Determination of Sulfate, Chloride, Bromide, and Nitrate Using the DIONEX Ion Chromatography System

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1. SCOPE AND APPLICATION

This SOP is applicable to the determination of chloride, sulfate, bromide, and nitrate in aqueous matrices (fresh, surface, ground, effluents, influents, and saline water).

- For chloride, the calibration range is 0.25 – 120 mg Cl/L, the MDL is 0.20 mg Cl/L, and the PQL is 0.50 mg Cl/L.
- For sulfate, the calibration range is 0.25 – 80 mg SO_4/L, the MDL is 0.20 mg SO4/L, and the PQL is 0.50 mg SO4/L.
- For bromide, the calibration range is 0.020 – 1.0 mg Br/L, the MDL is 0.013 mg Br/L, and the PQL is 0.040 mg Br/L.
- For nitrate, the calibration range is 0.080 – 10 mg N/L, the MDL is 0.050 mg N/L, and the PQL is 0.15 mg N/L.

The calibration ranges may be extended by dilution.

Tests covered by this SOP are W-CL-IC(-F), W-SO4-IC(-F), W-Br-IC(-F), and W-NO3-IC(-F).

2. SUMMARY OF THE METHOD

2.1. This method is based on EPA method 300.0, Rev. 2.1 (1993).

2.2. Anions of interest are separated in an ion chromatograph using an anion-exchange column and potassium hydroxide eluent. Once separated, the anions pass through an anion suppressor where they are converted to their more conductive acid forms and the potassium hydroxide eluent is converted to less conductive water. The separated anions in their acid forms are measured by a conductivity detector and are identified based on their retention times as compared to standards.

2.3. Depending on the needs of the analysis, two analysis methods are available: chloride, sulfate, and nitrate analysis on the AS18-4 µm 2x150 mm analytical column, and chloride, sulfate, and bromide analysis on the AS19-4 µm 2x250 mm analytical column. In both methods, the injection volume is set to 2.5 µL. Potassium hydroxide is used in both systems. Each system uses a column guard to protect the analytical column. For the AS18 method, a Carbonate Removal Device (CRD) is installed after the suppressor to remove carbonate from the anion mixture. The AS19 method does not use the CRD.

2.4. Ion chromatography eliminates the need to use hazardous reagents and it effectively distinguishes among the halides (Br^-, Cl^-, and F^-) and the oxy-anions (SO_3^-, SO_4^-, NO_2^-, and NO_3^-).

2.5. For a description of possible interferences, see 300.0, Rev. 2.1 (1993).

3. EQUIPMENT AND SUPPLIES

3.1. Instrumentation – Dionex Ion Chromatography System (ICS-5000+):

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Component	Manufacturer	Model	Application Notes
Pump (DP)	ThermoSci Dionex	ICS-5000+ Dual Pump	Flow rate: 0.38 mL/min
Eluent Generator (EG)	ThermoSci Dionex	ICS-5000+ EG	Reagent Free IC(RFIC) automated eluent generation
Detector/Chromatography Module (DC)	ThermoSci Dionex	ICS-5000+ DC	High-pressure 6-port valves Temperature: 30°C Dionex ICS-5000 Conductivity Detector
Autosampler (AS)	ThermoSci Dionex	AS-AP	2.5 µL injection volume
Software	ThermoSci Dionex	Chromeleon 7.2	

3.2. Equipment:

Equipment	Manufacturer/Vendor[1]	Model/Catalog Number
Autosampler J-clip	ThermoSci Dionex	074976
Ultrasonic Bath	Fisherbrand	CPX8800
Analytical Balance	Mettler Toledo	XSR105
Analytical Balance	Denver Instrument	XS-410
Stirrer/Hot Plate	Corning	PC-420
Stir bar	Fisher (SR)	14-513-61
Drying Oven	Thermo Scientific	Lindberg Blue M
Desiccator Cabinet	Fisherbrand	08-645-6
Adjustable Pipette, 2–20 µL	Eppendorf/Fisher	Research Plus, 3123000039
Adjustable Pipette, 10–100 µL	Eppendorf/Fisher	Research Plus, 3123000047
Adjustable Pipette, 100–1000 µL	Eppendorf/Fisher	Research Plus, 3123000063
Adjustable Pipette, 1–10 mL	Eppendorf/Fisher	Research Plus, 3123000080
Volumetric Flask, Class A, Assorted	Fisher (SR)	10-212B; 10-210-5D; 10-210-5E; 10-212E; 10-212-5L
Or Equivalent		
[1]**Note:** (SR) designates equipment is typically ordered through the in-house stockroom		

3.3. Consumables and frequently replaced supplies:

Supplies/Consumables	Vendor[1]	Catalog Number
Analytical Column, AS-18-4µm, 2x150 mm	ThermoSci Dionex	076036
Analytical Column, AS-19-4µm, 2x250 mm	ThermoSci Dionex	083223
Guard Column, AG-18-4µm, 2x30 mm	ThermoSci Dionex	076037
Guard Column, AS-19-4µm, 2x50 mm	ThermoSci Dionex	083225
Eluent Generator Cartridge, EGC 500 KOH	ThermoSci Dionex	075778 (see 4.1.2.)
Anion Trap Column, CR-ATC 500	ThermoSci Dionex	075550
Suppressor, ADRS 600 2 mm	ThermoSci Dionex	088667
Carbonate Removal Device, CRD 200 2mm	ThermoSci Dionex	062986
6-port High Pressure Valve Kit	ThermoSci Dionex	075974
Autosampler Syringe, 250 µL	ThermoSci Dionex	074306
Autosampler Syringe, 1000 µL	ThermoSci Dionex	074307

