VILNIUS UNIVERSITY

ASTA ORENTIENĖ

INVESTIGATION OF CHROMATOGRAPHIC BEHAVIOUR OF IMIDAZOLIUM IONIC LIQUID CATIONS BY ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY TECHNIQUES

Summary of doctoral dissertation

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VILNIAUS UNIVERSITETAS

ASTA ORENTIENĖ

IMIDAZOLIO JONINIŲ SKYSČIŲ KATIJONŲ ATSKYRIMO ULTRAEFEKTYVIOSIOS SKYSČIŲ CHROMATOGRAFIJOS METODAIS YPATUMŲ TYRIMAS

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1. INTRODUCTION

Ionic liquids (ILs) are a class of salts composed of organic nitrogen-containing cation (e.g., imidazolium, pyridinium, pyrrolidinium) and a halogen-based organic or inorganic anion (e.g., Cl⁻, I⁻, $[PF_6]^-$, methylsulfate, bis(trifluoromethylsulfonyl)imide) which have melting points below 100 °C. The combination of these ions has considerably expanded the number of possible salts with easily tunable physical and chemical properties available by synthetic means. Among these, alkylmethylimidazolium salts have recently shown potential as unique solvents with a wide range of solubility, miscibility, and other interesting properties such as negligible vapour pressure, high thermal stability, good conductivity and wide electrochemical window. These properties make ILs an attractive alternative to volatile organic solvents as environmentally benign media in many industrially important chemical processes. Hence, they have been recently proposed as solvents in various chemical reactions in synthesis, catalysis, bioprocesses and as electrolytes for batteries and fuel cells. In analytical chemistry, ILs are used as alternative solvents in liquid-liquid extraction as well as in spectral and electrochemical analysis, as stationary phases in gas chromatography, as mobile phase additives in liquid chromatography and as carrier electrolytes in capillary electrophoresis. The intensely increasing applicability of ILs can ultimately lead to their industrial production and utilization. Consequently, simple and fast analytical techniques for the determination of ILs in various matrices will be of great significance. Analytical methods are also necessary to assess the purity of ILs as well as to determine their physical and chemical properties. The most popular technique in this field is high-performance liquid chromatography (HPLC) with its various separation modes (cation-exchange or ion pair (IP), reversed-phase (RP) and hydrophilic interaction chromatography (HILIC). However, till now there are no studies devoted to exhaustive evaluation and comparison of the separation capabilities of ILs in different HPLC techniques. In addition, the ability of HPLC to increase efficiency, separation selectivity and speed of the separations can be further enhanced by ultra performance liquid chromatography (UPLC), which utilizes columns with 1.7 μm particles and separations at ultrahigh pressures (up to 1034 bar). Finally, the properties of imidazolium cations

(stability, different functional groups, conjugated π-system, charge) make them very attractive as model analytes for the investigation of a new separation modes.

The aim of this work was to evaluate and compare the separation of imidazolium IL cations by using different UPLC techniques and to investigate the chromatographic behaviour of imidazolium ILs in HILIC and per-aqueous liquid chromatography (PALC).

The following main tasks were set to achieve the aim:

1. Optimisation and comparison of the RP-UPLC, IP-UPLC and HILIC techniques for the separation of imidazolium IL cations.

2. Investigation of the effect of acetonitrile (ACN) content in the mobile phase on the retention behaviour of imidazolium cations using ethylene bridged hybrid (BEH) silica stationary phase.

3. Evaluation of the importance of different experimental variables (buffer concentration and pH, acid additive, organic solvent, column temperature and mass overload) on the retention, efficiency and separation selectivity of imidazolium ILs under PALC and HILIC conditions.

4. Evaluation of the retention mechanism of imidazolium ILs in HILIC and PALC separation modes.

Statements for defense:

1. HILIC is the most powerful chromatographic technique for ILs separations in terms of analysis time, separation efficiency and resolution.

2. Operation with both acetonitrile-rich and water-rich mobile phases on BEH silica stationary phase provides the possibility of selecting between the two retention modes with opposite separation selectivity, simply changing the composition of the mobile phase.

3. Imidazolium ILs demonstrate mixed mode retention mechanism on the BEH silica column using different content of ACN in the mobile phase. In PALC mode the mechanism is likely to be a mixture of RP and ion-exchange interactions, whereas in HILIC separation mode partitioning and ion-exchange interactions are responsible for the retention of cationic solutes.

4. HILIC displays considerably higher resistance to mass overload in comparison to PALC.

2. EXPERIMENTAL

Separations were performed on a Waters Acquity UPLC system (Waters, Milford MA) equipped with an Acquity UPLC photodiode array detector (PDA) detector and column oven enabling temperature control of the column. Following columns maintained at 30°C were used in the experiments: Acquity UPLC BEH C18, Acquity UPLC BEH C8, Acquity UPLC BEH Phenyl, Acquity UPLC BEH Amide and Acquity UPLC BEH HILIC (100 mm×2.1 mm I.D., 1.7 μm, Waters). The PDA detector was set at 210 nm, with a sampling rate of 20 points sec^{-1} and time constant of 0.1 sec. Data collection and management was performed by Empower 2 build 2154 software (Waters).

The following ionic liquids were used: 1-butyl-2,3-dimethylimidazolium iodide (BDMIM), 1-butyl-3-methylimidazolium tetrafluoroborate (BMIM), 1,2,3-trimethylimidazolium iodide (TMIM), 1,3-dimethylimidazolium methylsulfate (DMIM), 1-ethyl-2,3-dimethyimidazolium chloride (EDMIM), 1-cyanomethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide (CMMIM), 2,3-dimethyl-1-propylimidazolium bis(trifluoromethylsulfonyl)imide (DMPIM), 3-methyl-1-propylimidazolium iodide (MPIM), 1-ethyl-3-methylimidazolium tetracyanoborate (EMIM), 1-hexyl-3-methylimidazolium chloride (HMIM), 1-(2-hydroxyethyl)-3-methylimidazolium bis(trifluoromethylsulfonyl)imide (HEMIM), 3-methyl-1-octylimidazolium chloride (MOIM).

3. RESULTS AND DISCUSSION

3.1. The separation of imidazolium ionic liquid cations by ultra performance liquid chromatography

Imidazolium ILs are molten salts, resulting from the combination of organic cation (alkilmetilimidazolium) and various anions. Since the cation is the main structure component responsible for chromatographic behaviour of ILs, we will focus on the separation of IL cations. Chromatographic separation of ILs are complicated process and depends on the properties of ILs, on the composition of mobile phase and on the nature of the stationary phase. The retention mechanism of imidazolium cations may involve different type of interaction: hydrophobic, inductive, ionic, π-π, hydrogen bonding (Fig. 1). Due to different interaction ability there is possibility to separate ILs using different types of LC methods. For better understanding of the chromatographic

behaviour of imidazolium ILs, three different methods (RP-UPLC, IP-UPLC and HILIC-UPLC) were investigated and compared for the separation of six imidazolium ILs.
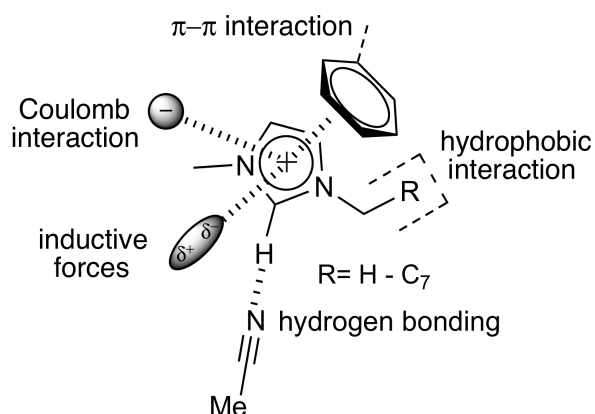

Fig. 1. 1-alkyl-3-methylimidazolium structure and its possible interactions.

RP-UPLC Separation. The use of RP-HPLC for the separation of IL cations is hampered by two important drawbacks: (1) weakly retained very polar cations with short alkyl chains on conventional RP columns; and (2) significant peak tailing due to the electrostatic interactions between cationic solutes and ionized residual silanol groups. Thus, in this study in the optimization of RP-UPLC separations special attention was paid to retentivity and peak efficiencies of IL cations.

In the first attempt, the retention properties of three commercial phases (C18, C8 and phenyl) were evaluated for a mixture of six imidazolium cations in the isocratic elution mode with a 10:90 (*v/v*) acetonitrile/water mobile phase at a flow rate of 0.4 mL min^{-1}. Comparable trends in elution orders and peak shapes (broad tailing peaks) were obtained on all three phases but the retention factors *k* differed considerably. As expected, the phenyl phase was more retentive for IL cations than the others. It delivered about 1.3-fold higher *k* values compared to C18 and almost 2-fold higher values compared to C8. The retentivity of IL cations using phenyl-bonded stationary phases was enhanced due to π-π aromatic interaction between imidazolium ring and phenyl ligand. In addition, the phenyl phase gave about 15% higher efficiency than the others, which is a larger variation than normal for columns of the same particle size. Thus, when evaluating both retentivities and peak efficiencies, phenyl phase was found to be the overall best performing system for the selected IL cation set. Although an increase in retention and higher efficiency obtained on the phenyl phase has lead to a slight improvement in

chromatographic resolution. However, even on this phase, the peaks showed significant tailing and selectivity was not sufficient to distinguish between DMIM and EMIM cations, which have short alkyl chains. In addition, for all IL cations the retention times were quite irreproducible. Peak tailing in this case was not a consequence of overloading of the column, because even after the reduction of injected amount the peak shapes were not improved. It is well known that residual ionized silanol groups on silica's surface can interact with positively charged groups. This interaction often results in tailed peaks, poor efficiency, and irreproducible retention. To improve the peak efficiency of organic cations four acids as mobile phase additives (hydrochloric acid (HCl), methanesufonic acid (MSA), trifluoroacetic acid (TFA) and perchloric acid ($HClO_4$)) were used. The effect of the mobile phase additives on RP-UPLC retention behaviour of the four most hydrophilic imidazolium cations is shown in Fig. 2.

