

# Novel characterization of *Radix Angelicae Dahuricae* before and after the sulfur-fumigation process by combining high performance liquid chromatographic fingerprint and multi-ingredients determination

Xiao Liu<sup>1,2,3,4</sup>, Jingjing Liu<sup>6</sup>, Hao Cai<sup>1,2,3</sup>, Songlin Li<sup>5</sup>, Xiaoqing Ma<sup>1,2</sup>, Yajing Lou<sup>1,2</sup>, Kunming Qin<sup>2,4</sup>, Hongyue Guan<sup>1,2</sup>, Baochang Cai<sup>1,2,3,4</sup>

<sup>1</sup>Department of Chinese Materia Medica, College of Pharmacy, <sup>2</sup>Engineering Center of State Ministry of Education for Standardization of Chinese Medicine Processing, and <sup>3</sup>National First-Class Key Discipline for Science of Chinese Materia Medica, Nanjing University of Chinese Medicine, <sup>4</sup>Nanjing Haichang Chinese Medicine Group Corporation, <sup>5</sup>Department of Pharmaceutical Analysis and Metabolomics, Jiangsu Province Academy of Chinese Medicine, Nanjing, <sup>6</sup>Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Jinan, People's Republic of China

Submitted: 05-01-2013

Revised: 16-02-2013

Published: 24-07-2014

## ABSTRACT

**Background:** Harmful sulfur-fumigation processing method is abused during *Radix Angelicae Dahuricae* preparation. However, the analytical technique characterizing *Radix Angelicae Dahuricae* before and after the sulfur-fumigation process is absent. **Materials and Methods:** The high performance liquid chromatography (HPLC) technique was adopted to develop methods combining finger-print analysis and multi-ingredients simultaneous determination for quality evaluation of *Radix Angelicae Dahuricae* before and after the sulfur-fumigation process. The chromatographic fingerprint method was established for qualitative analysis coupled with statistical cluster analysis basing on Euclidean distance. Additionally, a determination method was developed for quantitative analysis, which was able to assay the concentrations of the major coumarins including imperatorin, isoimperatorin, xanthotoxin, xanthotoxol, isoimipinellin, oxypeucedanin, and bergapten in *Radix Angelicae Dahuricae* simultaneously. The separations of the two methods were both achieved on a Hypersil octadecylsilyl C<sub>18</sub> column (250 mm × 4.6 mm, 5 μm) at 35 °C under different strategic gradient elution programs. The detection wavelength was set at 254 nm all the time. Method validation data indicated that the methods were both reliable and applicable. They were then used to assay different *Radix Angelicae Dahuricae* samples collected from good agricultural practice (GAP) bases and local herbal markets. **Results:** The successful application demonstrated that the combination of HPLC fingerprint and simultaneous quantification of multi-ingredients offers an efficient approach for quality evaluation of *Radix Angelicae Dahuricae* before and after the sulfur-fumigation process. **Conclusion:** In order to discriminate *Radix Angelicae Dahuricae* before and after the sulfur-fumigation process, oxypeucedanin, and xanthotoxol were the most sensitive biomarkers and should be determined.

**Key words:** Coumarins, fingerprint analysis, high performance liquid chromatography, multi-ingredients simultaneous determination, *Radix Angelicae Dahuricae*, sulfur-fumigation process

## INTRODUCTION

Traditional Chinese medicine (TCM), most of which is usually made from the parts of some plants, needs

sun-drying or drying in the shade after harvesting. It would take a very long-time for some of the fresh roots or rhizomes of herbal drugs to be dried. In recent decades, sulfur-fumigation process was applied to the crude drugs processing as an alternative method. It has really curtailed the dryness duration as well as played a role of pest controller and good looking giver. However, the fumigation process was usually achieved through sulfur combustion in a closed cabinet, generating a lot of SO<sub>2</sub>,

### Address for correspondence:

Prof. Hao Cai, Department of Chinese Materia Medica, College of Pharmacy, Nanjing University of Chinese Medicine, Nanjing - 210023, People's Republic of China.  
E-mail: haocai\_98@126.com

### Access this article online

#### Website:

www.phcog.com

#### DOI:

10.4103/0973-1296.137376

#### Quick Response Code:



a kind of chemically active compound. As a kind of reducing agent, SO<sub>2</sub> could easily react with the constituents containing hydroxyl in their chemical structures.<sup>[1,2]</sup> The fact that efficacy of TCM is closely related to its inherent multi-components has already been widely accepted. Hence, the sulfur-fumigation process, which has been commonly used in crude herbal drugs preparation over recent decades, was indeed a challenge to the quality of TCM. Furthermore, the commercial mineral sulfur used for fumigation process usually contains some contaminants, causing lots of residues of poisonous heavy metals which would be harmful to health after drug processing, such as arsenic, mercury, and lead. Recently, as this subject became hotter and hotter day by day, researchers began to pay attention to it, and the results suggested that sulfur-fumigation process had a severely destructive effect on the inherent quality of some herbal drugs.<sup>[3,4]</sup>

