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Characterization of Perchlorate in a New Frozen Human Urine Standard Reference Material

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Abstract

Perchlorate, an inorganic anion, has recently been recognized as an environmental contaminant by the U.S. Environmental Protection Agency (EPA). Urine is the preferred matrix for assessment of human exposure to perchlorate. Although the measurement technique for perchlorate in urine was developed in 2005, the calibration and quality assurance aspects of the metrology infrastructure for perchlorate are still lacking in that there is no certified reference material (CRM) traceable to the International System of Units (SI). To meet the quality assurance needs in biomonitoring measurements of perchlorate and the related anions that affect thyroid health, the National Institute of Standards and Technology (NIST) in collaboration with the Centers for Disease Control and Prevention (CDC) developed Standard Reference Material (SRM) 3668 Mercury, Perchlorate, and Iodide in Frozen Human Urine. SRM 3668 consists of perchlorate, nitrate, thiocyanate, iodine, and mercury in urine at two levels that represent the 50th and 95th percentiles, respectively, of the concentrations (with some adjustments) in the U.S. population. It is the first CRM being certified for perchlorate. Measurements leading to the certification of perchlorate were made collaboratively at NIST and CDC using three methods based on liquid or ion chromatography tandem mass spectrometry (LC-MS/MS or IC-MS/MS). Potential sources of bias were analyzed and results were compared for the three methods. Perchlorate in SRM 3668 Level I urine was certified to be 2.70 μg L^{-1} ± 0.21 μg L^{-1}, and for SRM 3668 Level II urine, the certified value is 13.47 μg L^{-1} ± 0.96 μg L^{-1}.

Keywords

perchlorate; urine; standard reference material; certified reference material

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Introduction

Perchlorate is an inorganic anion that can occur naturally at low levels in the environment (e.g., precipitation contains ~ 0.1 μg/L of perchlorate) [1]. Perchlorate is also a mass produced commodity used in oxidant formulations for a variety of products such as road flares, explosives, pyrotechnics, and solid rocket propellant [1]. Human activities in manufacture, handling, and use of perchlorate and perchlorate-containing natural products, e.g. Chilean nitrate fertilizer [2], have resulted in widespread environmental contamination [3]. As of November 2005, perchlorate was detected in 4.1 % of drinking water samples taken from community water supplies in 26 different states, with levels ranging from the method detection limit of 4 $\mu g\ L^{-1}$ to a maximum at 420 $\mu g\ L^{-1}$ [3]. The prevalence of perchlorate in the environment and its potential adverse effects on the public health led to the decision by the U.S. Environmental Protection Agency (EPA) to regulate perchlorate under the Safe Drinking Water Act [4].

Exposure to perchlorate in the environment is of potential health concern because perchlorate is known to modify thyroid function by competitively inhibiting iodide uptake at the sodium iodide symporter (NIS) [5]. Sustained inhibition of iodide uptake is a cause of hypothyroidism [6], which can lead to many adverse health effects in adults and children. Hypothyroidism can cause metabolic problems in adults and abnormal development during gestation and infancy [7]. Severe hypothyroidism resulting from iodine deficiency during pregnancy is a preventable cause of cretinism, a permanent cognitive impairment of the developing fetus [8]. Mild hypothyroidism during pregnancy has been associated with subtle cognitive deficits in children [9, 10].

The National Health and Nutrition Examination Survey (NHANES) is a program designed to assess the health and nutritional status of adults and children in the United States. An important component of NHANES is biomonitoring of many environmental chemicals that affect human health, such as perchlorate. Perchlorate has been monitored since 2001 in NHANES that is conducted continuously by the Centers for Disease Control and Prevention (CDC). The results are published in the biennial National Report on Human Exposure to Environmental Chemicals [11]. Data from NHANES surveys provided important information on the scope of perchlorate exposure in the U.S. population, which formed the scientific foundation for state and federal agencies debating appropriate regulatory limits for perchlorate in drinking water.

Urine is the preferred biomonitoring matrix for assessing human exposure to perchlorate because of the non-invasive nature of urine collection and because 70 – 95% of perchlorate intake is excreted unchanged in the urine with a half-life of about 8 h [12]. To ensure the accuracy of NHANES measurements so that the perchlorate data are comparable contemporarily and longitudinally, the National Institute of Standards and Technology (NIST) and the CDC collaborated to develop a new Standard Reference Material (SRM) for quality assurance of perchlorate measurements. The result is SRM 3668 Mercury, Perchlorate, and Iodide in Frozen Human Urine. Intended as a tool to validate methods and measurement processes in assessment of environmental factors affecting the normal function of thyroid, SRM 3668 was characterized for a panel of anions at two concentration levels including nitrate, thiocyanate, and iodine in addition to perchlorate. Similar to perchlorate, nitrate and

thiocyanate found in food and the environment can competitively inhibit iodide uptake [13]. Mercury, a toxic element unrelated to the thyroid health, was also certified in the SRM [14].

