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Problems in Urinary Iodine Determination Methods and an Automated Kinetic Assay As a Solution

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Abstract: Accurate, fast and economical urinary iodine measurement is very important in diagnosing iodine deficiency disorders. The urinary iodine determination method using ammonium persulfate digestion based on the Sandell-Kolthoff reaction was optimized and modified to kinetic and automated assay.

Ammonium persulfate digestion was performed at +95 ºC in a water-bath to ± 0.1 ºC precision. The performance of both the Sandell-Kolthoff reaction at 37 ºC and its kinetic measurement at 340 nm was tested in a random access automated analyzer. For method comparison, urinary iodine concentrations were measured using both the conventional chloric acid digestion method and the kinetic automated method in 66 randomly selected apparently healthy peoples' urine samples and five working iodine calibrators.

The method agreed well with the conventional chloric acid digestion method (n = 66; r = 0.937 ;y = 0.895x + 0.149; Sy/x = 0.136). The detection limit of assay was 0.10 µmol/L. The mean recovery of iodine was 97% (87-107%). The intra- and interassay CVs for samples with iodine concentrations between 0.20 and 3.14 µmol/L were ≤ 10%.

Our study suggested that urinary iodine should be determined by kinetic reading at 340 nm wavelength in an automated analyzer instead of by manual endpoint measurement at 410 nm.The kinetic procedure presented here therefore offers an easier,faster,more accurate,and more economical method.

Key Words: Iodine, ammonium persulfate digestion, photometric assay, urine, automation

Introduction

The determination of urinary iodine is the most commonly used tool to monitor dietary iodine intake. However, urinary iodine determination assays involve many difficulties and manual steps. There is no automated urinary iodine measurement method for screening wide populations. Most of the popular procedures for urinary iodine determination are based on the Sandell-Kolthoff reaction (1). To eliminate interfering substances, various digestion procedures were performed. Chloric acid digestion was reported to be highly effective among these techniques (2). However, the acid digestion method uses a strong and irritating acid and produces toxic gases that must be eliminated by a specially designed fume-hood. Recently, an ammonium persulfate digestion method was developed (3), which seems harmless and economical but is manual. The assay procedure was also designed with an endpoint reading at 410 nm, which is far beyond the absorbance peak and likely to have inherent difficulty in accuracy and precision.

In iodine determination, these problematic steps negatively impact on the wide use of methods in clinical laboratories. For this reason, we aimed (i) to obtain a more efficient digestion step, (ii) to make a more precise and accurate reading step after digestion and (iii) to automate the assay in order to avoid errors originating from manual handling.

Materials and Methods

Urinary iodine was measured by both automated kinetic assay and conventional chloric acid digestion methods in 66 randomly selected apparently healthy peoples' spot urine samples. Urine samples were placed into clean urine cups and kept in frozen at –70 °C until processing. Digestions were performed in a ±0.1 °C sensitive water-bath (Heto HMT 200, Denmark 1998) or a heating block (Stuart Scientific, UK). A Beckman-Coulter Synchron LX-20 (Beckman-Coulter, USA) analyzer was used for automated photometric assays. Spectrophotometric analyses were

performed in a scanning spectrophotometer (Philips UV/VIS spectrophotometer, UK).

Ammonium persulfate, perchloric acid and sulfuric acid were obtained from Carlo Erba, arsenic trioxide and sodium chloride were obtained from Merck and potassium iodate was obtained from Sigma Chemical Co. USA.

Reagents:

Sulfuric acid (2.5 M) was prepared in an ice bath by carefully adding 280 mL of concentrated sulfuric acid to 1000 mL of bidistilled water and diluting to 2000 mL.

Ceric ammonium sulfate (0.0158 mol/L) was prepared in 1.25 mol/L sulfuric acid.

Ammonium persulfate (1 mol/L) was prepared by dissolving 228.2 g of ammonium persulfate in 700 mL of water and bringing it up to a volume of 1000 mL.

Arsenious acid (0.0253 mol/L) was prepared in a 3000 mL flask by heating on a hot plate a mixture of 5 g of arsenic trioxide, 25 g of sodium chloride and 200 mL of 2.5 mol/L sulfuric acid until dissolved. After cooling, the mixture was diluted to 1000 mL with water.

The stock iodine calibrator (A) was prepared by dissolving 168.6 mg of potassium iodate in 100 mL of water, resulting in an iodine concentration of 7.87 mmol/L. For stock B, 1 mL of stock A was diluted in 100 mL of water with an iodine concentration of 78.74 µmol/L. Five working calibrators were prepared by diluting 1 mL, 2 mL, 3 mL, 4 mL and 5 mL of stock B calibrator with water to 100 mL.

