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ORIGINAL

Differentiation of Furanocoumarin Isomers with Ratio of Relative Abundance of Characteristic Fragment Ions and Application in *Angelicae dahuricae* Radix

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Abstract C-5-substituted and C-8-substituted furanocoumarin isomers, two important kinds of furanocoumarin, are widely documented as the main active constituents in *Angelicae dahuricae* radix. Due to the similar polarity and mass fragmentation pathways of such isomers, it is difficult to distinguish them using mass spectrometric methods. To address this issue, we developed a strategy employing combined full scan and product ion scan modes on an ultra high performance liquid chromatography–quadrupole time-of-flight tandem mass spectrometry (UHPLC-Q-TOF-MS) platform to differentiate four pairs of furanocoumarin isomer, viz. xanthotoxin and bergapten, imperatorin and isoimperatorin, psoralen and isopsoralen, and impinellin and isoimpinellin. A novel method using the ratio of relative abundance (RRA) of characteristic fragment ions was established to distinguish C-5-substituted and C-8-substituted furanocoumarin isomers, using the formula $R = M/N$, where M and N represent the ratios of relative abundance of characteristic fragment ions of a pair of furanocoumarin isomers. For R value greater than 1, compound M is substituted at C-5, whereas for R value less than 1, compound M is substituted at C-8. This method with good repeatability was applied to identify five pairs of isomeric furanocoumarins in *Angelicae dahuricae* radix. This is the first method to distinguish C-5-substituted and C-8-substituted furanocoumarin isomers, and can be used in complex matrix.

Keywords UHPLC-Q-TOF-MS · Furanocoumarin isomers · Relative abundance · Characteristic fragment ions · *Angelicae dahuricae* radix

Introduction

Furanocoumarins are widely distributed in higher plants. Many studies have shown that furanocoumarins display various pharmacological effects including antiinflammatory, antipyretic/analgesic, antimicrobial, antioxidative, antiproliferative, and antiviral actions [1–8]. The basic framework of furanocoumarins consists of benzo-α-pyranone and a furan ring, divided into two types: linear and angular. This kind of compound often coexists in isomeric forms, among which C-5-substituted and C-8-substituted furanocoumarin isomers are very common. Because of the identical molecular weight and similar structure and physicochemical properties of such isomers, they are often difficult to distinguish based on their chromatographic behavior or simple fragmentation pathways.

Some assays based on collision-induced dissociation (CID) methods have been described for the fragmentation pathways of single furanocoumarins such as psoralen, isopsoralen, imperatorin, isoimperatorin, phellopterin,

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xanthotoxin, bergapten, oxypeucedanin, and byakangelicol [9, 10]. Some singly substituted furanocoumarins have been distinguished based on the rule of retention time, the theory of an intermediate eight-membered ring, and characteristic ions at *m/z* 175 and *m/z* 159 [11, 12]. However, those reports mainly focus on identification of furanocoumarins, while distinguishing furanocoumarin isomers based on their mass fragments was not considered. Although Liu et al. [13] and Sun et al. [14] compared four pairs of isomeric furanocoumarins using mass spectrometry, a common rule for differentiating C-5-substituted and C-8-substituted furanocoumarin isomers was not found, and there was no methodological development or application for real samples. Isomers of some compounds, such as isoflavonoids and sulfated oligosaccharides, can be compared based on the relative abundance of fragment ions [15, 16], indicating that it may be possible to develop a method to distinguish C-5-substituted and C-8-substituted furanocoumarin isomers based on the relative abundance of their fragments.

Angelicae dahuricae radix, called *Baizhi* in China, is a widely used traditional Chinese medicine. It is obtained from the dried roots of *Angelicae dahuricae* (Fisch. ex Hoffm.) Benth. et Hook. f or *Angelica dahurica* (Fisch. ex Hoffm.) Benth. et Hook. f. var. formosana (Boiss.) Shan et Yuan and has been frequently used for treatment of headache, toothache, abscess, furunculosis, and boils [17] in Chinese clinic. Many studies have shown that furanocoumarins are the main bioactive components in *Angelicae dahuricae* radix [18–21]. Thus, identification of furanocoumarin isomers could improve understanding of the material basis of *Baizhi*.

In this study, the ratio of relative abundance (RRA) of characteristic fragment ions method was established on an UHPLC-Q-TOF-MS platform to analyze the fragmentation of four pairs of furanocoumarin isomers, viz. xanthotoxin and bergapten, imperatorin and isoimperatorin, psoralen and isopsoralen, and impinellin and isoimpinellin. The method was then successfully applied to identify the furanocoumarin isomers present in *Angelicae dahuricae* radix.

Experimental

Chemicals and Reagents

Reference standards of imperatorin, isoimperatorin, and psoralen were obtained from the National Institutes for Food and Drug Control (Beijing, China). Xanthotoxin, bergapten, isopsoralen, impinellin, and isoimpinellin were purchased from Jiangsu Yongjian Pharmaceutical Technology Co., Ltd. (Jiangsu China).

Methanol of HPLC grade was obtained from TEDIA Company, Inc. (USA). Ammonium acetate of MS grade was obtained from Fisher Scientific (USA). Absolute methanol of analytical grade was purchased from Yongda Chemical Reagent Co., LTD (Tianjin, China). Purified water was purchased from Wahaha Group Co., Ltd. (Hangzhou, China). Standard solutions of furanocoumarins were prepared in water–methanol (v/v, 3:7) at concentration of 1.00 μg/mL, respectively.

Angelicae dahuricae radix was obtained from Sinopharm Le-Ren-Tang Hebei Medicine Co., Ltd. (Shijiazhuang, China). The crude material was pulverized into powder and passed through a 60-mesh sieve. Powder (6.0 g) was extracted using 30.0 mL water–methanol (v/v, 1:3) by sonication for 45 min in ice water. The supernatant was filtered and condensed by rotary evaporator. The dried residues were dissolved in 5.0 mL water–methanol (v/v, 1:1). The solution was centrifuged at 14,000 rpm for 10 min. The supernatant was filtered through a 0.22-μm nylon membrane filter. Final supernatant (5.0 μL) was used for sample analysis.

