Quantitative Analysis of Marker Compounds in *Angelica gigas*, *Angelica sinensis*, and *Angelica acutiloba* by HPLC/DAD

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ABSTRACT

Although Danggui is the root of Angelica gigas Nakai in the Korean Pharmacopoeia, it is determined that Danggui is also the root of Angelica sinensis (Oliv.) Diels in China and Hong Kong, as well as the root of Angelica acutiloba Kitagawa in Japan. Accordingly, we tried to develop an identification method using the main compounds in A. gigas, A. sinensis, and A. acutiloba through HPLC/DAD. This method was fully validated for linearity, accuracy, precision, recovery, and robustness. Multivariate analysis was also implemented after pattern analysis and monitoring. As a result, each compound pattern of A. gigas, A. sinensis, and A. acutiloba was identified, making it possible to distinguish them from each other.

Key words: Angelica gigas; Angelica acutiloba; Angelica sinensis; HPLC/DAD
Introduction

Danggui (Angelicae Gigantis Radix) is the root of Angelica gigas Nakai, which is the most commonly used Umbelliferae medicinal plant and a perennial herb, in Korea. Its 1- to 2-year-old dried roots have been used in traditional medicine for the treatment of several diseases like a common cold, headache, neuralgia, arthralgia, menstrual disorders, amenorrhea, dysmenorrheal, anemia, and premenstrual syndrome in Korea.1-2) In the Korean Pharmacopoeia (KP), it is officially stated that Danggui, Angelica gigas root, has a characteristic odor and slightly bittersweet taste, as its medicinal properties.3) There are three representative species of Danggui, which has been stipulated differently in several Asian countries: the roots of Angelica gigas Nakai (Cham-Danggui) in the KP, the roots of Angelica acutiloba Kitagawa and Angelica acutiloba Kitagawa var. sugiyamae Hikino in the Japanese Pharmacopoeia (JP), the roots of Angelica sinensis (Oliv.) Diels in the Chinese Pharmacopoeia (ChP) and Hong Kong Chinese Materia Medica Standards (HKCMMS), and the roots of Angelica acutiloba Kitagawa and Angelica sinensis (Oliv.) Diels in the Vietnamese Pharmacopoeia (VP).4-7) Danggui has been distributed with the same name because of similarities in shapes, although it has different origin species and scientific names, such as A. gigas, A. sinensis, and A. acutiloba respectively, owing to local, climatic, and cultural differences.8) Therefore, the specificities of A. gigas, A. sinensis, and A. acutiloba are needed to be checked for differentiation in order to ensure an appropriate use for therapeutic purposes as well as the prevention of misuse in a distribution process. Therefore, it is needed to contribute to the determination of origin species using marker compounds for a suitable use of Danggui.

It is reported that the chemical constituents include decursin, decursinol, decursinol angelate, nodakenin, n-butyldienephthalide, and umbelliferone, as well as volatiles, steroid and polyacetylene in A. gigas; senkyunolide I, senkyunolide H, sedanenolide, butylphthalide, (E)-ligustilide, (Z)-ligustilide, ferulic acid, and coniferylferulate in A. sinensis; and ligustilide, n-butyldienephthalide, butylphthalide, senkyunolide E, senkyunolide F, senkyunolide H, and senkyunolide I in A. acutiloba.9-18) The plants of A. sinensis and A. acutiloba are quite similar in their constituents except for the content of compounds. In spite of the fact that A. gigas, A. sinensis, and A. acutiloba belong to the same genus, there are definite differences in the components and medicinal effects each species has. For example, A. gigas contains decursin and decursinol angelate as its peculiar components, while the other two species contain (Z)-
ligustilide, \textit{n}-butyldienephthalide, and butylphthalide. That is why the three species should not be distributed as a same medicinal herb. There have been many previous studies that researched each herb separately, but only a few have tried to analyze them in an integrated manner.\textsuperscript{19-21)}

In this study, at first, we expected to establish a comparative and concurrent method using HPLC/DAD analysis with markers in Danggui. Also, we intended to not only set up a method to confirm appropriacy but also conduct the validation for the respective marker compounds, in terms of linearity, accuracy, precision, recovery, and robustness about column type, column temperature, and flow rate. Finally, we will provide basic data to get accurate discernment and to identify the medicinal effects through the content analysis of marker compounds in \textit{A. gigas}, \textit{A. sinensis}, and \textit{A. acutiloba}. We hope that this study would be helpful in suitably using Danggui for therapeutic purposes.

