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ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 46 (2008) 258–266
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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The simultaneous determination of coumarins in *Angelica gigas* root by high performance liquid chromatography–diode array detector coupled with electrospray ionization/mass spectrometry

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Received 14 June 2007; received in revised form 19 September 2007; accepted 21 September 2007
Available online 26 September 2007

Abstract

An high performance liquid chromatography–diode array detector coupled with electrospray ionization/mass spectrometry (HPLC–DAD/MS) based method has been developed for the simultaneous determination of nine coumarin compounds, nodakenin (**1**), peucedanone (**2**), marmesin (**3**), decursinol (**4**), 7-hydroxy-6-(2*R*-hydroxy-3-methylbut-3-enyl)coumarin (**5**), demethylsuberosin (**6**), decursin (**7**), decursinol angelate (**8**) and isoimperatorin (**9**) in the Korean medicinal herb, Cham-Dang-Gui, the dried root of *Angelica gigas* (Umbelliferae). The methanol extracts were analyzed by HPLC using a reversed-phase C18 column (5 μm, 4.5 mm × 250 mm) using a gradient acetonitrile–water solvent system at a flow rate of 1.0 ml/min.

The analysis of six coumarins (**1**, **3**, **4** and **6**–**8**) with DAD was done at 330 nm and showed excellent linearity ($r^2 = 0.998$–0.999) in a range of 0.2–250 μg/ml for all the compounds. The average recoveries ($n = 3$) were between 96.5% and 110.8%. Identification of each peak was also discussed with the electrospray ionization multi-stage tandem mass spectroscopy (ESI-MS^n). The amount of these coumarin compounds was evaluated in *A. gigas* samples. Meanwhile, three coumarins (**2**, **5** and **9**) could not been quantified by DAD because these peaks were overlapped with others. Determination of these compounds could be successfully accomplished with the HPLC–ESI/MS in selected ion monitoring/selected reaction monitoring mode.

Keywords: *Angelica gigas*; Coumarins; High performance liquid chromatography–diode array detector coupled with electrospray ionization/mass spectrometry; Simultaneous determination; Electrospray ionization multi-stage tandem mass spectroscopy

1. Introduction

A Korean traditional herbal medicine, Cham-Dang-Gui (Korean Angelica, the dried root of *A. gigas* Nakai), has been widely used in the treatment of dysmenorrhea, amenorrhea, menopausal syndromes, anemia, abdominal pain, injuries, migraine headaches and arthritis [1]. It is also known that this herbal medicine ensures healthy pregnancies and easy deliveries, and that coumarins such as decursin and decursinol angelate are the major constituents of this plant [2–4].

Recently, it has been reported that decursinol and decursin exhibited significant neuroprotecitve activity against glutamate-induced neurotoxicity in primary cultures of rat cortical cells and improved scopolamine-induced amnesia *in vivo* with another coumarin constituent, nodakenin [5–7]. Anticancer activities of decursin have been also reported against human prostate carcinoma cells, human K562 erythroleukemia and U937 myeloleukemia cells [8,9].

Meanwhile, Dang-Gui, one of the most important traditional herbal medicines in Asia, is also marketed as a functional food product for women's health care in Europe and America. However, three different *Angelica* species are respectively recorded in Chinese, Korean and Japanese pharmacopoeia. Korean Angelica (the root of *A. gigas*) is quite distinct in having deep purple flowers while Chinese Angelica (the root of *A. sinensis* (Oliv.)

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doi:10.1016/j.jpba.2007.09.020

Diels) and Japanese Angelica (the root of *A. acutiloba* Kitagawa or *A. acutiloba* Kitagawa var. *sugiyama* Hikino) have white ones. Chinese Angelica and Japanese Angelica are sold in Korean markets with Korean angelica as just 'Dang-Gui'. Although the three roots are known to have similar pharmacological efficacy, they show variation in their chemical compositions and pharmacological properties. Instead of coumarins in Korean Angelica, phthalides are the principal components in the former two herbal drugs [10–12]. Coumarins have not been found in those herbal medicines [13]. Therefore, coumarins could be standard compounds to differentiate between Korean Angelica and Chinese or Japanese Angelica and to characterize the Korean Angelica's own biological activity.

Reversed phase high performance liquid chromatography (HPLC) methods are generally used to quantify coumarins with UV detector because coumarins have strong UV chromophore [3]. Detection and fragmentation patterns of these compounds by electrospray ionization-mass spectrometry (ESI-MS) have been recently reported [14–16]. Meanwhile, the two dehydropyranocoumarins, decursin and decursinol angelate are principal secondary metabolites in Korean Angelica, and the amounts exceed 3%, 2.5% of the dried root, respectively [3,17]. This aspect makes it difficult to quantify the other coumarins simultaneously, and previous analytical studies with Korean Angelica have been focused on determination of only decursin or/and decursinol angelate. There is no report about the analytical study of other coumarin constituents with HPLC. In addition, buffer eluent has been usually chosen to separate the two peaks of decursin and decursinol angelate because these two compounds are structural isomers of each other and it is difficult to divide their peaks [3,18]. However, to use the non-volatile salt solution forbids an HPLC system to be connected with MS detector.

