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A pharmacokinetic study on twenty-one compounds in rat plasma by integrating UPLC-QQQ-MS/MS with GC-MS after oral administration of Suxiao Jiuxin pill

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ABSTRACT

Suxiao Jiuxin pill (SX) was a commonly used traditional Chinese medical preparation for treating acute myocardial infarction. However, fewer studies have been reported for comprehensively investigating the pharmacokinetic of the non-volatile and volatile chemical constituents in SX. For current study, a comprehensive and sensitive quantitation of twenty-one compounds in rat plasma was developed and validated by integrating UPLC-QQQ-MS/MS with GC-MS after oral administration of SX. The pharmacokinetic study was performed under acceptable specificity, linearity, sensitivity, precision and accuracy, recovery, matrix effect and stability. Ultimately, borneol, isoborneol, senkyunolide A, senkyunolide I, ligustilide, ferulic acid, neocnidilide, senkyunolide H and levistolide A were detected. Their ranges of T_{max}, C_{max}, $T_{1/2z}$ and AUC $_{(0-\infty)}$ were 0.07 ~ 0.33 h, 5.24 ~ 683.35 μg/L, 0.29 ~ 1.08 h and 6.55 ~ 474.62 μg/L*h, respectively. This study would be meaningful in better revealing the therapeutic material basis of SX. Furthermore, it could provide reference on pharmacological effect of SX against acute myocardial infarction.

1. Introduction

Acute myocardial infarction, a serious and life-threatening cardiovascular disease, was usually due to myocardial hypoxia induced by coronary artery stenosis or embolism (Lu et al., 2019). Unfortunately, even with timely pharmacological, catheter-based and surgical reperfusion, patients were at the constant risks of complications such as pseudoaneurysm, free wall rupture, ventricular septal defect, *etc* (Damluji et al., 2021, Gong et al., 2021). Clinically, aspirin and nitroglycerin were usually thought as the drug of choice for the treatment because of their powerful therapeutic effect on thrombolysis and vasodilatation (Anderson and Morrow, 2017). Nevertheless, allergy, gastric mucosal injury and gout usually occurred after administration of aspirin (Ben Salem et al., 2017, Cortellini et al., 2017, Oncel et al., 2021). As well, nitroglycerin might trigger syncope and migraine attacks. In addition, resistance and susceptibility to inactivation also constrained their application (van Oosterhout et al., 2020). Traditional Chinese medicines (TCMs) have been widely used in the treatment of acute cardiovascular disease because of the significant curative effect and fewer side effects (Xu et al., 2017, Ruan et al., 2018, Li et al., 2020, Wang et al., 2021).

Suxiao Jiuxin pill (SX) was a presentative preparation of cardiovascular protection. It was composed of *Chuanxiong Rhizome* and *Borneolum Syntheticum*. According to the *meta*-analysis, SX was more effective than salvia tablet and nitroglycerin in improving ECG results (Ren et al., 2018). SX could exert cardioprotective effects in rat models of acute myocardial infarction by regulating focal adhesion and platelet activation pathways (Song et al., 2022). *Chuanxiong Rhizome*, also named chuanxiong in China, was the dried rhizome of *Ligusticum chuanxiong* Hort. As a famous blood-activating and stasis-resolving herbal medicine,

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chuanxiong possessed significant therapeutic on cardiovascular disease (Chen et al., 2018). Chuanxiong could significantly downregulate VEGF/VEGFR2 pathway to reduce angiogenesis and upregulate PI3K-Akt pathway to release nitric oxide (NO). It maintained the contractile function of the vascular endothelium and reversed reactive oxygen species (ROS) produced by H_2O_2 to reducing oxidative stress (Li et al., 2014, Ni et al., 2014, Yuan et al., 2018, Li et al., 2019). *Borneolum Syntheticum* was considered as an "upper guiding drug" that could gather therapeutic effect at the upper-jiao (Mei et al., 2023). There were many studies showed *Borneolum Syntheticum* possessed multiple therapeutic effect on cardiovascular disease, for instance, atherosclerosis, myocardial injury, hypertension, thrombosis, *etc* (Liu et al., 2021). Phthalidic, phenolic and terpenoidic ingredients were the main compounds in SX. Ligustilide could produce vasodilation via blocking voltage dependent calcium channel and receptor mediated Ca^{2+} influx and release (Cao et al., 2006). Senkyunolide A was able to significantly decrease the effect of tetraethylammonium on the relaxation of isolated aorta (Chan et al., 2007). Furthermore, the potent anti-inflammatory and antioxidant activities of chlorogenic acid, caffeic acid and ferulic acid have been validated through abundant vivo and vitro assays (Mubarak et al., 2012, Naveed et al., 2018, Afnan et al., 2022). Borneol, as a neutral antioxidant and antiinflammatory agent, could downregulate LPS-induced inflammatory infiltration by reducing levels of NO, TNF-α and IL-6.

The combined action of multiple compounds and targets was responsible for the efficacy of SX. Pharmacokinetic studies described how a drug was absorbed, distributed, metabolized and excreted. It played a key role in elucidating the pharmacological mechanism and clinical application of medicine (Huang et al., 2021, Yang et al., 2021). However, it was worth noting that corresponding study has not yet been reported in literature for comprehensively investigating the pharmacokinetic of the non-volatile and volatile chemical constituents in SX. Recently, lots of analytical methods were developed for pharmacokinetic study, for example, high performance liquid chromatogram tandem ultraviolet detection (HPLC-UV) (Wei et al., 2014), high performance liquid chromatogram tandem electrochemical detection (HPLC-ECD) (Liu and Chen, 2012), ultra-performance liquid chromatogram tandem mass spectrometry (UPLC-MS/MS) (Yang et al., 2021), gas chromatogram tandem mass spectrometry (GC-MS) (Li et al., 2018), *etc*. UPLC-MS/MS and GC-MS were broadly used in pharmacokinetic study with the advantages of high sensitivity, selectivity and rapidity.

Consequently, a rapid, sensitive and comprehensive quantitative method of twenty-one compounds (Fig. 1) in rat plasma was developed by integrating UPLC-QQQ-MS/MS with GC-MS. This validated method was successfully applied in pharmacokinetic study on SX. This work would provide a worthwhile foundation to explain the therapeutic material basis of SX.

2. Material and methods

2.1. Reagents, chemicals, and materials

LC-grade methanol and acetonitrile were purchased from Fisher Chemical (Fairlawn, OSU, USA). LC-grade formic acid was bought from Anaqua Chemicals Supply (Wilmington, DE, USA). Ultrapure water was produced by Milipore Milli-Q water purification system (Milford, MA, USA). Neochlorogenic acid, protocatechuic acid, chlorogenic acid, cryptochlorogenic acid, caffeic acid, vanillic acid, vanillin, scopoletin, isochlorogenic acid A, ferulic acid, isochlorogenic acid C, senkyunolide I, senkyunolide H, senkyunolide A, neocnidilide, angelicide, levistolide

