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Certification of Standard Reference Material® 3223:

Inorganic Constituents in Cigarette Tobacco Filler

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Abstract

The National Institute of Standards and Technology (NIST) Standard Reference Material® (SRM®) 3223 Inorganic Constituents in Cigarette Tobacco Filler is intended for use in the validation of procedures and methods for the determination of inorganic constituents in cigarette tobacco filler or similar matrices. Air-cured low-nicotine tobacco was processed commercially using the normal procedures used for the production of cigarette tobacco filler. The product was cryogenically milled, irradiated, and packaged into individual SRM units. Value-assigned measurements were made collaboratively at the Centers for Disease Control and Prevention (CDC) and NIST. This publication documents the production, analytical measurements, and statistical evaluations leading to the certification of the SRM.

Keywords

Inorganic; Elements; Tobacco; Cigarette; Arsenic (As); Beryllium (Be); Cadmium (Cd); Chromium (Cr); Cobalt (Co); Lead (Pb); Manganese (Mn); Mercury (Hg); Nickel (Ni); Selenium (Se); Uranium (U); Standard Reference Material (SRM).

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1. Introduction

The Food, Drug, and Cosmetics Act (FD&C Act), specifically Section 904(a)(3), amended by the Family Smoking Prevention and Tobacco Control Act, requires tobacco manufacturers and importers to report to the Food and Drug Administration (FDA) the levels of Harmful and Potentially Harmful Constituents (HPHCs) in their products and smoke. This mandatory report includes chemicals identified by the FDA that cause or could cause harm [1]. Among the HPHCs listed by FDA are ten inorganic elements: arsenic (As), beryllium (Be), cadmium (Cd), chromium (Cr), cobalt (Co), mercury (Hg), nickel (Ni), lead (Pb), selenium (Se), and uranium (U). Additionally, manganese (Mn) is listed in the Environmental Protection Agency (EPA)'s Health and Environmental Research Online (HERO) database, because toxicological research indicates Mn compounds are potential asthma inducers and respiratory irritants [2].

The Centers for Disease Control and Prevention (CDC) has been conducting measurements of tobacco products in support of FDA's regulatory efforts. To support measurements of the elemental HPHC content of tobacco-based products, development of SRM 3223 Inorganic Constituents in Cigarette Tobacco Filler was initiated after a 2017 technical meeting between CDC and the National Institute of Standards and Technology (NIST). Excess tobacco leaves from the production of SRM 3222 Cigarette Tobacco Filler [3] were repurposed for the production of SRM 3223.

Measurements contributing to SRM 3223 characterization and value-assignments were conducted by CDC and NIST.

The following Sections of this document describe the production, moisture (water plus other volatiles) content, homogeneity assessment, and (As, Be, Cd, Co, Cr, Hg, Mn, Ni, Pb, Se, and U) content of SRM 3223.

2. Materials and Production

The tobacco leaves for producing SRM 3223 come from the excess materials used for producing SRM 3222 Cigarette Tobacco Filler. The air-cured, low-nicotine tobacco was commercially processed using normal procedures for the production of cigarette tobacco filler. The dried and chopped leaves (referred to as “cut rag”; 30 cuts per inch) were blended and enclosed in plastic bags that were placed in cardboard cartons containing approximately 68.2 kg (150 lb) of tobacco. These materials were stored at –20 °C in Gaithersburg, MD, prior to shipping at ambient temperature to Charleston, SC, to initiate production. The pre-production material is pictured in Fig. 1.

Fig. 1. SRM 3223 Tobacco Filler Material Prior to Milling.

2.1. Material Homogenization

In 2021, two small test portions of excess tobacco from the production of SRM 3222 were homogenized in a polytetrafluoroethylene (PTFE) disk mill at different temperatures. One portion was milled at ambient temperature (≈ 20 °C) and the other was frozen at < -150 °C overnight before being cryogenically milled (cryomilled) the next day using identical equipment setup. Both homogenized materials were stored at room temperature before preliminary measurements. The particle size distributions (PSDs) of the two portions were analyzed with a laser-diffraction particle size analyzer fitted with a wet dispersion unit, and the elemental contents were analyzed with an inductively coupled plasma mass spectrometer (ICP-MS).

The elemental homogeneity of the two test portions was not significantly different according to ICP-MS results. However, debris were found accumulating on the mill components when milling at room temperature, and the homogenized product of the portion milled at room temperature had a broader PSD than the cryomilled portion. Consequently, cryomilling was adopted and the acceptance range of PSD was set to be between that of the room temperature milled and the cryomilled materials.

The chopped leaves with a combined mass of 27.2 kg were cryomilled in January 2022 at NIST's Cryogenic Reference Material Production Facility (CRMPF) in Charleston, SC according to NIST SP 1196r1 [4]. Briefly, material was pre-frozen in vapor-phase liquid nitrogen (LN_2) freezers (< -150 °C) then cryohomogenized through the Palla VM-KT Vibrating Cryomill.

Three randomly selected samples were used to assess the PSD of the milled material. The 10^{th}, 50^{th}, and 90^{th} percentiles of the PSD and the acceptance range are listed in Table 1. The PSD of each sample was found to be within the acceptance range, indicating that the PSD of the milled material was within the acceptance range. The post-milling PSDs and a sample of the milled material are displayed in Fig. 2. When cryohomogenization and PSD analysis were complete, the material was allowed to return to room temperature then transported to NIST Gaithersburg in February 2022 for further processing.

Table 1. Particle Size Distribution.

Batch	Dv10, µm	Dv50, µm	Dv90, µm
Sample 1	9.67 ±0.07	57.6 ±0.7	287 ±12.7
Sample 2	6.63 ±0.06	39.7 ±0.6	169 ±5.6
Sample 3	7.75 ±0.04	45.1 ±0.3	162 ±3.2
Acceptance Range	4.96 to 9.81	26 to 75.1	118 to 362

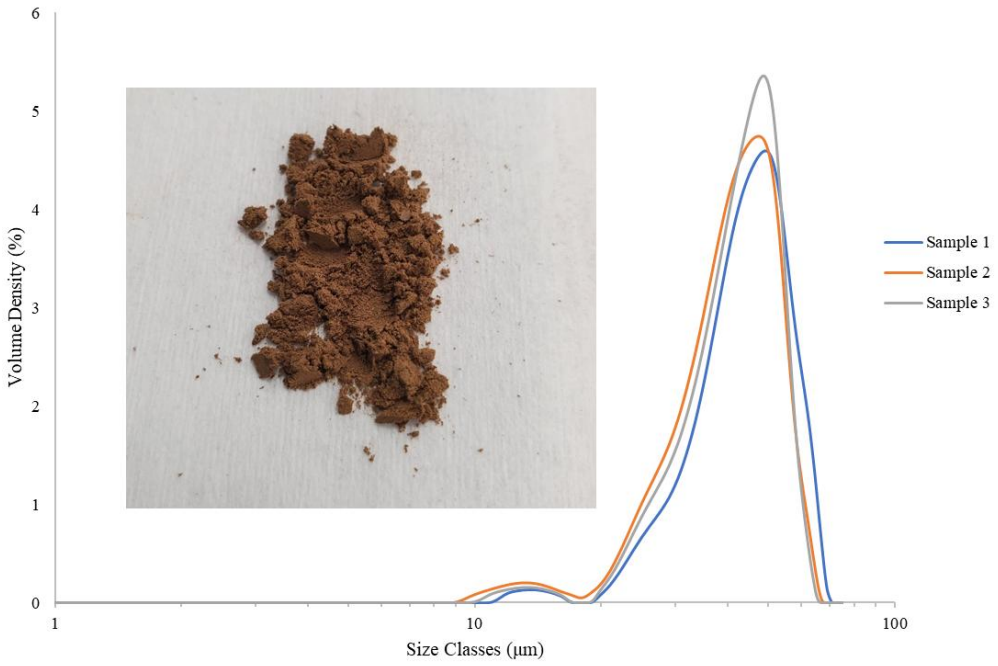

Fig. 2. Particle Size Distributions for the SRM 3223 Material After Cryomilling.

2.2. Irradiation

The milled tobacco leaves were placed in polyethylene bags, each bag containing approximately the same volume of tobacco leaves. The bags were placed inside 0.25 m by 0.25 m by 0.25 m cardboard boxes in preparation for irradiation. Eight boxes were irradiated at Neutron Products, Inc (Dickerson, MD) for microbial control. A minimum of 25 kGy of absorbed radiation was chosen for the irradiation, based on historical absorbed dosages used to stabilize NIST plant-based SRMs intended for method validation of inorganic constituents. A maximum 30 kGy dose was set based on Code of Federal Registrations Title 21 recommendation for microbial disinfection of dehydrated aromatic vegetable substances for flavoring or aroma [5]. The contractor reported an absorbed dose of between 29 kGy to 30 kGy.

2.3. Blending and Bottling

The irradiated tobacco leaves were pooled and blended in a 0.21 m^3 ceramic-lined double-cone blender for about 30 min. The blended leaves were transferred to 125 mL amber-colored high-density polyethylene (HDPE) bottles via a PTFE-coated hopper. Each bottle contains a minimum of 30.5 g of tobacco. The bottle caps were hand-tightened. Each bottle cap was sealed with a heat-shrink plastic ring. The bottle was heat-sealed inside an aluminized polyethylene bag. The bottles were identified with their production order, 1 to 835.

The finished units are stored at (21 ± 3) °C.

3. Moisture (Oven Volatiles) Determination

3.1. Drying Temperature

To aid the development of an oven drying method, the content of the oven-volatile components (referred to hereafter as “moisture”) in SRM 3223 was assessed using a thermal gravimetric analyzer (TGA) on duplicate 1 g samples. The temperature of the samples was ramped in 15 minutes to the target temperatures at (80, 85, 100, 105, and 160) °C. The samples were held at each target temperature for 5 h to produce a curve of mass loss as a function of temperature and time as shown in Fig. 3.

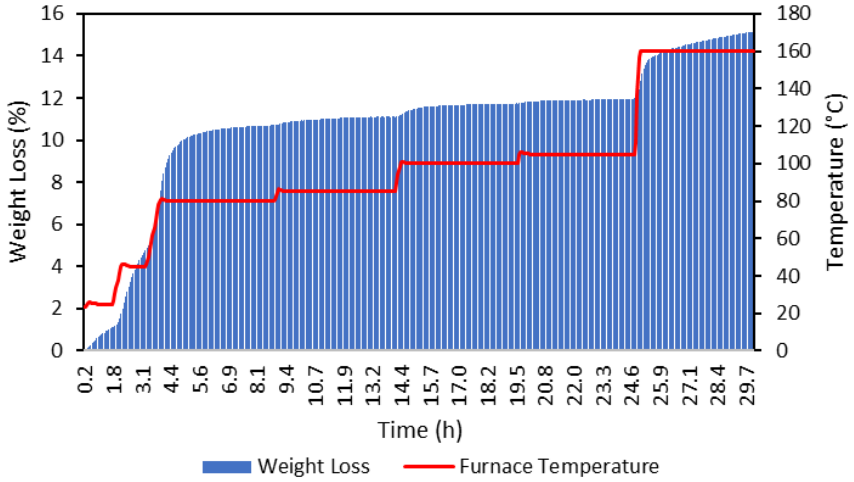

Fig. 3. Mass Loss on Drying Profile.

