1-Hexyl-3-methyl imidazolium tetrafluoroborate: An efficient column enhancer for the separation of basic drugs by reversed-phase liquid chromatography

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Abstract

Ionic liquids are dual modifiers composed by a large anion and a large cation, which interact with both the hydrophobic alkyl-bonded phase and the anionic residual silanols in C18 columns. The deactivation of the silanol groups has important implications on the chromatographic analysis of basic drugs, being the improvement of peak profiles and shorter retention times the most noticeable features. However, other characteristics as selectivity or resolution are not usually considered, or are only examined for selected chromatographic conditions. In this work, the effect of the addition of the ionic liquid 1-hexyl-3-methyl imidazolium tetrafluoroborate to acetonitrile-water mixtures in a wide range of concentrations, using three C18 columns (Zorbax SB-C18, Nucleosil and Spherisorb) was studied for the separation of a group of β-adrenolytic drugs. The extremely poor chromatographic performance was highly improved in the presence of the ionic liquid, giving rise to shorter analysis times, smaller consumption of acetonitrile, significantly narrower and more symmetric peaks, and high resolution. The improvement was especially noteworthy for the Spherisorb column, which yielded the poorest results in the absence of additive.

Keywords: Reversed-phase liquid chromatography; 1-Hexyl-3-methyl imidazolium tetrafluoroborate; silanol activity; basic drugs; chromatographic performance

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1. Introduction

The reversed-phase liquid chromatographic (RPLC) analysis of basic drugs is still a challenge for analysts. When protonated, basic drugs suffer slow-kinetics ion-exchange interaction with residual silanols on the alkyl-bonded RPLC packings. This results in asymmetric peaks, low efficiencies and strong retention [1–5], which affect peak resolution and analysis time. The addition of a reagent to the aqueous-organic mobile phases to block the silanol sites and reduce these deleterious effects is an efficient and widespread strategy. However, the selection of the appropriate additive is not an easy task, since the intrinsic nature of both stationary phase and additive can lead to widely different results.

Additives with an ionic character have been traditionally used in RPLC as silanol suppressors. Among these, ionic liquids have received special attention in recent years as efficient enhancers of the chromatographic performance of basic drugs [6–12]. As mobile phase additives in RPLC, they behave just as dissociated salts with hydrophobic cations and anions [11]. This confers them a dual nature that makes their behavior more complex with regard to amines or anionic surfactants added as column enhancers [12–14]. The cation is attracted to the anionic silanols, and both cation and anion can be adsorbed on the alkyl-bonded chains through hydrophobic interaction. This creates a bilayer, which is positively or negatively charged depending on the relative strength of the adsorption of the cation and the anion, respectively. In this environment, the retention of a basic drug is the consequence of the combination of a mixed mechanism that involves ion-pairing, ion-exchange, and hydrophobic partitioning. The extension of these interactions depends on the selected ionic liquid [8]. In recent work, 1-hexyl-3-methyl imidazolium tetrafluoroborate ($HMIM \cdot BF_4$) showed as an efficient peak profile enhancer of basic drugs. The adsorption of $HMIM^+$ on a C18 stationary phase is significantly stronger than that for BF_4^- [7]. This is translated in shorter retention times [12].

Previous studies with ionic liquids were carried out with a single column, and/or a reduced number of mobile phases that contained a fixed organic solvent content and a narrow concentration range of ionic liquid [6–12]. However, in order to appraise the performance or

convenience of ionic liquids as column enhancers, more comprehensive information is needed, provided by a wider range of chromatographic conditions. With this purpose, the chromatographic performance of HMIM·BF_4 added to acetonitrile-water mixtures was explored for the separation of a group of β-adrenolytic drugs, using three C18 stationary phases (Zorbax SB-C18, Nucleosil and Spherisorb). The search of the optimal separation conditions was carried out using an interpretive optimization approach. The improvement of column performance in the analysis of basic drugs was examined in terms of analysis time, peak profile and resolution.

2. Experimental

2.1. Chemicals and apparatus

The probe compounds were ten β-adrenolytic drugs (acebutolol, alprenolol, atenolol, metoprolol, pindolol, propranolol, timolol, celiprolol, esmolol, and oxprenolol). The mobile phases were prepared with water and acetonitrile (Scharlab, Barcelona), in the absence and presence of 1-hexyl-3-methylimidazolium tetrafluoroborate (Merck, Darmstadt, Germany), and were buffered at pH 3 with 0.01 M citric acid monohydrate and sodium hydroxide (Panreac, Barcelona).

The liquid chromatograph (Agilent, Waldbronn, Germany) was equipped with an isocratic pump (Series 1200), an autosampler, and a UV-visible detector (Series 1100) set at 254 nm (except for timolol, which was detected at 300 nm). An HPChemStation (Agilent, B.02.01) and MATLAB R2012A (The Mathworks, Natick, MA, USA) were used for data acquisition and mathematical treatment, respectively. Other details are given elsewhere [15].