The first peak eluted near the void volume corresponds to iodide anion, a counterion of the PMIM cation. It was surprising to see that the retention times of all four IL cations decreased upon addition of acid. Clearly, all four additives are relatively strong acids and the pH of mobile phase independently of the acid type could be generally referred to as pH 3 or lower. Possibly, decreased retention in acidic mobile phases may be due to suppression of residual silanol ionization and subsequent elimination of the charge-charge interaction sites from the stationary phase.

On the other hand, the anion resulting from the dissociation of acid can act as an ion pairing agent in the mobile phase and thus can enhance the retention of cations. As seen in Fig. 2, the retention of IL cations varied slightly with the type of acid and could be explained by ion pairing ability. The retention times of IL cations decrease in the order $HClO_4$ > TFA > MSA > HCl. Clearly, the ion pairing process has little effect on the retention of imidazolium cations because small anions are not sufficiently strong ion pairing agents to form ion pairs with relatively hydrophilic imidazolium cations. Similar retention behaviour was also observed for HMIM and MOIM cations.

The best peak shapes with acceptable resolution were obtained with $HClO_4$, the strongest ion pairing agent. Although compared to initial separations with pure ACN/water mobile phase the retentivity of the highly hydrophilic imidazolium cations was not enhanced, a general overall peak shape improvement was sufficient to achieve an adequate retention range which would allow a flexible separation optimization.
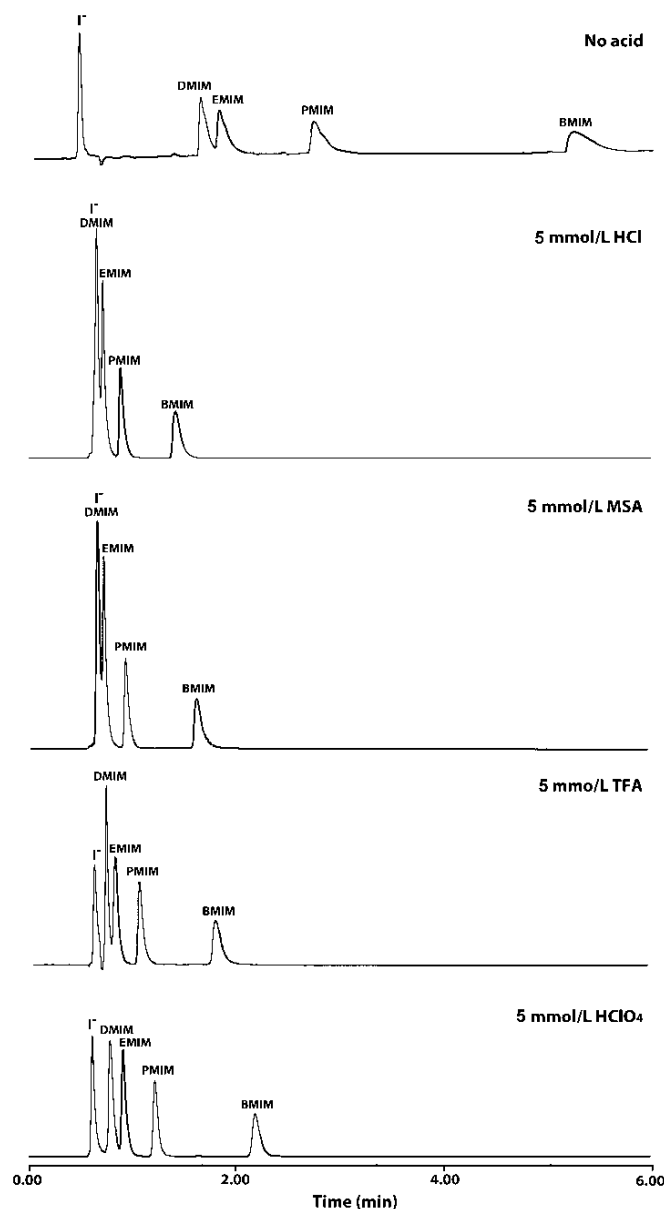
Fig. 2. Effect of acid on RP-UPLC elution profile of four imidazolium cations. The column was Acquity UPLC BEH Phenyl. The isocratic mobile phase was 10:90 (v/v) ACN/H_2O containing appropriate acid. The flow rate was 0.4 mL min^{-1}.

Next, the influence of the $HClO_4$ concentration on retention and peak shape for imidazolium cations was investigated. However, a variation in the acid concentration in the range of 1-10 mmol L^{-1} did not generate retention shifts to a relevant extent.

Due to the large difference in the retention factors (the retention factors between the most retained MOIM and the least retained DMIM differed almost 50-times) fast RP-UPLC separation of all six imidazolium cations using isocratic elution is impossible. The separation achieved under optimized gradient conditions is demonstrated in Fig. 3. As seen, near perfectly symmetrical peaks and high efficiencies for all IL cations studied were obtained. The small peaks obtained in the chromatogram belong to the impurities present in the MOIM salt. Compared to isocratic elution, the gradient elution clearly

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provides several advantages: (i) considerably faster separation; (ii) better peak shapes; (iii) higher detection sensitivity. The total cycle time for a gradient RP-UPLC method was reduced to six minutes (1 min for returning to the initial composition and 2 min for reequilibration).
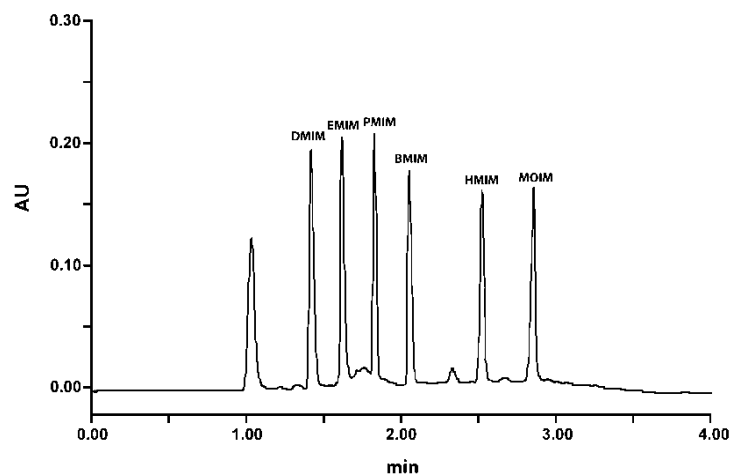

Fig. 3. Optimized RP-UPLC separation of six imidazolium cations. The column was Acquity UPLC BEH Phenyl. Mobile phase A was water containing 2.5 mmol L^{-1} $HClO_4$. Mobile phase B was 90:10 (*v/v*) ACN/H_2O containing 2.5 mmol L^{-1} $HClO_4$. The linear gradient was from 5 to 75% B in 3 min. The flow rate was 0.25 mL min^{-1}.

IP-UPLC Separation. An alternative approach to increase the retention of charged compounds and achieve adequate separation is to use ion pair chromatography. In this study, sodium 1-octanesulfonate (NaOS) was chosen as ion pairing additive to the ACN-water solutions. All separations were performed on an Acquity UPLC BEH C18 column at a flow rate of 0.25 mL min^{-1}. This IP-UPLC system involves the formation of neutral ion pairs which improves hydrophobic interactions with the stationary phase and/or leads to a dynamic ion-exchange between surface adsorbed OS^- anions and eluted solute cations.

To find the appropriate ion pairing reagent concentration, varying concentrations (0.1-5.0 mmol L^{-1}) of NaOS in a buffered (5 mmol L^{-1} ammonium formate, pH 3.2) ACN/water (20:80 *v/v*) mobile phase were tested as eluents under isocratic conditions. All imidazolium cations showed typical IP chromatographic behaviour: with increasing NaOS concentration in the mobile phase from 0.1 to about 1.0-1.2 mmol L^{-1} the retention of IL cations gradually increased. As the concentration of the ion pairing reagent was increased further, there was a clear leveling off in retention. Less hydrophilic compounds

responded to a change in the NaOS content to a higher degree. As expected, retention of IL cations increased with the alkyl chain length following their increasing hydrophobicity.

Additionally, the pH of the mobile phase was investigated in the range of pH 3.0-7.0 using appropriate phosphoric acid/phosphate and acetic acid/acetate buffer solutions. It was found that this parameter did not generate retention and/or selectivity shifts to a relevant extent: for all solutes the retention time and peak efficiency fluctuated slightly (less than 10% variation) with pH most likely due to the lack of change in the ionization state of the solutes in the examined pH range. Based on these results buffer free mobile phase was selected for further experiments.

