



Certificate of Analysis

Standard Reference Material[®] 3232

Kelp Powder (*Thallus laminariae*)

This Standard Reference Material (SRM) is intended primarily for use in validating methods for determining elements, arsenic species, vitamin K₁, and proximates in kelp and similar matrices. This SRM can also be used for quality assurance when assigning values to in-house control materials. A unit of SRM 3232 consists of three heat-sealed, aluminized pouches, each containing approximately 5 g of material.

Certified Mass Fraction Values: The certified mass fraction values reported on a dry-mass basis are provided for elements 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]. The certified mass fraction values in this material are the unweighted means of the NIST method means. The expanded uncertainty is calculated as $U = ku_c$, where the combined uncertainty u_c incorporates the observed difference between method results, the method uncertainties, and an uncertainty component for moisture correction, consistent with the ISO/JCGM Guide and its Supplement 1 [2-4]. The coverage factor, k , corresponds to an approximately 95 % level of confidence. Each measurand in Table 1 is the total mass fraction of the element and is metrologically traceable to the SI derived unit of milligrams per kilogram on a dry-mass basis.

Non-Certified Mass Fraction Values: The non-certified mass fraction values reported on a dry-mass basis are provided for the additional elements in Table 2, phylloquinone (vitamin K₁) and arsenic species in Table 3, and proximates in Table 4; a non-certified value for calories on a dry-mass basis is also provided in Table 4. A NIST non-certified value is the best estimate of the true value based on available data; however, the value does not meet the NIST criteria for certification and is provided with an uncertainty that may not include all sources of uncertainty [1]. The non-certified values are derived from results reported by NIST or collaborating laboratories. The non-certified values are the unweighted means of the NIST or collaborator-reported method means. The expanded uncertainty is calculated as $U = ku_c$, where the combined uncertainty u_c incorporates the observed difference between method results, the method uncertainties, and an uncertainty component for moisture correction, consistent with the ISO/JCGM Guide and its Supplement 1 [2-4]. The coverage factor, k , corresponds to an approximately 95 % level of confidence.

Value of Potential Interest: The mass fraction value of potential interest reported on a dry-mass basis is provided for arsenous acid in Table 5. A NIST value of potential interest is considered to be a value that may be of interest to the SRM user, but insufficient information is available to assess the uncertainty associated with the value [1]. Values of potential interest cannot be used to establish metrological traceability.

Expiration of Certification: The certification of SRM 3232 is valid, within the measurement uncertainty specified, until **31 January 2031**, provided the SRM is handled and stored in accordance with 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.

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Coordination of the technical measurements leading to the certification of this SRM was performed by L.L. Yu of the NIST Chemical Sciences Division.

Support for the development of SRM 3232 was provided in part by the Office of Dietary Supplements of the National Institutes of Health (NIH-ODS). Technical consultation was provided by J.M. Betz (NIH-ODS). Acquisition and preparation of the material were coordinated by K.E. Sharpless of the NIST Chemical Sciences Division.

Analyses at NIST were performed by C.Q. Burdette, G.C. Caceres, M. Ellisor, R.L. Paul, and L.L. Yu, of the NIST Chemical Sciences Division and J.F. Browning, K.D. Chieh, W.C. Davis, B.L. Kassim, S.E. Long, K.E. Murphy, R. Oflaz, L.J. Wood, and R. Zeisler, 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 SRM were coordinated through the NIST Office of Reference Materials.

NOTICE TO USERS: SRM 3232 IS INTENDED FOR RESEARCH USE; NOT FOR HUMAN OR ANIMAL CONSUMPTION.

SOURCE AND PREPARATION

The material used to produce the kelp powder (*Thallus laminariae*) was harvested from the East China Sea when the kelp was approximately 6 months old. The kelp powder was blended and packaged at High-Purity Standards (Charleston, SC) into packets. A packet contains approximately 5 g of kelp powder sealed in a nitrogen flushed plastic bag, which was sealed inside a nitrogen-flushed aluminized polyethylene bag with two packets of silica gel. The packets of kelp powder were irradiated at Neutron Products (Dickerson, MD) to absorbed doses of 5.9 kGy to 7.6 kGy, and then packaged into units of SRM 3232 at NIST (Gaithersburg, MD).

INSTRUCTIONS FOR STORAGE AND USE

Storage: The SRM should be stored at controlled room temperature (20 °C to 25 °C) in the original unopened packets. From the date the packet is opened, the certification is valid for one year for elements, two weeks for arsenic species and proximates, and 24 h for vitamin K₁ and its isomers, provided that the remaining kelp material is resealed inside the plastic bag and the aluminized polyethylene bag, and the resealed packet is stored in controlled room temperature of 20 °C to 25 °C.

