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

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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

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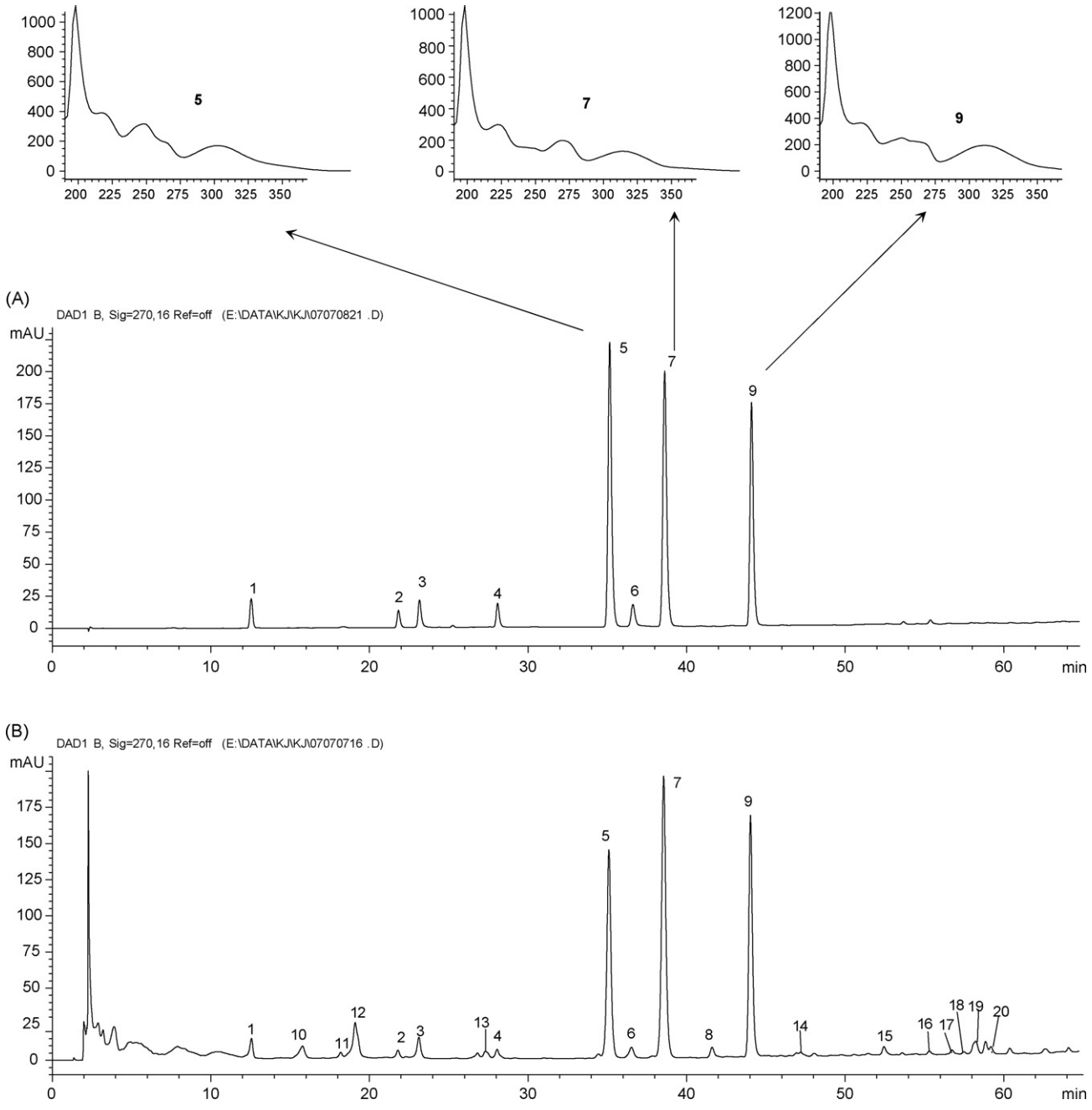


**Fig. 1.** The HPLC chromatograms of mixed standard compounds (A) and Baizhi samples collected from Sichuan I (B). (The serial number of peaks corresponding to that of compounds.)

