applied sciences

MDPI

Article

Simultaneous Determination of 12 Marker Components in Yeonkyopaedok-san Using HPLC–PDA and LC–MS/MS

Chang-Seob Seo and Hyeun-Kyoo Shin *

Herbal Medicine Research Division, Korea Institute of Oriental Medicine, Daejeon 34054, Korea; csseo0914@kiom.re.kr

* Correspondence: hkshin@kiom.re.kr; Tel.: +82-42-868-9464

Received: 5 February 2020; Accepted: 26 February 2020; Published: 2 March 2020

Abstract: Yeonkyopaedok-san is a traditional Korean medicine used in the early treatment of boils. In the present study, its 12 marker components for quality control were determined using high-performance liquid chromatography (HPLC) with photodiode array detection and ultra-performance liquid chromatography–mass spectrometry with tandem mass spectrometry (UPLC–MS/MS). The investigated 12 marker components of Yeonkyopaedok-san were as follows: 3-caffeoylquinic acid, cimifugin 7-glucoside, liquiritin apioside, ferulic acid, narirutin, 5-*O*-methylvisammioside, naringin, neohesperidin, oxypeucedanin hydrate, arctigenin, glycyrrhizic acid, and 6-gingerol. The analytical column used for the separation of the 12 marker analytes in Yeonkyopaedok-san was a Waters SunFire C18 column (4.6 mm × 250 mm, 5 μm). The two mobile phases used were 0.1% (*v*/*v*) aqueous formic acid and 0.1% (*v*/*v*) formic acid in acetonitrile. In the UPLC–MS/MS analysis, all components were separated using a Waters ACQUITY UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 μm). The two mobile phases used were 0.1% (*v*/*v*) aqueous formic acid and acetonitrile. The coefficient of determination of the calibration curves in both analysis systems showed good linearity (>0.99). The amounts of the 12 marker components in Yeonkyopaedok-san determined using HPLC–photodiode array detection and UPLC–MS/MS analyses were found to be 0.14–9.00 mg/g and 2.35–853.11 μg/g, respectively.

Keywords: HPLC–PDA; LC–MS/MS; simultaneous determination; traditional herbal formula; Yeonkyopaedok-san

1. Introduction

Traditional Korean medicine (TKM), traditional Chinese medicine (TCM), and Kampo medicine (KM) generally use chemically active complexes composed of two or more medicinal herbs. They ave been used for thousands of years for the prevention or treatment of various diseases. However, scientific evidence pertaining to the safety and standardization of TKM, TCM, and KM is lacking; this information is now required. Yeonkyopaedok-san (YPS), also known as Lian Qiao Bai Du San in Chinese, is a TKM. It is made up of extracts from the following 14 herbs: Forsythiae Fructus, Lonicerae Flos, Schizonepetae Spica, Saposhnikoviae Radix, Osterici seu Notopterygii Radix et Rhizoma, Araliae Continentalis Radix, Bupleuri Radix, Cnidii Rhizoma, Aurantii Fructus Immaturus, Platycodonis Radix, Poria Sclerotium, Glycyrrhizae Radix et Rhizoma, Menthae Herba, and Zingiberis Rhizoma Recens [1].

As reported in the Dongeuibogam (representative source of the Korean medicine), YPS has been used in the initial treatment of boils. It has anti-inflammatory effects (i.e., for treatment of colds, allergies, and dermatitis) and antiatherogenic effects [1–3]. Recently, Chen et al. [4,5] reported a

high-performance liquid chromatography (HPLC)–diode array detection and micellar electrokinetic chromatography assays for quality control of Lianqiao Baidu pills (LBP). In China, LBP is used for the treatment of diseases similar to those treated by YPS in Korea [4,5]. However, LBP is a completely different herbal formulation, consisting of 19 herbal medicines [6]. Although some studies on the efficacy of the TKM and YPS have been reported, there are no documented standardization studies which include a quality evaluation.

The chemical composition of the main 14 raw materials constituting YPS is as follows: lignans (e.g., arctigenin (ARC) and pinoresinol) from Forsythiae Fructus [7,8], phenylpropanoids (e.g., chlorogenic acid) from Lonicerae Flos [9], monoterpenoids (e.g., pulegone) from Schizonepetae Spica [10], flavonoids (e.g., cimifugin) and chromones (e.g., cimifugin 7-glucoside (CFG) and 5-*O*-methylvisammioside (5-MVS)) from Saposhnikoviae Radix [11], coumarins (e.g., oxypeucedanin hydrate (OPDH) and imperatorin) from Osterici seu Notopterygii Radix et Rhizoma [12], diterpenoids (e.g., kaurenoic acid and continentalic acid) from Araliae Continentalis Radix [13], triterpenoid saponins (e.g., saikosaponin A) from Bupleuri Radix [14], phenylpropanoids (e.g., ferulic acid (FA)) from Cnidii Rhizoma [15], flavonoids (e.g., naringin (NAG)) and flavonoid glucosides (e.g., NAG, and neohesperidin (NHES)) from Aurantii Fructus Immaturus [16], triterpenoid saponins (e.g., platycodin D and platycodin D2) from Platycodonis Radix [17], triterpenoids (e.g., polyporenic acid C and pachymic acid) from Poria Sclerotium [18], triterpenoid saponins (e.g., glycyrrhizinic acid) and flavonoids (e.g., liquiritin apioside) from Glycyrrhizae Radix et Rhizoma [19], monoterpenoids (e.g., menthone and menthol) from Menthae Herba [20], and phenols (e.g., 6-, 8, and 10-gingerol) from Zingiberis Rhizoma Recens [21].

In this study, a simultaneous analysis of the 12 marker compounds for a quality assessment of YPS was carried out using HPLC–photodiode array detection (PDA) and an ultra-performance liquid chromatography–mass spectrometry with tandem mass spectrometry (UPLC–MS/MS). The investigated 12 marker compounds were as follows: 3-caffeoylquinic acid (3-CQA, Lonicerae Flos); CFG and 5-MVS (Saposhnikoviae Radix); liquiritin apioside (LIQA) and glycyrrhizic acid (GA) (Glycyrrhizae Radix et Rhizoma); FA (Cnidii Rhizoma); narirutin (NAR), NAG, and NHES (Aurantii Fructus Immaturus); OPDH (Osterici seu Notopterygii Radix et Rhizoma); ARC (Forsythiae Fructus); and 6-gingerol (6-GIN, Zingiberis Rhizoma Recens).

