DIONEX

Application Note 37

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The Determination of Iodide in Milk Products

INTRODUCTION

Trace levels of iodide are necessary for normal physical and mental development; however, excess iodide can lead to thyroid disorders. Common sources of iodide include iodized table salt and seafood, but other food products also contain iodide. Within the dairy industry, iodophors are used as disinfectants, which can also lead to increased iodide consumption by the public.[1] A concern over high iodide levels in the diet has led to a nutritional labeling requirement for iodide/iodine.

In this Application Note, ion chromatography coupled with pulsed amperometric detection is used to determine iodide in milk products. This method is specific, sensitive, and rapid. Iodide is separated on the IonPac® AS11 column, which contains a hydrophilic, anion-exchange resin that is well suited to the chromatography of the relatively hydrophobic iodide anion. Using a nitric acid eluent, the iodide ion elutes from the column in less than 5 minutes. Although iodide can be detected by direct current (dc) amperometry on a silver working electrode, a pulsed amperometric waveform is used in this Application Note to improve the reproducibility of iodide analysis.[2] Like dc amperometry, the detection limit of iodide using pulsed amperometric detection is in the low µg/L range.

EQUIPMENT

Dionex DX-500 Chromatography system consisting of:
- GP40 Gradient Pump with vacuum degas option
- LC25 or LC30 Liquid Chromatography Module
- ED40 Electrochemical Detector
- EO1 Eluent Organizer
- AS3500 Autosampler

Dionex PeakNet Chromatography Workstation
Whatman 2V Filters, 185 mm (Whatman)
OnGuard®-II-RP Sample Pretreatment Cartridges (Dionex P/N 057083)

REAGENTS AND STANDARDS

Deionized water, 17.8 MΩ-cm resistivity or better
Concentrated nitric acid, ultrapure (J.T. Baker)
Glacial acetic acid (J.T. Baker)
Potassium iodide (Fisher Scientific)

CONDITIONS

Columns:	IonPac AS11 Analytical, 4 x 250 mm (P/N 44076) IonPac AG11 Guard, 4 x 50 mm (P/N 44078)
Expected Operating Pressure:	6.5 MPa (950 psi)
Degas Interval:	10 min
Injection Volume:	50 µL
Injection Loop:	100 µL
Eluent:	50 mM Nitric acid
Flow Rate:	1.5 mL/min
Detection:	Pulsed amperometry, silver working electrode, Ag/AgCl reference

Waveform for the ED40 Detector:

Time (sec)	Potential (V)	Integration
0.00	+0.1	
0.20	+0.1	Begin
0.90	+0.1	End
0.91	–0.8	
0.93	–0.3	
1.00	–0.3	

Collection Rate:	1 Hz
Background:	7–20 nC (typical)
Temperature:	30 °C
Autosampler:	11 min cycle time
Injection Mode:	Pull
Needle Height:	2 mm
Flush Volume:	400 µL

PREPARATION OF SOLUTIONS AND REAGENTS

50 mM Nitric Acid

Add 6.25 mL of concentrated nitric acid to approximately 1000 mL of degassed 17.8 MΩ-cm deionized water in a 2-L volumetric flask. Dilute to the mark with degassed deionized water.

Iodide Standards

Prepare a 1000 mg/L standard by dissolving 1.31 g of potassium iodide in 1000 mL of deionized water. This primary standard was used to prepare a 10 mg/L secondary standard, which was appropriately diluted for linearity studies. Both the primary and secondary standards were stored frozen. Because iodide is light-sensitive, exposure to light should be minimized. All standards prepared from the 10 mg/L stock solution should be used on the day they are prepared.

Electrode Preparation

Polish the silver electrode with the white fine polishing compound. Rinse the electrode well with deionized water and wipe with a damp paper towel. After this initial polish, the electrode should only be polished if it becomes discolored or if it has not been used for a month or longer.

SAMPLE PREPARATION

OnGuard-RP Preparation

Pass 5 mL of methanol, followed by 10 mL of deionized water, through the cartridge at 4 mL/min. To save time, up to 12 cartridges can be prepared at one time using the OnGuard Sample Prep Station (P/N 39599).

Milk Sample Preparation

Prepare the infant formula as suggested for feeding. Prepare the nonfat dried milk as recommended for serving (10 mL of water for every 0.95 g of milk powder).

