Improvement in the Dietary Environment by Optimizing Official Methods for Trace Elements Present in Infant Formula

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Abstract

A wholesome dietary environment is indispensable for a safe and healthy diet. An international trade on food is essential to improve the dietary environment. The Codex standards and the Codex test methods set by the Codex Committee are used to determine whether food is safe and contains the required ingredients. In recent years, multiple Codex methods of analysis have been registered for the same test item, and there were concerns that this would limit a smooth international trade.

To overcome this limitation, AOAC International (AOACI) launched Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN), which is a project with an aim to develop and unify the official methods used in the international trade of infant formula and adult nutrients. SPIFAN is currently working with International Organization for Standardization (ISO) and International Dairy Federation (IDF) and is accelerating the unification of official methods, while rapidly broadening its scope.

The leading members of the official methods formulation projects are food, raw-material, and equipment manufacturers from western countries, who exercise strong influence in these projects. The official methods adopted in these projects are therefore strongly influenced by the leading members involved, and the concerns of the minor members and the other states and organizations are not fully reflected in the scientific and decision-making processes. These inadequacies are reflected in some official methods, which are not sufficiently optimized in terms of reliability and versatility.

In this context, the unnecessary disposal of food products due to deviations from standard analysis values caused by insufficient optimization of Codex methods is a great challenge. In addition, some official methods cannot be adopted without employing equipment produced by manufactures from western countries, who are key stakeholders

in SPIFAN. The objective of this study was to prevent the unnecessary disposal of food products due to deviations from standard analysis values. To this end, studies on improvement of the AOAC Official Method of Analysis (AOAC-OMA), which is the basis of Codex methods for selenium and iodine, were conducted. Simultaneously, the similar problem was recognized for the arsenic test method being developed as a Codex test method by the Stakeholder Panel on Strategic Food Analytical Methods (SPSFAM). Therefore, the improvement of the test method was also examined as an additional study.

Firstly, the author examined the possibility for improvement of versatility and for expansion of the measurement range for AOAC Official Method 2015.06 (AOAC 2015.06), which is an Official Methods of Analysis of AOAC International (AOAC-OMA) for selenium in infant formulas. Due to poor sensitivity, this method cannot be applied to infant formula analysis without the use of selenium additives. Furthermore, AOAC 2015.06 specifies the use of hydrogen as the cell gas for Inductively Coupled Plasma Mass Spectrometry (ICP-MS) measurements, which is a strong limitation, as instruments which incorporate this use are limited. To address these limitations, the author carried out improvement studies to expand the model selectivity of AOAC 2015.06, to facilitate measurements in the absence of selenium additives. While carrying out improvement studies, the author decided to select helium for use as the ICP-MS cell gas. Even though it improves method applicability, its reduced sensitivity is a known disadvantage for selenium measurements when compared to the use of hydrogen. In this study, by optimizing the sample amount and the amount during the measurement at the time of final test solution adjustment, it was possible to accurately measure the elemental levels in infant formula without the need for selenium additives even when helium gas was adopted. Since AOAC 2015.06 was successfully improved, the author decided to

improve AOAC Official Methods for iodine based on these ideas.

Next, the author examined the improvement of AOAC Official Method 2012.15 (AOAC 2012.15), which is AOAC-OMA for iodine in infant formula. The amount of iodine present in infant formulas prepared in Japan is close to the lower limit of the standard defined by Codex Alimentarius Commission (CAC) and Standard Method Performance Requirement (SMPR) 2012.008, due to which, the possibility that the method might be inapplicable due to measurement sensitivity challenges for metals like selenium was considered at first. However, it became clear that the signal enhancement (a phenomenon wherein the measured value is higher than the actual value) is affected by each country's infant formula. This result suggests that the risk of deviation from the standard value derived from the test method was small; however, the product might be distributed with deviation from the standard value. In this study, the author hypothesized that the addition of a carbon source at an optimum concentration to the blank or standard, and the final test solutions could solve these problems and could improve the reliability of AOAC 2012.15. When methanol was adopted as a carbon source, and the measurement was conducted after setting its optimum concentration (5%), the author found that the measured values were close to the actual value for the infant formulas of each country, which thereby provides the solution for the aforementioned problems.

Finally, the author examined the improvement of AOAC Official Method 2015.01 (AOAC 2015.01), which is AOAC-OMA that is slated to become the Codex method for arsenic in infant formulas. AOAC 2015.01 specifies a wet ashing method using a microwave oven for pretreatment. The number of samples that can be processed at once under this approach is limited when compared to the dry ashing method which employs a muffle furnace. Further, the microwave oven and the equipment are relatively

expensive. Besides, in AOAC 2015.01 for arsenic, oxygen is specified as the cell gas of ICP-MS used for measurement, which imposes an equipment limitation as with the case of selenium. In this study, the author adjusted the final test solution pretreatment process by dry aching using a muffle furnace after addition of magnesium nitrate solution for preventing arsenic volatilization. Furthermore, the author adopted helium as the cell gas, which is highly versatile for by ICP-MS measurements but has a lower sensitivity than that of oxygen for arsenic measurement. By incorporating these modifications to AOAC 2015.01, the author was able to carry out accurate arsenic measurements in the infant formulas from each country.

Through this research, the author successfully optimized the Codex test method for almost all trace elements for which the challenges to be addressed in SPIFAN were recognized. Moreover, as an additional study, the challenge of the arsenic test method, which was recognized by SPSFAM, was solved. By overcoming the challenges related to the implementation of SPIFAN and SPSFAM in related organizations based on the results of this study, testing organizations in all countries and organizations, particularly developing countries, will be able to introduce the Codex test method more easily. Moreover, the author believe that this study will be an opportunity to establish an environment in which food products such as infant formulas from countries/organizations other than western countries can be consistently supplied and distributed through international trade.

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Abbreviation and Acronyms

AAS: Atomic Absorption Spectrometry

AOACI: AOAC International

AOAC-OMA: Official Method of Analysis of AOAC International

AOAC 2012.15: AOAC Official Method 2012.15

AOAC 2015.01: AOAC Official Method 2015.01

AOAC 2015.06: AOAC Official Method 2015.06

CAC: Codex Alimentarius Commission

CRC: Collision and Reaction Cell

FAO: Food and Agriculture Organization

FOS: Fructo Oligo Saccharide

GOS: Glacto Oligo Saccharide

HGAAS: Hydride Generation Atomic Absorption Spectrometry

HPLC-ECD: High Performance Liquid Chromatography Electrical Chemical Detection

ICP-AES: Inductively Coupled Plasma Atomic Emission Spectrometry

ICP-MS: Inductively Coupled Plasma Mass Spectrometry

IDF: International Dairy Federation

INCA: Infant Nutrition Council of America

ISE: Ion Selective Electrode

ISO: International Organization for Standardization

LOQ: Limit of Quantitation

MLT: Multi Laboratory Testing

NIST: National Institute of Standards and Technology

RF: Radio Frequency

RSD: Relative Standard Deviation

RSD_r: Relative Standard Deviation of repeatability

RSD_{IM}: Relative Standard Deviation of intermediate precision

SD: Standard Deviation

SD_r: Standard Deviation of repeatability

SD_{IM}: Standard Deviation of intermediate precision

SDGs: Sustainable Development Goals

SDOs: Standard Development Organizations

SMPR: Standard Method Performance Requirement

SPIFAN: Stakeholder Panel on Infant Formula and Adult Nutritionals

SPSFAM: Stakeholder Panel on Strategic Food Analytical Methods

SRM: Standard Reference Material

UN: United Nations

WHO: World Health Organization

List of Publications

Hieda, N. Nagatoshi, M. Ikeuchi, Y. Iga, Y. and Goto, T., Improvement of Versatility and Analytical Range of AOAC Official Method 2015.06 for Selenium. J AOAC Int. 2018. **101**(4): p. 1215-1218.

Hieda, N. Ikeuchi, Y. and Matsuno, I., Improvement in the Reliability of AOAC Official Method 2012.15 for Iodine. J AOAC Int. 2019. **102**(2): p. 673-676.

Hieda, N. Ikeuchi, Y. and Matsuno, I., Enhanced Versatility of AOAC Official Method 2015.01 for Arsenic Determination in Infant Formula and Dairy Products. Jpn J Food Chem Safety. 2019. **26**(3): p. 153-159.

Chapter 1 General introduction

1.1 Importance of infant formula analysis

Since the first analysis of the critical chemical components of mammalian milk, the composition of infant formula has been extensively modified to make it similar to that of human breast milk Therefore, infant formula has become one of the most regulated foods whose compositional innovations must be verified and product quality and above-mentioned safety measures must be maintained [1]. To comply with global regulations and ensure the highest quality products, infant formula companies manufacture their products to adhere to the most stringent specifications. Analytical methods used for analyzing infant formula need extraordinary precision and accuracy to be effectively monitor such products [2]. In addition, high-performance infant formula analysis must be conducted to ensure that infant formula meets the Codex standards, which is a requirement for international trade. To improve the dietary environment, the international trade in food is necessary and safe and nutritious food must be supplied without deceit. FAO and World Health Organization (WHO), which is one of the specialized agencies of UN, have jointly organized the Codex Alimentarius Commission (CAC) that prepares Codex standards in order to protect consumer health and ensure fairness. To check whether the Codex standard is satisfied, Codex methods authorized by the CAC are used (Figure 1.1).

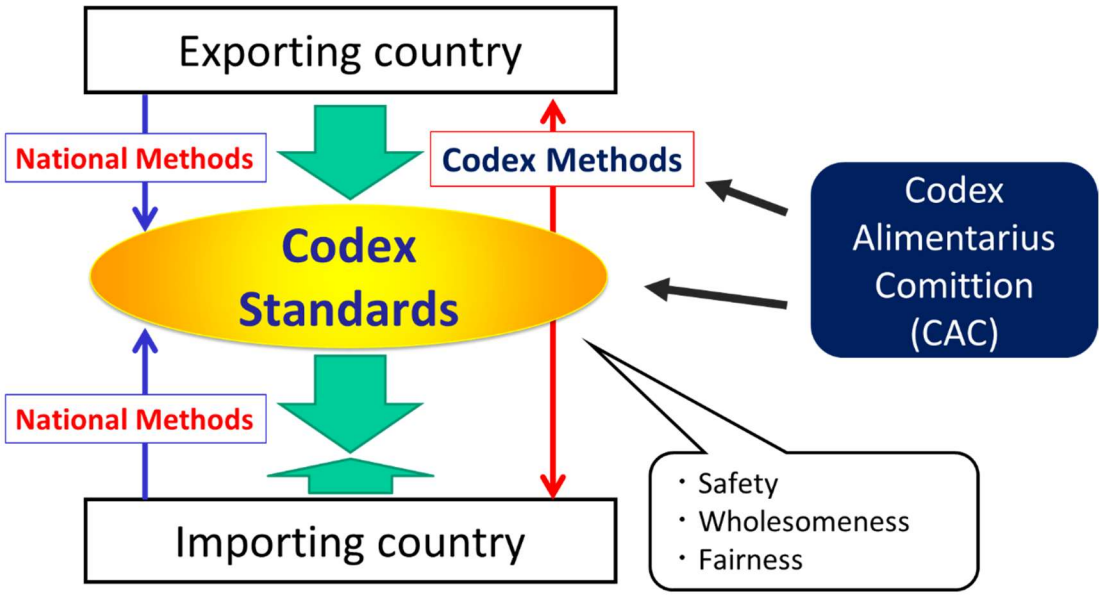


Figure 1.1 Rules in international trade of food.

1.2 The current state-of-the-art and official methods for infant formula analysis

As the manufacturing of various commercial brands of infant formulas and associated the analytical techniques have evolved, several testing methods for these products have been proposed by Standard Development Organizations (SDOs), such as AOAC International (AOACI), International Organization for Standardization (ISO), and International Dairy Federation (IDF). Some testing methods proposed by these SDOs have been authorized by CAC. While significant progress in the testing of infant formulas using these methods has been achieved, several official methods exist in the same field, and only a few of them have been updated periodically. These factors further complicate the manufacturing and trade of infant formulas globally. This tendency is remarkable for micronutrients contained in infant formula, which need to ensure advanced measurement techniques.

Under these circumstances, AOACI, a non-profit scientific association that publishes standardized analytical methods for improving the confidence in the chemical and microbiological analysis results in the USA, established the Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN) project to modernize the AOAC-OMA standards applied in the analysis of infant formula with Infant Nutrition Council of America (INCA) in 2010. In addition, a cooperation agreement was signed in 2012 between AOACI and ISO. This new partnership has facilitated the joint development and approval of common official methods while focusing on priority areas through SPIFAN. This existing agreement between AOACI and ISO also involves collaboration with IDF relating to their joint program of work toward the development of methods for the analysis and sampling of milk and milk products [3].

Furthermore, SPIFAN promotes the unification of official methods with significant financial support from key stakeholders, who primarily are manufacturers of food, raw materials, and analysis equipment in European and American countries.

1.3 Changes in the processes for formulation of official methods

The formulation of conventional processes of official methods involves the proposal and development of an analytical method for a specific purpose by project leaders in one of the organizations, which is then evaluated for suitability as the official method through an evaluation of the draft analytical method at a meeting or a circular, by collaborative study. However, formulation of these procedures requires several years and typically involves organizational challenges due to which analytical methods for specific purposes which do not benefit the proposer, are not formulated as the official methods.

SPIFAN began with the objective of reviewing the formulations resulting from the above-described processes and has expanded its scope to the formulation standardization of the necessary analytical methods. After selecting the required details, AOACI creates Standard Method Performance Requirements (SMPRs) that describe the requirements for conducting an AOAC-OMA [3]. In other words, in order to register a specific test method in AOAC-OMA, it is necessary to satisfy the conditions described in SMPR. SMPRs are developed by attaining consensus among voluntary stakeholders who need and use the methods, and they reflect the analytical requirements of the user community, taking into account technology considerations, compliance requirements, and other issues deemed critical by the stakeholders [3]. The application, analytical technic, measurement concentration range, reproducibility, accuracy, and the related parameters which need to be fit-for-the-purpose are described in SMPRs (Table 1.1) [4]. Besides, for

compounds such as vitamins and Galacto Oligo Saccharide (GOS), which may be present in a large number of derivatives and as isomers, the definitions of the compounds are also included in SMPRs [5-14].

Table 1.1 Example of the Standard Method Performance Requirement (SMPR).

Method performance requirements[a,b,c]	Selenium
Analytical range	10-500
Limit of quantitiation (LOQ)	≤4
Repeatability (RSD_r)	≤5%
Recovery	90 to 110% of mean spiked recovery over the range of the assay
Reproducibility (RSD_R)	≤15%

[a] From SMPR 2011.009 [4]

[b] Concentrations apply to: (1) "ready-to-feed" liquids "as is"; (2) reconstituted powders (25 g into 200 g water); and (3) liquid Concentrates diluted 1:1 by weight.

[c] μg/kg reconstituted final product.

Once SMPRs are determined, an expert panel will gather information on the existing or newly developed analysis methods from around the world and will select several analysis methods as AOAC-OMA first actions. Next, the expert panel prepares samples of the infant formula or the nutritious food that falls under the scope of application and conduct Multi Laboratory Testing (MLT). After further evaluations, discussion, and voting, the analytical methods with satisfactory results will be adopted as AOAC-OMA final actions.

