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Temperature-assisted ionic liquid dispersive liquid–liquid microextraction combined with high performance liquid chromatography for the determination of anthraquinones in Radix et Rhizoma Rhei samples

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ABSTRACT

In this article, a novel method termed as temperature-assisted ionic liquid dispersive liquid–liquid microextraction (TA IL-DLLME) combining high performance liquid chromatography with diode array detection (HPLC-DAD) was developed for the determination of anthraquinones in Radix et Rhizoma Rhei samples. The ionic liquid (1-hexyl-3-methylimidazolium hexafluorophosphate) was used to replace volatile organic solvent as an extraction solvent for the extraction of anthraquinones (aloe-emodin, rhein, emodin, chrysophanol and physcion) from Radix et Rhizoma Rhei. Several important parameters influencing the extraction efficiency of TA IL-DLLME such as the type and volume of extraction solvent and disperser solvent, sample pH, extraction time, extraction temperature, centrifugation time as well as salting-out effects were optimized. Under the optimal conditions, the spiked recovery for each analyte was in the range of 95.2–108.5%. The precisions of the proposed method were varied from 1.1% to 4.4% (RSD). All the analytes exhibited good linearity with correlation coefficients (r^2) ranging from 0.9986 to 0.9996. The limits of detection for all target analytes were ranged from 0.50 to 2.02 $\mu g\,L^{-1}$ (S/N = 3). The experimental results indicated that the proposed method was successfully applied to the analysis of anthraquinones in Radix et Rhizoma Rhei.

1. Introduction

As a well-known Chinese herbal medicine, Radix et Rhizoma Rhei has been used for thousands of years in China. It is officially listed in Chinese pharmacopoeia, containing three species, *Rheum palmatum* L., *Rheum tanguticum* Maxim. ex Balf. and *Rheum officinale* Baill. [1]. Radix et Rhizoma Rhei has the effects of purgation, purging heat, removing heat from the blood, promoting blood circulation and removing blood stasis [2]. Radix et Rhizoma Rhei has many pharmacological actions such as antifungal [3], antiviral [4], antioxidant [5], anticancer [6] and antimutagenicity [7,8]. There are various kinds of constituents isolated from Radix et Rhizoma Rhei, which can be classified as anthraquinones, flavonoids, polyphenols, organic acids and vitamins [9]. Among them, anthraquinone derivatives, including emodin, chrysophanol, rhein, aloe-emodin, physcion, and their glucosides, are thought to be the major active components. Therefore, the quality of Radix et Rhizoma Rhei is evaluated by the determination of the active components in Radix et Rhizoma samples. The chemical structures of these anthraquinones (AQs) are presented in Table 1.

The method of reflux extraction was utilized to extract AQs from Radix et Rhizoma Rhei, the extracting solution was pretreated by appropriate pretreatment method before its analysis. The technique of liquid–liquid extraction (LLE) was usually applied for the preconcentration of AQs with organic solvent from the extracting solution [10,11]. As a classical pretreatment method, LLE has many drawbacks, including time-consuming, loss of target analytes and emulsification [12]. In particular, the consumption of a great deal of organic solvents was dangerous for human health and environment [13]. Therefore, we try to establish a miniaturized, simple and reliable method to replace LLE for reducing of solvent consumption and saving time.

As an alternative sample preparation method, dispersive liquid–liquid microextraction (DLLME), which was introduced by Rezaee et al. in 2006 [14], has attracted much attentions in recent years. DLLME is based on a ternary component solvent system including disperser solvent, extraction solvent and aqueous samples containing analyte of interest [14,15]. The advantages of the DLLME technique are as follows: simplicity of operation; rapid extraction; high enrichment factor (EF); high recovery and low

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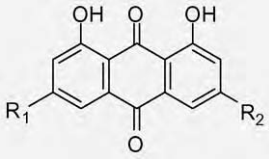
Table 1
The structures of five anthraquinones.

