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Silanol suppressing potency of alkyl-imidazolium ionic liquids on C18 stationary phases

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ABSTRACT

Residual silanols on C18 columns yield undesirable slow-kinetics ion-exchange interactions with positively charged basic compounds that result in asymmetrical peaks, low efficiencies and long retention times. The purity of the silica employed as supporting material, and the technique used to form the bonded phase, which varies with the brand and manufacturer, give rise to different amounts of residual silanols in the packings, and consequently, different chromatographic performance. One of the most efficient and widespread strategies to reduce or even eliminate the different performance among columns is the addition of a reagent to the mobile phase to block the silanol sites. However, the intrinsic nature of both stationary phase and additive leads to particular results. In this work, a group of basic compounds were analysed using six C18 stationary phases (Zorbax SB-C18, X-Terra MS C18, Kromasil, Lichrospher, Nucleosil, and Spherisorb) and acetonitrile–water mixtures. Two ionic liquids (ILs), 1-butyl- and 1-hexyl-3-methyl-imidazolium tetrafluoroborates, were added to the mobile phases to evaluate their silanol suppressing potency, based on the decreased retention of the basic compounds when the silanols are blocked (described by the Horváth equation), and the improvement in peak profile (described by the plots of the peak half-widths at diverse retention times). The suppressing potency based on the retention can be misleading when the adsorption of the IL anion is not negligible, since the anion attracts the cationic basic compounds increasing the retention. However, the accessibility of basic compounds to the silanols is prevented by both IL cation and anion, improving the peak profiles for all stationary phases. This was especially remarkable for Spherisorb, which in the absence of additive yielded by far the worst performance. 1-Hexyl-3-methyl-imidazolium tetrafluoroborate was the best additive in terms of retention and peak profile (width and asymmetry).

1. Introduction

Under the general term “silica-based C18”, a large number of diverse stationary phases are commercially available for reversed-phase liquid chromatographic (RPLC) analysis [1–3]. Conventional C18 columns are popular because they are able to separate a wide variety of compounds going from polar (including those ionisable) to highly apolar compounds. Unfortunately, the existence of residual silanols (negatively charged) on the packing material yields slow-kinetics ion-exchange interactions with the positively charged basic compounds that result in asymmetrical peaks, low efficiencies and long retention times [4–10]. This affects also considerably the selectivity and peak resolution. The purity of the silica employed as supporting material, and the technique used to form the bonded phase, which varies with the brand and manufacturer, give rise to different amounts of residual silanols in the RPLC packings, and consequently, different performance.

Several strategies have been proposed to reduce or suppress the deleterious effects of the strong affinity between silanol groups and basic compounds, thus improving the chromatographic performance [5,6,10,11]. These include the use of acidic mobile phases to protonate the silanols, the use of columns where silanols are deactivated, and the addition of reagents to the mobile phase to block the silanol sites. The latter strategy is one of the most efficient and widespread [12,13]. However, the intrinsic nature of the stationary phase and the additive leads to particular results.

The additives traditionally used in RPLC as suppressors of residual silanols have an ionic character. The cation and/or the anion can interact with the stationary phase blocking the ion-exchange processes with the basic compounds, according to the following mechanisms: (i) electrostatic attraction of the cation in the additive to the anionic silanols and (ii) hydrophobic interaction of the cation or the anion in the additive with the alkyl chains in the stationary phase, which forms a charged bilayer that prevents the penetration of the basic compounds to reach the silanol sites.

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Table 1
Physico-chemical properties of the C18 stationary phases used in this work.[a]

	Zorbax SB	X-Terra MS	Kromasil	Nucleosil	Lichrospher	Spherisorb ODS-2
Pore size (Å)	80	120	100	120	100	80
Particle size (μm)	5	5	5	5	5	5
Surface area (m^2/g)	180	175	340	200	350	220
Total carbon (wt%)	10	12	20	11	21.6	12
Surface coverage (μmol/m^2)	Not available	2.4	3.1	Not available	3.9	2.8
Endcapping	Yes	Yes	Yes	Yes	No	Yes
Dimensions (mm × mm)	150 × 4.6	150 × 4.6	150 × 4.6	150 × 4.6	150 × 4.6	150 × 4.6
pH stability range	1.0–7.5	1.0–12.0	1.5–10.0	3.0–7.5	2.0–7.5	3.0–7.5

[a] Data obtained from Refs. [34–36].

Amines have been used since long as silanol suppressors [14–17]. At the acidic pH of the mobile phase, these additives are positively charged and can interact through electrostatic attraction with the free silanols. Also, the molecules may be adsorbed on the alkyl chains with the ammonium groups oriented away from the surface. This would yield a positively charged stationary phase. Both processes decrease the retention, especially the second one through the repulsion of the basic compounds, which would elute with the void volume. With a Kromasil column, we observed only a small decrease in the retention [18], which supports the first mechanism.

Another effective silanol suppressor has been reported: the anionic surfactant sodium dodecyl sulphate (SDS) [19,20]. The long hydrophobic chain of SDS monomers is inserted in the bonded phase, with the sulphate group oriented outside [21]. This gives rise to a negatively charged stationary phase. The cationic basic compounds can interact hydrophobically with the alkyl-bonded layer, or electrostatically with the adsorbed anionic surfactant monomers, which seems to be the main mechanism. The kinetics of the electrostatic association of the basic compounds with the sulphate group seem to be more facile in comparison to the ion-exchange processes involving the silanols. This is concluded from the chromatographic peaks observed in the presence of SDS, which are almost symmetrical [20]. An interesting fact is that the suppression of the silanol effect with SDS is not due to a direct electrostatic interaction with the free silanols (as is the case of amines), but to the protecting coverage of the stationary phase by the surfactant. Unfortunately, a detrimental effect occurs concomitantly: the remarkably increase in the retention times due to the electrostatic attraction of the basic compounds to the modified stationary phase. However, once the stationary phase is saturated with the surfactant, the excess remains in the mobile phase and attracts the basic compounds, decreasing the retention [19].

The behaviours of amines and SDS are interesting to understand the interaction of ionic liquids (ILs), which have attracted some attention recently as efficient candidates to reduce or suppress the silanol activity on RPLC columns [18,22–32]. ILs are composed entirely of ions (cations and anions), and have the special feature of melting usually below 100 °C. Although ILs are extensively used as green solvents with a non-molecular nature that can replace pollutant organic solvents [33], they behave just as dissociated salts when used as mobile phase additives in RPLC, where both the cation and the anion are able to interact with the stationary phase. They have, thus, a dual character [18,27].

