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Validation of the use of spectrophotometer (WYD iodine checker) for the determination of iodine in food salt

Carmelle Mizehoun-Adissoda[1,2,3], Victoire Agueh[4], Achille Yemoa[5*], Boris Segla[1], Florence Alihonou[4], Roger Gérard Jossè[6], Dismand Houinato[2,7], Jean-Claude Desport[2,3] and André Bigot[1]

[1]Faculté des Sciences de Santé de Cotonou, Université d'Abomey Calavi, FSS/UAC, 01 BP 188 Cotonou, Bénin.
[2]INSERM, UMR_S 1094, Tropical Neuroepidemiology, Institute of Neuroepidemiology and Tropical Neurology, CNRS FR 3503 GEIST, Limoges, France.
[3]CHU Limoges, Unit of Nutrition, Limoges, France.
[4]Department of Health Promotion, Regional Institute of Public Health, 01 BP 918 Ouidah, Benin.
[5]Laboratoire de Chimie Analytique et Analyse des Médicaments (LCAM), Faculté des Sciences de Santé de Cotonou, Université d'Abomey Calavi, 01 BP 494 Cotonou, Bénin.
[6]Laboratory of Physicochemical Analysis of Aquatic Environments (LAPMIA/FAST/UAC), BP 526 Cotonou, Benin.
[7]Laboratory of Non-Communicable and Neurologic Diseases Epidemiology (LEMACEN), Faculty of Health Science, University of Abomey-Calavi, Cotonou, Benin.

The iodine content in food salt is generally determined qualitatively using rapid test kits or quantitatively by iodometric titration (reference method). Spectrophotometric analysis is one of the recent developed quantitative methods, which has the advantage of being simple, robust and more convenient for the laboratory technician. However, there are few comparative studies between this method and the reference method. The aim of this study was to evaluate the agreement between spectrophotometric and iodometric titration methods. From May to October 2013, 117 salt samples were collected in the households of Glazoué's town (Benin), through a three-stage sampling. Samples were assayed by iodometric titration and by a portable spectrophotometer (WYD Iodine Checker). The agreement between results of the two methods was performed using Bland-Altman plots. The mean levels of iodine in salt samples were 28.2±14.0 and 28.4±14.0 ppm by iodometric titration and spectrophotometry respectively. There is an excellent correlation between the results of both methods (r= 0.97, p< 0.001). The agreement between the two methods gave a mean difference of d= 0.2 ppm, within the limits: d ± 2 sd= -6.2 and 6.5 ppm. This study showed that the spectrophotometric method can replace the iodometric titration for iodine analysis in dietary salt. This method is more convenient, uses simple laboratory procedures and can be popularized.

Key words: Spectrophotometry, Iodometric titration, food salt, Iodine.

INTRODUCTION

The universal iodization of dietary salt is the least expensive and most effective strategy to prevent and control iodine deficiency disorders (Farebrother et al., 2015). The World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) recommend an iodine content of cooking salt between 15 and 40 ppm (WHO, 2007). In 2006, the majority of countries had a salt iodization program (UNICEF, 2008). However, each country reserves the right to define its regulations on the levels of iodine during import, production and distribution. The evaluation of effectiveness of these programs is based among other reasons, on the iodine determination of salt throughout the distribution chain and at the household level (Jooste and Strydom, 2010).

Several methods allow the assay of iodine in the food salt. Iodine can be qualitatively determined using rapid test kits to monitor its presence in adequate quantities or not in salt. Quantitatively, iodine content is determined by iodometric titration, the most common and most precise method. However this method takes time for laboratory technicians normally spending an average of 20 min per one salt sample (Jooste and Strydom, 2010; Khazan et al., 2013; Zahidi et al., 2016).

On the other hand, technological advances have allowed the development of other quantitative methods for the determination of iodine in food salts. There are for example the use of portable spectrophotometer and potentiometric methods (WHO, 2007; Rohner et al., 2015; Yadav et al., 2015). Dearth-Wesley et al. (2004) showed in an american study that iodine assay in salt using the spectrophotometer (WYD Iodine checker) gave similar results to those of iodometric titration, and it was a precise tool, sensitive, affordable cost, easy to use and based on a simple methodology and laboratory procedures (Dearth-Wesley et al., 2004). However, there are few number of comparison studies between the spectrophotometric method and iodometric titration.

The objective of this study was to evaluate the agreement between the results obtained by spectrophotometric assay and by iodometric titration during the determination of iodine content of salt samples from Glazoué town in Benin.

