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Liquid Chromatography Tandem Mass Spectrometry for the Simultaneous Quantification of Eleven Phytochemical Constituents in Traditional Korean Medicine, Sogunjung Decoction

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Abstract: The Sogunjung decoction (SGJD) is a traditional herbal formula that has been used to treat constipation and improve the constitution of infirm children in Korea. In this study, simultaneous quantification of gallic acid (**1**), magnoflorine (**2**), albiflorin (**3**), paeoniflorin (**4**), liquiritin apioside (**5**), liquiritin (**6**), liquiritigenin (**7**), coumarin (**8**), cinnamaldehyde (**9**), benzoylpaeoniflorin (**10**), and glycyrrhizin (**11**) was conducted using fast and sensitive liquid chromatography–tandem mass spectrometry (LC–MS) multiple-reaction monitoring to develop a quality-control protocol for the SGJD. A Waters Acquity UPLC BEH C_{18} column (2.1 × 100 mm, 1.7 µm) was used for the chromatographic separation of the 11 marker compounds in the SGJD using two mobile phases (5 mM ammonium acetate in distilled water containing 0.1% (*v/v*) formic acid, and acetonitrile). The MS parameters for a simultaneous analysis were capillary voltage (3.0 kV), source temperature (150 °C), desolvation temperature (500 °C), desolvation gas flow (700 L/h), and cone gas flow (50 L/h). The developed LC–MS method was validated by the evaluation of linearity, limits of detection, limits of quantification, recovery and precision. By using the developed and validated assay, the 11 marker components in the SGJD were detected in amounts of 0.01–51.83 mg/g.

Keywords: liquid chromatography tandem mass spectrometry; simultaneous quantification; Sogunjung decoction

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1. Introduction

Traditional Korean medicine (TKM), traditional Chinese medicine (TCM), and Kampo medicine (KM) have been widely used in Asian countries such as Korea, China, and Japan to enhance human health and prevent various diseases. They are traditionally prescribed in the form of a combination of two or more herbal medicines based on a traditional medicine philosophy and have mostly been used as a decoction [1,2].

The Sogunjung decoction (SGJD) is a TKM called *Xiao Jian Zhong Tang* in China and *Shokenchuto* in Japan and was recorded for the first time in China in the *Shanghanlun*, a famous medical book of Chinese herbal medicine, written by Zhong-Jing Zhang in the third century during the Later Han Dynasty [3]. In addition, the SGJD is included in the *Donguibogam*, the definitive text of traditional Korean medicine, written by Jun Heo during the Joseon Dynasty and used to treat children's weakness, fatigue, chronic gastroenteritis, and bedwetting. The SGJD is a herbal formula made by mixing six medicinal herbs (*Paeonia lactiflora* Pallas, *Cinnamomum cassia* J. Presl, *Glycyrrhiza uralensis* Fischer, *Zingiber officinale* Roscoe, *Zizyphus jujube* Miller var. *hoonensis* T. B. Lee and *Oryza sativa* Linnè) in a defined ratio (7.5:4.5:1.5:1.0:16:15.0) [4].

The SGJD has been reported to exhibit anti-allergy and immunomodulatory effects [5,6] and genotoxicity based on Ames and micronucleus tests [7]. Recently, clinical studies on the efficacy of SGJD granules for heartburn patients have been reported [8].

Today, many analytical methods for quality control using high-performance liquid chromatography (HPLC) and liquid chromatography–tandem mass spectrometry (LC–MS) have been reported for various kinds of compounds that are contained in herbal medicines or herbal prescriptions [9–15]. Recently, a simultaneous analysis of the major components of the SGJD was reported using HPLC coupled with a photodiode array detector (PDA) [16]. This study focused on simultaneous quantification for quality control of the SGJD using HPLC–PDA. However, studies using LC–MS have not been reported.

