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Simultaneous characterization and quantitation of 11 coumarins in *Radix Angelicae Dahuricae* by high performance liquid chromatography with electrospray tandem mass spectrometry

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ABSTRACT

A high performance liquid chromatography with electrospray tandem mass spectrometry (HPLC–ESI-MS/MS) method has been developed to characterize and quantify 11 coumarin compounds in *Radix Angelicae Dahuricae* simultaneously. By using this HPLC–ESI-MS/MS method, all 11 coumarins were separated and determined within 10 min. These coumarins were detected by ESI^+ ionization method and quantified by multiple reaction monitor (MRM). The linear regressions were acquired with $r^2 > 0.995$, respectively. The precision was evaluated by intra- and inter-day tests, and relative standard deviation (R.S.D.) values were reported within the range of 1.14–4.42% and 0.37–4.00%. The recovery studies for the quantified compounds were observed over the range of 92.1–105.6% with R.S.D. values less than 4.55%. It demonstrated that the method developed was successfully applied for identification and quantification of 11 coumarins in *Radix Angelicae Dahuricae*. The results showed that the contents of coumarins in *Radix Angelicae Dahuricae* were processed differently and varied significantly.

1. Introduction

Radix Angelicae Dahuricae, dried radix of *Angelica dahurica* (Fisch. ex Hoffm.) Benth. et Hook. f. and *Angelica dahurica* (Fisch. ex Hoffm.) Benth. et Hook. f. var. *formosana* (Boiss.) Shan et Yuan, is a well-known traditional Chinese medicine (TCM) for over 2000 years for the treatments of cold, headache, toothache, coryza, psoriasis, leucorrhea, etc. [1].

Up to now, *Radix Angelicae Dahuricae* is known to contain a large number of compounds including volatile oil, coumarins and glycosides. Among these compounds, coumarins are generally considered as the major components, such as oxypeucedanin, bergapten, imperatorin, cnidilin, isoimperatorin, xanthotoxol, byakangelicin, etc. [2–5]. Pharmacological studies and clinical practice demonstrated that coumarins had remarkable activities as antihistamine [6], spasmolysis [7], inhibition of insulin-induced lipogenesis [8], anticancer [9], antibacterial [10,11]. In the Chinese Pharmacopoeia, imperatorin and isoimperatorin have been used as the chemical marker for quality control of *Radix Angelicae Dahuricae*. However, simple quantitative analysis of one or two active components in herb could not represent its integral quality. Consequently, simultaneous quantitative analysis of active components is the most direct and important method for quality control of TCM.

To date, there have already been some preliminary researches about the quantitative analysis of coumarins in the different plant material, for example, simultaneous determination of 5 furocoumarin in *Angelica dahurica* by HPLC–UV [12], simultaneous determination of mono-coumarins by CE–UV in *Chrysanthemum segetum* or by CE–ILIFD in *Fructus sophorae japonicae* and *Herb sarcandrae* [13,14], simultaneous determination of some mono-coumarins and pyranocoumarins in *Angelica gigas* root by HPLC–DAD–ESI/MS in SIM/SRM mode[15], qualitative and quantitative determination of the major coumarins in *Zushima* [16], simultaneous determination of five furocoumarins in *Angelicae dahuricae Radix* [17]. To the best of our knowledge, there has been no method for simultaneous characterization and quantitation of mono-coumarins and furocoumarins in *Radix Angelicae Dahuricae* by HPLC–MS/MS in MRM mode by now. As there are a lot of compounds in *Radix Angelicae Dahuricae* with much lower concentration (such as scopoletin, xanthotoxol, xanthotoxin, psoralen and isoimpinellin), it is difficult to detect them with HPLC–UV and CE–UV due to their low sensitivity [12,13]. In the previous

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Table 1
Summary of the tested samples of *Radix Angelicae Dahuricae*.

