National Institute of Standards and Technology
U.S. Department of Commerce

Date of Issue:
20 July 2022

Standard Reference Material® 3234

Soy Flour

CERTIFICATE OF ANALYSIS

Purpose: The certified values delivered by this Standard Reference Material (SRM) are intended for validating methods for determining elements in soy flour and similar materials and can be used for quality assurance, such as when assigning values to in-house control materials.

Description: A unit of SRM 3234 consists of one bottle containing approximately 50 g of defatted soy flour prepared by a commercial manufacturer. Each bottle is sealed inside an aluminized pouch.

Certified Values: These values are traceable to International System of Units (SI) derived unit of mass fraction, expressed as milligrams per kilogram. The values are reported on a dry-mass basis [1].

Element	Mass Fraction[(a)] (mg/kg)
Calcium (Ca)	3191 ± 56
Copper (Cu)	15.34 ± 0.26
Iron (Fe)	80.3 ± 2.7
Magnesium (Mg)	3487 ± 60
Manganese (Mn)	36.78 ± 0.88
Phosphorus (P)	8080 ± 210
Potassium (K)	25010 ± 560
Zinc (Zn)	48.9 ± 1.1

[(a)] Values are expressed as $x \pm U_{95\%}(x)$, where x is the certified value and $U_{95\%}(x)$ is the expanded uncertainty of the certified value. The true value of the analyte lies within the interval $x \pm U_{95\%}(x)$ with 95 % confidence. To propagate this uncertainty, treat the certified value as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$.

Non-Certified Values: Non-certified values are provided in Appendix A.

Additional Information: Additional information is provided in Appendices B and C.

Period of Validity: The certified values delivered by **SRM 3234** are valid within the measurement uncertainty specified until **20 August 2032**. The certified values are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

Maintenance of Certified Values: NIST will monitor this SRM over the period of its validity. If substantive technical changes occur that affect the certification, NIST will issue an amended certificate through the NIST SRM website (https://www.nist.gov/srm) and notify registered users. SRM 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 SRM. 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).

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Safety: SRM 3234 is intended for research use; not for human consumption. Consult the Safety Data Sheet (SDS) for hazard information.

Storage: The original unopened bottles of SRM 3234 should be stored at room temperature (20 °C ± 5 °C). An open bottle can be reused for elemental analyses until the material reaches its expiration date, provided that the open bottle is resealed and stored at room temperature (20 °C ± 5 °C).

Use: Before use, the contents of the unopened bottle should be mixed thoroughly by rotating and/or rolling then allowed to settle for one minute prior to opening to minimize the loss of fine particles. Homogeneity of the material has not been evaluated for sample sizes smaller than those used by NIST methods described below. Therefore, the certified values may not be valid for test portions smaller than 0.5 g for determination of elements. Test portions should be analyzed as received and results converted to a dry-mass basis by determining moisture content on a separate test portion.

Source and Preparation: Three hundred twenty kilograms (from fourteen 50-pound bags) of defatted soy flour were blended and bottled by High-Purity Standards (Charleston, SC). The soy flour was placed in 4-ounce amber bottles that had been flushed with nitrogen. The bottles were capped and sealed with heat-shrink tape, then individually sealed in Mylar bags. Following bottling, SRM 3234 was irradiated by Neutron Products, Inc. (Dickerson, MD) to an absorbed dose of 7 kGy to 10 kGy.

Analysis: Value assignment of the mass fractions of the elements in SRM 3234 was based on the combination of measurements provided by NIST using inductively coupled plasma optical emission spectrometry (ICP-OES) and the median of mean results provided by collaborating laboratories.

NIST Analyses: The mass fractions of calcium, copper, iron, potassium, magnesium, manganese, phosphorus, and zinc were measured by ICP-OES using duplicate 0.5 g test portions taken from each of 12 bottles of SRM 3234. Samples for ICP-OES were digested in a nitric acid/hydrofluoric acid mixture using a microwave sample preparation system. Indium was added as an internal standard, and quantification for all elements was based on the method of standard additions using the SRM 3100 series single element standard solutions.

Collaborating Laboratories' Analyses: Analysts at fourteen collaborating laboratories (Appendix B) analyzed SRM 3234 as part of an interlaboratory comparison exercise of the Grocery Manufacturers Association (GMA) Food Industry Analytical Chemists Committee (FIACC). Collaborating laboratories were asked to use their usual methods to make measurements on single test portions taken from each of two bottles of SRM 3234. Methods reported by collaborating laboratories are described in Appendix B.