SRM 3668 has recently become available from NIST. It is the first reference material certified for perchlorate. We describe the preparation and characterization that led to the certification of perchlorate in the SRM.

Materials and methods*

SRM preparation

The urine used for the preparation of SRM 3668 was collected anonymously at CDC from healthy male and female volunteers in the spring of 2007. The protocol for collection of samples was reviewed and approved by the Institutional Review Board of the CDC. Each urine specimen was collected in a sterile, 4.5 oz polypropylene specimen container with a screw cap (American Precision Plastics, Northglenn, CO). Prior to use, the urine cups were screened for and found to be free of trace elements of interest. For preservation of the specimens, donors were instructed to place specimens in a Styrofoam box of 35 L capacity with 38 mm walls, containing three refrigerant gel packs (15 cm × 7.5 cm × 2.5 cm) chilled to −20 °C. Styrofoam boxes were collected at the end of every day and the specimens were transferred to a refrigerator for storage at 4 °C. Approximately 500 cups of urine specimen were collected. Each urine specimen was screened for trace elements and creatinine to determine the suitability of the specimen before being combined with other specimens in one of the two 20 L polyethylene carboys for either low or high elemental contents. Neither the individual urine samples nor the urine pools were screened for microbiological pathogens. All carboys and other containers for the urine pools were pre-cleaned with 5 % by volume of nitric acid in water and then rinsed with deionized water. While stirring, the urine pools were acidified slowly to contain 0.02 mol/L sulfamic acid prepared from ACS grade reagent (GFS Chemicals, Powell, OH). Acidification of the urine pools resulted in less than 1 % dilution of the urine. Urine from each pool was transferred to 700 mL polycarbonate bottles and centrifuged at 3700 g_n for approximately 60 min in a Beckman Coulter (Fullerton, CA) model J6-HC refrigerated centrifuge chilled to 10 °C. Precipitates in the urine were removed by decanting, and the concentrations of trace elements and anions in the two pools were adjusted to the target levels (see below) with additions of appropriate amounts of NIST SRM 3100 series single-element standard solutions or commercial standards similar to the SRMs. On the day of production of the SRM units, the urine pools were stirred constantly while aliquots of 1.8 mL from each urine pool were dispensed into 2 mL cryovials under class 100 clean room conditions. The vials were capped, heat-sealed in aluminized polyester bags, stored at −70 °C at CDC and at NIST. Transferal to NIST was via shipment with dry ice.

The concentrations of anions and Hg in Level I and Level II of the SRM were designed to represent 50th and 95th percentiles, respectively, of the concentrations (with some adjustments) in the U.S. population based primarily on data from the National Report on Human Exposure

*Disclaimer: Certain commercial items are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology or the Centers for Disease Control and Prevention, nor does it imply that the equipment identified is necessarily the best for the purpose.

to Environmental Chemicals (2005-2006 National Health and Nutrition Examination Survey) [11].

Homogeneity assessment

Measurements to assess the homogeneity of perchlorate were made at CDC using an ion chromatography tandem mass spectrometry (IC-MS/MS) method described previously [15]. Duplicate samples were measured from fourteen vials randomly selected from the production sequence.

Stability assessment

Stability assessment of perchlorate in SRM 3668 was conducted at CDC at an interval of approximately six months for a period of more than two years. On the day of measurement, SRM 3668 samples were retrieved from the −70° C storage and thawed at the room temperature. Perchlorate in the samples was measured using an IC-MS/MS method described previously [15].

Certification measurements

Validation of perchlorate calibrant—Certification measurements of perchlorate in SRM 3668 were made collaboratively at NIST and CDC. Perchlorate calibration standards traceable to the International System of Units (SI) are not available from National Metrology Institutes (NMIs). Therefore, commercial perchlorate calibration standards were purchased from AccuStandard (New Haven, CT) and Inorganic Ventures (Christiansburg, VA). SI traceability of the certificate values for perchlorate was validated, and the validated standards were used for calibration at NIST and CDC. To validate the mass concentration of perchlorate in a solution, total chlorine and non-perchlorate sourced chlorine in the solution were measured.

