Reliable measurement of silanol suppression potency in alkyl-bonded stationary phases

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Summary

The design of strategies to reduce the activity of residual silanols in conventional alkyl-bonded columns is still one of the main challenges in current separation of basic compounds by reversed-phase liquid chromatography. The addition of reagents, such as amines, surfactants and ionic liquids, to the hydro-organic mobile phases is a handy option that provides enhanced results. The effectiveness of an additive to suppress the silanol activity can be measured based on the produced changes in retention or peak shape. The former option cannot be applied when the anion in the additive is adsorbed on the stationary phase, at least partially. Also, we should not forget that the main interest in blocking the residual silanols is the enhancement in peak shape. We describe here a simple, reliable, practical and illustrative way of characterising the peak performance using the information provided by the plots of the left and right peak half-widths versus the retention time. The approach is applied to a variety of C18 columns and additives.

The silanol problem and solutions

A significant fraction of the drugs used in modern therapy and a large number of compounds of biomedical and biological significance are basic compounds, which are protonated at the pH conventionally used in reversed-phase liquid chromatography (RPLC) (between 3 and 7). The cationic species interact with the anionic residual silanols on the conventional alkyl-bonded RPLC packings (Figure 1a), increasing the retention times (in some cases excessively), but the most negative aspect is that the analyte desorption is a slow process, which gives rise to skewed peaks with low efficiencies.[1]

Several strategies have been proposed to reduce the silanol effect: (i) the use of an acidic pH to protonate the silanols, (ii) the use of deactivated columns, where silanols are eliminated, and (iii) the addition of different reagents (additives) to the hydro-organic mobile phases to block the residual silanols.

Use of additives to mask the silanols

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

Amines, such as triethylamine, have been used since long as silanol suppressors.[2] At an acidic pH, amines are positively charged and can interact through electrostatic attraction with

the silanols, which are blocked (Figure 1b). Also, the molecule can be adsorbed on the alkyl-bonded chains, with the ammonium group oriented away from the surface. This would give rise to a positively charged stationary phase. Both processes will decrease the retention.

More recently, the anionic surfactant sodium dodecyl sulphate (SDS) has been reported as an effective silanol suppressor.[3] The long hydrophobic chain of SDS monomers is inserted in the bonded phase, with the sulphate group oriented outside. Above the critical micelle concentration, the surfactant monomers also aggregate forming micelles (Figure 1c). A negatively charged stationary phase is obtained. The cationic basic drugs can interact hydrophobically with the alkyl-bonded layer, or through electrostatic attraction with the adsorbed anionic surfactant monomers, which seems to be the main mechanism. The kinetics of the electrostatic association of the basic drugs with the sulphate group seems to be more facile in comparison to the ion-exchange processes with the silanols. This can be concluded from the almost symmetrical peaks with high efficiencies when SDS is added to a hydro-organic mobile phase (Figure 2). It is interesting to note that the suppression of the silanol effect is not due to a direct electrostatic interaction with the free silanols, as is the case of amines, but to the protection of the stationary phase by the surfactant monomers, which prevents the silanol activity.

Recently, ionic liquids have attracted some attention to reduce the undesirable silanol activity in RPLC.[4] These compounds have the special features of being made of a cation and an anion, and of having a low melting temperature and high thermal stability. Ionic liquids are known especially as green solvents that can replace pollutant organic solvents. However, as mobile phase additives in RPLC, they behave just as dissociated salts, where both cation and anion are able to interact with the stationary phase. They have, thus, a dual character. The behaviour of ionic liquids is obviously more complex than the behaviour of amines or the anionic surfactant, since the cation can be attracted to the anionic silanols, and both cation and

anion can be adsorbed on the alkyl-bonded chains through hydrophobic interaction, creating a bilayer, positively or negatively charged, depending on the relative adsorption of the cation and anion (Figure 1d).

The interactions that take place inside the column in the presence of additives give rise to changes in the chromatographic behaviour related to the retention time and peak shape, which can be used to measure the suppression potency of silanols.

