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Determination of Mogroside V in Luohanguo Extract for Daily Quality Control Operation Using Relative Molar Sensitivity to Single-Reference Caffeine

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Mogroside V is one of the characteristic and effective components of luohanguo extract, a food additive used as a sweetener in Japan as per Japan’s Standards and Specifications for Food Additives (JSFA; 9th ed.). JSFA stipulates that the quantitative determination for mogroside V content in luohanguo extract applies HPLC using analytical standard mogroside V. However, no mogroside V reagents with proven purities are commercially available. Therefore the current JSFA determination method is not particularly suited for daily quality control operations involving luohanguo extract. In this study, we applied an alternative quantitative method using a single reference with relative molar sensitivity (RMS). It was possible to calculate the accurate RMS by an offline combination of 1H-quantitative NMR spectroscopy (1H-qNMR) and an HPLC/variable-wavelength detector (VWD). Using the RMS of mogroside V to a commercial certified reference material grade caffeine, the mogroside V contents in luohanguo extracts could be determined using HPLC/VWD without analytical standard mogroside V. There was no significant difference between the mogroside V contents in luohanguo extracts determined using the method employing single-reference caffeine with the RMS and using the JSFA method. The absolute calibration curve for the latter was prepared using an analytical standard mogroside V whose purity was determined by 1H-qNMR. These results demonstrate that our proposed method using a single reference with RMS is suitable for quantitative determination of mogroside V in luohanguo extract and can be used as an alternative method to the current assay method in JSFA.

Key words relative molar sensitivity (RMS); single-reference HPLC; mogroside V; caffeine; 1H-quantitative NMR spectroscopy; luohanguo extract

Introduction

Many useful natural products, such as crude drugs and food additives, are standardized to control their efficacy and quality for safe use. Monographs for food additives including their definition, contents of constituents, identification tests, and standards for their use, are set in Japan’s Standards and Specifications for Food Additives (JSFA; 9th ed., 2018). Generally, HPLC with a variable-wavelength detector (HPLC/VWD) is employed as a quantitative method for natural products because of its high separation capability and sensitivity. Effecting HPLC/VWD requires an analytical standard that is identical to the analyte employed and that can be certified in terms of its exact purity. However, there are few commercially available certified reference materials (CRMs) with purities metrologically calculated by appropriate methods.1 In many cases, reagents conforming to the standards of manufacturers are used as analytical standards due to lack of CRMs. The purities of these reagents are guaranteed only by the standards employed by their manufacturers and are not necessarily metrologically accurate. This substitution gives rise to unreliable quantification. Furthermore, most of the constituents derived from natural products are not commercially available, including CRMs. In these cases, quantitative methods cannot be established for quality control, thereby preventing development of specification for food additives, particularly for those that are naturally derived.

Luohanguo extract, a sweetener obtained from the fruit of the luohanguo plant (Siraitia grosvenorii [Swingle] C. Jeffrey ex A.M. Lu and Zhi Y. Zhang [Cucurbitaceae]), is a food additive listed in JSFA.2 In dried luohanguo extract, mogroside V(I) has to be present at a rate of more than 20%2 (see Fig. 1). To obtain the required quality, quantitative assay of mogroside V by chromatography is required. Commercially available mogroside V reagents vary in price from ¥11000 to ¥110000 per 100mg (as of February 2020), and their grade varies from reagent to analytical standard. When a reagent without exact
purity is used as a standard for quantitative determination by HPLC, the purity of the reagent is generally regarded as 100%. Even if an identical sample is quantified, the resulting value without correction by purity of the standard may vary depending on the reagents used as standard. For quantitative determination according to JSFA, mogroside V standard must conform to the reagent specification in these guidelines. Conforming reagents are necessarily more expensive because reagent manufacturers are responsible for the certification of their reagents in accordance with JSFA specifications. Quality control of luohanguo extract is routinely carried out; therefore using expensive reagents for quantitative assay represents a heavy burden for manufacturers of food additives. Furthermore, even if reagents conforming to specifications are used, accurate quantification of mogroside V cannot be achieved because the specification does not consist of testing for determining the exact purity of the reagent. It is uncertain whether mogroside V standards that conform to specifications will be stably provided in the future. However, determination of mogroside V for quality control must be continued, as long as luohanguo extracts are used as sweeteners in Japan. This is a common problem concerning the constituents of natural products.

