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Ionic Liquid-Based Microwave-Assisted Extraction and HPLC Analysis of Dehydrocavidine in *Corydalis saxicola* Bunting

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Summary. An efficient ionic liquid-based microwave-assisted (IL-MAE) method has been developed for extraction of dehydrocavidine from *Corydalis saxicola* Bunting *(C. saxicola)* for subsequent rapid analysis by high-performance liquid chromatography (HPLC). The yield of dehydrocavidine reached 9.446 mg g^{-1} within 10 min under the optimum IL-MAE conditions (1.5 mol L^{-1} [hmim]Br as extraction solvent, liquid-to-solid ratio 20:1 (mL:g), and extraction temperature 70°C). Compared with conventional procedures, the proposed IL-MAE method has many advantages, for example high extraction yield, short extraction time, low solvent consumption, no use of volatile organic solvents, and no further sample clean-up before HPLC analysis. The method was validated for limit of detection (LOD) and quantification (LOQ), linearity, precision, recovery, and reproducibility. The calibration range was 5.0–200 mg L^{-1} and the correlation coefficient, *r*, was 0.9996. The LOD and LOQ were 0.035 and 0.12 mg L^{-1}, respectively. The relative standard deviations of intra-day and inter-day assays were below 2.6% and 6.5%, respectively. Recovery was between 93.8% and 109.3% with RSD values below 5.0%. The method can be used for rapid and effective extraction and analysis of active components from medicinal plants.

Key Words: ionic liquids, microwave-assisted extraction, dehydrocavidine, *Corydalis saxicola* Bunting, HPLC

Introduction

Corydalis saxicola Bunting (*C. saxicola*, named *Yanhuanglian* in Chinese), an important component of a variety of prescriptions in traditional Chinese medicine, grows mainly in calcareous mountain areas of southern China [1, 2]. Clinically, it is commonly used in China for the treatment of hepatitis and liver cirrhosis and can also be used for alleviating fever, detoxification,

and as a painkiller. *C. saxicola* has multiple therapeutic effects and pharmacological activity, including antibacterial, antiviral, antitumor, and potential hepatoprotective activity from hepatitis virus B and A viral damage, mainly owing to the active alkaloids it contains [3–5]. Dehydrocavidine (*Fig. 1*), the major bioactive alkaloid in *C. saxicola*, has antinociceptive, spasmolytic activity and hepatoprotective effects [5–7]. To extract and determine dehydrocavidine in *C. saxicola* is important for quality control and quantitative assessment of the herb.

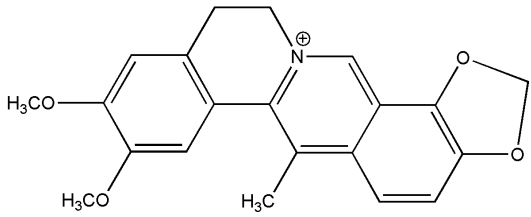

Fig. 1. The chemical structure of dehydrocavidine

Techniques reported for the extraction of dehydrocavidine from *C. saxicola* include heating under reflux, infiltration, and ultrasound-assisted extraction with organic solvents, including methanol, ethanol and acetonitrile [7–10]. Compared with conventional extraction methods, microwave-assisted extraction (MAE) can substantially reduce both extraction time and solvent consumption, and has thus been widely used for extraction of components of interest from a wide variety of samples [11–13]. MAE has also been used as an alternative sample preparation technique for several HPLC analyses [14–19].

Ionic liquids (IL) have recently been used as promising alternatives to the traditional organic solvents widely used in synthesis, catalysis, polymerization, separation, and extraction [20–24]. Although some reports indicate that some commonly used IL are toxic [25], more novel applications will still be developed [26, 27]. Because of their unique chemical and physical properties, especially their negligible vapor pressures, good extraction of a variety of target analytes, and the fact that many compounds are highly soluble in them, IL are very promising in sample-pretreatment techniques [28, 29]. For example, IL have been used in the MAE of polyphenolic compounds and alkaloids from traditional medicinal plants [14–17]; the results obtained indicated that aqueous solutions of IL were effective solvents for MAE of the active constituents from medicinal plants, and the sample preparation techniques proposed were attractive and rapid, combining the

excellent properties of IL with the advantages of microwave-assisted heating.

