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Comparative Tissue Distribution Study of Multiple Bioactive Components of Xiao-Yao Powder in Young and Old Rats

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Abstract: Xiao-Yao Powder (XYP) has been used for the treatment of liver function disorder, depression and comprehensive syndromes of perimenopausal women for over 2000 years. The present work aims to reveal the tissue distribution characteristics of XYP in young and old female rats contrastively to evaluate rationality and compatibility of herbal medicine, in terms of efficacy and toxicity. An ultra-performance liquid chromatography–tandem mass spectrometry method was used to detect the 14 representative components (albiflorin, paeoniflorin, ferulic acid, senkyunolide I, quercetin, isoliquiritigenin, atractylenolide III, ligustilide, atractylenolide II, liquiritin, liquiritigenin, saikosaponin c, glycyrrhizic acid, and saikosaponin a) in both 2-month-old and 18-month-old female rats. After oral administration of XYP extract (4 g/kg), rats' tissues (heart, liver, spleen, lung, kidney, brain, stomach, uterus, ovary and small intestine) were taken at time points immediately. The detection was performed on a triple-quadrupole tandem mass spectrometer equipped in positive and negative ionization modes by multiple reaction monitoring (MRM) and an electrospray ionization source. All validation parameters met the acceptance criteria according to regulatory guidelines. The tissue distribution study of XYP confirmed that 14 analytes from the two age groups were mainly distributed in ovary, uterus and liver, which is consistent with the characteristics of XYP in treating liver depression and perimenopausal syndrome. Compared with the young rats, the tissue concentrations of most analytes in old rats group decreased, with the exception of senkyunolide I and Atractylenolide III showing higher levels in brain. The identified target tissues of XYP may help in understanding its pharmacological action *in vivo*.

Keywords: Xiao-Yao Powder (XYP), UPLC-MS/MS, Tissue Distribution

1. Introduction

XYP is a traditional Chinese medicine prescription consisted of *Radix Bupleuri, Radix Angelicae Sinensis, Radix Paeoniae Alba, Rhizoma Atractylodis Macrocephalae, Poria, Radix Glycyrrhizae, Herba Menthae and Rhizoma Zingiberis Recens* [1], in a ratio of 5:5:5:5:5:4:1:1. XYP has been widely used as a treatment for liver damage, and postmenopausal symptoms since Song Dynasty of China [2-5]. In the past decades, XYP exerts a variety of biological effects on senior women with perimenopausal symptoms including anti-liver damage, anti-functional uterine bleeding, anti-menopausal syndrome [6], anti-anovulatory infertility [7], and anti-neuro degenerative diseases. With increasing knowledge of the pharmacological effects and molecular biological mechanisms of XYP, it is essential to achieve further insight on its pharmacokinetic profiles and tissue distribution characteristics, since pharmacokinetic studies are fundamental to evaluate the efficacy and/or toxicity of compounds based on their bodily absorption, distribution, metabolism, and excretion.

In the previous experiment, we studied the pharmacokinetics of 14 main ingredients in rat plasma after oral administration of XYP (albiflorin, paeoniflorin, ferulic acid, senkyunolide I, quercetin, isoliquiritigenin, atractylenolide III, ligustilide, atractylenolide II, liquiritin, liquiritigenin, saikosaponin c, glycyrrhizic acid, and saikosaponin a), which are responsible for the pharmacological efficacy of original herbs and XYP. Quercetin, saikosaponin a and saikosaponin c are the effective bioactive components of *Radix Bupleuri* for liver disease and menstrual disorders [8-10]. Ligustilide is an effective ingredient in *Radix Angelicae Sinensis*, which can reduce brain damage and improve cognitive function [11]. Another active ingredient in *Radix Angelicae Sinensis* is ferulic acid, which has a protective effect against CCl_4-induced liver injury in mice [12]. Senkyunolide I is also an active ingredient in *Radix Angelicae Sinensis*, which can protect the rat brain against focal cerebral ischemia-reperfusion injury [13]. Liquiritin, liquiritigenin, isoliquiritigenin, and glycyrrhizic acid have the effect of protecting the liver, and these are the effective ingredients of *Radix Glycyrrhizae* [14-17]. Paeoniflorin and albiflorin are the most abundant bioactive component of *Radix Paeoniae Alba* [18, 19]. Atractylenolide II and atractylenolide III are the effective bioactive component of *Rhizoma Atractylodis Macrocephalae* [20, 21].

However, the XYP tissue biodistribution research on either normal or aged animals has not been well studied, and literature data are reported for only a few low polar extract of XYP [22]. As we all know, neither a single herb nor a single component plays a role in XYP completely, but the outcome of a group of herbs combining multiple action does. Thus, the tissue distribution of these 14 constituents would represent the distribution characteristics of XYP in the rat after oral administration [23, 24]. Since the significant treatment effects on senior women, the comparative tissue distribution study of XYP is critical to explore the pharmacological action *in vivo.*

In the present study, a simultaneous and rapid assay of the 14 representative compounds of XYP in multi tissues of both young and old rats after oral administration was established based on ultra-performance liquid chromatography coupled with electrospray ionization triple-quadrupole tandem mass spectrometry (UPLC-ESI-MS). It was expected that the obtained results would be helpful for further study of the reliable basis for systematic research of XYP.

2. Materials and Methods

2.1. Chemicals and Reagents

Albiflorin, paeoniflorin, ferulic acid, senkyunolide I, quercetin, isoliquiritigenin, atractylenolide III, ligustilide, atractylenolide II, liquiritin, liquiritigenin, saikosaponin c, glycyrrhizic acid, saikosaponin a and naringenin as an internal standard (IS) (≥ 98% purity) were purchased from Chengdu Herbpurify Co., Ltd.

Acetonitrile (HPLC grade) was purchased from Fisher (Fisher, USA). Ultrapure water was prepared from a Milli-Q water purification system (Millipore, Bedford, USA). All other chemicals were analytical grade.

2.2. Animals

2-month-old and 18-month-old female Sprague-Dawley (SD) rats (220 ± 20 g) were purchased from the Animal Center of Heilongjiang University of Chinese Medicine (Harbin, China), and were bred in an environmentally controlled room (22 ± 2°C, relative humidity 50 ± 5%, natural light-dark cycle) with free access to standard diet and water. All animals fasted 12 hours before the experiment was carried out. All animal experiments were reviewed and followed the guidelines of the Laboratory Animal Management Committee of Heilongjiang University of Traditional Chinese Medicine.

2.3. Chromatographic Conditions

For the tissue distribution study, the UPLC-MS/MS system was Waters® Micromass® Quattro Premier™ XE triple-quadrupole tandem mass spectrometer (Waters Corp., Milford, MA, USA), composed of ACQUITY UPLCTM BEH C18 column (1.7 μm, 50 mm × 2.1 mm; Waters Corp., Milford, MA, USA) and ESI source. Mass Lynx™ NT 4.1 software (Waters, USA) was equipped for data acquisition and analysis. The injection volume was 5μL, and the column temperature was maintained at 35°C. The mobile phase (flow rate 0.3 ml/min) was composed of water containing 0.1% formic acid (A) and acetonitrile (B). The gradient elution program was referred to the precious analysis [25].

The mass spectrometric data was obtained in positive or negative ion modes with MRM under an optimized condition. The mass spectrometers and precursor/product ion pairs setting of 14 analytes were set in according with our previous work [25]. Under these optimal conditions, 14 active ingredients and IS were separated and detected efficiently in 14.5 min.

2.4. Preparation of XYP Extraction

The crude plant materials were purchased from Tongren Tang (Harbin, China) and identified by Professor Zhang Ning of Heilongjiang University of Chinese Medicine by the Chinese Pharmacopoeia (2020). The identification results were preserved in Heilongjiang University of Chinese Medicine. As the same as the procedure reported in the previous publication [25], The contents of albiflorin, paeoniflorin, ferulic acid, senkyunolide I, quercetin, isoliquiritigenin, atractylenolide III, ligustilide, atractylenolide II, liquiritin, liquiritigenin, saikosaponin c, glycyrrhizic acid, saikosaponin a in XYP extract were 0.62, 1.7, 5.46, 1.45, 0.70, 0.13, 1.42, 0.14, 1.01, 1.42, 1.23, 0.10, 1.12, 0.09 mg/g, respectively.

2.5. Preparation of Standards and Quality Control (QC) Samples

The stock solutions of the 14 analytes and naringenin were prepared in methanol at a final concentration of 1.0 mg/mL. A series of mixed stock solutions were diluted with methanol as QC samples. All the solutions were stored at 4°C.

The calibration standards samples in the concentration range of 0.02–30 μg/mL in tissue were prepared by adding 50 μL of mixed standard solutions to 150 μL of blank various tissue homogenates (heart, liver, spleen, lung, kidney, brain, stomach, uterus, ovary and small intestine). The mixture was then treated following the sample extraction procedure described above. The QC samples, which were used in the validation, were prepared in the same way as the standard calibration samples. QC samples (high, medium, low) were prepared by the same method. Moreover, the concentration of IS was 20 ng/mL in all the above-prepared solutions.

2.6. Tissue Sample Preparation

In the distribution study of 14 components in tissues, the oral administration XYP dose was 4 g/kg, the same as the dose in the previous study of the pharmacokinetics. The tissue samples (heart, liver, spleen, lung, kidney, brain, stomach, ovary, uterus and small intestine) were taken immediately at 0.25, 0.5, 1h and 4h after dosing. All the biological samples were rinsed three times with physiological saline to wash out the blood or content. The tissues were blotted on filter paper, weighed rapidly, and then stored at -20°C until analysis. The tissue samples of heart, liver, spleen, lung, kidney, brain, stomach, uterus, ovary, and small intestine were ground and homogenized with a 5-fold volume of distilled water. After centrifugation for 10 min at 14,000 g, 20 μL IS solution (200 ng/mL) was added to 300 μL sample. Then the samples were vortex-mixed with 1 mL methanol for 2 min and centrifuged at 14,000 × g for 10 min. The supernatants were collected and evaporated to dryness by nitrogen. The residue was re-dissolved in 100 μL of with methanol vortex-mixing for 1 min. After centrifugation for 10 min at 14,000 g, the supernatants were determined by UPLC-MS/MS analysis.

2.7. Method Validation

The method validation was conducted according to the currently accepted Chinese State Food and Drug Administration (SFDA) bioanalytical method validation guidance. The precision, extraction recovery, matrix effect, selectivity, and linearity tests were determined. All the assays were based on the ten blank tissues (heart, liver, spleen, lung, kidney, brain, stomach, ovary, uterus and small intestine).

2.7.1. Sensitivity

The specificity of the method was evaluated by comparing the chromatograms of plasma samples at 0.5h after an oral dose, blank rat plasma and plasma samples spiked with the IS. These samples are used to assess whether 14 analytes have endogenous interference. As shown in Figure 1.

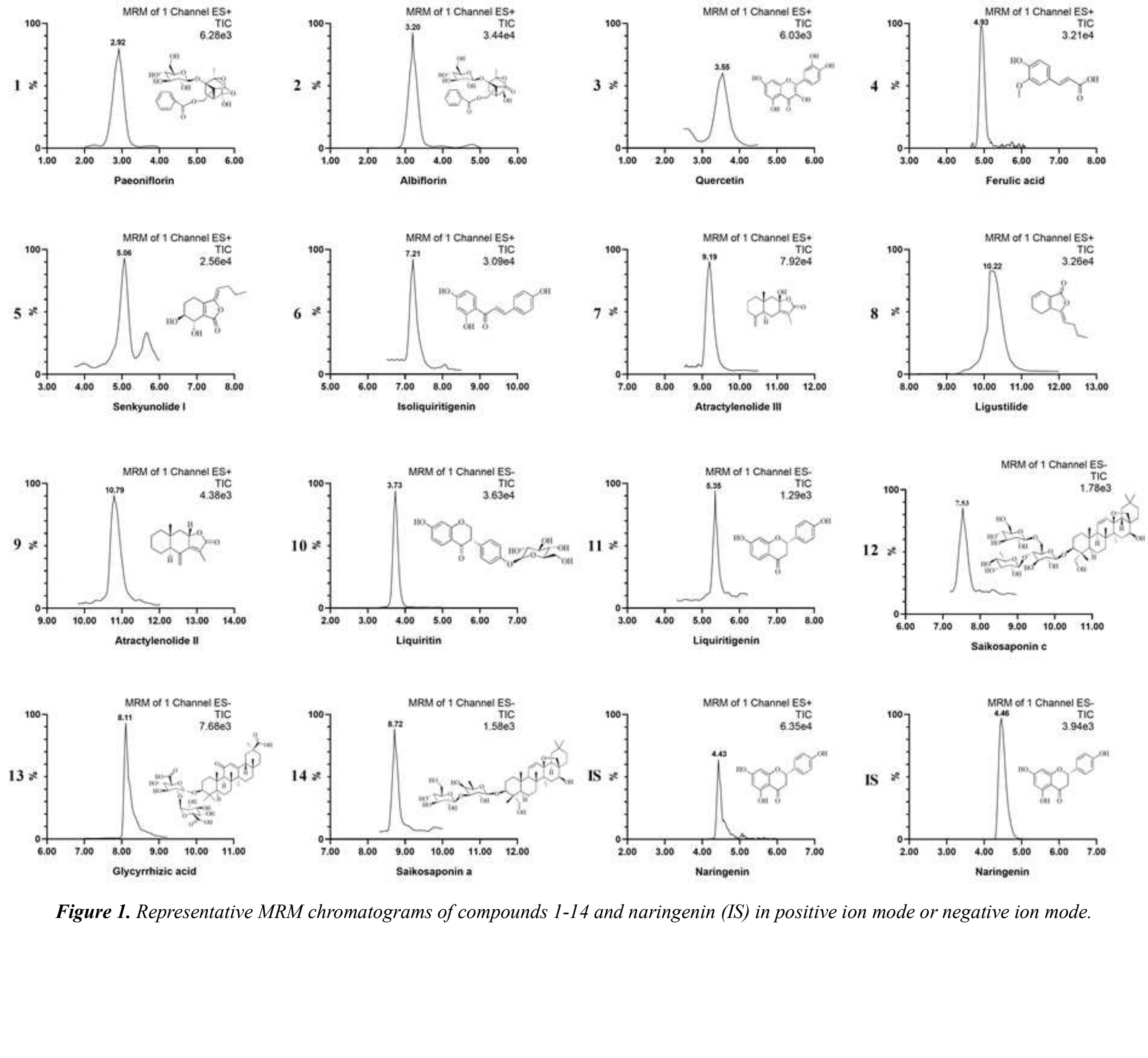


Figure 1. *Representative MRM chromatograms of compounds 1-14 and naringenin (IS) in positive ion mode or negative ion mode.*

