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Ultra-Performance Liquid Chromatography with Tandem Mass Spectrometry for Simultaneous Analysis of 22 Analytes of Oncheong-Eum, a Traditional Korean Herbal Formula

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Abstract: Oncheong-eum (OCE) is a traditional Korean herbal formula comprising eight medicinal herbs for treating skin disorders, including eczema and skin rashes. Here, we sought to simultaneously analyze 22 analytes of OCE using ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS). All analytes were separated on a Waters Acquity UPLC BEH C_{18} column (2.1 mm × 100 mm, 1.7 µm) maintained at 45 °C by gradient elution with a mobile phase of 0.1% (*v*/*v*) aqueous formic acid–acetonitrile. By applying a multiple reaction monitoring method, we rapidly determined the various analytes simultaneously. The coefficient of determination of the regression equation prepared in the tested concentration range of each authentic reference standard was ≥0.9950 and showed good linearity. The accuracy ranged from 84.23% to 115.47%, and the relative standard deviation values for intra- and interday precisions ranged from 0.84% to 9.57%, respectively. Analysis of OCE samples using this method showed that they contained up to 27.10 mg/g of active ingredients. The method can provide data to improve the consistency and, thus, the future quality of OCE preparations and other traditional herbal formulas.

Keywords: UPLC–MS/MS; simultaneous analysis; traditional herbal formula; Oncheong-eum

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1. Introduction

Traditional herbal formulas (THFs) are composed of two or more herbal medicines and show the therapeutic characteristics of multicomponent and multitarget preparations [1,2]. However, THFs have the disadvantage that quality control is a complex problem. Therefore, standardization and efficacy assurance of THFs is important.

Oncheong-eum (OCE; Wenqing-yin in Chinese, Unsei-in in Japanese) is a traditional herbal formula comprising eight medicinal herbs (*Angelica gigas* Nakai, *Cnidium officinale* Makino, *Paeonia lactiflora* Pall., *Rehmannia glutinosa* (Gaertn.) DC., *Coptis chinensis* Franch., *Scutellaria baicalensis* Georgi, *Phellodendron chinensis* C.K.Schneid., and *Gardenia jasminoides* Ellis) in equal weight proportions [3,4]. OCE is used to treat skin diseases such as itching caused by eczema and skin rashes [3–5]. The formula has been reported to have various therapeutic effects, such as relief of edema and inflammation, cytotoxicity, promotion of skin regeneration, wrinkle improvement, whitening, and moisturization [6–9].

To date, researchers have conducted extensive studies on the detailed composition of herbal medicines (HMs) or THFs using various analytical techniques, such as high-performance capillary electrophoresis, high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC), UPLC–tandem mass spectrometry (MS/MS), and gas chromatography–mass spectrometry [10–16]. Among these techniques, HPLC or LC–MS/MS techniques have been the most widely applied to the quality control and chemical profiling of HMs or THFs. For OCE, for example, Yeh et al. [17] reported the simultaneous quantification of six components (baicalin, berberine, genoposide, hydroxymethoxylfurfural, paeoniflorin, and ferulic acid) using HPLC coupled to a diode array

detector (DAD) for quality control of OCE. Recently, we reported the result of a simultaneous analysis of 19 components from OCE samples using an HPLC–DAD system [18]. However, this HPLC analysis method is limited in that the analysis time is longer than that of LC–MS/MS analysis.

In general, it is important to quantify as many active components as possible for any HMs or THFs to guarantee efficacy and safety. Therefore, in this study, we describe an analytical method for quality control of OCE by simultaneously determining 22 components with a UPLC–MS/MS system that is more sensitive and accurate than previously described HPLC methods. The components were gallic acid, gardenoside, oxypaeoniflorin, chlorogenic acid, 5-hydroxymethylfurfural, geniposide, albiflorin, paeoniflorin, nodakenin, ferulic acid, jatrorrhizine chloride (Cl), coptisine Cl, baicalin, palmatine Cl, berberine Cl, wogonoside, benzoylpaeoniflorin, baicalein, wogonin, *Z*-ligustilide, decursin, and decursinol angelate.

2. Materials and Methods

2.1. Plant Materials, Chemicals, and Reagents

Information on each medicinal herb comprising our OCE preparation (OCE–1) is described in detail in a previously reported article [18].

As analytes for quality control of OCE samples, we selected 22 authentic reference standards (Figure S1) that were variously purchased from commercial suppliers: Merck (Darmstadt, Germany), Biopurify Phytochemicals (Chengdu, China), Shanghai Sunny Biotech (Shanghai, China), Wuhan ChemNorm Biotech (Wuhan, China), Fujifilm Wako Pure Chemical (Osaka, Japan), and Tokyo Chemical Industry (Tokyo, Japan). Detailed composition information is presented in Table S1. Methanol, acetonitrile, water (LC–MS grade), dimethyl sulfoxide (DMSO, ACS reagent, ≥99.9%), and formic acid (LC–MS grade) were purchased from Thermo Fisher Scientific (San Jose, CA, USA) and Merck.

2.2. Sample Preparation for Simultaneous Analysis by UPLC–MS/MS

We prepared an OCE water decoction extract as previously described [18,19]. Briefly, 625.0 g of each dried OCE herb (total 5 kg) was mixed in a COSMOS-660 extractor (Kyungseo E&P, Incheon, Republic of Korea), and boiled for 2 h at 100 °C in 50 L of distilled water. The extract was freeze-dried to obtain 1232.6 g of powder (OCE–1, yield 24.7%). Other samples (OCE–2 to OCE–5) were commercially available products purchased from different pharmaceutical companies.

2.3. Preparation of Sample and Standard Stock Solutions for Simultaneous Analysis by UPLC–MS/MS

To determine the 22 analytes, approximately 50 mg of each OCE sample was accurately weighed into sufficient 70% methanol (approximately 10 mL) to achieve a final concentration of 5 mg/mL after sequential ultrasonic extraction (5 min) and vortex mixing (1 min).

We prepared standard stock solutions of each analyte at 100 mg/L methanol [20–23] and stored them at 4 °C. The standard stock solution of each analyte was diluted as required and used as a working standard solution.

The solutions were filtered through a hydrophobic polytetrafluoroethylene membrane filter (0.22 μm; SSOL Korea, Daejeon, Republic of Korea) before analysis.

