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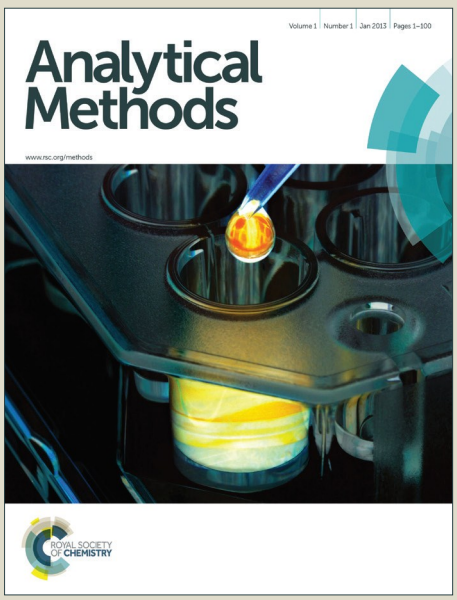

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Hydrophilic interaction liquid chromatography for separation and determination of pyrrolidinium ionic liquid cations

Chao Guan, Hong Yu*

College of Chemistry and Chemical Engineering, Harbin Normal University, Harbin 150025, China; *E-mail address*: yuhonghsd@126.com; Fax: 86-451-88060571.

Abstract A convenient and versatile method was developed for the separation and detection of pyrrolidinium ionic liquid cations by hydrophilic interaction liquid chromatography (HILIC) with indirect ultraviolet detection. Chromatographic separation was achieved on a hydrophilic column using background ultraviolet absorption reagents and organic solvents as mobile phase. HILIC mechanisms were investigated by studying the effects of the background ultraviolet absorption reagents, detection wavelength, organic solvents, and pH of the mobile phase on the separation and determination of pyrrolidinium cations. Good retention of very polar ionic liquid cations was obtained in HILIC. The optimized chromatographic conditions were selected by using 0.8 mmol L^{-1} 4-aminophenol hydrochloride aqueous solution (pH 3.0) / acetonitrile (25/75, v/v) as mobile phase, detection wavelength of 230 nm. The method is simple and selective and is believed to be applicable for pyrrolidinium ionic liquids synthesized in the laboratory. This is an improved analytical method for ionic liquid cations. Compared to reversed phase liquid chromatography, HILIC method provides an alternative approach for enhancement of retention of polar ionic liquid cations.

Keywords: hydrophilic interaction liquid chromatography, indirect ultraviolet detection, pyrrolidinium ionic liquid cations, 4-aminophenol hydrochloride

1. Introduction

Ionic liquids (ILs) are collectively known as organic salts, which represent a new class of non-molecular ionic solvents. In most cases, ILs are composed of organic cations (e.g., imidazolium, pyrrolidinium, pyridinium, ammonium, phosphonium) and various anions.[1-5] With the increasing awareness of environmental protection, ILs and their unique properties have attracted the interest of many researchers.[6-8] Due to their extremely low vapor pressure, ILs have been widely considered as "green solvents" that may be used to replace conventional molecular solvents.[9,10] In the development of processes based on ionic liquids, a simple, effective, and reliable analysis method is especially important for the detection and monitoring of their transport.[11,12] Currently, the methods for the determination of ionic liquid cations mainly included reversed phase liquid chromatography (RPLC),[13,14] ion-pair chromatography (IPC),[15-17] ion chromatography (IC)[18,19] and hydrophilic interaction chromatography (HILIC).[20-22] HILIC[23] has been developed as a chromatographic technique is used to improve poor retention behavior of polar substances in RPLC. To achieve the separation, HILIC method applied the strong polar stationary phase, combined with the high ratio of the organic phase and the low ratio of aqueous phase.

