



**THE EFFECT OF THE CONCENTRATION OF THE IONIC LIQUID
DIDECYLDIMETHYLAMMONIUM LACTATE
ON THE RETENTION AND SEPARATION OF PYRIMIDINE BASES**

Marcin SZYMAŃSKI^{1*}, Joanna ZAPART², Grażyna FLOREK²,
Arkadiusz SZYMAŃSKI²

¹Department of Pharmacognosy, Poznań University of Medical Sciences
Święcickiego 4, 60-781 Poznań, Poland

²Faculty of Chemistry, Adam Mickiewicz University
Umultowska 89b, 61-614 Poznań, Poland

ABSTRACT

Ionic liquids (IL) are new materials with a wide spectrum of use. Being a variable combination of an organic cation and an organic or inorganic anion, they have controllable properties. The effect of the ionic liquid added to the mobile phase on the separation of uracil derivatives is studied and demonstrated in this paper. The ionic liquid used was didecyldimethylammonium lactate, which has an advantageous effect on the separation of the analytes studied. Pyrimidine bases which were used as the test compounds are characterized by alkaline properties due to which so-called peak tailing is observed during separation. A methanol/water mixture was used as the mobile phase in the chromatographic analyses. Optimum separations were obtained for 8% levels of the organic modifier. The best separations of the test isomers were obtained for 0.01% IL added to the mobile phase. The findings obtained are evidence that the use of ionic liquids in HPLC is an efficient method for the separation of polar compounds: the duration of the analysis is shorter and the resulting peaks are sharp and symmetrical.

Keywords: ionic liquids, reversed-phase high performance liquid chromatography, additives

* Corresponding author: Marcin Szymański; e-mail: mszymanski@ump.edu.pl

INTRODUCTION

For over three decades now, ionic liquids have been raising remarkable interest in a number of research centers. This is caused by their unique physical and chemical properties, such as: high thermal stability, high viscosity, low volatility, and non-flammability. Ionic liquids are able to dissolve both organic and inorganic compounds and are useful as solvents for syntheses. They are liquids at room temperatures (up to 100°C). Potential combinations are numerous, making it possible to use a suitably selected, pre-planned combination of an organic cation and an organic or inorganic anion. This enables ionic liquids to be “designed” to suit specific needs. Their low volatility helps prevent vapour penetration into the natural environment. This is one reason why ionic liquids are regarded as environmentally friendly materials. Their properties comply with “green chemistry” rules, promoting a new approach to the use of chemical compounds.

Owing to their unique properties, ionic liquids have numerous applications in analytical chemistry, as evidenced by surveys [1-3] and other reports. They are useful in chromatography, as shown in papers [4-8]. There are a large number of literature reports on the use of ionic liquids as stationary phases in gas chromatography [5, 6], liquid chromatography [7, 8] and electrophoresis [9, 10].

More and more often is High-Performance Liquid Chromatography (HPLC) used in investigating analyzing such compounds [11, 12]. Ionic liquids are also useful as an additive to modify the mobile phase [13, 14].

The use of ionic liquids in the mobile phase has a favorable effect on chromatographic separation. When used in a reversed phase system, they dynamically modify the silica stationary phase, thereby blocking any unreacted silanols which may be present on its surface [13, 15]. They have a specific, favorable effect on the retention of alkaline compounds, reducing the time of analysis and considerably improving peak symmetry [16, 17].

This is confirmed by studies, carried out for a number of uracil derivatives. Uracils, or pyrimidine bases, are heterocyclic compounds derived from pyrimidine, having alkaline properties. They are part of nucleic acids, nucleotides and nucleosides: 5-methyluracil (thymine) is a component of DNA while RNA contains uracil in place of thymine.

Uracil derivatives, such as uracil, 6-methyluracil, 5-methyluracil (thymine), 5-ethyluracil, 5-propyluracil and 5-butyluracil, were used in the chromatographic study. This work was intended to investigate the effect of addition of the ionic liquid didecyltrimethylammonium lactate to the mobile phase on the retention of the uracil isomers.