Conventional chloric digestion was performed according to Zak et al. (2)

Firstly, we evaluated the digestion step at +95 °C for 30 mins with ammonium persulfate by comparing the heating block and water-bath. The temperature differences as measured in each well of the heating block (setted at +95 °C) by a thermometer were about 5-6 ºC.

Then we evaluated Sandell-Kolthoff reaction's kinetics in a scanning spectrophotometer. The peak absorbance of reaction was at the 317 nm (Figure 1) wavelength and the reaction had a kinetic pattern (ΔAbsorbance/min = -0.62). Therefore we changed the reading parameters to a rate measuring at 340 nm instead of an endpoint reading of 410 nm.

The assay was adapted to a Beckman-Coulter Synchron LX-20 automated analyzer by using new

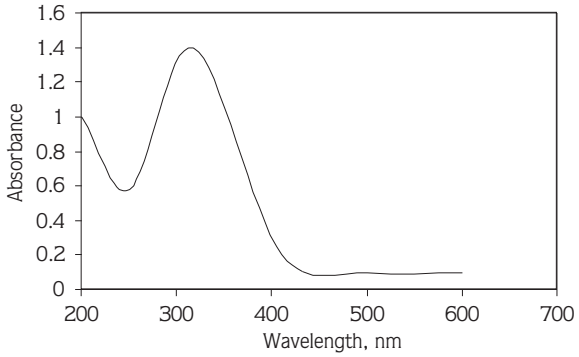

Figure 1. Absorbance spectrum of the endproduct of the Sandell-Kolthoff reaction. Maximum absorbance was observed at 317 nm.

parameters (Table 1). We also changed the final concentrations of reagents in the reaction mixture (8 µL of ceric ammonium sulfate , 200 µL of a mixture of 0.0253 mol/L arsenious acid + 1.25 mol/L sulfuric acid + bidistilled water and 40 µL of sample) by a carrying out a large number of trials to find optimum concentrations. The device calculated results in linear mathematical model with no need for a multipoint calibration curve. Reagents' performances were checked by measuring standards on consecutive days, and calibration was found to be stable for weeks.

For method comparison, linear regression and Pearson's correlation coefficients were calculated with SPSS software (Ver. 10.0) on a personal computer.

Results

The iodine recoveries for different iodine concentrations (0.39-3.14 µmol/L) in urines were 75-130% for the heating block and 85-107% for the water-bath.

The recovery of iodine (Table 2) was estimated to be between 87 and 107% (mean recovery was 97%). The assay was linear for urinary iodine concentrations from 0.20 up to 3.14 µmol/L. Intra- and interassay variability at different urine concentrations are shown in Table 3. These samples were assayed at the low, middle, and upper end of the calibration curve. Each of the samples was assayed 10 times on the same day and seven times on different days.

The detection limit of urinary iodine was 0.10 µmol/L. The assay was linear for urinary iodine concentrations from 0.2 up to 3.14 µmol/L .

Table 1. User– defined chemistry parameters of a Beckman Coulter Synchron LX 20 automated analyzer for iodine determination.

Reaction type:	Rate 1			
Calculation factor:	1.000			
Units:	µmol/L			
No. of calibrators:	2			
Precision:	x.xx			
Setpoints	1: 0.000			
	2: 2.340			
Reaction direction:	Negative			
Math model:	Linear			
Primary wavelength:	340			
Secondary wavelength:	700			
Cal time Limit:	336 h			
	First Inject	Second Inject	Third Inject	
Component	A	None	B	
Dispense volume	200 µL		8 µL	
Inject time			180 s	
Sample volume:	40 µL			
Reagent	Blank	Reaction 1	Reaction 2	Usable result range
Start read	120 s	200 s	------	Lower limit: 0.000
End read	150 s	450 s	-----	Upper limit: 10.000

Table 2. Iodine recovery with addition of iodate into urine.

