National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material 1507a

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. SRM 1507a consists of four bottles of freeze-dried urine: three bottles, each containing a different certified concentration of THC-9-COOH and one bottle of a urine blank. The contents of a bottle is to be reconstituted with 20.0 mL of organic-free or HPLC grade water.

Certified Concentration

The certified concentrations of THC-9-COOH in the reconstituted urine are given below with estimated uncertainties based on a statistical evaluation of random errors plus allowances for possible systematic error and possible degradation with time. No THC-9-COOH was 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. [Ref: "Detection in Analytical Chemistry - Importance, Theory, and Practice," ACS Symposium Series 361, pp 10, Lloyd A. Currie, Editor, 1988.]

Concentration Level	Concentration, ng/mL
Low (1507a-1)	13.5 ± 1
Medium (1507a-2)	31.6 ± 2
High (1507a-3)	49.4 ± 6
Blank (1507a-0)	X_D: <1

The certified concentration and uncertainty apply only to urine reconstituted as specified under "Reconstitution Procedure". The certified concentration and uncertainty are based on the results of measurements made at NIST by gas chromatography/mass spectrometry (GC/MS) and liquid chromatography (LC).

The overall direction and coordination of the preparation and technical measurements leading to the certification of this SRM were performed under the direction of M.J. Welch and W.E. May of the NIST Organic Analytical Research Division.

Analytical measurements were performed by L.C. Sander, NIST Organic Analytical Research Division, and S.S.-C. Tai, NIST Research Associate, College of American Pathologists.

Statistical consultation was provided by R.C. Paule, NIST National Measurement Laboratory.

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

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Gaithersburg, MD 20899

William P. Reed, Acting Chief
Standard Reference Materials Program

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Reconstitution Procedure

In order for the certified concentration to be valid, the SRM must be reconstituted as follows. Twenty (20.0) mL of organic-free or HPLC grade water at room temperature must be added to each bottle. Allow the bottles to stand with occasional swirling for 30 mintues to ensure complete dissolution. Do not shake. Vigorous shaking causes foaming which leads to inhomogeneous distribution of the analyte within the bottle. After completion of the reconstitution procedure, samples should be extracted or processed within one hour for the certified concentration to be valid within the specified uncertainty.

Notice and Warnings to User

This material is for laboratory use only. SRM 1507a may contain hazardous substances. The reconstituted material should be handled with precautions suitable for fresh urine samples.

Storage and Stability

Prior to reconstitution, SRM 1507a should be stored in the dark at a temperature between -10 and 5 °C. If properly stored, it is expected to be stable for at least one year from date of purchase. NIST will continue to monitor this SRM and purchasers will be notified if evidence indicates a change in the certified concentration. Please return attached registration card to facilitate notification. The material is certified for use for one year from the date of purchase.

Trademarks

The use of a trademark in this certificate is for identification only and does not imply endorsement of the product by the National Institute of Standards and Technology.

Source of the Material

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

Calibration Solutions

All analytical measurements, both GC/MS and HPLC, were based on calibration solutions prepared from weighed quantities of THC-9-COOH obtained from Research Triangle Institute, Research Triangle Park, NC.

GC/MS Measurement of THC-9-COOH in SRM 1507a

Two series of measurements, separated by approximately one year, were performed. For the first series, eight bottles of each level were analyzed in duplicate. Of the bottles selected, two were from the beginning of the production run, two from the middle, two from the end, and two randomly selected. For the second series, four randomly selected bottles were analyzed in duplicate. The contents were reconstituted as described above and two 8-mL aliquots were withdrawn from each. 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 trimethyl-silyl derivative for analysis.

GC/MS was performed using a standard 30-m, non polar, bonded phase capillary column interfaced to a quadrupole mass spectrometer operated in the electron ionization mode at 70 e.v. Molecular ions at M/Z 488 and 491 for the unlabeled and labeled forms, respectively, were monitored.

One additional set, consisting of six samples of the high level, was measured by GC/MS with the following modifications: (1) the whole bottle of the reconstituted urine was spiked with the isotopically labeled internal standard and a different solid-phase extraction was used; (2) the THC-9-COOH was converted to a t-butyl-dimethylsilyl derivative; and (3) methane chemical ionization was used to generate $(CM+H)^+$ ions at M/Z 573 and 576 for measurement.

HPLC Measurement of THC-9-COOH in SRM 1507

Four sets of SRM 1507a (consisting of three THC-9-COOH levels) were analyzed. Urine samples were rconstituted, 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 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 50:50 ACN:H_2O for 7.5 minutes; step (2) injection; step (3) linear gradient from initial conditions to 100% ACN over 30 minutes; step (4) hold at 100% ACN for 15 minutes; step (5) linear gradient from 100% ACN to 50:50 ACN-H_2O over 2 minutes. 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 1507a 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 1507a samples were calculated from the regression line.