Applying Four-Steps Characteristic Ion Filtering with HPLC-QE Orbitrap MS Approach to Rapid Characterize Compound Structures and Determine Major Representative Components in Modified Tabusen-2 Decoction

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Research

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Abstract

Background: Modified Tabusen-2 decoction (MTBD) is Traditional Chinese Mongolia Medicine, it is consist of “Lancitou” “Duzhong” “Sanqi” and “Honghua”. MTBD is mainly used to treat osteoporosis. However, the precise material basis of this prescription is not yet fully elucidated, which affects its modernization development

Methods: We establish a new HPLC-QE Orbitrap MS technology with four-steps characteristic ion filtering (FSCIF) strategy to quickly and effectively identify the structural features of MTBD, and determine the representative compounds content. Then the quantitative method of nineteen main components in MTBD were built, and eight batches MTBD were determined as well.

Results: By using the FSCIF strategy, a total of 143 compounds were unambiguously or tentatively identified, including 5 compounds were first reported in MTBD. Nineteen representative components were simultaneously quantified by HPLC-QE Orbitrap MS, and it is suitable for eight batches of MTBD. Methodology analysis showed that the assay method of 19 compounds (including three pairs of isomers) with a good repeatability, accuracy, and stability.

Conclusion: The method established above was successfully applied to assess the quality of MTBD extracts, which prove that the concentrations of 19 compounds varied significantly in different batches of MTBD extracts. Collectively, our findings enhance our molecular understanding of the MTBD formulation and will allow us to control its quality in a better way. At the same time, this study can promote the development and utilization of ethnic medicine.

Introduction

Modified Tabusen-2 decoction (MTBD), is developed on the basis of Tubson-2 decoction (TBD), which consist of “Lancitou” (inflorescences of *Echinops latifolius* Tausch (ELT)), “Duzhong” (barks of *Eucommia ulmoides* Oliver (EU)), “Sanqi” (roots of *Panax notoginseng* (PN)) and “Honghua” (flowers of *Carthamus tinctorius* L. (CT)) [1]. The Mongolian nationality is called a nation on horseback, osteoporosis as a common disease, especially common in the Mongolian nationality. TBD is a traditional classic prescription, it has been used to treat osteoporosis for centuries[2]. On this basis, MTBD has a better therapeutic effect, it mainly has the effects of promoting blood circulation, dispersing healing, reducing swelling and relieving pain, and renewing tendons. The chemical compositions of each herb are various, which has different pharmacological effects according to past reports. ELT, a traditional Mongolia herb, contained iochlorogenic acid A (ICGAA), chlorogenic acid and other phenylpropanoids [3, 4], which are the main active components in herb. The pharmacological mitigation of ELT on osteoporosis of postmenopausal women was also reported [5]. EU is enriched with lignans and iridoids, including geniposidic acid (GPA) and pinoresinol diglucoside (PDG), which has obvious antihypertensive effect [6]. In recent years, EU has attracted considerable attention because of its antiosteoporosis, anti-senile dementia, anti-aging, anti-inflammatory, antithrombotic, and antitumor activities [7, 8]. The flavonoids are the main active components of CT, with the efficacy of promoting blood circulation, removing blood stasis and relieving pain [9, 10]. Varieties of natural pigments isolated from CT, such as yellow pigments and red pigments [11], not only have pharmacological functions, but also have some nutritive value. Furthermore, triterpenoid saponins are main active constituents in PN, which are widely used for promoting blood clotting, relieving swelling, and alleviating pain [12, 13]. Although there have been many reports on the compounds in these medicinal materials, it is not enough to explain the overall structure of MTBD due to the interaction between temperature and herbs in the process of decoction. The chemical components of traditional Chinese medicine are the key to the study of its pharmacological activity, so in order to promote the development of traditional prescriptions, this research is aims to establish a new HPLC-QE Orbitrap MS technology to quickly identify the structural features of MTBD.

In accord with Traditional Chinese medicine (TCM), Traditional Mongolia medicine (TMM) is characterized with multiple components, multiple targets and played different roles in clinical therapy. This means that it is a great challenge to explain the main chemical composition of MTBD by traditional analytical methods. Especially, the irrelevant interference information makes it more difficult to identify the microscale detection. In order to solve this problem, some researchers have used the methods of mass defect, relative mass defect, neutral loss filtering (NLF), mass defect filtering and precursor ion to characterize the chemical structure in TCM or TMM prescription [14–18]. It has vital- important reference value for our following experiment. With the promotion of high-resolution mass spectrometry [19, 20], we propose a FSCIF strategy for substructure recognition, which can significantly improve the detection effectiveness, accuracy, and sensibility. This analysis program shows obvious efficiency (reduce data processing time) and intelligence (simplify the process of structural identification).

Nineteen representative components were selected for quantitative study. Methodology analysis showed that the assay method of nineteen compounds (including three pairs of isomers) with a good repeatability and stability. In terms of good linearity, acceptable recoveries and reproducibility, the method established above was successfully applied to quality assessment of eight batches MTBD extracts. The result prove that the concentrations of nineteen compounds varied significantly in different batches of MTBD extracts, which could facilitate the better development of quality evaluation technique in MTBD and will help to further its quality control exploration. This experiment explored the material basis of MTBD, promoted the development and utilization of traditional classic prescriptions, and provided new ideas for the analysis of complex prescriptions.

Methods

Materials and reagents

A total four batches of ELT were collected from various areas of Inner Mongolia (including Hohhot, Ordos, Xilingol, Ulan Hot) in August 2020. Different batches of EU, CT and PN powder were purchased from Bozhou Pharmaceutical Co. Ltd. (Anhui, China) and Guoda drugstore (Hohhot, Inner Mongolia). All herbs were authenticated by Professor Bi Qu. These specimens were preserved in the Department of general investigation of traditional Chinese medicine resources, Inner Mongolia Medical University.

Isochlorogenic acid A (ICGAA), 1,5-dicaffeoylquinic acid (1,5-DQA), genistein (GE), apigenin (APG), luteolin (LT), kaempferol (KPF), quercetin (QC), apigenin-7-O-glucuronide (A-7-O-G), rutin (RU), hydroxysafflor yellow A (HSYA), notoginsenoside R₁ (NG-R₁), ginsenoside Re (G-RE), ginsenoside Rg₁ (G-RG₁), ginsenoside Rb₁ (G-RB₁), caffeic acid (CA), ferulic acid (FA), geniposidic acid (GPA), chlorogenic acid (CGA), pinoresinol diglucoside (PDG) and digoxin (internal standard, IS) were purchased from Cybertech Limited (Beijing, China), with HPLC purity $\geq$ 98%. The chemical structures of these 19 compounds were displayed in Fig. 1. LC-MS grade methanol, acetonitrile and formic acid were achieved from Fisher Scientific (Hampton, NH, USA). Deionized water was prepared on a Millipore water purification system (Billerica, MA, USA).

MTBD Sample and standard solutions preparation

Sample preparation was a critical step for precise and convincing detection by the HPLC-QE Orbitrap mass method. The MTBD samples were prepared under the mature extraction process, and the whole operation process was in line with the basic operation safety regulations of the laboratory. EU, ELT and CT herbal materials were powdered and sieved through a 40-mesh for later extraction. A total 3.6 g of MTBD powders were accurately weighed (including 1.6 g of EU, 1.2 g of ELT, 0.8 g of CT) and placed in a 250 mL round-bottomed flask. These powders were immersed in 50mL ethanol: water (6:4, V/V) mixture and weighed, then reflux extracted twice, 90 min for each reflux. Taking into account the recovery rate of PN powder, add 0.4 g PN before the last extraction. After merging and mixing, the solution was filtered through a 0.45 μ m microporous membrane. This filtrate was diluted 40 times for HPLC-QE Orbitrap mass injection.

ICGAA 20.05 mg, 1,5-DQA 11.92 mg, GE 4.03 mg, APG 2.15 mg, LT 4.03 mg, KPF 1.30 mg, QC 2.02 mg, A-7-O-G 3.85 mg, RU 4.23 mg, HSYA 19.80 mg, NG-R₁ 10.02 mg, G-Re 19.40 mg, G-Rg₁ 10.17 mg, G-Rb₁ 20.49 mg, CA 2.07 mg, FA 1.05 mg, GPA 4.05 mg, CGA 18.90 mg, PDG 23.40 mg were accurately weighted and transferred into 2 mL volumetric flask, respectively. Owing to the solubility of these compounds, methanol was applied to prepare the standard solution. In order to improve the precision and accuracy of the content, digoxin was selected as the internal standard. These standard solutions were diluted with mobile phase to final concentration before injection into HPLC-QE Orbitrap mass.

Chromatography and mass spectrometry conditions

The characterization and quantification of MTBD sample extracts were analyzed using a Thermo HPLC-QE Orbitrap mass system (HPLC, UltiMate 3000, mass system, Quadrupole Exactive Orbitrap™). The qualitative analytical conditions were as follows: HPLC column, COSMOSIL C18 (250mm $\times$ 4.6mm ID, 5 μ m); Solvent system, methanol (A) and water containing 0.1% (v/v) formic acid (B); Gradient program, 0–5 min, 2%-5%A; 5–10 min, 5%-10%A; 10–15 min, 10%-18%A; 15–25 min, 18%-23%A; 25–35 min, 23%-28%A; 35–55 min, 28%-33%A; 55–60 min, 33%-39%A; 60–70 min, 39%-43%A; 70–75 min, 43%-46%A; 75–85 min, 46%-60%A; 85–100 min, 60%-65%A; 100–105 min, 65%-75%A; 105–110 min, 75%-100%A; 110–130 min, 100%-100%A. Flow rate, 0.6 mL/ min. Column temperature, 30°C. Sample injection volume, 10 μ L. The quantitative analysis of MTBD sample extracts was separated on an ACE C18-PFP (100 $\times$ 3.0mm ID, 3 μ m) column. The mobile phase consisted of methanol (A) and water containing 0.3% (v/v) formic acid (B). A gradient program was used as follows: 0–6 min, 40%-40%A; 6–15 min, 40%-90%A; 15–16 min, 90%-10%A; 16–21 min, 10%-10%A; 21–22 min, 10%-40%A; 22–25 min, 40%-40%A. The flow rate was set as 0.3 mL/ min. The column temperature was kept at 30°C. Sample injection volume was 2 μ L.

