Quantitative analysis of the eight major compounds in the Samsoeum using a high-performance liquid chromatography coupled with diode array detection and electrospray ionization mass spectrometer

Jin Bae Weon¹, Hye Jin Yang¹, Bohyoung Lee¹, Jin Yeul Ma², Choong Je Ma¹,³

¹Department of Biomaterials Engineering, Division of Bioscience and Biotechnology, ²Research Institute of Biotechnology, Kangwon National University, Chuncheon 200-701, Korea, ³TKM Converging Research Division, Korea Institute of Oriental Medicine, 483 Exporo, Yuseong-gu, Daejeon 305-811, Korea

Submitted: 03-06-2014 Revised: 19-06-2014 Published: 12-03-2015

ABSTRACT

Background: Samsoeum was traditionally used for treatment of a respiratory disease. Objective: The simultaneous determination of eight major compounds, ginsenoside Rg3, caffeic acid, puerarin, costunolide, hesperidin, naringin, glycyrrhizin, and 6-gingerol in the Samsoeum using a high-performance liquid chromatography (HPLC) coupled with diode array detection (DAD) and an electrospray ionization mass spectrometer was developed for an accurate and reliable quality assessment. Materials and Methods: Eight compounds were qualitative identified based on their mass spectra and by comparing with standard compounds and quantitative analyzed by HPLC-DAD. Separation of eight compounds was carried out on a LUNA C₁₈ column (S-5 µm, 4.6 mm i.d. × 250 mm) with gradient elution composed of acetonitrile and 0.1% trifluoroacetic acid. Results: The data showed good linearity ($R^2 > 0.9996$). The limits of detection and the limits of quantification were <0.53 µg and 1.62 µg, respectively. Inter- and Intra-day precisions (expressed as relative standard deviation values) were within 1.94% and 1.91%, respectively. The recovery of the method was in the range of 94.24–107.90%. Conclusion: The established method is effective and could be applied to quality control of Samsoeum.

Key words: High-performance liquid chromatography-diode array detection, high-performance liquid chromatography-mass spectrometer, marker constituents, samsoeum, simultaneous determination, validation

INTRODUCTION

Over the years, traditional herbal medicines (THM) have considerable attraction in many countries due to their high therapeutic effects in various diseases.¹,² Samsoeum, a THM was used for treatment of a respiratory disease such as chronic bronchitis, bronchitis, and cold. Samsoeum has been shown to have anti-allergic effect and anti-inflammatory effects.³⁻⁴ It is composed of 13 herbs, Panax ginseng, Perilla frutescens, Angelica decursiva, Pinellia ternate, Pueraria lobata, Poria cocos, Aucklandia lappa, Citrus unshiu, Playtocodon grandiflorum, Citrus aurantium, Glycyrrhiza uralensis, Zingiber officinale, and Zizyphus jujube. Most of THM are used in the complex formulas of many herbs. The quality of THM is closely related to the amount of their bioactivity compounds, which is slightly different according to culture environment and manufacturable condition. In general, chromatography and relative techniques are used to analysis of THM and plants. High-performance liquid chromatography (HPLC) is the most frequently used separation technique. Liquid chromatography-mass spectrometer (LC-MS)/MS technique was applied to qualitative and quantitative analysis of THM as a new method.⁵⁻⁶ Currently, many analytical techniques have been developed and reported to quality control of THM or herbs.⁶⁻⁹

In this study, reliable and accurate quantitative HPLC method for simultaneous determination of eight compounds, ginsenoside Rg3 of *P. ginseng*, caffeic acid of *P. frutescens*, puerarin of *P. lobata*, costunolide of *A.
Liquid chromatography-electrospray ionization-mass spectrometer condition

Liquid chromatography-ESI-MS analysis was conducted using TSQ Quantum Ultra Triple Stage Quadrupole MS (Thermo). Analysis was performed at 25°C on Atlantis dC18 column (150 × 2.0 mm i.d., 3 µm). The mobile phase was the same as HPLC-DAD analysis. The linear gradient was used as follows: 0–10 min, 15% → 20% A; 10–20 min, 20% → 25% A; 20–30 min, 25% → 50% A; 30–40 min, 50% A; 40–50 min, 50% → 25% A. Eluent A was acetonitrile. The flow rate was 200 µL/min. Mass spectrometry conditions were optimized to provide the highest sensitivity. All analytes were monitored under positive ionization mode. The ion spray voltage was 4,700 V, and the vaporizer temperature was 320°C. The other conditions were as follows: Sheath gas pressure, 60 psi; aux gas pressure, 30 psi; capillary temperature, 320°C.

