

Retention Mechanism of Selected Ionic Liquids On a Pentafluorophenylpropyl Polar Phase: Investigation using RP-HPLC

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Abstract

This study investigated the retention mechanism of a congeneric group of imidazolium ionic liquid cations with alkyl functional groups of different lengths, an aryl substituent, and one pyridinium cation on a pentafluorophenylpropyl silica-based stationary phase. The influence of organic modifier type and content in the mobile phase on the retention mode of alkylimidazolium ionic liquid cations shows a 'U-shaped' relationship. The use of this stationary phase gives excellent linearity and detection limits in the ppt range. The method is also readily applicable to the highly selective analysis of plant extracts previously spiked with ionic liquids.

Introduction

Ionic liquids are a new class of compounds usually consisting of an organic cation and a variety of organic or inorganic anions. Liquid below 100°C and generally with a negligible vapour pressure, these compounds make ideal non-volatile solvents for a variety of industrial chemical syntheses (1,2). Because of their widespread application in industrial processes, ionic liquids should be evaluated before being allowed to enter the environment. It has already been found that even small amounts of impurities significantly affect the performance of ionic liquids, thus analytical methods determining their purity will be very much in demand. In addition, research of considerable practical importance, such as investigations into the toxicity, eco-toxicity, and persistency of room temperature ionic liquids (RTIL) in the environment, will also require relatively simple and reproducible analytical techniques. These methods must not only be applicable to different natural matrices but also to the very low concentrations likely to be present in biological and environmental systems (3). Most of the methods reported so far for separating and determining ionic liquid cations involve high-performance liquid chromatography (HPLC) with chemically bonded phases (4–6). Separation of ionic liquids in these modes is, however, less selective, especially where highly hydrophilic solutes are concerned. Nevertheless, it can be improved by invoking interactions between solutes and non-hydrophobic separation phases. A number of other interactions are also possible between an ionic

liquid cation and a surface (dispersive, electrostatic, dipole–ion, ion–ion, or $\pi\cdots\pi$).

Identification of solute-stationary phase interactions can be useful for assessing their relative importance in affecting retention on different columns, column selectivity, and the ability of various columns to separate different compounds. Using chromatography, Kowalska et al. (7) investigated the retention mechanism of ionic liquid cations on stationary phases with different structural properties: these authors tested packings containing cholesterol ligands chemically bonded to silica (SG-CHOL), and mixed stationary phases (SG-MIX) containing cyanopropyl, aminopropyl, phenyl, and octadecyl ligands. Our recent study of the phenyl-bonded phase demonstrated the potential usefulness of $\pi\cdots\pi$ interactions between solutes and stationary phase ligands in the selective separation of imidazolium and pyridinium ionic liquid cations with extended π -electron systems (8).

Fluorinated, silica-based stationary phases are becoming increasingly popular alternatives to traditional alkyl phases owing to their differential selectivity and retention for a variety of analyte classes. In addition to dispersive interactions available on common C18 and C8 phases, dipole interactions of fluoro-substituted ligands, π - π interactions between solutes and phenyl-ligands as well as charge transfer and ion-exchange interactions also have a considerable impact on the selectivity of pentafluorophenylpropyl (PFPP) columns (9–11). Moreover, PFPP phases exhibit increased steric resistance to solute penetration into the stationary phase, which might be the result of greater bulk of the ligands and/or less ordered arrangement in comparison with phenyl or alkyl stationary phases (12). Several authors have noted that perfluorinated phases demonstrate a unique selectivity in that they retain polar solutes other than hydrophobic phases. For polar analytes, these columns exhibit both reversed- and normal-phase retention, which turns out to

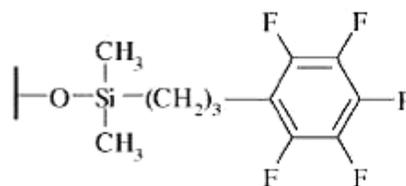


Figure 1. Structure of Discovery HS F5 stationary phase

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be dependent on the mobile phase composition. At lower concentrations of organic modifier solute, retention tends to be that of a reversed-phase system, but at higher concentrations, behavior typical of normal-phase separation is observed (13–15). Therefore, fluorinated phases can also be applied as in normal-phase chromatography. Perfluorinated stationary phases have also shown a unique selectivity in other column classification studies. Neue et al. (16) separated fluorinated phases from C18 and cyanopropyl phases claiming differences in extended polar selectivity and phenolic selectivity. Euerby and Petersson (17) investigated 135 commercially available stationary phases and observed significant selectivity differences for a set of fluorinated columns. In their further studies they noted orthogonal selectivity of the fluorinated phases in comparison with phenyl- and