\textbf{Experimental Section}

\textbf{Plant material} Forty five samples corresponding to fifteen \textit{A. gigas} (G01–G15), fifteen \textit{A. sinensis} (S01–S15), and fifteen \textit{A. acutiloba} (A01–A15) samples cultivated in different regions were provided by the National Center for Herbal Medicine Resources and were supplied from Prof. Ho-Young Choi of KyungHee University, Korea.

\textbf{Chemicals and reagents} Decursin (9) and decursinol angelate (10) were purified together from ethanol extracts of the root of \textit{A. gigas} by silica column chromatography as described with modifications.\textsuperscript{22)} Their purities were above 95\% as determined by HPLC analysis (data not shown); their structures are shown in Fig. 1. Chlorogenic acid (1), ferulic acid (2), nodakenin (3), decursinol (4), xanthotoxin (5), and \textit{\alpha}-asarone (I.S.) were purchased from Sigma-Aldrich Co. Demethylsuberosine (7) and (Z)-ligustilide (8) were purchased from the Institute for Korea Traditional Medical Industry and ChromaDex, respectively. Coniferylferulate (6) was purchased from Sichuan Provincial Administration of Food and Drug, China.

\textbf{Sample preparation} Dried root powder was used to determine the content of the ten marker compounds in each extract of \textit{A. gigas}, \textit{A. sinensis}, and \textit{A. acutiloba}. Powdered Danggui was sieved through 100-mesh, and about 1.0 g of the powder was accurately
weighed; 10 mL of 70% ethanol and α-asarone (I.S., 200 ppm) were then added, the weight was accurately measured and the sample was sonicated for 45 min at room temperature. The solution was weighed again, and the loss in weight was made up with 70% ethanol. The solution was filtered through a 0.45-μm membrane filter, and the filtrated was used as the test solution. Sample solution of 10 μL was injected into the HPLC system.

**HPLC/DAD conditions** The HPLC equipment was a Waters HPLC system (Waters, 2695) with a Waters binary pump, an auto-sampler, a column oven, and a Waters 2996 photodiode array detector. The YMC C18 analytical column (250 × 4.6 mm, 5 μm, YMC ODS) was tested with a guard column that was filled with the same stationary phase. A (H2O : HCOOH = 1000 : 1) and B (AcN : HCOOH = 1000 : 1) were used as the mobile phase under gradient conditions (0 min, 88% A; 10 min, 82% A; 25 min, 80% A; 30 min, 50% A; and 60 min, 50% A) to analyze the samples. The mobile phase was filtered under vacuum through a 0.45-μm membrane filter and degassed prior to use. The analysis was carried out at a flow rate of 1.0 mL/min with the detection wavelength set at 325 nm; the total run time was 60 min. All compounds could be resolved with baseline separation at 325 nm with maximum absorption. Hence, characteristic chromatographic patterns were obtained at 325 nm. The chromatograms were processed using Empower Pro software, build 1154 (Waters, Milford, MA).

**Analytical method validation** The standards were accurately weighed and then dissolved with 70% ethanol to produce stock standard solutions [chlorogenic acid (1), 100 ppm; ferulic acid (2), decursinol (4), xanthotoxin (5), and coniferylferulate (6), 50 ppm; nodakenin (3), 300 ppm; demethylsuberosine (7) and (Z)-ligustilide (8), 400 ppm; and decursin (9) and decursinol angelate (10), 2000 ppm]. The internal standard (α-asarone) of 2 mg was accurately weighed and then dissolved with 10 mL of 70% ethanol to produce a stock solution of 200 ppm. The calibration curves were made by diluting the stock solutions with 70% ethanol. The reference solution of the ten marker compounds at concentrations of 0.1 – 1000 μg/mL was analyzed by HPLC/DAD. The regression equations were calculated in the form of \( y = ax + b \), where \( y \) and \( x \) correspond to peak area ratio for an internal standard and compound concentration, respectively. The recovery tests were executed by mixing a powdered sample (1.0 g) with the reference compounds at three control levels (near the LOQ, medium, and higher concentrations from the calibration). The mixture was then extracted by sonication with 10 mL of 70% ethanol for 45 min. The extract solution was filtered through a 0.45-μm membrane. The HPLC/DAD analysis experiments were performed in triplicate for...
each control level. The data from the standard solution and the extracted sample were compared. Precision and accuracy were determined by multiple analysis (n=6) of quality control samples prepared at low, medium, and high concentrations spanning the calibration range.