2.7.2. Linearity and Calibration Curve

Calibration curves were calculated by plotting the peak area ratios (y) of the analytes to IS against the concentration (x) of analyte and evaluated by weighted least-squares linear regression using a weighting factor of $W=1/x^2$. The lower limit of quantification (LLOQ) for each analyte is defined by the signal-to-noise ratio S/N=10.

2.7.3. Precision

The intra-day precision was established by analyzing six reduplicate QC samples (n=6) under three concentrations (high concentration, medium concentration, low concentration) on the same day. The same procedure was conducted once a day for three consecutive days to assess inter-day precision along with the standard calibration curve by three analytical batches (high concentration, medium concentration, low concentration). The result is expressed as the relative standard deviation (RSD) (%) value of the sample, and the precision is required to be within 15%.

2.7.4. Recovery, Matrix Effects and Stability

Extraction recovery and matrix effect were evaluated by comparing the peak areas of analytes between three types of samples: (a) tissue samples spiked with known amount of analytes before sample preparation; (b) tissue samples spiked with known amount of analytes after sample preparation; (c) standard solution of analytes at three concentrations (high concentration, medium concentration, low concentration). Each tissue was tested in parallel six times, and the peak area was recorded and analyzed separately. Extraction recovery = peak area of group a / peak area of group c, matrix effect = peak area of group b / peak area of group c.

The stability of analytes was executed to evaluate the experimental conditions that the biological samples might be exposed during storage and handling. The freeze/thaw stability was evaluated by using three types of QC samples, stored at 4°C for 0 h, 12 h after 0.5 h of oral administration, stored at −20°C for 0 d, 30 d after 0.5 h of oral administration, frozen at −20°C and thawed at room temperature and the cycle was repeated three times.

3. Results and Discussion

3.1. Method Validation

The MRM chromatograms of the 14 analytes Naringenin (IS) were shown in Figure 1. Due to the high selectivity of MRM mode, there was no interference from endogenous substances.

The linearity regression equation, correlation coefficients, and linear ranges of the various tissues were calculated and summerized in Table 1. Precisions, extraction recoveries, matrix effects and stabilities were shown in Table 2. All the 14 analytes displayed good linearity in tissues (0.02μg/ml-30μg/ml, r^2 > 0.99). The LLOQ value were suitable for quantitative detection of analytes in tissue distribution. Intra-day and inter-day precision tests were assessed by measuring three different QC samples (n=6) in ten blank tissue (Table 2). The results were represented by the RSD (%) value: heart 1.08-13.85%, liver 1.30-12.97%, spleen 0.62-14.01%, lungs 0.83-13.11%, kidney 0.13-13.83%, brain 0.94-14.86%, stomach 0.20-13.87%, uterus 0.20-13.21%, ovary 0.64-14.89%, small intestine 0.60-14.09%, respectively. Both intra-day and inter-day precision data were within the acceptable standard range of ±15%, indicating that the precision is acceptable.

The extraction recoveries ranged from 82.80 to 113.83% for 14 analytes, respectively, which showed that the range of extraction recoveries was precise. All the analytes of the matrix effect were in the range of 83.68 to 105.78%, respectively, which indicated that there was no significant matrix effect for 14 analytes in the various tissue sample for this UPLC-MS/MS determination.

The stability range results of the 14 analytes were expressed by relative error (RE) (%). The stability of each tissue was calculated and listed in Table 2. The results demonstrated that 14 analytes were stable in various tissues homogenate after three freeze-thaw cycles (the RE range of 14 analytes is -20.10 – 15.33%.), in auto-sampler (12 h) at 4°C (the RE range of 14 analytes is -12.60 – 13.99%.), and in a long term freezer set at −20°C for 30 days (the RE range of 14 analytes is -14.46 – 14.37%.). It is rather remarkable that no significant degradation of the 14 analytes was observed during the lab procedure and storage.

Table 1. *Linearity regression equation, correlation coefficients, and linear ranges of analytes in various tissues.*

Biological samples	Analytes	Regression equation	Correlation coefficient	Linear range (*μg*/mL)	LOQ (*ng*/mL)	LOD (*ng*/mL)
Heart	Albiflorin	$y = 2064.8x - 74.18$	$R^2 = 0.9979$	0.02-6	18.14	6.35
	Paeoniflorin	$y = 1631.7x - 73.67$	$R^2 = 0.9986$	0.02-6	14.17	4.47
	Ferulic acid	$y = 4414.2x - 42.62$	$R^2 = 0.9989$	0.02-6	14.87	3.76
	Senkyunolide I	$y = 4251.2x - 66.47$	$R^2 = 0.9953$	0.02-6	13.57	4.33
	Quercetin	$y = 2102.6x + 25.856$	$R^2 = 0.9977$	0.2-35	152.27	53.98
	Isoliquiritigenin	$y = 4985.2x + 16.688$	$R^2 = 0.9990$	0.02-8	15.84	4.98
	Atractylenolide III	$y = 8429.8x + 730.23$	$R^2 = 0.9965$	0.02-6	17.28	6.57
	Ligustilide	$y = 4162.2x + 62.998$	$R^2 = 0.9966$	0.2-30	17.49	5.98
	Atractylenolide II	$y = 11095x - 203.81$	$R^2 = 0.9978$	0.02-8	16.30	5.16
	Liquiritin	$y = 538.9x + 100.36$	$R^2 = 0.9960$	0.02-8	12.38	4.49
	Liquiritigenin	$y = 760.32x + 73.717$	$R^2 = 0.9988$	0.02-6	19.04	6.36
	Saikosaponin c	$y = 1384.6x + 233.01$	$R^2 = 0.9985$	0.02-3	16.95	4.44
	Glycyrrhizic acid	$y = 4465.6x - 79.56$	$R^2 = 0.9969$	0.02-3	16.83	5.78
	Saikosaponin a	$y = 12195x + 74.354$	$R^2 = 0.9966$	0.02-8	14.48	3.02

Biological samples	Analytes	Regression equation	Correlation coefficient	Linear range (μg/mL)	LOQ (ng/mL)	LOD (ng/mL)
Liver	Albiflorin	$y = 1983.4x - 16.762$	$R^2 = 0.9988$	0.02-6	17.47	5.09
	Paeoniflorin	$y = 1580.8x - 29.343$	$R^2 = 0.9976$	0.02-6	17.09	4.26
	Ferulic acid	$y = 4386.5x - 71.23$	$R^2 = 0.9984$	0.02-6	16.72	3.46
	Senkyunolide I	$y = 4242.3x - 80.94$	$R^2 = 0.9980$	0.02-6	12.90	4.33
	Quercetin	$y = 1986.7x + 351.64$	$R^2 = 0.9982$	0.2-35	183.67	56.87
	Isoliquiritigenin	$y = 4970.1x - 96.69$	$R^2 = 0.9995$	0.02-8	16.56	4.56
	Atractylenolide III	$y = 8716.8x - 171.86$	$R^2 = 0.9998$	0.02-6	12.94	3.88
	Ligustilide	$y = 4090.5x + 186.21$	$R^2 = 0.9971$	0.2-30	174.47	6.57
	Atractylenolide II	$y = 11045x + 186.56$	$R^2 = 0.9989$	0.02-8	14.95	4.45
	Liquiritin	$y = 518.3x + 113.51$	$R^2 = 0.9979$	0.02-8	13.07	3.88
	Liquiritigenin	$y = 741.53x + 114.02$	$R^2 = 0.9982$	0.02-6	12.18	4.05
	Saikosaponin c	$y = 1542.8x - 28.048$	$R^2 = 0.9980$	0.02-3	15.29	5.45
	Glycyrrhizic acid	$y = 4573.3x - 90.84$	$R^2 = 0.9987$	0.02-3	9.94	3.09
	Saikosaponin a	$y = 11953x + 1334.2$	$R^2 = 0.9979$	0.02-8	16.75	5.66
Spleen	Albiflorin	$y = 1987.2x + 7.1068$	$R^2 = 0.9983$	0.02-6	17.77	5.46
	Paeoniflorin	$y = 1589.4x - 25.597$	$R^2 = 0.9975$	0.02-6	8.58	2.18
	Ferulic acid	$y = 4178.6x - 17.21$	$R^2 = 0.9994$	0.02-6	6.90	2.37
	Senkyunolide I	$y = 4335.9x - 96.85$	$R^2 = 0.9980$	0.02-6	14.64	3.64
	Quercetin	$y = 2017.7x + 420.41$	$R^2 = 0.9976$	0.2-35	17.38	5.61
	Isoliquiritigenin	$y = 4812.6x - 67.37$	$R^2 = 0.9984$	0.02-8	15.38	5.43
	Atractylenolide III	$y = 8811.7x - 145.03$	$R^2 = 0.9997$	0.02-6	8.99	3.19
	Ligustilide	$y = 4105.5x + 429.26$	$R^2 = 0.9972$	0.2-30	8.56	2.25
	Atractylenolide II	$y = 10672x - 79.695$	$R^2 = 0.9970$	0.02-8	12.77	4.44
	Liquiritin	$y = 524.04x + 211.62$	$R^2 = 0.9920$	0.02-8	13.27	4.37
	Liquiritigenin	$y = 802.09x + 12.889$	$R^2 = 0.9976$	0.02-6	19.57	6.10
	Saikosaponin c	$y = 1516.4x - 21.34$	$R^2 = 0.9900$	0.02-3	16.28	5.41
	Glycyrrhizic acid	$y = 4621.1x - 81.97$	$R^2 = 0.9982$	0.02-3	16.44	6.35
	Saikosaponin a	$y = 12410x - 222.88$	$R^2 = 0.9993$	0.02-8	14.32	3.00
Lung	Albiflorin	$y = 2114.1x - 28.202$	$R^2 = 0.9986$	0.02-6	17.09	4.17
	Paeoniflorin	$y = 1541.7x - 17.28$	$R^2 = 0.9970$	0.02-6	17.70	6.36
	Ferulic acid	$y = 4388.3x - 84.38$	$R^2 = 0.9981$	0.02-6	16.47	5.45
	Senkyunolide I	$y = 4288.5x - 49.61$	$R^2 = 0.9978$	0.02-6	13.31	4.55
	Quercetin	$y = 1963.9x + 370.97$	$R^2 = 0.9985$	0.2-35	152.15	56.17
	Isoliquiritigenin	$y = 4844.6x - 73.104$	$R^2 = 0.9988$	0.02-8	15.46	5.56
	Atractylenolide III	$y = 8811.9x - 176.07$	$R^2 = 0.9989$	0.02-6	17.37	6.52
	Ligustilide	$y = 4195.4x - 53.855$	$R^2 = 0.9980$	0.2-30	17.47	6.47
	Atractylenolide II	$y = 11015x - 216$	$R^2 = 0.9988$	0.02-8	16.36	5.77
	Liquiritin	$y = 557.22x + 33.406$	$R^2 = 0.9975$	0.02-8	14.04	4.21
	Liquiritigenin	$y = 827.37x + 2.767$	$R^2 = 0.9982$	0.02-6	17.92	5.99
	Saikosaponin c	$y = 1604x - 19.63$	$R^2 = 0.9987$	0.02-3	15.29	5.46
	Glycyrrhizic acid	$y = 4679.4x - 68.92$	$R^2 = 0.9974$	0.02-3	8.00	1.36
	Saikosaponin a	$y = 12474x - 161.99$	$R^2 = 0.9975$	0.02-8	8.17	2.84
Kidney	Albiflorin	$y = 2207x - 16.02$	$R^2 = 0.9976$	0.02-6	12.66	4.64
	Paeoniflorin	$y = 1672.6x - 29.25$	$R^2 = 0.9988$	0.02-6	13.37	5.29
	Ferulic acid	$y = 4573.8x - 48.61$	$R^2 = 0.9984$	0.02-6	19.09	6.95
	Senkyunolide I	$y = 4201x - 71.83$	$R^2 = 0.9980$	0.02-6	16.37	5.04
	Quercetin	$y = 2021.8x + 311.97$	$R^2 = 0.9972$	0.2-35	16.55	6.73
	Isoliquiritigenin	$y = 4972x - 17.219$	$R^2 = 0.9984$	0.02-8	14.47	4.66
	Atractylenolide III	$y = 9026.9x - 180.3$	$R^2 = 0.9981$	0.02-6	17.16	5.25
	Ligustilide	$y = 4412.6x - 570.49$	$R^2 = 0.9985$	0.2-30	160.04	53.58
	Atractylenolide II	$y = 11122x - 123.5$	$R^2 = 0.9984$	0.02-8	6.37	2.04
	Liquiritin	$y = 696.09x - 10.9$	$R^2 = 0.9980$	0.02-8	7.47	3.75
	Liquiritigenin	$y = 827.37x + 2.767$	$R^2 = 0.9982$	0.02-6	9.07	3.95
	Saikosaponin c	$y = 1637.8x - 113.37$	$R^2 = 0.9977$	0.02-3	16.99	5.63
	Glycyrrhizic acid	$y = 4739.9x - 54.19$	$R^2 = 0.9981$	0.02-3	14.26	5.56
	Saikosaponin a	$y = 12724x - 189.2$	$R^2 = 0.9988$	0.02-8	17.37	6.34
Brain	Albiflorin	$y = 2291.2x + 492.96$	$R^2 = 0.9983$	0.02-6	17.03	5.33
	Paeoniflorin	$y = 1731.7x - 22.73$	$R^2 = 0.9989$	0.02-6	16.33	6.47
	Ferulic acid	$y = 4543.7x - 53$	$R^2 = 0.9990$	0.02-6	9.07	2.65
	Senkyunolide I	$y = 4153.9x - 159.18$	$R^2 = 0.9987$	0.02-6	7.67	3.96
	Quercetin	$y = 2275x - 153.32$	$R^2 = 0.9989$	0.2-35	175.42	44.78
	Isoliquiritigenin	$y = 5170.2x - 106.78$	$R^2 = 0.9985$	0.02-8	5.38	1.98
	Atractylenolide III	$y = 9194.2x - 137.84$	$R^2 = 0.9986$	0.02-6	6.49	2.22

