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Analysis of the constituents in the rat plasma after oral administration of Yin Chen Hao Tang by UPLC/Q-TOF-MS/MS

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Abstract

A UPLC/Q-TOF-MS/MS method for analyzing the constituents in rat plasma after oral administration of Yin Chen Hao Tang (YCHT), a traditional Chinese medical formula, has been established. The UPLC/MS fingerprints of the samples were established first *in vitro and in vivo*, with 45 compounds in YCHT and 21 compounds in rat plasma after oral administration of YCHT were detected. Of the 45 detected compounds *in vitro*, 30 were identified, and all of the 21 compounds detected in rat plasma were identified either by comparing the retention time and mass spectrometry data with that of reference compounds or by mass spectrometry analysis and retrieving the reference literatures. Of the identified 21 compounds in rat plasma, 19 were the original form of compounds absorbed from the 45 detected compounds *in vitro*, 2 were the metabolites of the compounds existed in YCHT. It is concluded that a rapid and validated method has been developed based on UPLC–MS/MS, which shows high sensitivity and resolution that is more suitable for identifying the bioactive constituents in plasma after oral administration of Chinese herbal medicines, and provides helpful chemical information for further pharmacology and active mechanism research on the Chinese medical formula.

Keywords: Yin Chen Hao Tang; UPLC/Q-TOF-MS/MS; Constituents in rat plasma; Metabolite

1. Introduction

Yin Chen Hao Tang (YCHT) is a widely used traditional Chinese Medicine (TCM) formula composed of Flos Artemisiae, Fructus Gardeniae Jasminoidis, and Radix et Rhizoma Rhei. It has a broad spectrum of applications as a hepatoprotective, anti-inflammatory, antioxidant, anti-tumor agent, antipyretic, cholagogue, choleretic and diuretic agent for liver disorders (and jaundice), as well as for treatment of diabetes, and other conditions [1,2].

Chemical studies on the composition herbs Flos Artemisiae (capillary wormwood), Fructus Gardeniae Jasminoidis (gardenia fruit) and Radix et Rhizoma Rhei (Chinese rhubarb) have been conducted, respectively and systematically. Constituents such as coumarin, flavone, chromone, anthraquinone, organic acids, enzyme, triterpene and sterides have been experimentally verified [3]. However, there has been no fully integrated study of the constituents in the formula which is not simply combinations of the individual herbs but a prepared decotion. It has been reported that 6,7-dimethylesculetin, chlorogenic acid, capillarisin, geniposide and rhein are the main bioactive components [4–9] of the formula, but previous analysis of bioactive constituents of Yin Chen Hao Tang (YCHT) only reveal rather sparse data. The classical research method for active constituents of herbal or medical formulas *in vitro* is by fractional extracting guided with bioeffect, and this is considered to be time intensive and expensive. In addition, the effectiveness cannot be elucidated due to the small amount of isolated compound and the lack of high throughput effects screening method. To resolve the drawbacks of the classic method, and make most of the obtained compound identified, our research group has established a new methodology for searching the effective constituents of Chinese herbal formulas, and it is named "Plasma pharmacochemistry" [10].

Plasma pharmacochemistry has been suggested according to the process of forming, absorbing, distributing and show-

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Table 1
Structures of the known constituents found in Flos Artemisiae [3]

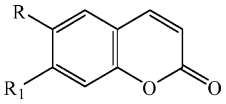
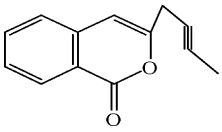
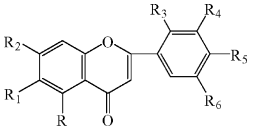
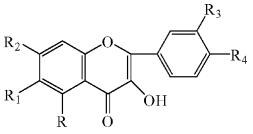
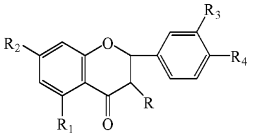
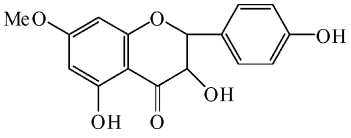
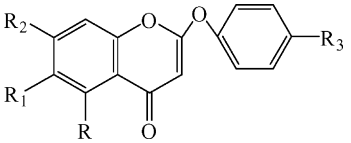
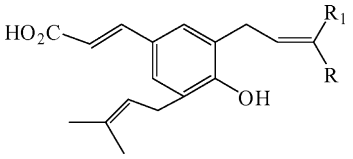
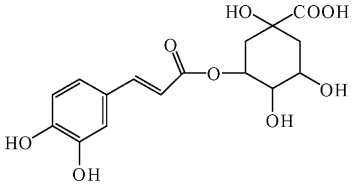
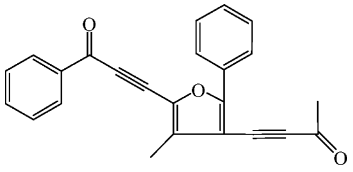
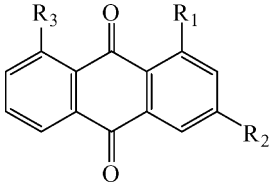
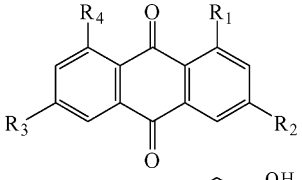
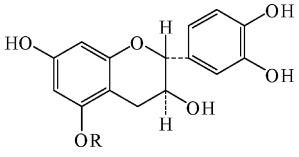
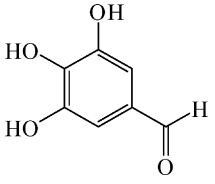
Type	Substituent group	Name of compound	Molecular weight
	R=R$_1$=OCH$_3$	6,7-Dimethylesculetin	206.20
	R=OH, R$_1$=OCH$_3$	7-Methylesculetin	192.17
	R=OCH$_3$, R$_1$=OH	6-Methylesculetin	192.17
	R=H, R$_1$=OCH$_3$	7-Methoxycoumarine	176.17
		Capillarin	198.22
	R=R$_5$=OH, R$_1$=R$_2$=OCH$_3$, R$_3$=R$_4$=R$_6$=H	Circimaritin	314.30
	R=R$_3$=R$_5$=OH, R$_1$=R$_2$=R$_6$=OCH$_3$, R$_4$=H	Arcapillin	360.32
	R=R$_1$=R$_5$=OH, R$_2$=R$_3$=R$_6$=OCH$_3$, R$_4$=H	Isoarcapillin	360.32
	R=R$_5$=OH, R$_1$=R$_2$=R$_4$=OCH$_3$, R$_3$=R$_6$=H	Cirsilineol	344.32
	R=R$_5$=OH, R$_2$=OCH$_3$, R$_1$=R$_3$=R$_4$=R$_6$=H	Genkwanin	252.27
	R=R$_4$=R$_5$=OH, R$_1$=R$_2$=OCH$_3$, R$_3$=R$_6$=H	5,3′,4′-Trihydroxy-6,7-dimeth-oxyflavone	330.30
	R=R$_4$=OH, R$_2$=OCH$_3$, R$_1$=R$_3$=H	Rhamnocitrin	300.27
	R=R$_4$=OH, R$_1$=R$_2$=OCH$_3$, R$_3$=H	Eupalitin	330.30
	R=R$_3$=R$_4$=OH, R$_1$=R$_2$=OCH$_3$	Eupatolitin	346.30
	R=R$_2$=R$_3$=R$_4$=OH, R$_1$=H	Quercetin	302.24
	R=R$_2$=R$_4$=OH, R$_3$=OCH$_3$, R$_1$=H	Isorhamnetin	316.27
	R=O–Glc, R$_1$=R$_2$=R$_3$=R$_4$=OH	Quercetin-3-*o*-glucoside	464.18
	R=O–Glc-Rha, R$_1$=R$_2$=R$_3$=R$_4$=OH	Rutin	610.53
	R=O–Gal–O–Glc, R$_1$=R$_2$=R$_3$=R$_4$=OH	Quercetin-3-gluco-galactoside	626.53
	R=O–Glc–Rha, R$_2$=O–Glc, R$_1$=R$_3$=R$_4$=OH	Quercetin-3,7-rutinoso-digalactoside	772.67
	R=O–Gal–Rha, R$_1$=R$_2$=R$_3$=R$_4$=OH	Quercetin-3-D-robinoside	610.53
	R=O–Gal, R$_1$=R$_2$=R$_3$=R$_4$=OH	Quercetin-3-*o*-D-galactoside	464.39
	R=O–Gal–Glc, R$_1$=R$_2$=R$_4$=OH, R$_3$=H	Kaempferol-3-gluco-galactoside	610.53
	R=O–Gal–Rha, R$_1$=R$_2$=R$_4$=OH, R$_3$=OCH$_3$	Isorhamnetin-3-*o*-D-robinoside	624.56
	R=O–Gal, R$_1$=R$_2$=R$_4$=OH, R$_3$=OCH$_3$	Isorhamnetin-3-*o*-D-galactoside	478.41
	R=O–Glc, R$_1$=R$_2$=R$_4$=OH, R$_3$=OCH$_3$	Isorhamnetin-3-glucoside	478.41
		7-Methyl-aromadendrin	302.39
	R=R$_2$=R$_3$=OH, R$_1$=OCH$_3$	Capillarisin	316.27
	R=R$_3$=OH, R$_1$=R$_2$=OCH$_3$	7-Methylcapillarisin	330.30
	R=R$_2$=OH, R$_1$=R$_3$=OCH$_3$	4′-Methylcapillarisin	330.30
	R=R$_2$=R$_3$=OH, R$_1$=H	6-Demethoxycapillarisin	286.24
	R=R$_2$=OH, R$_3$=OCH$_3$, R$_1$=H	6-Demethoxy-4′-methycapillarisin	300.27