Pyrrolidinium ionic liquids are important and widely application in organic synthesis, catalytic chemistry and electrochemistry. There are usually no UV absorption groups in their molecular structure. In previous reports, the determinations of ionic liquids principally focus on imidazolium cations. However, the research reports for determination of pyrrolidinium cations were fewer. Several studies have reported about the separation and determination of pyrrolidinium ionic liquid cations using RPLC[24] and IC.[19,25] The RPLC method show clear limitations for separation of short alkyl chains ionic liquid cations in terms of retention and selectivity, thus adding ion pair reagent was needed. Therefore, HILIC method was performed, which provides an alternative approach for enhancement of retention of polar ionic liquid cations.[20,21] IC with conductivity detector used the specialized ion chromatograph. As a general application in laboratory, UV detector has been widely paired with liquid chromatography to detect compounds with UV absorbance groups. For analytes with no UV absorbance groups, indirect ultraviolet (IUV) detection method has been the suitable method.[26] The simple method was achieved by adding substances having UV absorption group as background reagents to the mobile phase.

The aim of this work was to develop a method to analyze pyrrolidinium ionic liquid cations which have side chain of carbon atoms less than 5 by HILIC with IUV detection. The good separation and IUV detection of pyrrolidinium cations were achieved on a hydrophilic column using 4-aminophenol hydrochloride and organic solvents as mobile phase without ion pair reagent and isocratic elution. The method is simple and selective and is believed to be applicable for pyrrolidinium ionic liquid samples synthesized by chemistry laboratory.

2. Experimental

2.1 Instrumentation

All experiments were carried out on an Agilent 1200 HPLC system (Agilent, USA), which consisted of a quaternary pump (Model Quat pump-G1311A), a detector (Model DAD-G1315D), an autosample injector (Model ALSG1329A), a column oven (Model TCC-G1316A) and a degasser system (Model Degasser-G1322A). The chromatographic system control, data acquisition and data analysis were performed using the Agilent Rev.B.04.01 workstation (Agilent, USA).

A Millipore Milli-Q water purification system (Millipore, Bedford, MA, USA) was used to purify distilled water, and the deionized water produced at 18.2 MΩ cm was prepared for mobile phases and sample solutions. A model PHSF-3F pH meter (Shanghai Precision and Scientific Instrument, Shanghai, China) was used for pH measurement. Before use, mobile phases were filtered through a 0.22 μm filter, and then degassed for 15 min with a Model DOA-P504-BN pump (IDEX, USA).

2.2 Chemicals

The ILs (99% purity) were N-methyl-N-ethyl pyrrolidinium bromide ([MEPy][Br] N-methyl-N-propyl pyrrolidinium bromide ([MPPy][Br]), N-methyl-N-butyl pyrrolidinium

bromide ([MBPy][Br]) purchased from Shanghai Chengjie Chemical Ltd. (Shanghai, China). 4-aminophenol hydrochloride, sulfosalicylic acid, nicotinamide and phthalic acid (analytical grade) were supplied by J&K Chemical Ltd. (Beijing, China). Methanol and acetonitrile (HPLC grade) were obtained from Dikma Technologies (Shanghai, China). Acetic acid and sodium hydroxide (analytical grade) were obtained from Shanghai reagent factory (Shanghai, China).

2.3 Solutions

Standard solutions of ionic liquid cations at a concentration of 1 g L^{-1} were prepared in acetonitrile and deionized distilled water (50:50, v/v), and then diluted to the concentration required for the experiment. It then filtered through 0.22 μm membrane.

Milli-Q water was also used to prepare the mobile phases. Aqueous solutions of 4-aminophenol hydrochloride were prepared in appropriate concentration. The mobile phases were filtered through a 0.22 μm filter

2.4 Chromatographic conditions

All separations were performed on a 4.6 mm i.d. × 250 mm TSK-GEL Amide-80 HR column (TOSOH, Japan). The optimal mobile phase was consisted of 0.8 mmol L^{-1} 4-aminophenol hydrochloride aqueous solution (pH 3.0) / acetonitrile (25/75, v/v). The flow rate was set at 1.0 mL min^{-1}. Column temperature was 30 ℃. Injection volume was 20 μL. IUV at 230 nm was employed.

