National Institute of Standards & Technology

# Certificate of Analysis

## Standard Reference Material® 3671

### Nicotine Metabolites in Human Urine (Frozen)

This Standard Reference Material (SRM) is intended primarily for use in evaluating the accuracy of procedures for the determination of nicotine metabolites in human urine. It is also intended for use in validating working or secondary reference materials. SRM 3671 was prepared from normal human urine collected from three different populations: nonsmokers without environmental exposure to tobacco smoke, nonsmokers with exposure to "secondhand" smoke, and smokers who smoke at least one pack of cigarettes per day. A unit of SRM 3671 consists of three vials, each containing 10 mL of frozen human urine, one bottle each of three levels.

**Certified Mass Fraction Values:** The certified values for nicotine, cotinine, and 3-hydroxycotinine 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 [1]. The certified values are based on the results of NIST measurements using isotope dilution liquid chromatography mass spectrometry (ID-LC-MS), isotope dilution liquid chromatography with tandem mass spectrometry (ID-LC-MS/MS), gas chromatography mass spectrometry (GC-MS), and measurements performed at the Centers for Disease Control and Prevention (CDC) using ID-LC-MS/MS methods.

**Reference Mass Fraction Values:** Reference values are provided 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 [1] 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 values were derived from results reported by NIST or collaborating laboratories.

**Information Values:** Information values for selected analytes are provided in Table 3. A NIST information value is considered to be a value that will be of use to the SRM user, but insufficient information is available to assess the uncertainty associated with the value or only a limited number of analyses were performed [1]. Information Values cannot be used to establish metrological traceability.

**Expiration of Certification:** The certification of **SRM 3671** is valid, within the measurement uncertainty specified, until **01 January 2020**, 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) will facilitate notification.

Coordination of the technical measurements leading to the certification of SRM 3671 was performed by L.C. Sander of the NIST Chemical Sciences Division.

Acquisition of the material was performed by K.E. Sharpless of the NIST Chemical Sciences Division. Certification measurements were performed by B.A. Benner, Jr., J.S. Pritchett, and J.L. Prendergast of the NIST Chemical Sciences Division, and by J. McGuffey and C. Sosnoff at the U.S. CDC.

Statistical consultation was provided by N.F. Zhang of the NIST Statistical Engineering Division.

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Certificate Issue Date: 14 March 2014

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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

SRM 3671 IS INTENDED FOR IN-VITRO DIAGNOSTIC USE ONLY. THIS IS A HUMAN-SOURCE MATERIAL. HANDLE PRODUCT AS A BIOHAZARDOUS MATERIAL CAPABLE OF TRANSMITTING INFECTIOUS DISEASE. This human urine-based product should be handled at the Biosafety Level 2 or higher as recommended for any POTENTIALLY INFECTIOUS HUMAN SPECIMEN in the CDC and National Institutes of Health Manual (NIH) [2].

## INSTRUCTIONS FOR STORAGE AND USE

**Storage:** The SRM is stored at –80 °C at NIST. The urine is shipped frozen (on dry ice) and, upon receipt, should be stored frozen until ready for use. A freezer temperature of –20 °C is acceptable for storage for up to one week. If a longer storage time is anticipated, the material should be stored at or below –60 °C. The SRM should not be exposed to sunlight or ultraviolet radiation. Storage of thawed material at room or refrigerator temperatures may result in changes in analyte concentrations.

**Use:** Bottles of the SRM to be analyzed should be removed from the freezer and thawed to room temperature (20 °C to 25 °C). After the material is thawed to room temperature, it should be used immediately. The material should be swirled gently to mix it before aliquots are withdrawn.

## SOURCE, PREPARATION, AND ANALYSIS[(1)]

**Source and Preparation:** SRM 3671 was prepared by Bioreclamation Inc. (Westbury, NY). The urine pool was prepared from a minimum of 10 donors each from three different populations: nonsmokers without environmental exposure to tobacco smoke, nonsmokers with exposure to "secondhand" smoke, and smokers who smoke at least one pack of cigarettes per day.

**Analysis:** Value assignment of the concentration of nicotine and nicotine metabolites in SRM 3671 was based on the results from NIST using ID-LC-MS, ID-LC-MS/MS, GC-MS, and measurements from CDC using ID-LC-MS/MS methods. Three approaches were utilized in preparing samples for analysis; in each case, internal standards were added prior to sample preparation. For ID-LC-MS, samples were processed with solid-phase extraction without hydrolysis to provide levels of free analytes. For ID-LC-MS/MS, samples were diluted and analyzed without further processing, and both free and conjugated analytes were determined. Samples determined by GC-MS were hydrolyzed with β-glucuronidase using the same conditions as with the CDC measurements to provide "total" levels of selected analytes.

**Homogeneity Analysis:** A stratified random sampling plan 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 [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]. For the certified values shown below, $k = 2$. The measurand is the total mass fraction for each analyte listed in Table 1. The certified values are metrological traceable to the SI unit of gram per gram, expressed as nanogram per gram.

