separations

MDPI

Article

Validation of the Ultra-Performance Liquid Chromatography with Tandem Mass Spectrometry Method for Simultaneous Analysis of Eighteen Compounds in the Traditional Herbal Prescription, Sanjoin-Tang

Chang-Seob Seo * and Hyeun-Kyoo Shin

KM Science Research Division, Korea Institute of Oriental Medicine, Daejeon 34054, Republic of Korea; hkshin@kiom.re.kr

* Correspondence: csseo0914@kiom.re.kr; Tel.: +82-42-868-9361

Abstract: Sanjoin-tang (SJIT) is an ancient oriental medicine prescription listed in the *Jinguiyaolue* that is mainly used for the treatment of primary insomnia. This study was conducted to develop and validate an ultra-performance liquid chromatography with tandem mass spectrometry (UPLC–MS/MS) simultaneous analysis method for the quality control of SJIT using 18 target compounds. The 18 analytes were separated on an Acquity UPLC BEH C_{18} column maintained at 45 °C using a mobile phase composed of distilled water and acetonitrile. The MS system was used to simultaneously detect all analytes using the multiple reaction monitoring (MRM) method of Xevo TQ-XS coupled with an electrospray ionization source. The concentrations of the 18 analytes investigated in the SJIT samples ranged from below the limit of detection to 9.553 mg/g. In conclusion, the validated UPLC–MS/MS MRM analysis method can be used to obtain basic data to establish chemical-nonclinical linkage efficacy and for the clinical research and quality evaluation of SJIT.

Keywords: simultaneous quantitative analysis; traditional herbal prescription; Sanjoin-tang; UPLC–MS/MS

Citation: Seo, C.-S.; Shin, H.-K. Validation of the Ultra-Performance Liquid Chromatography with Tandem Mass Spectrometry Method for Simultaneous Analysis of Eighteen Compounds in the Traditional Herbal Prescription, Sanjoin-Tang. *Separations* **2023**, *10*, 411. https://doi.org/10.3390/separations10070411

Academic Editors: Chao Kang and Ronald Beckett

Received: 23 June 2023
Revised: 12 July 2023
Accepted: 14 July 2023
Published: 18 July 2023

1. Introduction

Insomnia is a common symptom in menopausal women and is one of the factors that determine quality of life [1]. Sanjoin-tang (SJIT, Suanzaoren-tang in Chinese, Sansoninto in Japanese) is one of the most frequently used prescriptions for treating insomnia in oriental medicine. SJIT was first recorded in *Jinguiyaolue* and consists of five traditional herbal medicines (*Ziziphus jujuba* Mill., *Cnidium officinale* Makino, *Anemarrhena asphodeloides* Bunge, *Poria cocos* Wolf, and *Glycyrrhiza uralensis* Fisch.) [1]. As a principal herb in SJIT, *Z. jujuba* has been reported to have insomnia, hypnotic, tranquilizing, and antianxiety effects [2–5]. In particular, jujubosides and spinosin, the main components of *Z. jujuba* seeds, have been reported to be effective for hypnosis and anxiety [2,6]. In addition, *G. uralensis* and *P. cocos*, which are other medicinal herbs that make up SJIT, have been reported to be effective for insomnia [7–10].

Various types of components such as flavonoids [11,12], terpenoids [13–15], alkaloids [13], miscellaneous [16,17], phenylpropanoids [17], xanthones [18], and steroids [19] were isolated from each medicinal herb of SJIT. Quantitative and qualitative analysis methods using high-performance liquid chromatography (HPLC), ultra-high-performance liquid chromatography, high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry, or ultra-performance liquid chromatography coupled with electrospray ionization (ESI) and quadrupole time-of-flight mass spectrometry have been developed for the efficient quality control of each herbal medicine based on the measurement of these isolated components [17,20–26]. As an analysis for the quality assurance of

SJIT, Zhu et al. [27] reported a component profiling analysis using liquid chromatography coupled with quadrupole time-of-flight mass spectrometry and liquid chromatography–ion trap–mass spectrometry. Although the efficacy of SJIT and analysis methods for each component of herbal medicine have been reported, no simultaneous analysis method for the quality assurance of SJIT has been validated to date.

Ultra-performance liquid chromatography with tandem mass spectrometry (UPLC–MS/MS) has high specificity, sensitivity, reproducibility, resolution, and accuracy, and its use for the quantitative analysis of traditional herbal medicines and herbal formulas has been increasing compared to other analytical instruments [28,29].

In this study, a simultaneous analysis method was validated for 18 compounds in SJIT samples using UPLC–MS/MS multiple reaction monitoring (MRM). The compounds examined were neomangiferin (**1**), mangiferin (**2**), magnoflorine (**3**), spinosin (**4**), liquiritin apioside (**5**), liquiritin (**6**), ferulic acid (**7**), 6‴-feruloyl spinosin (**8**), isoliquiritin (**9**), ononin (**10**), liquiritigenin (**11**), jujuboside A (**12**), isoliquiritigenin (**13**), glycyrrhizin (**14**), jujuboside B (**15**), *Z*-ligustilide (**16**), dehydropachymic acid (**17**), and pachymic acid (**18**).

2. Materials and Methods

2.1. Chemicals and Reagents

The 18 standard compounds used for the evaluation of quality control of SJIT were purchased from professional high-purity phytochemical manufacturers: neomangiferin (CAS No. 64809-67-2, purity: 98.3%, catalog No. BP0993), mangiferin (CAS No. 4773-96-0, purity: 99.1%, catalog No. BP0922), spinosin (CAS No. 72063-39-9, purity: 99.3%, catalog No. BP1336), liquiritin (CAS No. 551-15-5, purity: 99.6%, catalog No. BP0874), 6‴-feruloyl spinosin (CAS No. 77690-92-7, purity: 99.7%, catalog No. BP1572), isoliquiritin (CAS No. 5041-81-6, purity: 98.6%, catalog No. BP0788), ononin (CAS No. 486-62-4, purity: 98.5%, catalog No. BP1031), liquiritigenin (CAS No. 578-86-9, purity: 99.8%, catalog No. BP0873), glycyrrhizin (CAS No. 1405-86-3, purity: 99.1%, catalog No. BP0682), and dehydropachymic acid (CAS No. 77012-31-8, purity: 98.8%, catalog No. BP1676) from Biopurify Phytochemicals (Chengdu, China); magnoflorin (CAS No. 2141-09-5, purity: 98.8%, catalog No. CFN98071) from ChemFaces (Wuhan, China); liquiritin apioside (CAS No. 74639-14-8, purity: 99.6%, catalog No. DR10690), jujuboside A (CAS No. 55466-04-1, purity: 99.1%, catalog No. D19052410), jujuboside B (CAS No. 565466-05-2, purity: 98.3%, catalog No. D102876), and pachymic acid (CAS No. 29070-92-6, purity: 99.7%, catalog No. DR11130) from Shanghai Sunny Biotech (Shanghai, China); ferulic acid (CAS No. 1135-24-6, purity: 98.0%, catalog No. 086-04282) from Fujifilm Wako Pure Chemical Co. (Osaka, Japan); and isoliquiritigenin (CAS No. 961-29-5, purity: 99.8%, catalog No. TB0235-0500) and *Z*-liqustilide (CAS No. 81944-09-4, purity: 99.6%, catalog No. TB0322-0100) from ChemNorm (Wuhan, China) (Figure S1). Solvents (acetonitrile, methanol, and distilled water) and reagent (formic acid) for analysis were LC–MS grade.