Structure	R_1	R_2	Compound
	CH_3	H	Chrysophanol
	CH_3	OH	Emodin
	CH_3	OCH_3	Physcion
	H	CH_2OH	Aloe-emodin
	H	COOH	Rhein

cost [14–16]. However, the commonly used high-density extraction solvents, such as chlorobenzene [17], chloroform [18], or carbon tetrachloride [19,20], were typically highly toxic. Room-temperature ionic liquids (RTILs) can be used as the extraction solvent instead of organic solvents due to their unique physicochemical properties, such as negligible vapor pressure, miscible with water and organic solvents, good solubility for organic and inorganic compounds, high thermal stability and environmental benignity [21,22]. Ionic liquid (IL) has been used for sample preparation of Chinese medicine. Du et al. used IL as solvent to extract alkaloids from *Nelumbo nucifera* Gaertn and polyphenolic compounds from the leaves of *Psidium guajava* by microwave-assisted extraction (MAE) [23,24]. Compared with regular extraction way, IL-based MAE could improve extraction efficiency and save time. IL-modified silica could be applied to the enrichment of tanshinones in *Salvia miltiorrhiza* Bunge, which showed a special affinity to analyte and higher recovery than conventional silica cartridges [25]. The cases mentioned above indicated that IL showed a good prospect in the sample preparation of Chinese medicine.

The method that IL was chosen as extraction solvent in dispersive liquid–liquid microextraction, was mainly focused on the analysis of organic compounds and inorganic analytes. The organic compounds pretreated with IL-DLLME included polycylic aromatic hydrocarbons (PAHs), aromatic amines and organophosphorus pesticides (OPPs) in water samples [26–28], pesticides and biogenic amines in food samples [29,30], non-steroidal anti-inflammatory drugs in urine sample [31]. IL-DLLME combined with electrothermal atomic absorption spectrometry (ET-AAS) was established for the determination of inorganic analytes, including cobalt, vanadium and cadmium in biological and environmental samples [32–34]. To the best of our knowledge, IL-DLLME has not been applied for the analysis of Chinese medicine.

For the separation and determination of AQs in Radix et Rhizoma Rhei sample, a variety of methods, such as high performance liquid chromatography (HPLC) [35–37], liquid chromatography–tandem mass spectrometry (LC-MS) [38] and high-speed counter current chromatography (HSCCC) [39], were proposed. Among them, HPLC method is the commonly used analytical technique and well compatible with IL. Hence, HPLC was utilized as the detection method.

In the present study, temperature-assisted ionic liquid dispersive liquid–liquid microextraction (TA IL-DLLME) followed by HPLC-DAD was applied for the determination of five anthraquinone derivatives (aloe-emodin, rhein, emodin, chrysophanol and physcion) in aqueous samples. The aim of this work is to simplify the analytical step and reduce the consumption of toxic solvents by comparing the developed pretreatment method with the method listed in Chinese pharmacopoeia. The effects of various experimental parameters, such as the kind and volume of extraction solvent and dispersive solvent, extraction time, sample solution pH as well as salt effect, were studied and optimized. The developed method was successfully applied to real sample analysis.

2. Experimental

2.1. Reagents and standards

Aloe-emodin, rhein, emodin, chrysophanol and physcion were purchased from the National Institute for Control of Biological and Pharmaceutical Products (Beijing, China). Acetonitrile and methanol (HPLC grade) were obtained from Shandong Yuwang Industrial Co., Ltd. (Yucheng, China). Purified water was supplied by Hangzhou Wahaha group Co., Ltd. Chloroform ($CHCl_3$), acetone, sodium chloride, acetic acid and phosphoric acid were of analytical grade and purchased from Tianjin Chemical Reagent Factory (Tianjin, China). The ionic liquids of 1-alkyl-3-methylimidazolium hexafluorophosphate ($[C_nMIM][PF_6]$, $n = 6$, 8 or 10) were obtained from Lanzhou Zhongke Kaidi Chemical New-tech Co., Ltd. (Lanzhou, China). The Radix et Rhizoma Rhei samples were collected from Gansu province in China.

2.2. Instrumentation

Chromatographic analysis was carried out on Agilent 1200 HPLC system equipped with diode array detection (DAD) system. A reversed phase C18 column (250 mm × 4.6 mm i.d., 5 μm) was obtained from Dalian Zhonghuida Scientific Instrument Co., Ltd. (Dalian, China). Agilent ChemStation for HPLC system was employed to acquire and process chromatographic data. The mobile phase consisted of 0.1% acetic acid in water (A) and 19% acetonitrile in methanol (B) in the ratio of 24:76 (v/v) with the flow rate of 1.0 mL min^{-1}. The injection volume and detection wavelength were 10 μL and 254 nm, respectively. The pH measurements were performed with a model PB-10 pH meter (Sartorius, Germany).

