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Short communication

Solid-phase microextraction–gas chromatography–mass spectrometric analysis of volatile compounds in a compounded Chinese medicinal prescription, Xiao-Cheng-Qi-Tang

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Abstract

In this paper, a headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography–mass spectrometry (GC–MS) was applied for the analysis of volatile compounds in a famous compounded Chinese medicinal prescription, Xiao-Cheng-Qi-Tang. Some parameters affecting the extraction efficiency such as stirring, extraction temperature, fiber exposure time and desorption time were optimized. The best results were obtained using a 100 μm PDMS fiber during headspace extraction at 90 °C with stirring at 1000 rpm for 20 min. Twenty-seven compounds were identified in Xiao-Cheng-Qi-Tang including some main compounds such as D-limonene and linalool. Inter- and intra-day relative standard deviations (R.S.Ds.) were less than 15.6%, showing that the method had a good reproducibility. The result might provide some foundation for building the convincing theory on the pharmacological activity of this prescription.

Keywords: Xiao-Cheng-Qi-Tang; Solid-phase microextraction; Gas chromatography–mass spectrometry; Volatile compounds

1. Introduction

Solid-phase microextraction (SPME) was introduced in 1990 by Arthur and Pawlizyn [1]. This technique is proved increasingly useful in volatile and semivolatile compounds because it is a rapid and simple procedure of extraction with a great capacity of concentration with no need of organic solvent. In recent years, this novel method has been widely adopted in many fields, including analyses of plant materials, environment, food, water, drugs and TCMs [2–11]. Indeed, its use has been extended to the analyses of a great variety of matrices (gas, liquid and solid). The fiber was usually transferred to the gas chromatography or gas chromatograph–mass spectrometer to desorb the adsorbed volatiles at high temperatures.

As natural materials, traditional Chinese medicines (TCMs) appeal to many people because of their high activity, low toxicity, and rare complications [12–14]. Xiao-Cheng-Qi-Tang, an ancient prescription of traditional Chinese medicine, has been safely used as a heat-clearing, intestine movement and cathartic herb for many centuries. It is composed of Dahuang (*R. officinale Baill*), Zhishi (*Citrus aurantium* L.) and Houpu (*Magnolia officinalis Rehd. Et Wils.*). In China, people usually boil the above prescription in water for certain time, and then get the concentrated liquid, namely "Xiao-Cheng-Qi-Tang". When drinking it, we could always smell the strong fragrance from the decoction. However, few studies on the analysis of volatile compounds in Xiao-Cheng-Qi-Tang have been reported yet.

In this paper, we developed a novel SPME technique for analyzing volatile constituents of Xiao-Cheng-Qi-Tang. Twenty-seven compounds were identified including some main compounds such as D-limonene, which may provide some foundation for building the convincing theory on the pharmacological activity of this prescription.

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2. Experimental

2.1. Materials

Raw materials of Dahuang (*R. officinale Baill*), Zhishi (*C. aurantium* L.) and Houpu (*M. officinalis Rehd. Et Wils.*) were purchased from Leiyunshang company (Shanghai), a long-established authorized traditional Chinese medicine trader. The induction cooker EF196 (PD19JA) was purchased from Midea company. An SPME holder with 100 μm polydimethylsiloxane (PDMS) were purchased from Supelco, Bellefonte, PA and was conditioned prior to use according to supplier's prescriptions.

2.2. Sample preparation, SPME extraction and analysis

Twelve grams of Dahuang, 6 g of Zhishi and 9 g of Houpu were mixed into a 1000 ml earthen container and 500 ml of distilled water was added. The mixture was boiled for 1 h using a home-used Midea EF196 (PD19JA) induction cooker with a power of 800 W. The decoction was poured down from the venthole of container and stored at −10 °C until use.

In our work, the headspace sampling was chosen for extraction of volatile components in Xiao-Cheng-Qi-Tang. Different parameters were studied including the effect of stirring, extraction temperature, fiber exposure time and desorption time.

The vial containing 5.0 ml of decoction sample was placed in a thermostated bath adjusted to the different temperatures tested and was sealed with a Black Viton septum (Supelco). Thermal desorption of the analytes from the fiber inside the GC injection port was carried out at a desorption temperature of 250 °C.

2.3. GC–MS analysis

Volatile compound desorption and analyses were carried out on a HP 6890 GC system, coupled with a HP MD5973 quadrupole mass spectrometer. The compounds were separated on a HP-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 μm film). Split injection was employed for SPME samples with a ratio of 10:1. The column oven temperature was programmed to rise from an initial temperature of 50 °C (1 min) to 200 °C at 5 °C/min, then to 270 °C at 15 °C/min. The injection temperature and ion source temperature were 250 °C 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 40–500 amu. Compounds were tentatively identified using the NIST Mass Spectral Search Program (National Institute of Standards and Technology, Washington, DC, USA).

