Arabian Journal of Chemistry (2022) **15**, 103825

King Saud University

Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com

REVIEW ARTICLE

Qualitative and quantitative analysis of multi-components in Xing-Su-Ning Capsules for quality improvement

Juanning Ren, Rongrong Wang, Wenjing Fan, Tong Li, Pengzhi Dong, Guixiang Pan, Ming Ren *, Miaomiao Jiang *

State Key Laboratory of Component-based Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin 301617, China

Received 3 December 2021; accepted 25 February 2022
Available online 4 March 2022

KEYWORDS

Xing-Su-Ning Capsules;
Constituent identification;
Quantitative analysis;
UHPLC-Q-Exactive-Orbitrap-MS;
UHPLC-QQQ-MS/MS

Abstract *Background:* Xin-Su-Ning Capsules (XSNC) is an effective prescription for the treatment of arrhythmia composed of eleven Chinese herbs. With the wide application of XSNC in clinic, its quality control issues have also received increasing attention. Based on the multi-components characteristics of Chinese herbal compound, there is an urgent need to establish a quality evaluation system.

Methods: Gas chromatography-mass spectrometry (GC-MS) and ultra high-performance liquid chromatography quadrupole electrostatic orbitrap high resolution mass spectrometry (UHPLC-Q-Exactive-Orbitrap-MS) were performed to identify the preliminary chemical profile of XSNC. Subsequently, a rapid ultra high-performance liquid chromatography coupled with electrospray ionization triple-quadrupole mass spectrometry (UHPLC-QQQ-MS/MS) method was developed to evaluate the quality of XSNC through a simultaneous determination of 16 components.

Results: A total of 21 volatile components and 59 non-volatile compounds were tentatively identified from the XSNC, each identified compound is marked on the corresponding chromatogram. Moreover, sixteen chemical constituents (sophocarpine, matrine, febrifugine, berberine, palmatine, Tangeratin, nobiletin, liensinine, neferine, scopoletin, isoliquiritigenin, liquiritigenin, naringenin, naringin, hesperidin and glycyrrhizic acid) were quantified by the developed UHPLC-QQQ-MS/MS method. The method validation of the sixteen compounds was performed with acceptable linearity (R^2, 0.9990-1.0000), precision (RSD, 0.25-2.06%), repeatability (RSD, 0.93-2.90%) and recovery (99.65%-104.03%, RSD≤4.35%).

Abbreviations: ESI, electrospray ionization; GC-MS, gas chromatography-mass spectrometry; TCM, Traditional Chinese medicine; UHPLC-Q-Exactive-Orbitrap-MS, ultra performance liquid chromatography tandem quadrupole orbitrap mass spectrometer; UHPLC-QQQ-MS/MS, ultra high-performance liquid chromatography coupled with electrospray ionization triple-quadrupole mass spectrometry; XSNC, Xin-Su-Ning Capsules

* Corresponding authors at: Tianjin University of Traditional Chinese Medicine, 10 Poyanghu Road, West Area, Tuanbo New Town, Jinghai District, Tianjin 301617, China.
E-mail addresses: renming2008@126.com (M. Ren), miaomiaojiang@126.com (M. Jiang).

https://doi.org/10.1016/j.arabjc.2022.103825

Conclusions: This qualitative analysis method sensitive and reliable for searching the volatile and non-volatile compounds from XSNC. The linearity, accuracy and precision of the quantitative analysis method were satisfactory. It is proposed that the methods described here can be applied for rapid evaluation, quality control and authenticity establishment of XSNC.

Contents

1. Introduction

Chinese herbal compound has definite curative effect and low side effects. It is the main drug for the clinical treatment of some complex diseases, chronic diseases and other diseases (Li and Du, 2015). Arrhythmia is a common and extremely dangerous cardiovascular diseases, it can not only aggravate the pre-existing heart disease, but also cause sudden death of patients (James and Calkins, 2016; Sossalla and Vollmann, 2018) With the limitation of anti-arrhythmic effect of chemical drugs, Chinese herbal compound represented by Xin-Su-Ning capsules (XSNC) has been more and more recognized in clinical practice because of its remarkable anti-arrhythmic effect in terms of multi-ion channel block and non-ion channel regulation(Li et al., 2019; Sun, 2017; Yao and Fang, 2017). XSNC consists of 11 Chinese herbs, including Coptidis Rhizoma, Pinelliae Rhizoma, Poria, Aurantii fructus Immaturus, Dichroae Radix, Nelumbinis Plumula, Sophorae flavescentis Radix, Artemisiae annuae Herba, Ginseng Radix et Rhizoma, Ophiopogonis Radix, Glycyrrhizae Radix et Rhizoma, it is a good prescription for the treatment of phlegm heat disturbance arrhythmia (Ma et al., 2006). Evidence-based medicine studies carried out from 2014 to 2017 have confirmed that XSNC has a definite clinical effect in treating cardiac arrhythmias caused by phlegm-heat (Zhaiet al., 2017). However, its chemical composition and quality control research are not in-depth, according to the instruction of Committee for the Pharmacopoeia of China in 2015, only berberine was indicated to be a index of XSNC for qualitative identification and quantitative analysis. In view of the complexity of chemical components of traditional Chinese medicine (TCM), its single qualitative and quantitative index is not enough to show the overall quality information of TCM, and it is difficult to fully reflect the effectiveness and safety of TCM. As a result, the comprehensive identification method of chemical composition is of great significance to the research of chemical composition of TCM. A new analytical method is needed to quantitatively determine various active components in XSNC.

In recent years, with the development of analytical technology, ultra high-performance liquid chromatography quadrupole electrostatic orbitrap high resolution mass spectrometry (UHPLC-Q-Exactive-Orbitrap-MS) is widely used in the analysis of TCM and compound prescription due to its fast separation speed, high sensitivity and strong determination accuracy (Eliuk and Makarov, 2015; Yang et al., 2020). Ultra high-performance liquid chromatography coupled with triple-quadrupole tandem mass spectrometry (UHPLC-QQQ-MS/MS) could provide simultaneous quantification of multiple components in the analysis of TCM (Liu et al., 2017, 2017; He et al., 2015).

To achieve the comprehensive chemical characterization of XSNC, we developed methods based on GC-MS and LC-MS to effectively analyze the chemical composition of XSNC. Then, an approach based on UHPLC-QQQ-MS/MS was developed to investigate the content of multi-components in XSNC. The quality of XSNC was comprehensively evaluated by quantifying the content of 16 compounds, which provided a reference for its quality evaluation, and laid a foundation for the later-stage drug-effective material basic research and clinical application.

2. Materials and methods

2.1. Materials and reagents

Methanol and acetonitrile (chromatographic purity) was purchased from Fisher company (USA), formic acid (MS grade) was purchased from ACS company (USA), and distilled water was purchased from Guangzhou Watsonsfood and beverage company (Guangzhou, China). Reference standards of sophocarpine, matrine, febrifugine, berberine, palmatine, Tangeratin, nobiletin, liensinine, neferine, scopoletin, isoliquiritigenin, liquiritigenin, naringenin, naringin, hesperidin and glycyrrhizic acid, jatrorrhizine were purchased from Sichuan Weikeqi Biotechnology Co., Ltd. or Shanghai Yuanye Biotechnology Co., Ltd. XSNC were supplied by Shanxi Momentum Pharmaceutical Co., Ltd (Shanxi, China).

