Determination of Total Cyanide in Municipal Wastewater and Drinking Water Using Ion-Exclusion Chromatography with Pulsed Amperometric Detection (ICE-PAD)

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Application Note 227

Introduction

Cyanide is a well known acute toxin that prevents cellular respiration by irreversibly binding with the iron in cytochrome C oxidase.[1,2] In addition, thiocyanate, which is metabolized from cyanide, interferes with iodine uptake by the thyroid gland, causing goiters and other long-term iodine deficiency diseases.[1] Cyanide is regulated as an environmental contaminant by the United States Environmental Protection Agency (EPA) for drinking water, surface water, and wastewater due to these health concerns.[3–5]

Total cyanide is defined by the EPA as free cyanide ion and complex cyanides that are converted to hydrocyanic acid (HCN) during strong acid digestion.[6] More recently, total cyanide also includes ferrocyanide and ferricyanide due to free cyanide formed by exposure to light.[7] For drinking and surface waters, the EPA has established a maximum contamination level (MCL) of 200 µg/L free cyanide determined by a total cyanide assay.[3] To determine total cyanide, the sample is digested with sulfuric acid to convert the cyanide to hydrogen cyanide gas, aspirated into a strong caustic solution, then assayed.

In wastewater, the EPA specifies cyanide discharge limits by industry and size of the facility (<38,000 or >38,000 liters per day).[8] The typical sources of cyanide contamination are industrial waste from plating and mining industries, burning coal and plastics, and effluent from publicly owned treatment works (POTW).[1,2] The EPA specifies 5.2 µg/L total cyanide continuous discharge limits for POTW and 22 µg/L maximum discharges into fresh water.[4,5] For salt water bodies, the continuous and maximum discharges are 1 µg/L total cyanide. The EPA defines these continuous (4 d) and maximum (1 h average) limits to ensure that aquatic life is unharmed.

Ninety percent of the cyanide in POTW influent and flow-through are attributed to the metal finishing and organic chemical industries.[2] However, many POTWs report that cyanide concentrations in wastewater effluents are higher than those from the influent levels.[9] Cyanide concentrations as high as 60 µg/L have been reported at discharge sites.[10] This cyanide generation is associated with chlorination and chloramination processes used for waste disinfection.[9,10] Nitrate formed from chlorination of ammonium creates unstable intermediates that degrade to cyanide during the harsh acid and temperature conditions typically used for acid-distillation in total cyanide determinations.

In EPA Methods 335.2, 335.3, and 335.4, samples are individually acid- or UV-digested to convert all cyanide compounds to hydrogen cyanide gas which is distilled into sodium hydroxide (pH 13). Total cyanide is then determined spectrophotometrically or by titration.[6,11,12] These methods are complicated, often requiring multiple distillation apparatuses, and they are subject to interference from high-pH solutions, oxidizers, and sulfur-containing compounds.[13] Chromatography methods, such as ion-exchange (IE) and ion-exclusion (ICE) can eliminate some of these interferences by separation. With IE chromatography, cyanide is not fully resolved from chloride and sulfide concentrations at mg/L levels. ICE is preferred because strong acid anions such as chloride and sulfate are excluded from the column, and cyanide is resolved from sulfide. Electrochemical detection by direct current (DC) amperometric, or pulsed amperometric detection (PAD), is sensitive, selective, and suitable

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for direct determinations of cyanide.[14,15] PAD is preferred over DC amperometry because in PAD, the working electrode is cycled through three or four voltage potentials every second, resulting in an electrode surface which is continually cleaned, whereas in DC amperometry, the working electrode can foul over time, leading to a loss in peak response.[13] In the previous PAD methods used to detect cyanide, the silver working electrode also detected chloride and was incompatible with samples containing mg/L concentrations of sulfide.[15] Using PAD with a Pt working electrode, chloride is not detected, and the Pt working electrode is stable with mg/L sulfide concentrations. None of the previous ICE-PAD methods were used to determine cyanide.[15–17] With this method, the authors combine the advantages of ICE with the sensitivity, selectivity, and stability of PAD using a Pt working electrode to directly detect cyanide without interferences from chloride and sulfide.

In this Application Note, the authors describe a method with PAD using a Pt disposable working electrode and a waveform optimized for determination of total cyanide in drinking and wastewater. Prior to analyses, the samples are acid distilled, trapped in 1 M NaOH, and diluted to 250 mM NaOH using the EPA-approved MICRO DIST® sample preparation system. This ICE-PAD method has the advantages of eluting cyanide before sulfide (Rs > 3) and excluding chloride and sulfate, which typically interfere in ion exchange methods. This ICE-PAD method provides a fast, reliable, sensitive, and selective method to directly determine µg/L to sub- µg/L concentrations of total cyanide in wastewater. The authors also demonstrate linearity, detection limits, accuracy, and precision for determination of total cyanide in drinking water and wastewater samples using the MICRO DIST system and ICE-PAD.