3. Results and discussion

3.1. Optimization of SPME conditions

In this study, HS-SPME is based on the equilibrium of analytes among the three system phases: the coated fiber, the headspace and the sample solution. The limiting step in this extraction is the diffusion of the analytes through the system [15]. Stirring is an important parameter that influences

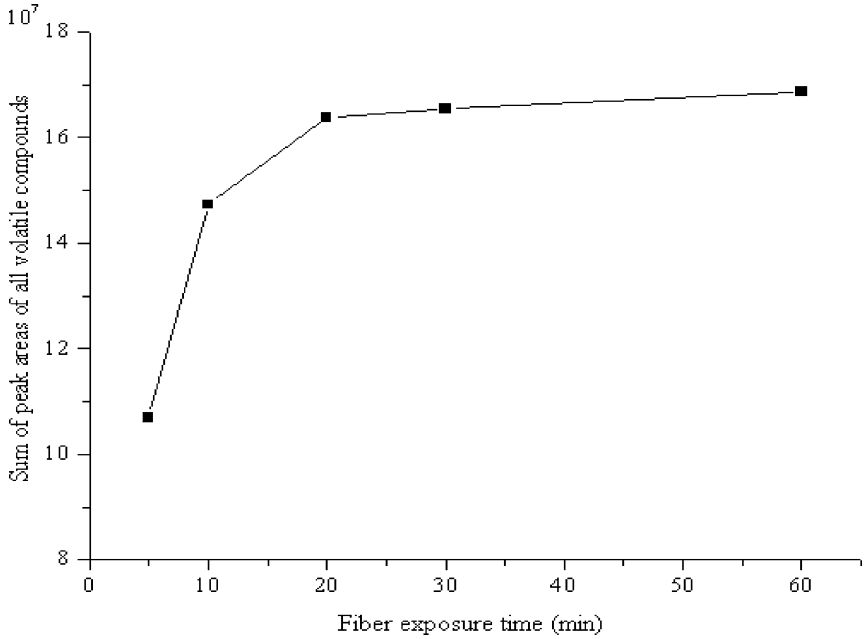

Fig. 1. Effect of the fiber exposure time on the sum of the peak areas of all volatile compounds of the Xiao-Cheng-Qi-Tang. Fiber: 100 μm PDMS; absorption temperature: 90 °C; stirring: 1000 rpm; and desorption conditions: 250 °C and 3 min.

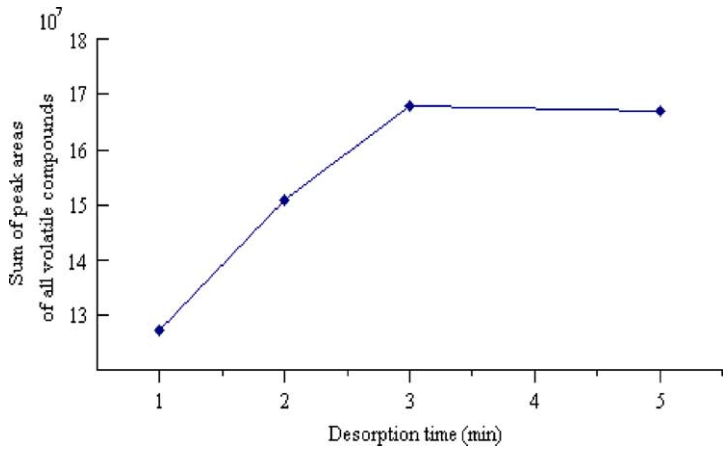

Fig. 2. Effect of the desorption time on the sum of the peak areas of all volatile compounds of the Xiao-Cheng-Qi-Tang. Fiber: 100 μm PDMS; absorption temperature: 90 °C; fiber exposure time: 20 min; stirring: 1000 rpm; and desorption temperature: 250 °C.

extraction, because it causes turbulence in the liquid and gaseous phases [16,17], which would shorten the balance time, especially the headspace/fiber balance of volatiles. Thus, constant stirring (1000 rpm) was applied to increase the efficiency of extracting free volatiles.