Like in the case of RP-UPLC, due to the large difference in the retention factors fast IP-UPLC separation of all six imidazolium cations using isocratic elution is impossible. A typical separation using the optimized gradient elution is demonstrated in Fig. 4.
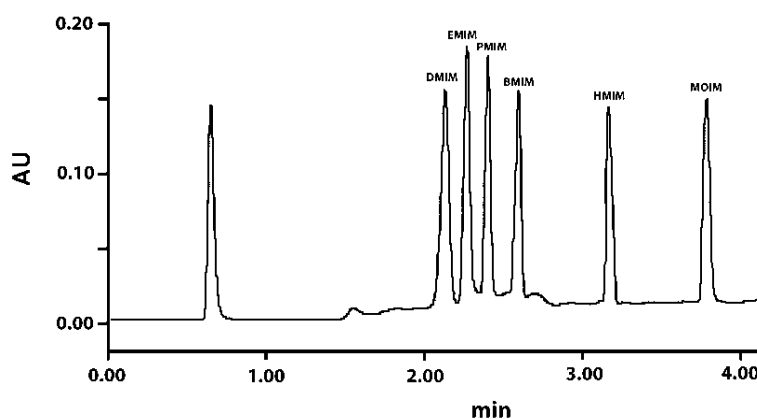

Fig. 4. Optimized IP-UPLC separation of six imidazolium cations. The column was Acquity UPLC BEH C18. Mobile phase A was water containing 1 mmol L^{-1} NaOS. Mobile phase B was 90:10 (*v/v*) ACN/H$_2$O containing 1 mmol aL^{-1} NOS. The linear gradient was from 15 to 60% B in 4 min. The flow rate was 0.25 mL min^{-1}.

As seen, imidazolium cations were all baseline separated in about 4 min. At least fivefold increase in sensitivity for late eluting HMIM and MOIM peaks was achieved using the gradient elution in comparison with isocratic elution. Due to the slow column equilibrium time-consuming column reequilibration is required in gradient IP-HPLC especially when a hydrophobic ion pairing reagent is used. Variation in retention time of the solutes between runs might occur when sample solution is injected before the column

is equilibrated. Therefore, we compared the repeatability ($n = 6$) of retention time under identical gradient conditions by applying reequilibration times of 3, 5, 10 and 15 min, respectively. Even with 5 min reequilibration of the column excellent retention time repeatability ($\pm$ 0.005 min) between runs was obtained for all imidazolium cations. Thus, for the present IP-UPLC to system, a minimum time of 5 min was required to reequilibrate the column and the total run time was 10 min.

HILIC-UPLC Separation. Similarly to RP separation, HILIC separation commonly employs water and ACN as the mobile phase, but requires much higher organic content (>60 %) to ensure significant hydrophilic interaction. The level of ACN in the mobile phase is the factor that has the largest influence on retention. In this investigation, the retention behaviour of imidazolium cations on an Acquity UPLC BEH HILIC column was studied in the range of 50-90 % ACN at a flow rate of 0.5 mL min^{-1} (Fig. 5). As can be seen, all imidazolium cations exhibited typical HILIC behaviour of decreasing retention with increasing water content in the mobile phase and leveled off when the ACN content further decreased to 50 %. As expected, the IL cations were eluted in opposite order to that obtained during RP-UPLC and IP-UPLC separations. However, the separation using pure water/ACN mobile phases was far from complete: all imidazolium cations gave broad and tailing peaks even with gradient elution.
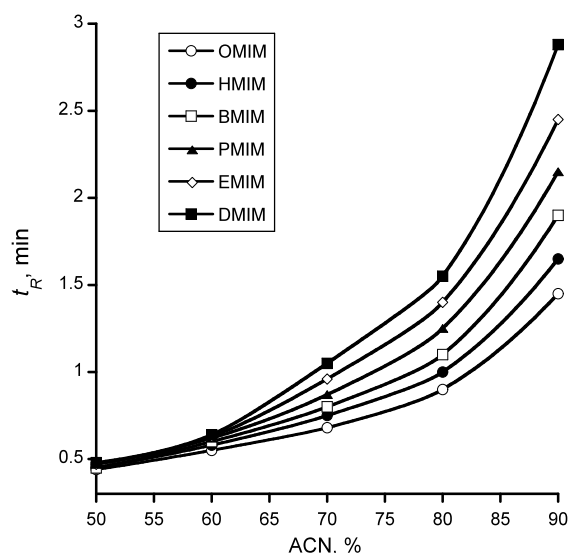

Fig. 5. Effect of ACN content on the retention of IL cations on Acquity UPLC BEH HILIC column maintained at 30°C. The flow rate was 0.5 mL min^{-1}.

Next, by using 85:15 ACN:H_2O (*v/v*) mobile phases containing different concentrations of ammonium formate buffer (pH 3.2) we investigated the effect of buffer concentration on the retention behaviour of IL cations. As seen in Fig. 6, the retention of the solutes decreased dramatically with increasing buffer concentration, and became almost constant for ammonium formate concentrations greater than 5 mmol L^{-1}. In addition to retention time effects, the increase in the buffer concentration resulted in more symmetrical and sharper peaks, due to suppression of silanol ionization in acidic mobile phase.
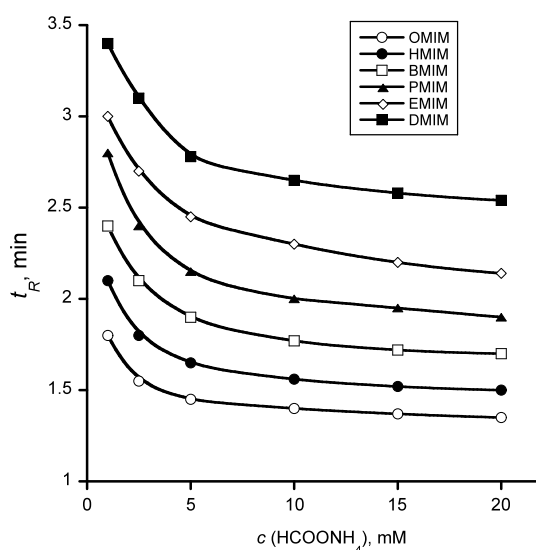

Fig. 6. Effect of ammonium formate concentration on the retention of IL cations. The column was Acquity UPLC BEH HILIC. The isocratic mobile phase was 85:15 (*v/v*) ACN/H_2O containing ammomium formate (pH 3.2). The flow rate was 0.5 mL min^{-1}.

On the basis of all of the above findings, the best results were obtained using elution at 0.5 mL min^{-1} with acetonitrile-water (85:15, *v/v*) mobile phase containing 5 mmol L^{-1} ammonium formate (pH 3.2). The chromatogram obtained under optimum conditions for a standard IL cations solution is shown in Fig. 7. An excellent separation was achieved within 2 min, hence demonstrating significant improvements, especially regarding the reduced separation time (in about 25-times) compared to the conventional HILIC technique. Another significant advantage is that all IL cations requiring gradient elution by RP-UPLC and IP-UPLC are eluted in an isocratic HILIC system, thereby simplifying the whole analysis.
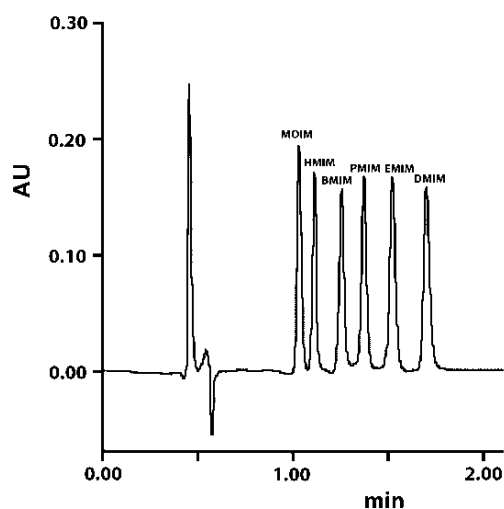
Fig. 7. Optimized HILIC-UPLC separation of six imidazolium cations. The column was Acquity UPLC BEH HILIC. The mobile phase was 85:15 (*v/v*) ACN/H_2O containing 5 mmol L^{-1} ammomium formate (pH 3.2). The flow rate was 0.5 mL min^{-1}.

3.2 Investigation of retention behaviour of imidazolium ionic liquid cations by hydrophilic interaction chromatography

Hydrophilic interaction chromatography (HILIC) is a technique, where analyte retention is believed to be caused by partitioning of the analyte between a water enriched layer that is partially immobilized on the surface of the stationary phase and a relatively hydrophobic bulk eluent, with the main components usually being 5-40 % water in ACN (Fig. 8).
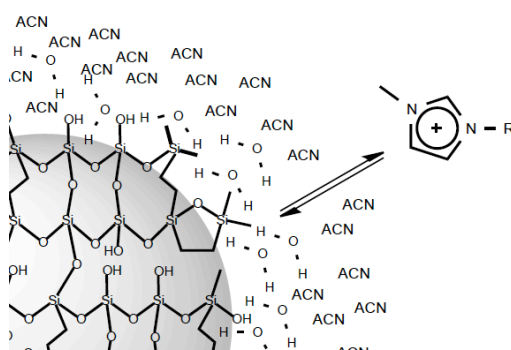

Fig. 8. The partitioning retention mechanism of alkylmethylimidazolium cations on hybrid silica in HILIC.

However, the water-layer is only dynamically immobilized on the surface and therefore cannot completely screen surface functional groups from possible interaction with solutes. The multimodal retention mechanisms have been proposed in the literature and include ion-exchange, hydrogen-bonding, hydrophobic and hydrophilic interactions.

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The predominant mechanism in a HILIC separation is not fixed and can vary from hydrophobic (water-rich mobile phase) to hydrophilic (ACN-rich mobile phase) interactions within the same column. When the water content is higher than 50 %, the retention of polar compounds increases with increasing their hydrophobicity, which is similar to RP-HPLC. Such HILIC system, where water is the main component of mobile phase was named per-aqueous liquid chromatography (PALC). Considering very little amount of data available about the retention mechanism operating in PALC, it was decided to conduct a detailed evaluation of the retention behavior of imidazolium ILs in both HILIC and PALC separation modes.