2. Materials and Methods

2.1. Plant Materials

Table S1 lists the 14 crude plant materials constituting YPS. They were purchased from Kwangmyungdang Medicinal Herbs (KMH; Ulsan, Korea) in November 2017. Dr. Seung-Yeol Oh, president of KMH, confirmed the origin of each ingredient based on "The Dispensatory on the Visual and Organoleptic Examination of Herbal Medicine" [22]. The voucher specimen (2017KE59–1~2017KE59–15) was deposited at the Herbal Medicine Research Division, Korea Institute of Oriental Medicine.

2.2. Chemicals and Reagents

The reference standard compounds were obtained from the following suppliers: 3-CQA (PubChem CID: 1794427, 99.6%), Acros Organics (Pittsburgh, PA, USA); CFG (PubChem CID: 14034912, 99.4%), ChemNorm Biotech Co., Ltd. (Wuhan, China); LIQA (PubChem CID: 10076238, ≥98.0%), NHES (PubChem CID: 232990, 98.4%), and ARC (PubChem CID: 64981, 99.4%), Shanghai Sunny Biotech (Shanghai, China); FA (PubChem CID: 445858, 98.0%), GA (PubChem CID: 14982, ≥99.0%), and 6-GIN (PubChem CID: 442793, 99.3%), Wako Chemicals (Osaka, Japan); NAR (PubChem CID: 442431, 99.5%) and 5-MVS (PubChem CID: 21670038, 99.9%), Biopurify Phytochemicals (Chengdu, China); NAG (PubChem CID: 442428, 95.0%), Merck KGaA (Darmstadt, Germany); and OPDH (PubChem CID: 17536, 98.0%), ChemFaces Biochemical Co., Ltd. (Wuhan, China). The chemical structures of these compounds are shown in Figure S1.

The HPLC-grade solvents like methanol, acetonitrile, and water, required for preparation of test solution and quantitative analysis, were purchased from JT Baker (Phillipsburg, NJ, USA). Formic acid (ACS reagent-grade, 98.0–100.0%) was purchased from Merck KGaA.

2.3. Preparation of the YPS Water Decoction

For the preparation of the YPS water decoction, 14 herbal medicines (Forsythiae Fructus, Lonicerae Flos, Schizonepetae Spica, Saposhnikoviae Radix, Osterici seu Notopterygii Radix et Rhizoma, Araliae Continentalis Radix, Bupleuri Radix, Cnidii Rhizoma, Aurantii Fructus Immaturus, Platycodonis Radix, Poria Sclerotium, Glycyrrhizae Radix et Rhizoma, Menthae Herba (each 368.5 g), and Zingiberis Rhizoma Recens (210.1 g)) were mixed, followed by the addition of distilled water (50 L). Then, using an electric extractor (COSMOS-660; Kyungseo Machine Co., Incheon, Korea), extraction was carried out for 2 h at 100 °C under pressure (98 kPa). Pretreatment of the extract was performed according to a previously reported method [23]. The extract was then filtered and lyophilized to obtain a freeze-dried powder sample (1150.0 g, 23.0%).

2.4. Preparations of Sample and Standard Solutions for HPLC Analysis

A sample solution for the simultaneous quantitative analysis of the 12 marker components (3-CQA, CFG, LIQA, FA, NAR, 5-MVS, NAG, NHES, OPDH, ARC, GA, and 6-GIN) was prepared as follows: 100 mg of lyophilized YPS sample was added to 10 mL of 70% methanol and then extracted for 60 min at room temperature using a Branson 8510 ultrasonicator (Denbury, CT, USA). The extracted solution was filtered through a 0.2 μm syringe filter (Pall Life Sciences, Ann Arbor, MI, USA) before sample injection for HPLC analysis.

A standard solution of each reference standard compound was prepared at a concentration of 1000 μg/mL using methanol or methanol:DMSO (1:1) solution. Prepared stock solutions were stored in a refrigerator (4 °C) until required for use.

2.5. HPLC Apparatus and Conditions for Simultaneous Analysis of the 12 Marker Components

The analytical method used for YPS analysis was a modification of the HPLC method used in earlier research [24]. Briefly, a HPLC system for simultaneous analysis was used, i.e., a Shimadzu Prominence LC-20A series HPLC system (Kyoto, Japan) equipped with PDA detector and the LC Solution software (Version 1.24, SP1) for data analysis and system control. Other analytical conditions are given in Table S2.

2.6. Validation of the HPLC Analytical Method

The optimized assay using HPLC was tested and validated with respect to parameters such as linearity, range, limit of detection (LOD), limit of quantification (LOQ), accuracy (recovery), and precision, according to the International Conference on Harmonisation guidance for Q2B Validation of Analytical Procedures [25]. Briefly, linearity was evaluated by the coefficient of determination (r^2), whereas the LOD and LOQ were calculated using the respective equations LOD = $3.3 \times \sigma/S$ and LOQ = $10 \times \sigma/S$ (where σ is the standard deviation of the y-intercept and S is the slope of the calibration curve). Accuracy was assessed by the recovery test and calculated using Equation (1).

$$\text{Recovery } (\%) = \frac{(\text{found amount} - \text{original amount})}{\text{spiked amound}} \times 100 \quad (1)$$

Precision was assessed by the relative standard deviation (RSD) values throughout the intra- and inter- day precision and repeatability.

$$\text{RSD } (\%) = \frac{\text{standard deviation (SD)}}{\text{mean}} \times 100 \quad (2)$$

The system suitability of the assay was evaluated using the parameters such as capacity factor (k'), selectivity factor (α), resolution (Rs), number of theoretical plates (N), and tailing factor (Tf) [26].

2.7. UPLC–MS/MS Apparatus and Conditions for Quantification of the 12 Marker Components

The apparatus used in the UPLC–MS/MS system for the quantification of the 12 marker components in YPS included an ACQUITY UPLC H-Class system (Waters, Milford, MA, USA) with a Xevo TQ-S micro triple quadrupole mass spectrometer (Waters) equipped with an electrospray ionization (ESI) interface. The MassLynx software (version 4.2, Waters) was used to carry out the data acquisition and processing. The analytical column used for chromatographic separation was a Waters ACQUITY UPLC BEH C_{18} column (2.1 mm × 100 mm, 1.7 μm). Two mobile phases were used: 0.1% (*v*/*v*) aqueous formic acid (solvent A) and acetonitrile (solvent B). The mobile phase was eluted at 0.3 mL/min. The gradient conditions used were as follows: 10% solvent B at initial to 1.0 min, 10–40% solvent B at 1.0 to 6.0 min, 40–95% solvent B at 6.0 to 10.0 min, 95% solvent B at 10.0 to 15.0 min, 95–10% solvent B at 15.0 to 15.1 min, and 10% solvent B at 15.1–18.0 min. The volume of autosampler injection for the quantification of all analytes was 2 μL, and the temperature of the column and sample tray were maintained at 40 °C and 5 °C, respectively. The 12 marker components in a MS system were detected in positive and negative ion mode using an ESI interface. Quantification was conducted using the multiple reaction monitoring (MRM) mode. Capillary voltage, source temperature, desolvation temperature, desolvation gas flow, and cone gas flow were set at 1.2 kV, 150 °C, 450 °C, 800 L/h, and 50 L/h, respectively. Apart from these common MS parameters, other optimized analytical parameters, such as collision energy, cone voltage, and the transition required for the analysis of each analyte are displayed in Table S3.