2.2. Sample solutions preparation

XSNC were completely removed the capsule and weighed 1.77 g of the powder precisely. The powder was placed in a headspace sample bottle and sealed with an aluminum cap. Then, it was injected in the headspace sampler for GC-MS analysis.

The contents of XSNC were extracted by cold leaching with methanol for 24 h and repeated three times, then ultrasonically extracted for three times with methanol, the extracts was combined, concentrated and freeze-dried to obtain freeze-dried powder of non-volatile components. The lyophilized powder was weighed and dissolved in methanol a concentration of 5 mg/mL, then the solution was centrifuged at about 14000 rpm for 10 min. The supernatant was filtered through a 0.22 μm syringe filter and the filtrate was stored at 4 °C ready for UHPLC-ESI-Q-Exactive-Orbitrap-MS qualitative analysis.

A total of 0.2 g powder of XSNC was accurately weighed, ultrasound (25 kHz, 35 °C, 300 W) for 30 min with 20 mL methanol room temperature. After cooling down, the lost volume of methanol was complemented. Then the extracted solution was centrifuged at 14000 rpm for 10 min and the supernatant was taken. The supernatant 1 mL was accurately measure, diluted it with methanol and constant volume to obtain a test solution diluted 500 times with XSNC extract. The solution was filtered through a 0.22 μm syringe filter and the filtrate was stored at 4 °C ready for UHPLC-QQQ-MS/MS quantitative analysis.

2.3. Standard solutions preparation

The standards for matrine, sophocarpine, jatrorrhizine, palmatine, berberine, liquiritigenin, isoliquiritigenin, Tangeratin, nobiletin, and scopoletin were weighed accurately and dissolved in methanol for preparation of 1 mg/mL single reference solutions. Each reference solution was drew 0.1 mL and obtained a 100 μg/mL mixed standard solution. The solutions were filtered with 0.22 μm syringe filters before UHPLC-ESI-Q-Exactive-Orbitrap analysis.

The standards for sophocarpine, matrine, febrifugine, berberine, palmatine, Tangeratin, nobiletin, liensinine, neferine, scopoletin, isoliquiritigenin, liquiritigenin, naringenin, naringin, hesperidin and glycyrrhizic acid were weighed accurately and dissolved in methanol for preparation of single reference substance mother solution. The concentrations of reference substance mother solutions were as follows: sophocarpine 20 μg/mL, matrine 20 μg/mL, febrifugine 1 μg/mL, berberine 20 μg/mL, palmatine 20 μg/mL, Tangeratin 1 μg/mL, nobiletin 1 μg/mL, liensinine 2 μg/mL, neferine 10 μg/mL, scopoletin 5 μg/mL, isoliquiritigenin 2 μg/mL, liquiritigenin 2 μg/mL, naringenin 10 μg/mL, naringin 300 μg/mL, hesperidin 20 μg/mL, and glycyrrhizic acid 40 μg/mL.

Preparation of the standard curve: The above-mentioned reference substance mother solution was taken 50 μL each and the volume was made up to 1 mL with methanol. It contains sophocarpine 1 μg/mL, matrine 1 μg/mL, and febrifugine 50 ng/mL, berberine 1 μg/mL, palmatine 1 μg/mL, Tangeratin 50 ng/mL, nobiletin 50 ng/mL, liensinine 100 ng/mL, neferine 500 ng/ mL, scopoletin 250 ng/mL, isoliquiritigenin 100 ng/mL, liquiritigenin 100 ng/mL, naringenin 500 ng/mL, naringin 15 μg/mL, hesperidin 1 μg/mL and glycyrrhizic acid 2 μg/mL the highest concentration of mixed standard solution. The highest concentration of mixed reference solution was diluted with methanol 1:1 (v: v) by 2, 4, 8, 16, 32, 64 times to obtain a series of mixed reference solution.

2.4. GC-MS analysis

Chromatographic analysis was performed on an Agilent 7890B gas chromatograph (American, Agilent). A HP-5 MS quartz capillary column was used for chromatographic separation. Injections were performed in a split mode (ratio 5:1). High-purity nitrogen was used as a carrier gas and injector temperature was 240 °C. The initial column temperature was maintained at 50 °C for 2 min, then raised to 200 °C at a rate of 4 °C/min and held isothermally for 2 min. The column flow was 10 ml/min. Mass spectrometry analysis was performed on an Agilent 5977B mass spectrometer (American, Agilent). EI ionization method was adopted; the ion source temperature and quadrupole temperature were 230 °C and 150 °C respectively; the full scan mode range m/z 40∼400.

2.5. UHPLC-Q-exactive-orbitrap-MS qualitative analysis

Chromatographic analysis was performed on a Thermo Scientific UltiMate 3000 Ultra Performance Liquid Chromatograph (Thermo Fisher Scientific, USA); A Waters ACQUITY UPLC BEH C18 (1.7 μm, 2.1×100 mm) maintained at 35 °C was used for chromatographic separation. The mobile phase consisted of water acidified with 0.1 % (v/v) formic acid (A) and acetonitrile (B), was delivered at flow rate of 0.2 mL/min using the following gradient program: 0-2 min, 5-10% B; 2-5 min, 10-15% B; 5-10 min, 15-25% B; 10-15 min, 25-30% B; 15-20 min, 30-45%; 20-25 min, 45-65% B; 25-30 min, 65-95% B.

The Q-Exactive-Orbitrap mass spectrometer (Thermo Fisher Scientific, USA) equipped with an electrospray ion source. The atomizing gas was nitrogen; the spraying voltage was 3.5 KV; the flow rate of the sheath gas and the aux gas was 35 L/h and 10 L/h, respectively; the capillary temperature and auxiliary heating temperature were 350 °C; the first level spectrum adopted the positive and negative ion full scan mode, the scan range was 100-1500 m/z, the full scan resolution was 70000 FWHM; the second level fragment spectrum used the

Table 1 Condition parameters of mass spectrometry analysis of 16 compounds in Xin-Su-Ning capsules.

Compound	Formula	Parent	Daughters	CV	CE	Detection mode
Sophocarpine	$C_{15}H_{22}N_2O$	247.14	136.13	82	28	positive
Matrine	$C_{15}H_{24}N_2O$	249.16	148.16	80	26	positive
Febrifugine	$C_{20}H_{18}NO_4$	336.16	320.24	20	28	positive
Berberine	$C_{21}H_{22}NO_4$	352.12	308.13	62	28	positive
Palmatine	$C_{16}H_{19}N_3O_3$	302.11	138.13	32	14	positive
Tangeratin	$C_{20}H_{20}O_7$	373.03	343.14	26	26	positive
Nobiletin	$C_{21}H_{22}NO_8$	403.10	373.15	84	26	positive
Liensinine	$C_{37}H_{42}N_2O_6$	611.40	206.17	100	34	positive
Neferine	$C_{38}H_{44}N_2O_6$	625.42	206.16	100	30	positive
Scopoletin	$C_{10}H_8O_4$	191.01	176.03	36	16	negative
Isoliquiritigenin	$C_{15}H_{12}O_4$	255.11	119.15	46	24	negative
Liquiritigenin	$C_{15}H_{12}O_4$	255.17	119.15	40	26	negative
Naringenin	$C_{15}H_{12}O_5$	271.04	151.07	30	20	negative
Naringin	$C_{27}H_{32}O_{14}$	579.21	151.05	80	46	negative
Hesperidin	$C_{28}H_{34}O_{15}$	609.22	301.18	48	24	negative
Glycyrrhizic acid	$C_{42}H_{62}O_{16}$	821.56	351.09	38	42	negative