Therefore, six coumarin compounds contained in Korean Angelica were simultaneously analyzed by a simple high performance liquid chromatography–diode array detector (HPLC–DAD) method in this study. Identification of each peak was also determined with the electrospray ionization multi-stage tandem mass spectroscopy (ESI-MS^n). The amount of these coumarin compounds was also evaluated in the commercial samples from Korea, authenticated as the roots of *A. gigas*. Determination of other three coumarin compounds not quantified by DAD could be successfully accomplished with the HPLC–ESI/MS in selected ion monitoring (SIM)/selected reaction monitoring (SRM) mode.

Table 1
Solvent gradient conditions for HPLC–DAD

Final time (min)	Flow rate (ml/min)	Water	MeCN
0	1.0	80	20
3	1.0	80	20
8	1.0	70	30
18	1.0	70	30
19	1.0	50	50
40	1.0	50	50
41	1.0	10	90
50	1.0	10	90

2. Experimental

2.1. Instrumentation

A Hewlett-Packard 1100 series HPLC system equipped with an autosampler, a column oven, a binary pump and a degasser (Hewlett-Packard, Waldbronn, Germany) was used. A 10 μl volume of standard or sample solutions was directly injected on a Shiseido Capcell Pak C_{18} UG120 (5 μm, 4.5 mm × 250 mm) with a compatible guard column (C_{18}, 5 μm, 4.6 mm × 7.5 mm) using a gradient acetonitrile–water solvent system at 35 °C. The flow rate was 1.0 ml/min and the solvent gradient conditions are shown in Table 1. The eluent was detected with DAD (diode

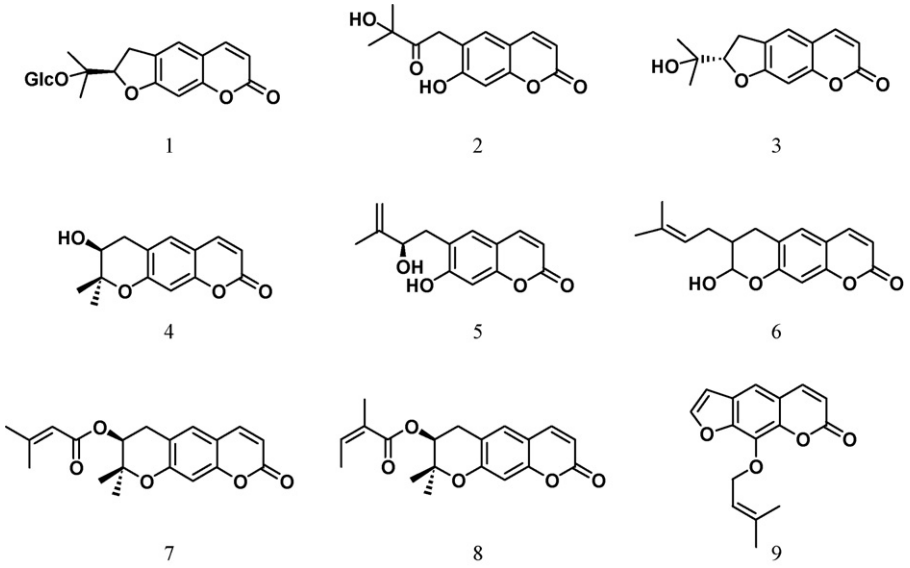

Fig. 1. Chemical structures of the coumarin compounds identified in the root of *Angelica gigas*. **1** = nodakenin; **2** = peucedanone; **3** = marmesin; **4** = decursinol; **5** = 7-hydroxy-6-(2*R*-hydroxy-3-methylbut-3-enyl)coumarin; **6** = demethylsuberosine; **7** = decursin; **8** = decursinol angelate; **9** = isoimperatorin.

array detector, UV 210, 230 and 330 nm). The Chemstation software (Hewlett-Packard, Avondale, CA, USA) was used to operate this HPLC–DAD system. A post-column microsplitter (Upchurch, WA, USA) was applied to restrict the flow to the mass spectrometer's source into 0.3 ml/min.

All ESI-MS and ESI-MS^n spectra were acquired using a Finnigan MAT LCQ ion-trap mass spectrometer (San Jose, CA, USA) equipped with a Finnigan electrospray source and capable of analyzing ions up to *m/z* 2000. Mass spectrometer conditions were optimized in order to achieve maximum sensitivity. The source voltage was set to +36.5 V and the capillary temperature to 200 °C. The other conditions were as follows: capillary voltage, +36.5 V; inter-octapole lens voltage, 10 V; sheath gas flow, 80 arbitrary units; auxiliary gas flow, 20 arbitrary units. Nitrogen (>99.999%) and He (>99.999%) were used as sheath and damping gas, respectively.

The precursor ions were isolated with an isolation width of 2 *m/z* units in positive mode and fragmented using collision energy of 45% for MS^2 experiments and 40% for MS^3 experiments.

The mass scale was calibrated in the positive-ion mode using a solution consisting of caffeine, the tetra-peptide MRFA, and Ultramark 1621 (Sigma, St. Louis, MO, USA) solution. The Xcalibur software (Finnigan MAT) was used for the operation.

2.2. Solvents and chemicals

All the solvents used in this experiment were HPLC-grade. Acetonitrile (MeCN) and water were purchased from Mallinckrodt (USA), and methanol (MeOH) from Fischer (USA). A membrane filter (MF3-13 PTFE, diameter—13 mm, pore size—0.50 μm, Advantec, CA, USA) was used to filter each sample.

