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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.[1] It has been used as an anti-inflammatory and anti-tussive agent in oriental medicine.[2] Moreover, it is distributed in many countries, such as Korea, Japan, China, Taiwan and Russia.[3] Traditionally, Adenophorae Radix has been used for discharging phlegm, enhancing immunity and being adopted to an anti-inflammatory agent.[4] Recent studies have discovered that Adenophorae Radix conveys some kinds of bioactive effects, such as anti-cancer,[5] anti-hyperglycemia,[6] anti-obesity,[7] anti-oxidant,[8] asthma relief[9] and immunomodulation.[10]

β-Sitosterol, one of the main triterpenoid components in Adenophorae Radix,[11] has anti-herpes,[12] anti-inflammatory,[13] cytotoxic[14] and immunomodulation[15] activities. Another study proved that β-sitosterol had an insulin sensitizing effect.[16] Lupenone, another major triterpenoid in Adenophorae Radix, has been reported for anti-herpes-1,2,[17] anti-oxidant and anti-inflammatory[18] activities. It was also proven that lupenone had a PTP1B inhibitory activity.[19]

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.[20] 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 (6*R*,7*R*)-*E*,*E*-tetradeca-4,12-

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diene-8,10-diyne-1,6,7-triol in order to quality control Adenophorae Radix; however, the 0.003% of content stipulation was too low to control the quality of Adenophorae Radix.[21] Another previous study attempted to check the purity of compounds isolated from ether and *n*-butanol fractions of Adenophorae Radix with HPLC-ELSD; yet, this study did not suggest the marker compounds and pattern analysis of Adenophorae Radix.[11]

Overall, there have been no studies stipulating the main components of Adenophorae Radix with GC-FID. Therefore, we suggest a suitable analytical method for quantitative and classification analyses of Adenophorae 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 Adenophorae 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.), nargingenin (**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 Adenophorae 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.

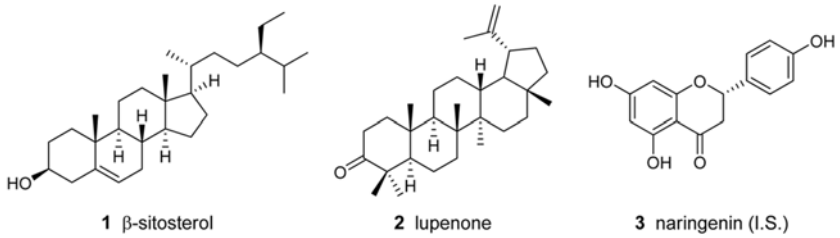

Fig. 1. Structures of standards and an internal standard.

The lowest concentration of working solution was diluted with appropriate concentrations, and the limits of detection (LOD) and quantification (LOQ) under the chromatographic conditions were separately determined at signal-to-noise ratios (S/N) of about 3 and 10, respectively. Precision and accuracy were determined by spiking three concentration levels of the β-sitosterol and lupenone standards, which were mixed with an Adenophorae Radix (A01) sample for subsequent extraction and filtration. Three concentrations of 1.0, 120.0, and 160.0 μg/mL for β-sitosterol, and 1.0, 90.0, and 170.0 μg/mL for lupenone were evaluated. All analytical experiments were performed in triplicate for each control level. Data from the standard solution and the extracted sample were compared. Precision and accuracy were determined by multiple analyses (n = 5) of quality control samples prepared at low, medium and high concentrations spanning the calibration range.

The robustness of the method was studied by introducing changes in the column pressure (*i.e.*, 98, 108, and 118 kPa), initial column oven temperature (*i.e.,* 190, 200, and 210 °C) and split ratio (*i.e.,*1 : 57, 1 : 80, and 1 : 120).

Classification analysis – A classification analysis was conducted to evaluate the phytochemical equivalency among the fifty-six samples (forty-two *A. triphylla* var. *japonica* Hara (A01-A42), and fourteen *C. lanceolata* (C01-C14) samples). We used two major marker compound peaks of β-sitosterol and lupenone, for the classification analysis using IBM SPSS Statistics Version 19 software (SPSS, Inc., Chicago, IL, USA).

