

Development of a Nutritional Supplement Certified Reference Material for Elemental Analysis

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Abstract : A certified reference material (CRM) for the analysis of inorganic nutrients in nutritional supplements has been developed. Accurate mass fractions of chromium (Cr), iron (Fe), copper (Cu), and zinc (Zn) were determined by isotope dilution inductively coupled plasma mass spectrometry (ID ICP/MS). The measurement results were used to assign certified values for the CRM, which were metrologically traceable to the definitions of the measurement units in the International System of Units (SI). Production of a candidate reference material (RM) and the certification processes are summarized. Each nutrient in the CRM showed good homogeneity, which was estimated using relative standard deviations of the measurement results of twelve bottles in a batch. This CRM is expected to be an important reference to improve reliability and comparability of nutrient analyses in nutritional supplements and related samples in analytical laboratories.

Keywords : certified reference material, isotope dilution mass spectrometry, elemental analysis, nutritional supplements, inductively coupled plasma mass spectrometry

Introduction

Nutrients are substances that are essential for sustaining life. Particularly, inorganic nutrients play critical roles in biochemical processes and should be included in diet to appropriate amounts. Recommended daily intakes of iron (Fe), copper (Cu), and zinc (Zn) are 10 mg, 0.8 mg, and 10 mg, respectively, for Korean males aged 19 to 49.¹ For chromium (Cr), the adequate intake is suggested as 35 mg per day, though scientific controversies exist regarding the recommendation.¹ To prevent nutrient deficiency and related diseases, a large percentage of the population frequently take nutritional supplements.² However, both nutritional deficiency and oversupply pose health risks, making it necessary to accurately evaluate the amount of

nutrients contained in supplements and assess risk and benefit associated with their consumption.

Certified reference materials (CRMs) are important measurement standards in chemical analysis. Validating a measurement procedure using a CRM whose specified property values, or the ‘certified values’, are traceable to a widely accepted reference, such as the International System of Units (SI),³ is necessary to ensure the comparability of measurement results.^{4,5} Production of a matrix CRM for chemical analysis is usually performed by homogenizing raw material, dividing the homogenized material into separate bottles, and analyzing the materials contained in a set of select bottles to estimate a property of the whole batch that is intended to be certified. Certification of CRMs are ideally performed using the so-called ‘primary methods of measurements’.⁶ Isotope dilution (ID) mass spectrometry (MS) is a potential primary ratio method⁶ with a broad scope of applications and often used to certify candidate reference materials (RMs).⁷ In ID, an isotope-enriched material (‘spike’) is added to a sample, and measuring the isotope ratios of the sample with and without spiking gives information on the amount of a substance contained in the sample. The mass fraction of the spike is usually characterized simultaneously by mixing with a standard solution with a certified mass fraction and measuring the resulting isotope ratios. Spiking both the sample and a standard solution as such is called a ‘double ID’ process,

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which provides measurement results with smallest uncertainties when operated at the exact-matching condition.⁸ In elemental analysis, the double ID process is most often combined with inductively coupled plasma (ICP) MS, which enables highly precise measurements of isotope ratios.

In this study, we report the development of a CRM for the analysis of elements in nutritional supplements. Double ID ICP/MS was used to certify the mass fractions of Cr, Fe, Cu, and Zn in the CRM to ensure the reliability of the measurement results while minimizing the measurement uncertainties. Production and certification of the CRM were performed using procedures previously established at KRISS⁹⁻¹² and were in accordance with ISO Guide 35.¹³ This matrix CRM is expected to be an important measurement standard for enhancing the confidence in the measurement of amount of nutrients in nutritional supplements and related samples.

Experimental

Chemicals

Monoelemental standard solutions with mass fractions traceable to the SI were produced by gravimetrically dissolving purity-assayed materials in acids. ⁵³Cr-, ⁵⁷Fe-, ⁶⁵Cu-, and ⁶⁸Zn-enriched materials were purchased from Oak Ridge National Laboratory (Oak Ridge, TN, USA). Nitric acid (electronic grade, ChemiTop Co., Ltd., Jincheon, Korea) purified by sub-boiling distillation (duoPUR, Milestone SRL, Sorisole, Italy) and deionized water (resistivity > 18.2 MΩ·cm, Milli-Q Integral 5, Millipore, Billerica, MA, USA) were used during the experiments.

