

Article

Feasibility of Salt Iodate Micro-Method Reagent (SIMR) Detection Kit for Salt Iodate Analysis

Mohd Azerulazree Jamilan ¹, Husniza Hussain ^{1,†}, Nurul Aznyda Norizan ¹, Ahmad Ali Zainuddin ²,
Noor ul-Aziha Muhammad ², Filza Noor Asari ³, Aswir Abd Rashed ¹ and Mohd Fairulnizal Md Noh ^{1,*}

- ¹ Nutrition, Metabolism and Cardiovascular Research Centre, Institute for Medical Research, National Institutes of Health, Ministry of Health Malaysia, No. 1, Jalan Setia Murni U13/52, Setia Alam, Shah Alam 40170, Selangor D.E., Malaysia; azerulazree@moh.gov.my (M.A.J.); husniza.hussain@yahoo.com (H.H.); nurul.aznyda@moh.gov.my (N.A.N.); aswir@moh.gov.my (A.A.R.)
- ² Centre for Nutrition Epidemiology Research, Institute for Public Health, National Institutes of Health, Ministry of Health Malaysia, No. 1, Jalan Setia Murni U13/52, Setia Alam, Shah Alam 40170, Selangor D.E., Malaysia; ahmadali@moh.gov.my (A.A.Z.); noorulaziha@moh.gov.my (N.u.-A.M.)
- ³ Centre for Communicable Disease Epidemiology Research, Institute for Public Health, National Institutes of Health, Ministry of Health Malaysia, No. 1, Jalan Setia Murni U13/52, Setia Alam, Shah Alam 40170, Selangor D.E., Malaysia; filza.a@moh.gov.my
- * Correspondence: fairulnizal@moh.gov.my
- † Author was employed at the respective affiliation at the time of this research.

Abstract: Although the Universal Salt Iodization (USI) program has been highly successful, it remains relevant due to the continued risk of Iodine Deficiency Disorders (IDDs) in vulnerable groups, such as children and pregnant women. This program empowers the relevant authority to continuously monitor iodine levels in iodized salt. Our study reports on the use of a Salt Iodate Micro-Method Reagent (SIMR) detection kit for this purpose. The kit was validated, with a linearity of 5.0–60.0 mg/Kg, at a detection limit of 6.8 mg/Kg, with excellent recovery ranging from 93.0 to 108.3%, whereas the repeatability, intermediate precision, and reproducibility achieved a mean coefficient of variation (CV) of 5.3%, 6.8%, and 5.9%, respectively. The stability of the reagents used in the kit was tested using freshly prepared iodine standard quality control (QC) samples of 20.0 mg/Kg and 40.0 mg/Kg, all of which were observed to be stable, within the range of the mean $\pm 2 \times$ (standard deviation, SD), for 10 days. The suitability of the kit was proven when no difference was found in the mean results of 70 salt samples, using a paired *t*-test and the Bland–Altman plot, compared to the reference method, at a 95% confidence interval (CI). Thus, the SIMR detection kit is a highly feasible alternative method for iodine monitoring, with a fast analysis time, as well as being cost effective, and environmentally friendly.

Keywords: universal salt iodization; salt fortification; iodized salt; table salt; portable kit; on-site kit; iodine detection



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1. Introduction

Iodine, a vital micronutrient for thyroid hormone production, plays a crucial role in regulating human metabolism, growth, and development; the most efficient and widespread way to ensure adequate iodine intake is through the consumption of iodized table salt (also known as iodized salt) [1]. Deficiencies of iodine can cause goiter, and, in more severe cases, impaired mental growth and stunting [2].

According to the Malaysia Food Regulation 1985 pursuant to the Malaysia Food Act 1983, iodized salt for human consumption must contain iodine within the range of

20 mg/Kg to 40 mg/Kg. The form of iodine is either potassium iodate, potassium iodide, sodium iodate, or sodium iodide [3]. The implementation of these rules is part of the Universal Salt Iodization (USI) program, a cost-effective measure, which has been instrumental and reassuring in regard to the prevention of Iodine Deficiency Disorders (IDDs).

Globally, approximately 2 billion people are at risk of iodine deficiency, with around 50 million exhibiting symptoms of IDDs [4]. Nearly 241 million school-age children worldwide are iodine deficient and, of these, 58 million (40%) are from Africa [5]. Based on a recent report [6], in Asia, the prevalence rate of IDDs was 2609.6 per 1,000,000 people in 2019, showing a 28.3% decrease since 1990, when the rate was 3639.2 per 1,000,000 people. Malaysia has shown a tremendous decrease in the IDD prevalence rate, decreasing by 69.1% from 5699.3 people in 1990 to 837.1 people in 2019, per 1,000,000 people. This positive trend is due to the implementation of salt iodization legislation.

However, it is concerning that despite the implementation of iodized salt consumption rules, an inadequate iodine status is still a prevalent issue, particularly among pregnant women in Sarawak, one of the states in Malaysia, even after 10 years of USI program implementation [7]. Therefore, consistently monitoring iodine levels in iodized salt is essential to mitigate the health risks. Monitoring iodine levels in iodized salt within the specified range is crucial for preventing the recurrence of IDDs, particularly in high-risk groups, such as children and pregnant women [8,9]. While conventional methods, such as iodometric titration [10] or the use of instrumentation of inductively coupled plasma mass spectrometry (ICP-MS) [11], are commonly employed for iodine analysis, these methods are often labor intensive, expensive, and require significant reagent usage [12]. These methods are generally suited to laboratory settings and are not ideal for point-of-care testing (POCT).

For instance, iodometric titration involves the preparation of reagents and two general procedures: (1) sample preparation and (2) titration using thiosulphate, with starch as a color-change indicator. Although less expensive, this method requires the involvement of an experienced technician; where less experienced technicians are involved, comprehensive training in regard to troubleshooting was essential, otherwise the results would suffer from human error [13]. On the other hand, ICP-MS is regarded as the most reliable tool for iodine detection [14,15]. However, the acquisition and maintenance of this instrument can be costly, making it unaffordable for some laboratory setups [16]. Additionally, the crystallization of salt in the plasma tubing of ICP-MS was prone to interrupting the analysis, due to clogged tubing [16,17]. This method, in turn, requires a highly skilled technician for the maintenance of the instruments.

This situation highlights the need for more efficient and cost-effective alternatives, such as the Salt Iodate Micro-Method Reagent (SIMR) detection kit. The SIMR detection kit (Figure 1), developed using a micro-method approach, only uses small volumes of reagents with diluted concentrations, making the kit more environmentally friendly and less hazardous. The use of diluted reagents significantly reduces the time taken for the reaction to occur, making the overall procedure for analysis remarkably faster compared to the conventional titration method. For instance, it requires less than 10 min for the SIMR detection kit to carry out the procedure versus 20 min for the conventional titration method. The detection kit is an improvement of our previously unreported in-house qualitative kit, known as the Institute for Medical Research (IMR) iodine rapid test kit (RTK), which was conducted qualitatively based on the appearance of the color-change reaction. The coloration observed was due to the presence of iodate, which forms a complex with the starch solution. On the other hand, using the same iodine–starch reaction principle in regard to the SIMR detection kit, the detection of iodate is based on the absorbance intensity measured by the photo absorbance reader provided in the kit.



