



Original article

High-performance liquid chromatography utilization of ionic liquids as mobile phase additives for separation and determination of the isomers of amino benzoic acids



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ABSTRACT

For the isomers of amino benzoic acid, including *o*-, *m*-, *p*-amino benzoic acid, the beneficial effects of using the ionic liquid, 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIm][BF₄]), as mobile phase additives on retention behavior and separation were investigated. Chromatographic separation of the *o*-, *m*-, *p*-amino benzoic acid was performed on a reversed-phase C18 column by ultraviolet detection at 245 nm. The effects of several chromatographic parameters, concentrations and pH values of [BMIm][BF₄] solutions, methanol concentration and length of alkyl chain on different ionic liquids, on the separation and determination of the isomers were evaluated. The optimized chromatographic conditions were established using an aqueous 0.5 mmol/L [BMIm][BF₄] solution (pH 3.0)/methanol (40:60, v/v) as mobile phase without need of gradient elution, with separation of three amino benzoic acids achieved within four min. The calibration curve showed good linearity over the tested range of 2 mg/L to 120 mg/L for the three isomers with a correlation coefficients of 0.9999. The recoveries of the three amino benzoic acids of spiked components were between 99.8% and 100%. The method has been successfully applied to the determination of *p*-amino benzoic acid in the pharmaceutical, Bromine Mitag Procaine Injection.

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1. Introduction

Ionic liquids (ILs), salts that are liquids at or approaching room temperature, are normally composed of relatively large organic cations (e.g. alkylammonium, alkylimidazolium, alkyipyridinium etc.) and inorganic (e.g. [Br]⁻, [PF₆]⁻, [BF₄]⁻ etc.) or organic anions (e.g. [(CF₃SO₂)₂N]⁻, [CF₃CO₂]⁻ etc.). Compared to normal organic solvents, ILs have prominent physicochemical properties, such as negligible vapor pressure, nonflammable, good thermal stability, good conductivity and miscibility with water and organic solvents. Utilizing these characteristic properties, applications of ILs in the fields involving extraction, synthesis, catalysis, electrochemistry, environment protection and analytical chemistry are becoming widely used as new “green chemistry” solvents [1,2]. More recently, the attention of ILs employed in chromatographic analysis have been growing rapidly [3,4]. ILs are primarily used

as gas chromatography (GC) stationary phases, high performance liquid chromatography (HPLC) mobile phase additives and stationary phases, as well as capillary electrophoresis (CE) running electrolytes.

An extensive effort has been made in elucidating the effects of ionic liquids on the retention behavior and the separation as mentioned in the following reports. Recently, Yu *et al.* [5] reported that the addition of ILs to a mobile phase had an advantageous effect on the separation of morpholinium in a HPLC system. The determination and separation of alkyl sulfonates [6] were investigated by reversed-phase HPLC using 1-ethyl-3-methylimidazolium salts as new mobile phase additives. The separation of naphazoline nitrate [7] was studied using ILs as mobile phase additives. The determination of a group of heterocyclic aromatic amines [8] was achieved using a group of alkylimidazolium-based ILs as mobile phase additives in HPLC with electrochemical detection. The *o*-amino benzoic acid (OABA), *m*-amino benzoic acid (MABA) and *p*-amino benzoic acid (PABA) are commonly used in compositions or as intermediates in pharmaceutical products. Similarly, the

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compendial limit of PABA, which is a degradation impurity in Hydrochloric Acid Procaine Injection is not allowed to exceed 1.2% using thin layer chromatography (TLC) in the 1995 China Pharmacopoeia. Since the method is by visual inspection comparing with the sizes of pigmentation and the depths of color, it is not easy to estimate levels, especially near or at the specification limit. Presently, the reported methods for analysis of aromatic carboxylic acids are mainly by HPLC [9], CE [10], ion exclusion chromatography [11] and liquid chromatography–mass spectrometry [12].

In this study, we developed a method for the separation and determination of three amino benzoic acid isomers by HPLC with ILs as mobile phase additives, and investigated the factors that impacted the separation of the three amino benzoic acids and the retention behavior in HPLC. The optimized chromatographic conditions were selected, and then a method for simultaneous determination of isomers of amino benzoic acid was established. This method was employed in the determination of PABA in the pharmaceutical, Bromine Mitag Procaine Injection.

2. Experimental

HPLC separation was performed on an Agilent 1200 system (Agilent Technologies, USA) equipped with a quaternary pump-G1311A, an autosample injector Model ALS-G1329A, a thermostatted (30 °C) column compartment Model TCC-G1316A, a diode-array multiple wavelength UV/vis detector Model DAD-G1315D and a degasser system Model Degasser-G1322A. A Model PHSF-3F pH meter (China) was used for pH measurements. A Millipore Milli-Q water purification system (Millipore, Bedford, MA, USA) was used to deionize distilled water. A Model DOA-P504-BN pump (IDEX, USA) and 0.22 μm membrane filter (China) were used to filter mobile phases and sample solutions.