Biological samples	**Analytes**	**Regression equation**	**Correlation coefficient**	**Linear range (*μg*/mL)**	**LOQ (*ng*/mL)**	**LOD (*ng*/mL)**
	Ligustilide	$y = 4314.3x + 183.9$	$R^2 = 0.9996$	0.2-30	9.66	3.04
	Atractylenolide II	$y = 11430x - 176.9$	$R^2 = 0.9989$	0.02-8	9.72	4.05
	Liquiritin	$y = 629.96x + 127.8$	$R^2 = 0.9986$	0.02-8	18.31	6.27
	Liquiritigenin	$y = 797.74x + 162.63$	$R^2 = 0.9981$	0.02-6	18.36	7.09
	Saikosaponin c	$y = 1487.2x + 233.72$	$R^2 = 0.9982$	0.02-3	15.09	4.93
	Glycyrrhizic acid	$y = 4754.6x + 233.72$	$R^2 = 0.9988$	0.02-3	12.77	4.32
	Saikosaponin a	$y = 13368x - 246.6$	$R^2 = 0.9989$	0.02-8	9.56	4.01
	Albiflorin	$y = 2568.3x + 443.85$	$R^2 = 0.9988$	0.02-6	19.56	7.28
	Paeoniflorin	$y = 2282.6x - 34.13$	$R^2 = 0.9980$	0.02-6	18.42	6.74
	Ferulic acid	$y = 4050.8x - 95.817$	$R^2 = 0.9987$	0.02-6	14.11	3.63
	Senkyunolide I	$y = 4109.2x + 152.21$	$R^2 = 0.9972$	0.02-6	14.06	4.47
	Quercetin	$y = 2429.7x - 411.1$	$R^2 = 0.9966$	0.2-35	153.99	53.22
	Isoliquiritigenin	$y = 4874x - 71.247$	$R^2 = 0.9982$	0.02-8	12.78	5.95
Uterus	Atractylenolide III	$y = 9086.9x - 148.2$	$R^2 = 0.9979$	0.02-6	15.33	5.37
	Ligustilide	$y = 4277.4x - 139.44$	$R^2 = 0.9984$	0.2-30	139.22	48.44
	Atractylenolide II	$y = 11430x - 176.9$	$R^2 = 0.9989$	0.02-8	17.56	5.85
	Liquiritin	$y = 629.96x + 127.8$	$R^2 = 0.9986$	0.02-8	16.09	6.32
	Liquiritigenin	$y = 797.74x + 162.63$	$R^2 = 0.9981$	0.02-6	12.47	5.17
	Saikosaponin c	$y = 1487.2x + 233.72$	$R^2 = 0.9982$	0.02-3	19.00	6.88
	Glycyrrhizic acid	$y = 4754.6x + 233.72$	$R^2 = 0.9988$	0.02-3	16.32	5.56
	Saikosaponin a	$y = 13368x - 246.6$	$R^2 = 0.9989$	0.02-8	14.22	4.55
	Albiflorin	$y = 2502x + 279.55$	$R^2 = 0.9977$	0.02-6	17.47	6.23
	Paeoniflorin	$y = 2163x + 73.039$	$R^2 = 0.9976$	0.02-6	16.88	5.47
	Ferulic acid	$y = 3978.3x - 66.53$	$R^2 = 0.9973$	0.02-6	6.70	3.03
	Senkyunolide I	$y = 3916.2x + 377.9$	$R^2 = 0.9981$	0.02-6	7.65	3.22
	Quercetin	$y = 2125.2x - 244.97$	$R^2 = 0.9970$	0.2-35	191.00	71.02
	Isoliquiritigenin	$y = 4364.4x + 492.26$	$R^2 = 0.9967$	0.02-8	16.00	5.67
Ovary	Atractylenolide III	$y = 8526.2x - 169.38$	$R^2 = 0.9976$	0.02-6	13.32	3.09
	Ligustilide	$y = 3931.9x + 149.73$	$R^2 = 0.9977$	0.2-30	121.09	56.75
	Atractylenolide II	$y = 10294x - 243.75$	$R^2 = 0.9983$	0.02-8	7.73	2.46
	Liquiritin	$y = 877.46x - 13.12$	$R^2 = 0.9977$	0.02-8	9.22	3.55
	Liquiritigenin	$y = 1015.8x - 17.15$	$R^2 = 0.9975$	0.02-6	16.19	5.78
	Saikosaponin c	$y = 1716.9x + 122.94$	$R^2 = 0.9962$	0.02-3	14.01	5.94
	Glycyrrhizic acid	$y = 4874.6x + 459.15$	$R^2 = 0.9981$	0.02-3	17.84	6.12
	Saikosaponin a	$y = 12298x - 197.8$	$R^2 = 0.9962$	0.02-8	17.57	4.45
	Albiflorin	$y = 2131.7x - 22.72$	$R^2 = 0.9978$	0.02-6	16.37	4.22
	Paeoniflorin	$y = 1496.6x + 278.51$	$R^2 = 0.9974$	0.02-6	9.09	3.65
	Ferulic acid	$y = 4133x - 147.3$	$R^2 = 0.9984$	0.02-6	17.96	5.36
	Senkyunolide I	$y = 4304.1x - 79.05$	$R^2 = 0.9982$	0.02-6	15.57	6.42
	Quercetin	$y = 2029.3x + 334.33$	$R^2 = 0.9977$	0.2-35	182.68	67.06
	Isoliquiritigenin	$y = 4956.8x - 62.86$	$R^2 = 0.9987$	0.02-8	8.96	2.31
Stomach	Atractylenolide III	$y = 9006.2x - 158.1$	$R^2 = 0.9987$	0.02-6	12.46	3.36
	Ligustilide	$y = 4105.5x + 273.75$	$R^2 = 0.9982$	0.2-30	16.75	49.38
	Atractylenolide II	$y = 11150x - 222.2$	$R^2 = 0.9984$	0.02-8	19.05	6.24
	Liquiritin	$y = 539.4x + 139.68$	$R^2 = 0.9974$	0.02-8	16.76	7.06
	Liquiritigenin	$y = 806.86x - 3.3403$	$R^2 = 0.9973$	0.02-6	16.64	5.57
	Saikosaponin c	$y = 1462x + 116.83$	$R^2 = 0.9970$	0.02-3	14.84	5.67
	Glycyrrhizic acid	$y = 4543.2x - 53.04$	$R^2 = 0.9989$	0.02-3	18.26	5.13
	Saikosaponin a	$y = 11363x + 1030.8$	$R^2 = 0.9971$	0.02-8	14.17	4.22
	Albiflorin	$y = 1817.7x + 316.77$	$R^2 = 0.9973$	0.02-6	14.35	3.47
	Paeoniflorin	$y = 1473.2x + 214.44$	$R^2 = 0.9962$	0.02-6	15.22	6.37
	Ferulic acid	$y = 3910.1x - 46.84$	$R^2 = 0.9962$	0.02-6	12.02	4.23
	Senkyunolide I	$y = 3921.9x - 33.73$	$R^2 = 0.9958$	0.02-6	15.78	5.77
	Quercetin	$y = 1949.3x + 66.203$	$R^2 = 0.9975$	0.2-35	130.56	46.09
	Isoliquiritigenin	$y = 4821.3x - 93.62$	$R^2 = 0.9978$	0.02-8	17.00	7.88
Small intestine	Atractylenolide III	$y = 8909.6x - 186.4$	$R^2 = 0.9965$	0.02-6	16.15	5.57
	Ligustilide	$y = 3913.2x + 294.92$	$R^2 = 0.9975$	0.2-30	120.35	42.06
	Atractylenolide II	$y = 10301x - 157.8$	$R^2 = 0.9974$	0.02-8	17.56	6.86
	Liquiritin	$y = 704.03x - 8.326$	$R^2 = 0.9977$	0.02-8	8.11	2.46
	Liquiritigenin	$y = 919.96x + 45.86$	$R^2 = 0.9963$	0.02-6	6.57	1.35
	Saikosaponin c	$y = 1564.3x + 98.06$	$R^2 = 0.9988$	0.02-3	14.66	4.37
	Glycyrrhizic acid	$y = 4301.2x - 57.11$	$R^2 = 0.9963$	0.02-3	17.44	7.04
	Saikosaponin a	$y = 10971x + 2594.2$	$R^2 = 0.9969$	0.02-8	9.08	3.33

Table 2. *Precisions, extraction recoveries, matrix effects and stabilities of analytes in various tissues.*