alkyl-based columns, which was especially evident for the retention of basic analytes (9). Bell and Jones (14) studied the molecular interactions contributing to retention on a pentafluorophenylpropyl stationary phase at high organic modifier content. It was found that retention of protonated bases at high percentages of organic modifier is determined by strong ion-exchange interactions with ionized surface silanol groups and additional non-ionic interactions. An important role in the special selectivity of perfluorinated phases is also played by the adsorbed organic solvent molecules on the stationary phase surface. Valko et al. (18) investigated the selectivity of C18 and perfluorinated stationary phases with acetonitrile, methanol, and 2,2,2-trifluoroethanol as organic mobile phase modifiers. Clearly different selectivity was observed for fluorinated phase with all the three solvents in comparison with the alkyl-bonded phase.

The aim of the present work was to investigate the retention mechanism of a congeneric group of imidazolium ionic liquid cations and one alkyipyridinium cation on a polar reversed-phase (a pentafluorophenylpropyl silica-based stationary phase). The retention and selectivity of a PFPP column should be useful in the selective separation of imidazolium and pyridinium ionic liquid cations, especially at higher concentrations of organic modifier. Also investigated was the effect of altering the mobile phase content of two solvents (methanol and acetonitrile) on the retention profiles of the compounds analyzed.

Materials and Methods

Chemicals

The ionic liquids for this study [1-ethyl-3-methylimidazolium (EMIM), 1-ethyl-3-ethylimidazolium (EEIM), 1-propyl-3-methylimidazolium (PMIM), 1-butyl-3-methylimidazolium (BMIM), 1-hexyl-3-methylimidazolium (HMIM), 1-octyl-3-methylimidazolium (OMIM), 1-benzyl-3-methylimidazolium (BzMIM), and 1-ethylbenzyl-3-methylimidazolium (EBzMIM) chlorides] were purchased from Merck KGaA (Darmstadt, Germany), and also obtained from the Ionic Liquids Collection of the working group of Prof. Bernd Jastorff (Centre for Environmental Research and Technology, UFT, University of Bremen). In addition, *N*-butyl-4-methylpyridinium chloride (MBPyr) was also used to compare the specific chromatographic behaviour between imidazolium and pyridinium ionic liquids.

All the ionic liquids were used as obtained, without additional pre-treatment. Standard stock solutions (1mM) were prepared by dissolving a weighed amount of the compounds in distilled water. Serial dilutions were made from

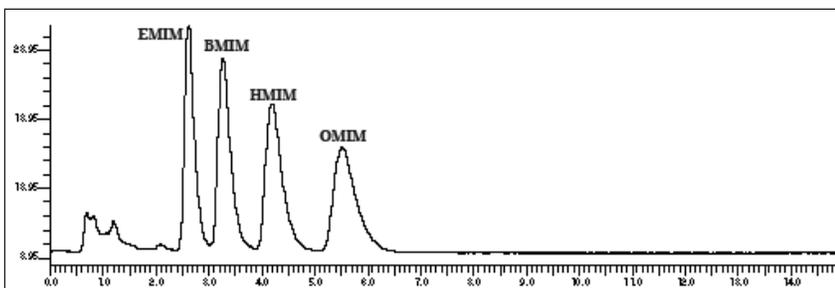


Figure 2. Exemplary chromatogram of selected alkylimidazolium ionic liquids mixture in gradient mode. 75–80% ACN, water phase: 10 mM KH_2PO_4 , pH = 3.0, flow: 0.25 mL/min. Column: Discovery HS F5 3.3 cm x 3 mm.