Experimental

Equipment

Thermo Scientific™ Dionex™ ICS-3000 Ion Chromatography system consisting of:

- Single Gradient Pump (SP) module with degas option
- Detector and Chromatography Module (DC) with single or dual heating zone, and 6-port injection valve
- Electrochemical Detector ED (P/N 061718)
- AS Autosampler with Sample Tray Temperature Controlling option, and 10 mL sample tray
- An electrochemical cell containing a combination pH–Ag/AgCl reference electrode (cell and reference electrode, P/N 061756) and a disposable (Pt) working electrode (package of six, P/N 064440)
- Thermo Scientific™ Dionex™ Chromeleon™ 6.8 Chromatography Data System (CDS) Workstation

Vial Kit, 10 mL polystyrene with caps and septa (P/N 055058)

Knitted reaction coil, 375 µL (P/N 043700), with two PEEK™ unions (¼-28 thread female to 10-32 thread female, P/N 042806)

MICRO DIST System for sample distillation (Lachat Instruments P/N MDD001) with user- filled tube kit (Hach Company, package of 100, P/N A17117), heating block, protective gloves, test tube racks, and a small mechanical press

Filter unit for vacuum filtration, 0.2 µm nylon (Thermo Scientific™ Nalgene™ Rapid-Flow™ with 90 mm filter, P/N 164-0020) or equivalent nylon filter

Vacuum pump

Syringe filter (Pall Gelman, GHP Acrodisc® 25 mm with 0.45 µm GHP membrane, P/N 4560T) or filter unit for sample filtration, 0.45 µm nylon (Nalgene Rapid Flow with 50 mm filter, P/N 153-0045) or equivalent nylon filter

PEEK Tubing:

- Red (0.127 mm or 0.005 in i.d., 5 ft, P/N 052310) tubing used for liquid line connections from injection valve to the guard and analytical columns, and cell
- Yellow (0.76 mm or 0.003 in i.d., 5 ft, P/N 052301) tubing used for system backpressure loop
- 50 µL PEEK sample loop (P/N 042950)

Reagents and Standards

Reagents

- Deionized water, Type 1 reagent grade, 18.2 MΩ cm resistivity, freshly degassed by ultrasonic agitation and applied vacuum
- Use only ACS reagent grade chemicals for all reagents and standards.
- Magnesium chloride, hexahydrate (VWR, P/N JT2444-1)
- Methanesulfonic acid (P/N 033478; Sigma-Aldrich, P/N 64280)
- pH 7 (yellow) buffer solution (VWR, P/N BDH5046)
- pH 4 (red) buffer solution (VWR, P/N BDH5018)
- Sodium cyanide, anhydrous (Sigma-Aldrich, P/N 20,522-2)
- Sodium hydroxide, 50% (w/w) (Fisher Chemicals, P/N SS254-500)
- Sulfuric acid (VWR, P/N JT9681-33)

For Interference Experiments

- Ammonium chloride (Sigma-Aldrich, P/N 213330, FW 53.49)
- Sodium cyanate (Sigma-Aldrich, P/N 185086, FW 65.01)
- Sodium sulfide, nonahydrate, >99.99% (Sigma-Aldrich, P/N 431648, FW 240.18)
- Sodium thiocyanate (Sigma-Aldrich, P/N 251410, FW 81.07)
- Sodium nitrate (Sigma-Aldrich, P/N SS506, FW 84.99)
- Sodium sulfate (Sigma-Aldrich, P/N 239313, FW 142.04)

Samples

Certified Wastewater Standard for cyanide, 40 µg/L total cyanide (20 µg/L free cyanide from potassium cyanide and 20 µg/L complexed cyanide from potassium ferricyanide in 0.5% potassium hydroxide) (High-Purity™ Standards, P/N CWW-CN-D).

Municipal wastewater effluent samples were collected at the same time and location. Sodium hydroxide was added to one of the samples immediately after collection.

A municipal drinking water sample stabilized with 2 g of 50% sodium hydroxide per 250 mL of sample.

Conditions

Column:	Thermo Scientific™ Dionex™ IonPac™ ICE-AG1 Guard column, 4 × 50 mm (P/N 067842) Dionex IonPac ICE-AS1 Analytical column, 4 × 250 mm (P/N 064198)
Flow Rate:	0.2 mL/min
Eluent:	50 mM methanesulfonic acid
Column Temperature:	30 °C
Tray Temperature:	10 °C
Inj. Volume:	50 µL
Detection:	PAD
Waveform:	See Table 1.
Reference Electrode:	pH-Ag/AgCl electrode (P/N 061879) in AgCl mode
Working Electrode:	Disposable Platinum
Typical Background:	70–120 nC
Typical System Backpressure:	2200 psi
Noise:	20–30 pC
Typical pH:	1.2–1.3
Run Time:	30 min

Table 1. Cyanide Detection Waveform Optimized for Acid Eluents17

Time (sec)	Potential vs. Ag/AgCl (V)	Gain Region[a]	Integration	Ramp[a]
0.00	+0.30	Off	Off	Ramp
0.31	+0.30	On	Off	Ramp
0.32	+1.15	On	Off	Ramp
0.64	+1.15	On	On (Start)	Ramp
0.66	+1.15	On	Off (End)	Ramp
0.67	–0.30	On	Off	Ramp
1.06	–0.30	Off	Off	Ramp
1.07	+0.30	Off	Off	Ramp

[a]The gain and ramp are instrument settings for the Dionex ICS-3000 IC electrochemical detector.

Preparation of Solutions and Reagents

When preparing eluents, it is essential to use high quality, Type 1 water (18.2 MΩ cm resistivity or better) that contains as little dissolved gas as possible. Dissolved gases can cause higher noise levels. Degas the deionized water before eluent preparation by using a Nalgene filter flask (P/N 164-0020) with 0.2 µm nylon filter with applied vacuum. Prepare 1 L of degassed Type 1 water weekly for the AS Autosampler flush solution.

Preparation of Eluent

To prepare 2 L of 50 mM methanesulfonic acid (MSA) eluent, pipette 4.5 mL (9.6 g) MSA (FW 96.10) into a 2 L glass eluent bottle containing 1993 g of Type 1 degassed, deionized water. Immediately cap the bottle, connect it to the Eluent A line, and place the eluent under ~4–5 psi of helium or other inert gas. Thoroughly mix the eluent solution and prime the pump with the new eluent.