The behaviour of ILs is more complex than the behaviour of amines or the anionic surfactant SDS, since the cation can be attracted to the anionic silanols, and both the cation and the anion can be adsorbed on the alkyl-bonded chains through hydrophobic interactions, creating a bilayer, positively or negatively charged depending on the relative strength of the adsorption of cation and anion, respectively. In previous work [18], the silanol suppressing effect of four commercially available alkyl-imidazolium ILs was compared, using a Kromasil C18 column, acetonitrile–water mixtures and a set of β-blockers. ILs containing the anions tetrafluoroborate (BF_4^-) and hexafluorophosphate (PF_6^-) showed two kinds of behaviour, similar to that observed with triethylamine (TEA) for the former, and the anionic surfactant SDS, for the latter. This work expands this research to six C18 stationary phases with different characteristics (type-A and type-B silica), by observing the effect of the tetrafluoroborate ILs, which showed the best performance as silanol suppressors.

2. Experimental

2.1. Reagents

Eight β-blockers were used as basic probe compounds: acebutolol, atenolol, metoprolol, pindolol, timolol (Sigma, St. Louis, MO, USA), celiprolol (Rhône-Poulenc Rorer, Alcorcón, Spain), esmolol (Du Pont-De Nemours, Le Grand Saconnex, Switzerland), and oxprenolol (Ciba-Geigy, Barcelona, Spain). Stock standards were prepared in aqueous solution containing a small amount of acetonitrile (Scharlab, Barcelona), and diluted with water to obtain concentrations of approximately 40 μg/mL.

The mobile phases were prepared with water, acetonitrile and an IL, either 1-hexyl-3-methylimidazolium tetrafluoroborate (HMIM·BF_4, Merck, Darmstadt, Germany) or 1-butyl-3-methylimidazolium tetrafluoroborate (BMIM·BF_4, Sigma). All mobile phases were buffered at pH 3 with 0.01 M citric acid monohydrate and sodium hydroxide (Panreac, Barcelona).

Nanopure water (Barnstead, Sybron, Boston, MA, USA) was used throughout. Solute working solutions and mobile phases were filtered through 0.45 μm Nylon membranes (Osmonics, Herental, Belgium), with a diameter of 47 mm (Magna) and 17 mm (Cameo), respectively.

2.2. Apparatus

The chromatograph (Agilent, Waldbronn, Germany) was equipped with an isocratic pump (Series 1200), an autosampler, and a UV–visible detector (Series 1100). The ILs absorbed below 230–240 nm. For this reason, the basic drugs were detected at 254 nm, except timolol, which was detected at 300 nm. An HPChemStation (Agilent, B.02.01) was used for data acquisition. The retention data were obtained at 25 °C using isocratic conditions with a flow rate of 1 mL/min. Duplicate injections of 20 μL were carried out.

2.3. Columns and working conditions

The behaviour of six C18 columns was examined: Zorbax SB-C18 (StableBonded, Agilent), X-Terra MS C18 (Waters, MA, USA), Kromasil (Análisis Vínicos, Ciudad Real, Spain), Lichrospher, Nucleosil, and Spherisorb (Scharlab) (Table 1). The analytical columns were preceded by similar 30-mm guard columns to protect them from the mobile phase. In order to examine exclusively the behaviour

Table 2
Retention times (min) in acetonitrile–water mixtures without additive.

Basic compound	Zorbax SB[a]	X-Terra MS[a]	Kromasil[a]	Nucleosil[a]	Lichrospher[b]	Spherisorb[b]
Atenolol	2.08	2.14	2.02	3.19	2.91	3.63
Pindolol	4.92	5.50	5.59	11.76	11.69	13.28
Timolol	8.28	9.65	10.22	19.02	14.41	22.22
Acebutolol	8.72	10.36	10.58	22.47	13.53	22.99
Metoprolol	9.69	11.65	12.18	23.79	16.05	26.69
Esmolol	17.08	20.38	21.32	40.08	24.44	48.98
Celiprolol	20.79	24.50	25.37	59.64	27.47	44.76
Oxprenolol	22.93	27.22	29.14	54.15	33.12	62.05

[a] Mobile phase: 15% acetonitrile.
[b] Mobile phase: 20% acetonitrile.

of the analytical column, the solutions of the probe compounds were injected after the guard columns. HMIM·BF_4 was added to acetonitrile–water mixtures at concentrations 0.01, 0.02 and 0.04 M, and BMIM·BF_4 at concentrations 0.01, 0.02, 0.04 and 0.06 M. Acetonitrile–water mixtures in the absence of the ILs were also assayed for comparison purposes. With the Zorbax, X-Terra, Kromasil, and Nucleosil (type-B) columns, a fixed concentration of 15% acetonitrile was used in the mobile phase to explore the effects of the addition of both ILs. The acetonitrile amount was increased to 20% with Lichrospher and Spherisorb (type-A), owing to the significantly longer retention. The selected percentages of organic solvent prevented extremely short retention times upon addition of BMIM·BF_4 or HMIM·BF_4 to the mobile phases. The dead times were: Zorbax (1.27 min), X-Terra (1.34 min), Kromasil (1.29 min), Nucleosil (1.43 min), Lichrospher (1.27 min), and Spherisorb (1.26 min).

3. Results and discussion

The interpretation of the interactions that take place inside the column through the addition of ionic reagents is relatively simple, since changes in the chromatographic behaviour related to both retention and peak profile are yielded. Thus, we have in theory, two ways of measuring the suppressing potency of the additives: through the changes in retention and in peak profile.

3.1. Retention times of the basic compounds eluted with acetonitrile–water mixtures without additive

The polarity of the compounds eluted from RPLC columns is a factor that affects the absolute and relative retention. However, basic compounds are protonated in acidic medium yielding cationic species, which experience strong interactions with the negatively charged free silanols on the columns [6]. This may result in an appreciable increase in their retention, when eluted with hydro-organic mixtures in the absence of reagents able to block the silanols. Therefore, the most appropriate content range of organic solvent in the mobile phase depends on the amount of available silanols in each particular column. On the other hand, as commented, basic compounds attracted to non-protected silanols experience slow desorption, which is the reason of the poor efficiencies.

