Analysis of *Rhioxma Curcumae Aeruginosae* Volatiles by Solid-phase Microextraction with Gas Chromatography-Mass Spectrometry

Yun F. Sha[a], Shun Shen[b], and Geng L. Duan[a,*]

[a] Department of Pharmacy, Fudan University, Shanghai 200032, People's Republic of China. E-mail: glduan@shmu.edu.cn

[b] Department of Chemistry, Fudan University, Shanghai 200433, People's Republic of China

* Author for correspondence and reprint requests

Z. Naturforsch. **59 c**, 533–537 (2004); February 24/April 1, 2004

In this paper, a headspace solid-phase microextraction (HS-SPME) method was applied to analyse the volatile compounds in a traditional Chinese medicine (TCM), *Rhioxma Curcumae Aeruginosae*. SPME parameters such as fibers, extraction temperature, extraction time and desorption time were investigated. Thirty-five volatile compounds were separated and identified. Relative standard deviations (RSDs) were less than 8.4%, showing that the method has a good reproducibility. The volatile constituents were also analyzed by steam distillation (SD) and thirty-seven compounds were identified. The similar results obtained by the two methods showed that SPME is a good alternative for the analysis of volatile constituents in *Rhioxma Curcumae Aeruginosae* samples and it is a relatively simple, rapid and solvent-free method.

Key words: Rhioxma Curcumae Aeruginosae Volatiles, Solid-phase Microextraction, Gas Chromatography-Mass Spectrometry

Introduction

Rhioxma Curcumae Aeruginosae, the dry rhizome of *Curcuma phaeocaulis* Valeton, *Curcuma kuangsiensis* S. G. Lee et C. F. Liang or *Curcuma wengyujin* Y. H. Chen et C. Ling, has been used for many centuries to enhance blood circulation, to remove blood stasis and as an acesodyne herb in China. Its essential oil called Zedolary Turmeric oil, including mainly β-elemene, curcumol and curzerenone, is effective in treating cancer (Li *et al.*, 2001). Therefore, the preparation of Zedolory Turmeric oil and glucose injection have been embodied in Chinese Pharmacopoeia (Chinese Pharmacopoeia Committee, 2000a).

The dry rhizome of *Curcuma phaeocaulis*, *C. kuangsiensis* and *C. wengyujin* contains about 1.2% of essential oil. Classical methods such as steam distillation and solvent extraction were applied to determine chemical constituents in its volatile oil (Li *et al.*, 2002; Yang and Wen, 2000). Super critical fluid extraction, a good method, was also used for analysis of volatile compounds of this traditional Chinese medicine (TCM) (Shu et al., 2000). Thirty-seven compounds were identified by steam distillation in the TCM. Some active compounds such as eucalyptol, camphor, β-elemene, curcumol, curzerene and curzerenone were found to be present in the volatile oil of the TCM.

However, these conventional techniques take hours to prepare a sample. Steam distillation (SD) requires complex glassware, the assembly, disassembly, and cleaning of which consume additional time. Moreover, steam distillation requires large amounts of sample. Solvent extraction has the disadvantage that nonvolatile resinous components extracted along with the essential oil affect GC columns. Super critical fluid extraction needs special and expensive equipment. Thus, it is necessary to develop a rapid, simple, inexpensive and sensitive method for qualitative analysis of volatile constituents in a *Rhioxma Curcumae Aeruginosae* sample.

Solid-phase microextraction (SPME), introduced by Pawliszyn's group in 1990, is a relatively new sampling and concentration technique (Arthur and Pawliszyn, 1990). It has been developed for determination of chemical components present in plant volatile compounds (Tasdemir *et al.*, 2003; Xiong *et al.*, 2003; Vereen *et al.*, 2000; Flamini *et al.*, 2003) and has been proved to be a simple, rapid, sensitive and solvent-free method for determination of volatiles in TCMs (Song *et al.*, 2003; Deng *et al.*, 2003).

In this work, SPME parameters such as fibers, extraction temperature, extraction time and desorption time were optimized. After optimization,

0939–5075/2004/0700–0533 $ 06.00 © 2004 Verlag der Zeitschrift für Naturforschung, Tübingen · http://www.znaturforsch.com · D

SPME-GC-MS was applied to investige the volatile constituents of *Rhioxma Curcumae Aeruginosae* from Sichuan in China.

Experimental

Material and reagents

Rhioxma Curcumae Aeruginosae samples were collected from Sichuan, China. The extraction fibers 100-μm polydimethylsiloxane (PDMS), 65-μm polydimethylsiloxane/divinylbenzene (PDMS/DVB), 65-μm carbowax/ divinylbenzene (CW/DVB) and 85-μm polyacrylate (PA) were purchased from Supelco (Bellefonte, PA, USA) and conditioned prior to use according to supplier's prescriptions.

