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Determination of Thiamine in Pharmaceutical Preparations by Reverse Phase Liquid Chromatography Without Use of Organic Solvent

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A novel green aqueous mobile phase modified with room temperature ionic liquids (RTILs) was employed in the absence of volatile organic solvents or ion-pairing reagents to analyze thiamine, a very polar compound, by reverse phase high performance liquid chromatography (RP-HPLC). Due to its strongly hydrophilic nature, thiamine was eluted near the column dead time (t_0) using a mobile phase without adding RTILs or ion-pairing reagents, even if a 100% aqueous mobile phase, which has weak elution power under reverse phase conditions, was used. Thus, 1-ethyl-3-methyl-imidazolium hexafluorophosphate ([EMIM][PF_6]), which has the strongest chaotropic effect, was selected as a mobile phase additive to improve retention and avoid baseline disturbances at t_0. Various mobile phase parameters such as cation moiety, chaotropic anion moiety, pH and concentration of RTILs were optimized to determine thiamine at the proper retention time. Method validation was performed to assess linearity, intra- and inter-day accuracy and precision, recovery and repeatability; all results were found to be satisfactory. The developed method was also compared to the current official United States Pharmacopoeia (USP) and Korean Pharmacopoeia (KP) methods using an organic mobile phase containing an ion-pairing reagent by means of evaluating various chromatographic parameters such as the capacity factor, theoretical plate number, peak asymmetry and tailing factor. The results indicated that the proposed method exhibited better efficiency of thiamine analysis than the official methods, and it was successfully applied to quantify thiamine in pharmaceutical preparations.

Key Words : Very polar compound, Thiamine, Room temperature ionic liquids (RTILs), Reverse phase high performance liquid chromatography (RP-HPLC)

Introduction

Thiamine, also known as vitamin B_1, is a water-soluble vitamin that belongs to a class of very polar chemical compounds. It plays an important role in carbohydrate metabolism,[1-3] being a coenzyme in three major enzyme complexes (α-ketoglutarate dehydrogenase, pyruvate dehydrogenase and transketolase), and it is necessary for the normal development and function of brain and nerves.[4-6] The recommended daily intake of thiamine for healthy adults is 1.5 mg.[7]

The high performance liquid chromatography (HPLC) analysis of very polar and ionic compounds is an important challenge in chemical, biomedical, pharmaceutical and environmental fields. In reverse phase high performance liquid chromatography (RP-HPLC) conditions, the mobile phase commonly consists of an organic solvent like methanol or acetonitrile mixed with water. Unfortunately, hydrophilic analytes are generally weakly retained and sometimes elute near the column dead time (t_0) under these conventional reverse phase conditions. This fact reduces the chromatographic resolution from complex matrix interferences, and baseline disturbance at t_0 can have a negative influence on the accurate quantification of analytes.

Thiamine contains a primary amine group on the pyrimidine moiety, and it is always ionized under acidic or neutral conditions (Figure 1). From an analytical point of view, these physicochemical properties make it difficult to analyze thiamine.[8] First, extremely polar thiamine is scarcely retained under general reverse phase conditions. Second, due to its amine structure, thiamine can strongly interact with free silanol groups on the surface of the stationary phase, which causes band broadening or tailing. These phenomena directly affect chromatographic resolution and quantitative analysis.

To solve these problems, several HPLC methods based on either pre- or post-column oxidation of thiamine to thiochrome allowing fluorescent detection (FLD) have been used.[9-13] However, the oxidation procedure is relatively time-consuming, and the product of derivatization, thiochrome, has chemical stability issues.[14] Reverse phase ion-pair chromatography with various counter ions (*e.g.*, alkanesulphonates) has also been used to separate thiamine from various matrices.[10,13,15-17] Ion-pairing reagents improve the chromatographic resolution and peak shape, particularly by reducing peak tailing. With these methods, the mixture of an

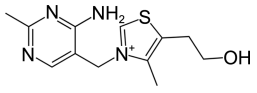

Figure 1. The chemical structure of thiamine.