To overcome this problem, we developed a single-reference HPLC method with relative molar sensitivity (RMS) (Fig. 2 (A)). In chromatography, the detector’s response is generally proportional to the amount, or concentration, of analyte, and the response intensity per unit amount is substance specific. The RMS is defined as the ratio of response intensity per mole of an analyte to that of a reference compound (i.e., a stable and appropriate compound that is different from the analyte). As long as the RMS is applied to quantitative assays under the same HPLC conditions as those for RMS determination, it can be used with a specific factor. Hence using the RMS of an analyte to the reference, the analyte content in a sample solution (the constant amount of single reference added in advance) can be quantified using HPLC/VWD, based on the relationship between the ratio of detector response intensities, the amount of the single-reference, and the RMS.

An accurate determination of the RMS can be achieved by the offline combination of $^1$H-quantitative nuclear magnetic resonance spectroscopy ($^1$H-qNMR) and chromatography. (Color figure can be accessed in the online version.)
an International System of Units (SI) traceable measurement for the quantitative analysis of organic compounds; it is, however, inferior to chromatography in terms of sensitivity and multi-compound separations. Because the detection sensitivity of 1H-qNMR is proportional only to the number of 1H nuclei, analyte purity can be accurately estimated by comparing the signal areas of the analyte with those of a CRM that is prepared for 1H-qNMR and used as an internal standard. Since exact purities of reagents at any grade can be calculated by 1H-qNMR, the accurate calibration curves of analytes can be constructed with HPLC/VWD. From the ratio of slopes of these accurate calibration curves of an analyte and a single-reference, an accurate RMS can be obtained. This offline combination of 1H-qNMR and HPLC/VWD enables the single-reference method with RMS, which does not require analytical standards corresponding to the analytes for quantitative assays. As shown in our previous studies, we applied the single-reference method with RMS to the quantitative determination of active constituents in various natural food additives.

In this study, we report that the single-reference HPLC method with RMS was applied for the quantitative determination of mogroside V in luohanguo extract using caffeine as a single reference. In the context of the official method, it is essential that anyone must be able to perform quantitative determination appropriately using any reagent and instrument, regardless of their manufacturers, as long as the reagent and instrument meet the specifications. In the single-reference HPLC method, accurate RMS determined by 1H-qNMR is provided alongside the procedure, and a compound available as a commercial standard with certified purity is selected as a reference compound. This method is sufficiently accurate to serve as an alternative for the quantitative assay defined by JSFA.

Results and Discussion

Selection of Single Reference and Establishment of HPLC Conditions At first, a single-reference compound was selected. It is essential for this reference compound to be chemically stable, to have certified purity, and to be low cost. It is also preferable for this reference compound to have the same or close absorption maximum ($\lambda_{\text{max}}$) to the analyte for reducing the influence brought on by differences in HPLC instruments. In this study, caffeine (2) was selected as a single-reference compound candidate to meet these requirements (Fig. 1); caffeine is available as a CRM grade reagent with certified purity. Moreover, the $\lambda_{\text{max}}$ of caffeine is close to that of mogroside V, the analyte, although the respective $\lambda_{\text{max}}$ do not exactly match each other.

Secondly, the HPLC conditions were determined. The JSFA prescribes HPLC analysis for the determination of mogroside V content in luohanguo extracts to be performed under isocratic elution condition with an amino column. This HPLC condition was changed into the appropriate isocratic conditions with caffeine as a single-reference compound. However, the retention time of caffeine was apart from that of mogroside V, leading to prolonged elution time for both compounds when they were analyzed under an HPLC isocratic elution condition. Considering this method is defined as a JSFA official method and used for daily operations, it is preferable for one analysis to be conducted within roughly 30 min. Therefore we propose a different HPLC condition for caffeine (which was used as an external standard) from that for mogroside V and luohanguo extract (Table 1).

