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Ultrasonication-assisted extraction and preconcentration of medicinal products from herb by ionic liquids

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ABSTRACT

Ionic liquid-based extraction of medicinal or useful compounds from plants was investigated as an alternative to supercritical fluid, cloud point and conventional organic solvent extractions. The method integrated extraction and preconcentration. Medicinal products were first extracted by an ionic liquid solution, part of which was then converted to a hydrophobic form by anion metathesis for preconcentration. The remaining soluble ionic liquid acted as a dispersive agent to enhance the efficiency of preconcentration. Protein in the extract was precipitated spontaneously without addition of further solvents. Ultrasonication assisted this method for extraction and preconcentration of cryptotanshinone, tanshinone I and tanshinone II A from *Salvia Miltiorrhiza* Bunge. 0.233 mg g^{-1}, 0.695 mg g^{-1} and 0.682 mg g^{-1} of each, respectively, were extracted using [OMIM][Cl], and preconcentrated in a [OMIM][PF_6] phase at respective concentrations of 148.1, 507.1 and 486.1 μg mL^{-1}. The method exhibited potential applicability with other medicinal products.

1. Introduction

Medicinal compounds in functional foods, herbs and nutraceuticals, such as polyphenolics, nitrogen compounds, vitamins, terpenoids and other endogenous metabolites, can prevent or even treat diseases [1,2]. While methods of analyzing these compounds have developed well [3–6], the preparation of samples has not seen such advances. Obtaining such medicinal products at adequate concentrations usually involves extraction with large amounts of toxic, organic solvents, followed by evaporation. With increasing demand for samples from environmental and pharmaceutical industries, less environmentally damaging techniques is required [7], prompting the development of supercritical fluid (SFE) and cloud point extractions [8–10]. The former uses pure or modified carbon dioxide (CO_2) to extract analytes; it is attractive for its short extraction time and use of non-toxic solvent. Disadvantages lie in its required sample sizes and high equipment costs [11]. Cloud point extraction (CPE), also efficient and green, is primarily based on nonionic and zwitterionic surfactants. However, extraction is significantly affected by the cloud point temperature, which may degrade thermally labile analytes [12]. Ionic liquid (IL)-based extraction was developed to overcome these deficiencies.

ILs have great potential for extraction [13–15]. They can improve the selectivity and extraction yields of bioactive compounds while polluting less than conventional organic solvents. However, most research focuses on extraction, giving scant regard to subsequent preconcentration. Determination of low concentration analytes is difficult due to the influence of the IL. Preconcentration of the analytes would therefore be necessary. Evaporation, addition of other hydrophobic solvents and solid-phase microextraction are complicated and not particularly efficient. Through their soluble Cl^- ions, ILs can be changed to hydrophobic liquids by anion metathesis, allowing analytes to be preconcentrated by the hydrophobic ILs. Due to their relatively high concentration in extracts, conversion of all the soluble ILs to hydrophobic form is inefficient. Instead, some Cl^- form ILs are converted to hydrophobic form, while the remaining soluble ILs can act as a dispersive agent (Fig. 1). Ultrasonication can aid extraction and dispersion [15]. By previous investigations, an integrated method is established for the extraction and preconcentration of medicinal products. According to the special interactions between ILs and aromatic or π-bonded compounds, the method was tested by extracting the slightly water-soluble compounds, cryptotanshinone (CT), tanshinone I (T I) and tanshinone II A (T IIA) (Fig. 2) from *Salvia Miltiorrhiza* Bunge (*SMB*).

