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Studies on the flavones using liquid chromatography–electrospray ionization tandem mass spectrometry

Wei Wu, Cunyu Yan, Li Li, Zhiqiang Liu*, Shuying Liu**

Laboratory of New Drug Research and Mass Spectrometry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, 5625 Renmin Street, Changchun 130022, PR China

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Abstract

Fragmentation pathways of nine flavone compounds have been studied by using electrospray ionization multi-stage tandem mass spectrometry (ESI-MS^n). Analyzing the product ion spectra of flavonoids and aglycones, we observed some diagnostic neutral losses, such as $^{\bullet}CH_3$, H_2O, residue of glucose and gluconic acid, which are very useful for the identification of the functional groups in the structures. Furthermore, specific retro Diels-Alder (RDA) fragments for flavones with different hydroxyl substitution have also been discussed. The information is helpful for the rapid identification of the location site of hydroxyl substitution on flavones. Fragmentation pathways of C-glycosidic flavonoid have also been discussed using ESI-MS^n, demonstrating ions $[M-H\text{-}60]^-$, $[M-H\text{-}90]^-$, $[M-H\text{-}120]^-$ are characteristic ions of C-glycosidic flavonoid. According to the fragmentation mechanism of mass spectrometry and HPLC–MS data, the structures of seven flavones in *Scutellaria baicalensis Georgi* have been identified on-line without time-consuming isolation. The HPLC–ESI-MS^n method for analyzing constituents in the *Scutellaria baicalensis Georgi* has been established.

Keyword: Flavones

1. Introduction

Flavones are a kind of polyphenolic compounds that contain a C6–C3–C6 flavone skeleton. They are structurally a rather diverse group of natural products distributed ubiquitously in the nature [1]. Many investigation results demonstrate that flavones have wide biological activities [2–5], such as anti-oxidants, anti-cancer, anti-inflammatory, anti-HIV, etc. For herbal medicine, it is important to ensure the reliability and reproducible for pharmacological and clinical research, and to improve product quality control, therefore, there is an increasing demand for methods of rapidly identifying and charactering constituents in herb.

With the advent of soft ionization techniques, mass spectrometry has become a powerful analytical tool in phytochemistry due to its sensitivity, rapidity, and low levels of sample consumption [6,7]. A review of the application of FAB/MS/MS to the study of flavonoid glycosides has appeared [8]. Thermospray LC/MS/MS has been used for characterization of flavonoids [9,10]. Ionspray LC/MS/MS [11,12] and APCI/MS/MS [13,14] have been also employed for the analysis of flavonoids. Recently, owing to the large number of flavonoids and commonly existing isomers in plants, high performance liquid chromatography (HPLC) coupled to electrospray ionization tandem mass spectrometry (ESI-MS^n) has been proved to be useful for rapid identification of flavones by comparison with retention time and the mass spectrometry fragmentation pathways of the synthetic standards [15–20]. Thus it appears important to us to investigate the fragmentation pathways of flavones to obtain important structure information.

Scutellaria bacalensis Georgi is one of a commonly used Traditional Chinese Herbal Medicine (TCHM) with clearing away heat effect, in which the main active constituents are

* Corresponding author. Tel.: 86 431 5262 236; fax: 86 431 5262 236.
** Co-Corresponding author.
E-mail address: liuzq@ns.ciac.jl.cn (Z. Liu).

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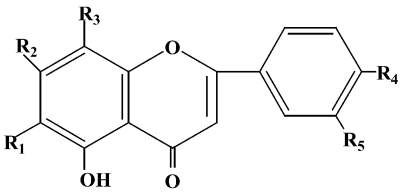


	Name	[M-H]⁻	R_1	R_2	R_3	R_4	R_5
1	apigenin	269	H	OH	H	OH	H
2	norwogonin	269	H	OH	OH	H	H
3	wogonin	283	H	OH	OCH_3	H	H
4	acacetin	283	H	OH	H	OCH_3	H
5	luteolin	285	H	OH	H	OH	OH
6	chrysin	253	H	OH	H	H	H
7	baicalin	445	OH	O-gluconic acid	H	H	H
8	wogonoside	459	H	O-gluconic acid	OCH_3	H	H
9	6-C-ara-8-C-glu chrysin	547	C-ara	OH	C-glu	H	H

ara=arabinose, glu=glucose

Fig. 1. Structures of the flavones (1–9) studied.

flavones compounds. It possesses anti-bacterial activity [21], and is applied in the treatment of diarrhea [22] and hepatitis [23].