MATERIALS AND METHODS

Type of study and sample

This was cross-sectional and analytical study. From May to October 2013, 117 salt samples were collected from households in Glazoué, a monitored area for endemic goitre since 1994 (Ministère de l'Agriculture de l'Elevage et de la Pêche (Bénin), 2011), by a three-stage sampling (Ardilly, 2006). The salt used in household food might be from local production or imported salt and was purchased in the surrounding markets. The size of sample was obtained by Schwartz formula (Sullivan et al., 1995). A sample of 50 g of salt was taken in selected households and was tightly stored in closed containers for analysis. The sample was systematically replaced by 1 kg of iodized salt offered to the household.

Sample analysis

Assays were conducted at the Laboratory of Biochemistry of the Faculty of Health Sciences (University of Abomey-Calavi; Benin).

Iodine content by iodometric titration

The reagents used were sodium thiosulfate solution 0.005 N ($Na_2S_2O_3$: Scharlau, Spain); concentrated sulfuric acid solution 2 N (H_2SO_4: Sigma Aldrich, Germany); potassium iodide solution at 10% (KI: Scharlau, Spain); soluble starch (Scharlau, Spain) and sodium chloride (NaCl: Analar, England). 10.0 g of salt sample was dissolved in 100.0 mL of distilled water. 1.5 mL of sulfuric acid and 5.0 mL of potassium iodide were added to the solution, and the mixture was stirred; the solution color turning to yellow was observed. After 10 min in dark storage, the mixture was titrated with sodium thiosulfate solution until yellow coloration was obtained. 2.0 mL of starch paste was added again and the color changed to purplish blue. Titration was continued until the disappearance of the blue purple color (Sullivan et al., 1995; Nepal et al., 2013). This assay was repeated five times for each sample, and for the control sample of salt (iodized salt sample at 40 ppm, bought from the Directorate of Food and Applied Nutrition of Benin). The iodine content of salt is determined by the following formula expressed in mg Kg^{-1} or ppm:

$$Iodine\ mg/kg = \frac{\text{Titration volume (mL)} \times (21.15) \times \text{normality of sodium thiosulfate} \times 1000}{\text{Salt sample weight(g)}}$$

Table 1. Iodine content of different food salts sampled at Glazoué (Benin) analyzed by iodometric titration and spectrophotometric methods.

Iodine content (ppm)	Spectrophotometric method			Iodometric titration		
	n	Percentage	Mean±sd	n	Percentage	Mean±sd
0	0	0	-	3	2.56	-
<15	18	15.38	-	17	14.53	-
15-40	80	68.38	-	74	63.25	-
>40	19	16.24	-	23	19.66	-
Total	117	100	-	117	100	-
Mean	-	-	28.42±14.02	-	-	28.23±14.0

The results consist of determination of iodine content of each sample by two different methods and the determination of the mean of the content of all 117 samples. Specifications are set to the following scale: adequate content: 15-40 ppm; inadequate content or out-of-specifications (OOS): <15 ppm and >40 ppm;

Table 2. Comparison of results as per the adequacy level between the two methods.

Spectrophotometric method	Titration method		
	Inadequate values (n)	Adequate values (n)	Global
Inadequate values (n)	66	1	67
Adequate values (n)	7	73	80
Global	73	74	147

Iodine content by spectrophotometry

A portable spectrophotometer WYD Iodine Checker (Salt Research Institute of China, National Salt Industry Corporation) was used. Assays were performed following the instructions in the user manual (Salt Research Institute) . The reagents used were: sodium chloride NaCl, sodium carbonate (Na_2CO_3: Scharlau, Spain); potassium iodate (KIO_3: Scharlau, Spain); Solution A composed of: soluble starch, potassium iodide (KI: Scharlau, Spain); monobasic potassium phosphate $K_2HPO_4.3H_2O$), tetra borate disodium ($Na_2B_4O_7.10H_2O$: UCB, Belgium); solution B composed of sulfuric acid (H_2SO_4: Sigma (Aldrich, Germany). The iodine concentration of the control solution was 15 ppm. 1.0g of iodized salt well mixed was introduced into a 50 mL tube containing (2 mL of solution A, 2 mL of solution B and distilled water). Reading was done at the wavelength of 585 nm. The iodine content was directly read on the screen of the spectrophotometer in ppm.