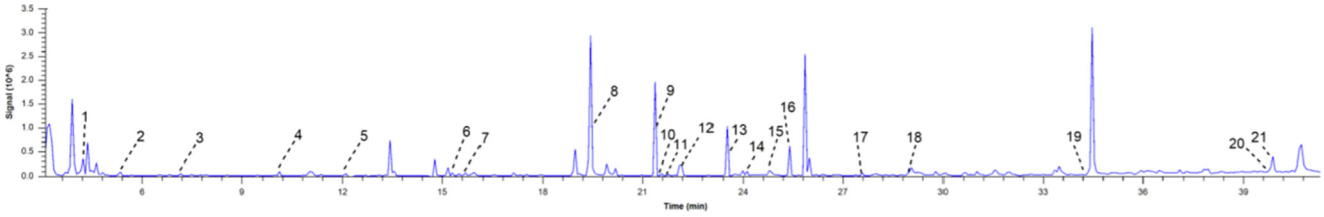

Fig. 1 The total ion current diagram of volatile components in Xin-Su-Ning capsules.

target ion detection mode, the resolution was 17500 FWHM; the collision induced dissociation energy gradient was set to 30/40/50 V.

2.6. UHPLC-QQQ-MS/MS quantitative analysis

Chromatographic analysis was performed on an ACQUITY UPLC Ultra Performance Liquid Chromatograph (American, waters company); Chromatographic separation was conducted on a Waters UPLC ACQUITY BEH C18 (1.7 μm, 2.1 mm×100 mm) maintained at 35 °C, the mobile phase consisted of 0.1% formic acid solution (A) and acetonitrile (B) using a gradient elution as following: 0-2 min, 5-10% B; 2-5 min, 10-20% B; 5-8 min, 20-25% B; 8-10 min, 25-30% B; 10-15 min, 30-45% B; 15-20 min, 45-95% B; the flow rate was kept at 0.3 mL/min.

Table 2 Identification List of volatile Components of Xin-Su-Ning capsules.

No	tR (min)	Compounds	Molecular formula	possibility	Forward match	Reverse match	CAS
1	4.249	2-Methylbutanal	$C_5H_{10}O$	85.02	910	918	96-17-3
2	5.382	Ethylpropenylether	$C_5H_{10}O$	65.08	876	888	928-55-2
3	7.184	2-Ethoxyoxolane	$C_6H_{12}O_2$	64.13	808	811	13436-46-9
4	10.114	(*Z*)-2-Butenoic acid ethyl ester	$C_6H_{10}O_2$	78.51	910	911	6776-19-8
5	12.116	Ethyl acetate	$C_8H_{16}O_2$	90.07	868	875	123-66-0
6	15.183	Methyl lactate	$C_4H_8O_3$	95.95	859	883	2155-30-8
7	15.716	Methylheptenone	$C_8H_{14}O$	73.86	778	865	110-93-0
8	19.984	Acetoxy-2-acetone	$C_5H_8O_3$	87.60	898	935	592-20-1
9	21.380	2-Acetylfuran	$C_6H_6O_2$	74.57	943	950	1192-62-7
10	21.581	Pyrrole	C_4H_5N	81.84	939	946	109-97-7
11	21.781	2,4-Dihydroxy-2,5-dimethyl-3	$C_6H_8O_4$	68.04	740	797	10230-62-3
12	22.113	Propionic acid	$C_3H_6O_2$	79.50	960	977	137-40-6
13	23.515	5-Methylfuran aldehyde	$C_6H_6O_2$	85.14	900	913	620-02-0
14	24.116	Hotrienol	$C_{10}H_{16}O$	73.52	789	811	20053-88-7
15	25.380	γ-Butyrolactone	$C_4H_6O_2$	60.08	961	966	96-48-0
16	25.849	Furfuryl alcohol	$C_5H_6O_2$	67.13	916	916	98-00-0
17	27.583	5-Methyl-2-furanmethanol	$C_6H_8O_2$	86.83	806	806	3857-25-8
18	29.048	2(5H)-Furanone	$C_4H_4O_2$	64.78	735	934	497-23-4
19	34.450	2-Acetylpyrrole	C_6H_7NO	77.55	930	935	1072-83-9
20	40.646	Ethyl palmitate	$C_{18}H_{36}O_2$	91.76	901	903	628-97-7
21	40.715	Ethyl hexadecanoate	$C_{18}H_{36}O_2$	71.12	700	721	628-97-7

MS detection was performed on Waters Xevo TQ-S Triple Quadrupole Mass Spectrometer (American, waterscompany). Quantification was performed using multiple reaction monitoring (MRM) mode. The optimized MS conditions for the positive ion mode were as follows: capillary voltage 3.0 KV, cone voltage 30 V, solvent removal temperature 350 °C. The optimized MS conditions for the negative ion mode were as follows: capillary voltage 2.0 KV, cone voltage 37 V, desolvation temperature 350 °C. The mass spectrometry analysis conditions of the 16 compounds were optimized and summarized in Table 1.

2.7. Method validation of UHPLC–QQQ–MS/MS

According to "2.6" analysis conditions and "2.2" extraction conditions, the linearity, limit of detection (LOD), limit of quantification (LOQ), precision, repeatability, stability and recovery rate of 16 compound markers were determined. The standard curve was drew with the concentration x (ng/mL) of the reference substance as the abscissa and the corresponding peak area y of each reference substance as the ordinate. Then linear regression was performed on the standard curve to examine the correlation coefficient and linear range of the resulting linear regression equation. LOD and LOQ were determined based on the standard deviation of response value and the slope of standard curve, calculation formula: $LOD = 3.3\delta/S$, $LOQ = 10\delta/S$ (δ, standard deviation; S, slope). XSNC was accurately weighed to test its precision, repeatability and stability. The test solution was continuously injected 6 times within 24 hours to evaluate the accuracy of the instrument, and 16 identical samples were prepared for repeatability analysis. The stability of the samples was studied after being placed at room temperature for 0, 2, 4, 8, 12 and 24 hours. The test solution and the reference solution were added at a