Total chlorine in the nominal 1000 mg L^{-1} commercial solutions was determined by instrumental neutron activation analysis (INAA). A sample for INAA measurement was prepared by depositing 0.2 g of a commercial solution onto a piece of Whatman No. 41 filter paper. Once dried at the room temperature, the filter paper was pressed into a pellet in a 13 mm diameter die under 4500 kg load. Each pellet was sealed in a pre-cleaned polyethylene bag for irradiation. Six replicate samples were prepared for each commercial solution. SRM 3182 Chloride Anion Standard Solution and SRM 919a Sodium Chloride (Clinical Standard) were used for calibration. Calibration standards and three procedure blanks were prepared on filter papers similarly as the samples. The pellets were irradiated for 60 s in the pneumatic tube RT-2 of the NIST reactor at reactor power of 20 MW. Counting was performed after 10 min decay for 10 min at 20 cm. Quantitative determination of chlorine was achieved using 1642.7 KeV and 2167.4 KeV gamma lines from decay of ^{38}Cl ($t_{1/2}$=37.24 min ± 0.05 min) [16]. Gamma spectrum data were converted to mass fraction values for the identified elements using commercially available software routines that determine peak areas, calculate the activity present at the end of the irradiation based on irradiation and decay times, and calculate mass fraction of element present in samples based on comparison with standards.

The purity of the 1000 mg L^{-1} perchlorate commercial solutions was assessed on a Dionex DX-600 ion chromatography (IC) system equipped with a 50 μL injection loop, a Dionex AS

autosampler, a GP 50 gradient pump, ASRS 300 2-mm anion self-regenerating suppressor, and ED50 electrochemical detector operating at 55 mA suppressor current. Measurement samples were prepared to contain approximately 150 mg L^{-1} perchlorate in water. The injection volume was 15 μL. A Dionex 2 mm × 250 mm IonPac AS16 column equipped with a 2 mm × 50 mm AG16 guard column was used with 35 mmol L^{-1} NaOH eluent at a flow rate of 0.25 mL min^{-1} under isocratic conditions. The column temperature was kept at 15 °C.

Perchlorate measurements at NIST—Perchlorate in SRM 3668 was measured using isotope dilution method and the standard addition method. One sample from each of 8 vials per level of SRM 3668 was measured with each method. SRM 3668 samples were thawed in a class 100 HEPA filtered workstation. For the isotope dilution measurements, approximately 0.5 g portion of each sample was weighed into a 1.5 mL autosampler vial. A 0.5 g solution containing approximately the same amount of perchlorate in sample as $Cl^{18}O_4^-$ (Cambridge Isotope Laboratories, Andover, MA) was added to the vial to yield a theoretical normal-to-enriched perchlorate ratio of 1. The mass fraction of $Cl^{18}O_4^-$ was calibrated by the method of reverse isotope dilution against the validated perchlorate calibration standard described above. Perchlorate in the sample was calculated from the peak area ratio of the normal and the enriched isotopes. For standard addition measurements, duplicate aliquots of 0.5 g from each vial of SRM 3668 were transferred into two 1.5 mL autosampler vials. To each autosampler vial was added 0.5 g solution containing an appropriate amount of $Cl^{18}O_4^-$ as an internal standard. To one of the autosampler vials was added 0.5 g validated calibration standard that approximately tripled the perchlorate contents in the vial. To the other vial was added 0.5 g of deionized water. The normal-to-enriched perchlorate peak area ratio for the spiked and the unspiked samples was used to calculate the analyte in the sample with the method of standard addition. Six quality assurance urine samples produced by CDC were processed similarly.

The isotope dilution samples and the standard addition samples were measured using the liquid chromatography tandem mass spectrometry (LC-MS/MS) method described below. The separation of anions was performed on an Agilent (Santa Clara, CA) 1200 series liquid chromatography (LC) system consisted of an autosampler, binary gradient pump, and column compartment. Samples were injected with a 100 μL syringe and the injection volume was 50 μL. A 2 mm × 250 mm Dionex (Sunnyvale, CA) AS16 column equipped with a 2 mm × 50 mm IonPac AG16 guard column was used. An eluent consisting of 50 mmol L^{-1} ammonium acetate in 1:1 mixture by volume of acetonitrile and water was pumped at a rate of 0.5 mL min^{-1} under isocratic conditions. The Agilent LC system and a Knauer K-501 LC pump were coupled to an Applied Biosystems (Foster City, CA) API 4000 triple quadrupole mass spectrometer via an Agilent column switching valve. The switching valve alternated to allow either the eluent from the Agilent pump or a rinse solution containing 1:1 mixture by volume of acetonitrile and water from the Knauer pump to reach the mass spectrometer. The eluent was allowed to reach the mass spectrometer from 3.5 min to 6.5 min of the 10 min chromatographic measurement cycle, the window that contained the perchlorate peak, to minimize fouling of the mass spectrometer by the salt from the urine and the eluent. Results were calculated from $^{35}ClO_4^- \rightarrow {}^{35}ClO_3^-$ transitions.