AOAC-OMA final actions are generally examined as joint AOAC-ISO/IDF methods by ISO/IDF, but when necessary data acquisition and evaluation is already complete, the final draft will be voted without much further discussion. Additionally, AOAC-OMA final actions will also be submitted as Codex methods at the CAC before the ISO/IDF review is complete. By conducting the above-described process, SPIFAN unifies official methods without missing necessary items and at high speed, which has not been realized in the past（Figure 1.2）.

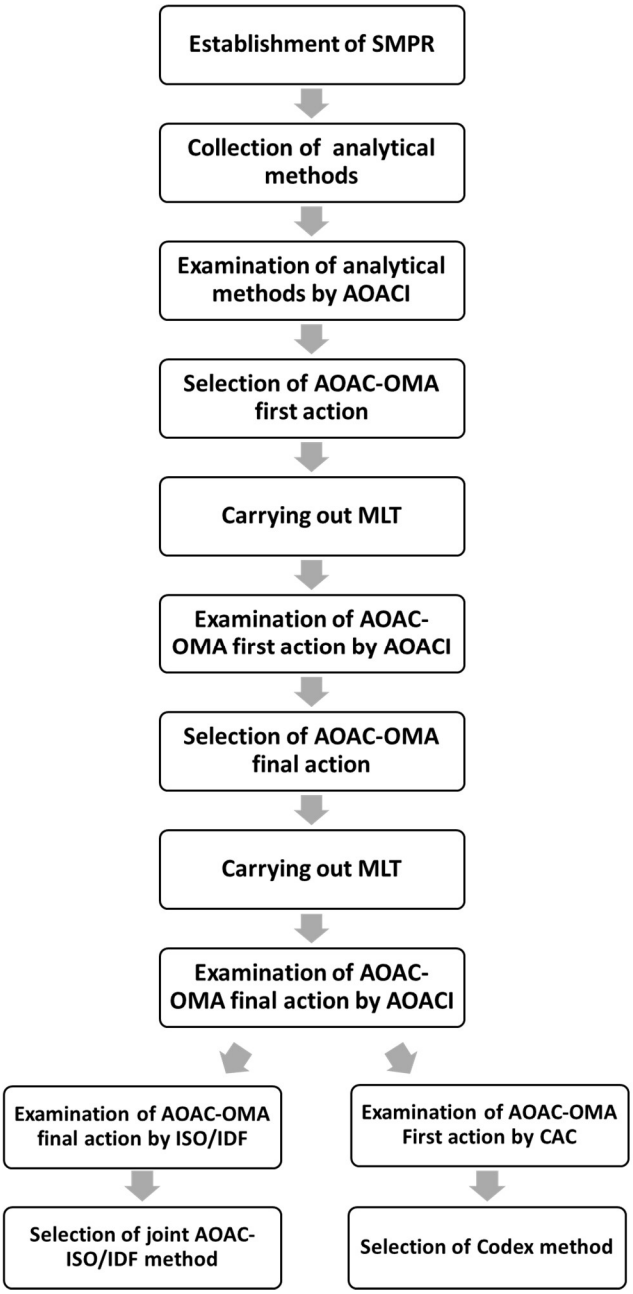


Figure 1.2 Stakeholder Panel on Infant Formula and Adult Nutritional (SPIFAN)'s official method unification process.

SMPR: Standard Method Performance Requirement; AOACI: AOAC International; AOAC-OMA: Official Method of Analysis of AOAC International; MLT: Multi Laboratory Testing; IDF: International Dairy Federation; ISO: International Organization for Standardization; CAC: Codex Alimentarius Commission

By the development of processes and the testing methods used during the import and export, the resolution of trade conflicts will be unified all over the world. SPIFAN has achieved the unification of half of the major test items available in the last 7 years after launching the project by reviewing the conventional formulation procedures (Table 1.2). As a result, AOACI signed an agreement in October 2018 to promote the standardization of official methods while working closely with AOACI and ISO, based on the results from SPIFAN. In the future, the unification of official methods will be further advanced while expanding the scope of the project to include official methods for general foods (Stakeholder Panel on Strategic Food Analytical Methods: SPSFAM) as well at SPIFAN.

Table 1.2 Progress of Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN) project (as of September 30, 2018).

Nutrient/nutrient category[a]	SMPR adopted	First Action Official Method℠	Final Action Official Method℠	Codex adoption
Vitamins A/E	X	X	X	X
Vitamin D	X	X	X	X
Vitamin B_{12}	X	X	X	X
Folate	X	X	X	X
Inositol	X	X	X	X
Nucleotides	X	X	X	X
Cr/Mo/Se	X	X	X	X
Vitamin C	X	X	X	X
Choline	X	X		
Pantothenic acid	X	X	X	X
Carnitine	X	X	X	
Iodine	X	X	X	X
Fatty acids	X	X	X	X
Biotin	X	X	X	X
Vitamin K	X	X	X	
FOS	X	X		
GOS	X			
Minerals	X	X	X	
Amino acids	X	X		
Carotenoids (alpha-carotene, beta-carotene, lutein, lycopene)	X	X		
Chloride	X	X	X	X
Fluoride	X	X		
Vitamin B_1	X	X	X	
Vitamin B_2	X	X	X	
Vitamin B_3	X	X	X	
Vitamin B_6	X	X	X	

[a] From http://stakeholder.aoac.org/SPIFAN/; accessed August 18, 2019

SMPR: Standard Method Performance Requirement; FOS: Fructo Oligo Saccharide; GOS: Glacto Oligo Saccharide

1.4 Challenges with the leadership of SPIFAN

SPIFAN will constitute the basis of analysis methods and standards for use India and China, which are projected to become a massive market in the future. The use of these unified official methods will facilitate smooth international trade in the future. Besides, for the leading members of the project, their participation will be advantageous to themselves and to the activities of the organization in the future.

The leading members of SPIFAN are food, raw-material, and equipment manufacturers in western countries, who exert a strong influence in both these projects. Therefore, the official methods adopted in these projects are strongly influenced by the interests of the leading members and do not fully reflect the interests of the minor member countries and organizations. Due to such reasons, some official methods are not sufficiently optimized in terms of reliability and versatility, which could potentially lead to unnecessary waste of food internationally, due to its deviation from the standard values set forth by some poorly drafted official methods. This is in part because the standards and criteria for infant formulas from countries/organizations other than western countries are not fully considered while formulating some official methods (Challenge 1). Furthermore, some official methods are designed for testing using equipment made by manufactures from western countries, who mainly take part in SPIFAN, and the lack of these equipment could lead to faulty results. Due to these reasons, some official methods cannot be used without adopting equipment made from manufactures in western countries, who are key stakeholders in SPIFAN (Challenge 2).

1.5 Review of trace elements and their challenge in SPIFAN

1.5.1 Reasons for minerals and trace elements selected by SMPRs at an early stage and their smoothly progression

SMPRs reflect the analytical requirements of the user community and are especially influenced by the interests of the leading members. Test items of SPIFAN are shown in Figure 1.3. Most of the nutrients of SMPRs are micronutrients, such as vitamins, minerals, and trace elements, and other novel nutrients such as nucleotides, Fructo Oligo Saccharide (FOS), and GOS. Based on these SMPRs, these kinds of the test methods are unified gradually [15-18]. In particular, SMPRs for minerals and trace elements were established at an early stage. Also, the development of the test methods was advanced [19].

Following are the two key reasons why minerals and trace elements were selected for SMPRs at an early stage and their analysis progressed smoothly. First, minerals and trace elements have a significant effect on the human body, even if present in small quantities. Moreover, they may be both essential and toxic depending on their content. Second, sample inorganic analysis process is relatively simple. Listed below is one such case that supports these reasons. Most of minerals and trace elements without choline were adopted as Codex methods.

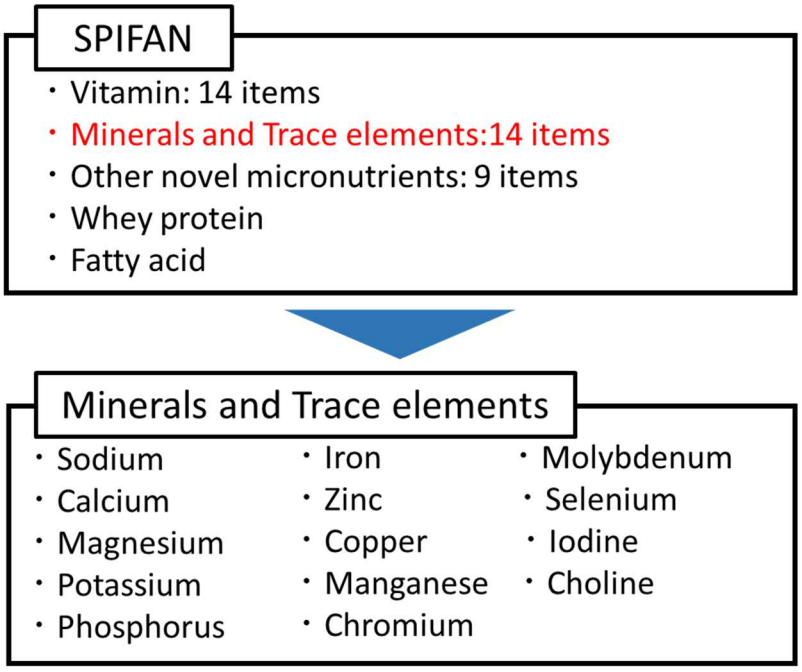


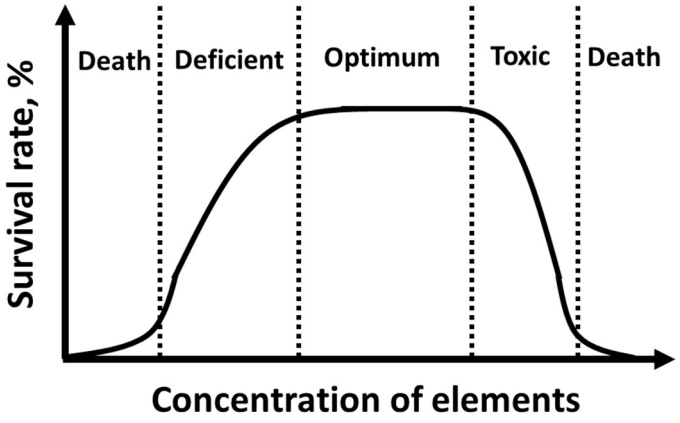
Figure 1.3 Test items of Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN).

1.5.2 About minerals and trace elements

Trace elements have a significant effect on the human body, even if present in trace amounts. Moreover, they may be both essential and toxic depending on their content. Minerals and trace elements are inorganic species or substances that are of neither animal nor vegetable origin [19]. Minerals (calcium, magnesium, sodium, potassium, phosphorus, and chlorine) and some essential trace elements (e.g., iron, zinc, copper, chromium, molybdenum, selenium, and iodine) account for about 3.5% and 0.5% of total human body, respectively. Moreover, trace elements play an important role as materials and regulators in numerous biological activities in body structure building. On the other hand, some trace elements such as arsenic, lead, cadmium, and mercury are regarded as toxic elements [19].

When the amount of most essential trace elements is low, deficiencies develop, and patients may die if deficiencies persist. On the other hand, when most essential elements are excessive, poisoning occurs, and depending on the amount, patients may die (Figure 1.4 A). The optimum concentration varies depending on the element. On the other hand, the high content of elements other than essential trace elements can cause poisoning symptoms and/or death depending on the amount (Figure 1.4 B).

A Essential trace elements



B Other than essential trace elements

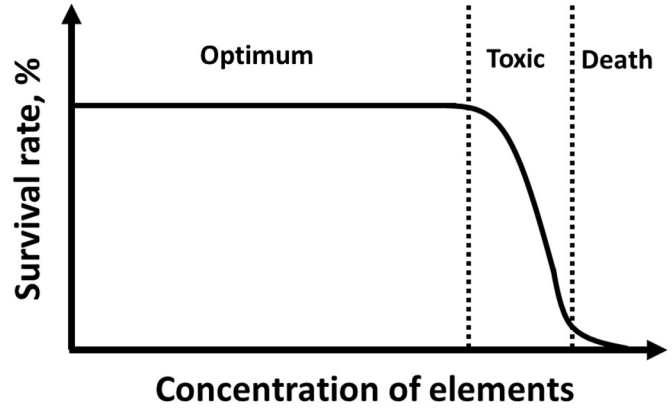


Figure 1.4 Relationship between concentration of elements and survival rate.

Therefore, Codex standards for trace elements in infant formula is strictly set. For example, the Codex standard and analytical range for selenium and iodine in infant formula are 1-9 μg/100 kcal and 10-60 μg/100 kcal, respectively [20]. From the above, it can be concluded that analytical methods used for testing infant formula need extraordinary precision and accuracy to effectively monitor contents of these trace elements.

1.5.3 Trace element analysis of infant formula

In order to strictly control the concentration of trace elements, sample preparation and measurement techniques have advanced. Regarding sample preparation, development of decomposition techniques such as acid dissolution of dry ash, wet acid, or alkaline digestion/extraction by conventional conductive or microwave heating enhanced inorganic analysis [19]. In particular, inorganic substances can remove most other main components by wet ashing. Moreover, the final solutions are easily amenable to any inorganic analytical technique [19]. Therefore, pretreatment of trace elements does not require complicated processing compared to that of other micronutrients listed in SPIFAN and SPSFAM.

Furthermore, Atomic Absorption Spectrometry (AAS) and Inductively Coupled Atomic Emission Spectrometry (ICP-AES) capable of simultaneous multi-analyte measurement have been designated as test methods of mineral and trace elements. Nowadays, Inductively Coupled Plasma Mass Spectrometry (ICP-MS), which currently has the highest sensitivity, is the main measurement method in trace element analysis in order to satisfy stricter nutritional and safety requirements. ICP-MS equipped with new Collision and Reaction Cell (CRC) technologies and an inert/reactive gas (e.g., helium,

hydrogen, ammonia, methane, or oxygen) is employed to minimize isobaric and polyatomic interferences [19]. Also, each ICP-MS manufacturer utilizes original CRC technology.

1.5.4 Participation in MLT

The author took part in MLT that minerals and trace elements in milk, milk products, infant formula, and adult/pediatric nutritional formula, ICP-MS method: collaborative Study, AOAC Final Action 2015.06, ISO/DIS 21424, IDF 243 as an analyst. The result of the MLT was good result [21]. Based on these results, it was registered as a Codex test method. After MLT, when the author tried to introduce AOAC 2015.06 in our laboratory. The relative standard deviation of selenium in infant formula distributed in Japan was scattering (12.3%). And AOAC 2015.06 describes that equipment conditions with specifications that were difficult to introduce when measuring only selenium are set [21].

1.5.5 Challenges in trace element analysis faced by SPIFAN

As described above, selenium, which are present in infant formula as nutrients, will face these challenges for trace element analysis. Listed below is one such case which describes these challenges.

By testing selenium according to the AOAC-OMA (AOAC 2015.06 [22]), which forms the basis of the Codex methods, the selenium content in an infant formula manufactured in Japan is around the lower limit of the standard defined by CAC [20], and is outside the analytical range of SMPR for selenium (SMPR 2011.009 [4], Figure 1.5). Currently, as sodium selenite is the only approved selenium additive in Japan since 2017,

and as it is also designated as a poison, the manufacturing of the infant formula while conforming to the results from the available analytical methods is highly challenging because the use of the generated material is not practical. Overall, the current methods present deviation risks from the standard values derived while using the official method (Challenge 1).

