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Influence of the Anionic Part of 1-Alkyl-3-Methylimidazolium-Based Ionic Liquids on the Chromatographic Behavior of Perazine in RP-HPTLC

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1-Alkyl-3-methylimidazolium-based ionic liquids (tetrafluoroborate, L-(+)-lactate and ethyl sulfate) were used as mobile phase additives to assess the effect of its anionic part on the retention mechanism of perazine in pharmaceutical formulation in reversed-phase high-performance thin-layer chromatography (RP-HPTLC) method. In all cases, significant changes and improvements in the retention factor were observed when ionic liquids were added to the mobile phase. We found that the chromatographic behavior of the basic analyte depends on the utilized ionic liquid as well as its various anions. Enhancement of separation confirms silanol suppressing potency of employed ionic liquids and their positive impact on chromatographic separation of basic drugs. Among selected ionic liquids, the optimum distribution parameters such as shape and quality of spots, high precision, and accuracy in qualitative and quantitative determination characterize the system with [EMIm][BF_4] as mobile phase modifier. Our proposed HPTLC method for determination of perazine in oral tablets was also subjected to subsequent validation procedure in accordance with ICH guidelines and proved to be suitable, inexpensive, and convenient method in a pharmaceutical analysis.

Keywords: basic drugs, ionic liquids (ILs), mobile phase modifiers, perazine, pharmaceutical analysis, reversed-phase high-performance thin-layer chromatography (RP-HPTLC)

Introduction

Reversed-phase liquid chromatography (RP-LC) of basic drugs may cause numerous difficulties in the chemical and pharmaceutical analysis due to the strong interactions between these compounds and silica-based stationary phases. Poor peak shape or peak tailing, band broadening, and change in retention time or retardation factor are typical examples of problems which may occur in liquid chromatography separation.[1–3]

Improvement of the quality of separation and enhancing its efficiency can be achieved using various types of mobile phase modifiers, such as amines. The most popular are ammonia and short chain organic amines such as diethylamine (DEA), triethylamine (TEA), or longer chain compounds as N,N-dimethyloctylamine (DMOA).[3–6] These additives were successfully used as tailing suppressors and chromatographic separation with their use resulted in shortened retention time and improvements in peak shapes of peptides and basic compounds like alkaloids, β-blockers, antipsychotic, and antidepressant drugs.[5–10] However, much more attention is recently directed toward ionic liquids (ILs) rather than ammonium compounds in the separation and analysis process.[1–3,11–13] In a comparative study reported by Ruiz-Angel et al.,[11] ILs have proven to be even better additive compared to TEA when assessing peak efficiency and its shape. Similarly, Kaliszan et al.[3] demonstrated the advantages of the use of imidazolium ILs in thin-layer system over the conventional chromatographic modifiers like NH_4OH, TEA, and DMOA. As shown in the aforementioned study, 1-alkyl-3-methylimidazolium-based ILs, particularly the imidazolium ring, significantly increased the mobility of basic analytes by their ability to compete with them and suppress free silanols on octadecylsilica TLC-plates and so remove the specific interactions between the negatively charged silanol groups and basic drugs.

ILs is a term generally related to the compounds made solely of ions: large asymmetric organic cations and inorganic or organic counterions, whose melting temperature are below 100° C or sometimes even below room temperature (RTILs).[1,2,12] ILs have negligible vapor pressure, therefore they do not release noxious substances into the environment and are classified as "green chemistry" materials.[12] The diversity of chemical structure of these compounds and the possibility of a combination of the cation–anion is truly enormous and can lead to many unique properties, which will reflect on their subsequent use in almost every field of modern chemistry: including organic synthesis, electrochemistry, catalysis, biocatalysis, chromatography, and others.[2,12]

Chromatographic determination of lipophilicity parameters might be of special importance for search for new drugs. The parameters obtained by use of liquid chromatography techniques can predict their potential application because correlate with drug bioactivity.[13] In practice, the most reliable chromatographic parameters for assessment lipophilicity of drugs are

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determined with the use of RP-LC.[14,15] Lipophilicity data from classical TLC are rather limited reproducibility.[16] The use of modern small-diameter particles stationary phase resulted in development of HPTLC. But correlation between retention parameters from HPTLC and reference lipophilicity parameters are often poor. Recently, imidazolium-based ILs additives appeared interesting from the point of view of determination of lipophilicity of ionized forms of basic drugs.[3,17]

In this study we have used commercially available ILs: 1-ethyl-3-methylimidazolium tetrafluoroborate, 1-ethyl-3-methylimidazolium L-(+)-lactate, and 1-ethyl-3-methylimidazolium ethyl sulfate in a chromatographic experiment to assess the influence of different ILs mobile phase additives on the final results of chromatographic separation of biologically active compounds.

