Research Article

Quality Assessment of Ojeok-San, a Traditional Herbal Formula, Using High-Performance Liquid Chromatography Combined with Chemometric Analysis

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Ojeok-san (OJS) is a traditional herbal formula consisting of 17 herbal medicines that has been used to treat various disorders. In this study, quantitative analytical methods were developed using high-performance liquid chromatography equipped with a photodiode array detector to determine 19 marker compounds in OJS preparations, which was then combined with chemometric analysis. The method developed was validated in terms of its precision and accuracy. The intra- and interday precision of the marker compounds were <3.0% of the relative standard deviation (RSD) and the recovery of the marker compounds was 92.74%–104.16% with RSD values <3.0%. The results of our quantitative analysis show that the quantities of the 19 marker compounds varied between a laboratory water extract and commercial OJS granules. The chemometric analysis used, principal component analysis (PCA) and hierarchical clustering analysis (HCA), also showed that the OJS water extract produced using a laboratory method clearly differed from the commercial OJS granules; therefore, an equalized production process is required for quality control of OJS preparations. Our results suggest that the HPLC analytical methods developed are suitable for the quantification and quality assessment of OJS preparations when combined with chemometric analysis involving PCA and HCA.

1. Introduction

Ojeok-san (OJS) is a traditional herbal formula used in Korean medicine that consists of 17 compositional herbal medicines: Atractylodis rhizoma, Ephedrae herba, Citri Unshiu pericarpium, Magnoliae cortex, Platycodonis radix, Aurantii Fructus Immaturus, Angelicae gigantis radix, Zingiberis rhizoma, Paeoniae radix, Poria sclerotium, Angelicae dahuricae radix, Cnidii rhizoma, Pinelliae tuber, Cinnamomi cortex, Glycyrrhizae radix et rhizoma, Zingiberis rhizoma recens, and Allii fistulosi bulbous. Traditionally, OJS has been used to treat disorders such as fever, anhidrosis, headache, whole body pain, contracture of the nape and neck, vomiting, abdominal and heart pain, and menstrual irregularities [1]. Recent studies have reported on the therapeutic effects of OJS against lumbago and inferior limb pain [2], primary dysmenorrhea [3], clastogenicity [4], and airway inflammation and pulmonary fibrosis [5]. Since a combination of multiple components is considered necessary to exhibit the therapeutic effects of the herbal formula, simultaneous determination of the compositional constituents has been developed for qualitative and quantitative analysis. Several previous studies have analyzed the chemical constituents of OJS using reversed-phase high-performance liquid chromatography (RP-HPLC) coupled with pulsed amperometric detection (PAD) or diode array detection (DAD) [6, 7].

Cluster analysis is a data analysis method used to assign similar objects belonging to the same group and is used in
a variety of practical applications like bioinformatics, using chemometric analyses, such as principal component analysis (PCA) and hierarchical clustering analysis (HCA) [8, 9].

PCA is an unsupervised pattern recognition technique and is a useful tool for visualizing similarities or differences in multivariate data [10]. PCA can represent objects or variables on a graph and is used to study the proximity of objects to classify them and to detect atypical objects [11]. HCA is a procedure that has a pyramid-like structure and is a very useful and widely adopted technique in information processing [12]. HCA determines similarities between samples by measuring the distance between all possible sample pairs in a high-dimensional space and any similarities between the samples are represented on two-dimensional diagrams [13]. The HPLC analytical method combined with chemometric analysis has been widely accepted for the quality control of herbal medicines, as it can be part of a powerful strategy to differentiate the source, location, or species in herbal medicines [14–16].

Recently, herbal formulas have been manufactured by pharmaceutical companies in diverse dosage forms, such as powder, granules, and tablets, as these are more convenient and easier to take than traditional decoction forms. However, the compositional herbal ratio or the origin of herbal components of a herbal formula may differ between different companies, and so the formula produced by each company may contain a variety of chemical constituents [17–19]. Such chemical inequalities cannot warrant equivalent therapeutic effects between different herbal formula preparations and may lead to a loss of innate characteristics of a given herbal formula.

Therefore, in this study, we developed analytical methods for the quantification of 19 marker compounds in a laboratory-produced water extract and in commercial granules of OJS using HPLC–PDA. In addition, chemometric analysis data were combined with the quantitative results and employed to assess the quality of OJS preparations via the Pearson correlation coefficient and PCA and HCA data.

2. Materials and Methods

2.1. Chemicals and Reagents. The HPLC-grade acetonitrile and water used were purchased from JT Baker Inc. (Phillipsburg, NJ, USA) and the guaranteed reagent grade acetic acid used was obtained from Junsei (Chuo-ku, Tokyo, Japan). The gallic acid (1), chlorogenic acid (3), ferulic acid (6), benzoic acid (8), neohesperidin (12), and cinnamic acid (15) used were obtained from Sigma-Aldrich (St. Louis, MO, USA). The protocatechuic acid (2) and nodakenin (9) used were purchased from ChromaDex (Irvine, CA, USA) and NPC BioTech (Geumsan, Chungnam, Korea), respectively. The albinorin (4), paeoniflorin (5), liquiritin (7), naringin (11), cinnamaldehyde (17), and glycyrrhizin (19) used were purchased from Wako Pure Chemical Industries (Chuo-ku, Osaka, Japan). The hesperidin (10), ononin (13), oxyxpeucedanin hydrate (14), byakangelicin (16), and benzoylpaeoniflorin (18) used were purchased from Chengdu Biopurify Phytochemicals (Chengdu, Sichuan, China).

