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Determination of rutin and quercetin in Chinese herbal medicine by ionic liquid-based pressurized liquid extraction–liquid chromatography–chemiluminescence detection

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ABSTRACT

A novel ionic liquid-based pressurized liquid extraction (IL-PLE) procedure coupled with high performance liquid chromatography (HPLC) tandem chemiluminescence (CL) detection capable of quantifying trace amounts of rutin and quercetin in four Chinese medicine plants including *Flos sophorae Immaturus*, *Crateagus pinnatifida Bunge*, *Hypericum japonicum Thunb* and *Folium Mori* was described in this paper. To avoid environmental pollution and toxicity to the operators, ionic liquids (ILs), 1-alkyl-3-methylimidazolium chloride ([$C_n$mim][Cl]) aqueous solutions were used in the PLE procedure as extractants replacing traditional organic solvents. In addition, chemiluminescence detection was utilized for its minimal interference from endogenous components of complex matrix. Parameters affecting extraction and analysis were carefully optimized. Compared with the conventional ultrasonic-assisted extraction (UAE) and heat-reflux extraction (HRE), the optimized method achieved the highest extraction efficiency in the shortest extraction time with the least solvent consumption. The applicability of the proposed method to real sample was confirmed. Under the optimized conditions, good reproducibility of extraction performance was obtained and good linearity was observed with correlation coefficients (r) between 0.9997 and 0.9999. The detection limits of rutin and quercetin (LOD, $S/N = 3$) were 1.1×10^{-2} mg/L and 3.8×10^{-3} mg/L, respectively. The average recoveries of rutin and quercetin for real samples were 93.7–105% with relative standard deviation (RSD) lower than 5.7%. To the best of our knowledge, this paper is the first contribution to utilize a combination of IL-PLE with chemiluminescence detection. And the experimental results indicated that the proposed method shows a promising prospect in extraction and determination of rutin and quercetin in medicinal plants.

1. Introduction

Traditional Chinese medicine (TCM) has a long therapeutic history over thousands of years in China. There are so many bioactive compounds, such as vitamins, polyphenolic compounds, nitrogen compounds, terpenoids and some other endogenous metabolites in various medicinal plants. So TCM have been used for pharmaceutical and dietary therapy for several millennia. *Flos sophorae Immaturus*, *Crateagus pinnatifida Bunge*, *Hypericum japonicum Thunb* and *Folium Mori* are four popular and important herbal medicines. Flavonoids were found to be the most important bioactive compounds in them. As the natural antioxidants, flavonoids can protect biomolecules against attacks caused by the free radicals, and they decrease the possibility of free radicals creation or even divert these radicals into less reactive or non-reactive products [1]. As the mutual flavonoids compounds in the three herbs, rutin and quercetin (Fig. 1) have received much attention because of their pharmaceutical use in phytotherapy [2–4]. The contents of rutin and quercetin are closely related to the efficacy and quality of the four herbs. Therefore, it is very important to develop a simple, fast, readily automated, sensitive and efficient method for the extraction and determination of rutin and quercetin in herbal drugs.

Usually before the normal analysis, the first and most tedious step is sample preparation, which includes the separation and preconcentration of target analytes from various complex matrices, especially environmental and biological samples. Extraction methods, such as Soxhlet extraction [5], heat-reflux extraction (HRE) [6] and ultrasonic-assisted extraction (UAE) [7,8] are commonly employed for the extraction of target analytes from solid

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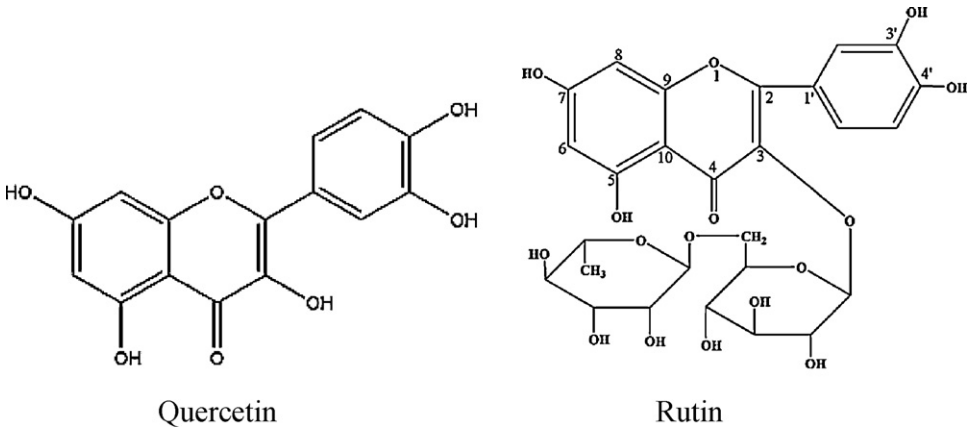

Fig. 1. Chemical structures of quercetin and rutin.