3. Results and discussion

3.1 Selection of background UV absorption reagent

Because pyrrolidinium cations have no UV absorption group in their molecular structure, the

addition of background UV absorbing reagents to the mobile phase can be used for detection by the IUV method. Phthalic acid,[27] sulfosalicylic acid,[28] nicotinamide[29] and 4-aminophenol hydrochloride [30] were used as background UV absorbing reagents in IUV method. The detections of pyrrolidinium cations were investigated using phthalic acid, sulfosalicylic acid, nicotinamide and 4-aminophenol hydrochloride as background UV absorbing reagents at their respective maximum wavelengths. The mobile phases used contained these reagents in aqueous solution / acetonitrile (20/80, v/v). As a result, when phthalic acid, sulfosalicylic acid and nicotinamide were in the mobile phase, there were no appeared chromatographic peaks of pyrrolidinium cations. Phthalic acid, sulfosalicylic acid and nicotinamide were not suitable for separation and detection of pyrrolidinium cations. When 4-aminophenol hydrochloride was used as the mobile phase, the chromatographic peaks of two pyrrolidinium cations appeared and the peak profiles were good, which the $[MEPy]^+$ peak has interference with system peak. Thus, 4-aminophenol hydrochloride was used as the background UV absorbing reagents to further study the effect of mobile phase on the separation and determination of pyrrolidinium cations.

3.2 Influence of mobile phase on the separation of pyrrolidinium cations

3.2.1 Effect of 4-aminophenol hydrochloride concentration

The effect of 4-aminophenol hydrochloride concentration on the separation of pyrrolidinium cations was investigated using 4-aminophenol hydrochloride aqueous solution / acetonitrile (20/80, v/v) as mobile phase. The concentrations of 4-aminophenol hydrochloride were investigated at 0.5, 0.8, 1.0 and 1.2 mmol L^{-1}, respectively. The results were shown in Fig. 1. In contrast to reversed-phase chromatography, the HILIC conditions led to an elution order in which the less polar substances were eluted first and the more polar substances would be eluted later. Alpert[31] confirmed that the reason for this elution order was that water in the mobile phase absorbed at the surface of the stationary phase, forming a dynamic "water-rich layer", in which the polar substances were more retained. Thus, retention of solutes in HILIC is achieved by their partition between a water-rich layer and the less polar mobile phase. The results showed that the retention

times of pyrrolidinium cations were shortened and the detection limits were increased with increasing the concentration of 4-aminophenol hydrochloride. Retention times were shortened by the increased concentration of 4-aminophenol hydrochloride, because of enhanced molecular dipole interactions between the solutes and the mobile phase additive, resulting in faster elution. The greater values of detection limits were caused by added 4-aminophenol hydrochloride as background UV absorption reagent. The higher the concentration is, the stronger is the background absorbance, it led to greater noise and greater values of detection limits. A 4-aminophenol concentration of 0.8 mmol L^{-1} was chosen for further analysis to take into account the chromatographic peak and system peak in a comprehensive comparison

3.2.2 Effect of organic solvent

The effects of organic solvents such as methanol and acetonitrile on separation of three pyrrolidinium cations were investigated using 0.8 mmol L^{-1} 4-aminophenol hydrochloride aqueous solution / methanol and 0.8 mmol L^{-1} 4-aminophenol hydrochloride aqueous solution / acetonitrile (20/80, v/v) as the mobile phase, respectively, as shown in Fig. 2. It showed that using methanol as an organic solvent, there were no peaks of three pyrrolidinium cations appeared. Because of methanol as a polar protic solvent which can form hydrogen bonds with the stationary phase, resulting in competitive adsorption with analytes in the HILIC, so that destructed the formation of water-rich layer. Because of this factor, the hydrophobicity of the stationary phase surface was enhanced, resulting in decreased retention of analytes. Therefore, it is better to choose acetonitrile as the organic component of mobile phase.

The effect of acetonitrile concentrations in mobile phase was studied for a range from 70% to 85%. The mobile phase was used 0.8 mmol L^{-1} 4-aminophenol hydrochloride aqueous solution. Fig. 3 showed the variation of retention factor (lg *k*) of pyrrolidinium cations as a function of the acetonitrile volume fractions. It showed that the lg *k* was increased with the increasing acetonitrile volume fractions. The reason is that water is very strong elution solvent in HILIC, with the amount of acetonitrile increased, water content was decreased in the mobile phase, the solute was not easy to be eluted, so the retention enhanced.[32] When 70% acetonitrile was used in mobile

phase, the $[MPPy]^+$ and $[MEPy]^+$ can not be completely separated and the peak profile of the cations was poor. Based on the consideration of separation and peak profile of pyrrolidinium cations, 75% acetonitrile was selected as optimum concentration.