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Autosampler Needle, PEEK coated	ThermoSci Dionex	069914
Autosampler Needle Cone and Bolt (black)	ThermoSci Dionex	062978; 074322
Autosampler Buffer Loop 1200 µL (green)	ThermoSci Dionex	074998
Autosampler Sample Transfer Line (blue)	ThermoSci Dionex	074990
Autosampler Lines' Bolts and Ferrules (tan and clear)	ThermoSci Dionex	071358; 052230; 062511
PEEK Tubing, Red, 0.005" id, 5 ft	ThermoSci Dionex	052310
PEEK Tubing, Black, 0.010" id, 5 ft	ThermoSci Dionex	052306
PEEK Tubing, Green, 0.030" id, 5 ft	ThermoSci Dionex	052305
HPIC bolt for PEEK tubing, Beige	ThermoSci Dionex	22000-98001
HPIC ferrule for PEEK tubing, Beige	ThermoSci Dionex	043276
Vial Kit, 1.5 mL Polypropylene (100/pk)	ThermoSci Dionex	079812 (incl. caps/septas)
Vial Kit, Polysytrene 10 mL (100/pk)	ThermoSci Dionex	074228 (incl. caps/septas)
Vials, 2 mL Polypropylene (100/pk)	Fisher (SR)	03-376-900 (vials only)
Sample Beakers, Polypropylene, Assorted	Fisher (SR)	08-732-119; 08-732-120; 08-732-122
Centrifuge Tubes, Polypropylene, 15 mL	Fisher (SR)	05-538-59A
Syringe, Polypropylene, Assorted	Fisher (SR)	22-204-500; B303134
Syringe Filters, Polypropylene, 25mm (0.45 µm pore size)	Fisher (SR)	05-713-404A
Standard Bottles, Nalgene, 250 mL	Fisher (SR)	05-719-359B
Standard Bottles, Nalgene Amber, 1L	Fisher (SR)	03-313-8F
Transfer Pipet, 5 mL	Fisher (SR)	13-711-5AM
Weigh Dish, Hexagonal, Assorted	Fisher (SR)	02-202-102; 02-202-100
Weigh Dish, Aluminum, 110 mm	Fisher (SR)	08-732-100
Parafilm	Fisher (SR)	13-374-12
Kimwipes, Assorted	Fisher (SR)	06-666A; 06-666C
Or Equivalent		
[1]**Note:** (SR) designates supply is typically ordered through the in-house stockroom		

4. REAGENTS AND STANDARDS (SEE DEP SOPS NU-041 AND NU-042)

Note: Prior to using the chemicals in this analysis, read their MSDS and the safety notes found in section 11 of this SOP.

Note: Reagent and standard preparations must be documented in the Laboratory Information Management System (LIMS) 'Standard Preparation Tracker' module. Unique ID numbers for each standard and reagent are generated in the module and documented in the standard and reagent sheets submitted with each analysis folder. If available, a Certificate of Analysis for each purchased standard or reagent is maintained on the Nutrients drive in: \REAGENT_PREP\IC\ folder. All containers holding standards or reagents must be labeled with their unique ID and expiration date.

Note: The volumes prepared for the reagents and standards may be altered by the analyst as necessary. The final concentrations of the constituents of a standard or reagent must not be changed.

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4.1. Chemicals:

	Chemical	Alias	Manufacturer[1]	Catalog Number	Purity	Expiration[2]	Storage
4.1.1.	DI water	H_2O	In house	N/A	N/A	N/A	N/A
4.1.2.	KOH Eluent Generator	KOH	Dionex	075778	Concentrated cartridge	See Label	Room Temp
4.1.3.	Potassium Chloride	KCl	Fisher Chemical	P217-500	ACS, ≥99%	See COA	Room Temp
4.1.4.	Potassium Sulfate	K_2SO_4	Fisher Chemical	P305-500	ACS, ≥99%	See COA	Room Temp
4.1.5.	Potassium Nitrate	KNO_3	Fisher Chemical	P383-100	See COA	See COA	Room Temp
4.1.6.	Bromide 1000 mg Br/L Stock Standard	Stock Bromide	Ricca	1180-4	See COA	See COA	Fridge
4.1.7.	Sodium Carbonate Anhydrous	Na_2CO_3	Fisher Chemical	S263-500	ACS, ≥95%	See COA	Room Temp
4.1.8.	Potassium Phosphate Monobasic	KH_2PO_4	Fisher Chemical	P285-500	ACS, ≥99%	See COA	Room Temp
4.1.9.	Fluoride 100 mg F/L Stock Standard	Fluoride Stock	Ricca	3171-32	ACS, ≥99%	See COA	Room Temp
4.1.10.	Nitrite 1000 mg N/L Stock Standard	NO2 ALT Stock	Ricca	R5444900	See COA	See COA	Fridge
4.1.11.	ALTQC Mineral Standard	ALTQC (Cl, SO_4, NO_3)	Inorganic Ventures	QCP-MIN-500	See COA	See COA	Fridge after opening
4.1.12.	Bromide ALTQC Stock Standard, WasteWatR	Br ERA Stock	ERA	769	See COA	See COA	Room Temp
	Potassium Bromide	KBr	Fisher chemical	P205	See COA	See COA	Room Temp
Or Equivalent [1]**Note:** (SR) designates chemical is typically ordered through the in-house stockroom							

4.2. Chloride stock standard, 10,000 mg Cl/L –

Potassium Chloride (4.1.3.)	21.0282 g
DI water (4.1.1.)	to 1000 mL

Before preparation, dry at least 23 g of KCl for 24 hours at 105ºC. Cool to room temperature in desiccator after drying period finished.

In a 1 L volumetric flask containing approx. 800 mL of DI water, dissolve 21.0282 g of dried KCl. Bring to volume with DI water and invert to mix thoroughly.

Store in refrigerator. Holding time, 180 days.

4.3. Sulfate stock standard, 10,000 mg SO_4/L –

Potassium sulfate (4.1.4.)	18.1402 g
DI water (4.1.1.)	to 1000 mL

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Before preparation, dry at least 20 g of K_2SO_4 for 24 hours at 105ºC. Cool to room temperature in desiccator after drying period finished.

