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Research Article

DRUG FORMULATIONS

Identification of Chemical Constituents in Zhizhu Pills Based on UPLC-QTOF-MS^E

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Abstract

Background: Zhizhu pills (ZZP) are a traditional Chinese medicine (TCM) prescription, mainly used for clinically treating digestive diseases such as functional dyspepsia, constipation, and peptic ulcer. However, the chemical constituents of ZZP have rarely been reported.

Objective: To establish an ultrahigh-performance liquid chromatography-quadrupole time of flight-mass spectrometry (UPLC-QTOF-MS^E) method for the identification of chemical constituents in ZZP, including individual herbs and a complicated Chinese medicinal formula.

Methods: The extracts of ZZP and its individual herb samples were analyzed by a UPLC-QTOF-MS^E method on an ACQUITY UPLC HSS T3 column (100 × 2.1 mm id, 1.8 μm particle size) using a gradient elution of 0.1% formic acid in acetonitrile - 0.1% formic acid water (v/v) at a constant flow rate of 0.4 mL/min. With the MS^E technique, both precursor ion and fragmentation information of compounds can be simultaneously acquired by alternating between low and high collision energy during a single chromatographic run. The data were analyzed on UNIFI.

Results: A total of 154 compounds, including 67 flavonoids, 17 coumarins, 11 terpenoids, 10 alkaloids, six limonoids, six sequiterpene lactones, and 37 other components, were ultimately identified based on accurate masses and fragmentation patterns in ZZP and its individual herbs.

Conclusions: This paper summarized fragmentation patterns of flavonoids, sequiterpene lactones, alkaloids, coumarins, and limonoids. A rapid, accurate, and comprehensive UPLC-QTOF-MS^E method has been developed for the identification of chemical compounds and applied to simultaneously evaluate the quality and effectiveness of ZZP.

Highlights: A total of 154 compounds were ultimately identified in ZZP and its individual herbs by UPLC-QTOF-MS^E; the fragmentation patterns of flavonoids, sequiterpene lactones, alkaloids, coumarins, and limonoids in ZZP and its individual herbs are summarized.

Zhizhu pills (ZZP) is a classical formula comprising two traditional Chinese medicines (TCM)—stri-baked Aurantii Fructus Immaturus (AFI) with wheat bran (WAFI) and raw Atractylodis Macrocephalae Rhizoma (AMR). Furthermore, the water extract of Nelumbinis Folium (NF) was also used in the preparation of ZZP. ZZP was initially described by Dongyuan Li in "Distinguishing the Confusion of Internal and External Injury" as a TCM prescription to cure distention and fullness (1). Nowadays, ZZP has been widely used to treat digestive diseases such as functional dyspepsia, constipation, and peptic ulcer (2). These therapeutic effects have been attributed to active components in the prescription formula of ZZP.

In the past few decades, chemical constituents of individual herbs in ZZP have been reported in much of the literature (3–6). However, the chemical analysis of ZZP remains poorly defined.

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Some researchers have identified many volatile compounds in ZZP (7, 8). In order to provide valuable information for quality control, it is necessary to develop a reliable and sensitive analytical method to identify and characterize chemical compounds in ZZP.

TCM preparations have numerous and extremely complex compositions with similar structure. Thus, compositional analysis of TCM preparations remains a challenge for TCM research. However, ultrahigh-performance liquid chromatography-quadrupole time of flight-mass spectrometry (UPLC-QTOF-MS) is an efficient analytical tool with high selectivity, specificity, and accuracy that is more suitable for compositional analysis of TCM preparations, especially the UPLC-QTOF-MS method combined with the acquisition mode of MS^E, which could provide parallel alternating scans for acquisition at either low collision energy to obtain precursor ion information, or ramping of high collision energy to obtain full-scan accurate mass fragment, precursor ion, and neutral loss information (9, 10). This method is commonly used to deduce chemical structures of compounds. In general, the same class of compounds have similar mother nuclei and mass spectral fragmentation pattern during cracking. For example, the main and typical fragmentation pathway of flavonoids is RDA reaction on the C-ring (11, 12). The benzyl of benzylisoquinoline alkaloids is always cleaved on the C-1 position (13, 14). The aporphine alkaloids have occurred mainly in the RDA reaction on the B-ring (15, 16). In addition, the coumarins can consecutive lost neutral CO group until all oxygen atoms are lost (17, 18), and so on. However, they also have some different fragmentation behavior due to compounds with different substituents. In this study, we identified and characterized chemical compounds in ZZP and its individual herbs by UPLC-QTOF-MS^E and summarized fragmentation patterns of flavonoids, sequiterpene lactones, alkaloids, coumarins, and limonoids in ZZP and its individual herbs. In a word, we are looking forward to providing a scientific basis for evaluating the overall quality of ZZP.

Experimental

Materials and Reagents

AFI, AMR, and NF were bought from Sichuan Neautus Traditional Chinese Medicine Co., Ltd. WAFI was prepared corresponding to the processing specification in the Chinese Pharmacopoeia. Atractylenolide I, narirutin, naringenin, sinensetin, tangeretin, astragaline, and nuciferine were from Chendu Mansite Biotech (Chengdu, China). Hesperidin and hesperetin were from Jiangsu Yongjian Medicine Technology Co., Ltd. (Jiangsu, China). Obacunone was from Dalian Meilun Biological Technology Co., Ltd. (Dalian, China). Formic acid of LC-MS grade was purchased from Merck KGaA (Darmstadt, Germany). Methanol of HPLC grade and acetonitrile of LC-MS grade were purchased from Thermo Fisher Scientific (Shanghai, China). Ultrapure water was produced through a Milli-Q ultrapure water system (Millipore, Billerica, MA).

Sample Extraction and Preparation

NF was decocted in water (40:1, v/w) for 1 h and filtered through filter paper. The residues were continuously decocted in water (20:1, v/w) for 1 h and filtered. The mixed solution was centrifuged at 12 000 r/min for 5 min and a concentration of 0.25 g/mL was made by evaporating water.

WAFI and AMR were individually crushed and sifted through an 80-mesh sieve and were accurately weighed in proportion (1:2) and then fully mixed. Finally, the decoction of NF was added and pelleted into ZZP.

Two grams of rough powders of WAFI, AMR, and ZZP were accurately weighed and soaked in methanol (12.5:1, v/w) for 30 min, and then ultrasonic-assisted extracted for 30 min. The solution was centrifuged at 12 000 r/min for 5 min, and then the supernatants were filtered through a 0.22 μm filter membrane, and all filtrates were stored at 4°C.

Preparation of the Mixed Standard Solution

The standard stock solutions of 10 reference standards (atractylenolide I, hesperidin, hesperetin, narirutin, naringenin, sinensetin, tangeretin, astragaline, nuciferine, and obacunone) were prepared by dissolving them in methanol, respectively. Then the appropriate amount of each standard stock solution was taken and mixed, and finally diluted to an appropriate concentration. The standard solution was filtered through a 0.22 μm filter membrane, and the filtrate was stored at 4°C.

Liquid Chromatographic and Mass Spectrometric Conditions

For the chromatographic conditions in this work, it was conducted on the basis of our previous study with minor revision (19). The chromatographic analysis was performed on a Waters ACQUITY I-Class UPLC system (Waters Corp.), consisting of a binary solvent delivery system, an autosampler, and a PDA detector system. An ACQUITY UPLC HSS T3 column (2.1 × 100 mm id, 1.8 μm particle size) was applied at a temperature of 30°C. The mobile phase system was acetonitrile with 0.1% formic acid (solvent A) and water with 0.1% formic acid (solvent B) in the following gradient elution: 0–2 min, 0.2–2% A; 2–6 min, 2–15% A; 6–8 min, 15–16% A; 8–9 min, 16–17% A; 9–12.5 min, 17–22% A; 12.5–13 min, 22–23% A; 13–17 min, 23–29% A; 17–18 min, 29–35% A; 18–18.4 min, 35–36% A; 18.4–20 min, 36–42% A; 20–21 min, 42–43% A; 21–23 min, 43–60% A; 23–28 min, 60–62% A; 28–30 min, 62–100% A. The flow rate of the mobile phase was 0.4 mL/min. The injection volume was 2 μL for each run.

The mass spectrometric detection was performed on a Waters XEVO G2-XS QTOF-MS (Waters Corp.) connected to the Waters ACQUITY UPLC system via an electrospray ionization (ESI) interface. High-purity nitrogen was used as the nebulizer and auxiliary gas, while argon was used as the collision gas. The QTOF-MS was operated in positive ion mode with a capillary voltage of 2.0 kV and negative ion mode with a capillary voltage of 2.5 kV. Both positive ion mode and negative ion mode were in a sampling cone voltage of 40 V and source offset of 80 V. The ESI source temperature was set at 100°C, and the cone gas flow was 100 L/h. In addition, the desolvation temperature was 400°C, and the desolvation gas flow rate was 800 L/h. The mode of data acquisition was MS^E; collision energy was 6 V in low energy mode and 20 V to 30 V in high energy mode. Continuum data were acquired for each sample from 50 to 1000 Da. In addition, the mass accuracy was maintained by using Lockspray. The $[M+H]^+$ ion of leucine-enkephalin at m/z 556.2771 was used as the lock mass in positive ESI mode. The $[M-H]^-$ ion of leucine-enkephalin at m/z 554.2615 was used as the lock mass in negative ESI mode. The concentration of leucine enkephalin was 1 ng/mL, and infusion flow rate was 10 μL/min. Masslynx v.4.1 (Waters Corp.) was used to control all of the data acquisition.

Data Analysis Strategy

The data were processed on UNIFI v.1.9.4 (Waters Corp.). First, a database of chemical compounds belonging to ZZP (>800

compounds) was established. Information on chemical constituents of ZZP and its individual herbs, including the names, molecular formulas, and chemical structures, was collected by comprehensively searching literature and databases, such as PubMed, CNKI, Chemspider, and so on. After data acquisition, the chemical components in ZZP and its individual herbs were identified by UNIFI software. The detail parameters were set as follows: the retention time range was 0–30.0 min; the mass tolerance was 5.0 ppm; intensity was >5000; the considered negative adducts were $[M-H]^-$ and $[M+HCOO]^-$; and the considered positive adducts were $[M+H]^+$ and $[M+Na]^+$.

Results and Discussion

Identification of the Main Constituents in ZZP

Under optimized chromatographic and MS conditions, a total of 154 compounds were ultimately identified in ZZP and its individual herbs. Ten of them were identified by comparing them with standard compounds. Since there are no commercial standard compounds, the rest of the compounds were confirmed mainly based on mass spectra data and previous literature research (20–27). The TIC chromatograms of ZZP in positive and negative ion modes are shown in Figures 1 and 2. The details of these identified peaks, such as retention times, molecular formulas, theoretical mass, detected masses, mass errors, fragment ions, sources, and type of compounds, are summarized in Table 1.

Fragmentation Patterns of Representative Constituents

The ZZP mainly includes many kinds of flavonoids, sequiterpene lactones, alkaloids, coumarins, limonoids, terpenoids, and other components. In order to analyze chemical compositions in ZZP and its individual herb effectively, the representative constituents were seriously selected and investigated by UPLC-QTOF-MS^E to determine their retention time, analyzing their mass spectral information and ultimately summarizing their fragmentation pathways.

Flavonoids

Most flavonoids in ZZP originate mainly from AFI and NF, which have similar mother nuclei and mass spectral fragmentation pattern during cracking. The RDA reaction usually occurred on the C-ring of flavonoids to produce corresponding "ion a" and "ion b" from the A-ring and B-ring, respectively. There are some flavonoids that have neither methoxyl substituents at the C-3, C-6, or C-8 positions nor isopentenyl substituents at each position in ZZP. The chemical structures of these flavonoids in ZZP are shown in Figure 3a. Take thtmof (peak 132, $t_R = 23.18$ min) as an example: it could yield protonated ion $[M+H]^+$ at m/z 361.0916 in the positive ionization mode. There are three fragmentation patterns under high collision energies: *(1)* the protonated ion produced fragment ions at m/z 343.0812 by losing a neutral H_2O group; *(2)* the protonated ion produced fragment ions at m/z 333.0980 and 305.1017, owing to the successive loss of a neutral CO group; *(3)* RDA cleavage of C1-C2 and C3-C4 bonds in the C ring, which generated "ion a" at m/z 183.0290, "ion b" at m/z 179.0340, and "ion c" $[C_9H_9O_3^+]$ at m/z 165.0549. Then "ion a" could further fragment by losing a CO group to form characteristic "ion a-28" at m/z 155.0472. However, "ion a" generated "ion a-30" by losing a CH_2O group that will be detected in other flavonoids. The MS fragmentation pathway and MS/MS spectra for the $[M+H]^+$ ion of thtmof are shown in Figure 4a.

Flavonoid glycosides are composed of flavones (flavanones) aglycone and glycosyl. There are two kinds of connection types between flavones (flavanones) aglycone and glycosyl. They were oxyside and carboside. An oxyside was glycosyl linked to aglycone via an oxygen atom, and the compound containing oxyside will be called O-glycoside. The O-glycoside could produce the ions corresponding to aglycone by losing 162 Da (-glucose, -Glc), 146 Da (-rhamnose, -Rha), 132 Da (-Xylose, -Xyl). Then the fragmentation pattern of aglycone was the same as flavones (flavanones). The chemical structures of flavonoid-O-glycoside in ZZP are shown in Figure 3a. Take astragaline (peak 56, $t_R = 12.51$ min) as example: it could yield $[M+Na]^+$ ion at m/z 471.0872 and protonated ion $[M+H]^+$ at m/z 449.1079 in the positive ionization mode. The protonated ion produced "M-28" ion at m/z 421.1391 and "M-162" ion at m/z 287.0554, owing to losing a CO_2 and -Glc group. After being further fragmented, it yielded fragment ions at m/z 259.0590, 231.0663 by successively losing a neutral CO group. Moreover, some of the characteristic fragment ions could be detected, such as "ion a" at m/z 153.0184, "ion b" at m/z 135.0442, and "ion a-28" at m/z 125.0234. This result was demonstrated by combining the fragmentation information with commercial standards.

