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Certification of Standard Reference Material® 917d D-Glucose (Dextrose)

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U.S. Department of Commerce
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Abstract

Standard Reference Material (SRM) 917d D-Glucose (Dextrose) is certified as a chemical substance of known high purity. It is intended for use in calibrating measuring systems for glucose determinations employed in clinical analysis. A unit of SRM 917d consists of one bottle containing 50 g of crystalline D-glucose. This publication documents the production, analytical methods, and computations involved in characterizing this product.

Keywords

D-Glucose; NIST PS1 Primary Standard for quantitative Nuclear Magnetic Resonance Spectroscopy; purity determination; quantitative proton nuclear magnetic resonance spectroscopy with internal standard ($^{1}H\{^{13}C\}$-qNMR); Standard Reference Material (SRM).

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Acknowledgments

The authors thank Carolyn Vallone of the NIST Office of Reference Materials for providing the 1990 thru 2002 sales history of SRMs 917a and 917b.

1. Introduction

Glucose measurements are used to monitor patients being treated for diabetes, making glucose perhaps the most commonly measured blood constituent [1]. Standard Reference Material® (SRM®) 917d D-Glucose (Dextrose) is a high-purity primary reference material for use as a calibration standard in measurement procedures for glucose determinations employed in clinical analysis.

The certified purity of SRM 917d is traceable to the International System of Units (SI) through calibration to NIST PS1 Primary Standard for quantitative NMR (Benzoic Acid) [2] via a primary ratio method [3]. The method uses quantitative proton nuclear magnetic resonance spectroscopy with NIST PS1 as the internal standard (q^1H-NMR$_{IS}$) [4,5]. Measurement results calibrated via SRM 917d can be established as metrologically traceable to the SI. SI-traceability is now recognized as essential to enabling comparison of clinical measurements across time and place [6,7].

SRM 917d is the fifth member of the SRM 917 series. SRM 917 [8], the first of the series, was issued in 1970 with a stated purity of (99.9 ± 0.1) %. It was followed by SRM 917a [9] in 1989 with stated purity of (99.7 ± 0.2) %, SRM 917b [10] also with a stated purity of (99.7 ± 0.2) %, and SRM 917c [11] with a stated purity of (99.7 ± 0.3) %. The sales of the SRM 917 series as a function calendar year are displayed in Fig. 1. The proportion of sales to customers in the US, Europe, Asia, and the rest of the world are displayed in Fig. 2.

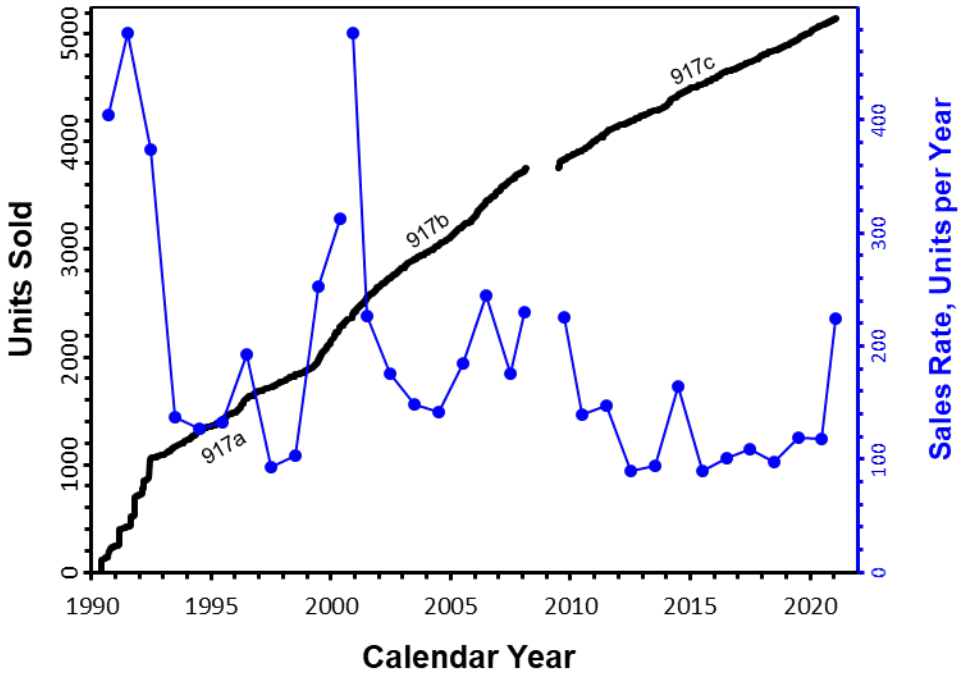

Fig. 1. Sales History of SRM 917, 1990 to 2021.

The thick black line depicts the cumulative distribution of sales as a function of the order date; it is plotted using the "Units Sold" axis at the left of the plot. The thin blue line depicts the total units sold per year; it is plotted using the "Sales Rate, Units per Year" axis to the right of the graph. There is no accessible record of SRM sales prior to August 1990.

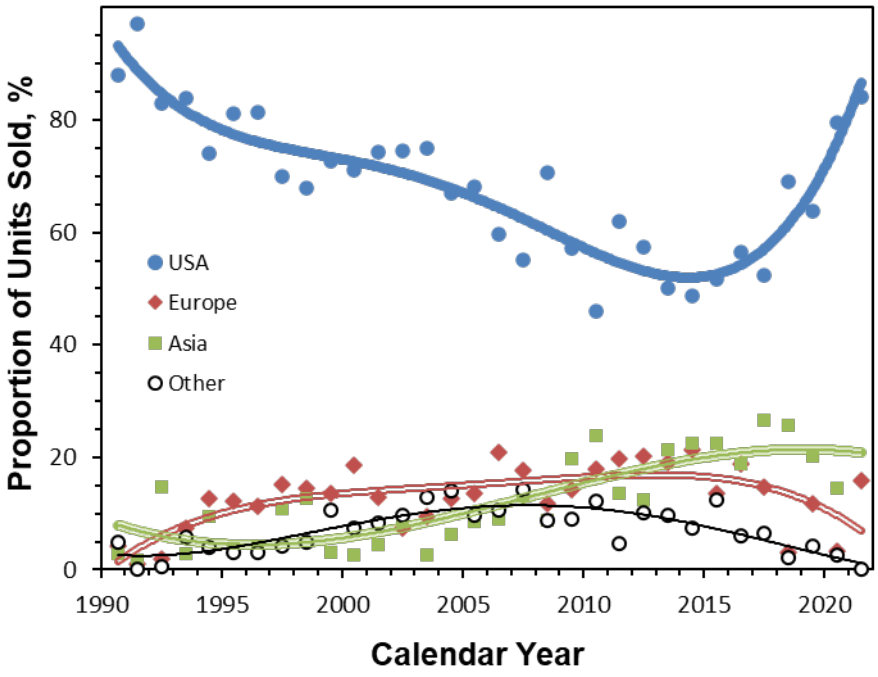

Fig. 2. Location of Customers for the SRM 917 Series Materials

The solid circles and the thick polynomial trendline display the proportion of sales to customers within the USA from the onset of currently accessible electronic records in 1990 to the date of the last unit sold in 2021. Solid diamonds and the double-line polynomial trendline display the proportion of units sold to customers in Europe (including the United Kingdom) customers; solid squares and the triple-line polynomial trendline display the proportion solid to customers in Asia. The open circles and thin single-line polynomial trendline display the proportion of units sold to customers elsewhere.

1.1. Production

Four (4) buckets of bulk crystalline D-glucose, each containing 25 kg of material, were purchased from Sigma-Aldrich[1]. All materials were from one lot. The approximately 100 kg of bulk material was not blended prior to bottling. A total of 1,954 units were bottled in April 2020 at NIST, Gaithersburg by the Office of Reference Materials, Materials and Physical Services Group. Each unit consists of approximately 50 g of D-glucose in a clear glass bottle sealed with a polytetrafluoroethylene-lined polymer screwcap.

Table 1 lists the first, last, and total number of units produced from each bucket.

Table 1. Units Produced

	Production Sequence		Number
Bucket	First	Last	Units
1	1	486	486
2	487	981	495
3	982	1467	486
4	1468	1954	487

[1] Certain commercial entities, equipment, or materials may be identified in this document in order to describe an experimental procedure or concept adequately. Such identification is not intended to imply recommendation or endorsement by the National Institute of Standards and Technology, nor is it intended to imply that the entities, materials, or equipment are necessarily the best available for the purpose.

2. Material Suitability Assessment

Prior to bottling the commercially-obtained bulk D-glucose, the material was evaluated to assess its suitability for use as SRM 917d D-Glucose (Dextrose).

2.1. Materials

An aliquot from each of the four buckets of crystalline D-glucose (labeled 1-4) was stored in glass vials at room temperature. SRM 84k Potassium Hydrogen Phthalate [12] was used as an internal standard and stored at room temperature in a desiccator.

Deuterated solvents with ≥ 99.8 % D-atom purity are typically used for qNMR applications. The neat chemical materials were diluted with D_2O ("99.96%" D atom purity) purchased from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA, USA).

2.2. Sample Preparation

Two replicate samples were prepared for each individual drum for a total of eight qNMR samples.

Glassware for sample preparation was cleaned with distilled water and organic solvents, baked in a furnace at 450 °C, and stored in a desiccator. Clean Bruker 600 MHz NMR tubes (5 mm internal diameter, 7-inch length) were stored in a desiccator prior to use. Sample mass determinations and preparation for ^1H NMR analysis were performed in accordance with established balance use and sample preparation procedures. Neat material masses were determined using a calibrated ultra-microbalance (Mettler Toledo XPR2U). Samples were dissolved in the NMR preparatory lab. Samples were diluted with approximately 0.7 mL of solvent (withdrawn from the ampoules by cleaned glass Pasteur pipettes). Samples were sonicated and vortexed several times to facilitate total dissolution. Both glucose and KHP readily dissolved in D_2O and no solubility issues were observed. Care was taken to ensure complete dissolution and that no crystals of the neat materials adhered to the weigh bottle walls.

2.3. Analysis

Experimental NMR data was acquired by a Bruker Avance II 600 MHz spectrometer equipped with a 5-mm broadband inverse (BBI) detection probe and operating with Topspin (Version 3.2) software. The ^1H experimental analyses, subsequent data processing and chemical purity determinations were performed according to established protocols.

One dimensional ^1H with ^{13}C decoupling ($^1H\{^{13}C\}$) experiments were conducted at 298 K. Ninety-degree ^1H excitation pulse widths were used and globally optimized alternating phase rectangular pulse (GARP) composite pulse ^{13}C decoupling was executed during FID acquisition. A long relaxation delay (D1) of 60 s was used. A T1 inversion recovery experiment performed for a sample of D-glucose with KHP in D_2O indicated that the longest T1 was 4.8 s; thus, a D1 of 60 s allowed net magnetization to return to practically 100 % of the equilibrium value between 90-degree excitation pulses. TopSpin 'baseopt' mode was used for signal digitization and apodization was performed via application of an exponential multiply (em) window function to achieve 0.3 Hz line broadening. For each analysis, 64 acquired data scans were averaged, 16

dummy scans performed, the spectral sweep width was set to 20.0276 ppm, and the transmitter frequency offset for ^{1}H (O1) and ^{13}C (O2) channels were set to 6.175 ppm and 120.000 ppm, respectively. Data acquisition time was 5.4525952 s for each scan to generate an FID with 131072 data points. The total elapsed time per sample was about 90 min.