2.3. Dispersive liquid–liquid microextraction procedure

The experimental procedure for TA IL-DLLME is illustrated in Fig. 1.

(1) A 5.0 mL of aqueous solution (acidified using H_3PO_4, overall pH 2.0) containing the target analytes (10 μL working solution or 10 μL sample solution) was placed in a 10 mL screw cap glass conical tube.
(2) Methanol (0.4 mL) containing 55 μL $[C_6MIM][PF_6]$ was injected rapidly into the sample solution using a 1.0 mL syringe, immediately, a cloudy solution was formed in the conical tube and then heated in a water bath with the temperature controlled at 60 ± 1 °C, the cloudy solution was gradually clarified.
(3) The tube was thereafter transferred to ice water for 5.0 min, the solution became turbid.
(4) Emulsions were disrupted by centrifugation at 3000 rpm for 10 min.
(5) The upper aqueous phase was removed with a syringe, the volume of the settled phase collected was 20 μL, meanwhile, 10 μL of which was aspirated into 50 μL Agilent microsyringe (Australia) and injected into HPLC system for analysis.

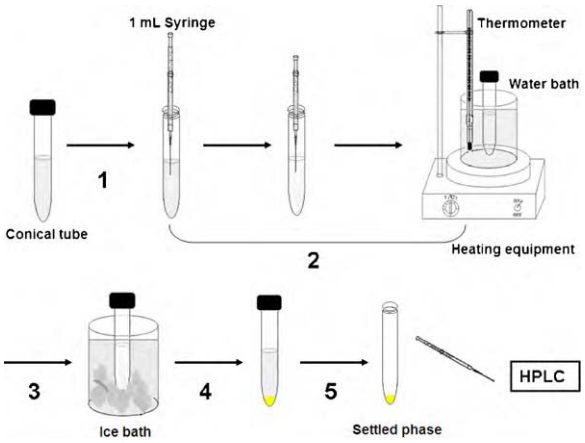


Fig. 1. The experimental process of TA IL-DLLME method.

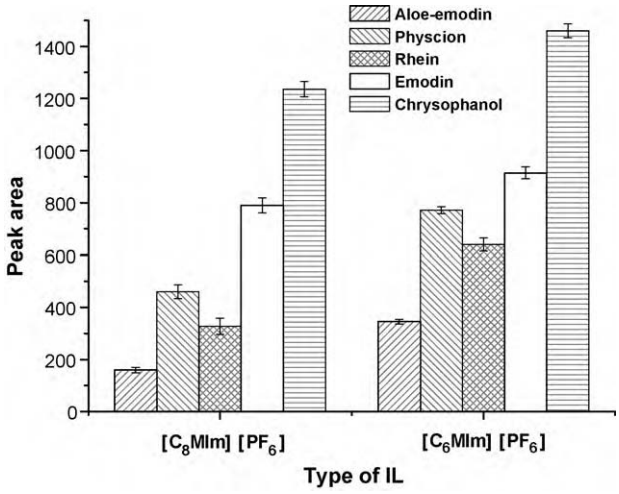


Fig. 2. Impact of type of IL on extraction efficiency ($n = 3$). Extraction conditions: sample volume, 5.0 mL; spiked working solution, 10 μL; disperser solvent, 0.6 mL; pH, 3; dispersive temperature, 50 °C; extraction time, 10 min; centrifugation time, 15 min.

2.4. Calibration of enrichment factor and extraction recovery

The enrichment factor (EF) and extraction recovery (ER) were calculated based on the following equations:

$$\mathrm{EF} = \frac{C_0}{C_{\mathrm{sed}}} \quad (1)$$

$$\mathrm{ER} = \frac{C_{\mathrm{sed}} \times V_{\mathrm{sed}}}{C_0 \times V_{\mathrm{aq}}} \times 100 \quad (2)$$

The C_{sed} and C_0 are the concentration of analyte in the settled phase and the initial analyte concentration in the sample solution, respectively. V_{sed} and V_{aq} are the volumes of settled phase and sample solution, respectively.

The C_{sed} was obtained by direct injection of AQs standard solution in the IL at the range of 1.716–17.16 μg mL^{-1} for aloe-emodin, 0.3432–3.432 μg mL^{-1} for rhein, 1.848–18.48 μg mL^{-1} for emodin, 1.716–17.16 μg mL^{-1} for chrysophanol and 0.396–3.96 μg mL^{-1} for physcion.