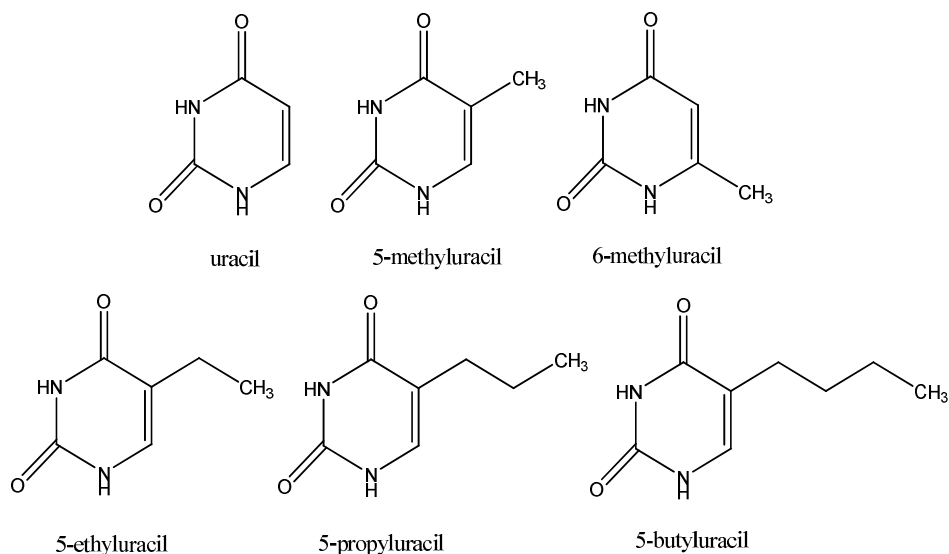


Fig. 1. The molecular structures of the compounds examined

EXPERIMENTAL

Reagents

Methanol was HPLC grade, supplied by Merck (Germany). Standards of uracil, 6-methyluracil, 5-methyluracil (thymine), 5-ethyluracil, 5-propyluracil (all purchased from Sigma Aldrich (USA)) and 5-butyluracil (own synthesis) were used for the preparation of an individual stock solution in methanol at the level of 1 mg/mL. Working solutions were obtained by the successive dilution of the standard solution using the mobile phase used in the chromatographic analysis. Figure 1 shows the molecular structures of the target analytes.

Apparatus

HPLC. A Hewlett Packard liquid chromatograph, Model 1050 (Waldbron, Germany) equipped with a quaternary pump, a variable-wavelength UV detector operated at 190-600 nm, and an injector, Model 7125 (Rheodyne) of a 20 μ L sample loop was used, together with a LiChrospher 100 RP-18 column (Merck, Germany) (250 mm x 4 mm i.d., 5 μ m particle size).

Mobile phase. A methanol/water mixture (8/92% vol.) was used as mobile phases. The ionic liquid, didecyldimethylammonium lactate, was synthesized by the team of Prof. Juliusz Pernak from the Faculty of Chemical Technology, Poznań University of Technology, Poland. The mobile phase flow rate was 1.0 mL/min.

Water. Ultrapure water was prepared using a Milli-Q filter system from Millipore (USA).

UV. UV spectra were obtained for the test compounds using the HP 8453 spectrophotometer from Hewlett Packard. The chromatographic analyses of the uracyls were performed at 280 nm.