In the present study, six flavone aglycones (1–6) and three flavonoids (7–9) standards (listed in Fig. 1) have been first run by electrospray ionization mass spectrometry (ESI-MS) in the negative ion mode to obtain the ions of the molecular species. Then MS^n spectra have been obtained by low energy collision induced dissociation (CID) from these $[M–H]^-$ ions and analyzed to propose plausible fragmentation pathway for each compound studied. Using these techniques, the extract of *Scutellaria baicalensis Georgi* has been analyzed using HPLC–ESI-MS^n. By comparing the retention time (t_R) and product ion spectra of the flavones with those of authentic standards or literature data, the valid identifications of seven kinds of flavones in *Scutellaria baicalensis Georgi* have been provided. The negative ion mode of ESI has been selected in the present study, as it easily provided flavone fragmentations information by CID.

2. Experimental

2.1. Apparatus

The high performance liquid chromatograph (HPLC) system is consisted of a waters (Milford, MA, USA) 2690 HPLC with a photodiode-array detector set at 280 nm. The chromatographic conditions are as follows: column, Dikma Diamonsil C18, 250 mm × 4.6 mm, 5 μm; eluent, (A) water (0.0034 M ammonium acetate and 0.2% acetic acid), (B) acetonitrile. The linear gradient is 0–60 min 30–88% B. The flow-rate is 0.5 ml/min and the temperature is 23 °C.

Mass spectrometry experiment was performed on a LCQ ion trap instrument (Finnigan MAT. San Jose, CA USA) with an electrospray source. The electrospray voltage is set to 5.0 kV. The capillary temperature is 260 °C. The HPLC is connected to the mass spectrometer via the UV cell outlet.

Mass spectrometry experiment at high mass resolution was performed on IonSpec Ultima 7.0 T FTICR-MS (IonSpec, USA) with an electrospray source. Probe heater is 120 °C. Source heater is 80 °C. Probe HV is set to 2.4 kV. Sample cone voltage is set to −30 V. Extractor cone is set to −4 V.

2.2. Standards of flavones

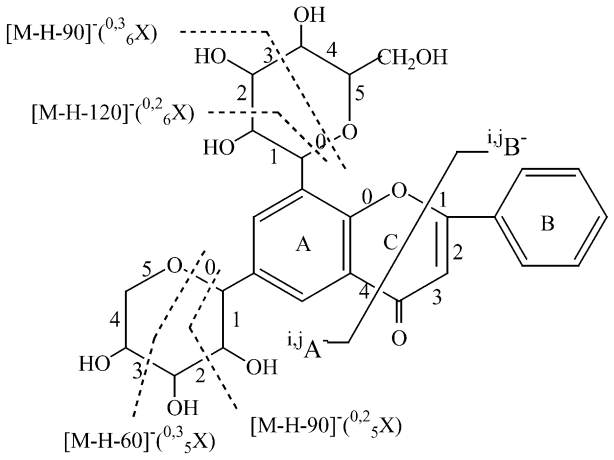
Standards of apigenin (1), wogonin (3), and baicalin (7) were purchased from the Chinese Authenticating Institute of Material Medical and Biological Products (Peking, China); acacetin (4), luteolin (5), and chrysin (6) were obtained from Sigma Chemical (Stlouis MO); wogonoside (8) was kindly provided by Pharmaceutical Academy of Jilin University; norwogonin (2) and 6-C-arabinose-8-C-glucose chrysin (9) were isolated from plants *Scutellaria baicalensis Georgi* by ourselves, which purity were checked by reversed-phase HPLC.

2.3. Extraction and separation

Scutellaria baicalensis Georgi purchased in the Changchun Drug Store was powered and extracted with 75% of ethanol. The extraction was repeated three times. After combining them together, the extract solution was passed through a 0.45 μm filter and 3 μl of the filtrate was then injected into the HPLC–MS system directly.

2.4. Nomenclature

The nomenclature adopted for the product ions containing intact A and B rings of flavone skeleton was adapted from the one proposed by Ma and coworkers [24,25]. The ${}^{i,j}A^-$ and ${}^{i,j}B^-$ represent product ions containing intact A and B rings, the superscripts *i* and *j* indicate the C-ring bonds of flavone skeleton that have been broken. For C-glycosidic flavonoids, the fragment ions were based on the description by Domon and Costello [26], Li and Claeys [27]. The ions produced by the cleavage of hexose are termed ${}^{i,j}{}_6X$, while the ions produced by the cleavage of pentose are termed ${}^{i,j}{}_5X$. The superscripts *i* and *j* indicate the C-bonds of sugar ring that have been broken. Scheme 1 shows the fragments observed in this study.



Scheme 1. Nomenclature adopted for various retrocyclization fragments observed in this study.