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 |

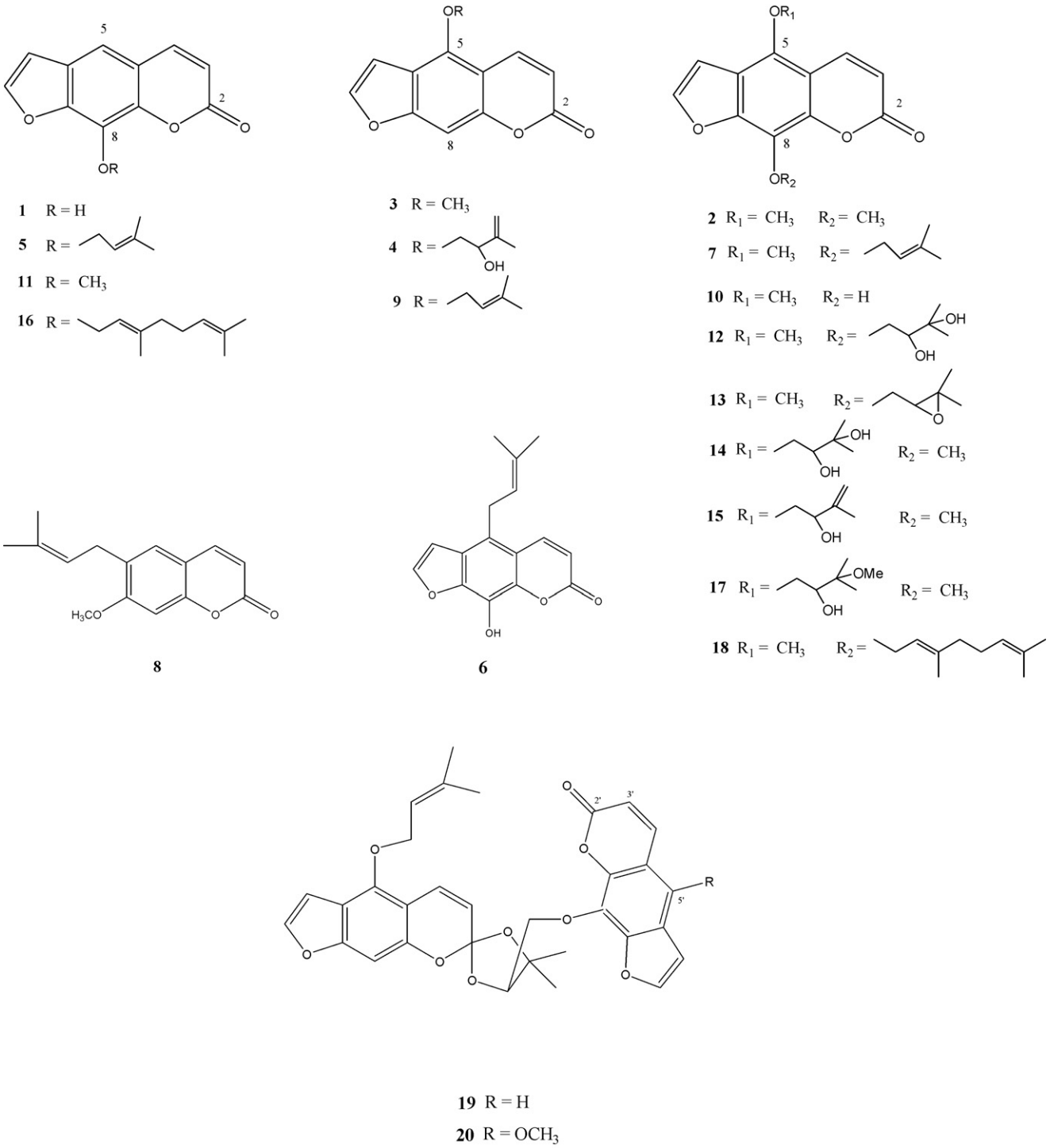


**Fig. 2.** Structures of furocoumarins identified or tentatively characterized from the roots of *Angelica dahurica*.

compartment. Chromatographic separation was carried out using an Agilent Zorbax Extend-$C_{18}$ column (5 μm, 250 mm × 4.6 mm, Agilent), operated at 25 °C.

The mobile phase consisted of methanol (A) and water (B) with a linear gradient elution at a flow rate of 1.0 mL/min. The elution program was as follows: 30–45% A (0–10 min); 45–60% A (10–25 min); 60–65% A (25–35 min); 65–75% A (35–45 min); 75–85% A (45–60 min); 85–90% A (60–65 min). The detection wavelength was at 270 nm.

### 2.2.2. HPLC/ESI-$MS^n$ instrumentation and chromatographic condition

For HPLC/ESI-$MS^n$ analysis, a Finnigan LCQ Advantage ion trap mass spectrometer (ThermoFinnigan, San Jose, CA, USA) was connected to the Agilent 1100 HPLC system via an ESI interface. The LC effluent was introduced into the ESI source in a post-column splitting ratio of 2:1. Ultra-high purity helium (He) was used as the collision gas and high purity nitrogen ($N_2$) as the nebulizing gas. The mass detector was optimized to obtain maximum yields of $[M+H]^+$ or $[M+Na]^+$ ions of the furocoumarins. The mass parameters in the

