Original Article
Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
DOI: https://doi.org/10.5327/fst.00018

Establishment of simultaneous analysis validation for *Perilla frutescens* var. *acuta* extract

So-Heon JEON[1]‡, Hyo Seung CHOI[2]‡, Soon-Ho YIM[1]*

Abstract

In this study, the UV spectra maximum absorption wavelengths and molecular weights of major compounds 1–3 were identified in *Perilla frutescens* var. *acuta* extract using high-performance liquid chromatography (HPLC)-DAD and HPLC-MS/MS. Luteolin-7-*O*-diglucuronide (1), scutellarin (2), and rosmarinic acid (3) were identified, and simultaneous analysis validation was performed for quantification using HPLC-DAD. All linearities showed R^2 values of 0.9993 or higher, and the limits of detection and quantification were measured in the ranges of 4.4802–5.4133 and 13.5764–16.4038 μg/mL, respectively. The relative standard deviation (RSD) values for accuracy ranged from 0.09 to 0.89%, and the recovery rates were in the range of 99.36–101.96%. The precision RSD values within the laboratory were in the range of 0.05–1.04%, and the RSD values for repeatability evaluation were below the guideline criteria of 2%. The content change of the three marker compounds within 24 h was 0.36–0.69%, confirming the chemical stability of the extract within 24 h, which was below the guideline criteria of 2.0% established by the Ministry of Food and Drug Safety guidelines. The content analysis of the extract according to ethanol concentration showed that luteolin-7-*O*-diglucuronide had the highest content of 302.22 μg/mL in the 0% ethanol extract, while scutellarin and rosmarinic acid showed the highest contents of 103.61 and 118.77 μg/mL, respectively, in the 60% ethanol extract.

Keywords: *Perilla frutescens* var. *acuta*; major compounds; validation.

Practical Application: basic data for the development of natural antioxidant products from *Perilla frutescens* var. *acuta*.

1 INTRODUCTION

As civilization advances, people's awareness on maintaining good health is increasing, leading to a mounting interest in various foods and natural medicines such as ginseng (Yoo et al., 2010). Therefore, there is a growing need for standardized manufacturing methods and quality specifications for various natural medicines and healthy foods to ensure quality control (KFDA, 2007). In particular, standardization and specification of raw materials are important in the development and production of healthy foods using medicinal herbs, and quality control methods using indicator components' content to scientifically prove the functionality and safety of raw materials (Kim et al., 2012). Such quality control methods are the most common way to minimize differences in indicator components present in raw materials to reach in final products, thus maintaining the desired components during the manufacturing process (Jeon et al., 2011). Indicator components are selected for standardization of raw materials, taking into consideration the availability, specificity, representativeness, safety, ease of analysis technology, and reliability (Ahn et al., 2017). The method of verifying indicator components involves the use of accredited or precision analysis methods to ensure reliable results, requiring validation of the analysis method (KFDA, 2004).

Perilla frutescens var. *acuta* is an annual or biennial herbaceous plant primarily cultivated in Asian countries. Its leaves are commonly referred to as "purple leaves" because both sides of the leaves have a purple coloration. It has been used in traditional medicine for asthma, cough, pharyngitis, indigestion, insomnia, and diabetes (Kim et al., 2007). The major components of *P. frutescens* var. *acuta* include rosmarinic acid, luteolin, apigenin, and caffeic acid, which are known to exhibit antioxidant, anti-inflammatory, antimicrobial, and anti-allergic effects (Bae et al., 2017). Although studies on the physiological activities and major components of *P. frutescens* var. *acuta* are well reported, there is a lack of validation studies on the reliability of analytical methods for its major components. In this study, we identified and characterized indicator components of *P. frutescens* var. *acuta* extract, which is known for its various functions, using high-performance liquid chromatography (HPLC)-DAD and LC-MS/MS. We developed an analytical method to simultaneously analyze the indicator components using HPLC-DAD and determine their content. To validate the method, we performed

Received May 11, 2022.
Accepted Aug. 3, 2023.
[1]*Dongshin University, Department of Pharmaceutical and Cosmetics, Jeonnam, Korea.*
[2]*Dongshin University, Department of Digital Contents, Jeonnam, Korea.*
*Corresponding author: virshyim@gmail.com
‡These two authors contributed equally to this study.
Funding: National Research Foundation of Korea, grant funded by the Korean government (No. 2022R1F1A1076398) and "Leaders in Industry-university Cooperation 3.0" Project.

validation tests for specificity, linearity, detection limit, quantification limit, accuracy, precision, and stability.

