Article

Quantitative Analysis of 18 Marker Components in the Traditional Korean Medicine, Cheongsangbangpung-Tang, Using High-Performance Liquid Chromatography Combined with Photodiode Array Detector

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Abstract: Cheongsangbangpung-tang (CSBPT) is a traditional herbal medicine that has been used in many regions of Asia, including Korea, China, and Japan, for the treatment of purulent inflammation and eczema on the face. In this study, a method for the simultaneous analysis of 18 marker components, geniposide (1), coptisine chloride (2), *prim-O*-glucosylcimifugin (3), berberine chloride (4), liquiritin apioside (5), liquiritin (6), ferulic acid (7), narirutin (8), 5-*O*-methylvisammisoid (9), hesperidin (10), arctigenin (11), baicalin (12), oxypeucedanin hydrate (13), wogonoside (14), baicalein (15), arctiin (16), glycyrrhizin (17), and pulegone (18), was developed for quality control of CSBPT. The novel approach, which is based on high-performance liquid chromatography (HPLC) separation coupled with photodiode array detection, was verified by the assessment of linearity, limit of detection, limit of quantification, accuracy, recovery, and precision. Analysis of CSBPT by using the established assay revealed that compounds 1–18 were present in concentrations of 0.27–18.31 mg/g.

Keywords: quantitative analysis; Cheongsangbangpung-tang; purulent inflammation; high-performance liquid chromatography

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1. Introduction

Oriental medical prescriptions consisting of a combination of two or more medicinal herbs and containing many components have been widely used in Asian countries, such as Korea, China, and Japan, to enhance human health and prevent various diseases. However, there is a limit to continuous use without standardization of these herbal medicine prescriptions. Therefore, standardization for their continuous efficacy and quality control must be performed.

Cheongsangbangpung-tang (CSBPT; “Qing-Shang-Fang-Feng-Tang” in Chinese and “Seijo-bofu-to” in Japanese) is an oriental medical prescription that has been used in the treatment of purulent inflammation including acne and boils on the face and head [1,2]. CSBPT was first recorded in Gogeumuigam of Gong Shin in the Ming Dynasty of China, and it has also been included in Dongeuibogam of Heo Jun in the Joseon Dynasty of Korea [1,2]. This prescription consists of a combination of 12 medicinal herbs: *Saposhnikovia Radix*, *Angelica Dahurica Radix*, *Forsythia Fructus*, *Platycodon Radix*, *Scutellaria Radix*, *Cnidii Rhizoma*, *Schizonepetae Spica*, *Gardenia Fructus*, *Coptidis Rhizoma*, *Aurantii Fructus Immaturus*, *Menthae Herba*, and *Glycyrrhizae Radix et Rhizoma*, in 2.5:2.7:2.7:2.7:2.3:2.3:1.7:1.7:1.7:1.7:1.0 ratio [2].

Studies have been reported on the efficacy of CSBPT with respect to its antibacterial effects on *Propionibacterium acnes* and *Staphylococcus aureus* [3–5], its effect on atopic allergy [6], skin regeneration, wrinkle improvement, whitening, and moisturizing effects [1]. Saruwatari et al. [7] assessed the safety of this prescription in a study on herbal drug interactions with cytochrome P450 (CYPs), *N*-acetyltransferase 2, and xanthine oxidase.

Recently, Yang et al. [8] conducted and reported a pilot study on the effectiveness and safety of chronic urticarial in combination with Xiao-Feng-San and Qing-Shang-Fang-Feng-Tang.

