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Chinese Journal of Natural Medicines 2019, **17**(11): 0803–0821
Chinese Journal of Natural Medicines

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Identification of constituents in Gui-Zhi-Jia-Ge-Gen-Tang by LC-IT-MS combined with LC-Q-TOF-MS and elucidation of their metabolic networks in rat plasma after oral administration

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Available online 20 Nov., 2019

[ABSTRACT] Gui-Zhi-Jia-Ge-Gen-Tang (GZJGGT) is a traditional Chinese medicine (TCM) prescription commonly used to treat cervical spondylopathy, scapulohumeral periarthritis, *etc*. Though it is widely applied in clinical practice, the effective constituents of GZJGGT remain unclear. This was the first report on the identification of the chemical constituents from GZJGGT *in vitro* and *in vivo* using LC-IT-MS combined with LC-Q-TOF-MS. A total of 141 constituents were detected in GZJGGT, and 77 were identified. These compounds mainly included flavonoid glycosides, triterpene saponins, monoterpene glycosides, puerosides, and organic acids. Among them, 12 compounds were unequivocally identified in comparison with reference substances. Additionally, a diagnostic base peak ion filtering strategy for rapid classification of flavonoid *O*-glycosides and *C*-glycosides was proposed. After gastrointestinal administration of GZJGGT to rats, 45 prototypes and 48 metabolites in rat plasma were speculated. In addition, the metabolic profile of GZJGGT was portrayed to understand interrelationship between metabolites.

[KEY WORDS] Gui-Zhi-Jia-Ge-Gen-Tang; LC-MS; Chemical constituents; Prototype compounds; Metabolites

[CLC Number] R917 **[Document code]** A **[Article ID]** 2095-6975(2019)11-0803-19

Introduction

Gui-Zhi-Jia-Ge-Gen-Tang (GZJGGT) is a traditional Chinese medicine (TCM) prescription from Shang-Han-Lun, a very famous medical work from the Eastern Han Dynasty of China. GZJGGT, one of the categorized prescriptions of Gui-Zhi-Tang, is composed of six crude drugs, *i.e.*, Cinnamomi Ramulus (Guizhi in Chinese, sovereign drug), Puerariae Lobatae Radix (Gegen in Chinese, ministerial drug), Paeoniae Radix Alba (Baishao in Chinese, ministerial drug), Jujubae Fructus (Dazao in Chinese, adjuvant drug), Zingiberis Rhizoma Recens (Shengjiang in Chinese, adjuvant drug), and Glycyrrhizae Radix et Rhizoma Preparata cum Melle (Zhigancao in Chinese, adjuvant drug and guide drug) [1]. GZJGGT is mainly used to treat cervical spondylopathy [2-4], scapulohumeral periarthritis [5], and carotid-cardiac syndrome [6] in clinical practice. In spite of its wide application, the effective constituents of GZJGGT remain unclear.

It is well known that only constituents absorbed into blood circulation could exert therapeutic effect, except for that directly acting on the gastrointestinal tract. In addition to prototypes, metabolites might contribute to pharmacological action [7]. Therefore, it is important to investigate the absorption and metabolism of chemical constituents of GZJGGT, so as to better understand the material basis for the action mechanism and clinical application of GZJGGT.

However, it is never an easy task to identify the prototypes and metabolites of TCM prescription. i) TCM prescription generally contains hundreds of compounds. The detection of the compounds *in vivo* might be disturbed by massive endogenous metabolites during LC-MS analysis [8], especially those compounds (prototype constituents or metabolites) with low abundance. ii) What makes metabolite identification more complicated is that metabolic reactions are various and multi-step metabolic reactions are commonly involved in formation of metabolites. This results in multiple metabolites coming from the same precursor compound, and on the other hand, a metabolite is produced by multiple precursor com-

[Received on] 28-Aug.-2019
[Research funding] This work was supported by Zhejiang Provincial Natural Science Foundation of China (No. LY17H280002).
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These authors have no conflict of interest to declare.

pounds. iii) It is still a challenge to differentiate some prototypes from metabolites, given that some prototypes could also be metabolized from the homologous compounds in TCM prescription [9]. They shared identical retention time, molecular formula and fragment ions in LC-MS analysis. iv) Some prototype constituents in blood are difficult to be detected, since they might transform into metabolites to the greatest extent possible. v) TCM prescription usually contains isomers that could not be distinguished by MS spectral analysis alone, hence, it was hard to correlate the parent compounds with metabolites.

In past decades, liquid chromatography-mass spectrometry (LC-MS) has become a powerful tool for the identification of chemical constituents *in vitro* [10-12] and *in vivo* [13]. Liquid chromatography-ion trap mass spectrometry (LC-IT-MS) is featured by providing multiple-stage fragment information that can reflect the structural characteristics of compounds with both sensitivity and rapidity. Due to the low accuracy of IT analyzer, liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-Q-TOF-MS) combined with LC-IT-MS is applied to obtain molecular formulae based on the exact molecular mass. Recently, these analysis techniques have been widely used in the study of TCM, such as for licorice. HUANG *et al.* [14] proposed a targeted strategy to profile the metabolites of licorice in rats from *in vitro* to *in vivo* by UHPLC combined with triple quadrupole-linear IT-MS. WANG *et al.* [15] identified metabolites of bioactive licorice compounds in rats by HPLC-DAD-ESI-MS^n and LC-IT-TOF-MS analyses.

In this work, 141 compounds were detected in GZJGGT by LC-IT-MS combined with LC-Q-TOF-MS. In total, 77 compounds were speculated, among which 12 compounds were confirmed by reference standards. Moreover, 45 prototype compounds and 48 metabolites were deduced in rat plasma. As far as we know, this was the first report on identification of the chemical constituents of GZJGGT *in vitro* and *in vivo*. A diagnostic ion filtering strategy for rapid structural classification of flavonoid *O*-glycosides and *C*-glycosides was recommended by MS analysis, and the metabolic network of GZJGGT in rat plasma after oral administration was delineated.

Experimental

Materials and reagents

Cinnamomi Ramulus and Glycyrrhizae Radix et Rhizoma Preparata cum Melle were purchased from Zhejiang Chinese Medical University Medical Pieces Co., Ltd. (Hangzhou, China). Puerariae Lobatae Radix and Paeoniae Radix Alba were acquired from the local pharmacy (Hangzhou, China), while Jujubae Fructus and Zingiberis Rhizome Recens were obtained from a local market. Cinnamomi Ramulus was from the dry twig of *Cinnamomum cassia* Presl, Jujubae Fructus was from the dry and ripe fructus of *Ziziphus jujuba* Mill., and Zingiberis Rhizome Recens was from the fresh rhizome of *Zingiber officinale* Rosc.. Puerariae Lobatae Radix and Paeoniae Radix Alba were from the dry radix of *Pueraria lobata* (Willd.) Ohwi and *Paeonia lactiflora* Pall., respectively. Glycyrrhizae Radix et Rhizoma Preparata cum Melle was made from the dry radix and rhizoma of *Glycyrrhiza uralensis* Fisch.. These six crude drugs were authenticated by associate professor CHEN Liu-Rong of College of Pharmaceutical Sciences, Zhejiang University.

Puerarin was purchased from Shanghai Winherb Medical Technology Co., Ltd. (Shanghai, China). Isoliquiritin apioside was acquired from Shanghai Yuanye Biological Technology Co., Ltd. (Shanghai, China). Genistoside was obtained from Chengdu Must Bio-technology Co., Ltd. (Chengdu, China). Authentic standards of 3'-methoxypuerarin, 4'-methoxypuerarin, liquiritin, glycyrrhizin, liquiritin apioside, ononin, isoviolanthin, albiflorin, and daidzein were isolated from Glycyrrhizae Radix et Rhizoma in our laboratory. Their structures were identified using UV, MS, and NMR analysis. The purity of each compound was determined to be greater than 96%.

HPLC-grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Formic acid (HPLC grade) was offered by ROE Scientific Inc. (Newark, USA). Deionized water was purified by Milli-Q water purification system (Millipore, Molsheim, France).

Standard solutions preparation

Standards of puerarin, isoliquiritin apioside, genistoside, 3'-methoxypuerarin, 4'-methoxypuerarin, liquiritin, glycyrrhizin, liquiritin apioside, ononin, isoviolanthin, albiflorin, and daidzein were dissolved in methanol at 1 mg·mL^{-1} and stored at 4 °C for convenient use. They were mixed to the appropriate concentration before qualitative analysis, and then filtered through 0.22 μm filter membranes.

Extract of GZJGGT prescription and its herbs

Samples for LC-MS analysis *in vitro*: GZJGGT consisted of six herbs in accordance with Shang-Han-Lun, *i.e.*, Cinnamomi Ramulus (6.0 g), Puerariae Lobatae Radix (12.0 g), Paeoniae Radix Alba (6.0 g), Jujubae Fructus (24.0 g), Zingiberis Rhizoma Recens (9.0 g), and Glycyrrhizae Radix et Rhizoma Preparata cum Melle (6.0 g). They were soaked in 630 mL (ten times their total mass) deionized water for 12 h. Then they were decocted to boil for 1 h by electric heater. After filtration, the residue was extracted by adding 504 mL (eight times of their total weight) deionized water in the same way for another 1 h, and then percolated. Finally, these two filtrates were blended, and concentrated to 0.2 g·mL^{-1} for LC-MS analysis after being filtered through 0.22 μm filter membrane. The individual extract of Cinnamomi Ramulus (6.0 g), Puerariae Lobatae Radix (12.0 g), Paeoniae Radix Alba (6.0 g), Jujubae Fructus (24.0 g), Zingiberis Rhizoma Recens (9.0 g), and Glycyrrhizae Radix et Rhizoma Preparata cum Melle (6.0 g) was prepared by means of the same extraction method of GZJGGT.

Samples for intragastric administration to rats: Cinnamomi Ramulus (60.0 g), Puerariae Lobatae Radix (120.0 g),

Paeoniae Radix Alba (60.0 g), Jujubae Fructus (240.0 g), Zingiberis Rhizoma Recens (90.0 g), and Glycyrrhizae Radix et Rhizoma Preparata cum Melle (60.0 g) were extracted with water twice (the first time using 6300 mL and the second time 5040 mL) as mentioned above. After incorporating the two batches of filtrates, they were concentrated by rotary vaporization at 40 °C under reduced pressure, and then dried by vacuum freeze drier. The extraction yield of GZJGGT was about 35%.

Animals and plasma samples preparation

Nine male Sprague-Dawley (SD) rats (200 ± 10 g) were purchased from the Laboratory Animal Center of Zhejiang University (Hangzhou, China). These rats were bred in an animal room with temperature of 24 ± 2 °C, humidity of 55% ± 15%, and a 12 h dark-light cycle for five days to allow acclimation to the environment. They were fasted with free access to water for 12 h before administration of the extract of GZJGGT. The animal feeds and facilities were supplied by the Laboratory Animal Center of Zhejiang University (Hangzhou, China).

The rats were randomly divided into three groups of three rats each. Groups A and B were administered extracts by gavage at a six-fold human equivalent dosage (6×2.3 g·kg^{-1}), and the blank group of rats were given normal saline. About 3 mL blood samples from each rat were obtained from the portal veins and collected in heparinized tubes. The blood of rats in groups A and B were collected at 0.25 and 0.5 h after oral administration, respectively. The blood samples acquired were centrifuged at 4 000 r·min^{-1} for 15 min at 4 °C to obtain plasma, and then stored at −80 °C before analysis. The plasma samples of six rats in group A and group B were mixed to eliminate individual differences and detect many more compounds *in vivo*, and this process repeated for blank samples.

800 μL methanol was added to 200 μL plasma and mixed for 1 min in a vortex mixer. Then the solution was centrifuged at 10 000 r·min^{-1} for 10 min. The supernatant was concentrated to dry at 35 °C. The residue was redissolved with 50 μL 20% methanol-water, and centrifuged at 10 000 r·min^{-1} for 10 min, and the supernatant was for HPLC-Q-TOF-MS analysis.

LC-IT-MS analysis

LC analysis was performed on an Agilent 1100 high performance LC system (Waldbronn, Germany), which was composed of a binary pump, a DAD detector, a column compartment, an auto-sampler, and an online degasser. The separation of samples was carried out on a Zorbax SB-C_{18} column (4.6 mm × 250 mm, 5 μm, Agilent) at 25 °C and a flow rate of 0.6 mL·min^{-1}. The mobile phases A and B were 0.05% formic acid−water and pure acetonitrile, respectively. The gradient eluent profile was: 0−35 min, 3%−41% B; 35−45 min, 41%−100% B; 45−55 min, 100%−100% B. The injection volume of samples was 10 μL. The full-scan range was from 190 to 400 nm, and real-time detection wavelengths were at 254 and 280 nm.

MS^n analysis was implemented on a Finnigan LCQ Deca XP^{plus} ion trap mass spectrometer (Thermo Finnigan, San Jose, CA, USA), combined with electrospray ionization (ESI) interface. The operation parameters were as follows: auxiliary/sweep gas, high-purity nitrogen flow, 20 L·min^{-1}; sheath gas (N_2) flow, 60 L·min^{-1}; collision gas, high-purity helium; ESI spray voltage, ESI^- (−3 kV) and ESI^+ (4 kV); capillary temperature, 350 °C; capillary voltage, ESI^- (−15 V) and ESI^+ (19 V); tube lens offset voltage, ESI^- (−30 V) and ESI^+ (25 V). The collision energy ranged from 30% to 55% for collision-induced dissociation (CID). The scan range was set from *m/z* 100−1500. Each sample was analyzed in positive and negative mode individually.

LC-Q-TOF-MS analysis

The LC-Q-TOF-MS analysis was operated on an Acquity^{TM} ultra performance LC system (Waters Corp., Milford, MA, USA), equipped with ESI source, and combined with a Triple TOF 5600$^+$ mass spectrometer (AB SCIEX, Framingham, MA, USA). The chromatographic conditions were the same as those used in the LC-IT-MS analysis. For Q-TOF-MS analysis, the samples were analyzed in positive and negative modes respectively, ranging from *m/z* 50−1500. The relative mass spectrum parameters for ESI/MS (−/+) were as follows: ion source GS1, 50 psi; ion source GS2, 50 psi; curtion gas (CUR), 30 psi; temperature, 550 °C (−)/600 °C (+); ionspray voltage floating (ISVF), −4500 V/+5500 V; declustering potential (DP), ±100 V; collision energy (CE), ±10 V. For ESI/MS^2 (−/+), the parameter settings were: high sensitivity; declustering potential (DP), ±100 V; collision energy (CE), ±35 V; collision energy spread (CES) at 10.0; ion release delay (IRD) at 67; ion release width (IRW) at 25. The PeakView® software incorporating an elemental composition calculator was used to process the obtained accurate masses.