Standard solutions and sample preparation

Each accurately weighed standard was dissolved in 10 mL of 60% methanol. Individual stock solutions were prepared at a concentration of 200 µg/mL for puerarin, 350 µg/mL for caffeic acid, 289.5 µg/mL for naringin, 175 µg/mL for hesperidin, 400 µg/mL for glycyrrhizin, 265 µg/mL for 6-gingerol, 1000 µg/mL for ginsenoside Rg3, and 170 µg/mL for costunolide. The analytical working solutions were prepared by appropriate dilution of the stock solution and mixed before HPLC analysis.

The Samsoeum powders were weighed accurately and added in 8 mL of 60% methanol. The sample solutions were filtered through a 0.45 µm membrane filter before analysis.

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions

To obtain the best separation condition, four different columns have been tested Dionex C18 column (150 mm × 4.6 mm i.d., 5 µm), LUNA C18 column (250 mm × 4.6 mm i.d., 5 µm), SHISHEDO C18 column (250 mm × 4.6 mm i.d., 5 µm) and XTerra™ RP18 (250 mm × 4.60 mm i.d., 5 µm). As a result of the
Weon, et al.: Quantitative analysis method of Samsoeum

Weon, et al.: Quantitative analysis method of Samsoeum

Pharmacognosy Magazine | April-June 2015 | Vol 11 | Issue 42

test, use of LUNA C18 column (250 mm × 4.60 mm i.d., 5 µm) resulted in a well separation of eight compounds. In mobile phase condition, buffer such as 0.1% TFA was added to improve peak shape and inhibit the ionization of compounds. The maximum UV wavelength of eight compounds was different. Thus, the detection UV wavelength was selected according to their maximum wavelength. 6-gingerol, ginsenoside Rg3, and costunolide were at 205 nm. Puerarin and glycyrrhizin were at 250 nm. Naringin and hesperidin were at 280 nm. Caffeic acid was at 330 nm. The chromatograms of the standard solution and Samsoeum sample were shown in Figure 2. It was appeared that a good separation was achieved under the established LC condition.

**Liquid chromatography-electrospray ionization-mass spectrometer analysis**

This method involved the use of LC-ESI-MS to identify the peaks of eight compounds found in HPLC chromatogram of Samsoeum. Accurate molecular mass of puerarin, caffeic acid, naringin, hesperidin, glycyrrhizin, 6-gingerol, ginsenoside Rg3, and costunolide were obtained by the LC-ESI-MS analysis. In positive ionization mode, the protonated molecular ions [M + H]+ were observed at m/z 181.06, 416.37, 610.56, 822.93, 785.01, and 294.38 for caffeic acid, puerarin, naringin, hesperidin, glycyrrhizin, and 6-gingerol, respectively.

Sodiated molecular ions [M + Na]+ were observed at m/z 603.22 and 807.69 for naringin and ginsenoside Rg3, respectively [Table 1 and Figure 3].

**Method validation**

**Linearity, limits of detection, and limits of quantification**

Good linear correlation and high sensitivity were evaluated by the correlation coefficient, limits of detection (LOD) and limits of quantification (LOQ). The linear calibration curves were plotted with diluted six different concentrations of standard solutions. Each concentration of compounds was analyzed in triplicate. The linear regression equations were calculated in the form of \( Y = ax + b \) (a is the slope of the calibration curve b is the intercept of calibration curve, x and Y are the concentration and peak area of compound, respectively). Calibration curves exhibited good linearity \( (R^2 > 0.9996) \) in the range of measured concentrations.

| Table 1: Identification of the 8 compounds |
|-----------------------------------------|
| Components                        | Exact mass | Molecular ions (m/z) |
|-----------------------------------------|
| Puerarin                            | 416.37     | [M+H]+ 417.12       |
| Caffeic acid                        | 180.16     | [M+H]+ 181.06       |
| Naringin                            | 580.54     | [M+Na]+ 603.22      |
| Hesperidin                          | 610.56     | [M+H]+ 611.25       |
| Glycyrrhizin                        | 822.93     | [M+H]+ 823.54       |
| 6-Gingerol                          | 294.38     | [M+H]+ 295.17       |
| Ginsenoside Rg3                     | 785.01     | [M+Na]+ 807.69      |
| Costunolide                         | 233.16     | [M+H]+ 233.16       |

**Figure 1:** Chemical structures of eight major compounds
Weon, et al.: Quantitative analysis method of Samsoeum

concentration for eight major compounds. LOD and LOQ were measured on the basis of the signal to noise ratio of 3 and 10, respectively. The LOD and LOQ were found to be in the range of 4.6–66.7 ng and 21.0–202.1 ng, respectively. The detailed descriptions of results were presented in Table 2.

