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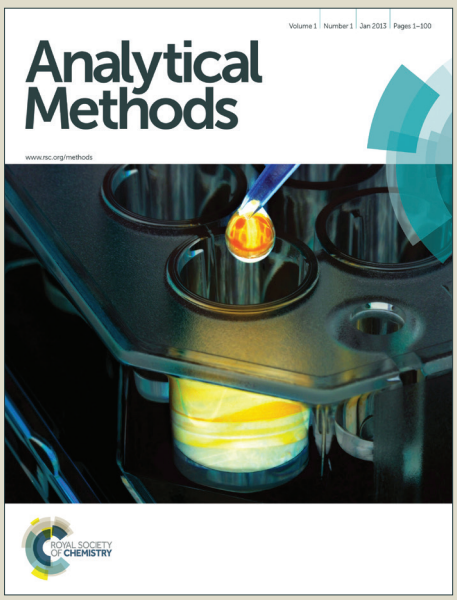

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ARTICLE

Rapid analysis of essential oils in fruits of *Alpinia oxyphylla Miq.* by microwave distillation and simultaneous headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry

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A method based on microwave distillation (MD) and simultaneous headspace solid-phase microextraction (HS-SPME), coupled with gas chromatography-mass spectrometry (GC-MS) was developed for the rapid determination of essential oils in fruits of *Alpinia oxyphylla Miq.*, a traditional Chinese medicine (TCM). HS-SPME conditions, including SPME fiber, desorption time, and microwave parameters (irradiation power and time) were optimized. The method accomplishes isolation, extraction and concentration of the essential oils simultaneously. Compared to the conventional steam distillation (SD) method, which could only recover 35 compounds, MD-SPME led to the separation and identification of 53 compounds in the essential oils of *Alpinia oxyphylla Miq.* fruits. The intra-day and inter-day relative standard deviation (RSD) values of MD-SPME-GC-MS method are all less than 8%, which shows that it has satisfactory precision. The MD-SPME-GC-MS method developed in this study is simple, rapid and solvent-free, and shows promise for routine analysis of essential oils in fruits of *Alpinia oxyphylla Miq.* and potentially other TCMs.

Keywords: *Alpinia oxyphylla Miq.* fruits; gas chromatography-mass spectrometry; microwave distillation; solid-phase microextraction; essential oils.

Introduction

Traditional Chinese medicines (TCMs) have played an important role in clinical therapy for thousands of years in China because of their high pharmacological activity, low toxicity and immune regulation [1,2]. Research on TCM compounds has attracted the interests of many pharmacologists. The mature fruits of *Alpinia oxyphylla Miq.*, which grows in the southern part of China, have long been used as an important TCM for the treatment of hypertension and cerebrovascular disorders. They are also widely used as a tonic, aphrodisiac, anti-salivation and anti-diarrhea [3-5].

Different mass spectrometric and chromatographic techniques have been developed for the identification of the essential oils, e.g. API-LC-MS [6-8], UMHE-HS-SPME [9] and MALDI-TOF-MS [10, 11]. Among the various analytical techniques, GC-MS has become the most preferred one for the identification and quantitative analysis of volatile compounds in TCMs [12]. GC-MS can produce two-dimensional data containing both chromatographic and spectral information from a single sample run. Qualitative and quantitative information for compounds can be derived from retention times, peak heights, peak areas and mass spectra [13, 14]. The conventional method for analysis of essential oils in TCMs involves oil isolation by steam distillation (SD) followed by GC-MS [15, 16]. This method requires large amounts of sample (50-1,000g) and toxic organic solvents. Furthermore, SD is rather time-consuming as the procedure typically requires 6-8 h of distillation [17-19]. Various alternative methods have been developed for the analysis of essential oils, such as hydro-distillation, Soxhlet extraction and solvent extraction [20-23]. However, loss of some volatile compounds, low extraction efficiency, and residues of toxic solvents in the extracts may be encountered in these methods. Moreover, they can also be time-consuming. For the quality evaluation of *Alpinia oxyphylla Miq.* fruits, a rapid, simple and sensitive analytical method that requires less solvent and preparation time is critically needed for investing the essential oils present in this TCM.

