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# The Determination of Iodide in Urine

## INTRODUCTION

Measuring the concentration of iodide in urine is useful for the diagnosis of transient thyroid dysfunction and iodine-induced hyperthyrosis. Patients with iodine-induced hyperthyrosis have 10- to 100-fold more urinary iodide than healthy patients.<sup>1</sup>

In this Application Update, ion chromatography coupled with pulsed amperometric detection is used to determine iodide in urine. This method is specific, sensitive, and rapid. Sample preparation involves removal of urine components with molecular weights greater than 10,000 daltons, sample dilution, and treatment with a polymeric, reversed-phase cartridge (OnGuard™-RP).

Iodide, a relatively hydrophobic anion, is separated on the IonPac® AS11 column, which contains a hydrophilic resin that is well suited to the chromatography of iodide. Using a nitric acid eluent, the iodide ion elutes from the column in under 5 minutes. Although iodide can be detected by direct current (dc) amperometry on a silver working electrode, a pulsed amperometric waveform improves reproducibility.<sup>2</sup> The linearity and reproducibility of iodide detection by pulsed amperometry has been demonstrated.<sup>3</sup> Because the detection limit of iodide using pulsed amperometric detection is in the low µg/L range, this technique can be used to determine iodide in urine.

## EQUIPMENT

Dionex DX-500 Chromatography system consisting of:  
GP50 Gradient Pump with vacuum degas option  
LC25 or LC30 Liquid Chromatography Module  
ED40 Electrochemical Detector  
EO1 Eluent Organizer  
AS3500 Autosampler

Dionex PeakNet Chromatography Workstation

## REAGENTS AND STANDARDS

Deionized water, 17.8 MΩ-cm resistivity or better  
Concentrated nitric acid (16 M), ultrapure (J.T. Baker)  
Potassium iodide (Fisher Scientific)

## CONDITIONS

Column: IonPac AS11 analytical, 4 x 250 mm,  
(P/N 44076)  
IonPac AG11 guard, 4 x 50 mm,  
(P/N 44078)

### Expected Operating

Pressure: 950 psi (6.5 MPa)  
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:

<u>Time (sec)</u>	<u>Potential (V)</u>	<u>Integration</u>
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	

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Collection Rate:	1 Hz
Expected Background:	7–20 nC
Temperature:	30 °C
Autosampler Cycle Time:	11 min
Injection Mode:	Pull
Needle Height:	2 mm
Flush Volume:	400 $\mu$ L

## **PREPARATION OF SOLUTIONS AND REAGENTS**

### **Eluent Preparation**

#### *50 mM Nitric Acid*

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

### **Iodide Standards**

Dissolve 1.31 g of potassium iodide in 1000 mL of deionized water to prepare a 1000-mg/L standard. Use this primary standard to prepare a 10-mg/L secondary standard from which working standards can be prepared on the day of analysis. Freeze and store the primary and secondary standards. Because iodide is light-sensitive, minimize exposure to light.

### **Electrode Preparation**

Polish the silver electrode with fine white polishing compound. Rinse the electrode well with deionized water and wipe with a damp paper towel. After this initial polishing, 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. Up to 12 samples at a time can be prepared using the OnGuard Sample Prep Station (P/N 39599).

### **Urine Sample Preparation**

Dilute 1 mL of human male urine from which components with molecular weights greater than 10,000 daltons have been removed (Sigma, P/N U6378) with 4 mL of water. Pass this sample through an OnGuard-RP cartridge

at 4 mL/min, discarding the first 3 mL. Inject this filtrate into the chromatograph. To determine recovery, add 0.5 mL of 1-mg/L iodide to the sample prior to dilution with water (add 3.5 mL of water). Prepare a 5-mL sample of 0.1-mg/L iodide using the above sample preparation procedure to calculate the iodide content of each sample. Prepare this standard in duplicate for each experiment.

### **Analysis of Electroactive Anions**

To determine the retention times of other electroactive anions, analyze 1-mg/L solutions of the sodium salts of sulfite, thiosulfate, cyanate, thiocyanate, and sulfide.<sup>4</sup>

## **RESULTS AND DISCUSSION**

### **Chromatography of Iodide**

Figure 1 shows the separation of 1-mg/L iodide on the AS11 column set using a 50 mM nitric acid eluent. Iodide elutes in under 4 min and is well separated from the void volume. Unlike other anion-exchange columns, the IonPac AS11 column contains a very hydrophilic, pellicular resin that produces improved peak shape for the hydrophobic iodide ion. The choice of a nitric acid eluent also improves peak shape. Chloride elutes at approximately 1.5 min. The dip in the baseline at approximately 8 min is due to dissolved oxygen. This dip is from the previous injection (elution time of approximately 19 min) and varies from column to column. An 11-min injection-to-injection time (autosampler cycle time) was chosen to place the dip where it does not interfere with iodide chromatography on either of the two column sets tested. Determine the dissolved oxygen elution time to ensure that 11 min 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.

