ESTIMATION OF MEASUREMENT UNCERTAINTY: A CASE STUDY FOR DETERMINATION OF CALCIUM (CA) CONTENT IN POWDERED TONIC FOOD DRINK USING ION CHROMATOGRAPHY

Estimasi Ketidakpastian Pengukuran: Studi Kasus untuk Penentuan Kalsium (Ca) Konten dalam Makanan Minuman Tonik Bubuk Menggunakan Ion Kromatografi

Harry Budiman and Nuryatini

Research Centre for Chemistry, Indonesian Institute of Sciences
Kawasan PUSPIPTEK Serpong, Banten 15314, Indonesia
e-mail: harry.budiman@lipi.go.id

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Abstract

In participation of the laboratory on Proficiency Testing Scheme: Analysis of Proximates (Moisture, Fat, Protein, and Ash) and Minerals (Iron, Calcium, and Sodium) in Powdered Tonic Food Drink held by Food Nutrition Research Institute, Department of Science and Technology, Philippines (FNRI-DOST), Research Centre for Chemistry-LIPI conducted the estimation of uncertainty measurement in the determination of calcium (Ca) content in powdered tonic food drink using ion chromatography. The estimation of uncertainty measurement of laboratory participant of proficiency is needed for comparison with the standard deviation for proficiency assesment (σ_P) that represents fitness-for-purposes over a whole application sector in proficiency testing scheme. The evaluation of the uncertainty measurement included the several stages of procedure which consisted of the specification of measurand, identification of source of uncertainty, calculation of standard uncertainty, combined uncertainty and expanded uncertainty. The measurement result shows that the Ca content in powdered tonic food drink sample was 541.4 mg/100g, with the expanded uncertainty measurement 41.1 mg/100g (coverage factor, k = 2, at confidence level 95%). The repeatability of method measurement gave the major contribution to the uncertainty of the final results. Furthermore, the measurement result was within the consensus value and its expanded uncertainty that indicates the “satisfactory” result of Research Centre for Chemistry-LIPI in this proficiency testing scheme.

Keywords: proficiency testing, uncertainty of measurement

Abstrak

Dalam partisipasinya pada skema uji profisiensi: Analysis of Proximates (Moisture, Fat, Protein, and Ash) and Minerals (Iron, Calcium, and Sodium) in Powdered Tonic Food Drink *yang diselenggarakan oleh* Food Nutrition Research Institute, Department of Science and Technology, Philippines (FNRI-DOST)*, laboratorium Pusat Penelitian Kimia-LIPI telah melakukan estimasi ketidakpastian pengukuran dalam pengujian kadar kalsium (Ca) dalam contoh powdered tonic food drink menggunakan metode ion kromatografi. Ketidakpastian pengukuran dari laboratorium peserta uji profisiensi dibutuhkan dalam upaya perbandingannya dengan standar deviasi untuk asesmen uji profisiensi yang merupakan indikasi kevalidan dan kelayakan dari seluruh tahapan dalam suatu skema uji profisiensi. Tahapan perhitungan ketidakpastian pengukurannya meliputi spesifikasi parameter yang diukur, investigasi sumber-sumber ketidakpastian pengukuran, perhitungan ketidakpastian pengukuran standar, ketidakpastian pengukuran gabungan ,dan ketidakpastian pengukuran diperluas. Hasil pengujian menunjukkan kadar Ca dalam contoh* powdered tonic food drink *adalah 541.4 mg/100g, dengan ketidakpastian pengukurannya ± 41.1 mg/100g pada tingkat kepercayaan 95% (faktor cakupan k=2). Repeatabilitas pengukuran memberikan kontribusi terbesar ke dalam hasil akhir perhitungan estimasi ketidakpastian pengukuran. Selanjutnya, hasil pengukuran ada dalam nilai konsensus dan ketidakpastian pengukuran yang diperluas, hal ini mengindikasikan bahwa dalam uji profisiensi ini, laboratorium Pusat Penelitian Kimia-LIPI memperoleh hasil yang memuaskan.*

Kata kunci*: uji profisiensi, ketidakpastian pengukuran*

1. INTRODUCTION

The nutrition scenario in the South-East Asian region has changed dramatically in the last two decades. Especially among the urban segments of the community, diet related chronic diseases have been on the rise. The main cause of the deaths in most of these developing countries in the region is coronary heart disease. With the increase in these diseases in the region, there has been greater focus on the role of nutrition in the disorders. Consumers are paying greater attention to the nutritional value of their diets. The food industries are also increasing concentration of some nutrients while decreasing some of the others. Government agencies have intensified efforts to promote healthy dietary practices.

Nutrition labeling of foods is one of the strategies adopted to assist consumers in adopting healthy dietary practices. The primary objective of nutrition labeling is to describe the nutritional qualities of food product factually and informatively. It is aimed at providing a means for conveying information of the nutrient content on the label, thereby assisting consumers in making better food choices when planning daily meals. Nutrition labeling is equally important to the food industry as labeling provide a means for food manufacturers and retailers to become more aware of the nutritional properties of their products, and be encouraged to emphasize these properties to the consumers. Food manufacturers have a social responsibility to contribute positively to the healthy lifestyle programs of the health authorities. There is increasing interest in developing nutrition labeling around the world, even for developing countries, and a wide range of types and approaches are now being practiced.

