National Institute of Standards & Technology

Report of Investigation

Reference Material 8187

Soy Protein Concentrate

This Reference Material (RM) is intended primarily for evaluation of methods for determining isoflavones in soy protein concentrates and in similar matrices. This RM can also be used for quality assurance when assigning values to in-house reference materials. A unit of RM 8187 consists of 5 packets, each packet containing approximately 10 g of powder.

The development of RM 8187 was a collaboration between the National Institute of Standards and Technology (NIST) and the National Institutes of Health, Office of Dietary Supplements (NIH-ODS).

Due to the lack of measurement capability at NIST to maintain the certified mass fractions values of isoflavones, SRM 3237 no longer meets international quality standards (ISO 17034) for serving as a certified reference material. However, the material continues to meet ISO 17034 standards as a reference material and is thus being offered as RM 8187.

Non-Certified Mass Fraction Values: The non-certified mass fraction values of isoflavones (as the total glycoside) in RM 8187 are provided in Table 1. A non-certified value for the mass fraction value for total protein (%) is provided in Table 2. Non-certified values are the best estimate of the true values based on available data; however, the values do not meet the NIST criteria for certification [1] and are provided with associated uncertainties that may reflect only measurement reproducibility, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods. Analyses for value assignment were performed by NIST, and non-certified values were calculated as the equally weighted mean of the mean values from NIST methods. The associated uncertainties are expressed at the 95 % level of confidence [2–4]. Values are reported on a dry-mass basis in mass fraction units [5]. Non-certified values are traceable to the measurement processes and standards used by NIST or the contract laboratory.

Period of Validity: RM 8187 is valid, within the measurement uncertainty specified, until **01 May 2031**, provided the RM is handled and stored in accordance with instructions given in this Report of Investigation (see "Instructions for Storage and Use"). This report is nullified if the RM is 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 Report of Investigation and notify the purchaser. Registration (see attached sheet or register online) will facilitate notification. However, before making use of any of the values delivered by this material, please check for the most recent documentation on the NIST SRM website at https://www.nist.gov/srm.

Support for the development of RM 8187 was provided in part by NIH-ODS. Technical consultation was provided by J.M. Betz of NIH-ODS.

Overall direction and coordination of the technical measurements leading to the value assignment of this RM were performed by M.M. Phillips, C.Q. Burdette, K.A. Lippa, L.C. Sander, and S.A. Wise of the NIST Chemical Sciences Division, and K.E. Sharpless of the NIST Special Programs Office.

Analytical measurements at NIST were performed by M. Bedner, M.A. Nelson, M.M. Phillips, and L.J. Wood of the NIST Chemical Sciences Division, and B.J. Porter, formerly of NIST.

Statistical analysis was provided by J.H. Yen of the NIST Statistical Engineering Division.

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

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NOTICE AND WARNING TO USERS

RM 8187 IS INTENDED FOR LABORATORY USE ONLY, NOT FOR HUMAN CONSUMPTION.

INSTRUCTIONS FOR STORAGE AND USE

Storage: The original unopened packets of RM 8187 should be stored at controlled room temperature (20 °C to 25 °C). Once the packet is opened, the long-term stability of all analytes in RM 8187 is unknown. Therefore, the value assignment only applies to the initial use, and the same results are not guaranteed if the remaining powder is used longer than two months after opening.

Use: Prior to removal of a test portion for analysis, the contents of a packet of material should be mixed thoroughly by shaking the packet and allowing the contents to settle for one minute prior to opening to minimize the loss of fine particles. For non-certified values to be valid, a test portion of at least 200 mg should be used; the homogeneity of test portions less than 200 mg has not been evaluated. Results obtained in analyses should include their own estimates of uncertainty and can be compared to the non-certified values using procedures described in reference 6. Test portions should be analyzed as-received and results converted to a dry-mass basis by determining moisture content (described below) on a separate test portion.