1H-qNMR Measurement for Purity Assessment of Mogroside V Standard The RMS was determined from the ratio of slopes in the calibration curves for the analyte and reference compound; here, horizontal and vertical axes in the calibration curves were molar concentration ($\mu$mol/mL) and peak area, respectively. To determine the accurate RMS value of mogroside V to caffeine, accurate calibration curves were needed. These could be constructed using reagents whose exact purities were available. The given purity of the caffeine reagent used as a single-reference compound in this study was reliable because of its availability as a CRM reagent, while that of mogroside V standard was unclear. To establish accurate calibration curves, purity of the mogroside V standard was determined by 1H-qNMR using internal standard. The 1H-qNMR spectrum of solution $^{1}\text{H-qNMR}$ of caffeine was shown in Fig. 3. In this spectrum, chemical shifts were defined as a 1H signal derived from the methyl group of DSS-$d_6$ calibrant at $\delta 0.0$ ppm. In addition, 1H signals derived from saturated hydrocarbons of aglycone moiety were detected at roughly $\delta 0.8$ to 3.0 ppm, whereas 1H signals derived from glycosides were observed at approximately $\delta 3.2$ to 5.0 ppm (Fig. 3). However, these signals were inappropriate as quantitative signals used for 1H-qNMR, due to overlapping with other 1H signals. The signal at $\delta 5.59$ ppm from H-6 of mogroside V, however, was accepted as suitable as the quantitative signal for mogroside V because it showed good separation from other signals derived from the analyte, 2,2-dimethyl-2-silapentane-5-sulfonate-$d_6$ sodium salt ($\text{DSS}-d_6$) calibrant, and impurities (Fig. 3). Using this signal in the 1H-qNMR spectra of solutions $^{1}\text{H-qNMR}$ from 1 to 3, the absolute purity of the mogroside V standard was determined as 93.8% (relative standard deviation (RSD) 0.2%).

| Table 1. Operational Conditions for HPLC/VWD |
|---------------------------------|
| Column | L-column2 ODS (5 µm, 4.6 × 250 mm) (CERI, Tokyo, Japan) |
| Column temperature | 40°C |
| Injection volume | 10 µL |
| Mobile phase | A: water/formic acid (1000 : 1), B: acetonitrile/formic acid (1000 : 1) |
| Mogroside V and luohanguo extract | A:B (78:22) |
| Caffeine | A:B (90:10) |
| Flow rate | 1.0 mL/min |
| Detector wavelength | 210 nm |
210 nm) and the $\lambda_{max}$ of mogroside V (under 200 nm). In many cases, the curve of the absorption spectra was relatively shallow near $\lambda_{max}$, reducing the influence of the difference in the absorption spectral resolution on the intensity of detector responses. However, for the official method, setting the detector wavelength below 200 nm was avoided; the reason for this was linked to perspectives of the influences caused by the absorption of mobile phase and differences in the sensitivity of detectors. Since differences in the RSD of RMS values due to difference of instruments were within a 5% margin in this study, a detector wavelength at 210 nm was considered appropriate for adopting the single-reference HPLC method as a quantitative assay in JSFA.

In the determination of mogroside V content in luohanguo extracts using the current method described in JSFA (JSFA method), the purity of any commercial mogroside V standard is regarded as being 100%. Employing the mogroside V standard used in this study (purity 93.8%), a minimum 6% higher content of mogroside V compared with true content will be calculated using the JSFA method. Since the purity of mogroside V standards can vary significantly according to lot number and manufacturer, different figures can be obtained for mogroside V content from identical luohanguo extract products, undermining their consistent quality control. In contrast, the single-reference HPLC method developed in this study required caffeine as a single reference. The CRM grade of caffeine was available, and as a result, mogroside V content was rarely affected by differences linked to lot numbers and manufacturers where reagents were concerned. Although RMS differences based on instruments affected the content of mogroside V, these were within a 5% margin for the condition.
tions in this study. These differences were smaller than those between the true amount of mogroside V and that calculated by the JSFA method using mogroside V standards. Moreover, the margin for RSD of RMSs obtained by the same instrument was within 1.2% (Table 2). These results indicate that the single-reference HPLC method developed in this study is suitable for daily operation in the quality control of luohanguo extract products, compared with the JSFA method.