3. Results and Discussion

3.1. Optimization of HPLC Chromatographic Conditions

The 12 marker components in YPS decoction (3-CQA, CFG, LIQA, FA, NAR, 5-MVS, NAG, NHES, OPDH, ARC, GA, and 6-GIN) were efficiently set to the separation conditions using a SunFire C_{18} column (250 mm × 4.6 mm, 5 μm) maintained at 40 °C and a mobile phase system of distilled water–acetonitrile and formic acid. Using the established assay, all analytes were separated within 50 min, with Rs values ≥4.16 (Figure 1).

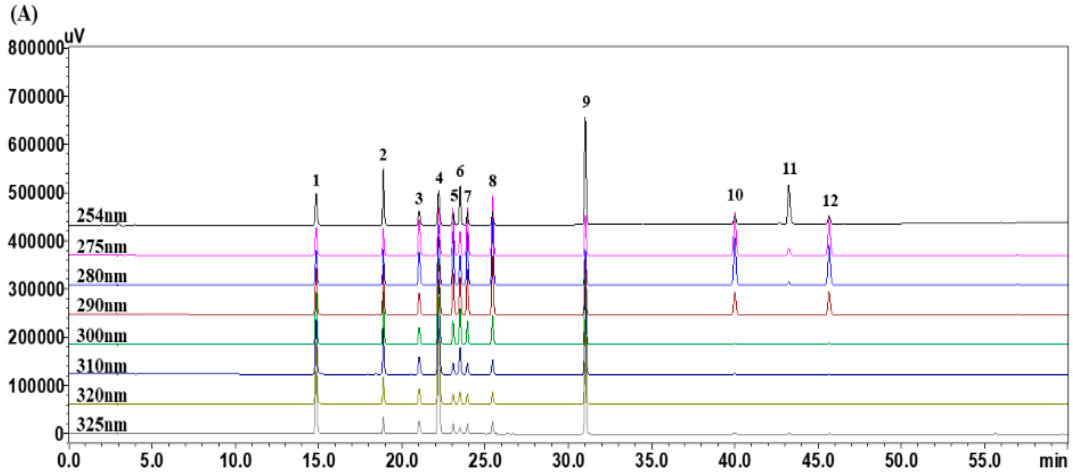

Figure 1. *Cont.*

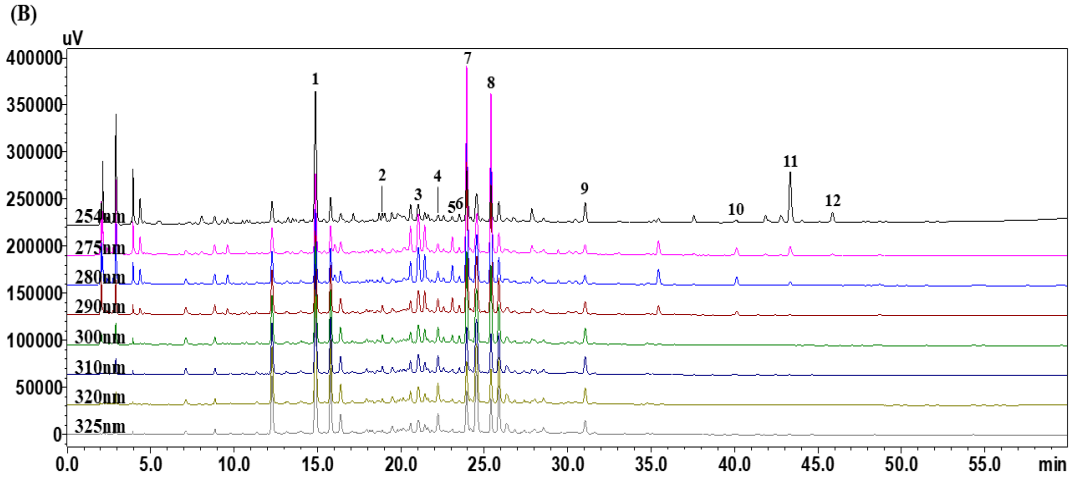

Figure 1. HPLC chromatograms of the standard solutions (**A**) and YPS sample (**B**). 3-CQA (**1**), CFG (**2**), LIQA (**3**), FA (**4**), NAR (**5**), 5-MVS (**6**), NAG (**7**), NHES (**8**), OPDH (**9**), ARC (**10**), GA (**11**), and 6-GIN (**12**).

3.2. Method Validation for Quantitative Analysis by HPLC

In the established HPLC analytical method, the values of the system suitability parameters for the 12 marker components, namely, *k*′, *α*, *N*, *Rs*, and *Tf* were 4.02–14.44, 1.02–1.34, 2.7624792 × 10^5–1.36925323 × 10^6, 4.16–50.58, and 1.09–1.29, respectively (Table S4). The calibration curve of each analyte was determined by plotting the peak area (y) versus the corresponding concentration (x) using the standard solution, in the different concentration levels (Table 1). The linearity of these components was ≥0.99. The results showed good linearity. The LOD and LOQ ranges were calculated to be 0.05–0.20 μg/mL and 0.16–0.60 μg/mL, respectively (Table 1). The accuracy (recovery) test of the marker components was conducted using the standard addition method, and the recoveries averaged 92.30–101.30% with the RSD (%) values of <3.00 (Table 2). Furthermore, the RSD (%) values of repeatability and intra- and inter-day precisions showed good precision within 4.79% (Table 3). These results demonstrated that the developed HPLC assay is suitable for the simultaneous analysis of the 12 marker components in YPS.

Table 1. Linear range, regression equation, r^2, LODs, and LOQs for marker compounds using HPLC–PDA (n = 3).

