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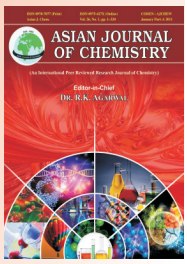

Determination of Micro-Iodate in Iodized Salt by High Performance Liquid Chromatography

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A novel method was developed to determine micro-iodate in iodized salt by high performance liquid chromatography *via* hydrazine hydrate reduction. Iodate was first reduced to iodide using 0.085 % hydrazine hydrate solution followed by HPLC analysis. The sample pretreatment process was simple without any significant interference from the large amount of chloride in the matrix sample during the final analysis. Linearity was validated in the range of 1 to 1000 µmol/L with high correlation coefficient (R^2 = 0.9995) and the limit of detection (LOD) was 0.214 mg/kg (KIO_3 content, S/N = 3). The relative standard deviation was less than 2 % (n = 5) and the spiked recoveries of four real samples were between 98.1 and 100.8 %. The proposed method was applicable for effective routine analysis of micro-iodate in iodized salt.

Keywords: Iodized salt, Iodate, Hydrazine hydrate reduction, HPLC.

INTRODUCTION

Iodine is an essential trace element for human beings and is also required for biosynthesis of the thyroid hormones, thyroxin and triiodothyronine. Iodine deficiency in the human body can not only cause a variety of diseases, such as endemic goiter, deaf-mute, disorders, but also lead to other severe complications during conception like miscarriage, premature birth and fetal congenital malformations, *etc*. As its most hazardous consequence, iodine lack is probable to affect the brain development of young people, leading to mental retardation. On the other hand, excessive intake of iodine can also cause thyroid disorders and finally have a certain hazards to human health[1].

Adding potassium iodate to edible salt is the mainly existing form of human supplementary iodine. Potassium iodate ingested into human body is captured from the blood into the thyroid gland where it is first deoxidized into iodine to be incorporated in the biosynthesis of different thyroid hormones including thyroxine (T4) and triiodothyronine (T3). The thyroid hormones act on nearly every cell in the body. They act to increase the basal metabolic rate, affect protein synthesis and neuronal maturation *etc*. The thyroid hormones are essential to proper development and differentiation of all cells of the human body.

Recently, several methods for determination of iodate were reported. The methods included redox titration using sodium thiosulfate ($Na_2S_2O_3$)[2], spectrophotometry[3], precipitation titrimetry[4], flow injection analysis with amperometric detection[5], ion chromatography[6,7], gas chromatography-mass spectrometry[8], liquid chromatography[1,9]. However, because of the high matrix chloride concentration, most of the mentioned methods suffered disadvantages like either needing a complicated pretreatment steps or the trend cost was so high. For example, the sample in the ion chromatography method[6] was necessarily filtered through two guard Ag cartridges to get rid of the high chloride background interference imposing a complexity as well as an added cost to the proposed approach. Besides, the liquid chromatography method[1] was based on trace iodate in the sample salt solution being deoxidized into iodine (I_2) by ferrous sulfate ($FeSO_4$). The produced iodine was then analyzed using C_{18} column with UV detection. While the solubility and stability of iodine in water are poor, the accuracy of the method might be affected. Again, the sample pre-treatment procedure in the GC-MC method[8] is much more complicated. In the present study, iodate in iodized salt solution has been determined *via* reaction with hydrazine hydrate and the produced iodide was determined by HPLC-UV. The method applies a convenient sample pretreatment procedure in order to effectively remove the interference from the high

chloride background interference in the sample matrix. The method proves to be simple, accurate, easy to handle and applicable for routine analysis of iodate in iodized salt samples.

EXPERIMENTAL

A Dionex (Dionex, Sunnyvale, CA, USA) Model Ultimate-3000 High Performance Liquid Chromatograph equipped with a LPG3400A quaternary pump, a WPS3000TSL autosampler, a TCC-3000 Column Heater and a VWD-3100 Ultraviolet Detector was used. The instruments control and data collection were performed with Chromeleon SR6.8 software (Dionex, USA). The analytical column was Dionex Acclaim Mixed-Mode WAX-1 (120 Å, 250 mm × 4.6 mm × 5 µm). HPLC-grade Acetonitrile (Sigma-Aldrich, USA) and de-ionized water of 18.2 MΩ cm^{-1} achieved by a Millipore water system (Millipore, Mosheim, France) was used throughout. Potassium iodide (KI), potassium phosphate monobasic (KH_2PO_4), sodium pyrophosphate decahydrate ($Na_4P_2O_7{\cdot}10H_2O$), 85 % hydrazine hydrate ($N_2H_4{\cdot}H_2O$), sodium hydroxide (NaOH) and phosphoric acid (H_3PO_4) were of analytical grade and obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Potassium iodate (KIO_3) crystalline was purchased from Institute of Chemical Reagent in Tianjing, China. 0.085 % hydrazine hydrate was prepared by appropriate dilution of 85 % hydrazine hydrate solution. Iodized refined salt, marine alga iodine salt, morton low salt and Healthy Balance salt real samples were purchased from local market.

