Research Article

Chemical Profiling of Xueshuan Xinmaining Tablet by HPLC and UPLC-ESI-Q-TOF/MS

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Xueshuan Xinmaining Tablet (XXT) is a widely used traditional Chinese medicine for the treatment of stroke, chest pain, coronary heart disease, and angina pectoris caused by blood stasis. Having a multiple-component preparation, it is still far from meeting the requirements of modernization and standardization because its detailed chemical basis and action mechanism have not been clarified. In this work, the different batches of XXT samples were analyzed by HPLC and the typical sample was analyzed by UPLC-ESI-Q-TOF/MS to understand its chemical profiling. As a result, 77 chromatographic peaks were detected, among which 63 constituents were identified or tentatively characterized based on the comparison of retention time and UV spectra with authentic compounds as well as by summarized MS fragmentation rules and matching of empirical molecular formula with those of published components. This is the first systematic report on the chemical profiling of the commercial XXT products, which provides the sufficiently chemical evidence for the global quality evaluation of XXT products.

1. Introduction

Xueshuan Xinmaining Tablet (XXT) is a widely used traditional Chinese medicine for the treatment of stroke, chest pain, coronary heart disease, and angina pectoris caused by blood stasis [1]. It is prepared from ten raw materials, including Chuanxiong (*Chuanxiong rhizoma*), Huaihua (*Sophorae flos*), Danshen (*Salviae miltiorrhizae radix et rhizoma*), Shuizhi (*Hirudo*), Maodongqing (*Hairy holly root*), Rengong Niu Huang (*Bovis calculus artifactus*), Rengong Shexiang (*Moschus artifactus*), Renshen Jingye Zongzaogan (Total ginsenoside of ginseng stems and leaves), Bingpian (*Borneolum syntheticum*), and Chansu (*Bufonis venenum*) [2]. Nowadays, several qualitative analyses have been reported concerning volatile constituents by GC-MS [3] and chemical

fingerprints by HPLC-ELSD [4] and UPLC-PDA [5]. However, having a multiple-component preparation, it is still far from meeting the requirement of modernization and standardization because its complicated ingredients lead to difficulty in clarifying therapeutic material basis, establishing quality control strategy, or selecting chemical markers for pharmacokinetic study.

Ultraperformance liquid chromatography coupled with electrospray ionization tandem quadrupole time-of-flight mass spectrometry (UPLC-ESI-Q-TOF/MS) has become one of the most effective tools for the on-line structural elucidation of multiple components of TCM, owing to its characteristics of accurate mass measurement, high resolution, and excellent sensitivity. MS^E (E represents collision energy) technology provides an automated strategy to decrease

analysis time and maximize duty cycles by using parallel alternating scans at low collision energy in the collision cell to obtain precursor ion information or at high collision energy to obtain accurate full-scan mass fragment, precursor ion, and neutral loss information. Therefore, both precursor and fragmentation data in exact mass mode were collected in a single run. This method has been proved to provide excellent chromatographic and MS efficiencies in the previous publications [6]. In the present investigation, a combination of HPLC and UPLC-Q-TOF/MS analyses was employed to find out and identify the common chemical profile in various batches of XXT samples. A total of 63 constituents were identified or tentatively characterized based on the comparison with reference substances, on-line UV spectra, and the fragmentation rules.

2. Materials and Methods

2.1. Materials and Reagents. Ten batches of Xueshuan Xinmaining Tablet (Lot. 160904, 160905, 161001-161008) were provided by *Jilin Huakang Stock Ltd., Company of Medicines* (Jilin, China). Reference substances of sodium danshensu (Lot. ZZS17032207), rosmarinic acid (Lot. ZZS17032201), salvianolic acid B (Lot. ZZS17032203), dihydrotanshinone (Lot. ZZS17032209), cryptotanshinone (Lot. ZZS17032204), and tanshinone IIA (Lot. ZZS17032206) were purchased from *Shanghai ZZBIO Co.* (Shanghai, China). Reference substances of neochlorogenic acid (Lot. X-014-140801), isochlorogenic acid B (Lot. Y-069-141122-1), and isochlorogenic acid C (Lot. Y-070-140801) were purchased from *Chengdu Herbpurify Co.* (Chengdu, China). Reference isochlorogenic acid A (Lot. 20130816) were purchased from *Shanghai Yuanye Bio-Technology Co.* (Shanghai, China). Reference substances of caffeic acid (Lot. 110885-200102), ginsenoside Rg₁ (Lot. 110703-201027), ginsenoside Re (Lot. 110754-200822), ginsenoside Rb₂ (Lot. 111715-200802), ginsenoside Rb₃ (Lot. 111686-201002), 20S-ginsenoside F₁ (Lot. 111763-200601), 20S-ginsenoside F₂ (Lot. 111764-200601), 20S-ginsenoside Rh₂ (Lot. 111748-200501), cinobufagin (Lot. 110803-200605), and resibufogenin (Lot. 110718-200507) were purchased from the National Institutes for Food and Drug Control (Beijing, China). Gamabufotalin, bufotalin, and bufalin were purified from the fresh toad venom (the purity of gamabufotalin was over 92.9% and the purity of bufotalin and bufalin was over 98% based on the area normalization by HPLC analysis). Ginsenoside Rd, chlorogenic acid, rutin, quercetin, protocatechualdehyde, ursodesoxycholic acid, and chenodeoxycholic acid were from the collection of our laboratory and their purities were over 98%. The structures of all reference compounds were shown in Figure 1.

HPLC grade acetonitrile and formic acid (Fisher, Fair Lawn, NJ, USA) and ultrapure water were used. All other chemical reagents were of analytical grade from *Beijing Chemical Corporation* (Beijing, China).

2.2. Reference Solution Preparation. Stock solution with a concentration of about 0.1 g·L⁻¹ was prepared by dissolving

an accurately weighed amount of each reference substance in methanol.

2.3. Sample Preparation

2.3.1. XXT Sample. 1.0 g (about 2.5 tablets) of pulverized XXT sample was extracted with methanol (25 mL) by ultrasonication (200 W, 53 kHz) for 30 min at room temperature, and the extract was centrifuged for 5 min at 3000 rpm. The supernatant was evaporated at 80°C and the residue was dissolved with aqueous methanol (MeOH-H₂O, 9:1). The obtained solution was centrifuged for 5 min at 12000 rpm. A volume of 10 µL of the supernatant was used for HPLC analysis.