2 MATERIALS AND METHODS

2.1 Preparation of materials and sample extracts

In February 2021, 600 g of *P. frutescens* var. *acuta* used in this study was purchased from Nongup Hwasa Corporation, Duson-Aeyakcho (Yeongcheon, Gyeongsangbuk-do, Korea). The purchased plant was separated into leaves and stems, and only the leaves were used for the experiments. The leaves were ground using a grinder (NFM-3561SN, NUC Co., Daegu, Korea). Notably, 10 g of the ground sample was mixed with 100 mL of distilled water, 20% ethanol, 40% ethanol, 60% ethanol, 80% ethanol, and 100% ethanol, respectively, and reflux-extracted for 1 h at 100°C. After extraction, the solution was filtered through a 185-mm Whatman filter paper (GE Healthcare Life Sciences, California, USA), and the filtrate was concentrated using a rotary evaporator (EYELA, N-1110, China) and then dried using a freeze-dryer (Hyper COOL HC3110, Hanil Scientific Inc., Gimpo, Korea). The samples were stored at 4°C until used for the experiments.

2.2 Preparation of samples and standards for analysis

The analysis samples were prepared by filtering the perilla extract through a syringe filter (0.45 μm, Hyundai Micro Co., Seoul, Korea). Standards for compounds **1–3** were dissolved in 100% ethanol at a concentration of 800 μg/mL and then diluted to concentrations of 25, 50, 100, 200, and 400 μg/mL through filtration with a syringe filter (0.45 μm, Hyundai Micro Co., Seoul, Korea) to prepare standard solutions of the marker compounds.

2.3 HPLC-DAD and HPLC-MS/MS analysis conditions for the identification of major components

To identify and establish an analysis method for the indicator components present in the *P. frutescens* var. *acuta* extract, we used an HPLC Agilent 1100 series HPLC (Agilent Technologies, California, USA) equipped with an autosampler and a DAD detector. The column used was SHISEIDO (UG 120, 4.6 × 250 mm, 5 μm), and the mobile phase consisted of 0.1% formic acid in third distilled water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The analysis time was set to be 0–25% (0–25 min), 25–40% (25–50 min), and 40–50% (50–60 min) for solvent B. A sample injection of 10 μL was performed, and the analysis was carried out at a flow rate of 0.5 mL/min and a detection wavelength of 280 nm.

For mass analysis of the major compounds **1–3** in the *P. frutescens* var. *acuta* extract, a triple quadrupole mass spectrometer (LCMS-8050, Shimadzu, Kyoto, Japan) coupled with an LC-30A liquid chromatography system (Shimadzu, Kyoto, Japan) was used. The same mobile phase and separation conditions used in the HPLC profiling analysis were employed. The samples were ionized using an electrospray ionization (ESI) source and analyzed under a collision-induced dissociation gas (argon) pressure of 270 kPa, with nebulizing gas, drying gas, and heating gas flow rates set at 3, 10, and 10 L/min, respectively. Other analysis conditions included setting the interface, desolvation line, and heat block temperatures to 300, 250, and 350°C, respectively. Mass analysis was performed in scan mode in the range of 100–1,200 *m/z*, and MS/MS analysis was performed at the confirmed molecular weight within the same range.

2.4 Validation of simultaneous analysis method for major components

In this study, the established simultaneous analysis method was validated for its specificity, linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, precision, and stability according to the pharmaceutical validation guidelines specified by the regulatory agency (KFDA, 2015).

2.4.1 Specificity

A standard mixture was prepared by mixing the sample and each reference compound, and the obtained chromatogram was visually evaluated. The UV spectrum maximum absorption wavelength pattern of the standard compounds was also evaluated by comparing it with the literature.

2.4.2 Linearity

The standard calibration curve and regression equation were evaluated using the results obtained from the analysis of indicator components diluted to concentrations of 25, 50, 100, 200, and 400 μg/mL, followed by three analyses.

2.4.3 Limit of detection and limit of quantitation

Using the method based on the average slope and standard deviation of the y-intercept obtained through linearity verification, the limit of detection (LOD) and limit of quantitation (LOQ) were calculated by substituting into the Equations 1 and 2:

$$\text{Limit of detection (LOD)} = 3.3 \times (\text{SD/slope of calibration curve}) \quad (1)$$

$$\text{Limit of quantitation (LOQ)} = 10 \times (\text{SD/slope of calibration curve}) \quad (2)$$

SD: standard deviation of the response.