3.2.3 Effect of the mobile phase pH

The pH ranges of the chromatographic column are from 2.0 to 7.5. The effects of the mobile phase pH adjusted with acetic acid or NaOH for 3.0, 3.5, 4.0, 4.5 (unadjusted pH of mobile phase) and 5.0 on separation of three pyrrolidinium cations were investigated using 0.8 mmol L^{-1} 4-aminophenol hydrochloride aqueous solution mixed with 75% acetonitrile as mobile phase, as shown in Fig. 4. The results indicated that with the increase of the mobile phase pH from 3.0 to 5.0, the retention times were gradually extended. The possible reason was the pH of the mobile phase will affect the degree of protonation of the solute, thereby affecting the solute retention. When the pH was gradually increased, the degree of ionization of the solute increased, resulting in hydrophilicity of solutes was enhanced, retention strengthened, which conform to the work mechanism of HILIC.[33] The mobile phase pH less than 3.0 was disadvantageous for the chromatographic column. When mobile phase pH was greater than 4.0, the chromatographic peak of $[MEPy]^+$ was interfered with system peak in Fig. 4. Fig. 4 also showed that as the pH value was 3, $[MEPy]^+$ was not affected from system peak and the three pyrrolidinium cations were all separated. Thus, pH value 3 was selected for the entire analysis.

3.3 Selection of detection wavelength

The maximum absorption wavelength of 4-aminophenol hydrochloride was about 240 nm. Therefore, the effect of detection wavelength was investigated by varying the wavelength from 200 to 250 nm for the determination of three pyrrolidinium cations by using 0.8 mmol L^{-1} 4-aminophenol hydrochloride aqueous solution (pH 3.0) / acetonitrile (25/75, v/v) as the mobile phase. The results were shown in Table 1. It was found that using the detection wavelength at 230

nm, the detection limits of the cations were low. Therefore, the suitable detection wavelength selected was 230 nm.

3.4 Effect of column temperature

Near room temperature was usually selected in HILIC analysis. The effect of column temperature was investigated for a range from 20 ℃ to 40 ℃. The mobile phase was 0.8 mmol L^{-1} 4-aminophenol hydrochloride aqueous solution (pH 3.0) / acetonitrile (25/75, v/v). Fig. 5 showed the variation of retention factor (lg *k*) of pyrrolidinium cations as a function of the column temperature. It showed that with the increase of column temperature, the retention factor (lg *k*) of analytes was gradually shortened, but the effect of temperature on retention of analytes was slight. When the column temperature is 30 ℃, the separation of pyrrolidinium cations were not affected from system peak. Therefore, 30 ℃ was selected as an appropriate temperature.

3.5 Carbon number rule of pyrrolidinium cations homologues

There are a carbon number rule between carbon atoms and homologue retention factor: lg $k = a\ n\mathrm{C} + b$[34] (*k* is the retention factor, *n*C is the homologue carbon atoms number). Under optimum chromatographic conditions, the carbon number rule curve of the cations ($[MEPy]^+$, $[MPPy]^+$, $[MBPy]^+$) was lg $k = -0.1149\ n\mathrm{C} + 0.9122$, $r = 0.9993$. The equation showed that lg*k* and *n*C has a good linear relationship, indicating that in these conditions, retention factor of pyrrolidinium cations conformed with the number of carbon. As seen from the formula, with the increase in homologues of carbon atoms, retention factor decreased, and it was contrary to reversed-phase chromatography retention rule.

3.6 Quantitative analysis

3.6.1 Optimum chromatographic conditions

According to the above discussion, the suitable chromatographic conditions for determination of three pyrrolidinium cations were described as follows. The analytes were determined using a TSK-GEL Amide-80 HR column with an IUV detection wavelength of 230 nm, 0.8 mmol L^{-1} 4-aminophenol hydrochloride aqueous solution (pH 3.0) / acetonitrile (25/75, v/v) as mobile phase, a flow rate of 1.0 mL min^{-1} and the column temperature controlled at 30 ℃. Under these conditions, the chromatogram of three pyrrolidinium cations was shown in Fig. 6.