2.4. UPLC–MS/MS Simultaneous Analysis Conditions

A UPLC–MS/MS multiple reaction monitoring (MRM) method for simultaneous analysis of the 22 analytes was created by modifying previously reported assay protocols [2,24]. Briefly, an LC–MS/MS system comprising a Waters Acquity UPLC I-Class system (Milford, MA, USA) and a Waters Xevo TQ-XS MS system was used. The analytes were separated on a Waters Acquity UPLC BEH C_{18} reverse phase column (2.1 mm × 100 mm, 1.7 μm) using gradient elution with a mobile phase of 0.1% (*v*/*v*) aqueous formic acid–acetonitrile. We

used an electrospray ionization (ESI) source. Detailed UPLC and MS operating conditions for simultaneous analysis are given in Table S2.

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

The method for each analyte was validated by evaluating linearity (coefficient of determination, r^2), sensitivity by limit of detection (LOD) and limit of quantification (LOQ), accuracy, and precision (intra- and interday precision, repeatability) [25].

Calibration curves for the 22 analytes were prepared over the following concentration ranges: 10.00–500.00 μg/L (oxypaeoniflorin, 5-hydroxymethylfurfural, nodakenin, ferulic acid, jatrorrhizine Cl, coptisine Cl, palmatine Cl, benzoylpaeoniflorin, wogonin, *Z*-ligustilide, decursin, and decursinol angelate); 100.00–2500.00 μg/L (chlorogenic acid and berberine Cl) and 250.00–5000.00 μg/L (gallic acid, gardenoside, geniposide, albiflorin, paeoniflorin, baicalin, wogonoside, and baicalein).

Linearity was evaluated by the r^2 value of the regression equation in the calibration curve of each analyte. Sensitivity values (LOD and LOQ) were calculated from signal-to-noise (S/N) ratios of 3:1 and 10:1, respectively. Accuracy was evaluated by adding three concentrations (low, medium, and high) of different standard solutions for each known analyte (original amount) in the OCE sample and calculating the accuracy (%, Equation (1)) from five replicate determinations. Validated accurate concentration ranges are shown in Table S3.

$$\text{Accuracy}(\%) = \frac{\text{found amount} - \text{orignal amount}}{\text{spiked amount}} \times 100 \quad (1)$$

The precision (intra- and interday precision, repeatability) of our UPLC–MS/MS assay method was verified for each component by its relative standard deviation (RSD %). The intra- and interday precision of each component was determined five times on one day and on three consecutive days using three concentrations of mixed standard solutions. Repeatability was evaluated by the RSD % of retention time and peak area of each analyte from six replicate determinations of a mixed standard solution comprising all 22 authentic standard reference compounds. The results were evaluated as the mean RSD % values calculated by Equation (2). Concentration ranges for precision validation are shown in Table S3.

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

2.6. Stability Test

We tested the stability of the 22 analytes for 3 days at room temperature (24 ± 1 °C) using standard solutions.

3. Results and Discussion

3.1. Optimization of Analytical Conditions for Simultaneous UPLC–MS/MS MRM Analysis

Analytical conditions were optimized for simultaneous analysis of the 22 analytes, which were chlorogenic acid, nodakenin, decursin, and decursinol angelate (from *A. gigas*); ferulic acid and *Z*-ligustilide (*C. officinale*); gallic acid, albiflorin, paeoniflorin, oxypaeoniflorin, and benzoylpaeoniflorin (*P. lactiflora*); 5-hydroxymethylfurfural (*R. glutinosa*); jatrorrhizine Cl, coptisine Cl, palmatine Cl, and berberine Cl (*C. chinensis* and *P. chinensis*); baicalin, wogonoside, baicalein, and wogonin (*S. baicalensis*) and gardenoside and geniposide (*G. jasminoides*) [21–23,26–30]. A previous HPLC method of analysis [18], depended on the retention time of each component, but using the MRM method in UPLC–MS/MS analysis has the advantage of being able to analyze many components simultaneously within a short time. Figure 1 shows that all analytes were eluted within 9 min, and Table 1 shows the optimal UPLC–MS/MS MRM parameters for simultaneous quantification. The flow rate and injection volume were 0.3 mL/min and 2.0 μL, respectively, and the analysis sample was maintained at 5 °C.