Acetonitrile content. As mentioned before, the level of ACN in the mobile phase is probably the factor that has the largest influence on retention. Thus, in the first attempt, the retention behaviour of imidazolium cations on an Acquity UPLC BEH HILIC column was studied in the range of 5-90 % ACN. The ammonium formate concentration was maintained constant at 5 mmol L^{-1} in each case with the pH in the aqueous component held at pH 3.2. Solutes were prepared in the mobile phase to avoid peak broadening caused by the use of solvents stronger than the mobile phase. Fig. 9 shows the plots of the retention factors k of four IL cations as a function of acetonitrile concentration in the mobile phase. As can be seen, all four cations (as well as all other solutes) show so-called "U-shape" retention profile with a minimum of retention for the ~40-50 % ACN.
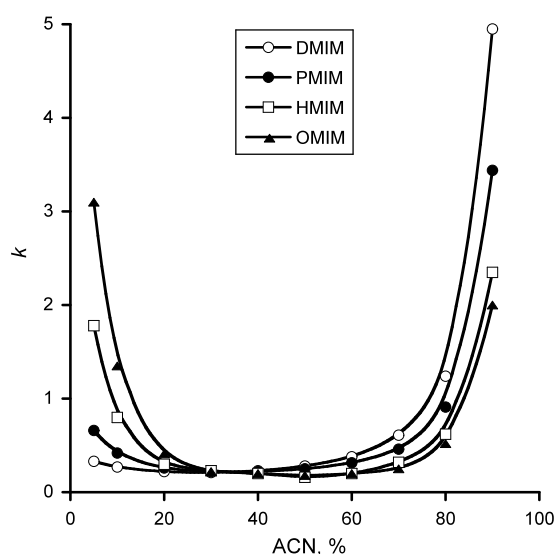

Fig. 9. Effect of ACN concentration on the retention of four imidazolium cations on Acquity UPLC BEH HILIC column. Conditions: flow rate, 0.5 mL min^{-1}; mobile phase, 5 mmol L^{-1} ammonium formate buffer (pH 3.2) in 5-90 % ACN.

In acetonitrile-rich (≥60 % ACN) mobile phases retention of solutes increases with increasing ACN content. This is an indication of HILIC retention behaviour. In contrast, in the water-rich mobile phases (≤30 % ACN) the retention increases with water content in the mobile phase and the solutes are eluted in opposite order, i.e. with increasing their hydrophobicity. The initial separations with high ACN content were much better than with water-rich mobile phases

For further investigation of the retention mechanism of imidazolium IL cations under HILIC and PALC conditions, some models of partitioning and surface adsorption were applied. The plot of log k *vs.* log φ (φ - volume fraction of stronger solvent in the mobile phase) should yield a straight line for an adsorption mechanism, whereas a linear plot of log k *vs.* φ indicates a partitioning mechanism. Neither log k - φ plots nor log k *vs.* log φ plots were satisfactory for the all studied imidazolium ILs under PALC and HILIC separation mode (Figs. 10 and 11). This serves as clear evidence that none of the pure above mechanisms is responsible for the retention of imidazolium cations. From the Fig. 10 and Fig. 11 it is clear that the contribution of the adsorption mechanism in both PALC and HILIC modes is more pronounced than partitioning. Thus, depending on the particular conditions employed (e.g., mobile phase pH) in both PALC and HILIC regions, positively charged solutes can also be additionally retained due to ion-exchange interactions with the ionized silanol groups on silica's surface.
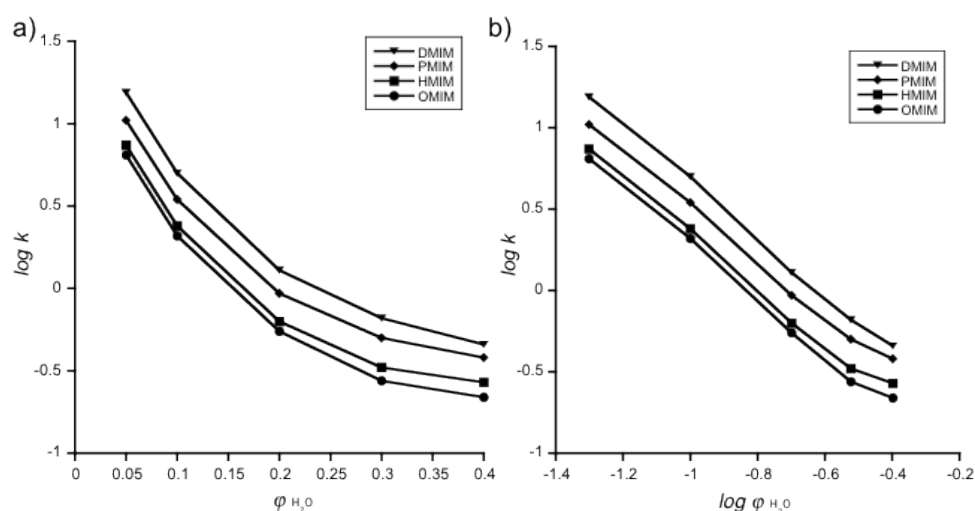

Fig. 10. Plots of log k *vs.* φ_{H2O} (a) and log k *vs.* log φ_{H2O} (b) for selected imidazolium cations in HILIC separation mode.
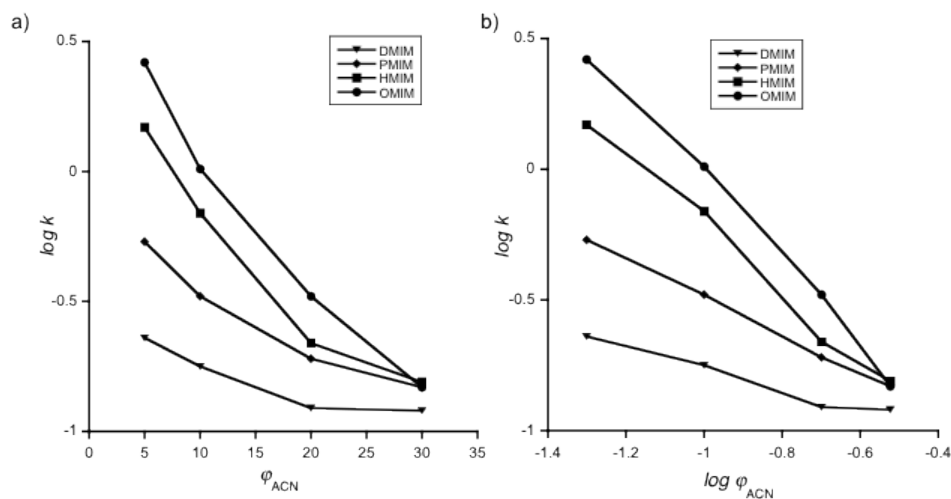
Fig. 11. Plots of log k *vs.* φ_{ACN} (a) and log k *vs.* log φ_{ACN} (b) for selected imidazolium cations in PALC separation mode.

Effect of mobile phase pH. From the previous results it is known that better peak shapes for cationic solutes are obtained at low pH, due to the suppression of the ionization of column silanol groups. The effect of mobile phase pH on the retention behaviour of IL cations in both PALC (2.5 % ACN) and HILIC (90 % ACN) modes was compared at pH 3.2 (5 mmol L^{-1} ammonium formate buffer) and pH 6.5 (5 mmol L^{-1} ammonium acetate buffer). The complete set of data from these experiments is summarized in Table 1.

Table 1. Retention factors (k), efficiencies (N) and asymmetry factors (A_s) for imidazolium cations in PALC (2.5 % ACN) and HILIC (90 % ACN) at pH 3.2 and 6.5

| Solute | PALC | | | | | | HILIC | | | | | |
| | pH 3.2 | | | pH 6.5 | | | pH 3.2 | | | pH 6.5 | | |
	K	N	A_s	k	N	A_s	k	N	A_s	k	N	A_s
CMMIM	0.2	5,100	1.2	1.0	4,200	1.3	3.2	18,100	1.2	5.4	17,500	0.9
HEMIM	0.3	5,200	1.4	1.4	4,800	1.5	5.3	17,600	1.2	9.2	16,800	1.3
DMIM	0.4	5,500	1.4	1.9	4,800	1.5	4.6	18,800	1.2	7.7	17,200	1.3
EMIM	0.6	5,200	1.5	2.8	4,500	1.7	3.8	20,000	1.1	6.3	18,900	1.1
TMIM	0.6	5,100	1.5	2.7	4,700	1.8	4.2	18,900	1.2	6.9	18,100	1.1
MPIM	1.0	4,400	1.8	3.9	4,100	2.1	3.2	19,400	1.1	5.3	18,300	1.0
EDMIM	1.0	4,500	1.8	4.0	3,800	2.2	3.5	18,500	1.1	5.9	18,300	1.1
BMIM	1.5	4,300	2.0	5.8	2,500	2.7	2.7	19,000	1.1	4.5	17,900	1.0
DMPIM	1.5	3,900	2.1	5.9	2,700	2.6	3.0	18,600	1.0	4.9	18,100	1.0
BDMIM	2.2	3,600	2.1	8.3	2,100	3.3	2.5	18,200	1.0	4.4	17,400	0.9
HMIM	3.2	2,100	2.4	12.2	860	3.9	2.1	19,500	1.0	3.6	18,700	0.9
MOIM	7.3	1,200	2.9	-	-	-	1.9	18,500	0.9	3.1	17,200	0.9

The efficiency in PALC at pH 6.5 was too low to allow reliable determination of plate count and asymmetry of strongly retained MOIM, due to poor signal-to-noise ratio, therefore some data points are missing in Table 1. As can be seen, in both separation modes the retention of IL cations increased upon increasing mobile phase pH. However, significantly weaker impact of the mobile phase pH on the retention was observed under HILIC conditions. In general, the contribution of ion-exchange to retention of basic compounds is expected to derive from the extent of ionization of both surface silanols and solute. Since in our case all the solutes remain completely ionized at both pHs the increased retention at the higher pH is, therefore, attributed to the increase in fraction of surface silanols in the ionized state.