The qualitative and quantitative mass parameters conditions were set up as follows: Aus gas heater temperature, 150°C; Capillary temperature, 350°C; Spray voltage, 3.5 kv; S-lens RF level, 50; Sheath gas flow rate, 40 L; Aux gas flow rate, 2 L. Scanning range, 100–1500 *m/z*. Mass spectrometry uses full scan mode for analysis in positive ion mode and negative ion mode.

Method Validation

The dependent variable was the ratio of the peak area of each analyte to the peak area of the internal standard, while the independent variable was set as the concentration value of each analyte, the least square regression was used to construct the standard curve equation. The lower limit of quantification (LLOQ) should be with signal-to-noise ratio > 10. The precisions and accuracies of intra-day and inter-day was assessed by analyzing each concentration level (low, medium, and high) of six repeated QC samples on the same day and three consecutive days, respectively. Sample stability was investigated after the extracts kept at room temperature for 0 h, 6 h, 12 h, 24 h. Add the mixed control solution equal to the content of each analyte in the sample to the MTBD sample, repeat the preparation of 6 solutions, and calculate the recovery according to the following formula.

Recovery (%) = (detected amount – original amount) / spiked amount $\times$ 100 %

Construction of the identification strategy

Each type of compounds has its similar core and skeleton. On this basis, the characteristic ion will be produced, which provides us with new ideas for identifying these structures. In addition, FSCIF is especially suitable for compounds with the same structural type contained similar fragmentation pathways with some characteristic ions. Correspondingly, a FSCIF-based and sub-structure scanning strategy will be used for rapid identification of MTBD structures. The analytical strategy is show in Fig. 2. The compounds in MTBD were characterized by HPLC-QE Orbitrap MS technology with FSCIF strategy, which including following steps: 1) established the self-building chemical database of MTBD according to literature and online database; 2) comprehensively summarized characteristic ions for each compound type to conduct global identification of the ingredients in MTBD; 3) rapidly screened relevant structure information by neutral loss fragments (NLF) to conform the sugar type, conjunction position, and other information; 4) concluded the precise compound structure through high-precision MSⁿ data. The typical total ion chromatograms (TICs) of MTBD by HPLC-QE Orbitrap mass system in positive and negative ion modes were shown in Fig. 3. 143 compounds were identified through high-precision MSⁿ data, including 51 triterpene saponins, 28 flavanoids, 20 phenylpropanines, 15 iridoids, 12 lignans, 11 polyphenols, and 6 other types (Table 1), in which 5 compounds were first reported in MTBD and 20 compounds were unambiguously identified by comparing with reference standards. These 143 components structures were shown in **Figure S1**.

Table 1
Characterization of chemical constituents of MTBD

No	tR(min)	Formula	Identification	Precursor ions (m/z)	Diff	Fragment (m/z)	Type	Reference standard
1	0.25	C ₂₇ H ₃₀ O ₁₆	Rutin	609.1461[M-H] ⁻	-3.414	301.0351, 300.0278, 283.0325, 271.0251, 255.0292, 227.0321, 151.0293	F	Yes
2	1.05	C ₁₅ H ₁₀ O ₇	Quercetin	301.0353[M-H] ⁻	3.724	273.0405, 257.0452, 229.0500, 178.9978, 151.0026, 121.0283, 107.0126	F	
3	1.68	C ₈ H ₆ O ₄	Vanillic acid	167.0349[M-H] ⁻	0.867	152.0105, 123.0438, 108.0203	PO	
4	2.35	C ₁₇ H ₂₀ O ₉	Methyl chlorogenic acid	367.1034[M-H] ⁻	5.239	191.0553, 173.0078	P	
5	3.57	C ₁₁ H ₁₄ O ₅	Genipin	225.0768[M-H] ⁻	2.738	207.0659, 147.0441, 123.0439, 101.0231	I	Yes
6	3.82	C ₆ H ₆ O ₃	Pyrogalllic acid	125.0244[M-H] ⁻	-1.604	107.4741, 97.0282	PO	
7	4.09	C ₁₆ H ₂₂ O ₁₀	Geniposidic acid	373.1140[M-H] ⁻	-6.227	211.0940, 193.0498, 167.0703, 149.0598, 123.0439	I	
8	4.47	C ₁₆ H ₂₂ O ₁₁	Deacetyl trifolate	389.1089[M-H] ⁻	3.167	227.0550, 209.0356, 191.0553, 183.0655, 165.0541, 147.0285, 139.0389	I	
9	4.85	C ₁₀ H ₁₄ O ₁₀	2-Methylsuccinyl-6'-O-glucoside	293.0514[M-H] ⁻	6.047	131.0450	PO	

F: Flavonoids, P: Phenolic acids, T: Triterpenoid saponins, L: Lignans, I: Iridoids, PO: Polyphenol, O: Other compounds

* first reported in MTBD

No	tR(min)	Formula	Identification	Precursor ions (m/z)	Diff	Fragment (m/z)	Type	Reference standard
10	4.92	C ₁₅ H ₂₂ O ₉	Aucubin	345.1191[M-H] ⁻	2.335	183.0660, 165.0543, 139.0391, 121.0285	I	
11	5.37	C ₁₇ H ₂₆ O ₁₁	Harpagide acetate	405.1402[M-H] ⁻	-1.364	191.0554, 147.0289, 119.0026, 101.0023	I	
12	6.23	C ₁₅ H ₁₀ O ₆	Kaempferol	285.0404[M-H] ⁻	4.615	257.0453, 243.1601, 239.1650, 229.0322, 199.0395, 185.0420	F	Yes
13	7.41	C ₂₃ H ₃₄ O ₁₅	Genipin gentian diglycoside	549.1824[M-H] ⁻	5.378	387.2035, 207.1128, 179.0551, 147.0298	I	
14	7.50	C ₂₅ H ₂₄ O ₁₁	3-Caffeoyl-5-coumaroyl-quinic acid	499.12458[M-H] ⁻	5.81	353.1080, 191.0554	P	
15	8.32	C ₁₉ H ₁₈ O ₁₁	Isomangiferin	421.0776[M-H] ⁻	-1.966	259.0224	F	
16	8.44	C ₉ H ₆ O ₃	Umbelliferone	161.0244[M-H] ⁻	-0.249	135.0441, 99.0438, 71.0124	P	
17	8.99	C ₇ H ₆ O ₅	Gallic acid	169.0142[M-H] ⁻	0.179	125.0232, 141.0914	PO	Yes
18	9.25	C ₆ H ₆ O ₄	2-hydroxyphenol	141.0193[M-H] ⁻	3.247	123.0175	PO	
19	9.76	C ₁₅ H ₁₄ O ₆	L-Epicatechin	289.0717[M-H] ⁻	7.333	271.0235, 245.0411, 205.2713, 179.0110	F	
20	10.56	C ₄ H ₄ O ₄	Maleic acid	115.0036[M-H] ⁻	-0.479	71.0124	PO	

F: Flavonoids, P: Phenolic acids, T: Triterpenoid saponins, L: Lignans, I: Iridoids, PO: Polyphenol, O: Other compounds

* first reported in MTBD

No	tR(min)	Formula	Identification	Precursor ions (m/z)	Diff	Fragment (m/z)	Type	Reference standard
21	11.43	C ₁₅ H ₂₄ O ₁₀	Harpagide	363.1296[M-H] ⁻	0.977	183.0652, 89.0228	I	
22	12.53	C ₁₆ H ₁₈ O ₉	Chlorogenic acid	353.0878[M-H] ⁻	0.854	191.0554, 179.0341, 173.0446, 161.0234, 155.0338, 137.0322, 135.0440, 93.0333	P	Yes
23	14.13	C ₈ H ₈ O ₄	Methyl protocatechuic acid	167.0349[M-H] ⁻	0.508	152.0106, 123.0439, 108.0203	PO	
24	15.84	C ₈ H ₈ O ₄	Isovanillic acid	167.0349[M-H] ⁻	-2.536	123.0439	PO	
25	18.23	C ₁₃ H ₁₆ O ₉	Protocatechuic acid-4-glucoside	315.0721[M-H] ⁻	-1.392	108.0204	PO	
26	19.56	C ₁₄ H ₁₈ O ₉	4-glucopyranoxy-3-benzoic acid	329.0878[M-H] ⁻	4.340	167.0340, 152.0105, 123.0439, 108.0204	O	
27	20.23	C ₉ H ₁₂ O ₅	Rehmaglutin C	199.0611[M-H] ⁻	3.316	155.0704, 137.0596	I	
28	20.39	C ₁₈ H ₂₄ O ₁₂	Asperuloside acid	431.1194[M-H] ⁻	-3.114	269.0198, 251.0098	I	
29	21.77	C ₁₆ H ₁₈ O ₉	Neochlorogenic acid	353.0878[M-H] ⁻	-0.699	191.0554, 179.0341, 135.0440	P	
30	22.36	C ₁₆ H ₁₈ O ₉	4-caffeoylquinic acid	353.0878[M-H] ⁻	3.941	191.0554, 179.0340, 135.1440	P	
31	23.28	C ₂₀ H ₂₄ O ₇	Cycloolivil	375.1449[M-H] ⁻	-2.075	327.1343, 297.1207, 257.1132, 151.0752	L	
32	24.28	C ₉ H ₈ O ₄	Caffeic acid	179.0349[M-H] ⁻	0.856	135.0440	P	Yes
33	24.36	C ₉ H ₁₀ O ₄	Dihydro caffeic acid	181.0506[M-H] ⁻	1.849	163.0390, 135.0441, 119.0488	P	

