



Certificate of Analysis

Standard Reference Material[®] 3672

Organic Contaminants in Smokers' Urine

(Frozen)

This Standard Reference Material (SRM) is 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 reference values are provided are naturally present in the urine. 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 Mass Fraction Values: Certified mass fraction values for hydroxylated PAHs 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 uncertainty have been investigated or taken into account [1]. The certified values are based on the agreement of results obtained at NIST, CDC, and Institut national de santé publique du Québec (INSPQ).

Reference Mass Fraction Values: Reference mass fraction values are provided in Table 2 for phthalate metabolites, Table 3 for phenol metabolites, Table 4 for VOC metabolites, and Table 5 for additional compounds. Reference values are noncertified values that are estimates of the true values; however, the values do not meet the NIST criteria for certification and are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods [1].

Expiration of Certification: The certification of **SRM 3672** is valid, within the measurement uncertainty specified, until **31 May 2030**, 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.

Overall direction and coordination of technical measurements leading to certification were performed by L.C. Sander of the NIST Chemical Sciences Division.

Evaluation of the data was provided by N.A. Heckert of the NIST Statistical Engineering Division.

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

Preparation of the urine was performed by Solomon Park Research Laboratories, Inc. (Kirkland, WA).

Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

Carlos A. Gonzalez, Chief
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Acquisition of the material was performed by K.E. Sharpless of the NIST Chemical Sciences Division. Analytical measurements at NIST were performed by B.A. Benner Jr., and S.S. Vander Pol of the NIST Chemical Sciences Division; and M.M. Schantz, Y. Vasquez, and M. Villegas formerly of NIST. Analytical measurements at CDC were performed by K.U. Alwis, B.C. Blount, A.M. Calafat, Z. Li, and A. Sjödin and at INSPQ by E. Gaudreau.

NOTICE AND WARNINGS TO USERS

SRM 3672 IS INTENDED FOR RESEARCH USE. THIS IS A HUMAN-SOURCE MATERIAL. HANDLE PRODUCT AS A BIOHAZARDOUS MATERIAL CAPABLE OF TRANSMITTING INFECTIOUS DISEASE. Accordingly, 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 Centers for Disease Control and Prevention/National Institutes of Health Manual [2].

INSTRUCTIONS FOR STORAGE AND USE

Storage: The SRM is stored at $-80\text{ }^{\circ}\text{C}$ 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 $-20\text{ }^{\circ}\text{C}$ is acceptable for storage for up to one week. If a longer storage time is anticipated, the material should be stored at or below $-60\text{ }^{\circ}\text{C}$. 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 ($20\text{ }^{\circ}\text{C}$ to $25\text{ }^{\circ}\text{C}$). After the material is thawed to room temperature, it should be used immediately. The material should be vortex mixed before aliquots are withdrawn.

SOURCE, PREPARATION, AND ANALYSIS⁽¹⁾

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

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 aliquotting into amber glass bottles that are capable of withstanding ultra-cold temperatures. Each bottle was filled with 10 mL of urine and stored at $-80\text{ }^{\circ}\text{C}$ 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 ¹³C₆, followed by the addition of sodium acetate and β -glucuronidase/aryl sulfatase enzyme. The samples were incubated at $37\text{ }^{\circ}\text{C}$ 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 $60\text{ }^{\circ}\text{C}$ for 30 min and analyzed using gas chromatography with mass spectrometry (GC/MS) with a 0.25 mm i.d. \times 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. [3]. 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 \times 0.25 mm \times 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.

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

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. [4] and Ye et al. [5], respectively. Bisphenol A was quantified at NIST using a method adapted from Arakawa et al. [6]. Test portions of approximately 1 g were taken from six vials of SRM 3672. An internal standard solution containing $^{13}\text{C}_{12}$ -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. \times 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. [7].

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 \times 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. [8]. 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. \times 30 m fused silica capillary column with a 50 % (mole fraction) trifluoropropyl methylpolysiloxane phase (0.25 μm film thickness; DB-210).

Metrological Traceability: Certified values are metrologically traceable to the SI derived unit for mass fraction (expressed as micrograms per kilogram) and the measurand is the total concentration of each analyte in Table 1. Reference values are based on the method used for each analyte listed in Tables 2 to 5, the measurand is the mass fraction for each analyte listed and are metrologically traceable to the SI derived unit for mass fraction (expressed as micrograms per kilogram or milligrams per kilogram).

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

Hydroxylated PAHs	Mass Fraction ($\mu\text{g}/\text{kg}$)
1-Naphthol ^(a,b,c,d)	33.8 \pm 4.3
2-Naphthol ^(a,b,c,d)	8.57 \pm 0.16
9-Hydroxyfluorene ^(a,b,c,e)	0.331 \pm 0.077
3-Hydroxyfluorene ^(a,b,c,d)	0.420 \pm 0.018
4-Hydroxyphenanthrene ^(a,b,c,d)	0.0480 \pm 0.0045
9-Hydroxyphenanthrene ^(a,c,d)	0.959 \pm 0.061
3-Hydroxyphenanthrene ^(a,b,c,d)	0.123 \pm 0.007
1-Hydroxyphenanthrene ^(a,b,c,e)	0.133 \pm 0.014
2-Hydroxyphenanthrene ^(a,b,c,e)	0.0825 \pm 0.0007
1-Hydroxypyrene ^(a,b,c,e)	0.170 \pm 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 \pm 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 $\mu\text{micrograms}$ per kilogram using the density of the urine (1.019 \pm 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 [9,10]. The expanded uncertainty is the half-width of a symmetric 95 % parametric bootstrap confidence interval [11], which is consistent with the ISO/JCGM Guide [12,13]. 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 [9]. The uncertainty listed with each value is an expanded uncertainty about the mean [9,10], with coverage factor $k = 2$, calculated by combining within-method variances with a between-method variance [14] following the ISO/JCGM Guide [12,13].

