National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 3222

Cigarette Tobacco Filler

This Standard Reference Material (SRM) is intended primarily for use in evaluating the accuracy of procedures for the determination of nicotine (NIC); tobacco specific nitrosamines (TSNAs), N-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK); and moisture in tobacco. It is also intended for use in validating working or secondary reference materials. SRM 3222 was prepared from air-cured, low nicotine tobacco [1]. A unit of SRM 3222 consists of 20 jars, each containing approximately 10 g of cigarette tobacco filler.

The development of SRM 3222 was through collaboration with the National Institute of Standards and Technology (NIST) and the Food and Drug Administration Center for Tobacco Products (FDA CTP).

Certified Mass Fraction Values: The certified mass fraction values for nicotine, NNN, NNK, and volatiles in SRM 3222 are 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 [2]. The certified values are based on the results of NIST measurements using isotope dilution liquid chromatography with tandem mass spectrometry (ID-LC-MS/MS) with different sample preparation techniques, and with measurements performed at the U.S. Centers for Disease Control and Prevention (CDC) and commercial laboratories.

Reference Mass Fraction Values: Reference mass fraction values for volatiles and moisture in SRM 3222, are reported on an as-received basis in Table 2. A NIST reference value is a noncertified value that is the best estimate of the true value based on available data; however, the value does not meet the NIST criteria for certification [2] and is 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. The reference mass fraction values were derived from results reported by NIST and/or commercial laboratories.

Expiration of Certification: The certification of **SRM 3222** is valid, within the measurement uncertainty specified, until **30 September 2021**, 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.

Coordination of the technical measurements leading to the certification of SRM 3222 was performed by L.C. Sander of the NIST Chemical Sciences Division. Coordination of other aspects of SRM development was performed by M. Walters, T. Phillips, and M. Holman of the FDA CTP. Acquisition of the material was performed by M. Walters.

Measurements were performed by J.S. Pritchett, Y. C. Daniels, L.C. Sander, L. Wood, and B. Lang of the NIST Chemical Sciences Division, and by G. Lee, J. Lisko, B. Lane, L. Valentin, B. Kimbrell, P. Kuklenyik, and S. Stanfill at the CDC.

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

Analyses were also performed by commercial laboratories: Enthalpy Analytical, Inc. (Richmond, VA), Global Laboratory Services, Inc. (Wilson, NC), and Microbac Laboratories, Inc. (Wilson, NC).

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Certificate Issue Date: 23 August 2016

Steven J. Choquette, Director
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Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

NOTICE AND WARNINGS TO USERS: For research use. Not for human consumption.

INSTRUCTIONS FOR STORAGE AND USE

Storage: SRM 3222 is stored at –20 ºC at NIST, and is shipped cold. Upon receipt, the material should be stored at controlled temperature (–20 °C to 0 °C), in unopened bottles, until required for use.

Use: Bottles of the SRM to be analyzed should be removed from the freezer and thawed to room temperature (20 °C to 25 °C). It is recommended that the contents of the jar be ground prior to removal of subsamples. One gram or larger test portions should be analyzed as received (i.e., without drying) and results converted to a dry-mass basis through a correction factor for volatiles.

SOURCE, PREPARATION, AND ANALYSIS[(1)]

Source and Preparation: Air-cured, low nicotine tobacco was processed and supplied by 22nd Century Group, Inc., (subcontracted by RTI International) using normal procedures used for the production of cigarette tobacco filler. The dried and chopped leaves (referred to as "cut rag"; 30 cuts per inch) were blended and enclosed in plastic bags that were placed in cardboard cartons containing approximately 68.2 kg (150 lbs) of tobacco. These materials were stored at –20 °C prior to packaging. Four ounce jars were filled to capacity with tobacco, without additional processing. A unit of SRM 3222 consists of a box containing 20 jars of cigarette tobacco filler, with each jar containing approximately 10 g of the bulk material.

Analysis: Value assignment of levels of nicotine and TSNAs in SRM 3222 was based on the results from measurements carried out at NIST using ID-LC-MS/MS methods. Different approaches were utilized in preparing samples for analysis; in each case, internal standards consisting of deuterated analogs of nicotine, NNN, and NNK were added prior to sample preparation.

One gram samples of tobacco were finely ground using an automated mortar and pestle. Aliquots of the labeled internal standard solutions were added to approximately 0.250 g portions of the ground material in 15 mL polypropylene tubes. Ten milliliters of 100 mM aqueous ammonium acetate were added to each tube, and then vortex mixed for 60 minutes to perform the extraction. After the extraction period, the samples were filtered using 0.45 µm PVDF membrane syringe filters and the filtrate was transferred into autosampler vials for analysis.

Samples were also extracted with methanol, using a different mixing approach. The contents of a jar were ground, and one gram subsamples were processed. After the addition of the internal standards, five milliliters of methanol were added to each sample, and the slurry was processed by rotational inversion mixing at 60 rpm, for a period of 18 h. After extraction, the slurries were centrifuged for 10 min at 3750 rpm (3210 g). Unfiltered aliquots of the supernatant solution were placed in autosampler vials for analysis. Samples were also extracted by the same method, but using 100 mM aqueous ammonium acetate.