**Precision and accuracy**

Precision of the method was evaluated by performing the inter- and intra-day test with eight major compounds at three different concentrations. The intra- and inter-day tests were, repetitively, conducted on the mixed standard solution five times once a day for 3 consecutive days (1, 3, 5 days) and a day, respectively.

Precision was determined as relative standard deviation (RSD). RSD values of the inter- and inter-day were within 1.94% (0.34–1.94%) and 2.00 (0.56–2.00%), respectively [Table 3].

In order to confirm the accuracy, a recovery experiment was performed. Three different concentrations of the eight major compounds were added into the Samsoeum sample in triplicate. The mean recoveries of investigated eight compounds ranged from 94.24% to 107.90% with RSD <1.92%. These results indicated that the established method had acceptable precision and accuracy.

### Table 2: Regression equation, the correlation coefficient ($R^2$), LOD, and LOQ for the 8 compounds

| Components    | Linear range (μg/mL) | Regression equation       | $R^2$ (n=6) | LOD (ng) | LOQ (ng) |
|---------------|----------------------|---------------------------|-------------|----------|----------|
| Puerarin      | 0.625-50.000         | $Y=1.2791x-0.2613$        | 0.9999      | 14.5     | 44.0     |
| Caffeic acid  | 0.547-43.750         | $Y=1.5255x-0.2570$        | 1.0000      | 5.0      | 16.4     |
| Naringin      | 0.452-36.188         | $Y=0.5148x-0.0384$        | 1.0000      | 13.2     | 40.1     |
| Hesperidin    | 0.273-21.875         | $Y=0.5248x+0.0565$        | 0.9999      | 24.3     | 73.5     |
| Glycyrrhizin  | 0.625-50.000         | $Y=0.1923x+0.0173$        | 0.9999      | 6.9      | 21.0     |
| 6-Gingerol    | 0.414-33.125         | $Y=1.3554x-0.0651$        | 1.0000      | 4.6      | 13.8     |
| Ginsenoside Rg3 | 1.563-125.000       | $Y=0.0684x-0.0404$        | 0.9996      | 44.8     | 135.8    |
| Costunolide   | 0.266-21.250         | $Y=1.1565x+0.0266$        | 0.9999      | 66.7     | 202.1    |

*Y: Peak area; x: Concentration (mg/mL). LOD: Limits of detection; LOQ: Limits of quantification*

### Table 3: Analytical results of intra-day and inter-day variability

| Components    | Concentration (μg/mL) | Intra-day (n=5) | Inter-day (n=5) |
|---------------|----------------------|-----------------|-----------------|
| Puerarin      | 3.13                 | 3.25±0.06       | 3.23±0.04       | 1.17  | 103.41 |
|               | 6.25                 | 6.22±0.03       | 6.25±0.12       | 1.88  | 100.08 |
|               | 12.50                | 12.40±0.16      | 12.45±0.14      | 1.09  | 99.63  |
| Caffeic acid  | 5.47                 | 5.52±0.05       | 5.16±0.10       | 1.91  | 94.31  |
|               | 10.94                | 10.72±0.11      | 10.58±0.21      | 1.99  | 96.70  |
|               | 21.88                | 22.28±0.24      | 21.42±0.21      | 0.97  | 97.89  |
| Naringin      | 4.52                 | 4.88±0.09       | 4.89±0.08       | 1.62  | 108.10 |
|               | 9.05                 | 9.51±0.05       | 9.75±0.12       | 1.23  | 107.74 |
|               | 18.09                | 18.06±0.28      | 18.60±0.18      | 0.99  | 102.81 |
| Hesperidin    | 2.73                 | 2.82±0.05       | 2.97±0.04       | 1.32  | 108.66 |
|               | 5.47                 | 5.76±0.03       | 5.95±0.07       | 1.10  | 108.78 |
|               | 10.94                | 11.48±0.21      | 11.85±0.13      | 1.13  | 108.30 |
| Glycyrrhizin  | 6.25                 | 6.25±0.10       | 6.35±0.10       | 1.60  | 101.53 |
|               | 12.50                | 12.34±0.08      | 12.68±0.19      | 1.51  | 101.47 |
|               | 25.00                | 24.75±0.36      | 25.58±0.44      | 1.71  | 102.33 |
| 6-Gingerol    | 4.14                 | 4.20±0.07       | 4.15±0.04       | 1.08  | 100.14 |
|               | 8.28                 | 8.15±0.06       | 8.36±0.14       | 1.62  | 100.98 |
|               | 16.56                | 16.60±0.27      | 16.62±0.26      | 1.55  | 100.34 |
| Ginsenoside Rg3 | 15.63              | 15.41±0.19      | 16.13±0.29      | 1.77  | 103.18 |
|               | 31.25                | 32.86±0.49      | 31.74±0.17      | 0.55  | 101.58 |
|               | 62.50                | 65.90±0.23      | 62.24±0.70      | 1.13  | 99.58  |
| Costunolide   | 2.66                 | 2.71±0.05       | 2.68±0.02       | 0.78  | 100.74 |
|               | 5.31                 | 5.70±0.11       | 5.42±0.11       | 2.00  | 102.01 |
|               | 10.63                | 10.95±0.12      | 10.97±0.11      | 0.96  | 103.24 |

