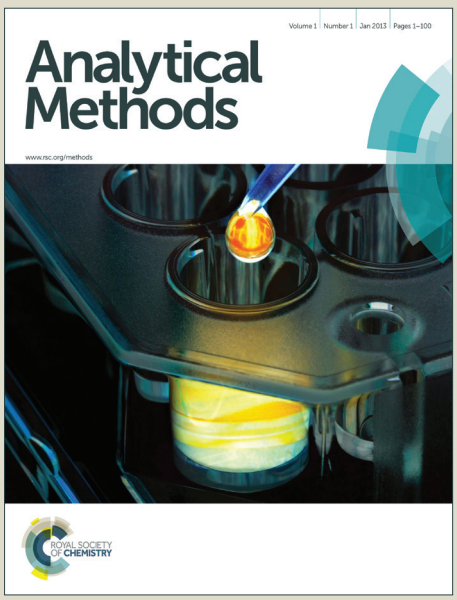
Analytical Methods

Accepted Manuscript



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UPLC-Q-TOF-MS/MS fingerprinting for rapid identification of chemical constituents of Ermiao Wan

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Abstract

In this work, a novel and efficient determination method for rapid identification of multi-class chemical constituents of Ermiao Wan has been developed. UPLC-Q-TOF-MS/MS fingerprinting coupled with data mining method to more efficiently detecting ion signals from accurate mass data. Ermiao Wan (EW) is a combination prescription of Cortex Phellodendri Chinensis and Rhizoma Atractylodis, commonly used to treat gout and hyperuricemia, described in State Pharmacopoeia of People's Republic of China. However, the chemical constituents of EW are still unclear to date. In the present study, the multi-compounds in this formula samples were extracted and simultaneously determined under the optimized conditions. Waters UPLC BEH C_{18} column was used to separate the target analytes, followed by tandem mass spectrometry (MS/MS) detection using an electro-spray ionization source in positive and negative mode. As a result, a total of 100 compounds (45 ions in positive mode, 55 ions in negative mode) were detected, among them, 93 components were tentatively identified by comparing the retention time and mass spectrometry and subsequent fragment ions. UPLC-Q-TOF-MS/MS analysis revealed the complexity of the chemical composition of this formula. It was demonstrated that the integration of the multivariate data mining method (MMA) with the UPLC-Q-TOF-MS/MS instrument could serve as a valuable strategy for rapid screening and identification of major constituents of EW.

Keywords

UPLC-ESI-Q-TOF-MS; Ermiao Wan; constituents; identification; multiple data processing approach; principal component analysis

1. Introduction

The therapeutic effects of herbal medicine are due to the contribution of multiple constituent and developing a rapid and reliable analytical method with high sensitivity for their identification is crucial to enhancing quality control [1-3]. With the tremendous expansion in the use of herbal medicines worldwide, there is an urgent need to develop high-throughput fingerprinting methods to rapid screening and monitoring phytochemical constituents [4]. Therefore, a robust and new method must be developed to facilitate the identification of the constituents. It was strongly felt necessary to develop simple, efficient, sensitive, selective and cost effective method. In spite of the enormous power, identification of herbal medicines and related products is very difficult and time-consuming process. Fortunately, multivariate data mining method (MMA) has been proved to be helpful to elucidate the effective constituents of herbal medicine, for assessing and controlling the overall quality [5-7].

Liquid chromatography-mass spectrometry (LC-MS) has become a major tool that provides a significant source of global constituent data [8]. LC-MS has enjoyed a growing popularity as the platform for herbal medicine studies due to its high throughput, soft ionization, and good coverage of metabolites, offer a robust, reliable and economical method for quantitative constituents analysis [9,10]. The advantages of coupling LC separation with MS detection include improved MS sensitivity and signal reproducibility by reducing sample complexity, enlarging the detected analytes in the widely used separation sciences [11]. Pattern-recognition programs have been developed to handle the acquired data and to search for the discriminating features from herbal medicine [12]. Experimental setup for LC/MS coupled with multiple data processing approach analysis was shown in Figure 1.

Ermiao Wan (EW) is a combination prescription of *Cortex Phellodendri Chinensis* and *Rhizoma Atractylodis*, commonly used to treat gout and hyperuricemia, described in State Pharmacopoeia of People's Republic of China [13,14]. Although modern pharmacological studies have revealed that it has been used in clinic widely, however, little study has focused on the phytochemical study of EW. Therefore, it is of very importance to develop a rapid and sensitive method to identify and characterize the systematic chemical profile of EW. This paper describes in detail about method development, identification and characterization of phytochemical constituents. Our experimental data demonstrated that the MMA in combination with UPLC/MS chemical fingerprinting is a simple, rapid, and robust methodology for pharmaceutical analysis, with promising prospects for separation and identification of herbal medicines and related products.

2. Material and methods

2.1 Chemicals and materials

Acetonitrile (HPLC grade) was purchased from Merck (Germany). Methanol (HPLC grade) was purchased from Fisher (USA). Distilled water was purchased from Watson’s Food & Beverage Co., Ltd. (Guangzhou, China). Leucine enkephalin was purchased from Sigma–Aldrich (MO, USA). Formic acid was purchased from (DIKMA,

USA). *Cortex Phellodendri Chinensis* and *Rhizoma Atractylodis* were purchased from Harbin Tongrentang Drug Store (Harbin, China), and authenticated by Prof. Xijun Wang, Department of Pharmacognosy of Heilongjiang University of Chinese Medicine. Voucher specimens were deposited at the authors' laboratory.

2.2 Preparation of EW samples for LC/MS analysis

According to the original composition and preparation method of EW recorded in 'Chinese Pharmacopeia', EW was prepared in the following procedure. *Cortex Phellodendri Chinensis* (100g) and *Rhizoma Atractylodis* (100g) were kept in 75% methanol (1000ml) reflux extraction for 90min twice. The extracted solution was filtered and concentrated using a rotary evaporator to 1/4 volume, and then made to freeze-drier powder. The powder (25mg) was accurately weighed, and added 25ml volume of methanol water (50:50) mixture (control group) for ultrasonic 15min, centrifuged at 13000rpm for 10min at 4℃. The solution was filtered through 0.22 um membranes (pore size) prior to use, and a 5 ul aliquot was injected for UPLC-MS analysis.

2.3 High-resolution accurate mass MS

A Waters Acquity™ Synapt mass spectrometer (Waters Corp., Manchester, UK) was connected to the UPLC system equipped with ESI source, and mass range was set at m/z 100–1000. For MS detection, the operating parameters were as follows: ESI^+ mode, capillary voltage of 3.0 kV, sampling cone voltage was 35.0 V, extraction cone voltage was 4.0 V, extraction cone voltage was 4.0 V; ESI^- mode, capillary voltage of 3.0 kV, sampling cone voltage was 35.0 V, extraction cone voltage was 4.0 V, extraction cone voltage was 4.0 V. The temperature was set at 110℃, desolvation gas temperature was 300℃, desolvation gas flow was 800L/h. Nitrogen was used as nebulizer and auxiliary gas. Data were collected in centroid mode and mass was corrected during acquisition using an external reference (Lock-Spray™) comprising a 200 pg/mL solution of leucine-enkephalin via a lockspray interface, generating a reference ion at 556.2771 Da ($[M+H]^+$) for positive ESI mode and, while at m/z 554.2615 Da ($[M-H]^-$) in negative ion mode. All the acquisition and analysis of data were controlled by the Ezinfo Software 2.0 (Waters Corp., Manchester, UK).

2.4 UPLC-Q-TOF-MS/MS system and conditions

Chromatographic separation for samples was performed using a Waters Acquity™ ultra performance LC systems (Waters Corporation, Milford, USA) controlled with Masslynx (V4.1). Separation was performed on a Waters ACQUITY UPLC™ BEH C_{18} (2.1mm×100mm, 1.7μm), and column temperature was maintained at 35 ◦C. The mobile phases were composed of acetonitrile with 0.1% formic acid (A) and water with 0.1% formic acid (B) using a multi-step linear gradient elution. The chromatographic conditions were as follows: 1-16 % A at 0-1.5 min, at

16-20% A 1.5-5 min, 20-25% A at 5-7 min, 25-35 % A at 7-10.0 min, and 35-99% A at 10.0-20.0 min with the flow rate kept at 0.3 mL/min. The sample volume injected was set at 5 uL.

2.5 Data mining methods

The centroid LC/MS data files were further processed with multiple-data processing approach using the Waters EZinfo 2.0 software (Waters Corp., Milford, MA, USA). For further confirmed the structure and the source of the chemical constituents of a Chinese herbal formula EW, all data matrices were introduced to MetaboLynx™ software. The ions which were present in the EW group and absent in the control group were extracted with the help of the corresponding loading plot, and further these ions were identified with a combination of elemental composition tool and MS/MS fragment mass spectra.

3. Results and discussion

3.1. Optimization of LC and MS conditions

Generally, LC-MS analysis gives more complete information about the composition of herbal medicines and therefore, complete identification of the ingredients is achieved when appropriate sample preparation and chromatographic separation techniques are used. In order to obtain chromatograms with good separation and strong total ion current, several mobile phase systems including methanol–water, acetonitrile–water, methanol with 0.1% formic acid and acetonitrile with 0.1% formic acid were selected to optimize the chromatographic conditions. As a result, acetonitrile with 0.1% formic acid on the optimized gradient mode showed a good separation and abundant signal response both in positive and negative ion scan mode. The MS conditions, capillary voltage, sampling cone voltage, extraction cone voltage, extraction cone voltage, desolvation gas temperature, and desolvation gas flow were optimized in order to achieve efficient separation and good responses to all chemical components in EW. Series of experiments were conducted to optimize the LC chromatographic and MS conditions as described in Section 2.3 and 4. And both positive and negative ion modes were employed to identify the corresponding signals.

