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

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Standard Reference Material® 3672
Organic Contaminants in Smokers' Urine
(Frozen)
CERTIFICATE OF ANALYSIS

Purpose: The certified values delivered by this Standard Reference Material (SRM) are intended for use in evaluating analytical methods for the determination of selected hydroxylated polycyclic aromatic hydrocarbons (hydroxylated PAHs) and phthalate, phenol, and volatile organic compound (VOC) metabolites in urine. All of the constituents for which certified and non-certified values are provided are naturally present in the urine.

Description: A unit of SRM 3672 consists of five vials each containing 10 mL of frozen urine. The development of SRM 3672 was a collaboration between the National Institute of Standards and Technology (NIST) and the Division of Laboratory Sciences, Centers for Disease Control and Prevention (CDC).

Certified Values: 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 uncertainty have been investigated or taken into account [1]. Certified mass fraction values for hydroxylated PAHs are provided in Table 1. The certified values are based on the agreement of results obtained at NIST, CDC, and Institut national de santé publique du Québec (INSPQ). These values are traceable to the International System of Units (SI) derived unit of mass fraction, expressed as micrograms per kilogram.

Table 1. Certified Mass Fraction Values for Hydroxylated PAHs in SRM 3672

Hydroxylated PAHs	Mass Fraction (µg/kg)
1-Naphthol[a,b,c,d]	33.8 ± 4.3
2-Naphthol[a,b,c,d]	8.57 ± 0.16
9-Hydroxyfluorene[a,b,c,e]	0.331 ± 0.077
3-Hydroxyfluorene[a,b,c,d]	0.420 ± 0.018
4-Hydroxyphenanthrene[a,b,c,d]	0.0480 ± 0.0045
9-Hydroxyphenanthrene[a,c,d]	0.959 ± 0.061
3-Hydroxyphenanthrene[a,b,c,d]	0.123 ± 0.007
1-Hydroxyphenanthrene[a,b,c,e]	0.133 ± 0.014
2-Hydroxyphenanthrene[a,b,c,e]	0.0825 ± 0.0007
1-Hydroxypyrene[a,b,c,e]	0.170 ± 0.010

[a] NIST GC/MS analysis.
[b] CDC GC/MS analysis; data were converted from nanograms per milliliter to micrograms per kilogram using the density of the urine (1.019 ± 0.024 g/mL). The urine density uncertainty was accounted in the expanded uncertainties.
[c] INSPQ GC/MS/MS analysis; data were converted from nanograms per milliliter to micrograms per kilogram using the density of the urine (1.019 ± 0.024 g/mL). The urine density uncertainty was accounted in the expanded uncertainties.
[d] The certified value is a weighted mean of average mass fractions, with one average from each of two or three analytical methods [2,3]. The expanded uncertainty is the half-width of a symmetric 95 % parametric bootstrap confidence interval [4], which is consistent with the ISO/JCGM Guide [5,6]. The effective coverage factor, *k,* is 2.
[e] The certified mass fraction value is a weighted mean of the mass fractions from three analytical methods [2]. The uncertainty listed with each value is an expanded uncertainty about the mean [2,3], with coverage factor $k = 2$, calculated by combining within-method variances with a between-method variance [7] following the ISO/JCGM Guide [5,6].

Non-Certified Values: Non-certified values and additional information are provided in Appendix A.

Period of Validity: The certified values delivered by **SRM 3672** are valid within the measurement uncertainty specified until **31 May 2030**. The certified values are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

Carlos A. Gonzalez, Chief
Chemical Sciences Division

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Office of Reference Materials

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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: SRM 3672 IS INTENDED FOR RESEACRCH USE. This is a human-source material. Handle product as a biohazardous material potentially capable of transmitting infectious disease. Accordingly, this human urine-based product should be handled at Biosafety Level 2 as recommended by the Centers for Disease Control and Prevention/National Institutes of Health's Biosafety in Microbiological and Biomedical Laboratories [8] for human-derived products where the presence of infectious agent(s) may be unknown.

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 to analyte concentrations.

Use: Vials of the SRM to be analyzed should be removed from the freezer and thawed completely 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 vortex mixed before aliquots are withdrawn.