Biological samples	Analytes	Concentration (*μg*/mL)	Intra-day precision RSD (%)	Inter-day precision RSD (%)	Extraction recovery (%)	Extraction recovery RSD (%)	Matrix effect (%)	Stability: Auto-sampler (4°C, 12h) RE (%)	Stability: Long-term (-20°C, 30 d) RE (%)	Stability: Freeze-thaw (-20°C -room temperature) RE (%)
heart	Albiflorin	0.056	5.345	4.863	96.11	7.43	86.94	-6.056	-7.722	-6.333
		0.603	13.163	9.650	95.99	7.38	91.66	-1.500	-7.111	-2.722
		5.398	3.279	7.079	94.14	6.61	92.00	-7.333	-5.944	-10.333
	Paeoniflorin	0.054	13.128	13.184	89.74	5.02	85.91	1.000	3.000	10.667
		0.482	2.881	2.555	100.50	5.10	90.36	-2.967	-1.500	-3.200
		4.637	3.267	1.636	95.06	5.91	89.54	-8.133	-8.700	-7.200
	Ferulic acid	0.054	13.128	13.854	93.72	4.90	85.23	0.333	-5.667	10.667
		0.484	2.803	1.904	96.97	3.00	86.47	-3.567	-2.600	-2.433
		4.793	3.832	2.034	98.47	6.12	91.64	-4.897	-3.597	-2.163
	Senkyunolide I	0.056	12.222	11.743	90.77	5.87	84.10	-1.000	-1.333	9.667
		0.468	5.169	9.650	103.25	3.04	90.25	-9.467	2.933	-6.440
		4.495	7.918	2.203	101.42	8.44	92.42	-8.833	-4.133	-12.200
	Quercetin	0.227	12.311	8.467	92.67	9.09	86.90	-1.533	-8.667	-11.800
		2.340	6.509	7.342	86.75	2.67	87.75	-5.633	-11.420	-6.433
		24.068	5.397	8.042	86.75	3.56	86.95	-1.953	1.101	-2.739
	Isoliquiritigenin	0.060	11.233	7.157	95.07	6.49	87.57	-4.056	1.900	1.472
		0.577	10.324	10.140	100.51	5.31	97.77	-7.667	-10.111	-5.611
		5.693	7.647	7.821	89.35	5.19	91.01	-5.811	-3.478	-7.144
	Atractylenolide III	0.057	4.904	10.546	89.59	7.19	90.09	-1.333	-5.667	11.667
		0.507	9.469	7.984	87.21	4.50	89.87	1.367	1.700	2.633
		4.828	3.220	2.069	92.19	2.37	90.77	-5.140	-5.273	-4.173
	Ligustilide	0.228	13.404	12.067	88.68	7.49	88.68	-6.067	1.567	-8.733
		2.339	4.783	7.061	85.53	3.58	89.13	-7.849	-11.382	-6.287
		23.887	7.642	6.120	85.48	3.99	85.14	-2.625	-0.566	-4.230
	Atractylenolide II	0.059	11.243	4.575	96.65	8.74	100.09	-6.667	-6.667	-3.611
		0.592	3.569	3.286	94.79	5.01	88.79	-0.861	-2.167	-3.333
		5.653	6.836	4.679	100.74	3.93	91.74	-3.172	-2.965	-6.908
	Liquiritin	0.057	5.610	6.416	105.98	10.88	95.29	-2.500	-4.444	-6.389
		0.548	10.123	10.726	100.45	4.28	91.29	-3.611	-3.583	-7.083
		5.708	3.786	4.999	95.43	11.23	92.69	-5.841	-6.524	-5.404
	Liquiritigenin	0.054	13.128	11.243	87.33	4.42	87.65	11.333	3.333	10.333
		0.547	10.326	8.903	96.16	8.19	94.49	-3.600	-1.067	7.267
		4.653	4.082	5.846	91.16	3.43	89.66	-0.717	-4.283	-7.230
	Saikosaponin c	0.019	4.720	7.056	97.27	8.86	91.07	-7.333	-2.750	-3.750
		0.189	7.022	9.504	92.68	9.54	88.85	-2.417	-1.583	-2.583
		2.307	6.352	4.618	87.48	3.42	92.48	-6.750	-6.167	13.333
	Glycyrrhizic acid	0.019	7.416	12.370	86.83	7.02	88.50	-2.333	-7.917	-7.083
		0.194	5.106	3.629	87.33	2.99	87.30	10.417	1.583	-2.250
		2.021	8.411	4.400	90.18	8.00	83.68	-4.667	-4.333	-0.200
	Saikosaponin a	0.058	3.846	1.081	89.52	7.30	92.02	-5.750	-5.033	-3.417
		0.583	3.445	2.734	88.10	4.68	91.31	-3.639	-4.378	-2.917
		5.984	3.999	2.622	90.20	3.31	86.13	0.239	-3.206	-0.304
liver	Albiflorin	0.056	9.573	8.667	90.43	5.75	101.50	-6.111	-4.722	-7.500
		0.598	7.920	5.521	93.62	3.08	100.50	-1.111	-4.278	-10.083
		5.657	1.781	4.561	98.20	8.21	97.39	-7.064	-6.717	-11.897
	Paeoniflorin	0.052	8.968	10.952	90.06	1.83	95.74	1.333	6.800	0.233
		0.491	3.814	3.925	87.74	3.13	99.07	-2.733	1.467	-7.086
		4.560	1.494	2.021	91.92	9.50	99.03	-8.140	-9.583	-9.577
	Ferulic acid	0.050	7.813	5.567	85.88	1.54	99.23	0.333	-12.667	0.667
		0.481	3.053	2.225	92.21	2.24	99.23	-2.400	-6.133	-7.053
		4.777	3.830	2.430	97.77	9.06	102.89	-4.913	-3.700	-7.800
	Senkyunolide I	0.052	9.650	4.649	85.29	0.71	95.05	0.000	-2.733	-3.000
		0.454	4.023	7.378	93.44	5.43	95.05	-11.667	14.367	-8.571
		4.697	10.313	3.947	94.78	4.73	101.14	-7.000	-0.420	-9.118
	Quercetin	0.227	12.391	6.646	88.92	1.57	89.16	-6.200	-6.913	-10.000
		2.373	4.369	9.547	91.24	6.90	87.83	-4.140	-7.540	-11.092
		24.995	4.817	3.892	95.31	12.29	88.23	-1.960	-3.375	-8.544
	Isoliquiritigenin	0.059	3.409	5.467	95.57	9.89	88.40	-8.500	-11.989	-20.103
		0.577	8.103	7.897	101.13	3.79	95.06	-6.944	-8.889	-16.725
		5.540	5.715	3.003	92.43	5.26	92.36	-5.656	-3.167	-4.378

Biological samples	Analytes	Concentration (μg/mL)	Intra-day precision RSD (%)	Inter-day precision RSD (%)	Extraction recovery (%)	Extraction recovery RSD (%)	Matrix effect (%)	Stability		
								Auto-sampler (4°C, 12h) RE (%)	Long-term (-20°C, 30 d) RE (%)	Freeze-thaw (-20°C -room temperature) RE (%)
	Atractylenolide III	0.053	12.971	12.107	92.93	5.32	95.38	1.000	-2.200	11.000
		0.506	9.659	7.142	90.26	3.17	95.38	4.833	2.000	3.246
		4.825	3.551	2.369	86.47	3.58	91.76	-3.614	-5.870	-7.107
		0.228	10.146	11.689	94.15	11.51	92.45	1.787	-3.420	-13.630
	Ligustilide	2.313	5.634	5.492	94.48	12.21	93.07	-6.173	-11.167	-11.311
		24.490	1.438	1.682	88.03	2.03	87.96	-1.339	-4.906	-6.742
		0.055	6.527	6.277	95.48	6.15	97.86	-7.083	-12.500	-14.306
	Atractylenolide II	0.586	3.472	1.814	96.24	5.40	98.87	-2.386	-4.111	-5.961
		5.642	6.907	4.662	93.07	10.22	102.87	-5.022	-8.716	-8.804
		0.057	5.367	5.975	95.52	7.78	98.11	-3.611	-10.278	-8.611
	Liquiritin	0.579	4.717	3.901	94.16	13.49	91.11	-3.083	-8.175	-10.672
		5.769	2.678	4.210	104.52	11.73	99.14	-5.576	-6.251	-8.229
		0.055	9.304	10.383	93.95	6.49	89.43	9.167	-12.000	1.000
	Liquiritigenin	0.493	5.455	8.378	91.11	0.55	96.09	1.067	-8.733	2.553
		4.780	9.251	6.151	87.81	1.06	92.15	-1.210	-10.379	-8.053
		0.019	6.686	6.549	92.50	3.68	89.10	-8.583	-4.333	-10.917
	Saikosaponin c	0.192	8.682	6.473	100.17	10.04	87.21	0.000	-4.750	-9.397
		1.875	5.190	4.343	93.71	12.85	87.62	-3.000	-5.433	5.088
		0.019	5.432	10.379	86.50	1.16	92.98	-3.583	-9.500	-10.750
	Glycyrrhizic acid	0.209	9.918	7.521	87.51	3.41	93.50	8.083	-0.667	-8.000
		1.875	4.316	2.390	86.68	8.76	88.52	-4.383	-7.158	-3.158
		0.058	2.868	1.304	93.68	1.66	88.63	-7.444	-6.564	-5.418
	Saikosaponin a	0.583	3.377	2.845	97.97	8.24	88.55	-5.333	-7.445	-4.389
		5.980	3.145	4.114	90.85	9.98	90.21	-0.728	-4.617	-2.634
		0.052	5.516	10.862	90.48	3.08	100.23	-4.458	-3.472	-6.944
	Albiflorin	0.560	11.710	4.045	97.06	7.26	91.31	0.094	-4.039	0.722
		5.537	1.058	3.588	102.50	3.44	93.31	-4.800	-6.008	-9.544
		0.051	5.698	7.358	89.24	3.13	98.40	7.517	1.467	5.333
	Paeoniflorin	0.480	0.985	1.956	90.39	7.44	98.66	-0.225	-5.487	-3.600
		4.587	1.314	1.630	90.67	5.24	97.83	-5.273	-9.753	-7.973
		0.051	5.698	9.914	97.84	2.24	98.00	6.800	-8.900	5.333
	Ferulic acid	0.477	2.240	1.962	95.39	4.95	98.59	1.635	-7.223	-4.267
		4.663	2.831	2.911	99.62	5.08	101.26	-1.060	-5.490	-5.600
		0.051	5.698	9.081	97.13	5.43	101.67	4.800	-4.567	8.333
	Senkyunolide I	0.469	4.268	4.045	91.89	6.54	104.59	-9.517	10.823	-5.540
		4.650	4.317	2.127	92.78	5.76	100.55	-10.518	-2.907	-11.160
		0.245	2.857	2.677	92.95	6.90	90.10	0.920	-8.900	-8.600
	Quercetin	2.420	4.693	4.519	94.64	12.80	88.27	-5.083	-11.567	-5.573
		22.990	2.476	9.392	93.08	1.58	91.52	1.471	-2.111	-6.225
		0.057	7.878	5.715	96.29	3.79	99.69	-3.208	-2.925	-1.333
	Isoliquiritigenin	0.555	12.704	5.322	94.91	7.50	98.17	-2.879	-5.783	-5.806
		5.929	6.057	11.189	94.74	9.20	101.79	-3.360	-5.597	-2.278
	Atractylenolide III	0.051	5.698	9.914	88.45	3.17	90.47	4.650	-1.233	13.333
spleen		0.513	6.718	9.775	84.92	1.93	88.53	5.278	1.113	1.467
		4.910	3.902	2.803	93.75	5.32	93.76	-2.865	-4.763	-4.077
		0.237	6.454	2.404	93.50	12.21	89.69	0.976	-6.933	-11.120
	Ligustilide	2.279	5.848	10.041	85.85	1.83	88.32	-8.193	-14.240	-7.185
		23.695	2.154	11.995	88.60	1.22	87.73	-0.323	-2.512	-5.436
		0.061	14.007	4.467	98.68	5.40	93.10	-4.250	-9.889	-3.028
	Atractylenolide II	0.603	4.224	0.945	89.76	6.54	91.42	1.374	-1.717	-0.639
		5.592	10.459	6.409	98.03	9.68	96.64	-1.619	-6.420	-5.831
		0.057	7.018	5.298	95.53	13.49	100.00	-1.042	-10.750	-4.167
	Liquiritin	0.536	2.616	13.208	111.16	5.35	101.14	0.087	-2.975	-6.594
		5.718	5.807	6.277	109.52	0.69	95.68	-2.905	-4.201	-4.603
		0.051	5.698	7.358	88.01	0.55	95.62	13.992	-5.967	4.333
	Liquiritigenin	0.477	7.368	9.126	90.00	4.85	93.16	-1.167	1.500	7.067
		4.789	3.478	6.133	93.49	9.31	101.21	0.790	-6.083	-6.553
		0.019	2.935	10.390	96.81	10.04	96.05	-4.342	-5.383	-6.583
	Saikosaponin c	0.183	2.478	12.689	97.43	13.38	98.52	4.100	-3.075	-6.578
		2.220	2.813	4.519	100.75	0.57	93.62	-3.217	-5.300	14.872
		0.018	5.441	13.752	88.52	3.41	92.00	-0.050	-12.133	-8.417
	Glycyrrhizic acid	0.194	7.533	5.311	82.80	2.37	88.20	9.308	-6.008	-3.800
		1.897	9.980	2.813	94.54	10.86	91.48	-2.993	-5.775	0.383