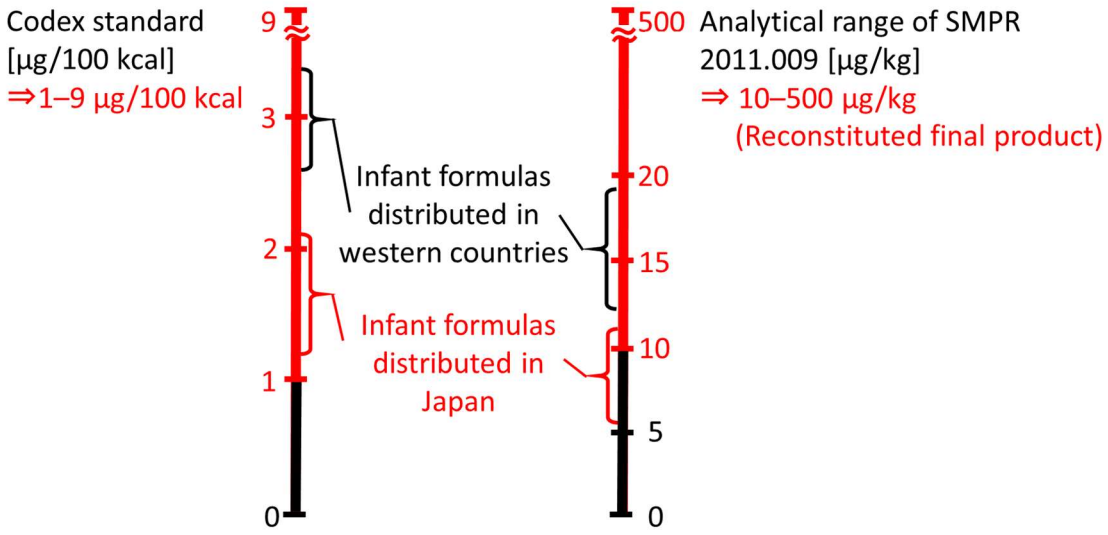


Figure 1.5 Codex standard and the analytical range of Standard Method Performance Requirement (SMPR) for selenium in infant formula.

Besides, for the separation elements using ICP-MS for selenium measurement according to AOAC 2015.06, hydrogen is designated as the cell gas. However, only specific manufacturers formally use hydrogen, and not all manufacturers employ ICP-MS. Due to such restrictions with the use of AOAC 2015.06 [22], there is a distinct possibility that a state or an organization may be excluded from the international trade due to lack of this equipment, which prevents the introduction of the Codex method based on AOAC 2015.06 [22] (Challenge 2).

In addition, the author considered that iodine could cause the same problems as selenium. Iodine is a key component in infant formulas and is present only in trace quantities like selenium and is an essential nutrient for infants. Therefore, a reliable measurement method is needed for its analysis. On the other hand, the use of iodine additives in infant formulas is not allowed in Japan, and the typical amount of iodine in infant formulas is around the lower limit of the standard defined by CAC [20] and SMPR for Iodine (SMPR 2012.008 [23], Figure 1.6). Therefore, when measuring the iodine content in infant formulas from Japan, the fluctuation of the measured value seemed to become significant, which presents increased risk of deviation from the standard value and could potentially lead to nonconformance and the disposal of the material (Challenge 1).

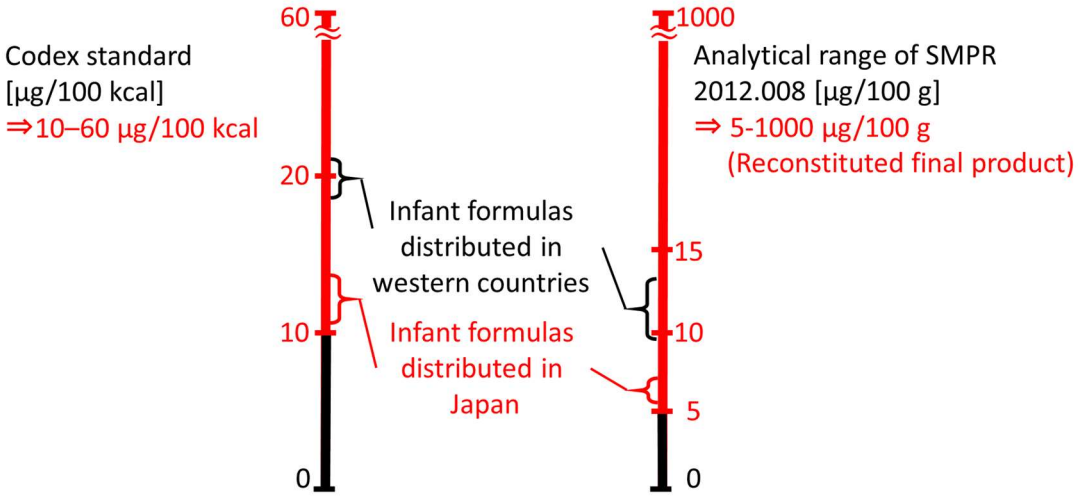


Figure 1.6 Codex standard and the analytical range of Standard Method Performance Requirement (SMPR) for iodine in infant formula.

As a result, these methods, which have become Codex test methods at SPIFAN, have problems with the versatility of the measurement method and the reliability of the measurement values, which may confuse international trade.

1.6. Development of solutions for the various presented issues

As mentioned above, projects such as SPIFAN is mainly conducted by western countries and organizations, while global partners are not actively involved in these projects. Besides, the analytical methods for testing infant formulas in Japan are defined by the Consumer Affairs Agency, Government of Japan. These analytical methods are far from global standards, and the Consumer Agency in Japan is not active in revising them, which poses additional challenges in testing.

While several test methods have been reported for such analyses, there have been no reported research for improving them by considering the aforementioned limitations. To address these limitations, the author conducted a study for analyzing infant formulas from the perspective of improving the reliability and versatility of the AOAC-OMA for the detection and determination of selenium and iodine, which form the basis of Codex methods because of the above-mentioned reasons.

In Chapter 2, the author examined improvement in the versatility and the expansion of the measurement range for AOAC 2015. 06, which is an AOAC-OMA for selenium in infant formulas, by initially optimizing sample preparation and changing the cell gas employed in ICP-MS to solve the challenges of SPIFAN. Since our results appeared promising, the author decided to continue to our studies of AOAC-OMA for iodine.

In Chapter 3, the author examined the improvement of reliability for AOAC 2012.15, which is AOAC-OMA for iodine in infant formulas. Because of the challenges described above, the possibility that the measurements from the method might be inapplicable, such as for the sensitivity of selenium (Chapter 2), was considered at first in order to solve challenge 1.

From the results of Chapter 2 and Chapter 3, the author succeeded at conducting optimizations of all Codex methods for trace elements formulated in SPIFAN. Moreover, the optimization of these test methods was easy to introduce and reliable, even for infant formula that cannot be supplemented with selenium and iodine additives, such as infant formula distributed in Japan. The discussion up through Chapter 3 has addressed the optimization of SPIFAN test methods that has been completed. On the other hand, the development of Codex test method AOAC 2015.01, which is a test method for heavy metals (arsenic, lead, cadmium, mercury) in foods, is currently underway in the SPSFAM project. Equipment conditions with specifications that are difficult to introduce at the time of measurement were set for arsenic, as in the selenium test method (Challenge 2).

Therefore, as discussed in Chapter 4, the author conducted additional examinations to improve the versatility of AOAC 2015.01, which is the AOAC-OMA slated to become the Codex method for arsenic in infant formula in SPSFAM, by adapting helium as the cell gas instead of oxygen gas for ICP-MS measurement, as in the case of selenium (Chapter 2) and employing the dry ashing method for sample preparation to solve challenge 2. the author decided to implement the versatility improvement before AOAC 2015.01 was adopted as a Codex test method based on the knowledge discussed in chapter 2 as an additional study.

By organizing these research results and sharing them with the concerned organizations, it will be the first opportunity for organizations around the world to introduce versatile and reliable Codex methods. These improved methods are expected to enhance the food environment by the supply of stable infant formula. Ultimately, these results will help achieve the Goal 3, which is to “Ensure healthy lives and promote well-being for all at all ages” and Goal 12, which is to “Ensure sustainable consumption and production patterns” among the 17 goals of the Sustainable Development Goals (SDGs) adopted by the United Nations in 2015 [24]

Chapter 2 Studies on improvement of the versatility and analytical range of AOAC Official Method 2015.06 for determination of selenium

2.1 Introduction

As described in Chapter 1, the author initially examined the improvement of the versatility and analytical range of AOAC 2015.06 for selenium analysis, which forms the basis for the Codex method for selenium testing in infant formulas to solve the challenges of SPIFAN, since AOAC 2015.06 encompasses both challenges (i.e., challenges 1 and 2).

Selenium is an essential trace element and is an integral part of many antioxidant enzymes such as glutathione peroxidase and selenoprotein P in humans and animals [25]. Deficiency of selenium leads to various clinical consequences, including cancer, cardiovascular diseases, type 2 diabetes, and lung disorders [25]. The infant selenium intake depends on the content of selenium in human milk and/or infant formula [26]. Selenium determination in human milk and/or infant formulas requires very sensitive techniques due to the low levels of selenium, which are of the order of a few micrograms per liter [26]. Therefore, it is important to select suitable measurement methods and conditions for reliable determination of selenium in infant formula.

The classical selenium decomposition method is thermal decomposition with the addition of nitric, nitric, and perchloric acid. However, in the decomposition method, if the nitric or sulfuric acid evaporates during the thermal decomposition and only organic substances and perchloric acid exist, there is a risk of explosion. Currently, a dry ashing method serves as one of the main decomposition methods, but selenium is not desirable because it easily becomes volatile at high temperatures. Nowadays, the most commonly used digestion technique for minerals and trace elements is wet ashing using a closed-vessel microwave system. This approach allows high sample throughput, significantly minimizes losses during oxidation, avoids any reaction between the minerals and vessels,

and reduces the contamination risk [19]. In the case of selenium, by using a closed-vessel microwave system, the volatilization of selenium is suppressed.

Hydride generation AAS (HGAAS) is famous as a selenium measurement method [19]. However, pre-processing in HGAAS is time-consuming and a high level of skill is required of the analysts. Recently, ICP-MS has been commonly used for inorganic analysis. ICP-MS technology is certainly the multianalyte technique of choice to obtain equivalent or better instrumental detection limits in solution. Moreover, ICP-MS detectors allow samples with varying analyte concentrations to be analyzed together because of their wide analytical working range [19]. Employing an ICP-MS after wet ashing by using a closed-vessel microwave system is currently the best option for mineral analysis in digested food samples [19]. AOAC 2011.19, which is applied for the determination of Cr, Se, and Mo in infant formulas and adult/pediatric nutritional formulas, adopted ICP-MS after wet ashing digestion [27].

AOAC 2015.06 is an extension of AOAC 2011.19 [21-22, 28] and is employed for determining nine additional elements (Na, Mg, P, K, Ca, Cr, Mn, Fe, Cu, Zn, Se, and Mo). AOAC 2015.06 has also been extended to support the analysis of 11 of the 12 elements, other than Cr, in dairy products (milk, milk powder, whey powder, whey protein concentrate, butter, and cheese) [21].

AOAC 2015.06 has been selected as the joint AOAC-ISO/IDF method in 2017 [29], and AOAC 2015.06 has been selected the Codex type II method in 2018 [30]. On the other hand, the content of selenium in infant formulas manufactured in Japan is around the lower limit of the standard defined by CAC [20] and is outside the analytical range of SMPR for selenium (SMPR 2011.009 [4]). Because sodium selenite, currently the only approved selenium additive in Japan since 2017, is also designated as a poison, which

renders its use and control during manufacturing and analysis highly challenging. From the above, there is a significant deviation risk from the standard value derived using AOAC 2015.06 that cannot be applied to infant formula in the absence of selenium additives (Challenge 1).

Additionally, AOAC 2015.06 specifies the use of hydrogen gas as the cell gas during ICP-MS analysis, and there are only a few manufacturers who have formally adopted hydrogen gas as the cell gas in such instrumentation. Due to these reasons, a distinct possibility exists where a nation or an organization which does not possess this equipment, may suffer removal from the international trade arrangements due to the introduction of the Codex method based on AOAC 2015.06 (Challenge 2).

To overcome these challenges, the author aimed to expand the lower limit of the analytical range of AOAC 2015.06. The author also attempted to evaluate alternative measurement instrumentation by optimizing the sampling size and the amount of final test solution, and by using helium gas, which has high model selectivity but lower sensitivity than hydrogen gas [31].

2.2 Materials and methods

2.2.1 Apparatus

(a) *Precision balance* — Model XSE204 (Mettler Toledo International Inc., Colombus, OH, USA).

(b) *Microwave oven* —TOPwave (Analytic Jena AG, Thuringia, Germany.)

Apparatus parameters employed were according to AOAC 2015.06 [22]

(c) *ICP-MS* — NexION 300D (Perkin Elmer, Inc., Waltham, MA, USA).

The apparatus parameters are as follows. Radio Frequency (RF) power was

1600 W. Sampling depth was 5 mm. Flow rate of the carrier gas was 0.99-1.07 mL/min. Nebulizer (Glass concentric) was Meinhard Type C. Interface cone was made from nickel. Helium cell gas flow rate was 4.5 mL/min. Nebulizer pump rate was 20 rpm. And the analyte/internal standard/gas mode was Selenium-78/Tellurium-130 in helium mode.

2.2.2 Reagents

(a) *Laboratory water* — Water purified with a Milli-Q® Integral 10 system (Merck KGaA, Darmstadt, Germany) with 18 MΩ was used.

(b) *Selenium standard (1000 mg/L) and tellurium standard solutions (1000 mg/L)* — Purchased from Kanto Chemical Co. (Tokyo, Japan).

(c) *Selenium standard stock solution (20 μg/L)* — The selenium standard solution (2 mL) was added to a 100 mL volumetric flask with glass pipet and was then diluted to volume with laboratory water (solution A). Then, solution A (10 mL) was added to a 100 mL volumetric flask with glass pipet, and the mixture was diluted to volume with laboratory water (solution B). In addition, solution B (1 mL) was added to a 100 mL volumetric flask with glass pipet, and the mixture was diluted to volume with laboratory water.

(d) *Internal standard stock solution (5 mg/L)*—The tellurium standard solution (0.5 mL) was added to a 100 mL volumetric flask with glass pipet, and was then diluted to volume with laboratory water.

(e) *Calibration Blank (Cal Blk) and calibration standard (Cal Std) solution set* — Cal Blk (0 μg/L), Cal Std 1 (0.4 μg/L), Cal Std 2 (2.0 μg/L), Cal Std 3 (8.0 μg/L), and Cal Std 4 (16.0 μg/L) standard solutions were prepared by pipetting 0 mL, 1 mL, 5 mL, 20 mL, and 40 mL, respectively, of the selenium standard stock solution into

separate 50 mL DigiTUBE®s (SCP SCIENCE, Baie D'Urfé, Canada) with a glass pipet. The internal standard stock solution (0.5 mL) was added with a digital pipet, followed by the addition of 5 mL of concentrated nitric acid, and 0.5 mL of methanol to each flask with the digital pipet. The volume was made up with laboratory water.