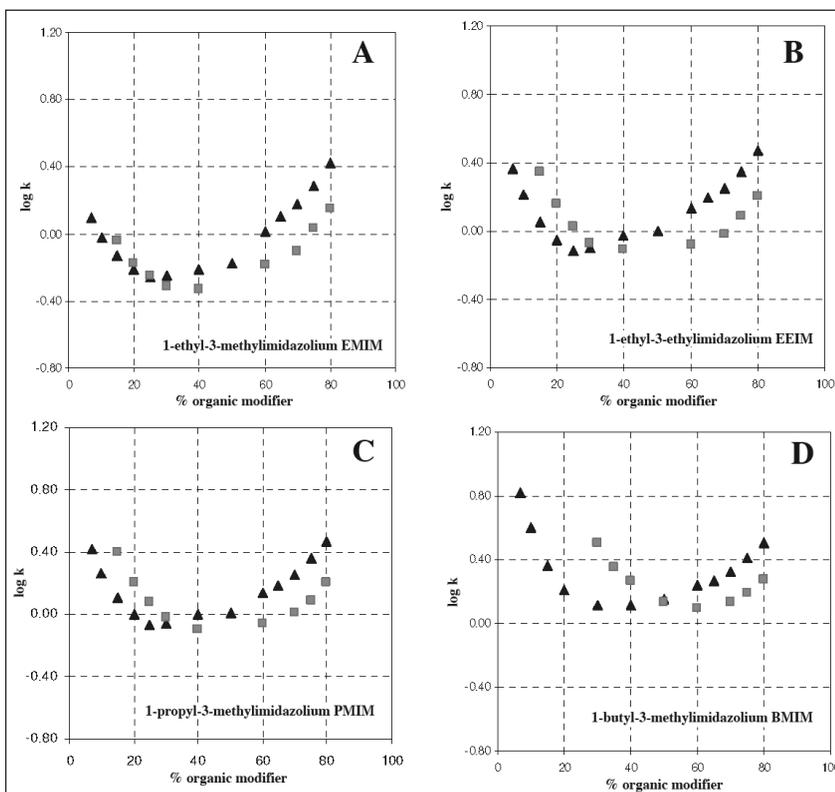


Figure 3A. The influence of the organic modifier type and content in the mobile phase on the retention profile of alkylimidazolium ionic liquid cations. Mobile phase: 7–80% of methanol (or acetonitrile)–water (10mM KH_2PO_4). pH = 3.0. Column: Discovery HS F5 150 x 4.6 mm, ■ = methanol in the mobile phase, ▲ = acetonitrile in the mobile phase. Retention factors are means from two measurements.

the stock solutions to achieve the final concentrations of 0.01–1 mM. The monopotassium phosphate and 85% *ortho*-phosphoric acid for the buffer were obtained from POCh (Gliwice, Poland). HPLC-gradient grade methanol and acetonitrile were supplied by Scharlau Chemie (Barcelona, Spain).

Apparatus and separation conditions

The analytical system was a Perkin Elmer Series 200 high performance liquid chromatograph (Waltham, MA) equipped with binary pump, UV–vis detector, vacuum degasser, and Rheodyne injection valve. The ionic liquids were separated on a Supelco-Discovery HS F5 150 × 4.6 mm column (Bellefonte, PA) (particle size = 3 μm pore size = Å 120, surface area = 300 m²g⁻¹, carbon load = 12%, calculated bonded phase coverage = 4 μmole m⁻², packing density = 0.58 g/mL) and on a Supelco-Discovery HS F5 3.3 cm × 3 mm column, packed with 5 μm particles with pore size of 120 Å. Figure 1 shows the structure of the PFPP phase.

Elution of single compounds was isocratic. Analyses were performed at ambient temperature at a flow rate of 0.5 mL/min; higher flow rate of the mobile phase caused high pressure in the studied system. Elution profiles were monitored at λ = 218 nm (this is the optimum for absorption of UV by ionic liquids) and the separation column was equilibrated with the mobile phase until baseline stabilisation. Sample injections (1 μL) were made at this point. The mobile phase was methanol or acetonitrile (7–80% v/v) mixed with 10 mM phosphate buffer (KH₂PO₄/H₃PO₄) adjusted to pH = 3. These conditions were chosen according to previously studies of our team (5,8). Retention factors (*k*) were calculated from $k = (t - t_0)/t_0$. The system dead time was measured by injecting pure methanol into the system.

The ionic liquid mixture was separated in both isocratic and gradient modes. The mobile phase consisted of acetonitrile and an aqueous solution of 10 mM phosphate buffer (KH₂PO₄/H₃PO₄) adjusted to pH = 3.

Table I. Analytical Performance Parameters for Selected Ionic Liquid Cations

	BMIM	HMIM	BzMIM	MBPy
R ² (calibration curve)	0.9996	1	0.9992	1
repeatability % (RT)	0.06–0.23	0.02–0.04	0.06–0.25	0–0.02
repeatability % (area)	0.09–3.37	0.1–3.14	0.06–1.83	0.13–2.55
LOD (ppb)	0.00278	0.00501	0.00462	0.00344
LOQ (ppb)	0.00556	0.01002	0.00924	0.00688

Results

The imidazolium ionic liquid cations, an aryl substituent, and one pyridinium cation, were selected in order to obtain the retention profiles on a pentafluorophenylpropyl stationary phase. Figure 2 shows a chromatogram of selected ionic liquids in gradient mode when the mobile phase was a mixture of acetonitrile and aqueous KH₂PO₄ (10 mM, pH = 3). The peaks of all the ionic liquid cations analyzed were somewhat distorted at lower concentrations of organic modifier, but exhibited greater symmetry at higher concentrations.

