



Certificate of Analysis

Standard Reference Material[®] 2380

Drugs of Abuse in Human Hair II

This Standard Reference Material (SRM) is intended primarily for use in validating methods for determining morphine (MOR), codeine (COD), 6-monoacetylmorphine (MAM), and tetrahydrocannabinol (THC) in human hair and similar materials. This SRM can also be used for quality assurance when assigning values to in-house control materials. The SRM is a drug-free human hair material, to which drugs of abuse have been added. A unit of SRM 2380 consists of one bottle containing approximately 100 mg of hair segments.

Certified Mass Fraction Values: The certified mass fraction values for the target compounds in SRM 2380 are provided in Table 1. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [1]. The certified concentration values for each level are based on the agreement of results from gas chromatography - mass spectrometry (GC-MS) and liquid chromatography - mass spectrometry (LC-MS). Values are reported on an as-received basis in mass fraction units.

Expiration of Certification: The certification of **SRM 2380** is valid, within the measurement uncertainty specified, until **01 January 2017**, provided the SRM is handled and stored in accordance with instructions given in this certificate (see "Instructions for Storage and Use"). However, the 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) will facilitate notification.

The overall direction and coordination of the analytical measurements leading to the certification of this SRM were performed by M.J. Welch of the NIST Chemical Sciences Division, and K.W. Phinney of the NIST Biomolecular Measurement Division.

Certification measurements were performed by J.L. Prendergast, L.T. Sniegowski, and S.S-C. Tai of the NIST Chemical Sciences Division. Additional measurements were performed by M.M. Schantz of the NIST Chemical Sciences Division and B.E. Lang of the NIST Biosystems and Biomaterials Division.

Statistical analysis was provided by N.-f. Zhang of the NIST Statistical Engineering Division.

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

Carlos A. Gonzalez, Chief
Chemical Sciences Division

Gaithersburg, MD 20899
Certificate Issue Date: 07 December 2012
Certificate Revision History on Last Page

Robert L. Watters, Jr., Director
Office of Reference Materials

NOTICE AND WARNING TO USERS

Warning: For laboratory use only.

INSTRUCTIONS FOR STORAGE AND USE

Storage: The SRM should be stored under refrigeration at temperatures between 4 °C and 8 °C.

Use: A quantity of hair appropriate for a particular analysis should be withdrawn and weighed on an analytical balance prior to extraction and analysis. Particular care must be exercised for determinations of morphine and monoacetylmorphine (MAM) because of the possible conversion of significant quantities of MAM to morphine during sample preparation. These effects can be minimized by spiking the sample with isotope-labeled internal standards at levels near those of the unlabeled analytes prior to extraction.

SOURCE, PREPARATION AND ANALYSIS⁽¹⁾

Preparation: SRM 2380 is human hair from a single individual. The hair was manually cut into short segments and then soaked for 17 days in equal volumes of water and dimethylsulfoxide containing the target analytes. The hair was removed from the mother liquor, rinsed several times with methanol, and allowed to air dry.

NIST Analyses For the Target Drugs of Abuse: For each of the analytes, measurements from two independent methods based on GC-MS and LC-MS were used. For the GC-MS method for the opiates [2], hair samples were spiked with deuterated analogs of each of the analytes and then extracted with 0.1 mol/L HCl at 45 °C for 24 h. The extracts were neutralized and put through commercial solid-phase extraction cartridges designed for urine drug testing following the manufacturer's directions. After the eluants were collected and dried, COD, MOR, and MAM were dissolved in N,O-bis(trimethylsilyl)acetamide and analyzed together. For the GC-MS measurements of THC, the hair samples were treated with 1 mol/L NaOH and heated at 95 °C for 30 min. The mixture was then acidified to pH 4–5 and put through a solid-phase extraction cartridge. The eluant was treated with N,O-bis(trimethylsilyl)acetamide to derivatize the THC for GC-MS analysis. For both methods, the GC-MS was equipped with a 30 m low-polarity GC column and measurements were performed using electron ionization and selected ion monitoring. For the LC-MS method for the opiates, hair samples were spiked with deuterated analogs of each of the analytes and extracted with 0.1 mol/L HCl at 45 °C for 24 h. The extracts were neutralized to pH 6.0 and put through solid-phase extraction cartridges that were different from those used for GC-MS. No derivatization was necessary, and the three opiate compounds were measured together in a single run. The LC was equipped with a deactivated C₁₈ column, and compounds were eluted using a gradient mobile phase of 0.1 % (volume fraction) acetic acid in water and methanol. For the LC-MS measurement of THC, the hair samples were treated with 1 mol/L NaOH and heated at 95 °C for 30 min. After the pH of the samples was adjusted to pH 3–4, THC was extracted with hexane:ethyl acetate (9:1, volume fractions). This extract was then dried and reconstituted in methanol for analysis by LC-MS. This method used a different deactivated C₁₈ column, but the mobile phase was the same as that used for the opiates. The electrospray ionization conditions were optimized for each analyte. For both GC-MS and LC-MS methods, three sets of samples were measured for all of the analytes. Each set consisted of two samples each from three bottles of hair. Calibration standards were run before and after the samples, and a standard curve was generated by a least-squares fit of all of the standards measurements for a particular set.