Preparation of Standards

Warning: Cyanide is a poison by inhalation, contact, and ingestion. Solutions containing cyanide can generate hydrogen cyanide gas at neutral or acidic pH, and must be stabilized with base. Read and follow the material safety data sheet (MSDS) instructions for personnel handling, exposure, and disposal information. Consult local safety personnel for regulations concerning the proper disposal of cyanide. Add 100 mL of 50% NaOH into the system waste container. Hydrogen cyanide gas is created during the acid digestion of cyanide-containing samples. Conduct the acid digestion sample preparation in a well-ventilated hood.

Use high quality, 50% (w/w) sodium hydroxide solution for diluent preparation. Sodium hydroxide pellets are coated with sodium carbonate and cannot be used for this application.

Preparation of 100 mM Sodium Hydroxide Diluent

To prepare 250 mL of 100 mM sodium hydroxide (NaOH) diluent, pipette 1.3 mL (2.0 g) of 50% NaOH into a 250 mL HDPE bottle containing 248.7 g degassed Type 1 deionized water. Swirl the bottle gently to thoroughly mix the solution. Use this solution as the diluent for all cyanide standards. Prepare a fresh solution daily or as needed.

Cyanide Standards

To prepare a 1000 mg/L stock solution, weigh 0.189 g of reagent grade sodium cyanide into a 100 mL polyethylene bottle and dissolve thoroughly in 100 g of 100 mM NaOH diluent. Prepare an intermediate 1.0 mg/L cyanide standard by pipetting 50 µL of the 1000 mg/L stock solution into a 50 mL polyethylene bottle and diluting with 100 mM NaOH to a final weight of 50.00 g.

To prepare 1.0, 2.0, 5.0, 10, and 25 µg/L cyanide working standards from the 1.0 mg/L intermediate standard, pipette 20, 40, 100, 200, and 500 µL respectively, of the intermediate standard into 20 mL polyethylene bottles. Dilute these working standards with 100 mM NaOH to 20.00 g total weight. The stock solution and the intermediate standard are stable for more than a month when refrigerated. The working standards should be prepared daily.

Standards for Interference Experiments

As a test for positive interferences of cyanide methods, the American Society for Testing and Materials (ASTM) D19.06 Cyanide Task Group devised an ASTM Challenge Matrix stock solution,18 containing 17.8 mM ammonium chloride (FW 53.49), 17.8 mM potassium nitrate (FW 101.10), 49.4 mM sodium sulfate (FW 142.04), 5.95 mM potassium cyanate (FW 81.12), 2.6 mM potassium thiocyanate (FW 97.18), and 12 mM NaOH (1 mL of 12 M NaOH in 1 L). The Challenge Matrix working solution is a 10-fold dilution of the stock solution.

Individual interference stock solutions (Table 2) were prepared at 10 times the concentration of the ASTM Challenge Matrix Stock to facilitate preparation of individual interference solutions. Sulfide causes a negative interference with cyanide determinations in some methods, and was therefore added to the interference testing solution. Sulfide was prepared at the same molar concentration (17.8 mM) as nitrate, ammonium, and chloride. To prepare individual stock solutions (ammonium, chloride, cyanate, nitrate, sulfate, sulfide, thiocyanate), add the amount of reagent grade compound (Table 2) to a 100 mL polyethylene bottle and dilute with 100 g of deionized water.

To prepare separate or combined interference standards, dilute the stock solutions 100-fold with 100 mM NaOH by pipetting 200 µL of the stock solutions into 19.8 g of 100 mM NaOH. Prepare the combined 5 µg/L cyanide/19 mg/L sulfide interference standard by pipetting 100 µL of the 1 mg/L cyanide intermediate standard and 200 µL of the 1900 mg/L sulfide stock solution into 19.7 g of 100 mM NaOH.

Sodium sulfide solutions degrade quickly upon exposure to air. Prepare sulfide solutions from a new bottle of sodium sulfide nonahydrate solid and store at 4 °C, as degradation accelerates as temperature increases. The 1900 mg/L sulfide stock solution must be prepared every 2 weeks when stored at 4 °C. Sulfide solutions at concentrations <1 mg/L should be prepared every two days. With the 1900 mg/L sulfide stock solution, long-term stability can only be achieved by freezing at –10 °C.

Table 2. Amount of Compound Used to Prepare 100 mL of Individual Stock Solutions

Ion	Compound	Mass (g)	Concentration mg/L (mM)
Ammonium	Ammonium chloride (NH_4Cl, FW 53.49)	0.954	3220 (178)
Chloride			6320 (178)
Cyanate	Sodium cyanate (NaOCN, FW 65.01)	0.387	2500 (59.5)
Nitrate	Sodium nitrate ($NaNO_3$, FW 84.99)	1.51	11,000 (178)
Sulfate	Sodium sulfate (Na_2SO_4, FW 142.04)	7.03	4750 (494)
Sulfide	Sodium sulfide, nonahydrate ($Na_2S•9H_2O$, FW 240.18)	4.28	1900 (178)
Thiocyanate	Sodium thiocyanate (NaSCN, FW 81.07)	0.209	1500 (26)

Sample Preparation

Free cyanide is reactive and unstable, and therefore water samples should be stabilized at the time of collection. Oxidizing agents decompose free cyanide and any free cyanide present at neutral pH will volatilize to hydrogen cyanide. Sodium hydroxide solution (2 g of 50% [w/w]) was added to ~250 g of municipal drinking water samples for preservation. The cyanide certified wastewater (CWW) sample was prepared according to the instructions then diluted 10-fold by combining 10 mL of the prepared CWW sample with 90 mL 100 mM NaOH diluent. The municipal wastewater effluent samples were filtered with 0.45 µm syringe filters prior to sample digestion to remove particulate matter and bacteria. Control samples of 100 mM NaOH blank and 5 µg/L cyanide standard samples were prepared in the same manner. To filter samples >50 mL, the authors used the 150 mL Nalgene filter apparatus (0.45 µm, nylon).