In addition to retention time effects, under PALC conditions the increase in the buffer pH resulted in the decrease in the peak efficiency for all solutes. Moreover, as the retention increased, the peak tailing considerably increased at both pHs. Such behaviour may be attributed to the increased sample overloading effects which occur more readily at higher k values. In order to prove this, ACN concentration in the mobile phase was adjusted to get similar k for a given solute at both pHs. In this case, at both mobile phase pH values the efficiencies for the same solute were similar or even slightly better in the less acidic mobile phase (results not shown).

In contrast to PALC, under HILIC conditions high efficiencies were achieved for all solutes and the peak shapes were excellent at both pH. Some fronting was obtained for the less hydrophilic and larger solutes whereas smaller ones showed a slight tailing.

Effect of buffer concentration. In chromatographic separations based on ionic interactions, a decrease in retention is normally seen as the salt concentration in the mobile phase is increased. The effect of buffer cation concentration (in the range of 2.5-15 mmol L^{-1}) on the retention of ILs in the PALC and HILIC separation modes at pH 3.2 and pH 6.5 is presented for three compounds among the 12 tested (Fig. 12).

For all mobile phases the retention of the solutes decreases with the increase in the concentration of the buffer cation $[NH_4]^+$ but the PALC and HILIC separation modes exhibit somewhat different dependencies. In PALC the linear relationships were observed for all IL cations ($R \geq 0.98$) at both pH values (Fig. 12b). Positive intercepts indicate that there is considerable retention by secondary reversed-phase mechanism. At pH 3.2 the effect of competing cation concentration on retention of solutes is hardly

pronounced indicating that in this case solutes are retained predominantly by reversed-phase mechanism. This is expected considering the reduced ionization of the surface silanols at lower pH.

In HILIC, the convex curves were obtained at both pH values as shown in Fig. 12a. Considerably higher retention factors and slopes observed at the higher pH confirm that there also is a significant ion-exchange contribution to retention. Although retention at pH 3.2 seems to be mainly due to partitioning, the non-linear dependences indicate that it is also affected by competing cation concentration. Under HILIC conditions, where the ACN concentration is relatively high, the dielectric constant of the mobile phase decreases, resulting in stronger ion pairing interactions between cationic solutes and buffer anions. Thus, it is possible that increasing the salt concentration might additionally affect retention of solutes e.g. by lowering their net positive charge and, consequently, reducing their hydrophilicity.
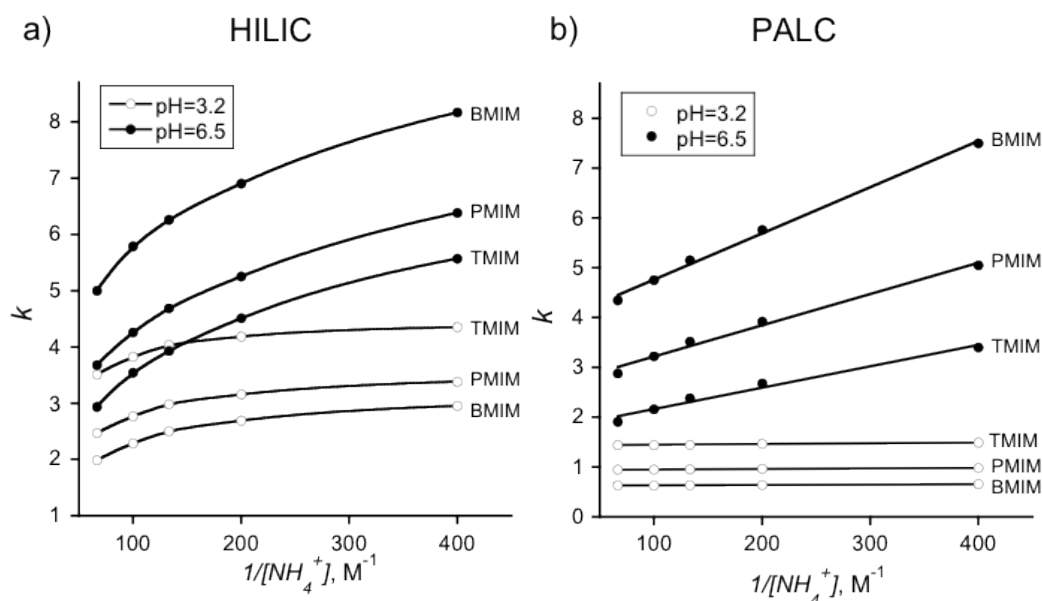

Fig. 12. Effect of buffer cation $[NH_4]^+$ concentration on the retention of selected imidazolium cations in HILIC (a) and PALC (b) separation modes. Conditions: Acquity UPLC BEH HILIC column, mobile phase: ammonium formate (pH 3.2) or ammonium acetate (pH 6.5) buffer in 2.5 % ACN (PALC) or 90 % ACN (HILIC).

In HILIC separation mode at both pH values the efficiency hardly varied over the range of $[NH_4]^+$ concentrations studied, indicating that the buffer concentration is not an important factor in determining efficiency. In contrast, using water-rich mobile phases the efficiency increased with increasing $[NH_4]^+$ concentration at the two pH conditions

employed. The increase in efficiency with increasing salt concentration was much more pronounced at higher pH. This is presumably due to the much larger retention decreases in less acidic mobile phase that results in reduction of peak tailing. For example, an increase in ammonium acetate (pH 6.5) concentration from 2.5 to 15 mmol L^{-1} caused between 1.5 and 4.0-fold increase in efficiency. However, the efficiency increased only about 10-15 % with the same increase in ammonium formate (pH 3.2) concentration.

Effect of acid additives. The above results demonstrate that under PALC conditions using conventional low pH formate buffer imidazolium cations are not sufficiently retained to be well separated. For that reason three acids were compared as mobile phase additives, namely MSA, TFA and $HClO_4$. Retention factors of solutes were measured with a 2.5:97.5 (v/v) ACN/water mobile phase in the presence of 1 and 10 mmol L^{-1} of each acid. All additives are relatively strong acids and the pH of mobile phase used in the present study independently of the acid type can be generally referred to as pH 3 or lower. Surprisingly, at 1 mmol L^{-1} concentration all three acids resulted in the retention of the solutes being reduced by 40-50 % compared with that obtained in ammonium formate buffer at pH 3.2. This effect is believed to be due in part to the stronger acidification of the mobile phase and higher degree of protonation of the more acidic silanols. At 10 mmol L^{-1} of each acid the retention of solutes slightly (~20-30 %) increased compared with that obtained at 1 mmol L^{-1}. This indicates that some ion pairing takes place.

The retention factors and peak efficiencies varied slightly (≤15 %) with the type of acid and no noticeable correlation between the retention factors and the acid type was observed. The results obtained indicate that ion pairing ability of the additives studied is not sufficiently strong to enhance the retention of ILs in water-rich mobile phases.

In HILIC separation mode (90:10 (v/v) ACN/water mobile phase) a more significant effect of acid additive on the retention was observed. The use of TFA and $HClO_4$ at both concentration levels resulted in weak or almost no retention, with retention factors ranging from 0.05 to 0.4 (i.e. ~10-25 times smaller than those obtained with ammonium formate buffer) and co-elution of most of the solutes. An explanation of this behaviour is the ion pairing which is favored in acetonitrile-rich mobile phases and results in a decrease of the hydrophilicity of the solutes. The use of MSA provided better retentivity (k values ranged from 0.5 to 1.6). This is attributed to the weaker ion pairing

ability of the MSA. On average, the efficiencies achieved in acidified mobile phases were similar to those obtained in ammonium formate buffer.

From the above results, it is evident that the ion pairing compounds have a greater effect on the retention of imidazolium cations at highly organic conditions.

Nature of organic modifier. A consideration of numerous papers published in recent years indicates that of the common polar solvents, ACN is the solvent of choice for HILIC, whatever the nature of the stationary phase.

To the best our knowledge, there are no studies evaluating the nature of organic modifier in PALC separation mode. For this reason, the effect of three alcohols (methanol (MeOH), ethanol (EtOH) and isopropanol (iPrOH) as the organic modifier of the mobile phase for PALC on the retention of IL cations was investigated. The retention behaviour of solutes was evaluated using organic modifier-water (5:95, v/v) mobile phase containing 5 mmol L^{-1} ammonium acetate buffer (pH 6.5). Retention factors of imidazolium cations increased in the order iPrOH < EtOH ≈ ACN < MeOH which is consistent with the eluotropic series of these solvents in conventional RP separations. Evaluation of the impact of the nature of organic modifier on separation efficiency revealed that for early eluting solutes (CMMIM, HEMIM, DMIM, EMIM, TMIM) all solvents yielded similar efficiencies (variations less than 25 %), but for the late eluting less hydrophilic IL cations peak efficiencies increased in the order MeOH < ACN ≤ EtOH < iPrOH. For example, substituting isopropanol by MeOH caused a 2.1- and 3.9-fold decrease in efficiency for EDMIM and BDMIM, respectively. In this case, column overload and peak tailing become more significant. Finally, no differences in selectivity were observed when alcohols were used as compared to ACN.