Figure 1. Overall illustration of SIMR detection kit, containing the complete content for on-site analysis, including: (1) pipet tips; (2) IMR iodine RTK-containing solution A, B, and C; (3) weighing boats; (4) Nitrile gloves; (5) plastic spatula; (6) mini pipet; (7) pestle and mortar; (8) SIMR reagent A; (9) 3 mL syringe; (10) 13 mm nylon filter (0.45 µm pore size); (11) 75% alcohol wipe; (12) disposal bag with zip tie; (13) portable photo absorbance reader; (14) 0.2 mL tubes for sample scooping; (15) rack with 5 mL sample tube containing pre-filled 4 mL of deionized water; and (16) 0.2 mL tube rack pre-filled with SIMR reagent B.

Our objective was to study the feasibility of the SIMR detection kit as an alternative method for salt iodate analysis. We aimed to demonstrate that it offers good accuracy and precision, comparable to the reference method, namely ultra-high performance liquid chromatography (UHPLC) [18]. A more straightforward iodine detection method using an SIMR detection kit would make monitoring iodine levels in iodized salt more feasible and scalable, especially in regions where laboratory-based methods are impractical due to cost or infrastructure constraints. This alternative method could significantly improve the USI program, making it more feasible in various settings.

2. Materials and Methods

2.1. Materials

2.1.1. Chemicals and Reagents

The deionized water used in the study was sourced from an ultra-purification system (18.2 MΩ grade, Sartorius, Göttingen, Germany), whereas all the chemicals, including 98% sulfuric acid, phosphoric acid, potassium iodate, potassium iodide, soluble starch, sodium chloride, sodium bisulfite, sodium phosphate monohydrate, and sodium pyrophosphate decahydrate, were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.1.2. Salt Samples

Three commercial iodized salts, labelled as sample S1, S2, and S3, obtained from the local market in Klang Valley, Malaysia, were used to validate the SIMR detection kit. The real samples were obtained from the samples collected by the National Health and Morbidity Survey (NHMS) 2024: Nutrition program, where approximately 30 g of the salt samples was collected from household kitchens throughout all 13 states and three federal territories in Malaysia. A total of 1429 salt samples were collected, which were immediately placed in airtight polythene bags. For this study, all 1429 salt samples were qualitatively screened to assess the type of iodine in the sample, using the IMR iodine RTK. Only the iodate form of salt samples was randomly selected for comparison with the

reference method. Eventually, 70 salt samples were selected. There were no specific criteria for the random selection.

2.2. Instruments

Ultra-high performance liquid chromatography (UHPLC), using a diode array detector (UltiMate 3000, Thermo Scientific, Waltham, MA, USA), was used as the reference method. The SIMR detection kit uses a photo absorbance reader (model PAS-110-YU, Pi-COEXPLORER, Yamato Scientific, Tokyo, Japan), with a red light-emitting diode (LED) set at 40% output, for the detection of the iodine concentration. This setting, adjusted based on the manufacturer's manual, ensures that the intensity reading for the blank measurement falls within the specified range from 30,000 to 60,000 in terms of the intensity reading, which immediately automatically converts to the absorbance reading.

2.3. Methods

2.3.1. Optimization of Scooping Weight Using 0.2 mL Tube

Two different salts were obtained from the local market to study the weight of one scoop using a 0.2 mL tube. The salts were ground using the pestle and mortar before the study was conducted. Each salt was scooped using the 0.2 mL tube, wherein the 0.2 mL tube was compressed and compacted using a finger, while, later on, the top of the scooped sample was flattened to align with the rim of the 0.2 mL tube (Figure 2). This procedure was conducted in regard to 12 replicates using 0.2 mL tubes, with each replicate weighed three times.

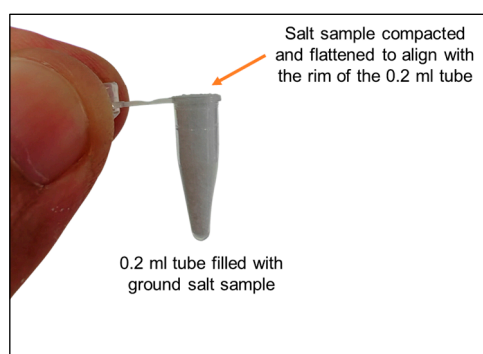


Figure 2. Illustration of scooped salt sample using a 0.2 mL tube, with flattened top, after being compressed and compacted using a finger.

2.3.2. Preparation of Stock Solution

- Sulfuric acid (1.0 M):

In a 15 mL tube, 552.0 μL of sulfuric acid (98%) was mixed with 9448.0 μL of deionized water in order to produce 1.0 M of sulfuric acid.

- Iodate stock (5.7 mM, or 1000 mg/L; and 571.7 μM , or 100 mg/L) from potassium iodate:

A total of 8.2 mg of potassium iodate was dissolved in 10.0 mL of deionized water in order to produce 5.7 mM of iodate stock. Then, 571.7 μM of stock was prepared by diluting 5.7 mM of iodate to a total volume of 10.0 mL using 1.0 mL of deionized water.

2.3.3. Preparation of Iodate Standard

A series of iodate concentrations were prepared for the calibration of the iodine concentration by the photo absorbance reader. A volume of 65.7 μL , 131.3 μL , 197.0 μL , 262.6 μL , and 328.3 μL of iodate stock (571.7 μM) were individually added to a 5 mL tube, containing one scoop of sodium chloride (using a 0.2 mL tube), to produce an iodine

concentration of 12.0 mg/Kg, 24.0 mg/Kg, 36.0 mg/Kg, 48.0 mg/Kg, and 60.0 mg/Kg, respectively. Then, deionized water was added to each of the tubes to achieve the final volume of 4.0 mL. The mixture was then mixed to form a homogeneous solution.

2.3.4. Preparation of QC Solution

Two QC solutions were used to monitor the stability of the SIMR detection kit, namely QC1 and QC2. QC1 was prepared by mixing 109.4 μ L of iodate stock (571.7 μ M) with 3890.6 μ L of deionized water, containing one scoop of sodium chloride, scooped using the 0.2 mL tube. A similar preparation was conducted for QC2, except 218.9 μ L of 100 mg/L iodate stock was mixed with 3781.1 μ L of deionized water instead. The QC solutions were the only solutions prepared daily for the stability study. The other solutions used in regard to the SIMR detection kit, including the solutions for the IMR iodine RTK (solution A, solution B, and solution C), SIMR reagent A, and SIMR reagent B, were only prepared once during the first day of the study and were kept at room temperature. This was to ensure the stability result was reflected throughout the process, due to the stability of the prepared reagents used in the SIMR detection kit.

2.3.5. Preparation of IMR Iodine RTK

- Solution A:

A total of 0.2 g of soluble starch was dissolved in 1.0 L of warm deionized water (80 °C). Then, another 0.2 g of oxalic acid was added and mixed into the solution after it had cooled down to room temperature.

- Solution B:

A total of 20.0 g of potassium iodide was added to 1.0 L of deionized water. The mixture was mixed until the solute had completely dissolved.

- Solution C:

A total of 20.0 g of potassium iodate was mixed with 1.0 L of deionized water until it was completely dissolved and which would be used as solution C.

2.3.6. Preparation of SIMR Reagents

- SIMR reagent A:

SIMR reagent A contains potassium iodide (192.8 mM, or 3.2% (*w/v*)). Moreover, 1.6 g of potassium was mixed with 50.0 mL of deionized water.