The main ingredients of each medicinal herb that makes up CSBPT are as follows: chromones (e.g., cimifugin, *prim-O*-glucosylcimifugin, and 5-*O*-methylvisammioside) from *Saposhnikovia Radix* [9], coumarins (e.g., imperatorin, phellopterin, and isoimperatorin) from *Angelica Dahurica Radix* [10], debenzylbutyrolactone lignans (e.g., matairesinol, arctiin, and arctigenin) from *Forsythia Fructus* [11], platycosides (e.g., platycodon D and platycodin D2) from *Platycodonis Radix* [12], flavonoids (e.g., baicalin, wogonoside, baicalein, and wogonin) from *Scutellaria Radix* [13], phenylpropanoids (e.g., ferulic acid) and miscellaneous (e.g., *z*-ligustilide) from *Cnidii Rhizoma* [14], essential oils (e.g., pulegone) from *Schizonepetae Spica* [15], iridoid glycosides (e.g., geniposide) from *Gardenia Fructus* [16], alkaloids (e.g., berberine chloride and coptisine chloride) from *Coptidis Rhizoma* [17], flavonoids (e.g., narirutin, naringin, and hesperidin) from *Aurantii Fructus Immaturus* [18], essential oils (e.g., menthol and menthone) from *Menthae Herba* [19], and triterpenes (e.g., glycyrrhizin) and flavonoids (e.g., liquiritin apioside and liquiritin) from *Glycyrrhizae Radix et Rhizoma* [20].

There have been many simultaneous analysis methods reported for the quality assessment of each constituent herbal medicine of CSBPT based on high-performance liquid chromatography (HPLC), gas chromatography, and liquid chromatography–mass spectrometry [9–20]. However, no method of analysis for the quality control of CSBPT has been established.

In this study, a simultaneous quantitative analysis method was developed using 18 selected marker analytes for the quality evaluation of CSBPT based on HPLC separation combined with photodiode array detection (PDA). The selected components were *prim-O*-glucosylcimifugin (3) and 5-*O*-methylvisammioside (9) from *Saposhnikovia Radix*, oxypeucedanin hydrate (13) from *Angelica Dahurica*, arctigenin (11) and arctiin (16) from *Forsythia Fructus*, baicalin (12), wogonoside (14), and baicalein (15) from *Scutellaria Radix*, ferulic acid (7) from *Cnidii Rhizoma*, pulegone (18) from *Schizonepetae Spica*, geniposide (1) from *Gardenia Fructus*, coptisine chloride (2) and berberine chloride (4) from *Coptidis Rhizoma*, narirutin (8) and hesperidin (10) from *Aurantii Fructus Immaturus*, and liquiritin apioside (5), liquiritin (6), and glycyrrhizin (17) from *Glycyrrhizae Radix et Rhizoma*.

2. Materials and Methods

2.1. Plant Materials

Twelve crude plant materials, shown in Table S1, were purchased from a pharmaceutical manufacturer of herbal medicine (Kwangmyungdang Medicinal Herbs (KMH); Ulsan, Korea) in November 2017. The origins of these plant materials were confirmed by Dr. Seung-Yeol Oh, president of KMH, according to the guideline “The Dispensatory on the Visual and Organoleptic Examination of Herbal Medicine” [21]. Voucher specimens (2018KE69–1 to 2018KE63–12) of the crude plant material were deposited at the Herbal Medicine Research Division, Korea Institute of Oriental Medicine (KIOM, Daejeon, Korea).

2.2. Chemicals and Reagents

The standards used in this study were supplied from manufacturers specializing in standard compounds: compounds 1 (CAS No. 24512-62-7, ≥98.0%), 2 (CAS No. 6020-1804, ≥98.0%), 6 (CAS No. 551-15-5, 99.6%), 7 (CAS No. 1135-24-6, 98.0%), 12 (CAS No. 21967-41-9, 98.0%), and 17 (CAS No. 1405-86-3, 99.4%) from Fujifilm Wako Pure Chemical Co., Ltd. (Osaka, Japan); compound 3 (CAS No. 80681-45-4, 99.4%) from ChemNorm Biotech Co., Ltd. (Wuhan, China); compounds 4 (CAS No. 633-65-8, 98.0%) and 15 (CAS No. 491-67-8, 98.0%) from KGaA (Darmstadt, Germany); compounds 5 (CAS No. 74639-14-8, ≥98.0%), 11 (CAS No. 7770-78-7, 99.4%), and 16 (CAS No. 20362-31-6, 98.1%) from Shanghai Sunny Biotech Co., Ltd. (Shanghai, China); compounds 8 (CAS No. 14259-46-2, 99.5%), 9 (CAS No. 84272-85-5, 99.9%), 10 (CAS No. 520-26-3, 98.6%), and 18 (CAS No. 89-82-7, 99.3%) from