Results and Discussion

The extract of GZJGGT and its plasma samples were analysed by LC-Q-TOF-MS; the total ion current (TIC) chromatograms of them by LC-Q-TOF-MS are shown in Fig. 1. A total number of 141 compounds were detected in the extract of GZJGGT, and the origins of these compounds were attributed to herbs by comparing the retention time and MS^n data of the extract of GZJGGT with each herb obtained using LC-IT-MS as listed in Table 1. Finally, 77 compounds were identified, including 47 flavonoids primarily from Puerariae Lobatae Radix and Glycyrrhizae Radix et Rhizoma Preparata cum Melle, 13 triterpene saponins belonging to Glycyrrhizae Radix et Rhizoma Preparata cum Melle, seven monoterpene glycosides originating from Paeoniae Radix Alba, three puerosides coming from Puerariae Lobatae Radix, six organic acids existing in multiple herbs, and one other type as displayed in Fig. 2. The main constituents of Guizhi (*e.g.*, aromatic and aliphatic volatile oil with low polarity) and Shengjiang (*e.g.*, terpenes volatile oil, gingerols and diphenyl heptanes) were lost or difficult to be extracted by boiling water. Therefore, only few compounds from Guizhi and Shengjiang were detected in GZJGGT. For Dazao, the main constituents are saccharides, saponins, flavonoids,

organic acids. Due to the poor response in mass analyzer (*e.g.*, saccharides), only nine compounds might come from Dazao in our study. However, some of them lacked of MS information, or their MS spectra or structural information were inconsistent with existing literature reports, and one compound was identified finally.

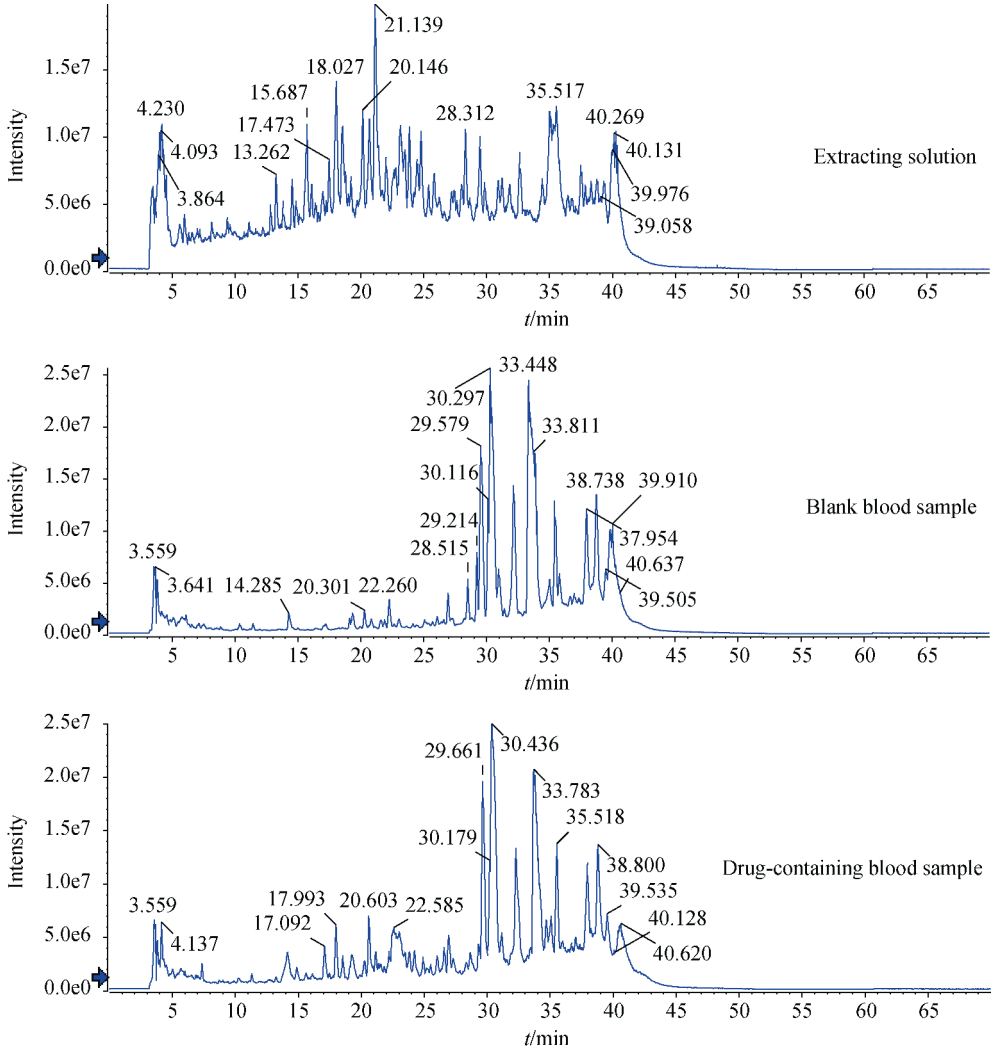


Fig. 1 Total ion chromatograms of GZJGGT extract, blank blood sample and drug-containing (*i.e.*, GZJGGT) blood sample by LC-Q-TOF-MS analysis in negative ion mode

By comparing the retention time and MS data of the extract of GZJGGT with those of plasma samples obtained using LC-Q-TOF-MS, 45 prototype compounds were found to be absorbed into blood circulation and 48 metabolites were speculated on the basis of their similarity of fragmentation pathway with that of prototype.

Identification of compounds from GZJGGT in vitro by LC-Q-TOF-MS and LC-IT-MS

Characterization of flavonoids

A total of 44 flavonoid glycosides were identified or assumed tentatively in our study, except that compounds **115**, **117**, and **121** were aglycones of flavonoids. By comparing with reference standards, compounds **48**, **51**, **67**, **78**, **83**, **88**, **91**, **99**, **104**, and **117** were ascertained to be puerarin, 3'-methoxypuerarin, isoviolanthin, liquiritin apioside, liquiritin, genistoside, 4'-methoxypuerarin, isoliquiritin apioside, ononin, and daidzein. By analyzing their structural features, fragmentation patterns, and typical base peak ions in MS^2 spectra, a diagnostic ion filtering strategy for rapid speculation of flavonoid glycosides by LC-IT-MS was proposed in Fig. 3. It could be summarized as follows and some specific compounds were taken as examples:

i) Flavonoid glycoside with 7-*O*-Glc, its pseudo-molecular ion could be $[M + HCOO]^-$ in MS^1 spectrum, instead of $[M - H]^-$.

The molecular formula of compound **60** was $C_{21}H_{20}O_9$ provided by LC-Q-TOF-MS. It generated adduct ion $[M + HCOO]^-$ at *m/z* 461 and base peak ion at *m/z* 253 $[M - Glc - H]^-$ in MS^2 spectrum, indicating the existence of 7-*O*-Glc. Both daidzin and daidzein-4'-*β*-D-glucopyranoside matched the molecular formula, however, only daidzin had a 7-*O*-Glc. Eventually, compound **60** was supposed to be daidzin.

Table 1 Characterization of compounds in GZJGGT by LC-IT-MS and LC-Q-TOF-MS in negative ion mode

No.	t_R/min	Identification	Formula	Detected *m/z*	Error (ppm)	Source
1	5.19	Unidentified	−	103 $[M-H]^-$	−	G
2	5.27	Unidentified	$C_{18}H_{32}O_{16}$	549.1672 $[M+HCOO]^-$	0.3	PaJ
3	6.28	Unidentified	$C_{18}H_{32}O_{16}$	549.1672 $[M+HCOO]^-$	−0.4	unknown
4	6.28	Unidentified	−	1052	−	J
5	6.44	Unidentified	$C_{25}H_{44}O_{23}$	711.2201 $[M-H]^-$	1.3	PuJG
6	6.80	Citric acid or isocitric acid	$C_6H_8O_7$	191.0197 $[M-H]^-$	3.5	PuPaG
7	7.18	Citric acid or isocitric acid	$C_6H_8O_7$	191.0197 $[M-H]^-$	3.5	CPuPaJZ
8	7.81	Desbenzoylpaeoniflorin	$C_{16}H_{24}O_{10}$	421.1347 $[M+HCOO]^-$	−1.1	Pa
9	8.40	Unidentified	$C_7H_{10}O_7$	205.0354 $[M-H]^-$	3.0	PuG
10	8.49	Unidentified	−	187 $[M-H]^-$	−	unknown
11	8.63	Unidentified	−	289 $[M+HCOO]^-$	−	CPuPaJZ
12	8.74	Unidentified	$C_7H_{10}O_7$	205.0354 $[M-H]^-$	2.6	unknown
13	8.91	Unidentified	−	429 $[M+HCOO]^-$	−	CJ
14	8.94	Unidentified	−	187 $[M-H]^-$	−	unknown
15	9.21	Unidentified	−	117 $[M-H]^-$	−	CPuPaJG
16	9.40	Unidentified	$C_{21}H_{20}O_7$	429.1250 $[M+HCOO]^-$	−1.1	J
17	10.25	Unidentified	$C_{30}H_{28}O_{14}$	611.1406 $[M-H]^-$	−6.1	PuJ
18	10.81	Unidentified	$C_{22}H_{34}O_{14}$	567.1931 $[M+HCOO]^-$	1.0	Pa
19	11.48	Unidentified	−	339 $[M-H]^-$	−	Pa
20	11.64	Unidentified	$C_{13}H_{16}O_9$	315.0720 $[M-H]^-$	−0.5	Pu
21	11.88	Unidentified	$C_{13}H_{16}O_9$	315.0722 $[M-H]^-$	0.1	Pu
22	11.99	1'-*O*-galloylsucrose	$C_{19}H_{26}O_{15}$	493.1199 $[M-H]^-$	−0.4	Pa
23	12.24	Unidentified	−	493$[M-H]^-$	−	Pa
24	12.36	Quinic acid	$C_7H_{12}O_6$	191.0561 $[M-H]^-$	3.1	Pu
25	13.57	6'-*O*-*β*-D-Apiofuranosyldianthoside	$C_{17}H_{24}O_{12}$	465.1250 $[M+HCOO]^-$	0.0	Pu
26	14.42	Unidentified	$C_9H_8O_6$	211.0248 $[M-H]^-$	3.3	J
27	14.70	Protocatechuic acid 3-glucoside	$C_{13}H_{16}O_9$	315.0722 $[M-H]^-$	0.8	Pu
28	15.04	Puerarin-7-*O*-*α*-isomaltoside	$C_{33}H_{40}O_{19}$	739.2091 $[M-H]^-$	1.6	Pu
29	15.34	8-*β*-D-Glucopyranosyl-3-[4-(*β*-D-glucopyranosyloxy)-3-hydroxyphenyl]-7-hydroxy-4*H*-1-benzopyran-4-one	$C_{27}H_{30}O_{15}$	593.1512 $[M-H]^-$	2.0	Pu
30	15.77	Puerarin-4'-*O*-*β*-D-glucopyranoside	$C_{27}H_{30}O_{14}$	577.1563 $[M-H]^-$	2.8	Pu
31	16.30	Mirificin-4'-*O*-glucoside	$C_{32}H_{38}O_{18}$	709.1985 $[M-H]^-$	3.6	Pu
32	16.37	8-*β*-D-Glucopyranosyl-3-[4-(*β*-D-glucopyranosyloxy)-3-methoxyphenyl]-7-hydroxy-4*H*-1-benzopyran-4-one	$C_{28}H_{32}O_{15}$	607.1668 $[M-H]^-$	1.7	Pu
33	16.55	Unidentified	$C_{12}H_{14}O_8$	285.0616 $[M-H]^-$	1.8	Pu
34	17.06	Unidentified	$C_{28}H_{32}O_{16}$	623.1618 $[M-H]^-$	3.0	unknown
35	17.22	Unidentified	$C_7H_{12}O_4$	205.0721 $[M+HCOO]^-$	1.6	Pu
36	17.55	Puerarin-4'-*O*-*β*-D-glucopyranoside isomer	$C_{27}H_{30}O_{14}$	577.1563 $[M-H]^-$	0.9	Pu
37	17.65	Unidentified	$C_{29}H_{34}O_{17}$	653.1723 $[M-H]^-$	1.8	unknown
38	18.20	Unidentified	$C_{33}H_{30}O_{18}$	713.1359 $[M-H]^-$	3.0	Pu
39	18.24	Genistein-6-*C*-glucoside or 3'-hydroxypuerarin	$C_{21}H_{20}O_{10}$	431.0984 $[M-H]^-$	0.8	Pu
40	18.63	Genistein-8-*C*-apiosyl-(1→6)-glucopyranoside or 3'-hydroxypuerarin-2"-*β*-D-xyloside or its isomer	$C_{26}H_{28}O_{14}$	563.1406$[M-H]^-$	1.4	Pu
41	19.07	Unidentified	$C_{34}H_{30}O_{18}$	725.1359 $[M-H]^-$	2.8	Pu
42	19.29	(*p*-Hydroxybenzyl)malonic acid	$C_{10}H_{10}O_5$	209.0455 $[M-H]^-$	5.0	G
43	19.44	Unidentified	$C_{28}H_{34}O_{16}$	625.1774 $[M-H]^-$	−	G

