

Determination of iodide in multivitamin-mineral supplements using ion chromatography

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Goal

To develop a method for the determination of iodide in multivitamins using an ion chromatography (IC) system with a Thermo Scientific™ Dionex™ IonPac™ AS20 column and electrochemical detection

Introduction

Iodine is an essential mineral for the body. The thyroid gland uses iodine to produce thyroid hormones that regulate many important biochemical reactions and are metabolically critical. It is known that iodine deficiency can lead to varying degrees of growth and developmental abnormalities in children and adults.¹⁻³ Excess iodine can lead to thyroid disorders.⁴ Therefore, an appropriate level of iodine is critical for the body to function properly. As the body does not produce iodine, it must be supplied by the diet (foods or dietary supplements). Multivitamin-mineral supplements containing iodine, usually in the form of



potassium iodide or sodium iodide, are one of the common sources of dietary iodine.

In the United States, accurate measurements of nutrients are needed to ensure compliance with Food and Drug Administration (FDA) regulations on the Nutrition Facts and Supplement Facts labels. The percent daily value of iodine must be listed on the label if iodine is added to a food.⁵ The current daily value for iodine is 150 µg for individuals aged ≥4 years. Determination of iodine in foods or dietary supplements is important and has been included in the National Institute of Standards and Technology (NIST) Dietary Supplement Laboratory Quality Assurance Program (DSQAP).⁶

Many analytical methods have been used for determining iodine in food matrices, including ion chromatography (IC) methods with electrochemical detection (ED).⁷⁻⁹ A Thermo Scientific™ application note (AN) demonstrated the determination of iodide and iodate in infant formulas using acid digestion, and a 4 mm Thermo Scientific™ Dionex™ IonPac™ AG11/AS11 column set with manually prepared nitric acid eluent and ED.⁷ This application note shows the development and validation of a different ED method with an alkaline eluent for the determination of iodide in multivitamin-mineral supplement samples. This ED method uses alkaline digestion and a 2 mm version of the Dionex IonPac AS20 column with electrolytically generated potassium hydroxide (KOH) eluent. Using an alkaline digestion sample preparation method and a dual channel IC system, the nutrient chlorine (as chloride) in the supplements can be determined by conductivity detection (CD) simultaneously, which will be reported in a different application document. Here we demonstrate using the CD channel to support ED method development. This document shows that the ED method is sensitive and accurate for determining iodide in multivitamin supplements for regulatory monitoring.

In addition, we also demonstrate using a palladium hydrogen (PdH) reference electrode rather than a silver/silver chloride (Ag/AgCl) reference electrode for iodide determination and compare its performance to that of the Ag/AgCl reference electrode. The PdH electrode is a solid-state reference electrode. It provides a more stable reference potential and offers other advantages, such as longer lifetime and less maintenance. Its value was previously demonstrated for carbohydrate determinations.¹⁰

Experimental

Equipment and consumables

- Thermo Scientific™ Dionex™ ICS-6000 Dual Channel HPIC™ system with RFIC™-EG module, conductivity and electrochemical detectors*

*For iodide determination, equivalent results can be achieved using a single-channel Thermo Scientific™ Dionex™ ICS-6000 system, Thermo Scientific™ Dionex™ ICS-5000+ system, or Thermo Scientific™ Dionex™ Integriion™ HPIC™ system with electrochemical detection.

- Thermo Scientific™ Dionex™ AS-AP autosampler with 250 µL syringe and tray temperature control (P/N 074926)
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software, version 7.2.10
- Thermo Scientific™ Dionex™ ICS-6000 ED Electrochemical Detector (P/N 072042) with Electrochemical Detector Cell (P/N 072044)

Consumables

- Thermo Scientific™ Dionex™ EGC 500 KOH Potassium Hydroxide Eluent Generator Cartridge (P/N 075778)
- Thermo Scientific™ Dionex™ CR-ATC 600 Continuously Regenerated Anion Trap Column (P/N 088662)
- Thermo Scientific™ Dionex™ AS20 Analytical Column, 2 × 250 mm, (P/N 063065)
- Thermo Scientific™ Dionex™ AG20 Guard Column, 2 × 50 mm (P/N 063066)
- Thermo Scientific™ Dionex™ AS-AP Autosampler Vials 10 mL (P/N 074228)
- Thermo Scientific™ Dionex™ Electrochemical Detector Ag/AgCl pH Reference Electrode (P/N 061879)
- Thermo Scientific™ Dionex™ Electrochemical Detector Palladium Hydrogen (PdH) Reference Electrode (P/N 072075)**
- Thermo Scientific™ Dionex™ Electrochemical Detector Silver Disposable Electrode (P/N 063003). Package includes 6 electrodes and 6 gaskets (0.002 in.).
- Fisherbrand™ Polypropylene Centrifuge Tubes, 50 mL (Cat. No. 05-539-13)
- Thermo Scientific™ Nalgene™ Syringe Filter 0.2 µm PES (P/N 725-2520)