In this study, ionic liquid-based microwave assisted extraction (IL-MAE) was combined with HPLC for rapid and effective extraction and analysis of dehydrocavidine in *C. saxicola*. The IL-MAE conditions, including the IL and their compositions, liquid-to-solid ratio, extraction temperature, and time, were systematically studied. The advantages of the approach were demonstrated by comparison with conventional methods.

Experimental

Materials and Reagents

Standard dehydrocavidine was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Dried *C. saxicola* were collected from Donglan, Guangxi, China, in August 2006 and were triturated to power (0.30–0.90 mm), passed through a stainless steel sieve, and stored in closed desiccators. HPLC grade acetonitrile used for the mobile phase was purchased from Merck (Darmstadt, Germany). Sodium dicyanamide was purchased from Sigma (St Louis, MO, USA). 1-Methylimidazole (99%) was obtained from Kaile Chemical Plant (Zhejiang, China), tetramethylammonium chloride from Shanghai Chemical Reagent Company (Shanghai, China), and sodium tetrafluoroborate, purity ≥98%, from Xiangyang Chemical Factory (Zhejiang, China). 1-Bromoethane, 1-chlorobutane, 1-bromobutane, 1-bromohexane, and methyl chloroacetate, minimum 98% purity, were purchased from Sinopharm Chemical Reagent Company (Shanghai, China). Pyridine and other reagents were of analytical grade and were supplied by Guangzhou Chemical Reagent Factory (Guangzhou, China).

Stock solution of dehydrocavidine at a concentration of 200 mg L^{-1} was prepared in 95% (v/v) ethanol containing 0.75 mol L^{-1} [hmim]Br. Standard solutions of different concentration were prepared when needed by appropriate dilution of the stock solution. All stock solutions and working solutions were stored under refrigeration and brought to room temperature before use.

Synthesis of IL

The ionic liquids studied were 1-butyl-3-methylimidazolium chloride ([bmim]Cl), 1-butyl-3-methylimidazolium bromide ([bmim]Br), 1-ethyl-3-

methylimidazolium bromide ([emim]Br), 1-hexyl-3-methylimidazolium bromide ([hmim]Br), *N*-butylpyridinium chloride (bPyCl), 1-ethyl-3-methylimidazolium tetrafluoroborate ([emim][BF_4]), 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim][BF_4]), 1-butyl-3-methylimidazolium dicyanamide ([bmim][$N(CN)_2$]), and 1-butyl-3-methylimidazolium dihydrogen phosphate ([bmim][H_2PO_4]). These ionic liquids were synthesized and fully characterized in previous work [15]. 1-Acetic acid-3-methylimidazolium chloride ([$HOOCH_2$-mim]Cl) and 1-butyl-3-methylimidazolium hydrogen sulfate ([bmim][HSO_4]) were synthesized according to experimental procedures described in the literature [30, 31]. All IL obtained were dried at 80°C for 12 h under vacuum and then checked by ^{1}H NMR and ^{13}C NMR spectrometry with Mercury-Plus 300 and Varian-Inova 500 spectrometers (Varian, USA). The spectra showed there were no organic impurities in the IL. Tests with silver nitrate showed there was no halide in [bmim][BF_4], [emim][BF_4], and [bmim][$N(CN)_2$].

Ionic Liquid-Based Microwave-Assisted Extraction (IL-MAE)

MAE experiments were performed with a MAS-I microwave oven (Sineo Microwave Chemistry Technology Company, Shanghai, China). The procedure has been described in detail elsewhere [14].

Accurately weighed *C. saxicola* (1.0 g) was extracted with 20 mL 1.50 mol L^{-1} aqueous IL solutions at 70°C for 10 min to study the effects of different IL on extraction of dehydrocavidine and thus to select the optimum IL. The IL-MAE conditions, including IL concentration, liquid-to-solid ratio, extraction temperature and time, were optimized by a univariate method. All extraction experiments were repeated three times. The extracts obtained were filtered through a sand funnel (Shanghai Siyuan, Shanghai, China) under vacuum at room temperature and then diluted to 50 mL with deionized water. All extracts were filtered through a 0.45-μm filter before chromatographic analysis. The yield of dehydrocavidine was defined as:

$$\text{Yield (mg g}^{-1}) = \frac{\text{Mass of dehydrocavidine in extraction solution (mg)}}{\text{Mass of sample (g)}}$$

Conventional MAE

The reference solvent for MAE extraction of dehydrocavidine from *C. saxicola* was 95% (*v*/*v*) ethanol. The extraction experiments were operated under the optimized conditions except for the solvent. After extraction, the ex-

tracts were cooled to ambient temperature and then diluted to 50 mL with 95% ethanol.

