

## Determination of Inorganic Anions by Ion Chromatography

### 1. Scope:

This document provides a procedure for analyzing liquid and solid fertilizer samples for inorganic anions using ion chromatography. The anions that are analyzed by this method include bromide, chloride, fluoride, nitrate, nitrite, phosphate, sulfate, and thiosulfate.

### 2. Principle:

Samples are prepared according to RA-SP-SMPL-PREP. Water soluble inorganic anions are extracted by sonication. Sample containing sulfates are digested in acid using a hot block. Anions are eluted on an ion chromatograph using a strong base solution and separated based on their affinity for the exchange sites on the analytical column. The separated anions are measured by an electrical conductivity detector and are identified based on their retention times compared to known standards.

### 3. Safety:

The SDS for all chemicals shall be read before performing any part of this method. Gloves, safety glasses, and a lab coat shall be worn when handling hazardous materials and reagents.

Hydrochloric acid is very toxic and extremely corrosive and shall be used in a fume hood. The acidification of samples containing reactive materials may result in the release of toxic gases and can be exothermic. Sample acidification and digestion shall be performed carefully in a fume hood.

### 4. Definitions:

**Initial Calibration Verification (ICV)** – A mid-level standard that is analyzed after the calibration standards that is obtained from a different vendor than calibration standards.

**Continuing Calibration Verification (CCV)** – A mid-level standard used to demonstrate the ongoing instrument performance and is analyzed after every 10 samples, and at the end of the analytical sequence.

**Calibration Blank (CB)** – Solution used to prepare calibration standards and is analyzed after the ICV and CCV standards to evaluate carryover.

**Matrix Blank/Reagent Blank (MB/RB)** – 1g of water that is subjected to the entire extraction process.

**Quality Control Sample (QC)** – A NIST or Magruder sample that is prepared and analyzed with each set to demonstrate the accuracy of the test.

**Method Detection Limit (MDL)** – The minimum concentration of an analyte that can be reported and measured with 99% confidence that the analyte concentration is greater than zero and is determined in a given matrix using this method.

**Reporting Limit (RL)** – The lowest defined or specified concentration that can be reported that accounts for variances in matrices with a reasonable degree of accuracy and precision.

## 5. Interferences:

- 5.1. Interference can be caused by substances with retention times that are similar and overlap those of the anion of interest. Large amounts of an anion can interfere with the peak resolution of an adjacent anion. Sample dilution can be used to solve most interference problems associated with retention times.
- 5.2. Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts or elevated baseline in ion chromatograms.

## 6. Equipment and Supplies (equivalents are acceptable):

- 6.1. Dionex ICS 6000 Ion Chromatograph (IC) System
- 6.2. Cation column (Dionex 088582)
- 6.3. Cation guard column (Dionex 088583)
- 6.4. Anion column (Dionex 076036)
- 6.5. Anion guard column (Dionex 076037)
- 6.6. Analytical balance capable of weighing 0.1 mg
- 6.7. Mechanical shaker (SPEX Sample Prep 2010)
- 6.8. Sonicator with temperature control (VWR 250HT)
- 6.9. Hot Block (Environmental Express SC151)
- 6.10. 100mL polypropylene sample bottles with caps (Environmental Express SC490)
- 6.11. 15mL polypropylene sample tubes with caps (VWR 10026-076)
- 6.12. 50mL polypropylene sample bottles (Environmental Express UC474)
- 6.13. Disposable watch glass (Environmental Express SC610)
- 6.14. FilterMate PTFE certified filter and plunger (Environmental Express SC0408)
- 6.15. Disposable funnels (Evergreen Scientific 208-5136-030)
- 6.16. 0.22µm membrane filter (GVS Filter Technology FJ13BNPNY002AD01)
- 6.17. 1mL syringe (VWR BD301025)
- 6.18. 1.5mL split septum sample vial kit (Dionex 055427)
- 6.19. pH indicator strips 4.5 – 10 (J.T.Baker 4395-01)