F: Flavonoids, P: Phenolic acids, T: Triterpenoid saponins, L: Lignans, I: Iridoids, PO: Polyphenol, O: Other compounds

* first reported in MTBD

No	tR(min)	Formula	Identification	Precursor ions (m/z)	Diff	Fragment (m/z)	Type	Reference standard
34	26.50	C ₇ H ₆ O ₄	Gentianic acid	153.0193[M-H] ⁻	-0.295	109.0282	PO	
35	27.23	C ₇ H ₆ O ₄	Protocatechuic acid	153.0193[M-H] ⁻	0.685	109.0283, 91.0175,	PO	
36	28.03	C ₄₂ H ₇₀ O ₁₂	Ginsenoside F ₄	789.4759[M + Na] ⁺	-1.837	707.1499, 643.4222, 349.1090	T	
37	28.55	C ₂₀ H ₂₄ O ₇	Oleoresin	375.1449[M-H] ⁻	-1.018	179.0341, 161.0233	L	
38	29.40	C ₁₆ H ₁₈ O ₉	Cryptochlorogenic acid	353.0878[M-H] ⁻	3.516	191.0554, 179.0340, 173.0446, 135.0440	P	
39	30.90	C ₂₀ H ₂₄ O ₇	Olivil	375.1449[M-H] ⁻	-2.635	327.1360, 195.1251, 179.0341, 161.0220	L	
40	32.26	C ₁₇ H ₂₄ O ₁₀	Geniposide	387.1296[M-H] ⁻	3.128	207.1025, 123.0444, 101.0232	I	
41	33.49	C ₃₃ H ₄₄ O ₁₉	Naringin dihydrochalcone 4-O-β- D-glucoside	743.2404[M-H] ⁻	3.128	373.1295, 313.1088, 181.0498, 151.0396	F	
42	34.26	C ₂₇ H ₃₂ O ₁₆	Hydroxysafflor yellow A	611.1617[M-H] ⁻	2.207	491.1200, 473.1092, 403.1042, 325.0720	F	Yes
43	37.58	C ₁₀ H ₁₀ O ₄	Ferulic acid	193.0506[M-H] ⁻	2.208	178.0264, 149.0598, 134.0362	P	Yes
44	38.11	C ₃₂ H ₄₂ O ₁₆	Pinoresinol diglucoside	681.2400[M-H] ⁻	2.039	519.5070, 357.1346, 151.0390, 136.0159	L	
45	39.59	C ₁₆ H ₁₈ O ₈	3-O-p-coumaroylquinic acid	337.0928[M-H] ⁻	6.688	191.0553, 173.0448, 163.0390	P	
46	40.21	C ₂₇ H ₃₆ O ₁₃	Citrusin B	567.2083[M-H] ⁻	-4.289	341.1384, 329.1394	L	

F: Flavonoids, P: Phenolic acids, T: Triterpenoid saponins, L: Lignans, I: Iridoids, PO: Polyphenol, O: Other compounds

* first reported in MTBD

No	tR(min)	Formula	Identification	Precursor ions (m/z)	Diff	Fragment (m/z)	Type	Reference standard
47	41.77	C ₂₇ H ₃₀ O ₁₇	Quercetin-3, 4'-O-di-β-glu copyranoside	625.1410[M- H] ⁻	0.831	463.0884, 301.0350, 271.0243	F	
48	42.23	C ₁₅ H ₂₆ O ₉	Eucommioside	349.1504[M- H] ⁻	-1.102	187.1528, 89.0230	I	
49	43.69	C ₁₀ H ₁₀ O ₃	Coniferyl aldehyde	177.0557[M- H] ⁻	-0.101	162.0312	P	
50	46.23	C ₂₀ H ₂₂ O ₇	Erythroglycerin-β- terpineol aldehyde ether	373.1292[M- H] ⁻	4.259	177.0548, 165.0547, 150.0308,	P	
51	48.38	C ₂₆ H ₂₈ O ₁₆	Quercetin 3-O-Sambu bioside	595.1305[M + H] ⁺	-0.638	301.0327	F	
52	49.62	C ₂₆ H ₃₂ O ₁₁	Pinoresinol-4'-O-β-D-glucopyranoside	519.1871[M- H] ⁻	-3.929	357.1345, 151.0390	L	
53	54.63	C ₂₆ H ₃₂ O ₁₁	Pinoresinol-β-D-glucoside;	519.1871[M- H] ⁻	3.334	357.1345, 342.1107, 311.1293, 151.0390, 136.0154	L	
54	55.16	C ₂₁ H ₂₀ O ₁₂	Isoquercitrin	463.0881[M- H] ⁻	3.623	301.0349, 271.0321, 255.0299	F	Yes
55	56.44	C ₂₂ H ₂₈ O ₁₄	5-(3'-o-caffeoylglucosyl) quinine	515.1406[M- H] ⁻	8.420	191.0555, 161.0234, 135.0440	P	
56	57.45	C ₂₂ H ₂₈ O ₁₄	1-O-(3'-o-caffeoylglucosyl) quinine	515.1406[M- H] ⁻	2.499	179.0341, 173.0446, 161.0233, 135.0440	P	
57*	57.73	C ₃₃ H ₄₀ O ₂₁	Quercetin 3-glucosyl- (1->3) -rhamnosyl- (1->6) - galactoside	771.1989[M- H] ⁻	2.070	609.1469, 463.0873, 301.0351	F	
58	58.66	C ₂₈ H ₃₆ O ₁₃	Syringaresionl-O-β-D-g1uc0pyranoside	579.2083[M- H] ⁻	3.298	417.1557	P	
59	59.50	C ₃₅ H ₆₀ O ₆	Daucosterol	575.4317[M- H] ⁻	2.329	397.7564	T	
60	62.59	C ₂₅ H ₂₄ O ₁₂	1,5-dicaffeoylqunic acid	515.1194[M- H] ⁻	2.499	353.0881, 191.0554, 135.0440	P	

F: Flavonoids, P: Phenolic acids, T: Triterpenoid saponins, L: Lignans, I: Iridoids, PO: Polyphenol, O: Other compounds

* first reported in MTBD

No	tR(min)	Formula	Identification	Precursor ions (m/z)	Diff	Fragment (m/z)	Type	Reference standard
61	63.34	C ₂₅ H ₂₄ O ₁₂	Isochlorogenic acid A	515.1194[M-H] ⁻	1.288	353.0881, 191.0554, 179.0341, 173.0446, 135.0440	P	
62	64.00	C ₄₂ H ₇₂ O ₁₅	6- O-β-D-glucopyranosyl-20-o-β-D-glucopyranosyl-3β,6β,12β,20 (S) 7-25-pentaphydroxydammar-23-eneDroginosenoside Rg ₁	839.4763[M + Na] ⁺	-2.644	659.4114	T	
63	65.10	C ₂₅ H ₂₄ O ₁₂	Isochlorogenic acid B	515.1194[M-H] ⁻	1.288	353.0881, 335.0777, 191.0554, 179.0341, 173.0446	P	
64	66.70	C ₉ H ₁₆ O ₄	Eucommitol	187.0975[M-H] ⁻	1.521	169.0861, 143.1068, 125.0960	I	Yes
65	66.72	C ₆ H ₄ O ₄	Coumalic acid	139.0036[M-H] ⁻	6.332	119.5097	O	
66	67.26	C ₁₈ H ₁₆ O ₅	Sideroxylin	311.0924[M-H] ⁻	2.177	267.0663	F	
67	67.68	C ₂₁ H ₂₀ O ₁₂	Hyperoside	463.0882[M-H] ⁻	4.206	301.03455, 151.00258	F	
68	68.78	C ₁₅ H ₂₆ O ₇	2-(5-hydroxyethyl-2,3-dimethyl-2-cyclopenten-1-yl)-glucopyranoside	317.1605[M-H] ⁻	4.580	243.1238, 225.1132	I	
69	69.40	C ₂₁ H ₂₀ O ₁₀	Apigenin-7-O-glucuronide	431.0983[M-H] ⁻	3.147	269.0376	F	
70	70.29	C ₉ H ₁₆ O ₃	1-deoxyeucommitol	171.1026[M-H] ⁻	0.930	127.1118, 125.0959	I	
71	72.63	C ₂₁ H ₂₀ O ₁₁	Astragalin	447.0933[M-H] ⁻	2.532	285.0395, 241.0829, 217.0886	F	
72	73.55	C ₂₁ H ₁₈ O ₁₁	Baicalin	445.0776[M-H] ⁻	4.724	269.0456	F	
73	74.23	C ₂₇ H ₃₀ O ₁₅	Nicotiflorin	593.1511[M-H] ⁻	3.681	285.0404, 255.0307, 227.0352	F	
74	74.88	C ₂₇ H ₃₀ H ₁₅	Safflor yellow (A)	593.1511[M-H] ⁻	3.884	285.0404	F	Yes
75	75.61	C ₁₈ H ₁₄ O ₆	Milletin C	325.0717[M-H] ⁻	2.650	310.0848	F	
76	76.26	C ₁₂ H ₁₆ O ₃	3-Butyl-4-hydroxy-4,5-dihydro-2-benzofuran-1(3H)-one	207.1026[M-H] ⁻	0.368	135.0443	O	