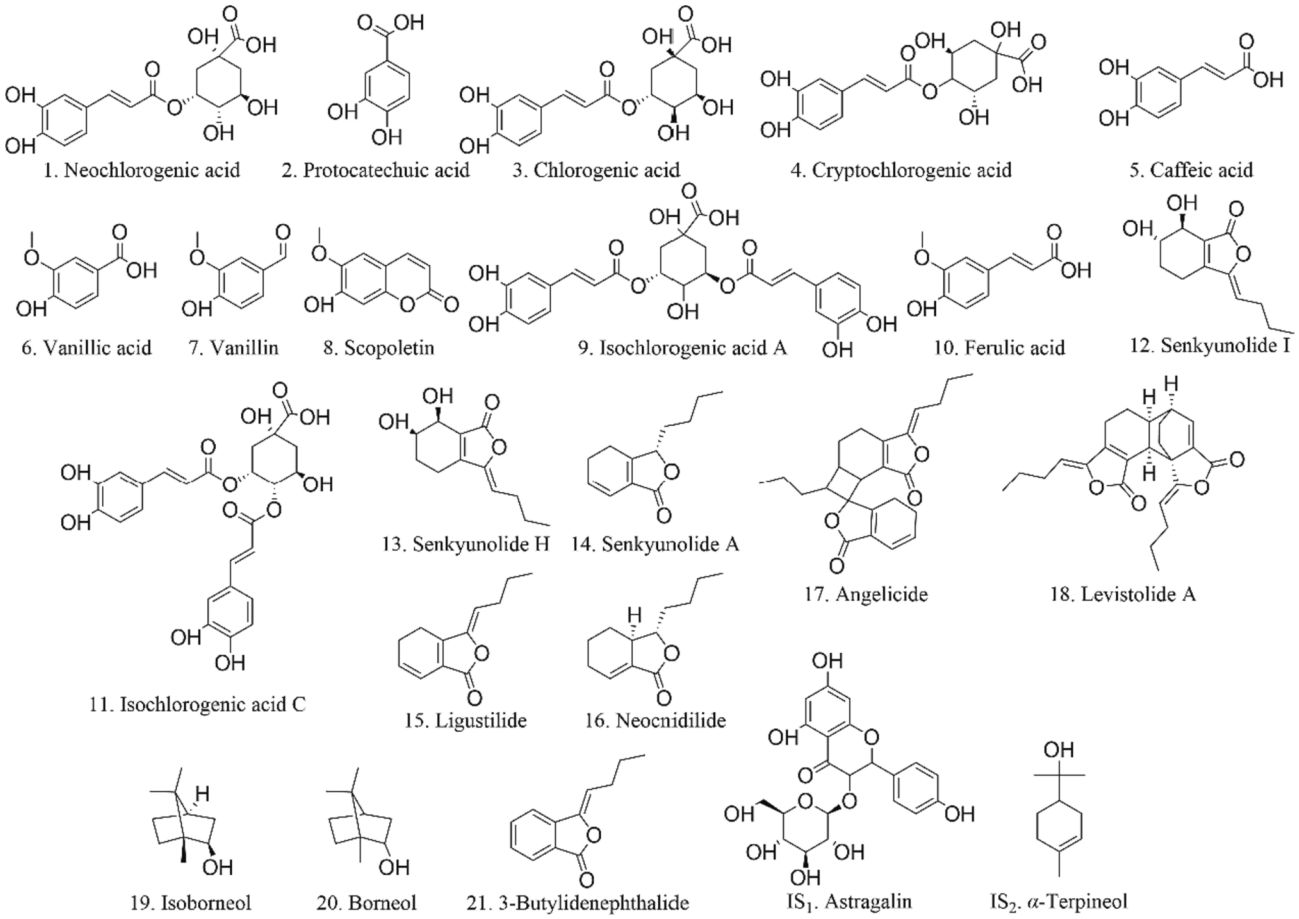

Fig. 1. The chemical structure of twenty-one analytes and two ISs.

A, 3-butylidenephthalide, astragalin (internal standard, IS_1) and α-terpineol (IS_2) were purchased from Chengdu Desite Bio-Technology Co., Ltd. (Chengdu, Sichuan, China). Ligustilide, isoborneol, and borneol were obtained from National Institutes for Food and Drug Control (Beijing, China). The purities of above standards were all more than 98 %. Suxiao Jiuxin pills (No.611323) were provided from Tianjin Pharmaceutical Da Ren Tang Group Corporation Limited NO.6 Traditional Chinese Medicine Factory (Tianjin, China).

2.2. UPLC-QQQ-MS/MS condition

Agilent 1290 UPLC system (Santa Clar, CA, USA) was employed in this work. A Waters ACQUITY UPLC®HSS T3 column (2.1 × 100 mm, 1.8 μm) was utilized to perform separation in a 23 ℃ thermostated column compartment. The mobile phase was composed of 0.1 % formic acid aqueous solution (A) and methanol (B). The gradient elution procedure was as follow: 0 ~ 1 min, 15 ~ 45 % B; 6 ~ 9 min, 86 ~ 95 % B; post run time was set as 5 min. The flow rate was set as 0.3 mL/min, injection volume was set as 5 μL.

Agilent 6470 QQQ-MS/MS system was equipped with an air jet spray electron spray ionization (AJS ESI) ion source. Positive and negative acquisition modes with using multiple reaction monitoring (MRM) were both utilized in this analysis. The ion source parameters were set as follow: drying gas, 300 ℃; drying gas flow rate, 7 L/min; nebulizer, 35 psi; sheath gas, 350 ℃; capillary voltage, 3.5 kV. The MRM ion transition parameters of eighteen targets and IS_1 were optimized (Table 1).

2.3. GC-MS condition

Shimadzu QP 2010 GC-MS (Kyoto, Japan) was equipped with an electron ionization (EI) ion source. The separation procedure was performed in a HP-5MS column (30 m × 0.25 mm × 0.25 μm). In the case of high-purity helium as carrier gas, the flow rate was set as 1.84 mL/min. The gradient heating procedure was as follow: the initial temperature was held at 110 ℃ for 4 min, and it was elevated to 140 ℃ at an increment of 40 °C/min, then elevated to 210 ℃ at an increment of 200 °C/min, thereafter, elevated to 230 ℃ at an increment of 31 ℃/min and remained for 1.2 min. The injection volume was set as 2 μL at a split ratio of 15:1.

For the mass spectrum module, the temperatures of EI ion source and the injector were set as 230℃ and 250℃, respectively. The solvent delay time was set as 5 min. The single ion monitoring (SIM) acquisition mode was applied for obtaining higher specificities of analyte (Table 2).

Table 2
The SIM parameters of GC-MS analysis.

Compound	Retention time (min)	Monitoring ion (*m/z*)	Reference ion (*m/z*)
Isoborneol	4.050	95.0	110.0, 93.0
Borneol	4.182	95.0	110.0, 93.0
3-Butylidenephthalide	6.784	159.0	146.0
α-Terpineol (IS_2)	4.453	93.0	95.0, 59.0

2.4. Preparation of standard and quality control (QC) samples

For the UPLC-QQQ-MS/MS analysis, the stock solutions of standard references were prepared by dissolving appropriate substance with methanol to a concentration of 1 mg/mL, respectively. The working solutions were prepared by mixing appropriate stock solution of each analyte and diluting into different concentrations with methanol. Calibration standard solutions were prepared by spiking with 20 μL working solution, 20 μL IS_1 (1000 ng/mL) and 20 μL formic acid into 100 μL blank plasma, subsequently, obtained mixture was extracted by the method same with "*2.5. Sample Pretreatment*". The final concentrations of calibration standard solutions were at the ranges of to the concentration ranges of 0.25 ~ 100 ng/mL (neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, caffeic acid, vanillic acid, vanillin, scopoletin, isochlorogenic acid A, isochlorogenic acid C, senkyunolide H, angelicide, levistolide A), 1.0 ~ 400 ng/mL (protocatechuic acid), 4 ~ 1600 ng/mL (ferulic acid, ligustilide), 2 ~ 800 ng/mL (senkyunolide I), 5.5 ~ 2200 ng/mL (senkyunolide A) and 0.35 ~ 140 ng/mL (neocnidilide), respectively. The QC samples were prepared in the same way at three concentration levels (low, medium, high). All the samples were stored in 4℃ before analysis.

For the GC-MS analysis, the stock solutions of standard references were prepared by dissolving appropriated substance with *n*-hexane to a concentration of 1 mg/mL, respectively. The working solutions were prepared by mixing appropriate stock solutions of each analyte and diluting into different concentration with *n*-hexane. Calibration standard solutions were prepared by spiking with 10 μL working solution and 10 μL IS_2 (5000 ng/mL) into 100 μL blank plasma, subsequently, obtained mixture was extracted by the method same with "*2.5. Sample Pretreatment*". The final concentrations of calibration standard solutions were at the ranges of 12.5 ~ 4000 ng/mL (isoborneol and 3-butylidenephthalide) and 25 ~ 8000 ng/mL (borneol), respectively. The QC samples were prepared in the same way at three concentration levels (low, medium, high). All the samples were stored in 4 ℃ before analysis.

Table 1
The MRM ion transition parameters of eighteen analytes and IS_1.