Instrumentation and Method

LC analyses were conducted on a Shimadzu UHPLC system (Kyoto, Japan) consisting of a LC-30AD solvent delivery system, SIL-30AC autosampler, CTO-30A column oven, DGU-20A_{5R} degasser, and CBM-20A controller. Chromatographic separation was achieved on Epic C_{18} column (150 mm × 2.1 mm I.D., particle size 3 μm, ES Industries, USA). The mobile phase was composed of water containing 1 mmol/L ammonium acetate (A) and methanol (B), and gradient elution was adopted as follows: 0–25 min, 20–55% B; 25–60 min, 55–95% B; 60–70 min, 95–95% B. The flow rate was 0.2 mL/min. The interval between consecutive injections was set as 10 min for system equilibration.

Mass spectrometric detection was performed on a Triple TOF™ 5600$^+$ system with Duo Spray source (AB SCIEX, Foster City, CA, USA) in positive electrospray ionization (ESI) mode. The MS conditions were as follows: ion spray voltage, 5500 V; ion source temperature, 550 °C; curtain gas, 30 psi; nebulizer gas (GS1), 55 psi; heater gas (GS2), 55 psi. The mass ranges were set at *m/z* 100–800 for TOF MS scan or 50–800 for TOF MS/MS experiments. The collision energy was set at 35 eV and the collision energy spread (CES) was 15 eV for MS/MS experiments. The 12 ions with greatest intensity were selected for MS/MS fragmentation analysis in full scan mode combined with information-dependent acquisition (IDA). For product ion scan mode detection, *m/z* 217.05 was selected for xanthotoxin and bergapten, *m/z* 271.10 was selected for imperatorin and isoimperatorin, *m/z* 187.04 was selected for psoralen and isopsoralen, and *m/z* 247.06 was selected for impinellin and isoimpinellin. Data were analyzed using MasterView software (AB SCIEX, Foster City, CA, USA).

Results and Discussion

Analysis of Fragmentation Characteristics of Four Pairs of Furanocoumarin Isomers

The standards of the four pairs of furanocoumarin isomers were detected in full scan mode separately, and their MS/MS spectra acquired. The dissociation pathways of the standards and their spectra are shown in Figs. 1, 2, 3, and 4. Based on analysis of the fragmentation patterns of the four pairs of furanocoumarin isomers, a rule was found indicating that successive loss of CO was the main fragmentation pathway for the furanocoumarins (substituents being lost first). Thus, the characteristic fragment ions for bergapten and xanthotoxin lay at *m/z* 202, 174, and 146, those for isoimperatorin and imperatorin lay at *m/z* 203, 175, and 147, those for psoralen and isopsoralen lay at *m/z* 187, 159, and 131, and those for isoimpinellin and impinellin lay at *m/z* 217, 189, and 161.

Proposed RRA Method

To determine mass fragmentation rules to distinguish the isomers, the relative abundance of characteristic fragment ions of the four pairs of furanocoumarin isomers was calculated. The results for the C-5-substituted and C-8-substituted furanocoumarin isomers revealed that, with respect to the fragment ion after loss of the substituent (denoted *F*0), the first two successive fragment ions (denoted *F*1 and *F*2) were