Perazine chemically defined as 10-[3-(4-methylpiperazin-1-yl)propyl]phenothiazine (Figure 1) served as the test compound. It is a widely used basic phenothiazine derivative, known for its antipsychotic potential to treat moderate or severe mental disorders. Perazine mechanism of action is associated mainly with its moderate antagonistic affinity with D_2-dopaminergic receptors, but also weak antagonistic activity with respect to D_1-dopaminergic, α_1-adrenergic, 5-HT_2-serotonergic, and also M_1-muscarinic receptors.[18,19] Various methods have been previously employed for the determination of perazine and other neuroleptic drugs in pharmaceutical formulation or biological samples. These methods include: chromatography,[20–22] capillary electrophoresis,[21] spectrofluorimetry,[23] and spectrophotometry.[24,25] The reported results mostly described methods of monocomponent formulation assay. Comparing with Polish Pharmacopoeia VI TLC method[22] for perazine determination our system is eco-friendly – we replaced organic phase into more aqueous – butan-1-ol/NH_4OH 17 g/l (5:1) vs. ACN/H_2O (6:4). Moreover, as mentioned above, ILs utilized in the study possess good physicochemical properties as they have no vapor pressure and are concerned as nonvolatile compounds and so are classified as "green" solvents. Though HPLC methods are very precise, accurate[20,21] – quantification limit level at ng/ml – they are time, solvents, and work-consuming. Here, we decided to develop rapid with minimal sample clean-up but still satisfactory HPTLC method which can be useful for simultaneously quantification of neuroleptics (like perazine). This is the first reported method with the use of IL as a modifier of mobile phase for perazine quantification by HPTLC method. For this purpose, densitometry scanning was used – non-destructive method with high repeatability – where it is easily to recreate it at any time, any place as silica plates can be stored for a long time. Our HPTLC analysis found to be easily accessible and can be considered as an alternative to spectroscopic methods of determination,[23–25] however when connected with MS-interface it could bring even more accurate analysis results. Hence, the proposed HPTLC method with the IL-based mobile phase might be of special importance for the simultaneous studies of mixtures of neuroleptics.

Here, we have developed and validated in accordance with ICH guidelines[26] method for determination of perazine in a commercially available pharmaceutical formulation using 1-alkyl-3-methylimidazolim-based ILs as mobile phase modifiers in reversed-phased high-performance thin-layer system. To our knowledge, this is the first planar liquid chromatography method published for any perazine drug formulation with the use of IL in the mobile phase system. However, the main aim of the study is to evaluate the effect of different 1-alkyl-3-methylimidazolium-based ILs counterions (tetrafluoroborate, L-(+)-lactate and ethyl sulfate) in the separation process. Because it should be noted that, due to their dual nature not only cationic but also anionic part of IL can affect the chromatographic results in a specific manner by suppressing interactions between the analyte and silanol groups of the stationary phase. Use of the two-retention site model proposed by Nahum and Horvàth enabled a reliable determination of the silanol-suppressing potency of ILs as mobile phase modifiers in HPLC and confirmed the influence of both the anion and the cation of IL on the retention as well as peak shape of basic drugs.[1,11,27–31] Also, other studies showed that the

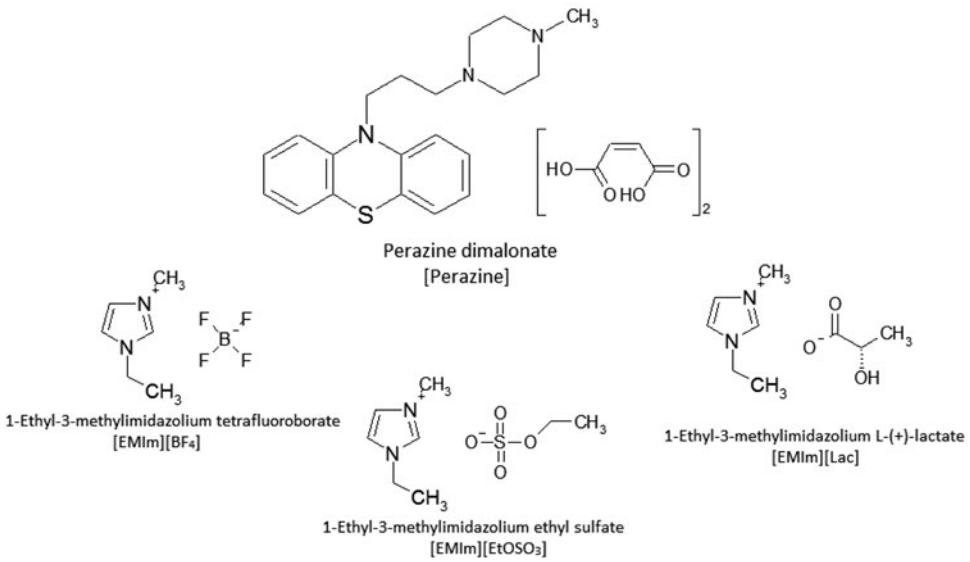

Fig. 1. The chemical structures and abbreviations of perazine and the imidazolium ionic liquids.

alkyl-imidazolium tetrafluoroborate class ILs with short alkyl-chain lengths are particularly suitable as modifiers in HPTLC.[32]

Experimental

Chemicals and Reagents

Perazine reference standard as perazine dimalonate was obtained from LGC Germany GmbH (Luckenwalde, Germany). 1-Ethyl-3-methylimidazolium tetrafluoroborate ([EMIm][BF_4]) and 1-ethyl-3-methylimidazolium L-(+)-lactate ([EMIm][Lac]) were from Sigma-Aldrich (St. Louis, MO, USA). 1-Ethyl-3-methylimidazolium ethyl sulfate ([EMIm][$EtOSO_3$]) was supplied by Solvent Innovation GmbH (Köln, Germany). Methanol and acetonitrile (HPLC grade) were from Sigma-Aldrich (St. Louis, MO, USA), whereas deionized water as a solvent was prepared by Millipore Milli-Q Integral Water Purification System.

