Original Article

Chemical material basis study of Xuefu Zhuyu decoction by ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry

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ABSTRACT

Xuefu Zhuyu decoction, a classic prescription in traditional Chinese medicine, has been widely used in the clinical treatment of cardiovascular and cerebrovascular diseases. In order to profile the chemical material basis of this formula, an ultra-performance liquid chromatography (UPLC) coupled with quadrupole time-of-flight mass spectrometry (Q/TOF MS) method has been established for rapid separation and structural characterization of compounds in the decoction. As a result, 103 compounds including phenolic acids, spermidines, C-glycosyl quinochalcones, terpenoids, flavonoids, saponins, and others were detected; 35 of them were unambiguously identified, and 68 were tentatively characterized by comparing the retention time, MS data, characteristic MS fragmentation pattern and retrieving the literature. In conclusion, the UPLC coupled with quadrupole time-of-flight mass spectrometry method developed in this work is an efficient approach to perform chemical material basis studies of traditional Chinese medicine formulae.

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1. Introduction

Cardiovascular and cerebrovascular diseases are common diseases of the elderly that have seriously threatened human health in recent years. Even when the most advanced and comprehensive treatment was applied, more than 50% of the survivors from cardiovascular and cerebrovascular incidents were still unable to provide for themselves completely. Every year around the world, as many as 15 million people die of cardiovascular and cerebrovascular diseases. They have become one of the primary causes of human death.

The traditional Chinese medicine formula Xuefu Zhuyu decoction (XFZYD) was first recorded in Yilin Gaicuo (*Correction of Medical Errors*, 1850) by Qingren Wang (1768−1831) [1]. The herbal combination is regarded as a modification of two famous classic prescriptions, Taohong Siwu decoction (Peach Seed and Safflower Decoction of Four Ingredients) and Sinisan (Powder for Regulating Liver and Spleen) [2], which comprises 11 herbs: *Semen prunus* (Taoren) 12 g, Radix *Angelicae sinensis* (Danggui) 9 g, Rhizoma chuanxiong (Chuanxiong) 4.5 g, *Flos carthami* (Honghua) 9 g, Radix *Paeoniae rubra* (Chishao) 6 g, Radix rehmanniae (Dihuang) 9 g, *Fructus aurantii* (Zhiqiao) 6 g, Radix Bupleuri (Chaihu) 3 g, Radix platycodonis (Jiegeng) 4.5 g, Radix *Achyranthis bidentatae* (Niuxi) 9 g, and Radix and Rhizoma glycyrrhizae (Gancao) 6 g [3,4]. XFZYD has been demonstrated to show definite protection in the cardiovascular and cerebrovascular system, and modern pharmacological studies have elucidated the protective mechanisms [5,6]. XYZFD could induce endothelial progenitor cell angiogenesis, hasten tube formation [7], and regulate blood lipid [8,9]. Satisfactory clinical efficiency has been achieved for cardiovascular and cerebrovascular diseases [10] such as atherosclerosis, hypertension, hyperlipidemia, thromboembolism, and angina pectoris.

It is well known that the therapeutic effects of herbal medicine are due to the synergistic contribution of multiple constituents [11]. Since XFZYD has centuries of clinical use and reliable curative efficacy, developing a feasible and rapid analytical method for characterizing the constituents in the decoction is valuable and vital to ensuring its reliability and safety in clinical therapy. Many researchers have made significant contributions to the studies of substance foundation in XFZYD. Zhang et al [12] and Liu et al [13] used high-performance liquid chromatography−mass spectrometry (HPLC-MS) methods to identify antiatherogenic constituents of the decoction. Gao et al [14] introduced an HPLC−evaporative light scattering detector method to quantify chemical constituents in the XFZY capsule. In our previous study, an ultra-performance liquid chromatography (UPLC) coupled with diode array detector tandem MS method was undertaken to perform quantitative and qualitative analysis of the constituents in XFZYD products [15].

In order to deeply unveil the chemical compositions of XFZYD, a UPLC coupled with quadrupole time-of-flight (Q/TOF) MS method was introduced and established in this work. A total of 103 constituents were unambiguously identified or tentatively characterized. This also provides a valuable reference for further research and development of this formula and its related medicinal products.

2. Methods

2.1. Reagents and materials

HPLC grade acetonitrile and methanol were purchased from Merck (Merck, Darmstadt, Germany) and Sigma (Sigma−Aldrich, St Louis, MO, USA), respectively. Formic acid and dimethyl sulfoxide were obtained from Meridian Medical Technologies (Columbia, MD, USA). Water used in the experiment was purified by a Milli-Q water purification system (Millipore, Billerica, MI, USA).

Reference compounds (gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, chlorogenic acid, caffeic acid, hydroxysafflor yellow A, amygdalin, albiflorin, paeoniflorin, *p*-hydroxycinnamic acid, ferulic acid, schaftoside, 6-hydroxy kaempferol-3-*O*-glucoside, liquiritin, rutin, isoquercitrin, verbascoside, astragalin, narirutin, β-ecdysterone, naringin, rhoifolin, hesperidin, neohesperidin, liquiritigenin, naringenin, kaempferol, platycodin D, isoliquiritigenin, formononetin, ginsenoside-Ro, 18β-glycyrrhizic acid, chikusetsu saponin Iva, nobiletin, and saikosaponin A) were obtained from the National Institute for Food and Drug Control (Beijing, China), Tianjin ZhongXin Pharmaceutical Group Co., Ltd. (Tianjin, China), and Top High Bio Technology Co., Ltd. (Nanjing, China). The purities of standards were >98%.

2.2. Preparation of standard solutions

Reference compounds were accurately weighed and directly prepared in methanol and dimethyl sulfoxide as individual standard stock solutions; following this mixed standard stock solutions containing all 35 standards were prepared. A working standard solution was prepared by diluting the mixed stock solution with water (v/v, 1:3) to obtain a suitable concentration.