Table 1 (*Continued*)

Type	Substituent group	Name of compound	Molecular weight
	$R=CH_2OH$, $R_1=CH_3$	Capillartemisin A	316.40
	$R=CH_3$, $R_1=CH_2OH$	Capillartemisin B	316.40
	$R=R_1=CH_3$	Artepillin C	316.40
		Chlorogenic acid	354.32
		Capillaridin A	352.11

ing the effect of active compounds after oral administration of a formula. The Chinese herbal formula's potentially effective constituents can be ascertained by analyzing compounds absorbed in the blood after oral administration. Because only the absorbed compounds have the chance to show the effects, and furthermore the compounds in the herbs is the secondary metabolites, some of them have no significant effects, they must be transformed to another form to show the bioactivity, therefore only the compounds in the blood have the probability become to the effective constituents. This pathway has provided

Table 2
Structures of the known constituents found in Radix et Rhizoma Rhei [14]

Type	Substituent group	Name of compound	Molecular weight
	$R_1=R_3=OH$, $R_2=CH_2OH$	Aloeemodin	270.24
	$R_1=R_3=OH$, $R_2=CH_2–O–Glc$	Aloeemodin-*o*-D-glucopyranoside	432.39
	$R_1=R_3=OH$, $R_2=CH_3$	Chrysophanol	254.24
	$R_1=R_3=OH$, $R_2=COOH$	Rhein	284.23
	$R_1=OH$, $R_2=CH_3$, $R_3=O–Glc$	Chrysophano-8-*o*-D-glucopyranoside	176.17
	$R_1=R_2=OH$, $R_3=CH_3$, $R_4=O–Glc$	Anthraglycoside B	432.39
	$R_1=R_2=R_4=OH$, $R_3=CH_3$	Emodin	270.24
	$R_1=R_4=OH$, $R_2=OCH_3$, $R_3=CH_3$	Physcion	284.27
	R=H	Epicatechin	290.28
	R=Glc	(+)-Catechin-5-*o*-glucoside	452.42
		Gallic acid	170.12

significant advantages in quickly screening *in vivo*, giving a high probability of hitting, and being consonant with characteristics of traditional Chinese medicine. Our group has carried out preliminary studies on the constituents in blood after oral administration of YCHT instructed by plasma pharmacochemical ideas [11,12]. However, due to limitations of UV detector and the resolving power of conventional HPLC with 5 μm C_{18} particle size column, the chemical information obtained under such chromatography conditions was not enough. Even though the chromatographic data was recorded for 80 min, only 12 chromatographic peaks were detected *in vitro*, and two main ingredients (6,7-dimethylesculetin and capillarisin) in plasma were detected after oral administration of YCHT.

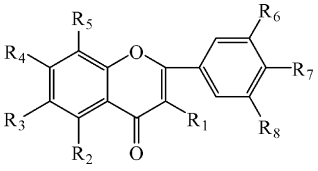
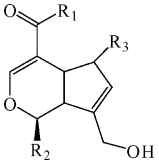
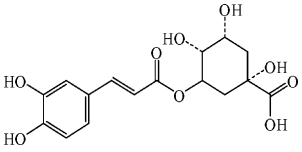
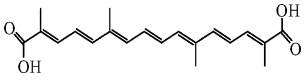
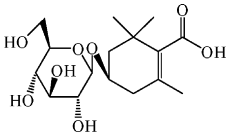
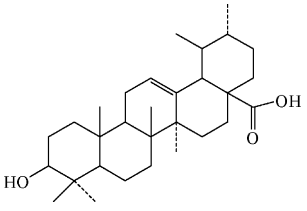
The present work is to identify the potential bioactive constituents as much as possible in the plasma after oral administration of YCHT, a UPLC–MS detector and C_{18} column with a 1.7 μm particle size was utilized, a thorough analysis of a great deal of information of the constituents in the rat plasma was undertaken. UPLC/Q-TOF/MS has compensate for the deficiency of conventional HPLC–UV detector and C_{18} column with a 5 μm particle size due to the high throughput and higher sensitivity and resolution. As a result, 45 peaks YCHT and 21 peaks in plasma after oral administration of YCHT were detected only in 20 min recorded chromatography. The structure identification of the detected compounds was confirmed by using UV spectrum, MS fragments and by comparing with them to standards. UPLC–MS technology coupled with plasma pharmacochemistry should enhance the elucidation of effective constituents of traditional Chinese herbal formula.

2. Experimental

2.1. Chemicals and materials

LC grade acetonitrile was purchased from Dikma Technology Inc. (Richmond Hill, ON, Canada). Deionized water was purified by the Milli-Q system (Millipore, Bedford, MA, USA). Formic acid and phosphoric acid was of an analytical grade pur-

Table 3
Structures of the known constituents found in Gardeniae Jasminoidis, Fructus [14]

Type	Substituent group	Name of compound	Molecular weight
	R_1=R_3=R_4=R_7=R_8=OCH_3, R_2=OH, R_5=R_6=H	Artemisetin	388.38
	R_1=R_2=R_3=R_7=R_8=OCH_3, R_4=R_6=OH, R_5=H	3′,5-Dihydroxy-3,4′,5′,6,7-pentamethoxy flavone	404.38
	R_1=R_3=R_6=H, R_5=OCH_3 R_2=R_4=R_7=R_8=OH,	4′-Dihydroxywogonin	316.37
	R_3=R_4=R_5=R_6=R_7=R_8=OCH_3 R_1=H, R_2=OH	Gardenin	418.40
	R_1=OCH_3, R_2=OH, R_3=H	Genipin	226.23
	R_1=OCH_3, R_2=O–Glc–Glc, R_3=H	Genipingentiobioside	550.52
	R_1=OCH_3, R_2=O–Glc, R_3=H	Geniposide	388.37
	R_1=OH, R_2=O–Glc, R_3=H	Geniposidic acid	374.35
	R_1=OCH_3, R_2=O–Glc, R_3=OH	Scandoside methylester	404.37
		Chlorogenic acid	354.32
		a-Crocetin	328.41
		Picrocrocinic acid	346.38
		Ursolic acid	456.72

chased from Beijing Reagent Company (Beijing, PR China). Gallic acid, chlorogenic acid, (−)epicatechin, geniposide, sennoside A, sennoside C, 6,7-dimethylesculetin, capillarisin, rhein, emodin, chrysophanol and kaempferide were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, PR China).

The Flos Artemisiae was purchased from Xinneitian Pharmaceutical Inc. (China Branch Office, Japan); Fructus Gardeniae Jasminoidis and Radix et Rhizoma Rhei were purchased from Harbin Tongrentang Drug Company (Harbin, China). All the crude drugs were of high quality and were authenticated by Prof. Xijun Wang of the Pharmacognosy Department, Heilongjiang University of Chinese Medicine. YCHT was prepared according to the method recorded in "*Shanghanlun*", an ancient traditional Chinese medical text [13], and the extract was freeze-dried.

The name and structure of the known constituents in Flos Artemisiae, Fructus Gardeniae Jasminoidis and Radix et Rhizoma Rhei are listed in Tables 1–3.

2.2. Animals

Male Wistar rats (200 ± 20 g) were obtained from the Laboratory Animal Center of Heilongjiang University of Chinese Medicine (Harbin, China). Animals were bred in a breeding room with temperature of 24 ± 2 °C, humidity of 60 ± 5%, and 12 h dark–light cycle. They were given tap water and fed normal food *ad libitum*. All the experiment animals were housed under the above conditions for 3 days acclimation, and were fasted overnight before the experiments. The animal facilities and protocols were approved by the Institutional Animal Care and Use Committee, Heilongjiang University of Chinese Medicine. All procedures were in accordance with the National Institute of Health's guidelines regarding the principles of animal care (2004).

2.3. Sample preparation

2.3.1. Preparation of samples for analysis in vitro

According to the original composition and preparation method of YCHT recorded in '*Shanghan Lun*', YCHT was prepared in the following procedure. Flos Artemisiae (18 g) was boiled in 1400 mL distilled water until the volume of water reduced to about 400 mL, then 9 g Fructus Gardeniae Jasminoidis and 6 g Radix et Rhizoma Rhei were added, and kept it boiling for 10 more minutes. The extracted solution was filtered through 5 layer gauzes and made to a concentration of 1 g crude drug per milliliter, and finally the solution was freeze-dried. Two hundred milligrams of the freeze-dried powder were extracted with 50 mL methanol for 30 min under ultrasonics. The methanol extraction was centrifuged at 13,000 rpm for 10 min at 4 °C, and the supernatant was filtered through a 0.20 μm-filter, the filtrate was used as UPLC sample.