Pharmaceutical formulation: *Perazin 0.1* tablets (labeled to contain 100 mg of perazine equivalent to 168.4 mg of perazine dimalonate) was purchased from the local pharmacy, brand name Hasco-Lek (Wrocław, Poland).

Structures of perazine and the ILs used in this study are shown in Figure 1.

Standards Preparations

Stock solution of perazine dimalonate containing 1.0 mg mL^{-1} was prepared by dissolving 15 mg of drug standard in 15 mL freshly prepared deionized water followed by 15 min of bath sonication until complete dissolution of the drug. The solution was further diluted with the same solvent to obtain standard solutions with different concentration in range of 0.20–0.55 mg mL^{-1}. These dilutions were stored at 4°C. Each solution was applied in triplicate in the volume of 4 µL on a plate yielding adequate amount of perazine per spot and an eight-point calibration curve was plotted in a range of 0.80–2.20 µg (Figure 6).

Equipment and Chromatographic Conditions

Reversed-phase high-performance thin-layer chromatography (RP-HPTLC) was performed on chromatographic plates precoated with octadecylsilica gel (60 RP-18 F_{254}, 20 × 10 cm) manufactured by Merck (Darmstadt, Germany) using densitometry scanning with a reflectance mode and extinction as an evaluation parameter by Desaga HPTLC CD 60 densitometer (Wiesloch, Germany) coupled with a Desaga ProQuant software (Wiesloch, Germany).

Perazine solutions were spotted on a RP-18 plates in the form of bands of 5 mm width with a HPTLC applicator AS 30 by Desaga (Wiesloch, Germany) and a 25 µL syringe from Innovative Labor Systeme GmBH (Stützerbach, Germany). Samples were applied at the constant rate 20 µL s^{-1} and 10 mm gap between them was kept. Plates were developed in glass chambers, previously saturated (for 25 min) with vapor of three various mobile phases containing acetonitrile and water but differing deployed IL modifier from a group of 1-ethyl-3-methylimidazolium-based ILs, and more precisely varying his anionic part (tetrafluoroborate, L-(+)-lactate or ethyl sulfate). The mean distance of a chromatographic run was 8 cm and its separation process was carried out at room temperature (20°C ± 1°C). The developed octadecylsilica gel plates with spots were dried with a help of air dryer for 5 min and followed by densitometric analysis using deuterium lamp as a light radiation source, with slit height and width set at 1.0 and 4.0, respectively. The detection process was performed at $\lambda_{max} = 247$ nm.

Linear regression was determined based on the data of peak areas and concentration of the corresponding standard per spot. The measurements of eight-point calibration curve were repeated three times (Table 1).

The analysis of chromatograms and developed RP-plates were performed using Desaga CabUV-VIS (Wiesloch, Germany). The plates were visualized by a digital camera Canon Power Shot G5 combined with Desaga ProViDoc 3.0 software (Wiesloch, Germany).

Tailing factor (T_f) was calculated according to the equation $T_f = (a + b)/2a$, where a and b are the front and back half-widths at the 5% of the peak height.

Preparation of Sample Solutions

Twenty tablets of *Perazin 0.1* were weighed and grounded in a mortar and an accurately weighed amount of powder

Table 1. Comparison of quantitative determination of perazine dimalonate in tablets with different mobile phase modifiers investigated by RP-HPTLC with densitometry scanning at 247 nm

	[EMIm][BF_4]	[EMIm][$EtOSO_3$]	[EMIm][Lac]
Mobile phase pH[a]	4.31	7.47	9.16
Range (µg $spot^{-1}$)[b]	0.80–2.20	0.80–2.20	0.80–2.20
Regression equation[a]	y = 1217.6x −265.83	y = 1502.1x −525.91	y = 1435.6x −739.65
R[c]	0.9979	0.9981	0.9747
Mean declared (ng)[d]	1.50	1.50	1.50
Mean found (ng)[e]	1.51	1.48	1.43
Recovery (%)	100.66	98.78	95.43
RSD (%)	3.66	4.13	6.97

[a]Average, n = 3.[b]Each concentration was obtained minimum from 9 assay (3 determinations/3 independent plates).
[c]Pearson's correlation coefficient.
[d]Label claimed from pharmaceutical formulation.
[e]Amount found from tablets extract.

corresponding to 37.5 mg of perazine dimalonate were extracted with 80 mL of water in a 100 mL flask. The powder was shaken for 20 min, diluted with water to the final volume, then filtrated using 0.45 µm sterile filters to remove any insoluble particles. The resulting solution of perazine dimalonate (0.375 mg mL^{-1}) was used for chromatographic analysis. Preceding extract was spotted on a silica-based plate (4 µL equivalent to 1.5 µg per spot) and analyzed by RP-HPTLC method.

