

Determination of Iodide and Iodate in Seawater and Iodized Table Salt by HPLC with UV Detection

INTRODUCTION

Iodine is an essential element naturally present in seawater and seafood. The most common forms of natural iodine in the diet are iodide and iodate, with additional iodized organic compounds providing a small fraction of bioavailable iodine. Kelp species have been known for centuries as effective iodine accumulators and most iodide in seawater is thought to be the result of biological reduction of the thermodynamically stable iodate species.¹

Dietary iodine deficiency affects thyroid function and leads to developmental diseases, neurological damage, goiter, and paralysis.² To combat disease, table salt is routinely iodized. Iodization levels vary greatly and can range from 5–100 µg/g (ppm) of iodine in salt depending on the country of manufacture and the storage conditions.³ Potassium iodide and potassium iodate are safe sources of dietary iodine used to iodize salt and prevent iodine deficiencies. In the United States and Canada, potassium iodide is frequently used to iodize salt whereas in many other countries, potassium iodate is preferred due to its greater stability.⁴

Iodide can be oxidized to iodine under many conditions, including exposure to humidity, reaction with existing moisture present in the salt, exposure to sunlight,

and exposure to heat. This conversion is also catalyzed by metal ions, particularly ferrous ions. Iodine readily sublimates, and is therefore easily lost from iodized salt. However, iodate is comparably more stable and is not lost by such pathways.

In this application note (AN), High Performance Liquid Chromatography (HPLC) coupled with photodiode array UV detection is used to determine iodide and iodate in seawater, synthetic sea salt, and table salt. This method is specific, sensitive, and rapid. Iodide and iodate are separated using the Acclaim[®] Mixed-Mode WAX-1, a silica-based column that incorporates hydrophobic, weak anion-exchange, and ion-exclusion properties. Consequently, retention of basic, neutral, and acidic molecules can be independently or concurrently adjusted by changing ionic strength, pH, and organic solvent content in the mobile phase. These properties confer adjustable and unique selectivity. The Acclaim Mixed-Mode WAX-1 column effectively separates anions with the high capacity necessary for the determination of iodide in saline matrices, which minimizes the need for sample pretreatment or dilution.

EQUIPMENT

Dionex UltiMate® 3000 HPLC system consisting of:
SRD-3200 Solvent Rack (Dionex P/N 5035.9250)
HPG-3200M pump (Dionex P/N 5035.0018)
WPS-3000TSL Micro autosampler
(Dionex P/N 5822.0025)
Sample Loop, 25 µL (Dionex P/N 6820.2415)
TCC-3200 column compartment
(Dionex P/N 5722.0025)
PDA-3000 detector (Dionex P/N 5080.0020)*
Chromeleon® 6.8 Chromatography Data System
Polypropylene vials with caps and septa, 0.30 mL
(Dionex P/N 055428)
Nalgene® 125 mL HDPE narrow mouth bottles
(VWR P/N 16057-062)
Nalgene 250 mL HDPE narrow mouth bottles
(VWR P/N 16057-109)
Nalgene 250 mL 0.2 µm nylon filter units
(VWR P/N 28199-371)
Nalgene 1000 mL 0.2 µm nylon filter units
(VWR P/N 28198-514)

* In this application, a PDA was used for detection; however, a VWD-3400RS (P/N 5074.0010) or a VWD-3100 (P/N 5074.0005) can be substituted.

REAGENTS AND STANDARDS

Deionized water, Type I reagent grade, 18 MΩ-cm resistivity or better
Potassium iodide (VWR, P/N VW5225-1)
Sodium iodate (Fluka P/N 71701)
Sodium phosphate, monobasic (SigmaUltra P/N 58282)
Tetrasodium pyrophosphate decahydrate (Fluka P/N 71515)
Phosphoric acid (EMD P/N PX0996-6)
Sodium chloride (JT Baker P/N 4058-05)
Sodium sulfate (VWR, P/N EM-SX0760-1)
Potassium chloride (Mallinckrodt P/N 6858)
Sodium bicarbonate (VWR, P/N EM-SX0320-1)
Potassium bromide (JT Baker P/N 2998-01)
Sodium fluoride (Fisher Scientific P/N S-299)
Boric acid (JT Baker P/N 0084-01)

