



National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material 1508

Cocaine and Metabolites in Freeze-Dried Urine

This Standard Reference Material (SRM) is intended primarily for validating methods used for the determination of cocaine and benzoylecgonine in human urine. The concentrations of cocaine and benzoylecgonine are certified. In addition to cocaine and benzoylecgonine, ecgonine methyl ester is present in the SRM but its concentration is not certified. SRM 1508 consists of four bottles of freeze-dried urine: one bottle each of three different levels of the analytes plus one bottle of blank urine. The freeze-dried urine in each bottle should be reconstituted with 10.0 mL of organic-free water.

Certified Concentrations

The certified values of cocaine and benzoylecgonine (as the free bases) are given in the table below. The values in this certificate dated October 25, 1990 reflect additional measurement data and material stability information. The certified values are reviewed annually as part of the NIST update certification program. The estimated uncertainty of each certified value is based on scientific judgment and is intended to approximate \pm two standard deviations except as noted with asterisk.

Concentration Level	Concentration, ng/mL	
	Cocaine	Benzoylecgonine
Low (1508-1)	90 \pm 14	*103 \pm 10
Medium (1508-2)	263 \pm 22	259 \pm 20
High (1508-3)	429 \pm 20	510 \pm 29
Blank (1508-0)	**Not detected	**Not detected

* The observed variability and uncertainty for this value was extremely small and unrealistic. However, based on scientific judgment, an uncertainty of 10 ng/mL measurement was assigned.

**The approximate limit of detection for cocaine was 5 ng/mL and for benzoylecgonine was 12 ng/mL.

The certified concentrations apply only to urine reconstituted as described under "Instructions for Use" and are based on the results from two independent methods. Brief descriptions of the methods are given under the section heading "Analytical Methods for Certification". Noncertified concentrations of ecgonine methyl ester are given in the Appendix.

Analytical measurements were performed by R. G. Christensen, L. C. Sander, and L. T. Sniegowski of the Organic Analytical Research Division and by P. Ellerbe and S. S.-C. Tai, Research Associates, College of American Pathologists.

Statistical consultation was provided by R. C. Paule of the National Measurement Laboratory.

October 25, 1990
Gaithersburg, MD 20899
(Revision of certificate dated 4-12-90)

William P. Reed, Acting Chief
Standard Reference Materials Program

(over)

The overall direction and coordination of the technical measurements leading to the certification of this SRM were performed by M. J. Welch, E. White V, and W. E. May of the Organic Analytical Research Division.

The technical and support aspects involved in the certification and issuance of this Standard Reference Material were coordinated by R. Alvarez, Standard Reference Materials Program.

Instructions for Use

In order for the certified concentrations to be valid, SRM 1508 must be reconstituted as follows. Ten (10.0) mL of high purity water at room temperature, 24 ± 3 °C, must be added to each bottle. The bottles should be allowed to stand at room temperature with occasional swirling for 30 minutes 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 one hour for the certified concentrations to be valid.

Notice and Warning to Users

This material is for laboratory use only. SRM 1508 may contain hazardous substances. The reconstituted urine should be handled with precautions suitable for fresh urine samples.

Storage and Stability

Prior to reconstitution, SRM 1508 should be stored in the dark at temperatures between -10 and 5 °C. If properly stored, it is expected to be stable for at least one year from the date of purchase. NIST will continue to monitor this SRM and purchasers will be notified if evidence indicates a change in the certified concentrations. Please return the attached registration form to facilitate registration. The certified values are valid for one year from the date of shipment.

Source of the Material

The material was prepared by Cone Biotech, Inc., Seguin, TX.

Urine collected from male employee donors of Cone Biotech was combined into a master pool, which was filtered through a sterile 0.22- μ m filter and divided into four subpools (one each for the blank and the three levels). Cocaine, benzoylecgonine, and ecgonine methyl ester were added to the three subpools.

Analytical Methods for Certification

Cocaine and benzoylecgonine were each determined by two independent methods, gas chromatography/mass spectrometry (GC/MS) and liquid chromatography (LC) for cocaine, and GC/MS and flow injection, thermospray mass spectrometry for benzoylecgonine. Although the GC/MS methods for the two analytes are very similar, they were performed separately on different bottles of sample. For both analytes, the samples were reconstituted as described in the "Instructions for Use" section. A total of nine bottles in three independent sets, were prepared for each level of each analyte. From each bottle a single 5-mL aliquot was taken, spiked with a known amount of the internal standard (cocaine-d₃ or benzoylecgonine-d₃), and mixed with phosphate buffer. The pH was adjusted to between 4 and 6, and the aliquot was passed through a solid-phase ion exchange cartridge. The analyte was eluted with a solution of methylene chloride, 2-propanol, and ammonium hydroxide, after which the solvent was evaporated. For GC/MS measurements, the residue was dissolved in N,O-bis(trimethylsilyl)acetamide. This solvent reacts with benzoylecgonine to form the trimethylsilyl (TMS) ester derivative; cocaine is underivatized in this solvent.

The GC/MS measurements were performed using a quadrupole mass spectrometer operated in the electron ionization mode with a 30-meter nonpolar fused silica capillary column connected directly to the ion source. The ions at m/z 82 and 85 were monitored for cocaine and cocaine-d₃, respectively. The ions at m/z 240 and 243 were monitored for benzoylecgonine-TMS and benzoylecgonine-d₃-TMS, respectively. Analyte concentrations were calculated by linear interpolation from calibration curves constructed independently for each set of samples.

For cocaine, ultra violet detection was used in the LC method. The contents of a bottle were reconstituted, as described previously, and spiked with a known amount of the internal standard, benzoylecgonine ethyl ester. Each sample was put through a solid-phase cyclodextrin column. Cocaine was eluted with methanol, which was evaporated under argon, and the residue dissolved in water for LC analysis.

For the LC measurements, a C₁₈ column was used with a linear gradient from 5% acetonitrile in pH 2.1 aqueous phosphate buffer to 20% acetonitrile in pH 2.1 aqueous buffer over 5 minutes. Detection was at 233 nm. Calibration curves were established from LC analyses of extracts from blank urine samples that had been spiked with known amounts of cocaine and the internal standard. Three samples at each of the three levels were analyzed on two different days.

For benzoylecgonine, the second method involved flow injection-thermospray mass spectrometry. The internal standard was benzoylecgonine-d₃. The solid-phase extraction used was identical to that for the LC method for cocaine except that the extract was not evaporated, but was directly injected. The thermospray device was interfaced to a quadrupole mass spectrometer and was operated without use of a filament or a glow discharge for ionization. The mobile phase was 0.1 M ammonium acetate. No column was used; injected samples were passed directly into the thermospray unit. Positive ions at m/z 290 and 293 were monitored. A small interference from the urine matrix was seen under these conditions. Because all three concentration levels had been prepared from the same pool, the response from the blank urine was subtracted from the responses of the other levels. Aqueous calibration mixtures consisting of known amounts of benzoylecgonine and the internal standard were subjected to the same extraction procedure and were run interspersed with the samples. One sample at each level was analyzed a total of 13 times on two different days.

Purity of the reference compounds used for calibration of all methods was assessed and appropriate corrections in the certified values were made.

APPENDIX

Information Values

The manufacturer of the material added to the subpools weighed amounts of ecgonine methyl ester corresponding to concentrations of 90, 250, and 475 ng/mL in levels 1508-1, 1508-2, and 1508-3, respectively. However, this constituent was not determined and is therefore not certified.