Analysis of Chemical Constituents of Guizhi Shaoyao Zhimu Granules by UPLC-Q-Orbitrap HRMS

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Research

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Abstract

Background: Guizhi Shaoyao Zhimu Granules (GSZG), which are derived from Guizhi Shaoyao Zhimu decoction in China, have good clinical efficacy in Rheumatoid arthritis(RA) especially. However, there is currently no reliable and systematic method to research the effective ingredients of the kind of prescription.

Methods: Vanquish Ultra High Performance Liquid Chromatography Q Exactive IV Pole-electrostatic field orbital trap high resolution mass spectrometer (UPLC-Q-Orbitrap HRMS), a quick and systematic method was established and apply to analysis the chemical component of GSZG. According to using reference standards comparsion, fragmentation rules elucidation and available databases like mzCloud and the OTCML to identify the components of GSZG.

Results: The results showed that it identified a total of 74 components, including 2 chromone, 23 flavonoids, 10 alkaloid, 9 phenolic acid, 8 nucleosides and nucleobases, and 22 other components, were characterized from GSZG.

Conclusion: this was the first time to systematically report the compounds of GSZG, the chemical basis inquiry of this work would have a profound impact on mechanism exploration and providing certain reference for the preparation and quality control of GSZG developed by China independently.

Background

Rheumatoid arthritis(RA) is a kind of chronic autoimmune disease which cause progressive damage, functional loss and comorbidity, what's more, it has a high global prevalence with an incidence rate of 0.5% to 1% [1-4]. The drugs currently used to treat rheumatoid arthritis include disease modifying antirheumatic drugs (DMARDs), non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, biological medicines, and surgery, *etd*[5-7], although it has a certain effect, long-term use will cause obvious adverse effects[8, 9]. In Asian countries and regions, traditional Chinese medicine has been used as a treatment method to maintain human health for thousands of years. Through the long-term experience of fighting the disease and the continuous summary of the treatment plan by countless outstanding doctors, many classic prescriptions with remarkable curative effects have been produced, including Guizhi Shaoyao Zhimu decoction(GSZD), which have unique effectiveness and safety in treating rheumatoid arthritis compared to western RA drugs[10-12].

GSZD, first appeared in "The Synopsis of the Golden Chamber" by Zhang Zhongjing, is composed with Cinnamomi Ramulus, Paeoniae Radix Alba, Anemarrhenae Rhizoma, Saposhnikovia Radix, Atractylodis Macrocephalae Rhizoma, Ephedrae Herba, Glycyrrhizae Radix et Rhizoma, Zingiberis Rhizoma Recens and Aconiti Lateralis Radix Praeparata sauted with sand (Figure 1). It is mainly used to treat patients suffering from Lijie disease, which is similar to RA in modern medicine[13]. Although GSZD has a significant effect in the treatment of RA, there is a lack of systematic research on its medicinal material basis. At present, there are only a few literatures on the effect of GSZD Chemical components were analyzed. Qing Zhang et al. [10] used HPLC-QqQ-MS to analyze the water extract of Guizhi Shaoyao Zhimu Decoction and obtained 13 main chemical components. Qin Yi[14] used UPLC-Q-TOF-MS to analyze the chemical components contained in GSZD and identified 28 compounds.

With the 11th edition of the "International Statistical Classification of Diseases and Related Health Problems" (ICD) released a detailed description of Chinese medicine [15], Chinese medicine has gained more and more widespread recognition worldwide, at the same time, in the outbreak of the COVID-2019, Chinese medicine has obvious advantages in treating diseases[16], however, the traditional decoction has the disadvantages of cumbersome decoction and not easy to carry, Chinese government has issued a series of related policies to encourage the development of classic prescriptions into modern preparations, including GSZD[17, 18]. At present, only Japan and Taiwan of China have developed corresponding products, but the effective ingredients of Guizhi Shaoyao Zhimu Granules (GSZG) have not been consulted in relevant literature. According to relevant regulations, GSZD can be developed into granules required to ensure the development principle of "based on the original formula, higher than the original formula", as much as possible to retain the chemical ingredients in the prescription. In this experiment, UPLC-Orbitrap-MS/MS was used for the first time to analyze GSZG produced by Japan Sanwa Co., Ltd in order to further clarify the effective material basis and provide certain reference for the preparation of GSZG and the establishment of quality control of GSZG.

Methods

Chemicals and reagents

Reference standards including 5-O-methylvisammioside (111523-201610, the purity is 96.10%), Paeoniflorin(110736-2018, the purity is 97.40%), Mangiferin (111607-201704, the purity is 98.10%), Prim-O-glucosylcimifugin (111522-201712, the purity is 96.20%), Liquiritin (111610-201607, the purity is 93.10%), Gallic acid (110831-201605, the purity is 90.80%), Ephedrine Hydrochloride (171241-201809) and Pseudoephedrine Hydrochloride (171237-201510), all were purchased from the National Institutes for Food and Drug Control (Beijing, China). Guizhi Shaoyao Zhimu Granules was collected from Japan Sanwa Co., Ltd (NLA02)

HPLC grade acetonitrile and formic acid were bought from Thermo Fisher Scientific (USA). The Watsons distilled water were used to prepare samples and mobile phase. All other reagents were of analytical grade.