The curve was used to guide the selection of a drying temperature for the SRM. Steep changes in sample mass were observed at temperatures <85 °C and >105 °C. The mass loss rate was moderate between (85 and 105) °C with the insignificant mass loss from (100 to 105) °C, which suggested selecting 85 °C and 100 °C for a timed drying study.

3.2. Drying Duration: Forced-Air Oven

Twelve 1 g samples from one unit of SRM 3223 were dried at 85 °C in the forced-air oven at time intervals spanning from 1 h to 15 h. Twelve 1 g samples from a second unit were similarly dried at 100 °C. The percentage mass loss due to moisture, $w_{\%moist}$ was calculated for each sample as:

$$w_{\%\mathbf{moist}} = 100\,\frac{m_{t(0)} - m_{t(1)}}{m_{t(0)} - m} \qquad (1)$$

where m, $m_{t(0)}$, and $m_{t(1)}$ are the masses of the weighing vessel, the weighing vessel with sample before drying, and the vessel with sample after drying, respectively. In all cases, the weighing vessels were covered with lids inside the oven within 1 min of the set time and transferred to an anhydrous magnesium perchlorate desiccator to allow cooling to ambient temperature. The mass loss due to moisture is formally expressed in mass fraction units of centigram per gram sample, cg/g, but is more conveniently expressed as the percentage of sample mass, %.

As shown in Fig. 4, the drying curve at 85 °C exhibits a visible increase in mass loss during the first 3 h, followed by a leveling off after 4 h. The drying curve at 100 °C appears flat from 1.5 h onward. Since the purpose of the drying is to establish a basis on which properties of the material can be compared, drying at 85 °C for more than 4 h or drying at 100 °C for more than 2.5 h suffices. The choice between the two drying conditions becomes a matter of convenience [6]. Drying at 100 °C for 3 h was chosen for its shorter measurement time and consistency with prevailing industrial and laboratory practices [7,8].

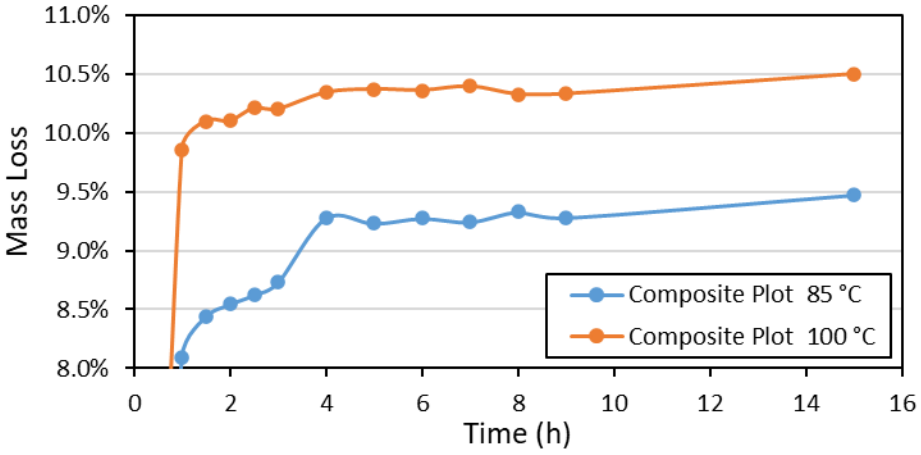

Fig. 4. Mass Loss on Forced-Air Drying at 85 °C and 100 °C.

3.3. Drying Duration: Gravity-Convection Oven

Eight 1 g samples from one unit of SRM 3223 were dried at 100 °C in a gravity convection oven. Samples were removed from the oven after (3, 4, 5, 6, 7, 8, 16, and 17) h. As shown in Fig. 5, the mass loss curve appears flat from 6 h onward.

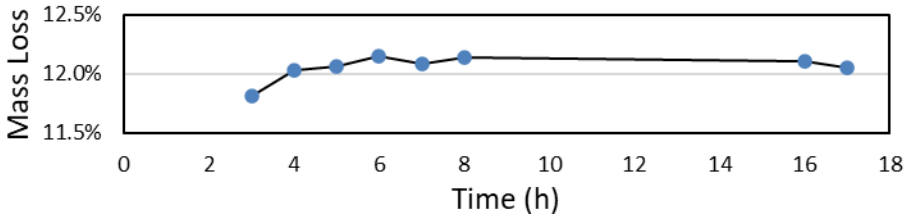

Fig. 5. Mass Loss on Gravity-Convection Drying at 100 °C.

3.4. Comparison of Forced-Air and Gravity Convection Drying

Twelve 1 g samples from one unit of SRM 3223 were used to compare forced-air with gravity convection drying at 100 °C. Six of the samples were dried in a preheated forced-air oven for 3 h and the remaining in a preheated gravity convection oven for 16 h. The mass fraction moisture in each sample was calculated using Eq. 1; the results are listed in Table 2,

Table 2. Comparison of Forced-Air and Gravity Convection Drying.

	Moisture, %	
Replicate	Forced-Air	Gravity Convection
1	12.43	12.44
2	12.41	12.39
3	12.36	12.34
4	12.37	12.31
5	12.40	12.33
6	12.38	12.30
Mean:	12.392	12.352
s:	0.026	0.053
%RSD:	0.21 %	0.43 %

where “Mean” is the arithmetic average, “*s*” is the standard deviation, and “%RSD” is the relative standard deviation expressed as a percentage: %RSD = 100(*s*/Mean).

The difference between the mean results, (12.392 ± 0.026) % – (12.352 ± 0.053) %, is not statistically significant, as shown by a score of 1.64 on the two-proportion Z-test.

3.5. Moisture by Forced-Air Drying

Moisture in SRM 3223 was determined using duplicate samples of approximately 1 g each from bottles 2, 193, 290, 408, 575, 616, 701, and 834. The samples were dried for 3 h in a forced-air oven preheated to 100 °C. The mass fraction moisture in each sample was calculated using Eq. 1. The results are listed in Table 3.

Table 3. Mass Fraction Moisture.

	Moisture, %		
Bottle	Dup_1	Dup_2	Mean
2	11.3	11.1	11.2
193	10.6	10.6	10.6
290	10.6	10.6	10.6
408	9.4	9.6	9.5
575	11.2	11.2	11.2
616	11.2	11.1	11.1
701	10.9	10.9	10.9
834	10.7	10.7	10.7
		n:	8
		Mean:	10.73
		s:	0.55
		%RSD:	5.2 %

One-way analysis of variance (ANOVA) of the duplicate samples indicates that there are statistically significant bottle-to-bottle differences in the mass fraction moisture. There is no apparent trend in the differences related to the production order of the bottles.

4. CDC Analyses

In accordance with a protocol designed by NIST staff, CDC staff analyzed duplicate samples from 12 bottles of SRM 3223. The bottles were selected following a stratified random design that included the first and last produced. Measurements were made over seven separate days, one measurement run per day.

Measurements of As, Be, Cd, Co, Cr, Mn, Ni, Pb, and U were made using a "triple quad" inductively coupled plasma mass spectrometer (QQQ-ICP-MS). Duplicate samples of (0.15 to 0.19) g were taken from each of the twelve bottles. Samples were digested at 200 °C in a microwave system using 9 mL concentrated ultrapure nitric acid (HNO_3), 0.5 mL hydrogen peroxide (H_2O_2), and 0.5 mL hydrofluoric acid (HF). Digests were diluted in 100 mL ultrapure water in Class A polyethylene volumetric flasks. The solutions were teed into the ICP-MS at a 1:1 ratio with the internal standard. Be, Pb, U were analyzed in single quadrupole mode with no gas. Cr and Co were measured as $[^{52}Cr(NH_3)_2]^+$ and $[^{59}Co(NH_3)_2]^+$ in triple quadrupole mode using an ammonia (NH_3)/helium (He) cell gas. Ni and As were measured as $[^{60}NiO]^+$ and $[^{75}AsO]^+$ in triple quadrupole mode using oxygen cell gas. Cadmium ($^{111}Cd^+$) was measured on mass in tandem (MS/MS) mode using oxygen cell gas to eliminate the $[^{95}Mo]^+$ interference. $^{55}Mn^+$ was measured on mass in kinetic energy discrimination (MS/MS KED) mode using helium cell gas.

Measurements of Hg were made using a direct mercury analyzer (DMA). Duplicate samples of (0.040 to 0.060) g were taken from each of the twelve bottles.

Commercial calibration standards traceable to NIST 3100 series standard solutions [9] were used to calibrate the QQQ-ICP-MS and DMA instruments.

4.1. Day 1 Measurements

The "Day 1" measurement campaign was designed to assess the SRM 3223 homogeneity with respect to 10 elemental analytes: As, Be, Cd, Co, Cr, Hg, Mn, Ni, Pb, and U. All measurements were accomplished in single runs under repeatability conditions. The moisture content of the material in each bottle was determined using a gravity convection oven with the temperature and duration conditions described in Section 3.4.

Table 4 lists the measurement results, corrected for moisture. The Cr and Ni results for the bottle 404 Dup_B solution were identified as statistically anomalous, thought to be due to a contaminated microwave cell used in sample digestion. All other results met the CDC's quality control criteria.

The maximum percent relative standard deviation, %RSD, for the ten elements is 8.1 % which meets the predetermined customers' need of less-than 10 % [10]. The between-bottle and within-bottle variance for each analyte was assessed using single-factor analysis of variance: the *p-values* for all analytes are greater than 0.05, suggesting no statistically significant between-bottle heterogeneity for the population.

Table 4. CDC Homogeneity Assessment Results for Ten Elements.