Statistical analysis

Data were entered in Epi data 3.1 (Epi Data Association, Odense, Denmark), and were analyzed using Stat-view 5.0 software (SAS Institute, Cary, USA). Quantitative variables were expressed as mean ± standard deviation. Qualitative variables were expressed in frequency. Correlation between iodometric titration and spectrometric results was assessed via the correlation coefficient. The agreement between these two methods was assessed by Bland-Altman plots. Bland-Altman plot was performed in Medcalc 14.8 (Acacialan 22-B8400 Ostend Belgium, 2014). The limits of

agreements were defined as: (1) d= mean difference between titration and spectrophotometric results, (2) sd: standard deviation which, when multiplied by 1.96 gives a confidence interval within 95% of the limits of validity. The significance threshold used was 5% for all the statistical analysis.

RESULTS

Iodine content in food salt

The average iodine content in different samples of food salt by iodometric titration was 28.2±14.0 ppm (0 to 74.0 ppm) versus 28.4±14.0 ppm (0.5 to 74.0 ppm) by the spectrophotometric method. 63.2% versus 68.3% of households had adequately iodized salt (15 to 40 ppm) according to WHO recommendations (WHO, 2007) (Table 1).

Comparison of iodometric and spectrophotometric methods

Comparison of results as per the adequacy level between titrimetric and spectrophotometric methods

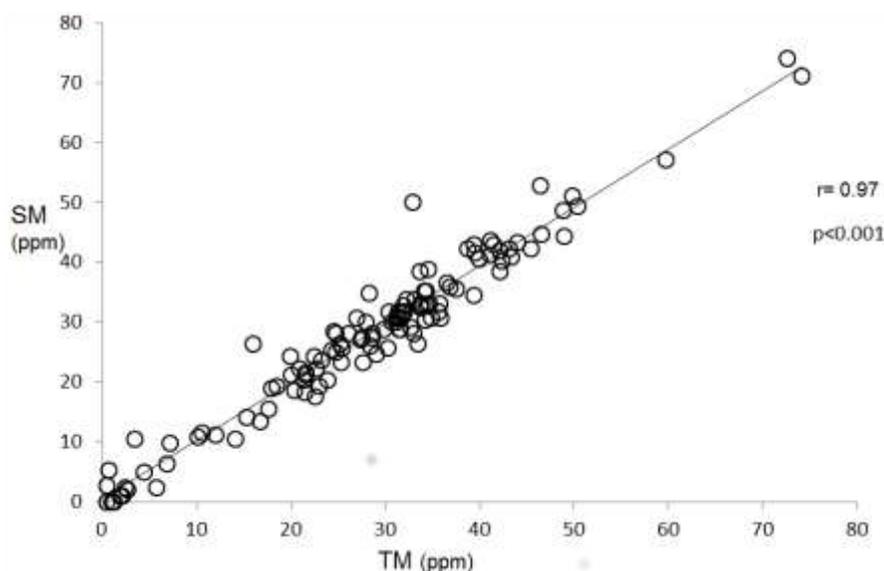Results are summarized in Table 2. The following scale:

Figure 1. Correlation between spectrophotometry and titration method.
SM: values obtained by spectrophotometric method; TM: values obtained by titration method.

adequate content: 15 to 40 ppm; inadequate content: < 15 ppm and > 40 ppm; was able to detect a sensitivity of 90.4% and a specificity of 98.6%.

Correlation between spectrophotometric and iodometric titration measures

There was a positive correlation between the two methods (r= 0.97, p< 0.0001) (Figure 1).

Agreement between spectrophotometric and iodometric titration measures

The concordance results between spectrophotometric and titration measurements by Bland-Altman plots were: d= 0.2 ppm, d±2 sdd= - 6.2 and 6.5 ppm, with a concordance coefficient: Intra-class correlation coefficient (ICC)= 0.9 (Figure 2). The trend graph derived from the Bland-Altman plots coincides with the horizontal axis at 0 ppm.

DISCUSSION

In this study, we compared two quantitative methods of assessing iodine content in edible salt: iodometric titration which is the reference method, the most common and

spectrophotometry, a recent analytical method using a single wavelength at 585 nm. The use of spectrophotometer has been recommended as an alternative to iodometric titration by WHO and the expected results of this method should be similar to those of the reference method (WHO, 2007). In order to verify the concordance between the results of these methods, we performed a Bland-Altman plot for the comparison of the two methods (Ancelle, 2011; Bland and Altman, 1986; Bland and Altman, 1995; Fuhrman and Chouaïd, 2004).

