Analysis of the influence of sulfur-fumigation on the volatile components of *Angelicae sinensis* Radix by comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry

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ABSTRACT

Background: Sulfur-fumigation of *Angelicae sinensis* Radix causes changes in the structure and composition of volatile components. These changes alter the curative effect and the quality of *A. sinensis* Radix. **Materials and Methods:** In this study, comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC × GC-TOFMS) was employed to investigate the influence of sulfur-fumigation on the volatile components, and to characterize and quantify the chemical composition of the volatile oil of *A. sinensis* Radix. **Results:** The present study has shown that sulfur-fumigated *A. sinensis* Radix samples had significant loss of the main active compounds and a more destructive fingerprint profile compared to non-fumigated samples. **Conclusion:** From this study, it can be concluded that the combination of GC × GC and TOFMS has potential as a quality monitoring tool in herbal medicine and food processing industries.

Key words: *Angelicae sinensis* Radix, comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry, quality control, sulfur-fumigation, volatile compounds

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INTRODUCTION

Angelicae sinensis Radix (Danggui in Chinese) is derived from the root of *A. sinensis* (Oliv.) Diels (reported in Chinese Pharmacopoeia, Edition 2010), which is one of the oldest and most frequently used Chinese herbs in oriental medicine. *A. sinensis* Radix has been traditionally used for tonifying the blood and for treatment of anemia, rheumatism, female menstrual disorders and amenorrhea.[1] Meanwhile, *A. sinensis* Radix has been used as a common health food supplement for women's care for 1000's of years in China.[2,3] Pharmacological studies and clinical practices have demonstrated that *A. sinensis* Radix possesses various bioactivities, including antibacterial, anti-amnestic, and antihypertensive effects,[4,5] inhibitory effect on acetylcholinesterase,[6] reduced cardiac contraction,[7] activation of protein kinase C,[8] and antitumor activity.[9] Regarding the chemical constituents of *A. sinensis* Radix, more than 70 compounds, including essential oils, phthalide dimers, organic acids and their esters, vitamins and amino acids, have been identified so far, and various biological activities of the compounds have been reported.[10]

For centuries, post-harvest processing of the root of *A. sinensis* (Oliv.) Diels has occurred naturally, i.e. sun-dried. However, in recent years, *A. sinensis* Radix has been reported to be sulfur-fumigated by herbal farmers or wholesalers during post-harvest handling and storage for the purpose of torrefaction, sterilization, mildew proof, insect prevention, and bleaching.[11] The sulfur dioxide extracted from sulfur heating acts as a strong reducing agent, which reacts with the components of the ketonic group and hydroxyl radical in *A. sinensis* Radix. As a result, this has an extremely negative effect on the character and taste of *A. sinensis* Radix, weakening its quality and curative effect.

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In recent years, gas chromatography (GC) and GC-mass spectrometry (GC-MS)[12] have been used for evaluating the quality of *A. sinensis* Radix and its processed products. Essential oils constitute the main active pharmacological components of *A. sinensis* Radix and most previous studies have been focused on its volatile components. However, to the best of our knowledge, there has been no report on the influence of sulfur-fumigation on the volatile components of *A. sinensis* Radix.

GC-MS is a powerful method that can be used to analyze volatile components of *A. sinensis* Radix. However, it is difficult for GC-MS to distinguish enantiomers unless a chiral separation is used. Typically, this results in poor component identification in the MS library as well as difficulty in obtaining accurate qualitative and quantitative results. These problems can however, be overcome using multi-dimensional GC. When compared to conventional GC, comprehensive GC × GC is a hyphenated technique that greatly improves the result of volatile component separation and identification with low concentrations in a shorter analytical period. The addition of time-of-flight mass spectrometry (TOFMS) provides a sensitive detector with full-scan MS capability and a high data density in the second dimension separation space. The combination of GC × GC-TOFMS has previously been shown to be very useful for many complex samples.[13-15]

The aim of the current study was to investigate the influence of sulfur-fumigation on the volatile components of *A. sinensis* Radix. A comprehensive two-dimensional gas chromatograph coupled to a time-of-flight mass spectrometer was employed to identify all individual components in complex *A. sinensis* Radix essential oils. The difference between main volatile components in sulfur-fumigated and non-fumigated *A. sinensis* Radix samples was then compared. Using the GC × GC-TOFMS method, we accurately and efficiently differentiated sulfur-fumigated and non-fumigated *A. sinensis* Radix from commercial samples and evaluated the quality of various *A. sinensis* Radix sources.

MATERIALS AND METHODS

Samples and sample preparation

Non-fumigated *A. sinensis* Radix samples were collected from Gansu province, China, and inspected by an expert in the field. Sulfur-fumigated samples were created from a subset of non-fumigated samples, following procedures similar to those employed by farmers and wholesalers: 1000 g of the non-fumigated *A. sinensis* Radix were wetted with 100 mL water then put to stand for 5 h, 100 g of sulfur powder was heated until burnt, the burning sulfur and the wetted non-fumigated *A. sinensis* Radix were carefully placed into the lower and upper layers of a desiccator, respectively. The desiccator was then kept closed for 24 h. After fumigation, the prepared *A. sinensis* Radix was dried in a ventilated drying oven at 40°C for 24 h.

