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

**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.⁵ Meanwhile, *A. sinensis* Radix has been used as a common health food supplement for women’s care for 1000’s of years in China.⁶,⁷ Pharmacological studies and clinical practices have demonstrated that *A. sinensis* Radix possesses various bioactivities, including antibacterial, anti-ammestic, and antihypertensive effects,⁸ inhibitory effect on acetylcholinesterase,⁹ reduced cardiac contraction,¹⁰ activation of protein kinase C,¹¹ and antitumor activity.¹² 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.¹³

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.¹⁴ 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.
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 6890N gas chromatograph with split-splitless injector, 7683 B Series auto sampler and time of flight mass spectrometer LECO Pegasus III. Major parameters were set as: 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 on 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
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 H₂SO₃ 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
| Name                                      | R.T. (s) | Formula     | Similarity | Non-fumigated (%) | Sulfur-fumigated (%) |
|-------------------------------------------|----------|-------------|------------|-------------------|----------------------|
| (R)-1-Hexen-3-ol                          | 312, 1.150 | C_{6}H_{10}O | 974        | 100               | 467.5               |
| Acetic acid, butyl ester                  | 372, 1.210 | C_{3}H_{6}O  | 962        | 100               | 147.14              |
| 2-Furancarboxaldehyde                     | 390, 1.430 | C_{4}H_{2}O  | 964        | 100               | 182.52              |
| 2-Hexenal                                 | 402, 1.300 | C_{5}H_{10}O | 924        | 100               | 5.68                |
| p-Xylene                                  | 420, 1.240 | C_{5}H_{6}O  | 948        | 100               | 8.45                |
| 2-Heptanone                               | 426, 1.270 | C_{6}H_{12}O | 934        | 100               | 3.55                |
| Heptanal                                  | 432, 1.280 | C_{7}H_{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_{7}H_{14}O | 914        | 100               | 2.18                |
| Octanal                                   | 504, 1.290 | C_{8}H_{16}O | 957        | 100               | 4.67                |
| Benzene, 1,2,3-trimethyl-                  | 504, 1.330 | C_{10}H_{16} | 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_{10}H_{16} | 911        | 100               | 12.91               |
| Neo-allo-ocimene                           | 528, 1.460 | C_{10}H_{16} | 891        | 100               | 1.2                 |
| Benzeneacetaldehyde                       | 540, 1.590 | C_{10}H_{16} | 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_{9}H_{20}O | 877        | 100               | 5.97                |
| Ethanol, 1-(3,4-dimethylphenyl)-           | 570, 1.530 | C_{10}H_{16} | 904        | 100               | 0.73                |
| Camphenone, 6-                            | 576, 1.590 | C_{11}H_{24} | 907        | 100               | 4.03                |
| Furan, 2-methyl-                           | 576, 1.600 | C_{8}H_{16}O | 827        | 100               | 29.75               |
| α-Campholenaldehyde                       | 594, 1.570 | C_{11}H_{20} | 877        | 100               | 15.81               |
| Isopropone                                 | 594, 1.670 | C_{12}H_{20} | 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_{10}H_{16} | 942        | 100               | 38.41               |
| Safranal                                   | 630, 1.730 | C_{10}H_{16} | 869        | 100               | 5.38                |
| Dodecane                                  | 636, 1.340 | C_{12}H_{26} | 937        | 100               | 6.47                |
| 4-Terpineol                               | 636, 1.650 | C_{12}H_{24} | 900        | 100               | 17.72               |
| Benzenemethanol, a, a, 4-trimethyl-        | 636, 1.800 | C_{12}H_{18}O | 850       | 100               | 31.71               |
| Benzaldehyde, 2,5-dimethyl-                | 642, 1.520 | C_{10}H_{16} | 918        | 100               | 6.36                |
| Decanal                                   | 648, 1.560 | C_{12}H_{24} | 918        | 100               | 19.11               |
| Naphthalene                               | 648, 2.010 | C_{10}H_{12} | 954        | 100               | 6.25                |
| cis-Carveol                               | 660, 1.810 | C_{10}H_{16} | 932        | 100               | 14.65               |
| D-Carvone                                 | 684, 1.980 | C_{10}H_{16} | 835        | 100               | 37.17               |
| 2-Decenal, (Z)-                           | 690, 1.520 | C_{10}H_{16} | 929        | 100               | 12.24               |