F: Flavonoids, P: Phenolic acids, T: Triterpenoid saponins, L: Lignans, I: Iridoids, PO: Polyphenol, O: Other compounds

* first reported in MTBD

No	tR(min)	Formula	Identification	Precursor ions (m/z)	Diff	Fragment (m/z)	Type	Reference standard
77	77.03	C ₁₁ H ₁₂ O ₄	Ethyl caffeate	207.0662[M-H] ⁻	3.162	179.0341, 161.0234, 135.0440	P	
78	77.96	C ₄₈ H ₈₂ O ₁₉	Notoginsenoside R ₆	985.5342[M + Na] ⁺	-2.049	365.1045, 305.0816	T	
79	78.56	C ₄₈ H ₈₂ O ₁₉	Notoginsenoside R ₃	985.5342[M + Na] ⁺	2.402	645.4159, 365.1044	T	
80*	79.20	C ₂₈ H ₃₂ O ₁₆	6-Methoxykaempferol 3-robinobioside	623.16175[M-H] ⁻	1.524	315.0509, 301.0320, 300.0276	F	
81*	79.40	C ₂₉ H ₃₆ O ₁₅	3,4',6'-Trihydroxy-4,2'-dimethoxychalcone 4'-O-rutinoside	623.19814[M-H] ⁻	6.18	315.0510, 301.0313, 300.0376	F	
82	79.58	C ₄₈ H ₈₂ O ₁₉	Notoginsenoside M	985.5342[M + Na] ⁺	-3.227	805.4688, 365.1047	T	
83	81.76	C ₄₈ H ₈₂ O ₁₉	Notoginsenoside N	985.5342[M + Na] ⁺	-2.983	805.4689	T	
84	82.77	C ₄₈ H ₈₂ O ₁₉	20-O-glucoginsenoside Rf	985.5342[M + Na] ⁺	-1.684	805.4689, 365.2320	T	
85*	83.83	C ₂₃ H ₂₂ O ₁₁	Apigenin 7- (2"-acetylglucoside)	473.1089[M-H] ⁻	1.569	413.0891, 269.0379	F	
86	84.34	C ₄₁ H ₆₈ O ₁₂	Notoginsenoside T ₅	775.4602[M + Na] ⁺	-2.385	692.0035, 643.3312, 463.3556, 335.0930	T	
87	84.47	C ₄₇ H ₈₀ O ₁₈	Notoginsenoside R ₁	931.5271[M-H] ⁻	0.633	799.4888, 637.4328, 475.3800, 391.0658	T	Yes
88	85.37	C ₁₅ H ₁₀ O ₆	Luteolin	285.0404[M-H] ⁻	4.720	257.0453, 151.0030	F	
89	85.76	C ₄₂ H ₇₂ O ₁₄	Majoroside F ₄	823.4814[M + Na] ⁺	-1.603	643.4166	T	
90	86.17	C ₄₂ H ₇₂ O ₁₄	3-O-β-D-glucopyranosyl-6-O-β-D-glucopyranosyl-20-(S)-protopanaxatriol	823.4814[M + Na] ⁺	-3.157	703.0069, 643.4163	T	
91	87.18	C ₄₂ H ₇₂ O ₁₄	Gynoside B	823.4814[M + Na] ⁺	-1.603	643.4164	T	
92	87.69	C ₄₂ H ₇₂ O ₁₄	Ginsenoside Rg ₁	823.4814[M + Na] ⁺	-1.603	643.4104	T	

F: Flavonoids, P: Phenolic acids, T: Triterpenoid saponins, L: Lignans, I: Iridoids, PO: Polyphenol, O: Other compounds

* first reported in MTBD

No	tR(min)	Formula	Identification	Precursor ions (m/z)	Diff	Fragment (m/z)	Type	Reference standard
93	88.21	C ₄₅ H ₇₄ O ₁₇	Malonyl ginsenoside Rg ₁	909.4818[M + Na] ⁺	-1.891	865.4895, 729.4166, 685.4270	T	
94	89.59	C ₄₈ H ₈₀ O ₁₉	Notoginsenoside G	983.5186[M + Na] ⁺	-2.897	803.4535	T	
95*	89.94	C ₃₀ H ₂₈ O ₁₂	4,2',3',4'-Tetrahydrochalcone 4'-O- (2"-O-p-coumaroyl) glucoside	579.1507[M-H] ⁻	0.622	271.0614, 151.0027, 107.0126	F	
96	90.05	C ₁₅ H ₁₀ O ₅	Genistein	269.0455[M-H] ⁻	4.572	225.0554, 201.0555, 151.0027, 117.0329, 107.0124	F	Yes
97	90.57	C ₁₅ H ₁₀ O ₅	Apigenin	269.0455[M-H] ⁻	4.572	225.0555, 201.0553, 151.0025, 117.0328, 107.0124	F	Yes
98	91.24	C ₄₁ H ₇₀ O ₁₃	Pseudoginsenoside RT ₃	793.4708[M + Na] ⁺	3.882	613.4072	T	
99	91.79	C ₄₄ H ₇₄ O ₁₅	Yesaninoside D	865.4919[M + Na] ⁺	-4.633	685.4267	T	
100	92.47	C ₃₀ H ₂₆ O ₁₂	Apigenin-7-O-(6"-coumaroyl) glucosid	577.1351[M-H] ⁻	1.390	431.0988, 269.0457	F	
101	92.96	C ₂₀ H ₂₂ O ₆	Epipinoresinol	357.1343[M-H] ⁻	1.216	151.1533, 136.0809, 121.0282	L	
102	94.52	C ₄₂ H ₇₂ O ₁₄	Ginsenoside Rf	823.4814[M + Na] ⁺	-2.113	661.5368, 641.4468, 365.1043	T	
103	95.39	C ₄₁ H ₇₀ O ₁₃	Notoginsenoside R ₂	793.4708[M + Na] ⁺	-2.583	661.4249, 481.3630, 335.0939	T	
104	95.89	C ₄₂ H ₇₂ O ₁₃	Ginsenoside Rg ₂	807.4865[M + Na] ⁺	-1.362	661.4281, 481.3676, 349.1101	T	
105	96.47	C ₃₆ H ₆₂ O ₉	Gypenoside LXXVI	661.4286[M + Na] ⁺	2.479	601.2890, 481.3620	T	
106	97.28	C ₃₆ H ₆₂ O ₉	Ginsenoside Rh ₁	661.4286[M + Na] ⁺	-1.769	481.3650, 413.2539	T	

F: Flavonoids, P: Phenolic acids, T: Triterpenoid saponins, L: Lignans, I: Iridoids, PO: Polyphenol, O: Other compounds

* first reported in MTBD

No	tR(min)	Formula	Identification	Precursor ions (m/z)	Diff	Fragment (m/z)	Type	Reference standard
107	98.42	C ₅₉ H ₁₀₀ O ₂₇	Ginsenoside Ra ₃	1263.6344[M + Na] ⁺	-2.327	789.4784, 497.1457, 437.1239	T	
108	99.42	C ₅₉ H ₁₀₀ O ₂₇	Notoginsenoside Fa	1263.6344[M + Na] ⁺	-0.688	921.5158	T	
109	100.51	C ₅₄ H ₉₂ O ₂₂	Notoginsenoside I	1115.5972[M + Na] ⁺	-1.470	773.4795, 365.1046	T	
110	101.91	C ₅₄ H ₉₂ O ₂₃	Ginsenoside Rb ₁	1107.5956[M-H] ⁻	0.589	945.5432, 783.4906, 621.4368, 459.3851	T	Yes
111	102.94	C ₄₂ H ₇₂ O ₁₃	Ginsenoside Rg ₃	807.4865[M + Na] ⁺	-0.904	365.1046	T	
112	103.85	C ₄₈ H ₈₂ O ₁₈	Ginsenoside Re	969.5393[M + Na] ⁺	-1.908	789.4742	T	Yes
113	105.07	C ₅₄ H ₉₂ O ₂₃	Yesaninoside E	1131.5921[M + Na] ⁺	-5.768	789.4737, 365.1045	T	
114	106.08	C ₃₈ H ₆₄ O ₁₀	6'-O-acetyl ginsenoside F ₁	703.4391[M + Na] ⁺	0.071	481.3647	T	
115	106.58	C ₅₆ H ₉₄ O ₂₄	Quinquenoside R ₁	1173.6027[M + Na] ⁺	-2.548	831.4845, 365.1044	T	
116	107.65	C ₅₆ H ₉₄ O ₂₄	6'''-O-acetyl ginsenoside Rb ₁	1173.6027[M + Na] ⁺	-2.326	831.4845, 789.4744, 407.1151, 347.0945	T	
117	108.75	C ₅₃ H ₉₀ O ₂₂	Ginsenoside Rb ₂	1101.5815[M + Na] ⁺	-1.479	789.4740, 335.0939	T	
118	109.76	C ₅₃ H ₉₀ O ₂₂	Notoginsenoside L	1101.5815[M + Na] ⁺	-1.479	789.4740	T	
119	110.33	C ₅₇ H ₉₄ O ₂₆	Malonyl ginsenoside Rb ₁	1217.5925[M + Na] ⁺	-2.727	1173.5993, 875.4738, 831.4844, 789.4738	T	
120	111.31	C ₄₈ H ₈₂ O ₁₇	Vina-ginsenoside R ₃	953.5444[M + Na] ⁺	-2.349	773.4788	T	
121	111.47	C ₄₈ H ₈₂ O ₁₈	Gypenoside XVII	969.5393[M + Na] ⁺	-0.908	365.1048	T	

