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Journal of Pharmaceutical and Biomedical Analysis 38 (2005) 326–331
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Short communication

Rapid analysis of essential oil from *Fructus Amomi* by pressurized hot water extraction followed by solid-phase microextraction and gas chromatography–mass spectrometry

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Received 1 December 2004; accepted 20 December 2004
Available online 10 February 2005

Abstract

In this paper, a simple, rapid, solvent-free and low-cost method of pressurized hot water extraction (PHWE) followed by headspace solid-phase microextraction (HS-SPME) and gas chromatography–mass spectrometry (GC–MS) was developed for the analysis of essential oil in a traditional Chinese medicine (TCM) of the dried ripe fruit of *Fructus Amomi* (Sha Ren). The essential oil in the TCM (0.050 g) was extracted by water at 50 bar and 150 °C, followed by extraction and concentration by SPME fibers at 80 °C for15 min and analysis by GC–MS. The PHWE and HS-SPME parameters were optimized. Thirty-five compounds in the TCM were identified by PHWE–HS-SPME. Among them, camphor, an active compound, in the TCM samples was quantitatively analyzed. The proposed method required little time to prepare the sample. Moreover, little sample mass and no organic solvent was needed. The precision of the present method was found to be good (R.S.D. <10.0%). It is shown that PHWE–SPME–GC–MS is an alternative method for the determination of volatile components in TCMs and can be used as a powerful tool for TCM quality assessment.

Keywords: Traditional Chinese medicine; *Fructus Amomi*; Pressurized hot water extraction; Headspace solid-phase microextraction; Essential oil

1. Introduction

Traditional Chinese medicines (TCMs) are gaining more and more attention in modern pharmaceutical institutes as they provide an important resource for drug development. Analysis of TCMs is an important subject in biochemical, pharmaceutical and clinical research [1]. For many TCMs, there are main active components in their essential oils [2]. Conventional techniques of steam distillation and solvent extraction, coupled with gas chromatography–mass spectrometry (GC–MS) have been applied to the determination of essential oils in TCMs. Supercritical CO_2 extraction was developed for the extraction of plant essential oils [3–11], however, this technique requires special and expensive equipment, and the sample preparation is a tedious and time-consuming procedure.

Recently, a simple and inexpensive technique of pressurized hot water extraction (PHWE) was developed for the extraction of volatile compounds in solid samples. PHWE has successfully been used for extraction of alkylbenzenes, PAHs and PCBs residues in soils and environmental solids, pesticide residues in soils, plant material and food [12–25]. It has also been applied to the analysis of essential oils in plant materials [26–30]. Recently, the application of PHWE to solid sample analysis was reviewed by Smith [31]. Since PHWE is a dynamic extraction method, the analytes in the aqueous extract have to be further extracted and concentrated before analysis. Solid-phase microextraction (SPME) is a sample extraction and simultaneous concentration technique with the advantages of simplicity, rapidness and no need for organic solvents. PHWE combined with SPME has

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doi:10.1016/j.jpba.2004.12.027

been developed for the analysis of residual solvents in pharmaceutical products and flavors in tablets [32–34].

Fructus Amomi, the dried ripe fruit of *Amomum villosum* Lour., *Amomum villosum* Lour. var. xanthioides T.L. Wu et Senjen or *Amomum longiligulare* T.L. Wu, is a common TCM. Its main active ingredient, essential oil, is often used to eliminate damp and improve appetite, to warm the spleen and check diarrhea, and to prevent abortion [35]. Due to the effective components in its oil, *Fructus Amomi* is usually added into tea and wine. Moreover, *Fructus Amomi* is also used as a food spice in China. It is, therefore, of interest to be able to analyse the essential oil in *Fructus Amomi*. The routine methods of steam distillation and solvent extraction have been applied to its volatile oil analysis [36,37]. As we know, extraction of essential oil by the two techniques is a tedious and time-consuming procedure. In our previous studies [38–42], SPME was developed for the rapid analysis of volatile constituents in TCMs. However, it is impossible to quantitatively analyze the volatile compounds in solid samples such as TCMs by SPME.