| 1-Butanone, 1-phenyl-                      | 690, 2.060 | C_{10}H_{20} | 903        | 100               | 16.74               |
| 6-Undecanone                              | 696, 1.670 | C_{11}H_{24} | 914        | 100               | 12.21               |
| 3,5-Dimethoxytoluene                      | 696, 2.120 | C_{12}H_{16}O | 861       | 100               | 13.4                 |
| 6-Undecanol                               | 708, 1.630 | C_{12}H_{20} | 898        | 100               | 16.84               |
| Phenol, 4-ethyl-2-methoxy-                | 708, 2.130 | C_{12}H_{16}O | 802       | 100               | 17.92               |
| 2-Undecanone                              | 714, 1.730 | C_{12}H_{20} | 836        | 100               | 13.32               |
| (E)-Solanoone                             | 714, 1.790 | C_{12}H_{20} | 826        | 100               | 12.08               |
| Tridecane                                 | 720, 1.460 | C_{13}H_{28} | 945        | 100               | 6.35                |
| Undecanol-3                               | 720, 1.650 | C_{12}H_{24} | 803        | 100               | 19.72               |
| (E)-trans-Pinocarvyl acetate              | 726, 1.990 | C_{12}H_{18} | 829        | 100               | 7.68                |
| 1,3-Benzodioxole, 5-{(2-propenyl)-        | 726, 2.210 | C_{12}H_{16}O | 918       | 100               | 15.11               |
| Indole                                    | 732, 2.770 | C_{8}H_{14}N | 933        | 100               | 1.64                |
| 2-Methoxy-4-vinylphenol                   | 738, 2.410 | C_{9}H_{14}O | 938        | 100               | 12.34               |
| 2,4-Decadienal                            | 744, 1.950 | C_{12}H_{20} | 908        | 100               | 17.1                |

Contd...
| Name                                                                 | R.T. (s) | Formula      | Similarity | Non-fumigated (%) | Sulfur-fumigated (%) |
|----------------------------------------------------------------------|---------|--------------|------------|-------------------|----------------------|
| Naphthalene, 2-methyl-                                               | 744, 2.320 | C₁₁H₁₀      | 905        | 100               | 11.13                |
| Benzaldehyde, 2,4,6-trimethyl-                                      | 750, 2.400 | C₁₃H₁₀O      | 872        | 100               | 22.76                |
| 2,4,6-Trimethyl-1,3,5-heptatriene                                   | 756, 1.950 | C₁₃H₁₆       | 821        | 100               | 10.55                |
| Naphthalene, 2-methyl-                                              | 756, 2.470 | C₁₃H₁₀       | 895        | 100               | 13.84                |
| n-Decanoic acid                                                     | 774, 1.880 | C₁₁H₂₀O₂     | 869        | 100               | 26.73                |
| 1-Phenyl-1-propanol-(1)                                             | 774, 2.270 | C₁₃H₁₀O      | 908        | 100               | 15.86                |
| 1-Pentanone, 1-phenyl-                                              | 786, 2.370 | C₁₃H₁₀       | 944        | 100               | 14.67                |
| 2 (3H)-Furanone, dihydro-5-pentyl-                                  | 786, 2.550 | C₁₃H₂₀O₂     | 924        | 100               | 5.61                 |
| Benzenecacetic acid, a-oxo-, methyl ester                          | 792, 2.490 | C₁₃H₁₀O      | 929        | 100               | 20.04                |
| Benzaldehyde, 2,4,5-trimethyl-                                      | 798, 2.620 | C₁₃H₁₀O      | 876        | 100               | 30.88                |
| 5-Tetradecene, (E)-                                                | 804, 1.650 | C₁₅H₂₈O      | 928        | 100               | 4.18                 |
| (-)-Isolideone                                                      | 810, 1.930 | C₁₅H₂₄       | 883        | 100               | 4.92                 |
| Tetradecane                                                         | 816, 1.590 | C₁₅H₂₄O      | 958        | 100               | 8.43                 |
| 6-Dodecyl-1-α                                                       | 816, 2.040 | C₁₃H₁₀O      | 848        | 100               | 58.82                |
| Benzene, 1,2-dimethoxy-4-(2-propenyl)-                              | 816, 2.660 | C₁₃H₂₀O₂     | 937        | 100               | 20.39                |
| Phenol, 4-pentyl-                                                   | 834, 2.480 | C₁₀H₁₆O      | 878        | 100               | 35.92                |
| Ethyl dl-mandelate                                                   | 840, 2.780 | C₁₁H₁₄O₂     | 865        | 100               | 20.1                 |
| 3-Methyl-2-butenoic acid, 3-tridecyl ester                          | 858, 1.990 | C₁₅H₂₄O      | 822        | 100               | 23.53                |
| (+)-β-Funebrene                                                     | 864, 2.120 | C₁₅H₂₄       | 887        | 100               | 6.59                 |
| Naphthalene, 1,3-dimethyl-                                           | 864, 2.800 | C₁₅H₂₆O      | 826        | 100               | 32.36                |
