Development of microwave-assisted extraction followed by headspace single-drop microextraction for fast determination of paeonol in traditional Chinese medicines

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Abstract
Paeonol is an important active component present in traditional Chinese medicines (TCMs), which was used for the treatment of many diseases such as eczema. In this work, microwave-assisted extraction (MAE) was firstly combined with headspace single-drop microextraction (HS-SDME), and applied to rapid determination of paeonol in two TCMs of Cynanchum paniculatum and Paeonia suffruticosa. In the proposed method, paeonol in TCMs was isolated by using MAE, followed by extraction and concentration by HS-SDME, and detected by gas chromatography–mass spectrometry (GC–MS). The experiment parameters of MAE and HS-SDME were discussed, and the method precision, recovery and detection limit were also studied. To further demonstrate the reliability of the quantification, both the proposed method and a standard method of steam distillation (SD) were simultaneously applied to quantitative analysis of paeonol in TCM samples from different growing areas. The experimental results show that MAE–HS-SDME is a simple and rapid method for the quantitative analysis of paeonol in TCMs, and is also a potential and alternative tool for quality monitoring for the two TCMs of C. paniculatum and P. suffruticosa.

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Keywords: Traditional Chinese medicines; Paeonol; Microwave-assisted extraction; Headspace single-drop microextraction; Gas chromatography–mass spectrometry; Quality monitoring

1. Introduction

Traditional Chinese medicines (TCMs) as we know it today has a long history dating back several thousands of years. So far, there have been 12,806 medical resources found in China, including 11,145 medicinal plants, 1581 medicinal animals and 80 medicinal minerals [1]. Furthermore, a total of 2375 products have been compiled in the Pharmacopoeia of People’s Republic of China (2000 edition) [2]. Due to their high pharmacological activity, low toxicity and rare complication, TCMs have played important roles in clinical therapy [3]. Recently, especially in Asia, more and more interest was re-attracted in this field. The quantitative analysis of active components in TCMs is an important research subject.

Cynanchum paniculatum and Paeonia suffruticosa are two common TCMs, which have been used to treat many diseases, such as eczema for thousand years [4–7]. Recently, it was found that the extract of C. paniculatum could inhibit the growth of human cancer cells [8]. It has been shown that paeonol is the main active constituent present in the two TCMs, which has anti-aggregatory, antioxidant and anti-inflammatory activities [9,10]. Therefore, it is very interesting to quantitatively determined paeonol concentration in TCMs. Many methods, such as micellar capillary electrophoresis [11], capillary zone electrophoresis and micellar electrokinetic chromatography (MEKC) [12], high performance liquid chromatography [13], and high performance liquid chromatography–electrospray ionisation–mass spectrometry [14] were developed for the determination of paeonol in the TCMs. Relatively, gas chromatography–mass spectrometry (GC–MS) is a very simple, rapid, and high-resolution tool for the analysis of the volatile constituents in TCMs [15–18]. Prior to the analyses, it was required to isolate and extract paeonol from the TCMs. Various extraction techniques can be used for that
purpose, e.g. hydro-distillation, steam distillation (SD), Soxhlet extraction, and solvent extraction [19–23]. However, losses of some volatile compounds, low extraction efficiency, and toxic solvent residue in the extract may be encountered using these extraction methods. Moreover, these extraction procedures are time-consuming. These shortcomings have led to the consideration of the new technique in essential oil extraction, which typically use less solvent, time, and energy, such as supercritical fluids, ultrasound and microwave.

There has recently been widespread interest in the application of microwave heating to the analysis of active compounds in plant herbs. Microwave-assisted extraction (MAE) was used for the isolation of volatile and active compounds from plant materials [24–26]. The main virtue of MAE is the reduction of extraction time and organic solvent. Prior to analysis, the analytes in the extracts obtained by MAE needed further extraction and concentration. So, a simple, rapid, and low-cost technique with extraction and concentration capacity is desirable.

Single-drop microextraction (SDME) introduced by Jeannot and Cantwell [27] has been shown to be a simple, inexpensive, fast, effective and virtually solvent-free sample pretreatment technique. In 2001, Jeannot and co-workers [28] developed headspace (HS) SDME for extraction and concentration of volatile compounds in an aqueous matrix. It was applied to ooped headspace (HS) SDME for extraction and concentration. So, a simple, rapid, and low-cost technique with extraction and concentration capacity is desirable.