Perchlorate measurements at CDC—Duplicate samples from each of 14 vials per level of SRM 3668 were measured. SRM 3668 samples were thawed to room temperature and mixed to suspend any particulate material. A 0.25 mL portion from each sample was transferred to an autosampler vial and diluted with 0.25 mL of deionized water. A measurement sample was prepared by adding to the vial a 0.50 mL aliquot of deionized water containing $Cl^{18}O_4^-$ internal standard. A set of nine calibration standards were prepared similarly by adding known amounts of analytes to a pooled urine containing labeled internal standards. Samples were measured using tandem mass spectrometry IC-MS/MS. Quantification was based on the ratio of analyte to stable isotope-labeled internal standard relative to those of the nine calibrators. The separation of anions was performed on a Dionex model ICS 3000 ion chromatography system equipped with a gradient pump, autosampler, thermal compartment, and an ASRS Ultra II 2-mm anion self-regenerating suppressor operated in the external water mode. A Dionex 2 mm × 250 mm IonPac AS16 column was used with a 25 μL injection loop and 50 mmol L^{-1} KOH eluent under isocratic conditions at a flow rate of 0.5 mL min^{-1}. An Applied Biosystems model API 4000 triple quadrupole mass spectrometer with electrospray interface was used for the detection of perchlorate [15].

The CDC procedure for perchlorate measurements was also used in the assessment of chlorate impurities in commercial perchlorate calibration standards. Chlorate standard from Inorganic Ventures (Christiansburg, VA) was used for calibration.

Results and Discussion

Validation of calibrant

The concentration of perchlorate in the commercial standard was validated with the determination of the chlorine from perchlorate in the standard. Because chlorine from perchlorate equals total chlorine minus chlorine from species other than perchlorate, the concentration of perchlorate in the commercial standard can be calculated from the total chlorine determined using INAA and chlorine from impurities determined using IC. The concentrations of chlorine in the commercial standards as determined by INAA are listed in Table 1.

IC chromatograms of the two commercial perchlorate standards show presences of trace amounts of impurities at the same retention time. Figure 1 shows a typical chromatogram of a 150 mg L^{-1} perchlorate sample prepared from the AccuStandard solution. A comparison between the 150 mg L^{-1} perchlorate and the blank indicated that the peak at 4.4 min is an impurity from the standard solution. Anion solutions of acetate (CH_3COO^-) , chloride (Cl^-), hypochlorite (ClO^-), chlorite (ClO_2^-), chlorate (ClO_3^-), bromide (Br^-), fluoride (F^-), nitrate (NO_3^-), phosphate (PO_4^-), sulfate (SO_4^{2-}), and thiocyanate (SCN^-) were analyzed in an attempt to identify the unknown peak by matching retention times. ClO_3^-, Br^-, and NO_3^- all eluted at 4.4 min. Slight separation of these three anions was achieved using a linear gradient program of increasing OH^- in the mobile phase from 1.5 mmol L^{-1} to 35 mmol L^{-1} over 20 min; however, the resolution was not sufficient for positive identification of the unknown peak. Based on the perchlorate validation scheme discussed before, only chlorine containing impurity will have an impact on the validation process. To determine whether the unknown peak was from ClO_3^- impurity, solutions containing 1 mg L^{-1} ClO_4^- standards were measured using

the CDC perchlorate measurement procedure. A 1 mg L^{-1} solution of ClO_3^- was used for calibration and peak identification. Figure 2 overlays the chromatograms of 1 mg L^{-1} ClO_4^- and 1 mg L^{-1} ClO_3^- solutions. The ClO_3^- in 1 mg L^{-1} ClO_4^- standards was below the detection limit of 0.01 μg L^{-1}, indicating that ClO_3^- as an impurity in the 1000 mg L^{-1} ClO_4^- standards to be < 0.01 mg L^{-1}. Table 1 lists the ClO_3^- impurity in terms of chlorine to be < 0.004 mg L^{-1} in the 1000 mg L^{-1} ClO_4^- standards.

The mass concentration and the associated uncertainty of chlorine as perchlorate were converted into the mass concentration and the associate uncertainty of perchlorate, and these values were compared to the certificate values of perchlorate provided by the producers. The perchlorate concentration from validation measurements agrees with the certificate values, as indicated by the overlap of the uncertainty intervals of the measured values and the certificate values. However, the mean of the validation measurement is greater than the certificate mean. The certificate mean was calculated from the gravimetric preparation of a solution from 99.99 + % pure potassium perchlorate; therefore, the results of the validation measurements may be positively biased albeit well within the measurement uncertainty. Consequently, the certificate mean was taken as the validated mean, and the expanded uncertainty for the validated mean was calculated as the sum of the certificate uncertainty and the validation measurement uncertainty in quadrature, as shown in the Table 1.