2.5. Preparation of standard solution

Standard stock solutions of aloe-emodin (260 μg mL^{-1}), rhein (52 μg mL^{-1}), emodin (280 μg mL^{-1}), chrysophanol (260 μg mL^{-1}) and physcion (104 μg mL^{-1}) were prepared in methanol and then diluted with methanol to the desired concentration. The concentrations of the working solution were 8.58 μg mL^{-1} for aloe-emodin, 1.72 μg mL^{-1} for rhein, 9.24 μg mL^{-1} for emodin, 8.58 μg mL^{-1} for chrysophanol and 1.76 μg mL^{-1} for physcion. All the solutions were stored at around 4 °C condition.

2.6. Preparation of sample solution

A 0.15 g sample of powdered Radix et Rhizoma Rhei (100 mesh) was extracted with 25 mL methanol by refluxing for 60 min. The weight loss of solution in the extraction procedure was compensated with methanol. After filtering, 5 mL filtrate was transferred into a flask and then evaporated to dryness. The residue dissolved with 10 mL of 8 M HCl was sonicated for 2 min. The hydrolyzed solution was heated on a water bath for 60 min, and evaporated to near-dryness under the reduced pressure. The residue was dissolved and transferred into the 10 mL volumetric flask.

To obtain the reference sample solution, 5 mL filtrate was used to prepare the hydrolyzed solution, according to the steps mentioned above. The hydrolyzed solution and 10 mL chloroform were added into flask and refluxed for 60 min on a water bath. The mixture of two phases was transferred into separatory funnel and extracted with 10 mL chloroform for three times, the combined extract was then evaporated. The residue was dissolved and transferred into a 10 mL volumetric flask with methanol. The solution was filtered through a 0.22 μm filter before injection.

3. Results and discussion

3.1. Optimization of temperature-assisted ionic liquid dispersive liquid–liquid microextraction

3.1.1. Selection of IL

The selection of an appropriate solvent is a pivotal step in the optimization of IL-DLLME conditions, some properties must be considered such as good chromatographic behavior, extraction capability of interested compound, appropriate water immiscibility and high density than water. In this study, three hydrophobic ILs, including [C_6MIM][PF_6], [C_8MIM][PF_6] and [C_{10}MIM][PF_6], were investigated. [C_{10}MIM][PF_6] was solid in ambient circumstance and inconvenient to use, so it was not chosen as extraction solvent. By comparing [C_8MIM][PF_6] with [C_6MIM][PF_6] as extraction solvent, it was observed that the cloudy solution was formed more readily by using [C_8MIM][PF_6] than [C_6MIM][PF_6], but the target analytes exhibited a better affinity for [C_6MIM][PF_6], the results are shown in Fig. 2. According to the results discussed above, [C_6MIM][PF_6] was selected as extraction solvent in the subsequent experiments.

3.1.2. Selection of disperser solvent

The disperser solvent must have the appropriate miscibility in both IL phase (extraction solvent) and aqueous sample in order to form a distinctly cloudy solution. For this purpose, three possible disperser solvents, such as methanol, acetonitrile and acetone, were tested. The cloudy solution was difficult to form by using acetonitrile as disperser solvent. The experimental data for different solvents are exhibited in Fig. 3. As can be seen, methanol displays the best extraction efficiency for target analytes, therefore, methanol was selected as the disperser solvent for further studies.

3.1.3. Effect of the volume of IL

In order to evaluate the effect of the volume of the extraction solvent on extraction efficiency of analytes, methanol with a constant volume (0.6 mL) containing different volumes of [C_6MIM][PF_6] (45, 50, 55, 60, 65, 70 μL) were tested with the proposed method. The results are shown in Fig. 4. With the increase of the volume of [C_6MIM][PF_6], the volume of settled phase and the extrac-

