Quantitative Assessment of Traditional Oriental Herbal Formulation Samhwangsasim-tang Using UPLC Technique

Amrit Poudel1, Se-Gun Kim1, Ramakanta Lamichhane1, Yun-Kyung Kim1, Hyung-Kwon Jo2 and Hyun-Ju Jung1 *

1Department of Oriental Pharmacy, College of Pharmacy and Wonkwang-Oriental Medicines Research Institute, Wonkwang University, Iksan, Jeonbuk, South Korea, and 2Hanpoong Pharm & Foods Co., Ltd., Jeonju, Jeonbuk, 333-24, South Korea

*Author to whom correspondence should be addressed. Email: hyun104@wku.ac.kr

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A specific and reliable ultra-performance liquid chromatography–diode array detection method has been developed and validated for the quantitative assessment of a traditional oriental herbal formulation, Samhwangsasim-tang (SST). A Halo reversed-phase amide column (2.7 µm, 4.6 × 150 mm) was used to separate marker compounds; detection was conducted by ultraviolet absorbance at 250 nm. The column temperature was maintained at 45°C. A mobile phase consisting of acetonitrile (A) and 0.1% trifluoroacetic acid in water (B) was found to be suitable for the separation, at a flow rate of 1.8 mL/min with gradient elution. Linearity, specificity, precision and recovery were calculated to validate the method and instrumentation. Under the described conditions, all marker compounds (rhaponticin, berberine, palmatine, baicalin, baicalein and wogonin) were collected within 25 min. All calibration curves of components showed good linearity (correlation coefficient > 0.9996). The limit of detection and limit of quantitation ranged from 0.08–3.05 and 0.23–8.12 μg/mL, respectively. The relative standard deviation (RSD) and repeatability values of intra-day and inter-day precision were less than 2.30, 2.99 and 1.82%, respectively. In the recovery test, the accuracy ranged from 97.56–103.30% with RSD values less than 2.63%. The developed method was simple, specific, sensitive, accurate, precise and reproducible for the quantification of the active chemical constituents of SST. The simultaneous analysis of the contents of marker compounds in different SST samples prepared by different extraction procedures and different commercial products was successfully evaluated.

Introduction

Herbal formulations are becoming more popular in the world for improving health conditions and preventing and healing diseases (1). In recent years, despite the growth in the global trade of herbal products, they are often unable to enter international markets because of inconsistencies in quality and effectiveness. Therefore, it is essential to develop analytical methods that allow the standardization of herbal formulations and study the stability of herbal medicines (2).

Traditional herbal formulations are typically used as a combination of many herbs. Their efficacy results from various constituents present in these herbs. The quality of the herbs is influenced by seasonal variation, collection, post-harvest processing, storage location and procedures of extraction (3). The extraction involves boiling, percolating or macerating the herb in water, ethanol or other solvents to release biologically active constituents from the cell matrices of the plant into the solvents (4). The extraction is influenced by various parameters like extraction apparatus, temperature, pressure, particle size, flush volume, static time and solid-to-solvent ratio (5). Therefore, optimization of extraction conditions is required to obtain the highest yield of bioactive compounds.

Recently, an improvement in chromatographic performance has been achieved by the introduction of ultra-performance liquid chromatography (UPLC). UPLC takes full advantage of chromatographic principles to run separations using columns packed with smaller particles; additionally, it offers the advantages of increased speed, improved sensitivity, selectivity and specificity compared with high-performance liquid chromatography (HPLC) (6, 7).

