Rapid Determination of Volatile Compounds Emitted from *Chimonanthus praecox* Flowers by HS-SPME-GC-MS

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A novel rapid, simple and solvent-free method was developed for determination of the volatile compounds from the flowers of *Chimonanthus praecox* Link using headspace solid-phase microextraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS). The SPME conditions were firstly optimized and applied to sampling of the volatile compounds emitted from living *Chimonanthus praecox* L. flowers and excised *Chimonanthus praecox* L. flowers. Thirty-one compounds emitted from living flowers were identified for the first time, which mainly included 4-methyl-1,3-pentadiene (2.0%), α-phellandrene (4.7%), benzyl methanol (11.1%), *trans*-linalool oxide (furanyl ring) (5.3%), α-linalool (36.0%), methyl salicylate (24.5%) and acetic acid benzyl ester (5.9%). Comparing the emission from living flowers and excised flowers, twenty-eight compounds were found to be detected in the two emissions, and three compounds, *n*-pentadecane, *n*-cetane and *n*-heptadecane, were only found in the emission from the living flowers, which shows that they might be biomarker compounds.

Key words: HS-SPME, GC-MS, Volatile Emission, *Chimonanthus praecox* Flowers

Introduction

In 1990, a new extraction technique, solid-phase microextraction (SPME), was introduced (Arthur and Pawliszyn, 1990). It has gained widespread acceptance in many areas in recent years (Arthur *et al.,* 1992; Pawliszyn, 1999; Zhang *et al.,* 1994). SPME combined with gas chromatography-mass spectrometry (GC-MS) has been used to investigate volatile constituents present in plant tissues (Kovacevic and Kac, 2001; Stashenko *et al.,* 2004; Flamini *et al.,* 2003b). Recently, investigation of volatile compounds emitted from living plants and plant tissues was performed using this technique (Zini *et al.,* 2001; Xu *et al.,* 2002). It is well documented that the scent of plant flower plays a major role in attracting pollinating insects. The floral scents can act both at long distances as attraction cues and at short distances as orientation cues among different flowers (William, 1983). Therefore, the investigation of volatile compounds emitted from flowers is very interesting. SPME and GC-MS were developed to analyze the floral scent (Flamini *et al.,* 2003a; Bartak *et al.,* 2003). In almost reports, flowers were excised from plant and extracted and analyzed. Only one paper reported that SPME was applied to analyze living flowers (Verdonk *et al.,* 2003).

In our previous studies, GC-MS and SPME were developed for analysis of volatile constituents present in plant leaves, fruit and flowers (Deng *et al.,* 2003a, b, c; Deng *et al.,* 2004a; Shang *et al.,* 2002). Recently, we developed SPME for investigation of the plant defense response to tobacco mosaic virus (TMV) by determination of volatile compounds emitted from living tomato plants (Deng *et al.,* 2004b).

The shrub *Chimonanthus praecox* Link belongs to the *Calycanthaceae* family. It occurs in Chinese montaine forest. Due to its fragrant flowers, it is widely cultivated in lots of countries. The white-yellow flowers appear before the leaves during late winter. Due to its exceptional fragrant flowers in winter, it has the common name of wintersweet. The aromatic volatiles from *Chimonanthus praecox* flowers are very pleasant to the human sensory system and have a potential application as components of perfumes. Moreover, in China, the flowers have been applied to the treatment of lots of diseases for a long time. However, up to date, the aromatic compounds present in the flowers are unclear.

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In this paper, SPME with GC-MS was developed for determination of the volatile compounds from the *Chimonanthus praecox* flowers.

Materials and Methods

SPME holder and fibers

A manual SPME holder and five commercial SPME fibers: 100-μm poly(dimethylsiloxane) (PDMS), 65-μm poly(dimethylsiloxane)-divinylbenzene (PDMS-DVB), 65-μm carbowax-divinylbenzene (CW-DVB), 85-μm poly(acrylate) (PA), 75-μm carboxen-poly(dimethylsiloxane) (CAR-PDMS) were purchased from Supelco company (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 250 °C in the GC injector. For the following analyses, 2 min of desorption after each extraction was used as conditioning time.

Living flowers

A 15-year-old *Chimonanthus praecox* Link shrub from the campus of Fudan University, Shanghai, China, was used in the present experiment. Its flowers appeared between January 15, 2004 and February 20, 2004. The experiment was carried out on January 28 and 29, 2004. A glass chamber was devised for sampling of the volatile compounds emitted from living *Chimonanthus praecox* flowers (Fig. 1). A branch with six flowers was introduced into the sample chamber. At the same time, six flowers were excised from the *Chimonanthus praecox* shrub and introduced into a 10-ml headspace vial.

