# Separation and Identification of Volatile Constituents in *Artemisia argyi* Flowers by GC–MS with SPME and Steam Distillation

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## Abstract

***Artemisia argyi*** **leaf is a widely used traditional Chinese medicine (TCM). In this work, for the first time, the separation and identification of volatile constituents in** ***Artemisia argyi*** **flowers is performed. Gas chromatography–mass spectrometry (GC–MS) with solid-phase microextraction (SPME) is developed for the fast analysis of volatile constituents in the flowers. Several headspace SPME parameters, including fiber coating, extraction temperature, and extraction time, are optimized. Forty-nine compounds in the flowers are re-identified by SPME-GC–MS. At the same time, in order to compare with the SPME, steam distillation is used for analysis of the volatile constituents in the flowers, and forty-seven are detected. The total fifty-three compounds in the flowers, which mainly include cylcofenchene, α-pinene, α-myrcene, D-limonene, caryophyllene, and germacrene D, are identified by the two methods. Compared to the volatile components in** ***Artemisia argyi*** **leaves, the main components (including the two active compounds of borneol and borneol acetate) are also found in** ***Artemisia argyi*** **flowers. These results show that** ***Artemisia argyi*** **flowers as well as leaves might be used as TCM.**

## Introduction

*Artemisia* species, widespread throughout the world, are important medicinal plants which are receiving phytochemical attention due to their biological and chemical diversities (1). *Artemisia argyi* is one of the most popular plants in Chinese traditional preparations and is frequently used as a common traditional Chinese medicine (TCM) for the treatment of diseases such as malaria, anaphylaxis, hepatitis, cancer, inflammation, and infections by fungi, bacteria, and viruses (1,2). It has been found that methanol extracts from *Artemisia argyi* leaves have strong antimutagenic activities against Trp-P-2 (3). Extensive studies of the chemical components of *Artemisia argyi* leaves have been performed, which has led to the identification of many compounds, such as monoterpenes, sesquiterpenes, triterpenes, eudesmanollides, and flavones, etc. (4,5). Sesquiterpene lactones from *Artemisia argyi* leaves were found to exhibit a wide range of biological activities (1,6).

In China, the dry leaf of *Artemisia argyi* has been used as a common TCM for long time. Some bioactive components such as borneol and borneol acetate had been found to be present in its essential oil. Gas chromatography–mass spectrometry (GC–MS) with steam distillation (SD) has been applied to isolation and identification of the essential oil (7), and 35 compounds in *Artemisia argyi* leaves were identified (7). In our previous work, a simple, rapid, and solvent-free method, GC–MS with headspace solid-phase microextraction (HS-SPME), was developed for the analysis of the essential oils in *Artemisia argyi* leaves and other TCMs (8–15).

In China, *Artemisia argyi* leaves instead of its flowers are used as a TCM. To date, identification of the volatile constituents in *Artemisia argyi* flowers has not been reported in the literature. In the present work, two methods, GC–MS with HS-SPME, and GC–MS with SD, were applied to the separation and identification of the volatile constituents in *Artemisia argyi* flowers.

## Materials and methods

### SPME holder, fibers, solvent, and plant materials

A manual SPME holder and four commercial SPME fibers (100-µm poly[dimethylsiloxane] [PDMS], 65-µm carbowax–divinylbenzene [CW–DVB], 85-µm poly[acrylate] [PA], 75-µm carboxen–poly[dimethylsiloxane] [CAR-PDMS]) were purchased from Supelco Company (Bellefonte, PA). The SPME fibers were conditioned as recommended by the manufacturer at some degree 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 250ºC in the GC injector. For the following analyses, 2 min of desorption after each extraction was used as conditioning time. HPLC-grade *n*-hexane was purchased from Shanghai Chemical Reagent Company (Shanghai, China).

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*Artemisia argyi* flowers growing in Fudan campus (Shanghai, China) were collected on November 3, 2004. The fresh *Artemisia argyi* flowers (4.0 g) were ground and introduced into 8-mL headspace vials. Eighty grams of *Artemisia argyi* flowers were used for steam distillation.