Biopurify Phytochemicals (Chengdu, China); compound **13** (CAS No. 2643-85-8, 98.0%) from ChemFaces Biochemical Co., Ltd. (Wuhan, China); and compound **14** (CAS No. 51059-44-0, 98.2%) from Tauto Biotech (Shanghai, China). HPLC-grade acetonitrile, water, and trifluoroacetic acid ($\geq 99.0\%$), and ACS reagent-grade formic acid ($\geq 98.0\%$), glacial acetic acid ($\geq 100.0\%$), and phosphoric acid (85.0%) were used in the mobile phase, and they were purchased from JT Baker (Phillipsburg, NJ, USA), Merck KGaA (Darmstadt, Germany), and Fujifilm Wako Pure Chemical Co., Ltd. (Osaka, Japan), respectively. Methanol and dimethyl sulfoxide (99.7%) used to prepare the test and standard solutions were purchased from JT Baker (Phillipsburg, NJ, USA) and Merck KGaA (Darmstadt, Germany), respectively.

2.3. Preparation of CSBPT Water Extract

CSBPT water extract was obtained from a formulation with the following weight ratio (total 5000 g): *Saposhnikovia* Radix (500.7 g), *Angelica* Dahuricae Radix (546.2 g), *Forsythia* Fructus (546.2 g), *Platycodon* Radix (546.2 g), *Scutellaria* Radix (477.1 g), *Cnidii* Rhizoma (477.1 g), *Schizonepeta* Spica (340.5 g), *Gardenia* Fructus (340.5 g), *Coptidis* Rhizoma (340.5 g), *Aurantii* Fructus Immaturus (340.5 g), *Mentha* Herba (340.5 g), and *Glycyrrhiza* Radix et Rhizoma (204.0 g), and it was prepared in KIOM according to the extraction method detailed in the previous study using an electric extractor, COSMOS-660 (Kyungseo E & P, Incheon, Korea) [22,23]. The prepared aqueous extract was lyophilized to obtain 1410.0 g of powder.

2.4. Preparation of Test Solution and Standard Solution for HPLC–PDA

To perform quantitative analysis on the 18 marker components of CSBPT using HPLC–PDA, a test solution was prepared at a concentration of 10 mg/mL using 70% methanol. The prepared solution was extracted using an ultra-sonicator at room temperature for 60 min and then filtered with a 0.2 μ m polypropylene (GHP) membrane (Pall Life Sciences, Ann Arbor, MI, USA).

Each of the standard solution for compounds **1–18** was prepared at a concentration of 1000 μ g/mL using either methanol or DMSO–methanol solution (1:1), and then stored at approximately 4 °C until use.

2.5. HPLC Equipment and Chromatographic Separation Conditions

The simultaneous analysis of the 18 marker compounds for quality evaluation of CSBPT was performed by using a modification of the previous methods [24,25]. A Shimadzu Prominence LC-20A series (Kyoto, Japan) HPLC system was used that combines a PDA for detection of the analyte and LabSolution software (Version 5.53, SP3, Kyoto, Japan) for data acquisition and processing. Other detailed analysis data are presented in Table S2.

2.6. Validation of the Analytical Method

The established HPLC assay was verified with respect to linearity, range, limit of detection (LOD), limit of quantification (LOQ), accuracy (recovery), and precision tests according to the International Conference on Harmonisation guidance [26]. Linearity of the response for the analysis of compounds **1–18** was evaluated based on the coefficient of determination (r^2) of the calibration curve prepared at different concentrations (0.31–20 μ g/mL for compounds **7**, **8**, **13**, **14**, and **18**; 0.78–50 μ g/mL for compounds **1–3**, **5**, **6**, **9**, **11**, **12**, and **15–17**; 1.25–80 μ g/mL for compound **4**; and 1.56–100 μ g/mL for compound **10**).

Accuracy was assessed by conducting extraction recovery tests using the standard addition method.

The precision of the assay was evaluated by plotting the relative standard deviation (RSD) of each parameter, such as repeatability and intra- and inter-day precisions. In other words, the repeatability was evaluated by the RSD value of the retention time and peak area of each marker component after six repeated measurements of a standard solution.

