National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1507b

11-Nor-Delta-9-Tetrahydrocannabinol-9-Carboxylic Acid in Freeze-Dried Urine

This Standard Reference Material (SRM) is intended primarily for verifying the accuracy of methods used for the determination of 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (THC-9-COOH) in human urine. A unit of SRM 1507b consists of three bottles of freeze-dried urine: two bottles, each containing a different certified concentration of THC-9-COOH and one bottle of a urine blank. The bottles are labeled as levels 1507b-1, 1507b-2, and 1507b-0, respectively. The contents of each bottle must be reconstituted with 20.0 mL of organic-free or HPLC-grade water.

Certified Concentrations: The certified concentrations and uncertainties apply only to urine reconstituted as specified under "Reconstitution Procedure". The certified concentrations and uncertainties are based on the results of measurements made at NIST by gas chromatography/mass spectrometry (GC/MS), gas chromatography/tandem mass spectrometry (GC/MS/MS), and liquid chromatography (LC) and measurements by GC/MS at five laboratories involved with the Department of Defense Drug Testing Program. All NIST analytical measurements were based on calibration solutions prepared from weighed quantities of THC-9-COOH obtained from Research Triangle Institute (Research Triangle Park, NC). Stability measurements have been performed using liquid chromatography/mass spectrometry (LC/MS).

The certified concentrations of THC-9-COOH in the reconstituted urine are given in Table 1 with estimated uncertainties. THC-9-COOH was not detected in the urine blank. The limit of detection, X_D, refers to the underlying true analyte concentration that the employed chemical measurement process is capable of detecting [1]. The measurand is the total concentration of THC-9-COOH in reconstituted urine and the values listed are metrologically traceable to the SI unit for concentration (expressed as nanograms per milliliter and micromoles per liter.

Table 1. Certified Concentrations for THC-9-COOH in SRM 1507b

Concentration Level		Concentration (ng/mL)	Concentration (mmol/L)
Low	(1507b-1)	11.7 ± 1.4	$(3.40 \times 10^{-5}) \pm (0.41 \times 10^{-5})$
Medium	(1507b-2)	24.1 ± 1.3	$(7.00 \times 10^{-5}) \pm (0.38 \times 10^{-5})$
Blank	(1507b-0)	X_D: < 1	X_D: (< 3) × 10^{-6}

Each certified concentration is a weighted average of results from each method, the weights being determined iteratively. Given the weights, the effective degrees of freedom are then calculated from the weights. Given the weighted average $\overline{x}$ and the effective degrees of freedom, df, the approximate 95 percent confidence interval is [2]:

$$\overline{x} \pm t(0.975, df)\,\hat{\sigma}(\overline{x})$$

For the two levels, the effective degrees of freedom are 3.5 and 3.9 respectively for levels 1507b-1 and 1507b-2. The standard errors of the weighted averages were 0.49 and 0.47.

Expiration of Certification: The certification of **SRM 1507b** is valid, within the measurement uncertainty specified, until **31 December 2026**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Instructions for Handling, Storage, and Use"). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

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Gaithersburg, MD 20899
Certificate Issue Date: 14 December 2016
Certificate Revision History on Last Page

Steven J. Choquette, Director
Office of Reference Materials

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 the preparation and technical measurements leading to the original certification of this SRM were performed under the direction of M.J. Welch and W.E. May formerly of the NIST Analytical Chemistry Division.

Coordination of the measurements for the reanalysis of the SRM was performed by M.J. Welch formerly of the NIST Analytical Chemistry Division.

Analytical determinations for the reanalysis of the SRM were performed by L.T. Sniegoski and S.S. Tai of the NIST Chemical Sciences Division.

The original analytical measurements were performed by L.C. Sander and S.S. Tai of the NIST Chemical Sciences Division.

Statistical consultation was provided by K.J. Coakley of the NIST Statistical Engineering Division.

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

NOTICE AND WARNINGS TO USER

SRM 1507b 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. No known test method can offer complete assurance that Hepatitis B virus, HIV, or other infectious agents are absent from this material.

INSTRUCTIONS FOR HANDLING, STORAGE, AND USE

Storage and Stability: Prior to reconstitution, SRM 1507b should be stored in the dark at a temperature between −10 °C and 5 °C.