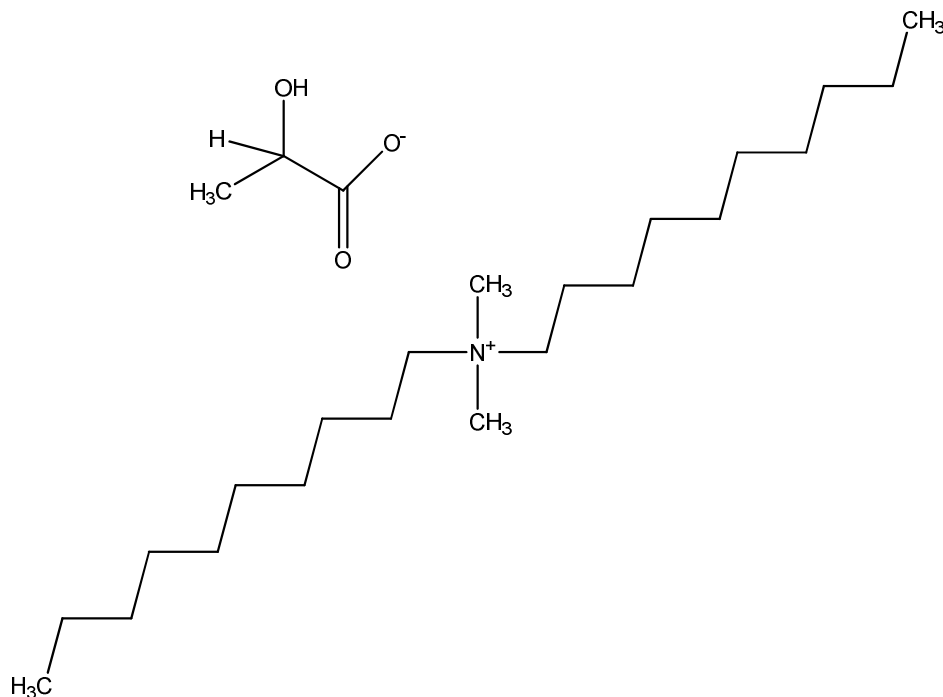


Fig. 2. Didecyltrimethylammonium lactate

RESULTS AND DISCUSSION

The effect of addition of didecyltrimethylammonium lactate to the mobile phase on the retention of the uracil derivatives

The results of a study on the chromatographic separation of the uracil derivatives are presented in this paper. The mobile phase containing a mixture of an organic solvent and water with an addition of ionic liquid was used. After optimizing the amount of the organic modifier, the dependence of the retention index on changes in the ionic liquid concentration in the mobile phase was investigated. Chromatographic separations were performed using methanol as the organic modifier for a constant concentration of 8% and for different didecyltrimethylammonium lactate concentrations in water. Lower organic phase concentrations tended to extend retention times excessively, extending the duration of analysis and affecting the separations of the test compounds. Higher concentrations of methanol in the mobile phase made it impossible to separate compounds with lower retention times. The diagram below (Fig. 3) shows curves

which represent changes in the retention index for uracil derivatives vs. changes in the concentration of the ionic liquid added to the mobile phase.