Homogeneity Assessment: The homogeneity of elements was assessed at NIST using the method and test portion size described. Analysis of variance did not show statistically significant heterogeneity. Other analytes have been treated as though they are homogeneously distributed in the material.

Value Assignment: For calculation of assigned values for analytes that were measured only by NIST, the mean of the NIST results was used. For calculation of assigned values for analytes that were measured only by the collaborating laboratories, the median of the laboratory means was used. For analytes that were measured by both NIST and collaborating laboratories, the mean of the NIST data and the median of the individual collaborating laboratory means were averaged, as appropriate. All assigned values were determined on an as-received basis and converted to a dry-mass basis using a conversion factor that is the inverse of the dry-mass proportion. The dry-mass-proportion of (0.9387 ± 0.0049) gram dry mass per gram as-received mass was determined by averaging results obtained at NIST by using (1) freeze drying to constant mass over 7 d; (2) drying over magnesium perchlorate in a desiccator at room temperature for 21 d; and (3) drying in a forced-air oven at 90 °C for 2 h. The uncertainty in the dry mass-proportion is an expanded uncertainty ($k = 2$) corresponding to a 95 % level of confidence. An uncertainty component for the conversion factor (0.26 %) obtained from the moisture measurements is incorporated in the uncertainties of the certified and non-certified values, reported on a dry-mass basis, that are provided in this certificate and its appendices.

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 Jul 2022).

Certificate Revision History: 20 July 2022 (Correction to units in Table 1; editorial changes); **13 May 2022** (Change of period of validity; removal of certified values for riboflavin and pantothenic acid based on potential instability and NIST's decision to no longer support these measurement capabilities in this matrix; correction of name of cysteine to cystine; updated format; editorial changes); **25 October 2017** (Change of expiration date; removal of certified values for thiamine, niacin, niacinamide, total vitamin B_3, pyridoxal hydrochloride, pyridoxamine dihydrochloride, pyridoxine hydrochloride, and total vitamin B_6 based on NIST's decision to no longer support these measurement capabilities in this matrix; removal of certified values for choline and carnitine based on observed instability; removal of reference values for fatty acids based on observed instability; correction of value for phenylalanine; editorial changes); **15 October 2014** (Addition of reference values for isoflavones; removal of reference value for solids; editorial changes); **08 July 2013** (Addition of certified values for forms of vitamin B_6; editorial changes); **28 September 2012** (Original certificate date).

Certain commercial equipment, instruments, or materials may be identified in this Certificate of Analysis 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 SRM should ensure that the Certificate in their possession is current. This can be accomplished by contacting the Office of Reference Materials 100 Bureau Drive, Stop 2300, Gaithersburg, MD20899-2300; telephone (301) 975-2200; e-mail srminfo@nist.gov; or the Internet at https://www.nist.gov/srm.

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APPENDIX A

Non-Certified Values: Non-certified values are suitable for use in method development, method harmonization, and process control but do not meet the NIST criteria for certification [1] nor provide metrological traceability to the International System of Units (SI) or other higher-order reference system. They 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 elements and isoflavones, determined at NIST and reported on a dry-mass basis, are provided in Table A1. Non-certified mass fraction values for proximates, amino acids, and calories, determined by collaborating laboratories participating in an interlaboratory comparison exercise of the GMA FIACC and reported on a dry-mass basis, are provided in Tables A2 and A3.

Table A1. Non-Certified Mass Fraction Values (Dry-Mass Basis) for Elements and Isoflavones in SRM 3234

Analyte	Mass Fraction[(a)] (mg/kg)
Sodium (Na)	2.52 ± 0.45
Daidzein	14.0 ± 3.0
Daidzin[(b)]	1680 ± 530
Genistein	15.49 ± 0.30
Genistin[(b)]	2080 ± 520
Glycitin[(b)]	245 ± 46

(a) These values are expressed as $x \pm U_{95\%}(x)$, where x is the noncertified value and $U_{95\%}(x)$ is the expanded uncertainty of the noncertified value. To propagate this uncertainty, treat the noncertified value as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$. These values are metrologically traceable to results from NIST measurement procedures.

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

NIST Analyses for Na Using ICP-OES: The mass fraction of sodium was measured by ICP-OES using duplicate 1.0 g test portions taken from each of 12 bottles of SRM 3234. Samples for ICP-OES were digested in a nitric acid/hydrofluoric acid mixture using a microwave sample preparation system. Indium was added as an internal standard, and quantification was based on the method of standard additions using SRM 3152a *Sodium (Na) Standard Solution*.