No.	Sources	Collection date	Process method
1	Liushuang Anguo Hebei	November 2007	Collected from field, sliced and dried under the sunshine
2	Chezhangzhuang Anguo Hebei	October 2007	Collected from field, sliced and dried under the sunshine
3	Dongwangqi Anguo Hebei	October 2007	Collected from field, sliced and dried under the sunshine
4	Magu Anguo Anguo Hebei	November 2007	Collected from field, sliced and dried under the sunshine
5	Liuchang Anguo Hebei	November 2007	Collected from field, sliced and dried under the sunshine
6	Xiwangqi Anguo Hebei	November 2007	Collected from field, sliced and dried under the sunshine
7	Liushuang Anguo Hebei	November 2007	Collected from field, sliced and fumigated by sulphur
8	Chezhangzhuang Anguo Hebei	October 2007	Collected from field, sliced and fumigated by sulphur
9	Anguo Hebei	November 2006	Purchased from local drug stores(has been fumigated by sulphur)
10	Anguo Hebei	October 2007	Purchased from local drug stores(has been fumigated by sulphur)
11	Shanxi Prov	November 2007	Purchased from local drug stores(has been fumigated by sulphur)
12	Sichuan Prov	November 2007	Purchased from local drug stores(has been fumigated by sulphur)

researches, a lot of them have much longer analytical time [15,16]. In my paper, most of them were furocoumarins and all 11 compounds have been eluted within 10 min, which was suitable for HPLC–MS. It also described a simple and reliable technique for isolation and purification of coumarins by preparative HPLC. The mass parameters for each analyte including fragmentation ions, lost ions were also analyzed, and it provided same reliable information for the identification of these compounds. The study was to optimize and establish a reliable and rapid HPLC–MS/MS method for the simultaneous analysis of 9 furocoumarins and 2 mono-coumarins in *Radix Angelicae Dahuricae* using MRM methods for quantification. We described the details of the HPLC–MS method in this paper, a powerful approach to solve the problems encountered in the routine analysis.

2. Experimental

2.1. Reagents and materials

HPLC grade methanol and formic acid were obtained from Tedia (Tedia, Fairfield, USA) and Dikma Pure (Dima, USA), respectively. Methanol used for extraction was supplied by Tianjin Chemical Reagent Corporation (Tianjin, China). Ultrapure water was produced by Heal Force Water System (Likang, Shanghai, China). Eight batches of samples were collected from different fields of Hebei province in PR China, four batches of samples were purchased from local drug stores in different provinces (Table 1). Scopoletin (**1**) and osthole (**10**) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Xanthotoxol (**2**), xanthotoxin (**3**) and isoimpinellin (**5**) were bought from Shanghai Tauto Biotech (Shanghai, China). Psoralen (**4**), bergapten (**6**), oxypeucedanin (**7**), imperatorin (**8**), cnidilin (**9**) and isoimperatorin (**11**) were isolated by the author from *Radix Angelicae Dahuricae*. The structures of 11 compounds are shown in Fig. 1.

2.2. Isolation and purification of coumarins

Air-dried root of *Radix Angelicae Dahuricae* (1 kg) was refluxed with 95% ethanol (10 L 3×) at 80 °C for 2 h. The combined organic solution was concentrated under vacuum. The residue was suspended in water and extracted with petroleum ether to remove lipids. The aqueous phase was then extracted with ethyl acetate and the resulting solution was evaporated vacuum and offered a brown residue. Then the crude extract was subjected to column chromatography (i.d. = 10 cm, height = 60 cm) on silica gel (A: petroleum ether-ethyl acetate, 5:1; B: petroleum ether-ethyl acetate, 4:1; C: petroleum ether-ethyl acetate 3:1; D: petroleum ether-ethyl acetate, 2:1; E: petroleum ether-ethyl acetate, 1:1). The fractions were examined by TLC and revealed that compound **11** was in fraction A, and compound **7** in fraction C. A portion of fraction B were subjected to preparative HPLC (Waters Prep LC Controller 600/Waters 2487) with MeOH–H_2O as the solvent system (75:25, 2.5 ml/min) and obtained compound **4**, **6**, **8** and **9**. The chromatogram was shown in Fig. 2. All these compounds were identified by direct comparison of their ^{1}H NMR, ^{13}C NMR and MS spectral data with those reported in the literature [2,18–20], and their purities were no less than 98% by HPLC analysis.

2.3. Instrument and chromatographic conditions

Liquid chromatography separation was performed using an Agilent 1200 HPLC system (Agilent, USA) equipped with an automatic degasser, a quaternary pump and an autosampler. Chromatographic separation was carried out on an Waters SunFire™ C_{18} column (150 mm × 4.6 mm, 5 μm) at 25 °C. The flow rate of mobile phase was maintained at 0.8 ml/min and the injection volume was 5 μl. The mobile phase was methanol/0.1% formic acid water (75:25, v/v).