**Both reference electrodes were evaluated in this application note. Only one is needed to run this method.

Reagent and standards

- Degassed deionized (DI) water, 18 MΩ·cm resistance or better
- Potassium iodide (>99.9%), A.C.S. reagent grade or better, for preparing iodide standards, Fisher Chemical™ (Fisher Scientific, P/N P410-100)
- Sodium hydroxide solution (50% w/w/Certified), Fisher Chemical™ (Fisher Scientific P/N SS254-500)

Samples

Four brands of multivitamin-products were purchased from local stores. The iodine contents (mg/kg) were calculated by the tablet weight and the label for each sample (Table 1).

IC setup and conditions

Figure 1 shows a schematic diagram of a Reagent-Free™ IC system for the determination of iodide. The dual channel setup used to develop the iodide method and measure chloride simultaneously on the second channel is shown in the Appendix. An autosampler with a diverter valve is used to serve both channels. For the determination of iodide, only one channel of the system with electrochemical detection, is required. In other words, only one pump of the DP or a single pump is used and the diverter valve is not needed for the AS-AP.

Table 1. Sample details

Multivitamin	Information	Iodine on label (mcg*/tablet)	Weight of 3 tablets (g)	Label iodine (mg/kg)
Sample 1 (MV1)	Brand 1, Multivitamin for men	150	4.7254	95.2
Sample 2 (MV2)	Brand 2, Multivitamin for women	150	4.9449	91.0
Sample 3 (MV3)	Brand 3, Organic multivitamin	N/A	4.9000	N/A
Sample 4 (MV4)	Brand 4, Multivitamin	150	3.8324	117.4