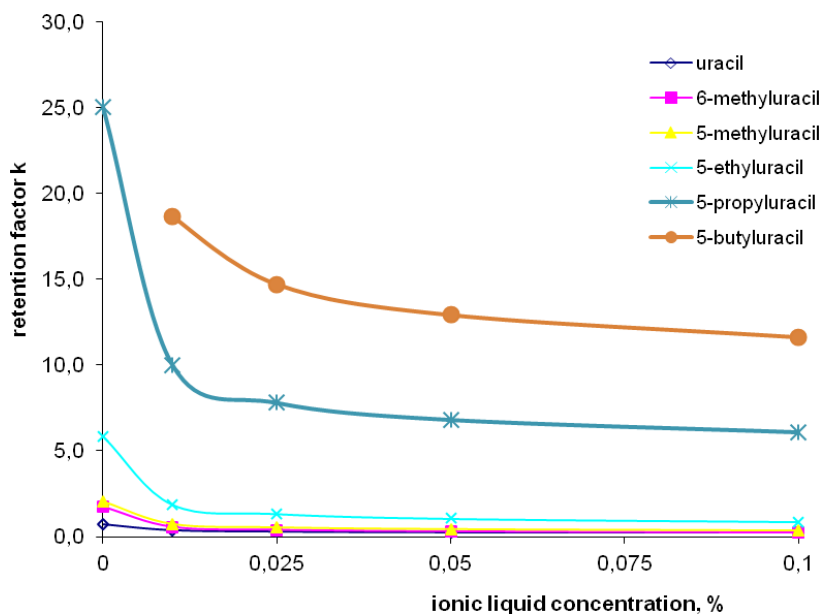
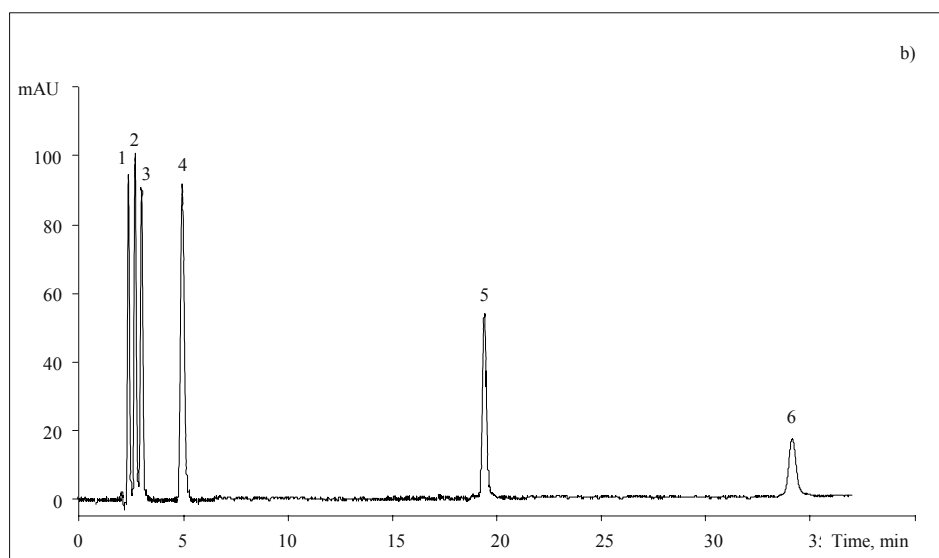
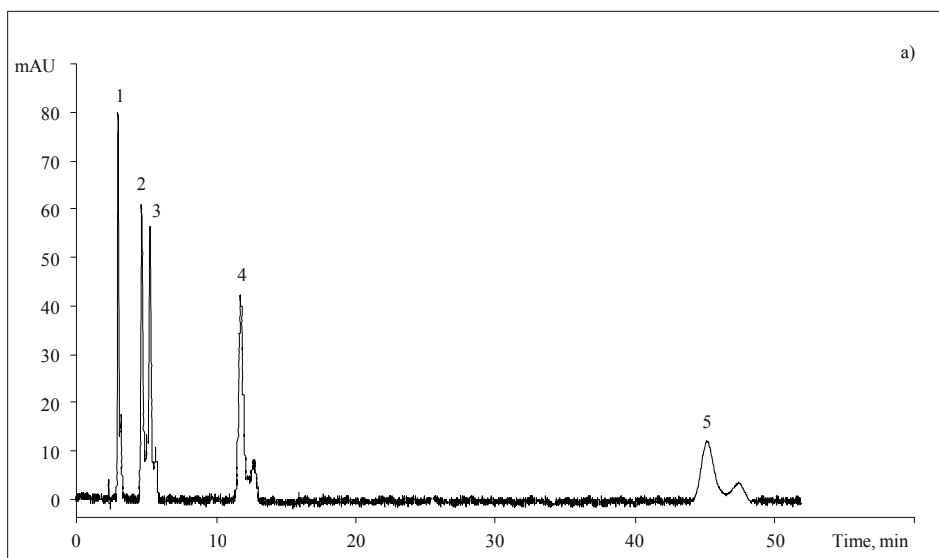