The standard stock solution (10 mmol/L) of potassium iodate (KIO_3) and potassium iodide (KI) were prepared by accurately dissolving 214 mg of KIO_3 or 166 mg of KI in 100 mL water. Other working solutions of 0.1, 1, 5, 10, 50, 100, 1000 µmol/L of KIO_3 and KI were prepared by appropriate dilution from the stock solutions.

The mobile phase was composed of a mixture of 50 mmol/L potassium phosphate monobasic (pH 6) and acetonitrile (volume ratio of 65:35). Flow rate was set at 1 mL/min. Column temperature was 30 °C. Injection volume was 10 µL.

The buffer component of the mobile phase was prepared by dissolving 6.80 g of KH_2PO_4 and 2.50 g of $Na_4P_2O_7{\cdot}10H_2O$ in 1000 mL water and the pH was adjusted to 6 using 1 mol/L NaOH. Finally, the solution was filtered through 0.45 µm membrane filter and ultrasonically degassed for 15 min prior to use.

Methods: Both the standards and sample solutions were firstly deoxidized using 0.085 % hydrazine hydrate according to the following procedure. 2 mL potassium iodate solution (or edible salt solution) followed by 5 mL 0.085 % hydrazine hydrate were transferred to a 20 mL stoppered glass tube, the mixture was then heated for 6 h in an oven at 90 °C. The produced equivalent iodide could be measured according to its UV absorption at 226 nm. The reaction can be represented by the following equation:

$$2IO_3^- + 3NH_2 \xrightarrow[6\ H]{90\ °C} 2I^- + 3N_2 + 6H_2O$$

Procedures for real samples were as follows: iodized refined salt, marine alga iodine salt and Morton low salt samples were prepared as 0.10 g /L and Healthy Balance salt as 0.025 g/mL in deionized water. 5 mL of the salt solution were transferred to a 20 mL stoppered glass tube and the procedure was completed as above metioned.

RESULTS AND DISCUSSION

The concentration of potassium iodate in iodized salt is only about 20-60 mg/kg. Hence, sodium chloride constitutes the main sample matrix. Chloride has no UV absorption, however, large amount of chloride can overload the column giving rise to a serious interference. Under the optimal conditions, the experimental results (Fig. 1) indicated that in the absence of a high background salt concentration, several common inorganic anions (except nitrite and bromide) could be well separated (if gradient elution was applied, seven kinds of anions could be baseline separated). On the other hand, spiking 0.1 g/mL iodized salt samples with similar concentrations of these anions would result in the anions peaks eluted before 10 min (iodate, bromate, nitrate, nitrite and bromide) being masked by the background chloride, but fortunately, those peaks of iodide and thiocyanate eluted after 10 min was not subject to any interference allowing accurate analytical peak interpretation. Possible reason is that the high concentration of chloride in the matrix occupied most of the active site of the column stationary phase resulting in column overload masking most of the earlier peaks (eluted before 10 min) while peaks eluted thereafter does not suffer any interference (Fig. 1). So from the above discussion it can be concluded that the analysis of potassium iodate in iodized salt samples was possible after reduction using hydrazine hydrate according to the proposed approach without interference and with much simpler pretreatment procedure.

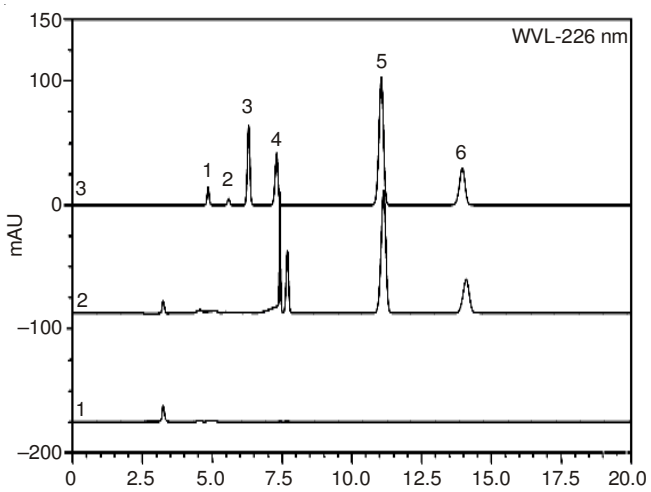

Fig. 1. Chromatograms of 7 common inorganic anions on column WAX-1.1, 0.1 g /mL iodized salt solution as a blank; 2, 0.1 g/mL iodized salt solution spiked with the 7 studied anions; 3, the 7 common anions standards plain peak profile. Peak identified: (1) iodate; (2) bromate; (3) nitrate; (4) nitrite and bromide; (5) iodide; (6) thiocyanate