Compound	Retention time (min)	Precursor ion (*m/z*)	Product ion (*m/z*)	Fragmentor voltage (V)	Collision energy (eV)	Ionization mode
Neochlorogenic acid	2.948	353.1	191.0	103	16	Negative
Protocatechuic acid	3.103	153.0	109.0	93	16	Negative
Chlorogenic acid	3.285	353.1	191.0	103	16	Negative
Cryptochlorogenic acid	3.324	353.1	173.0	113	12	Negative
Caffeic acid	3.677	179.0	135.0	98	16	Negative
Vanillic acid	3.693	167.0	152.0	80	12	Negative
Vanillin	3.958	153.1	65.1	80	28	Positive
Scopoletin	4.133	193.0	132.9	141	24	Positive
Isochlorogenic acid A	4.135	515.1	353.1	118	12	Negative
Ferulic acid	4.305	193.1	134.0	93	16	Negative
Isochlorogenic acid C	4.534	515.1	353.1	128	16	Negative
Senkyunolide I	4.921	225.1	207.0	70	4	Positive
Senkyunolide H	5.151	225.1	207.0	70	4	Positive
Senkyunolide A	6.846	193.1	91.0	80	28	Positive
Ligustilide	7.528	191.1	173.0	113	16	Positive
Neocnidilide	7.581	195.1	149.1	80	8	Positive
Angelicide	8.331	381.2	191.0	146	8	Positive
Levistolide A	8.522	381.2	191.0	93	16	Positive
Astragalin (IS_1)	5.093	447.1	284.0	194	28	Negative

2.5. Sample preparation

For UPLC-QQQ-MS/MS analysis, 20 μL IS_1 (1000 ng/mL), 20 μL formic acid and 20 μL methanol were successively added into 100 μL plasma and vortexed for 1 min. The analytes extraction was operated by protein precipitation method with 800 μL acetonitrile. The mixture was vortexed for 5 min and centrifuged for 10 min at 14000 rpm. Thereafter, the supernatant was dried under flow nitrogen gas. The residue was dissolved with 100 μL methanol and vortexed for 5 min. The reconstituted mixture was centrifuged for 10 min at 14000 rpm. A liquid of 5 μL supernatant was injected into UPLC-QQQ-MS/MS system for analysis.

For GC-MS analysis, 10 μL IS_2 (5000 ng/mL) and 10 μL *n*-hexane were successively added into 100 μL plasma and vortexed for 1 min. The analytes extraction was operated by liquid–liquid extraction method with 130 μL *n*-hexane. The mixture was vortexed for 5 min and centrifuged for 10 min at 14000 rpm. A liquid of 2 μL *n*-hexane fraction was injected into GC-MS system for analysis.

2.6. Method validation

The specificity, linearity, sensitivity, precision and accuracy, recovery, matrix effect and stability were evaluated for ensuring a high reliable quantitative method according to the US Food and Drug Administration (Xu et al., 2019, Tang et al., 2020, Zhang et al., 2022). Specificity was evaluated by comparing the chromatograms among blank plasma, medium concentration level QC sample and the plasma at 5 min after oral administration of SX. The linearity of calibration curve was plotted by using the peak areas of the analytes to IS versus concentration with a $1/X^2$ as weighting factor. The sensitivity was assessed by limit of quantitation (LOQ) that concentration at signal-to-noise (S/N) of 10. Six replicates of QC samples at each concentration level on the same day and three consecutive days were prepared and determined. The relative standard deviation (RSD) and relative error (RE) were calculated for expressing the intra- and inter-day precision and accuracy, respectively. The recovery was investigated by comparing the peak area of analytes in pre-treatment QC samples with corresponding post-treatment spiked sample. The matrix effect was calculated by the peak area ratio of analytes extracted form QC samples to the equal concentration standard solution. The stability was evaluated by QC samples stored in room temperature for 4 h, autosampler for 12 h, −80℃ refrigerator for 7 days and three cycles of freeze and thaw, respectively.

2.7. Pharmacokinetic study

The male Sprague–Dawley rats (220 ± 10 g) were purchased from Beijing Huafukang Bio-Technology Co., Ltd. (Beijing, China). The animals were housed in 40 ~ 60 % humidity, 23 ~ 27 ℃, and 12 h dark-light cycle at the animal center of Traditional Chinese Medicine of Tianjin University. Free access to water and food were permitted to animals until 12 h before the experiment. All animals were randomly divided into two groups for LC-MS/MS and GC-MS analysis, respectively. The rats were received SX at the dosage of 600 mg/kg (dissolved by pure water to the concentration of 80 mg/mL) by oral administration after fasted for 12 h. The contents of analytes in SX were determined by the established UPLC-QQQ-MS/MS integrated with GC-MS method (Table 3). A liquid of 200 μL blood sample was obtained from ophthalmic venous plexus into heparinization centrifuged tube at predose, 0.03, 0.08, 0.17, 0.25, 0.33, 0.5, 0.75, 1 h, 2 h, 4 h, 6 h, 10 h and 24 h post dosing, respectively. All blood samples were directly centrifuged at 7000 rpm for 10 min. The plasma layer was then transferred into clean tube and stored at −80℃ before use. All animal studies were conducted under the guidance of Laboratory Animal Ethics Committee of Tianjin University of Traditional Chinese Medicine (TCM-LAEC2022028).

Table 3
The content of twenty-one analytes in Suxiao Jiuxin pill ($n = 3$, mean ± SD).

Compound	Content (μg/g)
Neochlorogenic acid	1.40 ± 0.04
Protocatechuic acid	4.60 ± 0.28
Chlorogenic acid	92.07 ± 4.67
Cryptochlorogenic acid	4.03 ± 0.34
Caffeic acid	22.92 ± 1.05
Vanillic acid	9.19 ± 1.12
Vanillin	55.15 ± 3.61
Scopoletin	4.81 ± 0.34
Isochlorogenic acid A	126.40 ± 7.89
Ferulic acid	535.80 ± 18.59
Isochlorogenic acid C	6.99 ± 0.75
Senkyunolide I	1104.26 ± 8.90
Senkyunolide H	169.41 ± 10.48
Senkyunolide A	4218.09 ± 89.76
Ligustilide	4792.16 ± 164.81
Neocnidilide	264.47 ± 11.80
Angelicide	263.27 ± 2.86
Levistolide A	658.00 ± 10.30
Isoborneol	88036.11 ± 3601.89
Borneol	143053.70 ± 5356.65
3-Butylidenephthalide	402.29 ± 24.52

2.8. Data analysis

The pharmaceutic parameters were calculated by Drug and Statistics software (DAS, version 3.0, Medical College of Wannan, China) with a non-compartment model.

3. Results and discussions

3.1. Optimization of UPLC-QQQ-MS/MS conditions and sample preparation

The stationary phase was extremely crucial for getting a satisfactory separation. The main chemical regredient of most TCMs were naturally organic products which were easily absorbed by reverse-phase stationary. For this work, two commonly used reverse stationary, Waters ACQUITY UPLC®BEH C18 (2.1 × 100 mm, 1.7 μm) and Waters ACQUITY UPLC®HSS T3 (2.1 × 100 mm, 1.8 μm), were utilized to optimize the separation of eighteen analytes. Comparatively, the Waters ACQUITY UPLC®HSS T3 chromatographic column showed a better retained performance. It was able to separate analytes within a short time. Besides, the mobile phase was also a key factor affecting the peak shape and resolution. Subsequent optimization was done by changing the kinds of mobile phase A (water and 0.1 % formic acid aqueous solution) and mobile phase B (methanol and acetonitrile). After adjusting the gradient elution program, a satisfactory separation was obtained using 0.1 % formic acid aqueous solution and methanol as mobile phase (Fig. 2).

Protein precipitation and liquid–liquid extraction methods were both investigated in this experiment. Meanwhile, in view of the carboxyl group of phenolic acid, which led to a strong absorption with plasma protein, the formic acid was applied in phenolic acid analytes extraction (Sun et al., 2013, Huang et al., 2017). The result showed acetonitrile possessed a higher extraction property on all analytes than methanol and ethyl acetate. For the ionization of ESI mode, the complex matrix of plasma could affect target compounds response (Ismaiel et al., 2008, Yadav et al., 2012). The optimization of redissolving reagent was a direct and effective method to minimize the interference from endogenous ingredients. As a result, methanol showed more pleasing result than acetonitrile and the mixture of methanol and acetonitrile (*v:v*, 1:1).