The uncertainty of the validated mean was dominated by the uncertainty of INAA measurements. The uncertainty in INAA measurements resulted primarily from the chlorine in the blank filter papers used to load the aqueous samples. To correct for this source of chlorine, each paper was weighed before the solution was loaded. Chlorine from the paper was corrected based on the mass of the paper using the mass fraction of chlorine calculated from the unloaded filter papers that served as procedure blanks. Because each filter paper contained Cl that amounted to ~ 25 % Cl of the sample, the variability of chlorine contents from filter to filter was a major source of uncertainty in INAA measurements.

The ~ 2 % relative expanded uncertainty of the resulting validated perchlorate standard is large compared to the typical ~ 0.3 % expanded uncertainty for the SRM 3100 series single element calibration standards produced by NIST. Despite the greater uncertainty, the validated standards fit the purpose for the certification measurements of perchlorate in SRM 3668 discussed below.

Homogeneity

The IC-MS/MS results for duplicate samples from 14 vials of SRM 3668 were analyzed using single factor analysis of variance (ANOVA). Table 2 lists the results of ANOVA and the variance or mean square (MSq) values. The fact that between-vial MSq is greater than within-vial MSq for perchlorate in both levels of the SRM suggests that perchlorate in the SRM is inhomogeneous. However, the *p*-values of 0.29 for Level I and 0.09 for Level II samples indicate that the inhomogeneity is statistically insignificant at the 95 % confidence level. Nevertheless, the statistical model used for data analysis treated SRM 3668 samples as inhomogeneous because particulates were observed in both levels of the SRM. The between-vial variance was incorporated into the expanded uncertainties of the certified values using

statistical Monte Carlo methods consistent with the methods suggested by Supplement 1 to the "Guide to the Expression of Uncertainty in Measurements" [17].

Stability

Four stability measurements were made for each level of perchlorate in SRM 3668. The mass concentration of perchlorate in Level I and Level II of SRM 3668 was plotted as a function of time in Figures 3 and 4, respectively. The certified intervals (discussed below) are shown as two horizontal lines in each plot. The fact that the value of each stability measurement overlaps the corresponding certified values indicates that perchlorate in SRM 3668 is stable over the period of ~ 1100 d.

Certification methods

Three sets of data were obtained for the determination of perchlorate in SRM 3668 with one from CDC and two from NIST as shown in Table 3. CDC used the locally developed IC-MS/MS method published in 2005 [15]. Pooled urine was used for the preparation of matrix matched calibration standards, and quantification based on the ratio to $Cl^{18}O_4^-$ internal standard further minimized the potential for matrix effects [15]. The method achieved specificity and sensitivity needed for determination of trace levels of perchlorate in clinical samples. The calibration transfer based on matrix matched calibration curve allowed high throughput required of a method for biomonitoring measurements such as those needed for NHANES. The instrumental method used at NIST was a variation of that developed at CDC [15]. The same model column was used at CDC and NIST for the separation of perchlorate. However, NIST used ammonium acetate as the mobile phase additive for compatibility with the Agilent LC system in the laboratory. The methods of standard addition and isotope dilution were used at NIST for calibration, which eliminated the concerns of commutability between calibrant and the sample because the calibration was prepared in the sample matrix. Compared to the matrix matched calibration implemented at CDC, the shortcoming of the standard addition and isotope dilution methods is the reduced sample throughput. For the purpose of data analysis, NIST methods were considered independent from the CDC method due to the divergent implementations for calibration and perchlorate separation.

The two sets of data from NIST were obtained by LC-MS/MS using isotope dilution in one case and standard addition in another for calibration. Despite the difference in names, there are many similarities between the two because both calibration methods used $Cl^{18}O_4^-$ and the validated perchlorate standard. With the use of $Cl^{18}O_4^-$ internal standard, the standard addition method gained the same robust characteristics of the isotope dilution method, i.e., freedom from multiplicative interferences, which diminished the differences between the two methods. The methods are considered the same if they are susceptible to the same influence factors to the same extent. For certification purposes, the datasets from the same method should be comingled. To determine if the two NIST methods can be considered the same, the influence factors on the two methods were compared. Mass spectrometric measurements are susceptible to the influence of the detector deadtime. By virtue of exact matching, the isotope dilution method is less susceptible to this influence than the standard addition method [18]. Second, isotope dilution methods are influenced by the mass bias effect although the exact matching isotope dilution method is affected to a lesser extent [18]. The standard addition method is

unaffected by the mass bias effect. Third, the results of standard addition are subject to a positive bias because the $Cl^{18}O_4^-$ from the sample is not considered, though the bias (~ same order as the $Cl^{18}O_4^-$ abundance in relative terms) is undetectable because of the negligible abundance of $Cl^{18}O_4^-$ (1.6×10^{-11}) in nature. In these respects, the two NIST methods differ from each other; therefore, the two datasets from NIST were treated as being from two methods.