In our previous studies, where the propylphenyl modification of the stationary phase was used, it was found that acetonitrile, in contrast to methanol, suppressed any available π...π interactions, and that the choice of organic modifier in the mobile phase is critical for the design of the selective separation (8). The influence of different contents (7–80%) of acetonitrile and methanol on the retention profiles of alkyl(aryl)imidazolium and pyridinium ionic liquid cations on a PFPP phase was also investigated in the present study. Higher concentrations of organic modifier could not be used because of buffer crystallisation.

Figure 3 illustrates the influence of the type and content of organic modifier in the mobile phase on the retention mode of alkylimidazolium ionic liquid cations; Figure 4 shows the corresponding data for arylimidazolium and alkylpyridinium ionic liquid cations. In each figure, the data are presented in the form of a plot of organic modifier content versus log of retention factors, in accordance with the classical chromatographic Snyder–Soczewinski relation represented by the equation:

$$\log k = \log k_w - S\phi$$

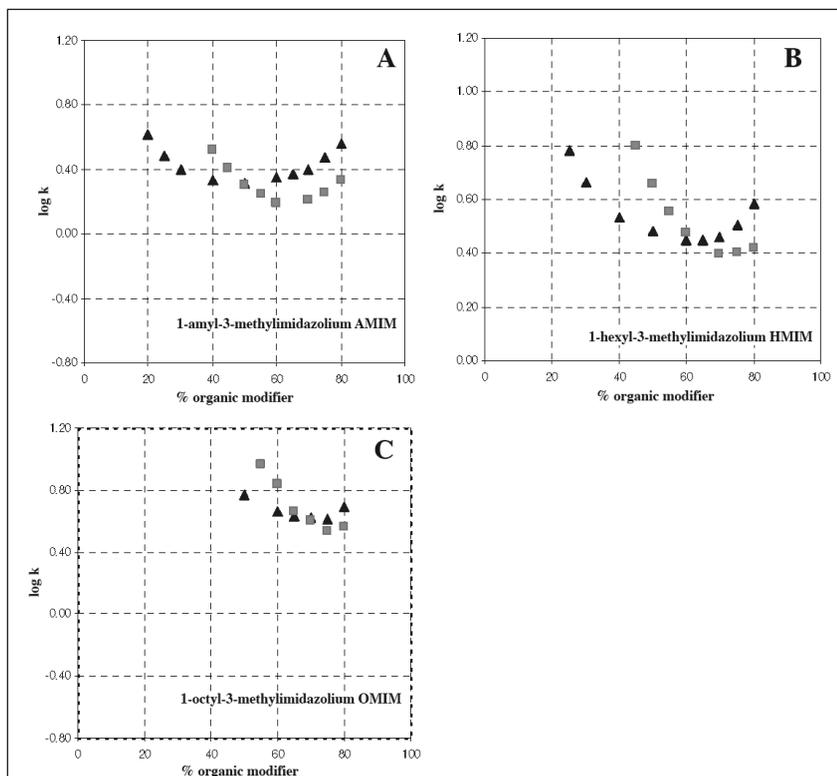


Figure 3B. The influence of the organic modifier type and content in the mobile phase on the retention profile of alkylimidazolium ionic liquid cations. Mobile phase: 7–80% of methanol (or acetonitrile)–water (10 mM KH₂PO₄), pH = 3.0. Column: Discovery HS F5 150 × 4.6 mm, ■ = methanol in the mobile phase, ▲ = acetonitrile in the mobile phase. Retention factors are means from two measurements.

where k is the retention factor; k_w is the intercept corresponding to k in pure water as the mobile phase, often described as a measure of the relative hydrophobicity of the analyzed solute; S is a constant for a given solute; and φ is the volume fraction of the organic modifier. The dependence of the organic modifier content in the mobile phase on the all ionic liquid cations retention profile was non-linear. At lower concentrations of organic modi-

fier, ionic liquid cation retention resembled that of the classical reversed-phase mode, but at higher concentrations, retention increased with increasing percentages of organic modifier, following normal-phase behaviour.