Pipet 10 mL of milk product into a 100-mL polypropylene beaker. Add 2 mL of 3% acetic acid and mix. Add 8 mL of deionized water and mix. Pass the sample through a Whatman 2V filter. Measure the filtrate volume and pass 5 mL of sample through the OnGuard-RP cartridge at 4 mL/min, discarding the first 3 mL of sample. Collect the remaining filtrate and inject an aliquot into the chromatograph. If the filtrate is cloudy, it should not be used. A cloudy filtrate suggests that a different sample preparation method is necessary.

To determine recovery, add 1 mL of 1 mg/L iodide to the sample prior to the addition of acetic acid and add only 7 mL of water prior to filtration. Calibration standards were prepared by subjecting them to the sample preparation procedure. 10 mL of 0.1 mg/L iodide was prepared in duplicate for each experiment.

RESULTS AND DISCUSSION

Chromatography of Iodide

Figure 1 shows the separation of 1 mg/L iodide on the IonPac AS11 column set using a 50 mM nitric acid eluent. Iodide elutes in less than 4 minutes and is well separated from the void volume. Compared to other ion-exchange columns, the IonPac AS11 contains a very hydrophilic pellicular resin that improves the peak shape of the hydrophobic iodide ion. The nitric acid eluent also improves peak shape.

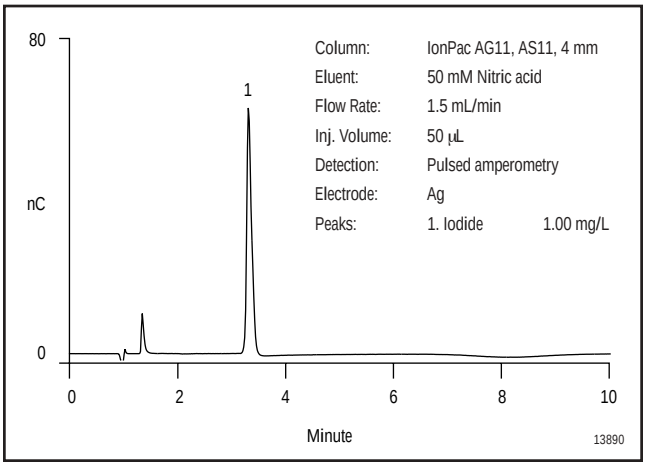

Figure 1 *Determination of iodide by ion chromatography with pulsed amperometric detection*

Fluoride, chloride, bromide, and iodate elute well before iodide. Chloride elutes at approximately 1.5 minutes. The dip in the baseline at approximately 8 minutes is due to dissolved oxygen. This dip is from the previous injection (elution time of approximately 19 minutes) and varies from column to column. An 11-minute injection-to-injection time (autosampler cycle time) places the dip where it does not interfere with iodide

chromatography on either of the two column sets tested. When installing a new column, the dissolved oxygen elution time should be determined to ensure that 11 minutes is an appropriate cycle time. Although the iodide peak elutes earlier using higher eluent concentrations, the separation is subject to interferences from early eluting compounds and consequently is not as reproducible as separations using lower eluent concentrations.

Amperometric detection with a silver working electrode is highly specific for iodide, and does not respond to most matrix components when analyzing milk products by ion chromatography. Potential interferences are therefore largely eliminated. The iodide from the sample combines with the silver of the working electrode surface to form silver iodide precipitate, oxidizing silver in the process. Pulsed amperometric detection allows for detection in the μg/L range and has high specificity for the iodide ion. Other halides are detected in the same manner, but less efficiently.

Because the formation of the AgI precipitate is reversible, a small dip is observed after iodide elution due to the dissolution of the AgI remaining on the electrode and concomitant reduction of silver. This dip is much smaller when using pulsed amperometry rather than dc amperometry. The dip should not be integrated as part of the iodide peak. Most importantly, standards and samples should be integrated in the same manner.

Figure 2 shows that the detection of iodide is linear over the concentration range of 25 to 10,000 μg/L (r^2 = 0.9999). Figure 3 shows a chromatogram of 10 μg/L iodide, which is greater than 10 times the signal to noise. When analyzing lower concentrations, be sure to check a blank injection, because as much as 1 μg/L carryover has been observed. Greater autosampler rinse volumes may reduce carryover. Lower concentrations can also be analyzed by increasing the injection volume.