Intra- and inter-day precisions were assessed by calculating the RSD value based on the data measured on one day and on three consecutive days, respectively.

System suitability in the established assay was verified by confirming parameters such as the capacity factor (k'), selectivity factor (α), resolution (R_s), number of theoretical plates (N), and tailing factor (T_f) values [27].

3. Results and Discussion

3.1. Confirmation of the Main Component in Each Raw Herbal Medicine

For the selection of marker components for the quality evaluation of CSBPT, we analyzed and confirmed the main components of 12 raw herbs as follows: *prim-O*-glucosylcimifugin and 5-*O*-methylvisammioside from *Saposhnikovia* Radix; imperatorin, isoimperatorin, oxypeucedanin, and oxypeucedanin hydrate from *Angelicae Dahuricae*; arctigenin and arctiin from *Forsythiae Fructus*; platycodin D and platycodin D2 from *Platycodonis Radix*; baicalin, wogonoside, baicalein, and wogonin from *Scutellariae Radix*; ferulic acid from *Cnidii Rhizoma*; pulegone from *Schizonepetae Spica*; gardenoside and geniposide from *Gardeniae Fructus*; berberine chloride, coptisine chloride, and palmatine chloride from *Coptidis Rhizoma*; narirutin, naringin, hesperidin, and neohesperidin from *Aurantii Fructus Immaturus*; menthol and menthone from *Menthae Herba*; and glycyrrhizin, liquiritigenin, liquiritin apioside, and liquiritin from *Glycyrrhizae Radix et Rhizoma*. The presence of these herbal medicines and constituents was confirmed by HPLC using a distilled water–acetonitrile mobile phase system containing formic acid (Supplementary Materials Figure S1).

3.2. Selection of Marker Components for Quality Assessment of CSBPT

The major components that were identified in each herbal medicine are described in Section 3.1 and were also identified in the CSBPT sample. Thus, the 18 compounds were detected in the CSBPT sample among a total 31 components, as shown in Figure S2. These 18 compounds were selected as suitable marker components for the quality control of CSBPT; the chemical structures of the components are shown in Figure S3.

3.3. Optimization of HPLC Chromatographic Separation Conditions

For efficient chromatographic separation of compounds 1–18, various parameters, such as make and type of column [SunFire™ (Waters, Milford, MA, USA), Gemini (Phenomenex, Torrance, CA, USA), Capcellpak UG120 (Shiseido, Tokyo, Japan), Quasar™ SPP (PerkinElmer, Shelton, CT, USA), and INNO (YoungJin Biochrom Co., Seongnam Korea)], acids (formic acid, trifluoroacetic acid, acetic acid, and phosphoric acid), and column temperatures (30, 35, 40, and 45 °C), were compared. Optimal analysis was achieved with a SunFire C₁₈ column (4.6 × 250 mm, 5 µm), a column temperature of 40 °C, and two-component mobile phase system of distilled water–acetonitrile, both containing 0.1% (*v/v*) formic acid. Under the optimized HPLC conditions, compounds 1–18 were baseline separated with resolution values ≥ 3.85 , with HPLC run-times within 45 min (Figure 1 and Table S3).

Figure 1. HPLC chromatograms of the standard solution (A) and test solution for quantification of 70% methanol extract of Cheongsangbangpung-tang (CSBPT) sample (B). Geniposide (1), coptisine Cl (2), *prim-O*-glucosylcimifugin (3), berberine Cl (4), liquiritin apioside (5), liquiritin (6), ferulic acid (7), narirutin (8), 5-*O*-methylvisammioside (9), hesperidin (10), arctigenin (11), baicalin (12), oxypeucedanin hydrate (13), wogonoside (14), baicalein (15), arctiin (16), glycyrrhizin (17), and pulegone (18).