Suppression potency measured based on changes in retention

The approach based on changes in retention considers a secondary equilibrium on the silanol groups, involving a cationic species, which is attracted to the anionic silanols (Figure 1b). Without additive, there are two contributions to the retention factor, corresponding to the hydrophobic (k_1) and silanophilic (k_2) interactions:

$$k = k_1 + k_2 \tag{1}$$

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 the silanols, K_A. This constant measures the suppression potency based on retention:

$$k = k_1 + \delta k_2 = k_1 + \frac{k_2}{1 + K_A[A]} \tag{2}$$

If both equations (Eq. (1) and (2)) are subtracted:

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

and the terms rearranged, another equation is obtained, which was proposed by Horváth to measure the ability of amines to block the silanol sites:[5]

$$\frac{[A]}{k_0 - k} = \frac{1}{k_2 K_A} + \frac{[A]}{k_2} \tag{4}$$

This approach has been also applied to some ionic liquids.[6,7] Thus, for 1-butyl- and 1-hexyl-3-methylimidazolium tetrafluoroborates, and a Kromasil column, the value of K_A was 36.5 and 436, respectively, indicating that the latter reagent is a better silanol suppressor. However, this approach cannot be used with anionic additives, such as SDS, or ionic liquids where the adsorption of the anion is not negligible, since K_A is considering two opposite processes: the direct interaction of the cation with the silanols, which decreases the retention and the attraction of the basic compounds to the adsorbed anion, which increases the retention. However, the access of the basic drugs to the silanols is prevented by both cation and anion, producing an improvement in peak shape.

Suppression potency measured based on changes in peak shape

The enhancement in peak shape itself offers another measurement of the suppression potency of the residual silanols. A simple, practical and illustrative way of characterising the peak performance is the plot of the left and right half-widths of chromatographic peaks at a given height versus the retention time (Figure 3).[3,7,8] This plot can be built with the peak half-widths for a compound eluted in different conditions, or for a set of compounds showing similar interactions with the chromatographic column eluted at a given condition. In the presence of additives, the stationary phase surface is modified, changing its nature. Therefore, the second approach should be used. β-Blockers are particularly suitable as test compounds. A useful set formed with compounds commercially available is the following: atenolol, pindolol, timolol, esmolol, acebutolol, celiprolol, metoprolol and oxprenolol.

The approach is based on the approximately linear correlation between both peak half-widths (*A* and *B*, see Figure 3) and the retention time, t_R:

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

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

where m_A and m_B are the slopes of the linear correlations, and A_0 and B_0 the corresponding intercepts. These equations allow the prediction of the peak half-widths at any retention time, and provide parameters useful to characterize the column performance. The sum of the slopes ($r_{pb} = m_A + m_B$) indicates the increasing rate of peak width with the retention time, which we have called the peak broadening rate. The ratio of slopes ($r_{A/B} = m_B/m_A$) measures the maximal peak skewness, reached at a sufficiently high retention time when the extra-column effects are neglected.

Figure 3 illustrates the meaning of the half-width plots for two chromatographic peaks. Measurement of the peak half-widths at 10% peak height is convenient to appraise the peak asymmetry, instead of the more usual measurement at 50% peak height. For low efficiencies and highly asymmetrical peaks, fitting of Eqs. (5) and (6) can be somewhat poor, due to the larger difficulty in the accurate measurement of the peak half-widths. However, the provided information can be still useful.

The approach has been applied in our laboratory to measure the suppression potency for several columns, additives and organic solvents, at several mobile phase compositions. Some results obtained with the set of β-blockers are shown in Table 1. Without additive, the peaks with a Kromasil column were broad and skewed. The peak shape was improved by the addition of different reagents that were adsorbed on the stationary phase. The addition of SDS to hydro-organic mixtures of methanol, ethanol, propanol or acetonitrile yielded significant peak shape enhancements (increased efficiencies and symmetrical peaks). With SDS, acetonitrile is superior to the alcohols in terms of peak symmetry.

Although all assayed ionic liquids ($HMIMBF_4$, $BMIMBF_4$, $EMIMPF_6$ and $BMIMPF_6$ (Table 1) yielded an improvement in the peak shape for the β-blockers, eluted from the Kromasil column, the best peaks were obtained with $HMIMBF_4$, which contains the most

hydrophobic cation and a weakly adsorbed anion. The improvement is superior with respect to triethylamine and similar to SDS, which gave rise to the most symmetrical peaks. Comparing the performance of six columns using $HMIMBF_4$, the improvement in peak shape was larger for the Lichrosphere and Spherisorb columns, with a substrate having a larger amount of free silanols (type A columns). It seems that the ionic liquid coverage is more efficient with these columns.

