An improved ultra-performance liquid chromatography-electrospray ionization/ quadrupole-time-of-flight high-definition mass spectrometry method for determining ingredients of herbal *Fructus corni* in blood samples

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ABSTRACT

Background: Fructus Corni (FC), a well-known traditional Chinese medicine (TCM), derived from the dry ripe sarcocarp of *Cornus offi cinalis Sieb. et Zucc* (Cornaceae), has been widely prescribed to treat disease in China for centuries. It has attracted increasingly much attention as one of the most popular and valuable herbal medicine in clinic. However, the systematic analysis of the chemical constituents of FC is diffi cult to determine and remain unclear. **Materials and Methods:** In this work, a rapid, sensitive, and reliable ultra-performance liquid chromatography-electrospray ionization/ quadrupole-time-of-fl ight high-defi nition mass spectrometry (UPLC-ESI/QTOF/MS) with automated data analysis (MetaboLynx™) in negative ion mode were established to characterize the chemical constituents of FC and simultaneously identify components in blood after oral administration of FC, respectively. The analysis was performed on a Waters UPLC™ HSS T_3 (2.1 × 100 mm, 1.8 μm) using gradient elution system. MS/MS fragmentation behaviors were proposed for aiding the structural identifi cation of the components. **Results:** With optimized conditions, a total of 34 peaks were obtained from FC, 23 of which were tentatively characterized by comparing the retention time and mass spectrometry data and retrieving the reference literatures. Of note, the 25 compounds were identifi ed after oral administration of FC, which might be the potential active components *in vivo*. **Conclusion:** The present study demonstrates the potential of UPLC-ESI/QTOF/MS approach for the rapid and reliable characterization of the metabolites of natural products.

Key words: Bioactive components, fructus corni, herbal medicine, identification, UPLC–ESI/QTOF/MS

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INTRODUCTION

Chinese herbal medicine is an essential part of the healthcare system in most Asian countries, and has attracted attention even in European and North American countries.[1,2] It has been widely used in clinical practice to treat diseases for thousands of years.[3] Fructus Corni, a famous traditional Chinese medicine (TCM), derived from the dry ripe sarcocarp of *Cornus officinalis Sieb. et Zucc*, was used for nourishing liver and kidney. It possesses a variety of pharmacological activities, including immune regulation, anti-oxidation, anti-tumor, and anti-aging effects.[4-7] Although pharmacological activities of FC have been extensively studied, very little is known about their systematic chemical constituents or characteristics *in vivo*.

According to the theory of plasma pharmacochemistry, only compounds absorbed into the blood have the possibility of showing pharmacological bioactivities.[8-12] Therefore, simultaneous identification of potential active components of FC in blood is indispensable. Despite

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the recent advancement of various analytical tools, the identification of bioactive components from TCM remains a great challenge. In the past few years, chromatographic separation coupled with mass spectrometry (MS) has become a powerful technique for component identification.[13,14] There is a boost in the use of the LC-MS technique because of the superior sensitivity, selectivity, and the ability to conclusively identify the compounds.[14] Ultra performance liquid chromatography (UPLC) is an attractive option for the determination of substance at low concentrations in shorter analytical period in comparison with conventional LC.[15] UPLC coupled with tandem MS has been proven to be an efficient tool for the rapid analysis of the known compounds and elucidation of unknown compounds in complex matrix, and has therefore become an important analytical tool in TCM research.[16,17]

It is of significance to systematically analyze the chemical constituents of FC so as to interpret its material basis for pharmacological effects. Given the chemical complexity of FC, UPLC and sub-2 um particle size columns were used to increase chromatographic resolution and to shorten analysis time.[18,19] In this study, UPLC-ESI/QTOF/MS was applied for the identification of phytochemical constituents of FC and multiple absorbed components in rat plasma. The developed method could be adopted to rapidly screen and identify potentially bioactive components contributing to the pharmacological effects of TCM.

MATERIALS AND METHODS

Chemicals and materials

HPLC grade methanol was purchased from Merck (Darmstadt, Germany). Distilled water was purchased from Watson's Food and Beverage Co., Ltd. (Guangzhou, China). Leucine enkephalin was purchased from Sigma-Aldrich (MO, USA). Formic acid was purchased from Tianjin Kermel Chemical Reagent Co., Ltd. (Tianjin, China). Fructus Corni was purchased from Harbin Shiyitang 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.

Preparation of FC samples for LC/MS analysis

Weighing 10 g of the powder of FC, suspend in 1000 mL water, and extract backflow for two times (each for 2h). The supernatant was subjected to macroporous resin (AB-8), and 10% ethanol eluate was collected and finally the solution was freeze-dried into a powder. The solution was filtered through 0.22-um membranes (pore size) prior to use, and a 5-ul aliquot was injected for analysis.

Animals handling

Male Sprague-Dawley rats (225 ± 25 g) were obtained from the Laboratory Animal Center of Heilongjiang University of Chinese Medicine. The rats were housed in an animal room (24 ± 2°C, 60 ± 5% relative humidity). A 12-h dark/light cycle was set, and the rats were given water and fed normal food *ad libitum* for 1 week before the experiment. The animal facilities and protocols were approved by Animal Care and Use Committee of Heilongjiang University of Chinese Medicine. All rats were randomly divided into 2 groups of 6 rats each: Control group and dosed group. All animals were fasted overnight before the experiments and had free access to water. The freeze-dried FC powder was dissolved in 0.5% CMC, and then the mixture was grinded adequately and sonicated for 30 min to prepare the decoction (3.2 mg/mL). The rats were orally administered with FC extract (1 mL/100 g). The control group was orally administrated with an equivalent volume of 0.5% CMC. After 90 min, the rats were anesthetized by intraperitoneal injection of 1% pentobarbital sodium (0.20 mL/100 g body weight).