## 7. Standards and Reagents (equivalents are acceptable):

- 7.1. Standard anion stock mix, 20µg/mL F<sup>-</sup>, 30µg/mL Cl<sup>-</sup>, 100µg/mL each Br<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup> and 150µg/mL each PO<sub>4</sub><sup>3-</sup> and SO<sub>4</sub><sup>2-</sup> (Inorganic Ventures IC-FAS-1A)

- 7.2. Secondary source calibration check anion standard mix, 20µg/mL F<sup>-</sup>, 30µg/mL Cl<sup>-</sup>, 100µg/mL each Br<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup> and 150µg/mL each PO<sub>4</sub><sup>3-</sup> and SO<sub>4</sub><sup>2-</sup> (Thermo Scientific 056933)
- 7.3. Thiosulfate calibration standard, 1000µg/mL (Inorganic Ventures ICS2O31)
- 7.4. Hydrochloric acid, ultrapure trace metal grade (Fisher A508-P500)
- 7.5. LCMS grade water (Fisher # W6-4) or 18 MΩ deionized water – **All water used in this method is LCMS grade water, 18 MΩ deionized water, or equivalent.**
- 7.6. HPLC grade ammonium hydroxide (1M)

## 8. Standard/QC Preparation:

- 8.1. Stock standards are purchased from an ISO 17034 or accredited vendor and are accompanied by a certificate of analysis. Standards are stored at room temperature.
- 8.2. Calibration standards are dilutions of stock standards and other calibration standards and are made with water. See Table 1 for suggested standard preparation.
- 8.3. The ICV solution is prepared by diluting 1.25mL of the anion standard solution mix from 7.1 into 20mL of water.
- 8.4. The CCV is a mid-level calibration standard and is run after every 10 samples.
- 8.5. A QC, RB and MB are prepared with every set.

## 9. Sample Extraction for fertilizer sample

- 9.1. Water soluble inorganic anions (F<sup>-</sup>, Cl<sup>-</sup>, Br<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup> and S<sub>2</sub>O<sub>3</sub><sup>2-</sup>).
  - 9.1.1. Mix each sample thoroughly before weighing. Weigh 0.5g of sample in a labelled 50mL plastic bottle.
  - 9.1.2. Add 50mL of water and cap bottle tightly.
  - 9.1.3. Shake sample using mechanical shaker at 750 rpm for ~10 minutes.
  - 9.1.4. Sonicate in the ultrasonic bath for ~10 minutes.
  - 9.1.5. Allow sample to cool to room temperature and dilute to volume with water if needed.

- 9.1.6. Use a syringe fitted with a 0.22 $\mu$ m filter to filter sample before injecting on the IC.
- 9.2. Hot Block acid digestion for sulfate ( $\text{SO}_4^{2-}$ ) anions
  - 9.2.1. Mix each sample thoroughly before weighing. Weigh 0.5g of sample in a labelled 100mL plastic bottle. For samples with high guarantees, it may be necessary to use less sample (eg., 0.3g)
  - 9.2.2. Add 15mL of water and 10mL of concentrated HCl to each sample. Cap and allow to pre-digest overnight in a fume hood.
  - 9.2.3. Turn on the hot block and set the temperature to 145°C.
  - 9.2.4. Remove the cap from the sample, place a watch glass on top and put in the hot block. Reflux for ~70 minutes without boiling (avoid drying).
  - 9.2.5. Remove samples from the hot block and allow to cool for ~5 minutes in a fume hood. (Note: Sulfur crystals could form if sample cools completely).
  - 9.2.6. Rinse watch glass with water and collect the rinsate in the digestion bottle. Discard the watch glass.
  - 9.2.7. While the sample is warm, carefully transfer to a 100mL volumetric flask. Rinse the digestion bottle at least 3 times with water and transfer rinsate to the flask. Add water to the 100mL mark.
  - 9.2.8. Use pH indicator strips to test the sample pH. Adjust the pH between 8-9 using 1M  $\text{NH}_4\text{OH}$  or an equivalent base solution.
  - 9.2.9. Samples may be stored at room temperature at this point if needed.
  - 9.2.10. Filter sample extract using 0.22 $\mu$ m membrane filter syringe before injecting on the IC.