(f) *Selenium standard solution for recovery factor (0.05 mg/L)* — Selenium standard solution (1 mL) was added to a 100 mL volumetric flask with glass pipet, and the mixture was diluted to volume with laboratory water (solution C). Furthermore, solution C (1 mL) was added to a 200 mL volumetric flask with glass pipet and was diluted to volume with laboratory water.

(g) *Other reagents* —Nitric acid (60%), hydrogen peroxide (30–35.5%), and methanol (>99.8%) were purchased from Kanto Chemical Co. (Tokyo, Japan).

2.2.3 Sample

(a) *Reference material* —NIST SRM® 1849 Infant/Adult Nutritional Formula was used.

(b) *Infant formula* —The infant formula with lower selenium content distributed in Japan was used.

2.2.4 Sample preparation

(a) *Reference material* —NIST SRM® 1849 samples were prepared according to the procedure described in AOAC 2015.06 [22].

(b) *Sample solution* — Sample solutions (10 %, w/w) were prepared by reconstituting 10 g of infant formula in 90 g of warm laboratory water (60 °C).

(c) *Control sample (test condition AOAC 2015.06)* —The control sample was prepared according to the procedure described in AOAC 2015.06 [22].

(d) *Test samples (test condition A-F)* —The procedure employed for the test sample preparation is described in Figure 2.1. The following quantities of the sample solutions were added into the digestion vessels: 3.0 g (test condition A, B, C, and F), 4.5 g (test condition D), and 6.0 g (test condition E). Selenium standard solution for recovery factor (0.5 ml) was added with glass pipet to the recovery factor sample (only test condition F). The following internal standard stock solutions were added with a micropipette: 0.5 mL (test condition A), 0.2 mL (test condition B, D, E, and F), and 0.1 mL (test condition C). Concentrated nitric acid (0.5 mL), hydrogen peroxide (2 mL) were added with a digital pipette. A heating program equivalent to that shown in AOAC 2015.06 [22] was executed. After digestion, the contents were transferred to 50 mL DigiTUBE®s, and the vessels were washed with laboratory water. Laboratory water was transferred to the washing the vessel. With the laboratory water, the following solutions were added: 0.5 mL (test condition A), 0.2 mL (test condition B, D, E, and F) of methanol and the mixtures were diluted to 50 mL (test condition A), and 20 mL (test condition B, D, E, and F) with laboratory water. In the case of test condition C, the author employed another procedure in order to concentrate the final test solution volume. After digestion, the contents were transferred into a laboratory quartz beaker, and the vessel was washed with water. The beaker was placed on a hot plate, which was set at 90 °C, and the mixture was maintained under this condition until almost no liquid remained. After concentration, the concentrate was suspended in 2 mL of laboratory water. The contents were transferred to 50 mL DigiTUBE®s, and the vessel was washed with laboratory water. Methanol (0.1 mL), and

concentrated nitric acid (1 mL) were added, and the mixture was diluted up to 10 mL with laboratory water.

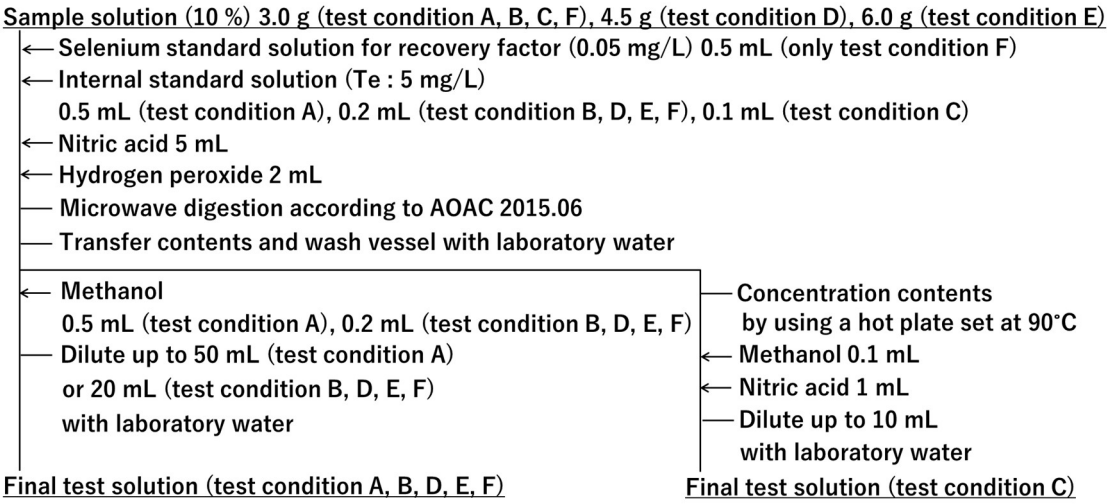


Figure 2.1 Diagram of the procedure for test sample preparation (test conditions A–F).

AOAC 2015.06: AOAC Official Method 2015.06

2.2.5 Linearity

Linearity was monitored as a coefficient of determination by constructing a calibration curve, which was prepared by using Cal Blk (0 μg/L), Cal Std 1 (0.4 μg/L), Cal Std 2 (2.0 μg/L), Cal Std 3 (8.0 μg/L), and Cal Std 4 (16.0 μg/L) standard solutions, and was compared to that of AOAC 2015.06 (>0.998) [22].

2.2.6 Limit of quantitation (LOQ)

The LOQ was defined as ten times the average standard deviation (SD), which was calculated from the result of the digested blank (unspiked) sample analyzed in decuplicate on a single day. LOQ was reconstituted as the final product (Formula 2.1), and was compared to that of SMPR 2011.009 (≤ 10 μg/kg reconstituted final product) [4].

$$LOQ, \frac{\mu g}{kg}(\text{final product}) = LOQ, \frac{\mu g}{L}(\text{digested blanks}) \cdot \frac{50\ mL}{0.2\ g} \cdot \frac{25\ g}{225\ g} \quad (2.1)$$

2.2.7 Analysis of reference material

Accuracy was tested in triplicate on a single day by using NIST SRM® 1849 Infant/Adult Nutritional Formula. Analytical results were expressed as mean ± Standard Deviation of intermediate precision (SD_{IM}). The obtained analytical result was compared with the certified value (783-841 μg/kg).

2.2.8 Analysis of calibration standard solution

Precision was tested in triplicate on a single day by using Cal Std 1 (0.4 μg/L), Cal Std 2 (2.0 μg/L), Cal Std 3 (8.0 μg/L), and Cal Std 4 (16.0 μg/L) standard solutions.

The obtained analytical results were expressed as mean ± SD, Relative Standard Deviation (RSD), and recovery factor ± SD.

2.2.9 Optimization of the pretreatment conditions – part 1

The control sample (test condition AOAC 2015.06) and test samples (test condition A-C) were analyzed in quintuplicate on a single day. Analytical results were expressed as mean ± SD and RSD. The obtained RSD was compared to that of SMPR 2011.009 (≤ 5.00 %) [4].

2.2.10 Optimization of the pretreatment conditions – part 2

The control sample (test condition AOAC 2015.06) and test samples (test condition B, D, and E) were analyzed in triplicate on 3 separate days. Analytical results were expressed as mean, Standard Deviation of repeatability (SD_r), Relative Standard Deviation of repeatability (RSD_r), SD_{IM}, and Relative Standard Deviation of intermediate precision (RSD_{IM}). The obtained RSD_{IM} was compared to that of SMPR 2011.009 (≤ 5.00 %) [4].

2.2.11 Recovery factor

Test samples (test condition B, and F) were analyzed in triplicate on 3 separate days. Analytical results were expressed as recovery factor, SDr, SD_{IM}, RSDr, and RSD_{IM}. The obtained recovery factor was compared to that of SMPR 2011.009 (90-110 %) [4].

2.3 Results and discussion

2.3.1 Linearity, LOQ, and analysis of reference material

To prove the validity of the test method, SPIFAN sets SMPRs for each test item. SMPR 2011.009 has LOQ, repeatability, and recovery performance requirements and demands that the accuracies of certified reference materials be checked [4]. Furthermore, although not described in the SMPR, linearity is an important item in confirming the validity of the test method. AOAC 2015.06 describes the performance requirements for linearity [22]. To confirm the performance requirements depending on the ICP-MS equipment conditions, the author changed the ICP-MS cell gas to helium and checked the linearity, LOQ, and reference material analysis results (NIST SRM® 1849).

Firstly, the author checked the linearity. The calibration curve of selenium displayed a coefficient of determination >0.999, which indicates good linearity and meets the requirement of AOAC 2015.06 (>0.998) [22]. Next, the author measured the digested blank (unspiked) sample, and calculated The LOQ. The LOQ was 0.44 μg/kg and meets the SMPR 2011.009 (≤4 μg/kg reconstituted final product) [22]. Finally, the author measured NIST SRM® 1849, and the result was determined to be 813 ± 25 μg/kg, and it was within the certified value range (783-841 μg/kg).

Based on the linearity, LOQ, and reference material analysis results, the author concluded that these results satisfied the performance requirements depending on the ICP-MS equipment conditions. Therefore, the author decided to investigate whether the repeatability of the results of infant formula measurement could be improved by using helium as the ICP-MS cell gas without adding selenium.

2.3.2 Analysis of calibration standard solutions

The author set the ICP-MS cell gas to helium and confirmed that it met the performance requirements of AOAC 2015.06, depending on the equipment conditions. Consequently, the author decided to optimize the sampling and final sample volumes so that stable measurements could be obtained with infant formula that did not contain selenium additives. Firstly, the author confirmed the repeatability of each standard solution to check the minimum concentration in the final test solution necessary to obtain a stable measurement value.

The repeatability of calibration standard solution studies is presented in Table 2.1. The RSD value of Cal Std 1 (9.42 %) was larger than that of the other calibration standard solutions (1.05-3.12 %). The range of recovery factor value of Cal Std 1 (108.4 ± 10.2 %) was also greater than that of the other calibration standard solutions (Cal Std 2 : 102.7 ± 3.2 %, Cal Std3 : 99.6 ± 2.1 %, Cal Std 4 : 101.3 ± 1.1 %). Therefore, the measurement of the selenium levels with high versatility and stable measurement precision is feasible by changing the cell gas stipulated by AOAC 2015.06 [22] (hydrogen) to helium, and the use of a final test solution with selenium concentration greater than 0.4 μg/L is recommended. Based on this result, I decided to optimize the pretreatment conditions.

Table 2.1 Repeatabilities of calibration standard solution for selenium (n=3).

Calibration standard solution concentration, µg/L	Mean ± SD, µg/L	RSD, %	Recovery ± SD, %
0.4	0.43 ± 0.04	9.42	108.4 ± 10.2
2.0	2.05 ± 0.06	3.12	102.7 ± 3.2
8.0	7.97 ± 0.17	2.09	99.6 ± 2.1
16.0	16.21 ± 0.17	1.05	101.3 ± 1.1

SD: Standard Deviation; RSD: Relative Standard Deviation

2.3.3 Optimization of the pretreatment conditions - part1

Based on analysis of calibration standard solutions, the author optimized the pretreatment conditions such as sampling size, as well as the final test volume. Accounting for the fact that it is used for analytical work, the author examined the pretreatment conditions not only to improve the repeatability but also for ease of introduction of the pretreatment method. The pretreatment conditions employed in this study are listed in Table 2.2.

To increase the selenium concentration in the final test solution, it was necessary to increase the sampling amount or the final test solution amount. When increasing the sampling amount under the current conditions (test portion size on dry basis: 0.2 g, final test solution volume: 50 mL), it is necessary to confirm whether the decomposition can be sufficiently performed under the microwave decomposition conditions specified in AOAC 2015.06. In inorganic analysis, the residual organic matter after pretreatment is considered to affect the value measured by ICP-MS. Therefore, organic substances should be decomposed by using microwaves as much as possible. When the amount of sampling at the time of microwave digestion exceeded 0.3 g, some contents after decomposition were not colorless and transparent, and consequently, some turbidity was observed. Therefore, the author considered the obtained pretreatment liquid to be insufficiently decomposed in organic matter. Consequently, the author set the test portion size on a dry base to 0.3 g; set the final volume to 50, 20, and 10 mL (test conditions A, B, and C, respectively), which are the volumes of the volumetric flasks typically used in the laboratory; and examined the optimum conditions.

Table 2.2 Test condition AOAC Official Method 2015.06 (AOAC 2015.06), A, B, and C for selenium.

Test condition	Test portion size on dry basis, g	Volume of final test solution, mL
AOAC 2015.06	0.20	50.0
A	0.30	50.0
B	0.30	20.0
C	0.30	10.0

The analytical results of samples prepared by test condition AOAC 2015.06, A, B, and C are shown in Table 2.3. The measurement precision of test condition A was improved to RSD of 6.22 % compared the 20.43 % obtained when test condition AOAC 2015.06 was used instead. However, these results did not satisfy the requirement of SMPR 2011.009. In addition, the measurement precision of test conditions B and C were improved further to RSDs of 4.56 % and 3.12 % compared to that of the 6.22 % value obtained when test condition A was used instead. These results meet the requirement of SMPR 2011.009 (≤5.0%) [4].

Furthermore, the selenium concentrations in the final test solutions corresponding to test conditions AOAC 2015.06 and A were 0.37 μg /L and 0.46 μg /L, respectively. Considering the results obtained with the calibration standard solution, the selenium concentration in the final test solution with Cal Std 1 (0.4 μg/L) indicates unstable measurement precision. On the other hand, the selenium concentrations in the final test solutions corresponding to test conditions B and C were 1.26 μg /L and 2.55 μg /L, respectively. Thus, by setting the selenium concentration in the final test solution to 1.26 μg/L or more, the author consider that the obtained RSD values indicate stable measurement precision.

The obtained results also suggest that the result from test condition C indicated the most stable measurement precision. However, test condition C required an additional step to concentrate the combined solution of the content and washing water after digesting, as the overall volume exceeded 10 mL. Due to the high volatility of selenium, its analysis at high temperatures is undesirable. However, at lower temperatures, the process takes a longer time, and on average requires about 3 hours by adopting the conditions of this study.

For obtaining superior precision and efficiency, test condition B is considered the optimal pretreatment condition. Based on these results, the author conducted further studies to confirm this consideration.

Table 2.3 Repeatabilities of samples prepared using test condition AOAC Official Method 2015.06 (AOAC 2015.06), A, B, and C for selenium (n=5).

Test condition	Mean ± SD, µg/kg	RSD %
AOAC 2015.06	93.01 ± 16.63	20.43
A	76.08 ± 4.73	6.22
B	84.30 ± 3.84	4.56
C	84.98 ± 2.65	3.12

SD: Standard Deviation; RSD: Relative Standard Deviation

2.3.4 Optimization of the pretreatment conditions - part2

Based on the study results described above, the author found that the final sample volume of 20 mL was useful in terms of work efficiency and measurement accuracy. In addition, when the sample amount was greater than 0.3 g under the microwave conditions of AOAC 2015.06, it was inferred that the measurement accuracy might be affected because the decomposition might be insufficient. In this study, to confirm that test condition B was the best for pretreatment, the author compared the repeatability of samples pretreated with various test conditions added with a sample amount of more than 0.3 g. The pretreatment conditions for this study are summarized in Table 2.4. After microwave-based digestion, some contents after decomposition under test conditions D and E were not colorless and transparent, and consequently, some turbidity was observed.