In a 1 L volumetric flask containing approx. 800 mL of DI water, dissolve 18.1402 g of dried K_2SO_4. Bring to volume with DI water and invert to mix thoroughly.

Store in refrigerator. Holding time, 180 days.

4.4. Nitrate stock standard, 1,000 mg N/L –

Potassium Nitrate (4.1.5.)	3.6091 g
DI water (4.1.1.)	to 500 mL

Before preparation, dry at least 4 g of KNO_3 for 24 hours at 105ºC. Cool to room temperature in desiccator after drying period finished.

In a 500 mL volumetric flask containing approx. 400 mL of DI water, dissolve 3.6091 g of dried KNO_3. Bring to volume with DI water and invert to mix thoroughly.

Store in refrigerator. Holding time, 180 days.

4.5. Bromide stock standard, 1,000 mg Br/L -

Potassium Bromide	1.4890 g
DI water	to 1000 mL

Before preparation, dry at least 2 g of KBr for 24 hours at 105ºC. Cool to room temperature in desiccator after drying period finished.

In a 1 L volumetric flask containing approx. 800 mL of DI water, dissolve 1.4890 g of dried KBr. Bring to volume with DI water and invert to mix thoroughly.

Store in refrigerator. Holding time, 180 days.

Note: Sodium bromide can also be used for this preparation: dissolve 1.2877 g of dried (at 105ºC for 24 hours) ACS grade sodium bromide in DI water and dilute to 1 L in a volumetric flask.

4.6. Bromide spiking solution, 100 mg Br/L –

1000 mg Br/L stock standard (4.1.6.)	10 mL
DI water (4.1.1.)	to 100 mL

In a 100 mL volumetric flask containing approx. 70 mL of DI water, pipette 10 mL of the purchased 1000 ppm bromide stock standard. Bring to volume with DI water and invert to mix.

Store in refrigerator. Holding time, 180 days.

4.7. Carbonate stock solution, 10,000 mg $CaCO_3$/L –

Sodium carbonate (4.1.7.)	1.0606 g
DI water (4.1.1.)	to 100 mL

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Before preparation, dry at least 2 g of sodium carbonate for 12-24 hours at 105ºC. Cool to room temperature in desiccator after drying period finished.

In a 100 mL volumetric flask containing approx. 70 mL of DI water, dissolve 1.0606 g of Na_2CO_3. Bring to volume with DI water and invert to mix.

Store in refrigerator. Holding time, 180 days.

4.8. Phosphate stock solution, 1,000 mg P/L –

Potassium phosphate (4.1.8.)	4.394 g
DI water (4.1.1.)	to 1000 mL

Before preparation, dry at least 5 g of KH_2PO_4 for 12-24 hours at 105ºC. Cool to room temperature in desiccator after drying period finished.

In a 1 L volumetric flask containing approx. 800 mL of DI water, dissolve 4.394 g of dried KH_2PO_4. Bring to volume with DI water and invert 12 times to mix thoroughly.

Store in refrigerator. Holding time, 180 days.

Note: This solution is also used for PO4 analysis (see DEP NU-070).

4.9. IC calibration and check standards –

Using the table volumes below, prepare each standard by pipetting the specified volumes of stock solutions of each analyte into volumetric flasks partially filled with DI water. Bring to volume with DI water and invert to mix thoroughly.

Store in refrigerator. Holding time, 30 days.

Calibration Standards and CCV		**Chloride**		**Sulfate**		**Nitrate**		**Bromide**	
Name/Alias (QC)	**Final Volume**	**Final conc.**	**Stock vol (4.2.)[1]**	**Final conc.**	**Stock vol (4.3.)**	**Final conc.**	**Stock vol (4.4.)**	**Final conc.**	**Stock vol (4.5.)**
STD1	250 mL	0.25 ppm	6.25 µL	0.25 ppm	6.25 µL	0.080 ppm	20.0 µL	0.020 ppm	50.0 µL **(4.6.)**
STD2 (PQCK5)	250 mL	0.50 ppm	12.5 µL	0.50 ppm	12.5 µL	0.10 ppm	25.0 µL	0.040 ppm	10.0 µL
STD3	250 mL	1.0 ppm	25.0 µL	1.0 ppm	25.0 µL	0.20 ppm	50.0 µL	0.050 ppm	12.5 µL
STD4 (CHCK1)	250 mL	2.5 ppm	62.5 µL	2.5 ppm	62.5 µL	0.50 ppm	125 µL	0.08 ppm	20.0 µL
STD5	250 mL	5.0 ppm	125 µL	5.0 ppm	125 µL	1.0 ppm	250 µL	0.20 ppm	50.0 µL
STD6	250 mL	12 ppm	300 µL	8.0 ppm	200 µL	1.0 ppm	250 µL	0.40 ppm	100 µL
STD7	250 mL	30 ppm	750 µL	20 ppm	500 µL	2.5 ppm	625 µL	0.50 ppm	125 µL
STD8 (CHCKHI4)	250 mL	120 ppm	3 mL	80 ppm	2 mL	10 ppm	2.5 mL	1.0 ppm	250 µL
CHCK2 (CCV)	1 L	20 ppm	2 mL	20 ppm	2 mL	0.80 ppm	800 µL	0.20 ppm	200 µL

4.10. Interference standard –

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Refer to the table below for volumes of the stock solutions used. Add all components to a 250 mL volumetric flask containing approx. 150 mL of DI water. Bring to volume with DI water and invert to mix thoroughly.

