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.

Continuously Regenerated Trap Column (CR-TC) – An electrolytically regenerated trap column that removes all ionic contaminants from the deionized feed water, providing low drift during gradient operations.

Detector/Chromatography Module (DC) – The DC provides two temperature-controlled compartments (upper and lower) for the chromatography components.

Eluent Generator (EG) – The EG generates high purity acid or base eluents from deionized water.

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

Reagent Blank (RB) – 1g of water not subjected to the extraction process.

Suppressor – The suppressor reduces the eluent conductivity and enhances the conductivity of the sample ions, thereby increasing detection sensitivity.

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^-, 30µg/mL Cl^-, 100µg/mL each Br^-, NO_3^-, NO_2^- and 150µg/mL each PO_4^{3-} and SO_4^{2-} (Inorganic Ventures IC-FAS-1A)

7.2. Secondary source calibration check anion standard mix, 20µg/mL F^-, 30µg/mL Cl^-, 100µg/mL each Br^-, NO_3^-, NO_2^- and 150µg/mL each PO_4^{3-} and SO_4^{2-} (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^-, Cl^-, Br^-, NO_3^-, NO_2^- and $S_2O_3^{2-}$).

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 LCMS grade or 18 MΩ deionized water and cap bottle tightly.

9.1.3. Sonicate in the ultrasonic bath for ~10 minutes.

9.1.4. Shake sample using mechanical shaker at 750 rpm for ~10 minutes.

9.1.5. Allow sample to cool to room temperature. Use a syringe fitted with a 0.22µm filter to filter sample before injecting on the IC.

9.2. Hot Block acid digestion for sulfate (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 rinseate 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 NH_4OH 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 0.22µm membrane filter syringe before injecting on the IC.

10. Diluting Samples:

10.1. The sample anion concentration must fall within the range of the minimum and maximum values of the calibration curve per respective anion:

Chlorides: 0.470 – 7.500 µg/mL
Nitrates: 1.565 – 25.000 µg/mL
Sulfates: 2.348 – 37.500 µg/mL

10.2. If the anion concentration of your sample is below the lower limit of the calibration range, analyze the sample again to confirm.

10.3. If the anion concentration of the sample exceeds the upper limit of the calibration range, the sample must be diluted to bring the anion concentration in range. Choose a target anion concentration that is mid-range in the calibration curve from step 10.1 (e.g. 4 µg/mL for Cl^{-}, 12 µg/mL for NO_3^{-}, 18 µg/mL for SO_4^{2-}). Divide the given anion concentration by the chosen target anion concentration to determine the dilution factor needed for this sample to fall within the calibration range. Refer to Table 2 when diluting the sample.

11. Instrument Preparation and Calibration:

11.1. Verify that the eluent reservoir bottle is filled with fresh LCMS grade water or 18 MΩ deionized water.

11.2. Empty the instrument waste container if needed.

11.3. Flush the buffer loop on the autosampler.

11.4. Prime the pump after opening the priming valve on the secondary pump head and setting the purge rate to 6.0 mL/min for 20 minutes (1200 seconds). After priming, close the priming valve. Do not overtighten.

11.5. Verify the pump is turned on and the flow rate is set to 0.25 mL/min.

11.6. Verify the EG is turned on and switch the CR-TC to ON.

11.7. Verify the DC is turned on and switch the suppressor and CD to ON.

11.8. Allow the instrument to warm up for ~10 minutes to ensure the baseline is stable. Verify the conductivity detector signal (CD) is less than 1 before proceeding with the analysis.

11.9. The instrument is calibrated each day of operation using the CB and at least 5 calibration standards to establish analytical curves.

12. Sample Analysis

12.1. IC Operating Conditions:

Flow rate: 0.25 mL/min
Injection Volume: 20 µL
ASRS: On
Conductivity Cell Temp: 30ºC

Gradient Eluent Schedule

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

12.2. 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.

12.3. 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.

12.4. 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.

13. QA/QC:

13.1. The correlation coefficient (r^2) of the curve shall be ≥ 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.

13.2. 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.

13.3. 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.

13.4. The retention time of a peak shall differ from the standards by no more than ±0.25 minutes.

13.5. QC recoveries shall be control charted with every batch and monitored for trends.

13.6. Any analytes detected in the RB or MB shall be below the reporting limit. If not, the problem must be resolved before continuing.

13.7. 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.

13.8. The reporting limit is determined from the 0.5g sample weight and the lowest calibration standard and is 0.1% for all anions.

14. Calculations:

14.1. 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)

14.2. ICV recoveries are calculated as follows:

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

14.3. CCV recoveries are calculated as follows:

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

14.4. To convert SO_4^{2-} concentration to sulfur from sulfate, divide the SO_4^{2-} concentration by 3 (MW sulfate / MW sulfur = 96 / 32 = 3).

14.5. To convert $S_2O_3^{2-}$ concentration to sulfur from thiosulfate, divide the $S_2O_3^{2-}$ concentration by 1.752 (MW thiosulfate / MW sulfur = 112.13 / 64 = 1.752).

14.6. To convert NO_3^- concentration to nitrogen from nitrate, divide the NO_3^- concentration by 4.43 (MW nitrate / MW nitrogen = 62 / 14 = 4.43).

14.7. No conversion is required for chloride

15. Instrument Maintenance:

15.1. As needed or on a weekly basis refill the eluent reservoir with deionized water.

15.2. Before use, check the instrument for leaks or spills. Wipe up spills, isolate and repair leaks.

15.3. Before use, check the columns and pump for leaks. The pump flow should be 0.25mL/min and the pressure above 2200 psi.

15.4. Before use, check fluid lines for crimping or discoloration. Relocate any pinched lines and replace damaged lines.

15.5. Replace the columns and eluent generator cartridge when chromatography degrades or when expired.

16. References:

16.1. 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

16.2. 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 (µg/mL)				
Std Level	**Final Vol. (mL)**	**Vol. *Anion Mix (mL)**	**** Vol. $S_2O_3^{2-}$ 100 µg/mL (mL)**	**F^-**	**Cl^-**	**SO_4^{2-} PO_3^{4-}**	**NO_3^-, NO_2^-, Br^-**	**$S_2O_3^{2-}$**
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 20µg/mL F^-, 30µg/mL Cl^-, 100µg/mL Br^-, NO_3^-, NO_2^- and 150µg/mL each PO_4^{3-} and SO_4^{2-}

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

Table 2: Dilution Factor reference table

Dilution Factor	**Water* (mL)**	**Sample (mL)**	**Total Volume (mL)**
x200	9.95	0.05	10
x100	9.90	0.10	10
x50	9.80	0.20	10
x20	9.50	0.50	10
x10	9.00	1.00	10
x5	8.00	2.00	10

*Water must be LCMS grade or 18 MΩ deionized water.

Approvals:

Written By:

Tad Naquin

Tad Naquin
Agricultural Biological Technician

08/16/2024
Date

Approved By:

Elsa Poon

Elsa Hei Man Poon
Senior Environmental Scientist (Supervisor)

8/16/24
Date

Teresa Bowers

Teresa Bowers
Environmental Program Manager I

8/16/2024
Date

Sarva Balachandra
Quality Assurance Officer

8/16/24
Date

Revision Log:

Date	What was Revised? Why?
8/15/2024	Section 4 - Added more definitions for analyst clarity
	Section 9 – specified water parameters for method operation, placed sonication step before shaking, removed dilution statement because a new section about dilutions has been added
	Section 10 – added section with instructions on how to dilute samples if needed
	Section 11 – clarified to better reflect IC startup procedure
	Table 2 – added dilution factor table for operator reference