Nine coumarins, nodakenin (**1**), peucedanone (**2**), marmesin (**3**), decursinol (**4**), 7-hydroxy-6-(2*R*-hydroxy-3-methylbut-3-enyl)coumarin (**5**), demethylsuberosin (**6**), decursin (**7**), decursinol angelate (**8**) and isoimperatorin (**9**) were isolated from the root of *A. gigas* through extraction and several column chromatography in our laboratory (Fig. 1). The purified standard compounds were identified by comparison of the MS, ^1H NMR and ^{13}C NMR data with published ones [19–21]. The purity was determined by HPLC–UV with two wavelengths (210 and 330 nm) and LC-MS, and was shown to be greater than 97%.

2.3. Plant materials

Three commercial products R1, R2 and R3 (the roots of *A. gigas*) were purchased from Korea Herbal Medicine Association. The other *A. gigas* samples (R4–R6) were purchased from Kyungdong traditional herbal market (Seoul, Korea). The samples were authenticated by Prof. J.H. Park in the College of Pharmacy, Pusan National University, Korea.

2.4. Preparation of standard solutions and samples

Stock solutions for standard compounds were prepared with HPLC grade methanol as solvent. Working calibration solutions were prepared by successive serial dilution of the stock solution with methanol and the final concentrations were 500, 250, 100, 50.0, 25.0, 12.5, 6.25, 3.13, 1.56, 0.781, 0.390, 0.195, 0.098 μg/ml, respectively.

The root of *A. gigas* was ground into powder and lyophilized. The finely pulverized powder was weighed (0.5 g), and 40 ml of methanol was added, and the mixture was extracted for 2 h at 90 °C, using a reflux. The extract was then filtered with filter papers (Whatman No. 40) and evaporated *in vacuo* followed by adding 10 ml of methanol. The sample solutions were centrifuged for 10 min at 4 °C, 5000 rpm, and filtered through a 0.45 μm membrane filter (Millipore, Nylon, 170 mm) prior to analysis.

3. Results and discussion

3.1. Selection of HPLC–DAD conditions

Decursin (**7**) and decursinol angelate (**8**) are two major components in the root of *A. gigas*, and they are structural isomers of each other. Their amounts reach about 3%, 2.5% of the dried root, respectively [3]. They showed similar retention times in liquid chromatography and analogous MS^n fragmentation patterns. Therefore, it is necessary to set up HPLC conditions to separate these two peaks for the analysis of this herbal drug.

Reversed phase columns have been usefully applied to analyze the components of natural resources. The resolutions of these two compounds were tested and compared with the reversed phase conditions using a variety of analytical columns including Capcell Pak C18 UG120 (5 μm, 4.5 mm × 250 mm), Capcell Pak C18 UG120 (5 μm, 4.5 mm × 150 mm), Capcell Pak C18 MG (5 μm, 4.5 mm × 150 mm), XTerra RP18 (5 μm, 4.5 mm × 150 mm), Luna C18 (5 μm, 4.5 mm × 150 mm) and Motor C18 (5 μm, 4.5 mm × 150 mm). The preferred chromatographic condition was found to be using Capcell Pak C18 UG120 (5 μm, 4.5 mm × 250 mm) column with acetonitrile–water. A gradient elution was chosen to obtain the efficient separation of peaks from the extracts (Table 1).

The optimum UV absorption wavelength was 330 nm. Strong UV absorption was found at this wavelength by examining the UV spectra of standard compounds (**1**–**9**).

In previous studies, non-volatile surfactants or buffer salts such as sodium lauryl sulfate and sodium phosphate have been added into the eluent to separate two peaks of decursin and decursinol angelate [3,17]. However, those additions were not conducted to the chromatographic condition in this study and this aspect made it possible to connect the HPLC–DAD with ESI/MS detector.

3.2. Method validation by HPLC–DAD

3.2.1. Linearity and range

Calibration curves were constructed on three consecutive days by analysis of a mixture containing nine coumarins at various concentration levels and plotting peak area against the concentration of each reference standard (Table 2). The curves showed good linearity and the correlation coefficients were found to be in the range of 0.998–0.999 for all the

Table 2
Linear ranges and correlation coefficients of calibration curves

Compounds	Range (μg/ml)	Slope (a)[a]	Intercept (b)[a]	Regression (r^2)	LOD (ng)
Nodakenin (**1**)	0.2–250	0.0118	−14.860	0.9980	∼1
Peucedanone (**2**)	0.2–250	0.0067	1.6624	0.9999	∼1
Marmesin (**3**)	0.2–250	0.0201	0.5417	0.9998	∼1
Decursinol (**4**)	0.2–250	0.0215	5.9749	0.9999	∼1
Compound **5**[b] (**5**)	0.2–250	0.0213	10.801	0.9976	∼1
Demethylsuberosine (**6**)	0.2–500	0.0254	−6.4463	0.9980	0.3
Decursin (**7**)	0.2–500	0.0218	−11.696	0.9999	0.3
Decursinol angelate (**8**)	0.2–500	0.0197	−8.1254	0.9999	0.3
Isoimperatorin (**9**)	0.4–250	0.0076	−0.7805	0.9998	∼1

[a] Slope and intercept represent a and b in $Y = ax + b$ linear model. Y means peak area and x, concentration.
[b] Compound **5**, 7-hydroxy-6-(2*R*-hydroxy-3-methylbut-3-enyl)coumarin.

compounds, over the concentration ranges, 0.2–250, 0.2–500 and 0.4–250 μg/ml for compounds **1**–**5**, **6**–**8** and **9**, respectively.