Preparation of serum samples

The blood samples were collected from hepatic portal vein at 90 min after administration, and the rats were sacrificed. Then, the serum was separated immediately by centrifuging at 13000 rpm for 15 min at 4°C. All samples were stored at −80°C until analysis. To 2.0 mL of the above supernatant, 40 uL phosphoric acid was added and then the solution was vortexed for 60 s. The mixed solution was applied to pre-actrbated OASIS HLB solid phase extraction C_{18} columns (Waters Corporation, USA). Before that, the column was washed with 4 mL of methanol and 4 mL of water. Then, 4 mL 30% methanol elutes were collected and dried under nitrogen gas at 35°C. The residues were re-dissolved in 100 ul of 50% methanol, centrifuged at 13000 rpm for 15 min at 4°C. The sample was filtered through a 0.22-um membrane, and a 3-ul aliquot was injected for UPLC-MS analysis.

Chromatography

Chromatographic analysis was performed in a Waters Acquity™ Ultra Performance LC systems (Waters Corporation, USA) controlled with Masslynx (V4.1). Separation was performed on an Waters ACQUITY UPLC™ HSS T_3 (2.1 × 100 mm, 1.8 μm) held at 50°C, and the flow rate was 0.3 mL/min. The optimal mobile phase consisted of A (HCOOH: H_2O = 0.1: 100, v/v) and B (HCOOH: CH_3CN = 0.1:100, v/v). The linear elution gradient program was used as follows: 0-3 min, 3-10% B; 3-5 min, 10-12% B; 5-8 min, 12-20% B; 8-12 min, 20-50% B; 12-15 min, 50-100% B.

Mass spectrometric characterization

UPLC was directly interfaced with a Waters Synapt™ High Definition MS System (Waters Corporation, Milford, USA) equipped with an electrospray ion source operating in negative ESI mode. The optimal conditions of analysis were as follows: ESI − mode, capillary voltage of 2.6 kV, sampling cone voltage was 30.0 V, extraction cone voltage was 3.5 V. The temperature was set at 110°C, desolvation gas temperature was 300°C, desolvation gas flow was 600 L/h. The mass spectrometer was calibrated using a solution of sodium formate before the experiment. The full-scan MS data were produced across the mass range of 50-1000 Da. Data were collected in centroid mode and mass was corrected during acquisition using an external reference (Lock-Spray™) comprising a 100 μL/min solution of leucine-enkephalin via a lockspray interface, generating a reference ion at 554.2615 Da ($[M-H]^-$) in negative ion mode.

RESULTS AND DISCUSSION

UPLC-MS characterization of chemical constituents from FC

All information of MS data obtained from the robust UPLC-ESI-Q-TOF-MS analysis, which was performed using the aforementioned protocol, indicated the retention time and precise molecular mass and provided the MS/MS data, which was necessary for the structural identification of the biomarkers. The precise molecular mass was determined within a reasonable degree of measurement error using Q-TOF, and the potential element composition, and unsaturation degree were also obtained. Global profiling in negative ion mode was analyzed by UPLC-ESI-Q-TOF-MS. Using the optimal UPLC-MS conditions described above, the total ion current chromatogram at negative ESI mode was shown in Figure 1. A total of 32 peaks were obtained, and 21 of these were identified or tentatively characterized [Table 1]. The chemical structures and potential fragmentation mechanism for loganin, morroniside, and sweroside are proposed in Figure 2, respectively.

Automated MetaboLynx analysis

UPLC-ESI-Q-TOF-MS data of all determined samples were further processed by Metabolynx XS version 4.1 (Waters Corp., Milford, USA), which employs a comprehensive list of potential biotransformation reactions with the elemental compositions of each possible metabolites and generates a sequence of extraction ion chromatograms (XICs). Comparison of the XICs of the samples after FC administration and blank samples allows for the identification of potential drug metabolites. The major analysis parameters were set as follows: Mass defect filter was set at ±50 mDa; the MS trace retention time range was from 0.00 to 15.00 min; for expected MS chromatograms, mass values were estimated from the parent molecule and expected metabolites, which was set with sulfate conjugation and glucuronide conjugation, unexpected metabolite chromatograms were presented from mass range chromatograms by a full acquisition mass ranging from 50 to 1000 Da; in the expected and unexpected mass chromatograms, the mass window was set at 0.05 Da; in false positive conditions, the retention time window was set to a 0.2 min matching with a blank sample, the ratio of analyte to control peak area was 10. Elemental composition for expected and unexpected metabolites peaks was created using an exhaustive list of potential formula of the metabolites.

