# Anion Determinations in Municipal Wastewater Samples Using EPA Method 300.1 (A) on an Integrated Ion Chromatography System

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#### **Key Words**

Environmental, Dionex IonPac AS22, anions, Dionex ICS-1100, Dionex ICS-1600

## Goal

Demonstrate inorganic anion determinations in wastewater samples according to U.S. EPA Method 300.1 (A) using a Dionex IonPac AS22 column and an integrated IC system

## Introduction

The determination of common inorganic anions in drinking, surface, and wastewaters is an important ion chromatography (IC) application worldwide. In the U.S. water quality is regulated under the Safe Drinking Water Act (SDWA) to control the safety and cleanliness of drinking water and the Clean Water Act (CWA) to minimize the pollutants discharged into surface or groundwaters.<sup>1,2</sup> IC has been approved for compliance monitoring of inorganic anions in drinking water since the 1980s through the U.S. EPA Method 300.0 and updated in 1997 under the U.S. EPA Method 300.1. In 1995, IC was adopted for wastewater analysis under the U.S. EPA Office of Water and the National Pollution Discharge Elimination System (NPDES) permits program.<sup>2-4</sup>

Thermo Scientific<sup>™</sup> Dionex<sup>™</sup> Application Note AN 135: Determination of Inorganic Anions in Wastewater by Ion Chromatography, was written in 2000 and discussed separations on a Thermo Scientific<sup>™</sup> Dionex<sup>™</sup> IonPac<sup>™</sup> AS14 column.<sup>5</sup> Here we discuss the use of a Dionex IonPac AS22 anion-exchange column to separate and quantify inorganic anions with manually-prepared carbonate/bicarbonate eluents on a Thermo Scientific Dionex ICS-1100 IC system. This method updates



AN 135 by reducing run time and increasing sample throughput from 34 to 40 samples per 8-h shift. The Dionex IonPac AS22 column is the latest column technology for this application, by providing better resolution and higher capacity. Here this method uses suppressed conductivity detection with either chemical suppression for greater ruggedness and lower noise in complex samples or electrolytic suppression for easeof-use and convenience in all but the most complex of samples. The Dionex ICS-1100 IC system further offers the flexibility advantages by operating either suppressor type mode as needed or desired.

## Equipment

- Dionex ICS-1100 Integrated IC system\*
- Thermo Scientific Dionex AS-DV Autosampler
- Thermo Scientific<sup>™</sup> Dionex<sup>™</sup> Chromeleon<sup>™</sup> Chromatography Data system (CDS), version 7.2
- \* This application can be run on any Dionex ion chromatography system capable of delivering 1.2 mL/min flow rates.



## **Reagents and Standards**

- 18 M $\Omega$ -cm resistivity degassed deionized water
- Thermo Scientific<sup>™</sup> Dionex<sup>™</sup> Combined Seven Anion Standard II, NIST traceable (P/N 057590)
- Thermo Scientific Dionex IonPac AS22 Carbonate/ Bicarbonate Concentrate (100×) (P/N 063965)

# For chemical regeneration only

• Thermo Scientific Dionex Anion Regenerant Solution Concentrate (20×) (P/N 039203)

#### **Samples**

Municipal wastewater samples

#### Conditions

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Columns	Dionex lonPac AG22 (4 $\times$ 50 mm), Dionex lonPac AS22 (4 $\times$ 250 mm)			
Eluent	4.5 mM Sodium carbonate, 1.4 mM sodium bicarbonate			
Flow Rate	1.2 mL/min			
Inj. Volume	5 µL			
Detection	Suppressed conductivity			
Suppressor	A: Thermo Scientific <sup>™</sup> Dionex <sup>™</sup> AERS <sup>™</sup> 500 Anion Electrolytic Regenerating suppressor, recycle mode, 31 mA			
	B: Thermo Scientific <sup>™</sup> Dionex <sup>™</sup> AMMS <sup>™</sup> 300 MicroMembrane <sup>™</sup> suppressor, chemical suppression mode; 25 mN sulfuric acid at 5–10 mL/min			
Background Conductance	~ 22 µS			
System backpressure	~ 1800 psi			

#### Standard and Sample Preparation

It is important to use 18 M $\Omega$ -cm resistivity, deionized (DI) water for standards and eluent. It is also recommended to degas the DI water intended for eluent used for anion determinations (an appropriate degassing method is vacuum filtration). Using less pure DI water can reduce sensitivity, introduce contamination, and affect calibration, thereby resulting in inaccurate quantification.