3.2.2. Specificity

The peak purity was determined by the photodiode array detector and ESI-MS (Table 3). A diode array detector and the corresponding computer software allow checking the singularity of each peak. In other words, the absorption spectrum of a single component remained invariable at each time point in one peak. The $[M+H]^+$ molecular ion and fragmentation patterns of each component were well matched with each chemical structure in its HPLC–ESI-MS spectra (Table 3). Nine standard compounds (**1**–**9**) were clearly isolated each other in standard mixture solution and six coumarins (**1**, **3**, **4** and **6**–**8**) were identified in the extracts of Korean Angelica (Fig. 2).

3.2.3. Precision and accuracy

The method reproducibility was evaluated by the intra-day and inter-day variability for three injections of standard solutions and three replicates analysis of sample solutions, respectively ($n = 21$). The coefficient of variance (CV) was less than 3.9%, which demonstrated good precision of this method.

The recovery of six coumarins was assessed by spiking sample with high, medium and low concentrations of each reference compound, namely: 250, 1000, 5000 ng. The average recoveries were between $96.5 \pm 2.1\%$ (mean ± CV, $n = 6$) and $110.8 \pm 3.5\%$ ($n = 6$).

3.2.4. Limits of detection (LOD) and quantification (LOQ)

The limits of detection and quantification were determined by means of serial dilution based on a signal-to-noise (S/N) ratio of 3:1 and 10:1, respectively. LOD of all nine coumarins were less than 1 ng, which showed a high sensitivity at this chromatographic condition (Table 2).

3.3. Analysis of commercial products by HPLC–DAD

The established method has been applied to the determination of nine coumarin components in the methanolic extract of Korean Angelica. For the preliminary study, sample was extracted with methanol:water (7:3 v/v), 100% methanol and ethanol:water (7:3 v/v), 100% ethanol, respectively. The amounts of decursin (**7**) and decursinol angelate (**8**) were lower in 70% methanol and 70% ethanol extracts than in others, while nodakenin (**1**) showed lowest content in 100% ethanol extract. Therefore, 100% methanol was recommended as an effective solvent for extraction of coumarins in the root of Korean Angelica and preparation of analyte solution.

Each peak was identified by comparison of retention time (t_R), UV spectrum and MS data with those of each standard compound (Fig. 2 and Table 2). Chromatographic separation of

Table 3
The on-line detected chromatographic data of nine compounds identified in the extracts of Korean Angelica

Peak no.	t_R (min)	MS (m/z)[a]	MS/MS (m/z)[b]	SIM/SRM mode	Identification
1	8.4	409	247, 229, 187	409	Nodakenin
2	10.1	263	245	263	Peucedanone
3	14.1	247	229, 175	247 → 229	Marmesin
4	15.6	247	229, 197, 175	247 → 229	Decursinol
5	16.3	247	229, 211	247 → 229	Compound **5**[c]
6	28.6	231	175, 97	231 → 175	Demethylsuberosine
7	39.7	329	247, 229, 123	329 → 229 → 211	Decursin
8	40.8	329	247, 229, 123	329 → 229 → 211	Decursinol angelate
9	41.7	271	203, 175	203	Isoimperatorin

[a] $[M+H]^+$.
[b] Ions derived from $[M+H]^+$.
[c] Compound **5**, 7-hydroxy-6-(2*R*-hydroxy-3-methylbut-3-enyl)coumarin.

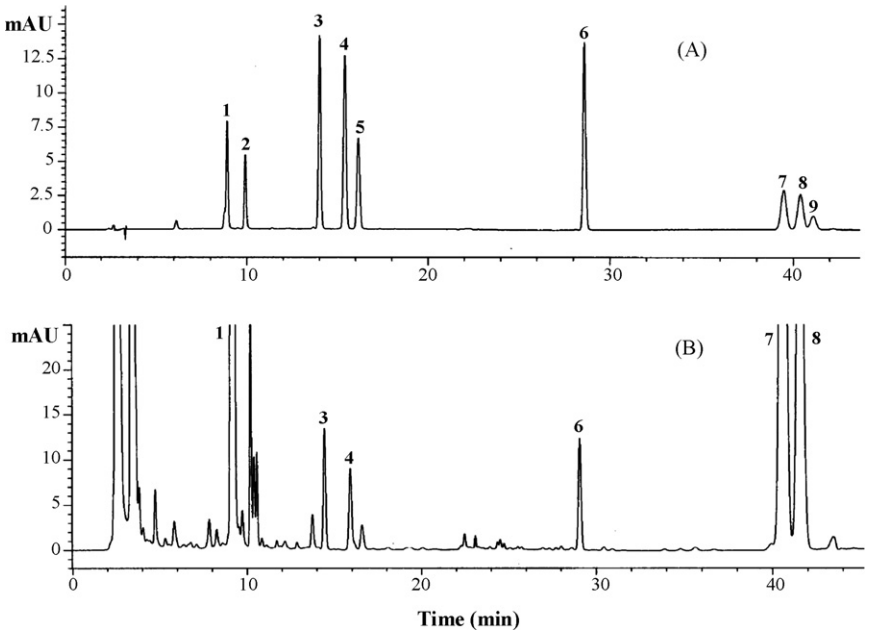

Fig. 2. HPLC chromatograms of target analytes (compounds **1–9**) and the methanol extract of *A. gigas*. (A) Chromatogram of the standard solution mixture (6250 ng/ml) and (B) chromatogram of the extract of Korean Angelica.

