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RESEARCH ARTICLE

Profile Analysis of Iodine in Powdered Infant Formula and Infant Complementary Foods by Inductively Coupled Plasma Mass Spectrometry

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Abstract

Background: Both iodine deficiency and excessive iodine intake are very detrimental to the development of the baby, it is necessary to accurately measure the iodine content in infant milk powder. A probing study to establish a reliable and robust method for determining the iodine concentration using the inductively coupled plasma mass spectrometry was performed in combination with a sample digestion step.

Methods: A sample was digested in 5% tetramethylammonium hydroxide at 85°C. This method adopted a shorter extraction time (3h) and used ^{186}Re as the internal standard, which exhibited greater stability in infant foods. The linearity range (R^2) was >0.998, limit of detection was 0.01 mg kg^{-1}, limit of quantification was 0.03 mg kg^{-1}, relative standard deviation was less than 4%, and recovery range was 94.8%–109%.

Results: The accuracy of the optimized method was evaluated by testing standard reference material SRM 1849a, and the results (1.17 ± 0.05 mg kg^{-1}, n = 6) were in good agreement with the certified value (1.29 ± 0.11 mg kg^{-1}).

Conclusions: The results of the validation process confirmed that ICP-MS is the gold standard for determination of low iodine concentrations in infant foods. In addition, profiling the iodine content of different infant foods allows the development of a rational diet for infants to promote healthy development.

Keywords: Iodine, Inductively Coupled Plasma Mass Spectrometry, Tetramethylammonium Hydroxide, Powdered Infant Formula, Matrix.

1. Introduction

The first 1000 days of life (from pregnancy to 2nd birthday) is the most critical period of human growth (the window period of children's nutrition improvement), and also the key period of intelligent development (such as sensory perception, motor coordination ability, language). Malnutrition during this period will have an irreversible and irreparable impact on children's health status and development potential (Beluska-Turkan et al., 2019). Both iodine deficiency and excessive iodine intake have negative effects on the human body (Tian et al., 2020). Too low iodine intake can lead to problems such as thyroid dysfunction, dwarfism, fetal cretinism, and impaired intelligence commonly referred to as iodine deficiency disorders, while too high intake may cause harm to the thyroid and other organs of the body (Choudhry and Nasrullah, 2018; Lee et al., 2021). Many infant

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formulas and complementary feeding are fortified with iodine. Although iodine excess is rare, the concentration of iodine in these products needs to be monitored because the limit for iodine concentration specified in national standards GB 10765-2021 and GB 10766-2021 is 3.6–14.1 μg/100 kJ (approximately 77–302 μg/100 g) (2021a, b). Meanwhile, the limit for iodine concentration specified in national standard GB 10767-2021 is 1.4–14.1 μg/100 kJ (approximately 27–278 μg/100 g) (2021c). Numerous studies have focused on the iodine content detection in infant foods. However, they measured the iodine content in one or a few foods. Further sorting and analyzing of a large number of published data on iodine content in infant foods will provide new information for the development of more scientific and reasonable iodine nutrition standards. The purpose of this study was to integrate the iodine content data of different brands and types of infant formula and infant complementary food. And this can provide guidance for parents and doctors to choose infant formula and infant complementary food suitable for infants.

According to China's national standards, the main methods used for iodine content analysis are inductively coupled plasma mass spectrometry (ICP–MS), redox titration, arsenic cerium catalytic spectrophotometry, and gas chromatography (GC) (2021d). GC is an accurate and reproducible method for the determination of iodine in infant foods, but it requires pre-treatment steps (SHAN et al., 2015). Compared to GC for the iodine determination, ICP-MS can obtain better results with higher sensitivity, more complete detection of multiple elements, faster and no pre-treatment. At present, studies have been conducted to determine the iodine content in milk powder using ICP-MS (Vance et al., 2018; Todorov and Gray, 2016). Therefore, ICP–MS will be adopted to detect the iodine content in this study.

