Validation of a user-friendly and rapid method for quantifying iodine content of salt

Fabian Rohner, Greg S. Garrett, Arnaud Laillou, Simone K. Frey, Ralf Mothes, Florian J. Schweigert, and Lorenzo Locatelli-Rossi

Abstract

Background. *Despite considerable progress made in the past decade through salt iodization programs, over 2 billion people worldwide still have inadequate iodine intake, with devastating consequences for brain development and intellectual capacity. To optimize these programs with regard to salt iodine content, careful monitoring of salt iodine content is essential, but few methods are available to quantitatively measure iodine concentration in a simple, fast, and safe way.*

Objective. *We have validated a newly developed device that quantitatively measures the content of potassium iodate in salt in a simple, safe, and rapid way.*

Methods. *The linearity, determination and detection limit, and inter- and intra-assay variability of this colorimetric method were assessed and the method was compared with iodometric titration, using salt samples from several countries.*

Results. *Linearity of analysis ranged from 5 to 75 mg/kg iodine, with 1 mg/kg being the determination limit; the intra- and interassay imprecision was 0.9%, 0.5%, and 0.7% and 1.5%, 1.7%, and 2.5% for salt samples with iodine contents of 17, 30, and 55 mg/kg, respectively; the interoperator imprecision for the same samples was 1.2%, 4.9%, and 4.7%, respectively. Comparison with the iodometric method showed high agreement between the methods ($R^2 = 0.978$; limits of agreement, –10.5 to 10.0 mg/kg).*

Conclusions. *The device offers a field- and user-friendly solution to quantifying potassium iodate salt content reliably. For countries that use potassium iodide in salt iodization programs, further validation is required.*

Key words: Iodization, iodine, monitoring, potassium iodate, quality control, rapid test kit, regulatory monitoring, salt

Introduction

Iodine deficiency has important consequences for mental development, intellectual capacity, and growth, particularly in children [1]. Most recent estimates state that globally over 2 billion people are adversely affected [2].

At approximately US$0.05 per person per year, salt iodization is among the most cost-effective and sustainable interventions to counter iodine deficiency and its disorders. Salt iodization programs have been implemented at large scale in many countries [3]. Most programs use potassium iodate (KIO_3) because of its better stability as compared with potassium iodide (KI). As a result of these programs, important progress in reducing iodine deficiency and its disorders has been made in the past two decades [4].

Nonetheless, based on urinary iodine concentrations in school-aged children as a proxy to determine iodine status in a population, 32 countries continue to show high levels of iodine deficiency [5]. Simultaneously, an increasing number of countries are starting to show iodine intakes that are above recommended levels [2]. Excessive iodine intake can paradoxically lead to hypothyroidism and goiter, but the consequences are less devastating than those of iodine-deficiency disorders [6].

To avoid iodine deficiency, but also to avoid potential excess, it is important to carefully monitor the iodine that is added to salt in salt iodization programs at the levels of production, importation, distribution, and consumption. To assess salt iodine content, several

Fabian Rohner, Greg S. Garrett, Arnaud Laillou, and Lorenzo Locatelli-Rossi are affiliated with the Global Alliance for Improved Nutrition (GAIN), Geneva. Fabian Rohner is also affiliated with GroundWork LLC, Crans-près-Céligny, Switzerland. Simone K. Frey, Ralf Mothes, and Florian J. Schweigert are affiliated with BioAnalyt GmbH, Teltow, Germany. Florian J. Schweigert is also affiliated with the University of Potsdam, Potsdam-Rehbrücke, Germany.

Please direct queries to the corresponding author: Fabian Rohner, GroundWork LLC, 40b Les Landes, 1299 Crans-près-Céligny, Switzerland; e-mail: fabian@groundworkhealth.org.

methods are available, but iodometric titration has become the widely accepted method. However, its use requires laboratory equipment, technical skills and basic training [4]. The introduction of qualitative or semiquantitative rapid test kits has simplified monitoring in the past, but these kits have the important limitation that they have a large margin of error when used for semiquantitative analysis or—if used qualitatively—will provide confirmation of the iodine content of salt, but not of the level of adequacy. More recently, a quantitative device (WYD iodine checker) was commercialized and successfully validated [7]. Despite several simplifications it offered in comparison to the titration method and its high precision, this device bears the disadvantage that the operator still needs to handle corrosive liquids (sulfuric acid) and operates in an open system, rendering the analysis susceptible to iodine contamination through the calibration solution.