Preparation of standard solutions

The reference standards of 5-O-methylvisammioside, Paeoniflorin, Mangiferin, Prim-O-glucosylcimifugin, Liquiritin, Gallic acid, Ephedrine Hydrochloride and Pseudoephedrine Hydrochloride were precisely weighed, dissolved in methanol to 10mL to obtain a single standard solution and stored in a refrigerator at 4°C, mix a certain amount of each single standard solution, dilute them with methanol to about 1µg/ml of each reference substance is before use.

Preparation of sample solutions

GSZG (0.5g) was put into a 50ml Erlenmeyer flask respectively after weighing accurately, added 25ml of 75% methanol, weighed, extracted using an ultrasonic bath (40kHz, 150W) for 30 minutes, cooled, adjusted to make the weight the same as before, shake well, and finally filtered each sample solution with a 0.22µm microporous membrane before being injected into UPLC instrument for analysis.

Instrument and analysis conditions

The experiments were performed on Vanquish Ultra High Performance Liquid Chromatography Q Exactive IV Pole-electrostatic field orbital trap high resolution mass spectrometer(Thermo Fisher Scientific, USA). Xcalibur software (version 3.0) was used to control instrument, collect data and analysis data.

Thermo Scientific Accucore™ C₁₈ column(100 × 3mm, 2.6 µm, Thermo Scientific) was used for chromatographic separation at column temperature 30°C. The gradient elution with mobile phase 0.1% formic acid in water(A) – acetonitrile(B) was set as follows: 5%-70%(B) from 0 to 20 min; 70%-90%(B) from 20 to 25 min; 90%-5%(B) from 25 to 30 min; 5%(B) from 30 to 35 min. The flow rate was 0.3 mL/min, and the injection volume was 2 µL.

Q Exactive Quadrupole-Electrostatic Field Orbital Trap combined with UPLC for positive and negative ion mode detection through ESI electrospray ion source. The spray voltage is 3.2kV, the ion source temperature is 350°C, the sheath gas flow rate is 35arb, the auxiliary gas flow rate is 10arb, and the ion transfer tube temperature is 320°C. The compound was scanned by using a full scan/data-dependent secondary scan method, the primary resolution was 70,000, the secondary resolution was 17,500, the scanning range from m/z=100 to m/z=1500, and the collision energy gradient was 20ev, 40ev, and 60ev.

Data processing

The collected raw data was imported into Compound Discoverer 3.0 software and MZmine (version 2.53) software[19], and the identification process of unknown compounds was established through its wizard settings and method templates. The software was performed peak alignment and peak extraction on the original data, so that the obtained molecular ion peaks, possible molecular formulas fitted by isotope, and the measured spectra of the secondary fragments were matched with the mzCloud network database and the local Chinese medicine component database OTCML respectively. Retain the compounds whose peak area threshold is greater than 80,000, the first- and second-level mass deviation is less than 5 ppm, and the matching score is greater than 85, and then compare with the reference substance and related literature to identify the compound.

Results

Identification of components in the sample by HPLC - Q-Orbitrap HRM

The (Figure 2) and (Figure 3) stands the total ion chromatogram of sample and mixed reference solution both in positive mode and negative mode respectively. Combined with the accurate relative molecular mass, fragmentation rules elucidation detected by the positive and negative ion mode, the molecular formula was calculated by the Compound Discoverer 3.0 software within the range of 5 ppm mass deviation, and match the available databases like mzCloud and the OTCML, at the same, referring to relevant literatures. Then, a total of 74 components, including 2 chromone, 23 flavonoids, 10 alkaloids, 9 phenolic acids, 8 nucleosides and nucleobases, and 22 other componets, were identified from GSZG. The results of identification were show in Table 1.