- SIMR reagent B:

SIMR reagent B comprises soluble starch (1.5 mM) and sulfuric acid (32.0 mM). In a 50 mL volumetric flask, 25.7 mg of soluble starch was mixed with 25.0 mL of warm deionized water (80 °C). Then, 1.6 mL of sulfuric acid (1.0 M) was added and mixed into the solution, before the solution was cooled down to room temperature. Later on, more deionized water was added to achieve a final volume of 50.0 mL. This reagent was pipetted (50 μ L) into 0.2 mL tubes, arranged in a 96-position rack.

2.3.7. Method Validation

The SIMR detection kit was validated according to linearity, the detection limit, method recovery, repeatability, intermediate precision, and reproducibility [19,20].

The linearity and method recovery were tested by analyzing a series of iodine concentrations, namely 5.0 mg/Kg, 10.0 mg/Kg, 20.0 mg/Kg, 30.0 mg/Kg, 40.0 mg/Kg, 50.0 mg/Kg, and 60.0 mg/Kg. These concentrations were prepared by dissolving 27.4 μ L,

54.7 μL , 109.4 μL , 164.2 μL , 218.9 μL , 273.6 μL , and 328.3 μL of iodine, in regard to iodine concentrations from 5.0 mg/Kg to 60.0 mg/Kg, respectively, the solutions of which had a total volume of 4.0 mL, containing one scoop of sodium chloride, scooped using a 0.2 mL tube. The detection limit was calculated based on the standard deviation (SD) of the y-intercept of the regression line.

Precision validation was performed using three commercial salt samples, namely sample S1, S2, and S3. The repeatability assessment was conducted by analyzing sample S1, S2, and S3 in regard to 10 replicates, with each replicate analyzed two times. The intermediate precision assessment was conducted by analyzing sample S1, S2, and S3, with two analyses of each sample, on three different days. The reproducibility assessment was carried out by a different technician, analyzing sample S1, S2, and S3, in regard to five replicates, with two analyses for each preparation.

2.3.8. Analysis of Salt Samples Using UHPLC

An analysis of the salt samples was also performed using UHPLC as a reference method, in order to be used as a comparison with the proposed method. The method was described in a previous study [18]. First, the salt sample was weighed (0.5 g) and mixed with sodium bisulfite (0.5 M), achieving a final volume of 10.0 mL. A total of 50 μL of the sample solution was ready for UHPLC injection and the following chromatographic conditions applied: isocratic mobile phase of sodium phosphate (120 mM), monobasic pH 3.0 (containing pyrophosphate, 560 μM) and methanol with a ratio of 1:1 (*v/v*); flow rate of 0.2 mL/min for 15 min; a Thermo Scientific Acclaim[®] Mixed-Mode WAX-1 analytical column, 2.1 mm $\times$ 150 mm, with a 5 μm particle size at 30 $^{\circ}\text{C}$; and an observed wavelength of 223 nm. The use of pyrophosphate during mobile phase A was intended to prevent the non-specific binding of iodide ions to the stainless steel tubing used in the UPLHC setup [21], which otherwise would produce a poor level of recovery in regard to the chromatogram, whereas a mixed-mode WAX-1 column was chosen for its ability to interact with iodide ions through weak anion-exchange interactions [22].

2.3.9. Iodine Qualitative Screening Using IMR Iodine RTK

The salt samples were screened qualitatively using the IMR iodine RTK, prior to the quantitative analysis. The IMR iodine RTK is an in-house kit that was previously used to determine the presence of iodine in the field, based on the color-change reading. It consists of solutions A, B, and C, according to which, when three drops of A are mixed with three drops of B on the salt sample, any appearance of a color change will indicate the presence of iodate, whereas when three drops of A are mixed with three drops of C on the salt sample, a color change will indicate the presence of iodide. The color appearance may range from light purple to dark grey or bluish-black. The SIMR detection kit can only be conducted if the color change is due to the presence of a form of iodate.

2.3.10. Iodine Quantitative Determination Using SIMR Detection Kit

The salt sample was meticulously ground using a pestle and mortar for several minutes, until it reached the desired fineness. The fineness of the ground salt was achieved when it had reached the optimal consistency and could not be made any finer. The ground salt sample was then carefully scooped using a 0.2 mL tube, ensuring it was pressed and compacted, and leveled to align with the rim of the tube using a finger. It was then mixed with 4.0 mL deionized water, pre-filled into the sample tube, and shaken until the salt had dissolved. SIMR reagent A was pipetted (50 μL) into a 0.2 mL tube that was pre-filled with SIMR reagent B. The sample solution was then pipetted (50 μL) into the same 0.2 mL tube; the mixture solution was pipetted several times to mix the solution in the 0.2 mL tube. The 0.2 mL tube was then inserted into the photo absorbance reading chamber, before

the iodine concentration reading was taken (Figure 3A). The representation of the process involving the photo absorbance reader is shown in Figure 3B.

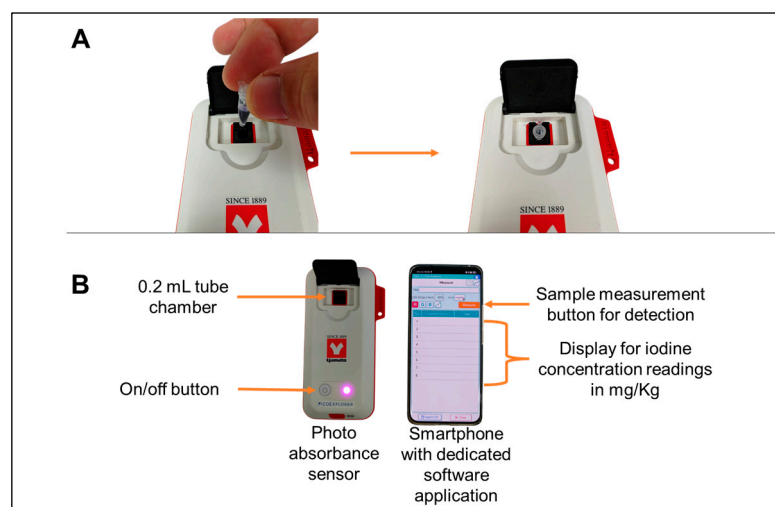


Figure 3. An overview of the process involving the photo absorbance reader used in the SIMR detection kit, where (A) demonstrates the 0.2 mL tube being placed in the tube chamber and (B) details the general notations in terms of the photo absorbance reader, which was linked with a smartphone.

2.3.11. Statistical Analysis

IBM SPSS statistics version 26 for Windows was used to analyze the sample results using a paired *t*-test, whereas the Bland–Altman plot was constructed using Windows Microsoft Office Excel 2019.

3. Results

3.1. Kit Optimization

3.1.1. Optimization of 0.2 mL Tube Scooping Weight

The study of weight optimization for one scoop of the ground salt sample, using a 0.2 mL tube, resulted in a weight of 396.9 ± 10.0 mg (Table 1). This value was used to back-calculate the concentration of iodate in the solution, with respect to the iodine concentration in the salt samples.

Table 1. Summary of the data on the weight of one scoop by a 0.2 mL tube, using two different salt samples, in regard to 12 replicates.