Continued

No.	t_R/min	Identification	Formula	Detected *m/z*	Error (ppm)	Source
44	19.53	Unidentified	−	757 $[M-H]^-$	−	G
45	19.82	6''-*O*-*α*-D-Glucopyranosylpuerarin	$C_{27}H_{30}O_{14}$	577.1563 $[M-H]^-$	1.4	Pu
46	20.03	Mirificin or 7-hydroxy-3-(4-hydroxyphenyl)-8-(6-*O*-*β*-D-xylopyranosyl-*β*-D-glucopyranosyl)-4*H*-1-benzopyran-4-one or its isomer	$C_{26}H_{28}O_{13}$	547.1457 $[M-H]^-$	1.1	Pu
47	20.34	4',7-Dihydroxy-3'-methoxyisoflavone-8-*C*-[*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranoside	$C_{28}H_{32}O_{15}$	607.1668 $[M-H]^-$	1.4	Pu
48*	20.60	Puerarin	$C_{21}H_{20}O_9$	415.1035 $[M-H]^-$	0.1	Pu
49	20.69	Mirificin or 7-hydroxy-3-(4-hydroxyphenyl)-8-(6-*O*-*β*-D-xylopyranosyl-*β*-D-glucopyranosyl)-4*H*-1-benzopyran-4-one or its isomer	$C_{26}H_{28}O_{13}$	547.1457 $[M-H]^-$	0.5	Pu
50	21.03	8-(6-*O*-D-Apio-*β*-D-furanosyl-*β*-D-glucopyranosyl)-5, 7-dihydroxy-3- (4-methoxyphenyl)-4*H*-1-benzopyran-4-one or 7-hydroxy-3-(4-hydroxy-3-methoxyphenyl)-8-(6-*O*-*β*-D-xylopyranosyl-*β*-D-glucopyranosyl)-4*H*-1-benzopyran-4-one	$C_{27}H_{30}O_{14}$	577.1563 $[M-H]^-$	1.6	Pu
51*	21.15	3'-Methoxypuerarin	$C_{22}H_{22}O_{10}$	445.1140 $[M-H]^-$	0.0	Pu
52	21.25	Unidentified	$C_{34}H_{32}O_{17}$	711.1567 $[M-H]^-$	2.6	Pu
53	21.37	Unidentified	$C_{25}H_{40}O_{12}$	577.2502 $[M+HCOO]^-$	0.7	unknown
54	21.77	Unidentified	$C_{35}H_{42}O_{24}$	845.1993 $[M-H]^-$	−3.0	unknown
55	21.90	6'-*O*-*β*-D-Glucopyranosylpaeoniflorin	$C_{29}H_{38}O_{16}$	687.2142 $[M+HCOO]^-$	2.5	Pa
56	22.48	Unidentified	$C_{13}H_{12}O_8$	295.0459 $[M-H]^-$	2.6	Pu
57*	22.84	Albiflorin	$C_{23}H_{28}O_{11}$	525.1614 $[M+HCOO]^-$	1.2	Pa
58	23.12	Genistein-8-*C*-apiosyl-(1→6)-glucopyranoside or 3'-hydroxy-puerarin-2''-*β*-D-xyloside or its isomer	$C_{26}H_{28}O_{14}$	563.1406 $[M-H]^-$	2.3	Pu
59	23.16	Unidentified	$C_{42}H_{38}O_{19}$	845.1935 $[M-H]^-$	1.8	unknown
60	23.35	Daidzin	$C_{21}H_{20}O_9$	461.1089 $[M+HCOO]^-$	1.2	Pu
61	23.40	Unidentified	$C_{34}H_{32}O_{17}$	711.1567 $[M-H]^-$	2.6	Pu
62	23.50	Unidentified	$C_{13}H_{12}O_8$	295.0459 $[M-H]^-$	2.6	Pu
63	23.85	Paeoniflorin	$C_{23}H_{28}O_{11}$	525.1682 $[M+HCOO]^-$	−	Pa
64	23.95	Genistein-8-*C*-apiosyl-(1→6)-glucopyranoside or 3'-hydroxy-puerarin-2''-*β*-D-xyloside or its isomer	$C_{26}H_{28}O_{14}$	563.1406 $[M-H]^-$	1.5	Pu
65	24.09	Unidentified	−	937	−	Pu
66	24.14	3'-Methoxydaidzin or its isomer	$C_{22}H_{22}O_{10}$	491.1195 $[M+HCOO]^-$	1.6	Pu
67*	24.34	Isoviolanthin	$C_{27}H_{30}O_{14}$	577 $[M-H]^-$	−	G
68	24.45	Mirificin or 7-hydroxy-3-(4-hydroxyphenyl)-8-(6-*O*-*β*-D-xylopyranosyl-*β*-D-glucopyranosyl)-4*H*-1-benzopyran-4-one or its isomer	$C_{26}H_{28}O_{13}$	547.1457 $[M-H]^-$	1.6	Pu
69	24.61	Unidentified	$C_{20}H_{18}O_{10}$	417.0827 $[M-H]^-$	1.4	G
70	24.72	Genistein-6-*C*-glucoside or 3'-hydroxypuerarin	$C_{21}H_{20}O_{10}$	431.0984 $[M-H]^-$	1.7	Pu
71	24.78	Pueroside A	$C_{29}H_{34}O_{14}$	605.1876 $[M-H]^-$	2.3	Pu
72	24.88	Unidentified	$C_{30}H_{52}O_{24}$	795.2776 $[M-H]^-$	−3.6	Pu
73	25.16	Mirificin or 7-hydroxy-3-(4-hydroxyphenyl)-8-(6-*O*-*β*-D-xylopyranosyl-*β*-D-glucopyranosyl)-4*H*-1-benzopyran-4-one or its isomer	$C_{26}H_{28}O_{13}$	547.1457 $[M-H]^-$	1.1	Pu
74	25.19	Unidentified	$C_{22}H_{22}O_{11}$	461.1089 $[M-H]^-$	0.6	Pu
75	25.26	Neopuerarin A/B	$C_{21}H_{20}O_9$	415.1035 $[M-H]^-$	2.0	Pu
76	25.67	Unidentified	$C_{30}H_{36}O_{15}$	681.2036 $[M-H]^-$	1.7	Pu
77	25.72	3'-Methoxypuerarin isomer	$C_{22}H_{22}O_{10}$	445.1140 $[M-H]^-$	0.4	Pu
78*	25.95	Liquiritin apioside	$C_{26}H_{30}O_{13}$	549.1614 $[M-H]^-$	1.2	G
79	26.09	Neopuerarin A/B	$C_{21}H_{20}O_9$	415.1035 $[M-H]^-$	1.3	Pu

Continued

No.	t_R/min	Identification	Formula	Detected *m/z*	Error (ppm)	Source
80	26.17	Unidentified	$C_{30}H_{36}O_{15}$	681.2036 $[M-H]^-$	1.7	Pu
81	26.50	3'-Methoxypuerarin isomer	$C_{22}H_{22}O_{10}$	445.1140 $[M-H]^-$	−1.2	Pu
82	26.53	Formononetin-8-*C*-[xylosyl(1→6)]-glucoside or 8-(6-*O*-D-apio-*β*-D-furanosyl-*β*-D-glucopyranosyl)-7-hydroxy-3-(4-methoxyphenyl)-4*H*-1- benzopyran-4-one	$C_{27}H_{30}O_{13}$	561.1614 $[M-H]^-$	1.0	Pu
83*	26.60	Liquiritin	$C_{21}H_{22}O_9$	417.1179 $[M-H]^-$	0.2	G
84	27.08	Unidentified	$C_{23}H_{24}O_{10}$	459.1297 $[M-H]^-$	1.2	unknown
85	27.15	Formononetin-8-*C*-[xylosyl(1→6)]-glucoside or 8-(6-*O*-D-apio-*β*-D-furanosyl-*β*-D-glucopyranosyl)-7-hydroxy-3-(4-methoxyphenyl)-4*H*-1- benzopyran-4-one	$C_{27}H_{30}O_{13}$	561.1614 $[M-H]^-$	2.0	Pu
86	27.32	6'-*O*-Galloyl paeoniflorin	$C_{30}H_{32}O_{15}$	631.1668 $[M-H]^-$	1.7	Pa
87	27.48	Unidentified	$C_{41}H_{48}O_{29}$	1003.2209 $[M-H]^-$	−1.6	Pu
88*	27.58	Genistoside	$C_{21}H_{20}O_{10}$	477.1039 $[M+HCOO]^-$	1.2	Pu
89	27.80	Unidentified	−	1151	−	Pu
90	28.00	Unidentified	$C_{40}H_{78}O_{26}$	973.4709 $[M-H]^-$	−2.0	Pu
91*	28.12	4'-Methoxypuerarin	$C_{22}H_{22}O_9$	429.1191 $[M-H]^-$	1.8	Pu
92	28.34	Unidentified	−	837 $[M-H]^-$	−	G
93	28.75	Puerol B *O*-apiosylglucoside	$C_{29}H_{34}O_{14}$	651.1931 $[M+HCOO]^-$	1.10	Pu
94	29.03	Naringenin-7-*O*-(2-*β*-D-apiofuranosyl)-*β*-D-glucopyranoside	$C_{26}H_{30}O_{14}$	565.1563 $[M-H]^-$	0.9	G
95	29.25	Unidentified	−	1358	−	unknown
96	29.98	Daidzin 6"-*O*-acetate	$C_{23}H_{22}O_{10}$	503.1195 $[M+HCOO]^-$	1.8	Pu
97	30.22	Albiflorin isomer	$C_{23}H_{28}O_{11}$	525.1614 $[M+HCOO]^-$	0.8	Pa
98	30.46	Unidentified	$C_{28}H_{32}O_{15}$	607.1668 $[M-H]^-$	1.7	PuG
99*	30.80	Isoliquiritin apioside	$C_{26}H_{30}O_{13}$	549.1614 $[M-H]^-$	0.8	G
100	31.00	Pueroside C	$C_{24}H_{26}O_{10}$	519.1508 $[M+HCOO]^-$	0.2	Pu
101	31.46	8-(6-*O*-D-Apio-*β*-D-furanosyl-*β*-D-glucopyranosyl)-5, 7-dihydroxy-3-(4-methoxyphenyl)-4*H*-1-benzopyran-4-one or 7-hydroxy-3-(4-hydroxy-3-methoxyphenyl)-8-(6-*O*-*β*-D-xylopyranosyl-*β*-D-glucopyranosyl)-4*H*-1-benzopyran-4-one	$C_{27}H_{30}O_{14}$	577.1563 $[M-H]^-$	1.9	Pu
102	31.95	Kudzu saponin SA_4	$C_{47}H_{74}O_{20}$	957.4701 $[M-H]^-$	2.3	Pu
103	32.30	Unidentified	−	905	−	PuG
104*	32.30	Ononin	$C_{22}H_{22}O_9$	475.1246 $[M+HCOO]^-$	0.5	PuG
105	32.66	Licorice glycoside $A/C_1/C_2$	$C_{36}H_{38}O_{16}$	725.2087 $[M-H]^-$	1.9	G
106	32.66	Licorice glycoside $B/D_1/D_2$	$C_{35}H_{36}O_{15}$	695.1981 $[M-H]^-$	1.1	G
107	32.89	Unidentified	$C_{12}H_{12}O_6$	251.0561 $[M-H]^-$	4.3	Pu
108	33.38	24-Hydroxy-licorice saponin A_3	$C_{48}H_{72}O_{22}$	999.4442 $[M-H]^-$	3.2	G
109	33.49	Unidentified	$C_{48}H_{76}O_{20}$	971.4857 $[M-H]^-$	1.8	Pu
110	33.64	Unidentified	$C_{42}H_{64}O_{18}$	855.4020 $[M-H]^-$	1.5	unknown
111	33.75	Unidentified	$C_{17}H_{22}O_{10}$	385.1140 $[M-H]^-$	2.0	Pu
112	33.87	Unidentified	−	1034	−	Pu
113	34.64	Unidentified	$C_{27}H_{30}O_{14}$	623.1618 $[M-H]^-$	1.0	Pu
114	34.91	Licorice glycoside E	$C_{35}H_{35}NO_{14}$	692.1985 $[M-H]^-$	1.9	G
115	35.09	Licochalcone B	$C_{16}H_{14}O_5$	285.0768 $[M-H]^-$	2.6	G
116	35.21	Licorice saponin A_3	$C_{48}H_{72}O_{21}$	983.4493 $[M-H]^-$	2.7	G
117*	35.47	Daidzein	$C_{15}H_{10}O_4$	253.0051 $[M-H]^-$	3.8	Pu
118	35.66	Unidentified	−	1119	−	Pu
119	35.76	Licorice glycoside $A/C_1/C_2$	$C_{36}H_{38}O_{16}$	725.2087 $[M-H]^-$	1.8	G

Continued

No.	t_R/min	Identification	Formula	Detected *m/z*	Error (ppm)	Source
120	35.84	Licorice glycoside B/D_1/D_2	$C_{35}H_{36}O_{15}$	695.1981 $[M-H]^-$	1.8	G
121	36.19	3'-Methoxydaidzein	$C_{16}H_{12}O_5$	283.0612 $[M-H]^-$	2.5	Pu
122	36.82	Unidentified	$C_{48}H_{74}O_{21}$	985.4650 $[M-H]^-$	1.9	unknown
123	37.06	Yunganoside K_1	$C_{48}H_{72}O_{21}$	983.4493 $[M-H]^-$	2.6	G
124	37.19	3'-Methoxydaidzin or its isomer	$C_{22}H_{22}O_{10}$	491.1195 $[M+HCOO]^-$	0.2	Pu
125	37.65	Yunganoside K_2 or its isomer	$C_{42}H_{62}O_{17}$	837.3914 $[M-H]^-$	3.1	G
126	38.32	Benzoylpaeoniflorin	$C_{30}H_{32}O_{12}$	629.1876 $[M+HCOO]^-$	0.8	Pa
127	38.51	Unidentified	−	1003	−	Pu
128	38.98	Yunganoside K_2 or its isomer	$C_{42}H_{62}O_{17}$	837.3914 $[M-H]^-$	3.2	G
129	39.91	Unidentified	−	973 $[M-H]^-$	−	Pu
130	40.13	Yunganoside K_2 or its isomer	$C_{42}H_{62}O_{17}$	837.3914 $[M-H]^-$	2.7	G
131	40.47	Yunganoside L_1	$C_{48}H_{72}O_{20}$	967.4544 $[M-H]^-$	2.5	G
132	40.86	Yunganoside G_1	$C_{48}H_{74}O_{21}$	985.4650 $[M-H]^-$	2.6	G
133	41.27	Yunganoside K_2 or its isomer	$C_{42}H_{62}O_{17}$	837.3914 $[M-H]^-$	2.6	G
134	41.52	Yunganoside K_2 or its isomer	$C_{42}H_{62}O_{17}$	837.3914 $[M-H]^-$	3.9	G
135*	41.63	Glycyrrhizin	$C_{42}H_{62}O_{16}$	821.3965 $[M-H]^-$	3.0	G
136	41.70	Unidentified	−	1233.42	−	G
137	41.95	Unidentified	$C_{36}H_{74}O_{29}$	969.4242 $[M-H]^-$	4.3	G
138	42.15	Unidentified	−	1001	−	PuG
139	42.26	Unidentified	−	987	−	Pu
140	42.36	Unidentified	−	1233	−	G
141	42.65	Licorice saponin H_2	$C_{42}H_{62}O_{16}$	821.3965 $[M-H]^-$	3.2	G

The data in MS^1 containing decimal point derived from LC-Q-TOF-MS, while the data containing no decimal point derived from LC-IT-MS, which could not be detected or provided appropriate molecular formula in Q-TOF-MS. It was important to point out that the retention time here originated from LC-IT-MS. C = Cinnamomi Ramulus, Pu = Puerariae Lobatae Radix, Pa = Paeoniae Radix Alba, J = Jujubae Fructus, Z = Zingiberis Rhizoma Recens, G = Glycyrrhizae Radix et Rhizoma Preparata. *: Identified with authentic compounds. −: No response under present conditions or this part of data was unprovided.

