

# Reference Material 8652

## Kudzu-Containing Solid Oral Dosage Form

### REFERENCE MATERIAL INFORMATION SHEET

**Purpose:** This reference material (RM) is intended for harmonizing measurements of analytical methods for the determinations of isoflavones in ground kudzu-containing solid oral dosage form and similar matrices.

**Description:** A unit of RM 8652 consists of five packets, each containing approximately 2.6 g of ground kudzu-containing solid oral dosage form.

**Non-Certified Values:** NIST non-certified values are the best estimates of the true values based on available data. However, they do not meet the NIST criteria for certification. Non-certified values should not be used to establish metrological traceability to the International System of Units (SI) or other higher-order reference system [1].

Non-certified values, on a dry-mass basis, are provided below. These non-certified values are metrologically traceable to the materials and procedures used in their determination

#### Non-Certified Mass Fraction Values for Isoflavones (Dry-Mass Basis) in RM 8652

	Mass Fraction <sup>(a)</sup> (mg/g)		
Daidzein	3.51	±	0.35
Daidzin	12.0	±	1.2
Puerarin	68.1	±	6.8

<sup>(a)</sup> Values are expressed as  $x \pm U_{95\%}(x)$ , where  $x$  is the non-certified value and  $U_{95\%}(x)$  is the expanded uncertainty of the non-certified value. The method-specific value of the analyte lies within the interval  $x \pm U_{95\%}(x)$  with 95 % confidence. To propagate this uncertainty, the non-certified value should be treated as a normally distributed random variable with mean  $x$  and standard deviation  $U_{95\%}(x)/2$  [2–4].

**Additional Information:** Additional information is listed in Appendices A and B.

**Period of Validity:** The non-certified values are valid within the measurement uncertainty specified until **01 December 2031**. The value assignments are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

**Maintenance of Non-Certified Values:** NIST will monitor this material to the end of its period of validity. If substantive technical changes occur that affect the non-certified values during this period, NIST will update this Reference Material Information Sheet and notify registered users. RM users can register online from a link available on the NIST SRM website or fill out the user registration form that is supplied with the RM. Registration will facilitate notification. Before making use of any of the values delivered by this material, users should verify they have the most recent version of this documentation, available through the NIST SRM website (<https://www.nist.gov/srm>).

**Safety:** RM 8652 IS INTENDED FOR RESEARCH USE; NOT FOR HUMAN CONSUMPTION. See the Safety Data Sheet (SDS) for additional information.

**Storage:** RM 8652 should be stored at controlled room temperature (20 °C to 25 °C) in the original unopened packet until required for use. The packet can be opened, test portions removed and analyzed, and then the packet can be resealed with test portions able to be removed for analysis up to one week after initial opening of packet.

**Use:** The contents of the package should be thoroughly mixed before each use. Allow the contents to settle for one minute prior to opening to minimize the loss of fine particles. To relate analytical determinations to the values in this report, a minimum test portion mass of 10 mg should be used. Test portions should be analyzed as received and results converted to a dry-mass basis. The moisture conversion factor given below (see “Determination of Moisture”) can be used for the sample(s) when using an unopened packet for the first time. If using a previously opened and resealed packet, moisture must be determined using one of the recommended techniques described below. Analytical test results should include their own estimates of uncertainty and can be compared to the certified values using procedures described in reference [5].

**Determination of Moisture:** Moisture content of RM 8652 was determined at NIST by (1) drying over magnesium perchlorate in a desiccator at room temperature for 28 d and (2) drying for 2 h in a forced-air oven at 80 °C. The means from both techniques were averaged to determine a dry-mass proportion of  $(0.9460 \pm 0.0018)$  gram dry-mass per gram as-received mass; the uncertainty shown on this value is an expanded uncertainty to represent a 95 % level of confidence. The conversion factor used to convert data from an as-received to a dry-mass basis is the inverse of the dry-mass proportion. A relative uncertainty component of 0.1 % for the conversion factor obtained from the moisture measurements is incorporated in the uncertainties of the assigned values, reported on a dry-mass basis, that are provided in this report.

## REFERENCES

- [1] Beauchamp, C.R.; Camara, J.E.; Carney, J.; Choquette, S.J.; Cole, K.D.; DeRose, P.C.; Duewer, D.L.; Epstein, M.S.; Kline, M.C.; Lippa, K.A.; Lucon, E.; Molloy, J.; Nelson, M.A.; Phinney, K.W.; Polakoski, M.; Possolo, A.; Sander, L.C.; Schiel, J.E.; Sharpless, K.E.; Toman, B.; Winchester, M.R.; Windover, D.; *Metrological Tools for the Reference Materials and Reference Instruments of the NIST Material Measurement Laboratory*; NIST Special Publication (NIST SP) 260-136, 2021 edition; U.S. Government Printing Office: Washington, DC (2021); available at <https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-136-2021.pdf> (accessed May 2022).
- [2] Thompson, A.; Taylor, B.N.; *Guide for the Use of the International System of Units (SI)*; NIST Special Publication 811; U.S. Government Printing Office: Washington, DC (2008); available at <https://www.nist.gov/pml/special-publication-811> (accessed May 2022).
- [3] JCGM 100:2008; *Evaluation of Measurement Data — Guide to the Expression of Uncertainty in Measurement* (GUM 1995 with Minor Corrections); Joint Committee for Guides in Metrology (JCGM) (2008); available at <https://www.bipm.org/en/publications/guides> (accessed May 2022); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297; U.S. Government Printing Office: Washington, DC (1994); available at <https://www.nist.gov/pml/nist-technical-note-1297> (accessed May 2022).
- [4] JCGM 101:2008; *Evaluation of Measurement Data — Supplement 1 to the “Guide to the Expression of Uncertainty in Measurement” — Propagation of Distributions Using a Monte Carlo Method*; JCGM (2008); available at <https://www.bipm.org/en/publications/guides> (accessed May 2022).
- [5] Sharpless, K.E.; Lippa, K.A.; Duewer, D.L.; Rukhin, A.L.; *The ABCs of Using Standard Reference Materials in the Analysis of Foods and Dietary Supplements: A Practical Guide*; NIST Special Publication 260-181; U.S. Government Printing Office: Washington, DC (2014); available at <https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-181.pdf> (accessed May 2022).

