National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material 2382

Morphine Glucuronide in Freeze-Dried Urine

This Standard Reference Material (SRM) is intended primarily for verifying the accuracy of methods used for the determination of morphine that is present as a glucuronide in human urine. SRM 2382 consists of four bottles of freeze-dried urine: one bottle each of three different levels of the analyte plus one bottle of blank urine.

Certified Concentration

The certified values for morphine, as the free base, 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 certification.

Level	Morphine	
	mmol/L	**ng/mL**
II	$(7.36 \pm 0.77) \times 10^{-4}$	210 ± 22
III	$(1.53 \pm 0.05) \times 10^{-3}$	437 ± 13
IV	$(3.03 \pm 0.14) \times 10^{-3}$	865 ± 41

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 two independent analytical methods. GC/MS analyses found no free (unconjugated) morphine in levels II and III, but free morphine accounted for approximately 0.1% of the total morphine found in Level IV. Brief descriptions of the methods are given under the "Analytical Methods" section on page 2.

SRM 2382 includes one bottle of Level I, "Freeze-Dried Urine Blank", for which there is no certified value. Morphine was not detected in the blank by GC/MS at a limit of detection of less than 4×10^{-6} mmol/L (1 ng/mL).

Notice and Warning to Users

This material is for laboratory use only. SRM 2382 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. Gills.

Gaithersburg, MD 20899
February 28, 1992

William P. Reed, Chief
Standard Reference Materials Program

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Storage and Stability

Prior to reconstitution, SRM 2382 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, unless purchasers are notified otherwise. 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 concentration 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 analyte 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, Texas. Drug-free human urine was spiked with appropriate quantities of morphine-3-ß-D-glucuronide for the three levels.

Analytical Methods

Certification of the concentration of morphine glucuronide in this material was based on two independent methods performed on separately prepared samples at NIST.

One of the methods used for certification involved gas chromatography/mass spectrometry (GC/MS). The 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 and treated with an enzyme, ß-glucuronidase/arylsulfatase from Helix pomatia, at 55 °C for 17 hours, to hydrolyze the morphine glucuronide. Each sample was then spiked with a known amount of the internal standard (morphine-d_3), and processed with a solid-phase extraction column using a mixed-mode retention mechanism of ion exchange and reversed-phase. The morphine was 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.

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

The second method for morphine glucuronide involved liquid chromatography/mass spectrometry (LC/MS). Four vials of each level were reconstituted as above and a single 5 mL aliquot taken from each vial. To each aliquot was added 0.6 mL of concentrated hydrochloric acid and the mixture was capped and heated at 121 °C for 20 minutes to hydrolyze the morphine glucuronide. After each sample was neutralized, it was spiked with a known amount of the internal standard (morphine-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 that provided good sensitivity and stability. The positively charged ions at m/z 286 and 289 were monitored for morphine and morphine-d_3, respectively. Analyte concentrations were calculated from comparison of measured ratios with response factors from standard mixtures.

Purity of the reference compound used for calibration of both methods was 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 ten laboratories returning results used acid hydrolysis to free morphine from the glucuronide and GC/MS methods to determine the morphine concentrations. Their results (mean and one standard deviation) are summarized below.

	Morphine (ng/mL)	
Level	mean	s
II	213	19
III	420	38
IV	822	79

These results demonstrate that laboratories which routinely use GC/MS methods to determine morphine glucuronide in urine can obtain results on this material (SRM 2382) that are in agreement with the NIST certified values.