The Food and Agriculture Organization of the United Nation (FAO) and the World Health Organization (WHO) has published the Codex Alimentarius standards and guidelines on food labeling that consists of collection of internationally adopted food standards, guidelines, codes of practice and other recommendation, with the purposes of protecting the health of consumers and ensuring fair practices in the food trade. In Indonesia, specific requirements for nutrition labeling in foods were provided in government regulation number 69:1999 pertaining to food labeling and advertisement. Nutrition labeling is mandatory for certain types of foods namely baby foods, dietary foods, milk and milk products and other foods as specified by the Director-General. The regulation also apply to foods making claims that they contain specific nutrients, including energy, protein, fat, and carbohydrate content, as well as levels of vitamins and minerals. Nutrition labeling is also mandatory for foods that required to be fortified or enriched with specific nutrients as required by the national legislations. The regulations are also applicable to the voluntary labeling of all other types of foods.

In order to support the implementation of the requirements of food labeling, the nutrients content in food product should be accurately determined using the certain analytical method by the food testing laboratories not only in industry but also in governmental agency that responsibility to monitor food products selling in public so that the valid information about the nutritional of food products can be provided to consumer factually. Therefore, the proficiency testing (PT) held by Food Nutrition Research Institute, Department of Science and Technology, Philippines (FNRI-DOST) covers seven nutrition labeling-related food components, namely moisture, fat, protein, ash, iron, calcium, and sodium. The objective of this PT is to evaluate the performance of participant laboratories in the analysis of moisture, fat protein, ash, iron, calcium, and sodium in powdered tonic food drink. This evaluation enables individual laboratories to compare their analytical result with those of other laboratories and demonstrate the validity and reliability of tests that they undertake. In addition, the regular participation of testing laboratories in well-organized PTs is one of the quality assurances procedures that are included in the requirements for sustained accreditation to the ISO/IEC 17025 standard.

Participants in a PT scheme are required to report quantity values consistent with the definition of the measurand, which means that they need to apply a correction to the quantity value obtained directly from measurement to compensate for any systematic effect associated with differences between the actual measurement conditions and those specified for the measurand. Thus, the uncertainty of measurement indicating the analytical variability of a result has to be evaluated. It demonstrates how well the result represents the value of the quantity being measured in the test portion and also allows an assessment of the reliability of the result. The uncertainty provides an interval within which the value of measurand is believed to lie with a higher level confidence.Therefore, PT can no longer be limited to ensuring that participants's quantity values have satisfactory accuracy; it must include an evaluation of the reliability of the reported uncertainty associated with each measurement.

This paper describes the evaluation of uncertainty of measurement in determination of calcium (Ca) content in powdered tonic food drink using ion chromatography. The specification of measurand, source of uncertainty, standard uncertainty, combined uncertainty and expanded uncertainty from this measurement were evaluated and accounted. The purpose of the evaluation of uncertainty in this measurement is to provide the bias taken place which depended on the various components or measurands that effect to the measurement.This is required for the laboratory participating in the Proficiency Testing Scheme: Analysis of Proximates (Moisture, Fat, Protein, and Ash) and Minerals (Iron, Calcium, and Sodium) in Powdered Tonic Food Drink held by Food Nutrition Research Institute, Department of Science and Technology, Philippines (FNRI-DOST). The estimation of uncertainty measurement of each laboratory is needed for comparison with the standard deviation for proficiency assesment (σ_P) that represents fitness-for-purposes over a whole application sector in proficiency testing scheme.

2. METHODOLOGY

2.1 Proficiency Testing Scheme

All the participant laboratories were requested to analyze the FNRI-DOST powdered tonic food drink test samples, for any or all the following analytes: moisture, fat, protein, ash, iron, calcium, and sodium, using the method of analysis routinely used in their respective laboratories. The participant laboratories were requested to report values with their corresponding measurement uncertainty, for all analytes. In order to ensure that the results obtained from this activity are valid and useful for subsequent analysis, participants were requested to adhere the instructions which contained the information related to proficiency testing such as sample, storage and handling, analysis, test method, reporting, laboratory code number, and submission of documents.

2.2 Analytical method selection

The preparation sample method of tonic food drink sample chosen by Research Centre for Chemistry-LIPI laboratories was dry digestion according to AOAC Official Method 999.11. Subsequently, the calcium content in sample was determined by using ion chromatography based on the proposed method for analysis ion potassium, sodium, calcium and magnesium by Basta, N.T, and Tabatabai, M.A., (1985). These analytical methods are used by Research Centre for Chemistry-LIPI laboratories for the routine analysis of calcium content in water and food samples.

3. EXPERIMENTAL

3.1 Reagents

All chemicals were purchased from MERCK and for analysis grade. NANOpure deionized water (17.8 MΩcm, Barnstead) was used for all solution preparation.