2.2. Columns and experimental designs

Three 150×4.6 mm i.d. C18 columns were examined: Zorbax SB-C18 (StableBonded, Agilent), Nucleosil and Spherisorb (Scharlab), which were preceded by similar 30×4.6 mm

i.d. C18 guard columns. Uracil was used as dead time marker. Zorbax SB-C18 and Nucleosil are made of purified silica (type-B silica). The former has a higher average surface coverage and was designed to reduce the strong absorption of basic compounds. Spherisorb is manufactured with type-A silica, which contains a significant amount of unprotected silanols and contaminating metals, giving rise to extremely poor peak profile for basic compounds.

Experimental designs were run with acetonitrile-water in the absence and presence of the ionic liquid HMIM·BF_4. In the former, the concentration of acetonitrile (v/v) was the following: 15, 17, 20, 25 and 30% for the Zorbax and Nucleosil columns, and 20, 30, 35 and 40% for the Spherisorb column (the elution strength for this column was appreciably smaller, which forced higher acetonitrile contents). The second experimental design consisted of five mobile phases prepared with acetonitrile and ionic liquid, which were located in the corners and center of a square according to the following ranges: 10–20% acetonitrile and 0.02–0.06 M HMIM·BF_4. For the Zorbax column, an additional mobile phase (10% acetonitrile/0.01 M HMIM·BF_4) was also assayed.

3. Results and discussion

3.1. Description of the retention behavior

In RPLC, the quadratic relationship between the logarithm of the retention factor, *k*, and the volume fraction of organic solvent in the aqueous-organic mobile phase, φ, is conventionally used to describe the retention:

$$\log k = c_0 + c_1 \varphi + c_{11} \varphi^2 \qquad (1)$$

where t_R is the retention time, and c_i regression coefficients with characteristic values for a given solute and column/solvent system. In the presence of two modifiers, polynomial models with two variables have been suggested. For a mobile phase containing organic solvent and ionic liquid:

$$\log k = c_0 + c_1 \varphi + c_2 [\mathrm{IL}] + c_{12} \varphi [\mathrm{IL}] \quad (2)$$

where [IL] is the molar concentration of ionic liquid.

The retention behavior of the probe compounds was modeled inside the established experimental domains in the absence and presence of HMIM·BF_4. Fig. 1 depicts the excellent descriptions using Eqs. (1) and (2), respectively. For the Zorbax, Nucleosil and Spherisorb columns, the global mean errors in the prediction of retention times, considering all mobile phases in the experimental domains, were 0.45, 0.13 and 0.65% without ionic liquid, and 3.6, 0.7 and 1.2% with ionic liquid, respectively.

3.2. Peak profile

Cationic basic drugs interact with anionic silanol groups in a slow process that give rise to poor peak profiles. Such interaction is prevented by the addition of an ionic liquid to the mobile phase, which decreases the peak width and asymmetry. The improvements can be visualized by building simple linear plots that represent the left (*A*) and right (*B*) halfwidths of chromatographic peaks versus the retention time [14,16]:

$$A = m_A t_R + A_0 \quad (3)$$

$$B = m_B t_R + B_0 \quad (4)$$

where m_A and m_B are the slopes of the linear correlations, and A_0 and B_0 the intercepts. The measurement of peak halfwidths are conveniently done at 10% peak height, where they are not affected by the baseline noise, and the asymmetry is evident. The combination of the parameters in Eqs. (3) and (4) characterizes the chromatographic system: the sum of slopes ($m_A + m_B$) represents the peak broadening rate inside the column at increasing retention time, and their ratio (m_B/m_A) the asymmetry of a peak eluting at a time where the extra-column contribution is non-significant. The equations allow the prediction of the halfwidths for peaks eluting at different retention times.

In order to compare the peak profiles for the three columns (Zorbax SB-C18, Nucleosil and Spherisorb), peak halfwidth plots were built in the absence and presence of HMIM·BF_4, considering the peaks of the ten probe compounds obtained with the whole set of mobile phases in the experimental designs (Fig. 2). In the absence of ionic liquid, the slope of the straight line for the right halfwidth (*B*) was always larger than for the left halfwidth (*A*), indicating significant tailing. The peak profile was significantly poorer for Spherisorb and Nucleosil. Point scattering in the plots for these columns, particularly for the right halfwidth, evidences the existence of more than one retention kinetics. It should be reminded that the amount of residual silanols for Nucleosil and Spherisorb is significantly larger than for the Zorbax column, especially for Spherisorb. For the Zorbax, Nucleosil and Spherisorb columns, the peak broadening rate ($m_A + m_B$) was 0.0724, 0.362 and 0.304, and the asymmetry ratio (m_B/m_A), 4.17, 3.99 and 6.12, respectively.

The addition of HMIM·BF_4 to the mobile phase enhanced appreciably the peak profiles. Both peak width and asymmetry decreased. For the Zorbax, Nucleosil and Spherisorb columns, the values for $m_A + m_B$ were 0.0463, 0.0635 and 0.0423, and for m_B/m_A, 1.09, 1.67 and 1.02, respectively. Note the significant improvement with respect to the values in the absence of additive, especially for the Spherisorb column. As observed in Fig. 2, in the presence of HMIM·BF_4, the peaks were narrow and symmetric, but more interestingly, the best peak profiles corresponded to the Spherisorb column, which yielded the poorest peaks in the absence of ionic liquid.