2.4.4 Accuracy and precision

Accuracy refers to the degree of proximity between the measured and known true values and was evaluated by measuring the recovery rate after measuring the concentrations of 25, 100, and 400 μg/mL three times each. Precision was determined by the relative standard deviation (RSD) of each measurement value obtained when measuring a single sample under predetermined conditions. The RSD of the results obtained from three repeated experiments with a mixed standard solution of three concentrations (i.e., 25, 100, and 400 μg/mL) was calculated to evaluate precision.

2.4.5 Stability

To confirm the chemical stability of the indicator components over the storage period, the content was measured after 24 h of storage at room temperature.

2.5 Analysis of Perilla frutescens *var.* acuta *extracts content*

Using the same analytical method, the average and standard deviation of the peak area values of the indicator components were determined by analyzing the extract of the reference sample at different ethanol concentrations. Then, the content was calculated by substituting the obtained regression equation from the linearity evaluation.

3 RESULTS AND DISCUSSION

3.1 Identification of compounds 1–3 from Perilla frutescens *var.* acuta *extracts*

Using the 60% ethanol extract with the best separation at different concentrations of reference samples, the molecular weights of compounds **1–3** were determined under LC-MS/MS ESI spectrum analysis conditions (Figure 1), and the maximum absorption wavelengths of compounds **1–3** were determined under HPLC-DAD analysis conditions for their UV spectra (Figure 2).

In the MS spectra of compound 1, the $[M\text{-}H]^-$ form at 637.1 *m/z* in the negative mode and the $[M+H]^+$ form at 639.1 *m/z* in the positive mode were observed, confirming that the molecular weight of the compound is 638. The MS/MS spectra generated from 637.1 *m/z* in the negative mode showed characteristic fragment ions at 285.1 and 351.1 *m/z* (Figure 1B). The UV spectrum showed maximum absorption wavelengths of 254, 267, and 347 nm (Figure 2B). Comparing these MS spectra and UV spectrum patterns with those in previous literature, the compound was identified as luteolin-7-*O*-diglucuronide (Cho et al., 2020).

In the MS spectra of compound 2, the $[M\text{-}H]^-$ form at 461.1 *m/z* in the negative mode and the $[M+H]^+$ form at 463.1 *m/z* in the positive mode were observed, confirming that the molecular weight of the compound is 462. The MS/MS spectra generated from 461.1 *m/z* in the negative mode showed a characteristic fragment ion at 285.1 *m/z* (Figure 1B). The UV spectrum showed maximum absorption wavelengths of 283 and 335 nm (Figure 2B). Comparing these MS spectra and UV spectrum patterns with those in previous literature, the compound was identified as scutellarin (Cui et al., 2015; Tang et al., 2014; Wong et al., 2022).

In the MS spectra of compound 3, the $[M\text{-}H]^-$ form at 359.1 *m/z* in the negative mode and the $[M+H]^+$ form at 361.1 *m/z* in the positive mode were observed, confirming that the molecular weight of the compound is 360. The MS/MS spectra generated from 359.1 *m/z* in the negative mode showed characteristic fragment ions at 161.1, 179.2, and 197.1 *m/z* (Figure 1B). The UV spectrum showed a maximum absorption wavelength of 332 nm (Figure 2B). Comparing these MS spectra and UV spectrum patterns with those in previous literature, the compound was identified as rosmarinic acid (Jeong et al., 2018; Jun et al., 2014; Wang et al., 2021).

3.2 Validation of simultaneous analysis method for major components

3.2.1 Specificity

When analyzing a mixture of standards for luteolin-7-*O*-diglucuronide **(1)**, scutellarin **(2)**, and rosmarinic acid **(3)** using the same method as the 60% ethanol extract from the tea leaves, the identical retention time was observed. Additionally, the UV spectrum maximum absorption patterns of the marker peaks in the 60% ethanol extract were compared with those in the literature, and they were found to match (Table 1).

Linearity, limit of detection, and limit of quantification

Values were measured by analyzing luteolin-7-*O*-diglucuronide, scutellarin, and rosmarinic acid diluted stepwise at concentrations of 25, 50, 100, 200, and 400 μg/mL in triplicate by HPLC-DAD and were used to construct calibration curves. The correlation coefficients (R^2) for luteolin-7-*O*-diglucuronide, scutellarin, and rosmarinic acid were all above 0.9993, indicating high linearity. The limits of detection (LOD) were 4.4802, 5.4133, and 4.6262 μg/mL, respectively, and the limits of quantification (LOQ) were 13.5764, 16.4038, and 14.0187 μg/mL, respectively (Table 2).