2.3. Plant material and sample preparation

The plant materials (Taoren, Danggui, Chuanxiong, Honghua, Chishao, Dihuang, Zhiqiao, Chaihu, Jiegeng, Niuxi, and Gancao) were purchased from Anguo (Hebei, China) and identified by Professor Tianxiang Li. All herbs were deposited in Tianjin State Key Laboratory of Modern Chinese Medicine.

According to the traditional formula, 11 plant materials (total weight of 78 g) were mixed and immersed in 600 mL deionized water for 1 hour at room temperature, and then refluxed for 2 hours twice. After filtration and concentration, aqueous extract was dried at 45°C in an oven under vacuum to give 30 g original extract powder. The yield of preparation was 38.5%. A 10 mL aliquot of methanol was added to 0.4 g of extract powder and sonicated for 30 min at room temperature. The solution was diluted with deionized water (v/v, 1:1) and then centrifuged at 17,968 g for 10 min. Finally, the supernatant was transferred to autosampler vials for UPLC-Q/TOF MS analysis.

2.4. UPLC-Q/TOF MS/MS analysis

Analysis was performed on a Waters ACQUITY UPLC system (Binary Solvent Manager, Sample Manger and thermostatically controlled column compartment; Waters Co., Parsippany, NJ, USA) coupled with Waters SYNAPT G2 high definition mass spectrometer (HDMS) with a LockSpray and an electrospray ionization (ESI) interface. The system was controlled under MassLynx V4.1 software (Waters).

Gradient elution was performed on an ACQUITY UPLC BEH C18 column (2.1 × 100 mm, 1.7 μm; Waters) at 50°C. The mobile phase was composed of 0.1% formic acid aqueous solution (A) and acetonitrile (B). The gradient program applied was as follows: 0–6.5 min, 3–11% B; 6.5–15 min, 11–20% B; 15–20 min, 20–36% B; 20–27 min, 36–48% B; 27–30 min, 48–55% B; 30–33 min, 55–74% B, and 33–35 min, 74–90% B. The flow rate was 0.3 mL/min and injection volume was 5 μL.

The analysis of mass spectra was performed in both positive and negative modes. And, the spectra were recorded in the range of m/z 100–1500 Da for full scan. The optimal MS parameters were: capillary voltage, −2.5 kV (negative ion mode), 3.0 kV (positive ion mode); capillary temperature, 120°C; desolvation temperature, 400°C; desolvation gas (N_2) flow, 700 L/h; cone gas (N_2) flow, 50 L/h; collision gas (Ar) flow rate, 0.20 mL/min; the MS and MS/MS acquisition rate was set at 0.2 s in centroid mode. Mass accuracy was maintained using a LockSpray with leucine–enkephalin for positive ion mode [$(M+H)^+$ = 556.2771] and negative ion mode [$(M-H)^-$ = 554.2615] at a concentration of 200 pg/mL.

2.5. Data processing

MS and MS/MS data obtained from the robust UPLC-Q/TOF MS were performed using the aforementioned protocol. The chemical profiling study was based on retention time, precise molecular mass, isotopic pattern, MS/MS data and MS fragmentation behavior. The mass accuracy of MS and MS/MS data should be < 10.0 ppm.

3. Results and Discussion

3.1. UPLC-MS characterization of chemical constituents from XFZYD

The representative base peak intensity chromatograms of XFZYD sample are presented in Fig. 1A and 1C corresponding to negative and positive ion mode, respectively. A total of 103 compounds (Fig. S1) including 7 phenolic acids, 4 spermidines, 7 terpenoids, 52 flavonoids, 29 saponins, and 4 others were characterized. Among them, 35 constituents were unambiguously identified by comparing retention time, MS, and MS/MS data with the base peak intensity chromatograms of authentic compounds (shown in Fig. 1B and 1D). A further 68 compounds were tentatively characterized by comparing accurate mass of quasimolecular and product ions, characteristic fragmentation patterns and related botanical biogenesis with the literature.

3.2. Fragmentation patterns study of XFZYD

The mass error for quasimolecular and product ions of all compounds identified by UPLC-Q/TOF MS was within ± 10 ppm. The exact identification of each group of components is outlined below.

3.3. Characterization of phenolic acids

Compounds P1, P2, P3, P4, P5, P6, and P7 were unequivocally identified as gallic acid, protocatechuic acid, p-hydroxybenzoic acid, chlorogenic acid, caffeic acid, hydroxycinnamic acid, and ferulic acid, respectively, by comparison with authentic compounds. Due to low ionization efficiency in positive ion mode, most phenolic acids were only presented in negative ion mode except chlorogenic acid. Compounds P1, P2, P3, P5, and P6 showed characteristic neutral loss of CO_2. Compound P4 exhibited ions at m/z 353.0858 $[M-H]^-$ and 377.0849 $[M+Na]^+$. The fragment ion at m/z 191.0533 $[M-H-C_9H_6O_3]^-$ and 215.0596 $[M+Na-C_9H_6O_3]^+$ corresponded to the loss of caffeoyl residue. Compound P7 produced a dominant deprotonated ion at m/z 193.0499 $[M-H]^-$ with typical consecutive loss of CH_3, CO, and CO_2. The fragment ions of P7 at m/z 178.0242 $[M-H-CH_3]^-$, 149.0543 $[M-H-CO_2]^-$, and 134.0350 $[M-H-CO_2-CH_3]^-$ were shown in the negative MS/MS spectrum.

3.4. Characterization of flavonoids

Flavonoids exist in both free aglycones and glycoside forms which include several classes of compounds with similar structure having a common C6–C3–C6 flavone skeleton [16]. The cleavage of aglycones in flavonoids can be divided into two broad categories: one is the retro Diels–Alder reaction for C ring, the other is the loss of small molecular fragments such as CO, CH_2O, CO_2 and C_2H_2O. The flavonoid glycosides have many isomers with the same molecular weight but different aglycones and sugars conjugating at multiple linkage positions. Flavonoid glycosides exist in two main glycosylation modes: O-glycosylation formed by the linkage of a carbon-–oxygen bond and C-glycosylation formed by the linkage of a carbon–carbon bond. These two flavonoid glycosides exhibit entirely different fragmentation behaviors. For the O-glycoside form, loss of sugar moiety is the characteristic fragmentation behavior. For the C-glycoside form, the stable carbon–carbon bond is resistant to cleavage, so cleavage of the sugar moiety is the typical MS fragmentation pattern.