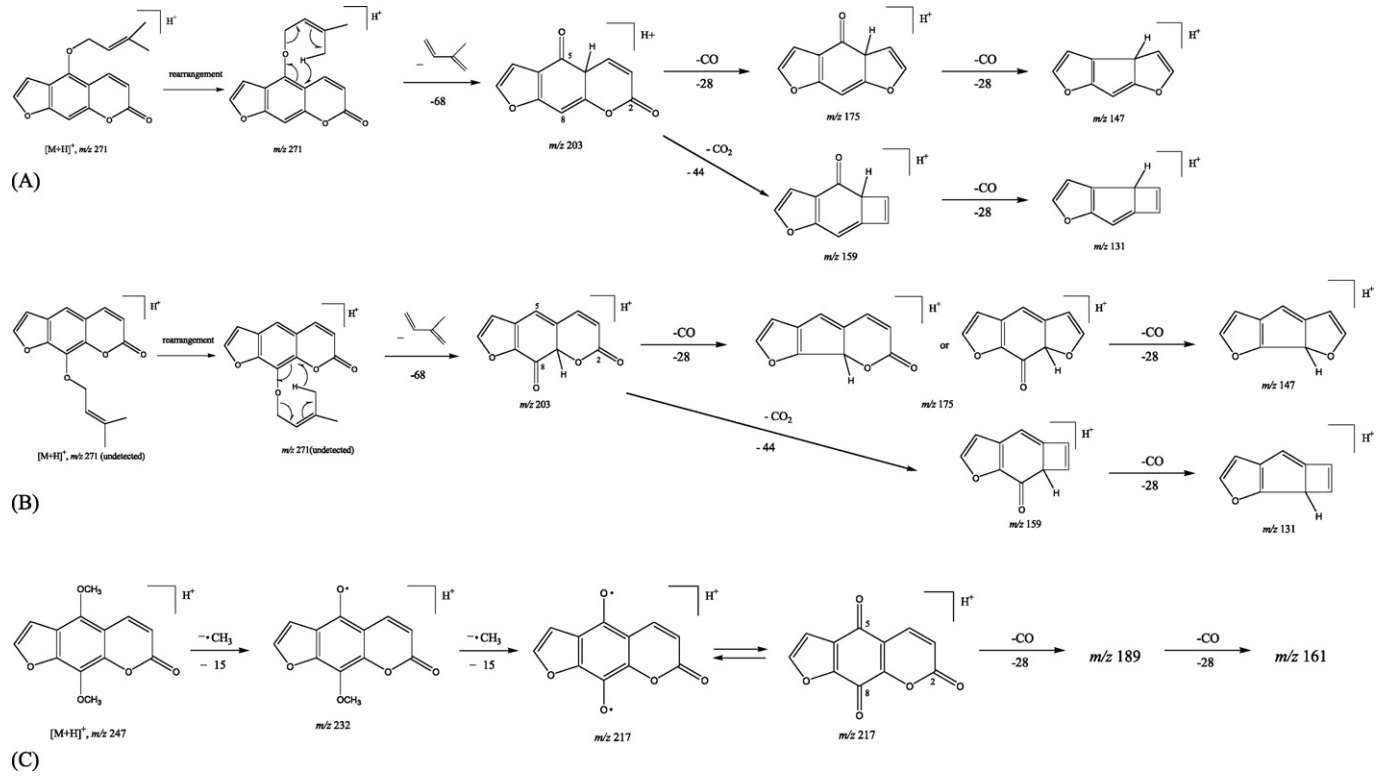


**Fig. 3.** Major MS fragmentation pathways proposed for compound **9** (isoimperatorin) (A). Major MS fragmentation pathways proposed for compound **5** (imperatorin) (B). Major MS fragmentation pathways proposed for compound **2** (isopimpinellin) (C).

**Table 2**
The RRT of nine common peaks in 13 batches of Baizhi samples

| Sample number | The RRT of common peaks | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Mean chromatogram | 0.3585 | 0.6214 | 0.6587 | 0.7992 | 1 | 1.0419 | 1.0984 | 1.1853 | 1.2544 |
| 1 | 0.3588 | 0.6224 | 0.6591 | 0.7993 | 1 | 1.0410 | 1.0982 | 1.1854 | 1.2548 |
| 2 | 0.3569 | 0.6209 | 0.6584 | 0.7990 | 1 | 1.0453 | 1.0985 | 1.1851 | 1.2547 |
| 3 | 0.3595 | 0.6214 | 0.6591 | 0.7994 | 1 | 1.0423 | 1.0983 | 1.1852 | 1.2543 |
| 4 | 0.3591 | 0.6210 | 0.6586 | 0.7989 | 1 | 1.0412 | 1.0985 | 1.1853 | 1.2538 |
| 5 | 0.3591 | 0.6219 | 0.6593 | 0.7994 | 1 | 1.0434 | 1.0986 | 1.1857 | 1.2547 |
| 6 | 0.3579 | 0.6210 | 0.6585 | 0.7992 | 1 | 1.0405 | 1.0983 | 1.1853 | 1.2544 |
| 7 | 0.3569 | 0.6218 | 0.6586 | 0.7990 | 1 | 1.0432 | 1.0984 | 1.1860 | 1.2548 |
| 8 | 0.3575 | 0.6206 | 0.6579 | 0.7987 | 1 | 1.0411 | 1.0984 | 1.1858 | 1.2545 |
| 9 | 0.3584 | 0.6214 | 0.6587 | 0.7991 | 1 | 1.0415 | 1.0986 | 1.1854 | 1.2546 |
| 10 | 0.3590 | 0.6213 | 0.6589 | 0.7992 | 1 | 1.0417 | 1.0983 | 1.1850 | 1.2541 |
| 11 | 0.3593 | 0.6216 | 0.6590 | 0.7992 | 1 | 1.0419 | 1.0982 | 1.1847 | 1.2540 |
| 12 | 0.3586 | 0.6213 | 0.6588 | 0.7992 | 1 | 1.0410 | 1.0983 | 1.1855 | 1.2548 |
| 13 | 0.3589 | 0.6208 | 0.6586 | 0.7990 | 1 | 1.0410 | 1.0981 | 1.1851 | 1.2543 |