Results and Discussion

Optimization of chromatographic conditions – Due to the high molecular weight and boiling point of maker compounds, the temperatures of the column oven and injector detector was set up high, close to the limit of the temperature. Then, GC conditions were selected in order to obtain a good resolution on the chromatograms within a short retention time. We investigated three different column pressures, 98, 108 and 118 kPa, in order to optimize the GC-FID chromatographic conditions. All of these three pressures presented good results; however, 108 kPa showed a better resolution and theoretical plate of each peak with regard to the robustness. In the case of the initial column oven temperature, we compared three different levels, 190, 200 and 210 °C, for optimization. As a result, three different levels of temperatures conveyed good results for maker compounds; however, an oven temperature of 200 °C showed better results in the resolution and theoretical plate regarding the robustness.

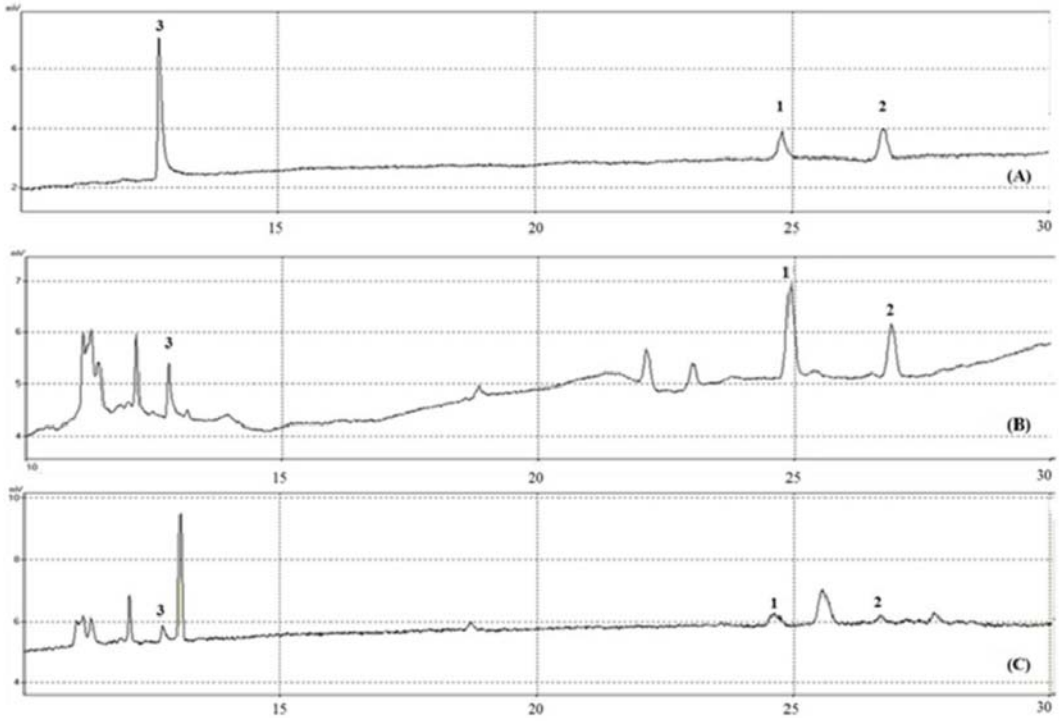

Fig. 2. GC-FID chromatograms of standard mixture (**A**), the sample of *A. triphylla* var. *japonica* Hara (**B**) and the sample of *C. lanceolata* (**C**). **1**: β-sitosterol, **2**: lupenone, **3**: naringenin.

Among three different values of the split ratio, 1 : 57, 1 : 80 and 1 : 120, 1 : 80 showed a better resolution and theoretical plate than those of other values. Ultimately, 108 kPa was selected for the column pressure and 200 ºC was selected for the initial column oven temperature; further, 1 : 80 of the split ratio was chosen for the split ratio of the GC-FID system. Typical chromatograms of the sample and standard mixtures are shown in Fig. 2. Overall, the target compounds, including an internal standard, were completely separated within 30 minutes. Naringenin was selected as the I.S. (Fig. 1).