*Radix Angelicae Dahuricae*, referred to as Baizhi in Chinese, is an important crude herb used in TCM. This herb has been used primarily to treat the headache caused by common cold,<sup>[5]</sup> as well as pain caused by swelling, migraine, contusions and strains.<sup>[6,7]</sup> It has been demonstrated that the major bioactive components in *Radix Angelicae Dahuricae* are coumarins, including, imperatorin, isoimperatorin, and scopoletin, a group of components, which are sensitive to heat and SO<sub>2</sub>.<sup>[8-14]</sup> Up to now, kinds of analysis methods have already been developed for quality control of *Radix Angelicae Dahuricae*, including high performance liquid chromatographic (HPLC)/electrospray ionization-mass spectrometry (ESI-MS),<sup>[15]</sup> capillary electrophoresis,<sup>[16]</sup> and high performance liquid chromatography (HPLC)/diode array detector (DAD)/electrospray ionization-mass spectrometry (ESI-MS).<sup>[17]</sup> However, the destructive effect caused by sulfur-fumigation was scarcely mentioned in these papers. Though, the destructive effects of sulfur-fumigation progress on *Radix Angelicae Dahuricae* has ever been suggested,<sup>[18]</sup> the efficient analytical method for discrimination and quality control is still absent. In fact, our previous study results revealed that almost no *Radix Angelicae Dahuricae* samples collected from TCM markets survived sulfur-fumigation after harvesting because of its juicy texture.

Unlike synthetic drugs, TCM generally exerts their therapeutic effects through the synergic effects of the multiple active ingredients and the multi-targets they are targeting, which makes it very difficult to evaluate the quality of herbal drugs and their preparations.<sup>[19]</sup> The HPLC fingerprint technique has been considered as a useful method for quality evaluation of a complex system such as herbal drugs with a quantitative degree of reliability in recent years. Compared with conventional

analytical approaches, fingerprint technique emphasizes on the integral characterization and can give an overall view of all components in TCM successfully.<sup>[20,21]</sup> However, one drawback is that it can only show results of similarity calculated based on the relative value using pre-selected marker peak as a reference standard, and minor differences between very similar chromatograms might not be distinguishable.<sup>[22]</sup> In this situation, chemical pattern recognition methods such as multi-ingredients quantitative analysis should be taken into consideration for reasonable addition.

In this paper, HPLC-DAD technique was adopted to set up the chromatographic fingerprint for *Radix Angelicae Dahuricae*; meanwhile, an analytical method for simultaneous determination of the major coumarins was also developed. The methods were then applied to test the *Radix Angelicae Dahuricae* samples collected from good agricultural practice (GAP) bases and local herbal markets. The overall quality evaluation of *Radix Angelicae Dahuricae* before and after the sulfur-fumigation process was accomplished, and the biomarkers for sulfur-fumigation characterization were finally discovered.

## MATERIALS AND METHODS

### Materials and reagents

HPLC grade methanol and formic acid were obtained from Tedia (Tedia, USA) and Dikma Pure (Dikma, USA), respectively. Ethanol used for extraction was supplied by Tianjin Chemical Reagent Corporation (Tianjin, China). Ultrapure water was used throughout the experiment. Imperatorin, isoimperatorin, and adenosine were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Xanthotoxin, xanthotoxol, isoimipinellin, oxypeucedanin, and bergapten were purchased from Yousi Biotechnology Co., Ltd. (Shanghai, China). The purities of the standard compounds were all above 98%, and their chemical structures are shown in Figure 1.

Nineteen batches of *Radix Angelicae Dahuricae* samples were collected in all, including four batches of sun-dried sample provided by GAP cultivation bases and fifteen batches of sulfur-fumigated ones purchased from different local markets [Table 1]. The botanical origin of materials was identified by Jianwei Chen, Professor of Pharmacognosy (Nanjing University of Chinese Medicine, China). The commercial samples were confirmed to have been treated by sulfur-fumigation using sulfite residue testing according to the state standard of sulfur dioxide residue (>150 ppm).

### Instrumentation and HPLC conditions

The analyses were performed using a Varian LC-920 HPLC system (Varian, Australia) equipped with a Prostar 240 quaternary pump, a DAD, a Prostar 410 autosampler, a column compartment and Galaxie Chemstation data station. Chromatographic separation was carried out on a Hypersil ODS C<sub>18</sub> column (250 mm × 4.6 mm, 5 μm) under 35°C.

The mobile phase consisted of water containing 0.1% formic acid (A) and methanol (B) with a linear gradient elution at a flow rate of 1.0 mL/min. For chromatographic fingerprint analysis the elution program was as follows: 0-50 min, 95% → 35% A; 50-70 min, 35% → 5% A; 70-75 min, 5% A. While for multi-ingredients quantitative analysis the elution program was as follows: 0-6 min, 70% → 40% A; 6-15 min, 40% A; 15-30 min, 5% A. The detection wavelength was set at 254 nm for determination and the ultraviolet (UV) spectra scanned by DAD for all the samples were also collected.

### Preparation of standard solutions

Mixed standard stock solution containing imperatorin, isoimperatorin, xanthotoxin, xanthoxol, isoimipinellin, oxypeucedanin, and bergapten was prepared in methanol. Working standard solutions were prepared by diluting the mixed standard stock solution with methanol to give different concentrations for calibration curves. All solutions were stored in a refrigerator under 4°C prior to use.