For this study, six C18 stationary phases containing different types of silica were selected: Lichrospher and Spherisorb, manufactured with type-A silica, and Zorbax SB, X-Terra MS, Kromasil, and Nucleosil, manufactured with type-B silica. Type-A packings are more acidic, due to the significant amount of non-protected silanols and contaminating metals (e.g. Fe and Al), which yields poorer peak profile for basic compounds [1]. In contrast, type-B packings are made of highly purified silica, with a higher average surface coverage [2]. X-Terra MS and Zorbax SB were specially designed to reduce the strong absorption of basic compounds, resulting in significant improvements in the peak profiles. X-Terra MS contains bonded hybrid particles, where methylsiloxanes replace one-third of the silica units, and Zorbax SB is made of a densely covered, sterically protected diisobutyl-*n*-octadecylsilane stationary phase bonded to a high purity porous silica microsphere.

The retention times of the β-blockers eluted with acetonitrile–water mixtures without additive were shorter for Zorbax SB, X-Terra MS, Kromasil and Nucleosil columns, with respect to Lichrospher and Spherisorb. The latter showed by far the longest retention (Table 2). The retention times were also longer for Nucleosil with respect to the other type-B packings. The higher retention is produced, at least partially, by the higher amount of silanols.

3.2. Effects of HMIM·BF_4 and BMIM·BF_4 on the retention of basic compounds with different C18 stationary phases

Both cation and anion in an IL added to a hydro-organic mobile phase have been demonstrated to interact with C18 stationary phases. This is supported by the adsorption isotherms measured by one of the authors in previous work [27]. Owing to the different adsorption of the cation and anion, an asymmetric bilayer with a net charge is created. This yields changes in the chromatographic system, due to the new electrostatic and/or hydrophobic interactions that can be established with the basic solutes. In the presence of an IL, the retention of a basic compound, which is cationic at the mobile phase pH, will be the result of the combination of a mixed mechanism that involves ion-exchange and hydrophobic partitioning, added to the ion-pair interactions with the IL anion in the mobile phase. The cation in the IL competes with the basic compounds in their interaction with the anionic silanols. The extension of all these interactions depends on the relative adsorption of the cation and the anion.

As commented, with a Kromasil column, in the presence of tetrafluoroborate ILs, the retention behaviour resembled that of amines, while in the presence of hexafluorophosphate ILs it resembled the anionic surfactant SDS [18]. This can be understood by considering the moderate adsorption of BF_4^- compared with PF_6^-. Thus, the retention factors of the basic compounds decreased at increasing concentration of HMIM·BF_4 and BMIM·BF_4. This indicates that the imidazolium cation interacts preferentially with the stationary phase (with the free silanols, and in minor extension with the octadecyl layer) with regard to BF_4^- (which only interacts with the octadecyl layer). Hexafluorophosphates exhibited a different behaviour: the retention times of the basic compounds were appreciably longer with respect to the absence of additive, which can be explained by the adsorption of PF_6^- on the stationary phase, which is stronger compared to the imidazolium cation. The stationary phase was observed to be saturated with HMIM·PF_6 and after reaching a maximum, the retention decreased, which should be explained by the ion-pair interactions with the IL in the mobile phase.

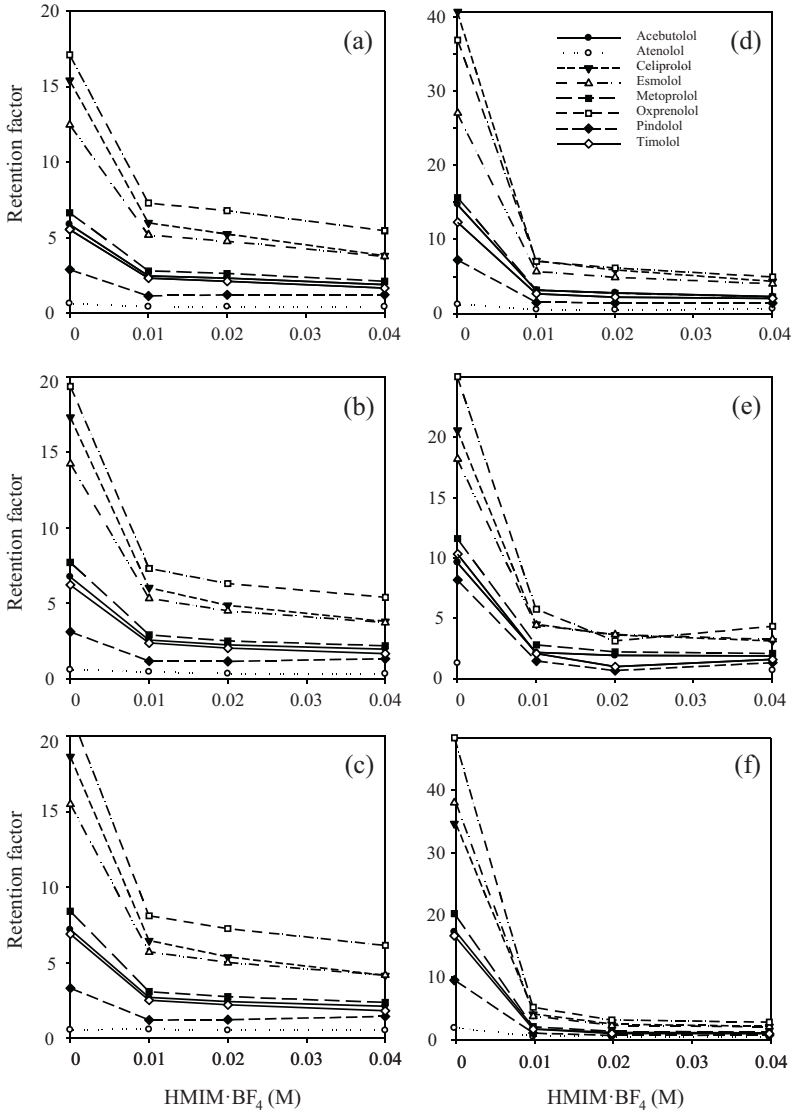

Fig. 1. Retention behaviour of the β-blockers for different C18 stationary phases using acetonitrile–water mixtures at several concentrations of HMIM·BF_4: (a) Zorbax, (b) X-Terra, (c) Kromasil, (d) Nucleosil, (e) Lichrospher, and (f) Spherisorb. The acetonitrile content was 15% for Zorbax, X-Terra, Kromasil and Nucleosil, and 20% for the Lichrospher and Spherisorb.

