National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 3035

Arsenic Species in Apple Juice

This Standard Reference Material (SRM) is intended primarily for validating analytical methods and measurements for the determination of arsenic species in apple juice. SRM 3035 was produced from commercial apple juice spiked with trace amounts of arsenous acid (AsIII), arsenic acid (AsV), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA). A unit of SRM 3035 consists of five pouches each containing 1.5 mL of spiked juice in a cryovial. SRM 3035 is shipped on dry ice, and it should be stored at –80 °C until use.

The development of SRM 3035 was a collaboration between NIST and the Centers for Disease Control and Prevention (CDC), National Center for Environmental Health, Division of Laboratory Sciences (Atlanta, GA).

Certified Values: The certified mass fraction values and the certified mass concentration values are provided for arsenic species and total arsenic in Table 1. The structural formulas of the arsenic species are shown in the Appendix. 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 reported certified values are the weighted means of the individual sets of measurements made by NIST and the National Metrology Institute of Japan (NMIJ), estimated using a Gaussian random effects model [2] and the DerSimonian-Laird procedure [3,4]. The associated measurement uncertainty was evaluated by the application of the parametric statistical bootstrap, consistent with the ISO/JCGM Guide and its Supplement 1 [5-7]. The expanded uncertainty, U, is calculated as $U = ku_c$, where u_c represents, at the level of one standard deviation, the combined effects of the between-laboratory and the within-laboratory components of uncertainty. The coverage factor, k, corresponds to an approximately 95 % level of confidence.

Expiration of Certification: The certification of **SRM 3035** is valid, within the measurement uncertainty specified, until **31 January 2027**, 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.

Coordination of the technical measurements leading to the certification was under the direction of L.L. Yu of the NIST Chemical Sciences Division.

Analytical measurements for certification of this SRM were performed by W.C. Davis, M.B. Ellisor, R.L. Paul, and L.L. Yu of the NIST Chemical Sciences Division; N.D. Hilliard and C.D. Ward of the CDC Inorganic and Radiation Analytical Toxicology Branch; and T. Narukawa of NMIJ (Tsukuba, Ibaraki, Japan).

Partial technical support for the development of this SRM was provided under the direction of R.L. Jones and K.L. Caldwell of the CDC Inorganic and Radiation Analytical Toxicology Branch.

Statistical consultation for this SRM was provided by D.D. Leber 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: 16 June 2017

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NOTICE TO USERS

SRM 3035 IS INTENDED FOR RESEARCH USE, NOT FOR HUMAN CONSUMPTION.

INSTRUCTIONS FOR STORAGE AND USE

Storage: The SRM should be stored at –80 °C in the original unopened package. The certification does not apply to contents of previously opened pouches as the stability of all species has not been investigated under such conditions.

Use: SRM 3035 should be thawed at room temperature. The material should be used within 4 h after being thawed, and unused or remaining material should be discarded. Once the pouches are cut open, each vial of the SRM should be homogenized by gently inverting the vial several times before a test portion is removed. The recommended minimum sample size is 0.2 g.

PREPARATION AND ANALYSIS[(1)]

Source and Preparation: The apple juice used for the preparation of SRM 3035 was purchased from a grocery store in 2014. A 10 L pool of apple juice was formed by combining 2.5 L from each of four 3.8 L bottles of apple juice. The day before the production, neat standard solutions of AsIII, AsV, MMA, and DMA were added to the pool to raise the mass fraction of these species to approximately (5, 5, 10, 5) µg/kg, respectively. The pool was stirred and was sparged continuously with nitrogen through the night. In the morning of the next day, the tubing for sparging was withdrawn to the surface to stop sparging while keeping the pool in the positively pressurized nitrogen environment. Aliquots of approximately 1.5 mL of apple juice from the pool were dispensed into 2 mL cryovials inside a glove box continuously purged with nitrogen to provide an anaerobic environment. The vials were heat-sealed in Mylar bags containing oxygen absorbers and stored at –80 °C.

Homogeneity: The homogeneity of the SRM was assessed using anion exchange liquid chromatography coupled with inductively coupled plasma mass spectrometry (LC-ICP-MS) [8]. Analyses of variance and graphical analyses of the data found no detectable inhomogeneity at approximately 95 % level of confidence.

Analysis: Value assignment of arsenic species in SRM 3035 was based on NIST measurements using cation exchange LC-ICP-MS and ion chromatography (IC) ICP-MS, and NMIJ measurements using reversed phase LC-ICP-MS. Value assignment of total arsenic in SRM 3035 was based on NIST measurements using ICP-MS and radiochemical neutron activation analysis (RNAA).

Cation exchange LC-ICP-MS method: Test portions of 1 g from 8 vials of SRM 3035 were diluted to 2 g using a water solution containing approximately 80 ng/g arsenobetaine (AB) as an internal standard. For calibration by the method of standard addition, a spiked sample and an unspiked sample were prepared each with 1 g of the diluted apple juice. Arsenic species in the spiked and the unspiked samples were measured using cation exchange LC-ICP-MS [9] operated in dynamic reaction mode using oxygen as a reaction gas. The signal intensity at 91 u was integrated for quantification by the method of standard addition. Quantitative determinations are traceable to the assigned arsenic mass fraction in *SRM 3103a Arsenic (As) Standard Solution*.

IC-ICP-MS method: Test portions of 0.9 g from 8 vials of SRM 3035 were diluted with 1 g water solution of AB serving as an internal standard. Spiked samples were prepared by the addition of 0.5 g of a mixed arsenic standard solution into a diluted apple juice, and the contents were diluted to 7 g with water. Unspiked samples were prepared by diluting the dilute apple juice to 7 g with water. Arsenic species in the spiked and the unspiked samples were measured using IC-ICP-MS [9] operated in collision cell mode using 8 % hydrogen in 92 % helium (volume fraction) as the collision gas. The signal intensity at 75 u was integrated for quantification by the method of standard addition. Quantitative determinations are traceable to the assigned arsenic mass fraction in *SRM 3103a Arsenic (As) Standard Solution*.