**Table 3**
The RPAs of nine common peaks in 13 batches of Baizhi samples

| Sample number | The RPAs of common peaks | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Mean chromatogram | 0.0570 | 0.0305 | 0.0906 | 0.0327 | 1 | 0.0381 | 1.0098 | 0.0459 | 0.6764 |
| 1 | 0.0868 | 0.0337 | 0.0755 | 0.0172 | 1 | 0.0507 | 1.0023 | 0.0321 | 0.6535 |
| 2 | 0.0198 | 0.0164 | 0.1134 | 0.0460 | 1 | 0.0317 | 1.2300 | 0.0288 | 0.7251 |
| 3 | 0.0476 | 0.1113 | 0.1716 | 0.0575 | 1 | 0.0322 | 1.0962 | 0.0789 | 0.7566 |
| 4 | 0.0684 | 0.0230 | 0.1025 | 0.0366 | 1 | 0.0414 | 0.9273 | 0.0508 | 0.6511 |
| 5 | 0.0373 | 0.0350 | 0.1062 | 0.0402 | 1 | 0.0513 | 1.3856 | 0.0764 | 0.8206 |
| 6 | 0.0888 | 0.0319 | 0.0962 | 0.0444 | 1 | 0.0634 | 1.4147 | 0.0486 | 1.0142 |
| 7 | 0.0994 | 0.0323 | 0.0644 | 0.0141 | 1 | 0.1450 | 0.9958 | 0.0449 | 0.8603 |
| 8 | 0.0258 | 0.0625 | 0.1069 | 0.0551 | 1 | 0.0277 | 0.8686 | 0.0777 | 0.7096 |
| 9 | 0.0314 | 0.0131 | 0.0690 | 0.0172 | 1 | 0.0433 | 1.3546 | 0.0409 | 0.9021 |
| 10 | 0.0699 | 0.0195 | 0.0707 | 0.0198 | 1 | 0.0413 | 1.0345 | 0.0346 | 0.6848 |
| 11 | 0.0489 | 0.0242 | 0.0738 | 0.0233 | 1 | 0.0261 | 0.8011 | 0.0345 | 0.4920 |
| 12 | 0.0747 | 0.0205 | 0.0770 | 0.0194 | 1 | 0.0338 | 0.8843 | 0.0409 | 0.7112 |
| 13 | 0.0725 | 0.0163 | 0.0983 | 0.0237 | 1 | 0.0224 | 0.6939 | 0.0255 | 0.4119 |

positive ion mode were optimized as follows: ion spray voltage, 4.5 kV; sheath gas ($N_2$) pressure, 50 a.u.; auxiliary gas ($N_2$) pressure, 10 a.u.; capillary temperature, 330 °C; capillary voltage, 5 V. For full-scan MS analysis, the spectra were recorded in the range of $m/z$ 80–600. A data-dependent program was used in the liquid chromatography coupled with tandem mass spectrometry analysis so that the two most abundant ions in each scan were selected and subjected to $MS^2$ and $MS^n$ ($n$ = 3–4) analyses. The collision energy for $MS^n$ was adjusted to 45% in LC/MS analysis. The isolation width of the precursor ions was 1.6 Th.

#### 2.2.3. Software

The software "Similarity Evaluation System for Chromatographic Fingerprint of TCM" was published by CPC (Version 2004A) and mainly applied to the similarity analysis of chromatographic patterns. The software about the calculation of correlation coefficients was mainly based on the peak area and retention time. The chromatograms should be introduced in the form of AIA (*.cdf), which include the information of peak areas and retention time. Then the marker peaks were chosen by the user and all peaks would be matched. Subsequently, the mean chromatogram was produced, and the correlation coefficients of all introduced chromatograms relative to that of mean chromatogram would be calculated. If the correlation coefficients were higher than 0.900, it suggested that the qualities of samples were relatively consistent. The mathematical theories of the software were based on principal component analysis (PCA) and fuzzy information analysis, which were suitable for complicated system. In a word, the software made the analysis method become accurate and rapid.

### 2.3. Sample preparation

An aliquot of 0.2 g drug powder was extracted with 2 mL of methanol by ultrasonic for 40 min and then filtered through a 0.45 μm filter membrane before use. A volume of 20 μL was injected into the HPLC system for analysis.

### 2.4. Data analysis of chromatogram

The correlation coefficients of chromatograms of 13 batches of Baizhi samples were calculated by the software "Similarity Evaluation System for Chromatographic Fingerprint of TCM". There were nine "common peaks" in Baizhi fingerprints (peaks 1–9, Fig. 1). The RRT and RPA of each "common peak" related to the reference peak were calculated in the chromatographic patterns.

## 3. Results and discussion

### 3.1. Optimization of HPLC/DAD/ESI-$MS^n$ system

To obtain chromatograms with good separation and strong total ion current (TIC), we used MeOH/$H_2O$ as the mobile phase, because MeOH could apparently improve ionization efficiency of the furocoumarins in positive ion mode. In addition, the retention behavior of furocoumarins on the HPLC column and the intensity of TIC were not remarkably improved by adding acid in mobile phase. Therefore, MeOH/$H_2O$ was the optimal choice in both HPLC and ESI-MS analyses.

The wavelength for the detection of compounds in the roots of *A. dahurica* was selected by DAD. It appeared that the chromatograms at 270 nm and 320 nm could represent the profile of the extract. The chromatogram at 270 nm and the UV spectra of three main furocoumarins, imperatorin (**5**), phelloptorin (**7**) and isoimperatorin (**9**) were all shown in Fig. 1.