Reconstitution Procedure: For the certified concentration to be valid within the specified uncertainty, the SRM must be reconstituted as follows: Add 20.0 mL of organic-free or HPLC grade water at room temperature to each bottle. Allow the bottles to stand with occasional swirling for 30 min to ensure complete dissolution. **Do not shake**. Vigorous shaking causes foaming which leads to inhomogeneous distribution of the analyte within the bottle. After the completion of the reconstitution procedure, samples should be extracted and processed within l h for the certified concentrations to be valid.

SOURCE, PREPARATION, AND ANALYSIS[(1)]

Source and Preparation of Material: SRM 1507b THC-9-COOH was prepared by Consolidated Technologies Inc. (Austin, TX). The urine used to prepare this material was collected from male donors tested and found negative for THC-9-COOH. 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 0.22 μm cellulose acetate filter. It was then fortified, and appropriate dilutions were made. THC-9-COOH in ethanol was used for the fortification. The THC-9-COOH solution was obtained from Research Triangle Institute (Research Triangle Park, NC). All levels were dispensed into amber glass vials (20.0 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.

[(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 National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Analytical Methods for Certification

GC/MS Analysis: Three series of measurements, separated by approximately one year, were performed at NIST using methods based on GC/MS. For the first series, a total of twelve randomly selected vials, in two independent sets, were analyzed for each level. Samples were reconstituted as described in the "Reconstitution Procedure", and a single 10.0 mL aliquot was withdrawn from each vial. For the second and third series, three randomly selected vials, in three independent sets, were analyzed. Two 8.0 mL aliquots were withdrawn from each vial for the second series and a single 10.0 mL for the third series. Each aliquot was spiked with an isotopically labeled internal standard, 5'-d_3-11-nor-delta-9-THC-9-carboxylic acid. The samples were processed using C_{18} solid-phase extraction cartridges to isolate the THC-9-COOH from the urine. The THC-9-COOH was converted to its trimethylsilyl derivative for analysis.

The GC/MS measurements were performed using a quadruple mass spectrometer operated in the electron ionization mode with a 30 m nonpolar fused silica capillary column connected directly to the ion source. Molecular ions at m/z 488 and 491 for the unlabeled and labeled forms, respectively, were monitored for the first two series. For the third series fragment ions at m/z 371 and 374 were monitored. Analyte concentrations were calculated by linear interpolation from calibration curves constructed for each set of samples.

GC/MS Analysis (Laboratories Affiliated with The Department of Defense Drug Testing Program): A standard Department of Defense procedure for determination of THC-9-COOH by GC/MS was used by laboratories affiliated with the Department of Defense Drug Testing Program. The method involved the addition of a deuterated internal standard to 10 mL of the reconstituted urine, followed by treatment with aqueous KOH to hydrolyze any THC-9-COOH esters. The solution is then buffered to a pH between 9 and 10 and put through a solid-phase extraction column. The eluent is evaporated, and iodomethane and tetramethylammonium hydroxide in dimethylsulfoxide are added to form the methyl ester of THC-9-COOH. The derivative is extracted into organic solvents for injection into a GC/MS instrument equipped with a nonpolar capillary column and set to monitor m/z 313, 357, and 372 from the derivative and 360 and 375 from its deuterated analog. Ion ratios obtained from samples are compared with those obtained from calibration standards to determine the concentration of THC-9-COOH in the sample. Each laboratory analyzed one vial of each level of SRM 1507b.

GC/MS/MS Analysis: Five vials of the low level, and three vials of the medium level were analyzed. The entire contents of each vial of the reconstituted urine were spiked with the labeled internal standard and processed by a solid-phase extraction cartridge using a mixed-mode retention mechanism of ion exchange and reverse phase to isolate the THC-9-COOH from the urine. The THC-9-COOH was converted to its tert-butyldimethylsilyl derivative for analysis.

The GC/MS/MS measurements were performed using a 30 m nonpolar fused silica capillary column interfaced to a triple quadrupole mass spectrometer. Electron ionization was used to generate molecular ions, m/z 572 and 575 for the unlabeled and labeled forms, respectively, which were subjected to collisions with argon in the middle quadrupole. The quadrupoles were operated in the neutral loss mode, with loss of 57 monitored for both ions. Analyte concentrations were calculated by linear interpolation from calibration curves constructed for the analyte.