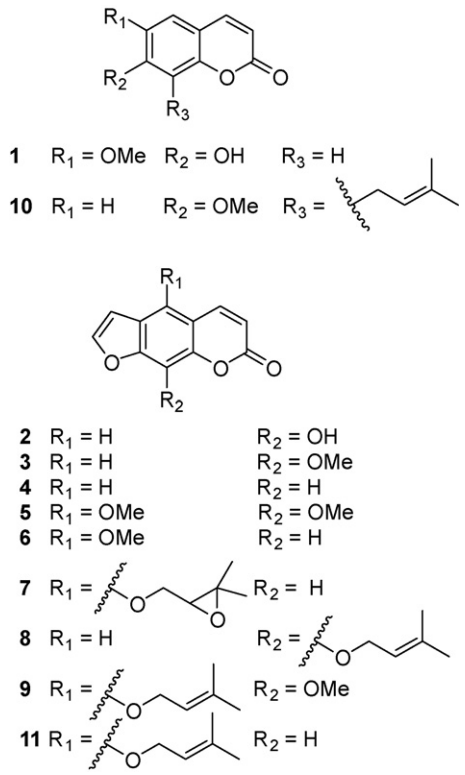

Fig. 1. Chemical structures of the 11 coumarins. scopoletin (**1**), xanthotoxol (**2**), xanthotoxin (**3**), psoralen (**4**), isoimpinellin (**5**), bergapten (**6**), oxypeucedanin (**7**), imperatorin (**8**), cnidilin (**9**), osthole (**10**) and isoimperatorin (**11**).

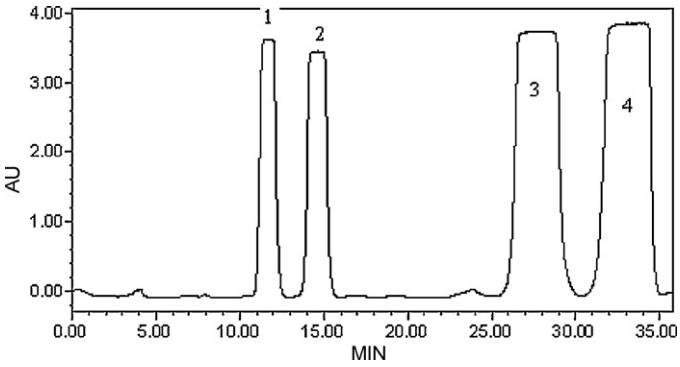

Fig. 2. Preparative HPLC chromatogram of four compounds. psoralen (**1**), bergapten (**2**), imperatorin (**3**) and cnidilin (**4**).

The HPLC system was connected to 3200 Q TRAP LC/MS/MS System, a hybrid triple quadrupole/LIT (linear ion trap) mass spectrometer equipped with an ESI ion source (Applied Biosystems/MDS Sciex, USA). The optimization of mass condition was achieved on infusing injection of each compound separately at a flow rate of 10 μl/min. The temperature was set at 400 °C in the positive-ion mode with ionspray voltage 5500 V. Ion source gas 1 and ion source gas 2 were 40 psi, collision gas was Medium, and curtain gas was set 20 psi. Detection was operated in the multiple reaction monitoring (MRM) scanning mode. The instrument control and data acquisition were carried out by the analyst 1.4.2 software.

2.4. Standard solution preparation

Each reference compound was accurately weighed (scopoletin 1.70 mg, xanthotoxol 1.95 mg, xanthotoxin 2.16 mg, psoralen 2.27 mg, isoimpinellin 1.98 mg, bergapten 4.44 mg, oxypeucedanin 8.4 mg, imperatorin 6.65 mg, cnidilin 1.86 mg, osthole 2.7 mg, isoimperatorin 3.68 mg), dissolved in 75% methanol and diluted to appropriate concentration respectively. A stock solution containing the 11 standards (scopoletin 34.0 μg/ml, xanthotoxol 6.24 μg/ml, xanthotoxin 21.6 μg/ml, psoralen 4.54 μg/ml, isoimpinellin 1.98 μg/ml, bergapten 22.2 μg/ml, oxypeucedanin 168.0 μg/ml, imperatorin 133.0 μg/ml, cnidilin 74.4 μg/ml, osthole 0.27 μg/ml, isoimperatorin 73.6 μg/ml) was prepared in 75% methanol. The standard stock solution was further diluted with 75% methanol to make 6 different concentrations at 1/100, 2/100, 4/100, 8/100, 8/50 and 8/25 of the original concentration. All solutions were stored in a refrigerator at 4 °C for analysis.