To clarify the active constituents responsible for the pharmacological action, it is necessary to know the chemical constituent profile *in vivo*.[20] Therefore, the rat plasma after oral administration of FC was analyzed by the same UPLC-ESI-Q-TOF-MS method. UPLC chromatograms of rat plasma samples collected after administered FC are shown in Figure 3. The retention time, MS data, and the fragments of the metabolites were listed in Table 2. Of note, a total of 34 peaks were obtained, and 25 of these were tentatively characterized [Table 2]. The present study is aimed at developing an approach for elucidating the phytochemical constituents of FC, conducted via UPLC-ESI-Q-TOF-MS. Although FC has been used in clinic widely, the bioactive ingredients of FC are not well understood. The classical method for searching bioactive components is by systematic separation guided with bioactivity tracking *in vitro*.[21-25] However, using this method, the screening results cannot represent the really effective components. To resolve the drawbacks, a new methodology for searching effective constituents of herbal medicine should be established. In this work, we utilized UPLC-ESI-Q-TOF-MS to rapidly and globally identify

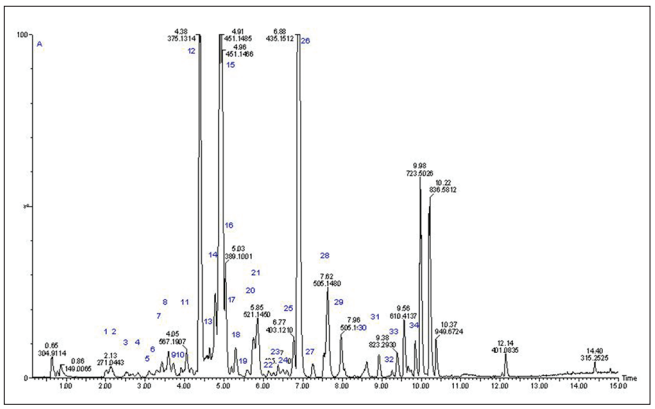

Figure 1: The UPLC-ESI/QTOF/MS BPI chromatogram of Fructus Corni in negative ion mode

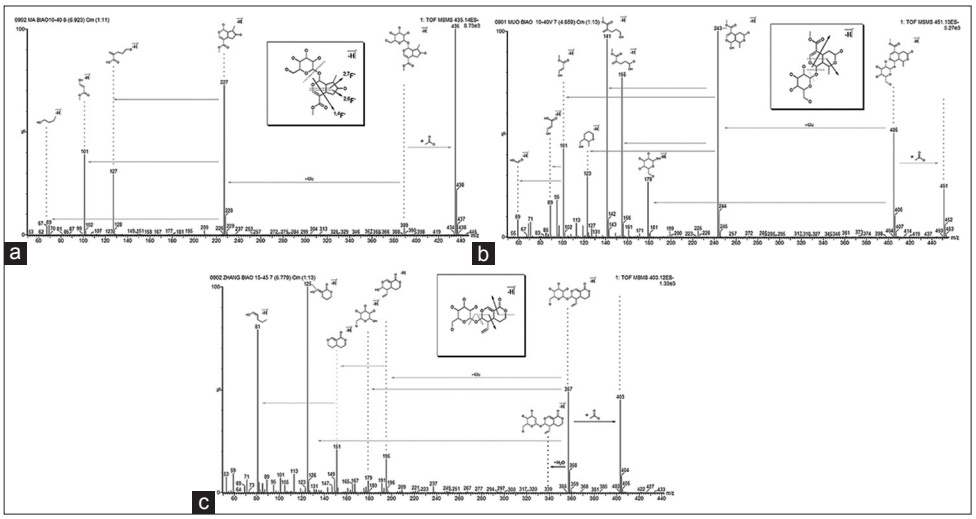

Figure 2: MS/MS spectrum and proposed fragmentation pathway of major constituents, loganin (a), morroniside (b), sweroside (c) in negative ion mode

Table 1: Identification information of *in vitro* constituents of Fructus Corni by UPLC–ESI/QTOF/MS

