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Rapid determination of essential oil in *Acorus tatarinowii Schott*. by pressurized hot water extraction followed by solid-phase microextraction and gas chromatography–mass spectrometry

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Abstract

A simple, rapid, efficient and inexpensive method for the determination of essential oil in a traditional Chinese medicine (TCM), *Acorus Tatarinowii Schott*. was developed by using pressurized hot water extraction (PHWE) combined with solid-phase microextraction (SPME) and gas chromatography–mass spectrometry (GC–MS). *Acorus Tatarinowii Schott*. (0.050 g) was subjected to dynamic extraction with water at 50 bar, 150 °C and 1.0 ml/min for 5.0 min, and then the essential oil in the aqueous extract were extracted by SPME fibers at 60 °C for 10 min, finally the compounds on the fiber were desorbed and analyzed by GC–MS. The PHWE and SPME parameters were studied. The key active compound of α-asarone in the TCM samples from three different growing areas was quantitatively analyzed by external standard method. Compared to steam distillation (SD), the proposed method required little time (only 15 min) to prepare sample. Moreover, little sample mass and no organic solvent was needed. The present method provided good repeatability (R.S.D. less than 13.0%) and recovery (92% for α-asarone). It has been shown that PHWE–SPME–GC–MS is an alternative method for determination of essential oils in TCMs and a potential tool for TCM quality assessment.

Keywords: Pressurized hot water extraction; Solid-phase microextraction; Essential oils; Extraction method; *Acorus Tatarinowii Schott.*

1. Introduction

Historically, especially in China, traditional Chinese medicines (TCMs) have played an important role in clinical therapy because of their high pharmacological activity, low toxicity and rare complication [1]. In recent years, more and more interests have been re-attracted in this field. For many TCMs, such as *Flos Chrysanthemi Indici*, there are active components in their essential oils. Analysis of active components in essential oils has used as a tool for TCM quality assessment. The routine methods for the analysis of TCM essential oils included steam distillation (SD), solvent extraction and Soxhlet extraction. These conventional techniques required long time and large amount of organic solvents for the extraction of the TCM essential oils. These shortcomings have led to the consideration of the use of supercritical fluids in essential oil extraction processes. CO_2 is the most commonly used supercritical fluid because of its modest critical conditions. Thus, the supercritical CO_2 extraction of essential oils from TCMs and other plant materials is well documented [2–11]. The green house effect caused by the emissions of carbon dioxide and the cost of the fluid with the required purity and specially its low dielectric constant make mandatory the searching for new solvents.

Water is non-flammable, non-toxic, readily available and cheap and as solvent environmentally benign. The dramatic change in its physico-chemical properties at elevated temperatures and pressures enhances its usefulness. Pressurized hot water (PHW) has been used to replace conventional organic solvents in a variety of extraction process. Temperatures below the critical value of water ($T_c = 374$ °C) but usually

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above 100 °C are employed. Pressurized hot water extraction (PHWE) has been applied to the analyses 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 used for the analysis of essential oils in plant materials [26–30].

The dry rhizome of *Acorus Tatarinowii Schott*. is a common TCM and used for treatment of lots of diseases, such as epilepsy [31]. It was found that α-asarone in its essential oil is a key active compound used for treatment of epilepsy [32]. Conventional methods of SD and SFE have been used for isolation of its essential oil [33–35]. In general, the TCM quality assessment can be done by using SD method. In our previous studies, a simple, rapid and solvent-free technique, solid-phase microextraction (SPME), was developed for the analyses of TCM essential oils [36,37]. Recently, we successfully developed SPME for quality assessment of a common TCM, *Flos Chrysanthemi Indici* [38]. As we know, it is difficult to quantitatively analyze the volatile essentials in plant materials by SPME method. The aim of this research was to develop a rapid, efficient and inexpensive method for the quantitative analysis of essential oil in the TCM. In the proposed method, the essential oil in *Acorus Tatarinowii Schott*. was firstly sampled by PHWE, followed by SPME and gas chromatography–mass spectrometry (GC–MS).