The dilute of HNO_3 solution was used in the preparation of powdered tonic food drink sample such as 0.5 M of solution. Standard of Ca of 1000 mg/L as stock solution was prepared by the dilution of 1.350 g $CaCl_2.2H_2O$. The test sample is powdered tonic food drink which prepared and distributed by FNRI-DOST. Solution of 1.8 mM methyl sulfonic acid was used as mobile phase for elution the component from the column of ion chromatograph. The flow rate of mobile phase was set to 1 mL/min as condition optimum of separation in ion chromatography.

3.2 Instrumental

Hotplate Cymarec 2 and muffle furnace Sibata were used in the preparation of powdered tonic food drink sample by means of dry digestion. Dionex Ion Chromatography equipped by cation exchange column of Dionex CS12 A (4x250 mm) and conductivity detector was utilized to determine the concentration of Ca contained in sample.

3.3 Sample Preparation

The powdered tonic food drink sample was prepared by dry digestion method according to AOAC Official Method 999.11.

Drying step. The 5 g of sample was weighed in the crucible and heated on a hot plate at temperature 100° C until the sample blacken was formed.

Ashing step. The crucible was put in the furnace at initial temperature not higher than 100° C and the temperature of furnace was slowly raised to 450° C at rate no more than 50° C/hour. The sample was ashed for at least 8 hour or overnight. Then, the crucible was taken out from the furnace and let it cool. The ash was wetted with 1-3 mL water and evaporated on hotplate. The crucible was put back in furnace with temperature set up the same as the first step of ashing above. The procedure was repeated until the sample was completely ashed, ash should be white/grey or slightly colored.

Dissolving step. The ash was dissolved in 5 mL of 0.5 M HNO_3. Then, the residue was filtered. Finally, the solution was transferred into volumetry flask and diluted by the aqua demineralized until 50 mL.

4. RESULTS AND DISCUSSION

4.1 Specification of Measurand

The specification of measurand, first step of estimation uncertainty processes, is carried out to describe the measurement procedure. This specification was conducted by a comprehensive description of the several stages of the analytical method and by providing the equation of measurand. The several stages to determine of Ca content in powdered tonic food drink using ion chromatography was shown in the following flowchart Figure 1.

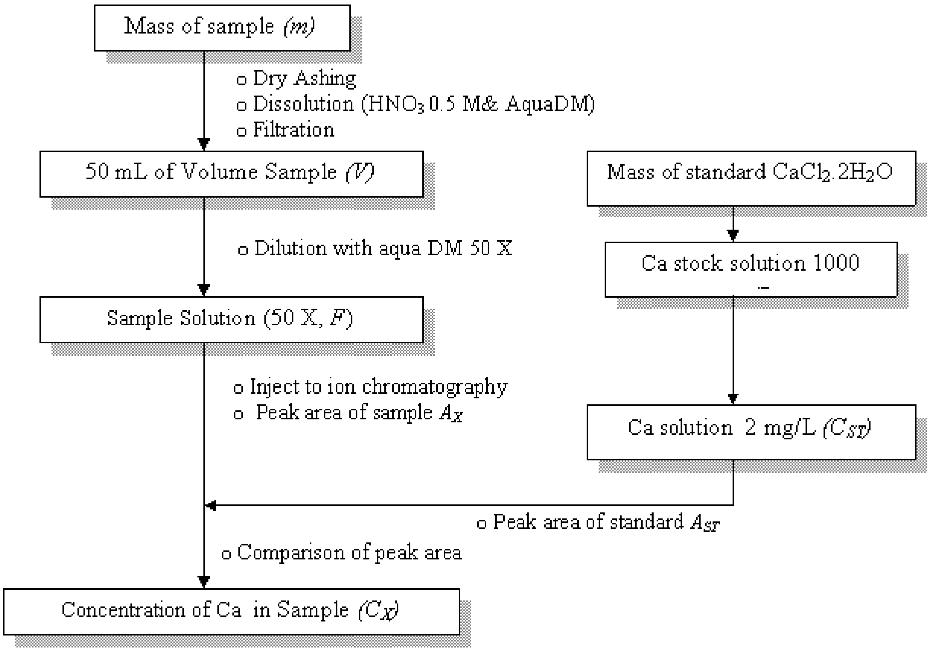

Figure 1 Determination of Ca Content in Powdered Tonic Food Drink Using Ion Chromatography

Based on the measurement procedure above, the measurand of the procedure can be determined. The measurand was the concentration of Ca in sample. It depends on peak area of Ca in sample and standard obtained from ion chromatography, concentration of standard Ca, volume of sample, dilution factor, and the mass of sample. The mathematic statement to determine the concentration of Ca in sample was shown in the following formula.

$$C_X = \frac{A_X \times C_{ST} \times V \times F}{A_{ST} \times m} \qquad (1)$$

where,

C_X : concentration of Ca in sample (µg/g)
A_X : peak area of Ca in sample
C_{ST} : concentration of standard Ca (mg/L)
A_{ST} : peak area of Ca in standard
V : Volume of sample (mL)
F : dilution factor
m : mass of sample (g)

4.2 Sources of Uncertainty

All of the possible uncertainty sources were identified an analyzed in determination of Ca content in powdered tonic food drink using ion chromatography. In order to describe the influence of uncertainty sources from each parameter to the value of the measurand, the cause-effect diagram or Ishikawa fishbone diagram was created, as shown in Figure 2. The uncertainty sources of determination Ca by ion chromatography comprises the uncertainties of peak area of Ca in sample and standard obtained from ion chromatograph, the uncertainties of volume of sample, the uncertainties of mass of sample, the uncertainties of dilution, the uncertainties of concentration of Ca standard, the uncertainties of linearity, and the uncertainties of repeatability.