* mcg = micrograms

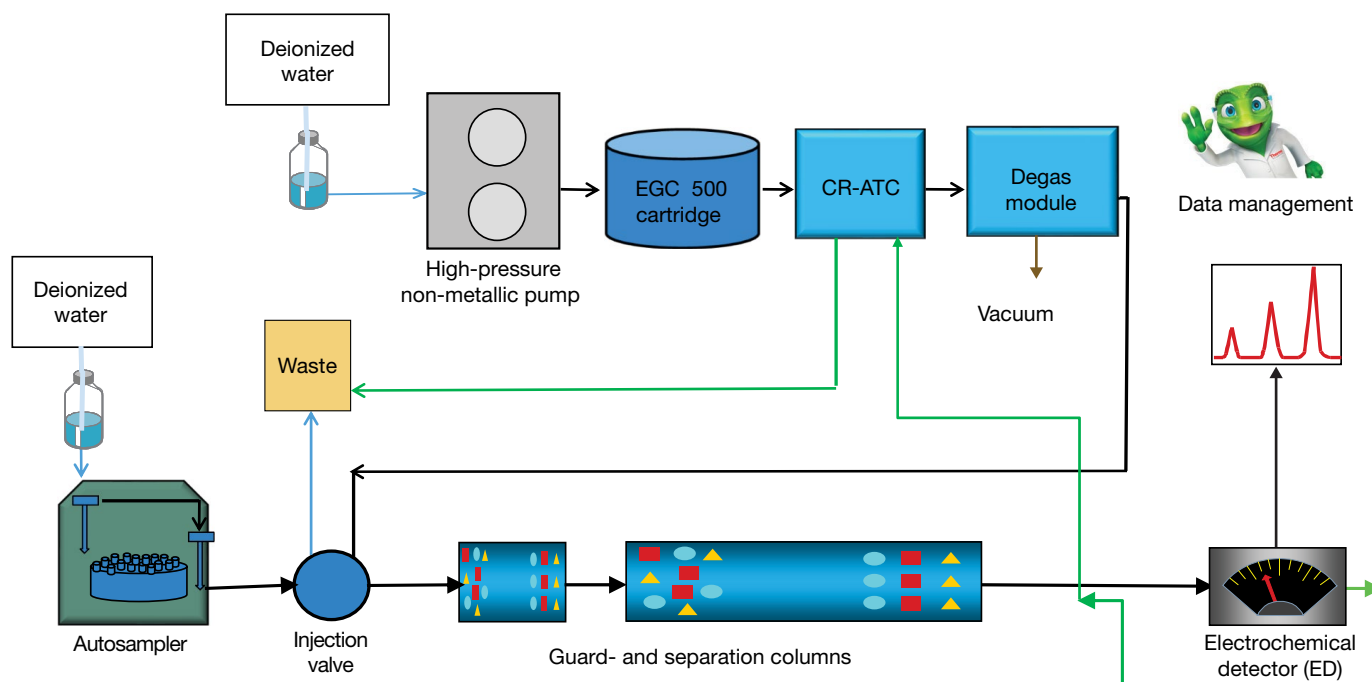


Figure 1. Schematic diagram of an RFIC system for the determination of iodide

Table 2. Chromatography conditions

Parameter	Value																					
Columns	Dionex IonPac AS20 analytical column, 2 × 250 mm Dionex IonPac AG20 guard column, 2 × 50 mm																					
Eluent	30 mM potassium hydroxide (KOH)																					
Eluent source	Thermo Scientific™ Dionex™ EGC 500 KOH cartridge with Thermo Scientific™ Dionex™ CR-ATC 600 and high-pressure degasser																					
Flow rate	0.35 mL/min																					
Injection volume	25 µL (full loop)																					
Column temp.	30 °C																					
Sample tray temp.	4 °C																					
Detection	Electrochemical detection: Pulsed amperometric detection (PAD) mode																					
Working electrode	Silver working electrode (disposable)																					
Reference electrode	Ag/AgCl pH reference electrode or PdH reference electrode																					
Run time	15 min																					
	Silver, (Sulfide, Cyanide, Iodide, Thiosulfate)																					
	<table border="1"> <thead> <tr> <th>Time (s)</th> <th>Potential (V)</th> <th>Integration</th> </tr> </thead> <tbody> <tr> <td>0.00</td> <td>-0.10</td> <td></td> </tr> <tr> <td>0.20</td> <td>-0.10</td> <td>Start</td> </tr> <tr> <td>0.90</td> <td>-0.10</td> <td>End</td> </tr> <tr> <td>0.91</td> <td>-1.00</td> <td></td> </tr> <tr> <td>0.93</td> <td>-0.30</td> <td></td> </tr> <tr> <td>1.00</td> <td>-0.30</td> <td></td> </tr> </tbody> </table>	Time (s)	Potential (V)	Integration	0.00	-0.10		0.20	-0.10	Start	0.90	-0.10	End	0.91	-1.00		0.93	-0.30		1.00	-0.30	
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Waveform (when using Ag/AgCl reference electrode)																						
Waveform (when using Palladium Hydrogen (PdH) reference electrode)																						

Preparation of solutions and reagents**Iodide stock standard solutions (2,000 mg/L)**

Iodide stock standard solution is prepared by dissolving 262.0 mg of dry potassium iodide salt in 100 mL of DI water. The stock standard is stable for at least six months when stored at 4 °C.

Calibration standards

Diluted calibration standard solutions are prepared from the 2,000 mg/L stock standard and DI water (Table 3).

Sample preparation**Extraction solution (0.02% (w/w) sodium hydroxide)**

Mix 0.4 g of 50% w/w NaOH with 999.6 g DI water in a plastic bottle.

Sample powder

Grind >30 tablets and mix the resulting powder thoroughly. Store at room temperature if analyzed within 2 days. Otherwise, store at 4 °C.

Prepare sample

1. Weigh about 0.500 g of multivitamin sample into a 50 mL conical centrifuge tube and record the exact weight.
2. Add 20 mL (g) of the extraction solution into the tube, shake and mix well.
3. Place the tube in an ultrasonic bath and sonicate for >2 h.
4. Centrifuge at 6,000-7,000 rpm for 30 min.
5. Filter the sample solution through a 0.2 µm PES syringe filter, discarding the first couple drops of the effluent.
6. Dilute sample solution 1 to 20 with the extraction solution before IC analysis.

Prepare spiked sample

1. Weigh about 0.500 g of multivitamin sample into a 50 mL conical centrifuge tube and record the exact weight.
2. Add known amounts (approximately 30% to 70% of the label amount) of iodide standards into the solid multivitamin sample in the centrifuge tube.
3. Follow steps 2 to 6 above.

Table 3. Calibration standards (mg/L)

Analyte	Level 1*	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7	Level 8
Iodide (mg/L)	0.001 (0.0025)	0.005	0.01	0.04	0.1	0.4	1.0	2.0

* Level 1 = 0.001 mg/L iodide when using a Ag/AgCl reference electrode; Level 1 = 0.0025 mg/L iodide when using a PdH reference electrode.