Results

Study of Chromatographic Conditions

Preliminary studies were aimed at determining the optimum separation conditions for analysis of perazine dimalonate using RP-HPTLC system. As shown and mentioned in earlier papers,[1,3] basic compounds such as perazine may cause chromatographic separation difficulties using RP-LC due to their strong affinity between their cationic and the anionic part of silanols. Some peak tailing, unsatisfactory elution, or distortion of spots size and image may occur during separation process. One of the strategies, aimed at reducing these interactions (electrostatic and/or hydrophobic), is the use of ILs.[1,3,12,33] In this study, among different ILs [EMIm][BF_4], a representative of imidazolium-based ILs has been selected as a mobile phase modifier..

As an initial study, the separation of perazine was examined with previously fixed mobile phase −1.5% (v/v) [EMIm][BF_4] with different ratios of acetonitrile (or methanol) and water in the mobile phase. Graphical relationships between the retardation factor (R_F) of perazine and various mobile phase volume compositions were plotted in Figure 2. Elution of tested substance without acetonitrile or methanol was insufficient due to the strong specific interaction between basic perazine and the stationary phase. The evident effect is observed at the organic solvent concentration of 40% (v/v) and higher. However, elution is much weaker when the methanol is added to the eluent. Because the shape of the spot is mostly symmetrical for 1.5% (v/v) [EMIm][BF_4] in acetonitrile:water (60:40, v/v), this mobile phase was used for further study.

Similar correlation was presented in Figure 3 with respect to the R_F values and the percentage additive of the selected IL in

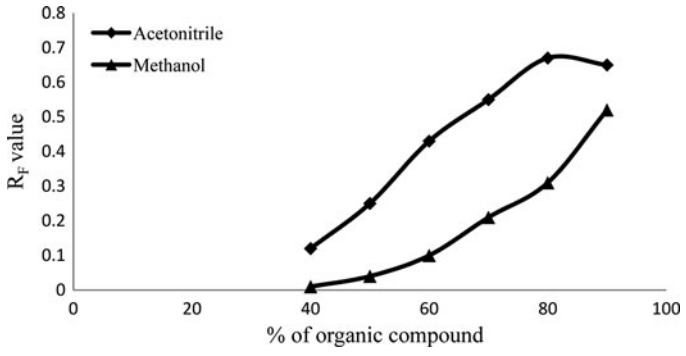

Fig. 2. Graphical representation of the relationship between the retention factor (R_F) of perazine and the volume of organic compound in the mobile phase with the use of 1.5% (v/v) [EMIm][BF_4].

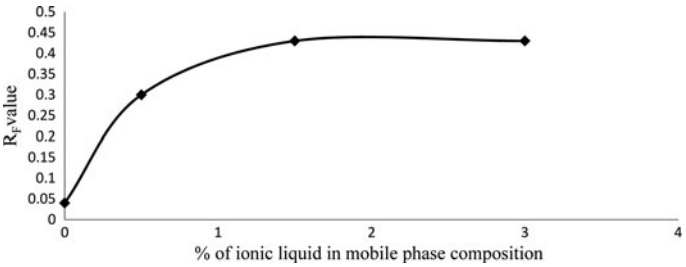

Fig. 3. Plot of retention factor (R_F) of perazine against the concentration of [EMIm][BF_4] in the mobile phase: acetonitrile:water (60:40, v/v).

the mobile phase. As is evident in the chart, elution of analyzed compound did not change significantly over 1.5% (v/v) of [EMIm][BF_4]. In both cases, the sample spots were clear and well separated at concentration ≥1.5% (v/v) of IL, whereas when 0.5% (v/v) was used some tailing was observed. Finally, a simple mixture of acetonitrile:water (60:40, v/v) with the addition of 1.5% (v/v) of the 1-ethyl-3-methylimidazolium IL was chosen as it was characterized by the best separation results: round spot shape, optimal retention ($R_F = 0{,}43 \pm 0{,}02$), and quite brief elution (15 min for 8 cm). Chromatographic system containing methanol as a mobile phase component was unsatisfactory and was discarded for further study due to the poor elution effect as R_F was no greater than 0.2 in the range of 40–70 ratio volume of methanol.

Perazine Assay Results

In order to compare the effects of ILs with 1-ethyl-3-methylimidazolium cation but different counterions, an attempt was made to determine perazine dimalonate in pharmaceutical formulation using RP-HPTLC system and different IL additive: [EMIm][BF_4], [EMIm][Lac], and [EMIm][$EtOSO_3$]. The study was to investigate the effect of the anionic part of IL on the hydrophobic interactions and the ability to assay perazine in tablets.

Calibration curves were plotted based on the relationship between peak area and drug quantity per spot. The equations derived from the data are shown in a linear regression (Table 1). Based on the quantitative and qualitative results, we have concluded that the optimum phase for the determination of pharmaceutical formulation would be the system consisting of 1.5% of tetrafluoroborate as it gave a high Pearson's correlation coefficient of 0.9979 (for the linear indications) and 100.66% recovery for tested drug. Admittedly, the system using ethyl sulfate also gave good results (even better $R = 0.9981$), but with slightly higher values scatter and deviations as it gave lower RSD of the analysis 4.13% comparing to 3.66% for [EMIm][BF_4] – which indicates that the use of tetrafluoroborate determines more precise indications. Recovery from the mobile phase with [EMIm][$EtOSO_3$] as a modifier was also worse than from the [EMIm][BF_4]. The separation of analyzed sample were generally worse when L-(+)-lactate was used, where the discrepancy between the results (RSD = 6.97%) and the smallest recovery could be observed.