Samhwangsasim-tang (SST) is a traditional herbal medicinal formula containing *Rhei rhizoma* (polygonaceae), *Coptidis rhizoma* (ranunculaceae) and *Scutellariae radix* (lamiaceae). SST has been used for thousands of years as a therapeutic formula in Korea, China and Japan, and is still widely used. The primary constituents of *Rhei rhizoma* are known to be stilbenes and anthraquinone derivatives (8). *Coptidis rhizoma* yields 6.71–13.78% of alkaloids, with 50–80% being berberine and small amounts of palmatine, coptisine, worenine and epi-berberine (9). The dried root of *Scutellariae radix* contains over 50 kinds of flavonoids; the primary component of the root is baicalin (10). Previous laboratory and clinical studies have suggested that SST plays a role in the treatment of various diseases, including gastric inflammatory symptoms, acute lung injury, septic shock, hypertension, neurodegenerative disorders and hepatoprotective and oxidative stress (9, 11–17).

The aim of the present study was to develop a UPLC–photodiode array (PDA) method for the identification and quantification of six marker compounds: rhaponticin from *Rhei rhizoma*, berberine and palmatine from *Coptidis rhizoma*, baicalin, baicalein and wogonin from *Scutellariae radix*; in different SST samples prepared with different extraction methods and commercial formulations. Validation of the linearity, specificity, precision and accuracy of the UPLC method were also performed to verify the present method.

Experimental

Materials and reagents

All organic solvents used for extraction and isolation were purchased from Dae-Jung Chemicals Co. (Siheung, South Korea). A reversed-phase (RP) ODS-A, 75 μm, was purchased from YMC Co. (Kyoto, Japan). Sephadex LH-20 was purchased from...
GE Healthcare Bio-Science (Uppsala, Sweden). A medium-pressure liquid chromatography (MPLC) Yamazen Pump 54 with Prep UV-10V detector (Osaka, Japan) was used. For structural elucidation, a Bruker FT-NMR spectrometer (Billerica, MA) was used. The powdered SST formulations were obtained from Hanpoong Pharm & Food Co. (Jeonju, South Korea). The commercial SST formulations were received from Professor Yun-Kyung Kim (Wongkwang University, South Korea). The marker compounds berberine, palmatin, baicalin were purchased from Chromadex Inc. (Irvine, CA); sennoside A, baicalein and wogonin were purchased from Korea Food and Drug Administration (KFDA) (Cheongwon, South Korea). The purity of these marker compounds was above 98%. The HPLC-grade solvents water and acetonitrile were purchased from Burdick and Jackson (Muskegon, MI). The analytical grade trifluoroacetic acid (TFA) was purchased from Samchun Pure Chemical Co. (Yeosu, South Korea).

**Extraction and isolation of Rhaponticin**

Commercially available *Rhei rhizoma* was refluxed with methanol for 3 h. The methanol extract of the plant was successively partitioned with hexane, ethyl acetate, butanol and water. The ethyl acetate fraction was subjected to reversed-phase column chromatography. The sub-fraction was further applied on a Sephadex LH-20 column. Purification from MPLC yielded a pale yellowish compound. The structural determination was conducted by proton nuclear magnetic resonance (1H-NMR), carbon-13 nuclear magnetic resonance (13C-NMR) and distortionless enhancement by polarization transfer (DEPT).

$^1$H-NMR (500 MHz, DMSO-$d_6$), δ: 7.01 (1H, d, J = 2.3 Hz, H-2$^\prime$), 6.89 (1H, d, J = 8.2 Hz, H-5$^\prime$), 6.95 (1H, dd, J = 2.3, 8.4 Hz, H-6$^\prime$), 6.83 and 6.98 (each 1H, d, J = 16.5 Hz, olefinic H), 6.56, 6.33 and 6.72 (each 1H, brs, H-2, H-4 and H-6), 4.80 (1H, d, J = 7.3 Hz, anomeric H), 3.77 (3H, s, –OCH$_3$), 3.1–3.7 (6H, m, sugar-H). $^{13}$C-NMR (125 MHz, DMSO-$d_6$) δ: 139.14 (C-1), 104.87 (C-2), 158.34 (C-3), 102.88 (C-4), 158.86 (C-5), 107.23 (C-6), 129.98 (C-1$^\prime$), 112.95 (C-2$^\prime$), 146.56 (C-3$^\prime$), 147.71 (C-4$^\prime$), 112.11 (C-5$^\prime$), 118.55 (C-6$^\prime$), 128.54 (C-β), 126.07 (C-α), 100.63 (C-1$^\prime$), 73.27 (C-2$^\prime$), 77.10 (C-3$^\prime$), 69.74 (C-4$^\prime$), 76.69 (C-5$^\prime$), 6.70 (C-6$^\prime$), 55.62 (–OCH$_3$). The values were confirmed by comparing with the literature values (18).