Tanshinones are abietanoid diterpenes found in the traditional medicinal herb *SMB* [16] and have been used to treat many diseases with their powerful antibacterial [17], anticancer [18,19], anti-inflammatory [20], antioxidant [21] and hepatic-protection [22] activities. Extraction of tanshinones from *SMB* by organic solvents has often been reported with subsequent analysis by HPLC [23,24] and spectrophotometry [25]. Despite CPE's attractiveness [26], its applicability is limited in such circumstances. In order to simplify

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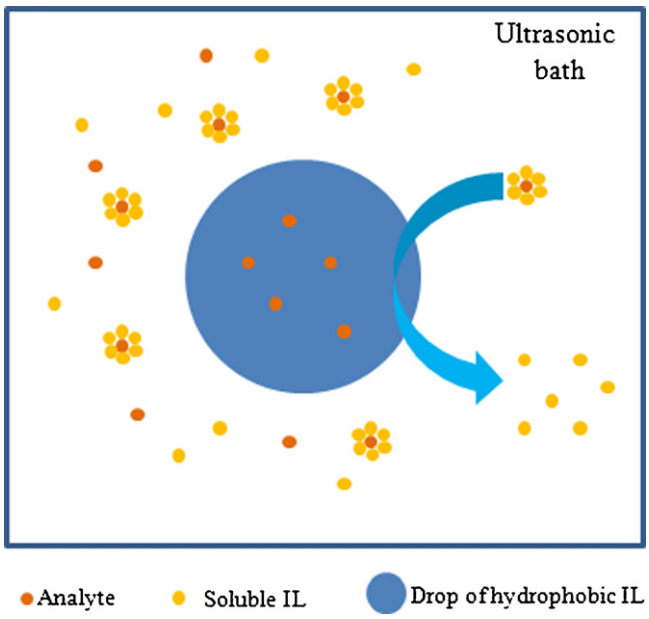

Fig. 1. Ultrasonic-assisted IL-based extraction.

extraction and increase its efficiency, the ILs were employed to extract and to preconcentrate tanshinones.

This work reports the systematic optimization of the use of aqueous IL solutions for ultrasonication-assisted extraction. Part of the IL was subsequently converted to hydrophobic form to achieve phase separation. All significant variables affecting preconcentration were studied, including the amount of anion metathesis agent, sodium chloride concentration, centrifuging duration, and ultrasonication power and duration. Higher concentrations of analytes were obtained in a simpler and less environmentally damaging manner than by conventional extraction and preconcentration.

2. Experimental

2.1. Materials

Cryptotanshinone (CT), tanshinone I (T I) and tanshinone II A (T IIA) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). 1-Ethyl-3-methylimidazolium chloride ([EMIM][Cl]), 1-butyl-3-methylimidazolium chloride ([BMIM][Cl]), 1-hexyl-3-methylimidazolium chloride ([HMIM][Cl]) and 1-oxyl-3-methylimidazolium chloride ([OMIM][Cl]) were supplied by the Kowoon Institute of Technology (Whasung-gu, Korea). Hexafluorophosphoric acid solution (*ca.* ~65 wt.% in water) was obtained from Sigma (St. Louis, MO, USA). Methanol, ethanol, ethyl acetate, dichloromethane n-hexane and acetone were purchased from Duksan Pure Chemical Co., Ltd. (Ansan, Korea). Water was twice distilled and filtered (FH-0.45 μm, Advantec MFS, Inc., Japan) using a decompressing pump (Division of Millipore, Waters, USA).

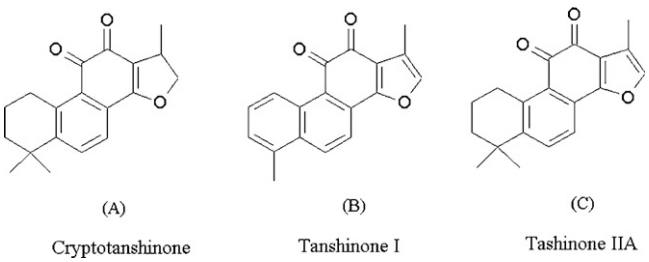

Fig. 2. Chemical structures of tanshinones.

2.2. Apparatus

The HPLC system comprised a M930 solvent delivery pump (Young Lin Co., Korea), a UV detector (M720 Absorbance Detector, Young Lin Co., Korea) and an integrated data system (Autochrowin. Ver. 1.42, Young Lin Co., Korea). Injection valves with 20.0 μL sample loops were used. The HPLC analysis was performed with a commercial C_{18} column (4.6 mm × 150 mm, 5.0 μm) purchased from RStech Co. (Daejeon, Korea). Ultrasonication was carried out using the ultrasonic bath (MIRAE ULTRASONIC TECH. Co., Bucheon, Korea, 35 kHz).