The volatile oils in 200 g of non-fumigated and sulfur-fumigated *A. sinensis* Radix were extracted with 2000 mL of water by using the steam distillation method described in the Chinese Pharmacopoeia (Edition 2010) for 4 h. Extraction yields of volatile oils for non-fumigated and sulfur-fumigated *A. sinensis* Radix were above 0.5% and 0.35%, respectively. The volatile oils obtained were dried over anhydrous sodium sulfate (Sigma Corp., St. Louis, MO, USA) and stored in dark glass bottles at 4°C for analyses.

Instrumentation, column system and conditions

The GC × GC-TOFMS analyses were performed using a Laboratory Equipment Corporaton (LECO) Pegasus 4D instrument (LECO Corp., St. Joseph, MI, USA), coupled to Agilent 6890 N gas chromatograph with split-splitless injector, 7683 B Series auto-sampler and time of flight mass spectrometer LECO Pegasus III. Major parameters were set at: Electron impact ionization 70 eV, acquisition rate 50 spectra/s, ion-source temperature 220°C, and transfer interface temperature 250°C. A column set with a non-polar stationary phase primary column and a medium-polar stationary phase secondary column was used. The first dimension chromatographic column was 30 m × 0.25 mm, 0.25 μm film thickness DB-5 ms (5% phenyl-substituted methyl polysiloxane, J and W Scientific, Folsom, CA, USA). The second dimension chromatographic column was 2 m × 0.1 mm, 0.1 μm film thickness DB-17 ht (14% cyanopropylphenylmethylpolysiloxane, J and W Scientific, Folsom, CA, USA). Helium was used as a carrier gas at a constant flow rate of 1 mL/min. The two columns were connected with a press-fit connector and individually installed in two separate ovens. Column 1's oven was heated to 50°C for 1 min, then increased at a rate of 15°C/min to 160°C and held for 10 min. The temperature was then further increased at a rate of 3°C/min to 260°C and held for 5 min. Column 2's oven was heated to 55°C for 1 min, then increased at a rate of 15°C/min to 160°C and held for 10 min. The temperature was then further increased at a rate of 3°C/min to 265°C and held for 5 min. The modulation period was set at 6.0 s. The data-acquisition rate was 100 Hz (scans/s) for the mass range of 45-550 amu. The detector voltage was −1850 V. The injection volume of sample solution was 1 μL at a split ratio of 200:1 in a 250°C inlet onto column 1.

Data processing

Data were processed with LECO Pegasus 4D software; including peak finding, mass spectrum deconvolution, and MS component identification using the NIST 08, Adams and Wiley 6 database libraries. Results of the analyses

were located in the peak table. All statistical analyzes were conducted with John's Mackintosh Program (JMP) version 7.0.1 (SAS Institute Inc., Cary, NC, USA). Figures and tables were generated with Microsoft Office Excel 2010 (Microsoft Corporation, Redmond, WA, USA).

RESULTS AND DISCUSSION

Qualitative analysis of *A. sinensis* Radix volatile oil

The non-fumigated and sulfur-fumigated *A. sinensis* Radix samples were analyzed using the optimized GC × GC-TOFMS method. The GC × GC-TOFMS contour plots of volatile oil in non-fumigated and sulfur-fumigated *A. sinensis* Radix under different column systems are depicted in Figure 1. With non-fumigated *A. sinensis* Radix as a reference, a total of 209 compounds with match quality >80% in both non-fumigated and sulfur-fumigated *A. sinensis* Radix samples were identified by TOFMS and quantified by flame ionization detection including hydrocarbons, ketones, aldehydes, esters, alcohols, acids, and other components. The major compounds identified in *A. sinensis* Radix volatile oil by GC × GC-TOFMS along with the first and the second dimension retention times, formula, similarity(S), and areas are presented in Table 1. It should be noted that the peak identification of components was based on mass spectra obtained from NIST 08 and Wiley 6 library databases. Identification based on a mass spectral library search using S was above 800. Compounds having lower search probabilities than these were classified as unknowns and disqualified for Kovats index comparison.

Differentiation of sulfur-fumigated *A. sinensis* Radix using volatile profiling

The established method has been successfully applied to analyze the influence of sulfur-fumigation on the volatile components of *A. sinensis* Radix. With non-fumigated *A. sinensis* Radix samples used as a reference, the major portions of volatile groups in sulfur-fumigated *A. sinensis* Radix samples were found to be significantly different, probably due to changes in medicinal properties resulting from the sulfur-fumigation process. In addition, the amount of H_2SO_3 in *A. sinensis* Radix was increased during sulfur-fumigation, and the *A. sinensis* Radix appeared whitened and accompanied by an acidic taste, resulting from a lower *pH* value. Moreover, sulfur dioxide further reacted with components in medicine and directly reduced the contents of volatile compounds. 36 volatile compounds were not found in *A. sinensis* Radix after sulfur-fumigation as shown in Table 2. Meanwhile, the majority of low-boiling fractions and esters were lower in sulfur-fumigated samples than in non-fumigated samples, as shown in Table 1.