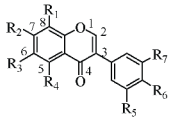
ii) Flavonoid glycosides without *C*-glucosides were inclined to lose the whole sugar moiety to generate base peak ions in MS^2 spectra, such as $[M-162-H]^-$ and $[M-162-132\text{ (pentosyl)}-H]^-$. Unlike flavonoid *O*-glucosides, flavonoid *C*-glucosides exhibited representative fragmentation behaviors in losing glycosyl segments [16-17]. In our research, flavonoid *C*-glucosides with one or two sugar units, tended to yield base peak ions, *e.g.*, $[M-120-H]^-$, $[M-120-132\text{ (pentosyl)}-H]^-$, and $[M-120-162\text{ (hexosyl)}-H]^-$ in MS^2 spectra.

Compound **45** had a molecular formula of $C_{27}H_{30}O_{14}$. It exhibited deprotonated molecule at *m/z* 577 in its MS^1 spectrum, as well as base peak ion at *m/z* 295 $[M-120-162-H]^-$ in its MS^2 spectrum. According to the strategy in Fig. 3, compound **45** contained *C*-Glc-hexosyl, and was proposed to be 6''-*O*-*α*-D-glucopyranosylpuerarin. Other fragment ions at *m/z* 397 $[M-162-H_2O-H]^-$ and *m/z* 267 $[M-120-162-CO-H]^-$ confirmed the above speculation.

iii) Flavonoid glycosides with *C*-glycosides and *O*-glycosides synchronously more readily lost a glycosyl segment of *C*-glycosides to yield a base peak ion in the MS^2 spectra, rather than getting rid of glycosyl at *O*-glycosides.

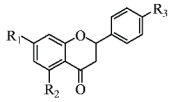
Compounds **30** and **36** were isomers sharing an identical molecular formula: $C_{27}H_{30}O_{14}$. By searching in the literature [18], only puerarin-4'-*O*-*β*-D-glucopyranoside isolated from Puerariae Lobatae Radix matched that molecular formula. On basis of the foregoing characteristic ion filtering strategy, compound **30** was classified into the type that containing *C*-Glc, with deprotonated molecule $[M-H]^-$ at *m/z* 577 and base peak ion $[M-120-H]^-$ at *m/z* 457 in the MS^2 spectrum. Besides, fragment ions at *m/z* 429 $[M-120-CO-H]^-$ (base peak ion), *m/z* 295 $[M-120-162-H]^-$, and *m/z* 267 $[M-120-162-CO-H]^-$ in MS^3 spectrum indicated the existence of *O*-Glc. Hence, compound **30** was supposed to be puerarin-4'-*O*-*β*-D-glucopyranoside. In contrast, compound **36** contained 7-*O*-Glc, since it gave adduct ion $[M+HCOO]^-$ at *m/z* 623 in the MS^1 spectrum and base peak ion $[M-Glc-H]^-$ at *m/z* 415 in the MS^2 spectrum; however, there was no compound isolated from Puerariae Lobatae Radix matching these characteristics, it was presumed to be puerarin-4'-*O*-*β*-D-glucopyranoside isomer.

Isoflavones



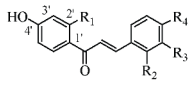
	R_1	R_2	R_3	R_4	R_5	R_6	R_7
28 Puerarin-7-*O*-*α*-isomaltoside	Glc	OGlc(6,1)-Glc	H	H	H	OH	H
29 8-*β*-D-Glucopyranosyl-3-[4-(*β*-D-glucopyranosyloxy)-3-hydroxyphenyl]-7-hydroxy-4*H*-1-benzopyran-4-one	Glc	OH	H	H	H	OGlc	OH
30 Puerarin-4'-*O*-*β*-D-glucopyranoside	Glc	OH	H	H	H	OGlc	H
31 Mirificin-4'-*O*-glucoside	Glc(6,1)-Api	OH	H	H	H	OGlc	H
32 8-*β*-D-Glucopyranosyl-3-[4-(*β*-D-glucopyranosyloxy)-3-methoxyphenyl]-7-hydroxy-4*H*-1-benzopyran-4-one	Glc	OH	H	H	H	OGlc	OCH_3
39/70 Genistein-6-*C*-glucoside	H	OH	Glc	OH	H	OH	H
or 3'-hydroxypuerarin	Glc	OH	H	H	H	OH	OH
40/58/64 Genistein-8-*C*-apiosyl-(1→6)-glucopyranoside	Glc(6,1)-Api	OH	H	OH	H	OH	H
or 3'-hydroxypuerarin-2''-β-D-xyloside	Glc(2,1)-Ara	OH	H	H	H	OH	OH
45 6''-*O*-*α*-D-Glucopyranosylpuerarin	Glc(6,1)-Glc	OH	H	H	H	OH	H
46/49/68/73 Mirificin	Glc(6,1)-Api	OH	H	H	H	OH	H
or 7-hydroxy-3-(4-hydroxyphenyl)-8-(6-*O*-*β*-D-xylopyranosyl-*β*-D-glucopyranosyl)-4*H*-1-benzopyran-4-one	Glc(6,1)-Ara	OH	H	H	H	OH	H
47 4',7-Dihydroxy-3'-methoxyisoflavone-8-*C*-[*β*-D-glucopyranosyl-(1→66)]-*β*-D-glucopyranoside	Glc(6,1)-Glc	OH	H	H	H	OH	OCH_3
48 Puerarin	Glc	OH	H	H	H	OH	H
50/101 8-(6-*O*-D-Apio-*β*-D-furanosyl-*β*-D-glucopyranosyl)-5,7-dihydroxy-3-(4-methoxyphenyl)-4*H*-1-benzopyran-4-one	Glc(6,1)-Api	OH	H	OH	H	OCH_3	H
or 7-hydroxy-3-(4-hydroxy-3-methoxyphenyl)-8-(6-*O*-*β*-D-xylopyranosyl-*β*-D-glucopyranosyl)-4*H*-1-benzopyran-4-one	Glc(6,1)-Ara	OH	H	H	H	OH	OCH_3
51 3'-Methoxypuerarin	Glc	OH	H	H	H	OH	OCH_3
60 Daidzin	H	OGlc	H	H	H	OH	H
66/124 3'-Methoxydaidzin	H	OGlc	H	H	H	OH	OCH_3
75/79 Neopuerarin A/B	Glucofuranosyl	OH	H	H	H	OH	H
82/85 Formononetin-8-*C*-[xylosyl(1→6)]-glucoside	Glc(6,1)-Ara	OH	H	H	H	OCH3	H
or 8-(6-*O*-D-apio-*β*-D-furanosyl-*β*-D-glucopyranosyl)-7-hydroxy-3-(4-methoxyphenyl)-4*H*-1-benzopyran-4-one	Glc(6,1)-Api	OH	H	H	H	OCH3	H
88 Genistoside	H	OGlc	H	OH	H	OH	H
91 4'-Methoxypuerarin	Glc	OH	H	H	H	OCH_3	H
96 Daidzin 6''-*O*-acetate	H	6-acetyl-OGlc	H	H	H	OH	H
104 Ononin	H	OGlc	H	H	H	OCH_3	H
117 Daidzein	H	OH	H	H	H	OH	H
121 3'-Methoxydaidzein	H	OH	H	H	H	OH	OCH_3

Flavanones



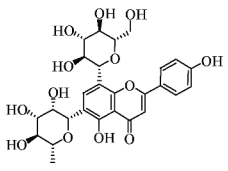
	R_1	R_2	R_3
78 Liquiritin apioside	OH	H	OGlc(2,1)-Api
83 Liquiritin	OH	H	OGlc
94 Naringenin-7-*O*-(2-*β*-OGlc(2,1)-Api D-apiofuranosyl)-*β*-D-glucopyranoside	OH	OH	
105/119 Licorice glycoside C_1/C_2	OH	H	OGlc(2,1)-feruloylapioside
106/120 Licorice glycoside D_1/D_2	OH	H	OGlc(2,1)-coumaroylapioside
114 Licorice glycoside E	OH	H	OGlc(2,1)-1*H*-indol-3-ylcarbonylapioside

Chalcones



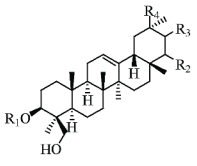
	R_1	R_2	R_3	R_4
99 Isoliquiritin apioside	OH	H	H	OGlc(2,1)-Api
105/119 Licorice glycoside A	OH	H	H	OGlc(2,1)-feruloylapioside
106/120 Licorice glycoside B	OH	H	H	OGlc(2,1)-coumaroylapioside
115 Licochalcone B	H	OCH_3	OH	OH

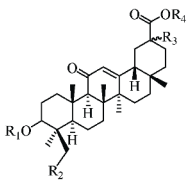
Flavones

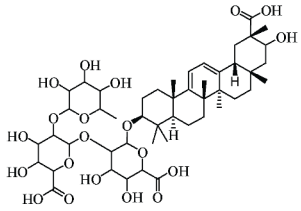


67 Isoviolanthin

Triterpene saponins





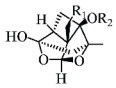


131 Yunganoside L_1

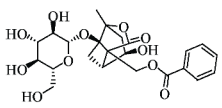
	R_1	R_2	R_3	R_4
102 Kudzu saponin SA_4	GluA(2,1)-GluA	Ara	OH	CH_3
123 Yunganoside K_1	Rha(2,1)-GluA(2,1)-GluA	oxo	H	COOH
125/128/130/133/134 Yunganoside K_2	GluA(2,1)-GluA	oxo	H	COOH
132 Yunganoside G_1	GluA(2,1)-GluA(2,1)-Rha	H	OH	COOH

	R_1	R_2	R_3	R_4
108 24-Hydroxy-licorice-saponin A_3	GluA(2,1)-GluA	OH	*β*-CH_3	Glc
116 Licorice saponin A_3	GluA(2,1)-GluA	H	*β*-CH_3	Glc
135 Glycyrrhizin	GluA(2,1)-GluA	H	*α*-CH_3	H
141 Licorice saponin H_2	GluA(2,1)-GluA	H	*β*-CH_3	H

Monoterpene glycosides

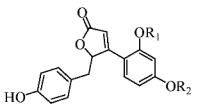


	R_1	R_2
8 Desbenzoylpaeoniflorin	OH	Glc
55 6'-*O*-*β*-D-Glucopyranosylpaeoniflorin	Benzoyl	Glc(6,1)-Glc
63 Paeoniflorin	Benzoyl	Glc
86 6'-*O*-Galloyl paeoniflorin	Benzoyl	Galloylglucoside
126 Benzoylpaeoniflorin	Benzoyl	Benzoylglucoside



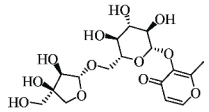
57 Albiflorin

Puerosides



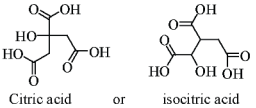
	R_1	R_2
71 Pueroside A	Glc(6,1)-Rha	H
93 Puerol B *O*-apiosylglucoside	Glc-Api	CH_3
100 Pueroside C	Glc	CH_3

Others

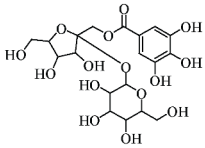


25 6'-*O*-*β*-D-Apiofuranosyl-dianthoside

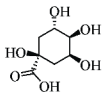
Organic acid



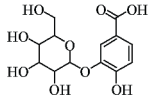
6/7 Citric acid or isocitric acid

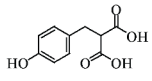


22 1'-*O*-galloylsucrose



24 Quinic acid





42 (*p*-Hydroxybenzyl)malonic acid

27 Protocatechuic acid 3-glucoside

Fig. 2 Chemical structures of compounds identified in GZJGGT *in vitro*

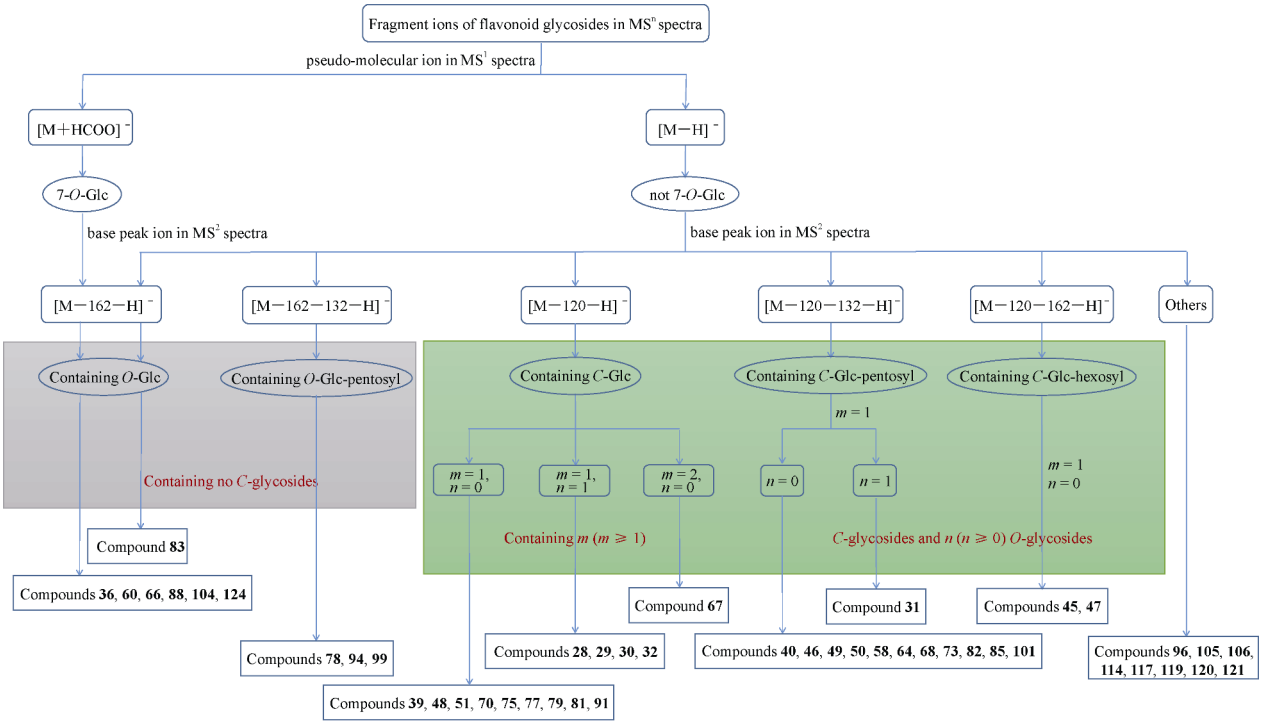


Fig. 3 A diagnostic base peak ion filtering strategy for rapid structural classification of flavonoid *O*-glycosides and *C*-glycosides by LC-IT-MS in negative ion mode

iv) Chalcones with *C*-2'-OH in its skeleton could convert to corresponding flavanone isomers by the way of RDA reaction, and then produced almost the same fragment ions as the latter [19], therefore, they could not be distinguished *via* MS spectral data alone.