2.3. Extraction of SMB

SMB (Incheon, Korea) from local market was oven-dried, sliced and crushed. Conventional solvent extraction was carried out by ultrasonic extraction of 0.05 g *SMB* samples with different solvents. Ultrasonic-assisted IL-based extraction was similar: 0.05 g samples were mixed with different concentrations of aqueous IL solutions. Extraction conditions were systematically optimized in this work. Extraction suspensions were filtered through a 0.45 μm filter and collected for preconcentration. Repeatability was tested by extracting *SMB* powders three times over a 5-day period. Two-sided *t*-tests were used to evaluate the data of independent samples.

2.4. Preconcentration

A 1.7 mL centrifuge tube was filled with 1.0 mL extract. Turbidity resulted upon addition of aqueous HPF_6. After ultrasonication, the turbid solution was centrifuged at 10,000 rpm (10,845 × *g*). The upper aqueous solution was removed with a pipette, and the IL residue enriched with analytes was withdrawn into a syringe and injected into a HPLC column. The syringe was then rinsed with methanol and acetonitrile repeatedly to remove residual analyte and IL. All significant variables were investigated.

2.5. HPLC analysis

The samples were directly injected into the liquid chromatography. The mobile phase was methanol/water (78/22, v/v, containing 0.5% acetic acid) and its flow rate was set at 0.5 mL min^{-1}. The injection volume was 10.0 μL and the wavelength of UV detector was set at 254 nm [27]. Each sample was injected 5 times to evaluate the precision and accuracy of the analysis.

3. Results and discussion

3.1. Extraction of tanshinones from SMB

3.1.1. Effect of ILs

ILs are strong solvents and were employed as solvents and co-solvents in the extraction of the analytes [28,29]. Their structures significantly influence their physicochemical properties and thus extraction efficiency. ILs with various cations and anions should be investigated to evaluate their performance in the extraction of tanshinones. However, analytes were to be preconcentrated using soluble ILs converted to hydrophobic form by anion metathesis, requiring Cl^- anions. Hence, [EMIM][Cl], [BMIM][Cl], [HMIM][Cl] and [OMIM][Cl], all with Cl^- anions were tested to investigate the influence of their cations (Fig. 3A). Increasing alkyl chain length