Use: Before use, the contents of the packet should be homogenized thoroughly by shaking the packet in the horizontal plane for 1 min. The contents should be allowed to settle for 1 min prior to opening to minimize the loss of fine particles. For certified values to be valid, test portion size should be based on descriptions below (See "Analysis"). Examples of how the measured values should be evaluated relative to those in the certificate are available in the literature [5]. The non-certified values of arsenic species in Table 3 and value of potential interest of arsenous acid in Table 5 are dependent on the extraction procedures. The extraction procedure described in the Analysis section must be followed to relate the measurement values to the arsenic species values of the certificate.

Correction to a Dry-Mass Basis: The SRM must be measured as received. A separate portion of the SRM must be used to determine the moisture contents for conversion of the analysis results from as-received to dry-mass basis. The portion of the SRM for moisture analysis must fill the glass weighing vessel to approximately 1 cm in depth and must be dried to a constant mass. The moisture content in mass fraction is calculated as the difference in mass of the test portion before and after drying divided by the mass of the test portion before drying. At NIST, the moisture content of SRM 3232 was determined by drying over magnesium perchlorate in a desiccator at room temperature for 28 d and by drying in a forced-air oven at 80 °C for 3 h. The means of the two methods were combined to derive the unweighted mean and the expanded uncertainty at approximately 95 % confidence of the dry-mass fraction of (0.9368 ± 0.0015) gram per gram of as-received mass.

ANALYSIS⁽¹⁾

Homogeneity Assessment: The homogeneity of elements and arsenic species was assessed using the methods and test portion sizes described below. Analyses of variance and graphical analyses of the data found no detectable

⁽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.

inhomogeneity at approximately 95 % level of confidence. For the values related to vitamin K₁, the uncertainty incorporates a component for possible inhomogeneity based on the standard deviation. Homogeneity of proximates was not assessed, although the data were treated as though the analytes were homogeneous.

Determination of Elements: Value assignment of the mass fractions of the elements in SRM 3232 was based on NIST measurements using inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS), isotope dilution ICP-MS (ID-ICP-MS), isotope dilution cold-vapor generation ICP-MS (ID-CV-ICP-MS), and instrumental neutron activation analysis (INAA).

ICP-OES and ICP-MS methods: Mass fractions of calcium, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc were measured by ICP-OES. Mass fractions of arsenic and iodine were measured by ICP-MS. For the determination of all elements except iodine, 0.5 g test portions from either six or ten packets of SRM 3232 were digested in a closed-vessel microwave system using nitric acid. For the determination of iodine, 0.2 g test portions from eight packets of SRM 3232 were digested in a closed-vessel microwave system using ammonium hydroxide. Quantification for all analyses was based on the method of standard additions using the SRM 3100 series single element standard solutions.

ID-CV-ICP-MS method [6]: The mass fraction of mercury was measured by ID-CV-ICP-MS. Test portions of 0.25 g from 6 packets were digested with enriched isotopes in a closed vessel microwave system using a mixture of hydrochloric acid and nitric acid. The digests were stored in a refrigerator overnight at 4 °C to allow degassing of excess nitrogen dioxide and carbon dioxide. The following day, mercury isotopes were measured in time-resolved analysis mode using cold vapor generation coupled with ICP-MS. Quantitative determinations are traceable to *SRM 1641d Mercury in Water*, a primary standard for mercury.

ID-ICP-MS methods: Mass fractions of cadmium, chromium, copper, lead, and molybdenum were measured by ID-ICP-MS. For the determination of chromium, copper, and molybdenum, test portions of 0.5 g from 6 packets were digested with enriched isotopes in a closed-vessel microwave system using a mixture of hydrofluoric acid and nitric acid. Isotopic measurements were made at mass resolution of approximately 4000 using a sector-field ICP-MS. For the determination of cadmium and lead, test portions of 0.6 g were digested with enriched isotopes in a closed-vessel microwave system using a mixture of hydrofluoric acid and nitric acid. Isotopic measurements were made using a quadrupole ICP-MS. Lead was measured in standard mode, and cadmium was measured in collision cell mode with kinetic energy discrimination using 8% hydrogen in balance helium as collision gas [7,8]. Quantitative determinations are traceable to the SRM 3100 series single element standard solutions.