Heating Extraction

Heating extraction (HE) was selected as a traditional reference method for extraction of dehydrocavidine from *C. saxicola*. Sample (1.0 g) and 1.5 mol L^{-1} (hmim)Br solution (30 mL) were placed in a flask (50 mL) and the suspensions were heated in a water-bath at 70°C for a set time with mechanical stirring. After extraction, the extracts were filtered through a sand funnel and then diluted to 50 mL with deionized water.

Maceration Extraction

Maceration extraction (ME) was also selected as a traditional reference method. Sample (1.0 g) was macerated in 20 mL 1.5 mol L^{-1} [hmim]Br solution at room temperature for 24 h with magnetic stirring. After extraction, the extracts were filtered through a sand funnel and then diluted to 50 mL with deionized water.

HPLC Analysis

Chromatography was performed with a Shimadzu (Japan) LC-10AT system equipped with an SPD-10A UV–visible dual-wavelength detector. Compounds were separated on a 250 mm × 4.6 mm i.d., 5-μm particle, Gemini C_{18} analytical column (Phenomenex, USA) at ambient temperature. The mobile phase was a gradient prepared from acetonitrile (component A) and an aqueous solution of 20 mmol L^{-1} KH_2PO_4 and 0.2% (v/v) H_3PO_4 (component B). The gradient elution program was: 0–16 min, 75% B; 16–25 min, 75–70% B, then held for 5 min. The flow rate was 1 mL min^{-1}. The aqueous extract solutions were filtered through a 0.45-μm microporous membrane and then 10 μL was injected. Elution was monitored at 268 nm and data collection and calculation were performed with Shimadzu CLASS-VP software.

Results and Discussion

Effect of IL Structure on MAE

The structures of IL affected their microwave-absorption properties, solvent power and extractabilities, leading to obvious difference between their extraction efficiency, whether pure or as aqueous solutions [15, 17]. To study the effects on extraction of IL with different cations and anions and to select the optimum IL for MAE of dehydrocavidine from *C. saxicola*, 11 different IL were tested.

According to *Fig. 2*, the structures of the IL had a substantial effect on the extraction, mainly because of the many different interactions between the IL and the target analytes [32, 33]. The yield of dehydrocavidine obtained by using [hmim]Br solution as solvent was higher than for six other IL, which indicated that [hmim]Br was more efficient than the other ten IL in the MAE of dehydrocavidine. For the 1-*n*-butyl-3-methylimidazolium based ionic liquids with Br^-, Cl^-, BF_4^-, $N(CN)_2^-$, $H_2PO_4^-$, and HSO_4^-, there were no obvious differences between the yields of dehydrocavidine when their aqueous solutions were used as extraction solvents, which indicated that the anions of IL investigated in this work had no significant effect on MAE of dehydrocavidine. With the same anion, Br^-, [emim]Br, [bmim]Br, and [hmim]Br resulted in significantly different extraction of dehydrocavidine from *C. saxicola*, and the yield increased with increasing alkyl chain length. The hydrogen-bond acidity of 2-position of the 1-alkyl-3-methylimidalizium ring and, especially, the hydrophobic interactions of the IL increased with increasing of alkyl chain length, resulting in an increase in the yield of dehydrocavidine. For $(CH_3)_4NCl$, [bmim]Cl, [$HOOCH_2$-mim]Cl, and bPyCl, the results obtained indicated that the yield of dehydrocavidine was affected by cation type. IL with cationic moieties with an electron-rich aromatic π-system interacted more strongly with solute molecules capable of participating in polar, π–π and n–π interactions [32, 33], leading to the higher yields. For $(CH_3)_4NCl$, however, there were no π–π or n–π interactions between the ammonium cation and the dehydrocavidine, and strong solvent power, which contributed to the lower yield. These results suggest that the yield of dehydrocavidine was mainly cation-dependent for the IL investigated in this work; this behavior is different from that observed in the MAE of polyphenolic compounds from medicinal plants [15].

On the basis of the yields shown in *Fig. 2*, [hmim]Br solution was selected as the optimum solvent for MAE of dehydrocavidine from *C. saxicola*.