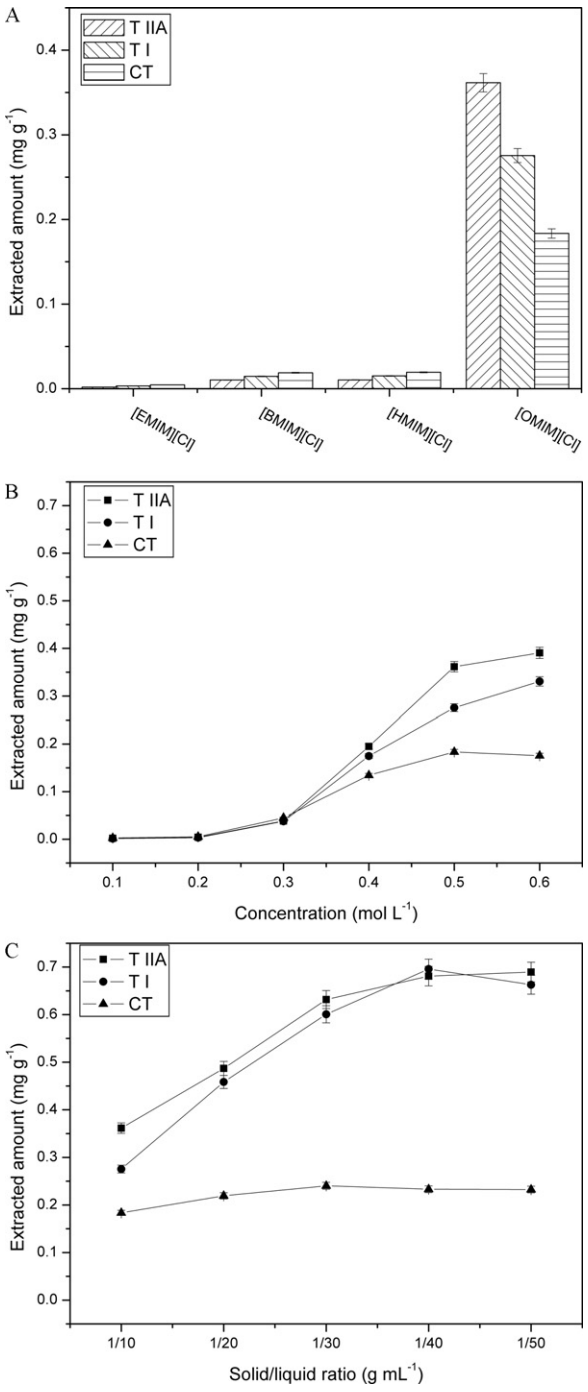

Fig. 3. Effect of ILs (A), concentration (B) and solid/liquid ratio (C) on the extracted amounts of tanshinones. (A) 0.50 mol L^{-1} IL in water, solid/liquid ratio (g mL^{-1}) = 1/10, ultrasonic power = 105.0 W, duration = 80.0 min, room temperature. (B) Solid/liquid ratio (g mL^{-1}) = 1/10, ultrasonic power = 105.0 W, duration = 80.0 min, room temperature. (C) 0.5 mol L^{-1} [OMIM][Cl] in water, ultrasonic power = 105 W, duration = 80.0 min, room temperature.

influenced the extraction, with [OMIM][Cl] being the most efficient IL. The hydrogen bond acidity [14] and the hydrophobicity of the cations increased from ethyl to oxyl at 1-position of the 1-alkyl-3-methylimidalizium ring, resulting in the strongest interactions of [OMIM][Cl] with the tanshinones. Further increases of alkyl chain length decreased extraction efficiency with high viscosity, but would also complicate the samples' injection during preconcentration. [OMIM][Cl] was selected for use in the subsequent experiments.

3.1.2. Effect of IL concentration

Different concentrations of the aqueous IL (from 0.10 to 0.60 mol L^{-1}) were used to extract tanshinones from *SMB* (Fig. 3B). The amounts of extracted tanshinones increased as IL concentration increased from 0.10 to 0.50 mol L^{-1}. However, the amount of extracted CT decreased at 0.60 mol L^{-1} due to changes of diffusion and transfer capacities. Therefore, 0.50 mol L^{-1} of [OMIM][Cl] was selected for the following experiments.

3.1.3. Effect of solid/liquid ratio

Analyte extraction also depended on the solid/liquid ratio. Large volumes of solvent are not only uneconomic but also wasteful. A series of extractions was carried out at different solid/liquid ratios (1/10, 1/20, 1/30, 1/40 and 1/50, g mL^{-1}) to evaluate its effect (Fig. 3C). Extracted amounts reached their highest at a solid/liquid ratio of 1/40 (g mL^{-1}). The solvation of medicinal components is a physical process. With increasing amounts of solvent, the chance of target molecules coming into contact with the solvent also increases, leading to higher leaching-out rates. The solid/liquid ratio of 1/40 (g mL^{-1}) was considered optimal given economic considerations.

3.1.4. Effect of ultrasonication power

The effect of ultrasonication power on extraction was examined, as it has been reported to influence interactions and equilibrium rates, and to affect the distribution of the analyte between the sample and extracting phases. Extractions were carried out at 15.0, 45.0, 75.0, 105.0 and 135.0 W for 80.0 min. Ultrasonication power above 105.0 W did not significantly influence extraction. Considering energy efficiency, 105.0 W was considered suitable.

3.1.5. Effect of extraction duration

To optimize the extraction duration, extractions were carried out at 105.0 W for 20.0–100.0 min. Extraction improved greatly as the extraction duration increased from 20.0 to 80.0 min. Above 80.0 min, no obvious increase in tanshinone extraction was observed.