Separate 1 µg/L cyanide spike recovery samples were prepared from the municipal drinking water samples by pipetting 20 µL of 1.0 mg/L cyanide standard into separate 20 mL polyethylene bottles containing 20 g of base-treated water sample. The 5 µg/L cyanide spiked samples of municipal wastewater effluent and the 10-fold dilution of the cyanide CWW samples were prepared similarly with 100 µL of 1.0 mg/L cyanide standard added into 20 g of sample.

Acid Digestion

The MICRO DIST sample preparation system uses a three-part tube (Figure 1) and a digestion block designed to hold 21 assembled tubes. The tube includes a polypropylene sample tube, hydrophobic membrane, and a polypropylene collector tube that contains the trapping solution and functions as a measuring tube. The membrane separates the sample tube from the collector tube and allows only the gaseous sample to pass into the trapping solution. During the initial experiments, both the prefilled (assembled with the trapping solution) and user-filled (unassembled without the trapping solution) tubes were tested. The user-filled collector tubes were used for the final development of this Application Note. During digestion at 120 °C, hydrogen cyanide gas is generated in the sample tube from the reaction of cyanide in the sample with 7.11 M sulfuric acid and 0.75 M magnesium chloride solution. Hydrogen cyanide gas passes through the sample membrane in the collector tube and is dissolved as cyanide in the 1 M NaOH trapping solution. After the 20 min digestion time, the tubes are removed from the heating block, the sample tube is discarded, and the collector tube is inverted to cool for 10 min. The condensate is collected off the walls of the collector tube by the trapping solution. To prepare the sample for dilution and analysis, the collector tube is broken at the breakaway point to yield a measuring tube (M). The distillation (D) half of the collector tube is discarded. The sample in M tube is diluted to 6 mL with deionized water for a final concentration of 250 mM NaOH.

MICRO DIST Solutions

Prepare the 7.11 M sulfuric acid/0.75 M magnesium chloride digestion and 1 M NaOH trapping solutions according to the MICRO DIST Cyanide-1 Method, 10-204-00-1-X19 instructions. Caution: Carefully prepare the sulfuric acid/magnesium chloride solution in the exhaust hood with the hood sash positioned between you and the acid. Concentrated sulfuric acid reacts exothermically with water, and at this concentration, the solution can exceed the boiling point of water and violently boil over and splatter. To minimize isolated hot spots and violent flashbacks, add the concentrated sulfuric acid (MW 98.08, 95.7%) slowly, in 50 mL increments, pouring down the side of the flask and mixing gently between additions. Cool to room temperature and dilute to the 1 L mark.

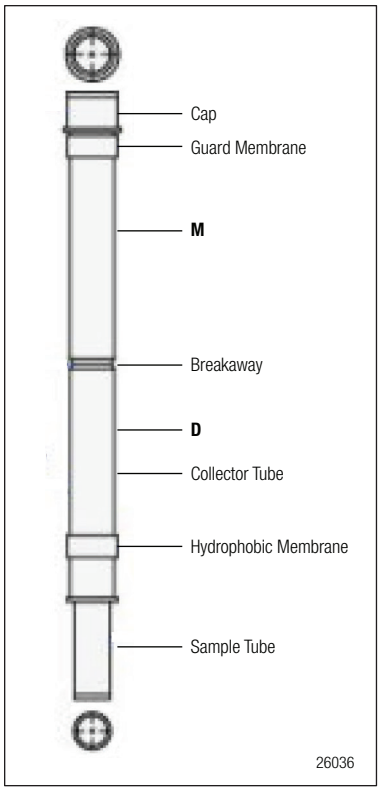

Figure 1. MICRO DIST tube assembly 14

MICRO DIST Acid Digestion

The cyanide samples, 100 mM NaOH blanks, and cyanide control standards were digested according to MICRO DIST Cyanide-1, Method 10-204-00-1-X. Each digestion experiment should include duplicate 100 mM NaOH blanks, control cyanide standards, and samples. Use the following procedure to digest the samples:

- Place the heater block in the exhaust hood, turn it on, and set the temperature to 120 °C. Allow at least 40 min for the heating block to stabilize.
- Rinse the MICRO DIST user-filled collector tubes (Figure 1) on both sides of the D side membrane with 1 mL each of acid and base solutions prior to use to minimize contamination.
- To assemble the collector tubes, first add 1.5 mL of 1 M NaOH trapping solution to the M side of the collector tubes, then cap the collector tube (M side) with the cap and a filter membrane. The cap must be securely attached and the filter must completely cover the top of the tube. The cap and filter are responsible for containing the final solution in the collector tube.
- Label the collector tubes on each side of the breakaway point.
- Place the collector tubes in the test tube rack with the M side up.
- Label the sample tubes, weigh 6.0 g sample into each tube, and place in the test tube rack.

The next three steps must be performed quickly:

- Add 0.6 mL of the 7.11 M sulfuric acid/0.75 M magnesium chloride to one sample tube and immediately place the assembled collector tube over the sample.
- To press-fit the tubes, place the tubes in the press (D side down), support the tubes around the breakaway mark, and pull the press lever down to smoothly press-fit the collector tube into place over the sample tube.
- Using the heat-protective gloves, place the fully assembled tube in the pre-stabilized heating block (D) side down, and digest at 120 °C for 30 min. Repeat with the other samples, blanks, and controls. The manufacturer recommends adding the tubes to the heat block within one minute.
- After the 30 min digestion, quickly remove the tube from the heating block using heat-protective gloves, remove the sample tube within 4 s, and quickly invert the collector tube (D side up). Discard the sample tube and the solution from the sample tube according to safety regulations. Remove the other tubes in the same manner.