six coumarins (**1**, **3**, **4** and **6–8**) in the extracts of *A. gigas* roots were well achieved by using the developed method. However, the other three coumarins, peucedanone (**2**), 7-hydroxy-6-(2*R*-hydroxy-3-methylbut-3-enyl)coumarin (**5**) and isoimperatorin (**9**) failed to show clear separation with other peaks. Their peaks were overlapped with the neighbouring peaks. Therefore, quantification with HPLC–DAD was accomplished on the six coumarin constituents in the root of *A. gigas*.

The content of decursin varied from 1.87% to 4.56% and for decursinol angelate, the content varied from 1.11% to 3.68% (Table 4). The overall quantity of six coumarins was much higher in R1 sample than in the other samples, except R5. More than two times greater amount was found in R1 sample than in R2 sample. The sample R5 showed similar pattern with that of R1 sample.

3.4. Analysis of commercial products by HPLC–ESI/MS

Multiple reaction monitoring (MRM) mode by ESI-MS was applied to determine the other three coumarins not identified by DAD. The most abundant fragment ion for each standard compound was chosen for quantification by SRM (Table 3). For compounds **3–5**, the fragment ion at *m/z* 229 $[M+H-H_2O]^+$ was prominent in MS^2 spectrum of the ion at *m/z* 247 $[M+H]^+$.

Table 4
Quantification of six coumarins in *Angelica gigas* roots (R1–R6) with HPLC–DAD (mg/g) ($n=21$)

Samples	Nodakenin (**1**)			Marmesin (**3**)			Decursinol (**4**)		
	Mean[a]	S.D.[a]	CV[b]	Mean	S.D.	CV	Mean	S.D.	CV
R1	13.1	0.04	0.3	0.592	0.006	1.0	0.301	0.01	3.9
R2	4.53	0.02	0.5	0.059	0.0003	0.5	0.083	0.001	1.7
R3	6.32	0.01	0.1	0.103	0.0001	0.1	0.157	0.002	1.4
R4	5.78	0.01	0.2	0.178	0.0001	0.1	0.267	0.0003	0.1
R5	12.5	0.05	0.4	0.148	0.0002	0.1	0.431	0.001	0.2
R6	4.93	0.10	2.1	0.099	0.0003	0.3	0.144	0.003	0.2

Samples	Demethylsuberosine (**6**)			Decursin (**7**)			Decursinol angelate (**8**)		
	Mean	S.D.	CV	Mean	S.D.	CV	Mean	S.D.	CV
R1	1.10	0.02	2.1	44.8	0.02	0.0	33.3	0.02	0.1
R2	0.539	0.0008	0.1	18.7	0.02	0.1	11.1	0.01	0.1
R3	0.544	0.006	1.2	25.0	0.006	0.0	27.2	0.02	0.1
R4	0.337	0.0003	0.1	32.4	0.06	0.2	35.8	0.002	0.0
R5	2.31	0.007	0.3	45.6	0.03	0.1	36.8	0.02	0.1
R6	0.412	0.0006	0.1	35.4	0.006	0.0	27.4	0.006	0.0

[a] Data are expressed as mean (the average value of content) and S.D. (the standard deviation value) of three independent experiments.
[b] CV: coefficient of variance (%) = (S.D./mean) × 100.