2.3.2. Preparation of sample for analysis in vivo

Freeze-dried powder of 'YCHT' was dissolved with distilled water as stock solution (0.8 g/mL). The above solution was orally administrated to male Wistar rats (1 mL/100 g body weight). Fifteen minutes after drug administration, the animals were anaesthetized by intraperitoneal injection of 1% pentobarbital sodium (0.15 mL/100 g body weight). The blood was collected from the hepatic portal vein and then centrifuged at 13,000 rpm for 5 min at 4 °C. The supernatant obtained were frozen immediately and stored at −26 °C, and thawed before analysis. Phosphoric acid (10 μL) and some internal standard kaempferide solution was added to 500 μL of the above supernatant and ultrasonicated for 1 min, and vortexed for 30 s. The mixed solution was applied to a pre-activated OASIS HLB solid phase extraction C_{18} column (30 μm, 60 mg, Waters Corporation, USA). The column was washed with 1 mL of water, 1 mL of 2% methanol and 2 mL of 100% methanol. The 100% methanol

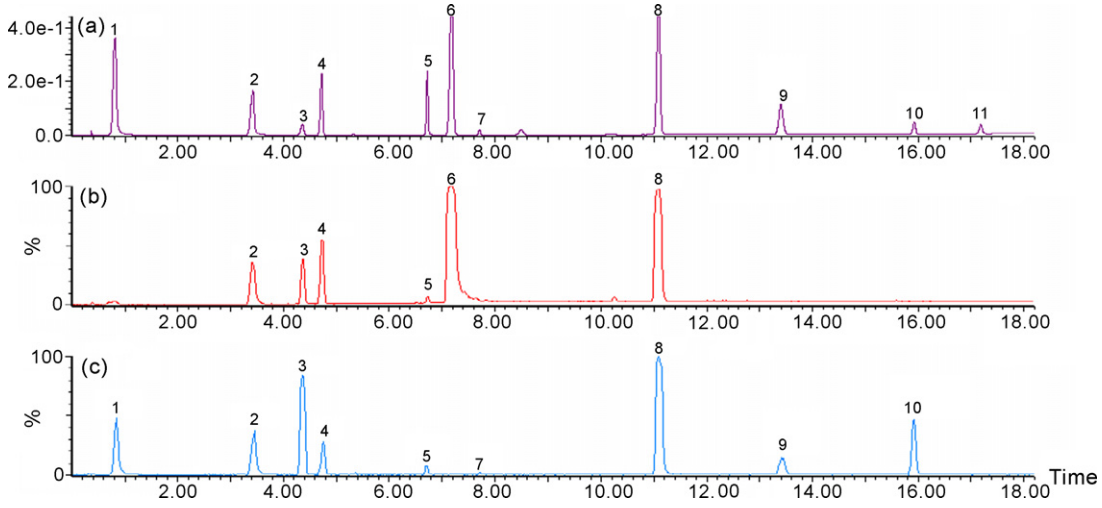


Fig. 1. Chromatograms of reference compounds by UPLC–UV-Q-TOF. (a) UPLC–UV chromatogram at 254 nm. (b) TIC chromatogram in positive ESI mode. (c) TIC chromatogram in negative ESI mode. Peaks (1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11) are of gallic acid, chlorogenic acid, (−)epicatechin, geniposide, sennoside A, 6,7-dimethoxy coumarin, sennoside C, capillarisin, rhein, emodin and chrysophanol, respectively.

elutes were collected and dried under nitrogen gas at 45 °C. The residues were re-dissolved in 350 μL of methanol and filtered through a 0.20 μm-filter, the filtrate was used as UPLC sample.

2.4. Instrumentation and conditions

2.4.1. UPLC–UV analysis

Waters Acquity^{TM} Ultra Performance LC system (Waters Corporation, Milford, USA) equipped with quaternary pump, vacuum degasser, autosampler, diode-array detector. The chromatographic condition was as follow: UPLC^{TM} BEH C_{18} column (1.7 μm, 2.1 mm × 50 mm); mobile phase: a linear gradient system of A (HCOOH:H_2O = 0.1:100) and B (HCOOH:CH_3CN = 0.1:100), the gradient program is shown in Table 4; flow rate: 0.4 mL/min; column temperature: 45 °C; detecting wavelength: 254 nm; injection volume: 5 μL.

Table 4
Solvent gradient program of UPLC analysis

Time (min)	Flow (ml/min)	*A* (%)	*B* (%)
0	0.400	99.0	1.0
1.0	0.400	99.0	1.0
1.5	0.400	97.0	3.0
13.5	0.400	68.0	32.0
18.5	0.400	40.0	60.0
20.5	0.400	1.0	99.0

2.4.2. UPLC/Q-TOF analysis

Waters Micromass Q-TOF-micro^{TM} (Manchester, UK) equipped with an electrospray ion source operating in either positive ion or negative ion mode, and with Masslynx data analysis system. The ion source temperature was set at 110 °C with

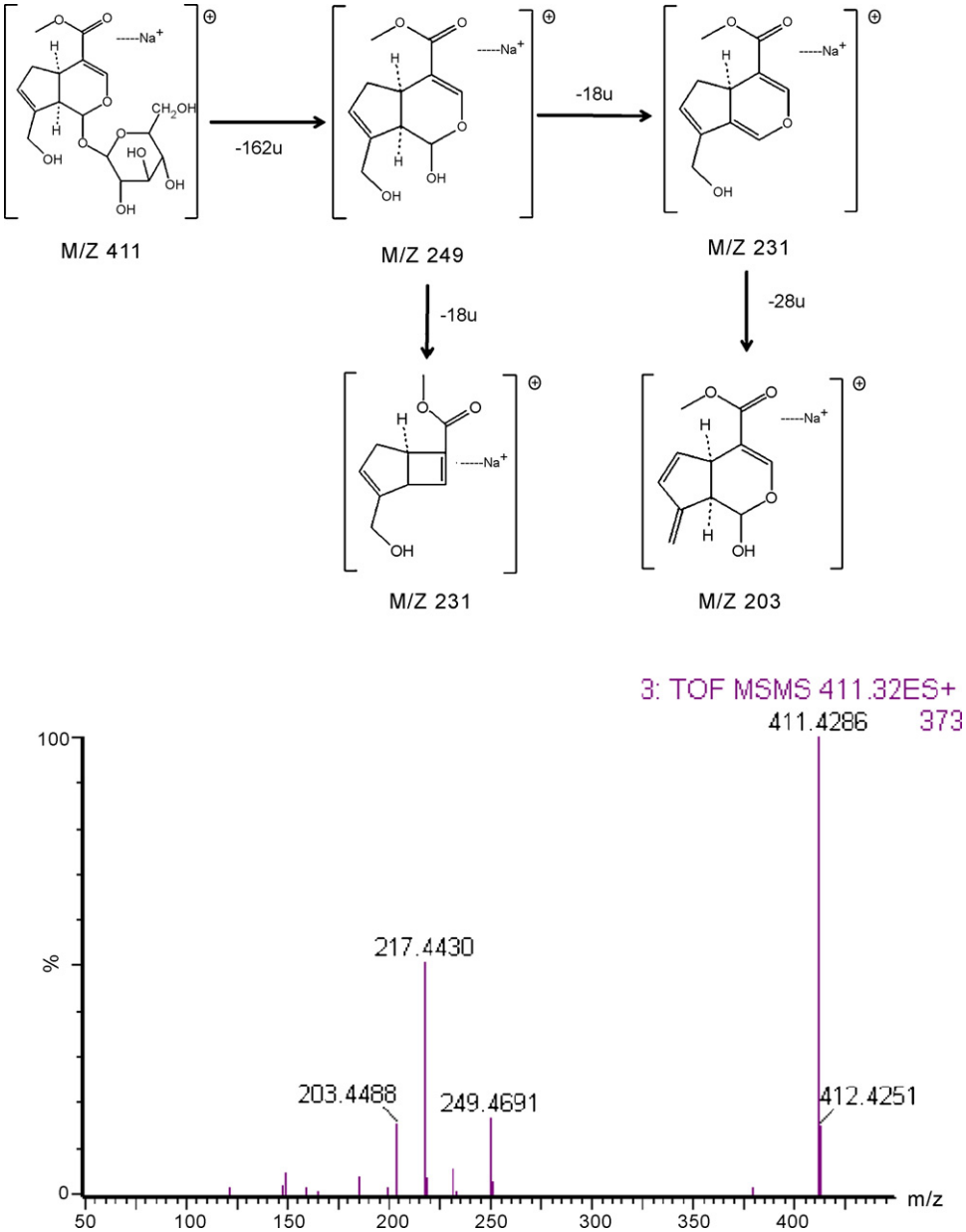


Fig. 2. Analysis of parent ion spectra and product ion spectra of geniposide (peak 4 in Fig. 1) using ESI-MS scanning in positive ion mode.

