National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 921

Cortisol (Hydrocortisone)

This Standard Reference Material is certified as a chemical of known purity. It is intended primarily for use in the calibration and standardization of procedures for cortisol determinations and for routine critical evaluation of the daily working standards used in these procedures.

Constituent	Percent
Cortisol	98.9
21-Dehydrocortisol	0.6
21-O-Acetylcortisol	0.2
21-Dehydrocortisone	0.1
Cortisone	0.1
Total Steroids	99.9
Ash	0.002
Insoluble Matter	0.001
Loss on Drying	0.08

Expiration of Certification: The certification of **SRM 921** is valid for 5 years from the date of shipment from NIST, within the measurement uncertainty specified, 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 this certificate, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Analyses were performed by R.F. Brady, Jr., A. Cohen, B. Coxon, M. Darr, W.D. Dorko, D.P. Enagonio, T.E. Gills, E.E. Hughes, W.P. Schmidt, and S.A. Wicks formerly of NIST.

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

This Certificate of Analysis has undergone editorial revisions. No attempt was made to reevaluate the values presented on this certificate.

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Gaithersburg, MD 20899
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Certificate Revision History on Last Page

Steven J. Choquette, Acting Director
Office of Reference Materials

NOTICE AND WARNINGS TO USERS

SRM 921 IS INTENDED FOR RESEARCH USE.

INSTRUCTIONS FOR STORAGE, AND USE

Storage: Keep this SRM refrigerated (4 °C) in a well-closed container and away from direct sunlight.

Use: A standard solution containing 5 µg per mL of cortisol may be prepared as follows. Transfer 50 mg of SRM 921 to a 50 ml volumetric flask and add 35 ml of absolute ethanol. When the cortisol is completely dissolved, dilute to the mark with absolute ethanol. Dilute l mL of this ethanol solution to 200 mL with distilled water [1,2]. Working standard solution may be made by appropriate dilution of aliquots of the 5 µg per mL solution with distilled water. The solution of l mg/mL of cortisol in ethanol as prepared above should be stored in a well-stoppered, all-glass container and kept in a refrigerator at 4 °C. The aqueous solution of 5 µg per mL cortisol should also be stored in a well-stoppered, all-glass container at 4 °C. Under these conditions both solutions should be stable for six months. The more dilute "working" standard solutions should be prepared daily. All constituted solutions of cortisol should be clear and display no turbidity.

PREPARATION AND ANALYSIS[(1)]

The cortisol used for this SRM was obtained from the Upjohn Company (Kalamazoo, MI). Identification and quantitation of the four steroid impurities were accomplished by Fourier transform proton magnetic resonance (PMR) spectroscopy and thin-layer chromatography (TLC) performed on fractions from the liquid chromatography of 100 mg of the material in ethanol on a column (91 x 0.8 cm) of poly(vinylpyridine) crosslinked with 8 % of divinylbenzene. Fractions were eluted with ethanol at a pressure of 4.7 $kg \cdot cm^2$. TLC of aliquots of the fractions was performed on silica gel GF_{254} using 9:1 (v/v) chloroform-methanol. Equivalent sensitivity of detection was obtained by fluorescence quenching and by spraying with aqueous 20 % sulfuric acid and heating at 120 °C. All the steroid impurities, except 21-dehydrocortisone, showed similar sensitivity. 4-Androsten-llβ-ol-3,17-dione was found in liquid chromatography fractions, but could not be demonstrated directly by TLC of up to 1 mg of the bulk material. Because this compound was readily resolved from mixtures prepared with it and the bulk material and detected in proportion to amounts used for reference, the compound was adjudged an artifact produced during liquid chromatography. On the other hand, the major impurity, 21-dehydrocortisol, did not arise by the known copper-catalyzed oxidation of cortisol, as shown by its unaltered proportion by TLC of the bulk material even after EDTA-treatment of the system to remove copper.

The quantitative proportion of each steroid present in the sample was estimated from the dry weight (W) of the residue of each liquid chromatographic fraction and the measured intensity of the Fourier transform generated methyl signals characteristic for each steroid, using the expression:

$$m_n M_n = \frac{W h_n M_n}{h_1 m_1 + h_2 M_2 \ldots + h_m M_m}$$

where m_n and M_n are the number of moles present and the molecular weight, respectively of the n_{th} component in a mixture of m components, h_n is the methyl signal intensity of the n_{th} component, and $h_1 \ldots h_m$ are the corresponding intensities of components l...m, obtained by measurement of the methyl peaks above the methylene envelope of the steroids.