The increased retention of basic compounds in the presence of hexafluorophosphate ILs is indeed not attractive. Thus, we continued our research with the tetrafluoroborate ILs (HMIM·BF_4 and BMIM·BF_4) added to acetonitrile–water mixtures, and studied their effect on C18 columns containing different amounts of silanols. When HMIM·BF_4 was added, the retention decreased for all columns, especially for Spherisorb, which surprisingly reached the shortest retention times (Fig. 1). This indicated that the HMIM cation interacts preferentially with the stationary phase with regard to BF_4^-. The behaviour with BMIM·BF_4 was rather different: for Zorbax, X-Terra and Kromasil (Fig. 2a–c), which contain a low amount of residual silanols, the changes in retention were small and with different trends. This suggests a similar strength in the adsorption of the cation and the anion in the IL. However, the retention times decreased again for Lichrospher, Nucleosil and Spherisorb (Fig. 2d–f), but in a smaller extent with respect to

Table 3
Affinity (K_A) of HMIM·BF_4 to the silanols for different C18 stationary phases.

Basic compound[a]	Zorbax SB	X-Terra MS	Kromasil	Nucleosil	Lichrospher	Spherisorb
Timolol	277	341	388	–[b]	–[b]	1887
Acebutolol	321	457	485	838	–[b]	1399
Metoprolol	305	448	470	873	1014	1853
Esmolol	284	363	401	825	859	1808
Celiprolol	246	330	331	765	845	1290
Oxprenolol	295	413	423	884	900	1561
K_A[c]	288 ± 26	392 ± 55	416 ± 55	837 ± 47	904 ± 77	1633 ± 253

[a] The compounds are ordered according to their retention times in acetonitrile–water mixtures.
[b] The value of K_A was too large and not considered in the mean.
[c] Mean and standard deviation.

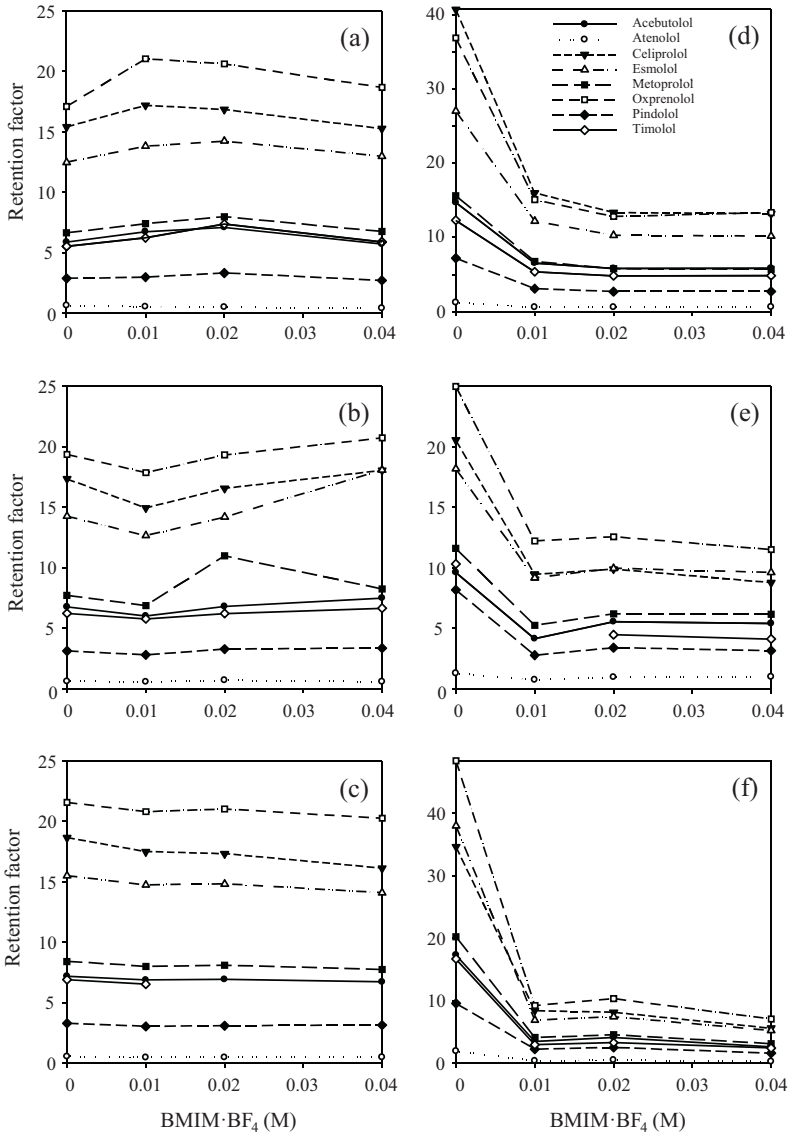

Fig. 2. Retention behaviour of the β-blockers for different C18 stationary phases using acetonitrile–water mixtures at several concentrations of BMIM·BF_4: (a) Zorbax, (b) X-Terra, (c) Kromasil, (d) Nucleosil, (e) Lichrospher, and (f) Spherisorb. See Fig. 1 caption for acetonitrile contents.

HMIM·BF_4 (Fig. 1d–f), since the adsorption of the BMIM cation is weaker than that of the HMIM cation.

These results indicate 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 (HMIM·BF_4 and BMIM·BF_4), whereas for the type-B stationary phases, the effect on retention is less significant.

3.3. Estimation of the suppressing potency of ionic liquids based on the retention

When a cationic additive is added to a hydro-organic mobile phase, the observed changes in retention for basic compounds with C18 columns can be explained by considering a single secondary equilibrium, involving the additive. Without additive, the retention factor (k_0) of a basic compound has two contributions corresponding to the hydrophobic (k_1) and silanophilic (k_2) interactions:

$$k_0 = k_1 + k_2 \quad (1)$$

Masking the silanols will decrease the retention in a factor that depends on the concentration of additive [A], and the affinity of the additive to block the silanol sites, K_A, namely the suppressing potency:

$$k = k_1 + \frac{k_2}{1 + K_A[A]} \quad (2)$$

Table 4
Affinity (K_A) of BMIM·BF_4 to the silanols for Kromasil and Spherisorb.