3.4.1. Characterization of O-glycosylation flavonoids
The structures of flavonoids were identified at both positive and negative ion modes, and some structures were further validated with standards. Compounds FN12 and FN17 were identified as naringin and naringenin. These two compounds showed typical retro Diels–Alder reaction for C ring in both positive and negative ion modes. In positive ion spectra of compound FN12, the fragment ion at m/z 457.1137 $[M+Na-C_6H_{10}O_4]^+$ was observed, which was attributed to the loss of rhamnose. While the ion at m/z 271.0578 $[M-H-C_{12}H_{20}O_9]^-$ in negative ion mode corresponded to the neutral loss of neohesperidose, the aglycone of compound FN12, compound FN17 showed fragment
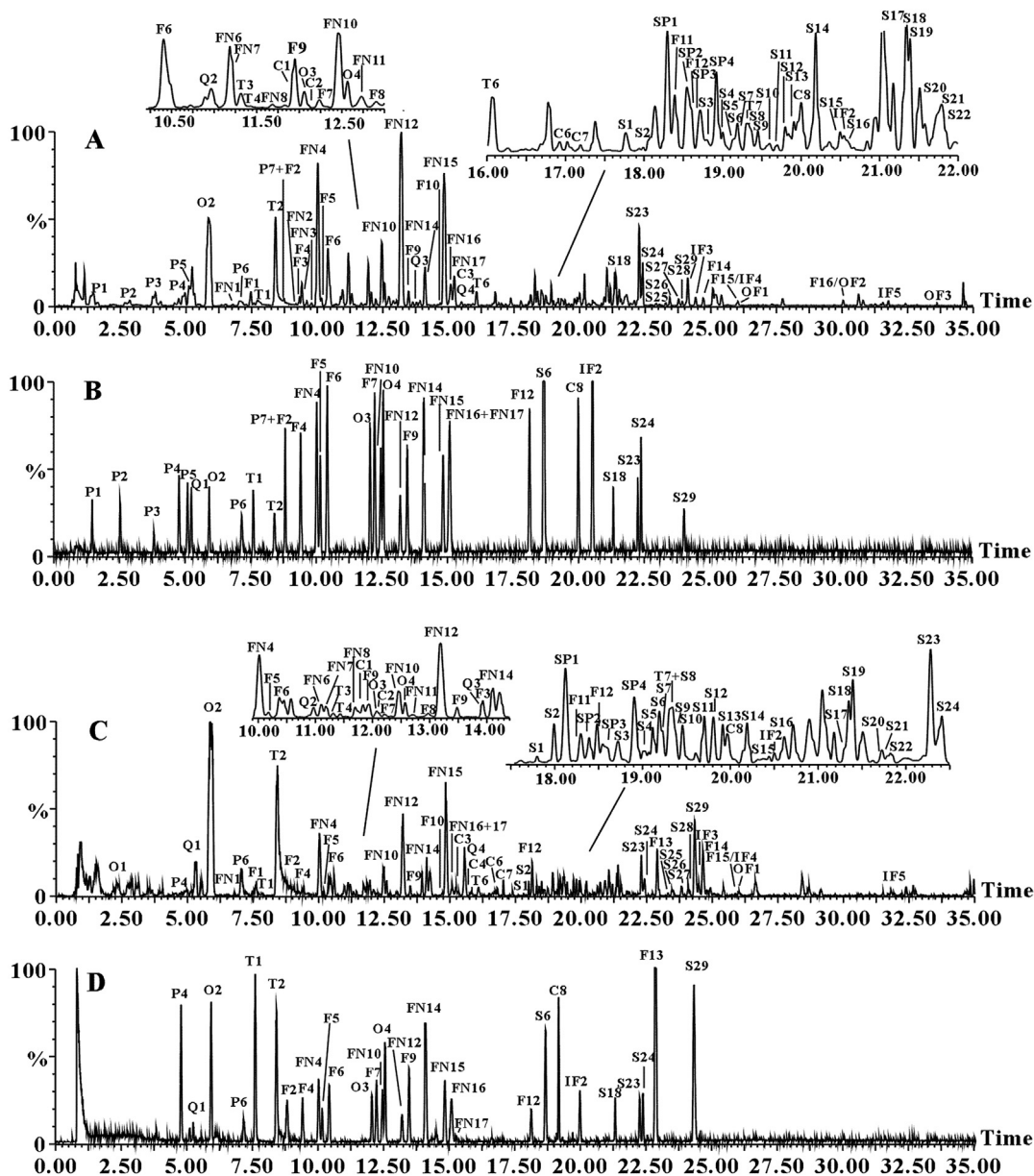
Fig. 1 — Base peak intensity chromatograms of Xuefu Zhuyu decoction extracts (A) in negative ion mode and (C) in positive ion mode, base peak intensity chromatograms of mixed standards (B) in negative ion mode and (D) in positive ion mode by ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. C = chalcone; F = flavone; FN = flavanone; IF = isoflavone; O = others; OF = other flavonoid; Q = C-glycosyl quinochalcone; P = phenolic acid; S = saponin; SP = spermidine; T = terpenoid.

ions at m/z 147.0446 $[M+H-C_6H_6O_3]^+$ and 119.0508 $[M+H-C_6H_6O_3-CO]^+$ in positive ion mode, which were reasonably attributed to the loss of benzene-1,3,5-triol and CO. The ions at m/z 177.0160 $[M-H-C_6H_6O]^-$, 151.0011 $[M-H-C_8H_8O]^-$, and 107.0118 $[M-H-C_8H_8O-CO_2]^-$ in negative ion mode corresponded to the loss of phenol, 4-vinylphenol, and CO_2.