Samples

Simulated seawater⁵
Iodized table salt
Iodized table salt aged at ambient conditions for 1 week
Commercial synthetic sea salt for aquarium use
Seawater collected at Pacifica, CA
Seawater collected at Half Moon Bay, CA

CONDITIONS

Column: Acclaim Mixed-Mode WAX-1,
2.1 × 150 mm
Eluent: 50/50 Methanol/120 mM sodium
phosphate, monobasic (pH=3.00)
Flow Rate: 0.20 mL/min
Temperature: 30 °C (column compartment)
Inj. Volume: 25 µL
Detection: UV, 223 nm
Noise: ~0.02–0.06 mAU
System
Backpressure: ~1200 psi

PREPARATION OF SOLUTIONS AND REAGENTS

Mobile Phase A

Prepare 120 mM sodium phosphate by dissolving 28.8 g of sodium phosphate, monobasic and 0.50 g of tetrasodium pyrophosphate decahydrate in 2000 g (2 L) of deionized water. Adjust the pH to 3.00 ±0.05 with HPLC grade phosphoric acid. Filter the phosphate solution through a 0.2 µm nylon filter unit. Transfer the solution to a clean, dry glass bottle. Check phosphate-based eluents regularly for biological contamination, and discard the solution if any sign of biological growth is observed. To improve retention time stability for iodide in samples of varying salinity, sodium phosphate was used to prepare the eluent. If potassium phosphate buffers are used as eluents, as described in the product manual,⁶ iodide retention times will shift due to the sample's sodium content. The extent of this shift increases with increasing sodium concentration.

Mobile Phase B

Transfer 1 L of degassed HPLC grade methanol into a clean, dry glass bottle for use as mobile phase B.

Stock Standard Solutions

Stock solutions of 1000 ppm of potassium iodide (KI) and sodium iodate (NaIO₃) were prepared. Potassium iodide was prepared by dissolving 131 mg in 100 mL (100.00 g) of DI water. Sodium iodate was prepared by dissolving 113 mg in 100 mL (100.00 g) of DI water. The solutions were stored in Nalgene HDPE bottles at < 6 °C.

Working Standard Solutions

Working standard solutions were prepared by diluting 100 µL (100 mg) of the stock solution of potassium iodide into 100 g (100 mL) of DI water to yield a 1.00 mg/L iodide intermediate stock solution. Calibration standards between 10 µg/L and 250 µg/L were prepared by appropriate dilution of the intermediate stock solution with DI water.

Combined iodide/iodate working standards were freshly prepared before use by spiking the prepared iodide calibration standards with volumes of 1000 mg/L iodate to prepare standards of iodate between 0.5 mg/L and 7.6 mg/L. The iodide concentration was corrected for the minor dilution by the added iodate.

Sample Preparation

Simulated Seawater

Simulated seawater was prepared by dissolving the salts listed in Table 1 into 1 L of deionized water (following the method of Kester et al.), with the exclusion of magnesium chloride, calcium chloride, and strontium chloride.⁵ These salts were excluded for anion analysis because they added only a small amount of additional chloride to the matrix and required preparation by drying, dissolution, and volumetric addition to the other salts. This yields a solution with a salinity of approximately 3.5%.

Synthetic Sea Salt

Commercially available synthetic sea salt was prepared following package directions (1/2 cup of salt per gallon of deionized water). A 1 L portion was prepared with 30 g of aquarium salt. A sea salt density of approximately 2.2 g/cm³ was used to convert the preparation directions to metric units.⁷

Fresh Iodized Table Salt

Solutions of table salt were initially prepared at a salinity similar to seawater. These solutions contained iodide in amounts that exceeded the standard curve and were further diluted to generate solutions of approximately

Reagent	Amount Added (mg)
Sodium Chloride	23900
Sodium Sulfate	400
Potassium Chloride	680
Sodium Bicarbonate	198
Potassium Bromide	95.4
Boric Acid	27.1
Sodium Fluoride	4.0

100 µg/L iodide. This was done by serial dilution as follows: Dissolve 1.92 g of table salt in 100 mL of DI. Dilute 6.0 mL (6.0 g) of this solution in 57.5 g of DI water.