**Chromatographic conditions**

The UPLC system (Waters Acquity H-class, Milford, MA) for sample analysis was composed of a pump (ACQ-QSM), an autosampler (ACQ-FTN), a column oven and a PDA detector (ACQ-PDA). The output signal of the detector was recorded using Empower 2 software. Chromatographic separation was conducted using a Halo RP-amide column (2.7 μm, 4.6 × 150 mm) and the column temperature was maintained at 45°C. The mobile phase consisted of acetonitrile (A) and 0.1% TFA in water (B) with gradient elution for better separation. The gradient solvent system was optimized and performed as follows: 10–20% A (0–10 min), 20–25% A (10–15 min), 25–30% A (15–20 min) and 30–52% A (20–25 min) at a flow rate of 1.8 mL/min. The detection was conducted at 250 nm and the injection volume of each sample was 4 μL.

**Preparation of standard solutions**

The stock solution was prepared in 50% methanol–water at a concentration of 1 mg/mL of each compound: sennoside A, rhaponticin, berberine, palmatine, baicalin, baicalein and wogonin (Figure 1). A serial dilution of the stock solution was conducted to establish the calibration curve. All solutions were filtered through 0.20 μm filters [polytetrafluoroethylene (PTFE), hydrophilic filter].

**Extraction method**

SST was formulated by mixing *Rhei rhizoma* (5.3 g), *Coptidis rhizoma* (5.3 g) and *Scutellariae radix* (2.7 g). Water was selected as the extraction solvent. The herbs were extracted with an herb-to-solvent ratio of 1:10 for 2 h at a temperature of 98 ± 3°C with a condenser. The extract was evaporated in vacuum evaporator at 60°C and a dry powder was prepared. These extraction parameters were kept constant in the following procedures unless otherwise mentioned. Procedure A: three individual herbal plants were extracted separately and mixed

![Figure 1. Chemical structures of standard compounds.](https://academic.oup.com/chromsci/article-abstract/52/2/176/315542/177?tab=abstract&anchor=52472176315542&guest=02March2019)
together (Ex A). Procedure B: all three herbal plants were mixed together and extracted (Ex B). Procedure C: the mixed formulation was extracted without a connected condenser until the solvent was halved (Ex C). Procedure D: the mixed formulation was extracted in pottery in the traditional way without a connected condenser until the solvent was halved (Ex D). Procedure E: the mixed formulation was extracted with 20 times more solvent (Ex E). Procedure F: the mixed formulation was extracted with high pressure at a temperature of 110 °C.

Preparation of sample solutions
The individual herbal extracts prepared from Ex A were accurately weighed and dissolved in 50% methanol–water at the concentration of 20 mg/mL in an ultrasonic water bath at a temperature of 50 °C for the quality assurance of herbs used for the SST formulation. The powders of SST prepared by different extraction procedures were also prepared at a concentration of 20 mg/mL by using a similar process. For commercial SST formulations, SST was first extracted with 50% methanol–water three times at an elevated temperature. It was then evaporated and a sample of 20 mg/mL was prepared in 50% methanol–water. All solutions were filtered through a 0.20 µm filter (PTFE, hydrophilic filter) before injection.

Method Validation
The method was validated for linearity, limit of detection (LOD) and limit of quantification (LOQ), specificity, precision (inter-day, intra-day and repeatability) and accuracy (recovery) following the international Conference on Harmonization (ICH) guideline (19) and some reports on analysis (20).