For proof of homogeneity, nine samples were withdrawn from the bulk SRM according to a statistical plan. They were analyzed in a commercial carbon-hydrogen-nitrogen microanalyzer and were found to be homogeneous with respect to carbon and hydrogen content within the limits of precision of the method. Solutions of the samples in 95 % ethanol at 25 °C showed an absorbance maximum at 242 nm with a molar extinction coefficient of 16.1 x 10^3 $liter \cdot cm^{-1} \cdot mol^{-1}$.

Elemental macroanalysis of the material showed carbon 69.49 % ± 0.10 % (2 SD of the mean); and hydrogen 8.39 % ± 0.05 % (2 SD of the mean); the calculated values for cortisol are 69.58 % and 8.34 %, respectively.

The SRM melted at 219.0 °C to 220.5 °C (corrected) when heated in an open capillary tube at 0.5 $°C \cdot min^{-1}$. The resulting pale yellow melt did not solidify on cooling. After sealing in a capillary tube under vacuum, the material melted at 220.5 °C to 221.5 °C without yellowing, but did not resolidify.

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(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.

Thermogravimetric analysis of samples heated under dry nitrogen at 2 °C·min^{-1} showed the initiation of loss of a large proportion of sample weight at 221 °C (uncorrected). However; for samples heated in air, loss of weight began at 204 °C. The attempted application of differential scanning calorimetry to samples heated under nitrogen gave thermograms that were very dependent on the rate and time of heating, and that were not reproducible.

The mass spectrum of the SRM obtained by electron induced ionization at 70 eV and a probe temperature of 220 °C showed strong ion currents at *m/e* 362 [M^+], 344 [M–18(H_2O)], 332 [M–30(CH_2O)], 303 [M–59($COCH_2OH$)], 302 [M–60(CH_2=C=O and H_2O)], 285 [M–59–18], 42 [CH_2=C=O], and 31 [(CH_2=0-H)$^+$]. The peaks at *m/e* 302 and 163 correspond to the molecular ion and key fragment, respectively, of the thermal degradation product of cortisol, namely 4-Androsten-llβ-ol-3,17-dione.

Optical rotations of solutions of the SRM in ethanol (c 1.00) were measured at 20 °C by means of automatic and high-precision, manual polarimeters and are as follows:

	$[\alpha]_\lambda^{20}$ (±2 SD of the mean)	
λ (nm)	degrees	radians
589	168.9 ± 0.1	2.948 ± 0.002
578	177.3 ± 0.1	3.094 ± 0.002
546	205.6 ± 0.1	3.588 ± 0.002
436	387.9 ± 0.4	6.770 ± 0.007
365	571.7 ± 0.6	9.978 ± 0.010

Insoluble matter was determined by filtration of solutions of the SRM (1.8 g to 2.0 g) in methyl sulfoxide (9 mL to 20 mL) through Millipore filters (1.5 µm).

The loss of weight on drying was determined after heating three samples (2.8 g each) of the material at 110 °C and 0.1 torr for 24 hours. No further weight loss occurred during heating of the samples for an additional 48 hours.

Ash was determined by heating of samples (10 g) of the SRM to 700 °C. Spectrometric analysis of the ash indicated calcium as a major constituent, and sodium, magnesium and silicon as minor constituents.

Destructive neutron activation analysis using SRM 1577, *Bovine Liver*, as a standard indicated that the Cortisol SRM contains 0.035 ppm ± 0.005 ppm (±2 SD of the mean) of copper.

REFERENCES

[1] Peterson, R.E., Hydrocortisone in Plasma; In *Standard Methods of Clinical Chemistry*, Vol. 3; Seligson D., Editor-in-Chief, Academic Press, Inc., New York, N.Y.; pp. 160–166 (1961).

[2] Tietz, N.W., *Fundamentals of Clinical Chemistry*, W.B. Saunders Co., Philadelphia, PA, pp. 722–726 (1970).

Certificate Revision History: 04 March 2016 (Updated storage conditions; editorial changes);**16 December 2015** (Editorial changes); **16 December 1973** (Editorial changes); **15 February 1973** (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.