		Arsenic, µg/g		Beryllium, ng/g		Cadmium, µg/g		Chromium, µg/g		Cobalt, µg/g	
Bottle	Moisture,%	Dup_A	Dup_B	Dup_A	Dup_B	Dup_A	Dup_B	Dup_A	Dup_B	Dup_A	Dup_B
1	10.30	0.2363	0.2347	39.1	34.4	1.010	0.963	1.34	1.16	0.708	0.574
89	10.19	0.2418	0.2254	38.6	36.3	1.042	1.010	1.35	1.40	0.686	0.674
147	10.35	0.2405	0.2416	37.6	39.7	1.034	1.057	1.34	1.56	0.664	0.663
261	9.52	0.2271	0.2251	40.2	35.4	0.997	1.024	1.40	1.45	0.627	0.668
312	10.15	0.2398	0.2175	41.4	46.4	1.036	1.105	1.48	1.48	0.645	0.661
404	9.85	0.2316	0.2441	42.0	41.7	1.080	1.092	1.50	*2.44*[a]	0.661	0.670
489	10.31	0.2389	0.2418	37.6	40.9	1.057	1.028	1.53	1.37	0.616	0.627
513	10.81	0.2368	0.2416	43.4	40.9	1.003	1.076	1.41	1.54	0.607	0.679
594	11.06	0.2487	0.2383	37.0	39.5	1.074	1.072	1.41	1.66	0.643	0.656
615	11.21	0.2274	0.2419	42.1	40.1	1.017	1.049	1.60	1.60	0.716	0.691
787	10.39	0.2317	0.2323	39.8	37.4	1.010	1.012	1.57	1.52	0.688	0.681
835	10.45	0.2317	0.2356	40.5	41.3	1.033	1.035	1.60	1.56	0.659	0.688
n	12	24		24		24		23		24	
Mean	10.38	0.2355		39.7		1.038		1.47		0.661	
s	0.47	0.0073		2.7		34		0.12		0.033	
RSD%	4.6 %	3.1 %		6.7 %		3.3 %		7.9 %		5.0 %	
	p[b]	0.51		0.12		0.080		0.10		0.56	

	Lead, µg/g		Manganese, µg/g		Nickel, µg/g		Uranium, ng/g		Mercury, ng/g	
Bottle	Dup_A	Dup_B	Dup_A	Dup_B	Dup_A	Dup_B	Dup_A	Dup_B	Dup_A	Dup_B
1	0.837	0.794	141.7	139.6	1.66	1.62	69.8	68.2	30.32	30.21
89	0.790	0.767	146.3	143.7	1.69	1.63	70.8	69.5	30.17	30.73
147	0.750	0.838	141.9	147.0	1.65	1.63	65.5	67.8	30.23	30.56
261	0.809	0.778	141.7	143.7	1.57	1.69	69.9	65.8	30.06	29.95
312	0.794	0.885	145.8	148.4	1.63	1.64	67.3	74.2	30.27	30.38
404	0.786	0.803	148.5	146.1	1.67	*2.08*[a]	68.3	65.1	30.39	30.39
489	0.763	0.786	143.7	144.2	1.67	1.59	69.1	67.0	30.44	30.44
513	0.766	0.814	144.9	147.1	1.68	1.69	68.2	67.4	30.61	30.72
594	0.839	0.801	146.6	147.4	1.70	1.68	67.5	70.3	30.69	30.47
615	0.914	0.795	148.4	147.1	1.62	1.64	69.2	67.5	30.63	30.52
787	0.782	0.778	145.6	143.6	1.61	1.56	68.7	66.0	30.47	30.24
835	0.785	0.772	146.3	142.7	1.60	1.52	66.4	66.8	30.49	30.6
n	24		24		23		24		24	
Mean	0.801		145.1		1.63		68.2		30.42	
s	0.038		2.4		0.05		2.0		0.21	
RSD%	4.8 %		1.7 %		2.9 %		3.0 %		0.67 %	
p[b]	0.61		0.051		0.24		0.64		0.070	

a Identified as anomalous and excluded from statistical summaries.
b *p*-value from single-factor analysis of variance.

The results are displayed as functions of the bottle production sequence in Fig. 6. There are no readily apparent production-related trends, other than a potential linear increase in the Cr content, which as shown in Fig. 7 is likely an artifact of the run as it is not observed in the results obtained from Day 2 though Day 7.

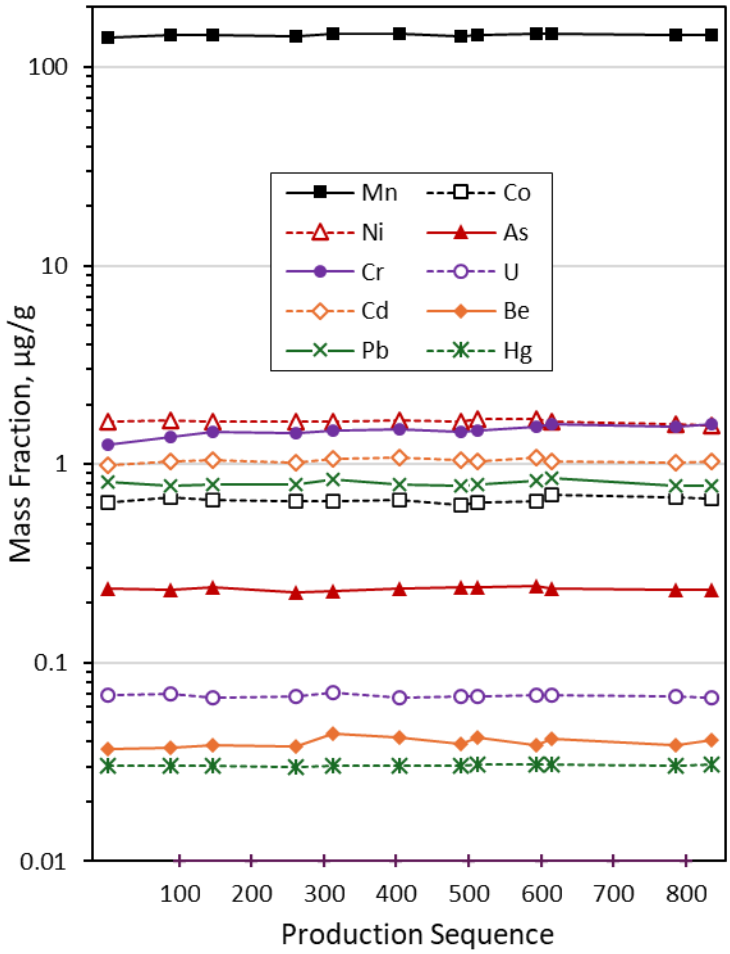

Fig. 6. Homogeneity Mass Fraction Results as Functions of Bottle Production Sequence.

4.2. Day 2 Through Day 7 Measurements

Table 5 lists the moisture-corrected measurement results from Day 2 through Day 7 measurement runs. All ten analytes were evaluated in four of the 24 duplicate solutions in each of the six runs. The randomized analysis run order minimized the impact of instrument drift. The results for Cr and Ni in Dup_B of bottle 404 are again statistically anomalous, confirming that the content of the solution is anomalous rather than the instrumental analysis.

While capturing only uncertainty components related to relatively short-term between-run instrumental calibration and sample replication, Table 5 also lists the standard and approximate 95 % level of confidence expanded uncertainties of the measurement means. These values are estimated as

$$\boldsymbol{u = s/\sqrt{n}\,;\; U = ku} \tag{2}$$

where *s* is the standard deviation, *n* is the number of independent measurements, and *k* is the critical 95 % Student's *t* value for *n*-1 degrees of freedom. These expanded uncertainties are therefore lower-bounds, preventing the mean results from being metrological traceable to the International System of Units (SI).

Table 5. CDC Day 2 through Day 7 Value Assessment Results for Ten Elements.

Bottle	Dup	Day	As µg/g	Be ng/g	Cd µg/g	Cr µg/g	Co µg/g	Pb µg/g	Mn µg/g	Ni µg/g	U ng/g	Hg ng/g
1	A	2	0.244	39.7	1.007	1.328	0.609	0.801	137.1	1.623	69.3	28.76
1	B	6	0.242	44.9	1.019	1.769	0.601	0.809	140.8	1.631	68.5	29.77
89	A	6	0.274	51.2	0.967	1.816	0.616	0.787	143.1	1.680	73.9	29.95
89	B	7	0.227	47.2	1.043	1.667	0.609	0.761	135.6	1.642	74.6	29.84
147	A	4	0.243	46.0	1.039	1.516	0.632	0.843	147.7	1.670	70.4	29.67
147	B	7	0.218	39.4	1.058	1.518	0.600	0.774	131.2	1.535	67.3	29.89
261	A	2	0.231	45.5	1.029	1.355	0.621	0.813	137.7	1.628	69.3	28.41
261	B	2	0.246	41.0	1.012	1.331	0.619	0.761	138.7	1.716	68.5	28.63
312	A	4	0.255	42.0	0.982	1.446	0.630	0.785	143.1	1.774	68.6	29.83
312	B	5	0.241	42.6	0.997	1.401	0.658	0.818	142.8	1.630	75.3	29.60
404	A	3	0.230	58.3	0.992	1.684	0.595	0.895	142.0	1.616	85.6	28.51
404	B	7	0.228	41.2	1.101	*2.550*[a]	0.627	0.784	136.4	*2.042*[a]	72.3	29.51
489	A	3	0.234	39.6	1.052	1.288	0.584	0.800	139.8	1.558	74.1	28.88
489	B	6	0.258	46.5	1.043	1.737	0.631	0.775	143.4	1.669	73.1	29.66
513	A	2	0.250	44.3	1.073	1.367	0.629	0.759	140.6	1.761	69.0	28.81
513	B	4	0.231	45.9	1.003	1.416	0.639	0.806	145.2	1.692	70.1	29.49
594	A	5	0.230	45.5	1.094	1.466	0.639	0.839	144.0	1.679	71.1	29.91
594	B	6	0.244	46.9	1.053	1.787	0.632	0.819	143.0	1.728	70.9	30.25
615	A	3	0.238	43.2	1.094	1.319	0.605	0.927	142.5	1.616	75.7	29.28
615	B	7	0.247	36.6	1.053	1.513	0.637	0.769	138.0	1.602	70.4	30.07
787	A	5	0.228	45.2	0.972	1.380	0.626	0.769	144.0	1.634	69.4	29.57
787	B	5	0.209	44.3	1.033	1.431	0.650	0.773	140.2	1.600	70.3	29.68
835	A	3	0.246	44.0	1.012	1.360	0.587	0.817	139.4	1.655	71.8	28.81
835	B	4	0.241	44.1	1.065	1.465	0.600	0.768	142.9	1.723	69.1	29.70
		n	24	24	24	23	24	24	24	23	24	24
		Mean	0.2389	44.38	1.0330	1.4939	0.6198	0.8022	140.80	1.6549	71.61	29.437
		s	0.0136	4.32	0.0379	0.1662	0.0194	0.0417	3.61	0.0597	3.80	0.536
		RSD%	5.7	9.7	3.7	11.1	3.1	5.2	2.6	3.6	5.3	1.8
		u	0.0028	0.88	0.0077	0.0347	0.0040	0.0085	0.74	0.0124	0.78	0.109
		$t_{0.05,n\text{-}1}$	2.069	2.069	2.069	2.074	2.069	2.069	2.069	2.074	2.069	2.069
		U	0.0058	1.82	0.0160	0.0719	0.0082	0.0176	1.52	0.0258	1.61	0.226

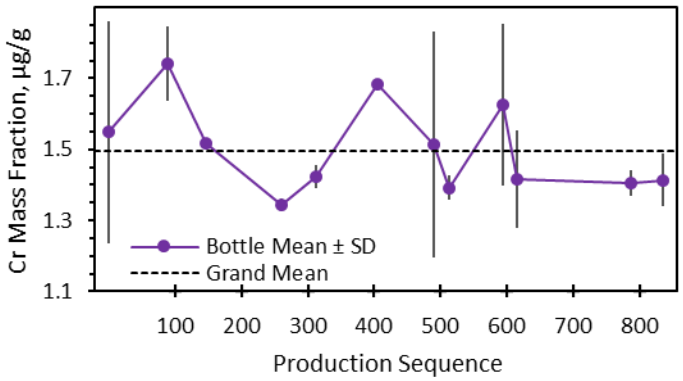

Fig. 7. Day 2 through Day 7 Chromium Mass Fraction Results as a Function of Bottle Production Sequence.