Overall, the most efficient conditions for extraction were 0.50 mol L^{-1} [OMIM][Cl] in water with an ultrasonication power of 105.0 W for 80.0 min, at a solid/liquid ratio of 1/40 (g mL^{-1}). Under these conditions, 0.233 mg g^{-1} CT, 0.695 mg g^{-1} T I and 0.682 mg g^{-1} T IIA were extracted from *SMB*.

3.1.6. Comparison of traditional solvent and IL-based extraction

To compare the efficiencies of IL-based and conventional solvent extractions, water, n-hexane, ethanol, methanol, dichloromethane, acetone and ethyl acetate were used to extract three tanshinones from *SMB* under optimal conditions. The tanshinones sparingly dissolve in water, but the IL-based method obviously increased their solubility (Fig. 4). Further comparison of the solvents demonstrated the excellent extraction efficiency of the aqueous IL solution, likely due to the multiple-interactions, such as π–π, dipole–π and hydrophobic interactions, between [OMIM][Cl] and the tanshinones. It may also be attributable to the IL acting as a surfactant to increase the solubility of analytes. Aqueous [OMIM][Cl] was shown to be effective for the extraction of tanshinones from *SMB*.

3.2. Preconcentration of tanshinones from extract

3.2.1. Selection of anion source for anion metathesis

During extraction, part of the IL in the extract converted to hydrophobic form by anion metathesis. This required an appropriate anion source, as it could affect the properties of the hydrophobic IL and the efficiency of preconcentration. [C_nMIM][PF_6] and [C_nMIM][Tf_2N] have been reported to be excellent hydrophobic extractants [30–32], therefore HPF_6 and $LiNTf_2$ were selected as

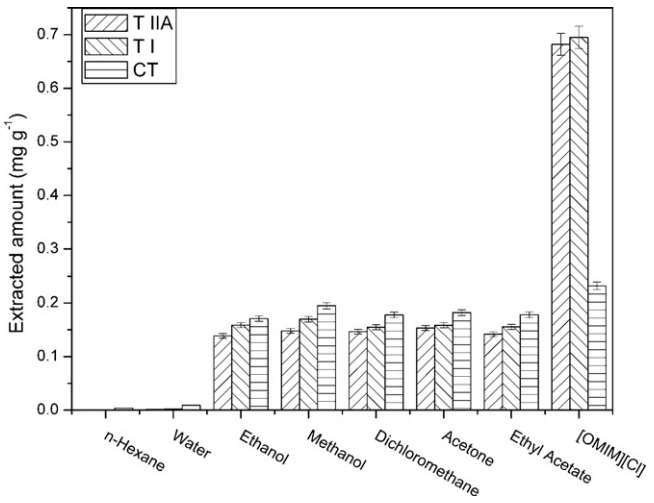

Fig. 4. Comparison of traditional solvent and ultrasonic-assisted IL-based extraction (0.5 mol L^{-1} [OMIM][Cl] in water, solid/liquid ratio (g mL^{-1}) = 1/40, ultrasonic power = 105.0 W, duration = 80.0 min, room temperature).

anion sources. Equimolar amounts of the anion sources were added for the preconcentration, with [OMIM][PF_6] (0.039 mg mL^{-1} CT, 0.113 mg mL^{-1} T I and 0.108 mg mL^{-1} T IIA in the IL phase) showing higher affinity to the tanshinones than [OMIM][Tf_2N] (0.033 mg mL^{-1} CT, 0.101 mg mL^{-1} T I and 0.101 mg mL^{-1} T IIA in the IL phase), because of the different miscibilities of tanshinones in the ILs. Therefore, [OMIM][PF_6] was chosen for subsequent experiments.