**Table 4**
The similarities (correlation coefficients) of 13 batches of Baizhi samples

| Sample number | Similarities |
|---|---|
| 1 | 0.989 |
| 2 | 0.970 |
| 3 | 0.975 |
| 4 | 0.985 |
| 5 | 0.973 |
| 6 | 0.962 |
| 7 | 0.975 |
| 8 | 0.981 |
| 9 | 0.967 |
| 10 | 0.980 |
| 11 | 0.988 |
| 12 | 0.986 |
| 13 | 0.981 |

### 3.2. Method validation

The injection precision was determined by replicated injection of the same sample six times in 1 day. The relative standard deviations (R.S.D.s) of retention time and peak areas of nine "common peaks" were lower than 0.10% and 3.45%, respectively.

The repeatability was evaluated by analyzing six independently prepared samples of Baizhi. The R.S.D.s of retention time and peak areas of nine "common peaks" were lower than 0.19% and 3.72%, respectively.

The stability test was assessed by successive injection of the same sample in 0 h, 2 h, 4 h, 6 h, 8 h, 10 h, and 24 h. The R.S.D.s of retention time and peak areas of nine "common peaks" were lower than 0.12% and 3.26%, respectively.

All the results indicated that the method for the fingerprint analysis of Baizhi was adequate and applicable.

### 3.3. HPLC fingerprints of roots of A. dahurica

To obtain the standardized fingerprint, 13 batches of Baizhi samples were analyzed. There were nine "common peaks" identified in the fingerprint of Baizhi (peaks 1–9, Fig. 1). Among them, the shape

**Table 5**
$^1$H NMR spectral data for compounds **1**–**7** and **9** in acetone-$d_6$ (400 MHz)

| Number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 9 |
|---|---|---|---|---|---|---|---|---|
| H-3 | 6.31d (10.0) | 6.25d (10.0) | 6.24d (10.0) | 6.23d (10.0) | 6.34d (9.6) | 6.30d (10.0) | 6.25d (10.0) | 6.25d (9.9) |
| H-4 | 8.01d (10.0) | 8.17d (10.0) | 8.19d (10.0) | 8.33d (10.0) | 8.03d (9.6) | 8.17d (10.0) | 8.18d (10.0) | 8.21dd (9.9,0.6) |
| H-5 | 7.42s | – | – | – | 7.61s | – | – | – |
| H-8 | – | – | 7.15s | 7.18s | – | – | – | 7.17t (0.6) |
| H-2′ | 7.91d (2.0) | 7.90d (2.4) | 7.86d (2.0) | 7.86d (2.4) | 7.94d (2.0) | 7.90brs | 7.89d (2.4) | 7.86d (2.4) |
| H-3′ | 6.96d (2.0) | 7.27d (2.4) | 7.31d (2.0) | 7.26d (2.4) | 7.00d (2.0) | 7.05brs | 7.26d (2.4) | 7.25dd (2.4,0.6) |
| H-1″ | – | – | – | 4.49m (2H) | 4.97d (2H, 5.1) | 3.78d (2H, 5.2) | 4.80d (2H, 7.2) | 5.03d (2H, 6.6) |
| H-2″ | – | – | – | 4.59m | 5.56t (5.1) | 5.17t (5.2) | 5.55 t (7.2) | 5.59 t (6.6) |
| H-4″ | – | – | – | 4.96s, 5.17s | 1.70s (3H) | 1.85s (3H) | 1.68s (3H) | 1.76s (3H) |
| H-5″ | – | – | – | 1.81s (3H) | 1.68s (3H) | 1.67s (3H) | 1.66s (3H) | 1.70s (3H) |
| $OCH_3$-5 | – | 4.23s (3H) | 4.33s (3H) | – | – | – | 4.23s (3H) | – |
| $OCH_3$-8 | – | 4.08s (3H) | – | – | – | – | – | – |

**Table 6**
$^{13}$C NMR spectral data for compounds **1**–**7** and **9** in acetone-$d_6$ (100 MHz)

| Number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 9 |
|---|---|---|---|---|---|---|---|---|
| C-2 | 160.4 | 160.3 | 160.7 | 160.8 | 160.4 | 160.3 | 160.3 | 160.8 |
| C-3 | 114.9 | 113.5 | 113.3 | 113.3 | 115.2 | 114.2 | 113.4 | 113.3 |
| C-4 | 145.8 | 140.1 | 139.8 | 140.3 | 145.5 | 142.5 | 146.5 | 140.0 |
| C-5 | 111.2 | 144.8 | 150.8 | 145.7 | 114.8 | 123.7 | 145.6 | 146.3 |
| C-6 | 126.6 | 115.6 | 113.5 | 114.7 | 126.8 | 126.1 | 115.4 | 115.0 |
| C-7 | 145.3 | 151.0 | 159.3 | 159.0 | 149.4 | 147.1 | 151.7 | 159.0 |
| C-8 | 130.1 | 128.8 | 93.9 | 94.3 | 132.1 | 129.8 | 127.3 | 94.3 |
| C-9 | 139.8 | 145.5 | 153.8 | 153.7 | 144.9 | 141.0 | 144.5 | 153.7 |
| C-10 | 117.2 | 108.2 | 107.0 | 107.9 | 117.6 | 114.7 | 108.2 | 108.1 |
| C-2′ | 148.0 | 146.6 | 146.2 | 146.4 | 148.1 | 147.4 | 140.1 | 146.4 |
| C-3′ | 107.7 | 106.2 | 106.3 | 106.1 | 107.7 | 106.7 | 106.2 | 106.2 |
| C-1″ | – | – | – | 74.5 | 70.4 | 28.2 | 70.5 | 70.4 |
| C–2″ | – | – | – | 76.8 | 120.9 | 123.8 | 121.0 | 120.3 |
| C-3″ | – | – | – | 150.1 | 139.8 | 132.6 | 139.6 | 150.0 |
| C-4″ | – | – | – | 112.6 | 25.8 | 25.7 | 25.8 | 25.8 |
| C-5″ | – | – | – | 18.8 | 18.0 | 18.1 | 18.0 | 18.2 |
| $OCH_3$-5 | – | 61.7 | 60.7 | – | – | – | 61.3 | – |
| $OCH_3$-8 | – | 61.3 | – | – | – | – | – | – |