3.6.2 Quantitative parameters

Under optimum chromatographic conditions, the standards solutions of three pyrrolidinium cations were determined. Linear regression equations were obtained from the relationship between peak area (integral value) and ionic concentration (mg L^{-1}). Detection limits were calculated with a tripled signal-to-noise ratio (S/N = 3), and noise of the baseline was 0.04774 mAU. Relative standard deviations of retention time and peak area (RSD_t/RSD_s) were obtained by five repeated measurements of a standard mixture solution of $[MEPy]^+$ (50 mg L^{-1}), $[MPPy]^+$ (50 mg L^{-1}) and $[MBPy]^+$ (50 mg L^{-1}) under the chromatographic conditions. The data were listed in Table 2. The results showed that the reproducibility and linearity of the method can meet the requirements of quantitative analysis.

3.7 Analysis of sample

This method was applied to the determination of pyrrolidinium ionic liquid samples synthesized by the chemistry lab, namely N-methyl-N-ethyl pyrrolidinium bromide ([MEPy][Br]), N-methyl-N-propyl pyrrolidinium bromide ([MPPy][Br]), N-methyl-N-butyl pyrrolidinium bromide ([MBPy][Br]). The sample weights of [MEPy][Br], [MPPy][Br] and [MBPy][Br] were 0.1701g, 0.1621g and 0.1562g, respectively, diluted to 100 mL as stock solutions. Then, 1.0 mL [MEPy][Br], 1.0 mL [MPPy][Br] and 1.0 mL [MBPy][Br] were taken from the stock solutions,

respectively, and dilute to 50 mL. After filtering through a 0.22 μm membrane filter, these sample solutions were used for the determination of pyrrolidinium cations under the selected conditions. Chromatograms were shown in Fig. 7. Recoveries were tested by the standard addition method. Analytical results and recoveries of pyrrolidinium cations in the ionic liquid samples are listed in Table 3. The data in Table 3 are average values (n = 5), and the RSD are less than 0.8%. Data from Table 3 showed the method were accurate and reproducible, and can meet the requirements of quantitative analysis of pyrrolidinium cations.

4. Conclusion

Our goal was to study the retention of pyrrolidinium cations and to develop a versatile method to separate them by HILIC. Good separation of very polar pyrrolidinium cations was obtained using a simple mobile phase without ion pair reagent and isocratic elution in HILIC. Compared to RPLC, HILIC method provides an alternative approach for enhancement of retention of polar ionic liquid cations. The method is simple and selective and is believed to be applicable for pyrrolidinium ionic liquid samples synthesized by chemistry laboratory, which prove that the method can meet quantitative analysis requirements of pyrrolidinium cations. The use of IUV method is simple and convenient, which is practical alternative for other routine analysis.

Acknowledgments

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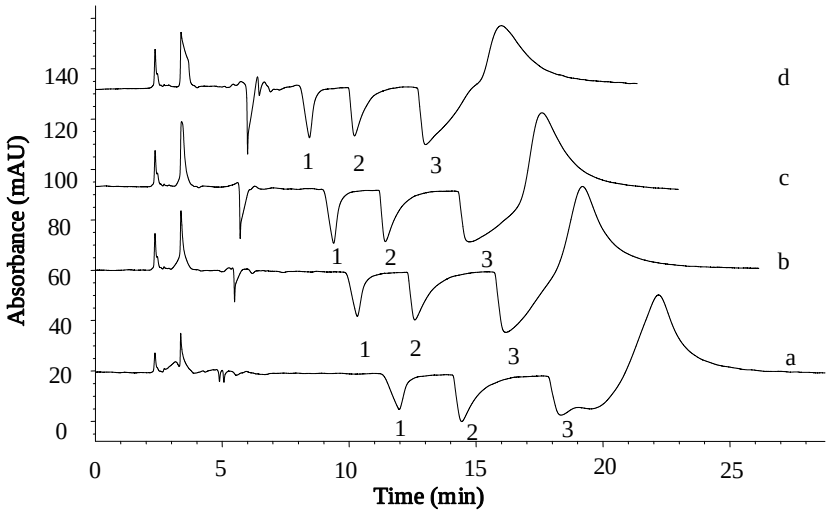