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. An extractor (a prototype designed and patented by Salvador and Merchan [39]), 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 0.25 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

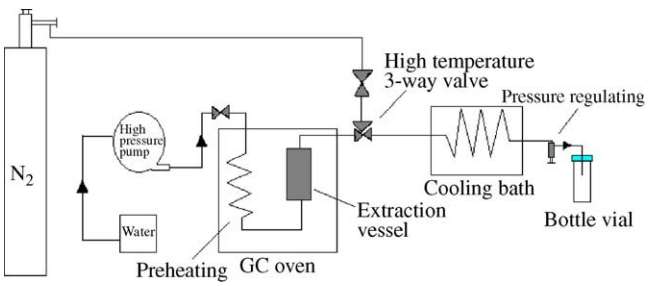

Fig. 1. PHWE equipment used in the experiments.

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

The dry rhizome of *Acorus Tatarinowii Schott*. from three growing areas (Guangxi, Hainan and Anhui, China) was purchased from a Chinese Herb Shop, Shanghai, China. Eucalyptol, camphor, terpinen-4-ol, linalool and α-asarone standards were all provided by the National Institute for the Control of Pharmaceuticals and Biological Products, Beijing, China. To verify the five compounds in the TCM, 1.0 μg/ml standard solution in water for each compound was prepared for SPME. Bis-distilled water purified through a Milli-Q deionizing unit (Millipore, Milwaukee, USA) was used as extractant. SPME manual holder and a 100-μm poly(dimethylsiloxane) (PDMS) fiber, a 65-μm poly(dimethylsiloxane)-divinylbenzene (PDMS-DVB), a 65-μm Carbowax-divinylbenzene (CW-DVB), a 75-μm Carboxen-poly(dimethylsiloxane) (CAR-PDMS) were purchased from Supelco (St. Louis, MO, USA). The SPME fibers were conditioned as recommended by the manufacturer at some degrees below each fiber's maximum temperature before they were used for the first time. Before the first daily analysis, the fibers were conditioned for 5 min at 270 °C in the GC injector. The magnetic stirrer was purchased from ShiLe Company, Shanghai, China.

2.3. Sample preparation

Acorus Tatarinowii Schott. samples were stored in the dark at 4 °C till used. The samples were ground to a fine powder. *Acorus Tatarinowii Schott*. (0.050 g) was used for PHWE, and 50 g was used for SD.

2.4. Calibration solution preparation

Standard stock solution (1.0 mg/ml) of α-asarone was prepared in methanol and stored at −4 °C. For quantitative analysis of α-asarone in *Acorus Tatarinowii Schott*. samples, working standard solutions, containing 0.05, 0.1, 1.0, 10 and 20 μg/ml α-asarone were prepared by dilution with bis-distlled water.

2.5. GC analysis

GC analyses were accomplished with an HP-5980 series II instrument equipped with HP-WAX and HP-5 capillary column (30 m × 0.25 mm i.d. × 0.25 μm film). The following temperature program was used: 60 °C, ramp of 10 °C/min up to 300 °C; injector temperature, 270 °C; detector temper-

ature, 300 °C; carrier gas, nitrogen (2 ml/min) detector dual FID; split ratio, 20:1; injection, 0.5 μl. Identification of the components was performed for both columns by comparison of their retention times with those of pure authentic samples and by means of their retention indices relative the series of *n*-hydrocarbons.

2.6. GC–MS analysis

Desorption analyses were performed on HP 6890 GC system, coupled with a HP MD5973 quadrupole mass spectrometer. A fused-silica capillary HP-5MS column with 30 m long, 0.25 mm i.d. and 0.25 μm film was from Agilent, USA, which was used for separation. The carrier gas was helium with flow-rate of 1.0 ml/min. Splitless (2.0 min) and split modes were used. The injector temperature was set as 270 °C. The column temperature was programmed to rise from an initial temperature of 60–160 °C at 10 °C/min, then to 300 °C at 12 °C/min, hold for 5 min. The temperature of mass spectrometer was 230 °C.

2.7. PHWE and SPME

Extraction of the essential oil in *Acorus Tatarinowii Schott*. was firstly performed using the assembly described above (Fig. 1). Bis-distilled water stored in a reservoir was pumped to the oven, where it reached the preheater and passed through the 1 ml extraction chamber, which contained 0.050 g sample powder. The aqueous extract was cooled in the refrigerant at 25 °C and, after passing through the variable restrictor, collected in a 10 ml-vial.

To obtain the optimum PHWE conditions, the extraction of *Acorus Tatarinowii Schott*. from Guangxi was carried out at three temperatures of 125, 150 and 175 °C and three pressures of 20, 50 and 80 bar. Extraction time of 5 min and flow-rate of 1.0 ml/min were used for each sample.