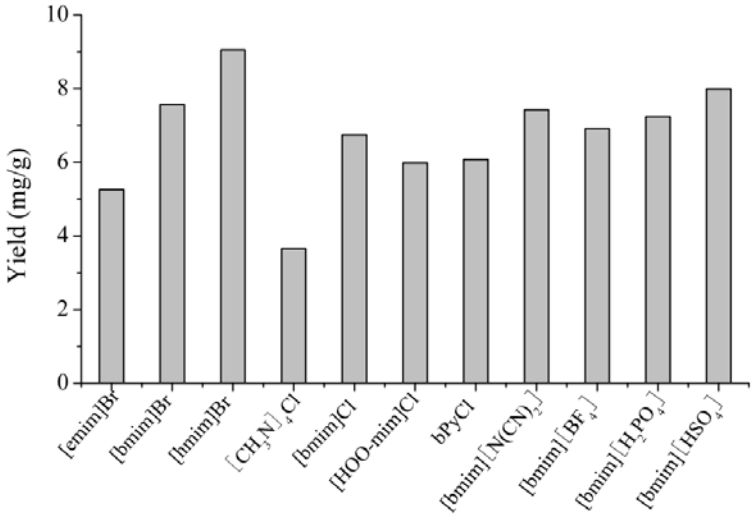

Fig. 2. Effect of IL structure on the yield of dehydrocavidine

Effect of IL Concentration on MAE

To evaluate the effect of IL concentration on extraction yield, additional experiments were performed using different concentrations of [hmim]Br for MAE extraction of dehydrocavidine from *C. saxicola* in 10 min at 70°C; the results are shown in *Fig. 3*.

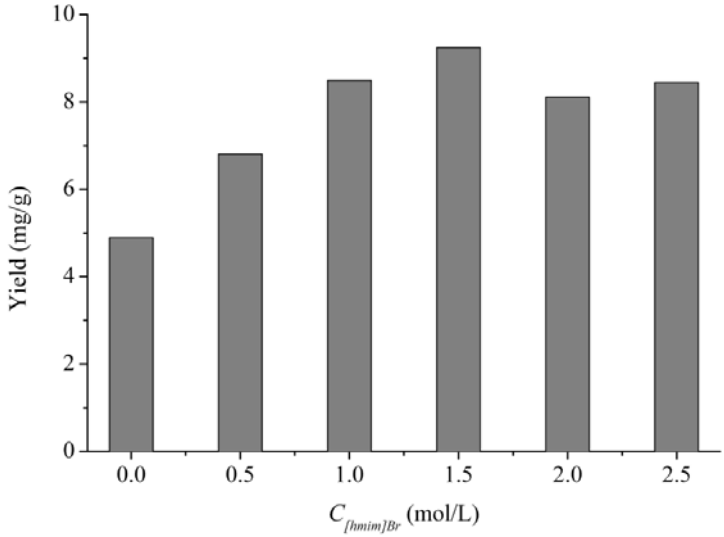

Fig. 3. Effect of [hmim]Br concentration on yield of dehydrocavidine

Fig. 3 shows that the yield of dehydrocavidine from *C. saxicola* increased with increasing [hmim]Br concentration from 0 to 1.5 mol L^{-1}. Addition of [hmim]Br improved the solvent properties of the aqueous solution of the ionic liquid for dehydrocavidine, because of the solvation power and multiple interactions of [hmim]Br. The strong interactions between the imidazolium cation and dehydrocavidine, especially hydrogen bonding, π–π, π–n, and dipolar, contributed substantially to this increase. When the concentration of [hmim]Br was above 1.5 mol L^{-1}, the yield decreased slightly, owing to the greater viscosity and stronger aggregation interaction with the increasing concentration [34]. Therefore, 1.5 mol L^{-1} [hmim]Br was adopted in further studies.

Effect of Liquid-to-Solid Ratio, Extraction Temperature, and Time on MAE

Because liquid-to-solid ratio, extraction temperature, and time affect extraction efficiency in most extraction processes, in this work these MAE conditions were further investigated by performing univariate design experiments.

