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**Development of low and elevated level Multivitamin and mineral supplement Certified Reference Materials: VITA-1 and VITB-1**

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**Abstract**

Two new multivitamin Certified Reference Materials have been issued by National Research Council Canada. VITA-1 is a low-level and VITB-1 is an elevated-level CRM, both of which were certified for minerals and vitamins. The proportion of nutrients are the same in each formula and they were prepared such a way that VITB-1 has twice the amount of minerals and vitamins as VITA-1. The availability of these two matrix-matched CRMs, with different concentrations for all analytes, will not only assist in the validation of procedures and the development of methods for the determination of respective analytes in multivitamin or samples of a similar matrices, but will also be useful for calibration purposes. Tablets were individually packaged in trilaminate foil pouches and values presented in the certificate of analysis are expressed on a "as is" basis, with no need for the determination of a moisture content on a separate test portion. In addition, certified/ reference values are provided per single tablet (mass fraction expressed as both mg/kg and µg/tablet) eliminating the need of a homogenization step prior to the analysis. Homogeneity and stability were assessed and included in the calculation of the certified values. Quantity value assignment for both VITA-1 and VITB-1 was based on the use of at least two independent methods, using data generated at NRC and data from external collaborators for confirmation purposes. As a result of this campaign, value assignment was possible for a total of 39 analytes: 24 trace elements and 15 vitamins. Among them, 17 are classified as certified values (16 trace metals and one vitamin), 12 as reference values (5 trace metals and 7 vitamins), and 10 information values (3 for trace metals and 7 for vitamins) for both VITA-1 and VITB-1.

Key words: Trace metals, vitamins, certified reference material, value assignment, stability, homogeneity, isotope dilution, HR-ICPMS, standard additions calibration.

## Introduction

The use of dietary supplements has been showing a rising interest in the last few decades as these products are used by a large fraction of the population in an effort to increase their daily nutrient intake and/or for overall health promotion as vitamins and minerals play essential role in metabolic pathways and the functioning of the human body[1, 2]. Vitamins and minerals are often added to food to address public health issues and maintain the nutritional quality of the food supply. For example, vitamin D is to milk to combat the childhood deficiency-disease of rickets, and folic acid added to flour to reduce birth defects.

Nearly 45% of Canadians (aged one year and older) used at least one nutritional supplement in 2015, with the highest percentile being females over 50 years old. [3]

Dietary supplements come in a variety of forms such as tablets, capsules, and gummies and they often contain a combination of vitamins and minerals. They are usually complex products and in Canada, they are controlled under the Natural Health Products Regulations which ensure that Canadian have access to safe, effective, and high quality natural health products. According to this regulation, all natural health products must be licensed by Health Canada before they are allowed to be legally sold in Canada . A quick search in the Licensed Natural Health Products Database from the Health Canada website [4] for multivitamins products shows about 100 different brand names for products that have been licensed by Health Canada. Those products range from multivitamins for toddlers to those for adults over 50 years old. It should be noted that each age group has different recommended daily intakes as set by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO). [5] Also, these recommendations may differ for men and women[5]. For instance, recommended magnesium intake varies from 60 mg/day for 1-3 year old children to 230 mg/day for males over 65 years[5]. Exceeding the recommended daily dosage of some vitamins and mineral supplements could be detrimental and lead not only to some considereably minor health issues such as nausea and diarrhea, but also to heart problems (vitamin D, calcium)[6], liver damage (vitamin A) [7 8], or nerve damage (vitamin B6) [9], among other serious health issues. Therefore, levels of vitamins and minerals in multivitamin products must be accurately assessed in order to guarantee that a dose meets the required daily intake. Also, earlier studies [10, 11 12 13]have shown that the actual amount of "active ingredient" in some multivitamins and supplements currently available on the market can vary significantly from the amount listed on the label. This may be due to the lack of uniformity of the ingredients added to the formulation. Thus there is a need for chemical metrology, not only from health and safety aspects but also to support/improve any future clinical studies which might rely on the use of commercially-available multivitamins. The ability to provide consumers with assurance of the precise concentrations of minerals and vitamins presented in those products depends

on the implementation of precise and accurate methods of analysis employing proper quality control (QC) procedures such as the use of Certified Reference Materials (CRMs) of multivitamins and similar matricies.

In recent years CRM producers have developed such reference materials. For example, the national metrology institute of Korea (KRISS) released two CRMs, both in powder form: one for nutritional supplements for elemental analysis (108-10-012) [14] which is certified for Se and a multivitamin powder for analysis of organic nutrients (108-10-010) [15] which is certified for seven water-soluble vitamins. The National Institute of Standards and Technology (NIST) released a standard reference material for multivitamin/ multiement tablets (SRM 3280), with certified and reference mass fraction values for 13 vitamins, 26 elements and 2 carotenoids. This material is offered as five units, each containing 30 tablets. During the certification campaign of SRM 3280, it was verified that the material used showed a large tablet-to-tablet variability, ranging from approximately 15 to 25% for measured element mass fractions presented in the certificate of analysis[16-18]. In order to use the standard reference material, users are instructed to grind at least 15 tablets to obtain a homogeneous sample prior to sampling a test portion for analysis. [16-18] For the vitamin analysis, it was also recommended to use of a freshly ground portion as some vitamins were observed to be unstable in the ground material. [16]This can significantly increase the cost of the analysis, specially for the vitamins.

National Research Council Canada (NRC) has produced two new CRMs for multivitamins: VITA-1 (low-level), and VITB-1 (elevated-level). Both materials have the same nutrient's proportion with VITB-1 having twice the amount of minerals and vitamins as VITA-1. These two matrix-matched CRMs, with different concentrations for all analytes, will assist not only the validation of procedures and development of methods for the determination of respective analytes in multivitamin or samples of similar matrices, but also for calibration purposes.

The NRC CRMs aim to achieve a higher degree of homogeneity and provide certified values as per single tablet (mass fraction expressed as both mg/kg and µg/tablet), eliminating the need of a homogenization step prior to the analysis. In addition, tablets were individually packaged in trilaminate foil pouches and values presented in the certificate of analysis are expressed on a wet weight basis, with no need for the determination of a moisture content on a separate test portion.

The certification campaign for both VITA-1 and VITB-1 is described here. Both materials were produced in compliance with the NRC Metrology Quality System, which conforms to the requirements of ISO/IEC 17025 and ISO Guide 34. Quantity value assignment for both VITA-1 and VITB-1 was based on the use of at least two independent methods, preferably one of them being a single primary method performed at NRC. Confirmation of these results by external collaborators' results (ISO/IEC 17025 accredited) was achieved.

## Results and Discussion

### *Preparation of VITA-1 and VITB-1 candidate CRMs*

Two candidate CRMs for multivitamins, named VITA-1 and VITB-1, were prepared at National Research Council Canada. VITA-1 is a low-level multivitamin and VITB-1 an elevated-level multivitamin. The proportion of nutrients is the same in each formula and they were prepared in such a way that VITB-1 has twice the amount of minerals and vitamins as VITA-1. They were both produced by a Canadian commercial nutraceutical manufacturer according to pharmaceutical standards using the product specification presented in the Table 1.

Non-medicinal ingredients such as lactose monohydrate, hydroxypropyl cellulose, silicon dioxide, microcrystalline cellulose, magnesium stearate, opadryl II white (polyvinyl alcohol, titanium dioxide, polyethylene glycol, talc) were also added to the formulations. Both materials were pressed into tablets, film-coated and individually packed into a trilaminate foil pouch. Each tablet of VITA-1 and VITB-1 weighs approximately 2.57 g and 2.62 g, respectively. Both materials have been stored in a dark, +4 °C temperature environment.

## Certification Campaign

Quantity value assignment for both VITA-1 and VITB-1 was based on the use of at least two independent methods, preferably one of them being a single primary reference method performed at NRC. Confirmation of these results by analysis performed by external collaborators (ISO/IEC 17025 accredited) was also used.