Linearity
The stock solutions of standard compounds were serially diluted into five concentrations: sennoside A (31.25–500 µg/mL), rhamnogalacturonan (2.5–1,000 µg/mL), berberine (62.5–100 µg/mL), paeoniflorin (15.62–250 µg/mL), baicalin (62.5–1,000 µg/mL), baicalein (31.25–500 µg/mL) and wogonin (7.81–125 µg/mL). These solutions were analyzed individually in triplicate for the establishment of calibration curves. Calibration equations were obtained by plotting the mean of the peak area (y-axis) versus concentration (x-axis) for each analyte in that range. Linearity was evaluated by correlation coefficient (R²) values (21).

Limits of detection and quantification
The LOD and LOQ were calculated based on the standard deviation (SD) and the slope (∆) of the calibration curve based on Equations (1) and (2) respectively (22). From the calibration curve of the peak area versus concentration, the SD was the SD of the response, which was estimated by the standard deviation of the intercepts of the regression line in calibration curve; ∆ was the slope of the calibration curve:

\[ \text{LOD} = \left( \frac{3.3 \times \text{SD}}{\Delta} \right) \]  
\[ \text{LOQ} = \left( \frac{10 \times \text{SD}}{\Delta} \right) \]  

Specificity
The specificity of the developed chromatographic method was determined by the peak purity of six marker compounds in the extract using the ultraviolet (UV) spectrum provided by diode array detection (DAD) with standards. The peak purity was confirmed by comparing the spectra on the upslope and downslope peaks in the samples (20).

Precision
Intra-day and inter-day variations and the repeatability of six samples were chosen to determine the precision of the developed assay. Intra-day precision was validated with three concentrations of all standard solutions under the optimized conditions within a day. Inter-day precision was validated with the standard solutions for three consecutive days. Repeatability was conducted by analyzing the extractive solution six times in the same day. All investigated components were expressed as relative standard deviation (RSD) (23).

Accuracy
The accuracy was assessed by recovery analysis by adding measured amounts of standards to an SST extractive sample solution. The recovery data were determined by the following formula (22):

\[ \text{recovery} = \left( \frac{\text{amount found} - \text{original amount}}{\text{amount spiked}} \right) \times 100 \]  

where original amount is the amount of individual marker compounds in the SST extractive sample solution and amount spiked is the additional amount of marker compounds added to the SST extractive sample solution. The experiments were performed in triplicate.

Quantification of extractive solution
The newly established analytical method was subsequently applied for the quality assessment of individual herbs and the simultaneous determination of six active compounds in different samples of SST. The marker compounds were quantified by linear regression of the standards. Each sample was analyzed in triplicate to determine the mean content.

Results
Optimization of chromatographic condition
The UPLC conditions were investigated with regard to column, mobile phase and detection wavelength for better chromatographic resolution. To obtain accurate, valid and optimal chromatographic conditions, various columns (Halo C18, 2.7 µm, 4.6 × 100 mm; Acquity C18, 1.7 µm, 2.1 × 50 mm; Halo PFP, 2.7 µm, 4.6 × 100 mm; Halo RP-amide, 2.7 µm, 4.6 × 150 mm), mobile phases (methanol–water and acetonitrile–water with different modifiers including acetic acid, formic acid, phosphoric acid and TFA), column temperature (20, 30, 40 and 45 °C), mobile phase flow rates (0.6, 1, 1.5 and 1.8 mL/min) and analysis time (15, 20 and 25 min) were examined. The method was optimized with a Halo RP-amide column (2.7 µm, 4.6 × 150 mm) column and gradient solvent system (acetonitrile–0.1% TFA) at...
a column temperature of 45°C with a flow rate of 1.8 mL/min and in analysis time of 25 min. The standards and SST samples were analyzed in the wavelength range of 200–400 nm. The choice of 250 nm as the detection wavelength allowed a high sensibility for all peaks. Figure 2 shows a typical chromatogram of the SST sample and the corresponding marker compounds.