of common peak 5 was symmetrical and its position was in the middle of the chromatogram (imperatorin, Fig. 1). So the peak 5 was used as reference to calculate the RRT (Table 2) and RPAs (Table 3). The calculating formulas of RRT and RPAs were $RRT = RT_{peak}/RT_{peak5}$ and $RPA = PA_{peak}/PA_{peak5}$, respectively. The significance of calculating RRT and RPAs was to make the various absolute values become stable, which could semi-quantitatively reflect the constituents displayed in the chromatographic profile of the extract of herbs.

The software "Similarity Evaluation System for Chromatographic Fingerprint of TCM" was used to evaluate these chromatograms. "Common peaks" 5, 7 and 9 were adopted as marker to match peaks. The correlation coefficients of chromatograms of 13 batches of Baizhi samples comparing with that of mean chromatogram are very close to 1 (Table 4). The data showed that the chromatograms of 13 batches of Baizhi samples were consistent, and the whole chromatograms provide applicable means of assessing the quality of Baizhi. Though the chromatograms of 13 batches of Baizhi samples were generally similar, the peak area ratios of the "common peaks" or some "small peaks" were distinctly different. The difference in HPLC fingerprint chromatograms displayed various qualities of different batches of herbal medicines. The HPLC fingerprint analysis played a significant role in the authentication and evaluation for the quality of herbal medicines.

### 3.4. Identification of furocoumarins in fingerprint chromatograms

#### 3.4.1. Identification of reference furocoumarins

The structures of eight reference furocoumarins were unambiguously identified by comparing their $^1$H, $^{13}$C NMR and MS spectra with recorded literatures, and they were identified as the known compounds xanthotoxol (**1**) [24], isopimpinellin (**2**) [24,25], bergapten (**3**) [25,26], pabulenol (**4**) [27,28], imperatorin (**5**) [29], alloimperatorin (**6**) [30], phelloptorin (**7**) [26], and isoimperatorin (**9**) [29]. Their $^1$H NMR and $^{13}$C NMR spectral data were shown in Tables 5 and 6.

#### 3.4.2. Tandem mass spectrometry of pure standards

The ESI-MS$^n$ spectra were detected in both positive and negative ion modes, while more abundant information was obtained in positive ion mode. Therefore, positive ESI was selected for the analysis.

The fragmentation behavior of linear-type furocoumarins in the roots of *A. dahurica* in an electrospray ion trap mass spectrometer was studied. Though only two positions (C-5 and C-8) of these compounds could be substituted, their fragmentation patterns were very different.

Most substituent groups of the linear-type furocoumarins in Baizhi were oxysubstituent groups, and substituent positions were at C-5 or C-8. According to their fragmentation patterns in ESI-MS$^n$ spectra, we proposed some rules on determining the substituent positions and summarized in the following paragraphs.

If isopentenoxy group was at C-5 and no substituent at C-8, such as isoimperatorin (**9**), the ESI-MS spectrum exhibited a $[M+H]^+$ ion of $m/z$ 271 as the base peak. The protonated molecular ion was further fragmented by neutral loss of a rearranged isopentenyl moiety (Fig. 3), leading to the formation of a predominant ion at $m/z$ 203. The ion at $m/z$ 203 $[M+H-68]^+$ was then subjected to MS$^3$ analysis and produced ions at $m/z$ 175 $[M+H-68-CO]^+$ and 159 $[M+H-68-CO_2]^+$. In particular, the relative abundance of $m/z$ 159 was larger than that of $m/z$ 175, which was diagnostic for identification of the compound possessing isopentenoxy group at C-5. The two ions, $m/z$ 175 and 159, further produced signals at $m/z$ 147 $[M+H-68-2CO]^+$ and 131 $[M+H-68-CO_2-CO]^+$ in MS$^4$ spectrum.

If isopentenoxy group was at C-8 and no substituent at C-5, such as imperatorin (**5**), the protonated molecular ion of $m/z$ 271 was not observed in ESI-MS spectrum. Instead, $[M+Na]^+$ ion at $m/z$ 293 and loss of a rearranged isopentenyl fragment ion at $m/z$ 203 $[M+H-68]^+$ were detected. The two ions were separately introduced to MS$^2$ experiment. Ion at $m/z$ 225 $[M+Na-68]^+$ was produced in MS$^2$ analysis of sodium adduct ion, which could only be rationalized by neutral loss of a rearranged isopentenyl moiety. In addition, the ion at $m/z$ 203 $[M+H-68]^+$ produced the same mass number ions at $m/z$ 175 $[M+H-68-CO]^+$ and 159 $[M+H-68-CO_2]^+$ in MS$^2$ spectrum as that of isoimperatorin (**9**) in MS$^3$ analysis under the same energy (Fig. 3). But the relative abundance of the two ions of imperatorin (**5**) was opposite to that of isoimperatorin (**9**): the relative abundance of $m/z$ 175 was larger than that of $m/z$ 159. The ratio of relative abundance of two ions combined with the fragmentation pattern of sodium adduct ion suggested the isopentenoxy group to be at C-8. The ions at $m/z$ 175 and 159 further separately produced signals at $m/z$ 147 $[M+H-68-2CO]^+$ and 131 $[M+H-68-CO_2-CO]^+$ in MS$^3$ equal to that of isoimperatorin (**9**) in MS$^4$ analysis.

