National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1508a

Benzoylecgonine (Cocaine Metabolite) in Freeze-Dried Urine

This Standard Reference Material (SRM) is intended primarily for validating methods used for the determination of benzoylecgonine (cocaine metabolite) in human urine. SRM 1508a consists of four bottles of freeze-dried urine: one bottle each of three different levels of benzoylecgonine plus one bottle of blank freeze-dried urine. The freeze-dried urine in each bottle should be reconstituted with 10.0 mL of organic-free water.

Certified Concentrations: The certified concentrations and uncertainties of benzoylecgonine (as the free base) are given in Table 1. The certified concentrations apply only to urine reconstituted as described (see "Instructions for Use") and are based on the results from two independent methods. 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 fully investigated or taken into account. Brief descriptions of the methods are given under the section heading "Analytical Methods for Certification."

Table 1. Certified Concentrations for Benzoylecgonine in SRM 1508a

Level	Concentration (ng/mL)
Low (1508a-1)	78.1 ± 4.0
Medium (1508a-2)	161.0 ± 6.8
High (1508a-3)	315 ± 15
Blank (1508a-0)	Not detected

The certified values are the weighted means of results obtained from two independent methods over an eleven year period. The means and expanded uncertainties of the certified concentrations are calculated using a Bayesian approach for combining results from multiple methods [1]. The expanded uncertainty $U = ku_c$, where u_c is the combined standard uncertainty calculated according to the ISO/JCGM Guide [2], and is intended to represent, at the level of one standard deviation, the combined effect of between-method variation, within-method variation, and other components of uncertainty; k is the coverage factor determined from the Student's t-distribution corresponding to the appropriate degrees of freedom and 95 % confidence for each level. The measurand is the total concentration of benzoylecgonine in reconstituted urine and the values listed are metrologically traceable to the SI unit for mass expressed as nanograms per milliliter.

Expiration of Certification: The certification of **SRM 1508a** is valid, within the measurement uncertainty specified, until **31 December 2019**, provided the SRM is handled and stored in accordance with instructions given in this certificate (see "Instructions for Use"). This 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 certification was under the direction of M.J. Welch of the NIST Chemical Sciences Division.

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Gaithersburg, MD 20899
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Certificate Revision History on Last Page

Robert L. Watters, Jr., Director
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Analytical measurements were performed at NIST in the Chemical Sciences Division by S. Tai and by P. Ellerbe, Research Associate, College of American Pathologists.

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

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

NOTICE AND WARNING TO USERS

SRM 1508a IS INTENDED FOR RESEARCH USE. THIS IS A HUMAN-SOURCE MATERIAL. HANDLE PRODUCT AS A BIOHAZARDOUS MATERIAL CAPABLE OF TRANSMITTING INFECTIOUS DISEASE. THE RECONSTITUTED URINE SHOULD BE HANDLED WITH PRECAUTIONS SUITABLE FOR FRESH URINE. Accordingly, this human source product should be handled at the Biosafety Level 2 or higher as recommended for any potentially infectious human serum or blood specimen by the Centers for Disease Control and Prevention (CDC) Office of Safety, Health, and Environment and the National Institutes of Health (NIH) [3].

INSTRUCTIONS FOR USE

Storage and Stability: Prior to reconstitution, SRM 1508a should be stored in the dark at temperatures between –10 °C and 5 °C.

Reconstitution Procedure: In order for the certified concentrations to be valid, SRM 1508a must be reconstituted as follows. Ten (10.0) milliliters of high purity water at room temperature (23 °C ± 3 °C) must be added to each bottle. The bottles should be allowed to stand at room temperature with occasional swirling for 30 min to ensure complete dissolution. **Do not shake.** Vigorous shaking causes foaming, which may lead to inhomogeneous distribution of the analytes within the bottle. After completion of the reconstitution procedure, samples should be extracted and processed within 1 h for the certified concentrations to be valid.