**Identification of rhaponticin**

The $^1$H-NMR (500 MHz, DMSO-$d_6$) spectrum showed the presence of a methoxy group ($\delta = 3.77$) and a trans-olefinic group ($\delta = 6.83, 6.98$, each 1H, $d, J = 16$ Hz). It showed the presence of two independent aromatic rings: one with a 1, 2, 4 trisubstituted system ($\delta = 7.01, 1$H, $d, J = 2.3$ Hz, H-2'; $\delta = 6.89, 1$H, $d, J = 8.2$ Hz, H-5' and $\delta = 6.95, 1$H, $dd, J = 2.3, 8.4$ Hz, H-6'), and the other with a 1, 3, 5 trisubstituted system ($\delta = 6.52, 6.33$ and 6.72 each, 1H, brs, H-2, H-4 and H-6). The $^1$H-NMR spectrum also exhibited a sugar anomeric proton doublet ($\delta = 4.80$, 1H, $d, J = 3.1–3.7$ Hz). Other sugar signals appeared in the range of $\delta = 3.1–3.7$. The presence of the glucose moiety was deduced from the examination of the $^{13}$C NMR spectrum. From these spectral data, the compound was assumed to be rhaponticin.

**Method validation**

UPLC analyses of linearity, specificity, precision and accuracy were performed to demonstrate that the present method is selective, precise and reproducible. To determine linearity, five concentrations of each standard solution were injected individually: sennoside A (31.25–500 μg/mL), rhaponticin (62.50–1,000 μg/mL), berberine (62.50–1,000 μg/mL), palmatine (15.62–250 μg/mL), baicalin (62.50–1,000 μg/mL), baicalien (31.25–500 μg/mL) and wogonin (7.81–125 μg/mL).

Calibration curve equations were obtained by plotting the peak areas versus concentrations. Because all of the $R^2$ values were $>0.9996$, linearity was verified. Detailed information regarding the calibration curve, linear range, LOD and LOQ is listed in Table I. Specificity was determined by comparing the peak purity of six marker compounds of the extract with

<table>
<thead>
<tr>
<th>Compound</th>
<th>Regression equation$^\dagger$</th>
<th>$R^2$</th>
<th>Linear range (μg/mL)</th>
<th>LOD (μg/mL)</th>
<th>LOQ (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sennoside A</td>
<td>$Y = 1613.8x + 962.46$</td>
<td>1</td>
<td>31.5–500</td>
<td>1.95</td>
<td>5.93</td>
</tr>
<tr>
<td>Rhaponticin</td>
<td>$Y = 2803.2x + 13356$</td>
<td>0.9996</td>
<td>62.50–1,000</td>
<td>2.88</td>
<td>8.12</td>
</tr>
<tr>
<td>Berberine</td>
<td>$Y = 705x + 31162$</td>
<td>0.9999</td>
<td>62.50–1,000</td>
<td>2.92</td>
<td>8.84</td>
</tr>
<tr>
<td>Palmatine</td>
<td>$Y = 5557.6x + 30507.7$</td>
<td>1</td>
<td>15.62–250</td>
<td>0.91</td>
<td>2.77</td>
</tr>
<tr>
<td>Baicalin</td>
<td>$Y = 2594x + 9094.9$</td>
<td>1</td>
<td>62.50–1,000</td>
<td>2.21</td>
<td>6.69</td>
</tr>
<tr>
<td>Baicalien</td>
<td>$Y = 5121.5x – 3981.7$</td>
<td>1</td>
<td>31.25–500</td>
<td>3.05</td>
<td>9.25</td>
</tr>
<tr>
<td>Wogonin</td>
<td>$Y = 4706.7x + 11044.4$</td>
<td>1</td>
<td>7.81–125</td>
<td>0.08</td>
<td>0.23</td>
</tr>
</tbody>
</table>

$^\dagger$ Note: Tests were conducted in triplicate.