F: Flavonoids, P: Phenolic acids, T: Triterpenoid saponins, L: Lignans, I: Iridoids, PO: Polyphenol, O: Other compounds

* first reported in MTBD

No	tR(min)	Formula	Identification	Precursor ions (m/z)	Diff	Fragment (m/z)	Type	Reference standard
122	112.04	C ₅₇ H ₉₄ O ₂₆	3-(β-D-glucopyranosyl-β-D-glucopyranosyl)-20-O-(6-O-malonyl-β-D-glucopyranosyl-β-D-glucopyranosyl)-3β,12β,20(S)-trihydroxydammar-24-ene	1217.5925[M + Na] ⁺	-1.07	1173.6008, 1131.5912, 875.4739, 831.4839, 789.4733, 451.1044, 407.1150	T	
123	112.53	C ₄₈ H ₈₂ O ₁₈	Ginsenoside Rd	945.5428[M-H] ⁻	0.857	783.4907, 621.4375, 459.3848, 375.3146	T	Yes
124	112.57	C ₃₆ H ₆₂ O ₈	Notoginsenoside R ₇	645.4336[M + Na] ⁺	-3.249	627.3813, 465.3691	T	
125	113.48	C ₃₆ H ₆₀ O ₈ C36H60O7	Ginsenoside Rh ₃	643.4180[M + Na] ⁺	-3.232	583.3644, 463.3514	T	
126	113.89	C ₅₁ H ₈₄ O ₂₁	Malonyl ginsenoside Rd	1055.5397[M + Na] ⁺	3.598	875.4738, 789.4740	T	
127	114.07	C ₄₈ H ₈₂ O ₁₈	Gypenoside LXXII	969.5393[M + Na] ⁺	-1.691	789.4739	T	
128	114.88	C ₃₆ H ₆₂ O ₁₁	Notoginsenoside T ₄	693.4184[M + Na] ⁺	-3.763	633.3707	T	
129	115.36	C ₄₇ H ₈₀ O ₁₇	3-O-[β-D-glucopyranosyl(1-2)-β-D-glucopyranosyl]-20-O-β-D-xylopyranosyl-3β,12β,20(s)-trihydroxydammar-24-ene	939.5287[M + Na] ⁺	-0.872	789.4735	T	
130	116.22	C ₁₅ H ₁₀ O ₅	Baicalein	269.0455[M-H] ⁻	4.238	197.1905	F	Yes
131	117.02	C ₄₇ H ₈₀ O ₁₈	6-O-[xylopyranosyl-β-D-glucopyranosyl]-3β,6β,12β,20(s),25-pentahydroxydammar	811.4814[M + Na] ⁺	1.208	793.3365, 751.2600, 679.2239, 499.1350, 412.1227, 335.0018	T	
132	117.70	C ₂₀ H ₂₂ O ₆	Pinoresinol	357.1343[M-H] ⁻	1.340	313.1811, 151.1520, 136.0819	L	
133	118.87	C ₄₂ H ₇₂ O ₁₃	Ginsenoside F ₂	807.4865[M + Na] ⁺	0.458	627.4217	T	
134	120.08	C ₄₂ H ₇₂ O ₁₃	Gypenoside LXXV	807.4865[M + Na] ⁺	7.789	365.1045	T	
135	121.53	C ₂₉ H ₄₂ O ₅	Ulmoidol	469.2959[M-H] ⁻	3.715	423.2238	T	
136	121.65	C ₂₈ H ₃₄ O ₄	Unknown	433.2384[M-H] ⁻	-0.685	433.2577	O	

F: Flavonoids, P: Phenolic acids, T: Triterpenoid saponins, L: Lignans, I: Iridoids, PO: Polyphenol, O: Other compounds

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No	tR(min)	Formula	Identification	Precursor ions (<i>m/z</i>)	Diff	Fragment (<i>m/z</i>)	Type	Reference standard
137	122.04	C ₃₆ H ₆₀ O ₉	Ginsenoside Rh ₇	659.4129[M + Na] ⁺	-0.349	599.3925	T	
138	122.50	C ₃₂ H ₄₂ O ₁₇	1-Hydroxypinoresinol-4,4"-di-O-β-D-glucopyranoside	697.2349[M-H] ⁻	0.112	535.1532, 373.0323	L	
139	122.99	C ₂₀ H ₂₄ O ₈	Threo-dihydroxydehydrodiconiferyl alcohol	391.1398[M-H] ⁻	-3.313	313.1747, 295.0882	L	
140	123.91	C ₁₆ H ₃₂ O ₂	Palmitic acid	255.2329[M-H] ⁻	1.978	241.3251	O	Yes
141	124.01	C ₂₀ H ₂₄ O ₈	Erythro-dihydroxydehydrodiconiferyl alcohol	391.1398[M-H] ⁻	-2.359	341.1587, 313.0930, 207.0832	L	
142	125.83	C ₁₈ H ₃₆ O ₂	Palmitic acid ethyl ester	283.2642[M-H] ⁻	3.859	89.0229	O	
143	127.10	C ₉ H ₁₂ O ₄	Eucommidiol	183.0662[M-H] ⁻	0.268	139.1124, 93.7235	I	
F: Flavonoids, P: Phenolic acids, T: Triterpenoid saponins, L: Lignans, I: Iridoids, PO: Polyphenol, O: Other compounds								
* first reported in MTBD								

Results

Identification of triterpenoid saponins

Triterpenoid saponins were typical bioactive components of PN, which were classified into two categories of protopanaxadiol (PPD) triterpene saponins and protopanaxatriol (PPT) triterpene saponins, the characteristic ions at *m/z* 459.39 [aglycones-H]⁻ and at *m/z* 475.38 [aglycones-H]⁻, corresponded to the PPD and PPT type ginsenosides [21]. In this study, most triterpene saponins (46 compounds) were detected [M + Na]⁺ in positive ion mode, other triterpenoid saponins (5 compounds) were detected [M-H]⁻ in negative ion mode, excimer ion peaks can produce different cleavage modes to provide structural information such as aglycone type, sugar type and its junction position and. Compounds **110** and **123** were filtered by characteristic ion *m/z* 459.39, which tentatively identified PPD type ginsenosides, compound **110** (C₅₄H₉₂O₂₃) [M-H]⁻ at *m/z* 1107.5956, its molecular ion peak successively lost the four molecules of glucose, and obtained *m/z* 945.5432, *m/z* 783.4906, *m/z* 621.4368, and *m/z* 459.3851. Compared with the standard, compound **110** was identified as ginsenoside Rb₁, the possible cleavage pathways of ginsenoside Rb₁ were shown in **Figure S2-A**. Similarly, [M-H]⁻ at *m/z* 945.5428 (Compound **123**) was tentatively identified Ginsenoside Rd, the main fragment ions were [M-H-glc]⁻ *m/z* 783.4907, [M-H-glc-glc]⁻ *m/z* 621.4375, and [M-H-glc-glc-glc]⁻ *m/z* 459.3848 [22]. Compound **87** were filtered by characteristic ion *m/z* 475.38, which tentatively identified PPT type ginsenoside. In the secondary mass spectrum, fragment ions *m/z* 799.4888, *m/z* 637.4328, *m/z* 475.3800 and *m/z* 391.0658 were [M-H-xyl]⁻, [M-H-xyl-glc]⁻, [M-H-xyl-glc-glc]⁻ and aglycon, the possible cleavage pathways of [M-H]⁻ were shown in **Figure S2-B**.

By using the FSCIF strategy, a total of eleven compounds (**107, 112, 113, 116, 117, 118, 119, 122, 126, 127, 129**) were detected by characteristic ion of 789.47 Da ([M + Na-glcglc⁶malonyl]⁺). The retention time of compound **122** was 112.04 min, the fragment ions *m/z* 451.1044 and *m/z* 789.4733 were a pair of complementary ions [glcglc⁶malonyl + Na]⁺ and [M + Na-glcglc⁶malonyl]⁺. In addition, the fragment ions were observed in *m/z* 1173.6008, 1131.5912, 875.4739, 831.4839 and 407.1150, which were assigned to [M + Na-CO₂]⁺, [M + Na-malonyl]⁺, [M + Na-glcglc]⁺, [M + Na-(glcglc + CO₂)⁺] and [glcglc⁶malonyl + Na-CO₂]⁺ fragment ions. The possible cleavage pathways of compound **122** are shown in **Figure S2-C**.

Additionally, the sugar type and its junction position and were concluded with the application of NLF strategy. The position of sugar fragments on the aglycon was relatively fixed (C3, C6 and C12), and the main types of sugars were glc (162.02 Da), rha (146.01 Da) and xyl (132.02 Da), the linkage between sugars is mainly 1-2, 1-6. In this experiment, nineteen compounds (**62, 82, 83, 84, 89, 90, 91, 92, 93, 94, 98, 99, 102, 105, 106, 120, 124, 125, 133**) detected by NLF with 162.02 Da. Compounds **36, 86, 103** and **104** filtered by 146.01 Da or 132.02 Da were obtained. Compound **103** (C₄₂H₇₂O₁₃) [M + Na]⁺ at *m/z* 793.4708 tentatively identified Notoginsenoside R₂, the main fragment ions were [M-H-xyl]⁻ *m/z* 661.4249, [M-H-xyl-glc]⁻ *m/z* 481.3630, and [M-H-xyl-glc-rha]⁻ *m/z* 335.0939. Compound **104** (C₄₂H₇₂O₁₃) [M + Na]⁺ at *m/z* 807.4865 tentatively identified Ginsenoside Rg₂. First, the ion at *m/z* 661.4281 was formed by the neutral loss of a rhamnose unit of the ion at *m/z* 807.4865. Second, the ion at *m/z* 481.3676 was formed by the neutral loss of a glucose unit of the ion at *m/z* 661.4281. Finally, ion at *m/z* 349.1101 was formed by the neutral loss of a xylose unit of the ion at *m/z* 481.3676.