Aged Iodized Table Salt

A sample of table salt aged for 1 week under ambient light and humidity conditions was prepared by dissolving 1.4 g of table salt in 100 mL of DI water. This solution was further diluted 1:5 to produce a solution that contained iodide within the standard curve.

Seawater Collected at Half Moon Bay and Pacifica, CA

Surface seawater was collected at Half Moon Bay, CA in a 250 mL HDPE Nalgene bottle that had been pre-cleaned prior to sample collection. The sample was stored in ice until it could be filter sterilized through a 250 mL, 0.2 µm nylon filter unit. After filtration, the sample was stored at <6 °C.

To check for potential loss of iodide during sample filtration, a 75 µg/L iodide standard was filtered through a 250 mL, 0.2 µm nylon filter unit. The concentration of iodide determined in the filtered standard was the same as the unfiltered standard, within the error of the method.

Precautions

Samples should be prepared for analysis as soon as possible to prevent loss of iodide by oxidation. Iodide will be lost from solution over time. This is especially noticeable in samples containing ≤10 µg/L of iodide. Samples should be analyzed within 24 h after collection. All solutions should be stored at <6 °C and the autosampler should be thermostatically controlled to at least 10 °C.

Care should be taken when filling the 300 μL vials. If they are overfilled, the first injection from a vial will not be at full volume, which greatly reduces method precision.

RESULTS AND DISCUSSION

The Acclaim Mixed-Mode WAX-1 column has a unique selectivity that is controlled by adjusting the percentage of solvent, the pH, and the ionic strength of the eluent. In addition to these options to control the column selectivity, sodium phosphate was used as an eluent rather than the potassium phosphate buffer described in the product manual.⁶ This eluent change gives further control over the selectivity for high salinity samples.

If potassium phosphate is used as an eluent, the retention time of iodide will shift dramatically with changes in total salinity. The use of a 120 mM sodium phosphate eluent minimizes retention time shifting of iodide in seawater samples compared to standards prepared in DI water. This is due to the effect of sodium on the column's stationary phase. The Acclaim Mixed-Mode WAX-1 column separates compounds by hydrophobic interaction, anion exchange, and electrostatic repulsion. Potassium and sodium differentially affect the electrostatic repulsion behavior of the Mixed Mode WAX-1 column. The smaller ionic radius of sodium compared to potassium allows greater interaction with the stationary phase's silanol groups. For samples that are high in sodium, such as seawater, the sodium injected with the sample affects the retention of other sample components. When potassium phosphate mobile phase was used, the sample's sodium would change the electrostatic repulsion behavior of the column compared to when standards were run. In this case, the sodium decreases the anion-repulsion effect of silanol groups, leading to increased retention times and improved peak shapes for iodide. By using a sodium phosphate buffer close in concentration to the sodium concentration expected in the sample, this effect can be made consistent.

In order to match the ionic strength of the samples, mobile phase A was prepared as 120 mM sodium phosphate. Compared to initial experiments using 50 mM potassium phosphate buffer at pH 6.0, the increased concentration of phosphate decreased the retention time of anionic analytes, leading to a decrease in the resolution of peaks. This retention time decrease was mitigated by reducing the eluent pH resulting in a rapid

high-resolution analysis for both iodide and iodate with stable retention times.

Figure 1 shows the separation of iodate and iodide on the Acclaim Mixed-Mode WAX-1 column using a sodium phosphate eluent at a pH of 3.00. A wavelength of 223 nm was determined by collecting data between 210–500 nm and choosing the wavelength with the best signal-to-noise (S/N) ratio for iodide. Iodide and iodate can be analyzed simultaneously with excellent peak shapes. In a sample matrix, iodate and iodide can be separated in less than 20 min, as shown in Figure 2. Iodide is well separated from all other peaks and iodate is easily identified.