- Allow to cool for 10 min.
- To rinse the condensate off the collector tube walls, gently tip the collector tube and the trapping solution until all of the condensate is collected. Tap the collector tube to collect any droplets clinging to the membrane.
- To break off the D side of the collector tube, firmly place both hands on both sides of the breakaway point and break the tube by pushing away. Place the M side of the collector tube into test tube rack. Discard the D side of the tube.
- Dilute to the 6 mL mark with deionized water. Swirl the sample to mix.
- Transfer the samples to the AS Autosampler sample vials.

As noted in the instructions, the digestion temperature and time, the condensation time, quick removal of the sample vial after digestion, and efficient rinsing of the condensate off the collector tube walls are critical to achieving good sample recovery.

System Preparation and Setup

The Dionex IonPac ICE-AS1 column should not exceed backpressure >1000 psi. Do not remove or install the ED module while the DC module is turned on, as power surges can cause internal damage to the ED module.

Configuring Virtual Channel to Monitor pH

It is useful to monitor and record the pH during sample analyses. To continuously record the pH during sample determinations, create a Virtual Channel in the Server Configuration program according to the instructions in AN 188.[15] (The pH virtual channel becomes one of the available signal channels.) More information on Virtual Channels can be found in the Chromeleon "help" program.

Plumbing the Chromatography System

CAUTION: Cyanide is converted to hydrogen cyanide, a toxic gas, at pH <9. Add concentrated NaOH to the waste container prior to starting the system to maintain the pH of the waste stream and to prevent evolution of gaseous hydrogen cyanide. Add 100 mL of 50% NaOH for each 5 gallons of waste. This will yield 5 gallons of NaOH at ~1–2x the MSA eluent concentration.

Use red PEEK (0.127 mm or 0.005 in i.d.) tubing for all eluent lines from after the injection valve to the cell inlet. Black PEEK (0.25 mm or 0.010 in i.d.) tubing can be used from the pump to injection valve. Install the Dionex IonPac ICE-AS1 column set according to the product manual.[20] Column pressure is typically ~850 psi, which is sufficiently below the recommended operating pressure limit of 1000 psi for this column. A 1000 psi backpressure loop can be installed between the pump and injection valve to further reduce system noise. Install the 375 µL knitted reaction coil between the Dionex IonPac ICE-AS1 column and the electrochemical cell as described in Dionex, now part of Thermo Fisher Scientific, Application Note (QN) 188: Determination of Glycols and Alcohols in Fermentation Broths Using Ion-Exclusion Chromatography and Pulsed Amperometric Detection.

Assemble the Electrochemical Cell

Assemble the electrochemical cell according the instructions in AN 188. In this application, the working electrode is a disposable platinum electrode. Typically, the background will stabilize within 10 min. However, a longer equilibration may be required when initially setting up the system.

Results and Discussion

Sample Preparation

Initial experiments with the MICRO DIST sample preparation system found total cyanide (1–2 µg/L) when 100 mM NaOH was used as a sample (blank) with either the prefilled collector tubes or as received user-filled tubes with lab prepared 1 M NaOH trapping solution. The source of the contamination was unknown but it is likely a non-cyanide contaminant related to the hydrophobic membrane in the collector tubes. As discussed previously, cyanide can be generated when nitrate and nitrite are present under acid-digestion conditions. This problem was eliminated for the user-filled collector tubes by pre-rinsing the sides of the tubes labled D and M with 1 mL of the 7.11 M sulfuric acid/0.75 M magnesium chloride solution and 1 M NaOH solution. These experiments illustrate the importance of control samples and standards in the acid-digestion sample preparation.

Separation

ICE achieves better separation of cyanide from other anions in the sample compared to ion-exchange chromatography. In ICE (also known as Donnan exclusion), the fully sulfonated ion-exchange resin acts as a semipermeable membrane with separating molecular species rather than ions.[21,22] Strong acid anions, such as chloride and sulfate, are excluded by Donnan exclusion on the stationary phase and pass quickly through the column.[22] While weak acid anions, such as cyanide and sulfide, are protonated by the strong acid eluent to neutral compounds. These neutral compounds are not excluded but instead partition in the aqueous phases within and between the resin beads and separate in the order of increasing pKas.[21]

With this method, cyanide was separated by ICE using a Dionex IonPac ICE-AS1, 4 × 250 mm column using 50 mM MSA at a flow rate of 0.2 mL/min and detected by PAD using a Pt disposable working electrode with an amperometric waveform optimized for acid eluents. Figure 2 shows the separation of 5 µg/L cyanide standard prepared in 100 mM NaOH. The cyanide peak is symmetrical (A_s = 1.1 (EP)) and elutes in 16 min.