The purity of the standard compounds was ≥98%; their chemical structures are shown in Figure 1. Compositional herbal medicines of OJS were purchased from the herbal medicine company, Kwangmyungdang Medicinal Herbs (Ulsan, Gyeongbuk, Korea). A voucher specimen (2012-KE04-1-17) was deposited in the Herbal Medicine Formulation Research Group of the Korea Institute of Oriental Medicine. Commercial OJS samples denoted as “OJS02–OJS10” were purchased from nine pharmaceutical companies located in Korea. The compositional herbal ratio was shown in Table 1.

2.2. Preparation of the OJS Water Extract and Commercial Granules. Dried herbal drugs consisting of OJS were mixed and extracted using a 10-fold volume of distilled water (w/v) at 100°C for 2 h under pressure (1 kgf/cm²) using an electric extractor (COSMOS-660, KyungSeo Machine Co., Incheon, Korea). The extracted decoction was filtered through a standard sieve (number 270, 53 μm, Chunggyesangongsa, Seoul, Korea) and freeze-dried to make OJS water extract powder denoted as “OJS01.”

Powdered OJS01 (200 mg) and commercial OJS granules (OJS02–OJS10, 500 mg) were dissolved in 10 mL of distilled water and the solutions were filtered through a 0.2 μm syringe filter (SmartPor, Woongki Science, Seoul, Korea) before being injected into the HPLC system.

2.3. Chromatographic Conditions. The HPLC system used was a Shimadzu LC-20A (Kyoto, Japan) chromatograph equipped with a solvent delivery unit (LC-20AT), an autosampler (SIL-20AC), a column oven (CTO-20A), a degasser (DGU-20A), and a photodiode array detector (SPD-M20A). Separation was conducted on a Gemini C18 column (4.6 × 250 mm, 5 μm; Phenomenex, Torrance, CA, USA). The column temperature was set at 40°C. The mobile phase consisted of water containing 0.1% formic acid (A) and acetonitrile (B). The composition of the mobile phase was 6%–20% (B) for 0–20 min, 20%–25% (B) for 25–30 min, 25%–40% (B) for 30–40 min, 40%–46% (B) for 40–50 min, and 46%–87% (B) for 50–55 min, held for 5 min and then reequilibrated to 6% (B) until the end of the analysis. The flow rate was 1.0 mL/min and the injection volume was 10 μL. The detection wavelengths of all standards and samples were in the UV at 230, 250, 260, 270, 275, 280, 290, 310, 325, and 335 nm.

2.4. Method Validation

2.4.1. Linearity. The S9 standard compounds were accurately weighed and dissolved in methanol to prepare stock solutions at a concentration of 1000 μg/mL. Stock solutions of the marker compounds were serially diluted to construct calibration curves. The diluted concentrations of marker compounds were plotted against the peak area on the calibration curves and the linearity was measured from the correlation coefficient.
Figure 1: Chemical structures of 19 marker compounds in Ojeok-san (OJS). (1) Gallic acid, (2) protocatechuic acid, (3) chlorogenic acid, (4) albiflorin, (5) paeoniflorin, (6) ferulic acid, (7) liquiritin, (8) benzoic acid, (9) nodakenin, (10) hesperidin, (11) naringin, (12) neohesperidin, (13) ononin, (14) oxypeucedanin hydrate, (15) cinnamic acid, (16) byakangelicin, (17) cinnamaldehyde, (18) benzoylpaeoniflorin, and (19) glycyrrhizin.
Table 1: Compositional ratio of herbal medicine consisting of Ojeok-san (OJS) samples.