INAA methods: Mass fractions of aluminum, arsenic, calcium, chlorine, cobalt, iodine, iron, potassium, magnesium, manganese, rubidium, sodium, and zinc were determined by INAA. Test portions of 0.2 g from 10 packets were made into 13 mm pellets, sealed in linear polyethylene bags, and irradiated at a reactor power of 20 MW. For the determination of aluminum, calcium, chlorine, iodine, potassium, magnesium, manganese, and sodium, the duration of the irradiation was 1 min. Gamma rays were counted for 5 min to 30 min after a 5 min to 3 h decay. For the determination of arsenic, cobalt, iron, rubidium, and zinc, the duration of the irradiation was 6 h. Gamma rays were counted for 2 h to 8 h after a 5 d to 1 month decay. Quantitative determinations are traceable to standards prepared from NIST SRMs and standards prepared from high purity metals from Alfa Aesar, Spex Industries, or Aldrich Chemicals.

Determination of Vitamin K₁: Value assignment of total vitamin K₁ as a sum of *trans*-vitamin K₁ and *cis*-vitamin K₁ was based on NIST measurements using an isotope dilution liquid chromatography atmospheric pressure chemical ionization tandem mass spectrometry (ID-LC-APCI-MS/MS) method. The ratio of vitamin K₁ isomers in SRM 3232 was determined with the assumption that the instrument response was the same for *trans*- and *cis*- isomers.

ID-LC-APCI-MS/MS method: The mass fractions of total and isomers of vitamin K₁ were determined by ID-LC-APCI-MS/MS. Test portions of 2.5 g from 10 packets were transferred into 50 mL polyethylene centrifuge tubes containing an appropriate amount of vitamin K₁-[²H₇] as an internal standard. For extraction, 30 mL of 30 mg/L butylated hydroxytoluene (BHT) in hexane was added. The samples were sonicated in a water bath for 1 h, mixed on a rotary mixer at 1 Hz for 1 h, centrifuged at 1400 g_n for 15 min, and the hexane was removed from the aqueous solution. Another 30 mL aliquot of hexane was added and the extraction process was repeated three more times. The hexane fractions from the four extraction steps were combined and 1 g magnesium sulfate was added. The contents were mixed and centrifuged. The hexane phase was decanted into a drying vessel, dried under nitrogen, and the residue was reconstituted in 1 mL of 1:1 volume ratio of ethanol:ethyl acetate that contained 30 mg/L BHT. The separation of vitamin K₁ isomers was accomplished by eluting with water:methanol gradient at 0.75 mL/min through a 150 mm X 3 mm C30 column. The LC-MS/MS was operated in positive ion mode and multiple reaction monitoring (MRM) mode. The transitions at *m/z* 451.4 → *m/z* 187.3 and at *m/z* 458.4 → *m/z* 194.4 were monitored

for vitamin K₁ and vitamin K₁-[²H₇], respectively. Quantitative determinations of vitamin K₁ are traceable to the molar absorptivity of vitamin K₁ [9].

Determination of Arsenic Species: Value assignment of the mass fractions of arsenic species in SRM 3232 was based on measurements at NIST using liquid chromatography followed by offline INAA determination (LC-INAA) and online ICP-MS determination (LC-ICP-MS).

NOTE: THE EXTRACTION PROCEDURE BELOW MUST BE FOLLOWED TO RELATE THE MEASURED VALUES TO THOSE IN THE CERTIFICATE.

Extraction procedure: Test portions of 1 g from 8 packets were transferred into 15 mL polypropylene test tubes, into which 10 mL of a solvent containing 50 % volume fraction of methanol in water was added. The contents were vortexed at 40 Hz for 1 min and then allowed to stand on the bench for 2 h at ambient temperature of 21 °C ± 1°C. The contents were vortexed at 40 Hz for 30 s and centrifuged at 3600 g_n for 30 min. The supernatant was used for speciation measurements by LC-INAA and LC-ICP-MS. The mass of the extracted arsenic species was calculated as the mass concentration of the species in the extract multiplied by the volume of the solvent.

LC-INAA method [10]: From the above extraction procedure, a 0.9 g aliquot of the extract was diluted in a 2 mL vial by weighing into the vial 0.9 mL water and 0.2 mL internal standard. A 5 µg/g monomethylarsonic acid (MMA) solution and a 5 µg/g trimethylarsine oxide (TMAO) solution were used as the internal standards for separation by anion exchange and cation exchange, respectively. A 50 µL aliquot of the extract containing the internal standard was injected onto a cation exchange or an anion exchange LC column and eluted under isocratic conditions using 30 mmol/L pyridine at pH 3.0 and 20 mmol/L ammonium carbonate at pH 9.0, respectively. Arsenic species were quantitatively collected in the fractions of the eluent. The solvent of each fraction was evaporated at 75 °C under a stream of nitrogen. The residue was reconstituted in water and quantitatively transferred to a filter paper, which was dried under an infrared lamp and pressed into a pellet for measurement using a procedure similar to the *INAA methods* described above. Quantitative determinations are traceable to standards prepared from SRM 3103a *Arsenic (As) Standard Solution*.