To further simplify quantitative monitoring of salt iodine content, BioAnalyt has developed a simple, portable device to rapidly quantify the potassium iodate concentration in salt (iCheck-iEx, hereafter referred to as the "portable device"). The objective of this study was to validate the performance of the portable device for salt iodine content analysis in a laboratory setting.

Materials and methods

The validation in this manuscript has been performed for potassium iodate but not for potassium iodide, because the former is used considerably more often than the latter. Salt samples fortified with potassium iodide require an oxidation to potassium iodate prior to analysis with the portable device. The method for the oxidation of iodide to iodate is treatment with an excess of bromine water. The excess of bromine is then removed by treatment with formic acid [8].

Portable device

Methods

The principle of this colorimetric method is based on the reaction of potassium iodate from a salt sample with potassium iodide added in excess; chemically, iodide (I^-) forms iodine (I_2) and triiodide (I_3^-), resulting in a blue-purple complex after addition of a starch solution. The absorption of the blue color is dependent on the concentration of the solution and is measured at 565 nm in the measuring unit. The results are displayed and saved in the measuring unit and can be downloaded via a universal serial bus (USB) as a text file (.txt) to a computer. The device can be battery-operated or connected to the mains (100 to 240 V). The device's software has an algorithm programmed that converts the absorption units of the blue-color reaction into milligrams per liter.

Material

The salt samples used for this work were commercially available salts from various countries (Croatia, France, Egypt, Germany, Ghana, Honduras, India, Morocco, Pakistan, Senegal, Switzerland, and Thailand) with varying iodine concentration, ranging from zero to approximately 135 mg/kg.

The portable device consists of two units, the measuring unit (iCheck) and the disposable reagent vial (iEx) in which the reaction is performed (**fig. 1**). A

FIG. 1. Prototype of the portable device that has been validated as described in this paper. As an indication of size, a 1-cent Euro coin has been placed in the picture

small weighing scale (TS-200, G&G, Neuss, Germany) and syringes to inject the salt solution, as well as the solution A (starch solution), are provided by the manufacturer. Additional materials that are not supplied but are required are distilled water and closable plastic flasks to weigh the salt and water for dissolution. Ideally, distilled water is used to avoid any risk of interference with the measurement. If distilled water is not available, the water to be used is to be tested by blank measurements.

Both the measuring unit and the reagent vials have been developed by BioAnalyt (www.bioanalyt.com). Currently, the shelf life of the vials at 25°C has been successfully verified for 12 months. For quality control purposes, the device conducts an auto-control to verify that the emitter and receptor are working correctly. An iodine calibrator or standard to control the device is not needed. During the production process, the measuring unit is calibrated, and good quality control procedures are used at all stages. Because the measuring unit uses a stable light source (LED), user calibration is not required for the anticipated shelf life of the portable device. Based on the half life of the light source of 30,000 hours, approximately 10 million measurements can be conducted. The device will be shipped along with the LED intensity information at the time of production, so the user can manually compare a potential deterioration of the emitter.

Sample preparation and procedure

The reagent vials and measuring unit were stored at room temperature (20° to 30°C) prior to analysis. Salt samples to be analyzed were carefully mixed in a closed plastic beaker to increase homogeneity.