| Herbal medicine                  | OJS01 | OJS02 | OJS03 | OJS04 | OJS05 | OJS06 | OJS07 | OJS08 | OJS09 | OJS10 |
|--------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Atractylodis rhizoma           | 0.133 | 0.212 | 0.215 | —     | 0.212 | 0.443 | 0.212 | 0.532 | 0.214 | 0.443 |
| Ephedrae herba                 | 0.067 | 0.045 | 0.046 | —     | 0.045 | 0.223 | 0.045 | 0.268 | 0.045 | 0.223 |
| Citri Unshiu persicarpium      | 0.067 | 0.091 | 0.090 | —     | 0.091 | 0.223 | 0.091 | 0.268 | 0.090 | 0.223 |
| Magnoliae cortex               | 0.053 | 0.018 | 0.018 | —     | 0.018 | 0.223 | 0.018 | 0.268 | 0.018 | 0.223 |
| Platycodonis radix            | 0.053 | 0.095 | 0.097 | —     | 0.095 | 0.223 | 0.095 | 0.268 | 0.097 | 0.223 |
| Aurantii Fructus Immaturus     | 0.053 | 0.069 | 0.070 | —     | 0.069 | —     | 0.069 | —     | 0.070 | —     |
| Angelicae gigantis radix      | 0.053 | 0.082 | 0.084 | —     | 0.082 | 0.223 | 0.082 | 0.268 | 0.083 | 0.223 |
| Zingiberis rhizoma            | 0.053 | 0.049 | 0.050 | —     | 0.049 | 0.223 | 0.049 | 0.268 | 0.050 | 0.223 |
| Paoniae radix                  | 0.053 | 0.060 | 0.061 | —     | 0.060 | 0.223 | 0.060 | 0.268 | 0.061 | 0.223 |
| Poria sclerotium              | 0.053 | 0.004 | 0.004 | —     | 0.004 | 0.223 | 0.004 | 0.268 | 0.004 | 0.223 |
| Cnidii rhizoma                | 0.047 | 0.069 | 0.067 | —     | 0.069 | 0.223 | 0.069 | 0.268 | 0.066 | 0.223 |
| Angelicaedahuricae radix      | 0.047 | 0.071 | 0.069 | —     | 0.071 | 0.223 | 0.071 | 0.268 | 0.069 | 0.223 |
| Pinelliae tuber                | 0.047 | 0.071 | 0.051 | —     | 0.051 | 0.223 | 0.051 | 0.268 | 0.050 | 0.223 |
| Cinnamomi cortex              | 0.047 | 0.011 | 0.011 | —     | 0.011 | 0.223 | 0.011 | 0.268 | 0.011 | —     |
| Glycyrrhiza radix et rhizoma   | 0.040 | 0.045 | 0.046 | —     | 0.045 | 0.223 | 0.045 | 0.268 | 0.045 | 0.223 |
| Zingiberis rhizoma crudus      | 0.067 | 0.027 | 0.028 | —     | 0.027 | —     | 0.027 | —     | 0.027 | —     |
| Poncri Fructus Immaturus       | —     | —     | —     | —     | 0.223 | —     | 0.268 | —     | 0.223 | —     |
| Zizyphi Fructus               | —     | —     | —     | —     | 0.223 | —     | 0.268 | —     | 0.223 | —     |
| Cinnamomi ramulus             | —     | —     | —     | —     | 0.223 | —     | 0.268 | —     | 0.223 | —     |
| Cyperi rhizoma                | —     | —     | —     | —     | —     | —     | 0.160 | —     | 0.133 | —     |
| Allii fistulosi bulbus         | 0.067 | —     | —     | —     | —     | —     | —     | —     | —     | —     |

Single dose 1 1 1 1 1 1 1 1 1

*aOJS01, Ojeok-san water extract from the laboratory and bOJS02–OJS10 = Ojeok-san granules from Chinese manufacturers.*

2.4.2. **LOD and LOQ.** Blank samples were analyzed in triplicate and the area of the noise peak was calculated as the response. The LOD and LOQ were calculated as LOD = 3.3 × SD/S and LOQ = 10 × SD/S, where SD is the standard deviation of the response and S is the slope of the calibration curve.

2.4.3. **Precision.** The precision was calculated by analyzing sample extracts containing low and high concentrations of the marker compounds. The precision was represented by the relative standard deviation (RSD), which was calculated using the equation RSD = (standard deviation/mean) × 100. The precision was measured three times in a single day (intraday precision) and over three consecutive days (interday precision).

2.4.4. **Recovery.** The accuracy of the method used was evaluated through the recovery test. Both low and high concentrations of the marker compounds were added to the samples. The recovery was calculated as follows: recovery (%) = ((detected concentration – initial concentration)/spiked concentration) × 100.

2.5. **Chemometric Analysis.** The relationship between OJS samples was evaluated using the Pearson coefficient of the amounts of the marker compounds. To cluster the OJS samples, PCA and HCA were performed based on the rows (OJS samples) and columns (the amounts of the 19 marker compounds). The evaluation of the Pearson coefficient and the clustering analysis (PCA and HCA) were carried out using the open-source software package R (v. 3.0.2).

3. Results and Discussion

3.1. **Optimization of Chromatographic Conditions.** The mobile phase modifier, gradient ratio, and UV detection wavelength were considered as the main factors for optimizing the conditions for the HPLC analysis of the OJS water extract. A C_{18} column was employed for the simultaneous determination of the 19 marker compounds in the OJS water extract, as it has been the most frequently used technique in the chemical analysis of herbal medicines [20, 21]. Two different modifiers, 1% acetic acid and 0.1% formic acid, were compared to find the optimal conditions for the separation of the 19 marker compounds. Peak resolution and shape of the marker compounds were considered better indicators when 0.1% formic acid was used as a modifier.

Various ratios of the components of the mobile phase (A : B) were tested using gradient elution, and the optimal separation was observed at the following gradient conditions: 6%–20% (B) for 0–20 min, 20%–25% (B) for 25–30 min, 25%–40% (B) for 30–40 min, 40%–46% (B) for 40–50 min, and 46%–87% (B) for 50–55 min, held for 5 min and then reequilibrated to 6% (B).

The UV wavelength in the range 190–400 nm was scanned to find the maximum absorption for each marker
compound. For albiflorin, paeoniflorin, benzoic acid, and benzoylpaeoniflorin, this occurred at 230 nm; for ononin and glycyrrhizin at 250 nm; for protocatechuic acid at 260 nm; for gallic acid and byakangelicin at 270 nm; for liquiritin and cinnamic acid at 275 nm; for hesperidin, naringin, and neohesperidin at 280 nm; for cinnamaldehyde at 290 nm; for oxypeucedanin hydrate at 310 nm; for chlorogenic acid and ferulic acid at 325 nm; and for nodakenin at 335 nm. For the conditions described above, the 19 marker compounds were reasonably separated on C$_{18}$ column for quantitative analysis (Figure 2).

