



Certificate of Analysis

Standard Reference Material[®] 912a

Urea

This Standard Reference Material (SRM) is intended primarily for calibrating apparatus and validating methods used in clinical and pathology laboratories. It is certified as a chemical of known purity. A unit of SRM 912a consists of 25 g of high purity urea contained in a glass bottle.

Physical Property	Weight Percent (%)		
Purity ^(a)	99.9	±	0.1
Moisture ^(b)	0.2	±	0.003
Biuret ^(c)	0.2	±	0.02
Ash ^(d)	0.001	±	0.000 7
Insoluble Matter ^(e)	0.0001	±	0.000 05

^(a) Differential scanning calorimetry [1] indicated a purity of 99.97 ± 0.01 mole percent (exclusive of moisture content).

^(b) The moisture content was determined separately by Karl Fischer titration of samples ranging from three grams to ten grams.

^(c) Biuret was estimated spectrometrically by use of a 6 mol/L solution of urea in aqueous, alkaline nickelous sulfate measured at the absorption maximum of biuret at 461 nm [2]. This determination was calibrated against dilute solutions of crystalline biuret, the purity of which was established by high resolution, pulse-Fourier transform, carbon-13 nuclear magnetic resonance (¹³C NMR) spectroscopy of 1 mol/L solution of the biuret in methyl sulfoxide-d₆.

Measured in a 2-cm cell, a 50 percent (w/v) solution of urea in water showed strong absorption (1.87 ± 0.01) at 220 nm, a weak absorption (0.036 ± 0.001) at 280 nm, and a very weak absorption (0.006 ± 0.001) at 461 nm.

^(d) The sulfated ash content was determined by volatilization of 25 g samples in porcelain crucibles, followed by moistening of the residue with concentrated sulfuric acid, further volatilization, and finally, ignition at 800 °C to constant weight.

^(e) Insoluble matter was determined by dissolution of 25 g samples in 100 mL aliquots of water, followed by filtration of the briefly boiled solutions through tared crucibles, which were then dried at 110 °C.

Expiration of Certification: The certification of **SRM 912a** is valid, within the measurement uncertainty specified, for five years from the date of shipping, provided the SRM is handled and stored in accordance with instructions given in this certificate (see "Instructions for Handling, 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, 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 was performed by B. Coxon and D.J. Reeder formerly of the NBS Organics Analytical Research Division.

This Certificate of Analysis has undergone editorial revision to reflect program and organizational changes at NIST and at the Department of Commerce. No attempt was made to reevaluate the certificate values or any technical data presented on this certificate.

Carlos A. Gonzalez, Chief
Chemical Sciences Division

Gaithersburg, MD 20899
Certificate Issue Date: 05 January 2016
Certificate Revision History on Last Page

Steven J. Choquette, Acting Director
Office of Reference Materials

Analytical measurements were performed by R.G. Christensen, B. Coxon, A.L. Cummings, J. Lee, D.J. Reeder, F.J. Savluk, and L.T. Sniegoski formerly of the NBS Organics Analytical Research Division.

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

NOTICE AND WARNING TO USERS

THIS SRM IS INTENDED FOR RESEARCH USE.

INSTRUCTIONS FOR HANDLING, STORAGE, AND USE

This material should be stored in a well-stoppered container at room temperature (30 °C or less). It should not be subjected to heat, moisture, or direct sunlight during storage. Refrigerated storage is recommended, but the material should be allowed to warm to room temperature before opening the container.

A standard solution containing 20 mg per 100 mL (0.2 mg per mL) of urea nitrogen may be prepared by weighing 0.429 g urea into a one-liter volumetric flask and making to volume with ammonia-free distilled water. A few drops of ACS Reagent-Grade chloroform are to be added as a preservative. The solution should be stored in a refrigerator [3]. The concentration of urea nitrogen in this solution is approximately that of the normal level in serum. An alternate procedure [4] recommends 0.1 g sodium azide per 100 mL of solution as a preservative. This standard urea nitrogen solution (20 mg/100 mL), is stable for three months when refrigerated at 4 °C in a well-stoppered all-glass container. All constituted solutions of urea should be clear and without indications of bacterial growth of any kind.

PREPARATION AND ANALYSIS

Stability: The homogeneity was verified by differential scanning calorimetry, ¹³C NMR spectroscopy, melting point determination, and moisture determination by the Karl Fischer method. Spectroscopy by ¹³C NMR of approximately 10 mol/L solutions of the urea in H₂O:D₂O(9:1 v/v), performed by signal averaging of 12 000 scans at 22.6 MHz showed only a single ¹³C resonance with a chemical shift, δ_c, of 161.6 from external tetramethylsilane, and thus indicates the absence of organic impurities by this test.

The melting range is 133.0 to 134.0 °C, as measured in an open capillary tube heated at 0.5 °C per min.

A 10 percent (w/v) solution of this SRM in water, free from carbon dioxide, showed a pH of 7.1 ± 0.2 at 23 °C.

REFERENCES

- [1] Plato, C.; Glasgow, A.R., Jr; *Differential Scanning Calorimetry as a General Method for Determining the Purity and Heat of Fusion of High-purity Organic Chemicals. Application to 95 Compounds*; Anal. Chem., Vol 41, pp. 330–336 (1969).
- [2] Reeder, D.J.; Savluk, F.J.; unpublished method.
- [3] Henry, R.D.; *Clinical Chemistry, Principles and Practice*; Hoeber Medical Division, Harper & Row: New York, NY, pp. 262–276 (1967).
- [4] Tietz, N.W.; *Fundamentals of Clinical Chemistry*; W.B. Saunders Co.: Philadelphia, PA, pp. 718–722 (1970).

Certificate Revision History: 05 January 2016 (Editorial changes); 05 December 1990 (Added analytical methods used to determine each physical property; editorial changes); 16 November 1979 (Original certificate date).
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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>.