Identification of chromone compounds

Chromone compounds are characteristic compounds of Saposhnikovia Radix, and it is reported in the literature that these compounds have the effect of inhibiting arthritis [20]. Through research and analysis, it is concluded that this type of compound has a greater abundance in the positive ion mode under the conditions of the first-level mass spectrum, mainly in the form of [M+H]⁺, by consulting related literature, it is found that the secondary mass spectrometry of this type of compound mainly loses neutral fragment ions such as glucoside (162), methyl (15), H₂O (18), CO (28), CO₂ (44), and , Retro-Diels-Alder (RDA) cracking will also occur. Compared with database search, related literatures and reference standards, this experiment identified two chromone compounds from GSZG. For example, compound 62 obtained the quasi-molecular ion m/z =453.17575[M+H]⁺in the positive ion mode, the predicted molecular formula was C₂₂H₂₈O₁₀. In the MS/MS spectrometry, this compound was first removed one molecule of glucose to become M/Z=291.12265 [M+H-C₆H₁₀O₅]⁺, and then separately yielded ions to m/z=273.11206 [M+H-C₆H₁₀O₅-H₂O]⁺, m/z=243.06525 [M+H-C₆H₁₀O₅-CH₄O₂]⁺, m/z=231.06529 [M+H-C₆H₁₀O₅-C₃H₈O]⁺, m/z=219.06546 [M+H-C₆H₁₀O₅-C₄H₈O]⁺, m/z=216.04190 [M+H-C₆H₁₀O₅-C₃H₈O-CH₃]⁺, m/z=203.07022 [M+H-C₆H₁₀O₅-C₃H₈O- CO]⁺ fragment ion by lossing some neutral fragment ions such as C₃H₈O, H₂O, C₄H₈O or generating the RDA cleavage. Summarizing database searches, related literatures and reference standards, the compound 62 was identified as 5-O-methylvisammioside[21-23], the MS/MS spectrum is shown in(Figure 4), and MS² fragmentation pathway of compound 62 is shown in (Figure 5)

Identification of Flavonoids compounds

Flavonoids are a kind of obvious biologically active compounds widely distributed in Chinese medicinal herbs. According to literature reports, these have many pharmacological efficacy like neuroprotective, anti-inflammatory, anti-oxidant and anti-tumor effects[24-27].Such compounds are often combined with pentose, Hexose, and hexuronic acid, they are mainly divided into flavonoids, flavonols, dihydroflavonoids, isoflavones, chalcones, etc depending on the type

of structure. Through research and analysis, it is concluded that this type of compounds is roughly similar in cleavage, except for isoflavones, these compounds can be detected in both positive and negative ion modes under the condition of first-order mass spectrometry, and the characteristic fragments of glycosides were mainly lost in the mass/mass spectrometry, such as pentose (132), Hexose (162), hexuronic acid (176), and then neutral molecular fragments such as CHO (15), H₂O (18), CO (28), CO₂ (44), in addition, RDA cleavage will occur. Compared with database, related literature and reference standards, it was identified 23 flavonoids from GSZG, mainly from *Anemarrhenae Rhizoma*, *Ephedrae Herba*, *Glycyrrhizae Radix et Rhizoma*. For example, compound 53 obtained the quasi-molecular ion m/z 417.11996 [M-H]⁻ in the negative ion mode, and the predicted molecular formula was C₂₁H₂₂O₉, in the MS/MS spectrum, this compound was first lost one molecule of glucose and become m/z =255.0664[M-H-C₆H₁₀O₅]⁻, thereby obtaining fragment ion like m/z =153.01900, m/z =135.00833, m/z =119.04967, m/z =91.1820 by losing some neutral fragment ions such as C₃H₈O, H₂O, C₄H₈O or generating the RDA cleavage, The database, reference substance and literature [28, 29] which provided evidence that compound 53 could be attributed to Liquiritin. Compound 67 obtained the quasi-molecular ion m/z 549.16272 [M-H]⁻ in the negative ion mode, and the predicted molecular formula was C₂₆H₃₀O₁₃, which was similar to the ion fragments obtained by the cleavage of compound 53, they both had 417, 255, 153, 135, 119 and 91 ion fragments. Searching database and comparing the literature [28, 30] it is speculated that compound 67 was Liguitigenin-7-O-β-D-apsiosyl-4'-O-β-D-glucoside. The MS² spectrum of compound 53 and 67 is shown in (Figure 6), and MS fragmentation pathway of compound 53 and 67 is shown in (Figure 7).