2.5. Sample preparation

The dried powders of *Radix Angelicae Dahuricae* samples (0.5 g, 75 mesh) were accurately weighed and extracted with 20 ml of 75% methanol in ultrasonic bath for 30 min. Then the resultant mixtures were adjusted to the original weights and aliquots of the supernatants were filtered through 0.45 μm membrane before HPLC injection.

3. Results and discussion

3.1. Extraction conditions

In order to optimize the extraction conditions, the extraction method, solvent and time were investigated. The results suggested that ultrasonic extraction was better than refluxing. Water, 30% methanol, 50% methanol, 75% methanol, 90% methanol and methanol were used as extraction solvents. It showed that 75% methanol was the most suitable extraction solvent. To determine

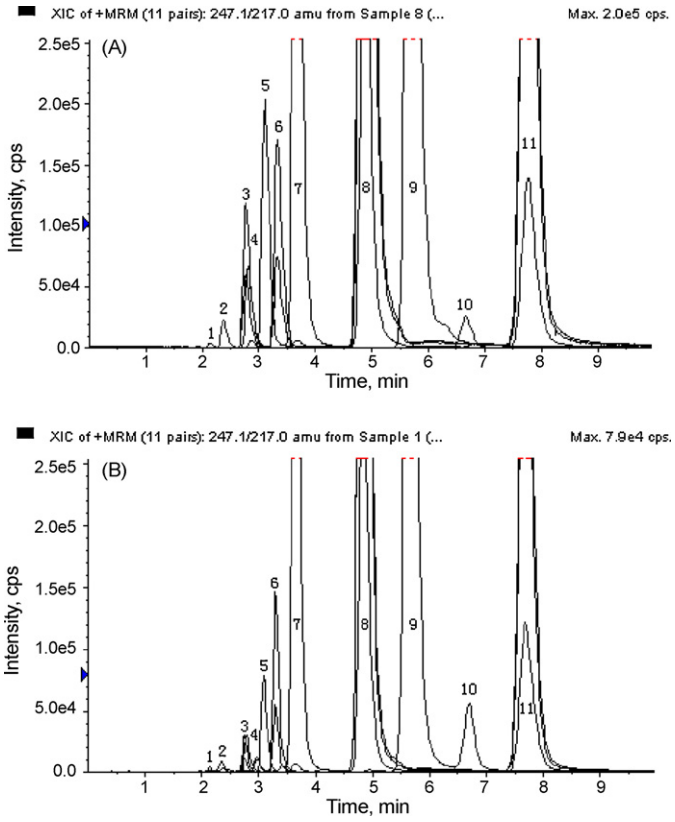

Fig. 3. Total-ion MRM chromatograms of standard solution (**A**) and the sample (**B**) obtained in positive-ion mode. scopoletin (**1**), xanthotoxol (**2**), xanthotoxin (**3**), psoralen (**4**), isoimpinellin (**5**), bergapten (**6**), oxypeucedanin (**7**), imperatorin (**8**), cnidilin (**9**), osthole (**10**) and isoimperatorin (**11**).

optimal extraction time, 0.5 g samples were extracted with 20 ml of 75% methanol by ultrasonic extraction for 10, 20, 30, 40 and 60 min, respectively. The compounds were almost completely extracted within 30 min.

3.2. Chromatographic conditions

Because of the similarity of these compounds in terms of physical property, good resolution must be obtained to meet the requirements. HPLC parameters including mobile phase (methanol–water, acetonitrile–water, methanol–acid aqueous solution, acetonitrile–acid aqueous solution) and flow rate of mobile phase (0.6, 0.8, 1.0 ml/min) were all examined and compared. Several mobile phase additives such as ammonium acetate, oxalic acid, formic acid and acetic acid were used to achieve the highest sensitivity. The experimental results showed that the presence of 0.1% aqueous formic acid in mobile phase could improve the ionization efficiency under the ESI^+ mode. It also can significantly improve the retention behavior of the different components. When acetonitrile was replaced by methanol and the flow rate was set 0.8 ml/min, the resolution was greatly improved and all the 11 compounds could be separated and eluted within 10 min. The typical chromatograms were shown in Fig. 3.

