U. S. Department of Commerce
Frederick B. Dent
Secretary
National Bureau of Standards
Richard W. Roberts, Director

# National Bureau of Standards
# Certificate of Analysis
## Standard Reference Material 920
## D-Mannitol

B. Coxon and R. Schaffer

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 triglycerides determinations employed in clinical analysis and for routine critical evaluation of the daily working standards used in these procedures.

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|---|---|
| D-Mannitol | 99.8 percent |
| D-Glucitol | 0.1 percent |
| Total Alditol | 99.9 percent |
| Loss on drying | <0.02 percent |
| Ash | <0.001 percent |
| Insoluble matter | <0.001 percent |

The value for the purity has an estimated inaccuracy of 0.1 percent.

The D-mannitol used for this Standard Reference Material was obtained from Pfanstiehl Laboratories, Inc., of Waukegan, Illinois. Analyses were performed by R. F. Brady, Jr., B. Coxon, B. A. Johnson, W. H. McCurdy, and W. P. Schmidt of the NBS Analytical Chemistry Division.

The overall direction and coordination of technical measurements leading to the certification was under the chairmanship of R. Schaffer.

The technical and support aspects concerning the preparation, certification, and issuance of this Standard Reference Material were coordinated through the Office of Standard Reference Materials by T. W. Mears.

Washington, D. C. 20234
January 17, 1972
Revised November 23, 1973

J. Paul Cali, Chief
Office of Standard Reference Materials

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The homogeneity of this Standard Reference Material was monitored by differential scanning calorimetry of 20 samples taken at random from the bulk material, and by polarimetry, gas-liquid chromatography, and thin-layer chromatography. Elemental microanalysis (±2 SD) showed carbon 39.51 ± 0.10 and hydrogen 7.86 ± 0.12; the calculated values are 39.56 and 7.75 percent, respectively. The Standard Reference Material melted at 167.0–168.0 °C (corr.) when heated in an open capillary at 0.5 °C·min$^{-1}$

The content of D-glucitol in the material was determined by gas-liquid chromatography of hexakis-*o*-(trifluoroacetyl) derivatives on a 10-ft. column of Gas Chrom Q (100-120 mesh) coated with 3 percent of GE-XE-60 liquid phase, using a flame-ionization detector. The derivatives were prepared by treating 200 mg of the D-mannitol with 2 ml of trifluoroacetic anhydride and 10 mg of sodium trifluoroacetate in 2 ml of nitromethane for 2 hours at 25-30 °C. The solution resulting was diluted to 5.0 ml with nitromethane, and 2-ml aliquots were injected into the gas chromatograph (inlet temperature, 135 °C; column, 145 °C; detector, 150 °).

The purity of the Standard Reference Material, as determined by differential scanning calorimetry, is 99.9 mole percent, and by phase solubility analysis, 99.8 weight percent. For differential scanning calorimetry, the D-mannitol (~0.5 mg) was heated from 380 to 436 K at 80 K·min$^{-1}$ and then from 436 to 442 K at 0.625 K·min$^{-1}$. Phase solubility analysis was conducted by equilibrating the material with either ethanol-water (1:1 v/v), or methanol-water (1:1 v/v) at 25 °C for six days.

No impurities were revealed by paper and thin-layer chromatography, infrared spectroscopy (KCl pellet), ultraviolet spectrophotometry (20-percent solution of the material in water), or by proton and carbon-13 magnetic resonance spectroscopy.

The proton magnetic resonance spectrum obtained at 90 MHz by accumulating 324 frequency sweeps of a solution of 0.1 g of the material in 0.5 ml of methyl sulfoxide-$d_6$ showed, as expected for D-mannitol, only a complex multiplet and a doublet at 4.4 and 4.2 ppm, respectively, from tetramethylsilane, due to six hydroxyl protons, and a complex multiplet at 3.5 ppm due to eight CH protons.

The proton-decoupled carbon-13 magnetic resonance spectrum, accumulated at 22.6 MHz from 1024 scans of a saturated solution of the material in water-hexafluoroacetone sesquihydrate (5:1 v/v), with heteronuclear, field-frequency stabilization on fluorine, showed only three singlets, at 122, 123, and 129 ppm from external, $^{13}$C-enriched carbon disulfide, owing to the two-fold axis of symmetry of D-mannitol.

In the absence of proton decoupling, 5650 scans of a similar solution gave a carbon-13 nmr spectrum that showed only two overlapping doublets ($J13_{C-H}$ 146 and 141 Hz), each overlapped by a triplet ($J13_{C-H}$ 142 Hz).

Optical rotations were measured by means of an automatic polarimeter and a high-precision, manual polarimeter, by using solutions of the material either in water, or in aqueous 4.7 percent ammonium molybdate solution (20.0 ml) diluted to 25.0 ml with 0.5 M sulfuric acid at 20 °C.

$[\alpha]^{20}_{D} = 139.0°$ (*c* 1.6 in acidified 3.8 percent ammonium molybdate solution)

$[\alpha]^{20}_{D} = -0.3°$ (*c* 16.0 in water)

On being heated at 70 °C under vacuum for 24 h, 7-g samples of the Standard Reference Material lost 0.001 to 0.002 percent of their weight; however, at 110 °C and 30 torr, constant weight was not reached during 80 h, at which time, 50-g samples of the material had decreased in weight by 0.02 percent, corresponding mainly to sublimation of the samples.

The residue insoluble in water and the ash content of the Standard Reference Material were each determined on 25-g samples.

This Standard Reference Material is intended for "in vitro" diagnostic use.

This material is for use as a standard in the determination of triglycerides in clinical chemistry. Since mannitol solutions require no preliminary handling prior to the automated part of the analysis they are useful in checking automated systems. A stock solution may be prepared by transfer of 182.2 mg of SRM 920 to a 1-liter volumetric flask, dissolving it in 0.2 N sulfuric acid and diluting to the mark with 0.2 N sulfuric acid. This solution contains 1 $\mu$mol/ml of mannitol. Working solutions of 0.050, 0.100, and 0.150 $\mu$mol/ml may be prepared by dilution of appropriate aliquots with 0.2 N sulfuric acid [1,2].

This Standard Reference Material should be stored in a well-closed container at room temperature (30 °C or less). It should not be subjected to heat or direct sunlight during storage. Under proper storage, experience at NBS indicates this material to be stable for at least 5 years. If the material degrades beyond the limits certified, purchasers will be notified by NBS. It is recommended that material not be used after 5 years from date of purchase.

The stock standard solution (1 $\mu$mol/ml) and the working solutions should be stored in a glass-stoppered, brown bottle at 4 °C. Under such conditions these standards should be stable for 6 months.

All constituted solutions of D-mannitol should be clear and display no turbidity.

References:

[1] Kanter, S. L., Mannitol as a primary standard in the determinations of triglycerides. Clin. Chim. Acta **16**, 177-178 (1967).

[2] Communication from Lipids Laboratory, Center for Disease Control, Atlanta, Ga., January 8, 1974.

This Standard Reference Material has been measured and certified at the laboratories of the National Bureau of Standards, Gaithersburg, Maryland. All inquiries should be addressed to:

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The date of issuance and certification of this Standard Reference Material was Janaury 17, 1972.