In this study, as in the previous study [16], most of the major constituents contained in *O. sativa* were saccharides, so they were excluded from the analysis. Thus, five of the raw SGJD herbal medicines, were mixed, extracted, and then used for LC–MS analysis. The present research focused on the simultaneous analysis of 11 marker compounds using LC–MS in a multiple-reaction monitoring (MRM) mode: gallic acid (**1**), albiflorin (**3**), paeoniflorin (**4**), and benzoylpaeoniflorin (**9**) from *P. lactiflora*, coumarin (**8**) and cinnamaldehyde (**10**) from *C. cassia*, liquiritin apioside (**5**), liquiritin (**6**), liquiritigenin (**7**), and glycyrrhizin (**11**) from *G. uralensis*, and magnoflorine (**2**) from *Z. jujube*.

2. Materials and Methods

2.1. Plant Materials

As shown in Table S1, five raw plant materials (*P. lactiflora*, *C. cassia*, *G. uralensis*, *Z. officinale* and *Z. jujube*) were purchased in November 2017 from Kwangmyungdang Medicinal Herbs (Ulsan, Korea), a herbal medicine supplier for pharmaceuticals. The origin of these herbal medicines was confirmed by Dr. Seung-Yeol Oh based on *The Dispensatory on the Visual and Organoleptic Examination of Herbal Medicine* [17]. Specimens of the five raw herbs (2017KE63–1 to 2017KE63–5) were deposited at the Korea Institute of Oriental Medicine.

2.2. Chemicals and Reagents

Eleven reference standard compounds (Figure S1) for the LC–MS/MS analysis of the SGJD were purchased from commercial suppliers: compounds **1** ($C_7H_6O_5$, 100.0%), **2** ($C_{20}H_{24}NO_4$, 99.0%) and **8** ($C_9H_6O_2$, 99.0%) from KGaA (Darmstadt, Germany); compounds **3** ($C_{23}H_{28}O_{11}$, 99.8%), **6** ($C_{21}H_{22}O_9$, 99.6%), **10** ($C_9H_8O_5$, 98.0%), and **11** ($C_{42}H_{62}O_{16}$, 99.4%) from Fujifilm Wako Pure Chemical Co., Ltd. (Osaka, Japan); compounds **4** ($C_{23}H_{28}O_{11}$, 99.4%), **5** ($C_{26}H_{30}O_{13}$, 98.0%), and **9** ($C_{30}H_{32}O_{12}$, 98.0%) from Shanghai Sunny Biotech Co., Ltd. (Shanghai, China); and compound **7** ($C_{15}H_{12}O_4$, 98.0%) from ChemFaces Biochemical Co., Ltd. (Wuhan, China). Methanol (MeOH; LC–MS-grade) and acetonitrile (ACN; HPLC-grade) were purchased from ThermoFisher Scientific (San Jose, CA, USA) and J.T. Baker (Phillipsburg, NJ, USA), respectively. Distilled water (DW) was produced with a Vivagen water purification system (EXL3 Analysis 16, Seongnam, Korea). Formic acid (FA; $\geq$99.5%) and ammonium formate (AF; 99.0%) were purchased from Fujifilm Wako Pure Chemical (Osaka, Japan) and Kanto Chemical Co., Inc. (Tokyo, Japan), respectively.

2.3. Preparation of SGJD Aqueous Extract

The SGJD was prepared from a formulation of the five raw herbs: *P. lactiflora* (2329.2 g), *C. cassia* (1397.5 g), *G. uralensis* (465.8 g), *Z. officinale* (310.6 g) and *Z. jujube* (496.9 g). Each herb was mixed and extracted according to the previously reported method [16]. The extract was freeze-dried to obtain an aqueous powder extract (887.1 g).

2.4. Preparation of Samples and Standard Solutions for LC–MS/MS MRM Analysis

A sample preparation for the quantification of compounds **1**–**11** in the SGJD by the LC–MS/MS MRM mode was prepared by adding 50 mL of 70% MeOH to 35.3 mg of a lyophilized and homogenized SGJD sample, followed by ultrasonic extraction with a Branson 8510 ultra-sonicator (Denbury, CT, USA) for 5 min at room temperature. The sample was then purified by vortexing for 1 min and filtering through a 0.2 µm hydrophobic filter. The filtrate was diluted 50 times with 70% MeOH and used for LC–MS/MS analysis.