Sample preparation

50 g of *Rhioxma Curcumae Aeruginosae* were ground to fine powder, and then put into a 1000-ml distillation flask. 500 ml of distilled water were added and a volatile oil distillation apparatus was set according to the Chinese Pharmacopoeia (Chinese Pharmacopoeia Committee, 2000b). The mixture was distilled for 6 h. The oil was collected from the condenser, dried over anhydrous sodium sulfate and the yield of the sample was 1.3%. The obtained essential oil was stored at – 10 °C until analysis.

HS-SPME sample preparation was as follows: 2.0 g of a *Rhioxma Curcumae Aeruginosae* sample was ground to fine powder, then introduced into a 15-ml headspace glass bottle. The bottle was immediately sealed by a silicone septum and stored at – 10 °C until use.

Optimization of SPME conditions

2.0 g of *Rhioxma Curcumae Aeruginosae* were used for investigation of the optimal extraction conditions. At first, selection of the optimum fiber was performed by extraction of the volatile compounds of the sample using PDMS, PDMS/DVB, CW/DVB and PA fibers simultaneously at the same conditions (extraction temperature of 70 °C and extraction time of 45 min). Next, optimization of extraction temperature (40 °C to 80 °C), extraction time (20 min to 45 min) and desorption time (1 min to 5 min) were carried out using the best fiber. These optimum conditions were all determined by the peak areas of five main active constituents: eucalyptol, camphor, β-elemene, curzerene and curzerenone.

SPME of the volatile constituents

The PDMS/DVB fiber was applied to extraction of the volatile constituents in the *Rhioxma Curcumae Aeruginosae* sample. Extraction was carried out at 70 °C for 30 min, and then the sample was introduced into the GC injection liner and desorbed at 250 °C for 3 min.

GC-MS analysis

Desorption and analysis of volatile compounds were carried out using 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 in diameter, 0.25 μm film thickness). Split injection was employed for both distillation and SPME samples with a ratio of 20:1. 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 250 °C at 10 °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 to 500 amu. Compounds were identified using the Wiley 6.0 Mass Spectral library (Wiley, New York, NY, USA).

Results and Discussion

Optimization of SPME parameters

In this study, the headspace solid-phase microextraction (HS-SPME) technique was applied to analyze essential oils in *Rhioxma Curcumae Aeruginosae* samples. It was found that the SPME fiber had a capability to concentrate the analytes, and provided the method to analyze volatile components. SPME parameters such as extraction fibers, extraction temperature, extraction time and desorption time were optimized for identification of the volatiles.

Comparison of different fibers

To select a more appropriate fiber, four different fibers were exposed for 45 min at 70 °C to 2.0 g of the *Rhioxma Curcumae Aeruginosae* sample from Sichuan in headspace mode. The peak

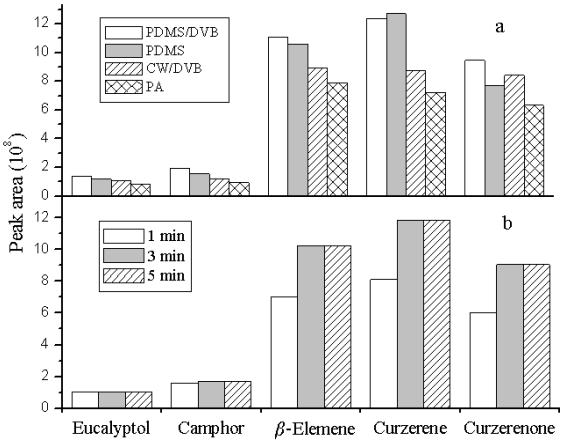

Fig. 1a. Extraction profile obtained with different fibers for five active compounds in *Rhioxma Curcumae Aeruginosae* from Sichuan. Extraction conditions: sample mass, 2.0 g; extraction time, 45 min; extraction temperature, 70 °C.
Fig. 1b. Desorption time profile for 65-μm PDMS/DVB fiber. Conditions: extraction temperature, 70 °C; extraction time, 30 min; sample mass, 2.0 g.