Table 2.4 Test condition AOAC Official Method 2015.06 (AOAC 2015.06), B, D, and E for selenium.

Test condition	Test portion size as a powder sample, g	Volume of final test solution, mL
AOAC 2015.06	0.20	50.0
B	0.30	20.0
D	0.45	20.0
E	0.60	20.0

The analytical results of samples prepared using test condition AOAC 2015.06, B, D, and E are shown in Table 2.5, and indicate that the measurement precision of the AOAC 2015.06 test condition tended to be highly scattered (RSD_r : 17.62 %, RSD_{IM} : 15.66 %). In addition, the measurement precision of the test condition B (RSD_r : 2.54 %, RSD_{IM} : 3.49 %) was lower than that obtained with the other test conditions (test condition D : RSD_r 5.96 % RSD_{IM} 5.75 %, test condition E : RSD_r 4.10 % RSD_{IM} 5.35 %). The result of test condition B meets the requirement of SMPR 2011.009 (≤5.0%) [4]. Furthermore, the selenium concentrations in the final test solutions for the AOAC 2015.06 test conditions and conditions B, D, and E were 0.37 μg/L, 1.20 μg/L, 1.93 μg/L, and 2.53 μg/L, respectively. Although the selenium concentrations in the final test solutions corresponding to test conditions D and E were higher than that corresponding to test condition B, RSDIMs did not satisfy SMPR. These results suggest that the lack of sample decomposition via microwave digestion may affect these results.

Overall, these results indicate that test condition B is optimal for pretreatment. Finally, the author confirmed the recovery factor by changing the pretreatment condition to test condition B.

Table 2.5 Repeatabilities of samples prepared using test condition AOAC Official Method 2015.06 (AOAC 2015.06), B, D, and E for selenium (n=3×3 days).

Test condition	Mean, µg/kg	SD_r	RSD_r, %	SD_{IM}	RSD_{IM}, %
AOAC 2015.06	93.60	16.49	17.62	14.65	15.66
B	79.97	2.03	2.54	2.79	3.49
D	85.71	5.10	5.96	4.93	5.75
E	84.49	3.46	4.10	4.52	5.35

SD_r: Standard Deviation of repeatability; SD_{IM}: Standard Deviation of intermediate precision; RSD_r: Relative Standard Deviation of repeatability; RSD_{IM}: Relative Standard Deviation of intermediate precision

2.3.5 Recovery factor

Analytical results by changing the pretreatment condition to test condition B obtained for the recovery factor were as follows. Recovery factor : 103.1 %, SD_r : 2.52 %. SD_{IM} :5.08 %, RSD_r : 2.45 %, and RSD_{IM} :4.93 %). These results met the requirement of SMPR 2011.009 (90–110%) [4] and obtained a good precision.

Therefore, by changing the pretreatment condition to test condition B, it is considered that infant formula samples with lower selenium content can be measured with high accuracy.

2.4 Conclusions

The author aimed to make AOAC 2015.06 more flexible and attempted to expand its measurement range by optimizing the sampling size and the amount of final test solution and evaluated the use of helium as the reaction cell gas. The author processed and analyzed NIST SRM® 1849 and infant formula distributed in Japan which is around the lower limit of the standard defined by CAC [20] and is outside the analytical range of SMPR for selenium (SMPR 2011.009 [4]), in the absence of any selenium additives by using modified method. Good linearity was obtained over the standard concentration range 0.4–16.0 μg/L, with the coefficient of determination exceeding 0.999 and the LOQ for the reconstituted final product being 0.44 μg/kg. The result of NIST SRM® 1849 was 813 ± 25 μg/kg, within the certified range (783–841 μg/kg). The precision of measurement was improved to an RSD_{IM} of 3.49%, and a recovery factor of 103.1% was achieved upon adapting test condition B (test portion size on dry basis = 0.3 g, final test solution volume = 20 mL). This study demonstrates that helium gas can be used as the cell gas in ICP-MS instruments. Notably, its use eases the restriction of the selection of

ICP-MS instruments. Furthermore, the method also expands applicability to lower selenium content infant formula samples by modifying the sample preparation method. The results of this study indicate that I found a key solution for solving the issues arising from the possibility that the results employing the official method for the infant formula analysis, which is outside the analytical range of SMPR for selenium (SMPR 2011.009 [4]), could lead to deviations and disposals (Challenge 1). Also, solutions to situations, wherein a nation or an organization which may have to be dropped from the international trade due to the lack of the required instrumentation or the device specified for selenium, have also been addressed (Challenge 2).

Since this study revealed a potential means of overcoming these challenges, the author performed an additional study on the AOAC-OMA for iodine, which seems to have the same problem as the selenium test method.

Chapter 3 Studies on improvement the reliability of AOAC Official Method 2012.15 for determination of iodine

3.1 Introduction

In Chapter 2, the author described the studies for improving the versatility, and analytical range of AOAC 2015.06, which the AOAC-OMA selected as the Codex type II method for selenium in infant formula by optimizing the sampling size and the amount of final test solution and using helium as the reaction cell gas for ICP-MS measurement. Since this study revealed a potential route to solving these challenges, the author continued the AOAC-OMA studies for iodine that seems to have the same problem as the selenium test method (Challenge 1).

Similar to selenium, iodine is present in trace quantities in infant formulas. Importantly, it is an essential component of the hormones produced by the thyroid gland and is therefore essential for mammalian life. Iodine deficiency can lead to multiple adverse conditions in humans, which are termed iodine deficiency disorders, and are expressed due to inadequate thyroid hormone production. Iodine deficiency during pregnancy and infancy may impair the growth and neurodevelopment of the offspring and increase infant mortality [32]. On the other hand, the use of iodine additives in infant formula is prohibited in Japan, and the typical amount of iodine in infant formulas distributed in Japan is around the lower limit of the standard defined by CAC [20] and SMPR for Iodine (SMPR 2012.008 [23]). Therefore, when measuring infant formulas from Japan, the fluctuation of the measured value seemed to become significant, which increases the risk nonconformance of iodine levels in Japanese infant formulas and might lead to their disposal due to deviation from the standard value (Challenge 1). This was one of the scenarios considered for selenium measurements.

Since iodine volatilizes easily, it is necessary to perform heat treatment in a stabilized state by adding an alkaline solution such as KOH for pretreatment during

analysis. As a decomposition method, it is possible to use a drying oven that can easily introduce the pretreatment liquid into the laboratory or a microwave oven with high decomposition ability. Inorganic iodide (I^-) has historically been and still is determined in infant formula by either potentiometry (AOAC 992.24) using ISE or by HPLC-ECD (AOAC 992.22) [19, 33-34]. Today the ICP-MS technique is used to determine the total iodine through the recently issued AOAC 2012.15 method because ICP-MS technology has better instrumental detection limits in solution [19, 35].

AOAC 2012.15 meets the requirements of the SMPR 2012.008 [23, 36-37], and it was also selected as the joint AOAC-ISO/IDF method in 2015 [38]. Furthermore, AOAC 2012.15 was selected as the Codex type II method in 2017 [39]. AOAC 2012.15 describes two sample digestion procedures (oven digestion and open vessel microwave digestion) which are based on alkaline dissolution using a KOH solution. Oven digestion is operationally easier when digesting samples than the open vessel microwave digestion. According to a multi-laboratory testing report, 10 out of 13 laboratories that participated in the study used the oven method for sample digestion [37].

However, it is known in some cases, that these sample digestion methods do not completely decompose the matrix, which leads to the incomplete removal of carbon. Signal enhancements for iodine, similar to those for arsenic, selenium, and other elements, have been reported when co-existing carbon is present during the analysis [40]. The most commonly accepted explanation for this phenomenon is that the ionization energy of iodine (10.45 eV) is close to that of the CH bond (10.64 eV), which contributes to the enhanced signal intensity [40]. According to the previous study, Todor and coworkers revealed that accurate iodine quantification using ICP-MS requires the inclusion 2-propanol at a minimum 3% level as to the internal standard solutions for serving as a

source of organic carbon [41]. AOAC 2015.06, to matrix match the samples, methanol is added as a carbon equivalent to both the standard solution and the final test solution before analysis, for preventing selenium signal enhancements due to the presence of carbon in the samples [22]. In contrast, AOAC 2012.15 does not use a carbon additive, and in the case of AOAC 2012.15, when preparing final test solutions, the digested sample solutions after filtering are usually diluted twice [35]. Diluting the sample is known to reduce the matrix load on the ICP-MS detector. Therefore, optimization of dilutions according to the sample characteristics could reduce the signal enhancement for iodine caused by co-existing carbon. However, greater dilutions would affect the reporting limits [35]. The results of iodine determinations in infant formulas from various countries, including Japan, indicate that AOAC 2012.15 is not a reliable method for this analysis. The previous study revealed that the original AOAC 2015.06 was at risk of deviating from standard values derived from test methods. On the other hand, for the reasons mentioned above, it was found that if the original AOAC 2012.15 is used in international trade, products that deviate from the Codex standard are more likely to be distributed.

Given this situation, the author aimed to develop a more accurate iodine quantification procedure for analysis of infant formulas including those made in Japan, in which, incomplete digestion of the matrix was observed by addition of carbon in the form of methanol to both the standard solution and the final test solutions.

3.2 Materials and methods

3.2.1 Apparatus

(a) *Precision balance* — Model XSE204 (Mettler Toledo International Inc., Colombus,

OH, USA).

(b) *Drying oven* — Model DX31 (Yamato Scientific, Co., Ltd. Tokyo, Japan).

(c) *ICP-MS* — NexION 300D (Perkin Elmer, Inc., Waltham, MA, USA).

Apparatus parameters are described below. RF power was 1600 W. The sampling depth was 5 mm. The carrier gas was set to 1.17–1.23 mL/min and was optimized every day. The nebulizer (glass concentric) was a Meinhard Type C. Interface cones were made from nickel. The nebulizer pump rate was 20 rpm. The analyte/internal standard/gas mode consisted of iodine-127/praseodymium-141 in standard (STD) mode.

The internal diameter (id) of the pump tubing used for the carrier solution was 0.38 mm. Additionally, the id of the pump tubing used for the internal standard solution was 0.19 mm.

3.2.2 Chemicals and reagents

(a) *Laboratory water* — Water (18 MΩ) was purified with a Milli-Q® Integral 10 system (Merck KGaA, Darmstadt, Germany).

(b) *Potassium iodide and praseodymium standard solution (1000 mg/L)* — Purchased from Kanto Chemical Co. (Tokyo, Japan).

(c) *Other reagents* — KOH pellets and sodium thiosulfate pentahydrate were purchased from FUJIFILM Wako Pure Chemical Co. (Osaka, Japan). Ammonium hydroxide (28–30%), Triton® X-100, nitric acid (60%), perchloric acid (70%), and methanol (>99.8%) were purchased from Kanto Chemical Co. (Tokyo, Japan).

3.2.3 Reagent solution preparation

The reagent solutions (5% KOH solution, stabilizer concentrate, and so forth)

were prepared according to the procedure provided in AOAC 2012.15 [35].

3.2.4 Standard solution preparation

(a) *Iodine standard stock solution (1000 mg/L)* — Potassium iodide was crushed in a glass mortar and was added (0.6535 g) into a volumetric flask (500 mL) with a spatula. The salt was dissolved and diluted to volume with laboratory water.

(b) *Internal standard stock solution (10 mg/L)* — Praseodymium standard solution (1 mL) was added to a 100 mL volumetric flask with a glass pipet, and was diluted to volume with laboratory water.

(c) *Iodine standard solution sets for recovery factor* —Solution A (0.9 mg/L of the infant formulas distributed in Japan), Solution B (1.1 mg/L for the infant formulas distributed in the USA), and Solution C (1.6 mg/L of the infant formulas distributed in Germany) were prepared by pipetting 4.5, 5.5, and 8 mL, respectively, of the intermediate stock standard iodine solution (10 mg/L) into separate 50 mL DigiTUBE®s (SCP SCIENCE, Baie D'Urfé, Canada). KOH solution (5 mL of a 5% solution) and stabilizer concentrate (1 mL) were added with a digital pipet to each 50 mL DigiTUBE®, and the samples were diluted to volume with laboratory water.

(d) *Intermediate stock standard iodine solutions, calibration standard iodine solutions, and internal standard (IS) solutions* —The solutions were prepared according to the procedure described in AOAC 2012.15 [35].

(e) *Calibration standard iodine solutions with 5% methanol (only for modified AOAC 2012.15)* — Refer to the AOAC 2012.15 for the procedure up to dilution to volume with laboratory water [35]. Before the dilution, methanol (2.5 mL) was added into the separate 50 mL DigiTUBE®s with a digital pipet.

3.2.5 Samples

(a) *Reference material* — NIST SRM® 1849 Infant/Adult Nutritional Formula was used.

(b) *Infant formula* — Infant formulas with different iodine contents distributed in Japan (Infant formula A), USA (Infant formula B), and Germany (Infant formula C) were used.

3.2.6 Sample preparation

(a) *Optimization of the methanol concentration with calibration standard iodine solutions including methanol* — Solutions with 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10% methanol concentrations were prepared in 1, 10, and 100 µg/L iodine solutions by pipetting 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 mL, respectively, of methanol into separate 10 mL volumetric flasks with a digital pipet. The intermediate stock standard iodine solution (10 µg/L, 1 mL) was added to the intermediate stock standard iodine solution made up with 1 mg/L concentration (0.1 mL), and the intermediate stock standard iodine solution made up with 1 mg/L (1 mL) using a glass pipet to each flask, respectively. Then, 5% KOH solution (1 mL) was added to the stabilizer concentrate (0.2 mL) with a digital pipet, and the solution was diluted to volume with laboratory water.

(b) *Reference material* — NIST SRM® 1849 sample was prepared according to the procedure described in AOAC 2012.15 [35] (only for original AOAC 2012.15). However, in the case of the modified AOAC 2012.15, the final solution included 5% of methanol.

(c) *Sample solution* — The sample solution (11.1%, w/w) was prepared by reconstituting 25 g of the infant formula in 200 g of laboratory water.

(d) *Test samples (test conditions of the original procedure and modified AOAC 2012.15)* — The procedure for the test sample preparation, shown in Figure 3.1, is as follows. The sample solution (6.0 g) was added into separate 50 mL DigiTUBE®s. Iodine standard solutions (0.5 mL) were added for the recovery factor (Solutions A–D) with a glass pipet (only for the recovery factor). Laboratory water (10 mL) was then added, and 5% KOH solution (5 mL) was added with a digital pipet. The contents were mixed with a vortex apparatus, and the samples were digested in an oven set to maintain 105 ± 5 °C for 1 h. After digestion, the samples were allowed to cool to room temperature, and the stabilizer concentrate (1 ml) was added with a digital pipet, followed by dilution using laboratory water to 50 mL. Each digested sample solution was filtered using a 0.45 μm disposable syringe membrane filter (GL Science, Japan) into a 15 mL polypropylene (PP) centrifuge tube (Pretreatment solution). All pretreatment solutions (5 mL) were added into 10 mL volumetric flasks with a glass pipet, and methanol (0.5 mL) was added with a digital pipet (only for modified AOAC 2012.15) followed by dilution using laboratory water to 10 mL.