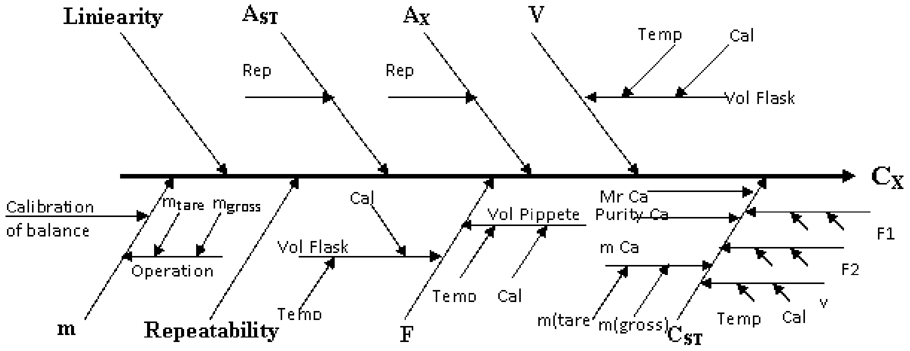

Figure 2 The Sources of Uncertainty in The Determination of Fe Content in Powdered Tonic Food Drink Using Graphite Furnace Atomic Absorption Spectrometry

4.3 Quantifying Uncertainty

4.3.1 Weighing sample

The 1.350 g of powdered tonic food drink samples were weighed by analytical balance. The weighing process was carried out by determination of the difference between container plus sample and empty container. The standard uncertainty of balance is ± 0.0003 g at 95% confidence level which is stated in the certificate of calibration. The standard deviation from balance was calculated by dividing 0.0003 by percentage point of the normal distribution at 95% confidence level. A 95% confidence interval is calculated using a value 1.96 ≈ 2, in equation (2). The contribution uncertainty from the balance was calculated by the equation (3). The value 0.00015, in equation (3), was multiplied by 2, considering two times weighing (container plus sample and empty container).

$$\mu_{Balance} = \frac{0.0003}{2} = 0.00015 \tag{2}$$

$$\mu(m) = \sqrt{2(\mu_{Balance})^2} = 0.00021213g \tag{3}$$

4.4 Dissolving sample

After dry ashing preparation, sample was dissolved with aqua DM until 50 mL in volumetric flask. The uncertainty of the internal volume of the 50 mL volumetric flask is stated by the manufacturer as ±0.09 mL. The assumption of rectangular distribution (divided by $\sqrt{3}$) was used in calculation the standard uncertainty of calibration of volumetric flask, as shown in equation 4, since the value of uncertainty ±0.09 mL is given without certain information about confidence level or distribution.

$$\mu_{CAL} = \frac{0.09}{\sqrt{3}} = 0.05196mL \tag{4}$$

According to the manufacturer, the volumetric flask has been calibrated at temperature of 20 °C, whereas the laboratory temperature varies between the limits of ±3°C when dissolving process. The uncertainty due to this temperature effect can be determined from the estimation of temperature difference and the coeficient of the volume expansion. The volume expansion of the liquid (2.1×10^{-4}°C^{-1} at 20°C, for water) is considerably greater than that flask (10×10^{-6}°C^{-1} for borosilicate glass flask), so only the former needs to be considered. The standard uncertainty due to the temperature effect was calculated using the assumption of rectangular distribution for the temperature variation, in equation (5).

$$\mu_{Temp} = \frac{50mL \times 3^{o}C \times 2.1\times10^{\;4} /^{o}C}{\sqrt{3}} = 0.018165mL \tag{5}$$

The two contributions of uncertainty, calibration and effect temperature, were combined to give the standard uncertainty µ(V) of the volume (V), in equation (6).

$$\mu(V) = \sqrt{(\mu_{CAL})^2 + (\mu_{Temp})^2}$$

$$\mu(V) = \sqrt{(0.05196)^2 + (0.018165)^2} = 0.05502mL$$

(6)

4.5 Dilution Factor

Due to high the concentration of sample, the sample solution was diluted 50 times with aqua demineralized in 50 mL volumetric flask to obtain the peak area that can be compared against the standard. Contribution of uncertainty due to the variation within specification limits and temperature effect were determined and combined for each type of glassware (1 mL of pippete and 50 mL of volumetry flask) Table 1. is a summary of the calculation of uncertainties arising from the variation within specification limits and temperature effect.