Separation and detection reproducibilities were determined by repetitive analyses of 1 mg/L and 0.1 mg/L iodide standards. Figure 4 shows every injection, over a 41-hour period, of a 1 mg/L iodide standard (the 8 injection gap was due to an empty vial). The peak area RSD of this analysis was 2.5% and the retention time RSD was 0.5%.

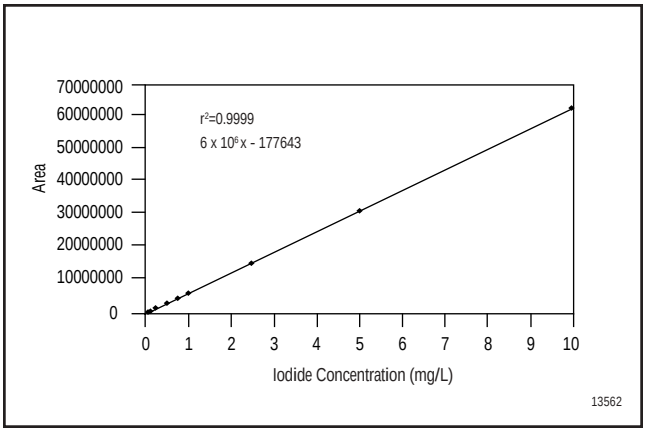

Figure 2 *Iodide linearity: 0.025–10 mg/L*

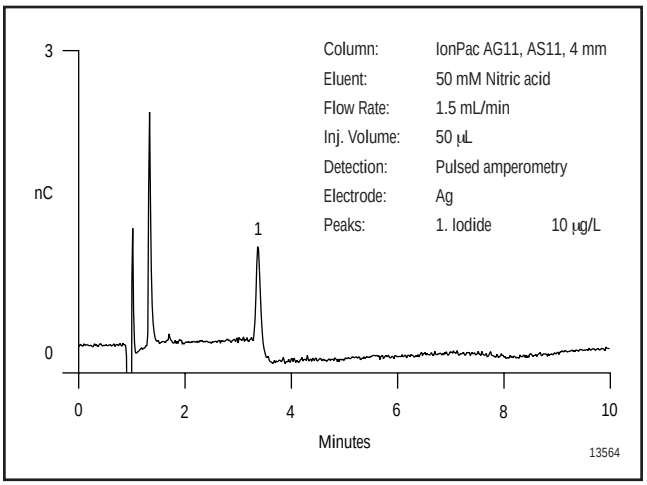

Figure 3 *Low-level determination of iodide by pulsed amperometry*

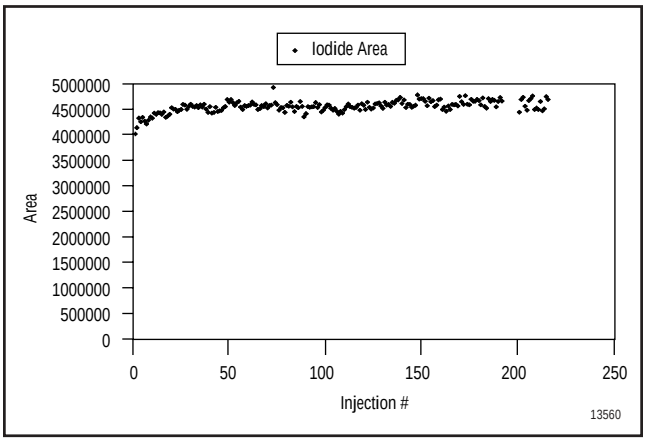

Figure 4 *41-hour reproducibility of iodide analysis by pulsed amperometry*

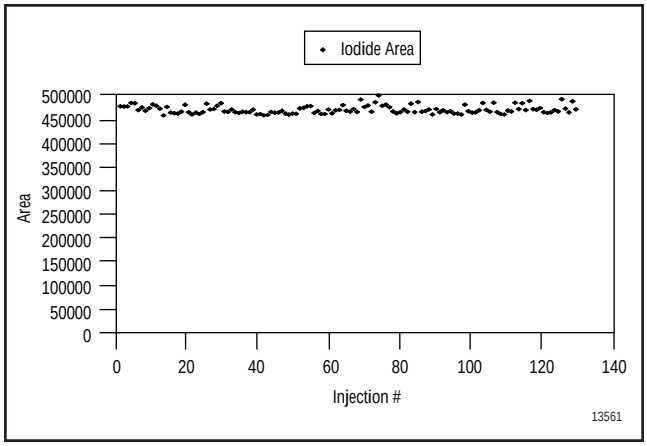

Figure 5 *24-hour reproducibility of iodide analysis, 100 ppb injected*

Figure 5 shows every injection of a 24-hour analysis of a 0.1 mg/L iodide standard. In this experiment the peak area and retention time RSDs were 1.8 and 0.3%, respectively. Temperature control of the electrochemical cell and on-line degassing were critical to obtaining these low peak area RSDs.