This SRM was developed after an appropriate human subjects research determination by NIST.

REFERENCES

[1] 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; U.S. Government Printing Office: Washington, DC (2021); available at https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-136-2021.pdf (accessed Dec 2022).

[2] DerSimonian, R.; Laird, N.; *Meta-Analysis in Clinical Trials*; Control Clin. Trials, Vol. 7, pp. 177–188 (1986).

[3] Rukhin, A.L.; *Weighted Means Statistics in Interlaboratory Studies*; Metrologia, Vol. 46, pp. 323–331 (2009).

[4] Efron, B.; Tibshirani, R.J.; *An Introduction to the Bootstrap*; Chapman & Hall: London, UK (1993).

[5] 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 https://www.bipm.org/en/committees/jc/jcgm/publications (accessed Dec 2022); 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 Dec 2022).

[6] 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 Dec 2022).

[7] Horn, S.A.; Horn, R.A.; Duncan, D.B.; *Estimating Hetroscedastic Variances in Linear Models*; J. Am. Stat. Assoc., Vol.70, pp. 380–385 (1975).

[8] CDC/NIH: *Biosafety in Microbiological and Biomedical Laboratories,* 6th ed.; HHS Publication No. (CDC) 300859; Meechan, P.J.; Potts, J.; Eds.; U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health; US Government Printing Office: Washington, DC (2020); available at https://www.cdc.gov/labs/BMBL.html (accessed Dec 2022).

[9] Li, X.; Romanoff, L.C.; Trinidad, D.A.; Hussain, N.; Jones, R.S.; Porter, E.N.; Patterson, D.G.; Sjödin, A.; *Measurement of Urinary Monohydroxy Polycyclic Aromatic Hydrocarbons Using Automated Liquid-Liquid Extraction and Gas Chromatography/Isotope Dilution High Resolution Mass Spectrometry*; Anal. Chem., Vol. 78, pp. 5744–5751 (2006).

[10] Silva, M.J.; Samander, E.; Preau, J.L.; Reidy, J.A.; Needham, L.L.; Calafat, A.M.; *Quantification of 22 Phthalate Metabolites in Human Urine*; J. Chromatogr. B, Vol. 860, pp. 106–112 (2007).

[11] Ye, X.; Kuklenyik, Z.; Needham, L.L.; Calafat, A.M.; *Automated On-Line Column-Switching HPLC-MS/MS Method with Peak Focusing for the Determination of Nine Environmental Phenols in Urine*; Anal. Chem., Vol. 77, pp. 5407–5413 (2005).
[12] Arakawa, C.; Fujimaki, K.; Yoshinaga, J.; Imai, H.; Serizawa, S.; Shiraishi, H.; *Daily Urinary Excretion of Bisphenol A*.; Environ. Health Prev. Med., Vol. 9, pp. 22–26 (2004).
[13] Alwis, K.U.; Blount, B.C.; Britt, A.S.; Patel, D.; Ashley, D.L.; *Simultaneous Analysis of 28 Urinary VOC Metabolites using Ultra High Performance Liquid Chromatography Coupled with Electrospray Ionization Tandem Mass Spectrometry (UPLC-ESI/MSMS)*; Anal Chim Acta, Vol. 750, pp. 152–160 (2012).
[14] Man, C.N.; Gim, L-H.; Ismail, S.; Lajis, R.; Awang, R.; *Simple, Rapid and Sensitive Method for Simultaneous Quantification of Urinary Nicotine and Cotinine Using Gas Chromatography-Mass Spectrometry*; J. Chromatogr. B, Vol. 844, pp. 322–327 (2006).

Certificate Revision History: 21 December 2022 (Correction of non-certified values for N-Acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine and N-Acetyl-S-(2-hydroxypropyl)-L-cysteine; updated format; editorial changes); 12 November 2021 (Correction of reference value for N-Acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine; editorial changes); 17 March 2020 (Change of expiration date; removal of 2-hydroxyfluorene certified value based on NIST's decision to no longer support the measurement capability in this matrix; editorial changes); 04 December 2015 (Editorial changes); 07 February 2014 (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 Values: Non-certified mass fraction values are provided in Table A1 for phthalate metabolites, Table A2 for phenol metabolites, Table A3 for VOC metabolites, and Table A4 for additional compounds. Non-certified values are suitable for use in method development, method harmonization, and process control but do not provide metrological traceability to the International System of Units (SI) or other higher-order reference system [1].