A primary or higher order method is the most preferred to obtain quantity values for a certification and could be applied to obtain certified quantity values on its own or with an additional confirmatory method. Unfortunately, there are a limited number of primary methods available. For this certification campaign, the primary method used for the determination of trace elements was double isotope dilution inductively-coupled plasma mass spectrometry (ID-ICP-MS) and it was applied for the determination of B, Cd, Cr, Cu, Fe, Pb, Hg, Mo, Se, and Zn in both VITA-1 and VITB-1 proposed candidate CRM samples using high resolution ICP-MS. Monoisotopic elements, such as As, Co, and Mn, were determined using a standard addition calibration strategy to account for any possible matrix effects. A second set of data was also produced at NRC by using standard addition calibration strategy and detection by triple quadrupole (QQQ) ICP-MS or ICP-atomic emission spectrometry (ICP-AES). For the determination of vitamins in both VITA-1 and VITB-1, only results for

a single method, liquid chromatography tandem mass spectrometry (LC-MS/MS), were produced at NRC, except for cyanocobalamin which was also determined by, LC-ICP-MS and ID-LC-MS/MS.

In addition to NRC's results, external collaborators provided measurements using their own internal procedures. Each collaborator received blinded samples that encompassed a QC sample of a similar matrix and several single tablets from each proposed candidate CRM (VITA-1 and VITB-1). For the determination of the trace elements, collaborators were asked to perform a complete digestion using a microwave-assisted acid digestion procedure.

Gravimetric data, i.e, the amount of each ingredient that was added to the formulations, is also available from the manufacturer of the proposed candidate CRM (VITA-1 and VITB-1) and was used during the certification as an independent data set.

The summary of the analytical methods used in the certification of VITA-1 and VITB-1 is presented in Table 2. For most analytes, three independent methods were used, two of them performed at NRC.

**Determination of trace metals by Double Isotope Dilution ICP-MS method at NRC**

The isotope dilution (ID) method is capable of compensating for matrix effects, instrument drift and any subsequent losses of analyte during sample preparation procedures, providing that isotopic equilibrium has been achieved prior to analysis. In addition, ID provides superior measurement accuracy and precision compared to other calibration strategies. For the certification of VITA-1 and VITB-1, double ID ([19] was applied for the determination of B, Cr, Cu, Fe, Pb, Hg, Mo, and Zn in the multivitamin samples using high resolution (HR) ICP-MS and Cd and Se using the ICP-QQQ-MS.

Tablets were digested "as it is", i.e, no subsampling was performed. A typical mass of a tablet is about 2.57 g and 2.62 g for VITA-1 and VITB-1, respectively. Individual whole vitamin tablets were accurately weighed into pre-cleaned Teflon microwave digestion vessels. The contents were then gravimetrically spiked with known masses of enriched isotopes to achieve an approximately 1:1 ratio in intensities of selected isotope pairs. The following reference/spike isotope pairs were used: $^{11}B/^{10}B$, $^{114}Cd/^{111}Cd$, $^{52}Cr/^{53}Cr$, $^{63}Cu/^{65}Cu$, $^{56}Fe/^{57}Fe$, $^{208}Pb/^{207}Pb$, $^{202}Hg/^{201}Hg$, $^{98}Mo/^{100}Mo$, $^{78}Se/^{82}Se$ and $^{66}Zn/^{67}Zn$. Similarly, three procedural blanks were prepared by addition of only 10 % of the mass of enriched isotope spike used for the samples. After addition of 15 mL $HNO_3$, 2 mL HF and 3 mL $H_2O_2$, contents were heated on a hot plate at 80°C for 24 hours. Then 3 mL of HCl was added prior to the microwave digestion using 5 min ramping to 360 W and hold 5 min, another 5 min ramping to 850 W and hold for 20 min, then 0 W for the cool down cycle (35 min). The contents were transferred into Teflon tubes and evaporated to 2-3 mL remained. Another 4 mL of $HNO_3$ and 12 mL of HCl were added to the tubes which were then heated at 60°C for 60 min. Contents were diluted to 500 g with deionized water (DIW). Sample solutions were left to stand for at least 5 days prior to analysis.

**Determination of trace metals by standard addition ICP-MS (As, Ca, Co, K, Mg, Mn, P) or Standard addition ICP-AES (B, Ca, Cr, Cu, Fe, Mg, Mn, Mo, P, K, Se, Zn) at NRC**

The same digestion procedure used for ID-ICP-MS method was applied for standard addition ICP-MS and ICP-AES methods, except isotopically-enriched standards were not added to samples. In this case, a three-point standard addition calibration was used. For that, three 20 g subsamples of digest were weighed into pre-cleaned polyethylene bottles. Appropriate masses of 0.20 and 0.40 g of the mixed standard solution were added to two subsamples so as to result in approximately a 1- and 2-fold increase in the analyte mass fractions, respectively. Equation 3 was used for the calculation of the mass fraction of analyte using standard addition calibration [20, 21]

$$\frac{m_{\text{std-i}} \cdot w_{\text{std}}}{m_{\text{s-i}}} \cdot \frac{m_{xf}}{m_x} = b \cdot I_i \cdot \frac{m_{sf-\text{i}}}{m_{\text{s-i}}} \cdot \frac{m_{\text{df-i}}}{m_{\text{d0-i}}} + a \quad \text{and} \quad w_x = -a \qquad (3)$$

where:

$w_x$ is the mass fraction of the analyte in the sample (μg $kg^{-1}$);

$w_{std}$ is the mass fraction of the analyte in the primary standard solution (μg $kg^{-1}$);

$I_i$ is the measured intensity in the prepared set of samples, i=0, 1, 2;

$m_{std-i}$ is the mass of natural abundance standard added to the spiked sample (g), i=1, 2;

$m_{s-i}$ is the mass of aliquot of diluted sample used to prepared spiked sample (g), i=1, 2;

$m_{sf-i}$ is the final mass of spiked sample (g), i=1, 2;

$m_{df-i}$ is the final mass of diluted set of samples (g), i=0, 1, 2;

$m_{d0-i}$ is the mass of aliquots of spiked samples for dilution (g), i=0, 1, 2;

$m_{d0-f}$ is the final mass of aliquots of spiked samples after dilution (g), i=0, 1, 2;

$m_x$ is the mass (g) of the original sample;

$m_{xf}$ is the final mass of the original sample after addition of enriched spikes and 1% $HNO_3$ (g).

In addition, that errors-in-variables regression was used to obtain uncertainty. [22]

All operations for both isotope dilution and standard addition ICP-MS/ ICP-AES methods were conducted in a class 100 clean room or in fume hoods of class 10 air quality.

Validation of the method at NRC was performed by analysis of established CRMs, NIST-3280, multivitamin/multielement tablets (for all elements/compounds except Hg, selenometionine and chromium picolinate), NRC CRM DORM-4 (for As, Cd, Co, Hg and Pb) and NRC CRM SELM-1 (for selenomethionine). Sample preparation is the same as described above except that for SRM 3280, a total of 25 tablets were grinded and 1 g sub-samples were used. For the other CRMs, 0.25 g of sample was used instead.

**Determination of water-soluble vitamins at NRC**

Cyanocobalamin (vitamin B12) was analyzed at NRC by two independent methods: LC-ICP-MS using standard addition strategy and isotope dilution LC-MS/MS. Information is presented in ref [23]. The ID LC-MS/MS method is based on high-precision quadruple isotope dilution for quantitation using isotopically enriched $^{13}C^{15}N$-B12 as internal standard. Purity assessment of the cyanocobalamin was determined by $^{1}H$-qNMR [23].

In short, tablets were dissolved in dilute acetic acid solution, then samples were sonicated at 25°C for 30 min, follow by centrifugation at 2000 rpm for 10 min. Supernatant was filtered through 0.45 mm nylon filter and concentrated by solid phase extraction (SPE). The SPE tubes (Strata™-X 33 mm Polymeric Reversed Phase, 500 mg/12 mL Phenomenex) were previously conditioned with methanol and DIW. The multivitamin extract was loaded on the SPE followed by a wash with 5% methanol in water for removing the minerals. cyanocobalamin was finally eluted with 10 mL methanol and evaporated to dryness under nitrogen. The solid residue was reconstituted with 1mL of water and diluted 1:10 in 1% acetic acid prior to LC-MS/MS analysis. Sample preparation for determination of cyanocobalamin by LC-ICPMS analysis was similar to the one just described, without the SPE step. There, quantitation was attained by standard addition. A C18 column was used and analytes were eluted in isocratic mode at 25°C in 15:85 acetonitrile: water mobile phase with 0.1% formic acid.