Optimization of the SPME conditions

The *Chimonanthus praecox* branch with six living flowers in the sample chamber was applied to optimize the SPME conditions. To obtain the optimum fiber, five commercially available fibers were simultaneously used for extraction of volatile compounds emitted from the living flowers at 15 °C for 30 min. The extraction time was also tested by adsorption of volatiles in emission at different adsorption times with the same temperature of 15 °C.

Headspace SPME of volatile compounds emitted from Chimonanthus praecox flowers

The optimized SPME conditions were applied to headspace extraction of volatile compounds emitted from the living *Chimonanthus praecox* flowers and excised *Chimonanthus praecox* flowers. The volatiles adsorbed on the fibers were desorbed at the GC injection port with the temperature of 250 °C for 3 min and analyzed by GC-MS. Four replicated analyses for each sample were performed.

GC-MS

A Finnigan Voyager gas chromatograph-mass spectrometer 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 Superlco, which was inserted directly into the ion source of the MS. The splitless mode was used. 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^{-1}, 300 °C was maintained for 5 min. Helium (99.999%) carrier gas had a flow-rate of 1 ml min^{-1}. The analysis was carried out under full-scan acquisition mode within the 41–450 a.m.u. range.

Precision

The four replicated extractions and analyses of volatile compounds emitted by the six living *Chimonanthus praecox* flowers were performed under the same conditions. The extraction was carried out at 15 °C for 20 min with CAR-PDMS fiber. GC-MS analysis conditions were described above.

Results and Discussion

SPME is a simple, rapid and solventless technique that permits the establishment of equilibrium between the sample matrix, the headspace above sample and a stationary phase coated on a fused silica fiber. The adsorbed analytes are then thermally desorbed from the fiber in the injector port of a gas chromatograph. This technique permits sampling of volatiles emitted by living plants in a fast and easy way. We have obtained noteworthy improvements with respect to procedures reported in the previous paper (Deng *et al.,* 2004b): The high concentration capability of SPME permits the use of considerably lower amounts of volatile compounds emitted from living tomato

plants; furthermore, the sampling time is less than 30 min, minimizing the possibility of contamination from environment. In the present work, the technique was further developed for analysis of volatile compounds emitted from living *Chimonanthus praecox* flowers and excised *Chimonanthus praecox* flowers. A sampling chamber was devised and applied to analysis of the living flowers (Fig. 1).

Optimizing SPME conditions

When optimizing extraction conditions in any SPME method, there are a number of variables that must be considered. The major factors studied in this work include extraction temperature, extraction time, and SPME fiber.

Headspace SPME of volatiles emitted from the living *Chimonanthus praecox* flowers was carried out outdoors. The outdoor temperature of 15 °C was used as extraction temperature. The optimum fiber was examined exposing five different fibers to the headspace of the *Chimonanthus praecox* flowers in a glass chamber for 30 min. The main six volatile compounds in the emission, methyl salicylate (a), acetic acid benzyl ester (b), α-linalool (c), *trans*-linalool oxide (furanyl ring) (d), benzyl methanol (e), α-phellandrene (f) (seen in Table I), were applied to determine the optimum fiber. The results are shown in Fig. 2, where peak areas of the six compounds are plotted against the different fibers. For five compounds, α-phellandrene, benzyl methanol, *trans*-linalool oxide (furanyl ring), methyl salicylate and acetic acid benzyl ester, the highest efficiencies were observed using the CAR-PDMS fiber. Only for α-linalool, the CAR-PDMS fiber has lower efficiencies than PDMS-DVB fiber. Therefore, the CAR-PDMS fiber was chosen as the optimum fiber for headspace extraction of the *Chimonanthus praecox* flower emission.

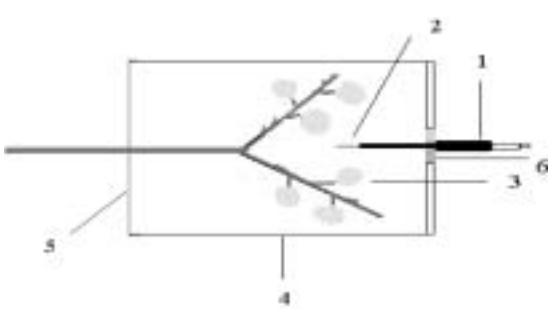

Fig. 1. Experimental design for headspace-SPME of volatile compounds emitted by living *Chimonanthus praecox* flowers. 1, SPME holder; 2, SPME fiber; 3, living *Chimonanthus praecox* flowers; 4, glass cylinder (120 mm wide, 60 mm diameter); 5, aluminium foil; 6, Teflon tape.