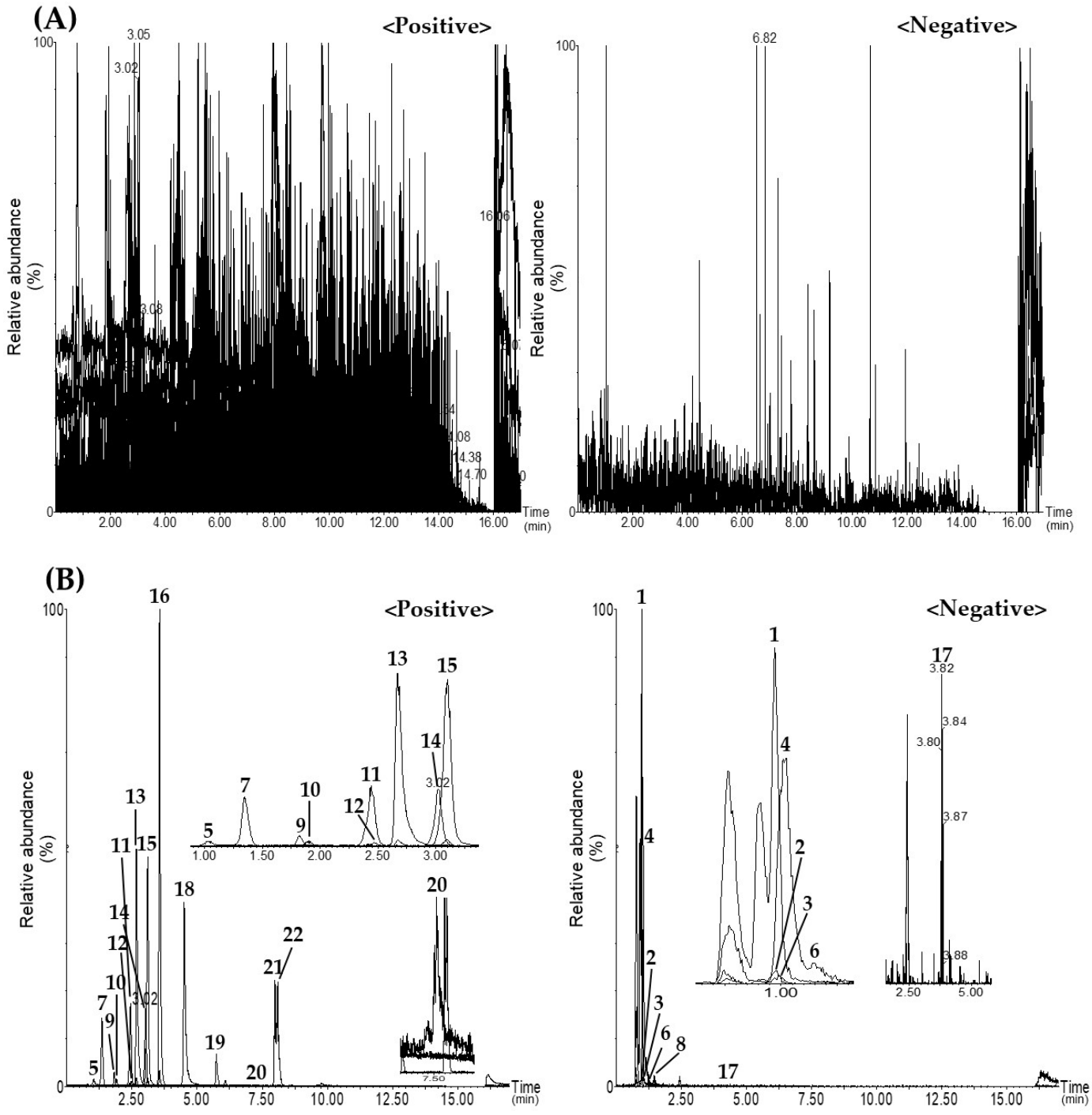

Figure 1. *Cont.*

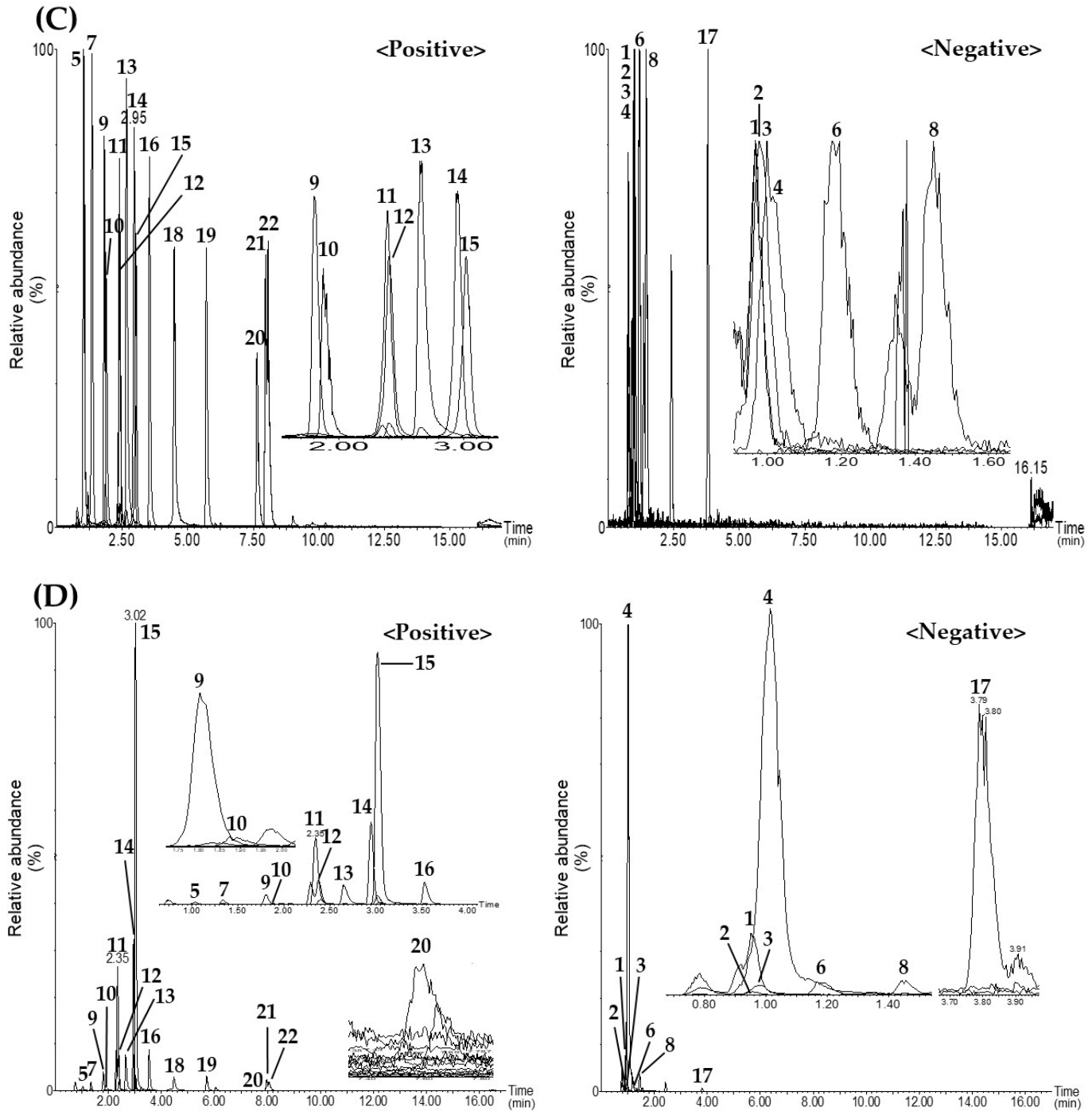

Figure 1. Representative total ion chromatograms of blank (**A**), LOQ the standard solution at the LOQ level (**B**), the standard solution at the high concentration level (**C**), and the 70% methanolic solution of the lyophilized OCE–1 sample (**D**) measured by the UPLC–MS/MS MRM method in positive and negative ion modes, Gallic acid (1), gardenoside (2), oxypaeoniflorin (3), chlorogenic acid (4), 5-hydroxymethylfurfural (5), geniposide (6), albiflorin (7), paeoniflorin (8), nodakenin (9), ferulic acid (10), jatrorrhizine Cl (11), coptisine Cl (12), baicalin (13), palmatine Cl (14), berberine Cl (15), wogonoside (16), benzoylpaeoniflorin (17), baicalein (18), wogonin (19), Z-ligustilide (20), decursin (21), and decursinol angelate (22).