2.2. Plant Materials and Preparation of the SJIT Water Extract

The five raw herbal medicines (Table S1) that make up SJIT were purchased from Kwangmyungdang Pharmaceutical (Ulsan, Korea) and used in the study after morphological identification by Dr. Goya Choi (Korea Institute of Oriental Medicine (KIOM), Naju, Republic of Korea). Each raw herbal medicine (KE–90–1 to KE–90–5) and SJIT water extract (KE90) were deposited at the Korean Medicine Science Research Division, KIOM (Daejeon, Republic of Korea).

After the SJIT water extract (SJIT–1) was mixed well according to the amount shown in Table S2 (total 5000.0 g: *Z. jujuba* 2666.67 g, *C. officinale* 666.67 g, *A. asphodeloides* 666.67 g, *P. cocos* 666.67 g, and *G. uralensis* 333.32), 50 L of primary distilled water was added and the mixture was extracted at 100 °C for 2 h using an electric extractor, COSMOS-660 (Kyungseo E&P, Incheon, Korea). The extract solution was filtered through a sieve (53 μm mesh) and freeze-dried using an LP100R freeze dryer (IlShinBioBase, Yangju, Korea) to obtain the powdered sample. Finally, 840.2 g (16.8% yield) of powdered SJIT water extract

was obtained. Other samples (SJT–2, SJT–3, and SJT–4) were purchased from different pharmaceutical companies.

2.3. Analytical Method for UPLC–MS/MS Analysis

The analytical method for the simultaneous analysis of the 18 markers from SJIT samples using the UPLC–MS/MS system, consisting of an Acquity UPLC I-Class system coupled with a Xevo TQ-XS MS, was conducted with the parameters summarized in Table S2. All markers were separated on a UPLC BEH C_{18} column (2.1 mm × 100 mm, 1.7 μm, Waters) with gradient elution of a 0.1% formic acid in distilled water–acetonitrile mobile phase. Temperatures of the column, sample, ion source, and ion desolvation were maintained at 45, 5, 150, and 500 °C, respectively. The injection volume was 0.2 μL and the flow rate was 0.3 mL/min. Details of the conditions for UPLC–MS/MS MRM analysis are presented in Table 1.

Table 1. UPLC–MS/MS MRM analytical conditions for simultaneous analysis of the 18 analytes in SJIT samples.

Analyte [1]	Ion Mode	Molecular Weight	Precursor Ion	Product Ion	Cone Voltage (V)	Collision Energy (eV)
1	−	584.5	583.3 $[M-H]^-$	331.0	45	35
2	−	422.3	421.1 $[M-H]^-$	301.0	30	20
3	+	342.4	342.4 $[M]^+$	297.2	30	20
4	+	608.5	609.5 $[M+H]^+$	327.2	40	25
5	−	550.5	549.3 $[M-H]^-$	255.0	45	30
6	−	418.4	417.4 $[M-H]^-$	255.2	30	15
7	+	194.2	195.0 $[M+H]^+$	177.0	15	10
8	+	784.7	785.5 $[M+H]^+$	327.1	35	25
9	+	418.4	419.3 $[M+H]^+$	257.0	35	15
10	+	430.4	431.3 $[M+H]^+$	269.0	25	15
11	+	256.3	257.2 $[M+H]^+$	137.0	35	25
12	+	1207.3	1225.1 $[M+H_2O]^+$	473.5	30	20
13	+	256.3	257.2 $[M+H]^+$	137.0	15	20
14	−	822.9	821.9 $[M-H]^-$	351.2	45	40
15	−	1045.2	1043.8 $[M-H]^-$	911.5	30	35
16	+	190.1	191.0 $[M+H]^+$	91.0	30	25
17	−	526.7	525.7 $[M-H]^-$	59.0	30	35
18	−	528.8	527.6 $[M-H]^-$	465.4	45	35

[1] Neomangiferin (**1**), mangiferin (**2**), magnoflorine (**3**), spinosin (**4**), liquiritin apioside (**5**), liquiritin (**6**), ferulic acid (**7**), 6‴-feruloyl spinosin (**8**), isoliquiritin (**9**), ononin (**10**), liquiritigenin (**11**), jujuboside A (**12**), isoliquiritigenin (**13**), glycyrrhizin (**14**), jujuboside B (**15**), Z-ligustilide (**16**), dehydropachymic acid (**17**), and pachymic acid (**18**).

2.4. Preparation of Standard Stock and Sample Solutions

Standard stock solutions of the 18 reference standards were prepared at a concentration of 1000 μg/mL using methanol and stored in a refrigerator (approximately 4 °C) until use. To prepare the sample solution of the SJIT samples, about 50 mg of the prepared SJIT water extract or commercially available SJIT granules was accurately taken and made to a concentration of 5 mg/mL using 70% methanol, followed by ultrasonic extraction (for 5.0 min) and vortexing (for 1.0 min). The extracted sample solution was filtered with a 0.2 μm hydrophobic filter (SSOLKOREA Co., Ltd., Daejeon, Korea) before UPLC–MS/MS analysis.

2.5. Validation of the UPLC–MS/MS MRM Analytical Method

The UPLC–MS/MS analytical method developed in this study was validated with respect to specificity, linearity, sensitivity, recovery, and precision evaluation [30]. That is, specificity was confirmed by verifying the presence or absence of an interference peak in the chromatogram of each component. The linearity was evaluated by the coefficient of determination (r^2) value in the regression equation of the calibration curve prepared at

different concentrations. The sensitivity of the method was verified by limit of detection (LOD) and limit of quantitation (LOQ) values and calculated as signal-to-noise (S/N) ratios of 3 and 10, respectively. Recovery (%) was evaluated by the standard addition method, which was evaluated by adding three different levels (low, medium, and high) of standard solutions to the known sample. This parameter was calculated using the following equation: Recovery (%) = found amount/spiked amount × 100. Precision was demonstrated by evaluating relative standard deviation (RSD) values of intraday (within a day) and interday (three consecutive days) precisions and repeatability. This parameter was calculated using the following equation: RSD (%) = standard deviation/mean × 100.

3. Results and Discussion

3.1. Selection of Target Marker Components for Simultaneous Analysis Using UPLC–MS/MS in SJIT Samples

From the SJIT consisting of five medicinal herbs, 18 components were selected from each raw material for simultaneous analysis using the UPLC–MS/MS system: magnoflorine, spinosin, 6‴-feruloyl spinosin, jujuboside A, and jujuboside B from *Z. jujuba*; ferulic acid and *Z*-ligustilide from *C. officinale*; neomangiferin and mangiferin from *A. asphodeloides*; dehydropachymic acid and pachymic acid from *P. cocos*; and liquiritin apioside, liquiritin, isoliquiritin, ononin, liquiritigenin, isoliquiritigenin, and glycyrrhizin from *G. uralensis* [11–19].