3.2 Multivariate data mining method for in vivo identification of EW

MMA provides an expert means of maximizing information recovery from complex MS data, as well as enables fast and easy data handling was explored. Experimental setup for UPLC-ESI-Q-TOF-MS coupled with multiple data processing approach analysis was shown in Figure 1. Principal component analysis (PCA) is the most widely used exploratory techniques in multivariate analysis. It converts the multidimensional and original data space into a low dimensional model plane. In order to gain the details of differences, the UPLC-MS datasets of the two groups were subjected to the PCA. In our work, PCA method was employed to phenotype the differences between the EW and control group. PCA of LC-MS spectra of EW (2M) group vs control group in positive and negative mode was shown

in Figure 2A and 2B. The 3-D PCA of UPLC-ESI-Q-TOF-MS spectra in positive and negative mode were shown in Figure 2C and 2D. VIP-plot can clearly display leading contributing markers that differentiate the two sample groups. Thus, the interest ions which were present only in the EW group and absent in the control group were extracted easily by MMA. As showed in Figure 2E and 2F, the points in the red frame were at higher level in EW group. As demonstrated above, 100 interested ions (45 ions in positive mode, 55 ions in negative mode) were extracted, among them, 93 components of EW were identified or tentatively characterized based on their retention times, exact mass measurement for each molecular ion and subsequent fragment ions, and their information was shown in Table 1 and 2, respectively. The mass error for molecular ions of all identified compounds was within ±6 ppm. 93 major constituents (Figure 3A and B) including alkaloids, phenylpropanoids, flavonoids, isoflavonoids, organic acids, amino acids, and saponins were identified or tentatively characterized according to their retention times and MS data obtained on-line (Table 1).

3.3 UPLC- MS/MS characterization of chemical constituents from EW

Global profiling in both ESI modes were analyzed using the optimal UPLC-ESI-Q-TOF-MS. In the full scan mass spectra, most of the authentic compounds exhibited $[M+H]^+$ ions in positive mode or $[M-H]^-$ in negative mode. In the MMA method file, both positive and negative adducts can be chosen to aid in identification. In order to understand better the MS fragmentation pattern of the constituents in EW, the TOF-MS/MS spectrum of peak 10 showed the $[M+H]^+$ ion at m/z 315.1504. Taking an example, the precise molecular weight is 315.1504, and the main fragment ions that were analyzed via the MS/MS screening were observed at m/z 298 $[M+H-C_2H_6N]^+$, 249 $[M+H-C_3H_{10}NO_2]^+$, 192 $[M+H-C_9H_{10}O_2]^+$, 177 $[M+H-C_{10}H_{14}NO]^+$, 145 $[M+H-C_{11}H_{19}NO_2]^+$ in the positive ion spectrum. Its molecular formula was speculated to be $C_{12}H_{19}N_7O_5$ based on the analysis of its elemental composition and fractional isotope abundance, ion 10 was inferred as tetrahydrojatrorrhizine. The chemical structure and mass fragment information of tetrahydrojatrorrhizine in positive mode are illustrated in Figure 4.

The new method using high-resolution LC-MS technology significantly improves the possibility to get the rich information among different chromatographic profiles [15]. With the help of this tool, the qualitative and quantitative information of chemical components within complicated constituents will be mined out effectively [16]. The present study is aimed at developing an approach for elucidating the phytochemical constituents of the EW, conducted via UPLC-ESI-Q-TOF-MS coupled with MMA. EW has been used in clinic widely, but the bioactive ingredients of EW are not well understood. To resolve the drawbacks, a new methodology for searching the constituents of herbal medicine should be established. We utilized UPLC-ESI-Q-TOF-MS combined with MMA to rapidly discover and identify the constituents of the EW. This is the first report on systematic analysis of chemical constituents of EW. MMA analysis methods together with LC/MS fingerprints are considered potential useful tools to select candidate drugs from herbal medicines. MMA provides a much faster analytical speed and reduces measurement time is one of

the attractive features, indicating that this technique is capable of high-throughput monitoring of the holistic chemical profile of herbal medicines and related products.

5. Conclusion

In this paper, we developed a reliable method to discover, screen and analyze the multiple components from EW. More importantly, as demonstrated in this study, this work proved to be a very valuable tool to address an important issue that EW is facing. Using MMA approach and UPLC/MS techniques, of note, a total of 100 interested ions were extracted, 93 components of EW were characterized tentatively. As a useful tool for rapidly monitoring the holistic chemical profile at the molecular level, MMA methodology presented here allow a rapid and efficient screening of 93 botanicals for quality control of EW. This identification and structural elucidation of the chemical compounds provided essential data for further pharmacological studies of EW. This will provide a type of validated rapid and higher throughput methodology for the identification of constituents for herb medicine. We expected that approach would be useful for the screening and characterization of compounds in other famous herb medicines.

Acknowledgments

This work was supported by grants from the Key Program of Natural Science Foundation of State (Grant No. 90709019, 81173500, 81373930, 81302905, 81102556, 81202639), National Key Technology Research and Development Program of the Ministry of Science and Technology of China (Grant No. 2011BAI03B03, 2011BAI03B06, 2011BAI03B08), National Key Subject of Drug Innovation (Grant No. 2009ZX09502-005).

Competing financial interests

The authors declare no competing financial interests.

Abbreviations

EW, Ermiao Wan; MMA, multivariate data mining methods; UPLC-ESI-Q-TOF-MS, ultra performance liquid chromatography coupled with electrospray ionization/quadrupole-time-of-flight mass spectrometry; LC-MS, Liquid chromatography-mass spectrometry; PCA, principal component analysis

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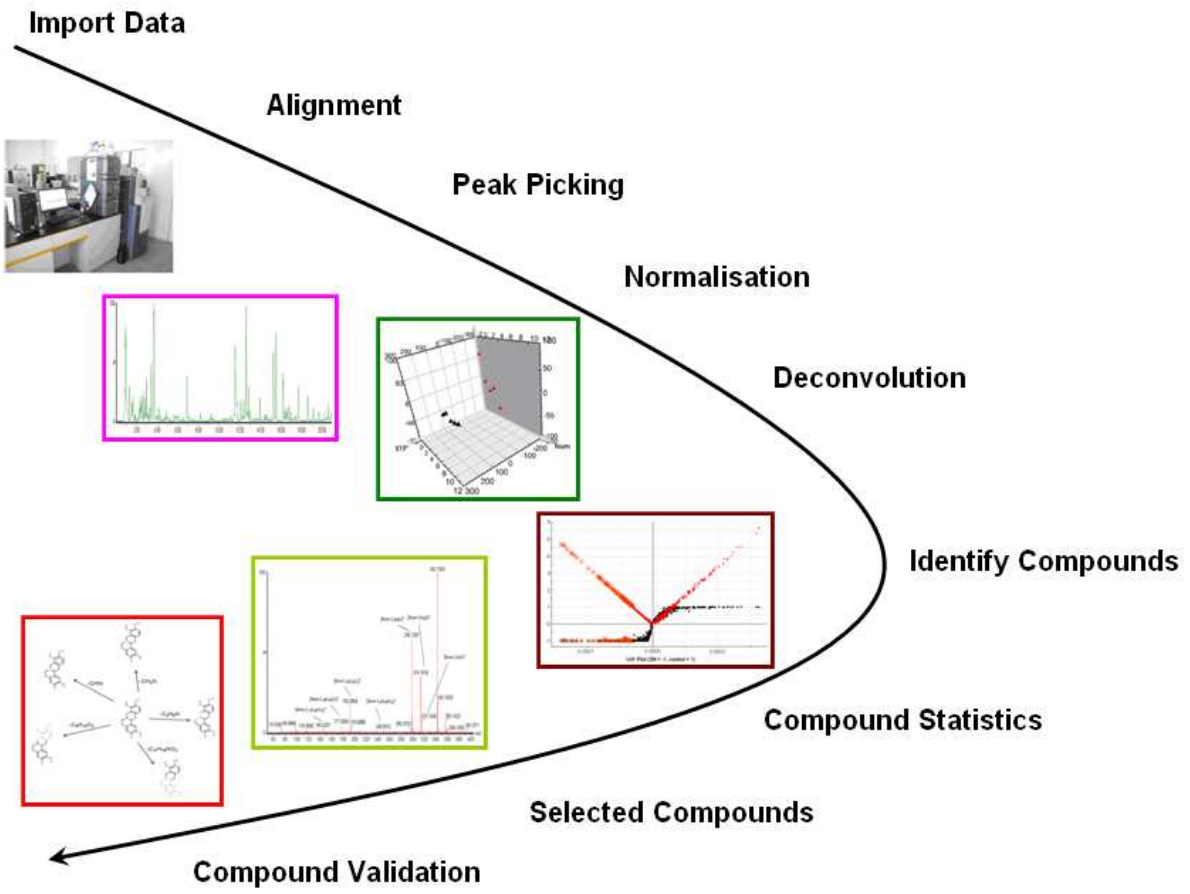


Fig. 1. Experimental setup for UPLC-ESI-Q-TOF-MS coupled with data mining approach analysis.