CRM production

Fifty bottles of commercial nutritional supplements produced using yeast extracts were used to produce the CRM. Approximately 13.75 kg of supplement tablets were ground using variable speed rotor mill (Pulverisette 14, FRITSCH, Idar-Oberstein, Germany) at 12,000 rpm and passed through a 0.5 mm sieve. Both rotor ribs and sieve ring were coated with titanium. A vibrating sifter (V/SIFTER-14, Daega Powder Systems, Seoul, Korea) equipped with a 100 μm nylon sieve was used to collect uniformly sized particles. Approximately 12.5 kg of powder containing particles of sizes smaller than 100 μm was used. A V-blender that was coated with Teflon (Daega Powder Systems, Seoul, Korea) was employed at 15 rpm for 10 h to homogenize the powder material. The homogenized material was divided into 600 acid-cleaned amber glass bottles (Fisher Scientific, Pittsburgh, PA, USA), each accommodating approximately 20 g of the powder. The sealed bottles were sterilized by irradiation with ⁶⁰Co γ-rays at a dose of approximately 25 kGy (Greenpia Technologies, Yeosu, Korea).

Sample pretreatment

Procedures at KRISS for certification of candidate RMs using double ID ICP/MS⁷ were described in detail previously.⁹⁻¹² KRISS has shown excellent measurement capabilities in a high number of key comparisons organized by the Consultative Committee for Amount of Substance (CCQM) using double ID ICP/MS,¹⁴⁻¹⁶ demonstrating the validity of the following established procedures. Preliminary analyses including brief ID were first performed to determine approximate mass fractions of elements in the candidate RM. Optimal target isotope ratios were calculated based on propagation of uncertainties,¹⁷ and dilution factors of elemental standard solutions and amounts of isotopic spike to be added were calculated based on the preliminary analysis results to achieve the target isotope ratios. For certification, approximately 0.2 g of powder was taken from each of the 12 select bottles of the candidate CRM to microwave-assisted digestion¹⁸ vessels. Isotope solutions diluted to appropriate mass fractions were spiked to the sample and standard solutions that had been diluted to appropriate mass fractions. Isotopic spike was not added to one of the powder subsamples to verify that the isotope ratios of elements in the sample are within the range of natural variations.^{19,20} Microwave digestion of the samples were performed either using a closed-vessel microwave-assisted digestion system (ETHOS PLUS, Milestone SRL, Sorisole, Italy) or a single reaction chamber microwave digestion system (UltraWAVE, Milestone SRL, Sorisole, Italy). In case of the former (for Fe, Cu, and Zn), 8 mL of nitric acid was added to each of the digestion vessels, which were left overnight for pre-digestion. 2 mL of hydrogen peroxide was added to each of the digestion vessels, which were then subjected to closed-vessel microwave-assisted digestion by increasing the temperature to 200°C in 15 min and maintaining the temperature for 15 min. In case of the latter (for Cr), 10 mL of nitric acid were added to each of the digestion vessels, which were placed in a chamber pressurized to 40 bar with nitrogen at room temperature. The samples were digested by increasing the temperature to 120°C in 8 min, 220°C in the next 8 min, and maintaining the temperature for 15 min. The digest solutions were diluted with deionized water and recovered in pre-cleaned low-density polyethylene bottles. For blank correction, three procedure blanks were prepared. For dry-mass correction, three subsamples of 0.5 g were taken from each of the 12 bottles simultaneously with the subsamples for ICP/MS experiments, which were placed over P₂O₅ in desiccators. The changes in mass were measured after one week.

ICP/MS

The isotope ratios were measured using a double focusing magnetic sector type ICP mass spectrometer (Element 2, Thermo Scientific Inc., Bremen, Germany) in medium-resolution mode. Nickel sampler and skimmer

Table 1. Comparison between certified values and associated expanded uncertainties (mg/kg) for the nutritional supplements and a related CRM. Relative associated expanded uncertainties and coverage factors at the 95% level of confidence are shown in parentheses

CRM name	KRISS 108-10-012	NIST SRM 3280
	Nutritional supplements for elemental analysis	Multivitamin/Multielement Tablets
Cr	139.7 ± 3.2 (2.3%, <i>k</i> = 2.1)	93.7 ± 2.7 (2.9%, <i>k</i> = 2.06)
Fe	3702 ± 104 (2.8%, <i>k</i> = 2.0)	12350 ± 910 (7.4%, <i>k</i> = 3.18)
Cu	559 ± 20 (3.6%, <i>k</i> = 2.0)	1400 ± 170 (12%, <i>k</i> = 2.12)
Zn	5840 ± 270 (4.6%, <i>k</i> = 2.0)	10150 ± 810 (8.0%, <i>k</i> = 2.00)

cones (Elemental Scientific, Omaha, NE, USA), cyclonic borosilicate spray chambers (Glass Expansion, Port Melbourne, Australia), and concentric borosilicate glass nebulizers (Glass Expansion, Port Melbourne, Australia) were used for the experiments. The radiofrequency (RF) power for ICP was 1250 W.

