Comprehensive Profiling and Characterization of The Absorbed Components and Metabolites in Mice Serum and Tissues Following Oral Administration of Qingfei Paidu Decoction by UHPLC-Q-Exactive-Orbitrap HRMS

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Research

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Abstract

Background: Qingfei Paidu decoction (QFPDD) is a Chinese medicine compound formula recommended for combating corona virus disease 2019 (COVID-19) by National Health Commission of the People's Republic of China. This study aims to identify the main constituents in QFPDD and the absorbed components (including prototypes and metabolites) in serum and tissues after oral administration of QFPDD to mice.

Methods: A practical and sensitive method of UHPLC-Q-Exactive-Orbitrap HRMS was developed to identify the chemical constituents in QFPDD and the absorbed prototypes as well as the metabolites in mice serum and tissues following oral administration of QFPDD.

Results: A total of 405 chemicals, including 40 kinds of alkaloids, 162 kinds of flavonoids, 44 kinds of organic acids, 71 kinds of triterpene saponins and 88 kinds of other compounds in the water extract of QFPDD were tentatively identified *via* comparison with the retention times and MS/MS spectra of the standards or refereed by literature. With the help of the standards and *in vitro* metabolites, 195 chemical components (including 104 prototypes and 91 metabolites) were identified in mice serum after oral administration of QFPDD. In addition, 165, 177, 112, 120, 44, 53 constituents were identified in the lung, liver, heart, kidney, brain, and spleen of QFPDD-treated mice, respectively.

Conclusions: An UHPLC-Q-Orbitrap HRMS based method was established for chemical profiling the constituents in QFPDD, while the absorbed prototypes and metabolites occurring in mice serum and tissues were investigated following oral administration of QFPDD. These findings provided key information and guidance for further investigation on the pharmacologically active substances and clinical applications of QFPDD.

Background

Corona Virus Disease 2019 (COVID-19), a newly emerged infective disease, has spread all over the world, with a long incubation period, high infectivity and general susceptibility to all types of people [1–3]. The COVID-19 global pandemic has brought significantly negative effects for human health, economic and social stability. Researchers are now trying to find effective medications (including western therapeutics and herbal medicines) for combating COVID-19, while some therapeutics and herbal medicines have been used for treating COVID-19 in clinical settings [4–6]. In China, several Chinese medicine compound formulas, such as Qingfei Paidu decoction (QFPDD), Jingyin granules and Lianhua Qingwen capsule, have been validated playing an active role in combating this epidemic, especially alleviating the symptoms of some moderate and mild patients [7]. Among all recommend Chinese medicines for combating COVID-19 in China, QFPDD has drawn much attention owing to its effectiveness of COVID-19. As the first Chinese medicine compound formula recommended by National Health Commission of the People's Republic of China for combating COVID-19, QFPDD has been used to treat thousands of COVID-19 patients with the total effective rate of 97% [8–10]. Following oral administration of QFPDD, the major symptoms and imaging manifestations of more than 60% patients were significantly improved, while the symptoms of 30% patients were stable and did not aggravate [11]. Early treatment with QFPDD is associated with favorable outcomes for patient recovery, viral shedding, hospital stay, and course of the disease. And early treatment with QFPDD may serve as an effective strategy in controlling the epidemic [12].

Unlike Western therapeutics, Chinese medicines are always the mixtures of a wide range of ingredients, these ingredients as well as their *in vivo* metabolites may interact with a wide range of targets in the human body in an extremely complex way [13, 14]. To better understand the material basis for efficacy of QFPDD, the chemical constituents in this anti-COVID-19 Chinese medicine and the key components (including prototypes and metabolites) occurring in serum and target organs (such as the lung and the liver) following QFPDD treatment should be carefully investigated. Notably, QFPDD is deriving from four classic Chinese medicine prescriptions (including Maxing Shigan decoction, Shengan Mahuang decoction, Xiaochaihu decoction, and Wuling powder) that are used for treating epidemic diseases and the related inflammatory symptoms for thousands of years [15]. As a super combination of 20 herbs and a mineral drug (*Gypsum Fibrosum*), QFPDD is composed by hundreds of ingredients and parts of them may be absorbed in circulation system and then exert their bioactivities, such as anti-inflammatory and anticoagulation effects [16, 17]. However, the chemical constituents in QFPDD and the absorbed components of this Chinese medicine have not been well-investigated yet, which strongly block further investigations on the pharmacological and toxicological studies, the quality control and clinical applications of this anti-COVID-19 Chinese medicine.

This study aims to investigate the main chemical constituents in QFPDD and the absorbed components (including prototypes and metabolites) in serum and tissues after oral administration of QFPDD to mice. To this end, an UHPLC-Q-Orbitrap HRMS based method was well-established for systematic profiling the constituents in QFPDD, while the absorbed components (including the prototypes and the metabolites) occurring in mouse serum and tissues were also identified *via* profiling the serum and tissue samples from QFPDD-treated mice. The prototype components in both QFPDD and mouse serum were tentatively identified *via* comparison with the retention times and MS/MS spectra of the standards or refereed by literature, while the *in vivo* metabolites were tentatively identified *via* comparison with the retention times and MS/MS spectra of the *in vitro* metabolites generated in mice liver microsomes (MLMs). Finally, a total of 405 chemicals (including 40 kinds of alkaloids, 162 kinds of flavonoids, 44 kinds of organic acids, 71 kinds of triterpene saponins and 88 kinds of other compounds) in the water extract of QFPDD were identified, while 195, 165, 177, 112, 120, 44, 53 constituents were identified in the serum, lung, liver, heart, kidney, brain, and spleen of QFPDD-treated mice, respectively. All these findings are very helpful for deep understanding the fates of the constituents in QFPDD, as well as the key information of the tissue distribution of the absorbed components of this anti-COVID-19 Chinese medicine.

Materials And Experiments

Chemicals and materials

Herba Ephedrae (Mahuang, No. 191119-1), *Ramulus Cinnamomi* (Guizhi, No. 190802-1), *Rhizoma Alismatis* (Zixie, No. 191031-1), *Polyporus* (Zhuling, No. 191109-1), *Flos Farfarae* (Kuandonghua, No. 191207-1), *Herba Asari* (Xixin, No. 191220-1) and *Rhizoma Dioscoreae* (Shanyao, No. 191023-1) were purchased from Shanghai Wanshicheng Pharmaceutical Co., Ltd.(Shanghai, China). *Gypsum Fibrosum* (Shengshigao, No. 190801), *Poria* (Fuling, No. 191218), *Radix*

Bupleuri (Chaihu, No. 200103), *Rhizoma Pinelliae* prepared with ginger juice (Jiangbanxia, No. 191009) and *Herba Agastachis* (Huoxiang, No. 190814) were purchased from Shanghai Hongqiao Traditional Chinese Medicine Decoction Piece Co., Ltd. (Shanghai, China). *Radix Glycyrrhizae Praeparata* (Zhigancao, No. 191213), *Rhizoma Belamcandae* (Shegan, No. 191022), *Fructus Aurantii Immaturus* (Zhishi, No. 200119), *Semen Armeniaceae Amarum* (Xingren, No. 200108) were purchased from Shanghai Kangqiao Traditional Chinese Medicine Decoction Piece Co., Ltd. (Shanghai, China). *Radix Scutellariae* (Huangqin, No. 19112307), *Radix Asteris* (Ziwan, No. 1909247) and *Pericarpium Citri Reticulatae* (Chenpi, No. 191119) were purchased from Shanghai Yanghetang Traditional Chinese Medicine Decoction Piece Co., Ltd. (Shanghai, China). *Rhizoma Atractylodis Macrocephalae* (Baizhu, No. 190830) was purchased from Shanghai Qingpu Traditional Chinese Medicine Decoction Piece Co., Ltd. (Shanghai, China). Fresh *Rhizoma Zingiberis Recens* (Shengjiang) was purchased from the supermarket. All of the herbs were authenticated by prof. Changhong Wang, from the Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine.

Arginine, uridine, uracil, tyrosine, leucine, isoleucine, adenine, 6-hydroxypurine, guanine, guanosine, adenosine, xanthosine, L-phenylalanine, tryptophan were purchased from Dalian Meilun Biotechnology Co. LTD. (Dalian, China). Neochlorogenic acid, caffeic acid, chlorogenic acid, cryptochlorogenic acid, L-amygdalin, prunasin, 1,3-O-dicaffeoylquinic acid, ferulic acid, isochlorogenic acid B, isochlorogenic acid A, resveratrol, isochlorogenic acid C, tilianin, 6-Gingerol, 8-gingerol, 10-gingerol, alisol B-23-acetate and pachymic acid were purchased from Chengdu Biopurify Phytochemicals Ltd. (Chengdu, China). Quinic acid, stachydrine, citric acid, succinic acid, gallic acid, synephrine, phenylpropanolamine, D-demethyl pseudoephedrine, vanillic acid, ephedrine, pseudoephedrine, methylephedrine, catechin, epicatechin, neoliquiritin, 3,4-dihydroxybenzaldehyde, luteolin-7-O- β -D-glucoside, liquiritin, vitexin, tectoridin, isovitexin, hyperin, scutellarin, quercetin, isoquercitrin, verbascoside, hesperetin, isoacetoside, hesperidin, astragalin, neohesperidin, cinnamaldehyde, liquiritigenin, isoliquiritin, ononin, naringin, luteolin, baicalin, cinnamic acid, naringenin, licochalcone A, licochalcone B, wogonoside, kaempferol, rosmarinic acid, isoliquiritigenin, baicalein, formononetin, sinensetin, irisflorethin, oroxylin A, nobiletin, atractylenolide-I, tangeretin, asarinin, glycyrrhizic acid, tussilagone and alisol A were provided by prof. Xinmiao Liang from Dalian Institute of Chemical Physics, Chinese Academy of Sciences (Dalian, China). The purities of those reference substances were more than 98%.

Glucose 6-phosphate (G-6-P), glucose-6-phosphate dehydrogenase (G-6-PDH), nicotinamide adenine dinucleotide phosphate disodium salt (β -NADP⁺), uridine di-phosphate glucuronic acid (UDPGA), alamethicin, S-adenosyl-L-methionine (SAM) and 3-phosphoadenosine 5-phosphosulfate (PAPS) were purchased from Sigma Aldrich Co. (St. Louis, MO, USA). Tris base and MgCl₂ were purchased from Majorbio Biotech Corp., Ltd. (Shanghai, China). Pooled liver microsomes from male ICR/CD-1 mouse (MLM, Lot no. STOM) was ordered from Research Institute for Liver Diseases (RILD, Shanghai, China). Acetonitrile, methanol and formic acid of HPLC grade were purchased from Fisher Scientific Co. (Santa Clara, USA). Deionized water was produced with a Milli-Q Academic System (Millipore, Billerica, MA). All other reagents and solvents were either analytical or HPLC grade.

Preparation of the Chinese multi-herbal formula QFPDD

The Chinese multi-herbal formula Qingfei Paidu decoction (QFPDD) was prepared according to the following procedure, pieces of *Herba Ephedrae* (Mahuang) 45 g, *Radix Glycyrrhizae Praeparata* (Zhigancao) 30 g, *Semen Armeniaceae Amarum* (Xingren) 45 g, *Gypsum Fibrosum* (Shengshigao) 75 g (fried first), *Ramulus Cinnamoni* (Guizhi) 45 g, *Rhizoma Alismatis* (Zixie) 45 g, *Polyporus* (Zhuling) 45 g, *Rhizoma Atractylodis Macrocephalae* (Baizhu) 45 g, *Poria* (Fuling) 75 g, *Radix Bupleuri* (Chaihu) 80 g, *Radix Scutellariae* (Huangqin) 30 g, *Rhizoma Pinelliae* prepared with ginger juice (Jiangbanxia) 45 g, *Rhizoma Zingiberis Recens* (Shengjiang) 45 g, *Radix Asteris* (Ziwan) 45 g, *Flos Farfarae* (Kuandonghua) 45 g, *Rhizoma Belamcandae* (Shegan) 45 g, *Herba Asari* (Xixin) 30 g, *Rhizoma Dioscoreae* (Shanyao) 60 g, *Fructus Aurantii Immaturus* (Zhishi) 30 g, *Pericarpium Citri Reticulatae* (Chenpi) 30 g and *Herba Agastachis* (Huoxiang) 45 g, were mixed together and macerated in 12 L deionized water for 40 min before being decocted. After then, 980 g of the herbal mixture was decocted for 45 min. A total of 100 batches of QFPDD were decocted repeatedly and then combined. After filtration, the decoction (~180 L) was further concentrated to 20 L by rotary evaporation at 40–50 °C in vacuum with a 93.98% concentrated yield. Finally, 6.52 kg powder was obtained by spray-drying with a 78.55% spray-dried yield.

Preparation of QFPDD extract

QFPDD (1.0 g) was weighed and dissolved in 125 mL deionized water by ultrasonic (250W, 50HZ) for 1 h. Then, the sample solution was centrifuged at 10000 × g for 15 min. The supernatant solution was filtered through a 0.22 μ m membrane for further analysis.

Animal handling and sampling

Twelve male C57BL/6J mice (SPF grade, weight of 20 ± 2 g) were purchased from Shanghai Slac Laboratory Animal Co. Ltd. (Shanghai, China) and were fed in Experimental Animal Center of Shanghai University of Traditional Chinese Medicine. All of animals were housed in an environment-controlled breeding room at 25 °C with a 12 hours dark/light cycle. All experiments performed in mice were in accordance with the State Committee of Science and Technology of P.R. of China, and the protocol for this study was approved and monitored by the Animal Ethics Committee of Shanghai University of Traditional Chinese Medicine (PZSHUTCM200717018).

After seven days of acclimatization, twelve male mice were randomly divided into two groups (6 mice for each group). QFPDD group was oral administered QFPDD (18 g dried material/kg); control group was oral administered deionized water. All animals were fasted overnight before the experiments and had free access to water. One hour after the once oral administration, all mice were anesthetized, blood samples were collected from the abdominal aorta, and their various tissues (lung, liver, heart, kidney, brain, spleen) were removed rapidly. Blood samples were placed at room temperature for 1 h until solidification. Then, serum samples were obtained by centrifuged at 5000 rpm for 15 min at 4 °C. All samples were stored at –80 °C until analysis. The serum samples (200 μ L) were added five times methanol (1 mL) was to the serum samples, vortexed and then, centrifuged at 10000 × g for 15 min. The supernatant (960 μ L) was dried with nitrogen gas. The residue was redissolved in 80 μ L 10% methanol, vortexed and then, centrifuged at 10000 × g for 15 min, and the filtrate was used as the LC/MS sample. The 100 mg various tissues (lung, liver, heart, kidney, brain, spleen) were homogenized in 1 mL methanol on ice, the homogenate of them was centrifuged at 4 °C at 10000 × g for 15 min, The supernatant (800 μ L) was dried with nitrogen gas. The residue was redissolved in 80 μ L 10% methanol,

vortexed and then, centrifuged at $10000 \times g$ for 15 min, and the filtrate was used as the LC/MS sample. 2 μL aliquot was injected for UHPLC-Q-Exactive Orbitrap HRMS analysis.

In vitro metabolism of the major constituents isolated from QFPDD in MLMs

Microsomal incubation with NADPH: The incubation was performed in a 50 mM Tris-HCl buffer (pH 7.4) containing MLMs (1.0 mg protein/mL), the NADPH-generating system contained 10 mM G-6-P, 1 mM NADP^+ , 4 mM MgCl_2 , and 1 unit/mL G-6-PDH, and 200 μM analyte (nobiletin) which was previously dissolved in methanol with concentration 200 μM in a total volume of 200 μL [18, 19]. The reactions were terminated with 600 μL ice-cold acetonitrile after 60 min incubation. The mixture was centrifuged at $10,000 \times g$ for 15 min, and then the supernatant (720 μL) was evaporated to dryness by a gentle stream of nitrogen (37 $^\circ\text{C}$). The residue was dissolved by 90 μL of 5% methanol and centrifuged at $10,000 \times g$ for 10 min. 2 μL of the supernatant was injected for UHPLC-Q-Exactive Orbitrap HRMS system. Control samples containing no NADPH or substrates were prepared. Incubation was performed in duplicate.

Microsomal incubation with NADPH and UDPGA: 200 μM analyte (baicalein, scutellarin, wogonin, norwogonin, formononetin, liquiritigenin, hesperetin, naringenin, quercetin, oroxylin A and caffeic acid, each compound was incubated separately) was mixed with alamethicin (25 $\mu\text{g}/\text{mg}$ protein) and the microsomes (1.0 mg protein/mL) in 100 mM Tris-HCl buffer (50 mM, pH 7.4). After 5 min of pre-incubation at 37 $^\circ\text{C}$, the incubation reaction was initiated by the addition of G-6-P (10 mM), G-6-PDH (1 unit/mL), NADP^+ (1.0 mM), and UDPGA (5.0 mM). The total incubation volume was 200 μL . The reactions were terminated with 600 μL ice-cold acetonitrile after 1 h of incubation. The mixture was centrifuged at $10,000 \times g$ for 15 min, and then the supernatant (720 μL) was evaporated to dryness by a gentle stream of nitrogen at 37 $^\circ\text{C}$. The residue was dissolved by 90 μL of 5% methanol and centrifuged at $10,000 \times g$ for 15 min. The supernatant (2 μL) was then injected for UHPLC-Q-Exactive Orbitrap HRMS system. Control samples without NADPH and UDPGA or substrates were prepared. Incubation was performed in duplicate.

Microsomal incubation with NADPH and PAPS: The incubation was performed in a 50 mM Tris-HCl buffer at pH 7.4 containing microsomes (1.0 mg protein/mL), the NADPH-generating system contained 10 mM G-6-P, 1 mM NADP^+ , 4 mM MgCl_2 , and 1 unit/mL of G-6-PDH, 100 μM PAPS, and 200 μM analyte (baicalein, wogonin, formononetin, liquiritigenin, hesperetin and caffeic acid, each compound was incubated separately) in a total volume of 200 μL . The reactions were terminated with 600 μL ice-cold acetonitrile after 60 min incubation. The next process was as same as above and the control samples containing no NADPH, no PAPS or substrates were prepared. Each of the incubations was performed in duplicate.

Microsoma incubation with NADPH and SAM: The incubation was performed in a 50 mM Tris-HCl buffer at pH 7.4 containing microsomes (1.0 mg protein/mL), the NADPH-generating system contained 10 mM G-6-P, 1 mM NADP^+ , 4 mM MgCl_2 , and 1 unit/mL of G-6-PDH, 100 μM SAM, and 200 μM analyte (baicalein, scutellarin, chlorogenic acid and caffeic acid, each compound was incubated separately) in a total volume of 200 μL . The reactions were terminated with 600 μL ice-cold acetonitrile after 60 min incubation. The next process was as same as above and the control samples containing no NADPH, no SAM or substrates were prepared. Each of the incubations was performed in duplicate.

Chromatography conditions for UHPLC-Q-Exactive Orbitrap HRMS

Chromatographic separation was performed on a UHPLC-Q-Exactive Orbitrap system (Thermo Fisher Scientific Inc., Grand Island, NY, USA). The UHPLC system consisted of a Thermo Scientific Dionex Ultimate 3000 Series RS pump coupled with a Thermo Scientific Dionex Ultimate 3000 Series TCC-3000RS column compartments and WPS-3000 autosampler controlled by Chromeleon 7.2 Software. The cooling autosampler was set at 4 $^\circ\text{C}$ and protected from light, and the column heater was set at 40 $^\circ\text{C}$. A Waters ACQUITY UPLC BEH C18 column (2.1 $\times$ 100 mm, 1.7 μm) was employed with the temperature set at 40 $^\circ\text{C}$. The mobile phase consisted of A (methanol) and B (0.1% formic acid) at a flow rate of $0.3 \text{ mL} \cdot \text{min}^{-1}$ and eluted with gradient elution: 0–4 min (4% A), 4–10 min (4%–12% A), 10–30 min (12%–70% A), 30–35 min (70% A), 35–38 min (70–95% A), 38–42 min (95% A), 42–45 min (4% A). The injection volume was 2 μL .

The mass spectrometer Q-Exactive Orbitrap system was connected to the UHPLC system *via* heated electrospray ionization and controlled by Xcalibur 4.1 software that was used for data collection and analysis. The electrospray ionization source was operated and optimized in negative and positive ionization mode. The optimized parameters of mass spectrometry were: capillary temperature: 325 $^\circ\text{C}$; sheath gas (N_2) flow rate: 45 arbitrary units; auxiliary gas (N_2) flow rate: 8 arbitrary units; sweep gas flow rate: 0 arbitrary units; spray voltage: 2.5 kV (negative), 3.5 kV (positive); S-lens RF level: 50V; auxiliary gas heater temperature, 300 $^\circ\text{C}$; scan mode: Full MS/SIM and Full MS/dd-MS2 mode, which includes 1 first-level full scan (resolution 70000 FWHM) and 1 data-dependent secondary scan (resolution 17500 FWHM) 2 events, the scanning range is 80–1200 m/z, and the collision energy gradient is 20V, 50V, 100V.

Establishment of QFPDD chemical database and chemical profiling

By searching databases, such as PubMed of the US National Library Medicine and the National Institutes of Health, SciFinder Scholar of American Chemical Society and the Chinese National Knowledge Infrastructure (CNKI) of Tsinghua University, the Encyclopedia of Traditional Chinese Medicine Database (ETCM, <http://www.tcmip.cn/ETCM/index.php/Home/Index/index.html>), Traditional Chinese Medicine Integrated Database (TCMID, <http://www.megabionet.org/tcmid/>), and all components reported in the literature on 21 herbs of QFPDD were summarized in a TraceFinder software (version 3.3) to establish a database, which includes the name, molecular formula, chemical structure and literature of each published known compound. After the sample was injected, the quasi-molecular ion information was obtained, and the molecular formula was fitted by Xcalibur 3.0 software, and matched with the CD (Compound discovery, 2.1) compound analysis and identification software and self-built database, and the possible chemical composition was inferred. Combined with the relative retention time and fragment ion information of reference substances and literature, the chemical composition was identified.

Results

Identification and characterization of chemical constituents from QFPDD

Under the optimal conditions described above, the constituents in the crude extract of QFPDD were comprehensively analyzed by UHPLC-Q-Exactive Orbitrap HRMS, with a running time of 45 min. The total ion chromatograms (TICs) of the extract of QFPDD under both positive and negative ion modes are shown in Fig. 1. The protonated molecular weights of all identified compounds were calculated within an error of 10 ppm. Following carefully comparison with the retention times and MS/MS spectra of the reference standards, reference literature, Chemical Book and self-bulit databases, a total of 405 chemicals were identified or tentatively characterized from QFPDD, including 40 kinds of alkaloids, 162 kinds of flavonoids, 44 kinds of organic acids, 71 kinds of triterpene saponins and 88 kinds of other compounds (Table 1). The detailed information of the identified chemical constituents in QFPDD including retention time, precise molecular weight, MS/MS fragment ions and the source of each constituent, have been listed in Table 1. Among all identified chemical constituents, ninety-one constituents are confirmed by the authentic standards.