Table 1. Optimized UPLC–MS/MS MRM conditions for simultaneous analysis of the 22 analytes in OCE samples.

Analyte [1]	Ion Mode	Molecular Weight	Precursor Ion (Q1)	Product Ion (Q3)	Cone Voltage (V)	Collision Energy (eV)	Retention Time (min)
1	−	170.1	169.0	125.0	25	15	0.97
2	−	404.4	403.2	241.0	35	10	0.97
3	−	496.5	495.4	137.0	40	25	0.98
4	−	354.3	353.2	191.0	20	20	1.02
5	+	126.1	127.0	109.0	20	10	1.02
6	−	388.4	387.2	123.0	25	15	1.20
7	+	480.5	481.4	197.1	20	15	1.33
8	−	480.5	479.2	121.0	32	25	1.44
9	+	408.4	409.4	247.2	30	15	1.81
10	+	184.2	195.0	177.0	15	10	1.89
11	+	373.8	338.4	322.3	30	30	2.38
12	+	355.8	320.1	290.0	45	25	2.39
13	+	446.4	447.3	271.0	25	15	2.65
14	+	387.9	352.1	336.0	40	30	2.95
15	+	371.8	336.1	320.0	35	30	3.02
16	+	460.4	461.3	285.1	30	20	3.54
17	−	584.6	583.4	121.0	40	25	3.80
18	+	270.2	271.1	123.0	40	30	4.47
19	+	284.3	285.1	270.0	40	20	5.70
20	+	190.1	191.0	91.0	30	25	7.66
21	+	328.4	329.2	229.0	35	20	7.96
22	+	328.4	329.2	229.0	35	20	8.07

[1] Gallic acid (1), gardenoside (2), oxypaeoniflorin (3), chlorogenic acid (4), 5-hydroxymethylfurfural (5), geniposide (6), albiflorin (7), paeoniflorin (8), nodakenin (9), ferulic acid (10), jatrorrhizine Cl (11), coptisine Cl (12), baicalin (13), palmatine Cl (14), berberine Cl (15), wogonoside (16), benzoylpaeoniflorin (17), baicalein (18), wogonin (19), *Z*-ligustilide (20), decursin (21), and decursinol angelate (22).

3.2. Setting of MRM Transition of Each Analyte for UPLC–MS/MS Analysis

Eleven components (5-hydroxymethylfurfural, albiflorin, nodakenin, ferulic acid, baicalin, wogonoside, baicalein, wogonin, Z-ligustilide, decursin, and decursinol angelate) were detected with m/z 127.0, 481.4, 409.4, 195.0, 447.3, 461.3, 271.1, 285.1, 191.0, 329.2, and 329.2, respectively, in the positive ion mode ($[M + H]^+$) (Figure 1 and Table 1). Four alkaloids (jatrorrhizine Cl, coptisine Cl, palmatine Cl, and berberine Cl) were detected with m/z 338.4, 320.1, 352.1, and 336.1 in the positive ion mode in their chloride-free form (Table 1). The other seven components (gallic acid, gardenoside, oxypaeoniflorin, chlorogenic acid, geniposide, paeoniflorin, and benzoylpaeoniflorin) were detected with m/z 169.0, 403.2, 495.4, 353.2, 387.2, 479.2, and 583.4, respectively, in the negative ion mode ($[M - H]^-$) (Figure 1 and Table 1).

The MRM transition conditions from the precursor ion (Q1) to the product ion (Q3) peaks for simultaneous analysis are also presented in Table 1. Namely, Q3 peaks of gallic acid and chlorogenic acid were set at m/z 125 ($[M–H–COO]^-$) and 191 ($[M–H–C_9H_7O_3]^-$), respectively. These transitions were generated by loss of the COO moiety and caffeoyl group from the Q1 peaks of m/z 169.0 ($[M–H]^-$) and m/z 353.2 ($[M–H]^-$), respectively [31,32]. Two iridoid glycosides, gardenoside and geniposide, had respective Q3 peaks at m/z 241.0 ($[M–H–Glc]^-$) and m/z 123.0 ($[M–H–Glc–C_4H_5O_2]^-$) [33]. The ion peak at m/z 123.0 was generated simultaneously in three positions from one double bond and two single bonds ($^{2,7,9}A_0$) [33]. The molecular ion peaks of the monoterpene glycosides oxypaeoniflorin, paeoniflorin, and benzoylpaeoniflorin were observed at m/z 495.4, 479.2, and 583.4 in the form of $[M–H]^-$ in the negative ion mode, while albiflorin was observed at m/z 481.4 in the form of $[M + H]^+$ in the positive ion mode. The Q3 peak of oxypaeoniflorin was selected by its p-hydroxybenzoic acid group observed at m/z 137.0 [34]. For paeoniflorin and benzoylpaeoniflorin, a benzoic acid group was observed at m/z 121.0, so this peak was selected as the Q3 peak of the two analytes [35,36]. The Q3 peak of albiflorin was set to

m/z 197.1 ([Aglycone–H]$^-$), which was produced by the cleavage of the benzoic acid and glucose groups from the albiflorin molecular ion peak [35]. 5-Hydroxymethylfurfural, a furan derivative, had a Q3 peak ([M + H–H_2O]$^+$) at m/z 109.0, where one water molecule was removed [37]. Nodakenin, decursin, and decursinol angelate are coumarin components of A. gigas, all of which were detected in positive ion mode ([M + H]$^+$). In the case of nodakenin, the Q3 peak at m/z 247.2 ([M + H–H_2O]$^+$) was generated by removing the molecule of glucose from the Q1 peak [38]. For both decursin and decursinol angelate, the Q3 peak at m/z 229.0 was generated by cleavage of the isoprenyl moiety (or angeloyl moiety) plus a water molecule at the C-3 position [39]. Ferulic acid exhibited a Q3 peak at m/z 177.0 in the form of [M + H–H_2O]$^+$ where one water molecule was lost [31]. Jatrorrhizine Cl, coptisine Cl, palmatine Cl, and berberine Cl, the main alkaloids of *C. chinensis* and *P. chinensis*, were observed in the chloride-free form at m/z 338.4, 320.1, 352.1, and 336.1, respectively. For jatrorrhizine Cl, palmatine Cl, and berberine Cl, the Q3 peaks observed at m/z 322.3, 336.0, and 320.0 ([M + H–CH_3]$^+$) were due to methyl radical elimination from the methoxy group at C-9 or C-10, respectively [40]. The Q3 peak of coptisine Cl was observed in the form of [M + H–2H–CO]$^+$ at m/z 290.0 (Scheme S1) [40]. The Q3 peaks of baicalin and wogonoside were observed at m/z 271.0 and 285.0, respectively, corresponding to the loss of glucuronic acid from the Q1 peak of [M + H]$^+$ [41]. The Q3 peak of wogonin was set to m/z 270.0 where the methyl group was lost [M + H–CH_3]$^+$ [31]. For baicalein, the Q3 peak was set to the trihydroxyphenyl moiety at m/z 123.0, generated by cleavage of the B ring [41]. The Q3 peak of Z-ligustilide was at m/z 91.0 ([M + H–H_2O–CO–C_4H_6]$^+$ [42]. MS transitions for the MRM analysis of the 22 analytes are summarized in Figure S2 and Scheme S1.