3.4.2. Characterization of C-glycosylation flavonoids

Compound F2 was identified as schaftoside. In positive ion mode, Compound F2 yielded a quasimolecular ion at m/z 565.1557 $[M+H]^+$. The fragment ions at m/z 547.1499 $[M+H-H_2O]^+$, 529.1407 $[M+H-2H_2O]^+$, and 511.1284 $[M+H-3H_2O]^+$ were attributed to successive dehydration at sugar alcohol; the ion at m/z 445.1608 $[M+H-C_4H_8O_4]^+$ corresponded to sugar bond cleavage. In negative ion mode, compound F2 produced a quasimolecular ion at m/z 563.1393 $[M-H]^-$. Subsequently, compound F2 yielded fragment ions at m/z 473.1020 $[M-H-C_3H_6O_3]^-$, 443.0919 $[M-H-C_4H_8O_4]^-$, 383.0731 $[M-H-C_4H_8O_4-C_2H_4O_2]^-$, and 353.0613 $[M-H-C_3H_6O_3-C_4H_8O_4]^-$: typical fragment ions (m/z 120, 90, and 60) for cleavage of sugar bonds, which are presented in Fig. 2.
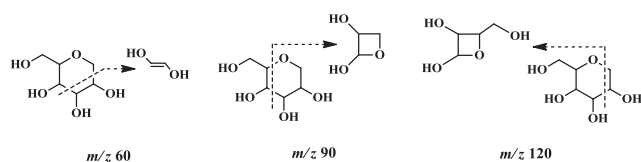
Fig. 2 – Fragmentation from glucose moieties of C-glycosyl flavonoids.

C-glycosyl quinochalcones are unique constituents in *Flos carthami*, which have the same quinochalcone skeleton with a hydroxyl and *C*-glucosyl group at the 5′-position [17]. *C*-glycosyl quinochalcones showed special fragmentation of glucose and branched groups in both positive and negative ion modes. These typical fragmentation pathways made it easy to characterize *C*-glycosyl quinochalcones in a complex sample. For example, compound Q1 showed characteristic internal cleavage of glucose, such as the loss of H_2O, CH_2O, $C_2H_4O_2$, and $C_4H_8O_4$. The fragmentation behaviors of compounds Q2–Q4 mainly occurred at branched chains. In positive ion spectra, compound Q4 yielded major fragment ions at *m/z* 517.0958 $[M+Na-C_8H_8O]^+$, 473.1053 $[M+Na-C_9H_8O_3]^+$, 353.0487 $[M+Na-C_9H_8O_3-C_8H_8O]^+$, and 311.0524 $[M+Na-C_9H_8O_3-C_8H_8O-C_2H_2O]^+$, which was reasonably attributed to the loss of *p*-vinyl phenol, 3-(4-hydorxyphenyl)acrylic acid, and ethynol at side chain. Compounds Q2 and Q3 were *C*-glycosyl quinochalcones containing a nitrogen atom. Their fragmentations showed a special cleavage at the C–C bond except for the characteristic cleavage at branched chain. Homolysis (radical cleavage) of the C–C bond occurred, which yielded a glucose free radical. By comparison with the reported compounds and MS information, compounds Q1–Q4 were tentatively characterized as hydroxysafflor yellow A, hydroxycartormin, cartormin or isomer, and safflomin C or isomer.

3.5. Characterization of saponins

In negative ion mode, triterpene saponins yielded a dominant quasimolecular ion $[M-H]^-$ and typical fragment ions at *m/z* 351.0564 [glucuro-glucuronic acid-H]$^-$ and 193.0348 [glucuronic acid-H]$^-$, or continuously lose sugars. In positive ion spectra, the characteristic MS fragmentation behavior of saponins is consecutive loss of sugars and H_2O. In addition, acetylated saponins often lose an acetyl group before the behaviors mentioned above. All these features were highly characteristic for the identification of triterpene saponins. Finally, a total of 29 triterpene saponins (Tables 1 and S1) were identified in XFZYD and showed similar MS behavior [18]. For example, ions at *m/z* 823.4116 $[M+H]^+$ and 821.3956 $[M-H]^-$ of compound S23 were found in positive and negative MS spectrum, respectively. The fragment ions at *m/z* 647.3810 $[M+H-C_6H_8O_6]^+$ and 453.3374 $[M+H-2C_6H_8O_6-H_2O]^+$ were produced directly from the precursor ion, corresponding to the neutral loss of a glucuronic acid unit, a glucuro-glucuronic acid unit, and H_2O. In negative mode, the fragment ions at *m/z* 351.0544 $[M-H-C_{30}H_{46}O_4]^-$ and 193.0343 $[M-H-C_{30}H_{46}O_4-C_6H_8O_6]^-$ corresponded to [glucuro-glucuronic acid-H]$^-$ and [glucuronic acid-H]$^-$, respectively.

Based on the MS/MS data and retention time compared with a reference compound, compound S23 was identified as 18β-glycyrrhizic acid.