In the work, for the first time, MAE and headspace single-drop microextraction (SDME) introduced by Jeannot and Cantwell [27] has been shown to be a simple, inexpensive, fast, effective and virtually solvent-free sample pretreatment technique. In 2001, Jeannot and co-workers [28] developed headspace (HS) SDME for extraction and concentration of volatile compounds in an aqueous matrix. It was applied to extraction and concentration of benzene, toluene, ethylbenzene and xylenes (BTEX) [29], methyl tert-butyl ether [30], poly-cyclic aromatic hydrocarbons [31], chlorobenzenes [32] in water samples, and aldehydes in blood [33]. More recently, Yamini and co-workers [34] successfully developed HS-SDME for the extraction and pre-concentration of the volatile components of plant sample (Iranian Pimpinella anisum anise seed). However, only semi-quantitative analysis can be obtained by using HS-SDME to analyze volatile components in such solid sample.

In the work, for the first time, MAE and headspace single-drop microextraction (SDME) were combined, and developed for quantitative analysis of paeanol in the two TCMs of C. paniculatum and P. suffruticos.

2. Materials and methods

2.1. TCM sample, chemicals and microwave oven

The dry roots of C. paniculatum from Anhui, Shangxi, and Ganshu, and P. suffruticos from Henan, Hebei and Guizhou, were purchased from Leiyingshang Company, Shanghai, China. Paeanol and menthol (internal standard) (Fig. 1) were obtained from the National Institute for the Control of Pharmaceuticals and Biological Products, Beijing, China. 1-Octanol, methanol (analytical grade) and sodium chloride were purchased from Chemical Agent Company, Shanghai, China. The microwave oven with a maximum delivered power of 700 W was purchased from Haier Company (Qingdao, China).

2.2. Calibration solution preparation

Stock solution (5.0 mg/ml) of paeanol was prepared by dissolving paeanol into methanol, and stored at 4 °C. The internal standard solution with 5.0 mg/ml was made by dissolving menthol in methanol. Working standard solutions with the paeanol concentration from 1.0 to 200 μg/ml were done by dilution of the paeanol stock solution with distilled water. For quantifying paeanol in the TCMs, 2 ml of working solutions and 10 μl of internal standard solution were introduced into 4 ml headspace vials.

2.3. GC–MS analysis

An HP 6890 GC system, coupled with a HP MD5973 quadrupole mass spectrometer was used. The extracted compounds were separated on an HP-5 capillary column (30 m length x 0.25 mm I.D., 0.25 μm film thickness). Split injection was employed for both distillation and SPME samples with a ratio of 20:1. The column oven temperature was programmed to rise from an initial temperature of 40 °C (1 min) to 200 °C at 8 °C/min, then to 280 °C at 15 °C/min. The injection temperature and ion source temperature were 250 and 230 °C, respectively. Helium was used as the carrier gas with a flow rate of 1 ml/min. The ionizing energy was 70 eV. All data were obtained by collecting the full-scan mass spectra within the scan range 40–350 amu. Compounds were identified using the Wiley 6.0 (Wiley, New York, NY, USA) Mass Spectral Library.

2.4. Optimization of MAE–HS-SDME parameters

In the proposed method, at first, paeanol was isolated from the TCMs using MAE. Next, the aqueous extract was further extracted and concentrated by suspending a solvent microdrop. The apparatus of MAE followed by HS-SDME for the active compound of paeanol from the TCMs is shown in Fig. 2. In this work, the experiment parameters of MAE (extraction solvent, stirring rate, sample temperature, extraction time, and salt effect) were studied. A C. paniculatum sample from Anhui, China, was used for this study. The average peak area of paeanol obtained by replicate four analyses was used to determine the optimal parameters.