3.3. Identification of 11 coumarins from *Radix Angelicae Dahuricae*

In this study, the mass spectral conditions were optimized in both positive- and negative-ion modes, and the positive-ion mode was found to be more sensitive. The 11 coumarins exhibited their quasi-molecular ions $[M+H]^+$, $[M+Na]^+$, $[M+NH_4]^+$, $[M+K]^+$

Table 2
Chromatographic, mass spectral data and optimized MS parameters for the target analytes using ESI^+ mode.

Compounds	Time (min)	MW	MS (*m*/*z*)		MS^n (*m*/*z*)	Lost ions	Quantitative daughter ion	DP (eV)	EP (V)	CE (V)
Scopoletin	2.13	192	193.1	M+H	178.1	193.1–178.1=CH_3	178.1	15	10	30
			210.1	M+NH_4	150.0	178.1–150.0=CO				
			215.1	M+Na	122.1	150.0–122.1=CO				
			231.1	M+K	94.1	122.1–94.1=CO				
Xanthotoxol	2.37	202	203.1	M+H	175.1	203.2–175.1=CO	147.1	20	10	25
			220.1	M+NH_4	147.0	175.1–147.0=CO				
			225.1	M+Na	131.0	175.1–131.0=CO_2				
			241.0	M+K						
Xanthotoxin	2.76	216	217.1	M+H	202.1	217.1–202.1=CH_3	202.2	10	10	25
			234.1	M+NH_4	174.1	202.1–174.1=CO				
			239.1	M+Na	161.1	217.1–161.1=2CO				
			255.0	M+K	146.1	161.1–146.1=CH_3				
					118.1	146.1–118.1=CO				
Psoralen	2.81	186	187.1	M+H	159.1	187.1–159.1=CO	131.0	35	10	30
			204.1	M+NH_4	143.1	187.1––143.1=CO_2				
			209.1	M+Na	131.1	159.1–131.1=CO				
			225.0	M+K	115.1	143.1–115.1=CO				
Isoimpinellin	3.10	246	247.1	M+H	232.1	247.1–232.1=CH_3	217.2	30	10	25
			264.1	M+NH_4	217.0	232.1–217.0=CH_3				
			269.1	M+Na	189.0	217.0–189.0=CO				
			285.0	M+K	161.1	189.1–161.1=CO				
Bergapten	3.32	216	217.1	M+H	202.1	217.1–202.1=CH_3	202.2	100	10	30
			234.1	M+NH_4	174.1	202.1–174.1=CO				
			239.1	M+Na	146.1	174.1–146.1=CO				
			255.0	M+K	118.1	146.1–118.1=CO				
Oxypeucedanin	3.66	286	287.1	M+H	203.1	287.1-203.1=C_5H_9O	203.1	60	10	24
			304.2	M+NH_4	159.1	203.1–159.1=CO_2				
			309.1	M+Na	147.1	203.1–147.1=2CO				
			325.1	M+K	131.1	159.1–131.1=CO				
			364.2	M+2K						
			382.2	M+2K+NH_4						
Imperatorin	4.86	270	271.1	M+H	215.1	271.1–215.1=2CO	203.1	25	10	15
			288.2	M+NH_4	203.1	271.1–203.1=C_5H_8				
			293.1	M+Na	175.1	203.1–175.2=CO				
			309.1	M+K	147.1	175.2–147.0=CO				
Cnidilin	5.70	300	301.2	M+H	245.1	301.1–245.1=2CO	233.1	15	10	15
			318.2	M+NH_4	233.1	301.1–233.1=C_5H_8				
			323.1	M+Na	218.1	233.1–218.1=CO				
			339.1	M+K						
Osthole	6.66	244	245.2	M+H	189.1	245.1–189.1=C_4H_8	189.1	30	10	15
			262.2	M+NH_4	159.1	189.1-159.1=CH_2O				
			267.2	M+Na	131.1	159.1–131.1=CO				
			283.1	M+K						
Isoimperatorin	7.75	271	271.2	M+H	203.0	271.2–203.0=C_5H_8	203.1	40	10	16
			288.2	M+NH_4	175.1	203.0–175.1=CO				
			293.1	M+Na	159.1	203.1–159.1=CO_2				
			309.1	M+K	147.1	175.1–147.1=CO				

Table 3
Calibration curves of the 11 coumarins.