matrices. These conventional methods are time and solvent consuming and require additional clean-up or filtration steps [9]. Recently, microwave assisted extraction (MAE) [10,11] has been employed to extract constituents in herbal drugs. It can significantly reduce the extraction time and the consumption of solvents. However, the microwave radiation should be handled carefully because the leaked microwaves are dangerous to human health [12] and additional clean-up and centrifugation steps are also necessary in MAE. Therefore, nowadays more efficient, easy operating, rapid and safe extraction methods are expected. Pressurized liquid extraction (PLE) is an efficient extraction procedure that uses solvents at high pressures and temperatures above the boiling points to extract substances from solid and semi-solid matrices. High temperature accelerates the extraction kinetics and decreases the viscosity of the liquid solvent, allowing a better penetration of the matrix and weakened solute–matrix interactions. Elevated pressure keeps the solvent in liquid state and forces the solvent into the matrix pores and hence facilitates the extraction of analytes [13,14]. With the advantages over conventional extraction techniques, such as the possibility of automation, simple operation, low solvent volume, and reduced extraction time, PLE has been used successfully to extract active ingredients from solid matrices [15–17]. However, the use of toxic, volatile, flammable organic solvents is highly problematic for the lab workers. Therefore, the exploration of safe and environmentally benign extraction solvents and processes is increasingly important in the development of sample pretreatment techniques.

Recently, ionic liquids (ILs) are used as promising alternatives to the traditional organic solvents employed in sample preparation. Owing to their unique properties [18], ILs have been extensively applied as substituents for conventional organic solvents in various extraction processes, such as liquid–liquid extraction (LLE) [19], liquid phase microextraction (LPME) [20,21], solid phase microextraction (SPME) [22,23], microwave-assisted extraction (MAE) [10,11,24,25] and ultrasonic-assisted extraction (UAE) [7,26]. Lately, to demonstrate the potentiality of IL solution as alternative solvents in the pressurized liquid extraction, our group [27] originally used the IL 1-octyl-3-methylimidazolium chloride ($[C_8mim][Cl]$) aqueous solution in the pressurized liquid extraction of preservatives from glace fruits.

For the last decades, high performance liquid chromatography (HPLC) coupled with chemiluminescence (CL) detection was widely used in analytical chemistry combining the advantages of high efficiency of separation of HPLC and the low detection limits inherent to CL systems [28,29]. Some researchers reported that reducing agents such as corticosteroids or rutin can produce CL when reacting with luminol (5-aminophthalylhydrazide) in the presence of a catalyst in alkaline solution [30,31]. Based on this CL system, rutin and quercetin were both determined in the present work and the parameters that affect the CL intensity were further optimized. Imidazolium-based ILs have significant absorption in the entire UV region, which can result in serious background interference for HPLC–UV analysis [32]. But if chemiluminescence detection was adopted for the analysis of analytes in IL phase, there is no significant interference in the chromatogram.

Up till now, there are no reports about IL-based pressurized liquid extraction (IL-PLE) of bioactive substances from herb samples. The purpose of the present study is to develop a rapid and effective IL-PLE procedure coupled with HPLC–CL for the simultaneous determination of rutin and quercetin in herbal medicines. The effect of ILs with different cation on extraction efficiency was systematically investigated. In addition, the extraction performance of IL-PLE was compared with HRE and UAE for the extraction of rutin and quercetin in real samples. What is more, the selectivity of the proposed method was investigated by comparing the chromatograms of real sample obtained with CL detector and UV detector. Parameters related to extraction and determination were optimized systematically, and the possible extraction mechanism of IL-PLE was also discussed for the first time.

2. Experimental

2.1. Chemicals and solutions

All chemicals used in this study were at least of analytical reagent grade. Both rutin and quercetin standards were obtained from Zhejiang Institute of Food and Drug Control (Hangzhou, China). Ionic liquids (ILs, 99%) were obtained from The Centre of Green Chemistry and Catalysis, LICP (Lanzhou, China) and used as received. Luminol was supplied by Sigma (St. Louis, MO, USA). Potassium ferrocyanide, potassium ferricyanide and sodium hydroxide were received from Shanghai Chemical Reagent Company (Shanghai, China). Raw herbs of *F. sophorae Immaturus*, *C. pinnatifida Bunge*, *H. japonicum Thunb* and *F. Mori* were purchased from a local drug store (Hangzhou, China). HPLC-grade methanol was purchased from Tedia Company (Fairfield, OH, USA). Millipore Milli-Q academic water-purification system (Molsheim, France) was used to provide high quality water for preparation of the mobile phase and running the analysis.