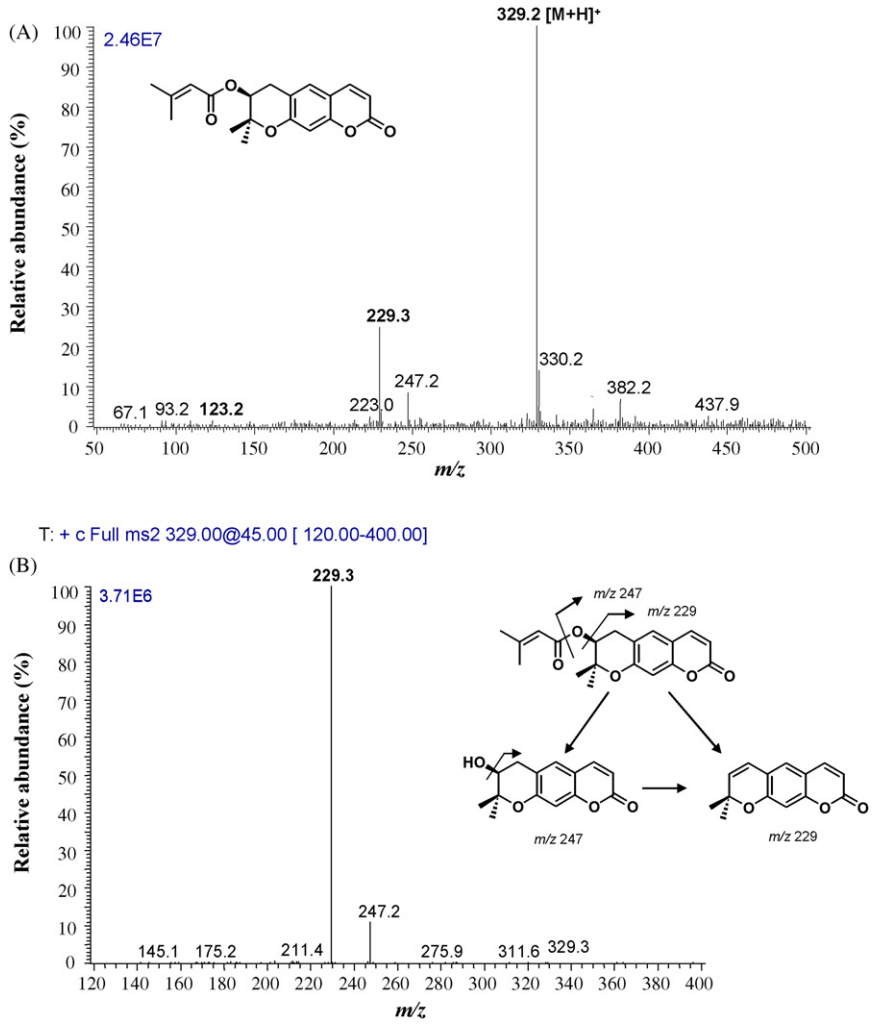

Fig. 3. ESI mass spectrum of decursin (A), MS/MS spectrum of the molecular ion at *m/z* 329 and MS fragmentation pattern of decursin (B).

A typical fragment ion of dihydropyranocoumarin was shown at *m/z* 175 $[M+H-C_4H_8]^+$ in MS^2 spectrum of the molecular ion of demethylsuberosine (**6**) [19].

Decursin (**7**) displayed a quasimolecular ion peak at *m/z* 329 $[M+H]^+$, and the MS/MS spectrum of the $[M+H]^+$ ion showed the peaks at *m/z* 247, 229 due to the successive loss of an isoprenyl moiety at C-3′ position (82 Da) and a hydroxy group as a water molecule (18 Da) (Fig. 3). The prominent peak resulting from another loss of a water molecule at C-1′ position was observed at *m/z* 211 in MS^3 spectrum of the ion at *m/z* 229 from MS^2 of the protonated and cationized decursin.

For decursinol angelate (**8**), it exhibited the same precursor ion peak at *m/z* 329 $[M+H]^+$ with decursin and the same fragment ion peaks at *m/z* 247, 229 due to the successive loss of an angeloyl moiety at C-3′ position (82 Da) and a hydroxy group as a water molecule (18 Da) (Fig. 4). In MS^3 spectrum of the ion at *m/z* 229, the major fragment ion at *m/z* 211 was also detected as in decursin.

From these results, the fragment ion at *m/z* 211 in MS^3 spectrum was chosen for quantification of decursin and decursinol angelate, which showed better resolution than the ion at *m/z* 229 in MS^2 spectrum.

The precursor ion was selected for nodakenin (**1**) and peucedanone (**2**) in SIM mode because intensity of the fragment ion was much low. The stronger fragment ion at *m/z* 203 was monitored for imperatorin (**9**) instead of the parent ion at *m/z* 271 in SIM mode (Table 3).

The calibration curves showed linearity ($r^2 = 0.995$, 0.995 and 0.999 for compounds **2**, **5** and **9**, respectively) in a range of 0.2–12.5 μg/ml for three standard compounds, and LOD were below 1 ng. As shown in Table 5, the repeatability values (coefficient of variance, CV) were ranged from 7.5% to 19.2%. The average recoveries were between $85.6 \pm 11.3\%$ (mean ± CV, $n = 3$) and $104.1 \pm 9.5\%$ ($n = 3$). Compared with the result by DAD, higher variation was observed. This variation could be improved by eliminating the flow fluctuation into MS source through the microflow splitter interface.

The developed HPLC–ESI/MS system has been applied to the determination of coumarins in the extracts of Korean Angelica, and made it possible to determine three coumarins (compounds **2**, **5** and **9**) which could not be detected with HPLC–DAD. The data showed that the content of three coumarins fell in the range of 0.009–0.150 mg/g. These values were much lower than those of the previous six coumarins. By the first monitoring in MRM