Basic compound[a]	Kromasil	Spherisorb
Timolol	47.9	477
Acebutolol	40.9	549
Metoprolol	39.0	635
Esmolol	29.7	779
Celiprolol	29.0	467
Oxprenolol	32.3	690
K_A[b]	36.5 ± 7.4	560 ± 124

[a] The compounds are ordered according to their retention times in acetonitrile–water mixtures.
[b] Mean and standard deviation.

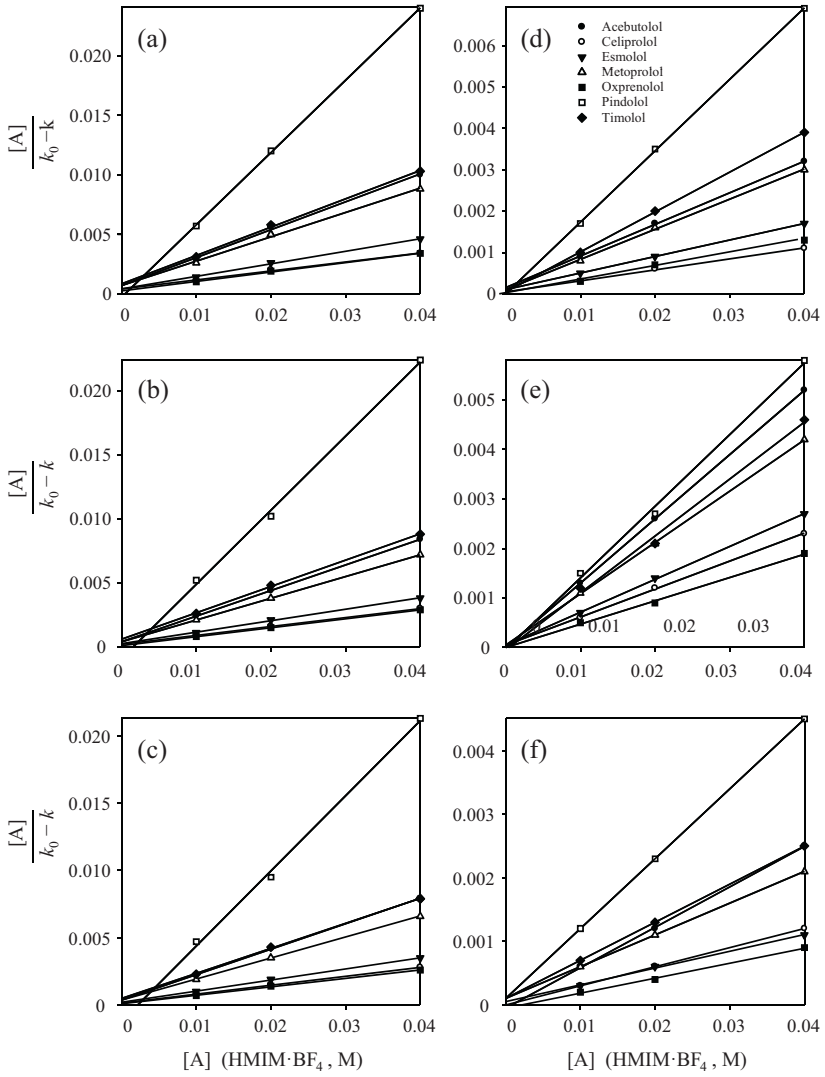

Fig. 3. Horváth plots (Eq. (3)) for several β-blockers eluted from different C18 stationary phases with acetonitrile–water mixtures containing HMIM·BF_4: (a) Zorbax, (b) X-Terra, (c) Kromasil, (d) Nucleosil, (e) Lichrospher, and (f) Spherisorb. See Fig. 1 caption for acetonitrile contents.

If Eqs. (1) and (2) are subtracted and the terms rearranged, an equation is obtained, which was first proposed by Horváth et al. to measure the ability of amines to block the silanol sites [37,38]:

$$\frac{[A]}{k_0 - k} = \frac{1}{k_2 K_A} + \frac{[A]}{k_2} \quad (3)$$

The suppressing potency K_A can be obtained by regressing $[A]/(k_0–k)$ versus $[A]$.

The Horváth equation has been also used to measure the suppressing potency of ILs [18,30]. However, in this case, it can only be strictly applied when the adsorption of the anion is negligible, or at least, much smaller than the adsorption of the cation. In other words, when no other secondary equilibria modify the retention of the basic compound. HMIM·BF_4 is close to this condition, since the cation is preferentially adsorbed. However, we cannot discard a certain adsorption of the anion [27]. The suppressing potency is thus measuring the combined effect of the cation and the anion on the retention, which oppose each other.

For BMIM·BF_4, the Horváth equation could not be applied to the stationary phases that contained a small amount of silanols. In this case, the adsorption of the cation and the anion were compensated, owing to the small extension of the adsorption of the cation. For the other stationary phases, the adsorption of the BMIM cation was stronger. However, the adsorption of the anion was relatively more significant than in the case of HMIM·BF_4, making the calculation of K_A from Eq. (3) arguable. Finally, for the hexafluorophosphate ILs, the suppressing potency K_A could not be calculated, due to the strong adsorption of the anion [18].

Therefore, the suppressing potency based on the retention (K_A) could only be measured for HMIM·BF_4 and BMIM·BF_4 (the latter only with some stationary phases). Note that K_A is obtained by comparison of the retention using mobile phases with the same amount of organic solvent in the presence and absence of additive. As a consequence, it is a relative value: the larger the amount of residual silanols, the larger this constant. Tables 3 and 4 show the suppressing potency for different stationary phases using HMIM·BF_4 and BMIM·BF_4, respectively. Fig. 3 depicts the linear plots for the fitted Hórvath equations for HMIM·BF_4.