The stock solutions (100 mg/L) of rutin and quercetin were prepared in methanol and diluted with water to obtain appropriate concentrations of working solutions. The ILs aqueous solutions were prepared in deionized water. The 1.0×10^{-2} mol/L luminol stock solution was prepared in 0.1 mol/L sodium hydroxide solution

and diluted to the appropriate concentration with 0.4 mol/L sodium hydroxide solution containing 0.1 mol/L potassium ferrocyanide. Potassium ferricyanide solution was prepared in deionized water. All the above standards were stored in a refrigerator at 4 °C.

2.2. Instrument

PLE was carried out using a Dionex ASE 150 automated extraction system (Sunnyvale, CA, USA) equipped with 34 mL stainless steel extraction cells and 250 mL Dionex vials for extract collection. KQ-5200DE ultrasonic water baths (Kunshan, China) and IKA RCT basic S25 magnetic stirrer (Konigswinter, Germany) were used in the UAE and HRE procedure, respectively.

HPLC analysis was performed with an Agilent Technologies 1200 liquid chromatographic system (Santa Clara, CA, USA) including a quaternary pump, a variable wavelength detector (VWD) and a manual injector valve provided with a 50 μL loop and a thermostatic column compartment. An Agilent Eclipse XDB-C18 column (4.6 mm × 150 mm, particle size 5 μm) was used for the separation of rutin and quercetin extracted in the IL phase. CL signal was detected by a chemiluminescence system consisting of a peristaltic pump (Qingpu, Huxi, Shanghai, China) to deliver potassium ferricyanide solution and luminol solution, a mixing tee, a model CL1000 CL detector (Najing, Xiamen, China) equipped with a glass coil (i.d. 1.2 mm, 15 cm length used as reaction coil and detection cell) and a photomultiplier. The data acquisition was performed by LabNet CL1000 ChemStation program (Najing, Xiamen, China).

2.3. Sample preparation

All medicinal herb samples were dried in a vacuum oven (Model 1400E, VWR Scientific Products, West Chester, PA, USA) set at 50 °C for 6 h, then ground and sieved with particle sizes in the range between 40 and 60 mesh. After homogenized, the dried powder of samples were collected and stored in a moisture controlled cabinet.

2.4. IL-based pressurized liquid extraction

1.5 g of dried sample was placed in a 250 mL clean glass beaker, and then approximate 1.6 g of diatomaceous earth as supporting material was added in and mixed with the sample. The mixture was subsequently placed into a 34 mL stainless steel extraction cell. The cell was then closed and put in the oven of the ASE 150 system. The extraction was performed under the optimized conditions: 1.0 mol/L 1-butyl-3-methylimidazolium chloride ([C_4mim][Cl]) aqueous solution was used as extraction solvent. The cell was heated to a temperature of 120 °C, and pressurized at 1500 psi. These conditions were maintained for 5 min, after which the cell was rinsed with fresh solvent (60% of the cell volume). Then the remaining solvent was displaced with purge gas for 60 s. Static cycle was set one time. The obtained extract (approximately 40 mL) was then transferred into a 100 mL volumetric flask and made up to volume with water. The obtained solution was filtered through the 0.22 μm nylon filter and diluted a certain times (1000 times for *F. sophorae Immaturus*, 20 times for *C. pinnatifida Bunge* and 50 times for *H. japonicum Thunb* and *F. Mori*) with water for the subsequent HPLC–CL analysis. The extraction yield of target analytes was determined as follows:

$$\text{yield (mg/g)} = \frac{\text{mean mass of target analytes in herb samples (mg)}}{\text{mean mass of the herb samples (g)}}$$

The mean mass of target analytes in herb samples was calculated for 3 subsequent sample determinations under the optimized conditions. The mean mass of the herb samples was the average mass of three samples before extraction.

2.5. Conventional reference extraction procedures

UAE and HRE were both selected as the reference methods for extraction of rutin and quercetin from the four herb samples. Preliminary experiments shown that the optimal extraction conditions of the two procedures are as follows:

In UAE procedure, 1.5 g of dried sample powder was mixed with 100 mL 1.0 mol/L [C_4mim][Cl] aqueous solution in a 250 mL glass conical flask, the suspension was extracted for 50 min under ultrasonication and centrifuged for 10 min at 5000 rpm subsequently. The supernatant was filtered through a nylon filter (pore size 0.22 μm) and diluted a certain times (1000 times for *F. sophorae Immaturus*, 20 times for *C. pinnatifida Bunge* and 50 times for *H. japonicum Thunb* and *F. Mori*) with water for subsequent HPLC–CL analysis.

In HRE procedure, 1.5 g of dried sample powder was mixed with 100 mL 1.0 mol/L [C_4mim][Cl] aqueous solution in a 250 mL round-bottomed flask. The sample was then extracted for 8 h under reflux at 120 °C using an oil bath with mechanical stirring. The extracts were centrifuged for 10 min at 5000 rpm and the supernatant was filtered through a nylon filter (pore size 0.22 μm) and diluted a certain times (1000 times for *F. sophorae Immaturus*, 20 times for *C. pinnatifida Bunge* and 50 times for *H. japonicum Thunb* and *F. Mori*) with water for subsequent HPLC–CL analysis.