Table 1 Uncertainties Due To The Manufacture's Spesification and Effect Temperature

Volumetric materials	**Uncertainty (Uncert.)**		**Combined Standard Uncert.**	**Standard Uncert (mL)**
	calibration	**Temperature effect**		
1 mL pippete	0.00462	0.000364	$\sqrt{(0.00462)^2+(0.000364)^2}$	0.00463
50 mL volumetry flask	0.0520	0.0182	$\sqrt{(0.0520)^2+(0.0182)^2}$	0.0551

There was an uncertainty associated with the initial and final volumes taken, so the dilution factor was associated with them. The uncertainty from the dilution factors, $\mu(F_d)$, were calculated as:

$$\frac{\mu(F_d)}{50}=\sqrt{\frac{\mu(v1)^2}{1^2}+\frac{\mu(v50)^2}{50^2}}$$

$$\mu(F_d)=50\times\sqrt{\frac{(0.00463)^2}{1^2}+\frac{(0.0551)^2}{50^2}}$$

$$\mu(F_d)=0.2381 \tag{7}$$

4.6 Concentration of Ca standard

Preparation of standard Ca of 1000 mg/L as stock solution involved several stages of procedure such as weighing and dissolving of standard $CaCl_2.2H_2O$, as described in Figure 1. Subsequently, the concentration of standard Ca was calculated by the following formula.

$$C_{Ca}=\frac{m_{Ca}}{Mr_{Ca}}\times\frac{1000}{V}\times purity \tag{8}$$

In addition, the dilution of standard Ca of 1000 mg/L solution was conducted for injection purposes in ion chromatograph. Hence, the uncertainty due to concentration of Ca standard depended on the estimation of standard uncertainty of weighing, dissolving, molecular weight, and purity of standard $CaCl_2.2H_2O$ as well as the dilution of standard Ca solution.

The same method in previous section was used for calculation of standard uncertainty of weighing, dissolving and dilution of standard Ca. Furthermore, the uncertainty of purity of $CaCl_2.2H_2O$ was determined by consideration of the purity information from the supplier. The purity of $CaCl_2.2H_2O$ quoted in the bottle is 99.5± 0.5%. There is no further information from the supplier related the uncertainty of purity in the bottle. Consequently, this uncertainty, 0.005, was taken as having a rectangular distribution and calculated as:

$$\mu(P_{Ca})=\frac{0.005}{\sqrt{3}}=0.0022867 \tag{9}$$

Moreover, the uncertainty in the molar mass of the $CaCl_2.2H_2O$ was determined by combining the uncertainty in the atomic weights of its constituent elements. The information of atomic weights including uncertainty estimates was obtained from the latest International Union of Pure and Applied Chemistry (IUPAC) Table. The standard uncertainty for each elements are generated by treating the IUPAC quoted uncertainty as rectangular distribution. Thus, the corresponding uncertainty is obtained by dividing those values by $\sqrt{3}$. The atomic weights and listed uncertainties for the constituent elements of $CaCl_2.2H_2O$ are shown in Table 2.

Table 2 The Atomic Weights and Listed Uncertainties for The Constituent Elements of $Cacl_2.2H_2O$

Element	**Atomic weight**	**Quoted uncertainty**	**Standard uncertainty**
Ca	40.078	±0.004	0.002309401
Cl	35.453	±0.002	0.001154701
H	1.00794	±0.00007	0.000040415
O	15.9994	±0.0003	0.000173205

The uncertainty of each element contributing to the molecular weight uncertainty is calculated by multiplying the standard uncertainty in the Table 2 by the number of atoms. The table 3 listed the separate element contribution to the molecular weight, together with the uncertainty contribution for each.

Table 3 The Separate Element Contribution to The Molecular Weight and Its Uncertainty Contribution

Element	Calculation	Result	Standard Uncertainty	Result
Ca	1 x 40.078	40.078	1 x 0.002309401	0.002309
Cl_2	2 x 35.453	70.906	2 x 0.001154701	0.002309
H_4	4 x 1.00794	4.03176	4 x 0.000040415	0.000162
O_2	2 x 15.9994	31.9988	2 x 0.000173205	0.000346
Molecular weight		147.0146		

From the Table 3, the standard uncertainty of molecular weight, μ(MW), was calculated by the square root of the sum of the squares of the uncertainty contribution from each element, as follows:

$$\mu(MW) = \sqrt{(\mu(Ca))^2 + (\mu(Cl_2))^2 + (\mu(4H))^2 + (\mu(2O))^2}$$
$$\mu(MW) = \sqrt{(0.002309)^2 + (0.002309)^2 + (0.000162)^2 + (0.00034641)^2} \qquad (10)$$
$$\mu(MW) = 0.003288 g / mol$$

So the uncertainty due to concentration of Ca standard was obtained by combining the standard uncertainty of weighing, dissolving, molecular weight, and purity of standard $CaCl_2.2H_2O$ as well as the dilution of standard Ca solution, as summarized in the Table 4.