Recently, the application of microwave in the analysis of essential oils has gained widespread interest. Microwave-assisted solvent extraction (MASE) has been used for the isolation of essential oils from plant materials [24, 25]. The main advantages of MASE are the significant reduction of extraction time and organic solvent consumption [26]. More recently, Stashenko et al. developed microwave-assisted hydrodistillation (MAHD) technique for the extraction of essential oils [27-29]. Chemat et al. developed a new technique combining microwave and dry distillation technique for the isolation of essential oils in fresh plant materials [30]. However, to isolate the essential oils these methods required further extraction using organic solvents and the preparation time was more than 30 min.

Solid-phase microextraction, introduced by Pawliszyn's group in 1990, is a relatively new sampling and concentration technique [31]. It combines sampling, preconcentration, and allows direct transfer of the analytes into a standard gas chromatography. This technique has been widely adopted for the determination of chemical components in plant essential oils. Application of SPME in the analysis of food, biomedical and environmental samples was recently reviewed in a number of papers [32-36]. In our previous studies, Headspace SPME (HS-SPME) coupled to gas chromatography-mass spectrometry (GC-MS) was used to analyze the essential oils in TCMs [13, 14]. HS-SPME requires about 30 min for the headspace extraction of the volatile compounds evaporated from TCMs, but some semi-volatile compounds in TCMs cannot be extracted efficiently.

In this work, we combine microwave distillation, HS-SPME and GC-MS for rapid extraction and analysis of essential oils in *Alpinia oxyphylla Miq.* fruits. This new analysis method can perform the isolation, extraction and concentration simultaneously. The extraction conditions, including microwave power, irradiation time, SPME fiber and desorption time were optimized. The performance of the MD-SPME-GC-MS method was also compared with the

conventional approach of SD extraction followed by GC-MS analysis.

Experimental

Materials, Chemicals and Apparatus

Alpinia oxyphylla Miq. fruits were obtained from the Leiyunshang Chinese Medicine Store, Shanghai, China. Anhydrous sodium sulfate was purchased from the Chemical Reagent Company, Shanghai, China. Hexane was supplied by the Dikma Technology, USA. The following SPME fibers (Supelco, Bellefonte, PA, USA) were used for the extraction: 75 μm Carboxen/polydimethylsiloxane (CAR/PDMS), 65 μm polydimethylsiloxane-divinylbenzene (PDMS/DVB), 85 μm polyacrylate (PA), 65 μm Carbowax/divinylbenzene (CW/DVB), 100 μm polydimethylsiloxane (PDMS). All fibers were conditioned prior to use according to supplier's instructions. Fig. 1 illustrates the home-made MD-SPME apparatus used in this study. The microwave oven with a maximum delivered power of 700 W was purchased from Haier Company, Qingdao, China. A hole with diameter of approximately 5 cm was drilled on the top of the microwave oven to allow installation of the distillation apparatus. Based on the wavelength of the microwave (12.2 cm for 2.45 GHz microwave), the chance for the microwave to escape from this hole is negligible because its wavelength is much larger than the size of the hole. All the MD-SPME experiments were carried out in a fume hood and a metal panel was used to protect the user from potential leak of microwave radiation, if any, from the hole at the oven top during operation.

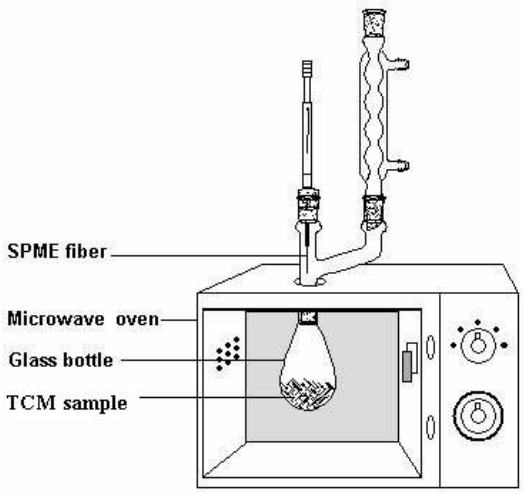

Fig.1 Schematic illustration of the home-made microwave distillation and solid-phase microextraction apparatus. The openable front panel is not shown here.