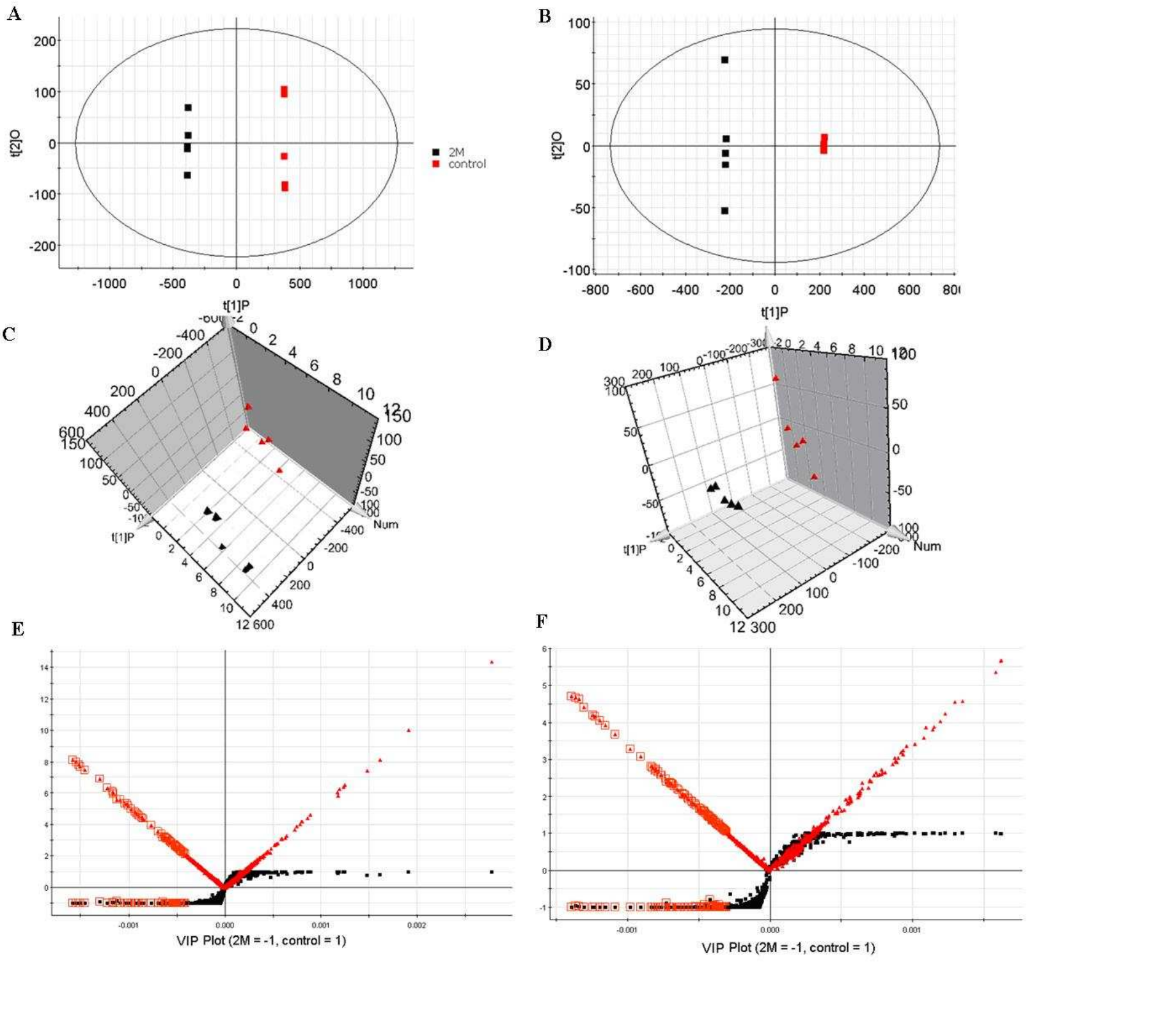


Figure 2. Multiple data processing approach for accurate mass UPLC/MS data of Ermiao Wan (2M) in positive mode. PCA of UPLC-MS spectra of EW group vs control group in positive mode (A) and negative mode (B); 3-D PCA of UPLC-MS spectra in positive mode (C) and negative mode (D); VIP-splot for accurate mass UPLC/MS data in positive mode (E) and negative mode (F) data.

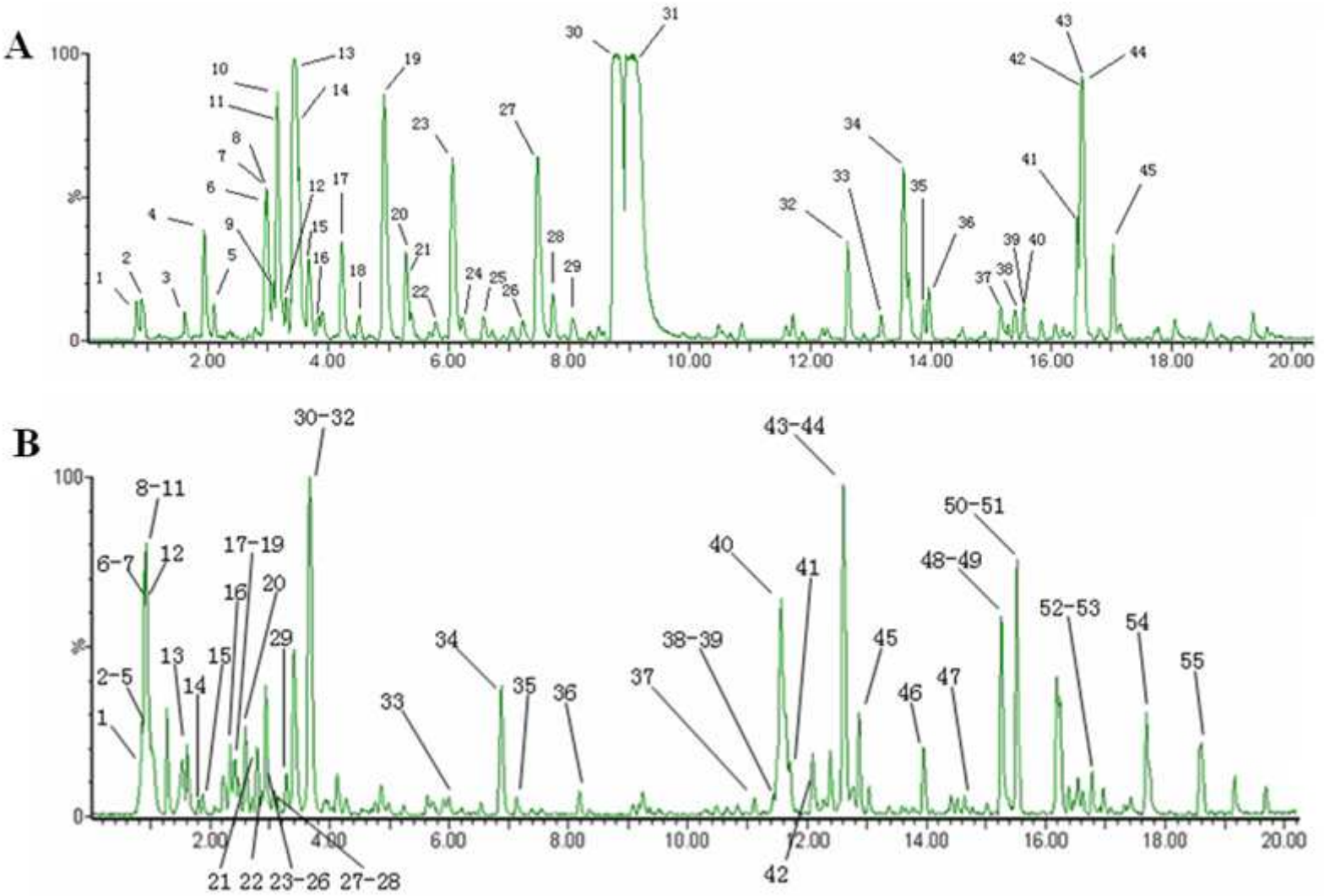
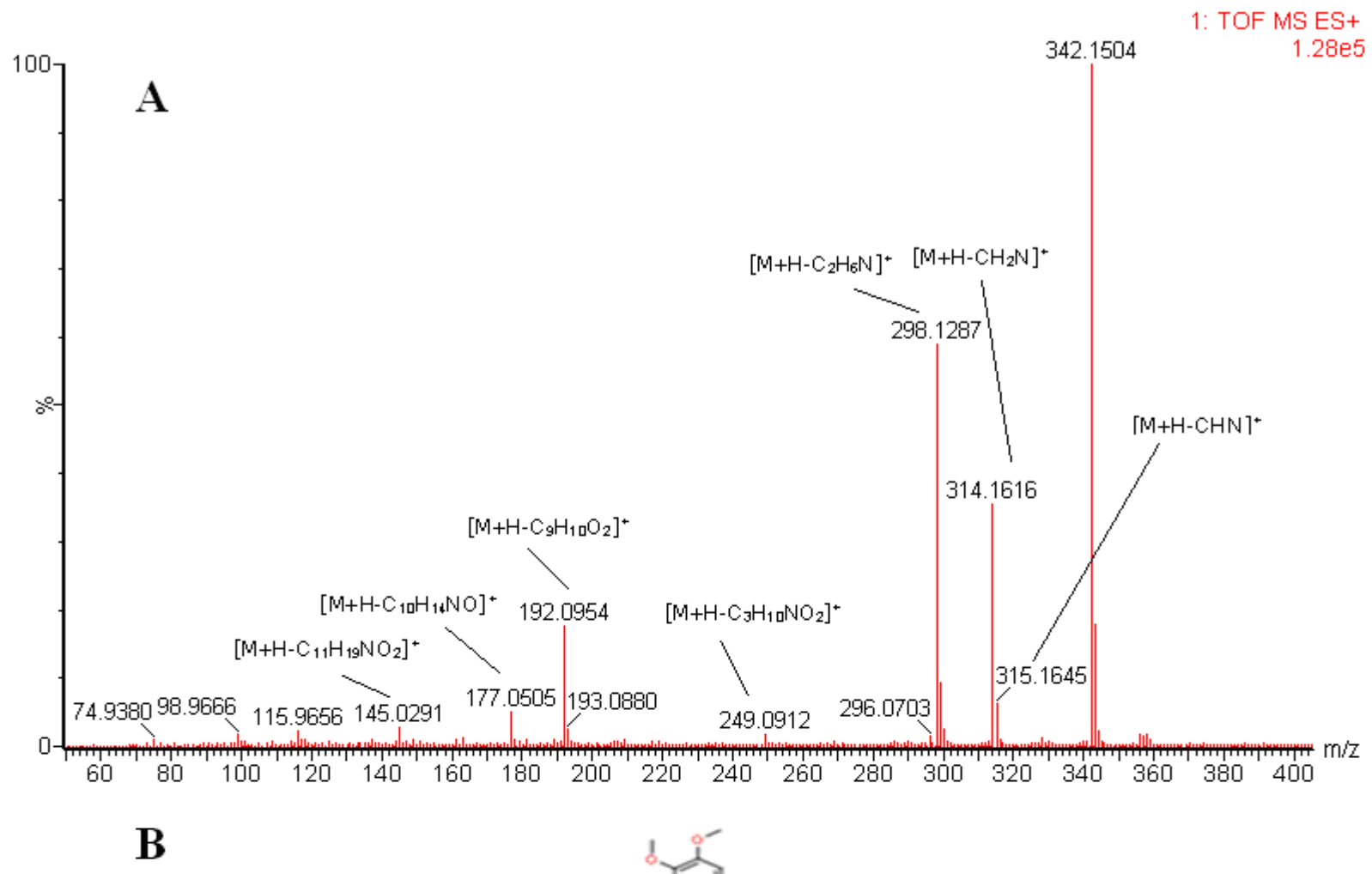


Figure 3. UPLC-MS BPI chromatograms of EW in positive mode (A) and in negative mode (B).