Accuracy

To verify accuracy, three concentration ranges (i.e., 25, 100, and 400 μg/mL) were measured and analyzed three times. The RSDs for each indicator component ranged from 0.09 to 0.89%, which was below the guideline standard of 2.0% set by the regulatory agency. The recovery rates were measured in the range of 99.36–101.96% (Table 3).

Precision

For intermediate precision, three concentrations of 25, 100, and 400 μg/mL of the three major compounds were analyzed in triplicate. The RSDs for intermediate precision ranged from 0.05 to 1.04%, which was below the guideline value of 2.0% set by the regulatory authority. In addition, repeatability was measured by analyzing the major compounds at a concentration of 100 μg/mL six times. The RSDs for repeatability ranged from 0.49 to 1.92%, which were also below the guideline value of 2% (Table 4).

Stability

To confirm the chemical stability of the three major compounds in the sample, the final concentration of 400 μg/mL was stored at room temperature for 0–24 h and the changes in the content were measured (Table 5). The results indicate that the content increased as time passed, and the change rate before and after 24 h was 0.36–0.69%, which was within the standard of 2% set by the Ministry of Food and Drug Safety guidelines. These results confirm the chemical stability of the three major compounds for 24 h.

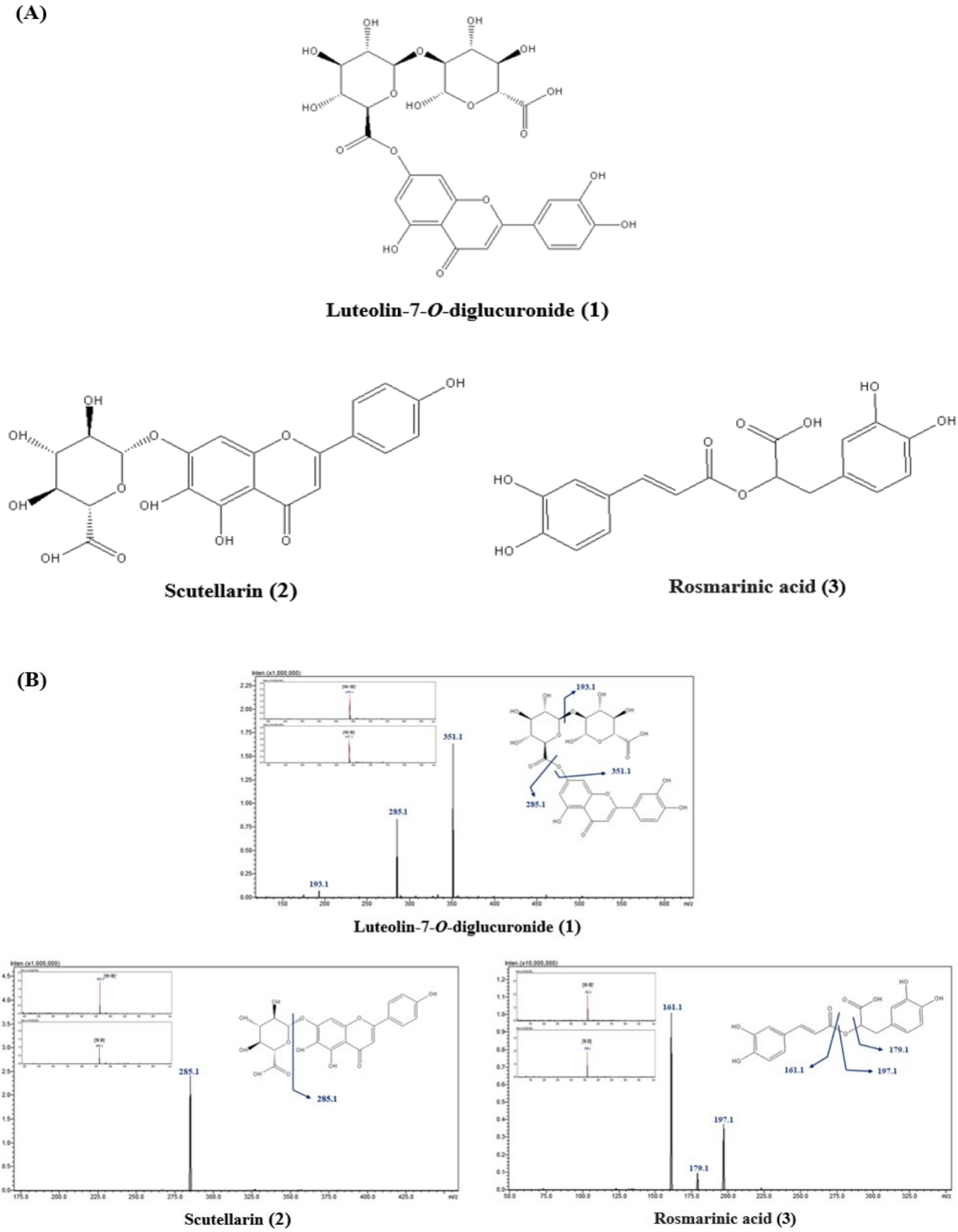

Figure 1. The structure of major compounds identified from *Perilla frutescens* var. *acuta* extracts. (A) Structure of compounds 1–3. (B) HPLC--MS/MS analysis results of compounds 1–3.

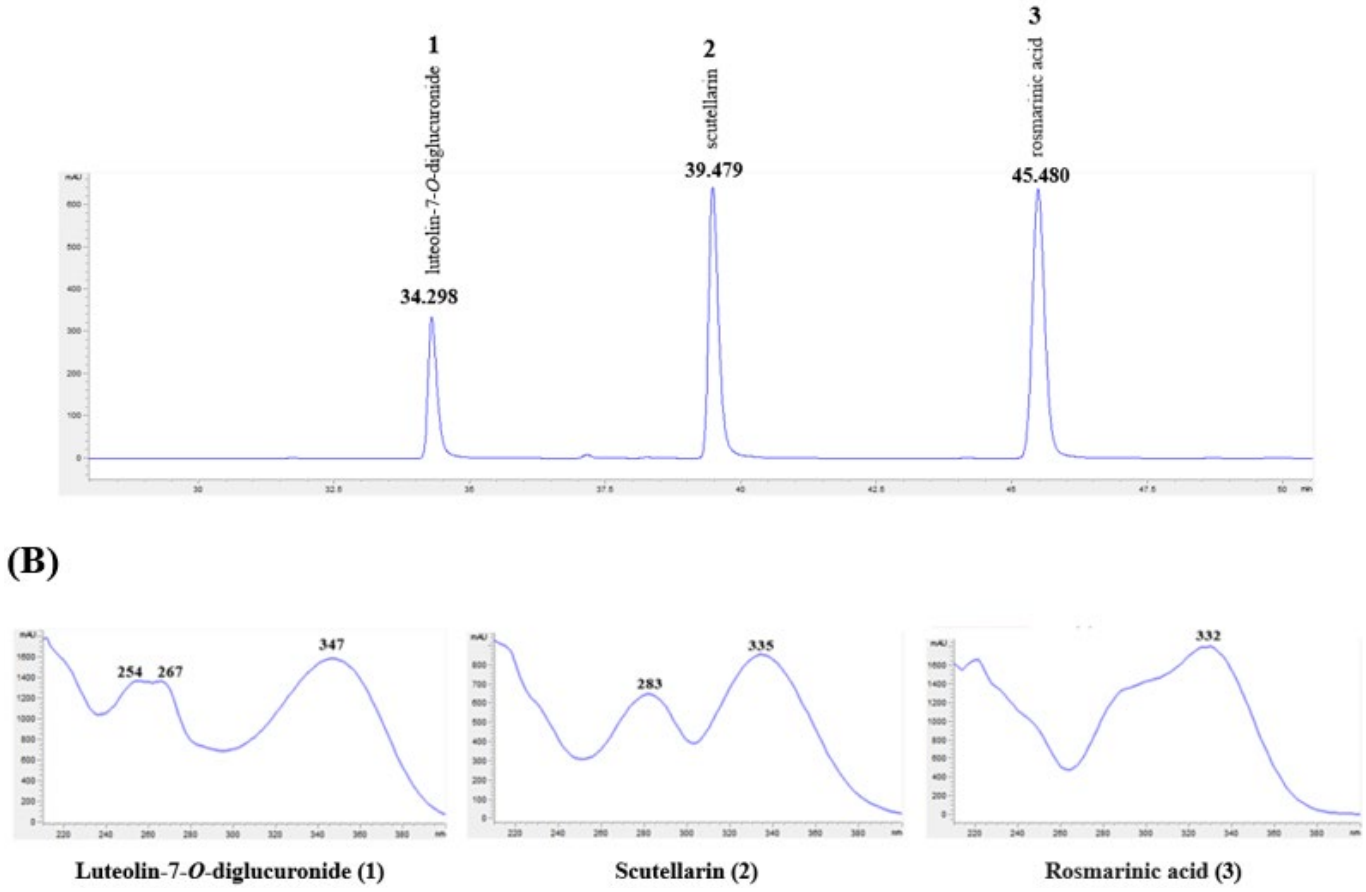

Figure 2. HPLC and UV-spectrum maximum absorption wavelength pattern analytical chromatogram of major compounds 1–3. (A) HPLC chromatogram of standards. (B) UV-spectrum maximum absorption wavelength.