Optimization of MD-SPME conditions

Four parameters of MD-SPME, namely, SPME fiber coating, desporption time, microwave power and irradiation time, were varied to optimize the extraction conditions. Sample of *Alpinia oxyphylla Miq.* fruits (5.0 g) was ground to fine powder, wetted by 0.5 mL of water, then introduced into a 25 mL glass flask for the investigation of MD-SPME conditions. At first, selection of the optimum fiber was performed by extracting the volatile compounds of the sample using CAR/PDMS, PDMS/DVB, PA, CW-DVB and PDMS fibers under the same conditions (microwave power of 700 W, irradiation time of 5 min, and desorption time of 5 min). Subsequently, the microwave power (200, 400 and 700 W), microwave irradiation time (1, 3, 5 and 7 min), and desorption time (1, 2, 3, 4 and 5 min) were systematically varied.

Extraction of essential oils in *Alpinia oxyphylla Miq.* fruits by MD-SPME

Based on the results of the optimization experiments, CAR/PDMS fiber was selected for extracting the essential oils in *Alpinia oxyphylla Miq.* fruits and the optimum MD-SPME conditions were determined. The wetted powder of *Alpinia oxyphylla Miq.* fruits was irradiated at microwave

power of 400 W for 5 min. The essential oil compounds extracted onto the CAR/PDMS fiber were then desorbed at 250 °C in the GC injection port for 3 min.

Extraction of essential oils in *Alpinia oxyphylla Miq.* fruits by SD

Fifty grams of *Alpinia oxyphylla Miq.* fruits were ground to fine powder, and then transferred into a 1,000 mL distillation flask, followed by addition of 500 mL of distilled water. The essential oil distillation apparatus was set according to the Chinese pharmacopoeia. The mixture was distilled for 6 hours and then the oils were collected from the condenser, dried over anhydrous sodium sulfate. The obtained essential oils were then dissolved with 5 mL hexane and stored at -10 °C until analysis.

Gas chromatography-mass spectrometry

A Finnigan Voyager GC-MS system with electron impact ionization was used in the analysis of the essential oil compounds extracted from *Alpinia oxyphylla Miq.* fruits. The analytes were separated using a 30 m × 0.25 mm fused-silica capillary column with a film thickness of 0.25 μm coated with polydimethylsiloxane. The following temperature program was used: initial temperature at 50 °C for 2 min, then raised to 300 °C at a rate of 10 °C min^{-1}. Helium (99.999%) was used as the carrier gas at a flow-rate of 1 $mL \cdot min^{-1}$. The split ratio was 20:1. The mass spectrometer was operated with electron impact (EI) ionization at 70 eV, and the mass range scanned was 41-400 a.m.u. in the full-scan acquisition mode. The compounds were tentatively identified using the NIST Mass Spectral Search Program (National Institute of Standards and Technology, Washington, DC, USA) or identified by comparison with the mass spectra obtained by analyzing standard solutions.

Under the optimum conditions, the repeatability of the developed MD-SPME-GC-MS method was studied by four replicate analyses of the essential oils in *Alpinia oxyphylla Miq.* fruits. The values of relative standard deviation (RSD) were calculated based the peak areas of the respective compounds.

Results and discussion

MD-SPME condition optimization

Fig. 2 shows the MD-SPME extraction efficiencies of spathulenol, cedrene epoxide, myristic acid and eremophila-1(10),11-dien-2-one using five different SPME fibers. PA fiber exhibited the poorest recoveries for all four essential oil compounds because its polyacrylate coating was most efficient at enriching polar compounds. CAR/PDMS and PDMS fibers showed comparable performance in the extraction of spathulenol, while the CAR/PDMS coated fiber exhibited the best efficiencies for the other three compounds. The porous solid dispersed in liquid polymer matrix gives the fiber with CAR/PDMS coating strong adsorption capacities for most volatile compounds.