Table 1. Certified Mass Fraction Values for Nicotine and Nicotine Metabolites in SRM 3671

| | Mass Fraction (ng/g) |
|---|---|
| **Level 2** | |
| Nicotine (free)[a,b,c,d] | 123.6 ± 5.6 |
| Cotinine (total)[e,f,g,h] | 315.3 ± 14.2 |
| **Level 3** | |
| Nicotine (free)[a,b,c,d] | 1498 ± 185 |
| Cotinine (total)[e,f,g,h] | 2233 ± 101 |
| 3-Hydroxycotinine (free)[a,b,c] | 4955 ± 213 |

[a] The designation “free” refers to analytes that are unconjugated as glucuronides or other species.
[b] LC/MS without sample hydrolysis
[c] LC/MS/MS without sample hydrolysis
[d] GC/MS without sample hydrolysis
[e] The designation “total” refers to analyte levels for hydrolyzed samples.
[f] GC/MS with sample hydrolysis using β-glucuronidase
[g] CDC LC/MS/MS Method A
[h] CDC LC/MS/MS Method B

**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/JCGFM 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]. For the reference values shown below, $k = 2$. The reference values are based on the method used for each analyte listed in Table 2, the measurand is the mass fraction for each analyte listed. Metrological traceability is to the SI unit of gram per gram, expressed as nanogram per gram.

Table 2. Reference Mass Fraction Values for Nicotine and Nicotine Metabolites in SRM 3671

| | Mass Fraction (ng/g) |
|---|---|
| **Level 1** | |
| Caffeine (free)[a,b] | 752.8 ± 83.9 |
| Theobromine (free)[a,b] | 4260 ± 584 |
| **Level 2** | |
| Nicotine glucuronide[c] | 77.9 ± 27.3 |
| Nicotine (total)[d,e] | 143.4 ± 9.4 |
| Cotinine glucuronide[c] | 194.2 ± 42.4 |
| 3-Hydroxycotinine (free)[a,c,f] | 481.8 ± 87.8 |
| 3-Hydroxycotinine (total)[d,e,g] | 717.4 ± 29.9 |
| Cotinine-N-oxide (total)[d,e] | 46.3 ± 5.2 |
| Nicotine-N-oxide (total)[d,e] | 68.4 ± 8.4 |
| Caffeine (free)[a,b] | 833.3 ± 58.3 |
| Ibuprofen (free)[a,b] | 1131 ± 122 |
| Theobromine (free)[a,b] | 5529 ± 704 |
| **Level 3** | |
| Nicotine (total)[d,e] | 1665 ± 96.3 |
| Cotinine (free)[a,b,c,f] | 1164 ± 294 |
| Cotinine glucuronide[c] | 1629 ± 441 |
| 3-Hydroxycotinine (total)[d,e,g] | 6752 ± 322 |
| Cotinine-N-oxide (total)[d,e] | 485.4 ± 37.6 |
| Nicotine-N-oxide (total)[d,e] | 627.0 ± 44.6 |
| Caffeine (free)[a,b] | 1086 ± 380 |
| Ibuprofen (free)[a,b] | 4469 ± 241 |
| Theobromine (free)[a,b] | 3311 ± 341 |

[a] The designation “free” refers to analytes that are unconjugated as glucuronides or other species.
[b] GC/MS without sample hydrolysis.
[c] LC/MS/MS without sample hydrolysis
[d] The designation “total” refers to analyte levels for hydrolyzed samples.
[e] CDC LC/MS/MS Method A
[f] LC/MS without sample hydrolysis
[g] CDC LC/MS/MS Method B

**Information Mass Fraction Values:** Information values are not certified and insufficient information exists to assess uncertainty. The data are provided to characterize the material, and are not intended for quantitative comparisons.

Table 3. Information Mass Fraction Values for Nicotine and Nicotine Metabolites in SRM 3671

| | Mass Fraction (ng/g) |
|---|---|
| **Level 1** | |
| Nicotine (free)[a,b] | 13 |
| Cotinine (free)[a,b,c] | 0.4 |
| Cotinine (total)[d,e,f] | 0.6 |
| 3-Hydroxycotinine (free)[a,c] | 1.2 |
| 3-Hydroxycotinine (total)[d,f] | 0.6 |
| **Level 2** | |
| Cotinine (free)[a,b,c,g] | 133 |
| 3-Hydroxycotinine glucuronide[g] | 160 |
| **Level 3** | |
| 3-Hydroxycotinine glucuronide[g] | 2244 |

[a] The designation "free" refers to analytes that are unconjugated as glucuronides or other species.
[b] GC/MS without sample hydrolysis
[c] LC/MS without sample hydrolysis
[d] The designation "total" refers to analyte levels for hydrolyzed samples.
[e] GC/MS with sample hydrolysis using β-glucuronidase
[f] CDC LC/MS/MS Method B
[g] LC/MS/MS without sample hydrolysis

## REFERENCES


[1] May, W.; Parris, R.; Beck, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definitions 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: Gaithersburg, MD (2000); available at http://www.nist.gov/srm/publications.cfm (accessed Mar 2014)

[2] CDC/NIH; *Biosafety in Microbiological and Biomedical Laboratories, 5th ed.*; Richardson, J.; Barkley, W.E.; Richmond, J.; McKinney, R.W., Eds.; U.S. Department of Health and Human Services, Public Health Service, CDC and NIH; US Government Printing Office: Washington, D.C. (2009); available at http://www.cdc.gov/biosafety/publications/index.htm (accessed Mar 2014)

[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 (2008); available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Mar 2014); 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://physics.nist.gov/Pubs/ (accessed Mar 2014).

[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*; (GUM 1995 with Minor Corrections); Joint Committee for Guides in Metrology (2008); available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_101_2008_E.pdf (accessed Mar 2014)


*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.*