Standard solutions of compounds **1**–**11** were made to a concentration of 100.0 μg/mL in MeOH, and stored in a refrigerator (ca. 4 °C) after preparation.

2.5. LC–MS/MS Equipment and Operating Conditions for Quantification of the Compounds **1**–**11**

Quantification of compounds **1**–**11** in the SGJD was applied by modifying the analysis method that was developed in the previously study [18]. Briefly, LC–MS/MS analysis was performed with a Waters Acquity UPLC system (Milford, MA, USA) consisting of two pumps, a degasser, column oven and auto-sampler and a Waters Xevo TQ–XS triple quadrupole mass spectrometry system (Milford, MA, USA) coupled with an electrospray ionization (ESI) source operating in either negative or positive ion mode. Chromatographic separation of the 11 markers was performed with an Acquity UPLC BEH C_{18} column (2.1 × 100 mm, 1.7 μm, Waters, Milford, MA, USA) maintained at 45 °C. The mobile phase was 5 mM AF in distilled water containing 0.1% (*v*/*v*) FA (solvent A) and ACN (solvent B), and gradient elution was 20–95% B in 0–14.0 min, 95–100% B in 14.0–15.0 min, 100–20% B in 15.0–15.1 min and 20% B in 15.1–18.0 min. The flow rate and injection volume were 0.3 mL/min and 2.0 μL, respectively.

The ionization mode, collision energy, cone voltage, and transition information (precursor ion and product ion) for an LC–MS/MS MRM analysis of compounds **1**–**11** are summarized in Table 1. Other parameters for an LC–MS/MS MRM analysis were capillary voltage (3.0 kV), source temperature (150 °C), desolvation temperature (500 °C), desolvation gas flow (700 L/h) and cone gas flow (50 L/h). The system and all data collection were controlled with Waters MassLynx software (Version 4.2, Milford, MA, USA).

Table 1. LC–MS/MS MRM conditions for the simultaneous quantification of compounds **1**–**11**.

Analyte	Ion Mode	Molecular Weight	Precursor Ion	Product Ion	Cone Voltage (V)	Collision Energy (eV)	Retention Time (min)
1	negative	170.12	169.0	125.0	25	15	1.01
2	positive	342.41	342.4	297.2	30	20	1.31
3	positive	480.46	481.4	197.1	20	15	1.40
4	negative	480.46	479.2	121.0	32	25	1.45
5	negative	550.51	549.3	255.0	45	30	1.53
6	negative	418.39	417.4	255.2	30	15	1.65
7	positive	256.25	257.2	137.0	35	25	2.99
8	positive	146.15	147.1	91.0	30	20	3.06
9	negative	584.57	583.4	121.0	40	25	2.31
10	positive	132.16	133.1	115.0	25	15	4.42
11	negative	822.93	821.9	351.2	45	40	5.20

2.6. Validation of the LC–MS/MS Analysis Method

The developed LC–MS/MS MRM analysis method was validated by establishing linearity, range, limit of detection (LOD), limit of quantification (LOQ), accuracy, repeatability and intra- and inter-day precisions. The linearity was evaluated as the coefficient of determination (r^2) of the calibration curve prepared in different concentration ranges: 50.00–800.00 ng/mL for compound **1**, 0.05–0.80 ng/mL for compound **2**, 100.00–1600.00 ng/mL for compounds **3**, **10**, and **11**, 250.00–4000.00 ng/mL for compound **4**, 25.00–400.00 ng/mL for compound **5**, 10.00–160.00 ng/mL for compounds **6** and **9**, 1.00–16.00 ng/mL for compound **7** and 5.00–80.00 ng/mL for compound **8**. LOD and LOQ were calculated based on signal-to-noise ratios of 3 and 10, respectively.