**Pattern recognition analysis** To evaluate the phytochemical equivalency among the fifteen *A. gigas*, fifteen *A. sinensis*, and fifteen *A. acutiloba* samples, pattern recognition analysis was conducted. We used ten marker compound peaks [chlorogenic acid (1), ferulic acid (2), nodakenin (3), decursinol (4), xanthotoxin (5), coniferylferulate (6), demethylsuberosine (7), (Z)-ligustilide (8), decursin (9), and decursinol angelate (10)] for the pattern recognition analysis. Pattern recognition analysis was conducted using software package R-2.11.0.

**Results and Discussion**

**Optimization of chromatographic conditions** The good separation conditions were selected according to the requirements for obtaining the chromatograms with a better resolution of the adjacent peaks within a short analysis time. For the optimization of chromatographic conditions, the effect of the mobile phase composition on separation was examined. Various mixtures of water and acetonitrile were used as the mobile phase, but resolution was not satisfactory to separate structurally similar compounds. Therefore, the addition of 0.1%, 1%, and 10% acids (acetic acid, formic acid, and phosphoric acid) to water and acetonitrile was tested as a suitable chromatographic solvent. The addition of 0.1% formic acid to the mobile phase resulted in good resolution, as well as appropriate peak symmetry and shape. The typical chromatograms of samples and standard mixture are shown in Fig. 2, including internal standard peak. All target compounds and internal standard are completely detected in 60 min. α-Asarone (I.S.) was selected as an internal standard. The chromatographic peaks of the analytes in sample solution were identified by comparing the retention times of reference compounds and were further confirmed by spiking samples with reference compounds. All compounds could be resolved with baseline separation at 325 nm with maximum absorption shown for the ten marker compounds. Hence, characteristics chromatographic patterns were obtained at 325 nm.

**Optimization of sample preparation conditions** Four extracting solvents–100% ethanol,
70% ethanol, 50% ethanol, and 25% ethanol—were compared with regard to the content of compounds after ultrasonic water bath for 45 min (700W, 50/60 Hz). In the extraction efficiency of the different solvents, when 70% ethanol was employed, the content of compounds was higher than in other extracting solvent samples. Therefore, we selected 70% ethanol as the extracting solvent throughout this work. Two extraction methods—ultrasonication and reflux using 70% ethanol as an extraction solvent—were compared with regard to the content of compounds. When the sonication extraction method was used, the content of compounds was higher than in the reflux method. To determine the time needed for complete extraction, samples were extracted for five different lengths of time (15, 45, 60, 90, and 120 min) using a 70% ethanol solvent and the sonication extraction method. When the extraction time was 45 min, the results were similar to those at 60 min. Hence, when the extraction time was 45 min, all of the compounds were sufficiently extracted (all extraction data not shown).

Validation of the method Each correlation coefficient ($r^2$) was $> 0.999$, as determined by the least square analysis, suggesting a good linearity between the peak area ratio and the compound concentrations (Table 1). The limits of detection (LOD) and limits of quantitation (LOQ) were evaluated based on the lowest detectable peak in the chromatogram having a signal-to-noise (S/N) ratio of 3 and 10, respectively. The standard solutions were diluted with a solvent to obtain apposite concentrations. The LOD and LOQ under our experimental conditions are listed in Table 1. The obtained values of both LOD and LOQ for these ten marker compounds were low enough to detect the traces of these compounds in either crude extract or its preparation.