### Preparation of sample solutions

An aliquot of drug powder (1 g for fingerprint analysis and 0.5 g for multi-ingredients quantitative analysis)

was accurately weighed and extracted with 70% ethanol (10 mL for fingerprint analysis and 20 mL for multi-ingredients quantitative analysis) of by ultrasonic for 30 min (250 W, 40 kHz). Then the resultant mixture was adjusted to the original weight and the supernatant was filtered through a 0.45 μm filter membrane. A volume of 20 μL was injected into the HPLC system for analysis.

## RESULTS AND DISCUSSION

### Optimization of chromatographic conditions and compounds identification

Various compositions of mobile phase were tried to obtain optimal chromatograms with good resolution and adjacent peaks, including, methanol-water, acetonitrile-water, methanol-water containing 0.1% phosphoric acid, acetonitrile-water containing 0.1% phosphoric acid, methanol-water containing 0.1% formic acid, and acetonitrile-water containing 0.1% formic acid. Finally, the elution system of methanol-water containing 0.1% formic acid in a gradient mode was chosen to give the desired separation with an acceptable running time of 70 min. In order to detect more peaks while achieving precise signals of quantitative components, the most appropriate wavelength was set at 254 nm. The typical chromatograms for the fingerprint analysis are shown in Figure 2. The HPLC-DAD analysis system made a systematic collection of chromatographic data and UV spectra for all the analytes. Seven coumarins compounds, as well as adenosine were identified by comparing their retention behaviors and UV characteristics with the reference compounds. Meanwhile the eight compounds, which are the inherent and the major bioactive ingredients of *Radix Angelicae Dahuricae*, were chosen as the “common peaks.” Among them, the peak of imperatorin was symmetrical and detectable in all the tested samples and was used as reference for relative retention time (RRT) and relative peak area (RPA) calculating. The aim to calculate RRT and RPA was to make the various absolute values become relatively stable, which could semi-quantitatively reflect the constituents displayed in the chromatographic profile of the samples. The formulas of RRT and RPA were as follows:

$$RRT = RT_{\text{peak}} / RT_{\text{reference}}; RPA = PA_{\text{peak}} / PA_{\text{reference}}$$

### HPLC method validation

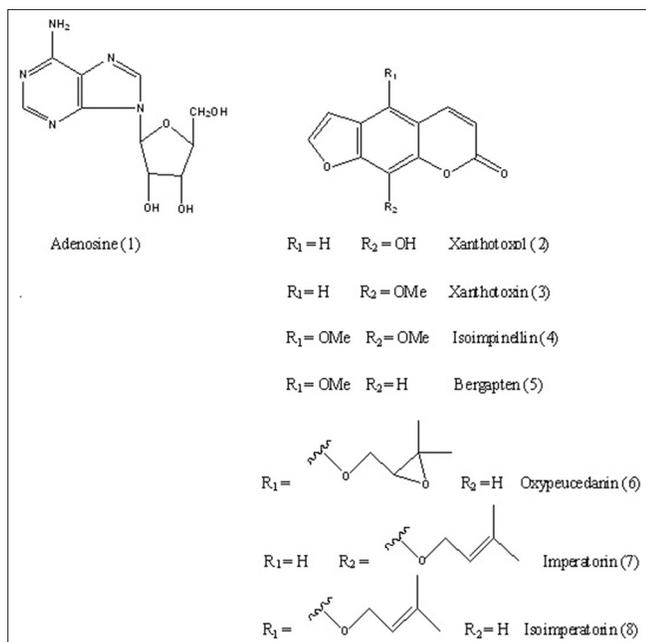
#### Precision

The injection precision was determined by replicated injection of the same sample 6 times in 1 day. The relative standard deviations (RSD) of retention time and peak areas of eight “common peaks” were lower than 0.2% and 3.6%, respectively.

**Table 1: Sources of nineteen batches of *Radix Angelicae Dahuricae* samples**

Source	Processing method
Hebei	Supplied by GAP base and dried under the sun
Hebei	Supplied by GAP base and dried under the sun
Anhui	Supplied by GAP base and dried under the sun
Anhui	Supplied by GAP base and dried under the sun
Zhejiang	Purchased from local market and fumigated by sulfur
Sichuan	Purchased from local market and fumigated by sulfur
Zhejiang	Purchased from local market and fumigated by sulfur
Anhui	Purchased from local market and fumigated by sulfur
Hebei	Purchased from local market and fumigated by sulfur
Zhejiang	Purchased from local market and fumigated by sulfur
Anhui	Purchased from local market and fumigated by sulfur
Hebei	Purchased from local market and fumigated by sulfur
Sichuan	Purchased from local market and fumigated by sulfur
Sichuan	Purchased from local market and fumigated by sulfur
Hebei	Purchased from local market and fumigated by sulfur
Sichuan	Purchased from local market and fumigated by sulfur
Anhui	Purchased from local market and fumigated by sulfur
Anhui	Purchased from local market and fumigated by sulfur
Sichuan	Purchased from local market and fumigated by sulfur

GAP: Good agricultural practice



**Figure 1:** Chemical structures of reference compounds

### Repeatability

The repeatability was evaluated by testing six samples prepared from the same batch of *Radix Angelicae Dahuricae* independently. The RSD of retention time and peak areas of eight “common peaks” were lower than 0.3% and 3.8%, respectively.