| Heptadecane, 2,6,10,14-tetramethyl-                                 | 882, 1.650 | C₁₅H₂₄O      | 874        | 100               | 48.75                |
| Aromadendrene                                                       | 888, 2.150 | C₁₅H₂₆O      | 899        | 100               | 38.77                |
| Widdrene                                                            | 888, 2.230 | C₁₅H₂₆O      | 870        | 100               | 8                    |
| 2′-Hydroxyvalerophenone                                              | 888, 2.670 | C₁₁H₁₄O₂     | 804        | 100               | 45.81                |
| Phenol, 2-methoxy-4-propyl-                                         | 888, 3.480 | C₁₀H₁₆O₂     | 905        | 100               | 35.12                |
| Oxirane, tetradeceyl-                                               | 900, 2.080 | C₁₃H₂₄O      | 836        | 100               | 39.77                |
| 2 (3H)-Furanone, 5-hexylhydro-                                      | 900, 2.890 | C₁₁H₂₀O₂     | 953        | 100               | 12.53                |
| E-2-Hexadecacenc-1-ol                                               | 906, 1.810 | C₁₅H₂₆O      | 907        | 100               | 14.79                |
| Pentadecane                                                         | 924, 1.740 | C₁₅H₂₆O      | 947        | 100               | 17.18                |
| 2-Pentadecanol                                                      | 930, 2.060 | C₁₅H₂₆O      | 896        | 100               | 35.36                |
| (E, E)-c-Farnesene                                                  | 936, 2.200 | C₁₅H₂₆O      | 810        | 100               | 14.22                |
| β-Bisabolene                                                        | 948, 2.230 | C₁₅H₂₆O      | 906        | 100               | 29.04                |
| Bicyclogermacrene                                                   | 954, 2.450 | C₁₅H₂₆O      | 892        | 100               | 0.4                  |
| Aromadendrene, dehydro-                                             | 960, 2.550 | C₁₅H₂₆O      | 808        | 100               | 5.23                 |
| β-Himachalene                                                       | 966, 2.450 | C₁₅H₂₆O      | 826        | 100               | 10.24                |
| β-Sesquiphellandrene                                                | 972, 2.320 | C₁₅H₂₆O      | 875        | 100               | 19.18                |
| δ-Cadinene                                                          | 972, 2.470 | C₁₅H₂₆O      | 856        | 100               | 29.07                |
| Benzoic acid, 2-propenyl ester                                      | 978, 2.770 | C₁₀H₁₆O₂     | 812        | 100               | 15.93                |
| Dibenzoferan                                                        | 990, 3.480 | C₁₀H₁₆O      | 908        | 100               | 30.5                 |
| d-Nerolidol                                                         | 1014, 2.430 | C₁₀H₁₆O₂     | 914        | 100               | 35.57                |
| 7-Hexadecene, (Z)-                                                 | 1032, 1.960 | C₁₀H₁₆O₂     | 909        | 100               | 30.69                |
| Isoisophthalenol                                                    | 1044, 2.800 | C₁₀H₁₆O₂     | 829        | 100               | 29.3                 |
| Palustrol                                                           | 1056, 2.650 | C₁₀H₁₆O₂     | 877        | 100               | 49.54                |
| (+) Spathulenol                                                     | 1056, 2.940 | C₁₀H₁₆O₂     | 925        | 100               | 600.15               |
| Hexadecane                                                          | 1062, 1.880 | C₁₀H₁₆O₂     | 942        | 100               | 19.04                |
| (-)-Spathulenol                                                     | 1062, 3.000 | C₁₀H₁₆O₂     | 945        | 100               | 22.26                |
| Globulol                                                            | 1074, 2.810 | C₁₀H₁₆O₂     | 897        | 100               | 37.82                |
| 9H-Fluorene                                                         | 1080, 3.870 | C₁₀H₁₆O₂     | 889        | 100               | 31.35                |
| 1,3-Isobenzofurandione                                              | 1092, 2.190 | C₁₀H₁₆O₂     | 822        | 100               | 21.31                |
| (+) Spathulenol                                                     | 1104, 3.120 | C₁₀H₁₆O₂     | 878        | 100               | 59.01                |
| Cedrol                                                              | 1116, 3.010 | C₁₀H₁₆O₂     | 867        | 100               | 6.51                 |
| Isoisophthalenol (isomer)                                           | 1134, 3.210 | C₁₀H₁₆O₂     | 909        | 100               | 20.16                |
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.