The extraction recovery test was performed by extracting a known amount of the ten marker compounds from the Danggui powder samples. The test was carried out as follows: the known amount of each compound at three different levels (low, medium, and high) was mixed with the sample powder and extracted with 70% ethanol, as described in the experimental section. The recovery rate of each standard ranged from 97.94 to 102.40 %, and the standard deviation (SD) was less than 4.78% (Table 2). The average recovery was represented by the formula: $R (%) = [(\text{amount from the sample spiked standard} - \text{amount from the sample})/\text{amount from the spiked standard}] \times 100$. Precision and accuracy tests were carried out by the intra-day and inter-day variability. Intra-day precision and accuracy were determined from the variability of multiple analysis ($n=6$) of quality control samples analyzed in the same analytical run. The quality control samples had an intra-day precision
below 4.63% and accuracy in the range of 98.72 to 101.67%. Inter-day precision and accuracy were evaluated from the variability of multiple analysis (n=6) of quality control samples analyzed on a single analytical run for consecutive 6 days. The quality control samples had an inter-day precision less than 4.76% and accuracy in the range of 98.46 to 101.37%. From the results of recovery, precision, and accuracy tests, it was known that the method manifested good precision and accuracy. Precision and accuracy data are presented in Table 3.

The robustness was determined in order to evaluate the reliability of the established HPLC method. All of the parameters were maintained so there would not be any interference with other peaks for Danggui. The experimental conditions, such as column temperature, column type, and flow rate, were intentionally altered. The theoretical plate (N), capacity factor (k'), separation factor (a), and resolution (Rs) were evaluated. To evaluate their suitability, three different columns–YMC, Phenomenex, and Shiseido–were compared with regard to four analytical factors (N, k', a, and Rs) at the column temperature of 30°C. In the assessment of inter-column performance, the range of standard deviations of each compounds for the value of N, k', a, and Rs were 4~3576 (mean: 21275), 0.01~0.24 (mean: 3.62), 0.01~0.06 (mean: 1.64), 0.01~0.23 (mean: 0.57), respectively. Hence, this result indicated that the four analytical factors did not differ greatly, depending on the column type. Three different column temperatures–30, 35, and 40°C–were compared with regard to these four analytical factors using the YMC. The result displayed that the range of standard deviations of each compounds for the value of N, k', a, and Rs were 7~1422 (mean: 22603), 0.02~0.65 (mean: 3.49), 0.02~0.13(mean: 1.63), 0.02~0.27(mean: 0.47), respectively. In addition, three different flow rates–0.8, 1.0 and 1.2 mL/min–were also compared with regard to the four analytical factors using the YMC column at 30°C. The result showed that the range of standard deviations of each compounds for the value of N, k', a, and Rs were 243~66710 (mean: 30958), 0.00~0.61 (mean: 3.49), 0.00~0.23(mean: 1.63), 0.00~2.43(mean: 0.49), respectively. In terms of the robustness study for the HPLC assay, four analytical factors did not differ greatly, when the column temperature and flow rate conditions were changed. We sought to optimize the chromatographic parameters, but the four analytical factors did not differ greatly when the conditions were changed. Therefore, these experimental conditions were sufficiently robust. The sample stability was tested with extraction solution on 0, 0.5, 1, 2, 5, 10, 15 and 30 days. During this period, the solution was stored in the dark at room
temperature or at 4°C. The resulting data indicated that all marker analytes remained stable during the experimental

Sample analysis The developed HPLC/DAD method was then applied to the simultaneous determination of the ten marker compounds in Danggui–chlorogenic acid (1), ferulic acid (2), nodakenin (3), decursinol (4), xanthotoxin (5), coniferylferulate (6), demethylsuberosine (7), (Z)-ligustilide (8), decursin (9), and decursinol angelate (10). The quantity of each compound present in samples was determined, and the results are summarized in Table 4. Each sample was analyzed in triplicate to ensure the reproducibility of the quantitative results. The quantitative results showed chlorogenic acid (1, 147–424 μg/mL), nodakenin (3, 16–121 μg/mL), decursin (4, 1–36 μg/mL), demethylsuberosine (7, 37–337 μg/mL), decursin (9, 909–2155 μg/mL), and decursinol angelate (10, 460–1580 μg/mL) in A. gigas; ferulic acid (2, 15–348 μg/mL), coniferylferulate (6, 21–112 μg/mL), and (Z)-ligustilide (8, 734–918 μg/mL) in A. sinensis; and chlorogenic acid (1, 34–153 μg/mL), xanthotoxin (5, 8–26 μg/mL), and (Z)-ligustilide (8, 59–308 μg/mL) in A. acutiloba. The most abundant components were decursin and decursinol angelate shown only in A. gigas. In comparing A. sinensis and A. acutiloba, the content of ferulic acid in A. sinensis (171 μg/mL, average value of S01–S15) was higher than that in A. acutiloba (6 μg/mL, average value of A01–A15). Also the content of (Z)-ligustilide in A. sinensis (829 μg/mL, average value of S01–S15) was higher than that in A. acutiloba (133 μg/mL, average value of A01–A15). However, the content of chlorogenic acid in A. acutiloba (59 μg/mL, average value of A01–A15) was higher than that in A. sinensis (43 μg/mL, average value of S01–S15) (Table 4). In the quantitative analysis, the samples of A. gigas, A. sinensis, and A. acutiloba were clustered into three groups significantly.

