



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. A unit of SRM 2382 consists of four bottles of freeze-dried urine: one bottle of blank urine, Level I, and one bottle each of three different analyte levels, Level II, III, and IV. The contents of each bottle must be reconstituted with 10.0 mL of organic-free or HPLC grade water at room temperature, 22 °C.

Certified Concentration: The certified concentrations in Table 1 apply only to urine reconstituted as specified under the “Reconstitution Procedure” section and are based upon the concordant results from two independent analytical methods. Brief descriptions of the methods are given under the “Analytical Methods” section. 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). 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. Drug-free human urine was spiked with appropriate quantities of morphine-3- β -D-glucuronide for the three levels.

Expiration of Certification: The certification of **SRM 2382** is valid, within the measurement uncertainty specified, until **13 March 2015**, provided the SRM is handled and stored in accordance with instructions given in this certificate (see “Instructions for Storage and Use”). This certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

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.

Coordination of the technical measurements leading to the certification of this SRM were performed by M.J. Welch and W.E. May of NIST.

Analytical measurements were performed by L.C. Sander S.S.-C. Tai of the NIST Chemical Sciences Division and R.G. Christensen formerly of NIST.

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

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

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NOTICE AND WARNINGS TO USER

SRM 2382 IS INTENDED FOR IN VITRO LABORATORY USE ONLY. THIS IS A HUMAN SOURCE MATERIAL AND SHOULD BE TREATED AS A BIOHAZARDOUS SUBSTANCE CAPABLE OF TRANSMITTING INFECTIOUS DISEASE. THE RECONSTITUTED URINE SHOULD BE HANDLED WITH PRECAUTIONS SUITABLE FOR FRESH URINE. Accordingly, this human urine based product should be handled at the Biosafety Level 2 or higher as recommended for any potentially infectious human specimen in the Centers for Disease Control and Prevention/National Institutes of Health Manual [1].

INSTRUCTIONS FOR STORAGE AND USE

Storage: Prior to reconstitution, SRM 2382 should be stored in the dark at temperatures between $-10\text{ }^{\circ}\text{C}$ and $5\text{ }^{\circ}\text{C}$. 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 or HPLC grade water at room temperature, ($22\text{ }^{\circ}\text{C}$) 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.

Certified Concentration Values: The material for this SRM was prepared by Cone Biotech, Inc. (Seguin, TX).⁽¹⁾ The certified concentrations for morphine glucuronide in the reconstituted urine are given in Table 1 as free morphine with estimated uncertainties. The morphine glucuronide 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 [2].

Table 1. Certified Concentrations for Morphine in SRM 2382

Concentration Level	Morphine	
	ng/mL	mmol/L
I	$X_D: <1$	$X_D: <4 \times 10^{-6}$
II	209 ± 20	$(7.32 \pm 0.70) \times 10^{-4}$
III	437 ± 21	$(1.53 \pm 0.07) \times 10^{-3}$
IV	853 ± 39	$(2.99 \pm 0.14) \times 10^{-3}$

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 \bar{x} and the effective degrees of freedom, df , the approximate 95 percent confidence interval is

$$\bar{x} \pm t_{.975, df} \sigma(\bar{x}) \quad [3]$$

For the three levels the effective degrees of freedom are 4.80, 4.97, and 3.95 respectively for levels II, III, and IV. The standard error of the weighted averages are 3.66, 8.09, and 14.2.

⁽¹⁾ Certain commercial instruments, materials, or processes are identified in this certificate to adequately specify 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 instruments, materials, or processes identified are necessarily the best available for the purpose.

Military Laboratory Round-Robin Study: A group of military laboratories involved in urine drug testing was twice sent samples of the SRM for evaluation and analysis. The 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.

Level	Morphine (ng/mL)			
	1991		1992	
	mean	σ	mean	σ
II	213	19	216	8
III	420	38	432	20
IV	822	79	857	55

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.

ANALYTICAL METHODS

Gas Chromatography/Mass Spectrometry (GC/MS): One of the methods used for certification involved GC/MS. The samples were reconstituted as described in the “Reconstitution Procedure” section. Two series of measurements were performed, separated by approximately one year. For the first series, a total of twelve vials, in two independent sets, were prepared for each level. For the second series, a total of six 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, β -glucuronidase/arylsulfatase from *Helix pomatia*, at 55 °C for 17 h, 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.

Liquid Chromatography/Mass Spectrometry (LC/MS): 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.

REFERENCES

- [1] CDC/NIH; Biosafety in Microbiological and Biomedical Laboratories, 5th ed.; Richardson, J.; Barkley, W.E.; Richmond, J.; McKinney, R.W., Eds.; U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention and National Institutes of Health; US Government Printing Office: Washington, D.C. (2009); available at http://www.cdc.gov/OD/OHS/biosfty/bmb15/BMBL_5th_Edition.pdf (accessed Apr 2015).
- [2] *Detection in Analytical Chemistry-Importance, Theory, and Practice*; ACS Symposium Series 361, Lloyd A. Currie, Editor, pp. 10 (1988).
- [3] Schiller, S.B.; Eberhardt, K.R.; *Combining Data From Independent Chemical Analysis Methods*; Spectrochimica Acta, Vol. 46 B, No. 12, pp. 1607–1613 (1991).

Certificate Revision History: **15 April 2015** (Change of expiration date, ended period of certification; editorial changes); **15 July 1993** (Updated certified values; editorial changes); **28 February 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, email srminfo@nist.gov; or via the Internet at <http://www.nist.gov/srm>.