Analytical performance parameters

Selected ionic liquids: BMIM, HMIM, BzMIM, and MBPyr were

chromatographed in the concentration range 0.01–1mM. The applied chromatographic conditions, where the mobile phase consists of 80% (v/v) of acetonitrile in water (10mM KH_2PO_4) were chosen on the basis of preliminary experiments. At higher concentrations of organic modifier in the mobile phase, the peaks of all analyzed compounds were characterized by greater symmetry. Acetonitrile was chosen as an organic modifier, which, if compared to methanol, resulted in sharper peaks. Figure 5 shows calibration graphs for these compounds; excellent linearity was obtained. The correlation coefficients for the resultant calibration plots ranged from 0.9992–1.0000 (Table I). Table I also presents the repeatability of the retention times (0–0.25%) and peak areas (0.06–3.37%) for these ionic liquid cations obtained with duplicate injection samples. The limit of detection (LOD) was evaluated by the measurement of 10 independent blank samples fortified at the lowest acceptable concentration: LOD was equal to three times the blank standard deviation of the measured signals. The lowest LOD value was noted for the BMIM cation (0.002 ppb); that of the remaining entities was 0.003–0.005 ppb. The limit of quantification (LOQ) was calculated as twice the LOD value and ranged from 0.006 ppb to 0.01 ppb

Applicability of the method

Our research group is currently working on predicting the environmental fate of ionic liquids, including their measurements in model batch soils or spiked plants (19–21). One of the challenges currently being faced is the direct determination of ionic liquid cations from acidic extracts in the presence of several interfering compounds being extracted simultaneously from an environmental sample. The applied reversed phase separation so far has often failed to separate the analyte from the matrix compound. It was, therefore, decided to test the applicability of the pentafluorophenyl phase in this respect. Figure 6 compares chromatograms of acidic extracts of rye, obtained using the following procedure.

The homogenized rye sample spiked with known amount of BMIM chloride was extracted with 0.1M of phosphoric acid and was sonicated for 1.5 h. Afterwards, the extract was cen-

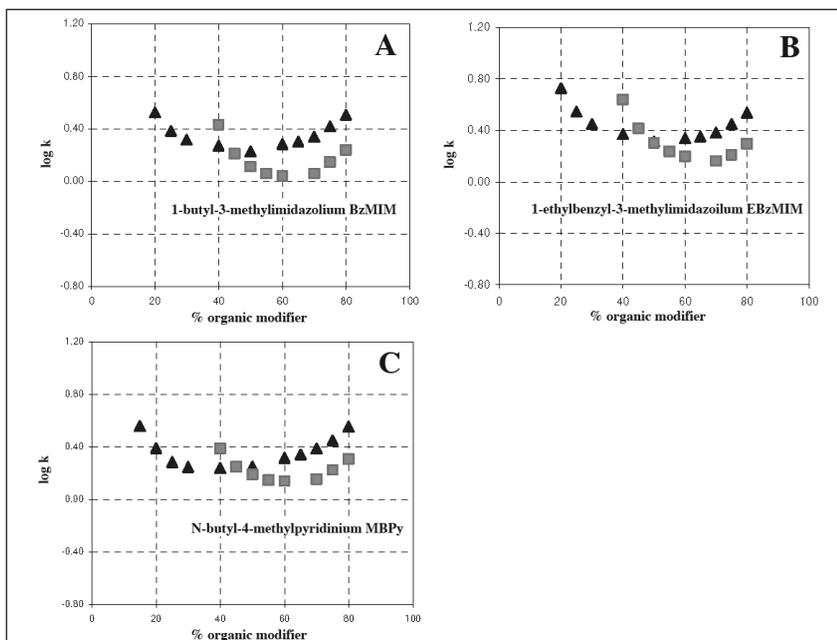


Figure 4. The influence of the organic modifier type and content in the mobile phase on the retention profile of arylimidazolium and alkylpyridinium ionic liquid cations. Mobile phase: 15–80% of methanol (or acetonitrile)–water (10mM KH_2PO_4), pH = 3.0. Column: Discovery HS F5 150 x 4.6 mm, \blacksquare = methanol in the mobile phase, \blacktriangle = acetonitrile in the mobile phase. Retention factors are means from two measurements.

