Analysis of Some β-Lactam Antibiotics Using Ionic Liquids as Mobile Phase Additives by RP-HPLC

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Abstract

The retentions of five β-Lactam antibiotics were analyzed by reversed-phased high-performance liquid chromatography. The mobile phase was acetonitrile/water (15/85, v/v) and the absorption wavelength was determined at 254 nm. When 1-butyl-3-methylimidazolium tetrafluoroborate was added into the mobile phase as additive, the samples were well separated with sharper peaks. The optimal concentration of 1-butyl-3-methylimidazolium tetrafluoroborate at pH 3 was 1.0 mM. The pH has large effect on the retention that the retention factor increasing with the pH of the mobile phase increasing. Three types of 1-alkyl-3-methylimidazolium-based ionic liquid (1-butyl-3-methylimidazolium tetrafluoroborate, 1-hexyl-3-methylimidazolium tetrafluoroborate, and 1-octyl-3-methylimidazolium tetrafluoroborate) were used and compared as additives in the mobile phase. The results showed that the retention time increased with increasing alkyl chain length. Among the three ionic liquids, the optimal effective additive was 1-butyl-3-methylimidazolium tetrafluoroborate.

Introduction

Room-temperature ionic liquids (ILs) are generally accepted as being environmentally benign solvents (1–3) with several unique properties (4–6), such as a wide liquid range, negligible vapor pressure, good thermal, chemical stability, and extraordinary dissolution properties of both organic and inorganic compounds. The application of ionic liquids in analytical chemistry has been reported in many areas, such as stationary phases in gas chromatography (7,8), buffer electrolytes in capillary electrophoresis (9), and mobile phase additives in reversed-phase liquid chromatography (RPLC) (10–13). In general, tailing problems associated with the poor separation reproducibility of basic analytes on chemically modified bonded-silica phases are often encounetred in RPLC. The addition of an ionic liquid could decrease this band tailing, reduce the band broadening, and improve the resolution. This is partly due to the competition between the imidazolium cations and polar groups of the analyte for the silanol group on the alkyl silica surface along with the formation of a weak bilayer electronic structure on the C18 column. Furthermore, the silanol-suppressing potency of ionic liquids is significantly higher than that of the standard alkylamine additives (11,14,15). β-Lactam antibiotics, including penicillins and cephalosporins, are widely used as pharmaceutical compounds in veterinary and human medicine. In veterinary practice, they are used for disease treatment and prevention. This can result in the presence of residues in edible tissues or derived products, which can lead to health problems in individuals who are hypersensitive and increase the incidence of microbial resistance against these compounds (16). Therefore, the development of rapid, specific and sensitive analytical methods to determine β-Lactam antibiotics is fundamental. Microbiological assays are the most commonly used techniques for detecting antibiotics in foods, and can provide an accept/reject decision at the farm level. However, their lack of specificity and precision makes them unsuitable for the quantitation and/or individual identification of antibiotics (17). The analyses of antibiotics by high-performance liquid chromatography (HPLC) have been widely reported (18–22). Previous researchers used a phosphate buffer solution as the mobile phase to determinate five β-Lactam antibiotics piperacillin, cefuroxim, ceftazidim, cefepim and meropenem in human plasma (23). However, the phosphate buffer was harmful to the HPLC column. Compared with the other methods, ionic liquid as a mobile phase additive can improve the resolution greatly with almost no damage to the C18 column. In addition, ionic liquids have much greater potential in future chromatographic separation due to their great variety. In this study, 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]), 1-octyl-3-methylimidazolium tetrafluoroborate ([OMIM][BF_4]), and 1-hexanyl-3-methylimidazolium tetrafluoroborate ([HMIm][BF_4]) which can reduce the interaction between β-Lactam antibiotics and stationary phase were first proposed. Several influencing factors, such as the types and concentration of ionic liquids and pH were investigated, and the optimum separation conditions were obtained.