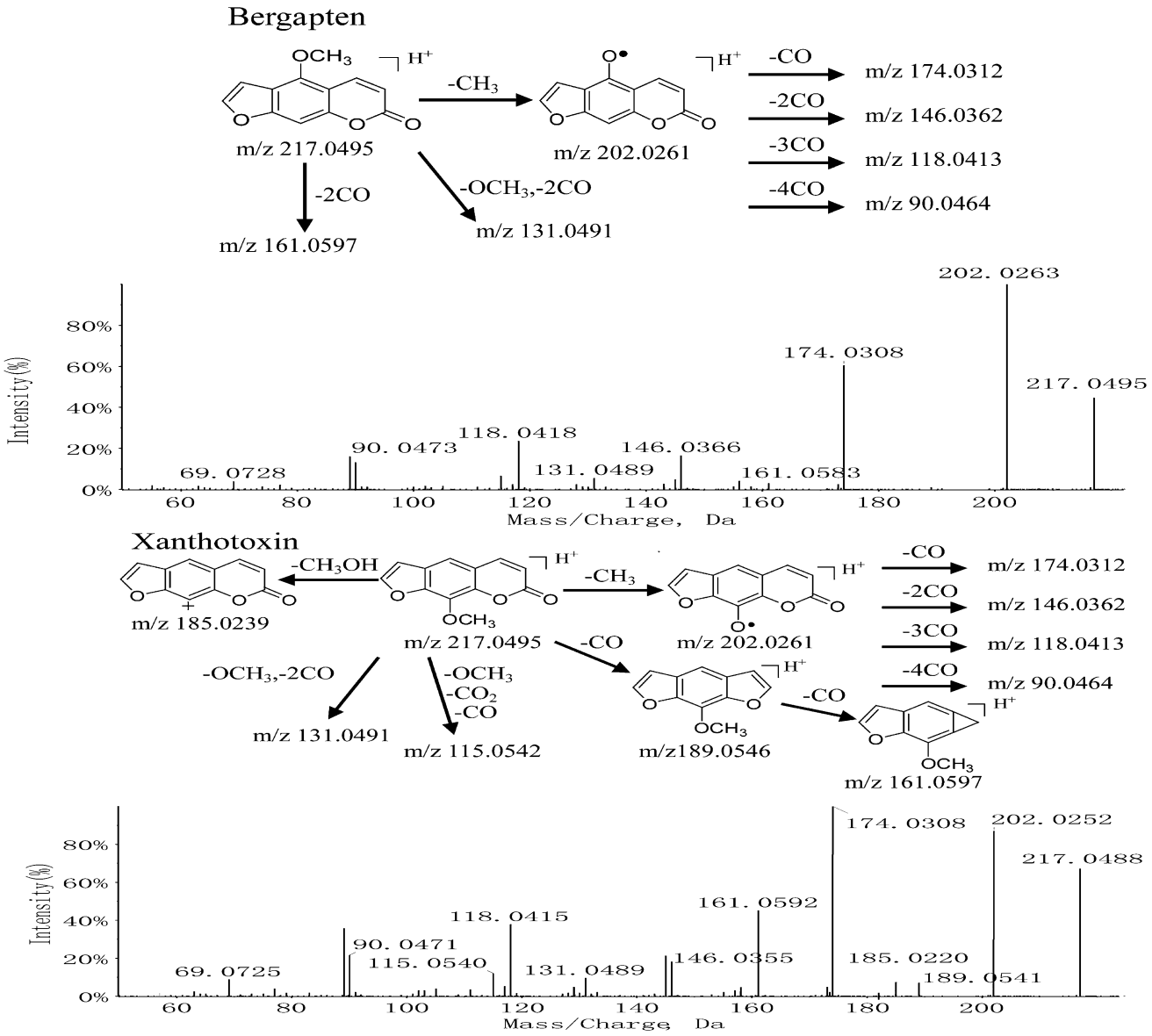

Fig. 1 MS/MS spectra and fragmentation pathways for bergapten and xanthotoxin

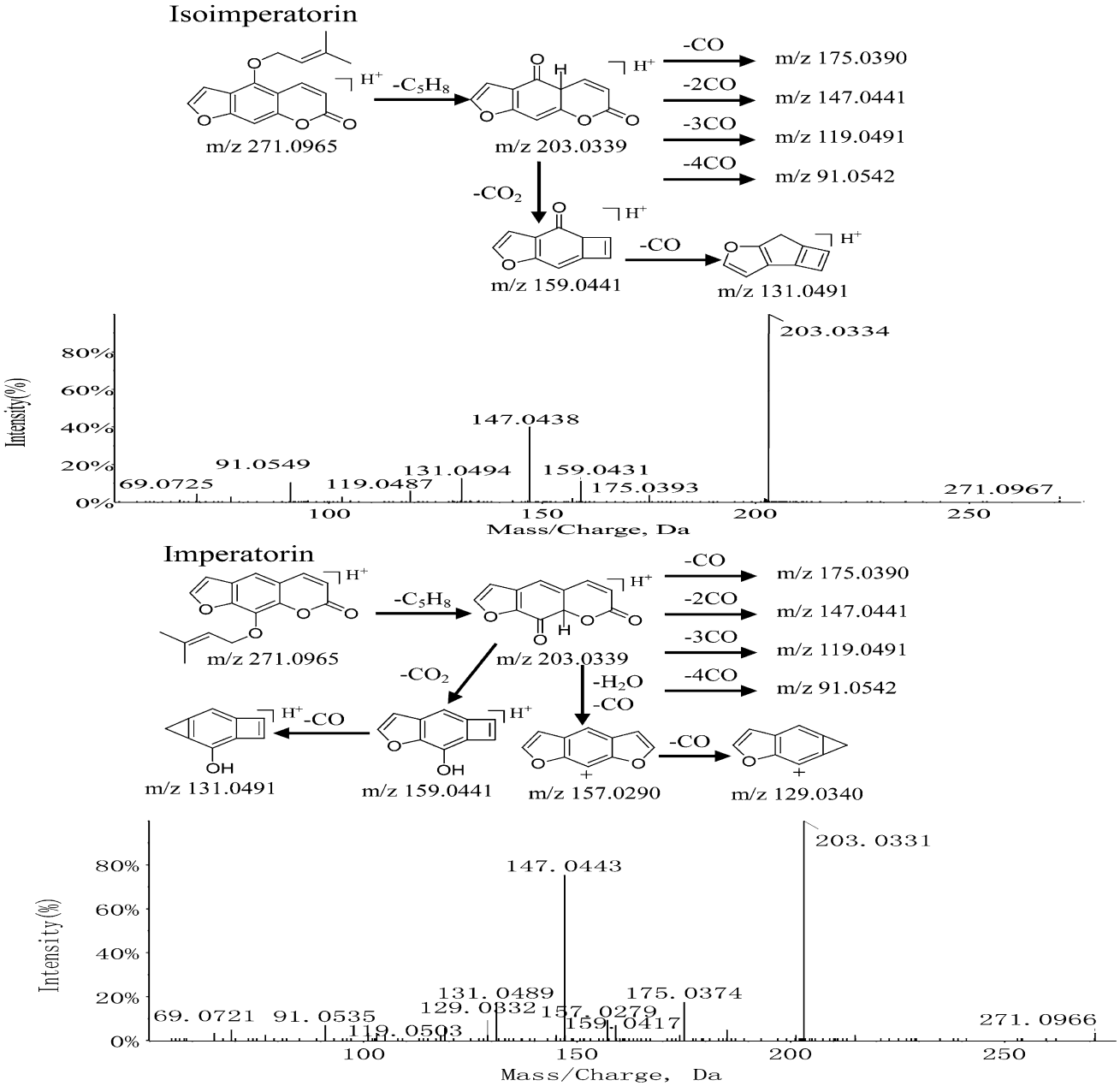

Fig. 2 MS/MS spectra and fragmentation pathways for isoimperatorin and imperatorin

obtained by successive loss of CO, resulting in two ratios of relative abundance in the MS/MS spectra with respect to the reference ion (*F*0). These two ratios for the C-5-substituted compound (denoted *M*1, *M*2) were then compared with the two ratios for the C-8 substituted compound (denoted *N*1, *N*2), yielding two new ratios denoted as *R*1 and *R*2. For both *R*1 and *R*2 greater than 1, the former compound is confirmed to be C-5 substituted while the latter compound is C-8 substituted. This approach is called the ratio of relative abundance (RRA) of characteristic fragment ions method. It was found that the polarity and mass fragmentation pathways were similar between the isomers, and the relative abundance of their characteristic fragment ions was distributed in a very similar manner. According to the formula $R = M/N$, the values for the four pairs of furanocoumarin isomers were greater than 1. Therefore, the cutoff is 1, and cannot be higher.