Identification of Alkaloid compounds

Alkaloid compounds are a kind of obvious biologically active ingredients widely distributed in Chinese medicinal materials, but most of these compounds have obvious side effects. According to literature reports, such compounds have significant anti-inflammatory and anti-tumor effects[31-33]. Through research and analysis, it is concluded that this type of compound has a greater abundance in the positive ion mode under the conditions of primary mass spectrometry, and mainly exists in the form of [M+H]⁺. Looking up related literature in the MS/MS spectrum, it is easy to lose the substituents, such as hydroxyl, amino, methoxy, acetyl, benzoyl, etc., thereby losing CO(28), H₂O(18), NH₂(16), CH₃OH(32), CH₃COOH(60), C₇H₆O₂ (122) and other neutral fragment molecules. In experiment, we identified 10 alkaloid compounds from GSZG, mainly from *Ephedrae Herba* and *Aconiti Lateralis Radix Praeparata*. For instance, compound 24 obtained the quasi-molecular ion m/z 166.12265 [M+H]⁺ in the positive ion mode, and the predicted molecular formula is C₁₀H₁₅NO. In the MS/MS spectrum, this compound first lost one molecule of H₂O and become m/z =148.11197[M+H-H₂O]⁺, and then lose the methyl, NH₂ and vinyl, resulting in fragmented ions such as m/z =133.08862, m/z =117.07005, m/z =91.05464. Summarizing database searches, related literatures and reference standards[34], it was concluded that compound 24 was Ephedrine. Compound 26 obtained the quasi-molecular ion m/z 166.12283 [M+H]⁺ in the positive ion mode, and the predicted molecular formula is also C₁₀H₁₅NO, which is similar to the ion fragments obtained by the cleavage of compound 24, with 148, 133, 117 and 91 ion fragments, through database search and literature comparison, it is inferred that compound 26 was Pseudoephedrine. For another example, compound 51 obtained the quasi-molecular ion m/z 632.3067 [M+H]⁺ in the positive ion mode, and the predicted molecular formula was C₃₃H₄₅NO₁₁, in the MS/MS, this compound was first removed one molecule of CH₃OH and CO to become m/z =572.2849[M+H-H₂O]⁺, then the CH₃, CH₃COOH and BzOH, H₂O also were lost, thus obtaining fragmentation ions like m/z =540.25800, m/z =512.26489, m/z =508.23422, m/z =480.24136, m/z =354.16934, m/z =105.03383. In addition, m/z =105.03383 was the compound obtained by losing one molecule of H₂O to the benzoyl, the molecular formula was [C₇H₅O]⁺. Through database search and comparison of literature [35, 36], Presumably it was Mesaconitine. The MS/MS spectrum, and MS fragmentation pathway of Compound 24 (Figure 8) (Figure 9) and 51 (Figure 10) (Figure 11) are shown.

Identification of Phenolic acid compounds

In this experiment, through comparing with database search, related literature and standard reference, 9 phenolic acid compounds were identified from GSZG, mainly from *Paeoniae Radix Alba*. Through research and analysis, it is concluded that the type of compound has a greater abundance in the negative ion mode under the conditions of the primary mass spectrum, and mainly exists in the form of [M-H]⁻, and It is easy to loss CO (28), H₂O (18), CO₂ (44), C₇H₆O₂ (122) and other neutral fragment molecules in the MS². For example, compound 28 obtained the quasi-molecular ion m/z 169.1385[M-H]⁻ in the negative ion mode, and the predicted molecular formula is C₇H₆O₅. In the MS/MS, this compound was first removed one molecule of CO₂ and became m/z =125.0237[M-H-CO₂]⁻, then lost CO and H₂O, and fragmentation ions such as m/z =97.02866, m/z =81.03369, m/z =69.03363 could be obtained, through databases and standard reference, it is concluded that compound 28 was gallic acid. Compound 42 obtained the quasi-molecular ion m/z 353.08881 [M-H]⁻ in the negative ion mode, and the predicted molecular formula was C₁₆H₁₈O₉, in the MS/MS, this compound appeared m/z 191.05589, m/z 179.03462, m/z 161.02374, and m/z 135.04454 ion fragments, referring to the previous literatures[37] and local database, the compound 42 was inferred as Neochlorogenic acid, the MS² spectrum of the compound 42 and 28, The MS/MS spectrum of Compound 28 and 42 are exhibited(Figure 12)(Figure 13), and the possible fragmentation pathway of compound 28 and 42 are exhibited(Figure 14). The RT= 20.165 (compound 40), it was also measured the quasi-molecular ion m/z 353.08875[M-H]⁻ in the negative ion mode, and the same ion fragments in MS² spectrum, based on the mzCloud and mzVault database, it was most likely to be Chlorogenic acid.

Identification of Nucleosides and nucleobases compounds

Nucleosides and nucleobases compounds are widely found in Chinese medicine herbs. Which led to ignored by researchers, however, these compounds have a wide range of biological activities. it has identified 8 from GSZG. The cleavage characteristic of this class of compounds is that losing one molecule of ribose, and the nucleobases was lost neutral molecular fragments such as NH₃. Compound 22 obtained the molecular m/z 268.1041 [M+H]⁺ in the positive ion mode, and the molecular formula was predicted to be C₁₀H₁₃N₅O₄. In the secondary mass spectrometry, only m/z 136.06178 strong fragment ions existed,

through database search and relevant literature[38] comparison, it was concluded that compound 22 was Adenosine. The secondary mass spectrum of the compound and the possible fragmentation pathway is exhibited (Figure 15) (Figure 16).