Results and discussion

Separation

The Dionex IonPac AS20 column is a hydroxide-selective, high-capacity anion-exchange column developed to determine anions that are strongly retained on other anion-exchange columns. The selectivity for these highly retained anions, such as iodide, allows them to be determined with lower ionic strength eluents and in less time compared to other anion-exchange columns. Figure 2 shows the separation of a 0.4 mg/L iodide standard on a Dionex IonPac AS20 column set using a 30 mM KOH eluent and ED detection. Iodide elutes in less than 10 min and is well resolved from the void volume and the baseline dip, which is due to dissolved oxygen, at approximately 12 min.

Columns: Dionex IonPac AG20, 2 × 50 mm and Dionex IonPac AS20, 2 × 250 mm
Eluent: Potassium hydroxide (KOH) 30 mM
Eluent source: Dionex EGC 500 KOH Cartridge with Dionex CR-ATC 600 and Dionex high pressure degasser
Flow rate: 0.35 mL/min
Inj. volume: 25 µL (Full loop)
Column temp.: 30 °C
Sampler temp.: 4 °C
Detection: Electrochemical detection (ED) with Ag/AgCl reference electrode and disposable silver working electrode

	min	mg/L	
Peak:	1. Iodide	8.5	0.4

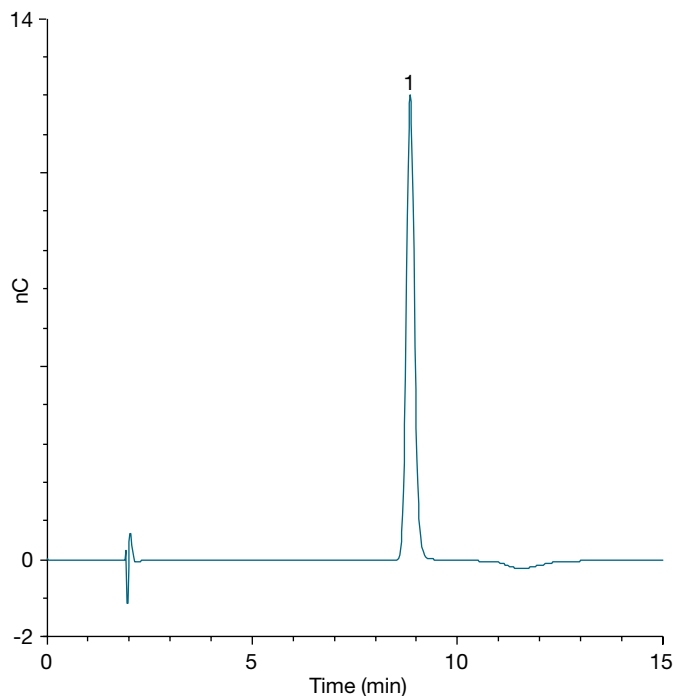


Figure 2. Chromatogram of 0.4 mg/L iodide

Figure 3 shows the separation of iodide in multivitamin sample 1. Most other anions in multivitamin samples retained by the Dionex IonPac AS20 column are not detected by ED. To ensure all anions elute and do not consume column capacity, multivitamin samples were analyzed using suppressed conductivity detection and the same separation conditions. That analysis (not shown) demonstrated that most anions elute well before iodide, with chloride eluting at approximately 3.3 min. Iodide elutes at 8.5 min and is well-resolved from other anions in the sample. The concentration of iodide in multivitamin sample 1 (MV1), however, is too low to be determined by suppressed conductivity detection.

Columns: Dionex IonPac AG20, 2 × 50 mm and Dionex IonPac AS20, 2 × 250 mm
Eluent: Potassium hydroxide (KOH) 30 mM;
Eluent source: Dionex EGC 500 KOH Cartridge with Dionex CR-ATC 600 and Dionex high pressure degasser
Flow rate: 0.35 mL/min
Inj. volume: 25 µL (Full loop)
Column temp.: 30 °C
Sampler temp.: 4 °C
ED detection: Electrochemical detection (ED) with Ag/AgCl reference electrode and disposable silver working electrode