NIST Analyses for Isoflavones Using ID-LC-MS: The mass fractions of daidzein, daidzin, genistein, genistin, and glycitin were measured by ID-LC-MS in duplicate 100 mg test portions taken from each of 12 bottles of SRM 3234. An aliquot of a mixed internal standard solution containing $^{13}C_6$-daidzin, $^{13}C_6$-daidzein, $^{13}C_6$-genistein, $^{13}C_6$-genistin, $^{13}C_6$-glycitein, and $^{13}C_6$-glycitin was added to each calibrant and sample. Analytes and internal standards were extracted from the sample into 80:20 volume fraction methanol:water, then hydrolyzed with sodium hydroxide 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 C1. Calibrants were prepared gravimetrically, at levels intended to approximate the levels of the isoflavones in the SRM following extraction. The purity of the isoflavone calibrant materials was determined at NIST using quantitative proton nuclear magnetic resonance spectroscopy (qNMR). A single internal standard solution was used for the calibrants and samples.

NIST Analyses for Isoflavones Using LC-absorbance: Mass fractions of daidzein, daidzin, genistein, genistin, and glycitin were measured by LC-absorbance in duplicate 200 mg test portions taken from each of 12 bottles of SRM 3234. An aliquot of an internal standard solution containing sissotrin was added, and analytes and the internal standard were extracted from the sample into 80:20 volume fraction methanol:water, then hydrolyzed with sodium hydroxide 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 C2. The separation was monitored and quantitation was performed using an absorbance detector at 254 nm. Calibrants were prepared gravimetrically, at levels intended to approximate the levels of the isoflavones in the SRM following extraction. The purity of the isoflavone calibrant materials was determined at NIST using qNMR. A single internal standard solution was used for the calibrants and samples.

Table A2. Non-Certified Mass Fraction Values (Dry-Mass Basis) for Proximates and Amino Acids in SRM 3234

Analyte	Mass Fraction[a] (g/100 g)	n[b]
Ash	6.77 ± 0.14	14
Protein	53.37 ± 0.36	14
Fat (sum of fatty acids as triglycerides)	1.49 ± 0.12	13
Carbohydrates	37.14 ± 0.69	14
Total Dietary Fiber	18.19 ± 0.37	10
Alanine	2.28 ± 0.16	6
Arginine	3.72 ± 0.31	6
Aspartic Acid	6.0 ± 1.2	6
Cystine	0.74 ± 0.15	4
Glutamic Acid	10.2 ± 1.4	6
Glycine	2.22 ± 0.15	6
Histidine	1.222 ± 0.089	6
Isoleucine	2.31 ± 0.23	6
Leucine	4.03 ± 0.42	6
Lysine	3.20 ± 0.25	6
Methionine	0.69 ± 0.13	6
Phenylalanine	2.54 ± 0.13	6
Proline	2.71 ± 0.23	6
Serine	2.69 ± 0.32	6
Threonine	2.02 ± 0.11	6
Tryptophan	0.66 ± 0.14	4
Tyrosine	1.76 ± 0.43	6
Valine	2.45 ± 0.41	6

[a] These values are expressed as $x \pm U_{95\%}(x)$, where x is the noncertified value and $U_{95\%}(x)$ is the expanded uncertainty of the noncertified value. To propagate this uncertainty, treat the noncertified value as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$. These values are metrologically traceable to results reported by participants of an interlaboratory comparison study sponsored by the GMA FIACC using their routine methods.

[b] Number of contributors providing technically valid results.

Table A3. Non-Certified Mass Fraction Values (Dry-Mass Basis) for Calories in SRM 3234

	Mass Fraction[a] (kcal/100 g)	n[b]
Calories	377.7 ± 3.7	13

[a] This value is expressed as $x \pm U_{95\%}(x)$, where x is the noncertified value and $U_{95\%}(x)$ is the expanded uncertainty of the noncertified value. To propagate this uncertainty, treat the noncertified value as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$. This value is metrologically traceable to results reported by participants of an interlaboratory comparison study sponsored by the GMA FIACC using their routine methods.

[b] Number of contributors providing technically valid results.

Collaborating Laboratories' Analyses: Analysts at many collaborating laboratories (Appendix B) analyzed SRM 3234 as part of an interlaboratory comparison exercise of the GMA FIACC. Collaborating laboratories were asked to use their usual methods to make measurements on single test portions of proximates, calories, and amino acids taken from each of two bottles of SRM 3234. Methods reported by collaborating laboratories are described in Appendix B.