Other types of compounds

In addition, this study also identified sugars, amino acids, coumarins, vitamins, and terpenoids from GSZG, which found in a variety of Chinese herbal medicines. In this experiment, through database comparison, five carbohydrate compounds were found. Under the negative ion mode, these compounds have the same fragment ions, such as $m/z=59$, $m/z=71$, $m/z=89$, $m/z=179$. However, some compounds, such as compound 45, compared with the reference substance, and it was concluded that the compound was paeoniflorin which only existed in *Paeoniae Radix Alba*, and the cleavage law mainly includes breaking glycosidic bonds, removing sugar, benzoic acid, etc. neutral molecules, forming characteristic fragment ions with $m/z=121$ and $m/z=121$ in the negative ion mode.

Discussion

UPLC-Q-Orbitrap HRMS has many advantages like high sensitivity, high scoregood resolution and quality stability[39], especially suitable for the screening, identification and rapid identification of the main chemical components in complex Chinese medicine prescriptions. GSZD was listed in the first batch of classic recipes and was approved to be developed into granules[18]. According to relevant policy requirements, the members of this research group conducted related studies on the material standards of GSZD and the development of GSZG in the early stage, for the chemical constituents, using UPLC-Q-TOF-MS method to analysis the GSZD[14, 40, 41]. Traditional Chinese medicine prescriptions work synergistically with their many medicinal herbs. In the process of torment, different medicinal materials act synergistically to produce many new compounds. In this study, it was found that there were identified some new components which never reported, like Afzelin, Sakuranetin. Furthermore, it was identified many amino acids, carbohydrates under experimental conditions, which inferred the sample preparation technology in some extent. These studies will offer helpful information for clarifying bioactive components and preparation technology of GSZG.

Conclusion

In the present work, a strategy was proposed to profile the chemical constituents found in GSZG with the help of UPLC-Q-Orbitrap HRMS. The finding revealed the fact that chemical constituents maybe the main ingredients existing in GSZG, followed by the provision of the unique data, the research provided more information of chemical components in GSZG in this field. In addition, at present, the research group is developing the classic Chinese prescription GSZD into granules. Now, through the analysis of the chemical composition of GSZG produced in Japan, it will help the research group to prepare GSZG, as well as set the quality standard for the prepared GSZG.

Abbreviations

Rheumatoid arthritis: RA

disease modifying antirheumatic drugs: DMARDs

non-steroidal anti-inflammatory drugs: NSAIDs

Guizhi Shaoyao Zhimu decoction: GSZD

Guizhi Shaoyao Zhimu granules: GSZG

Declarations

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Not applicable.

Authors' contributions

Qinwan Huang and Yongxiang Gao are both the corresponding authors on the study. Tingna Li and Hairong Zeng are first authors and responsible for screening experiment, processing the data and writing the paper. Xiaorui Zhang, Yuanyuan Ren and Yijia Zeng helped in organizing the information and giving some suggestion. 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.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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Tables