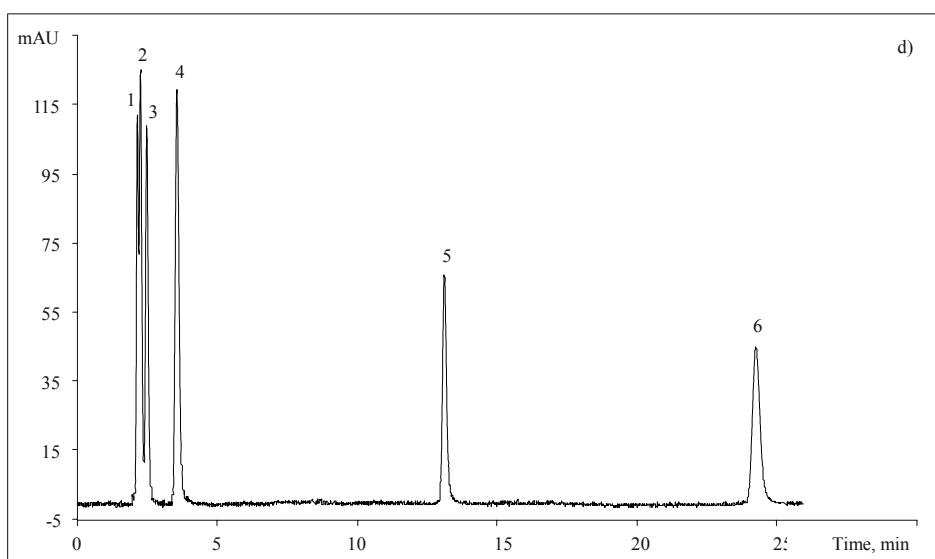
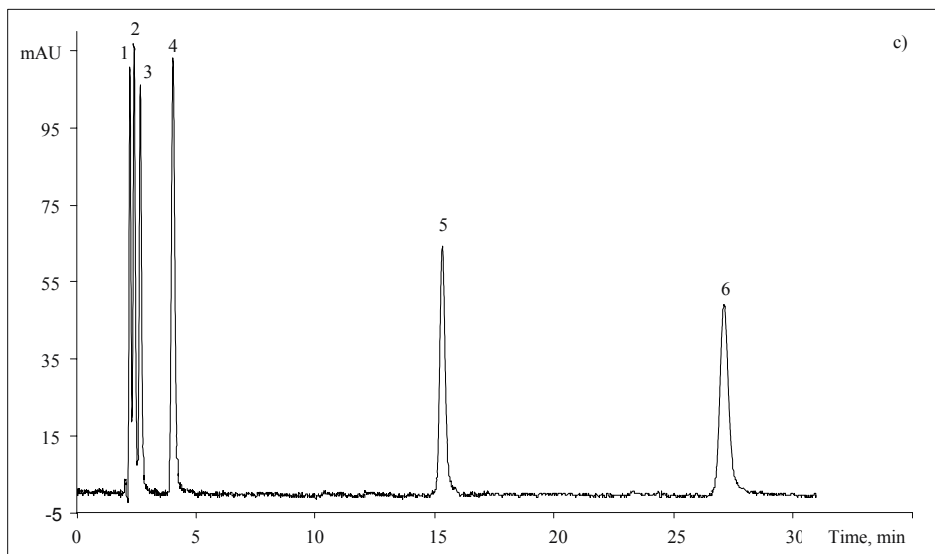