3.2. Multiple Reaction Monitoring Conditions for UPLC–MS/MS Analysis of SJIT Samples

As mentioned in Section 2.3, MRM conditions for the simultaneous analysis of the 18 marker components in SJIT were explored using a UPLC–MS/MS system. An ESI attachment was used as the ion source. Eight compounds (**1**, **2**, **5**, **6**, **14**, **15**, **17**, and **18**) showed high intensity in the $[M–H]^-$ form, and eight compounds (**4**, **7**–**11**, **13**, and **16**) showed good intensity in the $[M+H]^+$ form. Magnoflorine (**3**) and jujuboside A (**12**) showed an $[M]^+$ peak and an adduct peak in the form of $[M+H_2O]^+$, respectively (Figure 1). In case of jujuboside A, the adduct peak ($[M+H_2O]^+$) observed at *m/z* 1225.1 was detected more strongly than $[M+H]^+$ during MS measurement (Figure S2).

To simultaneously quantify the 18 components in SJIT samples using the UPLC–MS/MS MRM analysis mode, parameters such as precursor ion (Q1) peak and product ion (Q3) peak, cone voltage, and collision energy for each component were set as shown in Table 1. The Q3 peaks of xanthone types, neomangiferin, and mangiferin were set to *m/z* 331.0 and 301.0 in the form of $[M–H–Glu–C_4H_8O_4]^-$ and $[M–H–C_4H_8O_4]^-$, respectively [31,32]. These components showed a common feature of the Q3 peak being formed due to the cleavage of the sugar ring. For magnoflorine and alkaloids, a Q3 peak was observed at *m/z* 297.2 ($[M–(CH_3)_2NH]^+$), corresponding to the loss of two methyl groups, and an amine group was lost from the ion generating the Q1 peak [33]. For spinosin, 6‴-feruloyl spinosin, and liquiritigenin, Q3 peaks were observed at *m/z* 327.2, 327.1, and 137.0, arising from the fragmentation of the Q1 peak to $[M+H–Glu–C_4H_8O_4]^+$, with the loss of a glucose and a $C_4H_8O_4$ molecule; $[M+H–Feryloyl–Glu–C_4H_8O_4]^+$, with the loss of a feruloyl functional group, one glucose, and a $C_4H_8O_4$ molecule; and $[M+H–C_8H_8O_8]^+$, with the loss of a $C_8H_8O_8$ molecule, respectively [33,34]. In both liquiritin and ononin, Q3 peaks were generated as $[M–H–Glu]^-$ and $[M+H–Glu]^+$ with one molecule of glucose removed [33,35]. In liquiritin apioside, the Q3 peak was detected in the form of an aglycone, in which apiose and glucose were removed from the Q1 peak [33]. The Q3 peaks of ferulic acid (phenylpropanoid-type) and *Z*-ligustilide (benzoquinone derivative) were set to *m/z* 177.0 ($[M+H–H_2O]^+$) and *m/z* 91 ($[M+H–H_2O–CO–C_4H_6]^+$), respectively [36]. In the MRM transition of isoliquiritin and isoliquiritigenin, which are chalcone-type compounds, Q3 peaks were observed at *m/z* 257.0 ($[M+H–Glu]^+$) and *m/z* 137.0 ($[M+H–C_8H_7O]^+$), respectively, where *m/z* 257.0 was produced by the release of one molecule of glucose from the Q1 peak, and *m/z* 137.0 was generated by the cleavage of the B ring in the molecular structure [37]. The Q3 peak of jujuboside A, triterpenoids, was observed at *m/z* 473.5 in the form of aglycone in which five sugars were cleaved from the Q1 peak [38], whereas

the Q3 peak of glycyrrhizin was observed at *m/z* 351.2 in the form of two glucuronic acid conjugates, excluding the aglycone [39]. For jujuboside B, the Q3 peak was observed at *m/z* 911.5 ([M–H–Xyl]$^-$), where one molecule of xylose was removed from the Q1 peak [40]. In dehydropachymic acid and pachymic acid, the highest intensities were observed at *m/z* 59.0 and 465.4, respectively, as each Q1 peak was fragmented by cone voltage and collision energy, and these were set as Q3 peaks [24]. Under the optimized UPLC–MS/MS MRM analysis conditions, all components were completely eluted within 13.0 min; representative total ion chromatograms (TICs) are shown in Figure 1. The TIC of the other samples (SJIT–2 to SJIT–4) is shown in Figure S3. In addition, the extracted ion chromatograms of the 18 reference markers and samples (SJIT–1 ~ SJIT–4) are presented in Figure S4.

