Analytical Methods

RSC Publishing

PAPER

View Article Online
View Journal | View Issue

Cite this: *Anal. Methods*, 2013, **5**, 2674

Dissolution determination of five components in Huoxiang Zhengqi tablets using partitioned dispersive liquid-liquid microextraction combined with HPLC-UV

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Received 26th January 2013
Accepted 24th March 2013

DOI: 10.1039/c3ay40153g

www.rsc.org/methods

The dissolution determination of multiple components in herbal preparations is a crucial method for controlling the quality of oral solid dosage forms. In order to improve the low detection sensitivity of dissolution samples, an efficient method for the extraction and concentration of multiple components in dissolution samples of Huoxiang Zhengqi tablets was developed by partitioned dispersive liquid-liquid microextraction (PDLLME). In the PDLLME process, 1.6 mL of THF as the disperser solvent and 100 μL of 1,2-dichloroethane as the extraction solvent were injected into 8 mL of the dissolution sample solution. Based on the partition coefficient, a portion of THF will transfer into the extraction solvent, then the multiple components in the Huoxiang Zhengqi tablet can be extracted into the mixture extraction phase of 1,2-dichloroethane and partitioned THF. In combination with HPLC-UV analysis, the enrichment factors reached to 43 to 119-fold for five of the target components (three of the more polar components were unknown). For honokiol and magnolol, a linear range from 10 to 1000 μg L^{-1} was obtained, and the recoveries at three spiking levels ranged from 90.2 to 99.4% with RSD less than 7.2% (n = 3). The proposed method may be applied for the extraction and concentration of multiple components in the dissolution samples of other herbal preparations.

1 Introduction

Herbal preparations have been widely used for thousands of years in many countries, such as China and India. Nowadays, herbal preparations have been incorporated into the pharmaceutical industry. Huoxiang Zhengqi is a Chinese patent medicine, containing Atractylodes, Tangerine Peel, Officinal Magnolia Bark and ten other herbal ingredients. It is widely used for treatment of the common cold, vomiting, headache and diarrhea.[1]

The dissolution test is important to guarantee the safety, efficacy and stability of medicines, and it can be used to monitor whether the dissolution status of different batches is similar.[2] Furthermore, the in vivo absorption and availability of drugs can also be predicted through in vivo/in vitro correlation analysis based on dissolution test results.[3,4] However, because most of the potentially active components are present in quite low concentrations, they are often difficult to detect directly by routine HPLC-UV methods, which makes the dissolution of herbal preparations extremely difficult to comprehensively investigate. Therefore, the establishment of a proper sample pretreatment method for the extraction and concentration of multiple components in herbal preparations is an imperative task.

In the past few years, methods such as liquid–liquid extraction (LLE), solid-phase extraction (SPE),[5] solid-phase microextraction (SPME),[6,7] and hollow fiber liquid-phase microextraction (HF-LPME)[8–10] have been widely used for the pretreatment of environmental samples. However, the above-mentioned methods are often time-consuming or/and require large amounts of toxic organic solvents and sample solution consumption, which make these methods unsuitable for dissolution tests on herbal preparations. Although dispersive liquid-liquid microextraction (DLLME) has the advantages of easy operation and low consumption of solvents, it is often used for the extraction of one type or a few target compounds with lower polarity. Partitioned dispersive liquid-liquid microextraction (PDLLME) is a newly developed sample pretreatment technique. It allows the partition of a disperser solvent transferred into organic extraction droplets, forming a mixture extraction phase (partitioned disperser solvent and extraction solvent). Therefore, more components can be extracted, even those with higher polarity.[11,12] This sample pretreatment method is applicable for dissolution tests on multiple components with a wide polarity range in oral solid herbal preparations.

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In this work, an efficient PDLLME method combined with HPLC-UV was established to extract and concentrate five components in dissolution samples of Huoxiang Zhengqi tablets.

