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Short communication

Reversed-phase liquid chromatographic method for the determination of selected room-temperature ionic liquid cations

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Abstract

The separation of selected 1-alkyl- and 1-aryl-3-methylimidazolium-based room temperature ionic liquid cations has been performed using reversed-phase high-performance liquid chromatography with electrospray ionization mass detection. The RP-HPLC method development started with the selection of a column taking into account especially the resolution of low molecular congeners of the selected group. Mobile phase composition was optimized for peak resolution, sensitivity and high reproducibility of retention values. The results of the method development were applied to the determination of exemplary ionic liquid species present in the medium used in cytotoxicity studies.
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1. Introduction

Room temperature ionic liquids are low melting salts, which represent a new class of non-molecular ionic solvents [1]. Their most common representatives are typically composed of a small inorganic, weakly coordinating anion and an unsymmetrically substituted bulky organic, nitrogen-containing heterocyclic cation, exhibiting no measurable vapor pressure [2]. Although, archetypal ionic liquids are *N*-butylpyridinium chloride–aluminium(III) chloride, [*N*Bupy]Cl–$AlCl_3$, and 1-ethyl-3-methylimidazolium chloride–aluminium(III) chloride, [emim]Cl–$AlCl_3$ systems, the range of possibly available anions and cations has expanded enormously up to one trillion (10^{18}) accessible room temperature ionic liquids [3]. Comprehensive information about their applications in chemical processes may be found in recent reviews by Welton [1], Holbrey and Seddon [3], Wasserscheid and Keim [4] and Gordon [5]. So far, no optimized procedures for separation of the most commonly used class of room temperature ionic liquids (differently substituted imidazolium cations) by high-pressure liquid chromatography have been reported to our knowledge. The aim of the present study was therefore to develop a reversed-phase high-performance liquid chromatography (RP-HPLC) procedure. Since research now is almost

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exclusively based upon the 1-ethyl-3-methylimidazolium cation $[emim]^+$ and derivatives thereof, our study was carried out on the R-methyl- and R-ethylimidazolium group of cations with varying alkyl chain lengths or aryl substituents. As counter anions bromide $[Br]^-$, chloride $[Cl]^-$, tetrafluoroborate $[BF_4]^-$ and hexafluorophosphate $[PF_6]^-$ were used.

2. Materials and methods

2.1. Chemicals and samples

Ionic liquids selected for these studies were synthesized in the group of Prof. B. Ondruschka, from the Institute of Technical Chemistry and Environmental Chemistry in Jena, Germany as described in Ref. [6]. Compounds, their names, structures and numbers chosen for the presented method development are listed in Table 1. Concentrations of the selected ionic liquids in the mixture were in the range of 0.2 to 0.4 m*M* in methanol–water (1:1, v/v) solution. Standard solutions in the range from 50 to 1000 μ*M* of 1-amyl(pentyl)-3-methylimidazolium hexafluorophosphate [AMIM][PF_6] were prepared in the methanol–water (1:1, v:v) solutions for preliminary quantitative studies. Acetonitrile-G Chromasolv®, super gradient grade and methanol-G Chromasolv® for gradient elution were purchased from Riedel-de-Haën (Germany), acetic acid p.a. from Fluka Chemika (Buchs, Switzerland) and ammonium acetate from Merck (Darmstadt, Germany).

2.2. RP-HPLC analysis

The experiments were performed on a Hewlett-Packard 1100 series unit equipped with a binary solvent pump, autosampler, vacuum degasser and variable wavelength detector. All analyses were performed at 0.8 ml min^{-1} flow-rate. The injection volume was 1 μl and the column temperature was 30 °C. Columns used in this study were C_{18} and C_8 LiChrospher Select 250×4.6 mm I.D. 5 μm (Merck) and C_8 MetaSil Basic 250×4.6 mm I.D. 5 μm (Varian).