LC-ICP-MS method: From the above extraction procedure, a 0.9 g aliquot of the extract was diluted in a 2 mL vial by weighing into the vial 0.9 mL of water and 0.2 mL of 5 µg/g TMAO solution serving as an internal standard. A 0.5 g aliquot of the resulting solution was diluted to 2 g with water to form a measurement sample. A 1 g aliquot of the measurement sample solution was spiked with a 0.3 g standard containing 95 ng/g arsenosugar 328 and 30 ng/g dimethylarsinic acid (DMA). An unspiked sample was prepared by mixing 1 g of the measurement sample with 0.3 g water. The spiked and the unspiked kelp extract samples were analyzed for arsenous acid, DMA, TMAO, and arsenosugar 328 by cation exchange LC-ICP-MS using 30 mmol/L pyridine in a gradient elution method [11]. Quantitative determinations are traceable to standards prepared from SRM 3103a *Arsenic (As) Standard Solution* and SRM 83d *Arsenic Trioxide (As₂O₃) Reductometric Standard*.

Determination of Proximates: The results for proximates were provided by Covance Laboratories (Madison, WI) and National Food Lab (Livermore, CA). The measurement methods for proximates are referenced in the footnote of Table 4.

Table 1. Certified Mass Fraction Values for Elements in SRM 3232

Element	Mass Fraction (mg/kg)	Coverage Factor, <i>k</i>
Arsenic (As) ^(a,b)	38.3 ± 1.3	2.00
Cadmium (Cd) ^(c)	0.4259 ± 0.0084	2.00
Calcium (Ca) ^(b,d)	12260 ± 680	2.00
Chromium (Cr) ^(c)	5.92 ± 0.52	2.57
Copper (Cu) ^(c)	3.875 ± 0.087	2.03
Iodine (I) ^(a,b)	944 ± 88	2.00
Iron (Fe) ^(b,d)	672 ± 13	2.00
Lead (Pb) ^(c)	1.032 ± 0.039	2.16
Magnesium (Mg) ^(b,d)	6130 ± 180	2.00
Manganese (Mn) ^(b,d)	24.6 ± 1.6	2.00
Mercury (Hg) ^(c)	0.1129 ± 0.0032	2.00
Molybdenum (Mo) ^(c)	0.2441 ± 0.0091	2.13
Potassium (K) ^(b,d)	76000 ± 1100	2.00
Sodium (Na) ^(b,d)	16330 ± 380	2.00
Zinc (Zn) ^(b,d)	27.4 ± 1.1	2.00

(a) ICP-MS

(b) INAA

(c) ID-ICP-MS

(d) ICP-OES

(e) ID-CV-ICP-MS

Table 2. Non-Certified Mass Fraction Values for Elements in SRM 3232

Element	Mass Fraction (mg/kg)	Coverage Factor, <i>k</i>
Aluminum (Al) ^(a)	1070 ± 110	2.07
Chlorine (Cl) ^(a)	35600 ± 1200	2.03
Cobalt (Co) ^(a)	0.307 ± 0.011	2.06
Phosphorus (P) ^(b)	4551 ± 51	2.05
Rubidium (Rb) ^(a)	28.44 ± 0.51	2.06

(a) INAA

(b) ICP-OES

Table 3. Non-Certified Mass Fraction Values for Vitamin K₁ and Arsenic Species^(a) in SRM 3232

	Mass Fraction (mg/kg)	Coverage Factor, <i>k</i>
Total Vitamin K ₁ ^(b)	0.431 ± 0.081	2.09
<i>cis</i> -Vitamin K ₁ ^(b)	0.0353 ± 0.0067	2.09
<i>trans</i> -Vitamin K ₁ ^(b)	0.396 ± 0.075	2.09
Arsenosugar 328 ^(c,d)	1.20 ± 0.14	2.00
Arsenosugar 392 ^(c)	14.06 ± 0.72	2.03
Arsenosugar 482 ^(c)	5.59 ± 0.51	2.00
Dimethylarsinic acid ^(c,d)	0.479 ± 0.077	2.00

(a) The quantity of a species is expressed as mass fraction of arsenic. Refer to the Appendix for nomenclature for arsenosugars.