The each peak number was consistent with Table 1 and 2, respectively.



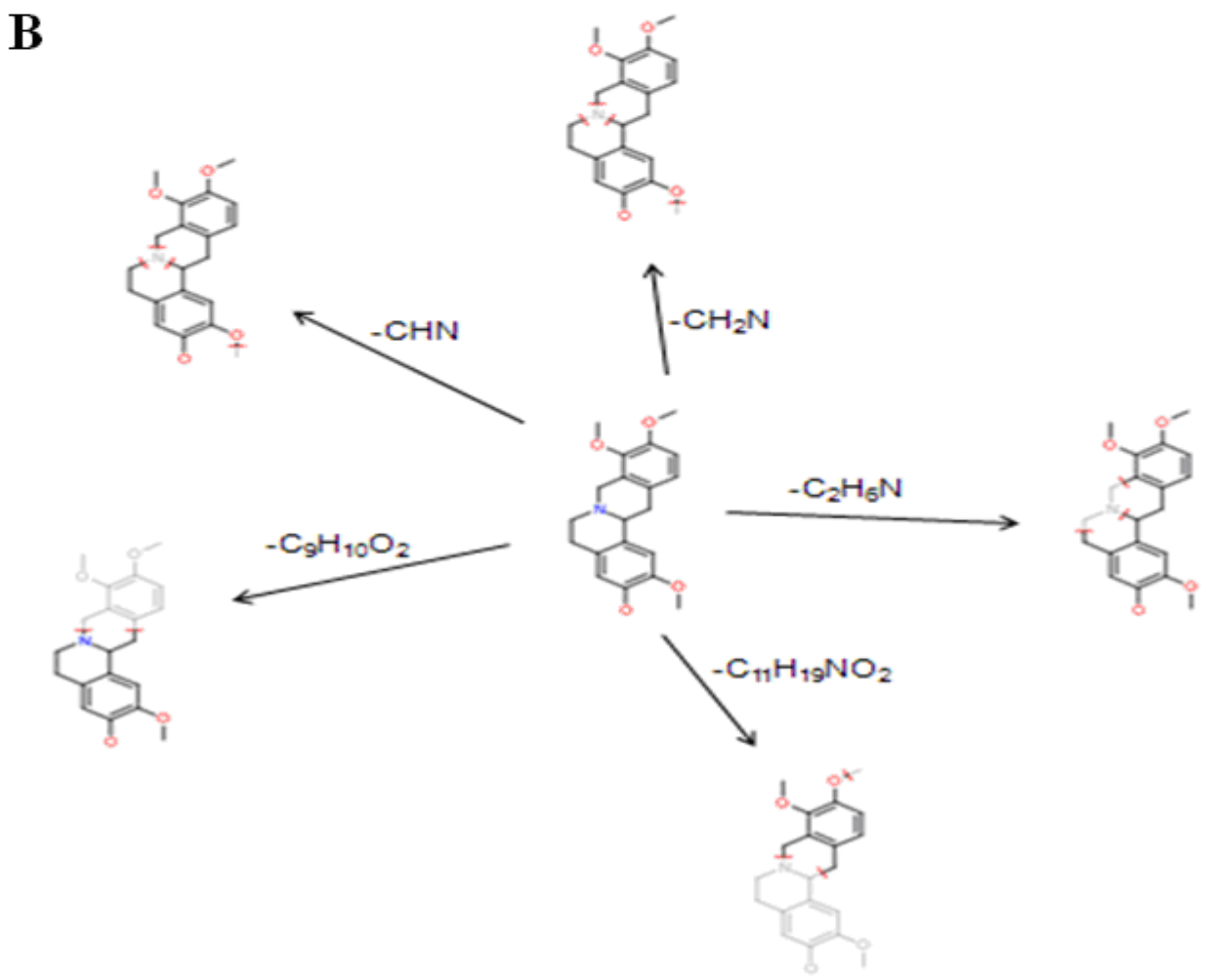


Figure 4. Chemical structure and mass fragment information of tetrahydrojatrorrhizine in positive mode.

Table 1. Characterization of chemical constituents of EW by UPLC-ESI-Q-TOF-MS in positive ionization mode.

No	RT (min)	m/z $[M+H]^+$	Error (ppm)	Molecular formula	MS/MS fragment ion (m/z)	Identification	Source
1.	0.81	337.1652	5.3	C19H20N4O2	308.1273 $[M+H-C2H5]^+$ 295.1195 $[M+H-C3H6]^+$ 277.1089 $[M+H-C3H8O]^+$ 200.0334 $[M+H-C10H17]^+$ 133.0653 $[M+H -C10H12N4O]^+$ 106.0419 $[M+H -C12H15N4O]^+$ 74.0156 $[M+H -C13H19N4O2]^+$	2-(2-Amino-5-morpholin-4-yl-phenyl)-4-methyl-2 H-phthalazin-1-one	RA
2.	0.9	325.1054	2.5	C14H16N2O7	279.0159 $[M+H-C_3H_{10}]^+$ 277.0097 $[M+H-C_3H_{12}]^+$ 247.0719 $[M+H-C_2H_6O_3]^+$ 225.0511 $[M+H-C_5H_8O_2]^+$ 217.0249 $[M+H-C_4H_{12}O_3]^+$	Dimethyl 2-(morpholin-4-yl)-5-nitroterephthalate	RA
3.	1.61	325.1072	-1.8	C20H12N4O	271.0871 $[M+H-C_2H_2N_2]^+$ 154.0387 $[M+H-C_9H_7N_4]^+$ 130.0531 $[M+H -C_{12}H_7N_2O]^+$	11-Methyl-7H,10H-benzo[de]imidazo[4',5':5,6]ben zimidazo[2,1-a]isoquinolin-7-one	RA
4.	1.94	180.128	-6.5	C11H18NO	150.0919 $[M+H-C_2H_7]^+$ 133.0528 $[M+H-C_3H_{12}]^+$ 122.0732 $[M+H-C_3H_9N]^+$ 119.0497 $[M+H-C_3H_{12}N]^+$ 70.0657 $[M+H-C_7H_{11}O]^+$	Candicine	CRC
5.	2.1	192.0958	-4.3	C10H9NO3	163.0390 $[M+H-CH_3N]$+ 160.0399 $[M+H-CH_4O]$+ 151.0395 $[M+H-C_2H_3N]$+ 60.0443 $[M+H-C_8H_4O_2]$+	Noroxyhydrastinine	CRC
6.	2.93	177.05	-4.6	C10H8O3	163.0395 $[M+H-CH_2]^+$ 151.0395 $[M+H-C_2H_2]^+$ 147.0446 $[M+H-CH_2O]^+$ 145.0290 $[M+H-CH_4O]^+$ 121.0653 $[M+H-C_2O_2]^+$ 118.0419 $[M+H-C_2H_3O_2]^+$ 109.0290 $[M+H-C_4H_4O]^+$ 89.0391 $[M+H-C_3H_4O_3]^+$	Hymecromone	CRC
7.	2.95	314.1611	5.1	C19H23NO3	281.1052 $[M+H-C_2H_9]$+ 189.0790 $[M+H-C_8H_{13}O]$+ 161.0477 $[M+H-C_{10}H_{17}O]$+ 150.0681 $[M+H-C_{10}H_{14}NO]$+ 146.0368 $[M+H-C_{10}H_{18}NO]$+ 121.0290 $[M+H-C_{12}H_{19}NO]$+ 107.0497 $[M+H-C_{12}H_{17}NO_2]$+	Evoeuropine	CRC
8.	2.98	298.1294	3.7	C14H19NO6	250.1093 $[M+H-CH_4O_2]$+ 223.0481 $[M+H-C_4H_{11}O]$+ 209.0688 $[M+H-C_4H_9O_2]$+ 177.0426 $[M+H-C_5H_{13}O_3]$+ 161.0477 $[M+H-C_5H_{13}O_4]$+ 89.0265 $[M+H-C_8H_{17}O_6]$+	3,4,5-pyridinetricarboxylic acid, 1,4-dihydro-2,6-dimethyl-, 3,5-diethyl ester	RA
9.	3.09	448.1869	0.7	C30H25NO3	357.1365 $[M+H-C_7H_7]$+ 343.1572 $[M+H-C_7H_5O]$+ 147.0446 $[M+H-C_{21}H_{19}NO]$+ 131.0497 $[M+H-C_{21}H_{19}NO_2]$+ 130.0055 $[M+H-C_{22}H_{24}NO]$+	8-[(dibenzylamino)methyl]-7-hydroxy-3-phenyl-4 H-chromen-4-one	CRC
10.	3.15	342.1504	2.9	C12H19N7O5	342.1504 $[M+H]$+ 298.1287 $[M+H-C_2H_6N]$+ 249.0916 $[M+H-C_3H_{10}NO_2]$+ 192.1025 $[M+H-C_9H_{10}O_2]$+ 177.0552 $[M+H-C_{10}H_{14}NO]$+ 145.0290 $[M+H-C_{11}H_{19}NO_2]$+	Tetrahydrojatrorrhizine	CRC
11.	3.16	192.0955	-4.1	C11H13NO2	175.0633 $[M+H-CH_5]$+ 151.0759 $[M+H-C_2H_3N]$+ 122.0368 $[M+H-C_4H_8N]$+ 109.0290 $[M+H-C_5H_9N]$+ 87.9949 $[M+H-C_5H_{14}NO]$+	Dehydroheliamine	CRC
12.	3.38	344.1774	-3.4	C15H25N3O6	343.1704 $[M+H-H]$+ 222.1004 $[M+H-C_4H_{12}NO_3]$+ 209.0562 $[M+H-C_6H_{17}NO_2]$+ 192.0773 $[M+H-C_6H_{16}O_4]$+ 163.0144 $[M+H-C_8H_{23}NO_3]$+ 95.0007 $[M+H-C_{11}H_{25}N_2O_4]$+	Methyl N-{[(2-methyl-2-propanyl)oxy]carbonyl}glycylpro lylglycinate	CRC
13.	3.46	342.1437	-3.6	C15H23N3O6	325.1400 $[M+H-H_2N]$+ 313.1274 $[M+H-C_2H_5]$+ 311.1481 $[M+H-CH_3O]$+ 298.1039 $[M+H-C_3H_8]$+ 293.1012 $[M+H-C_2H_9O]$+ 290.1505 $[M+H-H_4O_3]$+ 287.1481 $[M+H-C_3H_3O]$+	2-{3,3-Bis[(2-hydroxyethyl)amino]-2-nitroprop-2-en-1-ylidene}-5,5-dimethylcyclohexane-1,3-dione	CRC
14.	3.5	274.1277	-3.3	C11H19N3O5	257.1376 $[M+H-HO]$+ 217.1063 $[M+H-C_3H_5O]$+ 189.0875 $[M+H-C_4H_7NO]$+ 179.0695 $[M+H-C_3H_{11}O_3]$+ 127.0395 $[M+H-C_5H_{13}N_3O_2]$+ 95.0735 $[M+H-C_5H_{11}N_2O_5]$+	5-[(1R,2S)-1,2-Dihydroxy-2-methyl-3-(4-morpholi nyl)propyl]-2,4-imidazolidinedione	CRC
15.	3.67	177.0453	4.2	C6H4N6O	149.0338 $[M+H-CH_2N]$ + 149.0212 $[M+H-C_2H_3]$ + 133.0514 $[M+H-N_2O]$ + 145.0263 $[M+H-CH_3O]$+ 117.0327 $[M+H-H_2N_3O]$+	5,7-Diamino[1,2,5]oxadiazolo[3,4-b]pyridine-6-ca rbonitrile	CRC
16.	3.83	312.1172	5.4	C20H13N3O	297.0902 $[M+H-CH_3]$+ 290.0354 $[M+H-CH_9]$+ 290.0480 $[M+H-H_7N]$+	6,7,12,13-Tetrahydro-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazol-5-one	CRC
17.	4.22	314.1634	5.7	C15H23NO6	219.0532 $[M+H-C_4H_{15}O_2]$+ 177.0552 $[M+H-C_5H_{15}NO_3]$+ 162.0681 $[M+H-C_5H_{14}NO_4]$+ 149.0239 $[M+H-C_7H_{19}NO_3]$+	Methyl 4-{3-[bis(2-hydroxyethyl)amino]- 2-hydr oxypropoxy}benzoate	RA

