\cdot}BF_4$, $C_4C_1IM{\cdot}PF_6$, $C_2C_1IM{\cdot}PF_6$ | 15% v/v acetonitrile-water and 0–6 mM TEA<br>15% v/v propanol-water and 0.02–0.15 M SDS | Kromasil $C_{18}$ / pH 3 / 25 °C / UV 254 nm | [58] |
| | 15 or 20% v/v acetonitrile-water and: 0–0.06 M $C_4C_1IM{\cdot}BF_4$, $C_6C_1IM{\cdot}BF_4$ | | Lichrospher $C_{18}$, Nucleosil $C_{18}$, Kromasil $C_{18}$, Spherisorb $C_{18}$, Zorbax Sb $C_{18}$, XTerra MS $C_{18}$ / pH 3 / 25 °C / UV 254 nm | [59] |
| | 10–40% v/v acetonitrile-water and: 0.02–0.06 M $C_6C_1IM{\cdot}BF_4$ | | Nucleosil $C_{18}$, Zorbax Sb $C_{18}$, Spherisorb $C_{18}$ / pH 3 / 25 °C / UV 254 nm | [60] |
| | 15% v/v acetonitrile-water and: 0–0.04 M $C_2C_1IM{\cdot}Cl$, $C_4C_1IM{\cdot}Cl$, $C_6C_1IM{\cdot}Cl$, $C_2C_1IM{\cdot}BF_4$, $C_4C_1IM{\cdot}BF_4$, $C_6C_1IM{\cdot}BF_4$ | 15% v/v acetonitrile-water and 0–4 mM TEA and 0–0.1 M DMOA | Kromasil $C_{18}$ / pH 3 / 25 °C / UV 254 nm | [61] |
| *Cathecolamines*: dopamine, epinephrine, norepinephrine | Buffered aqueous solution and: 2.8–45 mM $C_4C_1IM{\cdot}Cl$, $C_2C_1IM{\cdot}BF_4$, $C_4C_1IM{\cdot}BF_4$, $C_4PY{\cdot}BF_4$ | | Chromatorex $C_{18}$ / pH 3 and 7 / 25°C / UV 280 nm | [40] |
| *Fangchinoline and tetrandrine* | 80% v/v methanol-water and: 50 mM $C_2C_1IM{\cdot}BF_4$, $C_4C_1IM{\cdot}BF_4$, $C_6C_1IM{\cdot}BF_4$, $C_6C_1IM{\cdot}Cl$ | 80% v/v methanol-water and 10 or 50 mM TEA | Spherigel $C_{18}$ / pH 3–8 / UV 280 nm | [62] |

**Table 1** (continued).

| Compounds | Mobile phases with ionic liquids | Mobile phases with other additives | Stationary phase / pH / Temperature / Detection | Ref. |
|---|---|---|---|---|
| *Heterocyclic aromatic amines*: 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole, 3-amino-1-methyl-5H-pyrido[4,3-b]indole, 2-amino-3-methyl-9H-pyrido[2,3-b]indole, 2-amino-9H-pyrido[2,3-b]indole, 1-methyl-9H-pyrido[4,3-b]indole, 9H-pyrido[4,3-b]indole | 5–50% v/v acetonitrile-water and: 0–12 mM $C_4C_1IM{\cdot}BF_4$, $C_6C_1IM{\cdot}BF_4$, $C_1C_8IM{\cdot}BF_4$ | 18% (v/v) acetonitrile -water and 50 mM ammonium acetate | TSK Gel® ODS-80TM, Nova-Pak® $C_{18}$, ABZ+ / pH 3.6 or 6.0 / electrochemical detection (+1000 mV) | [63] |
| | acetonitrile (Solvent B) and: 1 mM $C_4C_1IM{\cdot}BF_4$ (Solvent A) Gradient program: Solvent B: 19% v/v (0 to 13 min), 19–30% (13.01–16 min), 30% v/v (16.01–22 min) | 18% (v/v) acetonitrile-water and 10 mM TEA | Nova-Pak® $C_{18}$ / pH 3.6 / UV 263 nm and fluorescence detection: excitation 264 nm, emission 410 nm | [64] |
| | 18% v/v acetonitrile-water and: 1–12 mM $C_4C_1IM{\cdot}BF_4$, $C_6C_1IM{\cdot}BF_4$, $C_1C_8IM{\cdot}BF_4$ | 18% v/v acetonitrile and 10 mM TEA | Nova-Pak® $C_{18}$ / pH 3.6 / fluorescence detection: excitation 264 nm, emission 410 nm | [65] |
| *Mandelic acid and derivatives*: 2-chloromandelic acid, mandelic acid, 4-methoxymandelic acid, 3,4,5-trimethoxymandelic acid | 20% v/v methanol-water-copper ion and: 0.5–1.5 mM isoleucine IL | | Kromasil $C_{18}$ / 10–30°C / UV 273 nm | [66] |