Fig. 3. The effect of didecyldimethylammonium lactate added to the mobile phase on the value of the retention index

Chromatographic separations

Figures 4a-e show the chromatograms of a mixture of the uracil derivatives. The mobile phase used in the separations comprised methanol at a concentration of 8% and various concentrations of didecyldimethylammonium lactate. As seen in the chromatograms, an increase in the ionic liquid concentration leads to shorter times of analysis and to incomplete separations of the compounds because the peaks partly cover one another. Also, in the absence of the ionic liquid in the mobile phase, the analytes have longer retention times, causing peak tailing and the presence of some extra peaks. The optimum result (a complete separation of six compounds), shown in the chromatogram in Fig. 4b, was obtained for a mobile phase comprising 0.01% of the ionic liquid. Chromatographic separation was not improved any further when the concentrations of the ionic liquid in the mobile phase were still lower.





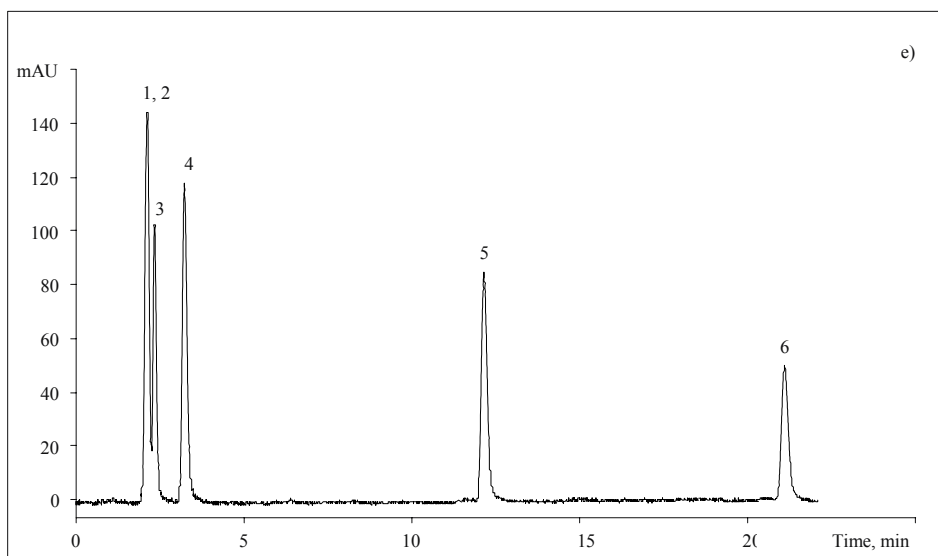


Fig. 4. Chromatograms for a mixture of 6 uracil derivatives

Separation conditions: mobile phase: methanol/water = 8/92% containing different ionic liquid concentrations:

a) 0%; b) 0.01%; c) 0.025%; d) 0.05% and e) 0.1%; flow rate 1 ml/min.

Test compounds: 1) uracil; 2) 6-methyluracil; 3) 5-methyluracil; 4) 5-ethyluracil; 5) 5-propyluracil; 6) 5-butyluracil

Examination of the effect of ionic liquid added to the mobile phase on the chromatographic separation of the uracil derivatives

The results obtained indicate that ionic liquids added to the mobile phase have a significant effect on the separation of the uracil derivatives. Increased concentrations in the eluent of the ionic liquid added led to lower retention values. A chromatographic column with RP-18 octadecyl packing having hydrophobic hydrocarbon chains was used in chromatographic separations. When this type of packing is used in analyzing alkaline compounds, additional peaks are often present or the so-called tailing is observed. Silica-based stationary phases have strong adsorption properties because there are free hydroxyl groups on their surfaces; such hydroxyl groups may be blocked, for instance, by adding an ionic liquid to the mobile phase. Therefore, didecyldimethylammonium lactate was used as an additive to the mobile phase. The didecyldimethylammonium cation probably interacts with silanol groups on the silica surface by means of electrostatic interactions. This blocks hydroxyl groups and prevents the occurrence of peak tailing or the formation of any extra peaks which are an effect of interactions between the analyte and any unreacted silanols. Furthermore, the ionic liquid used has long alkyl substitutes which may potentially be used by the retention mechanism which is typical of a reversed phase system, determined by dispersion interactions. Moreover, hydrophobic interactions may occur between

alkyl groups which are present in the cation and the alkyl ligand on the stationary phase. When the ionic liquid was not present in the mobile phase, the separated compounds were characterized by longer retention times and the extra peaks were present. Optimum results of the chromatographic separation were obtained for a didecyldimethylammonium lactate added in the amount of 0.01% to the mobile phase. This is confirmed by the chromatogram in Fig. 4b, showing well separated, symmetrical, sharp peaks. Increased concentrations of the ionic liquid added to the mobile phase lead gradually to lower analyte retentions though separations are not complete in effect.

CONCLUSIONS

Summing up, the results of this study indicate that the ionic liquid added to the mobile phase has a favorable effect on the separation of the uracil derivatives. Even as low a concentration of didecyldimethylammonium lactate as 0.01% in the eluent eliminates the effect of adsorptive interactions with silanols, which greatly improves chromatographic separation.