3.3. Resolution capability

Optimal chromatograms for the mixture of the ten β-adrenolytic drugs, obtained with the Zorbax, Nucleosil and Spherisorb columns, and acetonitrile-water mobile phases without and with HMIM·BF_4, are depicted in Figs. 3 and 4. Fig. 4 shows predicted and experimental chromatograms for the Spherisorb column. The former were obtained by simulation [17]. For this purpose, the retention times were predicted through Eqs. (1) and (2) for the mobile phases in the absence and presence of ionic liquid, respectively, and the peak profiles from the fitted halfwidth plots in Fig. 2. The agreement between predicted and experimental chromatograms was always highly satisfactory, as Figs. 1 and 2 pointed out.

Without ionic liquid, the resolution (measured as peak purity, *P* [17]) was unsatisfactory, especially for the Nucleosil and Spherisorb columns, with *P* = 0.012 and 0.041, against *P* = 0.345 for the Zorbax column (*P* values range between 0 and 1). In contrast, in the presence of HMIM·BF_4 the resolution was highly satisfactory for the three columns, especially for Nucleosil and Spherisorb. Global peak purities for these columns were *P* = 0.997 and 0.971, against *P* = 0.945 for the Zorbax column. Note that alprenolol and propranolol, which appear fully overlapped without ionic liquid for the three columns, are completely resolved in the presence of HMIM·BF_4 (Figs. 3 and 4). The resolution improvements cannot be explained only by changes in the selectivity (indeed, the relative retentions and even the elution order for several probe compounds changed), but especially because of the significant enhancement in peak profile upon addition of HMIM·BF_4.

Robustness, considered as the influence on peak resolution of small changes in mobile phase composition, was also checked. In the absence of ionic liquid, as commented, global resolution was low for the three columns, but also decreased rapidly at smaller or larger acetonitrile concentration with respect to the optimal one (Fig. 5). In the studied range, the resolution map for Zorbax and Spherisorb showed two maxima with *P* = 0.345 and 0.045 for

the former column, and P = 0.075 and 0.041 for the latter. It should be indicated that the chromatogram in Fig. 4 (left) corresponds to the secondary maximum, since the analysis time for the main maximum was above 5 hours. Critical compounds were besides alprenolol/propranolol, acebutolol/timolol for Zorbax, acebutolol/metoprolol/timolol for Nucleosil, and pindolol/acebutolol/timolol and celiprolol/esmolol for Spherisorb.

The resolution maps for the mobile phases containing the ionic liquid considered the concentration of both modifiers as factors (Fig. 5). In contrast to the absence of ionic liquid, the three columns exhibited robust optima in wide regions of the experimental domain. For the Zorbax column, satisfactory resolution (P > 0.95) was found in two regions: a big one at low elution strength (below 10.0% acetonitrile and between 0.02 and 0.055 M HMIM·BF_4), and a rather small one (delimited by 12.7–13.2% acetonitrile and 0.020–0.022 M HMIM·BF_4). For the Nucleosil column, satisfactory resolution was obtained from below 10 to 12% acetonitrile and between 0.020 and 0.045 M HMIM·BF_4. The Spherisorb column gave rise to three main robust regions of high resolution, at the lowest elution strength in the considered range. It should be noted that for the three columns, the changes in resolution were more intense for acetonitrile than for HMIM·BF_4. Also, the regions of high resolution were found in the neighborhood of 10% acetonitrile.

4. Conclusions

This work shows the significant improvement in the chromatographic performance of C18 columns for the analysis of basic drugs with acetonitrile-water mixtures, by addition of the ionic liquid HMIM·BF_4. In the absence of additive, the results are extremely poor owing to the activity of residual silanols. The cation and anion in HMIM·BF_4 are adsorbed on the alkyl-bonded phase. However, the strength of the cation adsorption is significantly larger. This blocks free silanols avoiding the interaction with the cationic basic compounds.

Consequently, these only (or mainly) experience hydrophobic interaction with the column, which yield significantly smaller retention times and enhanced peak profiles. These favorable features give rise to a noteworthy improvement in the chromatograms and a significant reduction in the consumption of acetonitrile (below 10−15%), especially for the Spherisorb column. Although it is expected that the ionic liquid covers the three stationary phases in a significant extent, these keep particular behaviors with respect to the analysis times, peak shape and selectivity (and consequently, different resolution).

A relevant observation is that the best chromatograms corresponded to the column with larger amounts of residual silanols (type-A silica), which in the absence of additive yielded the poorest results. In this column, the larger proportion of free silanols results in a larger amount of adsorbed ionic liquid cation ($HMIM^+$), as the percentage of decreased retention revealed [15]. Halfwidth plots indicated that there is no change in the kinetic behavior within the studied concentration range of $HMIM{\cdot}BF_4$. This points out that residual silanols are sufficiently blocked using concentrations of this ionic liquid of at least 0.02 M.

Acknowledgments

This work was supported by Project CTQ2010–16010/BQU (Ministerio de Economía y Competitividad, Spain), and FEDER funds.