Sample Preparation

Sample preparation should involve minimal dilution because the concentration of iodide in milk can be near the method detection limit (i.e., in the low to mid μg/L range). Here, 2 mL of 3% acetic acid is added to 10 mL of sample to precipitate protein, which is then removed by filtration. After filtration, sample volumes range from 11 to 14 mL. The volume of a standard treated in the same manner ranges from 16 to 17 mL. To remove fat, 5 mL of the filtrate is passed through an OnGuard-RP cartridge. Failure to remove fat will lead to greater column backpressure, loss of column capacity, and eventual column failure. The chromatographic method in this Application Note should be applicable to any sample preparation method that yields a clear filtrate from which fat has been removed.

Sample Analysis

Figures 6 and 7 show typical chromatograms of milk samples and a 100 μg/L standard prepared with the sample preparation method described above. Chromatograms A and B in Figure 6 and chromatogram A in Figure 7 show milk (2% milkfat), infant formula, and nonfat dried milk, respectively. The identity of iodide was confirmed by adding 10 μL of 0.1 M silver nitrate to 200 μL of sample and analyzing for the disappearance of the iodide peak.[3] The identity of the peak at 5.2 minutes, present in all milk

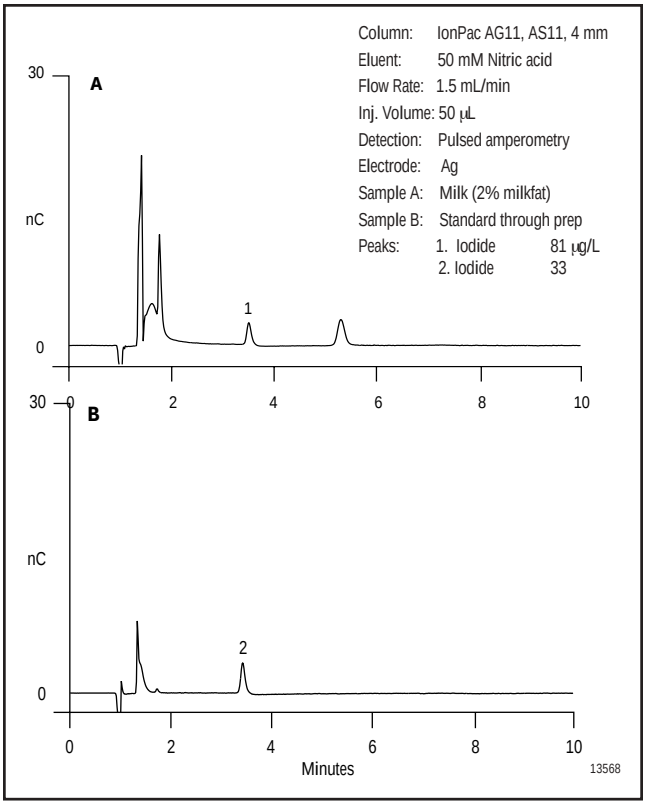

Figure 6 *Analysis of iodide in milk samples by IC with pulsed amperometric detection*

