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 910
Sodium Pyruvate

This Standard Reference Material (SRM) is certified as a chemical of known purity. It is intended primarily for use in the calibration and standardization of procedures for pyruvate, lactic dehydrogenase, and glutamic-pyruvic transaminase determinations in clinical analyses, and for critical evaluation of the routine working or secondary reference materials used in these procedures.

Sodium Pyruvate[1]	98.7	± 0.2	weight percent
Sodium 2-hydroxy-4-keto-2-methylpentanedioate[1] (parapyruvate)	0.9	± 0.2	weight percent
Moisture[2]	(0.28)		weight percent
Methanol	(0.21)		weight percent
Water-insoluble matter	(0.004)		weight percent

[1]The certified pyruvate and parapyruvate concentrations were obtained by taking weighted averages for results from two separate methods of analysis. Measurements were made at NBS using high-performance liquid chromatography (HPLC) and high-field, proton NMR spectroscopy at 400 MHz. For each certified average, the relative weights were calculated based on the internal (within-method) and the between-method variance components. The standard errors of the certified concentrations were also calculated using combinations of both the variance components. The individual results for each method of analysis were: NMR - pyruvate, 98.83 ± 0.05; parapyruvate, 0.68 ± 0.05; HPLC - pyruvate, 98.50 ± 0.03; parapyruvate, 1.02 ± 0.03 weight percent.

[2]Values in parentheses are not certified because they were determined by one method only.

All reported uncertainties are stated as plus or minus one standard error of the listed value.

NOTICE AND WARNINGS TO USERS

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

Storage:

SRM 910 should be stored in the tightly capped bottle at 2-6 °C. It should be allowed to warm to room temperature before opening. Under proper storage, this material should be stable for at least 5 years. If the purity of the material degrades beyond the limits certified, purchasers will be notified by NBS. This material is not certified for use after 5 years from date of purchase.

The sodium pyruvate used for this SRM was obtained from the Sigma Chemical Company, St. Louis, Missouri. Analyses and physical determinations were performed at NBS in the Organic Analytical Research Division by B. Coxon, A. Cummings, J. Lee, S. Margolis, and L. Sniegoski.

The statistical analysis of the data was made by R. Paule, NBS National Measurement Laboratory.

The overall direction and coordination of the technical measurements leading to the certification were under the chairmanship of S. Margolis and B. Coxon.

The technical and support aspects involved in the preparation, certification, and issuance of this Standard Reference Material were coordinated through the Office of Standard Reference Materials by R. Alvarez.

Washington, D.C. 20234
May 15, 1981

George A. Uriano, Chief
Office of Standard Reference Materials

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Liquid chromatography of 3-mg samples of this SRM, dissolved in water, was performed by using a 150 x 0.9 cm column packed with Sephadex G-15, operated at a pressure of 500 kPa. The eluant was 0.03 mol/L sodium phosphate buffer at pH 8.0. Elution was monitored by measurement of refractive index and transmittance at 254 nm. A major peak and two minor peaks with retention times of 0.65 and 0.80 relative to the major peak were detected. The material corresponding to each peak was collected, lyophilized, checked for chromatographic purity by chromatography on a Sephadex G-15 column, and characterized by both proton and carbon-13 (^{13}C) NMR spectroscopy. Chromatography on Sephadex G-15 of chromatographic fractions before and after lyophilization showed that no changes occurred on lyophilization of the separated materials nor did new products form during the process of chromatography.

The major peak exhibited proton and ^{13}C NMR spectra that are characteristic of sodium pyruvate: at pH 6 and pH 8, proton NMR indicated that approximately 7.5 percent and 4.7 percent, respectively, of the pyruvate is hydrated at the C-2 position. The impurity at the relative retention time 0.80 (the larger of the minor peaks) appears to be a single compound which exhibits an NMR spectrum characteristic of the linear form of the parapyruvate. The impurity at the relative retention time of 0.65 (present at only trace levels) was located at the void volume of the column, and NMR analysis of the lyophilized material suggested that this material was a mixture of oligomers of sodium pyruvate.

The parapyruvate content was found to be 0.68 ± 0.05 percent from methyl proton NMR peak intensities and 1.02 ± 0.03 percent from chromatographic measurements. The reasons for the discrepancy in the estimates by NMR and HPLC is unclear. It does not appear to be attributable to a contaminant in that HPLC peak, to differences in the extinction coefficients of the pyruvate and parapyruvate, or to differences in the intensities of the NMR signals of the methyl group of these compounds.

From signal measurements, ^{13}C NMR spectroscopy indicated the presence of less than 0.6 mol percent of organic impurities other than parapyruvate and methanol in the SRM. Proton NMR spectroscopy at 400 MHz was performed to determine the content of parapyruvate and methanol. The spectroscopy was performed at ambient temperature using a data set of 16,384 points, a 30° pulse, and a relaxation delay of 6.26 s between pulses.

Samples for proton NMR analysis were prepared by dissolving 240 mg of the SRM in 0.5 mL of 9:1 v/v water-deuterium oxide. No significant differences in parapyruvate or methanol content were observed for solutions that were analyzed by signal averaging over periods ranging from 7 minutes to 3 hours. After drying the SRM at 93 °C and 2.5 kPa for 15 hours, the methanol content was 0.16 percent, indicating that methanol is not readily removed by such treatment.

The structure of the parapyruvate was proven by means of ^{13}C NMR spectroscopy at 22.6 MHz using a data set of 8,192 points, a 30° or 45° pulse, a relaxation delay of 5 s, and solutions prepared by dissolving 1-g portions of the SRM in 2-mL aliquots of 9:1 v/v water-deuterium oxide. ^{13}C spectral assignments were confirmed by the use of off-resonance proton decoupling techniques, proton coupled spectra, and measurements of the spin-lattice relaxation times of sodium pyruvate and parapyruvate, using the fast inversion recovery method.

The ultraviolet absorption spectrum of sodium pyruvate in distilled water at pH 5.9 exhibited an absorbance maximum at 316 nm and a minimum at 289 nm. The molar extinction coefficient at 316 nm is 18.14 ± 0.04 L mol^{-1} cm^{-1}. Parapyruvate exhibited an absorbance maximum at 326 nm and a minimum at 301 nm. The molar extinction coefficient is 25 L mol^{-1} cm^{-1}. The absorbance of the SRM above 400 nm is less than 0.3 percent of the absorbance at 316 nm.

Microchemical analysis yielded these values, in percent: carbon, 32.64 ± 0.08; hydrogen, 2.84 ± 0.02; sodium, 20.73 ± 0.03; and oxygen, 43.49 ± 0.06. Calculated percentages based on $C_3H_3NaO_3$ are 32.74, 2.74, 20.89, and 43.91, respectively.

The homogeneity of the SRM, as determined by liquid chromatography and by proton and ^{13}C NMR spectroscopy, was found to be satisfactory.

This SRM is intended for "in vitro" diagnostic use only.

This material is for use as a standard in clinical chemistry. Stock solutions should be prepared daily with distilled water. The pH of a solution of the SRM in distilled water at a concentration of 250 mg/mL is 5.90 ± 0.06. Such solutions are stable for several weeks in the frozen state at −20 °C. However, at 20 °C and at an initial pH 5.90, the concentration of parapyruvate doubles in five days, and triples within 24 hours if the pyruvate is dissolved in 0.25 mol/L potassium phosphate at pH 8.0 and 20 °C. With the same solvent adjusted to pH 9, 50 percent of the pyruvate dimerizes within 24 hours.