Biological samples	**Analytes**	**Concentration (μg/mL)**	**Intra-day precision RSD (%)**	**Inter-day precision RSD (%)**	**Extraction recovery (%)**	**Extraction recovery RSD (%)**	**Matrix effect (%)**	**Stability**		
								Auto-sampler (4°C, 12h) RE (%)	**Long-term (-20°C, 30 d) RE (%)**	**Freeze-thaw (-20°C -room temperature) RE (%)**
		0.056	2.581	0.619	96.48	8.24	91.84	-2.514	-7.271	-5.014
	Saikosaponin a	0.577	1.637	2.696	89.32	11.39	88.75	-1.907	-9.386	-3.028
		5.989	3.477	2.937	90.38	6.83	93.34	1.607	-2.689	-1.897
		0.059	7.388	8.040	102.51	2.45	93.64	-5.389	1.509	-8.333
	Albiflorin	0.580	10.488	9.938	105.30	2.51	90.64	-1.022	-1.359	-3.444
		5.260	2.515	6.847	95.31	11.61	88.64	-6.786	-0.998	-8.850
		0.057	7.865	4.548	92.72	3.71	99.06	9.933	11.294	2.000
	Paeoniflorin	0.492	2.964	2.588	93.86	12.01	98.16	-1.093	2.558	-4.967
		4.687	4.588	1.928	100.33	7.26	96.39	-6.083	-5.018	-7.240
		0.056	11.710	11.060	95.73	4.73	98.69	4.320	-2.102	2.000
	Ferulic acid	0.492	2.588	1.708	96.67	1.18	101.60	-1.324	-0.833	-3.333
		4.923	2.588	0.829	95.59	2.98	97.92	-2.956	0.789	-4.833
		0.055	10.276	10.660	90.42	4.11	104.59	1.880	-0.335	5.000
	Senkyunolide I	0.448	3.420	12.486	99.00	7.43	100.55	-1.440	14.332	-7.373
		4.740	2.854	2.698	104.59	1.53	104.92	-10.763	3.250	-11.660
		0.216	5.458	4.617	95.64	11.98	88.75	0.467	-1.159	-7.067
	Quercetin	2.260	7.121	9.215	91.28	5.41	89.39	-5.813	-6.383	-7.707
		24.947	1.213	3.738	85.80	3.32	85.47	0.612	2.560	-5.578
		0.063	12.665	7.079	92.02	8.01	101.17	-3.917	6.590	-3.861
	Isoliquiritigenin	0.599	8.258	13.110	96.92	13.84	100.47	-3.556	1.081	-8.000
		5.458	7.737	4.215	100.18	6.58	90.51	-4.189	-0.639	-4.558
	Atractylenolide III	0.057	7.957	10.422	88.25	4.01	90.86	3.600	12.081	11.667
		0.517	7.975	5.702	92.64	7.67	94.42	4.333	10.196	-0.167
lung		4.747	1.287	1.672	85.86	3.67	92.86	-3.617	4.788	-4.210
		0.236	12.915	3.668	85.51	1.74	86.61	-0.227	-3.040	-6.453
	Ligustilide	2.400	2.087	4.527	88.09	2.31	88.37	-8.980	-7.456	-7.632
		23.080	9.531	4.701	86.30	4.04	86.13	-1.107	7.175	-2.776
		0.057	8.772	5.298	93.77	12.08	95.69	-5.222	-1.056	-4.417
	Atractylenolide II	0.580	1.302	2.214	98.35	8.99	100.58	0.639	7.642	-1.056
		5.715	2.478	3.678	91.38	1.99	101.41	-2.279	2.962	-6.117
		0.057	5.391	8.621	113.83	1.80	100.79	-1.611	-4.461	-5.278
	Liquiritin	0.550	13.008	7.391	105.87	8.42	90.51	-0.850	7.185	-9.094
		5.697	1.375	4.334	99.98	3.80	89.77	-3.669	6.078	-4.493
		0.055	11.026	1.063	88.43	6.66	100.83	13.067	-1.203	1.000
	Liquiritigenin	0.513	1.125	5.650	93.96	10.33	100.54	-2.200	11.792	6.733
		4.517	1.844	2.324	92.49	4.85	92.49	-0.063	3.972	-6.687
		0.019	6.406	3.577	95.43	11.40	95.18	-5.150	4.028	-7.167
	Saikosaponin c	0.196	8.703	1.117	98.84	3.49	85.62	2.733	6.498	-5.828
		2.127	2.222	4.469	95.18	1.77	84.73	-4.167	4.775	12.205
		0.019	9.483	6.903	89.49	9.48	85.47	-0.900	-3.205	-9.000
	Glycyrrhizic acid	0.187	1.722	1.986	94.81	10.29	91.43	8.717	-1.133	-3.383
		2.078	10.022	8.327	88.18	3.30	93.85	-3.792	3.171	2.267
		0.059	2.596	1.586	85.51	4.93	87.35	-3.350	1.395	-5.597
	Saikosaponin a	0.588	4.924	2.078	90.51	6.53	96.08	-2.678	-2.276	-4.472
		5.978	5.282	2.724	87.43	6.77	91.786	0.908	5.952	-0.335
		0.056	4.725	2.846	98.45	3.44	99.16	-6.111	-5.556	-6.111
	Albiflorin	0.596	8.383	3.978	96.18	13.27	94.16	-1.111	-5.444	-2.056
		5.613	0.914	0.710	91.31	4.78	93.64	-7.064	-5.311	-9.406
		0.049	6.516	9.932	93.34	5.24	97.19	1.333	2.667	8.000
	Paeoniflorin	0.482	0.936	3.267	101.07	2.39	94.52	-2.733	-1.833	-4.033
		4.550	0.440	1.641	98.66	5.68	96.06	-8.140	-8.413	-8.673
		0.049	6.278	7.525	97.26	5.08	95.57	0.333	-8.667	8.000
	Ferulic acid	0.471	0.804	2.784	95.64	4.01	94.80	-2.400	-4.200	-3.567
kidney		4.653	2.918	2.911	98.59	2.37	99.92	-4.913	-4.333	-4.900
		0.050	7.623	6.000	91.18	5.76	101.44	0.000	-3.000	11.000
	Senkyunolide I	0.459	4.361	2.508	100.08	1.75	105.78	-10.000	7.333	-7.467
		4.950	1.325	0.751	104.59	1.53	101.59	-8.333	0.040	-10.760
		0.238	0.131	5.461	92.00	1.58	93.74	-2.200	-9.067	-8.133
	Quercetin	2.417	5.019	4.949	89.02	2.91	91.12	-5.867	-11.240	-6.840
		24.060	2.057	4.595	88.27	1.71	87.60	-2.017	0.643	-5.425
		0.058	3.859	4.145	93.21	9.20	101.61	-5.444	1.067	-5.778
	Isoliquiritigenin	0.559	11.090	4.465	98.16	7.97	93.96	-7.361	-8.889	-8.333
		5.403	8.085	4.281	98.17	3.10	89.18	-5.611	-3.167	-3.894

Biological samples	Analytes	Concentration (μg/mL)	Intra-day precision RSD (%)	Inter-day precision RSD (%)	Extraction recovery (%)	Extraction recovery RSD (%)	Matrix effect (%)	Stability		
								Auto-sampler (4°C, 12h) RE (%)	Long-term (-20°C, 30 d) RE (%)	Freeze-thaw (-20°C -room temperature) RE (%)
	Atractylenolide III	0.049	6.516	6.611	93.80	5.32	94.12	-0.667	-5.333	13.667
		0.507	7.897	9.651	90.53	3.09	95.79	0.433	0.300	4.233
		4.917	4.190	3.597	88.53	1.93	92.19	-4.737	-4.237	-3.810
	Ligustilide	0.230	4.348	5.698	86.83	1.22	90.47	-4.427	0.367	-5.787
		2.370	3.867	4.240	87.92	5.71	91.17	-8.773	-12.267	-7.839
		24.612	2.082	1.755	88.32	1.98	86.16	-2.823	-0.666	-3.036
	Atractylenolide II	0.055	8.449	2.087	95.40	9.68	95.58	-5.833	-7.500	-1.639
		0.596	4.611	0.945	90.48	2.14	99.52	0.056	-0.750	-0.806
		5.583	10.619	6.221	91.42	2.06	100.41	-3.183	-3.255	-6.608
	Liquiritin	0.058	6.216	4.389	106.21	0.69	97.62	-3.056	-5.833	-3.611
		0.579	4.886	3.491	96.69	4.05	93.45	-3.778	-3.750	-11.067
		5.781	3.974	5.075	101.14	4.84	90.77	-5.576	-5.346	-4.913
	Liquiritigenin	0.053	7.578	6.668	90.40	9.31	99.13	8.500	0.333	7.000
		0.496	4.495	8.137	92.53	6.82	90.79	-2.200	0.000	10.067
		4.637	13.831	5.626	93.16	5.82	89.83	-0.810	-5.860	-7.087
	Saikosaponin c	0.018	1.462	8.199	100.36	0.57	98.29	-8.167	-3.500	-5.167
		0.182	3.496	3.144	95.66	2.44	91.60	-1.417	-3.000	-8.578
		1.823	1.142	5.784	98.52	7.17	87.58	-6.667	-6.000	13.288
	Glycyrrhizic acid	0.019	6.954	11.002	94.31	10.86	91.20	-2.333	-9.500	-6.167
		0.225	6.662	6.050	91.77	4.32	96.60	10.500	0.583	-4.300
		1.840	4.981	2.142	88.20	3.27	97.18	-5.342	-4.867	-1.200
	Saikosaponin a	0.056	1.546	1.625	88.88	6.83	89.88	-5.472	-5.061	-4.764
		0.578	1.493	2.859	90.34	9.35	93.70	-3.778	-5.450	-3.139
		6.100	1.148	5.036	88.75	5.32	89.20	0.261	-2.500	-0.424
	Albiflorin	0.056	11.274	8.304	96.36	13.27	88.73	-6.944	-4.167	-3.889
		0.570	14.035	5.884	91.31	4.78	93.64	-4.222	-3.556	1.444
		5.447	2.325	6.907	93.73	0.17	101.50	-7.203	-4.533	-9.644
	Paeoniflorin	0.051	5.698	8.921	100.66	2.39	99.96	4.333	0.333	8.667
		0.480	0.939	1.484	98.66	5.68	96.06	-2.567	-3.533	-3.467
		4.670	4.299	1.597	102.62	1.99	95.74	-7.740	-8.880	-6.680
	Ferulic acid	0.051	5.698	13.804	97.61	4.01	95.97	3.333	-8.667	6.000
		0.476	1.905	1.888	98.59	2.37	99.92	-1.133	-4.900	-3.567
		4.777	1.781	2.834	96.73	2.13	99.23	-3.080	-4.667	-3.767
	Senkyunolide I	0.051	5.698	14.394	98.63	1.75	104.09	2.333	-2.333	9.667
		0.472	3.487	5.884	104.59	1.53	101.59	-10.333	9.900	-2.640
		4.350	9.375	2.126	99.76	1.75	95.05	-12.600	-0.760	-6.593
	Quercetin	0.217	1.742	9.459	89.74	2.91	87.74	-5.333	-9.000	-7.600
		2.417	4.937	4.505	88.27	1.71	87.60	-5.800	-11.160	-7.453
		23.337	4.979	6.544	90.35	1.31	89.16	-1.623	0.783	-2.643
	Isoliquiritigenin	0.063	11.825	5.563	102.15	7.97	93.80	-5.444	-0.461	-1.028
		0.559	13.707	6.694	98.17	3.10	89.18	-8.583	-7.222	-2.833
		5.676	2.265	8.016	101.45	2.66	88.40	-5.028	-3.278	-4.908
brain	Atractylenolide III	0.051	5.698	11.759	87.57	3.09	96.72	1.667	-3.000	13.000
		0.483	11.971	1.894	88.53	1.93	92.19	1.167	3.233	0.433
		4.877	4.722	2.803	95.06	2.38	95.38	-4.937	-3.737	-4.303
	Ligustilide	0.217	11.615	3.519	91.19	5.71	88.67	-2.427	-2.000	-9.267
		2.282	6.116	10.037	88.32	1.98	86.16	-10.107	-13.867	-5.560
		24.000	4.421	2.250	87.98	2.18	92.45	-2.881	-0.712	-5.681
	Atractylenolide II	0.055	10.434	4.725	94.34	2.14	103.84	-4.444	-7.222	-1.667
		0.590	3.891	2.896	91.42	2.06	100.41	-0.972	-0.250	-0.639
		5.552	9.863	4.873	99.90	6.85	97.86	-4.292	-4.278	-4.237
	Liquiritin	0.055	3.808	5.172	95.52	4.05	92.39	-4.722	-6.944	-5.556
		0.616	10.634	9.279	101.14	4.84	90.77	-2.472	-2.389	-6.417
		5.723	5.825	4.904	96.55	7.28	98.11	-5.816	-4.118	-4.924
	Liquiritigenin	0.051	5.698	12.735	92.63	6.82	89.85	8.500	-1.000	5.667
		0.503	4.136	9.090	93.16	5.82	89.83	-2.533	3.333	9.333
		4.665	1.090	5.911	98.18	8.03	89.43	-0.043	-3.027	-7.907
	Saikosaponin c	0.019	3.801	10.158	93.70	2.44	85.53	-7.000	-3.417	-5.417
		0.193	9.981	12.110	98.52	7.17	87.58	2.167	-2.000	-4.300
		2.417	4.937	5.146	92.23	16.58	89.10	-5.333	-5.083	11.167
	Glycyrrhizic acid	0.017	2.601	14.864	94.10	4.32	89.35	-2.000	-11.083	-7.083
		0.195	7.179	4.628	88.20	3.27	97.18	8.000	-1.333	-3.133
		1.954	6.277	2.781	83.94	1.89	92.98	-4.842	-3.950	0.625