Experimental

Materials and reagents

Authentic standards (pharmaceutical grade) were purchased from National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China. [BMIM][BF_4], [OMIM][BF_4]

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and [HMIm][BF_4] were offered by Professor Yoon-Mo Koo, Department of Bioengineering, Inha University, Korea (The structures of the five β-Lactam antibiotics and three ILs are shown in Figure 1). Acetonitrile (ACN) and other reagents were of analytical grade and purchased from Duksan Pure Chemicals Co., Ltd., Ansansi, Korea. The water used was doubly-distilled.

Stock standard solutions of ampicillin, cefazolin, ceftriaxone, cephradine, and cefotaxime were prepared in methanol. Working standard solutions containing each of the five compounds were prepared by diluting the stock solutions with methanol to proper volumes. The concentration of each sample in the mixture was prepared to 0.2 mg/mL, and the injection volume of mixture was 10 mL. The standard stock solutions and working solutions were all prepared in dark brown calibrated flasks and stored at 4°C until analysis.

Apparatus and chromatographic conditions

The HPLC system was equipped with Waters 600S solvent delivery system including 616 solvent delivery pump, 600S controller and the 2487 UV dual channel detector and an injector (0.02 mL sample loop) of Rheodyne (Waters, Milford, MA). The data acquisition system was Millenium32 (Waters) installed in a Sumsung PC.

Separation was accomplished on C18 column (4.6 × 250 mm, 5 µm) from RS Tech Co., Ltd., Daejeon, Korea. The flow rate was set at 0.5 mL/min. The pH of the mobile phases was adjusted using a 10% (v/w) hydrochloric acid solution or 0.01 *M* sodium hydroxide and measured by PH-211 meter (Sechang Instruments Co., Ltd, Seoul, Korea). The column temperature was maintained at room temperature.

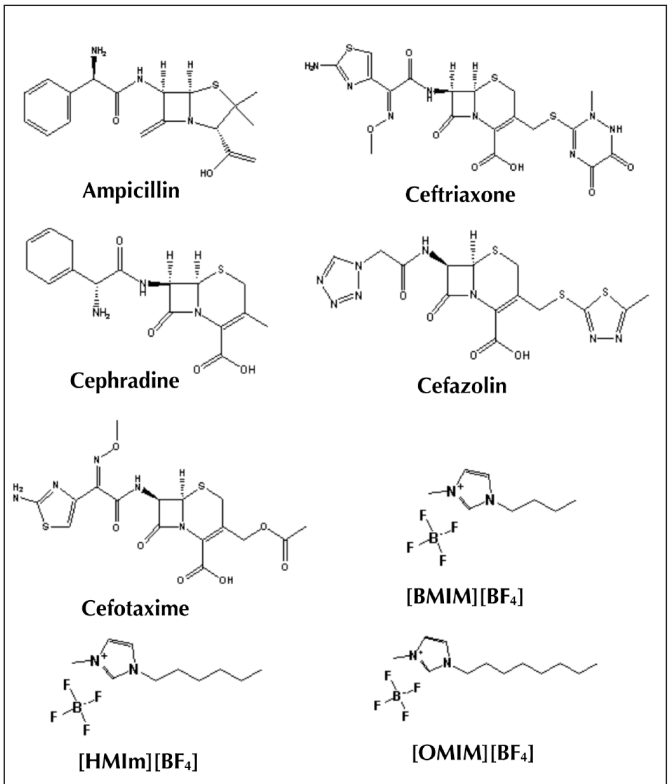

Figure 1. The structure of five kinds of β-Lactam antibiotics and three kinds of IL.

Preparation of standard solutions

Stock standard solutions of ampicillin, cefazolin, ceftriaxone, cephradine, and cefotaxime were prepared in methanol. Working standard solutions containing each of the five compounds were prepared by diluting the stock solutions with methanol to proper volumes. The concentration of each sample in the mixture was prepared to 0.2 mg/mL, and the injection volume of mixture was 10 mL. The standard stock solutions and working solutions were all prepared in dark brown calibrated flasks and stored at 4°C until analysis.