The three dimensional graph on Fig. 3 shows the effect of microwave power and irradiation time on extraction of essential oil compounds from *Alpinia oxyphylla Miq.* fruits by MD-SPME. The total amounts of essential oil compounds extracted increased almost linearly with irradiation time at microwave power of 200 W. However, significant reduction in the amounts of essential oils extracted occurred with irradiation time greater than 5 min at both 400 and 700 W. This is attributed to thermal degradation of the essential oil compounds under more intense microwave irradiation. For the irradiation time of 7 min, the total amounts of extracted essential oils decreased greatly as the microwave power was increased from 200 to 400, and then to 700 W, further indicating the occurrence of essential oils under excessive microwave heating. Overall, microwave power of 400 W and irradiation time of 5 min yielded the highest total amount of essential oil compounds and thus were deemed as optimum for MD-SPME.

To compare the performance of MD-SPME with SD, the desorption temperature was kept the same as the injection port temperature (250 °C) for the analysis of the samples obtained by SD. Fig. 4 depicts the effect of desorption time on desorption efficiency of the essential oil compounds from the CAR/PDMS fiber. The results indicate that 3 min was enough to complete the desorption process,

and no improvement in the desorption efficiency occurred with prolonged desorption time (5 min), indicating near complete desorption of the extracted essential oils within 3 min at 250 °C. As a result, the desorption time was set at 3 min.

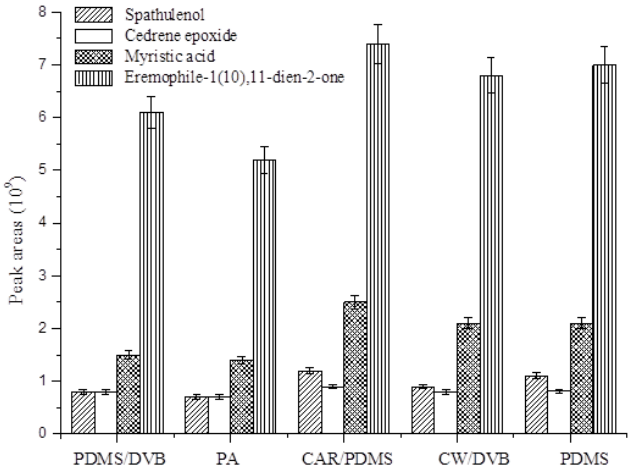

Fig.2 Effect of fiber coating on extraction efficiencies of spathulenol, cedrene epoxide, myristic acid and eremophila-1(10),11-dien-2-one by MD-SPME. Extraction conditions: microwave power of 700 W and irradiation time of 5 min. Desorption conditions: desorption time of 5 min at 250 °C.

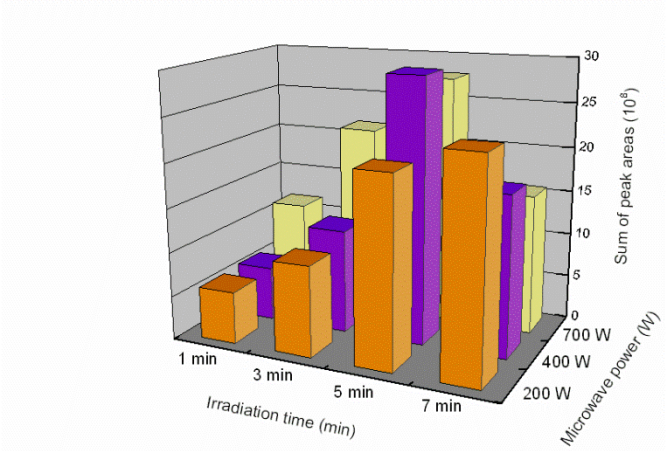

Fig.3 Effect of microwave power and irritation time on the sum of peak areas of essential oils in *Alpinia oxyphylla Miq.* fruits extracted by MD-SPME.