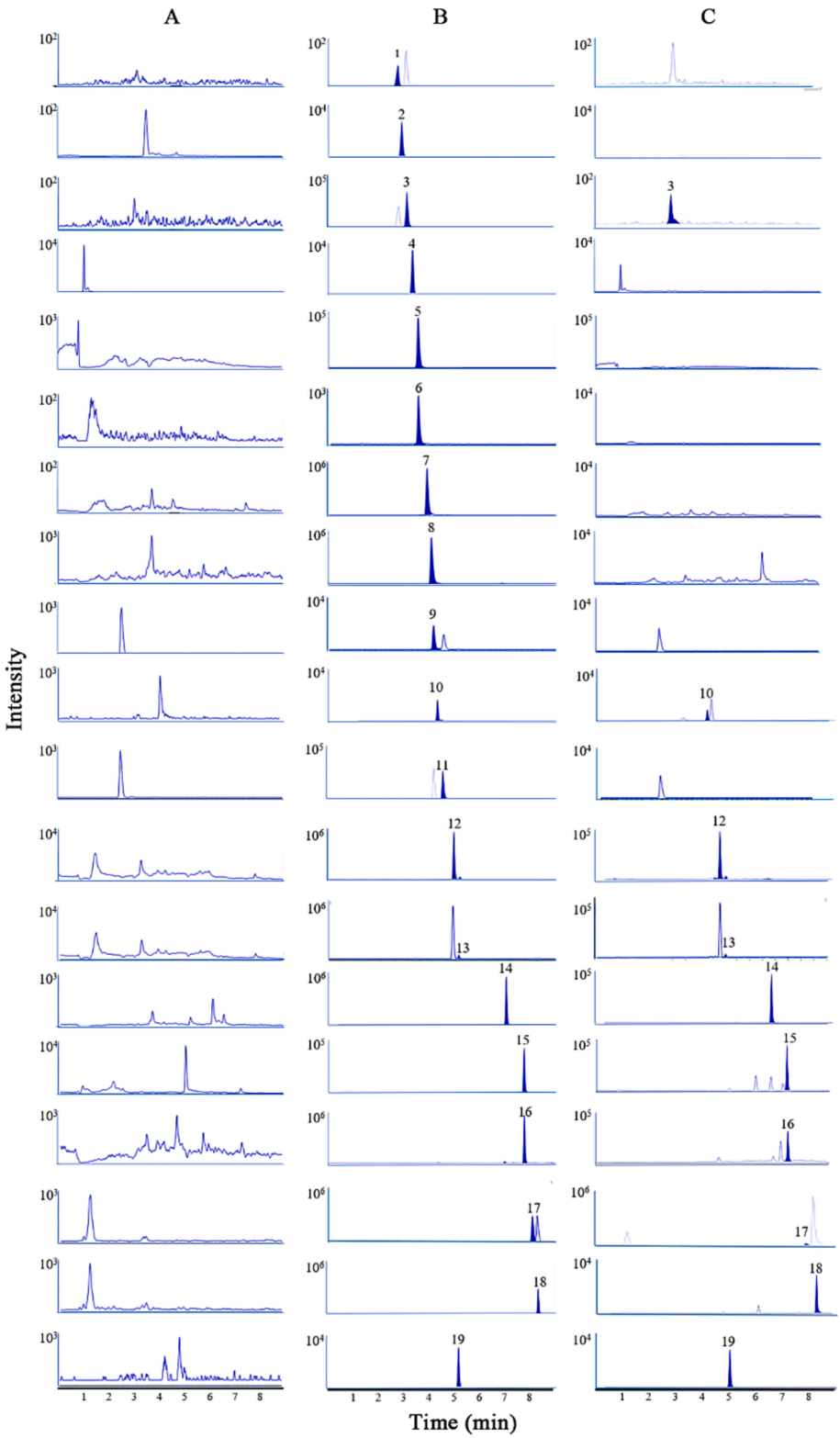

Fig. 2. The MRM chromatograms of eighteen analytes and IS_1 in blank plasma (A), blank plasma spiked with medium concentration QC (B), and plasma sample at 5 min after oral administration Suxiao Jiuxin pill (C).

3.2. Method validation

The specificity has been investigated by comparing the chromatograms among blank plasma, blank plasma spiked with medium concentration QC, and plasma sample at 5 min after oral administration of SX. Obviously, there were no significant interferences at the same retention time of analytes and internal standards (Figs. 2 and 3). It indicated a good specificity of these detection methods.

The calibration curves, correlation coefficients (*R*), linear ranges and LOQs of twenty-one analytes were exhibited in Table 4. All the calibration curves of analytes possessed fine correlation coefficients more than 0.995 within a certain concentration range. The LOQs of all

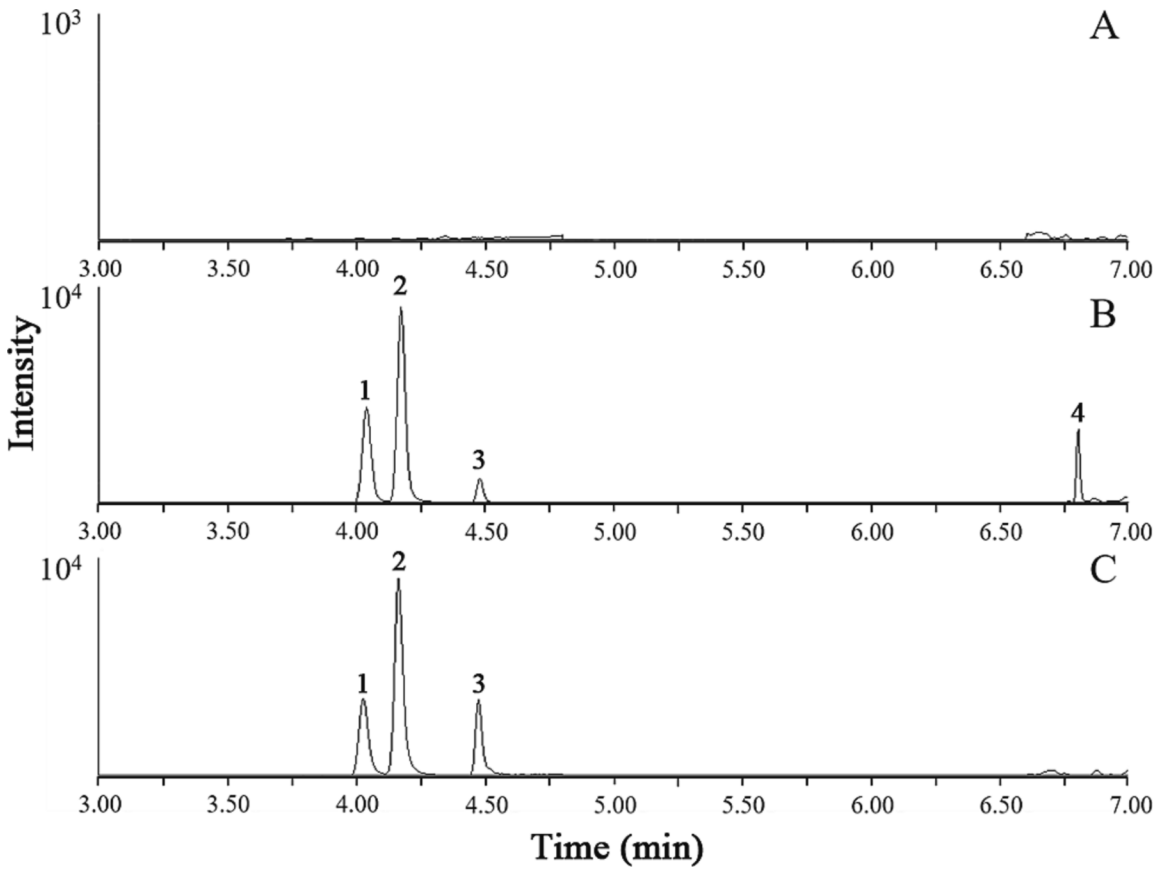

Fig. 3. The SIM chromatograms of three analytes and IS_2 in blank plasma (A), blank plasma spiked with medium concentration QC (B), and plasma sample at 5 min after oral administration Suxiao Jiuxin pill (C).

Table 4
The calibration curves, correlation coefficients (*r*), linear ranges, and LOQs of twenty-one analytes.