Table 1
Ingredient identification of QFPDD by UHPLC-Q-Exactive Orbitrap HRMS

No.	RT /min	Ion model	Measured mass /Da	Calculated mass /Da	Error /ppm	Molecular formula	Identification	Fragment ions
1*	0.85	[M-H] ⁻	173.1035	173.1033	1.085	C ₆ H ₁₄ N ₄ O ₂	Arginine	131.0825; 96.9672
2	0.88	[M + FA-H] ⁻	195.0502	195.0499	1.491	C ₅ H ₁₀ O ₅	Arabinose	195.0561; 179.0589; 165.04151.0635; 135.0313; 75.00971.0117;
3	0.89	[M + H] ⁺	118.0865	118.0863	0.205	C ₅ H ₁₁ NO ₂	Betaine	58.0655
4	0.90	[M-H] ⁻	149.0080	149.0081	-0.084	C ₄ H ₆ O ₆	Tartaric acid	149.0092; 87.0087; 72.992759.0134
5	0.90	[M-H] ⁻	157.0356	157.0356	-0.017	C ₄ H ₆ O ₃ N ₄	Allantoin	59.03; 97.00; 71.03;
6	0.90	[M-H] ⁻	179.0552	179.0550	0.980	C ₆ H ₁₂ O ₆	Glucose	179.0570; 161.0461; 87.00971.0137
7	0.91	[M + H] ⁺	138.0549	138.0550	-0.085	C ₇ H ₇ NO ₂	Trigonelline	92.05; 78.03; 65.04; 51.02
8*	0.92	[M-H] ⁻	191.0553	191.0550	1.389	C ₇ H ₁₂ O ₆	Quinic acid	191.0563; 85.0297
9*	0.95	[M + H] ⁺	144.1018	144.1019	-0.125	C ₇ H ₁₃ NO ₂	Stachydrine	144.1022; 84.0807; 56.0491
10	0.96	[M-H] ⁻	207.0141	207.0135	2.543	C ₆ H ₈ O ₈	Hydroxycitric acid	207.0082; 189.0049; 127.0083.0146
11	0.98	[M-H] ⁻	341.1091	341.1078	3.788	C ₁₂ H ₂₂ O ₁₁	Sucrose	179.0563; 161.0456; 119.0389.0249
12	1.02	[M + FA-H] ⁻	549.1677	549.1661	2.767	C ₁₈ H ₃₂ O ₁₆	Raffinose	503.1624; 221.0672; 179.05
13	1.05	[M-H] ⁻	115.0023	115.0026	-2.479	C ₄ H ₄ O ₄	Maleic acid	71.0142
14	1.05	[M-H] ⁻	133.0129	133.0131	-0.230	C ₄ H ₆ O ₅	Malic acid	115.0054; 71.0150
15	1.06	[M + NH ₄] ⁺	136.0617	136.0604	1.276	C ₄ H ₆ O ₄	Succinic acid	136.0609; 92.0239; 72.992465.0131
16	1.13	[M + FA-H] ⁻	711.2212	711.2190	3.130	C ₂₄ H ₄₂ O ₂₁	Maltotetraose isomer	665.2108; 485.1475; 443.14383.1172; 341.1066
17	1.14	[M + H] ⁺	118.0864	118.0863	0.175	C ₅ H ₁₁ NO ₂	Valine	72.0805; 55.0539
18	1.27	[M + Na] ⁺	851.2632	851.2639	-0.778	C ₃₀ H ₅₂ O ₂₆	Pentaose	851.2523; 527.1444
19	1.28	[M-H] ⁻	827.2685	827.2663	2.638	C ₃₀ H ₅₂ O ₂₆	Maltopentaose	647.2048; 503.1619; 341.10323.1004; 179.0565; 161.04
20	1.31	[M + H] ⁺	123.0554	123.0553	0.131	C ₆ H ₆ N ₂ O	Nicotinamide	78.03; 63.01; 53.04
21*	1.34	[M-H] ⁻	191.0183	191.0186	1.052	C ₆ H ₈ O ₇	Citric acid	191.0203; 111.0100; 87.009
22	1.39	[M + H] ⁺	127.0390	127.0390	0.049	C ₆ H ₆ O ₃	5-Hydroxymethylfurfural	127.0384; 109.0276; 81.03368.9974
23	1.42	[M-H] ⁻	1151.3737	1151.3720	1.481	C ₄₂ H ₇₂ O ₃₆	Fructo-oligosaccharide Dp7/Gf6	1151.3765; 1031.3220; 989.827.2672
24	1.44	[M-H] ⁻	827.2687	827.2663	2.868	C ₃₀ H ₅₂ O ₂₆	Fructo-oligosaccharide Dp5/Gf4	827.2575; 503.1631; 179.05
25	1.46	[M-H] ⁻	989.3212	989.3191	2.122	C ₃₆ H ₆₂ O ₃₁	Maltohexaose,fructo-oligosaccharide Dp6/Gf5	989.3319; 827.2717; 665.21503.1650; 341.1082
26	1.48	[M + H] ⁺	130.0499	130.0499	0.010	C ₅ H ₇ NO ₃	Pyroglutamic acid	84.0441; 56.0490
27	1.55	[M + FA-H] ⁻	711.2216	711.2190	3.650	C ₂₄ H ₄₂ O ₂₁	Maltotetraose	665.2161; 545.1857; 341.10179.0555; 89.0249

No.	RT /min	Ion model	Measured mass /Da	Calculated mass /Da	Error /ppm	Molecular formula	Identification	Fragment ions
28*	1.57	[M-H] ⁻	243.0621	243.0612	3.692	C ₉ H ₁₂ N ₂ O ₆	Uridine	128.0393; 110.0238
29*	1.58	[M + H] ⁺	113.0348	113.0346	2.177	C ₄ H ₄ N ₂ O ₂	Uracil	113.0369; 96.0086; 80.9743 68.0126; 52.0186
30	1.61	[M + FA-H] ⁻	549.1677	549.1661	2.876	C ₁₈ H ₃₂ O ₁₆	Melezitose	503.1603; 323.0967; 179.05 89.0243
31*	1.64	[M + H] ⁺	182.0811	182.0812	-0.438	C ₉ H ₁₁ NO ₃	Tyrosine	134.06; 107.05; 91.05; 77.04 65.04
32*	1.72	[M-H] ⁻	117.0197	117.0182	-2.266	C ₄ H ₆ O ₄	Succinic acid	73.0294; 55.0216
33	1.79	[M + Na] ⁺	289.1391	289.1410	-6.780	C ₁₅ H ₂₂ O ₄	4-Gingerol	177.1019; 163.0854; 145.07 133.0757
34	1.8	[M-H] ⁻	115.0023	115.0026	-2.218	C ₄ H ₄ O ₄	Fumaric acid or its isomers	115.004; 99.9255; 83.9301; 53.0040
35	1.85	[M + NH ₄] ⁺	194.1175	194.1176	-0.079	C ₁₁ H ₁₂ O ₂	Ethyl cinnamate	115.0548; 103.0547; 77.0391
36*	1.95	[M + H] ⁺	132.1019	132.1019	-0.025	C ₆ H ₁₃ NO ₂	Leucine or Isoleucine	132.1016; 86.0961
37	1.96	[M + H] ⁺	152.1070	152.1070	-0.041	C ₉ H ₁₃ NO	N-Methyltyramine	103.0549; 91.0546; 77.0387
38	2.06	[M + H] ⁺	166.1225	166.1226	-0.111	C ₁₀ H ₁₅ NO	Hordenine	121.0671; 103.0556; 91.055
39*	2.08	[M + H] ⁺	136.0616	136.0618	-0.142	C ₅ H ₅ N ₅	Adenine	136.0616; 119.0355; 92.023
40*	2.09	[M-H] ⁻	169.0133	169.0131	0.948	C ₇ H ₆ O ₅	Gallic acid	169.0127; 151.0020; 125.02 97.0277; 81.0327; 69.0327
41*	2.29	[M + H] ⁺	137.0457	137.0458	-0.067	C ₅ H ₄ N ₄ O	6-Hydroxypurine	137.0442; 119.0353; 110.03 94.0384
42*	2.29	[M + H] ⁺	152.0565	152.0567	-0.196	C ₅ H ₅ N ₅ O	Guanine	152.0670; 135.0294
43*	2.30	[M-H] ⁻	282.0846	282.0833	4.733	C ₁₀ H ₁₃ N ₅ O ₅	Guanosine	282.0879; 150.0432; 133.01 108.0211
44*	2.61	[M-H] ⁻	266.0896	266.0884	4.696	C ₁₀ H ₁₃ N ₅ O ₄	Adenosine	134.0485; 107.0382; 92.025
45*	2.91	[M + H] ⁺	168.1018	168.1019	-0.507	C ₉ H ₁₃ NO ₂	Synephrine	168.10; 150.09; 135.07; 119 91.05
46*	3.26	[M-H] ⁻	283.0685	283.0673	4.237	C ₁₀ H ₁₂ N ₄ O ₆	Xanthosine	283.0676; 151.0267; 108.02
47*	3.79	[M + H] ⁺	166.0861	166.0863	-0.165	C ₉ H ₁₁ NO ₂	L-Phenylalanine	120.0798; 103.0532; 91.053 79.0539; 77.0377;
48	3.81	[M + H] ⁺	120.0809	120.0808	0.144	C ₈ H ₉ N	N-Benzylidenemethylamine	120.0804; 103.0524; 91.053
49	4.10	[M-H] ⁻	305.0669	305.0656	4.428	C ₁₅ H ₁₄ O ₇	Leucocyanidin	305.0627; 179.0433; 167.03
50	4.33	[M-H] ⁻	153.0182	153.0182	-0.065	C ₇ H ₆ O ₄	Protocatechuic acid	109.0290; 108.0220; 91.019
51	4.70	[M-H] ⁻	315.0725	315.0711	4.512	C ₁₃ H ₁₆ O ₉	Gentisic acid glycoside	315.0716; 152.0116; 108.02
52*	5.18	[M + H] ⁺	152.1069	152.1070	-0.101	C ₉ H ₁₃ NO	Phenylpropanolamine	152.0574; 135.0303; 121.06 77.0377
53*	6.15	[M + H] ⁺	152.1069	152.1070	-0.131	C ₉ H ₁₃ NO	D-Demethyl pseudoephedrine	134.0962; 115.0555; 91.048 65.0371
54	6.16	[M-H] ⁻	331.1038	331.1024	4.233	C ₁₄ H ₂₀ O ₉	2,6-Dimethoxy-4-hydroxyphenol-1-O-β-D-glucopyranoside	168.0414; 153.0187; 123.01
55*	6.66	[M-H] ⁻	167.0340	167.0339	0.687	C ₈ H ₈ O ₄	Vanillic acid	152.0116; 108.0183

No.	RT /min	Ion model	Measured mass /Da	Calculated mass /Da	Error /ppm	Molecular formula	Identification	Fragment ions
56*	7.30	[M + H] ⁺	166.1225	166.1226	-0.171	C ₁₀ H ₁₅ NO	Ephedrine	166.1225; 148.1119; 133.08117.0701; 91.0548
57	7.71	[M + FA-H] ⁻	520.1673	520.1661	2.344	C ₂₀ H ₂₉ NO ₁₂	L-α-[(6- <i>O</i> -β-D-Glucopyranosyl-β-D-glucopyranosyl)oxy]benzeneacetamide	474.1613; 150.0584
58*	7.75	[M-H] ⁻	203.0819	203.0815	2.146	C ₁₁ H ₁₂ N ₂ O ₂	Tryptophan	203.0784; 159.0957; 142.06116.0516; 74.0254
59*	7.77	[M-H] ⁻	353.0880	353.0867	3.601	C ₁₆ H ₁₈ O ₉	Neochlorogenic acid	353.0857; 191.0563; 179.03135.0454
60	7.99	[M-H] ⁻	151.0390	151.0390	0.029	C ₈ H ₈ O ₃	Vanillin	109.3493; 125.0762; 65.005
61	8.05	[M-H] ⁻	181.0497	181.0495	1.131	C ₉ H ₁₀ O ₄	Syringaldehyde	135.9790; 117.0358
62*	8.33	[M + H] ⁺	166.1225	166.1226	-0.141	C ₁₀ H ₁₅ NO	Pseudoephedrine	148.110; 133.08777; 117.0691.0534
63	8.49	[M + FA-H] ⁻	520.1672	520.1661	2.228	C ₂₀ H ₂₉ NO ₁₂	D-α-[(6- <i>O</i> -β-D-Glucopyranosyl-β-D-glucopyranosyl)oxy]benzeneacetamide	474.1635; 313.1103; 150.05
64*	8.62	[M + H] ⁺	180.1381	180.1383	-1.281	C ₁₁ H ₁₇ NO	Methylephedrine	180.1393; 1262.1267; 147.1117.0702; 91.0537
65	8.73	[M-H] ⁻	285.0618	285.0605	4.582	C ₁₂ H ₁₄ O ₈	Uralenneoside	153.0183; 108.0215
66	8.79	[M + H] ⁺	447.1470	447.1497	-6.044	C ₁₉ H ₂₆ O ₁₂	Cinnacasolide A	317.0819;
67	8.92	[M-H] ⁻	165.0546	165.0546	0.009	C ₉ H ₁₀ O ₃	Phloretic acid	119.0506; 93.0343
68	9.22	[M-H] ⁻	577.1357	577.1341	2.924	C ₃₀ H ₂₆ O ₁₂	Procyanidin B1	425.0916; 407.0778; 289.07219.0668; 205.0510; 161.02137.0277; 125.0255;
69	9.39	[M-H] ⁻	475.1460	475.1446	2.910	C ₂₀ H ₂₈ O ₁₃	L-Amygdalinic acid	475.1452; 431.1560; 269.10161.0453; 101.0250
70	10.43	[M + H] ⁺	272.1278	272.1281	-1.359	C ₁₆ H ₁₇ NO ₃	Higenamine	107.0512; 115.0564; 143.05161.0626
71	10.53	[M-H] ⁻	121.0282	121.0284	-0.246	C ₇ H ₆ O ₂	4-Hydroxybenzaldehyde	121.0305; 92.0251
72	10.65	[M-H] ⁻	577.1358	577.1341	3.028	C ₃₀ H ₂₆ O ₁₂	Procyanidin B3	451.1160; 407.0712; 289.07125.0252;
73*	10.86	[M-H] ⁻	289.0719	289.0707	4.377	C ₁₅ H ₁₄ O ₆	Catechin	289.0702; 245.0818; 123.04109.0297
74	10.99	[M + H] ⁺	217.0970	217.0972	-0.572	C ₁₂ H ₁₂ N ₂ O ₂	Anoectochine	217.0973; 169.0758; 141.11144.0827; 127.0560;
75	11.08	[M-H] ⁻	463.1462	463.1446	3.439	C ₁₉ H ₂₈ O ₁₃	Cinnacassoside C	293.1080; 233.0642; 149.04139.0032;
76	11.60	[M-H] ⁻	303.0512	303.0499	4.128	C ₁₅ H ₁₂ O ₇	(2R,3R)-3,5,7,2',6' - Pentahydroxyflavanone	303.0511; 217.0537; 177.02149.0245; 125.0250
77	11.9	[M-H] ⁻	299.0775	299.0761	4.468	C ₁₃ H ₁₆ O ₈	Salicylic acid 2- <i>O</i> -β-D-glucoside	299.0766; 137.0248; 93.034
78	11.95	[M-H] ⁻	475.1459	475.1446	2.700	C ₂₀ H ₂₈ O ₁₃	D-Amygdalinic acid	475.1545; 431.1581; 151.03101.0259
79*	12.03	[M-H] ⁻	179.0340	179.0339	0.865	C ₉ H ₈ O ₄	Caffeic acid	135.045
80	12.06	[M + H] ⁺	137.1072	137.1073	-0.145	C ₈ H ₁₂ N ₂	Ligustrazine	137.1072; 122.0850; 96.08080.0483; 69.0692; 55.0549
81*	12.51	[M-H] ⁻	353.0880	353.0867	3.516	C ₁₆ H ₁₈ O ₉	Chlorogenic acid	353.0883; 191.0549; 179.03173.0442; 161.0228; 155.03135.0435
82	12.59	[M + FA-H] ⁻	535.1671	535.1657	2.436	C ₂₁ H ₃₀ O ₁₃	Tectoruside	165.0578
83	12.98	[M + FA-H] ⁻	373.1142	373.1129	3.502	C ₁₅ H ₂₀ O ₈	Androsin	165.0574; 150.0343

No.	RT /min	Ion model	Measured mass /Da	Calculated mass /Da	Error /ppm	Molecular formula	Identification	Fragment ions
84	13.18	[M-H] ⁻	367.1035	367.1024	3.082	C ₁₇ H ₂₀ O ₉	3-Feruloylquinic acid	134.0387; 193.0530; 117.03191.0588
85	13.51	[M-H] ⁻	577.1355	577.1341	2.508	C ₃₀ H ₂₆ O ₁₂	Procyanidin B2	451.1007; 425.0845; 407.08289.0729; 161.0264; 125.02
86	13.62	[M + FA-H] ⁻	293.1244	293.1231	4.421	C ₁₁ H ₂₀ O ₆	Pent-4-enyl-D-glucopyranoside	293.1216;
87*	13.74	[M-H] ⁻	353.0880	353.0867	3.516	C ₁₆ H ₁₈ O ₉	Cryptochlorogenic acid	353.0891; 191.0572; 179.03173.0470; 135.0459
88	13.80	[M-H] ⁻	443.1924	443.1912	2.655	C ₂₁ H ₃₂ O ₁₀	Dihydrophaseic acid 4'-O-β-glucopyranoside	443.1951; 119.0356; 89.02459.0144
89	13.82	[M + FA-H] ⁻	493.2294	493.2280	2.873	C ₂₁ H ₃₆ O ₁₀	Atractyloside A	447.2232; 285.1700; 179.05131.0359; 119.0362;
90*	13.93	[M + FA-H] ⁻	502.1568	502.1555	2.616	C ₂₀ H ₂₇ NO ₁₁	Amygdalin	502.1567; 323.0972; 263.07221.0661; 179.0550; ; 161.0119.0335; 101.0229; 89.022
91	14.07	[M + FA-H] ⁻	477.1616	477.1603	2.709	C ₁₉ H ₂₈ O ₁₁	Benzyl-β-gentiobioside	431.157269.103161.04510
92	14.16	[M-H] ⁻	431.1559	431.1548	2.463	C ₁₉ H ₂₈ O ₁₁	Osmanthuside H or isomer	431.1150; 125.0234
93*	14.22	[M-H] ⁻	294.0985	294.0972	4.510	C ₁₄ H ₁₇ NO ₆	Prunasin	161.0435; 101.0242; 85.03071.0141
94	14.38	[M + H] ⁺	193.0857	193.0859	-0.936	C ₁₁ H ₁₂ O ₃	Myristicin	133.0628; 147.0830
95	14.73	[M + H] ⁺	179.0337	179.0339	-0.923	C ₉ H ₆ O ₄	4,7-Dihydroxycoumarin	179.03354; 163.03850; 145.02814
96*	15.04	[M-H] ⁻	289.0720	289.0707	4.481	C ₁₅ H ₁₄ O ₆	Epicatechin	289.0723; 245.0813; 188.04123.0453
97	15.22	[M + H] ⁺	179.0336	179.0339	-1.481	C ₉ H ₆ O ₄	6,7-Dihydroxycoumarin	179.03357; 163.03873; 145.02832
98*	15.29	[M-H] ⁻	515.1197	515.1184	2.499	C ₂₅ H ₂₄ O ₁₂	1,3-O-Dicaffeoylquinic acid	515.1199; 353.0858; 335.07191.0562
99	15.36	[M + H] ⁺	303.0495	303.0499	-1.317	C ₁₅ H ₁₀ O ₇	Herbacetin or 3,5,7,2,6-pentahydroxyflavone	303.0483; 257.0466; 229.04153.0187; 149.0241
100	15.55	[M + H] ⁺	251.1024	251.1026	-1.089	C ₁₂ H ₁₄ N ₂ O ₄	Crotonine A	251.1026; 233.0930; 215.08
101	15.58	[M-H] ⁻	163.0391	163.0390	0.139	C ₉ H ₈ O ₃	p-Coumaric acid	119.0518; 93.0347
102	15.79	[M + FA-H] ⁻	502.1570	502.1555	2.975	C ₂₀ H ₂₇ NO ₁₁	Amygdalin isomer	456.1532; 323.0988; 221.06161.0460; 119.0357; 89.024
103	15.89	[M-H] ⁻	295.1189	295.1176	4.355	C ₁₅ H ₂₀ O ₆	Rosin	161.0453; 133.0663; 113.02101.0239
104	15.92	[M-H] ⁻	161.0233	161.0233	0.009	C ₉ H ₆ O ₃	Umbelliferone	133.0308; 105.0341; 77.03989.0406; 65.0037
105	16	[M-H] ⁻	207.0293	207.0288	2.561	C ₁₀ H ₈ O ₅	Fraxetin	207.0323; 164.5855; 108.02
106	16.02	[M + FA-H] ⁻	711.2151	711.2190	-5.447	C ₂₄ H ₄₂ O ₂₁	Stachyose	665.2138; 485.1515; 341.10179.0557
107	16.2	[M-H] ⁻	421.0779	421.0765	3.330	C ₁₉ H ₁₈ O ₁₁	Mangiferin	331.0461; 301.0361; 272.03
108	16.29	[M-H] ⁻	327.1090	327.1074	3.932	C ₁₅ H ₂₀ O ₈	Dihydrocinnacasside	165.0569; 147.0449; 121.06106.0451;
109	16.34	[M + H] ⁺	625.1759	625.1763	-0.659	C ₂₈ H ₃₂ O ₁₆	Stellarin-2	625.1707; 607.1640; 589.15487.1207
110	16.38	[M + H] ⁺	336.1803	336.1805	-0.653	C ₁₈ H ₂₅ NO ₅	Senecionine	308.1788; 138.0912; 120.0894.0642

No.	RT /min	Ion model	Measured mass /Da	Calculated mass /Da	Error /ppm	Molecular formula	Identification	Fragment ions
111	16.49	[M-H] ⁻	367.1036	367.1024	3.491	C ₁₇ H ₂₀ O ₉	4-Feruloylquinic acid	193.0522; 191.0578; 173.04134.0387; 93.0348
112	16.65	[M + H] ⁺	566.2010	566.2012	-0.401	C ₂₅ H ₃₂ N ₅ O ₈ Cl	Astin L	566.2131; 423.1469
113	16.75	[M-H] ⁻	367.1036	367.1024	3.491	C ₁₇ H ₂₀ O ₉	5-Feruloylquinic acid	191.0583; 173.0485; 134.0393.0346;
114	16.75	[M-H] ⁻	577.1357	577.1341	2.924	C ₃₀ H ₂₆ O ₁₂	Procyanidin B4	559.1291; 451.1081; 425.08407.0738; 289.0696; 273.04201.0526; 125.0234;
115	16.82	[M + H] ⁺	193.0494	193.0495	-0.753	C ₁₀ H ₈ O ₄	Isoscooletin	178.0294; 150.0337; 133.03122.0383
116	17.03	[M-H] ⁻	593.1517	593.1501	2.653	C ₂₇ H ₃₀ O ₁₅	Vicenin-li	593.1520; 503.1167; 473.10383.0748; 353.0645
117*	17.36	[M + H] ⁺	195.0649	195.0652	-1.258	C ₁₀ H ₁₀ O ₄	Ferulic acid	177.0534; 145.0281; 117.0389.0380
118	17.36	[M + H] ⁺	179.0336	179.0339	-1.593	C ₉ H ₆ O ₄	5,7-Dihydroxycoumarin	179.03358; 163.03880; 145.02818
119	17.5	[M + H] ⁺	251.1387	251.1642	-1.398	C ₁₅ H ₂₂ O ₃	1β,11-Dihydroxy-β-cyperone	72.0801; 84.9591; 110.0605140.9194; 198.1117; 219.04180.9492;
120	17.60	[M + H] ⁺	625.1758	625.1763	-0.850	C ₂₈ H ₃₂ O ₁₆	4'-Methoxy-lucenin-2	625.1632; 607.1759; 589.15487.1216
121	17.66	[M-H] ⁻	459.1149	459.1133	3.382	C ₁₉ H ₂₄ O ₁₃	Parishin E	307.0650; 208.0377; 164.05151.0398; 101.0250;
122	17.69	[M + H] ⁺	223.0600	223.0601	-0.672	C ₁₁ H ₁₀ O ₅	Saikochromone A or its isomers	223.0623; 207.0304; 190.02179.0358; 162.0332; 134.03105.0341; 78.0469
123	17.73	[M + H] ⁺	403.1360	403.1387	-6.832	C ₂₁ H ₂₂ O ₈	Hexamethoxyfalcone	403.1390; 388.1170; 373.01327.0863
124	17.85	[M + H] ⁺	366.1909	366.1911	-0.612	C ₁₉ H ₂₇ NO ₆	Senkirkine or its isomer	366.1893; 168.1009; 150.09122.0960
125	17.86	[M-H] ⁻	563.1410	563.1395	2.589	C ₂₆ H ₂₈ O ₁₄	8/6-C-Hexosyl-6/8-C-pentasy1 apigenin	563.1415; 473.1092; 383.07353.0667; 297.0743
126	17.99	[M-H] ⁻	435.0934	435.0922	2.878	C ₂₀ H ₂₀ O ₁₁	7-O-Methylmangiferin	315.0524; 287.0221; 272.03
127*	18.01	[M-H] ⁻	417.1195	417.1180	3.527	C ₂₁ H ₂₂ O ₉	Neoliquiritin	417.1194; 255.0803; 135.00119.0497
128	18.15	[M-H] ⁻	581.2243	581.2229	2.465	C ₂₈ H ₃₈ O ₁₃	(+)-Lyoniresinol 9'-O-glucoside	419.1663; 404.1436; 343.13233.0878; 202.0693; 153.05119.0358;
129*	18.23	[M-H] ⁻	137.0232	137.0233	-0.111	C ₇ H ₆ O ₃	3,4-Dihydroxybenzaldehyde	137.0242;
130	18.24	[M-H] ⁻	295.0459	295.0448	3.580	C ₁₃ H ₁₂ O ₈	Coumaroyltartaric acid	173.0106; 154.9996; 111.00
131*	18.30	[M-H] ⁻	447.0935	447.0922	3.024	C ₂₁ H ₂₀ O ₁₁	Luteolin-7-O-β-D-glucoside	447.0961; 357.0596; 327.04285.0434; 133.0314
132	18.40	[M-H] ⁻	563.1409	563.1395	2.376	C ₂₆ H ₂₈ O ₁₄	Schaftoside	563.1418; 503.1114; 383.07353.0676
133	18.51	[M-H] ⁻	415.1613	415.1599	3.316	C ₁₉ H ₂₈ O ₁₀	Icariside Dc	397.2059; 233.0752; 191.05161.0462; 149.0445; 119.0399.0075; 89.0250;
134	18.51	[M-H] ⁻	549.1617	549.1603	2.682	C ₂₆ H ₃₀ O ₁₃	Naringenin 7-O-(2-β-D-Apiofuranosyl)-β-D-glucopyranoside	549.1621; 429.1106; 255.06135.0100
135*	18.59	[M-H] ⁻	417.1195	417.1180	3.527	C ₂₁ H ₂₂ O ₉	Liquiritin	417.1195; 255.0654; 153.01135.0071; 119.0484
136	18.71	[M + H] ⁺	366.1907	366.1911	-1.131	C ₁₉ H ₂₇ NO ₆	Senkirkine or its isomer	366.1892; 168.1018; 150.09122.0595