### GC–MS

A Finnigan Voyager GC–MS was used in EI mode. Analytes were separated using a HP-5MS capillary column of 30 m × 0.25 mm with a phase thickness of 0.25 µm from Agilent (Palo Alto, CA). The injector temperature was set at 250°C. Desorption of the SPME fibers was performed in splitless mode for 2 min. The oven temperature program was as follows: initial temperature was 50°C for 2 min, which was increased to 300°C at 10°C/min, 300°C was maintained for 5 min. Helium (99.999%) carrier gas had a flow-rate of 1.0 mL/min at constant flow mode . The analysis was carried out under full-scan acquisition mode within the 41–350 amu range. The quadrupole temperature, transfer line temperature, and MS source temperature were 150°C, 280°C, and 230°C, respectively.

### Optimization of the SPME conditions

The fresh *Artemisia argyi* flower sample (4.0 g) was ground and put into an an 8-mL headspace vial, and used to optimize the SPME conditions. Firstly, to obtain the optimum fiber, four commercial fibers were tested, and used for the extraction of volatile constituents in its flowers at 25°C for 5 min. The extracted analytes on the fibers were desorbed in the GC injector in splitless mode at 250°C for 2 min, analyzed by GC–MS. The optimal fiber was determined by the peak areas of the main compound in the flower sample. Next, using the optimal fiber of the CAR-PDMS fiber, the extraction temperature and time was also studied by adsorption of the volatiles at different adsorption temperature (25°C, 40°C, 60°C, and 80°C) with different extraction times (5, 10, 15, 20, and 30 min) for each temperature. The analytes absorbed on the fibers were desorbed and analyzed by GC–MS.

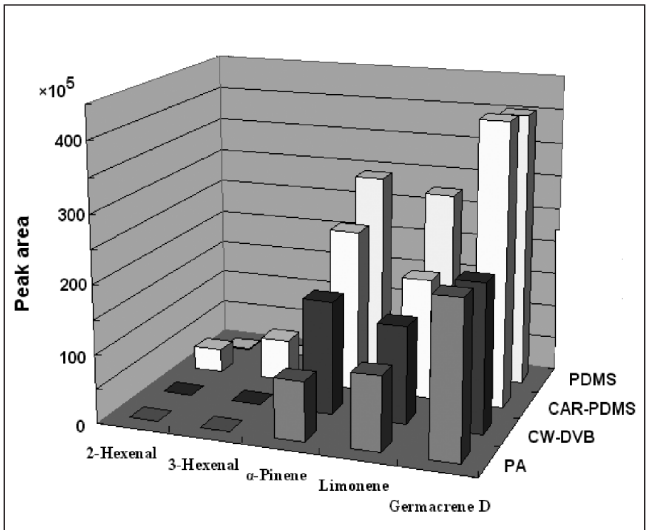


**Figure 1.** The effect of fiber coating on the extraction efficiencies of 2-hexenal, 3-hexenal, α-pinene, limonene, and germacrene D.

### Determination of volatile compounds in *Artemisia argyi* flowers by GC–MS following HS-SPME

The fresh *Artemisia argyi* flower sample with a mass of 4.0 g was ground and introduced into an 8-mL headspace vial. The volatile compounds in the sample were headspace extracted using the CAR-PDMS fiber at the optimal extraction conditions of 60°C for 15 min. The volatiles adsorbed on the fiber were desorbed at the GC injection port with a temperature of 250°C for 2 min and analyzed by GC–MS. Compounds were identified using the Wiley 6.0 mass spectra library. Further verification of the compounds in the flowers was performed by comparing the retention time and mass spectra with those of the same compounds in its leaves (7,8).