The studied amino benzoic acids were OABA, MABA and PABA, purchased from Bailingwei Chemicals (China). Ionic liquids (purity ≥ 99.0%) were obtained from Shanghai Chengjie Chemical Ltd. (China). The ILs were 1-ethyl-3-methylimidazolium tetrafluoroborate ([EMIm][BF₄]), 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIm][BF₄]), 1-hexyl-3-methylimidazolium tetrafluoroborate ([HMIm][BF₄]). Methanol (HPLC grade) was obtained from Shanghai Xingke Biochemistry Co., Ltd. (China). Acetic acid and NaOH were supplied by Shanghai Chemical Reagent Factory (China). Bromine Mitag Procaine Injection was marketed drug. The pH of ionic liquid solutions was adjusted with acetic acid.

Solutions were prepared using 18.2 MΩ/cm water. Standard stock solutions at a concentration 500 mg/L were prepared: accurately weighed OABA, MABA, and PABA at 0.0250 g, respectively, dissolved with 20% methanol solution and diluted to 50 mL, then preserved in refrigerator. Working standard solutions from each respective stock solution were prepared on a daily basis as required.

All separations were performed on a ZORBAX ODS column (250 mm × 4.6 mm i.d., 5 μm). The mobile phase was 0.5 mmol/L [BMIm][BF₄] solution (pH 3.0 adjusted with acetic acid)/methanol (40:60, v/v) without need of gradient elution. UV/vis detection (245 nm) was employed. Flow rate was 1.0 mL/min. Injection volume was 20 μL. Column temperature was 30 °C. The chromatographic system control, data acquisition and data analysis were performed using the Agilent Rev.B.04.01 workstation (Agilent, US). In order to eliminate the reagents absorbed on the chromatographic column, the chromatographic column should be flushed using at least 30 mL water–methanol (95:5, v/v) eluent after analysis everyday.

3. Results and discussion

Ionic liquids were used as mobile phase additives that possess exceptional properties. In reversed phase HPLC, the silanol groups attract the polar groups of a solute molecule through specific electrostatic interactions, while the alkyl groups attract the alkyl groups of a solute molecule through hydrophobic interactions. Such behavioral interactions between the analytes and the stationary phase can be interfered (disrupted) when ionic liquids are used as mobile phase additives, since the addition of ionic liquids to mobile phase leads to competition between cations of the ionic liquid and polar groups of the analytes for the polar silanol groups of the alkyl silica surface, and thus can reduce the retention of acids and cause bases to become more retained [13,14]. In our study, [BMIm][BF₄] was added to the mobile phase to separate three amino benzoic acids. All these organic substances have different affinities to silanol groups. Thus, all the employed solutes were apt to migrate along the chromatographic column due to the mixed retention mechanism.

The maximum absorbances of the isomers of amino benzoic acid each had a different response by UV detection. Further observation and comparison determined that all three amino benzoic acids had ultraviolet absorbance and high sensitivity when the detection wavelength was 245 nm. Therefore, the wavelength of 245 nm was selected for the detection of amino benzoic acids in the following investigations.

The effects of varying the concentration of [BMIm][BF₄] from 0.5 mmol/L to 5 mmol/L on the determination of amino benzoic acids were investigated. In Fig. 1, the results showed that the retention time of amino benzoic acids increased as the concentration of [BMIm][BF₄] solution was gradually reduced. The retention of three amino benzoic acids was improved when the concentration of [BMIm][BF₄] solution was 1.0 mmol/L, but the resolution of amino benzoic acids was worse than at a concentration of 0.5 mmol/L. Consequently, the appropriate concentration of the [BMIm][BF₄] solution was 0.5 mmol/L.

The effects of the methanol concentration on the retention of amino benzoic acids were also investigated, when the volume fractions of methanol were 40%, 50%, 60% and 70% with 0.5 mmol/L [BMIm][BF₄] solution as the mobile phase. As a general trend, the retention times of three amino benzoic acids decrease with increasing the concentration of methanol. But the separation of amino benzoic acids was poor with an increasing concentration of methanol. Upon comprehensive consideration of the resolution and retention times, the optimum content of methanol was selected at 60%.

The effect of the pH of the [BMIm][BF₄] solution on the separation of amino benzoic acids was investigated by changing

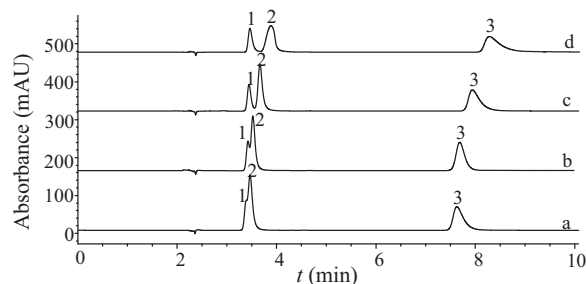


Fig. 1. Chromatograms of separation of the amino benzoic acids with mobile phases containing different concentrations of [BMIm][BF₄]. Concentrations of [BMIm][BF₄]: (a) 5.0 mmol/L; (b) 3.0 mmol/L; (c) 1.0 mmol/L; (d) 0.5 mmol/L. Chromatographic conditions: mobile phase, [BMIm][BF₄] solution (pH 3.0)/methanol (60:40, v/v); ZORBAX ODS column (250 mm × 4.6 mm i.d.); UV/vis detection, 245 nm; flow rate, 1.0 mL/min; column temperature, 30 °C; inject volume, 20 μL. Peaks: 1, PABA; 2, MABA; 3, OABA.

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