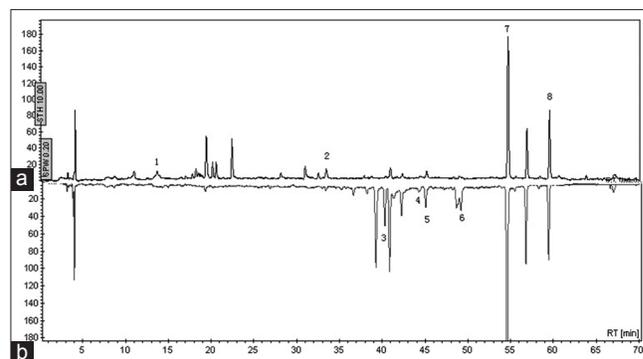
### Stability

The standard solution stability was assessed by injection of the same standard solution in 0, 2, 4, 6, 8, 10, and 24 h. The RSD of retention time and peak areas of eight “common peaks” were lower than 0.2% and 3.5%, respectively.

The results above suggested that the chromatographic fingerprint method for qualitative analysis of *Radix Angelicae Dahuricae* was applicable.

### Sample analysis and data acquisition

The software “Similarity Evaluation System for Chromatographic Fingerprint of TCM” was published by Chinese Pharmacopoeia Commission (Version 2004A) and mainly used to evaluate the similarity of chromatographic patterns. The software about the calculation of correlation coefficients was based on the peak areas and retention time. Its mathematical theories were principal component analysis and fuzzy information analysis, which were suitable for complicated system analysis.<sup>[23,24]</sup> Because of the software, the analytical process became quick and accurate. The chromatograms of nineteen batches of *Radix Angelicae Dahuricae* samples were introduced into the software “Similarity Evaluation System for Chromatographic Fingerprint of TCM,” and then the RPs



**Figure 2:** Typical high performance liquid chromatographic chromatograms of fingerprint analysis of the sulfur-fumigated sample (a), and the sun-dried sample (b): (1) Adenosine, (2) xanthoxol, (3) xanthoxin, (4) isoimpinellin, (5) bergapten, (6) oxypeucedanin, (7) imperatorin, (8) isoimperatorin

were obtained, the data were then calculated by the statistic software Statistical Product and Service Solutions (IBM SPSS) 15.0 to give the final cluster analytical result basing on Euclidean distance as shown in Figure 3.

Basing on the result of fingerprint analysis, the chromatograms of the sun-dried *Radix Angelicae Dahuricae* samples differed greatly from those of the sulfur-fumigated ones. The relatively higher contents of coumarins were found in the sun-dried samples supplied by GAP bases while the lower contents of coumarins were found in the sulfur-fumigated samples collected from the local markets. This phenomenon was obvious for xanthoxin, isoimpinellin, bergapten, and imperatorin, especially for oxypeucedanin, which could hardly be found in the sulfur-fumigated samples. Meanwhile, there were a lot of peaks for the newly produced compounds by fumigation process within the retention time range of 18-30 min. Cluster analytical result indicated that the tested samples were divided into two clusters regarding to their processing methods obviously. It seemed that sulfur-fumigation process did have an effect on the chemical composition of *Radix Angelicae Dahuricae*. Aiming to evaluate the differences more accurately and objectively, the multi-ingredients quantitative analysis of the major coumarins was carried out subsequently.

### Calibration curves, limits of detection, and limits of quantification

Standard stock solutions containing the seven analytes were prepared and diluted to appropriate concentrations for plotting the calibration curves. Six concentrations of the standard solutions containing the seven analytes were analyzed in triplicate, and then the calibration curves were constructed by plotting the peak areas versus the concentration of each analyte. The calculated results are given in Table 2. All the analytes showed good linearity ( $r^2 > 0.999$ ) in a relatively wide concentration

range. The LOD and LOQ were determined at concentrations giving a signal-to-noise ratio of 3 and 10, respectively [Table 2].

### Precision, recovery, and stability

The precision of the method was validated by determination of intra- and inter-day variance. The intra-day precision was determined by replicate analysis ( $n = 6$ ) of standard solutions of the seven analytes at low, medium, and high concentrations in a single day while the inter-day values was obtained by duplicating the experiment on 3 consecutive days. To further evaluate the repeatability of the developed method, six different solutions prepared from the same sample obtained from Anhui province were analyzed. The RSD was taken as a measure of precision and repeatability. The results are shown in Table 2, indicating that the intra-, inter-day and repeatability RSD values of the seven compounds were less than 2.7%, which showed good reproducibility of the developed method.

Recovery tests were carried out to investigate the accuracy of the method by spiking known amounts of the mixed standard solutions to approximately 0.25 g of the testing sample. The resultant samples were then extracted and analyzed with the described method. The average

percentage recoveries were evaluated by calculating the ratio of detected amount versus added one. The recovery of the method was in the range of 96.6-102.0%, with RSD values less than 2.8%.