Interference standard			
Component	**Stock concentration**	**Stock volume**	**Final concentration**
Chloride	10000 mg Cl/L	62.5 µL	2.5 ppm
Sulfate	10000 mg SO4/L	62.5 µL	2.5 ppm
Nitrate	1000 mg N/L	125 µL	0.50 ppm
Bromide	1000 mg Br/L	20.0 µL	0.08 ppm
Fluoride	1000 mg F/L	250 µL	1 ppm
Nitrite	1000 mg N/L	250 µL	1 ppm
Carbonate	10000 CaCO3/L	250 µL	10 ppm
Phosphate	1000 mg P/L	250 µL	1 ppm

4.11. A certified quality control (ALTQC) sample is prepared following the manufacturer's instructions. See the manufacturer's certificate for concentration and expiration date. Depending on the certified concentration, The ALTQC sample may be further diluted to bring its concentration within the calibration range. Store in the refrigerator.

5. SAMPLE PREPARATION PROCEDURE (SEE DEP SOP NU-033)

5.1. All samples are stored on ice or at ≤ 6°C (not frozen). Samples to be analyzed for bromide and/or chloride **only** do not require the above storage. Samples analyzed with improper preservation are reported with the Y qualifier.

5.2. Samples should be analyzed for chloride, bromide, or sulfate within 28 days of collection date. Samples should be analyzed for nitrate within 48 hours of collection. If samples are analyzed outside these hold times, results must be reported with the Q qualifier code and appropriate comment.

5.3. Remove the samples from the receiving room refrigerator and allow them to come to room temperature.

5.4. All samples should be pre-filtered with a 0.45 µm syringe filter. Add "_F" after the sample ID in the sequence to document filtration.

5.5. Samples for the IC should be pre-diluted based on their conductivity or salinity. This can be done either manually or through auto-dilutions. Refer to the table below for guidance.

Conductivity (µS/cm)	Salinity (PPTh)	Dilution Factor
0 - 500	0 – 0.20	None
500 – 999	0.21 – 0.40	2
1000 – 2999	0.41 – 2.0	10
3000 – 9999	2.1 – 4.3	20
10,000 – 14,999	4.4 – 8.6	40
15,000 – 19,999	8.7 – 10.7	50

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20,000 – 29,999	10.8 – 21	100
30,000 – 40,000	22 – 43	200
40,000+	Above 43	400

5.6. For each batch (maximum of 20 samples), prepare:

5.6.1. One method blank (MBLK or MBLK_F) (8.5.1)

5.6.2. One matrix spike for every 10 samples to calculate accuracy.

- A sample is selected to represent the samples in the batch as the QC sample. For a batch of 11 to 20 samples, two samples will be selected.
- Add the appropriate amount of spiking solution (see table below) into a 10 mL portion of each selected sample. (Spike code: N3)

Analyte	Spike Level	Stock conc. (mg/L)	Amount of Stock (µL)
Chloride	30	10,000 (4.2.)	30
Sulfate	30	10,000 (4.3.)	30
Nitrate	5.0	1,000 (4.4.)	50
Bromide	0.5	100 (4.5.)	50

5.6.3. A sample in duplicate or matrix spike in duplicate to calculate precision.

5.6.4. One lab fortified blank (LFB) containing *in-house* DI water and appropriate amount of spiking solution following the same procedure but substituting.

5.6.5. Matrix spikes and LFBs are named using the following format: SampleID_Cl spike code_SO4 spike code_NO3 spike code_Br spike code. For example, 1234567 N3 N3 N3. If an analyte is not analyzed in the batch, the corresponding spike code is omitted.

5.7. Effluents and influents are batched separately.

6. SAMPLE ANALYSIS/QUANTITATION

6.1. Preparing the Instrument:

6.1.1. Ensure that the pump, eluent generator compartment, DC compartment, and the autosampler are powered on.

6.1.2. The DC compartments are kept at a constant temperature of 30°C. The compartment doors should be kept shut at all times except when performing maintenance. The DC compartment doors should never be opened during analysis.

6.1.3. Connect all the compartments to Chromeleon 7.2.

6.1.3. Perform daily or weekly maintenance detailed in sections 10.2 and 10.3.

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6.1.4. Once temperature of the DC compartment stabilizes at 30°C, if needed, change the pump flow rate, eluent concentration, and suppressor to the corresponding initial settings of the method to be used (see table below). The pump flow rate should be increased gradually, in increments of 0.5 mL/min. See the weekly maintenance for guidance.

Analytical Method/Column	AS18-4µm		AS-19-4µm	
	Time (min)	Value	Time (min)	Value
Temperature	All	30.0 °C	All	30.0 °C
Flow Rate	All	0.38 mL/min	All	0.38 mL/min
Eluent Concentration[1]	Initial	17.0 mM	Initial	20.0 mM
	0 → 6.5	17.0 mM	0 → 9	20.0 mM
	6.5	30.0 mM	9.0	30.0 mM
	6.5 → 7.0	30.0 mM	9.0 → 10.0	30.0 mM
	7.0	60.0 mM	10.0	60.0 mM
	7.0 → 8.0	60.0 mM	10.0 → 11.0	60.0 mM
	8.0	17.0 mM	11.0	20.0 mM
	8.0 → 11.0	17.0 mM	11.0 → 16.0	20.0 mM
Suppressor[2]	All	57 mA	All	57 mA

[1]**Note:** Gradient step time interval and concentrations must be kept consistent throughout an analytical run. However, as the column ages, analytes retention times change. The timings of the eluent concentration changes have to be adjusted accordingly to maintain optimal eluent conditions.

[2]**Note**: As the suppressor ages, the suppressor current may be increased to improve its performance (see Dionex Suppressor Manual)

6.2. Preparing a Sample Queue:

6.2.1. Click the "Data" Module. All instrument data folders are organized in two folders: System_1 and System_2.

Note: It is important that all system folders, sequences, methods, and processing methods are kept together. Do not mix system 1 with system 2 or vice versa.

6.2.2. To create a run sequence, right-click on the appropriate subfolder in the Sequences folder and select New Sequence.

6.2.3. Alternatively, copy one of the templates available in the Templates folder and paste it in the appropriate subfolder of the Sequences folder for the same System.

6.2.3. Go to the last run of the system, then copy and paste the instrument method and the processing method into the new sequence.