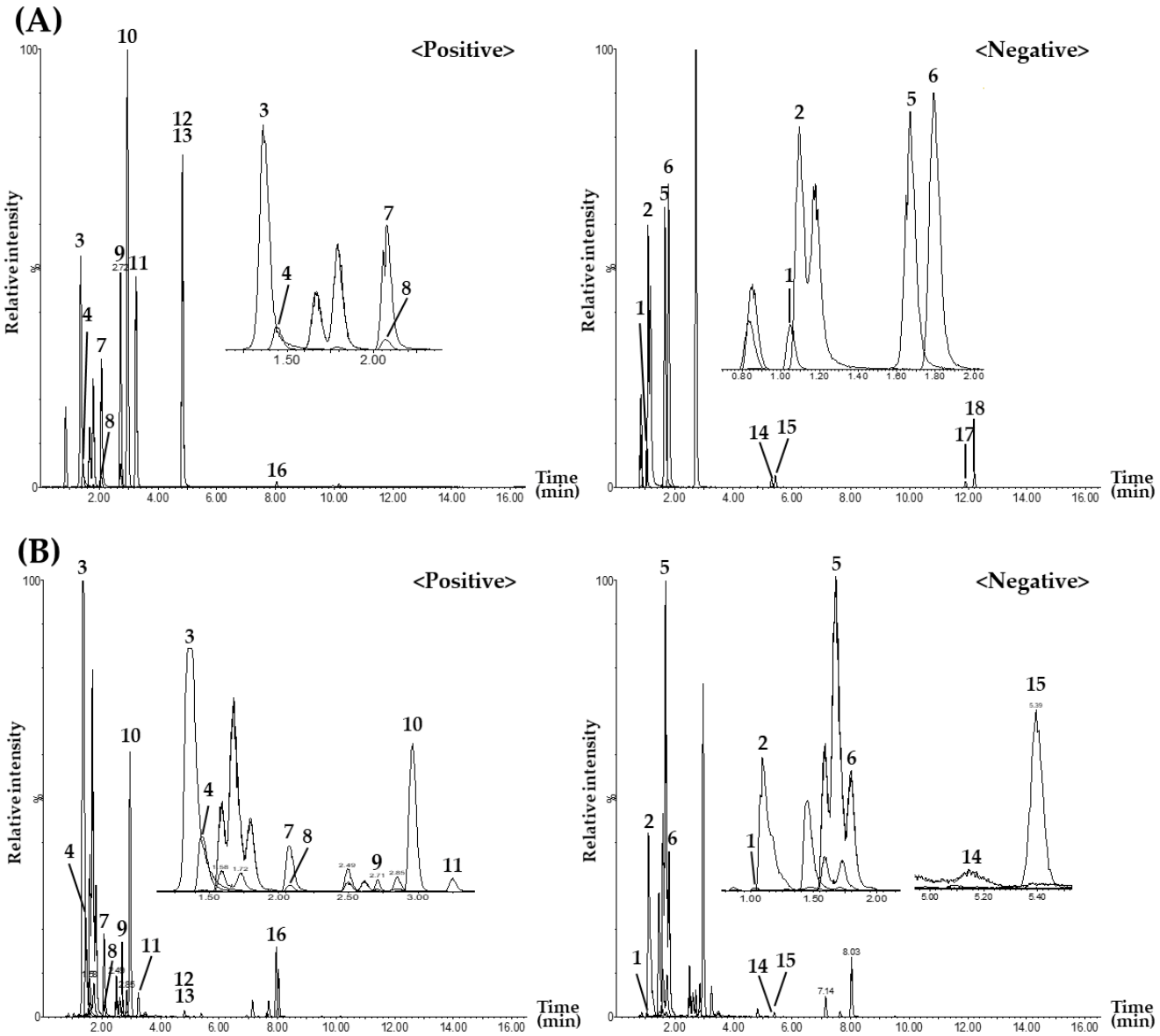

Figure 1. Representative total ion chromatograms of the standard mixtures (**A**) and SJIT–1 sample (**B**) by UPLC–MS/MS MRM analytical method mode. Neomangiferin (**1**), mangiferin (**2**), magnoflorine (**3**), spinosin (**4**), liquiritin apioside (**5**), liquiritin (**6**), ferulic acid (**7**), 6‴-feruloyl spinosin (**8**), isoliquiritin (**9**), ononin (**10**), liquiritigenin (**11**), jujuboside A (**12**), isoliquiritigenin (**13**), glycyrrhizin (**14**), jujuboside B (**15**), Z-ligustilide (**16**), dehydropachymic acid (**17**), and pachymic acid (**18**). The concentration of each compound in the standard mixtures (A) was 100.00 μg/L.

3.3. Validation of the Developed UPLC–MS/MS MRM Analytical Method

The developed UPLC–MS/MS MRM simultaneous analysis method was validated by assessing specificity, linearity, sensitivity (LOD and LOQ), recovery, and precision. Specificity results ensured the absence of any significant interference at the retention time of each component (Figure S4). The linearity was evaluated by the coefficient of determination (r^2). As shown in Table 2, all components showed acceptable linearity in the assay, with r^2 values $\geq$0.9968 in the regression equations tested at 10.00–500.00 and 250.00–5000.00 μg/L. In addition, LOD and LOQ values for sensitivity check were calculated as S/N ratios and were 0.002–11.825 and 0.005–35.474 μg/L, respectively (Table 2). Recovery tests for the 18 compounds in SJIT samples were performed to assess the accuracy of the developed analytical assay. Recovery (%) tested at three different concentration levels (low, medium, and high) was measured to be 85.26–114.21% with RSD values <10.0% for all marker components (Table 3). The tolerance of the recovery test was set within ±15% and the recovery of 85.26–114.21% proved that the newly developed UPLC–MS/MS method was acceptable. Precision results evaluated by RSD values are presented in Table 4. The intraday precision, evaluated by measuring five times within one day, was 0.69–9.94%, and the interday precision, evaluated by measuring on three consecutive days, was 2.11–9.24%. The repeatability of peak retention time and peak area was evaluated from the RSD values calculated by measuring the standard solution six times; the components had RSD values of 0.45–1.82% and 1.10–8.73%, respectively. The tolerance of precision (% RSD) was set to less than 15%, and the test results demonstrated that the developed UPLC–MS/MS method was suitable for less than 10%. Finally, based on all the validation results above, the developed UPLC–MS/MS MRM method proved to be suitable and precise as an analytical method for the simultaneous analysis of SJIT.

Table 2. Parameters for simultaneous quantification of the 18 analytes in SJIT samples using the UPLC–MS/MS MRM method.

Analyte [1]	Retention Time (min)	Linear Range (μg/L)	Regression Equation [2] $y = ax + b$	r^2	LOD (μg/L)	LOQ (μg/L)
1	1.06	10.00–500.00	$y = 296.00x + 3082.84$	0.9992	0.071	0.213
2	1.08	10.00–500.00	$y = 1550.26x + 24{,}896.50$	0.9980	0.058	0.174
3	1.35	10.00–500.00	$y = 23{,}534.20x + 432{,}415.00$	0.9996	0.011	0.032
4	1.45	10.00–500.00	$y = 2543.55x - 4216.33$	0.9968	0.017	0.050
5	1.65	10.00–500.00	$y = 2188.31x + 13{,}166.60$	0.9993	0.073	0.218
6	1.79	10.00–500.00	$y = 3298.34x + 23772.50$	0.9992	0.010	0.030
7	2.06	10.00–500.00	$y = 11{,}957.40x + 44{,}248.70$	0.9988	0.075	0.224
8	2.07	10.00–500.00	$y = 1041.87x + 3654.02$	0.9989	0.118	0.353
9	2.69	10.00–500.00	$y = 19{,}090.10x + 157{,}649.00$	0.9991	0.028	0.083
10	2.92	10.00–500.00	$y = 37{,}870.70x + 471{,}200.00$	0.9990	0.002	0.005
11	3.25	10.00–500.00	$y = 19{,}416.40x + 221{,}079.00$	0.9994	0.015	0.045
12	4.83	250.00–5000.00	$y = 0.30x - 49.79$	0.9978	11.825	35.474
13	4.83	10.00–500.00	$y = 28{,}495.50x + 368{,}896.00$	0.9992	0.008	0.024
14	5.26	10.00–500.00	$y = 71.87x + 65.45$	0.9985	0.975	2.925
15	5.42	10.00–500.00	$y = 78.53x + 378.80$	0.9989	2.148	6.444
16	8.04	10.00–500.00	$y = 609.03x + 5526.79$	0.9991	6.276	18.827
17	11.91	10.00–500.00	$y = 44.84x + 99.89$	0.9985	1.207	3.622
18	12.21	10.00–500.00	$y = 109.76x + 523.95$	0.9980	1.582	4.747

[1] Neomangiferin (**1**), mangiferin (**2**), magnoflorine (**3**), spinosin (**4**), liquiritin apioside (**5**), liquiritin (**6**), ferulic acid (**7**), 6‴-feruloyl spinosin (**8**), isoliquiritin (**9**), ononin (**10**), liquiritigenin (**11**), jujuboside A (**12**), isoliquiritigenin (**13**), glycyrrhizin (**14**), jujuboside B (**15**), *Z*-ligustilide (**16**), dehydropachymic acid (**17**), and pachymic acid (**18**). [2] y: peak area of each reference compound; x: concentration (μg/L) of each reference compound.

Table 3. Recovery (%) of the 18 target components using the UPLC–MS/MS MRM method.