Multiplicity-edited ^{1}H-^{13}C heteronuclear single quantum coherence (HSQC) experiments were conducted at 298 K. The following parameters were used: 1024 data points and spectral width of 13.0179 ppm was collected for the F2 axis (^{1}H); 256 data points and 165 ppm spectral width was collected for the F1 (^{13}C) axis; 8 scans and 16 dummy scans were performed; 64 µs dwell time; 6.012 ppm F2 frequency offset; 90 ppm F1 frequency offset.

2.4. Identification

D-glucose consists of α- and β anomers. Anomers are epimers of cyclic monosaccharides that differ from each other only in the configuration of the carbon that comprises the carbonyl carbon in the acyclic form (anomeric carbon) [13]. The anomeric carbon of D-glucose is C1. Anomers in aqueous solution can interconvert through transitory ring-opening.

The HSQC spectrum of a representative sample of the D-glucose material is displayed in Fig. 3. All D-glucose peaks are present; there are no unexpected peaks.

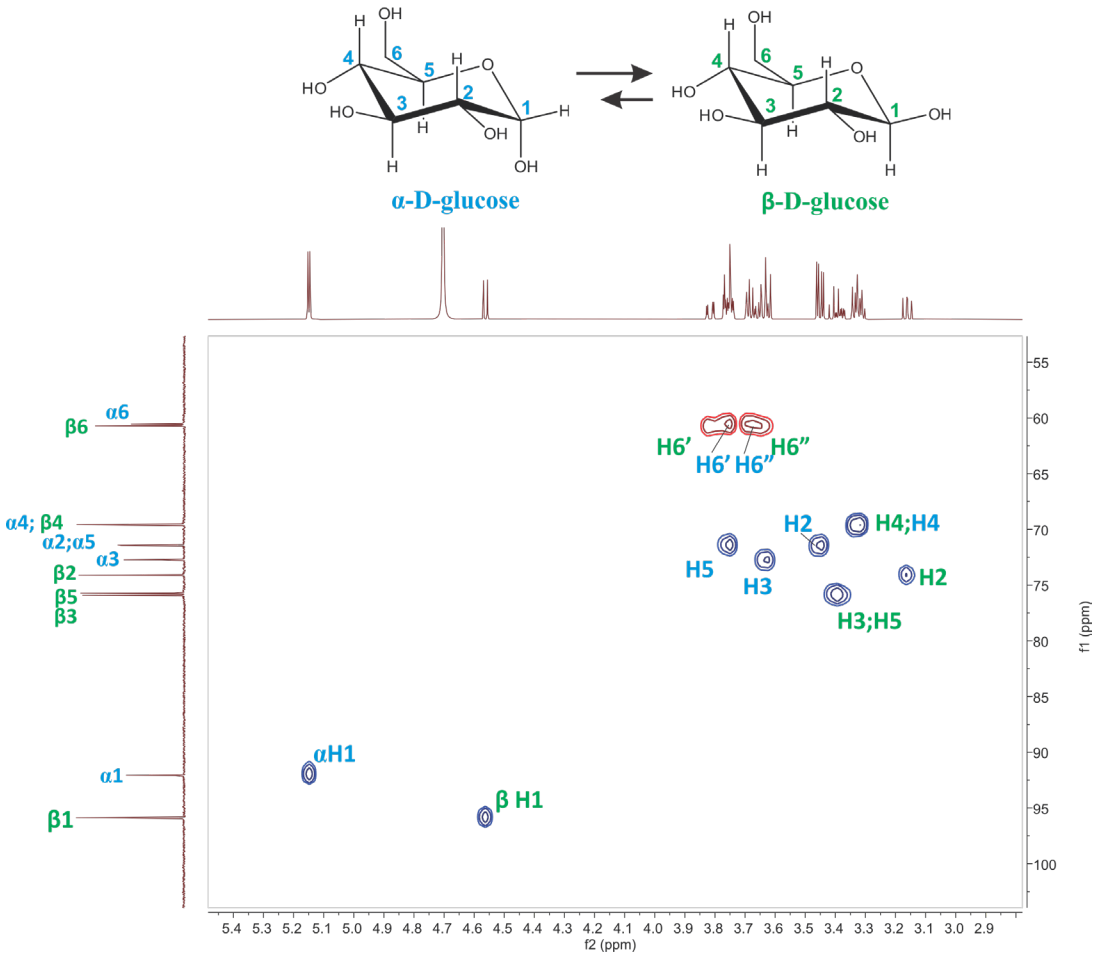

Fig. 3. ^{1}H-^{13}C HSQC of D-Glucose in D_2O with Labeled Peak Assignments.

2.5. Quantitation

The glucose purity (w_P, %) of each analysis sample is related to the q^{1}H{^{13}C}-NMR experimental data according to the following measurement function:

$$w_p = \left(\frac{N_I}{N_P}\right) \times \left(\frac{M_P}{M_I}\right) \times \left(\frac{A_P}{A_I}\right) \times \left(\frac{m_I}{m_C}\right) \times P_I \quad (1)$$

where: N_p = multiplicity (# H/peak) of the integrated glucose peaks,
N_I = multiplicity (# H/peak) of the integrated KHP internal standard peaks,
M_p = relative molar mass (molecular weight, g/mol) of glucose,
M_I = relative molar mass (molecular weight, g/mol) of KHP,
A_p = integrated area of the glucose peaks,
A_I = integrated area of the KHP internal standard peaks,
m_C = mass (g) of the candidate SRM 917d D-Glucose,
m_I = mass (g) of the SRM 84k KHP internal standard, and
P_I = purity (%) of the SRM 84k KHP internal standard.

The multiplicity of the peaks used is assumed to be exactly determined and has zero associated uncertainty.

Using the authoritative molecular weight calculator implemented by the IUPAC Commission on Isotopic Abundances and Atomic Weights [14], the molecular weights and standard uncertainties for KHP ($KC_8H_5O_4$) and D-glucose ($C_6H_{12}O_6$) are (204.221 ± 0.005) g/mol and (180.156 ± 0.004) g/mol, respectively.

The associated uncertainty of the integrated areas is determined by replication.

From the calibration certificate for the ultra-microbalance, the expected standard uncertainty for masses weighted is 0.5 µg.

From the Certificate of Analysis for SRM 84k KHP, the purity of the KHP is 99.9911 % with an 95 % expanded uncertainty of ± 0.0054 % [12]. Assuming that the distribution of the uncertainty is approximately normal, the standard uncertainty is ± 0.0027 %.

2.5.1. Peak Selection

An example ^{1}H spectrum of a D-glucose sample with added KHP internal is given in Fig. 4. The peak assignments correspond to the peaks integrated for q^{1}H-NMR analysis, identified in Table 2. A ^{1}H spectrum of SRM 917d in D_2O, with D-glucose peak assignments is presented in Fig. 5. The very tall peak at 4.7 ppm is the D_2O solvent.

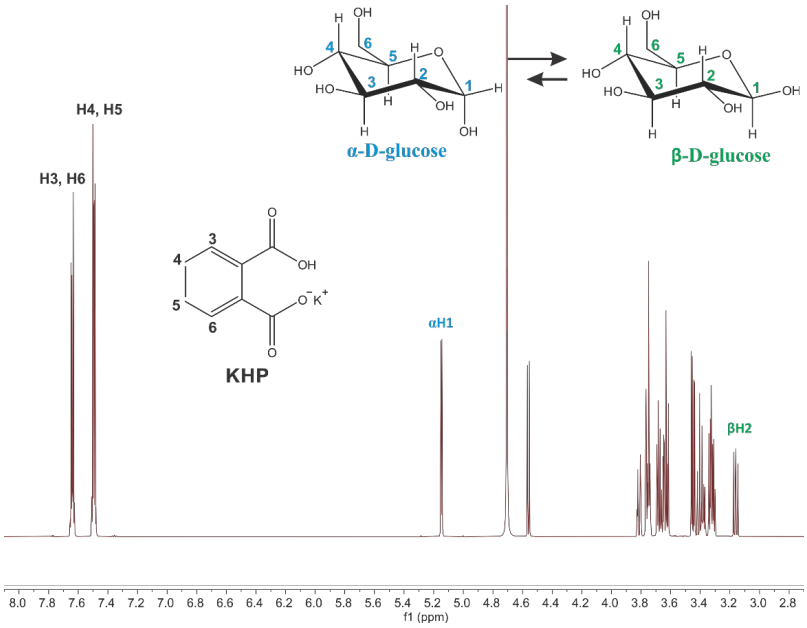

Fig. 4. Exemplary ^{1}H Spectrum of D-Glucose and KHP in D_2O.

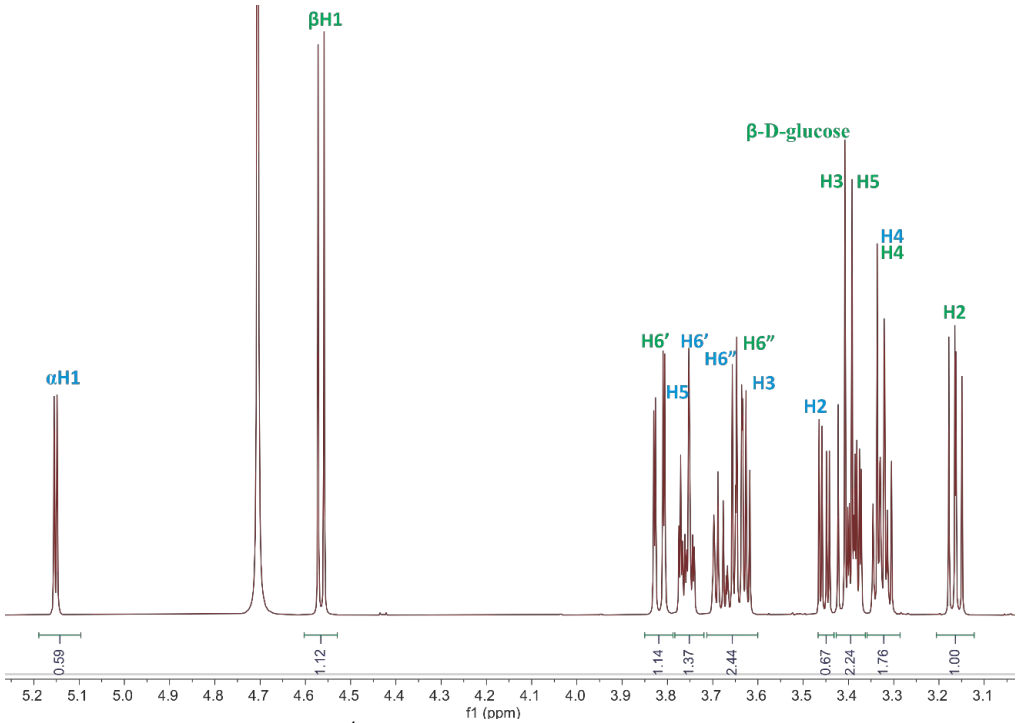

Fig. 5. Exemplary ^{1}H Zoomed-In Spectrum of D-Glucose in D_2O.