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

The optimized UPLC–MS/MS MRM analytical method for the simultaneous analysis of the 22 analytes selected in OCE samples was validated by evaluating the linearity, sensitivity (LOD and LOQ), accuracy, and precision (intraday and interday precision, repeatability). In the regression equation, the r^2 value of each analyte was $\geq$0.9950, showing good linearity in the tested range (Table 2). The accuracy test for all analytes using the standard addition method was calculated at 84.23–115.47% (RSD $\leq$ 9.65%) by Equations (1) and (2) (Table 3). The acceptance of accuracy was set at $\pm$20%, so in this respect, the assay is adequate. The acceptable range of precision was also set at $\pm$20%. The results are summarized in Table 4, as calculated by Equation (2). Satisfactory results were obtained with less than 10% variation in intra- and interday precision and repeatability tests. Precision was verified as adequate because the relative RSDs of intra- and interday precision, and repeatability were all <10.0%. Matrix effects could not be tested because it was difficult to find samples that did not contain target analytes to serve as blanks. However, the matrix effects are thought to be insignificant in this experiment, based on the results in Tables 3 and 4. Overall, the optimized UPLC–MS/MS MRM analytical method was found to be satisfactorily valid.

Table 2. Parameters for simultaneous analysis of the 22 analytes in OCE samples using the UPLC–MS/MS MRM assay.

Analyte [1]	Linear Range (μg/L)	Regression Equation [2] $y = ax + b$	r^2	LOD (μg/L)	LOQ (μg/L)
1	250.00–5000.00	$y = 366.26x + 35{,}869.50$	0.9967	1.88×10^{-1}	6.21×10^{1}
2	250.00–5000.00	$y = 2.96x + 418.22$	0.9950	1.64×10^{1}	5.40×10^{1}
3	10.00–500.00	$y = 128.97x + 849.98$	0.9986	4.93×10^{-1}	1.63
4	100.00–2500.00	$y = 523.14x + 20{,}049.70$	0.9996	2.96×10^{-1}	9.78×10^{-1}
5	10.00–500.00	$y = 5852.87x + 50{,}893.70$	0.9992	3.85×10^{-1}	1.27
6	250.00–5000.00	$y = 1.15x + 181.45$	0.9976	2.12×10^{1}	6.99×10^{1}
7	250.00–5000.00	$y = 2936.14x + 106{,}224.00$	0.9993	4.64×10^{-2}	1.53×10^{-1}

Table 2. *Cont.*

Analyte [1]	Linear Range (μg/L)	Regression Equation [2] $y = ax + b$	r^2	LOD (μg/L)	LOQ (μg/L)
8	250.00–5000.00	$y = 4.65x + 398.67$	0.9975	2.31×10^1	7.62×10^1
9	10.00–500.00	$y = 21{,}943.90x + 137{,}062.00$	0.9992	9.09×10^{-3}	3.00×10^{-3}
10	10.00–500.00	$y = 6981.34x + 17{,}442.00$	0.9975	9.52×10^{-2}	3.14×10^{-1}
11	10.00–500.00	$y = 80{,}723.40x + 1{,}592{,}260.00$	0.9972	7.58×10^{-3}	2.50×10^{-2}
12	10.00–500.00	$y = 7396.96x + 158{,}880.00$	0.9969	3.33×10^{-2}	1.10×10^{-1}
13	250.00–5000.00	$y = 13{,}141.30x + 668{,}576.00$	0.9983	3.09×10^{-2}	1.02×10^{-1}
14	10.00–500.00	$y = 96{,}358.10x + 1{,}478{,}850.00$	0.9987	1.82×10^{-3}	6.00×10^{-3}
15	100.00–2500.00	$y = 32{,}388.10x + 3{,}056{,}940.00$	0.9969	2.42×10^{-3}	8.00×10^{-3}
16	250.00–5000.00	$y = 19{,}403.60x + 2{,}923{,}960.00$	0.9952	2.73×10^{-3}	9.00×10^{-3}
17	10.00–500.00	$y = 20.86x + 83.16$	0.9986	3.20×10^{-1}	1.06
18	250.00–5000.00	$y = 9583.75x + 352{,}523.00$	0.9992	1.26×10^{-1}	4.15×10^{-1}
19	10.00–500.00	$y = 36{,}776.20x + 294{,}755.00$	0.9992	2.12×10^{-3}	7.00×10^{-3}
20	10.00–500.00	$y = 267.38x + 2420.96$	0.9977	5.68	1.88×10^1
21	10.00–500.00	$y = 58{,}707.80x + 602{,}086.00$	0.9994	4.55×10^{-3}	1.50×10^{-2}
22	10.00–500.00	$y = 83{,}487.50x + 830{,}806.00$	0.9993	4.24×10^{-3}	1.40×10^{-3}

[1] Gallic acid (1), gardenoside (2), oxypaeoniflorin (3), chlorogenic acid (4), 5-hydroxymethylfurfural (5), geniposide (6), albiflorin (7), paeoniflorin (8), nodakenin (9), ferulic acid (10), jatrorrhizine Cl (11), coptisine Cl (12), baicalin (13), palmatine Cl (14), berberine Cl (15), wogonoside (16), benzoylpaeoniflorin (17), baicalein (18), wogonin (19), Z-ligustilide (20), decursin (21), and decursinol angelate (22). [2] y: peak area of each reference standard compound; x: concentration of each reference standard compound.

Table 3. Accuracy results of the 22 tested analytes by the optimized UPLC–MS/MS MRM assay.