2 Experimental

2.1 Materials and reagents

Tetrachloroethylene, ethanol, formic acid, hydrochloric acid (HCl) and sodium chloride (NaCl) were purchased from Huaxin Chemical Reagent Co. Ltd (Baoding, China). Chlorobenzene, carbon tetrachloride, 1,2-dichloroethane, dichloromethane and tetrahydrofuran (THF) were obtained from Jinfeng Chemical Co. Ltd (Tianjin, China). Methanol was purchased from Xingke Biochemistry Co. Ltd (Shanghai, China). Huoxiang Zhengqi tablets were purchased from local pharmacies. Honokiol and magnolol were obtained from Shanghai Chinatural Biotech Co. Ltd.

2.2 Instrumentation and LC conditions

Chromatographic analysis was carried out on an HPLC system (Chuangxin Tongheng Technology Co. Ltd, Beijing, China) equipped with two P3000 solvent delivery pumps and a UV3000 detector. A CXTH-3000 workstation was used for controlling the system and data acquisition. A Welchrom-C18 column (4.6 mm × 150 mm, 5 μm, Welch Materials Inc., USA) was used for separation. The gradient elution program was set at a flow rate of 1 mL min^{-1} using mobile phase A (0.1% formic acid aqueous solution) and mobile phase B (methanol) with the following gradient: 0–40 min (A: 70% → 30%), maintained for 15 min, 55–57 min (A: 30% → 70%), then 5 min of equilibration. The detection wavelength was 248 nm. The injection volume was 20 μL and the HPLC system was run at room temperature. The dissolution test of Huoxiang Zhengqi tablets was accomplished using an intelligent dissolution apparatus (D-800L, Tianjin University Radio Factory, Tianjin, China). A low speed centrifuge (L500, Xiangyi Lab Instrument Development Co. Ltd, Hunan, China) was used to accelerate diphase separation. A nitrogen blowing instrument (SE812J, Ferren Science & Technology Co. Ltd, Beijing, China) was used to evaporate the organic phase, and the residues were redissolved with the assistance of a vortex mixer (QL-901, Qilin Medical Instrument Factory, Jiangsu, China).

2.3 Extraction procedure

The PDLLME procedure included six steps: (1) an aliquot of 8 mL of the dissolution sample solution was placed in a 15 mL centrifuge tube with a conical bottom. (2) 1.6 mL of THF (the disperser solvent) and 100 μL of 1,2-dichloroethane (the extraction solvent) were injected into the sample solution. (3) The mixed solution was shaken for a few seconds to form a homogeneous cloudy solution. (4) The target components were extracted into the mixed extraction phase of 1,2-dichloroethane and partitioned THF and further separated by centrifugation at 4000 rpm for 10 min. (5) The upper aqueous phase was removed with a syringe while the sediment phase (about 150 μL) was transferred into a 1.5 mL conical-bottomed centrifuge tube. (6) The sediment phase was evaporated to dryness by a nitrogen stream. The residues were redissolved in 60 μL of methanol/water (4 : 1), and 20 μL of the sample solution were injected into the HPLC system for further analysis.

3 Results and discussion

3.1 Selection of the extraction solvent

The extraction solvent should have higher density than that of water, high extraction capability for the target components, and be immiscible with water. Based on these characteristics, chlorobenzene, carbon tetrachloride, 1,2-dichloroethane, dichloromethane and tetrachloroethylene were selected for preliminary study. Compared with the other extraction solvents, the extraction recoveries using chlorobenzene and carbon tetrachloride were low. Therefore, 1,2-dichloroethane, dichloromethane and tetrachloroethylene were tested further as the extraction solvent in this study. The results in Fig. 1 show that 1,2-dichloroethane dissolved with THF had the best extraction efficiency for all five components. Therefore, 1,2-dichloroethane was employed in further work.