Table 1. Identified compounds of GSZG by UPLC-Q-Orbitrap HRMS

NO.	RT [min]	Molecular Formula	Identification	Measured mass (m/z)	Accuracy mass (m/z)	Error (ppm)	Selective ion	Prominent MS2 Fragmentations(m/z)
1	1.601	C ₉ H ₂₀ N ₂ O ₂	N6, N6, N6-Trimethyl-L-lysine	189.15993	189.15972	1.11	[M+H] ⁺	60.0815,84.08135,130.08641
2	1.655	C ₆ H ₉ N ₃ O ₂	L-Histidine	154.06175	154.0622	-2.92	[M-H] ⁻	93.04499,110.07165,137.03508
3	1.703	C ₆ H ₁₃ N O ₅	D-Glucosamine	180.08661	180.08717	-3.11	[M+H] ⁺	60.04510,72.04505,84.04497,98.060
4	1.986	C ₅ H ₁₁ N ₃ O ₂	4-Guanidinobutyric acid	146.09259	146.09239	1.37	[M+H] ⁺	60.05628,86.06053,87.04453,104.0
5	2.039	C ₄ H ₅ N ₃ O	Cytosine	112.05087	112.05054	2.95	[M+H] ⁺	70.06571,95.02435
6	2.095	C ₄ H ₈ N ₂ O ₃	Asparagine	133.06105	133.0609	1.13	[M+H] ⁺	74.02423,87.05576,116.03442
7	2.128	C ₅ H ₁₀ N ₂ O ₃	D- (-)-Glutamine	145.06128	145.0619	-4.27	[M-H] ⁻	84.04463,101.07125,109.04003,127
8	2.332	C ₇ H ₇ N O ₂	Trigonelline	138.05505	138.05496	0.65	[M+H] ⁺	94.06560,110.06036
9	2.342	C ₁₂ H ₂₂ O ₁₁	α, α-Trehalose	341.1092	341.1092	0.00	[M-H] ⁻	59.01290,71.01293,89.02358,101.0
10	2.363	C ₆ H ₁₄ O ₆	Dulcitol	181.0714	181.0718	-2.21	[M-H] ⁻	59.01288,71.01292,89.02358,101.0
11	2.4	C ₁₈ H ₃₂ O ₁₆	D-Raffinose	503.16257	503.162	1.13	[M-H] ⁻	71.01298,89.02364,101.02371,161.0
12	2.631	C ₆ H ₆ O ₃	5-Hydroxymethyl-2-furaldehyde	127.03909	127.039	0.71	[M+H] ⁺	53.03933,55.01858,69.03417,81.03
13	2.717	C ₁₈ H ₃₂ O ₁₆	Manninotriose	503.16293	503.1614	3.04	[M-H] ⁻	59.01288,71.01292,89.02359,101.0
14	3.034	C ₇ H ₁₂ O ₆	D- (-)-Quinic acid	191.05608	191.0555	3.04	[M-H] ⁻	85.02864,93.03379,127.03932
15	3.349	C ₇ H ₁₀ O ₅	Shikimic acid	173.04544	173.0448	3.70	[M-H] ⁻	73.02864,93.03384,111.04452,137.0
16	3.788	C ₄ H ₆ O ₅	DL-Malic acid	133.01379	133.0142	-3.08	[M-H] ⁻	71.01298,72.99225,89.02362,115.00
17	3.978	C ₆ H ₅ N O ₂	Nicotinic acid	124.03958	124.03931	2.18	[M+H] ⁺	80.05000,96.04480
18	4.058	C ₆ H ₈ O ₇	Isocitric acid	191.0194	191.0197	-1.57	[M-H] ⁻	73.02858,85.02864,111.00803,154.9
19	4.933	C ₁₀ H ₁₄ N ₅ O ₇ P	Adenosine 5'-monophosphate	348.07086	348.071	-0.40	[M+H] ⁺	97.02876,136.06171
20	4.977	C ₆ H ₉ N O ₅	N-Acetylaspartic acid	174.04066	174.041	-1.95	[M-H] ⁻	58.02886,88.03954,114.01900,115.0
21	5.491	C ₉ H ₁₂ N ₂ O ₆	Uridine	243.06274	243.062	3.04	[M-H] ⁻	66.03403,80.96436,82.02901,110.0
22	5.687	C ₁₀ H ₁₃ N ₅ O ₄	Adenosine	268.1041	268.104	0.37	[M+H] ⁺	136.06178
23	5.73	C ₁₀ H ₁₃ N ₅ O ₃	2'-Deoxyadenosine	252.10928	252.1093	-0.08	[M+H] ⁺	136.06174
24*	5.916	C ₁₀ H ₁₅ N O	Ephedrine	166.12265	166.12264	0.06	[M+H] ⁺	91.05464,117.07005,133.8862,148.
25	5.955	C ₆ H ₈ O ₇	Citric acid	191.0197	191.0192	2.62	[M-H] ⁻	85.02862,87.00790,111.00800
26*	6.324	C ₁₀ H ₁₅ N O	Pseudoephedrine	166.12283	166.12264	1.14	[M+H] ⁺	91.05473,117.07014,133.08873,148