Stability of herbal sample solution was tested at room temperature. The herbal sample solution was analyzed in triplicate every 2 h within 24 h. The analytes were found to be stable in ethanol solution (RSD < 2.7%) over the test period.

### Sample analysis

The developed quantitative analysis method was subsequently applied to test nineteen batches of *Radix Angelicae Dahuricae* samples collected from GAP bases and local herbal markets. The results demonstrated a successful application of this HPLC-DAD assay for the quantification of seven major coumarins in the different samples. All the seven compounds have been eluted within 30 min, giving good separation and acceptable tailing factors. Representative HPLC-DAD chromatograms of standard solutions and sample solutions for quantitative analysis are shown in Figure 4. The contents, summarized in Table 3, were calculated with external standard method.

The results of quantitative analysis were consistent with the results of chromatographic fingerprint analysis. Furthermore, some more detail information was obtained. As for the seven major coumarins in *Radix Angelicae Dahuricae*, four of them were lost significantly ( $P < 0.001$ ) after the sulfur-fumigation process, including, xanthotoxin, isoimpinellin, bergapten, and oxypeucedanin. The contents of xanthotoxin, isoimpinellin, and bergapten in sulfur-fumigated samples were 12.3%, 5.4%, and 27.1% as much as those of sun-dried ones, respectively. As the most sensitive one to sulfur-fumigation, oxypeucedanin was even unable to be determined in the fumigated samples because of its too low concentration below the linear range of calibration. The contents of imperatorin and isoimperatorin also tended downwards after being fumigated, though the differences were not significant ( $P > 0.05$ ). Different from the others, xanthotoxol, a kind of toxic compound, which

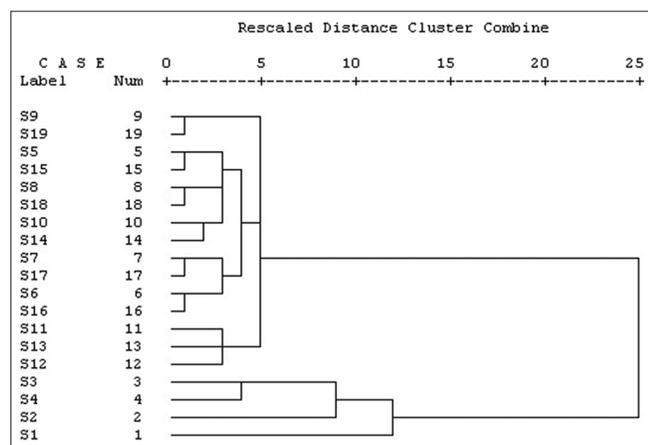
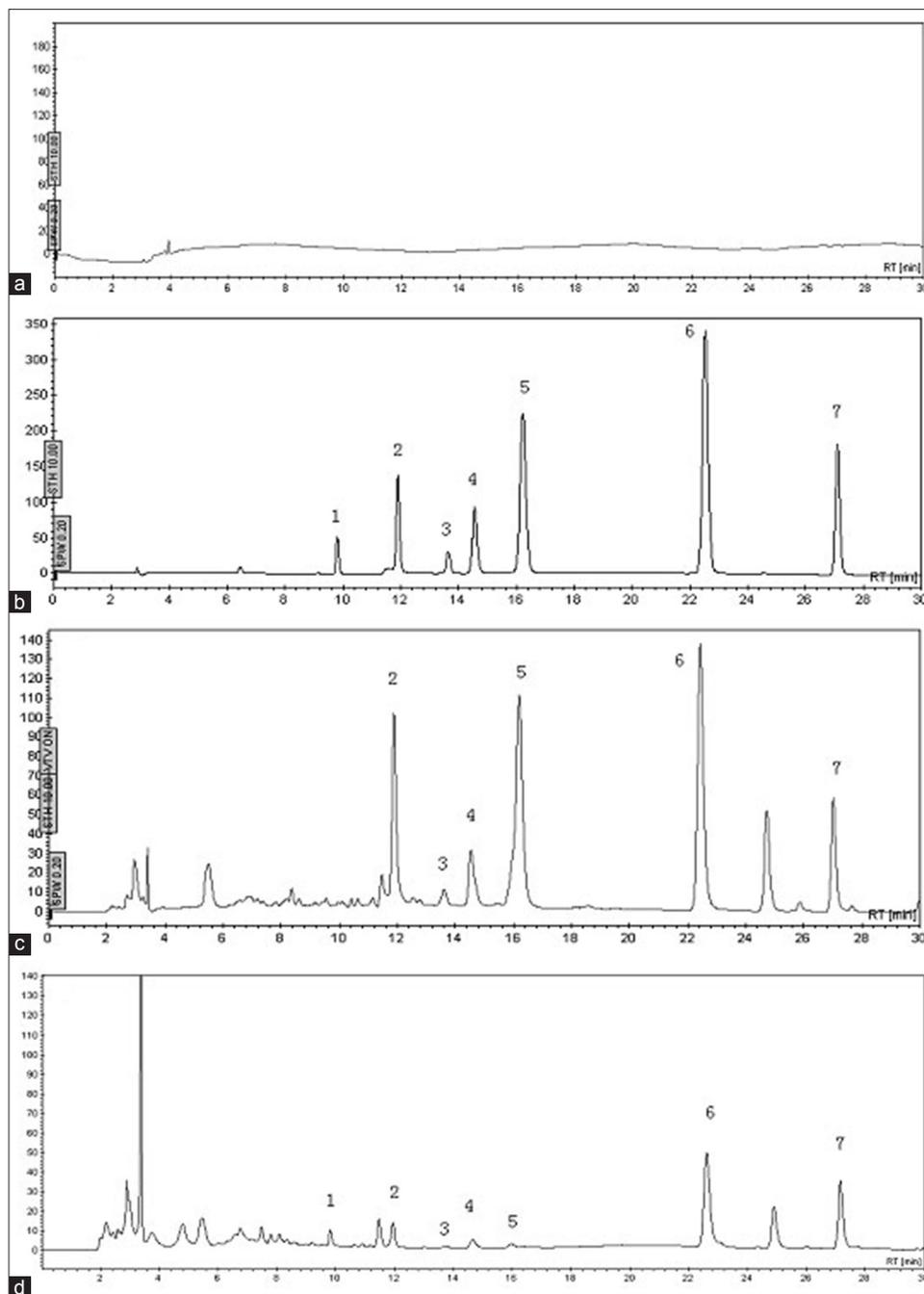