Furthermore, a carboside was glycosyl linked to aglycone via a carbon atom, and the compound containing carboside will be called C-glycoside. The fragmentation pathways of C-glycoside are different from O-glycoside. For instance, in positive ionization mode, isovitexin (peak 59, $t_R = 12.86$ min) is linked to glycosyl on C6, and it yielded protonated ion $[M+H]^+$ at m/z 433.1126. The protonated ion produced "M-148" ion at m/z 285.0333 and "M-149" ion at m/z 284.0311, owing to the loss of $C_5H_8O_5$ and $C_5H_9O_5$ groups.

The flavonoid was substituted at the C-6 position with methoxyl, which could lose a CH_3 group and produced free electrons on the C-6 position. The "M-CH3" ion was generated because the free electrons will combine with oxygen-free electrons of pyrones to produce p-quinones. The chemical structures of methoxyl-substituted flavonoids on the C-6 position in ZZP are shown in Figure 3b. Take 5-demethylsinensetin (peak 134, $t_R = 23.56$ min) as example: in the positive ionization mode, it could yield ion $[M+Na]^+$ at m/z 381.0958 and protonated ion $[M+H]^+$ at m/z 359.1122. There are three fragmentation patterns under high collision energies: *(1)* the protonated ion produced fragment ions at m/z 328.3213 by losing a CH_2OH group; *(2)* the protonated ion generated "M-CH_3" ion at m/z 344.0878; *(3)* RDA cleavage in the C-ring generated "ion a" at m/z 198, "ion b" at m/z 163.0393, and "ion c" $[C_9H_9O_3^+]$ at m/z 165.0695. In addition, "ion a" could yield "ion a-15" at m/z 182.0160 by losing a CH_3 group. The MS fragmentation pathway and MS/MS spectra for the $[M+H]^+$ ion of 5-demethylsinensetin are shown in Figure 4b. Moreover, sinensetin (peak 119, $t_R = 21.01$ min) was confirmed by using a reference standard.

The flavonoid was substituted at the C-8 position with methoxyl, which is more likely to lose a CH_3 group. It may be the result of steric hindrances and the stability of the o-quinone structure. The chemical structures of methoxyl-substituted flavonoids on the C-8 position in ZZP are shown in Figure 3c. Take tangeretin (peak 131, $t_R = 23.14$ min) an as example: it could yield ion $[M+Na]^+$ at m/z 395.1094 and protonated ion $[M+H]^+$ at m/z 373.1280 in the positive ionization mode. There are three fragmentation patterns under high collision energies: *(1)* the protonated ion produced fragment ions

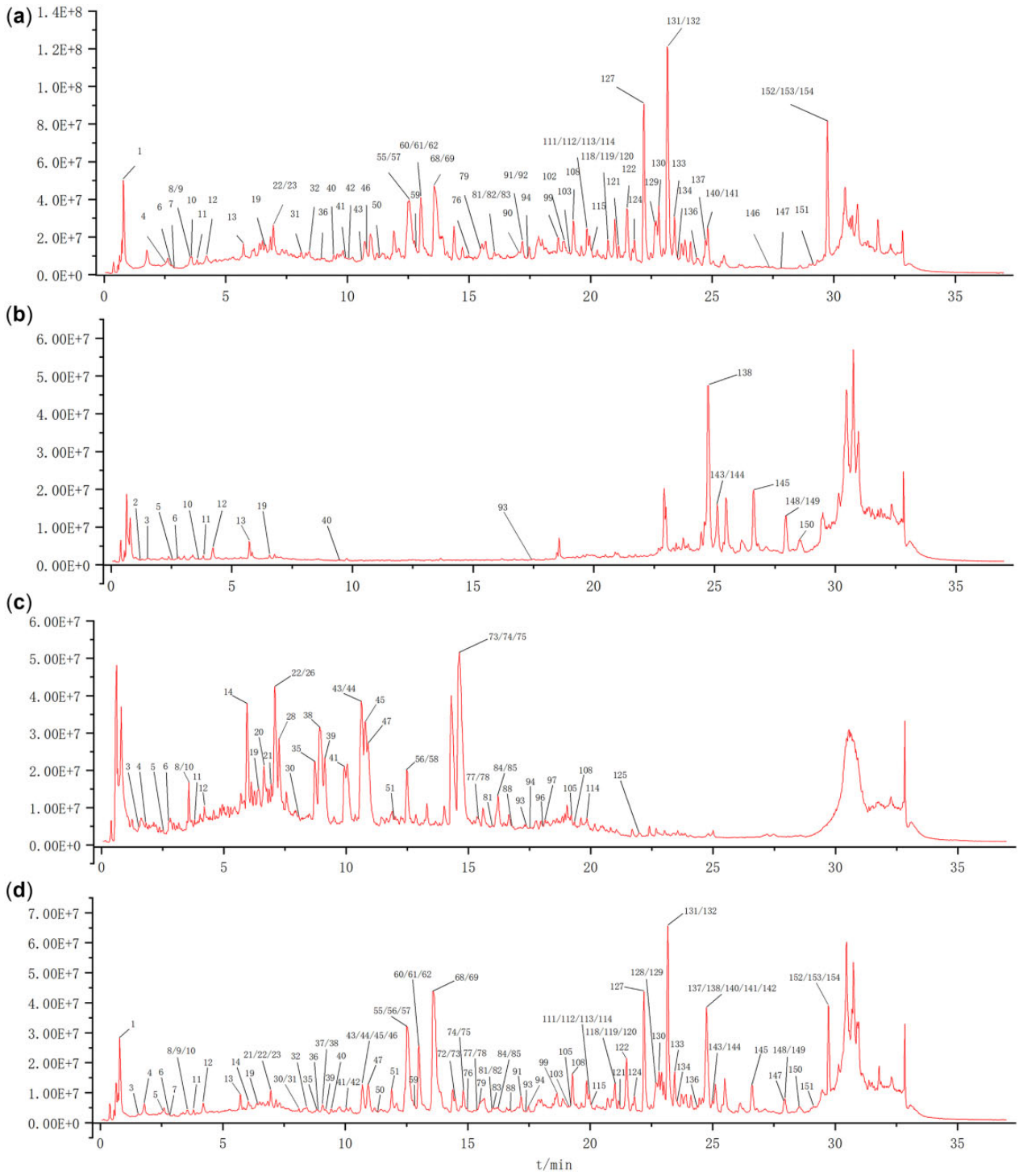

Figure 1. The TIC chromatograms of ZZP in positive ion modes (a: WAFI; b: AMR; c: NF; d-ZZP).

at m/z 342.0680 by losing a CH_2OH group; *(2)* the protonated ion generated "M-CH_3" ion at m/z 358.1044, and after being further fragmented, it yielded "ion [M-CH_3-CO]$^+$" at m/z 330.0660; *(3)* RDA cleavage in the C-ring generated "ion a" at m/z 241.0754, "ion b" at m/z 133.0647, and "ion c" [$C_8H_7O_2^+$] at m/z 135.0440. Moreover, "ion a" could yield two predominant ions at m/z 226.0584 and 198.0652 by losing CH_3 and CO, respectively. And "ion c" produced "ion c-28" at m/z 107.0494, owing to losing neutral CO. This result was demonstrated by combining the fragmentation information with commercial standards. The MS fragmentation pathway and MS/MS spectra for the [M+H]$^+$ ion of tangeretin are shown in Figure 4c.

The flavanones of ZZP have mainly occurred in RDA reaction and loss of B-ring. The chemical structures of flavanones in ZZP are shown in Figure 3d. Take isosakuranetin (peak 91, $t_R = 17.19$ min) as an example: in positive ionization mode, it yielded protonated ion [M+H]$^+$ at m/z 287.0917. There are two fragmentation patterns under high collision energies: *(1)* the

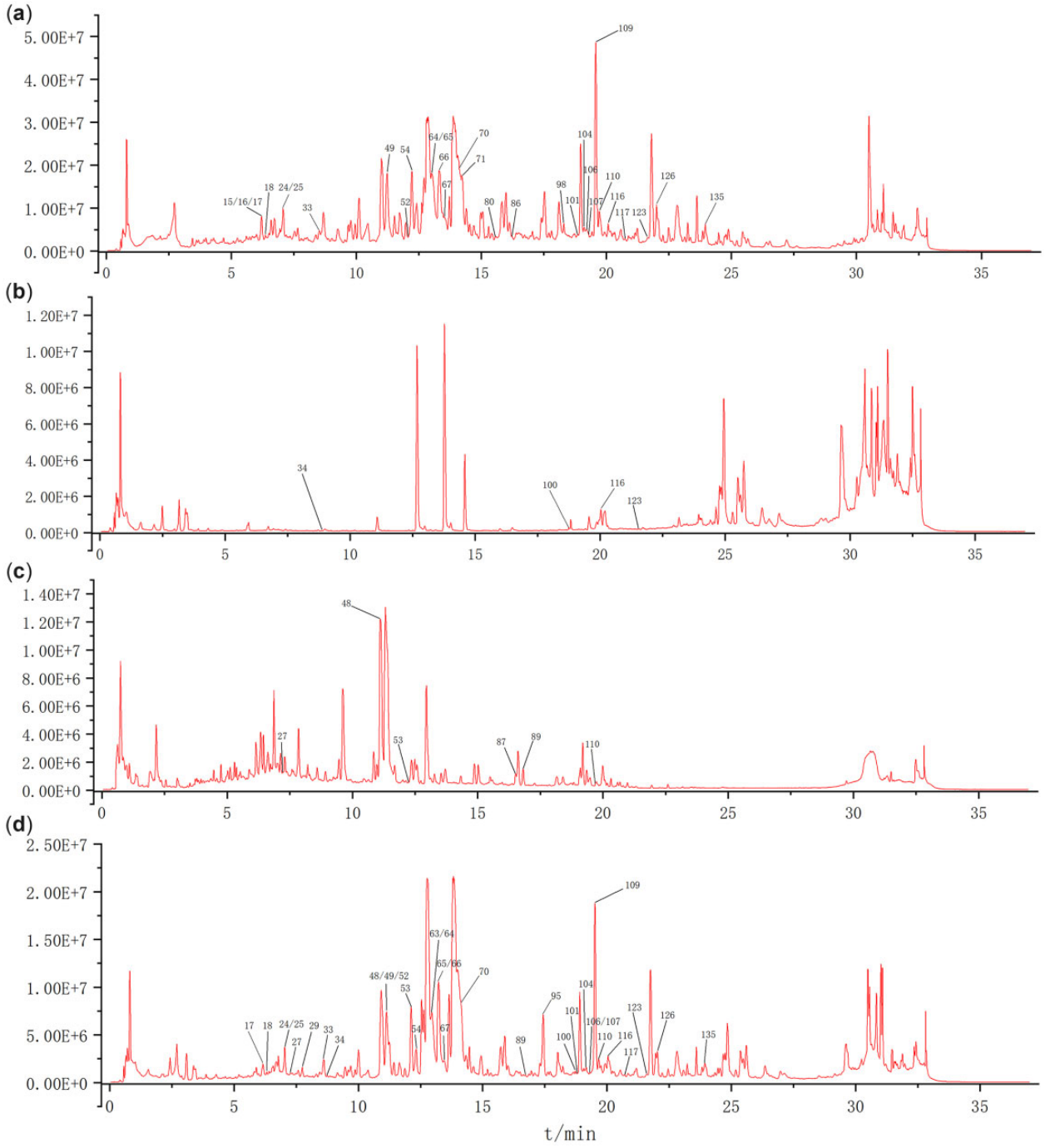

Figure 2. The TIC chromatograms of ZZP in negative ion modes (a: WAFI; b: AMR; c: NF; d-ZZP).

protonated ion produced two predominant ions at m/z 179.0339 and 109.0648 by losing B-ring; *(2)* RDA cleavage in the C-ring generated "ion a" at m/z 153.0183 and "ion b" at m/z 135.0804. Furthermore, "ion a" could yield "ion a-43"at m/z 125.0232 by losing neutral CO. The MS fragmentation pathway and MS/MS spectra for the $[M+H]^+$ ion of isosakuranetin are shown in Figure 4d. Furthermore, narirutin (peak 54, $t_R = 12.23$ min), hesperidin (peak 61, $t_R = 13.01$ min), naringenin (peak 99, $t_R = 18.65$ min), and hesperetin (peak 109, $t_R = 19.57$ min) were confirmed by using commercial standards.