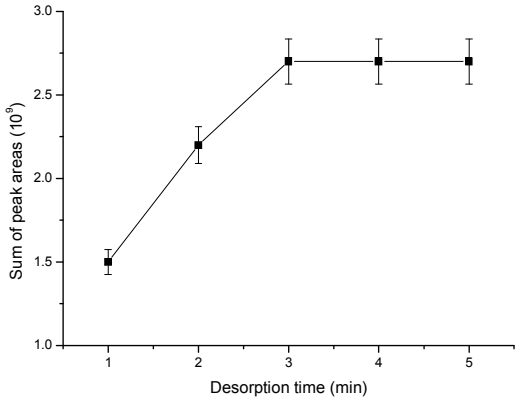

Fig.4 Effect of desorption time on the sum of peak areas of essential oils in *Alpinia oxyphylla Miq.* fruits extracted by MD-SPME. Extraction conditions: microwave power of 700 W and irradiation time of 5 min.

Determination of essential oils in *Alpinia oxyphylla Miq.* fruits by MD-SPME

Fig. 5a and 5b show the total ion chromatograms of essential oils in *Alpinia oxyphylla Miq.* fruits extracted by MD-SPME under the optimum conditions and SD, respectively. The chemical components in the essential oils were identified by comparison with the Mass Spectral Library. Fig. 6 shows the mass spectrum of the over-lapped peak at 16.051 minute compared to the NIST standard spectrum. Fifty-three compounds (Tab. 1), whose fit factor and reverse fit factor were both more than 800, were identified in the extract of MD-SPME. The relative amounts of the essential oil compounds were calculated from their peak area ratios. The major essential oil compounds in *Alpinia oxyphylla Miq.* fruits were found to be 3-furancarboxaldehyde, corylon, α-panasinsen, spathulenol, cadina-1,3,5-triene, cedrene epoxide, longiverbenone, myristic acid, and eremophila-1(10),11-dien-2-one. In contrast, only a total of thirty-five volatile compounds were recovered from *Alpinia oxyphylla Miq.* fruits by SD (Tab. 1).

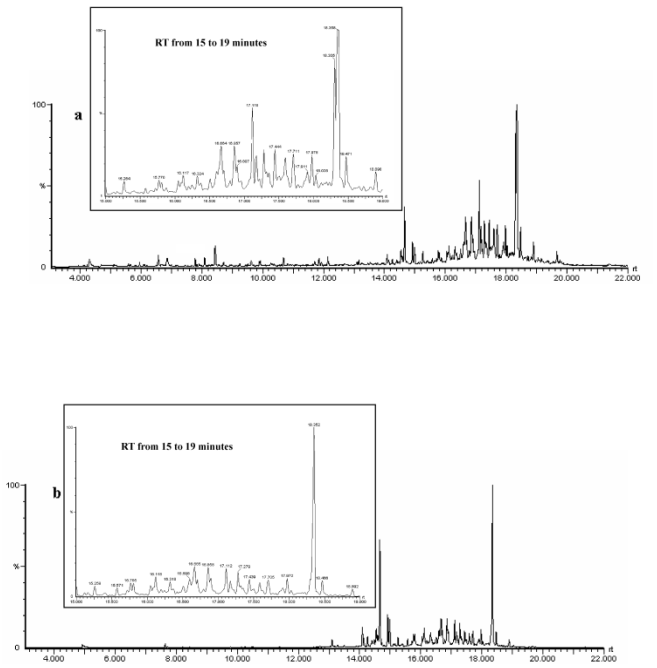

Fig.5 Total ion chromatograms of essential oils in *Alpinia oxyphylla Miq.fruits* extracted by MD-SPME with a CAR/PDMS fiber (a) and SD (b) respectively. The inserts show the magnified views for the chromatograms between retention times of 15 and 19 min.