The salt sample was dissolved in distilled water in order to achieve an iodine concentration in the range of 1 to 15 mg/L (e.g., into a flask containing 1 g of salt, 4 g of water is added, corresponding to an iodine concentration of 5 to 75 mg/kg). The dilution factor will depend on the expected iodine content, but the above dilution factor will allow measurement within a range of up to 75 ppm, which will be sufficient for most salt samples. A net weight of at least 1 g of salt must be used to minimize variation, but a larger sample is recommended to reduce variability coming from sampling inhomogeneity, in particular for coarse salt; this subject has previously been investigated, and the current recommendations are to use at least 10 g of salt for the analysis [4]. Subsequently, the iEx vials were prepared for usage by injecting 200 μL of solution A in each vial. The addition of solution A to the vials was done with all vials that were used in the same test run, meaning for analyses performed within 2 hours. Then, 1 mL of the salt solution was injected into the iEx vial and mixed and left to stand for 5 minutes. The iodine concentration was measured with the photometer iCheck. The syringe was cleaned twice with distilled water between samples.

Reference method

Method

The reference method, iodometric titration, was conducted according to the Association of Analytical Chemists (AOAC) description [9], with minor modifications. In brief, 30 mL of the solution containing the salt sample (previously used for the portable device) was mixed with 1 mL of 5% potassium iodide solution and 1 mL of 1 M sulfuric acid (H_2SO_4), and transferred into a beaker. The reaction mixture was kept for 5 minutes to reach the optimal reaction time and then titrated with 0.01 M sodium thiosulfate solution, using starch as an external indicator, until the solution turned colorless. From the volume of the used titrant, the iodate concentration was calculated against a series of standards.

Method validation of the portable device

Both the portable device and the titrimetric method use saline solutions as the measurement medium, and thus one obtains a concentration in milligrams per liter that will need to be converted into milligrams per kilogram. For the sake of simplicity, the unit used in this manuscript is milligrams per kilogram, with a few exceptions, meaning that the concentration has been converted by multiplying the result in milligrams per liter by the dilution factor.

In the following section, we present the validation steps conducted to assess the performance of the portable device. The Results section below follows this same structure:

Linearity of the portable device was determined by measuring in duplicate six standard solutions (potassium iodate in 20% sodium chloride) with iodate concentrations of 0, 3.0, 6.0, 9.0, 12.0, and 15.0 mg/L iodate; when a dilution rate of 1 to 4 is maintained, this corresponds to a salt iodine content of 0 to 75 mg/kg.

Limits of detection and determination: unfortified salt was dissolved in purified water, and the solution was measured 10 times. Limit of detection: mean +3 SD of the measurements; limit of determination: mean +10 SD of the measurements [10].

Intra-assay imprecision was assessed by preparing solutions of three salt samples of varying concentration (16.6, 30.0, and 55.0 mg/kg) and measuring them in 10 replicates; the coefficient of variation (CV) for each level was calculated.

Interassay imprecision was determined by one technician conducting five analyses of each of three salt samples of varying concentration (16.6, 30.0, and 55.0 mg/kg) over 3 days; the CV for each level was calculated.

Interoperator imprecision: three technicians measured the solutions of three salt samples of varying concentrations (16.6, 30.0, and 55.0 mg/kg) in five replicates on the same day; the CV for each level was

calculated.

Method recovery: seven standard solutions (potassium iodate in 20% sodium chloride) with iodate concentrations of 5, 10, 20, 30, 40, 50, and 100 mg/kg iodine were measured and compared with the expected concentration (expected/observed * 100).

Comparison of the portable device with the reference method: the concentration of iodate was measured in 56 salt samples from different countries of origin with the portable device, and the results were compared with those from iodometric titration. For this, a solution of each of the samples was prepared and then analyzed by the two methods, each in duplicate measurements.

Statistical analysis

For the laboratory validation of the method, standard protocols were followed, unless otherwise described. Data processing and statistics were conducted using Microsoft Excel 2007. For the method comparison, besides plotting the two data sets and calculating the Spearman coefficient and the regression equation, the Bland and Altman plot was used [11]. The absolute differences of the values in the samples analyzed by the two methods were calculated.

Limits of agreement (LOA) were calculated using

$$\Delta - 2s = \mathrm{LOA}_{\mathrm{low}}$$

$$\Delta + 2s = \mathrm{LOA}_{\mathrm{high}}$$

where Δ is the mean of the difference between the two methods and s is the standard deviation (SD) of this difference.