After the addition of HPF_6 to the extract, protein was unexpectedly precipitated as [OMIM][PF_6] formed (Fig. 5B). This may have been due to the generation of HCl after anion metathesis (equilibrium (1)). Thus, preconcentration of tanshinones and precipitation of protein can be achieved simultaneously, simplifying the process and avoiding blockage of the LC column by protein (Fig. 5):

$$[OMIM][Cl] + HPF_6 \leftrightarrow [OMIM][PF_6] + HCl \quad (1)$$

3.2.2. Effect of HPF_6 volume

The volume of added HPF_6 can significantly affect the amount of [OMIM][PF_6], influencing preconcentration efficiency. Less [OMIM][PF_6] will lead to a higher concentration of tanshinones in it. However, quite a small amount of IL caused the extraction to become difficult and insufficient, likely reducing accuracy and reproducibility. Conversely, more [OMIM][PF_6] implies less remaining [OMIM][Cl] dispersive agent, which would then result in inefficient extraction to the [OMIM][PF_6] phase. Therefore, the effect of HPF_6 volume on the tanshinones' concentrations in [OMIM][PF_6] was investigated from 10.0 to 68.0 μL mL^{-1} (Fig. 6A). Tanshinones' concentrations decreased with increasing HPF_6 volume. Although the highest tanshinone concentrations

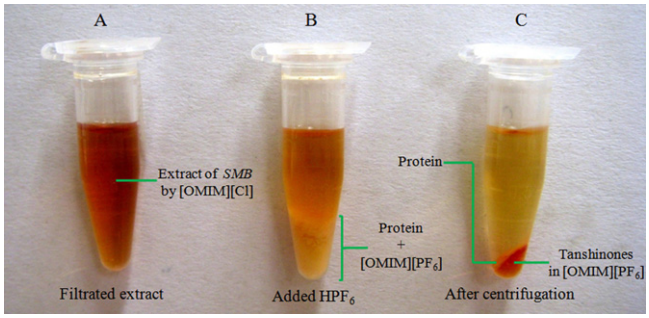

Fig. 5. Scheme of preconcentration process.