## 10. Instrument Preparation and Calibration:

- 10.1. If the instrument is turned off, prime the pump to ensure there is no gas entering the column.
- 10.2. Verify that the eluent reservoir bottle is filled with fresh water.
- 10.3. Empty the instrument waste container if needed.
- 10.4. Verify the pump, the eluent generator, and the conductance cell is turned on.

- 10.5. At instrument start up, verify the pump is on and the flow rate is 0.25mL/min with a pressure of at least 2200 psi.
- 10.6. Allow the instrument to warm up for at least 30 minutes to ensure the baseline is stable. Verify the conductivity detector signal (CD) is less than 1 before proceeding with the analysis.
- 10.7. The instrument is calibrated each day of operation using the CB and at least 5 calibration standards to establish analytical curves.

## 11. Sample Analysis

### 11.1. IC Operating Conditions:

Flow rate: 0.25 mL/min  
Injection Volume: 20  $\mu$ L  
ASRS: On  
Conductivity Cell Temp: 30°C

#### Gradient Eluent Schedule

0 to 9	min	20 mM
9 to 9.1	min	45 mM
9.1 to 18	min	20 mM

- 11.2. The analytical sequence should follow this format to meet all quality control criteria:
  - Calibration Standards
  - CB
  - ICV
  - RB
  - MB
  - QC
  - Up to 10 samples
  - CCV
  - RB
  - Can repeat up to 10 samples followed by a CCV and RB as many times as necessary if instrument remains in calibration.
- 11.3. After the initial calibration is completed and the ICV and CB results are within the acceptance criteria, samples may be run. The acceptance criteria are discussed in section 12.
- 11.4. If the response for the peak exceeds the working range of the system, dilute the sample with an appropriate amount of reagent water and reanalyze. Flush the system with reagent water to prevent carryover.

## 12. QA/QC:

- 12.1. The correlation coefficient ( $r^2$ ) of the curve shall be  $\geq 0.990$ . If the calibration fails, repeat the calibration and any sample analysis. If it fails a second time, investigate, prepare the standards again and recalibrate.
- 12.2. The ICV recovery shall be 90-110%. It may be reanalyzed twice but must be successful twice in succession. If it fails, recalibrate and repeat the analysis. If it fails, investigate, and prepare a fresh ICV standard, recalibrate, and repeat the analysis.
- 12.3. The QC recovery shall be within 2 standard deviations of the certified value for Magruder sample. For spiked matrix, recovery shall be within 20%. If the QC fails it is reinjected. If it is still outside the acceptable range, the set is re-extracted.
- 12.4. The retention time of a peak shall differ from the standards by no more than  $\pm 0.25$  minutes.
- 12.5. QC recoveries shall be control charted with every batch and monitored for trends.
- 12.6. Any analytes detected in the RB or MB shall be below the reporting limit. If not, the problem must be resolved before continuing.
- 12.7. The CCV recovery shall be 85-115%. If it fails, the analysis can be repeated. If it fails again, the instrument must be recalibrated and samples re-analyzed.
- 12.8. The reporting limit is determined from the 0.5g sample weight and the lowest calibration standard and is 0.1% for all anions.

## 13. Calculations:

- 13.1. Anion concentration (final concentration) is calculated as follows:

$$\text{Anion Conc. } (\mu\text{g/mL}) = \frac{C \times V \times D}{W}$$

Where     C = Concentration from instrument  
             V = Final volume (mL) after sample extracted and brought to volume  
             D = Dilution factor (if applicable)  
             W = Initial weight of sample (grams)

- 13.2. ICV recoveries are calculated as follows:

$$\text{ICV\%} = \frac{\text{ICV recovery } \mu\text{g/mL}}{\text{ICV theoretical } \mu\text{g/mL}} \times 100$$

13.3. CCV recoveries are calculated as follows:

$$\text{CCV}\% = \frac{\text{CCV recovery } \mu\text{g/mL}}{\text{CCV theoretical } \mu\text{g/mL}} \times 100$$

13.4. To convert  $\text{SO}_4^{2-}$  concentration to sulfur from sulfate, divide the  $\text{SO}_4^{2-}$  concentration by 3 (MW sulfate / MW sulfur = 96 / 32 = 3).