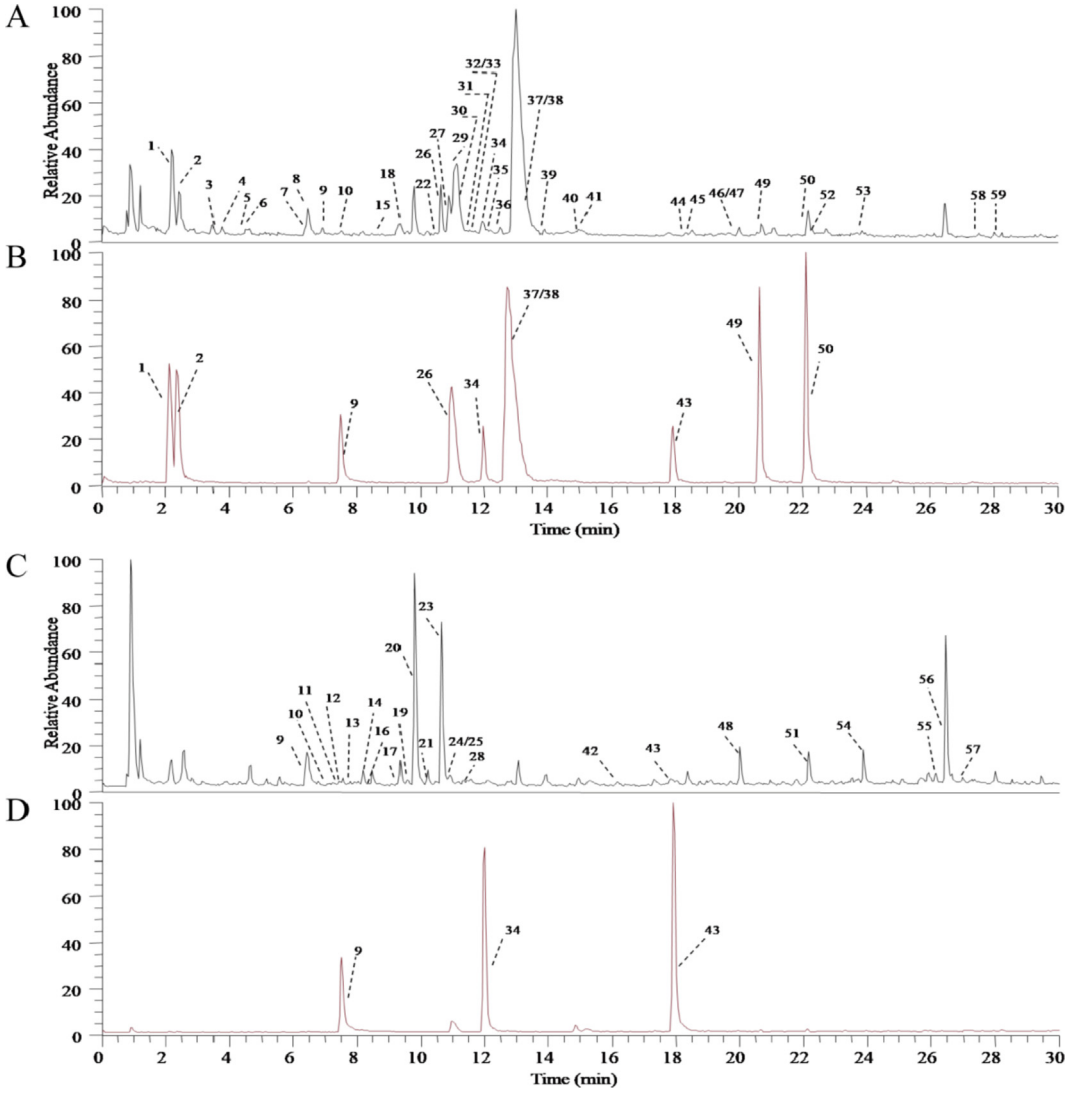

Fig. 2 Total ion current diagram of non-volatile components in Xin-Su-Ning capsules (A: positive ion mode Xin-Su-Ning capsules extract TIC; B: positive ion mode reference substance TIC; C: negative ion mode Xin-Su-Ning capsules extract TIC; D: negative ion mode reference substance TIC).

Table 3 Identification List of non-volatile Components of Xin-Su-Ning capsules.