acetate buffer with 1-octanesulfonate and organic solvents (acetonitrile and methanol) was selected as the mobile phase for the pharmaceutical analysis of thiamine in both United States Pharmacopoeia (USP)[18] and Korean Pharmacopoeia (KP)[19] monographs. Nevertheless, the ion-pairing reagents can result in irreversible damage to the column in terms of performance, including either a change in selectivity or longer equilibration times, due to adsorption of the counter ions onto the stationary phase.[20] Recently, several applications with specific stationary phases used for increasing retention time of thiamine were reported. A C_{30}-based stationary phase was adopted to determine water-soluble vitamins including thiamine in various food products,[21] and hydrophilic interaction liquid chromatography (HILIC) stationary phase was also employed for quantifying thiamine in meat sausages.[22] These methods can improve chromatographic separation of thiamine without the use of ion-paring reagents. However, with the C_{30} column, thiamine could not be completely separated from the baseline disturbance, and HILIC had some problems including peak broadening or tailing of thiamine. Also, the HILIC method generally requires a large consumption of hazardous organic solvents.

Nowadays, it is desirable to work with environmentally benign solvents, which means materials free of organic solvents or those that contain only a small amount of them. In this respect, room temperature ionic liquids (RTILs) have been globally introduced as green mobile phase additives.[23,24] RTILs are a type of semi-organic salt containing both cations and chaotropic anions exhibiting a melting point below 100 °C.[25] They have nonvolatile, nonflammable, thermally stable, and recyclable properties.[26] When used as mobile phase modifiers in RP-HPLC, RTILs improve basic compounds separation because they influence solute resolution and peak shape. This effect is explained in that their cation moieties suppress free silanol groups on the silica surface of stationary phase, thus the interaction between amine compounds and silanol group decreases.[25-27] Furthermore, chaotropic anion moieties of RTILs can act like ion-pairing reagents to enhance solute retention behaviors by chaotropic effect.[27,28] In contrast to other ion-pairing reagents, the use of RTILs which have short alkyl chains does not reduce column performance.[29] Various kinds of RTIL additives have already been investigated to separate amine compounds, and the object of those experiments was mainly to improve resolution and suppress the band tailing of analytes.[23,24,29-31] However, there is no scientific research using RTILs to enhance the retention of extremely hydrophilic and ionic compounds eluting close to the column dead volume under common reverse phase mobile phase conditions.

In this paper, a new RP-HPLC method was developed to increase retention of very polar thiamine using green aqueous mobile phase modified with hexafluorophosphate (PF_6) anion-based RTILs, which have the strongest chaotropic effect. By varying cation components, chaotropic anion components, pH and RTILs concentrations, optimal chromatographic conditions were established. To evaluate the effect of RTILs as mobile phase additives, the developed method was compared to the current official USP (KP) method with respect to capacity factor, theoretical plate number, peak asymmetry and tailing factor. As a result, this environmentally-friendly analytical technique showed better efficiency of thiamine analysis than the official method, and it was successfully applied to the quantification of thiamine in pharmaceutical preparations.

Experimental

Chemicals and Reagents. Thiamine hydrochloride and its tablet samples were obtained from Sigma-Aldrich (St. Louis, MO, USA) and Sinil Pharm Ltd. (Seoul, South Korea). RTILs: 1-ethyl-3-methylimidazolium chloride ([EMIM][Cl]), 1-ethyl-3-methylimidazolium bromide ([EMIM][Br]), 1-ethyl-3-methylimidazolium hydrogen sulfate ([EMIM][HSO_4]), 1-ethyl-3-methylimidazolium tetrafluoroborate ([EMIM][BF_4]), 1-ethyl-3-methylimidazolium hexafluorophosphate ([EMIM][PF_6]), 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]), 1-hexyl-3-methyl imidazolium hexafluorophosphate ([HMIM][PF_6]), 1-octyl-3-methylimidazolium hexafluorophosphate ([OMIM][PF_6]) and 1-butylpyridinium hexafluorophosphate ([Bpy][PF_6]) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan) except for [EMIM][Br], which was from Fluka (Buchs, Switzerland). Acetic acid (≥ 99.7%) and ammonium formate (≥ 97%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Formic acid and ammonium acetate (≥ 98%) were purchased from Fluka (Buchs, Switzerland) and Merck (Darmstadt, Germany), respectively. HPLC-grade water, acetonitrile and methanol were from Fisher Scientific Korea (Seoul, South Korea). All other reagents were of analytical grade except those used for HPLC, which were HPLC grade.

Apparatus and HPLC Analysis. The HPLC system was equipped with a Series 200 pump, a Series 200 column oven, a Series 200 vacuum degasser (PerkinElmer, Waltham, MA, USA), a 717 plus autosampler and a 966 photo diode array detector (Waters, Milford, MA, USA). Data acquisition, integration and processing were performed using Empower software (version 5.00.00.00, Waters).