3.2 Selection of the disperser solvent

When only extraction solvents are used, target components with greater polarity cannot be effectively extracted. The added disperser solvent should be miscible with both the organic extraction solvent and the aqueous phase to form a homogeneous emulsion. Thus, methanol, ethanol and THF were investigated as the disperser solvent in this experiment. The sediment phase volume was obviously different, and only THF could increase the volume of the sediment phase with 1,2-dichloroethane as the extraction solvent. The probable reason was that THF could disperse into the extraction phase and form a mixture with the extraction phase (1,2-dichloroethane + partitioned THF). It also noted that adding THF would increase the

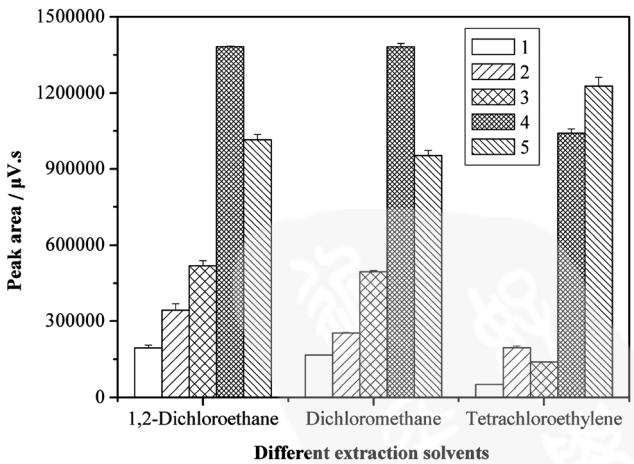

Fig. 1 Effect of different extraction solvents on extraction efficiency. Experimental conditions: sample volume, 8 mL; disperser solvent volume, 1.6 mL of THF; extraction solvent volume, 100 μL. Components: 1–3 were unknown components in this study, 4 and 5 were honokiol and magnolol, respectively.

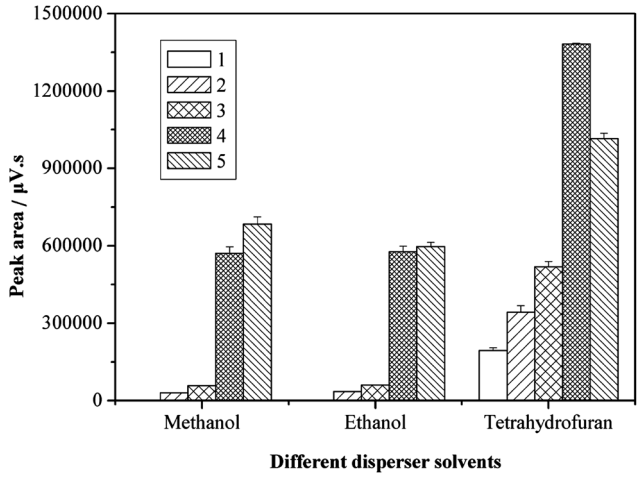

Fig. 2 Effect of different disperser solvents on extraction efficiency. Experimental conditions: sample volume, 8 mL; extraction solvent volume, 100 μL of 1,2-dichloroethane; disperser solvent volume, 1.6 mL. Components: 1–3 were unknown components in this study, 4 and 5 were honokiol and magnolol, respectively.