Compounds	Regression equation	r^2	Linear range (ng/ml)	LOD (ng)	LOQ (ng)
Scopoletin	$y = 2.22x + 290$	0.9992	340–10,880	0.22	0.52
Xanthotoxol	$y = 90.1x + 81$	0.9976	62.4–1996	0.08	0.30
Xanthotoxin	$y = 169x + 4590$	0.9984	216–6912	0.11	0.49
Psoralen	$y = 456x + 983$	0.9995	45.4–1452	0.27	0.67
Isoimpinellin	$y = 2490x + 1930$	0.9976	19.8–635	0.25	0.81
Bergapten	$y = 110x + 1520$	0.9985	222–7104	0.06	0.30
Oxypeucedanin	$y = 156x + 11{,}000$	0.9986	1680–53,760	0.20	0.42
Imperatorin	$y = 850x + 254{,}000$	0.9983	1330–42,560	0.08	0.25
Cnidilin	$y = 713x + 49{,}700$	0.9979	744–23,808	0.36	0.86
Osthole	$y = 2580x + 1015$	0.9993	2.7–87.6	0.10	0.38
Isoimperatorin	$y = 497x + 12{,}900$	0.9966	735–23,520	0.25	0.73

Table 4
Intra-day and inter-day precision of the 11 coumarins.

Spiked	Intra-day ($n=6$)		Inter-day ($n=3$)	
	Measured concentration (ng/ml)	R.S.D. (%)	Measured concentration (ng/ml)	R.S.D. (%)
Scopoletin				
680	663	2.96	674	2.09
2720	2756	1.94	2662	1.47
5440	5290	3.64	5369	2.69
Xanthotoxol				
124	119	2.34	119	2.76
499	491	2.83	494	2.13
998	964	2.37	988	1.96
Xanthotoxin				
432	442	3.79	445	1.06
1728	1710	2.49	1716	1.99
3456	3356	2.41	3426	1.17
Psoralen				
90	92	1.87	94	1.67
363	364	1.50	358	1.34
726	719	1.70	709	2.54
Isoimpinellin				
39	41	3.52	41	3.36
158	155	2.52	156	2.71
317	311	2.32	310	3.04
Bergapten				
444	459	2.85	455	0.54
1776	1774	2.41	1759	2.22
3552	3482	1.88	3477	0.51
Oxypeucedanin				
3360	3286	2.70	3234	1.16
13,440	12,903	1.14	13,049	2.26
26,880	26,750	1.19	26,950	4.00
Imperatorin				
2660	2782	3.32	2775	0.39
10,640	10,376	1.49	10,583	0.84
21,280	20,383	2.83	20,936	3.41
Cnidilin				
1488	1453	1.96	1414	1.14
5952	5795	3.03	5881	1.34
11,904	11,509	2.06	11,911	1.81
Osthole				
5.47	5.35	2.84	5.39	3.62
21.55	21.43	2.59	21.82	0.37
43	42	4.42	41.62	1.45
Isoimperatorin				
1470	1494	2.57	1424	1.83
5880	5704	1.93	5735	0.88
11,760	1156	3.02	11,580	2.37

and fragment ions $[M+H-CO]^+$, $[M+H-C_5H_9O]^+$, $[M+H-C_5H_8]^+$, $[M+H-C_5H_8-CO]^+$, $[M+H-C_5H_8-CO_2]^+$, $[M+H-CH_3]^+$, and most of them were in good agreement with the literature [21]. Mass parameters for each analyte including retention time, MW, MS MS^n fragmentation ions, lost ions, quantitative daughter ion, declustering potential (DP), entrance potential (EP), collision energy (CE) are summarized in Table 2.

3.4. Calibration curves, limits of detection and quantification

A stock solution containing the 11 standards was prepared as stated in Section 2.4. At least six concentrations were analyzed and the calibration curves were constructed by plotting the peak areas versus the concentration of each standard. Limits of detection (LOD) and quantification (LOQ) under the chromatographic conditions used were separately determined at signal-to-noise ratios (S/N) of 3 and 10, respectively. The results are given in Table 3. All the analytes showed good linearity ($r^2 > 0.99$) in a relatively wide concentration range.

