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

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Standard Reference Material® 3222
Cigarette Tobacco Filler
CERTIFICATE OF ANALYSIS

Purpose: The certified values delivered by this Standard Reference Material (SRM) are 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. They are also intended for use in validating working or secondary reference materials. SRM 3222 was prepared from air-cured, low nicotine tobacco [1].

Description: A unit of SRM 3222 consists of 20 jars, each containing approximately 10 g of cigarette tobacco filler.

Certified 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. 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 [3,4]. 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 [3]. NIST certified values are traceable to the International System of Units (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.

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

Additional Information: Additional information is available in Appendix B.

Period of Validity: The certified values delivered by **SRM 3222** are valid within the measurement uncertainty specified until **09 July 2029**. The certified values are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

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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).

Safety: For research use. Not for human consumption.

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.

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] 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; National Institute of Standards and Technology, Gaithersburg, MD (2021); available at https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-136-2021.pdf (accessed Apr 2023).

[3] 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 https://www.bipm.org/en/committees/jc/jcgm/publications (accessed Apr 2023); 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 https://www.nist.gov/pml/nist-technical-note-1297 (accessed Apr 2023).

[4] 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 https://www.bipm.org/en/committees/jc/jcgm/publications (accessed Apr 2023).

[5] 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).

Certificate Revision History: 13 April 2023 (Change of period of validity; updated format; editorial changes); **23 August 2016** (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 of Analysis in their possession is current. This can be accomplished by contacting the Office of Reference Materials 100 Bureau Drive, Stop 2300, Gaithersburg, MD 20899-2300; 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 Mass Fraction Values: Non-certified mass fraction values for volatiles and moisture in SRM 3222, are reported on an as-received basis in Table A1. 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 [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 non-certified mass fraction values were derived from results reported by NIST and/or commercial laboratories.

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 [3,4]. 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 [3].

Table A1. Non-Certified 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.

Period of Validity: The non-certified values are valid within the measurement uncertainty specified until **09 July 2029.** The value assignments are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

Maintenance of Non-Certified Values: NIST will monitor this material to the end of its period of validity. If substantive technical changes occur that affect the non-certified values during this period, NIST will update this Appendix 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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APPENDIX B

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).

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 [5].

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.

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