3. Results and discussion

3.1. Analysis of standards by ESI-MS^n

3.1.1. Fragmentation of flavonoids and aglycones

The ESI-MS^2 data of $[M–H]^-$ ions in flavones, summarized in Table 1, display some common features, such as the loss of neutral molecules CO (28 Da) and CO_2 (44 Da). It has been demonstrated that the loss of CO and CO_2 from the $[M–H]^-$ ion is due to the contraction of C ring [15], and meanwhile the fragmentation pathways of some flavones exhibit characteristic neutral loss.

Apigenin (1) and norwogonin (2) are a pair of isomers which contain hydroxyl substitution on different positions. The ESI-MS^2 spectra of apigenin and norwogonin are shown in Fig. 2. Analyzing the spectra, the characteristic ions for each compound have been found. Norwogonin (2) is trihydroxylated on A ring, whereas the three hydroxyl groups are distributed between A and B rings in apigenin (1). Norwogonin (2) with two OH groups in *ortho* positions gave the ion of the loss of H_2O (18 Da) at *m/z* 251 from the $[M–H]^-$ ion at *m/z* 269 in the MS^2 spectrum (Fig. 2a) while the corresponding ion in Apigenin (1) has not been observed (Fig. 2b), which is consistent with the result of literature [15]. So according to the loss of H_2O (18 Da), the existence of two OH groups in *ortho* positions can be determined, which is proved by means of the analysis of luteolin and chrysin displayed in Table 1. In addition, the ion at *m/z* 171 (Fig. 2a), the loss of $C_2H_2O_3$ (98 Da), is the characteristic ion of norwogonin with trihydroxyl on A ring. For apigenin (1), the most notable ions are the ion at *m/z* 149 attributed to a $^{1,4}B^-$ + 2H fragment and

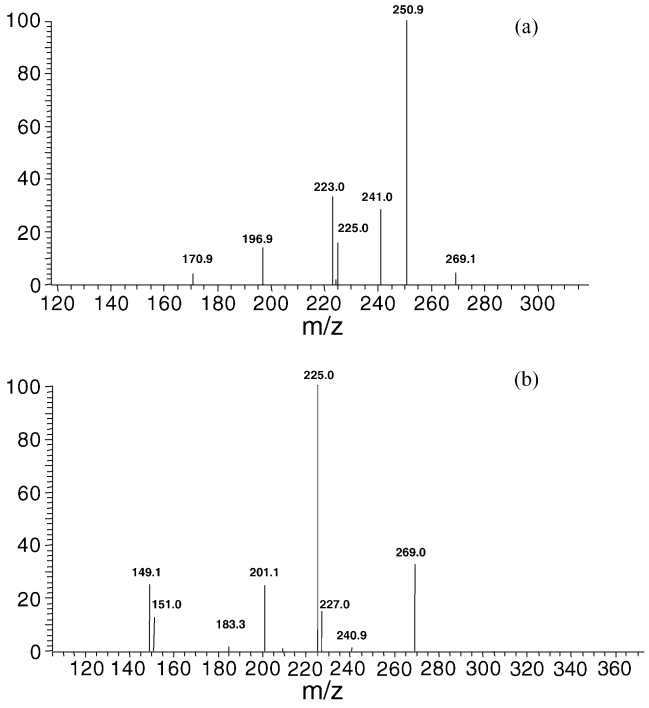


Fig. 2. MS^2 spectrum of the ion $[M–H]^-$ of (a) nowogonin and (b) apigenin.

Table 1
ESI-MS^2 product ions obtained from the $[M–H]^-$ ions of flavones 1–8

	1	2	3	4	5	6	7	8
$[M–H]^-$	269	269	283	283	285	253	445	459
$[M–H–CH_3]^-$	–	–	268	268	–	–	–	–
$[M–H–H_2O]^-$	–	251	–	–	267	–	–	–
$[M–H–CO]^-$	241	241	–	–	257	225	–	–
$[M–H–CO_2]^-$	225	225	–	–	241	–	–	–
$[M–H–H_2O–CO]^-$	–	223	–	–	239	–	–	–
$[M–H–C_2H_2O]^-$	227	–	–	–	243	–	–	–
$[M–H–C_2H_2O–CO_2]^-$	183	–	–	–	199	–	–	–
$[M–H–C_3O_2]^-$	201	–	–	–	217	–	–	–
$[M–H–A]^-$	–	171	–	–	–	–	–	–
$[M–H–CO_2–CO]^-$	–	197	–	–	213	–	–	–
$^{1,3}A^-$	151	–	–	–	151	–	–	–
$^{1,3}A–CO_2$	–	–	–	–	107	–	–	–
$^{1,4}B^-$ + 2H	149	–	–	–	–	–	–	–
$^{1,3}B^-$	–	–	–	–	133	–	–	–
$[M–Glu^a]^-$	–	–	–	–	–	–	269	283

[a] Gluconic acid.