Table 2. Reference Mass Fraction Values for Selected Phthalate Metabolites in SRM 3672

Phthalate Metabolites	Mass Fraction ^(a,b) ($\mu\text{g}/\text{kg}$)	
Mono-carboxynonyl phthalate isomers ^(c)	1.92	\pm 0.06
Mono-carboxyoctyl phthalate isomers ^(d)	21.3	\pm 1.1
Mono-(2-ethyl-5-carboxypentyl) phthalate	35.2	\pm 1.7
Mono-(2-ethyl-5-hydroxyhexyl) phthalate	24.8	\pm 0.4
Mono-(2-ethyl-5-oxohexyl) phthalate	14.9	\pm 0.4
Mono-(2-ethylhexyl) phthalate	4.13	\pm 0.15
Mono-(3-carboxypropyl) phthalate	2.99	\pm 0.20
Monobenzyl phthalate	8.37	\pm 0.18
Monoethyl phthalate	94.5	\pm 3.0
Mono-isobutyl phthalate	6.40	\pm 0.28
Mono- <i>n</i> -butyl phthalate	10.6	\pm 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 reference 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 3. Reference Mass Fraction Values for Selected Phenol Metabolites in SRM 3672

Phenol Metabolites	Mass Fraction ($\mu\text{g}/\text{kg}$)	
Bisphenol A (BPA) ^(a,b,c)	3.05	\pm 0.16
2,5-Dichlorophenol ^(b,d)	1.77	\pm 0.06
Benzophenone-3 ^(b,d)	191	\pm 5
Methyl Paraben ^(b,d)	113	\pm 2
Ethyl Paraben ^(b,d)	8.12	\pm 0.20
Propyl Paraben ^(b,d)	17.6	\pm 0.3
Butyl Paraben ^(b,d)	11.1	\pm 0.2
Triclosan ^(b,d)	17.7	\pm 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 reference value is a weighted mean of average mass fractions, with one average from each of two analytical methods [9,10]. The expanded uncertainty is the half-width of a symmetric 95 % parametric bootstrap confidence interval [11], which is consistent with the ISO/JCGM Guide [12,13]. The effective coverage factor, k , is 2.

^(d) The reference 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 4. Reference Mass Fraction Values for Selected VOC Metabolites in SRM 3672

VOC Metabolites	Mass Fraction ^(a,b) ($\mu\text{g}/\text{kg}$)
<i>Trans,trans</i> -muconic acid	173 \pm 21
<i>N</i> -Acetyl-S-(2-carboxyethyl)-L-cysteine	200 \pm 10
2-Aminothiazoline-4-carboxylic acid	117 \pm 17
<i>N</i> -Acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine	30.0 \pm 5.2
<i>N</i> -Acetyl-S-(2-carbamoylethyl)-L-cysteine	122 \pm 1
<i>N</i> -Acetyl-S-(2-hydroxyethyl)-L-cysteine	2.52 \pm 0.61
<i>N</i> -Acetyl-S-(3,4-dihydroxybutyl)-L-cysteine	277 \pm 24
<i>N</i> -Acetyl-S-(<i>N</i> -methylcarbamoyl)-L-cysteine	340 \pm 26
2-Thioxothiazolidine-4-carboxylic acid	20.6 \pm 2.3
<i>N</i> -Acetyl-S-(3-hydroxypropyl)-L-cysteine	1080 \pm 40
<i>N</i> -Acetyl-S-(2-hydroxypropyl)-L-cysteine	305 \pm 12
Mandelic acid	242 \pm 9
<i>N</i> -Acetyl-S-(2-cyanoethyl)-L-cysteine	126 \pm 8
<i>N</i> -Acetyl-S-(2-hydroxy-3-butenyl)-L-cysteine	2.01 \pm 0.11
<i>N</i> -Acetyl-(4-hydroxy-2-buten-1-yl)-L-cysteine	54.8 \pm 8.3
<i>N</i> -Acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine	1670 \pm 10
Phenylglyoxylic acid	290 \pm 18
2-Methylhippuric acid	73.5 \pm 4.8
<i>N</i> -Acetyl-S-(<i>n</i> -propyl)-L-cysteine	16.4 \pm 0.4
3-Methyl & 4-Methylhippuric acids	531 \pm 7
<i>N</i> -Acetyl-S-(1-phenyl-2-hydroxyethyl)-L-cysteine and <i>N</i> -Acetyl-S-(2-phenyl-2-hydroxyethyl)-L-cysteine	0.97 \pm 0.32
<i>N</i> -Acetyl-S-(phenyl)-L-cysteine	1.08 \pm 0.27
<i>N</i> -Acetyl-S-(benzyl)-L-cysteine	5.17 \pm 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 reference 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 5. Reference Mass Fraction Values for Additional Analytes in SRM 3672

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

^(a) NIST analysis.

^(b) The reference 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.

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Certificate Revision History: 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).

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 <https://www.nist.gov/srm>.