6.2.4. In the Instrument Method column, select the method that was pasted into the sequence, then press F9 to populate all the rows with this method.

6.2.5. In the Processing Method column, select the processing method that was pasted into the sequence, then press F9 to populate all the rows with this processing method.

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6.2.6. Name the sequence with the format YYMMDD_S#. For AS-19 method, the file should be named YYMMDD_S2_AS19. If there are more than one sequence used on the same date, add a letter "A", "B", "C," etc. after the date to differentiate between the sequences, e.g. 180605A_S1.

6.2.7. Beginning with the calibration curve, enter your run sequence.

An example run sequence includes:

- **STD1-STD8** (Calibration standards, 4.9.)
- **BLK** (DI water, 4.1.1.)
- **INT_STD** (Interference check standard, 4.10.)
- **CHCK1(_F)** (STD4, 4.9.)
- **PQCK5** (STD2, 4.9.)
- **ALTQC** (4.11.)
- **CHCKHI4** (STD8, 4.9.)
- **BLK**
- **CHCK2** (CCV, 4.9.)
- **BLK** (CCB)
- Batched samples (up to 20), including an **MBLK(_F), LFB, matrix spike(s)**, and **sample or matrix spike duplicate** (see 5.6.) with bracketing checks and blanks for every 10 samples.
- **CHCK2**
- **BLK**

6.2.8. In the "Type" column, the calibration standards (4.8.) should be set to "Calibration Standard."

6.2.9. The "Level" column indicates whether a given vial contains a calibrant. Set each standard to its corresponding level. (STD 1 is level 1, STD 2 is level 2, etc.)

6.2.10. The initial QC should be set to "Check Standard" in the Type column.

6.2.11. All samples should have a status of "Idle." It will change to "Finished" when the sample analysis has completed.

6.2.12. Save sequence. Press "Start" or "Resume" at the top when the sequence is ready to be run.

6.3. Running over multiple days:

6.3.1. The IC can run over multiple days, as long as it does not run out of DI water used for eluent generation. The ID water reservoirs can be easily re-filled while the run is in progress with sonicated Di water.

6.3.2. If running over multiple days, perform the daily maintenance indicated in (10.3) at the beginning of every day.

6.3.3. When running samples over multiple days, run the PQCK5 standard once every 24 hours (see 8.6.). If it fails, stop the run and begin again with a new calibration curve.

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6.3.4. When creating a sequence that will use a previously generated calibration, find the sequence that contains the original calibration data and copy the calibration rows into the "Insert Calibration" row of the new sequence.

6.3.5. If the instrument running has been briefly interrupted, for example the pump was shut down and re-started, the calibration must be checked before running new samples. This is done by running a CCV, a PQCK5 standard, and a CCB. If the instrument conditions are expected to change after the interruption, a new calibration must be analyzed.

6.3.6. If a run requires more cups than the maximum capacity of the autosampler, simply begin again at the first completed vial on the autosampler – replace the injected vials with new vials.

6.4. Shutdown:

6.4.1. For normal day to day operations and over the 2-day weekend, the IC system should not be shut off or the analytical methods initial parameters changed.

The instrument will continue to draw water from the eluent water reservoirs. Make sure to refill the reservoirs before leaving for weekend to ensure the system doesn't run dry and ruin the components.

Consult your supervisor if you are planning to leave the instrument unattended for more than two days!

6.4.2. For holiday weekends where the water reservoirs will not be refilled before running out, the parameters can be lowered to conserve resources. See the table below for these settings.

Weekend Method	AS18-4µm	AS-19-4µm
Temperature	30.0 °C	30.0 °C
Flow Rate	0.15 mL/min	0.15 mL/min
Eluent Concentration	17.0 mM	20.0 mM
Suppressor	7 mA	8 mA

<u>Note:</u> No analytical data should be collected using these methods.

- This can be done manually by lowering each parameter in the 'Instruments' module.
- Alternatively, at the end of the analytical sequence, add another sample line (named BLK_Weekend) and change the method for **only** that line to the corresponding system's weekend method ("S#_Weekend").

6.4.3. If the IC system is not expected to be used for multiple days or in case of uncertain weather, the IC system can be turned off in the following order: Suppressor → CR-TC → EG → Pump.

6.5. Quantitation/Calculations:

6.5.1. The instrument will give analysis results for: Chloride in mg Cl^-/L, Sulfate in mg SO_4^{2-}/L, Nitrate in mg N/L, and Bromide in mg Br^- mg/L.

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6.5.2. The QC Manager calculates recoveries for spikes and QC standards as well as relative percent differences (RPD) for duplicate samples and spikes.

6.5.3. The individual calibration standards are prepared according to section 4. The calibration is calculated by the instrument software for each component and is included in the analysis report. It also includes a correlation coefficient to evaluate the fit. The fit is cubic. The analyst is responsible for calculating calibrant recoveries and recording them on the Results report.

7. POST-RUN PROCEDURE

7.1. After the sequence has finished, double click the first chromatogram with the analytes. This will open the "Data Processing" tab.

7.2. Check the retention time windows and processing method parameters:

7.2.1. As a column ages, the retention times of all peaks shift. Review the chromatogram of the calibration and initial QC standards, especially the INT_STD and STD8.

7.2.2. Check that the retention time windows, and the processing method parameters are positioned appropriately in relationship to the peaks. Ask your supervisor to adjust them if necessary.

7.3. Check the calibration coefficient:

7.3.1. In 'Presets' at the top, click the 'Calibration and Results' window. Click the first analyte peak of interest in the chromatogram and then click the 'Calibration' tab at the bottom.

7.3.2. Check the correlation coefficient, then repeat for other analytes of interest by selecting another peak.

7.4. Review each chromatogram to check for baseline, peak shape, integration, and peak results:

7.4.1. The tab "Peak Results" at the bottom will also display the results of the selected chromatogram. Alternatively, after selecting the peak of the first analyte of interest, the "Summary" tab will show the results of that analyte for the entire sequence. To see the results for a different analyte, click the corresponding peak on the chromatogram or click on next injection icon.

