Determination of Betaine in Fructus Lycii Using Hydrophilic Interaction Liquid Chromatography with Evaporative Light Scattering Detection

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Received November 17, 2011, Accepted December 12, 2011

A simple new method was developed for the determination of betaine in Fructus Lycii using hydrophilic interaction liquid chromatography with evaporative light scattering detection (HILIC-ELSD). Good chromatographic separation and reasonable betaine retention was achieved on a Kinetex HILIC column (2.1 × 100 mm, 2.6 µm) packed with fused-core particle. The mobile phase consisted of (A) acetonitrile and (B) 10 mM ammonium formate (pH 3.0)/acetonitrile (90/10, v/v). It was used with gradient elution at a flow rate of 0.7 mL/min. The column temperature was set at 27.5 °C and the injection volume was 10 µL. The ELSD drift tube temperature was 50 °C and the nebulizing gas (nitrogen) pressure was 3.0 bar. Stachydrine, a zwitterionic compound, was used as an internal standard. Calibration curve over 10-250 µg/mL showed good linearity (R² > 0.9992) and betaine in the 70% methanol extract of Fructus Lycii was well separated from other peaks. Intra- and inter-day precision ranged from 1.1 to 3.0% and from 2.4 to 5.3%, respectively, while intra- and inter-day accuracy ranged from 100.0 to 107.0% and from 94.3 to 103.9%, respectively. The limit of quantification (LOQ) was 10 µg/mL and the recoveries were in the range of 98.2-102.7%. The developed HILIC-ELSD method was successfully applied to quantitatively determine the amount of betaine in fourteen Fructus Lycii samples from different locations, demonstrating that this method is simple, rapid, and suitable for the quality control of Fructus Lycii.

Key Words: Fructus Lycii, Betaine, Hydrophilic interaction liquid chromatography, Evaporative light scattering detection

Introduction

Fructus Lycii is the dried fruit of Lycium chinense Miller and L. barbarum Linne (Solanaceae). It has been gaining worldwide popularity and is widely used in East Asia for its benefits to stamina, anti-aging, vision, and liver and kidney nourishment.¹ Fructus Lycii has little or no side effects and is generally recognized as safe (GRAS).² It is ordinarily taken by tea or traditional liquor. Recent studies show that it possesses polysaccharides,¹ carotenoids,³ vitamins,⁴ flavonoids,⁵ and amino acids⁶ such as taurine, γ-aminobutyric acid, and betaine. Because Fructus Lycii has been commonly used in East Asia, several Asian regulatory authorities set quality standard of the herbal medicine. For example, Korea and China provide the content criteria for Fructus Lycii in their pharmacopoeia. The Korean pharmacopoeia (KP) stipulates that Fructus Lycii should contain more than 0.5% betaine while the Chinese pharmacopoeia (CP) sets this number at more than 0.3%.⁷,⁸ Several reports reveal that betaine works as an osmolyte and as a methyl donor in the human body. It has shown positive results for applications in fatty liver and cardiovascular diseases.⁹,¹⁰ Betaine is a naturally occurring compound that exists in zwitterionic form at neutral pH. A zwitterion is a molecule with both a positively and a negatively charged functional group (Figure 1). Generally, zwitterionic compounds have two pKₐ values. One is derived from a cationic group and the other is derived from an anionic group. However, betaine has only one pKₐ (1.84) derived from its carboxylic acid group because it has a permanently positive quaternary amine group. Betaine has a very low n-octanol/water partition coefficient value (logP = −4.93) and lacks UV-chromophore in its structure. Considering its physicochemical properties, betaine is inappropriate for analysis by the conventional reversed-phase high performance liquid chromatography with UV (RP-HPLC-UV). This is because of its very short retention time and low detection sensitivity.