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FIGURE CAPTIONS

Fig. 1. Accuracy in the prediction of the retention times for the ten β-adrenolytic drugs eluted from the three assayed columns with acetonitrile-water, in the absence (Eq. 1), and presence of the ionic liquid HMIM·BF_4 (Eq. 2).

Fig. 2. Halfwidth plots for the three assayed columns in the analysis of the β-adrenolytic drugs with acetonitrile-water, in the absence and presence of HMIM·BF_4. Symbols: Left halfwidth (*A*, ●) and right half-width (*B*, ○).

Fig. 3. Experimental chromatograms for optimal conditions using the Zorbax and Nucleosil columns, in the absence and presence of HMIM·BF_4 (global peak purity is given in parenthesis). Zorbax without ionic liquid: 15.8% acetonitrile (P = 0.345); Zorbax with ionic liquid: 10.0% acetonitrile and 0.0232 M HMIM·BF_4 (P = 0.945); Nucleosil without ionic liquid: 18.1% acetonitrile (P = 0.012); Nucelosil with ionic liquid: 10.0% acetonitrile and 0.0244 M HMIM·BF_4 (P = 0.997).

Fig. 4. Predicted and experimental chromatograms for optimal conditions using the Spherisorb column, in the absence and presence of HMIM·BF_4 (global peak purity is given in parenthesis). Without ionic liquid: 36.5% acetonitrile (P = 0.041); with ionic liquid: 10.5% acetonitrile and 0.0418 M HMIM·BF_4 (P = 0.971).

Fig. 5. Resolution maps (measured as peak purity) using acetonitrile-water mixtures without ionic liquid (one variable: acetonitrile), and in the presence of HMIM·BF4 (two variables: acetonitrile and HMIM·BF4. The right diagrams are contour maps, where the shadowed regions correspond to global peak purities $P > 0.95$.