Sequiterpene Lactones

Most sequiterpene lactones in ZZP are derived primarily from AMR. The sequiterpene lactones in ZZP are mainly 12, 8-eudesmanolides. Fragmentation is always produced "ion M-15" when

Table 1. Identification of chemical compounds by UPLC/Q-TOF-MS/MS

No.	Component name	T_R/min	Formula	Theoretical mass, Da	Detected mass, m/z	Mass error, ppm	MS^E ions	Source	Type
1	Stachydrine	0.80	$C_7H_{13}NO_2$	143.0946	144.1021	1.1	144.1021$[M+H]^+$, 116.0707$[M+H\text{-}CO_2]^+$	WAFI, ZZP	Other
2	Lactose	1.19	$C_{12}H_{22}O_{11}$	342.1162	387.1135	−2.3	387.1135$[M+COOH]^-$, 341.1080$[M\text{-}H]^-$	AMR	Other
3	Adenine	1.52	$C_5H_5N_5$	135.0550	136.0620	2.0	136.0620$[M+H]^+$, 119.0355$[M+H\text{-}NH_3]^+$	AMR, NF, ZZP	Other
4	Nicotinamide	1.79	$C_6H_6N_2O$	122.0480	123.0553	0.0	123.0553$[M+H]^+$, 106.0652$[M+H\text{-}NH_3]^+$	NF, ZZP	Other
5	Fumaric acid	2.54	$C_6H_{13}NO_2$	131.0946	132.1020	0.6	132.1020$[M+H]^+$	WAFI, AMR, NF, ZZP	Other
6	L-Tyrosine	2.76	$C_9H_{11}NO_3$	181.0739	182.0814	1.1	182.0814$[M+H]^+$, 165.0549$[M+H\text{-}NH_3]^+$, 147.0446$[M+H\text{-}NH_3\text{-}H_2O]^+$, 103.0543$[M+H\text{-}NH_3\text{-}H_2O\text{-}CO_2]^+$	WAFI, AMR, NF, ZZP	Other
7	Dimethyl anthranilate	2.82	$C_9H_{11}NO_2$	165.0790	166.0863	0.3	166.0863$[M+H]^+$, 123.0683$[M+H\text{-}CH_2NH]^+$	WAFI, ZZP	Other
8	Acetophenone	3.53	C_8H_8O	120.0575	121.0649	0.6	121.0649$[M+H]^+$	WAFI, NF, ZZP	Other
9	N-Methyltyramine	3.53	$C_9H_{13}NO$	151.0997	152.1069	−0.4	152.1069$[M+H]^+$, 121.0649$[M+H\text{-}CH_2NH_3]^+$, 103.0542$[M+H\text{-}CH_2NH_3\text{-}H_2O]^+$	WAFI, ZZP	Other
10	Adenosine	3.57	$C_{10}H_{13}N_5O_4$	267.0968	268.1044	1.3	268.1037$[M+H]^+$, 136.0621$[M+H\text{-}C_5H_8O_4]^+$, 119.0360$[M+H\text{-}C_5H_8O_4\text{-}NH_3]^+$	WAFI, AMR, NF, ZZP	Other
11	Guanine	3.80	$C_5H_5N_5O$	151.0494	152.0569	1.4	152.0569$[M+H]^+$, 135.0304$[M+H\text{-}NH_3]^+$	WAFI, AMR, NF, ZZP	Other
12	L-phenylalanine	4.20	$C_9H_{11}NO_2$	165.0790	166.0862	−0.1	166.0862$[M+H]^+$,149.0602$[M+H\text{-}NH_3]^+$, 120.0808$[M+H\text{-}CO_2]^+$	WAFI, AMR, NF, ZZP	Other
13	L-tryptophan	5.72	$C_{11}H_{12}N_2O_2$	204.0900	205.0970	−0.7	205.0970$[M+H]^+$	WAFI, AMR, ZZP	Other
14	Norcoclaurine	6.01	$C_{16}H_{17}NO_3$	271.1208	272.1283	0.6	272.1283$[M+H]^+$, 164.0697$[M+H\text{-}C_7H_8O]^+$, 107.0493$[M+H\text{-}C_{10}H_{13}NO_2]^+$	NF, ZZP	Alkaloids
15	5,7-Dihydroxychromone	6.18	$C_9H_6O_4$	178.0266	177.0185	−4.7	177.0185$[M\text{-}H]^-$, 123.0442$[M+H\text{-}2CO]+$	WAFI	Other
16	Coniferin	6.21	$C_{16}H_{22}O_8$	342.1315	387.1292	−1.3	387.1292$[M+HCOO]^-$, 343.1025$[M\text{-}H]^-$, 181.0498$[M\text{-}H\text{-}Glc]^-$	WAFI	Other
17	Androsin	6.24	$C_{15}H_{20}O_8$	328.1158	327.1077	−2.5	373.1148$[M+HCOO]^-$, 327.1077$[M\text{-}H]^-$, 165.0547$[M\text{-}H\text{-}Glc]^-$	WAFI, NF, ZZP	Other
18	Icariside F2	6.34	$C_{18}H_{26}O_{10}$	402.1526	401.1441	−3.1	401.1441$[M\text{-}H]^-$, 287.1564$[M\text{-}H\text{-}C_5H_6O_3]^-$, 125.0234$[M\text{-}H\text{-}C_5H_6O_3\text{-}Glc]^-$	WAFI, ZZP	Other
19	Chlorogenic acid	6.56	$C_{16}H_{18}O_9$	354.0951	355.1027	0.9	377.0836$[M+Na]^+$, 355.1027$[M+H]^+$, 337.0606$[M+H\text{-}H_2O]^+$, 163.1229$[M+H\text{-}C_7H_{12}O_6]^+$, 145.0277$[M+H\text{-}C_7H_{12}O_6\text{-}H_2O]^+$	WAFI, AMR, NF, ZZP	Other
20	Catechin	6.68	$C_{15}H_{14}O_6$	290.0790	291.0865	0.6	291.0865$[M+H]^+$, 153.1379$[M+H\text{-}C_7H_8O_3]^+$, 139.0391$[C_7H_7O_3]^+$	NF	Other
21	4–(1-hydroxybut-1-en-1-yl)-3,5,5-trimethylcyclohex-2-en-1-one	6.94	$C_{13}H_{20}O_2$	208.1463	209.1531	−2.4	209.1531$[M+H]^+$, 181.0961$[M+H\text{-}CO]^+$	NF, ZZP	Other
22	5,7-Dihydroxycoumarin	6.97	$C_9H_6O_4$	178.0266	179.0339	0.2	179.0339$[M+H]^+$, 123.0442$[M+H\text{-}2CO]^+$	WAFI, NF, ZZP	Coumarins
23	Fabiatrin	6.97	$C_{21}H_{26}O_{13}$	486.1373	487.1443	−0.6		WAFI, ZZP	Coumarins

(continued)

Table 1. (continued)

No.	Component name	T_R/min	Formula	Theoretical mass, Da	Detected mass, m/z	Mass error, ppm	MS^E ions	Source	Type
							509.1259[M+Na]$^+$, 487.1443[M+H]$^+$, 178.0499[M+H-Xyl-Glc-CH_3]$^+$, 150.0553[M+H-Xyl-Glc-CH_3-CO]$^+$, 122.0607[M+H-Xyl-Glc-CH_3-2CO]$^+$		
24	Citric acid	6.98	$C_6H_8O_7$	192.0270	191.0190	−4.0	191.0190[M-H]$^-$, 173.0078[M-H-H_2O]$^-$	WAFI, ZZP	Other
25	Taxifolin 7-rhamnoside	7.06	$C_{21}H_{22}O_{11}$	450.1162	449.1084	−1.1	449.1084[M-H]$^-$, 303.0881[M-H-Rha]$^-$, 151.0023[M-H-$C_7H_4O_4$]$^-$	WAFI, ZZP	Flavonoids
26	Coclaurine	7.16	$C_{17}H_{19}NO_3$	285.1365	286.1439	0.6	286.1439[M+H]$^+$, 178.0838[M+H-C_7H_8O]$^+$, 163.0747[M+H-C_7H_8O-CH_3]$^+$, 162.0665[M+H-C_7H_8O-CH_3-H]$^+$, 107.0493[M+H-$C_{10}H_{13}NO_2$]$^+$	NF	Alkaloids
27	Procyanidin B1	7.23	$C_{30}H_{26}O_{12}$	578.1424	577.1348	−0.7	577.1348[M-H]$^-$, 289.0711[$C_{15}H_{13}O_6$]$^-$, 151.0390[M-H-$C_7H_8O_3$]$^-$	NF, ZZP	Other
28	N-Methylcoclaurine	7.30	$C_{18}H_{21}NO_3$	299.1521	300.1596	0.5	300.1596[M+H]$^+$, 192.1007[M+H-C_7H_8O]$^+$, 179.0864[M+H-C_7H_8O-CH_3]$^+$, 178.0830[M+H-C_7H_8O-CH_3-H]$^+$, 149.0596[M+H-C_7H_8O-CH_3-CO]$^+$, 107.0493[M+H-$C_{11}H_{15}NO_2$]$^+$	NF	Alkaloids
29	Catechin	7.77	$C_{15}H_{14}O_6$	290.0790	289.0710	−2.6	289.0710[M-H]$^-$, 151.0390[M-H-$C_7H_8O_3$]$^-$, 139.0389[$C_7H_7O_3^+$]	ZZP	Flavonoids
30	4–(1,3-butadienyl)-3,5,5-trimethyl-2-cyclohexen-1-one	8.06	$C_{13}H_{18}O$	190.1358	191.1431	0.1	191.1431[M+H]$^+$, 163.0863[M+H-CO]$^+$	NF, ZZP	Terpenoids
31	Narcissoside	8.12	$C_{28}H_{32}O_{16}$	624.1690	625.1766	0.5	625.1766[M+H]$^+$, 165.0734[M+H-Rha-Glc-$C_7H_4O_4$]$^+$	WAFI, ZZP	Flavonoids
32	Vanillic acid	8.43	$C_8H_8O_4$	168.0423	169.0495	−0.2	169.0495[M+H]$^+$, 151.0391[M+H-H_2O]$^+$, 125.0597[M+H-CO_2]$^+$	WAFI, ZZP	Other
33	Naringenin-4′-glucose-7-neohesperidoside	8.52	$C_{33}H_{42}O_{19}$	742.232	787.2306	0.5	787.2306[M+COOH]$^-$, 741.2243[M-H]$^-$, 579.1714[M-H-Glc]$^-$, 271.0602[M-H-Glc-Rha-Glc]$^-$, 151.0024[M-H-Glc-Rha-Glc-C_8H_8O]$^-$, 119.0333[M-H-Glc-Rha-Glc-$C_7H_4O_4$]$^-$	WAFI, ZZP	Flavonoids
34	Apiin	8.80	$C_{26}H_{28}O_{14}$	564.1479	563.1397	−1.6	563.1397[M-H]$^-$, 471.1419[M-H-Rha]$^-$	AMR, ZZP	Flavonoids
35	Armepavine	8.81	$C_{19}H_{23}NO_3$	313.1678	314.1753	0.8	314.1753[M+H]$^+$, 206.1178[M+H-C_7H_8O]$^+$, 191.0848[M+H-C_7H_8O-CH_3]$^+$, 190.0940[M+H-C_7H_8O-CH_3-H]$^+$, 162.0919[M+H-C_7H_8O-CH_3-CO]$^+$, 107.0493[M+H-$C_{12}H_{17}NO_2$]$^+$	NF, ZZP	Alkaloids
36	Coumalic acid	8.91	$C_9H_8O_3$	164.0473	165.0550	2.1	165.0550[M+H]$^+$, 147.0443[M+H-H_2O]$^+$	WAFI, ZZP	Other
37	N-norarmepavine	9.05	$C_{18}H_{21}NO_3$	299.1521	300.1596	0.6	300.1596[M+H]$^+$, 192.1010[M+H-C_7H_8O]$^+$, 177.0905[M+H-C_7H_8O-CH_3]$^+$, 176.0818[M+H-C_7H_8O-CH_3-H]$^+$, 148.0763[M+H-C_7H_8O-CH_3-CO]$^+$, 107.0493[M+H-$C_{11}H_{15}NO_2$]$^+$	ZZP	Alkaloids

(continued)

Table 1. (continued)

No.	Component name	T_R/min	Formula	Theoretical mass, Da	Detected mass, m/z	Mass error, ppm	MS^E ions	Source	Type
38	Myricetin-3-O-glucoside	9.06	$C_{21}H_{20}O_{13}$	480.0904	481.0975	−0.4	481.0975$[M+H]^+$, 319.0455$[M+H\text{-}Glc]^+$,291.0440$[M+H\text{-}Glc\text{-}CO]^+$, 263.0555$[M+H\text{-}Glc\text{-}2CO]^+$, 167.0766$[M+H\text{-}Glc\text{-}C_7H_4O_4]^+$, 153.0183$[M+H\text{-}Glc\text{-}C_8H_6O_4]^+$	NF, ZZP	Flavonoids
39	Quercetin 3-O-sambubioside	9.21	$C_{26}H_{28}O_{16}$	596.1377	597.1458	1.4	619.1278$[M+Na]^+$, 597.1458$[M+H]^+$, 463$[M+H\text{-}Xyl]^+$, 303.0506$[M+H\text{-}Xyl\text{-}Glc]^+$, 275.0539$[M+H\text{-}Xyl\text{-}Glc\text{-}CO]^+$, 247.0605$[M+H\text{-}Xyl\text{-}Glc\text{-}2CO]^+$, 153.0184$[M+H\text{-}Xyl\text{-}Glc\text{-}C_8H_6O_3]^+$	NF, ZZP	Flavonoids
40	Umbelliferone	9.44	$C_9H_6O_3$	162.0317	163.0392	1.6	163.0392$[M+H]^+$, 135.0442$[M+H\text{-}CO]^+$, 107.0493$[M+H\text{-}2CO]^+$	WAFI, AMR, ZZP	Coumarins
41	Ferulic acid	9.93	$C_{10}H_{10}O_4$	194.0579	195.0655	1.6	195.0655$[M+H]^+$, 177.0549$[M+H\text{-}H_2O]^+$, 151.1224$[M+H\text{-}CO_2]^+$, 146.0330$[M+H\text{-}H_2O\text{-}OCH_3]^+$	WAFI, NF, ZZP	Other
42	Eriocitrin	10.02	$C_{27}H_{32}O_{15}$	596.1741	597.1813	−0.2	619.1624$[M+Na]^+$, 597.1813$[M+H]^+$, 435.1281$[M+H\text{-}Rha]^+$, 289.0705$[M+H\text{-}Rha\text{-}Glc]^+$, 180.0371$[M\text{-}M+H\text{-}Rha\text{-}Glc\text{-}C_6H_6O_2]^+$, 153.0187$[M+H\text{-}Rha\text{-}Glc\text{-}C_8H_8O_2]^+$, 137.0599$[M+H\text{-}Rha\text{-}Glc\text{-}C_7H_4O_4]^+$	WAFI, ZZP	Flavonoids
43	2-hydroxy-5-methoxy-α,α,4-trimethylbenzenemethanol	10.63	$C_{11}H_{16}O_3$	196.1099	197.1173	0.3	197.1173 $[M+H]^+$, 179.1076$[M+H\text{-}H_2O]^+$	WAFI, NF, ZZP	Other
44	Isoquercitrin	10.69	$C_{21}H_{20}O_{12}$	464.0955	465.1028	0.1	487.0844$[M+Na]^+$, 465.1028$[M+H]^+$, 303.0506$[M+H\text{-}C_6H_{10}O_5]^+$, 275.0544$[M+H\text{-}C_6H_{10}O_5\text{-}CO]^+$, 247.0606$[M+H\text{-}C_6H_{10}O_5\text{-}2CO]^+$, 153.0185$[M+H\text{-}C_6H_{10}O_5\text{-}C_8H_6O_3]^+$, 151.0382$[M+H\text{-}C_6H_{10}O_5\text{-}C_7H_4O_4]^+$, 125.0239$[M+H\text{-}C_6H_{10}O_5\text{-}C_8H_6O_3\text{-}CO]^+$	NF, ZZP	Flavonoids
45	Floribundine	10.75	$C_{18}H_{19}NO_2$	281.1416	282.1493	1.5	282.1493$[M+H]^+$, 265.1212$[M+H\text{-}OH]^+$, 251.1070$[M+H\text{-}OCH_3]^+$, 239.1056$[M+H\text{-}CH_2NH_3]^+$, 222.0998$[M+H\text{-}CH_2NH_3\text{-}OH]^+$, 208.0882$[M+H\text{-}CH_2NH_3\text{-}OCH_3]^+$	NF, ZZP	Alkaloids
46	Rutin	10.78	$C_{27}H_{30}O_{16}$	610.1534	611.1638	5.1	611.1638$[M+H]^+$, 465.2244$[M+H\text{-}Rha]^+$, 153.0687$[M+H\text{-}Rha\text{-}Glc\text{-}C_8H_6O_3]^+$, 151.0547$[M+H\text{-}Rha\text{-}Glc\text{-}C_7H_4O_4]^+$	WAFI, ZZP	Flavonoids
47	Miquelianin	10.90	$C_{21}H_{18}O_{13}$	478.0747	479.0818	−0.4	501.0639$[M+Na]^+$, 479.0818$[M+H]^+$, 303.0508$[M+H\text{-}Glu]^+$, 247.0163$[M+H\text{-}Glu\text{-}2CO]^+$, 153.0182$[M+H\text{-}Glu\text{-}C_8H_6O_3]^+$	NF, ZZP	Flavonoids
48	Leucoside	11.10	$C_{26}H_{28}O_{15}$	580.1428	579.1352	−0.6	625.1424$[M+COOH]^-$, 579.1352$[M\text{-}H]^-$, 285.0387$[M\text{-}H\text{-}Xyl\text{-}Glc]^-$, 151.0388$[M\text{-}H\text{-}Xyl\text{-}Glc\text{-}C_8H_6O_2]^-$	NF, ZZP	Flavonoids