### Determination of volatile constituents in *Artemisia argyi* flowers by GC–MS following SD

Eighty grams of fresh flowers were put into a 1000-mL distillation flask. 500 mL of distilled water were added and volatile oil distillation apparatus was set according to the Chinese Pharmacopoeia (18). The mixture was distilled for 6 h. Oil was collected from the condenser and dried over anhydrous sodium sulfate. The obtained essential oil was introduced into a 5-mL volumetric flask, and the final volume of the extract was adjusted to 5.0 mL with *n*-hexane. One microliter essential oil was injected into the GC–MS for analysis. These compounds were also identified using the Wiley 6.0 mass spectra library.

## Results and Discussion

### Optimization of HS-SPME conditions

Several parameters that can affect the extraction efficiency in this work, such as, fiber coating, extraction temperature, and time were investigated. It is very important to investigate the SPME conditions. First, fiber coating was studied. The five main compounds of 2-hexenal, 3-hexenal, α-pinene, limonene, and germacrene D (Table I) in the flower sample were identified by the Wiley 6.0 mass spectra library and the literatures (7,8), and used for the determination of the optimal fiber coating. Figure 1

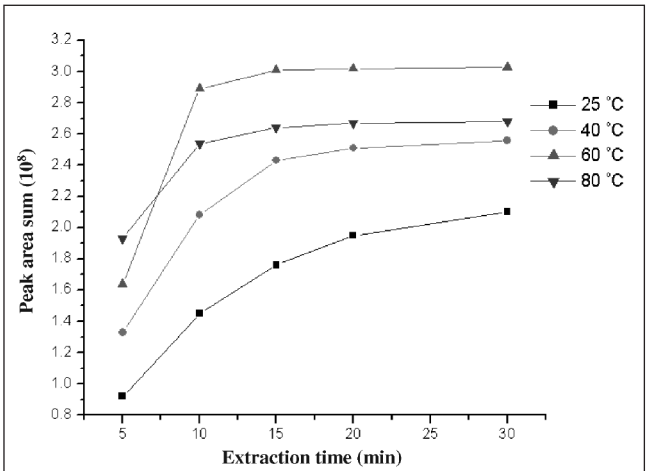


**Figure 2.** The effect of the extraction temperature and time on extraction efficiency.

**Table I. Identification of Volatile Compounds in *Artemisia argyi* Flowers by HS-SPME-GC–MS and SD-GC–MS***

| No. | Retention time | Compound | Molecular weight | Mass Spectra (relative abundance) | Relative content (%) SPME | SD |
|---|---|---|---|---|---|---|
| 1 | 3.69 | 2-Hexenal | 98 | 41(100),55(42),69(38),98(12) | 2.43 | ND |
| 2 | 3.71 | *n*-Caproaldehyde | 100 | 44(100),56(85),57(58),41(50) | 1.99 | ND |
| 3 | 4.47 | Butyl-cyclobutane | 112 | 56(100),55(55),43(51),42(39) | 0.22 | ND |
| 4 | 4.58 | 3-Hexenal | 98 | 55(100),41(96),69(82),98(25) | 4.28 | ND |
| 5 | 4.64 | 3-Hexen-1-ol | 100 | 67(100),41(89),55(70),100(8) | 1.33 | ND |
| 6 | 4.82 | 2-Hexen-1-ol | 100 | 57(100),41(40),82(30),100(7) | 0.17 | ND |
| 7 | 5.90 | 3-Thujene | 136 | 93(100),91(56),77(45),136(15) | 0.15 | 0.18 |
| 8 | 6.02 | Cylcofenchene | 136 | 93(100),91(48),92(46),136(13) | 6.46 | 25.23 |
| 9 | 6.30 | Camphene | 136 | 93(100),121(62),79(42),136(21) | 0.19 | 0.37 |
| 10 | 6.72 | α-Pinene | 136 | 93(100),91(38),77(30),136(16) | 4.06 | 9.09 |
| 11 | 6.79 | α-Phellandrene | 136 | 93(100),69(31),136(29),41(28) | 1.08 | 3.28 |
| 12 | 7.00 | α-Myrcene | 136 | 93(100),69(68),41(58),136(8) | 16.44 | 9.35 |
| 13 | 7.26 | 3-Methylene-6-Methylethyl-cyclohexene | 136 | 93(100),91(62),77(42),136(38) | 0.30 | 1.61 |
| 14 | 7.68 | Limonene | 136 | 68(100),93(85),67(80),136(29) | 12.22 | 19.79 |
| 15 | 7.98 | α-*cis*-Ocimene | 136 | 93(100),91(50),79(48),136(8) | 1.05 | 0.61 |
| 16 | 8.18 | 2,3-Dihydro-1H-indene | 136 | 93(100),79(61),91(59),77(53) | 0.01 | 0.31 |
| 17 | 8.34 | Sabinenehydrate | 154 | 71(100),43(62),93(56),154(11) | 0.02 | 0.09 |
| 18 | 8.68 | 4-Carene | 136 | 93(100),121(82),136(68),91(51) | 1.05 | 1.38 |
| 19 | 8.84 | Borneol | 154 | 95(100), 110(18),93(52),154(15) | 0.08 | 0.19 |