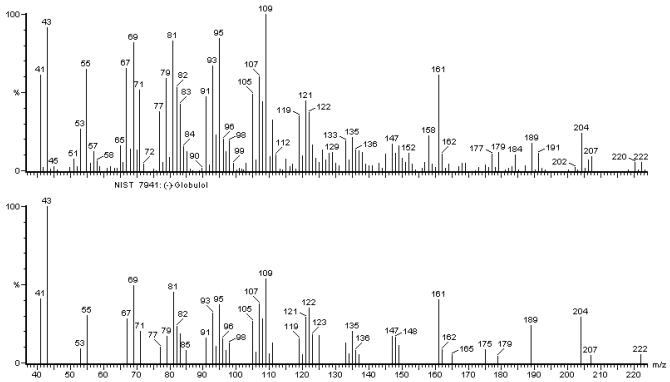

Fig.6 Comparison of the mass spectrum for the over-lapped peak at 16.051 min with the NIST standard spectrum.

Precision of MD-SPME

The repeatability of the MD-SPME method was studied by four replicate analyses of the volatile compounds in *Alpinia oxyphylla Miq.* fruits under the optimum conditions. The method precision was expressed as RSD values calculated on the basis of the peak areas obtained by replicate analyses. As shown in Tab. 1, the intra-day and inter-day RSD values were less than 8%, indicating good repeatability could be achieved by MD-SPME coupled with GC-MS.

Comparison of MD-SPME and SD for analyzing essential oils in *Alpinia oxyphylla Miq.fruits*

Tab. 1 shows that more components were isolated and extracted from *Alpinia oxyphylla Miq.* fruits by MD-SPME than by SD. Twenty-eight common compounds were recovered by both SD and MD-SPME, while additional twenty-five compounds, most of which have low boiling points, were extracted only by MD-SPME. This might be due to loss or decomposition of these compounds components during the relatively long period of the SD process. Microwave heats the wetted sample of *Alpinia oxyphylla Miq.* fruits almost instantly and the compounds volatilized could be collected by SPME, thus minimizing their degradation. As a result, MD-SPME method is more suitable for extraction of compounds with low boiling points than SD.

In the proposed method, microwave irradiation, distillation and headspace extraction are combined into one process and sample preparation takes as less as 5 min. In contrast, the conventional SD approach takes 6 h to isolate the essential oils. Compared with SD, MD-SPME requires less complicated sample preparation and lower amount of sample, and significantly reduces the overall analysis time. The method repeatability and recovery show that it is promising for routine analysis of essential oils in TCMs.

Safety considerations

MD-SPME performed satisfactorily using the setup modified from a domestic microwave oven in this study. On the other hand, it should be pointed out that the use of domestic microwave ovens in laboratory experiments is not advised because of safety concerns and performance [37]. Due to electromagnetic shielding, microwave with wavelength (12.2 cm) much larger than the size of the drilled hole (~5 cm) is unlikely to pass through in our home built MD-SPME setup. The leakage of microwave power was checked around the modified area and was found to be far below

the safety limit (10 mW/cm^2) under the maximum microwave power. Even though no flammable or toxic solvent was involved in the MD-SPME, the setup was placed inside a fume hood and a metal panel was used to shield the user during its operation. Meanwhile, the microwave field inside domestic microwave ovens is well known to be inhomogeneous, which may affect the reproducibility of extraction performance. Therefore, commercial focused microwave instrument is strongly recommended for application of MD-SPME in routine analysis.

Conclusions

In the work, MD combined with SPME for analysis of essential oils in *Alpinia oxyphylla Miq*. fruits was proven to have several advantages, including simplicity, rapidity and need of no solvent. Fifty-three compounds were identified in the *Alpinia oxyphylla Miq*. fruits using the proposed method. The results demonstrated that MD-SPME-GC-MS is a good alternative method for the determination of essential oils in TCMs and can be potentially used for the quality assessment of other TCMs.

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Notes and references

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Table 1. Volatile compounds in the essential oils of *Alpinia oxyphylla Miq.* fruits recovered by MD-SPME and SD and identified by GC-MS.