a cone gas flow of 100 L h^{-1}, a desolvation gas temperature of 300 °C and a desolvation gas flow of 600 L h^{-1}. The capillary voltage was set at 3.1 kV for positive ion mode and 2.8 kV for negative ion mode; the cone voltage was up to 35 V. A scan time of 0.48 s with an inter-scan delay of 0.1 s was used throughout, with collision energy of 3 eV and a collision gas pressure of $\sim 2.8 \times 10^{-3}$ mbar argon. A lock-mass of leucine enkephalin at a concentration of 0.5 ng μL^{-1}, in 50:50 acetonitrile:water (0.1% formic acid) for positive ion mode ($[M+H]^+ = 556.2771$) and 1 ng μL^{-1}, in 50:50 acetonitrile:water for negative ion mode ($[M-H]^- = 554.2615$), was employed at a flow rate of 60 $\mu L\ min^{-1}$ via a lockspray interface. Systematic data was collected in centroid mode, the lockspray frequency was set at 5 s and the lock-mass data was averaged over a 10 s scan for correction. The mass spectrometric data was collected in full scan mode, the *m/z* were from 100 to 900 in positive and negative ion.

3. Results and discussions

3.1. Optimization of MS conditions

To obtain better detection, the MS conditions should be optimized and a statistical orthogonal design was employed for optimization [15]. The effects of formic acid concentration, desolvation temperature, source temperature, capillary voltage, and desolvation gas flow were examined. In the middle of gradient elution, capillarisin, one of the reference compound, was used as a test compound, since capillarisin showed ideal ion signals in either positive ion mode or negative ion mode. The experiments

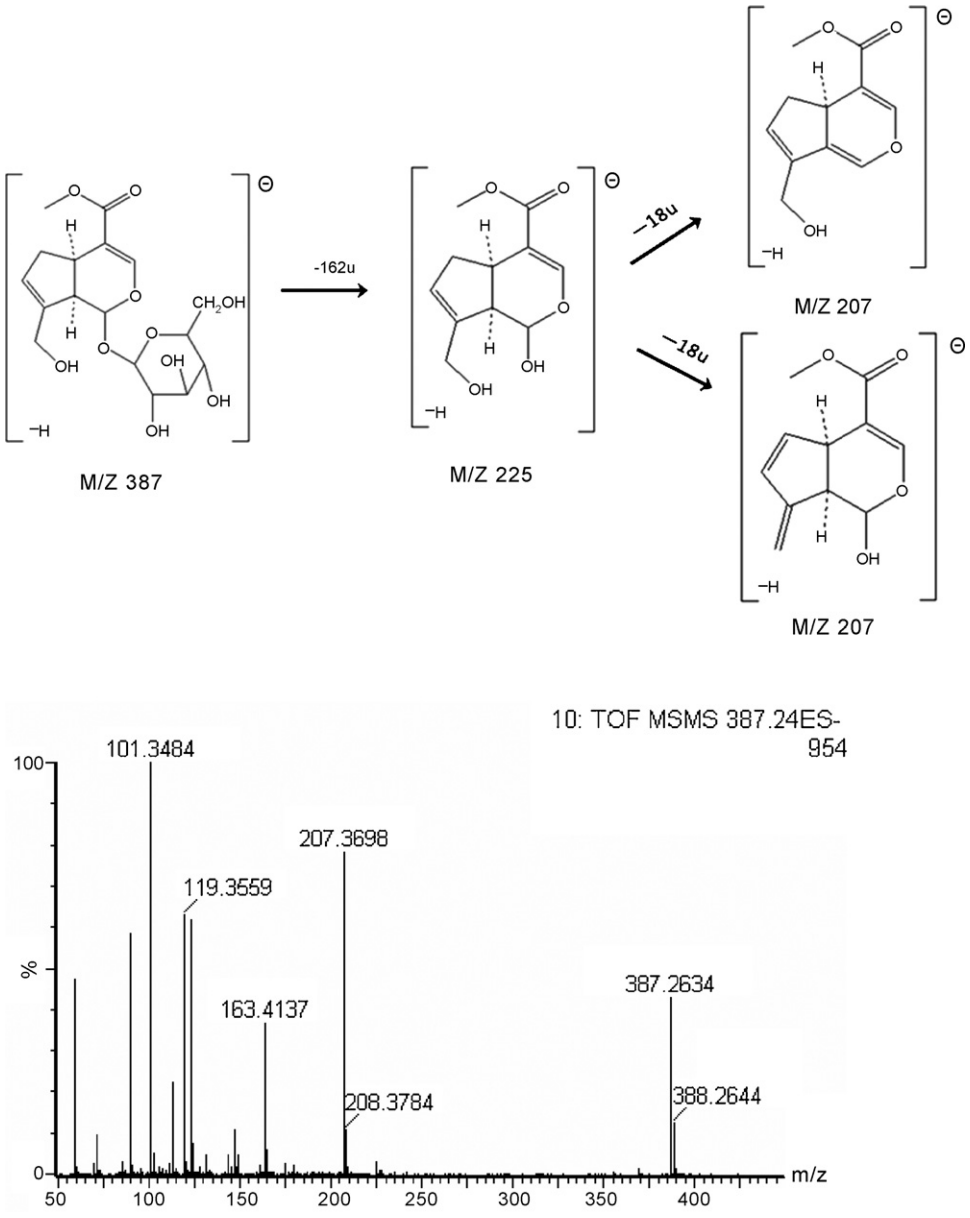


Fig. 3. Analysis of parent ion spectra and product ion spectra of geniposide (peak 4 in Fig. 1) using ESI-MS scanning in negative ion mode (peak 4).

were arranged according to $L_{16}(4^5)$ orthogonal design table: formic acid concentration (0.005%, 0.01%, 0.05%, 0.1%), desolvation temperature (250 °C, 275 °C, 300 °C, 325 °C), source temperature (100 °C, 105 °C, 110 °C, 115 °C), capillary voltage (positive ion ESI mode 2.6 kV, 2.8 kV, 3.0 kV, 3.2 kV; negative ion ESI mode 2.2 kV, 2.4 kV, 2.6 kV, 2.8 kV), desolvation gas flow (500 L h^{-1}, 550 L h^{-1}, 600 L h^{-1}, 650 L h^{-1}), and the peak area was taken as criteria for optimization. The statistical results indicated that the influence of the factors on the ionization were as follows: desolvation temperature and desolvation gas flow were statistical significant at $p < 0.05$. Formic acid concentration, capillary voltage, and source temperature were insignificant at $p > 0.05$. The optimum conditions were decided as follows: 0.1% formic acid, desolvation temperature 300 °C, source temperature 110 °C, desolvation gas (N_2) 600 L h^{-1}, capillary voltage 3.0 kV for positive ion ESI mode and 2.8 kV for negative ion ESI mode.

Ten reference compounds were detected by except chrysophanol of peak 11, 10 other reference compounds were well detected by UPLC–UV-Q-TOF under the above optimum MS conditions. The chromatogram (Fig. 1) of the reference compounds provide the information of their retention time and mass spectra for the comparison with those of peaks detected in the plasma sample after oral administration of YCHT.

In negative ion ESI mode, $[M-H]^-$ were observed, in positive ion ESI mode, $[M+H]^+$ and $[M+Na]^+$ were observed. The information of $[M-H]^-$, $[M+H]^+$ and $[M+Na]^+$ were used to determine molecular weight. These compounds were identified by referring to their MS and MS–MS spectra. For example, geniposide of peak 4, based on the detailed fragmentation in spectra of geniposide in (−)ESI-MS and (+)ESI-MS (Figs. 2 and 3), and also the reference [16], the molecular ions, $[M+Na]^+$ at *m/z* 411, $[M-H]^-$ at *m/z* 387, were observed, respectively. The MS–MS fragment ions of peaks 4 at *m/z* 249 $[M+Na-Glc+H_2O]^+$, 231 $[M+Na-Glc]^+$ and 203 $[M+Na-Glc-CO]^+$ in positive ion ESI mode were observed; the MS–MS fragment ions of 225 $[M-H-Glc+H_2O]^-$ 207 $[M-H-Glc]^-$ in negative ion ESI mode were observed.