Take compounds **105** and **119** with different retention times (t_R) as examples. They shared the same deprotonated molecule [M – H]$^-$ at *m/z* 725 and molecular formula of $C_{36}H_{38}O_{16}$. Besides, they generated similar fragment ions, such as *m/z* 549 [M – feruloyl – H]$^-$, *m/z* 531 [M – feruloyl – H_2O – H]$^-$, *m/z* 399 [M – feruloyl – Api – H]$^-$, *m/z* 255 [M – feruloyl – Api-Glc – H]$^-$ in MS^2 spectra. Licorice glycoside C_1/C_2 and licorice glycoside A matched the aforementioned features, and they were flavanone and chalcone severally. Although their base peak ions (*m/z* 549 and 531, respectively) were different in their MS^2 spectra, it was difficult to distinguish them in MS^3 and MS^4 spectra. As a result, compounds **105** and **119** were assumed to be licorice glycoside C_1/C_2 or licorice glycoside A. Likewise, compounds **106** and **120** were presumed as licorice glycoside B or licorice glycoside D_1/D_2.

Characterization of triterpene saponins

A total of 13 triterpene saponins were deduced in GZJGGT, all belonging to oleanane-type triterpene saponins. In negative ion mode, most of these saponins tended to lose the aglycon to generate base peak ions in MS^2 spectra. Besides, the sugar moiety of these saponins would lose 44 Da (CO_2) and 18 Da (H_2O) simultaneously in the MS^3 spectra. These phenomena were consistent with literature report [16]. In contrast with the reference standard, compound **135** was authenticated as glycyrrhizin. According to the fragmentation regularity of base peak ions in negative ion mode, they were categorized into three groups:

Group I yielded a base peak ion at *m/z* 351 [GluA-GluA – H]$^-$ in MS^2 spectra, which indicated the existence of a GluA-GluA unit at *C*-3 of A ring, *e.g.*, compounds **102**, **125**, **128**, **130**, **133**, **134**, **135** and **141**; group II produced base peak ion at *m/z* 497 [Rha-GluA-GluA – H]$^-$ in MS^2 spectra, which resulted from a Rha-GluA-GluA unit attaching to *C*-3 of A ring, compounds **123**, **131**, and **132** for instance; group Ⅲ generated base peak ion [M – Glc – H]$^-$ in MS^2 spectra with a glucosyl linking to E ring, as well as gave base peak ion at *m/z* 351 [GluA-GluA – H]$^-$ with a GluA-GluA unit at *C*-3 of A ring in MS^3 spectra, such as compounds **108** and **116**.

Take compound **102** for example. It belonged to group I with a base peak ion at *m/z* 351 [GluA-GluA – H]$^-$. LC-Q-TOF-MS analysis gave its molecular formula as $C_{47}H_{74}O_{20}$, and the deprotonated molecule [M – H]$^-$ of compound **102** was at *m/z* 957. It produced fragment ions at *m/z* 939 [M – H_2O – H]$^-$, *m/z* 895 [M – H_2O – CO_2 – H]$^-$, *m/z* 781 [M – GluA – H]$^-$, *m/z* 289 [GluA-GluA – H_2O – CO_2 – H]$^-$ in MS^2 spectrum, and *m/z* 193 [GluA + H_2O – H]$^-$, *m/z* 157 [GluA – H_2O – H]$^-$ in MS^3 spectrum. Kudzu saponin SA_4 [20], the major constituent of Puerariae Lobatae Radix, could match the above features. Thus, compound **102** was proposed to be kudzu saponin SA_4.

Compound **131** was classified as group II, with its base peak ion at *m/z* 497 [Rha-GluA-GluA – H]$^-$. It generated [M – H]$^-$ at *m/z* 967 and had molecular formula $C_{48}H_{72}O_{20}$. More-

over, it produced fragment ion at *m/z* 833 [M – $C_4H_6O_5$ – H]$^-$ by losing a partial glycosyl segment, and then produced a fragment ion at *m/z* 645 [M – $C_4H_6O_5$ – $C_8H_{12}O_5$ – H]$^-$ with very low abundance. Other fragment ions in the MS^2 spectrum included *m/z* 949 [M – H_2O – H]$^-$, *m/z* 905 [M – H_2O – CO_2 – H]$^-$, *m/z* 479 [Rha-GluA-GluA – H_2O – H]$^-$, *m/z* 435 [Rha-GluA-GluA – H_2O – CO_2 – H]$^-$, *m/z* 339 [Rha-GluA + H_2O – H]$^-$, and *m/z* 321 [Rha-GluA – H]$^-$. By exploring another literature [21], both yunganoside L_1 and yunganoside J_1 matched the molecular formula $C_{48}H_{72}O_{20}$, however, yunganoside J_1 could scarcely yield fragment ion at *m/z* 645. Hence, compound **131** was extrapolated to be yunganoside L_1.

Compound **108** had molecular formula $C_{48}H_{72}O_{22}$, as well as base peak ions at *m/z* 837 [M – Glc – H]$^-$ in its MS^2 spectrum and *m/z* 351 [GluA-GluA – H]$^-$ in the MS^3 spectrum, which was in accordance with group III. Besides, it generated fragment ions at *m/z* 879 [M – $C_4H_8O_4$ – H]$^-$ by partly losing glucose, *m/z* 819 [M – Glc – H_2O – H]$^-$, *m/z* 775 [M – Glc – H_2O – CO_2 – H]$^-$, and *m/z* 661 [M – Glc – GluA – H]$^-$ in MS^2 or MS^3 spectra. Therefore, it was supposed as 24-hydroxylicorice-saponin A_3.

Characterization of other types of compounds

Seven monoterpene glycosides were characterized in our study, among which compound **57** was identified as albiflorin by comparison with an authentic standard. All these compounds gave adduct ions of [M + HCOO]$^-$ in MS^1 spectra, instead of deprotonated molecule [M – H]$^-$, *e.g.*, compound **55**. It had molecular formula $C_{29}H_{38}O_{16}$ and generated an adduct ion at *m/z* 687 [M + HCOO]$^-$, along with fragment ions at *m/z* 641 [M – H]$^-$, *m/z* 623 [M – H_2O – H]$^-$, *m/z* 611 [M – CH_2O – H]$^-$, *m/z* 593 [M – CH_2O – H_2O – H]$^-$, *m/z* 489 [M – CH_2O – benzoyl – H]$^-$, and *m/z* 445 [M – $C_6H_{10}O_6$ – H_2O – H]$^-$ in the MS^2 spectrum. Finally, compound **55** was deduced to be 6'-*O*-*β*-D-glucopyranosylpaeoniflorin.

Only three compounds (compounds **71**, **93**, and **100**) belonged to puerosides. They were the representative ingredients of Puerariae Lobatae Radix. Among which, compound **71** gave a deprotonated molecule at *m/z* 605 [M – H]$^-$ and molecular formula $C_{29}H_{34}O_{14}$. It yielded base peak ion at *m/z* 297 [M – Glc-Rha – H]$^-$, as well as a fragment ion at *m/z* 253 [M – Glc-Rha – CO_2 – H]$^-$ in the MS^2 spectrum. Eventually, compound **71** was assigned as pueroside A.

Identification of prototypes and metabolites in rat plasma samples after oral administration of GZJGGT

It is important to investigate the prototypes and metabolites of GZJGGT *in vivo* to find potential effective compounds. Rats are most commonly used as the animal model to evaluate pharmaceutical effect because they are similar to human in many ways and are easily obtained. The preparation of the extract administrated to rats was in accordance with Shang-Han-Lun, *i.e.*, the herbs were extracted with water under refluxing. To increase the concentration of constituents absorbed into blood, rats were administered with extract by gavage at six-fold human equivalent dosage; hepatotoxicity and renal toxicity were not observed by biochemical indicator detection in rats at that dosage.

Identification of prototype compounds in rat plasma

Drug-containing plasma samples at different sampling times (0.25, 0.5, 1.0, 1.5 and 2.0 h) were analyzed by LC-Q-TOF-MS to observe the peak numbers and their response in TIC chromatograms. It was found that there were more peaks in 0.25 and 0.5 h blood samples, and some peaks were different. Thus, blood samples at 0.25 and 0.5 h were collected and mixed for LC-MS analysis to detect as many prototypes and metabolites as possible.

A total of 45 prototype compounds were presumed, including 28 flavonoid glycosides, 10 triterpene saponins, two monoterpene glycosides, two puerosides, two organic acids, and one other type (Table 2).

Isoflavonoids were regarded as mainly active constituents of Puerariae Lobatae Radix. Take compound **p-85** for example, it generated deprotonated molecule [M − H]$^-$ at *m/z* 561, along with characteristic fragment ions at *m/z* 339 [M – Glc – 60 − H]$^-$, *m/z* 309 [M – Glc – 90 − H]$^-$, *m/z* 281 [M – Glc – 90 – CO − H]$^-$, and *m/z* 266 [M – Glc – 90 – CO – ·CH_3 − H]$^{\cdot-}$ by elimination of glycosyl segments, which was in keeping with compound **85** *in vitro*. As a result, compound **p-85** was regarded as prototype of compound **85**, *i.e.*, formononetin-8-*C*-[xylosyl(1→6)]-glucoside or 8-(6-*O*-D-apio-*β*-D-furanosyl-*β*-D-glucopyranosyl)-7-hydroxy-3-(4-methoxyphenyl)-4*H*-1- benzopyran-4-one.

Similarly, compound **p-30** shared analogical elution time and identical ions in MS^2 spectra with compound **30** *in vitro*, such as *m/z* 577 [M − H]$^-$, *m/z* 457 [M – 120 − H]$^-$, *m/z* 429 [M – 120 − CO − H]$^-$, *m/z* 294 [M – 120 – ·$C_6H_{11}O_5$ − H]$^{\cdot-}$ and *m/z* 266 [M – 120 – ·$C_6H_{11}O_5$ − CO − H]$^{\cdot-}$. For this reason, compound **p-30** was deemed to be puerarin-4'-*O*-D- glucopyranoside, *i.e.*, the prototype of compound **30**.

Triterpene saponins were another major group of bioactive components in GZJGGT, and the majority of them were absorbed into blood. For example, compound **p-135** gave molecular formula $C_{42}H_{62}O_{16}$ and deprotonated molecule at *m/z* 821 [M − H]$^-$, along with distinctive fragment ions at *m/z* 351 [GluA-GluA – H]$^-$, and *m/z* 193 [GluA + H_2O − H]$^-$ in MS^2 spectrum. Besides, the retention time of compound **p-135** was similar to that of compound **135**, which was validated by authentic substance *in vitro*. Therefore, compound **p-135** was considered as prototype of glycyrrhizin.

Only two monoterpene glycosides (compounds **p-8** and **p-63**) were found to be absorbed into blood circulation. Take compound **p-63** for instance. It had the same retention time with compound **63** *in vitro*, as well as adduct ion [M + HCOO]$^-$ at *m/z* 525, along with a base peak ion at *m/z* 449 [M − CH_2O − H]$^-$ in MS^2 spectrum, and typical fragment ions at *m/z* 327 [M − CH_2O − benzoyl − H]$^-$, *m/z* 165 [M − benzoyl − 2CH_2O − $C_5H_8O_4$ − H]$^-$ in the MS^n spectra. Finally, compound **p-63** was inferred as paeoniflorin, prototype of compound **63**.

