National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material 2381

Morphine and Codeine in Freeze-Dried Urine

This Standard Reference Material (SRM) is intended primarily for verifying the accuracy of methods used for the determination of morphine and codeine in human urine. SRM 2381 consists of four bottles of freeze-dried urine: one bottle each of three different levels of the analytes plus one bottle of blank urine.

Certified Concentration

The certified values for morphine and codeine, as the free bases, are given in the table below with an estimated uncertainty corresponding to approximately ± two standard deviations of the certified value based on a statistical evaluation of random errors plus an allowance for possible systematic error in the analytical methods used for the certification.

	Morphine	
Level	**mmol/L**	**ng/mL**
II	$(4.84 \pm 0.46) \times 10^{-5}$	138 ± 13
III	$(1.03 \pm 0.05) \times 10^{-3}$	293 ± 15
IV	$(2.03 \pm 0.05) \times 10^{-3}$	578 ± 15

	Codeine	
Level	**mmol/L**	**ng/mL**
II	$(4.48 \pm 0.37) \times 10^{-5}$	134 ± 11
III	$(9.45 \pm 0.50) \times 10^{-4}$	283 ± 15
IV	$(1.97 \pm 0.20) \times 10^{-3}$	591 ± 60

The certified concentrations apply only to urine reconstituted as specified under the "Reconstitution Procedure" section on page 2 and are based upon the concordant results from three different analytical methods. Brief descriptions of the methods are given under the "Analytical Methods" section on page 2.

SRM 2381 includes one bottle of Level I, "Freeze-Dried Urine Blank", for which there are no certified values. Morphine and codeine were not detected by GC/MS at limits of detection of less than 4×10^{-6} mmol/L (1 ng/mL) and 3×10^{-6} mmol/L (1 ng/mL), respectively.

Notice and Warning to Users

This material is for laboratory use only. SRM 2381 may contain hazardous substances. The reconstituted urine should be handled with precautions suitable for fresh urine samples.

Analytical measurements were performed by R.G. Christensen, L.C. Sander, and S.S.-C. Tai of the Organic Analytical Research Division.

Statistical consultation was provided by R.C. Paule, Statistical Engineering Division.

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 NIST Organic Analytical Research Division.

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 and T.E. Gills.

Gaithersburg, MD 20899
February 28, 1992

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William P. Reed, Chief
Standard Reference Materials Program

Storage and Stability

Prior to reconstitution, SRM 2381 should be stored in the dark at temperatures between -10 and 5 °C. If the SRM is properly stored, the certified values are valid for one year from the date of shipment. NIST will continue to monitor this SRM and purchasers will be notified if evidence indicates a significant change in the certified concentrations.

Reconstitution Procedure

In order for the certified concentrations to be valid, the SRM must be reconstituted as follows. Ten (10.0) mL of organic-free water at room temperature (22 °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 used within one hour for the certified concentration to be valid within the specified uncertainty.

Source of the Material

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

Analytical Methods

Certification of the concentrations of morphine and codeine in this material was based on three independent methods performed on separately prepared samples at NIST.

For both morphine and codeine, one of the methods used for certification involved gas chromatography/mass spectrometry (GC/MS). Samples were reconstituted as described in the "Reconstitution Procedure" section above. A total of twelve vials, in two independent sets, were prepared for each level. From each vial, a single 5 mL aliquot was taken, spiked with known amounts of the internal standards (morphine-d_3 and codeine-d_3), and processed with a solid-phase extraction column using a mixed-mode retention mechanism of ion exchange and reversed-phase. The analytes were eluted with a solvent consisting of 2% concentrated ammonium hydroxide in methylene chloride: 2-propanol (80:20), and the solvent evaporated. For GC/MS measurements, the residue was dissolved in N,O-bis(trimethylsilyl)acetamide. This solvent reacts with morphine to form the bis(trimethylsilyl)(TMS) ether derivative and with codeine to form the mono(trimethylsilyl) ether.

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 429, 432, 371, and 374 were monitored for morphine, morphine-d_3, codeine and codeine-d_3, respectively. Analyte concentrations were calculated by linear interpolation from calibration curves constructed independently for each analyte for each set of samples.

The second method for both morphine and codeine involved liquid chromatography/mass spectrometry (LC/MS). Three vials of Levels II and IV and six vials of Level III were reconstituted as above and the entire contents of each vial spiked with a known amount of the internal standards, (morphine-d_3 and codeine-d_3). Each sample was processed with a solid-phase extraction column similar to the type used for the GC/MS method, using the same solvent mixture. The residue was reconstituted in water for the LC/MS analyses.

For the LC/MS measurements a monomeric C_8 column was used with an isocratic mobile phase consisting of 0.2% trifluoroacetic acid and 0.1 M ammonium acetate in water: methanol (3:1). The thermospray interface was operated with the discharge and electron ionization off, and temperatures were set to conditions optimized for sensitivity and stability. Positively charged ions at m/z 286, 289, 300, and 303 were monitored for morphine, morphine-d_3, codeine, and codeine-d_3, respectively. Analyte concentrations were calculated from comparison of measured ratios with response factors from standard mixtures.

The third method involved direct probe tandem mass spectrometry (MS/MS). Two vials of each level were prepared, spiked, and processed as was done for the GC/MS analyses, except that no derivatization was done; samples were dissolved in methanol. Approximately 5 microliters of these methanol solutions were placed in aluminum crucibles and dried with gentle heating. The crucibles were individually inserted into a temperature controlled direct probe, which was then inserted into the source of a triple quadrupole mass spectrometer. A temperature program was used to reproducibly heat the probe. Electron ionization was used to generate molecular ions which were subjected to collisions with argon in the middle quadrupole. The quadrupoles were operated in the neutral loss mode, with losses of 123 and 137 monitored for morphine and codeine, respectively. Analyte concentrations were calculated by linear interpolation from calibration curves constructed independently for each analyte.

Purities 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 was sent samples of the SRM for evaluation and analysis. All nine laboratories returning results used GC/MS methods. Their results (mean and one standard deviation) are summarized below.

ng/mL

	Morphine		**Codeine**	
Level	mean	s	mean	s
II	148	10	137	11
III	297	17	268	24
IV	583	35	545	40

These results demonstrate that laboratories which routinely use GC/MS methods to determine opiates (morphine and codeine) in urine can obtain results on this material (SRM 2381) that are in agreement with the NIST certified values.