Peak NO.	tR (min)	Formula	Measured (*m*/*z*)	Detected (*m*/*z*)	Delta (ppm)	Fragments	Identification	class	source
1#	2.21	$C_{15}H_{24}N_2O$	248.1889	249.1959	–0.642	148.1116 150.1271 112.0760	Matrine	Alkaloids	Sophora flavescens
2#	2.48	$C_{15}H_{22}N_2O$	246.1732	247.1804	–0.283	229.1705 179.1541 150.1275 136.1132	Sophocarpine	Alkaloids	Sophora flavescens
3	3.51	$C_{15}H_{22}N_2O$	246.1732	247.1804	–0.525	148.1121 136.1125 112.0763	Sophocarpine Isomers	Alkaloids	Sophora flavescens
4	3.76	$C_{15}H_{24}N_2O_2$	264.1838	265.1909	–0.394	150.1278 138.1277	Hydroxylated matrine	Alkaloids	Sophora flavescens
5	4.06	$C_{16}H_{19}N_3O_3$	301.1421	302.1497	–0.556	284.1398 203.0816 138.0914	Febrifugine	Alkaloids	Changshan
6	4.52	$C_{19}H_{23}NO_3$	313.1678	314.1748	–0.732	269.1172 237.0911 107.0495	Lotusine	Alkaloids	Lotus Seed Heart
7	6.48	$C_{20}H_{24}NO_4$	342.1705	342.1696	–1.241	265.0858 297.1119	Magnoflorine	Alkaloids	Coptis
8	7.45	$C_{27}H_{30}O_{14}$	578.1636	579.1706	–0.418	271.0599 195.0287 219.0287 153.0181	Rhoifolin	Flavone	Citrus aurantium
9#	7.53	$C_{10}H_8O_4$	192.0423	193.0495	–0.131	178.0261 133.0284 137.0597 145.0958	Scopoletin	Coumarin	Citrus aurantium
10	8	$C_{27}H_{32}O_{15}$	596.1741	595.1671	0.347	459.1097 287.0555 269.0454 135.0436	Eriocitrin	Flavone	Citrus aurantium
11	8.16	$C_{26}H_{30}O_{13}$	550.1686	549.1609	–0.754	297.0073 255.0655 153.0180	Glycyrrhizin-4'-apirin	Flavone	Citrus aurantium
12	8.36	$C_{27}H_{30}O_{16}$	610.1484	609.1465	0.611	301.0341 269.0451 201.0556 151.0022 88.9862	Rutin	Flavone	Citrus aurantium
13	8.45	$C_{27}H_{32}O_{15}$	596.1741	595.1669	0.146	459.1134 339.0707 287.0556 235.0556	Neoeriocitrin	Flavone	Citrus aurantium
14	8.73	$C_{27}H_{30}O_{15}$	594.1585	593.1513	0.197	447.0923 327.0599 285.0395	Lonicerin	Flavone	Citrus aurantium
15	9.3	$C_{19}H_{15}NO_4$	322.1079	322.1071	–0.914	307.0837 294.0759	Greenland Xanthine	Alkaloids	Coptis
16	9.32	$C_{27}H_{32}O_{14}$	580.1792	579.1718	–0.309	339.9276 295.0617 151.0023	Narirutin	Flavone	Citrus aurantium
17	9.75	$C_{27}H_{32}O_{14}$	580.1792	579.1714	0.162	459.1171 271.0607 151.0022 119.0487	Naringin	Flavone	Citrus aurantium
18	9.8	$C_{15}H_{12}O_5$	272.0685	273.0753	–1.794	153.0182 147.0040 171.0287 177.0546	Naringenin isomers	Flavone	Citrus aurantium
19	9.85	$C_{21}H_{22}O_{10}$	434.1163	433.1134	–1.432	363.3987 271.0607 151.0022 83.0123	Prunin	Flavone	Citrus aurantium
20	10.17	$C_{28}H_{34}O_{15}$	610.0898	609.1822	–0.498	325.0722 301.0710 164.0102 151.0022	Hesperidin	Flavone	Citrus aurantium
21	10.41	$C_{28}H_{32}O_{15}$	608.1691	607.167	0.34	329.1388 299.0554 242.0673 164.0103 125.0228	Neogeranin	Flavone	Citrus aurantium
22	10.64	$C_{16}H_{14}O_6$	302.0785	303.0858	–1.764	153.0182 285.0758 322.1056	Hesperetin	Flavone	Citrus aurantium
23	10.7	$C_{28}H_{34}O_{15}$	610.0898	609.18119	–0.892	555.9461 325.0710 301.0711 286.0476 151.0022	Neohesperidin isomers	Flavone	Citrus aurantium
24	10.81	$C_{22}H_{24}O_{11}$	464.1319	463.1247	0.227	301.0709 286.0476 242.0575 151.0021	Hesperetin-7-O-β-D-glucoside	Flavone	Citrus aurantium
25	10.89	$C_{28}H_{32}O_{15}$	608.1691	607.167	0.242	489.1394 343.0817 301.0710 267.0657 151.0023	Geranidin	Flavone	Citrus aurantium
26#	10.9	$C_{20}H_{20}NO_4$	338.1392	338.1383	–1.108	294.1133 97.1016 83.0861	Jatrorrhizine	Alkaloids	Sophora flavescens
27	11.01	$C_{20}H_{18}NO_4$	336.1236	336.1226	–1.232	320.0917 292.0962 292.0966	Dihydroberberine	Alkaloids	Coptis
28	11.08	$C_{26}H_{30}O_{13}$	550.1686	549.16156	0.357	399.1046 255.0655 153.0179 135.0072	Isoliquiritin glucocelium	Flavone	Licorice
29	11.09	$C_{19}H_{14}NO_4$	320.0923	320.0913	–1.232	277.0729 262.0871	Coptisine	Alkaloids	Coptis
30	11.17	$C_{20}H_{20}NO_4$	338.1392	338.1385	–2.202	323.1144 294.1122 308.0920	Tetrandrine isomers	Alkaloids	Coptis
31	11.48	$C_{21}H_{22}O_9$	418.1264	419.1335	–0.426	257.0806 137.0233 239.0702	Liquiritin	Flavone	Licorice
32	11.71	$C_{20}H_{20}NO_4$	338.1392	338.1385	–0.487	323.1147 294.1121	Tetrandrine isomers	Alkaloids	Coptis
33	11.96	$C_{19}H_{15}NO_4$	322.1079	322.1072	–0.635	307.0838 279.0880	Berberrubine	Alkaloids	Coptis
34#	12	$C_{15}H_{12}O_4$	256.0736	257.0808	–0.332	211.0753 147.0441 137.0234 119.0494	Liquiritigenin	Flavone	Licorice
35	12.23	$C_{21}H_{20}NO_4$	350.1386	350.1387	–0.042	334.1072 322.0706 306.1126	13-methylepiberberine	Alkaloids	Coptis
36	12.8	$C_{16}H_{12}O_5$	284.0679	285.0756	–0.648	270.052 253.0493 225.0546	Calycosin	Flavone	Sophora flavescens
37#	12.96	$C_{20}H_{18}NO_4$	336.1236	336.1227	–1.055	321.0984 292.0966 306.0757	Berberine	Alkaloids	Coptis
38#	13.27	$C_{21}H_{22}NO_4$	352.1549	352.1539	–1.149	337.1296 322.1074 308.1280	Palmatine	Alkaloids	Coptis
39	13.91	$C_{28}H_{34}O_{14}$	594.1943	595.2021	0.03	287.0911 153.0182	Poncirin	Flavone	Citrus aurantium
40	14.93	$C_{15}H_{12}O_5$	272.0685	273.0756	–0.476	153.0182 147.0440 119.0494	Naringenin	Flavone	Citrus aurantium
41	15.08	$C_{21}H_{20}NO_4$	350.1387	350.1386	–1.358	335.1142 320.0918 306.1123 292.0971 254.0569	13-methylberberine	Alkaloids	Coptis
42	16.18	$C_{16}H_{14}O_6$	302.079	301.0718	0.261	286.0479 257.0841 242.0574 233.0796	Hesperetin	Flavone	Sophora flavescens
43	17.95	$C_{15}H_{12}O_4$	256.0736	255.0661	–0.162	211.0753 135.0072 119.0487	Isoliquiritigenin	Flavone	Licorice
44	18.33	$C_{16}H_{12}O_4$	268.073	269.0805	–1.358	237.0542 137.0233 118.0414	Formononetin	Coumarin	Citrus aurantium
45	18.53	$C_{15}H_{16}O_4$	260.1043	261.1114	–2.74	189.0545 243.1012 159.0440 131.0492	Hesperitone	Coumarin	Citrus aurantium

Table 3 *(continued)*

Peak NO.	tR (min)	Formula	Measured (*m/z*)	Detected (*m/z*)	Delta (ppm)	Fragments	Identification	class	source
46	19.67	$C_{26}H_{30}O_8$	470.1935	471.2009	–0.414	425.1963 339.1952 213.0911 161.0598 95.0132	Limonin	Flavone	Citrus aurantium
47	19.64	$C_{19}H_{18}O_6$	342.1098	343.1172	–0.335	313.0704 285.0755 181.0129 373.0918	4',5,7,8-tetramethoxyflavonoid	Flavone	Citrus aurantium
48	19.97	$C_{42}H_{62}O_{16}$	822.4038	821.3964	–0.108	683.7745 513.6673 443.4119 351.0563 175.0234	Glycyrrhizic acid	Flavone	Citrus aurantium
49#	20.7	$C_{21}H_{22}O_8$	402.1315	403.1383	–0.404	388.1163 373.0916 355.0822	Nobiletin	Flavone	Citrus aurantium
50#	22.17	$C_{20}H_{20}O_7$	372.1209	373.1277	–1.397	343.0811 358.1042 325.0703	Tangeratin	Flavone	Citrus aurantium
51	22.09	$C_{26}H_{30}O_6$	438.2042	437.1969	–0.142	301.1429 151.0386 91.0539	Kurarinone	Flavone	Sophora flavescens
52	22.21	$C_{16}H_{35}NO_2$	273.2662	274.2737	–1.261	256.2634	Cetyl-Dihydrosphingosine	Alcoholamines	Pinellia
53	23.67	$C_{27}H_{32}O_6$	452.2193	453.2268	–0.85	329.1025 303.1590 197.0440	2'-Methoxymatrine	Flavone	Sophora flavescens
54	23.84	$C_{25}H_{30}O_6$	424.1886	423.1816	0.634	261.1491 161.0231 109.0281	Kushenol E isomers	Flavone	Sophora flavescens
55	25.97	$C_{25}H_{30}O_6$	424.1886	423.1967	–0.553	261.1491 161.0231 109.0281	Kushenol E isomers	Flavone	Sophora flavescens
56	26.54	$C_{26}H_{30}O_6$	438.2042	437.1967	–0.553	275.1648 161.0230 109.0277	Kuraridin	Flavone	Sophora flavescens
57	27.18	$C_{30}H_{46}O_4$	470.3391	469.3312	–0.326	425.3423 409.3109	Glycyrrhetinic acid	Triterpene	Licorice
58	27.47	$C_{20}H_{43}NO_2$	329.3288	330.3361	–1.623	312.3257 106.0866 88.0762	2-amino-1,3-eicosanediol	Alcoholamines	Pinellia
59	28.21	$C_{19}H_{22}O_3$	298.1563	299.1633	–0.871	189.0542 163.0389 119.0494	Grapefruit lactone	Coumarin	Citrus aurantium

Note: # stands for comparison with standard products.

ratio of 1:1, and six parts were measured in parallel to calculate the recovery rate of each component.

