

Effect of 1-alkyl-3-methylimidazolium-based ionic liquids as the eluent on the separation of ephedrine by liquid chromatography

Lijun He, Wenzhu Zhang, Liang Zhao, Xia Liu, Shengxiang Jiang*

Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Tianshui Road 342, Lanzhou 730000, China

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Abstract

The chromatographic behavior of ephedrine (norephedrine, ephedrine, pseudoephedrine and methylephedrine) on a C_{18} column was studied with different concentrations of 1-butyl-3-methylimidazolium tetrafluoroborate ionic liquids as the eluent at pH 3.0. The addition of ionic liquid has great effects on the separation of these basic compounds: decreasing band tailing, reducing band broadening, and improving resolution. The retention times of the analytes increase at first and then decrease with the increase in the concentration of ionic liquid. This effect may be attributed to the competition between imidazolium cations and the polar groups of the analytes for the silanol group on the alkylsilica surface, and also to the formation of weak bilayer electronic structure on the C_{18} column. Several ionic liquids with different alkyl substituents on the imidazolium cations or with different counterions as the eluents were compared.

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Keywords: Ionic liquid; Mobile phase composition; 1-Alkyl-3-methylimidazolium; Ephedrine

1. Introduction

Ionic liquids have recently gained recognition as possible environmentally benign solvents because of their favorable properties [1,2]. Typically, ionic liquids having lower melting points than common ionic salts consist of nitrogen-containing organic cations (e.g. alkylammonium, 1-alkyl-3-methylimidazolium, *N*-alkylpyridinium etc.) and a variety of inorganic anions (e.g. BF_4^- , PF_6^- , Cl^- etc.) (Fig. 1). They are nonvolatile, nonflammable, and are good solvents for a wide range of both inorganic and organic compounds, and have a wide liquid

range with negligible vapour pressure. In the past few years, ionic liquids have been extensively used in different chemical fields such as synthesis [3,4], catalysis [5,6] and electrochemistry [7,8].

Previously, alkylammonium salts containing weak nucleophilic anions, liquid at room temperature, were frequently used as a stationary phase in gas chromatography [9,10] or mobile phases in liquid chromatography [11,12]. Tetra-*n*-butylphosphonium salts, having low-melting points, can be used as efficient

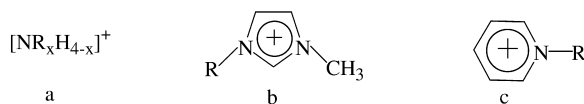


Fig. 1. Structure of alkylammonium (a), 1-alkyl-3-methylimidazolium (b), *N*-alkylpyridinium (c). $R=C_nH_{2n+1}$.

*Corresponding author. Tel.: +86-931-827-7652; fax: +86-931-827-7088.

E-mail address: sxjiang@ns.lzb.ac.cn (S. Jiang).

column packings in gas–liquid chromatography [13]. Recently, Yanes et al. [14] reported the successful capillary electrophoresis (CE) separation and identification of some phenolic constituents of grape seed extract using 1-alkyl-3-methylimidazolium ionic liquids as the main electrolyte solutions. However, no report on the application of 1-alkyl-3-methylimidazolium ionic liquids in high-performance liquid chromatography (HPLC) has been published.

Norephedrine ($C_6H_5-CHOH-CH(CH_3)-NH_2$), ephedrine ($C_6H_5-CHOH-CH(CH_3)-NH(CH_3)$), pseudoephedrine ($C_6H_5-CHOH-CH(CH_3)-NH(CH_3)$) and methylephedrine ($C_6H_5-CHOH-CH(CH_3)-N(CH_3)_2$) (Table 1) are potential stimulants of the central nervous system and are, therefore, forbidden by the International Olympic Committee for sports participants. Many HPLC methods, especially conventional HPLC methods with aqueous–organic mobile phases, have been applied extensively to the separation and determination of ephedrines. In these procedures, binary mobile phases containing methanol–water [16,17], acetonitrile–water [18,19], or ternary mobile phase of acetonitrile–methanol–water [20,21] etc. are commonly used. Nevertheless, these organic solvents are volatile and harmful to the environment. On the other hand, ephedrines are basic compounds with polar functional groups, so that these compounds may cause severe band tailing, band broadening and low plate numbers in HPLC analysis. For this reason, the separation and determination of ephedrines require reversed-phase packings based on silicas with low silanol activity, and mobile phase with high ionic strength and amine modifiers. To find a solvent which has a benign effect on the environment and can be used in HPLC efficiently and with good separation for polar and non-polar compounds is one of the greatest challenges for research workers in this field.