Peak no.	Rt (min)	Systematic name	Molecular formula	Molecular weight (Da)	$[M-H]^-$		Fragmentions (m/z)
					measured value (Da)	Error (ppm)	
1	2.022	Gallic acid	$C_7H_6O_5$	170.0215	169.0133	−2.4	125.0239 (−4.0 mDa) $C_6H_5O_3$ ($-CO_2$)
1	2.022	Gemin D	$C_{27}H_{22}O_{18}$	634.0806	633.0749	3.3	300.9984 (+2.7 mDa) $C_{14}H_5O_8$ ($-C_{13}H_{16}O_{10}$) 275.0192 (+1.0 mDa) $C_{13}H_7O_7$ ($-C_{14}H_{14}O_{11}$)
2	2.117	7-O-galloyl-D-sedoheptulose	$C_{14}H_{18}O_{11}$	362.0849	361.0764	−1.9	271.0463(+3.3 mDa) $C_{11}H_{11}O_8$
2	2.161	1,2-di-O-galloyl-β-D-glucose	$C_{20}H_{20}O_{14}$	484.0853	483.0785	2.1	331.0665 (−1.3 mDa) $C_{13}H_{15}O_{10}$ ($-C_7H_4O_4$) 313.0560 (+7.9 mDa) $C_{13}H_{13}O_9$ ($-C_7H_6O_5$) 176.0321 (+7.7 mDa) $C_6H_8O_6$ ($-C_{14}H_{11}O_8$) 169.0137 (−2.4 mDa) $C_7H_5O_5$ ($-C_{13}H_{14}O_9$) 151.0606 (−3.5 mDa) $C_5H_{11}O_5$ ($-C_{15}H_8O_9$)
3	2.538	Unknown	$C_{18}H_{26}O_{12}$	434.1424	433.1332	−3.2	
4	2.790	1,2-di-O-galloyl-D-glucose	$C_{20}H_{20}O_{14}$	484.0853	483.0775	0.0	331.0665 (−0.1 mDa) $C_{13}H_{15}O_{10}$ ($-C_7H_4O_4$) 313.0560 (+2.7 mDa) $C_{13}H_{13}O_9$ ($-C_7H_6O_5$) 169.0137 (−2.4 mDa) $C_7H_5O_5$($-C_{13}H_{14}O_9$) 125.0239 (−10.4 mDa) $C_6H_5O_3$ ($-C_{14}H_{14}O_{11}$)
5	3.291	1,7-di-O-galloyl-D-sedoheptulose	$C_{21}H_{22}O_{15}$	514.0959	513.0861	−3.7	361.0771 (+0.3 mDa) $C_{14}H_{17}O_{11}$ 169.0137 (−3.9 mDa) $C_7H_5O_5$
8	3.594	(1'S,2'R)-Guaiacyl glycerol 3'-O-β-D glucopyranoside	$C_{16}H_{24}O_{10}$	376.1370	375.1287	−1.1	213.0763 (−1.0.mDa) $C_{10}H_{13}O_5$ ($-C_6H_{10}O_5$) 179.0556 (−3.0 mDa) $C_6H_{11}O_6$ ($-C_{10}H_{12}O_4$) 169.0865 (−2.6 mDa) $C_9H_{13}O_3$ ($-C_7H_{10}O_7$)
9	3.731	1,7-di-O-galloyl-D-sedoheptulose	$C_{21}H_{22}O_{15}$	514.0959	513.0904	4.7	271.0454 (−8.9 mDa) $C_{11}H_{11}O_8$ 241.0348 (−19.9 mDa) $C_{10}H_9O_7$ 211.0243(−0.5.mDa) $C_9H_7O_6$ 169.0137 (−3.9 mDa) $C_7H_5O_5$
10	3.927	1,7-di-O-galloyl-D-sedoheptulose	$C_{21}H_{22}O_{15}$	514.0959	513.0858	−4.3	169.0137 (+0.7 mDa) $C_7H_5O_5$
11	4.052	Methyl (1S,4aS,8S,8aS)-8-{[6-O-(β-D-glucopyranosyl)-β-D-glucopyranosyl] oxy}-3-hydroxy-1-methyl-4,4a,8,8a-tetrahydro-1H,3H-pyrano[3,4-c] pyran-5-carboxylate	$C_{23}H_{36}O_{16}$	568.2003	567.1907	−3.2	405.1397 (−4.4 mDa) $C_{17}H_{25}O_{11}$ ($-C_6H_{10}O_5$) 285.0974 (−0.8 mDa) $C_{13}H_{17}O_7$ ($-C_{10}H_{18}O_9$) 243.0869 (+1.4 mDa) $C_{11}H_{15}O_6$ ($-C_{12}H_{20}O_{10}$) 143.0344 (−3.0 mDa) $C_6H_7O_4$ ($-C_{17}H_{28}O_{12}$) 101.0239 (−0.4 mDa) $C_4H_5O_3$ ($-C_{19}H_{30}O_{13}$)
12	4.377	Loganic acid	$C_{16}H_{24}O_{10}$	376.1370	375.1290	−0.3	213.0763 (−9.2 mDa) $C_{10}H_{13}O_5$ ($-C_6H_{10}O_5$) 169.0865 (−3.9 mDa) $C_9H_{13}O_3$ ($-C_7H_{10}O_7$) 151.0759 (+3.5 mDa) $C_9H_{11}O_2$ ($-C_7H_{12}O_8$) 136.0524 (−3.8 mDa) $C_8H_8O_2$ ($-C_8H_{15}O_8$) 125.0239 (−5.9 mDa) $C_6H_5O_3$ ($-C_{10}H_{18}O_7$) 115.0395 (+2.7 mDa) $C_5H_7O_3$ ($-C_{11}H_{16}O_7$) 110.0368 (+9.5 mDa) $C_6H_6O_2$ ($-C_{10}H_{17}O_8$) 99.0446 (−3.3 mDa) $C_5H_7O_2$ ($-C_{11}H_{16}O_8$) 83.9847 (−3.4 mDa) C_3O_3 ($-C_{13}H_{23}O_7$) 70.0419 (−3.4 mDa) C_4H_6O ($-C_{12}H_{17}O_9$)

Contd...

Table 1: Contd..