Optimization of the sample preparation conditions – Four extracting solvents, including methylene chloride, methanol, ethanol and acetone, were compared in the sample assays after extraction by sonication for 30 min at room temperature. When the samples were extracted with 100% methanol, the lupenone peak area was higher than the other solvent samples. In the case of *β*-sitosterol peak, 92% ethanol showed the highest area, but the gap between 99.8% methanol and 92% ethanol was not so big; when extracted with 92% ethanol, the lupenone peak area was lowest. Therefore, we employed 99.8% methanol as the extracting solvent throughout this study (Fig. 3). Ultrasonication and reflux using 99.8% methanol extraction solvent were compared as the extraction methods in the sample assays. Extraction by reflux showed better results than extraction by sonication (Fig. 4). In order to determine the time needed to complete the extraction, samples were extracted for 10, 20, 30 and 60 min. When the extraction time was set to 30 min, the sample assay results were similar to those of others. Therefore, all of the compounds were sufficiently extracted when the extraction time was 30 min (Fig. 5). Consequently, extracting by reflux for 30 minutes with 99.8% methanol was chosen for the sample extracting condition.

Linearity, calibration range, and limits of detection and quantification – The calibration curves portrayed good linearity ($r^2 > 0.99$) within the test ranges, as shown in Table 1. The stock solution containing the reference compound was diluted with methanol in order to give a series of appropriate concentrations; further, the aliquots of the diluted solutions were injected. The LOD (S/N = 3) and LOQ (S/N = 10) values for *β*-sitosterol and lupenone

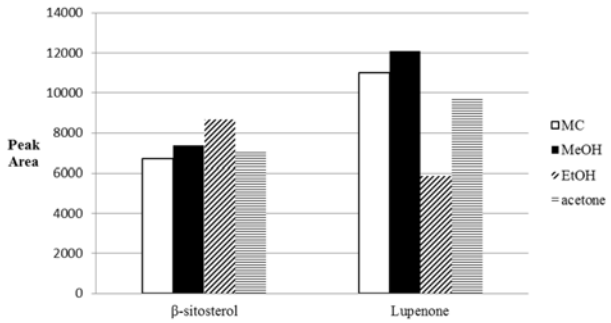

Fig. 3. Comparison of the extraction solvents for extraction efficiencies of marker compounds (n = 3, w/w%).

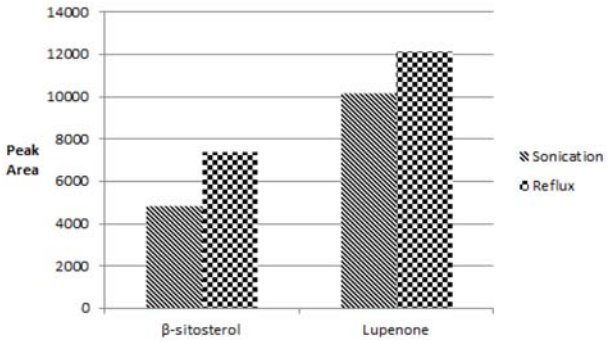

Fig. 4. Comparison of the extraction methods (sonication and reflux) for extraction efficiencies of marker compounds (n = 3, w/w%).

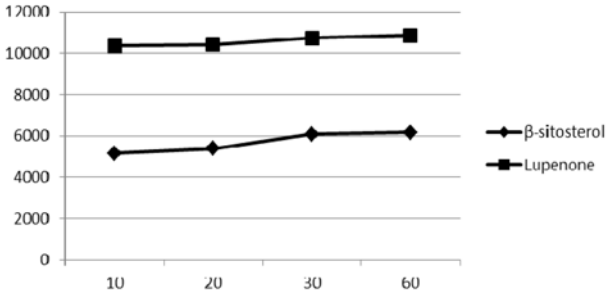

Fig. 5. Comparison of the extraction time for extraction efficiencies of marker compounds (n = 3, w/w%).

are presented in Table 1. The values for both LOD and LOQ for these two standards were sufficiently low to detect traces of these compounds in either a crude extract or its preparation.