3.6. Characterization of terpenoids

Monoterpene glycosides are the major bioactive constituents in Radix Paeoniae Rubra. The chemical structure of their aglycones is generally a *cage-like* pinane skeleton, and glucose was the only hexose hitherto reported in monoterpene glycosides of paeonia species [19]. The monoterpene glycosides are usually esterified with an aromatic acid such as benzoic acid, *p*-hydroxybenzoic acid, or gallic acid. A total of five monoterpene glycosides were identified based on their mass spectra. For example, compound T2 was observed in high abundance and was confirmed as paeoniflorin by comparison with a reference standard. In positive ion spectra, the quasi-molecular ion $[M+H]^+$ was too weak to be observed. Instead, compound T2 yielded a prominent adduct ion at *m/z* 503.1525 $[M+Na]^+$. In negative mode, a fragment ion at *m/z* 449.1378 $[M-H-CH_2O]^-$ was observed, which was reasonably attributed to the loss of a HCHO (formaldehyde) and assigned as the 5′-hydroxymethyl radical of the glucose residue [20]. The ion at *m/z* 327.1041 $[M-H-CH_2O-C_7H_6O_2]^-$ originated from the loss of HCHO and benzoic acid. The product ion at *m/z* 121.0276 $[M-H-CH_2O-C_{15}H_{20}O_8]^-$ indicated the presence of a benzoyl group. Compound T1, an isomer of paeoniflorin, was unequivocally identified as albiflorin by comparison with reference standard. In positive ion mode, it showed a dominant adduct ion at *m/z* 503.1540 $[M+Na]^+$. The fragment ions at *m/z* 341.0930 $[M+Na-C_6H_{10}O_5]^+$ and 219.0615 $[M+Na-C_6H_{10}O_5-C_7H_6O_2]^+$ probably originated from the successive losses of a hexose residue and benzoic acid. The negative ion spectrum showed adduct ion at *m/z* 525.1608 $[M-H+HCOOH]^-$. The product ion at *m/z* 121.0278 $[M-H+HCOOH-HCOOH-C_{16}H_{22}O_9]^-$ indicated the presence of a benzoyl group. Compound T3 and T4 were induced as galloyl-paeoniflorin or isomer. The product ions at *m/z* 169.0145 $[M-H-C_{23}H_{26}O_{10}]^-$ and 169.0191 $[M-H-C_{23}H_{26}O_{10}]^-$ suggested the presence of galloyl radical; the productions at *m/z* 313.0562 $[M-H-C_{17}H_{18}O_6]^-$ and *m/z* 313.0547 $[M-H-C_{17}H_{18}O_6]^-$ corresponded to a [galloyl glucose residue-H]$^-$.

3.7. Miscellaneous

Four isomers of spermidines (SP1–4) were detected and identified as N1,N5,N10-(E)-tri-*p*-coumaroylspermidine, N1,N5,N10-(Z)-tri-*p*-coumaroylspermidine, safflospermidine A, and safflospermidine B from *F. carthami* [21]. These four spermidines exhibited the same fragmentation pathways. For instance, compound SP1 yielded a quasimolecular ion at *m/z* 582.2606 $[M-H]^-$ in negative ion mode. The fragment ions at *m/z* 462.2029 $[M-H-C_8H_8O]^-$ and 342.1451 $[M-H-2C_8H_8O]^-$ originated from the loss of 4-vinylphenol. In positive ion mode, a quasimolecular ion at *m/z* 584.2761 $[M+H]^+$ was observed. The product ions at *m/z* 438.2390 $[M+H-C_9H_6O_2]^+$, 420.2283 $[M+H-C_9H_6O_2-H_2O]^+$, and 275.1764 $[M+H-C_9H_6O_2-C_9H_9NO_2]^+$ originated from the successive losses of 4-(3-oxoallylidene)cyclohexa-2,5-dienone, H_2O, and 3-(4-hydroxyphenyl)acrylamide.

Table 1 – The retention time, mass spectrometry data and characterization of compounds of Xuefu Zhuyu decoction.