Prepare standards by diluting the NIST-traceable Dionex Combined Seven Anion Standard II with DI water. Concentrations for nitrate and nitrite should be reported as mg/L nitrogen ( $NO_3$ -N and  $NO_2$ -N, respectively). *o*-Phosphate concentrations are reported as mg/L phosphorous ( $PO_4$ -P).

The samples were filtered  $(0.2 \ \mu m)$  and diluted with DI water prior to injection.

# **Eluent Preparation**

Using manually prepared eluent is an economical approach for IC. However, manually prepared eluents can create variability due to day-to-day and operator-tooperator differences. Care must be taken to maintain fresh eluent concentrates and to properly dilute concentrates. An alternative to manually prepared eluents is Eluent Generation which electrolytically generates precise and accurate eluent inline for isocratic or gradient separations. Eluent Generation is applied in Thermo Scientific Dionex Application Note AN 154: Determination of Inorganic Anions in Environmental Waters Using a Hydroxide-Selective Column<sup>6</sup> and is also approved for compliance monitoring for U.S EPA Methods 300.0 and 300.1. Eluent Regeneration can also be used for this application following the conditions in this Application Update.

#### Table 1. Consumables list.

Product name	Description	Part Number				
Dionex IonPac AG22 guard column	Anion-exchange guard column, $4\times50~\text{mm}$ for carbonate eluent separations	064139				
Dionex IonPac AS22 column	Anion-exchange analytical column, $4\times250$ mm, for carbonate eluent separations	064141				
Using Electrolytic Suppression						
Dionex AERS 500 suppressor	Anion suppressor for 4 mm i.d. columns using electrolytic suppression	082540				
Using Chemical Suppression						
Dionex AMMS 300 suppressor	Anion suppressor for 4 mm i.d. columns using chemical suppression mode	064558				
Anion regenerant solution concentrate	0.5 N sulfuric acid regenerant concentrate solution. Dilute 20-fold with deionized water.	039203				
External regeneration installation kit	Kit for chemical regenerant suppressor mode. Includes a 4 L regenerant reservoir, pressure regulator, tubing, and fittings.	038018				
Thormo Scientific Diopox AS DV Autocomplet viale 5 ml	PolyVials and 20 $\mu m$ filter caps, 250 each	038141				
	PolyVials and plain caps, 250 each	038008				
4 L eluent bottle	4 L eluent bottle recommended for convenience	066019				

# 4.5 mM Sodium Carbonate, 1.4 mM Sodium Bicarbonate Eluent Preparation

To prepare 4 L of eluent, pipet 20 mL of the Dionex IonPac AS22 Carbonate/Bicarbonate Eluent Concentrate into a 2 L volumetric flask containing ~1000 mL of previously degassed 18.2 MΩ-cm resistivity DI water. Gently swirl to mix. Dilute to the 2 L mark with degassed DI water, stopper, and invert the flask at least three times to mix. Transfer the eluent to a 4 L eluent bottle and prepare another 2 L of eluent in the same manner. Connect the bottle to the instrument eluent line and prime the pump to draw the new eluent through the eluent line and pump. For the best chromatography, equilibrate the system at starting conditions for 30 min prior to running samples and check standards. If running Eluent Regeneration the system can be left running for up to 28 days non-stop with a single 4 L bottle of eluent to ensure the system is always at equilibration. See the RFIC-ER Startup Kit documentation (Doc. No. 065295) for further details.