2.6. Analysis of the extracts by HPLC and CL detection

The diluted extracts were directly injected into the system. The mobile phase was a binary mixture of 0.25% aqueous phosphoric acid (A)–methanol (B) at a flow rate of 1.0 mL/min. The gradient elution program was as follows: 43% B (0–5 min), 43–63% B (5–6 min), and kept at 63% to 11 min, and returned back to 43% B in 5 min. The injection volume was 50 μL and the column temperature was set at 25 °C. The column effluent was mixed with the combined stream of luminol and potassium ferricyanide solution at a mixing tee. The flow rates of luminol and potassium ferricyanide were both 2.3 mL/min. CL was monitored under the optimized conditions by the photomultiplier tube. The concentration of luminol, potassium ferricyanide and potassium ferrocyanide were 5.0×10^{-4}, 5.0×10^{-5}, 0.1 mol/L, respectively. High voltage was 750 V. The peak identification was carried out by comparing their retention times with those of the corresponding peaks obtained by the standard solution. The quantitative determination was based on the CL intensity $I = I_S - I_0$, where I_S is the CL intensity in the presence of rutin and quercetin and I_0 is the CL intensity of blank signal. Comparison of the chromatogram of rutin and quercetin with CL detector obtained from standard solution and each sample extract is shown in Fig. 2. No effects attributable to the ILs were observed on peak resolution, elution order and elution time. Moreover, as an example, the chromatogram of rutin and quercetin obtained with UV detector from sample extract of *F. sophorae Immaturus* powder is also shown in Fig. 2 to compare the selectivity of CL detector and UV detector.

3. Results and discussion

3.1. Optimization of IL-PLE

In order to optimize the extraction conditions, some important parameters that would influence the extraction efficiency of two analytes were carefully investigated by taking *F. sophorae Immaturus* as an example.

3.1.1. Selection of extraction solvent

As the alkyl chain length of the imidazolium ring of IL has a significant influence on its physical and chemical