1 mL of the sample solution for HPLC analysis was diluted to a volume of 10 mL and used for UPLC-ESI-Q-TOF/MS analysis.

2.3.2. Raw Materials. The ethanolic and aqueous extracts of individual herb (Danshen, Maodongqing, Shuizhi, Chuanxiong, and Huaihua) were prepared according to the manufacturing processes of XXT (Figure S1) described in the current Chinese Pharmacopoeia [2]. The ethanolic and aqueous extracts along with Rengong Shexiang, Rengong Niu Huang, Renshen Jingye Zongzaogan, Bingpian, and Chansu were dissolved with aqueous methanol solution (MeOH-H₂O, 9:1) according to the method described in Section 2.3.1 and used for the comparative analyses on the possible contribution from individual herb to general chromatographic profile of XXT samples.

2.4. Qualitative HPLC Analyses of 10 Batches of XXT Samples. The analyses were performed on a Shimadzu HPLC system (Shimadzu, Japan) equipped with an LC-20AT binary pump, a DGU-20A5 degasser, an SIL-20AC autosampler, a CTO-20AC column oven, and an SPD-M20A photodiode array detector. The samples were separated on a Waters XTerra C₁₈ column (4.6×250mm, 5µm). The mobile phase consisted of water containing 0.1% acetic acid (A) and acetonitrile (B) using a gradient elution program as follows: 0 min, 10% B; 20 min, 25% B; 30 min, 26% B; 45 min, 30% B; 60 min, 60% B; and 95 min, 80% B. The flow rate was 1.0 mL·min⁻¹ and the column temperature was 30°C. The PDA detector recorded UV spectra in the range from 190 nm to 400 nm and HPLC chromatogram was monitored at 251 nm.

2.5. UPLC-ESI-Q-TOF/MS Analysis. To comprehensively identify the chemical constituents in XXT samples, a UPLC-ESI-Q-TOF/MS experiment was performed using a Waters Xevo G2-S spectrometer (Waters, America), connected to a Waters UPLC system (Waters, America). The samples were separated on a Waters ACQUITY BEH C₁₈ column (2.1×50 mm, 1.7 µm). The mobile phase consisted of water containing 0.1% formic acid (A) and acetonitrile (B) using a gradient elution program as follows: 0 min, 10% B; 4 min, 25% B; 7 min, 25.6% B; 8 min, 36% B; 14 min, 60% B; 18 min, 80% B; and 20 min, 80% B. The mass spectrometer was operated in both positive and negative modes using the following parameters: capillary voltage of 1.8 kV, sample cone voltage

FIGURE 1: Structures of the identified compounds in XXT sample.

of 30 V (ESI+) or 40 V (ESI-), source temperature of 120°C, desolvation temperature of 600°C, and cone gas flow 50 L/h. In MS^E mode, the trap collision energy for the low-energy function was set at 6 eV, while the ramp trap collision energy for the high-energy function was set at 25-50 eV. Argon was used as the collision gas for collision-induced dissociation (CID) in MS^E and MS² modes. To ensure mass accuracy and reproducibility, the mass spectrometer was calibrated over a range of 100-2000 Da for MS and 50-2000 Da for MS². Leucine-enkephalin (m/z 556.2771 in positive ion mode; m/z 554.2615 in negative ion mode) was used as an external reference for the LockSpray and was infused at a constant flow of 5 $\mu\text{L}\cdot\text{min}^{-1}$. The data were recorded in centroid type during acquisition.

3. Results and Discussion

3.1. Qualitative Analyses of XXT Samples by HPLC. In order to obtain better detection of XXT samples, HPLC conditions were optimized. The acetonitrile-water system displayed more powerful separation ability for the main constituents in all samples than the methanol-water system. When organic acid was added to the mobile phase, the symmetry of most chromatographic peaks was improved. Under optimized conditions, ten batches of XXT samples, together with the reference compounds, were examined and their HPLC chromatograms were shown in Figure 2. High similarity in the number, type, and amount of chemical constituents was observed in the HPLC profile of each individual sample, which suggested the chemical consistency of different batches of XXT samples. The characteristic peak 4 (rutin) represents an important active component in XXT sample with a consistently high content, which was chosen as the reference peak for the calculation of the relative retention time ratio and relative peak area ratio of other common chromatographic peaks. For the validation of the assay procedure, the results of precision and repeatability were indicated by RSDs that were less than 4.9% ($n=6$) for seventeen peaks (Tables S1~S4). The stability test suggested that these peaks were stable in the sample solution within 24 hr (Tables S5~S6).

To identify the origin of these characteristic peaks from individual raw material, a comparative study was carried out by using various extract of raw materials and XXT samples. Considering the chromatogram recorded at the wavelength of 251 nm, main chromatographic peaks in the HPLC profile were attributed to the following five raw materials: Danshen, Huaihua, Maodongqing, Chuanxiong, and Chansu (Figure S2). The contribution of other raw materials was not manifested, either because of the absence of UV absorption of active compounds or due to the existence of no potent components at the detection condition. More information is expected to further confirm the contribution of each raw material to the general chromatographic profile of XXT sample.

3.2. Identification of Chemical Constituents in XXT Sample by UPLC-ESI-Q-TOF/MS. MS^E analyses in the positive ion mode and negative ion mode were selected for obtaining

FIGURE 2: HPLC profiles of Xueshuan Xinmaining Tablet samples at 251 nm (from top to bottom: Lot. 160904, 160905, 161001-161008).

extensive structural information via collision-induced dissociation. For the purpose of determining the optimized CE to generate fragment ions for structural elucidation and characterization, MS/MS experiments at different CE values were performed. Under optimized parameters, the total ion chromatogram of XXT samples are shown in Figure 3.

The fragmentation behaviors of reference compounds were investigated, and these rules were applied for the structural elucidation of their derivatives with the same basic skeleton. A total of 63 compounds in XXT sample were detected and tentatively characterized by comparing the retention times, MS fragmentation behavior, and literature information. Among these compounds, there were twenty saponins, fifteen phenolic acids, ten quinones, eight steroids, four bile acids, four flavonoids, one amino acid, and one lactone. Their chemical structures are provided in Figure 1. For better understanding of the fragmentation behavior, 63 compounds were divided into several groups and deduced as follows. The chromatographic and mass data of the identified constituents were summarized in Table 1.