The optimum temperature was investigated by varying the temperature during the extraction of the volatile constituents of the decoction. The PDMS fiber was exposed in the headspace of the decoction at three temperatures of 30, 60 and 90 °C for the same time of 60 min with stirring

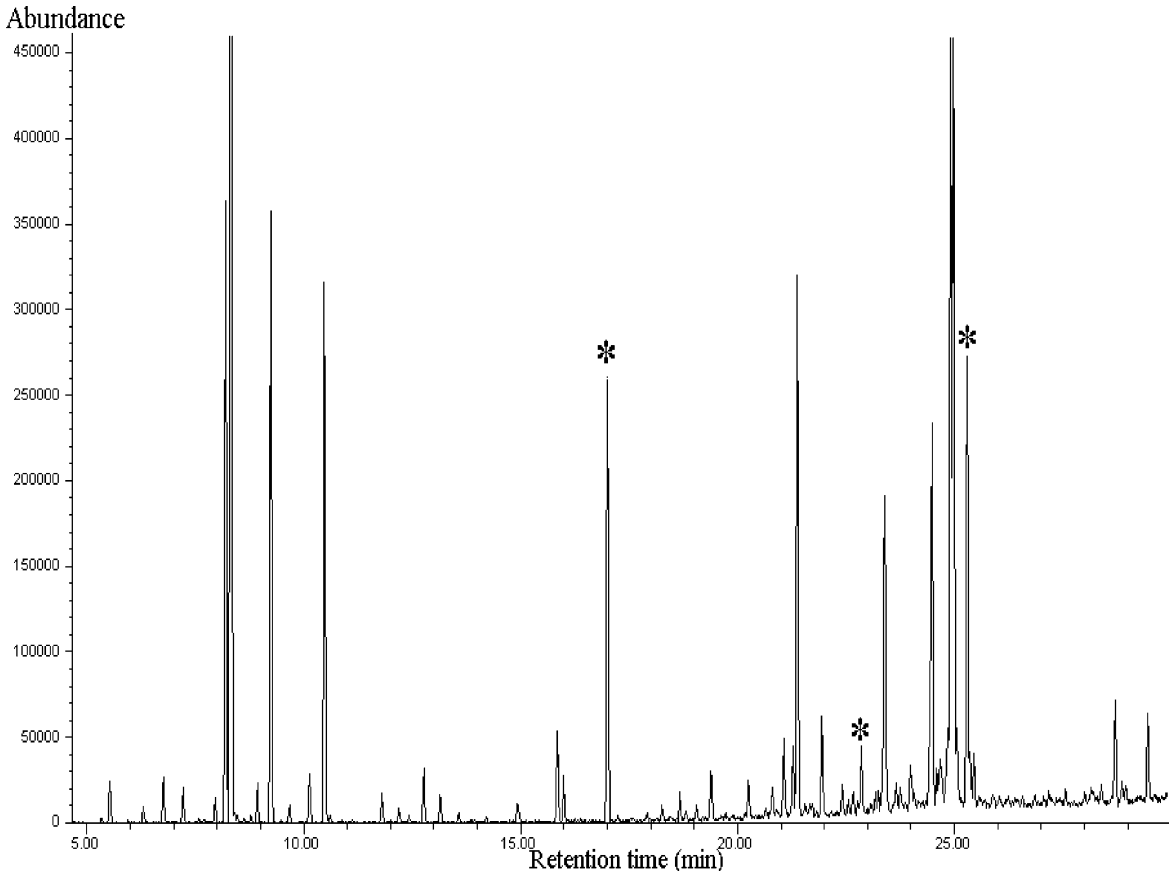

Fig. 3. A typical total ion chromatogram of volatile constituents of Xiao-Cheng-Qi-Tang. Extraction conditions: PDMS fiber, 1000 rpm, 20min and 90 °C, sample volume: 5.0 ml; desorption conditions: 250 °C and 3 min. The marked asterisk (∗) represents some silicoorganic compounds.

Table 1
GC–MS identification of volatiles of Xiao-Cheng-Qi-Tang and peak area percents

Number	Retention time (min)	Compounds[a]	Molecular weight	Relative area (%)
1	5.54	α-Pinene	136	0.44
2	6.30	Benzaldehyde	106	0.15
3	6.77	β-Pinene	136	0.43
4	7.22	β-Myrcene	136	0.26
5	7.96	α-Phellandrene	136	0.20
6	8.20	1-Methyl-4-(1-methylethyl)-benzene	134	5.79
7	8.30	D-Limonene	136	31.76
8	8.93	3,7-Dimethyl-1,3,7-octatriene	136	0.32
9	9.257	1-Methyl-4-(1-methylethyl)-1,4-cyclohexadiene	136	5.86
10	10.13	4-Carene	136	0.56
11	10.49	Linalool	154	4.78
12	11.81	Camphor	152	0.26
13	12.77	Menthol	154	0.47
14	13.15	α-Terpineol	154	0.26
15	14.93	4-(2-Propenyl)-phenol	134	0.24
16	15.84	Bornyl acetate	196	0.87
17	15.98	2-Methyl-5-(1-methylethyl)-phenol	150	0.37
18	18.26	Copaene	204	0.14
19	18.67	β-Elemene	204	0.22
20	19.40	Caryophyllene	204	0.51
21	20.26	α-Caryophyllene	204	0.38
22	21.38	Humulene	204	0.75
23	21.94	Eudesma-4(14),11-dinene	204	0.90
23	23.40	Caryophyllene oxide	220	3.96
24	24.49	Selinene	204	6.03
25	24.91	[2R-(2α,4aα,8aα)]-Decahydro-α,α,4a-trimethyl-8-methylene-2-naphthalenemethanol	222	6.90
26	24.98	[2R-(2α, 4aα,8aα)]-1,2,3,4,4a,5,6,8a-Octahydro-α,α,4a,8-tetramethyl-2-naphthalenemethanol	222	7.57
27	29.45	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	278	0.98