#### **Regenerant Preparation, 25 mN Sulfuric Acid**

In chemical regeneration mode, a 25–50 mN sulfuric acid chemical regenerant is needed at 5–10 L/min for the 4 mm Dionex AMMS 300 suppressor. To prepare the 25 mN sulfuric acid regenerant solution, dilute the Dionex Anion Regenerant Solution ( $0.5 \text{ N H}_2\text{SO}_4$ ) 20-fold by transferring 100 mL into a 2 L volumetric flask containing ~1 L of DI water. Swirl gently to mix, dilute to the 2 L mark with DI water, cap, and invert the flask to mix the solution. Transfer to the 4 L regenerant reservoir. Prepare and mix an additional 2 L in the same manner and transfer to the regenerant reservoir. Install the pressurized regeneration kit as described in the Instrument Setup section.

Note: The 25 mN sulfuric acid regenerant solution is not needed when using a Dionex AERS 500 suppressor with electrolytic suppression.

# **Instrument Setup**

# **Electrolytic Suppression**

Hydrate the Dionex AERS 500 suppressor according to the product manual instructions.<sup>7</sup> Allow 20 min for the suppressor membranes to fully hydrate.

#### **Chemical Suppression**

To set up this application using the Dionex AMMS 300 suppressor, install the chemical regenerant reservoirs pressurized with air or gas to generate a 5-10 mL/min flow rate. Connect 1/4" i.d. tubing from the regenerant reservoir to the Regen In port on the suppressor and a similar length tubing directed from the Regen Out port on the suppressor to waste. Hydrate the 4 mm Dionex AMMS 300 suppressor according to the product manual and Quick Start instructions by pumping 5 mL of eluent through the eluent channel and allowing 5 mL of regenerant to flow through the regenerant channel.8 Turn off the pump and the pressure regulator on the regenerant reservoir. Allow 20 min for the suppressor membranes to fully hydrate. When using chemical suppression, the regenerant solution should always be flowing through the suppressor when the IC pump is on.

# Installation of Column and Suppressor

Install and condition the columns for 30 min while temporarily directing the eluent leaving the column to waste. Install the fully hydrated suppressor and a 25 psi backpressure loop at the CD conductivity cell Eluent Out port. Connect one end of a waste line to the backpressure loop and direct the free end to a waste container. Complete the installation of the columns in the Dionex ICS-1100 or ICS-1600 Integrated IC system according to Figure 1. For more detailed instructions, refer to the cited product and operator's manuals.<sup>7-12</sup>



\*\* Recycle mode with Dionex AERS 500 suppressor

Figure 1. Flow diagram for the Dionex ICS-1100 or Dionex ICS-1600 (shown) Integrated IC system.

#### **Results and Discussion**

In this method, seven inorganic anions in wastewater samples were separated on a 4 mm i.d. Dionex IonPac AS22 carbonate eluent anion-exchange column and detected by suppressed conductivity. This column was designed for faster separations to meet compliance monitoring of inorganic anions using U.S. EPA Method 300.1 and for those customers preferring carbonate eluent separations. The ion-exchange chemistry was optimized to provide excellent resolution of the inorganic anions while maintaining fast isocratic separations (12 min run time). This method saves 2 min of run time compared to the Dionex IonPac AS14A column, which allows an increase of sample throughput from 34 to 40 samples per 8 hour day. The Dionex IonPac AS22 column replaces the Dionex IonPac AS4A-SC, Dionex IonPac AS12A, Dionex IonPac AS14, and Dionex IonPac AS14A columns, which are also approved for compliance monitoring. The listed columns, with the exception of Dionex IonPac AS4A-SC column, improved upon the separation of acetate and fluoride which was problematic on the initial U.S. EPA Method 300.0 designated column, the Dionex IonPac AS4A column.