Saponin Compounds. Saponin compounds have no UV absorption, but they could be well detected in both positive and negative ionization modes. Usually, the adduct ions such as $[\text{M}+\text{Na}]^+$ (ESI+) or $[\text{M}+\text{HCOO}]^-$ (ESI-) were detected in MS spectrum. In MS/MS spectrum, a series of

TABLE 1: Retention time (*t_R*), UV, and MS data of the identified compounds in XXT samples.

No.	<i>t_R</i> /min	UV <i>λ</i> _{max} /nm	Identified compounds	Formula	[M+H] ⁺ /[M+Na] ⁺	Calc.mass	Δppm	[M-H] ⁻	Calc.mass	Δppm	[M+HCOO] ⁻ /[2M-H] ⁻	Calc.mass	Δppm	Source ^a
1	1.03	282	danshensu	C ₉ H ₁₀ O ₅		197.0446	-2.5	197.0450	395.0978	-1.0	/395.0974	395.0978	-1.0	DS
2	1.22	295	neochlorogenic acid	C ₁₆ H ₁₈ O ₉		353.0869	-1.1	353.0873						DS/MDQ
3	1.59		suberyl arginine	C ₁₄ H ₂₆ O ₅ N ₄		329.1819	-1.8	329.1825						CS
4	1.83	320	chlorogenic acid	C ₁₆ H ₁₈ O ₉	331.1977/ 355.1026/	331.1981 355.1029	-1.2 -0.8	353.0876 137.0235/	707.1823	2.4	/707.1840	707.1823	2.4	DS/MDQ
5	1.83	220, 318	protocatechuic aldehyde	C ₇ H ₆ O ₃	/377.0854			353.0871						DS
6	1.99	233	cryptochlorogenic acid					179.0341						DS/MDQ
7	2.28	232, 318	caffeic acid	C ₉ H ₈ O ₄	/465.1384			441.1395						DS
8	3.41	244, 329	unidentified											
9	3.68	254, 352	rutin	C ₂₇ H ₃₀ O ₁₆	611.1618/ 595.1652/	611.1612 595.1663	1.0 -1.8	609.1458 593.1505	1219.2989	2.5	/1219.3020	1219.2989	2.5	HH
10	4.31	264, 343	kaempferol-3-O-	C ₂₇ H ₃₀ O ₁₅	/617.1472	617.1482	-1.6	517.1346						HH
			rutinoside		517.1331/ /539.1177	517.1346 539.1165	-2.9 2.2	515.1188						DS/MDQ
11	4.35	244, 327	isochlorogenic acid B	C ₂₅ H ₂₄ O ₁₂										
12	4.39	244, 327	salvianolic acid H	C ₂₇ H ₂₂ O ₁₂	539.1149/ 625.1754/	539.1190 625.1769	-7.6 -2.4	537.1031						DS
13	4.42	252, 339	isorhamnetin 3-O-rutinoside	C ₂₈ H ₃₂ O ₁₆	/647.1578	647.1588	-1.5	623.1616						HH
14	4.50	329	isochlorogenic acid A	C ₂₅ H ₂₄ O ₁₂	/539.1154	539.1165	-2.0	515.1193						DS/MDQ
15	4.90	244, 326	isochlorogenic acid C	C ₂₅ H ₂₄ O ₁₂	517.1359/ /539.1176	517.1346 539.1165	2.5 2.0	515.1192						DS/MDQ
16	5.02	244, 329	rosmarinic acid	C ₁₈ H ₁₆ O ₈	341.0666/	341.0661	1.5	359.0766	719.1612	-0.3	/719.1610	719.1612	-0.3	DS
17	5.13	250, 306	salvianolic acid G	C ₁₈ H ₁₂ O ₇										DS
18	5.17	251, 317	salvianolic acid A	C ₂₆ H ₂₂ O ₁₀	403.2483/ 417.2273/	403.2484 417.2277	-0.2 -1.0	493.1134	447.2383	-0.4	447.2381/	447.2383	-0.4	DS
19	5.28		gamabufotalin	C ₂₄ H ₃₄ O ₅	207.1024/	207.1021	1.4							CS
20	5.32		bufarenogin	C ₂₇ H ₁₄ O ₃										CS
21	5.43	276	4-hydroxyl-3-butylphthalide	C ₁₇ H ₁₄ O ₃										CX
22	5.53	232	salvianolic acid B	C ₂₆ H ₃₀ O ₁₆	/823.4792	823.4820	-3.4	717.1475						DS
23	6.17		ginsenoside Rg ₁	C ₄₂ H ₇₂ O ₁₄	/969.5378	969.5399	-2.2	945.5450	845.4899	0.7	845.4905/	845.4899	0.7	RSZZG
24	6.24		ginsenoside Re	C ₄₈ H ₈₂ O ₁₈	417.2271/	417.2277	-1.4	415.2111	991.5478	7.1	991.5548/	991.5478	7.1	RSZZG
25	6.44		arenobufagin	C ₂₄ H ₃₂ O ₆	303.0506/	303.0505	0.3	301.0346	461.2175	-1.5	461.2168/	461.2175	-1.5	CS
26	6.45		quercetin	C ₁₅ H ₁₀ O ₇	765.2371									HH
27	6.71		unidentified		/677.4221									
28	6.99		unidentified											
29	7.17	245, 328	hellebrigenin	C ₂₄ H ₃₂ O ₆	417.2274	417.2277	-0.7		461.2175					CS

TABLE 1: Continued.