Column selection: In conventional reversed-phase C_{18} column, iodate and iodide can not be retained. Therefore, a weak anion-exchange column (Acclaim Mixed-Mode WAX-1, 120 Å, 4.6 × 250 mm × 5 µm) was used in this study to

retain iodate and iodide. Since the chromatographic mechanism of the weak anion column (Acclaim Mixed-Mode WAX-1) was based on hybrid separation, three types of separation models could be obtained *via* reversed-phase, weak anion-exchange and hydrophilic interaction (HILIC), *etc*. The experiments showed that I^-, SCN^-, IO_3^-, Br^-, NO_3^- and other common anions could be efficiently retained by this column giving a sharp peak results in a reasonable time. The bonding type of Acclaim Mixed-Mode WAX-1 was represented in Fig. 2.

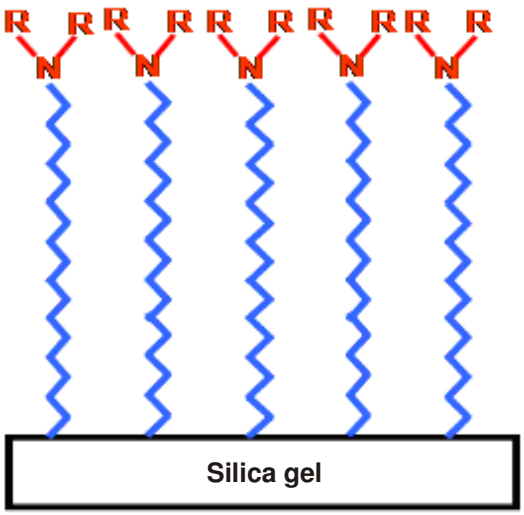

Fig. 2. Bonding type of Acclaim Mixed-Mode WAX-1

Selection of reducing agent: In addition to hydrazine, other reducing reagents were tried including sodium thiosulfate, Sodium bisulfite and bromine, *etc*. However, NH_2-NH_2 was chosen in this study as its oxidation byproduct includes only nitrogen without any other ions being generated decreasing any unnecessary additional burden to the analytical column. However, considering hydrazine hydrate solution to be significantly alkaline, too high concentration should be avoided as it may also affect the column. Finally, a concentration of 0.085 % (17 mmol/ L) was chosen as optimum in this study.

Selection of reduction temperature: To improve the yield of iodate with hydrazine hydrate reaction, production rate effective factor at different reduction temperatures was calculated. The experimental results (Fig. 3) showed that peak area ratio of iodate to iodide decreased gradually as the oven temperature was increased. At 90 °C, the reaction rate was noticed to be almost stable. So 90 °C was used as the optimal reduction temperature in this method.

Reduction time: Reaction time was also found to affect the yield of iodate hydrazine reaction. In order to study this factor, the reaction temperature was set at 90 °C and the peak area ratio of iodate to iodide was calculated at different reaction time. Results indicated (Fig. 4) that the peak area ratio decreased gradually as the reaction time increases being stable when the reaction time was close to 6 h referring to a maximum reaction yield. Therefore, 6 h was chosen as optimal reaction time.

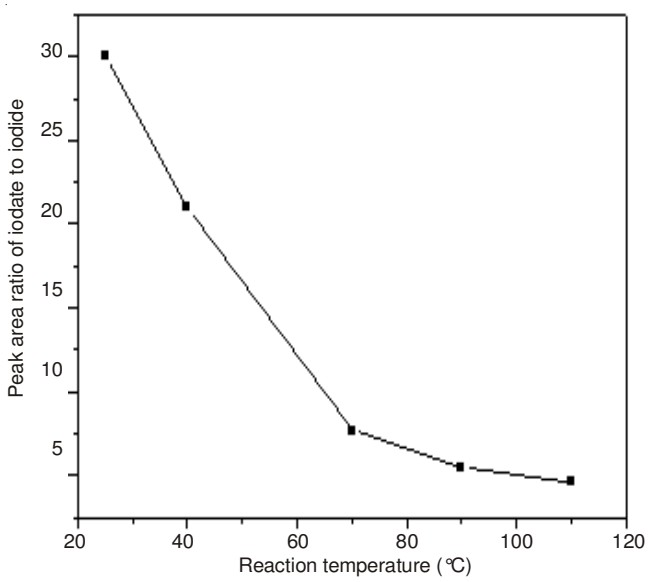

Fig. 3. Effect of temperature on the reduction rate, iodate concentration 100 µmol /L, reaction time 2 h