4.3. CDC Quality Assurance

The CDC used two materials for quality control (QC): North Carolina State University Smokeless Tobacco Reference Product 1S3 Moist Snuff [11] and Institute of Nuclear Chemistry and Technology INCT-PVTL-6 Polish Virginia Tobacco Leaves [12]. One sample of each of the two QC materials was measured once at the beginning and the end of every measurement run. The acceptability of the QC results was computed by a SAS program within CDC's Laboratory Information Management System (LIMS) using a modified Westgard Rule [13].

5. NIST Analysis of Cadmium, Nickel, and Lead

Cadmium (Cd), nickel (Ni), and lead (Pb) were determined at NIST using Isotope Dilution Inductively Coupled Plasma Mass Spectrometry (ID-ICP-MS). ID-ICP-MS is recognized as a higher-order reference measurement procedure suitable for the certification of NIST measurement results [14,15]. Measurements were calibrated using SRM 3100 single element standard solutions.

5.1. Materials

Eight bottles of SRM 3223 were selected by stratified random sampling across the production sequence. One bottle of SRM 1573a Tomato Leaves [16], two bottles of SRM 3246 *Ginkgo biloba* (Leaves) [17] were used as control materials. SRM 3328 Lead (Pb) Isotopic Standard Solution [18], SRM 3108 Cadmium (Cd) Standard Solution [19], SRM 3128 Lead (Pb) Standard Solution [20], SRM 3134 Molybdenum (Mo) Standard Solution [21], SRM 3136 Nickel (Ni) Standard Solution [22], SRM 3162a Titanium (Ti) Standard Solution [23], and SRM 3169 Zirconium (Zr) Standard Solution [24] were used to prepare calibration or interference correction solutions. Enriched isotopic compounds of ^{111}Cd, ^{62}Ni, and ^{206}Pb were obtained from Oak Ridge National Laboratory. Optima grade HF and HNO_3 were used in sample preparation.

5.2. Sample Preparation

Two sets of samples were prepared to quantify Cd, Ni, and Pb in SRM 3223. Each sample bottle was mixed by a gentle rotation for ten turns in a horizontal position before the bottle was opened and a subsample was taken.

The first set of samples was prepared to estimate the mass fraction of Cd, Ni, and Pb in SRM 3223 and to determine the molar mass of lead in samples and standards. One 1 g portion each from two bottles of SRM 3223 and two bottles of SRM 3246, and two 1 g portions from one bottle of SRM 1573a were weighed into microwave vessels into which 1 mL of HF and 9 mL of HNO_3 were added. Four procedural blanks were prepared similarly. The microwave vessels were loosely capped, and the contents were left in a fume hood overnight. The next day, the cap on each vessel was sealed with a torque wrench and the samples were digested in the microwave oven. The oven was ramped from ambient to 190 °C in 15 min at 100 % of 800 W power, held at 190 °C for 20 min, then ramped to 200 °C at 85 % of 1600 W power and held for 15 min. The digests were transferred to PTFE beakers and the contents evaporated to near dryness on hotplates at about 190 °C surface temperature. The residues were reconstituted in 1.5 % volume fraction HNO_3 and transferred to a 60 mL low-density polyethylene (LDPE) bottle where the content was diluted to about 50 g with 1.5 % volume fraction HNO_3.

The second set of samples was spiked with enriched isotopes for the quantification of Cd, Ni, and Pb. One 1 g sample from each of the first seven bottles of SRM 3223, two 1 g portions from the 8th bottle of SRM 3223, two 0.8 g portions each from two bottles of SRM 3246, and four 0.7 g portions from one bottle of SRM 1573a were transferred into microwave vessels. One 1 g aliquot of a spike solution containing approximately 0.6 µg ^{111}Cd, 0.8 µg ^{206}Pb, and 0.8 µg ^{62}Ni

was added into each vessel. The target ratios for $^{113}Cd/^{111}Cd$, $^{60}Ni/^{62}Ni$, and $^{208}Pb/^{206}Pb$ in samples are 0.16, 0.40, and 0.41 for error multiplication factors of 1.2, 1.1, and 1.3, respectively. Six procedural blanks were prepared similarly except that the amount of enriched isotope added to each vessel was a tenth of that added to the samples. After adding 1 mL HF and 9 mL HNO_3 into each vessel, the vessels were loosely capped for digestion at room temperature overnight. The samples were microwave digested the next day in the same way as was applied to the first set of samples, evaporated on hotplates, and diluted to 50 g in 60 mL LDPE bottles. A one-to-one dilution with 1.5 % HNO_3 was made for each sample before measurement.

A 1 g portion from each bottle of SRMs 3223 and SRM 3246 and two 1 g portions from the bottle of SRM 1573a were weighed into glass weighing vessels for assessment of moisture. The SRM 3223 samples were dried in the forced-air oven at 100 °C for 3 h (see Section 3.2). Samples of SRMs 1573a and SRM 3246 were dried at room temperature over fresh anhydrous magnesium perchlorate in desiccators for (5 and 12) d, respectively, in accordance with the instructions in their certificates [16,17].

The mass fraction of ^{111}Cd, ^{62}Ni, and ^{206}Pb in the spike solution was calibrated against the 3100 series of single-element standard solution SRMs using reverse-isotope dilution mass spectrometry (IDMS). Four spike calibration samples were prepared to have isotope ratios similar to the analytical samples and were diluted to produce the same ICP-MS count rate as the analytical samples. Two sets of Cd, Ni, and Pb single element working standard solutions were made by independent serial dilutions from the 3100 series of SRM calibration solutions to prepare the four spike calibration samples. The mass concentration of each working standard solution contained 5.0 µg/g of the analyte. Two reverse-IDMS calibration solutions were prepared from each set of single-element working standard solutions, resulting in four reverse-IDMS calibration solutions.

Each reverse-IDMS calibration solution was prepared by transferring 4.0 g of the IDMS spike solution, 0.83 g of Cd working standard solution, 0.97 g of Ni working standard solution, and 0.64 g of Pb working standard solution into a 125 mL LDPE bottle and diluting the contents to 100 g with 1.5 % HNO_3. A mass bias correction standard solution was prepared in an identical manner, but without the IDMS spike.

Single-element solutions containing 50 ng/g Mo and 17 ng/g Zr were used for isobaric interference corrections in Cd measurement. A single-element solution containing 160 ng/g Ti was used for isobaric interference correction in Ni measurement.

5.3. Instrumental Method

All samples were measured on an ICP-MS system using peak jump data acquisition with one point per peak. Three separate measurements were performed for Pb isotopic abundance measurement on the first set of samples, Cd and Pb isotope dilution measurements on the second set of samples, and Ni isotope dilution measurements on the second set of samples. Pb isotopic abundance measurements and Cd and Pb isotope dilution measurements were made

in single quadrupole mode without cell gas. Ni isotope dilution measurements were made in MS/MS mode using hydrogen (H_2)/helium (He) collision/reaction gas.

5.4. Quantitation

The mass fraction of analyte in the spike solution, c_{spk}, is calculated from the reverse-IDMS measurement of the spike calibration (SC) samples

$$\boldsymbol{c_{spk} = \frac{A - R_{SC}B}{R_{SC}b - a} \times \frac{m_{cal}M_{spk}}{m_{spk}}} \quad (3)$$

where A and a are the abundances of the reference isotope (^{113}Cd, ^{60}Ni, or ^{208}Pb) for the natural and the spike solutions, respectively; B and b are the abundances of spike isotopes (^{111}Cd, ^{62}Ni, or ^{206}Pb) for the natural and the spike solutions, respectively; R_{SC} the measured reference-to-spike isotope ratio for the SC sample; m_{cal} amount analyte in mole from SRM 3100 series calibration standard; M_{spk} the molar mass of the spike; m_{spk} the mass of the spike solution added to the SC sample.

The mass of analyte in a sample or a blank, m, is calculated from the isotope dilution equation

$$\boldsymbol{m = \frac{R_S b - a}{A - R_S B} \times \frac{c_{spk}w_{spk}M_s}{M_{spk}}} \quad (4)$$

where R_S is the measured reference-to-spike isotope ratio for the sample, w_{spk} is the mass of the spike solution added to the sample, and M_s is the molar mass of the analyte in the sample.

The mass fraction of analyte in the sample, w_{sam}, is calculated after the blank is subtracted

$$\boldsymbol{w_{sam} = \frac{m_s - m_b}{m_{sam}(1 - w_{\%moist}/100)}} \quad (5)$$

where m_s and m_b are the mass of analyte found in the sample and the blank, respectively, m_{sam} is the mass of the sample, and $w_{\%moist}$ is the fraction moisture in the sample expressed as a percentage (Eq. 1).

The reported values were calculated using the atomic weights and isotopic compositions compiled in [25].

5.5. Isobaric Interference Corrections

Table 6 lists Pb isotopic abundance and molar mass results for the two SRM 3223 and SRM 3246 samples, the single samples of SRM 3128 and the ORNL enriched ^{206}Pb spike solution, and the certificate values for SRM 3328. The "±" values represent 95 % level of confidence expanded uncertainties.

Table 6. Lead Isotopic Abundance and Molar Mass.

Analyte	Unit	SRM 3223 Result	SRM 3246 Result	SRM 3128 Result	^{206}Pb Spike Result	^{206}Pb Spike Certificate	SRM 3328 Certificate
^{208}Pb	mol/mol	0.5203 ± 0.0002	0.5231 ± 0.0000	0.5221	0.0006	0.0006	0.52347 ± 0.00010
^{207}Pb	mol/mol	0.2108 ± 0.0004	0.2148 ± 0.0001	0.2138	0.0020	0.0020	0.22083 ± 0.00007
^{206}Pb	mol/mol	0.2553 ± 0.0000	0.2484 ± 0.0003	0.2504	0.9971	0.9969	0.24144 ± 0.00006
^{204}Pb	mol/mol	0.0135 ± 0.0002	0.0137 ± 0.0002	0.0138	0.0003	0.0004	0.01426 ± 0.00001
Molar Mass	g/mol	207.20 ± 0.00*	207.21 ± 0.00*	207.21	205.98	205.97	207.2152 ± 0.0002

*an uncertainty is shown as 0.00 as a result of rounding a value <0.005.

The correction of ^{204}Hg isobaric interference at 204 u was negligible at < 0.001 % for the SRM 3128, ORNL ^{206}Pb spike, and SRM 3328 calibration materials. The correction was about 2.0 % for the SRM 3246 samples, consistent with the SRM's low (23.08 ± 0.17) ng/g certified Hg content [17]. The correction for the 3223 samples was 3.8 %, also consistent with the low ≈30 ng/g Hg content (Section 6). Note: the non-italic "u" is the symbol for the unified atomic mass unit.