(continued)

Table 1. (continued)

No.	Component name	T_R/min	Formula	Theoretical mass, Da	Detected mass, m/z	Mass error, ppm	MS^E ions	Source	Type
49	Prunin	11.19	$C_{21}H_{22}O_{10}$	434.1213	433.1134	−1.3	479.1196[M+COOH]$^-$, 433.1134[M-H]$^-$, 271.0601[M-H-Glc]$^-$	WAFI, ZZP	Flavonoids
50	Homoplantaginin	11.32	$C_{22}H_{22}O_{11}$	462.1162	463.1231	−0.8	463.1231[M+H]$^+$	WAFI, ZZP	Flavonoids
51	Luteolin	11.95	$C_{15}H_{10}O_6$	286.0477	287.0554	1.2	287.0554[M+H]$^+$, 259.0595[M+H-CO]$^+$, 135.0445[M+H-$C_7H_4O_4$]$^+$	NF, ZZP	Flavonoids
52	Pinoresinol-4-O-glucoside	12.06	$C_{26}H_{32}O_{11}$	520.1945	519.1867	−1	565.1924[M+COOH]$^-$, 519.1867[M-H]$^-$, 357.1337[M-H-Glc]$^-$	WAFI, NF, ZZP	Other
53	Guaijaverin	12.21	$C_{20}H_{18}O_{11}$	434.0849	433.0775	−0.3	433.0771[M-H]$^-$, 301.0340[M-H-Xyl]$^-$, 273.0368[M-H-Xyl-CO]$^-$, 151.0027[M-H-Xyl-$C_8H_6O_3$]$^-$	NF, ZZP	Flavonoids
54	Narirutin	12.23	$C_{27}H_{32}O_{14}$	580.1792	579.1724	0.8	625.1778[M+HCOO]$^-$, 579.1724[M-H]$^-$, 271.0610[M-H-Rha-Glc]$^-$, 177.0182[M-H-Rha-Glc-C_6H_6O]$^-$, 151.0033[M-H-Rha-Glc-C_8H_8O]$^-$, 119.0500[M-H-$C_7H_4O_4$]$^-$	WAFI, ZZP	Flavonoids
55	Hesperetin-4′-rhamnose-7-rutinoside	12.44	$C_{34}H_{44}O_{19}$	756.2477	757.2551	0.2	779.2372[M+Na]$^+$, 757.2551[M+H]$^+$, 611.1968[M+H-Rha]$^+$, 597.1807[M+H-$C_7H_{12}O_4$]$^+$, 449.1435[M+H-Rha-Glc]$^+$, 179.0696[M+H-Rha-Glc-$C_7H_{12}O_4$-$C_6H_6O_2$]$^+$, 136.1128[M+H-Rha-Glc-$C_7H_{12}O_4$-$C_7H_4O_4$]$^+$	WAFI, ZZP	Flavonoids
56	Astragaline	12.51	$C_{21}H_{20}O_{11}$	448.1006	449.1079	0.1	471.0872[M+Na]$^+$, 449.1079[M+H]$^+$, 421.1391[M+H-CO]$^+$, 287.0554[M+H-Glc]$^+$, 259.0590[M+H-Glc-CO]$^+$, 231.0663[M+H-Glc-2CO]$^+$, 153.0184[M+H-Glc-$C_8H_6O_2$]$^+$, 135.0442[M+H-Glc-$C_7H_4O_4$]$^+$, 125.0234[M+H-Glc-$C_8H_6O_2$-CO]$^+$	NF, ZZP	Flavonoids
57	Isoliquiritoside	12.55	$C_{21}H_{22}O_9$	418.1264	419.1336	−0.2	419.1336[M+H]$^+$, 257.1022[M+H-Glc]$^+$, 163.0623[M+H-Glc-C_6H_6O]$^+$, 147.0447[M+H-Glc-$C_6H_6O_2$]$^+$, 137.0234[M+H-C_8H_8O]$^+$, 120.0526[M+H-$C_7H_6O_3$+1]$^+$, 119.0492[M+H-$C_7H_6O_3$]$^+$	WAFI, ZZP	Chalcone
58	Kaempferol-3-O-glucuronide	12.56	$C_{21}H_{18}O_{12}$	462.0798	463.0873	0.4	463.0873[M+H]$^+$, 287.0554[M+H-Glu-Glc]$^+$, 231.0287[M+H-Glu-2CO]$^+$, 153.0184[M+H-Glu-$C_8H_6O_2$]$^+$, 135.0442[M+H-Glu-$C_7H_4O_4$]$^+$, 125.0234[M+H-Glu-$C_8H_6O_2$-CO]$^+$	NF	Flavonoids
59	Isovitexin	12.86	$C_{21}H_{20}O_{10}$	432.1057	433.1126	−0.7	433.1126[M+H]$^+$, 285.0333[M+H-$C_5H_8O_5$]$^+$, 284.0311[M+H-$C_5H_9O_5$]$^+$, 119.0492[M+H-$C_5H_9O_5$-$C_8H_5O_4^+$]$^+$	WAFI, ZZP	Flavonoids
60	Diosmin	13.00	$C_{28}H_{32}O_{15}$	608.1741	609.1816	0.3	609.1816[M+H]$^+$, 463.1234[M+H-Rha]$^+$, 301.0710[M+H-Rha-Glc]$^+$, 153.0183[M+H-Rha-Glc-$C_9H_8O_2$]$^+$, 149.0598[M+H-Rha-Glc-$C_7H_4O_4$]$^+$, 125.0602[M+H-Rha-Glc-$C_9H_8O_2$-CO]$^+$	WAFI, ZZP	Flavonoids

(continued)

Table 1. (continued)

No.	Component name	T_R/min	Formula	Theoretical mass, Da	Detected mass, m/z	Mass error, ppm	MS^E ions	Source	Type
61	Hesperidin	13.01	$C_{28}H_{34}O_{15}$	610.1898	611.1970	−0.1	633.1797[M+Na]$^+$, 611.1970[M+H]$^+$, 465.1387[M+H-Rha]$^+$, 303.0864[M+H-Rha-Glc]$^+$, 179.0340[M+H-Rha-Glc-$C_7H_8O_2$]$^+$, 153.0183[M+H-Rha-Glc-$C_9H_{10}O_2$]$^+$, 151.0755[M+H-Rha-Glc-$C_7H_4O_4$]$^+$, 125.0601[M+H-Rha-Glc-$C_9H_{10}O_2$-CO]$^+$	WAFI, ZZP	Flavonoids
62	(Cis-head-to-head)-limettin dimer	13.01	$C_{22}H_{20}O_8$	412.1158	413.1227	−0.9	413.1227[M+H]$^+$, 207.0284[M+H-$C_{11}H_{10}O_4$]$^+$, 145.0285[M+H-$C_{11}H_{10}O_4$-2OCH$_3$]$^+$, 117.0336[M+H-$C_{11}H_{10}O_4$-2OCH$_3$-CO]$^+$	WAFI, ZZP	Other
63	Quercitrin	13.01	$C_{21}H_{20}O_{11}$	448.1006	447.0924	−1.9	447.0924[M-H]$^-$, 301.0707[M-H-Rha]$^-$	ZZP	Flavonoids
64	Rhoifolin	13.1	$C_{27}H_{30}O_{14}$	578.1636	577.1564	0.2	623.1619[M+HCOO]$^-$, 577.1564[M-H]$^-$, 269.0453[M-H-Rha-Glc]$^-$, 151.0039[M-H-Rha-Glc-C_8H_6O]$^-$, 117.0349[M-H-Rha-Glc-$C_7H_4O_4$]$^-$	WAFI, ZZP	Flavonoids
65	Hesperetin-4′-glucose-7-neohesperidoside	13.15	$C_{34}H_{44}O_{20}$	772.2426	771.2352	−0.1	817.2413[M+COOH]$^-$, 771.2352[M-H]$^-$, 609.1830[M-H-Glc]$^-$, 463.0877[M-H-Glc-Rha]$^-$, 123.0439[M-H-Glc-Rha-Glc-$C_9H_6O_4$]$^-$	WAFI, ZZP	Flavonoids
66	Homoeriodictyol	13.26	$C_{16}H_{14}O_6$	302.079	301.0712	−1.7	301.0712[M-H]$^-$, 143.0485[M-H-$C_7H_6O_4$]$^-$	WAFI, ZZP	Flavonoids
67	Neoeriocitrin	13.51	$C_{27}H_{32}O_{15}$	596.1741	595.1672	0.6	595.1672[M-H]$^-$, 449.1229[M-H-Rha]$^-$	WAFI	Flavonoids
68	Neohesperidin	13.61	$C_{28}H_{34}O_{15}$	610.1898	611.1977	1.1	635.1906[M+Na]$^+$, 611.1977[M+H]$^+$, 465.1394[M+H-Rha]$^+$, 303.0868[M+H-Rha-Glc]$^+$, 179.0342[M+H-Rha-Glc-$C_7H_8O_2$]$^+$, 153.0185[M+H-Rha-Glc-$C_9H_{10}O_2$]$^+$, 151.0753[M+H-Rha-Glc-$C_7H_4O_4$]$^+$, 125.0599[M+H-Rha-Glc-$C_9H_{10}O_2$-CO]$^+$	WAFI, ZZP	Flavonoids
69	Isosakuranin	13.62	$C_{22}H_{24}O_{10}$	448.1370	449.1444	0.3	449.1444[M+H]$^+$, 287.0657[M+H-Glc]$^+$, 179.0341[M+H-Glc-C_7H_8O]$^+$, 153.0185[M+H-Glc-$C_9H_{10}O$]$^+$, 135.0438[M+H-Glc-$C_7H_4O_4$]$^+$, 125.0234[M+H-Glc-$C_9H_{10}O$-CO]$^+$, 109.0284[M+H-Glc-$C_9H_6O_4$]$^+$	WAFI, ZZP	Flavonoids
70	Limocitrin-3-O-(3-hydroxy-3-methylglutarate)-glucoside	14.10	$C_{29}H_{32}O_{17}$	652.164	651.1572	0.8	651.1590[M-H]$^-$, 507.1145[M-H-$C_6H_8O_4$]$^-$, 345.0611[M-H-$C_6H_8O_4$-Glc]$^-$, 181.0127[M-H-$C_6H_8O_4$-Glc-$C_9H_8O_3$]$^-$	WAFI, ZZP	Flavonoids
71	Hesperitin-7-O-β-D-glucoside	14.18	$C_{22}H_{24}O_{11}$	464.1319	463.1243	−0.7	463.1243[M-H]$^-$, 301.0711[M-H-Glc]$^-$, 177.0178[M-H-Glc-$C_7H_8O_2$]$^-$, 151.0029[M-H-Glc-$C_9H_{10}O_2$]$^-$, 149.0602[M-H-Glc-$C_7H_4O_4$]$^-$	WAFI	Flavonoids

(continued)

Table 1. (continued)