3.5. Precision, accuracy, repeatability 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 11 coumarins at low, medium and high concentrations in a single day, whilst the inter-day values obtained over three consecutive days. The concentration of each solution was determined using a calibration curve prepared on the same day. The intra- and inter-day precisions calculated as R.S.D. were within the range of 1.14% to

Table 5
Recoveries of the 11 coumarins.

Compounds	Initial amount (ng)	Added amount (ng)	Detected amount (ng)	Recovery (%)	R.S.D. (%)
Scopoletin	48,567	35,577	82,827	96.3	3.05
		47,436	95,007	97.9	2.14
		59,296	110,531	104.5	1.56
Xanthotoxol	1333	955	2250	96.0	3.52
		1273	2626	101.6	3.12
		1592	2832	94.2	2.25
Xanthotoxin	11,532	7948	19,607	101.6	4.14
		10,598	21,790	96.8	3.75
		13,248	24,462	97.6	3.58
Psoralen	4298	3142	7691	108.0	2.96
		4190	8605	102.8	2.56
		5238	9510	99.5	2.01
Isoimpinellin	2109	1526	3679	102.9	2.10
		2035	4107	98.2	1.58
		2544	4696	101.7	1.62
Bergapten	38,557	29,990	67,257	95.7	3.46
		39,987	79,304	101.9	2.52
		49,984	87,941	98.8	3.43
Oxypeucedanin	534,106	390,720	893,959	92.1	4.55
		520,960	1,041,000	97.3	3.23
		651,200	1,220,471	105.4	2.38
Imperatorin	212,442	156,134	372,635	102.6	2.90
		208,179	412,086	95.9	2.75
		260,224	477,089	101.7	3.76
Cnidilin	179,325	132,019	308,967	98.2	1.64
		176,025	349,013	96.4	2.57
		220,032	389,455	95.5	2.12
Osthole	551	420	939	92.4	4.25
		560	1098	97.7	3.57
		700	1290	105.6	3.68
Isoimperatorin	179,257	128,160	308,954	101.2	3.02
		170,880	359,364	105.4	2.58
		213,600	393,284	100.2	1.11

4.42% and 0.37% to 4.00%. The results were presented in Table 4.

Recovery was used to further evaluate the accuracy of the method. Known amounts of each standard solution (20 ml, 75% methanol) at three different concentration levels were mixed with known amounts of *Radix Angelicae Dahuricae* samples (0.25 g), the samples were then extracted and analyzed with the above-established method. The experiments were repeated three times at each level. The overall recovery rates of these coumarins were in the range of 92.1–105.6% with R.S.D. from 1.11 to 4.55%. Details have been listed in Table 5.

Six samples of *Radix Angelicae Dahuricae* from the same source were extracted and analyzed using the above-established method. The R.S.D. values were calculated as a measurement of method repeatability. R.S.D. values of 11 compounds were from 1.22% to 2.70%, which showed high repeatability.

Stability of sample solution was tested at room temperature. The sample solution was analyzed within 24 h. The analytes were found to be very stable in 75% methanol solution (R.S.D. < 2.55%) over the tested period. When the stock solution was stored at 4 °C, it was stable for at least 15 days.

3.6. Sample analysis

The developed analytical method was applied to analyze 11 coumarins in 12 samples of *Radix Angelicae Dahuricae* including plant material and cut crude drug from different places. The contents, summarized in Table 6, were calculated with external standard method.

The results demonstrated a successful application of this HPLC–MS assay for the quantification of major coumarins including mono-coumarins and furocoumarins in different samples. All the 11 compounds have been eluted within 10 min, which was suitable for HPLC–MS. The results showed that content of the total coumarins fell in the range 1327.7–5892.9 μg/g. It also showed that in all plant samples, oxypeucedanin was the highest component, whose mean content was 2024.5 μg/g, followed by imperatorin at 1071.2 μg/g. However, in the cut crude drug samples, isoimperatorin (555.8 μg/g) was the most dominant constituent, followed by cnidilin (512.2 μg/g). Osthole and scopoletine the mono-coumarins, whether in plant or in cut crude drug, were the lowest ingredients.