Pattern recognition analysis To evaluate the phytochemical equivalency between fifteen A. gigas, fifteen A. sinensis, and fifteen A. acutiloba samples, pattern recognition analysis was conducted. In this study, we used ten marker compound peaks [chlorogenic acid (1), ferulic acid (2), nodakenin (3), decursinol (4), xanthotoxin (5), coniferylferulate (6), demethylsuberosine (7), (Z)-ligustilide (8), decursin (9), and decursinol angelate (10)] for the pattern recognition analysis. The seven compounds, including chlorogenic acid (1), ferulic acid (2), xanthotoxin (5), coniferylferulate (6), (Z)-ligustilide (8), decursin (9), and decursinol angelate (10), in Danggui were selected as major marker compounds because of difficulties in the existence and content. In the pattern analysis of PAM (Partitioning Around Medoids), all

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of the samples were clustered into three groups: A (G01-G15, A. gigas), B (S01-S15, A. sinensis), and C (A01-A15, A. acutiloba) (Fig. 3). Hence, this pattern analysis results could be used for the quality control of Danggui.

Conclusions

This was the first report on the simultaneous determination using HPLC with the major compounds in the three samples of Danggui–A. gigas, A. sinensis, and A. acutiloba. A rapid and optimized chromatographic method with UV detection was designed for the quality control of the three samples. Validation results showed that the analytical method we used in this study is suitable for measuring the concentrations of ten marker compounds to applicate to the pattern recognition analysis of A. gigas, A. sinensis, and A. acutiloba. The HPLC/DAD method for quantitative analysis of marker compounds and pattern recognition analysis can provide a promising prospect for the comprehensive quality control of A. gigas, A. sinensis, and A. acutiloba. To conclude, chlorogenic acid (1), ferulic acid (2), xanthotoxin (5), coniferylferulate (6), (Z)-ligustilide (8), decursin (9), and decursinol angelate (10) can serve as marker compounds to distinguish between A. gigas, A. sinensis, and A. acutiloba. In the pattern recognition analysis, we indicated that all of the samples were largely clustered into three groups A (A. gigas), B (A. sinensis), and C (A. acutiloba).

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Conflict of Interest

The authors declare no conflict of interest.
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Fig. 1. Chemical structures of compounds 1 – 10 and I.S.

chlorogenic acid (1) 

ferulic acid (2) 

nodakenin (3) 

decursinol (4) 

xanthotoxin (5) 

coniferyl ferulate (6) 

demethylsuberosine (7) 

(Z)-ligustilide (8) 

decursin (9) 

decursinol angelate (10) 

α-asarone (I.S.)
Fig. 2. HPLC chromatograms: (A) Standard mixture, (B) A. gigas, (C) A. sinensis, (D) A. acutiloba

Peak assignment according to Fig. 1.