Thiamine was eluted on a Luna C18 column (150 × 4.6 mm, 5.0 μm, Phenomenex, Torrance, CA, USA). The chromatographic separation was performed using mobile phase which consisted of 10 mmol/L ammonium formate (pH 3.5 adjusted with formic acid) with 30 mmol/L [EMIM][PF_6]. Retention data were recorded by an isocratic elution system at a flow rate of 1.0 mL/min, a column temperature of 30 °C and injection volume of 10 μL. The autosampler temperature was 5 °C to avoid degradation of thiamine, and thiamine was detected at 254 nm.

Preparation of Standard Solutions and Pharmaceutical Preparations. The stock standard solution of thiamine was obtained in 0.01 mol/L hydrogen chloride at a concentration of 2000 μg/mL and stored in a freezer at −80 °C. The working solutions were prepared daily by diluting the stock standard solutions with water prior to use.

The thiamine tablet samples were prepared by reference to

a KP monograph. Twenty tablets were accurately weighed and crushed into a fine powder. The powder equivalent to 20 mg of thiamine was shaken with 60 mL of 0.01 mol/L hydrogen chloride for 10 min on a rotary shaker and diluted to 100 mL with methanol. After centrifugation of the sample solution at 2500 rpm for 5 min, 25 mL of supernatant liquid was diluted to 50 mL with water for RP-HPLC analysis.

Results and Discussion

The retention time is one of the most important factors in the proper determination of analytes, and the capacity factor (k) can be an indicator of reverse phase chromatographic development, that is $k = (t_R - t_0)/t_0$ where t_R and t_0 are analyte retention time and column dead time, respectively. When the capacity factor is too small ($k < 0.5$), an analyte band may be adversely affected by any initial baseline disturbance at t_0 or an early-eluting impurity band. When the capacity factor is too large, other problems such as excessive band broadening and run times that are too long can be encountered.[20] In the case of very polar and ionic compounds like thiamine, capacity factors are usually less than 0.5, making it difficult to separate and quantify analytes. Therefore, this study set a goal to improve the thiamine capacity factor ($k > 5$) and adjust its proper retention time within 10 min using a RTIL additive. In addition, an aqueous solvent was employed as a mobile phase to realize green chromatography.

Optimization of Mobile Phase Modified with RTILs. In order to optimize the proposed aqueous mobile phase modified with RTILs, several experimental parameters affecting the capacity factor of thiamine were studied, including the type of chaotropic anions, type of cations, pH and RTILs concentrations. From pre-optimization of analytical parameter, the effects of each parameter were investigated. When one parameter varied for optimization, other parameters were fixed at the preselected optimal values. Testing samples were prepared by spiking 50 μg/mL of thiamine in pure water, and all experiments were performed in triplicate (n = 3).

Effect of Chaotropic Anion on Retention Behavior: Selecting a suitable chaotropic anion is a major consideration on retention control of a very polar compound. Varying chaotropic anion (SO_4^{2-}, Cl^-, Br^-, BF_4^- and PF_6^-), five [EMIM]-based RTILs were tested. The dependencies of retention behavior of thiamine on different chaotropic anions are presented in Figure 2. The retention of thiamine increased in the following order: [EMIM][PF_6] > [EMIM][BF_4] > [EMIM][Br] > [EMIM][Cl] > [EMIM][HSO_4], and the shapes of these relationships depended on anion chaotropicity according to the Hofmeister classification (PF_6^- > BF_4^- > Br^- > Cl^- ~ SO_4^{2-}).[28,32] The possible mechanisms of increasing analyte retention using chaotropic anions are as follows: firstly, chaotropic anions can destroy the solvation shells surrounding the polar and positively charged analyte and increase hydrophobic interaction between the analyte and stationary phase. Secondly, ion pair formation between the chaotropic anion and cationic analyte is feasible to form a neutral ion complex. Thirdly, the anion may also be adsorbed on the stationary phase surface and retard the analyte by a dynamic ion exchange mechanism. Among the chaotropic anions, only [EMIM][PF_6], which has the strongest chaotropicity, satisfactorily retained thiamine at about 8.1 min (k : 5.7), while the other [EMIM]-based RTILs did not retain thiamine for longer than 3 min ($k < 1.3$). It was thought that the repulsion phenomenon between RTILs cations and the analyte might take precedence over anion chaotropicity in the absence of PF_6^-. Therefore, in this case, there was no alternative choice, and PF_6^- was selected as the chaotropic anion of RTILs in the mobile phase.