Compound	Linear Range (μg/mL)	Regression Equation [a] y=ax+b	r^2	LOD (μg/mL)	LOQ (μg/mL)
3-CQA	1.56–100.00	$y = 3.168268 \times 10^4x - 2.279203 \times 10^4$	0.9986	0.19	0.57
CFG	0.78–50.00	$y = 1.589998 \times 10^4x - 3.69893 \times 10^4$	0.9993	0.13	0.41
LIQA	1.56–100.00	$y = 1.435196 \times 10^4x - 6.7471 \times 10^3$	0.9991	0.16	0.49
FA	0.78–50.00	$y = 7.557159 \times 10^4x - 1766964 \times 10^4$	0.9991	0.09	0.26
NAR	0.78–50.00	$y = 1.698577 \times 10^4x - 4.07642 \times 10^3$	0.9990	0.05	0.16
5-MVS	0.78–50.00	$y = 1.850794 \times 10^4x - 4.67958 \times 10^3$	0.9990	0.11	0.32
NAG	2.34–150.00	$y = 1.779584 \times 10^4x - 1.182341 \times 10^4$	0.9991	0.09	0.27
NHES	1.56–100.00	$y = 2.215239 \times 10^4x - 1.007198 \times 10^4$	0.9991	0.10	0.31
OPDH	0.78–50.00	$y = 2.949819 \times 10^4x - 6.27553 \times 10^3$	0.9994	0.15	0.47
ARC	0.78–50.00	$y = 1.019838 \times 10^4x - 2.73577 \times 10^3$	0.9989	0.16	0.49
GA	1.56–100.00	$y = 8.69885 \times 10^3x - 3.5352 \times 10^2$	0.9993	0.20	0.60
6-GIN	0.78–50.00	$y = 8.57485 \times 10^3x - 2.32865 \times 10^3$	0.9990	0.06	0.18

[a] y: peak area (mAU) of compounds; x: concentration (μg/mL) of compounds.

Table 2. Recovery test for the assay of 12 components in YPS using HPLC–PDA.

Compound	Original Amount (μg/mL)	Spiked Amount (μg/mL)	Found Amount (μg/mL)	Recovery (%)	SD	RSD (%)
3-CQA	44.98	8.00	52.65	95.87	1.44	1.51
		20.00	64.02	95.16	2.85	2.99
		40.00	82.68	94.24	0.56	0.60
CFG	5.55	1.00	6.50	95.28	2.35	2.47
		2.00	7.52	98.62	2.00	2.02
		4.00	9.49	98.61	1.37	1.39
LIQA	30.28	6.00	36.05	96.14	1.59	1.65
		15.00	44.73	96.33	1.61	1.67
		30.00	59.70	98.05	0.91	0.93
FA	3.38	1.00	4.36	98.22	1.77	1.80
		2.00	5.28	94.85	0.68	0.72
		4.00	7.29	97.85	1.43	1.47
NAR	11.81	2.00	13.83	101.22	1.35	1.34
		5.00	16.81	100.14	1.87	1.87
		10.00	21.85	100.41	0.60	0.60
5-MVS	6.01	1.00	6.98	97.34	2.38	2.45
		2.00	7.96	97.66	1.63	1.67
		4.00	9.83	95.58	2.53	2.65
NAG	44.07	8.00	52.09	100.32	2.90	2.89
		20.00	63.74	98.39	0.95	0.97
		40.00	84.25	100.46	1.26	1.25
NHES	31.45	6.00	37.06	93.48	1.33	1.42
		15.00	45.51	93.71	0.33	0.35
		30.00	59.14	92.30	0.97	1.05
OPDH	6.35	1.00	7.31	96.39	1.42	1.47
		2.00	8.26	95.73	1.29	1.34
		4.00	10.26	97.69	0.36	0.37
ARC	9.17	2.00	11.16	99.56	2.80	2.81
		5.00	14.08	98.38	1.44	1.46
		10.00	18.80	96.38	1.04	1.08
GA	29.28	6.00	34.91	93.76	1.11	1.19
		15.00	43.30	93.41	1.05	1.13
		30.00	56.99	92.36	0.67	0.72
6-GIN	1.14	1.00	2.15	101.30	1.31	1.29
		2.00	3.12	99.26	2.44	2.45
		4.00	5.11	99.29	2.49	2.51

3.3. UPLC–MS/MS Confirmation

Table 3 shows that five compounds (3-CQA, LIQA, NAR, NAG, and GA) were detected in the negative ion mode ($[M–H]^-$) at *m/z* 353.2, 549.3, 579.3, 279.2, and 821.6, respectively, whereas seven compounds (CFG, FA, 5-MVS, NHES, OPDH, ARC, and 6-GIN) were detected in the positive ion mode ($[M+H]^+$) at *m/z* 469.3, 195.0, 453.0, 611.1, 304.9, 373.2, and 295.3, respectively. The precursor ion (Q1) and product ion (Q3) for the LC–MS/MS MRM method are shown in Table S3. Briefly, the Q3 peak of 3-CQA was detected at *m/z* 191.0 [M–H–caffeoyl]$^-$ by elimination of the caffeoyl group from the Q1 peak [27]. CFG and 5-MVW were set at the Q3 peaks at *m/z* 307.2 and 291.3 in the form of $[M+H–Glc]^+$, and these components released one glucose group at the Q1 peak [28,29]. FA and GA were detected at *m/z* 177.0 $[M+H–H_2O]^+$ and 351.2 $[M–H–2Glc]^-$ by the loss of one H_2O and two glucose groups from the Q1 peak, respectively [30,31]. LIQA was detected at *m/z* 549.3 (Q1) as $[M–H]^-$, formed by the loss of the apiosy-glucosyl function group from Q1 [32]. NAR, NAG, and NHES were apt to lose the rutinose to give aglycone ions of *m/z* 271.2 [M–H–rutinose]$^-$, 271.0 [M–H–rutinose]$^-$, and 303.2 [M+H–rutinose]$^+$, respectively [33–35]. The characteristic MS fragmentations of OPDH, ARC, and 6-GIN were observed at *m/z* $[M+H–C_5H_9O–OH]^+$, 137.0 [dimethoybenzyl group–H]$^+$, and

177.0 $[M+H–C_6H_{12}O_2]^+$; this was assigned to the loss of a C_5H_9O molecule and OH group, and $C_{13}H_{15}O_4$ and $C_6H_{12}O_2$ molecules from the Q1 peak, respectively [36–38]. Our analysis carried out using the MRM conditions gave an r^2 value of >0.99 for the calibration curve showing good linearity. Furthermore, the LOD and LOQ values of these components were calculated to be in the ranges of 0.002–1.135 µg/mL and 0.005–3.404 µg/mL, using signal-to-noise ratios (S/N) of 3.3 and 10, respectively. These results are presented in Table S5.

Table 3. Precision assay for 12 marker compounds in YPS using HPLC–PDA.