The other water soluble vitamins, i.e, thiamine, riboflavin, niacinamide, pantothenic acid, biotin, folic acid, pyridoxine, riboflavin, and thiamine, were analyzed at NRC by LC-MS/MS using external calibration. For that, tablets were dissolved in dilute acetic acid. Following this, samples were sonicated at 25°C for 30 min, followed by centrifugation at 2000 rpm for 10 min. Supernatant was filtered and diluted accordingly prior to the LC-ESI-MS/MS analysis. A C18 column was used. An ammonium acetate/methanol gradients were used for the determination of thiamine, pyridoxine, pantothenic acid and riboflavin. For niacinamide, a diluted formic acid in water/ diluted formic acid in acetonitrile gradient was used instead and pyridoxic acid was used as internal standard. Measurements were traceable to NIST and US Pharmacopea standards.

**Determination of Br, I, Cl by ICP-MS (external collaborator)**

Br, I and Cl were analyzed using a microwave-induced combustion method. In short, sample pellets were placed together with the filter paper on the quartz holder and 50 µL of ammonium nitrate solution (6 mol $L^{-1}$) were added to the paper. The sample holder was introduced into the quartz vessel, previously charged with 6 mL of absorbing solution (100 mmol $L^{-1}$ $NH_4OH$). After closing the vessels and capping of the rotor, vessels were pressurized with 20 bar of oxygen. The rotor was placed inside the oven and the selected microwave heating program was started. The microwave irradiation program was 900 W for 5 min and 0 W for 60 min (cooling

step). After digestion, the pressure of each vessel was carefully released. The resultant solution was diluted with water up to 25 mL in volumetric vessels. Final digests were analyzed by inductively coupled plasma mass spectrometry (ICP-MS), measuring isotopes $^{35}Cl$, $^{79}Br$, and $^{129}I$. NIST SRM 3280 multivitamin/multielement tablets, NIST SRM 1566a oyster tissue, and NIST RM 8435 whole milk powder were used as QC samples.

**Determination of trace metals by ICP-MS (external collaborator)**

Samples were digested using a closed vessel microwave system using a mixture of mineral acids following EPA Method 3052. Analytes were determined by external calibration ICP-MS. NIST SRM 3280 multivitamin/multielement tablets and NIST SRM 1566a oyster tissue were used as QC samples.

**Determination of water soluble vitamins by LC-MS/MS (external collaborator)**

The determination of water soluble vitamins by LC-MS/MS was based on the extraction of water soluble vitamins using an acidic aqueous-solvent mixture, followed by the addition of a small amount of alkaline solution to precipitate proteins and aid in chromatography. Determination was performed using LC-MS/MS. Stable isotope internal standards of each B-vitamin were used in the quantitation and an external calibration strategy was used.

**Microbiological assay (external collaborator)**

The microbiological assay on VITA-1 and VITB-1 was performed by an external laboratory according to AOAC 952.20 and 960.46 official methods of analysis for vitamin B12. The test consists in incubating the sample, formerly inoculated with *Lactobacillus leichmanii*, for 16 to 24 h and measuring the developed turbidity with a photometer. A blind sample of NIST 3280 SRM multivitamin tablets was supplied along with VITA-1 and VITB-1 for quality control purposes.

**Assessment of Homogeneity**

It is well known that in the pharmaceutical industry, production of homogenized products in the powder form is challenging [24] as optimum mixing is crucial, especially for low dose products like multivitamins. Homogeneity is a factor of the particle size, shape, and density, among other parameters[25]. During the certification of NIST SRM 3280 multivitamin/multielement tablets, it was verified that the material, which was produced by a manufacturer of multivitamin/multieelement tablets using their normal procedure, presented an individual tablet-to-tablet variability, ranging from 15 to 25%; therefore, at least 15 tablets of the material must be grinded to obtain a

homogenous sample prior to analysis. [18] In light of this, special attention was given during the production of VITA-1 and VITB-1 in order to achieve a better homogeneity in order to permit the use of single tablets for analysis.

An initial homogeneity assessment was carried out during production by selecting four water soluble vitamins (pyridoxine HCl, riboflavin, thiamine HCl, and niacinamide) as model analytes and using LC-UV for the respective analyte determination. Eight single tablets of VITA-1 and four single tablets of VITB-1, selected across the entire production, were using for this initial homogeneity assessment. Figure 1 shows the individual results for VITA-1. RSD values of about 5, 2.5, 7 and 1% (VITA-1) and 9.5, 2.5, 3 and 1.6% (VITB-1) were obtained respectively for pyridoxine HCl, riboflavin, thiamine HCl and niacinamide.

Tablet-to-tablet homogeneity for both metals and vitamins in VITA-1 and VITB-1 was assessed at NRC across the entire CRM production series (about 45,000 units for each product). For that, individual tablets were randomly selected and analyzed. The between-tablet variation comprises sample heterogeneity ($u_{hom}$) and variation due to batch characterization ($u_{char}$). Results for representative elements are presented in Figure 2 and refer to the digestion of the whole tablet.

Both ICP-MS and ICP-AES measurements were used for the homogeneity assessment. For most analytes, homogeneity was assessed with the analysis of at least 50 tablets. The relative standard deviation (RSD) values were less than 5 % for all analytes for VITA-1 except for Mg and Se in VITA-1 and Se in VITB-1, which were 6.2 % (measured by ICP-OES), 6.3 % (ID, ICP-MS) and 5.4 % (ID, ICP-MS) respectively.

Magnesium values come from contribution of both magnesium oxide (active ingredient) and magnesium stearate, a metallic salt of fatty acids that was added to the formulation to act as a lubricant in order to reduce friction by enhancing the powder flow during the manufacturing process [26]. A second data set for Mg at NRC using ICP-MS has a much smaller RSD (1.4% for the analysis of 15 single tablets) which shows that the higher RSD for Mg for the first method is related to the variation due to characterization rather than the sample heterogeneity.

In relation to selenium, it should be noted that its determination by ICP-MS suffers from several polyatomic interferences ($^{40}Ar^{40}Ar^{+}$, $^{40}Ca^{40}Ar^{+}$, $^{39}K^{37}Cl^{+}$, $^{60}Ni^{16}O^{+}$, $^{65}Cu^{17}O^{+}$, $^{64}Zn^{18}O^{+}$). In this certification, a triple quadrupole ICP-MS in oxygen mode was used to remove such interferences. A second dataset for Se determination at NRC (SA, ICP-AES, n=52) also showed slightly higher RSD but signal intensities were very low in the sample. Similar to Mg, we believe that this variation is also related to the characterization procedure.

The uncertainty component due to homogeneity was evaluated, for both VITA-1 and VITB-1, using the DerSimonian-Laird random effects model [27]. In the framework of this statistical model, the observed result for any analyte, $x_i$, is modeled as a juxtaposition of the true (unknown) value, the random effect due to homogeneity and random effect due to measurement. Results from the homogeneity assessment were included in the calculation of the certified values.

## Stability

A short-term stability study was carried out at NRC using an isochronous experimental design [28] to simulate the effect of the potential elevated temperatures experienced during shipment. For that, multivitamin samples were placed in four different environments (freezer at -20°C, fridge at +4°C, laboratory at +20°C, and oven at + 37°C) for 14 days. The reference temperature was +4°C (which is the storage condition for both VITA-1 and VITB-1). After 14 days, samples were analysed. No measurable degradation was observed during this period at any temperature as exemplified in Figure 3 for some water-soluble vitamins in VITA-1.

The long-term stability was assessed over a period of two years for quantity values for metals and over a period of one year for the water-soluble vitamins (biotin, folic acid, pantothenic acid, pyridoxine HCl, riboflavin, thiamine HCl, niacinamide, and cyanocobalamin). Samples were stored at +4°C. No change in measured mass fraction for metals and vitamins was observed over this period. Uncertainty components for long and short-term stability were thus considered negligible and are thus not included in the uncertainty budget. A shelf life of 2 years was assigned for both materials. Long-term stability will continue to be monitored at NRC.

## Validation of methods for trace elements at NRC

Validation of the methods used at NRC was performed by analysis of QC samples with similar matrices and results obtained were in agreement with the certified values. Figure 4 presents the ratio between the measured and certified value for various analytes following the analysis of NIST SRM 3280. Results were obtained using ID-ICP-MS, SA-ICP-MS, and SA-ICP-AES at NRC. Error bars represent one standard deviation (SD). It could be noted that most of the results are within the 10% range of the certified values, with exception of As (method 2, HG, SA-ICP-MS, certified value 0.132 ± 0.044 µg/g), and Se (method 2, SA, ICP-AES, certified value 17.42 ± 0.45 µg/g).