Maintenance of Non-Certified Values: NIST will monitor this material to the end of the period of validity listed on the first page of the Certificate of Analysis. If substantive technical changes occur that affect the non-certified values during this period, NIST will update this Appendix. 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.

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APPENDIX B

Contributors to the Development and Value Assignment of SRM 3234

Coordination of Development, Production, and Technical Measurements
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Support and Technical Consultation for Value Assignment of Isoflavones
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Analytical Measurements at NIST
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Isoflavones: M. Bedner (NIST Chemical Sciences Division)
M.M. Phillips (NIST Chemical Sciences Division)
Moisture: L.J. Wood (NIST Chemical Sciences Division)
Chemical Purity: M.A. Nelson (NIST Chemical Sciences Division)

Statistical Analysis
J.H. Yen (NIST Statistical Engineering Division)

Collaborating Laboratories Contributing Data to Value Assignment through GMA FIACC
Campbell Soup Company (Camden, NJ, USA)
Conagra Foods (Omaha, NE, USA)
Covance Laboratories, Inc. (Madison, WI, USA)
Del Monte Foods (Walnut Creek, CA, USA)
Eurofins Central Analytical Laboratories (Metairie, LA, USA)
Eurofins Scientific (Des Moines, IA, USA)
General Mills, Inc. (Golden Valley, MN, USA)
Hormel Foods Corporation (Austin, MN, USA)
Krueger Food Laboratories (Billerica, MA, USA)
Land O'Lakes (Arden Hills, MN, USA)
Schwan Food Company (Salina, KS, USA)
Silliker (Crete, IL, USA)
The J.M. Smucker Co. (Orrville, OH, USA)
The National Food Laboratory (Livermore, CA, USA)

Methods Reported by Collaborating Laboratories Contributing Data to Value Assignment

Analyte	Method
Elements	Inductively coupled plasma optical emission spectrometry
	Absorption spectrophotometry
Ash	Weight loss after ignition in a muffle furnace
	Thermogravimetric analysis
Fat	Sum of fatty acids by gas chromatography
Protein	Kjeldahl (nitrogen results converted to protein using a factor of 6.25)
	Thermal conductivity (nitrogen results converted to protein using a factor of 6.25)
	Pyrolysis gas chromatography (nitrogen results converted to protein using a factor of 6.25)
Dietary Fiber	Enzymatic digestion and gravimetry
Carbohydrates	Calculation as [solids – (protein + fat + ash)]
Calories	Calculation as [(9 x fat) + (4 x protein) + (4 x carbohydrate)]
Amino Acids	Hydrolysis and derivatization followed by liquid chromatography