It should be noted that K_A is obtained from the retention data of individual solutes. However, K_A is measuring the strength of the interaction between the cation in the additive and the silanols, and therefore, should be a single value corresponding to the stationary phase. In spite of this, a certain scattering was observed in the values of K_A for different β-blockers, which can be explained by:

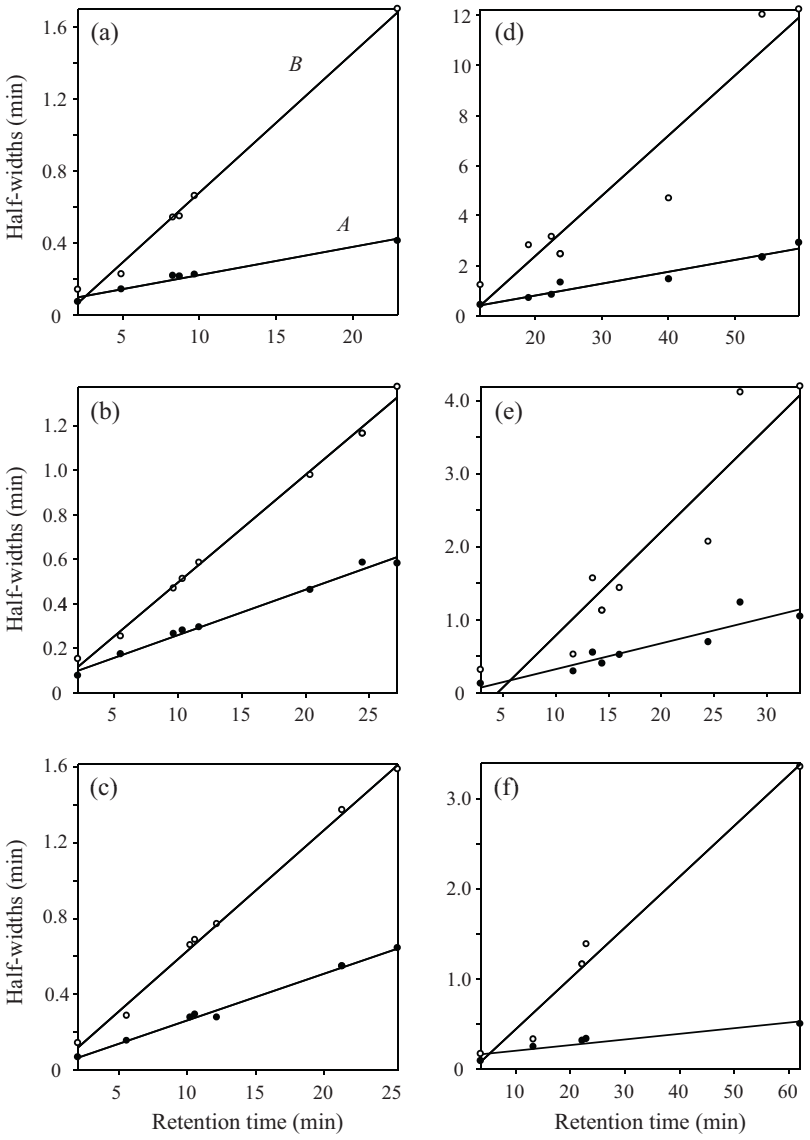

Fig. 4. Peak half-width plots built from the peaks of the β-blockers eluted with acetonitrile–water mixtures without additive: (a) Zorbax, (b) X-Terra, (c) Kromasil, (d) Nucleosil, (e) Lichrospher, and (f) Spherisorb. Half-widths: (●) *A*, left and (○) *B*, right.

(i) the different accuracy in the measurement of the retention times and (ii) the particular contributions of each basic compound to the retention.

Indeed, the least retained compounds, atenolol and pindolol, were more susceptible to experimental errors in the measurement of retention times, and yielded negative intercepts for the type-B packings, which offered shorter retention. For this reason, we decided to leave out these compounds for the calculation of K_A. On the other hand, even considering particular contributions of each basic compound to the retention, the mean K_A values given in Table 3 can be taken as representative of the ability of HMIM·BF_4 to block the residual silanols. It can be observed, as expected, that K_A was larger for Nucleosil, Lichrospher and Spherisorb, which contain a larger amount of unprotected silanol groups.

For BMIM·BF_4, the K_A values should be smaller. However, K_A could not be estimated for Zorbax and X-Terra, since the interaction of the cation and the anion were similar (see Fig. 2a and b). Also, the K_A values for different probe compounds were too scattered for Nucleosil and Lichrospher. Therefore, we could only estimate K_A for Kromasil (type-B silica) and Spherisorb (type-A silica) (Table 4), whose values can be compared with those in Table 3 for HMIM·BF_4. The small K_A value for BMIM·BF_4 using Kromasil evidences the reduced change in retention achieved with this stationary phase/IL combination. For Spherisorb, the reduction in retention was more significant.

3.4. Estimation of the suppressing potency of ionic liquids based on the peak profile

We should not forget that the main interest in blocking the residual silanols is the enhancement in peak profile. In order to measure the suppressing potency under this standpoint, a simple and practical tool that characterises the chromatographic peaks can be used: the plots of the left and right half-widths at a given peak height ratio versus the retention time. The half-widths are more conveniently measured at 10% peak height (instead of at 50% peak height), due to the larger sensitivity to the peak skewness. These plots can be built with the half-widths of either the peaks obtained for several compounds experiencing similar interactions with a chromatographic column at the same mobile phase composition (as the β-blockers in this work), or the peaks of a compound eluted with mobile phases at several compositions, provided there are no changes in the