The data also presented that the contents of coumarins in the plant differed from those in cut crude drug significantly ($P < 0.01$). The relatively high content coumarins were found in the samples which collected in the fields, while the relatively low content was determined in the samples purchased from local drug stores. For example, the contents of compound **1** and **4** in the plant were 5 times more than those in the cut crude drug. In particular, the content of compound **7** has reached 21 times. However, the content of the compound **2** in the cut crude drug was higher than those in the plant, which reached 6 times. These results indicated that the processing method probably affected the stability of these components and lead to decreasing of them. The difference between the sample process methods may also play their parts on the coumarin contents. The plants collected were sliced and dried under the sunshine while the cut crude drugs purchased from local drug stores were fumigated by sulphur. After the plant was fumigated by sul-

Table 6
Amounts (μg/g) of 11 coumarins in 12 samples from different parts of China.

Samples	1[b]	2	3	4	5	6	7	8	9	10	11	Total
1[a]	358.7	9.5	61.9	20.3	12.1	177.3	1858.7	905.7	664.8	0.7	733.8	4803.4
2	193.0	5.1	40.1	16.2	8.3	158.4	2053.6	839.8	692.9	2.2	687.1	4696.6
3	396.6	7.1	57.1	23.2	10.9	166.0	2079.2	985.7	743.4	1.1	790.9	5261.1
4	385.8	7.5	25.4	14.3	12.2	139.8	2056.2	1418.6	782.6	0.7	827.9	5671.1
5	272.3	4.2	31.6	18.4	8.0	173.3	2076.7	990.4	760.2	2.1	860.1	5197.2
6	378.6	5.6	95.4	49.0	14.8	217.3	2022.8	1286.9	861.2	1.2	860.1	5892.9
7	26.1	41.1	33.7	5.4	3.3	25.6	90.0	355.1	312.5	0.5	364.4	1327.7
8	52.3	55.0	23.1	3.1	7.5	52.5	106.9	576.3	503.8	0.4	485.9	1866.7
9	27.0	67.9	40.8	3.3	11.1	94.7	94.6	642.2	495.4	0.7	497.2	1974.8
10	38.8	22.8	99.0	5.5	2.6	30.4	81.3	378.2	397.8	0.5	632.4	1689.2
11	80.4	23.5	25.9	6.0	9.6	75.1	104.9	505.7	894.8	0.3	900.4	2626.6
12	40.4	37.9	49.1	4.4	10.9	75.1	81.8	477.5	469.0	0.6	454.5	1701.1
Mean ± S.D.[c] (1–6)	330.8 ± 81.0	6.5 ± 1.9	51.9 ± 25.6	23.6 ± 12.8	11.1 ± 2.6	172.0 ± 25.8	2024.5 ± 83.7	1071.2 ± 228.9	750.8 ± 69.4	1.3 ± 0.7	793.3 ± 70.6	5253.7
Mean ± S.D. (7–12)	44.2 ± 20.2	41.4 ± 17.7	45.3 ± 28.0	4.6 ± 1.2	7.5 ± 3.8	58.9 ± 27.5	93.2 ± 11.0	489.1 ± 111.1	512.2 ± 200.8	0.5 ± 0.1	555.8 ± 189.6	1864.4

[a] The samples numbers are the same as in Table 1.
[b] The compounds numbers are the same as in Fig. 1.
[c] $P < 0.01$.

phur, it was able to improve the appearance, dry easily, reduce odor of drug and pest control. Several papers have reported that the contents of coumarins in *Radix Angelicae Dahuricae* seriously lost after being fumigated by sulphur [22,23]. The same processing method has also been used to treat other Chinese crude drugs (such as paeonia). It suggested that most of paeoniflorin was converted to paeoniflorin sulfite in paeonia [24,25]. So it was possible that some coumarins could be destroyed after being fumigated by sulphur.

4. Conclusion

HPLC–ESI-MS has been proved as a reliable and powerful technique for the simultaneous quantification and confirmation of 11 coumarins in *Radix Angelicae Dahuricae*. The samples were divided into two clusters based on their source and processing methods and it has been found that there were significant differences. The cut crude drug could be easily differentiated from the plant by its low total coumarins. The developed method was simple, sensitive and reproducible. It demonstrated that qualitative and quantitative analysis in plant material and commercial products was of great importance and it could be used for the comprehensive evaluation of *Radix Angelicae Dahuricae*.

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