Over the past few decades, numerous studies have been carried out to determine the betaine content in plants and biological samples. Various analytical methods have been introduced for this purpose, including ion exchange chromatography, fast atom bombardment mass spectrometry (FAB-MS), high performance liquid chromatography-refractive index detector (HPLC-RI), HPLC-UV, HPLC-MS, and
HPLC-MS/MS. However, due to inherent instrumental limitations and tedious sample preparation, these methods are not suitable for the routine analysis of betaine in Fructus Lycii. For example, refractive index detection exhibits poor sensitivity, and HPLC-MS and -MS/MS require expensive instrumentation. In addition, sample preparation for these methods requires complicated ion exchange processes to remove most of the amino acids that could interfere with betaine quantification.

Betaine has no chromophore, and its polar, zwitterionic character causes unsuitable retention on reversed-phase columns (e.g., C18). For this reason, Lee et al. have recently developed and validated a new HPLC method using an evaporative light scattering detector (ELSD) instead of a conventional UV detector for the quantitative HPLC analysis of betaine. An ELSD is a universal detector providing a stable baseline even with a gradient elution and can detect non-volatile analytes (e.g., sugars, amino acids, steroids) not absorbing UV above 200 nm regardless of their spectral and physicochemical characters. Though Lee et al. developed and validated a new HPLC-ELSD method, a C18 column with a mobile phase that contained an ion-pairing reagent was used (perfluoropentanoic acid, PFPA, pKs = −2.29). PFPA may irreversibly adsorb to the C18 stationary phase because of its alkyl chain.

Another recent study used stable-isotope dilution ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) to simultaneously quantify acetylcholine, betaine, choline, and dimethylglycine in plasma and urine. The separation used a hydrophobic interaction liquid chromatography (HILIC) column to achieve the accepted retention of four choline related compounds. These compounds are so hydrophilic that conventional RP columns (e.g., C18) can hardly retain them. Thus, analysis was usually performed with a small amount of organic solvent added in the mobile phase. HILIC is a type of normal-phase (NP) chromatography because it has the same polar stationary phase. However, it differs in that HILIC employs more polar organic mobile phases than NP. UPLC-MS/MS can be a powerful tool for determining trace amounts of betaine in biological fluids samples. However, Fructus Lycii contains a relatively large amount of betaine. For this reason, such expensive and sophisticated equipment is unnecessary in the small laboratories of developing countries.

Betaine is not retained well on reversed-phase columns due to its high polarity. Using a very polar stationary phase with an aqueous mobile phase of high organic content provided an alternative for separating betaine from herbal medicine. Hydrophilic interaction liquid chromatography (HILIC) can retain highly polar analytes using large amounts of organic solvent (> 80%) as the mobile phase. The HILIC column was more effective for the chromatographic separation of betaine. Also, the HILIC column simplified the sample preparation to a sonication extraction because it enabled betaine to separate completely from Fructus Lycii. For these reasons, the HILIC column was chosen as the stationary phase for betaine determination in Fructus Lycii.

In the present study, we developed and validated hydrophilic interaction liquid chromatography-evaporative light scattering detection (HILIC-ELSD) for the determination of betaine in Fructus Lycii. To the best of our knowledge, this is the first report of a method that uses HILIC with ELSD to measure betaine. In addition, we tested the method for the quantitative betaine determination of fourteen Fructus Lycii samples.

**Experimental**

**Materials and Reagents.** Fourteen Fructus Lycii samples were obtained from the Kyungdong herbal medicine market (Seoul, South Korea). Glycine betaine, acetic acid, formic acid, and phosphoric acid (85%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The internal standard, stachydrine, was kindly supplied by the Seoul National University (Seoul, South Korea). Acetonitrile, methanol, and water were HPLC grade and purchased from Fisher Scientific (Fair Lawn, NJ, USA). Ammonium formate (97%) and ammonium acetate (98%) were purchased from Fluka (Buchs, Switzerland). Nylon membrane filters (0.45 and 0.22 μm) were purchased from Whatman (Maidstone, England). The pH values were measured by an Orion 710A pH meter from Orion Research Inc. (Beverly, MA, USA). All weighing was done with a Mettler Toledo AT460 Delta-Range from Mettler-Toledo Inc. (Columbus, OH, USA).