0.2 mL Tube	Salt Weight (mg)	
	Mean (<i>n</i> = 3)	SD
1	381.8	16.2
2	376.8	9.1
3	375.9	10.1
4	387.2	5.8
5	382.4	7.5
6	378.5	10.0
7	417.8	20.3
8	397.6	6.2
9	414.6	8.2
10	416.0	9.6
11	411.9	6.3
12	422.5	11.2
Total mean	396.9	10.0

3.1.2. Device Calibration and Detection Limit

The photo absorbance reader was calibrated according to the iodate standard, with an iodine concentration of 12.0 mg/Kg, 24.0 mg/Kg, 36.0 mg/Kg, 48.0 mg/Kg, and 60.0 mg/Kg (Figure 4A). The calibration curve was obtained by using a four-parameter logistic (4-PL) to get a better representation of the results (Figure 4B). Thus, it was found that the calibration ($R^2 = 0.9986$) follows the formula below:

$$Y = 0.1981 + \frac{-0.0002 - 0.1981}{1 + \left(\frac{X}{25.7504}\right)^{1.5521}} \quad (1)$$

which follows the 4-PL general formula:

$$Y = d + \frac{a - d}{1 + \left(\frac{X}{c}\right)^b} \quad (2)$$

where a is the theoretical response at zero concentration, b is the slope factor, c is the mid-range concentration (inflection point), and d is the theoretical response at an infinite concentration. Y is the absorbance reading in A.U., and X is the independent variable of the iodine concentration in mg/Kg unit.

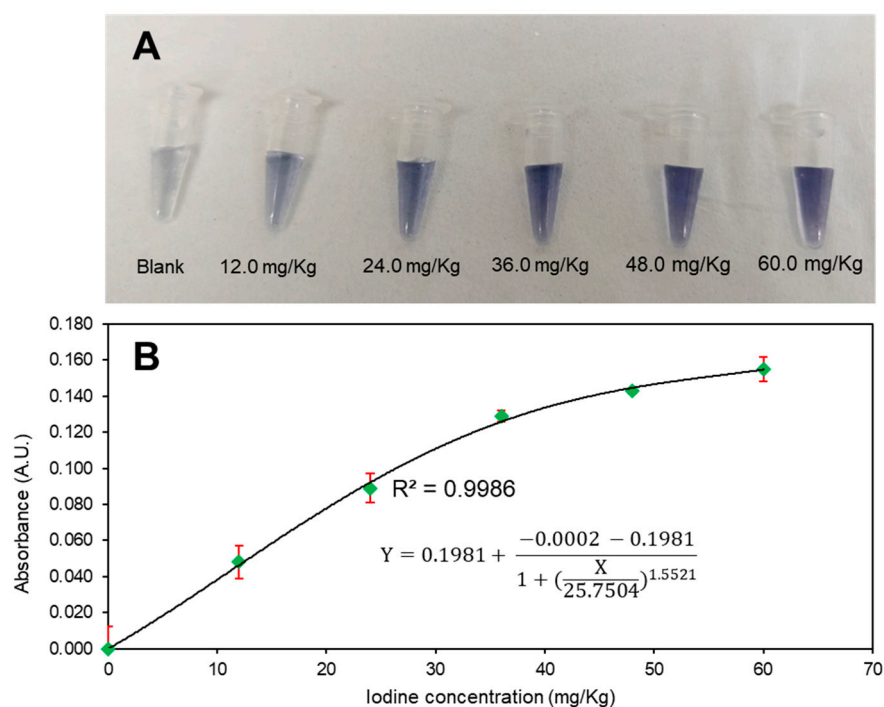


Figure 4. The calibration of the iodine standard for blank, 12.0 mg/Kg, 24.0 mg/Kg, 36.0 mg/Kg, 48.0 mg/Kg, and 60.0 mg/Kg, with (A) a comparison of the color intensity for different levels of iodine concentration, and (B) a calibrated curve of the iodine concentration from 12.0 mg/Kg to 60.0 mg/Kg using 4-PL.

Also, the detection limit of the reading was calculated based on the SD of the background response of the blanks. It was found that the curve produced a detection limit of 6.2 mg/Kg in terms of the iodine concentration.

3.2. Method Validation

3.2.1. Linearity and Detection Limit

The linearity of the detection was tested by analyzing the iodine standard according to various concentrations, including 5.0 mg/Kg, 10.0 mg/Kg, 20.0 mg/Kg, 30.0 mg/Kg, 40.0 mg/Kg, 50.0 mg/Kg, and 60.0 mg/Kg. The iodine readings were plotted against the respective expected concentrations (Figure 5) and the readings were found to be highly correlated due to a calibrated R^2 of 0.9811.

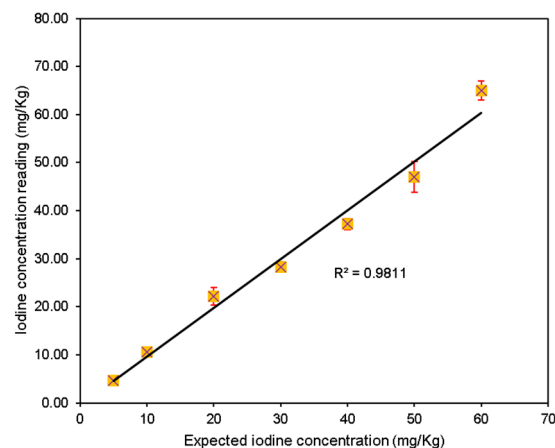


Figure 5. The correlation between the readings in terms of the iodine concentration by the SIMR detection kit and the expected iodine concentration from 5.0 mg/Kg to 60.0 mg/Kg.

Additionally, the detection limit was obtained based on the y-intercepts' SD of the regression line. It was found that the validated detection limit was 6.8 mg/Kg of iodine.

3.2.2. Method Recovery

The study of the method recovery was performed to validate the iodine concentration range. For instance, iodine concentrations of 5.0 mg/Kg, 10.0 mg/Kg, 20.0 mg/Kg, 30.0 mg/Kg, 40.0 mg/Kg, 50.0 mg/Kg, and 60.0 mg/Kg were studied (Table 2) and it was found that the iodine concentration showed a good recovery of 93.9%, 106.6%, 97.2%, 94.1%, 93.0%, 94.0%, and 108.3%, respectively.

Table 2. Recovery of iodine concentration, from 5.0 mg/Kg to 60.0 mg/Kg.

Iodine Concentration (mg/Kg)	Readings (mg/Kg)		Recovery (%)
	Mean	SD	
5.0	4.7	0.3	93.9
10.0	10.7	0.3	106.6
20.0	19.4	2.1	97.2
30.0	28.2	1.0	94.1
40.0	37.2	1.1	93.0
50.0	54.0	6.8	94.0
60.0	65.0	1.9	108.3

3.2.3. Repeatability

The repeatability assessment was conducted to oversee the variation in the results when performed repeatedly. This was to ensure the accuracy and precision of the SIMR detection kit. For this study, 10 replicates of samples S1, S2, and S3 were analyzed, and it was found that the coefficient of variation (CV) for S1 was 4.9%, S2 was 5.5%, and S3 was 5.5% (Table 3). The mean CV was 5.3%.

Table 3. Comparison of the mean and SD of 10 replicates of samples S1, S2, and S3 in order to study their CVs.