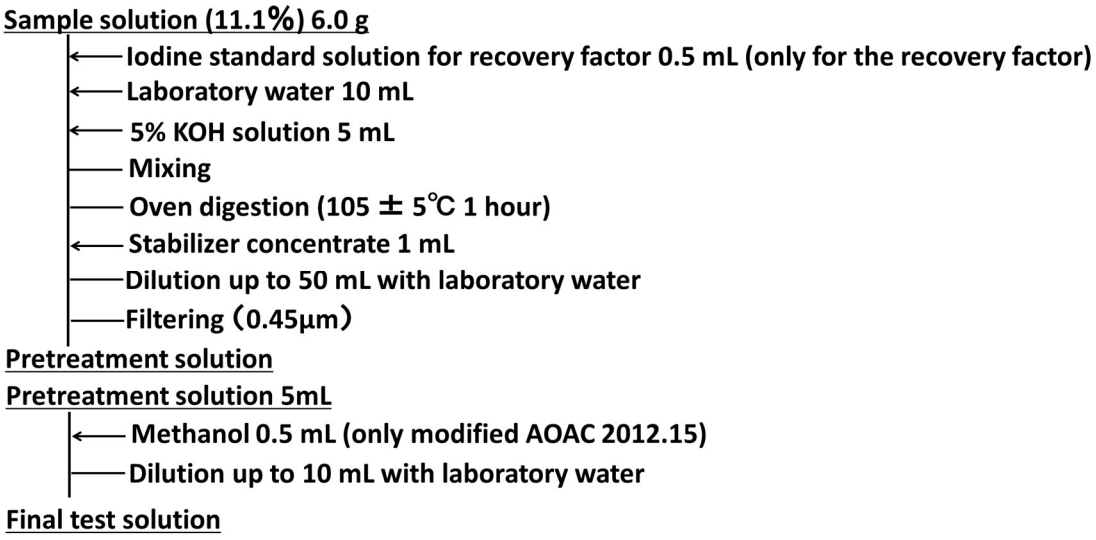


Figure 3.1. Diagram of the procedure for test sample preparation.

AOAC 2012.15: AOAC Official Method 2012.15

3.2.7 Optimized concentration of methanol

The optimized concentration of methanol, which was added to both the standard solutions and final solutions, was determined by comparisons of the enhancement factor for iodine-127 in calibration standard iodine solutions including methanol during optimization of the methanol concentration. The enhancement factor was computed as follows (Formula 3.1):

$$Enhancement\ \ factor\ \ of\ \ iodine-127 = \frac{ICP-MS\ \ signal\ \ of\ \ the\ \ calibration\ \ standard\ \ iodine\ \ solution\ \ including\ \ methanol\ \ from\ \ 0\ \ to\ \ 10\%, cps}{ICP-MS\ \ signal\ \ of\ \ the\ \ calibration\ \ standard\ \ iodine\ \ solution, cps} \quad (3.1)$$

3.2.8 Linearity

Linearity was monitored after the completion of the calibration curve. Calibration curves were prepared by using calibration standard iodine solutions, which included 5% methanol, and were compared to that of AOAC 2012.15 (>0.998) [35].

3.2.9 LOQ

The LOQ was defined as ten times the average standard deviation (SD), which was calculated from the result of the digested blank (unspiked) sample analyzed in decuplicate on a single day. The LOQ was computed for the reconstituted final product (Formula 3.2), and the results were compared to those of SMPR 2012.008 (≤5 μg/kg in the reconstituted final product) [23].

$$LOQ, \frac{\mu g}{100\ g}(\text{final product}) = LOQ, \frac{\mu g}{L}(\text{digested blanks}) \cdot 2(\text{dilution factor}) \cdot \frac{50\ mL}{0.667\ g} \cdot \frac{25\ g}{225\ g} \cdot 0.1 \quad (3.2)$$

3.2.10 Analysis of reference material

Accuracy was tested in triplicate on a single day by using the final test solutions of NIST SRM® 1849 Infant/Adult Nutritional Formula (original test condition and modified AOAC 2012.15). Analytical results were expressed as mean ± SD_{IM}. The analytical result was compared with certified value (118-140 μg/100 g).

3.2.11 Repeatability

Test samples (original test condition and modified AOAC 2012.15) were analyzed in triplicate on three separate days. The analytical results were expressed as the mean, SD_r, RSD_r, SD_{IM}, and RSD_{IM}. The RSD_{IM} was compared to that of SMPR 2012.008 (≤8.00%) [23].

3.2.12 Recovery factor

Test samples (original test condition and modified AOAC 2012.15) for assessment of the recovery factor were analyzed in triplicate on three separate days. The analytical results were expressed as the recovery factor, SD_r, and SD_{IM}. The recovery factor was compared to that of SMPR 2012.008 (90–110%) [23].

3.3 Results and discussion

3.3.1 Optimized concentration of methanol

To develop a more accurate iodine quantification procedure for the analysis of infant formulas, including those made in Japan, with incomplete digestion of the matrix, I firstly optimized the concentration of methanol for both the standard solution and final test solutions.

The analytical results for the calibration standard iodine solutions which include the added methanol are shown in Figure 3.2, which indicates that the enhancement factors of iodine-127 were 2.2–2.5 times higher when methanol was present in the 5% level when compared to that with 0%. Furthermore, this tendency did not change in cases with over 5% methanol. On the basis of these results, the author determined that the optimal concentration of methanol, which was added to both standard solutions and final solutions, was 5% (test conditions for modified AOAC 2012.15).

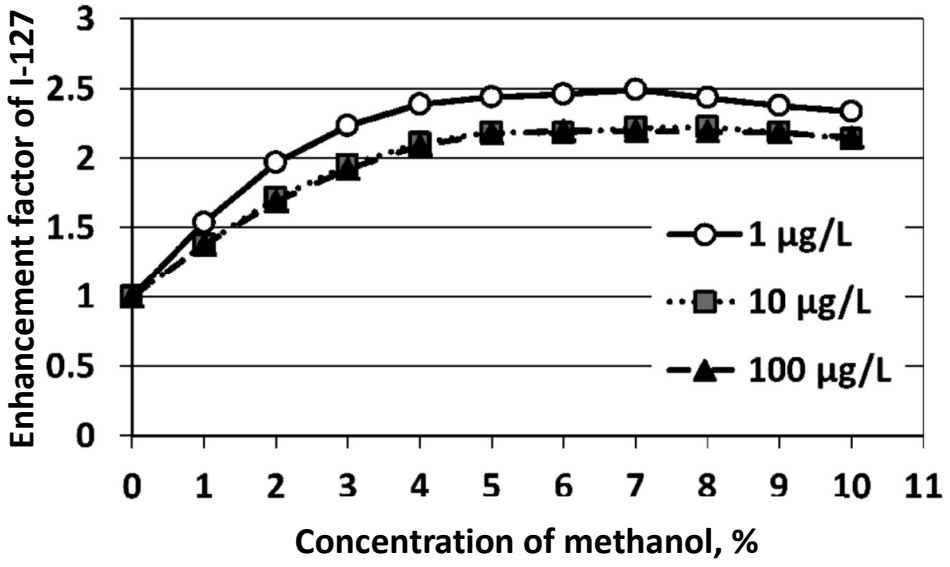


Figure 3.2 Dependence of the enhancement factor of selenium on the concentration of methanol.

3.3.2 Linearity, LOQ, and analysis of reference material

SMPR 2012.008 has LOQ, repeatability, and recovery performance requirements and demands that the accuracy of certified reference materials be checked [25]. Furthermore, AOAC 2012.15 describes the performance requirements for linearity [35]. To confirm the performance requirements depending on the ICP-MS equipment conditions, the author checked the linearity, LOQ, and reference material (NIST SRM® 1849) analysis results by using the original test condition and modified AOAC 2012.15.

Firstly, the author checked the linearity. The calibration curves for the original test conditions and modified AOAC 2012.15 produced coefficient of determination values >0.999 and the results thus exhibited good linearity and meets the requirement of AOAC 2012.15 (>0.998) [35]. Next, the author measured the digested blank (unspiked) sample, and calculated The LOQs of the original test conditions and modified AOAC 2012.15. The LOQs of the original test conditions and modified AOAC 2012.15 were 0.12 and 0.19 μg/100 g, respectively, and these values met the requirements of SMPR 2012.008 (≤5 μg/100 g reconstituted final product) [23]. Finally, I measured NIST SRM® 1849 by using the original test conditions and modified AOAC 2012.15. The results of NIST SRM® 1849 of the original test conditions and modified AOAC 2012.15 were 130.05 ± 5.77 and 120.43 ± 5.30 μg/100 g, respectively, and are within the certified value range (118-140 μg/100 g).

Based on the linearity, LOQ, and reference material analysis results, the author concluded that these results satisfied the performance requirements depending on the ICP-MS equipment characteristics under each test condition.

3.3.3 Repeatability

The author measured three kind of infant formula by the original test conditions and modified AOAC 2012.15. The analytical results of samples prepared by using the original test conditions and modified AOAC 2012.15 are shown in Table 3.1. The RSD_{IMS} of the original test conditions and modified AOAC 2012.15 samples were 1.72–5.28% and 2.62–3.76%, respectively, and these values met the requirements of SMPR 2012.008 (≤8.0%) [23]. These results confirm that there is no problem in the repeatability of the measured values of infant formulas distributed in several countries, including Japan. Furthermore, there is no problem with repeatability even with the modified AOAC 2012.15.

Table 3.1 Repeatabilities for samples prepared using original test conditions and modified AOAC Official Method 2012.15 (AOAC 2012.15) conditions (n = 3 × 3 days).

Sample name	Test condition	Mean, μg/100 g	SD_r	RSD_r, %	SD_{IM}	RSD_{IM}, %[c]
Infant formula A	original	78.45	1.21	1.54	1.35	1.72
	modified	67.52	0.98	1.46	2.17	3.21
Infant formula B	original	117.27	1.21	1.04	5.39	4.60
	modified	103.70	2.96	2.86	3.90	3.76
Infant formula C	original	130.01	0.84	0.64	6.87	5.28
	modified	113.41	2.59	2.29	2.97	2.62

SD_r: Standard Deviation of repeatability; SD_{IM}: Standard Deviation of intermediate precision; RSD_r: Relative Standard Deviation of repeatability; RSD_{IM}: Relative Standard Deviation of intermediate precision

3.3.4 Recovery factor

Finally, to confirm the signal enhancement due to the presence of carbon in the samples and the signal enhancement suppression by the addition of carbon in the form of methanol to both the standard and final test solutions, the author measured several kinds of IF by using the original test condition and modified AOAC 2012.15 and calculated the recovery factor. The recovery factors of samples prepared by using the original test conditions and modified AOAC 2012.15 are shown in Table 3.2. Analytical results (mean ± SD_{IM}) for the original test conditions in AOAC 2012.15 were as follows: Infant formula A, 118.1 ± 3.4%; Infant formula B, 119.5 ± 6.7 %; and Infant formula C, 118.8 ± 6.8%. These results did not meet the requirements of SMPR 2012.008 (90–110%) [23].In contrast, analytical results (mean ± SD_{IM}) for the test conditions of modified AOAC 2012.15 were as follows: Infant formula A, 102.1 ± 4.6%; Infant formula B, 101.7 ± 5.5%; and Infant formula C, 104.2 ± 5.8%. These results met the requirements of SMPR 2012.008 (90–110%). Therefore, by changing the test conditions in the modified AOAC 2012.15 procedure, the accuracy of iodine measurements could be improved for some infant formulas, which differed in their iodine and matrix material contents.

Table 3.2 Recovery factors for samples prepared by original test conditions and modified AOAC Official Method 2012.15 (AOAC 2012.15) conditions (n = 3 × 3 days).

Sample name	Spiked level, μg/L	Test condition	Mean, %	SD_r, %	SD_{IM}, %
Infant formula A	4.5	original	118.1	3.5	3.4
	4.5	modified	102.1	5.5	4.6
Infant formula B	5.5	original	119.5	2.1	6.7
	5.5	modified	101.7	5.9	5.5
Infant formula C	8.0	original	118.8	3.7	6.8
	8.0	modified	104.2	4.2	5.8

SD_r: Standard Deviation of repeatability; SD_{IM}: Standard Deviation of intermediate precision

3.4 Conclusions

The author aimed to make AOAC 2012.15 more reliable by adding carbon in the form of methanol to both the standard solutions and the final test solutions. I processed and analyzed NIST SRM® 1849 and the three kinds of infant formulas including that distributed in Japan which is around the lower limit of the standard defined by CAC [20] and the analytical range of SMPR for selenium (SMPR 2011.008 [23]).

Optimization of the addition of carbon additives showed that a minimum of 5% methanol was necessary to achieve a constant ratio of iodine. The results exhibited good linearity (coefficient of determination >0.999) when the standard concentrations ranged from 0.25 to 100 μg/L, and the LOQ was 0.19 μg/100 g for the reconstituted final product. The result of NIST SRM® 1849 was 120.43 ± 5.30 μg/100 g, within the certified range (118–140 μg/100 g). The measurement precision had an RSD_{IM} of 2.62–3.76%, and the recovery factor improved from 118.1–119.5% to 101.7–104.2% by the addition of carbon in the form of methanol to both the standard solutions and the final test solutions. This study demonstrates that methanol, which is added to both standard and final solutions, acts as an effective matrix matching agent and contributes to more accurate iodine quantification in infant formulas from each county, including Japan, which typically suffer from incomplete digestion of the matrix when employing the conventional method.

From the results presented in Chapters 2 and 3, the author was able to optimize AOAC 2015.06 and AOAC 2012.15. Consequently, these test methods can be easily introduced into laboratories and can be measured even for infant formula that does not contain selenium or iodine additives. Therefore, the author successfully optimized all Codex methods for minerals and trace elements formulated in SPIFAN (Figure 3.3).

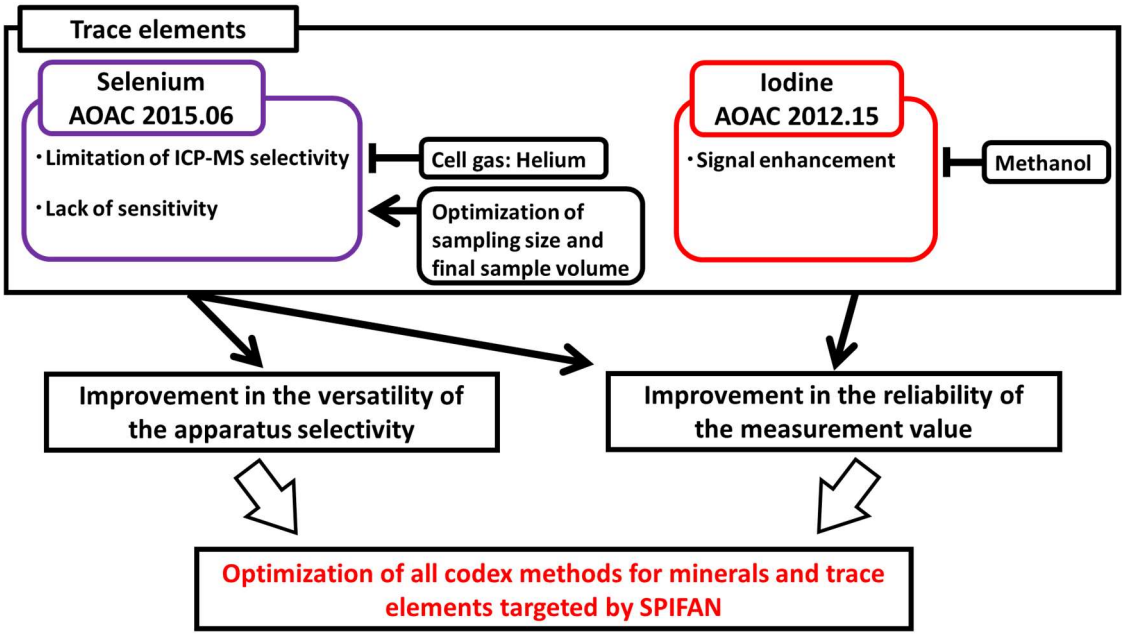


Figure 3.3 Flow and results of this study.