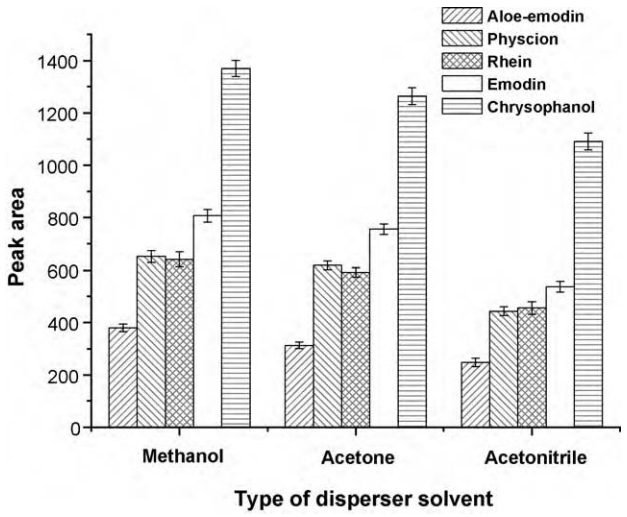


Fig. 3. Effect of various disperser solvents on extraction efficiency ($n = 3$). Extraction conditions: sample volume, 5.0 mL; spiked working solution, 10 μL; [C_6MIM][PF_6], 50 μL; pH, 3; dispersive temperature, 50 °C; extraction time, 10 min; centrifugation time, 15 min.

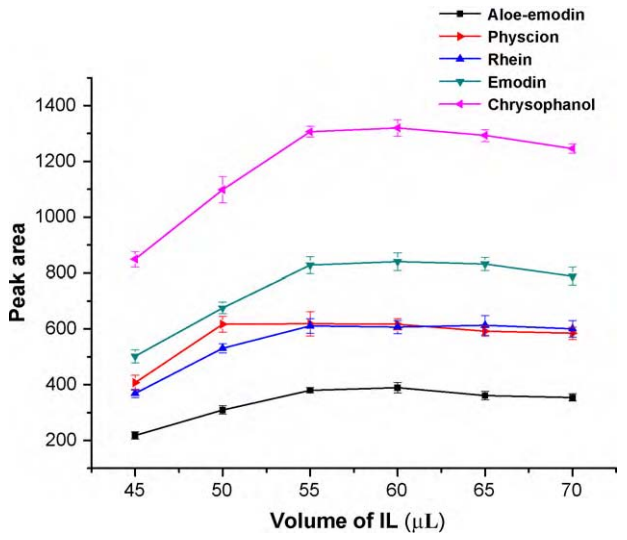


Fig. 4. Effect of the volume of IL on extraction efficiency ($n = 3$). Extraction conditions: sample volume, 5.0 mL; spiked working solution, 10 μL; disperser solvent, 0.6 mL; pH, 3; dispersive temperature, 50 °C; extraction time, 10 min; centrifugation time, 15 min.

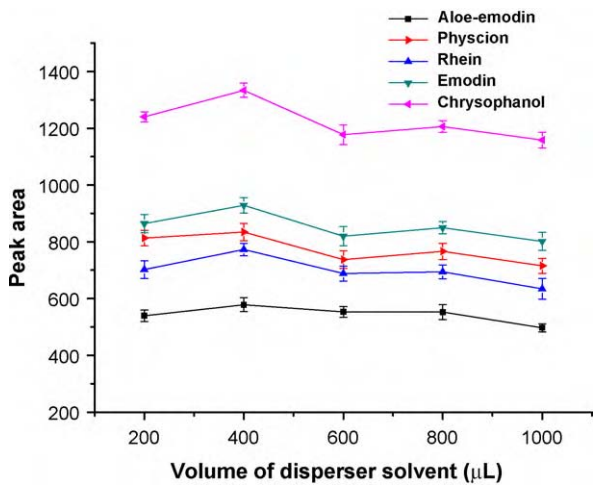


Fig. 5. Effect of the volume of disperser solvent on extraction efficiency ($n = 3$). Extraction conditions: sample volume, 5.0 mL; spiked working solution, 10 μL; [C_6MIM][PF_6], 55 μL; pH, 3; dispersive temperature, 50 °C; extraction time, 10 min; centrifugation time, 15 min.

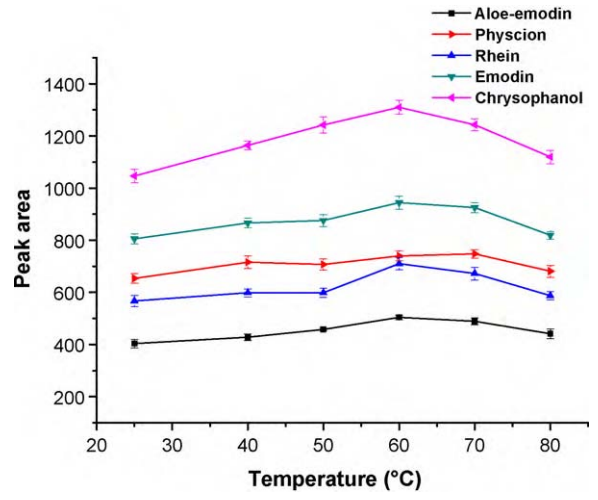


Fig. 6. Effect of the temperature on extraction efficiency ($n = 3$). Extraction conditions: sample volume, 5.0 mL; spiked working solution, 10 μL; [C_6MIM][PF_6], 55 μL; disperser solvent, 0.4 mL; pH, 3; extraction time, 10 min; centrifugation time, 15 min.