| 20 | 9.10 | 2-Ethenyl-1,1-dimenthyl-3-methylene-cyclohexane | 150 | 69(100),41(36),79(35),150(21) | 0.18 | 0.04 |
| 21 | 10.13 | *p*-Menth-1-en-4-ol | 154 | 71(100),43(62),93(56),154(11) | 0.03 | 0.55 |
| 22 | 10.32 | *p*-Menth-1-en-8-ol | 154 | 59(100),93(70),121(58),136(50) | 0.13 | 0.07 |
| 23 | 11.74 | Borneol acetate | 196 | 95(100),111(450),93(48),154(21) | 1.24 | 1.92 |
| 24 | 11.98 | *n*-Undecanal | 170 | 57(100),43(80),82(78),55(72) | 0.02 | 0.09 |
| 25 | 12.50 | 2-iso-Propenyl-1-vinyl-*p*-menth-3-ene | 204 | 121(100),93(88),136(82),204(5) | 0.48 | 0.26 |
| 26 | 12.66 | α-Cubebene | 204 | 105(100),119(89),161(94),204(21) | 0.11 | 0.05 |
| 27 | 13.06 | Copaene | 204 | 119(100),161(94),105(92),204(22) | 0.14 | 0.06 |
| 28 | 13.21 | 1,2,3,3a,3bα,4,5,6,6aα,6bα-Decahydro-1α-isopropyl-3aα-methyl-6-methylene-cyclobuta[1,2,3,4] dicyclopentene | 204 | 81(100),80(80),123(76),204(6) | Tr | 0.07 |
| 29 | 13.24 | 2,3,3aα,3bα,4,5,6,7-octahydro-4α-isopropyl-7α-methyl-3-methylene-$^{1}$H-Cyclopenta[1,3] cyclopropa[1,2]benzene | 204 | 161(100),41(50),119(49),204(21) | 1.57 | 0.78 |
| 30 | 13.68 | Caryophyllene | 204 | 93(100),69(82),133(80),204(13) | 2.76 | 2.34 |
| 31 | 13.80 | ς-Muurolene | 204 | 161(100),105(50),93(49),204(31) | 0.54 | 0.17 |
| 32 | 14.12 | Humulen-(v1) | 204 | 93(100),161(40),91(38),204(17) | 0.42 | 0.23 |
| 33 | 14.24 | Bicyclosesquiphellandrene | 204 | 161(100),105(32),204(28),119(26) | 0.52 | 0.04 |
| 34 | 14.39 | Germacrene B | 204 | 161(100),105(75),91(68),204(18) | Tr | 0.22 |
| 35 | 14.48 | Germacrene D | 204 | 161(100),105(78),91(62),204(25) | 28.73 | 18.48 |
| 36 | 14.67 | Isocaryophillene | 204 | 93(100),105(62),69(60),204(26) | 0.97 | 0.37 |
| 37 | 14.87 | ς-Cadinene | 204 | 161(100),105(35),91(32),204(27) | 0.31 | 0.17 |
| 38 | 15.00 | Cadina-3,9-Diene | 204 | 161(100),119(62),134(58),204(52) | 0.39 | 0.45 |
| 39 | 15.45 | 1α,4αH,10αH-Guaia-5,11-diene | 204 | 121(100), 161(62),105(58),204(50) | 1.08 | 0.21 |
| 40 | 15.49 | 2,6-Octadien-1-ol,3,7-dimethyl-propionate | 210 | 69(100),93(87),57(62),41(56) 1.07 0.20 | | |
| 41 | 15.74 | *n*-Cetane | 226 | 57(100),71(82),43(78),226(5) | 0.87 | 0.19 |
| 42 | 16.42 | .tau.-Muurolol | 222 | 95(100),161(82),121(65),222(5) | Tr | 0.35 |
| 43 | 16.57 | -Cadinol | 222 | 95(100),121(63),204(32),222(8) | Tr | 0.49 |
| 44 | 16.90 | *n*-Heptadecane | 240 | 57(100),43(81),71(52),240(4) | 0.33 | 0.06 |
| 45 | 16.96 | *trans*-Longipinocarveol | 220 | 109(100),91(82),79(78),220(12) | Tr | 0.33 |
| 46 | 17.09 | Pentadecanal | 226 | 82(100),57(87),41(79),96(75) | 0.14 | Tr |
| 47 | 18.51 | Hexahydrofarnesyl acetone | 268 | 58(100),43(70),71(58),268(4) | Tr | 0.13 |
| 48 | 19.68 | Hexadecanoic acid | 256 | 73(100),43(96),60 (90),256(43) | 0.15 | 3.52 |
| 49 | 20.80 | Stearic acid allyl ester | 324 | 43(100),100(98),57 (96),41(90) | Tr | 0.25 |
| 50 | 21.18 | Phytol | 296 | 71(100),43(40),123(36),57(30) | ND | 0.09 |
| 51 | 21.41 | Octadec-9-enoic acid | 282 | 57(100),43(81),71(52),240(4) | ND | 0.34 |
| 52 | 21.45 | 9-Octadecenoic acid | 282 | 55(100),69(73),83(56),282(8) | ND | 1.07 |
| 53 | 21.62 | Stearic acid | 284 | 43(100),73(80),60(78),284(48) | ND | 1.01 |