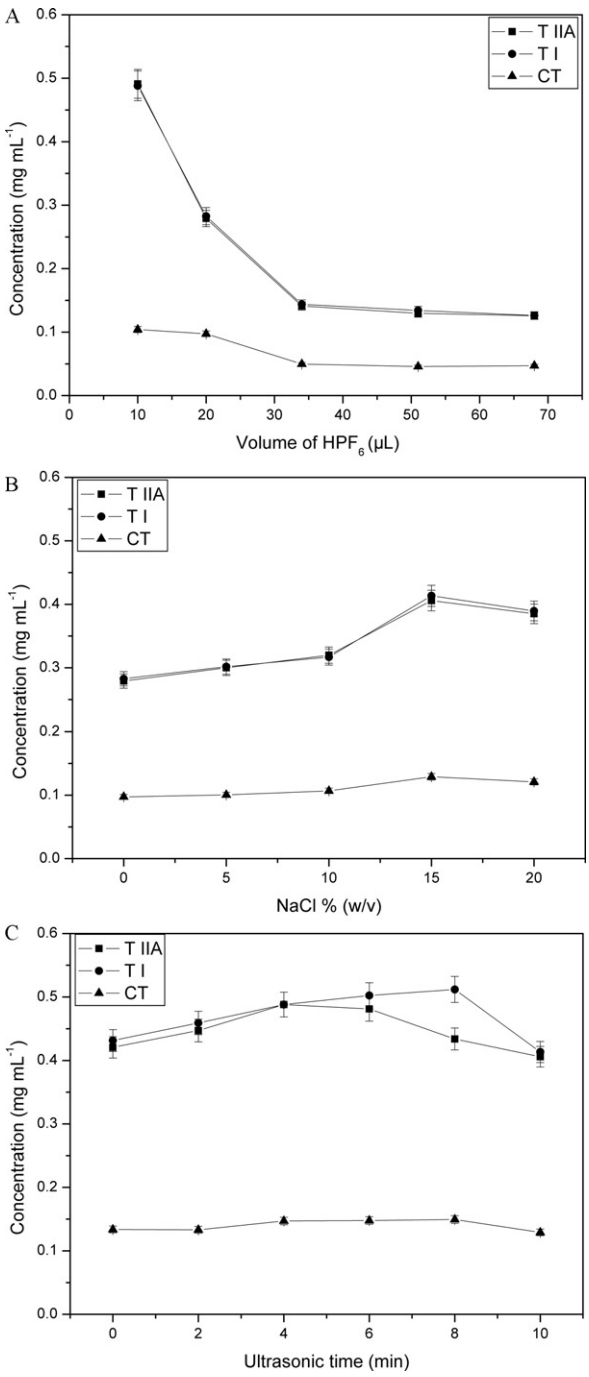

Fig. 6. Effect of volume of HPF_6 (A), NaCl (B) and ultrasonic duration (C) on tanshinones concentrations in [OMIM][PF_6]. (A) Ultrasonic power = 135.0 W, ultrasonic duration = 10.0 min, centrifuging duration = 5.0 min, room temperature. (B) 20 μL HPF_6, ultrasonic power = 135.0 W, ultrasonic duration = 10.0 min, centrifuging duration = 5.0 min, room temperature. (C) 20 μL HPF_6, ultrasonic power = 135.0 W, centrifuging duration = 5.0 min, room temperature.

(0.104 mg mL^{-1} CT, 0.488 mg mL^{-1} T I and 0.491 mg mL^{-1} T IIA) were obtained when 10.0 μL mL^{-1} HPF_6 was added, the enrichment factor (the ratio of tanshinone concentration in the IL and tanshinone concentration in the original extract) of CT (17.9) was significantly less than those of T I (28.1) and T IIA (28.8). The different enrichment factors were due to the relatively low hydrophobicity of CT. To avoid this, 20.0 μL mL^{-1} HPF_6 was selected to achieve similar enrichment factors of CT (16.7), T I (16.3) and T IIA (16.4).

3.2.3. Effect of added salt

The addition of salt during microextraction can modify the ionic strength and decrease the solubility of molecules in water. The effect of salt in this study was tested by adding various amounts of NaCl from 0.0 to 20.0% (w/v). The tanshinones' concentrations increased with increasing NaCl concentration from 0.0 to 15.0% (w/v) and decreased with further increases of NaCl concentration (Fig. 6B). This was because salting-in and salting-out effects predominate at lower concentrations. However, high concentration of NaCl influenced the ion exchange, affecting the amount of sedimented IL phase (equilibrium (1)). The addition of high concentrations of salt greatly increased the viscosity of the solution, possible reducing the transfer of the analytes. High concentration of salt also decreased the solubility of [OMIM][Cl] in water, making more [OMIM][Cl] dissolve in the [OMIM][PF_6], and so increasing the volume of the hydrophobic phase. Hence, 15.0% NaCl was considered optimal.

3.2.4. Effect of ultrasonication duration

Dispersion duration is very important to the efficiency of analyte preconcentration during microextraction. In this study, some tanshinones were removed from the aqueous phase by [OMIM][PF_6] during hydrophobic IL formation. The remaining tanshinones in the extract required time to distribute in the [OMIM][PF_6] phase assisted by [OMIM][Cl]. Therefore, dispersion time was studied in the range of 0.0–10.0 min (Fig. 6C). The highest concentrations of tanshinones were obtained by 6.0 min ultrasonication, and then the concentrations decreased with increasing time. The decrease of concentration can be attributed to heat generated during ultrasonication which could have dissolved the [OMIM][PF_6] or released analytes from the IL phase. Therefore, 6.0 min was selected for subsequent experiments.

3.2.5. Effect of ultrasonication power

Similar to its duration, ultrasonication's power affects analytes' dispersion. Higher power allows sooner equilibration, but generates more heat that would decrease the analytes' concentrations. Thus, the effect of ultrasonic power on concentration was investigated in the range of 45.0–175.0 W. The highest concentration was obtained at 135.0 W and was thus used in following tests.

3.2.6. Effect of centrifugation duration

Centrifugation was required to obtain three distinguishable phases (water, protein and IL phases) in the extraction tubes. Longer centrifugation duration should result in more [OMIM][PF_6] with higher preconcentration efficiency, however it also generates heat. Therefore, the influence of centrifugation duration was investigated from 2.0 to 25.0 min with the highest concentrations attained at 5.0 min for all analytes; longer times decreased their concentrations. Therefore, 5.0 min centrifugation was considered best.