Compound	Regression equation	*R*	Linear range (ng/mL)	LOQ (ng/mL)
Neochlorogenic acid	$Y = 1.657780X + 8.893346E^{-4}$	0.9966	0.25 ~ 100	0.25
Protocatechuic acid	$Y = 0.552811X + 8.162655E^{-4}$	0.9961	1.00 ~ 400	1.00
Chlorogenic acid	$Y = 3.280862X + 0.004356$	0.9956	0.25 ~ 100	0.25
Cryptochlorogenic acid	$Y = 0.852090X + 0.001596$	0.9954	0.25 ~ 100	0.25
Caffeic acid	$Y = 2.769898X + 0.006427$	0.9967	0.25 ~ 100	0.25
Vanillic acid	$Y = 0.044511X + 8.968622E^{-4}$	0.9952	0.25 ~ 100	0.25
Vanillin	$Y = 3.469706X + 0.023108$	0.9970	0.25 ~ 100	0.25
Scopoletin	$Y = 15.317656X + 0.006282$	0.9950	0.25 ~ 100	0.25
Isochlorogenic acid A	$Y = 1.598709X + 0.002493$	0.9963	0.25 ~ 100	0.25
Ferulic acid	$Y = 0.501721X + 0.004658$	0.9970	4.00 ~ 1600	0.10
Isochlorogenic acid C	$Y = 1.201293X + 8.632763E^{-4}$	0.9957	0.25 ~ 100	0.25
Senkyunolide I	$Y = 35.683544X + 0.122851$	0.9974	2.00 ~ 800	0.20
Senkyunolide H	$Y = 31.909741X + 0.044144$	0.9965	0.25 ~ 100	0.25
Senkyunolide A	$Y = 8.304275X + 0.016639$	0.9991	5.50 ~ 2200	0.10
Ligustilide	$Y = 3.134035X + 0.013888$	0.9975	4.00 ~ 1600	0.60
Neocnidilide	$Y = 6.170325X + 0.009155$	0.9954	0.35 ~ 140	0.35
Angelicide	$Y = 10.949451X-0.002706$	0.9960	0.25 ~ 100	0.10
Levistolide A	$Y = 28.335176X-0.007983$	0.9959	0.25 ~ 100	0.10
Isoborneol	$Y = 9.080319E^{-3}X + 1.538444E^{-2}$	0.9989	12.5 ~ 4000	12.5
Borneol	$Y = 9.182486E^{-3}X + 4.95145E^{-2}$	0.9987	25.0 ~ 8000	12.5
3-Butylidenephthalide	$Y = 2.714534E^{-3}X-4.088886E^{-3}$	0.9976	12.5 ~ 4000	12.5

analytes were as low as 12.5 ng/mL.

The QC samples at three concentration levels were used to assess the extraction recovery and matrix effect (Table5). The extraction recoveries of analytes were at the range of 61.2 ~ 107.0 % with RSDs no more than 11.7 %. It suggested that the extraction method was parallel to obtain analytes from plasma within a wide concentration range. For the matrix effect analysis, excepted neochlorogenic acid, protocatechuic acid, chlorogenic acid, vanillic acid and scopoletin, other analytes did not possess obvious endogenous interference that matrix effect ranged from 70.7 % to 114.8 % with RSDs less than 13.7 %. For the chemical ingredients with matrix interferences, their RSDs were less than 13.7 %, which indicating a stable endogenous interference in the sample solution. In addition, the acceptable LOQs (less than 1 ng/mL) suggested the interference was not sufficient to impede their highly sensitive

Table 5
The extraction recovery and matrix effect of twenty-one analytes ($n = 6$).

Compound	Concentration (ng/mL)	Extraction recovery (%)	RSD (%)	Matrix effect (%)	RSD (%)
Neochlorogenic acid	0.5	90.7	8.6	70.4	11.0
	5	81.0	9.1	69.4	3.8
	80	75.9	11.7	63.2	12.9
Protocatechuic acid	2	84.0	5.5	41.2	5.6
	20	86.7	6.2	31.9	4.9
	320	86.1	5.6	30.1	7.4
Chlorogenic acid	0.5	88.1	5.1	82.3	13.7
	5	85.6	5.5	70.3	10.8
	80	81.2	4.6	67.0	6.2
Cryptochlorogenic acid	0.5	94.8	8.8	97.8	9.4
	5	87.0	6.3	82.2	13.7
	80	85.5	3.1	71.8	8.0
Caffeic acid	0.5	89.4	6.4	88.6	9.9
	5	87.3	10.1	70.7	7.6
	80	86.1	7.4	71.1	7.4
Vanillic acid	0.5	78.5	9.7	65.8	13.4
	5	73.2	9.6	67.6	12.7
	80	71.9	5.5	60.6	8.3
Vanillin	0.5	76.9	7.7	109.4	8.6
	5	65.4	2.5	80.5	3.8
	80	61.2	5.5	84.3	11.9
Scopoletin	0.5	85.9	10.2	67.6	6.8
	5	89.0	3.1	71.7	2.0
	80	93.0	2.8	77.1	6.0
Isochlorogenic acid A	0.5	106.4	8.7	107.4	13.6
	5	90.4	6.8	110.1	12.1
	80	90.2	3.6	82.9	5.5
Ferulic acid	8	102.5	11.0	81.8	13.3
	80	87.5	5.2	72.6	4.4
	1280	92.2	3.6	71.7	5.9
Isochlorogenic acid C	0.5	99.8	7.4	110.8	12.9
	5	94.5	9.8	104.9	6.6
	80	86.5	8.9	97.0	9.7
Senkyunolide I	4	91.2	3.6	88.5	5.4
	40	95.3	3.8	88.6	1.6
	640	97.7	4.3	79.6	8.9
Compound	Concentration (ng/mL)	Extraction recovery (%)	RSD (%)	Matrix effect (%)	RSD (%)
Senkyunolide H	0.5	107.0	9.2	92.3	12.7
	5	100.3	8.4	84.8	4.0
	80	101.3	5.8	81.6	6.4
Senkyunolide A	11	75.6	4.1	96.2	5.0
	110	76.9	4.8	88.3	2.2
	1760	81.4	3.9	79.3	9.5
Ligustilide	8	75.2	3.8	89.8	3.8
	80	66.2	3.8	80.9	3.7
	1280	73.9	6.8	71.6	5.1
Neocnidilide	0.7	93.2	11.0	96.8	11.0
	7	80.6	7.0	88.5	6.3
	112	80.1	8.3	76.8	10.1
Angelicide	0.5	98.9	10.5	107.9	8.5
	5	83.5	7.2	101.2	10.0
	80	86.7	10.4	83.9	9.5
Levistolide A	0.5	95.5	5.6	100.1	12.9
	5	87.0	5.3	114.8	4.2
	80	83.8	8.3	100.8	9.3
Isoborneol	25	82.4	2.3	104.6	4.9
	250	88.9	4.9	89.3	3.9
	3200	93.3	2.0	87.9	3.7
Borneol	50	81.6	1.4	103.9	7.7
	500	85.8	4.3	92.9	4.8
	6400	91.6	2.2	89.5	3.8
3-Butylidenephthalide	25	100.9	3.4	94.1	7.1
	250	94.3	6.6	94.9	11.4
	3200	91.2	5.6	88.4	4.5

determination.

The intra- and inter-precision and accuracy were assayed by six replicates of QC samples at three concentration levels on the same day (intra-day) and after three consecutive days (inter-day). The calibration curve constructed on the same testing day were used for assessment. The ranges of intra- and inter-precision were 0.4 ~ 7.9 % and 0.8 ~ 9.0 %, respectively, with accuracy within ± 12 % for all QC samples (Table 6).

The results of the stability stored in room temperature for 4 h, autosampler for 12 h, −80℃ refrigerator for 7 days, and three cycles of freeze and thaw were listed in Table 7. All values were within the

Table 6
The intra- and inter-precision and accuracy of twenty-one analytes ($n = 6$).

