



National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material[®] 3237

Soy Protein Concentrate

This Standard Reference Material (SRM) is intended primarily for validation of methods for determining isoflavones in soy protein concentrates and in similar matrices. This SRM can also be used for quality assurance when assigning values to in-house reference materials. A unit of SRM 3237 consists of 5 packets, each containing approximately 10 g of material.

The development of SRM 3237 was a collaboration between the National Institute of Standards and Technology (NIST) and the National Institutes of Health, Office of Dietary Supplements (NIH-ODS).

Certified Mass Fraction Value: The certified mass fraction value for daidzin (as the total glycoside) in SRM 3237 is provided in Table 1. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [1]. Analyses for value assignment were performed by NIST, and 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].

Reference Mass Fraction Values: The reference mass fraction values for genistin and glycitin (as the total glycosides) are provided in Table 2. Reference values are noncertified values that 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.

Expiration of Certification: The certification of **SRM 3237** is valid, within the measurement uncertainty specified, until **15 August 2019**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see “Instructions for Storage and Use”). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Support for the development of SRM 3237 was provided in part by NIH-ODS. Technical consultation was provided by J.M. Betz of NIH-ODS.

The overall direction and coordination of the technical measurements leading to the certification of this SRM were performed by M.M. Phillips, L.C. Sander, K.E. Sharpless, and S.A. Wise of the NIST Chemical Sciences Division.

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

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

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

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Certificate Issue Date: 17 October 2014

Robert L. Watters, Jr., Director
Office of Reference Materials

NOTICE AND WARNING TO USERS

SRM 3237 IS INTENDED FOR LABORATORY USE ONLY, NOT FOR HUMAN CONSUMPTION.

INSTRUCTIONS FOR STORAGE AND USE

Storage: The original unopened packets of SRM 3237 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 SRM 3237 is unknown. Therefore, the certification 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 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 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: SRM 3237 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, SRM 3237 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 SRM. 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 ¹³C₆-daidzin, ¹³C₆-genistin, and ¹³C₆-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 ¹³C₆-daidzin were monitored at *m/z* 417 and *m/z* 423, respectively. Genistin and ¹³C₆-genistin were monitored at *m/z* 433 and *m/z* 439, respectively. Glycitin and ¹³C₆-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 SRM. 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 SRM 3237 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 conversion factor of (0.942 ± 0.003) gram dry mass per gram as received mass, which was used to convert data from an as-received to a dry-mass basis; the uncertainty shown on this value is an expanded uncertainty. An uncertainty component for the conversion factor (0.15 %) obtained from the moisture measurements is incorporated in the uncertainties of the certified values, reported on a dry-mass basis, that are provided in this certificate.

⁽¹⁾ Certain commercial equipment, instruments or materials are identified in this certificate 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.

Homogeneity Assessment: The homogeneity of isoflavones in the SRM 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.

Certified Mass Fraction Value for Daidzin: The certified mass fraction value for daidzin is 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 is a coverage factor corresponding to approximately 95 % confidence [2-4]. The measurand is the total mass fraction of daidzin in soy protein concentrate. The certified value is metrologically traceable to the SI unit of mass.

Table 1. Certified Mass Fraction Value (Dry-Mass Basis) for Daidzin in SRM 3237

	Mass Fraction (mg/kg)	Coverage Factor, k
Daidzin ^(a)	7.79 ± 0.34	2.00

^(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).

Reference Mass Fraction Values for Genistin and Glycitin: The reference mass fraction values for 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 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 is a coverage factor corresponding to approximately 95 % confidence [2-4]. The measurands are the mass fractions of genistin and glycitin in soy protein concentrate as determined by the methods indicated in this certificate. The reference values are metrologically traceable to the SI unit of mass.

Table 2. Reference Mass Fraction Values (Dry-Mass Basis) for Genistin and Glycitin in SRM 3237

	Mass Fraction ^(a) (mg/kg)	Coverage Factor, k
Genistin	12.3 ± 2.1	2.00
Glycitin	0.81 ± 0.14	2.00

^(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).