Validation of the methods developed for the determination of cyanocobalamin, selenomethionine, and chromium picolinate in multivitamins can be found elsewhere. [13] [23] [29]

It should be noted that the observed bias between results obtained for the QC samples and the certified values were compared to the respective measurement uncertainty and, if necessary, incorporated into the overall uncertainty budget.

## Value assignment

Tables 3 and 4 present the results obtained for each method as well the consensus values and the category of the value.

Quantity values presented in the NRC certificate of analysis fall into three categories: certified, reference

and information values. Certified values are considered to be those for which the National Research Council Canada (NRC) has the highest confidence in accuracy and that all known and suspected sources of bias have been taken into account and are reflected in the stated expanded uncertainties. Certified values are the best estimate of the mean and uncertainty. As a result, certified values were provided for 16 elements (As, B, Ca, Cr, Co, Cu, Fe, Pb, Mg, Mn, Hg, Mo, P, K, Se, Zn) and cyanocobalamin in both VITA-1 and VITB-1.

Reference values are those for which insufficient data are available to provide a comprehensive estimate of uncertainty. Br, Cl, chromium picolinate, iodine, selenomethionine, biotin, folic acid, niacinamide, pantothenic acid, pyridoxine HCl, riboflavin, and thiamine HCl were presented in VITA-1 and VITB-1 certificates as reference values. Br, Cl and I analysis were not performed at NRC but by an expert laboratory along with several QC samples. From the vitamins listed before, folic acid was not determined by NRC but good agreement was observed between two data from external collaborators and the gravimetry data.

At last, information values are those for which insufficient data are available to provide any estimate of uncertainty. They usually have results from either one technique or from two techniques that were not sufficiently independent as required for certification, or if the disagreement among the methods was greater than expected for certified values. Mass fraction of Cd, Na, and Sr were presented in both VITA-1 and VITB-1 certificates as information value as well quantity values for ascorbic acid, alpha tocopherol, beta carotene, ergocalciferol, lutein, phylloquinone, and retinol acetate.

For the characterization of the proposed candidate CRMs VITA-1 and VITB-1, value assignments were determined by combining measurement results obtained internally with external collaborators' results and are presented as consensus values and expanded uncertainties ($U_{(k=2)}$). Individual sets of data are presented as mean and characterization uncertainty ($u_{(k=1)}$). As many as five data sets were combined to calculate the assigned value. As mentioned before, several blinded samples from each candidate CRM were provided to the external collaborators along with blinded QC samples to support the validity of the analyte measurement data.

All data reported was regarded as being equal and were evaluated without weighting. Each set of measurement results had to prove their capabilities by analyzing QC samples. If their results for the QC samples were not in agreement with the certified value, their uncertainties for characterization would be inflated accordingly.

The consensus values were obtained by combining the results from individual methods using a random effects statistical model (DerSimonian-Laird). Details can be consulted elsewhere[27]. Note that the uncertainties of the individual method results were adjusted (expanded) based on the QC results.

**Uncertainty evaluation**

The expanded uncertainty ($U$) is equal to $U = ku_c$ where $u_c$ is the combined standard uncertainty calculated according to the JCGM Guide [1,2] and $k$ is the coverage factor. A coverage factor of two (2) was applied. It is

intended that $U_{CRM}$ accounts for every aspect that reasonably contributes to the uncertainty of the measurement.

The standard combined uncertainty, $u_c$, was determined using the following equation:

$$u^2_c = u^2_{char} + u^2_{hom} + u^2_{method}$$

where:

$u_{char}$ is the batch characterization,

$u_{hom}$ uncertainties related to possible between-tablet variation

$u_{method}$ uncertainties related to inconsistency between the various measurement methods.

Expressed as standard uncertainties, these components are listed in Table 5 for VITA-1 and table 6 for VITB-1

Consensus values for certified elements in both VITA-1 and VITB-1 are traceable to the SI through gravimetrically-prepared primary standards whose purities were established by glow discharge mass spectrometry (GDMS) at NRC[30], international measurement intercomparisons and to NIST SRM 3280 [18]. The purity of cyanocobalamin was assigned traceable to NIST SRM 350b (benzoic acid), and the purity of selenomethionine to NIST SRM 84L (potassium hydrogen phthalate), both via proton quantitative nuclear magnetic resonance spectroscopy ($^1$H-qNMR), validated through international measurement inter-comparisons.

**Conclusions**

Two new multivitamin Certified Reference Materials are now available from National Research Council Canada [31, 32]. VITA-1 [31] and VITB-1 [32] are, respectively, low- and elevated-level multivitamin Certified Reference Materials that are certified for minerals and vitamins. These Certified Reference Materials are intended for the calibration of procedures and the development of methods for the determination of trace, matrix constituents and vitamins in multivitamins materials or similar matrices.

Individual tablets could be analyzed without the need of homogenization of the sample. Quantity values for both VITA-1 and VITB- are presented as mg/kg as well as µg/tablet. Homogeneity and stability were assessed and included in the calculation of the certified values. Quantity value assignment was based on the use of at least two independent methods, using data generated at NRC and data from external collaborators for confirmation purposes. As a result of this campaign, value assignment was possible for a total of 39 analytes: 24 trace elements and 15 vitamins. Among them, 17 are classified as certified values (16 trace metals and one vitamin), 12 as reference values (5 trace metals and 7 vitamins), and 10 information values (3 for trace metals

and 7 for vitamins) for both VITA-1 and VITB-1.

**Conflicts of interest**

There are no conflicts to declare.

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**Table 1.** Product specification for VITA-1 and VITB-1*

| | VITA-1 | VITB-1 |
|---|---|---|
| ***Physical tests*** | | |
| punch size | Modified oval | Modified oval |
| average wt. of tablet | 2.57 g/tablet | 2.62 g/tablet |
| thickness | 11.5 (10.9-12.1)mm | 11.0 (10.45-11.55)mm |
| disintegration | < 60 min | < 60 min |
| hardness | 27.0 (22.0-32.0) kP | 27.0 (22.0-32.0) kP |
| friability | < 0.7% | < 0.7% |
| ***Input (µg/tablet)*** | | |
| beta carotene 20% (DC/AF, HNBC20B) | 150 (143-158) | 300 (285-315) |
| retinol acetate (500,000 IU/g) | 300 (285-315) | 600 (570-630) mcg/tablet |
| thiamine HCl (DC 98%) | 750 (713-788) | 1500 (1425-1575) |
| riboflavim (96% DC) | 850 (808-893) | 1700 (1615-1785) |
| niacinamide (USP DC 99%) | 10000 (9500-10500) | 20000 (19000-21000) |
| D-calcium pantothenate (USP 98%) | 5000 (4750-5250) | 10000(9500-10500) |
| pyridoxine hydrochloride (DC Grade, 98%) | 1000 (950-1050) | 2000 (1900-2100) |
| biotin, (min 97.5%) | 20 (19-21) | 40 (38-42) |
| folic acid (USP 97%) | 200 (190-210) | 400 (380-420) |
| cyanocobalamin, (USP 97%) | 3 (2.85-3.15) | 6 (5.7-6.3) |
| ascorbic acid (DC grade, 97%) | 30000 (28500-31500) | 60000 (57000-63000) |
| ergocalciferol | 5.00 (4.75-5.25) | 10.00(9.50-10.50) |
| D-Alpha Tocopheryl Acetate (700 IU/g) | 5000 (4750-5250) | 10000 (9500-10500) |
| phylloquinone | 15.00 (14.25-15.75) | 30.00 (28.50-31.50) |
| lycopene (5%) | 750 (713-788) | 1500 (1425-1575) |
| lutein (5%) | 150 (143-158) | 300 (285-315) |
| co-enzyme Q10 (powder 98%) | 3500 (3325-3675) | 7000 (6650-7350) |
| boron (as boron citrate, 5% B) | 100 (95-105) | 200 (190-210) |
| calcium (as calcium carbonate, 37% Ca) | 10307 (9792 – 10822) | 20614 (19583-21645) |
| calcium (as dicalcium phosphate anydrous 29.6% Ca, 22.8% P) | 64693 (61456 – 67928) | 129386 (122917-135855) |
| phosphate (as dicalcium phosphate | 50000 (47500 – 52500) | 100000 (95000 – 105000) |

| | | |
|---|---|---|
| anhydrous 29.6% Ca, 22.8% P | | |
| chromium (as chromium picolinate 12% Cr) | 25 (23.75-26.25) | 50 (47.5-52.5) |
| copper (as copper (II) oxide 75% Cu) | 250 (238-263) | 500 (475-525) |
| iodide (as potassium iodide 76.45%) | 75 (71.25-78.75) | 150 (143-158) |
| iron (as iron (II) fumarate 30% Fe) | 9000 (8550-9450) | 18000 (17100 – 18900) |
| magnesium (as magnesium oxide 58% Mg) | 45000 (42750-47250) | 90000 (85500 – 94500) |
| manganese (as manganese sulfate 32.5% Mn) | 1000 (950-1050) | 2000 (1900-2100) |
| molybdenum (as molybdenum amino acid chelated 0.2% Mo) | 37.50 (35.63-39.38) | 75 (71.25-78.75) |
| potassium (as potassium citrate 35.6% K) | 30000 (28500-31500) | 60000 (57000-63000) |
| selenium (as yeast 1220 µg/g) | 10 (9.5-10.5) | 20 (19-21) |
| zinc (as zinc oxide) | 6000 (5700-6300) | 12000 (11400-12600) |