Data analysis

The commutability of the three methods was evaluated with respect to the measurement of SRM 3668. However, the results in Table 3 cannot be use directly for comparison, because the uncertainty from sample inhomogeneity was not included in the results of each method. To compare the values from each method, an expanded uncertainty for each method was calculated using a prediction interval to account for the uncertainty from sample inhomogeneity. The means and expanded uncertainties for perchlorate in Level I and Level II samples of SRM 3668 from the three methods are plotted in Figures 5 and 6, respectively. The results of the three methods agreed well as indicated by the overlap of the expanded uncertainties at each level. Yet, small differences between the three methods were indicated as CDC results were lower than NIST isotope dilution results, which in turn were lower than NIST standard addition results. While this trend is observed in both levels of the SRM, its significance is unclear as the differences fall within the expanded uncertainties that are largely driven by the uncertainty due to material inhomogeneity. Whether biases existed between methods is under investigation. Table 4 lists the results for perchlorate in the quality assurance samples using the NIST standard addition method. No significant bias was detected as the measured values overlapped with the target values for both low level quality assurance (QL) and high level quality assurance (QH) samples.

Perchlorate in SRM 3668 is certified [14] based on results from a NIST primary method, i.e., the isotope dilution method, and two validated and independent isotopic internal standardized methods from CDC [15] and NIST, respectively. Table 3 lists the certified values as well as the results of the three methods from which they were derived. As mentioned earlier, *s*, the replication standard deviation of the measurement, does not include the uncertainties due to inhomogeneity of the material nor that from validation of the calibrant. These sources of uncertainties were accounted for in the calculation of the certified value. The certified value of perchlorate in each level was calculated as the weighted mean of results of the three methods by leveraging a linear, Gaussian random effects statistical model [19,20] and the methods of maximum likelihood estimation [21,22]. The estimation procedures were supplemented by the parametric bootstrap [23] for uncertainty propagation to include the uncertainty due to material inhomogeneity.

The uncertainties from validation of calibrant (u_{cal}), inhomogeneity of the SRM material (u_{inhomo}), the between-method and within-method variances ($u_{btw\,meth}$), and the certified values are also listed in Table 3. The uncertainty from validation of standards is the smallest when compared to the uncertainties from material homogeneity and between method variances. Inhomogeneity and the between method variance are the first and second largest sources, respectively, of uncertainties in the certified values. For this work, the uncertainty of validation

accounts for less than 2 % of the expanded uncertainty for the certified value. Therefore, the standard fits for the purpose of the certification measurements.

Conclusion

SRM 3668 is the first SRM certified for perchlorate, an emerging environmental contaminant and a potential health hazard. In addition to materials for quality assurance, the heightened interest in biomonitoring measurements of perchlorate also highlights the urgent needs for SI traceable calibration standard of the analyte. The method used for validating the commercial perchlorate standard may serve as a path to develop SI traceable calibration standards. However, the chlorine blank in the INAA method must be lowered by either finding low Cl paper or an alternative to paper to achieve better accuracy.

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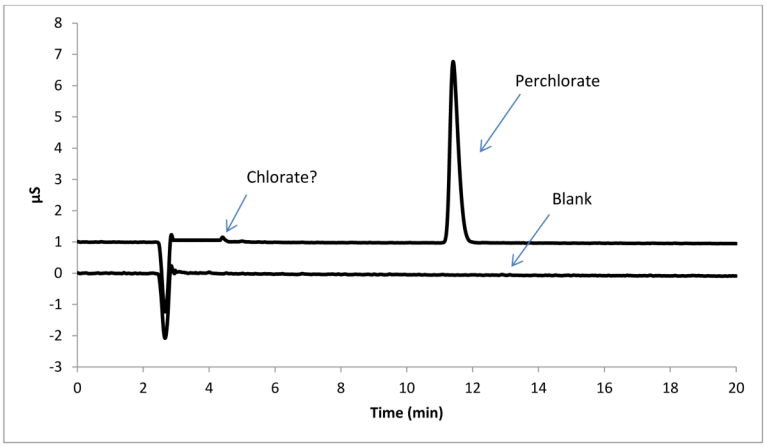

Figure 1.

Chromatograms of a 150 mg L^{-1} perchlorate and a blank. The perchlorate solution is shifted up by 1 μS for better comparison.