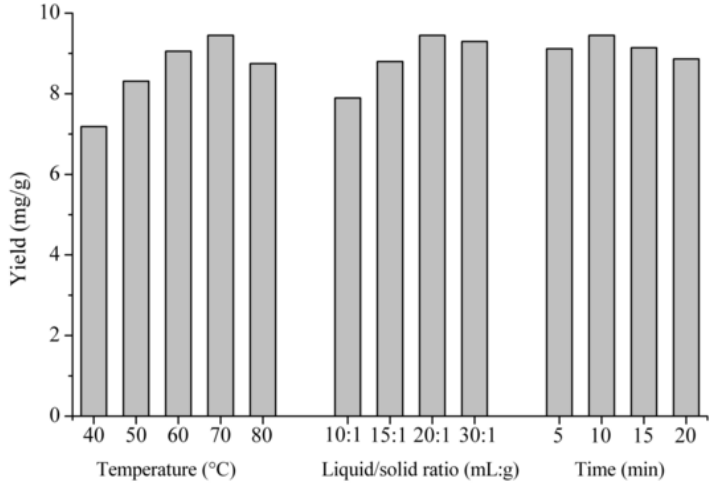

Fig. 4. Effects of liquid-to-solid ratio, extraction temperature, and time of MAE on yield of dehydrocavidine

Fig. 4 shows that the yield of dehydrocavidine increased with increasing liquid-to-solid ratio in the range from 10:1 to 20:1, and then remained constant with further increase of the liquid-to-solid ratio. Thus, 20:1 liquid-to-solid ratio was adopted in this work. The yield also increased with in-

creasing temperature in the range 40–70°C. From 70 to 80°C, the yield of dehydrocavidine from *C. saxicola* decreased slightly, which indicated that higher temperature probably caused degradation of dehydrocavidine. The extraction temperature selected was 70°C. The yield of dehydrocavidine was almost constant with increasing extraction time ranging from 5 to 20 min, attributed to rapid and effective microwave-assisted heating [11]. It seems that longer extraction time had a negative effect in the MAE of dehydrocavidine from *C. saxicola*. Extraction for 10 min was sufficient.

Briefly, the optimum conditions for MAE of dehydrocavidine from *C. saxicola* were: 20:1 of liquid-to-solid ratio, 70°C of extraction temperature, and 10 min of extraction time.

Comparison of IL-MAE with Conventional Procedures

The proposed IL-MAE approach under the optimum experimental conditions was compared with conventional thermal extraction (HE) and maceration extraction (ME). The results presented in *Table I* indicate that longer extraction times were needed in HE and ME to obtain a high yield comparable with that of MAE. The different results from MAE and HE were mainly because of the unique mechanism of microwave heating. Superheating, mass heating, and rapid heating were achieved by microwave-assisted heating but not by conventional heating [35]. In the ME process, the target analyte was extracted as a result of diffusion between sample and solvent, so, the rate of extraction was slow. MAE furnished higher yields with less solvent consumption and in a shorter time than with conventional extraction methods.

Table I. Comparison of the yield of dehydrocavidine under different extraction conditions (n = 3)[a]

Method	Solvent	Liquid-to-solid ratio (mL g^{-1})	Extraction temperature (°C)	Extraction time (min)	Yield (mg g^{-1})	RSD (%)
MAE	95% (v/v) ethanol	20:1	70	10	9.176	4.0
	1.5 mol L^{-1} [hmim] Br	20:1	70	10	9.446	4.7
HE	1.5 mol L^{-1} [hmim] Br	30:1	70	10	8.509	3.1
			70	240	8.346	1.9
ME	1.5 mol L^{-1} [hmim] Br	20:1	20±5	1440	7.722	2.7

[a]Each value is the mean from three independent experiments

To compare IL-MAE with regular MAE for extraction of dehydrocavidine from *C. saxicola,* 95% (*v*/*v*) ethanol was used as solvent. The results shown in *Table I* indicate that the [hmim]Br solution and 95% ethanol as solvent had no remarkable difference on extraction of dehydrocavidine. IL are therefore suggested as alternatives to conventional solvents in MAE of dehydrocavidine from traditional Chinese herbs.

Validation of the Method

Dehydrocavidine in extracts was identified by comparison of its retention time with that of the authentic standard. Chromatograms obtained from the standard and from extracts are shown in *Fig. 5*. Peak resolution, order of elution, and elution times are no different among *Figs 5B–5D*, which indicates that, similar to water or ethanol as solvent, the IL solution could simultaneously extract other alkaloids in *C. saxicola*.