Table 1. HPLC-MS/MS analysis of major compounds 1–3 identified from *Perilla frutescens* var. *acuta* extracts.

Peak No.	Identification	Chemical formula	t_R (min)	UV λ_{max} (nm)	$[M+H]^+$ (*m/z*)	$[M-H]^-$ (*m/z*)	MS/MS fragments (*m/z*)	Reference
1	Luteolin-7-*O*-diglucuronide	$C_{27}H_{26}O_{18}$	34.1	254, 267, 347	639	637	285, 351	(10)
2	Scutellarin	$C_{21}H_{18}O_{12}$	38.9	283, 335	463	461	285	(11-13)
3	Rosmarinic acid	$C_{18}H_{16}O_8$	44.6	332	361	359	161, 179, 197	(14-16)

Table 2. Calibration curve, linearity, limit of detection (LOD), and limit of quantitation (LOQ) of three major compounds.

Compound	Concentration (μg/mL)	Regression equation[1]	R^2	LOD (μg/mL)	LOQ (μg/mL)
Luteolin-7-*O*-diglucuronide	24.3–388.0	y = 10.412x - 6.0417	1	4.4802	13.5764
		y = 10.410x + 17.171	0.9997		
		y = 10.463x + 19.608	0.9998		
Scutellarin	24.8–396.0	y = 21.160x + 46.125	0.9998	5.4133	16.4038
		y = 21.122x + 101.42	0.9993		
		y = 21.267x + 110.23	0.9993		
Rosmarinic acid	24.5–392.0	y = 26.363x + 33.617	0.9999	4.6262	14.0187
		y = 26.242x + 98.121	0.9997		
		y = 26.404x + 96.992	0.9996		

[1]y: peak area; x: concentration.

Table 3. Accuracy of three major compounds.

Compound	Spiked amount (μg/mL)	Measured amount (μg/mL)	RSD[1] (%)	Recovery average[2] (%)
Luteolin-7-*O*-diglucuronide	24.3	24.34 ± 0.15	0.63	100.16 ± 0.63
	97.0	98.90 ± 0.88	0.89	101.96 ± 0.91
	388.0	386.66 ± 1.69	0.44	99.65 ± 0.43
Scutellarin	24.8	25.23 ± 0.09	0.35	101.71 ± 0.36
	99.0	100.89 ± 0.10	0.10	101.91 ± 0.11
	396.0	393.46 ± 2.04	0.52	99.36 ± 0.51
Rosmarinic acid	24.5	24.53 ± 0.04	0.17	100.11 ± 0.17
	98.0	97.98 ± 0.09	0.09	99.98 ± 0.09
	392.0	390.42 ± 1.14	0.29	99.60 ± 0.29

[1]Relative standard deviation; [2]recovery average (%) = (measured amount/spiked amount) × 100%.

Table 4A. Precision of three major compounds: Inter-day and intra-day precision of three major compounds.

Peak No.	Concentration (μg/mL)	Inter-1 day		Inter-2 day	
		Mean ± SD[1]	RSD[2] (%)	Mean ± SD	RSD (%)
1	24.3	24.24 ± 0.20	0.81	24.34 ± 0.15	0.63
	97.0	98.11 ± 1.02	1.04	98.90 ± 0.88	0.89
	388.0	389.12 ± 0.27	0.07	386.66 ± 1.69	0.44
2	24.8	25.27 ± 0.09	0.37	25.23 ± 0.09	0.35
	99.0	101.73 ± 0.26	0.26	100.89 ± 0.10	0.10
	396.0	396.20 ± 0.20	0.05	393.46 ± 2.04	0.52
3	24.5	24.56 ± 0.06	0.26	24.53 ± 0.04	0.17
	98.0	101.42 ± 0.53	0.53	97.98 ± 0.09	0.09
	392.0	391.83 ± 0.22	0.06	390.42 ± 1.14	0.29

[1]Values are mean ± standard deviation (n = 3); [2]relative standard deviation.

Table 4B. Precision of three major compounds: repeatability of three major compounds

Compound	Concentration (μg/mL)	Mean ± SD[1]	RSD[2] (%)
Luteolin-7-*O*-diglucuronide	97	98.50 ± 0.96	0.97
Scutellarin	99	101.31 ± 0.49	0.49
Rosmarinic acid	98	99.70 ± 1.92	1.92

[1]Values are mean ± standard deviation (n = 6); [2]relative standard deviation.