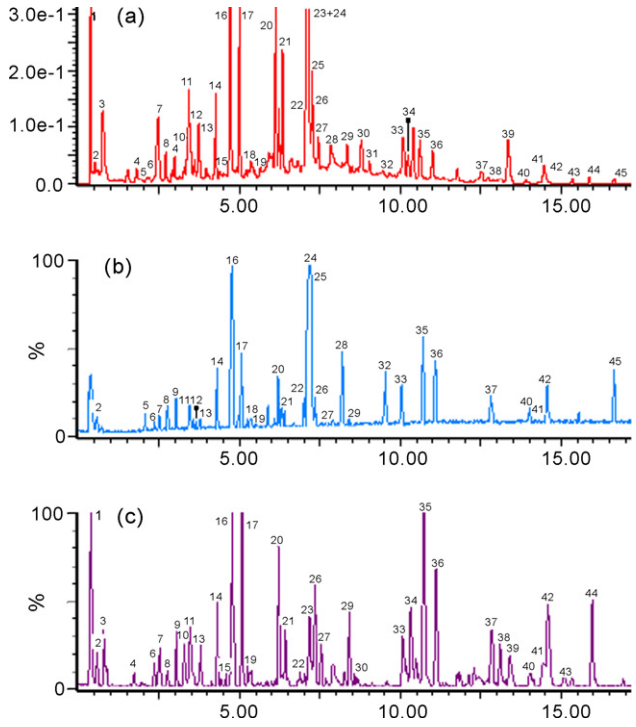


Fig. 4. Chromatograms of Yin Chen Hao Tang by UPLC–UV-Q-TOF. (a) UPLC–UV chromatogram at 254 nm. (b) TIC chromatogram in positive ESI mode and (c) TIC chromatogram in negative ESI mode.

3.2. UPLC–UV-Q-TOF analysis of YCHT

The chromatogram of the sample is shown in Fig. 4. The constituents in YCHT were well separated by using the UPLC–UV-Q-TOF method with 45 peaks and detected, the structures of the identified compounds are shown in Table 5. The MS data of (+)ESI-MS spectra and (−)ESI-MS spectra are shown in Table 6. For most of the constituents, $[M-H]^-$, $[M+HCOO]^-$, and $[M+Na]^+$ were observed. These results provided reliable information for confirming molecular weight

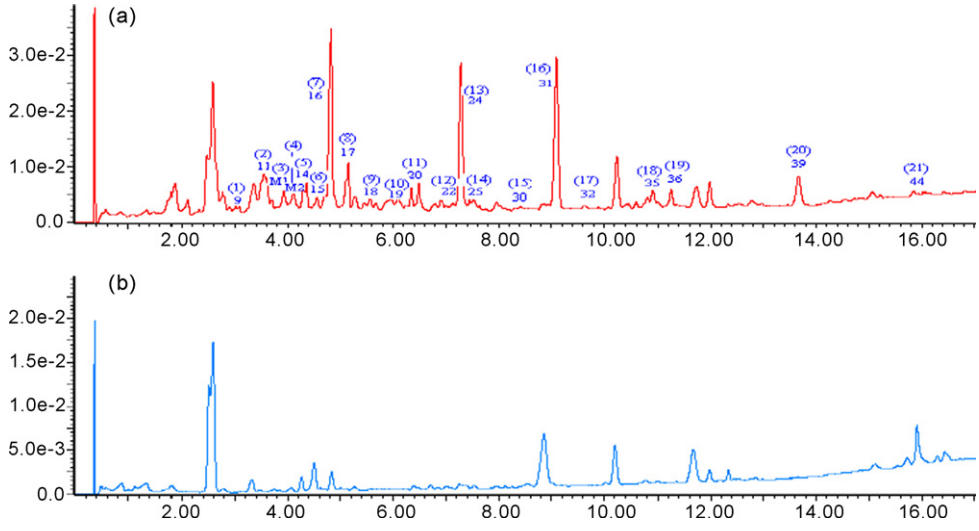


Fig. 5. Chromatograms of rat plasma by UPLC–UV detected at 254 nm. (a) Plasma after oral administration of YCHT and (b) control serum.

Table 5
MS data of (+)ESI-MS spectra and (−)ESI-MS spectra, and the identification results of the constituents of YCHTE *in vitro* or *in vivo*

No.	Identified compounds		T_R	Negative ion (*m*/*z*)		Positive ion (*m*/*z*)		Molecular weight (Da)	Elemental composition
	In vitro	*In vivo*		Indicated	Actual	Indicated	Actual		
1	Gentiobiose	–	0.37	341.1083	341.1084	–	–	342.1162	$C_{12}H_{22}O_{11}$
2	Pyroglutamic acid	–	0.56	128.0345	128.0348	130.0500	130.0504	129.0426	$C_5H_7NO_3$
3	Gallic acid	–	0.77	169.0137	169.0137	–	–	170.0215	$C_7H_6O_5$
4	Protocatechuic acid-3-glucoside	–	1.72	315.0718	315.0716	–	–	316.0794	$C_{13}H_{16}O_9$
5	Not identified	–	2.06	–	–	186.1291	186.1283	–	$C_{13}H_{15}N$
6	Villosolside	–	2.35	361.1495	361.1499	385.1538 $[M+Na]^+$	385.1475	362.1577	$C_{16}H_{26}O_9$
7	Neochlorogenic acid/isochlorogenic acid	–	2.53	353.0872	353.0873	377.0938 $[M+Na]^+$	377.0849	354.0951	$C_{16}H_{18}O_9$
8	Gardenoside B	–	2.74	403.1237 449.1254 $[M-H+46]^-$	403.1240	427.1252 $[M+Na]^+$	427.1216	404.1319	$C_{17}H_{24}O_{11}$
9 (1)[a]	Picrocrocininc acid	Picrocrocininc acid	3.04	345.1563	345.1549	369.158 $[M+Na]^+$	369.1525	346.1628	$C_{16}H_{26}O_8$
10	Not identified	–	3.28	289.0710	289.0712	–	–	–	$C_{15}H_{14}O_6$
11 (2)	Chlorogenic acid	Chlorogenic acid	3.46	353.0876	353.0873	377.0927 $[M+Na]^+$	377.0849	354.0951	$C_{16}H_{18}O_9$
12	Not identified	–	3.63	–	–	369.1619	369.1549	–	$C_{18}H_{24}O_8$
13	Isochlorogenic acid/neochlorogenic acid	–	3.78	353.0867	353.0873	377.0940 $[M+Na]^+$	377.0849	354.0951	$C_{16}H_{18}O_9$
M1 (3)	–	6-methoxy coumarin-7-hydroxyl sulfate	3.88					270.9922	$C_{10}H_8O_7S$
M2 (4)	–	7-methoxy coumarin-6-hydroxyl sulfate	4.05					270.9922	$C_{10}H_8O_7S$
14 (5)	Genipingentiobioside	Genipingentiobioside	4.31	549.1779 595.1921 $[M-H+46]^-$	549.1819	573.1730 $[M+Na]^+$	573.1795	550.1898	$C_{23}H_{34}O_{15}$
15 (6)	Not identified	Not identified	4.53	353.0866	353.0873	377.0920 $[M+Na]^+$	377.0849	354.0951	$C_{16}H_{18}O_9$
16 (7)	Geniposide	Geniposide	4.77	387.1329 433.1343 $[M-H+46]^-$	387.1291	411.1275 $[M+Na]^+$	411.1267	388.1369	$C_{17}H_{24}O_{10}$
17 (8)	Safflor yellow A	Safflor yellow A	5.08	593.1448	593.1506	595.1693	595.1663	594.1585	$C_{27}H_{30}O_{15}$
18 (9)	Capillaridin A	Capillaridin A	5.48	–	–	353.1209	353.1178	352.1099	$C_{24}H_{16}O_3$
19 (10)	5,6-Dimethoxy-7-hydroxy coumarin	5,6-dimethoxy-7-hydroxy coumarin	5.99	221.0449	221.0450	223.0611	223.0606	222.0528	$C_{11}H_{10}O_5$
20 (11)	Quercetin-3-*o*-glycoside	Quercetin-3-*o*-glycoside	6.20	463.0889	463.0877	487.0923 $[M+Na]^+$	487.0852	464.0955	$C_{21}H_{20}O_{12}$
21	Isoquercitrin/quercetin-3-*o*-glycoside	–	6.40	463.0884	463.0877	487.0921	487.0852	464.0955	$C_{21}H_{20}O_{12}$
22 (12)	6,8-Dimethoxy-7-hydroxycoumarin	6,8-dimethoxy-7-hydroxycoumarin	7.00	221.0450	221.0450	223.0613	223.0606	222.0528	$C_{11}H_{10}O_5$
23	3,4-Di-*o*-caffeoylquinic acid	–	7.16	515.1200	515.1190	–	–	516.1268	$C_{25}H_{24}O_{12}$
24 (13)	6,7-Dimethylesculetin	6,7-Dimethylesculetin	7.16	–	–	207.0655	207.0657	206.0579	$C_{11}H_{10}O_4$
25 (14)	2,5-Dimethyl-7-hydroxy chromone	2,5-Dimethyl-7-hydroxy chromone	7.23	–	–	191.4302	191.4253	190.0630	$C_{11}H_{10}O_3$
26	Isorhamnetin-3-glucoside	–	7.34	477.1043	477.1033	501.1056 $[M+Na]^+$	501.1009	478.1111	$C_{22}H_{22}O_{12}$
27	Cacticin	–	7.54	477.1041	477.1033	501.1047 $[M+Na]^+$	501.1009	478.1111	$C_{22}H_{22}O_{12}$
28	Not identified	–	8.19	–	–	349.1340	349.1499	–	$C_{15}H_{24}O_9$
29	6″-*o*-*p*-Coumaroylgenipingentiobioside	–	8.41	695.2194	695.2187	719.2181 $[M+Na]^+$	719.2163	696.2265	$C_{32}H_{40}O_{17}$
30 (15)	Not identified	Not identified	8.52	483.1032	483.0775	–	–	484.0853	$C_{20}H_{20}O_{14}$
31 (16)	Not detected	Not detected	9.04	–	–	–	–	–	–
32 (17)	Chimaphylin	Chimaphylin	9.52	–	–	187.0807	187.0759	186.0681	$C_{12}H_{10}O_2$
33	Naringenin	–	10.04	271.0600	271.0606	273.0870	273.0763	272.0685	$C_{15}H_{12}O_5$