Table 2 shows the comparison of the Dionex IonPac AS22 column to the Dionex IonPac AS14 column (AN 135). The Dionex IonPac AS22 column has greater than 3× the capacity of the Dionex IonPac AS14 column with a shorter run time. This increased capacity allows high resolution separations especially with high ionic strength samples or large loop injections.

Table 2. Comparison of Dionex columns.

	Dionex IonPac AS14 column	Dionex IonPac AS22 column			
Bead Type	Macroporous	Supermacroporous			
Bead size	9 µm	6 µm			
Porosity	1000 Å	2000 Å			
Cross-linking	55% Divinylbenzene (DVB)	55% DVB			
Capacity	65 µeq	210 µeq			

Figure 2 compares the same concentration standard separated with the optimum conditions for the Dionex IonPac AS14 and Dionex IonPac AS22 columns. In Figure 2A a 50  $\mu$ L sample was separated at 1.2 mL/min with all eight anions eluting within 14 min. In contrast, the Dionex IonPac AS22 column requires two minutes less to elute the same anions (Figure 2B). The elution order for both columns is the same, indicating similar selectivity, which allows easy implementation of this method. The Dionex IonPac AS22 column chemistry was optimized to elute the carbonate peak so that it is well resolved from any other inorganic anion peak, whereas the carbonate peak previously eluted prior to chloride and often resulted in interference with the chloride peak.



Figure 2. Separation of nine anions.

In Thermo Scientific Dionex Application Update AU 196: Anion Determinations in Municipal Drinking Water Samples Using EPA Method 300.1 (A) on an Integrated IC System,<sup>13</sup> the chromatograms of eight inorganic anions were compared on the Dionex IonPac AS14 and Dionex IonPac AS22 columns using suppressed conductivity detection and electrolytic suppression. Here, we show separations with an alternate method using chemical suppression. Using an electrolytic suppressor, such as the Dionex AERS 500 electrolytically regenerated suppressor, has the advantages of continuous suppression with greater thermal stability and faster recoveries after stopping and restarting operations. These advantages are obtained without the labor intensive preparation of chemical regenerants. Using a chemical suppressor, such as the Dionex AMMS 300 micromembrane suppressor, has the advantages of improved chemical robustness and low noise.

#### Method Evaluation

To evaluate the method, the Linear Calibration Ranges (LCR), method reporting limits (MRL), 4-day reproducibility, and spike recoveries in samples and the laboratory blank (LB) were determined. MRL standards were prepared by fortifying the LB with a concentrated standard to achieve a  $3-5 \times$ S/N response and the MRLs were calculated according to the U.S. EPA Method 300.1(A) by multiplying the standard deviation (n = 7) and 3.14 t-test factor. The MRL results were similar to those report in AU 196: 0.035 mg/L fluoride, 0.072 mg/L chloride, 0.073 mg/L NO<sub>2</sub>-N, 0.25 mg/L bromide, 0.055 mg/L NO<sub>2</sub>-N, 0.29 mg/L PO<sub>4</sub>-P, and 0.22 mg/L sulfate. To evaluate the reproducibility of this method, a 5-fold dilution of Dionex Combined Seven Anion Standard II (4 mg/L fluoride; 20 mg/L chloride, nitrite, nitrate, and sulfate; 40 mg/L phosphate) was injected continuously for four days while monitoring the retention times and peak areas for all seven analytes. The reproducibilities were similar to those reported in AU 196 with RSDs <0.6 and <0.7 for retention times and peak areas, respectively.

# Sample Analysis

The method was applied to six wastewater samples from two municipal water treatment facilities (Figure 3, Table 3). The results show that the samples primarily contain chloride and sulfate with <1 mg/L of fluoride and phosphate. As part of the wastewater treatment process, toxic ammonium is oxidized to less toxic nitrate and nitrite to minimize environmental disturbance during discharge. As expected, nitrate-N is present in the treatment and effluent samples.



Figure 3. Separation of anions in wastewater samples.