Most analytical techniques used for iodine determinations require that samples are in liquid form. Hence, solid samples must be extracted/digested to transfer the iodine species into a liquid phase. The commonly used alkaline solutions include ammonia, tetramethylammonium hydroxide (TMAH), sodium hydroxide, and water-soluble tertiary amines solution. Among these extraction approaches, extraction of iodine by TMAH solution at high temperatures in a closed container is the most commonly used sample treatment method (Hwang et al., 2020). In addition, the selection of an appropriate internal standard is essential for the accurate determination of iodine content in infant foods by ICP-MS. The commonly used internal standards include Te, Rh, Sb, In, and other elements (Todorov and Gray, 2016; Pacquette et al., 2012). The effect of different internal standards on the results can be assessed by selecting indicators such as the reproducibility/spiked recovery of the system (Winter et al., 2023). Compared with other internal standards, Rhenium (Re) is a relatively stable element. Studies have used Re as an internal standard to determine the iodine content in milk powder (Vance et al., 2018). Therefore, Re was selected as the internal standard in this study.

Given these premises, this work explored the difference of iodine content in infant formula and infant complementary foods in different literatures, products and databases. Then, this study developed an effective sample digestion method for the determination of total iodine concentration in infant foods, utilizing TMAH digestion, and Re as the internal standard. Finally, the iodine content in different matrix powdered infant formulas (PIFs) was analyzed by this method. It can help consumers to understand the iodine content level and its difference in different PIF products, so that they can select the suitable products for their babies in a targeted manner, thus ensuring their health and preventing potential harm caused by nutritional deficiencies.

2. Materials and Methods

2.1 Data Collection

The iodine content data in infant foods were collected from various literature, products label, and published database (Ershow et al., 2022). The search terms used were " ("infant formula" OR "baby powder") AND ("infant complementary food" OR "infant complementary feeding") AND ("iodine content" OR "iodine concentrate") AND ("inductively coupled plasma mass spectrometry" OR "ICP-MS")", and similar search terms were used for the web of science and PubMed databases. The references from relevant primary papers and review articles were searched to identify relevant studies. The data on iodine content were extracted manually from the above compiled literature to create a data table on iodine content in infant formula and infant complementary foods. Then, further analysis of the data was performed.

2.2 Reagents and Standards

The following reagents were used: pure water (H_2O, 18.2 MΩ cm; Milli-Q, Merk Millipore, Darmstadt, Germany), tetramethylammonium hydroxide

(TMAH, 25%; aladdin, Shanghai, China), isopropanol (Yifang, Tianjin, China), and potassium iodide (Sigma Aldrich, CAS: 7681-11-0, 99.99%, Sigma Aldrich, Saint Louis, USA). Iodine stock solutions (1000 mg L^{-1}) were prepared by dissolving potassium iodine in water. Working standard solutions (0, 0.1, 1, 5, 10, 15, 20, and 25 µg L^{-1}) were prepared from iodine stock solutions by diluting with 0.5% TMAH. Rhenium (Re) solution (10 µg L^{-1}) was used as an internal standard; 2 mL of isopropanol was added to 100 mL of the internal standard solution.

2.3 Samples Preparation

The applicability of the methodology proposed in this study was tested using PIF samples with different matrices, including PIF (milk based), formula powder (soybean based), children's milk powder, full nutrition formula containing flavor milk powder, and special medical formula. The TMAH digestion and ICP–MS methods were used to determine the iodine concentration in each sample.

PIF samples (0.3 g) were accurately weighed into centrifuge tubes (50 mL), to which 5% TMAH (5 mL) was added; the samples were then were dissolved. Each sample was digested in a constant temperature oven at 85 °C ± 5 °C for 3 h (vortex per 30 min) (Huynh et al., 2015). The centrifuge tubes were cooled to room temperature; following which each sample was diluted to 50 mL with ultrapure water. The undissolved residues were removed by centrifugation and then filtered, using a 0.45 µm syringe filter, into a sample bottle for testing.

2.4 Instrumental Measurements

The samples were digested using a constant temperature oven (Yiheng, Shanghai, China), weighed using an electronic balance (METTLER TOLEDO, Zurich, Switzerland), and purified using a centrifuge (Xiangyi, China). ICP–MS was performed using Thermo Scientific ICAPQ (USA) as the ICP–MS model. The RF power was 1.50 kW; the flow rate of plasma, makeup, and carrier gases was 15, 0.40, and 0.80 L min^{-1}, respectively; and the sampling depth was 8 mm. There were approximately three determination points per peak. Analyses were repeated three times, the nebulizer type was a concentric nebulizer, and its temperature was 2°C; ^{186}Re was used as the internal standard.