Precision and accuracy – The precision and accuracy

Table 1. Linearity, linear ranges, LOD and LOQ

Analytes	Regression equation	Linearity range (μg/mL)	Correlation coefficient (r^2)	LOD (μg/mL)	LOQ (μg/mL)
β-sitosterol	0.0161x + 0.1501	7.815 – 250.0	0.9989	0.52	1.74
lupenone	0.0210x + 0.2156	7.815 – 250.0	0.9984	0.38	1.25

Table 2. Precision and accuracy of analytical results

Analytes	Spiked conc. (μg/mL)	Intra-day (n = 5)				Inter-day (n = 3)			
		Observed (μg/mL)	SD	Accuracy (%)	Precision (%)	Observed (μg/mL)	SD	Accuracy (%)	Precision (%)
β-sitosterol	1.0	1.03	0.03	103.72	3.82	1.03	0.01	103.5	0.99
	120.0	118.23	2.04	98.53	1.72	119.09	0.48	99.25	0.40
	160.0	165.94	4.52	103.72	2.72	169.82	1.45	104.55	0.85
lupenone	1.0	1.01	0.46	100.96	4.52	1.03	0.05	103.96	4.78
	90.0	89.95	3.67	99.94	4.09	87.98	0.59	97.77	0.67
	170.0	171.36	7.77	100.81	4.54	169.13	0.38	99.49	0.22

Table 3. Effects of initial column oven temperature on system suitability (n=3)

Conditions	Analytes			
	β-sitosterol (**1**)		lupenone (**2**)	
	Mean	SD	Mean	SD
Theoretical plate (N)				
190 ºC	88954.9	43316.3	101144.9	7432.8
200 ºC	52590.6	6938.7	78610.4	10678.2
210 ºC	48402.6	4666.1	67352.1	7078.5
Capacity factor (k')				
190 ºC	10.393	0.0681	21.062	0.1100
200 ºC	10.052	0.0292	20.582	0.0777
210 ºC	9.636	0.0480	16.314	6.4885
Separation factor (γ)				
190 ºC	1.936	0.0027	1.079	0.00073
200 ºC	1.953	0.0019	1.080	0.00038
210 ºC	1.977	0.0004	1.081	0.00053
Resolution (Rs)				
190 ºC	48.903	4.5728	6.307	0.6340
200 ºC	41.954	2.1930	5.413	0.3570
210 ºC	40.122	1.9797	5.597	0.2420

were assessed by extracting a known amount of compounds from Adenophorae Radix powder samples. The known amounts of each standard compound at three levels were mixed with the sample powder and then extracted with 99.8% methanol. The average recovery was calculated by the formula: R (%) = [(amount from the sample spiked standard – amount from the sample)/amount from the spiked standard] × 100. Intra-assay precision and accuracy were determined from the variability obtained from multiple analyses (n = 5) of the quality control samples analyzed within the same analytical run. The quality control samples had an intra-assay precision ≤ 3.82% and an accuracy of 98.53 - 103.72%. Inter-assay precision and accuracy were evaluated from the differences in multiple analyses (n = 3) of the quality control samples analyzed for 3 consecutive days. The quality control samples had an inter-assay precision of ≤ 4.54% and an accuracy of 99.94 - 100.81%. Thus, the method was highly reproducible. The precision and accuracy data are presented in Table 2.

Robustness – The robustness was determined in order to evaluate the reliability of the established HPLC method. The experimental conditions, such as initial column oven temperature, split ratio and column press, were purposely altered; further, the theoretical plate (*N*), retention factor (*k*), separation factor (α) and resolution (*Rs*) were evaluated. The four analytical factors did not differ greatly when the conditions were changed (Tables 3 - 5); therefore, the experimental conditions were sufficiently robust.

Table 4. Effects of split ratio on system suitability (n = 3)

Conditions	Analytes			
	β-sitosterol (**1**)		lupenone (**2**)	
	Mean	SD	Mean	SD
Theoretical plate (N)				
1 : 57	59935.5	11207.4	99768.0	24752.8
1 : 80	52590.6	6938.7	78610.4	10678.2
1 : 120	41604.3	5436.0	62762.4	4978.0
Capacity factor (k')				
1 : 57	10.093	0.1052	20.664	0.1969
1 : 80	10.052	0.0292	20.582	0.0777
1 : 120	9.905	0.0087	20.317	0.0120
Separation factor (γ)				
1 : 57	1.953	0.0008	1.080	0.00049
1 : 80	1.953	0.0019	1.080	0.00038
1 : 120	1.955	0.0006	1.080	0.00017
Resolution (Rs)				
1 : 57	46.205	5.2056	6.377	0.6169
1 : 80	41.954	2.1930	5.413	0.3570
1 : 120	37.510	1.4922	4.998	0.2917

Table 5. Effects of column press on system suitability (n=3)