The accuracy of the developed method was evaluated based on extraction recovery tests. Briefly, the recovery (%) was measured by adding a standard compound (low, medium, and high) of a known concentration to the SGJD sample and calculated from Equation (1):

$$\text{Recovery } (\%) = \frac{\text{measured amount}}{\text{spiked amount}} \times 100. \quad (1)$$

To evaluate the precision of the developed LC–MS/MS MRM analysis method, repeatability and intra- and inter-day precisions were measured, and the relative standard deviation (RSD) value of each precision was measured. The repeatability of the method was established by repeated measurements of a mixed standard solution, and the RSD values of the retention time and peak area of each compound were calculated. Intra- and inter-day precision was established by calculating the RSD of the analysis of a mixed standard solution on one day and on three consecutive days, respectively. The RSD was calculated by Equation (2):

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

3. Results and Discussion

3.1. Optimization of LC–MS/MS Conditions

The analysis conditions for the LC–MS/MS MRM method were optimized for the simultaneous quantification of the 11 marker components for quality control of the SGJD. The optimal analysis was established with an Acquity UPLC BEH C_{18} column (2.1 × 100 mm, 1.7 μm), a column temperature of 45 °C, two mobile-phase system (0.1% (*v/v*) FA and 5 mM AF in DW, and ACN) and a flow rate of 0.3 mL/min, respectively. By using the optimized LC–MS/MS MRM method, compounds **1–11** were detected within 6 min (Figure 1, Table 1).