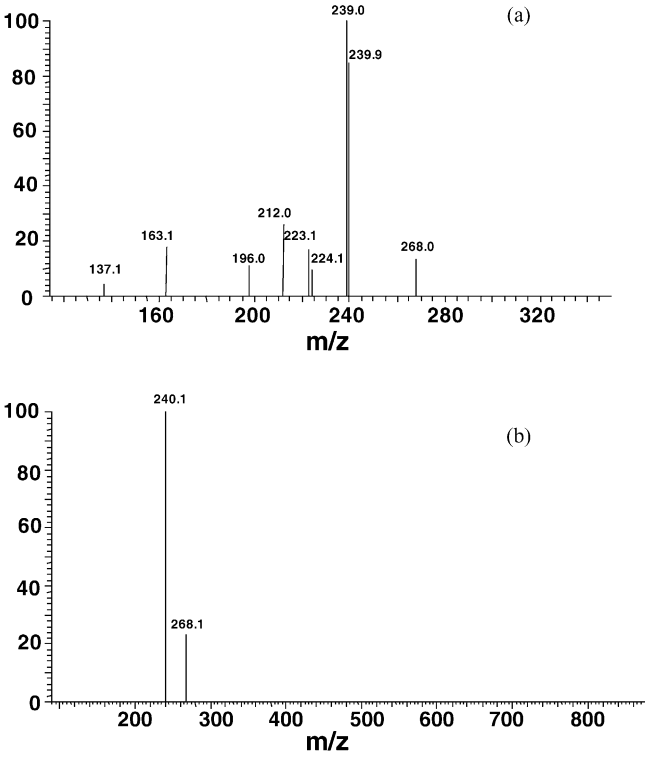


Fig. 3. MS^3 spectrum of the ion at *m/z* 268 of (a) wogonin and (b) acacetin.

ion at *m/z* 151 attributed to a $^{1,3}A^-$ fragment, which were formed from the cleavage of C ring. The compound luteolin (5) had the analogical fragmentation mechanism (Table 1), also having the ions at *m/z* 151 ($^{1,3}A^-$), *m/z* 133 ($^{1,3}B^-$), *m/z* 107 ($^{1,3}A–CO_2$). According to these ions, the location of hydroxyl substitution can be rapidly deduced, and then the isomers can be distinguished. Compared the spectra data of these four compounds (1), (2), (5) and (6), it can be concluded that when the substitution of hydroxyl on B ring, the cleavage of C ring is easy to happen, and the more number of OH groups in the structure, the easier to obtain the fragment ions.

The methoxylated flavones wogonin (3) and acacetin (4) exhibit a significant $[M–H–CH_3]^{-\bullet}$ radical anion as base peak and no other fragmentation in the MS^2 spectra have been observed (Table 1). The loss of a CH_3 radical (15 Da) from the $[M–H]^-$ ion explained the presence of a methoxy group. In MS^3 experiment of the $[M–H–CH_3]^{-\bullet}$ ion of wogonin, the seven ions were observed, which is shown in Fig. 3a. Low signal intensity ions at *m/z* 163 ($^{0,2}A^-$) and 137 ($^{1,4}A^-$) were produced by the cleavage of C ring. The ion at *m/z* 240 has been produced by the loss of CO from 4-position, and ion at *m/z* 224 was produced by loss of CO_2 from 1-O and 4-CO as discussed in the literature [15]. The notable ions are *m/z* 239 and 223, and the ion at *m/z* 239 was the base peak. It is proposed that these ions are due to the respective losses of $COH^\bullet$ and $CO_2H^\bullet$. The loss of $COH^\bullet$ (29 Da) has been described previously [28,29]. The mechanism for the loss of $COH^\bullet$ may be loss from 4-CO and one hydrogen atom from the molecular, thus the molecular is easy to form steady conjugated system. As to the loss of $CO_2H^\bullet$, we do not demonstrate the loss position. The neutral losses of CO, CO_2, $COH^\bullet$ and $CO_2H^\bullet$ were examined by using FTICR–MS at high mass resolution which allowed unambiguous determination of the elemental composition, displayed in Table 2. The ion at *m/z* 212 has been produced owing to the loss of two CO groups, but the position is not sure. As to acacetin (4), the MS^3 spectrum of the $[M–H–CH_3]^{-\bullet}$ ion was very simple (Fig. 3b). Only forming one ion at *m/z* 240 from the loss of 4-CO, thus it is easy to differentiate the methoxylated flavones isomers.