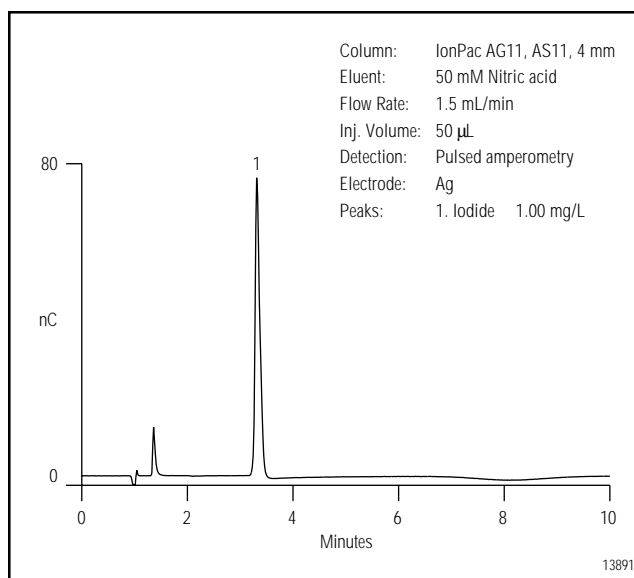
Iodide is detected using an amperometric detector with a silver working electrode. The iodide from the sample combines with the silver of the working electrode surface to form silver iodide precipitate, oxidizing silver in the process. Pulsed amperometric detection has high specificity for the iodide ion and allows for detection in the  $\mu$ g/L range. The other halides are detected in the same manner, but less efficiently. The formation of the silver iodide precipitate is reversible, so a small dip is observed after iodide elution due to the dissolution of the silver iodide

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. Temperature control of the electrochemical cell and on-line degassing are critical to obtaining good sample and standard reproducibility.

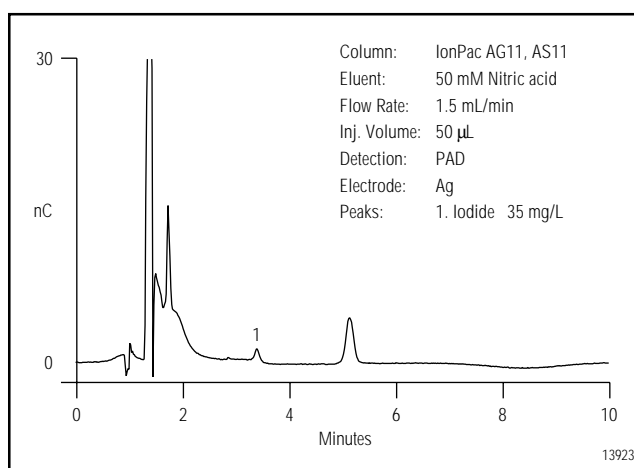
### Sample Analysis

Figure 2 shows the analysis of iodide in urine. This diluted sample contains enough iodide (35 µg/L) to be easily detected. The peak area RSD of eight sample injections was 6.9% and the retention time RSD was 0.3%. Table 1 shows the analysis of four sample preparations of urine. The values are consistent with those determined in a published analysis.<sup>5</sup> The values in Table 1 were calculated using duplicate 100-µg/L iodide standards treated with the OnGuard-RP cartridge. The recovery of the standard through the cartridge was 90%. Two additional urine samples were spiked with 100-µg/L iodide prior to OnGuard-RP treatment. There was a 114% recovery of the spike.

The samples analyzed in this Application Update had compounds with molecular weights greater than 10,000 daltons removed. Therefore, untreated samples should be treated with an ultrafiltration cartridge with a 10,000 molecular weight cut-off. Prior to analysis, the urine was diluted and treated with the OnGuard-RP, a polymeric reversed-phase sample pretreatment cartridge. A sample not treated with the OnGuard-RP was quantified using an untreated 100-µg/L standard. The iodide concentration of this sample (150 µg/L ± 7.8%) was similar to the treated samples, but there was reduced peak area response of standards analyzed immediately after the untreated sample. This indicates that untreated samples cause fouling of the working electrode surface and require frequent working electrode cleaning. When analyzing treated samples, the iodide peak area response in the first standard injection following sample analysis is often reduced about 5–10%, but peak area is recovered by the second or third injection.



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



**Figure 2** Determination of iodide in urine

Table 1 Analysis of Iodide in Urine			
Sample Number	Number of Injections	Concentration (µg/L)	RSD (%)
1	8	156	6.9
2	8	140	2.4
3	8	157	7.0
4	8	154	9.0

### Analysis of Other Electroactive Anions

Sulfite and cyanide were not eluted or detected with this method. Thiocyanate eluted at 5.2 min, approximately the same time as an unknown peak in the urine sample. Thiosulfate eluted at approximately 13 min. Sulfide, which usually is not present in urine, coeluted with iodide but was only detected with about one-fiftieth the sensitivity.

### PRECAUTIONS AND RECOMMENDATIONS

The IonPac AS11 column is packed in sodium hydroxide. The column should be flushed with water for at least 30 min before equilibrating with the nitric acid eluent. If there is a loss of iodide retention time or peak efficiency, 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 cell during column cleaning. Changing the inlet column frit or the guard column may also restore retention time and efficiency.

Installation of a 4-L eluent bottle (P/N 39164) allows unattended operation for longer periods. Dionex recommends replacement of the Ag/AgCl reference electrode every 6 months.

### REFERENCES

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