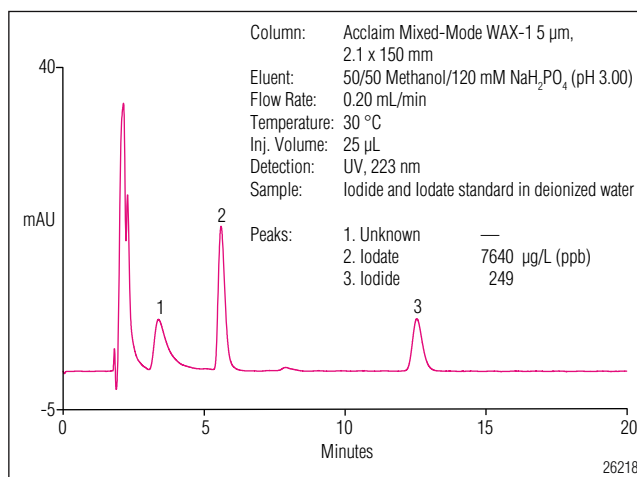


Figure 1. Separation of an iodide and iodate standard in deionized water.

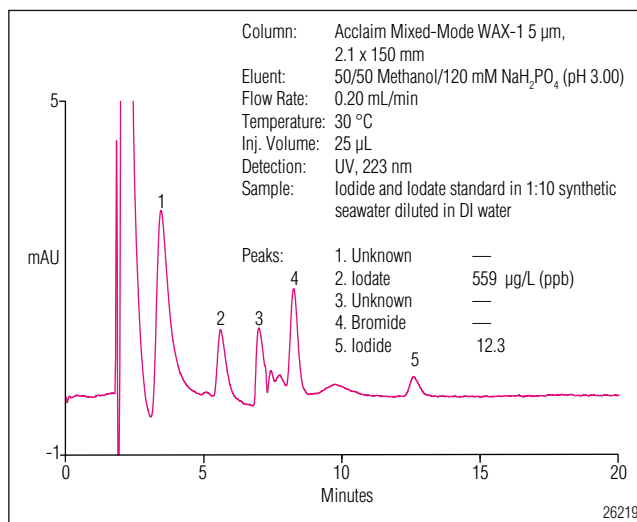


Figure 2. Separation of iodide and iodate in synthetic sea salt.

Linear Range, Limit of Quantitation, and Limit of Detection

Seawater typically contains 50–60 µg/L iodide with a wide range of observed concentrations. The iodide concentration can range from less than 1 µg/L, to greater than 60 µg/L with observed concentration affected by water depth, oxygen present, and the biological mediation of the iodide/iodate equilibrium.⁸ To determine the method linearity, calibration standards were injected in triplicate, covering the expected concentration range of iodide in seawater (10–250 µg/L). The LOD for iodide was estimated at three times the signal-to-noise ratio (S/N) to be 3.3 µg/L, which was confirmed by injecting 3.3 µg/L iodide standard in DI water. The LOQ was estimated at 10 times S/N to be 9.9 µg/L, which was also confirmed by injecting a standard prepared at this concentration.

Iodate weakly absorbs in the UV between 200–230 nm, leading to limited detection sensitivity of this anion. Iodate was not detected in any of the samples analyzed in this study, therefore the calibration curve of iodate was determined by the detection limits of the method. The LOD was determined to be 60 µg/L, with an LOQ of 200 µg/L. The linear range, LOQ, and LOD for iodate and iodide are presented in Table 2. Detection of iodate at lower wavelengths increases the peak height, but also increases the noise, leading to no improvement in detection limits.

Accuracy and Precision

The method performance was initially evaluated with seven replicate injections of a standard. Table 2 shows the peak retention time and peak area RSDs. The peak area RSD was < 1 with peak retention time RSDs of < 0.05 for seven injections of 100 µg/L iodide and 1.5 mg/L iodate. Precision of analysis in samples was similar to the precision for standards and is discussed in the section below.

Method accuracy was determined by calculating the recoveries of iodide from five different sample matrices. Samples were spiked with iodide that was nearly double the initial amount found in the sample, if any. Samples without iodide were spiked at 50, 141, and 244 µg/L. The recoveries ranged from 93–113%, which is excellent given the wide variety of samples (Table 3). All of the environmental samples, synthetic seawater, table salt, and lab-prepared simulated seawater showed good

Table 2. Precision, LOD, and LOQ for Iodate and Iodide Determination

Analyte	Retention Time (min)	Range (µg/L)	Correlation Coefficient (r ²)	LOD (µg/L)	LOQ (µg/L)	Retention Time Precision (RSD)	Peak Area Precision (RSD)*
Iodate	5.61	500-7600	0.9994	60	200	0.05	0.95
Iodide	12.54	10-250	0.9985	3.3	9.9	0.04	0.69

*Precision data determined from 7 injections of 100 µg/L iodide and 1.5 mg/L iodate in deionized water.