Sample: Multivitamin sample 1 (MV1)
Peak: 1. Iodide

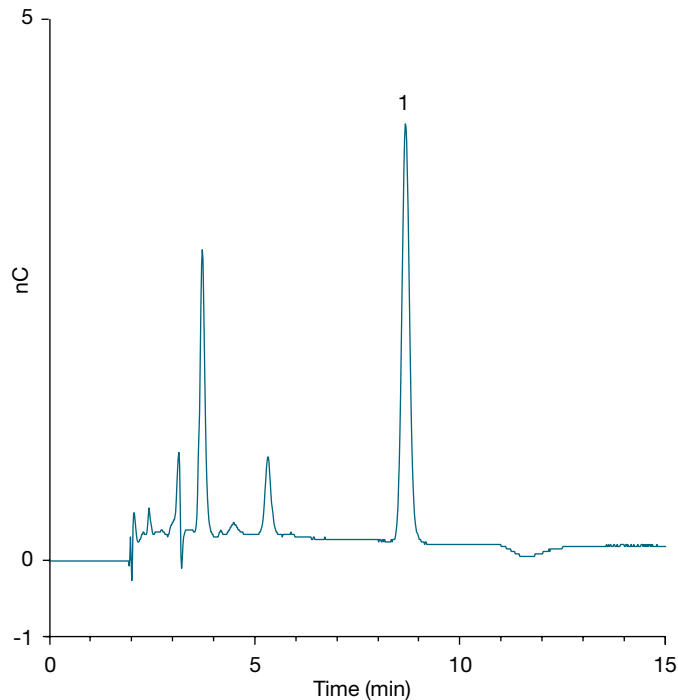


Figure 3. Chromatogram of multivitamin sample 1

Table 4. Linearity and method detection limit for iodide determination using ED detection

Reference electrode	Analyte	Injection volume (µL)	Range (mg/L)	Coefficient of determination* (r ²)	Calculated** MDL (mg/L)
Ag/AgCl	Iodide	25	0.001–2	0.9995	0.0005
PdH	Iodide	25	0.0025–2	0.9991	0.0018

* Calibration type is linear and forced through the origin.

**MDL = (t) × (S)

Where t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom (t= 3.14 for seven replicates)

S = standard deviation of the replicate analyses

Linearity and method detection limit

The method linearity was determined by triplicate injections of eight levels of calibration standards (Table 3). The method detection limit (MDL) was determined by performing seven replicate injections of the lowest level calibration standard, which is at a concentration of three to five times the estimated detection limit. Two different reference electrodes, a combination Ag/AgCl pH reference electrode for which we are using the Ag/AgCl half cell potential, and a PdH reference electrode, were studied for iodide determination. The results are shown in Table 4 and Figures 4 and 5. The study shows that peak area response is linear over the concentration ranges evaluated for each reference electrode with a coefficient of determination of 0.9995 for the Ag/AgCl reference electrode, and 0.9991 for the PdH reference electrode. The method is sensitive for the determination of iodide with MDL = 0.0005 mg/L when using the Ag/AgCl reference electrode and MDL = 0.0018 mg/L when using the PdH reference electrode.

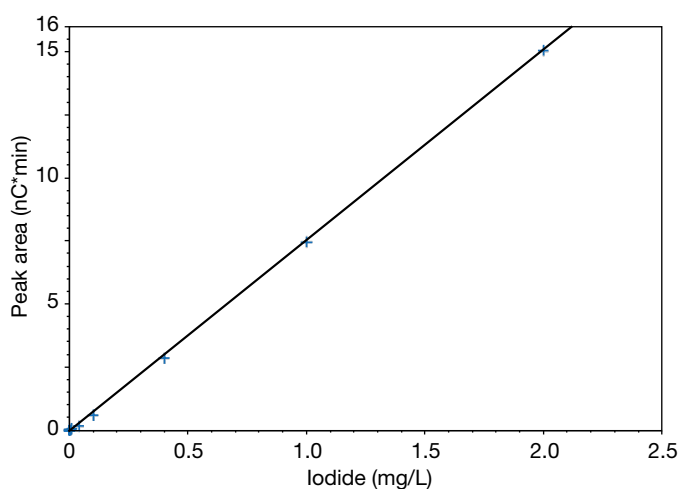


Figure 4. Calibration plot for iodide using a Ag/AgCl reference electrode with 25 µL injection volume

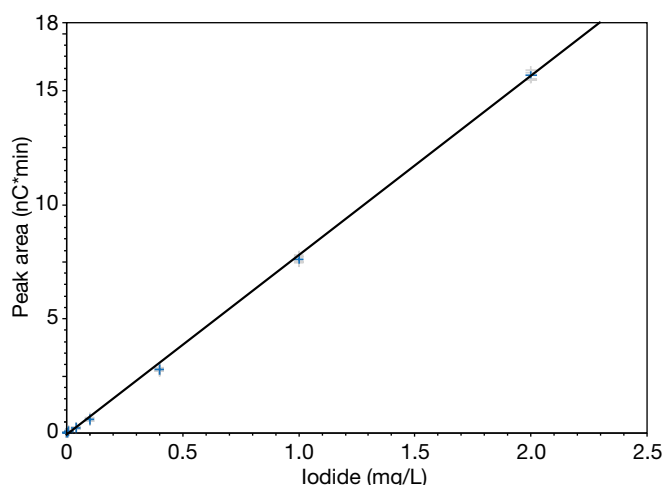


Figure 5. Calibration plot for iodide using a PdH reference electrode with 25 µL injection volume

Precision and accuracy

The method precision was evaluated by measuring iodide in a multivitamin sample and expressed as the relative standard deviation (RSD) of the results (Table 5). Intraday precision was evaluated by four samples independently prepared on a single day with triplicate injections for each sample. Interday precision was evaluated by comparing the results over four days with new sample preparations each day. The method shows an intraday precision = 1.3%, and an interday precision = 1.6%.