It seems that ionic liquids may have this potential.

Table 1
Properties of ephedrines

Compound	Molecular mass	pK_a [15]	Water solubility
Norephedrine	151.21	–	Immiscible
Ephedrine	165.23	10.1	Miscible
Pseudoephedrine	165.23	9.9	Miscible
Methylephedrine	179.26	–	Immiscible

In the present study, by using only water containing 5.2–20.8 mM 1-butyl-3-methylimidazolium tetrafluoroborate ionic liquids at pH 3.0 as the eluents, we successfully separated ephedrines in 17 min. The aim of this study is to present the effect of different concentrations and various kinds of ionic liquids on the separation of ephedrines, and to explore the potential for the application of ionic liquid, as an environmentally friendly solvent, to HPLC separation. This is the first report involving 1-butyl-3-methylimidazolium-based ionic liquids as eluents for the separation of ephedrines in HPLC.

2. Experimental

2.1. Apparatus

The HPLC system used was composed of a model 302 pump (Gilson, France), a 7125 injector with a 20- μ l sample loop (Rheodyne, USA), and a 785A UV–Vis detector (Perkin-Elmer, USA). An ODS column (silica particle size: 5 μ m, 100 \times 4.6 mm I.D.) was used (Chromatorex, Japan). The chromatograms were recorded on a Sepu 3000 Chemstation (Puhui Technology, China).

2.2. Reagents

Norephedrine (NE), ephedrine (E), pseudoephedrine (PE), methylephedrine (ME) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The 1-ethyl-3-methylimidazolium tetrafluoroborate ([emim]BF₄), 1-propyl-3-methylimidazolium tetrafluoroborate ([pmim]BF₄), 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim]BF₄), 1-butyl-3-methylimidazolium chloride ([bmim]Cl) were synthesized according to the literature procedures [22,23]. Double distilled water was used throughout.

2.3. Chromatographic conditions

NE, E, PE and ME were dissolved in a drop of methanol and were further diluted by distilled water to 400, 1600, 800, 800 μ g ml⁻¹, respectively. The mobile phases were prepared as molar concentrations by dissolving known amounts of ionic liquids in

distilled water or diluting to the specified concentration according to requirements. The pH values of mobile phases were adjusted with either 10% (v/w) hydrochloric acid or 0.01 M sodium hydroxide. The mobile phases were filtered through a 0.45- μm nylon membrane filter. All chromatograms were obtained at room temperature at a flow-rate of 1.0 ml min⁻¹. UV detection at 252 nm was used for the entire study.

3. Results and discussion

3.1. Effect of [bmim]BF₄ on retention behavior of ephedrines

Fig. 2 shows the chromatograms of ephedrines with 2.6 mM (0.05% (v/v) or 0.59 g l⁻¹) [bmim]BF₄ at pH 3.0 used as the eluent (b) and water at pH 3.0 used as the eluent (a), respectively. It is obvious the addition of ionic liquid to water results in a better separation of NE, E, PE and ME. First, the resolutions of four ephedrines were improved, especially for E and PE. Although the separation of PE and ME was incomplete, it was evidently superior to that using only water as the eluent. Second, the retention of all analytes decreased when [bmim]BF₄ was added to water. Third, after the addition of [bmim]BF₄ to water, the separation efficiency and symmetry of the four chromatographic peaks also improved greatly.

As for basic compounds with polar functional groups, severe band tailing, band broadening and low plate numbers in the chromatogram often occur because of the residual silanols on C₁₈ column. Imaz and co-workers [24,25] used triethylamine (TEA) as one of the composition of mobile phase to improve the peak shape of ephedrines on account of its ability to shield residual silanols. Yanes et al. [14] noted that the positively charged imidazolium groups not only existed in the bulk solution but also was coated on the capillary wall when ionic liquids were used as the electrolytes additives in CE. Thus, it can be concluded that when ionic liquid is used as the mobile phase additive in HPLC, it exists in the solution and is also coated on the C₁₈ column. Imidazolium cations can interact with silanol groups (Fig. 3) and compete for the silanol groups on the