### Table 1: Contd

| Name | R.T. (s) | Formula | Similarity | Non-fumigated (%) | Sulfur-fumigated (%) |
|------|----------|---------|------------|-------------------|----------------------|
| 1-(But-3-enyl) indan-1-ol | 1164, 4.250 | C19H19O | 824 | 100 | 18.18 |
| 8-Heptadecene | 1176, 2.100 | C17H34 | 926 | 100 | 45.63 |
| n-Tridecan-1-ol | 1176, 2.460 | C17H34 | 926 | 100 | 45.63 |
| Elemol | 1182, 3.190 | C16H32O | 840 | 100 | 28.91 |
| Ledene oxide-(II) | 1194, 3.290 | C18H32O | 832 | 100 | 21.61 |
| 1 (3H)-Isobenzofuranone, 3-Butylidenel | 1194, 4.440 | C17H30O | 870 | 100 | 39.68 |
| Ledene oxide-(II) | 1206, 3.280 | C17H30O | 851 | 100 | 17.14 |
| 2-Pentadecanol | 1218, 2.400 | C16H32O | 890 | 100 | 45.03 |
| n-Tridecan-1-ol | 1338, 2.610 | C16H32O | 922 | 100 | 42.48 |
| 3-n-Butylphthalide | 1392, 5.050 | C17H34O2 | 885 | 100 | 8.81 |
| Pentadecanoic acid, methyl ester | 1410, 2.640 | C16H32O2 | 850 | 100 | 8.78 |
| Pentadecanoic acid | 1464, 2.850 | C17H34O2 | 922 | 100 | 3.97 |
| 11-Hexadecen-1-ol, (Z)- | 1476, 2.880 | C17H34O | 918 | 100 | 36.09 |
| Hexadecanoic acid, methyl ester | 1584, 2.730 | C17H34O2 | 909 | 100 | 12.56 |
| n-Hexadecanoic acid | 1638, 3.010 | C16H32O2 | 920 | 100 | 26 |
| Dibutyl phthalate | 1638, 4.530 | C19H22O4 | 951 | 100 | 34.21 |
| cis-7-Tetradecen-1-ol | 1644, 2.990 | C17H30O2 | 918 | 100 | 30.64 |
| Falcarinol | 1776, 3.990 | C17H24O | 860 | 100 | 37.89 |
| 11-Hexadecen-1-ol, (Z)- | 1818, 3.040 | C17H34O2 | 939 | 100 | 33.02 |
| 9,12-Octadecadienoic acid, methyl ester | 1872, 3.200 | C18H32O2 | 928 | 100 | 8.56 |
| 9,12-Octadecadienoic acid (Z, Z)- | 1932, 3.510 | C18H32O2 | 933 | 100 | 0.16 |
| 9,12,15-Octadecatrienoic acid, (Z, Z, Z)-trans-13-Octadecenoic acid | 1944, 3.640 | C18H32O2 | 886 | 100 | 1.55 |
| 9,12-Octadecadienoic acid (Z, Z)- | 1950, 3.250 | C18H32O2 | 841 | 100 | 2.01 |
| 1,3-Cyclohexadiene, 1,5,5,6-tetramethyl- | 2058, 3.620 | C19H32O2 | 865 | 100 | 6.38 |
| 9-Octadecenamide, (Z)- | 2106, 4.410 | C19H36NO | 860 | 100 | 25.71 |
| 2. Eight compounds on co‑eluting peaks, the fingerprint information of 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.