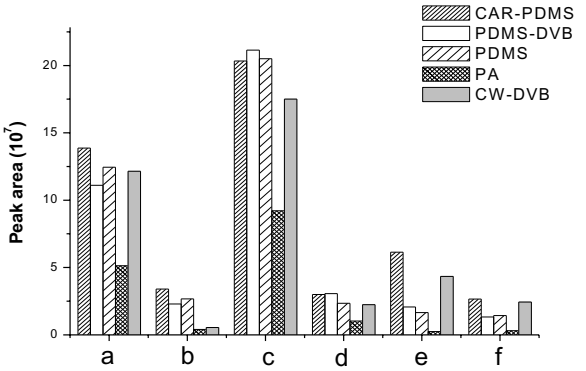

Fig. 2. Effect of fiber coating on the peak areas of six main compounds, methyl salicylate (a), acetic acid benzyl ester (b), α-linalool (c), *trans*-linalool oxide (furanyl ring) (d), benzyl methanol (e), α-phellandrene (f), in the *Chimonanthus praecox* flower emission.

The optimum extraction time for exposing the CAR-PDMS fiber to the headspace of the living *Chimonanthus praecox* flowers at 15 °C was examined by measuring the sum of peak area of the volatile compounds emitted from the flowers. An extraction equilibrium was found to be at 20 min.

Based on these experimental results, the sampling conditions for HS-SPME were set at a tem-

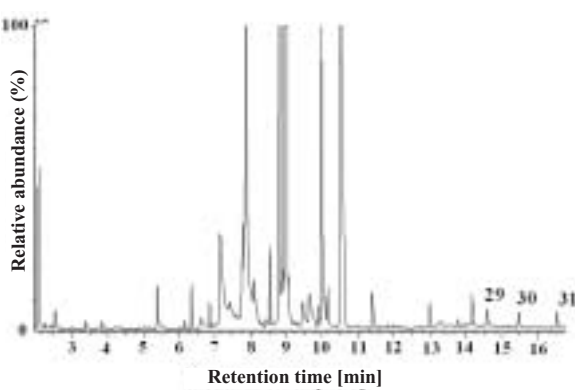

Fig. 3. Total ion chromatogram of volatile compounds emitted from living *Chimonanthus praecox* flowers by HS-SPME with GC-MS. The extraction fiber was CAR-PDMS, extraction temperature was 15 °C, and extraction time was 20 min. Identified compounds are listed in Table. I. No 29 (*n*-pentadecane), 30 (*n*-cetane) and 31 (*n*-heptadecane) are biomarker compounds.

perature of 15 °C and at an exposure time of 20 min, with the CAR-PDMS fiber as the optimum fiber.

Determination of volatile compounds emitted from the living flower

The CAR-PDMS fiber was exposed on the headspace of the six *Chimonanthus praecox* flowers in the sampling chamber (Fig. 1) at 15 °C for 20 min. The total ion chromatogram of the emission from living *Chimonanthus praecox* flowers was obtained and shown in Fig. 3. More than thirty-three compounds were extracted by the SPME fiber, separated by capillary GC, and thirty-one compounds among them were identified by NIST library and the retention indices. Thirty compounds were identified for the first time (Table I), which mainly included esters, alcohols, terpenes, carbonyls and alkane compounds. Their relative content values were calculated by peak areas, which are also listed in Table I.

Determination of volatile compounds emitted from the excised flower

The CAR-PDMS fiber with the optimum extraction conditions was also used to investigate volatiles emitted from the excised *Chimonanthus praecox* flowers. It was found that three compounds, *n*-pentadecane, *n*-cetane and *n*-heptadecane, were detected only in the emission from living *Chimonanthus praecox* flowers, while other compounds were found in both living flower and excised flowers. The results show that the three alkane compounds might be biomarker compounds for living *Chimonanthus praecox* flowers. For most compounds except of *trans*-linalool oxide (furanyl ring), their relative contents were found to be very close. The relative content of *trans*-lina-

Table I. Identification of volatile compounds emitted from living *Chimonanthus praecox* flowers by HS-SPME-GC-MS.