					147.0082 [M+H-$C_7H_{21}NO_3$]+ 116.9926 [M+H-$C_8H_{23}NO_4$]+		
18.	4.52	260.1238	-3.2	C11H13N7O	243.1232 [M+H-HO]+ 188.0698 [M+H-$C_2H_6N_3$]+ 177.1028 [M+H-CHN_5]+ 163.0871 [M+H-$C_2H_3N_5$]+ 161.0589 [M+H-$C_3H_7N_4$]+ 120.0813 [M+H-$C_3H_4N_6O$]+ 105.0578 [M+H-$C_4H_7N_6O$]+	1,3-Dimethyl-5-[(2H-tetrazol-5-ylamino)methyl]-1,3-dihydro-2H-benzimidazol-2-one	CRC
19.	4.93	356.1688	3.8	C13H21N7O5	261.0736 [M+H-$C_3H_{13}NO_2$]+ 260.1022 [M+H-$C_2H_{10}NO_3$]+ 242.0916 [M+H-$C_2H_{12}NO_4$]+ 177.0525 [M+H-$C_7H_{17}NO_4$]+	2-Amino-N-(3-amino-2-hydroxypropyl)adenosine	CRC
20.	5.29	304.1436	4.3	C19H17N3O	286.0889 [M+H-CH_6]+ 260.0773 [M+H-C_3H_8]+	7,8-dihydroxyrutaecarpine	CRC
21.	5.37	328.1846	-2.4	C16H21N7O	326.1022 [M+H-H]+ 313.1777 [M+H-HN]+ 313.1651 [M+H-CH_3]+	[4,6-Bis(dimethylamino)-1,3,5-triazin-2-yl](2-phenoxyethyl)cyanamide	CRC
22.	5.78	324.1187	-3.8	C19H17NO4	296.1287 [M+H-CO]+ 283.1208 [M+H-C_2HO]+ 202.0868 [M+H-$C_7H_6O_2$]+ 147.0446 [M+H-$C_{10}H_{11}NO_2$]+ 145.0290 [M+H-$C_{10}H_{13}NO_2$]+ 89.0027 [M+H-$C_{13}H_{17}NO_3$]+ 81.0704 [M+H-$C_{13}H_9NO_4$]+	Tetrahydrocoptisine	CRC
23.	6.04	356.1705	-4.8	C21H25NO4	324.1236 [M+H-C_2H_8]+ 147.0446 [M+H-$C_{12}H_{19}NO_2$]+ 130.0055 [M+H-$C_{13}H_{24}NO_2$]+	Tetrahydropalmatine	CRC
24.	6.22	368.144	-2.6	C17H17N7O3	354.1339 [M+H-CH_2]+ 352.1176 [M+H-CH_4]+ 337.0847 [M+H-C_2H_7]+	Ethyl{5-[ethyl(phenyl)amino][1,2,5]oxadiazolo[3,4-e][1,2,4]triazolo[4,3-a]pyrazin-8-yl}acetate	CRC
25.	6.58	396.7945	-1.8	C22H28N4O3	356.1848 [M+H-C_3H_5]+ 249.1239 [M+H-$C_9H_{12}N_2$]+ 161.0477 [M+H-$C_{13}H_{22}N_3O$]+ 100.0762 [M+H-$C_{17}H_{19}N_3O_2$]+ 69.9929 [M+H-$C_{20}H_{29}N_3O$]+	Dictamnine	CRC
26.	7.23	338.1337	-3.6	C20H19NO4	267.1259 [M+H-$C_3H_3O_2$]+ 209.0966 [M+H-$C_5H_7NO_3$]+ 105.0704 [M+H-$C_{12}H_{11}NO_4$]+ 100.0762 [M+H-$C_{15}H_{10}O_3$]+ 93.0215 [M+H-$C_{15}H_{17}O_3$]+ 79.0548 [M+H-$C_{14}H_{13}NO_4$]+ 69.9929 [M+H-$C_{18}H_{20}O_2$]+	-	CRC
27.	7.47	338.1204	-3.1	C20H20NO4	209.0477 [M+H-$C_7H_{14}O_2$]+ 167.0007 [M+H-$C_{10}H_{20}O_2$]+ 88.0187 [M+H-$C_{14}H_{19}O_4$]+	Jatrorrhizine	CRC
28.	7.73	370.1936	-3.2	C17H27N3O6	343.1743 [M+H-C_2H_3]+ 328.1872 [M+H-C_2H_2O]+ 261.1113 [M+H-$C_4H_{13}O_3$]+ 177.0426 [M+H-$C_8H_{21}N_2O_3$]+ 114.0919 [M+H-$C_{11}H_{16}N_2O_5$]+ 81.0578 [M+H-$C_{12}H_{21}N_2O_6$]+	2-{2-[4-(4,5-Dimethoxy-2-nitrobenzyl)-1-piperazinyl]ethoxy}ethanol	CRC
29.	8.06	218.2068	-2.6	C12H27NO2	200.1776 [M+H-H_3N]+ 170.0970 [M+H-$H_{15}O_2$]+ 149.0265 [M+H-$CH_{24}O_2$]+ 137.0477 [M+H-C_5H_{20}]+ 100.0762 [M+H-$C_7H_{17}O$]+	Ammonium laurate	RA
30.	8.8	352.1227	-4.8	C21H22NO4	338.1392 [M+H-CH_3]+ 324.1236 [M+H-C_2H_5]+ 169.0528 [M+H-$C_{10}H_{16}O_3$]+ 161.0603 [M+H-$C_{11}H_{14}NO_2$]+ 147.0446 [M+H-$C_{12}H_{16}NO_2$]+ 121.0290 [M+H-$C_{14}H_{18}NO_2$]+ 93.0340 [M+H-$C_{15}H_{18}NO_3$]+	Palmatine	CRC
31.	8.94	336.0902	0.9	C20H18NO4	145.0290 [M+H- $C_{11}H_{14}NO_2$]+ 133.0290 [M+H-$C_{12}H_{14}NO_2$]+ 88.0313 [M+H-$C_{13}H_{15}NO_4$]+	Berberine	CRC
32.	12.62	471.1917	-2.1	C26H30O8	435.1808 [M+H-H_4O_2]+ 425.1964 [M+H-CH_2O_2]+ 347.1859 [M+H-$C_6H_4O_3$]+ 205.0501 [M+H-$C_{15}H_{22}O_4$]+	Obaculactone	CRC
33.	13.17	352.1126	-2.7	C20H17NO5	309.0763 [M+H- C_2H_5N]+ 217.0739 [M+H- $C_8H_7O_2$]+ 163.0395 [M+H-$C_{11}H_{11}NO_2$]+ 147.0446 [M+H-$C_{11}H_{11}NO_3$]+	Oxyberberine	CRC
34.	13.55	274.2622	0.7	C13H31N5O	230.2232 [M+H-CH_4N_2]+ 172.1576 [M+H-$C_4H_{12}N_3$]+ 158.1293 [M+H-$C_6H_{16}N_2$]+ 130.1106 [M+H-$C_7H_{18}N_3$]+	N-[3-({4-[(3-aminopropyl)amino]butyl}amino)propyl]-β-alaninamide	RA
35.	13.88	231.1314	-2.1	C15H18O2	190.0994 [M+H-C_3H_5]+ 187.1123 [M+H-C_2H_4O]+ 175.0759 [M+H-C_4H_8]+ 161.0966 [M+H-C_4H_6O]+ 119.0861 [M+H-$C_6H_8O_2$]+ 105.0704 [M+H-$C_7H_{10}O_2$]+ 69.0704 [M+H-$C_{10}H_{10}O_2$]+	Atractylenolide-1	RA
36.	13.97	455.1982	-5.3	C26H30O7	439.1757 [M+H-CH_4]+ 419.1859 [M+H-H_4O_2]+ 395.1859 [M+H-$C_2H_4O_2$]+ 359.1859 [M+H-$C_5H_4O_2$]+ 333.2066 [M+H-$C_6H_2O_3$]+ 249.1491 [M+H-$C_{11}H_{10}O_4$]+ 163.0759 [M+H-$C_{16}H_{20}O_5$]+	Obacunone	CRC
37.	15.16	302.2996	5.2	C15H35N5O	158.1217 [M+H-$C_8H_{20}N_2$]+ 130.1106 [M+H-$C_9H_{22}N_3$]+ 130.1232 [M+H-$C_8H_{20}N_4$]+ 85.0402 [M+H-$C_{12}H_{31}N_3$]+	-	RA