**Chromatographic Conditions.** The HPLC system was equipped with a PerkinElmer Series 200 pump, a PerkinElmer Series 200 column oven (Waltham, MA, USA), a Waters 717 plus autosampler (Milford, MA, USA), and a SEDERE SEDEX 75 ELSD (Alfortville, France). Three different columns were used and compared: Waters Atlantis HILIC (3.0 × 100 mm, 5 μm), Phenomenex Kinetex HILIC (2.1 × 100 mm, 2.6 μm), and HALO HILIC (2.1 × 100 mm, 2.7 μm). The mobile phase was pumped into the system using a gradient elution of (A) acetonitrile and (B) 10 mM ammonium formate (pH 3.0)/acetonitrile (90/10, v/v) with 15% solvent B in 0-1 min, 15-25% solvent B in 1-16 min and 25-40% solvent B in 16-17 min. The flow rate was 0.7 mL/min and the injection volume was 10 μL with a column temperature of 27.5 °C. The ELSD parameters of drift tube temperature and nebulizing gas pressure were optimized at 50 °C and 3.0 bar, respectively.

**Preparation of Standard Solutions and Sample Solutions.** The standard stock solution was prepared by dissolving 10 mg of betaine into 10 mL of 70% methanol and then diluting with 70% methanol to obtain the appropriate concentration of working solution. The internal standard solution was prepared by dissolving 10 mg of stachydrine into 100 mL of methanol. All solutions were filtered through a 0.45 μm nylon membrane filter and stored at −70 °C before use.

Fructus Lycii samples were finely ground and strained through a sieve (50 mesh). Then 200 mg of the powder was weighed, blended with 10 mL of 70% methanol, and sonicated in the ultrasonic bath (25 °C for 40 min). The extract...
was centrifuged at 4000 rpm for 10 min and the supernatant was filtered through a 0.45 μm nylon membrane filter. 0.2 mL of the internal standard (stachydrine) solution was added to 0.8 mL of the sample solution and an aliquot of 10 μL was injected into the HILIC-ELSD.

Method Validation. The developed method was validated regarding linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), and recovery according to the guidelines of the Korea Food and Drug Administration (KFDA).

Calibration Curve and Linearity: By considering the amount of betaine in Fructus Lycii extracts, the calibration curve was composed of six concentration levels ranging from 10 to 250 μg/mL (10, 20, 50, 100, 150, and 250 μg/mL). The curve was constructed by plotting the logarithm of the peak area ratio (betaine/IS) against the logarithm of six different concentration values. Linearity was evaluated with the correlation coefficient (R²) of the calibration curve.

Intra- and Inter-day Precision and Accuracy: The intra-day precision and accuracy was assessed by analyzing five replicates at four different concentration points (10, 20, 100, and 250 μg/mL) within one day, whereas the inter-day precision and accuracy were estimated by analyzing one measurement at each of four concentrations for five consecutive days. Accuracy was expressed as the observed value’s percentage of the true value. Precision was expressed as the relative standard deviation (coefficient of variance, CV).

Limit of Detection (LOD) and Limit of Quantification (LOQ): The LOD and LOQ were determined as the concentrations at which the signal-to-noise ratios (S/N ratio) were larger than 3 and 10, respectively. The LOQ was accepted when precision CV was less than 20% and accuracy percentage was between 80 and 120% for both intra- and inter-day assays.

Recovery: The recovery assay was performed at three different concentration levels (10, 20, and 100 μg/mL) according to the standard addition procedure. Each 200 mg of the pulverized Fructus Lycii samples was spiked with three concentrations of betaine standard and prepared as described below. Recovery was estimated by the following equation:

\[
\text{Recovery (\%)} = \frac{(\text{Amount from sample spiked with standard} - \text{Amount from sample})}{\text{Amount from standard}} \times 100
\]

To determine extraction recovery, six replicates were measured at each concentration.

Quantification of Fructus Lycii Extracts: The developed HILIC-ELSD method was applied to the quantification of betaine in fourteen Fructus Lycii samples (one of them originated from *L. barbarum* while the rest were from *L. chinense*). The prepared samples were analyzed (n=3) and the peak area ratios (betaine/IS) from the HILIC-ELSD chromatograms were applied to the calibration curve to calculate betaine contents.