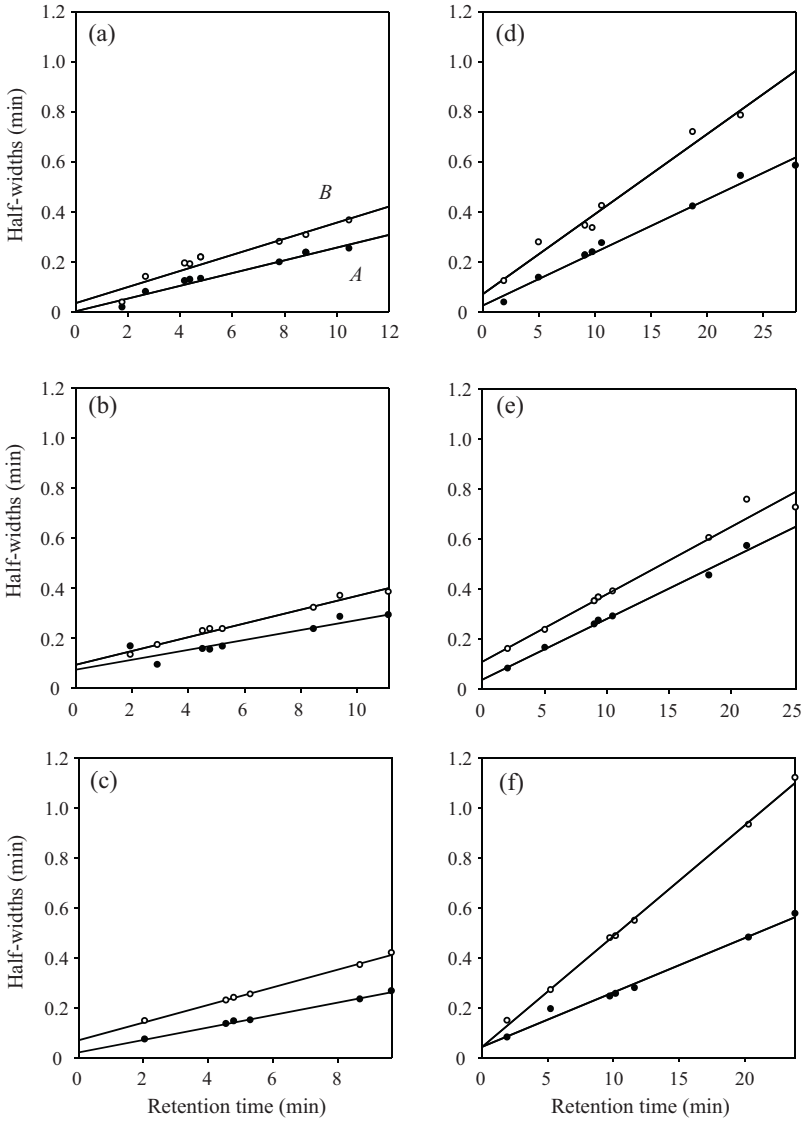

Fig. 5. Peak half-width plots built from the peaks of the β-blockers eluted with a mobile phase containing 15% acetonitrile and 0.01 M HMIM·BF_4 (a–c), or 0.01 M BMIM·BF_4 (d–f): (a and d) Zorbax, (b and e) X-Terra, and (c and f) Kromasil. Half-widths: (●) *A*, left and (○) *B*, right.

Table 5
Half-width parameters for the C18 stationary phases, using acetonitrile–water mixtures without additive and with an IL.

	Zorbax SB	X-Terra MS	Kromasil	Nucleosil	Lichrospher	Spherisorb
Slope of the left half-width plot (r_A)						
Without additive	0.0162	0.0204	0.0247	0.0466	0.0354	0.0214
0.01 M HMIM·BF_4	0.0254	0.0244	0.0249	0.0238	0.0226	0.0207
0.01 M BMIM·BF_4	0.0212	0.0243	0.0216	0.0204	0.0227	0.0177
Slope of the right half-width plot (r_B)						
Without additive	0.0776	0.0482	0.0668	0.121	0.0856	0.0549
0.01 M HMIM·BF_4	0.0321	0.0276	0.0354	0.0305	0.0226	0.0209
0.01 M BMIM·BF_4	0.0320	0.0269	0.0427	0.0389	0.0249	0.0255
Sum of slopes ($r_A + r_B$)						
Without additive	0.0938	0.0686	0.0915	0.168	0.121	0.0763
0.01 M HMIM·BF_4	0.0575	0.0520	0.0603	0.0543	0.0452	0.0416
0.01 M BMIM·BF_4	0.0532	0.0512	0.0643	0.0593	0.0476	0.0432
Slopes ratio (r_B/r_A)						
Without additive	4.8	2.4	2.7	2.6	2.4	2.6
0.01 M HMIM·BF_4	1.3	1.15	1.4	1.3	1.0	1.0
0.01 M BMIM·BF_4	1.5	1.1	2.0	1.9	1.1	1.45

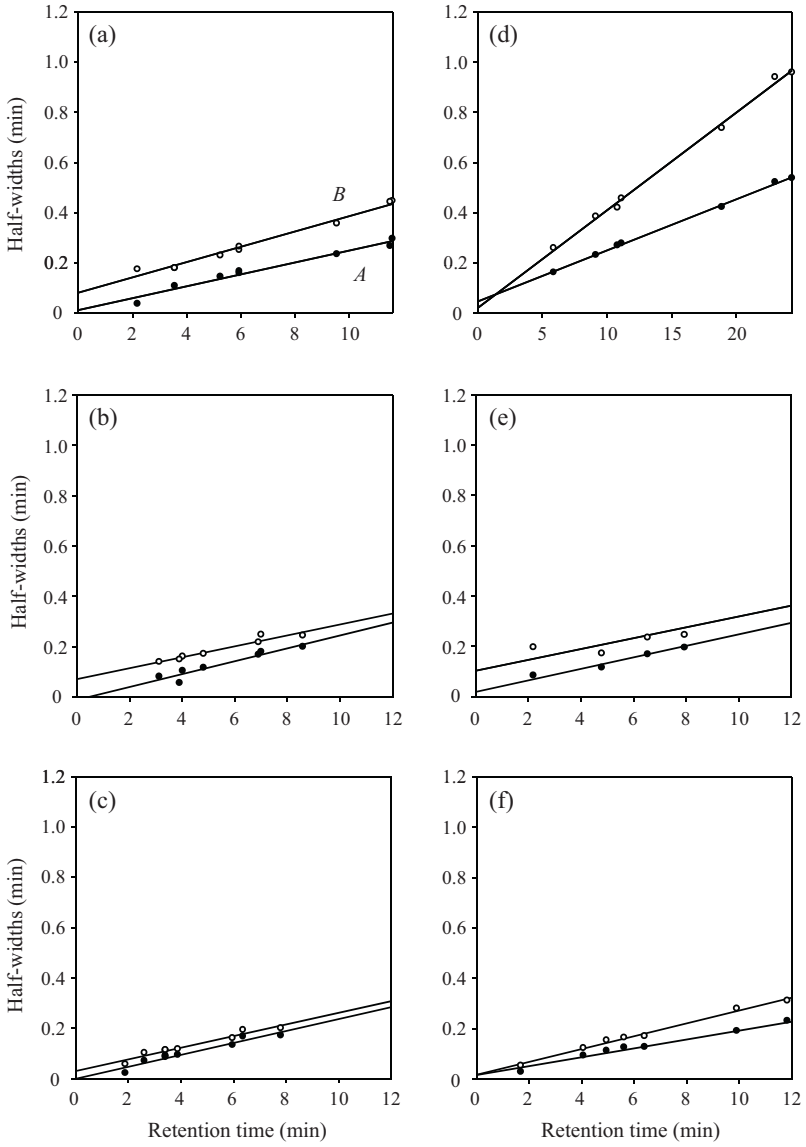

Fig. 6. Peak half-width plots built from the peaks of the β-blockers eluted with a mobile phase containing 15% (a and d) and 20% (b, c, e, and f) acetonitrile and 0.01 M HMIM·BF_4 (a–c), or 0.01 M BMIM·BF_4 (d–f): (a and d) Nucleosil, (b and e) Lichrospher, and (c and f) Spherisorb. Half-widths: (●) *A*, left and (○) *B*, right.

chemical interactions with the column [20,39]. Since, in this work, the column was modified with an additive, the first approach was used.