3. Results

3.1. Identification of chemical composition of Xin-Su-Ning capsules

3.1.1. Analysis of volatile components

The volatile components in XSNC were analyzed by GC-MS. The total ion flow diagram was shown in Fig. 1. A total of 21 volatile components were identified by searching with NIST mass spectrometry database (Table 2).

3.1.2. Analysis of non-volatile components

UHPLC-MS was used to analyze the non-volatile components in XSNC. The total ion flow diagram of the sample solution and the reference solution were shown in Fig. 2. For the compounds with chemical standards, according to the retention time, as well as accurate and high-resolution mass and tandem mass spectra, as a results, 10 compounds (peak 1, 2, 9, 26, 34, 37, 38, 43, 49 and 50) were identified as matrine, sophocarpine, scopoletin, jatrorrhizine, liquiritigenin, berberine,palmatine, isoliquiritigenin, nobiletin and Tangeratin, respectively. For the compounds without chemical standards, based on the retention time, exact mass data, fragment information, and molecular formula reported in the literatures, a total of 49 compounds were detected. Take hesperidin as an example to illustrate the fragmentation process, peak 20 exhibited the precursor ion $[M-H]^-$ ion at *m/z* 609.1822 in the negative mode and $[M+H]^+$ ion at *m/z* 611.1976 in the positive mode. It was speculated that the relative molecular weight of the compound was 610 and the predicted molecular formula was $C_{28}H_{34}O_{15}$. In the first-order mass spectrum of positive ion mode, there were fragments of *m/z* 449.1439 and *m/z* 465.1389, and in the second-order mass spectrum, there were fragment ions of *m/z* 303.0861 $[M+H–Rha–Glc]^+$, *m/z* 153.0182 $[M+H–Rha–Glc–C_9H_{10}O_2]^+$, which was consistent with the fragments of hesperidin in the literature, and speculated that the compound was hesperidin (Chen et al., 2012). All in all, a total of 59 chemical constituents were tentatively identified including 18 alkaloids, 33 flavonoids, 5 coumarins, 2 alcoholamines and 1 triterpenoid (Table 3). Among these compounds, sophocarpine, matrine, febrifugine, berberine, palmatine, Tangeratin, nobiletin, liensinine, neferine, scopoletin, isoliquiritigenin, liquiritigenin, naringenin, naringin, hesperidin and glycyrrhizic acid were mainly active constituents with reported bioactivities. As a result, the quantitative analysis of these 16 constituents was performed in XSNC extracts.

3.2. Quantitative analysis

Through LC-MS multiple reaction detection mode, the test solution was prepared according to the method under "2.2", and the prepared test solution was determined under the detection conditions "2.6". Aiming at the problem of large difference in mass spectrum response and content of various types of compounds in the complex system of XSNC, through the multiple dilution method, the same sample was prepared by preparing a low dilution ratio test solution to detect compo-

Table 4 Linear equation, linear range of, LOD and LOQ sixteen analytes in UHPLC-QQQ-MS/MS.

Compounds	Regression equation	R^2	Linearity range (ng/mL)	LOD (ng/mL)	LOQ (ng/mL)
Sophocarpine	y =8033.50x + 112717.00	R^2 = 0.9992	15.625-1000	4.910	14.880
Matrine	y = 3834.20x- 25.18	R^2 = 0.9997	15.625-1000	3.362	10.187
Febrifugine	y =2782.70x- 1136.30	R^2 = 0.9994	0.781-50	0.146	0.442
Berberine	y = 13757.00x + 16757.00	R^2 = 0.9999	15.625-1000	3.722	11.280
Palmatine	y = 16658.00x - 3566.10	R^2 = 1.0000	15.625-1000	3.236	9.805
Tangeratin	y = 92881.00x + 5240.10	R^2 = 0.9994	0.781-50	0.320	0.971
Nobiletin	y = 73488.00x + 16786.00	R^2 = 0.9998	0.781-50	0.498	1.509
Liensinine	y = 721.52x - 757.60	R^2 = 0.9990	1.563-100	0.429	1.299
Neferine	y = 1079.90x - 3426.70	R^2 = 0.9993	7.813-500	2.296	6.959
Scopoletin	y = 148.42x - 293.17	R^2 = 0.9995	3.906-250	1.230	3.729
Isoliquiritigenin	y = 418.49x - 279.76	R^2 = 0.9994	1.563-100	0.461	1.395
Liquiritigenin	y = 233.48x - 115.91	R^2 = 0.9992	1.563-100	0.158	0.480
Naringenin	y =295.30x - 599.14	R^2 = 0.9996	7.813-500	2.435	7.378
Naringin	y = 156.15x + 1022.20	R^2 = 1.0000	234.375-15000	60.074	182.043
Hesperidin	y = 433.93x + 109.30	R^2 = 0.9992	15.625-1000	4.831	14.641
Glycyrrhizic acid	y = 213.75x - 2960.30	R^2 = 0.9993	31.250-2000	5.541	16.792

Table 5 The results of precision, repeatability, stability, and recovery in UHPLC-QQQ-MS/MS (n = 6).

Compounds	Precision RSD (%)	Repeatability RSD (%)	Stability RSD (%)	Recovery	
				Mean	RSD (%)
Sophocarpine	0.77	0.93	0.44	100.43	4.35
Matrine	0.28	0.93	0.30	101.14	2.15
Febrifugine	1.94	2.04	2.17	101.58	1.32
Berberine	0.82	1.26	0.81	99.92	0.43
Palmatine	1.65	1.18	1.02	100.35	1.25
Tangeratin	0.77	1.23	0.54	101.65	0.25
Nobiletin	0.49	1.25	0.51	101.19	1.03
Liensinine	1.90	2.34	1.29	101.98	2.79
Neferine	2.06	1.22	1.05	99.65	1.92
Scopoletin	0.45	2.61	0.49	101.81	1.47
Isoliquiritigenin	0.68	2.29	0.73	99.71	0.77
Liquiritigenin	1.98	2.90	1.82	101.50	2.09
Naringenin	1.11	1.90	2.42	99.71	2.50
Naringin	0.37	2.73	0.80	104.03	0.60
Hesperidin	0.25	2.47	0.84	99.89	1.27
Glycyrrhizic acid	0.47	1.90	1.14	101.06	1.27

nents with low mass spectrometry response and low content (including febrifugine, liensinine, neferine, scopoletin, isoliquiritigenin, liquiritigenin, naringenin, naringin, hesperidin, and glycyrrhizic acid); then low dilution ratio sample was diluted by times to obtain the test solution with high dilution ratio to detect the components with high mass response and high content (including sophocarpine, matrine, berberine, palmatine, Tangeratin and nobiletin). The rapid detection of various components in the sample was realized by different dilution methods.