2.5. Quantification of paeanol in TCM samples from different growing areas by MAE–HS-SDME

C. paniculatum and P. suffruticosa samples from different growing areas, were ground to fine powder. Fifty milligrams for
Fig. 2. The apparatus of microwave-assisted extraction followed by headspace single-drop microextraction.

each TCM sample was extracted with 4 ml distilled water under microwave irradiation at 400 W for 3.0 min. After MAE, 2.0 ml the aqueous extract was introduced into 4 ml headspace vial, and 10 μl internal standard solution was added. Paeonol in the extracts was headspace extracted and concentrated by 1.0 μl 1-octanol, with the optimal HS-SDME conditions (stirring rate of 700 rpm, extraction temperature of 50 °C, extraction time of 4 min, 30% NaCl). Finally, the analyte in the microdrop solvent was analyzed by GC–MS.

To quantify paeonol in the TCMs, 2 ml calibration solutions (1.0–200 μg/ml) were sampled by HS-SDME with the optimal SDME conditions, and analyzed by GC–MS.

2.6. Precision, recovery and detection limit

The method precision was studied by four replicate analyses of paeonol in C. paniculatum from Anhui by MAE–HS-SDME under the optimum conditions. The relative standard deviation (RSD, %) was calculated on basis of the obtained peak areas. Recovery was also investigated by adding 20 μl of standard stock solution (5.0 mg/ml) and 20 μl internal standard solution (5.0 mg/ml) to a C. paniculatum sample (50 mg) containing known amounts of paeonol. Triplicate measurements were performed by MAE–HS-SDME–GC–MS. The detection limit was evaluated by replicate analyses of the calibration solution with a low paeonol concentration (1 μg/ml, 2 ml) by the proposed method.

2.7. Quantification of paeonol in TCM samples from different growing areas by SD

Forty grams of C. paniculatum and P. suffruticosa samples was ground to fine powder, and then put into a 1000 ml distillation flask. Five hundred milliliters of distilled water was added and volatile oil distillation apparatus was set according to the Chinese pharmacopoeia [2]. The mixture was distilled for 6 h. Oil was collected from the condenser, dried over anhydrous sodium sulfate, and the yield of the sample was 0.47%. The obtained essential oil was stored at −10 °C until analysis. According to Chen’s method [36], paeonol in the TCMs was quantitatively analyzed by GC–MS.

3. Results and discussion

3.1. Optimization of MAE–HS-SDME parameters

The paeonol in the TCMs was isolated by MAE, and further extracted and concentrated by using SDME. As we know, SDME has headspace and immerse modes. Considering interference from the matrix, headspace SDME was used in the work. The experiment parameters of MAE (extraction solvent, microwave power and irradiation time) and HS-SDME (headspace solvent, stirring rate, sample temperature, extraction time, and salt effect) were studied. A C. paniculatum sample (50 mg) from Anhui, China, was used.

3.1.1. MAE parameters

At first, the extract solvent of MAE was considered. Fifty milligrams C. paniculatum sample was introduced into a 10 ml glass bottle (Fig. 2). Four milliliters water–methanol mixture with the different methanol concentration (0, 25, 50 and 100%) was used as the extracted solvent of MAE, respectively. MAE of the TCM sample was performed at 700 W for 4 min. The MAE extract (2 ml) was further extracted and concentrated by using 1.0 μl 1-octanol microdrop at 25 °C for 6 min, with the stirring rate of 700 rpm. Finally, the extracts in 1-octanol were injected and analyzed by GC–MS. We found that the extracted amount of paeonol was very close by water–methanol mixture with different methanol concentration. Hence, water was selected as the MAE solvent of paeonol. Next, microwave power (200, 400 and
The effect of microwave power and irradiation time on extraction efficiency of paeonol was investigated. As seen from Fig. 3, with microwave power of 200, 400 and 700 W, the extraction efficiency increased with the exposure time. However, the extraction efficiency decreased when the irradiation time was more than 4 min. The best extraction efficiency was obtained at microwave power of 400 W and irradiation time of 3 min. This may be due to that high microwave power and long irradiation time can lead to the decomposition of the analyte. Hence, in the further work, MAE was performed at microwave power of 400 W, and irradiation time 3 min.

3.1.2. HS-SDME parameters

The HS-SDME parameters were also studied. *C. paniculatum* sample was extracted under the optimized MAE conditions. Two milliliters aqueous extract obtained by MAE was introduced into 4 ml headspace vial with 1 cm stirring bar, and was used for HS-SDME.