Analyte [1]	Spiked Amount (μg/L)	Found Amount (μg/L)	Accuracy (%)	SD [2]	RSD (%)
	400.00	3.95×10^2	98.69	6.62	6.71
1	800.00	9.24×10^2	115.47	4.77	4.13
	1600.00	1.83×10^3	114.13	4.82	4.22
	600.00	5.15×10^2	85.75	4.83	5.63
2	1200.00	1.18×10^3	97.96	5.11	5.22
	2400.00	2.41×10^3	100.35	5.15	5.14
	8.00	6.74×10^1	84.23	5.27	6.25
3	160.00	1.50×10^2	93.99	5.79	6.16
	320.00	2.94×10^2	91.86	8.57	9.32
	600.00	5.24×10^2	87.38	5.32	6.09
4	1200.00	1.14×10^3	95.39	2.87	3.01
	2400.00	2.18×10^3	90.85	3.67	4.04
	20.00	2.09×10^1	104.32	8.13	7.80
5	40.00	3.93×10^1	98.17	7.53	7.67
	80.00	7.68×10^1	96.06	8.02	8.35
	400.00	3.63×10^2	90.69	4.59	5.06
6	800.00	7.56×10^2	94.55	2.87	3.04
	1600.00	1.52×10^3	94.76	5.72	6.03
	800.00	8.00×10^2	99.98	6.60	6.61
7	1600.00	1.60×10^3	100.27	2.93	2.92
	3200.00	3.05×10^3	95.38	4.19	4.39
	1000.00	1.01×10^3	100.73	5.55	5.51
8	2000.00	2.25×10^3	112.56	1.01	0.90
	4000.00	4.42×10^3	110.42	2.45	2.22
	20.00	2.02×10^1	101.21	2.91	2.87
9	40.00	4.08×10^1	101.89	1.34	1.31
	80.00	8.04×10^1	100.50	1.22	1.21
	80.00	7.83×10^1	94.16	3.13	3.33
10	160.00	1.57×10^2	97.99	2.97	3.04
	320.00	3.23×10^2	101.01	3.84	3.80

Table 3. *Cont.*

Analyte [1]	Spiked Amount (μg/L)	Found Amount (μg/L)	Accuracy (%)	SD [2]	RSD (%)
11	20.00	2.04×10^1	102.02	9.84	9.65
	40.00	4.26×10^1	106.60	5.33	5.00
	80.00	8.14×10^1	101.72	6.50	6.39
12	40.00	3.64×10^1	90.98	4.16	4.57
	80.00	8.31×10^1	103.88	0.86	0.83
	160.00	1.65×10^2	102.93	1.80	1.75
13	1200.00	1.13×10^3	94.57	5.76	6.10
	2400.00	2.46×10^3	102.38	4.53	4.43
	4800.00	4.80×10^3	99.90	4.91	4.92
14	40.00	3.55×10^1	88.86	4.43	4.98
	80.00	7.95×10^1	99.38	3.28	3.30
	160.00	1.56×10^2	97.69	2.04	2.09
15	400.00	3.58×10^2	89.57	3.07	3.42
	800.00	8.78×10^2	109.78	2.56	2.33
	1600.00	1.75×10^3	109.30	1.89	1.73
16	600.00	5.31×10^2	88.50	3.51	3.96
	1200.00	1.12×10^3	93.03	3.20	3.44
	2400.00	2.23×10^3	93.04	2.34	2.51
17	40.00	3.66×10^1	91.39	7.46	8.16
	80.00	8.35×10^1	104.35	7.22	6.92
	160.00	1.67×10^2	104.17	1.62	1.56
18	600.00	5.28×10^2	87.99	6.20	7.05
	1200.00	1.27×10^3	105.42	9.95	9.44
	2400.00	2.46×10^3	102.63	5.52	5.38
19	20.00	2.01×10^1	100.33	2.23	2.22
	40.00	4.15×10^1	103.71	2.83	2.73
	80.00	8.12×10^1	101.55	2.08	2.05
20	20.00	1.98×10^1	99.06	3.87	3.91
	40.00	3.85×10^1	96.16	4.19	4.36
	80.00	7.65×10^1	95.58	4.59	4.80
21	40.00	3.95×10^1	98.68	7.90	8.01
	80.00	7.79×10^1	97.32	4.24	4.35
	160.00	1.50×10^2	93.73	2.89	3.08
22	40.00	3.99×10^1	99.72	3.54	3.55
	80.00	8.19×10^1	102.43	4.49	4.38
	160.00	1.63×10^2	101.70	3.01	2.96

[1] Gallic acid (1), gardenoside (2), oxypaeoniflorin (3), chlorogenic acid (4), 5-hydroxymethylfurfural (5), geniposide (6), albiflorin (7), paeoniflorin (8), nodakenin (9), ferulic acid (10), jatrorrhizine Cl (11), coptisine Cl (12), baicalin (13), palmatine Cl (14), berberine Cl (15), wogonoside (16), benzoylpaeoniflorin (17), baicalein (18), wogonin (19), Z-ligustilide (20), decursin (21), and decursinol angelate (22). [2] SD: Standard deviation.

Table 4. Precision test results for simultaneous analysis of the 22 analytes by the optimized UPLC–MS/MS MRM assay.

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 (%)	Retention Time (RSD, %)	Peak Area (RSD, %)
1	400.00	4.07×10^2	9.45	101.73	3.94×10^2	7.29	98.47	3.89	7.00
	800.00	9.20×10^2	2.97	115.06	9.25×10^2	3.33	115.61		
	1600.00	1.80×10^3	3.22	112.34	1.83×10^3	3.45	114.38		
2	600.00	5.48×10^2	9.09	91.39	5.20×10^2	7.69	86.60	3.87	5.48
	1200.00	1.20×10^3	5.63	100.39	1.20×10^3	5.21	100.07		
	2400.00	2.37×10^3	4.50	98.56	2.46×10^3	4.40	102.52		