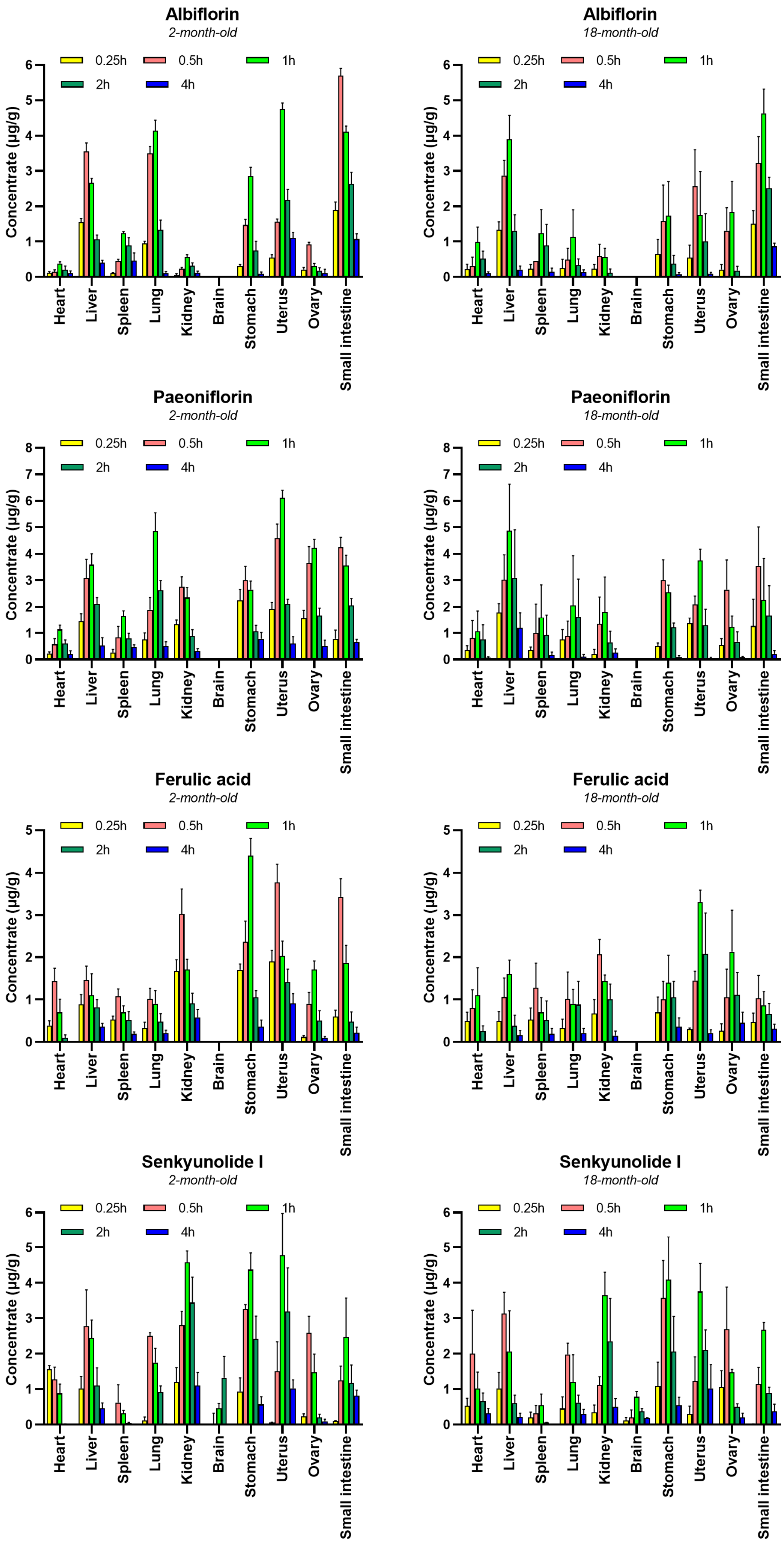
Biological samples	Analytes	Concentration (μg/mL)	Intra-day precision RSD (%)	Inter-day precision RSD (%)	Extraction recovery (%)	Extraction recovery RSD (%)	Matrix effect (%)	Stability		
								Auto-sampler (4°C, 12h) RE (%)	Long-term (-20°C, 30 d) RE (%)	Freeze-thaw (-20°C -room temperature) RE (%)
stomach	Saikosaponin a	0.057	1.123	1.119	93.66	9.35	91.34	-4.500	-5.489	-4.167
		0.577	1.671	2.749	88.75	5.32	89.20	-3.972	-6.311	-3.878
		6.004	3.073	2.597	87.51	2.78	88.63	-0.739	-1.028	-0.301
	Albiflorin	0.053	8.562	3.704	93.64	5.40	96.98	-4.444	-6.389	-8.611
		0.582	4.649	3.773	94.67	5.83	90.27	-2.944	-4.833	2.389
		5.677	1.940	6.311	88.64	1.23	88.03	-6.839	-4.989	-9.758
	Paeoniflorin	0.049	6.516	9.914	99.06	6.13	95.73	3.667	-2.000	11.000
		0.482	0.951	0.776	102.31	1.28	92.80	-3.133	-3.967	-3.200
		4.527	0.556	2.058	96.39	5.13	86.40	-7.447	-9.807	-6.807
	Ferulic acid	0.049	6.278	8.290	98.69	2.34	101.02	0.333	-8.333	10.667
		0.477	2.128	2.583	99.02	2.85	97.61	-3.900	-4.533	-0.567
		4.750	1.474	3.169	97.92	3.83	85.39	-3.800	-5.533	-2.747
	Senkyunolide I	0.050	6.928	5.000	104.59	1.53	97.25	-0.333	-2.667	14.000
		0.469	2.352	4.333	100.41	1.75	90.06	-5.400	5.733	-8.307
		4.850	2.632	1.928	104.92	11.10	85.21	-8.927	-2.493	-12.533
	Quercetin	0.216	2.084	4.997	88.75	0.93	89.42	-5.067	-9.133	-8.333
		2.416	5.092	5.132	85.96	3.22	88.32	-5.387	-11.813	-8.067
		24.407	4.485	5.795	85.47	1.95	86.41	-2.017	-0.751	-2.077
	Isoliquiritigenin	0.060	3.673	4.823	101.17	4.65	88.85	-4.889	-2.711	0.861
		0.556	10.568	4.863	97.56	5.46	88.68	-7.667	-10.806	-1.417
		5.460	8.596	4.415	90.51	6.30	85.92	-4.917	-2.444	-5.528
	Atractylenolide III	0.049	6.516	1.386	90.86	2.17	91.19	1.333	-1.667	13.000
		0.480	12.450	1.812	92.80	0.96	89.52	0.700	3.200	3.167
		4.890	4.887	3.393	92.86	3.13	93.25	-4.303	-4.743	-3.904
	Ligustilide	0.217	11.615	4.675	86.61	1.56	85.49	-5.267	-1.853	-5.093
		2.367	4.060	5.252	86.94	3.56	85.70	-10.067	-14.460	-5.759
		24.609	2.089	1.496	86.13	3.77	85.85	-2.784	-0.808	-4.278
	Atractylenolide II	0.055	8.449	1.818	95.69	6.98	99.41	-6.111	-7.194	-4.722
		0.585	4.289	2.017	100.97	1.52	94.58	-0.472	-0.278	-2.306
		5.536	9.923	4.873	101.41	5.48	105.05	-4.571	-4.019	-6.597
	Liquiritin	0.055	2.761	5.094	100.79	5.45	96.77	-5.556	-4.722	-5.833
		0.583	4.422	3.846	93.97	3.68	105.57	-2.472	-4.233	-8.278
		5.783	3.969	4.778	89.77	7.67	95.76	-4.903	-4.763	-4.009
	Liquiritigenin	0.051	5.698	3.762	100.83	8.80	90.83	5.000	-1.333	10.833
		0.499	3.194	3.512	100.50	9.44	88.79	-2.867	4.400	10.667
		4.650	13.869	1.727	92.49	4.85	87.23	-1.527	-2.540	-6.310
	Saikosaponin c	0.018	1.470	7.643	95.18	11.73	88.18	-6.917	-4.333	-5.083
		0.184	2.999	4.722	84.23	0.37	90.36	-0.250	-3.944	-1.133
		1.820	1.454	6.143	84.73	2.23	91.03	-5.250	-4.545	11.667
	Glycyrrhizic acid	0.019	6.783	12.006	85.47	1.54	91.52	-3.583	-10.500	-5.917
		0.215	10.657	5.834	86.82	2.06	95.32	6.917	-1.333	-4.217
		1.833	4.919	1.698	93.85	6.47	94.84	-4.700	-3.000	1.417
uterus	Saikosaponin a	0.057	0.203	1.480	87.35	2.91	88.20	-4.806	-5.808	-3.806
		0.577	1.513	2.916	95.72	2.22	94.11	-4.906	-5.489	-3.444
		5.963	3.698	3.382	91.86	3.52	95.47	-0.056	-1.728	-1.429
	Albiflorin	0.057	2.696	5.706	88.03	4.59	91.98	-6.667	-3.889	-6.944
		0.591	9.609	5.996	88.17	5.36	93.64	-2.222	-4.444	0.556
		5.573	0.452	2.526	91.02	9.58	97.01	-7.119	-4.756	-10.028
	Paeoniflorin	0.051	10.870	10.195	86.40	3.79	93.39	4.667	3.667	8.333
		0.485	0.546	5.259	86.06	3.31	89.06	-2.700	-2.500	-3.533
		4.597	1.811	1.673	91.96	0.01	96.19	-7.607	-8.813	-7.267
	Ferulic acid	0.050	11.641	5.057	85.39	3.49	85.59	4.000	-7.333	8.333
		0.481	4.619	2.418	84.06	1.97	86.02	-1.100	-4.833	-3.200
		4.747	5.960	2.312	88.01	1.18	92.86	-3.613	-3.933	-4.133
	Senkyunolide I	0.050	7.623	3.139	85.21	3.19	87.58	2.000	-3.333	11.667
		0.450	5.879	9.839	84.40	2.21	91.48	-11.667	10.733	-6.307
		4.920	2.344	2.840	89.16	7.74	91.64	-12.433	-0.793	-10.100
	Quercetin	0.245	12.745	6.226	86.41	3.84	86.47	-1.400	-7.400	-9.133
		2.360	5.508	2.266	88.62	1.26	90.42	-7.333	-10.627	-6.400
		24.530	3.037	3.766	93.31	1.52	89.55	-1.030	-0.377	-3.727
	Isoliquiritigenin	0.057	0.202	5.021	85.92	2.41	99.47	-5.500	0.950	0.361
		0.555	10.932	9.723	89.69	7.48	97.40	-5.250	-5.944	-3.889
		5.673	2.367	2.410	97.46	3.23	90.86	-5.722	-3.889	-5.111