Assignment of certified values

The model equation used to estimate the measurand was as follows:

$$c_x = D \cdot c_z \cdot \frac{m_{y,xy} m_z}{w \cdot m_x m_{y,yz}} \cdot \frac{R_y - R_{xy}}{R_{xy} - R_x} \cdot \frac{R_{yz} - R_z}{R_y - R_{yz}} \cdot \frac{\sum_i R_{x,i}}{\sum_i R_{z,i}} - B \quad (1)$$

where *x*, *y*, and *z* represent the sample for analysis, an isotope-enriched material, and a standard solution with a certified mass fraction, respectively. These are combined to make the sample blend (*xy*) and calibration blend (*yz*). The variables *c*, *m*, and *R* denote respectively the mass fraction, mass, and isotope ratio of those represented by the subscripts. $\sum_i R_{x,i}$ and $\sum_i R_{z,i}$ denote the sum of isotope ratios in the sample (*x*) and the certified standard (*z*), respectively, for all of the isotopes of the element being analyzed. *B* is the contribution of the blank to the measurement result, which is estimated using procedure blanks. *D* is the dilution factor of the elemental standard solution and *w* is the dry-mass correction factor. Isotope ratios were the ratios of an enriched isotope (^{53}Cr , ^{57}Fe , ^{65}Cu , or ^{68}Zn) to a reference isotope (^{52}Cr , ^{56}Fe , ^{63}Cu , or ^{66}Zn). Homogeneity evaluation was performed based on the between-bottle variations of individual measurement results.²¹⁻²⁴

Results and Discussion

Means of the measured mass fraction for each of the 12 bottles were assigned as the certified value of each element for the nutritional supplement CRM. All known sources of

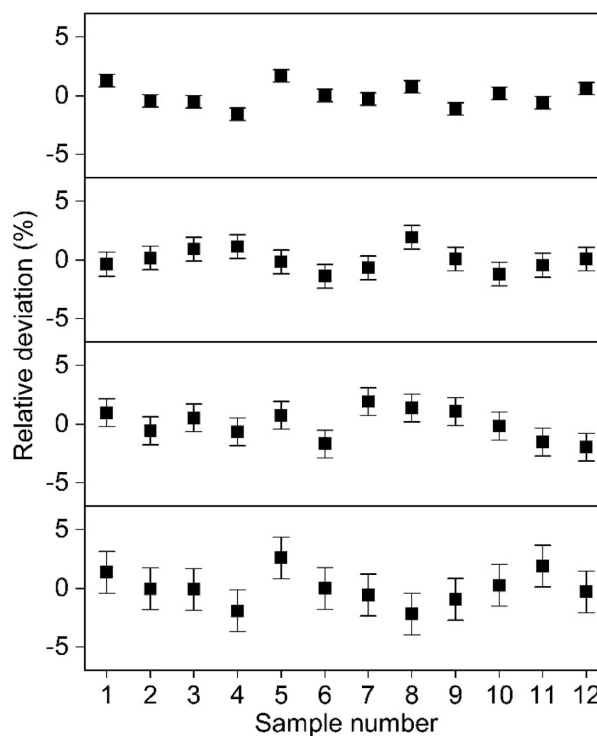


Figure 1. Measured mass fractions of Cr, Fe, Cu, and Zn (from top to bottom) for samples from individual bottles. The error bars denote the uncertainties from systematic effects and repeatability of the measurement results for each individual sample.

uncertainties were considered during uncertainty evaluation in accordance to the Guide to the Expression of Uncertainty in Measurement (GUM).²⁵ As summarized in Table 1, the certified values for Cr, Fe, Cu, and Zn and their associated expanded uncertainties at the 95% level of confidence were (139.7 ± 3.2) mg/kg, (3702 ± 104) mg/kg, (559 ± 20) mg/kg, and (5840 ± 270) mg/kg, respectively, where the coverage factor *k* were 2.1, 2.0, 2.0, and 2.0, respectively. The corresponding relative expanded uncertainties were 2.3%, 2.8%, 3.6%, and 4.6%, respectively. Measurement results for samples from individual bottles are illustrated in Figure 1. The between-bottle variations of the measurement results encompass the contribution from repeatability of the measurement procedure and between-bottle inhomogeneity of the CRM, and can be regarded as the upper limit of the inhomogeneity of the CRM. Relative standard deviations of the 12 measurement results were 0.96%, 0.96%, 1.29%, and 1.41% for Cr, Fe, Cu, and Zn, respectively.