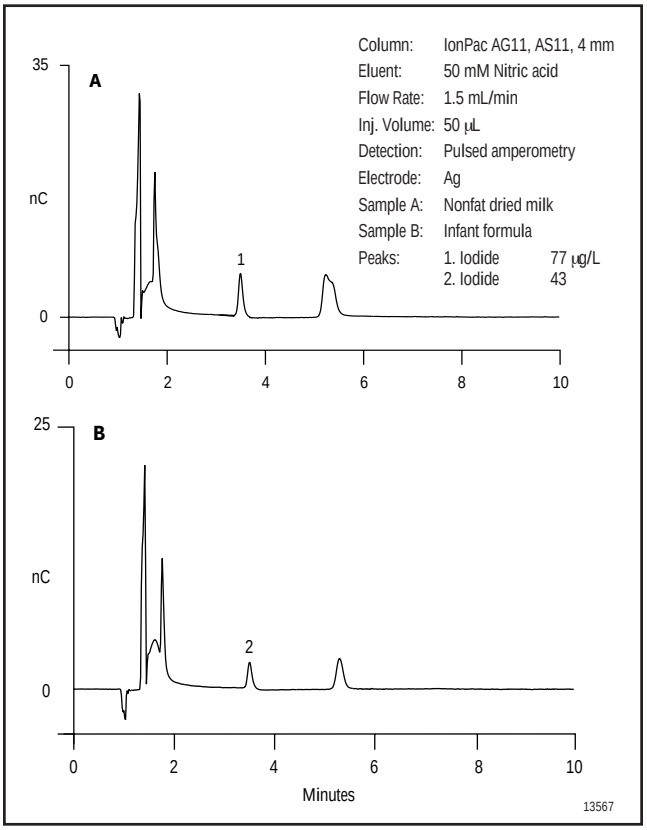

Figure 7 *Analysis of iodide in milk samples by IC with pulsed amperometric detection*

Table 1 Iodide in milk products				
Sample	# of Samples	Conc. (μg/L)	RSD	% Recovery
Milk (2%) #1	2	152	7.9	82
Milk (2%) #2	2	134	1.1	ND
Nonfat Dried Milk	4	154	5.8	81
Infant Formula	6	66	1.3	85

samples analyzed, is unknown. The reported concentrations are relative to an external 100 μg/L standard and have not been adjusted for sample dilution. The concentrations of all analyzed samples were between 10 and 100 μg/L. The iodide concentrations in the milk samples are reported in Table 1. These values were calculated using the average of two 100 μg/L standards prepared in the same manner as the samples and then adjusted for sample dilution.

Recovery was determined by preparing four samples and adding standard to two of the samples prior to sample preparation. Recovery was greater than 80% for all samples. Milk (2% milkfat) numbers 1 and 2 represent two different bottles of milk. The labeled value for the iodide in the powdered infant formula is equal to 61 μg/L.

For each analysis, 8 injections of each sample were analyzed. The area RSD for 8 sample injections was typically under 5%. When 50 μL of 0.1 mg/L iodide was added to 200 μL of a milk (2% milkfat) sample that had been prepared for analysis, and then analyzed, the recovery was 100%. This suggests that after preparation, the matrix does not inhibit iodide detection. Figure 8 shows that the analysis of milk samples (the blank injections) does not alter the detection of the 1 mg/L iodide standard. The iodide peak area and retention time RSDs are 1.4% and 0.4%, respectively.

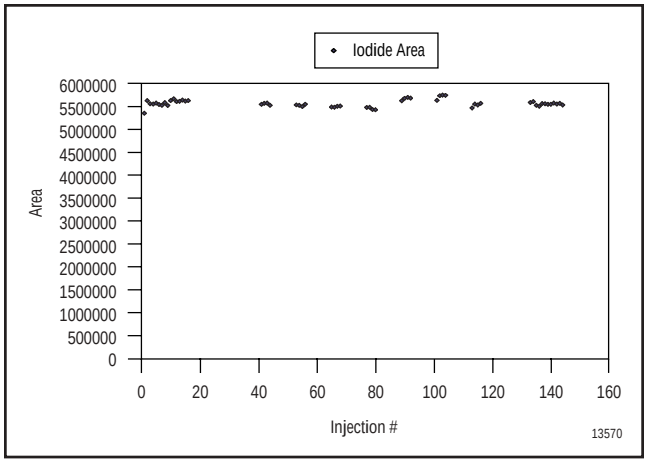

Figure 8 *Reproducibility of an iodide standard (1 mg/L) during sample analysis*

PRECAUTIONS AND RECOMMENDATIONS

The IonPac AS11 column is packed in sodium hydroxide solution, so the column should be flushed with water for at least 30 minutes before equilibrating with the nitric acid eluent. If iodide retention time and peak efficiency start to decrease, the column can be washed with a stronger nitric acid eluent. The AS11 column is stable in the 0–14 pH range, so strong base eluents can also be used for column cleaning. It is best to disconnect the column set from the detector during column cleaning. Changing the inlet column frit or the guard column may be a faster way to restore retention time and efficiency. Installation of a 4-L eluent bottle (P/N 39164) maximizes unattended operation. For best results, the Ag/AgCl reference electrode should be replaced every 6 months.

REFERENCES

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3. Chadha, W.J. and Lawrence, J.F. *J. Chromatogr.* **1990**, *518*, 268–272.

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