No.	Component name	T_R/min	Formula	Theoretical mass, Da	Detected mass, m/z	Mass error, ppm	MS^E ions	Source	Type
72	N-Nornuciferine	14.45	$C_{18}H_{19}NO_2$	281.1416	282.1492	1.1	282.1492[M+H]$^+$, 267.1288[M+H-CH_3]$^+$, 253.1162[M+H-CH_2=NH]+, 251.1024[M+H-OCH_3]$^+$	ZZP	Alkaloids
73	Pronuciferine	14.52	$C_{19}H_{21}NO_3$	311.1521	312.1595	0.4	312.1595[M+H]$^+$, 281.1173[M+H-OCH_3]$^+$	NF, ZZP	Alkaloids
74	Nuciferine	14.76	$C_{19}H_{21}NO_2$	295.1572	296.1648	1.1	296.1648[M+H]$^+$, 281.1547[M+H-CH_3]$^+$, 265.1225[M+H-OCH_3]$^+$, 253.1211[M+H-CH_2NH_3]$^+$, 222.1027[M+H-CH_2NH_3-OCH_3]$^+$	NF, ZZP	Alkaloids
75	Roemerine	14.83	$C_{18}H_{17}NO_2$	279.1259	280.1334	0.6	280.1334[M+H]$^+$	NF, ZZP	Alkaloids
76	Natsudaidain 3–(4-O-3-hydroxy-3-methylglutaroylglucoside)	14.97	$C_{33}H_{40}O_{18}$	724.2215	725.2297	1.3	747.2149[M+Na]$^+$, 725.2297[M+H]$^+$, 419.1305[M+H-$C_6H_8O_4$-Glc]$^+$, 404.1441[M+H-$C_6H_8O_4$-Glc-CH_3]$^+$, 376.0715[M+H-$C_6H_8O_4$-Glc-CH_3-CO]$^+$	WAFI, ZZP	Flavonoids
77	1,3-diisopropenyl-6-methyl-cyclohexene	15.37	$C_{13}H_{20}$	176.1565	177.1638	0.2	177.1638[M+H]$^+$	NF, ZZP	Terpenoids
78	3,5,9-trimethyl-2,4,8-decatrien-1-ol	15.37	$C_{13}H_{22}O$	194.1671	195.1745	0.6	195.1745[M+H]$^+$	NF, ZZP	Terpenoids
79	Methyl hesperidin	15.48	$C_{29}H_{36}O_{15}$	624.2054	625.2133	1.0	647.1953[M+Na]$^+$, 625.2133[M+H]$^+$, 479.1541[M+H-Rha]$^+$, 317.1123[M+H-Rha-Glc]$^+$, 153.0184[$C_8H_8O_3$]$^+$, 151.0753[M+H-Rha-Glc-$C_8H_6O_4$]+, 139.0759[M+H-Rha-Glc-C_9H_{10}O2-CO]$^+$, 125.0234[M+H-Rha-Glc-$C_{10}H_8O_4$]$^+$	WAFI, ZZP	Flavonoids
80	Citrusin A	15.58	$C_{26}H_{34}O_{12}$	538.2050	537.1972	−1.0	537.1972[M-H]$^-$	WAFI	Other
81	Neodiosmin	16.00	$C_{28}H_{32}O_{15}$	608.1741	609.182	0.9	631.1664[M+Na]$^+$, 609.1820[M+H]$^+$, 463.1224[M+H-Rha]$^+$, 244.0708[M+H-CO-Rha-Glc-CHO]$^+$, 153.0184[M+H-Rha-Glc-$C_9H_8O_2$]$^+$	WAFI, NF, ZZP	Flavonoids
82	Eriodictyol	16.01	$C_{15}H_{12}O_6$	288.0634	289.0711	1.3	289.0711[M+H]$^+$, 179.0338[M+H-$C_6H_6O_2$]$^+$, 153.0184[M+H-$C_8H_8O_2$]$^+$, 111.0082[M+H-$C_9H_6O_4$]$^+$	WAFI, ZZP	Flavonoids
83	Aviprin	16.04	$C_{16}H_{16}O_6$	304.0947	305.1024	1.3	305.1024[M+H]$^+$, 203.0342[M+H-$C_5H_{12}O_2$]$^+$, 175.0389[M+H-$C_5H_{12}O_2$-CO]$^+$, 147.0442[M+H-$C_5H_{12}O_2$-2CO]$^+$, 119.0492[M+H-$C_5H_{12}O_2$-3CO]$^+$	WAFI, ZZP	Coumarins
84	Isorhamnetin	16.26	$C_{16}H_{12}O_7$	316.0583	317.0662	1.9	317.0662[M+H]$^+$, 261.0756[M+H-2CO]$^+$, 165.0627[M+H-$C_7H_4O_4$]$^+$	NF, ZZP	Flavonoids
85	Rhamnetin-3-O-β-D-glucopyranoside	16.26	$C_{22}H_{22}O_{12}$	478.1111	479.1187	0.5	501.1006[M+Na]$^+$, 479.1187[M+H]$^+$, 317.0660[M+H-Glc]$^+$, 289.0888[M+H-Glc-CO]$^+$, 261.0747[M+H-Glc-2CO]$^+$, 165.0637[M+H-Glc-$C_7H_4O_4$]$^+$, 153.0725[M+H-Glc-$C_9H_8O_3$]$^+$, 125.0231[M+H-Glc-$C_9H_8O_3$-CO]$^+$	NF, ZZP	Flavonoids

(continued)

Table 1. (continued)

No.	Component name	T_R/min	Formula	Theoretical mass, Da	Detected mass, m/z	Mass error, ppm	MS^E ions	Source	Type
86	Chrysoeriol 7-β-rutinoside	16.28	$C_{28}H_{32}O_{15}$	608.1741	607.1670	0.3	607.1670[M-H]$^-$, 299.0556[M-H-Rha-Glc]$^-$	WAFI	Flavonoids
87	Atractyloside A	16.46	$C_{21}H_{36}O_{10}$	448.2309	493.2286	−1	493.2286[M+HCOO]$^-$, 327.1077[M-H]$^-$, 331.0422[M-H-Glc]$^-$	NF	Terpenoids
88	Quercetin	16.74	$C_{15}H_{10}O_7$	302.0427	303.0505	1.9	303.0505[M+H]$^+$, 275.0534[M+H-CO]$^+$, 247.0603[M+H-2CO]$^+$, 153.0183[M+H-$C_8H_6O_3$]$^+$, 151.0393[M+H-$C_7H_4O_4$]$^+$	NF, ZZP	Flavonoids
89	Isomucronulatol 7-O-glucoside	16.74	$C_{23}H_{28}O_{10}$	464.1683	463.1609	−0.3	509.1633[M+COOH]$^-$, 463.1609[M-H]$^-$, 301.1075[M-H-Glc]$^-$	NF, ZZP	Flavonoids
90	Acetyl-O-isonaringin	17.06	$C_{29}H_{34}O_{15}$	622.1898	623.1977	1.1	645.1787[M+Na]$^+$, 623.1977[M+H]$^+$, 477.1356[M+H-Rha]$^+$, 315.0882[M+H-Rha-Glc]$^+$, 273.0762[M+H-Rha-Glc-C_2H_4O]$^+$, 153.0713[M+H-C_8H_8O]$^+$	WAFI	Flavonoids
91	Isosakuranetin	17.19	$C_{16}H_{14}O_5$	286.0841	287.0917	1.2	287.0917[M+H]$^+$, 179.0339[M+H-C_7H_8O]$^+$, 153.0183[M+H-$C_9H_{10}O$]$^+$, 135.0804[M+H-$C_7H_4O_4$]$^+$, 125.0232[M+H-C_9H_{10}O-CO]$^+$, 109.0648[M+H-$C_9H_6O_4$]$^+$	WAFI, ZZP	Flavonoids
92	Poncirin	17.19	$C_{28}H_{34}O_{14}$	594.1949	595.2026	0.8	617.1844[M+Na]$^+$, 595.2026[M+H]$^+$, 449.1437[M+H-Rha]$^+$, 287.0916[M+H-Rha-Glc]$^+$, 179.0339[M+H-Rha-Glc-C_7H_8O]$^+$, 153.0183[M+H-Rha-Glc-$C_9H_{10}O$]$^+$, 135.0805[M+H-Rha-Glc-$C_7H_4O_4$]$^+$, 125.0236[M+H-Rha-Glc-C_9H_{10}O-CO]$^+$, 109.0650[M+H-Rha-Glc-$C_9H_6O_4$]$^+$	WAFI	Flavonoids
93	Bicyclo[4.4.0]dec-5-ene, 1,5-Dimethyl-3-hydroxy-8–(1-methylene-2-hydroxyethyl-1)	17.36	$C_{15}H_{24}O_2$	236.1776	237.1849	0.0	237.1849[M+H]$^+$, 219.1746[M+H-H_2O]$^+$	AMR, NF, ZZP	Other
94	Dihydroalatamide	17.42	$C_{16}H_{17}NO_2$	255.1259	256.1333	0.4	278.1150[M+Na]$^+$, 256.1333[M+H]$^+$, 135.0441[M+H-C_7H_7NO]$^+$, 122.0670[M+H-$C_9H_{12}O$]$^+$	WAFI, NF, ZZP	Other
95	Neoponcirin	17.46	$C_{28}H_{34}O_{14}$	594.1949	639.1930	−0.1	639.1930[M+COOH]$^-$, 593.1874[M-H]$^-$, 447.1288[M-H-Rha]$^-$, 285.0760[M-H-Rha-Glc]$^-$, 177.0180[M-H-Rha-Glc-C_7H_8O]$^-$, 151.0031[M-H-Rha-Glc-$C_9H_{10}O$]$^-$	ZZP	Flavonoids
96	Sakuranetin	18.02	$C_{16}H_{14}O_5$	286.0841	287.0917	1.0	287.0917[M+H]$^+$, 153.0184[M+H-$C_9H_{10}O$]$^+$, 109.0287[M+H-$C_9H_6O_4$]$^+$	NF	Flavonoids
97	Chrysoeriol-7-O-β-D-glucopyranoside	18.14	$C_{22}H_{22}O_{11}$	462.1162	463.1238	0.6	463.1238[M+H]$^+$, 301.0709[M+H-Glc]$^+$, 273.0757[M+H-Glc-CO]$^+$, 153.0183[M+H-Glc-$C_9H_8O_2$]$^+$	NF	Flavonoids
98	Lonicerin	18.25	$C_{27}H_{30}O_{15}$	594.1585	593.1518	1.0	593.1518[M-H]$^-$, 447.1291[M-H-Rha]$^-$, 285.0763[M-H-Rha-Glc]$^-$	WAFI	Flavonoids

(continued)

Table 1. (continued)