3.2. Method Validation

3.2.1. Linear Regression, LOD, and LOQ. The linearity of the calibration curve was measured using the correlation coefficient ($r^2$), which ranged in value from 0.9993 to 1.0000 for each compound. The LOD and LOQ values were 0.004–0.090 µg/mL and 0.012–0.272 µg/mL, respectively (Table 2).

3.2.2. Precision and Recovery. The intra- and interday precision, which were represented by the RSD values, were RSD < 3.0% for the two concentration levels (Table 3). The recoveries of the 19 marker compounds were in the range 92.74%–104.16%, with RSD < 4.0% at different spiked concentrations (Table 4). These results indicate that the developed analytical method was precise, accurate, and reliable for the analysis of the 19 marker compounds in the OJS samples.

3.3. Quantification of the Marker Compounds in the OJS Samples. The method we established was successfully applied to determine the 19 reference compounds in the OJS water extract (OJS01) and commercial OJS granules (OJS02–OJS10). There was wide variation observed in the contents of the marker compounds in the 10 OJS samples. While OJS01 contained the 19 marker compounds, the commercial OJS granules showed lack of one or more of the following compounds: protocatechuic acid, chlorogenic acid, ferulic acid, nodakenin, hesperidin, neohesperidin, and cinnamaldehyde.

Moreover, variation in the content of these compounds was apparent between the OJS samples: 2.8–16.6-fold for gallic acid, 1.1–3.5-fold for protocatechuic acid, 1.4–37.0-fold for chlorogenic acid, 2.0–64.0-fold for albiflorin, 2.1–8.7-fold for paoniflorin, 1.1-fold for ferulic acid, 5.9–571-fold for liquiritin, 1.1–78-fold for benzoic acid, 5.0–224.5-fold for nodakenin, 5.5–624.9-fold for hesperidin, 1.6–8.5-fold for naringin, 2.4–73.0-fold for neohesperidin, 3.5–13.8-fold for ononin, 3.8–71.8-fold for oxypeucedanin hydrate, 3.7–21.3-fold for cinnamic acid, 5.0–124.0-fold for byakangelicin, 1.5-fold for cinnamaldehyde, 3.8–112.6-fold for benzoylpaeoniflorin, and 3.1–8.6-fold for glycyrrhizin (Table 5).

This result implies that the water extract and commercial granules of OJS were not chemically equivalent because of the variation in the content of the marker compounds.

3.4. Evaluation of Correlation between the OJS Samples Using Chemometric Analysis. Similarities between the OJS samples were assessed using the Pearson correlation coefficient ($r^2$), which is a measurement of the distance between two samples and shows the degree of their relationship: a stronger correlation is observed when $r^2$ is closer to a value of 1 [22]. The average value of $r^2$ for OJS01 was the lowest, followed by OJS04, while the values of the other OJS samples were in the range 0.5 < $r^2$ < 0.8 (Figure 3). This means that OJS01 and

![Figure 2: Chromatograms of (a) the standard marker compounds and (b) OJS samples at a detection wavelength of UV 254 nm. (1) Gallic acid, (2) protocatechuic acid, (3) chlorogenic acid, (4) albiflorin, (5) paoniflorin, (6) ferulic acid, (7) liquiritin, (8) benzoic acid, (9) nodakenin, (10) hesperidin, (11) naringin, (12) neohesperidin, (13) ononin, (14) oxypeucedanin hydrate, (15) cinnamic acid, (16) byakangelicin, (17) cinnamaldehyde, (18) benzoylpaeoniflorin, and (19) glycyrrhizin. OJS01, Ojeok-san water extract from the laboratory; OJS02–OJS10, Ojeok-san granules from Korean manufacturers.](image)

![Figure 3: Pearson correlation coefficient ($r^2$) between the OJS samples. OJS01, Ojeok-san water extract from the laboratory; OJS02–OJS10, Ojeok-san granules from Korean manufacturers.](image)
Table 2: Regression, correlation coefficient ($r^2$), LOD, and LOQ of the marker compounds of OJS.