Comparison of Single-Reference HPLC Method with JSFA Method

For determining mogroside V content, luohanguo extract products C2101 and C2102 were analyzed using the single-reference HPLC method. Quantitative determination with the single-reference HPLC method was performed using three instruments (chromatographs A to C). Figure 5 shows the chromatograms of mogroside V, caffeine, and two luohanguo extract products analyzed by chromatograph A. For a more exact quantitative analysis, the concentration of the caffeine standard solution used as an external standard was corrected using certified purity (99.9%) and the contents of mogroside V in luohanguo extract products were determined (see Table 3).

To verify the single-reference HPLC method, the mogroside V contents of C2101 and C2102 were also analyzed using not only the JSFA method (Fig. 6) but also a calibration curve of mogroside V instead of RMS, using the same conditions as the single-reference HPLC method (absolute calibration curve method). The resultant figures were compared with those using the single-reference HPLC method (Table 3).

The content of mogroside V in C2101 determined by the JSFA method and the absolute calibration curve method was 33.6% and 31.5%, respectively. In the case of C2102, the mogroside V content determined by these two methods was 61.6% and 57.2%, respectively. Although the HPLC elution conditions were different between the JSFA method and the absolute

Table 3. Mogroside V Content of Luohanguo Extract Determined by Single-Reference HPLC Method, Absolute Calibration Curve Method, and JSFA Method

| A. C2101 | Chromatograph | A     | B     | C     | Average | RSD (%) |
|----------|---------------|-------|-------|-------|---------|---------|
|          | Single-reference HPLC method | 33.0% | 30.4% | 32.1% | 31.8%  | 1.3%    |
|          | Absolute calibration curve method | 33.6% | 34.2% | 33.9% | 33.9%  | 0.3%    |
|          | Absolute calibration curve method (corrected by 1H-qNMR) | 31.5% | 32.1% | 31.8% | 31.8%  | 0.3%    |
|          | JSFA method | 33.6% | —     | —     | 33.6%  | —       |
|          | JSFA method (corrected by 1H-qNMR) | 31.1% | —     | —     | 31.1%  | —       |

| B. C2102 | Chromatograph | A     | B     | C     | Average | RSD (%) |
|----------|---------------|-------|-------|-------|---------|---------|
|          | Single-reference HPLC method | 59.8% | 54.0% | 56.7% | 56.8%  | 5.1%    |
|          | Absolute calibration curve method | 61.0% | 60.9% | 60.0% | 60.6%  | 0.9%    |
|          | Absolute calibration curve method (corrected by 1H-qNMR) | 57.2% | 57.1% | 56.3% | 56.8%  | 0.9%    |
|          | JSFA method | 61.6% | —     | —     | 61.6%  | —       |
|          | JSFA method (corrected by 1H-qNMR) | 57.0% | —     | —     | 57.0%  | —       |
calibration curve method, the content of mogroside V was almost identical, indicating that the elution condition developed in this study was appropriate for quantitative determination. However, the mogroside V content obtained using these two methods was likely higher than the actual content because the purity of mogroside V standard used was regarded as 100%.

For a more exact quantitative analysis, the concentration of mogroside V standard solutions used as an external standard in the JSFA method and for the calibration curve in the absolute calibration curve method was corrected using the purity of the mogroside V standard determined by $^1$H-qNMR. The content of mogroside V in C2101 determined by the modified JSFA method and modified absolute calibration curve method (both methods with correction by $^1$H-qNMR purity) was 31.1 and 31.8%, respectively, whereas that in C2102 determined by these two modified methods was 57.0 and 56.8%, respectively (Table 3). From the average of these figures, the exact content of mogroside V in the two luohanguo extract products used in this study was accepted as 31.5% in C2101 and 56.9% in C2102 (we defined these values as “the exact content”).