Volatile components were determined by multiple methods. Approximately 0.5 g samples of tobacco were placed into weighed glass containers to an approximate depth of two centimeters. For determination by oven drying, the samples were placed in a forced-air oven at either 80 ºC or 100 ºC for three hours, removed and allowed to cool in a desiccator, and reweighed. For determination by desiccator drying, samples were placed in a desiccator over magnesium perchlorate. Masses were recorded at seven day intervals until a constant mass was achieved at 35 days. Moisture measurements were performed with a coulometric Karl Fischer oven method [3].

Homogeneity Analysis: A stratified random sampling scheme was devised to test for homogeneity across the lot of bottles. There was no apparent trend in the data when plotted against the sequence in which the bottles were prepared.

[(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.

Certified Mass Fraction Values: The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence using statistical methods consistent with the ISO/JCGM Guide and with its Supplement 1 [4,5]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is the combined standard uncertainty and k is a coverage factor corresponding to approximately 95 % confidence for each analyte [4]. The measurand is the total mass fraction for each analyte listed in Table 1. Metrological traceability is the SI derived unit of mass fraction (expressed as milligrams per gram, nanograms per gram, or grams per gram).

Table 1. Certified Mass Fraction Values for Nicotine, TSNAs, and Volatiles in SRM 3222

	Mass Fraction[(a)] (as-received)			Mass Fraction[(b)] (dry-mass basis)			k
Nicotine	0.117 mg/g	±	0.018 mg/g	0.132 mg/g	±	0.021 mg/g	2.00
NNN	1440 ng/g	±	90 ng/g	1630 ng/g	±	110 ng/g	2.00
NNK	31.3 ng/g	±	2.5 ng/g	35.4 ng/g	±	2.8 ng/g	2.00
Volatiles[(c)]	0.115 g/g	±	0.002 g/g				2.00

[(a)] Values are reported on an "as-received" basis.
[(b)] Values are reported on a dry-mass basis using the certified value for volatiles as a conversion factor.
[(c)] Volatiles are reported based on data obtained for oven drying at 80 ºC for three hours and desiccator drying over magnesium perchlorate for 35 days.

Reference Mass Fraction Values: The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence using statistical methods consistent with the ISO/JCGM Guide and with its Supplement 1 [4,5]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is the combined standard uncertainty and k is a coverage factor corresponding to approximately 95 % confidence for each analyte [4]. The measurand is the mass fraction for each analyte listed based on the indicated method. Metrological traceability is the SI derived unit of mass fraction (expressed as a percent).

Table 2. Reference Mass Fraction (As-Received Basis) Values for Volatiles and Moisture in SRM 3222

	Mass Fraction (%)	k
Volatiles (mass loss)		
Forced-Air Oven Drying at 80 °C for 3 h	11.6 ± 0.1	2.05
Forced-Air Oven Drying at 100 °C for 3 h	12.1 ± 0.6	2.00
Desiccator Drying[(a)]	11.4 ± 0.1	2.05
Hearson Tobacco Oven for 16 h	11.9 ± 0.1	2.23
Moisture (water; Karl Fischer)[(b)]	10.6 ± 0.9	2.23

[(a)] Values represent mass loss upon desiccator drying over magnesium perchlorate to constant mass, achieved after 35 days.
[(b)] Values represent water determined using the Karl Fischer oven distillation method.

REFERENCES

[1] *Title 7 Agriculture Part 30 – Tobacco Stocks and Standards*, U.S. Code of Federal Regulations, 7 CFR 30, pp. 168–175 (1974).

[2] May, W.; Parris, R.; Beck II, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definition of Terms and Modes Used at NIST for Value-Assignment- of Reference Materials for Chemical Measurements*; NIST Special Publication 260-136; U.S. Government Printing Office: Washington, DC (2000); available at http://www.nist.gov/srm/upload/SP260-136.PDF (accessed Aug 2016).

[3] Margolis, S.A.; *Systematic Errors in Measurement of Moisture by Karl Fischer Methods*; Third International Symposium on Humidity and Moisture, Vol. 2, pp. 133–140 (1998).

[4] JCGM 100:2008; *Evaluation of Measurement Data – Guide to the Expression of Uncertainty in Measurement*; (GUM 1995 with Minor Corrections), Joint Committee for Guides in Metrology (JCGM) (2008); available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Aug 2016); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297; U.S. Government Printing Office: Washington, DC (1994); available at http://www.nist.gov/pml/pubs/index.cfm (accessed Aug 2016).

[5] JCGM 101:2008; *Evaluation of Measurement Data – Supplement 1 to the Guide to the Expression of Uncertainty in Measurement – Propagation of Distributions Using a Monte Carlo Method*; JCGM (2008); available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_101_2008_E.pdf (accessed Aug 2016).

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.