No.	RT /min	Ion model	Measured mass /Da	Calculated mass /Da	Error /ppm	Molecular formula	Identification	Fragment ions
137	18.82	[M-H] ⁻	595.1673	595.1657	2.610	C ₂₇ H ₃₂ O ₁₅	Neeroicitrin	595.1671; 459.1117; 287.05151.0031; 135.0442
138	18.85	[M + H] ⁺	550.2059	550.2063	-0.786	C ₂₅ H ₃₂ N ₅ O ₇ Cl	Astin H or astin E	550.2035; 131.0473
139	18.86	[M-H] ⁻	741.2260	741.2237	3.096	C ₃₃ H ₄₂ O ₁₉	Naringenin-7-O-triglycoside	741.2304; 621.1685; 339.07271.0611; 151.0034
140	18.94	[2M-H] ⁻	279.0146	279.0135	3.750	C ₆ H ₄ O ₄	Coumaric acid	132.0226; 104.0295; 95.950
141	18.96	[M-H] ⁻	549.1617	549.1603	2.573	C ₂₆ H ₃₀ O ₁₃	Liquiritin apioside	549.1631; 255.0669; 135.00
142*	18.98	[M-H] ⁻	431.0985	431.0973	2.799	C ₂₁ H ₂₀ O ₁₀	Vitexin	431.0943; 283.0475; 249.06181.0308; 102.9854
143*	19.24	[M-H] ⁻	515.1196	515.1184	2.383	C ₂₅ H ₂₄ O ₁₂	Isochlorogenic acid B	515.1236; 353.0895; 191.05179.0356; 173.0463
144	19.28	[M-H] ⁻	595.1673	595.1657	2.610	C ₂₇ H ₃₂ O ₁₅	Eriocitrin	595.1664; 459.1188; 287.05
145	19.34	[M-H] ⁻	547.1461	547.1446	2.637	C ₂₆ H ₂₈ O ₁₃	Chrysin 6-C-Pen-8-C-Hex	547.1464; 457.1224; 427.10367.0862; 337.0725;
146	19.36	[M-H] ⁻	463.1252	463.1235	3.654	C ₂₂ H ₂₄ O ₁₁	Hesperetin-O-glucoside	463.1165; 337.0927; 160.01125.0244; 83.0141
147	19.44	[M-H] ⁻	563.1409	563.1395	2.483	C ₂₆ H ₂₈ O ₁₄	Isoschaftoside	563.1441; 44.1003; 353.068297.0770
148	19.51	[M-H] ⁻	547.1459	547.1446	2.418	C ₂₆ H ₂₈ O ₁₃	Chrysin-6-C-α-L-Arabinoside-8-C-β-D-glucoside	547.1482; 457.1150; 427.09367.0823; 337.0723;
149	19.52	[M + H] ⁺	502.2657	502.2660	-0.697	C ₂₅ H ₃₅ N ₅ O ₆	Astin G	502.2646; 270.1459; 155.12
150	19.6	[M + H] ⁺	301.0700	301.0707	-2.075	C ₁₆ H ₁₂ O ₆	Pratensein	285.0422; 151.0406; 134.03153.0194
151	19.6	[M + H] ⁺	301.0704	301.0707	-2.075	C ₁₆ H ₁₂ O ₆	Rhamnocitrin	231.0673; 167.0357
152	19.60	[M + H] ⁺	463.1228	463.1235	-1.485	C ₂₂ H ₂₂ O ₁₁	Diosmetin-7-O-glucoside	463.0650; 301.0707; 286.04
153*	19.61	[M-H] ⁻	461.1093	461.1078	3.128	C ₂₂ H ₂₂ O ₁₁	Tectoridin	461.1084; 299.0548; 283.02255.0275
154	19.67	[M-H] ⁻	147.0440	147.0441	-0.046	C ₉ H ₈ O ₂	2-Hydroxycinnamaldehyde	119.0516
155*	19.69	[M + H] ⁺	433.1122	433.1129	-1.577	C ₂₁ H ₂₀ O ₁₀	Isovitexin	397.0962; 313.0708; 283.05
156*	19.70	[M + H] ⁺	465.1026	465.1028	-0.328	C ₂₁ H ₂₀ O ₁₂	Hyperin	465.1013; 303.0503; 169.01
157	19.73	[M-H] ⁻	771.2359	771.2342	2.178	C ₃₄ H ₄₄ O ₂₀	Alpha-glucosyl hesperidin	771.2362; 301.0737
158*	19.74	[M-H] ⁻	515.1193	515.1184	1.781	C ₂₅ H ₂₄ O ₁₂	Isochlorogenic acid A	353.0903; 191.0578; 135.04
159	19.75	[M + H] ⁺	449.1077	449.1078	-0.307	C ₂₁ H ₂₀ O ₁₁	Kaempferol-3-O-glucoside	449.1073; 287.0536
160	19.77	[M + H] ⁺	463.1229	463.1235	-1.356	C ₂₂ H ₂₂ O ₁₁	Tectorigenin 4'-glucoside	301.0773; 286.0538
161	19.84	[M-H] ⁻	341.1243	341.1231	3.623	C ₁₆ H ₂₂ O ₈	Coniferin	179.0587
162	19.98	[M-H] ⁻	577.1567	577.1552	2.700	C ₂₇ H ₃₀ O ₁₄	Isoviolanthin	577.1571; 413.0846; 293.04
163*	19.99	[M-H] ⁻	461.0728	461.0715	2.880	C ₂₁ H ₁₈ O ₁₂	Scutellarin	461.0726; 285.0405
164*	20.00	[M + H] ⁺	303.0494	303.0499	-1.911	C ₁₅ H ₁₀ O ₇	Quercetin	303.0488; 229.0475; 153.01
165*	20.00	[M + H] ⁺	465.1023	465.1028	-1.059	C ₂₁ H ₂₀ O ₁₂	Isoquercitrin	465.1026; 303.0481; 289.06

No.	RT /min	Ion model	Measured mass /Da	Calculated mass /Da	Error /ppm	Molecular formula	Identification	Fragment ions
166	20.01	[M-H] ⁻	609.1466	609.1450	2.625	C ₂₇ H ₃₀ O ₁₆	Rutin	609.1458; 301.0358; 271.0225.0320
167	20.02	[M + H] ⁺	611.1598	611.1607	-1.425	C ₂₇ H ₃₀ O ₁₆	Lucenin-2	557.1270; 353.0646; 473.09
168*	20.05	[M-H] ⁻	623.1984	623.1970	2.107	C ₂₉ H ₃₆ O ₁₅	Verbascoside	623.2017; 461.1658; 179.03161.0240; 135.0455
169	20.06	[M + H] ⁺	493.1336	493.1341	-0.958	C ₂₃ H ₂₄ O ₁₂	Iristectorin B	343.0798; 331.0802; 316.05301.0328
170	20.09	[M-H] ⁻	433.1140	433.1129	2.090	C ₂₁ H ₂₂ O ₁₀	Chalconaringenin 4-O-glucoside	271.0599; 177.0182; 151.00119.0492
171*	20.20	[M + H] ⁺	229.0856	229.0859	-1.269	C ₁₄ H ₁₂ O ₃	Resveratrol	152.061; 107.0472; 91.0527
172	20.21	[M-H] ⁻	597.1828	597.1814	2.350	C ₂₇ H ₃₄ O ₁₅	Phloretin-3',5'-di-C-glucoside	387.1080; 357.0981
173	20.27	[M + H] ⁺	581.1857	581.1865	-1.397	C ₂₇ H ₃₂ O ₁₄	Isoliquiritigenin-4,4'-diglucoside	257.0854
174	20.31	[M-H] ⁻	579.1725	579.1708	2.700	C ₂₇ H ₃₂ O ₁₄	Narirutin	579.1727; 543.2543; 295.06271.0613; 151.0020
175	20.43	[M + H] ⁺	465.1025	465.1028	-0.521	C ₂₁ H ₂₀ O ₁₂	Quercetin-4-O-β-D-glucoside,Herbacetin-7-O-β-D-glucoside	465.0985; 303.0488
176	20.57	[M-H] ⁻	547.1459	547.1446	2.290	C ₂₆ H ₂₈ O ₁₃	Chrysin 6-C-Pen-8-C-Hex	547.1432
177	20.59	[M-H] ⁻	505.0999	505.0977	-2.415	C ₂₃ H ₂₂ O ₁₃	Viscidulin li 2'-O-β-D-glucuronide	505.1091; 329.0665; 314.04299.0170
178	20.66	[M + H] ⁺	563.1754	563.1759	-0.883	C ₂₇ H ₃₀ O ₁₃	Glycyroside	269.0824; 254.0583
179	20.69	[M-H] ⁻	459.1298	459.1286	2.650	C ₂₃ H ₂₄ O ₁₀	6'-Acetyliquiritin	459.1308; 255.0659; 135.00
180*	20.70	[M-H] ⁻	301.0718	301.0707	3.804	C ₁₆ H ₁₄ O ₆	Hesperetin	301.0682; 286.0490; 242.06215.0487; 163.1426; 164.01
181*	20.78	[M-H] ⁻	577.1566	579.1708	2.388	C ₂₇ H ₃₂ O ₁₄	Naringin	579.1759; 459.1184; 313.07271.0620; 151.0046
182*	20.82	[M-H] ⁻	623.1985	623.1970	2.316	C ₂₉ H ₃₆ O ₁₅	Isoaceteoside	623.2040; 461.1658; 161.02
183	20.83	[M + H] ⁺	249.1483	249.1485	-0.887	C ₁₅ H ₂₀ O ₃	4-Shogaol	137.0617; 122.0376; 94.042
184	20.88	[M-H] ⁻	581.1885	581.1865	3.541	C ₂₇ H ₃₄ O ₁₄	2,4,6-Trihydroxydihydrochalcone-3-C-β-D-glucoside-6-O-β-D-glucoside	581.1880; 461.1468; 329.110299.0928; 167.0358
185	20.88	[M-H] ⁻	635.1622	635.1607	2.470	C ₂₉ H ₃₂ O ₁₆	Multiflorin A	591.1745; 255.0658; 135.00
186	20.92	[M-H] ⁻	329.0669	329.0656	3.829	C ₁₇ H ₁₄ O ₇	Iristectorigenin A	314.0429; 299.0200; 282.01255.0319; 243.0316; 271.02227.0369; 215.0270; 199.04187.0426; 171.0471; 161.99134.0021;
187	20.92	[M + H] ⁺	493.1336	493.1341	-0.837	C ₂₃ H ₂₄ O ₁₂	Iristectorin A	331.0797; 316.0564; 301.03
188	21.17	[M-H] ⁻	431.0985	431.0973	2.799	C ₂₁ H ₂₀ O ₁₀	kaempferol-3-rhamnoside	255.0230; 227.0374
189*	21.19	[M-H] ⁻	609.1828	609.1814	2.304	C ₂₈ H ₃₄ O ₁₅	Hesperidin	609.1875; 343.0831; 325.07301.0728; 286.0496
190	21.21	[M + H] ⁺	361.0911	361.0918	-2.032	C ₁₈ H ₁₆ O ₈	Isoirigenin	361.0854; 301.0707
191	21.21	[M + H] ⁺	523.1442	523.1446	-0.740	C ₂₄ H ₂₆ O ₁₃	Iridin	361.0922; 346.0675; 301.07
192	21.22	[M-H] ⁻	463.1250	463.1235	3.200	C ₂₂ H ₂₄ O ₁₁	Hesperetin-O-glucoside	301.0720; 286.0450; 240.05164.0105

No.	RT /min	Ion model	Measured mass /Da	Calculated mass /Da	Error /ppm	Molecular formula	Identification	Fragment ions
193	21.28	[M-H] ⁻	547.1460	547.1446	2.527	C ₂₆ H ₂₈ O ₁₃	Chrysin 6-Cβ -D-glucoside-8-Cβ -L-arabinoside	547.1465; 457.1157; 427.10367.0822; 337.0714
194	21.38	[M-H] ⁻	161.0233	161.0233	-0.071	C ₉ H ₆ O ₃	Folic acid	91.0554; 62.0163
195*	21.38	[M-H] ⁻	515.1194	515.1184	1.898	C ₂₅ H ₂₄ O ₁₂	Isochlorogenic acid C	515.1216; 353.0884; 191.05179.0345; 173.0453
196*	21.51	[M-H] ⁻	447.0935	447.0922	3.024	C ₂₁ H ₂₀ O ₁₁	Astragalin	327.0475; 284.0347; 255.02227.0349;
197	21.53	[M-H] ⁻	693.2769	693.2753	2.255	C ₃₄ H ₄₆ O ₁₅	Nominin-17-β-D-glucoside	693.2793; 565.2748; 517.15271.1715; 175.0416
198	21.58	[M + H] ⁺	534.2108	534.2114	-1.036	C ₂₅ H ₃₂ N ₅ O ₆ Cl	Astin N	449.1598; 131.0482; 106.06
199*	21.59	[M-H] ⁻	609.1828	609.1814	2.304	C ₂₈ H ₃₄ O ₁₅	Neohesperidin	609.1797; 301.0726
200	21.63	[M-H] ⁻	813.2103	813.2084	2.375	C ₃₅ H ₄₂ O ₂₂	Limocitrin-3-O-(5-α-glucosyl-HMG)-β-glucoside	651.1520; 345.0613
201	21.65	[M + H] ⁺	579.1702	579.1708	-1.039	C ₂₇ H ₃₀ O ₁₄	Violanthin	417.1044
202	21.68	[M-H] ⁻	547.1461	547.1446	2.637	C ₂₆ H ₂₈ O ₁₃	Chrysin 6-CHex-8-CPen	547.1501; 457.1147; 427.10367.0826; 337.0715
203*	21.73	[M + H] ⁺	133.0647	133.0648	-0.081	C ₉ H ₈ O	Cinnamaldehyde	115.0538; 105.0673; 86.100
204	21.83	[M + H] ⁺	845.2338	845.2346	-1.010	C ₃₆ H ₄₄ O ₂₃	limocitrol-3-O-(5-α-glucosyl-HMG)-β-glucoside	377.0854; 362.0621
205*	21.91	[M-H] ⁻	255.0662	255.0652	3.939	C ₁₅ H ₁₂ O ₄	Liquiritigenin	255.0708; 135.0090; 119.0591.0192
206	21.95	[M-H] ⁻	623.1623	623.1607	2.614	C ₂₈ H ₃₂ O ₁₆	Isorhamnetin-3-O-rutinoside	623.1634; 461.1724; 315.04161.0253
207	21.97	[M-H] ⁻	549.1617	549.1603	2.682	C ₂₆ H ₃₀ O ₁₃	Isoliquiritin Apioside	549.1605; 255.0650; 135.00
208	22.01	[M-H] ⁻	463.1253	463.1235	3.934	C ₂₂ H ₂₄ O ₁₁	Hesperetin-O-glucoside	463.1230; 301.0712; 257.08164.0100
209	22.05	[M-H] ⁻	201.0186	201.0182	1.964	C ₁₁ H ₆ O ₄	Xanthotoxol	201.0196; 145.0314
210*	22.07	[M-H] ⁻	417.1191	417.1180	2.712	C ₂₁ H ₂₂ O ₉	Isoliquiritin	417.1149; 255.0695; 148.01135.0080
211	22.17	[M + H] ⁺	609.1810	609.1814	-0.602	C ₂₈ H ₃₂ O ₁₅	Diosmin or neodiosmin	301.0697; 286.0462
212*	22.26	[M + FA-H] ⁻	475.1249	475.1235	2.867	C ₂₂ H ₂₂ O ₉	Ononin	267.252
213	22.36	[M-H] ⁻	475.0884	475.0871	2.752	C ₂₂ H ₂₀ O ₁₂	5,7,2'-Trihydroxy-6-methoxy flavone-7-O-glucuronide	475.0933; 299.0580; 284.03
214	22.39	[M + H] ⁺	532.2398	532.2402	-0.826	C ₂₅ H ₃₃ N ₅ O ₈	Asterinin A	532.1802; 514.2322; 338.17235.1084; 131.0480
215	22.48	[M-H] ⁻	353.1033	353.1020	3.782	C ₂₀ H ₁₈ O ₆	Licoisoflavone A	353.1060; 227.0704; 125.0283.0125
216	22.49	[M-H] ⁻	549.1616	549.1603	2.463	C ₂₆ H ₃₀ O ₁₃	Licuraside	549.1569; 255.0698; 135.01
217	22.57	[M-H] ⁻	447.0935	447.0922	3.024	C ₂₁ H ₂₀ O ₁₁	Baicalein- 7-O-β-D-glucopyranoside	447.0950; 271.0612; 243.06225.0559
218	22.57	[M-H] ⁻	651.1574	651.1556	2.725	C ₂₉ H ₃₂ O ₁₇	3',3,5-Hydroxy-4',5'-methoxyflavone-O-glucoside-orhamnoside	651.1634; 589.1628; 549.13507.1189
219	22.58	[M + H] ⁺	347.0756	347.0761	-1.509	C ₁₇ H ₁₄ O ₈	Iristectorigenin B	331.0797; 316.0574; 273.03
220	22.76	[M + H] ⁺	317.0652	317.0656	-1.227	C ₁₆ H ₁₂ O ₇	Irilin D	317.0668; 302.0418; 245.04168.0044

No.	RT /min	Ion model	Measured mass /Da	Calculated mass /Da	Error /ppm	Molecular formula	Identification	Fragment ions
221	22.78	[M-H] ⁻	621.1465	621.1450	2.356	C ₂₈ H ₃₀ O ₁₆	Wogonin-7-O-glu-glu acid	621.1494; 445.1134; 430.09283.0600; 268.0349; 175.02
222	22.87	[M + H] ⁺	586.1820	586.1830	-1.655	C ₂₅ H ₃₃ N ₅ O ₇ Cl ₂	Astin B isomer	586.1751; 501.1269; 336.04249.0186; 194.0147; 106.06
223*	22.89	[M + H] ⁺	287.0544	287.0550	-2.001	C ₁₅ H ₁₀ O ₆	Luteolin	287.0548; 153.0192; 135.04
224*	22.90	[M-H] ⁻	445.0776	445.0765	2.274	C ₂₁ H ₁₈ O ₁₁	Baicalin	445.0786; 269.0466
225	22.98	[M-H] ⁻	263.1289	263.1278	4.045	C ₁₅ H ₂₀ O ₄	(+)-Absciscic acid	219.1463; 204.1170; 153.09122.0382; 109.0276;
226	23.05	[M-H] ⁻	621.1829	621.1814	2.356	C ₂₉ H ₃₄ O ₁₅	Isocrenatoside or isomer	203.0362; 179.0374; 161.02
227	23.07	[M-H] ⁻	695.1989	695.1970	2.594	C ₃₅ H ₃₆ O ₁₅	Licorice-glycoside B/D1/D2	549.1584; 531.1510; 255.06135.0104
228	23.07	[M-H] ⁻	725.2090	725.2076	1.887	C ₃₆ H ₃₈ O ₁₆	Licorice-glycoside A/C1/C2	549.1600; 531.1486; 255.06
229*	23.24	[M-H] ⁻	147.0439	147.0441	-0.186	C ₉ H ₈ O ₂	Cinnamic acid	103.0553
230	23.47	[M + H] ⁺	586.1826	586.1830	-0.615	C ₂₅ H ₃₃ N ₅ O ₇ Cl ₂	Astin B	586.1946; 409.9996; 338.06251.0331; 106.0645
231	23.59	[M-H] ⁻	591.1726	591.1708	3.008	C ₂₈ H ₃₂ O ₁₄	Liquiritigenin-4'-O-[β-D-3-O-acetyl-apiofuranosyl-(1-2)]-β-D-glucopyranoside	591.1762; 471.1338; 403.10307.0604
232	23.69	[M-H] ⁻	711.2881	711.2859	3.133	C ₃₄ H ₄₈ O ₁₆	Nomilinic acid glucoside	651.2662; 607.2740
233	23.70	[M-H] ⁻	505.0994	505.0977	3.372	C ₂₃ H ₂₂ O ₁₃	Viscidulin li 2'-O-β-D-glucuronide isomer	505.1024; 329.0651; 314.04299.0187
234	23.72	[M + H] ⁺	534.2110	534.2114	-0.726	C ₂₅ H ₃₂ N ₅ O ₆ Cl	Astin D	534.2099; 449.1561; 302.08215.0562
235	23.82	[M + H] ⁺	179.0704	179.0703	-1.288	C ₁₀ H ₁₀ O ₃	2-Methoxycinnamic acid	118.0416; 90.0466; 77.0388
236	23.88	[M-H] ⁻	445.0777	445.0765	2.746	C ₂₁ H ₁₈ O ₁₁	Norwogonin 7-O-β-D-glucuronide	445.0811; 269.0464
237*	23.92	[M-H] ⁻	271.0613	271.0601	4.243	C ₁₅ H ₁₂ O ₅	Naringenin	151.001
238	23.92	[M-H] ⁻	753.2254	753.2237	2.316	C ₃₄ H ₄₂ O ₁₉	Brutieridin	753.2278; 691.2259; 651.19609.1844; 301.0733
239	24.05	[M + H] ⁺	163.0752	163.0754	-0.206	C ₁₀ H ₁₀ O ₂	2-Methoxycinnamaldehyde	145.0659; 131.0496; 115.05105.0697; 91.0533; 77.0383
240*	24.07	[M + H] ⁺	133.0647	133.0648	-0.051	C ₉ H ₈ O	Cinnamaldehyde isomer	133.0644; 115.0538
241	24.14	[M + H] ⁺	570.1876	570.1881	-0.887	C ₂₅ H ₃₃ N ₅ O ₆ Cl ₂	Astin C	570.1876; 485.1351; 337.08251.0333; 223.0388
242	24.14	[M + H] ⁺	595.1696	595.1657	6.407	C ₂₇ H ₃₀ O ₁₅	Luteolin-7-O-rutinoside	449.1167; 287.0527
243	24.28	[M + H] ⁺	516.2448	516.2453	-0.900	C ₂₅ H ₃₃ N ₅ O ₇	Astin J	498.2315; 338.1695; 235.10218.0802; 193.0955; 151.08131.0477
244	24.31	[M-H] ⁻	429.0827	429.0816	2.510	C ₂₁ H ₁₈ O ₁₀	Chrysin7-O-β-D-glucuronide	429.0829; 253.0511; 175.02113.0249
245	24.32	[M + H] ⁺	255.0645	255.0652	-2.765	C ₁₅ H ₁₀ O ₄	Daidzein	137.0245
246	24.32	[M + H] ⁺	471.2010	471.2013	-0.752	C ₂₆ H ₃₀ O ₈	Limonin	471.2020; 453.1931; 425.19367.1914; 213.0920; 161.05
247	24.34	[M-H] ⁻	593.1877	593.1865	2.020	C ₂₈ H ₃₄ O ₁₄	Didymin	327.0881; 285.0785; 241.08164.0130
248	24.40	[M-H] ⁻	473.0726	473.0715	2.363	C ₂₂ H ₁₈ O ₁₂	Cichoric acid	149.0123

