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Chromatographic fingerprint analysis and characterization of furocoumarins in the roots of *Angelica dahurica* by HPLC/DAD/ESI-MS^n technique

Jie Kang, Lei Zhou, Jianghao Sun, Jian Han, De-An Guo*

The State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University Health Science Center, No. 38 Xueyuan Road, Beijing 100083, PR China

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ABSTRACT

A high performance liquid chromatography–diode array detection–electrospray ionization tandem mass spectrometry (HPLC/DAD/ESI-MS^n) method was used for the chromatographic fingerprint analysis and characterization of furocoumarins in the roots of *Angelica dahurica* for the first time. Nine "common peaks" were identified by comparing with the retention time, UV and MS spectra of reference furocoumarins. The software "Similarity Evaluation System for Chromatographic Fingerprint of TCM" was used to evaluate the similarities of 13 batches of Baizhi samples collected from Henan, Zhejiang, Sichuan and Anhui provinces of China. The results indicated that the samples from different batches had similar HPLC fingerprints, and the method could be applied for the quality control of the roots of *Angelica dahurica*. In addition, a total of 20 furocoumarins were identified or tentatively characterized by HPLC/DAD/ESI-MS^n technique, and their fragmentation patterns in an electrospray ion trap mass spectrometer were also summarized.

1. Introduction

Angelica dahurica, belonging to Umbelliferae family, is a widely used traditional Chinese medicine (TCM). Its dry roots, Baizhi in Chinese, have been frequently used for the treatment of acne, ulcer, carbuncle, rheumatism, headache and toothache in Chinese clinics [1–3].

The HPLC fingerprint technique has been considered as a useful method in identification and quality evaluation of herbs and their related finished products in recent years, because the HPLC fingerprint could systematically and comprehensively exhibit the types and quantification of the components in the herbal medicines [4–7]. Till now, HPLC coupled with electrospray ionization tandem mass spectrometry has been a powerful technique for analysis and identification of chemical constituents in complex TCM systems [8–21]. One could simultaneously obtain UV and tandem mass spectra of the individual compound in a short time, then the components of herbal medicine will be identified or tentatively characterized based on the fragmentation patterns and reference to the literatures. Therefore, comparing with the traditional methodologies on TCM separation and isolation, the on-line combined technique had remarkable advantage in efficiency and economy.

In this paper, we established a HPLC fingerprint analysis method to control the quality of the roots of *A. dahurica*. There were nine "common peaks" identified in the fingerprint of Baizhi (peaks 1–9, Fig. 1). The correlation coefficients were obtained by a software "Similarity Evaluation System for Chromatographic Fingerprint of TCM" developed by Chinese Pharmacopeia Commission (CPC) [22-23]. The relative retention time (RRT) and relative peak areas (RPAs) of each common peak related to the reference peak were calculated, which could semi-quantitatively reflect the ingredients displayed in the chromatographic profile of the extract of herbs.

Moreover, a total of 20 furocoumarins were identified or tentatively characterized from the roots of *A. dahurica* by HPLC/DAD/ESI-MS^n technique (Fig. 2). The fragmentation patterns of the furocoumarins in an electrospray ion trap mass spectrometer were also summarized (Fig. 3).

To our knowledge, this is the first report on the chromatographic fingerprint analysis and characterization of furocoumarins in the roots of *A. dahurica* by HPLC/DAD/ESI-MS^n method.

2. Experimental

2.1. Materials and reagents

The samples of the roots of *A. dahurica* were collected from Henan, Zhejiang, Sichuan and Anhui provinces of China at different time period. The various sources of samples were shown in Table 1.

Xanthotoxol (**1**), isopimpinellin (**2**), bergapten (**3**), pabulenol (**4**), imperatorin (**5**), alloimperatorin (**6**), phelloptorin (**7**), and

* Corresponding author. Tel.: +86 10 82802024; fax: +86 10 82802700.
E-mail address: gda@bjmu.edu.cn (D.-A. Guo).