Identification of main co-eluting peaks in *A. Sinensis* Radix by GC × GC-TOFMS

The essential oil in herbal medicine and food are very complex and it should be emphasized that the analyses of complex essential oil samples by one-dimensional GC may fail or be unsatisfactory. In particular, when considering the well-known limitation of one-dimensional GC and GC-MS techniques as being inherently unable to separate and identify the multitude of compounds present in low concentrations and co-eluting. The analysis of essential oil should provide not only sufficient separation, but also accurate qualitative information of all individual components. Therefore, GC × GC-TOFMS with high resolving power (peak capacity) and high sensitivity has been applied to the analysis of complex co-eluting peak clusters in essential oil.

In order to further explain automatic peak search

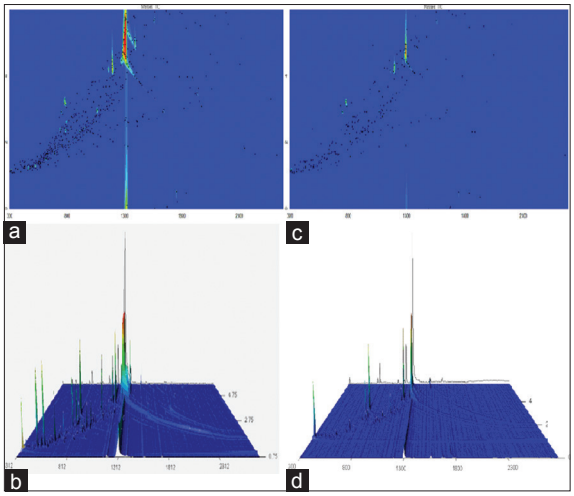

Figure 1: Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry and three-dimensional chromatograms of non-fumigated (a/b) and sulfur-fumigated (c/d) *Angelicae sinensis* Radix volatile oils

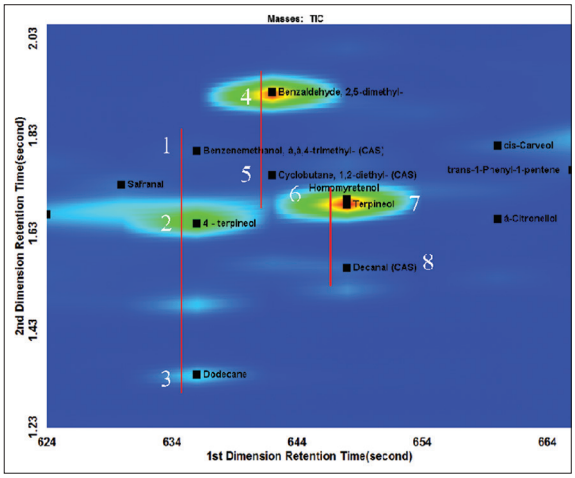

Figure 2: Typical contour plot of main co-eluting peaks in comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry chromatogram of non-fumigated *Angelicae sinensis* Radix sample from 624s to 654s

Table 1: Major volatile components identified in both non-fumigated and sulfur-fumigated *Angelicae sinensis* Radix samples