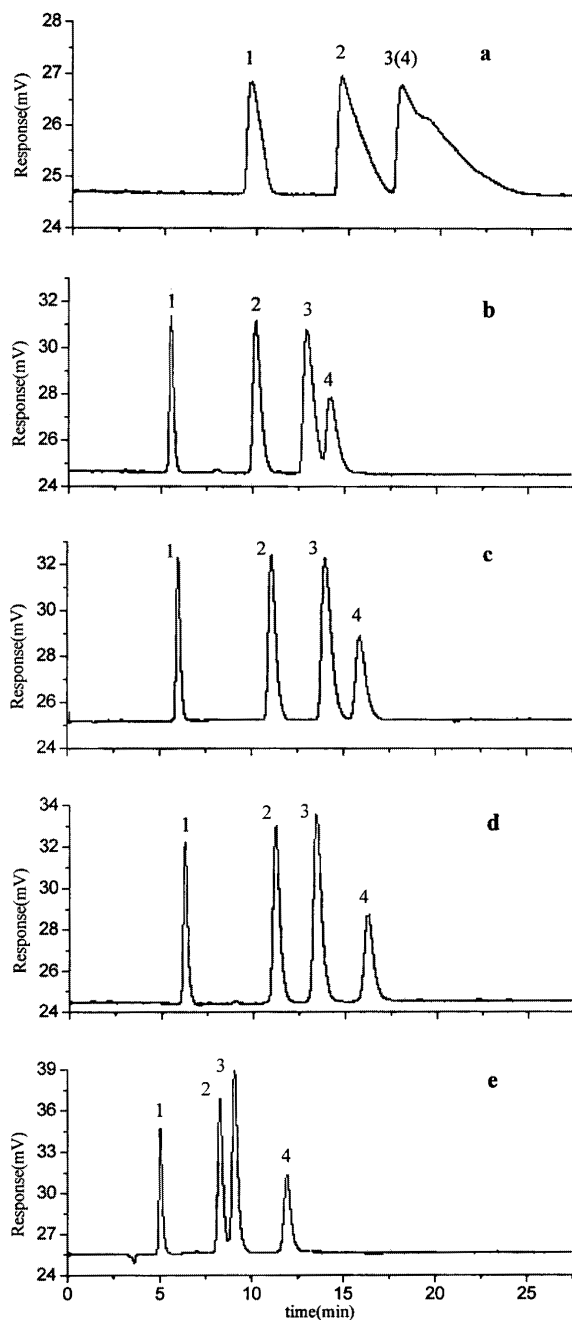


Fig. 2. Chromatograms of ephedrines with a mobile phase containing different concentrations of [bmim]BF₄ at pH 3.0. (a) 0, (b) 2.6, (c) 5.2, (d) 20.8, and (e) 62.4 mM. Chromatographic conditions: column: C₁₈ (5 μm , 100 \times 4.6 mm I.D.); rate-flow: 1.0 ml min⁻¹; detection: 252 nm. Peaks: (1) NE, (2) E, (3) PE, (4) ME.

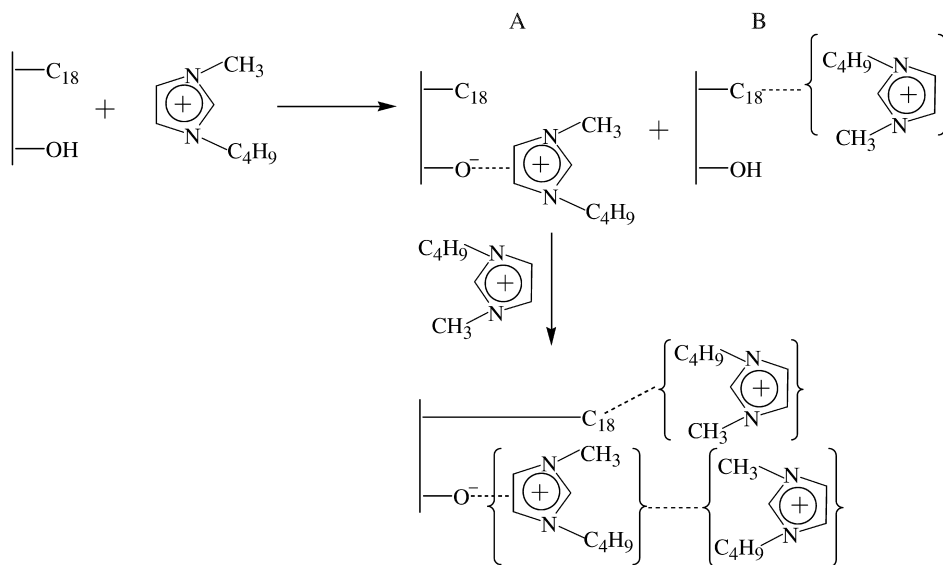


Fig. 3. Proposed scheme of interaction for [bmim]BF₄ on modified silica surface.

alkylsilica surface with the polar group of the analytes. Therefore, it can effectively shield the residual silanols and improve the peak shapes, while also decrease the retention time of the analytes.