Table 2. Validation of quantitative analysis of perazine dimalonate by HPTLC densitometry with a use of [EMIm][BF_4] ionic liquid as a mobile phase modifier

Parameters	[EMIm][BF_4]
Specificity	Specific
Range (μg $spot^{-1}$)	0.80 – 2.20
Regression equation	y = 1217.6x −265.83
R	0.9979
Limit of detection [LOD] (μg $spot^{-1}$)	0.11
Limit of quantification [LOQ] (μg $spot^{-1}$)	0.34
Accuracy	
80%	103.04%
100%	99.04%
120%	99.75%

Validation of the HPTLC Assay

The validation procedure of qualitative and quantitative determination of perazine in pharmaceutical formulation for the chosen mobile phase composition containing tetrafluoroborate IL additive (Table 2) was conducted in accordance with the ICH guidelines and tested protocols.[26,34]

Specificity

Proposed RP-HPTLC method proved to be an adequate for a separation of perazine dimalonate as no excipients from the pharmaceutical formulation (*Perazin 0.1* tablets) and any other impurities were observed when comparing tracks and densitograms of developed plates (Figures 4 and 5); $\lambda = 247$ nm, R_F values (0.43 ± 0.02) of reference substance and sample were both equal.

Linearity

The relationship between peak area (AU) and drug quantities per spot found to be linear at a range of 0.80–2.20 μg (n = 8). The regression equation determined by the method of least squares was y = 1217.6x −265.83, with high correlation where R was 0.9979. The linear correlation was checked and evaluated by plotting residuals against drug quantity applied per spot. The residuals showed no trends and were randomly dispersed above and below the zero residual line, as it was shown in Figure 6.

Limit of Detection and Quantification

The limits of detection (LOD) and quantification (LOQ) are given in Table 2 and their values were determined based on the standard deviation of the response (σ) and the slope (S) of the achieved calibration curve for perazine. Detection and quantification limit were evaluated according to the equations: 3.3 σ/S and 10 σ/S and their values were LOD = 0.11 and LOQ = 0.34 [μg $spot^{-1}$].

Accuracy

Accuracy was appointed by means of recovery through the use of synthetic mixtures of the drug. The solutions were made by spiking a reference solution of perazine in a range of 80.0%, 100.0%, and 120.0% (concentrations, respectively, 1.2, 1.5, 1.8 μg mL^{-1}) with a lactose monohydrate, which is one of the excipients and matrix component used in the tablet. The analytical procedure for preparation of these solutions was performed as with the extracts and assessed within the specified range (3 concentrations/3 replicates each). The values of proposed method in sequence 103.04%, 99.04%, and 99.75% found to be satisfactory and indicated good accuracy of the proposed method.

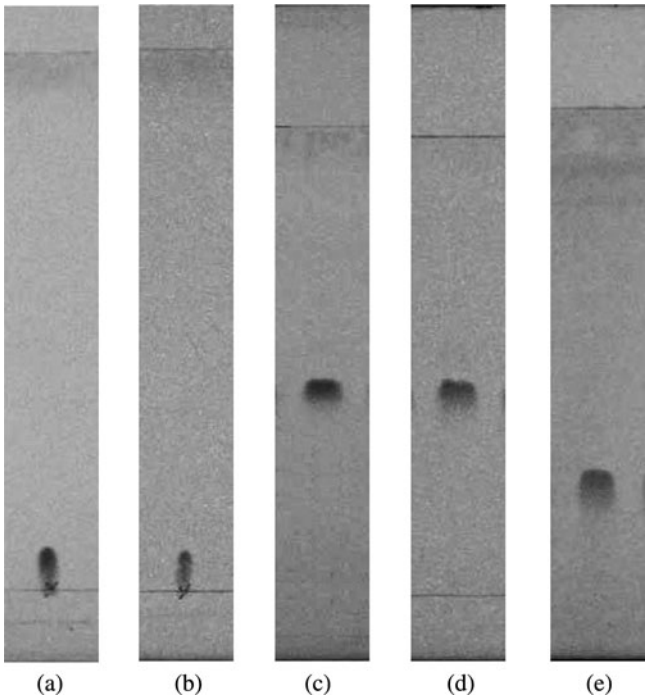

Fig. 4. Reference plates for perazine developed under various chromatographic conditions. Mobile phase: (a) acetonitrile:water (60:40, v/v), (b) acetonitrile:water (60:40, v/v) with decreased pH to 4.32, (c) acetonitrile:water (60:40, v/v) with 1.5% of [EMIm][BF_4], (d) acetonitrile:water (60:40, v/v) with 1.5% of [EMIm][$EtOSO_3$], and (e) acetonitrile:water (60:40, v/v) with 1.5% of [EMIm][Lac].

Precision

The values of relative standard deviation (% RSD) were established by analyzing three different standard drug solutions applied on a plate as spots containing, respectively, 1.0, 1.4, 1.8 μg three times within the same day and operating conditions (intra-day) and by repeating studies in the next two following days (inter-day). Data are shown in Table 3. The low results obtained (not greater than 3.46% at the lowest concentration) indicated well precision of carried out determination.

Robustness

Our proposed method has proven to be robust, as changes in temperature (20 ± 3°C), size of chromatographic chamber (20×20 cm or 10×15 cm) or the wavelength in the study did not change substantially the results obtained in the analysis.