					85.0528 [M+H-$C_{11}H_{29}N_4$]+ 81.0340 [M+H-$C_{10}H_{31}N_5$]+ 57.0578 [M+H-$C_{12}H_{29}N_4O$]+		
38.	15.36	233.1482	4.7	C15H20O2	232.1525 [M+H-H]+ 191.1038 [M+H-C_3H_6]+ 178.0970 [M+H-C_4H_7]+ 119.0867 [M+H-$C_6H_{10}O_2$]+ 79.0526 [M+H-$C_9H_{14}O_2$]+	Costunolide	RA
39.	15.55	331.17	3.3	C18H34O5	317.2328 [M+H-CH_2]+ 293.2117 [M+H-H_6O_2]+ 278.1882 [M+H-CH_9O_2]+ 229.1076 [M+H-C_7H_{18}]+ 182.0943 [M+H-$C_8H_{21}O_2$]+ 157.0865 [M+H-$C_{10}H_{22}O_2$]+ 109.0290 [M+H-$C_{12}H_{30}O_3$]+ 81.0340 [M+H-$C_{13}H_{30}O_4$]+	Sanleng acid	CRC
40.	15.55	717.3044	0.4	C39H44N2O11	497.2135 [M+H-$C_{16}H_{12}O$]+ 453.1999 [M+H-$C_{17}H_{14}NO_2$]+ 405.1186 [M+H-$C_{19}H_{24}N_2O_2$]+ 398.1366 [M+H-$C_{17}H_{23}N_2O_4$]+ 382.1740 [M+H-$C_{21}H_{19}O_4$]+ 264.0746 [M+H-$C_{27}H_{33}O_6$]+ 241.0824 [M+H-$C_{29}H_{32}O_6$]+ 200.0837 [M+H-$C_{26}H_{33}N_2O_9$]+ 179.0920 [M+H-$C_{32}H_{30}N_2O_6$]+ 145.0501 [M+H-$C_{33}H_{36}N_2O_7$]+ 99.0446 [M+H-$C_{34}H_{38}N_2O_9$]+	2',3'-O-Isopropylidene-5'-O-(3,4,6-tri-O-benzyl-α-D-glucopyranosyl)uridine	RA
41.	16.44	231.1267	-1.7	C10H18N2O4	201.0875 [M+H-C_2H_6]+ 185.1290 [M+H-CH_2O_2]+ 170.0817 [M+H-C_2H_7NO]+ 167.0821 [M+H-$C_2H_8O_2$]+ 105.0664 [M+H-$C_7H_{10}O_2$]+ 57.0704 [M+H-$C_6H_{10}N_2O_4$]+	4-Boc-piperazine-2-carboxylic acid	RA
42.	16.47	165.0612	5.1	C6H12O5	149.0429 [M+H-CH_4]+ 131.0359 [M+H-CH_6O]+ 121.0459 [M+H - C_2H_4O]+ 81.0044 [M+H- $C_2H_{12}O_3$]+	Rhamnose	RA
43.	16.51	325.1165	0.3	C12H18N6O5	263.1256 [M+H-CH_4O_3]+ 211.0943 [M+H -$C_5H_8O_3$]+ 166.0603 [M+H -$C_7H_{13}O_4$]+	-	RA
44.	16.51	341.1042	5.3	C20H12N4O2	180.0813 [M+H -$C_7H_3N_3O_2$]+ 149.0589 [M+H -$C_{13}H_6NO$]+ 100.0187 [M+H -$C_{13}H_{11}N_3O_2$]+	4-[2-(9H-Fluoren-9-ylidene)hydrazino]-3-nitrobenzonitrile	RA
45.	17.02	219.1651	0	C10H22N2O3	205.1650 [M+H -CH_2]+ 202.1364 [M+H -CH_5]+ 175.1005 [M+H -C_3H_8]+ 161.1178 [M+H -$C_2H_6N_2$]+ 147.0770 [M+H -C_5H_{12}]+ 81.0578 [M+H -$C_5H_{16}NO_3$]+	4-hydroxy-5-(pentyloxy)pentanehydrazide	CRC

Note: CRC, Cortex Phellodendri Chinensis; RA, Rhizoma Atractylodis.

Table 2. Characterization of chemical constituents of EW by UPLC-ESI-Q-TOF-MS in negative ionization mode.

No	RT (min)	m/z $[M+H]^+$	Error (ppm)	Molecular formula	MS/MS fragment ion (m/z)	Identification	Source
1.	0.81	131.0449	-3.7	C4H8N2O3	118.0504 $[M-H-N]^-$ 115.0269 $[M-H-H_2N]^-$ 104.0348 $[M-H-CHN]^-$ 88.0399 $[M-H-CHNO]^-$ 70.0293 $[M-H-CH_3NO_2]^-$ 59.0133 $[M-H-C_2H4N_2O]^-$	-	RA
2.	0.83	293.0978	0.0	C10H18N2O8	292.0907 $[M-H-H_2]$- 290.0750 $[M-H-H_4]$- 281.0985 $[M-H-CH]$- 281.0046 $[M-H-H_{13}]$- 280.1032 [M-H-N]-	N-L-β-Aspartyl-β-D-glucopyranosylamine	RA
3.	0.84	317.0525	-0.3	C13H10N4O6	280.0444 $[M-H-C_3H]^-$ 275.0542 $[M-H-CNO]^-$ 257.0311 $[M-H-C_2H_4O_2]^-$ 243.0154 $[M-H-C_3H_6O_2]^-$ 239.0569 $[M-H-CH_2O_4]^-$ 227.0178 $[M-H-C_6H_4N]^-$ 225.0022 $[M-H-C_6H_6N]^-$ 223.0229 $[M-H-C_5H_4NO]^-$	3,5-Dinitro-4-[(3-pyridinylmethyl)amino]benzoic acid	RA
4.	0.85	195.0475	-4.6	C3H4N10O	181.0732 $[M-H-O]^-$ 113.0338 $[M-H-CN_5]^-$ 85.0388 $[M-H-C_2N_5O]^-$	1,3-Di-2H-tetrazol-5-ylurea	RA
5.	0.85	207.0499	-2.4	C14H8O2	207.0446 [M-H-H]- 202.0055 $[M-H-H_6]$-	9,10-phenanthraquinone	RA
6.	0.88	439.08	-0.5	C22H12N6O5	422.0764 $[M-H-HO]^-$ 391.0791 $[M-H-C_4]^-$ 379.0917 $[M-H-C_4N]^-$ 319.0467 $[M-H-C_6H_4N_2O]^-$ 277.0362 $[M-H-C_8H_6N_2O_2]^-$ 243.0154 $[M-H-C_{12}H_8N_2O]^-$	N'-[(11Z)-11H-Indeno[1,2-b]quinoxalin-11-ylidene]-3,5-dinitrobenzohydrazide	RA
7.	0.89	191.0487	-3.5	C7H12O6	191.0556 $[M-H-H]^-$ 173.0450 $[M-H-H_2O]^-$ 93.0340 $[M-H-CH_6O_5]^-$ 85.0290 $[M-H-C_3H_6O_4]^-$ 59.0133 $[M-H-C_5H_8O_4]^-$	Kinic acid	RA
8.	0.92	179.0544	-5.0	C6H12O6	162.0528[M-H-HO]$^-$ 150.0528 $[M-H-CHO]^-$ 146.0579 $[M-H-HO_2]^-$ 144.0423 $[M-H-H_3O_2]^-$ 132.0423 $[M-H-CH_3O_2]^-$ 129.0188 $[M-H-CH_6O_2]^-$ 126.0317 $[M-H-H_5O_3]^-$ 121.0501 $[M-H-C_2H_2O_2]^-$ 116.0473 $[M-H-CH_3O_3]^-$ 114.0317 $[M-H-CH_5O_3]^-$	Fructose	RA
9.	0.92	503.1583	-5.2	C18H32O16	473.1507 $[M-H-CH_2O]$- 456.1843 $[M-H-O_3]$- 445.1557 $[M-H-C2H2O_2]$- 383.1190 $[M-H-C_4H_8O_4]$- 371.1190 $[M-H-C_5H_8O4]$-	Melezitose	RA
10.	0.92	665.2163	0.3	C24H42O21	665.2140 [M-H-H]- 657.1514 $[M-H-H_9]$- 651.1045 $[M-H-H_{15}]$- 646.0654 $[M-H-H_{20}]$- 642.1280 $[M-H-CH_{12}]$- 641.1565 $[M-H-H_9O]$- 638.1906 $[M-H-C_2H_4]$- 636.2113 [M-H-CHO]-	a-D-Glucopyranosyl-(1->4)-a-D-glucopyranosyl-(1->4)-a-D-glucopyranosyl-(1->4)-a-D-glucopyranose	RA
11.	0.93	341.0992	2.6	C15H14N6O4	293.0423 $[M-H-C_2H_8O]$- 207.0518 $[M-H-C_7H_6N_2O]$- 173.0589 $[M-H-C_6H_6N_3O_3]$- 145.0402 $[M-H-C_7H_8N_4O_3]$- 128.0222 $[M-H-C_{11}H_9N_4O]$- 113.0225 $[M-H-C_{12}H_{10}N_3O_2]$- 85.0276 $[M-H-C_{13}H_{10}N_3O_3]$-	5-[(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)hydrazono]pyrimidine-2,4,6(1H,3H,5H)-trione	CRC
12.	0.95	133.0134	0	C4H6O5	119.0344 [M-H-O]- 115.0031 $[M-H-H_2O]$- 104.0473 $[M-H-O_2]$- 99.0082 $[M-H-H_2O_2]$-	Malic acid	RA
13.	1.61	818.2643	3.8	C47H61N7O6	803.4370 $[M-H-CH_3]^-$ 766.3605 $[M-H-CH_{13}N_2]^-$ 761.3901 $[M-H-C_4H_9]^-$ 752.3560 $[M-H-C_3H_{14}O]^-$ 740.3237 $[M-H-H_{19}N_2O_2]^-$ 737.4265 $[M-H-C_5H_5O]^-$ 729.3639 $[M-H-C_5H_{13}O]^-$ 728.3812 $[M-H-C_{44}H_{50}N_5O_5]^-$ 728.3924 $[M-H-C_4H_{10}O_2]^-$	Methyl [(2S)-1-({(2S,3S,5S)-5-{[(2S)-3,3-dimethyl-2-{3-[(6-methyl-2-pyridinyl)methyl]-2-oxo-1-imidazolidinyl}butanoyl]amino}-3-hydroxy-6-phenyl-1-[4-(2-pyridinyl)phenyl]-2-hexanyl}amino)-3,3-dimethyl- 1-oxo-2-butanyl]carbamate	RA
14.	1.8	200.0563	-3.5	C4H7N7O3	196.0219 $[M-H-H_5]$- 167.0079 $[M-H-H_6N_2]$- 164.0321 $[M-H-H_5O_2]$- 151.0130 $[M-H-H_5N_2O]$- 135.0181 $[M-H-H_5N_2O_2]$- 130.0729 $[M-H-CN_2O_2]$- 125.0100 $[M-H-CH_5N_3O]$-	3-(Nitroamino)-1,2,4a,5,7,7a-hexahydro-6H-imidazo[4,5-e][1,2,4]triazin-6-one	RA
15.	1.87	339.129	3.5	C13H24O10	309.1186 $[M-H-CH_2O]$- 293.0873 $[M-H-C_2H_6O]$-	2,3,6-Trihydroxy-5-(hydroxymethyl)cyclohexyl hexopyranoside	RA