a) The concentrations of analytic standard (1 - 10) were 100, 50, 300, 50, 50, 50, 400, 400, 500 and 500 μg/mL, respectively.
Fig. 3. PAM of *A. gigas* (G01 – G15), *A. sinensis* (S01 – 15), and *A. acutiloba* (A01 – 15)
| Compound | Relative retention time (min) | Linearity range (μg/mL) | Regression equation | Correlation coefficient | Limit of detection (LOD) (ng/mL) | Limit of quantification (LOQ) (ng/mL) |
|----------|-----------------------------|------------------------|---------------------|------------------------|----------------------------------|--------------------------------------|
| 1        | 0.18±0.001                  | 0.1 - 200.0            | y=0.0127x-0.0913    | 0.9996                 | 12                               | 64                                   |
| 2        | 0.41±0.001                  | 0.1 - 200.0            | y=0.0150x-0.2803    | 0.9999                 | 28                               | 56                                   |
| 3        | 0.47±0.002                  | 0.1 - 400.0            | y=0.0054x+0.3056    | 0.9996                 | 24                               | 63                                   |
| 4        | 0.76±0.001                  | 0.1 - 100.0            | y=0.0094x+0.0616    | 0.9996                 | 11                               | 43                                   |
| 5        | 0.81±0.001                  | 0.1 - 200.0            | y=0.0061x-0.0654    | 0.9998                 | 15                               | 46                                   |
| 6        | 0.90±0.002                  | 0.1 - 200.0            | y=0.0056x-0.0641    | 0.9999                 | 23                               | 65                                   |
| 7        | 0.94±0.001                  | 0.1 - 800.0            | y=0.0037x+0.4569    | 0.9996                 | 17                               | 57                                   |
| I.S.     | 1.00                        | -                      | -                   | -                      | -                                | -                                    |
| 8        | 1.14±0.002                  | 0.1 - 400.0            | y=0.0029x-0.0892    | 0.9998                 | 16                               | 43                                   |
| 9        | 1.21±0.001                  | 0.1 - 2000.0           | y=0.0041x+6.9159    | 0.9997                 | 32                               | 78                                   |
| 10       | 1.21±0.001                  | 0.1 - 2000.0           | y=0.0043x+6.4666    | 0.9996                 | 29                               | 76                                   |

Where y = peak area, x = concentration of the compound μg/mL.

a) RRT is retention time of characteristic peak/retention time of marker peak of I.S. (α-asarone). The values of RRT is expressed as mean ± S.D.
Table 2. Recovery of marker compounds through standard addition \((n=6)\)

| Compound | Fortified conc. \((\mu g/mL)\) | Observed conc. \((\mu g/mL)\) | Recovery Mean \((\%)\) | Recovery S.D. \((\%)\) |
|----------|-------------------------------|-----------------------------|----------------------|-----------------------|
| 1        | 1.00                          | 1.01                        | 101.20               | 0.42                  |
|          | 50.00                         | 50.30                       | 100.60               | 0.97                  |
|          | 100.00                        | 100.54                      | 100.54               | 1.22                  |
|          | 1.00                          | 1.00                        | 100.00               | 0.74                  |
| 2        | 25.00                         | 25.37                       | 101.48               | 0.78                  |
|          | 50.00                         | 50.38                       | 100.76               | 0.97                  |
|          | 1.00                          | 1.02                        | 102.00               | 0.81                  |
| 3        | 150.00                        | 149.87                      | 99.91                | 0.79                  |
|          | 300.00                        | 296.86                      | 98.95                | 2.34                  |
|          | 1.00                          | 0.99                        | 98.60                | 1.08                  |
| 4        | 25.00                         | 24.87                       | 99.48                | 1.63                  |
|          | 50.00                         | 49.87                       | 99.74                | 2.99                  |
|          | 1.00                          | 1.02                        | 102.40               | 1.03                  |
| 5        | 25.00                         | 25.21                       | 100.84               | 0.68                  |
|          | 50.00                         | 50.38                       | 100.76               | 1.61                  |
|          | 1.00                          | 0.99                        | 98.70                | 0.96                  |
| 6        | 25.00                         | 24.92                       | 99.68                | 0.69                  |
|          | 50.00                         | 49.79                       | 99.58                | 1.98                  |
|          | 1.00                          | 0.99                        | 98.80                | 0.49                  |
| 7        | 200.00                        | 202.01                      | 101.01               | 1.66                  |
|          | 400.00                        | 403.47                      | 100.87               | 3.46                  |
|          | 1.00                          | 1.02                        | 102.00               | 1.24                  |
| 8        | 200.00                        | 202.01                      | 101.01               | 1.24                  |
|          | 400.00                        | 403.47                      | 100.87               | 2.85                  |
|          | 1.00                          | 0.98                        | 97.94                | 2.82                  |
| 9        | 1000.00                       | 987.68                      | 98.77                | 4.24                  |
|          | 2000.00                       | 1989.35                     | 99.47                | 4.78                  |
|          | 1.00                          | 0.98                        | 98.30                | 2.08                  |
| 10       | 1000.00                       | 989.68                      | 98.97                | 2.82                  |
|          | 2000.00                       | 1979.39                     | 98.97                | 4.44                  |
Table 3. Precision and accuracy of analytical results