The α and β anomers of D-glucose dissolved in D_2O are known to interconvert over the time required for a single qNMR analysis [15]. Since quantitative characterization of one sample requires about 90 min, the impact of interconversion on the proportion of each anomer in solution was investigated. The results of the time course study that was performed overnight on a sample of glucose in D_2O are given Fig. 6.

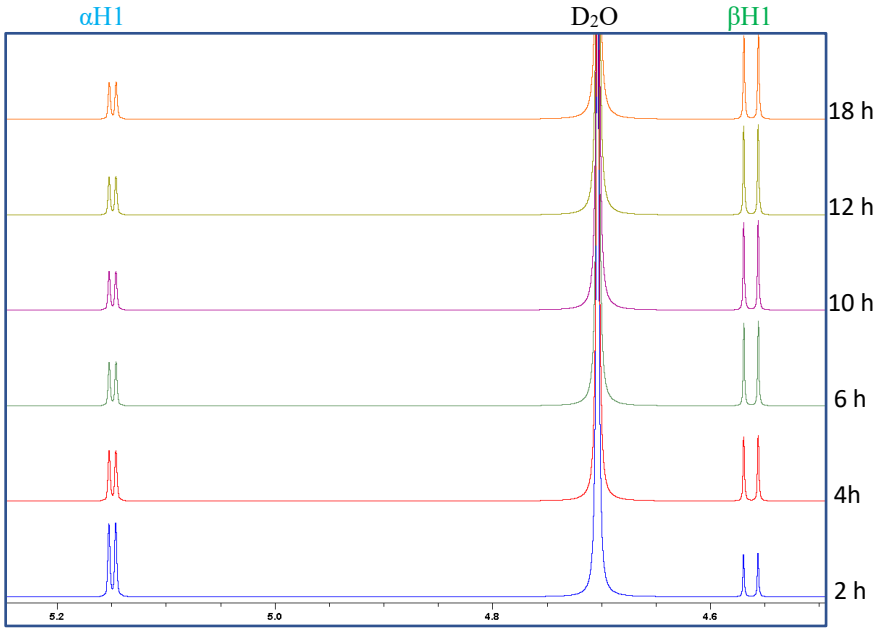

Fig. 6. $^{1}H\{^{13}C\}$-NMR Zoomed-In Spectra of D-Glucose in D_2O Over Time.

The times along the right edge of the plot represent approximate number of hours that lapsed between dilution of the glucose sample in D_2O and the completion of the respective NMR experiment. The assignment of the relevant peaks is provided along the top edge.

To correct for the anomerization when quantifying, the two integrals for the peaks at 5.1 ppm (αH1) and 3.1 ppm (βH2) were summed and treated as corresponding to a cumulative ^{1}H multiplicity of $N_p = 1$. The proton at 4.5 ppm (βH1) was not considered since it was on the shoulder of the 4.7 ppm D_2O peak, making it difficult to correct the baseline.

Table 2 lists the chemical shift regions chosen for use.

Table 2. ^{1}H NMR integration regions for purity assessment

Compound	Chemical Shift Region (ppm)	T_1 (s)	^{1}H Moiety	Proton Multiplicity (N)
D-(+)-glucose	5.2 to 5.0 3.2 to 3.0	3.6 3.3	αH1 βH2	1 1
KHP	7.8 to 7.3	5	H3, H4, H5, H6	4

2.5.2. Purity Estimates

The samples were reevaluated two to 15 days from their first analysis to ensure that the anomer composition reached equilibrium. The mass fraction purities for the two sets of experiments (same day and delayed) are listed in Table 3. The purity values are estimated individually using Eq. 1. With high confidence, the delays did not affect the analytical results.

Table 3. Comparison of Purity Results Between Original and Delayed Analyses

Sample	Original %	Delayed Days	Delayed %	Difference %
1.1	99.68	2	99.70	0.02
1.2	99.81	2	99.65	-0.16
2.1	99.55	15	99.83	0.28
2.2	99.48	15	99.77	0.29
3.1	99.65	15	99.54	-0.11
3.2	99.61	15	99.71	0.10
4.1	99.88	2	99.75	-0.13
4.2	99.49	2	99.73	0.24
Mean	99.64		99.71	0.07
SD	0.14		0.09	0.19

Based on the original measurements, the mean of these closed-form mass-fraction purity estimates of the bulk material is 99.64 %. This value is compatible with the (99.7 ± 0.3) % certified purity of SRM 917c.

2.5.3. Comparison to SRM 917c

The $^{1}H\{^{13}C\}$-NMR spectra for fully equilibrated samples of SRM 917c and SRM 917d are compared in Fig. 7 over the region relevant to D-glucose. The spectra are essentially identical.

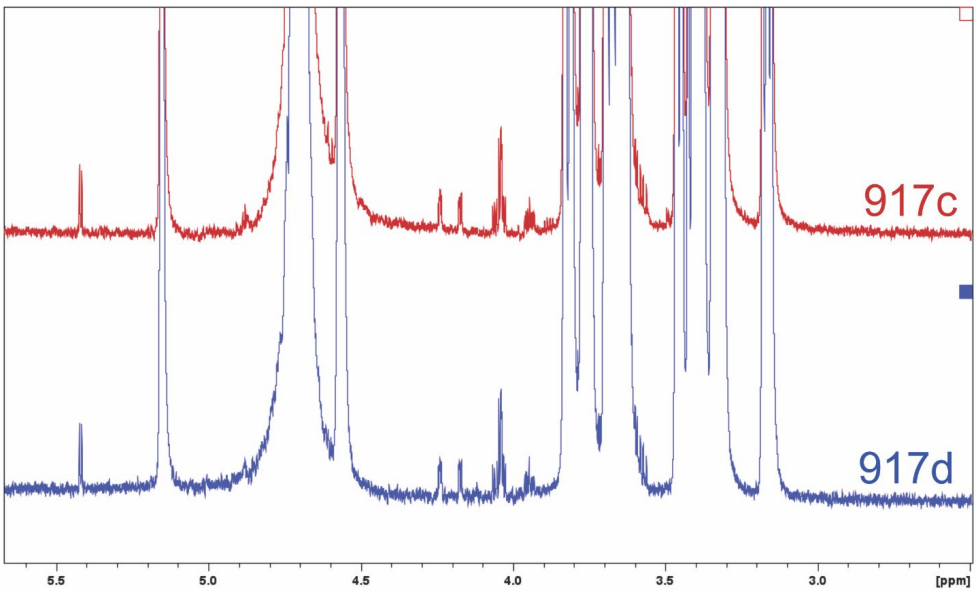

Fig. 7. Comparison of $^{1}H\{^{13}C\}$-NMR Spectra of SRM 917c and SRM 917d in D_2O.

The intensity axis is zoomed to demonstrate consistency of the observed organic impurity profiles.

3. Homogeneity and Purity Assessment

After bottling, the homogeneity of the prepared units of SRM 917d was assessed.

3.1. Materials

From the 1954 units of the production lot, seventeen were sampled for characterization of SRM 917d. The units sampled were from across the bottling order and each of the respective buckets of bulk material from which they were produced. Table 4 lists the units evaluated.

Table 4. Homogeneity Sampling Scheme

Bucket	Number of Units Evaluated	Production Sequence of Units Evaluated
1	4	1, 125, 250, 375
2	4	500, 625, 750, 875
3	4	1000, 1125, 1250, 1375
4	5	1500, 1675, 1750, 1875, 1954

One sub-sample (approximately 5 mg to 10 mg) was taken from each of the seventeen units and analyzed, with two additional sub-samples prepared from units #625 and #750. The bottled units were stored at room temperature.

SRM 84k Potassium Hydrogen Phthalate (KHP) was used as an internal standard for the qNMR analysis and stored at room temperature in a desiccator. The purity value associated with this material is traceable to the certified purity value of NIST PS1 Primary Standard for qNMR (Benzoic acid).

Deuterated solvents with ≥ 99.8 % D-atom purity are typically used for qNMR applications. The SRM 917d and SRM 84k samples were diluted with Cambridge Isotope Laboratories D_2O ("99.96%" D-atom purity).

3.2. Sample Preparation

The sample preparation and subsequent analyses were performed across multiple dates. A total of five sample sets, each containing two to six samples, were prepared and analyzed on separate days. Table 5 lists sample preparation and analysis dates.

Table 5. Analysis Scheme

Date Prepared	Date Analyzed	Unit(s) Analyzed
1/13/2022	1/13/2022	125, 1954, 625, 250
	1/14/2022	750, 1
1/21/2022	1/21/2022	375, 500, 1675
	1/22/2022	1375
2/24/2022	2/24/2022	1250
	2/25/2022	1125, 1500
	2/25/2022	1500
	2/27/2022	1000
	2/28/2022	1750

Date Prepared	Date Analyzed	Unit Analyzed
3/17/2022	3/17/2022	1875
	3/18/2022	875
4/14/2022	4/14/2022	625
	4/15/2022	625
	4/18/2022	750
	4/19/2022	750

Samples were prepared as described in Section 2.2.

3.3. Analysis

Samples were analyzed as described in Section 2.3, with exception that the D-glucose identity of the material packaged as SRM 917d was confirmed with ^{1}H-^{1}H correlation spectroscopy (COSY) and ^{1}H-^{13}C heteronuclear multi-bond coherence (HMBC) NMR experiments in addition to the ^{1}H, ^{13}C, and ^{1}H-^{13}C HSQC NMR experiments used in the suitability assessment. All of the identity studies were performed at 298 K.

3.4. Identity

The chemical identity of dextrose was confirmed using ^{1}H (Fig. 4 and Fig. 5), ^{13}C, ^{1}H multiplicity edited ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC (Fig. 8), and ^{1}H-^{1}H COSY spectra (Fig. 9).