References

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Figure captions

Figure 1. Solute environment in a chromatographic system with a C18 stationary phase, and a mobile phase without additive (a), and containing triethylamine (b), SDS forming micelles (c), and $HMIMBF_4$ (d). BH^+ is the analyte (the protonated basic compound), and K_A the equilibrium constant that measures the interaction between triethylamine and the free silanols.

Figure 2. Chromatograms obtained for a set of β-blockers eluted from a Kromasil column, and a mobile phase at pH 3 containing: (a) 15% (v/v) acetonitrile, and (b) 45% (v/v) acetonitrile/0.1125 M SDS. Compounds: (1) atenolol, (2) carteolol, (3) pindolol, (4) timolol, (5) acebutolol, (6) metoprolol, (7) esmolol, (8) celiprolol, (9) oxprenolol, and (10) labetalol.

Figure 3. Half-width plots for skewed and symmetrical chromatographic peaks.

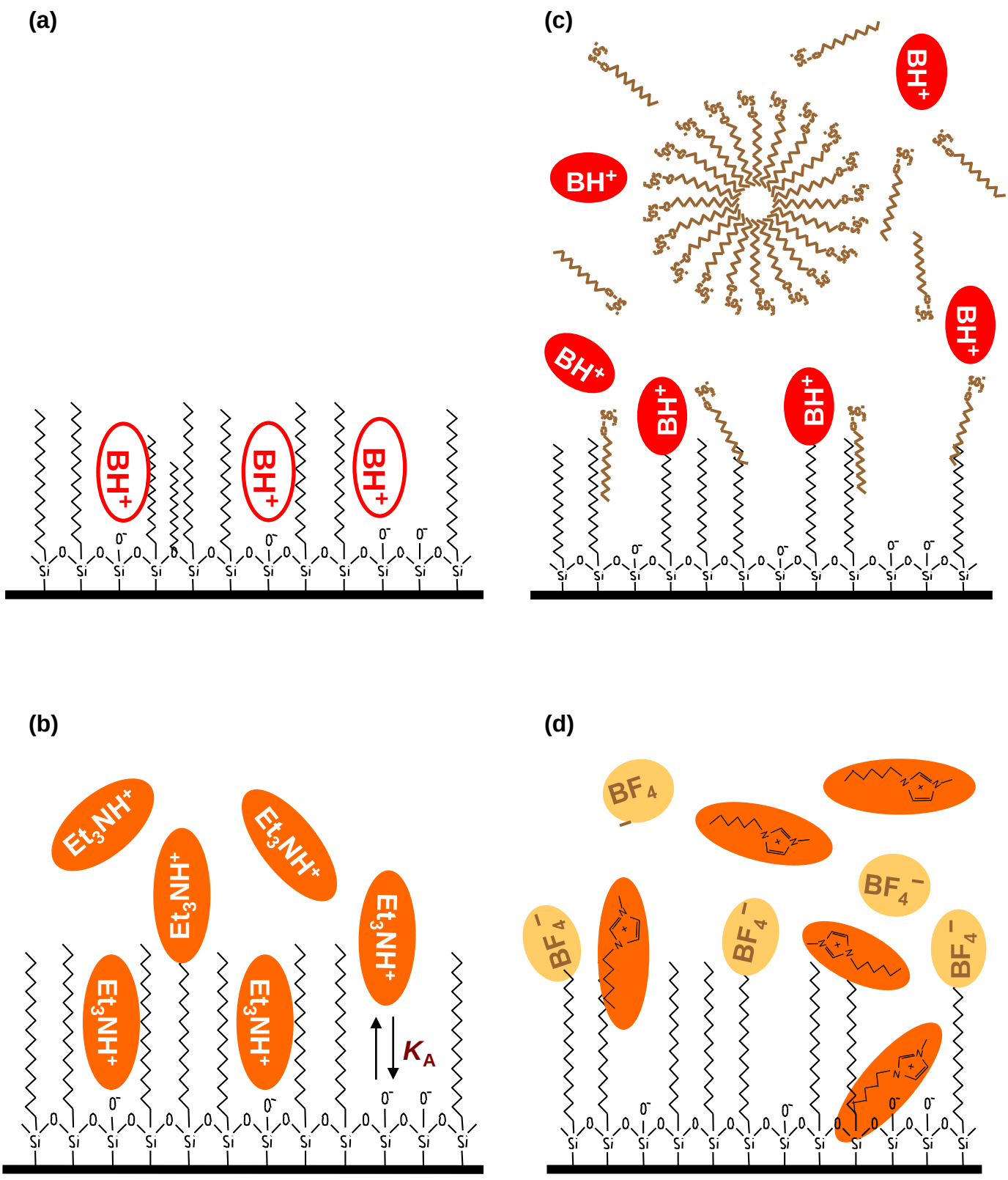
(a)
BH+
BH+
BH+
(c)
BH+
BH+
BH+
BH+
BH+
(b)
Et3NH+
Et3NH+
Et3NH+
Et3NH+
Et3NH+
KA
(d)
BF4−
BF4−
BF4−
BF4−
BF4−