No.	t_R /min	UV λ_{max} /nm	Identified compounds	Formula	$[M+H]^+/[M+Na]^+$	Calc.mass	Δ ppm	$[M-H]^-$ 677.1508	Calc.mass	Δ ppm	$[M+HCOO]^-/[2M-H]^-$	Calc.mass	Δ ppm	Source ^a
30	7.25		unidentified											
31	7.49		tanshinol A	$C_{18}H_{16}O_5$	313.1079/ /335.0901	313.1076	1.0							DS
32	8.14		ethyl lithospermate	$C_{29}H_{26}O_{12}$	567.1498/ /589.1320	567.1503	-0.9	565.1342	565.1346	-0.1				DS
33	8.49		unidentified		/755.1589	589.1322	-0.3	731.1622						
34	8.65		unidentified		/1117.5740						1139.5891/ 1007.5457/ 1007.5427	1007.5427	3.0	RSZZG
35	9.01		20S-glc-ginsenoside Rf	$C_{48}H_{82}O_{19}$	/985.5332	985.5348	-1.6							RSZZG
36	9.15		beads ginseng saponins F ₁	$C_{48}H_{82}O_{19}$	/985.5319	985.5348	-2.9							RSZZG
37	9.26		notoginsenoside R ₃	$C_{48}H_{82}O_{19}$	/985.5339	985.5348	-0.9				1007.5459/ 973.5042/ /1029.5789	1007.5427	3.2	RSZZG
38	9.35		unidentified		/951.4901									
39	9.52		unidentified		/538.2797			514.2904						
40	9.64		ginsenoside Rf	$C_{42}H_{72}O_{14}$	801.4985/ /823.4802	801.5000	-1.9				845.4923/ 489.2489/ 815.4818/ 815.4793	845.4899	2.8	RSZZG
41	9.72		bufotalin	$C_{26}H_{36}O_6$	445.2588/ /793.4705	823.4820	-2.2							CS
42	9.93		notoginsenoside R ₂	$C_{41}H_{70}O_{13}$	793.4714	445.2590	-0.4				815.4820/ 1123.5900	815.4793	2.5	RSZZG
43	10.07		ginsenoside F ₃	$C_{41}H_{70}O_{13}$	/793.4700	793.4714	-1.8							RSZZG
44	10.10		ginsenoside Rc	$C_{53}H_{90}O_{22}$	/1101.5831	1101.5821	0.9				829.4968/ 1123.5952/ 1123.5940/ 683.4393/ 991.5539/ 991.5509/ 961.5390/ 453.2857/ /815.5698 437.2902/ 783.5796 /783.5778	829.4949	2.3	RSZZG
45	10.20		ginsenoside Rg ₂	$C_{53}H_{90}O_{22}$	/807.4908	807.4871	4.3							RSZZG
46	10.31		ginsenoside Rb ₂	$C_{53}H_{90}O_{22}$	/1101.5798	1101.5821	-2.1							RSZZG
47	10.38		ginsenoside Rb ₃	$C_{53}H_{90}O_{22}$	/1101.5797	1101.5821	-2.2							RSZZG
48	10.49		unidentified		466.3162/ /661.4288			464.3032						
49	10.75		20S-ginsenoside F ₁	$C_{36}H_{60}O_9$	661.4288	661.4292	-0.6							RSZZG
50	10.73		bufalin	$C_{24}H_{34}O_4$	387.2531/ /969.5361	387.2535	-1.0							CS
51	10.86		unidentified		503.3368/ /969.5374	969.5399	-3.9	501.3224						
52	10.99		unidentified		969.5399	969.5399	-2.6							
53	11.27		ginsenoside Rd	$C_{48}H_{82}O_{18}$	/939.5284	939.5293	-1.0							RSZZG
54	11.61		gypenoside IX	$C_{47}H_{80}O_{17}$	385.2385/ 443.2435/ /431.2778	385.2379	1.6							RSZZG
55	11.68		resibutogenin	$C_{24}H_{32}O_4$		443.2434	0.2							CS
56	11.71		cinobufagin	$C_{26}H_{34}O_6$		431.2773	1.2	407.2803	407.2797	1.5				CS
57	11.79		cholic acid	$C_{24}H_{40}O_5$										CS
58	12.03		ursodeoxycholic acid	$C_{24}H_{40}O_4$				391.2851	391.2848	0.8				RGNH
59	12.41		1-oxo tanshinone IIA	$C_{19}H_{16}O_4$	309.1134/ /789.4742	309.1127	2.3							DS
60	12.59		ginsenoside Rk ₁	$C_{42}H_{70}O_{12}$	/807.4860	789.4765	-2.9							RSZZG
61	12.66		20S-ginsenoside F ₂	$C_{42}H_{70}O_{13}$	337.1431/ 279.1033/ /301.0849	807.4871	-1.4							RSZZG
62	12.89		neotanshinone D	$C_{21}H_{20}O_4$		337.1440	-2.7							DS
63	13.25		dihydrotanshinone	$C_{18}H_{14}O_3$	/301.0849	279.1021	4.3							DS
64	13.52		20R-ginsenoside Rg ₃	$C_{42}H_{72}O_{13}$	/807.4858	301.0841	2.7							
65	13.71		20S-ginsenoside Rg ₃	$C_{42}H_{72}O_{13}$	/807.4852	807.4871	-2.1							RSZZG
66	13.76		tetrahydro-tanshinone I	$C_{18}H_{16}O_3$	281.1189/ /303.1004	281.1178	3.9							DS
67	13.85		hydoxycholic acid	$C_{24}H_{40}O_4$		303.0997	2.3	391.2852	391.2848	1.0				RGNH

TABLE 1: Continued.

No.	t _R /min	UV λ _{max} /nm	Identified compounds	Formula	[M+H] ⁺ /[M+Na] ⁺	Calc.mass	Δppm	[M-H] ⁻	Calc.mass	Δppm	[M+HCOO] ⁻ /[2M-H] ⁻	Calc.mass	Δppm	Source ^a
68	13.92		unidentified		503.3368/ 339.1240/ /361.1054	339.1232	2.4	501.3222			783.5792 /1003.6544	783.5775	2.2	
69	13.98		methyl tanshinonate	C ₂₀ H ₁₈ O ₅		361.1052	1.4							DS
70	14.15		chemodeoxycholic acid	C ₂₄ H ₄₀ O ₄				391.2848	391.2848	0.0	437.2901/ /783.5789	437.2903 783.5775	-0.5 1.8	RGNH
71	15.05		cryptotanshinone	C ₁₉ H ₂₀ O ₃	297.1508/ /319.1313	297.1491	5.7							DS
72	15.62		ilxoside A/D	C ₄₂ H ₇₀ O ₁₂	/789.4742	789.4765	-2.9							MDQ
73	15.69	293	methylene tanshinquinone	C ₁₈ H ₁₄ O ₃	279.1030/ /301.0848	279.1021	3.2				811.4847/ /	811.4844	0.4	DS
74	16.03		20S-ginsenoside Rh ₂	C ₃₆ H ₆₂ O ₈		301.0841	2.3				667.4425/ 540.3302/ /	667.4421	0.6	RSZZG
75	16.39		unidentified		496.3405/ 295.1361/ /317.1165	295.1334	9.1							DS
76	16.81	267	tanshinone IIA	C ₁₉ H ₁₈ O ₃	283.1709/ /305.1525	317.1154	3.5							DS
77	17.31		miltirone	C ₁₉ H ₂₂ O ₂		283.1698	3.9							DS

^a Danshen, Huaihua, Maodongqing, Chansu, Chuanxiong, Renshen Jingye Zongzaogan, and Rengong Niu Huang are abbreviated as DS, HH, MDQ, CS, CX, RSZZG, and RGNH, respectively.