Figure 1

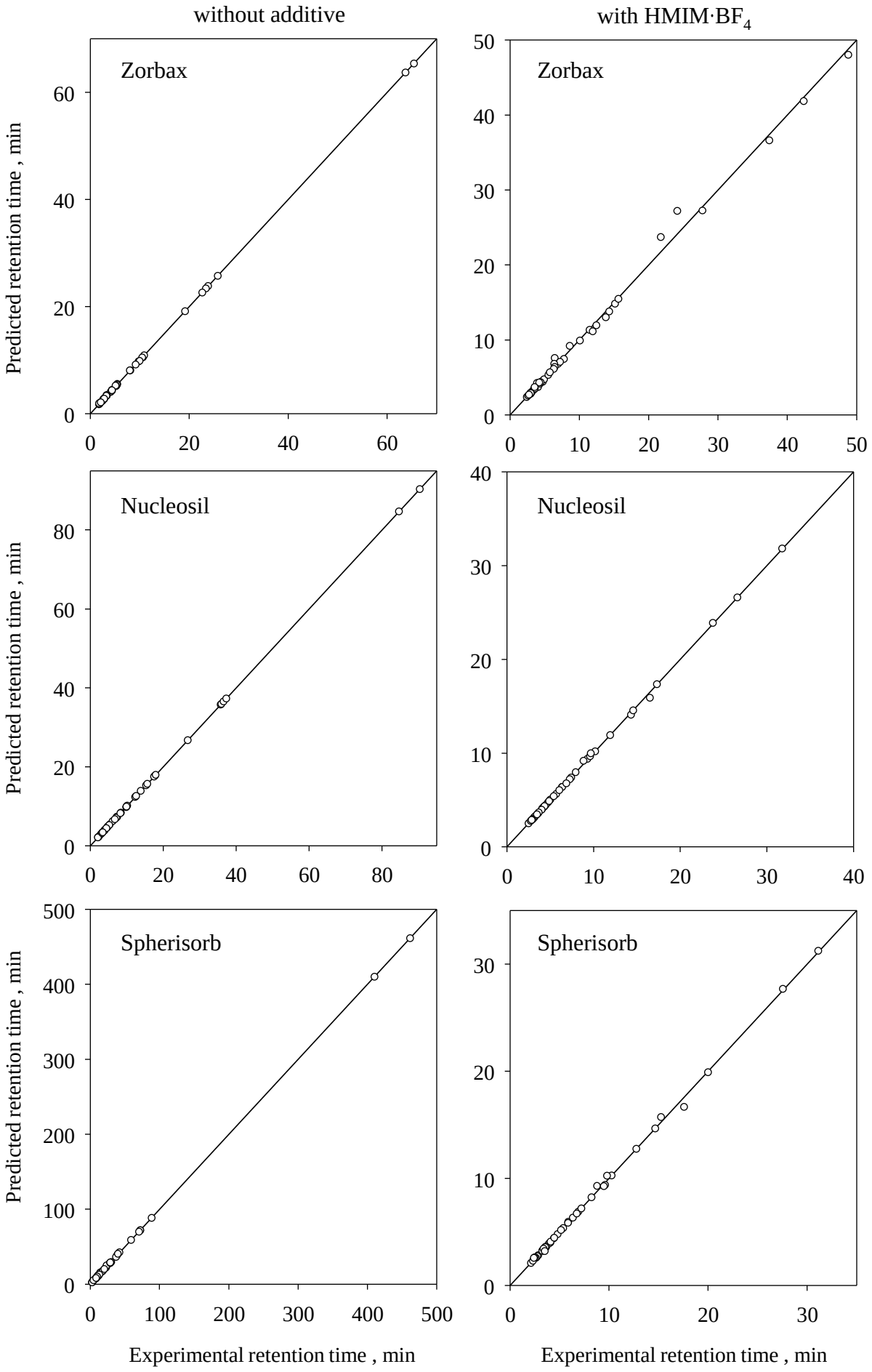

Figure 1

Figure 2

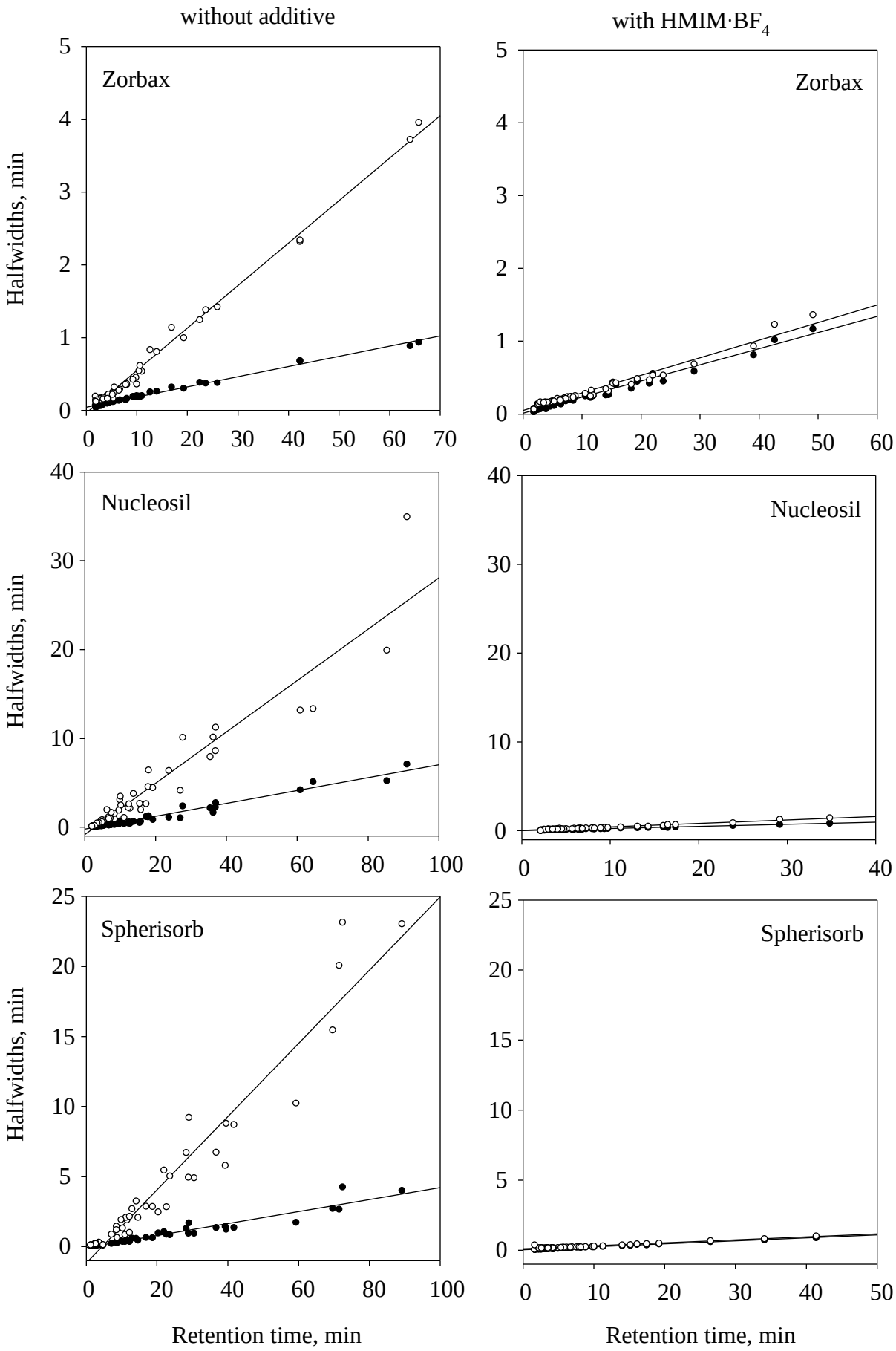

Figure 2

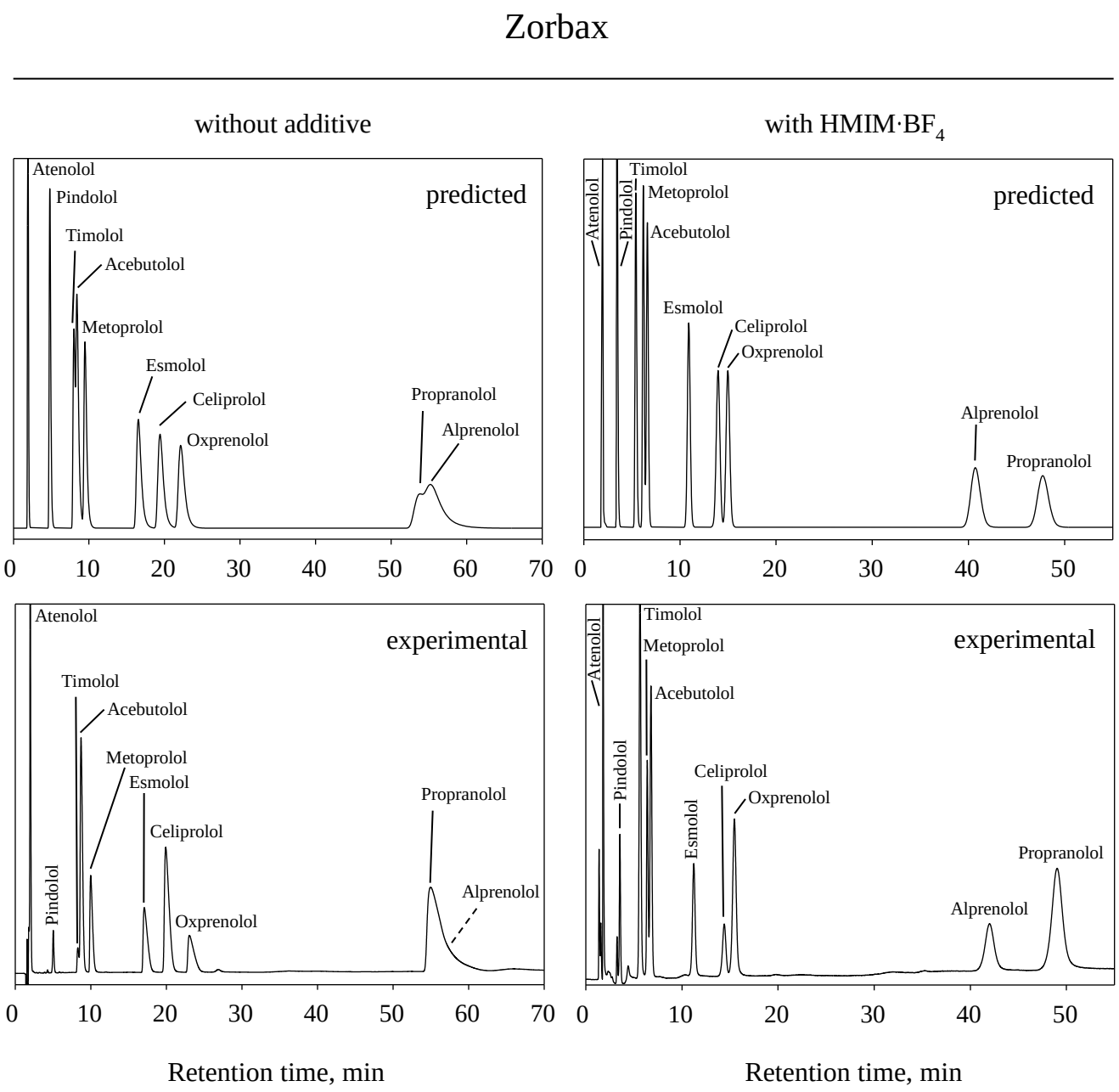
Zorbax
without additive
with HMIM·BF4
predicted
predicted
experimental
experimental
Atenolol
Pindolol
Timolol
Acebutolol
Metoprolol
Esmolol
Celiprolol
Oxprenolol
Propranolol
Alprenolol
0
10
20
30
40
50
60
70
Retention time, min
Retention time, min