For the isotope dilution measurement of Pb and Cd in the second set of samples, there were no known isobaric interferences at Pb masses, but $^{111}Cd^+$ and $^{113}Cd^+$ were interfered with by isobars $^{95}Mo^{16}O^+$ and $^{94}Zr^{16}O^1H^+$ at 111 u and $^{97}Mo^{16}O^+$, $^{113}In^+$, and $^{96}Mo^{16}O^1H^+$ at 113 u. $^{112}Cd^+$ and $^{114}Cd^+$ were monitored to confirm the results from $^{113}Cd^+$, but they were not used for value assignment. The overall isobaric corrections to SRM 3223 samples' count rate at 111 u and 113 u were < 0.5 % and < 0.6 %, suggesting a negligible effect of the isobaric interferences and the corrections on the results. For SRM 1573a, the quality assurance sample, isobaric interference corrections at 111 u and 113 u were negligible at < 0.1 %.

For the isotope dilution measurement of Ni in the second set of samples, the intensity ratios of 62/60 u/u for samples of SRM 3223 and SRM 1573a were biased low and high, respectively, relative to that of a 10 ng/g Ni neat standard solution, suggesting the presence of isobaric interferences at both Ni masses. Analysis of the isobars at 60 u indicated that the presence of $^{44}Ca^{16}O^+$ can cause a low bias for the 62/60 u/u intensity ratio. Based on previous experience, cell gas flow rates of 1 mL/min H_2 and 4 mL/min He minimized the interference with only about 20 % loss of count rate at Ni masses. $^{46}Ti^{16}O^+$ interferes with $^{62}Ni^+$ measurements, causing a high bias to the 62/60 u/u intensity ratio that persisted irrespective of cell gas mixture and flow rates. This interference was corrected by using the 49/62 u/u intensity ratio of a 160 ng/g single-element Ti standard solution measured concurrently with the samples. The corrections for TiO^+ interference at 62 u were about 0.33 % for SRM 3223 and 0.08 % SRM 1573a samples, which were consistent with the Ti contents of 100 µg/g and 20 µg/g found in these two materials by semiquantitative measurement.

5.6. Quality Assurance

The measured values for Cd and Ni in SRM 1573a and Pb in SRM 3246 are compared to their certified values in Fig. 8. The overlapping of the measured and the certified error bars demonstrates that there is no statistically significant difference between the two values, and hence, no detectable bias in the measurement.

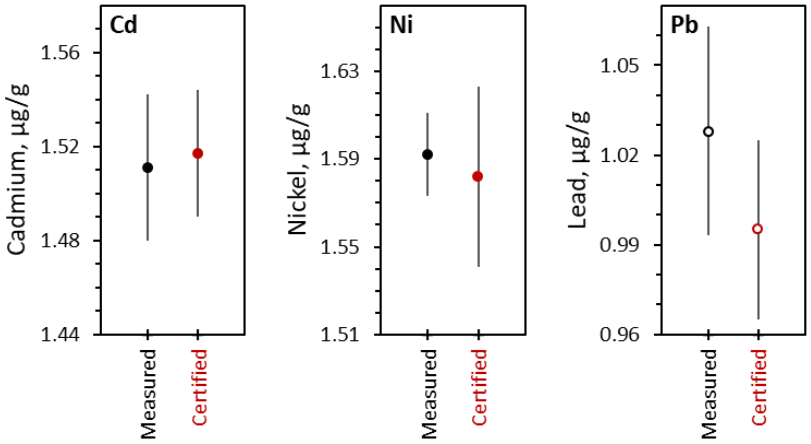

Fig. 8. Results for Cd and Ni in SRM 1573a and Pb in SRM 3246 Compared to Certified Values.

Each panel displays results for one element. The mean measurement result and the SRM certified values are plotted as functions of "Measured" and "Certified". Solid circles denote SRM 1573a values; open circles denote SRM 3246 values. Error bars represent 95 % level of confidence uncertainty intervals. The vertical axis of each panel is scaled to span ± 5 % around the midpoint of the displayed values.

5.7. Value Assessment

Table 7 lists the measurement replication and summary statistics of the moisture, Cd, Pb, and Ni content in SRM 3223.

Table 7. Mass Fraction of Moisture, Cadmium, Lead, and Nickel in SRM 3223.

Bottle	Moisture, %	Cd, µg/g	Ni, µg/g	Pb, µg/g
1	10.54	1.016	1.414	0.847
2	10.74	1.012	1.413	0.851
3	10.88	1.014	1.399	0.828
4	10.98	1.022	1.415	0.841
5	11.08	1.014	1.413	0.845
6	11.00	1.021	1.402	0.858
7	10.35	1.020	1.451	0.863
8a	11.00	1.006	1.407	0.865
8b		1.010	1.437	0.844
n	8	9	9	9
Mean	10.82	1.0150	1.417	0.849
s	0.26	0.0054	0.017	0.011
%RSD	2.4	0.53	1.2	1.4

Table 8 defines the components and factors used to estimate the 95 % level of confidence expanded uncertainty for Cd, Ni, and Pb in SRM 3223. Table 9 provides the quantitative values for uncertainty components and factors.

Table 8. Uncertainty Components for ID-ICP-MS Analysis.

Symbol	Source	Basis	*v*
u_{sam}	Measurement replication	Standard uncertainty of mean value based on replicate measurements, estimated as $s_{sam}/\sqrt{n_{sam}}$.	8
u_{blk}	Blank correction	Standard uncertainty of blank correction based on replicate measurements, estimated as $s_{blk}/\sqrt{n_{blk}}$.	5
$u_{\%moist}$	Moisture correction	Standard uncertainty of moisture content based on replicate measurements, estimated as $s_{\%moist}/\sqrt{n_{\%moist}}$.	7
u_{SC}	Spike Calibration	Standard uncertainty of spike calibration based on replicate measurements, estimated as $s_{SC}/\sqrt{n_{SC}}$.	3
B_{Cal}	Calibrant	Standard uncertainty of the calibrant, one-half of the SRM 3100 series 95 % expanded uncertainty.	60
B_{Disc}	Mass Discrimination	Relative standard certainty of instrument mass discrimination, temporal drift, and isobaric interference correction. Estimated as 0.5 %.	60
B_{Td}	Instrument Deadtime Correction	Relative standard uncertainty of instrument detector dead-time measurement. Estimated as 0.2 %.	60
B_{Bkg}	Instrument Background Correction	Relative standard uncertainty of instrument background intensity correction. Estimated as 0.004 % for Cd and Pb, and 0.003 % for Ni.	60
B_w	Weighing Uncertainty	Standard uncertainty of weighing due to the calibration of the balance. Estimated 0.1 mg for each weighing, normalized by dividing by $\sqrt{3}$.	60

Symbol	Definition	Description
v	Degrees of freedom	Experimentally determined components are assigned one less than the number of independent measurements. Literature or experience-based components have “large” large value. Note: the Student’s 95 % coverage critical value for 60 degrees of freedom is equal to 2.
u	Standard uncertainty	Square root of sum of squares of standard uncertainties
v_{eff}	Effective degrees of freedom	Welch-Satterthwaite estimate [26, Section B.3]
k	Expansion factor	Student’s *t* critical value for 95 % coverage with v_{eff} degrees of freedom
U	95 % level of confidence expanded uncertainty	$U = ku$

Table 9. Uncertainty Assessment of Cadmium, Lead, and Nickel in SRM 3223.

Factor	Cd, µg/g	Ni, µg/g	Pb, µg/g
u_{sam}	0.0018	0.0056	0.0038
u_b	0.000017	0.00012	0.0000061
u_{SC}	0.0026	0.0011	0.00090
$u_{\%moist}$	0.0010	0.0015	0.00087
B_{Cal}	0.0014	0.0018	0.00059
B_{Disc}	0.0029	0.0041	0.0025
B_{Td}	0.0012	0.0016	0.00098
B_{Bkg}	0.000026	0.000025	0.000020
B_w	0.00032	0.00045	0.00026
u	0.0048	0.0076	0.0049
v_{eff}	32	26	20
k	2.04	2.06	2.09
U	0.010	0.016	0.010

5.8. Metrological Traceability

NIST's ID-ICP-MS results for Cd, Ni, and Pb are metrologically traceable to the International System of Units (SI) derived unit for mass fraction, expressed as micrograms per gram (µg/g), based on calibrations using SRM 3108 [19], SRM 3128 [20], SRM 3136 [22], and SRM 3328 [18] and the quantitative characterization of all known sources of variability and bias.

6. NIST Analysis of Mercury

The mercury (Hg) content in SRM 3223 was determined at NIST using isotope dilution cold-vapor inductively coupled plasma mass spectrometry (ID-CV-ICP-MS). ID-CV-ICP-MS is recognized as a higher-order reference measurement procedure suitable for the certification of NIST Hg measurement results [15]. Direct combustion atomic absorption spectrometry (DC-AAS) was used to confirm that the ID-CV-ICP-MS results were not significantly biased. Both measurement methods were calibrated using SRM 3133 Mercury (Hg) Standard Solution [27].

6.1. Direct Combustion Atomic Absorption Spectrometry (DC-AAS)

The mass fraction of total mercury in the samples was determined with a direct Hg analyzer, a direct combustion atomic absorption spectrophotometer based on mercury vaporization, amalgamation, desorption, and analysis using an absorbance spectrophotometer and external calibration.

Samples are first dried at 200 °C and then heated in stages to 650 °C, causing organic materials to be decomposed and mercury to be vaporized in a carrier gas of oxygen which is introduced into a quartz catalyst tube. A continuous flow of oxygen carries the decomposition products through a hot catalyst bed where halogens, nitrogen, and sulfur oxides are trapped. All mercury species are reduced to mercury oxide and are then carried along with reaction gases to a gold amalgamator where the mercury is deposited on gold-covered molecular sieves. All non-mercury vapor and decomposition products are carried out of the system with the continuous gas stream. The mercury deposits are then desorbed as the amalgamator is heated. Vaporized mercury is transported to the spectrophotometer for analysis.

The spectrophotometer uses a mercury vapor lamp as its light source. Light from the lamp is directed through an excitation filter before it irradiates the vaporized mercury contained in a cuvette block with a dual-cell arrangement. The detector utilizes two sequential cells positioned along the optical path of the spectrophotometer: one for low concentration samples (cell 1) and the other for high concentration samples (cell 2). Light which is not absorbed by the mercury vapors then passes through an emission filter before being measured by the detector. Absorbance is measured at 253.7 nm as a function of mercury content.

6.1.1. Materials

Eight bottles of SRM 3223 were selected by stratified random sampling across the production sequence. One ampoule of SRM 3133 [27] was used to make calibration solutions. Two bottles of SRM 1573a [16] were used as a control material. Nickel weigh boats of nominal mass 1 g were used as procedural blanks.