Peak no.	Rt (min)	Systematic name	Molecular formula	Molecular weight (Da)	[M-H]⁻		Fragmentions (m/z)
					measured value (Da)	Error (ppm)	
13	4.631	Unknown	$C_{18}H_{26}O_{13}$	450.1373	449.1300	1.1	
14	4.710	1,7-di-O-galloyl-D-sedoheptulose	$C_{21}H_{22}O_{15}$	514.0959	513.0905	4.9	361.0771 (−4.1 mDa) $C_{14}H_{17}O_{11}$ 271.0472 (1.8 mDa) $C_{11}H_{11}O_8$ 241.0339(−0.9 mDa) $C_{10}H_9O_7$ 211.0243 (5.2 mDa) $C_9H_7O_6$ 169.0137 (0.0 mDa) $C_7H_5O_5$
15	4.942	Morroniside	$C_{17}H_{26}O_{11}$	406.1475	405.1401	1.0	243.0869 (+1.7 mDa) $C_{11}H_{15}O_6$ ($-C_6H_{10}O_5$) 179.0556 (+2.5 mDa) $C_6H_{11}O_6$ ($-C_{11}H_{14}O_5$) 155.0344 (−1.1 mDa) $C_7H_7O_4$ ($-C_{10}H_{18}O_7$) 141.0552 (−1.8 mDa) $C_7H_9O_3$ ($-C_{10}H_{16}O_8$) 123.0446 (−3.6 mDa) $C_7H_7O_2$ ($-C_{10}H_{18}O_9$) 85.0290 (+1.1 mDa) $C_4H_5O_2$ ($-C_{13}H_{20}O_9$) 59.0133 (+2.8 mDa) $C_2H_3O_2$ ($-C_{15}H_{22}O_9$)
16	5.057	Unknown	$C_{23}H_{18}O_6$	390.1103	389.1013	−3.1	
17	5.231	Unknown	$C_{20}H_{28}O_{14}$	492.1479	491.1411	2.0	
18	5.297	Unknown	$C_{20}H_{28}O_{14}$	492.1479	491.1396	−1.0	
19	5.607	Caffeic acid	$C_9H_8O_4$	180.0423	179.0339	−2.8	135.0446 (−2.0 mDa) $C_8H_7O_2$ ($-CO_2$)
20	5.747	Unknown	$C_{21}H_{30}O_{15}$	522.1585	521.1486	−3.8	
21	5.906	Unknown	$C_{21}H_{30}O_{15}$	522.1585	521.1481	−4.8	
22	6.138	Unknown	$C_{21}H_{30}O_{15}$	522.1585	521.1498	−1.5	
23	6.216	Unknown	$C_{21}H_{30}O_{15}$	522.1585	521.1497	−1.7	
24	6.353	Unknown	$C_{18}H_{27}O_{12}$	435.1503	435.1497	−1.4	
25	6.789	Sweroside	$C_{16}H_{22}O_9$	358.1264	357.1180	−1.7	195.0657 (+1.0 mDa) $C_{10}H_{11}O_4$ ($-C_6H_{10}O_5$) 179.0556 (+4.1 mDa) $C_6H_{11}O_6$ ($-C_{10}H_{10}O_3$) 125.0239 (−4.9 mDa) $C_6H_5O_3$ ($-C_{10}H_{16}O_6$) 81.0340 (+1.4 mDa) C_5H_5O ($-C_{11}H_{16}O_8$)
26	6.877	Loganin	$C_{17}H_{26}O_{10}$	390.1526	389.1439	−2.3	389.1448 (−0.3 mDa) $C_{17}H_{25}O_{10}$ 227.0919 (−5.2 mDa) $C_{11}H_{15}O_5$ ($-C_6H_{10}O_5$) 127.0395 (−2.3 mDa) $C_6H_7O_3$ ($-C_{11}H_{18}O_7$) 101.0239 (−1.3 mDa) $C_4H_5O_3$ ($-C_{13}H_{20}O_7$) 67.0184 (−0.8 mDa) C_4H_3O ($-C_{13}H_{22}O_9$)
28	7.535	Cornuside	$C_{21}H_{30}O_{14}$	506.1636	505.1550	−1.4	227.0919 (−1.2 mDa) $C_{11}H_{15}O_5$ ($-C_6H_{10}O_5$) 135.0446 (−1.1 mDa) $C_8H_7O_2$ ($-C_9H_{18}O_8$) 128.0473 (+7.4 mDa) $C_6H_8O_3$ ($-C_{11}H_{17}O_7$) 101.0239 (+1.2 mDa) $C_4H_5O_3$ ($-C_{13}H_{20}O_7$) 67.0184 (+0.4 mDa) C_4H_3O ($-C_{13}H_{22}O_9$)
29	7.963	Cornuside	$C_{21}H_{30}O_{14}$	506.1636	505.1552	−0.5	227.0919 (−3.3 mDa) $C_{11}H_{15}O_5$ ($-C_6H_{10}O_5$) 127.0395 (−3.1 mDa) $C_6H_7O_3$ ($-C_{11}H_{18}O_7$) 101.0239 (−1.8 mDa) $C_4H_5O_3$ ($-C_{13}H_{20}O_7$) 67.0184 (+0.8 mDa) C_4H_3O ($-C_{13}H_{22}O_9$)
31	8.919	7-dehydrologanin	$C_{17}H_{24}O_{10}$	388.1370	387.1291	4.6	225.0763 (+20.5 mDa) $C_{11}H_{13}O_5$ ($-C_6H_{10}O_5$) 155.0708 (−18.2 mDa) $C_8H_{11}O_3$ ($-C_9H_{12}O_7$) 127.0759 (−27.7 mDa) $C_7H_{11}O_2$ ($-C_{10}H_{12}O_8$) 95.0133 (+12.8 mDa) $C_5H_3O_2$ ($-C_{12}H_{20}O_8$) 79.0184 (+17.4 mDa) C_5H_3O ($-C_{12}H_{20}O_9$)
33	9.846	Cornuside	$C_{34}H_{50}O_{20}$	778.2896	777.2832	1.9	617.2446 (−4.5 mDa) $C_{28}H_{41}O_{15}$ ($-C_6H_8O_5$) 319.1393 (−1.0 mDa) $C_{14}H_{23}O_8$ ($-C_{20}H_{26}O_{12}$) 239.0556 (−5.7 mDa) $C_{11}H_{11}O_6$ ($-C_{23}H_{38}O_{14}$) 228.0998 (+0.4 mDa) $C_{11}H_{16}O_5$ ($-C_{23}H_{33}O_{15}$) 227.0919 (+3.2 mDa) $C_{11}H_{15}O_5$ ($-C_{23}H_{34}O_{15}$) 175.0243 (−1.2 mDa) $C_6H_7O_6$ ($-C_{28}H_{42}O_{14}$) 155.0344 (−1.0 mDa) $C_7H_7O_4$ ($-C_{27}H_{42}O_{16}$) 123.0446 (−7.4 mDa) $C_7H_7O_2$ ($-C_{27}H_{42}O_{18}$)
34	9.861	Cornuside	$C_{24}H_{30}O_{14}$	542.1636	541.1537	−3.7	379.1029 (+0.4 mDa) $C_{18}H_{19}O_9$ ($-C_6H_{10}O_5$) 169.0137 (−3.9 mDa) $C_7H_5O_5$ ($-C_{17}H_{24}O_9$) 125.0239 (−3.4 mDa) $C_6H_5O_3$ ($-C_{18}H_{24}O_{11}$)