2.5 Limit of Detection (LOD) and the Limit of Quantification (LOQ)

The LOD is the lowest concentration that can be detected but cannot be accurately quantified, whereas the LOQ is the lowest concentration that can be accurately quantified. The LOQ was calculated by testing 10 sample blanks.

2.6 Recovery

Samples with three different iodine concentrations, equivalent to approximately 50%, 100%, and 150% of an endogenous iodine sample, were used. Each gradient recovery dataset was tested at six levels (Suzuki et al., 2019). To confirm the accuracy of the method, standard reference material (SRM) 1849a, a milk-based nutritional powder, was also analyzed. The percentage of recovery was calculated as the ratio of the difference between the observed concentration and original concentration to the added amount using the following equation:

Recovery (%) = (observed conc. - original conc.) / spiked conc. × 100;

where, observed conc. is the sample concentration after adding the standard solution, original conc. is the concentration of the sample itself, and spiked conc. is the concentration of the standard solution added.

3.Results

3.1 Iodine Content Profile Analysis

In this study, information of iodine content in different infant foods was collected from a wide variety of resources ranging from products label and database (Ershow et al., 2022) to literature. Figure 1 is a tree diagram obtained by classifying 293 literatures collected by us according to different research objects. The different colors correspond to the different food groups. The values represent the number of studies in these 293 articles. It can be seen that there are relatively more articles on the content of iodine in milk. When we look up the literature, an interesting phenomenon is that there are few literatures on the comprehensive study of infant complementary foods. And there are slightly more studies on a certain type of infant complementary foods. In addition, most of the current studies mainly use ICP-MS to determine iodine content in foods. As some studies have reported (Dold et al., 2016), ICP-MS is the gold standard for determination of low iodine concentrations in complex matrices and the preferred method for infant formula.

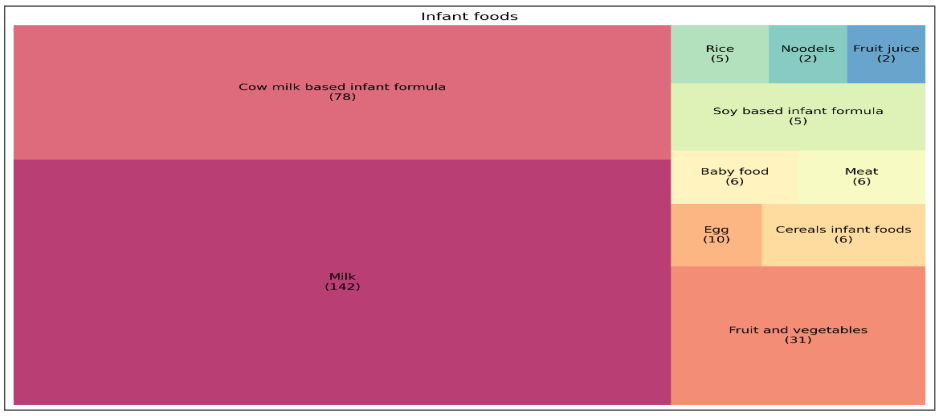

Figure 1. *A tree diagram obtained by classifying the 293 papers collected according to the different subjects studied.*