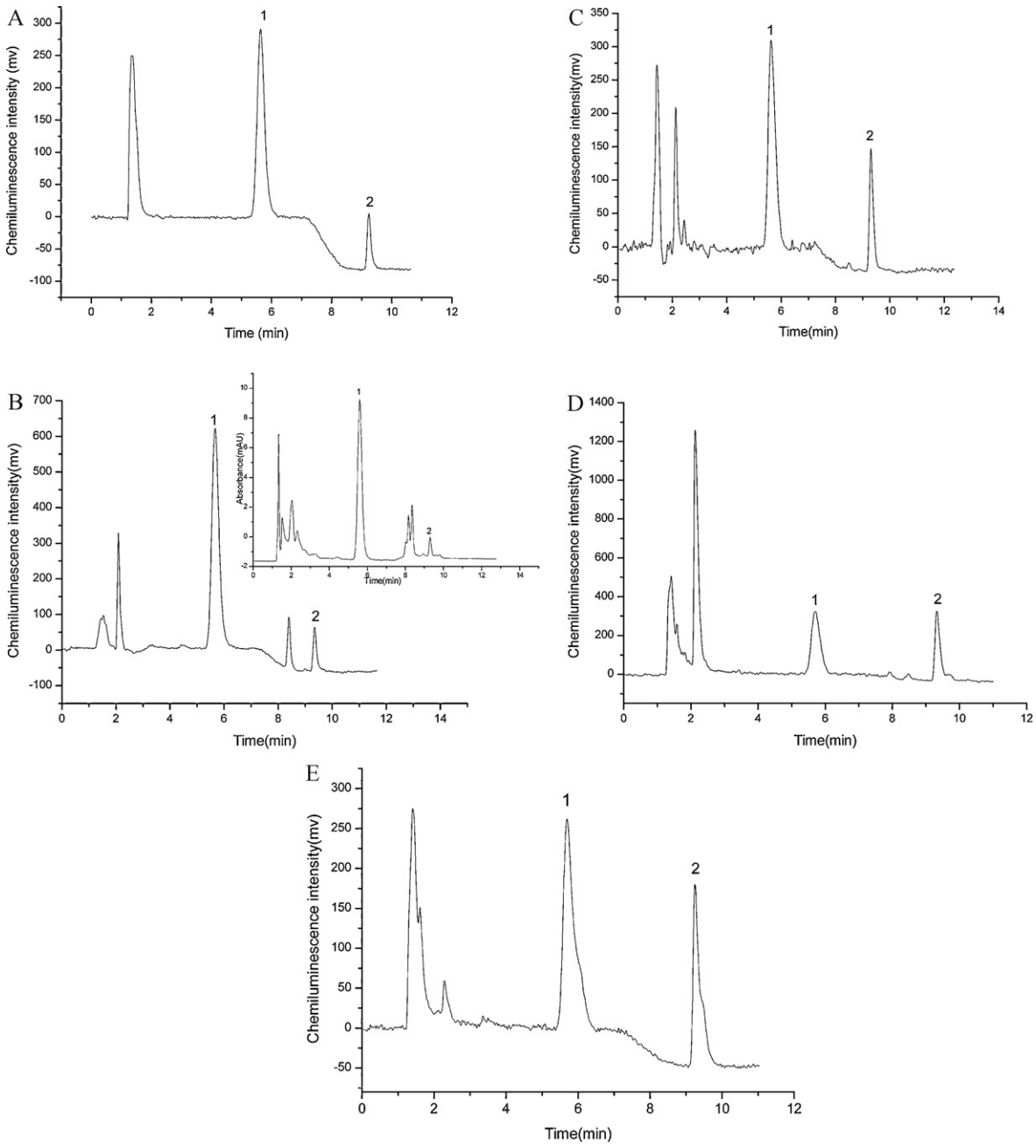

Fig. 2. Chromatograms of 0.5 mg/L rutin standard solution and 0.05 mg/L quercetin standard solution (A) and extracts of four herbs by [C_4mim][Cl] solution under the optimal conditions with CL detector: (B) *Flos sophorae Immaturus*, (C) *Crateagus pinnatifida Bunge*, (D) *Hypericum japonicum Thunb*, and (E) *Folium Mori*. Inset: chromatogram of extract of *Flos sophorae Immaturus* under the same conditions with UV detector. Peaks: (1) rutin, (2) quercetin.

properties, such as density, viscosity and dissolving ability [33,34], the extraction yields of the analytes will be consequently affected by the structure of the IL. In order to evaluate the performance of 1-alkyl-3-methylimidazolium-type ILs in PLE process, the effects of the alkyl chain length of the cation on the extraction efficiency were studied in this work. Three ILs: 1-butyl-3-methylimidazolium chloride ([C_4mim][Cl]), 1-hexyl-3-methylimidazolium chloride ([C_6mim][Cl]) and 1-octyl-3-methylimidazolium chloride ([C_8mim][Cl]) were used as PLE extractants. Water and methanol were selected as the compared extraction solvents for evaluating the extraction efficiency of the developed procedure. The results shown in Fig. 3 clearly indicated that the addition of ILs to water obviously improved the extraction yields of rutin and quercetin from *F. sophorae Immaturus* compared with water as solvent in PLE. The possible reason was related to the solubility of the polyphenolic compounds in extraction solvent. Quercetin and rutin are poorly soluble in water, the addition of ILs improved the extraction yields owing to the solvation power and multiple interactions between analytes and ILs. The strong interactions between imidazolium cation and phenolic compounds [35,36], especially hydrogen bonding, π–π, π–n, ionic/charge–charge and dipolarity, contributed greatly to the better extractability of the ILs. Moreover, the decreasing alkyl chain length distinctly increased the extraction efficiency. The [C_4mim][Cl] was more efficient than the other two ILs in the PLE of rutin and quercetin from *F. sophorae Immaturus*. And it is noteworthy that the extraction yields of the two analytes using [C_4mim][Cl] was almost the same as using methanol which was

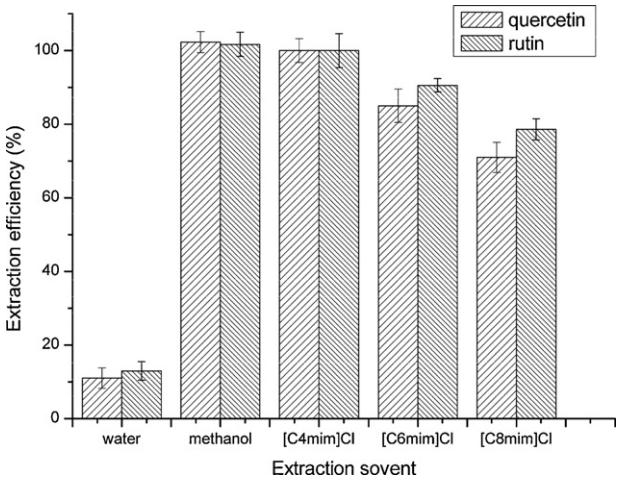

Fig. 3. Effect of different extraction solvents on extracting rutin and quercetin from *Flos sophorae Immaturus*. Sample: 1.5 g; [C_4mim][Cl], [C_6mim][Cl] and [C_8mim][Cl], 1.0 mol/L, respectively; oven temperature, 120 °C; pressure, 1500 psi; static extraction time, 5 min; rinse volume, 60%; purge time, 60 s; one static cycle. The extraction efficiency is expressed as the observed values of target analytes and the selected amount in this assay was taken to be 100%.