SOURCE, PREPARATION, AND ANALYSIS[(1)]

Source and Preparation: RM 8187 is a soy protein isolate prepared by a manufacturer of food and agricultural products. The product was packaged into single-use, nitrogen-flushed pouches, each containing 10 g of powder. Following packaging, RM 8187 was irradiated (Neutron Products, Inc., Dickerson, MD) to an absorbed dose of 6.0 kGy to 10.0 kGy.

NIST Analyses for Isoflavones Using Isotope Dilution Liquid Chromatography with Mass Spectrometric Detection (ID-LC/MS): Mass fractions of daidzin, genistin, and glycitin were measured at NIST using ID-LC/MS. Calibrants were prepared gravimetrically, at levels intended to approximate the levels of the isoflavones in the RM. Internal standards were employed; a single solution was used for the calibrants and samples. Duplicate 700 mg test portions of powder from each of 6 packets were accurately weighed into 15 mL polyethylene centrifuge tubes. An aliquot of a mixed internal standard solution containing $^{13}C_6$-daidzin, $^{13}C_6$-genistin, and $^{13}C_6$-glycitin was added. Analytes were extracted from the sample, then hydrolyzed to convert acetyl- and malonyl-glycosides to free glycosides, neutralized, diluted, and centrifuged prior to injection. Details of the separation and a typical chromatogram are provided in Figure 1. The separation was monitored using an absorbance detector at 260 nm, but MS was used for quantitation. Daidzin and $^{13}C_6$-daidzin were monitored at *m/z* 417 and *m/z* 423, respectively. Genistin and $^{13}C_6$-genistin were monitored at *m/z* 433 and *m/z* 439, respectively. Glycitin and $^{13}C_6$-glycitin were monitored at *m/z* 447 and *m/z* 453, respectively.

NIST Analyses for Isoflavones Using Liquid Chromatography with Absorbance Detection (LC/absorbance): Mass fractions of daidzin, genistin, and glycitin were measured at NIST using LC/absorbance. Calibrants were prepared gravimetrically, at levels intended to approximate the levels of the isoflavones in the RM. An internal standard approach was utilized with a single solution used for the calibrants and samples. Duplicate 200 mg test portions of powder from each of 10 packets were accurately weighed into 15 mL polyethylene centrifuge tubes. An aliquot of an internal standard solution containing sissotrin was added. Analytes were extracted from the sample, then hydrolyzed to convert acetyl- and malonyl-glycosides to free glycosides, neutralized, diluted, and centrifuged prior to injection. Details of the separation and a typical chromatogram are provided in Figure 2. The separation was monitored and quantitation performed using an absorbance detector at 254 nm.

Determination of Moisture: Moisture content of RM 8187 was determined at NIST (see "Instructions for Storage and Use") by (1) freeze drying to constant mass over 8 days; (2) drying over magnesium perchlorate in a desiccator at room temperature for 26 days; and (3) drying for 2 h in a forced air oven at 90 °C. The results obtained using all three techniques were averaged to determine a dry-mass proportion of (0.942 ± 0.003) gram dry-mass per gram as-received mass; the uncertainty shown on this value is an expanded uncertainty (expansion factor $k = 2$) representing 95 % 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 0.15 % for the conversion factor obtained

[(1)] Certain commercial equipment, instruments or materials are identified in this report 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.

from the moisture measurements is incorporated in the uncertainties of the non-certified values, reported on a dry-mass basis, that are provided in this report.

Homogeneity Assessment: The homogeneity of isoflavones in the RM was assessed at NIST using the methods and test portion sizes described above and analyses of variance with 5 % significance level; because of possible inhomogeneity, an uncertainty component for heterogeneity is incorporated in the combined uncertainty of each value.

Value Assignment: The equally weighted mean of NIST results provided by LC/absorbance and ID-LC/MS were used to calculate assigned values.

Non-Certified Mass Fraction Values for Isoflavones: The non-certified mass fraction values for daidzin, genistin and glycitin are the mean from the combination of the mean results provided by LC/absorbance and ID-LC/MS by NIST. The uncertainty provided is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c incorporates the observed difference between the results from the methods and their respective uncertainties, and uncertainty components related to moisture correction and to possible inhomogeneity, consistent with the ISO/JCGM Guide and with its Supplement 1, and $k = 2$ as a coverage factor corresponding to approximately 95 % confidence [2–4]. The measurands are the total mass fractions of isoflavones in soy protein concentrate.