Effect of Cation on Retention Behavior: To evaluate the effect of RTIL cations on retention behavior, four kinds of 1-alkyl-3-methylimidazolium hexafluorophosphate ([C_nMIM][PF_6], n = 2, 4, 6, 8), each having different alkyl chain lengths, and 1-butylpyridinium hexafluorophosphate, ([Bpy][PF_6], n = 4), were investigated (Figure 3). With increasing alkyl chain length in the 1-alkyl-3-methylimidazolium group, the capacity factor of thiamine rapidly decreased and approached zero when the number of carbon atoms (C_n) of the alkyl chain was greater than six ($[HMIM]^+$ and $[OMIM]^+$). Moreover, when $[HMIM]^+$ and $[OMIM]^+$ were used as additives, a certain amount of organic solvent (methanol) had to be included in the mobile phase because of their low solubility in pure water. This made analyte retention much more difficult. Even though both are having same four carbon atoms

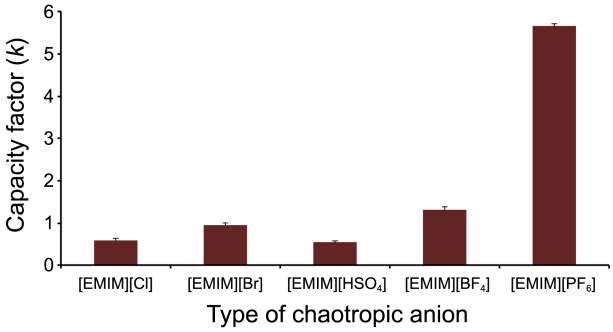

Figure 2. Effect of chaotropic anion type on the capacity factors (k) of thiamine. Mobile phase: 10 mmol/L ammonium formate (pH 3.5) with the addition of different 30 mmol/L [EMIM]-based RTILs.

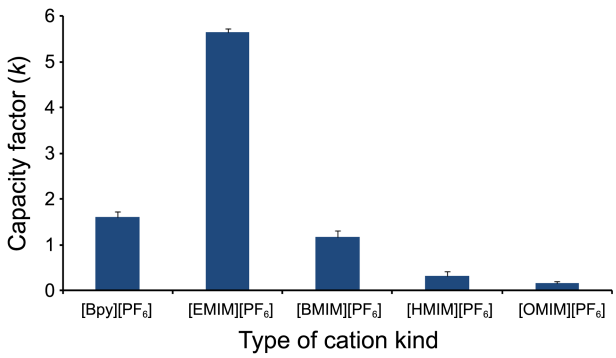

Figure 3. Effect of cation type on the capacity factors (k) of thiamine. Mobile phase: 10 mmol/L ammonium formate (pH 3.5) with the addition of different 30 mmol/L [PF_6]-based RTILs. Methanol was added into the mobile phase when [HMIM][PF_6] and [OMIM][PF_6] were used as mobile phase additives.

(C_4), $[Bpy]^+$ showed a slightly higher capacity factor than $[BMIM]^+$. However, the retention of thiamine was still insufficient to analyze (k < 1.6). $[EMIM]^+$ improved analyte retention approximately 2.5 times compare to $[Bpy]^+$. The results indicated that the hydrophobic interactions of the RTIL cations with thiamine were predominant to chaotropicity of PF_6^- when the alkyl chain length was longer than the ethyl moiety. In addition, the cations of RTILs with a long alkyl chain could dynamically coat more C18 groups of the stationary phase and then reduce the adsorption probability of the analyte due to charge repulsion. Thus, a low hydrophobic cation with a short alkyl chain length was more suitable to properly increase the retention of thiamine, and $[EMIM]^+$ was chosen as the cation of RTILs in the mobile phase. Not only increased retention, improved peak shape of thiamine was observed. This may be due to a suppression of the free silanol group of the stationary phase by $[EMIM]^+$, which efficiently blocked the interaction between thiamine and the silanol group.