**Results and Discussion**

Optimization of the Chromatographic Conditions. Various stationary phases, including bare silica gel and silica-based amino, amido, cyano, carbamate, diol, and polyol can

![Figure 2.](image-url)
be used in HILIC separation. This study tested three HILIC columns with bare silica stationary phases from different manufacturers: Waters Atlantis HILIC Silica (3.0 × 100 mm, 5 μm), Phenomenex Kinetex HILIC (2.1 × 100 mm, 2.6 μm) and HALO HILIC (2.1 × 100 mm, 2.7 μm). Among them, Phenomenex Kinetex HILIC and HALO HILIC, which were packed with unique 2.6-2.7 μm fused-core particles, showed a low back pressure compared to the conventional particulate columns. The separation factor (α) was used to evaluate the performance of the three columns by the equation "Separation factor (α) = k_2/k_1", where the k_2 and k_1 are the capacity factors of betaine and its nearest matrix peak, respectively. When using the Atlantis HILIC column, the separation factor was 1.2, betaine was poorly retained, and the peak shape was unsatisfactory. With the HALO HILIC column, the separation factor was 1.0, the betaine retention time was too long, and severe peak tailing occurred. The best separation was achieved with the Kinetex HILIC column which gave a separation factor of 1.6, a more satisfactory retention time, and a good shape of betaine peak (Figure 2(a)).

With the HILIC mode, the type of organic modifier can have a large effect on analyte retention. The elution power of organic solvents is generally known as follows: methanol > ethanol > 2-propanol > tetrahydrofuran > acetonitrile. Because it is aprotic, acetonitrile enhances the retention of polar compounds on the polar stationary phase, and consequently, is the most frequently used solvent for HILIC. In contrast, methanol solvates the surface of the polar stationary phase, which causes competitive solvation between methanol and water. As the ratio of methanol in the mobile phase increases, the retention time of the polar analyte is shortened. The organic modifier appropriate for betaine was determined by comparing acetonitrile and methanol. In accordance with previous studies, methanol eluted betaine too early and it showed poor separation from the matrix peaks of the Fructus Lycii extract. Conversely, betaine was properly retained when acetonitrile was used.

To obtain satisfactory separation of betaine, several types of mobile phase buffers including acetic acid, formic acid, ammonium acetate, and ammonium formate were compared in terms of separation factor (α). The results showed that ammonium formate gave the best resolution during chromatographic separation (Figure 2(b)). In addition, we evaluated the effects of ammonium formate concentration and buffer solution pH on the chromatographic separation (Figure 2(c) and 2(d)). Ultimately, the optimal mobile phase was a mixture of acetonitrile and 10 mM ammonium formate (pH 3.0)/acetonitrile (90/10, v/v) in the gradient elution mode. HILIC-ELSD chromatograms derived from the optimized chromatographic condition are presented in Figure 3.

**Optimization of the ELSD Conditions.** The evaporative light scattering detector (ELSD) operates according to three steps: nebulization of the effluent into droplets, evaporation

![Figure 3. Optimized HPLC-ELSD chromatograms of betaine standard (a) and Fructus Lycii extract (b) using a Kinetex HILIC column (2.1 × 100 mm, 2.6 μm) and a mobile phase composed of acetonitrile and 10 mM ammonium formate (pH 3.0)/acetonitrile (90/10, v/v) in gradient mode.](image-url)

![Figure 4. Optimization of the ELSD parameters. Drift tube temperature (a), nebulizing gas (N₂) pressure (b), and nitrogen gas purity (c).](image-url)
of the mobile phase from the droplets, and laser light-scattering detection of non-volatile analytes in the droplets.\textsuperscript{18,22} The ELSD response can be adjusted by varying the drift tube temperature, the nebulizing gas pressure,\textsuperscript{26,27} and the purity of the nebulizing gas.\textsuperscript{14} These factors are responsible for the formation of proper droplets and the sensitivity of the detector.

