



# National Institute of Standards & Technology

## Certificate of Analysis

### Standard Reference Material<sup>®</sup> 1508a

#### Benzoyllecgonine (Cocaine Metabolite) in Freeze-Dried Urine

This Standard Reference Material (SRM) is intended primarily for validating methods used for the determination of benzoyllecgonine (cocaine metabolite) in human urine. SRM 1508a consists of four bottles of freeze-dried urine: one bottle each of three different levels of benzoyllecgonine 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 benzoyllecgonine (as the free base) are given in Table 1. The certified concentrations apply only to urine reconstituted as described under “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 investigated or accounted for by NIST. Brief descriptions of the methods are given under the section heading “Analytical Methods for Certification.”

Table 1. Certified Concentrations for Benzoyllecgonine in SRM 1508a

Concentration 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 and NIST Guides [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.

**Expiration of SRM Certification:** The certification of this SRM is valid until **31 December 2009**, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given in this certificate. However, the certification is nullified if the SRM is contaminated or 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.

The coordination of the technical measurements leading to certification was under the direction of M.J. Welch of the NIST Analytical Chemistry Division.

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Certificate Issue Date: 03 August 2004  
*See Certificate Revision History on Last Page*

Robert L. Watters, Jr., Acting Chief  
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Analytical measurements were performed at NIST in the Analytical Chemistry 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.

The support aspects involved in the issuance of this SRM were coordinated through the NIST Standard Reference Materials Program by B.S. MacDonald of the NIST Measurement Services Division.

## NOTICE AND WARNING TO USERS

**SRM 1508a IS INTENDED FOR LABORATORY USE ONLY. THIS IS A HUMAN SOURCE MATERIAL. HANDLE PRODUCT AS A BIOHAZARDOUS MATERIAL CAPABLE OF TRANSMITTING INFECTIOUS DISEASE.** No known test method can offer complete assurance that Hepatitis B virus, HIV, or other infectious agents are absent from this material. The reconstituted urine should be handled with precautions suitable for fresh urine samples.

**Storage and Stability:** Prior to reconstitution, SRM 1508a should be stored in the dark at temperatures between  $-10\text{ }^{\circ}\text{C}$  and  $5\text{ }^{\circ}\text{C}$ .

**Reconstitution Procedure:** In order for the certified concentrations to be valid, SRM 1508a must be reconstituted as follows. Ten (10.0) mL of high purity water at room temperature ( $23\text{ }^{\circ}\text{C} \pm 3\text{ }^{\circ}\text{C}$ ) 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<sup>1</sup>

**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. The master lot was 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) [3] 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 trimethylsilyl (TMS) ester derivative.

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<sup>1</sup>Certain commercial equipment, instruments, or materials are identified in this certificate in order to specify adequately 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.

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- $d_3$ , 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  $\times$  2.1 mm, 5  $\mu$ m 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] ISO; *Guide to the Expression of Uncertainty in Measurement*, ISBN 92-67-10188-9, 1st ed.; International Organization for Standardization: Geneva, Switzerland (1993); 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).
- [3] 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).

<b>Certificate Revision History:</b> 03 August 2004 (This revision changes the certified values and extends the certification date); 22 December 2003 (This version extends the certification date); 05 March 1999 (Original certificate date).
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*Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-6776; fax (301) 926-4751; e-mail [srminfo@nist.gov](mailto:srminfo@nist.gov); or via the Internet at <http://www.nist.gov/srm>.*