*Value in parenthesis represents limits for 5% tolerance

**Table 2.** Analytical methods used for the certification of VITA-1 and VITB-1

| | Method 1 (NRC) | Method 2 (NRC) | Method 3 | Method 4 | Method 5 |
|---|---|---|---|---|---|
| arsenic | SA, ICP-MS ($O_2$ mode)[a] | HG, ICP-MS (HR mode) [b] | EC, ICP-MS[c] | EC, ICP-MS[c] | |
| boron | ID, ICP-MS (HR mode)[b] | SA, ICP-AES | EC, ICP-MS[c] | EC, ICP-MS[c] | |
| bromine | ND | ND | EC, ICP-MS[c] | | |
| cadmium | ID, ICP-MS ($O_2$ mode)[a] | | | | |
| calcium | SA, ICP-MS (HR mode)[b] | SA, ICP-AES | EC, ICP-MS[c] | Gravimetry[c] | |
| chlorine | ND | ND | EC, ICP-MS[c] | | |
| chromium | ID, ICP-MS (MR mode)[b] | SA, ICP-AES | EC, ICP-MS[c] | Gravimetry[c] | |
| chromium picolinate (as Cr) | SA, LC-ICP-MS | ND | Gravimetry[c] | | |
| cobalt | SA, ICP-MS (HR mode)[b] | SA, ICP-MS[a] | EC, ICP-MS[c] | | |
| copper | ID, ICP-MS (MR mode)[b] | SA, ICP-AES | EC, ICP-MS[c] | EC, ICP-MS[c] | Gravimetry[c] |
| iodine | ND | ND | EC, ICP-MS[c] | Gravimetry[c] | |
| iron | ID, ICP-MS (MR mode)[b] | SA, ICP-AES | EC, ICP-MS[c] | Gravimetry[c] | |
| lead | ID, ICP-MS (LR mode)[b] | SA,ICP-MS | EC, ICP-MS[c] | | |
| magnesium | SA, ICP-MS (MR mode) [b] | SA, ICP-AES | EC, ICP-MS[c] | Gravimetry[c] | |
| manganese | SA, ICP-MS (HR mode)[b] | SA, ICP-AES | EC, ICP-MS[c] | EC, ICP-MS[c] | Gravimetry[c] |
| mercury | ID, ICP-MS (LR mode)[b] | SA, ICP-MS[a] | | | |
| molybdenum | ID, ICP-MS (MR mode)[b] | SA, ICP-AES | EC, ICP-MS[c] | | |

| | | | | | |
|---|---|---|---|---|---|
| phosphorus | SA, ICP-MS (HR mode)[b] | SA, ICP-AES | EC, ICP-MS[c] | | |
| potassium | SA, ICP-MS (HR mode)[b] | SA, ICP-AES | EC, ICP-MS[c] | EC, ICP-MS[c] | Gravimetry[c] |
| selenium | ID, ICP-MS ($O_2$ mode)[a] | SA, ICP-AES | EC, ICP-MS[c] | EC, ICP-MS[c] | |
| selenomethionine | SA,LC-ICP-MS | | | | |
| sodium | ND | ND | EC, ICP-MS[c] | EC, ICP-MS[c] | |
| strontium | ND | ND | EC, ICP-MS[c] | EC, ICP-MS[c] | |
| zinc | ID, ICP-MS (MR mode)[b] | SA, ICP-AES | EC, ICP-MS[c] | EC, ICP-MS[c] | Gravimetry[c] |
| ascorbic acid | ND | ND | HPLC-UV[c] | Gravimetry[c] | |
| alpha tocopherol | ND | ND | HPLC-FLD[c] | Gravimetry[c] | |
| biotin | EC, IS, LC-MS/MS | ND | Microbiological[c] | Gravimetry[c] | |
| beta carotene | ND | ND | HPLC-UV[c] | Gravimetry[c] | |
| cyanocobalamin | SA,LC-ICP-MS | ID,LC-MS/MS | LC-MS/MS | Microbiological [c] | Gravimetry[c] |
| ergocalciferol | ND | ND | LC-MS[c] | | |
| folic acid | ND | ND | LC-MS/MS[c] | Microbiological[c] | Gravimetry[c] |
| niacinamide | EC,LC-MS/MS | HPLC-UV[c] | HPLC-UV[c] | Gravimetry[c] | |
| lutein | ND | ND | HPLC-UV[c] | | |
| pantothenic acid | EC,LC-MS/MS | ND | HPLC-UV[c] | Gravimetry[c] | |
| phylloquinone | | ND | LC-MS[c] | Gravimetry[c] | |
| pyridoxine HCl | EC, LC-MS/MS | HPLC-UV[c] | HPLC-UV[c] | LC-MS-MS[c] | Gravimetry[c] |
| retinol acetate | | ND | LC-MS[c] | | |
| riboflavin | EC, LC-MS/MS | HPLC-UV[c] | HPLC-UV[c] | LC-MS-MS[c] | Gravimetry[c] |
| thiamine HCl | EC, LC-MS/MS | HPLC-UV[c] | HPLC-UV[c] | LC-MS-MS[c] | Gravimetry[c] |

EC external calibration, HG hydride generation, ID double isotope dilution, IS internal standard, SA standard addition calibration, LR low resolution, MD middle resolution, HR high resolution, ND not determined

[a] triple quadrupole ICP-MS, [b] Double focusing sector field ICP-MS, [c] external collaborators