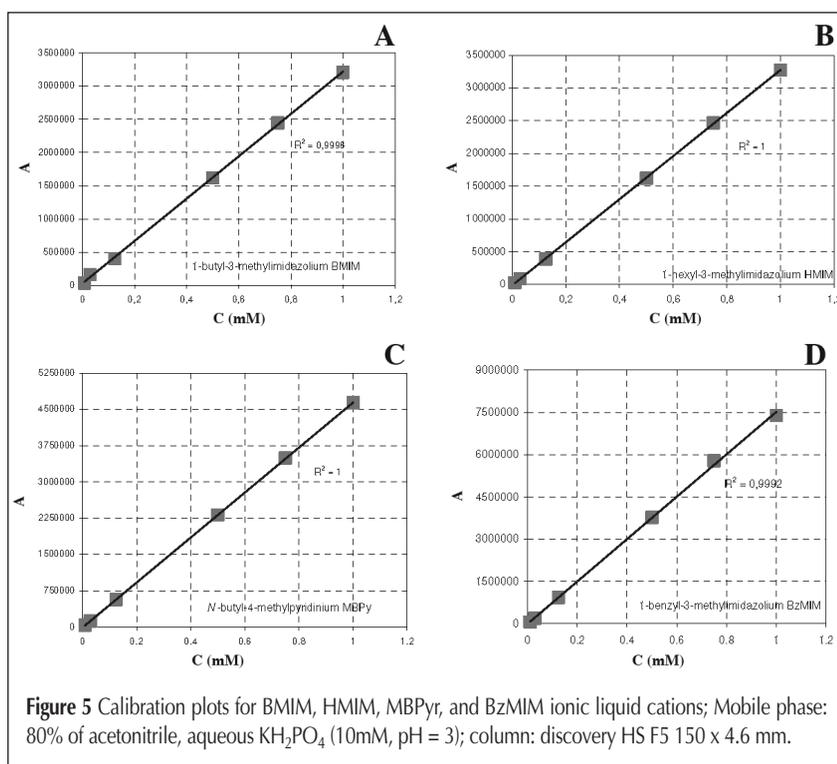


Figure 5 Calibration plots for BMIM, HMIM, MBPyr, and BzMIM ionic liquid cations; Mobile phase: 80% of acetonitrile, aqueous KH_2PO_4 (10mM, pH = 3); column: discovery HS F5 150 x 4.6 mm.

trifuged, filtered, and directly analyzed by HPLC. This analytical procedure was assisted with the extraction of a blank sample in order to evaluate the possible background interferences. The chromatogram obtained with the pentafluorophenyl stationary phase reveals a very good and highly selective separation of the ionic liquid from matrix interferences and were free from interfering peaks at the analyte retention time, suggesting the specificity of the method. The detection limits and excellent linearity of analytical performance obtained are highly suitable for this type of analysis.

Discussion

With regard to the chemical structure of the PFPP bonded phase, several interactions with varying intensity, depending on the mobile phase composition, can occur in this system. As in propylphenyl bonded phases, the presence of an aromatic ligand in the PFPP phase offers a potential $\pi\cdots\pi$ interaction with the aromatic system in the imidazolium or pyridinium ring of ionic liquid cations (7,8). Dipole interactions with fluoro-substituted ligands, dispersion interactions on the propyl phase spacer, and hydrogen bonding are also possible. A greater symmetry of the peak shapes of all ionic liquid cations at higher contents of organic modifier were observed in this study. This probably indicates that available ionized surface silanol concentrations are also higher, which ensures equal access to the surface silanol groups on the PFPP phase (14). Water can interact strongly with surface silanols; therefore, in the reversed-phase region, where the water concentration is relatively high, peak tailing results from an overload of the limited concentration of available ionized silanols on the PFPP phase.

It was found that for all ionic liquid cations, a combination of

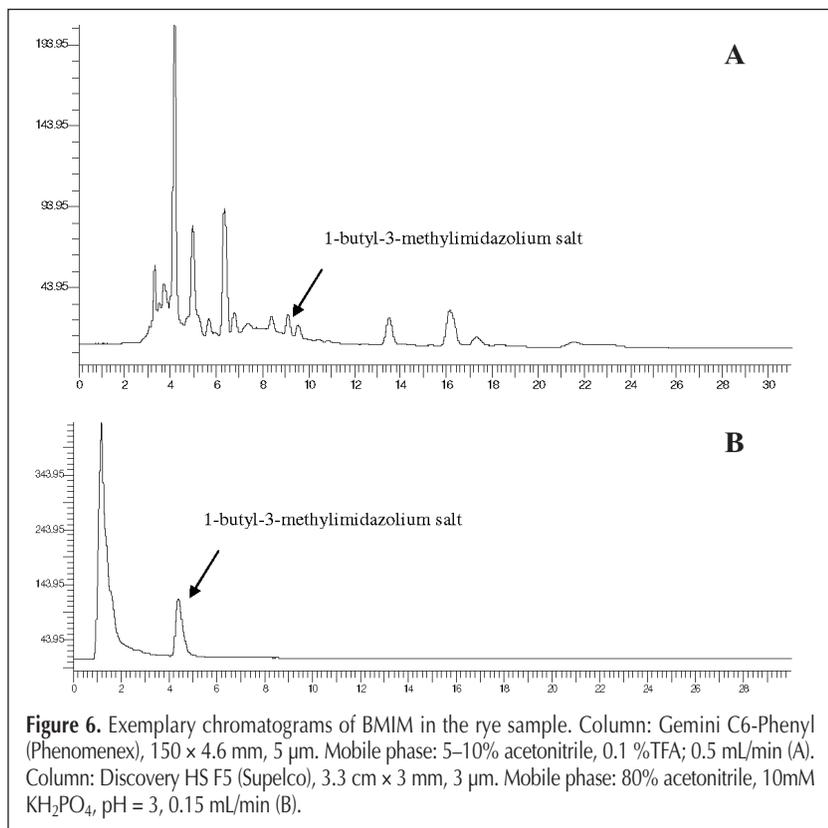
reversed- and normal-phase retention mode gave rise to a “U-shaped” relationship between the retention profile and the organic modifier content. Such a dual retention mechanism on the pentafluorophenylpropyl phase has also been noted by other authors studying the retention of basic compounds (e.g. 14,15).