Discussion

Commercially available pharmaceutical formulation was successfully determined with the proposed RP-HPTLC method. No interference has been observed from the tablet excipients. No additional spots were observed during analysis, whereas

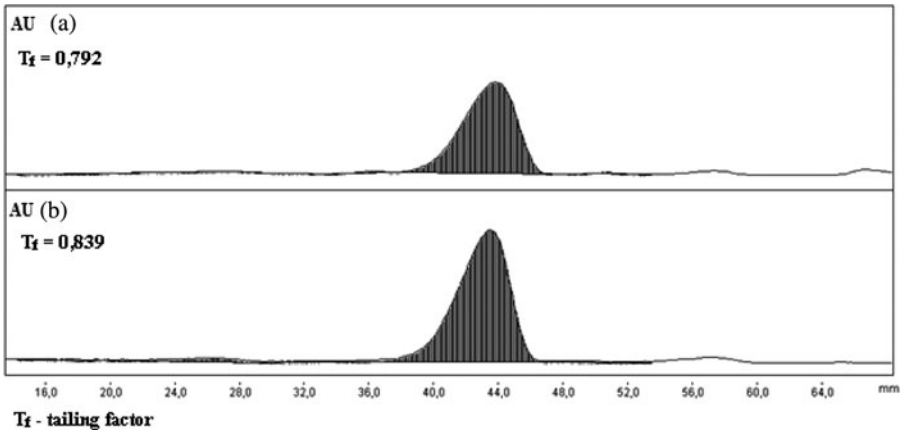

Fig. 5. Densitogram for perazine standard (a) and tablets (b) by RP-HPTLC, with a mobile phase of acetonitrile:water (60:40, v/v) and 1.5% addition of [EMIm][BF_4], showing no interference with the excipients in the analysis and the compatibility of R_F values of (a) and (b). The difference in peak height and area is associated with scanning of randomly selected standard spot.

Table 3. Precision of validated RP-HPTLC method

	Intra-day		Inter-day	
Concentration (μ g mL)	Mean[a] ±SD	RSD (%)	Mean[a] ±SD	RSD (%)
1.0	944.3 ± 32.65	3.46	730.57 ± 24.27	3.32
1.4	1356.29 ± 26.03	1.92	1102.90 ± 28.67	2.60
1.8	1731.45 ± 40.73	2.35	1454.12 ± 28.64	1.97

[a]n = 3 replicates each.

λ_{max} (247 nm) and R_F values (respectively, 0.43, 0.43, and 0.23 ± 0.02 for [EMIm][BF_4], [EMIm][$EtOSO_3$], and [EMIm][Lac]) were equal both for a reference substance and a test sample. Aforementioned representative RP-HPTLC plates are presented in Figure 4.

Comparison of retention and obtained quantitative data in the RP-HPTLC system confirmed the positive effect of the use of ILs in liquid chromatography and separation of basic compounds. However, the use of different ILs resulted in a slightly different peak symmetry and shape. Good symmetrical peaks were observed when [EMIm][BF_4] and [EMIm][$EtOSO_3$] were used, whereas utilization of [EMIm][Lac] caused peak distortion showing some tailing and asymmetry. It may be related to the occurrence of a background, which could interfere with silica gel as a carrier and thus lead to inaccurate determinations using reflectance densitometry scanning. This phenomenon occurred only with the use of [EMIm][Lac] as an additive.

In all cases, significant change in the pH values was observed before and after the addition of a mobile phase modifier (Table 1), hence the influence of the mobile phase pH on the retention behavior was also studied. In the first experiment, it was revealed that the mobile phase acetonitrile:water (60:40, v/v) without any additive (pH ca. 6.0) caused neither clear separation nor elution of studied compounds (Figure 4a). Next, after positive results in the separation process using [EMIm][BF_4] an experiment was conducted in which pH of the mobile phase was decreased from 6.0 to 4.3 (corresponding to [EMIm][BF_4], with the use of citric acid monohydrate). But even the

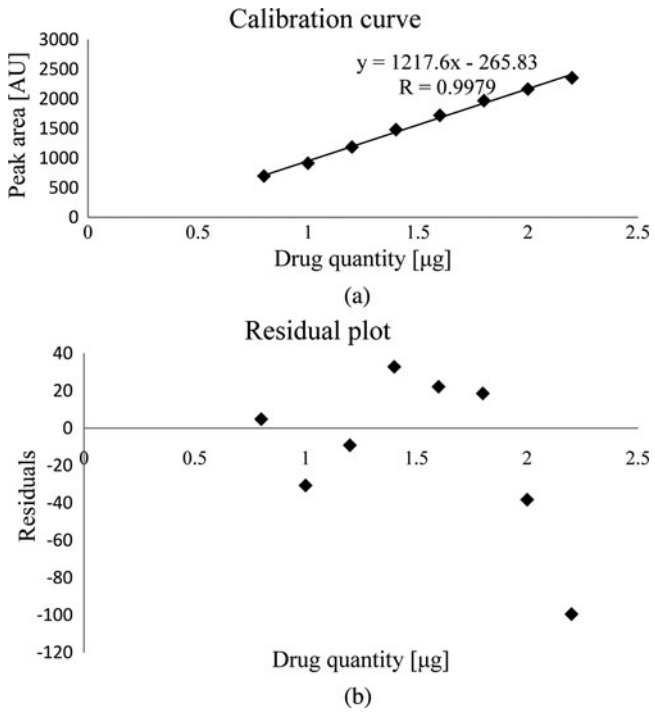

Fig. 6. Linear calibration curve (a) under optimized chromatographic condition and its residuals plot (b).