Aqueous extract (2.0 ml) and 1-cm stir bar were introduced into an 8 ml headspace vial for optimization of the SPME conditions. A stirring ratio of 1100 rpm was used. Extraction conditions, including fiber coating, extraction temperature and extraction time were firstly studied.

The optimum PHWE and SPME conditions were used for extraction of the TCM essential oil, and then the analytes were desorbed at 270 °C for 2 min and analyzed by GC–MS.

To quantitatively analyze α-asarone in *Acorus Tatarinowii Schott*., 2 ml working solutions were introduced into 8 ml headspace vials. Headspace extraction and GC–MS analyses were performed under the same conditions described above.

2.8. Recovery and repeatability

The repeatability was studied by four replicate analyses of the essential oil in *Acorus Tatarinowii Schott*. from Guangxi by PHWE and SPME at the optimum conditions. The relative standard deviation (R.S.D., %) values were calculated on basis of the obtained peak areas.

Recoveries were also investigated by adding 0.1 ml standard stock solution (1.0 mg/ml) to the TCM sample (50 mg), containing known amounts of α-asarone. Triplicate measurements were performed by PHWE–SPME–GC–MS.

2.9. Steam distillation

Acorus Tatarinowii Schott. (50.0 g) was put into a 1000 ml distillation flask. Distilled water (500 ml) was added and volatile oil distillation apparatus was set according to the Chinese pharmacopoeia (Chinese pharmacopoeia committee publishing house of people's Health, 2000, Part I: Appendix 64). The mixture was distilled for 6 h. Oil was collected from the condenser, dried over anhydrous sodium sulfate. The obtained essential oil was introduced into 5 ml volumetric flask, and the final volume of the extract was adjusted to 5.0 ml with *n*-hexane. The isolated oil by SD was analyzed by GC–MS, according to Wu et al.'s method [35].

3. Results and discussion

3.1. Optimization of PHWE conditions

Temperature and pressure of extraction can affect PHWE extraction efficiencies of volatile oils [26–30]. A mass amount of 0.050 g *Acorus Tatarinowii Schott*. from Guangxi, a short extraction time of 5 min and a flow-rate of 1.0 ml/min were used for this study.

To obtain the optimal extraction temperature and pressure, the essential oil in *Acorus Tatarinowii Schott*. was extracted at the temperatures of 125, 150 and 175 °C with the pressures of 20, 50 and 80 bar for each temperature. After PHWE, 2.0 ml aqueous extract was further headspace adsorbed by a CW-DVB fiber at 25 °C for 5 min, with a stirring ratio of 1100 rpm. SPME desorption was carried out at 270 °C for 2.0 min. The key active compound of α-asarone was used for determination of the optimal PHWE temperature and pressure. The peak areas of α-asarone obtained at different extraction conditions are shown in Table 1. It is shown that the maximum peak area of α-asarone can be obtained at 150 °C and 50 bar. Therefore, 150 °C and 50 bar are selected as the optimal PHWE conditions.

Table 1
Peak area of α-asarone obtained at by PHWE at different extraction temperatures and pressures

Peak area			
Temperature (°C)	Pressure (bar)		
	20	50	80
125	7.65×10^8	7.86×10^8	7.92×10^8
150	8.13×10^8	8.14×10^8	8.12×10^8
175	7.98×10^8	7.91×10^8	8.06×10^8