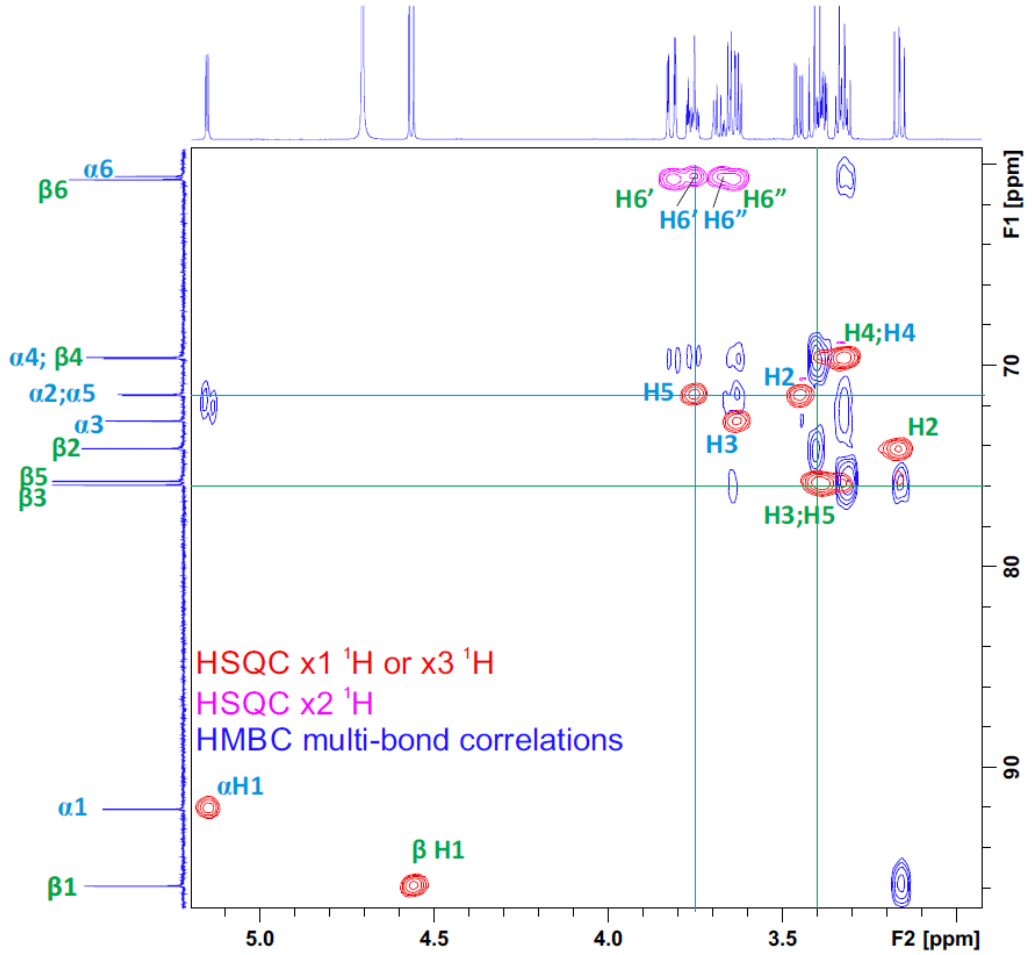

Fig. 8. ^{1}H-^{13}C HMBC and ^{1}H-^{13}C HSQC of SRM 917d D-Glucose in D_2O with HSQC Peak Assignments.

Overlay of multiplicity-edited ^{1}H-^{13}C HSQC and HMBC spectra of SRM 917d in D_2O, with assignment of ^{1}H-^{13}C HSQC correlations for α-D-glucose and β-D-glucose. HSQC correlations are depicted in red (multiplicity = 1 or 3) and magenta (multiplicity = 2) and HMBC correlations are blue. A ^{13}C-NMR spectrum with peak assignments is projected on the F1 axis and a 1H spectrum is projected on the F2 axis to serve as references for the correlation signals.

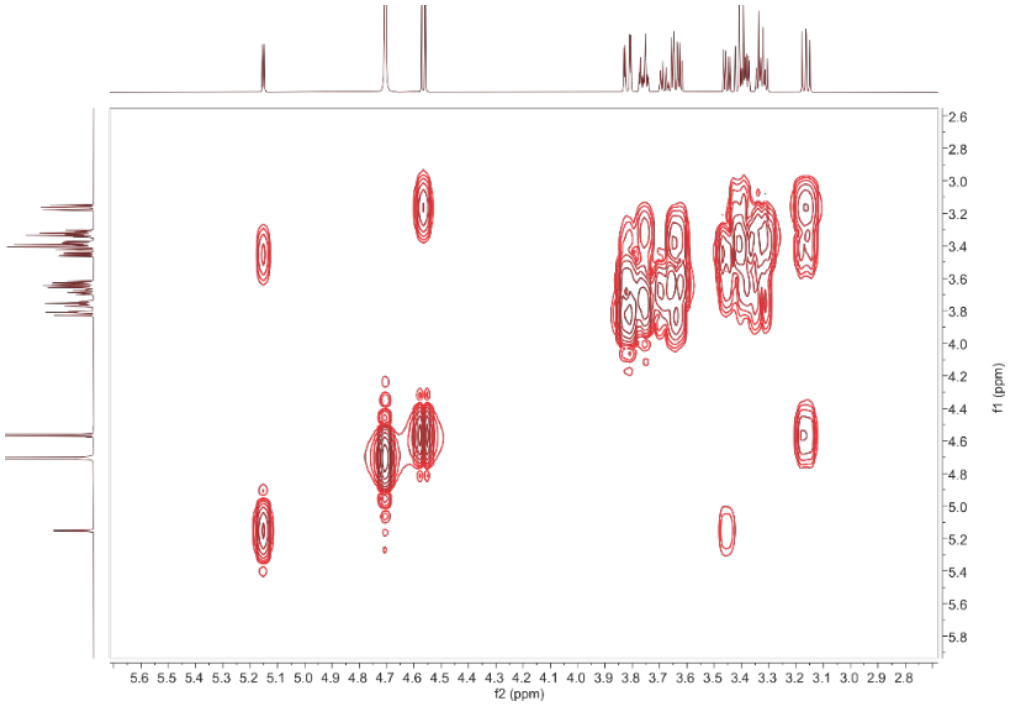

Fig. 9. ^{1}H-^{1}H COSY NMR Spectrum of SRM 917d Glucose in D_2O.

No significant differences were observed between the spectra for bulk material prior to bottling and for the bottled units. All D-glucose peaks are present; there are no unexpected peaks.

3.5. Quantitation

The estimate of total D-glucose was determined using the sum of integrals for the αH1 (α-D-glucose) and βH2 (β-D-glucose) ^{1}H spectral peaks. Equilibrium of the anomer composition was established several hours after sample dilution, as the proportion of β-D-glucose relative to the total D-glucose content increased to approximately 62 %. As described in Section 2, consistent total D-glucose purity results were obtained from analysis of samples both before and after anomer equilibrium was achieved.

Table 6 lists the measured values for each sample analysis along with estimated purities calculated via closed-form solution of Eq. 1. Table 7 provides a representative uncertainty budget for the closed-form calculations. The uncertainties assigned to the peak area integrations, particularly for the D-glucose peaks, are by far the largest contributors.

Table 6. $^{1}H\{^{13}C\}$-qNMR$_{IS}$ Measurement Data and Closed-Form Purity Estimates.

Bucket	Unit	m_C g	m_I g	A_P[a] a.u	A_I[b] a.u.	w_P[c] %	$u(w_P)$[d] %
1	1	0.0066647	0.0051620	1.7271461	1.1836239	99.69	0.11
1	125	0.0045674	0.0043987	1.1950844	1.0198058	99.55	0.11
1	250	0.0076223	0.0063847	1.9018469	1.4114074	99.56	0.11
1	375	0.0083789	0.0070364	2.0430767	1.5207657	99.52	0.11
2	500	0.0074944	0.0053657	1.8883216	1.1984835	99.50	0.11
2	625	0.0041837	0.0038727	1.1241221	0.9191643	99.86	0.11
2	625	0.0052209	0.0047017	1.2215859	0.9750581	99.52	0.11
2	625	0.0033971	0.0038815	1.2723881	1.2840090	99.87	0.11
2	750	0.0071234	0.0086005	1.6911860	1.8122934	99.38	0.11
2	750	0.0029103	0.0032263	1.1375502	1.1147523	99.79	0.11
2	750	0.0049527	0.0042403	1.1732692	0.8900295	99.55	0.11
2	875	0.0056146	0.0043677	1.4718948	1.0145607	99.55	0.11
2	875	0.0052283	0.0056375	1.1720299	1.1178482	99.72	0.11
3	1000	0.0042083	0.0043993	1.1089517	1.0264918	99.62	0.11
3	1125	0.0048324	0.0050281	1.2082011	1.1137842	99.56	0.11
3	1250	0.0072352	0.0082852	1.7910649	1.8181844	99.50	0.11
3	1375	0.0046618	0.0038383	1.1604996	0.8466346	99.55	0.11
4	1500	0.0042857	0.0044165	1.1109501	1.0130053	99.69	0.11
4	1675	0.0041858	0.0043754	1.0857480	1.0044326	99.67	0.11
4	1750	0.0073404	0.0078038	1.7925658	1.6881168	99.58	0.11
4	1875	0.0075866	0.0086565	1.8249031	1.8442436	99.59	0.11
4	1954	0.0055543	0.0056888	1.4044020	1.2751884	99.50	0.11

a Integral values are normalized to the ^{1}H multiplicity of D-glucose moieties αH1 and βH2 ($N_P = 1$). The standard uncertainty of the value is asserted to be 0.1 % of the value.

b Integral values are normalized to the ^{1}H multiplicity of KHP moieties H3, H4, H5, and H6 ($N_I = 4$). The standard uncertainty of the value is asserted to be 0.05 % of the value.

c Calculated as the closed-form solution to Eq. 1 using the constant values: $P_I = (99.9911 \pm 0.0027)$ %, $M_P = (180.156 \pm 0.004)$ g/mol, $M_I = (204.221 \pm 0.005)$ g/mol, $u(m_C) = 0.5$ µg, and $u(m_I) = 0.5$ µg.

d Standard uncertainties estimated as the square-root of the sum of the squared relative uncertainties, multiplied by the estimated purity [16]

Table 7. Representative Budget for Closed-Form Uncertainty Estimates.

Factor	Relative Uncertainty, %	Contribution to Total, %
P_I	0.0027	0.06
M_P	0.0022	0.04
M_I	0.0024	0.05
m_C	0.0089	0.63
m_I	0.0092	0.67
A_P	0.1000	78.85
A_I	0.0500	19.71
w_P	0.1126	100.00

3.6. Trend Assessment

The closed-form purity estimates as functions of bottling and analysis order are displayed in Fig. 10. No substantial, meaningful trend was observed across either variable.

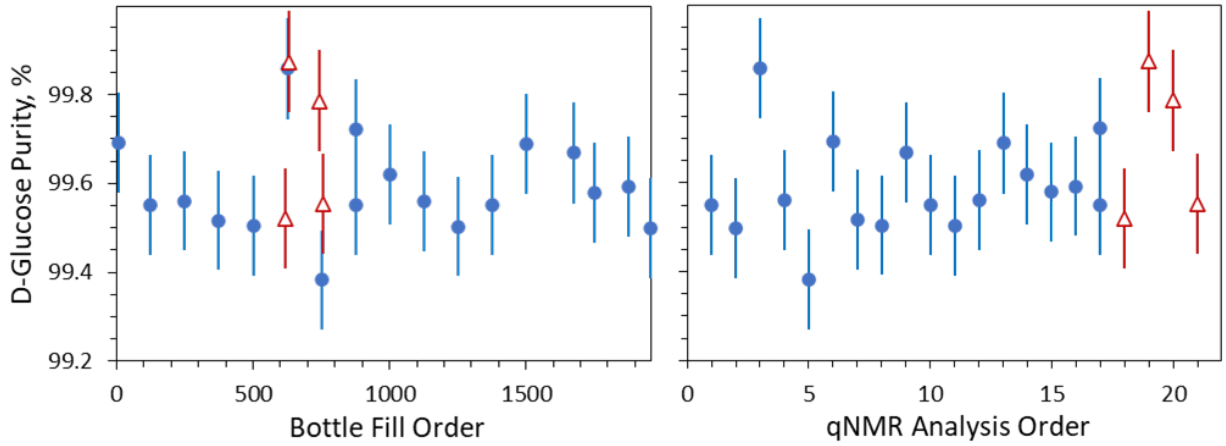

Fig. 10. Closed-Form Purity Estimates as Functions of Bottle Fill and qNMR Analysis Order.