LC Analysis: Four sets of SRM 1507b (consisting of one vial each of the two THC-9-COOH levels) were analyzed. Urine samples were reconstituted and spiked with an internal standard solution of Δ8-THC in methanol. The contents of each bottle were extracted using a commercially available solid-phase extraction cartridge specifically designed for extraction of THC-9-COOH from urine. THC-9-COOH and Δ8-THC were eluted from the cartridge with methanol.

THC-9-COOH was determined using a reversed-phase LC separation performed on a Zorbax[1] C_{18} column with gradient elution. Phosphoric acid was added to both water and acetonitrile (ACN) components (1.00 mL of 85 % phosphoric acid per liter of solvent). The following program was employed: step (1) equilibration with 50:50 ACN:H_2O for 7.5 min; step (2) injection; step (3) linear gradient from initial conditions to 100 % ACN over 30 min; step (4) hold at 100 % ACN for 15 min; step (5) linear gradient from 100 % ACN to 50:50 ACN-H_2O over 2 min. Column temperature was fixed at 40 °C, and mobile phase flow rate was 2 mL/min. Measurements were carried out using UV detection at 210 nm. Urine standards and SRM 1507b samples were processed identically using the method described above. Samples and standards were run alternately throughout the analysis procedure. A linear regression fit (using peak areas) was made of the urine standards, and the concentrations of THC-9-COOH in SRM 1507b samples were calculated from the regression line.

LC/MS Analysis: Stability measurements for this SRM have been performed using a method based on isotope-dilution LC/MS [3]. The samples were spiked with an isotopically labeled internal standard, 5'-d_3-11-nor-delta-9-THC-9-carboxylic acid and processed using C_{18} solid-phase extraction cartridges to isolate the THC-9-COOH from the urine. After the solvent from the extraction was evaporated, the residue was dissolved in the mobile phase used for the LC/MS (40 mM ammonium acetate in methanol:water [80:20]).

A commercial LC/MS instrument with an electrospray ionization source and a quadrupole mass analyzer was used for the analysis. The LC separation was carried out using a commercial C-18 column with an isocratic mobile phase at a flow rate of 0.3 mL/min. Electrospray ionization in the negative ion mode was used to monitor the $(M\text{-}H)^-$ ions at m/z 343 and 346 for THC-9-COOH and its labeled internal standard, respectively. Analyte concentrations were calculated by linear interpolation from calibration curves constructed for each set of samples.

REFERENCES

[1] American Chemical Society; *Detection in Analytical Chemistry - Importance, Theory, and Practice*; Currie, L.A., Ed; ACS Symposium Series: New York, Vol. 361, p. 10 (1988).

[2] Schiller, S.B.; Eberhardt, K.R.; *Combining Data From Independent Chemical Analysis Methods*; Spectrochemica Acta, Vol. 46B, No. 12, pp. 1607–1613 (1991).

[3] Tai, S.S.C.; Welch, M.J.; *Determination of 11-Nor-Delta-9-Tetrahydrocannabinal-9-carboxylic Acid in a Urine-based Standard Reference Material by Isotope Dilution-Liquid Chromatography/Mass Spectrometry with Electrospray Ionization*; J. Anal. Toxicol., Vol. 24, pp. 385–389 (2000).

Certificate Revision History: 14 December 2016 (Change of expiration date; editorial changes); 25 May 2011 (Extension of certification period; editorial changes); 05 January 2009 (Extension of certification period.); 01 December 2004 (This revision reflects the decertification and removal of the high level material); 29 September 2003 (This revision establishes an expiration date based upon a reanalysis of the SRM that demonstrated stability of THC-9-COOH in the material.); 01 February 1999 (This revision establishes an expiration date based upon a reanalysis of the SRM that demonstrated stability of THC-9-COOH in the material.); 16 November 1994 (Editorial changes); 12 May 1993 (This technical revision reports a correction in the certified data for all concentration levels.); 20 March 1992 (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 http://www.nist.gov/srm.