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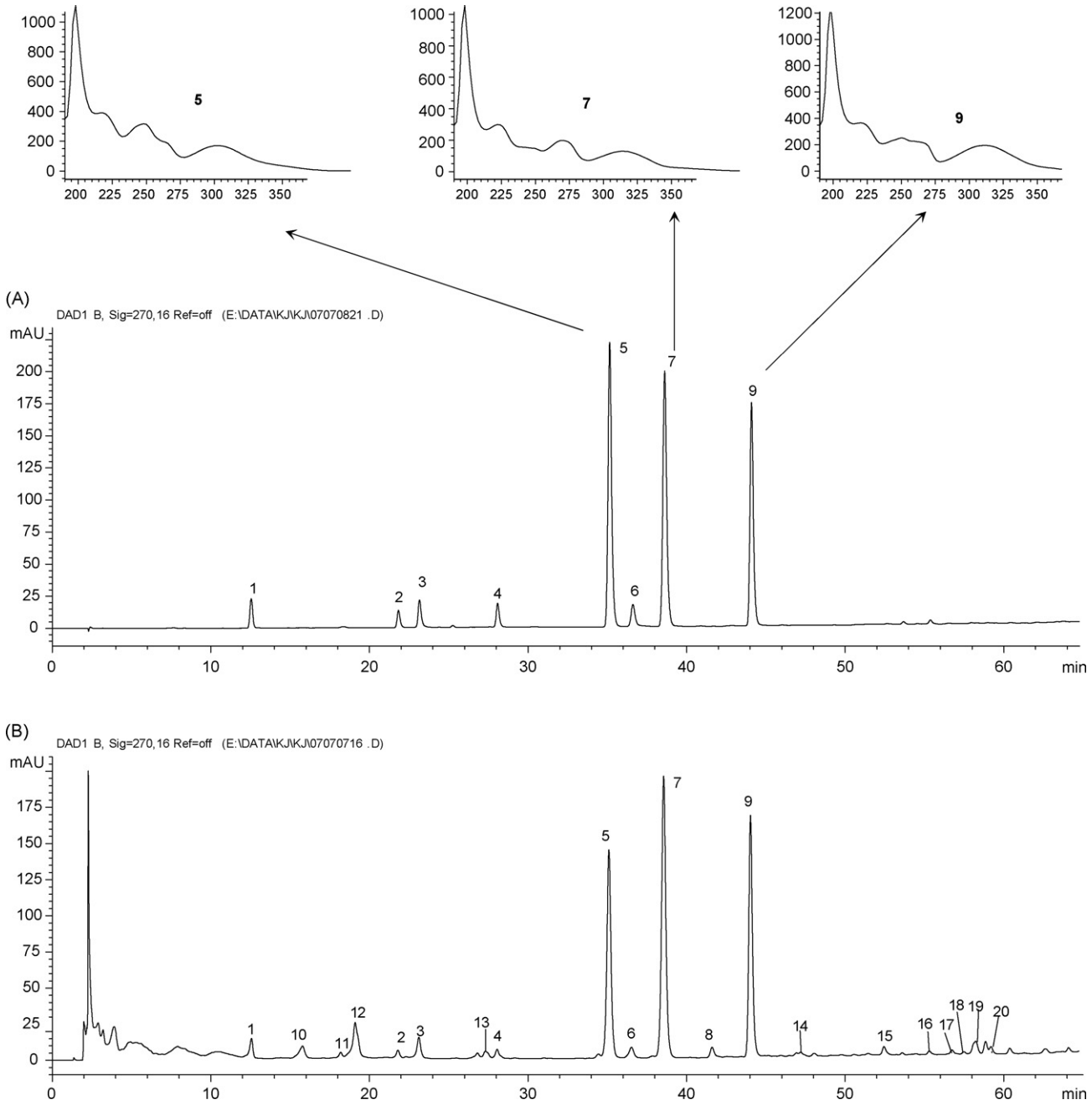

Fig. 1. The HPLC chromatograms of mixed standard compounds (A) and Baizhi samples collected from Sichuan I (B). (The serial number of peaks corresponding to that of compounds.)

isoimperatorin (**9**) were isolated from the roots of *A. dahurica* by the author, and their purities were over 98% determined by HPLC/UV analysis. Their structures were unambiguously identified by comparison of their ^{1}H, ^{13}C NMR and MS spectra with recorded literatures [24–30].

HPLC-grade methanol, purchased from Beijing Chemical Corporation (Beijing, China) and ultra-pure water were used for all analyses.

2.2. Instrumentation and chromatographic condition

2.2.1. HPLC instrumentation and chromatographic condition

The analyses were performed using an Agilent series 1100 HPLC system (Agilent, Waldbronn, Germany) equipped with a quaternary pump, a diode array detector (DAD), an autosampler, and a column

Table 1
Sources and collection dates of 13 batches of Baizhi samples

Sample number	Sources	Collection dates
1	Henan I	09/2006
2	Henan II	03/2007
3	Zhejiang I	11/2006
4	Zhejiang II	04/2007
5	Zhejiang III	02/2007
6	Sichuan I	03/2007
7	Sichuan II	10/2006
8	Sichuan III	12/2006
9	Sichuan IV	11/2006
10	Sichuan V	04/2007
11	Sichuan VI	01/2007
12	Anhui I	12/2006
13	Anhui II	03/2007