The compounds baicalin (7) and wogonoside (8) are O-glucuronide. In ESI–MS/MS negative ion mode, the glucosidic bond of O-glucuronide was easily cleaved to generate daughter ions of $[M–H–176]^-$ at *m/z* 269 and 283, respectively (Table 1), which resulted from the neutral loss of a gluconic acid residue from $[M–H]^-$ ions at *m/z* 445 and 459. This neutral loss is useful for the identification of O-glucuronides. In the MS^3 experiment, ion at *m/z* 269 produced the same ions as compound (2) discussed earlier (Fig. 4a). For wogonoside, in the MS^3 experiment, ion at *m/z* 283 produced only one even ion at *m/z* 268 (Fig. 4b), by losing of 15 Da, which is obviously corresponding to the loss of a $^\bullet CH_3$ group. In the MS^4 experiment (Fig. 4c), ion at *m/z* 268 produced the same ions as compound (3) discussed before.

3.1.2. Fragmentation of C-glycosidic flavonoid

C-glycosidic flavonoid Compound (9) was studied by ESI-MS/MS. In the negative ion mode full mass spectrum, the C-glycosidic flavonoid showed only the deprotonated ion $[M–H]^-$ at *m/z* 547, so it is easy to determine the molecular mass (548 Da). Fig. 5 showed the ESI-MS^2 spectrum of $[M–H]^-$ ion at *m/z* 547, giving a different fragmentation pattern from those of the O-glucosides, collision energy

Table 2
Observed and calculated mass of proposed neutral loss of the ion at *m/z* 268.03932 of wogonin in FTICR-MS^3

Produced ions (*m/z*)	Proposed neutral loss	Observed mass (Da)	Calculated mass (Da)	Error (ppm)
240.04421	CO	27.99511	27.99492	7.0
239.03642	COH	29.00290	29.00274	5.5
224.04924	CO_2	43.99008	43.98983	5.7
223.04143	CO_2H	44.99789	44.99766	5.1
212.04912	C_2O_2	55.99008	55.98983	4.5
196.05413	C_2O_3	71.98519	71.98474	6.3
163.00466	C_7H_5O	105.03446	105.03404	5.9

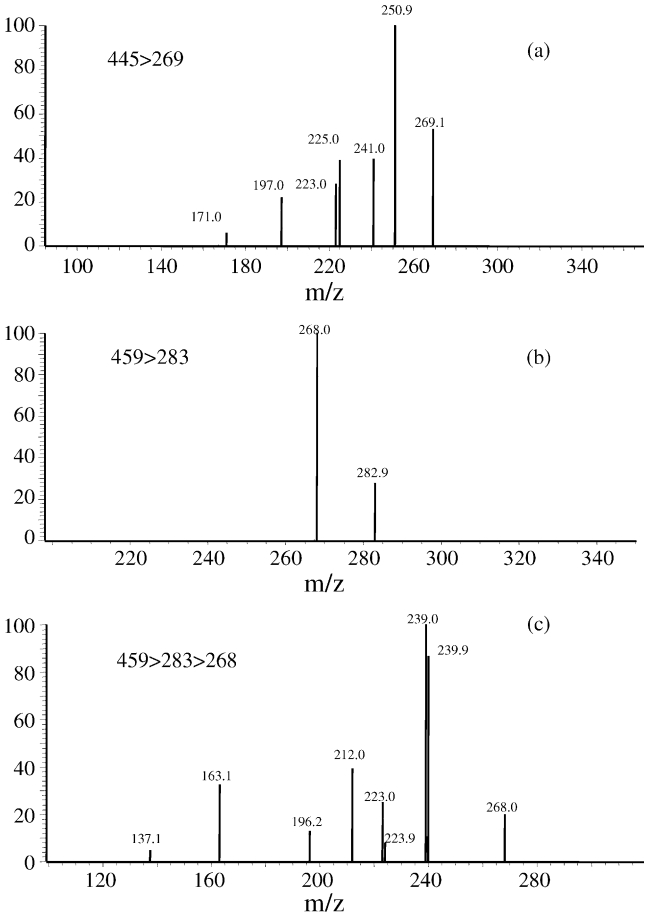


Fig. 4. Mass spectrum of the ion of (a) baicalin 445 > 269 (b) wogonoside 459 > 283 and (c) wogonoside 459 > 283 > 268.