Table 1. Non-Certified Mass Fraction Values (Dry-Mass Basis) for Isoflavones in RM 8187

	Mass Fraction (mg/kg)
Daidzin[(a)]	7.79 ± 0.34
Genistin	12.3 ± 2.1
Glycitin	0.81 ± 0.14

[(a)] Value was determined using a hydrolysis approach, and therefore represents total glycosides (sum of glycoside, malonyl-glycoside, and acetyl-glycoside present in the material).

Non-Certified Mass Fraction Value for Total Protein (%): The non-certified mass fraction value for total protein (%) was determined by the mean of the measurement results obtained from a modified AOAC 968.06 Protein (Crude) in animal feed (Dumas method) performed by a contract laboratory. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c incorporates the observed difference between the results from the methods and their respective uncertainties, and uncertainty components related to moisture correction and to possible inhomogeneity, consistent with the ISO/JCGM Guide and with its Supplement 1, and $k = 2.07$ as a coverage factor corresponding to approximately 95 % confidence [2–4]. The measurand is the total mass fraction of protein in soy protein concentrate.

Table 2. Non-Certified Mass Fraction (Dry-Mass Basis) for Total Protein (%) in RM 8187

	Mass Fraction (%)
Total Protein[(a)]	70.3 ± 0.3

[(a)] Value was determined using a modified AOAC 968.06 Protein (Crude) in animal feed (Dumas method).