7.4.2. Be particularly careful to check chromatograms where peak quantitation is marked as "n.a." The software will assign the "n.a." quantitation to both non-detected peaks and peaks with areas above calibration curve. Check the chromatogram and the peak area to distinguish between the two.

7.4.3. **Chromatograms with large peaks should be reviewed with particular care:** such peaks may interfere with quantitation and identification of

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neighboring peaks. Additionally, large peaks may appear with shifted retention time and be misidentified as another analyte.

7.5. Accessing, printing, and exporting the data and printouts for the report folder:

The report folder must contain:

- The 'Summary' that has the peak results for the sequence
- The sequence reported in the 'Bench Sheet' that
- The peak results located in the "Summary" tab.
- A printout of all calibration curves in the "Calibration" tab.
- The chromatograms of all the initial QC in the "Peak Analysis" tab.

When printing, make sure everything is in black and white and double sided. For all printouts (except the initial QC chromatograms), click the Chromeleon head at the top left of the screen, choose print, select "Current Injection," and make sure the right sheet is selected to be printed.

For the initial QC chromatograms, follow the same procedure but click "Current Sequence" instead of "Current Injection" and make sure the yellow vial with the asterisk and the green vial with the check mark are selected. Then print.

7.6. To print the method, click the "Instrument Method" tab above "Data Processing." Then print using the Chromeleon icon at the top left. If method has been modified, mark the notification on the printout. The only type of modifications allowed are adjustments to the gradient timing to account for retention time shifts due to column aging.

7.7. To print the processing method, click the "Data Processing" tab, Click "Calib. & PM" tab in Presets. Then click the "Detection" tab. Use the Chromeleon head to print and uncheck all options to print except for the "Detection" page. Click "OK" to print.

7.8. Generate an export file for QC Manager using the "Summary" tab of Report Designer. Click the Chromeleon head, Export, and "Current Injection." Select the appropriate parent folder in the Nutrients drive Select the "Text format (Results)(.txt)" option needed for QC Manager. Click "OK." The export file will appear in the selected folder under its own folder named in the same way as the sequence, e.g. 180605A_S2_AS19.

In the folder, give the text file a name made of the sequence name with an addition of an analyte code, e.g. 180605A_S2_AS19_CS. Use the following analyte code: "_CS" for chloride and sulfate, "_N" for nitrate, and "_B" for bromide.

7.9 Export the chromatogram files to the Nutrients network folder. In the "Data" module, click the sequence then File → Send to. Select the appropriate folder for the IC instrument used at \\FLDEP1\DEAR\LABS\Lab3\Chem_data\Inorganic \Nutrients\Dionex_IC\ \S1\Raw_data\ or \\FLDEP1\DEAR\LABS\Lab3\Chem_data\Inorganic \Nutrients\ Dionex_IC\ S2\Raw_data\. Place the directory into the appropriate subdirectory for month

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and year. Uncheck the "Include 3D and MS raw data" option and then click "Start."

7.10. Manual integrations should be infrequent. Discuss each instance with the supervisor. If a chromatogram must be manually reprocessed, mark the affected result(s) with "M" on the Results report and add date and initials. Print out a copy of the chromatogram with and without manual reprocessing applied, add explanations if needed, and include the printouts with the report folder. For the chemistry section's policy on manual integration, see DEP SOP CM-018.

8. QUALITY CONTROL/DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES (SEE DEP SOPS NU-038 AND NU-043)

8.1. For method performance and acceptance criteria for QC measures, corrective action for out-of-control data, and contingencies for handling out-of-control or unacceptable data, refer to the following DEP SOPs NU-038 and NU-043.

8.2. A new calibration is performed at the beginning of every analytical run (typically once a week or as needed.) The working calibration range is verified every time a new calibration is performed. The correlation coefficient for every component reported must be equal to or greater than 0.995. The acceptance limits for the recoveries for each calibrant above the PQL are 90 - 110 %. For the PQL-level standard acceptance limits are 70 - 130%. Acceptance limits below the PQL are 50 – 150%, with the additional requirement that the result is above MDL. This results in the effective acceptance limits of 80 – 150% for 0.25 ppm Cl and SO4 and 65 – 150% for 0.02 ppm Br.

8.3. Certain QC samples must be run after each new calibration. These are:

8.3.1. A second source (ALTQC, 4.11.). The acceptance limits for the recovery of the ALTQC are 90 - 110 %.

8.3.2. A PQL-level check standard (STD2, 4.9) is run at the beginning of each run and every 24 hours. Acceptance limits for the recovery of the PQL are 70 - 130 %. The PQCK5 standard serves both as a PQL-level check and as a spike for the ongoing MDL/PQL verification for chloride, sulfate, and bromide.

8.3.4. An Interference Check Standard (4.10) has acceptance limits of 85 - 115 % recovery. This should be run with a CHCK1 (STD4, 4.9), which has the same levels of each analyte. In case of failures, comparison between the two can help distinguish between an interference and a calibration problem. The acceptance limits on the CHCK1 are 90 - 110 % recovery.

8.3.5. A blank to check for carryover. This is simply DI water, and is run immediately after the highest calibration standard (CHCKHI4). The carryover blank has the same acceptance limits as other blanks.

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8.3.6. A CCV with the same values as the upper limit of calibration that is called CHCKHI4 (STD8, 4.8). The acceptance limits for CHCKHI4 are the same acceptance limits as the other CCVs, 90 - 110 % recovery.

8.4. A bracketing CCV (CHCK2) and a CCB (DI water) should be added at the beginning and the end of each run and every 10 samples.

8.4.1. Acceptance limits for the recovery of the check standards are 90 -110 %.

8.4.2. Results for all blanks (MBLK/CCB/BLK) must be less than the MDL and greater than -MDL.

8.5. Certain QC must be run for every batch of up to 20 samples:

8.5.1. A method blank (MBLK). This is a DI water that is prefiltered with a syringe filter if samples are filtered. Results for all blanks (MBLK/CCB/BLK) must be less than the MDL and greater than -MDL.