Table 5 (*Continued*)

No.	Identified compounds		T_R	Negative ion (*m/z*)		Positive ion (*m/z*)		Molecular weight (Da)	Elemental composition
	In vitro	*In vivo*		Indicated	Actual	Indicated	Actual		
34	Aloeemodin-w-*o*-β-D-glucopyranoside/Emodin-1-*o*-β-D-glucopyranoside	–	10.32	431.0956	431.0978	–	–	432.1056	$C_{21}H_{20}O_{10}$
35 (18)	6-Demethoxycapillarisin	6-Demethoxycapillarisin	10.72	285.0385	285.0399	287.0611	287.0556	286.0477	$C_{15}H_{10}O_6$
36 (19)	Capillarisin	Capillarisin	11.11	315.0511	315.0505	317.0707	317.0661	316.0583	$C_{16}H_{12}O_7$
37	Cirsimaritin	–	12.83	313.0715	313.0712	315.0929	315.0869	314.0790	$C_{17}H_{14}O_6$
38	5,3′,4′-Trihydroxy-6,7-dimethoxyflavone	–	13.10	329.2329	329.2328	–	–	330.0740	$C_{17}H_{14}O_7$
39 (20)	Rhein	Rhein	13.42	283.0242	283.0243	–	–	284.0321	$C_{15}H_8O_6$
40	5-Dehydroxy-6-demethylcapillarisin	–	14.02	285.0754	285.0763	287.1006	287.0919	286.0477	$C_{15}H_{10}O_6$
41	Not identified	–	14.46	207.1384	207.1385	209.1635	209.1542	–	$C_{13}H_{20}O_2$
42	Rhamnocitrin	–	14.58	299.0555	299.0556	301.0787	301.0712	300.0634	$C_{16}H_{12}O_6$
43	Not identified	–	15.10	293.1760	293.1753	–	–	–	$C_{17}H_{26}O_4$
44 (21)	Emodin	Emodin	15.96	269.0451	269.0450	–	–	270.0528	$C_{15}H_{10}O_5$
45	Not identified	–	16.64	–	–	359.2462	359.2434	–	$C_{19}H_{34}O_6$

[a] Number in parenthesis represents the no. of peak detected in serum after oral administration of YCHT.

and structure of the constituents. Peaks 11, 16, 24, 36, 39 and 44 were attributed to chlorogenic acid, geniposide, 6,7-dimethylesculetin, capillarisin, rhein and emodin, respectively by comparing the retention time and mass data with those of the reference compounds (Table 5). Other peaks were identified from several aspects, utilizing Elemental Composition of software MassLynx to carefully study their MS and MS–MS spectra, comparing with the literature data [17] (and taking possible structures into consideration as well). For example, a series of fragment ions of peak 14 were given in negative and positive ion ESI mode, 549 at $[M-H]^-$ and 573 at $[M+Na]^+$, fragments, their molecular weight could be confirmed to be 550, $C_{23}H_{34}O_{15}$, $C_{30}H_{30}O_{10}$ and $C_{36}H_{70}O_3$ were possible molecular composition being deduced by elemental composition. Accordingly, the literature data peak 14 was deduced as genipingentiobioside, 6,6′-diacetyl-2,2′,4,4′,5,5′-hexamethoxy-1,1′-binaphthalene-7,7′-diol or stearic anhydride [14]. Among three compounds; peak 14 was perhaps genipingentiobioside, which have similar fragment ion spectra, 549 $[M-H]^-$, 225 $[M-H–2Glc]^-$, 207 $[M-H–2Glc–H_2O]^-$ and 179 $[M-H–2Glc–H_2O–CO]^-$ were determined in the (−)ESI-MS–MS spectra. Some peaks had same *m/z* value, such as peaks 7, 13 and 15, peaks 20 and 21. They had the same *m/z* value of quasimolecular ion in the MS spectra and a similar fragment ion in MS–MS spectra, respectively, but the retention times of them are different, and the structural identification of these compounds are in progress. Peak 31 was detected at UV spectrum, however there is no signal in MS spectrum, and the further identification is in progress.

3.3. UPLC–UV-Q-TOF analysis of plasma sample after oral YCHT administration

Study on the adaptability of 6,7-dimethylesculetin, capillarisin, geniposide emodin and rhein in the rat blood after oral administration was carried out. The limit of detection of them was determined to be 41.44 ng/L, 102.20 ng/L, 301.80 ng/L, 4.70 ng/L, 31.56 ng/L, respectively (S/N ≥ 3). Kaempferol was as an internal standard. The extraction recoveries were more than 60% (relevant data will be published elsewhere in detail).

The typical chromatograms of the samples were shown in Figs. 5–7. The constituents in rat plasma after oral administration of YCHT were well separated and identified by using their retention time and mass spectra. By comparing the chromatograms of plasma containing drug and control plasma, 21 peaks were identified in plasma. As a result, peaks 2, 8, 9, 10, 11, 12, 13, 15, 17, 18, and 19 were original form compounds existing in Flos Artemisiae; peaks 1, 2, 5 and 7 came from Gardeniae Jasminoidis, Fructus; peaks 9, 14, 16, 20 and 21 resulted from Radix et Rhizoma Rhei; and peak 3 and peak 4 were metabolites of 6,7-dimethylesculetin. The MS data of (+)ESI-MS spectra and (−)ESI-MS spectra, and the identification results are shown in Table 5. Two (peak 3 and peak 4) of the 21 compounds were not an original form of compounds existed in the YCHT, they were identified as 6-methoxy coumarin-7-hydroxyl sulfate and 7-methoxy coumarin-6-hydroxyl sulfate by analyzing the mass

Table 6
MS/MS data of (+)ESI-MS spectra and (−)ESI-MS spectra, and the identification results of the constituents of YCHTE