Name	R.T. (s)	Formula	Similarity	Non-fumigated (%)	Sulfur-fumigated (%)
(R)-1-Hexen-3-ol	312, 1.150	$C_6H_{12}O$	974	100	467.5
Acetic acid, butyl ester	372, 1.210	$C_6H_{12}O_2$	962	100	147.14
2-Furancarboxaldehyde	390, 1.430	$C_5H_4O_2$	964	100	182.52
2-Hexenal	402, 1.300	$C_6H_{10}O$	924	100	5.68
p-Xylene	420, 1.240	C_8H_{10}	948	100	8.45
2-Heptanone	426, 1.270	$C_7H_{14}O$	934	100	3.55
Heptanal	432, 1.280	$C_7H_{14}O$	900	100	5.36
α-Pinene, (-)-	462, 1.240	$C_{10}H_{16}$	960	100	3.61
Camphene	474, 1.210	$C_{10}H_{16}$	954	100	7.68
β-Myrcene	492, 1.240	$C_{10}H_{16}$	909	100	3.61
Furan, 2-pentyl-	498, 1.270	$C_9H_{14}O$	914	100	2.18
Octanal	504, 1.290	$C_8H_{16}O$	957	100	4.67
Benzene, 1,2,3-trimethyl-	504, 1.330	C_9H_{12}	917	100	20.24
1,3,6-Octatriene, 3,7-dimethyl-, (E)-	522, 1.310	$C_{10}H_{16}$	973	100	2.71
(R, E)-1-Phenylnon-2-en-1-ol	522, 1.420	$C_{15}H_{22}O$	911	100	12.91
Neo-allo-ocimene	528, 1.460	$C_{10}H_{16}$	891	100	1.2
Benzeneacetaldehyde	540, 1.590	C_8H_8O	805	100	19.63
Undecane	564, 1.240	$C_{11}H_{24}$	943	100	5.47
5-Undecene, (E)-	564, 1.260	$C_{11}H_{22}$	907	100	3.91
2-Nonanone	564, 1.390	$C_9H_{18}O$	877	100	5.97
Ethanone, 1-(3,4-dimethylphenyl)-	570, 1.530	$C_{10}H_{12}O$	904	100	0.73
Camphenone, 6-	576, 1.590	$C_{10}H_{14}O$	907	100	4.03
Furan, 2-methyl-	576, 1.600	C_5H_6O	827	100	29.75
α-Campholene aldehyde	594, 1.570	$C_{10}H_{16}O$	877	100	15.81
Isophorone	594, 1.670	$C_9H_{14}O$	914	100	7
(3E,5Z)-1,3,5-Undecatriene	600, 1.420	$C_{11}H_{18}$	826	100	28.08
Benzene, pentyl-	618, 1.570	$C_{11}H_{16}$	942	100	38.41
Safranal	630, 1.730	$C_{10}H_{14}O$	869	100	5.38
Dodecane	636, 1.340	$C_{12}H_{26}$	937	100	6.47
4-Terpineol	636, 1.650	$C_{10}H_{18}O$	900	100	17.72
Benzenemethanol, a, a, 4-trimethyl-	636, 1.800	$C_{10}H_{14}O$	850	100	31.71
Benzaldehyde, 2,5-dimethyl-	642, 1.920	$C_9H_{10}O$	918	100	6.36
Decanal	648, 1.560	$C_{10}H_{20}O$	918	100	19.11
Naphthalene	648, 2.010	$C_{10}H_8$	954	100	6.25
cis-Carveol	660, 1.810	$C_{10}H_{16}O$	932	100	14.65
D-Carvone	684, 1.980	$C_{10}H_{14}O$	835	100	37.17
2-Decenal, (Z)-	690, 1.720	$C_{10}H_{18}O$	929	100	12.24
1-Butanone, 1-phenyl-	690, 2.060	$C_{10}H_2O$	903	100	16.74
6-Undecanone	696, 1.670	$C_{11}H_{22}O$	914	100	12.21
3,5-Dimethoxytoluene	696, 2.120	$C_9H_{12}O_2$	861	100	13.4
6-Undecanol	708, 1.630	$C_{11}H_{24}O$	898	100	16.84
Phenol, 4-ethyl-2-methoxy-	708, 2.130	$C_9H_{12}O_2$	802	100	17.92
2-Undecanone	714, 1.730	$C_{11}H_{22}O$	836	100	13.32
(E)-Solanone	714, 1.790	$C_{13}H_{22}O$	826	100	12.08
Tridecane	720, 1.460	$C_{13}H_{28}$	945	100	6.35
Undecanol-3	720, 1.650	$C_{11}H_{24}O$	803	100	19.72
(-)-trans-Pinocarvyl acetate	726, 1.990	$C_{12}H_{18}O_2$	829	100	7.68
1,3-Benzodioxole, 5-(2-propenyl)-	726, 2.210	$C_{10}H_{10}O_2$	918	100	15.11
Indole	732, 2.770	C_8H_7N	933	100	1.64
2-Methoxy-4-vinylphenol	738, 2.410	$C_9H_{10}O_2$	938	100	12.34
2,4-Decadienal	744, 1.950	$C_{10}H_{16}O$	908	100	17.1

Contd...