In this work, we developed PHWE with headspace (HS) SPME for the determination of the essential oil in *Fructus Amomi*. Parameters of PHWE and SPME, and method validation were studied, Quantitative analysis of camphor in the TCM samples was performed by the proposed method.

2. Experimental

2.1. PHWE instruments

PHWE was performed using the following assembly (Fig. 1): a Shimadzu LC10AD pump was used to propel the water used as extractant through the system. A nitrogen cylinder was attached to an extractor by three-way valve. The extractor (a prototype designed and patented by Salvador and Merchan [43]), consisting of a stainless steel cylindrical extraction chamber (8 cm × 3 mm i.d.), closed with screws at either end that permit the circulation of the leaching fluid through them, was used. The screw caps also contain stainless steel filter plates (2 μm in thickness and 1/4 in. i.d.; 1 in. = 2.54 cm) to ensure that the plant material remains in the extraction chamber. This chamber, together with a stainless steel preheater, is located in an oven, designed to work up to 300 °C and controlled using a Toho TC-22 temperature controller. A cooler system, (consisting of a coil coupled to an Ultraterm 6000383 P-Selecta recirculation bath) was used to cool the fluid from the oven to a constant temperature close to 25 °C, thus avoiding the losses of volatiles caused by the hot water. The outlet of this coil was coupled to a stainless steel variable restrictor that was used to control the pressure in the system in order to maintain the extractant water in liquid state.

2.2. Materials

Fructus Amomi samples (the dried ripe fruit of *Amomum villosum* Lour.) from two growing areas of Hainan and Guangdong, China, were purchased from the Leiyunshang Company (Shanghai, China). The extraction fibers: 100 μm polydimethylsiloxane (PDMS), 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB), 65 μm carbowax/divinylbenzene (CW/DVB) and 75 μm carboxen poly(dimethylsiloxane) (CAR/PDMS) were purchased from Supelco, Bellefonte, PA and USA. Camphor (purity, 98%) was obtained from Flavor and Spice Company, Shanghai, China.

2.3. Sample and calibration solution preparation

Fructus Amomi samples were stored in the dark at 4 °C till used. The samples were ground to fine powder. An amount of sample powder 0.050 g was used for PHWE.

Standard stock solution (1.0 mg/ml) of camphor was prepared in methanol. Working standard solutions of 0.05, 0.1, 1.0, 10 and 20 μg/ml were prepared by dilution with doubly distilled water.

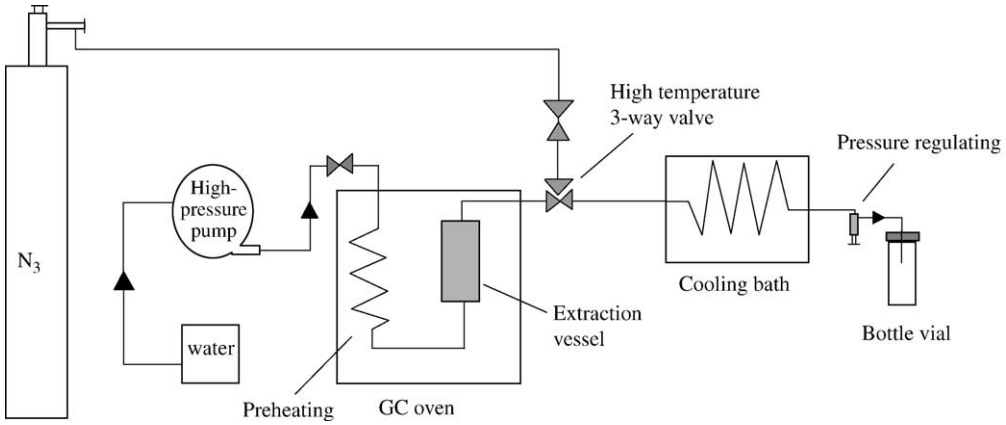

Fig. 1. PHWE equipment used in the experiments.