Compounds	QC concentration (ng/mL)	Intra-precision Measured concentration (ng/mL)	RSD (%)	RE (%)	Inter-precision Measured concentration (ng/mL)	RSD (%)	RE (%)
Neochlorogenic acid	0.5	0.51 ± 0.02	3.8	2.0	0.51 ± 0.02	3.7	1.2
	5	4.95 ± 0.31	6.3	−1.0	4.95 ± 0.14	2.9	−1.0
	80	84.14 ± 2.29	2.7	5.2	85.58 ± 5.55	6.5	11.3
Protocatechuic acid	2	2.06 ± 0.02	1.2	3.1	2.04 ± 0.03	1.7	2.0
	20	19.42 ± 0.39	2.0	−2.9	19.51 ± 0.54	2.8	−2.5
	320	341.17 ± 13.65	4.0	6.6	346.39 ± 8.48	2.4	6.9
Chlorogenic acid	0.5	0.52 ± 0.02	4.0	4.2	0.53 ± 0.03	5.3	5.8
	5	4.86 ± 0.10	2.1	−2.9	4.75 ± 0.37	7.8	−4.9
	80	86.96 ± 2.99	3.4	8.7	85.70 ± 6.30	7.4	5.8
Cryptochlorogenic acid	0.5	0.51 ± 0.01	2.4	2.8	0.51 ± 0.02	3.2	1.5
	5	4.88 ± 0.12	2.4	−2.5	4.82 ± 0.16	3.4	−3.6
	80	87.86 ± 2.92	3.3	9.8	85.25 ± 6.17	7.2	6.9
Caffeic acid	0.5	0.51 ± 0.02	4.0	1.3	0.50 ± 0.02	3.4	0.3
	5	4.86 ± 0.15	3.0	−2.7	4.87 ± 0.16	3.4	−2.7
	80	84.79 ± 3.17	3.7	6.0	83.75 ± 2.56	3.1	7.2
Vanillic acid	0.5	0.51 ± 0.02	4.1	2.0	0.51 ± 0.02	4.3	2.9
	5	4.92 ± 0.09	1.8	−1.6	4.84 ± 0.08	1.6	−3.1
	80	85.97 ± 4.30	5.0	7.5	84.74 ± 4.26	5.0	5.2
Vanillin	0.5	0.52 ± 0.02	3.2	3.6	0.52 ± 0.01	2.3	3.6
	5	4.88 ± 0.11	2.3	−2.5	4.88 ± 0.12	2.4	−2.4
	80	86.00 ± 2.61	3.0	7.5	87.88 ± 3.27	3.7	10.3
Scopoletin	0.5	0.53 ± 0.02	4.2	5.4	0.51 ± 0.02	4.4	2.7
	5	4.86 ± 0.14	2.9	−2.7	4.59 ± 0.26	5.6	−8.3
	80	85.17 ± 2.33	2.7	6.5	87.39 ± 2.20	2.5	11.7
Isochlorogenic acid A	0.5	0.52 ± 0.02	3.0	3.1	0.51 ± 0.01	2.7	2.1
	5	4.74 ± 0.11	2.2	−5.2	4.74 ± 0.25	5.2	−5.3
	80	87.34 ± 3.75	4.3	9.2	84.53 ± 7.59	9.0	4.9
Ferulic acid	8	8.32 ± 0.45	5.4	4.0	8.09 ± 0.10	1.2	1.1
	80	75.99 ± 1.76	2.3	−5.0	75.84 ± 1.99	2.6	−5.2
	1280	1296.80 ± 43.41	3.3	1.3	1317.94 ± 54.60	4.1	5.4
Isochlorogenic acid C	0.5	0.51 ± 0.02	3.3	2.1	0.51 ± 0.02	3.2	2.9
	5	4.84 ± 0.11	2.2	−3.2	4.83 ± 0.10	2.0	−3.4
	80	85.78 ± 3.12	3.6	7.2	81.68 ± 5.36	6.6	4.6
Senkyunolide I	4	3.97 ± 0.01	0.4	−0.6	3.95 ± 0.03	0.8	−1.2
	40	38.55 ± 1.55	4.0	−3.6	38.05 ± 1.72	4.5	−4.9
	640	666.27 ± 15.42	2.3	4.1	672.99 ± 14.05	2.1	5.9
Compounds	QC concentration (ng/mL)	Intra-precision Measured concentration (ng/mL)	RSD (%)	RE (%)	Inter-precision Measured concentration (ng/mL)	RSD (%)	RE (%)
Senkyunolide H	0.5	0.50 ± 0.03	5.4	0.1	0.52 ± 0.02	3.8	3.4
	5	5.00 ± 0.11	2.3	0.0	5.01 ± 0.16	3.2	0.3
	80	83.67 ± 2.91	3.5	4.6	83.01 ± 3.82	4.6	7.9
Senkyunolide A	11	11.23 ± 0.61	5.4	2.1	11.06 ± 0.60	5.4	0.5
	110	117.49 ± 4.74	4.0	6.8	118.57 ± 1.45	1.2	7.8
	1760	1653.86 ± 55.95	3.4	−6.0	1677.45 ± 40.75	2.4	−1.3
Ligustilide	8	8.44 ± 0.37	4.3	5.5	8.04 ± 0.22	2.8	0.5
	80	86.13 ± 2.20	2.5	7.7	85.48 ± 3.54	4.1	6.9
	1280	1241.47 ± 38.21	3.1	−3.0	1269.69 ± 54.96	4.3	3.9
Neocnidilide	0.7	0.73 ± 0.04	5.4	4.8	0.70 ± 0.02	2.8	−0.3
	7	7.07 ± 0.42	6.0	0.9	7.11 ± 0.51	7.2	1.6
	112	115.28 ± 4.11	3.6	2.9	118.69 ± 6.19	5.2	8.6
Angelicide	0.5	0.54 ± 0.01	1.7	7.7	0.53 ± 0.02	4.3	5.9
	5	4.84 ± 0.09	1.9	−3.2	4.94 ± 0.24	4.9	−1.1
	80	83.98 ± 2.12	2.5	5.0	85.99 ± 3.21	3.7	4.7
Levistolide A	0.5	0.53 ± 0.03	5.6	6.8	0.53 ± 0.04	7.6	6.4
	5	4.90 ± 0.16	3.2	−2.1	5.07 ± 0.16	3.2	1.4
	80	83.34 ± 2.90	3.5	4.2	87.43 ± 3.08	3.5	6.3
Isoborneol	25	25.08 ± 0.89	3.6	0.3	25.06 ± 0.78	3.1	0.2
	250	248.38 ± 4.20	1.7	−0.6	249.58 ± 4.19	1.7	−0.2
	3200	3114.59 ± 29.02	0.9	−2.7	3152.00 ± 73.30	2.3	−1.5
Borneol	50	49.58 ± 1.81	3.7	−0.8	49.59 ± 1.48	3.0	−0.8
	500	502.12 ± 7.11	1.4	0.4	499.28 ± 10.62	2.1	−0.1
	6400	6229.72 ± 56.50	0.9	−2.7	6300.37 ± 134.74	2.1	−1.6
3-Butylidenephthalide	25	25.65 ± 2.02	7.9	2.6	25.42 ± 1.18	4.6	1.7
	250	245.34 ± 12.16	5.0	−1.9	256.23 ± 4.27	1.7	2.5
	3200	3391.50 ± 77.92	2.3	6.0	3429.98 ± 90.46	2.6	7.2

Table 7
The stabilities of twenty-one analytes ($n = 6$).