8.5.2. An LFB and matrix spike(s) (at least one out of each ten samples should be spiked). Acceptance limits for LFB and MS recoveries are 90 - 110 %. If the concentration of the analyte in a QC sample is greater than four times the spiked amount, the corresponding matrix spike recovery is not reported.

8.5.3. A duplicate sample or duplicate matrix spike to assess the precision. The control limit for results above PQL is < 20 % RPD.

8.6. If samples are filtered, positive and negative filtration controls should be included on the run. This is typically accomplished by filtering the CHCK1 and the MBLKs for the batches that include filtered samples. Filtration is indicated by adding "_F" at the end of the sample/standard name.

9. DATA ARCHIVAL

9.1. The raw data is archived in paper format. A report folder is created for each analytical run. More than one run may be in one folder. The folder is labeled with the test (IC-SO4, Cl, etc.).

9.2. Each report folder should contain the following items - results report(s), bench sheet(s), backlog(s), reagents and standard sheet, the calibration reports, chromatograms for initial QC, method parameters, chromatogram processing parameters and QC assessments uploaded from QC Manager to LIMS at the time of test authorization. If dilutions were prepared, the pipette and dilutions sheet should also be included.

9.3. The report folders are on file with the nutrients and general chemistry supervisory personnel. Periodically, the files are moved to the State Records Center for long time storage according to DEP SOP LB-006.

9.4. Instrument raw data files, *.cmbx, are backed up and stored on the Nutrients drive under \\FLDEP1\DEAR\LABS\Lab3\Chem_data\Inorganic \Nutrients\Dionex_IC\.

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9.5. IC instrument data excel transfer files for upload to QC manager are stored in electronic format located on the Nutrients drive: \\FLDEP1\DEAR\Labs\Lab3\Chem_Data\Inorganic\Nutrients\Dionex_IC\.

9.6. If available, a Certificate of Analysis for each purchased standard or reagent is maintained in the \\FLDEP1\DEAR\Labs\lab3\Chem_Data\Inorganic\Nutrients\REAGENT_PREP\IC\Certificates of Analysis folder.

9.7. Method validation data is on file with the supervisor. Digital copy is also stored on the network, under an appropriate sub-folder in \\fldep1\dear\Labs\Lab5\Chemistry\Inorganic\Nutrient\Validations.

10. MAINTENANCE

Note: All major maintenance must be recorded in the instrument maintenance manual.

10.1. Keep the instrument clean and free of dust as much as possible. If eluent or sample is spilled, be sure to clean it up immediately.

10.2. Weekly maintenance. Perform the following maintenance procedures before you calibrate the instrument for the first time in a calendar week:

10.2.1. Check the expiration date and the EGC % remaining in the Eluent Generator tab of the Instruments module of the Chromeleon Console.

10.2.2. Turn the system off using the controls in the Home tab of the Instruments module of the Chromeleon Console in the following order: Suppressor → CR-TC → EG → Pump. The Compartment and Column temperature should remain on at 30° C. Turn the temperature on if it is off.

Important: Make sure the EG is turned off right away after the CR-TC has been turned off. Running the EG while the CR-TC is on will deplete and ruin the CR-TC.

10.2.3. Empty, rinse and refill the DI water reservoirs that supply water to the pumps and AS-AP. The DI water used for this purpose should be sonicated for 30 min prior to use to reduce air and carbonate content. High content or air/carbonate in DI water may result in formation of bubbles, which in turn can lead to unstable injection volume, baseline noise, and inconsistent analyte recoveries.

10.2.4. Prime the pump to prevent air from going through the system:

Open the corresponding system's purge valve in the ICS-5000 Dual Pump compartment, go to the "Pump" tab for that system, click "Prime," then "Execute despite warning." Wait a few minutes until all the bubbles go to waste. Click "Stop," **close the purge valve**, set the pump to 0.15 mL/min and then turn it on.

10.2.5. Turn the system back on using the following procedure:

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Wait till the pump pressure at 0.15 mL/min is stable. Turn the EG on at 17 mM for AS18 method or at 20 mM for AS19 method. Immediately turn the CR-TC on after turning the EG on. Turn the suppressor on at 57 mA for AS18 method or at 71 mA for AS19 method. Turn the pump up to 0.20 mL/min. Wait for the pressure to stabilize. Repeat at 0.25 mL/min → 0.30 mL/min → 0.35 mL/min → 0.38 mL/min.

Note: Always turn the pump and the Dionex Suppressor Control on and off at the same time. Eluent flow through the Suppressor is required for proper operation. Flowing eluent without current will cause the membranes, resin, and screens in the suppressor to become exhausted resulting in small analyte peak areas.

10.2.6. Prime the AS-AP:

10.2.6.1. Priming the AS-AP normally.

Ensure there are no bubbles in the syringe. Click the "Sampler" tab. Then "Prime Syringe" for 10 cycles. Then "Wash Needle and Fluidics" for 1500 µL. Then "Wash Sample Loop" with 1500 µL. Check there are no bubbles in the syringe afterwards. If so, manually prime the syringe.

10.2.6.2. To manually prime the syringe:

Go to the Sampler tab. Click "Wellness" then "Start Change." Once the syringe has stopped moving, unscrew the mounting bolt first and then the syringe.
Using DI water in a beaker, manually prime the syringe until all the bubbles have been expelled. Ensure that there is a white o-ring in the syringe valve. While holding the syringe vertically, push a small amount of DI water out of the top.
Screw in the syringe and then the mounting bolt finger tight. Click "Finish Change," close out the Wellness window, and then click the "OK" at the top of the screen to finish changing the syringe and return it to home position. Go through the normal priming steps (10.2.6.1) after manually priming the syringe.

