National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1511

Multi-Drugs of Abuse in Freeze-Dried Urine

This Standard Reference Material (SRM) is intended primarily for verifying the accuracy of methods used for the determination of morphine, codeine, cocaine metabolite (benzoylecgonine), marijuana metabolite (THC-9-COOH), and phencyclidine in human urine. SRM 1511 consists of three (3) bottles of freeze–dried urine with all of the analytes included in each bottle. (See reconstitution procedure for reconstitution to 25 mL.) There is no blank urine with this SRM.

Certified Concentration: A NIST certified value is a value for which NIST has the highest confidence in its accuracy and that all known or suspected sources of bias have been investigated or accounted for by NIST [1]. The certified values and uncertainties for the analytes, as the free bases, are given below.

Analyte	Concentration (mmole/L)	Concentration (ng/mL)
Morphine	$1.08 \times 10^{-3} \pm 0.07 \times 10^{-3}$	309 ± 20
Codeine	$9.62 \times 10^{-4} \pm 0.37 \times 10^{-4}$	288 ± 11
Benzoylecgonine	$5.60 \times 10^{-4} \pm 0.28 \times 10^{-4}$	162 ± 8
THC-9-COOH	$4.09 \times 10^{-5} \pm 0.23 \times 10^{-5}$	14.1 ± 0.8
Phencyclidine[(a)]	$8.51 \times 10^{-5} \pm 0.82 \times 10^{-5}$	20.7 ± 2.0

The certified concentrations apply only to urine reconstituted as specified under "Reconstitution Procedure" and are based upon the equally weighted results from two different analytical methods for each analyte. For benzoylecgonine, morphine, codeine, and phencyclidine, gas chromatography mass spectrometry (GC/MS) and liquid chromatography mass spectrometry (LC/MS) data were used and the uncertainty is a 95 % confidence interval for the mean. For the THC-9-COOH, the mean concentration was computed from GC/MS and gas chromatography tandem mass spectrometry mass spectrometry (GC/MS/MS) measurements taken at NIST and the uncertainty is also a 95 % confidence interval for the mean. However, this confidence interval also includes variability observed between NIST and five military laboratories which had been used to demonstrate the suitability of the material. It is assumed that systematic errors are very small compared to random errors. The expanded uncertainties, U, equal k times the standard uncertainties (u_c) with $k = 2$, and conform with ISO and NIST Guides [2].

[(a)] For phencyclidine, the original results were combined with new measurements using LC/MS to generate a prediction value of the concentration from 2005 to 2010 with the expanded uncertainty U for the 95 % prediction interval.

This material also contains amphetamine and methamphetamine, but these analytes were **NOT** certified as analytical results indicated probable degradation of these constituents with time.

Expiration of Certification: The certification of this SRM lot is valid until **01 January 2010**, 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 invalid if the SRM is damaged, contaminated, or modified.

The overall direction and coordination of the technical measurements leading to the certification of this SRM were performed by M.J. Welch of the NIST Analytical Chemistry Division.

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See Certificate Revision History on Last Page

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The analytical measurements for certification of this SRM were performed by R.G. Christensen, P. Ellerbe, C.S. Phinney, L.C. Sander, and S.S.–C. Tai of the NIST Analytical Chemistry Division. Stability measurements were performed by S.S.-C. Tai.

Statistical analysis was provided by K.J. Coakley and N.F. Zhang of the NIST Statistical Engineering Division.

The support aspects involved in the issuance of this SRM were coordinated through the NIST Measurement Services Division.

INSTRUCTIONS FOR USE

Reconstitution Procedure: In order for the certified concentration to be valid, the SRM must be reconstituted as follows: twenty-five milliliters (25.0 mL) of organic-free water at room temperature 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 one h in order to achieve the certified concentration within the specified uncertainty range.

Storage and Stability: Prior to reconstitution, SRM 1511 should be stored in the dark at temperatures between –10 °C and 5 °C. NIST will continue to monitor this SRM and purchasers will be notified if evidence indicates a change in the certified concentrations.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

NOTICE AND WARNING TO USERS

This material is for laboratory use only. SRM 1511 may contain hazardous substances. The reconstituted urine should be handled with precautions suitable for fresh urine samples.

Source of the Material: The material was prepared by Consolidated Technologies, Inc. (Austin, TX).[1]

Analytical Methods

Method 1: Certification of the concentrations of analytes in this material was based on two independent methods performed on separately prepared samples. These methods have been described in more detail in references 3 through 9. For all of the analytes, one of the methods used for certification involved gas chromatography/mass spectrometry (GC/MS). Separate samples were prepared and separate analyses performed for each analyte except for morphine and codeine, which were prepared and analyzed together. Samples were reconstituted as described in the "Reconstitution Procedure" section. For the GC/MS analyses, the number of independently prepared sets and the number of samples per set for each analyte are as follows: morphine/codeine, 3 and 6; benzoylecgonine and phencyclidine, 3 and 5; and THC-9-COOH, 4 and 6. From each vial, a single aliquot was taken, spiked with known amounts of the corresponding deuterated internal standards, and put through a solid-phase column. The analytes were eluted following the column manufacturer's directions and the solvent evaporated. For GC/MS measurements, the residue was dissolved in N,O–bis(trimethylsilyl)acetamide to form the trimethylsilyl derivatives, except in the case of phencyclidine, which was dissolved in ethyl acetate and not derivatized. The GC/MS measurements were performed using either a quadrupole mass spectrometer or an ion trap 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 monitored for each analyte and its deuterated analog were as follows: morphine, m/z 429 and 432; codeine, m/z 371 and 374; benzoylecgonine, m/z 240 and 243; THC-9-COOH, m/z 371 and 374; and phencyclidine, m/z 200 and 205, respectively. Analyte concentrations were calculated by linear interpolation from calibration curves constructed independently for each analyte for each set of samples.