Table 2
Chemical components in *Acorus Tatarinowii Schott*. essential oil

No.	Retention time (min)	Compounds	Retention index[a]	Identification method	Relative content (%)		R.S.D. (%)
					PHWE–SPME	SD	
1	4.242	α-Pinene	939	RT MS	0.21	0.16	6.1
2	4.455	Camphene	954	RT MS	0.24	0.19	5.9
3	4.848	β-Pinene	974	RT MS	0.17	0.11	6.3
4	4.992	2-Pentyl-furan	978	RT MS	0.06	0.04	7.3
5	5.396	2-Carene	1001	RT MS	0.07	0.06	6.0
6	5.500	*p*-Cymene	1028	RT MS	0.14	0.11	6.1
7	5.569	Limonene	1032	RT MS	0.04	0.01	5.7
8	5.621	Eucalyptol	1033	RT GC MS	0.06	0.03	8.3
9	5.661	Ocimene	1041	RT MS	0.08	0.04	7.5
10	6.001	γ-Terpinene	1064	RT MS	0.09	0.08	5.9
11	6.578	Linalool	1102	RT GC MS	0.09	0.07	10.3
12	7.328	Camphor	1146	RT GC MS	0.04	0.01	10.6
13	7.778	Terpinen-4-ol	1165	RTGC MS	0.06	0.04	11.3
14	7.963	α-Terpineol	1177	RT MS	0.08	0.02	12.3
15	8.061	1-Methoxy-4-(2-propenyl)-2-benzene	1221	RT MS	0.26	0.27	12.9
16	10.247	α-Copaene	1376	RT MS	0.05	0.09	11.4
17	10.547	β-Caryophyllene	1418	RT MS	0.24	0.21	5.9
18	10.680	Cedrene	1422	RT MS	0.14	0.09	6.8
19	10.761	γ-Elemene	1433	RT MS	0.11	0.10	8.6
20	10.859	1,2-Dimethoxy-4-(2-propenyl)-benzene	1437	RT MS	8.76	8.81	9.6
21	11.170	α-Guaiene	1439	RT MS	0.35	0.36	10.8
22	11.234	β-Humulene	1456	RT MS	0.15	0.12	11.5
23	11.522	1,2-Dimethoxy-4-(1-propenyl)-benzene	1462	RT MS	6.51	6.60	6.4
24	11.609	γ-Gurjunene	1473	RT MS	0.24	0.26	5.9
25	11.839	Germacrene D	1480	RT MS	0.13	0.09	12.1
26	12.111	β-Selinene	1491	RT MS	0.25	0.23	6.4
27	12.676	1,2,3-Trimethoxy-5-[2-propenyl]-benzene	1514	RT MS	0.43	0.42	8.9
28	13.466	α-Asarone	1587	RT GC MS	29.65	24.09	8.0
29	14.020	β-Asarone	1596	RT MS	44.76	49.16	10.1
30	14.159	3,8-Dimethyl-5-(1-methylethylethyldiene)-1,2,3,4,5,6,7,8-octahydroazulene-6-one	1608	RT MS	0.37	0.78	6.9
31	14.262	1,4-*trans*-1,7-*cis*-Acorenone	1632	RT MS	0.19	0.46	7.4

[a] Retention indices relative to C_9–C_{17} *n*-alkanes on the HP-5MS column. RT, comparison of the relative retention time with those obtained from the NIST/NBS, Wiley libraries spectra and those reported by Wu et al. [35]; GC, gas chromatographic coelution with pure standard; MS, mass spectrometry.

3.2. Optimization of SPME conditions

Extraction temperature and time can affect SPME efficiencies [36,37]. Aqueous extract (2.0 ml) from the TCM (Guangxi) was obtained by PHWE under the optimum PHWE conditions and used for the optimization of SPME conditions.

At first, selection of the optimum fiber was carried out. Four fibers of PDMS, CW-DVB, CAR-PDMS and DVB-PDMS were simultaneously used for extraction of the analytes in the aqueous extract. The extraction was performed at the temperature of 25 °C and time of 10 min. The optimal fiber was determined by the extraction efficiencies of the four main compounds of 1,2-dimethoxy-4-(2-propenyl)-benzene (DPB2), 1,2-dimethoxy-4-(1-propenyl)-benzene (DPB1), α-asarone (AA) and β-asarone (BA) in The TCM (Table 2). The data obtained by using the four fibers are shown in Fig. 2. It can be seen from Fig. 2 that for these compounds except β-asarone, PDMS-DVB fiber has the highest efficiencies. Comprehensive considered, PDMS-DVB was regarded as the optimal fiber.

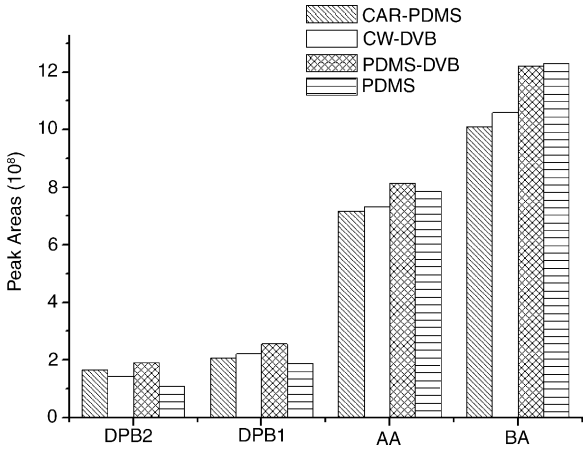

Fig. 2. The effect of fiber coating on extraction efficiencies of 1,2-dimethoxy-4-(2-propenyl)-benzene (DPB2), 1,2-dimethoxy-4-(1-propenyl)-benzene (DPB1), α-asarone (AA) and β-asarone (BA), SPME conditions were: extraction temperature, 20 °C; time, 5 min; stirring ratio, 1100 rpm; sample volume, 2.0 ml.