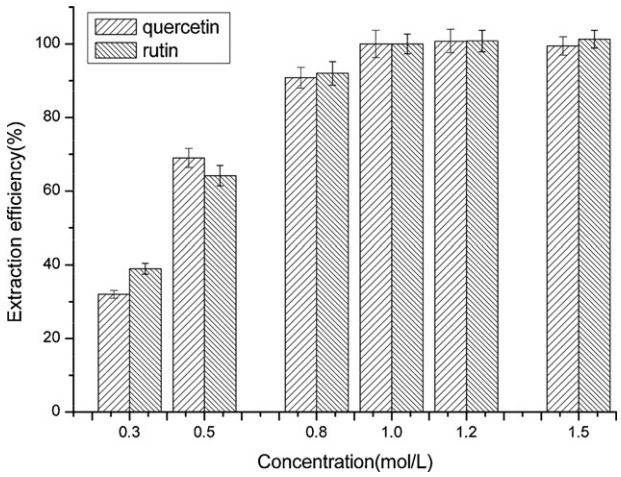

Fig. 4. Effect of concentrations of [C_4mim][Cl] on extraction yield of rutin and quercetin from *Flos sophorae Immaturus*. Sample: 1.5 g; oven temperature, 120 °C; pressure, 1500 psi; static extraction time, 5 min; rinse volume, 60%; purge time, 60 s; one static cycle. The extraction efficiency is expressed as the observed values of target analytes and the selected amount in this assay was taken to be 100%.

volatile, flammable and harmful to human beings. The obtained results revealed that the extractability of rutin and quercetin increased with decreasing length of the alkyl chain on the cation of the ILs, which could be interpreted by the fact that the increasing hydrophobicity of the cation may enhance the repulsion between ILs and rutin [36]. On the other hand, the ILs viscosity increased with increasing the alkyl chain length, so the ILs with too long alkyl chain length were not conducive to the extraction of rutin and quercetin from real samples. Therefore [C_4mim][Cl] was chosen as extraction solvent in the following studies.

3.1.2. Effect of IL concentration

To evaluate the effect of [C_4mim][Cl] concentration on extraction efficiency, additional experiments were performed using different concentrations of [C_4mim][Cl] to extract 1.5 g *F. sophorae Immaturus* sample. The results shown in Fig. 4 indicated that the extraction yields increased remarkably with increasing [C_4mim][Cl] concentration in the range 0.3–1.0 mol/L. Above this concentration level, the extraction yields remained nearly constant. It may be deduced that compromising the little increase in diffusion and transfer capability of extracting solution with its progressively grown viscosity change and decreased penetration power of [C_4mim][Cl] molecules into the interior of sample matrix above 1.0 mol/L [C_4mim][Cl] concentration gained a little regarding the extraction yield that remained nearly constant. Considering the above results, 1.0 mol/L [C_4mim][Cl] solution was selected for extracting rutin and quercetin from *F. sophorae Immaturus*. Furthermore, it is worth noticing that the optimized concentration of [C_4mim][Cl] of 1.0 mol/L was much lower than the selected concentrations of similar chemical structure ILs used to extract rutin and quercetin from medicinal plants [11,24,37]. This is important because less consumption of ILs means lower cost and less interference in the following HPLC–CL analysis. It may be a benefit from the combination of PLE and ILs. High temperature used during the PLE process causes not only an increase in solubility of analytes and diffusion rates but also weakening and disruption of strong interactions between analytes and matrix components. And it also leads to a decrease in viscosity and surface tension of the IL solvent. On the other hand, high pressures improve the extraction efficiency also by "pushing" the solvent into matrices and in this way making the analytes available [38].

3.1.3. Optimization of PLE conditions

The optimization of PLE was performed using dried *F. sophorae Immaturus* as sample. The pressure was set at 1500 psi. Other parameters, including temperature (60 °C, 80 °C, 100 °C, 120 °C, 130 °C and 140 °C) and static extraction time (1 min, 3 min, 5 min, and 7 min), and cycle (1, 2, 3) were optimized using univariate approach and shown in the Supplementary information Figs. S1, S2 and S3.

Temperature is an important parameter in PLE. In the light of Fig. S1, it can be observed that with the increase of temperatures from 60 °C to 120 °C, the extraction yields of rutin and quercetin are also increased obviously. While when the temperature range from 120 °C to 140 °C, a slight decrease of the extraction yield of analytes was observed. Since the amount of rutin and quercetin that can be extracted reached maximum at 120 °C. Thus, 120 °C was used as the optimum extraction temperature.

Extraction time is another factor that would influence the extraction yield in most extraction processes, in the present work, the range of static extraction time from 1 to 7 min was investigated. As shown in Fig. S2, the extraction yield of analytes increased with time over the range of 1–5 min. And an increase in the extraction time above 5 min produced ignorable effects on the extraction efficiency and increased the analysis time. Accordingly, the static extraction time of 5 min was adopted in the following studies.