AOAC 2015.01: AOAC Official Method 2015.01; AOAC 2012.15: AOAC Official Method 2012.15

The text up through Chapter 3 has discussed the optimization of test methods related to SPIFAN that has been completed. On the other hand, the development of the Codex test method of AOAC 2015.01, which is a test method for heavy metals (arsenic, lead, cadmium, and mercury) in foods, is currently underway in the SPSFAM project [42]. Equipment conditions with specifications that are difficult to introduce at the time of measurement were set for arsenic, as in the selenium test method (Challenge 2). Therefore, the author decided to implement versatility improvement before AOAC 2015.01 was adopted as a Codex test method based on the knowledge described in Chapter 2 as an additional study.

Chapter 4 Studies on improvement the versatility of AOAC Official Method 2015.01 for determination of arsenic

4.1 Introduction

From Chapters 2 and 3, the author optimized the test methods related to SPIFAN. However, the development of the Codex test method based on AOAC 2015.01, which is a test method for heavy metals (arsenic, lead, cadmium, and mercury) in foods, is currently underway in the SPSFAM project. the author confirmed that equipment conditions that are difficult to introduce were set in AOAC 2015.01 in the case of arsenic. Thus, the author examined the improvement of the versatility of AOAC 2015.01 as an additional study (Challenge 2).

Arsenic is different from selenium and iodine, and elemental arsenic and most arsenic compounds are toxic to the human body. Besides, Arsenic is abundant in nature and inadvertently contaminates soil, water, and foods [43]. Elemental arsenic and most arsenic compounds are toxic to the human body. For example, cardiovascular effects, pulmonary disorders, reproductive effects, and neurological effects have been reported in adults and children due to arsenic exposure [44]. Thus, to ensure food safety, careful measurement of the residual arsenic compounds that are inadvertently contained in food is critical.

As pretreatment methods for heavy metals such as arsenic, the primary methods employed are the dry ashing method using a muffle furnace and the wet ashing method using a microwave decomposition apparatus. As in the case of selenium, HGAAS is famous as an arsenic measurement method. Official techniques are available in which HGAAS is utilized after dry-ashing or wet-ashing to determine the total arsenic in foodstuffs (AOAC 986.15) [19, 45]. However, AOAC 986.15 is not adopted as a Codex method.

AOACI established SMPR 2012.007 [46], which presents the standard method requirements for determination of heavy metals, such as arsenic in food samples. AOAC 2015.01 is a test method that is assumed to meet the requirements of SMPR 2012.007 [42, 46]. After further MLT, discussion and voting, it will be determined whether AOAC 2015.01 will be adopted as AOAC-OMA final action. In the future, AOAC 2015.01 may be registered as a Codex test method.

AOAC 2015.01 specifies that samples must be prepared using a wet ashing method with microwave digestion [42]. AOAC 2015.01 also specifies that oxygen must be used as the reaction cell gas for ICP-MS-based analysis of arsenic [42]. Compared to dry ashing in a muffle furnace, only a limited number of samples can simultaneously be processed in a microwave digester. The microwave digester and related equipment are also comparatively expensive. Besides, oxygen is specified as the reaction cell gas in AOAC 2015.01, and ICP-MS equipment that allows its use is limited, just as in the case of selenium (Challenge 2).

Due to these limitations for carrying out this method, it is possible that a nation or an organization, which is equipment-limited, may be excluded from the international trade of its food products due to non-conformance of arsenic levels.

The author aimed to address these concerns by developing a more versatile version of the AOAC 2015.01 method for the analysis of arsenic in infant formula. The author adapted the dry ashing method for sample preparation and employed helium, which is a highly versatile cell gas for the ICP-MS measurements.

4.2 Materials and methods

4.2.1 Apparatus

(a) *Precision balance* — Model XSE204 (Mettler Toledo International Inc., Colombus, OH, USA).

(b) *Muffle furnace* — Model FP410 (Yamato Scientific Co. Ltd., Tokyo, Japan).

(c) *Hot plate* — Model EA-DB10 (Zojirushi Corporation, Osaka, Japan).

(d) *Cooking heater* — Model HP-103K (Toshiba Corporation, Tokyo, Japan).

(e) *ICP-MS* — NexION 300D (Perkin Elmer, Waltham, MA, USA).

RF power was set to 1600 W, and the sampling depth was 5 mm. The carrier gas was set to 1.06–1.07 mL/min and was optimized every day. The nebulizer (glass concentric) was a Meinhard Type C, and the interface cones were made from nickel. The nebulizer pump rate was 20 rpm, and the analyte/internal standard/gas mode consisted of arsenic-75/rhodium-103 in Helium mode. The internal diameter (id) of the pump tubing was 0.38 mm for the carrier solution and was 0.19 mm for the internal standard solution.

4.2.2 Chemicals and reagents

(a) *Laboratory water* — Water (18 MΩ) was used purified with a Milli-Q® Integral 10 system (Merck, Darmstadt, Germany).

(b) *Arsenic (100 mg/L) and rhodium standard solutions (1000 mg/L)* — Purchased from Kanto Chemical Co. (Tokyo, Japan).

(c) *Other reagents* — Magnesium nitrate hexahydrate [$Mg(NO_3)_2$ $6H_2O$] (99%), ethanol (99.8%), nitric acid (60%), hydrochloric acid (35-37%), and acetic acid (97%) were obtained from Kanto Chemical Co., Inc. (Tokyo, Japan).

4.2.3 Reagent solution preparation

(a) *2% magnesium nitrate hexahydrate solution* — Magnesium nitrate (4 g) was added to a 500 mL volumetric flask with a spatula and was dissolved and diluted to volume with ethanol.

(b) *6 mol/L hydrochloric acid* — Laboratory water (250 mL) was added to a beaker (500 mL) followed by the addition of hydrochloric acid (250 mL), which was measured using a graduated cylinder.

(c) *1 mol/L nitric acid* — Laboratory water (250 mL) was added to a beaker (500 mL) followed by the addition of nitric acid (3.5 mL) which was measured with a digital pipette. The solution was diluted to volume with laboratory water.

(d) *12% nitric acid* — Laboratory water (250 mL) was added to a beaker (500 mL), and nitric acid (100 mL) was added with a graduated cylinder, and the solution was diluted to volume with laboratory water.

4.2.4 Standard solution preparation

(a) *Arsenic standard stock solution sets* — Arsenic standard solution (0.5 mL) was added into a 50 mL DigiTUBE® (SCP SCIENCE, Baie D'Urfé, Canada) with a glass pipet, and the solution was diluted to volume with laboratory water (Arsenic standard stock solution A: 1 mg/L). Arsenic standard solution (5 mL) was added into a 50 mL DigiTUBE® with a glass pipet and was diluted to volume with laboratory water (Arsenic standard stock solution B: 100 μg/L).

(b) *Internal standard stock solution (10 mg/L)* — Rhodium standard solution (1 mL) was added into a volumetric flask (100 mL) with a glass pipet and was diluted to volume with laboratory water.

(c) *Calibration Blank (Cal Blk) and calibration standards (Sal Std) solution sets* — Iodine standard stock solution A (1 mL) and nitric acid (2.5 mL) were added into a 50 mL DigiTUBE® with a glass pipet and a digital pipet, respectively, and were diluted to volume with laboratory water (Cal Std 1: 20 µg/L). Iodine standard stock solution B (2.5 mL) and nitric acid (2.5 mL) were added into a 50 mL DigiTUBE® with a glass pipet and a digital pipet, respectively, and were diluted to volume with laboratory water (Cal Std 2: 5 µg/L). Cal Std2 (5.0 mL) and nitric acid (2.5 mL) were added into a 50 mL DigiTUBE® with a glass pipet and a digital pipet respectively and were diluted to volume with laboratory water (Cal Std 3: 0.5 µg/L). Cal Std2 (1.0 mL) and nitric acid (2.5 mL) were added into a 50 mL DigiTUBE® with a glass pipet and a digital pipet, respectively and were diluted to volume with laboratory water (Cal Std 4: 0.1 µg/L). Cal Std3 (2.0 mL) and nitric acid (2.5 ml) were added into a 50 mL DigiTUBE® with a glass pipet and a digital pipet, respectively and were diluted to volume with laboratory water (Cal Std 5: 0.02 µg/L). Cal Std3 (1.0 mL) and nitric acid (2.5 mL) were added into a 50 mL DigiTUBE® with a glass pipet and a digital pipet, respectively, and were diluted to volume with laboratory water (Cal Std6: 0.01 µg/L). Nitric acid (10 mL) was added into a volumetric flask (200 mL) with a digital pipette and was diluted to volume with laboratory water (Cal Blk).

(d) *Arsenic standard solution sets for repeatability and recovery factor* — Arsenic standard stock solution A (5 mL) and nitric acid (2.5 mL) were added into a 50 mL DigiTUBE® with a glass pipet and a digital pipet, respectively and were diluted to volume with laboratory water (Arsenic standard solution for recovery factor A: 0.1 mg/L). Arsenic standard solution for repeatability and recovery factor A (2.5 mL) and nitric acid (2.5 mL) were added into a 50 mL DigiTUBE® with a glass pipet and a

digital pipet, respectively, and were diluted to volume with laboratory water (Arsenic standard solution for repeatability and recovery factor B: 0.005 mg/L).

(e) *Internal standard solution (40 μg/L)* — Internal standard stock solution (2 mL), laboratory water (100 mL), and acetic acid (100 mL) were added into a volumetric flask (500 mL) with a glass pipet, a graduated cylinder, and a digital pipet, respectively, and were diluted to volume with laboratory water.

4.2.5 Samples

(a) *Proficiency test sample* — Test sample for the eleventh proficiency test of trace elements in rice powder organized by KANSO CO., LTD. (PTP-1801WR) (Osaka, Japan.) was used.

(b) *Infant formula* — Infant formulas distributed in Japan (Infant formula A), USA (Infant formula B), and Germany (Infant formula C) were used.

4.2.6 Sample preparation

Test samples — Figure 4.1 shows the procedure for test sample preparation, and the details are as follows. The sample (0.25 g) was added into the beakers made of quartz with a spatula, and 0.5 mL of the arsenic standard solution sets for repeatability and recovery factors A and B were added with a glass pipet, respectively, into each sample (only test condition for repeatability and recovery factor). Magnesium nitrate hexahydrate solution (5.0 mL, 2 % solution) was added with a digital pipette, and the beakers were put on a hot plate, which was maintained at approximately 90 °C until no liquid remained. The beakers were then placed on an electric stove until samples converted into ashes, and these were then transferred to an electric furnace set at 550 °C and were maintained for

12 hours. Nitric acid (2 mL, 1 mol/L) was added with a digital pipet, and the beakers were placed on a hot plate set at 90 °C until no liquid remained, which was followed by maintenance in an electric furnace at 550 °C for 2 hours. Hydrochloric acid (3.5 mL, 6 mol/L) was added with a digital pipet, and the beakers were placed on a hot plate set at 140 °C until no liquid remained. Nitric acid (5 mL, 12%) was added with a digital pipet, and the beakers were placed on a hot plate which was set at 90 °C for 30 seconds, which was followed by a transfer the contents to a 50 mL DigiTUBE®. The beaker was washed with 12% nitric acid, and the washings were transferred to another beaker. The resulting solution was diluted to 20 mL with 12% nitric acid. All sample solutions (5 mL) were added to a 50-mL DigiTUBE® using a glass pipet, and were diluted to 20 mL using laboratory water.

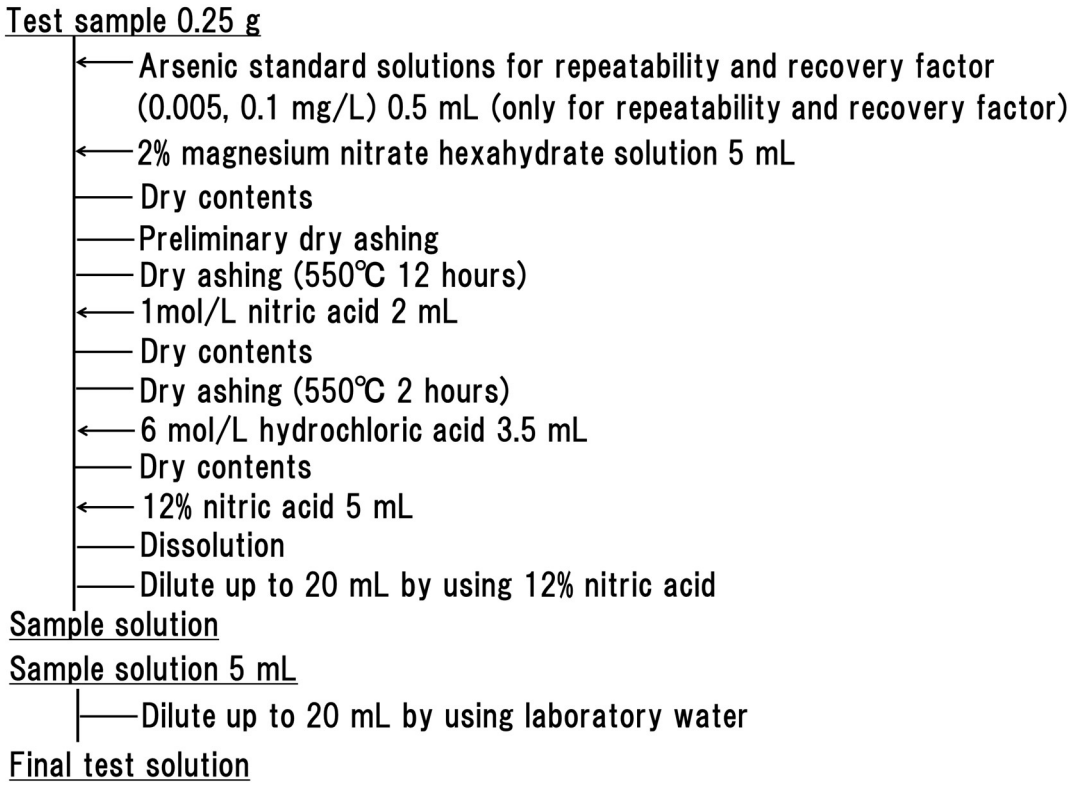


Figure 4.1 Diagram of the procedure for sample preparation.