Results and Discussion

Determination of absorption wavelength and mobile phase

The absorbance maxima for these antibiotics ranged from 226 and 270 nm, but a fixed wavelength was used to monitor the eluate without any significant loss in sensitivity. In ACN–H_2O (15:85, v/v) mobile phase, 0.2 mg/mL mixed samples were injected at several UV wavelengths: 226, 230, 235, 240, 254, 270, and 280 nm. The optimal absorption wavelength of 254 nm was determined from a comparison of the peak-area and peak-height of each sample at different wavelengths.

To optimize the composition of the mobile phase, different concentrations of ACN–H_2O solutions: 5:95, 10:90, 15:85, 20:80, and 50:50 (v/v) were compared. According to the retention time and resolution, all the samples showed a large peak in the chromatogram when the ratio of ACN–H_2O > 15:85, and the retention time is long when the ratio < 15:85. Therefore, 15:85 of ACN–H_2O was selected as the mobile phase.

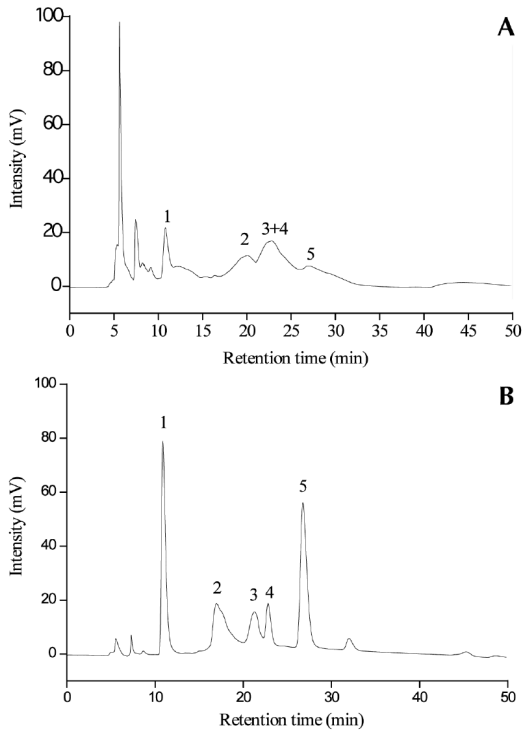

Figure 2. Chromatograms of the five β-Lactam antibiotics with a mobile phase containing different concentrations of (BMIM)(BF4) at pH 3.0.

Effect of the concentration of [BMIM][BF_4]

Figure 2 shows chromatograms of the five samples in the ACN–H_2O (15:85, v:v) mobile phase without [BMIM][BF_4] and with 1.0 mM [BMIM][BF_4] at pH 3, respectively. Obviously, the five β-Lactam antibiotics could not be separated without [BMIM][BF_4], but could be separated well in the [BMIM][BF_4] containing mobile phase. The resolution of the five samples was as follows: R12 = 3.06, R23 = 1.52, R34 = 0.91, and R45 = 2.17 (the peak names have been shown in the description for Figure 2). In addition, all peaks were sharper after IL had been added to the mobile phase. A previous study (11) of the mechanism for the action of [BMIM][BF_4] in a column reported that an ionic liquid exists in both solution and is coated on the column when used as the mobile phase additive in HPLC. Imidazolium cations can interact with silanol groups and compete for the silanol groups on the alkyl silica surface with the polar group of the analytes. Therefore, when a low amount of [BMIM][BF_4] was added to the mobile phase, the imidazolium cations could effectively shield the residual silanols then improve the peak shapes and decrease the retention time of the analytes. Figure 3 shows the retention factors of the five samples at the concentration of [BMIM][BF_4] used (0.5–3.0 mM) in the mobile phase at pH 3. The retention factor of all five samples initially decreased rapidly, then increased slowly with increasing [BMIM][BF_4] concentration. This is because when the concentration of [BMIM][BF_4] in the mobile phase is low, it will compete with the sample solutes adsorbing on the alkyl silica surface in the column and reduce their retention times. However, when much more [BMIM][BF_4] is added, it would not only affect with alkyl silica surface, but also effect with the sample solutes in the mobile phase, which would increase their retention time. However, the effect of the ILs and sample solute in increasing of the retention time is not very obvious. In addition, the retention time of the same solutes reached a minimum at 1.0 mM with increasing [BMIM][BF_4] concentration.