Table 1: Contd

Name	R.T. (s)	Formula	Similarity	Non-fumigated (%)	Sulfur-fumigated (%)
Naphthalene, 2-methyl-	744, 2.320	$C_{11}H_{10}$	905	100	11.13
Benzaldehyde, 2,4,6-trimethyl-	750, 2.400	$C_{10}H_{12}O$	872	100	22.76
2,4,6-Trimethyl-1,3,6-heptatriene	756, 1.950	$C_{10}H_{16}$	821	100	10.55
Naphthalene, 2-methyl-	756, 2.470	$C_{11}H_{10}$	895	100	13.84
n-Decanoic acid	774, 1.880	$C_{10}H_{20}O_2$	869	100	26.73
1-Phenyl-1-propanol-(1)	774, 2.270	$C_9H_{12}O$	908	100	15.86
1-Pentanone, 1-phenyl-	786, 2.370	$C_{11}H_{14}O$	944	100	14.67
2 (3H)-Furanone, dihydro-5-pentyl-	786, 2.550	$C_9H_{16}O_2$	924	100	5.61
Benzeneacetic acid, a-oxo-, methyl ester	792, 2.490	$C_9H_8O_3$	929	100	10
Benzaldehyde, 2,4,5-trimethyl-	798, 2.620	$C_{10}H_{12}O$	876	100	30.88
5-Tetradecene, (E)-	804, 1.650	$C_{14}H_{28}$	928	100	4.18
(-)-Isoledene	810, 1.930	$C_{15}H_{24}$	883	100	4.92
Tetradecane	816, 1.590	$C_{14}H_{30}$	958	100	8.43
6-Dodecen-1-al	816, 2.040	$C_{12}H_{22}O$	848	100	58.82
Benzene, 1,2-dimethoxy-4-(2-propenyl)-	816, 2.660	$C_{11}H_{14}O_2$	937	100	20.39
Phenol, 4-pentyl-	834, 2.480	$C_{11}H_{16}O$	878	100	35.92
Ethyl dl-mandelate	840, 2.780	$C_{10}H_{12}O_3$	865	100	20.1
3-Methyl-2-butenoic acid, 3-tridecyl ester	858, 1.990	$C_{18}H_{34}O_2$	822	100	23.53
(+)-β-Funebrene	864, 2.120	$C_{15}H_{24}$	887	100	6.59
Naphthalene, 1,3-dimethyl-	864, 2.800	$C_{12}H_{12}$	826	100	32.36
Heptadecane, 2,6,10,14-tetramethyl-	882, 1.650	$C_{21}H_{44}$	874	100	48.75
Aromadendrene	888, 2.150	$C_{15}H_{24}$	899	100	38.77
Widdrene	888, 2.230	$C_{15}H_{24}$	870	100	8
2'-Hydroxyvalerophenone	888, 2.670	$C_{11}H_{14}O_2$	804	100	45.81
Phenol, 2-methoxy-4-propyl-	888, 3.480	$C_{11}H_{14}O_2$	905	100	35.12
Oxirane, tetradecyl-	900, 2.080	$C_{16}H_{32}O$	836	100	39.77
2 (3H)-Furanone, 5-hexyldihydro-	900, 2.890	$C_{10}H_{18}O_2$	953	100	12.53
E-2-Hexadecacen-1-ol	906, 1.810	$C_{16}H_{32}O$	907	100	14.79
Pentadecane	924, 1.740	$C_{15}H_{32}$	947	100	17.18
2-Pentadecanol	930, 2.060	$C_{15}H_{32}O$	896	100	35.36
(E, E)-α-Farnesene	936, 2.200	$C_{15}H_{24}$	810	100	14.22
β-Bisabolene	948, 2.230	$C_{15}H_{24}$	906	100	29.04
Bicyclogermacrene	954, 2.450	$C_{15}H_{24}$	892	100	0.4
Aromadendrene, dehydro-	960, 2.550	$C_{15}H_{22}$	808	100	5.23
β-Himachalene	966, 2.450	$C_{15}H_{24}$	826	100	10.24
β-Sesquiphellandrene	972, 2.320	$C_{15}H_{24}$	875	100	19.18
δ-Cadinene	972, 2.470	$C_{15}H_{24}$	856	100	29.07
Benzoic acid, 2-propenyl ester	978, 2.770	$C_{10}H_{10}O_2$	812	100	15.93
Dibenzofuran	990, 3.480	$C_{12}H_8O$	908	100	30.5
d-Nerolidol	1014, 2.430	$C_{15}H_{26}O$	914	100	35.57
7-Hexadecene, (Z)-	1032, 1.960	$C_{16}H_{32}$	909	100	30.69
Isospathulenol	1044, 2.800	$C_{15}H_{24}O$	829	100	29.3
Palustrol	1056, 2.650	$C_{15}H_{26}O$	877	100	49.54
(+) Spathulenol	1056, 2.940	$C_{15}H_{24}O$	925	100	600.15
Hexadecane	1062, 1.880	$C_{16}H_{34}$	942	100	19.04
(-)-Spathulenol	1062, 3.000	$C_{15}H_{24}O$	945	100	22.26
Globulol	1074, 2.810	$C_{15}H_{26}O$	897	100	37.82
9H-Fluorene	1080, 3.870	$C_{13}H_{10}$	889	100	31.35
1,3-Isobenzofurandione	1092, 2.190	$C_8H_4O_3$	822	100	21.31
(+) Spathulenol	1104, 3.120	$C_{15}H_{24}O$	878	100	59.01
Cedrol	1116, 3.010	$C_{15}H_{26}O$	867	100	6.51
Isospathulenol (isomer)	1134, 3.210	$C_{15}H_{24}O$	909	100	20.16

Contd...

Table 1: Contd

Name	R.T. (s)	Formula	Similarity	Non-fumigated (%)	Sulfur-fumigated (%)
1-(But-3-enyl) indan-1-ol	1164, 4.250	$C_{13}H_{16}O$	824	100	18.18
8-Heptadecene	1176, 2.100	$C_{17}H_{34}$	915	100	34.8
n-Tridecan-1-ol	1176, 2.460	$C_{13}H_{28}O$	926	100	45.63
Elemol	1182, 3.190	$C_{15}H_{26}O$	840	100	28.91
Ledene oxide-(II)	1194, 3.290	$C_{15}H_{24}O$	832	100	21.61
1 (3H)-Isobenzofuranone, 3-Butylidene-	1194, 4.440	$C_{12}H_{12}O_2$	870	100	39.68
Ledene oxide-(II)	1206, 3.280	$C_{15}H_{24}O$	851	100	17.14
2-Pentadecanol	1218, 2.400	$C_{15}H_{32}O$	890	100	45.03
n-Tridecan-1-ol	1338, 2.610	$C_{13}H_{28}O$	922	100	42.48
3-n-Butylphthalide	1392, 5.050	$C_{12}H_{14}O_2$	885	100	8.81
Pentadecanoic acid, methyl ester	1410, 2.640	$C_{16}H_{32}O_2$	850	100	8.78
Pentadecanoic acid	1464, 2.850	$C_{15}H_{30}O_2$	922	100	3.97
11-Hexadecen-1-ol, (Z)-	1476, 2.880	$C_{16}H_{32}O$	918	100	36.09
Hexadecanoic acid, methyl ester	1584, 2.730	$C_{17}H_{34}O_2$	909	100	12.56
n-Hexadecanoic acid	1638, 3.010	$C_{16}H_{32}O_2$	920	100	26
Dibutyl phthalate	1638, 4.530	$C_{16}H_{22}O_4$	951	100	34.21
cis-7-Tetradecen-1-ol	1644, 2.990	$C_{14}H_{28}O$	918	100	30.64
Falcarinol	1776, 3.990	$C_{17}H_{24}O$	860	100	37.89
11-Hexadecen-1-ol, (Z)-	1818, 3.040	$C_{16}H_{32}O$	939	100	33.02
9,12-Octadecadienoic acid, methyl ester	1872, 3.200	$C_{19}H_{34}O_2$	928	100	8.56
9,12-Octadecadienoic acid (Z, Z)-	1932, 3.510	$C_{18}H_{32}O_2$	933	100	0.16
9,12,15-Octadecatrienoic acid, (Z, Z, Z)-	1944, 3.640	$C_{18}H_{30}O_2$	886	100	1.55
trans-13-Octadecenoic acid	1950, 3.250	$C_{18}H_{34}O_2$	841	100	2.01
9,12-Octadecadienoic acid (Z, Z)-	2058, 3.620	$C_{18}H_{32}O_2$	865	100	6.38
1,3-Cyclohexadiene, 1,5,5,6-tetramethyl-	2106, 4.410	$C_{10}H_{16}$	860	100	25.71
9-Octadecenamide, (Z)-	2304, 4.360	$C_{18}H_{35}NO$	856	100	6.3