Results

Method validation of the portable device

Linearity: The highly linear range of the portable device was determined to be 5 to 75 mg/kg. Over this range, R^2 was 0.999, and the regression equation was

$$y = 0.994x + 0.079.$$

Limits of detection and determination: the limit of detection was 0.3 mg/kg and the limit of determination was 1.0 mg/kg.

Intra-assay imprecision: the CVs of the 10 measurements conducted within 1 day by one technician for the three concentrations (16.6, 30.0, and 55.0 mg/kg) were 0.9%, 0.5%, and 0.7%, respectively.

Interassay imprecision: the CVs for the results from one person measuring the three samples over 3 days were 1.5%, 1.7%, and 2.5%, respectively.

The interoperator imprecision, obtained by calculating the CV from three technicians measuring the three concentrations (16.6, 30.0, and 55.0 mg/kg) of the salt samples in triplicate was 1.2%, 4.9%, and 4.7%, respectively.

The recoveries for 5, 10, 20, 30, 40, 50, and 100 mg/kg iodide were 123%, 120%, 106%, 108%, 104%, 103%, and 97%, respectively. The mean recovery across all concentrations was 109%.

Comparison between the portable device and the reference method: the mean CVs for duplicate analysis of the samples by each method were 2.5% and 4.5% for the portable device and the reference method, respectively. The results of the comparison are presented graphically in **figure 2**. The equation of the correlation is $y = 0.941x + 1.303$, with y being the reference iodometric method. The corresponding Spearman coefficient for the relationship is $R^2 = 0.978$. The limits of agreement are as follows: $\mathrm{LOA}_{\mathrm{low}}$ is –10.5 mg/kg and $\mathrm{LOA}_{\mathrm{high}}$ is 10.0 mg/kg, the mean difference between the methods being – 0.30 mg/kg.

The Bland-Altman plot (**fig. 2**) indicates that there are data points that are outside the 2SD line, and although tests for identifying outliers exist, no such outliers were excluded. One salt sample containing approximately 250 mg/kg was excluded from analysis because this concentration is outside the measuring range and would render graphic presentation less readable.

Discussion

Precise measurement of salt iodine concentration at the production site, throughout the distribution chain, at national borders, and at the households of consumers is crucial for monitoring program progress. Although large salt producers and national laboratories usually have the capacity to measure salt iodine levels quantitatively using titration, this may not be the case for small producers or regulatory institutions at decentralized levels, at borders, and for surveys collecting household samples. For the latter, a rapid test kit has been widely used in the past, but this test provides only semiquantitative results at best and is recommended to be used for qualitative assessment only [12]. Another method, described in the literature as being accurate and relatively simple, is the WYD iodine checker [7]. Despite the reliability, accuracy, and low cost of this method, it still requires some level of technical skill, since solutions need to prepared and concentrated sulfuric acid needs to be handled. Furthermore, there is a risk of contamination through inappropriate handling of the iodine-containing calibration solution.

To overcome these methodologic challenges, the portable device described here has been developed, and the method has been validated in the laboratory by skilled laboratory technicians on various salt samples, including those of lower quality (coarse, moist, or containing impurities). One limitation of the validation is

that for the instrument validation, fine salt was used. If coarse salt is used, the performance may be lower, but this would be related to problems of handling and inhomogeneity rather than the instrument's performance.

The comparison of the reference method and the portable device has yielded good correlation and agreement, with the portable device measuring slightly (0.3 mg/kg) lower than the reference method. The limits of agreement show that 97.5% of the results by the two methods do not differ by more than 20.5 mg/kg, regardless of the iodine concentration (within the range of 5 to 150 mg/kg); the larger discrepancies are found with higher iodine contents (**fig. 2**).

The linear range of the portable device was determined to be 5 to 75 mg/kg iodine (as iodate), a range that is sufficient for most salt iodization levels globally. Yet, when the proposed dilution ratio is used, linearity remains high up to a salt iodine content of 150 mg/kg. The validation of the instrument showed that the intra-assay imprecision was below 1% and the interassay imprecision was below 2.5%. The inter-operator imprecision was also low, at 4.9% or less.