Figure 1

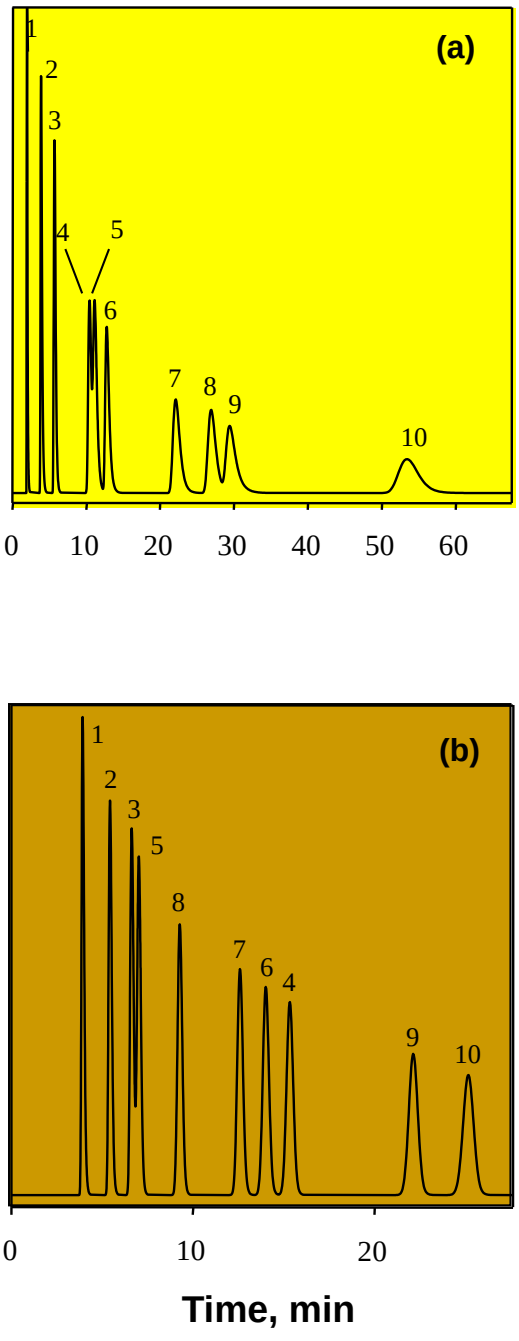

Figure 2

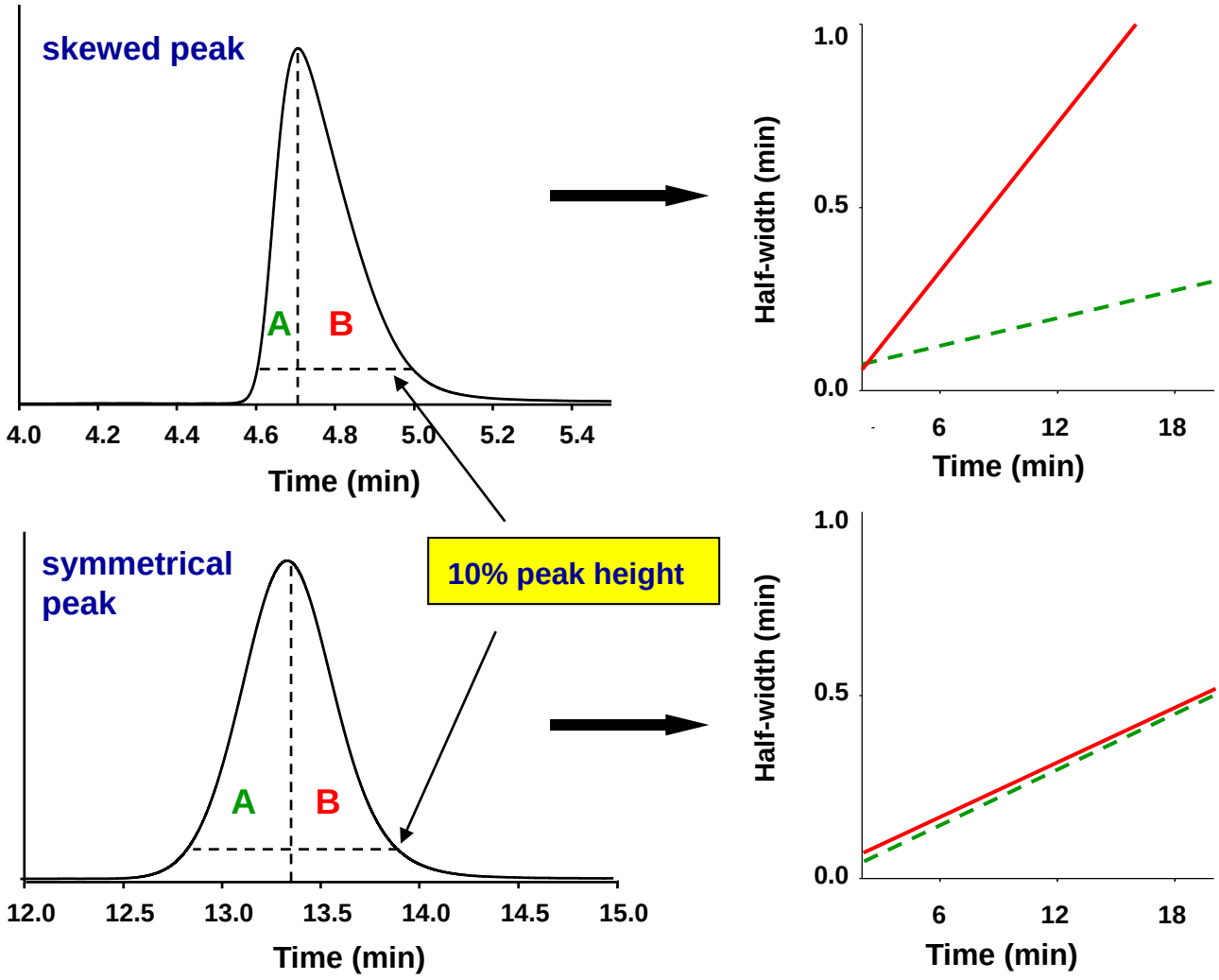
skewed peak
A
B
4.0
4.2
4.4
4.6
4.8
5.0
5.2
5.4
Time (min)
1.0
0.5
0.0
Half-width (min)
6
12
18
Time (min)
symmetrical peak
10% peak height
A
B
12.0
12.5
13.0
13.5
14.0
14.5
15.0
Time (min)
1.0
0.5
0.0
Half-width (min)
6
12
18
Time (min)

Figure 3

Table 1. Silanol suppression potency measured as the peak broadening rate (r_{pb}) and skewness at high retention time ($r_{A/B}$), measured from the half-width plots under different conditions.[a]

Kromasil column						
No additive	20% methanol	15% ethanol	15% propanol	15% acetonitrile		
r_{pb}	0.085	0.106	0.087	0.089		
$r_{A/B}$	3.2	3.2	5.7	2.6		
0.075M SDS	60% methanol	15% ethanol	15% propanol	15% acetonitrile		
r_{pb}	0.056	0.073	0.070	0.071		
$r_{A/B}$	0.9	1.0	0.9	1.1		
Ionic additives[b]	$HMIMBF_4$	$BMIMBF_4$	$EMIMPF_6$	$BMIMPF_6$	TEA	SDS
r_{pb}	0.058	0.064	0.065	0.066	0.068	0.057
$r_{A/B}$	1.4	1.75	1.65	1.5	2.3	1.0
Other columns						
0.01M $HMIMBF_4$ [b]	Zorbax	X-Terra	Kromasil	Nucleosil	Lichrospher	Spherisorb
r_{pb}	0.047	0.052	0.058	0.054	0.044	0.042
$r_{A/B}$	1.3	1.15	1.4	1.3	0.95	1.0

[a] SDS = sodium dodecyl sulphate; TEA = triethylamine; HMIM = 1-hexyl-3-methylimidazolium; BMIM = 1-butyl-3-methylimidazolium; EMIM = 1-ethyl-3-methylimidazolium.

[b] Aqueous mobiles phases containing acetonitrile and the indicated additive.