Compound	Conc. (µg/mL)	Intra-Day (n = 5)			Inter-Day (n = 5)			Repeatability (n = 6)	
		Observed Conc. (µg/mL)	Precision (%) [a]	Accuracy (%)	Observed Conc. (µg/mL)	Precision (%)	Accuracy (%)	RSD (%) of Retention Time	RSD (%) of Peak Area
3-CQA	25.00	24.68	1.03	98.73	24.95	0.98	99.79	0.23	0.35
	50.00	49.95	0.60	99.90	50.23	1.74	100.46		
	100.00	99.00	1.04	99.00	98.21	1.05	98.21		
CFG	12.50	12.23	1.19	97.83	12.14	1.53	97.12	0.06	0.33
	25.00	25.16	1.50	100.62	24.70	2.72	98.81		
	50.00	49.04	1.48	98.08	47.92	2.41	95.85		
LIQA	25.00	25.16	1.48	100.65	25.66	2.30	102.64	0.06	0.33
	50.00	49.97	1.58	99.94	50.88	2.06	101.75		
	100.00	97.95	1.20	97.95	98.67	1.44	98.67		
FA	12.50	12.46	2.52	99.71	12.25	2.28	97.99	0.06	0.36
	25.00	24.84	0.50	99.35	24.51	2.46	98.02		
	50.00	48.86	1.30	97.71	47.89	2.36	95.78		
NAR	12.50	12.24	1.28	97.95	12.25	2.60	98.00	0.04	0.34
	25.00	24.76	0.44	99.03	24.48	2.61	97.91		
	50.00	48.73	1.39	97.45	47.62	2.36	95.24		
5-MVS	12.50	12.29	1.31	98.28	12.22	1.74	97.76	0.08	0.33
	25.00	24.77	0.49	99.10	24.49	2.57	97.96		
	50.00	48.69	1.35	97.38	47.72	2.32	95.43		
NAG	37.50	36.84	1.31	98.23	36.56	1.45	97.50	0.08	0.35
	75.00	74.60	0.60	99.47	73.66	2.64	98.22		
	150.00	146.12	1.45	97.41	144.12	2.93	96.08		
NHES	25.00	24.59	1.22	98.36	24.38	1.47	97.52	0.06	0.35
	50.00	49.69	0.48	99.39	49.27	2.65	98.54		
	100.00	99.25	4.79	99.25	96.49	4.16	96.49		
OPDH	12.50	12.26	1.39	98.04	12.18	2.00	97.48	0.05	0.35
	25.00	24.75	0.51	99.02	24.48	2.60	97.94		
	50.00	48.64	1.39	97.27	47.47	2.39	94.94		
ARC	12.50	12.26	1.37	98.06	12.15	1.56	97.23	0.13	0.47
	25.00	24.71	0.43	98.83	24.40	2.61	97.59		
	50.00	48.55	1.35	97.10	47.46	2.33	94.91		
GA	25.00	24.32	1.43	97.30	24.12	1.52	96.49	0.10	0.33
	50.00	49.64	0.58	99.28	49.03	2.70	98.05		
	100.00	97.98	1.41	97.98	95.86	2.34	95.86		
6-GIN	12.50	11.85	1.42	94.81	11.74	1.51	93.90	0.07	0.07
	25.00	24.14	0.38	96.57	23.79	2.16	95.16		
	50.00	47.81	1.36	95.62	46.71	2.39	93.42		

[a] Precision is expressed as RSD (%) = (SD/mean) × 100.

3.4. Quantitative Analysis of the 12 Marker Components in YPS Samples Using HPLC–PDA

The 12 marker components (3-CQA, CFG, LIQA, FA, NAR, 5-MVS, NAG, NHES, OPDH, ARC, GA, and 6-GIN) were analyzed in the YPS samples using the developed and validated HPLC analytical method. Quantification of these components was carried out at 254 nm for GA; 275 nm for LIQA; 280 nm for NAR, NAG, NHES, ARC, and 6-GIN; 290 nm for 5-MVS; 300 nm for CFG; 310 nm for OPDH; 320 nm for FA; and 325 nm for 3-CQA, based on the λ_{max} of the ultra-violet (UV) spectra. The peak of each analyte was identified by comparing the UV spectrum and the retention time of the reference standard. The amounts of the 12 marker components in the three batches of the YPS samples were calculated using the calibration curves of each analyte. The amounts of these analytes in the samples were found to be in the range of 0.14–9.00 mg/g (Table 4).

Table 4. Amount of the 12 marker components in YPS using HPLC–PDA (n = 3).

Compound	Batch No.					
	1		2		3	
	Mean (mg/g) ± SD ($\times 10^{-1}$)	RSD (%)	Mean (mg/g) ± SD ($\times 10^{-1}$)	RSD (%)	Mean (mg/g) ± SD ($\times 10^{-1}$)	RSD (%)
3-CQA	4.28 ± 0.66	1.53	4.22 ± 0.56	1.32	4.15 ± 0.83	1.99
CFG	0.62 ± 0.05	0.80	0.61 ± 0.07	1.19	0.60 ± 0.12	2.03
LIQA	3.05 ± 0.26	0.84	2.90 ± 0.65	2.24	2.96 ± 0.39	1.31
FA	0.32 ± 0.04	1.23	0.30 ± 0.07	2.27	0.30 ± 0.08	2.74
NAR	1.30 ± 0.15	1.19	1.27 ± 0.25	1.93	1.24 ± 0.24	1.95
5-MVS	0.59 ± 0.03	0.49	0.58 ± 0.10	1.71	0.50 ± 0.15	2.97
NAG	9.00 ± 0.74	0.82	8.93 ± 0.07	0.08	8.84 ± 0.65	0.74
NHES	6.48 ± 0.42	0.65	6.39 ± 0.59	0.92	6.28 ± 0.94	1.50
OPDH	0.69 ± 0.05	0.78	0.67 ± 0.10	1.53	0.64 ± 0.17	2.64
ARC	0.94 ± 0.09	0.96	0.92 ± 0.03	0.31	0.92 ± 0.12	1.28
GA	6.02 ± 0.51	0.85	5.95 ± 0.37	0.61	5.85 ± 0.95	1.62
6-GIN	0.15 ± 0.02	1.19	0.15 ± 0.04	2.65	0.14 ± 0.02	1.16

3.5. Simultaneous Determination of the 12 Marker Components in YPS Sample Using UPLC–MS/MS MRM Mode

For a quality evaluation of YPS, quantification of the 12 marker components was carried out using the optimized UPLC–MS/MS MRM assay. The retention times (min) of the 12 marker components were as follows: 3-CQA, 1.98; CFG, 4.30; FA, 4.65; LIQA, 4.66; NAR, 5.12; 5-MVS, 5.30; NAG, 5.30; NHES, 5.61; OPDH, 6.58; GA, 8.10; ARC, 8.20; and 6-GIN, 8.94 min (Figure 2). The amounts of these analytes ranged from 2.35 to 853.11 μg/g (Table 5).