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 Certificate of Analysis 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).

Table A1. Non-Certified Mass Fraction Values for Selected Phthalate Metabolites in SRM 3672

Phthalate Metabolites	Mass Fraction[a,b] (µg/kg)
Mono-carboxynonyl phthalate isomers[c]	1.92 ± 0.06
Mono-carboxyoctyl phthalate isomers[d]	21.3 ± 1.1
Mono-(2-ethyl-5-carboxypentyl) phthalate	35.2 ± 1.7
Mono-(2-ethyl-5-hydroxyhexyl) phthalate	24.8 ± 0.4
Mono-(2-ethyl-5-oxohexyl) phthalate	14.9 ± 0.4
Mono-(2-ethylhexyl) phthalate	4.13 ± 0.15
Mono-(3-carboxypropyl) phthalate	2.99 ± 0.20
Monobenzyl phthalate	8.37 ± 0.18
Monoethyl phthalate	94.5 ± 3.0
Mono-isobutyl phthalate	6.40 ± 0.28
Mono-*n*-butyl phthalate	10.6 ± 0.5

[a] CDC analysis; data were converted from nanograms per milliliter to micrograms per kilogram using the density of the urine (1.019 ± 0.024 g/mL). The urine density uncertainty was accounted in the expanded uncertainties.

[b] The non-certified mass fraction value is the mean of results obtained using one analytical technique. The expanded uncertainty, U, is calculated as $U = ku_c$, where u_c is one standard deviation of the analyte mean, and the coverage factor, k, is determined from the Student's t-distribution corresponding to the associated degrees of freedom and a 95 % confidence level for each analyte. The effective coverage factor, k, is 2.20.

[c] Mono-(2,7-dimethyl-7-carboxyheptyl) phthalate was used as the calibrant for the quantification of the mono-carboxynonyl phthalate isomers.

[d] Mono-(2,6-dimethyl-6-carboxyhexyl) phthalate was used as the calibrant for the quantification of the mono-carboxyoctyl phthalate isomers.

Table A2. Non-Certified Mass Fraction Values for Selected Phenol Metabolites in SRM 3672

Phenol Metabolites	Mass Fraction (µg/kg)
Bisphenol A (BPA)[a,b,c]	3.05 ± 0.16
2,5-Dichlorophenol[b,d]	1.77 ± 0.06
Benzophenone-3[b,d]	191 ± 5
Methyl Paraben[b,d]	113 ± 2
Ethyl Paraben[b,d]	8.12 ± 0.20
Propyl Paraben[b,d]	17.6 ± 0.3
Butyl Paraben[b,d]	11.1 ± 0.2
Triclosan[b,d]	17.7 ± 0.5

[a] NIST analysis GC/MS.

[b] CDC analysis; data were converted from nanograms per milliliter to micrograms per kilogram using the density of the urine (1.019 ± 0.024 g/mL). The urine density uncertainty was accounted in the expanded uncertainties.

[c] The non-certified value is a weighted mean of average mass fractions, with one average from each of two analytical methods [2,3]. The expanded uncertainty is the half-width of a symmetric 95 % parametric bootstrap confidence interval [4], which is consistent with the ISO/JCGM Guide [5,6]. The effective coverage factor, *k,* is 2.

[d] The non-certified mass fraction value is the mean of results obtained using one analytical technique. The expanded uncertainty, U, is calculated as $U = ku_c$, where u_c is one standard deviation of the analyte mean, and the coverage factor, k, is determined from the Student's t-distribution corresponding to the associated degrees of freedom and a 95 % confidence level for each analyte. The effective coverage factor, *k,* is 2.20.