3.2. Effect of [bmim]BF₄ concentration

The dependencies of retention factors (*k*) on concentration of [bmim]BF₄ (2.6–62.4 mM) (0.05–1.2%, v/v or 0.59–14.1 g l⁻¹) in the eluent are shown in Fig. 4, and similar dependencies are obtained for all analytes. The increase of the [bmim]BF₄ concentrations in the eluents causes an increase in retention of all analytes first, then a decrease at 20.8 mM for NE, E, ME and 10.4 mM for PE, respectively.

The relationship between the concentration of [bmim]BF₄ and the retention may be attributed to the following factors. As mentioned above, the addition of ionic liquids to water, which causes the competition between imidazolium cations and polar group of analytes for silanol groups on alkylsilica surface, results in a drastic decrease in the retention of the analytes. When the concentrations of ionic liquids increase slightly, as illustrated in Fig. 3, interactions of imidazolium cations with the silanol groups on

alkylsilica surface by electrostatic interaction (A) or with the alkyl groups by hydrophobic interaction (B) gradually strengthen, which results in an increase in the carbon content of stationary phase, thus increases the retention of analytes. Because of the strong electrostatic interaction, the possibility of producing A is greater than that of B. With further increase of the concentration of [bmim]BF₄, imidazolium cations interact with A through electrostatic interaction, and produce a weak bilayer electronic structure that

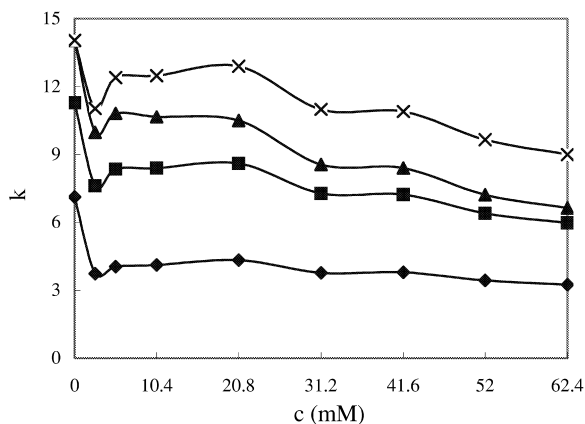


Fig. 4. Effect of concentration of [bmim]BF₄ in the eluent at pH 3.0 on retention factors of ephedrine derivatives. Chromatographic conditions as in Fig. 2. Symbols: ♦=NE, ■=E, ▲=PE, ×=ME.

repulses the basic analytes, and interact with alkyl group through hydrophobic interaction, so the retention of analytes decreases under the repulsive and hydrophobic interactions.

Concentration remarkably affects the retention and separation of the analytes (Fig. 2a–e). It is interesting to note that without [bmim]BF₄ the peaks of ME and PE are almost completely overlapped, whereas after the addition of only 2.6 mM [bmim]BF₄ to water PE and ME are partially resolved. Complete separation of PE and ME is achieved at the concentration of [bmim]BF₄ ≥ 5.2 mM. But with a further increase in the concentration of [bmim]BF₄, the resolution of E and PE decreases and almost incomplete separation of E and PE occurs at the concentration of [bmim]BF₄ ≥ 62.4 mM. Excellent separation of NE, E, PE and ME is achieved in 17 min with an eluent of 5.2–20.8 mM [bmim]BF₄ at pH 3.0.

As mentioned above, in HPLC, amine additive such as TEA is generally used as the screening agent for the separation of basic compounds but a distinct effect between [bmim]BF₄ and TEA on separation is observed in this study (Fig. 5). After the addition of 5.2 mM TEA to water, the separation for NE and E is improved slightly, but the peaks of PE and ME

still overlap, while successful separation is achieved using [bmim]BF₄ under the same condition. With a further increase of the concentration of TEA to 62.4 mM, the separation efficiency and symmetry of the four peaks are greatly improved, which indicates TEA has the ability to shield residual silanols, but PE and ME are only partially resolved. Comparing Fig. 5 with Fig. 2, it is evident that [bmim]BF₄ is superior to TEA in the separation of ephedrine such as in analytical time, separation efficiency, resolution, and the amount of reagent consumed.