[1] Certain commercial equipment, instrumentation, or materials are identified in this certificate to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the NIST, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Method 2: The second method for all of the analytes except THC-9-COOH involved liquid chromatography/mass spectrometry (LC/MS). For each analyte, six vials were reconstituted as described in "Reconstitution Procedure" at one time and a single 10.0 mL aliquot from each vial was spiked with a known amount of the corresponding deuterated internal standard. Each sample was put through a solid-phase column that was different than the one used for GC/MS analyses but similar in separation mechanism, following the manufacturer's directions. The residue was reconstituted in water for the LC/MS analyses, except for phencyclidine, which was dissolved in the LC mobile phase (see below). For the LC/MS measurements of morphine, codeine, and phencyclidine, a commercial monomeric C_{18} column was used with an isocratic mobile phase consisting of either 0.75 % glacial acetic acid and 0.1 M ammonium acetate in water:methanol (85:15) (morphine and codeine) or methanol:water (55:45) with 1 mM heptanesulfonic acid, 20 mM ammonium acetate, and 3.5 % glacial acetic acid (phencyclidine). For the LC/MS measurements of benzoylecgonine, a custom column with a triethyl bonded phase and an isocratic mobile phase of water:methanol (70:30) with 2 % glacial acetic acid and 0.1 M ammonium acetate were used. The thermospray interface was operated with the discharge and electron ionization off and temperatures were set to conditions optimized for sensitivity and stability. The ions monitored for each analyte and its deuterated analog were as follows: morphine, m/z 286 and 289; codeine, m/z 300 and 303; benzoylecgonine, m/z 290 and 293; and phencyclidine, m/z 244 and 249, respectively. Analyte concentrations were calculated from comparison of measured ratios with response factors from standard mixtures.

Method 2 for THC-9-COOH: The second method for THC-9-COOH involved GC/MS/MS. Four vials were prepared, spiked, and processed as was done for the GC/MS analyses, except that a different solid-phase extraction was used. Measurements were performed on an ion trap mass spectrometer with specially modified software to permit optimum excitation of parent ions. Electron ionization was used to generate ions at m/z 371 and 374, which were subjected to resonance excitation, yielding daughter ions at m/z 305 and 308 for the analyte and its deuterated analog, respectively. Analyte concentrations were calculated by linear interpolation from calibration curves.

The purity of the reference compounds used for calibration of all methods were assessed and appropriate corrections were made when calculating the certified values.

Military Laboratory Round-Robin Study: A group of military laboratories involved in urine drug testing were sent samples of the SRM for their evaluation. Five laboratories returning results used GC/MS methods. Their results (mean and one standard deviation) are summarized below.

Analyte	Concentration (ng/mL)
Morphine	307 ± 14
Codeine	297 ± 10
Benzoylecgonine	161 ± 3
THC-9-COOH	15.0 ± 0.7
Phencyclidine	23.1 ± 1.2

These results were not used in calculating the certified values (except as noted above in the "Certified Concentration" section); they were used to demonstrate that this material was suitable for its intended purpose in field laboratories.

Stability Testing: Stability measurements were performed using LC/MS with electrospray ionization operated in the positive ion mode for morphine, codeine, benzoylecgonine, and phencyclidine and in the negative mode for THC−9−COOH. Samples were reconstituted and prepared as described for LC/MS measurements. Analytes were separated by LC on a C18 column (15 cm × 2.1 mm, 5 μm particle diameter). A gradient mobile phase consisting of 0.1 % acetic acid in water:methanol was used for the positive ion measurements. The gradient was initially set at water:methanol (92:8) for 7.5 min, ramped to water:methanol (75:25) at 7.6 min, and held for 17.4 min. The ions monitored are those described above for LC/MS measurements. For THC−9−COOH, an isocratic mobile phase of 50 mmol/L ammonium acetate in water:methanol (32:68) was used for separation and measurements were made using m/z 343 and 346 for the unlabeled and deuterated forms, respectively.

REFERENCES

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[2] ISO; *Guide to the Expression of Uncertainty in Measurement*; ISBN 92–67–10188–9, lst 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); available at http://physics.nist.gov/Pubs/.

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Certificate Revision History: 05 April 2006 (This revision reflects an editorial change); 19 September 2005 (This revision reflects a change in the certified value of phencyclidine based upon measurements in 2005); 13 September 1994 (Original certificate date).

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; email srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.