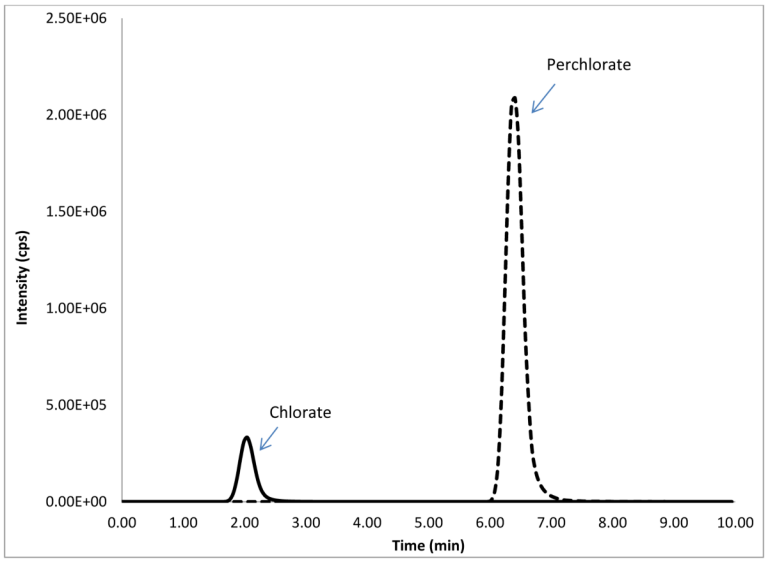

Figure 2.

A chromatogram of ClO_4^- standard at 1 mg L^{-1} concentration (dotted trace) shows no detectable chlorate. The solid trace is a 1 mg L^{-1} ClO_3^- solution.

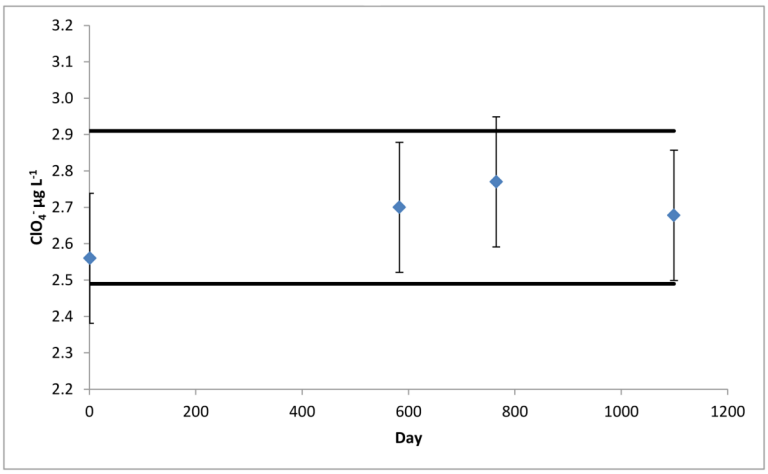

Figure 3.

Stability plot for perchlorate in SRM 3668 Level I. The horizontal lines mark the certified interval. The error bars show the expanded uncertainty of the measurement.

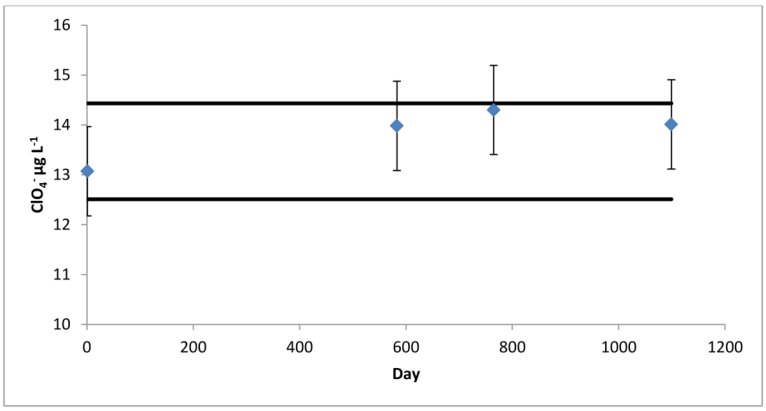

Figure 4.

Stability plot for perchlorate in SRM 3668 Level II. The horizontal lines mark the certified interval. The error bars show the expanded uncertainty of the measurement.