Solid circles represent the original analysis of each sample; open triangles represent sample replicates prepared for bottles 625 and 750. Error bars are standard uncertainties.

Units 625 and 750 were reanalyzed because results for the initial samples prepared from these two units were the most disperse among those for all sampled units. Though the results were not statistical outliers, further investigation was considered prudent to confirm that the variation characterizes random variability of the qNMR analysis, rather than localized heterogeneity across the bottling order interval bound by the position of these two successively sampled units. The results for the re-samplings, depicted by the open triangle symbols in Fig. 10, confirm the random nature of the variation between results for bottles 625 and 750.

3.7. Purity

An estimate of w_p that combines all of the q^{1}H{^{13}C}-NMR$_{IS}$ measurements of the seventeen SRM 917d bottles was calculated using a Bayesian statistical procedure modeled on "observation equations", in accordance with Eq.1, employing Markov Chain Monte Carlo (MCMC) sampling techniques. The model used for this assessment is congruous with the approaches described in [4,17], whereby the result is constrained to have a mass-fraction value no greater than 100 %. Measurement data were grouped into four blocks, each corresponding to the bucket of bulk material from which the respective analyzed samples originated. An estimate of purity was calculated for samples from each of the four drums using a hierarchical model and these estimates were combined via linear pooling.

For each variable term of Eq. 1, the measurement data for each sample was treated as having a normal distribution. Parameter values for the mean (μ) and standard deviation (σ) were specified by the respective data inputs to the statistical model provided in Appendix B. Standard uncertainties, treated as the σ, were evaluated as follows: the combined glucose ^{1}H-normalized peak integrals (for αH1and βH2) were assigned a Type B relative standard uncertainty, $u(A_P)$, of 0.15 %; the KHP internal standard ^{1}H-normalized peak integrals, $u(A_I)$, were assigned a Type B

relative standard uncertainty of 0.05 %; the $u(m_C)$ and $u(m_I)$ were each assigned a value of 0.5 µg; the $u(P_I)$ was assigned a value of 0.0001 g/g, a more conservative treatment of uncertainty in the potassium hydrogen phthalate mass fraction value than is expressed on the SRM 84k Certificate of Analysis; the M_I, $u(M_I)$, M_P, and $u(M_I)$ were calculated using [14]; no uncertainty was considered for the proton multiplicities of the primary component (N_P) and internal standard (N_I).

The four bucket block estimates were not related using a hierarchical model. Rather, they were blended via a linear pool procedure to determine the result of the over-all purity measurement. The posterior distribution of values attributed to the purity of candidate SRM 917d D-Glucose (Dextrose) is displayed in Fig. 11. The median, 0.9958 g/g, and standard deviation, 0.0011 g/g, of values sampled from the asymmetric posterior are reported, respectively, as the central value of the distribution and standard uncertainty of the result. The shortest 95% coverage interval of values attributable to the mass fraction (g/g) of D-glucose in the material is [0.9931, 0.9976] g/g.

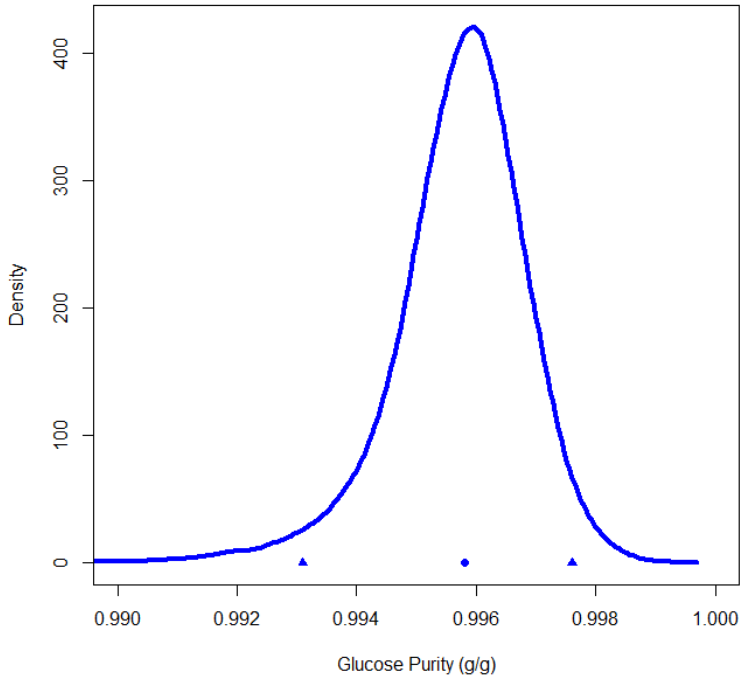

Fig. 11. Probability density plot of posterior distribution for mass fraction of glucose, g/g

The mass fraction purity is here expressed in units of g/g rather than percent. The boundaries of the shortest 95% coverage interval [0.9931, 0.9976] g/g are indicated by triangles and the median MCMC sample value (0.9958 g/g) is indicated by a circle.

The calculation of uncertainty in this fashion is a hybrid "top-down", "bottom-up" approach that accounts for variability associated with the terms of the measurement function (Eq. 1), analysis of the twenty-one samples taken from seventeen bottles representative of the entire the production lot, and variation between the four buckets. The Bayesian statistical model used for this analysis is implemented in the OpenBUGS software system [18,19]. Appendix B provides the model and data used to estimate the D-glucose purity of SRM 917d.

Box plots of purity values sampled from the posterior distribution for each block are shown in Fig. 12. The four estimates of purity are consistent. The production lot is confirmed as

sufficiently homogenous with respect to the certified purity value, using a minimum sample size of 10 mg.

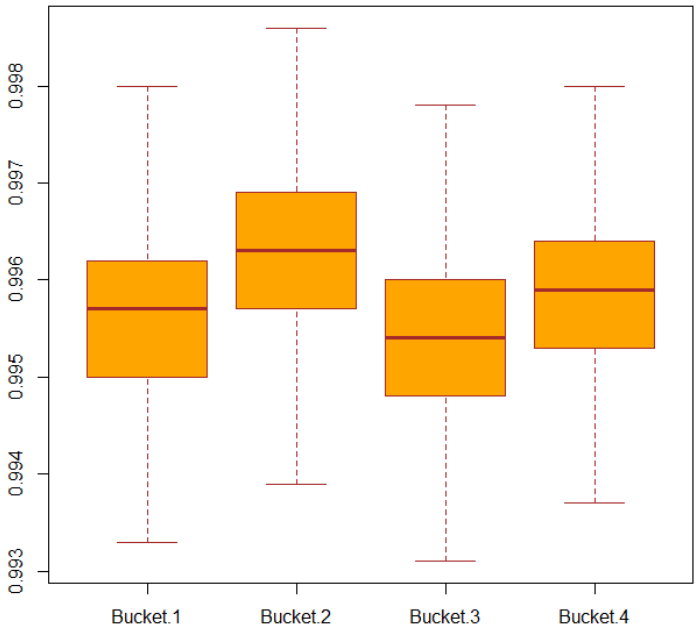

Fig. 12. Purity Estimates for Units Bottled from the Four Buckets of Bulk D-Glucose, g/g

The mass fraction purity is here expressed in units of g/g rather than percent. The distributions are defined by the posterior distributions of each block of samples. The central horizontal lines denote medians, the filled box spans the central 50 % of the MCMC samples, the whiskers span the central 95 % of each distribution.

To simplify practical usage of the delivered certified purity value, it is recommended that the uncertainty designate an interval of values attributable to the measurand that is symmetric about the central value. For this reason, the recommended certified value for purity of SRM 917d, expressed as a mass fraction in percent form, is (99.6 ± 0.3) %, where the number after the ± symbol is the uncertainty at a level of confidence of approximately 95 percent.

4. Water and Ash

The mass fractions of water and non-volatile impurities in SRM 917d were evaluated to ensure that they are compatible with the $^{1}H\{^{13}C\}$-qNMR$_{IS}$ purity estimate.

4.1. Karl Fischer Water Analysis

Twelve units of SRM 917d were evaluated for water content using volumetric Karl Fischer titration moisture analysis.

4.1.1. Materials

The 12 units were a subset of the 17 units sampled to determine the D-glucose purity described in Section 3. They included the first and last units produced. Table 8 lists the SRM 917d units analyzed.

Table 8. Karl Fischer Sampling Scheme

Bucket	Number of Units Evaluated	Production Sequence of Units Evaluated
1	2	1, 125
2	4	500, 625, 750, 875
3	4	1000, 1125, 1250, 1375
4	2	1750, 1954

The reagents used in the Karl Fischer system were Hydranal composite 2 (Fluka, lot SZBD3390V), methanol (Fisher, lot 161607), and formamide (Fluka, lot SZBD2980V). Additional reagents used were one bottle of anhydrous 1-octanol obtained from Sigma-Aldrich (lot SHBF8161V) and one bottle of LC-MS ultra chromosolve grade water obtained from Sigma-Aldrich (lot BCBQ8032V).

SRM 917c and SRM 2890 Water Saturated 1-Octanol [20] were used as controls.

4.1.2. Sample Preparation

The D-glucose from the SRM 917d bottles was used as received.

4.1.3. Method

The analysis was performed using a volumetric Karl Fischer system with Hydranal composite 2 as the Karl Fischer reagent. The working solvent for the titration is a 1:1 (vol:vol) mixture of methanol and formamide. Approximately 80 mL of the working solvent was added to the Karl Fischer vessel. The entire apparatus is enclosed in a glove bag and is purged with dry nitrogen to minimize water uptake when the solid samples are added to the Karl Fischer cell. The Karl Fischer system was run overnight to fully equilibrate.

On the day of the analyses, the titer (volume of solution delivered per milligram of water consumed) of the Hydranal composite 2 solution was determined from several injections of an in-house standard of water saturated 1 octanol (WSO). The WSO was prepared in 2010 and

stored on the benchtop at 22 °C, where the organic phase is used for the calibration. The WSO solution is periodically checked against gravimetrically prepared water in octanol solutions, and against SRM 2890 to confirm traceability [21]. A recent verification demonstrated that the material is still fit for purpose. A minimum of three calibration measurements using 40 mg (nominal) of WSO were made by injecting the WSO into the Karl Fischer titration vessel through a silicone septum via a gas-tight syringe. Samples of the WSO were weighed out on a Sartorius MC 210 S analytical balance having 0.01 mg readability. The amount of WSO injected into the Karl Fischer cell was determined by weighing the injection syringe before and after the injection.

Following the calibration measurements, test portions of SRM 917d (or control samples) were measured on the Karl Fischer system. The samples were introduced into the Karl Fischer system by briefly opening the fill port and adding the test portion via a glass weigh boat. The amount introduced into the Karl Fischer cell was determined from the mass difference of the weigh boat with and without the sample test portion. The D-glucose samples were run as sequential duplicates.