the constituents in rat serum after oral administration of FC, and potential bioactive compounds in FC may be discovered. The screening results of bioactive components in FC will be helpful for the further research of its action mechanism on molecular level. Most importantly, this work provided a method for rapid and global discrimination

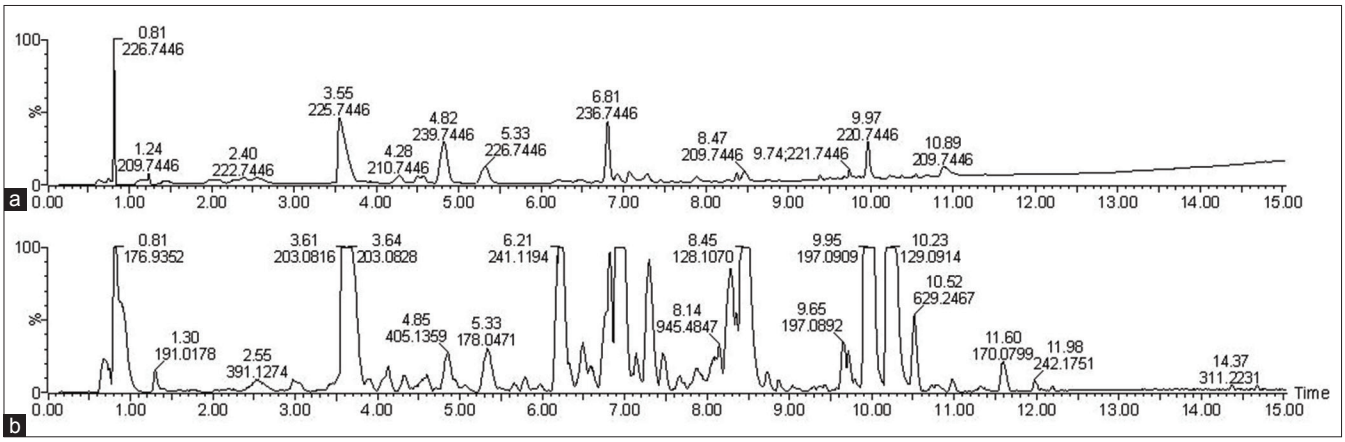

Figure 3: Analysis of the *in vivo* constituents of Fructus Corni with Metabolynx software. (a) Control serum; (b) Serum after oral administration of Fructus Corni