3.4. Method Validation of the Developed HPLC Analytical Method

For quality control of CSBPT based on compounds 1–18, the optimized analysis method was verified by evaluating factors such as system suitability, linearity, LOD, LOQ, and precision (repeatability, and intra- and inter-day precisions). The values of the system suitability factors k' , α , N , R_s , and T_f were calculated to be 3.23–13.80, 1.02–1.18, 247,354.40–1,161,300.13, 3.85–36.85, and 1.01–1.22, respectively (Table S3). The r^2 values of compounds 1–18 calculated from the corresponding calibration curves were ≥ 0.9996 , which confirmed the excellent linearity of the analysis assay. The LOD and LOQ values of compounds 1–18 were 0.07–0.36 $\mu\text{g/mL}$ and 0.21–1.10 $\mu\text{g/mL}$, respectively. Regression equation, r^2 , LOD, and LOQ data are presented in Table 1. The extraction recovery of compounds 1–18 for evaluation of the accuracy of the developed assay was 97.03–104.04%, and the RSD (%) value was <3.00 (Table 2). The RSD values of repeatability, and intra- and inter-day precisions of compounds 1–18 were 0.01–3.09%, showing good precision results (Table 3). These data confirmed that the developed HPLC assay was suitable for the quality control of CSBPT.

Table 1. Linear range, regression equation, r^2 , limit of detection (LOD), and limit of quantification (LOQ) values for HPLC analysis of compounds 1–18 ($n = 3$).

Analyte	Detection (nm)	Linear Range (µg/mL)	Regression Equation ^a $y = ax + b$	r^2	LOD (µg/mL)	LOQ (µg/mL)
1	235	0.78–50	$y = 22,979.62x + 2649.51$	1.0000	0.09	0.28
2	355	0.78–50	$y = 39,831.26x - 5918.67$	0.9996	0.20	0.60
3	300	0.78–50	$y = 19,703.59x + 3163.52$	1.0000	0.16	0.48
4	345	1.25–80	$y = 47,572.65x + 12858.14$	1.0000	0.31	0.95
5	275	0.78–50	$y = 17,486.99x + 3289.39$	1.0000	0.20	0.61
6	275	0.78–50	$y = 21,989.62x + 4049.96$	1.0000	0.20	0.60
7	320	0.31–20	$y = 65,199.18x + 4792.95$	1.0000	0.08	0.25
8	280	0.31–20	$y = 17,012.50x + 1238.65$	1.0000	0.07	0.21
9	290	0.78–50	$y = 17,739.58x + 3428.71$	1.0000	0.20	0.60
10	280	1.56–100	$y = 16,112.67x + 6984.59$	1.0000	0.36	1.10
11	280	0.78–50	$y = 7271.72x + 1344.77$	1.0000	0.20	0.60
12	275	0.78–50	$y = 38,381.96x + 6880.25$	1.0000	0.20	0.61
13	310	0.31–20	$y = 53,174.23x + 3697.05$	1.0000	0.09	0.28
14	275	0.31–20	$y = 48,111.17x + 3026.11$	1.0000	0.09	0.28
15	275	0.78–50	$y = 57,618.21x + 3704.23$	1.0000	0.15	0.45
16	280	0.78–50	$y = 10,789.77x + 2166.35$	1.0000	0.20	0.61
17	250	0.78–50	$y = 8540.20x + 1806.55$	1.0000	0.21	0.64
18	250	0.31–20	$y = 18,446.27x + 1845.86$	1.0000	0.07	0.22

^a y : peak area of compounds; x : concentration (µg/mL) of compounds.**Table 2.** Extract recovery test of compounds 1–18 in the developed analytical assay.

Analyte	Measured Amount (µg/mL)	Found Amount (µg/mL)	Recovery (%)	SD	RSD (%)
1	1.00	1.01	100.81	1.73	1.72
	2.00	1.99	99.41	0.94	0.95
	4.00	3.93	98.23	1.17	1.19
	2.00	2.07	103.52	0.98	0.94
2	4.00	3.99	99.70	1.00	1.00
	8.00	8.27	103.36	0.59	0.57
	1.00	1.02	101.52	1.50	1.48
3	2.00	1.99	99.58	0.36	0.36
	4.00	3.96	99.07	0.82	0.83
	4.00	4.04	100.90	2.80	2.77
4	10.00	9.92	99.24	2.97	2.99
	20.00	19.41	97.03	1.13	1.17
	2.00	1.98	99.19	1.86	1.88
5	5.00	4.97	99.45	1.11	1.12
	10.00	9.91	99.13	1.40	1.41
	1.00	1.00	100.31	1.24	1.24
6	2.00	2.01	100.43	1.48	1.47
	4.00	4.04	101.04	0.85	0.84
	1.00	1.01	100.56	1.79	1.78
7	2.00	1.99	99.73	0.67	0.67
	4.00	3.91	97.79	0.44	0.45
	1.00	1.04	104.04	2.36	2.27
8	2.00	1.96	98.25	2.56	2.60
	4.00	3.97	99.32	1.50	1.51

Table 2. Cont.

