U. S. Department of Commerce
Malcolm Baldrige
Secretary

National Bureau of Standards
Ernest Ambler, Director

National Bureau of Standards

Certificate of Analysis

Standard Reference Material 2671a

Freeze-Dried Urine Certified for Fluoride

This Standard Reference Material (SRM) is intended primarily for use as an analytical standard for the determination of fluoride in urine. SRM 2671a consists of four bottles of freeze-dried urine containing fluoride, two bottles each at low and elevated levels. The certified values are based on analysis of the reconstituted urine, resulting from the addition of 20 mL of pure water to each bottle.

Material	Fluoride, mg F^- / L
Low level	0.55 ± 0.03
Elevated level	5.7 ± 0.3

The certified values are based upon the determination of the fluoride content of samples, randomly selected from the lot, by an NBS method utilizing a fluoride ion selective electrode and the procedure of standard additions for quantitation. Additional analyses, made by an independent laboratory using an ion selective electrode method and a technique using an autoanalyzer, agreed with the certified values.

The certified values are the means of 16 measurements of samples at the low level and 17 measurements of samples at the elevated level.

The uncertainties of the certified values are the statistical tolerance intervals, at the 95% confidence level, for coverage of 99% of the samples of SRM 2671a. With a 95% confidence, the F^- concentration in at least 99% of the samples of this SRM should be included in all of the above intervals.

Use: This Standard Reference Material should be reconstituted by the addition of 20 mL of pure water to each bottle. The water used should be free of fluoride ion, or suitable blank corrections should be made for its fluoride content. The reconstituted material may be considered as fresh urine and should be handled under the same conditions as such samples. When reconstituted, the specific gravity of SRM 2671a is 1.0117 ± 0.0002 g/mL at 23 °C.

Notice to Users: It is recommended that SRM 2671a be stored under refrigeration or freezer conditions. The physical and chemical stability of SRM 2671a has not been rigorously assessed. NBS will continue to monitor this SRM and if the certification becomes invalid, the purchasers will be notified.

The certified values are based upon analyses made at NBS by W.F. Koch and J.W. Stolz of the Inorganic Analytical Research Division.

The overall direction and coordination of technical measurements leading to certification were performed by E.L. Garner, Chief, Inorganic Analytical Research Division.

The technical and support aspects involved in the certification and issuance of this Standard Reference Material were coordinated through the Office of Standard Reference Materials by T.E. Gills.

Washington, D.C. 20234
December 29, 1982

George A. Uriano, Chief
Office of Standard Reference Materials

The amount of peptide was calculated from the equation:

$$\frac{X}{X + M} = \frac{L_s \times P_{st}}{L_{st} \times P_s}$$

where X = μmol of peptide in the ampoule, M = μmol of phenylalanine added, L_s = height of leucine peak in the spiked sample, P_{st} = height of phenylalanine peak in the standard amino acid mixture, L_{st} = height of leucine peak in the standard amino acid mixture, and P_s = height of phenylalanine peak in the spiked sample. The samples had a mean weight of 526 μg and a standard error of 10 μg. The calculated coefficient of variation between ampoules was 4.0%. Similar results were obtained when the peak height of arginine was substituted for that of leucine. These results, in conjunction with the ampoule-filling study, indicate that users wishing to obtain very highly accurate measurements must weigh the angiotensin I before use.

The amino acid composition of nine randomly selected samples was determined by the chromatographic method of Benson and Hare (Proc. Natl. Acad. Sci., U.S. 72: 139, 1975). The molar ratios of the amino acids were: aspartic acid (asp) 1.01, arginine (arg) 0.95, valine (val) 1.05, tyrosine (tyr) 0.97, isoleucine (ile) 1.01, phenylalanine (phe) 1.05, leucine (leu) 1.04, and histidine (his) 1.94. This method did not permit the determination of imino acids.

However, the determination of the amino acid composition of 6 randomly selected samples by integration of selected identified resonances from the proton NMR spectrum of Angiotensin I enabled quantification of proline (pro) in addition to the other amino acids. The molar ratios of the constituent amino acids determined by this method were: his 9, 0.99 ± 0.03; his 6, 0.99 ± 0.05; phe, 1.00 ± 0.03; tyr, 1.03 ± 0.03; leu, 0.98 ± 0.04; pro, 1.00 ± 0.04; asp, 1.04 ± 0.04; ile, 0.98 ± 0.04; leu + arg, 1.98 ± 0.04; and ile + val 2.10 ± 0.04. These results independently confirm the results obtained by the method of Benson & Hare.

The D-amino acid composition of the acidic and neutral amino acids of ten randomly selected samples was determined by the method of Engel and Hare (Carnegie Inst. Yearbook, 1981). The percent D isomer content was: val 0.66 ± 0.30, ile 0.54 ± 0.18, pro 1.11 ± 0.40, leu 0.35 ± 0.14, asp 2.17 ± 1.00, phe 2.17 ± 0.41, and tyr < 1.0. These levels of the D isomers are consistent with the generation of D-amino acids during the hydrolysis and derivatization procedure. The D-amino acid content of the basic amino acids could not be determined by this method. However, the lack of other peptide peaks in the HPLC chromatograms, a method that resolves D-amino acid containing peptides (NBSIR 79-1947, Development of a Standard Reference Material for Angiotensin I) suggests that the levels of D isomers of the basic amino acids are comparable to those of the other D-amino acids.

Five angiotensin I samples were examined for non-peptide impurities by low resolution mass spectrometry. The measurements were made on a high resolution, double focussing, mass spectrometer operated at an ionizing energy of 70 eV and a source temperature of 250 °C. The following impurities were tentatively identified: acetic acid, low levels of alkyl groups containing up to at least 8 carbon atoms, very low levels of phthalate esters in three of five samples and extremely low levels of dimethylsilicone polymers.