No.	Retention time (min)	Compounds Name	MW.	Mass spectrum data	Similarity factor	Relative contents (%)		RSD for MD-SPME	
						MD-SPME	SD	Intra-day	Inter-day
1	3.020	Acetol	74	43(100)74(9)45(3)44(2)	924	1.06	nd	4.3	4.5
2	3.953	*tert*-Butylcarbinol	88	57(100)42(11)43(10)88(10)	931	0.16	nd	5.6	6.3
3	4.227	Butane-2,3-diol	90	45(100)94(28)43(17)90(1)	988	0.09	nd	6.3	5.8
4	4.307	3-Furancarboxaldehyde	96	95(100)96(94)67(9)42(5)	921	1.25	nd	4.2	4.5
5	4.928	Acetdimethylamide	87	44(100)87(97)43(42)42(32)	960	nd	1.06	nc	nc
6	5.580	2-Methyl-2-cyclopemtenone	96	67(100)96(90)53(42)41(13)	934	0.16	nd	6.0	5.8
7	5.654	2-Furyl methyl ketone	110	95(100)110(41)43(11)67(5)	990	0.13	nd	6.5	8.0
8	5.947	*(E)*-2-Hexene	84	55(100)42(83)84(49)41(38)	892	0.25	nd	6.5	6.6
9	6.567	5-Methyl-2-furaldehyde	110	110(100)109(97)53(66)51(17)	910	1.04	nd	4.5	3.5
10	6.861	Phenol	94	94(100)66(34)65(28)55(8)	925	1.50	nd	4.3	4.4
11	6.994	α-Methyl-ç-crotonolactone	98	69(100)41(98)98(88)42(22)	830	0.12	nd	6.2	6.7
12	7.774	Corylon	112	112(100)55(52)69(44)83(28)	982	0.55	0.35	5.6	4.9

13	7.901	2,3-Demethyl-2-cyclopenten-1-one	110	67(100)110(78)95(24)54(16)	870	0.23	nd	6.2	6.1
14	8.095	o-cresol	108	108(100)107(96)79(48)77(37)	882	0.70	nd	5.2	5.4
15	8.222	3,7,7-Trimethyl-bicyclo[4.1.0]hept-3-ene	136	93(100)91(56)77(42)136(31)	839	nd	0.06	nc	nc
16	8.435	*p*-Cresol	108	107(100)108(80)79(31)77(28)	902	1.99	0.07	5.1	3.9
17	8.715	*o*-Methoxy-phenol	124	109(100)124(84)81(66)53(20)	940	0.30	nd	6.5	6.4
18	8.869	*α*-Linalool	154	71(100)93(81)43(58)55(55)	932	nd	0.03	nc	nc
19	9.248	3-Methyl-2-hydroxy-2-crclopenten-1-one	126	126(100)55(60)83(48)43(45)	923	0.26	nd	6.3	6.2
20	9.508	Benzyl cyanide	117	117(100)90(50)116(39)89(29)	901	0.15	nd	6.9	7.6
21	9.615	2,5-Xylenol	122	107(100)122(99)121(53)43(24)	945	0.62	nd	5.9	5.6
22	9.895	*p*-Ethyl-phenol	122	107(100)122(34)77(18)108(7)	942	0.29	0.10	6.1	6.4
23	9.922	2,3-Xylenol	122	107(100)122(77)77(28)60(15)	924	0.38	0.03	5.8	5.4
24	10.082	2,6-Xylenol	122	107(100)122(85)77(29)121(24)	934	0.11	nd	7.0	7.7
25	10.143	Citral	152	69(100)84(71)41(27)152(7)	940	nd	0.08	nc	nc