Table 5. Reproducibility of determination of iodide in multivitamin sample 2*

Intraday (n=4)		Interday (Over 4 days)	
Iodide (mg/kg)	RSD	Iodide (mg/kg)	RSD
91.1 ± 1.2	1.3	90.1 ± 1.5	1.6

* Ag/AgCl reference electrode and 25 µL injection

The method accuracy was validated by recovery experiments. Spiked samples were analyzed together with non-spiked samples. The recovery percentages were calculated using the formula shown below:

$$\% \text{ Recovery} = (\text{Total iodide in the spiked sample} - \text{Iodide in the sample before spiking}) / \text{Iodide added}$$

*Calculated based on the result of non-spiked samples of 91.1 mg/kg.

Table 6 summarizes the recovery results. The method is accurate with recovery ranging from 94% to 101%.

Table 6. Spiked recovery of iodide in multivitamin at different spiked levels

Spiked in iodide/ iodide in sample (%)	Sample weight (g)	Iodide added (mg)	Recovery (%)
30	0.50620	0.016	100
50	0.50361	0.024	94
70	0.50167	0.032	101

Determination of iodide in multivitamin preparations

To demonstrate the method's application for regulatory monitoring, four brands of multivitamin samples were tested using a Ag/AgCl reference electrode. Each multivitamin sample was tested at least three times (independently prepared samples on separate days). The results are listed in Table 7. The test results were consistent, with an RSD range from 0.9 to 2.5%. Multivitamin quality varied when judged by comparing results to the labeled values. Within the four brands tested, MV2's result agrees well with the label (99%), while MV4 is 47% higher than its claim. Determination of iodine in supplements is important and was included in NIST Dietary Supplement Laboratory Quality Assurance Program. This easy-to-use IC-ED method is suited for regulatory monitoring of iodine (as iodide) in these products.

Table 7. Determination of iodide in multivitamin samples

Multivitamin	Measured iodine* (mg/kg) (n≥3)	RSD	Label iodine (mg/kg)	Measured/label (%)
MV1	106 ± 1.4	1.3	95.2	111%
MV2	90.1 ± 1.5	1.6	91.0	99%
MV3	13.3 ± 0.1	0.9	N/A	N/A
MV4	173 ± 4.3	2.5	117.4	147%

*Data used Ag/AgCl reference electrode

To compare Ag/AgCl and PdH reference electrodes, a set of samples was tested using both reference electrodes (Figures 6 and 7). The results were similar regardless of which reference electrode was used for iodide determination. While we did not run as many experiments with the PdH reference electrode as we did the Ag/AgCl reference, we believe that either reference electrode can be used for this application.

Columns: Dionex IonPac AG20, 2 × 50 mm and Dionex IonPac AS20, 2 × 250 mm
 Eluent: Potassium hydroxide (KOH) 30 mM
 Eluent source: Dionex EGC 500 KOH Cartridge with Dionex CR-ATC 600 and Dionex high pressure degasser
 Flow rate: 0.35 mL/min
 Inj. volume: 25 µL (Full loop)
 Column temp.: 30 °C
 Sampler temp.: 4 °C
 Detection: Electrochemical detection (ED) with Ag/AgCl reference electrode and disposable silver working electrode
 Samples: Four brands of multivitamin iodine value (mg/kg)