The isomers bergapten and xanthotoxin are taken as an example to explain this rule. Bergapten is 5-methoxypsoralen (denoted *M*), while xanthotoxin is 8-methoxypsoralen (denoted *N*). The main fragmentation pathway for both isomers was methyl group loss to generate fragment ion *m*/*z* 202, followed by sequential loss of two

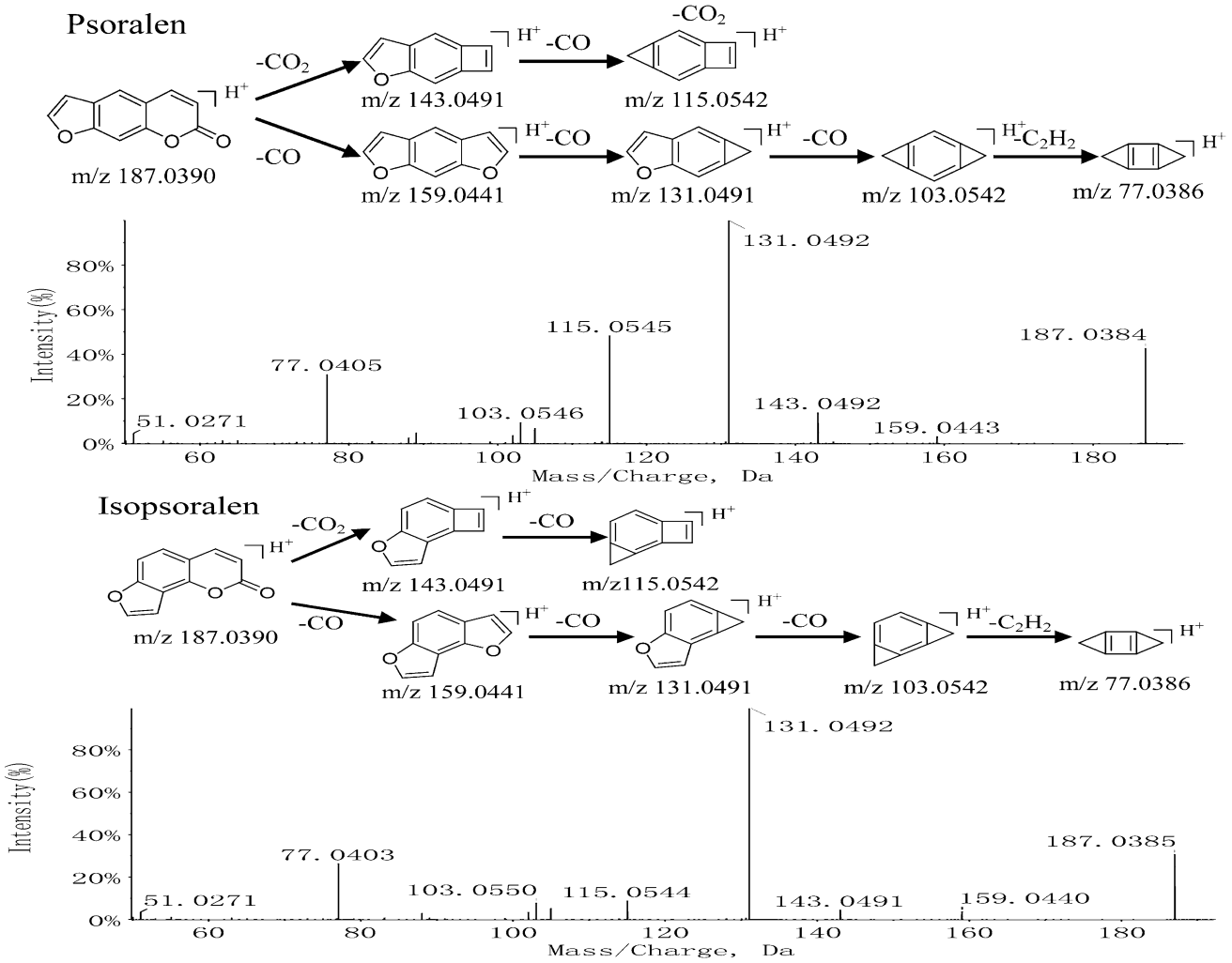

Fig. 3 MS/MS spectra and fragmentation pathways for psoralen and isopsoralen

CO, generating fragment ions *m*/*z* 174 and *m*/*z* 146. The ion at *m*/*z* 202 was used as a reference ion (*F*0), and the fragment ions obtained from it are denoted as *F*1 and *F*2. The relative abundance of *F*0 to that of *F*1 and *F*2 for bergapten are denoted as *M*1 and *M*2, while for xanthotoxin they are denoted as *N*1 and *N*2. The ratios of *M*1 and *M*2 to the corresponding *N*1 and *N*2 are denoted as *R*1 and *R*2, calculated using the formula $R = M/N$. For *R* greater than 1, compound *M* is substituted at C-5 while compound *N* is substituted at C-8, whereas for *R* value less than 1, compound *M* is substituted at C-8 while compound *N* is substituted at C-5. In addition, isopsoralen and impinellin are angular furanocoumarins that can be considered to be C-8 substituted. The isomers psoralen and isopsoralen as well as impinellin and isoimpinellin also followed this rule. Detailed information regarding the standards is presented in Table 1.