The content of mogroside V in C2101 and C2102 as determined by the single-reference HPLC method was 31.8 and 56.8%, respectively. These results were almost identical to the exact content. When using the same instrument, the content determined by the single-reference HPLC method was found to be closer to the exact content than that determined by the absolute calibration curve method without correction by $^1$H-qNMR purity. These results demonstrate the single-reference HPLC method developed in this study as a superior quantitative method to the JSFA approach and the absolute calibration curve method, in which the purity of the mogroside V standard was regarded as 100%.

The RSD of the content determined by the single-reference HPLC method using three chromatographs was 4 to 5%, which was larger than the RSD achieved by the absolute calibration curve method. Although the RMS determined in this study was 0.127, RMSSs of chromatograph A and B were 0.133 and 0.121, respectively, and these differed by 0.006 from the RMS used in this study. Since the contents were obtained by multiplying the reciprocal of RMS (Eqs. (3) and (4)), the larger value was calculated from chromatograph A and the smaller value from chromatograph B. In contrast, for the absolute calibration curve method, calibration curves were constructed using the same mogroside V standard among three chromatographs. This means that the difference between chromatographs had been corrected and the difference in amounts derived from three chromatographs was narrowed. Practically, the lot numbers and manufacturers of mogroside V standards are not always the same; therefore the differences between instruments were thought to be larger than the results achieved in this study. In addition, the difference between the exact content and the measured value was 4.6% maximum in the single-reference HPLC method but 8.5% in the absolute calibration curve method (C2101). Although the influence of different instruments on quantification by the single-reference HPLC method will be one of the issues for future research, its influence on daily operations for quality control of luohanguo extract products appears to be small.

Using analytical standards with exact purities, accurate calibration curves can be constructed correctly to quantify mogroside V content. However, as long as standards with exact purities are not commercially available, analysts themselves will have to determine the exact purity of the standard using proper methods such as $^1$H-qNMR. Since NMR instruments are less widely available than HPLC instruments, it would be difficult for some manufacturers to determine the exact purities of standards in daily operations for quality control of their products. Using the single-reference HPLC method developed in this study, more analysts can determine the exact content of mogroside V in luohanguo extract products using only HPLC instruments and without the exact purities of mogroside V standards. This method is suitable as a quantitative assay for luohanguo extract and can substitute for the current JSFA method.

Conclusion

This study aimed to introduce the single-reference HPLC method for determining the content of mogroside V in luohanguo extracts (without using mogroside V standards) as an official method in the JSFA. Caffeine, which is commercially available as a CRM, was selected as a reference compound. The RMS of mogroside V to caffeine was determined using an offline combination of $^1$H-qNMR and HPLC/VWD. Using the RMS of 0.127, the quantitative determinations of mogroside
V in two luo-hanguo extract products were performed with three chromatographs. More exact results were obtained using the developed method compared with the JSFA method. Data variation was within a 5% margin. A CRM grade caffeine reagent is available at ¥18800 per 100 mg, whereas a mogroside V standard without exact purity is ¥30000 per 50 mg (as of February 2020). The single-reference HPLC method with RMS using caffeine as an external standard was shown to be superior to the current JSFA method in terms of the accuracy of quantitative values and the cost of reagents. This method is thus suitable as a quantitative determination involving luo-hanguo extract and can be used as a substitute for the JSFA method.