Column temperature. As a further parameter the effect of the column temperature was investigated in the range of 20-70 °C using a 5 mmol L^{-1} ammonium acetate buffer (pH 6.5) in 2.5 and 90 % (v/v) ACN. In both separation modes the obtained ln k versus 1/T plots (van't Hoff plots) showed a linear relationship for all IL cations ($R \geq 0.99$) as exemplified in Fig. 13 for four selected solutes.

In PALC mode (Fig. 13a) all the solutes showed so called "normal behaviour", that is, decrease in retention with increasing temperature with no significant effect on resolution. As expected, the efficiency slightly improved for all solutes upon increasing column temperature. These improvements ranged from approximately 10 % for CMMIM

to about 40 % for HMIM. Further, this was accompanied by small reduction (~10-25 %) in peak asymmetry for all the solutes. Observed increases in efficiency are generally attributed to a reduction in the viscosity of the mobile phase with temperature and thus increased solute diffusivity. In addition, changing the temperature changes k for the solutes, and this in itself could give rise to changes in efficiency. However, the pairs of EMIM/TMIM, MPIM/EDMIM and BMIM/DMPIM were not resolved under all temperature conditions. From each plot, the standard enthalpy ($\Delta H°$) of the solute transfer from mobile to stationary phase was calculated from the slope. Obtained $\Delta H°$ values range from -8.5 to -29 kJ mol^{-1} (exothermic process) from CMMIM to OMIM and this correlates with increasing size of the solute. Similar patterns of van't Hoff plots were obtained in PALC at pH 3.2 (results not shown), with the overall retentivity being significantly weaker.
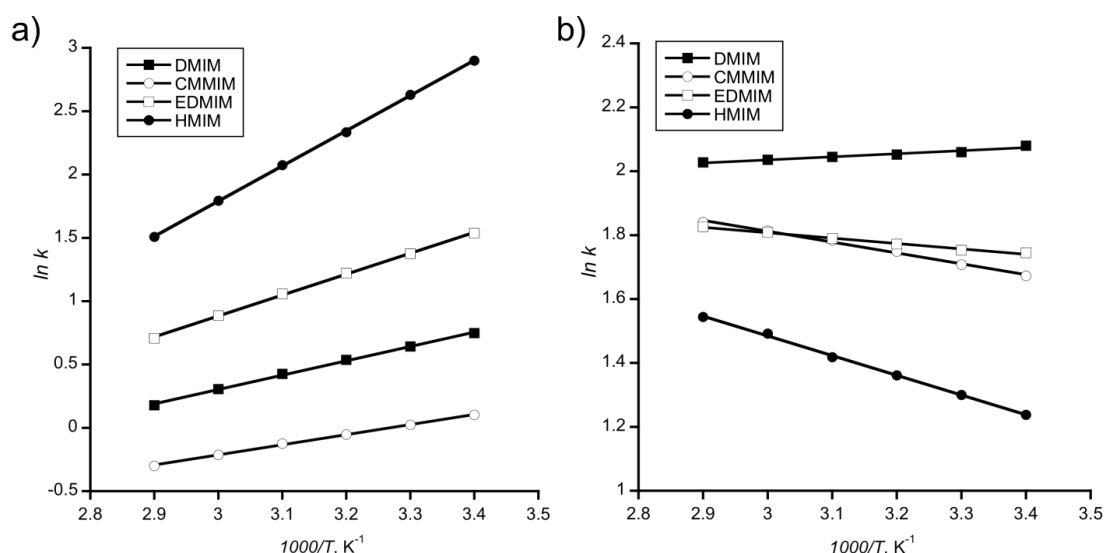

Fig. 13. Effect of column temperature on the retention of selected imidazolium cations in PALC (a) and HILIC (b) separation modes. Conditions: Acquity UPLC BEH HILIC column, mobile phase: 5 mmol L^{-1} ammonium acetate (pH 6.5) in 2.5 % (PALC) or 90 % (HILIC) ACN.

Under HILIC conditions different effects were observed for the different solutes when temperature was increased. Fig. 13b shows the van't Hoff plots for selected solutes. A decrease in retention was obtained for DMIM as the column temperature increased. Such "normal behaviour" was also observed for other stronger retained (i.e. more hydrophilic) HEMIM and TMIM cations (results not shown). In contrast, nine less hydrophilic solutes showed an increased retention with the temperature and, with the

exception of CMMIM, the negative slopes of their van't Hoff plots increased with increasing size of the solute.

At pH 6.5, the retention of cationic solutes is governed by both ion-exchange and HILIC separation mechanisms. Thus, it is quite possible that for less hydrophilic compounds such as OMIM, HMIM, DMPIM, ion-exchange interactions predominate over the hydrophilic partitioning causing an increase in the retention with temperature. In order to prove this, the influence of the column temperature on the retention of solutes was also evaluated at pH 3.2 (5 mmol L^{-1} ammonium formate buffer) and at pH 2.0 (10 mmol L^{-1} MSA). The obtained ln k vs. 1/T plots for three selected solutes are presented in Fig. 14.
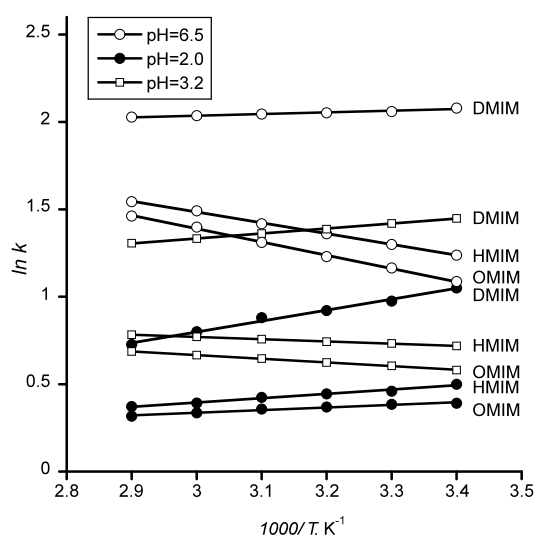

Fig. 14. Van't Hoff plots for three imidazolium cations under HILIC conditions at different mobile phase pHs. Conditions: Acquity UPLC BEH HILIC column, mobile phase: 5 mmol L^{-1} ammonium acetate (pH 6.5), 5 mmol L^{-1} ammonium formate (pH 3.2) or 10 mmol L^{-1} MSA in 90 % ACN.

As can be seen both largest solutes (OMIM and HMIM) at pH 3.2 show a smaller negative slope than at pH 6.5 and even a positive slope at pH 2.0, whereas the positive slope of smallest DMIM gradually increases with increasing mobile phase acidity. Calculated ΔH° values of the IL cations in HILIC at three different pHs are compared in Table 2. For all solutes retention enthalpies show more negative values when the pH of the mobile phase decreases. In the most acidic mobile phase all the compounds studied exhibit exothermic behaviour (decreasing retention with increasing temperature). Based on the above observations it is clear that the enthalpy changes are related to decreased ion-exchange contribution to retention. The changes in efficiency and peak asymmetry

with temperature were small (≤10 %) and did not follow a uniform trend at both pHs. Depending on the type of solute, a change in temperature affected N and A_s values in a positive or negative manner.

Table 2. Retention enthalpies of imidazolium cations in HILIC at different pHs

Solute	$\Delta H°$		
	pH 6.5	pH 3.2	pH 2.0
OMIM	6.27	1.79	-1.18
HMIM	5.13	1.07	-1.77
BDMIM	3.93	0.21	-2.25
BMIM	3.22	-0.19	-2.72
DMPIM	2.74	-0.71	-2.84
MPIM	1.86	-1.03	-3.35
CMMIM	2.81	0.56	-1.91
EDMIM	1.35	-1.55	-3.38
EMIM	0.49	-1.70	-3.95
TMIM	-0.01	-2.25	-3.78
DMIM	-0.87	-2.39	-4.31
HEMIM	-2.02	-2.68	-3.97

The selectivity of IL cations decreased at elevated temperatures, with a few exceptions. As indicated in Fig. 13b, the resolution of the pair EDMIM/CMMIM decreased at first, but increased above 60 °C with the reversal of the elution order. The resolution of the critical pair CMMIM/MPIM gradually increased when temperature was raised (results not shown). The best resolution was achieved at 30°C.

Finally, retention times in the HILIC mode were significantly less affected by a change in temperature than those obtained under PALC elution conditions. For example, as the temperature was increased from 20 to 70 °C (pH 6.5) the retention time change ranged from 0.67 (OMIM) to 0.78 min (HEMIM) and from 0.24 (CMMIM) to 27.3 min (OMIM) for HILIC and PALC, respectively.

Overloading effects. Peak shape and width are very important in chromatography for resolution enhancement and separation improvement. Mass overload is perhaps one of the main reasons of the deterioration in column performance in the RP separations of ionized basic compounds. It is therefore of interest to compare overload in PALC and HILIC separation modes on the same BEH column. Since sample overloading occurs more readily at high than at low k, different ACN concentrations were chosen for each solute to get k values in the range of 3-4.