3.2.1. Methodology validation

LC-MS was used for the quantitative analysis of sophocarpine, matrine, febrifugine, berberine, palmatine, Tangeratin, nobiletin, liensinine, neferine, scopoletin, isoliquiritigenin, liquiritigenin, naringenin, naringin, hesperidin and glycyrrhizic acid . The 16 index components had a good linear relationship within the corresponding concentration range, and their R^2 were all greater than 0.999, the LOD and LOQ were 0.146-60.074 ng/mL and 0.442-182.043 ng/mL, the results were shown in Table 4. The relative standard deviation (RSD) values of accuracy, repeatability, and stability were all less than 2.90 %, indicating that the instrument had good precision, the method had high repeatability, and the sample solution was stable for 24 h at room temperature. The sample recovery rate was between 99.65%-104.03%, and the RSD value was less than 4.35 %, indicating that the recovery rates of the 16 compounds in XSNC were good, and the established method had sufficient reliability and accuracy, the results were summarized in Table 5 (Supporting information Table S1-S5).

3.2.2. Determination of sample content

Multiple reaction monitoring (MRM) is a highly specific and sensitive mass spectrometry technique for quantifying predefined compounds of interest. The UHPLC–MS/MS analysis method described above was subsequently used to simultane-

Table 6 Contents of 16 compounds in 10 batches of Xin-Su-Ning capsules.

Sample batch	Sophocarpine (mg/g)	Matrine (mg/g)	Febrifugine (mg/g)	Berberine (mg/g)	Palmatine (mg/g)	Tangeratin (mg/g)	Nobiletin (mg/g)	Liensinine (mg/g)	Neferine (mg/g)	Scopoletin (mg/g)	Isoliquiritigenin (mg/g)	Liquiritigenin (mg/g)	Naringenin (mg/g)	Naringin (mg/g)	Hesperidin (mg/g)	Glycyrrhizic acid (mg/g)
220201	3.06 ± 0.27	11.62 ± 0.75	0.05 ± 0.00	31.05 ± 1.31	7.56 ± 0.32	0.46 ± 0.01	0.55 ± 0.02	0.06 ± 0.01	0.38 ± 0.01	0.36 ± 0.03	0.11 ± 0.01	0.15 ± 0.01	0.43 ± 0.04	28.01 ± 0.01	9.86 ± 0.21	1.66 ± 0.03
220202	2.65 ± 0.10	11.65 ± 0.21	0.06 ± 0.01	31.66 ± 0.64	7.79 ± 0.06	0.46 ± 0.00	0.55 ± 0.01	0.05 ± 0.00	0.37 ± 0.01	0.40 ± 0.01	0.12 ± 0.00	0.17 ± 0.00	0.52 ± 0.01	32.64 ± 0.05	10.99 ± 0.23	1.59 ± 0.04
210701	4.39 ± 0.20	15.90 ± 1.62	0.06 ± 0.00	34.25 ± 1.44	8.77 ± 0.21	0.45 ± 0.01	0.55 ± 0.01	0.07 ± 0.00	0.45 ± 0.11	0.69 ± 0.02	0.12 ± 0.00	0.24 ± 0.01	0.77 ± 0.02	47.02 ± 0.94	13.24 ± 0.13	1.60 ± 0.04
220101	2.52 ± 0.29	10.38 ± 0.99	0.17 ± 0.02	34.96 ± 0.93	8.82 ± 0.03	0.36 ± 0.00	0.43 ± 0.01	0.06 ± 0.01	0.41 ± 0.03	0.42 ± 0.02	0.20 ± 0.01	0.23 ± 0.01	0.44 ± 0.01	34.38 ± 0.36	13.46 ± 0.08	1.25 ± 0.04
210901	3.15 ± 0.05	12.16 ± 0.07	0.15 ± 0.01	25.35 ± 1.21	6.31 ± 0.26	0.27 ± 0.00	0.30 ± 0.01	0.05 ± 0.00	0.33 ± 0.03	0.53 ± 0.02	0.10 ± 0.00	0.16 ± 0.01	0.37 ± 0.02	24.66 ± 0.49	6.77 ± 0.10	1.66 ± 0.04
210502	4.05 ± 0.03	14.39 ± 0.77	0.10 ± 0.01	37.07 ± 0.54	8.79 ± 0.13	0.59 ± 0.02	0.58 ± 0.01	0.07 ± 0.01	0.55 ± 0.02	0.45 ± 0.01	0.19 ± 0.00	0.20 ± 0.01	0.42 ± 0.03	26.34 ± 0.84	7.57 ± 0.21	1.59 ± 0.04
210301	1.72 ± 0.08	9.20 ± 0.39	0.04 ± 0.01	20.60 ± 0.62	5.18 ± 0.17	0.37 ± 0.01	0.41 ± 0.01	0.05 ± 0.00	0.40 ± 0.01	0.32 ± 0.01	0.12 ± 0.01	0.15 ± 0.01	0.36 ± 0.01	19.03 ± 0.01	5.11 ± 0.05	1.72 ± 0.03
210501	3.96 ± 0.05	11.79 ± 0.03	0.07 ± 0.01	30.90 ± 1.30	7.50 ± 0.22	0.46 ± 0.01	0.53 ± 0.01	0.08 ± 0.01	0.68 ± 0.01	0.29 ± 0.01	0.12 ± 0.01	0.18 ± 0.00	0.61 ± 0.02	35.63 ± 0.40	9.46 ± 0.03	2.97 ± 0.02
201001	2.86 ± 0.06	10.56 ± 0.76	0.02 ± 0.00	17.13 ± 0.78	4.46 ± 0.15	0.35 ± 0.01	0.39 ± 0.01	0.04 ± 0.01	0.32 ± 0.01	0.35 ± 0.02	0.05 ± 0.00	0.09 ± 0.00	0.32 ± 0.02	17.33 ± 0.33	3.31 ± 0.05	0.55 ± 0.01
211101	2.16 ± 0.03	9.40 ± 0.03	0.05 ± 0.00	26.73 ± 0.49	6.34 ± 0.26	0.34 ± 0.01	0.37 ± 0.00	0.07 ± 0.01	0.66 ± 0.01	0.37 ± 0.00	0.09 ± 0.00	0.16 ± 0.01	0.40 ± 0.02	23.38 ± 0.34	6.40 ± 0.14	1.89 ± 0.09

Note: values are expressed as the mean ± SD of three parallel samples.

ously quantify 16 compounds in 10 collected batches. Every sample was analyzed in triplicates to acquire the average contents of the constituents. The results were shown in Table 6.