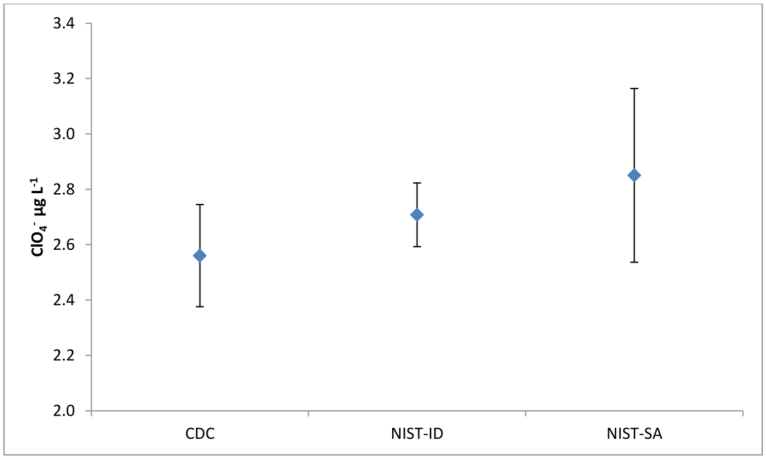

Figure 5.
Results of perchlorate in Level I sample of SRM 3668 by the three methods. The error bars show the expanded uncertainty of the measurement.

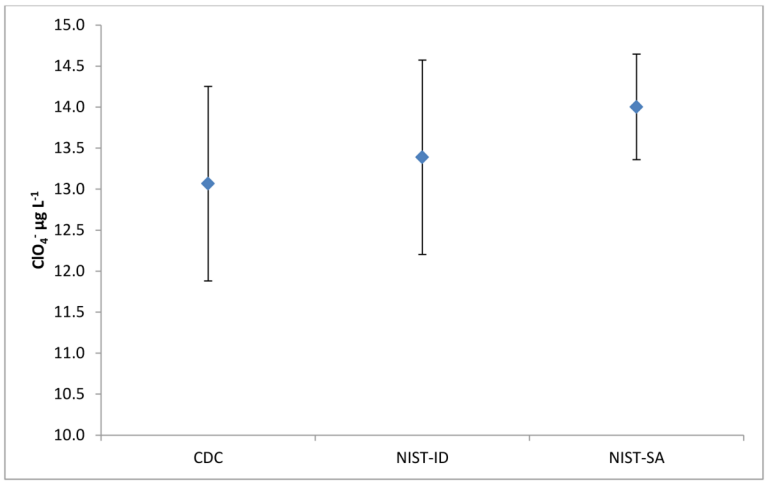

Figure 6.

Results of perchlorate in Level II sample of SRM 3668 by the three methods. The error bars show the expanded uncertainty of the measurement.

Table 1

Total Cl measured with NAA and Cl impurity measured with IC for the validation of ClO_4^- standards. All values are in mg L^{-1} units.

	AccuStandard		Inorganic Ventures	
	Mean	U^a	Mean	U^a
Cl total[b]	363	7	364	8
Cl impurity[c]	< 0.004	--	< 0.004	--
Cl (ClO_4^-)[d]	363	7	364	8
ClO_4^-[e]	1017	20	1020	22
Certificate	1000	5	999	3
Validated	1000	20	999	23

[a] *U* is the expanded uncertainty at approximately 95 % confidence.

[b] Measured with INAA.

[c] Measured with IC and IC-MS/MS.

[d] Calculated from *b* and *c*.

[e] Calculated from *d*.

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Table 2

Homogeneity assessment of perchlorate in SRM 3668 by ANOVA.

	Between-Vial MSq (*df* = 13)	**Within-Vial MSq (*df* = 14)**	***P*-Value**
Level I	0.013	0.009	0.29
Level II	0.32	0.15	0.09

Table 3

Measured and certified values for perchlorate in SRM 3668. All values have units of μg L^{-1}, except k, which has no units.

	Level I			Level II		
	Mean	***s***	***N***	**Mean**	***s***	***N***
CDC[a]	2.556	0.080	14	13.07	0.40	14
NIST ID[a]	2.708	0.026	8	13.39	0.46	8
NIST SA[a]	2.851	0.129	8	14.00	0.23	8
u_{cal}	0.027			0.13		
u_{inhomo}	0.080			0.40		
$u_{btw\ meth}$	0.069			0.25		
Certified[b]	2.70 ± 0.21, k = 1.95			13.47 ± 0.96, k = 2.00		

[a] CDC, NIST ID, and NIST SA stand for CDC method, NIST isotope dilution method, and NIST standard addition method, respectively.

[b] The certified value is expressed as the weighted mean ± U. The expanded uncertainty is calculated as $U = ku_C$, where u_C is intended to represent, at the level of one standard deviation, the combined effects of between-laboratory, within-laboratory, and inhomogeneity components of uncertainty. The coverage factor (k) corresponds to an approximate level 95 %.

Table 4

Quality assurance for perchlorate measurements at NIST with standard addition method. Values are perchlorate in $\mu g\ L^{-1}$ units.

Sample ID	QL	QH
1	3.31	75.3
2	3.22	72.9
3	3.32	71.8
Average ± *U*	**3.28 ± 0.43**	**73.3 ± 1.6**
Target Value	3.2	72