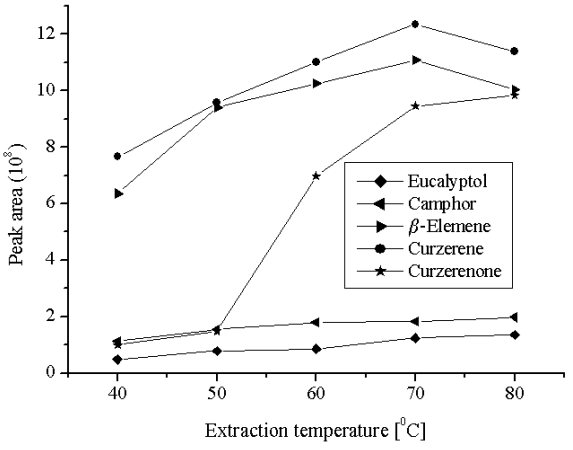

Fig. 2. Headspace SPME-GC-MS analysis of *Rhioxma Curcumae Aeruginosae* volatiles. Peak areas for five main active components (eucalyptol, camphor, β-elemene, curzerene and curzerenone) at different extraction temperatures. Conditions: extraction fiber, PDMS/DVB; extraction time, 45 min; sample mass, 2.0 g.

areas of the five main active components (eucalyptol, camphor, β-elemene, curzerene and curzerenone) were obtained; they are presented in Fig. 1a. As observed, the highest peak areas were obtained with the PDMS/DVB, which was selected for the future studies.

Extraction temperature

The extraction temperature profile obtained using a PDMS/DVB fiber (2.0 g of sample and 45 min of extraction time) is shown in Fig. 2. The fiber concentration of β-elemene and curzerene increases with extraction temperature rising from 40 °C to 70 °C, and achieves a maximum at 70 °C, then it decreases with rising extraction temperature. However, the fiber concentration of curzerenone keeps going up as temperature increases. The mass of eucalyptol and camphor absorbed on the fiber shows few changes if the extraction temperature increases. As we know, a higher extraction temperature could accelerate mass transfer flux through the sampling system, but any changes in the composition of the absorbed phase were due to competition between different volatiles with different affinities towards the fiber. Comprehensively considered, an extraction temperature of 70 °C was used as the best choice.

Extraction time

Since a short time of analysis was desired in sample pretreatment, a series of extraction times (20, 30, 45 min) were investigated at 70 °C. The results showed that 30 min was good enough for analysis of the five target constituents in respect that the extraction efficiency of 30 min is similar to that of 45 min. For the PDMS/DVB fiber, the equilibrium condition for the absorption of the most analytes was almost realized in 30 min. Besides, the total GC-MS analysis time was 33 min, so an extraction time of 30 min was selected as the optimal time for analysis of essential oils in *Rhioxma Curcumae Aeruginosae* samples.

Desorption time

Five main active components were still selected for investigation of the desorption time, and the result obtained using the 65-μm PDMS/DVB fiber was shown in Fig. 1b. A desorption period of 3 min was long enough to desorb the analytes from the PDMS/DVB fiber (temperature of GC injection port, 250 °C). Therefore, 3 min of desorption period was used for this fiber.

Determination of volatile compounds

As shown in Table I, thirty-five constituents of *Rhioxma Curcumae Aeruginosae* volatiles from

Table I. GC-MS identification of Rhioxma Curcumae Aeruginosae volatiles and peak area percentage.

No.	Retention time [min]	Compound	Relative content (%)	
			SPME 65-μm PDMS/DVB	SD
1	5.976	α-Pinene	0.85	0.58
2	6.374	Camphene	1.63	1.49
3	7.159	β-Pinene	1.44	1.26
4	7.592	β-Myrcene	0.32	0.23
5	7.949	α-Phellandrene	0.48	0.23
6	8.105	3-Carene	0.37	0.19
7	8.624	Limonene	0.88	0.41
8	8.714	Eucalyptol	1.57	1.32
9	8.890	(*E*)-3,7-Dimethyl-1,3,6-octatriene	0.10	0.06
10	9.455	1-Methyl-4-(1-methylethyl)-1,4-cyclohexadiene	0.09	tr
11	10.245	4-Carene	0.16	0.04
12	10.355	2-Nonanone	0.12	0.05
13	10.467	2-Nonanol	0.11	0.47
14	11.734	Camphor	2.42	2.28
15	12.057	Isoborneol	0.70	1.14
16	12.282	Borneol	0.23	0.56
17	12.570	Menthol	0.08	0.09
18	12.905	α-Terpineol	0.16	0.14
19	15.164	Isoborneol acetate	0.02	0.09
20	15.251	2-Undecanone	nd	0.05
21	15.424	2-Tetradecanol	nd	0.03
22	16.401	(3*R-trans*)-4-Ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-cyclohexene	3.41	1.13
23	16.643	α-Cubebene	0.11	0.09
24	17.145	Copaene	0.11	0.06
25	17.658	β-Elemene	14.40	6.86
26	17.952	Isocaryophyllene	0.16	0.06
27	18.218	β-Caryophyllene	2.57	0.92
28	18.500	γ-Elemene	1.66	0.60
29	18.702	Epizonarene	0.54	0.10
30	18.956	α-Caryophyllene	4.09	4.09
31	19.868	Curzerene	15.77	9.31
32	20.041	(*S*)-1-Methyl-4-(5-methyl-1-methylene-4-hexenyl)-cyclohexene	2.27	0.52
33	20.381	β-Sesquiphellandrene	8.87	2.18
34	21.085	1-Ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)-cyclohexane	2.23	2.36
35	21.999	Curzerenone	12.47	15.30
36	23.738	β-Elemenone	4.96	7.97
37	24.136	Curdione	4.20	9.12

tr: Relative content less than 0.01%.
nd: Not detected.