Table 3. Recoveries of Iodate and Iodide from Saline Samples (n=3)

Sample	Analyte	Average Amount Found (µg/L)	Amount Added (µg/L)	Recovery (%)
Simulated Seawater	Iodate*	530 ± 6.0	490	108 ± 1.2
	Iodide	52 ± 0.4	50	104 ± 0.8
		160 ± 0.8	141	113 ± 0.6
		255 ± 0.6	244	104 ± 0.2
Synthetic Sea Salt	Iodate*	550 ± 5.0	520	104 ± 0.9
	Iodide	197 ± 2.7	96.6	93 ± 1.3
Table Salt, fresh	Iodide	196 ± 1.8	99.1	103 ± 0.9
Seawater, Pacifica, CA	Iodide	21.5 ± 0.7	14.4	101 ± 3.3
Seawater, Half Moon Bay, Ca	Iodide	20.5 ± 1.6	14.0	93 ± 7.3

*Recoveries of iodate were from samples that had been diluted 1:10 in DI water.

recovery. The environmental seawater samples, which contained iodide at low levels compared to the other samples, showed good recoveries for spiking at nearly double the native concentration of the anion. Figures 3 and 4 illustrate the clear separation of iodide from the matrix components in these environmental samples. This recovery data in multiple sample matrices shows that the matrix does not affect quantification of iodide and that the method performed well for samples of varying salinity.

Recovery of iodate from two samples was performed. Iodate was not detected in any of the samples prior to spiking. If the samples are not diluted, recoveries are very low, ranging between 24–39% and excessive peak tailing is observed. Samples diluted 1:5 with DI water showed recoveries between 93–110%. However, the peak tailing was still evident. Both low recoveries and distorted peak shapes are typical signs of poor column performance due to overloading. Samples were diluted 1:10 with DI water to prevent column overloading. This produced recoveries in the range of 104–108% in the simulated

seawater and a solution of commercial synthetic sea salt. The peak asymmetry was equivalent to that observed in iodate standards prepared in DI water. Results from these recovery experiments are detailed in Table 3.

Samples were analyzed for iodide by direct injection of sample solutions. Figure 5 shows the separation of iodide in table salt. Fresh table salt from a new container contained an average of 47.8 $\mu\text{g/g}$ of iodide. To evaluate loss of iodide from table salt, a sample was aged at ambient conditions for one week. When analyzed, the iodide determined in the sample had decreased to 43.4 $\mu\text{g/g}$. As expected, the aged sample lost nearly 10% of the iodide during one week of exposure to light and ambient humidity. Both these samples had peak area RSDs of <1.0% for triplicate injections as detailed in Table 4.

Synthetic seawater prepared from synthetic sea salt was also analyzed as an example of a more complicated matrix. This sample contained 122 $\mu\text{g/L}$ of iodide. The peak area RSD for these injections was 1.2%. This synthetic seawater is a much more complex matrix, yet the precision is similar to that of table salt solutions.

The two environmental seawater samples collected at Half Moon Bay and Pacifica (CA) contained 7.6 and 9.3 $\mu\text{g/L}$ iodide, respectively. The peak area RSDs for these samples (Table 4) were 6.6% for triplicate injections. The peak area precision found is good when taking into consideration the low concentration of iodide in these samples. The iodide concentration determined in these samples is at or below the measured LOQ and precision would be expected to be reduced compared to the precision measured at higher concentrations of iodide.

CONCLUSION

This application note describes the use of the Acclaim Mixed-Mode WAX-1 column to determine iodide in seawater and other saline matrices by a direct injection, and iodate with sample dilution. The high capacity and unique selectivity of this column allow the determination of iodide without dilution or sample pretreatment to remove chloride in high salinity matrices. Standards can be prepared in DI water without the added complexity of matching the sample matrix. Recoveries of iodide from these matrices are excellent, showing the method is accurate. The precision of the method is good providing peak area RSDs $\leq 1.2\%$, with a maximum of 6% RSD at the LOQ. These data show the advantage of the high capacity and adjustable specificity of the Acclaim Mixed-Mode WAX-1 column to determine analytes in high ionic strength samples.