Table A3. Non-Certified Mass Fraction Values for Selected VOC Metabolites in SRM 3672

VOC Metabolites	Mass Fraction[a,b] (µg/kg)
Trans,trans-muconic acid	173 ± 21
N-Acetyl-S-(2-carboxyethyl)-L-cysteine	200 ± 10
2-Aminothiazoline-4-carboxylic acid	117 ± 17
N-Acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine	15.7 ± 1.6
N-Acetyl-S-(2-carbamoylethyl)-L-cysteine	122 ± 1
N-Acetyl-S-(2-hydroxyethyl)-L-cysteine	2.52 ± 0.61
N-Acetyl-S-(3,4-dihydroxybutyl)-L-cysteine	277 ± 24
N-Acetyl-S-(*N*-methylcarbomoyl)-L-cysteine	340 ± 26
2-Thioxothiazolidine-4-carboxylic acid	20.6 ± 2.3
N-Acetyl-S-(3-hydroxypropyl)-L-cysteine	1080 ± 40
N-Acetyl-S-(2-hydroxypropyl)-L-cysteine	152 ± 4
Mandelic acid	242 ± 9
N-Acetyl-S-(2-cyanoethyl)-L-cysteine	126 ± 8
N-Acetyl-S-(2-hydroxy-3-butenyl)-L-cysteine	2.01 ± 0.11
N-Acetyl-(4-hydroxy-2-buten-1-yl)-L-cysteine	54.8 ± 8.3
N-Acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine	915 ± 17
Phenylglyoxylic acid	290 ± 18
2-Methylhippuric acid	73.5 ± 4.8
N-Acetyl-S-(*n*-propyl)-L-cysteine	16.4 ± 0.4
3-Methyl & 4-Methylhippuric acids	531 ± 7
N-Acetyl-S-(1-phenyl-2-hydroxyethyl)-L-cysteine and *N*-Acetyl-S-(2-phenyl-2-hydroxyethyl)-L-cysteine	0.97 ± 0.32
N-Acetyl-S-(phenyl)-L-cysteine	1.08 ± 0.27
N-Acetyl-S-(benzyl)-L-cysteine	5.17 ± 0.83

[a] CDC analysis; data were converted from nanograms per milliliter to micrograms per kilogram using the density of the urine (1.019 ± 0.024 g/mL). The urine density uncertainty was accounted in the expanded uncertainties.

[b] The non-certified mass fraction value is the mean of results obtained using one analytical technique. The expanded uncertainty, U, is calculated as $U = ku_c$, where u_c is one standard deviation of the analyte mean, and the coverage factor, k, is determined from the Student's t-distribution corresponding to the associated degrees of freedom and a 95 % confidence level for each analyte. The effective coverage factor k is 4.30.

Table A4. Non-Certified Mass Fraction Values for Additional Analytes in SRM 3672

Analytes	Mass Fraction[a,b] (mg/kg)			k
Creatinine	734	±	5	2.07
Nicotine[c]	0.731	±	0.018	2.16
Ibuprofen[c]	0.122	±	0.005	2.16
Caffeine[c]	2.65	±	0.05	2.16
Cotinine[c]	1.09	±	0.02	2.16
Theobromine[c]	3.31	±	0.09	2.16
3-Hydroxycotinine[c]	3.46	±	0.09	2.16

[a] NIST analysis.
[b] The non-certified mass fraction value is the mean of results obtained using one analytical technique. The expanded uncertainty, U, is calculated as $U = ku_c$, where u_c is one standard deviation of the analyte mean, and the coverage factor, k, is determined from the Student's t-distribution corresponding to the associated degrees of freedom and a 95 % confidence level for each analyte. The effective coverage factor k is given in the table.
[c] Mass fraction of non-conjugated species only.

Additional Information: Partial support for the development of SRM 3672 was provided by the Division of Laboratory Sciences, Organic Analytical Toxicology Branch, CDC (Atlanta, GA).

Sample Preparation: Solomon Park Research Laboratories, Inc. acquired a 25 L pool of smokers' urine from donors who smoked more than one pack of cigarettes per day. The urine was filtered prior to aliquoting into amber glass bottles that are capable of withstanding ultra-cold temperatures. Each bottle was filled with 10 mL of urine and stored at –80 °C prior to shipping on dry ice to NIST.

