National Institute of Standards and Technology
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# Reference Material 8664
# Ginseng-Containing Solid Oral Dosage Form
# REFERENCE MATERIAL INFORMATION SHEET

**Purpose:** This Reference Material (RM) is intended primarily for evaluation of analytical methods for the determinations of ginsenosides in ground ginseng-containing solid oral dosage form and similar matrices. RM 8664 provides a common matrix to those in the botanical supplements community who may wish to conduct research studies.

**Description:** A unit of RM 8664 consists of five packets, each containing approximately 2.6 g of ground ginseng-containing solid oral dosage form.

**Non-Certified Mass Fraction Values:** NIST non-certified values do not meet the NIST criteria for certification [1] and are the best estimates of the true values based on available data. The values are provided with an uncertainty that may reflect only measurement reproducibility, may not include all sources of uncertainty, and/or may reflect a lack of sufficient statistical agreement among multiple analytical methods.

Non-certified mass fraction values for ginsenosides in RM 8664, reported on a dry-mass basis, are provided in Table 1 and are the mean of results provided by NIST measurements. 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 $U_{95\%}(x)/2$ [2,3]. The measurands are the total mass fraction of each analyte in Table 1. The non-certified mass fraction values are metrologically traceable to the materials and method used in its determination.

**Table 1. Non-Certified Mass Fraction Values for Ginsenosides in RM 8664**

| | Mass Fraction (mg/g) | | |
|---|---|---|---|
| Ginsenoside Rb1 | 2.64 | ± | 0.15 |
| Ginsenoside Rb2 | 0.312 | ± | 0.016 |
| Ginsenoside Rc | 0.334 | ± | 0.015 |
| Ginsenoside Rd | 0.674 | ± | 0.026 |
| Ginsenoside Re | 0.930 | ± | 0.038 |
| Ginsenoside Rf | 0.639 | ± | 0.023 |
| Ginsenoside Rg1 | 0.583 | ± | 0.035 |

**Period of Validity:** The non-certified values delivered by **RM 8664** are valid within the measurement uncertainty specified until **01 February 2030**. 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. Before making use of any of the values delivered by this material, users should obtain the most recent version of this documentation, available free of charge through the https://www.nist.gov/srm website.

Carlos A. Gonzalez, Chief
Chemical Sciences Division

Steven J. Choquette, Director
Office of Reference Material

**Safety:** RM 8664 IS INTENDED FOR RESEARCH USE; NOT FOR HUMAN CONSUMPTION.

**Storage and Handling:** RM 8664 should be stored at controlled room temperature (20 °C to 25 °C) in the original unopened packet until required for use. The package can be opened for removal of test portions and resealed for one week after initial opening of package.

**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 test portion mass of 0.03 g should be used (see "Appendix A: Source, Preparation, and Analysis" below). 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 non-certified values using procedures described in reference 4.

**Determination of Moisture:** Moisture content of RM 8664 was determined at NIST by (1) drying over magnesium perchlorate in a desiccator at room temperature for 22 d, and (2) drying for 1 h in a forced-air oven at 100 °C. The means from both techniques were averaged to determine a dry-mass proportion of (0.95164 ± 0.00017) 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 (1.05082± 0.00010) is the inverse of the dry-mass proportion; the uncertainty shown on this value is an unexpanded uncertainty. A relative uncertainty component of 0.01 % 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.; Phinney, K.W.; Polakoski, M.; Possolo, A.; Sharpless, K.E.; Sieber, J.R.; 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 260 136; U.S. Government Printing Office: Washington, DC (2020); available at https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-136-2020.pdf (accessed Aug 2021).

[2] 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 Aug 2021); 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 Aug 2021).

[3] 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 Aug 2021).

[4] 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 260-181r1; U.S. Government Printing Office: Washington, DC (2014); available at https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-181r1.pdf (accessed Aug 2021).


*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; or the Internet at https://www.nist.gov/srm.*

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# APPENDIX A

**Source and Preparation:** Tablets containing ginseng were obtained from several different commercially available sources and purchased by NIST for production of RM 8664. The material was homogenized using a Teflon disc mill and sieved through an 80-mesh screen. The following quantity, 8.4 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) using $^{60}Co$ to an absorbed dose of 6.8 kGy to 8.5 kGy.

**Homogeneity Assessment:** The homogeneity of ginsenosides was assessed at NIST using the method and test portion sizes described below; analysis of variance with a 5 % significance level and graphical analyses showed no evidence of statistically significant box effects.

**Analytical Approach for Determination of Ginsenosides:** Value assignment of the mass fractions of ginsenosides Rb1, Rb2, Rc, Rd, Re, Rf, and Rg1 in RM 8664 was based on measurements provided by NIST using liquid chromatography with tandem mass spectrometry (LC-MS/MS).

*NIST Analyses for Ginsenosides using LC-MS/MS:* The mass fractions of ginsenosides Rb1, Rb2, Rc, Rd, Re, Rf, and Rg1 were measured by LC-MS/MS in 30 mg test portions taken from each of ten packets of RM 8664; and 4-methylestradiol was added to each test portion as an internal standard. Ginsenosides were extracted in equal portions of 60 % methanol/water and 60 % methanol/0.4 M potassium hydroxide by end-over-end rotation for 20 min followed by ultrasonication for 60 min. After centrifugation for 10 min at 3000 rpm, ginsenosides in the sample extracts were separated using a C18 column and monitored by tandem mass spectrometry in negative ion mode. The 4-methylestradiol internal standard was monitored by tandem mass spectrometry in positive ion mode. Calibrants were prepared from SRM 3389 *Ginsenosides Calibration Solutions* at levels intended to approximate the levels of the ginsenosides in the RM following extraction. The purity of the neat calibrant materials used to prepare SRM 3389 was determined by NRC Canada using quantitative NMR (qNMR). A single internal standard solution was used for the calibrants and samples.

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# APPENDIX B

**Coordination:** C.A. Rimmer and L.J. Wood of the NIST Chemical Sciences Division.

**Analytical Measurements:** C.A. Barber, H.V. Hayes, and L.J. Wood of the NIST Chemical Sciences Division.

**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.

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