SD: Standard deviation; RSD: Relative standard deviation
Sample analysis
The developed HPLC method was applied to analyze eight compounds, puerarin, caffeic acid, naringin, hesperidin, glycyrrhizin, 6-gingerol, ginsenoside Rg3, and costunolide in the prepared Samsoeum sample and the seven commercial samples. Contents of the eight compounds in the samples are listed in Table 5. Table 5 shows that their contents in the samples were slightly different. Among of the compounds, puerarin was the main compound. The highest content of puerarin was 10.91 µg/mg, but the lowest was 10.27 µg/mg. The contents of caffeic acid were the lowest in Samsoeum samples and not were detected in some samples. Costunolide could not be detected in all samples. The quality of traditional medicine and the content of bioactive compounds were affected by the different processing procedures for manufacturing Samsoeum and the year of the plant cultivation, harvest time, plant origins, climate, and environment. Therefore, efficient analysis method to control the quality of traditional medicine like Samsoeum was needed. This HPLC method may be used as a protocol to evaluate the quality of Samsoeum.

CONCLUSION
Samsoeum, THM is a remedy for the treatment of respiratory disease. In the study, accurate, sensitive, and precise HPLC-DAD and LC-ESI-MS method for the

Table 4: Results of recovery of the 8 compounds

| Components       | Spiked amount (µg/mL) | Measured amount (µg/mL) | Recovery (%) | RSD (%) |
|------------------|-----------------------|-------------------------|--------------|---------|
| Puerarin         | 1.56 ± 0.03           | 1.65 ± 0.03             | 105.65       | 1.92    |
|                  | 3.13 ± 0.25           | 3.17 ± 0.05             | 101.36       | 1.71    |
|                  | 6.25 ± 0.02           | 6.39 ± 0.02             | 102.18       | 0.38    |
| Caffeic acid     | 2.73 ± 0.04           | 2.87 ± 0.04             | 97.71        | 1.66    |
|                  | 5.47 ± 0.05           | 5.40 ± 0.05             | 98.74        | 0.99    |
|                  | 10.94 ± 0.06          | 10.31 ± 0.06            | 94.24        | 0.54    |
| Naringin         | 2.26 ± 0.01           | 2.25 ± 0.01             | 99.32        | 0.52    |
|                  | 4.52 ± 0.07           | 4.66 ± 0.07             | 102.97       | 1.55    |
|                  | 9.05 ± 0.13           | 9.16 ± 0.13             | 101.20       | 1.44    |
| Hesperidin       | 1.37 ± 0.01           | 1.43 ± 0.01             | 104.50       | 0.22    |
|                  | 2.73 ± 0.02           | 2.80 ± 0.02             | 102.58       | 0.61    |
|                  | 5.47 ± 0.10           | 5.90 ± 0.10             | 107.90       | 1.70    |
| Glycyrrhizin     | 3.13 ± 0.01           | 3.12 ± 0.01             | 99.94        | 0.30    |
|                  | 6.25 ± 0.04           | 6.44 ± 0.04             | 103.07       | 0.63    |
|                  | 12.5 ± 0.03           | 11.92 ± 0.03            | 95.33        | 0.22    |
| 6-Gingerol       | 2.07 ± 0.02           | 1.97 ± 0.02             | 94.94        | 1.14    |
|                  | 4.14 ± 0.05           | 4.11 ± 0.05             | 99.36        | 1.24    |
|                  | 8.28 ± 0.07           | 8.60 ± 0.07             | 103.82       | 0.76    |
| Ginsenoside Rg3  | 7.81 ± 0.08           | 7.98 ± 0.08             | 102.18       | 1.04    |
|                  | 15.63 ± 0.05          | 15.00 ± 0.05            | 95.98        | 0.33    |
|                  | 31.25 ± 0.53          | 29.62 ± 0.53            | 94.79        | 1.80    |
| Costunolide      | 1.33 ± 0.01           | 1.33 ± 0.01             | 99.93        | 0.24    |
|                  | 2.66 ± 0.04           | 2.69 ± 0.04             | 101.29       | 1.41    |
|                  | 5.31 ± 0.04           | 5.28 ± 0.04             | 99.38        | 0.84    |