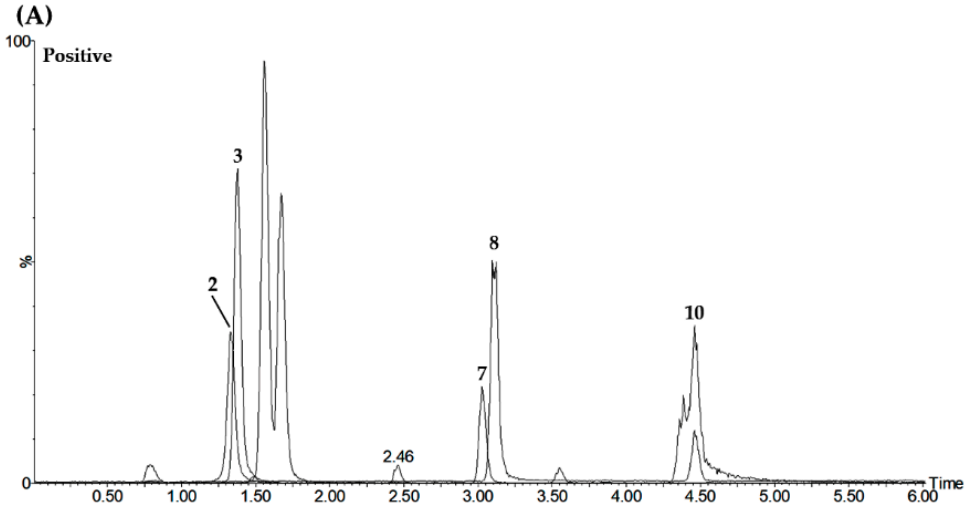

Figure 1. *Cont.*

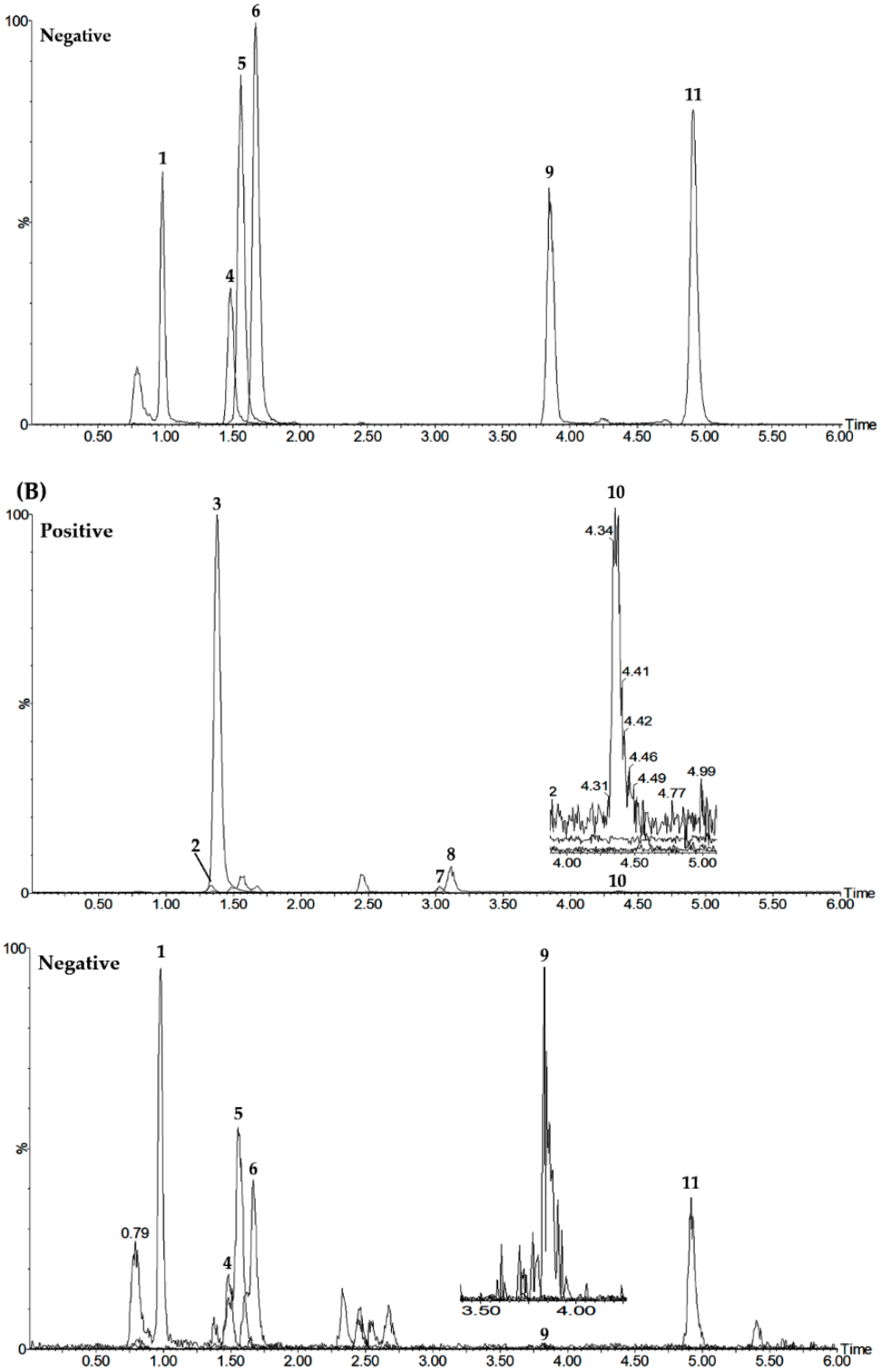

Figure 1. Total ion chromatograms of mixtures of the compounds **1**–**11** (**A**) and 70% methanol extract of the freeze-dried Sogunjung decoction (SGJD) sample (**B**) measured by LC–MS/MS MRM in positive and negative ion modes. Gallic acid (**1**), magnoflorine (**2**), albiflorin (**3**), paeoniflorin (**4**), liquiritin apioside (**5**), liquiritin (**6**), liquiritigenin (**7**), coumarin (**8**), cinnamaldehyde (**9**), benzoylpaeoniflorin (**10**) and glycyrrhizin (**11**).

3.2. Identification of Compounds **1**–**11** *for LC–MS/MS MRM Assay*

The LC–MS/MS analysis of compounds **1**–**11** in MRM mode was performed using the ESI source (negative and positive ion modes). Precursor ions for compounds **1**, **4**–**6**, **9** and