Peak No.	Serial No.	t_R (min)	Positive (+) ion mode			Negative (−) ion mode			Formula	Identification
			[M+H]+/ [M+Na]+	Theoretical [M+H]+/ [M+Na]+	Error (ppm)	[M−H]−/ [M−H+HCOOH]−	Theoretical [M−H]−/ [M−H+HCOOH]−	Error (ppm)		
*P1	1	1.485	—	—	—	169.0134	169.0137	−1.8	$C_7H_6O_5$	Gallic acid
O1	2	2.255	166.0865	166.0868	−1.8	164.0693	164.0712	−5.5	$C_9H_{11}NO_2$	Phenylalanine
*P2	3	2.563	—	—	—	153.0485	153.0488	−2.0	$C_7H_6O_4$	Protocatechuic acid
*P3	4	3.829	—	—	—	137.0236	137.0239	−2.2	$C_7H_6O_3$	p-Hydroxybenzoic acid
*P4	5	4.790	377.0847	377.0849	−0.5	353.0858	353.0873	−4.2	$C_{16}H_{18}O_9$	Chlorogenic acid
*P5	6	5.121	—	—	—	179.0339	179.0344	−2.8	$C_9H_8O_4$	Caffeic acid
*Q1	7	5.267	635.1578	635.1588	−1.6	611.1619	611.1612	1.1	$C_{27}H_{32}O_{16}$	Hydroxysafflor yellow A
*O2	8	5.864	480.1482	480.1482	0.0	456.1495	456.1506	−2.4	$C_{20}H_{27}NO_{11}$	Amygdalin
FN1	9	7.116	473.1028	473.1060	−6.8	449.1076	449.1084	−1.8	$C_{21}H_{22}O_{11}$	Neocarthamin
*P6	10	7.182	—	—	—	163.0391	163.0395	−2.5	$C_9H_8O_3$	p-Hydroxycinnamic acid
F1	11	7.472	595.1670	595.1663	1.2	593.1503	593.1506	−0.5	$C_{27}H_{30}O_{15}$	Vicenin-2
*T1	12	7.626	503.1540	503.1529	2.2	525.1608	525.1608	0.0	$C_{23}H_{28}O_{11}$	Albiflorin
*T2	13	8.433	503.1525	503.1529	−0.8	479.1541	479.1553	−2.5	$C_{23}H_{28}O_{11}$	Paeoniflorin
*P7	14	8.835	—	—	—	193.0499	193.0501	−1.0	$C_{10}H_{10}O_4$	Ferulic acid
*F2	15	8.835	565.1581	565.1557	4.2	563.1393	563.1401	−1.4	$C_{26}H_{28}O_{14}$	Schaftoside
FN2	16	9.290	765.2225	765.2218	0.9	741.2211	741.2242	−4.2	$C_{33}H_{42}O_{19}$	Naringinenin-4′-O-glucoside-7-O-rutinoside or Naringinenin-4′-O-glucoside-7-O-neoheperidoside
F3	17	9.309	633.1410	633.1432	−3.5	609.1556	609.1534	3.6	$C_{27}H_{30}O_{16}$	6-Hydroxy kaempferol-3-O-rutinoside
*F4	18	9.429	487.0862	487.0852	2.1	463.0871	463.0877	−1.3	$C_{21}H_{20}O_{12}$	6-Hydroxy kaempferol-3-O-glucoside
FN3	19	9.779	441.1137	441.1162	−5.7	417.1181	417.1186	−1.2	$C_{21}H_{22}O_9$	Neoliquiritin
*FN4	20	10.045	—	—	—	417.1186	417.1186	0.0	$C_{21}H_{22}O_9$	Liquiritin
*F5	21	10.176	633.1434	633.1432	0.3	609.1461	609.1456	0.8	$C_{27}H_{30}O_{16}$	Rutin
FN5	22	10.414	573.1589	573.1584	0.9	549.1612	549.1608	0.7	$C_{26}H_{30}O_{13}$	Liquiritin apioside
*F6	23	10.439	—	—	—	463.0835	463.0877	−9.1	$C_{21}H_{20}O_{12}$	Isoquercitrin
Q2	24	10.902	616.1622	616.1642	−3.2	592.1658	592.1666	−1.4	$C_{27}H_{31}NO_{14}$	Hydroxycartormin
FN6	25	11.173	473.1031	473.1060	−6.1	449.1083	449.1084	−0.2	$C_{21}H_{22}O_{11}$	4′,5,6,7-Tetrahydroxyl flavanone-6-O-glucoside
FN7	26	11.207	619.1642	619.1639	0.5	595.1667	595.1663	0.7	$C_{27}H_{32}O_{15}$	Neoeriocitrin
T3	27	11.320	655.1631	655.1639	−1.2	631.1666	631.1663	0.5	$C_{30}H_{32}O_{15}$	Galloyl-paeoniflorin or isomer
T4	28	11.541	655.1624	655.1580	6.7	631.1645	631.1663	−2.9	$C_{30}H_{32}O_{15}$	Galloyl-paeoniflorin or isomer
FN8	29	11.677	595.2019	595.2027	−1.3	593.1890	593.1870	3.4	$C_{28}H_{34}O_{14}$	Neoponcirin
C1	30	11.857	676.2595	676.2605	−1.5	674.2450	674.2449	0.1	$C_{33}H_{41}NO_{14}$	Isoliquiritigenin-4-O-apiosyl-(1→2)-[2-(2-piperidyl) acetyl]-glucoside or isomer
FN9	31	11.932	617.1864	617.1846	2.9	593.1907	593.1870	6.2	$C_{28}H_{34}O_{14}$	Poncirin
*O3	32	12.069	647.1943	647.1952	−1.4	623.1978	623.1976	0.3	$C_{29}H_{36}O_{15}$	Verbascoside
C2	33	12.082	676.2595	676.2605	−1.5	674.2424	674.2449	−3.7	$C_{33}H_{41}NO_{14}$	Isoliquiritigenin-4-O-apiosyl-(1→2)-[2-(2-piperidyl) acetyl]-glucoside or isomer
*F7	34	12.249	471.0902	471.0903	−0.2	447.0915	447.0927	−2.7	$C_{21}H_{20}O_{11}$	Astragalin
*FN10	35	12.479	603.1680	603.1690	−1.7	579.1716	579.1714	0.3	$C_{27}H_{32}O_{14}$	Narirutin
*O4	36	12.573	503.2960	503.2985	−5.0	479.2997	479.3009	−2.5	$C_{27}H_{44}O_7$	Ecdysterone
FN11	37	12.724	457.1096	457.1111	−3.3	433.1130	433.1135	−1.2	$C_{21}H_{22}O_{10}$	Naringenin-7-O-glucoside
F8	38	12.867	649.1355	649.1381	−4.0	625.1417	625.1405	1.9	$C_{27}H_{30}O_{17}$	6-Hydroxy kaempferol-di-O-glucoside
*FN12	39	13.215	603.1694	603.1690	0.7	579.1718	579.1714	0.7	$C_{27}H_{32}O_{14}$	Naringin
*F9	40	13.485	579.1717	579.1714	0.5	577.1538	577.1557	−3.3	$C_{27}H_{30}O_{14}$	Rhoifolin