Replicate	Iodine Concentration Readings (mg/Kg)					
	Sample S1		Sample S2		Sample S3	
	Mean (n = 3)	SD	Mean (n = 3)	SD	Mean (n = 3)	SD
1	29.6	0.8	23.6	2.2	24.6	1.7
2	26.1	1.0	28.3	1.7	28.3	2.6
3	27.0	2.2	26.1	0.3	26.1	1.2
4	28.2	1.0	23.8	0.4	28.9	2.1
5	33.5	0.0	26.8	1.8	24.0	0.7
6	28.8	0.8	29.0	0.0	28.9	1.7
7	30.1	2.3	27.4	2.2	32.6	0.0
8	33.3	2.8	31.6	2.8	29.9	2.4
9	27.7	1.3	29.6	1.9	29.5	2.8
10	30.2	2.2	26.5	1.8	32.6	0.6
Mean	29.5	1.4	27.3	1.5	28.5	1.6
CV (%)	4.9		5.5		5.5	
Mean CV (%)	5.3					

3.2.4. Intermediate Precision

The analysis of samples S1, S2, and S3 was performed on three different days using the SIMR detection kit in order to study the intermediate precision and the results, detailed in Table 4 below, were realized. It was discovered that the mean CV of samples S1, S2, and S3 were 7.5%, 8.2%, and 4.9%, respectively, and the mean CV for all the samples was 6.8%.

Table 4. Comparison of CVs for samples S1, S2, and S3 measured on three different days.

Day	Iodine Concentration Readings (mg/Kg)					
	S1		S2		S3	
	Mean (n = 3)	SD	Mean (n = 3)	SD	Mean (n = 3)	SD
1	26.6	2.0	26.2	5.1	28.4	1.6
2	27.0	2.2	26.1	0.3	26.1	1.2
3	26.3	1.7	26.5	1.0	26.6	1.2
Mean	26.6	2.0	26.3	2.1	27.0	1.3
CV (%)	7.5		8.2		4.9	
Mean CV (%)	6.8					

3.2.5. Reproducibility

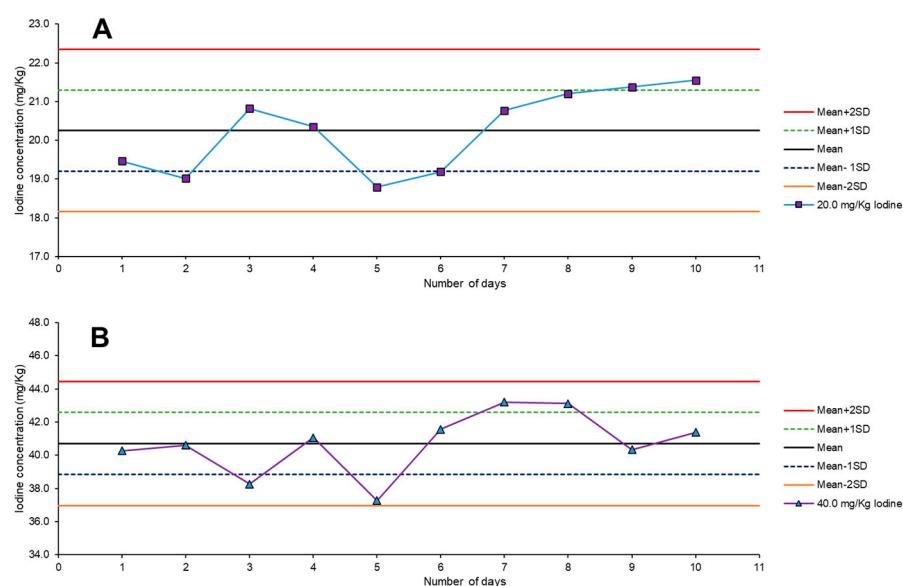
A different technician was employed to perform the analysis of samples S1, S2, and S3, using the SIMR detection kit, in regard to five replicates. As shown in Table 5, it was found that the CV for sample S1 was 5.0%, S2 was 6.4%, and S3 was 6.4%, resulting in a mean CV for all the samples of only 5.9%.

Table 5. Tabulated data on the iodine concentration in five replicates of sample S1, S2, and S3, with their respective CVs.

Replicate	Iodine Concentration Readings (mg/Kg)					
	Sample S1		Sample S2		Sample S3	
	Mean (<i>n</i> = 3)	SD	Mean (<i>n</i> = 3)	SD	Mean (<i>n</i> = 3)	SD
1	26.6	0.2	21.6	1.0	24.8	2.3
2	23.7	2.1	26.7	0.9	24.2	1.4
3	20.6	0.3	26.8	2.0	27.4	1.6
4	24.4	1.2	25.4	2.3	31.8	1.9
5	26.9	2.3	27.4	2.0	28.6	1.6
Mean	24.4	1.2	25.6	1.6	27.4	1.8
CV (%)	5.0		6.4		6.4	
Mean CV (%)	5.9					

3.3. Stability of the SIMR Reagents

The stability of the prepared reagents, i.e., SIMR reagent A and SIMR reagent B, was observed for 10 days, based on the iodine readings obtained for the QC1 and QC2 solutions, which represent an iodine concentration of 20.0 mg/Kg (Figure 6A) and 40.0 mg/Kg (Figure 6B), respectively. SIMR reagent A and SIMR reagent B were only prepared once, whereas the QC1 and QC2 solutions were prepared daily, for this purpose. The QC1 and QC2 results remained stable for 10 days within the range of the mean $\pm$ 2 SD of 20.3 ± 2.1 mg/Kg for QC1 and 40.7 ± 3.7 mg/Kg for QC2.

**Figure 6.** Charts on the 10 days of iodine concentration monitoring for the iodate standard that was equivalent to an iodine concentration of (A) 20.0 mg/Kg as QC1 and (B) 40.0 mg/Kg as QC2.

3.4. Real Sample Analysis

A paired sample *t*-test was performed to compare the iodine concentration results of the 70 salt samples measured using the UHPLC method ($n = 4$) and the SIMR detection kit ($n = 2$), as presented in Table 6. At a 95% confidence interval (CI), the analysis yielded a *p*-value of 0.091, greater than the significant level ($\alpha = 0.05$). This indicates that the null hypothesis (no significant difference between the two methods) cannot be rejected.

Table 6. Comparison of the iodine concentration results from the UHPLC method with the SIMR detection kit, using 70 salt samples.

Sample Number	Iodine Concentration (mg/Kg)			
	UHPLC		SIMR Detection Kit	
	Mean (<i>n</i> = 4)	SD	Mean (<i>n</i> = 2)	SD
1	10.9	2.5	12.8	1.8
2	23.1	1.8	30.6	1.5
3	16.1	1.3	14.6	2.1
4	31.9	0.6	28.9	0.2
5	23.5	2.4	15.8	0.1
6	11.4	1.4	10.0	1.0
7	4.2	0.1	2.7	0.0
8	21.4	0.9	26.9	4.1
9	21.1	0.2	21.8	0.9
10	24.7	1.1	31.8	0.1
11	24.3	1.4	22.9	0.3
12	32.7	4.2	35.0	0.2
13	7.5	3.3	17.6	2.2
14	28.2	4.3	32.8	0.2
15	22.1	2.6	23.8	1.3
16	24.8	2.5	23.5	0.0
17	27.2	7.2	24.2	0.2
18	2.2	0.0	0.5	0.0
19	17.7	6.9	23.9	0.2
20	14.3	0.3	25.0	0.1
21	18.6	2.2	29.3	0.2
22	23.5	0.9	34.1	0.0
23	23.2	1.3	28.1	0.9
24	20.4	0.4	28.8	0.2
25	22.5	1.1	33.4	2.1
26	20.5	0.1	28.2	0.1
27	20.1	2.3	34.1	0.0
28	27.7	1.8	42.8	0.1
29	9.7	0.5	16.0	3.7
30	17.5	1.0	22.9	1.2
31	17.8	0.5	33.5	2.9
32	17.6	0.2	27.6	1.6
33	0.0	0.0	0.6	0.9
34	25.5	0.2	25.0	1.3
35	25.3	0.9	28.6	3.7
36	11.1	0.3	12.8	3.1
37	23.7	1.3	20.8	0.4
38	16.7	0.5	40.1	5.3
39	15.5	0.7	14.2	0.1
40	32.3	2.6	43.4	0.1
41	21.0	5.1	26.5	0.1
42	12.6	3.3	13.0	0.0
43	22.9	0.3	22.6	0.2
44	17.4	2.1	20.5	3.7
45	25.5	1.1	23.6	1.3
46	25.6	0.3	25.2	1.3
47	12.5	2.4	14.2	1.3
48	19.9	1.9	26.6	2.3
49	30.6	0.9	33.5	1.6
50	32.5	2.8	16.9	0.9

Table 6. Cont.