Effect of pH

Figure 3 shows the retention factors of the five samples in the ACN/H_2O (15:85, v:v) mobile phase containing [BMIM][BF_4] (0.5–3.0 mM) at pH 4 and 3, respectively. It is obvious that the pH of the mobile phase has a large effect on the retention of β-Lactam antibiotics with the retention times increasing with decreasing pH of the mobile phase. In addition, at pH 3, the resolution of the five β-Lactam antibiotics was better than at pH 4. In the mobile phase, [BMIM][BF_4] is ionized to $(BMIM)^+$ and $(BF_4)^-$. $(BF_4)^-$ is a chaotropic ion that can disrupt the sheath of water molecules around the basic analytes and decrease the eluting power of the analytes (24). Therefore, $(BF_4)^-$ can decrease the eluting power of the mobile phase by interacting with $(H)^+$ through a chaotropic effect, until all the $(BF_4)^-$ changes to HBF_4. At pH 3, there was much more $(H)^+$ in the mobile phase than at pH 4. So the interaction between $(BF_4)^-$ and $(H)^+$ was stronger. In addition, the interaction decreased the eluting power of the mobile phase, which increased the retention time.

In addition, at pH 4, the trend of the retention factors increased initially and then decreased. The situation was the opposite at pH 3. This shows that pH (i.e., hydrogen bonding) has a large effect on the retention, when the other conditions are the same. Figure 4 shows the resolutions (R) of the five samples using [BMIM][BF_4] as an additive at pH 4. Most of the resolution values were < 1.5, which means that the samples could not be

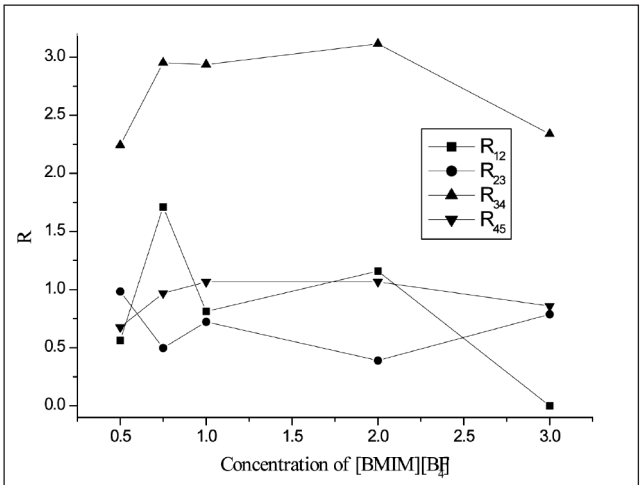

Figure 4. The error bar of resolutions (*R*) among the five samples using [BMIM][BF_4] as an additive at pH 4.

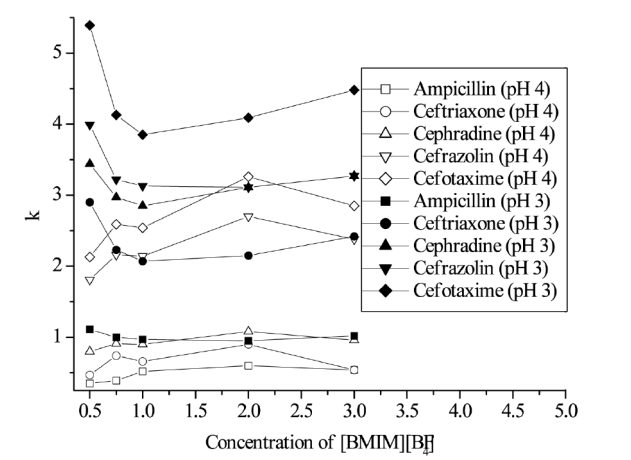

Figure 3. Effect of the (BMIM)(BF_4) concentrations on the retention factors (*k*) of the five samples at pH 4 and pH 3.