No.	RT /min	Ion model	Measured mass /Da	Calculated mass /Da	Error /ppm	Molecular formula	Identification	Fragment ions
249	24.40	[M-H] ⁻	475.0882	475.0871	2.374	C ₂₂ H ₂₀ O ₁₂	5,7,8-Trihydroxy-6-methoxy flavone-7- <i>O</i> -glucuronide	475.0954; 299.0567; 284.03
250	24.40	[M-H] ⁻	547.1456	547.1446	1.851	C ₂₆ H ₂₈ O ₁₃	Chrysin 6- <i>C</i> -β-D-glucoside-8- <i>C</i> -α-L-arabinoside	547.1488; 457.1156; 427.10367.0826; 337.0722
251	24.44	[M-H] ⁻	445.1142	445.1129	2.936	C ₂₂ H ₂₂ O ₁₀	Wogonin-5- <i>O</i> -β-D -glucoside	445.1167; 430.0944; 283.06267.0312; 239.0361
252	24.48	[M-H] ⁻	461.1094	461.1078	3.410	C ₂₂ H ₂₂ O ₁₁	(2 <i>S</i>)-5,7-Dihydroxy-6-methoxyflavanone-7- <i>O</i> -β-D-glucuronide	461.1128; 285.0775; 270.05165.9913; 113.0255
253*	24.50	[M-H] ⁻	285.0768	285.0758	3.718	C ₁₆ H ₁₄ O ₅	Licochalcone B	285.0674; 270.0507; 177.01150.0310; 121.0272
254	24.5	[M-H] ⁻	635.1627	635.1607	3.241	C ₂₉ H ₃₆ O ₁₆	Campneoside II or its isomer	635.1625
255	24.67	[M-H] ⁻	459.0936	459.0922	3.076	C ₂₂ H ₂₀ O ₁₁	Oroxylin A 7- <i>O</i> -glucuronide	459.0970; 283.0621; 268.03
256	24.7	[M + H] ⁺	219.1013	219.1016	-1.099	C ₁₃ H ₁₄ O ₃	6-Acetyl-2,2-dimethylchroman-4-one	163.0376; 107.0491; 77.038
257	24.75	[M-H] ⁻	299.0561	299.0550	3.697	C ₁₆ H ₁₂ O ₆	7,2',4'-Trihydroxy-5-methoxy-3-arylcoumarin	255.0323; 227.0373
258*	24.77	[M-H] ⁻	269.0820	269.0808	4.440	C ₁₆ H ₁₄ O ₄	Echinatin	237.0611; 183.0856; 161.01133.0308; 120.0216; 92.026
259	24.77	[M + H] ⁺	301.0718	301.0707	3.605	C ₁₆ H ₁₂ O ₆	Tectorigenin	301.0700; 286.0462; 168.00
260	24.77	[M-H] ⁻	301.0718	301.0707	4.004	C ₁₆ H ₁₄ O ₆	Tetrahydroxymethoxychalcone	149.9976; 108.0216
261	24.77	[M-H] ⁻	445.0778	445.0765	2.881	C ₂₁ H ₁₈ O ₁₁	Norwogonin 8- <i>O</i> -β-D-glucuronide	445.0785; 269.0454
262	24.88	[M-H] ⁻	475.0883	475.0871	2.500	C ₂₂ H ₂₀ O ₁₂	5,6,7-Trihydroxy-8-methoxyflavone-7- <i>O</i> -glucuronopyranoside	475.0899; 299.0555; 284.03
263*	24.90	[M + FA-H] ⁻	491.1196	491.1184	2.377	C ₂₂ H ₂₂ O ₁₀	Tilianin	491.2810; 283.0620; 268.04
264	24.9	[M + FA-H] ⁻	491.1196	491.1184	2.377	C ₂₂ H ₂₂ O ₁₀	Trifolirhizin	283.0626; 268.0397; 239.03211.0431
265	24.94	[M-H] ⁻	857.2365	857.2346	2.212	C ₃₇ H ₄₆ O ₂₃	7,4-Dihydroxy-5,6,8,3-tetramethoxyflavonol-3- <i>O</i> -(5-α-glucosyl-HMG)-β-glucoside	593.1484; 551.1380; 389.08
266*	25.07	[M-H] ⁻	459.0935	459.0922	2.815	C ₂₂ H ₂₀ O ₁₁	Wogonoside	459.1119; 283.0631; 268.03
267	25.43	[M + Na] ⁺	231.0624	231.0628	-1.472	C ₁₁ H ₁₂ O ₄	Ethyl Caffate	153.0172; 77.0380
268*	25.44	[M + H] ⁺	287.0545	287.0550	-1.729	C ₁₅ H ₁₀ O ₆	Kaempferol	287.0548; 231.0650; 153.01
269	25.51	[M + H] ⁺	331.0807	331.0812	-1.719	C ₁₇ H ₁₄ O ₇	Iristectorigenin A	316.0639; 301.0407; 273.04245.0494
270	25.56	[M-H] ⁻	445.0779	445.0765	2.960	C ₂₁ H ₁₈ O ₁₁	Baicalein 6- <i>O</i> -β-D-glucuronide	445.0811; 269.0450
271	25.59	[M + H] ⁺	221.1898	221.1900	-1.049	C ₁₅ H ₂₄ O	Alismol	105.0695; 91.0538; 79.057367.0542; 119.0842
272	25.62	[M + H] ⁺	361.0920	361.0918	-1.672	C ₁₈ H ₁₆ O ₈	Irigenin	361.0909; 331.0449; 232.03169.0131
273*	25.63	[M-H] ⁻	359.0776	359.0761	-3.450	C ₁₈ H ₁₆ O ₈	Rosmarinic acid	359.0774; 197.0469; 179.03161.0244; 135.0468; 123.04
274*	25.73	[M-H] ⁻	269.0456	269.0444	4.349	C ₁₅ H ₁₀ O ₅	Norwogonin	269.0458; 232.8405; 78.959
275	25.88	[M-H] ⁻	671.1628	671.1607	3.246	C ₃₂ H ₃₂ O ₁₆	6"- <i>O</i> -Vanilloyliridin	359.0766; 344.0538
276	25.91	[M + H] ⁺	711.2124	711.2131	-0.971	C ₃₂ H ₃₈ O ₁₈	monohydroxypentamethoxyflavonol-3- <i>O</i> -HMG-β-glucoside	405.1159; 390.0933; 375.06

No.	RT /min	Ion model	Measured mass /Da	Calculated mass /Da	Error /ppm	Molecular formula	Identification	Fragment ions
277	25.93	[M + H] ⁺	547.3603	547.3629	-4.824	C ₃₂ H ₅₀ O ₇	16-Oxo-alisol A-24-acetate	487.3420; 469.3346; 451.32415.2827; 189.1615;
278	25.94	[M-H] ⁻	547.1479	547.1446	-0.347	C ₂₆ H ₂₈ O ₁₃	Chrysin-6-CHex-8-CPen	547.1469; 457.1168; 427.10367.0839; 337.0699
279	25.98	[M + FA-H] ⁻	533.1302	533.1290	2.294	C ₂₄ H ₂₄ O ₁₁	Agastachoside	487.1302; 283.0624; 268.03
280	26.00	[M-H] ⁻	345.0617	345.0605	3.612	C ₁₇ H ₁₄ O ₈	Viscidulin lii	345.0624; 330.0392; 315.01287; 369
281	26.00	[M-H] ⁻	489.1042	489.1028	2.939	C ₂₃ H ₂₂ O ₁₂	5,7-Dihydroxy-8,2'-dimethoxyflavone7-Oβ-D-glucuronide	489.1039; 313.0726; 289.04283.0255
282*	26.10	[M-H] ⁻	255.0663	255.0652	4.370	C ₁₅ H ₁₂ O ₄	Isoliquiritigenin	255.0678; 119.0499; 91.018
283	26.18	[M + H] ⁺	887.2806	887.2816	-1.109	C ₃₉ H ₅₀ O ₂₃	Natsudaiddain-3-O-(5-α-glucosyl-HMG)-β-glucoside	419.1345; 404.1107; 389.08
284	26.22	[M + H] ⁺	728.3973	728.3978	-0.649	C ₃₆ H ₅₃ N ₇ O ₉	Citrusin III	710.3892; 700.4052; 587.31474.2331; 138.1269
285*	26.79	[M-H] ⁻	269.0458	269.0444	4.832	C ₁₅ H ₁₀ O ₅	Baicalein	269.0455; 241.04564; 223.0
286	26.85	[M-H] ⁻	243.1026	243.1016	4.110	C ₁₅ H ₁₆ O ₃	Batatasin Ⅱ/Ⅲ	243.1027; 137.0610; 122.03116.9939; 78.9609
287*	27.09	[M-H] ⁻	267.0664	267.0652	4.586	C ₁₆ H ₁₂ O ₄	Formononetin	267.0683; 223.0434; 178.99
288	27.10	[M + H] ⁺	373.1279	373.1282	-1.070	C ₂₀ H ₂₀ O ₇	Isosinensetin	373.1275; 357.0924; 343.08
289	27.14	[M-H] ⁻	837.3927	837.3903	2.834	C ₄₂ H ₆₂ O ₁₇	Licorice saponin P2	837.4260; 351.0647; 193.03
290	27.14	[M + FA-H] ⁻	843.4760	843.4737	2.712	C ₄₂ H ₇₀ O ₁₄	Hydroxysaikosaponin A	843.4743; 797.4672; 635.41
291	27.14	[M + H] ⁺	333.1692	333.1697	-1.292	C ₁₉ H ₂₄ O ₅	Marmin	333.16861; 315.15884
292	27.20	[M-H] ⁻	723.2152	723.2131	2.848	C ₃₃ H ₄₀ O ₁₈	Melitidin	723.2244; 417.1237; 402.09125.0249; 99.0462
293	27.21	[M + H] ⁺	359.0756	359.0761	-1.459	C ₁₈ H ₁₄ O ₈	Dichtomitin	359.0761; 326.0421; 270.05256.0364; 181.0130
294	27.21	[M + H] ⁺	419.1332	419.1337	-1.214	C ₂₁ H ₂₂ O ₉	Natsudaiddain	419.1327; 389.0903
295	27.44	[M + H] ⁺	545.3465	545.3473	-1.431	C ₃₂ H ₄₈ O ₇	Alisol M 23-acetate	467.3163; 387.2516; 299.19201.1624; 145.0996; 133.10119.0860;
296	27.44	[M-H] ⁻	895.3979	895.3958	2.328	C ₄₄ H ₆₄ O ₁₉	Uralsaponin F	351.0572
297	27.52	[M + H] ⁺	221.1898	221.1900	-1.003	C ₁₅ H ₂₄ O	10α-Hydroxy-1αH,5βH-guaia-3,6-diene	105.0695; 91.0538; 79.057367.0542; 119.0842
298	27.53	[M-H] ⁻	313.0720	313.0707	4.234	C ₁₇ H ₁₄ O ₆	3',4'-Dihydroxy-7,5'-dime thoxyflavone	313.0734; 298.0487; 283.02276.8391; 164.9813; 117.03
299	27.53	[M + FA-H] ⁻	521.3121	521.3109	2.370	C ₂₈ H ₄₄ O ₆	Polyporusterone B	475.3211; 313.0729;
300*	27.68	[M + H] ⁺	373.1277	373.1282	-1.312	C ₂₀ H ₂₀ O ₇	Sinensetin	373.1290; 358.1057; 357.09343.0827
301	27.70	[M + H] ⁺	403.1381	403.1387	-1.622	C ₂₁ H ₂₂ O ₈	Hexamethoxyfalfvone	403.1427; 387.1069; 373.09355.0892; 327.0889
302*	27.74	[M + H] ⁺	387.1069	387.1074	-1.483	C ₂₀ H ₁₈ O ₈	Irisflorentin	387.1098; 372.0863; 357.06326.0799
303	27.76	[M-H] ⁻	853.3872	853.3852	2.307	C ₄₂ H ₆₂ O ₁₈	22-Hydroxy-licorice saponin G2	853.3958; 351.0544
304	28.04	[M + FA-H] ⁻	523.3278	523.3265	2.437	C ₂₈ H ₄₆ O ₆	Polyporusterone A	477.3126; 433.1752; 343.14

No.	RT /min	Ion model	Measured mass /Da	Calculated mass /Da	Error /ppm	Molecular formula	Identification	Fragment ions
305	28.09	[M-H] ⁻	373.0929	373.0918	2.965	C ₁₉ H ₁₈ O ₈	5,7-Hydroxy-3',4',5',6-tetramethoxyflavone	373.0934; 358.0701; 343.04328.0224
306	28.12	[M + H] ⁺	531.3649	531.3680	-5.920	C ₃₂ H ₅₀ O ₆	Alisol N 23-acetate	453.3347; 381.2838; 229.14207.1747;
307	28.13	[2M-H] ⁻	295.0976	295.0965	3.641	C ₉ H ₈ O ₂	E-cinnamic acid	295.0971
308	28.15	[M-H] ⁻	487.1248	487.1235	2.735	C ₂₄ H ₂₄ O ₁₁	Agastachoside isomer	487.1285; 283.0613; 268.03
309	28.23	[M-H] ⁻	285.0769	285.0758	4.034	C ₁₆ H ₁₄ O ₅	Homobutein	123.0457; 117.0348; 108.02
310	28.27	[M-H] ⁻	819.3818	819.3798	2.536	C ₄₂ H ₆₀ O ₁₆	Licorice saponin E2	819.3898; 351.0554
311	28.36	[M + H] ⁺	531.3653	531.3680	-5.111	C ₃₂ H ₅₀ O ₆	16β-Hydroxyalisol B 23-acetate	213.1665; 147.1158;
312*	28.53	[M + H] ⁺	285.0751	285.0758	-2.385	C ₁₆ H ₁₂ O ₅	Oroxylin A	285.0764; 270.0526; 242.05153.0187
313*	28.7	[M + Na] ⁺	317.1718	317.1723	-1.326	C ₁₇ H ₂₆ O ₄	6-Gingerol	317.1711; 217.0801
314	28.75	[M-H] ⁻	879.4030	879.4009	2.341	C ₄₄ H ₆₄ O ₁₈	22-Acetoxyglycyrrhizin	879.4120; 351.0628
315	29.05	[M + H] ⁺	704.3972	704.3978	-0.756	C ₃₄ H ₅₃ N ₇ O ₉	Citrusin I	686.3947; 555.3062
316	29.06	[M-H] ⁻	343.0825	343.0812	3.675	C ₁₈ H ₁₆ O ₇	3',4',5'-Trimethoxyflavone	343.0837; 328.0560; 313.04
317	29.06	[M-H] ⁻	863.4076	863.4060	1.869	C ₄₄ H ₆₄ O ₁₇	22β-Acetoxyglycyrrhaldehyde	801.4087; 351.0576
318	29.06	[M-H] ⁻	983.4501	983.4482	1.927	C ₄₈ H ₇₂ O ₂₁	Licorice saponin A3	983.4489
319	29.08	[M-H] ⁻	879.4029	879.4009	2.273	C ₄₄ H ₆₄ O ₁₈	22β-Acetoxyglycyrrhizin or uralsaponin M	351.0578
320*	29.30	[M + H] ⁺	403.1382	403.1387	-1.474	C ₂₁ H ₂₂ O ₈	Nobiletin	403.1385; 388.1151; 373.09355.0811; 327.0856
321	29.38	[M + H] ⁺	505.3517	505.3524	-1.416	C ₃₀ H ₄₈ O ₆	16-Oxoalisol A	505.3501
322	29.42	[M + H] ⁺	285.0752	285.0758	-1.929	C ₁₆ H ₁₂ O ₅	Batatasin I	285.0752; 270.0912; 254.09253.0897; 225.0911
323	29.42	[M + H] ⁺	547.3623	547.3629	-1.152	C ₃₂ H ₅₀ O ₇	16-Oxo-alisol A-23-acetate	451.3238; 415.2820; 353.24241.1619; 149.0962;
324	29.43	[M-H] ⁻	837.3922	837.3903	2.177	C ₄₂ H ₆₂ O ₁₇	Yunganoside K2 or Macedonoside A	837.3912; 351.0547
325*	29.53	[M + H] ⁺	285.0750	285.0758	-2.806	C ₁₆ H ₁₂ O ₅	Acacetin	285.0768; 270.0506; 242.05153.0191
326	29.53	[M-H] ⁻	329.2335	329.2323	3.673	C ₁₈ H ₃₄ O ₅	Pinellic acid	329.2385; 229.1465; 211.13
327	29.57	[M + H] ⁺	531.3654	531.3680	-4.885	C ₃₂ H ₅₀ O ₆	Alisol F 24-acetate	513.1300; 163.1156;
328	29.65	[M + H] ⁺	489.3570	489.3575	-0.860	C ₃₀ H ₄₈ O ₅	Alisol F or isomer	471.3458; 453.3369; 399.28219.1701;
329	29.66	[M + FA-H] ⁻	971.5229	971.5210	1.939	C ₄₈ H ₇₈ O ₁₇	Saikosaponin C	971.5364; 779.4565; 617.44
330	29.80	[M-H] ⁻	329.2336	329.2323	4.220	C ₁₈ H ₃₄ O ₅	Pinellic acid isomer	293.2103; 229.1433; 171.10139.1133; 127.1152;
331	29.81	[M + H] ⁺	433.1488	433.1493	-1.105	C ₂₂ H ₂₄ O ₉	3,5,6,7,8',3',4'-Heptemthoxyflavone	433.1453; 418.1216; 417.11403.1007; 385.0913
332*	29.98	[M + H] ⁺	231.1377	231.1380	-0.979	C ₁₅ H ₁₈ O ₂	Atractylenolide- β	231.1381; 213.1315; 203.11185.1321
333	30.18	[M + H] ⁺	419.1333	419.1337	-0.927	C ₂₁ H ₂₂ O ₉	Gardenin A	419.1322; 404.1076; 389.08371.0741; 165.0535

No.	RT /min	Ion model	Measured mass /Da	Calculated mass /Da	Error /ppm	Molecular formula	Identification	Fragment ions
334	30.19	[M-H] ⁻	837.3925	837.3903	2.548	C ₄₂ H ₆₂ O ₁₇	Licorice saponin G2 isomer	837.3966; 351.0610
335	30.38	[M + FA-H] ⁻	973.5385	973.5367	1.842	C ₄₈ H ₈₀ O ₁₇	Saikosaponin F	973.5410; 927.5316; 781.47619.4630
336	30.45	[M + H] ⁺	329.1016	329.1020	-1.139	C ₁₈ H ₁₆ O ₆	Monohydroxytrimethoxyflavone	329.1027; 314.0788; 296.06268.0744; 240.0783
337	30.55	[M + H] ⁺	274.2736	274.2741	-1.699	C ₁₆ H ₃₅ NO ₂	Hexadecyl dihydrosphingosine	274.2739; 256.2890
338*	30.76	[M + H] ⁺	373.1284	373.1282	-1.580	C ₂₀ H ₂₀ O ₇	Tangeretin	373.1929; 358.1043; 343.08
339	30.89	[M + FA-H] ⁻	531.3330	531.3316	2.484	C ₃₀ H ₄₆ O ₅	16-Oxo-11-anhydroalisol A	485.3298; 467.3206; 367.26245.1579;
340	30.91	[M-H] ⁻	329.2336	329.2323	3.947	C ₁₈ H ₃₄ O ₅	Pinellic acid isomer	229.1451; 211.1352; 183.14171.1041; 118.9584; 99.082
341*	30.94	[M + Na] ⁺	377.0991	377.0994	-1.165	C ₂₀ H ₁₈ O ₆	Asarinin	377.0990
342	30.98	[M-H] ⁻	837.3923	837.3903	2.321	C ₄₂ H ₆₂ O ₁₇	Licorice saponin Q2	837.3956; 351.0559; 193.03
343	31.02	[M-H] ⁻	367.1188	367.1176	3.337	C ₂₁ H ₂₀ O ₆	Glycycoumarin	367.1208; 309.0429; 297.04284.0338
344	31.2	[M + FA-H] ⁻	533.3488	533.3473	2.756	C ₃₀ H ₄₈ O ₅	Alisol F	487.3427; 353.2485; 203.11
345	31.26	[M-H] ⁻	353.1399	353.1384	4.275	C ₂₁ H ₂₂ O ₅	Gancaonin I	335.1210; 150.0335
346	31.39	[M-H] ⁻	353.1034	353.1020	4.121	C ₂₀ H ₁₈ O ₆	Licoisoflavone A	353.1027; 316.8315; 269.04
347	31.40	[M + H] ⁺	403.1383	403.1387	-1.002	C ₂₁ H ₂₂ O ₈	Hexamethoxyfalfvone	403.1393; 388.1228; 373.09355.0789; 301.0740
348	31.61	[M + FA-H] ⁻	573.3438	573.3422	2.817	C ₃₂ H ₄₈ O ₆	Alisol C 23-acetate	527.3486; 509.3313; 449.31
349	31.62	[M + H] ⁺	529.3519	529.3524	-0.936	C ₃₂ H ₄₈ O ₆	16-Oxo-11-anhydroalisol A 24-acetate	511.3391; 469.3290; 451.32433.3083; ; 203.1442;
350	31.63	[M + H] ⁺	487.3429	487.3418	2.317	C ₃₀ H ₄₆ O ₅	Alisol C	469.3331; 415.2848; 217.15161.0951; 119.0853;
351	31.63	[M + FA-H] ⁻	533.3484	533.3473	2.531	C ₃₀ H ₄₈ O ₅	13β,17β-epoxyalisol B	487.3427; 353.2485; 203.11
352	31.63	[M-H] ⁻	837.3925	837.3903	2.548	C ₄₂ H ₆₂ O ₁₇	Licorice saponin G2	837.4026; 351.0586; 193.04
353	31.65	[M-H] ⁻	967.4555	967.4533	2.263	C ₄₈ H ₇₂ O ₂₀	Rhaoglycyrrhizin	967.4653; 497.1171
354	31.85	[M + H] ⁺	471.3463	471.3469	-1.287	C ₃₀ H ₄₆ O ₄	16,23-Oxido-alisol B	453.3332; 399.2878; 425.34201.1686; 177.1286; 155.08
355*	31.86	[M-H] ⁻	821.3973	821.3954	2.347	C ₄₂ H ₆₂ O ₁₆	Glycyrrhizic acid	821.3973; 645.3638; 469.33351.0563; 193.0341; 175.02
356	31.86	[M-H] ⁻	823.4034	823.4111	-9.330	C ₄₂ H ₆₄ O ₁₆	Uralsaponin C	823.4174; 351.0595
357	31.89	[M + H] ⁺	531.3658	531.3680	-4.208	C ₃₂ H ₅₀ O ₆	Alisol F 24-acetate isomer	495.3526; 453.3417; 276.94217.1596; 173.1322; 145.10121.1027;
358*	31.96	[M-H] ⁻	337.1447	337.1434	3.780	C ₂₁ H ₂₂ O ₄	Licochalcone A	266.9918; 216.9262; 198.9196.9702; 78.9587
359	32.21	[M-H] ⁻	351.0876	351.0863	3.718	C ₂₀ H ₁₆ O ₆	Licoisoflavone B	351.0878; 336.0748; 307.09283.1001; 265.0873; 199.07
360*	32.26	[M + Na] ⁺	345.2033	345.2034	-1.016	C ₁₉ H ₃₀ O ₄	8-Gingerol	345.2031
361*	32.30	[M - H] ⁻	353.1032	353.1032	2.612	C ₂₀ H ₁₈ O ₆	Isolicoflavonol	353.1022