*ND = not detected; Tr = the relative content less than 0.01%.

shows the peak areas of the five main compounds obtained by using the four different fibers. For the two compounds of 2-hexenal and 3-hexenal, the CAR-PDMS fiber had better extraction efficiencies than the other fibers. The extraction amounts of α-pinene, limonene, and germacrene D by the CAR-PDMS fiber were only less than those by the PDMS fiber. Comprehensively, the CAR-PDMS fiber was the optimal fiber and used for further work. However, the CAR-PDMS fiber can very strongly retain the analytes and causes a memory effect; it is necessary to make the carry-over desorbed by keeping the fiber on the inlet of the GC for 2 min after every run. Subsequently, the extraction temperature and time were also studied, using the CAR-PDMS fiber. Figure 2 shows the effect of the extraction temperature and time on the peak area sum of the five compounds. It can be seen from Figure 2 that the best extraction amount was obtained at 60ºC, and the extraction reached equilibrium after exposure for 15 min. Therefore, the optimal HS-SPME conditions are: CAR-PDMS fiber, extraction temperature of 60ºC, and time of 15 min.

### Determination of volatile constituents in *Artemisia argyi* flowers by GC–MS following HS-SPME

The volatile compounds in *Artemisia argyi* flower sample were headspace extracted by SPME at the optimal conditions, followed by desorption and analysis with GC–MS. Figure 3A shows the GC–MS total ion chromatogram of the volatile compounds in *Artemisia argyi* flowers by HS-SPME. Forty-nine compounds were extracted by SPME and identified through GC–MS according to our previous method (8). Their relative content values were calculated by peak area ratio (Table I). The identified compounds mainly included 2-hexenal (2.43%), n-caproaldehyde (1.99%), 3-hexenal (4.28%), 3-hexen-1-ol (1.33%), cylcofenchene (6.46%), α-phellandrene (4.06%), α-pinene (16.44%), limonene (12.22%), borneol acetate (1.24%), caryophyllene (2.76%), and germacrene D (28.73%). In addition, a few alkyl, aldehyde, esters, alcohols, terpenes, and acid compounds with low concentrations were also found.