Figure 4

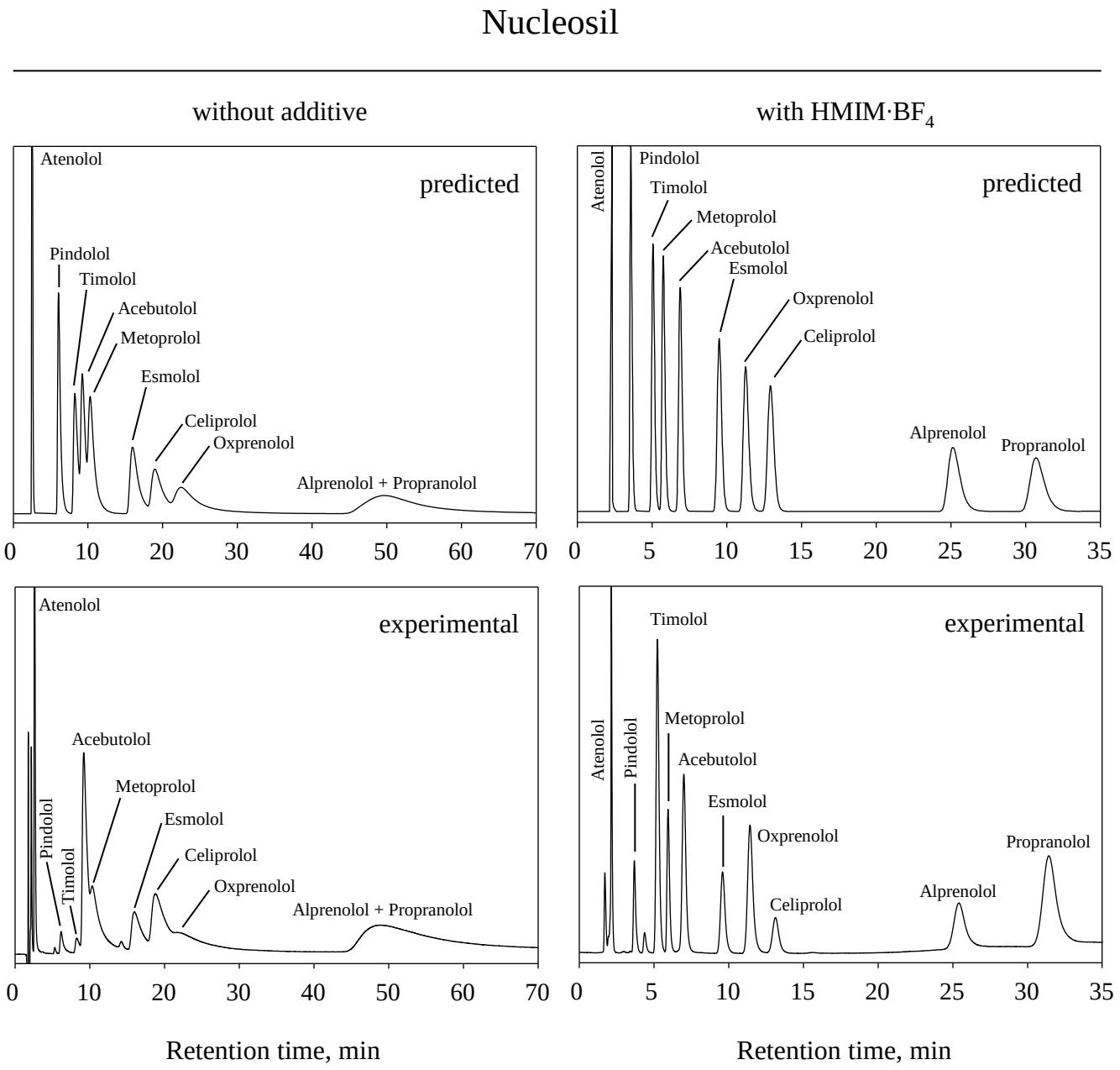

Figure 4

Figure 5