**Experimental**

**Materials and Reagents** Two commercial powdered luo-hanguo extracts, i.e., C2101 and C2102, used as food additives were obtained through the Japan Food Additive Association. According to product reports, the mogroside V content of these products was 32.57 (C2101) and 54.40% (C2102). Mogroside V standard (Cat. no. 131-16571) was purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). CRM grade caffeine (Cat. no. 56396-100MG) was obtained from Merck KGaA (Darmstadt, Germany), and its certified purity was 99.9% (Lot. no. BCBS0789V). In addition, 2,2-dimethyl-2-silapentane-5-sulfonate-6d, sodium salt (DSS-6d, Cat. no. 044-31671, certified purity 92.3 ± 0.7%, k = 2 (Lot. no. KPEI040), which was used as reference material for qNMR, was obtained from FUJIFILM Wako Pure Chemical Corporation Deuterium oxide (D 2O) (Cat. no. 151882-1KG) was purchased from Isotec (Miamisburg, OH, U.S.A.). Water was prepared using a water purification system (PURiC-εω, Organo Corp., Tokyo, Japan). All solvents used in this study were of HPLC or special grade.

**Instruments** A 600 MHz NMR spectrometer (JNM-ECA600; JEOL Ltd., Tokyo, Japan) was used to determine the purity of mogroside V standard. Chromatographic separations were performed with three HPLC-VWD instruments (chromatographs A to C). Chromatograph A was a Prominence ultrafast LC system comprising a degasser (DGU-20 A 3g), a solvent delivery unit (pump, LC-20AT), an autosampler (SIL-20AC), a column oven (CTO-20AC), and an UV-visible detector (SPD-20A) (Shimadzu Corp., Kyoto, Japan). Chromatograph B was an Agilent 1100/1200 Series comprising a degasser (G1322A), a binary pump (G1321A), an autosampler (G1329A), a column compartment (G1316A), and a VWD (G1314A) (Agilent Technologies, Inc., Santa Clara, CA, U.S.A.). Chromatograph C was a Dionex UltiMate 3000 comprising a degasser (SRD-3600), a dual-gradient standard pump (DPG-3600SD), an autosampler (WPS-3000TRS), a column compartment (TCC-3000SD), and a VWD (VWD-3400RS) (Thermo Fisher Scientific, Inc., Waltham, MA, U.S.A.). An ultramicroweighing balance (SCP-20U; Mettler Toledo International Inc., Columbus, OH, U.S.A.) was used for accurate measurement of weight.

**Purity Measurement of Mogroside V by 1H-qNMR** Mogroside V standard was dried at 105°C for 2 h so as accurately to weigh out 40 mg into a vial. In the same vial, 1 mg DSS-6d was accurately weighed and dissolved in 4 mL of D2O to obtain a sample solution for 1H-qNMR (solution6H). This solution was assessed using 1H-qNMR. The operating conditions of 1H-qNMR were as follows: probe, Royal Probe (JEOL Ltd.); digital resolution, 0.25 Hz; spectral width, −5 to 15 ppm; spinning, off; pulse angle, 90°; 1C decoupling, on; relaxation delay, 60 s; number of scans 128; temperature, room temperature. Three sample solutions for 1H-qNMR were prepared (solutions3MNDR, 1, and 3), and each sample solution was measured three times. As previously reported,11-13 all 1H-qNMR data were analyzed using Alice 2 for qNMR (JEOL Ltd.) and the purity of mogroside V (PM) was calculated using the following Eq. (1):

\[ PM = \left( \frac{I_M \times H_M}{M_DSS \times M_M \times W_DSS} \right) \times P_DSS \]

where I is the 1H signal area, H is the number of 1H nuclei in one molecule contributing to I, M is molecular weight, W is the amount (mg) of reagent, and P is the certified value of purity (%). Subscripted M and DSS refer to mogroside V and DSS-6d, respectively.