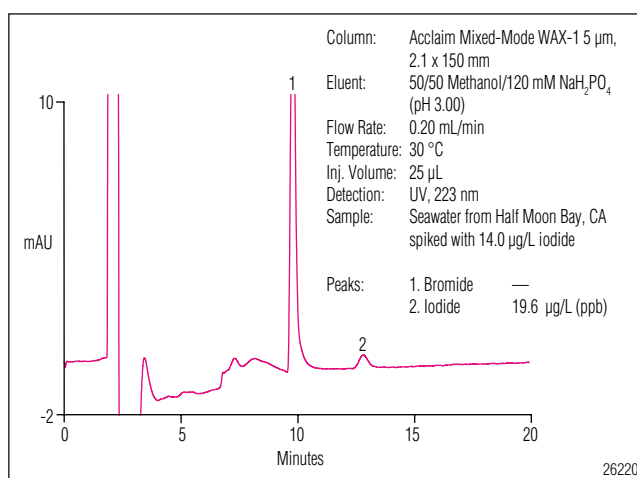


Figure 3. Separation of iodide spiked into seawater collected at Half Moon Bay, CA.

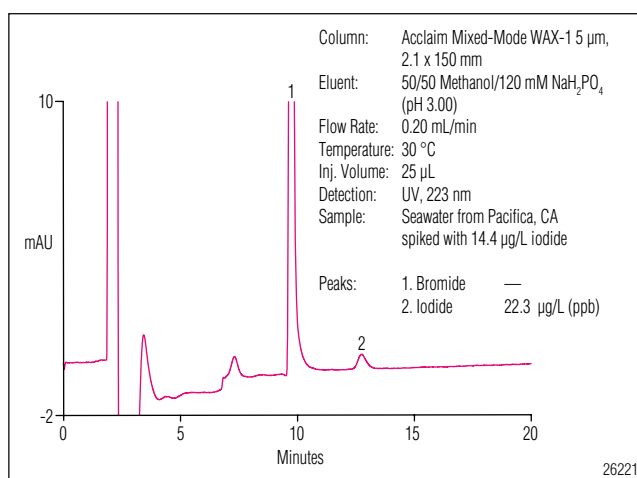


Figure 4. Separation of iodide spiked into seawater collected at Pacifica, CA.

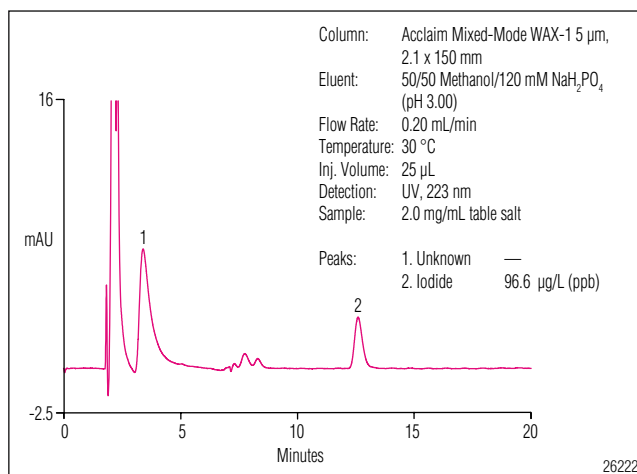


Figure 5. Separation of iodide in table salt on the Acclaim Mixed-Mode WAX-1 column.

Table 4. Determination of Iodide in Saline Samples (n=3)

Sample	Retention Time (min)	Retention Time Precision (RSD)	Peak Area Precision (RSD)	Amount Found (µg/L)	Amount in dry salt (µg/g)
Synthetic sea salt (30.4 mg/mL)	12.63	0.22	1.18	122 ± 1.4	4.0 ± 0.05
Fresh Table Salt (1.68 mg/mL)	12.74	0.21	0.11	80.3 ± 0.1	47.8 ± 0.05
Aged Table Salt (2.74 mg/mL)	12.67	0.06	0.96	119 ± 1.1	43.4 ± 0.4
Seawater (Pacifica, CA)	12.85	0.51	6.62	9.3 ± 0.6	—
Seawater (Half Moon Bay, CA)	12.66	0.24	6.69	7.6 ± 0.5	—

LIST OF SUPPLIERS

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