To obtain optimal sensitivity from the ELSD, several experiments that changed these three parameters were performed. The performances were evaluated by the betaine signal-to-noise (S/N) ratios. The drift tube temperature varied from 35 °C to 60 °C (Figure 4(a)). Within the temperature range of 35 °C to 50 °C, the S/N ratio values increased. The highest S/N ratio was at 50 °C, and from that point the S/N ratio values began decreasing. The pressure (flow-rate) of nebulizing gas was optimized by changing the value from 2.4 to 3.3 bar (Figure 4(b)), and the optimal pressure was found to be 3.0 bar. To evaluate the effect of nebulizing gas (N\textsubscript{2}) purity on detector sensitivity, three types of N\textsubscript{2} gas were employed (Figure 4(c)): normal purity (99.9%), high purity (99.999%) and ultra-high purity (99.9999%). As the gas purity increased, the S/N ratio of betaine peak increased. Because of this, ultra-high purity gas was used.

Validation of the Analytical Method. The developed method was validated with regard to linearity, intra- and inter-day precision and accuracy, limit of detection (LOD), limit of quantification (LOQ), and recovery.

The ‘power function’ is usually used to create a calibration curve for ELSD responses.\textsuperscript{28-32} The equation is as follows:

\[
A = a \times m^b
\]

where \(a\) and \(b\) are constants, \(A\) is the ELSD response, and \(m\) is the mass of injected sample. Using the power function, the betaine calibration curve was constructed ranging from 10 to 250 µg/mL (Table 1), where \(y\) is the peak ratio (betaine/IS) and \(x\) is the concentration (µg/mL) of the injected standard. As shown in Figure 5, a linear calibration curve was obtained after logarithmic transformation of the concentration values. The LOQ and LOD were 10 and 3 µg/mL, respectively (Table 1).

Table 2 shows the results for intra- and inter-day precision and accuracy. Intra- and inter-day precision ranged from 100.0 to 107.0% and from 94.3 to 103.9%, respectively. Intra- and inter-day accuracy ranged from 1.9 to 2.5% and from 2.4 to 3.0%, respectively. All values were within the acceptable range.

Results of the recovery assay are presented in Table 3. All measured recovery values were also within the acceptable range.

Quantification of Fructus Lycii Extracts. The developed and validated HPLC-ELSD method using an HILIC column was successfully applied for the quantification of betaine in fourteen Fructus Lycii samples. One sample was from the dried fruit of \textit{L. barbarum} from China (C1). Among the thirteen Fructus Lycii samples that originated from \textit{L. chinense}, six samples were from China (C2-C7), five samples were from South Korea (SK1-SK5), and two samples were from North Korea (NK6 and NK7). The results are expressed as a percentage of dry weight and are listed in Table 4. Sample SK4 contained the least betaine (0.52%) while sample C2 contained the most (1.04%). The betaine content of all fourteen Fructus Lycii samples was greater than the KP and CP criteria given previously.

Principal Component Analysis (PCA). To classify Fructus Lycii according to geographic origins (South Korea, North Korea, and China), principal component analysis (PCA)

**Table 1.** Calibration curve, LOD and LOQ of betaine (n=5)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Range (µg/mL)</th>
<th>Power function, (A=(a±SD)\times m^{b(SD)})</th>
<th>Coefficient of determination (R(^2))</th>
<th>LOD (µg/mL)</th>
<th>LOQ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Betaine</td>
<td>10-250</td>
<td>(y=(7.98\times10^{-4}±0.58\times10^{-4})\times x^{(1.62±0.01)})</td>
<td>0.9992</td>
<td>3</td>
<td>10</td>
</tr>
</tbody>
</table>