Figure 3: Result of cluster analysis

**Table 2: Linear regression data, LOD, LOQ, precision and repeatability of seven coumarins compounds in *Radix Angelicae Dahuricae* (n=6)**

Analyte	Regression equation	$r^2$	Linear range ( $\mu\text{g/mL}$ )	LOD ( $\mu\text{g/mL}$ )	LOQ ( $\mu\text{g/mL}$ )	Precision RSD (%)		Repeatability RSD (%)
						Intra-day	Inter-day	
Imperatorin	$Y=67.11 X-82.56$	0.9998	4.544-145.4	0.12	0.39	0.3	0.5	2.7
Isoimperatorin	$Y=56.29 X-26.95$	0.9997	2.261-72.36	0.05	0.16	0.8	1.2	2.0
Isoimpinellin	$Y=26.43 X+1.973$	0.9996	0.2875-9.200	0.07	0.29	0.5	1.8	2.5
Xanthotoxol	$Y=35.95 X+5.479$	0.9994	0.2735-8.750	0.04	0.15	0.9	1.6	1.7
Xanthotoxin	$Y=91.70 X+4.638$	0.9995	0.8172-26.15	0.05	0.15	0.7	1.1	1.9
Bergapten	$Y=65.46 X-5.386$	0.9992	1.091-34.90	0.03	0.10	1.1	1.5	1.7
Oxypeucedanin	$Y=43.36 X-61.35$	0.9997	4.806-153.8	0.09	0.30	1.6	2.0	2.7

LOD: Limits of detection; LOQ: Limits of quantification; RSD: Relative standard deviations



**Figure 4:** Typical high performance liquid chromatographic chromatograms of multi-ingredients simultaneous determination of blank solution (a), standard solution (b), the sun-dried sample solution (c), and the sulfur-fumigated sample solution (d): (1) Xanthotoxol, (2) xanthotoxin, (3) isoimipinellin, (4) bergapten, (5) oxypeucedanin, (6) imperatorin, (7) isoimperatorin

could not be determined in the sun-dried samples, was relatively rich in the sulfur-fumigated samples.

From the results combining chromatographic fingerprint analysis and quantitative analysis, it can be seen that the sensitivities of coumarins in *Radix Angelicae Dahuricae* were quite different. Xanthotoxin, isoimipinellin, bergapten, oxypeucedanin, as well as xanthotoxol contributed to the significant difference between the sulfur-fumigated and

the sun-dried samples mainly. However, imperatorin and isoimperatorin were relatively resistant to sulfur-fumigation process. Because coumarins have already been proved to be the main bioactive composition of *Radix Angelicae Dahuricae*,<sup>[25,26]</sup> the lost of their contents would inevitably reduce the clinical effect of this herbal drug. Thus, it seems that the method documented in Chinese Pharmacopoeia for quality control of *Radix Angelicae Dahuricae* simply focusing on imperatorin content,<sup>[27]</sup> might not be so reasonable. In