Analyte [1]	Spiked Amount (μg/L)	Found Amount (μg/L)	Recovery (%)	SD [2]	RSD (%)
1	40.00	37.15	92.86	8.87	9.55
	80.00	81.21	101.52	4.82	4.75
	160.00	168.60	105.37	4.70	4.46
2	120.00	119.47	99.56	4.22	4.24
	240.00	250.58	104.41	8.73	8.36
	480.00	525.41	109.46	6.23	5.69
3	40.00	38.28	95.71	6.98	7.30
	80.00	82.12	102.65	6.59	6.42
	160.00	170.78	106.74	4.11	3.85
4	40.00	39.64	99.10	9.13	9.21
	80.00	89.88	112.35	7.36	6.55
	160.00	172.27	107.67	7.90	7.34
5	80.00	80.70	100.88	1.17	1.16
	160.00	170.10	106.31	1.85	1.74
	320.00	344.46	107.65	4.54	4.22
6	80.00	78.89	98.61	3.32	3.36
	160.00	169.20	105.75	2.88	2.72
	320.00	343.38	107.31	3.80	3.54
7	10.00	9.78	97.81	6.26	6.40
	20.00	20.73	103.63	7.33	7.08
	40.00	42.51	106.28	4.34	4.09
8	8.00	8.16	102.06	6.69	6.55
	16.00	17.12	106.99	0.94	0.88
	32.00	33.49	104.65	4.54	4.34
9	80.00	82.71	103.39	2.56	2.48
	160.00	168.82	105.51	1.83	1.73
	320.00	333.96	104.36	1.86	1.78
10	4.00	3.94	98.60	5.32	5.39
	8.00	8.18	102.19	3.49	3.42
	16.00	16.43	102.71	2.89	2.81
11	80.00	78.72	98.39	3.91	3.98
	160.00	162.03	101.27	3.91	3.86
	320.00	332.91	104.03	3.93	3.78
12	600.00	526.21	87.70	8.04	9.16
	1200.00	1200.96	100.08	9.37	9.36
	2400.00	2741.03	114.21	4.83	4.23
13	20.00	20.28	101.42	1.56	1.54
	40.00	40.46	101.16	1.62	1.60
	80.00	79.62	99.52	4.00	4.02
14	200.00	191.87	95.94	3.62	3.78
	400.00	399.28	99.82	4.39	4.39
	800.00	828.35	103.54	5.10	4.93
15	100.00	109.39	109.39	6.00	5.48
	200.00	211.04	105.52	6.13	5.81
	400.00	396.60	99.15	4.10	4.13
16	80.00	84.39	105.49	2.99	2.84
	160.00	171.35	107.10	3.93	3.67
	320.00	337.40	105.44	4.00	3.80
17	20.00	17.05	85.26	8.02	9.41
	40.00	41.81	104.54	6.59	6.30
	80.00	87.37	109.21	4.01	3.67
18	20.00	18.54	92.72	8.45	9.11
	40.00	41.77	104.43	1.55	1.49
	80.00	85.50	106.88	7.62	7.13

[1] Neomangiferin (**1**), mangiferin (**2**), magnoflorine (**3**), spinosin (**4**), liquiritin apioside (**5**), liquiritin (**6**), ferulic acid (**7**), 6‴-feruloyl spinosin (**8**), isoliquiritin (**9**), ononin (**10**), liquiritigenin (**11**), jujuboside A (**12**), isoliquiritigenin (**13**), glycyrrhizin (**14**), jujuboside B (**15**), *Z*-ligustilide (**16**), dehydropachymic acid (**17**), and pachymic acid (**18**).
[2] Standard deviation.

Table 4. Precision test of the 18 target components using the UPLC–MS/MS MRM method.

Analyte [1]	Conc. (μg/L)	Intraday (n = 5)			Interday (n = 5)			Repeatability (n = 6)	
		Observed Conc. (μg/L)	Precision (RSD, %)	Accuracy (%)	Observed Conc. (μg/L)	Precision (RSD, %)	Accuracy (%)	RSD (%) of Retention Time	RSD (%) of Peak Area
1	40.00	39.57	3.67	98.93	39.20	7.08	97.88	1.67	3.94
	80.00	82.34	4.32	102.93	84.10	4.29	105.17		
	160.00	174.14	5.00	108.84	175.40	4.44	109.61		
2	120.00	122.09	5.22	101.74	119.90	6.09	99.92	1.82	8.05
	240.00	263.26	4.93	109.69	259.70	6.08	108.21		
	480.00	522.62	4.86	108.88	526.20	4.96	109.62		
3	40.00	37.43	7.34	93.58	38.60	7.13	96.49	1.21	4.17
	80.00	85.02	5.89	106.28	86.10	5.68	107.58		
	160.00	170.04	5.65	106.27	175.30	4.21	109.55		
4	40.00	36.00	8.20	90.01	39.00	7.54	97.55	1.15	8.73
	80.00	77.88	6.15	97.35	87.50	4.57	109.36		
	160.00	153.80	8.73	96.12	170.90	5.81	106.80		
5	80.00	79.53	3.95	99.41	79.40	3.19	99.26	1.31	4.24
	160.00	169.62	2.78	106.01	167.50	2.21	104.67		
	320.00	340.06	3.41	106.27	338.70	3.28	105.83		
6	80.00	78.11	3.19	97.64	79.57	3.91	99.46	1.14	6.01
	160.00	169.50	2.34	105.94	170.58	2.11	106.61		
	320.00	341.85	1.95	106.83	342.48	3.20	107.02		
7	10.00	9.53	5.30	95.33	9.80	5.33	98.34	1.43	4.75
	20.00	19.96	8.27	99.80	20.20	6.50	101.18		
	40.00	39.47	5.70	98.67	40.60	6.01	101.52		
8	8.00	7.74	3.98	96.76	8.10	4.69	100.96	1.15	7.87
	16.00	16.36	3.10	102.26	16.60	2.39	104.02		
	32.00	33.04	3.71	103.24	33.40	3.06	104.37		
9	80.00	77.81	3.24	97.27	73.90	4.70	92.42	1.09	3.85
	160.00	172.29	0.69	107.68	164.60	3.78	102.86		
	320.00	329.53	2.64	102.98	328.30	3.92	102.61		
10	4.00	4.05	3.53	101.33	4.00	4.52	99.07	1.13	4.45
	8.00	8.28	3.72	103.54	8.20	2.98	102.27		
	16.00	16.55	2.51	103.43	16.70	2.44	104.21		
11	80.00	81.95	0.86	102.44	81.00	2.45	101.25	1.11	5.74
	160.00	166.62	2.54	104.14	164.10	3.99	102.53		
	320.00	333.34	3.16	104.17	329.80	3.98	103.07		
12	600.00	618.67	9.71	103.11	540.90	9.24	90.15	0.83	7.52
	1200.00	1245.69	3.77	103.81	1232.90	7.54	102.74		
	2400.00	1958.18	7.55	81.59	2441.50	7.09	101.73		
13	20.00	19.76	3.33	98.80	19.90	2.71	99.45	0.92	1.10
	40.00	41.96	1.84	104.90	41.60	2.53	104.11		
	80.00	82.51	3.05	103.14	82.20	3.66	102.77		
14	200.00	208.51	2.10	104.25	199.40	2.49	99.72	0.72	4.48
	400.00	442.18	2.55	110.55	414.30	3.79	103.56		
	800.00	898.90	3.48	112.36	835.60	3.64	104.45		
15	100.00	103.36	9.94	103.36	74.60	7.08	100.76	0.73	6.57
	200.00	205.13	6.23	102.56	150.10	6.60	103.41		
	400.00	441.67	1.12	110.42	301.40	3.68	103.52		
16	80.00	84.93	4.79	106.17	82.40	3.66	102.97	0.82	3.54
	160.00	175.47	4.59	109.67	169.70	3.50	106.06		
	320.00	332.33	1.46	103.85	333.20	2.52	104.12		
17	20.00	18.50	8.46	92.51	18.00	8.96	90.09	0.46	4.85
	40.00	44.91	3.44	112.28	43.00	5.52	107.60		
	80.00	84.25	8.21	105.32	87.20	5.35	108.99		
18	20.00	21.47	3.57	107.34	20.30	5.72	101.47	0.45	8.15
	40.00	42.54	3.67	106.36	41.90	3.42	104.73		
	80.00	82.83	6.02	103.54	83.40	5.88	104.22		