Table 4. *Cont.*

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 (%)	Retention Time (RSD, %)	Peak Area (RSD, %)
	80.00	7.83×10^1	3.99	97.91	6.96×10^1	4.74	87.02		
3	160.00	1.68×10^2	5.09	104.72	1.53×10^2	5.89	95.65	4.07	7.44
	320.00	3.33×10^2	3.89	104.21	3.02×10^2	6.16	94.25		
	600.00	5.97×10^2	3.78	99.55	5.35×10^2	4.74	89.22		
4	1200.00	1.24×10^3	5.30	103.02	1.16×10^3	3.81	96.57	3.64	7.21
	2400.00	2.32×10^3	4.13	96.55	2.23×10^3	3.85	92.88		
	20.00	1.71×10^1	6.10	85.32	1.97×10^1	6.70	98.62		
5	40.00	3.95×10^1	3.66	98.84	4.06×10^1	5.75	101.46	3.89	8.31
	80.00	7.66×10^1	2.45	95.79	8.12×10^1	4.17	101.48		
	400.00	3.83×10^2	5.96	95.78	3.60×10^2	5.16	90.00		
6	800.00	7.75×10^2	5.09	96.82	7.64×10^2	4.13	95.55	3.41	3.90
	1600.00	1.60×10^3	6.97	100.28	1.59×10^3	6.24	98.04		
	800.00	7.50×10^2	1.30	93.78	7.76×10^2	5.18	97.06		
7	1600.00	1.48×10^3	4.84	92.22	1.57×10^3	3.65	98.34	2.90	7.91
	3200.00	2.94×10^3	4.12	91.76	3.10×10^3	4.42	96.95		
	1000.00	9.56×10^2	6.48	95.59	6.46×10^2	4.91	96.07		
8	2000.00	2.14×10^3	2.69	107.17	1.44×10^3	2.60	104.27	2.92	8.67
	4000.00	4.26×10^3	3.49	106.58	2.83×10^3	3.83	103.91		
	20.00	2.08×10^1	4.12	104.06	2.00×10^1	4.40	99.90		
9	40.00	4.17×10^1	1.80	104.14	4.10×10^1	1.53	102.39	2.71	5.68
	80.00	8.36×10^1	5.60	104.48	8.24×10^1	3.17	103.04		
	80.00	8.07×10^1	4.44	100.87	7.84×10^1	3.96	97.95		
10	160.00	1.58×10^2	1.08	98.54	1.59×10^2	2.32	99.49	3.09	4.92
	320.00	3.13×10^2	3.25	97.86	3.22×10^2	2.86	100.59		
	20.00	2.10×10^1	9.57	104.89	2.02×10^1	7.44	101.16		
11	40.00	4.29×10^1	2.68	107.23	4.29×10^1	3.63	107.16	2.40	7.01
	80.00	8.37×10^1	2.08	104.56	8.37×10^1	4.54	104.64		
	40.00	3.97×10^1	0.84	99.24	3.75×10^1	2.98	93.77		
12	80.00	8.55×10^1	1.91	106.81	8.36×10^1	1.60	104.47	2.49	7.31
	160.00	1.68×10^2	1.61	105.04	1.66×10^2	1.59	103.79		
	1200.00	1.26×10^3	3.12	104.62	1.17×10^3	4.85	97.30		
13	2400.00	2.71×10^3	2.08	112.80	2.58×10^3	3.11	107.40	2.20	6.45
	4800.00	5.29×10^3	2.34	110.12	5.09×10^3	3.37	105.96		
	40.00	3.30×10^1	2.01	82.38	4.92×10^1	2.95	90.11		
14	80.00	7.58×10^1	1.56	94.71	1.06×10^2	2.11	98.98	1.98	7.05
	160.00	1.53×10^2	2.96	95.53	2.14×10^2	1.99	99.52		
	400.00	3.62×10^2	2.45	90.40	3.58×10^2	3.45	89.37		
15	800.00	8.45×10^2	2.62	105.57	8.65×10^2	2.23	108.09	2.07	8.55
	1600.00	1.68×10^3	3.64	104.93	1.73×10^3	2.19	107.97		
	600.00	6.16×10^2	1.73	102.67	5.64×10^2	2.36	96.15		
16	1200.00	1.22×10^3	0.97	101.63	1.16×10^3	2.10	104.31	1.65	4.26
	2400.00	2.48×10^3	2.85	103.35	2.33×10^3	2.21	105.00		
	40.00	3.90×10^1	4.05	97.51	3.87×10^1	6.06	96.67		
17	80.00	7.97×10^1	6.30	99.59	8.15×10^1	6.07	101.90	1.59	7.36
	160.00	1.62×10^2	4.88	101.16	1.63×10^2	3.09	101.79		
	600.00	6.26×10^2	4.29	104.31	5.81×10^2	4.68	96.89		
18	1200.00	1.28×10^3	6.56	106.75	1.27×10^3	6.56	105.73	1.54	3.50
	2400.00	2.49×10^3	5.93	102.59	2.49×10^3	5.40	103.74		
	20.00	2.09×10^1	2.05	104.40	1.94×10^1	2.43	97.14		
19	40.00	4.27×10^1	2.34	106.81	4.12×10^1	2.62	102.96	1.39	5.73
	80.00	8.62×10^1	2.62	107.79	8.55×10^1	2.91	106.84		

Table 4. *Cont.*

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 (%)	Retention Time (RSD, %)	Peak Area (RSD, %)
20	20.00	2.02×10^1	2.84	100.91	1.94×10^1	3.57	96.92	1.17	3.93
	40.00	4.07×10^1	2.14	101.77	3.94×10^1	3.46	98.42		
	80.00	8.05×10^1	5.03	100.65	8.02×10^1	4.87	100.29		
21	40.00	3.80×10^1	3.65	95.05	3.86×10^1	5.67	96.41	1.02	4.98
	80.00	7.24×10^1	2.48	90.50	7.65×10^1	3.60	95.58		
	160.00	1.45×10^2	3.99	90.44	1.51×10^2	3.62	94.55		
22	40.00	4.35×10^1	1.42	108.67	4.09×10^1	3.35	102.25	1.00	3.52
	80.00	8.58×10^1	4.26	107.31	8.29×10^1	4.01	103.67		
	160.00	1.67×10^2	5.51	104.54	1.65×10^2	4.24	103.39		

[1] Gallic acid (1), gardenoside (2), oxypaeoniflorin (3), chlorogenic acid (4), 5-hydroxymethylfurfural (5), geniposide (6), albiflorin (7), paeoniflorin (8), nodakenin (9), ferulic acid (10), jatrorrhizine Cl (11), coptisine Cl (12), baicalin (13), palmatine Cl (14), berberine Cl (15), wogonoside (16), benzoylpaeoniflorin (17), baicalein (18), wogonin (19), Z-ligustilide (20), decursin (21), and decursinol angelate (22).

3.4. Stability of the Analytes

The stability of all analytes tested for 3 days ranged from 82.70% to 116.230% (RSD < 10.00).