The study results showed that the averages iodine contents in salts samples were 28.4±14.0 by spectrophotometry and 28.2±14.0 ppm by iodometric titration. This suggests that the averages given by these two methods are equivalent (p= 0.9131). By spectrophotometry and titration respectively, 68.3% versus 63.2% of the samples had adequate iodine content and 0% versus 2.5% were below the low limit of quantitation (LLOQ). This means as spectrophotometry method tends to slightly overestimate the results compared to the reference method, or spectrophotometry is a more sensitive method to low levels of iodine present in salt samples. It can also be explained by the fact that spectrophotometry requires only a small amount of salt (1 g) for analysis while by titration; a sample of 10g is necessary.

The concordance results showed very good agreement between the two methods with a mean difference at 0 (d= 0.2) and acceptable limits of agreement (d ± 2 sdd= - 6.2
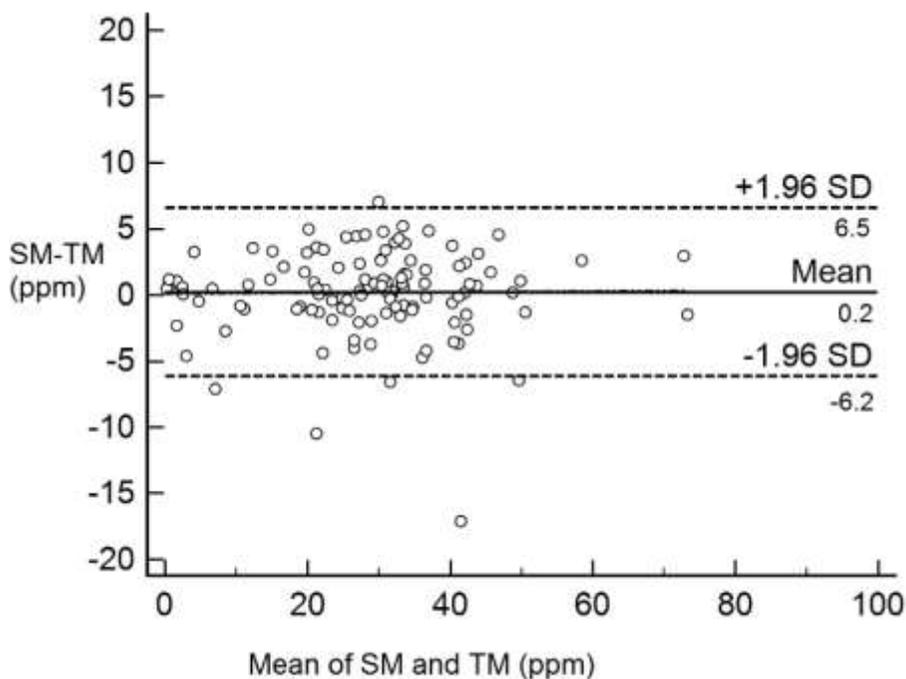
Figure 2. Concordance between spectrophotometric and titration measurements. Mean difference: d = 0.2 ppm; 95%CI: -0.4 to 0.7;SD=3.2 ppm; Lower limit: -6.2; 95% CI: -7.1 to -5.1; Upper Limit: 6.5; 95% CI: 5.5 to 7.5; Intra-class correlation coefficient (ICC) =0.97; 95% CI: 0.96 to 0.99; SM: Spectrophotometric method; TM: Titration method.

and 6.5 ppm) in addition to an excellent correlation (r= 0.97, p< 0.001). This result shows that the spectrophotometer (WYD Iodine Checker) can validly replace the iodometric titration. It can therefore be used for the determination of iodine in salt for the epidemiological monitoring of iodized salt consumption to prevent disorders due to iodine deficiency.

The few study already published on this topic was reported by Dearth-Wesley et al. (2004) who evaluated the concordance between spectrophotometry (WYD Iodine Checker) and iodometric titration results using Bland-Altman plots. They found a mean difference at d= 3.4 ppm, with agreements limits: d ± 2sdd= - 20.5 and 27.4 ppm with an excellent correlation (r= 0.92). These authors concluded that the spectrophotometry method was an alternative to titration and encouraged further studies to confirm the use of this portable spectrophotometer.

The mean difference at 3.4 ppm is a satisfactory result and suggests that this method is good for salt epidemiological studies. However, the agreement limits of Bland-Altman found in their study seem hightous for this method to be used in assaying an isolated sample salt. These limits much higher than those found in our study,

may be due to a smaller sample size (n= 47). Further studies need to be conducted with larger samples size.

Conclusion

This concordance study showed that spectrophotometric assay can validly replace iodometric titration method in epidemiological studies to assess iodine content in salt. However, the results may be slightly biased if this is an isolated sample of salt. The use of spectrophotometer (WYD Iodine Checker) is convenient and less burdensome for the laboratory technician.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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