| Compound          | UV wavelength | Regression equation | Linear range (µg/mL) | $r^2$  | LOD (µg/mL) | LOQ (µg/mL) |
|-------------------|---------------|---------------------|----------------------|--------|-------------|-------------|
| Gallic acid       | 270 nm        | 39,349              | 4,315                | 0.63–5.00 | 0.9995 | 0.015 | 0.047 |
| Protocatechuic acid | 260 nm       | 42,285              | 2,576                | 0.31–5.00 | 0.9994 | 0.014 | 0.043 |
| Chlorogenic acid  | 325 nm        | 32,676              | 9,500                | 1.56–25.00 | 0.9994 | 0.019 | 0.056 |
| Albiflorin        | 230 nm        | 10,703              | 2,218                | 1.56–25.00 | 0.9998 | 0.057 | 0.172 |
| Paeoniflorin      | 230 nm        | 15,956              | –377                 | 4.69–75.00 | 1.0000 | 0.038 | 0.115 |
| Ferulic acid      | 325 nm        | 44,533              | 17,050               | 1.56–25.00 | 0.9993 | 0.014 | 0.041 |
| Liquiritin        | 275 nm        | 24,585              | 12,410               | 4.69–75.00 | 0.9999 | 0.025 | 0.075 |
| Benzoic acid      | 230 nm        | 38,560              | 11,910               | 1.56–25.00 | 0.9998 | 0.016 | 0.048 |
| Nodakenin         | 335 nm        | 34,254              | 17,529               | 4.69–75.00 | 0.9999 | 0.018 | 0.054 |
| Hesperidin        | 280 nm        | 18,406              | 25,320               | 10.94–175.00 | 0.9999 | 0.033 | 0.100 |
| Naringin          | 280 nm        | 15,468              | 20,566               | 10.94–175.00 | 0.9999 | 0.039 | 0.119 |
| Neohesperidin     | 280 nm        | 25,094              | 24,132               | 7.81–125.00 | 0.9999 | 0.024 | 0.073 |
| Ononin            | 250 nm        | 58,807              | 3,317                | 0.31–5.00 | 0.9994 | 0.010 | 0.031 |
| Oxypeucedanin hydrate | 310 nm    | 16,087              | 2,865                | 0.56–25.00 | 0.9993 | 0.038 | 0.114 |
| Cinnamic acid     | 275 nm        | 93,234              | 10,584               | 0.63–10.00 | 0.9998 | 0.007 | 0.020 |
| Byakangelin      | 270 nm        | 23,738              | 5,392                | 1.56–25.00 | 0.9994 | 0.026 | 0.077 |
| Cinnamaldehyde    | 290 nm        | 156,619             | 8,846                | 0.33–5.25 | 0.9996 | 0.004 | 0.012 |
| Benzoypaeoniflorin | 230 nm       | 28,272              | 274                  | 0.16–2.50 | 0.9997 | 0.021 | 0.065 |
| Glycyrrhizin      | 250 nm        | 6,765               | 1,864                | 1.56–25.00 | 0.9993 | 0.090 | 0.272 |

OJS04 were weakly correlated with the other OJS granules, which showed a mild correlation between samples [23].

Clustering is a partitioning process of objects set into disjoint clusters: objects in the same cluster are similar, while objects belonging to different clusters differ considerably according to their attributes [24], to which PCA and HCA can then be applied.

The 10 OJS samples were distributed on a PCA plot using their PC1 and PC2 scores, as these had higher eigenvalues and, thus, contained the chemically relevant variance [25]. OJS01 and OJS04 had a negative PC1 score, while the other samples had a positive PC1 score, and these were further divided by their PC2 score. The laboratory OJS water extract was differentiated from the commercial OJS granules, except for OJS04, by its PC1 score, which was the most influential factor for clustering the samples. Moreover, the distribution of the commercial OJS samples, especially OJS03 and OJS07, was not located close to each other but spread wide by their PC2 score. Therefore, this was a lower influential factor on the clustering samples after the PC1 score. The marker compounds contributing to the distribution of OJS samples were mainly cinnamic acid, cinnamaldehyde, albiflorin, and benzoypaeoniflorin, which are denoted by the red-colored arrows in the PCA plot in Figure 4.

HCA is a method used to measure the distance between objects and find the underlying structure. It uses an iterative procedure that either associates or dissociates a group object by object to classify objects [26]. New clusters are produced by measuring the smallest increase in the sum of the squared within-cluster distances between all the possible clusters, and these are represented by dendrograms [27]. The 10 OJS samples were classified using Ward’s method employing the Euclidean distance as a measurement for the HCA. OJS01 showed an exclusively close correlation with OJS04 and formed a separate cluster from the other commercial samples. These were segregated at a height around a value of 11. Under a height value around 5, the commercial OJS samples were further divided into two groups, namely OJS03 and OJS07, and OJS02, OJS05, OJS06, and OJS08–OJS10, which is similar to the results from the PCA analysis (Figure 5).

Taking the results of the quantification and chemometric analyses together, the OJS water extract (OJS01) produced in the laboratory showed little correlation with the commercially manufactured OJS granules from a chemical perspective. This result demonstrates that the low correlation between the OJS samples, particularly the laboratory-produced water extract and the commercial granules, can presumably be ascribed to the different ratios of the compositional herbal medicines, herbal resources, or extraction procedures of the OJS preparations between different pharmaceutical companies.
Table 3: Intra- and interday precision of the marker compounds of OJS.