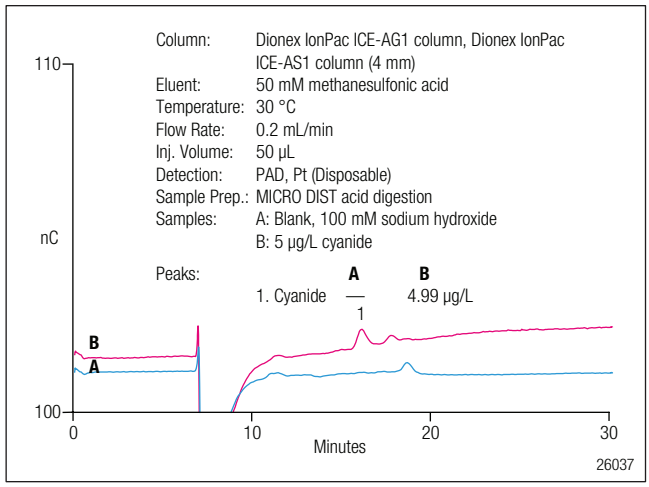

Figure 2. Comparison of A) Blank and B) 5 µg/L cyanide standard

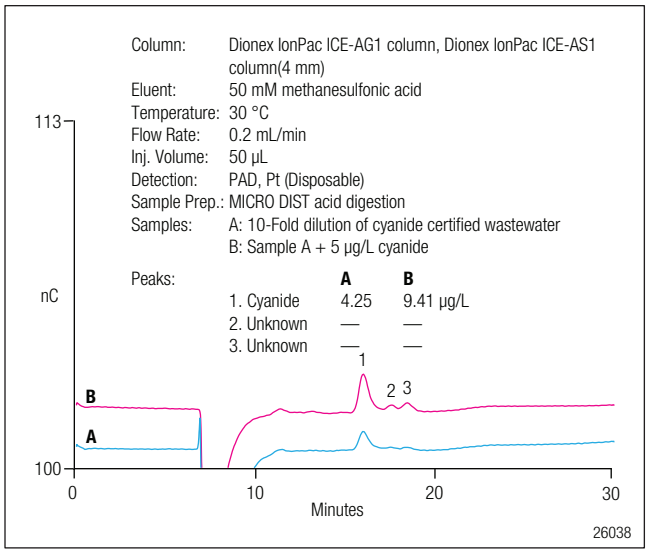

Figure 3. Comparison of A) 10-fold dilution of cyanide certified wastewater sample and B) Sample A with 5 µg/L cyanide added

Method Qualification

The authors determined the linearity and estimated limit of detection (LOD) to qualify the method. To determine the LOD, the peak to peak noise was determined per min in three consecutive runs of deionized water for 60 min each, resulting in an average noise of 19.8 pC. The LOD of 0.27 µg/L was determined by multiplying the Student's *t*-test value of 3.14 for 99% confidence limits and the standard deviation (0.0085) of seven replicate injections of 0.50 µg/L cyanide standard. The linearity of cyanide detection was determined by calibrating with triplicate injections of five standards from 1.0 to 25 µg/L cyanide and comparing the peak area response to concentration (r^2 = 0.9999).

Samples

The authors applied the method to acid-digested samples of CWW, municipal drinking water, and municipal wastewater effluent. To determine total cyanide in the CWW sample, the sample was diluted 10-fold to a certified concentration of 4 µg/L total cyanide. Recovery was 4.25 ± 0.07 µg/L cyanide, 6.3% higher than the total cyanide certified value (Figure 3, chromatogram A). The cyanide peak has similar peak shape as in the prepared standard in Figure 2 with two small unknown peaks eluting at approximately 17–18 min. Determination of total cyanide was also evaluated in the municipal drinking water and wastewater effluent samples. In this study, 0.67 ± 0.02 µg/L (n = 6) total cyanide was detected in the municipal drinking water after acid digestion (Figure 4, chromatogram A). Because municipal wastewater effluent samples are known to have high levels of bacteria and other particulates, both samples of the municipal wastewater effluent (with and without NaOH added) were filtered prior to acid digestion. Solutions of 100 mM NaOH and 5 µg/L cyanide prepared in 100 mM NaOH were also filtered as controls. The municipal wastewater effluent samples with and without base added during collection showed 5.99 ± 0.09 µg/L cyanide and no detectable cyanide (Figure 5), respectively. These results agree with previous reports that chloramine and chlorine disinfectant treatments used in POTWs generate unstable cyanide intermediates and that NaOH may stabilize these intermediate compounds.[9,10]

To determine the method precision, six replicate injections were performed using a 5 µg/L cyanide standard, a 10-fold dilution of CWW sample, and the same sample spiked with 5 µg/L cyanide. The calculated relative standard deviations (RSDs) ranged from 0.57 to 2.9%. The accuracy of the method was evaluated over three days by adding known concentrations of cyanide to the samples prior to acid digestion (Figures 3, 4, 5, chromatograms A, B, and C, respectively). Table 3 summarizes the results of this study. As shown, the method demonstrated good accuracy with average recoveries of 97.4–102%.

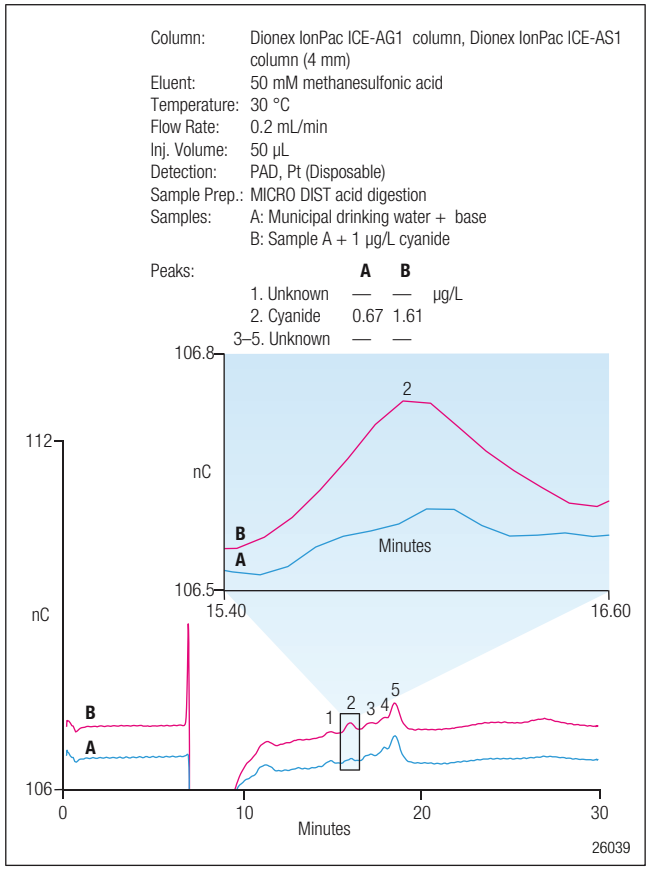

Figure 4. Comparison of A) Municipal drinking water and B) Sample A with 1 µg/L cyanide added