Validation of RRA Methodology

The standard solution and sample results were acquired in product ion scan mode using the same procedure as described above. Psoralen, xanthotoxin, bergapten, imperatorin, isoimperatorin, and isoimpinellin were detected in the sample solution with good specificity (see Supplementary Fig. S1).

The repeatability of six independent analyses of the standard mix solution and sample was determined in product ion scan mode using the same procedure as described above. The relative abundance of the main fragmentation ions for each analyte in standard solution and sample solution was recorded, showing relative standard deviations (RSDs) of the relative abundance variations and the ratios of the relative abundance variations less than 10%.

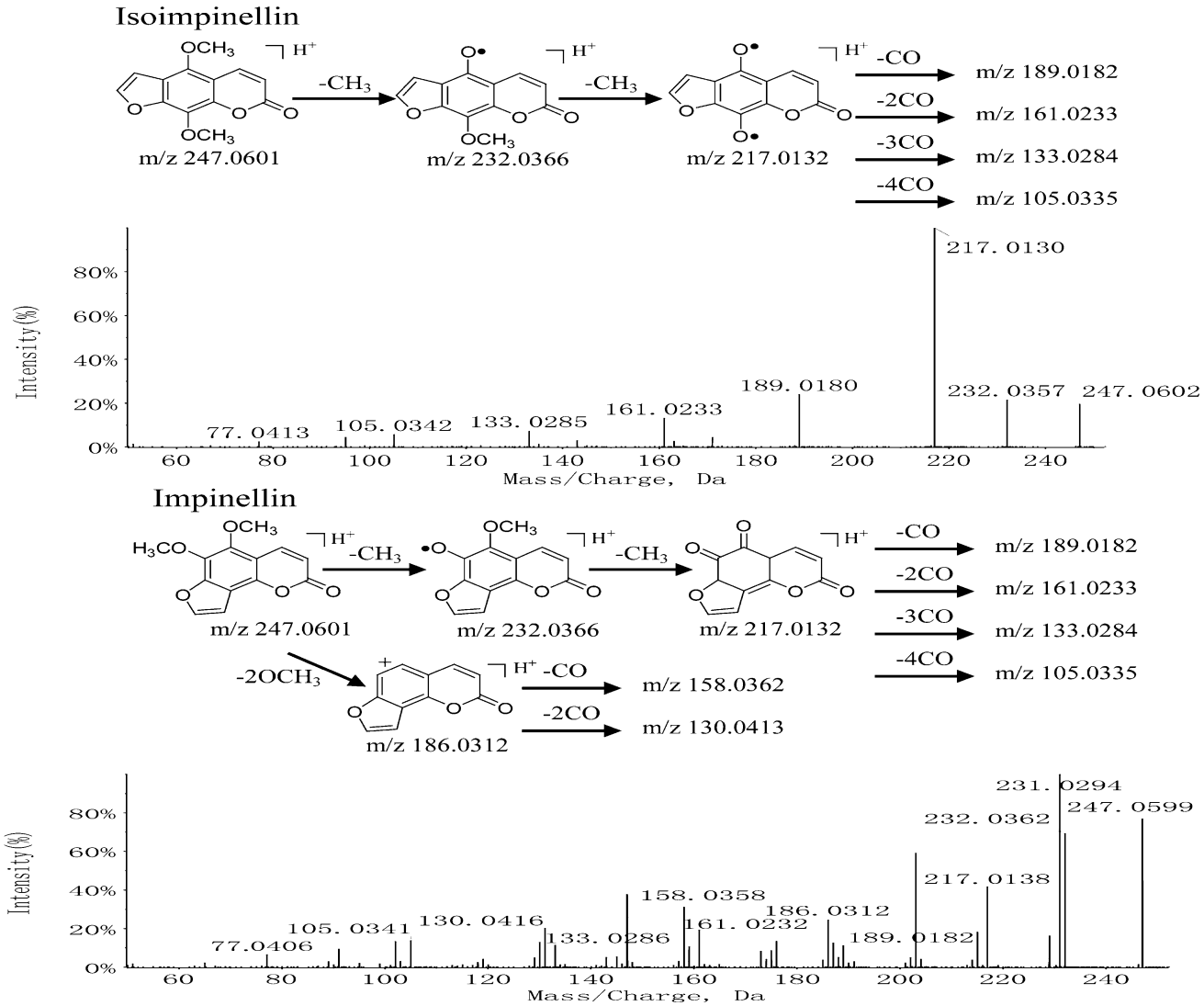

Fig. 4 MS/MS spectra and fragmentation pathways for isoimpinellin and impinellin

Selection of Scan Modes

The combination of the high separation efficiency of UHPLC with the high scanning speed, high sensitivity, and good selectivity of Q-TOF can enable accurate determination of analyte mass, the exact precursor, and product ions. Rich fragment ions can be acquired in full scan mode combined with information-dependent acquisition (IDA), as widely applied for analysis of components of Chinese herbal medicines. As far as we know, various data processing methods can be used to screen and analyze known or predicted compounds. Using these methods, the structure of compounds can be suggested by combining MS/MS information, fragmentation pathways, and retention time obtained in full scan mode. However, this mode cannot provide a stable response for fragment ions. Meanwhile, product ion scan mode can provide a stable intensity response for fragment ions, because they can be acquired all the time. The combination of these two modes enables identification of chemical constituents of Chinese medicinal materials and to obtain stable relative abundance values for fragment ions. When the collision energy (CE) was set at 35 eV and the collision energy spread (CES) was 15 eV in the MS/MS experiments, a reasonable proportion of precursor ions and product ions could appear for most furanocoumarins.

Application of RRA Method for Identification of Furanocoumarin Isomers in *Angelicae dahuricae* Radix

Firstly, sample solution results were acquired in full scan mode according to the above chromatographic conditions.