Effect of RTIL Concentration on Retention Behavior: The concentration of RTILs is able to affect the retention and separation of analytes. By varying the $[EMIM][PF_6]$ concentration (0, 5, 15, 25, 30, 35, 50, and 70 mmol/L) in the mobile phase, the retention performance of thiamine was tested. As shown in Figure 4, the capacity factor of thiamine increased sharply in the 0-15 mmol/L concentration range and gradually increased in the 15-30 mmol/L concentration range. A further increase in the concentration of $[EMIM][PF_6]$ did not lead to a continued increase in the capacity factor, which actually decreased when the concentration of $[EMIM][PF_6]$ was greater than 50 mmol/L. These phenomena were thought to arise from the multiple chemical reactions due to the complexity of the RTIL structure. At a low concentration of $[EMIM][PF_6]$, the chaotropic effect is predominant because $[EMIM][PF_6]$ consists of a strong lyotropic anion (PF_6^-) and a relatively weak hydrophobic cation ($[EMIM]^+$). However, at high concentrations of $[EMIM][PF_6]$, the hydrophobicity of $[EMIM]^+$ seems to be greater than that of the analyte. Therefore the competition between $[EMIM]^+$ and analyte for adsorption of C18 groups on the surface of the stationary phase intensifies. This leads to a repulsive force of

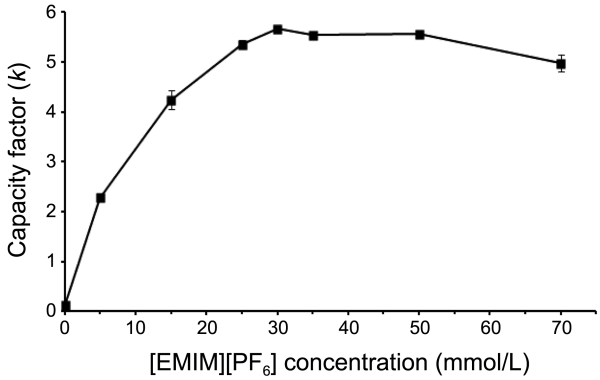

Figure 4. Effect of $[EMIM][PF_6]$ concentration on the capacity factors (k) of thiamine. Mobile phase: 10 mmol/L ammonium formate (pH 3.5) with different concentrations of $[EMIM][PF_6]$.

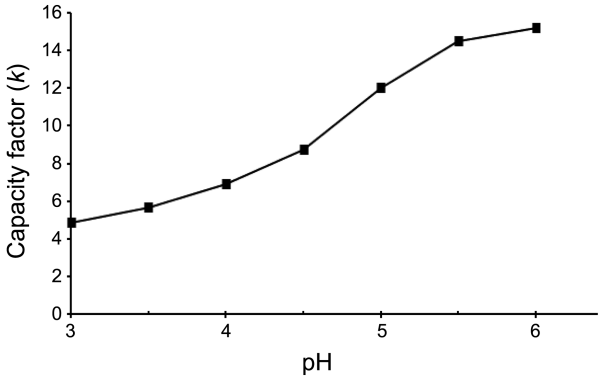

Figure 5. Effect of mobile phase pH on the capacity factors (k) of thiamine. Mobile phase: 10 mmol/L ammonium formate (pH 3.0 to 6.0) with 30 mmol/L $[EMIM][PF_6]$.

$[EMIM]^+$ stronger than the chaotropicity of PF_6^-. In this work, the highest capacity factor of thiamine was obtained at a concentration of 30 mmol/L, and that concentration seemed to be a turning point in the balance of the retention power between cation and anion. Therefore, 30 mmol/L was selected as the optimum concentration of $[EMIM][PF_6]$.

Effect of pH on Retention Behavior: The retention of polar and ionic compounds depends on the mobile phase pH, which affects the degree of ionization of the analytes in reverse phase chromatography. Therefore, the effect of mobile phase pH on retention behaviors of thiamine was examined using various pH values (pH 3.0-6.0). The pH adjustment was performed using an ammonium formate buffer at pH 3.0-4.5 and an ammonium acetate buffer at pH 4.5-6.0 by adding formic acid and acetic acid, respectively. The results are plotted in Figure 5. With an increase of the mobile phase pH, the retention of thiamine increased, but the theoretical plate number decreased. When pH was raised without adding $[EMIM][PF_6]$, the mobile phase not only decreased the theoretical plate number, but peak symmetry also rapidly deteriorated (data not shown). It is believed these results were caused by increasing the amount of ionized silanol groups on stationary phase in the absence of of RTILs, and increasing the incomplete ionization of thiamine while the pH of mobile phase increases as the pK_a of the analyte is 5.5. Hence, the mobile phase should be adjusted to a pH below 4.5. Considering both the appropriate capacity factor (k > 5) and satisfactory peak shape of thiamine, the pH value of the mobile phase was adjusted to 3.5 in the following experiments.