**Determination of RMS of Mogroside V to Caffeine** Solution3MNDR was diluted with 70 vol% methanol to obtain mogroside V standard solutions for HPLC/VWD analyses at nine concentrations from 40 to 1300 µg/mL. In a volumetric flask, 22.1 mg of caffeine was accurately weighed and dissolved in water to create exactly 50 mL, followed by dilution to obtain caffeine standard solutions at nine concentrations from 1.1 to 36.4 µg/mL. These standard solutions were subjected to HPLC/VWD under the conditions shown in Table 1. A calibration curve of mogroside V was constructed using the concentrations corrected by purity, as determined by 1H-qNMR, while that of caffeine was constructed with corrected concentrations by certified purity. Using the slope of the calibration curves (horizontal axis was corrected molar concentration (µmol/mL); the vertical axis was peak area), the RMS of mogroside V to caffeine was determined as follows:

\[ RMS = a_M /a_C \]

where a is the slope of the calibration curve. Subscripted M and C refer to mogroside V and caffeine, respectively. In the same manner, RMSs were calculated using solutions3MNDR and 2 and 3.

**Determination of Mogroside V Content in Luohanguo Extracts**

Preparation of Sample and Standard Solutions

The luohanguo extract was dried at 105°C for 2 h, and 0.2 g thereof was accurately weighed and suspended in 70 vol% methanol to create exactly 100 mL. The suspension was clarified through a membrane filter (0.45 µm pore size) to obtain the filtrate as a sample solution.

For preparation for the mogroside V standard solution, 5 mg of mogroside V standard (dried at 105°C for 2 h) was accurately weighed and dissolved in 70 vol% methanol to make exactly 10 mL.

One of the caffeine standard solutions prepared in “Determination of RMS of Mogroside V to Caffeine,” the concentration of which was 18 µg/mL, was used as an external standard solution for quantitative determination.

**Quantitative Determination of Mogroside V Content in Luohanguo Extracts Using RMS (Single-Reference HPLC Method)**

Sample and standard solutions prepared in “Preparation of Sample and Standard Solutions” were subjected to HPLC
under the conditions shown in Table 1. A peak of mogroside V in a sample solution was identified by comparing it with the peak observed in the mogroside V standard solution. Using the slope of the calibration curve \(a\) (horizontal axis was corrected caffeine concentration \(\mu\text{mol/mL}\); vertical axis was peak area) and the peak area of mogroside V in the sample solution \(A_{M}\), the concentration (mg/mL) of mogroside V \(C_{M}\) in the sample solution was determined as follows:

\[
C_{M} = \frac{A_{M}/a_{C} \times M_{M}/1000 \times 1/\text{RMS}}{1000 \times 1/\text{RMS}}
\tag{3}
\]

where \(M\) is the molecular weight.

Using the concentration of mogroside V \(C_{M}\) obtained above, mogroside V content in luohanguo extract was calculated using the following Eq. (4):

\[
\text{Content (\%)} = \frac{C_{M} 	imes V}{W 	imes 100} \times 100
\tag{4}
\]

where \(V\) is the volume (mL) of the sample solution and \(W\) is the amount (g) of luohanguo extract.

**Quantitative Determination of Mogroside V by the Assay Defined in JSFA (JSFA Method)** Sample and mogroside V standard solutions prepared in "Preparation of Sample and Standard Solutions" were subjected to HPLC according to the luohanguo extract assay listed in JSFA. The HPLC condition is shown as follows: column, Shodex Asahipak NH2P-50 4E (5 \(\mu\text{m}, 4.6 \times 250 \text{mm}\) (Showa Denko K.K., Tokyo, Japan); column temperature, 40 °C; injector volume, 20 \(\mu\text{L}\); mobile phase, acetonitrile/water (37 : 13); flow rate, 1.0 mL/min; and detector wavelength, 203 nm.

Using the peak areas \(A_{T}\) and \(A_{S}\) of mogroside V for the sample solution and mogroside V standard solution, the content of mogroside V was determined as follows:

\[
\text{Content (\%)} = \frac{W_{M} \times P/1000}{W_{M} \times (A_{T}/A_{S}) \times 10 \times 100}
\tag{5}
\]

where \(W_{M}\) is the amount (g) of mogroside V standard, \(W\) is the amount (g) of luohanguo extract, and \(P\) is the purity (%) of the mogroside V standard determined by \(^1\text{H}-q\text{NMR}.

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**Conflict of Interest** The authors declare no conflict of interest.

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