[1] Neomangiferin (**1**), mangiferin (**2**), magnoflorine (**3**), spinosin (**4**), liquiritin apioside (**5**), liquiritin (**6**), ferulic acid (**7**), 6‴-feruloyl spinosin (**8**), isoliquiritin (**9**), ononin (**10**), liquiritigenin (**11**), jujuboside A (**12**), isoliquiritigenin (**13**), glycyrrhizin (**14**), jujuboside B (**15**), *Z*-ligustilide (**16**), dehydropachymic acid (**17**), and pachymic acid (**18**).

3.4. Simultaneous Quantitation of the Eighteen Targets in SJIT Samples by the UPLC–MS/MS MRM Method

The UPLC–MS/MS MRM analytical method developed and validated in this study was applied for the quantitative analysis of SJIT samples. As shown in Table 5, for samples

SJIT–1 to SJIT–4, the concentrations of the components ranged from below the LOD to 9.553 mg/g. Liquiritin apioside (**5**) and glycyrrhizin (**14**), markers of *G. uralensis*, and neomangiferin (**1**) and mangiferin (**2**), markers of *A. asphodeloides*, were detected in relatively large amounts compared with other components. Dihydropachymic acid (**17**), a marker of *P. cocos*, was detected only in SJIT–4 (0.030 mg/g). Previously, Li et al. and Miyaoka et al. performed simultaneous analysis and fingerprinting to evaluate the quality of SJIT using HPLC [41,42]. In their assay, active ingredients such as mangiferin, liquiritin, ferulic acid, and glycyrrhizin were detected. Among the components detected, glycyrrhizin was most abundant, which showed similar results to our analysis. In the simultaneous analysis of SJIT, it is considered that an accurate and sensitive UPLC–MS/MS analysis method with a short operation time compared to previous HPLC analysis results is considered to be effective.

Table 5. Quantification (mg/g) of the 18 components in SJIT–1 to SJIT–4 samples by the UPLC–MS/MS MRM analytical method.

Analyte [1]	SJIT–1 [2]		SJIT–2		SJIT–3		SJIT–4	
	Mean (mg/g)	RSD (%)	Mean (mg/g)	RSD (%)	Mean (mg/g)	RSD (%)	Mean (mg/g)	RSD (%)
1	2.265	4.687	0.043	5.906	0.648	7.163	0.406	4.768
2	9.553	2.823	0.087	3.694	0.510	0.486	3.751	5.533
3	2.885	0.539	0.096	7.088	0.100	1.462	1.128	5.727
4	1.481	1.204	0.218	4.676	0.053	4.677	0.368	2.905
5	3.368	3.073	0.260	1.309	0.246	0.470	0.960	2.167
6	0.911	1.188	0.062	6.332	0.032	2.652	0.055	8.212
7	0.429	1.702	0.015	6.049	0.037	4.512	0.226	6.107
8	0.412	1.693	0.076	6.829	0.023	3.091	0.216	3.549
9	0.016	4.267	0.007	5.049	0.005	6.585	0.061	9.730
10	0.325	3.114	0.048	5.819	0.027	0.911	0.052	5.128
11	0.035	3.444	0.002	6.671	0.001	0.886	0.173	0.323
12	0.162	4.013	ND	–	ND	–	0.140	4.147
13	0.006	5.085	0.002	3.399	ND	–	0.032	2.157
14	9.084	1.003	1.097	9.726	0.816	2.173	2.564	2.621
15	0.050	7.549	ND	–	0.007	7.890	0.077	2.755
16	2.962	4.361	ND	–	0.174	0.651	2.019	1.653
17	ND [3]	–	ND	–	ND	–	0.030	3.253
18	ND	–	ND	–	0.001	4.623	0.080	5.340

[1] Neomangiferin (**1**), mangiferin (**2**), magnoflorine (**3**), spinosin (**4**), liquiritin apioside (**5**), liquiritin (**6**), ferulic acid (**7**), 6‴-feruloyl spinosin (**8**), isoliquiritin (**9**), ononin (**10**), liquiritigenin (**11**), jujuboside A (**12**), isoliquiritigenin (**13**), glycyrrhizin (**14**), jujuboside B (**15**), *Z*-ligustilide (**16**), dehydropachymic acid (**17**), and pachymic acid (**18**). [2] SJT–1: SJT water extract manufactured by Korea Institute of Oriental Medicine (KIOM), SJT–2 and SJT–3: commercial granules produced by Korean pharmaceutical companies, SJT–4: commercially available granule produced by Japanese pharmaceutical company. [3] ND: not detected.

4. Conclusions

In the present study, we developed a simultaneous analysis method using UPLC–MS/MS to quantify 18 components for the efficient quality control of SJIT, which is a prescription medicine used for insomnia. The developed assay was validated with respect to linearity, LOD, LOQ, recovery, and precision. The established analytical method was then applied to the simultaneous analysis of real SJIT samples, and the content of the 18 target components was analyzed simultaneously. Among them, neomangiferin, mangiferin, liquiritin apioside, glycyrrhizin, and Z-ligustilide, which are the main components of *A. asphodeloides*, *G. uralensis*, and *C. officinale*, were found to be relatively abundant compared with the other components. The method is therefore concluded to be suitable for collecting basic data for future clinical and efficacy studies.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/separations10070411/s1, Figure S1: Chemical structures of the eighteen target components in SJIT; Figure S2: Precursor ion spectrum of jujuboside A; Figure S3: Total ion chromatograms of SJIT–2 to SJIT–4 samples by the UPLC–MS/MS MRM method. Neomangiferin (**1**), mangiferin (**2**), magnoflorine (**3**), spinosin (**4**), liquiritin apioside (**5**), liquiritin (**6**), ferulic acid (**7**), 6‴-feruloyl spinosin (**8**), isoliquiritin (**9**), ononin (**10**), liquiritigenin (**11**), jujuboside A (**12**), isoliquiritigenin (**13**), glycyrrhizin (**14**), jujuboside B (**15**), *Z*-ligustilide (**16**), dehydropachymic acid (**17**), and pachymic acid (**18**); Figure S4: Extracted ion chromatograms of standard compounds (A) and SJIT–1 to SJIT–4 samples (B–E) by UPLC–MS/MS method. Compounds **1**–**18** as in Figure S2; Table S1: Composition of SJIT; Table S2: LC–MS/MS MRM analysis conditions for quantification of markers in SJIT.