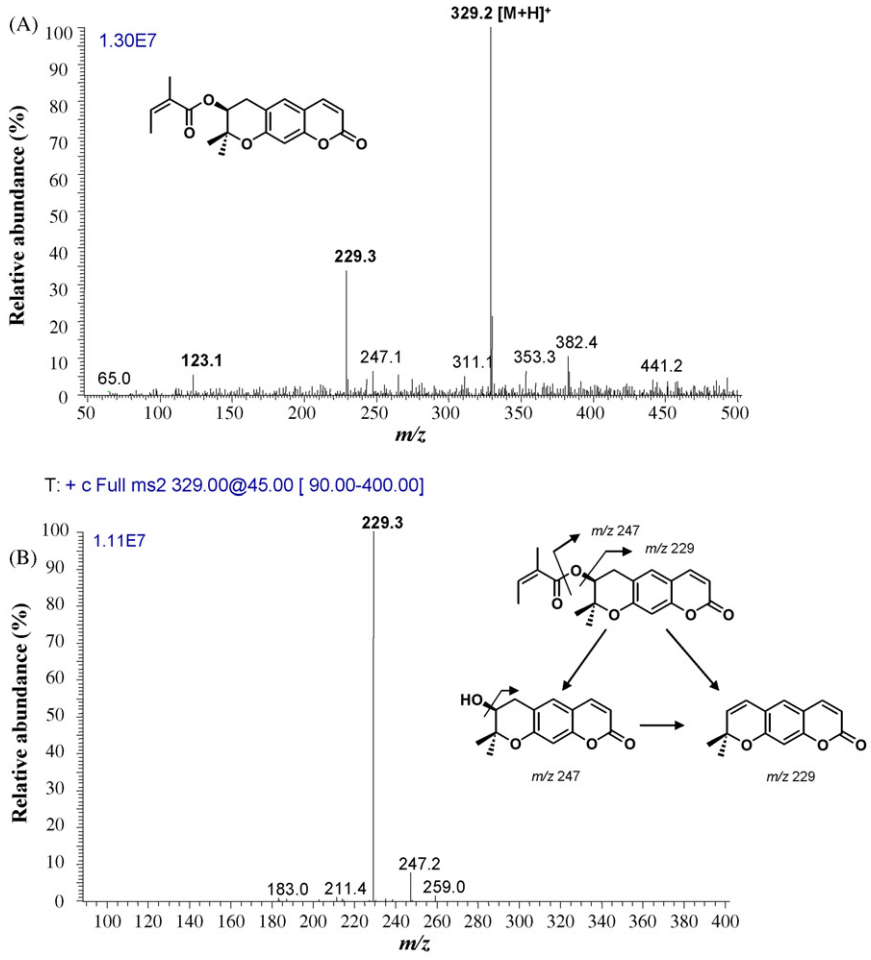

Fig. 4. ESI mass spectrum of decursinol angelate (A), MS/MS spectrum of the molecular ion at *m/z* 329 and MS fragmentation pattern of decursinol angelate (B).

mode, compound **5** showed similar patterns with those of the six coumarins determined by UV (Fig. 5 and Table 5). However, the content of peucedanone (**2**) was slightly high in R2 sample with the second running in SIM mode. Moreover, the quantity of a furanocoumarin, imperatorin (**9**) was highest in R2 sample (Fig. 6 and Table 5). These results were contrary to the fact that the amount of the most coumarins was much higher in R1 and R5 samples than in the other samples. Characteristically, the area of an unknown peak at 35.1 min was much bigger in R3 sample than in the others (Fig. 6). This compound (**10**) could be estimated to be a furanocoumarin, xanthotoxin or bergapten, which has the same chemical skeleton with imperatorin (a fragment ion peak at *m/z* 203 in SIM mode) and molecular weight (216 Da) [20,21]. The loss of a methoxy as a methylene group (14 Da) could show its fragment ion peak at *m/z* 203 $[M + H - CH_2]^+$ in this MS condition. These characteristic patterns of commercial herbs by MRM mode in this study gave useful information not obtained by UV detector.

Table 5
Quantification of three coumarins in Korean Angelica samples (R1–R6) with HPLC–MS (mg/g) ($n = 6$)

Samples	Peucedanone (**2**)			Compound (**5**)[c]			Isoimperatorin (**9**)		
	Mean[a]	S.D.[a]	CV[b]	Mean	S.D.	CV	Mean	S.D.	CV
R1	0.054	0.010	19.2	0.024	0.003	13.1	0.012	0.001	9.1
R2	0.075	0.011	14.9	0.016	0.002	15.3	0.150	0.029	19.2
R3	0.063	0.008	12.5	0.022	0.002	8.1	0.007	0.001	17.3
R4	0.053	0.004	7.5	0.018	0.003	16.7	0.011	0.001	9.1
R5	0.047	0.006	12.8	0.031	0.003	9.7	0.009	0.001	11.1
R6	0.066	0.005	7.6	0.023	0.004	17.4	0.058	0.007	12.1

[a] Data are expressed as mean (the average value of content) and S.D. (the standard deviation value) of three independent experiments.
[b] CV: coefficient of variance (%) = (S.D./mean) × 100.
[c] Compound **5**, 7-hydroxy-6-(2*R*-hydroxy-3-methylbut-3-enyl)coumarin.