$^\dagger$: peak area; x: concentration (μg/mL).

Figure 2. UPLC chromatograms: standard mixture (A); SST extract (B). Peaks: rhaponticin (1), baicalin (2), berberine (3), palmatine (4), baicalien (5), wogonin (6).
standards. Figure 3 shows that the peaks were pure and lacked interference by impurities.

The precision of the developed method was determined by intra-day and inter-day variation, along with repeatability of six samples. The RSDs of intra-day and inter-day analysis were in the ranges of 0.03–2.30% and 0.03–2.99%, respectively (Table II). Moreover, the RSD of repeatability was in the range of 0.39–1.82% (Table III). These data revealed that the described method had an acceptable degree of precision.

The accuracy was measured by determining the recovery of standard addition. Low, medium and large amounts of standards were added to the known sample and the percentage of recovery and RSD were calculated. The measured data showed a recovery of 97.56–103.30% and RSD of 0.22–2.63% (Table IV).

### Table II

Analytical Results of Intra-Day and Inter-Day Variability*

<table>
<thead>
<tr>
<th>Standards</th>
<th>Concentration (µg/mL)</th>
<th>Intra-day Mean ± SD (µg/mL)</th>
<th>RSD (%)</th>
<th>Inter-day Mean ± SD (µg/mL)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhaponticin</td>
<td>1,000</td>
<td>992.59 ± 1.62</td>
<td>0.16</td>
<td>994.16 ± 0.93</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>513.81 ± 1.44</td>
<td>0.28</td>
<td>512.92 ± 2.13</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>255.96 ± 0.96</td>
<td>0.38</td>
<td>250.15 ± 0.77</td>
<td>0.31</td>
</tr>
<tr>
<td>Berberine</td>
<td>1,000</td>
<td>1,001.80 ± 3.93</td>
<td>0.39</td>
<td>1,001.26 ± 0.90</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>511.30 ± 1.21</td>
<td>0.24</td>
<td>498.43 ± 1.97</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>251.31 ± 1.26</td>
<td>0.50</td>
<td>246.17 ± 1.08</td>
<td>0.44</td>
</tr>
<tr>
<td>Palmatine</td>
<td>125</td>
<td>125.37 ± 0.65</td>
<td>0.52</td>
<td>125.83 ± 0.39</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>62.5</td>
<td>62.99 ± 0.82</td>
<td>1.31</td>
<td>62.51 ± 0.73</td>
<td>1.17</td>
</tr>
<tr>
<td>Baicalin</td>
<td>1,000</td>
<td>991.72 ± 2.39</td>
<td>0.24</td>
<td>1,001.12 ± 0.25</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>496.90 ± 1.64</td>
<td>0.33</td>
<td>496.67 ± 0.85</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>251.92 ± 1.33</td>
<td>0.53</td>
<td>251.42 ± 1.95</td>
<td>0.78</td>
</tr>
<tr>
<td>Baicalein</td>
<td>125</td>
<td>125.86 ± 0.04</td>
<td>0.03</td>
<td>125.48 ± 1.40</td>
<td>1.11</td>
</tr>
<tr>
<td></td>
<td>62.5</td>
<td>62.99 ± 0.82</td>
<td>1.31</td>
<td>62.51 ± 0.73</td>
<td>1.17</td>
</tr>
<tr>
<td>Wogonin</td>
<td>31.25</td>
<td>31.76 ± 0.16</td>
<td>0.51</td>
<td>31.42 ± 0.10</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>15.625</td>
<td>15.88 ± 0.04</td>
<td>0.26</td>
<td>15.67 ± 0.03</td>
<td>0.21</td>
</tr>
<tr>
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<td>7.8125</td>
<td>7.75 ± 0.04</td>
<td>0.57</td>
<td>7.74 ± 0.17</td>
<td>2.26</td>
</tr>
</tbody>
</table>

*Note: Tests were conducted in triplicate.