Homogeneity Assessment: For the sample sizes used for the certification measurements (20 mg to 25 mg), there was no evidence of within-bottle inhomogeneity. There also was no correlation between fill order and concentrations measured for any of the analytes.

Stability Measurements: Analyses of the target analytes were performed using the GC-MS and LC-MS approaches previously described. The certified concentration values for codeine, morphine, and 6-monoacetylmorphine were updated based upon the stability measurements.

⁽¹⁾ Certain commercial equipment, instruments or materials 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 materials or equipment identified are necessarily the best available for the purpose.

Certified Mass Fraction Values: The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence, consistent with the ISO Guide and with its Supplement 1 [3–7]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is the combined uncertainty, and k is a coverage factor corresponding to approximately 95 % confidence for each analyte [3]. For the certified values shown below, $k = 2$.

Table 1. Certified Concentration Values for Analytes in SRM 2380

Analyte	Mass Fraction (ng/mg)
Codeine	8.80 ± 0.39
Morphine	9.31 ± 0.51
6-Monoacetylmorphine	2.10 ± 0.26
Tetrahydrocannabinol	0.99 ± 0.10

REFERENCES

- [1] May, W.; Parris, R.; Beck, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260-136, U.S. Government Printing Office, Gaithersburg, MD (2000); available at <http://nist.gov/srm/publications.cfm> (accessed Dec 2012).
- [2] Welch, M.J.; Sniegowski, L.T.; Allgood, C.C.; Habram, M.; *Hair Analysis for Drugs of Abuse: Evaluation of Analytical Methods, Environmental Issues, and Development of Reference Materials*; J. Anal. Toxicol.; Vol. 17, pp. 389–398 (1993).
- [3] JCGM 100:2008; *Evaluation of Measurement Data — Guide to the Expression of Uncertainty in Measurement* (ISO GUM 1995 with Minor Corrections); Joint Committee for Guides in Metrology (2008); available at http://www.bipm.org/utis/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Dec 2012); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297; U.S. Government Printing Office: Washington, DC (1994); available at <http://physics.nist.gov/Pubs/> (accessed Dec 2012).
- [4] JCGM 101:2008; *Evaluation of measurement data – Supplement 1 to the Guide to the Expression of Uncertainty in Measurement – Propagation of Distributions Using a Monte Carlo Method*; JCGM (2008); available at http://www.bipm.org/utis/common/documents/jcgm/JCGM_101_2008_E.pdf (accessed Dec 2012).
- [5] Rukhin, A.L.; *Weighted Means Statistics in Interlaboratory Studies*; Metrologia, Vol. 46, pp. 323–331 (2009).
- [6] Schiller, S.B.; Eberhardt, K.E.; *Combining Data from Independent Chemical Analysis Methods*; Spectrochim. Acta, Vol. 46B, pp. 1607–1613 (1991).
- [7] Levenson, M.S.; Banks, D.L.; Eberhardt, K.R.; Gill, L.M.; Guthrie, W.F.; Liu, H-k.; Vangel, M.G.; Yen, J.H.; Zhang, N.F.; *An Approach to Combining Results from Multiple Methods Motivated by the ISO GUM*; J. Res. Natl. Inst. Stand. Technol., Vol. 105, pp. 571–579 (2000).

Certificate Revision History: 07 December 2012 (This revision contains updated certified mass fraction values for codeine, morphine, and 6-monoacetylmorphine, an extension of the certification period, and editorial changes); 29 October 2002 (Original certificate issue 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; e-mail srminfo@nist.gov; or via the Internet at <http://www.nist.gov/srm>.