No.	Identified compounds	T_R (min)	Negative ion (*m/z*)	Positive ion (*m/z*)	Molecular weight (Da)	Elemental composition
1	Gentiobiose	0.37	341 $[M-H]^-$, 179 $[M-H-(Glc-H_2O)]^-$, 161 $[(Glc-H_2O)-H]^-$	–	342.1162	$C_{12}H_{22}O_{11}$
2	Pyroglutamic acid	0.56	128 $[M-H]^-$	130 $[M+H]^+$, 84 $[M-HCOO]^+$	129.0426	$C_5H_7NO_3$
3	Gallic acid	0.77	169 $[M-H]^-$, 125 $[M-HCOO]^-$	–	170.0215	$C_7H_6O_5$
4	Protocatechuic acid-3-glucoside	1.72	315 $[M-H]^-$, 153 $[M-H-(Glc-H_2O)]^-$, 109 $[M-H-(Glc-H_2O)-COO]^-$	–	316.0794	$C_{13}H_{16}O_9$
5	Not identified	2.06	–	186 $[M+H]^+$	–	$C_{13}H_{15}N$
6	Villosolside	2.35	361 $[M-H]^-$, 181 $[M-H-Glc]^-$, 137 $[M-H-Glc-COO]^-$	385 $[M+Na]^+$	362.1577	$C_{16}H_{26}O_9$
7	Neochlorogenic acid/isochlorogenic acid	2.53	353 $[M-H]^-$, 191 $[M-C_9H_7O_3]^-$, 179 $[M-C_7H_{11}O_5]^-$, 135 $[M-H-C_7H_{10}O_5-COO]^-$	377 $[M+Na]^+$, 215 $[M+Na+H-C_9H_7O_3]^+$, 163 $[C_9H_7O_3]^+$	354.0951	$C_{16}H_{18}O_9$
8	Gardenoside B	2.74	403 $[M-H]^-$, 449 $[M+HCOO]^-$, 241 $[M-H-(Glc-H_2O)]^-$, 223 $[M-H-(Glc-H_2O)-H_2O]^-$, 193 $[M-H-(Glc-H_2O)-H_2O-]^-$	427 $[M+Na]^+$, 265 $[M+Na-(Glc-H_2O)]^+$, 233 $[M+Na-(Glc-H_2O)-CH_3OH]^+$	404.1319	$C_{17}H_{24}O_{11}$
9	Picrocrocininc acid	3.04	345 $[M-H]^-$, 165 $[M-H-Glc]^-$, 179 $[Glc-H]^-$, 119 $[M-H-Glc-HCOOH]^-$, 89 $[M-H-Glc-HCOOH-2CH_3]^-$	369 $[M+Na]^+$	346.1628	$C_{16}H_{26}O_8$
10	Not identified	3.28	289 $[M-H]^-$	–	–	$C_{15}H_{14}O_6$
11	Chlorogenic acid	3.46	353 $[M-H]^-$, 191 $[M-C_9H_7O_3]^-$	377 $[M+Na]^+$, 215 $[M+Na+H-C_9H_7O_3]^+$, 163 $[C_9H_7O_3]^+$	354.0951	$C_{16}H_{18}O_9$
12	Not identified	3.63	–	369 $[M+H]^+$	–	$C_{18}H_{24}O_8$
13	Isochlorogenic acid/neochlorogenic acid	3.78	353 $[M-H]^-$, 191 $[M-C_9H_7O_3]^-$, 179 $[M-C_7H_{11}O_5]^-$, 135 $[M-H-C_7H_{10}O_5-COO]^-$	377 $[M+Na]^+$, 215 $[M+Na+H-C_9H_7O_3]^+$, 163 $[C_9H_7O_3]^+$	354.0951	$C_{16}H_{18}O_9$
14	Genipingentiobioside	4.31	549 $[M-H]^-$, 595 $[M+HCOO]^-$, 387 $[M-H-(Glc-H_2O)]^-$, 225 $[M-H-2(Glc-H_2O)]^-$	573 $[M+Na]^+$, 248 $[M+Na-2(Glc-H_2O)]^+$	550.1898	$C_{23}H_{34}O_{15}$
15	Not identified	4.53	353 $[M-H]^-$, 191 $[M-C_9H_7O_3]^-$	377 $[M+Na]^+$, 215 $[M+Na+H-C_9H_7O_3]^+$	354.0951	$C_{16}H_{18}O_9$
16	Jasminoidin	4.77	387 $[M-H]^-$, 433 $[M+HCOO]^-$, 207 $[M-H-Glc]^-$, 163 $[M-H-Glc-COO]^-$, 119 $[M-H-Glc-2COO]^-$	411 $[M+Na]^+$, 249 $[M+Na-(Glc-H_2O)]^+$, 217 $[M+Na-(Glc-H_2O)-CH_3OH]^+$	388.1369	$C_{17}H_{24}O_{10}$
17	Safflor yellow A	5.08	593 $[M-H]^-$, 473 $[M-H-C_8H_8O]^-$	595 $[M+H]^+$	594.1585	$C_{27}H_{30}O_{15}$
18	Capillaridin A	5.48	–	353 $[M+H]^+$ 249 $[M+H-C_7H_5O]^+$	352.1099	$C_{24}H_{16}O_3$
19	5,6-Dimethoxy-7-hydroxy coumarin	5.25	221 $[M-H]^-$, 206 $[M-H-CH_3]^-$, 191 $[M-H-2CH_3]^-$, 163 $[M-H-2CH_3-CO]^-$, 119 $[M-H-2CH_3-CO-COO]^-$	223 $[M+H]^+$, 208 $[M+H-CH_3]^+$, 190 $[M+H-CH_3-H_2O]^+$, 162 $[M+H-CH_3-H_2O-CO]^+$, 134 $[M+H-CH_3-H_2O-2CO]^+$, 106 $[M+H-CH_3-H_2O-3CO]^+$,		

Table 6 (*Continued*)

No.	Identified compounds	T_R (min)	Negative ion (*m/z*)	Positive ion (*m/z*)	Molecular weight (Da)	Elemental composition
78 $[M+H–CH_3–H_2O–4CO]^+$	222.0528	$C_{11}H_{10}O_5$				
20	Quercetin-3-*o*-glycoside/isoquercitrin	6.20	463 $[M-H]^-$, 301 $[M-H–(Glc–H_2O)]^-$, 300 $[M-H–(Glc–H_2O)–H]^-$, 271 $[M-H–(Glc–H_2O)–H–HCO]^-$	487 $[M+Na]^+$, 325 $[M+Na–(Glc–H_2O)]^+$, 185 $[Glc–H_2O+Na]^+$	464.0955	$C_{21}H_{20}O_{12}$
21	Isoquercitrin/quercetin-3-*o*-glycoside	6.40	463 $[M-H]^-$, 301 $[M-H–(Glc–H_2O)]^-$, 300 $[M-H–(Glc–H_2O)–H]^-$, 271 $[M-H–(Glc–H_2O)–H–HCO]^-$	487 $[M+Na]^+$, 325 $[M+Na–(Glc–H_2O)]^+$, 185 $[Glc–H_2O+Na]^+$	464.0955	$C_{21}H_{20}O_{12}$
22	6,8-Dimethoxy-7-hydroxycoumarin	7.00	221 $[M-H]^-$, 206 $[M-H–CH_3]^-$, 191 $[M-H–2CH_3]^-$, 163 $[M-H–2CH_3–CO]^-$, 119 $[M-H–2CH_3–CO–COO]^-$	223 $[M+H]^+$, 208 $[M+H–CH_3]^+$, 190 $[M+H–CH_3–H_2O]^+$, 162 $[M+H–CH_3–H_2O–CO]^+$, 134 $[M+H–CH_3–H_2O–2CO]^+$, 106 $[M+H–CH_3–H_2O–3CO]^+$, 78 $[M+H–CH_3–H_2O–4CO]^+$	222.0528	$C_{11}H_{10}O_5$
23	3,4-Di-*o*-caffeoylquinic acid	7.16	515 $[M-H]^-$, 353 $[M-H–C_9H_6O_3]^-$, 191 $[M-H–2C_9H_6O_3]^-$	–	516.1268	$C_{25}H_{24}O_{12}$
24	6,7-Dimethoxy coumaric	7.16	–	207 $[M+H]^+$, 192 $[M+H–CH_3]^+$, 191 $[M+H–CH_3–H]^+$, 163 $[M+H–CH_3–H–CO]^+$	206.0579	$C_{11}H_{10}O_4$
25	2,5-Dimethyl-7-hydroxy chromone	7.23	–	191 $[M+H]^+$, 151 $[M+H–C_3H_4]^+$	190.0630	$C_{11}H_{10}O_3$
26	Isorhamnetin-3-glucoside	7.34	477 $[M-H]^-$, 315 $[M-H–(Glc–H_2O)]^-$, 314 $[M-H–(Glc–H_2O)–H]^-$, 299 $[M-H–(Glc–H_2O)–H–CH_3]^-$, 271 $[M-H–(Glc–H_2O)–H–CH_3–CO]^-$, 243 $[M-H–(Glc–H_2O)–H–CH_3–2CO]^-$	501 $[M+Na]^+$, 339 $[M+Na–(Glc–H_2O)]^+$, 338 $[M+Na–(Glc–H_2O)–H]^+$, 185 $[Glc–H_2O+Na]^+$	478.1111	$C_{22}H_{22}O_{12}$
27	Cacticin	7.54	477 $[M-H]^-$, 315 $[M-H–(Glc–H_2O)]^-$, 314 $[M-H–(Glc–H_2O)–H]^-$, 299 $[M-H–(Glc–H_2O)–H–CH_3]^-$, 271 $[M-H–(Glc–H_2O)–H–CH_3–CO]^-$, 243 $[M-H–(Glc–H_2O)–H–CH_3–2CO]^-$	501 $[M+Na]^+$, 339 $[M+Na–(Glc–H_2O)]^+$, 338 $[M+Na–(Glc–H_2O)–H]^+$, 185 $[Glc–H_2O+Na]^+$	478.1111	$C_{22}H_{22}O_{12}$
28	Not identified	8.19	–	349 $[M+H]^+$	–	$C_{15}H_{24}O_9$
29	6″-*o*-*p*-Coumaroylgenipingentiobioside	8.41	695 $[M-H]^-$, 469 $[M-H–C_{11}H_{13}O_5–H]^-$, 225 $[C_{11}H_{13}O_5]^-$, 163 $[C_9H_7O_3]^-$	719 $[M+Na]^+$, 556 $[M+Na–C_9H_7O_3]^+$	696.2265	$C_{32}H_{40}O_{17}$
30	Not identified	8.52	483 $[M-H]^-$	–		$C_{20}H_{20}O_{14}$
31	Not detected		9.04	–	–	–
32	Chimaphylin	9.52	–	187 $[M+H]^+$	186.0681	$C_{12}H_{10}O_2$
33	Naringenin	10.04	271 $[M-H]^-$, 151 $[M–C_7H_4O_4]^-$, 119 $[C_8H_7O]^+$	273 $[M+H]^+$, 153 $[M–C_8H_8O]^+$	272.0685	$C_{15}H_{12}O_5$
34	Aloeemodin-w-*o*-β-D-glucopyranoside/emodin-1-*o*-β-D-glucopyranoside	10.32	431 $[M-H]^-$, 269 $[M-H–(Glc–H_2O)]^-$, 225 $[M-H–(Glc–H_2O)–COO]^-$	–	432.1056	$C_{21}H_{20}O_{10}$