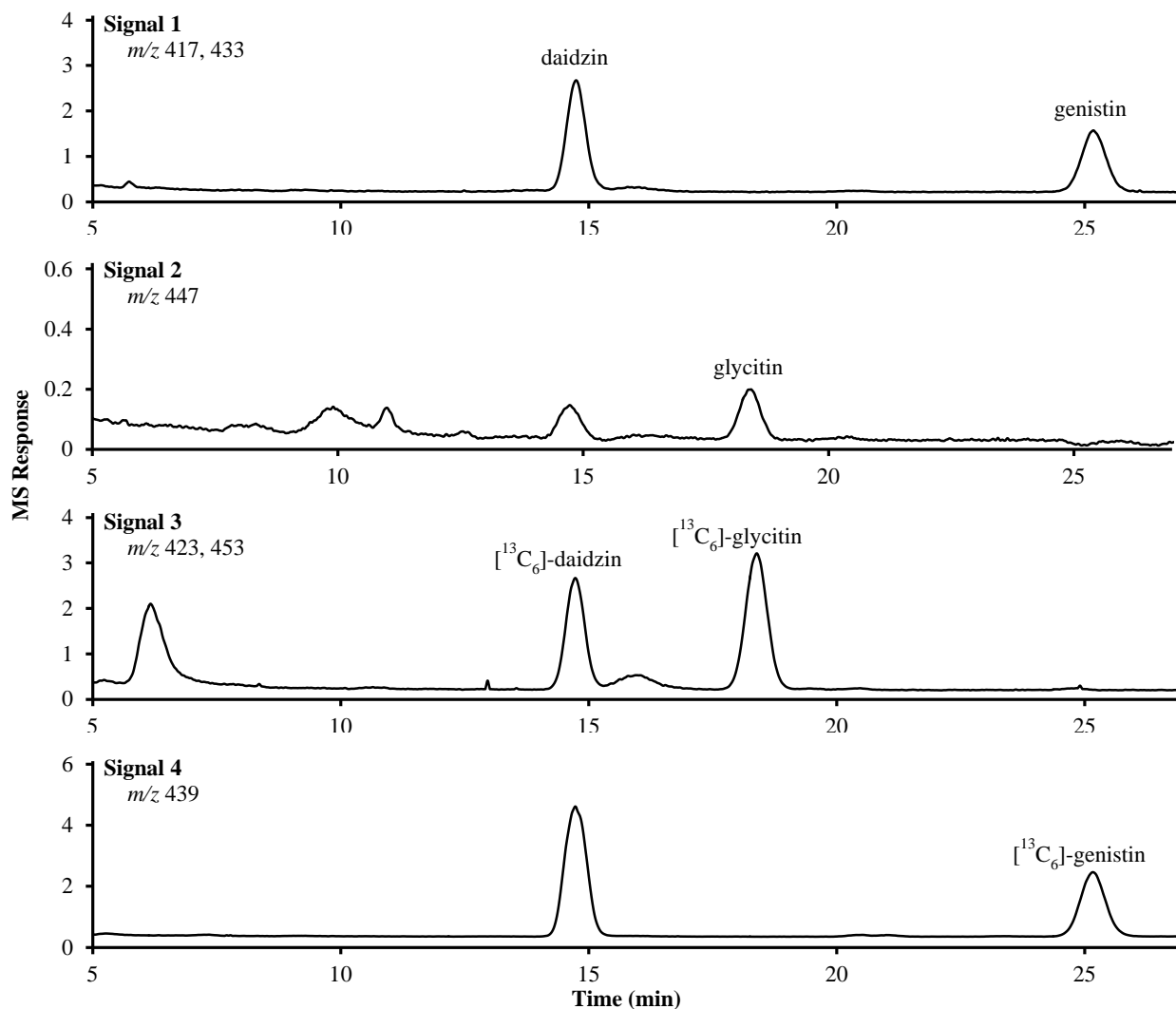


Figure 1. Chromatograms showing separation and detection of isoflavones in SRM 3237 using ID-LC/MS. For this method, a Zorbax SB-CN column (250 mm \times 4.6 mm, 5 μm particle size; Agilent Technologies, Wilmington, DE, USA) was held at 23 $^{\circ}\text{C}$. The separation was performed using a gradient 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.

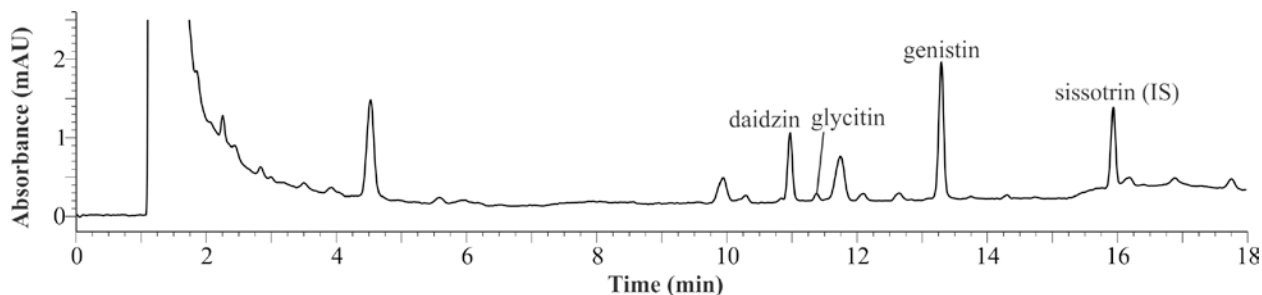


Figure 2. Chromatogram showing separation and detection of isoflavones in SRM 3237 using LC/absorbance. For this method, an Ascentis Express RP-Amide column (150 mm \times 4.6 mm, 2.7 μ m particle size; Supelco, Bellefonte, PA, USA) was held at 35 $^{\circ}$ C. The separation was performed using a gradient 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 SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or via the Internet at <http://www.nist.gov/srm>.