Sample Number	Iodine Concentration (mg/Kg)			
	UHPLC		SIMR Detection Kit	
	Mean (<i>n</i> = 4)	SD	Mean (<i>n</i> = 2)	SD
51	31.6	3.8	31.4	1.5
52	23.1	4.9	25.9	3.6
53	32.8	0.7	24.6	0.2
54	25.9	1.7	22.3	2.4
55	23.2	1.5	22.7	1.6
56	19.8	0.9	20.9	0.1
57	19.6	1.8	12.6	0.2
58	34.7	4.0	33.9	0.2
59	38.3	1.7	34.2	1.3
60	18.2	1.4	17.8	0.7
61	20.2	1.5	21.2	0.2
62	30.7	1.7	28.2	1.5
63	29.0	1.9	21.5	2.6
64	18.9	3.7	17.2	0.1
65	18.0	0.2	13.7	3.3
66	27.0	4.3	20.8	3.8
67	45.2	2.9	31.0	4.4
68	33.8	0.2	26.7	3.7
69	42.5	1.2	30.5	2.4
70	29.6	1.5	15.7	0.2

Moreover, a Bland–Altman plot was constructed, as shown in Figure 7, to visualize the agreement in the results of the methods, between the UHPLC reference method and the SIMR detection kit, based on the bias (−1.5 mg/Kg) and limit of agreement (LOA) from −15.7 mg/Kg to 12.7 mg/Kg, calculated as bias $\pm$ 1.96 $\times$ SD at a 95% CI. It was observed that all of the comparison data points were within the allowable LOA, within the outlier border.

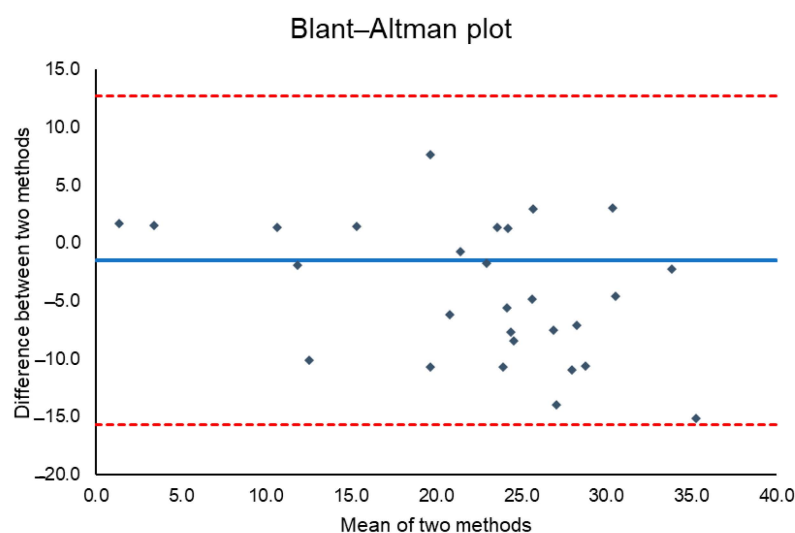


Figure 7. The Bland–Altman plot for the UHPLC method versus the SIMR detection kit in terms of the results for 70 salt samples.

4. Discussion

The micro-method technique is a sustainable solution that uses a small sample volume with a diluted concentration, making it more environmentally friendly. As the name

implies, the SIMR detection kit, developed based on this concept, uses reagents with a diluted concentration, with a total volume of 150 μL , when mixed prior to detection, using a photo absorbance reader. For instance, SIMR reagent A uses only 192.8 mM of potassium iodate, while SIMR reagent B contains only 1.5 mM of soluble starch and 32.0 mM of sulfuric acid. These reagents, each requiring a volume of 50 μL only, are not only cost effective, but also environmentally friendly, as they produce less waste. The slightly high volume of 4.0 mL of deionized water used to dissolve the salt samples can be safely disposed of due to its non-hazardous nature, further enhancing the sustainability of the method.

The development of the SIMR detection kit aimed to miniaturize the kit for maximum total analysis with the shortest analysis time. The miniaturization of the kit was conducted by minimizing the size of the consumables required for complete on-site analysis. For example, the use of a smaller sized pestle and mortar and a mini pipet minimized the space required in the kit box. However, the principle of detection in terms of the photo absorbance reader was to detect the intensity of the color change due to the presence of iodine in the samples; the challenge was to detect colored salt samples, such as pink rock salt. To ensure the accuracy of the results, we introduced a syringe and a nylon filter to filter the colored salt samples before the analysis. While this solution necessitates additional consumables, it highlights the study's commitment to thoroughness and ensures both process efficiency and the accuracy of the results.

During the development of the SIMR detection kit, the uniformity of the weighing technique for the salt samples was a critical factor in determining the trueness of the results. The salt samples, regardless of their grain size, had to be ground first to minimize error. The ground salt samples were then scooped using a 0.2 mL tube, compacted with a finger, and the top of the scooped sample was leveled to align with the rim of the 0.2 mL tube. Our study found the weight of the scooped ground salt sample, 396.9 ± 10.0 mg, to be a key determinant in establishing the expected concentration of iodine in the solution, relative to its concentration in the salt sample (mg/Kg).

Another one of the crucial steps in obtaining accurate readings is the proper mixing of SIMR reagent A, SIMR reagent B, and the sample solution. This is a task of utmost importance, before the 0.2 mL tube, filled with these three mixtures, is placed on the photo absorbance reader chamber for measurement. Improper mixing can lead to the introduction of air gaps at the bottom of the 0.2 mL tube, resulting in an error in reading the iodine concentration. This is because the absorbance reading is performed at the bottom of the 0.2 mL tube. The diagram below (Figure 8) provides several examples to demonstrate the correct way of placing the sample solution in the 0.2 mL tube, reiterating the importance of this procedure in obtaining accurate readings.

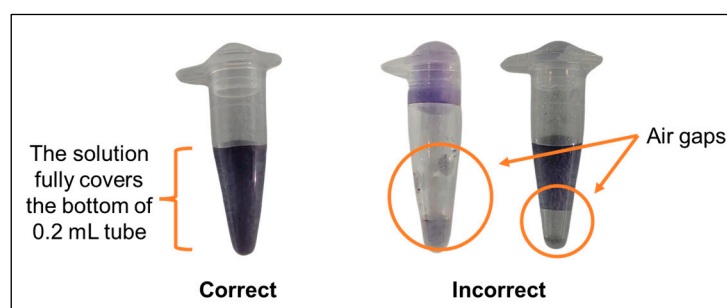
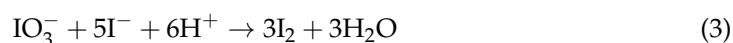


Figure 8. A comparison of the correct versus incorrect way of placing the sample solution in a 0.2 mL tube for detection.