3.3. Effect of various 1-alkyl-3-methylimidazolium-based ionic liquids

3.3.1. Effect of alkyl group on the imidazolium cations

The effect of different alkyl groups on imidazolium cations was investigated. Addition of the same concentration of three ionic liquids with different alkyl groups ([emim]BF₄, [pmim]BF₄, [bmim]BF₄) was used to study this effect on the separation of ephedrine (Fig. 6). Some of their properties are listed in Table 2. It is apparent that [bmim]BF₄ provides the best resolution, the shortest retention time, and the minimal peak half-width (W_h). Under the same condition, PE and ME can not be completely separated when [emim]BF₄ and [pmim]BF₄ are used. With the increase of alkyl chain length on the imidazolium cations, the retentions of all compounds are decreased and the peak shapes are improved.

As shown above, [emim]BF₄ and [pmim]BF₄ do not allow successful separation of ephedrine at a concentration of 10.4 mM, but [bmim]BF₄ does. However, in comparison with water, [emim]BF₄ and [pmim]BF₄ improve significantly the peak shape and decrease the peak tailing—the reason may be that [emim]BF₄ and [pmim]BF₄ effectively shield the residual silanols as with [bmim]BF₄. It seems that [bmim]BF₄ is most effective in shielding residual silanols—perhaps this is the reason why peak half-widths decrease and resolutions improve with the increase in alkyl chain length.

3.3.2. Effect of [bmim]⁺ counterion

To investigate the effect of the counterion, [bmim]⁺ with different counterions (BF₄⁻, Cl⁻) were

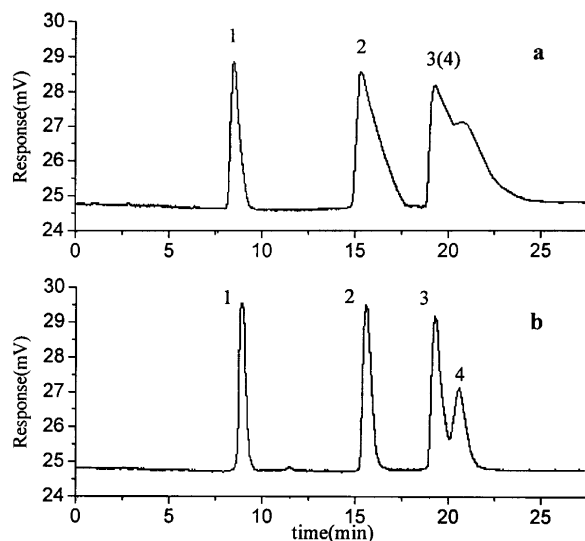


Fig. 5. Chromatograms of ephedrine with a mobile phase containing 5.2 (a) and 62.4 mM (b) TEA at pH 3.0. Other chromatographic conditions as in Fig. 2. Peaks: (1) NE, (2) E, (3) PE, (4) ME.