The choice of an appropriate extraction solvent is essential for the HS-SDME. The extraction solvent has to meet three requirements: to have low volatility in order to be stable at the extraction period, to extract analytes well and to be separated from the analyte peaks in the chromatogram [35]. In order to determine which solvent would be optimal for the extraction of paeonol, three solvents including tetradecane, 1-octanol, and dodecane were examined. Among different solvents examined, 1-octanol gave the best extraction efficiency, and its peak was easily separated from the sample peaks. Therefore, 1-octanol was chosen as extracting solvent in this investigation.

HS-SDME is based on the equilibrium between three phases. It was shown that both slow mass transfers in aqueous phase, and diffusion of analytes into the microdrop are limiting steps in the overall extraction process [29]. Stirring the solution improves mass transfer in the aqueous phase and induces the convection in the headspace. Therefore, equilibrium between the aqueous and vapor phases can be achieved more rapidly. So, sample stirring reduces the time required to reach the equilibrium and extraction time by enhancing the diffusion of the analytes towards the microdrop. In this study, the MAE extract with the volume of 2 ml was continuously agitated at 25 °C at different stirring rates with a 1.0 cm magnet on a stirrer plate. 1.0 μl 1-octanol was exposed on the headspace for 6 min. As can be seen in Fig. 4, the signal increases with increase in stirring speed up to 700 rpm. At elevated rates more than 900 rpm, the spattering of solution occurs which damages the drop and causes signal decreasing. Thus, 700 rpm was selected as optimum stirring rate.

When the sample temperature is increased, the vapor pressure of the analyte and consequently the concentration of analyte in the headspace are increased. This can improve the extraction efficiency. However, by increasing the solution temperature, the temperature of the headspace and accordingly the temperature of the microdrop will increase. This may be lead to the loss of the extraction solvent and decrease of extraction efficiency. The amount of extracted analyte is expected to increase with enhancing the microdrop exposure time in the headspace of the stirred sample solution. However, the HS-SDME is not an exhaustive extraction method and for optimum repeatability of the extraction, it is necessary to choose a time in which equilibrium between the extracting microdrop, the headspace and the sample solution is reached [35]. Therefore, sample temperature and extraction time are the two key parameters for HS-SDME. In this study, sample temperature (25, 40, 50 and 60 °C), and extraction time (2–10 min) were studied. The effect of sample temperature and extraction time on extraction efficiency is shown in Fig. 5. It was found that when sample
temperature was increased, sequentially, the concentration of analyte in the headspace was increased, and better extraction efficiency was obtained. However, high sample temperature can make the microdrop temperature increase, and lead to the loss of extraction solvent and decrease of extraction efficiency when the exposure time was more than 6 min. It can be seen from Fig. 5 that the best extraction efficiency was achieved at the sample temperature of 50 °C in a short exposure time of 4 min.

The influence of ionic strength on the efficiency of HS-SDME was also studied. For this purpose, the ionic strength of solutions was modified by addition of sodium chloride. In order to investigate the effect of ionic strength, a series of spiked samples with various concentrations of NaCl in the range of 0–30% were prepared by adding of calculated weight of NaCl into a 2 ml volume of MAE extract. Plots of relative peak area versus ionic strength have been shown in Fig. 6. According to the curves, it is clear that the addition of ionic strength promotes the transport of the analyte to the headspace and hence to the extracting drop. Thus, the followed measurements were carried out at a NaCl concentration of 30%.

3.2. Determination of paeonol in C. paniculatum and P. suffruticosa samples from different growing areas by MAE–HS-SDME

With microwave irradiation (400 W, 3 min), paeonol was isolated from the TCM samples from different growing areas, followed by further extraction and concentration by HS-SDME, with the optimal experimental parameters. Fig. 7 shows the GC–MS total ion chromatograms of C. paniculatum (a) and P. suffruticosa (b) by MAE–HS-SDME.