tion efficiency of the method increase. The extraction efficiency reaches a constant value when the volume of IL is in the range of 55–70 μL, but the enrichment factor gradually decreases. Therefore, to achieve optimal extraction efficiency for analytes and high enrichment factors for improving the sensitivity of the method, the volume of IL must be chosen appropriately. Based on these facts, the extraction volume of 55 μL was adopted as the optimum volume of [C_6MIM][PF_6] in the following studies.

3.1.4. Effect of the volume of the disperser solvent

The volume of disperser solvent could directly affect the solubility of IL in water solution and the volume of settled phase. To acquire the optimal volume, experiments were performed with different methanol volumes (0.2, 0.4, 0.6, 0.8, 1.0 mL) containing 55 μL [C_6MIM][PF_6]. As shown in Fig. 5, the extraction efficiency increases firstly and then decreases by increasing the volume of methanol. It seemed, at low volume of methanol, cloudy suspension of IL droplets was not formed well. Furthermore, if the volume proportion of the IL to methanol was improper, the mixed process of IL and methanol need much time to obtain a uniform mixture. At a high volume of methanol, the volume of settled phase and extraction efficiency of target analytes were decreased due to the increased solubility of IL in the aqueous sample. Consequently, 0.4 mL of methanol was chosen as the optimal disperser volume.

3.1.5. Effect of temperature

Temperature acts an important role as an assisted factor in the extraction process, which could affect the mass transfer rates of analytes and increase the contact area between IL and aqueous solution. In this work, the effects of extraction temperature were evaluated in the range of 25–80 °C with an extraction time of 5 min. The results are exhibited in Fig. 6. As can be seen from the results, the extraction efficiency increases before the temperature reaching at 60 °C and then decreases with the further increase of temperature. The high temperature could cause the loss of settled phase, which resulted in the reduction of extraction efficiency. Therefore, the temperature was fixed at 60 °C.

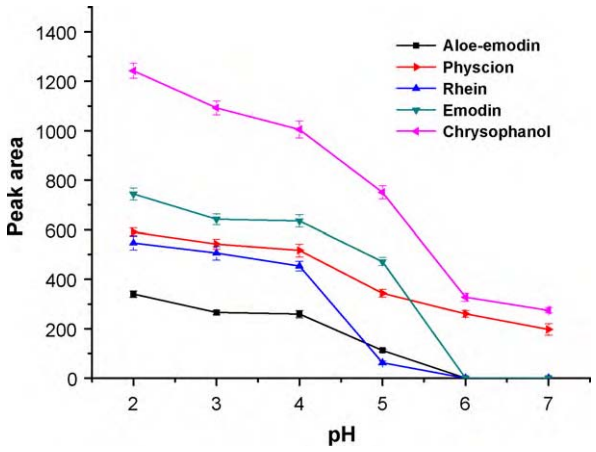


Fig. 7. Effect of the sample pH on extraction efficiency ($n = 3$). Extraction conditions: sample volume, 5.0 mL; spiked working solution, 10 μL; [C_6MIM][PF_6], 55 μL; disperser solvent, 0.4 mL; dispersive temperature, 60 °C; extraction time, 10 min; centrifugation time, 15 min.

3.1.6. Effect of sample pH

The pH value of sample solution determines the existing form of the analytes (as ions or neutral form), which thereafter could affect extraction efficiency. All the AQs are weakly acidic compounds, so they must exist as neutral form in the acid medium and then could be extracted with IL. The effects of sample pH were evaluated in the range of 1–7 by adding appropriate phosphoric acid or sodium hydroxide to aqueous samples. When the sample pH was at 1, the emulsive phenomenon was not occurred in the mixture, and the volume of settled phase was sharply reduced. Therefore, this pH value was inappropriate for further investigation. Fig. 7 shows the results studied within the range of 2–7. It can be seen that the peak areas of the target analytes decrease with the increase of pH value. Aloe-emodin, rhein and emodin were not detected by HPLC when the sample pH was higher than 6, the reason was that these analytes were existed as ionic form at a weakly acidic environment. Hence, pH 2 was selected in the following studies.