Table 2 Characterization of prototype compounds of GZJGGT in rat plasma by LC-Q-TOF-MS in negative ion mode

No.	t_R/min	UPLC-MS^1(*m/z*)	Prototype compounds	Fragment ions in MS^2 spectra
p-8	6.54	421.1345 [M + HCOO]$^-$	Desbenzoylpaeoniflorin	89.0271, 123.0447, 165.0541, 345.1174
p-25	11.10	465.1245 [M + HCOO]$^-$	6'-*O*-*β*-D-Apiofuranosyldianthoside	71.0147, 89.0248, 125.0271, 149.0432, 233.0649, 293.0788
p-27	12.20	315.0725 [M − H]$^-$	Protocatechuic acid 3-glucoside	109.0301, 153.0182, 315.0733
p-28	12.50	739.2103 [M − H]$^-$	Puerarin 7-*O*-*α*-isomaltoside	266.0576, 295.0577, 415.1092, 619.1891, 739.2234
p-29	12.83	593.1520 [M − H]$^-$	8-*β*-D-Glucopyranosyl-3-[4-(*β*-D-glucopyranosyloxy)-3-hydroxyphenyl]-7-hydroxy-4*H*-1-benzopyran-4-one	282.0543, 310.0493, 430.0938, 473.1135, 593.1585
p-30	13.27	577.1569 [M − H]$^-$	Puerarin-4'-*O*-D-glucopyranoside	266.0594, 294.0544, 429.1221, 457.1176, 577.1614
p-31	13.80	709.2006 [M − H]$^-$	Mirificin-4'-*O*-glucoside	266.0592, 294.0563, 429.1219, 457.1182, 709.2109
p-32	13.85	607.168 [M − H]$^-$	8-*β*-D-Glucopyranosyl-3-[4-(*β*-D-glucopyranosyloxy)-3-methoxyphenyl]-7-hydroxy-4*H*-1-benzopyran-4-one	295.0654, 309.0432, 324.0647, 487.1282, 607.1708
p-36	15.26	577.1567 [M − H]$^-$	Puerarin-4'-*O*-D-glucopyranoside isomer	267.0666, 295.0620, 577.1649
p-40	16.07	563.1414 [M − H]$^-$	Genistein-8-*C*-apiosyl-(1→6)-glucopyranoside or 3'-hydroxypuerarin-2"-*β*-D-xyloside or its isomer	227.0726, 255.0679, 283.0626, 311.0565, 323.0573, 341.0713, 563.1399
p-42	16.65	209.0460 [M − H]$^-$	(*p*-Hydroxybenzyl)malonic acid	59.0215, 93.0398, 119.0546, 121.0618, 165.0552
p-45	17.24	577.1568 [M − H]$^-$	6"-*O*-*α*-D-Glucopyranosylpuerarin	253.0504, 295.0639, 307.0620, 577.1602
p-48	17.98	415.1035 [M − H]$^-$	Puerarin	223.0747, 253.0511, 267.0660, 277.0509, 295.0609, 415.1035
p-49	18.07	547.1462 [M − H]$^-$	Mirificin or 7-hydroxy-3-(4-hydroxyphenyl)-8-(6-*O*-*β*-D-xylopyranosyl-*β*-D-glucopyranosyl)-4*H*-1-benzo- pyran-4-one or its isomer	267.0654, 277.0511, 295.0611, 325.0730, 547.1500
p-50	18.48	577.1569 [M − H]$^-$	8-(6-*O*-D-Apio-*β*-D-furanosyl-*β*-D-glucopyranosyl)-5, 7-dihydroxy-3-(4-methoxyphenyl)-4*H*-1-benzopyran-4- one or 7-hydroxy-3-(4-hydroxy-3-methoxyphenyl)-8-(6-*O*-*β*-D-xylopyranosyl-*β*-D-glucopyranosyl)-4*H*-1-benzopyran-4-one	282.0546, 297.0775, 325.0733, 355.0880, 577.1613
p-51	18.55	445.1140 [M − H]$^-$	3'-Methoxypuerarin	225.0560, 253.0510, 282.0535, 297.0775, 310.0494, 325.0724, 445.1160
p-60	20.69	461.1092 [M + HCOO]$^-$	Daidzin	195.0443, 208.0501, 223.0452, 239.0300, 253.0513, 415.1045
p-63	21.12	525.1618 [M + HCOO]$^-$	Paeoniflorin	77.0433, 121.0307, 165.0559, 327.1090, 449.1456
p-64	21.26	563.1406 [M − H]$^-$	Genistein-8-*C*-apiosyl-(1→6)-glucopyranoside or 3'- hydroxypuerarin-2"-*β*-D-xyloside	283.0629, 311.0568, 341.0687, 563.1468
p-66	21.41	491.1197 [M + HCOO]$^-$	3'-Methoxydaidzin or its isomer	211.0425, 268.0392, 271.0957, 283.0612, 430.0957, 445.1168
p-68	22.49	547.1464 [M − H]$^-$	Mirificin or 7-hydroxy-3-(4-hydroxyphenyl)-8-(6-*O*-*β*-D-xylopyranosyl-*β*-D-glucopyranosyl)-4*H*-1-benzo-pyran-4-one or its isomer	253.0588, 267.0688, 295.0625, 547.1629
p-71	22.05	605.1886 [M − H]$^-$	Pueroside A	253.0871, 297.0785, 605.1924
p-78	23.15	549.1618 [M − H]$^-$	Liquiritin apioside	135.0093, 255.0670, 549.1672
p-79	22.76	415.1038 [M − H]$^-$	Neopuerarin A/B	223.0411, 253.0517, 415.1054
p-81	23.01	445.1142 [M − H]$^-$	3'-Methoxypuerarin isomer	166.0043, 211.0379, 240.0401, 252.0433, 283.0620, 325.0771, 365.0336, 445.1964
p-83	23.87	417.1192 [M − H]$^-$	Liquiritin	119.0507, 135.0091, 255.0663
p-85	24.44	561.1620 [M − H]$^-$	Formononetin-8-*C*-[xylosyl(1→6)]-glucoside or 8-(6-*O*-D-apio-*β*-D-furanosyl-*β*-D-glucopyranosyl)-7-hydroxy-3-(4-methoxyphenyl)-4*H*-1-benzopyran-4-one	266.0589, 281.0832, 309.0789, 339.0904, 561.1687
p-91	25.39	429.1196 [M − H]$^-$	4'-Methoxypuerarin	237.0566, 266.0572, 276.0432, 281.0829, 309.0778, 429.1184
p-94	26.22	565.1568 [M − H]$^-$	Naringenin-7-*O*-(2-*β*-D-apiofuranosyl)-*β*-D-gluco-pyranoside	113.0268, 150.0317, 165.0571, 195.0649, 227.0734, 271.0616, 389.1637, 565.1674
p-99	27.99	549.1618 [M − H]$^-$	Isoliquiritin apioside	119.0505, 135.0087, 255.0659, 297.0773, 549.1660
p-100	28.31	519.1507 [M + HCOO]$^-$	Pueroside C	252.0801, 267.1049, 311.0937

Continued

NO.	t_R/min	UPLC-MS^1(*m/z*)	Prototype compounds	Fragment ions in MS^2 spectra
p-101	28.68	577.1567 $[M-H]^-$	8-(6-*O*-D-Apio-*β*-D-furanosyl-*β*-D-glucopyranosyl)-5, 7-dihydroxy-3-(4-methoxyphenyl)-4*H*-1-benzopyran-4- one or 7-hydroxy-3-(4-hydroxy-3-methoxyphenyl)-8-(6-*O*-*β*-D-xylopyranosyl-*β*-D-glucopyranosyl)-4*H*-1- benzopyran-4-one	282.0548, 310.0508, 325.0728, 355.0877, 577.1635
p-102	29.34	957.4716 $[M-H]^-$	Kudzu saponin SA_4	351.0569, 957.4871
p-115	32.15	285.0771 $[M-H]^-$	Licochalcone B	93.0349, 121.0302, 150.0316, 177.0183, 270.0497
p-116	32.65	983.4513 $[M-H]^-$	Licorice saponin A_3	351.0579, 821.4084, 983.4662
p-117	32.51	253.0051 $[M-H]^-$	Daidzein	91.0195, 132.0250, 180.0567, 208.0598, 223.0393, 253.0530
p-121	33.23	283.0618 $[M-H]^-$	3'-Methoxydaidzein	91.0199, 120.0203, 135.0084, 148.0169, 156.0581, 167.0484, 183.0438, 195.0449, 211.0398, 223.0404, 239.0346, 251.0358, 268.0387, 283.0754
p-123	34.44	983.4512 $[M-H]^-$	Yunganoside K_1	321.0908, 487.2506, 497.1244, 983.4663
p-125	35.06	837.3929 $[M-H]^-$	Yunganoside K_2 or its isomer	351.0591, 837.4041
p-128	36.38	837.3914 $[M-H]^-$	Yunganoside K_2 or its isomer	351.0557, 837.4045
p-130	37.51	837.3933 $[M-H]^-$	Yunganoside K_2 or its isomer	351.0589, 837.4023
p-131	37.85	967.4565 $[M-H]^-$	Yunganoside L_1	339.0940, 471.2549, 497.1128, 905.4484, 967.4660
p-132	38.31	985.4650 $[M-H]^-$	Yunganoside G_1	339.0952, 497.1233, 985.4865
p-134	39.32	837.3931 $[M-H]^-$	Yunganoside K_2 or its isomer	193.0343, 351.0599, 837.4047
p-135	40.21	821.3965 $[M-H]^-$	Glycyrrhizin	193.0357, 351.0584, 821.4065

p-n: compounds with corresponding NO. *in vitro* appeared in rat plasma as well (p means prototype components, and n was in agreement with the NO. in Table 1).

In the same manner, compound **p-100** in rat plasma and compound **100** *in vitro* provided an identical molecular formula $C_{24}H_{26}O_{10}$ and adduct ion at *m/z* 519 $[M + HCOO]^-$. Additionally, they produced similar fragment ions in MS^n spectra, *e.g.*, *m/z* 311 $[M - Glc - H]^-$, *m/z* 267 $[M - Glc - CO_2 - H]^-$, and *m/z* 252 $[M - Glc - CO_2 - {\cdot}CH_3 - H]^{\cdot-}$. In the light of literature [22], compound **100** was assumed as pueroside C, which had a possibility as prototype of compound **p-100**.

There were two organic acids (compounds **p-27** and **p-42**) that were absorbed into blood. Using compound **p-42** as an example, it shared the same $[M - H]^-$ at *m/z* 209 and similar fragmentation behavior with compound **42** in MS^2 and MS^3 spectra, *e.g.*, *m/z* 165 $[M - CO_2 - H]^-$, *m/z* 121 $[M - 2CO_2 - H]^-$, *m/z* 119 $[M - CO_2 - CH_2O_2 - H]^-$, and *m/z* 93 $[M - 2CO_2 - C_2H_4 - H]^-$. Hence, compound **p-42** was identified as (*p*-hydroxybenzyl)malonic acid, *i.e.*, the prototype of compound **42**.

Identification of metabolites in rat plasma

As an organism, the metabolism *in vivo* should follow a certain rule; otherwise metabolic disorder would happen. So far, the regularity of metabolic pathways in the body has not been clarified completely [23]. When a drug is absorbed into the blood, it undergoes phase I metabolic reactions, *e.g.*, hydroxylation, hydrogenation, demethylation, *etc*, to be added a polar group or exposes its polar groups, or decreases its toxicity. If the drug contains polar groups, it might conduct phase II metabolic reactions directly. During the course of phase II reactions, the precursor drug would conjugate with endogenous molecules, such as glucuronic acid, sulfuric acid and so on, to increase its water solubility and facilitate excretion *via* the kidney. These sequential metabolic pathways generate diverse metabolic products.

In order to discriminate endogenous metabolites from drug-related metabolites, the TIC chromatograms of drug-containing plasma were compared with that of blank plasma. Endogenous substances could then be eliminated and exogenous compounds could be constructed by manual analysis. By contrasting these compounds with already known compounds *in vitro*, searching for the same compounds and validating them *via* fragment comparison, we ascertain the nature of drug-related metabolites finally. MetabolitePilot^{TM} software incorporated in LC-Q-TOF-MS could process the data acquired, provide information about group changes from prototypes to metabolites by means of exact molecular weight, and provide elementary compositions thereof. These were beneficial to determine prototypes and metabolites in biological samples, and hence conjecture potential metabolic pathways. In this way, a total of 48 metabolites were presumed (Table 3). A variety of metabolic reactions, such as deconjugation, hydroxylation, glucuronidation, sulfation, methylation, hydrogenation, demethylation and loss of CH_2O, were involved in the metabolism of GZJGGT. Among which, part of components underwent two-step or multi-step reactions. The metabolic network of GZJGGT in rat plasma was intricate given the complexity of constituents in prescription and diversity of metabolic responses *in vivo*, as shown in Fig. 4.

Give the identification of metabolite **M8**, an isoflavone, as an example. It produced deprotonated molecule $[M - H]^-$ at *m/z* 511, along with base peak ion at *m/z* 311 $[M - SO_3 - 120 - H]^-$, as well as characteristic fragment ions at *m/z* 431 $[M - SO_3 - H]^-$, *m/z* 341 $[M - SO_3 - 90 - H]^-$, *m/z* 323 $[M - SO_3 - 90 - H_2O - H]^-$, and *m/z* 283 $[M - SO_3 - 120 - CO - H]^-$ in the MS^2 spectrum by LC-Q-TOF-MS. Metabolite **M8** could be produced from a total of 12 compounds (compounds **28**, **29**, **31**, **36**, **39**, **45**, **46**, **48**, **49**, **68**, **70**, and **73**) by one-step or successive metabolic reactions. Among them, compounds **39** and **70**, which were isomers providing a base peak ion at *m/z* 311 $[M - 120 - H]^-$ and typical fragment ion

Table 3 The metabolites of constituents of GZJGGT in rat plasma after intragastric administration