No.	RT /min	Ion model	Measured mass /Da	Calculated mass /Da	Error /ppm	Molecular formula	Identification	Fragment ions
362	32.35	[M-H] ⁻	985.4661	985.4639	2.207	C ₄₈ H ₇₄ O ₂₁	Yunganoside D1 or Yunganoside G1	985.4770; 497.1194
363	32.36	[M + H] ⁺	529.3497	529.3524	-5.092	C ₃₂ H ₄₈ O ₆	Alisol Q 23-acetate	451.3220;
364	32.39	[M-H] ⁻	807.4185	807.4161	2.938	C ₄₂ H ₆₄ O ₁₅	Licoricesaponin B2	807.4230; 351.0564; 193.03
365	32.50	[M-H] ⁻	837.3925	837.3903	2.619	C ₄₂ H ₆₂ O ₁₇	Uralsaponin N	837.3998; 351.0582; 193.03
366	32.66	[M-H] ⁻	807.4184	807.4161	2.790	C ₄₂ H ₆₄ O ₁₅	22-Dehydroxyural saponin	807.4214; 351.0548; 193.03
367	32.80	[M-H] ⁻	821.3976	821.3954	2.712	C ₄₂ H ₆₂ O ₁₆	Licorice saponin H2	821.4034; 351.0593; 193.03
368	32.81	[M + H - H ₂ O] ⁺	471.3464	471.3469	-1.096	C ₃₀ H ₄₈ O ₅	13B,17B-Epoxyalisol B	381.2803; 310.2266; 159.11 133.1009;
369	32.99	[M + FA-H] ⁻	867.4747	867.4737	1.231	C ₄₄ H ₇₀ O ₁₄	3"-O-Acetylsaikosaponin A	867.4879; 821.4796; 779.46 761.4559; 617.4142
370	33.12	[M + H] ⁺	248.2005	248.2009	-1.575	C ₁₆ H ₂₅ NO	N-Isobutyl-dodecatetraenamide	267.1306; 152.1072; 107.08 96.0442; 79.0538
371	33.16	[M + H] ⁺	531.3665	531.3680	-2.815	C ₃₂ H ₅₀ O ₆	Alisol D	435.3309; 337.2465; 163.10
372*	33.30	[M-H] ⁻	353.1032	353.1020	3.612	C ₂₀ H ₁₈ O ₆	Licoflavonol	353.1076; 297.0401; 214.87 151.0039
373	33.41	[2M + H] ⁺	437.3407	437.3414	-1.548	C ₁₅ H ₂₂ O	Nootkatone or its isomers	437.3442; 346.2790; 274.27 265.1953; 187.1491; 105.06
374	33.42	[M + FA-H] ⁻	779.4589	779.4576	2.951	C ₄₂ H ₆₈ O ₁₃	Saikosaponin A	779.4589; 617.4060; 471.34 161.0446; 145.0495; 113.04 101.0229
375	33.42	[M-H] ⁻	865.4600	865.4580	2.251	C ₄₅ H ₇₀ O ₁₆	Malonysaikosaponin A	865.4662; 821.4754; 779.46 761.4580; 617.4110
376	33.56	[M-H] ⁻	821.3977	821.3954	2.798	C ₄₂ H ₆₂ O ₁₆	Licroice Saponin K2	821.4061; 351.0591; 193.03
377	33.60	[M + H] ⁺	487.3433	487.3418	3.117	C ₃₀ H ₄₆ O ₅	(8α,9β,11β,14β,16β,23S,24R)-16,23:24,25-Diepox-11,20-dihydroxy-dammar-13(17)-en-3-one	469.3302; 451.3198; 397.27 353.2457; 253.1536; 201.12
378	33.60	[M + FA-H] ⁻	867.4758	867.4737	2.499	C ₄₄ H ₇₀ O ₁₄	4"-O-Acetylsaikosaponin A	867.4834; 821.4784; 779.46 761.4489; 617.1039
379	34.87	[M-H] ⁻	865.4600	865.4580	2.320	C ₄₅ H ₇₀ O ₁₆	Malonysaikosaponin D	865.4725; 821.4783; 779.45 761.4546; 617.4083
380	34.90	[M + H] ⁺	529.3503	529.3524	-3.940	C ₃₂ H ₄₈ O ₆	Alisol C	511.3422; 451.3223; 397.27 355.2664; 205.1599; 187.14 145.1003;
381	34.93	[M + FA-H] ⁻	825.4655	825.4631	2.935	C ₄₂ H ₆₈ O ₁₃	Saikosaponin G	825.4774; 779.4692; 617.41
382	34.94	[2M + H] ⁺	437.3410	437.3414	-0.862	C ₁₅ H ₂₂ O	nootkatone or its isomers	437.3464; 201.1661; 163.14
383	35.05	[M + FA-H] ⁻	867.4759	867.4737	2.568	C ₄₄ H ₇₀ O ₁₄	3"-O-Acetylsaikosaponin D	867.4855; 821.4813; 779.46 761.4565; 617.4124
384	35.15	[M + FA-H] ⁻	809.4707	809.4682	3.109	C ₄₂ H ₆₈ O ₁₂	Saikosaponin M	809.4758; 763.4699; 601.41 161.0452
385	35.26	[M-H] ⁻	823.4132	823.4111	2.609	C ₄₂ H ₆₄ O ₁₆	Licorice Saponin J2	823.4224; 351.0595; 193.03
386	35.26	[M + FA-H] ⁻	867.4758	867.4737	2.430	C ₄₄ H ₇₀ O ₁₄	6"-O-Acetylsaikosaponin A	867.4855; 821.4813; 779.46 761.4565; 617.4124
387	35.68	[M + H] ⁺	219.1741	219.1743	-1.149	C ₁₅ H ₂₂ O	Isolongifolen-9-one	105.07; 91.05; 77.04; 65.04
388	35.81	[M + FA-H] ⁻	825.4655	825.4631	2.935	C ₄₂ H ₆₈ O ₁₃	Saikosaponin B2	825.4748; 779.4655; 617.40

No.	RT /min	Ion model	Measured mass /Da	Calculated mass /Da	Error /ppm	Molecular formula	Identification	Fragment ions
389	36.31	[M + H] ⁺	219.1741	219.1743	-1.104	C ₁₅ H ₂₂ O	(Z)-Nuciferol	219.1728
390*	36.43	[M + Na] ⁺	413.2296	413.2298	-0.715	C ₂₃ H ₃₄ O ₅	Tussilagone	413.2287
391*	37.18	[M + Na] ⁺	373.2349	373.2348	-0.189	C ₂₁ H ₃₄ O ₄	10-Gingerol	373.2347; 217.0815
392	37.30	[M + FA-H] ⁻	867.4761	867.4737	2.856	C ₄₄ H ₇₀ O ₁₄	2"-O-Acetylsaikosaponin D	867.4847; 821.4798; 779.46761.4625; 617.4105
393	37.55	[M + H] ⁺	515.3728	515.3731	-0.584	C ₃₂ H ₅₀ O ₅	25-Anhydroalisol A 11-acetate	455.3519; 437.3452; 383.29339.2678; 219.1736; 159.11121.1009;
394	37.60	[M + FA-H] ⁻	825.4655	825.4631	2.850	C ₄₂ H ₆₈ O ₁₃	Saikosaponin D	825.4671; 779.4666; 617.41
395	38.23	[M + H] ⁺	513.3547	513.3575	-5.398	C ₃₂ H ₄₈ O ₅	Alisol O	453.3349; 435.3240; 399.28177.1263; 163.1105;
396*	38.24	[M + FA-H] ⁻	535.3643	535.3629	2.596	C ₃₀ H ₅₀ O ₅	Alisol A	475.3159; 415.0352; 329.17299.0619; 269.1501; 167.05
397	38.31	[M + H] ⁺	529.3495	529.3524	-5.432	C ₃₂ H ₄₈ O ₆	Alismaketone-C 23-acetate	433.3067; 379.2671; 233.15145.1009;
398	38.59	[M + FA-H] ⁻	867.4762	867.4737	2.925	C ₄₄ H ₇₀ O ₁₄	4"-O-Acetylsaikosaponin D	867.4873; 821.4802; 779.46761.4575; 617.4072
399	38.70	[M + Na] ⁺	555.3651	555.3656	-0.901	C ₃₂ H ₅₂ O ₆	Alisol A 24-acetate	555.3658
400	38.72	[M + H-H ₂ O] ⁺	515.3729	515.3731	-0.351	C ₃₂ H ₅₂ O ₆	Alisol A 23-acetate	497.3585; 437.3370; 383.29365.2855; 339.2698;
401	38.78	[M + H] ⁺	511.3391	511.3418	-5.380	C ₃₂ H ₄₆ O ₅	Alisol L 23-acetate	493.3340; 451.3210; 433.31397.2771; 353.2435; 283.19171.1182;
402*	39.29	[M + FA-H] ⁻	559.3644	559.3629	2.592	C ₃₂ H ₅₀ O ₅	Alisol B-23-acetate	537.3542; 477.3315
403	39.30	[M + H] ⁺	515.3727	515.3731	-0.817	C ₃₂ H ₅₀ O ₅	11-Deoxy-13β,17β-epoxyalisol B 23-acetate	383.2960; 339.2673; 341.28203.1418; 121.1004;
404	39.37	[M + FA-H] ⁻	517.3536	517.3524	2.463	C ₃₀ H ₄₈ O ₄	Alisol B	517.3535
405*	40.04	[M + H] ⁺	529.3873	529.3886	-2.798	C ₃₃ H ₅₂ O ₅	Pachymic acid	493.36; 451.36; 433.35; 355295.24

* means that the ingredient was confirmed by the reference substance.

RT-retention time; MH means *Herba Ephedrae* (Mahuang), GC means *Radix Glycyrrhizae Praeparata* (Zhigancao), XR means *Semen Armeniaceae Amarum* (Xingren), *Ramulus Cinnamoni* (Guizhi), ZX means *Rhizoma Alismatis* (Zixie), BZ means *Rhizoma Atractylodis Macrocephalae* (Baizhu), FL means *Poria* (Fuling), CH means *Radix Bupleuri* (Chaihu), HQ means *Radix Scutellariae* (Huangqin), BX means *Rhizoma Pinelliae* prepared with ginger juice (Jiangbanxia), SJ means *Rhizoma Zingiberis Recens* (Shengjiang), ZW means *Radix Asteris* (Ziwan), KDH means *Flos Farfarae* (Kuandonghua), SG means *Rhizoma Belamcandae* (Shegan), XX means *Herba Asari* (Xixin), SY means *Rhizoma Dioscoreae* (Shanyao), ZS means *Fructus Aurantii Immaturus* (Zhishi), CP means *Pericarpium Citri Reticulatae* (Chenpi) 30 g and GHX means *Herba Agastachis* (Huoxiang)

It is well-accepted that deep understanding of the fragmentation pattern of each authentic standard or its analogous is very helpful for the identification of herbal constituents by using HRMS [17]. In this study, the fragmentation pathways of various constituents (ephedrine, amygdalin, nobiletin, liquiritin, gallic acid, chlorogenic acid, saikosaponin A and glycyrrhizic acid) in QFPDD were carefully studied and proposed in Fig. 2 and Fig. 3. For example, ephedrine ($t_R = 7.30$ min), an abundant alkaloid distributed in *Herba Ephedrae*, could be easily detected in the positive ion mode with a quasi-molecular ion $[M + H]^+$ at m/z 166.1226 (C₁₆H₁₆NO). MS/MS fragmentation analysis demonstrated that this natural compound possessed a neutral loss of H₂O, subsequently by the cleavage of C-N bond moiety (Fig. 2A), evidenced by the product ions of m/z 148.1119 $[M + H-H_2O]^+$, m/z 133.0886 $[M + H-H_2O-CH_3]^+$, m/z 117.0701 $[M + H-H_2O-CH_5N]^+$ and m/z 91.0548 $[M + H-H_2O-CH_5N-C_2H_2]^+$. The compound **56** ($t_R = 7.30$ min) in Table 1 were detected in the positive ion mode at the same retention time with the identical m/z of 166.1225 (C₁₆H₁₆NO), thus was characterized as ephedrine. amygdalin ($t_R = 13.93$ min), an abundant alkaloid distributed in *Semen Armeniaceae Amarum*, could be easily detected in the negative ion mode with a quasi-molecular ion $[M + HCOOH-H]^-$ at m/z 502.1567

(C₂₁H₂₈NO₁₃). It was inclined to lose the whole sugar moiety to generate base peak ions m/z of 323.0972 [M + HCOOH-H-C₆H₁₁O₆]⁻ and 179.0550 [C₆H₁₁O₆]⁻ in MS² spectra (Fig. 2B). Subsequently by cleavage of the functional group on the second glucose, such as m/z of 263.0772 [M + HCOOH-H-C₆H₁₁O₆-C₂H₄O₂]⁻ and 221.0661 [M + HCOOH-H-C₆H₁₁O₆-C₇H₄N]⁻. Simultaneously the fragmentation signal of glucose was detected, its MS/MS data were shown as m/z of 161.0444 [C₆H₁₁O₆-H₂O]⁻, 119.0335 [C₆H₁₁O₆-H₂O-C₂H₄O₂]⁻, 101.0229 [C₆H₁₁O₆-H₂O-C₂H₄O₂-H₂O]⁻ and 89.0229 [C₆H₁₁O₆-H₂O-C₂H₄O₂-CH₂O]⁻. The compound **90** in Table 1 were detected in the negative ion mode at 13.93 min with the identical m/z of 502.1568 (C₂₁H₂₈NO₁₃), thus this peak was characterized as amygdalin. Similar to the identification process above, a total of 40 alkaloids were identified from QFPDD, including pseudoephedrine, methylephedrine, ligustrazine, prunasin, et al.

Nobiletin (t_R = 29.30 min), a flavonoid distributed in *Fructus Aurantii Immaturus* and *Pericarpium Citri Reticulatae*, could be easily detected in the positive ion mode with a quasi-molecular ion [M + H]⁺ at m/z 403.1385 (C₂₁H₂₃O₈). MS/MS fragmentation analysis demonstrated that this natural compound possessed demethylation, dehydration, and CO, evidenced by the product ions of m/z 388.1151 [M + H-CH₃]⁺, 373.0916 [M + H-CH₃-CH₃]⁺, 355.0811 [M + H-2CH₃-H₂O]⁺ and 327.0856 [M + H-2CH₃-H₂O-CO]⁺ (Fig. 2C). The compound **321** listed in Table 1 were detected in the positive ion mode at 29.30 min with the identical m/z of 403.1382 (C₂₁H₂₃O₈), thus this peak was characterized as nobiletin. Liquiritin (t_R = 18.59 min), a flavonoid glycoside distributed in *Radix Glycyrrhizae Praeparata*, which was detected in the negative ion mode with a quasi-molecular ion [M-H]⁻ at m/z of 417.1195 (C₂₁H₂₁O₉). Its MS/MS data were shown as m/z of 255.0654 [M-H-C₆H₁₀O₅]⁻, 153.0178 [M-H-C₆H₁₀O₅-C₈H₆]⁻, 135.0071 [M-H-C₆H₁₀O₅-C₈H₈O]⁻ and 119.0484 [M-H-C₆H₁₀O₅-C₇H₄O₃]⁻ (Fig. 2D). The compound **135** listed in Table 1 could be easily detected in the negative ion mode with the same R_t (at 18.59 min) and the identical m/z of 417.1195 (C₂₁H₂₁O₉), this peak was then characterized as liquiritin. Similar to the identification process above, a total of 162 flavonoids were identified from QFPDD, including isoliquiritin, neoliquiritin, kaempferol, luteolin, quercetin, rutin, hesperidin, neohesperidin, formononetin, isoflavin, formononetin et al.

Gallic acid (t_R = 2.09 min), an organic acid distributed in a variety of herbs, which was detected in the negative ion mode with a quasi-molecular ion [M-H]⁻ at the m/z of 169.0127 (C₇H₅O₅). MS/MS fragmentation analysis demonstrated that this natural compound possessed neutral loss of H₂O, CO₂ and/or CO (Fig. 3A), evidenced by the product ions of m/z 151.0020 [M-H-H₂O]⁻, 125.0226 [M-H-CO₂]⁻, 97.0277 [M-H-CO₂-CO]⁻, 81.0327 [M-H-2CO₂]⁻ and 69.0327 [M-H-CO₂-2CO]⁻. Compound **40** listed in Table 1 could be detected in the negative ion mode with the same R_t (at 2.09 min) and the identical m/z of 169.0133 (C₇H₅O₅), this compound was then characterized as gallic acid. Chlorogenic acid (t_R = 12.51 min), an organic acid which was produced from caffeic acid and quinic acid, it was distributed in variety of herbs. It could be easily detected in the positive ion mode with a quasi-molecular ion [M-H]⁻ at m/z 353.0883 (C₁₆H₁₇O₉). MS/MS fragmentation analysis demonstrated that it was easily broken down into caffeic acid and quinic acid to generate base peak ions m/z of 191.0549 [M-H-C₉H₆O₃]⁻ and 179.0337 [M-H-C₇H₁₀O₅]⁻ in MS² spectra (Fig. 3B). Subsequently by neutral loss of H₂O or CO₂, evidenced by the product ions of m/z 173.0442 [M-H-C₉H₆O₃-H₂O]⁻, 161.0228 [M-H-C₇H₁₀O₅-H₂O]⁻, 155.0335 [M-H-C₉H₆O₃-2H₂O]⁻ and 135.0435 [M-H-C₇H₁₀O₅-CO₂]⁻. Compound **81** listed in Table 1 could be easily detected in the negative ion mode with the same R_t (at 12.51 min) and the identical m/z of 353.0880 (C₁₆H₁₇O₉), thus this compound was characterized as chlorogenic acid. Similar to the identification process above, a total of 44 organic acids were identified from QFPDD, including tartaric acid, quinic acid, citric acid, succinic acid, caffeic acid, vanillic acid isochlorogenic acid A, isochlorogenic acid B, isochlorogenic acid C, coumaroyltartaric acid, et al.

Saikosaponin A (t_R = 33.42 min), a triterpene saponins distributed in *Radix Bupleuri*, could be easily detected in the negative ion mode with a quasi-molecular ion [M-H]⁻ at m/z 779.4589 (C₄₂H₆₇O₁₃). It was inclined to lose the sugar moiety to generate base peak ions m/z of 617.4060 [M-H-C₆H₁₀O₅]⁻, 471.3493 [M-H-C₆H₁₀O₅-C₆H₁₀O₄]⁻ and 179.0560 [C₆H₁₁O₆]⁻ in MS² spectra (Fig. 3C). Simultaneously the fragmentation signal of glucose was detected, its MS/MS data were shown as m/z of 161.0446 [C₆H₁₁O₆-H₂O]⁻, 145.0495 [C₆H₁₁O₆-H₂O-O]⁻, 113.0495 [C₆H₁₁O₆-H₂O-C₂H₄O₂]⁻ and 101.0229 [C₆H₁₁O₆-H₂O-C₃H₄O₂]⁻. Compound **374** listed in Table 1 could also be detected in the negative ion mode at the same R_t (at 33.42 min) with the m/z of 825.4652 (C₄₂H₆₇O₁₃), thus this compound was characterized as saikosaponin A. Glycyrrhizic acid (t_R = 31.86 min), a triterpene saponins distributed in *Radix Glycyrrhizae Praeparata*, could be detected in the negative ion mode with a quasi-molecular ion [M-H]⁻ at m/z of 821.3956 (C₄₂H₆₁O₁₆) in Fig. 3D. MS/MS fragmentation analysis demonstrated that this natural compound possessed loss of glucuronic acid group and glucosyl group, evidenced by the product ions of m/z 645.3638 [M-H-C₆H₈O₆]⁻, 469.3304 [M-H-C₆H₈O₆-C₆H₈O₆]⁻. Simultaneously the fragmentation signal of glucuronic acid group and glucosyl group was detected, its MS/MS data were shown as m/z of 351.0563 [M-H-C₃₀H₄₆O₄]⁻, 193.0341 [M-H-C₃₀H₄₆O₄-C₆H₆O₅]⁻ and 175.0232 [M-H-C₃₀H₄₆O₄-C₆H₆O₅-H₂O]⁻. Compound **355** listed in Table 1 could also be detected in the negative ion mode at the same R_t (31.86 min) with the identical m/z of 821.3973 (C₄₂H₆₁O₁₆) and fragment ions, thus this compound was characterized as glycyrrhizic acid. Similar to the identification process above, a total of 71 triterpene saponins were identified from QFPDD, including saikosaponin B2, saikosaponin C, saikosaponin D, saikosaponin F, saikosaponin M, licorice saponin A3, licorice saponin B2, licorice Saponin G2, alisol A, alisol B, alisol C, alisol O, alisol A-23-acetate, alisol B-23-acetate, et al.

The remaining 88 kinds of chemical constituents were identified from QFPDD, including amino acids, nucleosides, sugars, phenols et al. they were tentatively characterized based on their retention times and mass spectrometric data, referring to the reference standards and previous literature. For example, MS² spectra of compound **203** (molecular ion at m/z [M + H]⁺ 133.0647) in Table 1 showed several characteristic fragment ions of m/z 115.0538 [M + H-H₂O]⁺, m/z 105.0673 [M + H-CO]⁺, m/z 86.10 [M + H-H₂O-C₂H₅]⁺. Thus, it corresponded to cinnamaldehyde by comparison with authentic standards and literature data [20]. Moreover, MS² spectra of compound **322** (molecular ion at m/z [M + H]⁺ 285.0752) in Table 1 gave characteristic fragment ions of m/z 270.0912 [M + H-CH₃]⁺, m/z 254.0943 [M + H-CH₃O]⁺, m/z 253.0897 [M + H-CH₃-OH]⁺ and m/z 225.0911 [M + H-CH₃O-CHO]⁺. Then it was identified as batatasin I by comparison with authentic standards and literature data [21].

3.2 Characterization of the absorbed chemical constituents in mice serum

3.2.1 Characterization of the absorbed prototype constituents in mice serum

Next, the serum samples collected from QFPDD-treated mice at different sampling times (1 h, 2 h and 4 h) were analyzed by UHPLC-Q-Exactive-Orbitrap HRMS to observe the peak numbers and their response in TIC chromatograms. It was found that more peaks could be detected from 1 h serum samples, suggesting that most absorbed prototype constituents or metabolite could be detected in this sample. Thus, the serum samples collected from QFPDD-treated mice at 1 h were collected and mixed for LC-MS analysis to detect the absorbed prototypes and metabolites as more as possible. With the help of the key information of the constituents in QFPDD (as listed in Table 1), a total of 104 prototype compounds were presumed, including 12 kinds of alkaloids, 55 kinds of flavonoids, 21 kinds of organic acids, 8 kinds of triterpene saponins and 8 kinds of other compounds (Table 2, Fig. 4 and Fig. 5). Among them, 44 ingredients are confirmed by comparison with the reference standards. Take compound **p9** for example, this peak generated quasi-molecular ion $[M + H]^+$ at m/z 166.1225, along with characteristic fragment ions at m/z 148.1117 $[M + H - H_2O]^+$, 133.0879 $[M + H - H_2O - CH_3]^+$, 117.0699 $[M + H - H_2O - CH_5N]^+$ and 91.0545 $[M + H - H_2O - CH_5N - C_2H_2]^+$, which was identical to the compound **62** in the extract of QFPDD (Table 1). Compound **p9** was thus identified as pseudoephedrine. Similarly, compound **p21** generated deprotonated molecule $[M - H]^-$ at m/z 294.0988, along with characteristic fragment ions at m/z 161.0445 $[C_6H_9O_5]^-$, 101.0229 $[C_6H_9O_5 - C_2H_4O_2]^-$, 85.0279 $[C_6H_9O_5 - C_2H_4O_3]^-$, 71.0123 $[C_6H_9O_5 - C_2H_4O_2 - CH_2O]^-$, which was identical to the product ions of compound **93** in QFPDD. Thus, compound **p21** was characterized as prunasin.