If alkoxy moieties at C-5 and C-8, like isopimpinellin (**2**), the protonated ion ($m/z$ 247) was shown in ESI-MS spectrum. In MS$^2$ and MS$^3$ spectra, the ions at $m/z$ 232 $[M+H-CH_3]^+$ and 217 $[M+H-2CH_3]^+$ were observed due to successive elimination of methyl radical moiety (Fig. 3). Then the MS$^4$ spectrum displayed ions at $m/z$ 189 $[M+H-2CH_3-CO]^+$ and 161 $[M+H-2CH_3-2CO]^+$ resulting from suc-

**Table 7**
Characterization of furanocoumarins in Bai-zhi by HPLC/ESI-MS$^n$

| Number | Retention time (min) | Assigned identity | UV $\lambda_{max}$ (nm) | (+)ESI-MS $m/z$ | HPLC–ESI-MS$^n$ $m/z$ (% base peak) | References |
|---|---|---|---|---|---|---|
| 1 | 12.81 | Xanthotoxol | 262, 308 | 203 $[M+H]^+$ | $MS^2$[203]: 175(100), 159(15), 147(60), 131(20) | [24] |
| 2 | 21.99 | Isopimpinellin | 268, 312 | 247 $[M+H]^+$ | $MS^2$[247]: 232(100)<br>$MS^3$[247 → 232]: 217(100)<br>$MS^4$[247 → 232 → 217]: 189(100) | [24,25] |
| 3 | 23.18 | Bergapten | 268, 312 | 217 $[M+H]^+$ | $MS^2$[217]: 202(100)<br>$MS^3$[217 → 202]: 174(100) | [25,26] |
| 4 | 28.20 | Pabulenol | 260, 310 | 287 $[M+H]^+$ | $MS^2$[287]: 203(100)<br>$MS^3$[287 → 203]: 175(30), 159(100), 147(28) | [27,28] |
| 5 | 35.14 | Imperatorin | 248, 302 | 293 $[M+Na]^+$<br>203 $[M+H-68]^+$ | $MS^2$[293]: 225(100)<br>$MS^2$[203]: 175(98), 159(30), 147(100), 131(10) | [29] |
| 6 | 36.87 | Alloimperatorin | 272, 318 | 271 $[M+H]^+$ | $MS^2$[271]: 229(100)<br>$MS^3$[271 → 229]: 211(100), 201(70), 183(30), 155(40) | [30] |
| 7 | 38.63 | Phelloptorin | 270, 315 | 323 $[M+Na]^+$<br>233 $[M+H-68]^+$ | $MS^2$[323]: 254(100)<br>$MS^3$[323 → 254]: 239(100)<br>$MS^2$[233]: 218(100)<br>$MS^3$[233 → 218]: 190(90), 162(100) | [26] |
| 8 | 41.83 | Suberosin | 266, 312 | 245 $[M+H]^+$ | $MS^2$[245]: 189(100)<br>$MS^3$[245 → 189]: 161(50), 131(100) | [31] |
| 9 | 44.10 | Isoimperatorin | 260, 312 | 271 $[M+H]^+$ | $MS^2$[271]: 203(100)<br>$MS^3$[271 → 203]: 175(50), 159(100), 147(80), 131(10) | [29] |
| 10 | 16.03 | 5-Methoxy-8-hydroxypsoralen | 274, 318 | 233 $[M+H]^+$ | $MS^2$[233]: 218(100)<br>$MS^3$[233 → 218]: 190(100)<br>$MS^4$[233 → 218 → 190]: 162(100) | [32] |
| 11 | 18.35 | Xanthotoxin | 264, 304 | 217 $[M+H]^+$ | $MS^2$[217]: 202(100)<br>$MS^3$[217 → 202]: 174(100) | [33] |
| 12 | 19.22 | Byakangelicin | 268, 312 | 357 $[M+Na]^+$ | $MS^2$[357]: 254(100)<br>$MS^3$[357 → 254]: 239(100) | [26] |
| 13 | 27.52 | Byakangelicol | 266, 312 | 339 $[M+Na]^+$ | $MS^2$[339]: 254(100)<br>$MS^3$[339 → 254]: 239(100) | [34] |
| 14 | 47.60 | Isobyakangelicin | 286, 312 | 335 $[M+H]^+$ | $MS^2$[335]: 317(100)<br>$MS^3$[335 → 317]: 233(100)<br>$MS^4$[335 → 317 → 233]: 218(100) | [35] |
| 15 | 52.74 | Apaensin | 258, 318 | 317 $[M+H]^+$ | $MS^2$[317]: 233(100)<br>$MS^3$[317 → 233]: 218(100) | [36] |
| 16 | 55.42 | 8-Geranoxypsoralen | 280, 322 | 361 $[M+Na]^+$ | $MS^2$[361]: 225(100) | [37] |
| 17 | 57.04 | Iso-*tert*-O-methylbyakangelicin | 290, 322 | 349 $[M+H]^+$ | $MS^2$[349]: 317(100)<br>$MS^3$[349 → 317]: 233(100) | [38] |
| 18 | 57.56 | 8-Geranoxy-5-methoxypsoralen | 284, 322 | 391 $[M+Na]^+$ | $MS^2$[391]: 254(100) | [39] |
| 19 | 58.49 | 5′-Demethoxy-isodahuribirin A | 292, 308 | 557 $[M+H]^+$ | $MS^2$[557]: 489(100)<br>$MS^3$[557 → 489]: 203(100) | [40] |
| 20 | 59.44 | Isodahuribirin A | 290, 332 | 587 $[M+H]^+$ | $MS^2$[587]: 519(100)<br>$MS^3$[587 → 519]: 233(100)<br>$MS^4$[587 → 519 → 233]: 218(100) | [40] |