[a] The matching ratios of every compound identified by GC–MS are all above 90%.

at a rate of 1000 rpm. The total ion chromatograms of the volatile constituents obtained at different extraction temperatures showed that more compounds were adsorbed at a higher temperature (90 °C). Therefore, 90 °C was chosen as the optimum extraction temperature.

In order to obtain the optimum extraction time, adsorption was performed at 90 °C for varying fiber exposure time from 5 to 60 min. The effect of adsorption time on the sum of peak areas is plotted in Fig. 1, which shows that optimum adsorption was achieved in 20 min. This also demonstrates that the use of stirring and higher temperature can shorten the sampling time.

The sample was extracted by PDMS fiber at the optimum extraction conditions. Desorption of the analytes was carried out in the GC injector at 250 °C for varying times from 1.0 to 5.0 min. The results presented in Fig. 2 showed that the optimal desorption time was 3 min.

3.2. SPME–GC–MS determination of volatile compounds of Xiao-Cheng-Qi-Tang

The optimum extraction conditions (1000 rpm, 90 °C and 20 min of extraction, 3 min of desorption) were applied to SPME of volatile constituents of Xiao-Cheng-Qi-Tang. The typical total ion chromatogram of the volatile compounds of Xiao-Cheng-Qi-Tang is shown in Fig. 3. Twenty-seven compounds were identified using the NIST Mass Spectral Search Program (Table 1). Their relative contents were calculated by the peak area ratio. The total area of 27 identified volatile compounds was more than 81.36% of the sum of chromatographic area. The main volatile compounds of decoction included monoterpene hydrocarbons such as D-limonene, sesquiterpene hydrocarbons such as selinene, and alcohols such as linalool, [2R-(2α,4aα,8aα)]-decahydro-α,α,4a-trimethyl-8-methylene-2-naphthalenemethanol, [2R-(2α,4aα,8aα)]-1,2,3,4,4a,5,6,8a-octahydro-α,α,4a,8-tetramethyl-2-naphthaleneme-thanol. Besides, there are many kinds of low concentration terpenes, alcohols and esters in the identified volatile constituents.

D-Limonene, an important active component, was the richest (31.76%) in the volatiles. This constituent might mainly originate from Zhishi (*C. aurantium* L.), because Zhishi was reported to contain more than 59% D-limonene of its volatiles [18], but only 0.77% in Houpu oils [5] and none in Dahuang.

Furthermore, in China, people have been accustomed to decocting TCMs in an earthen container since ancient times. Therefore, an earthen container, rather than a metal one or

other materials, was used in this experiment. Consequently, some silicoorganic compounds were also detected, which was confirmed to derive from the earthen pot. These compounds were marked as asterisk (∗) in Fig. 3.

Last but not least, the individual herb of Dahuang, Zhishi and Houpu was also investigated after decoction about their volatile compounds using the above SPME–GC–MS method. However, there seems to be no significant volatile variations compared with Xiao-Cheng-Qi-Tang. In our laboratory, the comparative study of water-soluble constituents for individual herb and their mixture is further carried out.

3.3. Repeatability

The repeatability of this method was determined via the percentage coefficient variation of within and between day variations by replicating three analyses of the three main compounds (D-limonene, selinene and linalool) under the optimized SPME conditions. The intra- and inter-day relative standard deviations (R.S.Ds.) of peak areas of these three compounds were: D-limonene 8.4% and 12.5%, selinene 9.3% and 15.6%, linalool 7.9% and 14.9%, respectively. The results showed that the SPME method had a good reproducibility.

4. Conclusion

An SPME–GC–MS technique was successfully established for determination of volatile compounds in a famous compounded Chinese medicinal prescription, Xiao-Cheng-Qi-Tang. Twenty-seven components were identified including some kinds of terpenes, alcohols and esters. Moreover, we believe that this novel technique is potentially useful for analysis of volatiles in other prescriptions.

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