Analyte	Measured Amount (µg/mL)	Found Amount (µg/mL)	Recovery (%)	SD	RSD (%)
9	1.00	1.00	99.65	1.40	1.41
	2.00	2.02	100.84	1.34	1.33
	4.00	4.06	101.62	0.84	0.83
	5.00	5.13	102.63	2.36	2.30
10	12.50	12.79	102.29	1.23	1.20
	25.00	25.09	100.36	1.36	1.36
	2.00	1.97	98.37	2.86	2.90
11	5.00	4.96	99.16	1.60	1.61
	10.00	9.86	98.57	1.01	1.03
12	3.00	2.96	98.74	0.97	0.98
	7.50	7.42	98.89	0.82	0.83
	15.00	15.44	102.95	0.72	0.70
	1.00	1.01	100.80	1.49	1.47
13	2.00	2.03	101.35	0.54	0.53
	4.00	4.05	101.20	0.67	0.66
	1.00	1.01	100.61	1.83	1.82
14	2.00	2.03	101.74	0.90	0.89
	4.00	4.14	103.59	0.88	0.85
	2.00	1.99	99.45	1.20	1.21
15	5.00	4.96	99.14	1.61	1.62
	10.00	9.82	98.25	1.79	1.82
	2.00	2.04	101.96	0.89	0.87
16	4.00	4.06	101.47	1.44	1.42
	8.00	8.04	100.53	1.64	1.63
	3.00	3.10	103.47	2.29	2.21
17	7.50	7.53	100.37	1.18	1.18
	15.00	14.99	99.90	0.34	0.34
	1.00	0.99	98.85	1.91	1.93
18	2.00	2.02	101.14	1.02	1.01
	4.00	4.10	102.45	0.57	0.56

Table 3. Precision validation of compounds 1–18 in the developed analytical assay.

Analyte	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
1	12.50	12.73	0.91	101.84	12.80	0.88	102.39	0.05	0.52
	25.00	25.91	1.40	103.64	25.99	1.24	103.96		
	50.00	51.13	0.36	102.25	51.25	0.68	102.51		
2	12.50	12.56	0.75	100.44	12.95	2.67	103.58	0.05	0.16
	25.00	24.99	1.23	99.98	25.41	2.21	101.64		
	50.00	51.03	2.81	102.05	50.98	2.69	101.97		
3	12.50	12.97	1.49	103.79	13.33	2.54	106.62	0.04	0.52
	25.00	26.05	1.65	104.18	26.57	2.56	106.28		
	50.00	51.50	1.51	103.00	51.72	1.79	103.44		
4	20.00	20.72	1.28	103.62	21.30	2.65	106.52	0.03	0.35
	40.00	41.64	1.67	104.11	42.25	2.10	105.63		
	80.00	82.13	1.11	102.66	82.43	1.40	103.03		
5	12.50	13.09	2.00	104.74	13.41	2.70	107.26	0.03	0.46
	25.00	26.27	2.10	105.09	26.63	2.27	106.53		
	50.00	51.73	1.50	103.47	51.85	1.46	103.70		
6	12.50	13.06	1.82	104.48	13.34	2.52	106.69	0.03	0.41
	25.00	26.27	1.96	105.06	26.59	2.01	106.34		
	50.00	51.67	1.31	103.34	51.73	1.20	103.46		

Table 3. Cont.