Reversed phase LC-ICP-MS method: Test portions of 1 g from 8 vials of SRM 3035 were diluted with 1 g water solution of AB serving as an internal standard. For calibration by the method of standard addition, a spiked sample and an unspiked sample were prepared each with 1 g of the diluted apple juice. Arsenic species in the spiked and the unspiked samples were measured using reversed phase LC-ICP-MS [10] operated in collision cell mode using helium as the collision gas. The signal intensity at 75 u was integrated for quantification by the method of standard addition.

[(1)]Certain commercial instruments, materials, or processes 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 instruments, materials, or processes identified are necessarily the best available for the purpose.

Quantitative determinations are traceable to the assigned values of the Japan Calibration Service System (JCSS) AsIII standard, and NMIJ certified reference materials (CRM) NMIJ 7912-a and NMIJ 7913-a.

ICP-MS method: Test portions of 0.9 g from 8 vials of SRM 3035 were spiked with 0.5 g yttrium as an internal standard. A duplicate 0.9 test portion from the same 8 vials of SRM 3035 were spiked with 0.5 g yttrium internal standard and 0.5 g arsenic standard. The samples were digested in a microwave system using 4 mL concentrated nitric acid and 1 mL hydrogen peroxide. The digests were diluted to 50 g with water, and 0.5 mL of 1-butanol was added to enhance the sensitivity. The spiked and the unspiked samples were measured for arsenic in the high resolution mode (R ≈ 10,000) using a sector-field ICP-MS. Quantitative determinations are traceable to the assigned arsenic mass fraction in *SRM 3103a Arsenic (As) Standard Solution*.

RNAA method [11]: Test portions of 0.7 g from 8 vials of SRM 3035 were transferred to quartz vials. Spiked samples containing 0.55 g sample and 0.10 g to 0.15 g of a 51 mg/kg arsenic standard were also prepared in quartz vials. The quartz vials were flame sealed and then sealed in polyethylene containers for irradiation. The samples and the standards were irradiated for 6 h in the NIST reactor pneumatic tube irradiation facility, RT-1, which provided a thermal neutron fluence rate of approximately 3×10^{14} $cm^{-2}s^{-1}$. After approximately a 2 d decay, the samples were transferred to beakers containing ^{77}As internal standard. The contents were digested on a hotplate in nitric acid and perchloric acid. The perchloric acid in the beakers was expelled with sulfuric acid by fuming, and the arsenic in the beakers was then reduced to AsIII with potassium iodide. AsIII in each beaker was extracted into chloroform using zinc diethyldithiocarbamate, cleaned up with a solution of zinc sulfate in sulfuric acid, and transferred to a 20 mL plastic liquid scintillation counting vial for the determination of ^{76}As and ^{77}As by gamma ray spectroscopy. The samples were counted approximately 5 d after irradiation for approximately 1 h to 2 h each. Quantitative determinations are traceable to the assigned arsenic mass fraction in *SRM 3103a Arsenic (As) Standard Solution*.

Table 1. Certified Values for Arsenic Species and Total Arsenic in SRM 3035

	Mass Fraction as Arsenic[a] (μg/kg)	Mass Concentration as Arsenic[b] (μg/L)	Coverage Factor, *k*
Arsenous acid (AsIII)[c,d,e]	5.17 ± 0.17	5.40 ± 0.18	2.07
Arsenic acid (AsV)[c,d,e]	6.35 ± 0.49	6.64 ± 0.51	2.11
Monomethylarsonic acid (MMA)[c,d,e]	9.38 ± 0.64	9.80 ± 0.67	2.09
Dimethylarsinic acid (DMA)[c,d,e]	5.23 ± 0.26	5.47 ± 0.27	2.07
Inorganic arsenic (iAs)[c,d,e,f]	11.53 ± 0.66	12.05 ± 0.69	2.10
Arsenic (As)[g,h]	24.92 ± 0.84	26.04 ± 0.88	1.98

[a] The measurand is the total mass fraction of arsenic or each arsenic species listed, and the certified value is metrologically traceable to the SI-derived unit for mass fraction, expressed as microgram per kilogram.

[b] The density of SRM 3035, measured with a density meter at 1.045 kg/L ± 0.001 kg/L (expanded uncertainty at 95 % confidence), is used to convert the certified mass fraction values to the certified mass concentration values, metrologically traceable to the SI-derived unit for mass concentration, expressed as microgram per liter.

[c] Cation exchange LC-ICP-MS.

[d] IC-ICP-MS.

[e] Reversed phase LC-ICP-MS.

[f] The mass fraction of iAs is defined as the sum of the mass fractions of AsIII and AsV as arsenic.

[g] ICP-MS.

[h] RNAA.

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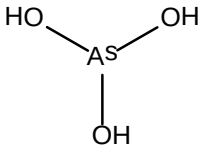
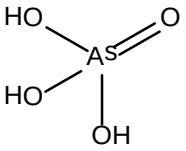
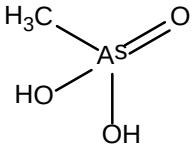
Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.

APPENDIX A

Table A1. Structural Formulas for Arsenic Species in SRM 3035

Name (Abbreviation)	CAS Registry Number	Structural Formula
Arsenous acid (AsIII)	13464-58-9	
Arsenic acid (AsV)	7778-39-4	
Monomethylarsonic acid (MMA)	124-58-3	
Dimethylarsinic acid (DMA)	75-60-5