No.	Retention time [min]	Compound	Main fragment ion (Relative abundance,%)	Relative content (%)	RSD* (%)
1	1.917	α-Methyl furan	82(100), 81(54), 53(52), 43(16)	0.29	3.11
2	2.084	4-Methyl-1,3-pentadiene	67(100), 82(53), 41(17), 65(16)	2.02	1.24
3	3.384	Toluene	91(100), 92(61), 65(18), 93(4)	0.12	1.06
4	3.843	*n*-Octane	43(100), 85(70), 57(51), 114(10)	0.10	1.96
5	5.385	Styrene	104(100), 78(49), 103(46), 77(20)	0.68	2.84
6	6.035	3-Thujene	93(100), 91(56), 77(38), 136(10)	0.04	2.6
7	6.160	α-Pinene	93(100), 91(40), 92(37), 136(8)	0.10	1.01
8	6.610	Benzaldehyde	106(100), 77(98), 105(83), 151(32)	0.40	2.95
9	6.852	α-Myrcene	93(100), 91(41), 77(34), 136(13)	0.25	3.64
10	7.135	α-Phellandrene	93(100), 41(81), 69(89), 136(11)	4.70	4.51
11	7.418	*cis*-Geraniol	93(100), 69(98), 41(76), 136(35)	0.83	5.60
12	7.768	2-Isopropylidene-5-methylhex-4-enal	41(100), 67(91), 137(87), 152(60)	1.43	6.24
13	7.860	Benzyl methanol	79(100), 108(90), 107(58), 77(56)	11.11	2.64
14	8.093	3-Carene	93(100), 91(51), 121(32), 136(16)	1.34	2.01
15	8.419	Acetophenone	105(100), 77(82), 120(43), 51(21)	0.15	2.71
16	8.535	*cis*-Linalool oxide (furanyl ring)	59(100), 94(56), 93(45), 111(39)	0.89	3.01
17	8.794	*trans*-Linalool oxide (furanyl ring)	59(100), 94(54), 43(48), 93(40)	5.30	1.00
18	8.994	α-Linalool	71(100), 93(80), 55(48), 154(3)	35.95	4.36
19	9.044	Myrcenol	71(100), 82(70), 43(49), 69(29)	0.43	3.69
20	9.969	Acetic acid benzyl ester	108(100), 91(50), 150(48), 79(31)	5.85	2.14
21	10.144	*trans*-Linalool oxide (pyranyl ring)	68(100), 94(63), 59(60), 67(51)	0.35	2.38
22	10.519	Methyl salicylate	120(100), 92(60), 152(53), 121(32)	24.50	2.45
23	11.377	Acetic acid phenethyl ester	104(100), 43(50), 91(30), 105(21)	0.76	3.10
24	11.619	Cinnamaldehyde	131(100), 132(71), 103(62), 77(49)	0.04	2.44
25	12.986	Benzenepropyl acetate	117(100), 118(90), 91(50), 43(41)	0.37	1.69
26	13.786	Caryophyllene	93(100), 69(89), 133(79), 204(17)	0.09	3.98
27	13.953	Cinnamyl alcohol acetate	115(100), 43(98), 134(50), 176(41)	0.05	4.62
28	14.170	Lyratyl acetate	91(100), 119(96), 134(90), 194(4)	0.41	0.94
29	14.595	*n*-Pentadecane	57(100), 71(73), 43(60), 212(4)	0.36	3.45
30	15.521	*n*-Cetane	57(100), 71(80), 43(61), 226 (5)	0.24	1.77
31	16.523	*n*-Heptadecane	57(100), 71(75), 43(68), 240(5)	0.27	2.06

* Relative standard deviation.

lool oxide (furanyl ring) in the living flower emission was much higher than that in the excised flower emission.

Precision of the method

The four replicated extractions and analyses of volatile compounds emitted by the living *Chimonanthus praecox* flowers were performed under the same conditions. Peak areas obtained were used to calculate the relative standard deviation (RSD) values. The RSD values are shown in Table I, which suggests that the present method has a good precision.

These results show that SPME with GC-MS is a simple, rapid and sensitive method suitable for analysis of the volatiles emitted from living flowers.

Arthur C.-L. and Pawliszyn J. (1990), Solid-phase microextraction with thermal-desorption using fused-silica optical fibers. Anal. Chem. 62, 2145–2148.