6.1.2. Analysis

The external calibration curve was prepared by gravimetrically aliquoting between (0.0493 and 0.8197) g of aqueous dilutions of SRM 3133 into quartz sample boats.

Mercury was measured in SRM 3223 and SRM 1573a samples by weighing approximately 120 mg of material into pre-cleaned nickel weigh boats and placing them into the instrument auto-sampler rotor. A single replicate was measured from each bottle of SRM 3223. Control material samples and procedural blanks were bracketed between blocks of candidate SRM 3223 samples to verify instrument calibration and monitor instrumental drift.

Moisture was determined for single 1 g samples from each of the eight SRM 3223 bottles with a gravity convection oven using the oven drying method described in Section 3.4. Moisture was determined for duplicated 1 g samples from each of the two bottles of SRM 1573a in accordance with the desiccate drying instructions in its certificate [16]. Hg mass fractions for SRMs 3223 and 1573a were corrected to dry-mass fractions on a sample-by-sample basis.

The {mercury mass, peak area} calibration points relevant for evaluating the SRM 3223 and 1573a materials are well estimated using a second-order (quadratic) polynomial model

$$A_{\mathrm{Hg},i} = a \times m_{\mathrm{Hg},i}^2 + b \times m_{\mathrm{Hg},i} + c \qquad (6)$$

where $m_{\mathrm{Hg},i}$ and $A_{\mathrm{Hg},i}$ are the mass of Hg and the resulting peak area for each of the series of n_{cal} calibrants. The model's *a*, *b*, and *c* coefficients are parameterized by regression over all the relevant ($m_{\mathrm{Hg},i}$, $A_{\mathrm{Hg},i}$) pairs. The calibration function is depicted in Fig. 9.

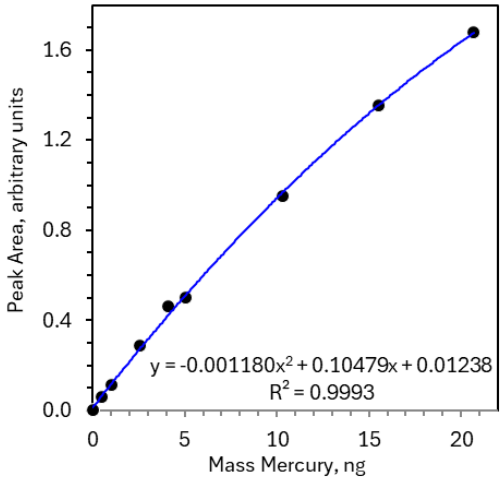

Fig. 9. DC-AAS External Calibration Function.

The curve represents the quadratic function. The symbols represent the ($m_{\mathrm{Hg},i}$, $A_{\mathrm{Hg},i}$) pairs used to parameterize the model coefficients.

The mass fraction Hg in each procedural blanks is estimated

$$w_{\text{blank},i} = \frac{-b \pm \sqrt{b^2 + 4\,a(c - A_{\text{Hg,i}})}}{2\,a\,m_{\text{blank}}} \quad (7)$$

where $A_{\text{Hg},i}$ is the measured peak area and m_{blank} is the 1 g nominal mass of a nickel weigh boat. The calculated $w_{\text{blank},I}$ were all negative and the samples were not blank corrected.

The mass fraction Hg in each SRM 3223 and 1573a sample is estimated

$$w_{\text{Hg},i} = \frac{-b \pm \sqrt{b^2 + 4\,a\,(c - A_{\text{Hg},i})}}{2\,a\,m_{\text{sam},i}(1 - w_{\%\text{moist},i}/100)} \quad (8)$$

where $A_{\text{Hg},i}$ is the measured peak area for a sample aliquot of mass $m_{\text{sam},i}$ and $w_{\%\text{moist},i}$ is the mass fraction moisture in the sample material expressed as a percentage (Eq. 1).

6.1.3. Value Assessment

Table 10 lists the measurement results and summary statistics of the moisture and Hg content (as estimated by DC-AAS) in SRM 3223.

Table 10. Mass Fraction of Moisture and DC-AAS Mercury Results for SRM 3223.

Bottle	Moisture, %	Hg, ng/g
1	11.2	33.7
2	11.5	29.9
3	11.4	33.6
4	11.5	32.6
5	11.1	30.8
6	11.5	30.3
7	11.8	29.0
8	11.2	29.9
n_{sam}	8	8
Mean	11.39	31.2
s_{sam}	0.22	1.8
%RSD	2.0	5.8

Table 11 provides the uncertainty factors used to estimate the 95 % level of confidence expanded uncertainty for the DC-AAS estimation of Hg in SRM 3223.

Table 11. Uncertainty Budget for DC-AAS Analysis of SRM 3223.

Symbol	Source	Basis	Value ng/g	*v*
u_{sam}	Measurement replication	Standard uncertainty of mean value based on replicate measurements, estimated as $s_{sam}/\sqrt{n_{sam}}$.	0.6	7
$u_{\%moist}$	Moisture correction	Standard uncertainty of moisture content based on replicate measurements, estimated as $s_{\%moist}/\sqrt{n_{\%moist}}$.	0.0008	7
B_{wgh}	Weighing Uncertainty	Standard relative uncertainty of balance calibration, drift (temporal and electrostatic) and relative impact on weighing measurements, estimated as 0.1 %	0.03	60
B_{SRM}	Calibrant	The 95 % level of confidence expanded uncertainty for SRM 3133 is 0.040 ng/g. This propagates through the dilutions as a relative standard uncertainty of ≈0.2 %.	0.06	12
B_{cal}	Calibration model	Root mean square deviation of the quadratic calibration model using n_{cal} calibrants	1.6	6
u	Standard uncertainty	Square root of sum of squares of standard uncertainties	1.7	8
U	95 % level of confidence expanded uncertainty	$U = ku$, where k = 2.4 is the Student's t critical value for 95 % coverage with 7 degrees of freedom	3.9	

6.2. Isotope Dilution Cold-Vapor Inductively Coupled Plasma Mass Spectrometry (ID-CV-ICP-MS)

The approximate mass fraction of total Hg in SRM 3223 was established by DC-AAS (Section 6.1). This information was used to optimize experimental parameters for the higher-order ID-CV-ICP-MS reference measurement procedure [28].

6.2.1. Materials

Eight bottles of SRM 3223 were selected by stratified random sampling across the production sequence. One bottle of SRM 3133 [27] was used to make calibration solutions. Two bottles of SRM 1573a [16] were used as a control material. Hg-201 spike (Batch 180691) was purchased from the National Isotope Development Center (NIDC) (Oak Ridge, TN). High-purity HNO_3 and high-purity hydrochloric acid (HCl) were used in sample preparation.

6.2.2. Sample Preparation

Single ≈0.5 g samples from each bottle of SRM 3223 and duplicate ≈0.5 g samples from each bottle of SRM 1573a were accurately weighed by difference in quartz vessels using a calibrated four-place analytical balance and spiked with an accurately weighed aliquot of ^{201}Hg, followed by the addition of 6 g HNO_3. Microwave digestion was carried out in a microwave system that uses sealed digestion vessels. The digestion program used was: 600 watts of power, 30 min ramp; 1200 watts of power, 15 min ramp and 15 min hold; and 0 watts of power, 15 min cool down.

After cooling to room temperature, the contents were transferred to 50 mL polypropylene centrifuge tubes and initially diluted with high-purity deionized water (18.3 Ω) to approximately 25 mL until diluted further on the day of measurement. Analytical measurements were completed within one day of final dilution to reduce the risk of external contamination and Hg losses from the solutions during storage. On the day of measurement, samples were diluted to approximately 0.2 ng/g ^{201}Hg, which was suitable for measurement by cold-vapor ICP-MS. About 1.0 g (approximately 2 % in final dilution) of HCl was added to each sample for additional Hg stabilization.

6.2.3. Instrumental Method

The Hg vapor was generated using tin (II) chloride reductant (10 % mass fraction in 7 % volume fraction HCl) and separated from the liquid phase using a commercial glass reaction/separator cell. The vapor was transferred to the ICP-MS using an argon carrier gas flow rate of approximately 100 mL/min. This gas stream was mixed with the plasma injector gas stream using a plastic T piece. The ICP-MS was operated in dry plasma mode. All samples were transferred to the instrument in manual sequence, and the timing of the sample uptake was adjusted to allow sufficient time to measure the instrument background prior to measurement of the sample. The ^{201}Hg and ^{202}Hg isotopes were monitored for a duration of 60 s in a pulse counting Time-Resolved-Analysis mode to recover the individual ion count rates. The isotope-time profiles were downloaded as CSV files to a spreadsheet for calculation of background corrected ^{201}Hg/^{202}Hg ratios using NIST Isotope Dilution Assistant (IDA) [29]. The instrument detector dead-time was 33 ns.

6.2.4. Quantitation

The working ^{201}Hg isotopic spike solution was prepared by accurate gravimetric dilution of a master stock solution, which was calibrated by reverse isotope dilution using SRM 3133. Stock solutions were prepared by serial dilution. Four spike calibration mixtures (approximately 0.2 ng/g ^{201}Hg) were prepared from these stock solutions (approximately 50 ng/g ^{201}Hg), and these were measured using cold-vapor ICP-MS, under the same conditions as the samples (double ID-MS [14]).

Moisture was determined for single 1 g samples from each of the eight SRM 3223 bottles with a gravity convection oven using the oven drying method described in Section 3.4. Moisture was determined for duplicated 1 g samples from each of the two bottles of SRM 1573a in accordance with the desiccator drying instructions in its certificate [16].

The individual sample ID-CV-ICP-MS mass fraction results were estimated:

$$w_{\mathrm{Hg},i} = \frac{\frac{m_{\mathrm{spk},i} M_{\mathrm{ref}}}{M_{\mathrm{spk}}} \frac{(A_{\mathrm{refspk}} - A_{\mathrm{spkspk}} R_i F)}{(A_{\mathrm{spknat}} R_i F - A_{\mathrm{refnat}})} - \bar{m}_{\mathrm{blk}}}{m_{\mathrm{sam},i}\left(1 - w_{\%\mathrm{moist},i}/100\right)} \qquad (9)$$

where $m_{spk,i}$ is the mass of ^{201}Hg spike added, M_{ref} is the molar mass of the reference isotope (^{202}Hg), M_{spk} is the molar mass of the spike isotope (^{201}Hg), A_{refspk} is the fractional abundance of the reference isotope in the spike, A_{spkspk} is the fractional abundance of the spike isotope in the spike, A_{refnat} is the natural fractional abundance of the reference isotope, A_{spknat} is the natural fractional abundance of the spike isotope, R_i is the detector dead-time corrected ^{202}Hg/^{201}Hg ratio, F is the discrimination correction factor, $\overline{m}_{\mathrm{blk}}$ is the absolute mean measured blank, $m_{sam,i}$ is the mass of sample, and $w_{\%moist,i}$ is the fraction moisture in the sample expressed as a percentage (Eq. 1).