No.	Component name	T_R/min	Formula	Theoretical mass, Da	Detected mass, m/z	Mass error, ppm	MS^E ions	Source	Type
99	Naringenin	18.65	$C_{15}H_{12}O_5$	272.0685	273.0761	1.4	273.0761$[M+H]^+$, 179.0340$[M+H\text{-}C_6H_6O]^+$, 153.0183$[M+H\text{-}C_8H_8O]^+$, 125.0239$[M+H\text{-}C_8H_8O\text{-}CO]^+$, 121.0649$[M+H\text{-}C_7H_4O_4]^+$	WAFI, ZZP	Flavonoids
100	Tetradecylcitric acid	18.74	$C_{20}H_{36}O_7$	388.2461	387.2399	2.7	387.2399$[M-H]^-$, 371.1090$[M\text{-}H\text{-}H_2O]^-$	AMR, ZZP	Other
101	Praealtin D	18.81	$C_{19}H_{24}O_6$	348.1573	347.1495	−1.4	347.1495$[M-H]^-$	WAFI, ZZP	Coumarins
102	12-Senecioyloxytetradeca-2E, 8E, 10E-trien-4,6-diyne-1-ol	18.95	$C_{19}H_{22}O_4$	314.1518	315.1590	−0.2	315.1590$[M+H]^+$	WAFI	Terpenoids
103	6′,7′-dihydroxybergamottin	19.10	$C_{21}H_{24}O_6$	372.1573	395.1471	1.4	395.1471$[M+Na]^+$, 373.1637$[M+H]^+$, 147.0444$[M+H\text{-}C_{10}H_{18}O_2\text{-}2CO]^+$	WAFI, ZZP	Coumarins
104	Apigenin	19.13	$C_{15}H_{10}O_5$	270.0528	269.0447	−3.2	269.0476$[M-H]^-$, 241.0492$[M\text{-}H\text{-}CO]^-$, 117.0334$[M\text{-}H\text{-}C_7H_4O_4]^-$	WAFI, ZZP	Flavonoids
105	Kaempferol	19.16	$C_{15}H_{10}O_6$	286.0477	287.0556	2.2	287.0556$[M+H]^+$, 259.0554$[M+H\text{-}CO]^+$, 231.0644$[M+H\text{-}2CO]^+$, 153.0184$[M+H\text{-}Glc\text{-}C_8H_6O_2]^+$, 135.1170$[M+H\text{-}C_7H_4O_4]^+$	NF, ZZP	Flavonoids
106	7-Hydroxy-3,5,6,3′,4′-pentamethoxyflavone	19.22	$C_{20}H_{20}O_8$	388.1158	387.1082	−0.8	387.1082$[M-H]^-$, 372.0841$[M\text{-}H\text{-}CH_3]^-$	WAFI, ZZP	Flavonoids
107	8–(6,7-dihydroxy-3,7-dimethy-loct-1en-3-yl)-5-hydroxy-6,7-furocoumarin	19.28	$C_{21}H_{24}O_6$	372.1573	371.1495	−1.3	371.1495$[M-H]^-$, 343.0819$[M\text{-}H\text{-}CO]^-$	WAFI, ZZP	Coumarins
108	Diosmetin	19.31	$C_{16}H_{12}O_6$	300.0634	301.0710	1.1	301.0710$[M+H]^+$, 244.0658$[M+H\text{-}CO\text{-}CHO]^+$, 153.0183$[M+H\text{-}C_9H_8O_2]^+$, 149.0597$[M+H\text{-}C_7H_4O_4]^+$, 125.0596$[M+H\text{-}C_9H_8O_2\text{-}CO]^+$	WAFI, NF, ZZP	Flavonoids
109	Hesperetin	19.57	$C_{16}H_{14}O_6$	302.0790	301.0716	−0.4	301.0716$[M-H]^-$, 177.0188$[M\text{-}H\text{-}C_7H_8O_2]^-$, 151.0033$[M\text{-}H\text{-}C_9H_{10}O_2]^-$, 149.0605$[M\text{-}H\text{-}C_7H_4O_4]^-$	WAFI, ZZP	Flavonoids
110	Eupafolin	19.69	$C_{16}H_{12}O_7$	316.0583	315.0503	−2.3	315.0503$[M-H]^-$, 300.0270$[M\text{-}H\text{-}CH_3]^-$, 272.0289$[M\text{-}H\text{-}CH_3\text{-}CO]^-$, 166.9969$[M\text{-}H\text{-}CH_3\text{-}CO\text{-}C_8H_6O_2\text{-}CH_3]^-$	WAFI, NF, ZZP	Flavonoids
111	β-Cyclocitral	19.86	$C_{10}H_{16}O$	152.1201	153.1274	0.3	153.1274$[M+H]^+$	WAFI, ZZP	Other
112	Epoxyaurapten	19.86	$C_{19}H_{22}O_4$	314.1518	315.1595	1.3	315.1595$[M+H]^+$, 163.0391$[M+H\text{-}C_{10}H_{18}]^+$, 135.1168$[M+H\text{-}C_{10}H_{18}O\text{-}CO]^+$, 107.0493$[M+H\text{-}C_{10}H_{18}O\text{-}2CO]^+$	WAFI, ZZP	Coumarins
113	Marmin	19.86	$C_{19}H_{24}O_5$	332.1624	355.1518	0.7	355.1518$[M+H]^+$, 163.0391$[M+H\text{-}C_{10}H_{10}O_2]^+$, 135.1168$[M+H\text{-}C_{10}H_{10}O_2\text{-}CO]^+$, 117.0573$[M+H\text{-}C_{10}H_{10}O_2\text{-}2CO]^+$	WAFI, ZZP	Coumarins
114	O-Cymene	19.86	$C_{10}H_{14}$	134.1096	135.1169	0.3	135.1169$[M+H]^+$	WAFI, NF, ZZP	Terpenoids
115	Deacetylnomilinic acid	20.05	$C_{26}H_{34}O_9$	490.2202	491.2275	−0.1	513.2082$[M+Na]^+$, 491.2275$[M+H]^+$	WAFI, ZZP	Limonoids
116	6,9-dihydroxy-3,3a-dihydro Atractylenolide III	20.07	$C_{15}H_{22}O_5$	282.1467	281.1387	−2.5	281.1387$[M-H]^-$, 251.1641$[M\text{-}H\text{-}2H_2O]^-$, 223.1698$[M\text{-}H\text{-}2H_2O\text{-}CO]^-$	AMR, ZZP	Sequiterpene lactones
117	Natsudaidain	20.72	$C_{21}H_{22}O_9$	418.1264	417.1186	−1.1	417.1186$[M-H]^-$, 402.0950$[M\text{-}H\text{-}CH_3]^-$, 374.0589$[M\text{-}H\text{-}CH_3\text{-}CO]^-$, 165.0178$[C_9H_9O_3^+]$	WAFI, ZZP	Flavonoids

(continued)

Table 1. (continued)

No.	Component name	T_R/min	Formula	Theoretical mass, Da	Detected mass, m/z	Mass error, ppm	MS^E ions	Source	Type
118	Deacetylnomilin	20.95	$C_{26}H_{32}O_8$	472.2097	473.2170	0.0	473.2170$[M+H]^+$, 377.1020$[M+H\text{-}C_5H_4O_2]^+$, 350.1764$[M+H\text{-}C_6H_3O_3]^+$	WAFI, ZZP	Limonoids
119	Sinensetin	21.01	$C_{20}H_{20}O_7$	372.1209	373.1280	−0.5	373.1280$[M+H]^+$, 358.1039$[M+H\text{-}CH_3]^+$, 330.0688$[M+H\text{-}CH_3\text{-}CO]^+$, 211.0858$[M+H\text{-}C_{10}H_{10}O_2]^+$, 163.0754$[M+H\text{-}C_{10}H_{10}O_5]^+$, 165.0547$[C_9H_9O_3]^+$	WAFI, ZZP	Flavonoids
120	6-Demethoxytangeretin	21.02	$C_{19}H_{18}O_6$	342.1103	343.1175	−0.5	343.1175$[M+H]^+$, 328.0940$[M+H\text{-}CH_3]^+$, 299.0557$[M+H\text{-}CH_3\text{-}CHO]^+$, 211.0236$[M+H\text{-}C_9H_8O]^+$, 196.1283$[M+H\text{-}C_9H_8O\text{-}CH_3]^+$, 168.0811$[M+H\text{-}C_9H_8O\text{-}CH_3\text{-}CO]^+$, 133.0649$[M+H\text{-}C_{10}H_{10}O_5]^+$	WAFI, ZZP	Flavonoids
121	5-Demethylnobiletin	21.17	$C_{20}H_{20}O_8$	388.1158	389.1228	−0.8	411.1046$[M+Na]^+$, 389.1228$[M+H]^+$, 374.0993$[M+H\text{-}CH_3]^+$, 165.0545$[C_9H_9O_3]^+$, 137.0235$[C_9H_9O_3\text{-}CO]^+$	WAFI, ZZP	Flavonoids
122	Limonin	21.49	$C_{26}H_{30}O_8$	470.1941	471.2014	0.0	493.1834$[M+Na]^+$, 471.2014$[M+H]^+$, 375.1685$[M+H\text{-}C_5H_4O_2]^+$, 348.1677$[M+H\text{-}C_6H_3O_3]^+$	WAFI, ZZP	Limonoids
123	6-hydroxy-3,3a-dihydro Atractylenolide III	21.59	$C_{15}H_{22}O_4$	266.1518	265.1436	−3.7	265.1436$[M\text{-}H]^-$, 201.0180$[M\text{-}H\text{-}2H_2O\text{-}CO]^-$	AMR, ZZP	Sequiterpene lactones
124	Trimethylapigenin	21.80	$C_{18}H_{16}O_5$	312.0998	313.1072	0.6	313.1072$[M+H]^+$, 285.1129$[M+H\text{-}CO]^+$, 181.1002$[M+H\text{-}C_9H_8O]^+$, 153.0678$[M+H\text{-}C_9H_8O\text{-}CO]^+$, 151.0761$[M+H\text{-}C_9H_8O\text{-}CH_2O]^+$, 133.0651$[M+H\text{-}C_9H_8O_4]^+$	WAFI, ZZP	Flavonoids
125	1,3,3-trimethyl-2–(3-methyl-2-methylene-3-butenylidene)-cyclohexanol	21.97	$C_{15}H_{24}O$	220.1827	221.1900	0.1	221.1900$[M+H]^+$	NF	Other
126	Nomilinic acid	22.04	$C_{28}H_{36}O_{10}$	532.2309	531.2233	−0.5	531.2233$[M\text{-}H]^-$	WAFI, ZZP	Limonoids
127	Hexamethylquercetagetin	22.20	$C_{21}H_{22}O_8$	402.1315	403.1386	−0.5	425.1203$[M+Na]^+$, 403.1386$[M+H]^+$, 370.1038$[M+H\text{-}CH_3\text{-}CO]^+$, 211.0238$[M+H\text{-}C_{11}H_{12}O_3]^+$, 193.01322$[M+H\text{-}C_{10}H_{10}O_5]^+$, 168.0051$[M+H\text{-}C_{11}H_{12}O_3\text{-}CH_3\text{-}CO]^+$	WAFI, ZZP	Flavonoids
128	Gardenin B	22.63	$C_{19}H_{18}O_7$	358.1053	359.1123	−0.5	381.0948$[M+Na]^+$, 359.1123$[M+H]^+$, 344.0876$[M+H\text{-}CH_3]^+$, 316.2841$[M+H\text{-}CH_3\text{-}CO]^+$, 227.1061$[M+H\text{-}C_9H_8O]^+$, 135.0807$[C_8H_7O_2]^+$, 133.1009$[M+H\text{-}C_{10}H_{10}O_6]^+$, 107.0853$[C_9H_9O_3\text{-}CO]^+$	ZZP	Flavonoids
129	Nomilin	22.64	$C_{28}H_{34}O_9$	514.2203	515.2274	−0.4	537.2075$[M+Na]^+$, 515.2274$[M+H]^+$, 419.1324$[M+H\text{-}C_5H_4O_2]^+$, 392.1977$[M+H\text{-}C_6H_3O_3]^+$	WAFI, ZZP	Limonoids

(continued)

Table 1. (continued)

No.	Component name	T_R/min	Formula	Theoretical mass, Da	Detected mass, m/z	Mass error, ppm	MS^E ions	Source	Type
130	3-Methoxynobiletin	22.82	$C_{22}H_{24}O_9$	432.1420	433.1486	−1.7	433.1486$[M+H]^+$, 418.1250$[M+H\text{-}CH_3]^+$, 390.1208$[M+H\text{-}CH_3\text{-}CO]^+$, 241.0726$[M+H\text{-}C_{11}H_{12}O_3]^+$, 193.0132$[M+H\text{-}C_{11}H_{12}O_6]^+$, 226.0480$[M+H\text{-}C_{11}H_{12}O_3\text{-}CH_3]^+$	WAFI, ZZP	Flavonoids
131	Tangeretin	23.14	$C_{20}H_{20}O_7$	372.1209	373.1280	−0.6	395.1094$[M+Na]^+$, 373.1280$[M+H]^+$, 358.1044$[M+H\text{-}CH_3]^+$, 330.0660$[M+H\text{-}CH_3\text{-}CO]^+$, 241.0754$[M+H\text{-}C_9H_8O]^+$, 226.0584$[M+H\text{-}C_9H_8O\text{-}CH_3]^+$, 198.0652$[M+H\text{-}C_9H_8O\text{-}CH_3\text{-}CO]^+$, 135.0440$[C_8H_7O_2]^+$, 133.0647$[M+H\text{-}C_11H_{12}O_6]^+$, 107.0494$[C_8H_7O_2^+\text{-}CO]$	WAFI, ZZP	Flavonoids
132	thtmof	23.18	$C_{18}H_{16}O_8$	360.0845	361.0916	−0.7	361.0916$[M+H]^+$, 343.0812$[M+H\text{-}H_2O]^+$, 333.0980$[M+H\text{-}CO]^+$, 305.1017$[M+H\text{-}2CO]^+$, 183.0290$[M+H\text{-}C_{10}H_{10}O_3]^+$, 179.0340$[M+H\text{-}C_8H_6O_5]^+$, 165.0549$[C_9H_9O_3]^+$, 155.0472$[M+H\text{-}C_{10}H_{10}O_3\text{-}CO]^+$	WAFI, ZZP	Flavonoids
133	Obacunone	23.46	$C_{26}H_{30}O_7$	454.1992	455.2062	−0.5	477.1901$[M+Na]^+$, 455.2062$[M+H]^+$, 359.1851$[M+H\text{-}C_5H_4O_2]^+$, 332.1366$[M+H\text{-}C_6H_3O_3]^+$	WAFI, ZZP	Limonoids
134	5-Demethylsinensetin	23.56	$C_{19}H_{18}O_7$	358.1053	359.1122	−0.8	381.0958$[M+Na]^+$, 359.1122$[M+H]^+$, 344.0878$[M+H\text{-}CH_3]^+$, 316.1903$[M+H\text{-}CH_3\text{-}CO]^+$, 182.0160$[M+H\text{-}C_{10}H_{10}O_2\text{-}CH_3]^+$,165.0695$[C_9H_9O_3]^+$,163.0393$[M+H\text{-}C_9H_8O_5]^+$	WAFI, ZZP	Flavonoids
135	Imperatorin	23.93	$C_{16}H_{14}O_4$	270.0892	269.0812	−2.9	269.0812$[M\text{-}H]^-$, 201.1079$[M\text{-}H\text{-}C_5H_{10}]^-$, 173.0222$[M\text{-}H\text{-}C_5H_{10}\text{-}CO]^-$, 145.0288$[M\text{-}H\text{-}C_5H_{10}\text{-}2CO]^-$, 117.0330$[M\text{-}H\text{-}C_5H_{10}\text{-}3CO]^-$	WAFI, ZZP	Coumarins
136	Osthole	24.38	$C_{15}H_{16}O_3$	244.1099	245.1174	0.7	267.1009$[M+Na]^+$, 245.1174$[M+H]^+$, 190.0582$[M+H\text{-}C_4H_7]^+$	WAFI, ZZP	Coumarins
137	Norhalkendin	24.71	$C_{12}H_8O_5$	232.0372	233.0447	1.3	233.0447$[M+H]^+$, 218.0210$[M+H\text{-}CH_3]^+$, 205.0901$[M+H\text{-}CO]^+$, 190.0258$[M+H\text{-}CH_3\text{-}CO]^+$, 162.0318$[M+H\text{-}CH_3\text{-}2CO]^+$, 134.0364$[M+H\text{-}CH_3\text{-}3CO]^+$, 106.0448$[M+H\text{-}CH_3\text{-}4CO]^+$	WAFI, ZZP	Coumarins
138	(+)-Alantolactone	24.77	$C_{15}H_{20}O_2$	232.1463	233.1538	1.0	233.1538$[M+H]^+$, 205.1584$[M+H\text{-}CO]^+$, 190.1627$[M+H\text{-}CH_3\text{-}CO]^+$	AMR, ZZP	Sequiterpene lactones

(continued)

Table 1. (continued)