Arthur C.-L., Killam L.-M., Motlagh S., Lim M., Potter D. W., and Pawliszyn J. (1992), Analysis of substituted benzene compounds in groundwater using solid-phase microextraction. Environ. Sci. Technol. **26**, 979–983.

Bartak P., Bednar P., Cap L., Ondrakova L., and Stransky Z. (2003), SPME – A valuable tool for investigation of flower scent. J. Sep. Sci. **26**,715–721.

Deng C.-H., Song G.-X., Hu Y.-M., and Zhang X.-M. (2003a), Analysis of the volatile constituents of *Schisandra chinesis* (Turz.) Bail by gas chromatography-mass spectrometry, using headspace solid-phase microextraction. Chromatographia **58**, 289–294.

Deng C.-H., Song G.-X., Zheng X.-H., Hu Y.-M., and Zhang X.-M. (2003b), Analysis of the volatile constituents of *Apium graveolens* L., and *Oenanthe* L. by gas chromatography-mass spectrometry, using headspace solid-phase microextraction. Chromatographia **57**, 805–809.

Deng C.-H., Song G.-X., Hu Y.-M., and Zhang X.-M. (2003c), Determination of the volatile constituents of Chinese *Coriandrum sativum* L. by gas chromatography-mass spectrometry with solid-phase microextraction. Chromatographia **57**, 357–361.

Deng C.-H., Zhang X.-M., Zhu W.-M., and Qian J. (2004a), Gas chromatography-mass spectrometry with solid-phase microextraction method for determination of methyl salicylate and other volatile compounds in leaves of *Lycopersicon esculentum*. Anal. Bioanal. Chem. **378**, 518–522.

Deng C.-H., Zhang X.-M., Zhu W.-M., and Qian J. (2004b), Investigation of tomato plant defence response to tobacco mosaic virus by determination of methyl salicylate with SPME-capillary GC-MS. Chromatographia **59**, 263–268.

Flamini G., Cioni P.-L., and Morelli I. (2003a), Use of solid-phase micro-extraction as a sampling technique in the determination of volatiles emitted by flowers, isolated flower parts and pollen. J. Chromatogr. A **998**, 229–233.

Flamini G., Cioni P.-L., and Morel I. (2003b), Differences in the fragrances of pollen, leaves, and floral parts of garland (*Chrysanthemum coronarium*) and composition of the essential oils from flowerheads and leaves. J. Agr. Food Chem. **51**, 2257–2271.

Kovacevic M. and Kac M. (2001), Solid-phase microextraction of hop volatiles – Potential use for determination and verification of hop varieties. J. Chromatogr. A **918**, 159–167.

Pawliszyn J. (1999), In: Applications of Solid Phase Microextraction (Pawliszyn, J., ed.). Royal Society of Chemistry, Cambridge.

Shang C.-Q., Hu, Y.-M., Deng C.-H, and Hu K.-J. (2002), Rapid determination of volatile constituents of *Michelia alba* flowers by gas chromatography-mass spectrometry with solid-phase microextraction. J. Chromatogr. A **942**, 283–288.

Stashenko E.-E., Jaramillo B.-E., and Martinez J.-R. (2004), Analysis of volatile secondary metabolites from *Colombian Xylopia aromatica* (Lamarck) by different extraction and headspace methods and gas chromatography. J. Chromatogr. A **1025**, 105–113.

Verdonk J.-C., de Vos C.-H. R., and Verhoeven H.-A. (2003), Regulation of floral scent production in petunia revealed by targeted metabolomics. Phytochemistry **62**, 997–1008.

William N.-H. (1983), Floral fragrances as cues in animal behavior. In: Handbook of Experimental Pollination Biology (Jones C. E. and Little R. J., eds.). Academic Press, New York, pp. 50–72.

Xu T., Zhou Q., and Xia Q. (2002), Effects of herbivore-induced rice volatiles on the host selection behavior of brown planthopper, *Nilaparvata lugens.* Chinese Sci. Bull. **47**,1355–1360.

Zhang Z., Yang M.-J., and Pawliszyn J. (1994), Solid-phase microextraction. Anal. Chem. **66**, A844-A853.

Zini C.-A., Augusto F., Christensen E., Smith B.-P., Caramao E.-B., and Pawliszyn J. (2001), Monitoring biogenic volatile compounds emitted by *Eucalyptus citriodora* using SPME. Anal. Chem. **73**, 4729–4735.