3.2.7. Preconcentration of tanshinones at optimal conditions

The above test found the optimal conditions: tanshinones in the filtrated extract were preconcentrated by anion metathesis reaction of [OMIM][Cl] and HPF_6 (20.0 μL). After adding 15.0% NaCl and dispersing for 6.0 min by 135.0 W ultrasonication, the extraction tube was centrifugated for 5.0 min for phase separation. Resulting concentrations were 0.148, 0.507 and 0.486 mg mL^{-1} for CT, T I and T IIA, respectively. Their respective enrichment factors were 25.4, 29.1 and 28.5. The optimal conditions allowed 84.3% CT, 96.2% T I and 94.3% T IIA to be extracted to the [OMIM][PF_6] phase. Fig. 7 shows the chromatogram.

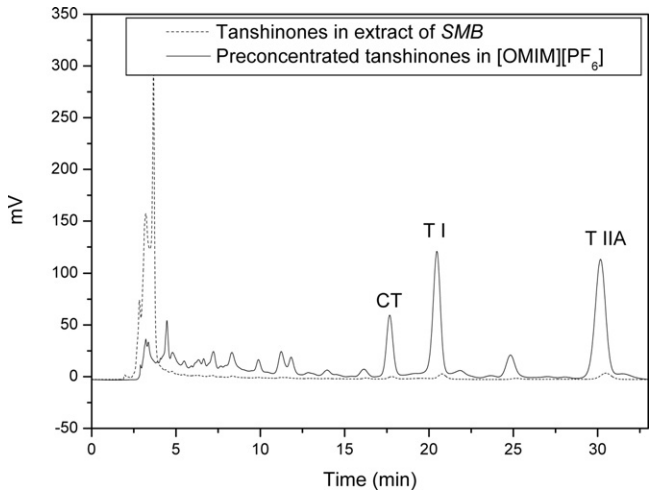

Fig. 7. Chromatogram of extract and preconcentrated tanshinones.

Table 1
Linear range, LODs and repeatability of the method.

Analyte	Linear range (mg mL^{-1})	r	RSD (%)	LODs (ng mL^{-1})
CT	$0.5–1.0 \times 10^{-3}$	0.9997	4.5	92.0
T I	$0.5–1.0 \times 10^{-3}$	0.9997	4.6	97.0
T IIA	$0.5–1.0 \times 10^{-3}$	0.9996	3.4	80.0

Linear range, LODs and repeatability of IL-based extraction.

3.3. Analytical performance

To evaluate the proposed IL extraction, a series of experiments were designed for obtaining linearity, precision, detection limits and other characteristics of the method under the optimized conditions. Table 1 shows that all the analytes had good linearity, with correlation coefficients (r) between 0.9996 and 0.9997. Precision was determined by extraction and preconcentration of a standard solution of a single concentration six times with the proposed method, and the RSD was 3.4–4.6%. Based on a signal-to-noise ratio of 3, the limits of determinations (LODs) of the three tanshinones were 80.0–97.0 ng mL^{-1}. These results show that the proposed method is stable, with the potential to be widely used in the determination of other medicinal products.

4. Conclusions

Ultrasonication-assisted IL-based extraction successfully separated and preconcentrated CT, T I and T IIA from *SMB*. The CT (0.233 mg g^{-1}), T I (0.695 mg g^{-1}) and T IIA (0.682 mg g^{-1}) were extracted by [OMIM][Cl], and then preconcentrated by anion metathesis. Compared with concentrations of these compounds in the original extraction solution (5.83, 17.4 and 17.0 μg mL^{-1}, respectively), the concentrations in the [OMIM][PF_6] phase increased to 148.1, 507.1 and 486.1 μg mL^{-1}, respectively. This process can simply and efficiently extract and preconcentrate tanshinones from *SMB* as well as precipitate the protein. Moreover, the whole process has a reduced environmental impact, and exhibited potential applicability to other medicinal products in functional foods, medicinal herbs and nutraceuticals.

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