| Compound             | Spiked concentration (μg/mL) | Intraday (n = 3) | Interday (n = 3) |
|----------------------|-----------------------------|-----------------|-----------------|
|                      | Detected concentration (μg/mL) | RSD (%) | Accuracy (%) | Detected concentration (μg/mL) | RSD (%) | Accuracy (%) |
| Gallic acid          | 1.00                        | 1.00           | 1.68           | 100.41 | 1.00 | 1.68 | 100.41 |
|                      | 2.00                        | 2.00           | 0.37           | 100.18 | 2.00 | 0.48 | 100.12 |
| Protocatechuic acid  | 1.00                        | 1.02           | 0.89           | 101.59 | 1.00 | 1.79 | 100.11 |
|                      | 2.00                        | 2.00           | 0.45           | 99.97  | 2.01 | 0.43 | 100.31 |
| Chlorogenic acid     | 2.00                        | 1.96           | 0.27           | 98.04  | 1.96 | 0.38 | 97.98  |
|                      | 4.00                        | 4.02           | 0.06           | 100.49 | 4.02 | 0.09 | 100.51 |
| Albulorin            | 2.00                        | 1.99           | 0.10           | 99.56  | 1.97 | 2.57 | 98.61  |
|                      | 4.00                        | 4.00           | 0.27           | 100.11 | 4.02 | 0.45 | 100.52 |
| Paeoniflorin         | 10.00                       | 10.22          | 1.36           | 102.20 | 10.22 | 1.37 | 102.20 |
|                      | 20.00                       | 19.89          | 0.35           | 99.45  | 19.89 | 0.35 | 99.45  |
| Ferulic acid         | 2.00                        | 2.01           | 0.56           | 100.53 | 1.99 | 1.10 | 99.62  |
|                      | 4.00                        | 4.00           | 0.26           | 100.09 | 4.00 | 0.27 | 100.10 |
| Liquiritin           | 5.00                        | 5.22           | 1.13           | 104.46 | 5.24 | 1.50 | 104.80 |
|                      | 10.00                       | 9.89           | 0.30           | 98.88  | 9.88 | 0.40 | 98.80  |
| Benzoic acid         | 3.00                        | 2.88           | 0.67           | 95.97  | 2.87 | 1.22 | 95.67  |
|                      | 6.00                        | 6.06           | 0.16           | 101.01 | 6.06 | 0.29 | 101.08 |
| Nodakenin            | 5.00                        | 5.01           | 0.42           | 100.12 | 5.03 | 0.28 | 100.51 |
|                      | 10.00                       | 10.00          | 0.11           | 99.97  | 9.99 | 0.16 | 99.93  |
| Hesperidin           | 20.00                       | 20.59          | 0.39           | 102.97 | 20.60 | 0.41 | 102.98 |
| Naringin             | 40.00                       | 39.70          | 0.10           | 99.76  | 39.70 | 0.11 | 99.25  |
| Neohesperidin        | 20.00                       | 20.83          | 0.30           | 104.15 | 20.83 | 0.31 | 104.15 |
|                      | 40.00                       | 39.58          | 0.08           | 98.96  | 39.58 | 0.08 | 98.96  |
| Ononin               | 15.00                       | 14.11          | 0.15           | 94.07  | 14.10 | 0.23 | 94.02  |
|                      | 30.00                       | 30.44          | 0.03           | 101.48 | 30.46 | 0.02 | 101.53 |
| Oxypeucedanin hydrate| 1.00                        | 0.98           | 0.26           | 97.60  | 0.98 | 0.24 | 97.60  |
|                      | 2.00                        | 2.01           | 0.06           | 100.60 | 2.01 | 0.06 | 100.60 |
| Cinnamic acid        | 1.00                        | 0.98           | 1.99           | 97.91  | 0.97 | 2.65 | 97.35  |
|                      | 2.00                        | 2.01           | 0.49           | 100.52 | 2.01 | 0.64 | 100.66 |
| Byakangelicin        | 1.00                        | 0.99           | 0.09           | 99.26  | 0.99 | 0.50 | 99.02  |
| Cinnamaldehyde       | 2.00                        | 2.00           | 0.02           | 100.19 | 2.00 | 0.12 | 100.24 |
| Benzoyleaoniflorin   | 1.00                        | 1.01           | 1.53           | 99.25  | 0.99 | 1.87 | 99.01  |
| Glycyrrhizin         | 2.00                        | 2.00           | 0.32           | 100.05 | 2.00 | 0.45 | 99.97  |
|                      | 3.00                        | 2.93           | 0.38           | 97.53  | 2.92 | 0.68 | 97.35  |
|                      | 6.00                        | 6.04           | 0.09           | 100.62 | 6.04 | 0.16 | 100.66 |

*RSD (%) = (SD/mean) × 100.

Therefore, verification of the herbal resources, using an identical combination ratio, or using a valid extraction process, is required to produce chemically equalized OJS preparations that can guarantee an equivalent therapeutic efficacy.