Q3	41	13.908	598.1504	598.1537	−5.5	574.1539	574.1561	−3.8	$C_{27}H_{29}NO_{13}$	Cartormin
FN13	42	13.922	779.2367	779.2374	−0.9	755.2375	755.2399	−0.8	$C_{34}H_{44}O_{19}$	Hesperetin-4′-O-rhamnoside-7-O-rutinoside
*FN14	43	14.106	611.2001	611.1976	4.1	609.1814	609.1819	−1.8	$C_{28}H_{34}O_{15}$	Hesperidin
F10	44	14.716	609.1842	609.1819	3.8	607.1652	607.1663	1.8	$C_{28}H_{32}O_{15}$	Diosmin or Neodiosmin
*FN15	45	14.853	611.1969	611.1976	−1.1	609.1830	609.1819	−0.8	$C_{28}H_{34}O_{15}$	Neohesperidin
*FN16	46	15.108	257.0808	257.0814	−2.3	255.0650	255.0657	−1.8	$C_{15}H_{12}O_4$	Liquiritigenin
*FN17	47	15.154	273.0793	273.0763	11.0	271.0605	271.0606	1.8	$C_{15}H_{12}O_5$	Naringenin
C3	48	15.249	419.1333	419.1342	−2.1	417.1176	417.1186	−2.7	$C_{21}H_{22}O_9$	Isoliquiritin
Q4	49	15.512	637.1505	637.1533	−4.4	613.1547	613.1557	−0.4	$C_{30}H_{30}O14$	Safflomin C or isomer
C4	50	15.640	615.1701	615.1690	1.8	591.1727	591.1714	−2.4	$C_{28}H_{32}O_{14}$	Acetyl-isoliquiritin apioside
FN18	51	15.644	581.1885	581.1870	2.6	579.1666	579.1714	−1.6	$C_{27}H_{32}O_{14}$	Isonaringin
T5	52	15.772	547.1685	547.1639	8.4	523.1673	523.1663	2.2	$C_{21}H_{32}O_{15}$	Rehmannioside A
IF1	53	15.813	269.0809	269.0814	−1.9	267.0656	267.0657	−8.3	$C_{16}H_{12}O_4$	Pallidiflorin
C5	54	15.971	441.1192	441.1162	6.8	417.1155	417.1186	1.9	$C_{21}H_{22}O_9$	Neoisoliquiritin
T6	55	16.294	623.1789	623.1741	7.7	599.1720	599.1765	−0.4	$C_{30}H_{32}O_{13}$	Benzoyl-hydroxyl-paeoniflorin
C6	56	16.944	719.3814	719.3892	−10.8	695.3956	695.4007	−7.4	$C_{35}H_{36}O_{15}$	Licoriceglycoside B
C7	57	17.211	749.2064	749.2058	0.8	725.2033	725.2082	−7.5	$C_{36}H_{38}O_{16}$	Licoriceglycoside A
S1	58	17.792	1277.5834	1277.5778	4.4	1253.5830	1253.5803	−7.3	$C_{58}H_{94}O_{29}$	Deapioplatycodin D3
S2	59	17.999	1409.6264	1409.6210	3.8	1385.6325	1385.6225	−6.8	$C_{63}H_{102}O_{33}$	Platycodin D2 or platycodin D3
SP1	60	18.300	584.2766	584.2761	0.9	582.2606	582.2604	2.2	$C_{34}H_{37}N_3O_6$	Safflospermidine A or safflospermidine B or N1,N5,N10-(Z)-tri-p-coumaroylspermine or N1,N5,N10-(E)-tri-p-coumaroylspermine
F11	61	18.382	617.1455	617.1482	−4.4	593.1478	593.1506	7.2	$C_{27}H_{30}O_{15}$	Kaempferol-3-O-rutinoside
SP2	62	18.589	584.2751	584.2761	−1.7	582.2601	582.2604	0.3	$C_{34}H_{37}N_3O_6$	Safflospermidine A or safflospermidine B or N1,N5,N10-(Z)-tri-p-coumaroylspermine or N1,N5,N10-(E)-tri-p-coumaroylspermine
*F12	63	18.691	287.0560	287.0556	1.4	285.0403	285.0399	−4.7	$C_{15}H_{10}O_6$	Kaempferol
SP3	64	18.716	584.2777	584.2761	2.7	582.2598	582.2604	−0.5	$C_{34}H_{37}N_3O_6$	Safflospermidine A or safflospermidine B or N1,N5,N10-(Z)-tri-p-coumaroylspermine or N1,N5,N10-(E)-tri-p-coumaroylspermine
S3	65	18.735	825.4304	825.4273	3.8	823.4091	823.4116	1.4	$C_{42}H_{64}O_{16}$	Uralsaponin C or isomer
SP4	66	18.915	584.2763	584.2761	0.3	582.2600	582.2604	−1.0	$C_{34}H_{37}N_3O_6$	Safflospermidine A or safflospermidine B or N1,N5,N10-(Z)-tri-p-coumaroylspermine or N1,N5,N10-(E)-tri-p-coumaroylspermine
S4	67	19.017	1115.5288	1115.5250	3.4	1091.5365	1091.5274	−3.0	$C_{52}H_{84}O_{24}$	Deapioplatycodin D
S5	68	19.103	1409.6280	1409.6210	5.0	1385.6316	1385.6225	−0.7	$C_{63}H_{102}O_{33}$	Platycodin D2 or Platycodin D3
*S6	69	19.195	1225.5851	1225.5853	−0.2	1223.5737	1223.5697	8.3	$C_{57}H_{92}O_{28}$	Platycodin D
S7	70	19.235	1451.6106	1451.6037	4.8	1427.6457	1427.6331	6.6	$C_{65}H_{104}O_{34}$	2″-O-Aceytlplatycodin D2 or 3″-O-aceytlplatycodin D2 or isomer
T7	71	19.307	607.1791	607.1791	0.0	583.1824	583.1816	3.3	$C_{30}H_{32}O_{12}$	Benzoyl-paeoniflorin
S8	72	19.318	1289.5876	1289.5778	7.6	1265.5905	1265.5803	8.8	$C_{59}H_{94}O_{29}$	Platycodin A or platycodin C
S9	73	19.389	897.4158	897.4120	4.2	895.4008	895.3964	1.4	$C_{44}H_{64}O_{19}$	22β-Acetoxy licorice saponin G2
S10	74	19.491	845.3996	845.3936	7.1	821.2969	821.2960	8.1	$C_{42}H_{62}O_{16}$	Uralsaponin B
S11	75	19.727	1451.6464	1451.6307	10.8	1427.6309	1427.6331	4.9	$C_{65}H_{104}O_{34}$	2″-O-Aceytlplatycodin D2 or 3″-O-aceytlplatycodin D2 or isomer
S12	76	19.817	1289.5904	1289.5778	9.8	1265.5800	1265.5803	1.1	$C_{59}H_{94}O_{29}$	Platycodin A or platycodin C
S13	77	19.934	985.4635	985.4644	−0.9	983.4548	983.4488	−1.5	$C_{48}H_{72}O_{21}$	Licoricesaponin A3
*C8	78	20.010	257.0815	257.0814	0.4	255.0651	255.0657	−0.2	$C_{15}H_{12}O_4$	Isoliquiritigenin

(continued on next page)

Table 1 − (continued)