All titrations were run for a set length of time (40 minutes) rather than a duration determined by the electrochemical potential of the cell alone. The drift of the instrument was calculated at the conclusion of every run over three successive 10-minute intervals to check for consistency in the baseline and to calculate the adjusted Karl Fischer signal due to system drift.

After every second measurement, an analysis blank titration was run by opening the fill port and mimicking introducing the sample using the weigh boat. On average, the blank correction for the Karl Fischer analysis is (25 ± 10) µL of Hydranal composite 2 or (29 ± 12) µg of water.

4.1.4. Quantification

The value for mass fraction of water in the sample, w_{H2O}, is calculated as a percentage:

$$w_{\mathrm{H2O}} = 1000\left(\frac{V_{\mathrm{s}} - V_{\mathrm{b}} - t \times R_{\mathrm{d}}}{m}\right) F \qquad (1)$$

where: V_{s} volume of titrant consumed by the D-glucose,
V_{b} volume of titrant consumed titrating a blank,
t titration time,
R_{d} drift rate,
m mass of D-glucose, and
F calibration factor determined by titrating WSO samples of known water content.

Table 9 reports the standard uncertainties for individual measurements that are associated with these factors [22]. When the individual components of uncertainty are combined in quadrature [16], the standard uncertainty for each of the Karl Fischer measurements is about 0.001 %.

Table 9. Components of Uncertainty in the Karl Fischer Measurements

		Uncertainty		
Factor	Value	Distribution [a]	*u*	Unit
Mass (*m*)	1 g	R[-0.03,0.03] [b]	0.024	mg
titration time (*t*)	40 min	N(0,1) [c]	0.5	s
		R[0,5] [c]	5	s
volume of reagent titrated (V_s and V_b)	10 mL	R[-7,7] [d]	4.04	µL
drift rate (R_d)	1 µL/min	R[-0.1,+0.1] [e]	0.05	µL/min
moisture in WSO (*F*)	48.3 mg/g	N(0,0.6)	0.3	mg/g

a The uncertainties are characterized as uniform distributions (R) along the interval [-α,+α], or normal distributions, N(0,σ).

b Mass is calculated from the difference of two weights so the uncertainty can be calculated from the linearity of the balance. The balance manufacturer gives the linearity as ±0.03 mg. The uncertainty for one measurement is $0.03/\sqrt{3} = 0.0173$ mg. This should be counted twice so the overall uncertainty is $u(m) = \sqrt{(2\times 0.0173^2)} = 0.024$ mg.

c The timing uncertainty has a random component estimated from observation and a time delay resulting in a strictly positive bias of up to about 5 seconds.

d The volume uncertainty is estimated from the instrument's stated maximum random error of values up to 10 mL: $u(V) = 7/\sqrt{3} = 4.04$ µL.

e The ±0.1 µL/min range is based on observation; it is larger than the uncertainty calculated from linear regression.

4.1.5. Analysis

The measured water content of the SRM 2890 control sample was in good agreement with the material's certified value [20]. The measured water content of the SRM 917c control sample was in excellent agreement with the non-certified reference value provided in that material's Certificate of Analysis [11]. These results confirm that the measurement process was in statistical control.

Table 10 lists the measured water mass fraction values, w_{ij} where *i* indexes the analyses and *j* indexes the replicate, and their uncertainties for each Karl Fischer analysis of the SRM 917d material, along with the mean, standard deviation, and standard uncertainty of the mean for the samples from each bottle. The results for each of the twelve bottles as functions of bottle fill order and Karl Fischer analysis order are displayed in Fig. 13.

Table 10. Karl Fischer Estimates of D-Glucose Water Content, %

Run$_i$ [a]	Bottle	w_{i1} [b]	$u(w_{i1})$ [c]	w_{i2} [b]	$u(w_{i2})$ [c]	$\bar{w}_i$ [d]	$s(w_i)$ [e]	$u(\bar{w}_i)$ [f]
1	1375	0.03817	0.00091	0.04034	0.00096	0.03926	0.00153	0.00131
2	1750	0.04985	0.00111	0.05224	0.00114	0.05105	0.00169	0.00147
3	625	0.04622	0.00109	0.04750	0.00102	0.04686	0.00091	0.00103
4	1250	0.05311	0.00103	0.05255	0.00106	0.05283	0.00040	0.00085
5	1000	0.05077	0.00110	0.04214	0.00094	0.04646	0.00610	0.00439
6	1125	0.05522	0.00117	0.05423	0.00121	0.05473	0.00070	0.00102
7	1	0.04419	0.00100	0.04575	0.00098	0.04497	0.00110	0.00109
8	500	0.04692	0.00100	0.04608	0.00098	0.04650	0.00059	0.00087
9	750	0.04364	0.00097	0.05010	0.00105	0.04687	0.00457	0.00332
10	125	0.04915	0.00105	0.05020	0.00106	0.04968	0.00074	0.00096
11	1954	0.04355	0.00093	0.05371	0.00107	0.04863	0.00718	0.00514
12	875	0.05908	0.00115	0.06199	0.00120	0.06054	0.00206	0.00170

a Run$_i$ designates the i^{th} pair of SRM 917d samples analyzed. Replicate samples were run sequentially.
b Result of Karl Fischer analysis for the jth replicate sample from the bottle analyzed during Run$_i$.
c Standard uncertainty of w_{ij}, estimated using Eq. 2.
d Mean of the Run$_i$ replicates, $\bar{w}_i = \sum_j^2 w_{ij}\,/2$.
e Standard deviation of the Run$_i$ replicates, $s(w_i) = \sqrt{\sum_j^2 (w_{ij} - \bar{w}_i)^2/(2-1)}$.
f Standard uncertainty of the mean of the Run$_i$ replicates, $u(\bar{w}_i) = \sqrt{(s^2(w_i) + \bar{u}^2(w_i))/2 + u_{cal}^2}$, where $\bar{u}(w_i) = \sqrt{\sum_j^2 u^2(w_{ij})\,/2}$ is the pooled standard deviation of the replicate measurements and u_{cal} = 0.000305 % is the standard uncertainty of the calibration standard.

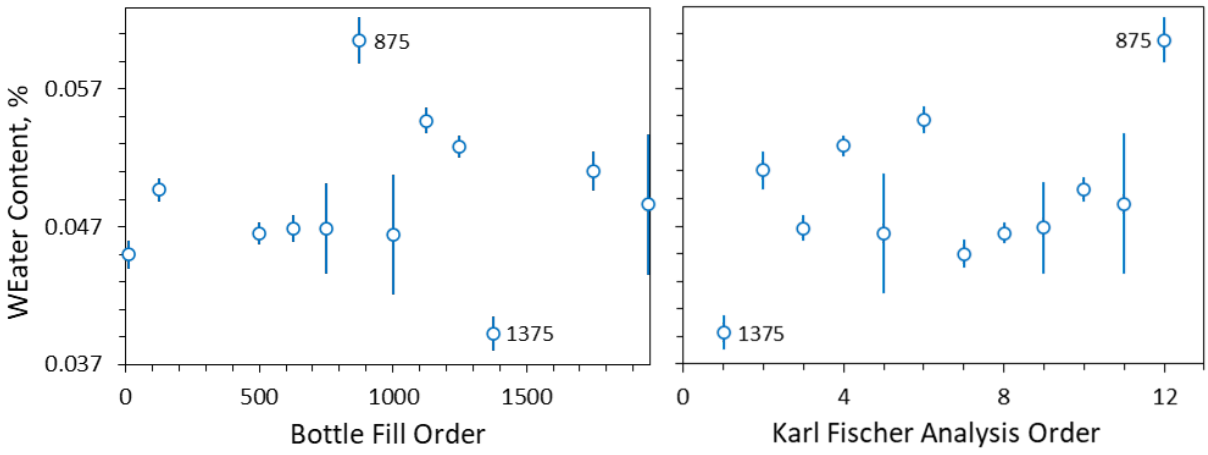

Fig. 13. Water Content as Functions of Bottle Fill and Karl Fischer Analysis Order

Open circles represent the mean of sequential duplicate Karl Fischer analyses for the 12 units of SRM 917d evaluated. Error bars represent standard uncertainties of the means. The bottle numbers are shown for the lowest and highest of the measured values.

There is no apparent trend related to the fill order of the bottles. However, the exceptionally low and high results being for the first and last bottles analyzed suggests a partial trend related to the order of analysis. The relatively large error bars for three of the bottles, reflecting atypical differences between the results of the replicate measurements for those bottles, suggests episodic measurement issues. The absence of substantial overlap among the error bars indicates the Karl Fischer variability among the results is not explained by just the known uncertainty components. The variability among the measurements for the twelve bottles could

arise from intrinsic heterogeneity in the water content of the bottled material, heterogeneity introduced by sample handling during their use in the qNMR measurements, Karl Fischer measurement issues, or some combination of causes.

The mean water content determined by Karl Fischer is 0.04903 % with a standard deviation of 0.00538 %. The pooled standard deviation of the replicate measurements (which for each bottle combines in quadrature the between-replicate standard deviation, the pooled standard uncertainty of the individual measurements, and the 0.00031 % standard uncertainty associated with the WSO calibration standard), is 0.00240 %. The standard deviation for the entire population of measurements can then be estimated as $\sqrt{(0.00538^2 + 0.00240^2)} = 0.00589$ %.

The standard uncertainty of the mean of these 12 bottles is $0.00589/\sqrt{12} = 0.00170$ %. The two-tailed Student's *t* 95 % confidence critical value for 12 independent measurements is 2.201; the 95 % confidence expanded uncertainty of the mean is then (2.201)(0.00170) = 0.00374 %. This suggests that the water content of SRM 917d is (0.0490 ± 0.0037) %.

The Karl Fischer estimates were also analyzed using the NIST Decision Tree [23]. A Cochran's test for homogeneity strongly suggested statistical heterogeneity among the results of the Karl Fischer analysis. A hierarchical gauss-gauss consensus estimation procedure was recommended and implemented via the Decision Tree to calculate the results of the water content analysis. The consensus mean is 0.0491 %, the standard uncertainty is 0.0018 %, and the shortest 95 % coverage interval is [0.0456, 0.0527] %. A summary of the water content analysis using the Decision Tree is shown in Appendix C. Since the interval is nearly symmetrical about the mean, the 95 % expanded uncertainty can be estimated as (0.0527 – 0.0456)/2 = 0.0036 %. The estimate for water in SRM 917d is thus (0.0491 ± 0.0036) %. The results calculated using either approach described herein are nearly identical given that the heterogeneity in Karl Fischer analysis estimates, conveyed through the estimate for "tau", is the largest source of variation in the data set.

4.2. Thermogravimetric Ash Analysis

Seven bottles of SRM 917d were evaluated for ash content by thermogravimetric analysis (TGA) using a LECO TGA701 (LECO Corp., St. Joseph, MI USA) analyzer.

4.2.1. Materials

The seven bottles were a subset of the 12 bottles used to determine water content (Section 4.1). They included the first and last units produced. Table 11 lists the SRM 917d units analyzed. Material from one bottle of SRM 917c D Glucose (Dextrose) was also evaluated.