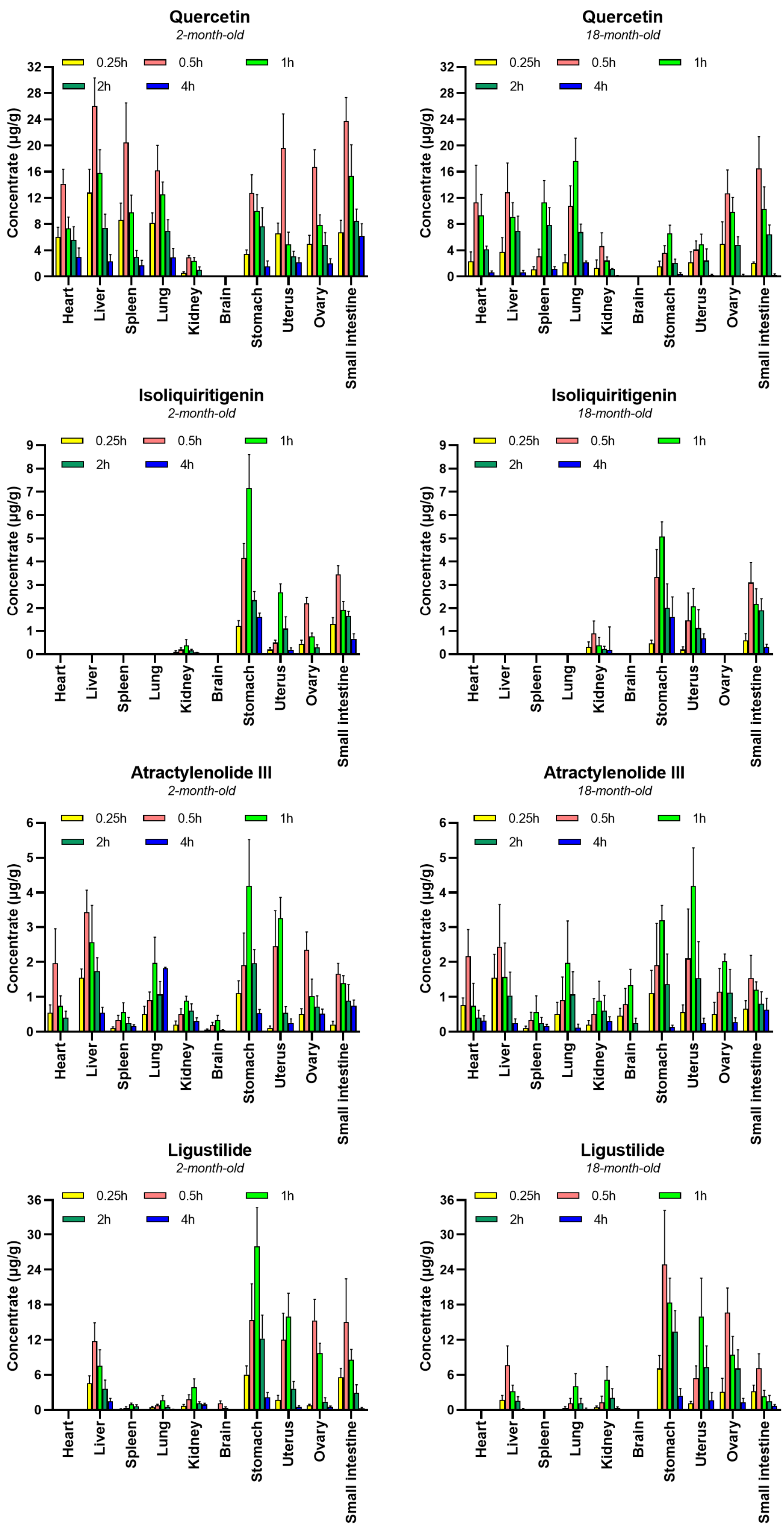
Biological samples	Analytes	Concentration (*μg*/mL)	Intra-day precision RSD (%)	Inter-day precision RSD (%)	Extraction recovery (%)	Extraction recovery RSD (%)	Matrix effect (%)	Stability: Auto-sampler (4°C, 12h) RE (%)	Stability: Long-term (-20°C, 30 d) RE (%)	Stability: Freeze-thaw (-20°C -room temperature) RE (%)
	Atractylenolide III	0.050	8.718	12.646	93.25	13.17	91.53	2.000	-1.333	13.667
		0.503	6.685	10.067	94.92	10.81	89.86	2.633	0.300	1.400
		4.767	1.474	2.343	95.85	4.35	91.81	-5.204	-3.503	-3.433
	Ligustilide	0.237	8.796	13.212	85.85	1.83	90.09	-1.760	-4.000	-8.667
		2.243	6.069	5.605	92.18	9.19	90.02	-10.507	-13.133	-6.425
		24.330	0.451	1.912	87.22	2.08	89.79	-2.736	-0.139	-4.450
	Atractylenolide II	0.056	7.174	1.025	105.05	11.70	88.05	-6.667	-7.222	-1.667
		0.599	3.721	1.522	102.10	9.82	89.36	-1.056	-0.444	-1.389
		5.811	3.510	4.595	94.81	13.24	91.15	-3.942	-2.823	-5.778
	Liquiritin	0.058	5.511	6.197	95.76	0.52	92.98	-3.333	-7.778	-5.278
		0.579	4.789	4.657	97.15	5.62	90.12	-2.417	-2.528	-8.667
		5.665	1.743	4.978	96.29	15.49	85.44	-5.269	-3.884	-4.871
	Liquiritigenin	0.054	8.501	11.136	87.23	0.08	92.49	11.167	-3.333	8.333
		0.479	5.169	10.128	89.76	5.07	97.49	-3.767	1.000	9.333
		4.977	4.824	8.833	92.33	7.50	92.62	-1.643	-4.727	-6.947
	Saikosaponin c	0.019	5.100	1.651	91.03	3.00	88.52	-6.667	-3.000	-4.917
		0.196	11.397	3.144	90.10	3.21	88.52	1.250	-2.333	-5.300
		1.867	4.461	4.354	93.04	3.49	85.55	-5.667	-4.667	15.333
	Glycyrrhizic acid	0.020	1.564	7.433	94.84	9.54	87.18	-2.500	-8.917	-7.417
		0.221	10.187	10.759	85.16	6.89	85.07	6.833	-3.000	-3.133
		1.900	1.823	2.972	85.95	6.69	84.55	-5.342	-4.617	1.050
	Saikosaponin a	0.057	3.089	1.611	95.47	11.51	95.19	-4.889	-5.033	-3.861
		0.577	1.572	4.103	88.18	3.50	93.34	-4.278	-6.719	-2.917
		6.017	3.554	5.494	90.41	2.29	86.53	-0.794	-2.639	-0.274
	Albiflorin	0.057	7.957	6.934	91.90	7.77	87.05	-3.611	-8.056	-6.667
		0.647	9.449	5.540	97.31	8.68	86.08	0.167	-6.722	-2.333
		5.420	4.954	0.800	93.64	8.74	91.98	-6.700	-5.675	-10.064
	Paeoniflorin	0.050	4.949	4.521	92.75	1.01	87.21	0.667	3.333	11.000
		0.491	3.166	5.203	95.73	4.73	88.40	-3.300	-1.267	-2.967
		4.550	2.014	1.298	95.26	5.05	93.39	-7.847	-8.707	-7.207
	Ferulic acid	0.053	13.072	6.516	90.49	5.37	86.59	-2.667	-5.667	10.667
		0.479	2.194	1.880	97.69	10.05	85.59	-5.167	-1.433	-1.267
		4.677	3.423	2.670	97.59	10.80	85.59	-5.633	-3.613	-2.180
	Senkyunolide I	0.054	13.354	6.345	90.59	4.58	96.20	-2.667	-0.333	10.667
		0.462	3.481	3.614	93.92	1.27	89.29	-5.067	2.400	-6.973
		4.717	7.515	5.541	102.92	14.49	87.58	-4.660	-3.633	-11.700
	Quercetin	0.229	2.912	3.101	86.14	4.05	89.02	-1.933	-9.333	-12.467
		2.403	4.583	3.584	86.08	3.98	87.42	-5.453	-11.653	-6.667
		24.423	2.485	2.647	88.00	1.65	86.47	-2.410	1.037	-2.803
	Isoliquiritigenin	0.059	7.397	7.046	93.91	6.11	93.75	-4.889	0.511	0.083
		0.577	13.058	5.507	92.08	4.24	98.75	-6.444	-9.806	-5.306
		5.970	5.228	8.453	91.85	2.33	99.47	-5.500	-3.278	-6.944
ovary	Atractylenolide III	0.053	7.578	6.345	90.96	2.12	91.02	-1.000	-5.000	12.333
		0.516	9.240	8.584	91.19	1.86	92.05	-0.033	0.767	1.700
		4.807	0.636	2.396	88.85	2.21	91.53	-4.103	-4.871	-3.771
	Ligustilide	0.223	11.268	9.910	88.20	7.21	85.22	-7.267	3.207	-7.093
		2.398	1.783	7.149	86.18	3.82	86.97	-8.733	-12.307	-7.212
		23.310	6.282	1.572	88.16	2.58	90.09	-2.726	-0.764	-4.428
	Atractylenolide II	0.061	1.883	9.123	89.84	5.39	96.39	-7.500	-5.833	-2.778
		0.607	2.771	2.262	94.08	9.13	91.47	0.556	-1.250	-2.417
		5.845	2.541	6.510	96.41	9.71	88.05	-3.462	-2.976	-6.919
	Liquiritin	0.059	3.508	6.216	87.91	7.83	92.67	-3.889	-5.000	-6.944
		0.534	7.224	3.649	86.43	5.31	93.33	-3.778	-3.750	-7.250
		5.656	1.860	4.725	90.77	7.80	92.98	-4.663	-6.259	-5.139
	Liquiritigenin	0.053	14.073	10.311	92.17	1.55	89.51	5.000	3.833	10.833
		0.526	12.742	7.324	90.83	2.05	91.17	-2.533	0.333	8.667
		4.897	7.598	3.933	87.49	6.48	92.49	-2.293	-4.377	-7.323
	Saikosaponin c	0.019	7.156	8.594	87.75	6.87	89.08	-8.083	-3.583	-4.583
		0.185	2.004	9.889	86.28	4.34	91.98	-3.833	-0.583	-1.583
		2.147	13.391	12.113	94.25	11.66	88.52	-6.583	-6.083	13.417
	Glycyrrhizic acid	0.020	2.786	11.300	88.39	6.60	84.24	-3.917	-7.917	-7.083
		0.205	14.890	4.249	91.70	7.75	88.01	9.417	1.667	-2.167
		1.965	6.563	5.733	88.52	7.17	87.18	-5.200	-5.008	-0.875

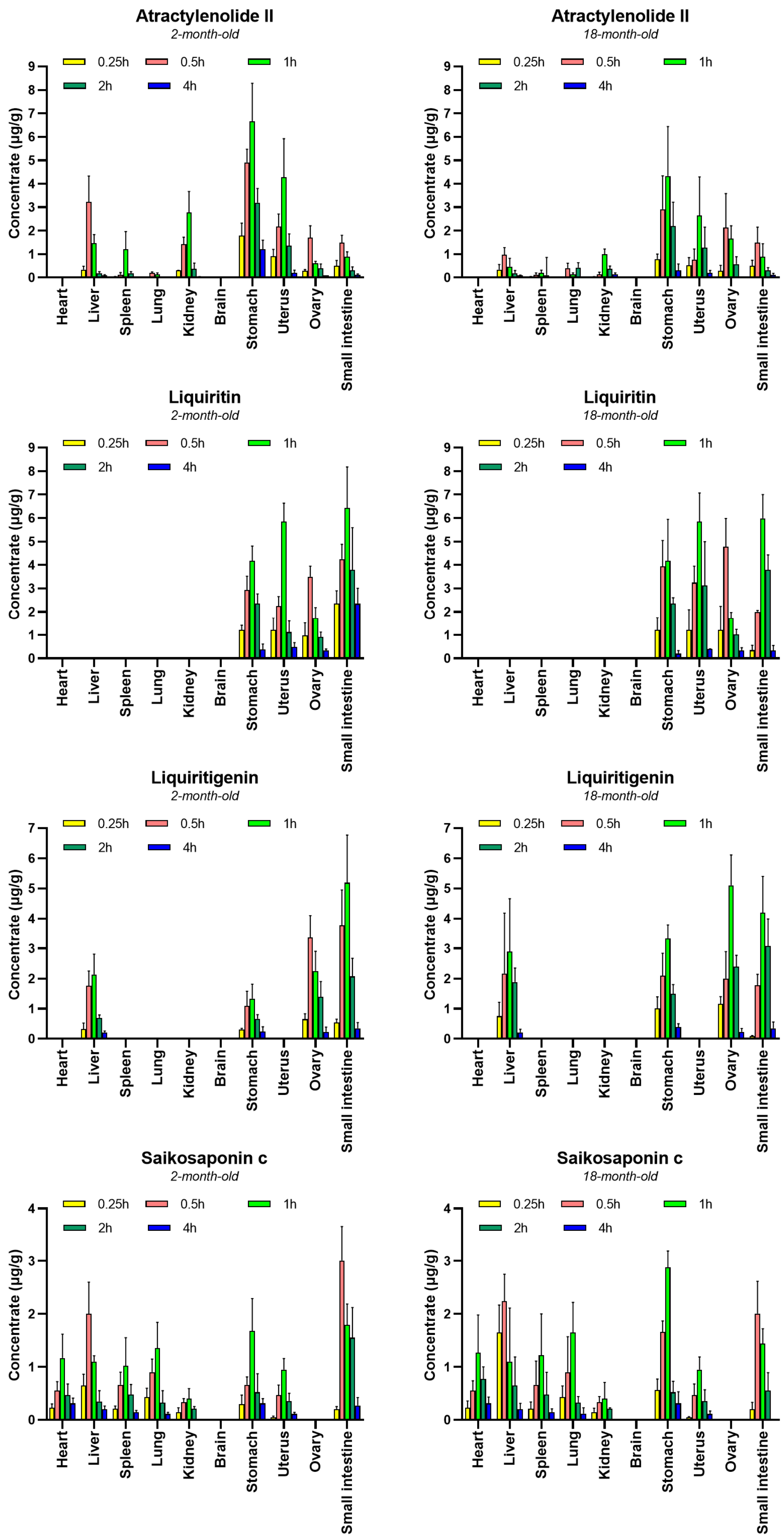
Biological samples	Analytes	Concentration (μg/mL)	Intra-day precision RSD (%)	Inter-day precision RSD (%)	Extraction recovery (%)	Extraction recovery RSD (%)	Matrix effect (%)	Stability: Auto-sampler (4°C, 12h) RE (%)	Stability: Long-term (-20°C, 30 d) RE (%)	Stability: Freeze-thaw (-20°C -room temperature) RE (%)
small intestine	Saikosaponin a	0.057	5.820	1.120	94.41	9.85	87.36	-5.778	-4.756	-3.139
		0.594	4.086	1.849	87.78	1.93	87.53	-4.711	-4.517	-3.056
		5.899	3.686	3.208	84.86	3.52	95.19	0.944	-3.183	-0.282
	Albiflorin	0.052	8.940	9.282	91.38	3.08	96.69	-4.167	-6.111	-6.389
		0.598	9.201	13.282	93.27	9.00	91.31	-0.944	-5.722	0.167
		5.470	2.011	9.144	98.36	2.66	98.76	-6.756	-5.211	-10.008
	Paeoniflorin	0.052	8.940	10.631	88.17	3.13	100.50	4.000	1.333	9.333
		0.477	1.904	1.759	91.79	9.49	98.66	-3.267	-2.933	-2.900
		4.623	1.801	2.256	95.90	5.08	98.91	-7.313	-9.740	-6.973
	Ferulic acid	0.052	8.940	10.631	94.95	2.24	92.18	1.000	-7.000	9.000
		0.488	4.000	2.560	99.25	7.99	98.59	-3.867	-4.467	-0.800
		4.760	5.916	2.984	97.61	7.08	100.10	-4.333	-4.800	-3.047
	Senkyunolide I	0.052	8.940	10.631	96.75	5.43	102.28	-0.667	-3.667	12.333
		0.457	6.690	13.282	96.05	2.87	104.59	-6.733	6.567	-7.573
		4.533	4.660	1.792	98.95	5.32	101.53	-8.760	-2.527	-10.267
	Quercetin	0.243	14.092	9.126	91.02	6.90	87.28	-1.133	-7.533	-7.867
		2.293	8.940	2.523	87.78	1.62	88.27	-6.920	-11.280	-7.867
		24.013	7.070	9.097	91.56	1.52	89.45	-1.423	-1.911	-2.250
	Isoliquiritigenin	0.055	2.592	5.712	96.05	3.79	102.97	-4.944	-1.300	-1.583
		0.547	12.817	13.511	92.64	4.91	98.17	-4.333	-9.528	-3.750
		5.713	11.838	4.473	94.64	5.85	98.46	-5.611	-3.056	-5.019
	Atractylenolide III	0.052	8.940	9.758	90.16	3.17	86.31	1.667	0.000	13.333
		0.503	4.663	10.130	87.69	4.22	88.53	2.167	0.267	0.900
		4.787	0.638	1.861	91.31	2.38	91.24	-4.570	-4.510	-4.937
	Ligustilide	0.247	10.202	12.421	97.42	12.21	88.42	-4.600	-3.853	-6.427
		2.330	4.709	4.713	86.56	3.75	88.32	-10.467	-13.727	-5.600
		24.525	3.859	8.115	88.79	4.96	88.56	-2.638	-0.235	-5.778
	Atractylenolide II	0.063	9.619	2.664	97.69	5.40	89.79	-8.333	-7.194	0.000
		0.603	4.321	4.276	97.38	4.54	91.42	-0.556	-0.472	-1.139
		5.814	3.530	4.779	96.04	1.54	97.24	-4.221	-2.563	-3.958
	Liquiritin	0.058	4.562	7.018	95.87	13.49	94.31	-4.167	-5.556	-4.722
		0.051	12.956	9.206	93.33	11.55	101.14	-2.417	-4.372	-6.417
		5.602	3.796	6.473	97.69	16.40	100.94	-4.356	-4.529	-5.837
	Liquiritigenin	0.052	8.940	3.297	90.37	0.55	93.34	7.667	-3.667	9.167
		0.477	7.368	13.004	89.75	3.02	93.16	-4.100	2.067	9.667
		4.702	5.656	8.194	91.67	0.96	101.00	-3.127	-4.240	-6.423
	Saikosaponin c	0.019	3.723	4.684	99.54	10.04	93.94	-6.583	-3.917	-5.500
		0.184	3.547	10.368	94.41	11.94	98.52	-1.167	-4.278	-1.883
		2.260	9.459	4.245	90.70	1.66	98.15	-5.583	-4.128	11.083
	Glycyrrhizic acid	0.019	5.010	12.523	85.39	3.41	94.23	-4.083	-8.333	-5.500
		0.193	7.898	4.882	90.88	9.16	88.20	5.750	-3.000	-2.050
		2.012	7.622	5.699	94.59	10.22	90.96	-5.200	-3.667	0.483
	Saikosaponin a	0.057	3.971	0.596	93.33	8.24	91.57	-5.194	-5.353	-3.861
		0.577	1.707	3.958	91.99	9.02	88.75	-5.211	-5.897	-2.944
		6.096	4.865	3.318	91.56	3.61	90.58	-0.111	-3.339	-0.985