Table 5. Analytical result of three major compounds stability test.

Compound	Concentration (μg/mL)		Difference[1] (%)
	0 h	24 h	
Luteolin-7-*O*-diglucuronide	386.66	389.12	0.63
Scutellarin	393.46	396.20	0.69
Rosmarinic acid	390.42	391.83	0.36

[1]Difference (%) = [(0 h Area–24 h Area)/0 h Area] × 100%.

3.3 Analysis of contents of Perilla frutescens *var.* acuta *extracts*

Using the same HPLC-DAD analysis method, the contents of tea extracts extracted with 0, 20, 40, 60, 80, and 100% ethanol were analyzed for luteolin-7-*O*-diglucuronide, scutellarin, and rosmarinic acid, and the results are shown in Table 6. Luteolin-7-*O*-diglucuronide had the highest content in the 0% ethanol extract at 302.22 μg/mL, while scutellarin and rosmarinic acid had the highest contents in the 60% ethanol extract at 103.61 and 118.77 μg/mL, respectively.

Table 6. Contents of three major compounds in *Perilla frutescens* var. *acuta* extracts.

Sample	Concentration (μg/mL)		
	Luteolin-7-O-diglucuronide	Scutellarin	Rosmarinic acid
0% EtOH	302.22 ± 7.58	59.58 ± 2.33	–
20% EtOH	223.78 ± 0.88	46.37 ± 0.21	30.90 ± 0.36
40% EtOH	263.42 ± 9.33	71.20 ± 2.84	64.36 ± 2.16
60% EtOH	244.68 ± 5.60	103.61 ± 4.69	118.77 ± 4.89
80% EtOH	29.61 ± 0.86	63.79 ± 1.90	71.75 ± 1.49
100% EtOH	–	–	40.37 ± 0.23

4 CONCLUSION

In this study, we identified the major compounds of ethanol extracts of *P. frutescens* var. *acuta* at different concentrations using HPLC-DAD and HPLC-MS/MS, namely, luteolin-7-*O*-diglucuronide (1), scutellarin (2), and rosmarinic acid (3). We also performed quantification and simultaneous analysis validation of the HPLC-DAD method. The linearity was confirmed by the regression equation and determination coefficient (R^2) values greater than 0.9993, and the detection and quantification limits were measured in the range of 4.4802–5.4133 and 13.5764–16.4038 μg/mL, respectively. The accuracy analysis

showed RSD values of 0.09–0.89% for each marker compound, and the recovery rates were measured in the range of 99.36–101.96%. The precision analysis within the laboratory showed RSD values in the range of 0.05–1.04%, and the RSD values in the range of 0.49–1.92% were confirmed in the repeatability evaluation, which met the criterion of the validation guidelines for drug testing methods of less than 2%. The 24-h changes in the content of the three major compounds were 0.36–0.69%, which confirmed the chemical stability within 24 h, meeting the guideline criterion of less than 2%. The quantification analysis of the ethanol extracts of *P. frutescens* var. *acuta* showed that luteolin-7-*O*-diglucuronide had the highest content in the 0% ethanol extract at 302.22 μg/mL, while scutellarin and rosmarinic acid had the highest content in the 60% ethanol extract at 103.61 and 118.77 μg/mL, respectively. Therefore, through the validation process of this analytical method, we confirmed that the HPLC-DAD method for luteolin-7-*O*-diglucuronide (1), scutellarin (2), and rosmarinic acid (3) is sufficient for quantitative analysis of *P. frutescens* var. *acuta* extracts.

REFERENCES

Ahn, E. M., Choi, S. A., & Choi, J.-Y. (2017). HPLC analytical method validation of Aralia elata extract as afunctional ingredients. *Korean Journal of Food Preservation*, *24*(6), 795-801.