adjustment of experimental pH to the desired level did not significantly affect the retention of compounds (Figure 4b). Lack of elution clearly pointed out that the obtained results are attributable only to the remarkable properties of ILs and their suppressing potency of free silanol.[3]

Conclusions

The evidence from presented study documents that 1-ethyl-3-methylimidazolium-based ILs are valuable and efficient suppressors of free silanols which are responsible for unwanted interactions of chromatographic stationary phases in chromatography of basic compounds. Comparison study between three different ILs with same cation but different counterions as additives to mobile phase proved also the significant influence of anionic part into the mechanism of action during the chromatography of perazine with the use of RP stationary phase. Moreover, the chromatographic separation produced by 1-ethyl-3-methylimidazolium ILs are not due to the pH change caused by the additive/different anion. In view of the results obtained here, both imidazolium cations as well as anions of ILs are responsible for effect of changing retention of perazine in RP-HPTLC.

References

1. Fernandez-Navarro, J. J.; Garcia-Alvarez-Coque, M. C.; Ruiz-Angel, M. J. The Role of the Dual Nature of Ionic Liquids in the Reversed-phase Liquid Chromatographic Separation of Basic Drugs. *J. Chromatogr. A* **2011**, *1218*, 398–407.
2. Polyakova, Y.; Koo, Y. M.; Row, K. H. Application of Ionic Liquids as Mobile Phase Modifier in HPLC. *Biotechnol. Bioproc. Eng.* **2006**, *11*, 1–6.
3. Kaliszan, R.; Marszall, M. P.; Markuszewski, M. J.; Baczek, T.; Pernak, J. Suppression of Deleterious Effects of Free Silanols in Liquid Chromatography by Imidazolium Tetrafluoroborate Ionic Liquids. *J. Chromatogr. A* **2004**, *1030*, 263–271.
4. Vervoort, R. J. M.; Maris F. A.; Hindriks, H. Comparison of High-performance Liquid Chromatographic Methods for the Analysis of basic Drugs. *J. Chromatogr. A* **1992**, *623*, 207–220.
5. Hamoir, T.; Verlinden, Y.; Massart, D. L. Reversed-phase Liquid-chromatography of Beta-adrenergic Blocking-drugs in the Presence of a Tailing Suppressor. *J. Chromatogr. Sci.* **1994**, *32*, 14–20.
6. Basci, N. E.; Temizer, A.; Bozkurt, A.; Isimer, A. Optimization of Mobile Phase in the Separation of Beta-blockers by HPLC. *J. Pharmaceut. Biomed.* **1998**, *18*, 745–750.
7. Baczek, T.; Marszall, M. P.; Kaliszan, R.; Walijewski, L.; Makowiecka, W.; Sparzak, B.; Grzonka, Z.; Wisniewska, K.; Juszczyk, P. Behavior of Peptides and Computer-assisted Optimization of Peptides Separations in a Normal-phase Thin-layer Chromatography System with and Without the Addition of Ionic Liquid in the Eluent. *Biomed. Chromatogr.* **2005**, *19*, 1–8.
8. Waksmundzka-Hajnos, M.; Matosiuk, D.; Petruczynik, A.; Kijkowska-Murak, U. Determination of the Lipophilicity of Selected Isoquinoline Alkaloids by RP-TLC. *Acta Chromatogr.* **2008**, *20*, 563–573.
9. Petruczynik, A. Effect of Ionic Liquid Additives to Mobile Phase on Separation and System Efficiency for HPLC of Selected Alkaloids on Different Stationary Phases. *J. Chromatogr. Sci.* **2012**, *50*, 287–293.
10. Andersson, M.; Hultin, U. K.; Sokolowski, A. Effects of Amine Additives on the Resolution of Antipsychotic and Antidepressant Drugs on a Cyanoalkyl HPLC Column. *Chromatographia* **1998**, *48*, 770–776.
11. Ruiz-Angel, M. J.; Carda-Broch, S.; Berthod, A. Ionic Liquids Versus Triethylamine as Mobile Phase Additives in the Analysis of Beta-blockers. *J. Chromatogr. A* **2006**, *1119*, 202–208.
12. Buszewski, B.; Studzinska, S. A Review of Ionic Liquids in Chromatographic and Electromigration Techniques. *Chromatographia* **2008**, *68*, 1–10.
13. Nasal, A.; Kaliszan, R. Progress in the use of HPLC for Evaluation of Lipophilicity. *Curr. Comput. Aided Drug. Des.* **2006**, *2*, 327–340.
14. Giaginis, C.; Theocharis, S.; Tsantili-Kakoulidou, A. Contribution to the Standardization of the Chromatographic Conditions for the Lipophilicity Assessment of Neutral and Basic drugs. *Anal. Chim. Acta* **2006**, *573*, 311–318.