The sum of the slopes of the plots for the left and right half-widths (r_A and r_B, respectively) indicates the peak width at increasing retention times, which has been called the "peak broadening rate" ($r_{pb} = r_A + r_B$), whereas the extra-column contributions are associated to the intercepts [20]. A smaller peak broadening rate implies narrower peaks at similar times, provided that the width is the same close to the dead time. On the other hand, a parameter that characterises the expected skewness for retained compounds is the slopes ratio. The coincidence of slopes (i.e. $r_A = r_B$) indicates that the peaks of compounds eluting at different retention times will be symmetrical.

Fig. 4 shows the half-width plots for the different columns studied in this work, using acetonitrile–water mixtures without additive. The behaviours after adding 0.01 M HMIM·BF_4 and 0.01 M BMIM·BF_4 are illustrated in Fig. 5 for Zorbax, X-Terra and Kromasil, and in Fig. 6 for Nucleosil, Lichrospher and Spherisorb. The corresponding slopes for the left and right half-width plots, their sum and ratio are compared for the six stationary phases in Table 5. Without additive, the peaks were broad and skewed, especially for Nucleosil, Lichrospher and Spherisorb (note the changes in scale in the plots in Fig. 4). Also, the angle between the plots for the left and right half-widths indicated that the peaks were significantly tailing. The peak profile was enhanced upon the addition of an IL, especially for HMIM·BF_4, and the Lichrospher and Spherisorb packings (Fig. 6b and c). The changes in the right half-width are more evident, in all cases.

These results should be complemented considering those obtained previously for Kromasil using four ILs: HMIM·BF_4, BMIM·BF_4, EMIM·PF_6 (1-ethyl-3-methylimidazolium perfluorophosphate), and BMIM·PF_6, together with TEA and SDS (Table 6). Although all assayed ILs yielded an improvement in the peak profile, the best peaks were obtained with HMIM·BF_4. Thus, for Kromasil, the peak broadening rate decreased from $r_{pb} = 0.091$ in the absence of additive to 0.060 for HMIM·BF_4, being close to 0.065 for the other ILs (similar to the value obtained for TEA, 0.068).

Table 6
Half-width parameters for Kromasil, using acetonitrile–water mixtures in the absence and presence of additives.[a]

Basic compound	Without additive	HMIM·BF_4	BMIM·BF_4	EMIM·PF_6	BMIM·PF_6	TEA	SDS
Slope of the left half-width plot (r_A)	0.0247	0.0249	0.0212	0.0245	0.0264	0.0206	0.0335
Slope of the right half-width plot (r_B)	0.0668	0.0354	0.0427	0.0405	0.0396	0.0474	0.0335
Sum of slopes ($r_A + r_B$)	0.0915	0.0603	0.0643	0.0650	0.0660	0.0680	0.0570
Slopes ratio (r_B/r_A)	2.7	1.4	2.0	1.65	1.5	2.3	1.0

[a] The cations in the ILs are: 1-hexyl-, 1-butyl- and 1-ethyl-3-methylimidazolium (HMIM, BMIM and EMIM, respectively).

The peak performance for SDS (0.057) was slightly better than the value for HMIM·BF_4. However, the retention was much longer with SDS.

4. Conclusions

The addition of an IL to a hydro-organic mobile phase in RPLC improves significantly the peak profile (width and skewness) of basic compounds. This has been observed independently of the relative adsorption of the cation and the anion. However, those ILs where the cation is preferentially adsorbed (as is the case of HMIM·BF_4 and BMIM·BF_4) are more convenient, due to the parallel reduction in retention times. On the contrary, ILs with an anion showing a strong adsorption (as is the case of hexafluorophosphates) should be discarded, due to the long retention times produced by the attraction of the cationic basic compounds to the adsorbed IL anion. Among the assayed ILs, the best performance corresponded to HMIM·BF_4, where the adsorption of the anion was significantly weaker with respect to the cation. This IL exhibits the most interesting features for RPLC analysis of basic compounds: short retention, a small peak broadening rate and almost symmetrical peaks.

The different amounts of free silanols in the C18 stationary phases yield significant differences in the chromatographic performance. These differences are reduced or even eliminated when a tetrafluoroborate IL is added to the mobile phase. Surprisingly, the best performance (at least related to the peak symmetry) was obtained with Lichrospher and Spherisorb, which are manufactured with type-A silica. It should be noted that these stationary phases yielded the worst performance with the acetonitrile–water mixtures in the absence of additives, and attracted the largest amount of IL cation, as seems to indicate the reduction in the retention times.

The measurement of the silanol suppressing potency for ILs through the changes in retention (when possible) is simpler, but can be misleading. This measurement is not correlated to the suppressing potency measured by the changes in the peak profiles. The reason is that the retention of the basic compounds in the presence of an IL is affected by two opposite processes: the direct interaction of the IL cation with the silanols, which are blocked decreasing the retention, and the attraction of the positively charged basic compounds to the adsorbed IL anion, which increases the retention. However, the accessibility of the basic compounds to the silanols is prevented by both the cation and the anion, giving rise to an improvement in the peak profile. This improvement provides clear information about the silanol suppressing potency of ILs.

In previous work, the chromatographic performance with ILs as blocking agents was compared with the performance of different amines [18,28,40]. The use of ILs seems to be advantageous. However, a thorough comparison of the effect of ILs on retention and peak shape with different amines is still needed. The consequences of the changes in retention and peak shape in the presence of ILs on the resolution are being currently under study in our laboratory. Finally, it should be said that the use of ILs as silanol suppressors complicates the use of a mass spectrometric detector, and may add noise or a background signal to UV detection.

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