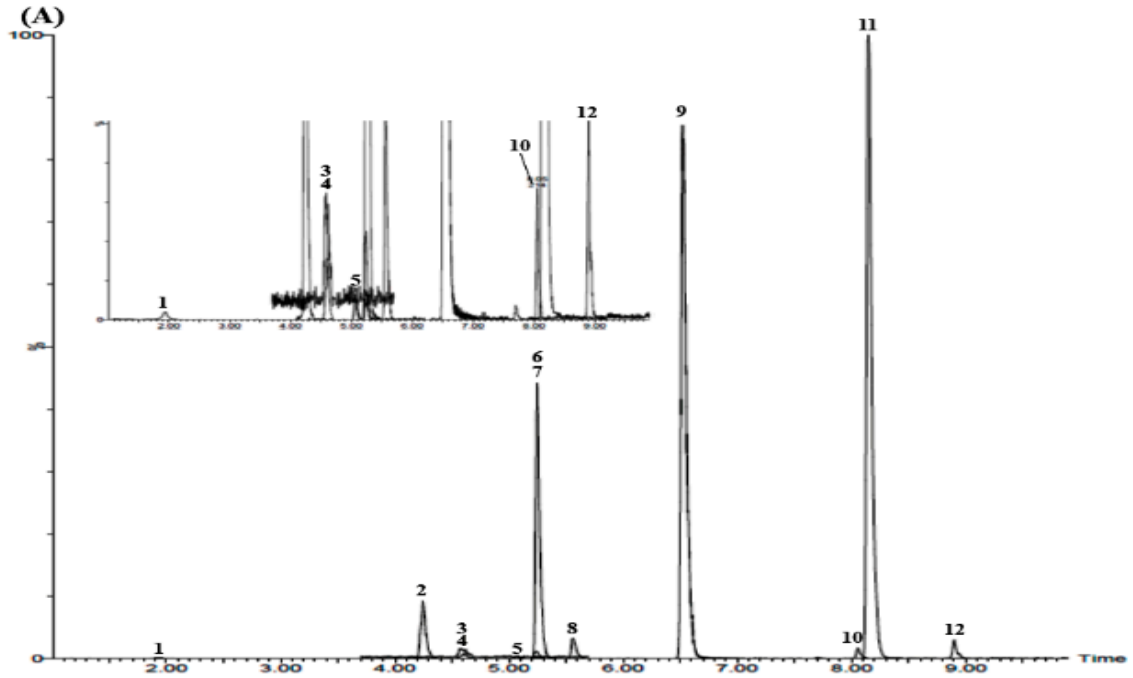

Figure 2. *Cont.*

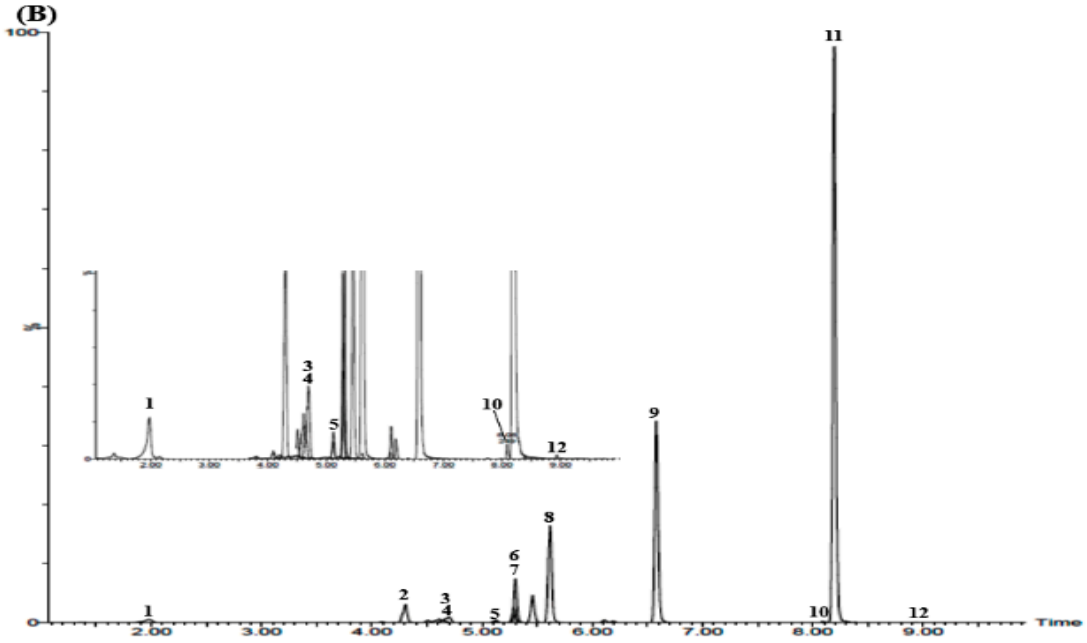

Figure 2. Total ion chromatograms of the reference standard (**A**) and YPS sample (**B**) by UPLC–MS/MS MRM mode. 3-CQA (**1**), CFG (**2**), FA (**3**), LIQA (**4**), NAR (**5**), 5-MVS (**6**), NAG (**7**), NHES (**8**), OPDH (**9**), GA (**10**), ARC (**11**), and 6-GIN (**12**).

Table 5. Amount of the 12 marker components in YPS by LC–MS/MS MRM mode (n = 3).

Compound	Amount (μg/g)		
	Mean	SD	RSD (%)
3-CQA	466.95	14.45	3.10
CFG	35.70	0.49	1.37
FA	26.91	0.78	2.89
LIQA	310.68	6.89	2.22
NAR	137.57	2.13	1.55
5-MVS	19.29	0.27	1.42
NAG	756.78	16.17	2.14
NHES	721.47	14.93	2.07
OPDH	56.13	1.48	2.64
GA	853.11	60.47	7.09
ARC	107.85	1.52	1.41
6-GIN	2.35	0.21	9.03

4. Conclusions

In this study, a simultaneous analysis for the quantification of the 12 marker components in YPS was carried out using an easy and convenient HPLC system, and a rapid and sensitive LC–MS/MS system, to construct basic data with which to assess the analytical quality of a commonly used, traditional Korean medicine. For the quality assessment of YPS, a simultaneous analysis was carried out using our established and optimized analytical method. These methods are expected to be very useful in quality assessments of YPS and other chemically related herbal prescriptions.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-3417/10/5/1713/s1, Figure S1: Chemical structures of 12 biomarker components in YPS, Table S1: Composition of YPS, Table S2: Chromatographic parameters for HPLC analysis of the 12 marker components in YPS, Table S3: MRM parameters for LC–MS/MS analysis of 12 marker components in YPS, Table S4: System suitability of the 12 marker compounds using HPLC–PDA, Table S5: The linear range, regression equation, r^2, LODs, and LOQs of the analytes from YPS using LC–MS/MS (n = 3).