Table 2: MS data and the fragments of the metabolites *in vivo*

Metabolite name	Formula	m/z found $[M-H]^-$	Error (mDa)	Time (min)	Fragment ions $[M-H]^-$	
Demethylation+hydroxylation+Glucoside conjugation	$C_{16}H_{24}O_{11}$	391.1263	2.2	2.55	113.0239 (+3.9.mDa) $C_5H_5O_3$ 81.0340 (−9.6.mDa) C_5H_5O	85.0290(+8.6.mDa) $C_4H_5O_2$ 59.0133 (−1.1.mDa) $C_2H_3O_2$
Glucuronide conjugation+Demethylation	$C_{15}H_{18}O_{10}$	357.0852	3.0	2.96	169.0501 (+1.2.mDa) $C_8H_9O_4$	137.0603(+4.9.mDa) $C_8H_9O_2$
Unknown	$C_{23}H_{30}N_4O_{14}S$	617.1411	1.0	3.05	Unknown	
Parent	$C_{16}H_{24}O_{10}$	375.1291	0.3	3.37	198.0528(+8.9.mDa) $C_9H_{10}O_5$ 151.0395 (+7.0.mDa) $C_8H_7O_3$	175.0395(+3.3.mDa) $C_{10}H_7O_3$ 59.0133 (+5.1.mDa) $C_2H_3O_2$
2 xHydroxylation+Glucoside conjugation	$C_{17}H_{26}O_{12}$	421.1375	2.9	3.82	179.0344 (−7.5.mDa) $C_9H_7O_4$ 119.0497 (+1.3.mDa) C_8H_7O	127.0395 (+4.9.mDa) $C_6H_7O_3$ 67.0184 (+8.5.mDa) C_4H_3O
Unknown	$C_9H_8O_3$	163.0379	−1.6	3.88	Unknown	
Demethylation	$C_9H_{10}O_4$	181.0482	−1.9	3.88	165.0552 (+3.1.mDa) $C_9H_9O_3$ 107.0497 (−0.2.mDa) C_7H_7O	119.0497 (+8.0.mDa) C_8H_7O
Glucuronide conjugation	$C_{16}H_{20}O_{10}$	371.1038	6.0	4.28	195.0657(−4.1.mDa) $C_{10}H_{11}O_4$ 134.0579 (+6.2.mDa) $C_5H_{10}O_4$ 113.0239 (+3.5.mDa) $C_5H_5O_3$	151.0759 (+5.7.mDa) $C_9H_{11}O_2$ 125.0603 (+3.7.mDa) $C_7H_9O_2$
Glucoside conjugation	$C_{16}H_{24}O_{10}$	375.1288	−0.3	4.33	195.0657 (−4.1.mDa) $C_{10}H_{11}O_4$ 69.0340 (+1.1.mDa) C_4H_5O	113.0239 (+2.9.mDa) $C_5H_5O_3$
Hydroxymethylene loss	$C_9H_{10}O_3$	165.0546	−0.6	4.47	125.0603 (+9.5.mDa) $C_7H_9O_2$	
Parent	$C_{23}H_{20}O_9$	439.1062	0.6	4.49	Unknown	
Parent	$C_{18}H_{26}O_{13}$	449.1299	0.4	4.49	Unknown	
2 x Hydroxylation+Desaturation	$C_{34}H_{48}O_{22}$	807.2563	0.4	4.50	403.1240(+1.4.mDa) $C_{17}H_{23}O_{11}$ 374.1213 (−6.4.mDa) $C_{16}H_{22}O_{10}$ 193.0501 (−8.7.mDa) $C_{10}H_9O_4$	389.1448(+4.2.mDa) $C_{17}H_{25}O_{10}$ 361.1499 (−3.6.mDa) $C_{16}H_{25}O_9$
Alkenes to dihydrodiol	$C_{34}H_{52}O_{22}$	811.2887	1.5	4.72	405.1397(+5.7.mDa) $C_{17}H_{25}O_{11}$ 280.1158(+5.1.mDa) $C_{11}H_{20}O_8$ 101.0239(+2.5.mDa) $C_4H_5O_3$	391.1240(+3.6.mDa) $C_{16}H_{23}O_{11}$ 243.0869 (−5.0.mDa) $C_{11}H_{15}O_6$
B: Glucoside conjugation	$C_{17}H_{26}O_{11}$	405.1363	−3.4	4.85	123.0446 (+9.9.mDa) $C_7H_7O_2$ 89.0239 (+7.2.mDa) $C_3H_5O_3$	101.0239 (+8.3.mDa) $C_4H_5O_3$ 59.0133 (+8.8.mDa) $C_2H_3O_2$
Unknown	$C_7H_8O_4$	155.0347	0.2	4.86	Unknown	
Glucoside conjugation+Glucuronide conjugation	$C_{23}H_{34}O_{17}$	581.1815	9.7	5.03	405.1397(+3.6.mDa) $C_{17}H_{25}O_{11}$ 243.0869 (−3.8.mDa) $C_{11}H_{15}O_6$ 193.0348 (−8.6.mDa) $C_6H_9O_7$	391.1604(+5.6.mDa) $C_{17}H_{27}O_{10}$ 101.0239(−2.2.mDa) $C_4H_5O_3$ 113.0239 (−0.4.mDa) $C_5H_5O_3$
Sulfate conjugation+2 x Glucoside conjugation	$C_{23}H_{36}O_{18}S$	631.1544	0	5.17	233.1025(−1.7.mDa) $C_{10}H_{17}O_6$ 130.0994 (−9.8.mDa) $C_7H_{14}O_2$ 107.0133 (0.0.mDa) $C_6H_3O_2$	153.0552 (+7.8.mDa) $C_8H_9O_3$ 123.0446 (−4.2.mDa) $C_7H_7O_2$ 101.0239 (−1.7.mDa) $C_4H_5O_3$
Unknown	$C_{10}H_{12}O_7S$	275.0266	4.0	5.27	Unknown	
(N-acetylcysteine conjugation) + Two sequential desaturations+Thioureas to Ureas	$C_{15}H_{15}NO_8$	336.0709	−1.1	5.37	293.1600 (+5.2.mDa) $-C_3O_2$	125.0603 (+9.6.mDa) $C_7H_9O_2$
Unknown	$C_9H_{10}O_6S$	245.0118	−0.2	5.49	Unknown	

Contd...

Table 2: Contd...