**Table 2.** Intra- and inter-day precision and accuracy (n=5)

<table>
<thead>
<tr>
<th>Nominal conc. (µg/mL)</th>
<th>Measured conc. (Mean ± SD)</th>
<th>Precision (% RSD)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-day (n=5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>10.1 ± 0.3</td>
<td>3.0</td>
<td>101.0</td>
</tr>
<tr>
<td>20</td>
<td>20.0 ± 0.5</td>
<td>2.4</td>
<td>100.0</td>
</tr>
<tr>
<td>100</td>
<td>107.0 ± 2.1</td>
<td>1.9</td>
<td>107.0</td>
</tr>
<tr>
<td>250</td>
<td>256.9 ± 2.9</td>
<td>1.1</td>
<td>102.8</td>
</tr>
<tr>
<td>Inter-day (n=5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>9.4 ± 0.5</td>
<td>5.3</td>
<td>94.3</td>
</tr>
<tr>
<td>20</td>
<td>19.7 ± 0.6</td>
<td>3.1</td>
<td>98.5</td>
</tr>
<tr>
<td>100</td>
<td>103.9 ± 2.5</td>
<td>2.4</td>
<td>103.9</td>
</tr>
<tr>
<td>250</td>
<td>248.4 ± 11.6</td>
<td>4.7</td>
<td>99.4</td>
</tr>
</tbody>
</table>

**Table 3.** Recovery assay of betaine (n=6)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Initial conc. (µg/mL)</th>
<th>Amount added (µg/mL)</th>
<th>Measured conc. (Mean ± SD)</th>
<th>Recovery (Mean ± RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Betaine</td>
<td>170.05</td>
<td>10</td>
<td>179.9 ± 0.8</td>
<td>98.2 ± 8.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>189.4 ± 2.5</td>
<td>96.5 ± 12.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>272.8 ± 5.0</td>
<td>102.7 ± 4.9</td>
</tr>
</tbody>
</table>
Table 4. Betaine content in the extracts of fourteen Fructus Lycii samples (n=3)

<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
<th>Content (g/g, %)</th>
<th>Betaine (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>Lycium barbarum L.</td>
<td>0.80 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>Lycium chinense Mill.</td>
<td>1.04 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>Lycium chinense Mill.</td>
<td>0.87 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>C4</td>
<td>Lycium chinense Mill.</td>
<td>0.71 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>C5</td>
<td>Lycium chinense Mill.</td>
<td>0.65 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>C6</td>
<td>Lycium chinense Mill.</td>
<td>0.63 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>C7</td>
<td>Lycium chinense Mill.</td>
<td>0.63 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>SK1</td>
<td>Lycium chinense Mill.</td>
<td>0.74 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>SK2</td>
<td>Lycium chinense Mill.</td>
<td>0.54 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>SK3</td>
<td>Lycium chinense Mill.</td>
<td>0.59 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>SK4</td>
<td>Lycium chinense Mill.</td>
<td>0.52 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>SK5</td>
<td>Lycium chinense Mill.</td>
<td>0.79 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>NK6</td>
<td>Lycium chinense Mill.</td>
<td>0.64 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>NK7</td>
<td>Lycium chinense Mill.</td>
<td>0.67 ± 0.01</td>
<td></td>
</tr>
</tbody>
</table>

Figure 6. PCA plot of 14 different Fructus Lycii samples based on HILIC-ELSD chromatographic data.

Based on the chromatographic peaks obtained from fourteen samples by established HPLC method was performed to extract major trends in a data set. The peak area of betaine and unknowns in the chromatograms were used as variables to calculate the PCA scores. The score plots derived from PCA are shown in Figure 6. It was interesting observation that all fourteen samples were classified into three clusters according to their geographic origins. Specifically, although the contents of betaine in the L. barbarum sample from China (C1) were similar to those in the other L. chinense samples, PCA could differentiate between L. barbarum and L. chinense samples. However, meaningfully large data sets may be required to demonstrate these PCA results more clearly.

Conclusion

A convenient HPLC-ELSD method based on the HILIC mechanism was developed and validated for the determination of betaine in Fructus Lycii. The developed method was successfully applied for the quantitative determination of betaine in fourteen Fructus Lycii samples. This method will be useful for quality control of the herbal medicine, Fructus Lycii.

Acknowledgments. This research was supported by the Chung-Ang University Research Scholarship Grant in 2010.

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