3.1.7. Effect of salting-out

The salting-out effect may adjust the ionic strength and improve the partition of analytes between aqueous phase and organic phase. The effects of the ionic strength on the extraction efficiency were examined by adding different amounts of sodium chloride (0–10%, w/v) into the aqueous samples. The results, the salt addition decreases extraction efficiency, could be attributed to the fact that the dissolution of sodium chloride in water increased the viscosity of the solution, which reduced the diffusion rates that target analytes diffused into extraction solvent. Furthermore, the addition of salt enhanced the solubility of IL in water. According to theses facts, the subsequent experiments were carried out without addition of salt.

3.1.8. Effect of extraction time

Extraction time is an important factor in the conventional extraction process, which could influence the extraction efficiency of target analytes. In the TA IL-DLLME, the extraction time is defined as the time interval between the conical tube being put into ice bath and starting to centrifugation. To study the effect of extraction time on extraction efficiency, the extraction time of 1.0, 2.0, 5.0, 10 and 15 min were examined, respectively. The results reveal that the extraction efficiency reaches a constant value when the extraction time exceeded 5 min. Because the temperature-assisted step could help IL dispersive completely in the aqueous phase, so additional time was needed to achieve the extraction equilibrium. Thus, the extraction time of subsequent experiments was set at 5 min.

3.1.9. Effect of centrifugation time

Centrifugation is an important procedure for separating IL phase from aqueous solution in the proposed method, and centrifugation time could affect the volume of settled phase. In order to attain the best extraction efficiency, the centrifugation time was optimized with the time span from 2 to 25 min at a rotation speed of 3000 rpm. The experimental results show that the best performance is obtained at 10 min. When the centrifugation time is shorter or longer than 10 min, the peak areas slowly decrease. The phenomenon could be explained like that the sediment of IL was not complete when the centrifugation time was too short. The settled phase was dissolved partly in the aqueous phase by the heat which was generated due to the centrifugation time was too long. Therefore, 10 min was chosen as the optimized centrifugation time.

3.2. Evaluation of method performance

To investigate the method performance of the proposed method for determining AQs in Radix et Rhizoma Rhei sample, a series of experiments were designed for evaluating the parameters including linearity, reproducibility, limits of detection and other characteristics of method under the optimized condition. Table 2 shows them. Linearity was observed in the range of 7.80–124.80 μg L^{-1} for aloe-emodin, 1.56–24.96 μg L^{-1} for rhein, 8.40–134.40 μg L^{-1} for emodin, 7.80–124.80 μg L^{-1} for chrysophanol and 3.20–101.10 μg L^{-1} for physcion. All the analytes exhibited good linearity with correlation coefficient (r^2) ranging from 0.9986 to 0.9996. The precision of this method was determined by six-time extraction and analysis of spiked aqueous sample. The relative standard deviations (RSDs) of the AQs ranged from 1.1% to 4.4% for intra-day precision and from 2.4% to 7.7% for inter-day precision. The limits of detection (LODs) were calculated in the range of 0.50–2.02 μg L^{-1} based on the ratio of signal-to-noise (S/N = 3). In addition, the experiment of ruggedness indicated that the method have a good ruggedness and could bear a small variance in some extent. These parameters indicated that the present approach with high sensitivity and reliability could be used to detect the concentration of AQs in Radix et Rhizoma Rhei samples.

Table 2
The performance characteristics of the proposed method.

Analyte	Regression equation	r^2	RSD (%)		LOD (μg L^{-1})	LOQ (μg L^{-1})	ER (%)	Enrichment factor
			Intra-day ($n = 6$)	Inter-day ($n = 6$)				
Aloe-emodin	$y = 13.184x + 12.281$	0.9996	3.7	6.3	1.23	7.80	63.8	176
Rhein	$y = 79.165x - 4.873$	0.9992	4.4	7.7	0.50	1.56	63.2	174
Emodin	$y = 23.596x + 11.201$	0.9992	3.0	4.4	2.02	8.40	76.1	209
Chrysophanol	$y = 55.720x - 27.884$	0.9996	1.1	2.4	1.87	7.80	70.0	192
Physcion	$y = 18.699x - 19.194$	0.9986	2.8	4.5	1.20	3.20	78.9	213

Table 3
Accuracy of the method (relative recoveries with their RSDs) for sample solution spiked at different concentrations.