**Table 3**. Summary of results for VITA-1, results reported as mass fraction mg/kg

| | Method 1, $u_{(K=1)}$ | Method 2, $u_{(K=1)}$ | Method 3, $u_{(K=1)}$ | Method 4, $u_{(K=1)}$ | Method 5, $u_{(K=1)}$ | Consensus mass fraction, mg/kg $U_{(K=2)}$ | Type of value |
|---|---|---|---|---|---|---|---|
| ***Element/ compound*** | | | | | | | |
| As | 0.047 ± 0.004 | 0.044 ± 0.006 | 0.073 ± 0.038 | | | 0.046 ± 0.008 | C |
| B | 86.3 ± 8.7 | 81.8 ± 6.6 | 84.0 ± 5.2 | | | 84 ± 10 | C |
| Br | ND | ND | 0.3 ± 0.0 | | | 0.28 ± 0.08 | R |
| Cd | 0.020 ± 0.01 | | | | | 0.02 | I |
| Ca | 29042 ± 445 | 27129 ± 785 | 28225 ± 960 | 29473 ± 737 | | 28 600 ± 4 400 | C |
| Cl | ND | ND | 222 ± 14 | | | 222 ± 32 | R |
| Cr | 10.52 ± 0.15 | 10.50 ± 0.14 | 9.78 ± 0.26 | 9.78 ± 0.26 | | 10.2 ± 0.6 | C |
| Cr picolinate (as Cr) | 9.7 ± 0.7 | ND | 9.7 ± 0.2 | | | 9.7 ± 0.4 | R |
| Co | 0.6 ± 0.0 | 0.6 ± 0.0 | 0.5 ± 0.1 | | | 0.573 ± 0.046 | C |
| Cu | 100.3 ± 1.7 | 99.8 ± 1.6 | 100.3 ± 1.9 | 93.0 ± 2.0 | 97.4 ± 2.4 | 98 ± 4 | C |
| I | ND | ND | 28.7 ± 3.6 | 29.2 ± 0.7 | | 29 ± 8 | R |
| Fe | 3636.3 ± 23 | 3476.3 ± 65 | 3550 ± 173 | 3505 ± 88 | | 3560 ± 280 | C |
| Pb | 0.06 ± 0.00 | 0.06 ± 0.02 | 0.07 ± 0.01 | | | 0.064 ± 0.008 | C |
| Mg | 19428 ± 429 | 17899 ± 253 | 18 775 ± 350 | 17 947 ± 449 | | 18 500 ± 1600 | C |
| Mn | 389.6 ± 4.6 | 413.0 ± 42.9 | 367.3 ± 11 | 443.3 ± 25.2 | 389.4 ± 9.7 | 389 ± 20 | C |
| Hg | 0.007 ± 0.002 | < 0.004 | | | | < 0.004 | C |
| Mo | 18.2 ± 0.3 | 17.6 ± 0.9 | 18.0 ± 2.2 | 17.0 ± 5.28 | | 18.1 ± 2 | C |
| P | 19209 ± 256 | 18825± 407 | 18333 ± 577 | | | 19 000 ± 800 | C |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| K | 12604 ± 604 | 10698 ± 371 | 12950 ± 520 | 13333 ± 1155 | 11691 ± 292 | 12 100 ± 1000 | C |
| Se | 4.1 ± 0.2 | 4.6 ± 0.9 | 4.4 ±1.0 | 5.0 ± 1.0 | | 4.2 ± 1.4 | C |
| SeMet (as Se) | 3.0 ± 0.3 | | | | | 3.0 ± 1.2 | R |
| Na | ND | ND | 1000± 0.00 | 990 ± 29.75 | | 1000 | I |
| Sr | ND | ND | 9.18 ± 0.05 | 8.00 ± 0.07 | | 9 | I |
| Zn | 2405 ± 138 | 2378 ± 69 | 2325 ± 50 | 2400 ± 54 | 2336 ± 58 | 2360 ± 80 | C |
| ***Vitamins*** | | | | | | | |
| ascorbic acid | ND | ND | 10700± 406 | 11682 ± 292 | | 11 190 | I |
| alpha tocopherol | ND | ND | 1580 ± 111 | 1947 ± 49 | | 1760 | I |
| biotin | 6.9 ± 0.6 | ND | 8.4 ± 0.7 | 8.3 ± 0.4 | 7.8 ± 0.2 | 7.9 ± 0.4 | R |
| beta carotene | ND | ND | 67.3 ± 1.6 | 58.4 ± 1.5 | | 60 | I |
| cyanocobalamin | 2.8 ± 0.3 | 2.6 ± 0.3 | 2.2 ± 0.1 | 2.5 ± 0.3 | | 2.4 ± 0.4 | C |
| ergocalciferol | ND | ND | 0.06 ± 0.01 | | | 0.06 | I |
| folic acid | ND | ND | 65 ± 13 | 70 ± 6 | 78 ± 2 | 76 ± 6 | R |
| lutein | ND | ND | 0.65 ± 0.15 | | | 0.7 | I |
| niacinamide | 3463± 258 | 3670 ± 130 | 4414 ± 771 | 3912 ± 38 | 3894 ± 97 | 3840 ± 172 | R |
| pantothenic acid | 1955 ± 72 | ND | 1970 ± 121 | 1880 ± 113 | 1947 ± 49 | 1940 ± 120 | R |
| phylloquinone | ND | ND | 5.48 ± 0.38 | 5.84 ± 0.15 | | 6 | I |
| pyridoxine HCl | 286 ± 46 | 464 ± 20 | 363 ± 12 | 414 ± 20 | 389 ± 10 | 390 ± 40 | R |
| retinol acetate | ND | ND | 48.6 ± 6.5 | | | 50 | I |
| riboflavin | 329 ± 6 | 328 ± 2 | 316 ± 40 | 414 ± 20 | 331 ± 8 | 342 ± 24 | R |
| thiamine HCl | 245 ± 12 | 296 ± 11 | 334 ± 41 | 326 ± 22 | 292 ± 7 | 290 ± 40 | R |

ND- not determined, C- certified, R- Reference, I- Information,

**Table 4.** Summary of results for VITB-1, results reported as mass fraction mg/kg

| | Method 1, $u_{(K=1)}$ | Method 2, $u_{(K=1)}$ | Method 3, $u_{(K=1)}$ | Method 4, $u_{(K=1)}$ | Method 5, $u_{(K=1)}$ | Consensus mass fraction, mg/kg $U_{(K=2)}$ | Type of value |
|---|---|---|---|---|---|---|---|
| ***Element/ compound*** | | | | | | | |
| As | 0.09 ± 0.02 | 0.10 ± 0.01 | 0.11 ± 0.02 | | | 0.103 ± 0.026 | C |
| B | 167 ± 17 | 162 ± 3 | 163 ± 6 | | | 163 ± 8 | C |
| Br | ND | ND | 0.4 ± 0.00 | | | 0.35 ± 0.08 | R |
| Cd | 0.04 ± 0.01 | | | | | 0.04 | I |
| Ca | 56809 ± 940 | 56540 ± 1084 | 59325 ± 2380 | 57843 ± 1446 | | 57 100 ± 2 400 | C |
| Cl | ND | ND | 319.1 ± 19.5 | | | 319 ± 42 | R |
| Cr | 20.09 ± 0.28 | 20.75 ± 0.28 | 18.75 ± 0.5 | 19.11 ± 0.48 | | 19.8 ± 1.2 | C |
| Cr picolinate (as Cr) | 18.81 ± 0.78 | ND | 19.11 ± 0.48 | | | 19 ± 0.8 | R |
| Co | 1.0 ± 0.0 | 1.0 ± 0.1 | 0.9 ± 0.0 | | | 0.97 ± 0.1 | C |
| Cu | 206.1 ± 3.5 | 201.2 ± 3.3 | 200.0 ± 4.7 | 186.7 ± 5.8 | 191.1 ± 4.8 | 198 ± 10 | C |
| I | ND | ND | 56.2 ± 4.6 | 57.3 ± 1.4 | | 57 ± 12 | R |
| Fe | 7029 ± 39 | 7274 ± 168 | 7067± 346 | 6878± 172 | | 7 030 ± 120 | C |
| Pb | 0.11 ± 0.01 | 0.10 ± 0.02 | 0.11± 0.01 | | | 0.11 ± 0.01 | C |
| Mg | 37975± 839 | 36082 ± 541 | 37675 ± 579.5 | 34806 ± 870 | | 36 700 ± 1600 | C |
| Mn | 831 ± 86.5 | 812 ± 9.7 | 785± 23.8 | 797 ± 32.6 | 764.2 ± 19.1 | 795 ± 32 | C |
| Hg | 0.005± 0.0006 | < 0.004 | | | | < 0.004 | C |
| Mo | 35.4 ± 0.6 | 35.9 ± 1.9 | 41.5 ± 3.0 | 34.7 ±10.8 | | 36.2 ± 3.2 | C |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| P | 38666 ± 502 | 37253 ± 731 | 36000 ± 1000 | | | 37 500 ± 1 600 | C |
| K | 24778± 1854 | 24835 ± 1505 | 26150 ±1212 | 25333 ± 3055 | 22945 ± 574 | 24 400 ± 1 800 | C |
| Se | 8.2 ± 0.3 | 7.4 ± 0.9 | 8.5 ± 0.8 | 9.0 ± 1.0 | | 8.2 ± 0.8 | C |
| SeMet (as Se) | 6.6 ± 1.3 | | | | | 6.6 ± 2.6 | R |
| Na | ND | ND | 1100 ± 0.00 | 990 ± 30 | | 1050 | I |
| Sr | ND | ND | 19.00 ± 0.82 | 13.67 ±1.15 | | 16 | I |
| Zn | 4631 ± 267 | 4515 ± 235 | 4550 ± 58 | 4600 ± 200 | 4585 ± 115 | 4560 ± 100 | C |
| ***Vitamins*** | | | | | | | |
| ascorbic acid | ND | ND | 21900 ± 830 | 22927± 573 | | 22 414 | I |
| alpha tocopherol | ND | ND | 3300 ± 233 | 3821 ± 95 | | 3 580 | I |
| biotin | 14.55± 1.31 | ND | 14.70 ± 1.19 | 16.1 ± 0.83 | 15.28 ± 0.38 | 15.3 ± 1.0 | R |
| beta carotene | ND | ND | 114± 2.66 | 115 ± 2.87 | | 110 | I |
| cyanocobalamin | 1.72 ± 0.16 | 1.75 ± 0.22 | 1.64 ± 0.06 | 1.74 ± 0.13 | | 1.7 ± 0.6 | C |
| ergocalciferol | ND | ND | 0.13 ±0.02 | | | 0.13 | I |
| folic acid | ND | ND | 149± 29.5 | 130 ± 11.5 | 152 ± 3.8 | 146 ± 16 | R |
| niacinamide | 7520 ± 267 | ND | 9155 ± 1600 | 7677 ± 126 | 7624 ± 191 | 7 650 ± 420 | R |
| lutein | ND | ND | 1.55 ± 0.36 | | | 1.6 | I |
| pantothenic acid | 3748 ± 139 | ND | 4020 ± 248 | 3960 ± 239 | 3821 ± 95 | 3830 ± 200 | R |
| phylloquinone | ND | ND | 9.79 ± 0.69 | 11.46 ± 0.29 | | 11 | I |
| pyridoxine HCl | 935 ± 150 | 900 ± 40 | 975 ± 32 | 902± 85 | 764 ± 19 | 890 ± 100 | R |
| retinol acetate | ND | ND | 83.20 ±.11.06 | | | 80 | I |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| riboflavin | 677± 5 | ND | 762 ± 98 | 688 ± 17 | 650 ± 16 | 674 ± 30 | R |
| thiamine HCl | 662 ± 25 | ND | 787 ± 96 | 655 ± 20 | 573 ± 14 | 642 ± 64 | R |