Bae, J. S., Han, M., Shin, H. S., Kim, M.-K., Shin, C.-Y., Lee, D. H., & Chung, J. H. (2017). Perilla frutescens leaves extract ameliorates ultraviolet radiation induced extracellular matrix damage in human dermal fibroblasts and hairless mice skin. *Journal of Ethnopharmacology*, *195*, 334-342. https://doi.org/10.1016/j.jep.2016.11.039

Cho, K., Choi, Y.-J., & Ahn, Y. H. (2020). Identification of Polyphenol Glucuronide Conjugates in Glechoma *hederacea* var. *longituba* Hot Water Extracts by High Performance Liquid Chromatography Tandem Mass Spectrometry (HPLC-MS/MS). *Molecules*, *25*(20), 4713. https://doi.org/10.3390%2Fmolecules25204713

Cui, X. B., Qian, X.-C., Huang, P., Zhang, Y.-X., Li, J.-S., Yang, G.-M., & Cai, B.-C. (2015). Simultaneous determination of ten flavonoids of crude and wine-processed Radix Scutellariae aqueous extracts in rat plasma by UPLC-ESI-MS/MS and its application to a comparative pharmacokinetic study. *Biomedical Chromatography*, *29*(7), 1112-1123. https://doi.org/10.1002/bmc.3398

Jeon, S. Y., Baek, J.-H., & Cha, Y.-J. (2011). Analytical Method Validation of Quercetin in Changnyeong Onion Extractas a Functional Ingredient for Functional Health Food. *Journal of Korean Society of Food Science and Nutrition*, *40*(4), 565-569.

Jeong, H. J., Xuan, S. H., Song, B. R., Lee, S. L., Lee, Y. J., & Park, S. N. (2018). Antimicrobial and Antioxidant Activities of Perilla frutescens var. acuta Extract and Its Fraction and Their Component Analyses. *Applied Chemistry for Engineering*, *29*(6), 716-725. https://doi.org/10.14478/ace.2018.1077

Jun, H. I., Kim, B.-T., Song, G.-S., & Kim, Y.-S. (2014). Structural characterization of phenolic antioxidants from purple perilla (Perilla frutescens var. acuta) leaves. *Food Chemistry*, *148*, 367-372. https://doi.org/10.1016/j.foodchem.2013.10.028

Kim, M. H., Kang, W. W., Lee, N. H., Kwoen, D. J., & Choi, U. K. (2007). Antioxidant activities of extract with water and ethanol of Perilla frutescens var. acuta kudo leaf. *Journal of Korean Society for Applied Biological Chemistry*, *50*, 327-333.

Kim, Y. J., Han, S.-G., Jeon, J.-Y., Hwang, M., Im, Y.-J., Chae, S.-W., Kim, M.-G. (2012). Method Development of Ellagic Acid as Marker Compound for Standardizationof Gochang Bokbunja (Rubus coreanus Miquel) as Functional Ingredient. *Journal of Korean Society of Food Science and Nutrition*, *41*(11), 1554-1558.

Korea Food and Drug Administration (KFDA) (2004). *Analytical method guideline about validation of drugs and etc*. Korea Food & Drug Administration.

Korea Food and Drug Administration (KFDA) (2007). *Guideline for standard of health functional food*. Korea Food and Drug Administration.

Korea Food and Drug Administration (KFDA) (2015). *Analytical method guideline about validation of drugs and etc*. Korea Food and Drug Administration.

Tang, H., Li, N.-G., Tang, Y.-P., Shi, Q.-P., Guo, J.-M., Zhang, W., Shen, M.-Z., & Duan, J.-A. (2014). Identification of scutellarein metabolites in rat using ultra performance liquid chromatography quadrupole-time-of-flight mass spectrometry. *Analytical Methods*, *6*(13), 4667-4673. https://doi.org/10.1039/C4AY00726C

Wang, X., Qian, X., Li, X., Jia, X., Yan, Z., Han, M., Qiao, M., Ma, X., Chu, Y., Zhou, S., & Yang, W. (2021). Rapid determination of rosmarinic acid and its two bioactive metabolites in the plasma of rats by LC–MS/MS and application to a pharmacokinetics study. *Biomedical Chromatography*, *35*(2), e4984. https://doi.org/10.1002/bmc.4984

Wong, W. L., Reis, A. C. C., Sousa, J. A. C., Vaz, L. B. A., Silva, B. M., Magalhães, C. L. B., Kohlhoff, M., Oliveira, A. B., & Brandão, G. C. (2022). High Resolution Mass Spectrometry Identification and Characterization of Flavonoids from Fridericia chica Leaves Extract with Anti-Arbovirus Activity. *Molecules*, *27*(18), 6043. https://doi.org/10.3390/molecules27186043

Yoo, D. S., Choi, Y. H., Cha, M. R., Choi, C. W., Kim, M. R., Yon, G. H., Hong, K. S., Lee, B. H., Kim, E. J., Cho, S. W., Kim, Y. S., Ryu, S. Y., & Kang, J. S. (2010). Variation of saponin contentsin the decoctions of Platycodi Radix(II). *Korean Journal of Pharmacognosy*, *41*(2), 147-152.