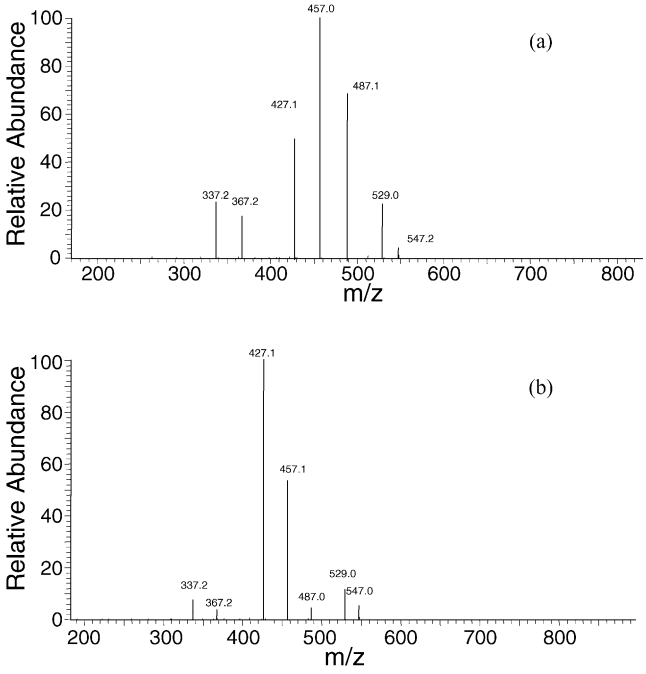


Fig. 5. MS^2 spectrum of the ion at *m/z* 547 of (a) 6-C-arabinose-8-C-glucose chrysin and (b) peak 2.

was 25%. Ions of [*M*–H-60]$^-$, [*M*–H-90]$^-$, [*M*–H-120]$^-$ were observed, which were demonstrated as characteristic ions of C-flavonoid by Becchi and Fraisse [30], who also demonstrated that characteristic fragment ions [*M*–H-60]$^-$ and [*M*–H-120]$^-$of [*M*–H]$^-$ allowed the differentiation between C-glycosylation at the 6- and 8-position. The proposed fragmentation is shown in Scheme 1. Analyzing the ESI-MS^2 spectra data of compound (9), [*M*–H-90]$^-$ ions at *m/z* 457 as base peak can be made up of $^{0,2}{}_5$X and $^{0,3}{}_6$X. Whereas [*M*–H-60]$^-$ ion at *m/z* 487 and [*M*–H-120]$^-$ ion at *m/z* 427 can be only produced by the cleavage of 0,3 bond of C-pentosyl ($^{0,3}{}_5$X) and 0,2 bond of C-hexosyl ($^{0,2}{}_6$X), respectively (Scheme 1). The intensity of $^{0,3}{}_5$X and $^{0,2}{}_6$X were displayed in Table 3. Experiments demonstrated the sugar substitution at the C6 position of flavone gives the most intense fragments [30]. So according to the relative abundance of ion [*M*–H-60]$^-$ ($^{0,3}{}_5$X) and ion [*M*–H-120]$^-$ ($^{0,2}{}_6$X), the linkage information of pentose and hexose can be deduced. The mass difference between *m/z* 547 and 529 is 18 Da, which suggested the loss of one H_2O. Ions at *m/z* 367 [*M*–H-180]$^-$ and *m/z* 337 [*M*–H-210]$^-$ can be made up of the cleavage of C-pentosyl ring and C-hexosyl ring. The relative intensities of these ions are summarized in Table 3.

The specific MS^n fragmentations mechanism of [*M*–H]$^-$ ion discussed above can be used for the identification of flavones in *Scutellaria baicalensis Georgi*.

3.2. Analysis of the extract of Scutellaria baicalensis Georgi by LC–MS/MS

An HPLC–DAD chromatogram and an HPLC–MS total ion chromatogram of the extract of *Scutellaria baicalensis Georgi* are shown in Fig. 6. In HPLC–DAD chromatogram, peaks 1–7 were identified, each peak had its corresponding peak in HPLC–MS, so it could be considered that the constituents in the extract have been well separated and detected. Table 4 lists the information of compounds identified in this paper. As shown in Table 4, peaks 1 and 2 are isomers with

Table 3
Relative intensity of ions in ESI-MS/MS of the two C-glycosidic flavonoid isomers

Ions in MS^2	[M−H-60]$^-$ (%)	[M−H-90]$^-$ (%)	[M−H-120]$^-$ (%)	[M−H-180]$^-$ (%)	[M−H-210]$^-$ (%)	[M−H-18]$^-$ (%)
Standard 9	70	10‘0	50	17	24	22
Peak 1	70	100	50	17	24	22
Peak 2	4	53	100	4	8	12

Table 4
Compounds identified in the present work in *Scutellaria baicalensis Georgi* by HPLC–MS^n