Identification of flavonoids

Most flavonoid aglycones were derivatives of quercetin, kaempferol and apigenin, so we set 301.03 Da, 285.04 Da, 269.04 Da as characteristic ions templates for these components identification, which contributes to the rapid identify flavonoids. A total of nine compounds (**1, 2, 47, 51, 54, 57, 67, 80, 81**) screened with 301.03 Da were found, compound **1** showed the $[M-H]^-$ at m/z 609.1461, which tentatively identified as rutin, its important fragment ion was 301.03 Da in secondary mass spectra, indicating the neutral loss of 308.11 Da ($C_{12}H_{20}O_9$). In addition, the occurrence of m/z 283.0325, m/z 255.0292, m/z 227.0321 were a better proof $[M-H-C_{12}H_{20}O_9-H_2O]^-$, $[M-H-C_{12}H_{20}O_9-H_2O-CO]^-$, and $[M-H-C_{12}H_{20}O_9-H_2O-2CO]^-$, which were the main peak, appeared in second mass spectra (**Figure S3-A**). More over, compounds **57, 80** and **81** were identified for the first time in MTBD. Compound **57** showed the $[M-H]^-$ at m/z 771.1989, the ion at m/z 609.1469 was formed by the neutral loss of a glucose unit of the ion at m/z 771.1989. Besides, the ion at m/z 463.0873 was formed by the neutral loss of axyllose unit of the ion at m/z 609.1469. Finally, ion at m/z 301.0351 was formed by the neutral loss of a glucose unit of the ion at m/z 463.0873. Hence, compound **57** was tentatively identified Quercetin 3-glucosyl- (1→3) -rhamnosyl- (1→6) -galactoside.

A total of five compounds (**12, 71, 73, 74, 88**) acquired with 285.04 Da were found. Compound **12** showed the $[M-H]^-$ at m/z 285.0404, m/z 257.0453, m/z 239.1650, m/z 229.0322, and m/z 185.0420, corresponding to $[M-H-CO]^-$, $[M-H-CO-H_2O]^-$, $[M-H-2CO]^-$, and $[M-H-2CO-CO_2]^-$, which were contribute to the crack of C2-C3 and C4-C10. In addition, the fracture of C4-C10 bond can also lead to the removal of C_2H_2O (42.02 Da), which was corresponds to m/z 243.1601. Next, the removal of CO_2 (44.01 Da) and result in the generation of m/z 199.0395. By using the FSCIF strategy, seven compounds (**69, 72, 85, 96, 97, 100, 130**) screened with 269.04 Da were found. Compound **97** was tentatively identified apigenin, a high abundance secondary mass spectrometer fragment ion m/z 225.0555 was formed after CO_2 (44.01 Da) loss, indicating that apigenin derivatives were easier to lose CO_2 . In addition, m/z 269.0455 lost one molecule C_3O_2 (68.02 Da), resulting in m/z 201.0553. Apigenin, which a flavonoid with double bond on the six membered ring, can also undergo ring opening reaction of C ring, resulting in fragment ions such as m/z 151.0025, m/z 117.0328, m/z 107.0124. These structural changes were also reflected at a Retro Diels-Alder reaction (RDA) [23]. Hence, the characteristic ion of RDA was set by 151.00 Da, compounds (**1, 2, 41, 67, 88, 95, 96, 97**) screened with 151.00 Da were found. Compound **95** was identified as 4,2',3',4'-Tetrahydroxychalcone 4'-O- (2"-O-p-coumaroyl) glucoside, which was being reported from MTBD for the first time. Its molecular ion peak m/z 269.0455 at $[M-H]^-$ was observed, the fragment ions m/z 271.0614, m/z 151.0027, m/z 107.0126 were proved that $[M-H-C_{15}H_{16}O_7]^-$, $[M-H-C_{15}H_{16}O_7-C_8H_8O]^-$, and $[M-H-C_{15}H_{16}O_7-C_8H_8O-C_9H_9O_2]^-$.

Identification of phenylpropanines

Phenylpropanoids and their derivatives, including monocaffeoylquinic acids, biscaffeoylquinic acids and acaffeoylquinic acid derivatives, were main components widely present in MTBD. Some papers [24] have previously shown that phenylpropanoids have multifaceted effects which include anti-inflammatory, antioxidant, antimicrobial, antidiabetic activities as well as exhibit renoprotective, hepatoprotective, and cardioprotective effects. By using the FSCIF strategy, twenty phenylpropanines were found, the ions at m/z 191.05 Da and 179.03 Da represented the base peaks of quinic acid, whereas ions at m/z 161.02 Da and 135.04 Da represented the base peaks of caffeic acid. Chlorogenic acid is an ester of caffeic acid and quinic acid, which indicates that chlorogenic acid contains the feature ions of both caffeic acid and quinic acid. A total of twelve compounds (**4, 14, 22, 29, 30, 38, 45, 55, 56, 60, 61, 63**) were detected by m/z 191.05 Da and 179.03 Da. Compound **22** was tentatively identified chlorogenic acids, produce m/z 191.0554, m/z 179.0341 (Compound **32**), m/z 173.0446, m/z 161.0234, m/z 155.0338, m/z 137.0322, m/z 135.0440 and m/z 93.0333, which were corresponding to $[M-H-C_9H_6O_3]^-$, $[M-H-C_7H_{10}O_5]^-$, $[M-H-C_9H_6O_3-H_2O]^-$, $[M-H-C_7H_{10}O_5-H_2O]^-$, $[M-H-C_9H_6O_3-2H_2O]^-$, $[M-H-C_9H_6O_3-3H_2O]^-$, $[M-H-C_7H_{10}O_5-CO_2]^-$ and $[M-H-C_9H_6O_3-3H_2O-CO_2]^-$. **Figure S3-B** has shown the main cracking pathways of chlorogenic acid. Compounds **16, 32, 33, 77** were filtered by m/z 161.02 Da and 135.04 Da, which were an indicative of caffeic acid derivatives. Take compound **77** ($C_{11}H_{12}O_4$) as an example, the $[M-H]^-$ at m/z 207.0662, and the secondary mass spectrometry were detected at m/z 179.0341 $[M-H-CO]^-$, m/z 161.0234 $[M-H-CO-H_2O]^-$, m/z 135.0440 $[M-H-CO-CO_2]^-$, which tentatively identified as ethyl caffeate. Compounds **32** and **33** also have a similar pyrolysis law.

Identification of iridoids

The most basic core of iridoids is iridoid alcohol, containing cyclic enethers and alcoholic hydroxyl groups, which imply the basic skeleton of iridoid glycosides contains a characteristic dihydropyran ring is cis connected to a cyclopentane unit structure. A total of 15 iridoids were detected $[M-H]^-$ in negative ion mode. In the ESI⁻ mode, the fragment ion $^{2,7}F_0^-$ ion at m/z 101.02 obtained by the fragmentation of the aglycon part of the excimer ion, which was a characteristic ion to identify the structure of the excimer ion [25, 26]. According to the literature [27], the ion at m/z 147.03 was the prominent ion of iridoids. Compounds **5, 8, 11, 13** and **40** were detected by characteristic ions 147.03 Da or 101.02 Da. Taking the derivation process of compound **8** as an example, the quasi-molecular ion peak of compound **8** was m/z 389.1089 $[M-H]^-$, yielded a formula of $C_{16}H_{22}O_{11}$. The $[M-H]^-$ ion of m/z 227.0550 was the absence of glucose neutral fragment from m/z 389.1089. The fragment ions m/z of 209.0356 and 183.0655 were losing one molecule of H_2O and one molecule of CO_2 from $[M-H]^-$ ion of m/z 227.0550. Then the $[M-H]^-$ ion of m/z 183.0655 continuous loss two molecule of H_2O , convert to the fragment ions of m/z 165.0543 and m/z 147.0285. Consistently, the dehydration of fragment ion m/z 209.0356 leads to the production of m/z 191.0553. And the fragment ion m/z 147.0285 was decarboxylation of m/z 191.0553. The cleavage detail of each ion was displayed in **Figure S3-C**.

In addition, iridoid glycosides are usually connected to a glucose at the C1 position, so they are easy to lose neutral fragments such as 162.02 Da (glc), 44.01 Da (CO_2) and 18.01 Da (H_2O) [28]. A total of five compounds (**7, 10, 21, 28, 48**) were filtered by 162.02 Da. Compound **7** ($C_{16}H_{22}O_{10}$) showed the $[M-H]^-$ at m/z 373.1140, the fragment ions determined from MS/MS spectra were m/z 211.0606 $[M-H-glc]^-$, m/z 193.0498 $[M-H-glc-H_2O]^-$, m/z 167.0703 $[M-H-glc-CO_2]^-$, and m/z 149.0598 $[M-H-glc-CO_2-H_2O]^-$, m/z 211.0940 (373.1140 Da - 162.02 Da) were characteristic fragments of compound **7**, which tentatively identified as geniposidic acid. To sum up, the iridoids were easier to lose the glucose neutral fragment ion 162.02 Da, and obtain aglycon fragment ions, and then the aglycon ions decarboxylated or dehydrated to become a series of fragments.