13.5. To convert  $\text{S}_2\text{O}_3^{2-}$  concentration to sulfur from thiosulfate, divide the  $\text{S}_2\text{O}_3^{2-}$  concentration by 1.752 (MW thiosulfate / MW sulfur = 112.13 / 64 = 1.752).

13.6. To convert  $\text{NO}_3^-$  concentration to nitrogen from nitrate, divide the  $\text{NO}_3^-$  concentration by 4.43 (MW nitrate / MW nitrogen = 62 / 14 = 4.43).

13.7. No conversion is required for chloride

#### 14. Instrument Maintenance:

- 14.1. As needed or on a weekly basis refill the eluent reservoir with deionized water.
- 14.2. Before use, check the instrument for leaks or spills. Wipe up spills, isolate and repair leaks.
- 14.3. Before use, check the columns and pump for leaks. The pump flow should be 0.25mL/min and the pressure above 2200 psi.
- 14.4. Before use, check fluid lines for crimping or discoloration. Relocate any pinched lines and replace damaged lines.
- 14.5. Replace the columns and eluent generator cartridge when chromatography degrades or when expired.

#### 15. References:

- 15.1. United States Environment Protection Agency. Method 300.0 Determination of Inorganic Anions by Ion Chromatography  
[https://www.epa.gov/sites/default/files/2015-08/documents/method\\_300-0\\_rev\\_2-1\\_1993.pdf](https://www.epa.gov/sites/default/files/2015-08/documents/method_300-0_rev_2-1_1993.pdf)
- 15.2. Dionex ICS-6000 Ion Chromatography System Operator's Manual  
<https://assets.thermofisher.com/TFS-Assets/CMD/manuals/man-22181-97002-ics-6000-man2218197002-en.pdf>

Table 1: Calibration Standard Preparation

Concentration ( $\mu\text{g/mL}$ )								
Std Level	Final Vol. (mL)	Vol. *Anion Mix (mL)	** Vol. $\text{S}_2\text{O}_3^{2-}$ 100 $\mu\text{g/mL}$ (mL)	$\text{F}^-$	$\text{Cl}^-$	$\text{SO}_4^{2-}$ $\text{PO}_3^{4-}$	$\text{NO}_3^-$ , $\text{NO}_2^-$ , $\text{Br}^-$	$\text{S}_2\text{O}_3^{2-}$
5	20	5	5	5	7.5	37.5	25	25
4	20	2.5	2.5	2.5	3.75	18.75	12.5	12.5
3	20	1.25	1.25	1.25	1.875	9.375	6.25	6.25
2	20	0.625	0.625	0.625	0.938	4.688	3.125	3.125
1	20	0.313	0.313	0.313	0.47	2.348	1.565	1.565

Note: Standards can be made at different concentrations and volumes as necessary.

\* Anion mix stock concentration 20 $\mu\text{g/mL}$   $\text{F}^-$ , 30 $\mu\text{g/mL}$   $\text{Cl}^-$ , 100 $\mu\text{g/mL}$   $\text{Br}^-$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$  and 150 $\mu\text{g/mL}$  each  $\text{PO}_4^{3-}$  and  $\text{SO}_4^{2-}$

\*\* 1:10 dilution of thiosulfate calibration standard from 7.3



**Approvals:**

**Written By:**

*Dinesh Chand*  
Dinesh Chand  
Environmental Scientist

10/11/21  
Date

**Approved By:**

*Stacy Aylesworth*  
Stacy Aylesworth  
Senior Environmental Scientist (Supervisor)

10/11/21  
Date

*Maryam Khosravifard*  
Maryam Khosravifard  
Environmental Program Manager I

10/11/21  
Date

*Sarva Balachandra*  
Sarva Balachandra  
Quality Assurance Officer

10/28/21  
Date