Table 1 Ratio of relative abundance of characteristic fragment ions of four pairs of furanocoumarin isomers in standard solution

Relative abundance	Relative abundance of *F*0	Relative abundance of *F*1	Relative abundance of *F*2	*M*1 or *N*1	*M*2 or *N*2	*R*1	*R*2
	202.0261	174.0312	146.0362			*M*1/*N*1	*M*2/*N*2
Bergapten (*M*)	100%	39.8%	14.1%	2.51	7.09	2.67	1.56
Xanthotoxin (*N*)	93.5%	99.5%	20.6%	0.94	4.54		
	203.0339	175.0390	147.0441			*M*1/*N*1	*M*2/*N*2
Isoimperatorin (*M*)	100%	2.3%	34.5%	43.48	2.90	5.65	1.62
Imperatorin (*N*)	100%	13%	56%	7.69	1.79		
	187.0390	159.0441	131.0491			*M*1/*N*1	*M*2/*N*2
Psoralen (*M*)	16.9%	2.3%	99.8%	7.35	0.17	1.75	1.31
Isopsoralen (*N*)	13.4%	3.2%	99.5%	4.19	0.13		
	217.0132	189.0182	161.0233			*M*1/*N*1	*M*2/*N*2
Isoimpinellin (*M*)	100%	14.1%	8.7%	7.09	11.49	1.06	2.76
Impinellin (*N*)	32%	4.8%	7.7%	6.67	4.16		

For compounds bergapten, isoimperatorin, psoralen, and isoimpinellin, *M*1 and *M*2 are the ratios of relative abundance of *F*0 to that of *F*1 and *F*2, respectively. For compounds xanthotoxin, imperatorin, isopsoralen, and impinellin, *N*1 and *N*2 are the ratios of relative abundance of *F*0 to that of *F*1 and *F*2, respectively

The data were analyzed using MasterView software, using the chemical formula reported in literature. Chromatographic peaks matching the molecular formula were selected. A pair of compounds within the error range of ±5 ppm were identified as furanocoumarin isomers, if they had the same fragmentation pathways and satisfied the furanocoumarin cleavage law. Then the stable relative abundance of fragment ions for each compound was obtained in product ion scan mode, and the ratio of relative abundance method was used to identify the furanocoumarin isomers in *Angelicae dahuricae* radix. Finally, five pairs of furanocoumarin isomers were identified (according to the flowchart shown in Fig. 5). The name, molecular weight, chromatographic retention time, and main fragment ions of the ten compounds can be found in Supplementary Table S1. The structures of all the compounds are shown in Fig. 6. The following inference process was used for the ten compounds:

Singly Oxygen-Substituted Furanocoumarin Isomers

1. Bergapten (1-1) and xanthotoxin (1-2): The extraction chromatograms and mass spectra of this pair of compounds are shown above. The stable relative abundance of fragment ions *m*/*z* 202, 174, and 146 was obtained in product ion scan mode. *F*0, *F*1, and *F*2 represent the characteristic fragment ions *m*/*z* 202, 174, and 146, respectively. The relative abundance of *F*0 to that of *F*1 and *F*2 is denoted as *M*1 and *M*2 for bergapten, and for xanthotoxin as *N*1 and *N*2. Using the formulas *R*1 = *M*1/*N*1 and *R*2 = *M*2/*N*2, both values *R*1 and *R*2 were greater than 1. Detailed information is presented in Table 2. Therefore, the peak at 32.560 min corresponds to bergapten (C-5 substituted) while the peak at 28.392 min corresponds to xanthotoxin (C-8 substituted). This result is consistent with that obtained from analysis of reference standards.
2. Isoimperatorin (2-1) and imperatorin (2-2): The extraction chromatograms and mass spectra of this pair of compounds are shown above. The stable relative abundance of fragment ions *m*/*z* 203, 175, and 147 was obtained in product ion scan mode. *F*0, *F*1, and *F*2 represent the characteristic fragment ions *m*/*z* 203, 175, and 147, respectively. The relative abundance of *F*0 to that of *F*1 and *F*2 is denoted as *M*1 and *M*2 for isoimperatorin, and for imperatorin as *N*1 and *N*2. Using the formulas *R*1 = *M*1/*N*1 and *R*2 = *M*2/*N*2, both values of *R*1 and *R*2 were greater than 1. Detailed information is presented in Table 2. Therefore, the peak at 45.871 min corresponds to isoimperatorin (C-5 substituted) while the peak at 40.978 min corresponds to imperatorin (C-8 substituted). This result is consistent with that obtained from analysis of reference standards.
3. Oxypeucedanin hydrate (3-1) and isooxypeucedanin hydrate (3-2): The molecular formula $C_{16}H_{16}O_6$ was input into MasterView software, and the selected chromatogram is shown in Fig. S2 (A). The errors on the protonated molecular ion $[M + H]^+$ for the two peaks at 20.377 min and 25.748 min were within ±5 ppm. Fragment ions *m*/*z* 305, 203, 175, 159, 147, 131, and 59 were found in the MS/MS spectra of both [Fig. S2 (B) and (C)]. It can be deduced that these two compounds are a pair of isomers with furanocoumarin parent ring and molecular weight of 304, namely oxypeucedanin hydrate and isooxypeucedanin hydrate. The stable relative abundance of fragment ions *m*/*z* 203,