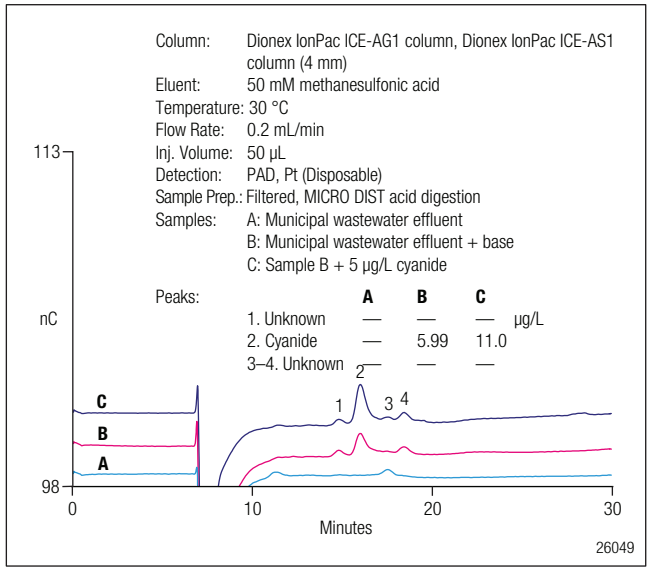

Figure 5. Comparison of A) Municipal wastewater effluent, B) second sample of A with base added, and C) Sample B with 5.0 µg/L cyanide added

Table 3. Average Cyanide Determinations Over Three Days

Sample	Amount Found (µg/L)[a]	Amount Added (µg/L)	Average Recovery[a] (%)
100 mM sodium hydroxide	<LOD	1.06	110 ± 6.4
Filtered 100 mM sodium hydroxide	<LOD	5.02	102 ± 1.0
10-fold dilution of certified cyanide wastewater sample (4.0 µg/L total cyanide)	4.25 ± 0.07	4.99	102 ± 0.9
Municipal drinking water	0.67 ± 0.02	0.99	97.4 ± 2.0
Filtered municipal waste-water effluent without base	<LOD	Not Tested	—
Filtered municipal waste-water effluent with base	5.99 ± 0.09	4.97	99.5 ± 1.0

[a]n = 6

Interferences

The ASTM Cyanide Task Group researched the effect of ions that can cause false positives for total cyanide and therefore developed a challenge matrix to evaluate results for various analytical methods.[18] The challenge matrix contains 95.4 mg/L ammonium chloride, 25 mg/L cyanate, 15 mg/L thiocyanate, 110 mg/L nitrate, and 475 mg/L sulfate. To determine the potential for false positives from the challenge matrix, the authors analyzed the challenge matrix samples for total cyanide and an undigested 25 µg/L cyanate sample for free cyanide. The experiments showed that the cyanide was generated from the acid digestion of ASTM challenge matrix primarily from cyanate (Table 4). No free cyanide was found in the undigested cyanate standards. Total cyanide concentrations increased when nitrate or thiocyanate was added to cyanate-containing samples then acid-digested. These results agree with the false positives previously reported in the literature and associated with acid-digestion and oxidation of thiocyanate and cyanate by nitrate to cyanide.[9,10] In wastewater treatment plants, nitrate is formed from chlorination of ammonium which reacts with other unstable intermediates to degrade to cyanide during acid digestion.

Sulfide is a known interferent with cyanide determinations and its presence in samples can yield poor recoveries. Sulfide concentrations at mg/L concentrations can foul the silver working electrode used in electrochemical methods and cause falsely high results in flow injection methods.[13] To determine whether sulfide interfered with accurate cyanide determinations, the authors analyzed a 19 mg/L sulfide sample spiked with 5.0 µg/L cyanide. Cyanide was fully resolved from sulfide, despite a cyanide-to-sulfide concentration ratio of 1:3800 (not shown), and the cyanide recovery was 99.2%. The results show that, unlike methods which use a silver working electrode or flow injection methods, sulfide does not interfere with accurate determinations of cyanide using the technique described in this application.

Table 4. Effect of Potential Interferences on Total Cyanide Determinations

Sample[a]	Average Cyanide Found (µg/L)
100 mM sodium hydroxide blank	<LOD
5 µg/L cyanide	5.03
ASTM challenge matrix	32.32
Ammonium chloride, cyanate, thiocyanate, and nitrate	36.20
Ammonium chloride, cyanate, and thiocyanate	21.29
Ammonium chloride, cyanate	26.31
Ammonium chloride, thiocyanate	0.44
Ammonium chloride	<LOD
Cyanate	16.21
Thiocyanate	<LOD

n = 2
[a]Interfering ions are the same molar concentrations as in the ASTM challenge matrix: 32.2 mg/L ammonium, 63.2 mg/L chloride, 25 mg/L cyanate, 110 mg/L nitrate, 47.5 mg/L sulfate, and 15 mg/L thiocyanate.[13]
ND is not detected.