and deconvolution of spectrograms in the software information processing of compounds with common outflow characteristics, sections of the identified chemical groups of *A. sinensis* Radix samples were included to elucidate the principle of relative position in the 2D chromatogram as shown in Figure 2. Eight compounds on that position extend along the direction of one dimension, and their qualitative results were finally yielded after the further separation of the second dimension column. This involved an automatic peak search and deconvolution of the corresponding spectrogram during the software image processing. According to these results, it can be seen that each compound was well identified with a high peak match. Furthermore, the eight components were separated independently without any influence from co-eluting peaks, the fingerprint information of light fraction such as propane remained intact, and the high quality spectrogram was given after the software deconvolution. As seen from the mass-spectrogram, Caliper was the unprocessed spectrogram, Peak True was the processed spectrogram (after software deconvolution), and Library Hit was the standard spectrogram. The structures and mass spectra of the eight compounds are shown in Figure 3a and b.

CONCLUSION

In the present study, GC × GC-TOFMS has been shown to be a powerful and effective method with high sensitivity and specificity in identifying individual volatile components. By employing this technique we were able to quantify all the individual components in the volatile oils of non-fumigated and sulfur-fumigated *A. sinensis* Radix for the first time. In addition, the established methodology was successfully applied to the rapid identification of sulfur-fumigated *A. sinensis* Radix in commercial samples, and also revealed the chemical changes of volatile components in *A. sinensis Radix* following sulfur-fumigation. We conclude that the GC × GC method is able to separate compounds in herbal medicine and food that heavily co-elute on a standard gas chromatograph system. Separation of analyses by volatility and polarity enables traditionally unresolved complex mixtures to be examined in greater detail and vastly increases the number of identified compounds. Therefore, the presently developed methodology could be used as a powerful and versatile tool for quality control and process monitoring tool for herbal medicine and food processing industries. Further, research involving biological activities of volatile components in *A. sinensis* Radix is, however, needed to fully explore its potential for practical application.