27	7.745	C ₁₀ H ₁₄ N ₅ O ₈ P	Guanosine monophosphate (GMP)	362.05197	362.0512	2.13	[M-H] ⁻	78.95815,96.96884,211.00140
28*	8.347	C ₇ H ₆ O ₅	Gallic acid	169.01385	169.0142	-2.07	[M-H] ⁻	69.03363,81.03369,97.02866,125.02866
29	8.729	C ₉ H ₁₃ N ₂ O ₉ P	Uridine monophosphate (UMP)	323.02969	323.0288	2.76	[M-H] ⁻	78.95815,96.96880,111.01925,211.01925
30	11.238	C ₂₄ H ₃₉ N O ₇	Fuziline	454.28027	454.2805	-0.51	[M+H] ⁺	58.06584,91.05495,108.08113,436.23813
31	11.396	C ₁₅ H ₁₄ O ₇	(-)-Galocatechin	305.0672	305.0677	-1.64	[M-H] ⁻	111.01923,125.02370,137.02383,137.02383
32	11.723	C ₂₄ H ₃₉ N O ₆	Neoline/Bullatine B	438.28485	438.2856	-1.71	[M+H] ⁺	58.06585,72.08144,154.12248,356.23813
33	11.795	C ₁₁ H ₁₂ N ₂ O ₂	DL-Tryptophan	205.09749	205.09715	1.66	[M+H] ⁺	118.06528,144.08078,146.05997,180.05997
34	11.864	C ₉ H ₁₇ N O ₅	D-Pantothenic acid	220.11826	220.1176	3.00	[M+H] ⁺	90.05545,184.09686,202.10739
35	12.931	C ₁₁ H ₁₅ N ₅ O ₃ S	5'-S-Methyl-5'-thioadenosine	298.09702	298.09683	0.64	[M+H] ⁺	136.06181
36	16.79	C ₁₅ H ₁₄ O ₇	Epigallocatechin	305.06769	305.0689	-3.97	[M-H] ⁻	125.02388,137.02390,139.03970,141.03970
37	17.344	C ₁₅ H ₁₄ O ₆	Catechin	291.08627	291.085	4.36	[M+H] ⁺	123.04415,139.03891,165.05458,201.05458
38	18.397	C ₃₀ H ₂₆ O ₁₂	Procyanidin B2	577.13727	577.13462	4.59	[M-H] ⁻	125.02375,289.07248,407.07782,421.07782
39	19.587	C ₂₅ H ₂₈ O ₁₆	Neomangiferin	583.13129	583.1316	-0.53	[M-H] ⁻	259.02521,271.02524,301.03583,331.03583
40	20.165	C ₁₆ H ₁₈ O ₉	Chlorogenic acid	353.08875	353.0881	1.84	[M-H] ⁻	93.03376,135.04451,173.04506,179.04506
41	20.815	C ₂₃ H ₂₈ O ₁₁	[(3R,5R,6S,8S)-3-(β-D-Glucopyranosyloxy)-6-hydroxy-8-methyl-9,10-dioxatetracyclo[4.3.1.02,5 03,8]dec-2-yl]methyl benzoate	479.15631	479.15543	1.84	[M-H] ⁻	105.03392,133.06493,151.07550,171.07550
42	21.818	C ₁₆ H ₁₈ O ₉	Neochlorogenic acid	353.08881	353.0878	2.86	[M-H] ⁻	93.03374,135.04454,137.02403,155.02403
43	22.336	C ₃₁ H ₄₃ N O ₁₀	Benzoylmesaconine	590.29657	590.2965	0.12	[M+H] ⁺	105.03376,508.23425,526.24207,540.24207
44	23.267	C ₃₂ H ₄₅ N O ₁₀	14-Benzoylaconine	604.3114	604.3121	-1.16	[M+H] ⁺	58.06588,105.03393,22.24713,540.24713
45*	24.008	C ₂₃ H ₂₈ O ₁₁	Paeoniflorin	479.15692	479.1559	2.13	[M-H] ⁻	121.02881,165.05524,327.10901
46	24.3	C ₂₇ H ₃₀ O ₁₅	Vicenin II	593.1521	593.1506	2.53	[M-H] ⁻	297.07730,353.06738,383.07779,471.07779
47	24.301	C ₃₁ H ₄₃ N O ₉	Benzoylhypaconine	574.30139	574.3016	-0.37	[M+H] ⁺	105.03392,510.24741,542.27515
48*	24.378	C ₁₉ H ₁₈ O ₁₁	Mangiferin	421.07886	421.07848	0.90	[M-H] ⁻	259.02515,271.02536,301.03586,311.03586
49	26.292	C ₂₆ H ₂₈ O ₁₄	Isoschaftoside	563.14197	563.1401	3.32	[M-H] ⁻	297.07761,325.07236,353.06760,383.06760
50	26.668	C ₂₆ H ₂₈ O ₁₄	Schaftoside	563.1424	563.1396	4.97	[M-H] ⁻	297.07767,325.07260,353.06781,383.06781