Information about parent compounds	No.	t_R/min	Measured *m/z*	Metabolic reactions	Metabolic pathway	Metabolic phase
p-27 ($C_{13}H_{16}O_9$, 316.07943, Protocatechuic acid 3-glucoside)	**M1**	10.20	345.046 14	Deconjugation + hydroxylation + glucuronidation	**p-27** − $C_6H_{10}O_5$ + O + $C_6H_8O_6$	I + II
	M2	11.45	329.051 46	Deconjugation + glucuronidation	**p-27** − $C_6H_{10}O_5$ + $C_6H_8O_6$	I + II
	M3	13.12	232.976 69	Deconjugation + sulfation	**p-27** − $C_6H_{10}O_5$ + SO_3	I + II
p-28 ($C_{33}H_{40}O_{19}$, 740.216 38, Puerarin-7-*O*-*α*-isomaltoside); **p-31** ($C_{32}H_{38}O_{18}$, 710.20582, Mirificin-4'-*O*-glucoside); **p-36** or **45** ($C_{27}H_{30}O_{14}$, 578.16356, Puerarin-4'-*O*-*β*-D-glucopyranoside isomer or 6"-*O*-*α*-D-gluco-pyranosyl puerarin); **46**/**p-49**/**p-68**/**73** ($C_{26}H_{28}O_{13}$, 548.15299, Mirificin or 7-hydroxy-3-(4-hydroxyphenyl)-8-(6-*O*-*β*-D- xylopyranosyl-*β*-D-glucopyranosyl)-4*H*-1-benzopyran-4-one or its isomer);	**M15**	18.03	547.145 71	Deconjugation	**p-31** − $C_6H_{10}O_5$	I
	M14	17.97	415.103 12	Deconjugation	**46**/[**p-49**/**M15**]/**p-68**/**73** − $C_5H_8O_4$/**p-36**/**45** − $C_6H_{10}O_5$	I
	M43	32.51	253.051 10	Deconjugation	**p-28** − $C_6H_{10}O_5$ − $C_6H_{10}O_5$ − $C_6H_{10}O_5$/[**p-48**/**M14**] − $C_6H_{10}O_5$	I
	M20	21.27	563.139 61	Hydroxylation	**46**/[**p-49**/**M15**]/**p-68**/**73** + O	I
	M4	14.92	591.134 56	Deconjugation + glucuronidation/ glucuronidation	**p-28** − $C_6H_{10}O_5$ − $C_6H_{10}O_5$ + $C_6H_8O_6$/[**p-48**/**M14**] + $C_6H_8O_6$	I + II/ II
	M28	24.42	561.160 67	Methylation (N, O, S)	**46**/[**p-49**/**M15**]/**p-68**/**73** + CH_2	II
	M30	25.35	429.118 75	Deconjugation + methylation (N, O, S)/methylation (N, O, S)	**p-28** − $C_6H_{10}O_5$ − $C_6H_{10}O_5$ + CH_2/[**p-48**/**M14**] + CH_2	I + II/ II
	M18	20.60	429.082 18	Glucuronidation	**M43** + $C_6H_8O_6$	II
	M34	26.59	333.007 67	Sulfation	**M43** + SO_3	II
p-48 ($C_{21}H_{20}O_9$, 416.110 73, Puerarin)	**M9**	15.91	627.101 77	Sulfation	**46**/[**p-49**/**M15**]/**p-68**/**73** + SO_3	II
	M45	35.81	267.066 55	Methylation (N, O, S)	**M43** + CH_2	II
	M10	16.39	607.129 54	Hydroxylation + glucuronidation	[**p-48**/**M14**] + O + $C_6H_8O_6$	I + II
	M8	15.86	511.054 31	Hydroxylation + sulfation/deconjugation + hydroxylation + sulfation	[**p-48**/**M14**] + O + SO_3/ **p-28** − $C_6H_{10}O_5$ − $C_6H_{10}O_5$ + O + SO_3	II/ I + II
	M33	26.42	349.002 55	Glucuronidation + sulfation	**M43** + $C_6H_8O_6$ + SO_3	II
	M44	33.21	283.061 64	Hydroxylation + methylation (N, O, S)	**M43** + O + CH_2	I + II
	M26	24.23	431.098 01	Hydrogenation + glucuronidation	**M43** + 2 × H + $C_6H_8O_6$	I + II
	M29	24.92	445.077 19	Hydroxylation + glucuronidation	**M43** + O + $C_6H_8O_6$	I + II
p-29 ($C_{27}H_{30}O_{15}$, 594.15847, 8-*β*- D-Glucopyranosyl-3-[4-(*β*-D-glucopyranosyloxy)-3-hydroxyphenyl]-7-hydroxy-4*H*-1-benzopyran-4-one); **39**/**70** ($C_{21}H_{20}O_{10}$, 431.0984, Genistein-6-C-glucoside or 3'-hydroxypuerarin)	**M7**	15.64	431.098 09	Deconjugation	**p-29** − $C_6H_{10}O_5$	I
	M48	38.92	269.046 18	Deconjugation	**M7**/**39**/**70** − $C_6H_{10}O_5$	I
	M8	15.86	511.054 31	Sulfation	**M7**/**39**/**70** + SO_3	II
	M16	18.54	445.113 65	Methylation (N, O, S)	**M7**/**39**/**70** + CH_2	II
	M44	33.21	283.061 64	Methylation (N, O, S)	**M48** + CH_2	II
	M10	16.39	607.129 54	Glucuronidation	**M7**/**39**/**70** + $C_6H_8O_6$	II
	M33	26.42	349.002 55	Sulfation	**M48** + SO_3	II
	M29	24.92	445.077 29	Glucuronidation	**M48** + $C_6H_8O_6$	II
	M22	22.21	621.109 24	2 × Glucuronidation	**M48** + 2 × $C_6H_8O_6$	II

Continued

Information about parent compounds	NO.	t_R/min	Measured *m/z*	Metabolic reactions	Metabolic pathway	Metabolic phase
p-32 or **47** ($C_{28}H_{32}O_{15}$, 608.17412, 8-*β*-D-Glucopyranosyl-3- [4-(*β*-D-glucopyranosyloxy)-3-methoxyphenyl]-7-hydroxy-4*H*-1-benzopyran-4-one or 4', 7-dihydroxy-3'-methoxyisoflavone-8-*C*-[*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranoside); **p-50**/**p-101** ($C_{27}H_{30}O_{14}$, 578.16356, 8-(6-*O*-D-Apio-*β*-D-furanosyl-*β*-D-glucopyranosyl)-5, 7-dihydroxy-3-(4-methoxyphenyl)-4*H*-1-benzopyran-4-one or 7-hydroxy-3-(4-hydroxy -3-methoxyphenyl)-8-(6-*O*-*β*-D-xylopyranosyl-*β*-D-glucopyranosyl)-4*H*-1-benzopyran-4-one); **p-51** or **77**/**p-81** or **p-66**/**124** ($C_{22}H_{22}O_{10}$, 446.1213, 3'-Methoxypuerarin or its isomer, or 3'-methoxydaidzin or its isomer); **p-121** ($C_{16}H_{12}O_5$, 284.06847, 3'-Methoxydaidzein)	**M16**	18.54	445.113 65	Deconjugation	**p-32** − $C_6H_{10}O_5$/ **p-50**/**p-101** − $C_5H_8O_4$	I
	M44	33.21	283.061 64	Deconjugation	**47**/[**p-51**/**M16**]/**p-66**/**77**/**p-81**/ **124** − $C_6H_{10}O_5$	I
	M20	21.27	563.139 61	Demethylation	**p-50**/**p-101** − CH_2	I
	M48	38.92	269.046 18	Demethylation	[**p-121**/**M44**] − CH_2	I
	M15	18.03	547.145 71	Hydrooxymethylene loss	**p-50**/**p-101** − CH_2O	I
	M43	32.51	253.051 10	Hydrooxymethylene loss	[**p-121**/**M44**] − CH_2O	I
	M17	18.68	579.135 38	Hydroxylation	**M20** + O	I
	M32	25.96	363.018 18	Sulfation	[**p-121**/**M44**] + SO_3	II
	M24	23.31	442.974 25	2 × Sulfation	[**p-121**/**M44**] + 2 × SO_3	II
	M5	15.57	525.069 86	Sulfation	**47**/[**p-51**/**M16**]/**77**/**p-81** + SO_3	II
	M21	21.37	459.092 75	Glucuronidation	[**p-121**/**M44**] + $C_6H_8O_6$	II
	M6	15.61	621.145 62	Glucuronidation	**47**/[**p-51**/**M16**]/**77**/**p-81** + $C_6H_8O_6$	II
	M29	24.92	445.077 19	Glucuronidation	**M48** + $C_6H_8O_6$	II
	M11	16.65	739.172 05	Glucuronidation	**M20** + $C_6H_8O_6$	II
	M10	16.39	607.129 54	Demethylation + glucuronidation	**47**/[**p-51**/**M16**]/**77**/**p-81** − CH_2 + $C_6H_8O_6$	I + II
	M31	25.63	475.08763	Hydroxylation + glucuronidation	[**p-121**/**M44**] + O + $C_6H_8O_6$	I + II
p-60 ($C_{21}H_{20}O_9$, 416.11073, Daidzin); **p-117** ($C_{15}H_{10}O_4$, 254.05791, Daidzein)	**M43**	32.51	253.051 10	Deconjugation	**p-60** − $C_6H_{10}O_5$	I
	M13	17.14	495.059 48	Sulfation	**p-60** + SO_3	II
	M12	17.03	593.150 69	Hydrogenation + glucuronidation	**p-60** + 2 × H + $C_6H_8O_6$	I + II
	M18	20.60	429.082 18	Glucuronidation	[**p-117**/**M43**] + $C_6H_8O_6$	II
	M34	26.59	333.007 67	Sulfation	[**p-117**/**M43**] + SO_3	II
	M26	24.23	431.098 01	Hydrogenation + glucuronidation	[**p-117**/**M43**] + 2 × H + $C_6H_8O_6$	I + II
	M29	24.92	445.077 19	Hydroxylation + glucuronidation	[**p-117**/**M43**] + O + $C_6H_8O_6$	I + II
	M45	35.81	267.066 55	Methylation(N, O, S)	[**p-117**/**M43**] + CH_2	II
	M44	33.21	283.061 64	Hydroxylation + methylation (N, O, S)	[**p-117**/**M43**] + O + CH_2	I + II
	M33	26.42	349.002 55	Hydroxylation + sulfation	[**p-117**/**M43**] + O + SO_3	I + II
p-71 ($C_{29}H_{34}O_{14}$, 606.19486, Pueroside A)	**M38**	28.32	311.092 79	Deconjugation + methylation (N, O, S)	**p-71** − $C_6H_{10}O_4$ − $C_6H_{10}O_5$ + CH_2	I + II
p-78 ($C_{26}H_{30}O_{13}$, 550.16864, Liquiritin apioside); **p-99** ($C_{26}H_{30}O_{13}$, 550.16864, Isoliquiritin apioside)	**M26**	24.23	431.098 01	Deconjugation + glucuronidation	**p-78**/**p-99** − $C_5H_8O_4$ − $C_6H_{10}O_5$ + $C_6H_8O_6$	I + II
	M37	28.31	335.023 36	Deconjugation + sulfation	**p-78**/**p-99** − $C_5H_8O_4$ − $C_6H_{10}O_5$ + SO_3	I + II
	M36	27.49	351.017 76	Deconjugation + hydroxylation + sulfation	**p-78**/**p-99** − $C_5H_8O_4$ − $C_6H_{10}O_5$ + O + SO_3	I + II
	M23	23.13	629.117 64	Sulfation	**p-78**/**p-99** + SO_3	II
82/**p-85** ($C_{27}H_{30}O_{13}$, 562.16864, Formononetin-8-*C*-[xylosyl(1→6)]-glucoside or 8-(6-*O*-D-apio-*β*-D-furanosyl-*β*-D-glucopyranosyl)-7- hydroxy-3-(4-methoxyphenyl)-4*H*-1-benzopyran-4-one); **p-91** ($C_{22}H_{22}O_9$, 430.12638, 4'-Methoxypuerarin); **104** ($C_{22}H_{22}O_9$, 430.12638, Ononin)	**M45**	35.81	267.066 55	Deconjugation	**82**/[**p-85**/**M28**] − $C_5H_8O_4$ − $C_6H_{10}O_5$/ [**p-91**/**M30**]/**104** − $C_6H_{10}O_5$	I
	M15	18.03	547.145 71	Demethylation	**82**/[**p-85**/**M28**] − CH_2	I
	M14	17.97	415.103 12	Deconjugation + demethylation/ demethylation	**82**/[**p-85**/**M28**] − $C_5H_8O_4$ − CH_2/[**p-91**/**M30**] − CH_2	I
	M16	18.54	445.113 65	Deconjugation + hydroxylation/ hydroxylation	**82**/[**p-85**/**M28**] − $C_5H_8O_4$ + O/ [**p-91**/**M30**] + O	I
	M43	32.51	253.051 10	Demethylation	**M45** − CH_2	I
	M20	21.27	563.139 61	Hydroxylation	**M15** + O	I

Continued

Information about parent compounds	No.	t_R/min	Measured *m/z*	Metabolic reactions	Metabolic pathway	Metabolic phase
82/**p-85** ($C_{27}H_{30}O_{13}$, 562.168 64, Formononetin-8-*C*-[xylosyl(1→6)]-glucoside or 8-(6-*O*-D-apio-*β*-D-furanosyl-*β*-D-glucopyranosyl)-7-hydroxy-3-(4-methoxyphenyl)-4*H*-1-benzopyran-4-one); **p-91** ($C_{22}H_{22}O_9$, 430.126 38, 4'-Methoxypuerarin); **104** ($C_{22}H_{22}O_9$, 430.126 38, Ononin)	**M17**	18.68	579.135 38	2 × Hydroxylation	**M15** + 2 × O	I
	M46	38.21	269.082 26	Hydrogenation	**M45** + 2 × H	I
	M47	35.81	347.023 28	Sulfation	**M45** + SO_3	II
	M39	29.81	443.09745	Glucuronidation	**M45** + $C_6H_8O_6$	II
	M4	14.92	591.134 56	Glucuronidation	**M14** + $C_6H_8O_6$	II
	M18	20.60	429.082 18	Glucuronidation	**M43** + $C_6H_8O_6$	II
	M11	16.65	739.172 05	Glucuronidation	**M20** + $C_6H_8O_6$	II
	M32	25.96	363.018 18	Hydroxylation + sulfation	**M45** + O + SO_3	I + II
	M21	21.37	459.092 75	Hydroxylation + glucuronidation	**M45** + O + $C_6H_8O_6$	I + II
	M31	25.63	475.087 63	2 × Hydroxylation + glucuronidation	**M45** + 2 × O + $C_6H_8O_6$	I + II
	M10	16.39	607.129 54	Hydroxylation + glucuronidation	**M14** + O + $C_6H_8O_6$	I + II
	M29	24.92	445.077 19	Hydroxylation + glucuronidation	**M43** + O + $C_6H_8O_6$	I + II
p-83 ($C_{21}H_{22}O_9$, 418.126 38, Liquiritin)	**M26**	24.23	431.098 01	Deconjugation + glucuronidation	**p-83** − $C_6H_{10}O_5$ + $C_6H_8O_6$	I + II
	M37	28.31	335.023 36	Deconjugation + sulfation	**p-83** − $C_6H_{10}O_5$ + SO_3	I + II
	M36	27.49	351.017 76	Deconjugation + hydroxylation + sulfation	**p-83** − $C_6H_{10}O_5$ + O + SO_3	I + II
	M12	17.03	593.150 69	Glucuronidation	**p-83** + $C_6H_8O_6$	II
88 ($C_{21}H_{20}O_{10}$, 432.105 65, Genistoside)	**M19**	21.25	435.129 11	2 × Hydrogenation	**88** + 4 × H	I
	M48	38.92	269.046 18	Deconjugation	**88** − $C_6H_{10}O_5$	I
	M29	24.92	445.077 29	Glucuronidation	**M48** + $C_6H_8O_6$	II
	M44	33.21	283.061 64	Methylation(N, O, S)	**M48** + CH_2	II
	M33	26.42	349.002 55	Sulfation	**M48** + SO_3	II
	M22	22.21	621.109 24	2 × Glucuronidation	**M48** + 2 × $C_6H_8O_6$	II
93 ($C_{29}H_{34}O_{14}$, 606.194 86, Puerol B O-apiosylglucoside); **p-100** ($C_{24}H_{26}O_{10}$, 474.1526, Pueroside C)	**M38**	28.32	311.092 79	Deconjugation	**93** − $C_5H_8O_4$-$C_6H_{10}O_5$/ **p-100**−$C_6H_{10}O_5$	I
	M42	32.33	391.049 05	Sulfation	**M38** + SO_3	II
	M25	24.04	663.156 47	2 × Glucuronidation	**M38** + 2 × $C_6H_8O_6$	II
	M40	29.98	487.123 85	Hydrogenation + glucuronidation	**M38** + 2 × H + $C_6H_8O_6$	II
p-115 ($C_{16}H_{14}O_5$, 286.084 12, Licochalcone B)	**M41**	32.21	365.033 86	Sulfation	**p-115** + SO_3	II
	M35	27.04	461.108 52	Glucuronidation	**p-115** + $C_6H_8O_6$	II
	M27	24.23	477.103 63	Hydroxylation + glucuronidation	**p-115** + O + $C_6H_8O_6$	I + II