**Table 3: Contents (µg/g) of seven coumarins in 19 batches of *Radix Angelicae Dahuricae***

Sample	Xanthotoxol	Xanthotoxin	Isoimipinellin	Bergapten	Oxypeucedanin	Imperatorin	Isoimperatorin
1	BI	136.7	56.2	237.9	4981.8	1620.0	857.5
2	BI	774.1	118.7	471.5	3982.3	2406.2	932.8
3	BI	220.3	49.1	115.9	2420.9	1605.4	605.9
4	BI	808.3	355.1	163.1	1244.9	1985.5	572.2
5	29.8	BI	14.2	BI	BI	713.2	601.2
6	77.9	BI	BI	55.6	BI	929.9	643.0
7	45.4	BI	BI	51.5	BI	1070.0	529.6
8	45.1	BI	12.7	73.6	BI	1083.6	704.3
9	82.9	31.8	BI	68.6	BI	908.7	626.9
10	55.6	106.7	13.5	82.5	BI	1710.2	615.0
11	124.8	133.7	BI	146.1	BI	2098.8	924.8
12	86.1	40.2	BI	50.4	BI	1914.4	582.6
13	44.7	39.0	BI	52.0	BI	1938.6	583.4
14	64.5	57.4	BI	61.9	BI	1734.8	544.6
15	53.2	47.5	BI	45.0	BI	1649.4	474.7
16	56.6	165.2	18.1	58.9	BI	1003.9	402.0
17	55.8	88.1	19.6	69.7	BI	2269.9	816.7
18	112.2	134.6	22.2	89.3	BI	1901.4	991.2
19	77.2	51.3	16.0	97.5	BI	3894.3	810.0
Mean±SD (1-4)	BI	484.9±355.7	144.8±143.7	247.1±157.8	3157.5±1654.2	1904.3±378.0	742.1±179.9
Mean±SD (5-19)	67.4±26.2 <sup>ab</sup>	59.7±54.3 <sup>a</sup>	7.8±8.8 <sup>a</sup>	66.8±31.5 <sup>a</sup>	BI <sup>ab</sup>	1654.7±795.9	656.7±164.4

BI: Below the lower limit of quantification; SD: Standard deviation. <sup>a</sup>*P*<0.001, compared with samples 1-4; <sup>b</sup>Sensitive biomarker

order to characterize *Radix Angelicae Dahuricae* before and after the sulfur-fumigation process, oxypeucedanin and xanthotoxol were the most sensitive biomarkers, whose contents should be determined.

## CONCLUSION

A novel, simple and informative HPLC method was developed for quality evaluation of *Radix Angelicae Dahuricae* before and after the sulfur-fumigation process. The combinative methods for chromatographic fingerprint analysis and multi-ingredients quantitative analysis were both reliable and applicable, which have been demonstrated by their successful application. Besides, the combination provided much more qualitative and quantitative information than any other singular method.

The assay results indicated that most of the coumarins in *Radix Angelicae Dahuricae* were sensitive to sulfur-fumigation process, which brought significant loss of these bioactive compounds and should be restricted during *Radix Angelicae Dahuricae* processing. The biomarkers for sulfur-fumigation characterization were finally discovered.

## ACKNOWLEDGMENTS

This research was financially supported by the National Natural Science Foundation of China (No. 81173546, No. 30940093, and No. 81202918), the Natural Science Foundation of Jiangsu Province, China (No. BK2009495), the International Science

and Technology Cooperation Project of Jiangsu Province, China (No. BZ2011053), the Project of Chinese Pharmacopoeia Commission, and the Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD) (No. 2011ZYX2-001)

## REFERENCES

- Li SL, Song JZ, Choi FF, Qiao CF, Zhou Y, Han QB, et al. Chemical profiling of *Radix Paeoniae* evaluated by ultra-performance liquid chromatography/photo-diode-array/quadrupole time-of-flight mass spectrometry. *J Pharm Biomed Anal* 2009;49:253-66.
- Li SL, Shen H, Zhu LY, Xu J, Jia XB, Zhang HM, et al. Ultra-high-performance liquid chromatography-quadrupole/time of flight mass spectrometry based chemical profiling approach to rapidly reveal chemical transformation of sulfur-fumigated medicinal herbs, a case study on white ginseng. *J Chromatogr A* 2012;1231:31-45.
- Kan WL, Ma B, Lin G. Sulfur fumigation processing of traditional Chinese medicinal herbs: Beneficial or detrimental? *Front Pharmacol* 2011;2:84-8.
- Cheng Y, Peng C, Wen F, Zhang H. Pharmacokinetic comparisons of typical constituents in white peony root and sulfur fumigated white peony root after oral administration to mice. *J Ethnopharmacol* 2010;129:167-73.
- Cottiglia F, Loy G, Garau D, Floris C, Casu M, Pompei R, et al. Antimicrobial evaluation of coumarins and flavonoids from the stems of *Daphne gnidium* L. *Phytomedicine* 2001;8:302-5.
- Kimura Y, Ohminami H, Arichi H, Okuda H, Baba K, Kozawa M, et al. Effects of various coumarins from roots of *Angelica dahurica* on actions of adrenaline, ACTH and insulin in fat cells. *Planta Med* 1982;45:183-7.
- Okuyama T, Takata M, Nishino H, Nishino A, Takayasu J, Iwashima A. Studies on the antitumor-promoting activity of naturally occurring substances. II. Inhibition