cessive loss of neutral CO. However, the ion of neutral loss of $CO_2$ was not observed.

*3.4.3. Fragmentation pattern*

For mono-oxysubstituting furocoumarins, if the isopentenoxy group was at C-5 or C-8, they could produce fragment ions at *m*/*z* 203 in MS or $MS^2$. The fragment ions (*m*/*z* 203) as the precursor subjected to the next stage ESI-MS analysis, produced ions at *m*/*z* 175 $[203\text{-}CO]^+$ and 159 $[203\text{-}CO_2]^+$. If isopentenoxy group was at C-5, the relative abundance of *m*/*z* 159 was larger than that of *m*/*z* 175. However, if isopentenoxy group was at C-8, the result was on the contrary. This was probably attributed to different π-conjugation extensions. If the isopentenoxy group was at C-5, its two carbonyl groups at C-2 and C-5 (*m*/*z* 203) did not form π–π-conjugation moiety (Fig. 3), while for isopentenoxy group at C-8, its two carbonyl groups at C-2 and C-8 (*m*/*z* 203) formed π–π-conjugation (Fig. 3). It was most likely easy for π-conjugation moiety losing CO at C-8 and non-π-conjugation moiety losing $CO_2$ at C-2. That is to say, when the isopentenoxy group was at C-8, it simultaneously had two fragmentation patterns to lose CO (C-8 and C-2), and the relative abundance of *m*/*z* 175 (loss of CO) was larger than that of *m*/*z* 159 (loss of $CO_2$); but when the isopentenoxy group was at C-5, only one fragmentation pathway of losing CO (C-2) was present, and the relative abundance of *m*/*z* 175 (loss of CO) was smaller than that of *m*/*z* 159 (loss of $CO_2$).

If the alkoxy moieties were both at C-5 and C-8, its fragmentation pattern was loss of CO, but loss of $CO_2$ was not observed. The reason why neutral loss of CO was the main fragmentation behavior was probably because its carbonyl groups at C-5 and C-8 (*m*/*z* 217) not only formed π–π-conjugation with carbonyl group at C-2, but also formed homo-quinone conjugation (Fig. 3).

The results suggested that the different conjugate modes caused different fragmentation patterns, and the method may be potential for application in the determination of the substituent positions of linear-type furocoumarins in the roots of *A. dahurica*.

*3.4.4. Identification of components in fingerprint chromatograms*

A total of 20 furocoumarins were identified or tentatively characterized (Table 7). Comparing the mass spectra and retention times of the components of the extract with those of the corresponding standards, peaks 1–7 and 9 could be unambiguously assigned as xanthotoxol (**1**), isopimpinellin (**2**), bergapten (**3**), pabulenol (**4**), imperatorin (**5**), alloimperatorin (**6**), phelloptorin (**7**), and isoimperatorin (**9**), respectively. The other 12 furocoumarins were tentatively identified according to their ESI-MS and $MS^n$ fragmentation rules based on the above standards, and also by studying their UV spectral characteristics and reported literatures [26,31–40]. Accordingly, the peaks 8 and 10–20 were ascribed to suberosin (**8**) [31], 5-methoxy-8-hydroxypsoralen (**10**) [32], xanthotoxin (**11**) [33], byakangelicin (**12**) [26], byakangelicol (**13**) [34], isobyakangelicin (**14**) [35], apaensin (**15**) [36], 8-geranoxypsoralen (**16**) [37], iso-*tert*-*O*-methylbyakangelicin (**17**) [38], 8-geranoxy-5-methoxypsoralen (**18**) [39], 5′-demethoxy-isodahuribirin A (**19**) [40], and isodahuribirin A (**20**) [40]. Among them, components **17**, **19** and **20** were new and **14**, **15**, **16**, **18** were first reported from the species.

## 4. Conclusions

A HPLC method was developed to obtain a fingerprint of the furocoumarins from the roots of *A. dahurica*. The similarities of 13 batches of Baizhi samples collected from Henan, Zhejiang, Sichuan and Anhui provinces were obtained with a standardized procedure. To our knowledge, this is the first report on the analysis of furocoumarins in Baizhi by HPLC/DAD/ESI-$MS^n$ technique, and the fragmentation patterns of the furocoumarins in an electrospray ion trap mass spectrometer were also discussed. A total of 20 furocoumarins were identified or tentatively characterized. All the results proved that the technique was useful in comprehensive quality evaluation of Baizhi.

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