Fig. 1 Chromatograms obtained with mobile phases containing different concentrations of 4-aminophenol hydrochloride

Concentrations of 4-aminophenol hydrochloride: a, 0.5 mmol L^{-1}; b, 0.8 mmol L^{-1}; c, 1.0 mmol L^{-1}; d, 1.2 mmol L^{-1}. Chromatographic conditions: mobile phase, 4-aminophenol hydrochloride aqueous solution / acetonitrile (20/80, v/v); column, TSK-GEL Amide-80 HR (4.6 mm i.d. × 250 mm, 5 μm); flow rate, 1.0 mL min^{-1}; column temperature, 30℃; indirect UV detection, 240 nm; inject volumn, 20 μL. Peaks (50 mg L^{-1}): 1, $[MBPy]^+$; 2, $[MPPy]^+$; 3, $[MEPy]^+$.

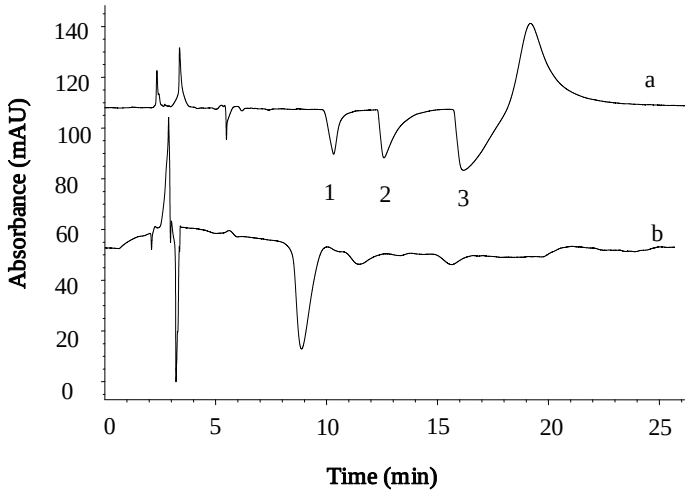

Fig. 2 Chromatograms obtained from different organic solvents of (a) acetonitrile and (b) methanol

Mobile phase: 0.8 mmol L^{-1} 4-aminophenol hydrochloride aqueous solution / organic solvents (20/80, v/v). The other chromatographic conditions and peak marks are same as in Fig.1.

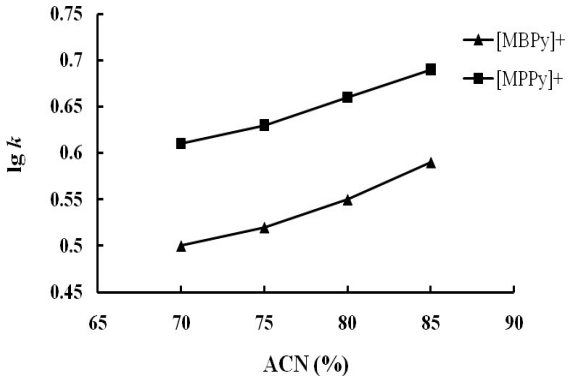

Fig. 3 Variation of lg *k* in function of volumic fraction of acetonitrile (ACN) in mobile phase

Mobile phase: 0.8 mmol L^{-1} 4-aminophenol hydrochloride aqueous solution / acetonitrile. The other chromatographic conditions are same as in Fig.1.