SOURCE, PREPARATION, AND ANALYSIS[(1)]

Source and Preparation of Material: SRM 1508a was prepared by Ciba Corning Diagnostics (Irvine, CA). The urine used to prepare this material was collected from donors tested and found negative for cocaine and its metabolites. Processing for this SRM was carried out under clean conditions. The bulk urine was processed as one master lot. The master lot of urine was filtered through a cellulose acetate filter and then divided into four separate concentration levels for fortification and filling. The first concentration level was the urine blank; the remaining three concentration levels were fortified with the appropriate amounts of benzoylecgonine. The benzoylecgonine used for the fortification was obtained from Sigma Diagnostics (St. Louis, MO). The fortified urines were homogenized for approximately one-half hour by gentle mixing. Mixing of each concentration level was continuous during the filling process. All levels were dispensed into amber glass vials (10.0 mL per vial) and freeze-dried. The net weight of the urine added to each vial varied by less than 1.0 % relative standard deviation over the entire filling range.

Analytical Methods for Certification

Benzoylecgonine was determined by two independent methods, one involving gas chromatography/mass spectrometry (GC/MS) [4] and the other involving liquid chromatography/mass spectrometry (LC/MS). The samples were reconstituted as described in the "Instructions for Use" section. For the GC/MS measurements, two bottles in each of three independent sets were prepared for each level of the SRM. From each bottle, a single aliquot was taken, spiked with a known amount of the internal standard, benzoylecgonine-d_3. The pH of the aliquot was adjusted and the material passed through a solid-phase ion exchange cartridge, according to the manufacturer's directions for benzoylecgonine in urine. The solvent was evaporated and the residue dissolved in N,O-bis(trimethylsilyl)acetamide. This solvent reacts with benzoylecgonine to form the trimethysilyl (TMS) ester derivative.

The GC/MS measurements were performed using a magnetic sector mass spectrometer operated in the electron ionization mode and at a resolution of 1000 with a 30-meter nonpolar fused silica capillary column connected directly to the ion source. The ions at m/z 240 and m/z 243 were monitored for benzoylecgonine and benzoylecgonine-d_3,

[(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.

respectively. Analyte concentrations were calculated by linear interpolation from calibration curves constructed independently for each set of samples.

For the second method, two bottles from each level were reconstituted and two aliquots from each bottle were spiked with benzoylecgonine-d_3 as above. For sample clean-up, the aliquots were put through a solid-phase extraction cartridge that was different than that used for the GC/MS method. After the solvent from the extraction was evaporated, the residue was dissolved in the mobile phase used for the LC/MS (0.085 volume % acetic acid in water/acetonitrile [85:15]).

A commercial LC/MS instrument with an electrospray ionization source and a quadrupole mass analyzer was used for the analysis. The LC separations were carried out using a commercial C_{18} column (15 cm × 2.1 mm, 5 μm particle diameter) with an isocratic mobile phase at a flow rate of 0.3 mL/min. Electrospray ionization in the positive mode was used with selected ion monitoring to measure the $(M + H)^+$ ions at m/z 290 and m/z 293 for benzoylecgonine and benzoylecgonine-d_3, respectively. Analyte concentrations were calculated by linear interpolation from calibration curves constructed independently for each set of samples.

Appropriate corrections in the certified values were made based on the purity of the benzoylecgonine reference compound used for calibration in each method.

REFERENCES

[1] Liu, H-k.; Zhang, N.F.; *Bayesian Approach to Combining Results from Multiple Methods*; Proceedings of the Section of Bayesian Statistical Science of American Statistical Society (2001).

[2] 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 Dec 2015); 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 Dec 2015).

[3] 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, Centers for Disease Control and Prevention and National Institutes of Health; US Government Printing Office: Washington, D.C. (2009); available at http://www.cdc.gov/OD/OHS/biosfty/bmbl5/BMBL_5th_Edition.pdf (accessed Dec 2015).

[4] Ellerbe, P.; Tai, S.S.-C.; Christensen, R.G.; Espinoza-Leniz, R.; Paule, R.C.; Sander, L.C.; Sniegoski, L.T.; Welch, M.J.; White, V.E.; *The Certification of Cocaine and Benzoylecgonine in a Human Urine Standard Reference Material*; J. Anal. Toxicol., Vol. 16, pp. 158–162 (1992).

Certificate Revision History: 01 December 2015 (Editorial changes); 10 April 2014 (Extension of certification period; editorial changes); 22 December 2009 (Extension of certification period); 03 August 2004 (Certified values changed; extension of the certification period); 22 December 2003 (Extension of certification period); 05 March 1999 (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 at: telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.