15. Giaginis, C.; Theocharis, S.; Tsantili-Kakoulidou, A. Octanol/Water Partitioning Simulation by Reversed-phase High Performance Liquid Chromatography for Structurally Diverse Acidic Drugs: Effect of n-Octanol as Mobile Phase Additive. *J. Chromatogr. A* **2007**, *1166*, 116–125.
16. Kaliszan, R. QSRR: Quantitative Structure-(Chromatographic) Retention Relationships. *Chem. Rev.* **2007**, *107*, 3212–3246.
17. Giaginis, C.; Tsantili-Kakoulidou, A. The Performance of 1-Ethyl-3-Methylimidazolium Tetrafluoroborate Ionic Liquid as Mobile Phase Additive in HPLC-based Lipophilicity Assessment. *Biomed. Chromatogr.* **2011**, *25*, 606–612.
18. Wojcikowski, J.; Pichard-Garcia, L.; Maurel, P.; Daniel, W. A. The Metabolism of the Piperazine-type Phenothiazine Neuroleptic Perazine by the Human Cytochrome P-450 Isoenzymes. *Eur Neuropsychopharm* **2004**, *14*, 199–208.
19. Leucht, S.; Helfer, B.; Hartung, B. Perazine for Schizophrenia. *Cochrane db Syst. Rev.* **2014**, *1*, CD002832.
20. Tanaka, E.; Nakamura, T.; Terada, M.; Shinozuka, T.; Hashimoto, C.; Kurihara, K.; Honda, K. Simple and Simultaneous Determination for 12 Phenothiazines in Human Serum by Reversed-phase High-performance Liquid Chromatography. *J. Chromatogr. B* **2007**, *854*, 116–120.
21. Madej, K.; Kala, M.; Wozniakiewicz, M. LC and Non-aqueous CE Determination of Phenothiazines in Autopsy Samples. *Chromatographia* **2005**, *62*, 533–538.
22. Polish Pharmaceutical Society. In Polish Pharmacopoeia VI, Warsaw, Poland, 560–561, 2002.
23. Mohamed, A. M. I.; Abdelmageed, O. H.; Salem, H.; Nagy, D. M.; Omar, M. A. Spectrofluorimetric Determination of Certain Biologically Active Phenothiazines in Commercial Dosage forms and Human Plasma. *Luminescence* **2013**, *28*, 345–354.
24. Stolarczyk, M.; Apola, A.; Krzek, J.; Sajdak, A. Validation of Derivative Spectrophotometry Method for Determination of Active Ingredients from Neuroleptics in Pharmaceutical Preparations. *Acta Pol. Pharm.* **2009**, *66*, 351–356.
25. Misiuk, W.; Halaburda, P. Flow Injection Spectrophotometric Determination of Perazine. *J. Trace Microprobe T* **2003**, *21*, 95–102.
26. The International Conference on Harmonisation - ICH. *Validation of Analytical Procedures: Text and Methodology Q2(R1)*; Geneva, Switzerland, 2005.
27. Nahum, A.; Horvath, C. Surface Silanols in Silica-bonded Hydrocarbonaceous Stationary Phases. 1. Dual Retention Mechanism in Reversed-phase Chromatography. *J. Chromatogr.* **1981**, *203*, 53–63.
28. Marszall, M. P.; Baczek, T.; Kaliszan, R. Evaluation of the Silanol-suppressing Potency of Ionic Liquids. *J. Sep. Sci.* **2006**, *29*, 1138–1145.
29. García-Alvarez-Coque, M. C.; Ruiz-Angel, M. J.; Berthod, A.; Carda-Broch, S. On the use of Ionic Liquids as Mobile Phase Additives in High-performance Liquid Chromatography. A Review *Anal. Chim. Acta* **2015**, *883*, 1–21.
30. Flieger, J.; Czajkowska-Zelazko, A. Ionic Liquids as Mobile Phase Additives in Reversed-phase High Performance Liquid Chromatography. *J. Liq. Chromatogr. Related Technol.* **2011**, *34*, 2224–2242.
31. Ubeda-Torres, M. T.; Ortiz-Bolsico, C.; Garcia-Alvarez-Coque, M. C.; Ruiz-Angel, M. J. Gaining Insight in the Behaviour of Imidazolium-based Ionic Liquids as Additives in Reversed-phase Liquid

Chromatography for the Analysis of Basic Compounds. *J. Chromatogr. A* **2015**, *1380*, 96–103.
32. Marszall, M. P.; Sroka, W. D.; Balinowska, A.; Mieszkowski, D.; Koba, M.; Kaliszan, R. Ionic Liquids as Mobile Phase Additives for Feasible Assay of Naphazoline in Pharmaceutical Formulation by HPTLC-UV-Densitometric Method. *J. Chromatogr. Sci.* **2013**, *51*, 560–565.
33. Fernandez-Navarro, J. J.; Torres-Lapasio, J. R.; Ruiz-Angel, M. J.; Garcia-Alvarez-Coque, M. C. Silanol Suppressing Potency of Alkylimidazolium Ionic Liquids on C18 Stationary Phases. *J. Chromatogr. A* **2012**, *1232*, 166–175.
34. Renger, B.; Vegh, Z.; Ferenczi-Fodor, K. Validation of Thin Layer and High Performance Thin Layer Chromatographic Methods. *J. Chromatogr. A* **2011**, *1218*, 2712–2721.