2.4. Pressurized hot water extraction and headspace solid-phase microextraction

Extraction of the essential oil in *Fructus Amomi* sample was performed using the assembly described above (Fig. 1). 0.050 g TCM sample was extracted at 150 °C and 50 bar. Extraction time of 5 min and flow-rate of 1.0 ml/min were used. The aqueous extract after PHWE was cooled in the refrigerant at 25 °C, and collected in a 10-ml vial. The analytes in the aqueous extract (2.0 ml) were headspace extracted by a PDMS-DVB fiber at 80 °C for 15 min, followed by GC–MS analysis.

To quantify camphor in the TCM samples, 2 ml working solutions were introduced into 8 ml headspace vials and HS-SPME was performed at the same conditions described above.

2.5. Recovery and repeatability

The repeatability of the method was studied by four replicate analyses of the essential oil in *Fructus Amomi* from Hainan by PHWE and HS-SPME at the optimum conditions. Recovery was investigated by adding 0.1 ml camphor stock solution (1.0 mg/ml) to a TCM sample containing a known amount of camphor. Triplicate measurements were performed by PHWE–HS-SPME.

2.6. GC–MS

Desorption and analysis of volatile compounds were carried out on an HP 6890 GC system, coupled with an HP MD5973 quadrupole mass spectrometer. The compounds were separated on a HP-5MS capillary column (30 m × 25 mm i.d. × 25 μm film). The carrier gas was helium with flow rate of 1.0 ml/min. Splitless (2 min) and split modes were used. The injector temperature was set as 270 °C. The column oven temperature was programmed to rise from an initial temperature of 50 °C (2 min) to 200 °C at 6 °C/min, then to 280 °C at 10 °C/min. The temperature of mass spectrometer was 230 °C. The ionizing energy was 70 eV. All data were obtained by collecting the full-scan mass spectra within the scan range 40–500 amu. Compounds were identified using the Wiley 6.0 (Wiley, New York, NY, USA) Mass Spectral library.

3. Results and discussion

3.1. Optimization of PHWE and HS-SPME conditions

To obtain the optimal PHWE conditions, *Fructus Amomi* (0.05 g) from Hainan was extracted by water at three temperatures of 125, 150 and 175 °C with the pressures of 20, 50 and 80 bar for each temperature. After PHWE, 2.0 ml of the aqueous extract was headspace adsorbed by a CW-DVB fiber at 25 °C for 5 min, with a stirring ratio of 1100 rpm. The peak area sum of eight main compounds of α-pinene, camphene, 1-myrcene, D-limonene, camphor, borneol, bornyl acetate and caryophyllene in the TCM at different PHWE conditions are

Table 1
Peak area sum of the eight main compounds in *Fructus Amomi* by PHWE at different extraction temperature and pressure

Peak area			
Temperature (°C)	Pressure (bar)		
	20	50	80
125	3.98×10^8	4.31×10^8	4.13×10^8
150	4.24×10^8	4.46×10^8	4.31×10^8
175	4.07×10^8	4.12×10^8	4.23×10^8

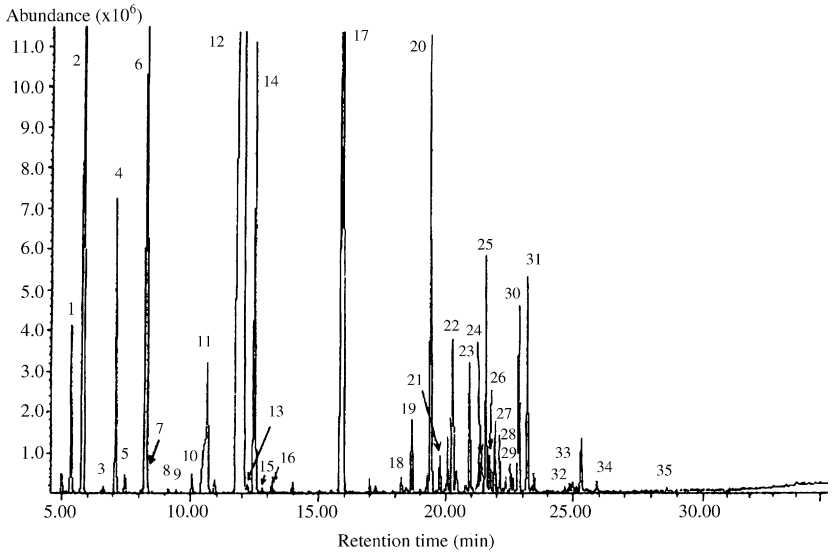

Fig. 2. The total ion GC–MS chromatogram of essential oil in *Fructus Amomi* from Hainan by PHWE–HS-SPME.

shown in Table 1, which indicated that 150 °C and 50 bar were the optimum PHWE conditions.