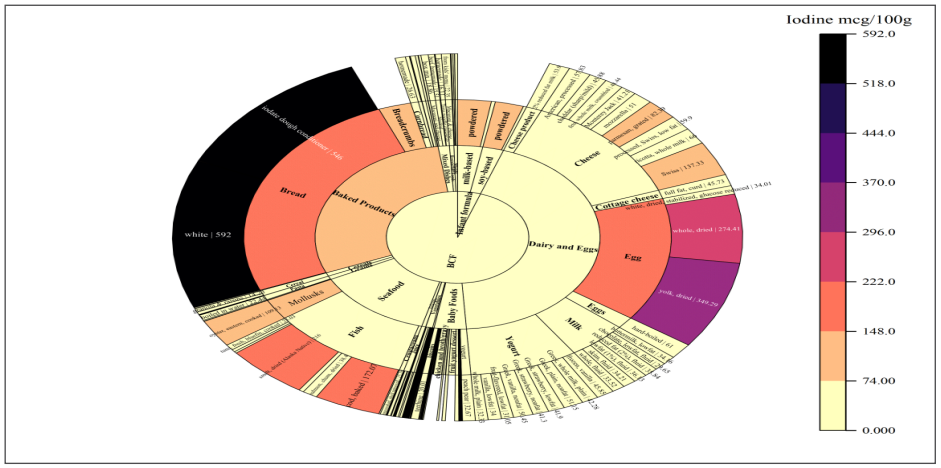

Figure 2. *Sunburst diagram of iodine content in different products.*

Figure 2 shows the Sunburst plot of iodine content in different food products. According to different food categories, it is divided into 19 categories. Each layer of circles represents a level, and the closer to the origin represents the higher level of the circle. The innermost circle represents the top of the hierarchy, and the further out, the lower the level and the finer the classification. The data in the outermost layer contains not only the food name but also the corresponding iodine content, and the darker the color of the graph, the higher the iodine content in the food.

3.2 Method Validation

3.2.1 Linearity

Linearity was monitored after the completion of each calibration curve by observing the linear correlation coefficients (R^2) value produced, calculated by the software. The calibration curve was extrapolated in the concentration range of 0-25 µg L^{-1}. For the calibration curve for iodine to be valid the R^2 value had to be 0.9995. The results show that the test method provided a good linear relationship. The relationship between the strength and concentration for standard was shown in figure 3.

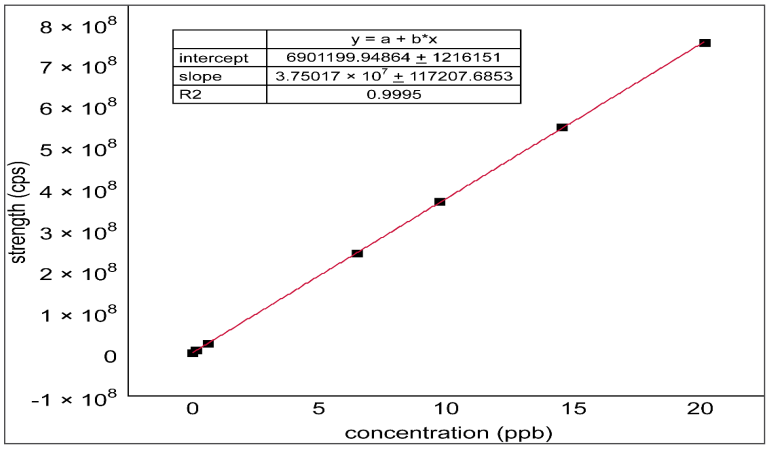

Figure 3. *Relationship between the strength and concentration for standard samples.*

3.2.2 LOD and LOQ

The LOD is the lowest detection threshold. The LOQ is the lowest concentration of the analyte in the sample that can be reliably quantified by the instrument. For iodine, the method exhibited an LOD of 0.01 mg kg^{-1} and an LOQ of 0.03 mg kg^{-1}.

3.2.3 Recovery

For the equivalent to approximately 50 % of an endogenous iodine sample, recoveries between 102 % and 109 % were achieved for solution spiked with iodine (n=6). The relative standard deviation (RSD) was 3.13 % (Table 1). For the equivalent to approximately 100 % of an endogenous iodine sample, recoveries between 94.8 % and 99.3 % were achieved for solution spiked with iodine. The RSD was 1.93 %. A recovery of 98.5 % ~ 101 % also found in the equivalent to approximately 150 % of an endogenous iodine sample. The RSD was 1.14 %.