Table 6 (*Continued*)

No.	Identified compounds	T_R (min)	Negative ion (*m/z*)	Positive ion (*m/z*)	Molecular weight (Da)	Elemental composition
35	6-Demethoxycapillarisin	10.72	285 $[M-H]^-$, 193 $[M–C_6H_5O]^-$, 192 $[M–C_6H_5O–H]^-$, 177 $[M–C_6H_5O–H–CH_3]^-$	287 $[M+H]^+$, 195 $[M+H–C_6H_4O]^+$	286.0477	$C_{15}H_{10}O_6$
36	Capillarisin	11.11	315 $[M-H]^-$, 300 $[M-H–CH_3]^-$, 207 $[M-H–CH_3–C_6H_5O]^-$	317 $[M+H]^+$, 302 $[M+H–CH_3]^+$, 209 $[M+H–CH_3–C_6H_5O]^+$	316.0583	$C_{16}H_{12}O_7$
37	Cirsimaritin	12.83	313 $[M-H]^-$, 298 $[M-H–CH_3]^-$, 283 $[M-H–2CH_3]^-$, 255 $[M-H–2CH_3–CO]^-$, 227 $[M-H–2CH_3–2CO]^-$, 117 $[C_8H_5O]^-$	315 $[M+H]^+$, 300 $[M+H–CH_3]^+$, 282 $[M+H–CH_3–H_2O]^+$, 254 $[M+H–CH_3–H_2O–CO]^+$, 226 $[M+H–CH_3–H_2O–2CO]^+$	314.0790	$C_{17}H_{14}O_6$
38	5,3′,4′-Trihydroxy-6,7-dimethoxyflavone	13.10	329 $[M-H]^-$, 299 $[M-H–2CH_3]^-$	–	330.0740	$C_{17}H_{14}O_7$
39	Rhein	13.42	283 $[M-H]^-$, 239 $[M-H–COO]^-$, 211 $[M-H–COO–CO]^-$, 183 $[M-H–COO–2CO]^-$	–	284.0321	$C_{15}H_8O_6$
40	5-Dehydroxy-6-demethylcapillarisin	14.02	285 $[M-H]^-$, 165 $[M-H–C_7H_4O_2]^-$, 119 $[C_7H_3O_2]^-$	287 $[M+H]^+$, 167 $[M+H–C_7H_4O_2]^+$	286.0477	$C_{15}H_{10}O_6$
41	Not identified	14.46	207 $[M-H]^-$	209 $[M+H]^+$	–	$C_{13}H_{20}O_2$
42	Rhamnocitrin	14.58	299 $[M-H]^-$, 284 $[M-H–CH_3]^-$, 271 $[M-H–CO]^-$, 255 $[M-H–CH_3–CO]^-$, 165 $[C_8H_5O_4]^-$	301 $[M+H]^+$, 286 $[M+H–CH_3]^+$, 258 $[M+H–CH_3–CO]^+$	300.0634	$C_{16}H_{12}O_6$
43	Not identified	15.10	293 $[M-H]^-$	–	–	$C_{17}H_{26}O_4$
44	Emodin	15.96	269 $[M-H]^-$, 241 $[M-H–CO]^-$, 240 $[M-H–CO–H]^-$, 225 $[M-H–CO–H–CH_3]^-$, 197 $[M-H–2CO–H–CH_3]^-$, 182 $[M-H–2CO–H–2CH_3]^-$	–	270.0528	$C_{15}H_{10}O_5$
45	Not identified	16.64	–	359 $[M+H]^+$	–	$C_{19}H_{34}O_6$

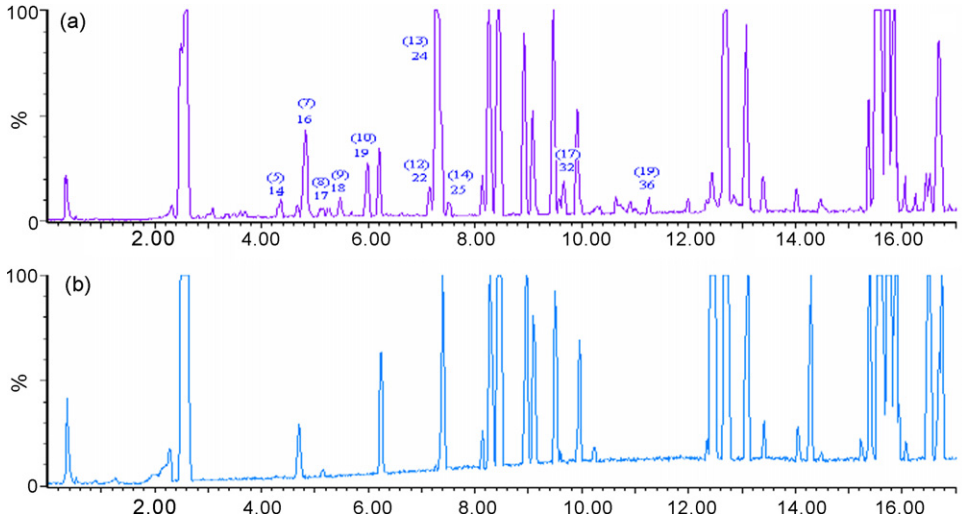


Fig. 6. TIC chromatograms of rat plasma by UPLC/Q-TOF in positive ESI mode. (a) Plasma after oral administration of YCHT and (b) control serum.

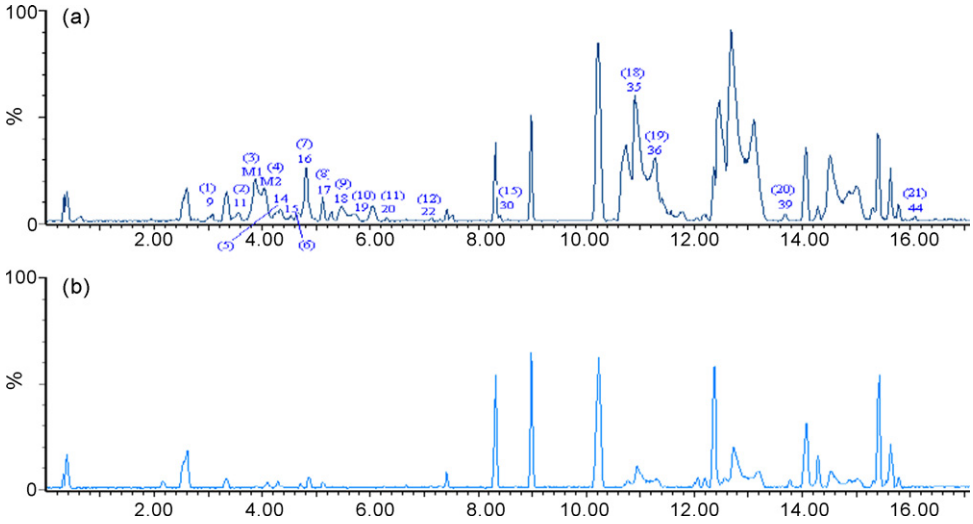


Fig. 7. TIC chromatograms of rat plasma by UPLC/Q-TOF in negative ESI mode. (a) Plasma after oral administration of YCHT and (b) control plasma.

data and corresponding data of recorded in literature [18], they were the metabolites of 6,7-dimethylesculetin.

4. Conclusion

By analyzing the constituents in rat plasma of YCHT based on LC–MS analysis, an identification method of the effective constituents in a traditional Chinese medicine (TCM) and formula have been established. The method has provided a methodological basis for elucidating the bioactive constituent of traditional Chinese medicine. In this study, 21 of the constituents in rat blood after oral administration of YCHT were identified by the UPLC–UV-Q-TOF system, which enhanced the speed and targeting of bioactive constituents analysis.

The research, compared with the previous studies or methods, maintained several differences or improvements. First, to obtain better detection, the MS conditions were optimized by a statistical orthogonal design which has seldom been introduced into this kind of study. Secondly, the compounds of YCHT, which were comprised of three herbs, have not been studied as an integrated whole before, the present research has filled a gap in this respect. Thirdly, the analysis based on the UPLC/Q-TOF system was a method of a rapid and accurate kind, allowing more ingredients to be found in the plasma when compared with the conventional HPLC–UV system. Therefore the established UPLC/Q-TOF system enriched the methodology of the plasma pharmacochemistry of the TCM. Moreover, in the present work, the study on the adaptability was performed, and the limit of detection of five main compounds was determined, the level of matrix effect which could lessen, weaken, or cover up the ion signal have been elucidated, the final results are much more reliable.

With the introduction of the UPLC/Q-TOF analytical system to the plasma pharmacochemistry of TCM, this will provide a type of validated rapid and higher throughput methodology for the identification of bioactive constituents for TCM and herbal drugs.

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