National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 921a

Cortisol (Hydrocortisone)

This Standard Reference Material (SRM) is certified as a neat chemical material of known purity. It is intended for use in the calibration and standardization of clinical measurement laboratory procedures for determining quantities of cortisol. A unit of SRM 921a consists of 1 g of high-purity crystalline cortisol.

Certified Cortisol Mass Fraction: 99.3 % ± 0.4 %

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 measurand is the mass fraction of cortisol (expressed as percent) [2] and the uncertainty is expressed as the 95 % confidence interval ($U_{95\%}$) [3,4]. The standard uncertainty (u) of the certified value is 0.2 %. Metrological traceability of the certified value is to the International System of Units (SI) through realization of measurement units for chemical mass fraction (expressed as percentage). The certified value was determined using a quantitative ^{1}H-nuclear magnetic resonance spectroscopy (^{1}H-qNMR) primary ratio measurement procedure [5,6].

Expiration of Certification: The certification of **SRM 921a** is valid, within the measurement uncertainty specified, until **31 December 2029**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Instructions for Storage and Use"). 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 the certificate, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Overall direction and coordination of the technical activities were under the chairmanship of M.A. Nelson of the NIST Chemical Sciences Division.

Analytical measurements at NIST were performed by A.S.P. Boggs, B.E. Lang, and M.A. Nelson of the NIST Chemical Sciences Division.

Statistical analysis was provided by B. Toman 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: 31 July 2020

Steven J. Choquette, Director
Office of Reference Materials

NOTICE AND WARNINGS TO USERS

SRM 921a IS INTENDED FOR RESEARCH USE.

INSTRUCTIONS FOR STORAGE AND USE

The SRM should be stored in its original container, tightly closed and refrigerated (2 °C to 8 °C), away from direct light. The SRM should always be protected from moisture and heat and be used without preliminary drying. Allow the material to equilibrate with ambient room temperature (between 20 °C and 30 °C) before opening the container. The minimum sample size is 5 mg.

SOURCE AND ANALYSIS

Source of Material: The SRM source material was obtained from a commercial supplier.

Analytical Approach and Heterogeneity Assessment: Analyses for chemical identity, purity, and heterogeneity were performed by NIST using eleven units, selected at regular intervals across the entire production lot. A ^{1}H-qNMR primary ratio measurement procedure using an internal standard approach was implemented for the determination of cortisol mass fraction with metrological traceability to SI units. Calculation of the 95 % coverage interval was performed under the Bayesian paradigm using a hierarchical measurement model for the ^{1}H-qNMR procedure [7,8]. No trend in mass fraction of cortisol was observed with respect to filling order and there is no significant heterogeneity at the 95 % confidence level. An identified cortisone impurity content was estimated via ^{1}H-qNMR as 0.2 % ± 0.1 % at the 95 % level of confidence. Supporting and confirmatory measurements for assessment of impurity components were made via liquid chromatography with ultraviolet detection, liquid chromatography with mass spectrometric detection, and thermogravimetric analysis.

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.; *Definition 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: Washington, DC (2000); available at https://www.nist.gov/system/files/documents/srm/SP260-136.PDF (accessed Jul 2020).

[2] Thompson, A.; Taylor, B.N.; *Guide for the Use of the International System of Units (SI)*; NIST Special Publication 811; U.S. Government Printing Office: Washington, DC (2008); available at https://www.nist.gov/pml/special-publication-811 (accessed Jul 2020).

[3] JCGM 100:2008; *Evaluation of Measurement Data — Guide to the Expression of Uncertainty in Measurement* (GUM 1995 with Minor Corrections); Joint Committee for Guides in Metrology (JCGM) (2008); available at https://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Jul 2020); 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 https://www.nist.gov/pml/nist-technical-note-1297 (accessed Jul 2020).

[4] JCGM 101:2008; *Evaluation of Measurement Data — Supplement 1 to the "Guide to Expression of Uncertainty in Measurement" — Propagation of Distributions Using a Monte Carlo Method*; JCGM (2008); available at https://www.bipm.org/utils/common/documents/jcgm/JCGM_101_2008_E.pdf (accessed Jul 2020).

[5] Milton, M.J.; Quinn T.J.; *Primary Methods for the Measurement of Amount of Substance*; Metrologia, Vol. 38, pp. 289−296 (2001).

[6] Jancke, H.; *NMR Spectroscopy as a Primary Analytical Method of Measurement*; Nachr. Chem. Tech. Lab., Vol. 46(7-8), pp. 720−722 (1998).

[7] Possolo, A.; Toman, B.; *Assessment of Measurement-Uncertainty via Observation Equations*; Metrologia, Vol. 44, pp. 464−475 (2007).

[8] Toman, B., Nelson, M.A.; Lippa, K.A.; *Chemical Purity Using Quantitative ^{1}H-Nuclear Magnetic Resonance: A Hierarchical Bayesian Approach for Traceable Calibrations*; Metrologia, Vol. 53, pp. 1193−1203 (2016).

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 https://www.nist.gov/srm.