11 were detected at *m/z* 169.0, 479.2, 549.3, 417.4, 583.4 and 821.9 in the negative ion mode ($[M–H]^-$), respectively, whereas compounds **2** (detected $[M]^+$), **3**, **7**, **8** and **10** were detected at *m/z* 342.4, 481.4, 257.2, 147.1 and 133.1 in the positive ion mode ($[M+H]^+$), respectively (Figure 1, Table 1). Under the optimized LC–MS/MS MRM conditions as shown in Table 1, the precursor ion (Q1) and product ion (Q3) of each marker compound were set and analyzed. The Q3 peak of compound **1** in LC–MS/MS MRM was detected at *m/z* 125.0 (Q3) as $[M-H]^-$. This Q3 ion, $[M-H-COOH]^-$, was formed by the loss of the carboxylic acid group from Q1 [19]. The Q3 peak for aporphine alkaloid, compound **2**, was detected at *m/z* 297.2 as $[M-(CH_3)_2NH]^+$, with the loss of a mass of a dimethyl amine group from the Q1 peak [20]. Monoterpenoid compound **3** was detected in the Q3 peak at *m/z* 481.4 (Q1) with *m/z* 197.1 in the form of [M+H–Glu–benzoic acid]$^+$, in which one glucose molecule and benzoic acid were eliminated [21]. Q3 signals from compounds **4** and **9**, both at *m/z* 121.0, were observed in the form of [benzoic acid$-$H]$^-$ in which 358 Da and 462 Da were eliminated from each of the Q1 peaks [21,22]. Compounds **5**, **6**, and **11** were detected at *m/z* 255.0 ($[M–H–Api–Glu]^-$), 255.2 ($[M-H-Glc]^-$) and 351.2 ($[M–H–2Glu]^-$), as Q3 peaks, respectively; these ions were formed by loss of the apiosyl glucoside, one glucose, and two glucose groups from each Q1 peak [23,24]. The Q3 peaks of compounds **7** and **8** were detected at *m/z* 137.0 ($[M+H–C_8H_8O_8]^+$) and 91.0 $[M+H–2CO]^+$, respectively; they were formed by the removal of $C_8H_8O_8$ and two CO molecules from each Q1 peak [24,25]. The Q3 peak of compound **10** was detected at *m/z* 115.0 $[M+H-H_2O]^+$, upon the elimination of one water molecule from the Q1 $[M+H]^+$ species at *m/z* 133.1 [26]. The Q1 and Q3 spectra of compounds **1–11** are shown in Figure S2.

3.3. Method Validation of the LC–MS/MS MRM Assay

The LC–MS/MS analysis developed in this study was verified with respect to linearity, LOD, LOQ, accuracy and precision. As shown in Table 2, the r^2 values of all compounds were >0.99, which showed that the linearity was good, and the LOD and LOQ values were also calculated as 0.004–32.468 ng/mL and 0.013–97.403 ng/mL, respectively. The extraction recovery test for evaluation of the accuracy of the analytical method calculated from Equation (1) (see Section 2.6) was 96.43–107.60 (Table 3). In the verification of repeatability and intra- and inter-day precisions, the RSD (%) values of compounds **1–11** were less than 9.00%, showing good precision (Table 4). These results suggest that the developed method is an appropriate analysis method for quality control of the SGJD.

Table 2. Linear range, regression equation, coefficient of determination (r^2), limit of detection (LOD), and limit of quantification (LOQ) for LC–MS/MS MRM analysis of compounds **1–11** (n = 3).

Analyte	Linear Range (ng/mL)	Regression Equation [a] y=ax+b	r^2	LOD (ng/mL)	LOQ (ng/mL)
1	50.00–800.00	$y = 143.39x - 308.07$	0.9951	5.892	17.675
2	0.05–0.80	$y = 48{,}051.20x + 142.79$	0.9980	0.004	0.013
3	100.00–1600.00	$y = 1365.54x + 1498.38$	0.9997	5.892	17.675
4	250–4000.00	$y = 4.46x - 284.30$	0.9977	26.596	79.787
5	25.00–400.00	$y = 164.29x + 467.48$	0.9977	3.970	11.911
6	10.00–160.00	$y = 502.82x + 116.66$	0.9986	1.089	3.267
7	1.00–16.00	$y = 5023.03x + 301.33$	0.9996	0.148	0.444
8	5.00–80.00	$y = 2964.74x + 5340.70$	0.9954	0.784	2.352
9	10.00–160.00	$y = 6.56x - 54.78$	0.9953	3.289	9.868
10	100.00–1600.00	$y = 18.81x + 1050.19$	0.9953	32.468	97.403
11	100.00–1600.00	$y = 33.10x + 124.16$	0.9985	2.323	6.969

[a] y: peak area of compounds; x: concentration of compounds.