Table 2

Identification of prototype compounds of QFPDD in rat serum and multiple tissue by UHPLC-Q-Exactive Orbitr

NO.	RT /min	Ion model	Measured mass /Da	Calculated mass /Da	Error /ppm	Molecular formula	Fragment ions	Identification	Peak Area (*10 ⁶)	
									Serum	Lung
p1	0.90	[M-H] ⁻	149.0081	149.0092	0.239	C ₄ H ₆ O ₆	149.0080; 87.0073; 72.9916; 59.0123	Tartaric acid	3.14	19.58
p2*	0.92	[M-H] ⁻	191.0555	191.0561	2.279	C ₇ H ₁₂ O ₆	191.0189; 111.0073; 87.0072; 85.0280; 57.0330	Quinic acid	88.17	406.21
p3*	0.97	[M + H] ⁺	144.1019	144.1019	-0.036	C ₇ H ₁₃ NO ₂	144.1017; 84.0812; 56.0503	Stachydrine	673.88	606.92
p4*	5.30	[M + H] ⁺	152.1070	152.1070	-0.007	C ₉ H ₁₃ NO	152.1068; 134.0962; 117.0700; 90.9481; 56.0503	Phenylpropanolamine	7.48	41.12
p5*	6.33	[M + H] ⁺	152.1070	152.1070	0.127	C ₉ H ₁₃ NO	134.0963; 117.0700; 104.9636; 91.0546; 56.0503	D-Demethyl pseudoephedrine	8.27	21.68
p6*	7.57	[M-H] ⁻	353.0883	353.0878	4.479	C ₁₆ H ₁₈ O ₉	353.0884; 191.0554; 179.0341; 135.0439; 85.0280	Neochlorogenic acid	1.47	10.28
p7*	7.74	[M + H] ⁺	166.1227	166.1226	0.176	C ₁₀ H ₁₅ NO	166.1223; 148.1118; 117.0699; 91.0545; 70.0658; 56.0502	Ephedrine	265.92	1579.7
p8	8.31	[M + FA-H] ⁻	520.1677	520.1672	3.036	C ₂₀ H ₂₉ NO ₁₂	474.1601; 312.1094; 150.0549; 101.0230; 71.0124	D-α-[(6-O-β-D-glucopyranosyl-β-D-glucopyranosyl)Oxy]Benzeneacetamide	2.26	14.42
p9*	8.66	[M + H] ⁺	166.1226	166.1226	-0.064	C ₁₀ H ₁₅ NO	166.1225; 148.1117; 133.0879; 117.0699; 91.0545; 70.0657; 56.0502	Pseudoephedrine	75.77	266.35
p10	8.82	[M + H] ⁺	180.1382	180.1383	-0.448	C ₁₁ H ₁₇ NO	180.1380; 162.1275; 135.0803; 117.0700; 57.0343	Methylephedrine	107.64	375.19
p11	9.36	[M-H] ⁻	475.1463	475.1457	3.605	C ₂₀ H ₂₈ O ₁₃	475.1428; 269.1033; 161.0445; 113.0231; 101.0230; 71.0124	L-Amygdalinic acid	0.69	8.86
p12	11.85	[M-H] ⁻	475.1463	475.1457	3.605	C ₂₀ H ₂₈ O ₁₃	475.1468; 314.1400; 269.1034; 181.0497; 135.0439; 101.0231	D-Amygdalinic acid	1.40	8.86
p13	11.96	[M + H] ⁺	137.1074	137.1073	0.182	C ₈ H ₁₂ N ₂	137.1070; 121.9662; 91.0546; 72.9377; 55.9352	Ligustrazine	0.06	2.42

NO.	RT /min	Ion model	Measured mass /Da	Calculated mass /Da	Error /ppm	Molecular formula	Fragment ions	Identification	Peak Area (*10 ⁶)	
									Serum	Lung
p14*	12.43	[M-H] ⁻	353.0884	353.0878	4.734	C ₁₆ H ₁₈ O ₉	353.0884; 191.0551; 179.0331; 173.0445; 161.0231; 155.0320; 135.0442	Chlorogenic acid	8.40	65.98
p15*	13.65	[M-H] ⁻	353.0884	353.0878	4.904	C ₁₆ H ₁₈ O ₉	353.0879; 251.0567; 191.0554; 179.0342; 173.0447; 135.0440; 93.0331	Cryptochlorogenic acid	2.18	20.53
p16	13.75	[M-H] ⁻	443.1933	443.1923	4.798	C ₂₁ H ₃₂ O ₁₀	443.1928; 375.0698; 245.0123; 195.0660; 165.0547; 113.0230; 101.0229	Dihydrophaseic acid 4'-O-β-glucopyranoside	0.52	3.26
p17	13.79	[M + FA-H] ⁻	502.1576	502.1566	4.070	C ₂₀ H ₂₇ NO ₁₁	323.0993; 245.0665; 221.0663; 179.0552; 161.0445; 119.0336; 101.0229	Amygdalin	28.57	203.76
p18	13.82	[M + FA-H] ⁻	493.2292	493.2291	2.569	C ₂₁ H ₃₆ O ₁₀	447.2232; 433.4934; 285.1710; 161.0448; 143.0337; 101.0230; 89.0230	Atractyloside A	0.31	2.24
p19	14.01	[M + FA-H] ⁻	477.1620	477.1614	3.610	C ₁₉ H ₂₈ O ₁₁	431.1563; 269.1034; 161.0445; 113.0231; 101.0230; 71.0123	Benzyl-β-gentiobioside	1.68	12.40
p20	14.03	[M-H] ⁻	431.1566	431.1559	4.179	C ₁₉ H ₂₈ O ₁₁	431.1562; 311.0566; 191.0552; 113.0230; 99.0072; 59.0123	Osmanthuside H or isomer	0.90	6.03
p21	14.14	[M-H] ⁻	294.0988	294.0983	5.462	C ₁₄ H ₁₇ NO ₆	294.0988; 161.0445; 101.0229; 85.0279; 71.0123	Prunasin	8.31	7.16
p22*	15.27	[M-H] ⁻	515.1197	515.1195	2.499	C ₂₅ H ₂₄ O ₁₂	485.9193; 434.3426; 353.0880; 285.1937; 191.0553; 161.0233; 135.0438	1,3-O-Dicaffeoylquinic acid	0.09	2.17
p23	15.39	[M + H] ⁺	303.0500	303.0499	0.201	C ₁₅ H ₁₀ O ₇	303.0493; 283.1068; 257.0439; 229.0490; 207.0285; 139.0386; 111.0078	Herbacetin	1.19	5.52
p24	16.20	[M-H] ⁻	327.1091	327.1085	5.154	C ₁₅ H ₂₀ O ₉	311.9916; 205.0349; 165.0548; 143.0338; 125.0231; 121.0646; 81.0331	Dihydrocinnacasside	6.18	11.36

NO.	RT /min	Ion model	Measured mass /Da	Calculated mass /Da	Error /ppm	Molecular formula	Fragment ions	Identification	Peak Area (*10 ⁶)	
									Serum	Lung
p25	17.00	[M-H] ⁻	593.1520	593.1512	3.770	C ₂₇ H ₃₀ O ₁₅	593.1522; 503.1204; 473.1093; 383.0779; 353.0672; 271.0611; 191.0342	Vicenin-li	1.43	8.82
p26	17.39	[M-H] ⁻	463.0891	463.0882	4.206	C ₂₁ H ₂₀ O ₁₂	463.0892; 341.0670; 300.0278; 271.0252; 178.9975; 139.0388; 89.0230	Carthamidin-7-O-glucuronide	18.23	4.24
p27*	17.43	[M-H] ⁻	193.0495	195.0652	0.485	C ₁₀ H ₁₀ O ₄	193.0493; 177.0542; 163.0387; 145.0281; 135.0439; 117.0335; 89.0388	Ferulic acid	15.28	4.94
p28	17.59	[M-H] ⁻	623.1632	623.1618	4.090	C ₂₈ H ₃₂ O ₁₆	623.1628; 533.1307; 503.1204; 463.0891; 413.0885; 383.0779; 312.0631	Isorhamnetin-3-O-rutinoside	0.15	3.16
p29	17.88	[M + H] ⁺	625.1758	625.1763	-0.850	C ₂₈ H ₃₂ O ₁₆	581.2217; 419.1717; 404.1479; 373.1295; 233.0816; 202.0629; 153.0546	4'-Methoxy-lucenin-2	0.10	3.11
p30	18.11	[M-H] ⁻	581.2245	581.2240	2.792	C ₂₈ H ₃₈ O ₁₃	581.2239; 53.1824; 419.1716; 404.1482; 373.1298; 355.1193; 223.0818	(+)-Lyoniresinol 9'-O-glucoside	0.13	2.06
p31	18.17	[M-H] ⁻	295.0463	295.0459	4.800	C ₁₃ H ₁₂ O ₈	295.1540; 259.0247; 221.0816; 189.0550; 163.0760; 143.0492; 121.0283	Coumaroyltartaric acid	1.99	5.88
p32*	18.21	[M-H] ⁻	137.0233	137.0244	-0.515	C ₇ H ₆ O ₃	137.0231; 93.0331; 84.8779; 70.7439	Salicylic acid	295.38	94.00
p33*	18.39	[M + H] ⁺	433.1130	433.1129	0.131	C ₂₁ H ₂₀ O ₁₀	433.1108; 415.1017; 397.0912; 337.0700; 313.0701; 271.0596; 206.0546	Isovitexin	2.84	0.00
p34*	18.57	[M-H] ⁻	417.1197	417.1191	4.103	C ₂₁ H ₂₂ O ₉	417.1188; 255.0664; 191.0560; 153.0183; 153.0183; 135.0076; 119.0488	Liquiritin	2.12	99.99
p35	18.82	[M-H] ⁻	595.1672	595.1668	2.409	C ₂₇ H ₃₂ O ₁₅	595.1689; 435.0936; 315.0723; 271.0615; 177.0186; 151.0026; 137.0233	Neoeriocitrin	0.08	11.56

NO.	RT /min	Ion model	Measured mass /Da	Calculated mass /Da	Error /ppm	Molecular formula	Fragment ions	Identification	Peak Area (*10 ⁶)	
									Serum	Lung
p36	18.84	[M + H] ⁺	366.1911	366.1911	-0.038	C ₁₉ H ₂₇ NO ₆	366.1905; 348.1799; 320.1843; 242.8689; 168.1017; 150.0912; 122.0600	Senkirkine /isomer	9.05	36.41
p37	18.86	[M-H] ⁻	741.2265	741.2248	3.838	C ₃₃ H ₄₂ O ₁₉	741.2263; 621.1686; 417.1567; 353.1033; 271.0615; 181.0499; 151.0062	Naringenin-7-O-triglycoside	0.37	8.96
p38	19.28	[M-H] ⁻	595.1679	595.1668	3.534	C ₂₇ H ₃₂ O ₁₅	595.1689; 435.0936; 315.0723; 271.0615; 177.0186; 151.0026; 137.0233	Eriocitrin	0.09	46.34
p39	19.50	[M-H] ⁻	547.1466	547.1457	3.532	C ₂₆ H ₂₈ O ₁₃	547.1465; 487.1251; 457.1147; 427.1038; 367.0827; 337.0723; 255.0665	chrysin-6-C-arabinosyl-8-C-glucoside	6.88	68.09
p40*	19.55	[M-H] ⁻	461.0734	461.0725	4.203	C ₂₁ H ₁₈ O ₁₂	461.1097; 446.0860; 341.0640; 298.0486; 255.0664; 167.0341	Scutellarin	1.28	6.20
p41*	19.61	[M-H] ⁻	461.1098	461.1089	4.320	C ₂₂ H ₂₂ O ₁₁	461.1094; 371.0776; 330.0383; 198.0483; 185.0407; 129.0179	Tectoridin	0.31	38.15
p42	19.72	[M-H] ⁻	771.2364	771.2353	2.814	C ₃₄ H ₄₄ O ₂₀	771.2362; 607.1677; 489.1410; 301.0719; 286.0484; 125.0231	Alpha-glucosyl hesperidin	0.23	6.42
p43*	19.74	[M-H] ⁻	515.1202	515.1195	3.451	C ₂₅ H ₂₄ O ₁₂	353.0883; 191.0554; 173.0447; 135.0439; 93.0330	Isochlorogenic acid B	0.89	91.68
p44	19.83	[M + H] ⁺	493.1317	493.1343	-4.811	C ₂₃ H ₂₄ O ₁₂	493.1729; 417.0955; 375.0841; 331.0807; 316.0572; 245.1202; 197.0441	Iristectorin A	0.68	10.92
p45	19.89	[M + H] ⁺	465.1031	465.1028	0.726	C ₂₁ H ₂₀ O ₁₂	465.1031	Hyperin	0.12	37.73
p46	19.98	[M-H] ⁻	577.1570	577.1563	3.219	C ₂₇ H ₃₀ O ₁₄	577.1570; 457.1139; 413.0885; 341.0673; 293.0461; 269.0458; 59.0124	Isoviolanthin	2.38	15.29
p47	19.99	[M + H] ⁺	449.1078	449.1078	-0.173	C ₂₁ H ₂₀ O ₁₁	426.0634; 398.0688; 369.0294; 353.0887; 338.0652; 165.0909	Naringenin-7-O-glucuronide	39.81	6.91

NO.	RT /min	Ion model	Measured mass /Da	Calculated mass /Da	Error /ppm	Molecular formula	Fragment ions	Identification	Peak Area (*10 ⁶)	
									Serum	Lung
p48*	19.99	[M-H] ⁻	463.0892	463.0882	4.530	C ₂₁ H ₂₀ O ₁₂	463.0892; 341.0670; 300.0278; 271.0252; 178.9975; 139.0388; 89.0230	Isoquercitrin	0.61	10.56
p49*	20.01	[M-H] ⁻	609.1467	609.1461	2.822	C ₂₇ H ₃₀ O ₁₆	609.1473; 487.1224; 431.0981; 300.0278; 269.0461; 178.9979	Rutin	0.16	20.92
p50*	20.03	[M-H] ⁻	515.1202	515.1195	3.451	C ₂₅ H ₂₄ O ₁₂	515.1160; 353.0884; 191.0554; 179.0337; 161.0234	Isochlorogenic acid A	0.87	10.05
p51	20.18	[M + H] ⁺	449.1078	449.1078	-0.173	C ₂₁ H ₂₀ O ₁₁	426.0634; 398.0688; 369.0294; 353.0887; 338.0652; 165.0909	Naringenin-4'-O-glucuronide	39.81	2.13
p52	20.57	[M-H] ⁻	547.1466	547.1457	3.532	C ₂₆ H ₂₈ O ₁₃	547.1463; 457.1144; 427.1038; 367.0829; 337.0724; 300.0280; 191.0339	chrysin-6-C-glucosyl-8-C-arabinoside	2.68	42.87
p53*	20.73	[M-H] ⁻	579.1728	579.1719	3.381	C ₂₇ H ₃₂ O ₁₄	579.1724; 459.1160; 415.1048; 313.0718; 271.0614; 235.0247; 151.0026	Naringin	2.44	1402.46
p54*	20.82	[M + H] ⁺	229.0860	229.0859	0.389	C ₁₄ H ₁₂ O ₃	229.0855; 211.0750; 183.0803; 145.0648; 135.0439; 119.0492; 107.0494	Resveratrol	1.04	0.29
p55	20.88	[M-H] ⁻	581.1899	581.1876	5.950	C ₂₇ H ₃₄ O ₁₄	581.1895	2,4,6-Trihydroxydihydrochalcone3-O-β-D-glucoside-6-O-β-D-glucoside	0.31	1.53
p56*	20.92	[M + H] ⁺	493.1342	493.1343	0.279	C ₂₃ H ₂₄ O ₁₂	493.2296; 373.0909; 331.0807; 271.0594; 177.0545	Iristectorin B	0.21	39.56
p57*	21.21	[M-H] ⁻	609.1837	609.1825	3.699	C ₂₈ H ₃₄ O ₁₅	609.1832; 489.1412; 343.0831; 301.0721; 217.0510	Hesperidin	1.10	246.73
p58*	21.38	[M-H] ⁻	515.1202	515.1195	3.452	C ₂₅ H ₂₄ O ₁₂	515.1160; 353.0884; 255.0663; 191.0554; 173.0447; 161.0234	Isochlorogenic acid C	0.23	66.60
p59*	21.58	[M-H] ⁻	609.1834	609.1825	3.305	C ₂₈ H ₃₄ O ₁₅	609.1829; 489.1410; 403.1042; 343.0825; 301.0720; 286.0485	Neohesperidin	1.98	983.38

NO.	RT /min	Ion model	Measured mass /Da	Calculated mass /Da	Error /ppm	Molecular formula	Fragment ions	Identification	Peak Area (*10 ⁶)	
									Serum	Lung
p60*	21.92	[M-H] ⁻	255.0660	255.0663	5.715	C ₁₅ H ₁₂ O ₄	255.0665; 213.4413; 153.0183; 135.0076; 119.0490; 91.0175	Liquiritigenin	0.89	0.00
p61	22.05	[M-H] ⁻	201.0188	201.0193	2.959	C ₁₁ H ₆ O ₄	201.0676; 183.1021; 169.0399; 144.0443; 131.03664; 116.0494; 116.0493	Xanthotoxol	0.05	11.43
p62*	22.06	[M-H] ⁻	417.1199	417.1191	4.462	C ₂₁ H ₂₂ O ₉	417.1203; 358.4896; 297.0761; 255.0663; 148.0155; 135.0076	Isoliquiritin	0.27	13.09
p63	22.12	[M-H] ⁻	443.0950	443.0983	-7.6	C ₂₂ H ₂₀ O ₁₀	267.0667; 252.0429; 227.0188; 175.0238	Formononetin-glucuronide	4.00	1.65
p64	22.26	[M + FA-H] ⁻	475.1253	475.1267	3.898	C ₂₂ H ₂₂ O ₉	329.0312; 299.0560; 267.0666; 151.0426; 151.0027	Ononin	0.10	10.49
p65	22.39	[M + H] ⁺	532.2408	532.2402	1.222	C ₂₅ H ₃₃ N ₅ O ₈	479.2282; 361.0911; 346.0677; 273.0752; 180.1016	Asterinin A	0.85	1.24
p66*	22.84	[M-H] ⁻	445.0783	445.0776	3.982	C ₂₁ H ₁₈ O ₁₁	355.0813; 269.0457; 243.0666; 175; 0241; 113.0230	Baicalin	217.59	974.47
p67	22.85	[M + H] ⁺	287.0548	287.0550	-0.817	C ₁₅ H ₁₀ O ₆	287.0545; 269.0440; 241.0492; 225.0545; 197.0594; 139.0022; 103.0546	Luteolin	13.44	65.73
p68	22.96	[M-H] ⁻	263.1292	263.1289	5.299	C ₁₅ H ₂₀ O ₄	263.0962; 219.1387; 204.1150; 151.0754; 139.0753; 79.9559	(+)-Absciscic acid	1.68	4.82
p69	23.13	[M + H] ⁺	361.0916	361.0930	-0.675	C ₁₈ H ₁₆ O ₈	361.0910; 346.0677; 301.0700; 286.0466; 219.1172; 174.0911; 117.0700	Isoirigenin	56.14	91.11
p70	23.92	[M-H] ⁻	271.0616	271.0612	5.350	C ₁₅ H ₁₂ O ₅	271.0614; 227.0712; 177.0184; 151.0025; 119.0489; 93.03331	Naringenin	1.33	102.41
p71	24.00	[M-H] ⁻	445.1147	445.1140	3.969	C ₂₂ H ₂₂ O ₁₀	445.1147; 430.0911; 325.0717; 283.0615; 268.0374; 113.0230	Wogonin-5-O-β-D -glucoside	7.33	1.44

NO.	RT /min	Ion model	Measured mass /Da	Calculated mass /Da	Error /ppm	Molecular formula	Fragment ions	Identification	Peak Area (*10 ⁶)	
									Serum	Lung
p72	24.28	[M-H] ⁻	429.0832	429.0827	3.582	C ₂₁ H ₁₈ O ₁₀	429.2076; 342.4299; 253.0507; 223.1342; 175.0239; 113.0229; 85.0279	Chrysin-7-O-β-D-glucuronide	11.43	65.77
p73	24.28	[M-H] ⁻	461.1099	461.1089	4.451	C ₂₂ H ₂₂ O ₁₁	461.1095; 440.0857; 299.0558; 283.0251; 268.0379; 211.0398; 113.0230	(2S)-5,7-Dihydroxy-6-methoxyflavanone-7-O-β-D-glucuronide	9.36	20.24
p74	24.40	[M-H] ⁻	473.0732	473.0725	3.715	C ₂₂ H ₁₈ O ₁₂	473.1093; 413.0858; 349.1303; 307.0831; 268.0380; 165.0547; 59.0123	Cichoric acid	0.22	0.57
p75*	24.67	[M-H] ⁻	459.0940	459.0933	3.991	C ₂₂ H ₂₀ O ₁₁	299.0563; 283.0613; 268.0379; 175.0238; 113.0230	Oroxylin A 7-O-glucuronide	33.80	290.88
p76	24.74	[M + H] ⁺	301.0706	301.0711	-0.248	C ₁₆ H ₁₂ O ₆	301.1040; 269.0439; 197.0448; 139.0021	Tectorigenin	15.18	46.57
p77	24.77	[M-H] ⁻	301.0722	301.0718	4.701	C ₁₆ H ₁₄ O ₆	301.0717; 284.0328; 257.0478; 164.0106; 151.0026; 123.0440; 80.9637	Hesperetin	0.06	29.45
p78	24.87	[M-H] ⁻	475.0887	475.0882	3.258	C ₂₂ H ₂₀ O ₁₂	475.0868; 433.8798; 299.0565; 284.0330; 153.0180; 113.0231	5,7,8-Trihydroxy-6-methoxy flavone-7-O-glucuronide	86.81	29.17
p79*	24.89	[M + FA-H] ⁻	491.1202	491.1195	3.741	C ₂₂ H ₂₂ O ₁₀	351.9693; 283.0614; 268.0377; 130.2955; 91.9576	Tilianin	0.76	237.40
p80*	25.06	[M-H] ⁻	459.0939	459.0933	3.795	C ₂₂ H ₂₀ O ₁₁	283.0610; 268.0379; 175.0235; 129.0180; 113.0230	Wogonoside	114.52	610.36
p81	25.43	[M + Na] ⁺	231.0627	231.0649	-0.217	C ₁₁ H ₁₂ O ₄	231.1370; 220.4256; 185.1321; 173.0960; 145.0645; 131.0851; 105.0696	Ethyl caffeate	0.38	26.29
p82*	25.54	[M-H] ⁻	445.0782	445.0776	3.645	C ₂₁ H ₁₈ O ₁₁	445.0753; 283.0615; 269.0458; 225.0559; 175.0245; 113.0231	Baicalein 6-β-D-glucuronide	142.76	25.71
p83*	25.63	[M-H] ⁻	359.0781	359.0772	5.336	C ₁₈ H ₁₆ O ₈	359.0777; 344.0541; 329.0308; 314.0082; 285.0403; 127.6942	Rosmarinic acid	0.68	82.92

NO.	RT /min	Ion model	Measured mass /Da	Calculated mass /Da	Error /ppm	Molecular formula	Fragment ions	Identification	Peak Area (*10 ⁶)	
									Serum	Lung
p84	25.73	[M-H] ⁻	269.0458	269.0455	4.944	C ₁₅ H ₁₀ O ₅	269.0457; 239.0349; 197.0600; 164.9820; 136.9867; 85.0282	Norwogonin	15.91	74.86
p85	25.93	[M-H] ⁻	489.1046	489.1039	3.675	C ₂₃ H ₂₂ O ₁₂	489.2673; 453.2310; 313.0721; 298.0486; 283.0254; 211.0406; 113.0232	5,7-Dihydroxy-8,2'-dimethoxyflavone 7-O-β-D-glucuronide	1.83	18.42
p86*	26.11	[M-H] ⁻	255.0665	255.0663	5.311	C ₁₅ H ₁₂ O ₄	255.0663; 237.1504; 221.0815; 171.1495; 153.0182; 135.0182; 119.0489	Isoliquiritigenin	0.57	17.94
p87	27.73	[M + H] ⁺	387.1073	387.1074	-0.449	C ₂₀ H ₁₈ O ₈	387.1065; 372.0831; 357.0619; 341.0647; 326.0788	Irisflorentin	2.18	108.69
p88	28.52	[M-H] ⁻	283.0615	283.0612	5.071	C ₁₆ H ₁₂ O ₅	283.0613; 268.0380; 255.0301; 215.1290; 164.01117; 133.0653; 87.0084	Wogonin	6.53	200.24
p89	28.77	[M-H] ⁻	313.0722	313.0718	4.809	C ₁₇ H ₁₄ O ₆	313.0720; 298.0485; 283.0250; 253.0506; 211.0397; 173.0594; 131.0705	3',4'-Dihydroxy-7,5'-dimethoxyflavone	1.11	4.21
p90	29.06	[M-H] ⁻	983.4514	983.4493	-5.459	C ₄₈ H ₇₂ O ₂₁	983.4497; 821.3976; 803.3886; 645.3657; 351.0573; 289.0569; 193.0347	Licorice saponin A3	1.02	2.54
p91	29.31	[M + H] ⁺	403.1387	403.1387	-0.109	C ₂₁ H ₂₂ O ₈	403.1381; 373.0911; 355.0809; 211.0220; 151.0752; 79.7506	Nobiletin	1.56	148.43
p92	29.37	[M + FA-H] ⁻	549.3441	549.3433	3.395	C ₃₀ H ₄₈ O ₆	549.3024; 485.3278; 467.3163; 367.2645; 249.1490; 191.1072; 139.0753	16-Oxo-alisol A	0.17	8.36
p93	29.42	[M + H] ⁺	285.0757	285.0758	-0.351	C ₁₆ H ₁₂ O ₅	285.0751; 270.0519; 242.0569; 153.0182; 133.0648; 62.4481	Oroxylin A	7.78	130.51
p94	29.53	[M-H] ⁻	329.2338	329.2333	4.706	C ₁₈ H ₃₄ O ₅	329.2338; 311.2223; 229.1443; 211.1335; 171.1015; 127.1015	Pinellic acid	2.95	74.22