ND- not determined, C- certified, R- Reference, I- Information

**Table 5. Uncertainty Components for VITA-1**

| | $u_c$, mg/kg | $u_{char}$, mg/kg | $u_{hom}$, mg/kg | $u_{method}$, mg/kg |
|---|---|---|---|---|
| ***Element/ compound*** | | | | |
| As | 0.004 | 0.003 | 0.003 | 0 |
| B | 5 | 4 | 3 | 0 |
| Br | 0.04 | 0.02 | 0.03 | 0 |
| Ca | 2200 | 300 | 2200 | 300 |
| Cl | 16 | 14 | 7 | 0 |
| Cr | 0.3 | 0.1 | 0.2 | 0.1 |
| Cr picolinate (as Cr) | 0.2 | 0.2 | 0 | 0 |
| Co | 0.023 | 0.017 | 0.016 | 0 |
| Cu | 2 | 1 | 2 | 1 |
| I | 4 | 1 | 4 | 0 |
| Fe | 140 | 20 | 130 | 40 |
| Pb | 0.004 | 0.003 | 0.002 | 0 |
| Mg | 800 | 200 | 700 | 300 |
| Mn | 10 | 4 | 8 | 5 |
| Mo | 1 | 0.3 | 1 | 0 |
| P | 400 | 200 | 300 | 100 |
| K | 500 | 200 | 300 | 400 |
| Se | 0.7 | 0.2 | 0.7 | 0 |
| selenomethionine | 0.6 | 0.3 | 0.5 | 0 |
| Zn | 40 | 30 | 30 | 0 |
| ***vitamins*** | | | | |
| biotin | 0.2 | 0.2 | 0.1 | 0.1 |
| cyanocobalamin | 0.2 | 0.1 | 0.2 | 0.1 |
| folic acid | 3 | 2 | 2 | 1 |
| niacinamide | 86 | 34 | 69 | 39 |
| pantothenic acid | 60 | 40 | 40 | 0 |
| pyridoxine HCl | 20 | 10 | 10 | 20 |
| riboflavin | 12 | 2 | 7 | 9 |
| thiamine HCl | 20 | 10 | 20 | 10 |

**Table 6. Uncertainty Components for VITB-1**

| | $u_c$, mg/kg | $u_{char}$, mg/kg | $u_{hom}$, mg/kg | $u_{method}$, mg/kg |
|---|---|---|---|---|
| ***Element/ compound*** | | | | |
| As | 0.013 | 0.009 | 0.009 | 0 |
| B | 4 | 3 | 3 | 0 |
| Br | 0.04 | 0.03 | 0.03 | 0 |
| Ca | 1200 | 600 | 1000 | 0 |
| Cl | 21 | 19 | 10 | 0 |
| Cr | 0.6 | 0.2 | 0.4 | 0.4 |
| Cr picolinate (as Cr) | 0.4 | 0.4 | 0 | 0 |
| Co | 0.05 | 0.03 | 0.03 | 0.02 |
| Cu | 5 | 2 | 3 | 3 |
| I | 6 | 1 | 6 | 0 |
| Fe | 60 | 40 | 40 | 0 |
| Pb | 0.004 | 0.004 | 0.001 | 0 |
| Mg | 800 | 300 | 500 | 600 |
| Mn | 16 | 8 | 12 | 6 |
| Mo | 1.6 | 0.6 | 1.4 | 0.6 |
| P | 800 | 400 | 400 | 600 |
| K | 900 | 500 | 500 | 500 |
| Se | 0.4 | 0.2 | 0.4 | 0 |
| selenomethionine | 1.3 | 1.3 | 0.3 | 0 |
| Zn | 50 | 50 | 0 | 0 |
| ***vitamins*** | | | | |
| biotin | 0.5 | 0.3 | 0.4 | 0 |
| cyanocobalamin | 0.3 | 0.1 | 0.3 | 0 |
| folic acid | 8 | 4 | 4 | 6 |
| niacinamide | 210 | 100 | 180 | 0 |
| pantothenic acid | 100 | 70 | 70 | 0 |
| pyridoxine HCl | 50 | 10 | 20 | 50 |
| riboflavin | 15 | 5 | 14 | 4 |
| thiamine HCl | 32 | 11 | 13 | 27 |

## Figure Captions

**Figure 1.** Initial homogeneity assessment for vitamins in VITA-1. Dotted line represents the specification for each water soluble vitamin (as indicated in Table 2) a) Pyridoxine HCl b) Thiamine HCl, c)Riboflavin and d) niacinamide

**Figure 2**. Homogeneity assessment of VITA-1 (blue) and VITB-1(orange). Solid columns refer to ICP-MS measurement, dashed columns refers to ICP-AES measurements, stripped columns refer to LC-MS-MS. The number on top of each column is the number of tablets that was used for the homogeneity assessment.

**Figure 3**. Short term stability for the following water soluble vitamins in VITA-1: thiamine HCl, niacinamide, riboflavin, ascorbic acid, and folic acid

**Figure 4**. Comparison of measured values obtained at NRC to certified values in NIST SRM 3280. Solid fill refers to the first method performed at NRC (HR-ICP-MS for most elements), open symbols refer to the second method performed at NRC (see table 2). Error bars represent one standard deviation (SD). Doted lines represents 10% range of certified values.