					272.0896 [M-H-CH7O3]- 266.1002 [M-H-C3H5O2]- 207.0869 [M-H-C5H8O4]- 200.0685 [M-H-C4H11O5]- 179.0556 [M-H-C7H12O4]- 111.0082 [M-H-C8H20O7]- 99.0446 [M-H-C8H16O8]-		
16.	2.37	529.1542	-0.4	C23H30O14	481.0982 [M-H-C2H8O]- 393.0822 [M-H-C5H12O4]- 359.0978 [M-H-C8H10O4]- 197.0450 [M-H-C14H20O9]- 151.0395 [M-H-C15H22O11]-	Pikuroside	CRC
17.	2.4	319.157	-3.8	C12H24N4O6	251.1144 [M-H-CH8O3]- 197.0562 [M-H-C4H14N2O2]- 179.0695 [M-H-C4H14NO4]- 85.0402 [M-H-C9H18N2O5]-	2,2',2"-Nitrilotris[N-(2-hydroxyethyl)acetamide]	RA
18.	2.4	493.2269	-2.2	C22H38O12	479.2129 [M-H-CH2]- 463.2179 [M-H-CH2O]- 385.1135 [M-H-C5H16O2]- 285.1702 [M-H-C7H12O7]- 191.0556 [M-H-C15H26O6]- 161.0450 [M-H-C16H28O7]- 89.0239 [M-H-C19H32O9]-	(1R,4S,6R)-1,3,3-Trimethyl-2-oxabicyclo[2.2.2]oct-6-yl 6-O-β-D-glucopyranosyl-β-D-glucopyranoside	RA
19.	2.41	447.2231	-0.7	C21H36O10	416.1683 [M-H-C2H7]- 393.1761 [M-H-C4H6]- 379.1393 [M-H-C2H12O2]- 361.1135 [M-H-C6H14]- 349.1499 [M-H-C6H10O]- 321.1549 [M-H-C7H10O2]- 253.1440 [M-H-C7H14O6]- 173.0450 [M-H-C14H26O5]- 113.0239 [M-H-C16H30O7]-	Geranyl 6-O-β-D-xylopyranosyl-β-D-glucopyranoside	RA
20.	2.59	431.1504	1.9	C15H24N6O9	389.1547 [M-H-CNO]- 353.0859 [M-H-CH8N3O]- 293.0886 [M-H-C3H10N2O4]- 193.0487 [M-H-C7H16N3O6]- 179.0695 [M-H-C7H14N3O7]- 134.0453 [M-H-C11H15N5O5]- 91.0058 [M-H-C10H22N5O8]-	-	CRC
21.	2.79	351.0737	5.4	C17H12N4O5	327.0729 [M-H-C2]- 313.0573 [M-H-C3H2]- 312.0495 [M-H-C3H3]- 269.0688 [M-H-C2N3O]- 216.0535 [M-H-C6H3N2O2]- 209.0926 [M-H-C7N2O2]- 193.0613 [M-H-C8H2N2O2]-	2-amino-5'-nitro-2',5-dioxo-1',2',5,6,7,8-hexahydrospiro[chromene-4,3'-indole]-3-carbonitrile	RA
22.	2.85	477.1618	-0.6	C20H30O13	447.1503 [M-H-CH2O]- 415.1604 [M-H-CH2O3]- 357.1186 [M-H-C4H8O4]- 311.0767 [M-H-C6H14O5]- 294.0951 [M-H-C9H11O4]- 205.0501 [M-H-C9H20O9]- 191.0556 [M-H-C13H18O7]- 137.0239 [M-H-C13H24O10]- 125.0239 [M-H-C14H24O10]- 85.0290 [M-H-C16H24O11]-	2-(3,4-Dihydroxyphenyl)ethyl-3-O-β-D-glucopyranosyl-β-D-glucopyranoside	CRC
23.	2.93	134.0369	-1.0	C6H5N3O	134.0354 [M-H-H]- 109.0276 [M-H-C2H2]- 108.0449 [M-H-N2]- 96.0198 [M-H-C3H3]- 75.0235 [M-H-HN3O]-	Hobt	CRC
24.	2.93	367.0977	-1.1	C12H20N2O11	296.1220 [M-H-C2O3]- 271.0930 [M-H-CH4O5]- 235.0692 [M-H-C4H6NO4]- 206.1267 [M-H-C4HO7]- 134.0453 [M-H-C8H11NO7]-	3,11-Bis(carboxymethyl)-4,7,10-trioxa-3,11-diazatridecane-1,13-dioic acid	CRC
25.	2.94	193.048	-2.3	C10H10O4	179.0344 [M-H-CH2]- 166.0630 [M-H-CO]- 149.0603 [M-H-CO2]- 135.0446 [M-H-C2H2O2]- 117.0340 [M-H-C2H4O3]- 59.0133 [M-H-C8H6O2]-	Ferulic acid	CRC
26.	2.94	312.1611	5.1	C19H23NO3	297.1365 [M-H-CH3]- 296.1287 $[M-H--CH_4]^-$ 293.1178 $[M-H-H_6N]$- 271.1334 $[M-H-C_2H_3N]^-$ 268.0974 $[M-H-C_3H_8]^-$ 245.1178 $[M-H-C_4H_5N]^-$ 233.0966 $[M-H-C_2H_{10}NO_2]^-$ 215.0582 $[M-H-C_{12}H_9NO_3]^-$ 206.1181 $[M-H-C_7H_6O]^-$	Evoeuropine	CRC
27.	3.05	446.1825	0.7	C30H25NO3	431.1885 [M-H-O]- 357.1365 [M-H-C7H5]- 271.0997 [M-H-C11H11O2]- 137.0239 [M-H-C23H19N]- 134.0368 [M-H-C22H18NO]- 61.9793 [M-H-C26H26NO2]-	8-[(dibenzylamino)methyl]-7-hydroxy-3-phenyl-4H-chromen-4-one	CRC
28.	3.12	340.1551	2.9	C20H23NO4	326.1392 [M-H-CH2]- 325.1440 [M-H-HN]- 229.1103 [M-H-C6H7O2]- 173.0477 [M-H-C10H15O2]- 121.0290 [M-H-C13H17NO2]-	Tetrahydrojatrorrhizine	CRC
29.	3.27	590.2217	1.9	C25H33N7O10	519.1866 [M-H-H7O4]- 495.1866 [M-H-C2H7O4]- 371.0839 [M-H-C12H17N3O]-	-	CRC