Analyte	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
7	5.00	5.20	1.44	104.07	5.34	2.33	106.90	0.03	0.37
	10.00	10.49	1.53	104.86	10.66	2.34	106.62		
	20.00	20.65	1.39	103.24	20.74	1.54	103.69		
8	5.00	5.21	1.87	104.22	5.37	3.04	107.48	0.03	0.40
	10.00	10.50	1.69	104.97	10.68	2.43	106.78		
	20.00	20.68	1.36	103.41	20.79	1.65	103.97		
9	12.50	12.89	1.73	103.12	13.20	2.33	105.63	0.03	0.25
	25.00	26.02	2.05	104.09	26.41	2.37	105.62		
	50.00	51.04	1.42	102.08	51.29	1.60	102.58		
10	25.00	26.14	1.83	104.56	26.96	3.05	107.85	0.03	0.35
	50.00	52.56	1.77	105.12	53.45	2.41	106.90		
	100.00	103.20	1.43	103.20	103.79	1.67	103.79		
11	12.50	13.09	1.79	104.73	13.52	3.09	108.17	0.02	0.39
	25.00	26.44	1.98	105.75	26.86	2.38	107.44		
	50.00	52.21	1.52	104.43	52.41	1.69	104.82		
12	12.50	12.85	1.67	102.80	13.29	3.07	106.32	0.02	0.43
	25.00	26.10	1.32	104.42	26.39	1.69	105.55		
	50.00	51.32	1.39	102.63	51.66	1.60	103.31		
13	5.00	5.22	1.74	104.38	5.35	2.53	107.03	0.02	0.33
	10.00	10.52	1.94	105.17	10.63	2.02	106.32		
	20.00	20.67	1.39	103.34	20.69	1.23	103.46		
14	5.00	5.19	1.73	103.87	5.37	3.08	107.31	0.02	0.35
	10.00	10.49	1.82	104.91	10.66	2.37	106.58		
	20.00	20.62	1.30	103.09	20.74	1.54	103.68		
15	12.50	12.87	1.50	102.94	13.13	2.83	105.03	0.02	0.39
	25.00	26.04	0.99	104.17	26.18	1.22	104.71		
	50.00	51.45	1.12	102.90	51.19	0.93	102.38		
16	12.50	13.07	1.81	104.60	13.47	2.95	107.79	0.02	0.38
	25.00	26.28	1.92	105.13	26.71	2.43	106.83		
	50.00	51.65	1.40	103.31	51.92	1.65	103.84		
17	12.50	13.07	1.83	104.55	13.36	2.06	106.85	0.01	0.32
	25.00	26.20	1.74	104.82	26.50	1.79	106.02		
	50.00	51.54	1.32	103.08	51.83	1.59	103.67		
18	5.00	5.18	1.18	103.58	5.31	2.22	106.24	0.01	0.27
	10.00	10.41	1.33	104.06	10.55	1.83	105.53		
	20.00	20.52	1.06	102.60	20.57	1.02	102.87		

^a Precision is expressed as RSD (%).

3.5. Quantification of Compounds 1–18 in CSBPT Samples

By using the developed and verified HPLC analysis method, quantification of the selected 18 marker components was performed for quality assessment of CSBPT samples. Quantification of compounds 1–18 was achieved by detection at the maximum absorption wavelength in the UV spectrum of each component. The amount of compounds 1–18 in the freeze-dried CSBPT sample was found to be 0.27–18.31 mg/g; among these analytes, compound 12, the major constituent of *Scutellariae Radix*, was identified as the most abundant (17.82–18.31 mg/g; Table 4).

Table 4. Concentration of compounds 1–18 in CSBPT according to sample batches ($n = 3$).