The total analysis time was mainly made up of the time taken to grind the salt samples and several steps in terms of the chemical reaction required for a color change-based

reaction to occur. The color change in the sample solution was due to the formation of an iodine–starch complex. This can be illustrated by the chemical reaction below:



where IO_3^- represents the iodate ion, I^- represents the iodide ion, H^+ represents the hydrogen ion, H_2O represents water, and I_2 represents the iodine compound. The iodate form of iodine in the salt samples necessitates the addition of excess iodide in an acidic medium to form iodine, which later on also requires the addition of starch for a color change to appear. These multiple steps in terms of the chemical reaction significantly extend the overall procedure of the analysis. Therefore, the pre-adding of SIMR reagent B, which contains an excess of starch solution in diluted sulfuric acid, into the 0.2 mL tube, was carried out in advance, highlighting the efficiency and productivity of this method in regard to saving time.

Finally, the developed and optimized SIMR detection kit was able to accommodate a total of 50 analyses, with one analysis requiring less than 10 min of analysis time only.

The SIMR detection kit was rigorously validated, including studies on the linearity, detection limit, method recovery and imprecision. The successful validation of the SIMR detection kit, with a linear detection range of 5.0–60.0 mg/Kg in terms of iodine concentration, a detection limit of 6.8 mg/Kg, and a recovery range between 93.0–108.3%, emphasizes its reliability and indicates the high accuracy of the method within the studied iodine concentration range. The detection limit was determined to be 6.8 mg/Kg and estimated based on the SD of the calibration curve's SD of the regression line [23], which was higher than the lowest iodine calibration concentration of 5.0 mg/Kg. This discrepancy was attributed to the lack of sensitivity of the photo absorbance reader [24], which resulted in a poor SD when comparing the measured iodine concentrations to the expected values. However, this limitation does not affect the performance of the SIMR detection kit, as it was still capable of detecting iodate in salt samples within the recommended range from 20.0 mg/Kg to 40.0 mg/Kg. On the other hand, the mean CVs for the repeatability, intermediate precision, and reproducibility of 5.3%, 6.8%, and 5.9%, respectively, demonstrate the high precision of the SIMR detection kit. In summary, the successful validation of the SIMR detection kit leaves no doubt about its effectiveness and reliability.

The prepared SIMR reagent A and SIMR reagent B demonstrated remarkable stability, remaining unchanged for a minimum of 10 days when tested with QC1 and QC2. The results were 20.3 ± 2.1 mg/Kg for QC1 and 40.7 ± 3.7 mg/Kg for QC2. These QCs represent iodine concentrations of 20.0 mg/Kg and 40.0 mg/Kg in the QC1 and QC2 salt samples, respectively. The excellent mean recovery of 101.5% for QC1 and 101.8% for QC2 further validates the effectiveness of our SIMR reagents.

The statistical comparison of the paired *t*-test (*p*-value = 0.091 > 0.05, 95% CI) and the Bland–Altman plot (bias = −1.5 mg/Kg; LOA: −15.7–12.7 mg/Kg, 95% CI) performed in regard to the 70 salt samples indicates the potential of the SIMR detection kit. It produces results similar to the reference method, suggesting that it could be a promising alternative for iodine concentration monitoring in salt samples.

Nevertheless, there were limitations to the methods used in regard to the SIMR detection kit, including the fact that it could only detect the iodate form of iodized salt. This limitation may restrict its applicability in regions where other forms of iodized salt are prevalent. However, most of the salt samples in Malaysia were in iodate form due to their stability [25], meaning that the method is still applicable to salt iodine monitoring. More studies need to be conducted to monitor the stability of the SIMR reagents over a longer

period of time. Extended stability is preferable, so it would be more feasible to be used as an on-site detection kit for salt iodization monitoring.

Based on the results from the salt samples obtained using the SIMR detection kit, the mean iodine concentration was 23.8 ± 1.8 mg/Kg. This indicates that the 70 salt samples collected fell within the recommended range of 20–40 mg/Kg, suggesting the initial success of the USI program in Malaysia. However, a more definitive conclusion should be based on a larger sample size that accurately represents the Malaysian population, specifically a total of 1429 salt samples collected as part of the NHMS 2024: Nutrition program, as mentioned in Section 2.1.2.

The SIMR detection kit allows for the rapid measurement of iodine in salt in the field, reducing the risk of iodine evaporation during the transportation of salt samples to laboratories. This helps to prevent the underestimation of iodine levels in samples, which should reflect the entire salt consignment at the port of entry. Additionally, high-risk groups, such as pregnant women and children, in particular, would benefit from the real-time, fast, and immediate measurements provided by the SIMR detection kit. These real-time data can help guide these individuals in adjusting their dietary intake to prevent the risks associated with IDD.

5. Conclusions

The development and optimization of the SIMR detection kit were conducted in order to provide an alternative salt iodate analysis method for on-site detection. The kit uses a color change-based reaction to quantify the iodate concentration in salt samples. It accommodated up to 50 analyses per kit, with a total run time of less than 10 min per analysis. The kit was well-validated, with a linearity of 5.0 mg/Kg to 60.0 mg/Kg, a detection limit of 6.8 mg/Kg, method recovery ranging from 93.0% to 108.3%, with a repeatability mean CV of 5.3%, an intermediate precision mean CV of 6.8%, and a reproducibility mean CV of 5.9%. The use of SIMR reagent A and reagent B remained stable within a mean ± 2 SD for at least 10 days when tested with freshly prepared QC1 (20.0 mg/Kg iodine) and QC (40.0 mg/Kg iodine). The real salt sample ($n = 70$) analyses demonstrated no difference between the SIMR detection kit and UHPLC (reference method) when tested statistically using a paired t -test (p -value = 0.091 at 95% CI) and a visual Bland–Altman plot (bias = -1.5 mg/Kg; LOA: -15.7 – 12.7 mg/Kg, 95% CI), which also suggests that there is a similarity between these two methods due to all the data points being within the bias $\pm$ LOA range. The salt iodate analysis by the SIMR detection kit, as an alternative method, was proven to have high accuracy and precision, comparable with the reference method. The fact that the kit uses a micro-method approach, offering portable and on-site detection, with a total analysis time of less than 10 min, makes it a more feasible method due to its cost effectiveness and environmentally friendliness. However, a more extended time period is necessary to assess the stability of the SIMR detection kit for effective long-term use, particularly in rural areas, where reliable infrastructure is often lacking.

6. Patents

The SIMR detection kit was patented by the Intellectual Property Corporation of Malaysia (MyIPO) (patent number PI 2018702654).