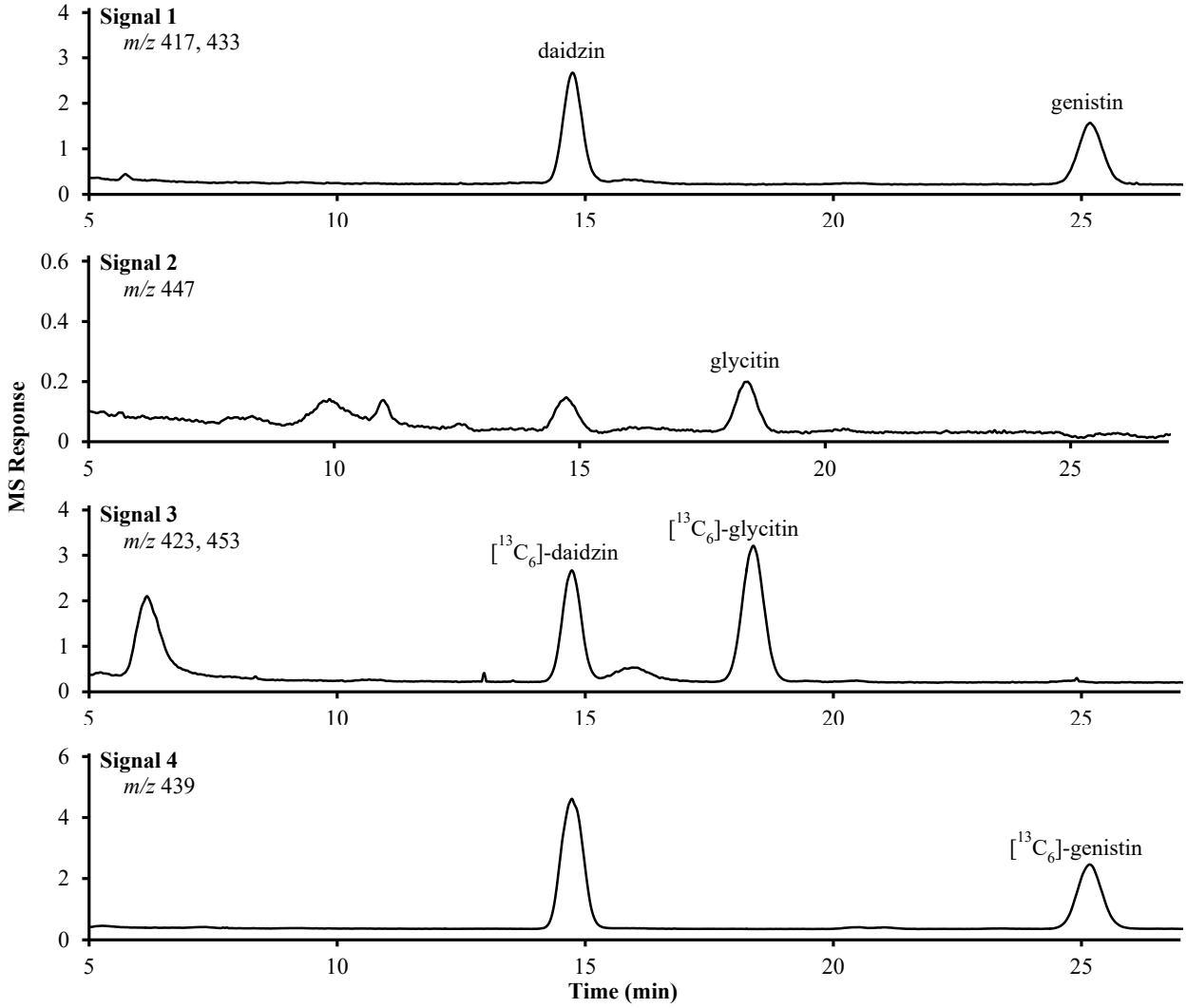

Figure 1. Chromatograms showing separation and detection of isoflavones in RM 8187 using ID-LC/MS. For this method, a Zorbax SB-CN column (250 mm × 4.6 mm, 5 µm particle size; Agilent Technologies, Wilmington, DE, USA) was held at 23 °C. The separation was performed using a gradient consisting of water and methanol, each containing 0.1 % formic acid (volume fraction). Mass spectrometric detection with electrospray ionization was utilized in the positive ion mode with selected ion monitoring as described in the text and within the figure.

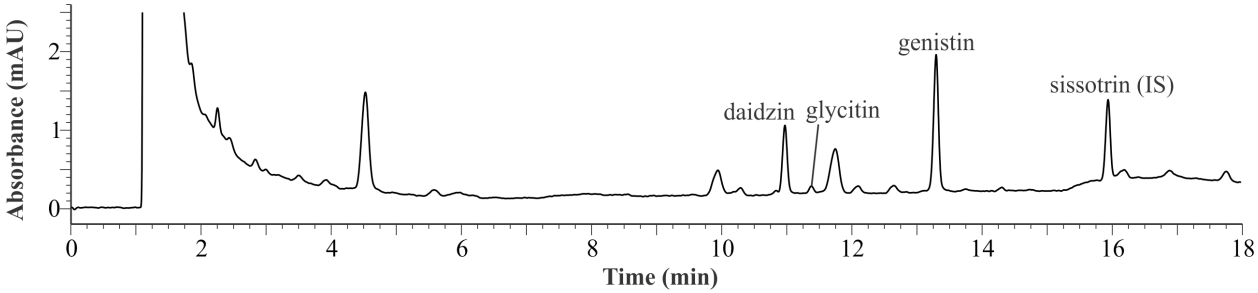

Figure 2. Chromatogram showing separation and detection of isoflavones in RM 8187 using LC/absorbance. For this method, an Ascentis Express RP-Amide column (150 mm × 4.6 mm, 2.7 µm particle size; Supelco, Bellefonte, PA, USA) was held at 35 °C. The separation was performed using a gradient consisting of 5 mmol/L ammonium acetate in water and acetonitrile. Absorbance detection was utilized at 254 nm.

REFERENCES

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[6] Sharpless, K.E.; Duewer, D.L.; *Standard Reference Materials for Analysis of Dietary Supplements*; J. AOAC Int., Vol. 91, pp. 1298–1302 (2008).

Users of this RM should ensure that the Report of Investigation in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; e-mail srminfo@nist.gov; or via the Internet at https://www.nist.gov/srm.