Method Validation. In order to validate the developed chromatographic method, analytical parameters including selectivity, linearity, intra- and inter-day accuracy, precision, recovery and repeatability were investigated. Method validation was performed by referring to the international conference on harmonization of technical requirements for registration of pharmaceuticals for human use (ICH) harmonized tripartite guideline (validation of analytical procedures: text and methodology Q2(R1), current step 4 version).[33]

Linearity: The linearity was evaluated within the concentration range of 80 to 120 μg/mL of thiamine. Calibration curves were created by plotting peak area against corre-

Table 1. The calibration data of thiamine, including regression equation, linear range and correlation coefficient

Analyte	Regression equation	Linear range (μg/mL)	Correlation coefficient (r^2)
thiamine	y = 20443x – 24220	80-120	0.9999

sponding concentration (μg/mL) of the analyte. The calibration data including linear range, the regression equation and correlation coefficient (r^2) are given in Table 1. Sufficient linearity was observed as a high r^2 value above 0.999.

Intra- and Inter-Day Accuracy and Precision: The intra- and inter-day accuracy and precision were assessed from repeated experiments (n = 5) at three different concentrations of thiamine within a linear range. The accuracy was expressed as an observed concentration relative to a nominal concentration, and the precision was calculated using the relative standard deviation (coefficient of variation, CV). The intra-day accuracy was 100.04-100.12%, and inter-day accuracy was 99.67-99.97%. The intra-day precision was 0.12-0.24%, and the inter-day precision was 0.29-0.37% (Table 2).

Recovery: The recovery was calculated according to a standard addition procedure at low, medium, and high concentrations (10, 20 and 40 μg/mL) of thiamine. Recovery was evaluated by spiking pharmaceutical preparation samples with a standard. To determine recovery, six replicates were measured at each concentration (n = 6). The mean recovery of the analyte was 98.6-102.1% (Table 3).

Repeatability: The repeatability was assessed from six repeated determinations (n = 6) of thiamine by calculating the relative standard deviation (coefficient of variation, CV) under the same operating conditions over a short interval of time. A CV value less than 1.0% was obtained, thus the repeatability was deemed acceptable.

Application in Pharmaceutical Preparations. To evaluate the applicability of the method, thiamine tablet samples were prepared by reference to a KP monograph and analyzed by a proposed optimum and validated chromatographic method. Three batches of the tablets were estimated (n = 3), and the amount of thiamine was calculated from the related linear regression equation. The retention time of thiamine was about 8.1 min, and no interference was observed at the retention time of the analyte. The mean amount of drug present per tablet was found to be 10.24 ± 0.10 mg, and there was good agreement between the amounts estimated and those claimed by the manufacturers (labeled content: 10 mg/tablet). Also, the test was repeated 50 times (n = 50) to determine whether pure aqueous eluents could damage the silica-based column (Figure 6). The results indicated that the capacity factor and peak symmetry remained unchanged during the tests, illustrating the robustness of the method.

Table 2. The intra- and inter-day precisions and accuracies of thiamine (n = 5)

Analyte	Concentration (μg/mL)	Precision (C.V, %)		Accuracy (%)	
		Intra-day	Inter-day	Intra-day	Inter-day
thiamine	80	0.12	0.37	100.12	99.67
	100	0.14	0.30	100.09	99.93
	110	0.24	0.29	100.04	99.97

Table 3. Recovery assay of the proposed method (n = 6)

Analyte	Initial amount (mg)	Amount added (mg)	Measured amount (mg)	Recovery (%)	
				Mean	RSD[a]
thiamine	10.00	1.00	10.99	98.6	1.6
		2.00	12.04	102.1	1.1
		4.00	13.98	99.6	0.3

[a]Relative Standard Deviation

Comparison with Current Official Analytical Methods. The developed chromatographic method was compared to the current official USP (KP) method using a mobile phase containing an organic solvent and ion-pairing reagent. For an official analytical method of thiamine, 0.005 mol/L sodium 1-octanesulfonate in 1% glacial acetic acid (solvent A) and the mixture of acetonitrile-methanol (60/40, v/v) (solvent B) were previously prepared, and the solvent A-solvent B mixture (77/23, v/v) was used as mobile phase. The typical chromatograms of thiamine employing the proposed method and the official method are shown in Figure 7, and the