### Determination of volatile constituents in *Artemisia argyi* flowers by GC–MS following SD

As we know, the extraction principle of SPME is very different to that of SD (12,16,17). In general, compounds with low-molecular weight and high volatility can be detected by the SPME method, while most of the compounds with low volatility can be isolated by SD. To find more compounds in the flowers, the SD method was also used for the analysis of volatile constituents in the flowers. Figure 3B shows the GC–MS total ion chromatogram of the volatile compounds in *Artemisia argyi* flowers by the SD method. Forty-seven compounds were identified and their relative contents were calculated (Table I). As seen in Table I and Figure 3, six low-molecular compounds of 2-hexenal, *n*-caproaldehyde, 1,1,3-trimethylcyclo-pentane, 3-hexenal, 3-hexen-1-ol, and 2-hexen-1-ol were detected by only by the SPME method, while phytol, octadec-9-enoic acid, 9-octadecenoic acid, and stearic acid were only detected by the SD method. Using the two methods, a total of fifty-three compounds were identified in the *Artemisia argyi* flowers. The comparison between the results of the SPME and SD method shows that SPME is better for the more volatile compounds, SD for the semi-volatile compounds.

Compared to the identified compounds in *Artemisia argyi* leaves (7,8), all compounds except acetic acid, 2,3-butanediol and cyclohexanol, and 2-methoxy-4-(2-propenyl)-phenol were also detected in its flowers. Moreover, the two active compounds of borneol and borneol acetate were found to be present in both leaves and flowers. These results show that *Artemisia argyi* flowers as well as its leaves might be used as a TCM for disease treatment. To demonstrate the hypothesis, the related pharmacology experiment is being performed in our lab.

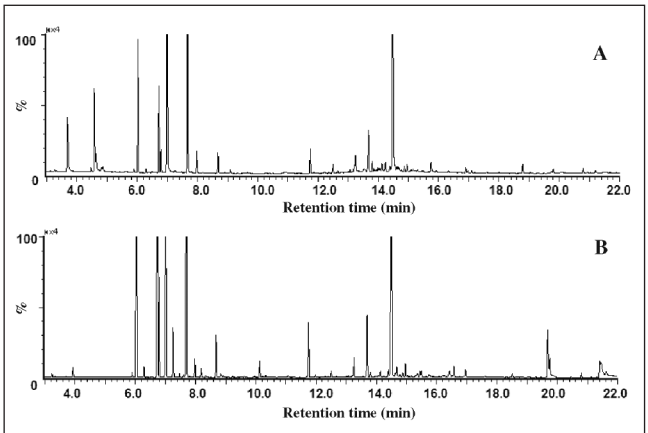


**Figure 3.** Total ion chromatogram of volatile compounds in *Artemisia argyi* flowers by GC–MS with HS-SPME (A), and GC–MS with SD (B), respectively. Identified compounds are listed in Table I.