Table 3. Extract recovery tests for compounds **1**–**11** in SGJD (n = 5).

Analyte	Spiked Amount (μg/mL)	Found Amount (μg/mL)	Recovery (%)	SD	RSD (%)
1	200.00	208.36	104.18	4.02	3.86
	400.00	408.88	102.22	2.92	2.86
	800.00	809.50	101.19	2.54	2.51
2	0.40	0.39	96.50	5.48	5.68
	0.80	0.80	99.75	3.47	3.48
	1.60	1.64	102.75	3.47	3.48
3	1000.00	1000.30	100.03	2.20	2.20
	2000.00	1945.72	97.29	1.99	2.05
	4000.00	3908.46	97.71	1.46	1.49
4	1000.00	1029.74	102.97	4.89	4.74
	2000.00	2024.48	101.22	2.05	2.02
	4000.00	4018.36	100.46	1.75	1.74
5	100.00	102.80	102.80	4.28	4.16
	200.00	199.78	99.89	6.22	6.23
	400.00	366.72	99.18	1.79	1.80
6	20.00	20.02	100.10	4.35	4.35
	40.00	40.58	101.45	4.35	4.29
	80.00	78.48	98.10	1.14	1.16
7	4.00	4.10	102.50	8.84	8.62
	8.00	7.84	98.00	3.38	3.45
	16.00	15.88	99.25	2.23	2.24
8	20.00	19.44	97.20	6.08	6.25
	40.00	39.46	98.65	5.16	5.23
	80.00	80.90	101.13	2.98	2.95
9	40.00	43.04	107.60	12.06	11.20
	80.00	77.14	96.43	7.14	7.40
	160.00	166.86	104.29	4.34	4.16
10	400.00	394.68	98.67	8.66	8.78
	800.00	798.48	99.81	1.41	1.41
	1600.00	1618.56	101.16	3.28	3.24
11	200.00	200.46	100.23	4.51	4.50
	400.00	410.68	102.67	2.81	2.74
	800.00	798.08	99.76	3.75	3.76

Table 4. Precision of LC–MS/MS MRM analytical method for compounds **1**–**11** in SGJD.

Analyte	Conc. (μg/mL)	Intraday (n = 5)			Interday (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
1	200.00	208.86	5.14	104.43	208.20	2.56	104.11	0.42	1.30
	400.00	408.32	2.76	102.08	409.70	1.62	102.43		
	800.00	807.32	2.14	100.92	809.90	1.67	101.24		
2	0.40	208.86	5.14	104.43	208.22	2.56	104.11	0.48	1.39
	0.80	408.32	2.76	102.08	409.72	1.62	102.43		
	1.60	807.32	2.14	100.92	809.92	1.67	101.24		
3	1000.00	0.39	4.96	97.00	0.40	4.81	99.83	0.30	1.68
	2000.00	0.77	4.43	96.50	0.81	1.50	101.00		
	4000.00	1.54	3.52	96.00	1.62	1.26	101.17		