Peak No.	Serial No.	t_R (min)	Positive (+) ion mode			Negative (−) ion mode			Formula	Identification
			[M+H]⁺/ [M+Na]⁺	Theoretical [M+H]⁺/ [M+Na]⁺	Error (ppm)	[M−H]⁻/ [M−H+HCOOH]⁻	Theoretical [M−H]⁻/ [M−H+HCOOH]⁻	Error (ppm)		
S14	79	20.201	881.4156	881.4171	−1.7	879.4031	879.4014	6.1	$C_{44}H_{64}O_{18}$	22β-Acetoxyl-glycyrrhizin or isomer
S15	80	20.509	839.4077	839.4065	1.4	837.3946	837.3909	−2.4	$C_{42}H_{62}O_{17}$	Yunganoside K2
*IF2	81	20.550	269.0808	269.0814	−2.2	267.0656	267.0657	1.9	$C_{16}H_{12}O_4$	Formononetin
S16	82	20.603	705.3814	705.3826	−1.7	681.3857	681.3850	4.4	$C_{36}H_{58}O_{12}$	3-O-Glucopyranosyl platycodigenin
S17	83	21.301	821.3887	821.3960	−8.9	819.3806	819.3803	−0.4	$C_{42}H_{60}O_{16}$	Licorice saponin E2
*S18	84	21.347	979.4777	979.4878	−10.3	955.4912	955.4903	1.0	$C_{48}H_{76}O_{19}$	Ginsenoside-Ro
S19	85	21.411	839.4058	839.4065	−0.8	837.3914	837.3909	0.4	$C_{42}H_{62}O_{17}$	Macedonoside A
S20	86	21.745	839.4077	839.4065	1.4	837.3898	837.3909	0.9	$C_{42}H_{62}O_{17}$	Macedonoside B
S21	87	21.824	949.5778	949.5137	67.5	925.5179	925.5161	0.6	$C_{48}H_{78}O_{17}$	Saikosaponin C
S22	88	21.854	887.4010	887.4041	−3.5	863.4072	863.4065	0.0	$C_{44}H_{64}O_{17}$	22β-Acetoxyl-glycyrrhaldehyde
*S23	89	22.275	823.4107	823.4116	−1.1	821.3956	821.3960	1.9	$C_{42}H_{62}O_{16}$	18β-Glycyrrhizic acid
*S24	90	22.399	817.4364	817.435	1.7	793.4322	793.4374	0.8	$C_{42}H_{66}O_{14}$	Chikusetsusaponin Iva
*F13	91	22.887	403.1385	403.1393	−2.0	—	—	—	$C_{21}H_{22}O_8$	Nobiletin
S25	92	23.297	809.4266	809.4323	−7.0	807.4196	807.4167	3.6	$C_{42}H_{64}O_{15}$	22-Dehydroxy-uralsaponin C[14]
S26	93	23.465	823.4104	823.4116	−1.5	821.3963	821.3960	0.4	$C_{42}H_{62}O_{16}$	Licorice saponin H2
S27	94	23.803	979.4902	979.4878	2.5	955.4955	955.4903	5.4	$C_{48}H_{76}O_{19}$	Yunganogenin A1/B1/C1 [24,26]
S28	95	23.822	823.4098	823.4116	−2.2	821.4030	821.3960	8.5	$C_{42}H_{62}O_{16}$	Licorice saponin K2
*S29	96	24.033	803.4545	803.4558	−1.6	779.4599	779.4582	2.2	$C_{42}H_{68}O_{13}$	Saikosaponin A
IF3	97	24.134	369.1329	369.1338	−2.4	367.1172	367.1182	−2.7	$C_{21}H_{20}O_6$	Glisoflavone
F14	98	24.731	355.1178	355.1182	−1.1	353.1008	353.1025	−4.8	$C_{20}H_{18}O_6$	Uralenin
F15/IF4	99	26.001	339.1218	339.1232	−4.1	337.1068	337.1076	−2.4	$C_{20}H_{18}O_5$	Eurycarpin A or glepidotin A [7] or 6-prenyl-5,7,4'-trihydroxyflavone or lupiwighteone
OF1	100	26.054	367.1131	367.1182	−13.9	365.1020	365.1025	−1.4	$C_{21}H_{18}O_6$	Glycyrol or isoglycyrol or neoglycyrol
F16/OF2	101	30.672	425.2003	425.1964	9.2	423.1848	423.1808	9.5	$C_{25}H_{28}O_6$	Gancaonin E or 3'-(γ,γ-dimethylallyl)-kievitone
IF5	102	31.780	423.1792	423.1808	−3.8	421.1644	421.1651	−1.7	$C_{25}H_{26}O_6$	Angustone A or isomer
OF3	103	33.641	439.2529	439.2484	10.2	437.2294	437.2328	−7.8	$C_{27}H_{34}O_5$	Licorisoflavan A

* Compared with authentic compounds.

In the positive ion spectra of compound O1, the fragment ion at m/z 120.0808 $[M+H-CH_2O_2]^+$ was observed, corresponding to the neutral loss of a formic acid via a rearrangement specific to amino acids. By comparing the MS data, elemental composition and fragmentation patterns with the literature data [22], compound O1 was tentatively identified as phenylalanine. Compound O2 was definitely assigned to amygdalin by comparison with an authentic standard. The highest intensity product ions at m/z 323.0948 $[M-H-C_8H_7NO]^-$ and 347.0946 $[M+Na-C_8H_7NO]^+$ were formed by the neutral loss of mandelonitrile from the parent ion. Compound O3 exhibited a quasimolecular ion at m/z 623.1978 $[M-H]^-$ in negative ion mode. The fragment ions at m/z 461.1594 $[M-H-C_9H_6O_3]^-$ of MS/MS corresponded to a loss of 2-hydroxy-4-(3-oxoallylidene)cyclohexa-2,5-dienone. The fragment ion at m/z 161.0222 $[M-H-C_{20}H_{30}O_{12}]^-$ also indicated the presence of 2-hydroxy-4-(3-oxoallylidene)cyclohexa-2,5-dienone. Compound O3 was identified as verbascoside by comparison with the authentic compound. In positive ion spectra, the adduction at m/z 647.1943 $[M+Na]^+$ could further support identification. Compound O4 was identified as β-ecdysterone by comparison with authentic compound.

4. Conclusion

A rapid and effective method based on UPLC-Q/TOF MS has been developed for separation and characterization of chemical profiles of XFZYD. This method successfully detected 103 major compounds in XFZYD, including 7 phenolic acids, 4 spermidines, 7 terpenoids, 52 flavonoids, 29 saponins, and 4 other compounds. The results could pave the way for the further study of XFZYD in pharmacology and mechanism.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Acknowledgments

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jfda.2015.06.004.

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