Compound	Peak no.	M_r	t_R (min)	Compared with standards	MS^n ions (*m*/*z*)
6-C-ara-8-C-glu chrysin	1	548	11.7	Yes	$547 \xrightarrow{MS^2} 529,487,457,427, 367,337$
6-C-glu-8-C-ara chrysin	2	548	12.8	No	$547 \xrightarrow{MS^2} 529,487,457,427, 367,337$
Baicalin	3	446	17.5	Yes	$445 \xrightarrow{MS^2} 269 \xrightarrow{MS^3} 251,241,225,\ 223,171,197$
Wogonin 5-O-D-glu	4	446	20.3	No	$445 \xrightarrow{MS^2} 283 \xrightarrow{MS^3} 268 \xrightarrow{MS^4} 240,239,224,223, 163, 212,196,137$
Wogonoside	5	460	22.2	Yes	$459 \xrightarrow{MS^2} 283 \xrightarrow{MS^3} 268 \xrightarrow{MS^4} 240,239,224,223, 163,212,196,137$
Norwogonin	6	270	34.0	Yes	$269 \xrightarrow{MS^2} 251,241,225, 223,171,197$
Wogonin	7	284	43.5	Yes	$283 \xrightarrow{MS^2} 268 \xrightarrow{MS^3} 240,239,224,223, 163,212,196,137$

ara: arabinose; glu: glucose.

molecular weight 548 Da. To further investigate the two isomers' structures, we employed tandem mass spectrum experiment on the two $[M–H]^-$ ions at *m*/*z* 547 separately. In the MS^2 spectra of the two compounds (Table 3, Fig. 5), Ions of $[M–H\text{-}60]^-$, $[M–H\text{-}90]^-$, $[M–H\text{-}120]^-$ were observed, consistent with the characteristic ions of C-flavonoid. Analyzing

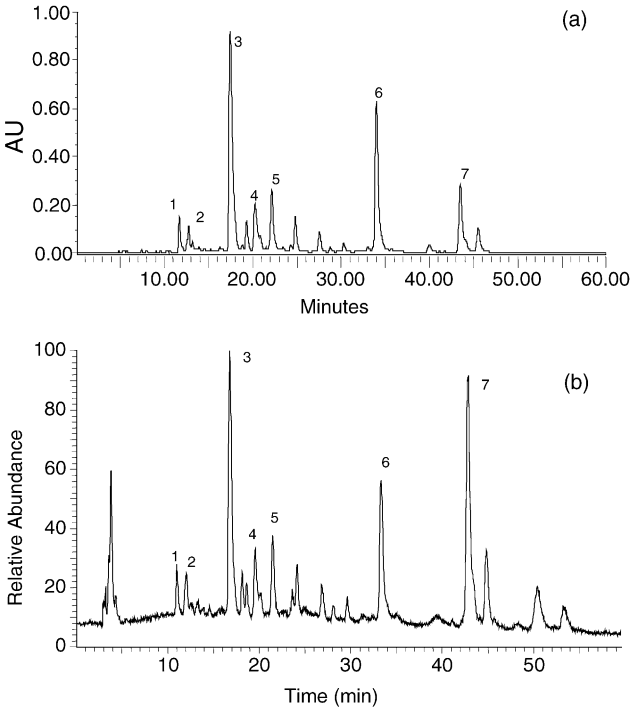


Fig. 6. (a) HPLC–DAD chromatogram of the extract of *Scutellaria baicalensis Georgi*, (b) HPLC–MS total ion chromatogram of the extract of *Scutellaria baicalensis Georgi*, 1 = 6-C-arabinose-8-C-glucose chrysin; 2 = 6-C-glucose-8-C-arabinose chrysin; 3 = baicalin; 4 = wogonin-5-O-glucoside; 5 = wogonoside; 6 = norwogonin; 7 = wogonin.

these data, although the two compounds have totally the same kinds of ions, the relative intensities of these ions are completely different, summarized in Table 3. Comparison with the results obtained for 6-C-arabinose-8-C-glucose chrysin (compound 9) suggested that peak 1 is 6-C-arabinose-8-C-glucose chrysin. As to peak 2, we found the relative intensity of the diagnostic ion $[M–H\text{-}60]^-$ at *m*/*z* 487 is low (4%), while the relative intensity of ion $[M–H\text{-}120]^-$ at *m*/*z* 427 is the base peak (100%) (Fig. 5b). According to the former discussion, we can conclude that the C-pentosyl substitution is on the C8, whereas the C-hexosyl substitution is on the C6. Combined with literature [31], the peak 2 can be identified as 6-C-glucose-8-C-arabinose chrysin. Peaks 3 and 4, eluted at 17.5 and 20.3 min, respectively in the HPLC–DAD system (Fig. 6a), have been studied by ESI-MS^n. In full scan negative ion mode MS, peaks 3 and 4 all exhibited as $[M–H]^-$ ion at *m*/*z* 445 and its dimer *m*/*z* 891(Table 4), which indicate that these two compounds are the isomers. They can be distinguished by using tandem mass spectrometry, the MS^2 spectrum of the $[M–H]^-$ ion at *m*/*z* 445 of peak 3 showed one ion $[M–H\text{-}176]^-$ at *m*/*z* 269 (Table 4), which indicated the loss of a gluconic acid moiety as discussed above. The MS^3 spectrum of the *m*/*z* 269 ion resulted in a fragmentation spectrum in which the main ions matched with the fragmentation spectrum of baicalin (Table 4), thus peak 3 at *m*/*z* 445 can be identified as baicalin compared with the t_R and MS^n data of standard. While in the MS^2 spectra (Fig. 7a), peak 4 yielded the ion $[M–H\text{-}162]^-$ at *m*/*z* 283, corresponding to the loss of a hexose sugar residue. The ion at *m*/*z* 283 in turn produced the ion at *m*/*z* 268 (Fig. 7b), indicating the loss of a CH_3 radical group, whose fragmentation pathways in MS^4 spectrum matched with those of wogonin (Fig. 7c). Combined with the literature data [31], peak 4 has been deduced as wogonin-5-O-glucoside. Peak 5 at t_R 22.2 min (*m*/*z* 459) gave ion at *m*/*z* 283 indicating the loss of gluconic acid residue