Recovery (%): (Concentration found−original concentration)/concentration spiked×100%. RSD: Relative standard deviation

Figure 2: The high-performance liquid chromatography chromatogram of eight standard compounds (a) and Samsoeum sample (b); (1) puerarin, (2) caffeic acid, (3) naringin, (4) hesperidin, (5) glycyrrhizin, (6) 6-gingerol, (7) ginsenoside Rg3, (8) costunolide
quantitative analysis and identification of the eight major compounds, puerarin, caffeic acid, naringin, hesperidin, glycyrrhizin, 6-gingerol, ginsenoside Rg3, and costunolide in Samsoeum. Up to now, simultaneous determination of the seven compounds (Puerarin, daidzin, liquiritin, naringin, hesperidin, neohesperidin, and glycyrrhizin) in the Samsoeum was reported.\[^1\,^2\] We analyzed more compounds of various herbs, and LC-ESI-MS method was additionally employed to verify the compounds in comparison to previous reported analysis method of Samsoeum. The developed method was successfully applied for simultaneous determination in the eight compounds in Samsoeum sample. Such results can form the basic method for improvement of quality of Samsoeum.

**ACKNOWLEDGMENT**

This research was supported by a grant [K11050] from the Korea Institute of Oriental Medicine.

**REFERENCES**

1. Lu AP, Jia HW, Xiao C, Lu QP. Theory of traditional Chinese medicine and therapeutic method of diseases. World J Gastroenterol 2004;10:1854-6.
2. Jiang WY. Therapeutic wisdom in traditional Chinese medicine: A perspective from modern science. Trends Pharmacol Sci 2005;26:558-63.
3. Lee SE, Shin JY, Lee SH. Anti-allergic effects of Shensuyin. J Korean Orient Intern Med 2005;26:119-28.
4. Kim SJ, Kim NH, Moon PD, Myung NY, Kim MC, Lee KT.
et al. Samsoeum inhibits systemic anaphylaxis and release of histamine, cytokine in vivo and in vitro. Orient Pharm Exp Med 2009;9:115-27.
5. Drasar P, Moravcova J. Recent advances in analysis of Chinese medical plants and traditional medicines. J Chromatogr B Analyt Technol Biomed Life Sci 2004;812:3-21.
6. Lu B, Liu Y, Yin L, Wang X, Peng J. Simple and reliable methods for the determination of sixteen marker components for quality control of Daochi pill by HPLC coupled with diode array detection. Phytochem Anal 2009;20:385-94.
7. Lee MK, Kim SH, Park JH, Cho JH, Kim DH, Baek JH, et al. Determination and identification of nine constituents in Siho-Gyeoji-Tang by HPLC/DAD and HPLC/MS/MS. J Liq Chromatogr Relat Technol 2009;32:2122-33.
8. Liang X, Zhang L, Zhang X, Dai W, Li H, Hu L, et al. Qualitative and quantitative analysis of traditional Chinese medicine Niu Huang Jie Du Pill using ultra performance liquid chromatography coupled with tunable UV detector and rapid resolution liquid chromatography coupled with time-of-flight tandem mass spectrometry. J Pharm Biomed Anal 2010;51:565-71.
9. Wang X, Sakuma T, Asafu-Adjaye E, Shiu GK. Determination of ginsenosides in plant extracts from Panax ginseng and Panax quinquefolius L. by LC/MS/MS. Anal Chem 1999;71:1579-84.
10. Heyrman AN, Henry RA. Importance of controlling mobile phase pH in reversed phase HPLC. Keystone Tech Bull 1999;99-06:1-7.
11. Dolan JW. The Importance of Temperature. Walnut Creek, California, USA: LC Resources Inc.; 2002.
12. Seo CS, Kim JH, Huang DS, Shin HK. Simultaneous determination of seven compounds in Samsoeum by HPLC-PDA. Korean J Orient Med Prescription 2010;18:95-103.

Cite this article as: Weon JB, Yang HJ, Lee B, Ma JY, Ma CJ. Quantitative analysis of the eight major compounds in the Samsoeum using a high-performance liquid chromatography coupled with diode array detection and electrospray ionization mass spectrometer. Phcog Mag 2015;11:320-6.

Source of Support: Nil, Conflict of Interest: None declared.