Fig. 15a shows the plots of column efficiency for DMIM and TMIM as a function of sample amount evaluated in the HILIC mode at two pH values. A comparison of the plots shows that loading capacity is roughly the same at either pH 3.2 or 6.5. As can be observed, a 10 % loss in efficiency was experienced in the HILIC separation for about 2-3 nmol of solutes, i.e at the levels up to ten times higher than those obtained in PALC. It should be noted that for most hydrophilic solutes increasing tailing in conjunction with decreasing efficiency occurred as sample amount was increased. In contract, less hydrophilic IL cations (e.g. OMIM, HMIM, BDMIM) showed increased fronting as sample load increased. Fronting could be due to anti-Langmuir sorption behaviour which arises when interactions between solute molecules are relatively strong. As the amount of solute injected increases, these solute-solute interactions draw further molecules into the stationary phase, giving fronting peaks (if the solute concentration is high enough) and subsequent increase in k with solute concentration. Clearly, such solute-solute interactions might be considerably stronger for larger IL cations.
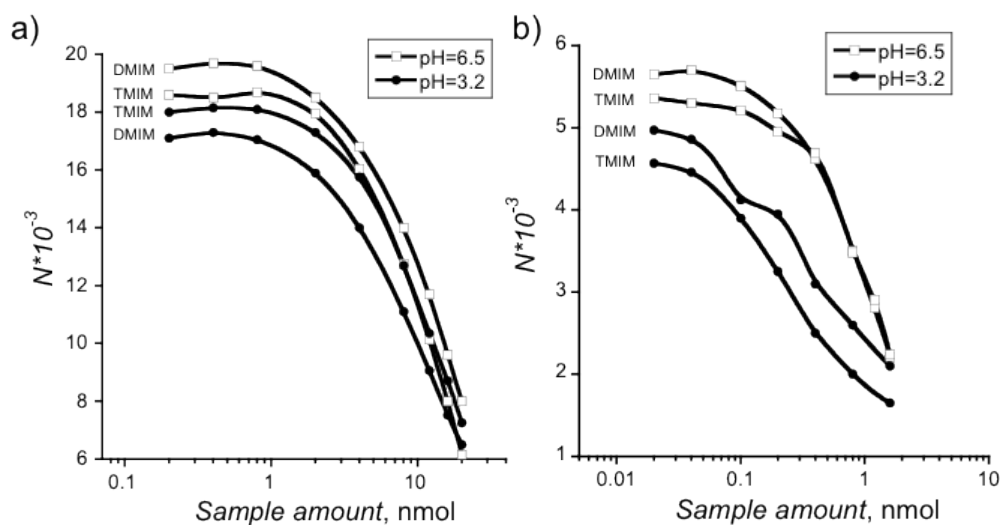

Fig. 15. Plots of column efficiency vs. sample amount for selected imidazolium cations in HILIC (a) and PALC (b) separation modes at two pH values. Conditions: Acquity UPLC BEH HILIC column, mobile phase, 5 mmol L^{-1} ammonium formate (pH 3.2) or 5 mmol L^{-1} ammonium acetate (pH 6.5) buffer in ACN/H$_2$O at different ratios.

Fig. 15b shows similar plots obtained for the two selected solutes under PALC conditions at both pH values For all solutes rapid overloading occurs with right-angled triangle peak shapes and shorter retention times. Such behaviour is typically observed in RP separations. At pH 3.2, about 10 % loss in efficiency is reached with ~0.2 nmol of

solute, compared with ~0.5 nmol at pH 6.5. Thus, in PALC mode overloading effects are less important in neutral mobile phases due to larger number of the ionized silanols.

Although sample loading effects are complex and require a more detailed study, the results obtained in this study indicate that HILIC exhibits considerably higher resistance to overload of IL cations in comparison with PALC.

Flow rate. The effect of mobile phase flow rate upon efficiency was briefly studied. Fig. 16 shows plots of plate height *vs* flow velocity for three imidazolium cations. Typical parabolic-type dependence was observed for the HILIC separation mode (Fig. 16a). At low mobile phase flow rates the predominate process is the longitudinal diffusion, which is more pronounced for the imidazolium cations with short alkyl group. By increasing the flow rate, the longitudinal diffusion effect is greatly reduced and the maximum column efficiency is reached at 0.3-0.4 mL min^{-1}. At higher flow rates efficiency decreased (*H* increased) due the higher resistance to mass transfer. As expected, this effect was more pronounced for bigger solutes. Optimum mobile phase velocity of 0.5 mL min^{-1} was chosen for the fast separation of all solutes with adequate resolution and efficiency.
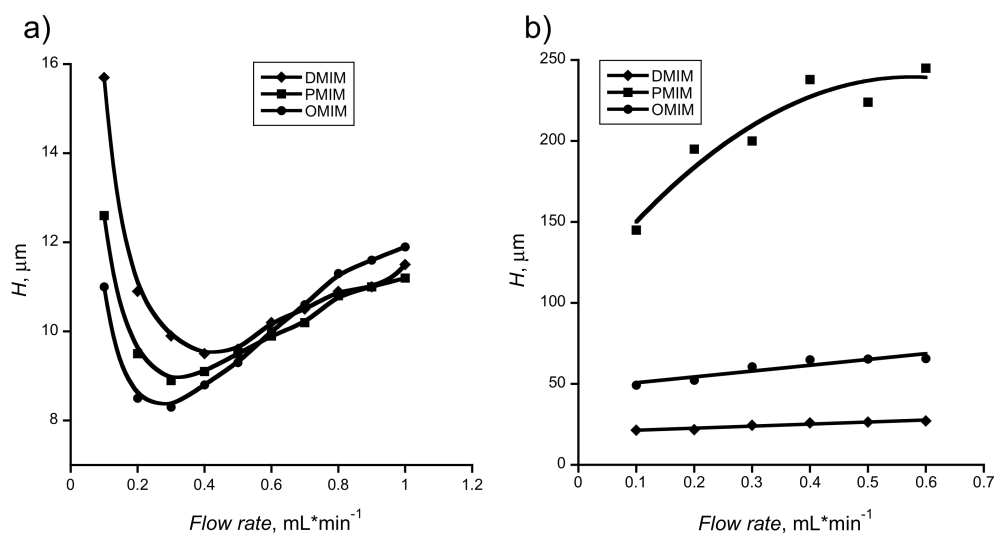

Fig. 16. Van Deemter plots for HILIC (a) and PALC (b) separation modes. Mobile phase 5 mmol L^{-1} ammonium formate in 2.5 % ACN (PALC) and 90% ACN (HILIC).

The effect of flow rate in the PALC separation mode was studied in the smaller range of velocities, because of the higher back-pressures resulted from the use of high viscosity aqueous mobile phases. The not typical dependencies were obtained indicating that in the entire flow rate range studied under PALC conditions the resistance to mass

transfer is the predominant diffusion process (Fig. 16b). One of the advantages of HILIC separation mode is the low back pressures which results from the low viscosity (high ACN content) of mobile phase and possibility of high flow rates.

Separation of IL cations. The fast and adequately efficient PALC separation of all twelve IL cations using isocratic elution is impossible. Thus, for the PALC elution of all twelve solutes gradient elution was necessary (Fig. 17a). In contrast, under HILIC conditions all 12 solutes were isocratically eluted in less than 4 min (Fig. 17b). It should be noted that the low back pressures enable to use higher flow rates (0.75 mL min^{-1}) in HILIC without loss in efficiency and resolution. Despite gradient elution the peak shapes obtained in PALC were considerably poorer and selectivity was not sufficient to distinguish between solutes with similar hydrophobicity (EMIM/TMIM, MPIM/EDMIM and BMIM/DMPIM pairs). Overall, however, obtained results demonstrate the excellent complement nature of the two separation modes.
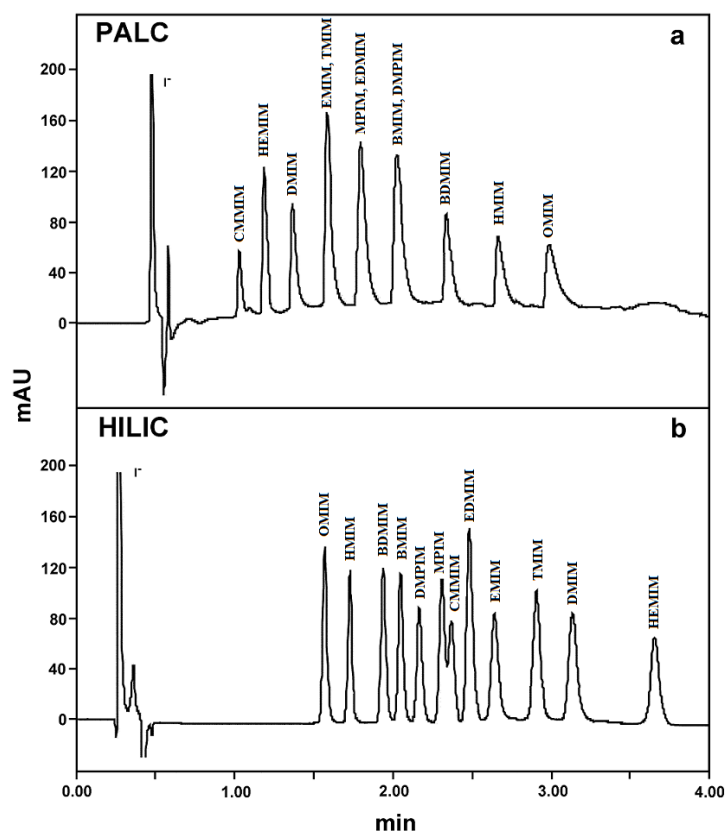

Fig. 17. Optimized separation of imidazolium cations in PALC (a) and HILIC (b) separation modes. Conditions: Acquity UPLC BEH HILIC column, flow rate, 0.5 ml min^{-1} in PALC and 0.75 ml min^{-1} in HILIC; mobile phase, A - 5 mmol L^{-1} ammonium acetate (pH 6.5) in 100 % H$_2$O, B - 5 mmol L^{-1} ammonium acetate in 90 % ACN, linear gradient from 2 to 30 % B in 3 min (PALC) and isocratic elution with 5 mmol L^{-1} ammonium formate buffer (pH 3.2) in 95 % ACN (HILIC).