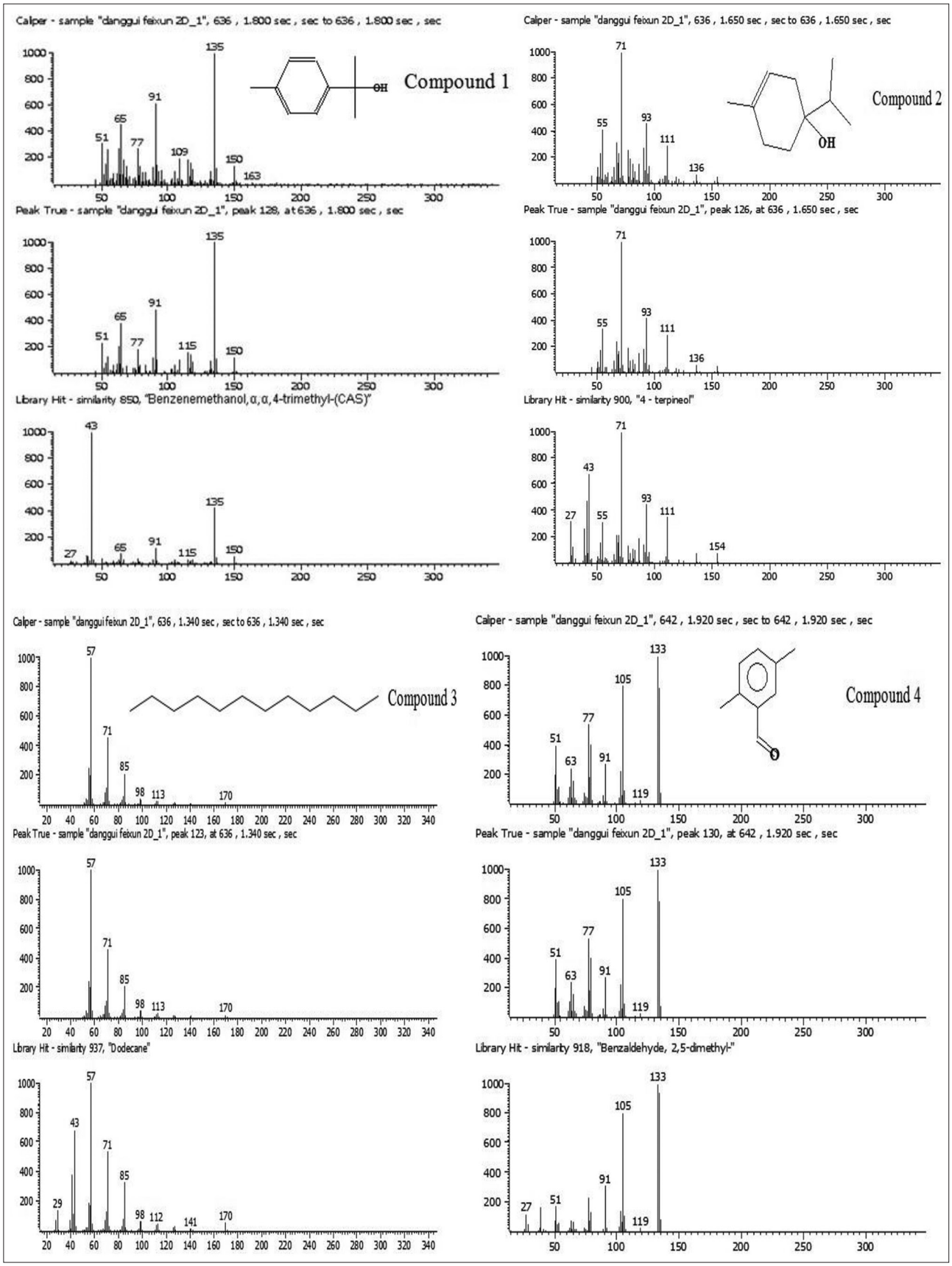

Figure 3a: The structures and mass spectra of the eight co-eluting peaks of *Angelicae sinensis* Radix volatile oil. Compound 1: Benzenemethanol, α,α,4-trimethyl-; Compound 2: 4-terpineol; Compound 3: Dodecane; Compound 4: Benzaldehyde,2,5-dimethyl-