HS-SPME conditions including fiber coating, extraction time and temperature were also studied, By comparing the extraction efficiencies of the eight compounds obtained at different HS-SPME conditions, the 100 μm PDMS fiber, extraction temperature of 80 °C and extraction time of 15 min were found to be the optimal HS-SPME conditions.

3.2. Determination the volatile components in Fructus Amomi by PHWE and HS-SPME

The *Fructus Amomi* sample from Hainan was extracted by PHWE equipment at the optimum conditions (extraction temperature: 150 °C; extraction pressure: 50 bar; flow-rate: 1.0 ml/min; extraction time: 5 min). Then, 2 ml PHWE of the aqueous extract was further headspace absorbed by using the PDMS fiber at 80 °C for 15 min. Finally, the analytes adsorbed on the fiber were desorbed at 270 °C for 2.0 min and analyzed by GC–MS. The GC–MS chromatogram of essential oil in *Fructus Amomi* from Hainan by PHWE–HS-SPME is shown in Fig. 2.Thirty-five compounds were separated and identified. The volatile constituents present in the TCM samples were mainly monoterpenes such as camphor, camphene and D-limonene as well as esters (borneol acetate) and sesquiterpene (caryophyllene) (Tab1e 2). The relative contents were obtained from their peak area ratios and these data are listed in Table 2. From Table 2, three active compounds, camphor, borneol and borneol acetate, were found to be present in the TCM essential oil [35–37].

Quality assessment is an important task for modernization of TCM. For many TCMs, due to the active components being present in their essential oils, assessing quality can be done by quantitative analysis of the active components by GC–MS. In this work, PHWE–HS-SPME was applied to quantitative analysis of the active compound camphor in *Frutus Amomi*.

To quantify the active compound, HS-SPME of the working solutions was performed. Three replicate measurements were carried put and a calibration curve for camphor was obtained. The quantitative equation is $Y = 1.48 \times 10^9$

Table 2
Determination of volatile constituents in *Fructus Amomi* by GC–MS–PHWE–HS-SPME