- of tumor-promoter-enhanced phospholipid metabolism by umbelliferous materials. *Chem Pharm Bull (Tokyo)* 1990;38:1084-6.
8. Hata K, Kozawa M, Ikeshiro Y. New coumarins isolated from the roots of *Angelica anomala* Lall. and *Angelica cartilaginomarginata* (Makino) Nakai (Umbelliferae). *Yakugaku Zasshi* 1967;87:1118-24.
  9. Kimura Y, Okuda H. Histamine-release effectors from *Angelica dahurica* var. *dahurica* root. *J Nat Prod* 1997;60:249-51.
  10. Kwon YS, Kobayashi A, Kajiyama S, Kawazu K, Kanzaki H, Kim CM. Antimicrobial constituents of *Angelica dahurica* roots. *Phytochemistry* 1997;44:887-9.
  11. Saiki Y, Morinaga K, Okegawa O, Sakai S, Amaya Y. Study on the coumarins of the roots of *Angelica dahurica* Benth. et Hook. *Yakugaku Zasshi* 1971;91:1313-7.
  12. Liang B, Xu LZ, Zou ZM, Yang SL. Chemical constituents isolated from *Angelica dahurica* var. *formosana*. *Chin Tradit Herb Drugs* 2005;36:1132-5.
  13. Wang MY, Jia MR, Ma YY, Tang SW, Jiang GH, Li XB. Studies on analgesic components of *Radix Angelicae Dahuricae*. *Chin Pharm J* 2005;40:583-5.
  14. Wei Y, Ito Y. Preparative isolation of imperatorin, oxypeucedanin and isoimperatorin from traditional Chinese herb "bai zhi" *Angelica dahurica* (Fisch. ex Hoffm) Benth. et Hook using multidimensional high-speed counter-current chromatography. *J Chromatogr A* 2006;1115:112-7.
  15. Zheng XG, Zhang XW, Sheng XN, Yuan ZF, Yang W, Wang Q, et al. Simultaneous characterization and quantitation of 11 coumarins in *Radix Angelicae Dahuricae* by high performance liquid chromatography with electrospray tandem mass spectrometry. *J Pharm Biomed Anal* 2010;51:599-605.
  16. Wang KT, Liu HT, Chen XG, Zhao YK, Hu ZD. Identification and determination of active components in *Angelica dahurica* Benth and its medicinal preparation by capillary electrophoresis. *Talanta* 2001;54:753-61.
  17. Kang J, Zhou L, Sun JG, Han J, Guo DA. Chromatographic fingerprint analysis and characterization of furocoumarins in the roots of *Angelica dahurica* by HPLC/DAD/ESI-MSn technique. *J Pharm Biomed Anal* 2008;47:778-85.
  18. Wang XH, Xie PS, Lam CW, Yan YZ, Yu QX. Study of the destructive effect to inherent quality of *Angelicae dahuricae* radix (Baizhi) by sulfur-fumigated process using chromatographic fingerprinting analysis. *J Pharm Biomed Anal* 2009;49:1221-5.
  19. Xie PS, Chen SB, Liang YZ, Wang XH, Tian RT, Upton R. Chromatographic fingerprint analysis – A rational approach for quality assessment of traditional Chinese herbal medicine. *J Chromatogr A* 2006;1112:171-80.
  20. Li Y, Wu T, Zhu J, Wan L, Yu Q, Li X, et al. Combinative method using HPLC fingerprint and quantitative analyses for quality consistency evaluation of an herbal medicinal preparation produced by different manufacturers. *J Pharm Biomed Anal* 2010;52:597-602.
  21. Wang TT, Jin H, Li Q, Cheng WM, Hu QQ, Chen XH, et al. The isolation and simultaneous determination of coumarin compounds in radix *Angelica dahurica*. *Chromatographia* 2007;65:477-81.
  22. Xu CJ, Liang YZ, Chau FT, Heyden YV. Pretreatments of chromatographic fingerprints for quality control of herbal medicines. *J Chromatogr A* 2006;1134:253-9.
  23. Wang TT, Chen XH, Hu QQ, Bi KS. RP-HPLC fingerprint for quality assessment of *Radix Angelicae dahuricae*. *Acta Pharm Sin* 2006;41:747-51.
  24. Wang SA, Ma HQ, Sun YJ, Qiao CD, Shao SJ, Jiang SX. Fingerprint quality control of *Angelica sinensis* (Oliv.) Diels by high-performance liquid chromatography coupled with discriminant analysis. *Talanta* 2007;72:434-6.
  25. Ban HS, Lim SS, Suzuki K, Jung SH, Lee S, Lee YS, et al. Inhibitory effects of furanocoumarins isolated from the roots of *Angelica dahurica* on prostaglandin E2 production. *Planta Med* 2003;69:408-12.
  26. Oh H, Lee HS, Kim T, Chai KY, Chung HT, Kwon TO, et al. Furocoumarins from *Angelica dahurica* with hepatoprotective activity on tacrine-induced cytotoxicity in Hep G2 cells. *Planta Med* 2002;68:463-4.
  27. Chinese Pharmacopoeia Committee. *Chinese Pharmacopoeia*. Vol. 1 (Edition 2010). Beijing: China Medical Science Press; 2010. p. 97-8.

**Cite this article as:** Liu X, Liu J, Cai H, Li S, Ma X, Lou Y, et al. Novel characterization of *Radix Angelicae Dahuricae* before and after the sulfur-fumigation process by combining high performance liquid chromatographic fingerprint and multi-ingredients determination. *Phcog Mag* 2014;10:338-45.

**Source of Support:** Nil, **Conflict of Interest:** None declared.