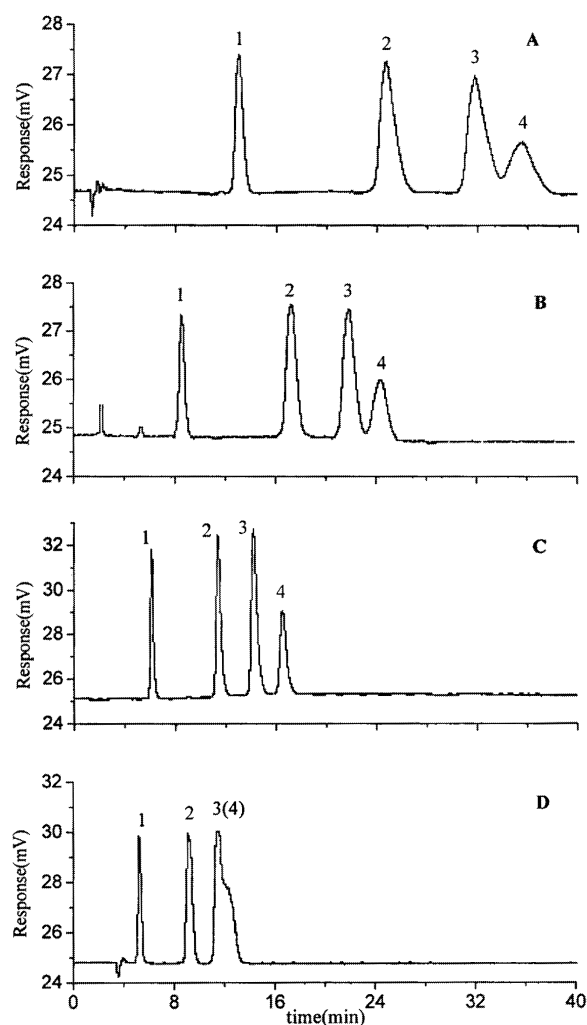


Fig. 6. Chromatograms of ephedrine using a mobile phase containing 10.4 mM various ionic liquids at pH 3.0: (A) [emim]BF₄, (B) [pmim]BF₄, (C) [bmim]BF₄, (D) [bmim]Cl. Other chromatographic conditions as in Fig. 2. Peaks: (1) NE, (2) E, (3) PE, (4) ME.

compared. In comparison with [bmim]BF₄ (Fig. 6C), [bmim]Cl (Fig. 6D) as eluent provides shorter retention times for all analytes but poorer resolution for PE and ME. It is worth noticing that the addition of [bmim]Cl to water can improve the peak shape, which also suggested that the addition of 1-alkyl-3-methylimidazolium-based ionic liquids certainly has an effective effect on the separation of ephedrine.

The different separations that resulted from two ionic liquids with different counterions as the eluents may be due to the association with solutes that are positively charged at pH 3.0, and it seems that [bmim]⁺ with BF₄⁻ is superior to that with Cl⁻ in the separation of ephedrine. Unfortunately, except for [bmim]BF₄, the amount of other 1-alkyl-3-methylimidazolium-based ionic liquids were not enough for further analysis. Furthermore, it is seen that [bmim]BF₄, as the eluent, presented a distinctive behavior for the separation of ephedrine in comparison with the other ionic liquids, so the effect of [bmim]BF₄ on the separation of ephedrine was investigated in detail.

In order to test the possible effects of ionic liquid on C₁₈ column, the column was evaluated before and after exposure to ionic liquid, using methanol–water as the mobile phase with the test mixture of benzene, biphenyl, naphthalene and anthracene (Table 3). The slight change in efficiency and peak tail factor (PTF) showed that the use of ionic liquid is not harmful to the column—this is different from tetra-alkylammonium which deteriorates the silica-based column when used as a component of the mobile phase [28].

4. Conclusion

In order to explore the effectiveness of 1-alkyl-3-

Table 2
Properties of ionic liquids [26,27]

Compound	Molecular mass	Density (g ml ⁻¹)	Melting point (°C)	Water solubility
[emin]BF ₄	197.97	1.29	6	Miscible
[pmin]BF ₄	212.00	1.23	–	Miscible
[bmin]BF ₄	226.03	1.17	–81	Miscible
[bmin]Cl	174.67	1.08	41	Miscible

Table 3
Chromatographic data of column before or after using ionic liquid as the eluent^a

Compound	Before the use of ionic liquid			After the use of ionic liquid		
	<i>k'</i>	N (m ⁻¹)	PTF ^b	<i>k'</i>	N (m ⁻¹)	PTF ^b
Benzene	0.83	25 660	1.55	0.82	25 790	1.56
Biphenyl	1.69	37 450	1.55	1.67	37 390	1.53
Naphthalene	2.49	45 820	1.17	2.45	47 030	1.18
Anthracene	4.01	55 880	1.08	3.91	56 170	1.08

^a Chromatographic conditions: column: C₁₈ (5 μm, 100×4.6 mm I.D.); eluent: CH₃OH/H₂O=85:15 (v/v), rate-flow: 1.0 ml min⁻¹; detection: UV 254 nm; water as unretained solute.

^b PTF=(A+B)/2A (A, B: the front or back part of peak width at 10% of peak height, respectively).

methylimidazolium-based ionic liquid—a “green chemistry” solvent—in HPLC, the separation of four polar compounds (NE, E, PE and ME) was carried out using several ionic liquids as the eluent. As a result, excellent separation of these compounds was achieved using 5.2–20.8 mM [bmim]BF₄ at pH 3.0 as the eluent. The separation mechanism may involve the competition between imidazolium cations and polar group of analytes for silanol groups, as well as the formation of weak bilayer electronic structure. These results strongly confirmed that the application of ionic liquids, especially, [bmim]BF₄, to HPLC was a simple effective way to separate polar compounds. The application of ionic liquids to separate other compounds is in progress.

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