**Table 1** (continued).

| Compounds | Mobile phases with ionic liquids | Mobile phases with other additives | Stationary phase / pH / Temperature / Detection | Ref. |
|---|---|---|---|---|
| *Mixtures of diverse drugs*: benzyltriethylammonium, chlorpromazine, diphenhydramine, fluphenazine, imipramine, naphazoline, *o*-toluidine, phenazoline, propiomazine quinine, thioridazine, tiamenidine, trifluopromazine | 30 or 50% v/v acetonitrile-water and: 0.125–3% v/v $C_1C_1IM{\cdot}CH_3SO_4$, $C_2C_1IM{\cdot}BF_4$ 3-heptyloxymethylo-1-hexylimidazolium tetrafluoroborate, 3-hexylomethylo-1-methyloimidazolium tetrafluoroborate | | LiChrospher RP-18 / pH 3 / 25, 40 and 60 °C / UV 254 nm | [42] |
| | 30% v/v acetonitrile-water and: 0–50 mM $C_2C_1IM{\cdot}Br$, $C_4C_1IM{\cdot}Cl$, $C_6C_1IM{\cdot}Cl$, $C_4C_1IM{\cdot}BF_4$, $C_6C_1IM{\cdot}BF_4$, $C_2C_1IM{\cdot}PF_6$, $C_4C_1IM{\cdot}PF_6$, $C_6C_1IM{\cdot}PF_6$, $C_8C_1IM{\cdot}PF_6$ | 30% v/v acetonitrile-water and: 0–60 mM NaCl, $NaBF_4$, $NaPF_6$ | Kromasil $C_{18}$ / pH 4 / UV 230 nm | [43] |
| | 50% v/v acetonitrile-water and: 3.2–128 mM $C_1C_1IM{\cdot}CH_3SO_4$, $C_2C_1IM{\cdot}Cl$, $C_2C_1IM{\cdot}Br$, $C_2C_1IM{\cdot}BF_4$, $C_2C_1IM{\cdot}CH_3CH_2SO_4$, $C_3C_1IM{\cdot}BF_4$, $C_4C_1IM{\cdot}Cl$, $C_4C_1IM{\cdot}Br$ 1-butyl-4-methylpyridinium chloride, $C_4C_1IM{\cdot}BF_4$, $C_4C_1IM{\cdot}CH_3(CH_2)_7SO_4$, $C_6C_1IM{\cdot}BF_4$, $C_8C_1IM{\cdot}Cl$, $C_8C_1IM{\cdot}BF_4$, 1-ethyl-3-methylimidazolium tosylate | 50% v/v acetonitrile-water and: 3.2–128 mM TEA | LiChrospher RP-18 / pH 3 / 25°C / UV 254 nm | [67] |
| *Morpholinium cations*: *N*-methyl ethylmorpholinium, *N*-methyl propylmorpholinium | 10–35% v/v methanol-water and: 0.3–1.0 mM $C_2C_1IM{\cdot}BF_4$, $C_3C_1IM{\cdot}BF_4$, $C_4C_1IM{\cdot}BF_4$, $C_5C_1IM{\cdot}BF_4$ | | Zorbax Eclipse XDB $C_{18}$ / 25–40°C / Indirect UV 210 nm | [68] |
| *Nitroaromatic explosives*: 1,3-DNB, 1,3,5-TNB, 2-A-4,6-DNT, 2,4-DNT, HMX, NB, RDX, TNT, 2NT, 3NT, 4NT, tetryl, 2,6-DNT, 4A-2,6-DNT | 0–20% v/v methanol-water and: 0–20 mM $C_4C_1IM{\cdot}BF_4$ | | GraceSmart $C_{18}$ / 23°C / UV 254 nm | [69] |