Metabolite name	Formula	m/z found $[M-H]^-$	Error (mDa)	Time (min)	Fragment ions $[M-H]^-$	
2 x Glucoside conjugation	$C_{23}H_{36}O_{15}$	551.2029	5.3	5.91	288.1209 (−6.5.mDa) $C_{13}H_{20}O_7$ 180.0634 (−2.5.mDa) $C_6H_{12}O_6$ 123.0446 (+6.8.mDa) $C_7H_7O_2$ 108.0211(+2.8.mDa) $C_6H_4O_2$	249.0974(+3.0.mDa) $C_{10}H_{17}O_7$ 142.0630 (+8.0.mDa) $C_7H_{10}O_3$ 113.0239 (+4.0.mDa) $C_5H_5O_3$ 103.0395 (0.1.mDa) $C_4H_7O_3$
Hydroxylation+desaturation+ Glucoside conjugation+ Methylation	$C_{18}H_{26}O_{11}$	417.1453	5.6	6.28	168.0786(+7.3.mDa) $C_9H_{12}O_3$ 123.0446 (+8.3.mDa) $C_7H_7O_2$ 101.0239(+6.0.mDa) $C_4H_5O_3$	135.0446 (−0.5.mDa) $C_8H_7O_2$ 113.0239 (+6.1.mDa) $C_5H_5O_3$
Methylation+2 x Hydroxylation	$C_{12}H_{18}O_7$	273.0999	8.9	6.40	227.0919(+1.2.mDa) $C_{11}H_{15}O_5$ 137.0239 (−5.1.mDa) $C_7H_5O_3$ 101.0239 (−0.4.mDa) $C_4H_5O_3$	167.0708 (+7.5.mDa) $C_9H_{11}O_3$ 110.0368 (−5.8.mDa) $C_6H_6O_2$
Acetylation+Glycine conjugation+Two sequential desaturation	$C_{15}H_{17}NO_8$	338.0866	−1	6.47	198.0892(+6.9.mDa) $C_{10}H_{14}O_4$ 187.0970 (−8.5.mDa) $C_9H_{15}O_4$	132.0211 (−0.1.mDa) $C_8H_4O_2$ 131.0708(−1.3.mDa) $C_6H_{11}O_3$
Glucoside conjugation	$C_{17}H_{24}O_{11}$	403.1269	2.8	6.68	357.1186(+4.5.mDa) $C_{16}H_{21}O_9$ 179.0556 (+0.6.mDa) $C_6H_{11}O_6$ 125.0239 (−1.2.mDa) $C_6H_5O_3$ 81.0340 (−2.7.mDa) C_5H_5O	195.0657(+3.0.mDa) $C_{10}H_{11}O_4$ 151.0759 (−8.7.mDa) $C_9H_{11}O_2$ 101.0239 (+5.9.mDa) $C_4H_5O_3$ 59.0133 (+2.5.mDa) $C_2H_3O_2$
Reduction	$C_{34}H_{52}O_{20}$	779.3005	3.1	6.70	389.1084(−2.9.mDa) $C_{16}H_{21}O_{11}$ 127.0395(+1.8.mDa) $C_6H_7O_3$	227.0919(+3.3.mDa) $C_{11}H_{15}O_5$ 101.0239 (−0.9.mDa) $C_4H_5O_3$
Unknown	$C_{15}H_{26}N_2O_1S$	425.1227	−0.3	6.81	Unknown	
Glucoside conjugation	$C_{18}H_{28}O_{12}$	435.1400	−10.3	6.82	389.1448(+5.8.mDa) $C_{17}H_{25}O_{10}$ 67.0184 (+8.0.mDa) C_4H_3O	127.0395 (+8.6.mDa) $C_6H_7O_3$
Unknown	$C_6H_8O_6$	175.0235	−0.8	6.83	Unknown	
Hydroxylation+desaturation+ Glucoside conjugation+ Hydroxylation+methylation	$C_{17}H_{22}O_{11}$	401.1070	−1.4	6.85	155.0344(−0.8.mDa) $C_7H_7O_4$ 113.0239 (+6.8.mDa) $C_5H_5O_3$	125.0239 (−6.6.mDa) $C_6H_5O_3$ 83.0497 (−8.7.mDa) C_5H_7O
Glucuronide conjugation	$C_{17}H_{24}O_{11}$	403.1235	−0.6	7.07	229.1076 (−0.2.mDa) $C_{11}H_{17}O_5$ 117.0340 (−4.0.mDa) C_8H_5O 101.0239 (+9.0.mDa) $C_4H_5O_3$ 67.0184 (+7.6.mDa) C_4H_3O 175.0243(+21.9.mDa) $C_6H_7O_6$	134.0368 (+9.8.mDa) $C_8H_6O_2$ 107.0497 (+4.9.mDa) C_7H_7O 97.0290 (+9.2.mDa) $C_5H_5O_2$ 113.0239 (+9.5.mDa) $C_5H_5O_3$
Glucoside conjugation+Glucuronide conjugation	$C_{23}H_{34}O_{16}$	565.1813	4.4	7.07	283.1393(+1.0.mDa) $C_{11}H_{23}O_8$ 161.0450 (+1.9.mDa) $C_6H_9O_5$ 113.0239(+2.3.mDa) $C_5H_5O_3$	227.0919 (−1.0.mDa) $C_{11}H_{15}O_5$ 101.0239 (−2.0.mDa) $C_4H_5O_3$
Acetylation+Desaturation	$C_{13}H_{16}O_7$	283.0822	0.4	7.50	150.0681(−3.7.mDa) $C_9H_{10}O_2$ 130.0419(−8.7.mDa) C_9H_6O 85.0290 (+4.0.mDa) $C_4H_5O_2$	108.0575 (−1.1.mDa) C_7H_8O 98.0368 (+8.7.mDa) $C_5H_6O_2$

constituents of herbal medicines in biological samples. In a word, UPLC-ESI-Q-TOF-MS is a powerful approach for *in vivo* bioactive compounds identification studies. This study may provide useful information for the further study of pharmacology and mechanism of FC. We expected that approach would be useful for the screening and characterization of compounds in other famous herb medicines and related medicinal products.

CONCLUSIONS

In this study, UPLC-ESI-Q-TOF-MS was developed for structural characterization of chemical constituents of FC after i.g. administration of FC. As a result, a total of 34 compounds were successfully separated and 23 compounds were identified. To screen the potentially bioactive components in FC, rat plasma samples were similarly investigated after the administration of FC. Of note, the 25 detected compounds were identified after oral administration of FC, which might be the potential active components *in vivo*. Our study demonstrated that UPLC-ESI-Q-TOF-MS followed by a more rapid and sensitive detection is a potentially powerful technique for achieving simultaneously rapid screening and analysis of multiple bioactive compounds in FC.

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