| Analytes | Fortified conc. (μg/mL) | Sample conc. (μg/mL) | Intra-day (n=6) | Inter-day (n=6) |
|----------|------------------------|----------------------|----------------|----------------|
|          | Observed (μg/mL)       | Accuracy (%)         | Precision (%)  |                |
| 1        | 25.00                  | 127.60 ± 0.27        | 100.50         | 102.47         |
|          |                        | 0.26                 | 102.47         | 127.61 ± 0.31  |
|          |                        | 0.20                 | 49.48          | 61.79 ± 0.10   |
|          |                        | 0.32                 | 49.33          | 74.70 ± 0.32   |
| 2        | 75.00                  | 382.58 ± 0.40        | 100.29         | 304.36         |
|          |                        | 0.40                 | 50.00          | 379.51 ± 0.30  |
|          |                        | 0.13                 | 52.12          | 77.00 ± 0.11   |
|          |                        | 0.11                 | 52.24          | 101.99 ± 0.10  |
| 3        | 12.50                  | 63.69 ± 1.26         | 100.21         | 52.17          |
|          |                        | 1.26                 | 64.68 ± 0.02   | 100.14         |
|          |                        | 0.13                 | 73.38 ± 0.22   | 100.38         |
|          |                        | 0.11                 | 78.40 ± 0.25   | 99.94          |
| 4        | 12.50                  | 65.92 ± 0.12         | 100.07         | 53.39          |
|          |                        | 0.12                 | 65.90 ± 0.13   | 100.10         |
|          |                        | 0.22                 | 78.40 ± 0.25   | 99.94          |
| 5        | 12.50                  | 73.21 ± 0.32         | 99.80          | 53.41          |
|          |                        | 0.33                 | 73.38 ± 0.22   | 100.38         |
|          |                        | 0.22                 | 78.40 ± 0.25   | 99.94          |
| 6        | 12.50                  | 103.15 ± 0.52        | 99.50          | 53.39          |
|          |                        | 0.53                 | 103.21 ± 0.64  | 99.64          |
| 7        | 100.00                 | 494.44 ± 0.21        | 99.90          | 394.79         |
|          |                        | 0.21                 | 494.80 ± 0.60  | 100.01         |
|          |                        | 0.73                 | 594.51 ± 0.51  | 99.79          |
| 8        | 100.00                 | 793.66 ± 2.83        | 99.20          | 396.84         |
|          |                        | 2.85                 | 796.40 ± 0.64  | 99.89          |
|          |                        | 0.32                 | 513.67 ± 0.56  | 100.12         |
| 9        | 500.00                 | 2492.69 ± 2.68       | 98.88          | 2484.57 ± 0.52 |
|          |                        | 2.71                 | 1998.32         | 97.25          |
|          |                        | 0.40                 | 1989.99         | 4.12           |
| 10       | 500.00                 | 2476.48 ± 3.98       | 98.88          | 2477.65 ± 0.19 |
|          |                        | 4.03                 | 1981.36         | 9.26           |
|          |                        | 0.32                 | 3981.81 ± 1.77 | 99.08          |
|          |                        | 4.57                 | 2991.43 ± 0.33 | 99.03          |
|          |                        | 4.63                 | 2001.12         | 4.61           |
|          |                        | 4.17                 | 1985.18         | 4.37           |

a) Mean ± S.D. (standard deviation; n=6)
Table 4. Content (µg/mL) of compounds 1 - 10 in A. gigas, A. sinensis, and A. acutiloba