(b) ID-LC-APCI-MS/MS

(c) LC-INAA

(d) LC-ICP-MS

Table 4. Non-Certified Mass Fraction Values for Proximates and Calories in SRM 3232^(a)

	Mass Fraction (g/100 g)	Coverage Factor, <i>k</i>
Ash ^(b)	23.55 ± 0.57	2.00
Carbohydrates ^(c)	56.7 ± 1.0	2.00
Protein ^(d)	14.48 ± 0.98	2.00
Total Fat ^(e)	2.4 ± 1.3	2.00
	Energy (kcal/100 g)	Coverage Factor, <i>k</i>
Calories ^(f)	306.2 ± 4.0	2.00

^(a) Measurements of proximates and calories were made at Covance Laboratories and National Food Lab.

^(b) Ash was determined using AOAC 923.03 Ash of Flour and AOAC 940.26 Ash of Fruits and Fruit Products methods and a 5 g sample size.

^(c) Carbohydrates were calculated as 100 g of the as-received sample minus the sum of moisture, protein, total fat, and ash in the sample, wherein the moisture was measured using AOAC 920.151 Solids (Total) in Fruits and Fruit Products and AOAC 925.09 Solids (Total) and Moisture in Flour.

^(d) Protein was determined using AOAC 992.15 Crude Protein in Meat and Meat Products Including Pet Foods method and a 0.3 g sample size.

^(e) Total Fat was determined using AOAC 922.06 Fat in Flour method and a 2 g sample size.

^(f) Calories were calculated as the sum of 9, 4, and 4 multiples of the measured fat, protein, and carbohydrates, respectively.

Table 5. Mass Fraction Value of Potential Interest for Arsenous Acid in SRM 3232

	Mass Fraction ^(a) (mg/kg)
Arsenous acid ^(b)	< 0.07

^(a) The quantity of arsenous acid is expressed as mass fraction of arsenic.

^(b) LC-ICP-MS

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Certificate Revision History: 24 March 2026 (Change of expiration date; editorial changes); 14 August 2019 (Removal of reference value for arsenic acid (AsV); editorial changes); 08 May 2017 (Original certificate issue date).

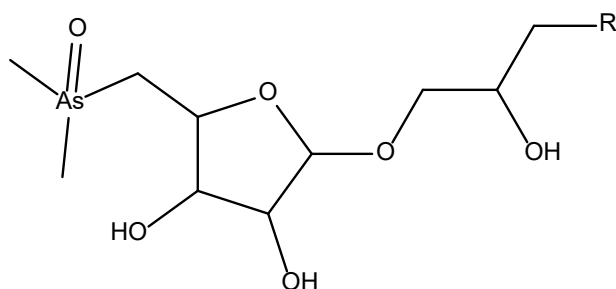
Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; e-mail srminfo@nist.gov; or via the Internet at <https://www.nist.gov/srm>.

Appendix A. Nomenclature for Arsenosugars

Table A1. Names of Arsenosugars in SRM 3232

Abbreviation	Name
Arsenosugar 328	IUPAC: 2-(2,3-dihydroxypropoxy)-5-(dimethylarsorylmethyl)oxolane-3,4-diol Common: 3-[5'-deoxy-5'-(dimethylarsinoyl)-β-ribofuranosyloxy]propylene glycol
Arsenosugar 392	IUPAC: 3-[5-(dimethylarsorylmethyl)-3,4-dihydroxyoxolan-2-yl]oxy-2-hydroxypropane-1-sulfonic acid Common: 3-[5'-deoxy-5'-(dimethylarsinoyl)-β-ribofuranosyloxy]-2-hydroxypropanesulfonic acid
Arsenosugar 482	IUPAC: 2,3-dihydroxypropyl [3-[5-(dimethylarsorylmethyl)-3,4-dihydroxyoxolan-2-yl]oxy-2-hydroxypropyl] hydrogen phosphate Common: 3-[5'-deoxy-5'-(dimethylarsinoyl)-β-ribofuranosyloxy]-2-hydroxypropyl 2,3-dihydroxypropyl hydrogen phosphate

Figure A1. Structural Formula of Arsenosugars in SRM 3232



Arsenosugar 328: R = OH

Arsenosugar 392: R = SO₃H

Arsenosugar 482: R = OPO₂(OH)CH₂CH(OH)CH₂OH