Table 11. Thermogravimetric Analysis Sampling Scheme

Bucket	Number of Units Evaluated	Production Sequence of Units Evaluated
1	2	1, 125
2	1	875
3	2	1000, 1250
4	2	1750, 1954

Three sets of high-purity gold wires served as bias correction controls; each set consisted of one large and one small gold piece with a combined weight of about 1 g.

4.2.2. Sample Preparation

The D-glucose from the SRM 917d bottles was used as received.

4.2.3. Method

TGA determination of ash content is based on the accurate evaluation of mass remaining after combustion in a thermogravimetric oven [24]. Test portions were removed from each bottle and heated in the TGA701 analyzer in an air atmosphere.

The analyzer consists of an electronics unit for furnace control and data management and a multiple sample furnace that allows up to 19 samples to be analyzed sequentially. The furnace holds 20 crucibles with one crucible designated as an empty reference crucible. After an analysis profile was created and selected, empty crucibles were loaded into the furnace carousel and tare weights were obtained. The run used about D-glucose from the seven SRM 917d bottles and the one SRM 917c control bottle. Each crucible containing a nominal 2 g test portion of D-glucose was transferred to the TGA to record an initial mass. The mass loss of each sample was monitored by the TGA and was recorded approximately every 4 min, The samples were heated to 107 °C and held for 3 hours, then heated to 800 °C and held for 3 hours. The output from the balance, a sequence of masses that changed over time, was recorded in a computer file and the data were downloaded from the instrument and analyzed off-line.

The accuracy and precision of the analyzer was monitored using the three sets of high-purity gold wire. Each set was added to one of the first three sample crucibles. After the initial mass of a set was recorded, the large piece of wire was removed, creating a known mass loss for that sample which could be compared to that determined by the instrument. Any gain or loss in mass of the gold wire serves as a measure of the high temperature buoyancy correction, c_b. The difference between the room temperature mass of gold, m_{rt}, and the mass of gold at 750 °C, m_{750}, was used to determine the buoyancy correction for the thermogravimetric analyzer at 750 °C:

$$c_b = m_{750} - m_{rt} . \quad [2]$$

The SRM 917d sample replicates were loaded in bottle-number sequence into the fourth through the 17th sample crucibles. The SRM 917c replicates were loaded into the 18th and 19th crucibles. Crucible 20 was the empty reference.

4.2.4. Quantification

The determinations of ash content were calculated from the final mass of the sample at 750 °C, m_f, minus the buoyancy correction, divided by the initial mass, m_i. The ash content, m_A is determined as a percent value:

$$m_A = 100\,(m_f - c_b)/m_i . \quad [3]$$

The sources of measurement uncertainty include sample repeatability, gold wire control repeatability, and weighing accuracy [24]. Between-replicate measurement repeatability was the only significant source.

4.2.5. Analysis

Table 12 lists the ash content of the D-glucose in the seven SRM 917d bottles as estimated from heating to 800 °C in the thermogravimetric analyzer, along with the mean and standard deviation of the replicate analyses. The results as a function of bottle fill order, which is also the sequence in which the samples were analyzed, are displayed in Fig. 14.

Table 12. TGA Estimates of D-Glucose Ash Content, %

Seq_i [a]	Bottle	w_{i1} [b]	w_{i2} [b]	w_i [c]	$s(w_i)$ [d]
1	1	0.01242	0.00635	0.00938	0.00429
2	125	0.00751	0.01024	0.00887	0.00193
3	875	0.00977	0.00569	0.00773	0.00288
4	1000	0.00145	0.00469	0.00307	0.00229
5	1250	0.00748	0.00649	0.00698	0.00070
6	1750	0.00278	0.00589	0.00433	0.00219
7	1954	0.00654	0.00188	0.00421	0.00330

a Seq_i designates the i^{th} pair of SRM 917d samples analyzed. Samples were run in bottle-number sequence.
b Result of TGA analysis for the jth replicate sample from the bottle analyzed during Seq_i.
c Mean of the Run_i replicates, $\bar{w}_i = \sum_j^2 w_{ij} /2$.
d Standard deviation of the Run_i replicates, $s(w_i) = \sqrt{\sum_j^2 (w_{ij} - \bar{w}_i)^2/(2-1)}$.

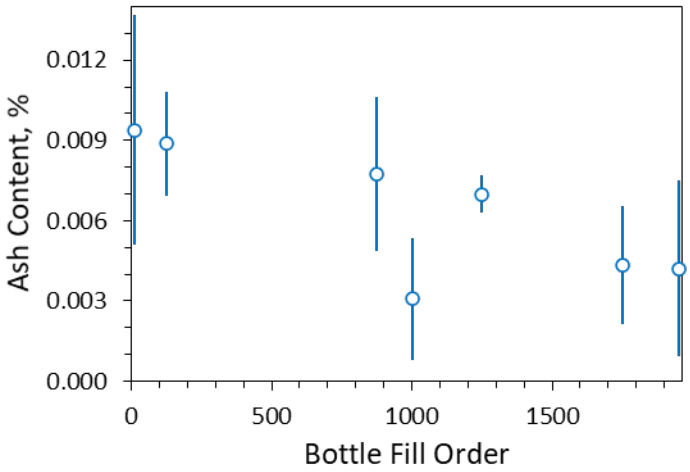

Fig. 14. Ash as a Function of Bottle Fill Order.

Open circles represent the mean of the sequential replicate analyses. Error bars represent standard uncertainties of the means.

The mean ash content is 0.00637 %. The standard deviation of the seven bottle means is 0.00249 % ($s_{between}$), and the pooled standard deviation of the seven replicate pairs is 0.00272 % (s_{within}). Since s_{within} is larger than $s_{between}$, it is reasonable to regard all measurements as effectively independent and to use the 0.00312 % standard deviation of the 14 measurements to characterize the population.

Assuming that SRM 917d is homogeneous with regard to ash content, the standard uncertainty of the mean of these 14 measurements is 0.00312/√14 = 0.00083 %. The two-tailed Student's *t* 95 % confidence critical value for 14 independent measurements is 2.160; the 95 % confidence expanded uncertainty of the mean is then (2.160)(0.00083) = 0.00179 %. This suggests an ash content for SRM 917d of (0.0064 ± 0.0018) %.

However, the measured ash content has some dependance on bottling/run order. Furthermore, there are too few TGA estimates of ash to reliably implement statistical tests for heterogeneity via the NIST Decision Tree. Given the apparent heterogeneity, yet low statistical power of the data, the NICOB's linear pool method [25], visualized in Fig. 15, may provide a more appropriate conservative estimate of measured ash content. The 0.0064 % is unchanged but the 0.0036 % standard uncertainty is much larger and the [-0.0004 to 0.0140] % 95 % coverage interval is much wider. Indeed, the interval includes values of less than zero ash. This suggests the ash content can be appropriately estimated as (0.01 ± 0.01) %.

The result for the second replicate of the SRM 917c material was lost due to technical issues. The 0.0026 % results for the initial replicate is compatible with the population of the SRM 917d results.

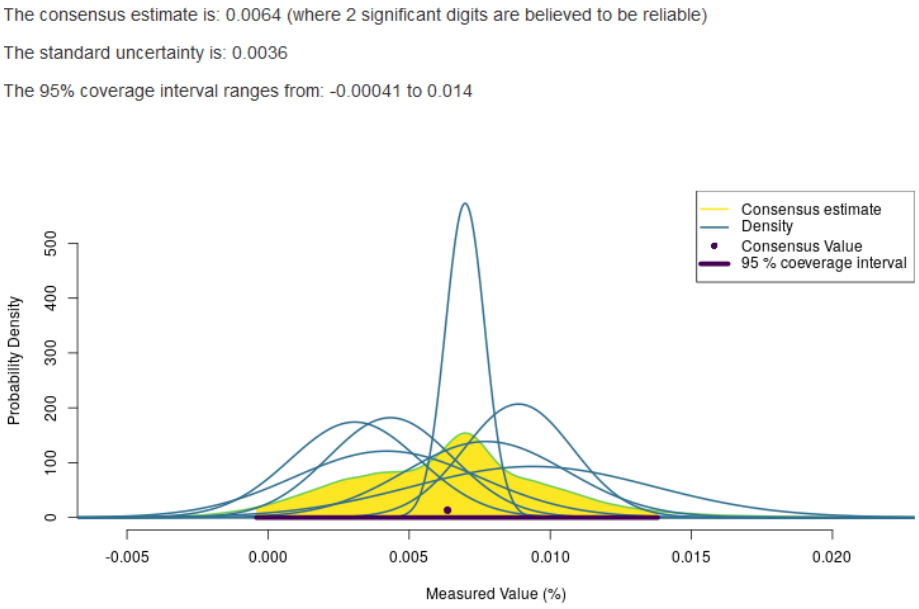

Fig. 15. Linear Pool Estimate of Ash Content.

The continuous blue curves represent Normal(x_i, $u(x_i)$) probability density functions. The continuous green curve represents the consensus pdf; the yellow band on top of the thick line horizontal line spans the 95 % confidence interval; the solid circle denotes the mean. The numerical results are given to the top left.

4.3. Summary

The conservative estimate of water content is (0.049 ± 0.004) %. Assuming that the inorganic ash is the oxidized remnant of metals and/or salts with anions that were heat-labile, the inorganic content in the SRM 917d material is unlikely to be much greater than the conservative estimate of (0.01 ± 0.01) %. The combined known impurity content can thus be estimated as the sum of the measured water and ash contents: (0.06 ± 0.01) %. This is quite compatible with the estimated D-glucose purity of (99.6 ± 0.3) % presented in Section 3.7.

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Appendix A. List of Symbols, Abbreviations, and Acronyms

μ	estimate of the mean
σ	estimate of standard deviation
$u(\cdot)$	standard uncertainty of a particular quantity
^{1}H-qNMR$_{IS}$	quantitative proton nuclear magnetic resonance spectroscopy using an internal standard
^{1}H{^{13}C}-NMR	one dimensional ^{1}H with ^{13}C decoupling NMR
BBI	broadband inverse (BBI) detection probe
COSY	correlated spectroscopy NMR
D1	relaxation delay
em	exponential multiply
F1	frequency used for the HSQC ^{1}H axis
F2	frequency used for the HSQC ^{13}C axis
FID	free induction decay
GARP	globally optimized, alternating phase, rectangular pulse ^{13}C decoupling
HMBC	heteronuclear multi-bond coherence NMR
HSQC	heteronuclear single quantum correlation NMR
IS	internal standard
KHP	potassium hydrogen phthalate
MCMC	Markov Chain Monte Carlo
NIST	National Institute of Standards and Technology
NMR	nuclear magnetic resonance spectroscopy
O1	transmitter frequency offset
qNMR	quantitative NMR
SD	standard deviation
SI	International System of Units (Système international d'unités)
SRM®	Standard Reference Material®
T1	spin lattice relaxation time
WSO	water saturated 1-octanol
zgig	90-degree single pulse excitation sequence

Appendix B. Bayesian Model

The following is the OpenBUGS code and data used to calculate combined D-glucose purity of SRM 917d D-Glucose (Dextrose). Commentary text is in bold font, following a # symbol.