| Sample | Compound |
|--------|----------|
|        | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 |
| G01    | 147 | 0  | 51 | 3  | 0  | 0  | 105| 0  | 1269| 755|
| G02    | 424 | 0  | 121| 21 | 0  | 0  | 289| 0  | 2155| 914|
| G03    | 206 | 0  | 75 | 12 | 0  | 0  | 67 | 0  | 909 | 727|
| G04    | 162 | 10 | 56 | 9  | 0  | 0  | 101| 0  | 1341| 612|
| G05    | 180 | 13 | 16 | 9  | 0  | 0  | 77 | 0  | 1331| 596|
| G06    | 304 | 15 | 48 | 19 | 0  | 0  | 126| 0  | 1255| 670|
| G07    | 276 | 14 | 31 | 36 | 0  | 0  | 202| 0  | 1149| 932|
| G08    | 222 | 15 | 49 | 17 | 0  | 0  | 337| 0  | 1643| 1580|
| G09    | 162 | 12 | 61 | 1  | 0  | 0  | 101| 0  | 1195| 678|
| G10    | 162 | 9  | 42 | 2  | 0  | 0  | 69 | 0  | 1094| 658|
| G11    | 214 | 11 | 49 | 1  | 0  | 0  | 86 | 0  | 1064| 460|
| G12    | 173 | 11 | 64 | 10 | 0  | 0  | 67 | 0  | 1081| 496|
| G13    | 248 | 13 | 67 | 1  | 0  | 0  | 86 | 0  | 1230| 660|
| G14    | 156 | 13 | 49 | 2  | 0  | 0  | 37 | 0  | 1017| 665|
| G15    | 153 | 15 | 63 | 5  | 0  | 0  | 151| 0  | 1383| 648|
| A01    | 48  | 9  | 0  | 0  | 10 | 0  | 0  | 117| 0  | 0  |
| A02    | 35  | 8  | 0  | 0  | 8  | 0  | 0  | 90 | 0  | 0  |
| A03    | 39  | 8  | 0  | 0  | 12 | 0  | 0  | 104| 0  | 0  |
| A04    | 35  | 8  | 0  | 0  | 8  | 0  | 0  | 141| 0  | 0  |
| A05    | 34  | 8  | 0  | 0  | 9  | 0  | 0  | 103| 0  | 0  |
| A06    | 90  | 8  | 0  | 0  | 24 | 0  | 0  | 195| 0  | 0  |
| A07    | 51  | 10 | 0  | 0  | 26 | 0  | 0  | 102| 0  | 0  |
| A08    | 38  | 8  | 0  | 0  | 15 | 0  | 0  | 171| 0  | 0  |
| A09    | 153 | 10 | 0  | 0  | 10 | 0  | 0  | 95 | 0  | 0  |
| A10    | 127 | 10 | 0  | 0  | 21 | 0  | 0  | 59 | 0  | 0  |
| A11    | 48  | 0  | 0  | 0  | 9  | 0  | 0  | 139| 0  | 0  |
| A12    | 43  | 0  | 0  | 0  | 10 | 0  | 0  | 135| 0  | 0  |
| A13    | 44  | 0  | 0  | 0  | 10 | 0  | 0  | 136| 0  | 0  |
| A14    | 43  | 0  | 0  | 0  | 10 | 0  | 0  | 101| 0  | 0  |
| A15    | 64  | 0  | 0  | 0  | 11 | 0  | 0  | 308| 0  | 0  |
| S01    | 60  | 119| 0  | 0  | 0  | 112| 0  | 750| 0  | 0  |
| S02    | 49  | 34 | 0  | 0  | 0  | 97 | 0  | 862| 0  | 0  |
| S03    | 22  | 15 | 0  | 0  | 0  | 54 | 0  | 822| 0  | 0  |
| S04    | 25  | 22 | 0  | 0  | 0  | 49 | 0  | 863| 0  | 0  |
| S05    | 24  | 15 | 0  | 0  | 0  | 50 | 0  | 742| 0  | 0  |
| S06    | 166 | 144| 0  | 0  | 0  | 76 | 0  | 780| 0  | 0  |
| S07    | 58  | 263| 0  | 0  | 0  | 59 | 0  | 790| 0  | 0  |
| S08    | 51  | 280| 0  | 0  | 0  | 85 | 0  | 856| 0  | 0  |
| S09    | 27  | 270| 0  | 0  | 0  | 74 | 0  | 882| 0  | 0  |
| S10    | 26  | 213| 0  | 0  | 0  | 49 | 0  | 865| 0  | 0  |
| S11    | 26  | 250| 0  | 0  | 0  | 66 | 0  | 883| 0  | 0  |
| S12    | 30  | 348| 0  | 0  | 0  | 58 | 0  | 918| 0  | 0  |
| S13    | 24  | 138| 0  | 0  | 0  | 37 | 0  | 896| 0  | 0  |
| S14    | 26  | 196| 0  | 0  | 0  | 21 | 0  | 734| 0  | 0  |
| S15    | 24  | 253| 0  | 0  | 0  | 50 | 0  | 789| 0  | 0  |

G01–G15: A. gigas; A01–A15: A. acutiloba and S01–S15: A. sinensis