Table 1. *Recovery data of iodine as determined by ICP–MS.*

Endogenous iodine (%)	Original conc. (mg/kg)	Spiked conc. (mg/kg)	Observed conc. (mg/kg)	Recovery (%)	Recovery relative standard deviation (RSD %, n = 6)
50%	1.34	0.65	2.03, 2.02, 2.00, 2.00, 1.99, 2.05	106, 104, 102, 102, 100, 109	3.13
100%		1.30	2.67, 2.62, 2.62, 2.61, 2.66, 2.65	99.3, 95.5, 95.5, 94.8, 98.5, 97.8	1.93
150%		1.95	3.26, 3.31, 3.31, 3.28, 3.30, 3.27	98.5, 101, 101, 99.5, 101, 99.0	1.14

The National Institute of Standards and Technology (NIST) SRM 1849a results (1.17 ± 0.05 mg kg^{-1}, n = 6) of this method were consistent with the standard values (1.29 ± 0.11 mg kg^{-1}) within the range of uncertainty.

3.2.4 Accuracy

The results obtained for the National Institute of Standards and Technology (NIST) milk standard using this method was 1.17 ± 0.05 mg kg^{-1}(n = 6) was in a close agreement with the certified value of 1.29 ± 0.11 mg kg^{-1}.

3.3 Quantification of Iodine in Different Matrix Samples by ICP–MS

The iodine contents in 6 different matrixes PIFs were determined using the TMAH/ICP-MS method. The results were presented in Table 2. The iodine concentration in PIFs can range widely with a minimum of 0.445 mg kg^{-1} and a maximum of 1.36 mg kg^{-1}. As a result of iodine quantification in PIFs, the group with the highest iodine content was infant formula group, and the group with the lowest iodine content was high protein formula powder. The iodine content in milk-based formula powder is higher than that in soybean-based formula powder. Furthermore, partial hydrolysis or complete hydrolysis of protein increases the content of iodine in infant formula. The protein in these formulas is provided by milk protein. Milk proteins in partially hydrolyzed protein formula decompose into small molecular milk proteins, peptides, and amino acids. A deep hydrolysis formula does not contain food proteins, and their amino acid sources cannot be derived from non-edible animal and plant raw materials; the concentrations of some minerals and vitamins can be adjusted appropriately. The average of the determined iodine concentrations in 1–9 groups ranged from 0.454 to 1.34 mg kg^{-1}. Several studies have reported similar results that kelp contains 1.09 - 1.503 mg kg^{-1} (Hwang et al., 2020). The RSD of triplicate determinations ranged from 1.56 % to 2.32 %.

Table 2. *Difference matrix test data.*

Sample	Matrix	Results (mg/kg)	Average (mg/kg)	RSD %
1	Infant formula	1.32, 1.36, 1.33, 1.35, 1.34, 1.36	1.34	1.56
2	Partially hydrolyzed milk protein infant formula	1.12, 1.16, 1.14, 1.15, 1.16, 1.13	1.14	1.97
3	Children's milk powder (age 3–7 years)	0.568, 0.568, 0.586, 0.578, 0.566, 0.576	0.574	1.88
4	Lactose free formula	0.71, 0.727, 0.749, 0.725, 0.738, 0.714	0.727	2.29

5	Soybean based formula powder	0.734, 0.712, 0.701, 0.712, 0.721, 0.715	0.716	2.32
6	Full nutrition formula containing flavored milk powder	0.684, 0.66, 0.663, 0.667, 0.659, 0.678	0.669	1.92
7	Milk-based formula powder	0.973, 0.991, 0.995, 0.983, 0.986, 0.991	0.987	1.18
8	High protein formula powder	0.462, 0.451, 0.448, 0.445, 0.447, 0.468	0.454	1.69
9	Deep hydrolyzed protein formula powder	0.82, 0.79, 0.808, 0.815, 0.782, 0.822	0.806	1.87

4. Discussion

Adequate iodine is essential for synthesizing thyroid hormones to ensure normal physical and neurological growth and development (Zhang et al., 2023). In order to determine whether infants and young children intake enough iodine, it is necessary to profile the iodine content in different PIF and infant complementary food. Iodine is an extremely important element in the human body, which has a crucial impact on physical development and thyroid function. Especially for pregnant women and infants, adequate iodine intake is essential for the intellectual development of the child and the healthy development of the immune system. Therefore, a profile analysis of iodine content in infant formula and infant complementary foods can help us understand the nutritional composition of the food, so as to ensure that infants and young children receive sufficient iodine to promote their healthy growth.In addition, the analysis of iodine content in different infant formulae and infant complementary foods can provide experimental data and relevant references for the establishment of an accurate method for determining iodine in infant foods. At the same time, in the actual production and quality control, the formula and process can also be optimized according to the iodine content in different infant foods to ensure the quality and safety of infant foods.