2.3. ESI-MS analysis

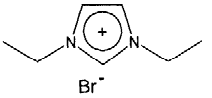
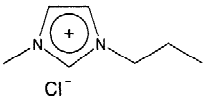
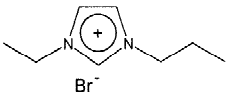
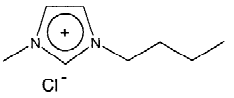
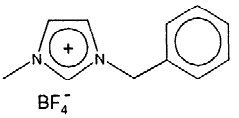
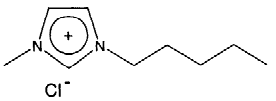
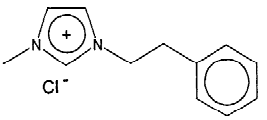
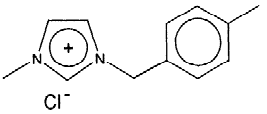
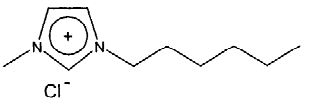
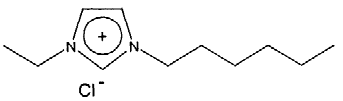
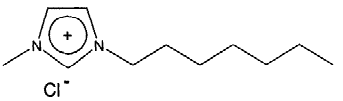
Some of the ionic liquids studied exhibit their absorption light maxima below 200 nm. Therefore, total ion current detection was used with a Bruker-EsquireLC electrospray ionisation mass detector (Brucker-Daltonic, Bremen, Germany). Mass spectra of partitioning cations were acquired in the positive ion mode in the scan range of m/z 50–200 and each component was confirmed with at least one $[M+H]^+$ ion. Different anions in the studied ionic liquids do not affect the chromatographic behaviour of the same cation. This we have confirmed by comparing the retention values of dissociated pairs of [BMIM][Br] with [BMIM][BF_4], [AMIM][Cl] with [AMIM][PF_6] and [HpMIM][Cl] with [HpMIM][PF_6]. The ESI source conditions were set to capillary voltage of 3500 V, drying gas flow-rate at 12 l min^{-1}, drying gas temperature at 360 °C and nebulizer pressure at 70 p.s.i.

3. Results and discussion

Using RP-C_{18} phase with methanol–water as a mobile phase very poor separation was obtained, especially for more polar components such $[EEIM]^+$, $[PMIM]^+$ and $[PEIM]^+$ cations observed also in Ref. [7]. Changing to a standard RP 8 column with less hydrophobic resolution strength using the methanol–water mobile phase did not improve the separation significantly, most likely due to a poor coverage of free silanols observed as a severe tailing for all studied solutes. Use of an RP 8 column with a higher carbon load resulted in satisfactory separation of early eluting compounds. Another observation was the low reproducibility of retention values, which was improved by stabilizing pH with an addition of 0.5 up to 1% acetic acid alone.

To compromise the better resolution and faster analysis, the organic modifier in the mobile phase was changed. A binary system was prepared where acetonitrile was mixed with 1% acetic acid solution in water. As is well known, if compared to methanol, peaks obtained in this mobile phase were much sharper and total time of analysis was shortened. Additionally, use of acetonitrile would improve future low wavelength UV detection of some of the

Table 1
Test mixture of selected ionic liquids for RP-HPLC method development

No.	Name	Formula	Structure	λ_{max}
1	1-ethyl-3-ethylimidazolium bromide	[EEIM][Br]		190 nm
2	1-n-propyl-3-methylimidazolium chloride	[PMIM][Br]		210 nm
3	1-n-propyl-3-ethylimidazolium bromide	[PEIM][Br]		190 nm
4	1-buthyl-3-methylimidazolium chloride	[BMIM][Cl]		212 nm
5	1-benzyl-3-methylimidazolium tetrafluoroborate	[BzMIM][BF_4]		*n.a.*
6	1-amyl(pentyl)-3-methylimidazolium chloride	[AMIM][Cl]		*n.a.*
7	1-(-2-phenylethyl)-3-methylimidazolium chloride	[EBzMIM][Cl]		203 nm
8	1-p-methylbenzyl-3-methylimidazolium chloride	[pMBzMIM][Cl]		*n.a.*
9	1-hexyl-3-methylimidazolium chloride	[HMIM][Cl]		*n.a.*
10	1-hexyl-3-ethylimidazolium chloride	[HEIM][Cl]		192 nm
11	1-heptyl-3-methylimidazolium chloride	[HpMIM][Cl]		*n.a.*

n.a., not available.