Urine samples	Iodine concentration, µmol/L			
	Water-added[a]	Iodate added[b]	Iodine recovery	Recovery%
1.	0.64 ± 0.02	1.03 ± 0.04	0.39	98
2.	0.53 ± 0.01	0.87 ± 0.02	0.34	87
3.	1.17 ± 0.08	1.44 ± 0.07	0.37	93
4.	0.55 ± 0.03	0.95 ± 0.08	0.40	101
5.	0.69 ± 0.10	1.04 ± 0.10	0.35	88
6.	0.43 ± 0.07	0.85 ± 0.09	0.42	107
7.	0.32 ± 0.04	0.73 ± 0.03	0.41	104
8.	2.03 ± 0.06	2.39 ± 0.01	0.36	91
9.	1.89 ± 0.11	2.27 ± 0.06	0.38	96
10.	1.50 ± 0.09	1.86 ± 0.13	0.36	91

[a] prepared by adding one part of water to nine parts of urine

[b] prepared by adding one part of iodate solution (3.94 µmol/L) to nine parts of urine

The automated kinetic method was compared with the conventional chloric acid digestion method by linear regression analysis. The correlation of methods was positive for 66 urinary iodine determinations ($r = 0.937$; $y = 0.895x + 0.149$; $Sy/x = 0.136$) (Figure 2). We also compared the method with the chloric acid digestion method by using the difference plot (Figure 3) recommended by Bland and Altman (4).

Discussion

Many methods have been employed for measuring urinary iodine, each with various disadvantages. An economical and rapid procedure for urinary iodine determination is required for screening wide populations. We showed the importance of heat stability for optimum digestion. It is better to use a precise water-bath instead of a heating block for digestion.

Table 3. Assay Precision.

	n	Urine I, µmol/L	CV%
Pool intraassay			
1	10	0.44 ± 0.04*	9.1
2	10	1.57± 0.04	2.5
3	10	3.14 ± 0.05	1.6
Pool interassay			
1	7	0.47 ± 0.05	10.6
2	7	1.48 ± 0.08	5.4
3	7	3.26 ± 0.09	2.7

*Mean ± SD

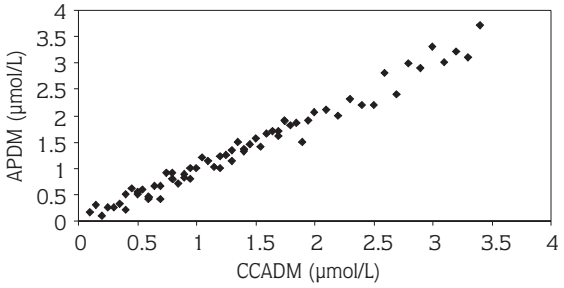

Figure 2. Comparison of the automated ammonium persulfate digestion method (APDM) with the conventional chloric acid digestion method (CCADM) (y = 0.895 x + 0.149; r = 0.937; Sy/x = 0.136; n = 66).

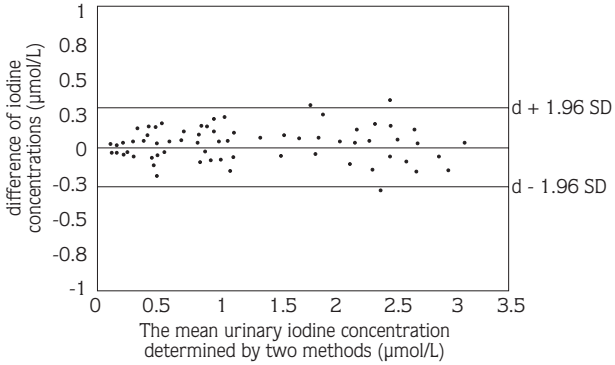

Figure 3. Difference plot for the comparison of the automated ammonium persulfate digestion method with the conventional chloric acid digestion method. (Mean of difference (d) = 0.03; SD = 0.14).

All methods have so far used endpoint readings at 410 nm wavelength. However, when the Sandell-Kolthoff reaction's kinetics were evaluated, it was clearly seen that these measuring parameters are not appropriate for accurate urinary iodine determinations, because the peak is at 317 nm and the reaction has a kinetic pattern.

Another problem that had been unsolved until now in determining urinary iodine is the risk of chemical contamination during procedures. It is neccessary to eliminate contamination in detecting iodine deficient situations. The source of contamination mainly originated from manual procedures. With this automated method, urinary iodine could be measured with few manual procedures. A method involving a microplate containing ammonium persulfate was reported by Ohashi et al. (5) employing an automated microplate reader. It is difficult to find this special device in all laboratories.

The toxic waste production deriving from arsenious acid is also a major problem during iodine determination. It was 100 µL per test, that is, the least toxic waste production among urinary iodine determination methods.

In this study we showed the problems in iodine determination methods and presented an automated kinetic assay at 340 nm with ammonium persulfate digestion in a precise water-bath.

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