No.	Component name	T_R/min	Formula	Theoretical mass, Da	Detected mass, m/z	Mass error, ppm	MS^E ions	Source	Type
139	Procerin	24.82	$C_{15}H_{18}O_2$	230.1307	231.1381	0.6	231.1381$[M+H]^+$, 213.1185$[M+H-H_2O]^+$	ZZP	Terpenoids
140	Isoimperatorin	24.83	$C_{16}H_{14}O_4$	270.0892	271.0968	1.3	271.0968$[M+H]^+$, 203.0341$[M+H-C_5H_{10}]^+$, 185.1320$[M+H-C_5H_{10}-H_2O]^+$, 175.0388$[M+H-C_5H_{10}-CO]^+$, 147.0442$[M+H-C_5H_{10}-2CO]^+$, 119.0492$[M+H-C_5H_{10}-3CO]^+$	WAFI, ZZP	Coumarins
141	Xanthotoxol	24.83	$C_{11}H_6O_4$	202.0266	203.0342	1.5	203.0342$[M+H]^+$, 175.0388$[M+H-CO]^+$, 147.0442$[M+H-2CO]^+$, 119.0492$[M+H-3CO]^+$	WAFI, ZZP	Coumarins
142	14-Acetoxy-12-β-methyl-butyryltetradeca-2E, 8E, 10E-trien-4,6-diyne-1-ol	24.85	$C_{21}H_{26}O_5$	358.1780	381.1666	−1.6	381.1666$[M+H]^+$	ZZP	Terpenoids
143	6-Hydroxy atractylenolide I	24.99	$C_{15}H_{20}O_3$	248.1412	249.1489	1.6	249.1489$[M+H]^+$, 231.1377$[M+H-H_2O]^+$, 203.1432$[M+H-H_2O-CO]^+$	AMR, ZZP	Sequiterpene lactones
144	8β-Methoxy atractylenolide I	25.01	$C_{16}H_{22}O_3$	262.1569	263.1565	−1.4	263.1565$[M+H]^+$, 233.1499$[M+H-2CH_3]^+$, 205.1544$[M+H-2CH_3-CO]^+$	AMR, ZZP	Sequiterpene lactones
145	Atractylenolide I	26.61	$C_{15}H_{18}O_2$	230.1307	231.1382	0.9	231.1382$[M+H]^+$, 216.1131$[M+H-CH_3]^+$, 188.0831$[M+H-CH_3-CO]^+$	AMR, ZZP	Sequiterpene lactones
146	2,6-Di-Tert-Butylquinone	27.30	$C_{14}H_{20}O_2$	220.1463	221.1536	−0.2	221.1536$[M+H]^+$	WAFI	Other
147	Scopoletin	27.89	$C_{10}H_8O_4$	192.0423	193.0495	0.0	193.0495$[M+H]^+$, 178.0260$[M+H-CH_3]^+$, 165.0697$[M+H-CO]^+$, 150.0309$[M+H-CH_3-CO]^+$, 122.0361$[M+H-CH_3-2CO]^+$	WAFI, ZZP	Coumarins
148	2-(4a, 8-dimethyl-2,3,4,4a, 5,6-hexahydro-naphthalen-2-yl)-prop-2-en-1-ol	27.95	$C_{15}H_{22}O$	218.1671	219.1744	0.5	219.1744$[M+H]^+$	AMR, ZZP	Terpenoids
149	1-Benzylidene-2,2,3-trimethylcyclopentane	27.95	$C_{15}H_{20}$	200.1565	201.1637	−0.4	201.1637$[M+H]^+$	AMR, ZZP	Other
150	3β-Acetoxyatractylon	28.51	$C_{17}H_{22}O_3$	274.1569	275.1646	1.5	275.1646$[M+H]^+$, 215.1432$[M+H-CH_3COOH]^+$, 200.1187$[M+H-CH_3COOH-CH_3]^+$, 172.0867$[M+H-CH_3COOH-CH_3-CO]^+$	AMR, ZZP	Terpenoids
151	Heraclenin	29.13	$C_{16}H_{14}O_5$	286.0841	287.0914	0.1	287.0914$[M+H]^+$, 203.0342$[M+H-C_5H_{10}O]^+$, 175.0385$[M+H-C_5H_{10}O-CO]^+$, 147.0445$[M+H-C_5H_{10}O-2CO]^+$	WAFI, ZZP	Coumarins
152	Auraptene	29.73	$C_{19}H_{22}O_3$	298.1569	299.1643	0.6	321.1264$[M+Na]^+$, 299.1643$[M+H]^+$, 163.03914$[M+H-C_{10}H_{18}]^+$, 135.0440$[M+H-C_{10}H_{18}-CO]^+$, 107.0493$[M+H-C_{10}H_{18}-2CO]^+$	WAFI, ZZP	Coumarins
153	Benzaldehyde	29.73	C_7H_6O	106.0419	107.0493	1.8	107.0493$[M+H]^+$	WAFI, ZZP	Other
154	Ocimene	29.73	$C_{10}H_{16}$	136.1252	137.1325	−0.2	137.1325$[M+H]^+$	WAFI, ZZP	Terpenoids

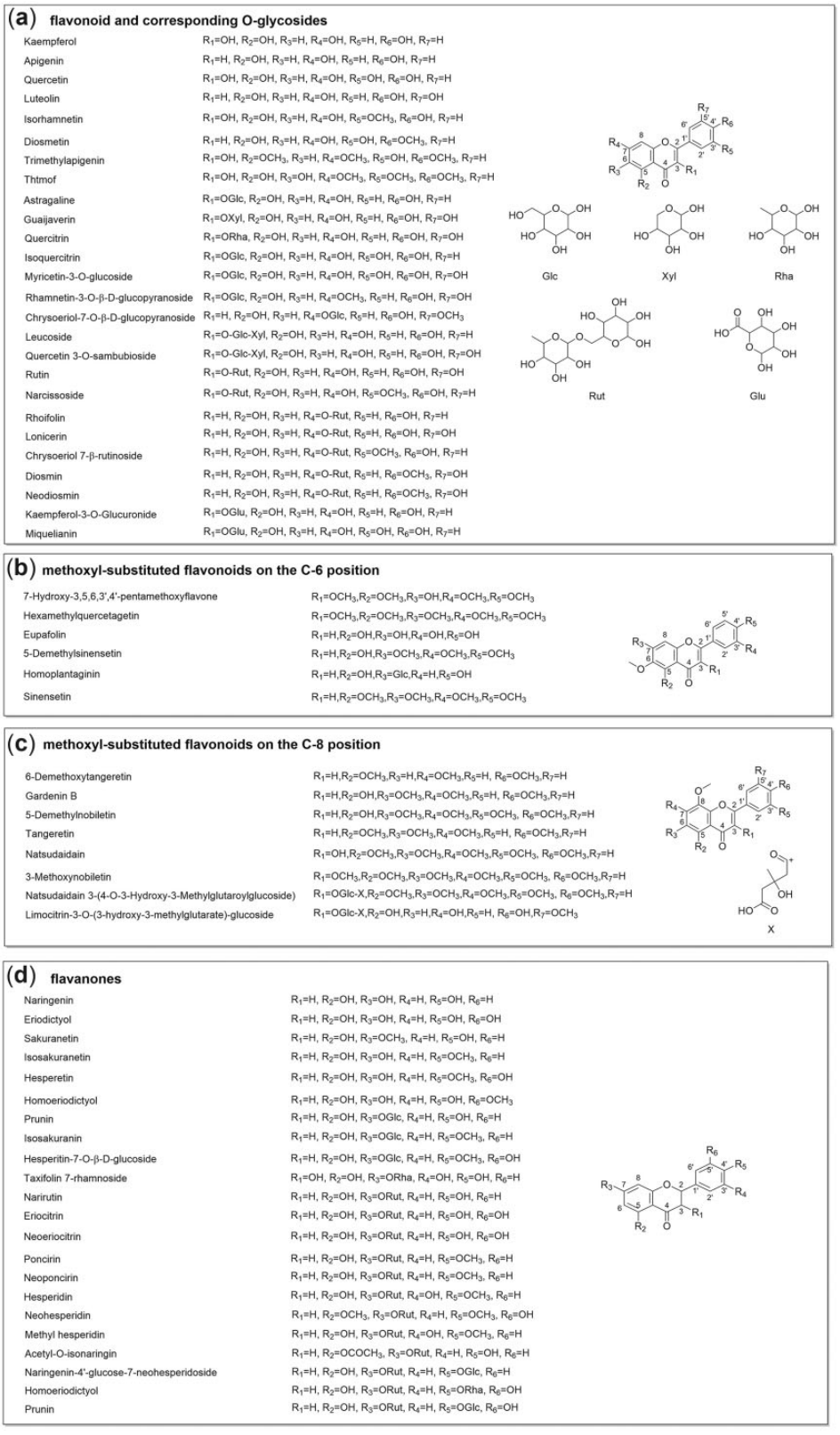

Figure 3. The chemical structures of sequiterpene lactones (a), benzylisoquinoline alkaloids (b), aporphine alkaloids (c), and coumarins and furocoumarines (d).

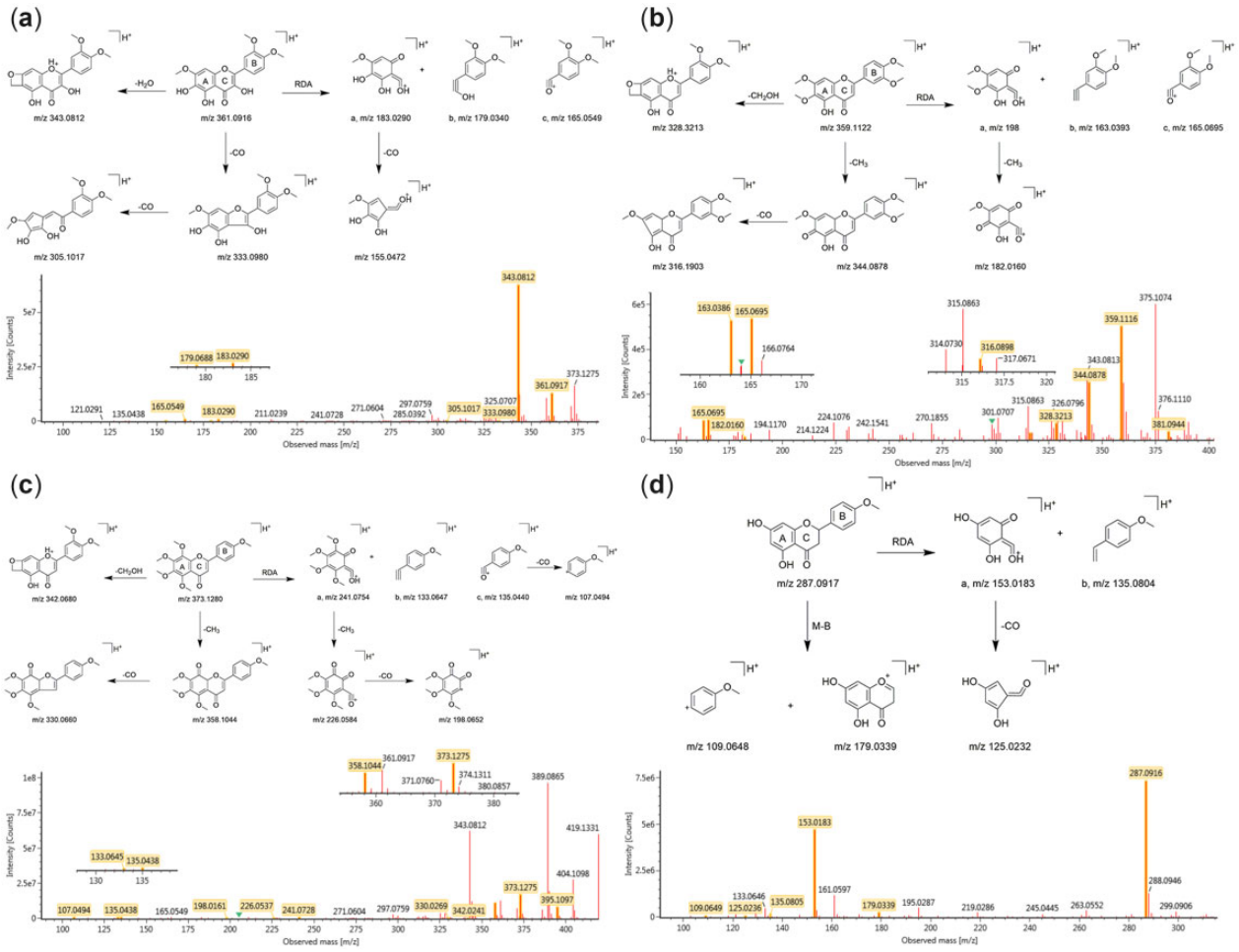

Figure 4. The MS fragmentation pathway and MS/MS spectra for the $[M+H]^+$ ion of thtmof (a), 5-demethylsinensetin (b), tangeretin (c), and isosakuranetin (d).

the C-10 position was substituted by a CH_3 group. The hydroxyl-substituted sequiterpene lactones yielded two characteristic ions ("ion M-18" and "ion M-46") through successive loss of H_2O and CO groups. This compound does not easily lose neutral CO_2. The chemical structures of sequiterpene lactones in ZZP are shown in Figure 5a. For example, in positive ionization mode, atractylenolide I (peak 145, $t_R = 26.61$ min) yielded protonated ion $[M+H]^+$ at m/z 231.1382. The protonated ion generated two characteristic ions at m/z 216.1131 and 188.0831 through successive losing CH_3 and CO. This result was demonstrated by combining the fragmentation information with commercial standards. The MS fragmentation pathway and MS/MS spectra for the $[M+H]^+$ ion of atractylenolide I are shown in Figure 6a.