To date, the portable device has been validated only for potassium iodate and not for potassium iodide or other iodine compounds. However, salt iodization programs increasingly use potassium iodate because it is more stable than potassium iodide. Thus, it is of primary importance to validate the former compound.

The cost per analysis is currently considerably higher than that of the WYD iodine checker or iodometric titration, but in settings that lack infrastructure or technical capacity, the higher cost is outweighed by the simple handling and rapid analysis of the portable device.

Because physicochemical characteristics of salt of different origins can vary considerably with regard to purity, humidity, grain size, and iodine content, we analyzed samples from several countries and found that the device performs well on salts across countries. A limitation may be salts with very high concentrations

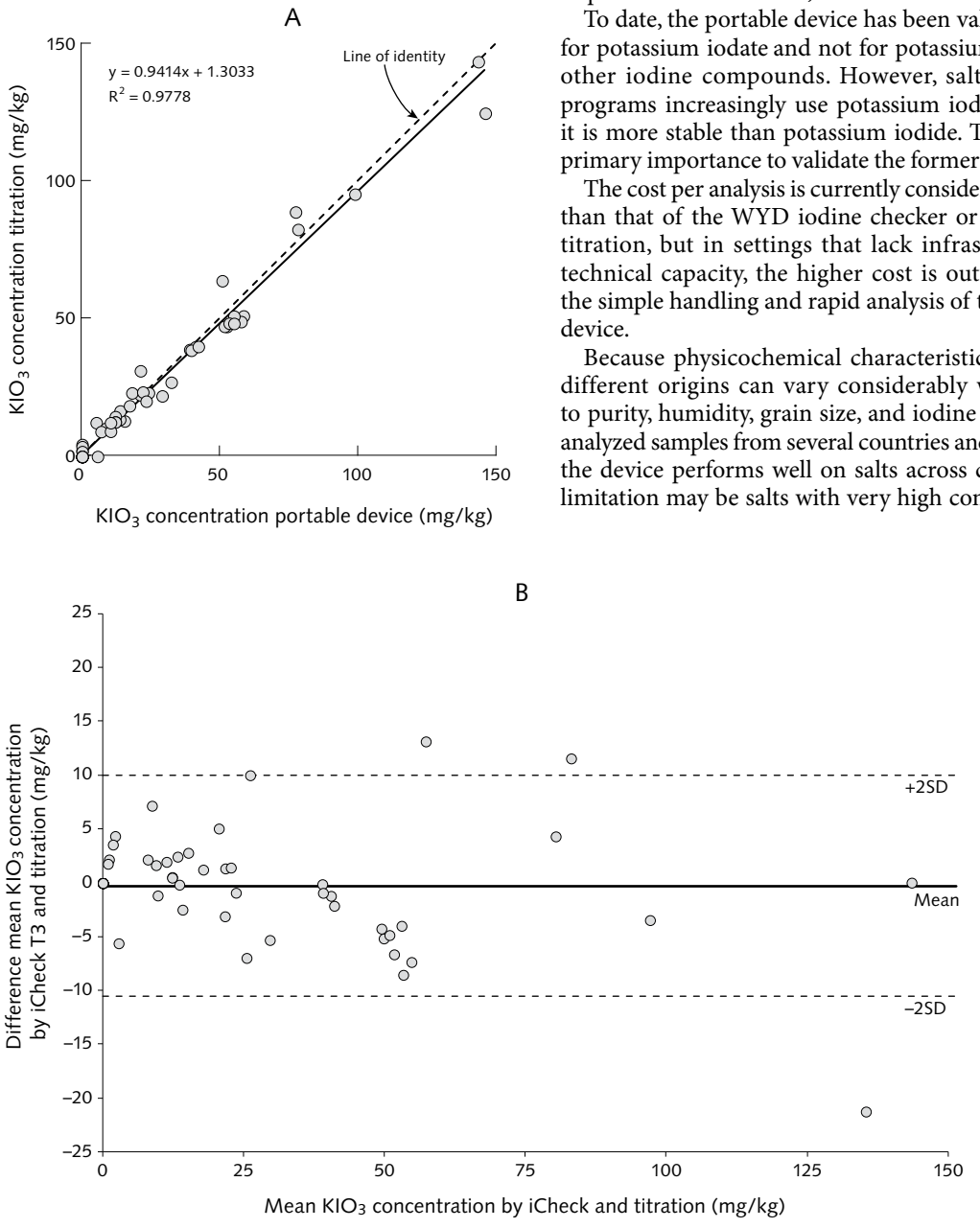

FIG. 2. Regression (A) and Bland and Altman plot (B) of the comparison between the portable device and the reference method

of interfering substances, such as the presence of Fe^{3+} or other strong oxidizing agents (mainly in doubly fortified salt). Further, no systematic assessment of the influence of other interfering substances was conducted, and it may be that alkalinity or other substances not assessed so far influence the performance of the method.

When compared with the WYD iodine checker, the use of hazardous reagents is reduced in volume in this system, and they are no longer hazardous once the measurement is completed (due to dilution of acid). The great advantage of the portable device over the WYD iodine checker is that none of these potentially dangerous liquids needs to be handled openly, as they come in a closed vial as the reaction chamber. Further, the duration of analysis with the portable device is 5 to 6 minutes, including the weighing and dissolution of the salt. If multiple samples are analyzed, the time per analysis will be reduced; the readout time of the device is less than 1 minute.

Conclusions