	Label	Measured
MV-1	95.2	106
MV-2	91.0	90.1
MV-3	N/A	13.3
MV-4	117.4	173

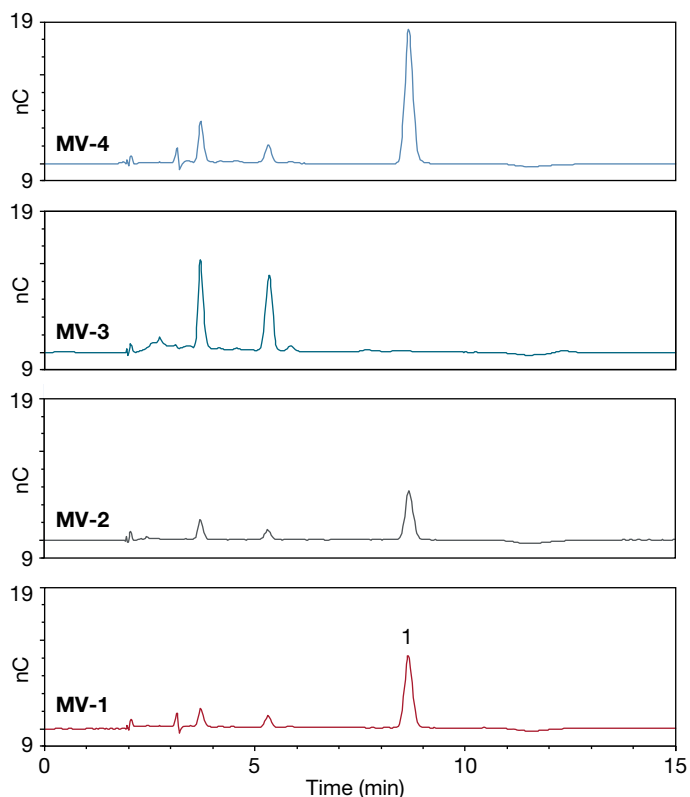


Figure 6. Chromatograms of multivitamin samples using a Ag/AgCl reference electrode

Columns: Dionex IonPac AG20, 2 x 50 mm and Dionex IonPac AS20, 2 x 250 mm
 Eluent: Potassium hydroxide (KOH) 30 mM
 Eluent source: Dionex EGC 500 KOH Cartridge with Dionex CR-ATC 600 and Dionex high pressure degasser
 Flow rate: 0.35 mL/min
 Inj. volume: 25 µL (Full loop)
 Column temp.: 30 °C
 Sampler temp.: 4 °C
 Detection: Electrochemical detection (ED) with PdH reference electrode and disposable silver working electrode

Samples: Four brands of multivitamin

	Iodine value (mg/kg)	
	Label	Measured
MV-1	95.2	108
MV-2	91.0	92.3
MV-3	N/A	14.0
MV-4	117.4	171

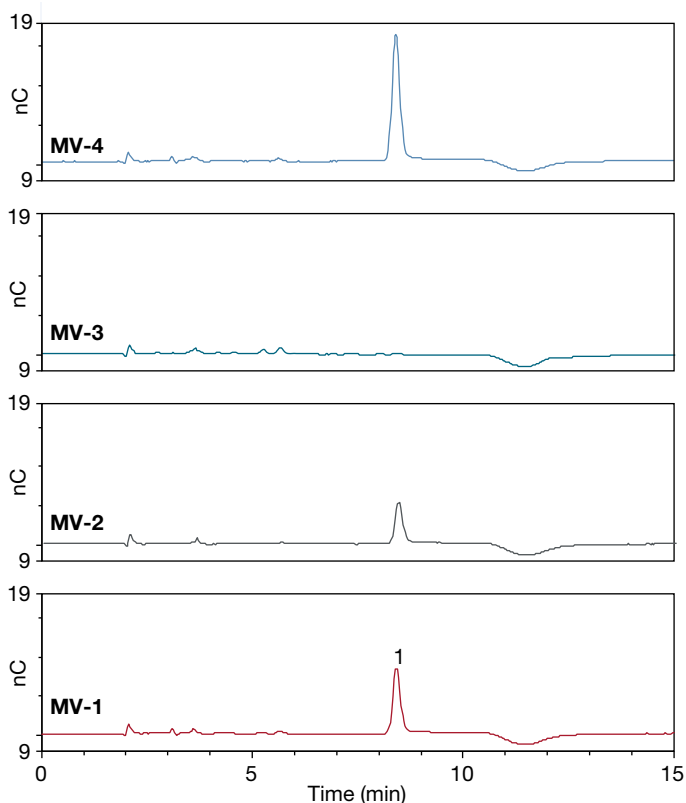


Figure 7. Chromatograms of multivitamin samples using a PdH reference electrode

Conclusion

This application note demonstrates the development and validation of a 15 min IC-ED method for the determination of iodide in multivitamin products. The method uses a 2 mm Dionex IonPac AG20/AS20 column set with electrolytically generated potassium hydroxide (KOH) eluent and a disposable silver working electrode. Using this method, iodide is well separated and resolved from all common anions and organic acids found in the multivitamin samples. The method has a linear calibration, is sensitive, precise, and accurate. Four different multivitamin samples were tested, and the data show this IC-ED method can be used for the regulatory monitoring of iodine in multivitamins.