FIGURE 3: TICs of Xueshuan Xinmaining Tablet in the negative and positive modes.

fragmentation ions were observed due to the neutral loss of glucose, rhamnose, arabinose, or xylose (see Figure 4). These fragmentation rules are in accordance with the previous data [7, 8]. Totally, 20 ginsenoside saponins were identified, and they are deduced from the raw material, total ginsenoside of ginseng stems and leaves. Among the identified saponins, eight compounds (**23**, **24**, **46**, **47**, **49**, **53**, **61**, and **74**) were unambiguously identified as ginsenoside Rg₁, ginsenoside Re, ginsenoside Rb₂, ginsenoside Rb₃, 20S-ginsenoside F₁, ginsenoside Rd, 20S-ginsenoside F₂, and 20S-ginsenoside Rh₂. Unknown ginsenoside saponins followed the similar dissociation pathways. For the ginsenosides of protopanaxadiol, the ions at m/z 407, 425, and 443 could be found in the positive ion mode. Meanwhile the ginsenosides of protopanaxatriol showed the ions at m/z 405, 423, and 441 in the positive ion mode.

Ginsenoside Rg₁ showed $[M+Na]^+$ ion at m/z 823.4824 ($C_{42}H_{72}O_{14}Na$) in the positive ion mode and $[M+HCOO]^-$ ion at m/z 845.4895 ($C_{43}H_{73}O_{16}$) in the negative ion mode. The fragmentation ions at m/z 621.4368 and 603.4259 were produced from the protonated ion $[M+H]^+$ by losing glucose and the further loss of H_2O . In the negative MS/MS analysis, $[M-H]^-$ at m/z 799.4838 gave the characteristic ion at m/z 637.4307 $[M-H-glc]^-$ and 475.3773 $[M-H-2glc]^-$. Compounds **23** and **40** showed the quasimolecular ions at m/z 823 $[M+Na]^+$ and 845 $[M+HCOO]^-$ in the full mass spectrum. Compound **23** has the same retention time and MS data as those of reference ginsenoside Rg₁ and it was identified as ginsenoside Rg₁. As for compound **40**, although the fragmentation ions at m/z 405, 423, or 441 could not be detected in the MS/MS spectrum, it was temporarily identified as ginsenoside R_f.

Ginsenoside Re showed $[M+Na]^+$ ion at m/z 969.5427 ($C_{48}H_{82}O_{18}Na$) in the positive ion mode and $[M+HCOO]^-$ ion at m/z 991.5482 ($C_{49}H_{83}O_{20}$) in the negative ion mode. The fragmentation ions at m/z 945.5428 $[M-H]^-$, 799.4845 $[M-H-rha]^-$, 783.4893 $[M-H-glc]^-$, and 637.1430 $[M-H-rha-glc]^-$ were found in MS/MS spectrum in the negative ion mode. Compound **24** showed similar retention time and MS data as those of reference ginsenoside Re and it was identified as ginsenoside Re.

FIGURE 4: Proposed fragmentation pathway of ginsenoside Rb₃.

As for reference ginsenoside Rb₂, $[M+Na]^+$ ion at m/z 1101.5868 ($C_{53}H_{90}O_{22}Na$) in the positive ion mode and $[M+HCOO]^-$ ion at m/z 1123.5908 ($C_{54}H_{91}O_{24}$) in the negative ion mode are shown in the full mass spectrum. The fragmentation ions at m/z 945.5425 $[M-H-ara]^-$ and 783.4889 $[M-H-ara-glc]^-$ were detected in MS². Reference ginsenoside Rb₃ (Figure 4) gave the adduct ions at m/z 1101.5844 $[M+Na]^+$ and 1123.5903 $[M+HCOO]^-$. The fragmentation ions at m/z 945.5430 $[M-H-xyl]^-$, 783.4881 $[M-H-xyl-glc]^-$, and m/z 621.4352 $[M-H-xyl-2glc]^-$ were observed in MS².

Compounds **44**, **46**, and **47** have the same molecular formula of $C_{53}H_{90}O_{22}$ according to the quasimolecular ions at m/z 1101 $[M+Na]^+$ and 1123 $[M+HCOO]^-$. Compounds **46** and **47** were unambiguously identified as ginsenoside Rb₂ and ginsenoside Rb₃ by comparing the retention time and MS data as those of references. Compound **44** was tentatively deduced as ginsenoside Rc.

20S-ginsenoside F₁ gave the adduct ions at m/z 661.4300 $[M+Na]^+$ ($C_{36}H_{62}O_9Na$) and 683.4375 $[M+HCOO]^-$ ($C_{37}H_{63}O_{11}Na$). In the negative MS/MS experiment, the fragmentation ion at m/z 475.3785 $[M-H-glc]^-$ was detected. Compound **49** showed similar retention time and MS data as those of reference 20S-ginsenoside F₁.

Reference ginsenoside Rd showed the adduct ions at m/z 969.5379 $[M+Na]^+$ and 991.5495 $[M+HCOO]^-$ in the full mass spectrum. The fragmentation ions at m/z 783.4892 $[M-H-glc]^-$, 621.4368 $[M-H-2glc]^-$, and 459.3842 $[M-H-3glc]^-$ were produced by losing glucose. Compound **53** was

identified as ginsenoside Rd by displaying the same retention time and MS information.

As for reference 20S-ginsenoside F₂, it showed the quasi-molecular ions at m/z 807.4890 [M+Na]⁺ and 829.4949 [M+HCOO]⁻. The deprotonated ion peak at m/z 783.4881 [M-H]⁻ produced the fragmentation ion at m/z 621.4355 [M-H-glc]⁻ in MS². Compounds **45**, **61**, **64**, and **65** gave the same adduct ions [M+Na]⁺ in positive MS and [M+HCOO]⁻ in negative MS, indicating the molecular formula as C₄₂H₇₂O₁₃. Compound **45** was deduced as protopanaxatriol type and the latter three compounds were deduced as protopanaxadiol type on the base of the fragmentation rules described above. Among them, compound **61** has similar ions in full mass spectrum and MS/MS experiment as those of reference 20S-ginsenoside F₂ and was unambiguously identified as 20S-ginsenoside F₂. The deprotonated ion of **45** at m/z 783.4910 [M-H]⁻ indicated the ion at m/z 475.3791 [M-H-rha-glc]⁻, supporting that **45** was ginsenoside Rg₂. Compounds **64** and **65** displayed same MS data; however, unfortunately, MS² information could not be detected and they were tentatively identified as 20R-ginsenoside Rg₃ and 20S-ginsenoside Rg₃.