Analyte	Original ($\mu g\,L^{-1}$)	Added ($\mu g\,L^{-1}$)	Found ($\mu g\,L^{-1}$)	Relative recovery (%)	RSD (%)
Aloe-emodin	16.67	12.48	29.39	102.0	1.7
		15.60	31.72	96.5	3.6
		18.72	35.98	103.2	0.9
Rhein	2.503	2.486	4.966	98.7	0.6
		3.120	5.471	95.2	4.1
		3.744	6.223	99.3	3.8
Emodin	17.54	13.44	31.05	100.5	1.6
		16.80	35.24	105.4	5.1
		20.16	37.94	101.2	2.2
Chrysophanol	16.19	12.48	28.34	97.4	1.4
		15.60	33.12	108.5	1.2
		18.72	36.11	106.4	2.1
Physcion	8.03	10.56	18.58	99.9	2.8
		12.80	20.90	100.5	1.6
		15.04	23.47	102.6	2.0

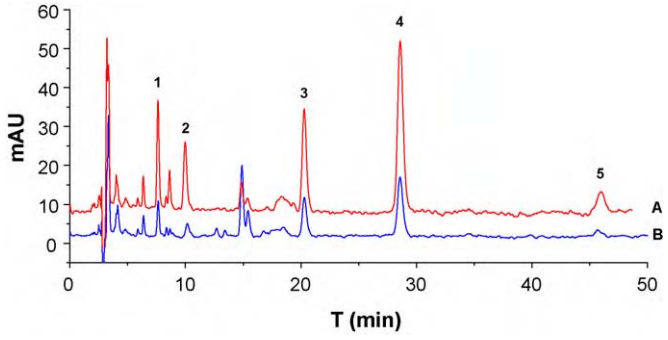


Fig. 8. Chromatogram of aqueous solutions spiked with AQs working solution 10 μL (A) and Radix et Rhizoma Rhei sample solution (B) after TA IL-DLLME at optimum conditions (compound numbers: 1. aloe-emodin; 2. rhein; 3. emodin; 4. chrysophanol; and 5. physcion).

3.3. Application to real samples

To assess the accuracy of the proposed method, the five AQs at three levels were spiked into the sample solution. As shown in Table 3, the recoveries of the five AQs ranged from 95.2% to 108.5% with the precision of 0.6–5.1% (RSD). The typical chromatogram of five AQs after TA IL-DLLME in Radix et Rhizoma Rhei sample and standard solution is illustrated in Fig. 8. To validate the applicability of proposed method in real sample analysis, the determination of AQs in Radix et Rhizoma Rhei sample collected from three different regions of Gansu province in China were performed (Lixian, I; Tianshui, II; Dangchang, III). The data are listed in Table 4. *F* test ($p = 0.95$, $n = 3$) was performed to compare the results obtained by two methods, there were not remarkable difference observed, the result of *F* test showed that the method of TA IL-DLLME could substitute the method in Chinese pharmacopoeia to prepare Radix et Rhizoma Rhei samples. Therefore, all the results displayed that the TA IL-DLLME was a precise and reliable method, which could instead of the conventional method to detect these AQs in real sample.

Table 4
The results of comparison of TA IL-DLLME and conventional extraction method.

Origin of Rhubarb sample	AQs ($mg\,g^{-1}$). TA IL-DLLME	AQs ($mg\,g^{-1}$). Extraction by $CHCl_3$
I	10.93	11.26
II	13.20	13.49
III	4.68	4.81

4. Conclusions

In this study, a new method of temperature-assisted ionic liquid dispersive liquid–liquid microextraction coupled to HPLC has been developed for the determination of anthraquinones in Radix et Rhizoma Rhei samples. The temperature could assist the analytes to transfer from aqueous solution to IL phase. The conditions for the extraction of analytes were investigated and optimized. Compared with the pretreatment technique mentioned in the Chinese pharmacopoeia, the TA IL-DLLME applies IL as extractant not only to reduce the consumption of volatile organic solvent but also to save the experimental cost. Furthermore, the TA IL-DLLME is firstly introduced in microextraction of anthraquinones in Radix et Rhizoma Rhei samples, the study also indicates that the new method reveals an excellent prospect in the field of sample pretreatment of Chinese medicine.

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