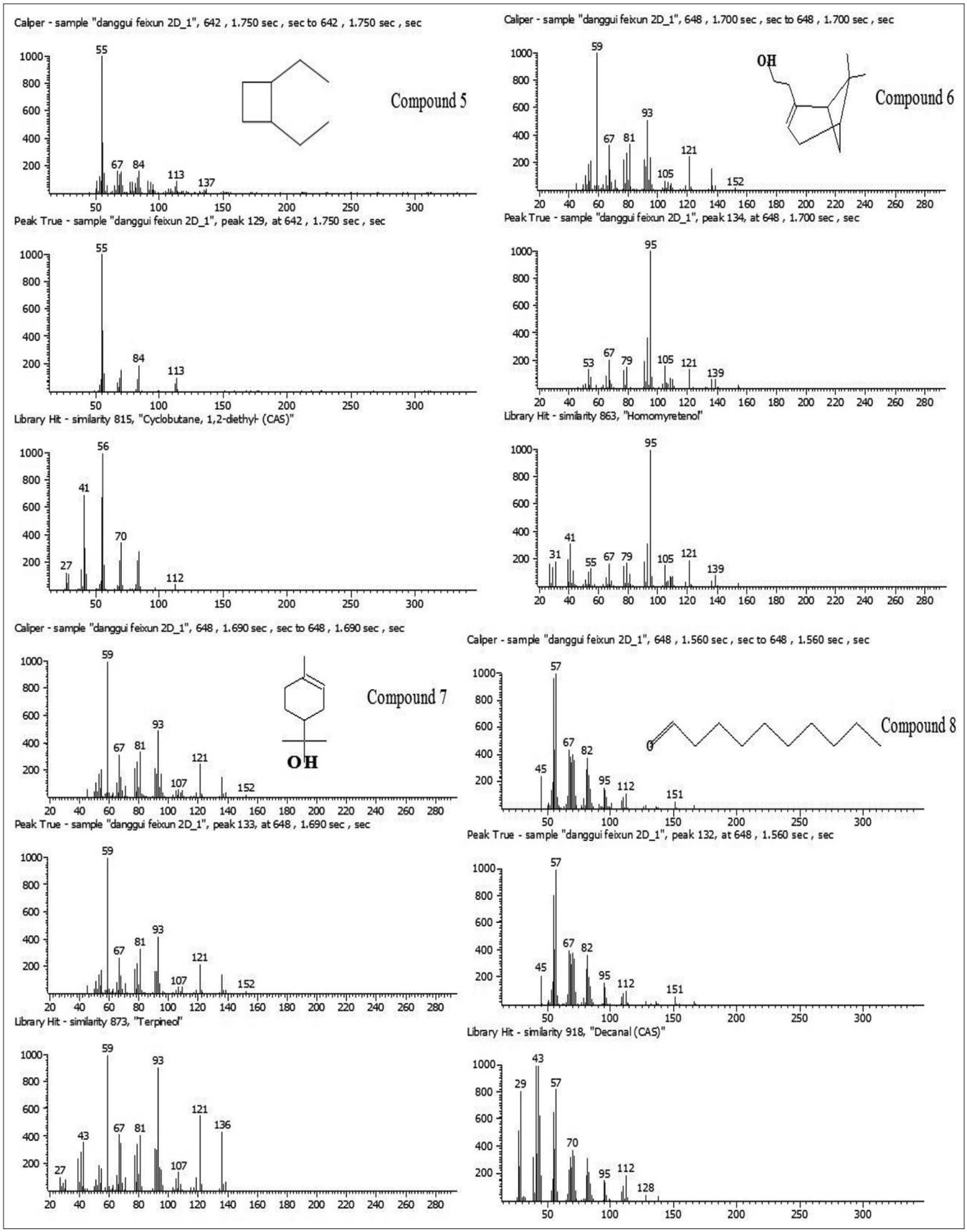

Figure 3b: The structures and mass spectra of the eight co-eluting peaks of *Angelicae sinensis* Radix volatile oil. Compound 5: Cyclobutane, 1,2-diethyl-; Compound 6: Homomyretenol; Compound 7: Terpineol; Compound 8: Decanal

Table 2: Using non-fumigated sample as a reference, 36 volatile compounds were not found in *Angelicae sinensis* Radix after sulfur-fumigation

Name	R.T. (s)	Formula	Similarity	Area
1-Hexanol	408, 1.260	$C_6H_{14}O$	821	30661
3-Hexene, 2,2-dimethyl-, (E)-	456, 1.320	C_8H_{16}	862	4468.4
2-Heptenal, (Z)-	474, 1.310	$C_7H_{12}O$	878	28737
Bicyclo [3.1.0] hex-2-ene, 4-methylene-1-(1-methylethyl)-	480, 1.220	$C_{10}H_{14}$	808	18197
Octane, 3,5-dimethyl-	498, 1.170	$C_{10}H_{22}$	834	12617
3-Octen-2-one, (E)-	528, 1.370	$C_8H_{14}O$	817	9485.4
3-Cyclohexen-1-one, 3,5,5-trimethyl-	534, 1.420	$C_9H_{14}O$	868	11076
2-Octenal	540, 1.390	$C_8H_{14}O$	927	53724
3-Oxatricyclo (4.1.1.0 [2,4]) octane, 2,7,7-trimethyl-	552, 1.380	$C_{10}H_{16}O$	802	59774
5,7-Dodecadiene, (E, Z)-	564, 1.300	$C_{12}H_{22}$	857	6599.9
Benzeneethanol, α-ethyl-	600, 1.610	$C_{10}H_{14}O$	810	12481
O, O, O-Triethyl thiophosphate	606, 1.710	$C_6H_{15}O_3PS$	890	15682
2-Nonenal, (E)-	612, 1.530	$C_9H_{16}O$	928	121134
Benzene, pentyl-	618, 1.540	$C_{11}H_{16}$	946	2447
Homomyretenol	648, 1.700	$C_{11}H_{18}O$	863	96517
β-Citronellol	660, 1.660	$C_{10}H_{20}O$	876	11823
trans-2-Caren-4-ol	690, 1.950	$C_{10}H_{16}O$	860	19203
Citronellyl acetate	762, 1.870	$C_{12}H_{22}O_2$	822	13105
Bicycloelemene	768, 1.840	$C_{15}H_{24}$	832	17699
α-Chamigrene	852, 2.020	$C_{15}H_{24}$	860	19451
Ethanone, 1-(2-hydroxy-4-methoxyphenyl)-	882, 3.190	$C_9H_{10}O_3$	815	12092
Phthalic anhydride	912, 2.520	$C_8H_4O_3$	902	50391
trans-α-Bisabolene	936, 2.260	$C_{15}H_{24}$	889	86274
Bicyclogermacrene	948, 1.660	$C_{15}H_{24}$	868	110509
Phthalic anhydride	984, 2.370	$C_8H_4O_3$	889	28448
δ-Elemene	996, 2.500	$C_{15}H_{24}$	843	33997
2-Tetradecanol	1068, 2.240	$C_{14}H_{30}O$	817	68391
1 (3H)-Isobenzofuranone, 3-propylidene-	1116, 4.200	$C_{11}H_{10}O_2$	833	16760
1-(3-Methyl-cyclopent-2-enyl)-cyclohexene	1164, 3.270	$C_{12}H_{18}$	821	64080
1 (3H)-Isobenzofuranone	1164, 4.230	$C_8H_6O_2$	804	8950818
p-Coumaric acid	1296, 5.330	$C_9H_8O_3$	844	17156
(R)-(+)-3-Hexyl-1,2-thiazinane 1,1-dioxide	1314, 5.710	$C_{10}H_{21}NO_2S$	820	6696.8
Isopropyl myristate	1410, 2.510	$C_{17}H_{34}O_2$	809	6197.8
Octadecanoic acid	1968, 3.090	$C_{18}H_{36}O_2$	834	55855
Squalene	2346, 1.840	$C_{30}H_{50}$	856	380459
1 (3H)-Isobenzofuranone, 3-butylidene-	2436, 1.650	$C_{12}H_{12}O_2$	805	13996

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