Alkaloids

Most alkaloids in ZZP are derived primarily from NF. The benzyl of benzylisoquinoline alkaloids is always cleaved on the C-1 position, which produced tetrahydroisoquinoline and benzyl, which was named as "ion a" and "ion b." The chemical structures of benzylisoquinoline alkaloids in ZZP are shown in Figure 5b. For instance, in positive ionization mode, N-methylcoclaurine (peak 28, $t_R = 7.3$ min) yielded protonated ion $[M+H]^+$ at m/z 300.1596. The protonated ion occurred benzyl cleavage under high collision energies, which produced "ion a" $[M+H\text{-}C_7H_8O]^+$ at m/z 192.1007 and "ion b" $[M+H\text{-}C_{11}H_{15}NO_2]^+$ at m/z 107.0493. The "ion a" was further split and provided three characteristic ions at m/z 179.0864, 178.0830, 149.0596 through successive loss of CH_3, H, and CO groups. The MS fragmentation pathway and MS/MS spectra for the $[M+H]^+$ ion of N-methylcoclaurine are shown in Figure 6b.

The aporphine alkaloids have occurred mainly in the RDA reaction on the B-ring. When an H atom in the N-H structure was substituted by a CH_3 group, the molecular ion of this compound will generate "ion M-CH_2=NCH_3" (M-43). The chemical structures of aporphine alkaloids in ZZP are shown in Figure 5c. Take floribundine (peak 45, $t_R = 10.75$ min) as an example: in positive ionization mode, it yielded protonated ion $[M+H]^+$ at m/z 282.1493. RDA cleavage in the B-ring generated "ion $[M+H\text{-}CH_2{=}NCH_3]^+$" at m/z 239.1056. The "ion $[M+H\text{-}CH_2{=}NCH_3]^+$" was further fragmented and produced two characteristic ions at m/z 222.0998 and 208.0882 by losing OH and OCH_3. In the meantime, the molecular ion yielded two characteristic ions at m/z 265.1212 and 251.1070. The MS fragmentation pathway and MS/MS spectra for the $[M+H]^+$ ion of floribundine are shown in Figure 6c. Furthermore, nuciferine (peak 74, $t_R = 14.76$ min) was confirmed by using a reference standard. However, N-nornuciferine (peak 72, $t_R = 12.45$ min) is an aporphine alkaloid that an H atom in the N-H structure was not substituted; its molecular ion will produce "ion M-CH_2=NH" (M-29).

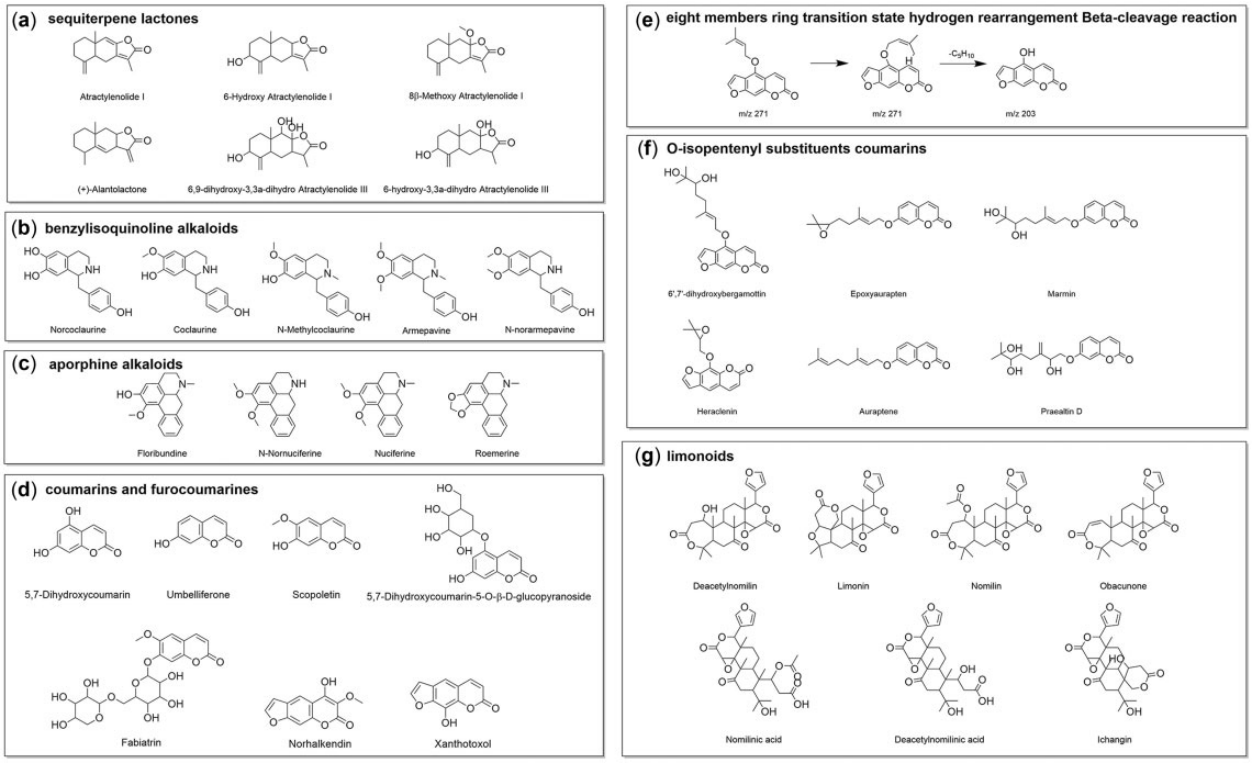

Figure 5. The chemical structures of sequiterpene lactones (a), benzylisoquinoline alkaloids (b), aporphine alkaloids (c), coumarins and furocoumarines (d), eight members ring transition state hydrogen rearrangement β-cleavage reaction (e), O-isopentenyl substituents coumarins (f), and limonoids (g) detected in ZZP.

Coumarins

Most coumarins in ZZP mainly originate from AFI. The coumarins can successive lost neutral CO group until all oxygen atoms are lost, and the fragmentation pattern of furocoumarines is the same as coumarins. The chemical structures of coumarins and furocoumarines in ZZP are shown in Figure 5d. Umbelliferone (peak 40, $t_R = 9.44$ min) as hydroxyl-substituted coumarin, in positive ionization mode, yielded protonated ion $[M+H]^+$ at m/z 163.0392. The protonated ion produced two predominant ions at m/z 135.0442 and 107.0493, owing to successive loss of neutral CO. The MS fragmentation pathway and MS/MS spectra for the $[M+H]^+$ ion of umbelliferone are shown in Figure 6d.

Scopoletin (peak 147, $t_R = 27.89$ min) is a methoxyl-substitued coumarin on the C-6 position. In positive ionization mode, it yielded protonated ion $[M+H]^+$ at m/z 193.0495. The protonated ion yielded two characteristic ions at m/z 178.0260 and 165.0697 by losing CH_3 and CO groups, respectively. They can generate "ion $[M+H\text{-}CH_3\text{-}CO]^+$" at m/z 150.0553 through losing CO or CH_3. After further fragmentation, the "ion $[M+H\text{-}CH_3\text{-}CO]^+$" was generated "ion $[M+H\text{-}CH_3\text{-}2CO]^+$" at m/z 122.0607. The MS fragmentation pathway and MS/MS spectra for the $[M+H]^+$ ion of scopoletin are shown in Figure 6e.

The O-isopentenyl substituents coumarins readily occurred eight members ring transition state hydrogen rearrangement *β*-cleavage reaction (http://www.jcmss.com.cn/CN/Y1998/V19/I2/5). Take isoimperatorin (peak 140, $t_R = 24.83$ min) as an example: in positive ionization mode, the typical eight members ring transition state hydrogen rearrangement *β*-cleavage reaction is shown in Figure 5e. Then the fragmentation was the same as corresponding original hydroxyl-substituted coumarins. The chemical structures of O-isopentenyl substituents coumarins in ZZP are shown in Figure 5f.

The isopentenyl-substituted coumarins generated some characteristic ions such as M-15, M-43, and M-55 through losing CH_3, C_3H_7, and C_4H_7 groups, respectively. For example, in positive ionization mode, osthole (peak 136, $t_R = 24.38$ min) could yield protonated ion $[M+H]^+$ at m/z 245.1174. The protonated ion will produce ion M-55 at m/z 190.0582 by losing C_4H_7. The MS fragmentation pathway and MS/MS spectra for the $[M+H]^+$ ion of osthole are shown in Figure 6f.

Limonoids

Most limonoids in ZZP are derived primarily from AFI. The lactone ring cleavage reaction readily occurred on the D-ring in the limonoids, which will produce corresponding "ion a," a furanyl-carbonyl ion, and "ion M-123" through hydrogen-atom transfer. The chemical structures of limonoids in ZZP are shown in Figure 5g. For example, in positive ionization mode, obacunone (peak 133, $t_R = 23.46$ min) could yield ion $[M+Na]^+$ at m/z 477.1901 and protonated ion $[M+H]^+$ at m/z 455.2062. The protonated ion generated "ion a" and "ion M-123" at m/z 359.1851, 332.1366. This result was demonstrated by combining the fragmentation information with commercial standards. The MS fragmentation pathway and MS/MS spectra for the $[M+H]^+$ ion of obacunone are shown in Figure 6g.

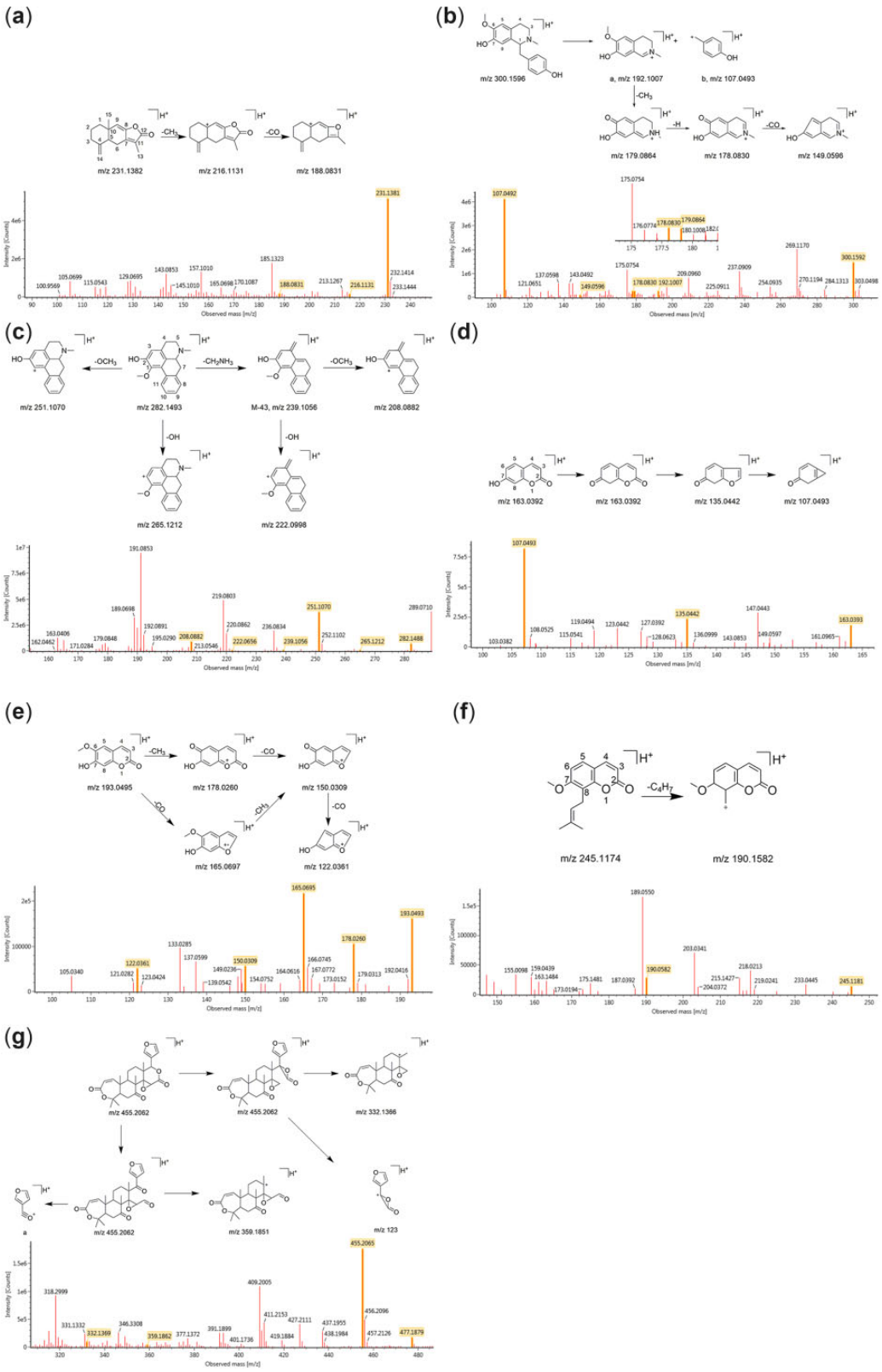

Figure 6. The MS fragmentation pathway and MS/MS spectra for the $[M+H]^+$ ion of atractylenolide I (a), N-methylcoclaurine (b), floribundine (c), umbelliferone (d), scopoletin (e), osthole (f), and obacunone (g).

Conclusions

In the present study, a reliable and sensitive analytical method was established to qualitative compounds in ZZP by UPLC-Q-TOF-MS^E. A total of 154 compounds were identified in ZZP and its individual herbs: 67 flavonoids, 17 coumarins, 11 terpenoids, 10 alkaloids, six limonoids, six sequiterpene lactones, and 37 other components. In previous studies, researchers focused only on the effect of AFI and AMR. In this work, we found that a great deal of components in ZZP are derived from NF. A preliminary analysis has revealed that the water extract of NF is not only an adhesive in the preparation of ZZP. Furthermore, the chemical analysis of ZZP could provide a reference for the research of pharmacodynamic substance basis and pharmacodynamic effects mechanism in the ZZP.

Conflict of Interest

There are no conflicts to declare.

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