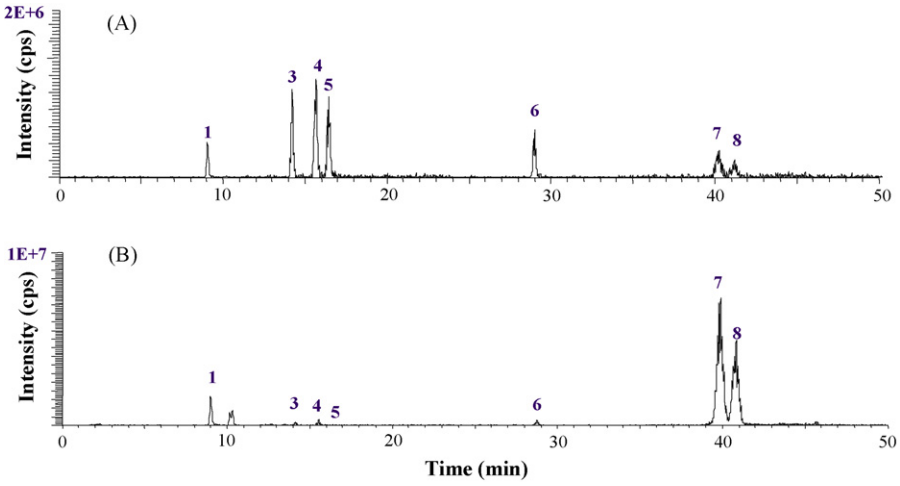

Fig. 5. Total ion chromatogram of the methanol extract of *A. gigas* root in MRM mode. (A) Chromatogram of the standard solution mixture (6250 ng/ml) and (B) chromatogram of the extract of *A. gigas*. Four segments were set as follows: 0–12 min, the ion at *m/z* 409 was selected; 12–23 min, the reaction *m/z* 247 → *m/z* 229 was monitored; 23–34 min and 34–50 min, the reactions *m/z* 231 → *m/z* 175 and *m/z* 329 → *m/z* 229 → *m/z* 211 were monitored, respectively.

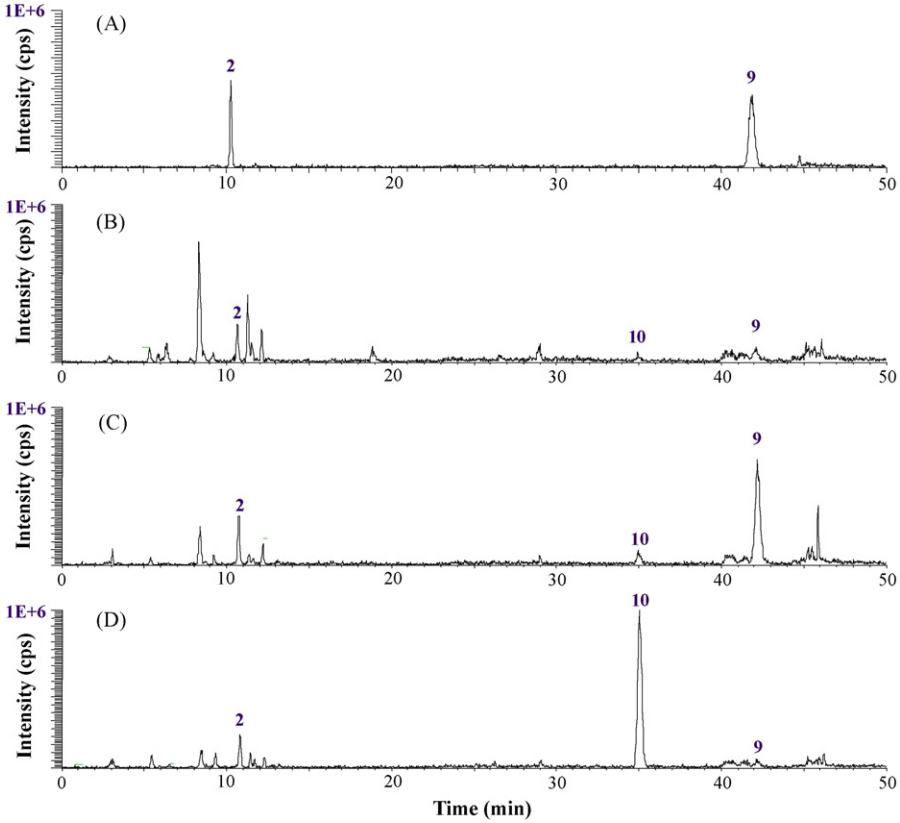

Fig. 6. Total ion chromatograms of the methanol extracts of *A. gigas* roots in SIM mode. (A) Chromatogram of the standard solution mixture (6250 ng/ml) and (B)–(D) chromatograms of the extracts of the samples R1, R2 and R3, respectively. In this condition, two segments were set as follows: the ion at *m/z* 263 was selected for the first 29 min period and the ion at *m/z* 203 was selected for the latter period.

4. Conclusions

A comprehensive quality assessment method for the root of *A. gigas*, was established in this study. Six coumarin compounds were simultaneously determined by reversed phase HPLC–DAD at 330 nm with good sensitivity, precision and accuracy.

The analytical method was applied to the determination of coumarins in the commercial *A. gigas* roots from Korea. The results confirmed that this method was sensitive enough to be used for quality control of Korean Angelica. In addition, this method focused on the characteristic coumarin compounds of this crude drug could be used to differentiate Korean Angelica from Chinese or Japanese Angelica.

Finally, this developed method using no salts allowed connecting MS detector with the typical UV detector. Therefore, determination of three coumarins, which showed overlapped peaks with others in HPLC–DAD, could be easily done with the HPLC–ESI/MS in SIM/SRM mode.

Acknowledgements

We wish to thank Do-Yoon Kim for isolation of standard compounds used in this study and Ok-Gyoung Choi for sample preparation. This research was supported by a grant from the National Center for Standardization of Herbal Medicines (05142-626) in Korea Food & Drug Administration.

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