National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material 1507

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 1507 consists of three bottles of freeze-dried urine: two bottles containing THC-9-COOH at the certified concentration and one bottle of a urine blank.

Certified Concentration

The certified concentration of THC-9-COOH in the reconstituted urine is given below with an estimated uncertainty based on a statistical evaluation of random errors plus an allowance for possible systematic error.

Certified concentration = 18 ± 2 ng/mL

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 verified by liquid chromatography with electrochemical detection.

SRM 1507 includes one bottle of "Freeze-Dried Urine Blank". There is no certified value associated with the blank. However, no THC-9-COOH was detected in the urine blank. The limit of detection for the THC-9-COOH was less than 1 ng/mL.

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 G.D. Byrd, R.G. Christensen, N.E. Craft, L.R. Hilpert, and M.J. Welch, 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.

Gaithersburg, MD 20899
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William P. Reed, Acting Chief
Standard Reference Materials Program

(over)

Reconstitution Procedure

In order for the certified concentration to be valid, the SRM must be reconstituted as follows. Twenty-five (25.0) mL of high purity water at room temperature must be added to each bottle. Allow the bottles to stand with occasional swirling for 30 minutes 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 1507 may contain hazardous substances. The reconstituted material should be handled with precautions suitable for fresh urine samples.

Storage and Stability

Prior to reconstitution, SRM 1507 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. The material is not certified for use after one year from the date of purchase.

Collection and Preparation of Material

The urine used to prepare SRM 1507 was collected from NIST staff members. Seventy-eight percent of the donated samples were from males and 22 percent were from females. These numbers refer to the percentage of samples donated and not the percentage of the sample volume.

The urine was processed under contract by Bell-More Laboratory, Hampstead, MD. Processing for SRM 1507, including aseptic filtering, bottling, and lyophilization, was carried out under sterile conditions. The bulk urine was processed in two lots; first lot (approximately 30 L) was the urine blank; the second lot (approximately 50 L) was fortified with THC-9-COOH. Each lot of urine was filtered through a 0.45 μm cellulose acetate filter. THC-9-COOH in ethanol was added to the second lot of filtered urine. The solution was prepared from THC-9-COOH obtained from Research Triangle Institute, Research Triangle Park, North Carolina. The fortified and blank urine samples were homogeneized for approximately one-half by swirling, allowed to stand for one hour, and mixed by swirling for an additional 15 minutes immediately prior to bottling.

The blank and fortified urine samples were dispensed into pre-cleaned 50-mL amber glass bottles (25 mL per bottle) and freeze-dried. The net weight of urine added to each bottle varied by less than 0.5% relative standard deviation over the entire filling sequence.

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

Representative bottles were selected at random and the material was reconstituted as described in the "Reconstitution Procedure" section above. A total of 63 bottles were analyzed in duplicate. One 10-mL aliquot of urine was removed from each bottle and spiked with an isotopically labeled internal standard, 5'-d_3-11-nor-delta-9-THC-9-carboxylic acid. The samples were processed separately using solid phase extraction to isolate the THC-9-COOH from the urine. The THC-9-COOH was converted to its trimethylsilyl derivative for analysis. The samples were processed and analyzed in random order.

The GC/MS analyses were carried out using a quadrupole mass spectrometer in the electron impact mode with a 30-meter DB-5 fused silica capillary column connected directly to the ion source. All samples were run in the selected ion monitoring mode. The ions at m/z 488 (M^+. for the trimethylsilyl derivative of THC-9-COOH) and 491 (M^+. for the labeled internal standard) were monitored. Response factor solutions, prepared from gravimetrically prepared solutions of the THC-9-COOH and the labeled internal standard solution, were analyzed to determine the response for the THC-9-COOH relative to the internal standard.

HPLC Measurement of THC-9-COOH in SRM 1507

Eleven representative bottles were selected for HPLC measurement. The freeze-dried urine in the bottles was reconstituted as described in the "Reconstitution Procedure" section. Aliquots of the reconstituted urine were spiked with an internal standard, 11-hydroxy-11-nor-delta-9-tetrahydrocannabinol (THC-11-OH), and processed separately using liquid-liquid extraction with 3% (V/V) isobutanol in hexane to isolate the THC-9-COOH from the urine. The organic phase was evaporated and the final extract was made up in methanol. The samples were processed and analyzed in random order.

Reversed-phase HPLC separation was performed on a monomerically bonded C_{18} analytical column. The analyte and internal standard were monitored by electrochemical detection in the oxidative mode (glassy carbon working electrode at + 1.0 V applied voltage and AgCl reference electrode). Quantitation was accomplished by the internal standard method using electronic measurement of peak heights.