6.2.5. Value Assessment

Table 12 lists the measurement replication and summary statistics of the moisture and Hg content in SRM 3223.

Table 12. Mass Fraction of Moisture and ID-CV-ICP-MS Mercury Results for SRM 3223.

Bottle	Moisture, %	Hg, ng/g
1	11.82	30.1
2	10.89	30.4
3	11.76	30.2
4	11.74	30.1
5	11.28	30.2
6	11.71	30.0
7	11.68	30.0
8	11.61	30.1
n_{sam}	8	8
Mean	11.56	30.16
s_{sam}	0.32	0.12
%RSD	2.8	0.4

The summary results for the CDC, DC-AAS, and ID-CV-ICP-MS are displayed in Fig. 10. The overlap of the CDC (Table 5) and DC-AAS (Table 10) results with the ID-CV-ICP-MS result confirm that the ID-CV-ICP-MS result provides a suitable basis for certifying the Hg content of SRM 3223.

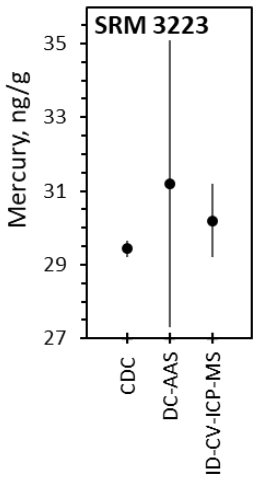

Fig. 10. Comparison of Results for Hg in SRM 3223.
Error bars represent 95 % level of confidence uncertainty intervals.

Table 13 defines the uncertainty components used to estimate the 95 % level of confidence expanded uncertainty for Hg in SRM 3223

Table 13. Uncertainty Components for ID-CV-ICP-MS Analysis of Mercury.

Symbol	Source	Basis	Value ng/g	*v*
u_{sam}	Measurement replication	Standard uncertainty of mean value based on replicate measurements, estimated as $s_{sam}/\sqrt{n_{sam}}$.	0.04	7
u_{blk}	Blank correction	Standard uncertainty of the method blanks as a percentage of the absolute mass of mercury in the sample trap.	0.007	3
$u_{\%moist}$	Moisture correction	Standard uncertainty of moisture content based on replicate measurements, estimated as $s_{\%moist}/\sqrt{n_{\%moist}}$.	0.001	7
u_{cal}	calibration of isotopic spike	Standard uncertainty of the reverse ID-MS spike calibration measurements using four independently prepared calibrants.	0.0003	3
$u_{MrefMspk}$	Natural / spike atomic weight ratio	Standard uncertainty of the IUPAC atomic weight for Hg divided by the atomic weight of the spike determined by isotopic measurements. Estimated as 0.0005 % relative.	0.0002	60
$u_{Arefspk}$	Abundance of ^{202}Hg in the spike	Standard uncertainty of the spike isotopic composition and its impact on the measurement of Hg by double ID-MS. Estimated as 1.1 % relative.	0.3	60
$u_{Aspkspk}$	Abundance of ^{201}Hg in the spike	Standard uncertainty of the spike isotopic composition and its impact on the measurement by double ID-MS. Estimated as 0.62 % relative.	0.2	60
$u_{Arefnat}$	Abundance of ^{201}Hg in the sample	Standard uncertainty of the IUPAC isotopic composition and its impact on the measurement of Hg by double ID-MS. Estimated as 0.02 % relative.	0.006	60
$u_{Aspknat}$	Abundance of ^{202}Hg in the sample	Standard uncertainty of the IUPAC isotopic composition and its impact on the measurement of Hg by double ID-MS. Estimated as 0.02 % relative.	0.006	60
u_R	^{201}Hg/^{202}Hg ratio measurement	Standard uncertainty of the dead-time corrected ICP-MS isotope ratio measurements based on pooled standard deviation of approximately 500 ratio measurement points and subtraction of the instrument blank counts on each isotope. Estimated as 0.75 %.	0.2	60
u_F	Mass discrimination correction	Standard uncertainty of the correction factor for the instrument mass bias/mass discrimination and its impact on the measured mass fraction of Hg using a double ID-MS approach. Estimated as 0.75 % relative.	0.2	60
u_w	Mass measurement of sample aliquot	Standard uncertainty due to weighing on a four-place balance, calculated using observed standard deviation and assuming a rectangular distribution. Estimated as 0.1 % relative.	0.06	60
u_{STD}	Mass of ^{201}Hg added	Standard uncertainty of SRM 3133 and its impact on the calibration. Estimated as 0.20 % relative.	0.1	11
u	Standard uncertainty	Square root of sum of squares of standard uncertainties.	0.50	60
U	95 % level of confidence expanded uncertainty	$U = ku$, where $k = 2.0$ is the Student's t critical value for 95 % coverage with 60 degrees of freedom	1.0	

6.3. Quality Assurance

The values for Hg in SRM 1573a from each measurement are compared to the certified values in Fig. 11. The certified Hg value was established using non-primary methods (cold-vapor atomic absorption and radiochemical neutron activation analysis) in 1993. Reanalysis in 2013 using ID-CV-ICP-MS, a primary method, suggests that the certified value may have a slightly high bias. The overlapping of the CDC, DC-AAS, and ID-CV-ICP-MS measurements with the 2013 analysis suggests that there is no statistically significant difference among the results and thus no detectable bias.

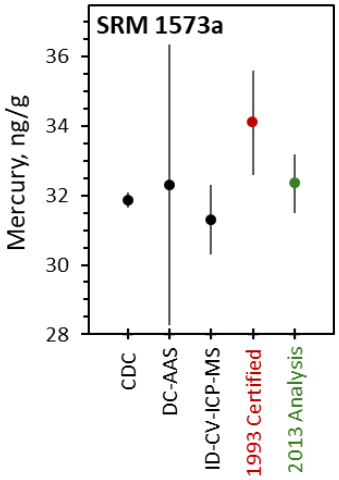

Fig. 11. Comparison of Results for Hg in SRM 1573a.

Error bars represent 95 % level of confidence uncertainty intervals.

7. NIST Analysis of Beryllium and Selenium

Beryllium (Be) and selenium (Se) were determined at NIST using ICP-MS and Inductively Coupled Plasma Tandem Mass Spectrometry (ICP-MS/MS). Measurements were calibrated using SRM 3100 single element standard solutions.

7.1. Materials

Eight bottles of SRM 3223 were selected by stratified random sampling across the production sequence. One bottle each of the single element standard solutions SRM 3105a Beryllium (Be) Standard Solution [30], SRM 3144 Rhodium (Rh) Standard Solution [31], SRM 3148a Scandium (Sc) Standard Solution [32], and SRM 3149 Selenium (Se) Standard Solution [33] were used to make the standard addition spike and internal standard solutions. One bottle of SRM 1570a Trace Elements in Spinach Leaves [34] and SRM 1643f Trace Elements in Water [35] were used as controls. Optima grade HF, Optima grade HNO_3, and in-house sub-boiling distilled water were used for all sample preparation.

7.2. Sample Preparation

7.2.1. Sampling

The SRM 3223 and SRM 1570a were thoroughly mixed by rotating bottles at 45° several times and allowed to settle for at least 5 minutes. Control 1643f was inverted several times and also allowed to settle.

For determination of moisture, one sample from each of the SRM 3223 bottles was dried following the procedure for forced-air oven drying described in Section 3.4. Triplicate samples from SRM 1570a were dried following the procedure on the Certificate of Analysis (COA) [34].

For determination of Be and Se, ≈1.0 g was sampled in duplicate from each bottle of SRM 3223 for a total of 16 samples, ≈1.0 g was sampled four times from the one bottle of SRM 1570a, and ≈2.0 g was sampled four times from the one bottle of SRM 1643f. Four blanks were prepared the same as samples. SRMs 3223 and 1570a were sampled using a reagent grade ethanol-cleaned spatula. SRM 1643f was sampled using a clean syringe. The sample masses were determined by difference using a calibrated 5-place analytical balance; all weights were to the nearest 0.01 mg.

7.2.2. Digestion

After quantitative transfer of sample into the PTFE microwave vessels, ≈8 mL concentrated HNO_3 and ≈2 mL of concentrated HF were added to each vessel. Blanks were treated the same as samples. The following microwave method was used: ramp from ambient temperature to 195 °C at 85 % of 800 W power over 15 min and hold for 20 min; ramp to 200 °C at 100 % of 1600 W power over 20 min and hold for 15 min; allow vessels to cool for at least 20 minutes. The samples were transferred into precleaned, prelabeled PTFE beakers. Each digestion vessel

was rinsed 3 to 5 times with water to ensure all the digested sample was quantitatively transferred from the vessels to the beaker.

Approximately 2 mL of concentrated HNO_3 and 5 mL of water were added to each sample. The beakers were covered with watch glasses and the contents were refluxed at 180 °C. Additional 2 mL HNO_3 aliquots were added when the samples reached near dryness, and the reflux continued until all particulates were dissolved. The beakers were removed from the hotplates and allowed to cool overnight. In the morning, the samples were checked again with a flashlight and a laser to see if any particulate remained. Once the samples were clear, they were dried down, and then the contents were dissolved in dilute HNO_3 and quantitatively transferred to 60 mL LDPE bottles to result in approximately 30 g of solution per sample. This entire process was repeated for the remaining samples.

7.2.3. Spiking and Dilution

Before completing the preparation of the samples for standard addition calibration methods, a semi-quantitative measurement was made on one sample of SRM 3223, SRM 1570a, SRM 1643f, and blanks to determine the approximate mass of Be and Se required for the calibration spikes. About 0.5 mL of each of the samples were volumetrically diluted to 5 mL with 1.5 % volume fraction HNO_3 and analyzed as described in Section 7.3. Based on these results, a spike solution containing ≈177 ng/g of Be and ≈428 ng/g of Se was prepared for the SRM 3223 and SRM 1570a samples; and a spike solution containing ≈108 ng/g of Be and ≈93 ng/g of Se was prepared for the SRM 1643f and blank samples.

The remaining samples were divided evenly into spiked and unspiked solutions. Approximately 0.5 g of the appropriate spike solution was added to a preweighed, prelabeled LDPE bottle then brought to ≈15 g by adding the corresponding unspiked solution.

7.3. Semiquantitative Analysis

The semiquantitative samples at approximately 1:10 dilution factor were analyzed using the “SemiQuant” method of an ICP-MS system. The measurement provides the concentration and associated intensity information needed to deliver the ideal number of counts to the detector and minimize the measurement uncertainty.

7.4. Quantitative Analysis

ICP-MS was used to determine Be by monitoring ^{9}Be with ^{103}Rh as the internal standard. The H_2 collision gas was delivered at 4 mL/min to reduce the number of polyatomic interferences by kinetically breaking apart the polyatomic molecules.