3.5. Simultaneous Analysis of the 22 Analytes in Various OCE Samples by the Optimized UPLC–MS/MS MRM Method

Our manufactured OCE and four commercial OCE preparations were compared using the new analytical method. With a few exceptions, all 22 components were identified in these preparations. Coptisine Cl was detected at <LOQ in OCE–2, and nodakenin, decursin, and decursinol angelate were detected at <LOQ in OCE–5 (Table 5); other analytes were detected at a maximum concentration of 27.10 mg/g. In previous HPLC analysis assays of OCE [17,18], geniposide, a major ingredient of *G. jasminoides*, showed the highest content, whereas berberine Cl was found to be the most abundant component in UPLC–MS/MS analyses. Comparing the earlier HPLC method with the current UPLC–MS/MS method, components such as berberine Cl, baicalin, geniposide, and paeoniflorin were found to be relatively more abundant than other components, showing a similar analytical pattern. However, the HPLC analysis method takes 90 min or more to complete, much slower than the new UPLC–MS/MS analysis method (<18.0 min for complete elution). Therefore, the UPLC–MS/MS MRM assay, which is equally sensitive and accurate, is more time-efficient for OCE analysis and probably other THFs.

Table 5. Amounts (mg/g) of the 22 analytes in OCE samples by the optimized UPLC–MS/MS MRM method (n = 3).

Analyte [1]	Amount									
	OCE–1 [2]		OCE–2		OCE–3		OCE–4		OCE–5	
	Mean (mg/g)	RSD (%)	Mean (mg/g)	RSD (%)	Mean (mg/g)	RSD (%)	Mean (mg/g)	RSD (%)	Mean (mg/g)	RSD (%)
1	0.18	0.85	0.15	0.95	0.68	2.62	0.47	0.84	0.15	3.76
2	1.91	5.32	1.13	9.86	1.29	9.73	0.47	4.59	1.33	9.93
3	0.23	4.45	0.05	6.13	0.05	1.83	0.05	0.77	0.25	1.57
4	2.77	1.06	0.29	0.92	0.09	3.65	0.05	0.87	0.87	1.98
5	1.00	7.23	0.09	4.53	0.04	6.02	0.01	5.09	0.02	5.74
6	17.39	5.51	3.15	8.34	3.80	6.57	3.56	8.06	6.60	2.73
7	5.17	0.30	1.65	0.91	1.78	0.74	1.64	0.16	2.74	0.66
8	6.24	1.29	3.32	3.41	2.80	1.93	3.28	2.46	4.97	2.40

Table 5. *Cont.*

Analyte [1]	Amount									
	OCE–1 [2]		OCE–2		OCE–3		OCE–4		OCE–5	
	Mean (mg/g)	RSD (%)	Mean (mg/g)	RSD (%)	Mean (mg/g)	RSD (%)	Mean (mg/g)	RSD (%)	Mean (mg/g)	RSD (%)
9	1.36	1.43	0.24	2.00	0.09	1.31	0.01	0.39	<LOQ	–
10	0.27	5.00	0.10	5.33	0.02	3.94	0.03	5.15	0.18	2.87
11	0.65	0.70	0.01	3.08	0.03	1.62	0.12	1.01	0.27	2.18
12	2.04	0.41	<LOQ	–	0.07	0.13	0.34	2.64	0.89	0.45
13	5.35	0.86	7.66	0.77	10.23	1.01	10.94	0.73	9.75	1.20
14	2.36	2.52	0.07	1.96	0.10	1.89	0.40	1.97	0.67	0.35
15	27.10	0.23	8.05	0.57	6.09	0.72	5.89	3.65	15.58	2.35
16	3.69	0.91	2.38	0.70	0.39	0.44	2.53	1.24	2.40	1.49
17	0.20	4.53	0.12	9.04	0.07	4.02	0.06	2.12	0.19	5.30
18	3.77	1.74	0.95	0.75	0.42	1.68	0.39	0.12	0.18	1.59
19	0.81	2.07	0.16	1.12	0.09	3.45	0.07	2.18	0.06	1.26
20	1.32	7.02	0.08	7.98	0.01	9.29	0.01	5.98	1.82	9.32
21	0.34	1.25	0.12	1.48	0.06	5.61	0.03	3.14	<LOQ	–
22	0.25	2.62	0.13	4.09	0.06	5.61	0.03	1.01	<LOQ	–

[1] Gallic acid (1), gardenoside (2), oxypaeoniflorin (3), chlorogenic acid (4), 5-hydroxymethylfurfural (5), geniposide (6), albiflorin (7), paeoniflorin (8), nodakenin (9), ferulic acid (10), jatrorrhizine Cl (11), coptisine Cl (12), baicalin (13), palmatine Cl (14), berberine Cl (15), wogonoside (16), benzoylpaeoniflorin (17), baicalein (18), wogonin (19), Z-ligustilide (20), decursin (21), and decursinol angelate (22). [2] OCE–1: The sample was prepared at the Korea Institute of Oriental Medicine; OCE–2 to OCE–5 were provided by different commercial pharmaceutical companies, Kyungbang, Jungwoo, Hankookshinyak, and Tsumura, respectively.

4. Conclusions

We established a method for simultaneous analysis of the 22 analytes in OCE preparations using a sensitive, accurate, and reliable UPLC–MS/MS MRM technique. Developed from a previous HPLC method, the new UPLC–MS/MS MRM analysis method for OCEs was tested and validated for parameters including linearity, sensitivity, accuracy, and precision. The new method is also likely to be useful for research on other THFs.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr11102906/s1, Figure S1: Representative extracted ion chromatograms of each authentic reference analyte (A) and the 70% methanolic solution of the lyophilized OCE–1 sample (B) measured by the UPLC–MS/MS MRM method in positive and negative ion modes. The concentration of each analyte in the standard solution was 1000.0 μg/L; Figure S2: Chemical structures of the 22 analytes selected for simultaneous analysis of OCE; Figure S3: MS fragmentation of each analyte for UPLC–MS/MS MRM analysis; Scheme S1: Fragmentation pathway of coptisine Cl; Table S1: Information of the 22 reference standard compounds for the simultaneous analysis in OCE samples using UPLC–MS/MS; Table S2: UPLC–MS/MS MRM analysis conditions for simultaneous analysis of the 22 analytes in OCE samples; Table S3: Concentration ranges for accuracy and precision validations in the optimized UPLC–MS/MS MRM assay.

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