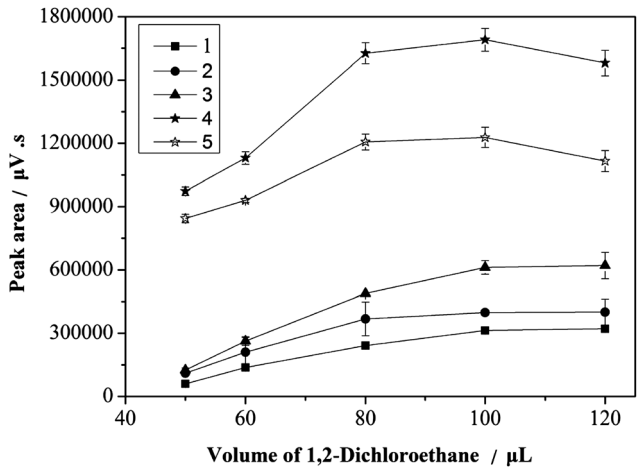

Fig. 3 Effect of extraction solvent volume on extraction efficiency. Experimental conditions: sample volume, 8 mL; disperser solvent volume, 1.6 mL of THF; extraction solvent, 1,2-dichloroethane. Components: 1–3 were unknown components in this study, 4 and 5 were honokiol and magnolol, respectively.

polarity of the mixed extraction phase. Thus, the partition coefficient of the components between the aqueous phase and the extraction phase would be changed. As can be seen in Fig. 2, THF as the disperser solvent had a high extraction efficiency for all five components. Therefore, THF was selected as the disperser solvent in this work.

3.3 Effect of extraction solvent volume

The effect of the extraction solvent volume (50, 60, 80, 100 or 120 μL of 1,2-dichloroethane) was investigated. The results in Fig. 3 show that the extraction efficiency for all target components increased with an increase in the volume of 1,2-dichloroethane from 50 μL to 100 μL. However, the extraction efficiency slight decreased for components 4 and 5 when 120 μL of 1,2-dichloroethane was used. Therefore, 100 μL of 1,2-dichloroethane was used for further experiments.

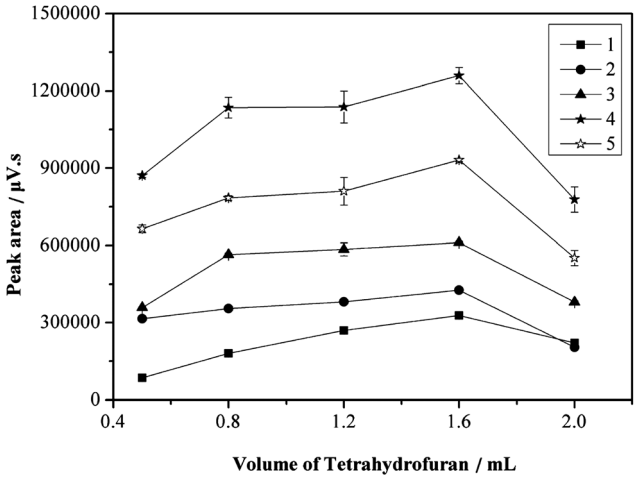

Fig. 4 Effect of disperser solvent volume on extraction efficiency. Experimental conditions: sample volume, 8 mL; extraction solvent, 100 μL of 1,2-dichloroethane; disperser solvent, THF. Components: 1–3 were unknown components in this study, 4 and 5 were honokiol and magnolol, respectively.

3.4 Effect of disperser solvent volume

The effect of the disperser solvent volume (0.5, 0.8, 1.2, 1.6 or 2.0 mL of THF) was also investigated. The results in Fig. 4 indicate that the extraction efficiency of all target components increased with an increase in the volume of THF from 0.5 to 1.6 mL and then slightly decreased. At the same time, the volume of the sediment phases increased. When the volume of THF was greater than 1.6 mL, the extraction phase was observed on top of the aqueous phase, which was due to the lower density of the mixed extraction phase than that of the aqueous phase. Therefore, 1.6 mL of THF was chosen for subsequent experiments.

3.5 Effect of ionic strength

To evaluate the influence of ionic strength on the extraction efficiency, different concentrations of NaCl in the range of 0–1% (w/v) were added. No noticeable variation in extraction efficiency was observed with an increase in the concentration of NaCl. If 1.5% or more NaCl was used, the mixed extraction phase occupied the upper layer of the extraction system. This phenomenon occurred since the density of the aqueous phase containing NaCl was higher than that of the mixed extraction phase. However, there was no influence of ionic strength on the extraction efficiency, so NaCl was not used in further PDLLME procedures.