Acknowledgements

This work was carried out as part of the Innovative Economy Operational Program, years 2007 – 2013, Action 1.4, Project No. UDA-POIG.01.04.00-30-012/10-00.

REFERENCES

- [1] Koel M., 2009. Ionic liquids in chemical analysis, In: *Analytical Chemistry Series*, CRC Press Taylor & Francis Group.
- [2] Sun P., Armstrong D.W., 2010. Ionic liquids in analytical chemistry. *Anal. Chim. Acta* 661, 1, 1-16.
- [3] Soukup-Hein R.J., Warnke M.M., Armstrong D.W., 2009. Ionic Liquids in Analytical Chemistry. *Ann. Rev. Anal. Chem.* 2, 145-168.
- [4] WangYe., Tian M., Bi W., Row K.H., 2009. Application of Ionic Liquids in High Performance Reversed-Phase Chromatography. *Int. J. Mol. Sci.* 10, 2591-2610.
- [5] Baltazar Q., Leininger S.K., Anderson J.L., 2008. Binary ionic liquid mixtures as gas chromatography stationary phases for improving the separation selectivity of alcohols and aromatic compounds. *J.Chromatogr. A* 1182, 119-127.
- [6] Poole C. F., Poole S.K., 2011. Ionic liquid stationary phases for gas chromatography. *J.Sep. Sci.* 34(8), 888-900.
- [7] Liu S.J., Zhou F., Xiao X.H., Zhao L., Liu X., Jiang, S.X., 2004. Surface confined ionic liquid—A new stationary phase for the separation of ephedrine in high performance liquid chromatography. *Chin. Chem. Lett.* 15, 1060-1062.
- [8] Wang Q., Baker G.A., Baker S.N., Colon L.A., 2006. Surface confined ionic liquid as a stationary phase for HPLC. *Analyst* 131, 1000-1005.
- [9] Mofaddel N., Krajian H., Villemin D., Desbène P.L., 2009. New ionic liquid for inorganic cations analysis by capillary electrophoresis: 2-hydroxy-N,N,N-trimethyl-1-phenylethanaminium bis(trifluoromethylsulfonyl)imide (phenylcholine NTf₂). *Anal. and Bioanal. Chem.* 393(5), 1545-1554.

- [10] Li J., Han H., Wang Q., Liu X., Jiang S., 2011. Polymeric ionic liquid-coated capillary for capillary electrophoresis. *J. Sep. Sci.* 34, 1555-1560.
- [11] Buszewski B., Studzińska S., 2008. A Review of Ionic Liquids in Chromatographic and Electromigration Techniques. *Chromatographia* 68(1-2), 1-10.
- [12] Studzińska S., Buszewski B., 2010. Study of retention mechanism of imidazolium-based ionic liquids in HPLC. *J. Sep. Sci.* 33, 225-238.
- [13] Polyakova Y., Jin Y., Zheng J., Row K.H., 2006. Effect of concentration of ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate for retention and separation of some amino and nucleic acids. *J. Liq. Chromatogr.* 29, 1687-1701.
- [14] Han D., Wang Y., Jin Y., Row K.H., 2011. Analysis of some B-lactam antibiotics using ionic liquids as mobile phase additives by RP-HPLC. *J. Chromatogr. Sci.* 49, 63-66.
- [15] Marszał M.P., Bączek T., Kaliszan R., 2006. Evaluation of the silanol-suppressing potency of ionic liquids. *J. Sep. Sci.* 29, 1138-1145.
- [16] He L.J., Zhang W.Z., Gu Y.L., Liu X., Jiang S.X., 2003. Effect of ionic liquids as mobile phase additives on retention of catecholamines in Reversed-Phase High-Performance Liquid Chromatography. *Anal. Lett.* 36, 827-838.
- [17] Xiao X., Zhao L., Liu X., Jiang S.X., 2004. Ionic liquids as additives in high performance liquid chromatography: Analysis of amines and the interaction mechanism of ionic liquids, *Anal. Chim. Acta* 519, 207-211.