Go to "Release Control" then click "Monitor Baseline". Go back to "Home" tab and wait for a stable baseline before starting a new run.

10.3. Daily maintenance for ongoing runs. Perform the following maintenance for every day in a calendar week after the first day that the instrument continues to run:

10.3.1 Top off DI water reservoirs. The system must not run out of DI water, have air go through it, or run dry. **This may damage instrument components.** The DI water used for this purpose can be sonicated for 30 min prior to use.

10.3.2. Check for leaks or unusually low pressure.

10.3.3. Check for air bubbles in syringe. If there are air bubbles, manually prime the syringe (10.2.6.2.)

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10.3.4. Click “Monitor Baseline” and watch for a stable baseline.

10.4. Routine replacement of parts:

10.4.1. The Eluent Generator Cartridge and Regenerating Anion Trap Column (CR-ATC 500) should be replaced when EGC % remaining is low.

10.4.2. The analytical column should be replaced when the pressure gets close to the maximum (5000 psi) or the peak resolution and baseline is too poor to continue use.

10.4.3. Aging suppressor will have reduced capacity for converting eluent to the less conductive water. Increased total signal and problems with baseline and changes in peak areas can result.

10.4.3. The suppressor or carbonate removal device (CRD) deterioration can cause an unstable baseline or a baseline that doesn’t recover well after a peak has gone through. This can be seen by tailing and extra peaks in the peak tail.

10.5. If any significant changes are made to the system, such as a new column, check the analytes’ retention time and any interfering anion retention times by running the interference check standard. All peaks should be clearly resolved and well within the established retention time windows. If necessary, the method isocratic window times may need to be adjusted (see 6.1.6.)

10.6. Record in the maintenance log any maintenance carried out. Write down the serial numbers of all parts removed from or installed on the system. Also, place all service visit and technical support calls records in the maintenance log.

11. SAFETY/HAZARDOUS WASTE MANAGEMENT/POLLUTION PREVENTION

11.1. Review the Laboratory Health and Safety Plan (accessible on the intranet at https://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents).

11.2. Safety glasses, gloves and lab coats should be worn at all times while handling samples and reagents.

11.3. Read the MSDS information for safe reagent and sample handling.

11.4. The IC analysis does not normally generate hazardous waste, so the eluent and sample waste can go down the drain. The Dionex may be set up to flush waste directly into a sink, or the waste can be directed into a plastic carboy that is emptied as needed.

12. REFERENCES/DEFINITIONS

For definitions see section 3 of the FDEP Quality Manual. All abbreviations used throughout the document are defined at least once in the document usually at the first time of use.

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12.1. Dionex AS-AP Autosampler Operator's Manual, Thermo Fisher Scientific Inc, 2012. Document No. 065361.

12.2. Dionex IonPac AS18-4µm Column Product Manual, Thermo Fisher Scientific Inc, 2014. Document No. 065499-02.

12.3. Dionex IonPac AS19-4µm Column Product Manual, Thermo Fisher Scientific Inc, 2014. Document No. 065532-03.

12.4. Dionex DRS 600 Suppressor, Thermo Fisher Scientific Inc, 2018. Document No. 031956-13.

12.5. Chromeleon Manuals:

12.5.1. Chromeleon 7.2 Operator Training: Level 1, Thermo Fisher Scientific Inc, 2015. Document CM 7.2 Level 1.

12.5.2. Chromeleon 7.2 Operator Training: Level 2, Thermo Fisher Scientific Inc, 2015. Document CM 7.2 Level 2.

12.6. EPA Method 300.0 Rev. 2.1 (1993).

12.7. DEP SOP NU-033, Summary of Sample Analysis Procedures for the General Chemistry and Nutrients workgroups.

12.8. DEP SOP NU-038, Nutrients and General Chemistry: Quality Assurance and Quality Control.

12.9. DEP SOP NU-041, Reagent Preparation Procedure.

12.10. DEP SOP NU-042, Standard Preparation Procedure.

12.11. DEP SOP NU-043, Nutrients and General Chemistry Quality Assurance and Data Authorization Guidelines

12.12. DEP SOP CM-018, Laboratory Policy Regarding Manual Chromatographic Peak Integration.

12.13. DEP SOP LB-006, Standard Operating Procedure for Records Maintenance and Storage.

12.14. Quality Manual, Bureau of Laboratories, FDEP.

12.15. Laboratory Health and Safety Plan.

APPENDIX OF SIGNIFICANT CHANGES

07/06/2018 Introduced the AS19 column method for the determination of chloride, sulfate, and bromide on Dionex IC 2. Elevated PQLs for chloride (0.6 mg/L), sulfate (0.6 mg/L), and nitrate (0.15 mg/L) to comply with the 2016 TNI standard. The MDL for bromide on the Dionex AS19 column is0.013 mg/L. Added bromide information to the standard preparation procedures. Clarified standard preparation descriptions. Added a note on the optional

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	carbonate standard. Revised CHCK1, INT STD preparation table. Removed references to CHCK5 standard. Added new guidelines for reporting matrix spike recoveries. Edited Instrument Methods table, added notes on changes to eluent program and suppressor current. Clarified Preparing a Sample Queue section. Changed location for the storage of the Certificates of Analysis for standards. Clarified explanations of data review, printing reports, assembling the report folder, and data archival. Expanded maintenance section. Clarified running over multiple days and Shutdown procedures. Moved system startup information from Sample Analysis section to Maintenance section. Added notes on chromatogram reviews.
02/06/2019	Revised Reagents and Standards chapter and removed references to use of MDL standard. Added reference to validation data. Added clarifications to Sample Analysis and Quantitation. Removed all references to sediment analysis of chloride, sulfate and nitrate.
01/03/2020	Changed PQL levels for Cl and SO4 to 0.5 ppm.
02/11/2020	Added control limits for the calibration points below PQL. Added requirement that DI water used for AS-AP and eluent is sonicated. Added clarifications to chapter 7.

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