Hydroxylated Polycyclic Aromatic Hydrocarbons: The approach used for the value assignment of hydroxylated PAHs in SRM 3672 consisted of combining results from analyses of the material at NIST, CDC, and INSPQ. At NIST, duplicate test portions of approximately 3 g from 10 vials were gravimetrically transferred to centrifuge tubes, spiked with a known amount of internal standard solution containing 1-naphthol-d_7 and 3-HO-phenanthrene $^{13}C_6$, followed by the addition of sodium acetate and β-glucuronidase/aryl sulfatase enzyme. The samples were incubated at 37 °C for 18 h and then eluted through a Strata X (Phenomenex, Torrance, CA) solid-phase extraction (SPE) column. Following washing and elution, the samples were concentrated to 0.5 mL in toluene and transferred to an autosampler vial. MSTFA (N-methyl-N-(trimethylsilyl)trifluoroacetamide) was added, and samples were heated at 60 °C for 30 min and analyzed using gas chromatography with mass spectrometry (GC/MS) with a 0.25 mm i.d. × 60 m fused silica capillary column with a 5 % (mole fraction) phenyl methylpolysiloxane phase (0.25 µm film thickness; DB-5, Agilent Technologies, Wilmington, DE).

The methods used for measurement of the hydroxylated PAHs at CDC are described in Li et al. [9]. At INSPQ, the urinary metabolites were hydrolyzed with β-glucuronidase enzyme followed by extraction with hexane at neutral pH. These extracts were then evaporated, derivatized with MSTFA, and analyzed using GC coupled with tandem mass spectrometry (GC/MS/MS) on a non-polar column (DB-XLB, 30 m × 0.25 mm × 0.10 µm film thickness). The multiple reaction monitoring (MRM) mode was used to quantify the hydroxylated PAHs.

Homogeneity Assessment for Hydroxylated PAHs: The homogeneity of SRM 3672 was assessed by analyzing duplicate test portions of 3 g from 10 vials selected by stratified random sampling. Test portions were processed and analyzed as described above for the NIST method. No differences among vials were observed for the hydroxylated PAHs at the 3 g test portion size.

Phthalate, Phenol, and Volatile Organic Compound Metabolites: The methods used at CDC for the quantification of phthalate and phenol metabolites are described in Silva et al. [10] and Ye et al. [11], respectively. Bisphenol A was quantified at NIST using a method adapted from Arakawa et al. [12]. Test portions of approximately 1 g were taken from six vials of SRM 3672. An internal standard solution containing $^{13}C_{12}$-bisphenol A was added followed by the addition of β-glucuronidase enzyme. Samples were incubated at 37 °C for 120 min. An amino SPE column was used to remove some of the potential interferences. MSTFA with 1 % (volume fraction) TMCS (trimethylchlorosilane) was added to the concentrated sample followed by incubation at 60 °C for 20 min prior to GC/MS analysis on a 0.25 mm i.d. × 30 m fused silica capillary column with a 5 % (mole fraction) phenyl methylpolysiloxane phase (0.25 µm film thickness; HP-5MS). The method used at CDC for the quantification of VOC metabolites is described in Alwis et al. [13].

Additional Analytes: Creatinine was quantified at NIST using liquid chromatography with mass spectrometry (LC/MS). Samples of the thawed urine were spiked with d_3-creatinine followed by the addition of water and a hydrochloric acid (HCl) solution to bring the final concentration to 0.01 mol/L HCl. LC/MS measurements utilized a Luna C18 column, 0.25 cm × 4.6 mm, 5 µm particle (Phenomenex, Torrance, CA) with single-ion monitoring.

The free (non-conjugated) levels of nicotine, ibuprofen, caffeine, cotinine, 3-hydroxycotinine, and theobromine in SRM 3672 were measured at NIST using liquid/liquid extraction with chloroform followed by GC/MS analysis similar to that described in Man et al. [14]. Isotopically labeled nicotine, ibuprofen, caffeine, cotinine, 3-hydroxycotinine, and theobromine were used as the internal standards. The GC/MS analysis used a 0.25 mm i.d. × 30 m fused silica capillary column with a 50 % (mole fraction) trifluoropropyl methylpolysiloxane phase (0.25 µm film thickness; DB-210).

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