26	10.262	*3*-Methyl-acetophenone	134	119(100)91(75)43(41)134(37)	908	0.29	0.08	6.3	6.8
27	10.682	Loumaran	120	120(100)91(44)119(16)65(16)	902	0.67	nd	5.8	6.3
28	10.815	5-(Hydroxymethyl)-2-furaldehyde	126	97(100)126(65)41(58)69(40)	941	0.20	nd	6.5	6.9
29	11.377	3,4,5-Trimethyl-bicyclo[4.3.0]non-3-ene	164	95(100)107(87)122(69)164(27)	869	nd	0.08	nc	nc
30	11.642	*p*-Ethylguaiacol	152	137(100)152(54)81(10)109(9)	876	0.10	nd	7.2	7.7
31	11.702	1-Indanone	132	132(100)104(91)103(49)78(36)	820	0.34	nd	6.3	6.1
32	11.856	3-Ethyl-benzenamine	117	117(100)90(37)89(31)63(11)	911	0.59	nd	5.5	5.3
33	12.143	4-Ethenyl-2-methxoy-phenol	150	150(100)135(88)77(44)107(39)	903	0.63	nd	6.1	6.5
34	12.704	α-Cubebene	204	105(100)119(99)161(96)204(26)	923	nd	0.17	nc	nc
35	13.103	Copaene	204	119(100)161(95)105(91)204(19)	914	0.21	0.93	6.7	6.8
36	13.156	3-Methyl-indole	131	130(100)131(52)77(13)65(9)	904	0.38	0.20	6.6	6.3
37	14.090	Cuaia-5,11-diene	204	91(100)105(87)93(82)204(32)	923	0.92	3.92	5.0	4.0
38	14.138	Caryophyllene-(l1)	204	105(100)133(98)163(63)204(21)	899	nd	0.88	nc	nc

39	14.550	Naphthalene	204	105(100)57(99)189(98)71(74)	920	1.34	3.70	5.3	5.9
40	14.657	Eremophila-1(10),11-diene	204	161(100)107(66)93(64)204(47)	908	4.30	15.83	4.8	4.5
41	14.923	4,5-Dehydro-isolongifolene	202	119(100)161(50)105(42)202(28)	912	1.51	3.99	5.8	5.4
42	14.990	α-Panasinsen	204	161(100)122(82)107(57)204(22)	933	1.20	3.75	5.5	5.5
43	15.264	Wieland-Michler ketone	178	135(100)157(88)178(82)79(80)	897	1.04	1.21	6.0	6.6
44	15.577	Bergamotol	220	107(100)43(74)79(58)220(6)	840	0.36	0.94	6.3	6.5
45	16.051	Globulol	222	109(100)95(90)81(87)222(8)	877	0.86	1.22	6.3	6.9
46	16.117	2,5,9-Trimethylcycloundeca-4,8-dienone	206	109(100)138(87)96(87)67(85)	902	1.96	3.36	5.2	5.5
47	16.324	Ledene oxide-(l)	220	121(100)125(89)143(84)220(9)	924	1.13	1.80	4.8	4.9
48	16.664	Eudesma-4(14),11-diene	222	121(100)100(46)125(43)55(35)	923	5.24	5.49	5.0	5.5
49	16.857	Spathulenol	220	159(100)79(92)177(88)93(86)	950	3.93	3.93	4.6	4.7
50	17.118	9-Hydroxy-isolongifolene	220	177(100)121(83)107(63)220(58)	924	5.81	3.60	4.3	4.9
51	17.178	Cadina-1,3,5-triene	202	159(100)131(53)160(49)202(3	930	2.17	2.20	4.9	4.9

				5)					
52	17.284	Cedrene epoxide	220	177(100)121(82)220(62)93(58)	910	3.06	3.05	4.5	5.8
53	17.444	Bisabolene epoxide	220	43(100)111(47)55(31)220(1)	923	2.42	2.02	5.2	5.7
54	17.591	3-oxo-ionone	206	164(100)43(76)121(51)206(47)	890	3.25	1.67	5.0	5.7
55	17.711	Longiverbenone	218	175(100)91(73)218(68)93(60)	842	3.25	2.50	5.3	5.0
56	18.305	Myristic acid	228	43(100)102(79)60(75)228(58)	958	8.87	nd	4.6	4.9
57	18.358	Eremophila-1(10),11-dien-2-one	218	79(100)147(96)91(88)218(25)	902	26.97	28.62	4.3	4.9
58	18.471	1,1,5,5-Tetramethyl-7H-2,4a-methanonaphthalen-7-one	218	175(100)176(96)95(56)218(24)	835	2.62	1.99	5.2	5.8
59	18.898	Illudol	222	55(100)43(94)102(92)222(2)	914	1.85	1.09	5.5	5.9
60	19.665	Hexadecanoic acid	256	73(100)60(89)43(75)256(32)	948	1.19	nd	5.8	5.6

* nd = not detected, nc = no calculated.