Sichuan could be identified using optimal SPME conditions (70 °C for 30 min and 2.0 g of sample mass) followed by GC-MS, representing more than 89.6% of the total oil. The main constituents were β-elemene (14.4%), curzerene (15.8%), β-sesquiphellandrene (8.9%) and curzerenone (12.5%). However, curcumol, an important anti-cancer substance, wasn't detected in this sample, which was in agreement with one reported literature (Li *et al.*, 2001).

Comparison of the results by SPME and SD

The traditional steam distillation method was also applied to analyze volatile constituents of *Rhioxma Curcumae Aeruginosae* followed by GC-MS, and thirty-seven components were detected (Table I). Compounds extracted by SD and SPME both contain the main active components such as eucalyptol, camphor, β-elemene, curzerene and curzerenone. Furthermore, the contents of these active constituents were higher than the 1% level.

But curcumol still could not be detected. Compared with the results obtained by the SPME technique, the SD method had the preference to extract all volatiles, which seemed more comprehensive. However, the main disadvantages of SD were: time consuming, possibility of solvent contamination, and laborious manipulation. While SPME showed enormous advantages: simplicity, rapid, solvent-free extraction, low cost, little interference, and suitable for routinely screening.

Repeatability

The repeatability of the method was determined by replicating three analyses of the five active compounds (eucalyptol, camphor, β-elemene, curzerene and curzerenone) under the optimized SPME conditions. The relative standard deviations (RSDs) of peak areas of these five active compounds were: eucalyptol 7.0%, camphor 6.9%, β-elemene 7.6%, curzerene 8.4% and curzerenone 6.9%, respectively. The result showed that the SPME method had a good reproducibility.

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

Chinese Pharmacopoeia Committee (2000a), Chinese Pharmacopoeia. Publishing House of People's Health, Beijing, part II, p. 697.

Chinese Pharmacopoeia Committee (2000b), Chinese Pharmacopoeia. Publishing House of People's Health, Beijing, part I, Appendix 64.

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

Flamini G., Cioni P.-L., and Morelli I. (2003), 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.

Li A.-Q., Huang X.-J., and Deng Y.-H. (2001), Chemical compounds of *Rhioxma Curcumae Aeruginosae* from Wenzhou in China. Chinese Tradit. Herbal Drugs **32**, 782–783.

Li A.-Q., Yang C.-S., and Huang X.-J. (2002), Comparison of volatile compounds of *Rhioxma Curcumae Aeruginosae* from different growing areas. J. Instrumental Anal. **21**, 67–69.

Shu L.-C, Jing Y., and Guo J.-W. (2000), Analysis of volatile compounds of *Rhioxma Curcumae Aeruginosae* by super critical fluid extraction. Chinese Tradit. Herbal Drugs **31**, 902–904.

Song G.-X., Deng C.-H., Wu D., and Hu Y.-M. (2003), Comparison of headspace solid-phase microextraction with solvent extraction for the analysis of the volatile constituents of leaf twigs of Chinese Arborvitae. Chromatographia **58**, 769–774.

Tasdemir D., Demirci B., Demirci F., Donmez A.-A., Baser K.-M. C., and Ruedi P. (2003), Analysis of the volatile components of five Turkish Rhododendron species by headspace solid-phase microextraction and GC-MS (HS-SPME-GC-MS). Z. Naturforsch. **58c**, 797–803.

Vereen D.-A., Paul McCall J., and Butcher D.-J. (2000), Solid phase microextraction for the determination of volatile organics in the foliage of Fraser fir (*Abies fraseri*). Microchem. J. **65**, 269–276.

Xiong G.-H., Goodridge C., Wang L.-M., Chen Y., and Pawliszyn J. (2003), Microwave-assisted headspace solid-phase microextraction for the analysis of bioemissions from *Eucalyptus citriodora* leaves. J. Agr. Food Chem. **51**, 7841–7847.

Yang L. and Wen J.-P. (2000), Analysis of volatile compounds of *Rhioxma Curcumae Aeruginosae* by different solvent extraction. Chinese Tradit. Herbal Drugs **30**, 457–460.