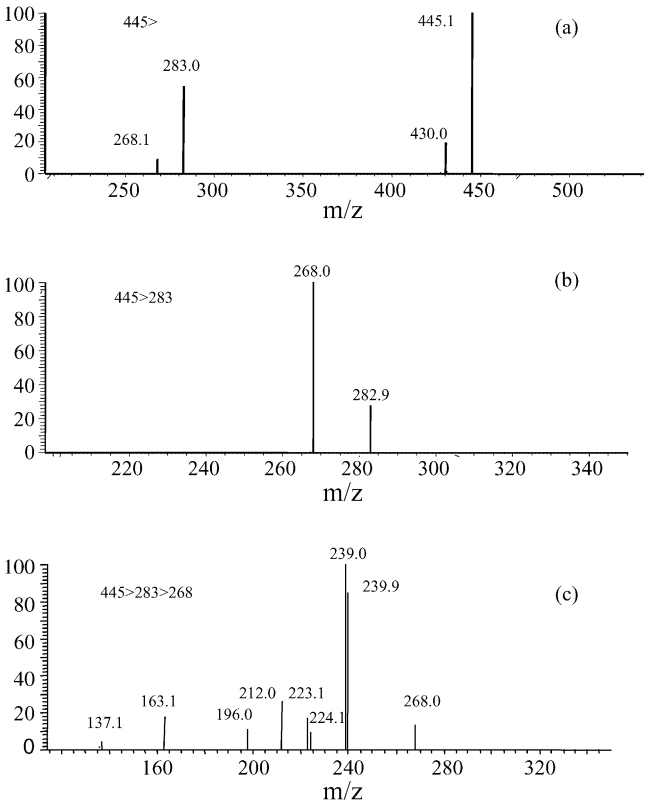


Fig. 7. (a) MS^2 spectrum of ion at *m/z* 445 of peak 4 (b) MS^3 spectrum of ion at *m/z* 445 of peak 4 and (c) MS^4 spectrum of ion at *m/z* 445 of peak 4.

(176 u) in the MS^2. So peak 5 was confirmed as wogonoside compared with t_R and MS^n spectrum data of standard (Table 4).

Peaks 6 and 7, detected at 34.0 and 43.5 min in HPLC–DAD chromatograph, were matched with the t_R of authentic norwogonin and wogonin compounds, respectively. MS^2 data of peak 6 exhibited the diagnostic neutral loss of H_2O (18 Da) resulting in the ion at *m/z* 251 as described above, whose fragmentation mechanism of mass spectrometry was consistent with the standard of norwogonin (Table 4). The mass spectrum of peak 7 exhibited the molecular ion at *m/z* 283, The MS^2 spectrum of $[M–H]^-$ ion at *m/z* 283 exhibited the methoxylated flavone characteristic loss of $^{\bullet}CH_3$ (15 Da) resulting in the ion at *m/z* 268, the MS^3 results matched with the standard of wogonin (Table 4). Thus peaks 6 and 7 were identified as norwogonin and wogonin, respectively.

4. Conclusions

In this study, the nine flavones have been characterized by using ESI-MS^n and the interesting losses of neutral molecules have been proposed, which are useful for the identification of flavone structures. An HPLC–ESI-MS^n method was developed to separate and identify flavone compounds. By using this method, the seven flavones in *Scutellaria baicalensis Georgi* have been identified. The experimental results demonstrate that both ESI-MS^n and HPLC–ESI-MS^n are powerful analytical tools for the rapid identification of the flavone structures without time-consuming isolation of compounds.

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