Compound	QC concentration (ng/mL)	Room temperature for 4 h		Autosampler for 12 h		Three cycles of freeze and thaw		−80℃ refrigerator for 7 days	
		Measured concentration (ng/mL)	RSD (%)	Measured concentration (ng/mL)	RSD (%)	Measured concentration (ng/mL)	RSD (%)	Measured concentration (ng/mL)	RSD (%)
Neochlorogenic acid	0.5	0.52 ± 0.03	5.6	0.52 ± 0.03	5.7	0.50 ± 0.02	3.0	0.49 ± 0.01	2.7
	5	5.00 ± 0.14	2.8	5.01 ± 0.03	0.6	4.96 ± 0.13	2.5	4.94 ± 0.13	2.6
	80	89.05 ± 4.99	5.6	82.67 ± 6.18	7.5	83.75 ± 4.54	5.4	82.72 ± 6.45	7.8
Protocatechuic acid	2	2.06 ± 0.10	4.8	2.02 ± 0.08	4.2	1.99 ± 0.06	2.9	2.01 ± 0.07	3.7
	20	20.66 ± 1.13	5.5	19.62 ± 0.44	2.2	19.55 ± 0.84	4.3	19.45 ± 0.32	1.7
	320	342.08 ± 10.62	3.1	334.34 ± 14.30	4.3	348.03 ± 10.45	3.0	344.46 ± 3.70	1.1
Chlorogenic acid	0.5	0.54 ± 0.03	5.6	0.54 ± 0.02	3.1	0.52 ± 0.04	7.2	0.52 ± 0.02	3.4
	5	5.08 ± 0.20	4.0	4.83 ± 0.23	4.8	4.82 ± 0.28	5.8	4.77 ± 0.15	3.2
	80	84.66 ± 3.94	4.7	82.19 ± 4.02	4.9	83.77 ± 4.21	5.0	81.48 ± 6.52	8.0
Cryptochlorogenic acid	0.5	0.52 ± 0.02	4.0	0.52 ± 0.03	5.3	0.53 ± 0.01	2.7	0.51 ± 0.02	3.1
	5	4.98 ± 0.12	2.4	4.76 ± 0.39	8.2	4.98 ± 0.11	2.1	4.98 ± 0.11	2.2
	80	85.50 ± 3.67	4.3	82.54 ± 2.14	2.6	82.80 ± 3.22	3.9	80.51 ± 4.33	5.4
Caffeic acid	0.5	0.52 ± 0.02	4.8	0.52 ± 0.02	3.6	0.52 ± 0.02	3.8	0.52 ± 0.04	6.8
	5	4.87 ± 0.13	2.7	4.90 ± 0.15	3.0	4.99 ± 0.09	1.7	4.88 ± 0.16	3.4
	80	85.78 ± 4.15	4.8	82.57 ± 2.37	2.9	83.22 ± 4.06	4.9	83.06 ± 1.49	1.8
Vanillic acid	0.5	0.51 ± 0.03	5.8	0.51 ± 0.02	4.5	0.50 ± 0.02	3.8	0.51 ± 0.02	3.3
	5	4.86 ± 0.15	3.1	4.97 ± 0.11	2.2	4.99 ± 0.09	1.7	4.93 ± 0.08	1.5
	80	84.18 ± 3.45	4.1	85.83 ± 0.98	1.1	86.14 ± 3.63	4.2	89.00 ± 6.61	7.4
Vanillin	0.5	0.51 ± 0.02	3.4	0.51 ± 0.02	4.5	0.50 ± 0.02	3.8	0.50 ± 0.02	4.2
	5	4.89 ± 0.10	2.1	4.92 ± 0.15	3.1	4.92 ± 0.15	3.0	4.95 ± 0.03	0.7
	80	88.27 ± 1.64	1.9	87.51 ± 4.34	5.0	88.77 ± 1.62	1.8	88.08 ± 3.68	4.2
Scopoletin	0.5	0.53 ± 0.02	3.6	0.51 ± 0.03	5.6	0.51 ± 0.04	7.5	0.53 ± 0.02	4.6
	5	4.56 ± 0.16	3.5	4.66 ± 0.09	1.9	4.80 ± 0.12	2.5	4.88 ± 0.30	6.1
	80	89.34 ± 1.48	1.7	87.03 ± 3.82	4.4	90.04 ± 2.98	3.3	87.59 ± 1.98	2.3
Isochlorogenic acid A	0.5	0.50 ± 0.01	2.9	0.51 ± 0.02	4.0	0.52 ± 0.02	4.6	0.52 ± 0.02	3.1
	5	4.76 ± 0.16	3.3	4.80 ± 0.05	1.0	4.89 ± 0.09	1.8	4.66 ± 0.12	2.5
	80	83.92 ± 4.93	5.9	81.53 ± 4.31	5.3	83.09 ± 4.84	5.8	79.27 ± 9.08	11.5
Ferulic acid	8	8.37 ± 0.33	3.9	7.99 ± 0.13	1.7	8.69 ± 0.77	8.8	8.74 ± 0.89	10.2
	80	77.67 ± 0.87	1.1	74.60 ± 2.38	3.2	76.17 ± 2.20	2.9	77.84 ± 1.07	1.4
	1280	1348.86 ± 22.80	1.7	1319.26 ± 34.02	2.6	1343.67 ± 27.72	2.1	1330.91 ± 24.13	1.8
Isochlorogenic acid C	0.5	0.50 ± 0.05	10.1	0.53 ± 0.02	4.2	0.51 ± 0.01	2.1	0.52 ± 0.02	4.8
	5	4.70 ± 0.15	3.1	4.72 ± 0.07	1.4	4.74 ± 0.10	2.2	4.82 ± 0.10	2.1
	80	83.69 ± 4.56	5.4	78.00 ± 4.30	5.5	80.77 ± 6.12	7.6	81.62 ± 4.27	5.2
Senkyunolide I	4	3.97 ± 0.06	1.5	3.81 ± 0.13	3.4	3.94 ± 0.13	3.4	3.97 ± 0.26	6.4
	40	38.25 ± 0.49	1.3	34.77 ± 1.08	3.1	36.77 ± 1.49	4.0	42.69 ± 1.27	3.0
	640	677.47 ± 13.15	1.9	670.73 ± 13.65	2.0	677.34 ± 19.14	2.8	679.18 ± 9.52	1.4
Senkyunolide H	0.5	0.52 ± 0.04	6.8	0.52 ± 0.03	4.9	0.52 ± 0.02	4.4	0.51 ± 0.02	3.5
	5	4.97 ± 0.15	3.1	4.77 ± 0.31	6.6	4.83 ± 0.19	4.0	5.11 ± 0.07	1.3
	80	86.30 ± 3.01	3.5	82.98 ± 3.64	4.4	83.74 ± 4.13	4.9	85.72 ± 3.11	3.6
Senkyunolide A	11	11.57 ± 0.26	2.2	11.03 ± 0.49	4.4	11.38 ± 0.65	5.7	11.16 ± 0.62	5.6
	110	118.29 ± 1.35	1.1	116.28 ± 3.44	3.0	117.62 ± 3.94	3.3	118.44 ± 2.66	2.2
	1760	1737.56 ± 48.37	2.8	1646.73 ± 47.86	2.9	1680.98 ± 41.92	2.5	1630.76 ± 49.26	3.0
Ligustilide	8	8.46 ± 0.38	4.5	8.18 ± 0.40	4.9	8.31 ± 0.21	2.6	9.02 ± 0.91	10.0
	80	85.96 ± 1.48	1.7	81.94 ± 2.73	3.3	87.57 ± 1.99	2.3	79.01 ± 3.90	4.9
	1280	1329.33 ± 45.90	3.5	1245.63 ± 37.54	3.0	1285.94 ± 35.48	2.8	1271.64 ± 44.50	3.5
Neocnidilide	0.7	0.70 ± 0.02	3.5	0.70 ± 0.02	2.9	0.74 ± 0.04	4.8	0.77 ± 0.02	2.7
	7	7.59 ± 0.15	2.0	6.78 ± 0.19	2.8	7.24 ± 0.30	4.1	7.67 ± 0.12	1.6
	112	121.60 ± 3.45	2.8	118.68 ± 3.19	2.7	122.69 ± 2.34	1.9	122.03 ± 3.99	3.3
Angelicide	0.5	0.54 ± 0.04	6.9	0.55 ± 0.03	6.0	0.57 ± 0.03	5.7	0.54 ± 0.02	4.3
	5	4.78 ± 0.10	2.1	4.91 ± 0.10	2.0	4.84 ± 0.07	1.4	5.14 ± 0.10	2.0
	80	83.78 ± 5.86	7.0	85.58 ± 1.76	2.1	84.56 ± 5.61	6.6	87.86 ± 4.78	5.4
Levistolide A	0.5	0.53 ± 0.04	7.4	0.53 ± 0.03	5.7	0.57 ± 0.02	3.7	0.54 ± 0.03	5.3
	5	5.15 ± 0.12	2.4	5.16 ± 0.04	0.7	5.05 ± 0.16	3.2	5.15 ± 0.19	3.7
	80	85.01 ± 4.36	5.1	84.69 ± 3.62	4.3	84.22 ± 8.85	10.5	91.39 ± 3.79	4.1

(*continued on next page*)

Table 7 (*continued*)

Compound	QC concentration (ng/mL)	Room temperature for 4h		Autosampler for 12h		Three cycles of freeze and thaw		−80℃ refrigerator for 7 days	
		Measured concentration (ng/mL)	RSD (%)	Measured concentration (ng/mL)	RSD (%)	Measured concentration (ng/mL)	RSD (%)	Measured concentration (ng/mL)	RSD (%)
Isoborneol	25	24.14 ± 1.17	4.9	25.66 ± 1.27	4.9	24.67 ± 1.94	7.9	25.48 ± 1.35	5.3
	250	252.12 ± 6.19	2.5	250.99 ± 7.18	2.9	247.24 ± 12.05	4.9	250.38 ± 5.20	2.1
	3200	3117.53 ± 25.01	0.8	3098.90 ± 32.22	1.0	3134.30 ± 25.12	0.8	3125.04 ± 32.53	1.0
Borneol	50	49.74 ± 2.80	5.6	50.53 ± 2.24	4.4	49.50 ± 2.99	6.0	49.70 ± 2.83	5.7
	500	509.30 ± 10.87	2.1	505.40 ± 12.91	2.6	501.61 ± 20.48	4.1	507.35 ± 11.47	2.3
	6400	6232.85 ± 55.11	0.9	6206.13 ± 68.75	1.1	6264.82 ± 51.79	0.8	6246.82 ± 65.27	1.0
3-Butylidenephthalide	25	25.73 ± 1.08	4.2	25.84 ± 1.81	7.0	25.70 ± 1.34	5.2	25.67 ± 1.43	5.6
	250	251.94 ± 8.28	3.3	242.82 ± 9.86	4.1	254.31 ± 11.84	4.7	242.58 ± 7.41	3.1
	3200	3347.64 ± 118.84	3.5	3469.18 ± 133.76	3.9	3423.95 ± 183.00	5.3	3407.04 ± 169.17	5.0

acceptable range of less than 12 %.