Author Contributions: Conceptualization, C.-S.S. and H.-K.S.; methodology, validation, formal analysis, investigation, data curation, writing—original draft preparation, writing—review and editing, C.-S.S.; project administration, funding acquisition, H.-K.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Korea Institute of Oriental Medicine (KSN2022310 and KSN1823311).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All the data in this study can be found in this paper.

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

References

1. Yeh, C.H.; Arnold, C.K.; Chen, Y.H.; Lai, J.N. Suan zao ren tang as an original treatment for sleep difficulty in climacteric women: A prospective clinical observation. *Evid.-Based Complement. Altern. Med.* **2011**, *2011*, 673813. [CrossRef] [PubMed]
2. Cao, J.X.; Zhang, Q.Y.; Cui, S.Y.; Cui, X.Y.; Zhang, J.; Zhang, Y.H.; Bai, Y.J.; Zhao, Y.Y. Hypnotic effect of jujubosides from *Semen Ziziphi Spinosae*. *J. Ethnopharmacol.* **2010**, *130*, 163–166. [CrossRef] [PubMed]
3. Wu, H.C.; Chen, Y.H.; Lai, J.N.; Hwang, J.S.; Wang, J.D. Improving sleep quality in climacteric women with insomnia: A randomized, head-to-head trial between *Jia-Wei-Shiau-Yau San* (*JWSYS*) and *Suan-Zao-Ren Tang* (*SZRT*). *Eur. J. Integr. Med.* **2011**, *3*, 143–151. [CrossRef]
4. Peng, W.H.; Hsieh, M.T.; Lee, Y.S.; Lin, Y.C.; Liao, J. Anxiolytic effect of seed of *Ziziphus jujuba* in mouse models of anxiety. *J. Ethnopharmacol.* **2000**, *72*, 435–441. [CrossRef] [PubMed]
5. Park, J.H.; Lee, H.J.; Koh, S.B.; Ban, J.Y.; Seong, Y.H. Protection of NDMA-induced neuronal cell damage by methanol extract of Zizyphi Spinosi Semen in cultured rat cerebellar granule cells. *J. Ethnopharmacol.* **2004**, *95*, 39–45. [CrossRef]
6. Kuang, X.; She, G.; Ma, T.; Cai, W.; Zhao, J.; Liu, B.; Xu, F. The pharmacology, pharmacokinetics, and toxicity of spinosin: A mini review. *Front. Pharmacol.* **2022**, *13*, 938395. [CrossRef]
7. Hoffmann, K.M.; Beltrán, L.; Ziemba, P.M.; Hatt, H.; Gisselmann, G. Potentiating effect of glabridin from *Glycyrrhiza glabra* on $GABA_A$ receptors. *Biochem. Biophys. Rep.* **2016**, *6*, 197–202.
8. Cho, S.M.; Shimizu, M.; Lee, C.J.; Han, D.S.; Jung, C.K.; Jo, J.H.; Kim, Y.M. Hypnotic effects and binding studies for $GABA_A$ and $5\text{-}HT_{2C}$ receptors of traditional medicinal plants used in Asia for insomnia. *J. Ethnopharmacol.* **2010**, *132*, 225–232. [CrossRef]
9. Shah, V.K.; Choi, J.J.; Han, J.Y.; Lee, M.K.; Hong, J.T.; Oh, K.W. Pachymic acid enhances pentobarbital-induced sleeping behaviors via $GABA_A$-ergic systems in mice. *Biomol. Ther.* **2014**, *22*, 314–320. [CrossRef]
10. Kim, H.; Park, I.; Park, K.; Park, S.; Kim, Y.I.; Park, B.G. The positive effects of *Poria cocos* extract on quality of sleep in insomnia rat models. *Int. J. Environ. Res. Public Health* **2022**, *19*, 6629. [CrossRef]
11. Lee, S.Y.; Lee, J.Y.; Kim, J.S.; Lee, J.H.; Kang, S.S. Flavonoids from the seeds of *Zizyphus jujuba* var. *spinosa*. *Korean J. Pharmacogn.* **2012**, *43*, 127–136.
12. Yang, Y.N.; Liu, Y.Y.; Feng, Z.M.; Jiang, J.S.; Zhang, P.C. Seven new flavonoid glycosides from the roots of *Glycyrrhiza uralensis* and their biological activities. *Carbohydr. Res.* **2019**, *485*, 107820. [CrossRef] [PubMed]
13. Li, L.M.; Liao, X.; Peng, S.L.; Ding, L.S. Chemical constituents from the seeds of *Ziziphus jujuba* var. *spinosa* (Bunge) Hu. *J. Intergr. Plant Biol.* **2005**, *47*, 494–498. [CrossRef]
14. 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 form the sclerotium of *Poria cocos*. *Arch. Pharm. Res.* **2004**, *27*, 829–833. [CrossRef]
15. Liu, Y.Y.; Yang, Y.N.; Feng, Z.M.; Jiang, J.S.; Zhang, P.C. Eight new triterpenoid saponins with antioxicant activity from the roots of *Glycyrrhaiza uralensis* Fisch. *Fitoterapia* **2019**, *133*, 186–192. [CrossRef] [PubMed]