<b>Information Sheet Revision History:</b> 19 May 2022 (Correction of isoflavone values in Table 1 to reflect purity of standards; editorial changes); 29 November 2021 (Original information sheet date).
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*Certain commercial equipment, instruments, or materials may be identified in this Reference Material Information Sheet to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.*

*Users of this RM should ensure that the Reference Material Information Sheet in their possession is current. This can be accomplished by contacting the Office of Reference Materials 100 Bureau Drive, Stop 2300, Gaithersburg, MD 20899-2300; telephone (301) 975-2200; e-mail [srminfo@nist.gov](mailto:srminfo@nist.gov); or the Internet at <https://www.nist.gov/srm>.*

**\* \* \* \* \* End of Reference Material Information Sheet \* \* \* \* \***

# APPENDIX A

## SOURCE, PREPARATION, AND ANALYSIS

**Source and Preparation:** Several commercially available products containing kudzu were purchased by NIST for production of RM 8652. The following quantity, 6.3 kg, was transferred to High-Purity Standards (Charleston, SC) where it was blended, aliquoted, and heat-sealed inside nitrogen-flushed 4 mil polyethylene bags, which were then sealed inside nitrogen-flushed aluminized plastic bags along with two packets of silica gel each. After packaging, the material was irradiated by Neutron Products, Inc. (Dickerson, MD) by  $^{60}\text{Co}$  to an absorbed dose of 7.4 kGy to 9.0 kGy.

**Homogeneity Assessment:** The homogeneity of isoflavones was assessed at NIST using a 10 mg test portion size and the methods described below; a Type B relative uncertainty component of 5 % was assigned to each isoflavone for possible inhomogeneity for measurements that might occur with this class of analytes for this group of materials.

**Analytical Approach for Determination of Isoflavones:** Value assignment of the mass fraction of puerarin in RM 8652 was based on measurements provided by NIST using liquid chromatography with ultraviolet absorbance detection (LC/UV-absorbance). Value assignment of the mass fractions of daidzin and daidzein in RM 8652 was based on measurements provided by NIST using liquid chromatography with mass spectrometry detection (LC-MS).

*NIST Analyses for Isoflavones using LC/UV-Absorbance and LC-MS:* The mass fraction of puerarin was measured by LC/UV-absorbance and daidzin and daidzein were measured by LC-MS in duplicate 10 mg test portions taken from each of ten packets of RM 8652. Methanol/water (80/20 (v/v)) and an internal standard solution of 0.60 mL caffeine, 0.50 mL  $^{13}\text{C}_6$ -daidzin, and 0.60 mL  $^{13}\text{C}_6$ -daidzein were added to each test portion as an internal standard and mixed well and extracted using ultrasonication and centrifugation. The supernatant was saved and the extraction process, using only methanol/water (80/20 (v/v)), was repeated with the supernatant added to the previous portion. Sodium hydroxide was added to the supernatant to convert acetyl- and malonyl-glycosides to free glycosides. A gradient mobile phase was used to separate the isoflavones, and caffeine was monitored at 274 nm and the puerarin at 251 nm. Daidzin and  $^{13}\text{C}_6$ -daidzin were monitored at  $m/z$  417 and  $m/z$  423, respectively and daidzein and  $^{13}\text{C}_6$ -daidzein were monitored at  $m/z$  255 and  $m/z$  261, respectively. Three stock calibration solutions were prepared gravimetrically at levels intended to approximate the levels of the isoflavones in the RM following extraction. The purity of the isoflavone calibrant materials was determined at NIST using quantitative proton nuclear magnetic resonance spectroscopy (qNMR).

\* \* \* \* \* End of Appendix A \* \* \* \* \*

# APPENDIX B

## RESPONSIBILITIES

**Coordination:** H.V. Hayes and C.A. Rimmer of the NIST Chemical Sciences Division and L.J. Wood, formerly of NIST.

**Analytical Measurements:** L.J. Wood, K.D. Chieh, and J.A. Lippert, formerly of NIST.

**Statistical Analysis:** J.H. Yen of the NIST Statistical Engineering Division.

**Institutional Support:** Support aspects involved in the issuance of this RM were coordinated through the NIST Office of Reference Materials.

Support for the development of RM 8652 was provided in part by the National Institutes of Health Office of Dietary Supplements (NIH-ODS).

\* \* \* \* \* End of Appendix B \* \* \* \* \*