Identification of lignans

A large number of the bisepoxylignans and monoepoxylignans combine with glucose to form monoglycoside or diglycoside. Therefore, the majority of them could lose glycosyl and methyl neutral fragments first, then lose one or two molecular of CH₂O, and finally formed 151.03 Da. Therefore, characteristic ion fragment 151.03 Da was used to identify lignans. Compound **31**, **44**, **52**, **53**, **101** and **132** were detected by FSCIF with 151.03 Da. Compound **44** showed the [M-H]⁻ at *m/z* 681.2400, the fragment ions *m/z* 519.5070, *m/z* 357.1346, *m/z* 151.0390, which were corresponding to [M-H-glc]⁻, [M-H-glc-glc]⁻ and [M-H-glc-glc-C₁₂H₁₄O₃]⁻. Subsequently, compound **37**, **39**, **138** and **139** filtered by NLF with ions of 162.02 Da, 44.01 Da or 18.01 Da.

Other compounds

A total of 11 polyphenol (**3**, **6**, **9**, **17**, **18**, **20**, **23**, **24**, **25**, **34**, **35**) were recognized by FSNLF analysis. Because of the presence of hydroxyl and carboxyl groups, these compounds were filtered by 18.01 Da (H₂O) and 44.01 Da (CO₂). In addition, six other compounds were identified by comparison with the literature.

Assay Validation

Quantification of 19 major compounds in MTBD

The 19 compounds quantified were the screening of osteoporosis targets by network pharmacology in the early stage of our laboratory, then the representative and top ranked compounds were selected. Methodology analysis showed that the assay method of 19 compounds (including three pairs of isomers) had a good repeatability and stability.

Specificity.

The chromatograms of blank sample, standard mixture sample, and MTBD extracts sample were presented in Fig. 4. Nineteen compounds in MTBD extracts were separated within 25 minutes, which baseline separation of each compound was achieved and no obvious signal noises were occurred around determinate peak. Additionally, no interferences were detected between the three isomers.

Linearity and lower limit of quantification.

Three batches of standard curve solutions with six different concentrations were prepared. The typical standard curves were assessed by using DAS 2.0 software with the quadratic weight ($W = 1 / C^2$). The standard curves, LLOQ and correlation coefficients were listed in Table 2, proved that the calibration curves of the components with a good linearity over the studied concentration range.

Table 2 Calibration curves, linear range, r^2 and LOQs of 19 compounds in MTBD				
Compound	Calibration curves	Linear range ($\mu\text{g/mL}$)	r^2	LLOQ ($\mu\text{g/mL}$)
ICGAA	$y = 647.937x + 1.476$	4.800–192.000	0.9994	4.800
1,5-DQA	$y = 364.018x - 0.810$	6.100–244.000	0.9991	6.810
GE	$y = 22386.297x - 0.191$	0.023–0.920	0.9982	0.025
APG	$y = 29960.140x - 0.562$	0.065–2.600	0.9989	0.073
LT	$y = 15454.517x - 0.090$	0.015–0.600	0.9986	0.015
KPF	$y = 9208.139x - 0.051$	0.010–0.400	0.9983	0.010
QC	$y = 8437.051x - 0.062$	0.011–0.440	0.9989	0.011
A-7-O-G	$y = 6901.196x + 11.968$	4.200–168.000	0.9962	4.200
RU	$y = 2560.803x - 0.093$	0.230–9.200	0.9992	0.250
HSYA	$y = 1104.734x - 0.935$	9.830–392.000	0.9984	10.930
NG-R ₁	$y = 8.300x - 0.016$	2.400–96.000	0.9964	2.400
G-Re	$y = 730.821x + 0.137$	1.010–40.400	0.9963	1.010
G-Rg ₁	$y = 21.700x + 0.029$	6.500–260.000	0.9983	6.700
G-Rb ₁	$y = 93.320x + 0.128$	5.660–226.400	0.9979	5.830
CA	$y = 183840.263x - 8.999$	0.260–10.400	0.9985	0.260
FA	$y = 2322.874x - 0.525$	0.390–15.600	0.9992	0.410
GPA	$y = 119.544x - 0.011$	0.800–32.000	0.9987	0.800
CGA	$y = 3414.355x - 5.245$	5.500–220.000	0.9978	5.660
PDG	$y = 10.556x - 0.018$	2.180–87.200	0.9987	2.180

Precision and accuracy.

Three batches of quality control samples were prepared according to three concentration levels. Each concentration was analyzed with 6 duplications. The precision values were between 1.13%~6.66% for intra-day, 2.42%~10.62% for inter-day and accuracy was range from 86.11–114.27%. The results above demonstrated the acceptable precision and accuracy of the present method.

Repeatability and stability.

Six MTBD sample extracts were prepared on the same day, the repeatability of 19 components was within 6.26% relative standard deviation (*RSD*). The stability results of 19 compounds were summarized in Table 3, the acceptability of the data within 3.92% deviation from the 0 h sample values, which indicated that a large number of samples could be stable in each analytical run.

Table 3
Precision, repeatability, stability and recovery of 19 compounds in MTBD

Compound	Inter-day precision			Intra-day precision			Repeatability	Stability	Accuracy	
	(RSD, n = 3)			(RSD, n = 3)					(n = 6, %)	
	Low	Middle	High	Low	Middle	High			(RSD, n = 6, %)	(RSD, n = 4, %)
ICGAA	4.74	5.61	6.45	1.24	2.34	3.39	2.62	3.13	107.36	1.76
1,5-DQA	3.37	4.31	5.18	1.13	0.59	1.63	6.26	3.48	106.46	2.11
GE	2.42	7.24	4.87	2.29	2.79	0.90	2.30	2.68	106.41	2.06
APG	3.34	5.34	3.64	3.22	2.58	2.82	1.70	2.18	106.73	2.23
LT	4.05	7.84	4.53	3.13	2.81	1.09	4.75	3.92	98.21	3.01
KPF	9.47	9.00	7.98	2.18	1.54	5.51	4.94	3.60	103.12	2.37
QC	8.67	6.94	5.52	6.66	2.25	1.35	4.68	2.81	103.42	4.10
A-7-O-G	4.17	3.42	7.38	4.15	3.05	3.13	2.09	2.38	104.56	1.87
RU	4.03	3.23	3.42	1.86	1.92	2.57	2.99	2.34	105.39	0.92
HSYA	3.81	4.97	5.67	2.25	1.13	4.78	3.40	2.56	104.80	1.13
NG-R1	8.22	4.19	5.85	1.63	2.07	4.55	4.05	0.75	104.87	2.81
G-RE	6.50	3.92	5.41	3.17	1.38	2.47	4.02	3.23	107.35	1.73
G-RG1	6.10	7.59	6.12	5.08	4.64	4.80	3.27	2.26	104.64	1.59
G-RB1	10.62	3.37	3.61	2.94	2.71	3.47	4.50	2.99	106.94	2.54
CA	6.29	5.83	4.08	5.45	3.62	4.31	2.15	2.43	101.10	0.53
FA	8.76	7.21	7.61	3.90	4.15	2.40	2.94	3.74	105.71	2.10
GPA	8.36	6.89	10.52	2.64	0.54	4.11	3.82	3.59	107.60	3.01
CGA	2.74	5.12	2.82	2.52	1.25	3.50	2.99	2.24	106.59	2.11
PDG	6.95	7.32	7.71	2.69	2.78	4.11	2.67	1.97	92.08	4.57

Application to samples Modified Tabusen-2 decoction (MTBD)

The method established above was successfully utilized to quantitative studies of MTBD extracts, as shown in Table 4. Eight batches of MTBD samples prepared with different herb sources were determined by using above mature method. The herb formulation of each batch was listed in **Table S1**. There is an indicative of that the concentrations of 19 compounds varied significantly in MTBD extracts, the content of flavonoids was the highest, followed by saponins (**Figure S4**), which attracted the attention of herb quality in picking as well as in circulating during the market. We should get the hints from the result of MTBD extracts quantitation research that strictly controlled the herb quality need to pay highly attention, which was a guarantee of their clinical efficacy and safety.

Table 4
The content of 19 compounds in the MTBD medicinal measured (μg , $\bar{X} \pm \text{SD}$, n = 3).