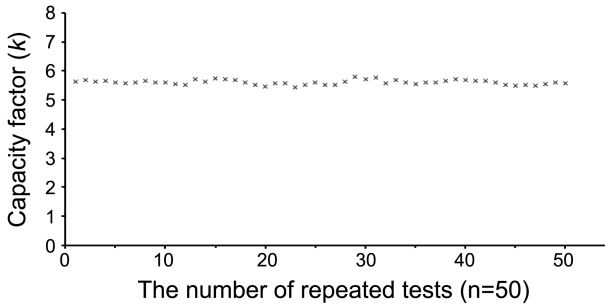

Figure 6. Dependence of capacity factors (k) of thiamine versus the number of repeated tests (n = 50) using aqueous 10 mmol/L ammonium formate (pH 3.5) with 30 mmol/L [EMIM][PF_6] as a mobile phase.

Table 4. Comparison of the proposed method with the current official method

Analytical method	Mobile phase components	Capacity factor (k)	Theoretical plate number (N)	Peak asymmetry factor (A_s)	Tailing factor (T_f)
Proposed method	Aqueous solvent with [EMIM][PF_6]	5.66	3687	1.27	1.13
USP (KP) method	23% organic solvent with sodium 1-octanesulfonate	5.37	1883	2.09	1.55

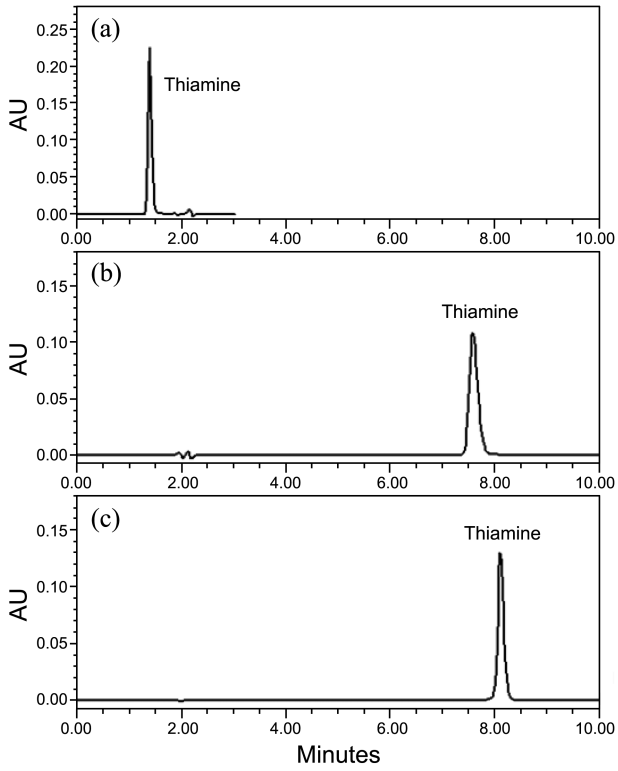

Figure 7. Chromatograms of thiamine with (a) an aqueous mobile phase without [EMIM][PF_6], (b) an organic mobile phase containing sodium 1-octanesulfonate (current official method) and (c) an aqueous mobile phase containing 30 mmol/L [EMIM][PF_6] (proposed method).

chromatographic parameters of the two methods, including mobile phase components, capacity factor, theoretical plate number, peak asymmetry and tailing factor, are listed in Table 4. Compared with the official methods, our method obtained a higher theoretical plate number and a lower peak asymmetry and tailing factor. Also, a volatile organic solvent which can be harmful to human health and the laboratory environment were not a component of the method. In summary, capacity factor values were in a similar range ($5 < k < 6$) for both methods. Overall, the proposed method might be a potential alternative for one of the current official methods.

Conclusion

In the present study, an effective RP-HPLC method using an aqueous mobile phase modified with RTILs has been described for the determination of thiamine. In spite of the analyte's strong polarity, the use of [EMIM][PF_6] in the mobile phase allowed complete control of the retention and separation of the analyte. Compared with current official methods (USP and KP) using an organic mobile phase with ion-pairing reagents, the developed method increased the theoretical plate number and reduced band tailing without the use of any toxic organic solvents or ion-pairing reagents. The proposed method was successfully utilized to quantify thiamine in pharmaceutical preparations, and it will be further tested as a novel green approach to replace the current methods to determine very polar compounds.

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