4. Conclusions

The analytical method developed using an HPLC-PDA with a reversed-phase C<sub>18</sub> column was precise, accurate, and reliable and was successfully applied to the simultaneous determination and quantification of 19 marker compounds for the quality assessment of OJS samples. The content of the marker compounds varied between the OJS samples. Moreover, a laboratory-produced OJS water extract was not closely related to the commercial OJS granules, which also showed a wide distribution in the results of chemometric analyses, such as the Pearson correlation coefficient, PCA, and HCA. Our results suggest that HPLC–PDA combined with chemometric analysis can be a useful strategy for...
| Compound          | Initial concentration (µg/mL) | Spiked concentration (µg/mL) | Detected concentration (µg/mL) | Recovery (%) | RSD (%) |
|-------------------|-------------------------------|-----------------------------|--------------------------------|--------------|---------|
| Gallic acid       | 1.90                          | 1.00                        | 2.88                           | 98.05        | 2.28    |
|                   | 2.00                          | 3.82                        | 96.15                          | 3.65         |         |
| Protocatechuic acid | 0.39                       | 1.00                        | 1.41                           | 102.22       | 1.75    |
|                   | 2.00                          | 2.47                        | 104.16                         | 0.88         |         |
| Chlorogenic acid  | 6.52                          | 2.00                        | 8.37                           | 92.74        | 1.21    |
|                   | 4.00                          | 10.36                       | 95.89                          | 1.08         |         |
| Albinflorin       | 3.62                          | 2.00                        | 5.57                           | 97.19        | 1.41    |
|                   | 4.00                          | 7.61                        | 99.59                          | 2.80         |         |
| Paeoniflorin      | 15.89                         | 10.00                       | 25.84                          | 99.54        | 1.86    |
|                   | 20.00                         | 34.91                       | 95.12                          | 0.78         |         |
| Ferulic acid      | 3.75                          | 2.00                        | 5.65                           | 95.33        | 1.09    |
|                   | 4.00                          | 7.53                        | 94.58                          | 0.24         |         |
| Liquiritin        | 16.01                         | 5.00                        | 20.98                          | 99.43        | 1.74    |
|                   | 10.00                         | 25.16                       | 91.55                          | 0.43         |         |
| Benzoic acid      | 6.72                          | 3.00                        | 9.59                           | 95.74        | 1.91    |
|                   | 6.00                          | 12.84                       | 101.90                         | 1.70         |         |
| Nodakenin         | 8.98                          | 5.00                        | 13.74                          | 95.28        | 1.03    |
|                   | 10.00                         | 18.40                       | 94.27                          | 0.77         |         |
| Hesperidin        | 61.70                         | 20.00                       | 81.66                          | 99.77        | 0.28    |
|                   | 40.00                         | 99.80                       | 95.26                          | 0.56         |         |
| Naringin          | 62.30                         | 20.00                       | 82.34                          | 100.19       | 0.72    |
|                   | 40.00                         | 99.63                       | 93.32                          | 0.38         |         |
| Neohesperidin     | 36.31                         | 15.00                       | 50.24                          | 92.90        | 0.18    |
|                   | 30.00                         | 66.90                       | 101.97                         | 0.07         |         |
| Ononin            | 0.56                          | 1.00                        | 1.50                           | 94.75        | 0.05    |
|                   | 2.00                          | 2.52                        | 98.29                          | 0.35         |         |
| Oxypeucedanin hydrate | 7.18                       | 1.00                        | 8.11                           | 93.19        | 2.25    |
|                   | 2.00                          | 9.14                        | 97.97                          | 2.43         |         |
| Cinnamic acid     | 0.94                          | 1.00                        | 1.92                           | 97.97        | 0.84    |
|                   | 2.00                          | 2.93                        | 99.43                          | 0.25         |         |
| Byakangelicin     | 4.95                          | 1.00                        | 5.94                           | 99.43        | 1.40    |
|                   | 2.00                          | 6.96                        | 100.58                         | 1.67         |         |
| Cinnamaldehyde    | 1.66                          | 1.05                        | 2.63                           | 92.64        | 1.97    |
|                   | 2.10                          | 3.66                        | 95.06                          | 1.17         |         |
| Benzyloypaeoniflorin | 0.38                        | 1.00                        | 1.39                           | 100.55       | 1.19    |
|                   | 2.00                          | 2.40                        | 100.85                         | 1.95         |         |
| Glycyrrhizin      | 23.08                         | 3.00                        | 26.00                          | 97.50        | 0.47    |
|                   | 6.00                          | 29.19                       | 101.91                         | 0.83         |         |

* RSD (%) = (SD/mean) × 100.
The average content is represented as mean ± SD.