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Appendix

Dual channel IC system with RFIC-EG module, conductivity and electrochemical detections

Figure 8 shows the schematic of the setup used for this study. An autosampler with a diverter valve is used to serve both channels. The multivitamin samples are analyzed by CD and ED simultaneously.

Besides the equipment and consumables for the ED channel, a diverter valve and suppressor are needed for the CD channel:

- Thermo Scientific™ Dionex™ AS-AP Autosampler with diverter valve (P/N 074123), 250 µL syringe, and tray temperature control
- Thermo Scientific™ Dionex™ ADRS 600 Anion Dynamically Regenerated Suppressor, 2 mm (P/N 088667)

Table 8. Chromatography conditions for the CD channel

Parameter	Value
Columns	Dionex IonPac AS20 analytical column, 2 × 250 mm Dionex IonPac AG20 guard column, 2 × 50 mm
Eluent	Isocratic, 30 mM potassium hydroxide (KOH)
Eluent source	Dionex EGC 500 KOH cartridge with CR-ATC 600 and Dionex high pressure degasser
Flow rate	0.35 mL/min
Injection volume	2.5 µL (full loop), 1/10 of ED channel
Column temperature	30 °C
Detection	Suppressed conductivity, Dionex ADRS 600 (2 mm) suppressor, recycle mode, 30 mA current
Detection temperature	25 °C
Sample tray temperature	4 °C
Run time	15 min

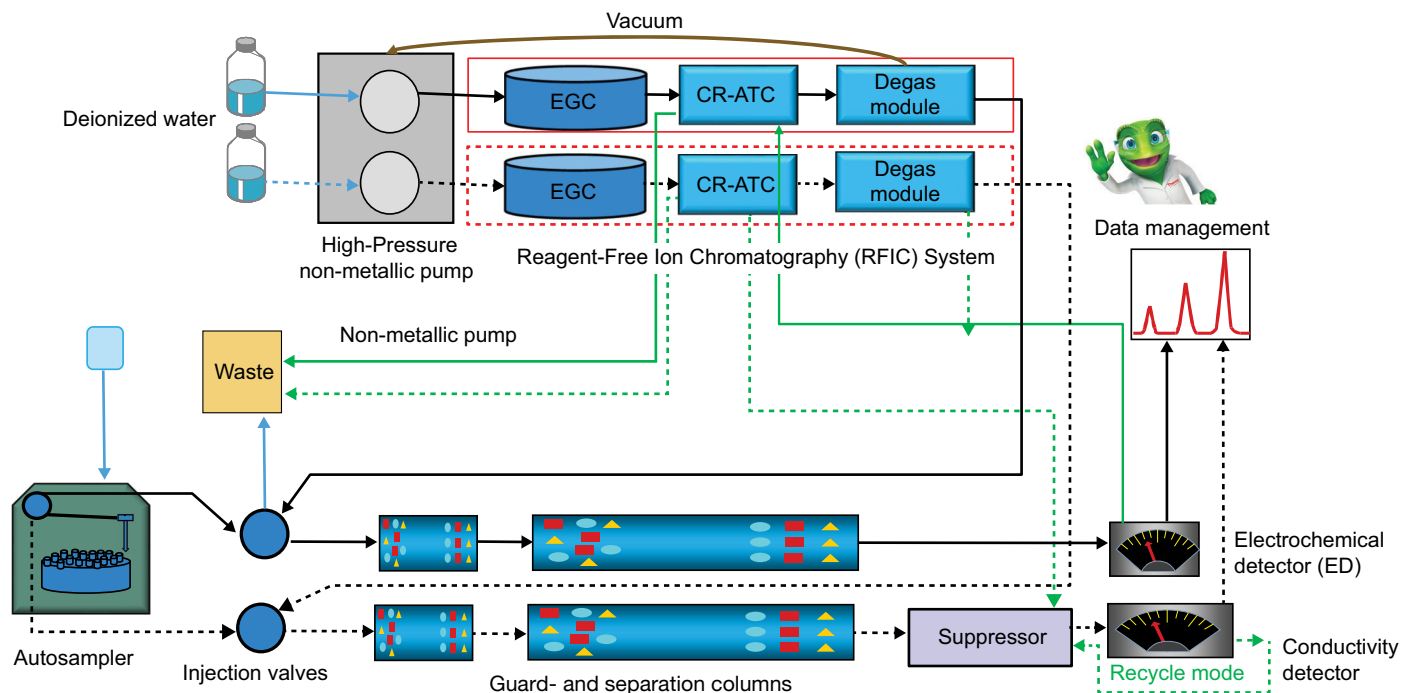


Figure 8. Illustration of the dual channel (ED+CD) RFIC system flow diagram

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