Conditions	Analytes			
	β-sitosterol (**1**)		lupenone (**2**)	
	Mean	SD	Mean	SD
Theoretical plate (N)				
98 kPa	54795.0	4820.3	81330.1	12839.2
108 kPa	52590.6	6938.7	78610.4	10678.2
118 kPa	34370.2	9575.4	58468.2	11508.2
Capacity factor (k')				
98 kPa	9.609	0.0464	20.070	0.0938
108 kPa	10.052	0.0292	20.582	0.0777
118 kPa	8.225	3.7989	16.765	7.3086
Separation factor (γ)				
98 kPa	1.986	0.0007	1.082	0.00019
108 kPa	1.953	0.0019	1.080	0.00038
118 kPa	1.926	0.0012	1.077	0.00070
Resolution (Rs)				
98 kPa	43.739	2.0747	5.779	0.3714
108 kPa	41.954	2.1930	5.413	0.3570
118 kPa	34.488	3.8360	4.604	0.2284

Sample analysis – The GC method was applied in order to analyze fifty-six samples corresponding to the roots of forty-two *A. triphylla* var. *japonica* Hara (A01-A42) and fourteen *C. lanceolata* (C01-C14) samples. The average contents (wt%) of *β*-sitosterol and lupenone are presented in Table 6. The average content of *β*-sitosterol (0.298%) in the *A. triphylla* var. *japonica* Hara samples was higher than that of *C. lanceolata* (0.178%). Similarly, the average content of lupenone (0.214%) in the *A. triphylla* var. *japonica* Hara samples was higher than that

Table 6. Average contents (wt%) of β-sitosterol and lupenone in Adenophorae Radix

	Mean ± S.D. (wt %)	
	β-sitosterol	lupenone
A.triphylla var. *japonica* Hara (n = 42)	0.2980 ± 0.1063	0.2138 ± 0.0914
C. lanceolata (n = 14)	0.1778 ± 0.0774	0.0455 ± 0.0178

Each value represents the mean ± S.D. (n = 3).

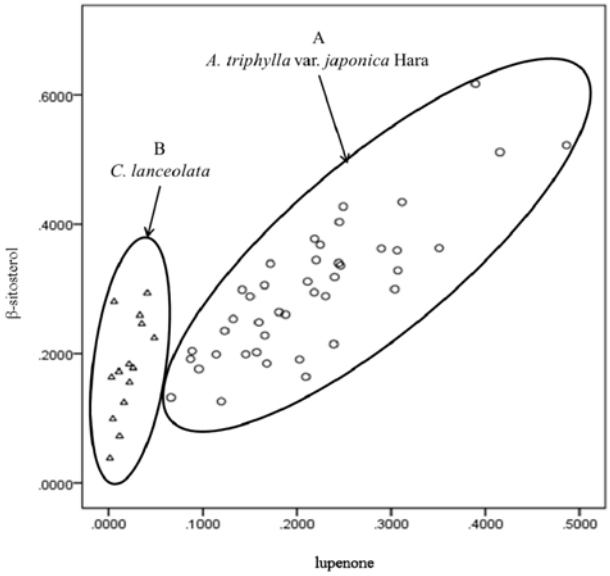

Fig. 6. PAM (Partitioning Around Medoids) of 56 authentic specimens. Group A (○): *A. triphylla* var. *japonica* Hara, Group B (△): *C. lanceolata*.

of *C. lanceolata* (0.046%).

This quantitative analysis result of β-sitosterol and lupenone will be reflected in the contents regulation of these two compounds for Adenophorae Radix in the next revision of the K.P.

Classification analysis – To evaluate the phytochemical equivalency among the roots of forty-two *A. triphylla* var. *japonica* Hara (A01-A42) and fourteen *C. lanceolata* (C01-C14) samples, the classification analysis was conducted using β-sitosterol and lupenone contents. Consequently, considering the concatenation of the two compounds, which was significantly different between the two groups of *A. triphylla* var. *japonica* Hara and *C. lanceolata,* all of the samples were divided into two groups, *A. triphylla* var. *japonica* Hara (A) and *C. lanceolata* (B), by the pattern analysis (Fig. 6).