| Compound                  | OJS01<sup>a</sup> | OJS02<sup>b</sup> | OJS03 | OJS04 | OJS05 | OJS06 | OJS07 | OJS08 | OJS09 | OJS10 |
|---------------------------|-------------------|-------------------|--------|--------|--------|--------|--------|--------|--------|--------|
| Gallic acid               | 0.096 ± 0.001     | 0.609 ± 0.023     | 0.978 ± 0.054 | 0.531 ± 0.005 | 0.273 ± 0.003 | 0.372 ± 0.004 | 0.371 ± 0.013 | 0.372 ± 0.023 | 1.592 ± 0.068 | 0.379 ± 0.006 |
| Protocatechuic acid       | 0.019 ± 0.000     | 0.063 ± 0.001     | ND<sup>c</sup> | 0.032 ± 0.000 | 0.022 ± 0.002 | 0.019 ± 0.000 | ND<sup>c</sup> | 0.018 ± 0.001 | 0.035 ± 0.001 | 0.036 ± 0.002 |
| Chlorogenic acid          | 0.326 ± 0.002     | 0.009 ± 0.002     | ND<sup>c</sup> | 0.333 ± 0.014 | 0.013 ± 0.000 | 0.062 ± 0.006 | ND<sup>c</sup> | 0.028 ± 0.001 | 0.032 ± 0.002 | 0.036 ± 0.004 |
| Albiflorin                | 0.184 ± 0.008     | 1.635 ± 0.020     | 5.950 ± 0.066 | 0.249 ± 0.010 | 0.243 ± 0.006 | 0.093 ± 0.011 | 5.060 ± 0.071 | 0.938 ± 0.017 | 1.072 ± 0.015 | 0.701 ± 0.040 |
| Paeoniflorin              | 0.796 ± 0.006     | 3.363 ± 0.030     | 2.457 ± 0.046 | 2.294 ± 0.030 | 0.385 ± 0.017 | 1.529 ± 0.034 | 2.190 ± 0.048 | 1.679 ± 0.005 | 2.522 ± 0.052 | 1.539 ± 0.010 |
| Ferulic acid              | 0.187 ± 0.000     | ND<sup>c</sup>    | ND<sup>c</sup> | 0.172 ± 0.004 | ND<sup>c</sup> | ND<sup>c</sup> | ND<sup>c</sup> | ND<sup>c</sup> | ND<sup>c</sup> | ND<sup>c</sup> |
| Liquiritin                | 0.800 ± 0.003     | 0.325 ± 0.008     | 0.259 ± 0.011 | 0.237 ± 0.009 | 0.083 ± 0.000 | 0.214 ± 0.005 | 0.04 ± 0.002  | 0.247 ± 0.002 | 0.236 ± 0.003 | 0.150 ± 0.003 |
| Benzoic acid              | 0.337 ± 0.003     | 0.950 ± 0.012     | 0.449 ± 0.003 | 0.371 ± 0.007 | 0.244 ± 0.001 | 0.275 ± 0.001 | 1.902 ± 0.103 | 0.998 ± 0.005 | 1.612 ± 0.005 | 0.476 ± 0.005 |
| Nodakenin                 | 0.449 ± 0.002     | ND<sup>c</sup>    | 0.037 ± 0.000 | 0.002 ± 0.001 | 0.000 ± 0.000 | ND<sup>c</sup> | ND<sup>c</sup> | 0.057 ± 0.001 | 0.045 ± 0.000 | 0.030 ± 0.001 |
| Hesperidin                | 3.086 ± 0.003     | 0.437 ± 0.013     | 0.348 ± 0.004 | 4.999 ± 0.010 | 0.376 ± 0.001 | 0.875 ± 0.006 | ND<sup>c</sup> | 0.044 ± 0.001 | 0.214 ± 0.002 | 0.008 ± 0.002 |
| Naringin                  | 3.115 ± 0.002     | 0.578 ± 0.007     | 1.084 ± 0.007 | 2.983 ± 0.001 | 0.631 ± 0.001 | 0.783 ± 0.002 | 0.366 ± 0.004 | 0.580 ± 0.001 | 1.276 ± 0.004 | 0.750 ± 0.003 |
| Neohesperidin             | 1.816 ± 0.001     | 0.277 ± 0.001     | 0.198 ± 0.001 | 3.139 ± 0.003 | 0.235 ± 0.000 | 0.592 ± 0.001 | ND<sup>c</sup> | ND<sup>c</sup> | 0.105 ± 0.000 | 0.043 ± 0.000 |
| Ononin                    | 0.028 ± 0.000     | 0.038 ± 0.000     | 0.022 ± 0.000 | 0.018 ± 0.000 | 0.014 ± 0.000 | 0.049 ± 0.000 | 0.004 ± 0.000 | 0.055 ± 0.000 | 0.033 ± 0.000 | 0.028 ± 0.000 |
| Oxypeucedanid hydrate     | 0.359 ± 0.001     | 0.021 ± 0.001     | 0.052 ± 0.000 | 0.165 ± 0.000 | 0.028 ± 0.001 | 0.057 ± 0.000 | 0.005 ± 0.000 | 0.028 ± 0.001 | 0.019 ± 0.000 | 0.080 ± 0.000 |
| Cinnamic acid             | 0.047 ± 0.000     | 0.033 ± 0.000     | 0.022 ± 0.000 | 0.055 ± 0.001 | 0.003 ± 0.000 | 0.011 ± 0.001 | 0.064 ± 0.002 | 0.013 ± 0.001 | 0.023 ± 0.000 | 0.041 ± 0.000 |
| Byakangelicin            | 0.248 ± 0.002     | 0.013 ± 0.000     | 0.046 ± 0.000 | 0.071 ± 0.001 | 0.000 ± 0.000 | 0.030 ± 0.000 | 0.002 ± 0.000 | 0.016 ± 0.000 | 0.015 ± 0.000 | 0.026 ± 0.000 |
| Cinnamaldehyde            | 0.083 ± 0.001     | ND<sup>c</sup>    | 0.122 ± 0.002 | ND<sup>c</sup> | ND<sup>c</sup> | ND<sup>c</sup> | ND<sup>c</sup> | ND<sup>c</sup> | ND<sup>c</sup> | ND<sup>c</sup> |
| Benzyloxyaeoniflorin      | 0.019 ± 0.001     | 0.563 ± 0.006     | 0.049 ± 0.001 | 0.058 ± 0.004 | 0.005 ± 0.000 | 0.049 ± 0.001 | 0.048 ± 0.002 | 0.107 ± 0.001 | 0.052 ± 0.000 | 0.038 ± 0.002 |
| Glycyrrhizin              | 1.155 ± 0.005     | 1.101 ± 0.032     | 0.959 ± 0.034 | 0.762 ± 0.044 | 0.248 ± 0.023 | 2.025 ± 0.015 | 1.179 ± 0.038 | 2.124 ± 0.028 | 0.790 ± 0.049 | 1.445 ± 0.037 |

The average content is represented as mean ± SD.

<sup>a</sup>OJS01, Ojeok-san water extract from the laboratory; <sup>b</sup>OJS02–OJS10 = Ojeok-san granules from Korean manufacturers, and <sup>c</sup>ND, not detected.
It is necessary to produce chemically equalized OJS preparations for better quality samples.

Conflict of Interests

The authors declare that they have no conflict of interests.

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