Sample	Fluoride (mg/L)	Chloride (mg/L)	ride NO <sub>3</sub> -N PO <sub>4</sub> -P /L) (mg/L) (mg/L)		Sulfate (mg/L)	
Influent, City 1	$0.155 \pm 0.009$	45.1 ± 0.3	45.1 ± 0.3 Not detected 0.977 ± 0.052		$5.01 \pm 0.06$	
RSD	5.49	0.60		5.37	1.15	
Process Sample, City 1	$0.202 \pm 0.006$	36.9 ± 0.1	7.22 ± 0.03	$0.830 \pm 0.010$	15.9 ± 0.1	
RSD	3.13	0.26	0.36	0.36 3.01		
Effluent, City 1	$0.235\pm0.008$	54.5 ± 0.1	1.45 ± 0.0 0.702 ± 0.031		$16.0 \pm 0.1$	
RSD	3.31	0.14	0.54	4.4	0.34	
Influent, City 2	$0.236 \pm 0.005$	$42.4 \pm 0.2$	Not detected	—	$19.2 \pm 0.075$	
RSD	2.3	0.43			0.39	
Process Sample, City 2	$0.233 \pm 0.012$	$44.4 \pm 0.2$	2.31 ± 0.01	—	$19.0 \pm 0.1$	
RSD	5.29	0.53	0.50		0.34	
Effluent, City 2	$0.235 \pm 0.008$	43.1 ± 0.1	2.55 ± 0.013	—	20.4 ± 0.1	
RSD	3.41	0.33	0.54		0.48	

Table 3. Analysis results of 5-fold diluted wastewater samples.

n = 7

#### Sample Accuracy

# To determine method accuracy, the Laboratory Blank (LB) and 5-fold diluted wastewater samples were fortified with known concentrations of fluoride, chloride, nitrate, phosphate, and sulfate and analyzed for the total concentrations (Table 4). The recovery results, 93 to 107%, are well within the U.S. EPA 300.1 accuracy requirements (Table 4) and indicate an accurate method.

This method, using the high capacity Dionex IonPac AS22 column, provides similar selectivity, but faster separations than previously shown in AN 135. This application meets U.S. EPA Method 300.1 (A) requirements for determinations of inorganic anions in municipal wastewater samples. Chemical suppression as shown here, is recommended for its ruggedness and lower noise, often needed when analyzing samples with complex matrices. In contrast, electrolytic suppression provides continuous suppression without the need for additional chemicals. Electrolytic suppression has the advantages of convenience, ease-of-use, and labor savings, and meets the needs of all but the most complex of sample matrices. Both suppressor types can be operated on the Dionex ICS-1100 IC integrated system, thus offering a low cost IC solution while having the flexibility to operate either chemical or electrolytic suppression.

	Fluoride		Chloride		NO <sub>3</sub> -N		P0₄-P		Sulfate	
	(%)	RSD	(%)	RSD	(%)	RSD	(%)	RSD	(%)	RSD
LB+	102	0.49	106	0.41	95.6	0.72	104	2.99	101	0.67
Influent, City 1+	99.9	0.73	105	0.23	103	0.25	113	2.45	97.4	0.26
Process Sample, City 1+	105	1.17	101	0.23	104	0.25	107	3.17	92.8	0.25
Final Effluent	99.3	0.71	111	0.54	101	0.33	107	3.06	99.8	0.24
MRL to 10 $\times$ MRL^{\scriptscriptstyle ++}	75 to 125% recovery									
10× MRL to highest standard <sup>++</sup>	85 to 115% recovery									

Conclusion

#### Table 4. Spike recoveries of wastewater samples.

<sup>+</sup> Added 0.9 mg/L fluoride, 35 mg/L chloride, 40 mg/L nitrate (9.03 mg/L nitrate-N), 2 mg/L phosphate (0.652 mg/L phosphate-P), and 20 mg/L sulfate.

++ Acceptable recovery ranges specified by U.S. EPA Method 300.1 (A).

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