Quantitative and Classification Analyses of Lupenone and β-Sitosterol by GC-FID in *Adenophora triphylla* var. *japonica* Hara and *Codonopsis lanceolata*

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Abstract – A simple GC method with a FID detector was developed in order to determine two main compounds (β-sitosterol and lupenone) for Adenophorae Radix. β-Sitosterol and lupenone were analyzed by the gradient thermal ramping method. Nitrogen was used as the carrier gas at 108 kPa. The flow rate of gas was 2.0 mL/min; 2 µL of filtered sample was injected at a split ratio of 1 : 80. This method was fully validated with respect to linearity, precision, accuracy and robustness. Further, this GC-FID method was applied successfully in order to quantify two compounds in an Adenophorae Radix extract. The GC analytical method for classification analysis was performed by repeated analysis of 59 reference samples in order to differentiate between *Adenophora triphylla* var. *japonica* Hara and 14 *Codonopsis lanceolata*. The results indicate that the GC-FID method is suitable and reliable for the quality evaluation of Adenophorae Radix.

Keywords – β-sitosterol, lupenone, Adenophorae Radix, GC-FID

Introduction

Adenophorae Radix refers to the dried roots of *Adenophora triphylla* var. *japonica* Hara or *Adenophora strica* Miq in the Korean Pharmacopoeia (K.P.), and belongs to the Campanulaceae family. It has been used as an anti-inflammatory and anti-tussive agent in oriental medicine. Moreover, it is distributed in many countries, such as Korea, Japan, China, Taiwan and Russia. Traditionally, Adenophorae Radix has been used for discharging phlegm, enhancing immunity and being adopted to an anti-inflammatory agent. Recent studies have discovered that Adenophorae Radix conveys some kinds of bioactive effects, such as anti-cancer, anti-hyperglycemia, anti-obesity, anti-oxidant, asthma relief and immunomodulation.

β-Sitosterol, one of the main triterpenoid components in Adenophorae Radix, has anti-herpes, cytotoxic and immunomodulation activities. Another study proved that β-sitosterol had an insulin sensitizing effect. Lupenone, another major triterpenoid in Adenophorae Radix, has been reported for anti-herpes-1,2,17 anti-oxidant and anti-inflammatory activities. It was also proven that lupenone had a PTP1B inhibitory activity.

The content regulation of Adenophorae Radix in C.P. is not stipulated yet. K.P. also has no stipulation on the main compounds contained in Adenophorae Radix. Hence, the purpose of this study was to establish a reliable gas chromatographic (GC) method in order to quantitatively analyze the major compounds in Adenophorae Radix as well as to suggest that the analytical method developed in this study could be adopted as the official analytical method in K.P. The dried roots of *Codonopsis lanceolata* is misused as Adenophorae Radix in Korean herbal market. Therefore, we also suggest analytical marker compounds in order to distinguish the roots of *A. triphylla* var. *japonica* Hara from those of *C. lanceolata*.

There are not many studies that quantify or identify the components in Adenophorae Radix. One previous study on HPLC-ELSD quantified (6R,7R)-E,E-tetradeca-4,12-
diene-8,10-diyne-1,6,7-triol in order to quality control Adenophora Radix; however, the 0.003% of content stipulation was too low to control the quality of Adenophora Radix. Another previous study attempted to check the purity of compounds isolated from ether and n-butanol fractions of Adenophora Radix with HPLC-ELSD; yet, this study did not suggest the marker compounds and pattern analysis of Adenophora Radix.

Overall, there have been no studies stipulating the main components of Adenophora Radix with GC-FID. Therefore, we suggest a suitable analytical method for quantitative and classification analyses of Adenophora Radix together with the establishment of appropriate marker compounds in order to distinguish between *A. triphylla* var. *japonica* Hara and *C. lanceolata*.

**Experimental**

**Reagents and materials** – The β-sitosterol (1), and lupenone (2) standards were kindly provided by the Adenophora Radix separation team of Korean National Center for Standardization of Herbal Medicines, which were separated from *A. triphylla* var. *japonica* Hara. The internal standards (I.S.), naringenin (3), was purchased from Sigma-Aldrich (St. Louis, MO, USA). The compound structures are shown in Fig. 1. The purities of these compounds were determined to > 99% by normalizing the peak areas detected by GC analyses. Methanol was purchased from Merck K GaA (Darmstadt, Germany). All other chemicals used were analytical grade. Distilled water was prepared using the Milli-Q purification system (Millipore, Bedford, MA, USA). This study adopted the root samples of forty-two *A. triphylla* var. *japonica* Hara (A01-A43) and fourteen *C. lanceolata* (C01-C14). Ten *A. triphylla* var. *japonica* Hara samples (A01-A07, A29, A30, A42) were originated from Korea and thirty-two *A. triphylla* var. *japonica* Hara samples (A08-A27, A28, A31-A40, A42) were originated from China. All *C. lanceolata* samples were originated from Korea. All of these samples were provided by Prof. Je Hyun Lee (College of Oriental Medicine, Dongguk University, Gyeongju, Korea).

**Sample preparation** – Each standard stock solution was prepared by adding 1.0 mg of β-sitosterol and lupenone to 1.0 mL of methanol containing 150 ppm of naringenin, respectively. A powdered sample of Adenophora Radix (5.0 g) was mixed with 50 mL of 100% methanol containing 50 ppm I.S. (naringenin) in a vial. Each mixture was refluxed for 30 min. The solution was weighed again, and the loss in weight was made up with methanol. The solution was filtered through a 0.45-µm membrane filter (Whatman). A 2 µL aliquot of the test solution was injected into the GC system.

**GC-FID conditions** – The GC equipment were Shimadzu GC-17A with FID and Shimadzu AOC-20i autosampler (Shimadzu, Kyoto, Japan). Agilent J&W DB-1 (30 m × 0.32 mm) was used as column to separate marker compounds (Agilent, California, USA). The column temperature was started at 200°C, and it was increased to 250°C for 1 minute. At 250°C, the temperature was maintained for 1 more minute. And the temperature was increased again to 305°C for 1 additional minute. At last, the temperature was maintained at 305°C for 27 more minutes. Chromatograms were acquired by Shimadzu Class-GC10 software (Shimadzu, Kyoto, Japan). Column flow was 2 mL/min and the head pressure was 108 kPa. Open split injection was conducted with split ratio of 1 : 80 and injection volume was 20 µL. Temperatures of injector and detector were 300°C and 320°C, respectively.

**Analytical method validation** – The developed GC method was validated according to Korea Food and Drug Administration (KFDA) guidelines for the following parameters: linearity, LOD, LOQ, accuracy, precision, and robustness. In linearity, a standard stock solution was prepared and diluted to an appropriate concentration to construct the calibration curves. The calibration curve was composed of six concentrations of 7.815, 15.625, 31.25, 62.5, 125.0 and 250.0 μg/mL. The calibration curve was constructed by plotting the peak area ratio (β-sitosterol / I.S., lupenone / I.S.) with six different concentration values.

![Fig. 1. Structures of standards and an internal standard.](image-url)