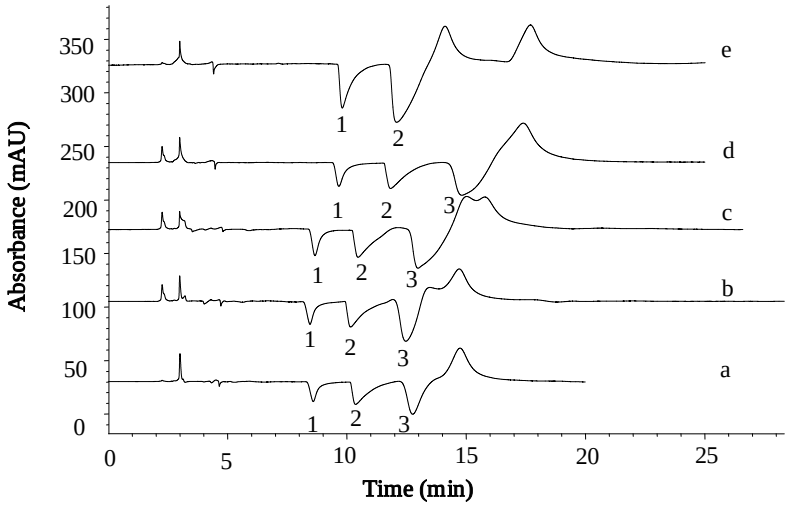

Fig. 4 Chromatograms obtained using mobile phases with different pH

pH: a, 3.0; b, 3.5; c, 4.0; d, 4.5; e, 5.0. Mobile phase: 0.8 mmol L^{-1} 4-aminophenol hydrochloride aqueous solution / acetonitrile (25/75, v/v). The other chromatographic conditions and peak marks are same as in Fig.1.

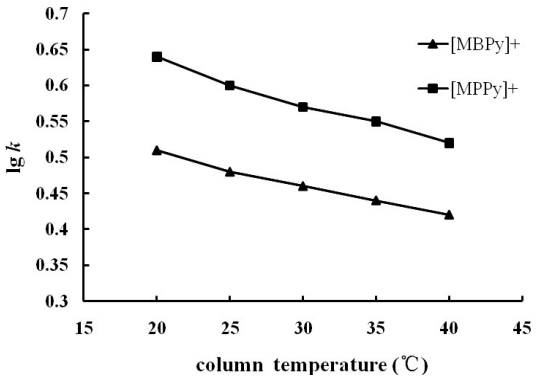

Fig. 5 Variation of lg *k* in function of column temperature

Mobile phase: 0.8 mmol L^{-1} 4-aminophenol hydrochloride aqueous solution (pH 3.0) / acetonitrile (25/75, v/v); indirect UV detection, 230 nm. The other chromatographic conditions are same as in Fig.1.

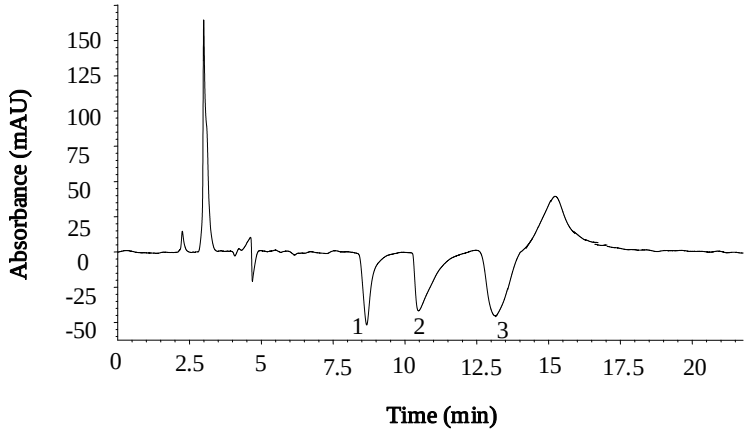

Fig. 6 Chromatogram of a standard mixture solution of pyrrolidinium ionic liquid cations

Chromatographic conditions: mobile phase, 0.8 mmol L^{-1} 4-aminophenol hydrochloride aqueous solution (pH 3.0) / acetonitrile (25/75, v/v); column, TSK-GEL Amide-80 HR (4.6 mm i.d. × 250 mm, 5 μm); flow rate, 1.0 mL min^{-1}; column temperature, 30 ℃; indirect UV detection, 230 nm; inject volumn, 20 μL. Peaks (50 mg L^{-1}): 1, $[MBPy]^+$; 2, $[MPPy]^+$; 3, $[MEPy]^+$.