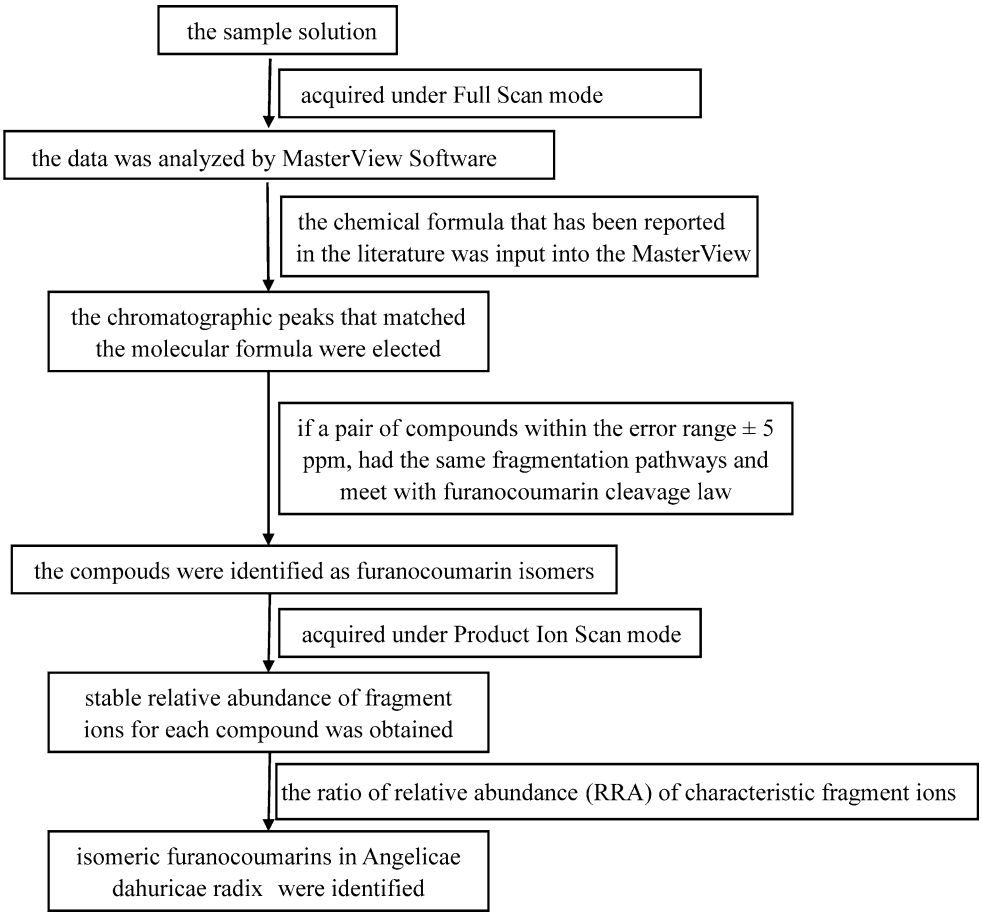

Fig. 5 Flowchart for identification of furanocoumarin isomers in *Angelicae dahuricae* radix

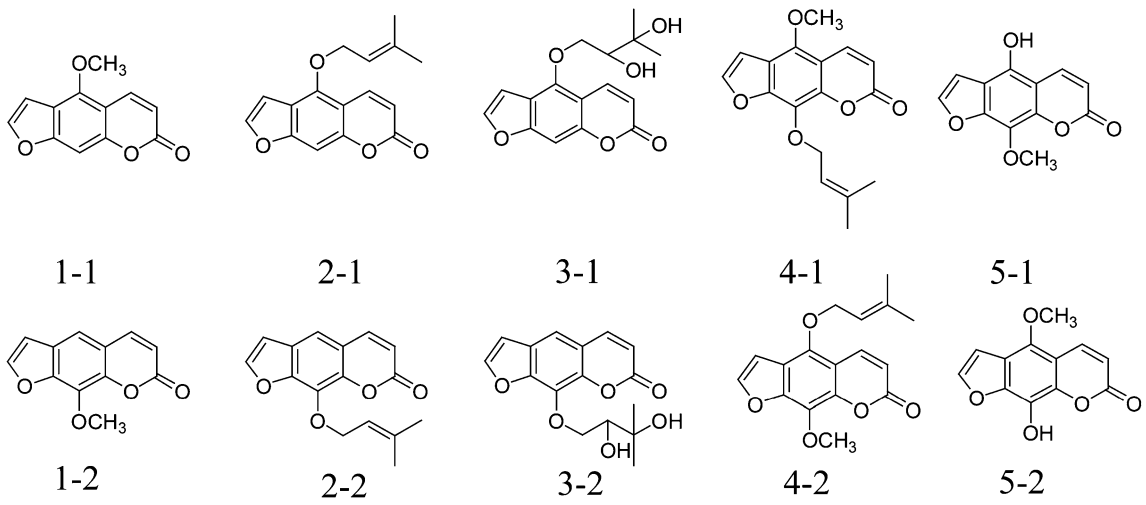

Fig. 6 Structures of the five pairs of furanocoumarin isomers

175, and 147 was obtained in product ion scan mode. *F*0, *F*1, and *F*2 represent the characteristic fragment ions *m*/*z* 203, 175, and 147, respectively. The relative abundance of *F*0 to that of *F*1 and *F*2 is denoted as *M*1 and *M*2 for the peak at 25.748 min, and those for the peak at 20.377 min are denoted as *N*1 and *N*2. Using the formulas $R1 = M1/N1$ and $R2 = M2/N2$, both values of *R*1 and *R*2 were greater than 1. Detailed information is presented in Table 2. Therefore, the peak at 25.748 min corresponds to oxypeucedanin hydrate

Table 2 Ratio of relative abundance of characteristic fragment ions of furanocoumarin isomers in *Angelicae dahuricae* radix