3.2. Tissue Distribution Study

The analytes were widely distributed in various tissues investigated after oral administration of 4 g/kg of XYP. The concentrations of the 14 analytes in 2-month-old and 18-month-old SD rats' tissues were shown in Figure 2, comparatively. The tissue distribution profile of compounds in certain target organs could be used as an evidence to explain why herbs can achieve certain effects.


Albiflorin
2-month-old
Albiflorin
18-month-old
Paeoniflorin
2-month-old
Paeoniflorin
18-month-old
Ferulic acid
2-month-old
Ferulic acid
18-month-old
Senkyunolide I
2-month-old
Senkyunolide I
18-month-old
0.25h
0.5h
1h
2h
4h
Concentrate (μg/g)
Heart
Liver
Spleen
Lung
Kidney
Brain
Stomach
Uterus
Ovary
Small intestine


Quercetin
2-month-old
Quercetin
18-month-old
Isoliquiritigenin
2-month-old
Isoliquiritigenin
18-month-old
Atractylenolide III
2-month-old
Atractylenolide III
18-month-old
Ligustilide
2-month-old
Ligustilide
18-month-old
0.25h
0.5h
1h
2h
4h
Concentrate (μg/g)
Heart
Liver
Spleen
Lung
Kidney
Brain
Stomach
Uterus
Ovary
Small intestine


Atractylenolide II
2-month-old
Atractylenolide II
18-month-old
Liquiritin
2-month-old
Liquiritin
18-month-old
Liquiritigenin
2-month-old
Liquiritigenin
18-month-old
Saikosaponin c
2-month-old
Saikosaponin c
18-month-old
0.25h
0.5h
1h
2h
4h
Concentrate (μg/g)
Heart
Liver
Spleen
Lung
Kidney
Brain
Stomach
Uterus
Ovary
Small intestine

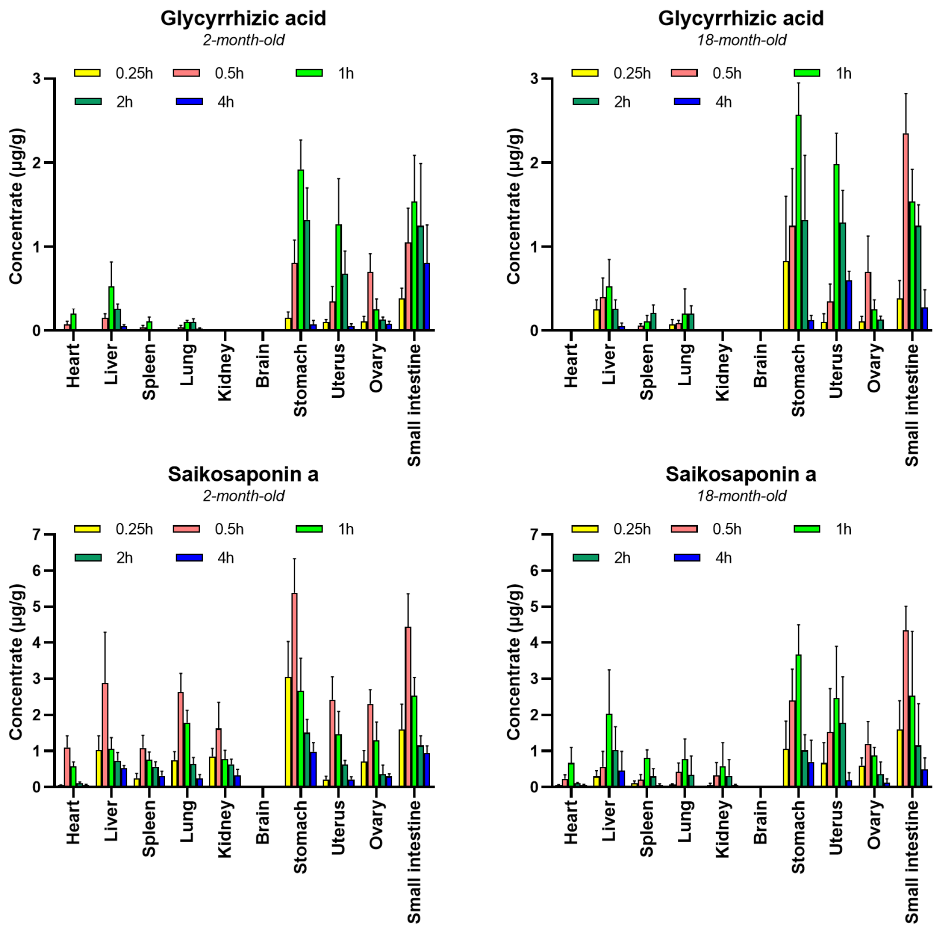


Figure 2. *Concentrations of 14 analytes in rat tissues at 0.25, 0.5, 1, 2 and 4 h after oral administration of XYP in 2-month-old rats (left) and 18-month-old rats (right).*

Albiflorin and paeoniflorin (monoterpene glycosides) are the effective ingredients of *Radix Paeoniae Alba* which was commonly used in China to regulate the liver and treat irregular menstruation. After oral administration of XYP, albiflorin and paeoniflorin showed similar wide distribution into rats' tissues except brain. Albiflorin and paeoniflorin were detected in the small intestine, liver, lung, uterus, and the stomach in both 2-month-old and 18-month-old rats. XYP is often used clinically to treat depression, hepatitis and irregular menstruation, indicating that albiflorin and paeoniflorin and could be the main components of XYP's pharmacological effects.

The peak concentration of ferulic acid was observed at 1 h. The highest tissue concentration of ferulic acid was detected in the stomach, followed by the uterus, the small intestine, the ovaries and the kidney in 2-month-old rats and 18-month-old rats. The biodistrubiton of ferulic acid showed similar results in the two groups. The only difference was that the concentrations of ferulic acid in old rats' stomach and small intestine were slightly lower young rats'. Ferulic acid showed highest concentration (C_{max} 632.47±244.04 ng/ml) in serum after oral administration [25], but lower concentration in tissues than other ingredients. Ferulic acid, as the effective ingredients of *Radix Angelicae Sinensis*, could reduce the liver injury induced by CCl_4, which was the same as the pharmacological effects of *Radix Angelicae Sinensis* and XYP in protecting the liver, indicating that ferulic acid is one of the main components of XYP.

Liquiritin, liquiritigenin and isoliquiritigenin originated from *Radix Glycyrrhizae* consistently distributed in uterus, ovary and small intestine. Compared with 2-month-old rats, higher concentrations of liquiritin and liquiritigenin in the ovary and higher liquiritigenin in liver were detected in 18-month-old rats. As Isoliquiritigenin and liquiritigenin are isomers, structural similarity led to their similar distribution profile *in vivo*. Another ingredient from *Radix Glycyrrhizae* is Glycyrrhizic acid which was detected in uterus and ovary except digestive organs.

Quercetin was the most abundant component in all of the tissues. The concentration of quercetin in these tissues maintained a high level in various periods. The peak concentration of quercetin was observed at 0.5 h in 2-month-old and 18-month-old rats. The highest tissue concentration of quercetin was detected in the liver, followed by the small intestine, the spleen, the uterus and the ovaries in 2-month-old rats. There are many pharmacological studies of quercetin on protecting liver, heart and lung [26-29]. The fact that quercetin distributed in the liver supported that quercetin could prevent hepatic fibrosis [26]. We also detected quercetin in heart and lung which indicated that quercetin was the active consituents of XYP on central hemodynamics, myocardial ischemia and redox-balance in pulmonary fibrosis [28, 29].

As lactones, senkyunolide I, atractylenolide III and ligustilide were all detected in brain in both 2-month-old and 18-month-old rats. This results indicated that the lactones with smaller molecular size could cross the blood-brain barrier. Senkyunolide I, atractylenolide III and ligustilide were presumed as the effective ingredients of XYP on brain diseased, such as Alzheimer's disease and glioma.

Atractylenolide III and atractylenolide II are representive constituents of *Rhizoma Atractylodis Macrocephalae* with similar chemical structure. However, atractylenolide II showed lower concentrations in various tissues and wasn't detected in brain. This might be caused by the lower concentration of atractylenolide II (1.01 mg/g) than atractylenolide III (1.42 mg/g) in XYP.

Radix Bupleuri is a principal herb in this formula for liver disorder treatment, and saikosaponin a and saikosaponin c from this herb were proved to be the effective ingredients [9, 10]. Saikosaponin a and saikosaponin c were widely distributed into the rats but brain. The peak concentration of saikosaponin a and saikosaponin c appeared at 0.5 h in 2-month-old rats, while 1 h in 18-month-old rats. This phenomenon proved that poor absorption rate of saikosaponins existed in old rats.

This is the first study to examine the tissue distribution profile of multiple bioactive components after oral administration of XYP. After the comparative biodistrubition study of XYP in young and old rats, the concentration levels of the 14 target compounds in multi tissues in 18-month-old rats were commonly lower than those in 2-month-old rats. This conclusion can be explained by the worse absorption of old rats. The tissue biodistribution regulation the 14 analytes in young and old rats almost stayed identically. The results of the tissue distribution study showed that the 14 interest analytes of XYP were distributed mainly into the gastrointestinal tract, uterus, ovary and liver, which provided the material basis for its pharmacological actions in clinical application.

4. Conclusion and Recommendation

The present study established an UPLC-MS/MS method for 14 analytes in the rats tissues, and was successfully applied for XYP after oral administration. The results showed that 14 representative components of XYP were mainly concentrated in gastrointestinal tract, uterus, ovary and liver, which was consistent with the pharmacological activities of XYP. This study could promote understanding the distribution of XYP in vivo and provide a basis for finding target organs, new targeting mechanisms and new pathways of action, and also provide the material basis for the dose regimen clinically. Future tissue distribution study can also focus on the treatment of the disease and conduct in-depth research.

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