ICP-MS/MS was used to determine Se because an oxide shift was required due to strong interferences formed by the argon plasma gas at 78 *m*/*z* from $^{40}Ar^{38}Ca^{+}$ and $^{40}Ar^{38}Ar^{+}$ and at 80 *m*/*z* from $^{40}Ar^{2+}$. An oxide shift overcomes these interferences by using oxygen gas to shift (+16

m/*z*) the ^{78}Se and ^{80}Se peaks to ^{78}Se^{16}O and ^{80}Se^{16}O. There are no interferences at 94 *m*/*z* and 96 *m*/*z*.

Single-point standard addition with use of an internal standard was used to quantify Be and Se. The mass fraction of the analyte in the i^{th} sample, $w_{sam,i}$, is estimated as

$$\boldsymbol{w_{sam}} = \left(\frac{\boldsymbol{R_u}}{\boldsymbol{R_{sp} - R_u}}\right)\left(\frac{\boldsymbol{m_{sp} w_{sp}}}{\boldsymbol{m_{spsolu}}}\right)\left(\frac{\boldsymbol{m_{solu}}}{\boldsymbol{m_{sam}}}\right)\left(\frac{\boldsymbol{1}}{\boldsymbol{1 - w_{\%moist}}}\right) - \overline{\boldsymbol{w_{blk}}} \quad (10)$$

where R_{sp} and R_u are the analyte-to-internal standard ratios in the spiked and the unspiked solutions, m_{sp} is the mass of spike solution, w_{sp} is the mass fraction of the analyte in the spike solution, m_{spsolu} is the mass of the solution that is spiked, m_{solu} is the final mass after the addition of the internal standard(s), $m_{sam,i}$ is the mass of sample present in the solution analyzed, $w_{\%moist}$ is the mass fraction moisture in the sample, and $\overline{w_{blk}}$ is the average mass fraction of procedural blanks.

7.5. Quality Assurance

The measured values for Be in SRM 1643f and Se in SRM 1570a are compared to their certified values in Fig. 12. The overlapping of the measured and the certified error bars demonstrates that there is no statistically significant difference between the two values, and hence, no detectable bias in the measurement.

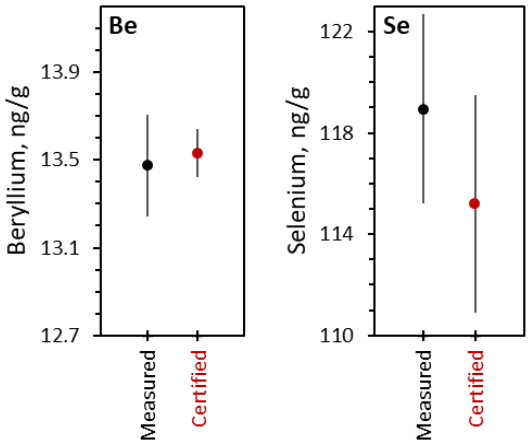

Fig. 12. Results for Be in SRM 1643f and Se in SRM 1570a Compared to Certified Values.

Each panel displays results for one element. The mean measurement result and the SRM certified values are plotted as functions of "Measured" and "Certified". Error bars represent 95 % level of confidence uncertainty intervals. The vertical axis of each panel is scaled to span ± 5 % around the midpoint of the displayed values.

7.6. Value Assessment

Table 14 lists the mass fraction of moisture, Be, and Se in SRM 3223 samples. A one-way ANOVA was applied to the two replicates from each bottle. The *p*-values of 0.41 and 0.46 for Be and Se, respectively, suggest no detectable between-bottle heterogeneity for the two elements.

Table 14. Mass Fraction of Moisture, Beryllium, and Selenium in SRM 3223.

Bottle	Rep	Moisture, %	Be, ng/g	Se, ng/g
1	A	9.96	38.2	113.7
	B		34.1	113.5
2	A	9.71	34.5	116.3
	B		26.3	113.2
3	A	9.90	33.3	115.1
	B		32.3	115.2
4	A	10.00	36.4	116.4
	B		33.7	105.3
5	A	9.81	31.6	124.1
	B		34.9	115.2
6	A	9.99	29.2	115.0
	B		35.1	118.0
7	A	10.03	32.2	116.0
	B		26.2	114.0
8	A	9.47	35.6	109.7
	B		36.3	113.0
	n	8	16	16
	Mean	9.859	33.12	113.59
	s	0.191	3.43	4.05
	%RSD	1.9	10.4	3.6

Table 15 defines the components and factors used to estimate the 95 % level of confidence expanded uncertainty for Be and Se in SRM 3223. Table 16 provides quantitative values for the uncertainty components and factors.

Table 15. Uncertainty Components for ICP-MS Analysis.

Symbol	Source	Basis	*v*
u_{sam}	Measurement replication	Standard uncertainty of mean value based on replicate measurements, estimated as $s_{sam}/\sqrt{n_{sam}}$.	15
u_{blk}	Blank correction	Standard uncertainty of blank correction based on replicate measurements, estimated as $s_{blk}/\sqrt{n_{blk}}$.	3
$u_{\%moist}$	Moisture correction	Standard uncertainty of moisture content based on replicate measurements, estimated as $s_{\%moist}/\sqrt{n_{\%moist}}$.	7
u_{STD}	Calibrant	Standard uncertainty of the calibrant, one-half of the SRM 3100 series 95 % expanded uncertainty.	60
u_{wgtSTD}	Mass Discrimination	Standard uncertainty of weighing calibration standard due to the readability of the balance. Estimated 0.01 mg for each weighing, normalized by dividing by $\sqrt{3}$.	60
u_{wgtsam}	Instrument Deadtime Correction	Standard uncertainty of weighing samples due to the readability of the balance. Estimated 0.01 mg for each weighing, normalized by dividing by $\sqrt{3}$.	60

Symbol	Definition	Description
v	Degrees of freedom	Experimentally determined components are assigned one less than the number of independent measurements. Literature or experience-based components have “large” large value. Note: the Student’s 95 % coverage critical value for 60 degrees of freedom is equal to 2.
u	Standard uncertainty	Square root of sum of squares of standard uncertainties
v_{eff}	Effective degrees of freedom	Welch-Satterthwaite estimate [26, Section B.3]
k	Expansion factor	Student’s *t* critical value for 95 % coverage with v_{eff} degrees of freedom
U	95 % level of confidence expanded uncertainty	$U = ku$

Table 16. Uncertainty Assessment of Beryllium and Selenium in SRM 3223.

Factor	Be, ng/g	Se, ng/g
u_{sam}	0.86	1.0
u_{blk}	0.0089	0.39
$u_{\%moist}$	0.022	0.074
u_{STD}	<0.0001	<0.001
u_{wghSTD}	0.0004	0.001
u_{wghsam}	0.0002	0.001
u	0.86	1.1
v_{eff}	15	18
k	2.13	2.10
U	1.8	2.3

As shown in Fig. 13, NIST’s (33.1 ± 1.8) ng/g SRM 3223 Be result is less than the CDC estimate of (44.4 ± 1.8) ng/g [Section 4.2, Table 5] and the curves do not overlap, indicating that one or both of the ICP-MS methods were significantly biased. The mean of the two results and its 95 %

level of confidence expanded uncertainty, (38.7 ± 11.4) ng/g, provides an estimate that is more robust because it incorporates differences between and within methods. The uncertainty of this combined mean was estimated using a bootstrap procedure based on a Gaussian random effects model for the between-method effects [26,36-38].

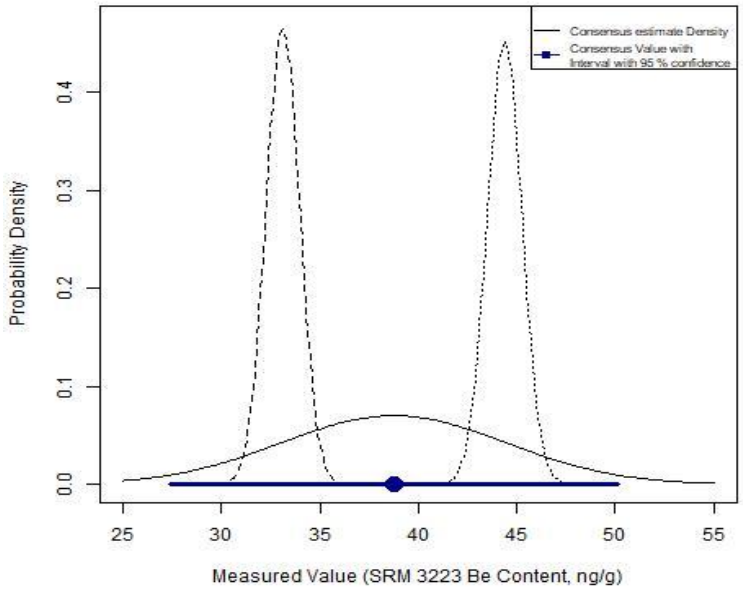

Fig. 13. Consensus Estimate of SRM 3223 Beryllium Content.

The tall curves represent estimated probability densities of the NIST (left) and CDC (right) method estimates. The wider curve represents the estimated density of the combined estimate. The thick horizontal line shows a 95 % coverage interval, with the combined value at its center.

8. Certificate of Analysis

The official results for this SRM are presented in the COA for SRM 3223 Inorganic Constituents in Cigarette Tobacco Filler. A NIST COA is defined: [39]

“In accordance with ISO 33401:2024, a NIST SRM certificate is a document containing the name, description, and intended purpose of the material, the NIST logo, the name of NIST as a certifying body, instructions for proper use and storage of the material, certified property value(s) with associated uncertainty(ies), method(s) used to obtain property values, the period of validity, if appropriate, and any other technical information deemed necessary for its proper use. A Certificate is issued for an SRM certified for one or more specific physical or engineering performance properties and may contain non-certified in addition to certified values. A Certificate of Analysis is issued for an SRM certified for one or more specific chemical properties.”

For the most current version of the COA for NIST SRM 3223, please visit: https://www-s.nist.gov/srmors/view_detail.cfm?srm=3223.

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Appendix A. List of Acronyms

ANOVA	analysis of variance
CDC	Centers for Disease Control and Prevention
COA	Certificate of Analysis
DC-AAS	direct combustion atomic absorption spectrometry
DMA	direct mercury analyzer
FDA	Food and Drug Administration
HDPE	high-density polyethylene
HERO	Health and Environmental Research Online
HPHC	Harmful and Potentially Harmful Constituent
ICP-MS	inductively coupled plasma mass spectrometry
ID-CV-ICP-MS	Isotope dilution cold vapor inductively coupled plasma mass spectrometry
ID-ICP-MS	isotope dilution inductively coupled plasma mass spectrometry
IDMS	isotope dilution mass spectrometry
LN_2	liquid nitrogen
LDPE	low-density polyethylene
LIMS	Laboratory Information Management System
NIST	National Institute for Standards and Technology
PSD	particle size distribution
PTFE	polytetrafluoroethylene
QQQ-ICP-MS	“triple quad” inductively coupled plasma mass spectrometer
QC	quality control
RSD	relative standard deviation
SC	spike calibration
SRM	Standard Reference Material
SI	Système International d'Unités (International System of Units)
TGA	thermal gravimetric analyzer