3.3. Pharmacokinetic study

The validated UPLC-QQQ-MS/MS integrated with GC-MS method was employed to quantify twenty-one compounds in rat plasma after oral administration of SX. The mean plasma concentration – time curves were visualized by GraphPad Prism 8.0.2 software (Fig. 4). Unfortunately, there were some compounds whose plasma concentration was too low to describe an intact pharmacokinetic profile, such as neochlorogenic acid, protocatechuic acid, chlorogenic acid, *etc*. It might be caused by their low content in Suxiao Jiuxin Pill. Ultimately, the pharmacokinetic parameters of nine analytes were analyzed by DAS software with non-compartment model (Table 8).

The maximum plasma concentration (C_{max}) was tightly bound to the clinical application of drug. Only when the plasma concentration reached therapeutic concentration could it take the effect. The C_{max} from high to low were borneol (683.35 μg/L), isoborneol (233.61 μg/L), senkyunolide A (200.51 μg/L), senkyunolide I (96.42 μg/L), ligustilide (88.74 μg/L), ferulic acid (50.07 μg/L), neocnidilide (13.79 μg/L), senkyunolide H (12.49 μg/L), and levistolide A (5.24 μg/L). Area under concentration–time curve ($AUC_{(0\text{-}tn)}$ and $AUC_{(0\text{-}\infty)}$) reflected the total exposure of analytes in plasma. The AUCs of senkyunolide I, senkyunolide A, ferulic acid, ligustilide, borneol and isoborneol were greater than 47.2 μg/L*h, which suggesting they possessed high exposure *in vivo*. It might be due to the higher content in SX. The time about reaching C_{max} (T_{max}) of all analytes were within 0.4 h, which indicated the active ingredients in SX could be rapidly absorbed into blood and build up to therapeutic concentration. Half-life ($T_{1/2z}$) represented the rate of drug clearance *in vivo*. All analytes possessed short $T_{1/2z}$ values ranging from 0.29 to 1.24 h. The rapid absorbed and distributed would provide a rapid effect on acute cardiovascular diseases. What's more, it could be found that the T_{max} and $T_{1/2z}$ values of borneol and isoborneol, ligustilide and neocnidilide were closely similar, but their doses were quite different. The reason for this phenomenon was probably due to their similar chemical structure.

4. Conclusion

In this study, a sensitive, effective and comprehensive method was developed to quantify twenty-one compounds in rat plasma for studying pharmacokinetic of SX. This validated method was successfully employed to describe pharmacokinetic profiles of nine chemical ingredients. After oral administration of SX at the dosage of 600 mg/kg, the C_{max} was 50.07 μg/L for ferulic acid, 96.42 μg/L for senkyunolide I, 12.49 μg/L for senkyunolide H, 200.51 μg/L for senkyunolide A, 88.74 μg/L for ligustilide, 13.79 μg/L for neocnidilide, 5.24 μg/L for levistolide A, 233.61 μg/L for isoborneol, 683.35 μg/L for borneol. The T_{max} of all analytes were lower than 0.53 h. Their $T_{1/2z}$ ranged from 0.29 ~ 1.08 h.

The pharmacokinetic property of SX were comprehensively investigated. This finding would provide scientific guide mechanism of action

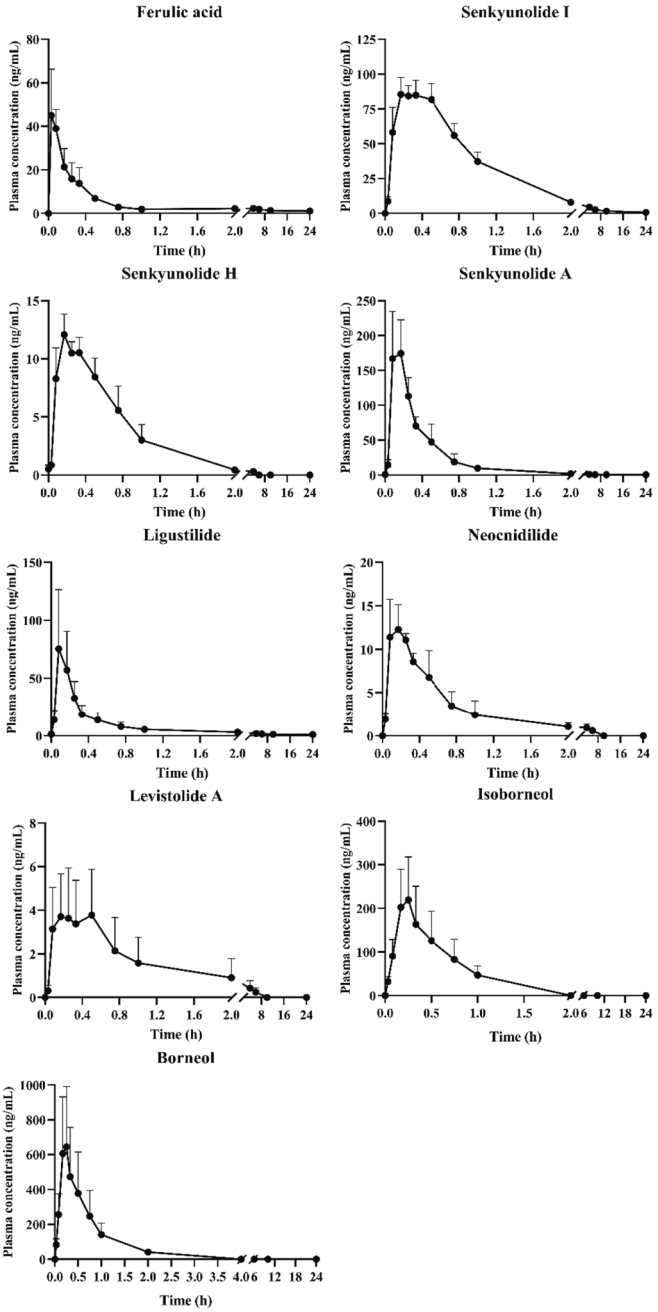

Fig. 4. The plasma concentration–time curves of analytes ($n = 6$, mean ± SD).

Table 8
The pharmacokinetic parameters of analytes after administrating of Suxiao Jiuxin pill ($n = 6$).

Compound	T_{max} (h)	C_{max} (μg/L)	$T_{1/2z}$ (h)	AUC $_{(0\text{-}tn)}$ (μg/L*h)	AUC $_{(0\text{-}\infty)}$ (μg/L*h)
Ferulic acid	0.07 ± 0.03	50.07 ± 16.24	0.63 ± 0.03	47.21 ± 10.86	58.49 ± 18.20
Senkyunolide I	0.35 ± 0.13	96.42 ± 5.91	0.58 ± 0.02	134.19 ± 17.30	140.15 ± 20.62
Senkyunolide H	0.22 ± 0.07	12.49 ± 1.32	0.85 ± 0.02	10.08 ± 1.15	10.08 ± 1.15
Senkyunolide A	0.10 ± 0.03	200.51 ± 33.27	0.35 ± 0.05	80.63 ± 31.02	94.54 ± 51.02
Ligustilide	0.10 ± 0.03	88.74 ± 45.52	0.70 ± 0.10	53.55 ± 41.27	70.16 ± 69.96
Neocnidilide	0.11 ± 0.04	13.79 ± 1.50	0.72 ± 0.05	12.91 ± 3.14	12.91 ± 3.14
Levistolide A	0.33 ± 0.19	5.24 ± 1.46	1.08 ± 0.68	6.55 ± 4.10	6.55 ± 4.10
Isoborneol	0.22 ± 0.04	233.61 ± 93.38	0.31 ± 0.02	139.41 ± 57.40	139.41 ± 57.40
Borneol	0.22 ± 0.04	683.35 ± 330.42	0.29 ± 0.01	474.62 ± 210.47	474.62 ± 210.47

and pharmacological effects on acute myocardial infarction of SX.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2023.105397.

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