3.6 Effect of ultrasound

The extraction equilibrium should be quickly reached among the extraction solvent, disperser solvent and aqueous phase in the PDLLME procedure. Recently, ultrasound has been reported

Table 1 Recoveries of honokiol and magnolol after the PDLLME procedure

	160/μg L^{-1}		130/μg L^{-1}		100/μg L^{-1}	
Compounds	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Honokiol	97.2	6.5	99.4	1.7	94.4	4.7
Hagnolol	90.2	7.2	94.6	1.7	96.7	6.8

to assist in the formation of an emulsion.[13–17] Therefore, the effect of ultrasound time (0, 2, 4, 6, 8 min) on the extraction efficiency was investigated. However, no noticeable variation in the extraction efficiency was observed. Therefore, ultrasound was not used in subsequent experiments.

3.7 Validation of the optimum PDLLME method

To validate the optimal PDLLME conditions, the linear range, correlation coefficient, accuracy and detection limits of honokiol (component 4) and magnolol (component 5) were investigated. Both honokiol and magnolol exhibited a good linear range of 10–1000 μg L^{-1} with a correlation coefficient (r^2) 0.9962 and 0.9995, and the recoveries (Table 1) at three spiking levels ranged from 90.2 to 99.4% with RSDs less than 7.2% ($n = 3$). The limits of detection (LODs) for honokiol and magnolol were 0.3 and 0.5 ng mL^{-1}, respectively, with a signal to noise ratio of 3. Furthermore, the precision, repeatability, and enrichment factors (EFs) of the five target components were also investigated. The precision test was carried out by injecting the standard solution five times consecutively, and the RSDs were in the range of 0.8–3.0%. The reproducibility of the method was determined by analyzing five samples on different runs, and the RSDs were in the range of 1.3–5.2%. EFs were calculated based on the ratio of the peak area before and after the PDLLME extraction procedure; they were 43, 75, 53, 119 and 87, respectively, for the five target components.

3.8 Application of the PDLLME technique

The dissolution tests of Huoxiang Zhengqi tablets from different manufacturers were conducted using the paddle method from the Appendix XC of the Pharmacopoeia of the People's Republic of China 2010. A 0.1 M HCl solution was used as the dissolution medium and the rotation speed was kept at 50 rpm, and 10 mL sample solutions were respectively collected at 5, 15, 30, 45, 60, 75 and 90 min (meanwhile, 10 mL of isothermal dissolution medium was used to compensate). Then, the sample solutions were filtered through a 0.45 μm membrane and the filtrate was extracted by the optimal PDLLME method described above. Fig. 5 shows the dissolution status curves of Huoxiang Zhengqi tablets from Manufacturers A and B. According to the results, there were significant differences in the dissolution status curves from different manufacturers. The dissolution amounts of honokiol and magnolol from different manufacturers at 90 min are summarized in Table 2.

Table 2 Dissolution results of honokiol and magnolol in Huoxiang Zhengqi tablets from different manufacturers at 90 min ($n = 6$)

Manufacturer	Honokiol (μg per tablet)	Magnolol (μg per tablet)
A	345	340
B	165	105
C	80	70
D	18	10

The representative chromatograms of Huoxiang Zhengqi tablets with and without the PDLLME procedure are shown in Fig. 6. It can be seen that almost no peaks could be observed without the enrichment procedure, which might have been due to the extremely low concentrations of the components. However, the detection sensitivity of most components was largely improved by the PDLLME technique. During the