The function for extraction cycle from 1 to 3 was also investigated at 120 °C, 1.0 mol/L [C_4mim][Cl], and static extraction time 5 min, respectively. From Fig. S3, it can be revealed that the increase of extraction yield of rutin and quercetin was considered negligible in the second and third cycles. So, the optimized extraction cycle was set at cycle 1.

Optimal PLE condition to obtain the highest extraction efficiency of rutin and quercetin in real samples were as follows: solvent, 1.0 mol/L [C_4mim][Cl]; temperature, 120 °C; static extraction time, 5 min; cycle 1.

3.2. Optimization of CL detection

The optimum CL condition was tested in the flow-injection-chemiluminescence system. 1.0 mg/L mixed rutin and quercetin standard solution was used to optimize the experimental conditions.

Table 1
Comparative study of extraction efficiency of rutin and quercetin using different extraction methods ($n = 3$).

Samples	Analytes	Proposed method	Conventional methods	
		IL-PLE	UAE ([C_4mim][Cl])	HRE ([C_4mim][Cl])
		Observed values[a] (Mean ± S.D.) (mg/g)		
Flos sophorae Immaturus	Rutin	196.3 ± 2.6	165.8 ± 3.1	193.5 ± 2.3
	Quercetin	5.18 ± 0.11	4.27 ± 0.18	5.08 ± 0.16
Crateagus pinnatifida Bunge	Rutin	0.768 ± 0.027	0.706 ± 0.024	0.776 ± 0.018
	Quercetin	0.203 ± 0.012	0.177 ± 0.010	0.205 ± 0.009
Hypericum japonicum Thunb	Rutin	2.35 ± 0.08	2.17 ± 0.08	2.33 ± 0.06
	Quercetin	1.17 ± 0.06	1.03 ± 0.05	1.20 ± 0.05
Folium Mori	Rutin	1.05 ± 0.05	1.01 ± 0.08	1.04 ± 0.04
	Quercetin	0.607 ± 0.038	0.557 ± 0.050	0.616 ± 0.036

[a] Each value was the mean and standard deviation (S.D.) of three independent experiments using the optimal conditions of the corresponding methods. Observed value (mg/g) = mass of analytes in extraction solution (mg)/mass of sample (g).

The effect of the flow rate on the CL reaction was evaluated in the range of 0.9–3.8 mL/min. The maximum signal-to-blank ratio was obtained at a flow rate of 2.3 mL/min.

As was well known, luminol affected the CL signal directly in the reaction, and the alkaline medium was necessary for luminol CL system. And then, sodium hydroxide was selected to constitute the alkaline reaction medium. The influence of the concentration of luminol and sodium hydroxide which was used in the luminol solution on the CL signal was examined in the range of 1.0×10^{-5} to 2.0×10^{-3} mol/L and 0.1–0.5 mol/L, respectively. As the concentration of luminol increased, the CL signal of the mixed standard and blank solutions both increased, while both decreased with increasing sodium hydroxide concentration. Therefore, 5.0×10^{-4} mol/L luminol and 0.4 mol/L sodium hydroxide were selected to get the maximum signal-to-blank ratio.

Potassium ferrocyanide was used in the CL reaction to inhibit the high blank signal. The optimal concentration of it was found to be 0.1 mol/L, which is lower than that reported in similar CL system in related literatures [30,31]. It was found that 5.0×10^{-5} mol/L was the optimum concentration of potassium ferricyanide to catalyze the CL reaction. If the concentration of it became higher or lower, the CL intensity would decrease obviously.

3.3. Comparison of the proposed IL-PLE with the conventional methods

To evaluate the extraction efficiency of different methods, the proposed IL-PLE approach under optimal conditions was compared with the optimized UAE and HRE methods. From the results shown in Table 1, it can be seen that the proposed approach was the most efficient one achieving the highest extraction yields with only approximately 40 mL solvent and 5 min to extract targets in the four herbs. Although the extraction efficiency of HRE was comparable with that of the proposed IL-PLE approach, more IL (100 mL) and longer time (8 h) were required in HRE process. In order to compare the extraction efficiency of ILs solution with volatile organic solvents in PLE process, methanol was used to extract rutin and quercetin from *F. sophorae Immaturus*. As shown in Fig. 3, the extraction efficiency of IL-PLE was just slightly lower than that of PLE using methanol as extractant. But in consideration of the toxicity and inflammability of methanol, the proposed IL-PLE procedure has great chance to probably even replace the traditional extraction method. Moreover, the proposed method has an advantage over the reference methods in that no additional centrifugation step is required, since the matrix components that are not dissolved in the ILs aqueous solution may be retained inside the extraction cell. This is very convenient for the purposes of automation and on-line coupling of the extraction and separation techniques. In conclusion, the proposed method has great potential to be a rapid, effective, readily automated and environmentally friendly approach for extraction of rutin and quercetin from the herbs.