Relative abundance	Relative abundance of *F*0	Relative abundance of *F*1	Relative abundance of *F*2	*M*1 or *N*1	*M*2 or *N*2	*R*1	*R*2
	202.0261	174.0312	146.0362			*M*1/*N*1	*M*2/*N*2
Bergapten (*M*)	100%	42%	16.6%	2.38	6.02	2.38	1.50
Xanthotoxin (*N*)	97.5%	97.5%	24.3%	1.00	4.01		
	203.0339	175.0390	147.0441			*M*1/*N*1	*M*2/*N*2
Isoimperatorin (*M*)	100%	1.8%	35%	55.56	2.86	15.97	1.47
Imperatorin (*N*)	100%	28.7%	51.2%	3.48	1.95		
	203.0339	175.0390	147.0441			*M*1/*N*1	*M*2/*N*2
Oxypeucedanin hydrate (*M*)	100%	1.25%	11%	80.00	9.09	1.54	1.10
Isooxypeucedanin hydrate (*N*)	99%	1.9%	12%	52.11	8.25		
	218.0210	190.0261	162.0312			*M*1/*N*1	*M*2/*N*2
Phellopterin (*M*)	100%	4.8%	10.1%	20.83	9.90	1.07	1.43
Cnidilin (*N*)	43%	2.2%	6.2%	19.55	6.94		
	218.0210	190.0261	162.0312			*M*1/*N*1	*M*2/*N*2
5-Hydroxy-8-methoxypsoralen (*M*)	100%	3.1%	11%	32.26	9.09	1.06	1.09
8-Hydroxy-5-methoxypsoralen (*N*)	100%	3.3%	12%	30.30	8.33		

For compounds bergapten, isoimperatorin, oxypeucedanin hydrate, phellopterin, and 5-hydroxy-8-methoxypsoralen, *M*1 and *M*2 are the ratios of relative abundance of *F*0 to that of *F*1 and *F*2, respectively. For compounds xanthotoxin, imperatorin, isooxypeucedanin hydrate, cnidilin, and 8-hydroxy-5-methoxypsoralen, *N*1 and *N*2 are the ratios of relative abundance of *F*0 to that of *F*1 and *F*2, respectively

(C-5 substituted), while the peak at 20.377 min corresponds to isooxypeucedanin hydrate (C-8 substituted).

Dioxygen-Substituted Furanocoumarin Isomers (C-5 and C-8 Substituted)

Xanthotoxol, xanthotoxin, and imperatorin are all C-8-substituted, by hydroxyl, methoxy, and isopentenoxy group, respectively. The elution order of these three compounds was: xanthotoxol, xanthotoxin, and imperatorin. Thus, the polarity strength of the three substituents lies in the order: hydroxyl > methoxy > isopentenoxy.

1. Phellopterin (4-1) and cnidilin (4-2): The molecular formula $C_{17}H_{16}O_5$ was input into MasterView software, and the selected chromatogram is shown in Fig. S3 (A). The errors on the protonated molecular ion $[M + H]^+$ for the two peaks at 43.357 and 44.795 min were within ±5 ppm. By comparison with the standard, the compound at 44.795 min was found to be cnidilin. Fragment ions *m*/*z* 301, 233, 218, 190, 173, 162, 134, and 69 were found in the MS/MS spectra of both compounds [Fig. S3 (B) and (C)]. The other compound can be deduced to be phellopterin. This pair of isomers has the same substituent groups but at different positions. The stable relative abundance of fragment ions *m*/*z* 218, 190, and 162 was obtained in product ion scan mode. *F*0, *F*1, and *F*2 represent the characteristic fragment ions of *m*/*z* 218, 190, and 162, respectively. The relative abundance of *F*0 to that of *F*1 and *F*2 is denoted as *M*1 and *M*2 for phellopterin, and for cnidilin as *N*1 and *N*2. Using the formulas $R1 = M1/N1$ and $R2 = M2/N2$, both values of *R*1 and *R*2 were greater than 1. Detailed information is presented in Table 2. As the former compound is phellopterin, when the *R* value is greater than 1, compound *M* is C-5 substituted with a substituent group of stronger polarity. On the other hand, an *R* value less than 1 indicates that compound *M* was C-8 substituted with a substituent group of stronger polarity.
2. 5-Hydroxy-8-methoxypsoralen (5-1) and 8-hydroxy-5-methoxypsoralen (5-2): The molecular formula $C_{12}H_8O_5$ was input into MasterView software, and the selected chromatogram is shown in Fig. S4 (A). The errors on the protonated molecular ion $[M + H]^+$ for the two peaks at 26.176 and 30.817 min were within ±5 ppm. Fragment ions *m*/*z* 233, 218, 190, 162, 134, and 78 were found in the MS/MS spectra of both [Fig. S4 (B) and (C)]. It can be deduced that these two compounds are a pair of isomers with furanocoumarin parent ring and molecular weight of 232, namely 5-hydroxy-8-methoxypsoralen and 8-hydroxy-5-methoxypsoralen. The stable relative abundance of fragment ions *m*/*z* 218, 190, and 162 was obtained in product ion scan mode. *F*0, *F*1, and *F*2 represent the characteristic fragment ions *m*/*z* 218, 190, and 162, respectively. The relative abundance of *F*0 to that of *F*1 and *F*2 is denoted as *M*1 and *M*2 for the peak at 26.176 min, and those for the peak at 30.817 min are denoted as *N*1 and

*N*2. Using the formulas $R1 = M1/N1$ and $R2 = M2/N2$, both values of *R*1 and *R*2 were greater than 1. Detailed information is presented in Table 2. Based on the rule for distinguishing dioxygen-substituted isomeric furanocoumarins described above, *R* value greater than 1 indicates that compound *M* was C-5 substituted with a substituent group of stronger polarity, so the peak at 26.176 min corresponds to 5-hydroxy-8-methoxypsoralen, while the peak at 30.817 min corresponds to 8-hydroxy-5-methoxypsoralen.

Conclusions

This is the first method to be established for differentiation of furanocoumarin isomers using the relative abundance of characteristic fragment ions by mass spectrometry. The method was validated, showing good stability and reliability, and was successfully used for characterization and differentiation of five pairs of furanocoumarin isomers in *Angelicae dahuricae* radix. This method is of great significance for differentiation of furanocoumarin isomers in complex systems of traditional Chinese medicine. This study also provides a concept for characterization of other isomeric compounds in complex matrices.

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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