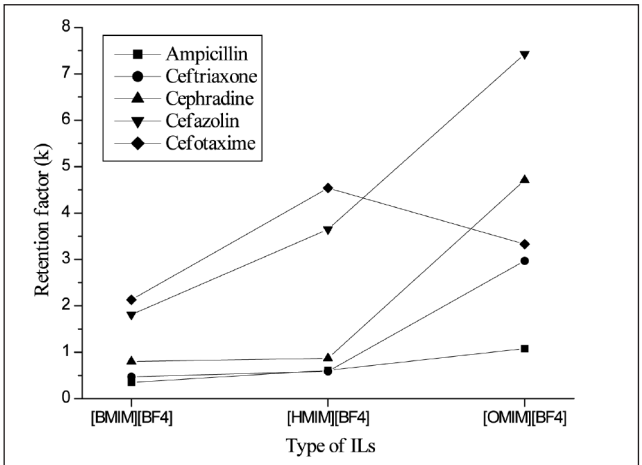

Figure 5. Effect of the different kinds of ionic liquids on the retention factors (k) of the five samples at pH 4.

separated completely at pH 4. Compared with the situation at pH 3, it is clear that a low pH value is beneficial to the resolution of the β-Lactam antibiotics.

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

To determine the effect of different alkyl groups on imidazolium cations, three types of 1-alkyl-3-methylimidazolium-based ionic liquids: [BMIM][BF_4], [HMIm][BF_4], and [OMIM][BF_4] were added to the mobile phase at 1 mM. Figure 5 shows the effects of these three types of IL on the retention of the samples are shown. The results show an opposite phenomenon from other basic compounds (12) in that the retention factors of the analytes increased with increasing alkyl chain length. It appears that the five β-Lactam antibiotics are not basic, but weak acids and the size of their structures is large. Therefore, their effects on alkyl silica or ILs are mainly through ion interactions. When the alkyl chain length of imidazolium cations is shorter, competition between the ILs and the sample solutes is larger, which results in a short retention time. Therefore, the optimal effective additive among the three ILs was [BMIM][BF_4]. In addition, cefotaxime appeared to be particularly different from the other samples using [OMIM][BF_4] as an additive, as shown in Figure 5. According to the structures in Figure 1, it is obvious that cefotaxime has a special ester group at the end, which is unlike the methyl or five or six-member ring of the other samples. Therefore, when using [OMIM][BF_4] as an additive, the ester group would increase the level of competition with it, which could decrease the retention time significantly.

Conclusion

The retention of five β-Lactam antibiotics was carried out by RP-HPLC. The separation conditions were determined to be a mobile phase of ACN–H_2O (15:85, v/v) and an absorption wavelength of 254 nm. The IL additive was effective on the retention of the five β-Lactam antibiotics. When [BMIM][BF_4] was added to the mobile phase, the samples could be separated well with sharper peaks. The optimal concentration of [BMIM][BF_4] at pH 3 was 1.0 mM. The pH has a large effect on the retention of the samples with pH 3 providing the best separation. Three types of 1-alkyl-3-methylimidazolium-based IL with three different alkyl chain lengths, [BMIM][BF_4], [HMIm][BF_4], and [OMIM][BF_4], were examined as additives in the mobile phase. The results showed that the retention factor increased with increasing alkyl chain length and the optimal effective additive among the three ILs is [BMIM][BF_4].

Acknowledgements

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