3.4. Selectivity of the CL detector

The selectivity of the CL detector was investigated by taking *F. sophorae Immaturus* as an example. In Fig. 2, it can be seen that the chromatogram of real *F. sophorae Immaturus* sample obtained with CL detector is cleaner than that obtained with UV detector, which indicates that less matrix compounds can respond to the CL detector. And the peaks of IL obtained with UV detector are more complex than that obtained with CL detector. So, the proposed method with CL detection has great potential to decrease the chance for possible interference from complex matrix, which is very important to the analysis of biological and natural product samples. Therefore, it has higher selectivity compared to UV detection.

3.5. Analytical figures of merit

Under the optimized conditions, some parameters such as linearity, limits of detection (LOD, $S/N = 3$), and reproducibility of the proposed method were investigated. Calibration curves were constructed by plotting the peak height versus the concentration of target analytes and obtained by the analysis of six concentrations of the standard solutions in triplicate. Table 2 summarized the linear ranges and limits of detection (LODs, based on signal-to-noise ratio of 3, $S/N = 3$). It described that good linearity was observed in the

Table 2
Calibration curves, correlation coefficients (r), limits of detection (LODs) of the method.

Analyte	Calibration curve[a]	r	Linear range (mg/L)	LODs (mg/L)
Rutin	$Y = (127.98 \pm 1.40^{b})X + (221.81 \pm 3.28^{b})$	0.9997	0.10–5.0	1.1×10^{-2}
Quercetin	$Y = (1001.8 \pm 7.0^{b})X + (37.27 \pm 1.63^{b})$	0.9999	0.01–0.5	3.8×10^{-3}

[a] X denotes concentration as mg/L and Y was the peak height.
[b] Slopes and intercepts were accompanied by their errors (± S.D.).

Table 3
Recovery of rutin and quercetin in the four herbs ($n = 5$).

Sample[a]	Analytes	Content (mg)	Added (mg)	Mean recovery (%)[b]	RSD (%)
Flos sophorae Immaturus	Rutin	196.3	50.0	98.3	3.0
			180.0	103	4.1
	Quercetin	5.18	2.00	95.1	3.5
			5.00	100	2.5
Crateagus pinnatifida Bunge	Rutin	0.768	0.200	98.4	1.6
			0.700	99.2	2.3
	Quercetin	0.203	0.080	105	3.4
			0.200	97.1	5.7
Hypericum japonicum Thunb	Rutin	2.35	0.70	98.0	3.5
			2.00	101	3.7
	Quercetin	1.17	0.40	103	2.7
			1.00	97.9	2.6
Folium Mori	Rutin	1.05	0.40	103	1.5
			1.00	101	2.5
	Quercetin	0.607	0.200	101	3.2
			0.600	93.7	2.3

[a] The amount of each sample was 1.00 g.
[b] Values were means of five recovery measurement.

concentration range of 0.1–5.0 mg/L for rutin and 0.01–0.5 mg/L for quercetin with correlation coefficients (r) above 0.9997. The LODs were 1.1×10^{-2} mg/L for rutin and 3.8×10^{-3} mg/L for quercetin. The reproducibility was investigated by the determination of five replicated extracts of the samples (1.5 g sample for each) under the optimized IL-PLE procedure and the relative standard deviation (RSD) value was 2.6%. Moreover, the recovery tests were performed by standard-addition method at different concentration levels. A good recovery ranged from 93.7% to 105% was obtained and the precision (expressed as RSD, $n = 5$) was lower than 5.7% (Table 3), which indicated that the present method was credible in the target analysis.

4. Conclusion

In the present work, the IL [C_4mim][Cl] was successfully used as an extractant for rutin and quercetin from *F. sophorae Immaturus*, *C. pinnatifida Bunge*, *H. japonicum Thunb* and *F. Mori* in the PLE process. In order to achieve high extraction efficiency as well as satisfactory sensitivity, the combination of IL-PLE method and CL detection has been developed for the first time. Compared to the traditional UAE and HRE procedures, the proposed IL-PLE approach can provide higher extraction yields of target analytes within shorter extracting time and provide more simplified easily automated extraction procedure eliminating the need for other exhaustive time consuming clean-up pretreatments. And if the smaller extraction cells, e.g. 5.0 mL-cell or 10 mL-cell, are available, the decrease of consumption of IL in the proposed extraction procedure would be expected. Additionally, the high selectivity of CL detection eliminates the interference rising from UV absorbing endogenous matrix in the analyzed samples and consequently simplify quantitation. Although the LOD of CL detection reported in this work is not superior than that of UV detector described in reference [11], it is also necessary to point out that the sensitivity of CL detector highly depend on the quality of the photomultiplier. Hence, if a high quality photomultiplier can be available, we believe that the better sensitivity will be acquirable. Therefore, as a whole, the proposed IL-PLE method is readily automated, fast, effective and environmentally benign and shows a great promising prospect in routine analyses of natural products and other complex biological samples.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2011.10.036.

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