### Table III

Repeatability Data of Six Marker Compounds in SST*

<table>
<thead>
<tr>
<th>Standards</th>
<th>Amount (µg/mL)</th>
<th>Intra-day Mean ± SD (µg/mL)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhaponticin</td>
<td>371.39</td>
<td>992.59 ± 4.56</td>
<td>0.48</td>
</tr>
<tr>
<td>Berberine</td>
<td>230.47</td>
<td>498.43 ± 1.97</td>
<td>0.40</td>
</tr>
<tr>
<td>Palmatine</td>
<td>125.83</td>
<td>496.90 ± 1.64</td>
<td>0.33</td>
</tr>
<tr>
<td>Baicalin</td>
<td>125.48</td>
<td>491.30 ± 1.40</td>
<td>0.41</td>
</tr>
<tr>
<td>Baicalein</td>
<td>125.83</td>
<td>496.90 ± 1.64</td>
<td>0.33</td>
</tr>
<tr>
<td>Wogonin</td>
<td>125.48</td>
<td>491.30 ± 1.40</td>
<td>0.41</td>
</tr>
</tbody>
</table>

*Note: Tests were conducted six times.

### Table IV

Recovery Data of the Analytical Method for the Determination of Six Marker Compounds*

<table>
<thead>
<tr>
<th>Standards</th>
<th>Original (µg/mL)</th>
<th>Added (µg/mL)</th>
<th>Found ± SD (µg/mL)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhaponticin</td>
<td>376.07</td>
<td>500</td>
<td>886.78 ± 1.15</td>
<td>102.14</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>630.13</td>
<td>101.62</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>Berberine</td>
<td>230.58</td>
<td>250</td>
<td>481.82 ± 0.62</td>
<td>100.50</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>357.13</td>
<td>101.24</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>Palmatine</td>
<td>87.47</td>
<td>62.5</td>
<td>294.84 ± 0.95</td>
<td>102.80</td>
<td>1.49</td>
</tr>
<tr>
<td>Baicalin</td>
<td>546.52</td>
<td>500</td>
<td>1032.52 ± 2.14</td>
<td>102.10</td>
<td>2.20</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>674.15</td>
<td>102.10</td>
<td>1.87</td>
<td></td>
</tr>
<tr>
<td>Baicalein</td>
<td>17.91</td>
<td>15.6</td>
<td>103.21 ± 0.19</td>
<td>100.91</td>
<td>1.19</td>
</tr>
<tr>
<td>Wogonin</td>
<td>4.94</td>
<td>3.9</td>
<td>87.48 ± 0.05</td>
<td>97.56</td>
<td>1.30</td>
</tr>
<tr>
<td></td>
<td>1.9</td>
<td>6.87 ± 0.04</td>
<td>101.59</td>
<td>2.53</td>
<td></td>
</tr>
</tbody>
</table>

*Note: Tests were conducted in triplicate.

**Quality assurance of herbs**

*Rhei rizoma*, *Coptidis rizoma* and *Scutellariae radix* were individually injected in the optimized validated method to check the quality of individual herbs. Sennoside A for *Rhei*
rhizoma, berberine for Coptidis rhizoma, and baicalein, baicalin, baicalin and wogonin for Scutellariae radix were tested as standards for the quality assurance of herbs (Figure 4). According to the Korean Pharmacopoeia, the content of sennoside A in Rhei rhizoma should be more than 0.25%; the content of berberine in Coptidis rhizoma should be more than 4.2%, and the sum of the contents of baicalin, baicalien and wogonin should be more than 10% in Scutellariae radix. The content of Sennoside A in Rhei rhizoma was found to be 0.29%; the content of berberine in Coptidis rhizoma was found to be 4.59%; the contents of baicalin, baicalien and wogonin in Scutellariae radix were found to be 9.53, 0.53 and 0.14%, respectively. Because all content values are within the limits of the Korean Pharmacopoeia, the quality of each herb was acceptable for the production of SST.