Reference ginsenoside Rh₂ showed the ions at m/z 1245.8995 [2M+H]⁺, 645.4348 [M+Na]⁺, and 667.4437 [M+HCOO]⁻. Compound **74** was identified as 20S-ginsenoside Rh₂ by comparing the retention time and MS data.

Compounds **35**, **36**, and **37** have the same molecular formula of C₄₈H₈₂O₁₉ and were tentatively characterized as 20S-glc-ginsenoside R_f, beads ginseng saponin F₁, and notoginsenoside R₃. They gave [M+Na]⁺ ions at m/z 985.5332, 985.5319, and 985.5339 in the positive ion mode and [M+HCOO]⁻ ions at m/z 1007.5457, 1007.5457, and 1007.5459 in the negative ion mode. In their MS/MS experiments, the fragmentation ions of **35** at m/z 799.4843 and 637.4273 were produced from the ion at m/z 961.5391 [M-H]⁻ by loss of glucose unit. Similarly, the fragmentation ions of **36** and **37** at m/z 799 and 637 were yielded. The retention order of three chromatographic peaks is determined according to the description in the literature [9].

The chemical formulas of compounds **42** and **43** are calculated as C₄₁H₇₀O₁₃ based on the ions at m/z 793.4705 [M+Na]⁺ in the positive ion mode and 815.4818 [M+HCOO]⁻ in the negative ion mode. Both compounds showed the fragmentation ions at m/z 405, 423, or 441 indicating that they are due to protopanaxatriol type. In MS/MS analysis, the ions at m/z 637.4326 [M-H-xy] and 475.3794 [M-H-xy-glc] for compound **42**, as well as 637.4319 [M-H-ara] and 475.3788 [M-H-ara-glc] for compound **43**, were detected from the deprotonated ions at m/z 769.4753 [M-H]⁻ and 769.4758 [M-H]⁻, respectively. Compounds **42** and **43** were identified as notoginsenoside R₂ and ginsenoside F₃.

Compound **54** showed the quasimolecular ions at m/z 939.5284 [M+Na]⁺ and 961.5390 [M+HCOO]⁻, indicating the molecular formula as C₄₇H₈₀O₁₇. The fragmentation ions 783.4901 [M-H-xy] and 621.4363 [M-H-xy-glc] were detected, supporting that **54** was gypenoside IX.

Compound **60** showed the quasimolecular ions at m/z 789.4742 [M+Na]⁺ and 811.4871 [M+HCOO]⁻ and the

molecular formula was calculated as C₄₂H₇₀O₁₂. In the MS/MS analysis, the deprotonated ion at m/z 765.4792 [M-H]⁻ gave the ions at m/z 603.4261 [M-H-glc] and 441.3730 [M-H-2glc]. Further loss of H₂O yielded the ions at m/z 423 and 405. It was identified as ginsenoside Rk₁.

Compound **34** showed [M+Na]⁺ ion at m/z 1117.5740 and [M+HCOO]⁻ ion at m/z 1139.5891, suggesting the molecule formula of C₅₃H₉₀O₂₃. Besides, it showed [M-H]⁻ ion at m/z 1093.5826 and fragment ions at m/z 961 [M-H- xyl/ara], 799 [M-H- xyl/ara-glc], and 637 [M-H- xyl/ara-2glc], concerning consecutive loss of 132, 294, and 456. These data were in accordance with those of floral ginsenoside P, floranotoginsenoside D, notoginsenoside FT₃, or yesanchinoside H [9], but its structure was not accurately determined based on the limited information.

Compound **72** showed [M+Na]⁺ ion at m/z 789.4742 and [M+HCOO]⁻ ion at m/z 811.4847, indicating the molecule formula as C₄₂H₇₀O₁₂. Besides, the ions at m/z 603.4251 [M-H-162] and 471.3466 [M-H-162-132] in the MS/MS analysis suggested that the glycoside chain of **72** consisted of a molecule of glucose and a molecule of arabinose or xylose. It could be one of ilexoside A or ilexoside D described in the literature [10].

Flavonoids. Flavonoids always present high sensitivity in the UV spectra. They could be well detected in both positive and negative ionization modes. Rutin, which has UV λ_{max} absorption at 255 and 353 nm, showed the protonated ion at m/z 611.1656 [M+H]⁺ and deprotonated ion at m/z 609.1461 [M-H]⁻ as well as the ion at m/z 1219.3008 [2M-H]⁻. Quercetin, with UV λ_{max} absorption at 253 and 356 nm, displayed the deprotonated ion at m/z 301.0376 [M-H]⁻. Four compounds (**9**, **10**, **13**, and **26**) were determined as flavonoid derivatives due to the typical UV absorption. Especially, compound **9** was one of main constituents of XXT. Compounds **9** and **26** were unambiguously identified as rutin and quercetin based on the direct comparison of their UV spectra, and mass spectra with those of the authentic compounds. Compound **10** has UV λ_{max} absorption at 264 and 343 nm. The molecular formula was calculated as C₂₇H₃₀O₁₅ by the quasimolecular ion at m/z 595.1652 [M+H]⁺, 617.1472 [M+Na]⁺, and 593.1505 [M-H]⁻. Other fragment ions at m/z 449.1083 [M+H-rha] and 287.1055 [M+H-rha-glc]⁺ were detected. Therefore, compound **10** was identified as kaempferol-3-O-rutinoside. Compound **13** has UV λ_{max} absorption at 252 and 339 nm. The molecular formula was calculated as C₂₈H₃₂O₁₆ by the quasimolecular ion at m/z 625.1754 [M+H]⁺, 647.1578 [M+Na]⁺, and 623.1616 [M-H]⁻, suggesting it was isorhamnetin 3-O-rutinoside [11].