Table 4 Uncertainties Due To The Concentration of Ca Standard

No	**Parameters**	**Uncertainty Mentioned**	**Standard Uncertainty**
1	Mass of Ca Standard (from balance)		
	Balance	0.0003 g (at confidence level 95%)	$\mu_{BALANCE} = \sqrt{2\left(\frac{0.0015}{2}\right)^2}$ $=0.000212$ g
	Repeatability* (experiment, 10 replication)	0.000322	$\mu(R_W) = \frac{0.000322}{\sqrt{10}}$ $\mu(R_W) = 0.00010198g$
			$\mu(m_{ST}) = \sqrt{(0.00021213)^2 + (0.00010198)^2}$ $\mu(m_{ST}) = 0.00023537g$
2	Standard Ca Purity (P_{Ca})	0.005	$\mu(P_{Ca})= 0.0022867$
3	Molecular weight		μ(MW)= 0.003288 g/mol
4	Standard Fe Volume (v)		
	Calibration of pippete	0.1 mL	$\mu_{CAL} = \frac{0.1}{\sqrt{3}} = 0.0577$
	Effect temperature	23°C	$\mu_{Temp} = \frac{100mL \times 3^oC \times 2.1\times10^{\ 4}/^oC}{\sqrt{3}}$ $\mu_{Temp} = 0.0364mL$
			$\mu(v) = \sqrt{(\mu_{CAL})^2 + (\mu_{Temp})^2}$ $\mu(v) = \sqrt{(0.0577)^2 + (0.0364)^2}$ $\mu(v) = 0.0682mL$
5	First dilution		μ(f1)= 0.020843

6	Second dilution		μ(f2)= 0.23810

Combined Standard Uncertainty

$$\mu(C_{ST}) = C_{ST} \times \sqrt{\frac{\mu(m_{ST})^2}{{m_{ST}}^2} + \frac{\mu(P_{Ca})^2}{{P_{Ca}}^2} + \frac{\mu(v)^2}{v^2} + \frac{\mu(f1)^2}{f1^2} + \frac{\mu(f2)^2}{f2^2} + \frac{\mu(MW)^2}{MW^2}}$$

$$\mu(C_{ST}) = 2 \times \sqrt{\frac{(0.000235)^2}{0.36738^2} + \frac{(0.002886)^2}{0.995^2} + \frac{(0.06823)^2}{100^2} + \frac{(0.02084)^2}{10^2} + \frac{(0.2381)^2}{50^2} + \frac{(0.003288)^2}{147.02^2}}$$

$$\mu(C_{ST}) = 0.01205 mg / L$$

4.7 Peak area of sample (A_X) and standard (A_{ST})

In chromatography, the quantification of concentration sample was carried out by comparison between peak area of sample (A_X) and standard (A_{ST}). The peak area of sample and Ca standard obtained from chromatogram of the ion chromatography instrument was shown in the Table 5.

Table 5 Peak Area of Sample and Standard Obtained from Chromatogram of Ion Chromatography Instrument

Replicate	**Peak Area of Sample**	**Peak Area of Standard**
1	979358	675133
2	985098	670416
3	970304	
4	988185	
5	998445	
6	998839	
7	963450	
Mean	983382.7143	672774.5
SD (S)	**13393.64005**	**3335.422687**

The standard uncertainty of peak area of sample and standard were simply determined by dividing their standard deviation of peak area with the square root of the amount of each replication, as calculated in Table 6.

Table 6 Standard Uncertainty of Peak Area From Sample and Standard

Equation	**Standard uncertainty of peak area**	
	sample	**standard**
$\mu(A_X) = \frac{S}{\sqrt{n}}$	$\mu(A_X) = \frac{13393.640}{\sqrt{7}}$ $\mu(A_X) = 5062.3201$	$\mu(A_{ST}) = \frac{3335.4227}{\sqrt{2}}$ $\mu(A_{ST}) = 2358.5$

4.8 Linearity

Since the quantification of concentration Ca in sample using one point calibration, the linearity of the ion chromatograph response in certain range concentration between standard and sample was determined. The non linearity response would contribute to the accuracy of result. A series of standard of Ca was prepared and analyzed by ion chromatograph to obtain the peak response from several concentration of standard, as summarized in Table 7.

Table 7 Linearity Verification of The Ion Chromatograph Using Standard of Ca

[Ca] made mg/L	**Mean Area**	**[Ca] standard mg/L**	**Area standard**	**[Ca] Analysis (after comparison) mg/L**	**Deviasi {[Ca}made-[Ca]analysis}**
1	332717.5			0.989	-0.0109
2	748607.5			2.225	0.225
3	1093671	2	672774.5	3.251	0.2512
4	1432288			4.258	_0.258*_
5	1749581			5.201	0.201

*maximum deviation (μ)

The highest difference between theoretical and observed value of Ca standards represent the deviation caused by the linearity of measurement. Table 7 shows that the maximum deviation is 0.258. The linearity contribution is assumed to show a rectangular distribution and is converted to a standard uncertainty by calculation in equation 11.

$$\mu(Lin) = \frac{0.258}{\sqrt{3}} = 0.148872 \qquad (11)$$

4.9 Repeatability

In order to investigate the standard uncertainty arising from random effects, the repeatability experiments was conducted and the standard deviation of the measured value was quantified. This standard deviation represents the uncertainty due to the variability observed of measurement result within a laboratory, over a short time, using a single operator, item of equipment etc. The result showed a repeatability of the determination of Ca by ion chromatography of 3.662% (as % rsd), as shown in Table 8.