No.	Retention time (min)	Compound	Mass spectrometric date	Relative content (%)	R.S.D. (%)
1	6.231	α-Pinene	93, 77, 41, 121, 105	1.52	4.5
2	6.600	Camphene	93, 121, 79, 41, 107	7.55	4.9
3	7.321	β-Pinene	93, 41, 69, 79, 121	0.05	5.2
4	7.678	β-Myrcene	41, 93, 69, 27, 53	2.72	5.6
5	8.036	α-Phellandrene	93, 77, 136, 41, 27	0.15	4.3
6	8.700	D-Limonene	68, 93, 41, 79, 136	5.85	6.5
7	8.786	Eucalyptol	43, 81, 108, 71, 154	0.17	6.2
8	9.448	*cis*-β-Terpineol	43, 71, 93, 55, 81	0.03	6.7
9	9.627	*cis*-Linaloloxide	59, 43, 94, 68, 111	0.01	7.9
10	10.059	(+)-4-Carene	121, 93, 136, 39, 79	0.15	4.2
11	10.701	3,7-Dimethyl-1,6-octadien-3-ol	71, 41, 93, 55, 80	2.93	7.9
12	12.045	Camphor	95, 81, 69, 108, 41	37.87	8.9
13	12.322	Isoborneol	95, 41, 55, 110, 67	0.04	8.3
14	12.622	Borneol	95, 41, 110, 55, 67	5.60	6.2
15	12.905	Menthol	71, 43, 93, 111, 55	0.04	5.1
16	13.274	α-Terpineol	59, 93, 43, 121, 136	0.07	8.4
17	15.916	Borneol acetate	95, 43, 121, 136, 108	15.46	3.7
18	18.276	Copaene	119, 105, 161, 93, 41	0.12	5.9
19	18.691	β-Elemene	81, 93, 68, 41, 107	0.58	4.1
20	19.424	Caryophyllene	93, 41, 69, 133, 79	5.51	8.9
21	19.781	α-Farnesene	41, 93, 69, 55, 107	0.29	9.2
22	20.278	α-Caryophyllene	93, 80, 41, 121, 147	1.78	7.4
23	20.941	Germacrene D	161, 105, 91, 41, 119	1.03	6.9
24	21.303	γ-Elemene	121, 93, 41, 107, 67	0.22	6.3
25	21.587	(S)-1-methyl-4-(5-methyl-methylene-4-hexenyl)-cyclohexene	69, 41, 93, 79, 109	1.99	7.1
26	21.730	α-Cubebene	161, 189, 119, 41, 105	0.36	8.9
27	21.931	β-Sesquiphellandrene	69, 41, 93, 55, 161	0.55	9.6
28	22.116	*trans*-γ-Bisabolene	93, 107, 119, 135, 79	0.43	8.1
29	22.556	Hedycaryol	59, 93, 161, 81, 107	0.19	6.5
30	22.902	(E)-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	69, 41, 93, 55, 107	1.57	7.1
31	23.220	1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene	81, 43, 123, 161, 55	1.87	6.6
32	24.660	tau-Cadinol	161, 43, 204, 105, 95	0.06	6.9
33	25.020	α-Cadinol	43, 95, 121, 204, 79	0.09	5.0
34	25.925	α-Santalol	93, 121, 107, 79, 41	0.49	4.6
35	28.613	Santalol	93, 121, 41, 79, 107	0.03	4.9

$X - 2.1 \times 10^7$ (Y: peak area; X: camphor concentration (μg/mg); R^2: 0.987). The camphor concentration in the TCM samples from Hainan and Guangdong was 2.4 and 1.7 μg/mg, respectively.

3.3. Repeatability and recovery

The method repeatability was studied by four replicate analyses of the essential oil in *Fructus Amomi* for Hainan by PHWE–SPME–GC–MS at the optimum conditions. The obtained peak areas were used for calculation of the relative standard deviation (%, R.S.D.) and R.S.D. valued form 4.3 to 9.8% were obtained (Table 2). The results show that the proposed method has a good repeatability. The analytical recovery was investigated by the replicate measurements of the camphor-added *Fructus Amomi* sample. The amount of the added camphor was calculated by the external standard method. The recovery valued of camphor was 90%, which was obtained by comparison of the real value with the calculation value. This indicates that the method provided a good analytical recovery.

PHWE is a powerful tool for extraction of volatile compounds in solid samples. Its major advantages are the low-cost and envormental friendliness of water. However, the PHWE aqueous extract requires further sampling and enrichment, prior to GC–MS analysis. As we know, SPME is an extraction and concentration sample technique. In this study, PHWE combined with HS-SPME was used for the extraction of TCM essential oils and quantitative analysis of the active compounds. In the proposed method, the sample preparation was simple, needed only 20 min (PHWE 5 min; SPME 15 min), which was much less than the time (about 6 h) required by steam distillation. Moreover, it required little sample mass (only 50 mg) and no organic solvent. It was shown that it is feasible to quantitatively analyze active components in TCMs by the proposed method.

4. Conclusions

The proposed method of PHWE–HS-SPME with GC–MS was shown to have several advantages of simplicity, rapidness and low-cost. It was demonstrated that PHWE–HS-SPME was an alternative method for the quantitative analysis of the active constituents in TCM essential oils and a potential tool for TCM quality assessment.

Acknowledgements

The project is supported by Project of the Economy committee of Shanghai (China), the National Natural Science Foundation (Project: 2047511) and the National Basic Research Priorities Programme (Project No. 001CB5102).

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