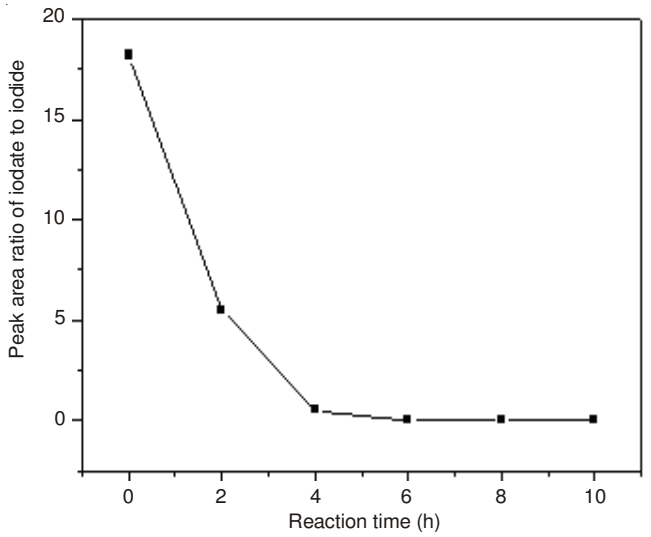

Fig. 4. Effect of reduction rate on different times, iodate concentration 100 µmol L^{-1}, reaction temperature 90 °C

Linearity, detection limit and reproducibility: Under the above optimal conditions, experiments proved a linear relationship for iodate determination in iodized salt samples in the range of 0.1-1000 µmol/L. The results showed good linearity as indicated by high correlation coefficients (R^2 = 0.9995) and a lower detection limit of 0.1 µmol/L (equivalent of 0.214 mg/kg of iodate in iodized salt). The regression equations was Y equal to 0.0593X - 0.0175 with the concentration of iodate standard solution as abscissa (X) and the corresponding peak area of iodide as vertical coordinate (Y).

Real sample analysis: To validate the applicability and accuracy of the proposed method for analysis of real samples, iodate was determined by the proposed method in four different types of iodized salt samples purchased from the local market. Because of Healthy Balance salt samples has low sodium, high calcium levels and low solubility in water, a sample solution of 0.025 g/mL instead of 0.1 g/mL was prepared. The results shown in Table-1 indicated a good reproducibility [RSD

TABLE-1
APPLICATION OF THE PROPOSED METHOD FOR ANALYSIS OF IODATE CONTENT IN IODIZED SALT REAL SAMPLES

Sample	Found (calculated as KIO_3, mg/kg)	RSD (n = 3%)	Add (mg/kg)	Recovery (%)
Iodized refined salt	57.9	1.9	107	100.4
Marine Alga iodine salt	42.6	1.2	107	98.1
Morton low salt	53.5	1.4	107	98.7
Health Balance salt	62.1	0.9	107	100.8

(n = 3) < 2 %], the recoveries were between 98.1-100.8 %. Sample chromatograms were shown in Fig. 5.

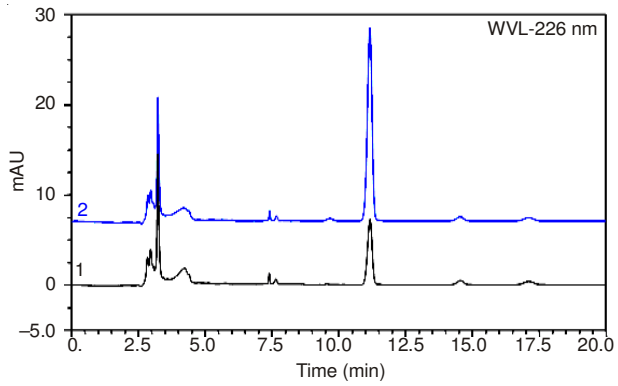

Fig. 5. Chromatograms for real and spiked samples; 1, 0.1g mL^{-1} Morton low salt; 2, 0.1 g mL^{-1} Morton low salt spiked with 107 mg kg^{-1} of potassium iodate

Conclusion

The method proposed in this study for analysis of iodate in iodized salt samples is simple, convenient and easy to apply. These, in addition satisfactory sensitivity (0.1 µmol /L) and reproducibility as well as a simple pretreatment procedure with low cost make the method applicable for regular accurate analysis of real salt samples.

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