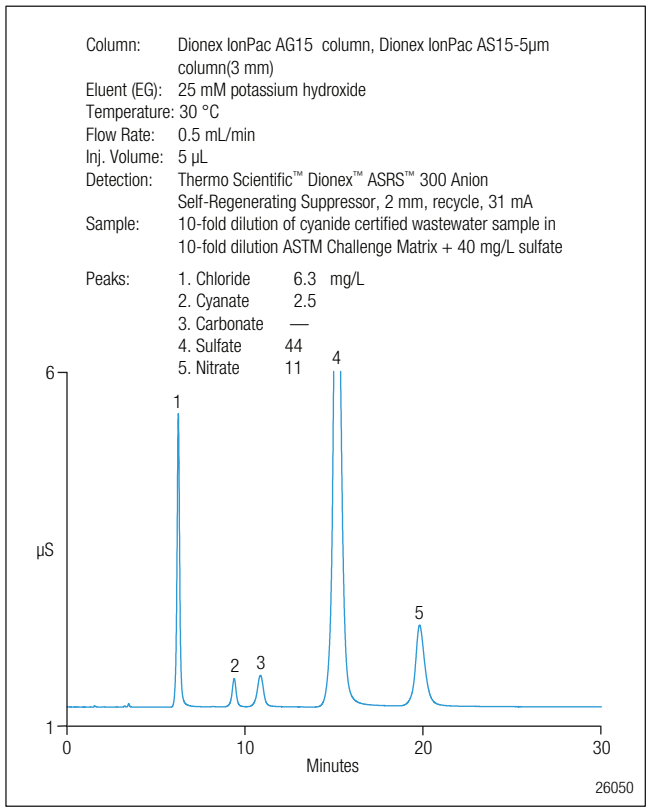

Figure 6. Determination of cyanate using a Reagent-Free™ IC system

The effect of nitrate, nitrite, cyanate, and thiocyanate interferences and other oxidizing agents can be minimized by pre-treating the samples with sulfamic acid or sodium arsenite prior to adding base for preservation and acid digestion.[23] The presence of interfering anions can be determined by IC with suppressed conductivity detection using Dionex, now sold under the Thermo Scientific brand, AN 154: Determination of Inorganic Anions in Environmental Waters Using a Hydroxide-Selective Column[24] for nitrite and nitrate, Dionex, now part of Thermo Fisher Scientific, AN 138: Determination of Thiosulfate in Refinery and Other Wastewaters[25] for thiocyanate, and AN 200[26] for cyanate determinations. Figure 6 shows the determination of 2.5 mg/L cyanate by the conditions in Dionex, now part of Thermo Fisher Scientific, AN 200: Direct determination of Cyanate in a Urea Solution and a Urea-Containing Protein Buffer, in a 10-fold dilution of the certified cyanide wastewater standard and ASTM matrix plus 40 mg/L of additional sulfate.

Robustness

To determine the robustness of the method, the authors evaluated the effects of Pt working electrodes (conventional and disposable electrodes within the same lot), eluent concentration, column temperature, and lot-to-lot column variation on 5.0 µg/L cyanide peak responses and retention times (Table 5). The results demonstrated that slight variations in eluent concentration, column temperature, and different working electrodes had little effect on the retention times of cyanide (<0.5%). Using a column from a different lot showed the greatest effect on retention time (+4.5%). In terms of the cyanide peak area, only nominal effects (<1.5%) were observed for the variables investigated in this study.

Conclusion

This Application Note describes an ICE-PAD method using the EPA-approved Lachat MICRO DIST acid digestion system to determine µg/L concentrations of total cyanide in municipal drinking water and municipal wastewater effluent. The method provides low detection limits and improvement of cyanide recoveries due to exclusion of chloride and resolution from sulfide. False positives from cyanate and thiocyanate in the presence of nitrate in POTW wastewater effluent are related to the POTW chloramination processes and the acid digestion conditions during sample preparation. The effect of these false positive interferences can be minimized by identifying the presence of nitrate and nitrite, thiocyanate, and cyanate by methods described in AN 154, AN 138, and AN 200, respectively, followed by pretreatment with sulfamic acid.

Parameter	Value	Retention Time[a] (min)	Difference (%)	Peak Area[a] (nC-min)	Difference (%)
Eluent Concentration (mM MSA)	47.5	15.92 ± 0.04	–0.3	0.377± 0.005	–0.8
	50	15.96 ± 0.02	—	0.380 ± 0.004	—
	52.5	15.91 ± 0.03	–0.3	0.380 ± 0.009	—
Column Temperature (°C)	28	16.01 ± 0.04	+0.3	0.385 ± 0.014	–1.3
	30	15.96 ± 0.02	—	0.380 ± 0.004	—
	32	15.89 ± 0.01	–0.4	0.373 ± 0.017	–1.8
Working Electrode	Conventional	15.96 ± 0.04	—	0.384 ± 0.006	+1.1
	Disposable Lot 080917	15.96 ± 0.02	—	0.380 ± 0.002	—
	Disposable Lot 080917	15.99 ± 0.03	+0.2	0.376 ± 0.005	–1.1
Column (Lot)	008-05-003	15.96 ± 0.02	—	0.380 ± 0.004	—
	008-05-092	16.68 ± 0.03	+4.5	0.385 ± 0.007	+1.3

[a]n = 6

Suppliers

Fisher Scientific, Pittsburgh, PA, USA, 800-766-7000 www.fisherscientific.com

Lachat Instruments, a Hach Company Brand, Loveland, CO, USA, 800-227-4224, www.hach.com

High-Purity Standards, Charleston, SC, USA, 843-767-7900, www.highpuritystandards.com

Sigma-Aldrich Corporation, St. Louis, MO, USA, 800-325-3010, www.sigmaaldrich.com

VWR International LLC, Radnor, PA, USA, 800-932-5000, www.vwrsp.com

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