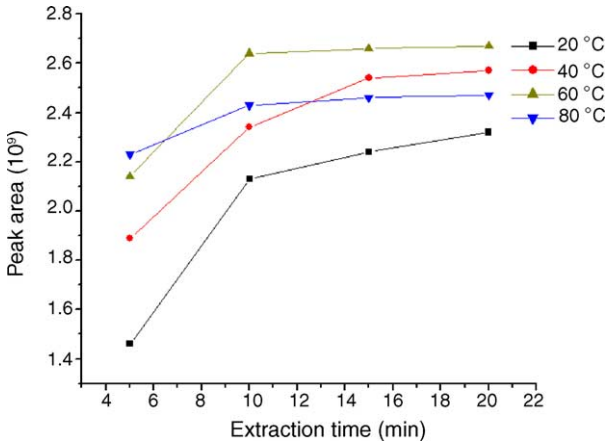

Fig. 3. The effect of SPME temperature and time on extraction efficiencies.

Next work was to optimize the SPME temperature and time by using the PDMS-DVB fiber. Extraction of the analytes was performed at extraction temperatures of 20, 40, 60 and 80 °C, with 5, 10,15 and 20 min for each temperature. The four main compounds of 1,2-dimethoxy-4-(2-propenyl)-benzene, 1,2-dimethoxy-4-(1-propenyl)-benzene, α- and β-asarone in the essential oil were used for determination of the optimum extraction temperature and time. The peak area sums of the four compounds at different conditions are shown in Fig. 3. It was shown that the best extraction efficiencies could be obtained at 60 °C for 10 min. Therefore, 60 °C and 10 min are regarded as the optimum SPME conditions.

3.3. Determination of the essential oil in Acorus Tatarinowii Schott. by PHWE and SPME

Acorus Tatarinowii Schott. (50 mg) from three growing areas (Guangxi, Hainan and Anhui, China) 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 aqueous extract was further headspace adsorbed by using a PDMS-DVB fiber at 60 °C for 10 min. Finally, the analytes adsorbed on the fiber were desorbed at 270 °C for 2.0 min and analyzed by GC–MS. Fig. 4a is the PHWE–SPME–GC–MS total ion chromatogram of essential oil in *Acorus Tatarinowii Schott*. from Guangxi, China. Compounds were identified by retention indices and Wiley 6.0 mass spectra library. Thirty-one compounds were identified in TCM samples, which mainly included 1,2-dimethoxy-4-(2-propenyl)-benzene, 1,2-dimethoxy-4-(1-propenyl)-benzene, α- and β-asarone (Table 1). Among them, eucalyptol, camphor, terpinen-4-ol, linalool and α-asarone were further verified by GC–MS analysis of their standards. The relative contents of the identified compounds in TCM from Guangxi were determined by peak area ratio. It has been shown that α-asarone in the TCM is a key active compound [32,35]. It is very important