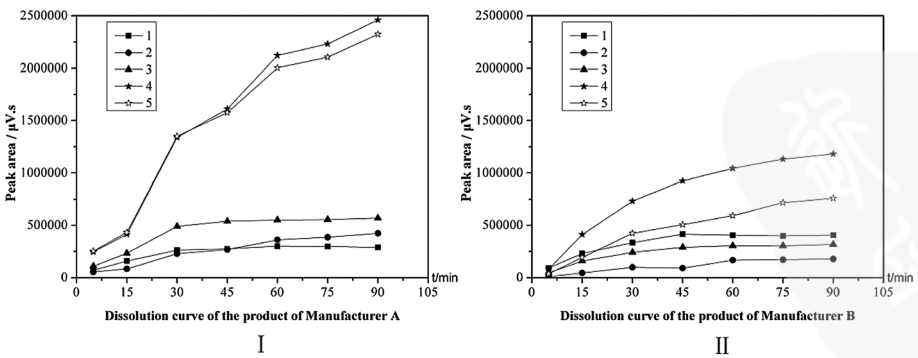

Fig. 5 Dissolution status curves of the products from manufacturer A and B. Components: 1–3 were unknown components in this study, 4 and 5 were honokiol and magnolol, respectively.

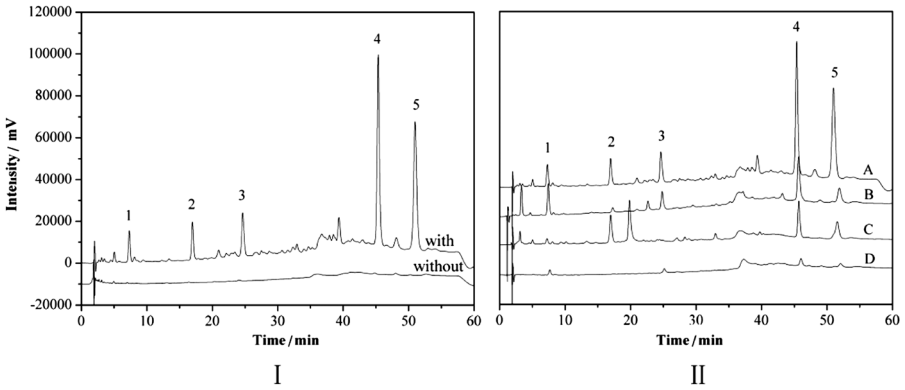

Fig. 6 Representative chromatograms of Huoxiang Zhengqi tablets from manufacturer A with and without PDLLME procedure at 60 min (I), and comparison of Huoxiang Zhengqi tablets from different manufacturers (A–D) using the PDLLME procedure (II). Experimental conditions were described in section "Extraction procedure". Peak identification: 1–3 were unknown components in this study, 4 and 5 were honokiol and magnolol, respectively.

PDLLME procedure, the disperser solvent could transfer into organic extraction droplets and form a mixed extraction phase with an extended soluble range. Therefore, components with a wider polarity range in the dissolution samples of Huoxiang Zhengqi tablet could be successfully enriched, resulting in improved detection sensitivity. In addition, a comparison of Huoxiang Zhengqi tablets from different manufacturers (A–D) using the PDLLME procedure was performed. The results show that the amounts and concentration of the dissolution samples of Huoxiang Zhengqi tablets from different manufacturers were significantly different. These results also demonstrate that the detection sensitivity of most potential active components was improved after using the PDLLME procedure.

4 Conclusions

In this study, an efficient PDLLME method was established and successfully used for the simultaneous extraction and concentration of five components in Huoxiang Zhengqi tablets after a dissolution process. Both a high enrichment factor and extraction recovery were obtained under the optimum conditions. By comparing the chromatograms and the dissolution results after the PDLLME procedure, a significant difference was observed between Huoxiang Zhengqi tablets from different manufacturers (A–D). These results demonstrated that PDLLME is a cost-effective and sensitive technique for the extraction and detection of five components in dissolution samples of Huoxiang Zhengqi tablets, and provides a new way to extract and concentrate multiple components in the dissolution determination of herbal preparations.

Acknowledgements

The project was supported by the National Natural Science Foundation of China (20905019), the Natural Science Foundation of Hebei (B2010000209), and the Natural Science Foundation of Hebei University (2007-111).

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