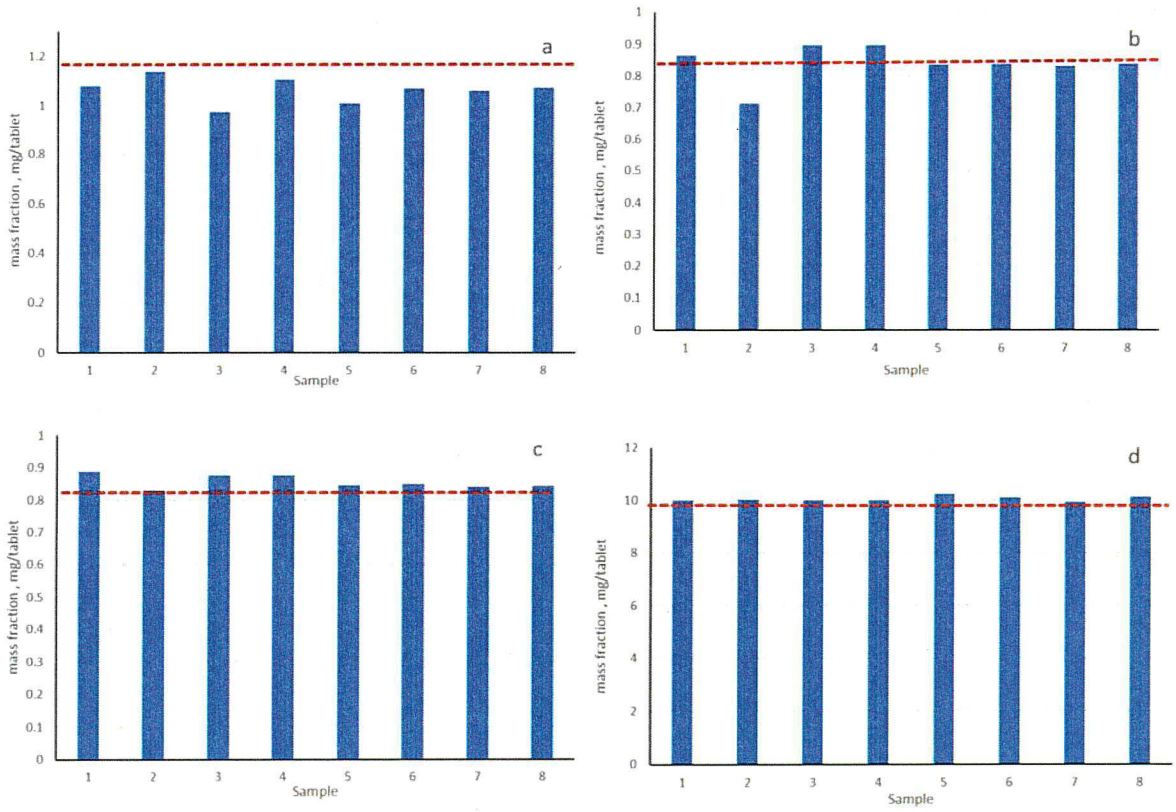


**Figure 1.** Initial homogeneity assessment for vitamins in VITA-1. Dotted line represents the specification for each water soluble vitamin (as indicated in Table 2) a) Pyridoxine HCl b) Thiamine HCl, c)Riboflavin and d) niacinamide

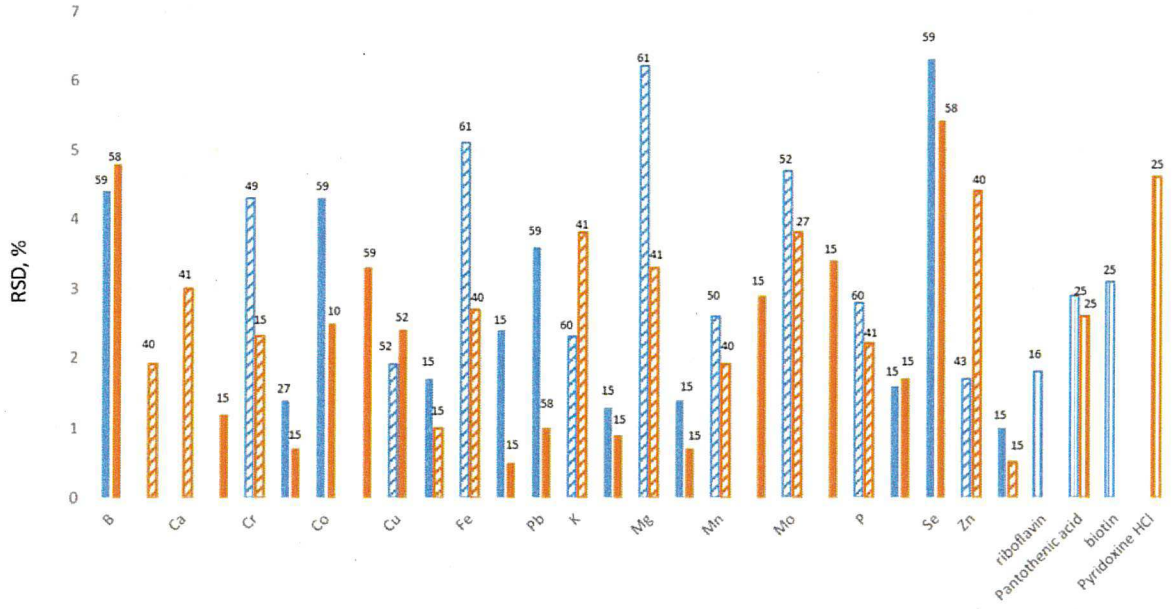


**Figure 2.** Homogeneity assessment of VITA-1 (blue) and VITB-1(orange). Solid columns refer to ICP-MS measurement, dashed columns refers to ICP-AES measurements, stripped columns refer to LC-MS-MS. The number on top of each column is the number of tablets that was used for the homogeneity assessment.

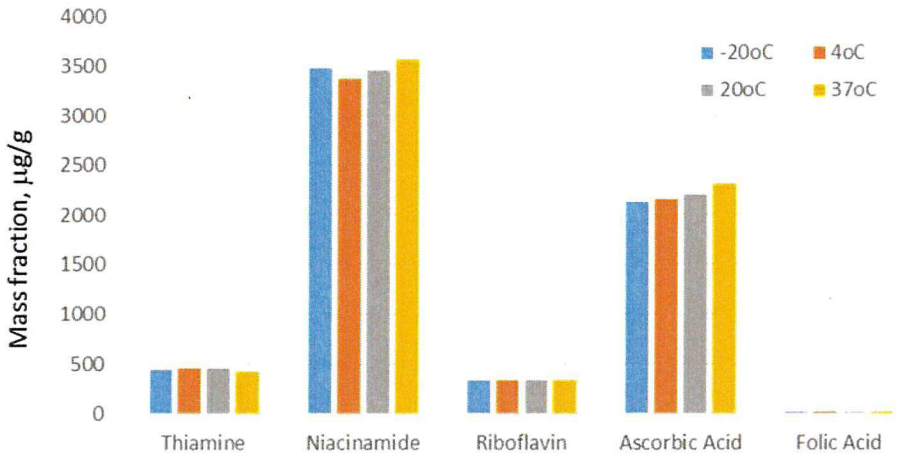


**Figure 3**. Short term stability for the following water soluble vitamins in VITA-1: thiamine HCl, niacinamide, riboflavin, ascorbic acid, and folic acid

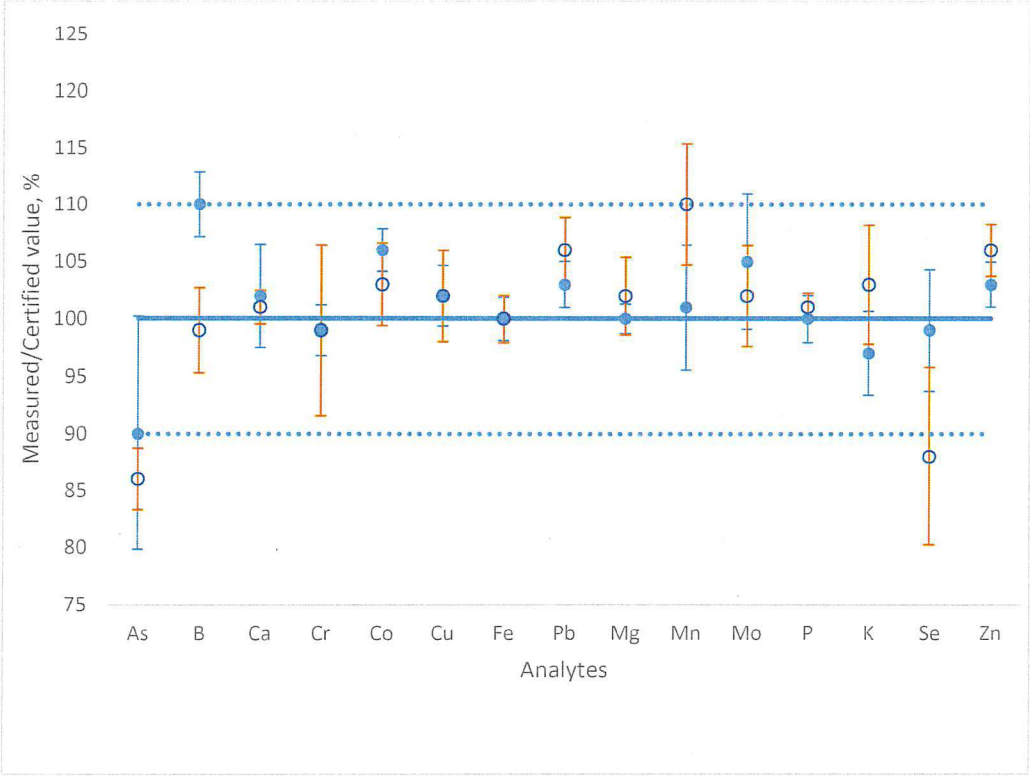


**Figure 4.** Comparison of measured values obtained at NRC to certified values in NIST SRM 3280. Solid fill refers to the first method performed at NRC (HR-ICP-MS for most elements), open symbols refer to the second method performed at NRC (see table 2). Error bars represent one standard deviation (SD). Doted lines represents 10% range of certified values.

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