Author Contributions: Conceptualization, C.-S.S. and H.-K.S.; performing experiments and analyzing data, C.-S.S.; writing—original draft preparation, C.-S.S.; funding acquisition, H.-K.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by a grant from the Korea Institute of Oriental Medicine (No. KSN1812240).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Heo, J. *Donguibogam*; Namsandang: Seoul, Korea, 2007; p. 540.
2. Yoon, D.; Hong, S.; Noh, H.; Yi, S.; Lee, I.; Lew, J. Observation of inflammatory marker levels in Sprague-Dawley rats with Youngyopaedoc-san-related anti-antherogenic effect. *J. Korean Med.* **2013**, *34*, 86–95. [CrossRef]
3. Khwag, N.G.; Kang, H.; Myung, E.G.; Park, S.M.; Shim, B.S.; Kim, S.H.; Choi, S.H.; Ahn, K.S. Effect of Yeongyupaedog-san on cytokine levels of mouse Th1/Th2 cells and anti-allergic activity in ovalbumin-sensitized allergic inflammation model. *Korean J. Orient. Physiol. Pathol.* **2006**, *20*, 844–852.
4. Chen, S.; Sun, G.; Yang, L.; Zhang, J. Micellar electrokinetic chromatography fingerprinting combined with chemometrics as an efficient strategy for evaluating the quality consistency and predicting the antioxidant activity of Lianqiao Baidu pills. *J. Sep. Sci.* **2017**, *40*, 2838–2848. [CrossRef] [PubMed]
5. Chen, S.; Sun, G.; Ma, D.; Yang, L.; Zhang, J. Quantitative fingerprinting based on the limited-ratio quantified fingerprint method for an overall quality consistency assesment and antioxidant activity determination of Lianqiao Baidu pills using HPLC with a diode array detector combined with chemometric methods. *J. Sep. Sci.* **2018**, *41*, 548–559. [PubMed]
6. Wu, L.; Yang, G.; Huang, F. Study on quality standard for Lianqiaobaidu pills. *Chin. J. Inf. Tradit. Chin. Med.* **2014**, *21*, 95–97.
7. Kang, H.S.; Lee, J.Y.; Kim, C.J. Anti-inflammatory activity of arctigenin from Forsythiae Fructus. *J. Ethnopharmacol.* **2008**, *116*, 305–312. [CrossRef]
8. Kim, H.Y.; Lim, J.K.; Choi, J.H.; Jung, J.Y.; Oh, W.Y.; Kim, D.C.; Lee, H.S.; Kim, Y.S.; Kang, S.S.; Lee, S.H.; et al. Hepatoprotective effect of pinoresinol on carbon tetrachloride-induced hepatic damage in mice. *J. Pharmacol. Sci.* **2010**, *112*, 105–112. [CrossRef]
9. Kao, S.T.; Liu, C.J.; Yeh, C.C. Protective and immunomodulatory effect of Flos Lonicerae Japonicae by augmenting IL-10 expression in a murine model of acute lung inflammation. *J. Ethnopharmacol.* **2015**, *168*, 108–115. [CrossRef]
10. Chun, M.H.; Kim, E.K.; Lee, K.R.; Jung, J.H.; Hong, J. Quality control of *Schizonepeta tenuifolia* Briq by solid-phase microextraction gas chromatography/mass spectrometry and principal component analysis. *Microchem. J.* **2010**, *95*, 25–31. [CrossRef]
11. Kim, M.K.; Yang, D.H.; Jung, M.; Jung, E.H.; Eom, H.Y.; Suh, J.H.; Min, J.W.; Kim, U.; Min, H.; Kim, J.; et al. Simultaneous determination of chromones and coumarins in Radix Saposhnikoviae by high performance liquid chromatography with diode array and tandem mass detectors. *J. Chromatogr. A* **2011**, *1218*, 6319–6330. [CrossRef]
12. Lee, K.; Park, G.; Ham, I.; Yang, G.; Lee, M.; Bu, Y.; Cim, H.; Choi, H.Y. Vasorelaxant effect of Osterici Radix ethanol extract on rat aortic rings. *Evid.-Based Complement. Alternat. Med.* **2013**, *2013*, 350964. [CrossRef] [PubMed]
13. Kim, H.S.; Moon, B.C.; Choi, G.; Kim, W.J.; Lee, A.Y. Ultra-performance convergence chromatography for the quantitative determination of bioactive compounds in *Aralia continentalis* Kitagawa as quality control markers. *J. Sep. Sci.* **2017**, *40*, 2071–2079. [CrossRef] [PubMed]
14. Han, D.S.; Lee, D.K. Separation and determination of saikosaponins in Bupleuri Radix with HPLC. *Korean J. Pharmacogn.* **1985**, *16*, 175–179.
15. Lu, G.H.; Chan, K.; Liang, Y.Z.; Leung, K.; Chan, C.L.; Jiang, Z.H.; Zhao, Z.Z. Development of high-performance liquid chromatographic fingerprints for distinguishing Chinese Angelica from related umbelliferae herbs. *J. Chromatogr. A* **2005**, *1073*, 383–392. [CrossRef] [PubMed]
16. Wang, C.; Pan, Y.; Fan, G.; Chai, Y.; Wu, Y. Application of an efficient strategy based on MAE, HPLC-DAD-MS/MS and HSCCC for the rapid extraction, identification, separation and purification of flavonoids from Fructus Aurantii Immaturus. *Biomed. Chromatogr.* **2010**, *24*, 235–244. [CrossRef] [PubMed]