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References

- Menon, K.; Skeaff, S. Iodine: Iodine Deficiency Disorders (IDD). In *Encyclopedia of Food and Health*; Caballero, B., Finglas, P., Toldra, F., Eds.; Academic Press: Oxford, UK, 2016; pp. 437–443, ISBN 9780123849533.
- Lisco, G.; De Tullio, A.; Triggiani, D.; Zupo, R.; Giagulli, V.A.; De Pergola, G.; Piazzolla, G.; Guastamacchia, E.; Sabbà, C.; Triggiani, V. Iodine Deficiency and Iodine Prophylaxis: An Overview and Update. *Nutrients* **2023**, *15*, 1004. [\[CrossRef\]](#) [\[PubMed\]](#)
- Codling, K.; Rudert, C.; Bégin, F.; Peña-Rosas, J.P. The Legislative Framework for Salt Iodization in Asia and the Pacific and Its Impact on Programme Implementation. *Public Health Nutr.* **2017**, *20*, 3008–3018. [\[CrossRef\]](#)
- Lazarus, J.H. The Importance of Iodine in Public Health. *Env. Geochem. Health* **2015**, *37*, 605–618. [\[CrossRef\]](#) [\[PubMed\]](#)
- Andersson, M.; Karumbunathan, V.; Zimmermann, M.B. Global Iodine Status in 2011 and Trends over the Past Decade. *J. Nutr.* **2012**, *142*, 744–750. [\[CrossRef\]](#) [\[PubMed\]](#)
- Wei, R.; Wang, Z.; Zhang, X.; Wang, X.; Xu, Y.; Li, Q. Burden and Trends of Iodine Deficiency in Asia from 1990 to 2019. *Public Health* **2023**, *222*, 75–84. [\[CrossRef\]](#)
- Kuay, L.K.; Chin, T.B.; Ying, C.Y.; Hussain, H.; Mahmud, N.A.; Kassim, M.S.A.; Harith, A.A.; Man, C.S.; Salleh, R.; Aris, T. Pregnant Women Are Iodine Deficient While School-Aged Children Demonstrate Adequate Iodine Status in Sarawak, Malaysia. *Food Nutr. Bull.* **2021**, *42*, 406–413. [\[CrossRef\]](#)
- Harith, A.A.; Kuay, L.K.; Azlan, M.S.; Mahmud, N.A.; Ismail, F. Impact of Universal Salt Iodization Programme Towards Iodine Status of Pregnant Women in Sarawak. *Int. J. Public Health Clin. Sci.* **2019**, *6*, 98–108. [\[CrossRef\]](#)
- Lim, K.K.; Chan, Y.Y.; Zainuddin, A.A.; Teh, C.H.; Ismail, H.; Lim, K.H.; Kee, C.C. Iodine Deficiency Disorder and Goitre among School Children in Sarawak—A Nationwide Study. *Int. J. Public Health Res.* **2014**, *4*, 419–424.
- Anteneh, Z.A.; Engidayehu, M.; Abeje, G. Iodine Content of Dietary Salt at Household Level and Associated Factors Using Iodometric Titration Methods in Dera District, Northwest Ethiopia. *BMC Nutr.* **2017**, *3*, 83. [\[CrossRef\]](#)
- Zergui, A.; Boudalia, S.; Joseph, M.L.; Venturi, S. Monitoring of Iodine Supplementation in Table Salts by ICP-MS and Possible Health Risks of Iodine Deficiency in Algeria. *Food Chem. Adv.* **2023**, *2*, 100230. [\[CrossRef\]](#)
- Khazan, M.; Azizi, F.; Hedayati, M. A Review on Iodine Determination Methods in Salt and Biological Samples. *Scimetr* **2013**, *1*, e14092. [\[CrossRef\]](#)
- Jooste, P.L.; Strydom, E. Methods for Determination of Iodine in Urine and Salt. *Best Pr. Res. Clin. Endocrinol. Metab.* **2010**, *24*, 77–88. [\[CrossRef\]](#)
- Niero, G.; Franzoi, M.; Vigolo, V.; Penasa, M.; Cassandro, M.; Boselli, C.; Giangolini, G.; De Marchi, M. Validation of a Gold Standard Method for Iodine Quantification in Raw and Processed Milk, and Its Variation in Different Dairy Species. *J. Dairy Sci.* **2019**, *102*, 4808–4815. [\[CrossRef\]](#)
- Long, S.E.; Catron, B.L.; Boggs, A.S.P.; Tai, S.S.C.; Wise, S.A. Development of Standard Reference Materials to Support Assessment of Iodine Status for Nutritional and Public Health Purposes. *Am. J. Clin. Nutr.* **2016**, *104*, 902S–906S. [\[CrossRef\]](#)
- Flores, E.M.M.; Mello, P.A.; Krzyzaniak, S.R.; Cauduro, V.H.; Picoloto, R.S. Challenges and Trends for Halogen Determination by Inductively Coupled Plasma Mass Spectrometry: A Review. *Rapid Commun. Mass Spectrom.* **2020**, *34*, e8727. [\[CrossRef\]](#)
- Falk, H.; Geerling, R.; Hattendorf, B.; Krengel-Rothensee, K.; Schmidt, K.P. Capabilities and Limits of ICP-MS for Direct Determination of Element Traces in Saline Solutions. *Fresenius J. Anal. Chem.* **1997**, *359*, 352–356. [\[CrossRef\]](#)
- Jamilan, M.A.; Abd Rashed, A.; Md Noh, M.F. Protocol for the Determination of Total Iodine in Iodized Table Salts Using Ultra-High-Performance Liquid Chromatography. *Chemosensors* **2025**, *13*, 46. [\[CrossRef\]](#)

19. Rohner, F.; Kangambega, M.O.; Khan, N.; Kargougou, R.; Garnier, D.; Sanou, I.; Ouaro, B.D.; Petry, N.; Wirth, J.P.; Jooste, P. Comparative Validation of Five Quantitative Rapid Test Kits for the Analysis of Salt Iodine Content: Laboratory Performance, User- and Field-Friendliness. *PLoS ONE* **2015**, *10*, e0138530. [[CrossRef](#)]
20. Chavan, S.D.; Desai, D.M. Analytical Method Validation: A Brief Review. *World J. Adv. Res. Rev.* **2022**, *16*, 389–402. [[CrossRef](#)]
21. Guimaraes, G.J.; Bartlett, M.G. Managing Nonspecific Adsorption to Liquid Chromatography Hardware: A Review. *Anal. Chim. Acta* **2023**, *1250*, 340994. [[CrossRef](#)]
22. Lämmerhofer, M.; Nogueira, R.; Lindner, W. Multi-Modal Applicability of a Reversed-Phase/Weak-Anion Exchange Material in Reversed-Phase, Anion-Exchange, Ion-Exclusion, Hydrophilic Interaction and Hydrophobic Interaction Chromatography Modes. *Anal. Bioanal. Chem.* **2011**, *400*, 2517–2530. [[CrossRef](#)] [[PubMed](#)]
23. Taleuzzaman, M. Limit of Blank (LOB), Limit of Detection (LOD), and Limit of Quantification (LOQ). *Org. Med. Chem. Int. J.* **2018**, *7*, 127–131. [[CrossRef](#)]
24. Fernandes, G.M.; Silva, W.R.; Barreto, D.N.; Lamarca, R.S.; Lima Gomes, P.C.F.; da S. Petrucci, J.F.; Batista, A.D. Novel Approaches for Colorimetric Measurements in Analytical Chemistry—A Review. *Anal. Chim. Acta* **2020**, *1135*, 187–203. [[CrossRef](#)]
25. Ekott, E.J.; Etukudo, U.I. Impact of Storage on Iodine Stability of Commercial Salt Brands. *Int. J. Eng. Tech. Res.* **2019**, *9*, 9–11. [[CrossRef](#)]

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