The nodal compounds were shown in square brackets.

at *m/z* 283 $[M - 120 - CO - H]^-$ in MS^2 spectra *in vitro*, could generate metabolite **M8** by sulfation. Compound **29** produced ions at *m/z* 430 $[M - Glc - \cdot H - H]^{\cdot -}$, *m/z* 310 $[M - Glc - 120 - \cdot H - H]^{\cdot -}$, and *m/z* 282 $[M - Glc - 120 - CO - \cdot H - H]^{\cdot -}$ in the MS^2 spectrum *in vivo*, and was also viewed as the parent compound of metabolite **M8** after undergoing deconjugation and sulfation. Compound **48** with only one Glc unit attached to C-8 of A ring, had been characterized as puerarin. It showed a deprotonated molecule at *m/z* 415 and base peak ion at *m/z* 295 $[M - 120 - H]^-$, as well as *m/z* 267 $[M - 120 - CO - H]^-$ in its MS^2 spectrum *in vivo*. Therefore, metabolite **M8** was regarded as the hydroxylation and sulfation product of puerarin. Likewise, compounds **28**, **31**, **36**, and **49**, which were detected in plasma samples, could produce identical ions to compound **48** after degrading 2 × $C_6H_{10}O_5$, $C_6H_{10}O_5$ + $C_5H_8O_4$, $C_6H_{10}O_5$, and $C_5H_8O_4$, respectively. Thus, compounds **28**, **31**, **36**, and **49** were also viewed as the parent compounds of metabolite **M8**. On the other hand, compound **45** (isomer of compound **36**) and compounds **46**/**68**/**73** (isomers of compound **49**), which could not be detected *in vivo*, might yield metabolite **M8** by a series of deconjugation, hydroxylation and sulfation reactions as well. Hence, they were deemed as potential precursor compounds.

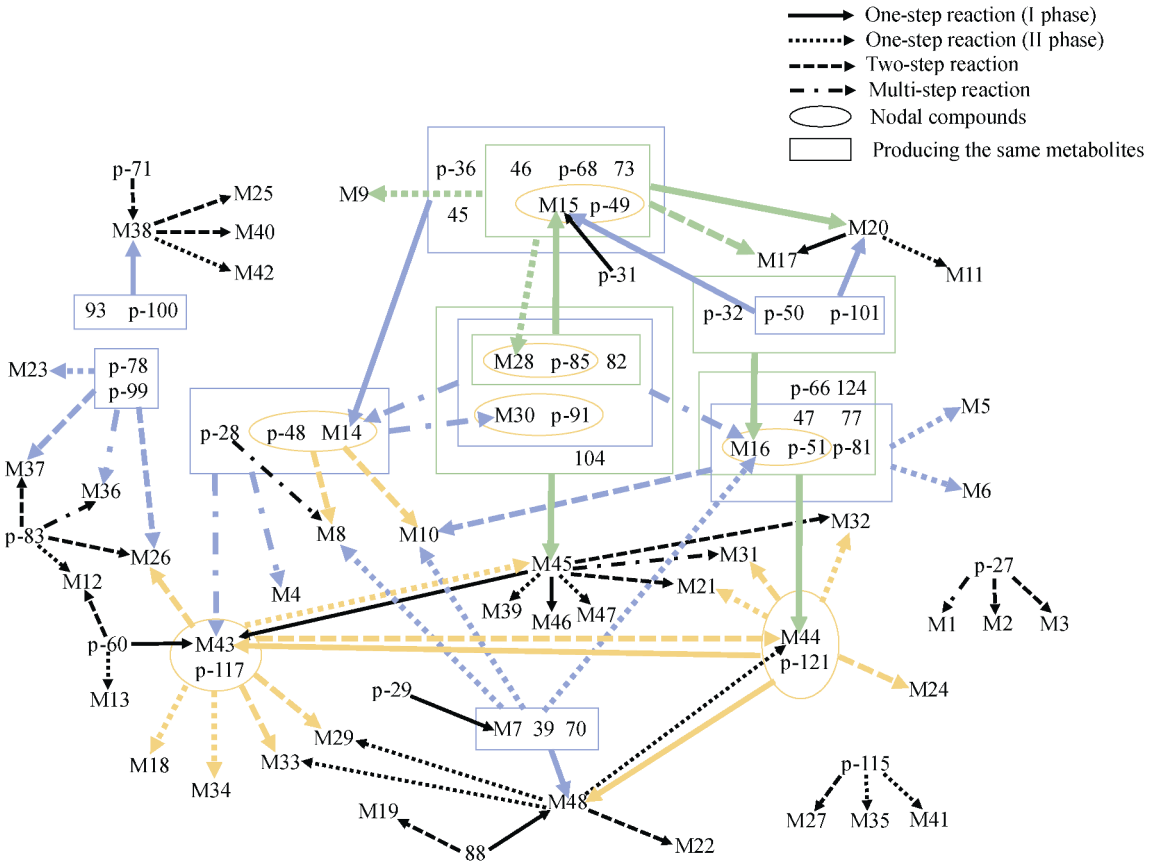


Fig. 4 The metabolic network of GZJGGT in rat plasma after intragastric administration. (black labels: one-to-one metabolism; orange labels: nodal compounds and their metabolism; blue or green labels: many-to-one metabolism, two colors were used to distinguish different groups.)

Take metabolite **M37** as another example. It gave a deprotonated molecule $[M - H]^-$ at *m/z* 335, as well as fragment ions at *m/z* 255 $[M - SO_3 - H]^-$, *m/z* 135 $[M - SO_3 - C_8H_8O - H]^-$, *m/z* 119 $[M - SO_3 - C_7H_4O_3 - H]^-$, and *m/z* 91 $[M - SO_3 - C_8H_8O - CO_2 - H]^-$ in the MS^2 spectrum. Metabolite **M37** could be yielded from three precursor compounds (**78**, **83**, and **99**) by two-step metabolic reactions. Among them, compounds **78** and **99** were isomers with molecular formula $C_{26}H_{30}O_{13}$, which were flavanones and chalcones, respectively. They produced deprotonated molecule at *m/z* 549, along with fragment ions at *m/z* 255 $[M - Glc\text{-}Api - H]^-$ and *m/z* 135 $[M - Glc\text{-}Api - C_8H_8O - H]^-$ in MS^2 spectra *in vivo*. Compounds **78** and **99** could generate metabolite **M37** by deconjugation and sulfation reactions. It was difficult to distinguish flavanones and chalcones *in vitro* by MS spectral data alone, as was the case *in vivo*. For compound **83**, it showed deprotonated molecule $[M - H]^-$ at *m/z* 417, and produced the same fragment ions as metabolite **M37** in MS^2 spectrum, *i.e.*, *m/z* 255 $[M - Glc - H]^-$, *m/z* 135 $[M - Glc - C_8H_8O - H]^-$, and *m/z* 119 $[M - Glc - C_7H_4O_3 - H]^-$.

Metabolite **M38** as a typical puerosides, provided deprotonated molecule $[M - H]^-$ at *m/z* 311, as well as characteristic fragment ions at *m/z* 267 $[M - CO_2 - H]^-$, *m/z* 252 $[M - CO_2 - \cdot CH_3 - H]^-$, *m/z* 237 $[M - CO_2 - CH_2O - H]^-$, *m/z* 161 $[M - CO_2 - C_7H_6O - H]^-$, *m/z* 119 $[M - CO_2 - C_9H_8O_2 - H]^-$, *m/z* 108 $[M - CO_2 - \cdot CH_3 - C_{10}H_8O - H]^-$, and *m/z* 93 $[M - CO_2 - C_{11}H_{10}O_2 - H]^-$. Three compounds (**71**, **93** and **100**) could be the parent compounds of metabolite **M38**. For compound **100**, it gave pseudo-molecular ion at *m/z* 519 $[M + HCOO]^-$, along with fragment ions at *m/z* 311 $[M - Glc - H]^-$, *m/z* 267 $[M - Glc - CO_2 - H]^-$, and *m/z* 252 $[M - Glc - CO_2 - \cdot CH_3 - H]^-$ in MS^2 spectrum *in vivo*, which could produce metabolite **M38** by deconjugation. Likewise, compound **93** might generate metabolite **M38** by deconjugation of Glc-Api. For compound **71**, it showed a deprotonated molecule at *m/z* 605, as well as fragment ions at *m/z* 297 $[M - Glc\text{-}Rha - H]^-$, and *m/z* 253 $[M - Glc\text{-}Rha - CO_2 - H]^-$ in MS^2 spectrum *in vivo*. Hence, it could metabolize into metabolite **M38** by deconjugation and methylation.

Only three metabolites (**M1**, **M2**, and **M3**) were produced from organic acid, *i.e.*, compound **27** protocatechuic acid 3-glucoside. For metabolite **M1**, it gave deprotonated molecule at *m/z* 345 and yielded a base peak ion at *m/z* 125 $[M - GluA - CO_2 - H]^-$, as well as fragment ions at *m/z* 183 $[M - C_5H_6O_6 - H]^-$, *m/z* 175 $[GluA - H]^-$, and *m/z* 169 $[M - GluA - H]^-$ in the MS^2 spectrum. On the other hand, com-

pound **27** provided deprotonated molecule $[M - H]^-$ at *m/z* 315, as well as fragment ions at *m/z* 153 $[M - Glc - H]^-$ and *m/z* 109 $[M - Glc - CO_2 - H]^-$ in the MS^2 spectrum. The MetabolitePilot^{TM} software analysis showed that metabolite **M1** could be produced from compound **27** by successive deconjugation, hydroxylation and glucuronidation. This analytical result could be proved by the above data.

With regard to some indistinguishable prototypes and metabolites, we defined herein these compounds as "nodal compounds" provisionally. Here, seven pairs of nodal compounds were found in rat plasma of GZJGGT: compounds **p-48** *vs* **M14**, compounds **p-49** *vs* **M15**, compounds **p-51** *vs* **M16**, compounds **p-85** *vs* **M28**, compounds **p-91** *vs* **M30**, compounds **p-117** *vs* **M43**, compounds **p-121** *vs* **M44**, respectively.

Take prototype compound **p-117** for instance. It could first metabolize into metabolites **M18**, **M26**, **M29**, **M33**, **M34**, **M44**, and **M45** *via* phase Ⅱ reaction or two-step reaction. On one hand, metabolite **M44** could go on metabolizing into metabolites **M21**, **M24**, **M31**, **M32**, **M43**, and **M48**. It was important to mention that compound **p-117** and metabolite **M43** were nodal compounds. In other words, metabolite **M44** could convert into metabolite **M43** through loss of CH_2O, and could be generated from metabolite **M43** by means of hydroxylation and methylation in turn. One the other hand, metabolite **M45** could transform into three metabolites (**M21**, **M31**, and **M32**), which could also be produced from **M44**, as well as metabolites **M39**, **M43**, **M46**, and **M47**. In a similar manner, metabolite **M43** could be produced by metabolite **M45** *via* a phase I demethylation reaction. On the other hand, metabolite **M45** could also be generated by metabolite **M43** through a phase Ⅱ methylation reaction. In this case, it was difficult to determine whether metabolite **M43** was transformed from metabolite **M44** or metabolite **M45**, partly or completely, or even not at all. Given that, an isotope labeling method might be useful here.

It was worthy of our attention that a certain metabolite could be produced from different parent compounds or its isomers. Take metabolite **M28** as an example: it could be generated *via* methylation metabolism from five precursor compounds, including prototype compounds **p-49** and **p-68**, metabolite **M15**, and compounds **46** and **73**. On one hand, metabolite **M15** could be produced by compounds **p-31**, **p-50** and **p-101** by means of deconjugation or loss of CH_2O. On the other hand, it could be yielded through demethylation by three precursor compounds, *i.e.*, nodal compounds **p-85**/**M28** and compound **82**. Likewise, metabolites **M15** and **M28** could be converted into each other by phase Ⅱ methylation metabolic reaction or phase I demethylation metabolism.

As mentioned above, the chemical constituents of GZJGGT were first analyzed by LC-MS and a total of 77 constituents were identified, either tentatively or unambiguously. After intragastric administration of GZJGGT to rats, it was found that almost all the type of compounds, including flavonoids, triterpene saponins, monoterpene glycosides, puerosides, and organic acids could be absorbed into blood.

The results showed that it was both complicated and laborious to identify metabolites in herbal formulae. It was too difficult to determine the metabolic pathways of TCM formulae accurately and completely based on the existing results. To elucidate the nature of the metabolic network, it was necessary to investigate the metabolites of individual compounds from GZJGGT.

Conclusion

In this work, the chemical constituents of GZJGGT were first analyzed by LC-MS and a total of 141 constituents were detected. By analyzing the MS^n fragmentation patterns and referring data in literatures, the structures of 77 compounds were deduced, including flavonoids, triterpenoids, monoterpene glycosides, puerosides and organic acids. Among which, twelve compounds were determined unequivocally by contrast with authentic standards. Besides, a base peak ion filtering method was proposed to classify flavonoid *O*-glycosides and *C*-glycosides structurally. As a result, 45 prototype compounds and 48 metabolites were found in rat plasma after intragastric administration of GZJGGT, and their metabolic network was described.

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Cite this article as: CHEN Lu-Lin, CHEN Can-Hui, ZHANG Xing-Xian, WANG Yi, WANG Shu-Fang. Identification of constituents in Gui-Zhi-Jia-Ge-Gen-Tang by LC-IT-MS combined with LC-Q-TOF-MS and elucidation of their metabolic networks in rat plasma after oral administration [J]. *Chin J Nat Med*, 2019, **17**(11): 803-821.