30.	3.67	173.0428	-5.8	C7H10O5	207.0406 [M-H-C16H25N5O6]- 195.0280 [M-H-C18H27N4O6]- 136.0035 [M-H-C19H30N6O7]- 157.0501 [M-H-O]- 137.0239 [M-H-H4O2]- 111.0810 [M-H-O4]- 111.0446 [M-H-CH2O3]- 94.0419 [M-H-CH3O4]- 71.0497 [M-H-C3H2O4]- 67.0184 [M-H-C3H6O4]-	Shikimic acid	CRC
31.	3.67	367.1005	-4.4	C17H20O9	325.0923 [M-H-C2H2O]- 195.0657 [M-H-C7H8O5]- 135.0446 [M-H-C9H12O7]- 134.0368 [M-H-C9H13O7]-	3-O-feruloylquinic acid	CRC
32.	3.76	297.0599	-1.0	C14H10N4O4	205.0362 [M-H-C6H4O]- 193.0487 [M-H-C6H2NO]- 179.0569 [M-H-C7H3O2]- 93.0300 [M-H-C13H5N2O]- 59.0133 [M-H-C12H7N4O2]-	1H-benzotriazol-1-yl(4-methoxy-3-nitrophenyl)methanone	CRC
33.	5.99	261.1339	3.1	C12H22O6	247.1182 [M-H-CH2]- 243.1232 [M-H-H2O]- 223.1182 [M-H-C3H2]- 223.0607 [M-H-CH11O]-	1,2:5,6-Bis-O-(1-methylethylidene)-D-mannitol	CRC
34.	6.87	187.0924	-4.1	C9H16O4	169.0865 [M-H-H_2O]$^-$ 141.0916 [M-H-CH_2O_2]$^-$ 126.1045 [M-H-CHO_3]$^-$ 125.0966 [M-H-CH_2O_3]$^-$ 97.0653 [M-H-$C_3H_6O_3$]$^-$ 57.0340 [M-H-$C_6H_{10}O_3$]$^-$	Nonanedioic acid	RA
35.	7.13	243.1222	-1.6	C12H20O5	227.1283 [M-H-O]- 211.1334 [M-H-O2]- 165.0552 [M-H-C3H10O2]- 150.0317 [M-H-C4H13O2]- 99.0082 [M-H-C8H16O2]-	Diethyl Tetrahydrofufurylmalonate	RA
36.	8.2	243.1227	-1.2	C14H16N2O2	243.1134 [M-H-H]- 225.0790 [M-H-H5N]- 207.0195 [M-H-C2H13]- 207.0320 [M-H-CH11N]- 199.0058 [M-H-H15NO]- 171.0109 [M-H-CH15NO2]-	Etomidate	RA
37.	11.12	485.1831	-4.7	C19H34O14	453.1244 [M-H-C2H8]- 441.1972 [M-H-CO2]- 397.2074 [M-H-C2O4]- 295.1029 [M-H-C8H14O5]- 265.1287 [M-H-C8H12O7]- 211.0818 [M-H-C12H18O7]-	Methyl 3,6-dideoxy-α-D-xylo-hexopyranosyl-(1->3)-[α-D-galactopyranosyl-(1->2)]-α-D-mannopyranoside	CRC
38.	11.44	327.0902	-4.3	C15H28N4O4	283.0593 [M-H-CH19N]- 244.9987 [M-H-CH27N2O]- 242.1379 [M-H-C5H9O]- 242.1392 [M-H-C3H7N3]- 209.0688 [M-H-C5H16N3]-	Peramivir	RA
39.	11.49	503.1897	-0.6	C26H32O10	387.1808 [M-H-C4H4O4]- 367.1393 [M-H-C8H8O2]- 327.2324 [M-H-C4HO8]- 305.1389 [M-H-C9H10O5]- 281.1389 [M-H-C11H10O5]- 96.9773 [M-H-C26H31O4]-	Dodecahydrodibenzo-octaoxacyclotetracosine-2,17-dicarbaldehyde	CRC
40.	11.55	329.2239	-3.3	C18H34O5	250.9981 [M-H-C4H31]- 224.9977 [M-H-C2H33O3]- 171.0657 [M-H-C10H23O]-	Sanleng acid	CRC
41.	11.71	329.2299	0.9	C14H30N6O3	266.1505 [M-H-CH9N3]- 264.1586 [M-H-CH9N2O]- 227.1270 [M-H-C4H12N3]- 225.1113 [M-H-C4H14N3]- 225.1239 [M-H-C3H12N4]- 201.1239 [M-H-C5H12N4]- 169.0851 [M-H-C7H18N3O]- 141.0790 [M-H-C7H18N5O]- 125.1079 [M-H-C7H16N4O3]-	2-({6-[(diaminomethylidene)amino]hexyl}amino)-2-oxoethyl(4-aminobutyl)carbamate	RA
42.	12.1	485.1782	-2.3	C26H30O9	407.1706 [M-H-C5H3O]- 313.1076 [M-H-C8H12O4]- 211.0970 [M-H-C15H14O5]- 209.0814 [M-H-C15H16O5]- 167.0344 [M-H-C18H22O5]- 61.9793 [M-H-C22H31O8]-	3,11-Bis(4-hydroxy-3-methoxyphenyl)-2,4,10,12-tetraoxadispiro-hexadecan-7-one	CRC
43.	12.61	469.1855	-2.1	C26H30O8	427.2121 [M-H-CO2]- 411.1444 [M-H-C_3H_6O]$^-$ 330.2195 [M-H-C_5O_5]$^-$ 283.1334 [M-H-$C_8H_{10}O_5$]$^-$ 196.9875 [M-H-$C_{15}H_{29}O_4$]$^-$ 61.9793 [M-H-$C_{22}H_{31}O_7$]$^-$	Obaculactone	CRC
44.	12.61	515.1823	-2.1	C26H30O8	427.2121 [M-H-CO2]- 411.1444 [M-H-C_3H_6O]$^-$ 330.2195 [M-H-C_5O_5]$^-$ 283.1334 [M-H-$C_8H_{10}O_5$]$^-$ 196.9875 [M-H-$C_{15}H_{29}O_4$]$^-$ 61.9793 [M-H-$C_{22}H_{31}O_7$]$^-$	Obaculactone-formic acid	CRC
45.	12.86	471.1975	0.8	C22H28N6O6	375.1668 [M-H-C4H4N2O]- 323.0780 [M-H-C6H16N2O2]- 307.1519 [M-H-C9H8O3]- 265.1049 [M-H-C12H14O3]-	Benzoic acid, 3,5-dimethoxy-, 2-[4-(2,3,6,7-tetrahydro-1,3-dimethyl-2,6-dioxo-1H-purin-8-yl)-1-piperazinyl]ethyl ester	CRC
46.	13.95	453.1884	-5.3	C26H30O7	291.1233 [M-H-C10H10O2]- 171.0082 [M-H-C16H26O4]-	Obacunone	CRC
47.	14.57	723.347	-1.7	C36H44N12O5	678.3629 [M-H-NO2]- 594.3067 [M-H-C3H5N4O2]-	-	RA

					423.1893 [M-H-C16H20N4O2]- 381.1437 [M-H-C17H24N7O]- 343.2121 [M-H-C19H18N5O4]- 337.2226 [M-H-C21H16N5O3]- 309.1913 [M-H-C23H20N5O3]- 300.1586 [M-H-C20H23N8O3]- 187.1083 [M-H-C28H28N10O2]- 124.0147 [M-H-C32H41N9O3]-		
48.	15.25	713.3132	-3.1	C38H50O13	680.2833 [M-H-CH5O]- 667.3482 [M-H-O3]- 645.2911 [M-H-C4H4O]- 649.3013 [M-H-CH4O3]- 623.0826 [M-H-C5H31]- 621.2700 [M-H-C3H8O3]- 613.3013 [M-H-C4H4O3]- 608.2622 [M-H-C4H9O3]- 605.2387 [M-H-C4H12O3]-	(2R,3R,3aR,4R,6S,7S,8S,10E,12S,13aR)-2,7,8-Triacetoxy-3,13a-dihydroxy-2,9,9,12-tetramethyl-4-[(2-methylbutanoyl)oxy]-5-methylene-13-oxo-2,3,3a,4,5,6,7,8,9,12,13,13a-dodecahydro-1H-cyclopenta[12]annulen-6-yl benzoate	RA
49.	15.25	723.3366	2.6	C46H48N2O6	678.3458 [M-H-CHO2]- 575.2672 [M-H-C9H10NO]- 325.1467 [M-H-C23H28NO5]-	(8Z,8'Z)-1,1',6,6'-Tetrahydroxy-5,5'-diisopropyl-3,3'-dimethyl-8,8'-bis{[(1-phenylethyl)amino]methylene}-2,2'-binaphthalene-7,7'(8H,8'H)-dione	RA
50.	15.5	593.2785	0.8	C27H46O14	505.3084 [M-H-C2O4]- 339.1291 [M-H-C14H22O4]- 307.1393 [M-H-C14H22O6]- 253.0923 [M-H-C18H28O6]-	3-O-[(1S)-1-Carboxy-2-cyclohexylethyl]-β-D-galactopyranosyl-(1->3)-[6-deoxy-α-L-galactopyranosyl-(1->4)]-1,5-anhydro-2,6-dideoxy-D-arabino-hexitol	RA
51.	15.5	677.3303	-0.9	C40H46N4O6	593.2764 [M-H-C_5H_8O]- 509.2315 [M-H-$C_9H_{14}NO_2$]- 491.2083 [M-H-$C_{10}H_{18}O_3$]- 391.1618 [M-H-$C_{22}H_{22}$]- 309.2052 [M-H-$C_{24}H_{18}NO_3$]- 265.1426 [M-H-$C_{27}H_{26}NO_3$]- 253.1063 [M-H-$C_{29}H_{30}NO_2$]- 101.0477 [M-H-$C_{36}H_{38}N_3O_4$]-	Galnon	RA
52.	16.76	293.2101	0.3	C18H30O3	289.1804 [M-H-H5]- 285.1491 [M-H-H9]- 272.1776 [M-H-H6O]- 267.1960 [M-H-C2H2]- 249.1491 [M-H-C3H8]-	2-{2-[4-(1,1,3,3-TETRAMETHYLBUTYL)PHENOXY]ETHOXY}ETHANOL	RA
53.	16.78	433.2361	0.2	C23H34N2O6	389.1713 [M-H-C3H8]- 378.1791 [M-H-C4H7]- 355.1869 [M-H-C6H6]- 337.2127 [M-H-C5H4O2]- 311.1607 [M-H-C8H10O]- 297.2430 [M-H-C5HN2O3]-	N,N'-1,3-Propanediyl Bis(4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]heptane-1-carboxamide)	RA
54.	17.73	761.3949	-0.7	C39H54N8O8	485.2638 [M-H-C14H18N3O3]- 433.1598 [M-H-C20H30N3O]- 353.1461 [M-H-C24H32N4O2]- 294.1580 [M-H-C24H31N6O4]- 265.1301 [M-H-C27H36N4O5]-	N-(pyrazin-2-ylcarbonyl)-D-valyl-D-valyl-(4R)-4-[(3,4-dihydroisoquinolin-2(1H)-ylcarbonyl)oxy]-N-[1,2-dioxo-1-(propan-2-ylamino)hexan-3-yl]-L-prolinamide	RA
55.	18.6	807.3986	-0.2	C44H60N2O12	353.0899 [M-H-C25H44NO6]- 309.1239 [M-H-C26H42O9]- 293.1389 [M-H-C28H38N2O7]- 265.1314 [M-H-C30H40NO8]-	(9E,19E)-2,15,17,27,29-Pentahydroxy-11-methoxy-3,7,12,14,16,18,22-heptamethyl-26-[(4-methyl-1-piperidinyl)methyl]-6,23-dioxo-8,30-dioxa-24-azatetracyclo[23.3.1.14,7.05,28]triaconta-1(29),2,4,9,19, 21,25,27-octaen-13-yl acetate	RA

Note: CRC, Cortex Phellodendri Chinensis; RA, Rhizoma Atractylodis.