Phenolic acids. Phenolic acids are the main constituents of XXT. This category of compounds was primarily derived from the ingredient herb, *Salviae miltiorrhizae radix et rhizoma* and *Hairy holly root*. In this study, 14 phenolic acids were found, among which 10 compounds (**1**, **2**, **4**, **5**, **7**, **11**, **14**, **15**, **16**, and **22**) were identified as danshensu, neochlorogenic acid, chlorogenic acid, protocatechuic aldehyde, caffeic acid, isochlorogenic acid A, isochlorogenic acid B, isochlorogenic acid C, rosmarinic acid, and salvianolic acid B by using reference substances. Other compounds (**12**, **17**, **18**, and **32**) were tentatively characterized by comparing UV absorption,

retention time, molecular formula, and mass fragmentation pathways [12–14].

Danshensu has UV $\lambda_{\max}$ absorption at 279 nm and retention time at 1.03 min. It showed the precursor ion at m/z 197.0443 and the molecular formula was calculated as $C_9H_{10}O_5$. In the full mass spectrum, the $[2M-H]^-$ ion at m/z 395.07949 was found. The MS/MS spectrum of $[M-H]^-$ exhibited an obvious fragment ion, $[M-H-H_2O]^-$ at m/z 179.0338, and further loss of COOH obtained ion at m/z 135.0438. Compound **1** was identified as danshensu.

Neochlorogenic acid has UV $\lambda_{\max}$ absorption at 323 nm. In the full mass spectrum, the deprotonated ion at m/z 353.0869 $[M-H]^-$ was found. The MS/MS spectrum of $[M-H]^-$ showed ions at m/z 191.0548, 179.0336, and 135.0438. Compound **2** was identified as neochlorogenic acid.

Chlorogenic acid has UV $\lambda_{\max}$ absorption at 325 nm and showed the quasimolecular ion at m/z 353.0872 $[M-H]^-$ and 707.1482 $[2M-H]^-$. The fragment ion at m/z 191.0553 was yielded by losing $C_9H_6O_3$. Compound **4** was identified as chlorogenic acid.

Protocatechuic aldehyde has UV $\lambda_{\max}$ absorption at 229, 278, and 310 nm. The $[M-H]^-$ ion at m/z 137.0236 was detected. In the MS/MS analysis, the ion at m/z 108.0204 was yielded by losing CHO and further losing oxygen produced from the ion at m/z 92.0256. Compound **5** was identified as protocatechuic aldehyde.

Caffeic acid has UV $\lambda_{\max}$ absorption at 238 and 322 nm. It showed $[M-H]^-$ ion at m/z 179.0339 in the full mass spectrum and the ion at m/z 135.0438 by losing CO_2 in the MS/MS experiment. Compound **7** was identified as caffeic acid.

The parent ions of three compounds (**11**, **14**, and **15**) at m/z 515 $[M-H]^-$ and 517 $[M+H]^+$ were easily located in the chromatogram of XXT, suggesting the molecular formula of $C_{25}H_{24}O_{12}$. They were assigned as dicaffeoylquinic acids by comparison with retention times. Besides, they gave the same fragment ions with those of chlorogenic acid, such as m/z 191, 179, 173, and 135, in MS/MS experiments. The fragmentation pathways are in accordance with those described in the literature [15]. Therefore, compounds **11**, **14**, and **15** were isochlorogenic acid B, A, and C.

Rosmarinic acid included a caffeic acid moiety and a molecule of danshensu. It showed $[M-H]^-$ ion at m/z 359.0759. The fragment ions at m/z 197.040 and 179.0332 were yielded by losing caffeic acid or danshensu moiety in MS^2 . Besides, the ion at m/z 161.0231 was yielded by further loss of H_2O . Compound **16** was identified as rosmarinic acid.

Salvianolic acid B showed $[M-H]^-$ ion at m/z 717.1464. Besides, the fragment ions at m/z 519.0934 ($[M-H-C_9H_{10}O_5]^-$), 339.0504, and 321.0392 were detected in the MS/MS analysis. The characteristic fragmentation pathway of losing $C_9H_{10}O_5$ is in accordance with those described in the literature [16]. Based on the retention time and the mass fragmentation pathway, compound **22** was identified as salvianolic acid B.

Compound **12** has UV $\lambda_{\max}$ absorption at 244 and 327 nm. The molecular formula was calculated as $C_{27}H_{22}O_{12}$ on the basis of the quasimolecular ions at m/z 539.1149 $[M+H]^+$ and 537.1031 $[M-H]^-$. In the MS/MS experiment, it indicated

the characteristic ion at m/z 339.0500 $[M-H-C_9H_{10}O_5]^-$, supporting that compound **12** was salvianolic acid H.

Compound **17** has UV $\lambda_{\max}$ absorption at 250 and 306 nm. It showed the ion at m/z 341.0666 $[M+H]^+$. In the MS/MS analysis, the ions at m/z 295.0617 and 279.0659 were yielded by losing CH_2O_2 and CH_2O_3 . Compound **17** was identified as salvianolic acid G.

Compound **18** has UV $\lambda_{\max}$ absorption at 251 and 317 nm. It showed $[M-H]^-$ ion at m/z 493.1134 and the molecular formula was calculated as $C_{26}H_{22}O_{10}$. In the MS/MS analysis, the fragment ion at m/z 295.0604 $[M-H-C_9H_{10}O_5]^-$ was detected, suggesting compound **18** was salvianolic acid A.

Compound **32** showed the ions at m/z 567.1498 $[M+H]^+$, 589.1320 $[M+Na]^+$, and 565.1342 $[M-H]^-$ in MS spectrum, revealing molecular formula of $C_{29}H_{26}O_{12}$. Besides, fragment ion at m/z 369.0971 $[M-H-C_9H_{10}O_5]^-$ was observed. Compound **32** was identified as ethyl lithospermate.

Bufadienolides. The MS/MS behaviors of bufadienolides have been extensively described [17, 18]. Briefly, for bufadienolides with only hydroxyl substituents, the fragmentation was characterized by successive eliminations of H_2O and CO molecules, and the profile of MS/MS product ions was correlated with the number of hydroxyl groups. If a C-16 acetoxyl group was present, the fragmentation of $[M+H]^+$ ions was triggered by initial loss of 60 Da (HOAc). The elimination of CO was significant for bufadienolides with a 19-formyl group, and the 19-hydroxyl group could be characterized by the loss of 30 Da (HCHO). These fragmentation rules were applied to the identification of bufadienolides in XXT sample. As shown in Table 1, eight bufadienolides were screened from XXT, six of which were unambiguously identified as gamabufotalin (**19**), arenobufagin (**25**), bufotalin (**41**), bufalin (**50**), resibufogenin (**55**), and cinobufagin (**56**) by comparison with reference substances isolated from toad venom. The other two bufadienolides were tentatively identified as bufarenogin (**20**) and hellebrigenin (**29**) [18, 19]. Taking gamabufotalin as a case, the fragmentation rules of bufadienolides were explained (Figure 5). Gamabufotalin showed UV $\lambda_{\max}$ absorption at 295 nm. In its full mass spectrum, $[M+H]^+$ ion at m/z 403.2486 was found and the fragment ions at m/z 385.2366 $[M+H-H_2O]^+$, 367.2262 $[M+H-2H_2O]^+$, and 349.2169 $[M+H-3H_2O]^+$ were detected in MS^2 .