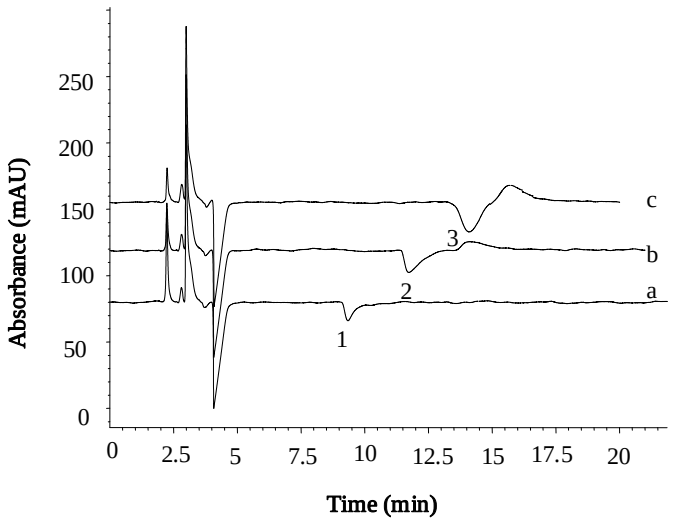

Fig. 7 Chromatograms of ionic liquid samples

a, The synthesized [MBPy][Br] sample; b, The synthesized [MPPy][Br] sample; c, The synthesized [MEPy][Br] sample. Chromatographic conditions are same as in Fig.6. Peaks: 1, $[MBPy]^+$; 2, $[MPPy]^+$; 3, $[MEPy]^+$.

Table 1 The relationship between the detection wavelength and baseline noise level, absorbance and detection limit

Detection wavelength (nm)	Baseline noise level (mAU)	Absorbance (mAU)			Detection limit (S/N = 3) (mg L^{-1})		
		$[MBPy]^+$	$[MPPy]^+$	$[MEPy]^+$	$[MBPy]^+$	$[MPPy]^+$	$[MEPy]^+$
200	2.0993	108.3	90.8	92.3	2.91	3.47	3.41
210	0.3702	100.6	67.9	68.1	0.55	0.82	0.81
220	0.2921	110.4	75.7	76	0.40	0.58	0.57
230	0.0477	51.6	41.4	46.6	0.14	0.17	0.15
240	0.0304	19.6	18.7	22.5	0.23	0.24	0.20
250	0.0279	12.2	11.7	13.0	0.34	0.36	0.32

Table 2 Linear regression equation, detection limit and RSD of retention time and peak area

Ion	Linear regression equation	Correlation coefficient (r)	Detection limit (mg/L, S/N = 3)	Linear range (mg L^{-1})	RSD_t/RSD_s (%, n = 5)
$[MBPy]^+$	$y = 20.74x - 17.50$	0.9998	0.14	0.47−100	0.25/0.30
$[MPPy]^+$	$y = 30.91x + 15.01$	0.9994	0.17	0.57−100	0.24/0.32
$[MEPy]^+$	$y = 30.46x + 34.51$	0.9997	0.15	0.50−100	0.14/0.31

y: peak area ; x : concentration (mg L^{-1})

Table 3 Analytical results and recoveries of pyrrolidiunium cations found in ionic liquid samples

Sample of ionic liquid	Cations	Original ρ_o (mg L^{-1})	Added ρ_A (mg L^{-1})	Found ρ_F (mg L^{-1})	Recovery R (%)	Content of cation in sample w (%)
[MBPy][Br]	$[MBPy]^+$	19.1	5	24.0	97.0	56.1
			40	57.8	96.8	
			80	98.1	98.8	
[MPPy][Br]	$[MPPy]^+$	19.4	5	24.3	98.0	59.8
			40	58.8	98.5	
			80	97.4	97.5	
[MEPy][Br]	$[MEPy]^+$	19.3	5	24.1	96.0	61.8
			40	58.3	97.5	
			80	96.3	96.3	

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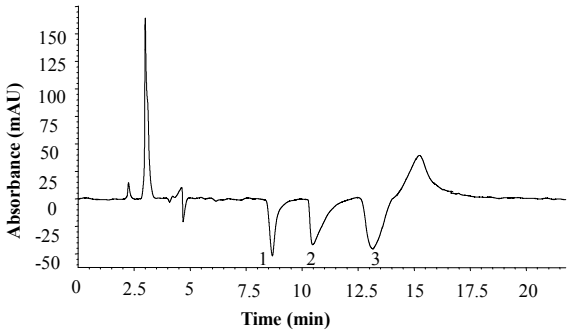

The separation and detection of pyrrolidinium cations by hydrophilic interaction liquid chromatography with indirect ultraviolet detection