Analyte	Batch No.					
	I		II		III	
	Mean (mg/g) $\pm$ SD ($\times 10^{-2}$)	RSD (%)	Mean (mg/g) $\pm$ SD ($\times 10^{-2}$)	RSD (%)	Mean (mg/g) $\pm$ SD ($\times 10^{-2}$)	RSD (%)
1	5.33 $\pm$ 1.02	0.19	5.35 $\pm$ 4.59	0.86	5.41 $\pm$ 3.41	0.63
2	0.70 $\pm$ 0.45	0.63	0.75 $\pm$ 0.31	0.41	0.71 $\pm$ 0.19	0.26
3	0.60 $\pm$ 0.87	1.45	0.59 $\pm$ 0.60	1.02	0.59 $\pm$ 0.18	0.31
4	2.30 $\pm$ 3.72	1.62	2.45 $\pm$ 2.10	0.86	2.30 $\pm$ 1.70	0.74
5	1.16 $\pm$ 1.41	1.22	1.16 $\pm$ 1.18	1.01	1.14 $\pm$ 0.97	0.86
6	0.54 $\pm$ 0.45	0.82	0.55 $\pm$ 0.41	0.75	0.54 $\pm$ 0.25	0.47
7	0.50 $\pm$ 0.20	0.39	0.50 $\pm$ 0.94	1.87	0.50 $\pm$ 0.29	0.58
8	4.55 $\pm$ 0.42	0.09	4.57 $\pm$ 2.82	0.62	4.56 $\pm$ 1.21	0.27
9	0.60 $\pm$ 0.32	0.53	0.60 $\pm$ 0.51	0.84	0.60 $\pm$ 0.24	0.40
10	2.51 $\pm$ 1.45	0.58	2.54 $\pm$ 1.66	0.65	2.51 $\pm$ 1.54	0.61
11	1.11 $\pm$ 1.04	0.94	1.16 $\pm$ 1.60	1.38	1.15 $\pm$ 2.10	1.83
12	17.82 $\pm$ 7.34	0.41	18.31 $\pm$ 3.76	0.21	18.19 $\pm$ 9.31	0.51
13	0.46 $\pm$ 0.03	0.06	0.47 $\pm$ 0.28	0.60	0.47 $\pm$ 0.23	0.50
14	4.40 $\pm$ 1.65	0.38	4.52 $\pm$ 2.19	0.48	4.48 $\pm$ 1.05	0.24
15	1.02 $\pm$ 2.23	2.19	1.05 $\pm$ 1.49	1.42	0.99 $\pm$ 2.01	2.02
16	0.82 $\pm$ 0.20	0.24	0.85 $\pm$ 0.25	0.30	0.82 $\pm$ 0.72	0.88
17	1.51 $\pm$ 0.82	0.54	1.52 $\pm$ 0.25	0.16	1.48 $\pm$ 0.29	0.20
18	0.27 $\pm$ 0.08	0.28	0.27 $\pm$ 0.05	0.17	0.27 $\pm$ 0.07	0.26

4. Conclusions

We developed a simultaneous analysis method for selected compounds 1–18 that enables the quality assessment of CSBPT based on a common and widely-used HPLC–PDA system. The developed assay was validated by evaluating linearity, LOD, LOQ, recovery, and precision, and it was successfully applied to the analysis of CSBPT samples. This HPLC–PDA analytical assay can be used as a framework to obtain basic data for quality assessment of CSBPT and other traditional Korean medicines in the future.

Supplementary Materials: The following are available online at <https://www.mdpi.com/2076-3417/11/1/14/s1>, Figure S1: HPLC chromatogram of constituent herbal medicine and its major components. A: *S. divaricate*; B: *A. dahurica*; C: *F. viridissima*; D: *P. grandiflorum*; E: *S. baicalensis*; F: *C. officinale*; G: *S. tenuifolia*; H: *G. jasminoides*; I: *C. japonica*; J: *C. aurantium*; K: *M. arvensis*; and L: *G. uralensis*, Figure S2: HPLC chromatograms of the standard solution (A) and 70% methanol extract of CSBPT sample (B). Gardenoside (1), geniposide (2), coptisine chloride (3), *prim-O*-glucosylcimifugin (4), berberine chloride (5), palmatine chloride (6), liquiritin apioside (7), liquiritin (8), ferulic acid (9), narirutin (10), 5-*O*-methylcissammoside (11), naringin (12), hesperidin (13), neohesperidin (14), arctigenin (15), platycodin D2 (16), baicalin (17), platycodin D (18), liquiritigenin (19), oxypeucedanin hydrate (20), wogonoside (21), baicalein (22), menthole (23), arctiin (24), glycyrrhizin (25), wogonin (26), oxypeucedanin (27), pulegone (28), menthone (29), imperatoxin (30), isoimperatorin (31), Figure S3: Chemical structures of compounds 1–18 in CSBPT, Table S1: Composition of CSBPT, Table S2: Chromatographic conditions for simultaneous quantification of compounds 1–18 in CSBPT, Table S3: System suitability of compounds 1–18.

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