studied solutes exhibiting absorption maxima below 210 nm as outlined in Table 1. Significant improvement in peak symmetry was also made by buffering mobile phase with ammonium acetate. At first, 10 m*M* concentration in water was used which when using isocratic mode gave us better reproducibility in retention values. However in the next step, buffer concentration was elevated to the fairly high concentration of 20 m*M* ammonium acetate, yielding stable identical retention times of solutes with different types of anions even if used in environmental or biological samples, where the presence of different anions could affect pH conditions. Finally, a gradient mode was used as shown in Fig. 1. Future method optimization from this point will mainly depend on the matrix of the environmental sample. Therefore the proposed mode will not give close and uniform distribution of standard peaks which allows the possibility of solute separation from matrix signals.

In order to verify method applicability, a sample of RPMI medium (WL glutamine, W/O $NaHCO_3$, with 1% penicillin/streptomycin, pH 7) used for cytotoxicity studies of the exemplaric ionic liquid [AMIM][PF_6] of known concentration at 62.5 μ*M* was tested. Fig. 2 shows a chromatogram with the well isolated and identified compound. A first attempt to construct a calibration curve was made. Under these particular detection conditions, curve shape has been found to fit a second order polynomial regression ($r^2 = 0.999$) which is likely to change with changing mass detector conditions, affecting response behavior.

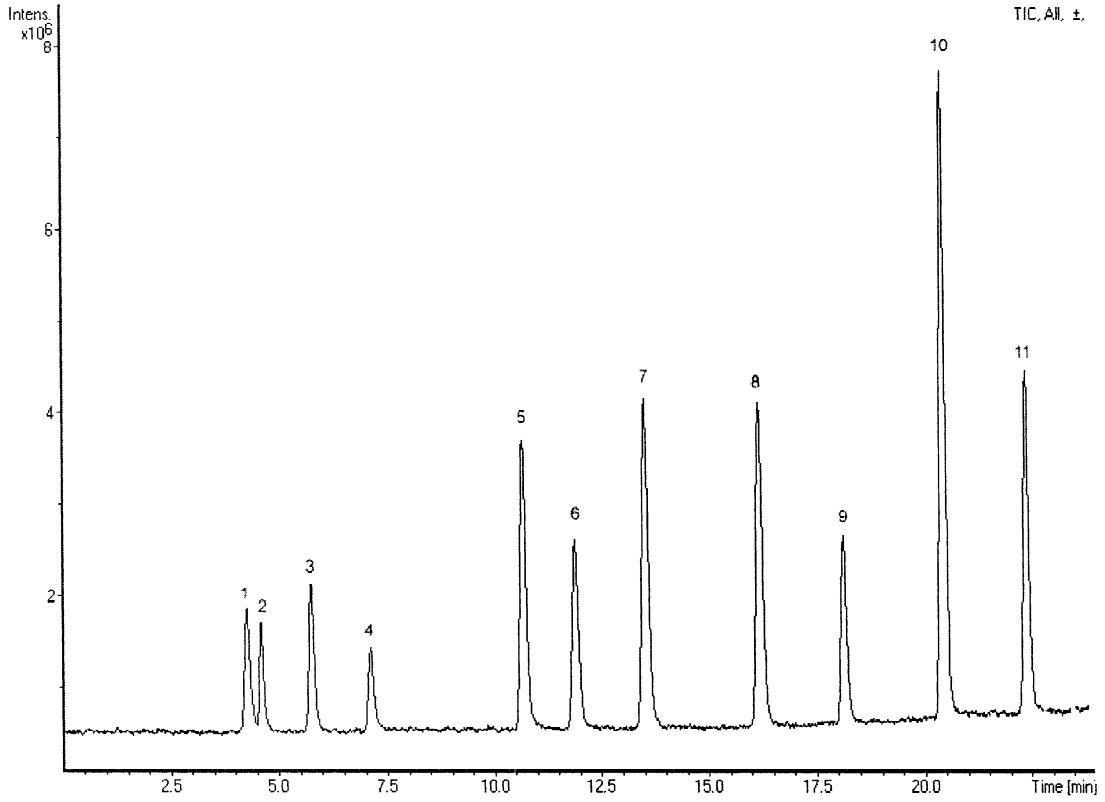


Fig. 1. Gradient separation of the test mixture. Column RP C_8 MetaSil Basic 250×4.6 mm I.D. 5 μm (Varian). Mobile phase A, acetonitrile; B, water (1% acetic acid/20 m*M* ammonium acetate) 0 min. 10% B, 10 min. 20% B, 25 min. 50% B. Compounds numbered according to Table 1.