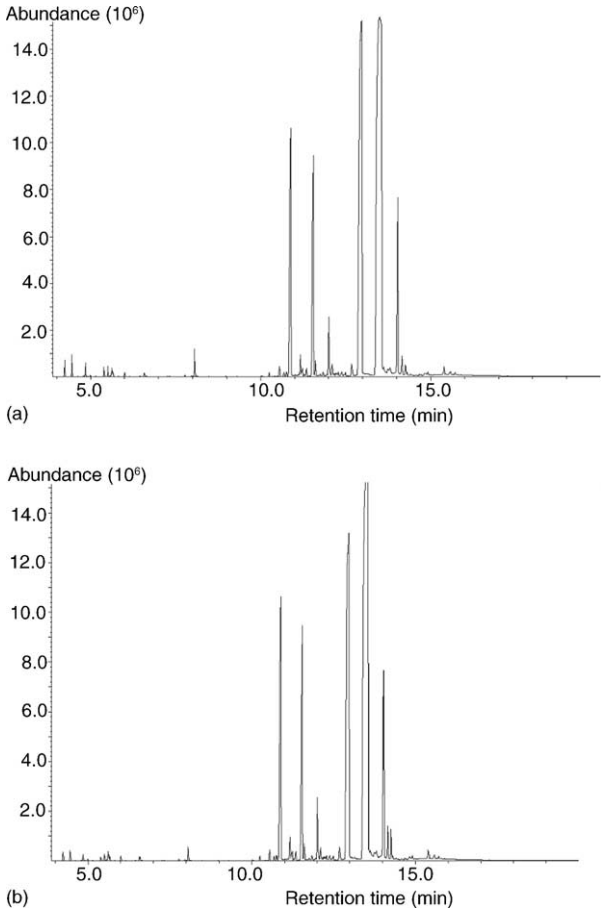

Fig. 4. The total ion chromatograms of the essential oil from *Acorus Tatarinowii Schott*. by PHWE–SPME with a PDMS-DVB fiber (a) and SD (b), respectively.

for TCM quality assessment to quantitatively analyze α-asarone in the TCM samples from different growing areas.

To quantify the active compound of α-asarone, headspace SPME of the working standard solutions was performed. Three replicate measurements were carried out and a calibration curve for α-asarone in the TCM was obtained. The quantitative equation is $Y = 9.8 \times 10^9 X + 2.1 \times 10^8$ (Y: peak area; X: α-asarone concentration, μg/mg; R^2: 0.986). The concentration of α-asarone in the TCM samples was determined by external standard method. The concentration of α-asarone in the TCM samples from Guangxi, Hainan and Anhui is 0.8, 0.1 and 1.3 μg/mg, respectively. This shows that the TCM quality from three areas is different. On basis of the key active compound of α-asarone, the TCM from Anhui was found to be the best.

3.4. Repeatability and recovery

The method repeatability was studied by four replicate analyses of the essential oil in *Acorus Tatarinowii Schott.* from Guangxi by PHWE and SPME at the optimum condi-

tions. Calculation of R.S.D. values was carried out by using the obtained peak areas. The R.S.D. data are shown in Table 2. The R.S.D. values were ranged from 5.7 to 12.9%. The values were close to those by SD [35]. It is shown that the proposed method has a good repeatability.

The analytical recovery was performed by the replicate measurements of the α-asarone-added *Acorus Tatarinowii Schott*. sample. The amount of the added α-asarone was calculated by external standard method. The recovery value of α-asarone was 92%, which was obtained by comparison of its real value with the calculation value. This indicates that the proposed method provided a good analytical recovery.

3.5. Comparison of SD and PHWE–SPME for analysis of the essential oil in Acorus Tatarinowii Schott.

SD method was also used for isolation of essential oil in *Acorus Tatarinowii Schott*. from the same growing areas. Fig. 4b is the total ion chromatogram of the essential oil in *Acorus Tatarinowii Schott*. from Guangxi, China. Thirty-one compounds were also identified in their essential oil (Table 2). It was found from Table 2; Fig. 4a and b that the same compounds in the TCM essential oil were obtained by the two methods. The relative contents in the TCM from Guangxi were calculated by their peak area ratio. The absolute concentration of α-asarone in the TCM samples from the three growing areas (Guangxi, Hainan and Anhui, China) was measured by SD, according to Wu et al.'s method [35]. The obtained value is 0.7, 0.1 and 1.4 μg/mg, respectively. The relative content values of α- and β-asarone by the two methods were found to be different (Table 2). As we know, SPME principle is very different with that of SD. This led to the difference in the relative content values of α- and β-asarone. However, the absolute concentration of α-asarone by PHWE–SPME was very close to those by SD. This suggests that PHWE–SPME is a good alternative method for determination of the essential oil in *Acorus Tatarinowii Schott*.

In the proposed method, two solvent-free techniques were combined and used for isolation of essential oil in TCMs. The major advantages of PHWE–SPME are the low cost and environmental friendliness of water. Compared with SD, the proposed method required simple sample preparation, little sample mass, little total analysis time. When SPME was used for determination of essential oils in plant materials, only semi-quantitative analysis can be done. In this work, SPME combined with PHWE was developed for quantitative analysis of essential oils in TCMs. The method repeatability, recovery and linearity have shown that it is feasible to quantitatively analyze of the active compounds in TCM essential oils by PHWE–SPME.

4. Conclusions

PHWE combined with SPME was proved to have several advantages of simplicity, rapidness and need of no solvent. It has been demonstrated that PHWE–SPME–GC–MS is a good alternative method for determination of essential oils in TCMs and is a potential tool for TCM quality assessment.

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