51	26.744	C ₃₃ H ₄₅ N O ₁₁	Mesaconitine	632.3067	632.3071	-0.63	[M+H] ⁺	95.04955,105.03383,354.16934,480
52*	27.251	C ₂₂ H ₂₈ O ₁₁	Prim-O-glucosylcimifugin	469.17029	469.1726	-4.92	[M+H] ⁺	159.04407,189.05478,235.06035,25
53*	28.336	C ₂₁ H ₂₂ O ₉	Liquiritin	417.11996	417.11801	4.67	[M-H] ⁻	91.01820,119.04967,135.00833,153
54	28.467	C ₁₅ H ₁₂ O ₅	Naringenin	273.07587	273.07568	0.70	[M+H] ⁺	91.05486,119.04942,147.04416,153
55	29.175	C ₂₁ H ₂₀ O ₁₀	Vitexin	433.11349	433.11249	2.31	[M+H] ⁺	121.02869,283.06018,284.06769,29
56	29.885	C ₁₃ H ₁₄ N ₂ O ₃	N-Acetyl-DL-tryptophan	245.09361	245.09315	1.88	[M-H] ⁻	58.02889,74.02384,98.02391,116.04
57	30.169	C ₂₇ H ₃₀ O ₁₄	(1S)-1,5-Anhydro-2-O-(6-deoxy-α-L-mannopyranosyl)-1-[5,7-dihydroxy-2-(4-hydroxyphenyl)-4-oxo-4H-chromen-6-yl]-D-glucitol	577.15808	577.1557	4.12	[M-H] ⁻	59.01288,175.00362,293.04614,413
58	30.876	C ₂₅ H ₂₄ O ₁₂	4,5-Dicaffeoylquinic acid	515.12067	515.1195	2.27	[M-H] ⁻	93.03387,135.04463,173.04517,179
59	31.018	C ₂₇ H ₃₀ O ₁₆	Rutin	609.14819	609.1461	3.43	[M-H] ⁻	151.00348,163.00388,243.02928,25
60	31.246	C ₂₁ H ₂₀ O ₁₂	Quercetin-3β-D-glucoside	463.08932	463.0879	3.07	[M-H] ⁻	301.0358
61	31.929	C ₁₄ H ₆ O ₈	Ellagic acid	300.99951	300.99899	1.73	[M-H] ⁻	185.02406,229.01453,283.99640
62*	31.95	C ₂₂ H ₂₈ O ₁₀	5-O-Methylvisammioside	453.17548	453.1767	-2.69	[M+H] ⁺	203.07022,216.04190,219.06546,23
63	32.288	C ₂₂ H ₂₂ O ₉	Ononin	431.13351	431.1337	-0.44	[M+H] ⁺	197.06004, 213.09105, 237.05453,2
64	32.3	C ₁₆ H ₁₂ O ₄	Formononetin	269.08054	269.0807	-0.59	[M+H] ⁺	213.09065,237.05464,253.04924
65	32.332	C ₁₅ H ₁₂ O ₄	Isoliquiritigenin	257.08102	257.08084	0.70	[M+H] ⁺	119.04943,137.02342,147.04413,23
66	32.333	C ₂₁ H ₂₂ O ₉	Isoliquiritin	419.13419	419.1337	1.17	[M+H] ⁺	137.02242,147.04411,257.08084
67	32.334	C ₂₆ H ₃₀ O ₁₃	Liquiritigenin-7-O-β-D-apiosyl-4'-O-β-D-glucoside	549.16272	549.16254	0.33	[M+H] ⁺	91.01807,119.04951,135.00814,153
68	32.691	C ₂₈ H ₃₂ O ₁₆	Narcissoside	623.16333	623.16066	4.28	[M-H] ⁻	243.03006,255.03008,271.02542,29
69	33.138	C ₁₆ H ₁₄ O ₅	Sakuranetin	287.09177	287.09152	0.87	[M+H] ⁺	107.04942,121.02855,193.04947,24
70	33.519	C ₁₂ H ₈ O ₄	Methoxsalen	217.04971	217.0495	0.97	[M+H] ⁺	161.05969,174.03114,189.05452,20
71	33.929	C ₂₁ H ₂₀ O ₁₀	Afzelin	431.09949	431.0984	2.53	[M-H] ⁻	227.03510,255.03023,284.03299,28
72	34.073	C ₁₆ H ₁₂ O ₅	Biochanin A	285.07584	285.0758	0.14	[M+H] ⁺	137.02325,213.05457,225.05464,25
73	34.199	C ₃₀ H ₃₂ O ₁₂	{{(3R,5R,6S,8S)-3-[(6-O-Benzoyl-β-D-glucopyranosyl)oxy]-6-hydroxy-8-methyl-9,10-dioxatetracyclo[4.3.1.02,5.03,8]dec-2-yl)methyl benzoate	585.19684	585.1989	-3.52	[M+H] ⁺	105.03395,151.07550,197.08104,24
74	34.667	C ₃₀ H ₃₂ O ₁₂	Benzoylpaeoniflorin	602.22327	602.2238	-0.88	[M+NH ₄] ⁺	105.03391,151.07552,179.07045,24

* : reference standards
a: chromone compounds, b: flavonoids compounds, c: alkaloid compounds, d: phenolic acid compounds, e: nucleosides and nucleobases compounds, f: other types of compounds

Figures

Figure 1
The composition of GSZD

Figure 2

The total ion chromatogram of sample solution both in positive mode and negative mode.

Figure 3

The total ion chromatogram of mixed reference solution both in positive mode and negative mode.

Figure 4

The MS/MS spectrum of compound 62

Figure 5

The possible fragmentation pathway of compound 62

The MS2 spectrum of compound 53 and 67

The possible fragmentation pathways of compound 53 and 67

The possible fragmentation pathway of compound 24

Figure 9

The MS/MS spectrum of compound 24

Figure 10

The possible fragmentation pathway of compound 51

Figure 11

The MS/MS spectrum of compound 51

Figure 12

The MS/MS spectrum of compound 28

Figure 13

The MS/MS spectrum of compound 42

Figure 14

The possible fragmentation pathways of compound 28 and 42

Figure 15

The MS/MS spectrum of the compound 22

Figure 16

The possible fragmentation pathway compound 22