The mean measured isotope ratios of the sample blends (R_{xy}) and calibration blends (R_{yz}) for Cr, Fe, Cu, and Zn, respectively, were 1.22 and 1.26; 1.09 and 1.08; 0.34 and 0.35; 0.35 and 0.34. The high similarities between R_{xy} and R_{yz} values demonstrate near-exact matching of isotope ratios, which enabled minimization of measurement

Table 2. Major sources of measurement uncertainties for the certified value of each element.

Element	Major sources of measurement uncertainties (uncertainty contribution, %)
Cr	Between-bottle random variation (77%)
Fe	Between-bottle random variation (47%)
Cu	Between-bottle random variation (54%)
Zn	Between-bottle random variation (43%), isotope ratios of the sample and the standard solutions (each 17%)

uncertainties.⁸ Table 2 shows that the major source of uncertainty for all of the elements was random variations between the measurement results of separate bottles. In case of Zn, uncertainties in isotope ratios also contributed significantly to the combined uncertainty, due to the relatively large variations in natural isotope ratios. In double ID ICP/MS, it is common to adopt ‘natural isotope ratios’ summarized by International Union of Pure and Applied Chemistry (IUPAC)^{19,20} owing to the difficulties in absolute isotope ratio measurements and as a conservative evaluation of measurement uncertainties. The relative uncertainties of the isotope ratios evaluated as such were the largest in case of Zn (0.70%), leading to a relatively large uncertainty contribution from systematic effects.

The overall certified results were well fitted for the intended use of the CRM, which is expected to be used for validation of analytical procedures and quality control of measurement results. According to ISO 13528, standard uncertainties of assigned values for proficiency testing (PT) samples ($u(x_{PT})$) can be considered negligible if the relation $u(x_{PT}) < 0.3\sigma_{PT}$ is fulfilled, where σ_{PT} is the standard deviation for proficiency assessment.²⁶ σ_{PT} is often assigned as the standard deviation predicted by the Horwitz equation (σ_H),²⁷ which describes typical interlaboratory reproducibility of measurement results (eq. 2).

$$\sigma_H = 0.02c^{0.8495} \quad (2)$$

where c is the mass fraction of the chemical species to be determined. For this CRM, the expected interlaboratory standard deviations are calculated as 10.6 mg/kg, 172 mg/kg, 34.5 mg/kg, and 253 mg/kg, for Cr, Fe, Cu, and Zn, respectively. The ratios of the standard uncertainties of the certified values (u_{CRM}) to the Horwitz standard deviations, u_{CRM}/σ_H , were 0.14, 0.30, 0.29, and 0.53, respectively. Therefore, except for Zn, measurement uncertainties of the certified values can be considered negligible for proficiency assessment of laboratories. Relatively high u_{CRM}/σ_H for Zn can be considered to originate from the high Zn contents in the CRM leading to small relative σ_H (σ_H/c), and relatively large natural variations in isotope ratios as described earlier. Nonetheless, u_{CRM} for Zn becomes a minor component of overall uncertainties if combined with σ_H , and thus the current uncertainties of the

CRM can be considered sufficient for most purposes.

Comparison of the certified values of the present CRM with those of an existing CRM for the analysis of nutritional supplements (NIST SRM 3280) shows that the present CRM generally has smaller uncertainties than formerly released CRM for certified elements. Thus, this new CRM is expected to be complementary to the existing CRM for validating measurement procedures for nutritional supplements or samples of similar matrices, and serve as a national measurement standard for confidence in chemical analysis.

Conclusions

A nutritional supplement CRM certified for nutritional elements has been developed. Certification of the CRM using a thoroughly validated procedure based on double ID ICP/MS allowed for the establishment of metrological traceability of the certified value to the SI. The production and measurement procedures for the CRM outlined in the present study are expected to be valuable for enhancing the reliability of measurements for samples of similar matrices.

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