17. Ha, Y.W.; Kim, Y.S. Preparative isolation of six major saponins from Platycodi Radix by high-speed counter-current chromatography. *Phytochem. Anal.* **2009**, *20*, 207–213. [CrossRef]
18. Li, G.; Xu, M.L.; Lee, C.S.; Woo, M.H.; Chang, H.W.; Son, J.K. Cytotoxicity and DNA topoisomerases inhibitory activity of constituents from the sclerotium of *Poria cocos*. *Arch. Pharm. Res.* **2004**, *27*, 829–833. [CrossRef]
19. Wu, Y.P.; Meng, X.S.; Bao, Y.R.; Wang, S.; Kang, T.G. Simultaneous quantitative determination of nine active chemical compositions in traditional Chinese medicine *Glycyrrhiza* by RP-HPLC with full-time five-wavelength fusion method. *Am. J. Chin. Med.* **2013**, *41*, 211–219. [CrossRef]
20. Cho, H.; Sowndhararajan, K.; Jung, J.W.; Jhoo, J.W.; Kim, S. Fragrance chemicals in the essential oil of *Mentha arvensis* reduce levels of mental stress. *J. Life Sci.* **2013**, *7*, 933–940. [CrossRef]
21. Wang, W.; Li, C.Y.; Wen, X.D.; Li, P.; Qi, L.W. Simultaneous determination of 6-gingerol, 8-gingerol, 10, gingerol and 6-shogaol in rat plasma by liquid chromatography-mass spectrometry: Application to pharmacokinetics. *J. Chromtogr. B* **2009**, *877*, 671–679. [CrossRef]
22. Lee, K.H. *The Dispensatory on the Visual and Organoleptic Examination of Herbal Medicine*; National Institute of Food and Drug Safety Evaluation: Seoul, Korea, 2013; pp. 24–706.
23. Seo, C.S.; Yoo, S.R.; Jeong, S.J.; Ha, H. Quantification of the constituents of the traditional Korea medicine, Samryeongbaekchul-san, and assessment of its antiadipogenic effect. *Saudi Pharm. J.* **2019**, *27*, 145–153. [CrossRef] [PubMed]
24. Jung, M.Y.; Seo, C.S.; Baek, S.E.; Lee, J.; Shin, M.S.; Kang, K.S.; Lee, S.; Yoo, J.E. Analysis and identification of active compounds from Gami-Soyosan toxic to MCF-7 human breast adenocarcinoma cells. *Biomolecules* **2019**, *9*, 272. [CrossRef] [PubMed]
25. International Conference on Harmonisation (ICH). *Q2B, Validation of Analytical Procedures: Methodology*; European Medicines Agency: Amsterdam, The Netherlands, November 1996.
26. U.S. Food and Drug Administration. *Reviewer Guidance, Validation of Chromatographic Methods*; Center for Drug Evaluation and Research: Rockville, MD, USA, November 1994.
27. Jaiswal, R.; Muller, H.; Muller, A.; Karar, M.G.E.; Kuhnert, N. Identification and characterization of chlorogenic acid, chlorogenic acid glycosides and flavonoids from *Lonicera henryi* L. (Caprifoliaceae) leaves by LC-MSn. *Phytochemistry* **2014**, *108*, 252–263. [PubMed]
28. Gui, Y.; Tsao, R.; Li, L.; Liu, C.M.; Wang, J.; Zong, X. Preparative separation of chromones in plant extract of *Saposhnikovia divaricata* by high-performance counter-current chromatography. *J. Sep. Sci.* **2011**, *34*, 520–526. [CrossRef]
29. Zhang, Z.; Li, Q.; Li, Q.; Du, S.; Zhou, Y.; Lv, C.; Zhao, Y.; Wang, Y.; Zhang, N. Simultaneous determination of nineteen major components in Qi She Pill by ultra-high-performance liquid chromatography-tandem mass spectrometry. *Acta Pharm. Sin. B* **2014**, *4*, 384–393. [CrossRef]
30. Wang, X.; Ma, X.; Li, W.; Chu, Y.; Guo, J.; Li, S.; Wang, J.; Zhang, H.; Zhou, S.; Zhu, Y. Simultaneous determination of five phenolic components and paeoniflorin in rat plasma by liquid chromatography-tandem mass spectrometry and pharmacokinetic study after oral administration of Cerebralcare granule®. *J. Pharm. Biomed. Anal.* **2013**, *86*, 82–91. [CrossRef]
31. Tan, G.; Zhu, Z.; Jing, J.; Lv, L.; Lou, Z.; Zhang, G.; Chai, Y. Characterization of constituents in Sini decoction and rat plasma by high-performance liquid chromatography with diode array detection coupled to time-of-flight mass spectrometry. *Biomed. Chromatogr.* **2005**, *25*, 913–924. [CrossRef]
32. Li, L.; Liang, S.; Du, F.; Li, C. Simultaneous quantification of multiple licorice flavonoids in rat plasma. *J. Am. Soc. Mass Spectrom.* **2007**, *18*, 778–782. [CrossRef]
33. Fu, C.; Xia, Z.; Liu, Y.; Lu, H.; Zhang, Z.; Wang, Y.; Fan, X. Qualitative analysis of major constituents from Xue Fu Zhu Yu Decoction using ultra high performance liquid chromatography with hybrid ion trap time-of-flight mass spectrometry. *J. Sep. Sci.* **2016**, *39*, 3457–3468. [CrossRef]
34. Xiong, X.; Jiang, J.; Duan, J.; Xie, Y.; Wang, J.; Zhai, S. Development and validation of a sensitive liquid chromatography-tandem mass spectrometry method for the determination of naringin and its metabolite, naringenin in human plasma. *J. Chromatogr. Sci.* **2004**, *52*, 654–660. [CrossRef]
35. Zheng, G.D.; Zhou, P.; Yang, H.; Li, Y.; Li, P.; Liu, E.H. Rapid resolution liquid chromatography-electrospray ionization tandem mass spectrometry method for identification of chemical constituents in Cirti Reticulatae Pericarpium. *Anal. Methods* **2013**, *136*, 604–611.

36. Tang, D.Q.; Zheng, X.X.; Chen, X.; Yang, D.Z.; Du, Q. Quantitative and qualitative analysis of common peaks in chemical fingerprint of Yuanhu Zhitong tablet by HPLC-DAD-MS/MS. *J. Pharm. Anal.* **2014**, *4*, 96–106. [CrossRef] [PubMed]
37. Wang, X.; Liang, J.; Wen, H.; Shan, C.; Liu, R. Simultaneous determination of five bioactive secolignans in rat plasma by LC-MS/MS for pharmacokinetic studies following oral administration of *Peperomia dindygulensis* Miq. extract. *J. Chromatogr. B* **2014**, *944*, 82–89. [CrossRef] [PubMed]
38. He, Q.; Ge, Z.W.; Song, Y.; Cheng, Y.Y. Quality evaluation of Cortex Moutan by high performance liquid chromatography coupled with diode array detector and electrospray ionization tandem mass spectrometry. *Chem. Pharm. Bull.* **2006**, *54*, 1271–1275. [CrossRef] [PubMed]