16. Bae, K.E.; Choi, Y.W.; Kim, S.T.; Kim, Y.K. Components of rhizome extract of *Cnidium officinale* Makino and their in vitro biological effects. *Molecules* **2011**, *16*, 8833–8847. [CrossRef] [PubMed]
17. Baek, M.E.; Seong, G.U.; Lee, Y.J.; Won, J.H. Quantitative analysis for the quality evaluation of active ingredients in Cnidium Rhizome. *Yakhak Hoeji* **2016**, *60*, 227–234. [CrossRef]
18. Xu, L.; Li, A.; Sun, A.; Liu, R. Preparative isolation of neomangiferin and mangiferin form Rhizoma Anemarrhenae by high-speed countercurrent chromatography using ionic liquids as a two-phase solvent system modifier. *J. Sep. Sci.* **2010**, *33*, 31–36. [CrossRef]
19. Yang, B.Y.; Zhang, J.; Liu, Y.; Kuang, H.X. Steroidal saponins from the Rhizomes of *Anemarrhena asphodeloides*. *Molecules* **2016**, *21*, 1075. [CrossRef]
20. Kim, W.I.; Zhao, B.T.; Zhang, H.Y.; Lee, J.H.; Son, J.K.; Woo, M.H. Quantitative and pattern recognition analyses of magnoflorine, spinosyn, 6‴-feruloyl spinosyn and jujuboside A by HPLC in Zizyphi Semen. *Arch. Pharm. Res.* **2014**, *37*, 1139–1147. [CrossRef]
21. Niu, C.; Zhang, J. Quantitative analysis and chromatographic fingerprinting of the semen zizyphi spinosae by ultra-high-performance liquid chromatography coupled with diode-array detector. *J. Sep. Sci.* **2011**, *34*, 2989–2996. [CrossRef] [PubMed]
22. Seo, C.S.; Ha, H.; Kim, Y.J.; Jung, J.Y. HPLC–PDA simultaneous determination and protective effect of *Anemarrhena asphodeloides* against acute renal failure. *Nat. Prod. Commun.* **2014**, *9*, 829–832. [CrossRef] [PubMed]
23. Peng, Y.; Zhao, L.; Lin, D.; Liu, Y.; Zhang, M.; Song, S.J. Determination of the chemical constituents of the different processed products of *Anemarrhena asphodeloides* Rhzomes by high-performance liquid chromatography quadrupole time-of-flight mass spectrometry. *Biomed. Chromatogr.* **2016**, *30*, 508–519. [CrossRef]
24. Wu, L.F.; Wang, K.F.; Mao, X.; Liang, W.Y.; Chen, W.J.; Li, S.; Qi, Q.; Cui, Y.P.; Zhang, L.Z. Screening and analysis of the potential bioactive components of *Poria cocos* (Schw.) Wolf by HPLC and HPLC–MS^n with the aid of chemometrics. *Molecules* **2016**, *21*, 227. [CrossRef] [PubMed]
25. Zhou, S.; Cao, J.; Qiu, F.; Kong, W.; Yang, S.; Yang, M. Simultaneous determination of five bioactive components in Radix Glycyrrhizae by pressurized liquid extraction combined with UPLC–PDA and UPLC/ESI–QTOF–MS confirmation. *Phytochem. Anal.* **2013**, *24*, 527–533. [CrossRef] [PubMed]
26. 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] [PubMed]
27. Zhu, Y.; Chen, L.; Gong, J.; Wang, S. Identification of constituents in Suanzaoren tang by LC-Q-TOF-MS and LC-IT-MS. *China J. Chin. Mater. Medica* **2014**, *39*, 457–465.
28. Pang, B.; Zhu, Y.; Lu, L.; Gu, F.; Chen, H. The applications and features of liquid chromatography-mass spectrometry in the analysis of traditional Chinese medicine. *Evid.-Based Complement. Altern. Med.* **2016**, *2016*, 3837270. [CrossRef]
29. Wang, X.; Zhang, A.; Yan, G.; Han, Y.; Sun, H. UHPLC-MS for the analytical characterization of traditional Chinese medicines. *Trends Anal. Chem.* **2014**, *63*, 180–187. [CrossRef]
30. International Conference on Harmonisation (ICH). *Guidance for Industry, Q2B, Validation of Analytical Procedures: Methodology*; Food and Drug Administration: Rockville, MD, USA, November 1996.
31. Ma, C.; Wang, L.; Tang, Y.; Fan, M.; Xiao, H.; Huang, C. Identification of major xanthones and steroidal saponins in rat urine by liquid chromatography–atmospheric pressure chemical ionization mass spectrometry technology following oral administration of Rhizoma Anemarrhenae decoction. *Biomed. Chromatogr.* **2008**, *22*, 1066–1083. [CrossRef]
32. Zhang, Y.Y.; Wang, Q.; Qi, L.W.; Qin, X.Y.; Qin, M.J. Characterization and determination of the major constituents in Belamcandae Rhizoma by HPLC–DAD–ESI–MS^n. *J. Pharm. Biomed. Anal.* **2011**, *56*, 304–311. [CrossRef] [PubMed]
33. Seo, C.S.; Shin, H.K. Liquid chromatography tandem mass spectrometry for the simultaneous quantification of eleven phyto-chemical constituents in traditional Korean medicine, Sogunjung decoction. *Processes* **2021**, *9*, 153. [CrossRef]
34. Yan, Y.; Liu, J.; Zhang, M.; Zhang, Y.; Shi, B.; Qin, X.; Du, C. A strategy to explore the quality markers of Ziziphi Spinosae semen by combining metabolic in vivo study with network pharmacology. *Biomed. Chromatogr.* **2023**, *37*, e5530. [CrossRef] [PubMed]
35. Xiao, H.B.; Krucker, M.; Albert, K.; Liang, X.M. Determination and identification of isoflavnolids in Radix *astragali* by matrix solid-phase dispersion extraction and high-performance liquid chromatography with photodiode array and mass spectrometric detection. *J. Chromatogr. A* **2004**, *1032*, 117–124. [CrossRef] [PubMed]
36. Chen, L.; Qi, J.; Chang, Y.; Zhu, D.; Yu, B. Identification and determination of the major constituents in traditional Chinese medicinal formula Danggui-Shaoyao-San by HPLC–DAD–ESI–MS/MS. *J. Pharm. Biomed. Anal.* **2009**, *50*, 127–137. [CrossRef]
37. Li, Z.; Wen, R.; Du, Y.; Zhao, S.; Zhao, P.; Jiang, H.; Rong, R.; Lv, Q. Simultaneous quantification of fifteen compounds in rat plasma by LC-MS/MS and its application to a pharmacokinetic study of Chaihu-Guizhi decoction. *J. Chromatogr. B* **2019**, *1105*, 15–25. [CrossRef]
38. Zhang, Y.; Xie, J.; Zhang, Y.; Zhang, M. Degradation kinetics of jujuboside A by rat intestinal flora and identification of the metabolites by HPLC-MS/MS. *Int. J. Food Prop.* **2014**, *17*, 1841–1849. [CrossRef]
39. Meng, X.; Li, H.; Song, F.; Liu, C.; Liu, Z.; Liu, S. Studies on triterpenoids and flavones in *Glycyrrhiza uralensis* Fisch. By HPLC-ESI-MS^n and FT-ICR-MS^n. *Chin. J. Chem.* **2009**, *27*, 299–305. [CrossRef]
40. Liu, X.; Zhu, X.; Zhu, H.; Xie, L.; Ma, J.; Xu, Y.; Zhou, Q.; Wu, Z.; Cai, B. Simultaneous quantification of six bioactive components in decoction of Ziziphi spinosae semen using ultrahigh performance liquid chromatography coupled with triple-quadrupole mass spectrometry. *J. Anal. Methods Chem.* **2018**, *2018*, 8397818. [CrossRef]

41. Li, J.; Wang, H.; Liu, J.; Du, C.; Yan, Y. Quality evaluation of Suanzaoren decoction by integrating fingerprint and quantitative analysis of multi-component. *Chin. Tradit. Herb. Drugs* **2022**, *53*, 4698–4708.
42. Miyaoka, T.; Kawano, K.; Furuya, M.; Wake, R.; Hashioka, S.; Liaury, K.; Limoa, E.; Tsuchie, K.; Fukushima, M.; Araki, T.; et al. Efficay and safety of Sansoninto in insomnia with psychiatric disorder: An open-label study. *Altern. Integr. Med.* **2015**, *4*, 181. [CrossRef]