### 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: Benzoaldehyde, 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 | C6H12O    | 821        | 30661    |
| 3-Hexene, 2,2-dimethyl-, (E)-    | 456, 1.320 | C6H12      | 862        | 4468.4   |
| 2-Heptenal, (Z)-                 | 474, 1.310 | C6H10O     | 878        | 28737    |
| Bicyclo [3.1.0] hex-2-ene, 4-methylene-1-(1-methylthyl)- | 480, 1.220 | C7H13      | 808        | 18197    |
| Octane, 3,5-dimethyl-             | 498, 1.170 | C6H12      | 834        | 12617    |
| 3-Octen-2-one, (E)-              | 528, 1.370 | C6H10O     | 817        | 9485.4   |
| 3-Cyclohexen-1-one, 3,5,5-trimethyl- | 534, 1.420 | C7H12O     | 866        | 11076    |
| 2-Octenal                        | 540, 1.390 | C6H10O     | 876        | 11823    |
| Octadecanoic acid (2 [2,4])      | 552, 1.380 | C8H18O2    | 802        | 53724    |
| 5,7-Dodecadiene, (E, Z)-         | 564, 1.300 | C7H12      | 857        | 6599.9   |
| Benzeneneethanol, α-ethyl-        | 600, 1.610 | C7H10O     | 810        | 12481    |
| O, O, O-Triethyl thiophosphate   | 606, 1.710 | C6H10O PS  | 890        | 15682    |
| 2-Nonenal, (E)-                  | 612, 1.530 | C6H12O     | 928        | 121134   |
| Benzene                           | 618, 1.540 | C6H10       | 946        | 2447     |
| Homomyretnol                      | 648, 1.700 | C7H12O     | 863        | 96517    |
| β-Citronellol                     | 660, 1.660 | C8H14O2    | 876        | 11823    |
| trans-2-Carene-4-ol              | 680, 1.950 | C8H14O2    | 860        | 19203    |
| Citronellyl acetate               | 762, 1.870 | C4H6O2     | 822        | 13105    |
| Bicycloelemene                    | 768, 1.840 | C10H16     | 832        | 17699    |
| α-Chamigrene                      | 852, 2.020 | C10H16     | 860        | 19451    |
| Ethanone, 1-(2-hydroxy-4-methoxyphenyl)- | 882, 3.190 | C7H12O3    | 815        | 12092    |
| Phthalic anhydride                | 912, 2.520 | C6H8O3     | 902        | 50391    |
| trans-α-Bisabolene                | 936, 2.260 | C10H24     | 889        | 86274    |
| Bicycloligermacrene               | 948, 1.660 | C10H18      | 868        | 110509   |
| Phthalic anhydride                | 984, 2.370 | C6H12O3    | 889        | 28448    |
| δ-Elemene                         | 996, 2.500 | C10H24     | 843        | 33997    |
| 2-Tetradecanal                  | 1068, 2.240 | C12H24     | 817        | 68391    |
| 1 (3H)-Isobenzo[1,3]furanone, 3-propylidene- | 1116, 4.200 | C12H18O    | 833        | 16760    |
| 1-(3-Methyl-cyclopent-2-enyl)-cyclohexene | 1164, 3.270 | C10H16     | 821        | 64080    |
| 1 (3H)-Isobenzo[1,3]furanone      | 1164, 4.230 | C4H8O2     | 804        | 8950818  |
| p-Coumaric acid                  | 1296, 5.330 | C8H8O3     | 844        | 17156    |
| (R)-(+)3-Hexyl-1,2-thiazinane 1,1-dioxide | 1314, 5.710 | C10H18NO2S | 820        | 6696.8   |
| Isopropyl myristate              | 1410, 2.510 | C10H22O8   | 809        | 6197.8   |
| Octadecanoic acid                | 1968, 3.090 | C18H36O2   | 834        | 55855    |
| Squalene                         | 2346, 1.840 | C20H30     | 856        | 300459   |
| 1 (3H)-Isobenzo[1,3]furanone, 3-butylidene- | 2436, 1.650 | C10H18O2   | 805        | 13996    |

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