**Sample analysis**

Using the newly established verified method, six compounds of different samples of SST were quantitatively analyzed. Each sample was analyzed in triplicate to determine the mean contents. The result was expressed as the content of marker compound per 13.3 g of the sample (Rhei rhizoma, 5.3 g + Coptidis rhizoma, 5.3 g + Scutellariae radix, 2.7 g) (Figure 5). The yields of various extraction methods were found to be 18.6% (Ex A), 26.7% (Ex B), 13.6% (Ex C), 13.2% (Ex D), 24.8% (Ex E) and 19.3% (Ex F). The results showed that baicalin is the major constituent in SST, as produced by all extraction procedures. Along with baicalin, rhaponticin and berberine were also found in significant amounts. Ex B produced high contents of baicalin and baicalein. Similarly, Ex E produced high contents of rhaponticin, berberine and palmatine. Although the detected

![Figure 4. UPLC chromatograms: Rhei rhizome (A); Coptidis rhizome (B); Scutellariae radix (C); marker compounds: rhaponticin (1), sennoside A (2), berberine (3), baicalin (4), baicalein (5), wogonin (6).](https://academic.oup.com/chromsci/article-abstract/52/1/176/315542)
amount of wogonin was quite low, Ex A produced a high content. The results showed that extraction without a connected condenser (Procedures C and D) produced low contents of active compounds. The commercial product of SST from each country was individually different (Figure 6). The contents of marker compounds were evaluated on the basis of each dose of the products. The product from the American market (A-V1) was found to have a higher content of biomarkers than other products. Products from Japan (J-V1 and J-V2) were found to have comparatively less contents of marker compounds.

Discussion
For the simultaneous analysis of marker compounds of SST, a reliable, fast and validated UPLC–DAD method was developed.
In SST, six marker compounds from three herbs were chosen for standardization. Rhaponticin was found to be a major constituent in *Rheum undulatum* (24). Literature has shown that rhaponticin possesses antiallergic, anticoagulative, antioxidant and hypoglycemic effects (25, 26). The major constituent of *Coptidis rhizoma*, berberine, was found to have various pharmacological activities, including antimicrobial, anticancer, antidiabetic, antihypertensive and anti-inflammatory (27). Similarly, baicalin was the major constituent of *Scutellariae radix* and was also found to be a major constituent in SST. An
extensive study on various biological activities of baicalin has been conducted; it was found to include anti-inflammatory, antioxidant, antiobesity and anticancer activities (28). The method was validated by various parameters like specificity, linearity, precision and accuracy; all parameters were within the ranges of ICH and KFDA regulations (19, 29). Different extraction procedures showed a variation in the contents of marker compounds. Result showed that extraction conducted with a connected condenser yielded more contents of active ingredients than without a connected condenser (Procedures C and D). Extraction with a larger material-to-solvent ratio (Procedure E: 20 times solvent) was found to produce more contents of rhapontin, berberine and palmatine. Also, baicalin and baicalein were found to be significantly high. This showed that the material-to-solvent ratio is an important parameter for extraction (30).

Worldwide consumption of herbal medicines has markedly increased; for the quality production of herbal formulations, the standardization of herbs and herbal formulations is of great importance (31). The results showed that rhapontcin was present in only Korean products; it was not detected in products from Japan, America and Taiwan. Rheum undulatum, which is also known as Korean rhubarb (32), is widely available in Korea and has been used for many years. Rheum officinale and Rheum palmatum, which are not cultivated in Korea, have been mixed with Rheum undulatum. This could be the primary reason that rhapontcin, which is a major constituent of Rheum undulatum, was found in Korean products, but not in products from Japan, America and Taiwan.

Conclusions
The developed simultaneous analysis method was simple, sensitive, accurate, precise, reproducible and specific for the determination of the active chemical constituents in SST. A comparative study on different extraction procedures showed that a 20-times solvent extraction procedure was significantly successful for the extraction of large contents of active constituents. It is expected that the method will help for the quality production of SST.

Acknowledgments
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