Table 4. *Cont.*

Analyte	Conc. (μg/mL)	Intraday (n = 5)			Interday (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
	1000.00	1025.88	1.30	102.59	1033.23	0.99	103.32		
4	2000.00	1923.94	1.97	96.20	1968.95	0.40	98.45	0.35	1.52
	4000.00	3837.44	1.85	95.94	3928.37	1.18	98.21		
	100.00	1037.62	6.66	103.76	1034.42	4.23	103.44		
5	200.00	2016.74	2.44	100.84	2047.77	1.17	102.39	0.33	1.89
	400.00	3966.68	2.55	99.17	4030.94	0.94	100.77		
	20.00	99.30	3.73	99.30	101.97	2.52	101.97		
6	40.00	195.08	3.59	97.54	203.38	2.14	101.69	0.31	0.92
	80.00	391.26	2.19	97.82	402.52	1.39	100.63		
	4.00	19.60	6.03	98.00	20.43	3.08	102.13		
7	8.00	37.54	2.70	93.85	40.89	2.11	102.23	0.25	4.88
	16.00	75.90	3.22	94.88	80.08	1.76	100.10		
	20.00	4.04	2.82	101.00	4.11	3.17	102.83		
8	40.00	8.22	2.00	102.75	8.05	2.34	100.58	0.24	3.94
	80.00	15.82	2.77	98.88	15.93	2.40	99.54		
	40.00	20.12	8.51	100.60	20.61	3.45	103.03		
9	80.00	38.48	3.96	96.20	40.22	2.93	100.55	0.21	2.07
	160.00	78.26	3.31	97.83	80.54	1.77	100.68		
	400.00	39.22	3.55	98.05	40.89	5.29	102.23		
10	800.00	75.76	3.05	94.70	79.60	1.79	99.50	0.26	3.84
	1600.00	161.86	0.55	101.16	165.99	1.37	103.74		
	200.00	386.18	1.32	96.55	388.75	2.77	97.19		
11	400.00	796.46	2.24	99.56	798.15	0.88	99.77	0.11	1.26
	800.00	1537.72	2.19	96.11	1575.34	1.85	98.46		

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

3.4. Quantification of Compounds **1**–**11** *in SGJD Samples by LC–MS/MS MRM Analysis*

The developed and validated LC–MS/MS analysis method was applied for quantitative analysis of compounds **1**–**11** in the SGJD. The retention times of compounds **1**–**11** in the sample were 1.01, 1.31, 1.40, 1.45, 1.53, 1.65, 2.99, 3.06, 2.31, 4.42 and 5.20 min, respectively (Figure 1 and Figure S3). As shown in Table 1, an analysis of the SGJD under the optimized MRM conditions for each compound showed that compounds **1**–**11** were present in the sample in amounts of 0.01–51.83 mg/g. Among these markers, compounds **3** and **4**, the major constituents of *P. lactiflora*, were found to be present in the highest amounts (25.38 mg/g and 51.83 mg/g, respectively) (Table 5). These results showed that the contents of compounds 3, 4 (the main components of *P. lactiflora*), and 11 (the main components of *G. uralensis*) were high in the same pattern as were the results analyzed by HPLC analysis, and this confirm the possibility of use as an analysis method for quality control of the SGJD.

Table 5. Amount of compounds **1**–**11** in SGJD determined by LC–MS/MS MRM analysis (n = 3).

Analyte	Amount		
	Mean (mg/g)	SD ($\times 10^{-1}$)	RSD (%)
1	5.69	3.73	6.54
2	0.01	0.01	4.76
3	25.38	4.19	1.65
4	51.83	5.56	1.07
5	3.37	1.58	4.71
6	0.79	0.15	1.87
7	0.10	0.04	4.22
8	0.66	0.42	6.45
9	1.69	0.90	5.29
10	8.02	7.27	9.05
11	9.28	4.25	4.58

4. Conclusions

In this study, a sensitive, accurate, and fast LC–MS/MS MRM analysis method for quality control of the SGJD was first developed using the selected compounds **1**–**11**. The method was verified by assessing the linearity, LOD, LOQ, accuracy, and precision parameters, and was successfully applied to sample analysis. The proposed LC–MS/MS MRM assay will be useful for the quality evaluation of the SGJD and for further research on other TKM, TCM, and KM along with the HPLC–PDA assay.

Supplementary Materials: The following are available online at https://www.mdpi.com/2227-9717/9/1/153/s1, Figure S1. Chemical structures of compounds **1**–**11** in SGJD, Figure S2. Precursor ion (Q1, A) and product ion (Q3, B) spectra of compounds **1**–**11**. Gallic acid (**1**), magnoflorine (**2**), albiflorin (**3**), paeoniflorin (**4**), liquiritin apioside (**5**), liquiritin (**6**), liquiritigenin (**7**), coumarin (**8**), cinnamaldehyde (**9**), benzoylpaeoniflorin (**10**), and glycyrrhizin (**11**), Figure S3. Extracted ion chromatograms of the reference standard (A) and SGJD sample (B) by LC–MS/MS MRM mode, Table S1: Composition of prepared SGJD.

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