Concluding remarks – We have provided a fully validated GC method for the quality control of Adenophorae Radix and classification analysis, which resulted in distinguishing between *A. triphylla* var. *japonica* Hara and *C. lanceolata*. The analytical conditions using a simple gradient thermal ramping GC system with a FID detector allowed for a concise experiment as well as enhanced the analytical conditions. Our results suggest that β-sitosterol and lupenone are marker compounds for quality evaluations of Adenophorae Radix. Currently, C.P. and J.P. do not stipulate marker compounds of Adenophorae Radix. From this study, we suggest that β-sitosterol and lupenone are reasonable and adoptable marker compounds for the quality evaluation of Adenophorae Radix.

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References

(1) Kang, M.; Ha, I. J.; Chun, J.; Kang, S. S.; Kim, Y. S. *Phytochem. Anal*. **2013**, *24*, 148-154.
(2) Gorovoi, P. G.; Ponomarchuk, G. I.; Stringna, L. I. *Phytochemistry* **1971**, *10*, 2419-2423.
(3) Hall, T.; Harvey, Y. *Curtis's Bot Mag*. **1999**, *16*, 90-97.
(4) Song, L. R.; Hong, X.; Ding, X. L. *Mordern Chinese Materia Medica Lexicon*; People Health Publishing House Press: China, 2001; p 1455.
(5) Lee, I. S.; Yang, E. J.; Kim, H. S.; Chung, S. K.; Furukawa, F.; Nishikawa, A. *Anticancer Res*. **2000**, *20*, 3227-3231.
(6) Kim, S. H.; Kwon, C. S.; Lee, J. S.; Son, K. H.; Lim, J. K.; Kim, J. S. *J. Food Sci. Nutr*. **2002**, *7*, 62-66.
(7) Lee, S. E.; Lee, E. H.; Lee, T. J.; Kim, S. W.; Kim, B. H. *Biosci. Biotechnol. Biochem*. **2013**, *77*, 544-550.
(8) Kim, J. H.; Hong, J. Y.; Shin, S. R.; Yoon, K. Y. *Int. J. Food Sci. Nutr*. **2009**, *60*, 150-161.
(9) Roh, S. S.; Kim, S. H.; Lee, Y. C.; Seo, Y. B. *Mediat. Inflamm.* **2008**, *2008*, 1-11.
(10) Shin, D. H.; Seo, Y. B. *Kor. J. Herbol*. **2000**, *15*, 31-43.
(11) Yao, S.; Liu, R.; Huang, X.; Kong, L. *J. Chromatogr. A*. **2007**, *1139*, 254-262.
(12) Shamsabadipour, S.; Ghanadian, M.; Saeedi, H.; Rahimnejad, M. R.; Mohammadi-Kamalabadi, M.; Ayatollahi, S. M.; Salimzadeh, L. *Iran. J. Pharm. Res*. **2013**, *12*, 759-767.
(13) Mahajan, S. G.; Mehta A. A. *Eur. J. Pharmacol*. **2011**, *650*, 458-464.
(14) Rathee, P.; Rathee, D.; Rathee, D.; Rathee, S. *Asian Pac. J. Trop. Med.* **2012**, 5, 225-230.
(15) Desai, F.; Ramanathan, M.; Fink, C. S.; Wilding, G. E.; Weinstock-Guttman, B.; Awad, A. B. *Int. Immunopharmacol.* **2009**, *9*, 153-157.
(16) Radika, M. K.; Viswanathan, P.; Anuradha, C. V. *Nitric Oxide* **2013**, *32*, 43-53.
(17) Madureira, A. M.; Ascenso, J. R.; Valdeira, L.; Duarte, A.; Frade, J. P.; Freitas, G.; Ferreira, M. J. *Nat. Prod. Res*. **2003**, *17*, 375-380.
(18) Jin, S. E.; Son, Y. K.; Min, B. S.; Jung, H. A.; Choi, J. S. *Arch.*

Pharm. Res. **2012**, *35*, 823-837.
(19) Na, M. K.; Kim, B. Y.; Osada, H.; Ahn, J. S. *J. Enzyme Inhib. Med. Chem.* **2009**, *24*, 1056-1059.
(20) Kim, J. Y.; Lee, Y. J. *Kor. J. Herbol.* **2007**, *22*, 121-126.
(21) Park, Y. K.; Yoo, H. H.; Baek, S. H.; Lee, S. H.; Kim, C. M.; Lee, K. S.; Park, M. K.; Park, J. H. *Kor. J. Pharmacogn*. **2003**, *34*, 10-13.

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