Table 8 Repeatability of Ca Measurement in Sample By Ion Chromatography

No	**[Ca] ug/g**
1	5481.21
2	5563.89
3	5954.02
4	5721.89
mean	5680.253
SD	208.0388
RSD	0.036625
% RSD	3.662493

This value was used directly for the calculation of the combined uncertainty associated with the different repeatability terms. The standard uncertainty of repeatability equals to the Relative Standard Deviation (RSD) of repeatability which was 0.0366 µg/g, equation (12).

$$\mu(\mathrm{Re}\ p) = RSD$$
$$\mu(\mathrm{Re}\ p) = 0.0366 \mu g / g \qquad (12)$$

4.10 Combined Standard Uncertainty

The value of parameters for calculation of Ca concentration in sample, equation (1), their standard uncertainties and their relative standard uncertainties were summarized in Table 9.

Table 9 Uncertainties in The Determination of Ca in Powdered Tonic Food Drink by Ion Chromatography

Symbol	**Value (X)**	**Standard Uncertainty (μ_X)**	**Relative Standard Uncertainty (μ_X/X)**	**Unit**
C_X	5414.240			µg/g
A_X	983382.714	5062.320	0.005148	
C_{ST}	2	0.01205	0.006026	mg/L
V	50	0.05505	0.001101	mL
F	50	0.2381	0.004762	
A_{ST}	672774.5	2358.5	0.003506	
m	1.34985	0.0002121	0.0001572	g
Rep	1	0.03663	0.03663 (μ_{REP}/1)	
Linearity		0.1489		

Using the values given in Table 9, the concentration of Ca in the sample can be calculated as follows.

$$C_X = \frac{983382.714 \times 2 \times 50 \times 50}{672774.5 \times 1.34985} = 5414.240 \mu g / g \approx 541.4 mg / 100 g \qquad (13)$$

For a multiplicative expression in equation (1) and equation (13), the combined standard uncertainties were used as follows

$$\mu(C_X) = C_X \times \sqrt{\frac{\mu(A_X)^2}{A_X^{\,2}} + \frac{\mu(C_{ST})^2}{C_{ST}^{\,2}} + \frac{\mu(V)^2}{V^2} + \frac{\mu(F)^2}{F^2} + \frac{\mu(A_{ST})^2}{A_{ST}^{\,2}} + \frac{\mu(m)^2}{m^2} + \frac{\mu(\mathrm{Re}\,p)^2}{\mathrm{Re}\,p^2}}$$

$$\mu(C_X) = 5414.2 \times \sqrt{(0.00515)^2 + (0.00949)^2 + (0.00110)^2 + (0.00476)^2 + (0.00351)^2 + (0.00016)^2 + (0.037)^2}$$

$$\mu(C_X) = 205.4849 \mu g / g \qquad (14)$$

The combined standard uncertainty given in equation 14 is not including the contribution of linearity into account. Hence, the following calculation was carried out to consider the uncertainty due to the linearity.

$$\mu(C_X) = \sqrt{(205.4849)^2 + (0.1489)^2} = 205.4850 \mu g / g \qquad (15)$$

4.11 Expanded Standard Uncertainty

Expanded Uncertainty of concentration Ca in sample, U(C_X), was obtained by multiplying the combined standard uncertainty by a coverage factor 2 (at confidence level 95%), in equation (16).

$$\mu = 2 \times 205.4850 \mu g / g$$

$$\mu = 410.9700 \mu g / g$$

$$U(C_X) = 2 \times 205.4850 \mu g / g = 410.9700 \mu g / g \approx 41.1 mg / 100g \qquad (16)$$

The contribution uncertainties of different parameters were shown in Figure 3. The largest contribution of uncertainty arises from the repeatability of method measurement of Ca by ion chromatography.

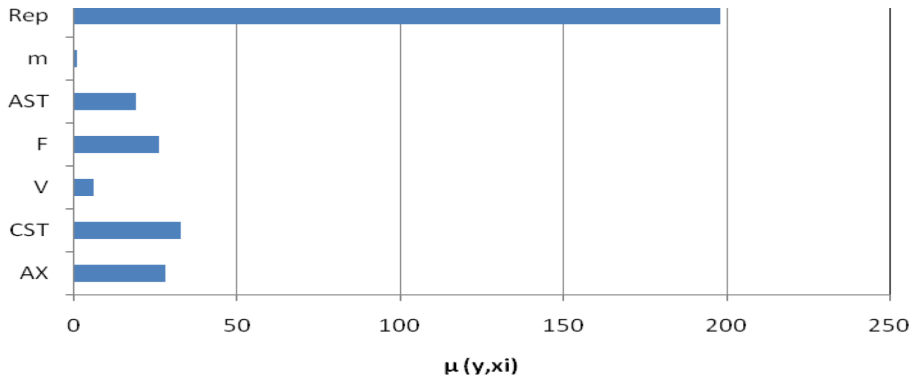

Figure 3 Uncertainty Contributions in The Determination of Ca Content in Powdered Tonic Food Drink Sample