**Table 1** (continued).

| Compounds | Mobile phases with ionic liquids | Mobile phases with other additives | Stationary phase / pH / Temperature / Detection | Ref. |
|---|---|---|---|---|
| *Phenols and basic compounds*: acetophenone, aniline, caffeine, *m*-cresol, *o*-cresol, *p*-cresol, methylparaben, phenol, pyridine | 5–20% v/v acetonitrile-water and: 3–9 mM $C_6C_1IM{\cdot}BF_4$, $C_8C_1IM{\cdot}BF_4$ | | Optimapak $C_{18}$ / pH 3.6 / 30°C / UV 254 nm | [70] |
| *Phenolic acids*: danshensu, protocatechuic acid, protocatechuic aldehyde, hydroxy safflower yellow A, ferulic acid | methanol (Solvent B)-water with 0.1% formic acid (Solvent A) and 0–0.6% v/v $C_4MIM{\cdot}Br$ Gradient program: Solvent B: 15% v/v (0–10 min), 15–25% v/v (10–25 min), 25–60% v/v (25–40 min), 60–100% v/v (40–55 min) | Methanol- $C_4C_1IM{\cdot}Br$ and 5.79–57.9 mg $mL^{-1}$ nano-gold particles with the same gradient program | TC-$C_{18}$ / pH 3 / 30°C / electrochemical detection +900 mV | [71] |
| *Phenoxy acid herbicides and phenols:* 4-chloro-2-methylphenol, 2,4-dichlorophenoxyacetic acid dichlorprop, mecoprop, 2-methyl-4-chlorophenoxyacetic acid, 4-nitrophenol | acetonitrile (Solvent B) and (Solvent A): 5–30 mM $C_4C_1IM{\cdot}Cl$, $C_8C_1IM{\cdot}BF_4$, $C_{10}C_1IM{\cdot}Cl$ Gradient program: Solvent B: 20–28% v/v (0–25 min), 28– 60% v/v (25–30 min), 60% v/v (30–40 min) | | TC-$C_{18}$ / pH 7 / UV 280 nm | [72] |
| *Proteins*: adenine dinucleotide, albumin, amyloglucosidase, glutamic dehydrogenase, lactatedehydrogenase, β-nicotinamide, sodium pyruvate, thyroglobulin | 10–50% v/v acetonitrile-water and: 10–50% v/v isopropyl-ammonium formate | | PRP-3 polymeric / fluorescence detection: excitation 270 nm, emission 350 nm | [73] |
| *Selenium species*: Se (IV), Se (VI), selenocystine, selenomethionine, Se-methylseleno-L-cysteine, selenoethionine | Aqueous mixtures of: $C_4C_1IM{\cdot}Cl$, $C_2C_1IM{\cdot}BF_4$, $C_4C_1IM{\cdot}BF_4$ 1-butyl-2,3-dimethylimidazolium tetrafluroborate, For Se(VI): 10–90% v/v For Se (IV) and selenoamino acids: 0.2–1.0% v/v | 0–5% v/v methanol | Shim-Pack CLC $C_{18}$ / pH 3–8 / 15–50°C / ICP-MS | [74] |
| *Stimulants*: ephedrine, methylephedrine, norephedrine, pseudoephedrine | Buffered aqueous solution and: 0–62.4 mM $C_4C_1IM{\cdot}Cl$, $C_2C_1IM{\cdot}BF_4$, $C_3C_1IM{\cdot}BF_4$, $C_4C_1IM{\cdot}BF_4$ | Buffered aqueous solution and: 5.2 and 62.4 mM TEA | Chromatorex ODS-2 / pH 3 / UV 252 nm | [42] |

**Table 2.** Affinity ($K_A$) of different amines and ILs to the silanols and half-width parameters (sum and ratio of the slopes in Eqs. [9] and [10]) for a Kromasil 5 µm column (data taken from Refs. [58,59,61]).

| Additive | $K_A$ | $m_A + m_B$ | $m_B / m_A$ |
|---|---|---|---|
| No additive | – | 0.051 | 2.9 |
| TEA | – | 0.041 | 1.2 |
| DMOA | 2875±820 | 0.056 | 1.0 |
| $C_2C_1IM{\cdot}Cl$ | – | 0.041 | 1.3 |
| $C_4C_1IM{\cdot}Cl$ | 185±145 | 0.039 | 1.0 |
| $C_6C_1IM{\cdot}Cl$ | 1610±485 | 0.036 | 0.8 |
| $C_2C_1IM{\cdot}BF_4$ | – | 0.040 | 1.2 |
| $C_4C_1IM{\cdot}BF_4$ | 95±90 | 0.042 | 1.1 |
| $C_6C_1IM{\cdot}BF_4$ | 330±165 | 0.039 | 0.8 |
| $C_2C_1IM{\cdot}PF_6$ | – | 0.065 | 1.6 |
| $C_4C_1IM{\cdot}PF_6$ | – | 0.066 | 1.5 |