4. Discussion

How to combine the basic requirements of "safe, effective and controllable quality" with the characteristics of TCM is the key problem of TCM quality research and control. In this context, Academician Liu Changxiao and his team put forward the concept of Chinese medicine quality marker (Q-Marker) (Liu, 2019; Liu et al., 2016). In view of this, a method for simultaneous quantitative analysis of 16 chemical components in XSNC was established. Berberine and palmatine are derived from the prince drug Coptidis Rhizoma. A large number of studies have shown that berberine mainly exerts anti-arrhythmic effects by affecting potassium ion channels (Chen et al., 2018); Palmatine and berberine have similar structures and have higher content in Coptidis Rhizoma, it also has better anti-arrhythmic activity (Liu et al., 2017, 2017). Sophocarpine and matrine are derived from the official medicine Sophorae flavescentis Radix, and liensinine, neferine come from the central medicine Nelumbinis Plumula. These alkaloids can exert their anti-arrhythmic effects by influencing myocardial cell ion channels and prolonging APD (Jain and Parmar, 2011). Hesperidin is derived from the adjuvant Aurantii Fructus Immaturus, and isoliquiritin is derived from the drug Glycyrrhizae Radix et Rhizoma. These ingredients are all antiarrhythmic active ingredients (Ojha et al., 2013). Another research report glycyrrhizic acid has a protective effect on the heart (Ding et al., 2018). Dichroae Radix has small poison and febrifugine is the active component of Dichroae Radix, the content of febrifugine is determined to ensure the safety of TCM compound preparation. The active ingredients and characteristic ingredients contained in each component of the TCM are selected as indicators for quantitative analysis, which can provide a better reference for the quality evaluation and the material basis of the medicinal effect of the traditional Chinese medicine compound.

The chemical components of XSNC are complex and it is difficult to achieve baseline separation by liquid chromatography. In this experiment, multi reaction monitoring technology (MRM) in LC-MS technology was selected for quantitative analysis of the selected 16 chemical components. MRM monitoring mode can detect and analyze specific compounds with strong specificity, high sensitivity and high accuracy. Different compounds have different mass spectrometric responses, and the content of each component is completely different. To solve this problem, a multiple dilution method was constructed to realize the simultaneous quantitative analysis of 16 components in XSNC.

In this study, qualitative analysis of the chemical components of XSNC was carried out by GC-MS and LC-MS techniques. A total of 21 volatile components and 59 non-volatile components were identified, which further clarified the chemical composition of XSNC. It provides a reference for the characterization of chemical components of other TCM preparations; on the basis of LC-MS technology, considering the active components and characteristic components contained in various components of XSNC, the quantitative analysis of 16 chemical components with great content difference in XSNC was realized by constructing the double ratio dilution method. The method has high sensitivity and good selectivity, which can provide experimental basis for formulating a comprehensive quality control method of XSNC.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the National Key Research and Development Plan of China (No.2018YFC1707403); the Science and Technology Program of Tianjin (No.20ZYJDJC00120 and 21ZYJDJC00080).

Funding information

National Key Research and Development Plan of China, Grant Number: 2018YFC1707403; Science and Technology Program of Tianjin, Grant Numbers: 20ZYJDJC00120 and 21ZYJDJC00080.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2022.103825.

References

ChenH, F., Zhang, W.G., Yuan, J.B., Li, Y.G., Yang, S.L., Yang, W. L., 2012. Simultaneous quantification of polymethoxylated flavones and coumarins in Fructus aurantii and Fructus aurantii immaturus using HPLC-ESI-MS/MS. Pharm. Biomed. Anal. 10, 0456–0463.

Chen, X., Guo, H., Li, Q., Zhang, Y., Liu, H., Zhang, X., Xie, K., Zhu, Z., Miao, Q., Su, S., 2018. Protective effect of berberine on aconite–induced myocardial injury and the associated mechanisms. Mol. Med. Rep. 18, 4468–4476.

Ding, S., Li, X., Han, Y., 2018. Inheritance and innovation of traditional Chinese medicine from the development of Xinsuning capsule. Chin. Traditional Patent Med. 40, 1875–1877.

Eliuk, S., Makarov, A., 2015. Evolution of orbitrap mass spectrometry instrumentation. Annu. Rev. Anal. Chem. 8, 61–80.

He, Y.J., Xin, D., Jia, X.X., 2015. Qualitative and quantitative analysis on chemical constituents from Curculigo orchioides using ultra high-performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry. J. Pharm. Biomed. Anal. 102, 236–245.

James, C.A., Calkins, H., 2016. Further progress in predicting life-threatening arrhythmias in patients with arrhythmogenic right ventricular cardiomyopathy. J. Am. Coll. Cardiol. 68, 2551–2553.

Jain, M., Parmar, H.S., 2011. Evaluation of antioxidative and anti-inflammatory potential of hesperidin and naringin on the rat air pouch model of inflammation. Inflamm. Res. 60, 483–491.

Li, Y., Du, Z., 2015. Overview on method and strategy of therapeutic material basisin traditional Chinese medicine by multidisciplinary approach.Zhongguo Zhongyao Zazhi. 40, 1644–1648.

Li, Y., Wang, J., Li, X., Ma, X., Li, Z., 2019. Mechanism of bradyarrhythmia and Current Traditional Chinese and Western Medicine Treatment. Med. Rev. 25, 3221-3226 + 3231.

Liu, C., Chen, S., Xiao, X., Zhang, T., Hou, W., Liao, M., 2016. Q-Marker: A New Concept for Quality Control of Chinese Medicine Products. Chin. Herbal Med. 47, 1443–1457.

Liu, C., 2019. Developmentof Q-marker theory, methods and strategies, and research to improve the level of science and technology of Chinese medicine. Acta Pharm. Sin. 54, 185–186.

Liu, D., Cao, G., Si, X., Chen, Q., Sun, H., 2017. Summary of the research on anti-arrhythmia of the alkaloids from Coptidis Rhizoma. Shandong J. Traditional Chin. Med., 36, 164-166 + 171.

Liu, G.D., Zhao, Y.W., Li, Y.J., 2017. Qualitative and quantitative analysis of major constituents from Dazhu Hongjingtian capsule by UPLC/Q-TOF-MS/MS combined with UPLC/QQQ-MS/MS. Biomed. Chromatogr. 31, 1–10.

Ma, Y.L., Bates, S., Gurney, A.M., 2006. The effects of paeonol on the electrophysiological properties of cardiac ventricular myocytes. Eur. J. Pharmacol. 545, 87–92.

Ojha, S., Golechha, M., Kumari, S., Bhatia, J., Arya, D.S., 2013. Glycyrrhiza glabra protects from myocardial ischemia-reperfusion injury by improving hemodynamic, biochemical, histopathological and ventricular function. Exp. Toxicol. Pathol. 65, 219–227.

Sossalla, S., Vollmann, D., 2018. Arrhythmia-Induced Cardiomyopathy. Dtsch Arztebl Int. 115, 335–341.

Sun, L., 2017. On the research progress of antiarrhythmic effects of traditional Chinese medicines. China Modern Med. Appl. 11, 194–195.

Yang, L., Zhai, Y., Chen, X., Zhang, Z., Gong, X., Zhou, D., Kong, J., Zhang, W., Zhang, Q., Niu, C., Wang, H., Sun, Z., 2020. Rapid identificationof chemical compositions in callicarpa kwangtungensisChun byultrahigh-performance liquid chromatography with Q Exactive hybrid quadrupole orbitrap highresolution accurate mass spectrometry. J. Sep. Sci. 43, 2487–2494.

Yao, L., Fang, F., 2017. Research progress of antiarrhythmic Chinese medicines acting on ion channels of cardiomyocytes. Chin. J. Integr. Med. Cardio-/Cerebrovasc. Dis. 15, 1057–1059.

Zhai, J., Yin, X., Yang, X., Zhang, J., 2017. Xinsuning capsule for the treatment of premature ventricualr contraction: a multicenter randomised clinical trial. The Lancet. 390, 61.