4.12 Proficiency testing result

Figure 4 shows the evaluation of results of proficiency testing by involving the uncertainty of measurement from each laboratory. Research Centre for Chemistry LIPI, coded as laboratory 38, demonstrated that the result of measurement was within the range of consensus value ± its expanded uncertainty (X±2μ_X: 526.1±28.6) which is 497.5-554.7 mg/100g. Therefore, the result of laboratory 38 was categorized as “satisfactory” or in-lier result in this proficiency testing.

Moreover, the expanded uncertainty of laboratory 38 was large and overlapped within the area of X±2μ_X. It indicated that overall of the sources of uncertainty especially the major contribution of uncertainty were taken into account to the measurement by the laboratory. Meanwhile, some of laboratories reported the results with the small uncertainty which no overlap with X±2μ_X such as laboratory 14 and 24, as shown in Figure 4. It could be happened when the laboratories was unable to identify all of the sources of uncertainty especially the uncertainty that majorly contribute to the measurement.

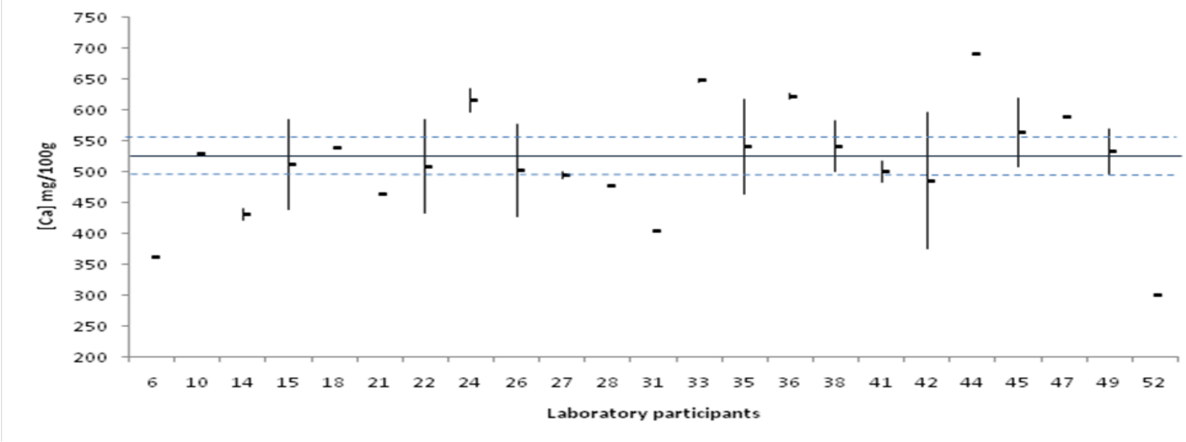

Figure 4 Graphic of Results Measurement Including Their Uncertainty in Proficiency Testing FNRI-DOST Ca In Powdered Tonic Food Drink

The methods and instruments used by the participant laboratories in the analysis of calcium are the following: (a) titration method (one lab), (b) Atomic Emission Spectroscopy (AES)/Inductively Coupled Plasma (ICP)/ICP-Optical Emission Spectrometry (OES) (three labs), (c) ion chromatography (Research Centre for Chemistry-LIPI lab), (d) Atomic Absorption Spectroscopy (AAS) (all other labs). Research Centre for Chemistry-LIPI laboratory that used ion chromatography for quantification of Ca in sample obtained “satisfactory” performance. It indicate that the method used by Research Centre for Chemistry-LIPI laboratory is reliable to determine calcium in food sample and comparable to other methods used by the participants in this proficiency testing.

5. CONCLUSION

The Ca content in powdered tonic food drink sample analysed by ion chromatography was.

541.4± 41.1 mg/100g

The result is expressed with an expanded uncertainty using coverage factor k=2 (at confidence level 95%). The Research Centre for Chemistry LIPI laboratory demonstrated the “satisfactory” result in the proficiency testing FNRI-DOST Ca in powdered tonic food drink. In addition, the method used by Research Centre for Chemistry-LIPI laboratory, ion chromatography, is comparable to other methods used by the participants in this PT.

The sources of uncertainty in the determination of Ca in sample by ion chromatography consist of the uncertainties of peak area of Ca obtained from ion chromatography in sample and standard, the uncertainties of volume of sample, the uncertainties of mass of sample, the uncertainties of dilution, the uncertainties of concentration of Ca standard, the uncertainties of linearity, and the uncertainties of repeatability.The uncertainty estimation of different sources in analysis Ca demonstrated that the repeatability of method measurement was the major contribution to the uncertainty of the final results.

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