The range of the reversed-phase retention mode increased with the hydrophobicity of the compounds being analyzed, showing the importance of dispersive interactions under reversed-phase conditions. Figure 7 presents the impact of the lipophilicity (e.g., expressed as the octanol–water partition coefficient $\log P$) of alkyimidazolium ionic liquid cations on the turning point (% of acetonitrile) between reversed- and normal-phase mode of retention. The $\log P$ values for alkyimidazolium ionic liquid cations were calculated according to (22). A similar relationship between the lipophilicity and the retention mode of basic and acidic compounds was observed by Marin and Barbas (15).

By fitting the linear function into the both plots of organic modifier content versus log of retention factors in the range of reversed-phase mode of elution, we calculated the slope values (S) in order to compare the influence of methanol and acetonitrile on the retention of all the ionic liquid cations. In contrast to methanol, acetonitrile suppressed any available $\pi\cdots\pi$ interactions between imidazolium and pyridinium cations and the aromatic $\pi\cdots\pi$ active moiety of the stationary phase. This is reflected by the much steeper slopes obtained for acetonitrile as the organic modifier in the case of short-chain hydrophilic entities like EMIM, EEIM, PMIM, and BMIM, as well as for MBPy cation. The range of the slope values for acetonitrile was from -0.021 to -0.046 and for methanol from -0.015 to -0.028 . For much longer alkyl chains in AMIM, HMIM, and OMIM that lead

to increased hydrophobicity as well as for aromatically substituted cations: BzMIM and EBzMIM, the predominant mode of retention will be due to hydrophobic interactions. In the case of these compounds, much steeper slopes for methanol (S values from -0.018 to -0.030) in comparison with acetonitrile (S values from -0.009 to -0.017) were noted. The $\pi\cdots\pi$ interactions here are limited because of the sterically unfavourable access of the π -system of the solute to the π -system in the pentafluorophenyl ligand of the stationary phase. According to Felix and Bertrand (11), the reduced π -basicity of the phenyl ligand by fluoro-substitution in the PFPP phase is also responsible for attenuation of the contribution of $\pi\cdots\pi$ interactions to PFPP column selectivity. As has been found in our earlier studies, in the case of aromatically substituted cations like BzMIM or EBzMIM (8) only one of the aromatic moieties takes part in $\pi\cdots\pi$ interactions, while the second is involved to a much weaker extent. This results in the similar retentions of those compounds in comparison with ionic liquid cations characterized by similar lipophilicities (AMIM and HMIM).

In a mobile phase rich in organic modifier, the extent of the normal-phase retention mode with



short-chain hydrophilic ionic liquid cations like EMIM, EEIM, PMIM, BMIM was the widest. According to Wang (23), it can be results of the unique C-F bonds within the pentafluorophenyl-propyl phase that provide a strong dipole, which enhance the phase's interactions with polar compounds. To compare the effect of high concentration of organic modifier in the mobile phase (methanol vs. acetonitrile) on the retention mechanism of imidazolium and pyridinium ionic liquid cations, we calculated $\Delta \log k$ values (difference in $\log k$ values obtained for both solvents at 80% concentration). Acetonitrile as the less polar organic modifier furnishes higher polar selectivity than methanol. Thus, the contribution of polar interactions to retention profiles of analyzed compounds can be expressed by $\Delta \log k$ values. As expected, the higher hydrophobicity of ionic liquid cations, the lower contribution of polar interactions to the retention mechanism was observed. It was reflected by the high $\Delta \log k$ values noted for EMIM, EEIM, PMIM, BMIM, and AMIM ($\Delta \log k$ between 0.27 and 0.22) and relatively low for long alkyl chain entities like HMIM ($\Delta \log k = 0.16$) and OMIM ($\Delta \log k = 0.13$). The $\Delta \log k$ values noted for pyridinium cation (0.24) and arylimidazolium cations: BzMIM (0.27) and EBzMIM (0.25) were com-

parable with short-chain hydrophilic ionic liquid cations.