Bile acids. Bile acid derivatives are in lack of conjugated system and their UV absorption is not obvious. However, they always present high sensitivity in the negative ion mode. The deprotonated ion $[M-H]^-$ and adduct ion $[M+HCOO]^-$ were obviously detected in MS spectrum. The loss of side chain was commonly observed in MS^2 . Four bile acid derivatives were detected from XXT sample, two of which were unambiguously identified as ursodeoxycholic acid (**58**, UDCA) and chenodeoxycholic acid (**70**, CDCA) based on the direct comparison of reference substances. The other two compounds were tentatively identified as cholanolic acid (**57**) and hyodeoxycholic acid (**67**). They are derived from the raw material, *Rengong Niu Huang* (*Bovis calculus artifactus*) [20].

Ursodeoxycholic acid showed the ions at m/z 391.2851 $[M-H]^-$, 437.2916 $[M+HCOO]^-$, and 783.5778 $[2M-H]^-$ in

FIGURE 5: Proposed fragmentation pathway of gamabufotalin.

the negative ion mode. In the positive ion mode, the ion at m/z 357.2807 $[M+H-2H_2O]^+$ was assigned as the loss of two molecules of H_2O . The ion at m/z 321.2587 represented its side chain loss in MS^2 . Compound **58** was identified as UDCA.

CDCA showed the ions at m/z 391.2872 $[M-H]^-$, 437.2907 $[M+HCOO]^-$, and 783.5798 $[2M-H]^-$ in the negative ion mode. In the positive ion mode, the loss of H_2O unit yielded $[M+H-2H_2O]^+$ ion at m/z 357.2809. The occurrence of the ion at m/z 321.2586 in MS^2 was due to side chain loss. Compound **70** was identified as CDCA.

Compound **57** showed $[M+Na]^+$ ion at m/z 431.2778 in positive mode, and $[M-H]^-$ ion at m/z 407.2803, $[M+HCOO]^-$ ion at m/z 453.2857, and $[2M-H]^-$ ion at m/z 815.5698 in the negative mode. The ions representing a series of H_2O loss was observed, such as 373.2749 $[M+H-2H_2O]^+$ and 355.2649 $[M+H-3H_2O]^+$. It was tentatively characterized as cholanolic acid.

Compound **67** showed $[M-H]^-$ ion at m/z 391.2852, $[M+HCOO]^-$ ion at m/z 437.2903, and $[2M-H]^-$ at m/z 783.5792 in the full mass spectrum. The molecular formula was calculated as $C_{24}H_{40}O_4$. It was tentatively deduced as hyodeoxycholic acid.

Quinones derivatives. Quinones derivatives are another kind of active constituents from Danshen and they were easily detected in XXT sample. Compounds **63**, **71**, and **76** were identified as dihydrotanshinone, cryptotanshinone, and tanshinone II A by comparison with reference substances. They displayed similar fragmentation pathways concerning successive eliminations of H_2O and CO molecules. Tanshinone IIA was used as an example to illustrate the fragmentation pathway of quinones constituents as shown in Figure 6. Based on these rules as well as empirical molecular formula, 7 compounds were characterized as tanshindiol A **31**, 1-oxo tanshinone IIA **59**, neotanshinone D **62**, tetrahydrotanshinone I **66**, methyltanshinonate **69**, methylenetanshinquinone **73**, and miltirone **77**. [12, 16, 20, 21].

Phthalide derivative. Compound **21** has UV absorption at 276 nm. Its molecular formula was calculated as $C_{12}H_{14}O_3$ on the basis of $[M+H]^+$ ion at m/z 207.1024. The above data were in accordance with those of 4-hydroxyl-3-butylphthalide in the literature [22]. Compound **21** was tentatively assigned as 4-hydroxyl-3-butylphthalide. It could be derived from individual herb *Chuanxiong rhizome* [23].

Among the identified compounds, most constituents were derived from the raw materials, Danshen and Renshen Jingye Zongzaogan, and a small proportion of compounds were considered from Huaihua, Maodongqing, Chuanxiong, Chansu, and Rengong Niu Huang. The other ingredients, Rengong Shexiang, Bingpian, and Shuizhi, were not characterized in the present HPLC and UPLC-QTOF/MS condition. This could be related to the prescription of raw materials and the manufacturing process employed. Usually, muscone, one of active constituents in Rengong Shexiang and borneol are detected by GC or GC-MS [3]. They are not easily detected in the liquid chromatography. Additionally, it is worth noting that no chemical information of Shuizhi, a famous traditional Chinese medicine originated from animal source, was found, although small molecule compounds with diverse structures have been reported from this drug [24]. More effort is made to explain its prescription role in XXT product.

4. Conclusion

In this work, HPLC analysis was employed to find out the common chromatographic peak in various batches of XXT samples and UPLC-Q-TOF/MS was used for the identification of main constituents in the typical XXT sample. As a result, a total of 63 constituents including twenty saponins, four flavonoids, fifteen phenolic acids, eight steroids, four bile acids, ten quinones, and other two compounds were identified or tentatively characterized based on the comparison of retention time and UV spectra with authentic compounds as well as by summarized MS fragmentation

FIGURE 6: Proposed fragmentation pathway of tanshinone IIA.

rules and matching empirical molecular formula with those of published components. The present investigation clearly understood the nonvolatile constituents in XXT and provided good basis for further study on the active substances and quality control of this preparation.

Data Availability

HPLC and UPLC-ESI-Q-TOF/MS data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

The pharmaceutical manufacture process of XXT described in current Chinese Pharmacopoeia is shown in Figure S1.

HPLC of XXT sample and extract of each raw material at 251 nm are shown Figure S2. Relative retention time ratio and relative area ratio of common characteristic peaks in precision, repeatability, and stability test for the HPLC method validation are shown in Tables S1~S6. (*Supplementary Materials*)

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