CONCLUSIONS

1. UPLC technique in RP, IP and HILIC separation modes is applicable for the separation of six imidazolium cations under optimized conditions. HILIC system appeared to be the most advantageous, where excellent separations was achieved within 2 min. Compared to current HPLC systems, UPLC affords considerably faster separations (up to 25-times) with better peak shapes.

2. Ethylene bridged hybrid silica column provides a dual retention mechanism with opposite separation selectivity depending on the composition of the mobile phase. In acetonitrile-rich ($\geq$60 % ACN) mobile phases the retention of solutes increases with increasing ACN content and hydrophilicity of solutes (HILIC mode). In contrast, in the water-rich mobile phases ($\leq$30 % ACN) the retention increases with water content in the mobile phase with opposite order of elution.

3. The adsorption and partitioning models were applied for HILIC and PALC separation modes and showed that retention behavior of imidazolium ionic liquids cannot be accurately described as neither pure partitioning nor pure adsorption mechanism.

4. The retention of the imidazolium cations decreases with the increase in the concentration of the buffer cation $[NH_4]^+$ under both HILIC and PALC conditions. It is clear that beside partitioning and adsorption, ion-exchange interactions are also operating between imidazolium cations and ionized silanol groups.

5. Retention factors of imidazolium cations in PALC mode increased in the order isopropanol < ACN $\approx$ EtOH < MeOH which is consistent with the eluotropic series of these solvents in conventional RP separations and confirms significant input of hydrophobic interactions to the retention mechanism.

6. In PALC mode, the decrease in retention was observed with increasing temperature (70 °C) for all the solutes. Under HILIC conditions different effects were observed for the different solutes when temperature was increased; the retention decreased for hydrophilic solutes, while less hydrophilic solutes showed an increased retention. The different behaviour depends on the predominant interaction (hydrophilic or ion exchange) between the solute and stationary phase.

7. HILIC exhibits considerably higher resistance to overload in comparison with PALC. In PALC separation mode sample overloading occurs at ~0.2 nmol (pH 3,2), or ~0.5 nmol (pH 6.5) of solute. In HILIC separation mode the mass overloading effect is seen at 2-3 nmol of solute, independent of the pH of mobile phase.

8. In PALC mode, gradient elution was required to achieve adequate retentivity of all solutes, however, selectivity was not sufficient to distinguish between solutes of very similar hydrophobicity (EMIM/TMIM, MPIM/EDMIM and BMIM/DMPIM pairs). In contrast, under HILIC conditions twelve solutes were almost completely resolved in less than 4 min by using isocratic elution. Compared to HILIC, PALC has an advantage of using low ACN contents and is thus attractive for the development of green chromatography.

The List of Original Publications by the Author

Articles in journals:

1. **A. Orentienė,** V. Olšauskaitė, V. Vičkačkaitė, A. Padarauskas. Ultra-Performance Liquid Chromatography: a Powerful Tool for the Separation of Imidazolium Ionic Liquid Cations. Chromatographia 73 (2011) 17-24.

2. **A. Orentienė,** V. Olšauskaitė, V. Vičkačkaitė, A. Padarauskas. Retention behaviour of imidazolium ionic liquid cations on 1.7 μm ethylene bridged hybrid silica column using acetonitrile-rich and water-rich mobile phases. Journal of Chromatography A 1218 (2011) 6884-6891.

Published contributions to academic conference:

1. V. Vičkačkaitė, **A. Orentienė**, A. Padarauskas. Ultra-performance liquid chromatography: a powerful tool for the separation of imidazolium ionic liquid cations. Abstracts of the 7th Aegean Analytical Chemistry Days AACD 2010, Lesvos, Greece, 2010, P3-33, p. 231.

2. V. Vičkačkaitė, **A. Orentienė**, V. Vičkačkaitė, A. Padarauskas. Hydrophilic interaction chromatography and per aqueous liquid chromatography for separation of imidazolium ionic liquid cations. IUPAC International Congress on Analytical Sciences 2011, Kyoto, Japan, 2011, 23P011.

3. **A. Orentienė**, V. Olšauskaitė, V. Vičkačkaitė, A. Padarauskas. Retention behaviour of imidazolium ionic liquid cations on 1.7 μm ethylene bridged hybrid silica column using acetonitrile-rich and water rich mobile phases. 5th International Scientific Conference The Vital Nature Sign, Kaunas, Lithuania, 2011, P-47, p. 83.

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Acknowledgments

IMIDAZOLIO JONINIŲ SKYSČIŲ KATIJONŲ ATSKYRIMO ULTRAEFEKTYVIOSIOS SKYSČIŲ CHROMATOGRAFIJOS METODAIS YPATUMŲ TYRIMAS

SANTRAUKA

Šioje daktaro disertacijoje apibendrintų mokslinių tyrimų tikslas – ištirti ir palyginti imidazolio joninių skysčių katijonų atskyrimą skirtingais ultraefektyviosios skysčių chromatografijos metodais bei nuodugniai ištirti imidazolio katijonų chromatografinę elgseną hidrofilinės sąveikos (HILIC) ir vandeninės skysčių (PALC) chromatografijos sąlygose.

Optimizuotos šešių imidazolio katijonų atskyrimo atvirkščių fazių (AF), jonų porų (JP) ir HILIC ultraefektyviosios chromatografijos (UESCh) metodais. Greitas ir efektyvus šešių imidazolio katijonų atskyrimas gautas AF-UESCh ir JP-UESCh metoduose pritaikius gradientinės eliucijos režimą. Tačiau tinkamiausias metodas imidazolio katijonų atskyrimui yra HILIC, kur greitai (per 2 min.) ir efektyviai atskirti visi tirti imidazolio katijonai izokratiniame režime. UESCh naudojimas pagreitino atskyrimą (pvz., HILIC metode net iki 25 kartų) ir pagerino smailių efektyvumus lyginant su ESCh.

Ištirta acetonitrilo (ACN) koncentracijos judrioje fazėje įtaką imidazolio katijonų chromatografinei elgsenai sistemoje hibridinis silikagelio sorbentas-H_2O/ACN. Nustatyta, kad imidazolio katijonų eliucijos tvarka ir sulaikymo mechanizmas priklauso nuo ACN/H_2O santykio judrioje fazėje. Kai ACN koncentracija judrioje fazėje >60 %, imidazolio katijonai sulaikomi hidrofiliškumo stiprėjimo tvarka, o vanduo pasižymi stipresne išstūmimo jėga (HILIC režimas). Kai ACN koncentracija mažesnė už 30 %, pasikeičia analičių eliucijos tvarka bei judrios fazės tirpiklių išstūmimo jėga (PALC režimas). Tiriamoms sistemoms pritaikius pasiskirstymo ir adsorbcijos modelius nustatyta, kad tiek HILIC, tiek ir PALC sąlygose imidazolio katijonų sulaikymas negali būti aprašytas nei grynu pasiskirstymo, nei grynu adsorbciniu mechanizmu.

Ištyrus judrios fazės pH ir amonio jonų koncentraciją judrioje fazėje abiejose sistemose nustatyta, kad analičių sulaikymas silpnėja didinant judrios fazės pH ir amonio jonų koncentraciją. Tai patvirtina, kad, nepriklausomai nuo eliucijos režimo, vyksta papildoma jonų mainų sąveika tarp imidazolio katijonų ir disocijavusių silanolinių grupių.

Įvertinta ir kitų eksperimentinių faktorių (kolonėlės temperatūra, masės perkrova, organinio tirpiklio prigimtis) įtaka imidazolio katijonų atskyrimui. Nustatyta, kad PALC sąlygose kolonėlės temperatūros didinimas (iki 70 °C) susilpnina visų analičių sulaikymą. HILIC režime keliant temperatūrą poliškiausių analičių sulaikymas silpnėja, o mažiau polinių – stiprėja. Tokią skirtingą temperatūros įtaką nulemia dominuojanti analitės sąveikos su nejudria faze rūšis (hidrofilinė sąveika ar jonų mainai). PALC sąlygose, ACN pakeitus kitais organiniais tirpikliais nustatyta, kad analičių sulaikymas stiprėjo tokia eile: izopropanolis<ACN≈EtOH< MeOH. Tai atitinka eliuotropinę šių tirpiklių eilę tradicinėje AFSCh ir patvirtina hidrofobinės sąveikos indėlį analičių sulaikymui. Taip pat nustatyta, kad HILIC sistema yra atsparesnė masės perkrovoms kolonėlėje. PALC metode kolonėlė perkraunama įleidžiamos analitės kiekiui viršijus 0,2 (pH 3,2) arba 0,5 nmol (pH 6,5). HILIC metode maksimalūs kiekiai nepriklauso nuo pH ir siekia apie 2-3 nmol.

Optimizuotos 12 imidazolio katijonų atskyrimo sąlygos PALC ir HILIC metodais. PALC režime, dėl smarkiai besiskiriančios skirtingo poliškumo analičių sąveikos su sorbentu, būtina gradientinė eliucija. Tačiau ir šiuo atveju sistemos atrankumas nepakankamas labai artimo hidrofobiškumo katijonų poroms (EMIM/TMIM, PMIM/EDMIM ir BMIM/DMPIM) atskirti. HILIC metodu izokratinėmis sąlygomis per 4 min beveik pilnai atskiriami visi 12 imidazolio katijonų. Pagrindinis PALC privalumas – sunaudojami labai maži ACN kiekiai, kas yra labai patrauklu ekologiškumo ir ekonomiškumo požiūriu.