To obtain the calibration curve, HS-SDME followed by GC–MS analysis of the calibration solutions ranged from 1.0 to 200 μg/ml. Replicated four analyses for each solution were performed. A calibration curve for quantifying paeonol in the TCMs was achieved: \( Y = 0.46X - 0.27, R^2 = 0.9941 \) (\( X \): peak area ratio of paeonol to IS; \( Y \): paeonol concentration in TCMs, mg/g). According to the calibration curve, paeonol concentration in the TCMs from different areas was calculated, and the analytical results are listed in Table 1. The data in Table 1 shows that the paeonol concentration in TCMs from different growing areas was very different. This may be due to different climate and soil. Paeonol is the main active component in the two TCMs, and plays key role in the course of disease treatment. It has been demonstrated in our previous studies that quality assessment cannot be performed according to the concentrations of active constituents in TCMs [15,17]. Therefore, the proposed method has the potential for quality monitoring of the two TCMs of C. paniculatum and P. suffruticosa.

3.3. Method precision, recovery and detection limit

The method precision was expressed by RSD value. Four replicate analyses of C. paniculatum from Anhui by MAE–HS-SDME were performed. By using paeonol peak areas, the RSD value was calculated as 10.5%. This shows that the proposed method has an acceptable precision. The analytical recovery was determined by replicate measurements on a paeonol-spiked C. paniculatum sample. The amount of added paeonol was obtained by calculation of paeonol in the TCM according to the internal standard method. The recovery of paeonol was 88%, which was obtained by comparison of its real value with the calculated data. To obtain the detection limit, replicate analyses of calibration solution with the concentration of 1.0 μg/ml were performed. The detection limit for paeonol was calculated on basis of S/N ratio of 3, the obtained value was 2.0 μg/ml. It was much lower than the paeonol concentration in TCMs. This shows that the proposed method is sensitive enough to analyze paeonol in TCMs.

3.4. Determination of paeonol in C. paniculatum and P. suffruticosa samples from different growing areas by MAE–HS-SPME

To demonstrate the reliability of the quantification by the proposed method, the conventional method, SD followed by GC–MS, was also used to quantitatively analyze paeonol in the six TCM samples. According to Chen’s method, paeonol in the TCMs was quantified and the analytical results are also listed in Table 1. It can be seen from Table 1 that the paeonol concentrations in the TCMs obtained by the proposed method is very close to those by SD, respectively. This shows that it is very reliable to determine the paeonol in the TCMs by MAE–HS-SDME.

![Fig. 6. The effect of ionic strength on HS-SDME extraction efficiency of paeonol.](image.png)

Table 1: The concentration of paeonol in six TCM samples by MAE–HS-SDME and SD

<table>
<thead>
<tr>
<th>TCM samples</th>
<th>Source</th>
<th>Paeonol concentration (mg/g)</th>
<th>MAE–HS-SDME</th>
<th>SD</th>
</tr>
</thead>
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<tr>
<td>Cynanchum paniculatum</td>
<td>Anhui, China</td>
<td>2.6 ± 0.1</td>
<td>2.4 ± 0.1</td>
<td></td>
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<tr>
<td>Cynanchum paniculatum</td>
<td>Shanghai, China</td>
<td>3.6 ± 0.2</td>
<td>3.8 ± 0.3</td>
<td></td>
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<tr>
<td>Cynanchum paniculatum</td>
<td>Guangdong, China</td>
<td>5.4 ± 0.4</td>
<td>5.2 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Paeonia suffruticosa</td>
<td>Henan, China</td>
<td>0.6 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Paeonia suffruticosa</td>
<td>Hebei, China</td>
<td>1.8 ± 0.2</td>
<td>1.9 ± 0.2</td>
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<tr>
<td>Paeonia suffruticosa</td>
<td>Guizhou, China</td>
<td>1.2 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td></td>
</tr>
</tbody>
</table>
4. Conclusions

The two sample preparation techniques, MAE and HS-SDME were successfully combined, and developed for isolation, extraction and concentration of the active component, paeonol from TCMs. Using the proposed method, paeonol in *C. paniculatum* and *P. suffruticosa* from different growing areas was fast determined. The results obtained for precision and recovery serve for method validation. All the results indicate that it is feasible to analyze the volatile bioactive components in TCMs by MAE–HS-SDME–GC–MS. Compared with SD, a short time and a small amount of sample were required in the proposed method. The extraction process was performed by using a home-made MAE equipment and a commercial microsyringe. Moreover, the low cost procedure is very straightforward.

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References