Compound	Batch 1		Batch 2		Batch 3		Batch 4		Batch 5		Batch 6		Batch 7	
	Content	RSD	Content	RSD	Content	RSD	Content	RSD	Content	RSD	Content	RSD	Content	RSD
	(μg)	(%)	(μg)	(%)	(μg)	(%)	(μg)	(%)	(μg)	(%)	(μg)	(%)	(μg)	(%)
ICGAA	16762.18 ± 199.87	1.19	11295.32 ± 149.83	1.33	13711.97 ± 574.45	4.19	14877.31 ± 411.70	2.77	18213.17 ± 604.40	3.32	17164.28 ± 553.79	3.23	16781.86 ± 743.30	2.2
1,5-DQA	13869.07 ± 653.18	4.71	12125.54 ± 324.70	2.68	3151.23 ± 42.20	1.34	9869.47 ± 186.95	1.89	14198.79 ± 468.05	3.30	15605.54 ± 768.51	4.92	5106.09 ± 189.07	3
GE	21.46 ± 1.26	4.73	12.26 ± 0.03	0.21	7.16 ± 0.17	2.35	29.67 ± 0.49	1.67	24.35 ± 1.46	6.03	18.19 ± 0.41	2.27	11.35 ± 0.46	4
APG	193.44 ± 7.91	4.09	30.00 ± 0.51	1.73	180.55 ± 7.09	3.93	61.60 ± 2.16	3.52	173.06 ± 9.52	5.50	26.22 ± 0.97	3.73	221.23 ± 5.14	2
LT	27.43 ± 1.31	4.80	9.46 ± 0.46	4.87	28.72 ± 1.06	3.70	11.38 ± 0.49	4.32	24.46 ± 0.85	3.49	6.33 ± 0.36	5.74	38.02 ± 1.77	4
KPF	8.76 ± 0.18	2.13	5.67 ± 0.10	1.90	7.52 ± 0.22	2.96	5.84 ± 0.27	4.76	11.25 ± 0.28	2.46	7.14 ± 0.24	3.31	15.15 ± 0.86	5
QC	5.88 ± 0.04	0.64	3.89 ± 0.06	1.46	5.65 ± 0.29	5.15	4.32 ± 0.16	3.77	8.00 ± 0.32	4.03	4.29 ± 0.21	4.79	9.29 ± 0.26	2
A-7-O-G	1338.14 ± 56.00	4.18	522.55 ± 12.41	2.37	2642.11 ± 111.36	4.21	1005.98 ± 28.87	2.87	1485.53 ± 73.13	4.92	418.74 ± 15.29	3.65	3759.35 ± 132.56	3
RU	278.13 ± 11.11	4.00	249.04 ± 5.18	2.08	224.49 ± 5.36	2.39	272.52 ± 8.27	3.03	299.01 ± 9.27	3.10	405.94 ± 10.02	2.47	353.85 ± 6.71	1
HSYA	36875.52 ± 584.45	1.58	32683.88 ± 1097.05	3.36	32605.85 ± 1720.72	5.28	36899.09 ± 1252.44	3.39	36566.99 ± 1173.24	3.21	37101.52 ± 865.43	2.33	35625.68 ± 1346.05	3
NG-R1	2286.91 ± 110.69	4.84	1761.26 ± 79.66	4.52	2534.44 ± 68.48	2.70	2245.17 ± 104.11	4.64	3040.46 ± 147.89	4.86	2719.07 ± 86.30	3.17	3063.47 ± 91.59	2
G-Re	115.34 ± 2.04	1.18	173.72 ± 2.04	1.18	138.15 ± 3.66	2.65	128.01 ± 2.95	2.30	129.25 ± 3.70	2.86	154.83 ± 4.72	3.05	117.29 ± 1.20	1
G-Rg1	16762.12 ± 199.87	1.19	21295.33 ± 149.83	1.33	13711.97 ± 574.45	4.19	14877.31 ± 411.70	2.77	18213.17 ± 604.40	3.32	17164.28 ± 553.80	3.23	18534.92 ± 541.17	2
G-Rb1	8989.89 ± 161.19	1.79	8170.53 ± 276.59	3.39	8514.05 ± 329.66	3.87	8002.95 ± 114.29	1.43	10322.41 ± 184.74	1.79	11080.66 ± 554.08	5.00	9488.45 ± 406.75	4
CA	684.33 ± 0.99	0.14	555.93 ± 19.10	3.44	980.54 ± 29.49	3.01	362.15 ± 16.41	4.53	601.07 ± 11.68	1.94	489.06 ± 13.84	2.83	953.46 ± 25.03	2
FA	142.20 ± 2.60	1.83	208.53 ± 6.40	3.07	211.98 ± 7.80	3.68	211.14 ± 9.22	4.17	271.42 ± 1.19	0.44	468.62 ± 8.42	1.80	532.06 ± 12.35	2
GPA	3146.68 ± 108.83	3.46	3163.53 ± 123.27	3.90	3112.44 ± 116.01	3.73	3045.59 ± 86.91	2.85	3163.44 ± 41.17	1.30	3284.01 ± 73.66	2.24	3173.78 ± 118.37	3
CGA	11069.83 ± 373.70	3.38	21193.91 ± 16.47	0.08	6736.66 ± 230.72	3.42	11708.75 ± 230.42	1.97	11213.11 ± 322.76	2.88	20884.47 ± 710.15	3.40	8027.72 ± 378.50	4

Compound	Batch 1		Batch 2		Batch 3		Batch 4		Batch 5		Batch 6		Batch 7	
	Content	RSD	Content	RSD	Content	RSD	Content	RSD	Content	RSD	Content	RSD	Content	RSD
	(μg)	(%)	(μg)	(%)	(μg)	(%)	(μg)	(%)	(μg)	(%)	(μg)	(%)	(μg)	(%)
PDG	8372.22 ±	0.79	7228.23 ±	3.11	8303.93 ±	0.90	8145.20 ±	3.00	7812.40 ±	4.17	6784.62 ±	3.79	8551.33 ±	2.4
	65.92		224.48		75.14		244.72		325.53		257.26		250.91	

Discussion

Although the isolation and purification before biological activity evaluation is a traditional strategy of exploring material basis in TMM, the time-consuming and labor intensive characteristics cannot be neglected. Ultraviolet (UV) detector is exceedingly common for flavanoids, phenylpropanines, and other UV-absorbing compounds [29], while is not applicable to the analysis of saponin. Although the detection of saponin could be enabled by evaporative light scattering detector (ELSD), the sensitivity during the test procedure should also be aware of [30]. Herein, in order to shorten the analysis time, improve the analysis sensitivity, and simultaneously determine UV- absorbing compounds and Non-UV-absorbing compounds, high performance liquid chromatography coupled with mass spectrometry (HPLC-QE Orbitrap MS) approach [31], as a high efficiency, FSCIF and sensitive technology, is employed in this study to simultaneous isolate and identify the material basis in MTBD. Additionally, the existence of isomer (ICGAA with 1, 5-DQA, GE with APG, LT with KPF) in MTBD increases the difficulty of separation and analysis [32]. The chromatographic conditions in quantitative analysis need to be optimized carefully during present research.

In order to achieve better separation effect for three pairs of isomers in MTBD, the mobile phase was screened in this experiment. The peak of each component was with symmetrical shape and no tailing phenomenon. Additionally, the influence of column temperature and flow rate was considered, and a better separation was achieved under column temperature of 30°C and flow rate of 0.3 mL/min. A variety of chromatographic columns were also optimized in this study. Comparing with ACE C18-PFP column (100×3.0mm ID 3μm), GRACE Alltima C18column (250mm×4.6nm 5μm), HITACHI La Chrom C18 column (250mm×4.6mm I.D. 5μm), Thermo ODS-2 HYPERSIL column (250mm×4.6mm, 5μm), ACE C18-PFP column performed better separation and resolution, especially for the three isomers.

It was found through analysis that the contents of the 19 components differ in MTBD prepared from different batches of crude drugs, this might be because the crude drugs of different batches were different in origin, growing environments. This has aroused our attention in all aspects of picking, transportation.

Conclusions

Based on HPLC-QE Orbitrap MS with FSCIF approach to rapid detect structure fragment and quantification of major representative components in MTBD, 143 compounds with seven chemical categories were unambiguously or tentatively identified. This study not only enriched the cleavage law of MTBD compounds, but also established a approach for the accurate search and discovery of active components from complex mixtures. The repeatability, accuracy, stability, linearity, recoveries, and reproducibility of quantitative analysis all meet the criteria for acceptability of quantitative studies. The determination of 19 compounds in MTBD extracts in different batches were obtained to monitor the quality of each prescription, which facilitates the better development of quality evaluation technique in MTBD and will help to further exploration of quality control of MTBD.The 19 compounds determined based on the qualitative and quantitative results are the major components of the MTDB, this work promotes the development and utilization of traditional prescriptions and lays the material foundation for their pharmacokinetics.

Abbreviations

MTBD: Modified Tabusen-2 decoction; TBD: Tabusen-2 decoction; EU: *Eucommia ulmoides* Oliver;EC: *Echinops latifolius* Tausch; CT: *Carthamus tinctorius* L; PN: *Panax Notoginseng*; TCM: Traditional Chinese Medicine; TMM: Traditional Mongolian Medicine; ICGAA: Isochlorogenic acid A; 1,5-DQA: 1,5-Dicaffeoylqunic acid; GE: Genistein; APG: Apigenin; LT: Luteolin; KPF: Kaempferol; QC: Quercetin; A-7-O-G: Apigenin-7-O-glucuronide; RT: Rutin; HSYA: Hydroxysafflor yellow A; NG-R₁: Notoginsenoside R₁;G-Re: Ginsenoside Re; G-RG₁: Ginsenoside Rg₁; G-RB₁: Ginsenoside Rb₁;CA: Caffeic acid; FA: Ferulic acid; GPA: Geniposidic acid; CGA: Chlorogenic acid; PDG: Pinoresinol diglucoside; IS: Internal standard.

Declarations

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Availability of data and material:All the data involved in this experiment is true and valid.

Author contributions:All authors contributed to the study conception and design. P F X and B Q conceived and designed the study; Y Z and X D performed the experiment and prepared the manuscript; Q X F analyzed the data, R D and R B graphed the picture.

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Figures

Figure 1

The chemical structures of nineteen analytes.

Figure 2

Analysis strategy of qualitative research of MTBD.

Figure 3

The typical total ion chromatograms (TICs) of MTBD. A: TIC in negative ion mode. B: Comparison with standard in negative ion mode. C: TIC in positive ion mode. D: Comparison with standard in positive ion mode.

Figure 4

Representative chromatograms of (A) blank, (B) 19 standard sample, and (C) 19 compounds in MTBD

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