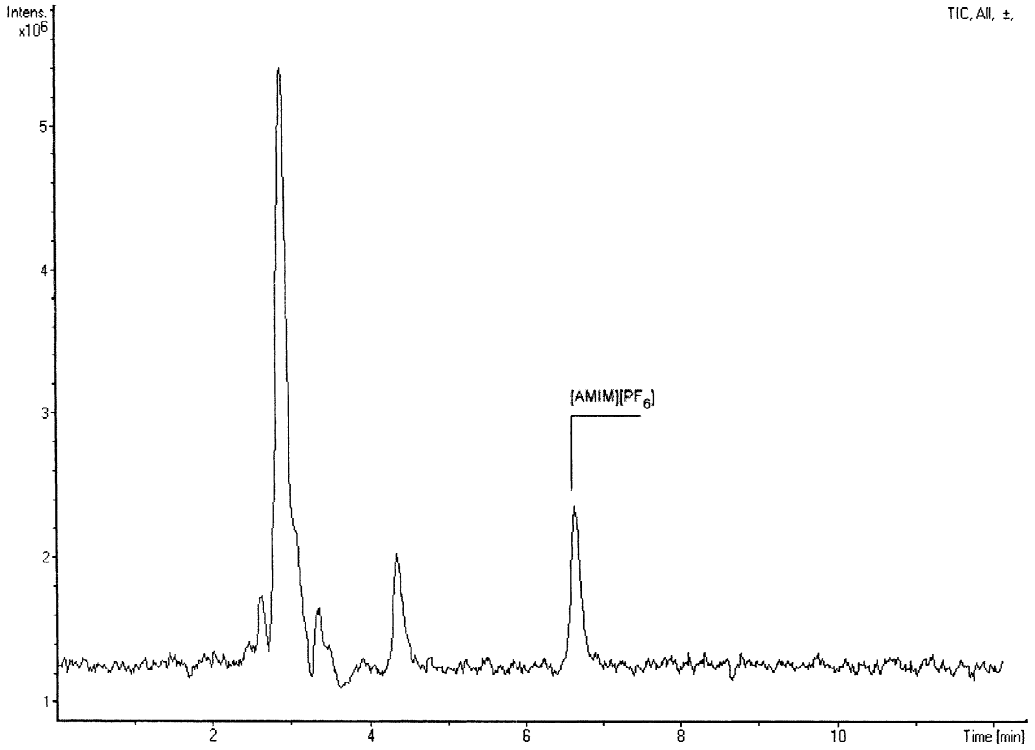


Fig. 2. Chromatogram of RPMI medium sample spiked with 62.5 μ*M* of 1-amyl-3-methylimidazolium hexafluorophosphate. Compound identification through retention and mass comparison to the standard sample. Column RP-C_8 MetaSil Basic 250×4.6 mm I.D. 5 μm (Varian). Mobile phase, isocratic acetonitrile–water (0.8% acetic acid/20 m*M* ammonium acetate) (20:80, v/v).

4. Conclusions

This paper reports the first published routine chromatographic method to analyse room temperature ionic liquids in environmental and biological studies. The method is simple and selective and is believed to be applicable for numerous ionic liquids having a similar basis of design. The method turns out to be stable with different types of biological samples, regardless of various pH modifiers present. Further investigations should be undertaken in particular with respect to the alternatives and limitations of different buffer systems such as triethylamine or cyclohexylamine typically used in nitrogen-containing compounds.

In the presented method development mass detection in the total ion current mode was used. However powerful, it is hardly proposed as a routine methodology for future applications. Future efforts should also concentrate on applicability of UV detection to the studied group of compounds. Additionally, studies with different mass detector conditions such as nebulizer pressure, drying gas flow and temperature which have a critical influence on response behavior of the electrospray detector should be undertaken. This development could lead towards optimization of a conductivity detection mode, which is also a low cost alternative for future routine analysis.

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