4.2.7 Linearity

Linearity as a coefficient of determination was monitored after the completion of the calibration curve, and were compared to that of AOAC 2015.01 (>0.995) [42].

4.2.8 LOQ

The LOQ was defined as ten times the average standard deviation (SD), which was calculated from the result of the digested blank (unspiked) sample analyzed in decuplicate on a single day. The LOQ was computed for the final product (Formula 4.1), and the result was compared with those obtained using SMPR 2012.007 (≤10 μg/kg for foods, ≤8 μg/kg for infant formula) [46].

$$LOQ, \frac{\mu g}{kg}(\text{final product}) = LOQ, \frac{\mu g}{L}(\text{digested blanks}) \cdot 4(\text{dilution factor}) \cdot \frac{50\ mL}{0.25\ g} \quad (4.1)$$

4.2.9 Analysis of the sample of proficiency test

The final solution of proficiency test sample (PTP-1801WR) was analyzed in triplicate on a single day. The result was reported as the mean ± SD, which were compared with the results of the eleventh proficiency test for trace element in rice powder organized by KANSO CO., LTD. (226±13 μg/kg).

4.2.10 Repeatability

Test samples were analyzed in triplicate on three separate days. The results were expressed as the mean, SD_r, RSD_r, SD_{IM}, and RSD_{IM}. The RSD_{IM} was compared to that

of SMPR 2012.007 (Range ≥8 ppb to 100 ppb, ≤15%; Range ≥100 ppb to 1 ppm, ≤11%) [46].

4.2.11 Recovery factor

Test samples assessed for the recovery factor were analyzed in triplicate on three separate days. The results were expressed as the mean, SD_r, and SD_{IM}. The mean was compared to that of SMPR 2012.007 (Range ≥8 ppb to 100 ppb, 60–115%; Range ≥100 ppb to 1 ppm, 80–115%) [46]. The means for the samples were calculated on each day by subtracting the mean of the blank (unspiked) samples from each measured spiked sample concentration and dividing the result by the theoretical spiked concentration.

4.3 Results and discussion

4.3.1 Linearity, LOQ, and analysis of the sample of proficiency test

To prove the validity of the test method, SPSFAM sets SMPRs for each test item. SMPR 2012.007 has LOQ, repeatability, and recovery performance requirements and demands that the accuracy of reference materials be checked when possible [46]. Furthermore, although not described in the SMPR, linearity is an important factor in confirming the validity of the test method. AOAC 2015.01 describes the performance requirements for linearity [42]. To confirm the performance requirements depending on the equipment conditions, the author changed the ICP-MS cell gas to helium and checked the linearity, LOQ, and proficiency test results of samples organized by KANSO Co., Ltd. (PTP-1801WR).

Firstly, the author checked the linearity. the calibration curves for the modified AOAC 2015.01 produced coefficient of determination values >0.999, and the results exhibited excellent linearity and met the requirement of AOAC 2015.01 (>0.995) [42]. Next, the author measured the digested blank (unspiked) sample, and calculated The LOQ. The LOQ of modified AOAC 2015.01 was 6.6 μg/kg, and this value met the requirements of SMPR 2012.007 (≤10 μg/kg for foods; ≤8 μg/kg for infant formula) [46]. Finally, the author measured proficiency test sample organized by KANSO CO., LTD. (PTP-1801WR). The result of the sample of proficiency test by using modified AOAC 2015.01 was 221 ± 12 μg/kg. It was close to the granted value by KANSO CO., LTD. (226 ± 13 μg/kg). These results exhibited excellent record. From the linearity, LOQ, and proficiency test analysis results of the samples, the author confirmed the performance requirements depending on the equipment conditions. Therefore, the author decided to investigate the repeatability of the measurement results of infant formulas distributed in several countries by adopting the dry ashing method for sample preparation after addition of magnesium nitrate solution to prevent arsenic volatilization and using ICP-MS cell gas as helium.

4.3.2 Repeatability

The author measured three kind of infant formula by modified AOAC 2015.01. Table 4.1 shows the results of the modified samples with AOAC 2015.01. The RSDIMs of the modified AOAC 2015.01 samples that spiked 10 μg/kg or 200 μg/kg, were 2.66–14.3% and 2.06–4.42%, respectively, and these values met the requirements of SMPR 2012.007 (Range ≥8 ppb to 100 ppb, ≤15%; Range ≥100 ppb to 1 ppm, ≤11%) [46].

In this study, the cell gas was changed from oxygen to helium. An inert collision cell gas like helium is highly versatile but tends to have lower interference removal

capability than oxygen as reaction cell gas [31]. Therefore, the author was concerned that the measured values did not meet the SMPR standard. However, the results demonstrated that the use of helium as collision cell gas is suitable and meets the requirements of the SMPR standard.

Table 4.1 Repeatabilities for samples prepared by modified AOAC Official Method 2015.01 (AOAC 2015.01) conditions for arsenic (n = 3 × 3 days).

Sample name	Spiking concentration, µg/kg	Measured As, µg/kg	SD_r	RSD_r, %	SD_{IM}	RSD_{IM}, %
Infant formula A	0	23.6	1.05	4.43	0.87	3.67
	10	31.7	0.95	3.01	0.84	2.66
	200	228	4.90	2.20	4.61	2.06
Infant formula B	0	10.3	1.02	10.0	0.98	9.56
	10	19.6	0.85	4.33	0.83	4.23
	200	208	5.22	2.51	5.07	2.45
Infant formula C	0	6.53	0.41	6.20	1.63	25.0
	10	17.0	0.69	4.08	2.43	14.3
	200	216	4.56	2.12	9.53	4.42

SD_r: Standard Deviation of repeatability; SD_{IM}: Standard Deviation of intermediate precision; RSD_r: Relative Standard Deviation of repeatability; RSD_{IM}: Relative Standard Deviation of intermediate precision

4.3.3 Recovery factor

Finally, the author measured three kind of infant formula by modified AOAC 2015.01, and confirmed recovery factors of infant formula in case of adopting the dry ashing method for sample preparation after addition of magnesium nitrate solution for preventing the arsenic volatilization and using ICP-MS cell gas as helium. The recovery factors of samples prepared by using the modified AOAC 2015.01 are shown in Table 4.2. The recovery factors of the modified AOAC 2015.01 that spiked 10 μg/kg or 200 μg/kg, respectively in each sample were 84.9–107% and 102–105%, respectively, and these values met the requirements of SMPR 2012.007 (Range ≥8 ppb to 100 ppb, 60–115%; Range ≥100 ppb to 1 ppm, 80–115%) [46].

In this study, the author employed dry ashing for sample processing. However, arsenic tends to be volatilized at high temperatures [47, 48], because of which, the recovery rate obtained could be lower than expected. The addition of magnesium nitrate before dry ashing has been reported to address such issues [47, 48]. Therefore, the author added it in the experiment and found that the results met the requirements of SMPR 2012.007 [46], thereby solving the potential problem.

Table 4.2 Recoveries from samples analyzed with modified AOAC Official Method 2015.01 (AOAC 2015.01) for arsenic (n = 3 × 3 days).

Sample name	Spiking concentration, µg/kg	Mean recovery, %	SD_r	SD_{IM}
Infant formula A	10	84.9	10.7	10.4
	200	102	0.7	1.4
Infant formula B	10	97.4	8.9	7.7
	200	103	3.2	2.9
Infant formula C	10	107	6.6	12.1
	200	105	2.4	4.8

SD_r: Standard Deviation of repeatability; SD_{IM}: Standard Deviation of intermediate precision

4.4 Conclusions

The author aimed to improve the flexibility of AOAC 2015.01 by employing dry ashing for sample processing and helium as the reaction cell gas. I also added magnesium nitrate solution before dry ashing to prevent the arsenic volatilization. The author processed and analyzed the proficiency test sample and several infant formulas, and these results were then compared to the requirements set forth in AOAC 2015.01. The results exhibited excellent linearity (coefficient of determination >0.999) and the LOQ was 6.6 μg/kg. The result of the proficiency test by using modified AOAC 2015.01 was 221 ± 12 μg/kg, close to the value accepted by KANSO CO., LTD. (226 ± 13 μg/kg). And the measurement precision had RSD_{IM}s of <14.3% and <4.42%. The recovery factors ranged from 84.9-107% and 102–105% in case of infant formula samples, which were spiked with 10 μg/kg or 200 μg/kg of arsenic per product.

This study demonstrated that several types of infant formula could be measured even under pretreatment by the dry ashing method using a muffle furnace followed by ICP-MS measurements using helium as the cell gas. Overall, by changing the test conditions in the modified AOAC 2015.01, the author improved the versatility of AOAC 2015.01 for determination of arsenic in infant formula. The results of this study are highly promising and point towards a solution when an organization or nation is excluded from the international trade for not adhering to the arsenic level requirements in infant formula.

Chapter 5 General conclusions

Ensuring a wholesome dietary environment is indispensable for a safe and healthy diet. An international trade on food is essential in improving the dietary environment. Codex standards and Codex test methods set by the Codex Committee are used to determine whether food is safe and contains the required ingredients. In recent years, multiple Codex test methods have been registered for the same test item, and there was concerns that this would restrict a smooth international trade.

Due to this circumstance, AOACI has led the way and, in cooperation with ISO and IDF, launched SPIFAN, a project to unify official methods used in the international trade of infant formula and adult nutrition. In the future, the unification of official methods will proceed at an accelerated pace while broadening the scope of SPIFAN. However, the project is strongly influenced by the manufacturers in the above-mentioned Western countries and does not sufficiently reflect the actual situation of the countries and organizations that were not deeply involved in the project. For this reason, some of the adopted Codex test methods are difficult to introduce, and products with individual specifications cannot be accurately measured, resulting in deviations from the standard values derived from the test method and unnecessary disposal of food. This may hinder a smooth international trade. In this context, optimizing the Codex method to facilitate international trade and to enable a stable supply of milk products, is highly necessary. So, the author conducted improvement studies on AOAC-OMA, which form the basis for the Codex methods of selenium, iodine for infant formula formulated in SPIFAN. At the same time, the same problem was recognized for the arsenic test method being developed as a Codex test method by SPSFAM. Therefore, the improvement of the test method was also examined as an additional study (Chapter 1).

From Chapter 2 onwards, each proposed theory, the experimental design, and studies for improvement of AOAC-OMA for selenium, iodine, and arsenic, in infant formula was described. Chapter 2 described our efforts for improving the versatility and for expanding the measurement range for AOAC 2015. 06, which is an AOAC-OMA for selenium in infant formula. As mentioned before, AOAC 2015. 06 cannot be applied to infant formula in the absence of selenium additives due to lack of sensitivity, and the method requires the use of hydrogen as cell gas for ICP-MS measurement. This equipment limitation is a major bottleneck which prevents the extensive use of this method. To address this limitation, the author carried studies for improving the model selectivity of AOAC 2015.06, which was successfully expanded, and the measurements were performed without any problems even in the absence of the selenium additives. While carrying out improvement studies, the author decided to select helium as ICP-MS cell gas. Although helium has high model selectivity, it has the disadvantage of lower sensitivity for selenium measurement when compared to hydrogen. In this study, by optimizing the sampling amount, and the amount of measurement at the time of final test solution adjustment, the accurate measurement of infant formula in the absence of the selenium additive was successfully demonstrated, even when helium gas was adopted for the ICP-MS analysis.

Chapter 3 summarized the improvement of AOAC 2012.15, which is AOAC-OMA for iodine in infant formula. Although iodine is present as a trace element in infant formula, it is essential for infants. While the use of iodine additives in infant formulas is not undertaken in Japan, the amount of iodine in infant formula is around the lower limit of the standard defined by CAC and SMPR 2012.008, which raises the distinct possibility that the method might be inapplicable due to measurement sensitivity limitations. While

the author considered these scenarios for selenium analysis at first, it quickly became evident that the signal enhancement (the phenomenon that the measured value is higher than the actual value) affected not only the domestically produced infant formula but also the ones from all countries. This suggests that the products deviate from the Codex standard might be distributed if original AOAC 2012.15 is used in international trade. The author assumed that the origin for this enhancement is from the carbon derived from the sample remaining in the final test solution subjected to the pretreatment, and on the basis of this assumption, the author hypothesized that the addition of a carbon source at an optimum concentration to the blank, standard, and the final test solutions would mitigate the aforementioned problems and would improve the reliability of AOAC 2012.15. After adopting methanol as the carbon source, the author conducted measurements after setting its optimum concentration (5%) and found that the measured values were close to the actual value obtained in the infant formulas of each country. Overall, this study provided solutions to all problems described for this method. From the results of Chapter 2 and Chapter 3, the author was able to optimize AOAC2015.06 and AOAC2012.15. As a result, these test methods can be easily introduced into laboratories and can be measured even for infant formula that does not contain selenium or iodine additives. Therefore, the author succeeded at conducting optimizations of all Codex methods for minerals and trace elements formulated in SPIFAN.

Chapter 4 summarized the improvement examinations for AOAC 2015.01, which is AOAC-OMA slated to become the Codex method for arsenic in infant formula. AOAC 2015.01 specifies a wet ashing method using a microwave oven for pretreatment. While the microwave oven exhibits superior performance, the number of samples that can be processed at once is limited under this approach, when compared to the use of dry

ashing method with a muffle furnace. Further, the microwave oven and equipment are relatively expensive. Besides, in AOAC 2015.01 for arsenic, oxygen is specified as the cell gas for the ICP-MS measurement, which further imposes equipment-limitations. In this study, the author adjusted the final test solution pretreatment by dry aching using a muffle furnace, which was easy to introduce and facilitated large volumes of pretreatment simultaneously. Furthermore, the author added magnesium nitrate solution before the dry ashing to prevent the arsenic volatilization. Additionally, the author evaluated helium, which is a highly versatile cell gas for ICP-MS measurements but is known to have lower sensitivity than that of oxygen for arsenic measurements. Consequently, the author was able to demonstrate the feasibility of accurate measurement of infant formulas from each country by modifying the AOAC 2015.01 condition, as described herein.

The author carried out various studies to improve the official methods for some trace elements present in infant formulas that will initially face the SPIFAN and SPSFAM formulation challenges by incorporating viewpoints and approaches of Japanese infant formula manufacturers, which were not considered during the drafting of the official methods in western countries. These reviews have already been submitted to relevant academic journals, which have been accepted [49-51]. In addition, the author is working on changing the flow of western-centered projects, by participating as an Expert Volunteer in a review project related to AOAC-OMA, which focused on infant formula-related Codex methods. From now on, it is imperative that all nations and organizations which do not have a voice in the framing of the official method formulation projects, participate as related stakeholder groups and become voting members for the formulation of these methods. In addition, the organization of more stakeholder groups that could proactively interact with AOACI is vital to ensure efficient analysis and food supply. Such coming

together of new stakeholders and organizations in various countries around the world will facilitate the introduction of official methods with high versatility and high reliability.

With these efforts, testing organizations in all countries and organizations, in particular developing countries, will be able to introduce the Codex test method more easily. the author believe this study will be an opportunity to establish an environment in which food such as infant formulas from countries/organizations other than western countries can be consistently supplied to and distributed around the world through international trade. Ultimately, these results will help achieve Goal 3: “Ensure healthy lives and promote well-being for all at all ages” and Goal 12: “Ensure sustainable consumption and production patterns” among the 17 goals of the SDGs adopted by the United Nations in 2015 [24].

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