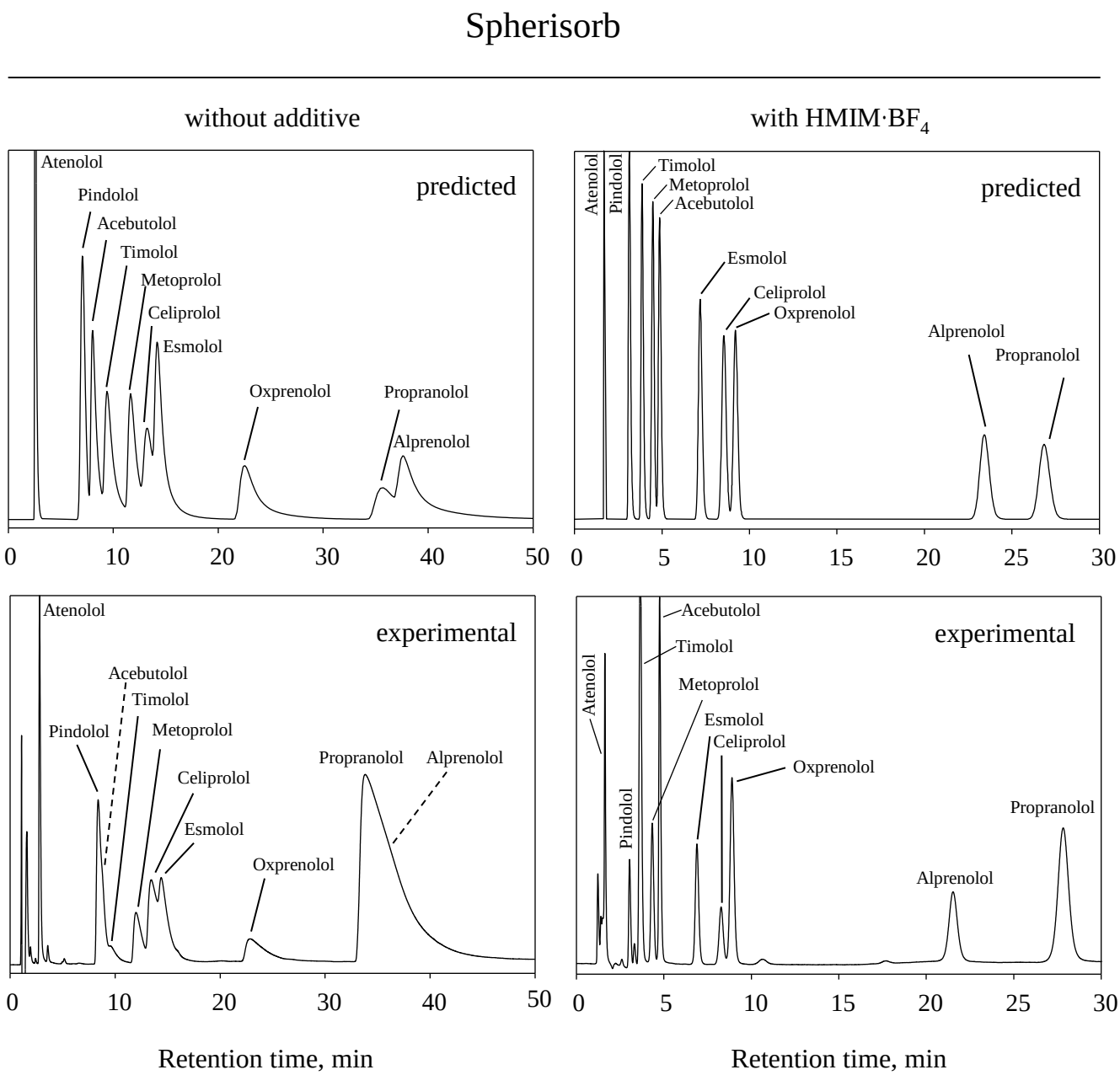

Figure 5

Figure 6

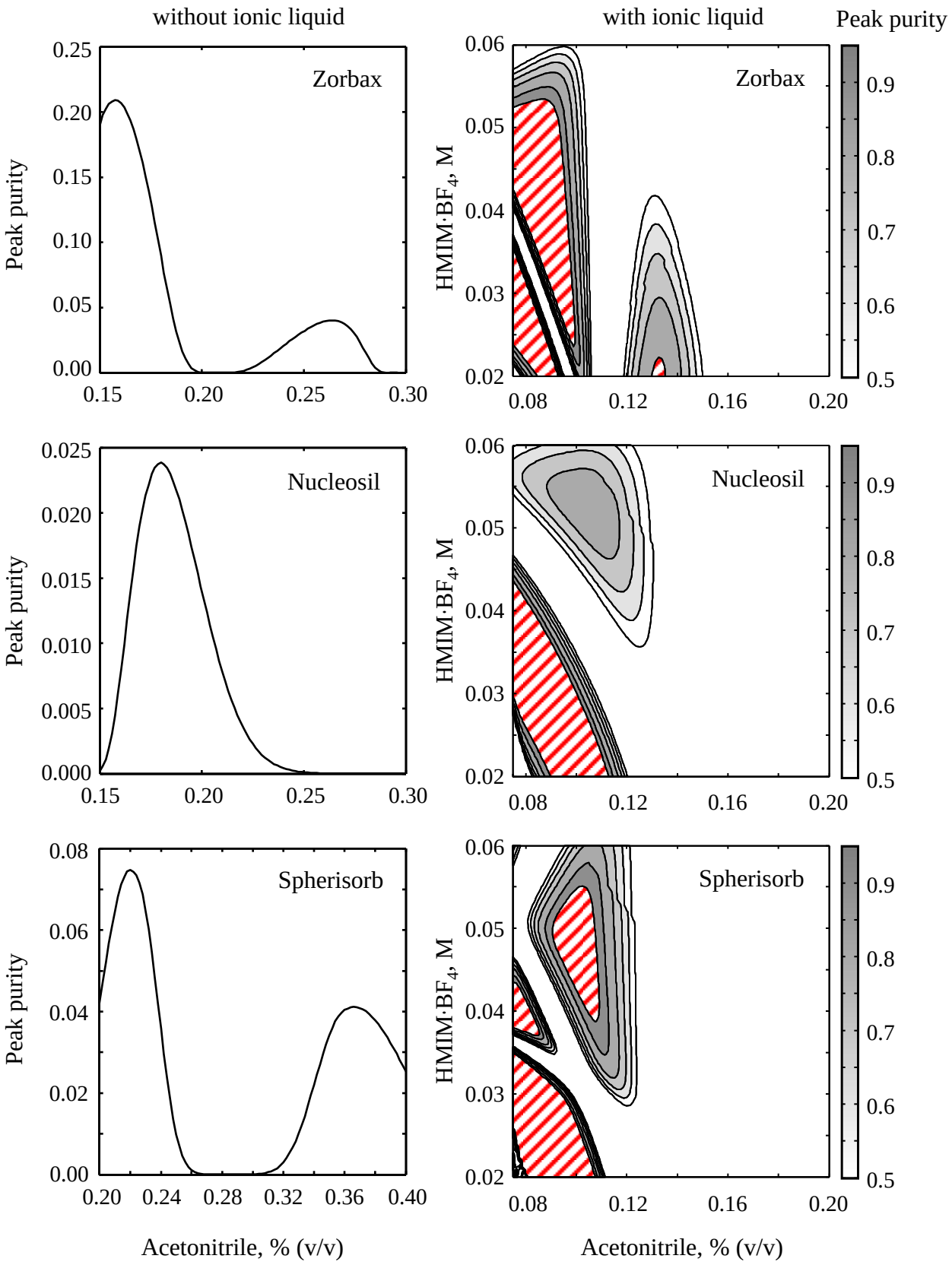

Figure 6