NO.	RT /min	Ion model	Measured mass /Da	Calculated mass /Da	Error /ppm	Molecular formula	Fragment ions	Identification	Peak Area (*10 ⁶)	
									Serum	Lung
p95	29.80	[M-H] ⁻	329.2339	329.2333	5.071	C ₁₈ H ₃₄ O ₅	329.2337; 311.2232; 293.2121; 211.1335; 171.1017; 139.1116; 99.0801	Pinellic acid isomer	2.46	35.22
p96	30.33	[M-H] ⁻	329.2338	329.2333	4.797	C ₁₈ H ₃₄ O ₅	329.2337; 311.2227; 251.1648; 211.1126; 171.1017; 139.1116; 99.0801	Pinellic acid isomer	2.46	13.65
p97	30.38	[M-H] ⁻	353.1033	353.1031	3.867	C ₂₀ H ₁₈ O ₆	353.1033; 285.1136; 285.1136; 254.0584; 193.0856; 125.0232	Licoisoflavone A	0.45	19.92
p98	30.76	[M + H] ⁺	373.1282	373.1282	-0.025	C ₂₀ H ₂₀ O ₇	373.1271; 343.0806; 325.0700; 297.0748; 217.0815; 121.0648	Tangeretin	3.84	101.45
p99	30.96	[M-H] ⁻	837.3931	837.3914	3.276	C ₄₂ H ₆₂ O ₁₇	837.3947; 775.3915; 661.3590; 485.3269; 351.0573; 193.0347; 71.0123	Licorice saponin Q2	2.55	8.08
p100*	31.84	[M-H] ⁻	821.3980	821.3965	3.090	C ₄₂ H ₆₂ O ₁₆	821.3978; 645.3635; 351.0574; 193.0347	Glycyrrhizic acid	14.12	40.48
p101*	33.42	[M -H] -	779.4603	779.4576	3.464	C ₄₂ H ₆₈ O ₁₃	779.4603; 617.4066; 471.3486; 179.0548; 161.0445; 145.0495; 101.0230	Saikosaponin A	0.08	14.77
p102	38.23	[M + H] ⁺	513.3551	513.3575	-4.580	C ₃₂ H ₄₈ O ₅	513.3542; 496.3501; 446.9593; 316.2172; 255.7422; 169.3950	Alisol O	46.16	106.65
p103*	38.24	[M + FA-H] ⁻	535.3648	535.3640	3.399	C ₃₀ H ₅₀ O ₅	535.3384; 475.3167; 415.0358; 329.1729; 299.0611; 269.1505; 167.0551	Alisol A	37.75	91.35
p104*	39.37	[M + FA-H] ⁻	517.3539	517.3535	3.043	C ₃₀ H ₄₈ O ₄	517.3328; 311.2019; 253.1591; 169.5029; 108.0066	Alisol B	31.79	17.44

p- is expressed as prototype; *-is expressed as prototype and confirmed by reference substance; RT-retention time

Flavonoids were major constituents in several herbs for preparing QFPDD, it is necessary to identify the absorbed flavonoids in serum from QFPDD-treated mice. In this study, a total of 54 flavonoids (as the absorbed prototype constituents) were detected in mice serum. Take compound **p87** for example, this peak generated a quasi-molecular ion [M + H]⁺ at *m/z* 387.1073, along with characteristic fragment ions at *m/z* 372.0831[M + H-CH₃]⁺, 357.0619[M + H-CH₃-CH₃]⁺, 341.0747[M + H-CH₃-CH₃O]⁺, 326.0788[M + H-CH₃-CH₂O₂]⁺, which was highly consistent with the product ions of compound **302** in QFPDD. As a result, compound **p87** was identified as the prototype of compound **302** (irisflorentin). Similarly, compound **p57** shared analogical elution time and identical ions in

MS² spectra with compound **188** in the extract of QFPDD, such as m/z 301.07[M-H-C₁₂H₂₀O₉]⁻, 286.05[M-H-C₁₂H₂₀O₉-CH₃]⁻, 257.08[M-H-C₁₂H₂₀O₉-CO₂]⁻, 242.06[M-H-C₁₂H₂₀O₉-CH₃-CO₂]⁻. For this reason, compound **p57** was then deemed to be hesperidin, *i.e.*, the prototype of compound **188**.

Triterpene saponins were another major group of bioactive components in QFPDD, and it was found that the majority of them could be absorbed into serum. For example, compound **p103** gave molecular formula C₃₀H₅₀O₅ and deprotonated molecule at m/z 535.3648 [M-H]⁻, along with distinctive fragment ions at m/z generated deprotonated molecule [M-H]⁻ at m/z 475.3167, 415.0358, 329.1729, 299.0611, 269.1505, 167.0551, which was in keeping with compound **396** in the extract of QFPDD. Then, compound **p103** was regarded as prototype of compound **396** Alisol A.

Similarly, compound **p50** (an organic acid) occurring in rat serum showed identical retention times and quasi-molecular ion (m/z 515.1202 [M-H]⁻) to compound **158** in QFPDD. Furthermore, they also produced near identical fragment ions in MS² spectra, *e.g.*, m/z 353.0884[M-H-C₉H₆O₃]⁻, 191.0554[M-H-C₉H₆O₃-C₉H₆O₃]⁻, 179.0337[M-H-C₉H₇O₄-C₉H₇O₄]⁻, 161.0234[M-H-C₉H₆O₃-C₇H₁₃O₅]⁻. Thus, compound **p50** was identified as the prototype of compound **158**, namely isochlorogenic acid A.

3.2.2 Identification of the metabolites in mice serum

Next, the metabolites in serum from QFPDD-treated mice that are derived from the constituents in QFPDD were tentatively characterized. To discriminate the endogenous metabolites from QFPDD-related metabolites, the TIC chromatograms of QFPDD-treated mice serum were compared with that of the blank serum. After then, the endogenous substances could be excluded, while the exogenous compounds could be identified by comprehensive analysis. By contrasting these compounds with already known compounds *in vitro*, searching for the same compounds and validating them *via* comparison of the retention time and the product ions. TraceFinder software incorporated in UHPLC-Q-Exactive-Orbitrap HRMS could process the data acquired, provide key information about group changes from prototypes to metabolites by means of exact molecular weight, and provide elementary compositions thereof. These analyses were beneficial to determine the prototypes and the metabolites in biological samples, and to conjecture potential metabolic pathways. Furthermore, in this study, parts of *in vivo* metabolites of the major constituents in QFPDD were identified *via* comparison with the retention times and MS/MS spectra of the *in vitro* metabolites generated in MLMs.

Taking metabolites **M27**, **M30** and **M65** as the examples, these three compounds gave similar quasi-molecular ion [M-H]⁻ at m/z 431.0983, as well as characteristic fragment ions at m/z 255.0665 [-176 Da, M-H-C₆H₈O₆]⁻, m/z 135.0090 [M-H-C₆H₈O₆-C₈H₈O]⁻, m/z 119.0501 [M-H-C₆H₈O₆-C₇H₄O₃]⁻ in the MS² spectrum. The fragment ions of these three metabolites were identical with that of compound **205** (liquiritigenin), a major constituent identified from the extract of QFPDD. *In vitro* metabolism of liquiritigenin in MLMs incubated with both NADPH and UDPGA, three mono-*O*-glucuronides could be easily detected. The retention times, product ions were consistent with the metabolites **M27**, **M30** and **M65**. In these cases, these three compounds were identified as liquiritigenin-*O*-glucuronide. Similarly, the *in vivo* metabolites **M34** and **M70** are identified as typical sulfates, both compounds displayed similar quasi-molecular ion [M-H]⁻ at m/z 335.0231, as well as characteristic fragment ions at m/z 255.0665 [-80 Da, M-H-SO₃]⁻, m/z 135.0090 [M-H-SO₃-C₈H₈O]⁻, m/z 119.0501 [M-H-SO₃-C₇H₄O₃]⁻ in the MS² spectrum, the fragment ions were identical with that of the authentic standard liquiritigenin. *In vitro* metabolism demonstrated that following incubation of liquiritigenin in MLMs in the presence of both NADPH and PAPS, two mono-*O*-sulfates could be easily detected. The retention times, product ions were well-matched with the metabolites **M34** and **M70**. Thus, these two peaks were identified as liquiritigenin-*O*-sulfate.

The metabolite **M21** and **M25** were produced from an organic acid. They gave similar quasi-molecular ion [M-H]⁻ at m/z 367.1034, as well as characteristic fragment ions at m/z 353.0883 [-15 Da, M-H-CH₃]⁻, m/z 191.0549 [M-H-CH₃-C₉H₆O₃]⁻, m/z 179.0337 [M-H-CH₃-C₇H₁₀O₅]⁻, m/z 173.0442 [M-H-C₉H₆O₃-H₂O]⁻, m/z 161.0228 [M-H-CH₃-C₇H₁₀O₅-H₂O]⁻ in the MS² spectrum, the fragment ions were identical with the compound **59** (neochlorogenic acid), **80** (chlorogenic acid), **87** (cryptochlorogenic acid) in the extract of QFPDD. *In vitro* metabolism demonstrated that following incubation of chlorogenic acid in MLMs in the presence of NADPH and SAM, two *O*-methylated metabolites were detected. The retention times, product ions were well-matched with that of the metabolites **M21** and **M25**. Thus, these two metabolites were identified as *O*-methylated chlorogenic acid. Previous study have reported that two *O*-methylated metabolites of chlorogenic acid were found in urine samples after intramuscular administration of chlorogenic acid injection to healthy adults, and the pure *O*-methylated metabolites were synthesized by chlorogenic acid and followed by isolation, purification and identification [22]. According to the chromatographic behavior of them, **M21** was tentatively identified as 3'-methyl-chlorogenic acid, and **M25** was tentatively identified as 4'-methyl-chlorogenic acid.

Following carefully metabolite identification as mentioned above, a total of 91 metabolites were tentatively identified (Table 3). A variety of metabolic reactions, such as hydroxylation, *O*-methylation, *O*-glucuronidation, *O*-sulfation, demethylation, were involved in the metabolism of the major constituents in QFPDD. Among them, 20 metabolites are fully confirmed by the *in vitro* metabolites generated in RLM.

Table 3

Identification of metabolites of QFPDD in rat serum and multiple tissues by UHPLC-Q-Exactive Orbitrap HRMS

NO	RT /min	Ion model	Measured mass /Da	Calculated mass /Da	Error /ppm	Molecular formula	Fragment ions	Identification	Peak Area (*10 ⁶)			
									Serum	Lung	Liver	Heart
M1	4.45	[M + H] ⁺	134.0590	134.0600	-7.8	C ₈ H ₇ NO	106.0629; 79.0536	Mandelonitrile	83.46	43.02	96.52	70.0
M2	8.16	[M-H] ⁻	477.0648	477.0674	-5.4	C ₂₁ H ₁₈ O ₁₃	477.1811; 343.0462; 301.0357; 193.0138; 151.0025; 125.0232	Quercetin-glucuronide	0.85	0.00	0.00	0.0
M3	8.49	[M-H] ⁻	371.0970	371.0983	-3.7	C ₁₆ H ₂₀ O ₁₀	371.0987; 309.0988; 248.9853; 223.0609; 195.0657; 157.0131; 113.0231	Caffeic acid-methylation-glucuronide-reduction	1.18	0.00	0.25	0.0
M4#	10.37	[M-H] ⁻	258.9918	258.9918	0.0	C ₉ H ₈ SO ₇	179.0342; 135.0439; 96.9587; 79.9558	Caffeic acid-sulfate	234.36	1.52	39.90	19.0
M5	11.25	[M-H] ⁻	637.1031	637.1046	-2.4	C ₂₇ H ₂₆ O ₁₈	285.0395	Scutellarein-di-glucuronide	0.02	0.00	0.00	0.0
M6	12.05	[M + FA-H] ⁻	293.1248	293.1242	5.786	C ₁₁ H ₂₀ O ₆	293.1243; 232.1332; 187.1077; 131.0687; 119.0332; 89.0217	Pent-4-enyl-D-glucopyranoside isomer	4.73	4.53	8.86	3.1
M7	12.68	[M-H] ⁻	477.0670	477.0674	-0.8	C ₂₁ H ₁₈ O ₁₃	477.1844; 372.8266; 301.0358; 283.0250; 193.0136; 151.0026	Quercetin-glucuronide	1.64	0.00	0.00	0.0
M8	13.09	[M-H] ⁻	256.9764	256.9761	1.2	C ₉ H ₆ SO ₇	239.2304; 198.4493; 177.0186; 159.1671; 133.0649	5,7-Dihydroxychromone-sulfate	18.35	1.95	1.41	2.0
M9	14.01	[M + FA-H] ⁻	293.1247	293.1242	5.445	C ₁₁ H ₂₀ O ₆	293.1245; 232.1336; 187.1082; 131.0701; 119.0337; 89.0229	Pent-4-enyl-D-glucopyranoside isomer	3.14	3.79	20.25	0.0
M10	14.40	[M-H] ⁻	294.0989	294.0983	5.564	C ₁₄ H ₁₇ NO ₆	251.0562; 161.0446; 121.0282; 113.0231; 85.0280; 71.0123	Prunasin isomer	8.31	1.17	8.43	8.4
M11	14.53	[M-H] ⁻	369.0820	369.0827	-2.0	C ₁₆ H ₁₈ O ₁₀	193.0543; 164.9292; 120.9383	Chlorogenic acid-methylation	0.60	0.00	1.65	0.0
M12	14.59	[M + FA-H] ⁻	293.1247	293.1242	5.581	C ₁₁ H ₂₀ O ₆	293.1244; 247.0244; 213.1745; 173.0812; 131.0701; 119.0336; 89.0229	Pent-4-enyl-D-glucopyranoside isomer	2.32	0.68	0.80	0.0
M13	14.97	[M-H] ⁻	593.1498	593.1512	-2.4	C ₂₇ H ₃₀ O ₁₅	593.1498; 417.1192; 369.0989; 255.0665; 175.0240; 135.0075	Liquiritin-glucuronide	3.71	0.17	0.38	0.0
M14	15.38	[M-H] ⁻	167.0342	167.0350	1.585	C ₈ H ₈ O ₄	167.0341	Vanillic acid isomer	0.55	5.52	1.18	0.0

NO	RT /min	Ion model	Measured mass /Da	Calculated mass /Da	Error /ppm	Molecular formula	Fragment ions	Identification	Peak Area (*10 ⁶)			
									Serum	Lung	Liver	He
M15	15.41	[M-H] ⁻	327.1090	327.1085	4.787	C ₁₅ H ₂₀ O ₈	327.1416; 266.1181; 211.1447; 164.0706; 147.0440; 113.0230; 89.0229	Dihydrocinnacasside isomer	3.76	3.71	4.81	0.0
M16	15.82	[M-H] ⁻	167.0342	167.0350	1.645	C ₈ H ₈ O ₄	167.0342	Vanillic acid isomer	3.04	0.00	0.78	0.0
M17	15.91	[M-H] ⁻	637.1017	637.1046	-4.6	C ₂₇ H ₂₆ O ₁₈	637.1015	Scutellarein-di- glucuronide	0.13	0.00	0.00	0.0
M18	16.05	[M-H] ⁻	511.0535	511.0552	-3.3	C ₂₁ H ₂₀ O ₁₃ S	431.0993; 335.0238; 255.0665; 217.0175; 175.0241; 135.0077	Liquiritin-sulfate	0.65	0.00	3.29	0.0
M19	16.27	[M-H] ⁻	371.0960	371.0983	-6.4	C ₁₆ H ₂₀ O ₁₀	371.0990; 293.0681; 243.1061; 195.1021; 180.0782; 129.0182; 113.0232	Caffeic acid- methylation- glucuronide-reduction	3.19	9.47	15.90	1.9
M20	16.28	[M-H] ⁻	497.0743	497.0759	-3.2	C ₂₁ H ₂₂ O ₁₂ S	497.0767; 417.1199; 323.0241; 255.0665; 243.0664;	Liquiritin-sulfate	0.07	0.00	1.06	0.0
M21#	16.48	[M-H] ⁻	367.1039	367.1034	1.2	C ₁₇ H ₂₀ O ₉	367.1052; 353.0871; 191.0543; 179.0315; 173.0444; 135.0424	3'- Methyl-chlorogenic acid	4.12	36.78	54.07	9.0
M22	16.52	[M-H] ⁻	137.0233	137.0244	-0.369	C ₇ H ₆ O ₃	137.0231; 93.0331; 84.8779; 70.7439	3,4- Dihydroxybenzaldehyde isomer	0.23	0.96	0.78	0.0
M23	16.85	[M-H] ⁻	379.0108	379.0129	-5.6	C ₁₆ H ₁₂ O ₉ S	299.0566; 284.0330; 216.1235; 203.1289; 169.0320	Wogonin-sulfate- hydroxylation	0.23	0.00	0.00	0.0
M24	16.97	[M-H] ⁻	629.1170	629.1182	-1.9	C ₂₆ H ₃₀ O ₁₆ S	629.1162; 255.0603	Liquiritin apioside- sulfate	0.03	0.00	0.82	0.0
M25#	17.41	[M-H] ⁻	367.1039	367.1034	1.2	C ₁₇ H ₂₀ O ₉	367.1043; 353.0889; 191.0537; 179.0321; 173.0451; 135.0435	4'- Methyl-chlorogenic acid	1.16	20.25	6.17	4.8
M26	17.43	[M-H] ⁻	369.0801	369.0827	-7.1	C ₁₆ H ₁₈ O ₁₀	193.0467; 120.9373	Chlorogenic acid- methylation- glucuronide	3.11	1.32	1.05	0.0
M27#	17.79	[M-H] ⁻	431.0995	431.0983	2.8	C ₂₁ H ₂₀ O ₁₀	255.0700; 135.0110; 119.0506	Liquiritigenin- glucuronide	51.44	4.01	33.19	7.2
M28	17.87	[M-H] ⁻	637.1033	637.1046	-2.0	C ₂₇ H ₂₆ O ₁₈	285.0425; 113.0312	Scutellarein-di- glucuronide	0.25	0.00	0.00	0.0
M29	18.21	[M-H] ⁻	557.0587	557.0606	-3.4	C ₂₂ H ₂₂ O ₁₅ S	557.0699; 477.1009; 301.0731; 284.9333; 216.9426; 148.9576	Hesperetin-glucuronide- sulfate	3.20	0.00	3.42	0.0
M30#	18.53	[M-H] ⁻	431.0968	431.0983	-3.5	C ₂₁ H ₂₀ O ₁₀	431.0992; 255.0700; 135.0118; 119.0515	Liquiritigenin- glucuronide	15.45	3.21	15.38	1.2

NO	RT /min	Ion model	Measured mass /Da	Calculated mass /Da	Error /ppm	Molecular formula	Fragment ions	Identification	Peak Area (*10 ⁶)			
									Serum	Lung	Liver	He
M31	18.94	[M-H] ⁻	653.1336	653.1359	-3.5	C ₂₈ H ₃₀ O ₁₈	477.1069; 415.1058; 301.0727; 175.0241; 113.0231	Hesperetin- <i>O</i> - diglucuronides	0.53	0.00	0.00	0.0
M32	19.06	[M-H] ⁻	785.2153	785.2146	0.9	C ₃₄ H ₄₂ O ₂₁	785.2206; 707.3138; 609.1837; 489.1429; 415.1046; 343.0828; 301.0722	Hesperidin-glucuronide or neohesperidin- glucuronide	0.45	0.00	0.24	0.0
M33	19.2	[M-H] ⁻	333.0057	333.0074	-5.2	C ₁₅ H ₁₀ O ₇ S	333.0082; 253.0508; 203.1109; 135.0076; 91.0180	Daidzein-sulfate	25.28	3.98	17.62	5.0
M34#	19.24	[M-H] ⁻	335.0215	335.0231	-4.8	C ₁₅ H ₁₂ O ₇ S	255.0699; 135.0102; 119.0498	Liquiritigenin-sulfate	9.25	6.15	0.00	2.9
M35	19.36	[M-H] ⁻	475.0858	475.0882	-5.1	C ₂₂ H ₂₀ O ₁₂	475.1029; 299.0536; 256.0377; 211.0419; 133.0297	Trihydroxy- methoxyflavone- glucuronide	33.79	24.02	54.73	9.6
M36	19.37	[M + H] ⁺	477.1012	477.1028	-3.3	C ₂₂ H ₂₀ O ₁₂	301.1382; 286.0488; 168.0059	Wogonin-hydroxylation- glucuronide	22.12	15.13	52.41	5.5
M37	19.44	[M-H] ⁻	651.1204	651.1203	0.2	C ₂₈ H ₂₈ O ₁₈	599.3586; 475.0865; 299.0565; 284.0330; 175.0241	Trihydroxy- methoxyflavone-di- glucuronide	4.73	0.62	0.36	0.0
M38	19.5	[M-H] ⁻	785.2146	785.2146	0.0	C ₃₄ H ₄₂ O ₂₁	785.2145; 609.1829; 489.1393; 415.1045; 301.0721; 284.0343; 175.0241	Hesperidin-glucuronide or neohesperidin- glucuronide	0.34	0.00	0.00	0.0
M39	19.58	[M-H] ⁻	621.1097	621.1097	0.0	C ₂₇ H ₂₆ O ₁₇	549.3765; 445.0777; 311.0574; 269.0459; 193.0349; 113.0231	Baicalein-di-glucuronide	2.08	0.18	0.00	0.0
M40	19.6	[M-H] ⁻	637.1021	637.1046	-3.9	C ₂₇ H ₂₆ O ₁₈	637.1035; 461.0659; 285.0406	Scutellarein-di- glucuronide	0.36	0.00	0.00	0.0
M41	19.63	[M-H] ⁻	333.0057	333.0074	-5.2	C ₁₅ H ₁₀ O ₇ S	333.0080; 270.1216; 253.0508; 205.0901; 171.1019	Daidzein-sulfate	25.28	1.08	17.59	0.0
M42	19.81	[M-H] ⁻	525.0313	525.0344	-6.0	C ₂₁ H ₁₈ O ₁₄ S	525.0321; 445.0763; 349.0031; 269.0460; 175.0241	Baicalin-sulfate	8.52	0.00	11.93	0.0
M43#	19.88	[M-H] ⁻	477.1003	477.1038	-7.3	C ₂₂ H ₂₂ O ₁₂	301.0721; 286.0483; 242.0571; 151.0239	Hesperetin-glucuronide	0.39	0.19	2.02	0.0
M44#	19.93	[M-H] ⁻	477.0657	477.0674	-3.6	C ₂₁ H ₁₈ O ₁₃	475.2026; 301.0720; 283.0614; 269.0459; 204.0074; 161.0234	Quercetin-glucuronide	3.31	0.00	0.00	0.0

NO	RT /min	Ion model	Measured mass /Da	Calculated mass /Da	Error /ppm	Molecular formula	Fragment ions	Identification	Peak Area (*10 ⁶)			
									Serum	Lung	Liver	He
M45	20.06	[M-H] ⁻	651.1191	651.1203	-1.8	C ₂₈ H ₂₈ O ₁₈	475.0876; 413.0883; 299.0565; 284.0330; 175.0240	Trihydroxy- methoxyflavone-di- glucuronide	0.28	0.00	0.00	0.0
M46	20.28	[M-H] ⁻	579.1727	579.1719	3.277	C ₂₇ H ₃₂ O ₁₄	417.1561; 295.0616; 271.0614; 181.0498; 151.0026; 93.0332	Liquiritigenin-7,4'- diglucoside isomer	1.87	211.94	404.51	0.0
M47	20.77	[M-H] ⁻	367.0109	367.0129	-5.5	C ₁₅ H ₁₂ O ₉ S	367.0128; 287.0570; 269.0465; 193.0859; 151.0027	Hesperetin- demethylation-sulfate	0.48	0.21	4.16	0.0
M48	20.86	[M + H] ⁺	375.1033	375.1074	-11.0	C ₁₉ H ₁₈ O ₈	375.1071; 360.0836; 345.0601; 329.0645; 163.0752	Nobiletin-di- demethylation	1.60	0.00	0.82	0.0
M49#	21.15	[M-H] ⁻	477.1007	477.1038	-6.5	C ₂₂ H ₂₂ O ₁₂	301.0722; 286.0484; 242.0589; 175.0241	Hesperetin-glucuronide	7.46	7.52	25.25	2.3
M50	21.17	[M-H] ⁻	621.1130	621.1097	5.3	C ₂₇ H ₂₆ O ₁₇	445.0783; 383.0786; 269.0459; 175.0243; 113.0232	Baicalein-di-glucuronide	70.49	11.47	26.47	1.0
M51	21.17	[M-H] ⁻	379.0120	379.0129	-2.4	C ₁₆ H ₁₂ O ₉ S	380.1751; 299.0564; 284.0330; 229.0178; 201.1126; 124.0060	Wogonin-sulfate- hydroxylation	26.81	32.52	143.23	15
M52	21.17	[M-H] ⁻	635.1234	635.1253	-3.0	C ₂₈ H ₂₈ O ₁₇	459.0945; 325.0716; 283.0615; 268.0378; 175.0239	Wogonin-di-glucuronide or Oroxylin A-di- glucuronide	3.56	0.29	3.36	0.0
M53	21.20	[M-H] ⁻	167.0341	167.0350	1.286	C ₈ H ₈ O ₄	167.0341	Vanillic acid isomer	4.30	0.00	2.86	0.0
M54	21.35	[M-H] ⁻	461.0702	461.0725	-5.1	C ₂₁ H ₁₈ O ₁₂	285.0400; 229.0507; 195.0445; 85.0288	Trihydroxyflavone- glucuronide	2.12	0.88	2.19	0.0
M55	21.42	[M + H] ⁺	609.1439	609.1509	-11.5	C ₂₇ H ₂₈ O ₁₆	573.6403; 447.0903; 314.0520; 271.0596; 226.4313	Baicalein-di-glucuronide	1.42	1.46	9.22	0.0
M56	21.46	[M + H] ⁺	623.1241	623.1243	-0.3	C ₂₇ H ₂₆ O ₁₇	447.0887; 285.0754; 271.0595	Norwogonin-di- glucuronide	9.74	1.76	2.22	0.0
M57#	21.72	[M-H] ⁻	381.0270	381.0286	-4.2	C ₁₆ H ₁₄ O ₉ S	381.0279; 301.0725; 286.0487; 229.0175; 193.0347	Hesperetin-sulfate	0.80	3.40	8.25	0.0
M58	21.73	[M-H] ⁻	525.0308	525.0344	-7.0	C ₂₁ H ₁₈ O ₁₄ S	525.2744; 445.0775; 349.0027; 269.0459; 175.0241	Baicalin-sulfate	12.25	0.00	52.63	0.0
M59#	22.04	[M-H] ⁻	477.1038	477.1038	0.0	C ₂₂ H ₂₂ O ₁₂	477.1039; 415.1037; 301.0722; 257.0823; 215.0710; 162.0221	Hesperetin-glucuronide	4.03	9.80	24.65	2.4