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APPENDIX C

Chromatograms Depicting Separation and Detection of Isoflavones in SRM 3234

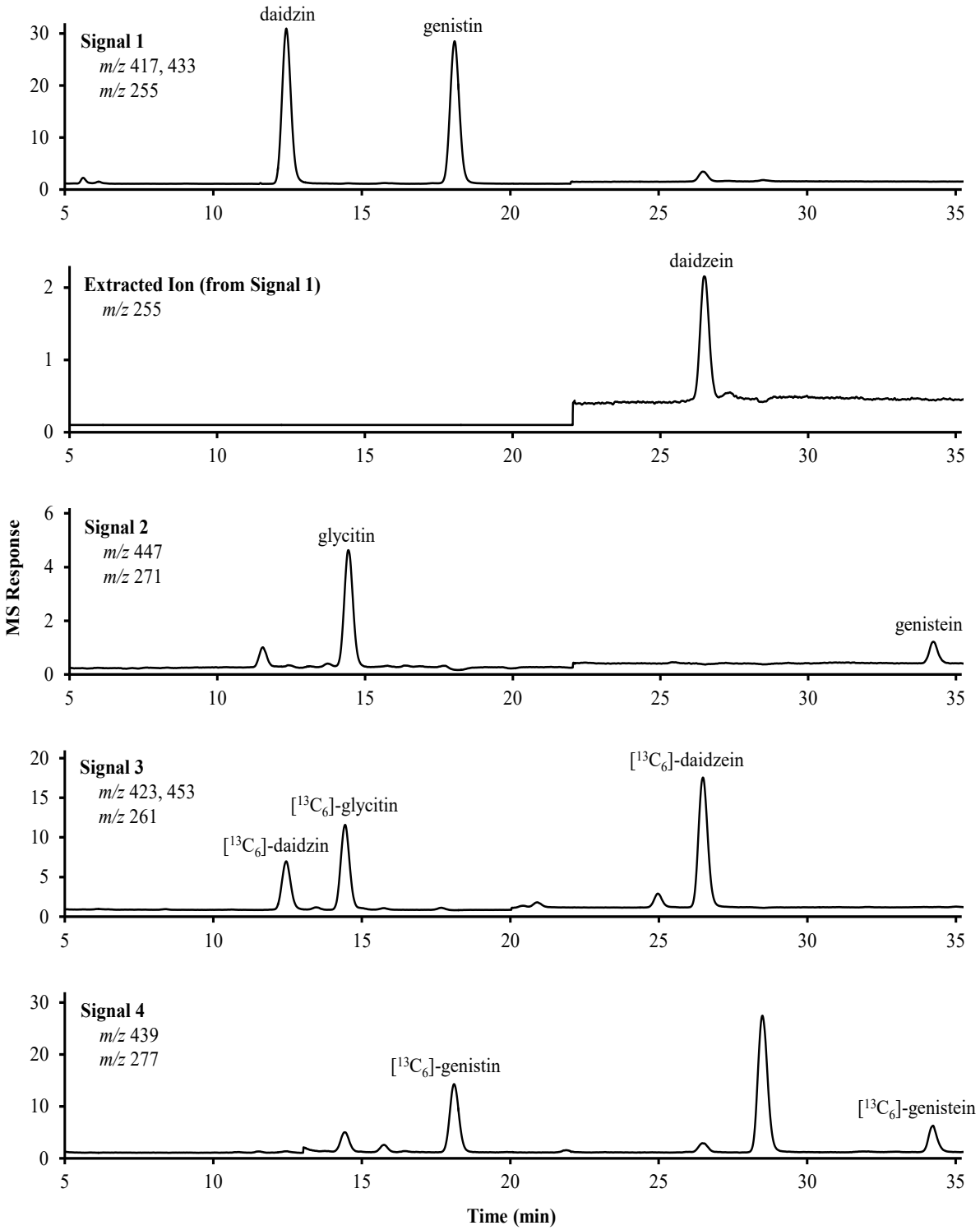

Figure C1. Chromatograms showing separation and detection of isoflavones in SRM 3234 using ID-LC-MS. For this method, a Zorbax SB-CN column (250 mm × 4.6 mm, 5 µm particle size; Agilent Technologies, Wilmington, DE) was held at 23 °C. The separation was performed using a gradient consisting of water 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. Daidzein and $^{13}C_6$-daidzein were monitored at *m/z* 255 and *m/z* 261, respectively. Daidzin and $^{13}C_6$-daidzin were monitored at *m/z* 417 and *m/z* 423, respectively. Genistein and $^{13}C_6$-genistein were monitored at *m/z* 271 and *m/z* 277, 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.

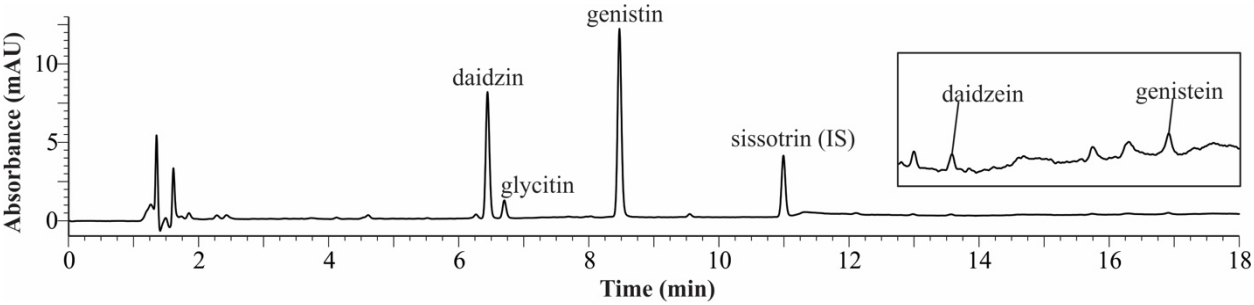

Figure C2. Chromatogram showing separation and detection of isoflavones in SRM 3234 using LC-absorbance. For this method, an Ascentis Express RP-Amide column (150 mm × 4.6 mm, 2.7 µm particle size; Supelco, Bellefonte, PA) was held at 35 °C. The separation was performed using a gradient consisting of 5 mmol/L ammonium acetate in water and acetonitrile. Absorbance detection was utilized at 254 nm.

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