## References


1. J.H. Kim, H.K. Kim, S.B. Jeon, K.H. Son, E.H. Kim, S.K. Kang, N.D. Sung, and B.M. Kwon. New sesquiterpene-monoterpene lactone, artemisolide, isolated from *Artemisia argyi*. *Tetra. Lett.* **43:** 6205–6208 (2002).
2. R.L. Bian. Antiasthmatic action and components of the Chinese herb *Artemisia-argyi*. *Euro. J. Pharm.* **183:** 226–227 (1990).
3. T. Nakasugi, M. Nakashima, and K. Komai. Antimutagens in gaiyou (Artemisia argyi Levl.et Vant.). *J. Agric. Food Chem.* **48:** 3256–3266 (2000).
4. R.X. Tan and Z.J. Jia. Eudesmanolides and other constituents from *Artemisia-argyi*. *Planta Med.* **58:** 370–372 (1992).
5. M. Yoshikawa, H. Shimada, H. Matsuda, J. Yamahara, and N. Murakami. Bioactive constituents of Chinese natural medicines.1. New sesquiterpene ketones with vasorelaxant effect from Chinese moxa, the processed leaves of *Artemisia argyi* Levl et Vant: Moxartenone and moxartenolide. *Chem. Pharm. Bull.* **44:** 1656–1662 (1996).
6. J.M. Seo, H.M. Kang, K.H. Son, J.H. Kim, C.W. Lee, S.I. Chang, and B.M. Kwon. Antitumor activity of flavones isolated from *Artemisia argyi*. *Planta Med.* **69:** 218–222 (2003).
7. F.Y. Yao, Q. Qiou, T.L. Liu, and X. Miao. Analysis of volatile components in *Artemisia argyi* leaves by gas chromatography–mass spectrometry steam distillation. *J. Instr. Anal.* **20:** 42–45 (2001).
8. X.H. Zheng, C.H. Deng, G.X. Song, and Y.M. Hu. Comparison of essential oil composition of *Artemisia argyi* leaves at different collection times by headspace solid-phase microextraction and gas chromatography–mass spectrometry. *Chromatographia* **59:** 729–732 (2004).
9. C.H. Deng, G.X. Song, and Y.M. Hu. Application of HS-SPME and GC–MS to characterization of volatile compounds emitted from

osmanthus flowers. *Annali Di Chimica* **94:** 921–927 (2004).
10. S. Shen, Y.F. Sha, C.H. Deng, X.M. Zhang, D.X. Fu, and J.K. Chen. Quality assessment of *Flos Chrysanthemi Indici* from different growing areas in China by solid-phase microextraction-gas chromatography–mass spectrometry. *J. Chromatogr. A* **1047:** 281–287 (2004).
11. C.H. Deng, G.X. Song, Y.M. Hu, and X.M. Zhang. Analysis of the volatile constituents of *Schisandra chinesis* (Turz.) bail by gas chromatography–mass spectrometry, using headspace solid-phase microextraction. *Chromatographia* **58:** 289–294 (2003).
12. C.H. Deng, X.Q. Xu, N. Yao, N. Li, and X.M. Zhang. Rapid determination of essential oil compounds in *Artemisia Selengensis* Turcz by gas chromatography–mass spectrometry with microwave distillation and simultaneous solid-phase microextraction. *Anal. Chim. Acta* **556:** 289–294 (2006).
13. C.H. Deng, G.X. Song, and Y.M. Hu. Rapid determination of volatile compounds emitted from Chimonanthus praecox flowers by HS-SPME-GC–MS. *Z. Naturforsch. C* **59:** 636–640 (2004).
14. C.H. Deng, N. Li, X.F. Yang, and X.M. Zhang. Rapid determination of C-6-aldehydes in tomato plant emission by gas chromatography–mass spectrometry and solid-phase microextraction with on-fiber derivatization. *J. Sep. Sci.* **28:** 172–176 (2005).
15. C.H. Deng, N. Li, and X.M. Zhang. Rapid determination of essential oil in *Acorus tatarinowii* Schott. by pressurized hot water extraction followed by solid-phase microextraction and gas chromatography–mass spectrometry. *J. Chromatogr. A* **1059:** 149–155 (2004).
16. C.H. Deng, J. Ji, N. Li, Y.J. Yu, G.L. Duan, and X.M. Zhang. Fast determination of curcumol, curdione and germacrone in three species of Curcuma rhizomes by microwave-assisted extraction followed by headspace solid-phase microextraction and gas chromatography–mass spectrometry. *J. Chromatogr. A* **1117:** 115–120 (2006).
17. P. Bartak, P. Bednar, L. Cap, L. Ondrakova, and Z. Stransky. SPME—A valuable tool for investigation of flower scent. *J. Sep. Sci.* **26:** 715–721 (2003).
18. *Chinese Pharmacopoeia.* Committee Publishing House of People's Health, 2000, Part II: Appendix 64.

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