The portable device presented in this paper and validated by our study offers a viable solution to the challenge of analyzing salt iodine contents simply, reliably, and safely, overcoming the current bottleneck. Further research and field use are warranted to gain more experience on possible drawbacks and to extend the validation to the analysis of potassium iodide as an iodine fortificant.

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References

1. Zimmermann MB, Jooste PL, Pandav CS. Iodine-deficiency disorders. Lancet 2008;372:1251–62.
2. de Benoist B, McLean E, Andersson M, Rogers L. Iodine deficiency in 2007: global progress since 2003. Food Nutr Bull 2008;29:195–202.
3. Horton S, Alderman H, Rivera JA. Copenhagen Consensus 2008 Challenge Paper: Hunger and malnutrition. Available at: http://siteresources.worldbank.org/EXTHDOFFICE/Resources/5485726-1239047988859/5995659-1239051886394/CP_Malnutrition_and_Hunger_-_Horton.pdf. Accessed 18 July 2012.
4. World Health Organization/International Council for the Control of Iodine Deficiency Disorders/UNICEF. Assessment of iodine deficiency disorders and monitoring their elimination: a guide for programme managers. Geneva: WHO, 2007.
5. Andersson M, Karumbunathan V, Zimmermann MB. Global iodine status in 2011 and trends over the past decade. J Nutr 2012;142:744-50.
6. Eastman CJ, Zimmermann MB. The iodine deficiency disorders. Thyroid Disease Manager, 2009. Available at: http://www.thyroidmanager.org/Chapter20/chapter20.pdf. Accessed 18 July 2012.
7. Dearth-Wesley T, Makhmudov A, Pfeiffer CM, Caldwell K. Fast and reliable salt iodine measurement: evaluation of the WYD Iodine Checker in comparison with iodometric titration. Food Nutr Bull 2004;25:130–6.
8. Guamuch M, Makhumula P, Dary O. Manual of laboratory methods for fortified foods (vitamin A, riboflavin, iron and iodine)—Part I. Arusha, Tanzania; A2Z, 2007.
9. AOAC. Official methods of analysis, Section 33. Gaithersburg, MD, USA. AOAC, 1984.
10. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline. Validation of analytical procedures: Text and methodology Q2(R1). 2005. Available at: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf. Accessed 18 July 2012.
11. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;1:307–10.
12. Pandav CS, Arora NK, Krishnan A, Sankar R, Pandav S, Karmarkar MG. Validation of spot-testing kits to determine iodine content in salt. Bull World Health Organ 2000;78:975–80.