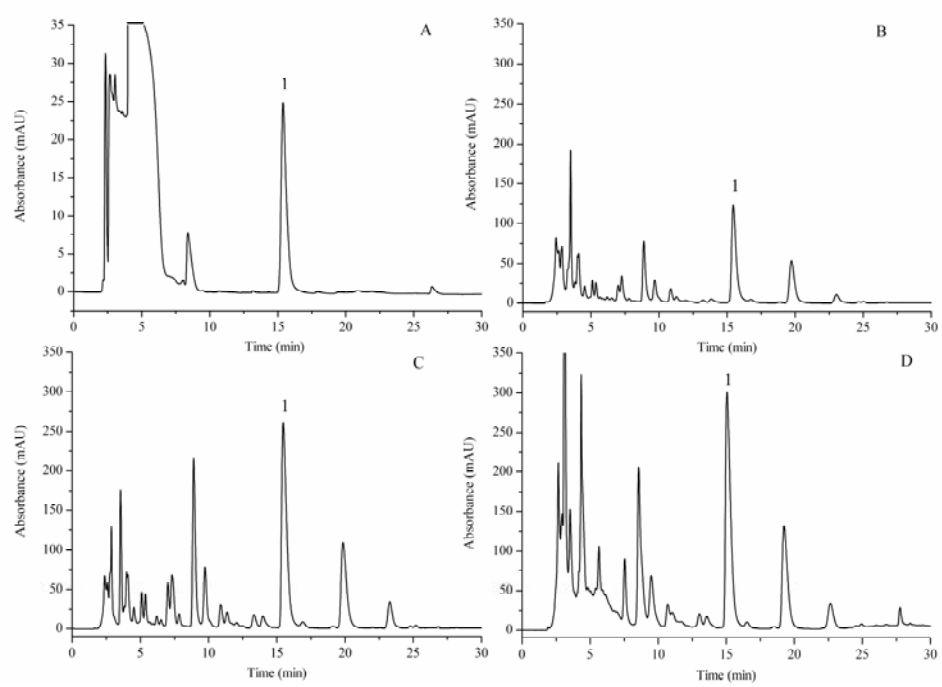

Fig. 5. Chromatograms obtained from a standard solution of dehydrocavidine: 20 mg L^{-1} in 0.75 mol L^{-1} [hmim]Br (A) and from aqueous (B), 95% (*v*/*v*) ethanol (C), and 1.50 mol L^{-1} [hmim]Br solution (D) extracts of *C. saxicola.* Peak 1 is dehydrocavidine

The external standard method was used for quantitative analysis of the analytes and all experiments were run in triplicate at least. The calibration plot for dehydrocavidine at 268 nm was $Y = 325758X + 47708$ ($r = 0.9996$) in the range 5.0 and 200 mg L^{-1}, where X was dehydrocavidine concentration (mg L^{-1}) and Y was peak area. The limit of detection (LOD), based on a signal-to-noise ratio of 3 ($S/N = 3$) of the method was 0.035 mg L^{-1} and the limit of quantification (LOQ, $S/N = 10$) was 0.12 mg L^{-1}. The precision was evaluated by performing seven repetitive analyses of a 10 mg L^{-1} standard solution of dehydrocavidine. The relative standard deviation (RSD) for intra-day retention time and peak area precision was <2.6% and the corresponding RSD for inter-day precision was <6.5%. These results indicated the repeatability of the method was good.

The accuracy of the method at different concentrations was investigated by measurement of recovery by the standard-addition method. Three different amounts (low, medium, and high) of dehydrocavidine standard were added to *C. saxicola* samples. In this study 4.00, 8.00, and 9.45 mg dehydrocavidine was added to three 1.0-g *C. saxicola* samples; these were then extracted and analyzed. Each set of additions was repeated in triplicate. Under the optimized IL-MAE conditions, recovery of dehydrocavidine was 109.3%, 93.8%, and 98.0%, respectively, at the three levels specified and the corresponding RSD values were <5.0%. The results from determination of recovery and reproducibility indicated the proposed method was credible.

Conclusions

In this work IL-MAE was successfully used for the extraction of dehydrocavidine from *C. saxicola*. This was followed by HPLC without removing the IL from the filtrate. The IL solution proved to be an effective alternative to conventional organic solvents for MAE of analytes from native samples. Under the IL-MAE conditions selected, the yield of dehydrocavidine was 9.446 mg g^{-1}, and recovery at different concentrations was from 93.8 to 109.3% with RSD <5.0%. All these results indicate the proposed method is simple, rapid, effective, and environmentally benign, and appropriate for quality control of alkaloids in herb samples.

Acknowledgments

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