NO	RT /min	Ion model	Measured mass /Da	Calculated mass /Da	Error /ppm	Molecular formula	Fragment ions	Identification	Peak Area (*10 ⁶)			
									Serum	Lung	Liver	He
M60	22.04	[M-H] ⁻	503.1166	503.1195	-5.8	C ₂₄ H ₂₄ O ₁₂	354.9995; 269.0461	Isoscutellarein-glucuronide	1.03	0.62	2.30	0.0
M61	22.14	[M-H] ⁻	635.1256	635.1253	0.5	C ₂₈ H ₂₈ O ₁₇	459.0936; 325.0712; 283.0615; 268.0380; 175.0240	Isowogonin-di-glucuronide	10.28	1.19	3.36	0.0
M62	22.36	[M-H] ⁻	461.0698	461.0725	-6.0	C ₂₁ H ₁₈ O ₁₂	285.0441; 139.0062; 61.9885	Trihydroxyflavone-glucuronide	0.65	0.12	0.00	0.0
M63	22.45	[M + H] ⁺	609.1440	609.1509	-11.3	C ₂₀ H ₃₂ O ₂₁	573.6403; 447.0903; 314.0520; 271.0596; 226.4313	Baicalein-glucosyl-glucuronide	0.27	7.93	4.34	0.0
M64	22.63	[M-H] ⁻	651.1189	651.1203	-2.2	C ₂₈ H ₂₈ O ₁₈	475.0886; 475.0886; 299.0565; 284.0330; 175.0241	Trihydroxy-methoxyflavone-di-glucuronide	3.37	0.20	0.18	0.0
M65#	22.64	[M-H] ⁻	431.0969	431.0983	-3.2	C ₂₁ H ₂₀ O ₁₀	255.0703; 135.0099; 119.0503	Liquiritigenin-glucuronide	4.76	0.00	17.17	0.0
M66	22.85	[M-H] ⁻	505.0960	505.0987	-5.3	C ₂₃ H ₂₂ O ₁₃	505.0964; 428.0726; 329.0672; 314.0437; 299.0199	Trihydroxy-dimethoxyflavone-glucuronide	47.70	24.23	72.26	9.5
M67	22.87	[M-H] ⁻	349.0008	349.0023	-4.5	C ₁₅ H ₁₀ O ₈ S	307.1511; 269.0459; 252.0426; 207.1397	Baicalein-sulfate	25.59	30.98	305.56	10
M68	23.62	[M-H] ⁻	533.1306	533.1300	1.1	C ₂₅ H ₂₆ O ₁₃	357.0987; 327.0515; 309.0418; 175.0238	Sinensetin-glucuronide demethylation	3.42	4.15	20.20	1.7
M69#	23.74	[M-H] ⁻	347.0221	347.0231	-2.9	C ₁₆ H ₁₂ O ₇ S	347.1715; 269.0457; 232.0168; 193.0348; 173.0997; 113.0232	Formononetin-sulfate	17.66	1.36	7.99	0.0
M70#	23.74	[M-H] ⁻	335.0216	335.0231	-4.5	C ₁₅ H ₁₂ O ₇ S	255.0897; 135.0104; 119.0511	Liquiritigenin-sulfate	3.63	2.50	2.04	4.2
M71	24.06	[M-H] ⁻	563.1377	563.1406	-5.1	C ₂₆ H ₂₈ O ₁₄	387.1091; 372.0855; 357.0622; 342.0382; 215.0739	Nobiletin-glucuronide-demethylation	2.55	5.71	27.24	2.4
M72#	24.16	[M-H] ⁻	349.0003	349.0023	-5.9	C ₁₅ H ₁₀ O ₈ S	269.0516; 229.0295; 117.0386	Baicalein-sulfate	20.92	25.15	95.15	2.6
M73	24.38	[M + H] ⁺	285.0739	285.0758	-6.5	C ₁₆ H ₁₂ O ₅	285.0753; 270.0518; 249.1475; 221.1528; 183.0287	Baicalein-methoxylation	5.36	0.29	23.62	2.5
M74	24.6	[M-H] ⁻	475.0858	475.0882	-5.1	C ₂₂ H ₂₀ O ₁₂	299.0565; 284.0330; 204.0077; 113.0231; 85.0281	Trihydroxy-methoxyflavone-glucuronide	6.49	20.09	21.93	1.2
M75	24.64	[M + H] ⁺	521.1271	521.1290	-3.6	C ₂₄ H ₂₄ O ₁₃	521.1315; 345.0964; 330.0723; 284.0662; 205.0488; 85.0286	Skullcapflavon I-glucuronide	1.27	0.29	3.13	0.0

NO	RT /min	Ion model	Measured mass /Da	Calculated mass /Da	Error /ppm	Molecular formula	Fragment ions	Identification	Peak Area (*10 ⁶)			
									Serum	Lung	Liver	He
M76	24.69	[M + H] ⁺	491.1160	491.1184	-4.9	C ₂₃ H ₂₂ O ₁₂	491.0766; 316.0604; 285.0440	Wogonoside-methoxylation	0.59	0.00	0.00	0.0
M77	24.75	[M-H] ⁻	299.0560	299.0561	-0.4	C ₁₆ H ₁₂ O ₆	299.0567; 284.0331; 236.9977; 219.0774; 175.0756;	Wogonin-hydroxylation	8.84	49.84	261.36	15
M78	25.07	[M + H] ⁺	461.1075	461.1078	-0.7	C ₂₂ H ₂₀ O ₁₁	285.0753; 270.0517; 153.0180; 113.0235	Baicalein-glucuronide methylation	98.36	0.00	1328.19	21
M79	25.07	[M + H] ⁺	285.0741	285.0758	-5.8	C ₁₆ H ₁₂ O ₅	285.0753; 270.0518; 183.0287; 167.0936; 133.1007	Baicalein-methoxylation	11.72	87.37	137.38	10
M80#	25.73	[M-H] ⁻	363.0180	363.0180	0.0	C ₁₆ H ₁₂ O ₈ S	283.0616; 268.0381; 163.0034; 137.0236	Wogonin-sulfate	35.90	54.16	98.83	25
M81#	26.31	[M-H] ⁻	387.1066	387.1085	-4.9	C ₂₀ H ₂₀ O ₈	357.0632; 342.0382; 268.0370; 162.9988; 78.9593	Nobiletin-demethylation	0.56	4.11	38.96	2.2
M82#	26.36	[M-H] ⁻	363.0176	363.0180	-1.1	C ₁₆ H ₁₂ O ₈ S	283.0610; 268.0375; 239.0374	Wogonin-sulfate	35.94	35.58	193.63	13
M83	26.76	[M-H] ⁻	529.1335	529.1351	-3.1	C ₂₆ H ₂₆ O ₁₂	353.1036; 329.1607; 273.0777; 125.0232	Licoisoflavone A-glucuronide	5.92	1.46	8.46	0.0
M84	27.39	[M-H] ⁻	529.1343	529.1351	-1.6	C ₂₆ H ₂₆ O ₁₂	353.1011; 125.0248; 61.9896	Licoisoflavone A-glucuronide	0.20	0.19	0.00	0.0
M85	27.76	[M-H] ⁻	527.1169	527.1195	-4.9	C ₂₆ H ₂₄ O ₁₂	527.2500; 351.0862; 323.0813; 151.0048	Semilicoisoflavone B-glucuronide	0.15	0.88	1.60	0.0
M86#	27.89	[M-H] ⁻	387.1066	387.1085	-4.9	C ₂₀ H ₂₀ O ₈	357.0632; 342.0382; 268.0370; 162.9988; 78.9593	Nobiletin-demethylation	2.11	0.00	1.58	1.1
M87	28.17	[M-H] ⁻	527.1160	527.1195	-6.6	C ₂₆ H ₂₄ O ₁₂	527.2577; 351.0877; 283.1005	Semilicoisoflavone B-glucuronide	0.39	0.00	0.71	0.0
M88	29.1	[M-H] ⁻	253.0511	253.0506	2.0	C ₁₅ H ₁₀ O ₄	253.0508; 207.1388; 189.1281; 163.0760; 143.0488	Liquiritigenin-dehydrogenation	0.42	33.04	297.05	10
M89#	29.32	[M + H] ⁺	285.0749	285.0758	-3.0	C ₁₆ H ₁₂ O ₅	285.0753; 270.0517; 201.1260; 183.0276; 159.1165	Baicalein-methoxylation	7.78	90.10	526.55	84
M90	29.36	[M + H] ⁺	527.3324	527.3367	-8.2	C ₃₂ H ₄₆ O ₆	527.3339; 509.3266; 407.1786; 375.1550; 290.3741	Alisol C-23 acetate-dehydrogenation	0.20	8.71	48.49	0.0
M91	34.45	[M-H] ⁻	485.3241	485.3272	-6.5	C ₃₀ H ₄₆ O ₅	485.3303; 467.2944; 439.3218; 423.3270	22-acetoxyl glycyrrhizin-deacetylated-dediglucuronide	2.79	0.52	9.36	0.0

M- means metabolite, #- means the metabolite that has been confirmed by microsomal incubation *in vitro*; RT-retention time.

3.3 Characterization of the absorbed prototypes and metabolites in mice tissues

With the help of the information about the absorbed prototypes and metabolites in mice serum, the absorbed prototypes and metabolites in various tissues from QFPDD-treated mice were explored. Subsequently, total of 165, 177, 112, 120, 44 and 53 prototypes and metabolites were identified in the lung, liver, heart, kidney, brain, and spleen of QFPDD-treated mice, respectively. The peak areas of absorbed prototypes and metabolites were summarized in Table 2 and Table 3, while Fig. 6 showed the heat map of the peak areas of both the absorbed components and the metabolites occurring in serum or six different tissues of QFPDD-treated mice.

On the basis of the peak area, the top ten components in the mice serum were ranked as follows, stachydrine (**p3**), salicylic acid (**p32**), ephedrine (**p7**), caffeic acid-sulfate (**M4**), baicalin (**p66**), baicalein 6-*O*- β -D-glucuronide (**p82**), wogonoside (**p80**), methylephedrine (**p10**), baicalein-glucuronide methylation (**M78**), quinic acid (**p2**).

The top ten components in the lung tissue were ranked as follows: ephedrine (**p7**), naringin (**p53**), neohesperidin (**p59**), baicalin (**p66**), wogonoside (**p80**), stachydrine (**p3**), quinic acid (**p2**), methylephedrine (**p10**), oroxylin A 7-*O*-glucuronide (**p75**), pseudoephedrine (**p9**).

The top ten components in the liver tissue were ranked as follows: ephedrine (**p7**), naringin (**p53**), neohesperidin (**p59**), baicalein-glucuronide methylation (**M78**), irisflorentin (**p87**), stachydrine (**p3**), wogonoside (**p80**), wogonin (**p88**), baicalin (**p66**), methylephedrine (**p10**).

The top ten components in the heart tissue were ranked as follows: ephedrine (**p7**), naringin (**p53**), stachydrine (**p3**), methylephedrine (**p10**), neohesperidin (**p59**), alisol O (**p102**), wogonoside (**p80**), pseudoephedrine (**p9**), baicalein-methoxylation (**M79**), alisol A (**p103**).

The top ten components in the kidney tissue were ranked as follows: ephedrine (**p7**), naringin (**p53**), neohesperidin (**p59**), baicalin (**p66**), wogonoside (**p80**), stachydrine (**p3**), methylephedrine (**p10**), quinic acid (**p2**), oroxylin A 7-*O*-glucuronide (**p75**), pseudoephedrine (**p9**).

The top ten components in the brain tissue were ranked as follows: ephedrine (**p7**), pseudoephedrine (**p9**), methylephedrine (**p10**), stachydrine (**p3**), mandelonitrile (**M1**), phenylpropanolamine (**p4**), salicylic acid (**p32**), alisol O (**p102**), D-demethyl pseudoephedrine (**p5**), oroxylin A (**p93**).

The top ten components in the spleen tissue were ranked as follows: ephedrine (**p7**), stachydrine (**p3**), methylephedrine (**p10**), pseudoephedrine (**p9**), oroxylin A (**p93**), baicalein -methoxylation (**M89**), pinellin acid isomer (**p96**), alisol O (**p102**), wogonin (**p88**).

From the above results, it was obvious that ephedrine (**p7**) and methylephedrine (**p10**) from *Herba Ephedrae*, and stachydrine (**p3**) from *Fructus Aurantii Immaturus* displayed relatively high exposure levels in mice serum and all tested tissues. Additionally, baicalin (**p66**), oroxylin A 7-*O*-glucuronide (**p75**), wogonoside (**p80**), wogonin (**p88**) and oroxylin A (**p93**) from *Radix Scutellariae*, alisol O (**p102**) and alisol A (**p103**) from *Rhizoma Alismatis* also showed relatively high exposure levels in most of mice tissues.

Discussion

To fight against COVID-19, some Chinese medicines were recommended by National Health Commission of the People's Republic of China or the local government in China, for alleviating the major symptoms of COVID-19 or preventing disease deterioration [9]. As the first Chinese medicine compound formula recommended for combating COVID-19, QFPDD is made by 20 herbs and a mineral drug, which is composed by hundreds of ingredients. In most cases, for treating COVID-19, the absorbed constituents (including the prototypes and the metabolites) into blood circulation can be delivered to the target organ (such as the lung) and then exerts various pharmacological effects, such as anti-inflammatory and anticoagulant effects [23, 24]. Therefore, it is necessary to investigate the absorbed constituents of QFPDD following oral administration of this super Chinese medicine compound formula, which will be very helpful for better understanding the key material basis of QFPDD, as well as for guiding the quality control and clinical applications of this formula.

In this study, an UHPLC-Q-Orbitrap HRMS based method was established for profiling the constituents in QFPDD and the absorbed constituents in QFPDD-treated mice. The research strategy and the key findings for clarifying the chemical composition and absorption components of QFPDD have been summarized in Fig. 7. The chemical constituents in QFPDD were tentatively identified *via* comparison with exact molecular weight, the retention times and MS/MS spectra of the standards or refereed by TCM databases and literature. The absorbed components (including the prototypes and the metabolites) occurring in mice serum and tissues were identified *via* profiling the serum and tissue samples from QFPDD-treated mice. The absorbed prototype components were tentatively identified *via* comparison with the retention times and MS/MS spectra of components in QFPDD. The *in vivo* metabolites were tentatively identified *via* comparison with fragment ions of the parent compounds or refereed by literature, while parts of *in vivo* metabolites of the major constituents were identified *via* comparison with the retention times and MS/MS spectra of the *in vitro* metabolites of each constituent generated in MLMs. Finally, a total of 405 chemicals (including alkaloids, flavonoids, organic acids, triterpene saponins and other compounds) in the extract of QFPDD were identified, while 195, 165, 177, 112, 120, 44, 53 constituents were identified in the serum, lung, liver, heart, kidney, brain, and spleen of QFPDD-treated mice, respectively. All these findings might be very helpful for deep understanding the fates of the constituents in QFPDD, as well as offered key information of the tissue distribution of the absorbed components of this anti-COVID-19 Chinese medicine.

It is well known that it is always a challenging task for identification of the prototypes and the metabolites of TCM prescription. For example, oroxylin A 7-*O*-glucuronide (**p75**) is a trace amount prototype constituent occurring in QFPDD, but this agent is a highly exposed component in the serum and various tissues of QFPDD-treated mice. The reasons may include i) produced by the *O*-glucuronidation of oroxylin A (**p103**) *in vivo*; ii) produced by baicalein (**p66**) through 6-*O*-methylation and *O*-glucuronidation. It is also suggested that it is very difficult to differentiate some components detected in QFPDD-treated mice as the prototypes or the metabolites, owing to that some *in vivo* components are derived from both the prototypes and the metabolites of the homologous

compounds occurring in this TCM prescription. Meanwhile, the exposure of some prototype constituents in blood are extremely low and thus are difficult to be detected, since these natural constituents could be readily transformed into the corresponding metabolites (glucuronides or sulfates) *in vivo*. For instances, trihydroxy-methoxyflavone-*O*-glucuronides (**M35** and **M60**) and daidzein-sulfates (**M33** and **M41**) can be easily detected in mice serum, but the related prototypes are hardly detected in mice serum. In addition, many nature products have isomers that cannot be distinguished by MS spectral analysis alone, thus it is hard to correlate the parent compounds with the metabolites, such as vanillic acid and its isomer.

Modern pharmacological studies have found that some of absorbed components of QFPDD displayed a wide range of beneficial effects or biological activities. Some pharmacological activities of them are considered to be beneficial for COVID-19 patients. For example, ephedrine, pseudoephedrine and amygdalin displayed significant anti-cough and anti-asthmatic effects [25], as well as anti-inflammatory [26, 27] and immunomodulatory effects [28, 29], which are considered as the major active components of TCM prescriptions for treating respiratory diseases. Zhang et al. [30] report that amygdalin can induce LPS by inhibiting NF- κ B and NLRP3 signaling pathways, while baicalin has antiviral, anti-inflammatory, antioxidant, and immunomodulatory pharmacological activities [31]. A recent study has reported that baicalin can significantly inhibit the catalytic activity of SARS-CoV-2 3CL^{Pro} and then exert antiviral activity [32]. The virtual screening of active ingredients of SARS-CoV-2 virus also suggests that hesperidin and baicalin have potential inhibitory effects [33]. The glycyrrhizin and glycyrrhizic acid in licorice have been found with extensive anti-inflammatory [34] and anti-damage effects [35]. Some previous studies have shown that glycyrrhizin can serve as an alternative agent for treating COVID-19 infection and its associated respiratory syndrome [36-38]. The extract of *Rhizoma Alismatis* processed exerts anti-inflammatory activity *via* inhibiting cytochrome P450 enzymes [39], the key enzymes responsible for the metabolism of arachidonic acid, thereby reducing the production of inflammatory factors [40-42].

Furthermore, some *in vivo* metabolites of QFPDD are more likely to be the active ingredients. Previous studies clearly demonstrated that a panel of *O*-glucuronides of flavonoids displayed good anticoagulant activities, and their anticoagulant activities are better than that of the prototypes [43]. Recent studies have shown that the blood of COVID-19 patients was in a hypercoagulable state, thus the *O*-glucuronides of flavonoids were more likely to promote blood circulation through anticoagulation and then relieve the symptoms of patients [44-46]. This study was not only found a considerable number of prototypes and metabolites in serum, but also found a large number of chemical components in lung, liver and other tissues. Among them, the active ingredients derived from *Herba Ephedrae* (ephedrine, pseudoephedrine, methylephedrine, phenylpropanolamine etc.), *Fructus Aurantii Immaturus* (stachydrine, naringin, hesperidin, neohesperidin, quinic acid etc.), *Radix Scutellariae* (baicalin, oroxylin A 7-*O*-glucuronide, wogonoside, wogonin, oroxylin A etc.) and *Rhizoma Alismatis* (alisol O, alisol A etc.) displayed relatively high exposure levels in most of organs. However, the kinds and exposure levels of each component in various tissues are much different. These findings suggest that these *in vivo* metabolites of QFPDD may play potential role in the treatment of COVID-19 *via* **targeting** multiple targets or pathways. In future, the bioactive ingredients and mechanisms of action can be explored.

Conclusion

In summary, a practical and sensitive UHPLC-Q-Exactive-Orbitrap HRMS approach was developed for chemical profiling of the constituents in QFPDD, as well as the absorbed prototypes and the metabolites in mice serum and tissues following oral administration of QFPDD. With the help of the authentic standards and *in vitro* metabolites generated by MLM, a total of 405 constituents (including 40 kinds of alkaloids, 162 kinds of flavonoids, 44 kinds of organic acids, 71 kinds of triterpene saponins and 88 kinds of other compounds) were identified from the water extract of QFPDD, while 195 components (including 104 prototypes and 91 metabolites) were identified from mice serum after oral administration of QFPDD to mice. Meanwhile, 165, 177, 112, 120, 44, 53 components were identified from the lung, liver, heart, kidney, brain, and spleen of QFPDD-treated mice, respectively. Additionally, the metabolic pathways of some major absorbed components occurring in the serum of QFPDD-treated mice were also described. All these findings provided key information and guidance for further investigations on the pharmacologically active substances and quality control of QFPDD.

Abbreviations

CMs, Chinese Medicines; COVID-19, Corona Virus Disease 2019; CYPs/P450s, cytochrome P450 enzymes; ESI, electrospray ionization; G-6-P, D-Glucose-6-phosphate; G-6-PDH, glucose-6-phosphate dehydrogenase;

MLMs, mice liver microsomes; β -NADP⁺, nicotinamide adenine dinucleotide phosphate disodium salt; PAPS, 3-phosphoadenosine 5-phosphosulfate; PBS, potassium phosphate buffer; QFPDD, Qingfei Paidu decoction; SAM, S-adenosyl-L-methionine; SARS, Severe Acute Respiratory Syndrome; UDPGA, uridine diphosphate glucuronic acid; UHPLC-Q-Orbitrap HRMS, ultra-high-performance liquid chromatography-Q exactive hybrid quadrupole orbitrap high-resolution accurate mass spectrometric.

Declarations

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Authors' contributions

WL and JH carried out the experiments; WL, FZ, and GG wrote the manuscript and analyzed the data; WL, FZ, CZ and RL analyzed the data; CW prepared the extract of QFPDD; GG revised the manuscript; GG, WZ and PL conceived of the study, contributed to the design and interpretation of the research. All authors read and approved the final manuscript.

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Availability of data and materials

The research data generated from this study is included within the article.

Ethics approval and consent to participate

Animal experimental was ratified by the Animal Care and Use Committee of Shanghai Institute of Food and Drug Control.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figure 1

The total ion chromatograms (TICs) of the extract of Qingfei Paidu decoction by UHPLC-Q-Exactive Orbitrap HRMS

Figure 2

The mass fragment and fragmentation pathway of ephedrine (A), amygdalin (B), nobiletin (C) and liquiritin (D)

Figure 3

The mass fragment and fragmentation pathway of gallic acid (A), chlorogenic acid (B), saikosaponin A (C) and glycyrrhizic acid (D)

Figure 4

Structures of the absorbed prototype compounds (alkaloids and flavonoids) in mice serum after oral administration of Qingfei Paidu decoction

Figure 5

Structures of the absorbed prototype compounds (organic acids, triterpene saponins, and others) in mice serum after oral administration of Qingfei Paidu decoction

Figure 6

The heat map of the peak areas of absorbed components and metabolites in mice serum and tissues after oral administration Qingfei Paidu decoction

Figure 7

Research strategy for clarifying the chemical composition and absorption components of Qingfei Paidu decoction based on UHPLC-Q-Exactive Orbitrap HRMS technology