B.1. Model

```
#Model:
{
KHP~dnorm(0.99991,100000000) #distribution of values attributable to purity (mass fraction KHP) of internal
                                standard
mImCp<-1/(mImCu*mImCu)

for(i in 1:4){ #i=4, number of buckets of bulk glucose
mwI[i]~dnorm(204.221, 27778) #distribution of values attributed to relative molar mass (g/mol) of KHP
mwP[i]~dnorm(180.156,111111.111111) #distribution of values attributed to relative molar mass (g/mol) of
                                glucose

#specification of prior (beta) distribution of purity values for samples from each bucket of bulk glucose:
mu[i]~dunif(0.9,1)
sd[i]~dunif(0,.05)
c[i]<-mu[i]/(sd[i]*sd[i])
d[i]<-(1-mu[i])/(sd[i]*sd[i])

  for(j in 1:N[i]){
pb[i,j]~dbeta(c[i],d[i]) #hierarchy placed on j sample analyses for each of i buckets. Node pb[i,j] is for purity
                        values calculated from data of the jth sample corresponding to the ith bucket

#specification of relationship between distributions corresponding to observed measurement data inputs,
according to Eq. 1 measurement function:
korig[i,j]~dunif(0,0.01); k.cut[i,j]<-cut(korig[i,j])
   mI[i,j]~dnorm(avgmI[i,j],mImCp); avgI[i,j]<-KHP*mI[i,j]/(mwI[i]*korig[i,j])
   AreaIp[i,j]<-1/(AreaIu[i,j]*AreaIu[i,j]); AreaI[i,j]~dt(avgI[i,j],AreaIp[i,j],2)
   mC[i,j]~dnorm(avgmC[i,j],mImCp); avgP[i,j]<-pb[i,j]*mC[i,j]/(mwP[i]*k.cut[i,j])
   AreaPp[i,j]<-1/(AreaPu[i,j]*AreaPu[i,j]); AreaP[i,j]~dnorm(avgP[i,j],AreaPp[i,j])}}

# Linear pool procedure for combining all i posterior estimates of purity and calculate glucose purity result (g/g)
for SRM 917d D-Glucose
for(i in 1:4){S[i]<-1}
R[1:4]~ddirich(S[]); T~dcat(R[]); PLP<-mu[T]}
```

B.2. Data

#Data inputs from 1H-NMR analysis of j number of samples of units from each of the i = 4 Buckets:
list(
mImCu=0.0000005, **#uncertainty associated with measured values of mass of internal standard and SRM 917d added to each sample**
N=c(4,8,4,5), **#array specifying the value of j corresponding to each of i buckets represented by the data inputs**

#mass of internal standard added to each sample:
avgmI=structure(.Data=c(
0.004399,0.006385,0.005162,0.007036,NA,NA,NA,NA, #for i=1
0.003873,0.008601,0.005366,0.004702,0.003882,0.003226,0.00424,0.005638, #for i=2
0.003838,0.008285,0.005028,0.004399,NA,NA,NA,NA, #for i=3
0.005689,0.004375,0.004417,0.007804,0.008657,NA,NA,NA),.Dim=c(4,8)), #for i=4

#mass of candidate SRM 917d added to each sample:
avgmC=structure(.Data=c(
0.004567,0.007622,0.006665,0.008379,NA,NA,NA,NA, #for i=1
0.004184,0.007123,0.007494,0.005221,0.003397,0.00291,0.004953,0.005228, #for i=2
0.004662,0.007235,0.004832,0.004208,NA,NA,NA,NA, #for i=3
0.005554,0.004186,0.004286,0.00734,0.007587,NA, NA,NA),.Dim=c(4,8)), #for i=4

#1H-NMR spectrum integral values for KHP internal standard:
AreaI=structure(.Data=c(
1.019806,1.411407,1.183624,1.520766, NA,NA,NA,NA, #for i=1
0.919164,1.812293,1.198483,0.975058,1.284009,1.114752,0.89003,1.117848, #for i=2
0.846635,1.818184,1.113784,1.026492,NA,NA,NA,NA, #for i=3
1.275188,1.004433,1.013005,1.688117,1.844244,NA, NA,NA),.Dim=c(4,8)), #for i=4

#u(AreaIi,j)
AreaIu=structure(.Data=c(
0.00051,0.000706,0.000592,0.00076,NA,NA,NA,NA, #for i=1
0.00046,0.000906,0.000599,0.000488,0.000642,0.000557,0.000445,0.000559, #for i=2
0.000423,0.000909,0.000557,0.000513,NA,NA,NA,NA, #for i=3
0.000638,0.000502,0.000507,0.000844,0.000922,NA, NA,NA),.Dim=c(4,8)), #for i=4

 # glucose 5.1 ppm and 3.1 ppm peak combined 1H-NMR spectrum integral values:
AreaP=structure(.Data=c(
1.195084,1.901847,1.727146,2.043077,NA,NA,NA,NA, #for i=1
1.124122,1.691186,1.888322,1.221586,1.272388,1.13755,1.173269,1.17203, #for i=2
1.1605,1.791065,1.208201,1.108952,NA,NA,NA,NA, #for i=3
1.404402,1.085748,1.11095,1.792566,1.824903,NA,NA,NA),.Dim=c(4,8)), #for i=4

#u(AreaPi,j)
AreaPu=structure(.Data=c(
0.001195,0.001902,0.001727,0.002043,NA,NA,NA,NA, #for i=1
0.001124,0.001691,0.001888,0.001222,0.001272,0.001138,0.001173,0.001172, #for i=2
0.00116,0.001791,0.001208,0.001109,NA,NA,NA,NA, #for i=3
0.001404,0.001086,0.001111,0.001793,0.001825,NA, NA,NA),.Dim=c(4,8))) #for i=4

Appendix C. NIST Decision Tree Analysis

The following is the NIST Decision Tree Analysis [23] of the Karl Fischer water analysis results presented in Table 10.

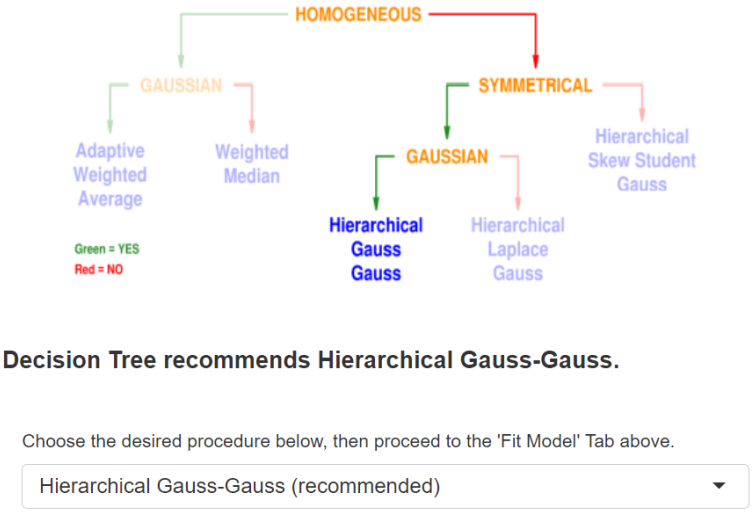

Fig. C-1. Decision Tree Consensus Estimation Procedure Recommendation

Pathway chosen in Decision Tree flow diagram, with "yes" or "no" decisions based on the results of statistical tests for homogeneity, symmetry, and normality implemented via the software (https://decisiontree.nist.gov/).

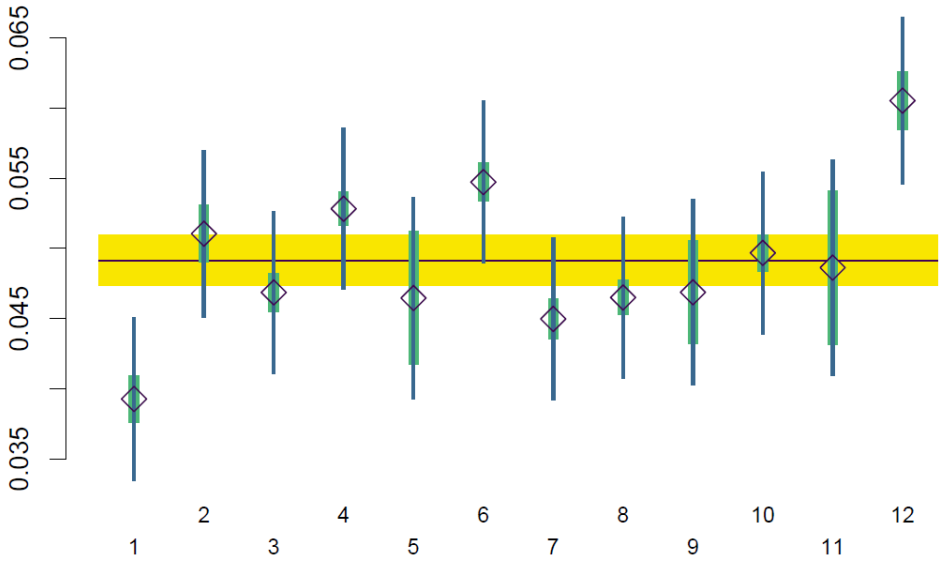

Fig. C-2. Bayesian Gauss-Gauss Consensus Estimate of Water Content

Karl Fischer estimates for the individual units are plotted as the respective mean (diamond) and standard uncertainty (wide green error bars). The estimate of dark uncertainty, tau, is plotted as the thin blue error bars. The consensus mean value, X, is plotted as the black horizontal line and the yellow plot region indicates the interval $X \pm u(X)$, where $u(X)$ is the standard uncertainty of the consensus estimate.

Include	Laboratory	Result	Uncertainty	DegreesOfFreedom
TRUE	1	0.03926	0.00131	10000
TRUE	2	0.05105	0.00169	10000
TRUE	3	0.04686	0.00103	10000
TRUE	4	0.05283	0.00085	10000
TRUE	5	0.04646	0.00439	10000
TRUE	6	0.05473	0.00102	10000
TRUE	7	0.04497	0.00109	10000
TRUE	8	0.04650	0.00087	10000
TRUE	9	0.04687	0.00332	10000
TRUE	10	0.04968	0.00096	10000
TRUE	11	0.04863	0.00514	10000
TRUE	12	0.06054	0.00170	10000

Date: 2022-09-19
Selected Procedure: Hierarchical Gauss-Gauss
Consensus estimate: 0.04914
Standard uncertainty: 0.001764
95% coverage interval: (0.04564, 0.05265)
Dark uncertainty (tau): 0.005579
Tau posterior 0.025 and 0.975 quantiles: (0.003556,0.008911)

Decision Tree Hypothesis test results

Cochran's test for Homogeneity:
p-value: $p < 0.001$
$Q = 181.9$ (Reference Distribution: Chi-Square with 11 Degrees of Freedom)
tau est. $= 0.004941$
tau/median(x) $= 0.1035$
tau/median(u) $= 4.117$

Shapiro-Wilk test for Normality: $p = 0.5144$

Miao-Gel-Gastwirth test of Symmetry: $p = 0.199$

Fig. C-3. NIST Decision Tree Analysis Summary