In this study, we have successfully validated a method for the assessment of iodine in infant foods and highly accurate, reproducible and precise. The method results demonstrate that good linearity of 0–25 µg L^{-1} for the iodine calibration curve. This method performed well. The memory effect of ICP–MS can be eliminated using diluted TMAH and an internal standard. The digestion of samples using TMAH is a sealed process, which reduces the possibility of loss of analytes. Furthermore, after digesting the sample with TMAH, TMAH can be completely eliminated from the ICP–MS apparatus without any residue. This is a critical step in the establishment of a successful detection method.

Another important influencing factor for the successful establishment of the method is the selection of the internal standard. The internal standard should usually have the following characteristics: have similar properties to the analyte. It has stable isotope abundance. Be independent of the matrix of the analyzed sample, i.e., the internal standard will not be affected by interference in the sample matrix. The concentration of the internal standard solution should be an order of magnitude or more higher than the concentration of the sample solution to ensure that the signal intensity of the internal standard is significantly higher than the signal of the analyte, thereby reducing errors.

In conclusion, this method performed well, with sensitivity and low detection limit. The modified method is able to recover over 98% of iodine from most spiked solutions, has a lower quantitation limit and has RSD well below 4%. This method represents a significant advance in the assessment of iodine concentrations in infant foods and its application will enable us to gain new insights. In the future, we will explore other faster digestion methods.

5. Conclusion

Iodine is one of the important nutrients necessary for the healthy development of infants. This study is significant in providing theoretical guidance for the development of a reasonable diet for infants by profiling the iodine content of different infant foods. In addition, we also successfully proposed a method for the determination of PIF with different matrices by ICP-MS using TMAH digestion of the samples and ^{186}Re as the internal standard. The results indicated that the digestion of a formula powder diluted with TMAH in a constant temperature oven could yield accurate results. TMAH can destroy the organic matter in PIF and extract the total iodine. Hence, TMAH digestion followed by ICP–MS exhibits stability and dependability in detecting iodine in different infant formula matrices. The results obtained by this method were very good, the R^2 ranged from 0.998 to 1.00, LOD was 0.01 mg kg^{-1}, LOQ was 0.03 mg kg^{-1}, repeatability range was 1.14%—3.13%, recovery range was 94.8%—109%. Complete sample digestion at 85 ± 5°C for 3 h was demonstrated using the SRM 1849a test. In addition, elemental Re proved to be effective as an internal standard; it could effectively

correct the matrix difference between the sample and standard solution, as well as for the state of the instrument. Analysis of different matrix samples confirmed that the proposed method can be used for different formula powders. In summary, this method is suitable for testing infant foods containing different concentrations of iodine. Accurate determination of iodine content in PIF can help parents and doctors better grasp the nutritional status of infants, ensure that infants get enough iodine, and can also play an important role in regulating whether the products of relevant enterprises meet the relevant regulatory standards.

Declaration of Conflicting Interest

The author(s) declare no potential conflicts of interest with respect to the research of Yunpeng Zheng, authorship and/or publication of this article.

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Notes

Availability of Data and Materials

All data and materials are available in this manuscript.

Authors' Contributions

Conceptualization: Yan Lu, Zhiqiang E, Xiaochun Zhang, Yang Li, Hailing Feng, Yongcheng Liu, Yingtao Liu,Huihui Yang and Yunpeng Zheng; Designing the study: Yan Lu and Yunpeng Zheng; Analyzing the data: Yan Lu , Zhiqiang E and Yunpeng Zheng; Drafting, writing, reviewing and approving the final manuscript: Yan Lu, Zhiqiang E, Xiaochun Zhang, Yang Li, Hailing Feng, Yongcheng Liu, Yingtao Liu,Huihui and Yunpeng Zheng.

All authors have read and approved the manuscript.

Consent for Publication

All the authors consent to the publication of the present paper.

Ethical Approval: Not Applicable.

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