It is also clear that increasing the organic content in the mobile phase causes decrease of the counter-ion concentration in the system. Lower counter-ion results in less competition for ionic sites on the surface of the stationary phase and, thus, enhances the ion exchange mechanism (24).

Also worthy of note is the reversal of elution order in the separation of BzMIM and EBzMIM and MBPyr salts in acetonitrile concentrations > 40%, which rendered those compounds inseparable (Figure 8). In particular, the retention factor of *N*-butyl-4-methylpyridinium cation increased with higher contents of acetonitrile: the free electron pair on the nitrogen atom in the pyridinium ring appears to be responsible for this effect. This observation is in agreement with Neue (25), who suggest that the retention of the PFPP phase is enhanced for electron-rich structures.

Conclusion

A wide range of imidazolium ionic liquid cations with alkyl functional groups of different lengths, an aryl substituent, and one pyridinium cation were chosen in order to obtain the retention profiles on a PFPP stationary phase. The symmetry of the peak shapes of all the ionic liquid cations was quite poor, with lower contents of organic modifier, but improved when its percentage was increased. The influence of the type and content of organic modifier in the mobile phase on the retention mode of alkyl(aryl)imidazolium and pyridinium ionic liquid cations shows a non-linear, 'U-shaped' relationship. This is the result of the combination of reversed- and normal-phase retention modes as well as the suppression of available $\pi \dots \pi$ interactions at higher concentrations of acetonitrile. Retention of short-chain hydrophilic ionic liquid cations took place largely in the normal-phase mode; in contrast, that of the more hydrophobic cations occurred mostly in the reversed-phase mode. The use of this stationary phase gives excellent linearity and detection limits in the ppt range. The method is also readily applicable to the highly selective analysis of plant extracts previously spiked with ionic liquids.

Acknowledgment

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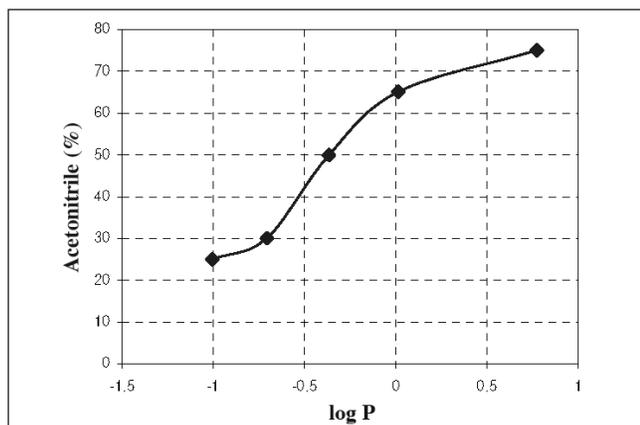


Figure 7. The influence of the lipophilicity ($\log P$) of arylimidazolium ionic liquid cations on the turning point (% of acetonitrile) between reversed- and normal-phase mode of retention.

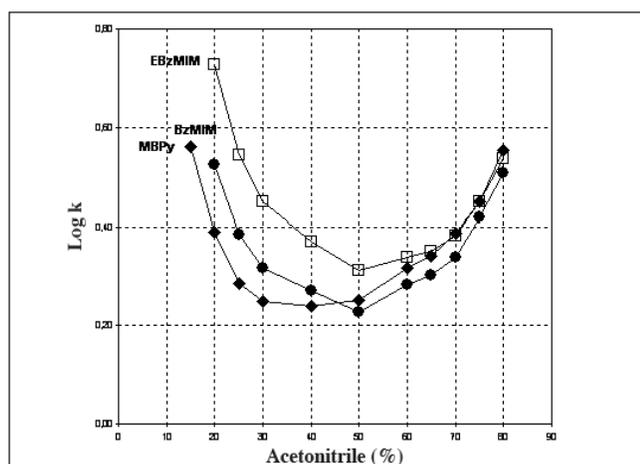


Figure 8. Reversal of elution order in the separation of EBzMIM and BzMIM and MBPyr salts. Mobile phase: 15–80% of acetonitrile–water (10mM KH_2PO_4). pH = 3.0. Column: Discovery HS F5 150 x 4.6 mm.

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