Protocol and Procedure: Analyzing Water Samples using Ion Chromatography

PREPARED BY	ORGANIZATION	DATE
Dr. Terrance Loecke	Loecke Lab, University of Kansas	04/20/2021

APPROVALS	ORGANIZATION	DATE

Change Record

REVISION	DATE	DESCRIPTION OF CHANGE
A_DRAFT	6/28/2016	Initial draft release.
B	4/24/2018	Minor formatting changes.
C	9/18/2019	Minor formatting changes. Standard and check protocols updated per feedback from NEON. Section added detailing how to pull and process data. Appendix added on preparing concentrated eluent solution.
D	1/10/2022	Updated template and responded to comments on clarification from NEON.

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1. Priming and Stabilizing the IC

Before starting an IC run, the pump must stabilize for at least 1 hour or, preferably, as long as possible. Therefore, the first steps should always be to ensure there is eluent and regenerant within the appropriate bottles, prime the pump if needed, and turn the pump on.

1.1 Refilling the Eluent and Regenerant:

Check to see that the eluent and regenerant are filled. These both need to be full to the shoulder of the bottle. If they are not, top the bottles off with eluent or regenerant that is stored in the flasks next to the IC. If there is not enough in these flasks to top off the eluent and regenerant, make more.

a. Any time eluent is added, tap on the bottom of the eluent bottle to dislodge any bubbles that have formed.
b. Be careful when filling the regenerant bottle as the bottle is opaque and can easily overflow. Do not fill past the line.

1.2 Making Eluent:

a. Using a 100 ml volumetric flask, measure 100 ml of 20x concentrated eluent solution.
    i. The 20x concentrated eluent solution can be found on the top shelf in the reagent refrigerator. If more 20x concentrated eluent solution needs to be made, see appendix*: "Preparing Concentrated Eluent Solution".*
b. Rinse the 2L volumetric "Eluent" flask with ultrapure water.
c. Pour the 100 mL of 20x concentrated eluent into the 2L "Eluent" flask. Fill the 2L "Eluent" flask the rest of the way with ultrapure water to dilute the 20x concentrated eluent solution to 2L.

1.3 Making Regenerant:

a. The regenerant uses 4N sulfuric acid, and a lab coat, goggles, and acid gloves are required when handling either the acid or the regenerant.
b. Rinse the 4L Erlenmeyer "Regenerant" flask with ultrapure water.
c. Measure out 50 mL of 4N sulfuric acid using a beaker and a 50 mL volumetric flask.
d. In the 4L Erlenmeyer "Regenerant" flask, add approximate 3L of ultrapure water. Slowly add the 50 ml of 4N Sulfuric Acid and dilute with ultrapure water to 4L (4000 mL).

1.4 Priming the Pump:

Priming the pump must be done if:

c. Eluent has been refilled.

d. The pump fails to stabilize.
e. Makes a clicking or banging noise.
f. Fails to build pressure (check the log to see the expected pressure).
g. There are noticeable air bubbles in the eluent line leading into the pump.

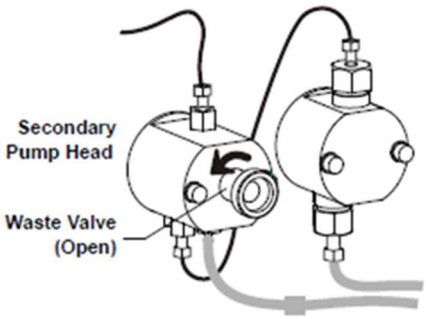
Priming the pump:

i. The pump should be off. If it is not, turn it off (this can be done from the instrument screen).

ii. On the IC's secondary pump head, turn the waste valve roughly 1/4 turn counterclockwise.

iii. After opening the waste valve, click "prime" on the instrument screen. A pop up message will appear to make sure that the waste valve has been opened. Click okay.

iv. The pump should now be priming.



Let the pump prime for at least 15 minutes, but preferably over 30 minutes. When finished:

i. On the instrument screen, turn the pump off.
ii. On the IC's secondary pump head, turn the waste valve clockwise until it is tight.
    a. NOTE: the waste valve's seal is extremely sensitive. Do not overtighten or under tighten. To ensure the correct seal, turn the valve gently and firmly until you no longer feel any 'clicks.'
    b. If the valve is under tightened, the pump will fail to build pressure when the pump is turned on.

1.5 Turn the Pump On

a. In the "Chromeleon Console" software, go to the instrument screen.
b. Increase the flow rate (if necessary) to 0.9ml/min
c. Select under the "Pump" section "On"

d. The pump should now be on. The pressure should rise into the 1600-1800 psi range. Look in the IC log to compare it to the pressure from previous runs.
    i. If the pressure will not raise, try the following:
    ii. Make sure that the flow rate is set to 0.90 mL/min. If it set lower, the pressure will not rise.
    iii. Make sure that the waste valve is properly tightened. If the pump was primed and then the pressure will not raise, an under tightened waste valve is probably the reason.
    iv. Bubbles could also be the cause of pressure not rising. If this is suspected, prime the pump again.
e. Wait for the baseline to stabilize- let the pump run for at least 1 hour with the gas on before starting the run.
    v. Select 'monitor baseline' to track the conductivity as the pump stabilizes.
    vi. When the pump has stabilized, the conductivity will be fluctuating by less than 0.01µS, and the pressure by less than 1psi
    vii. *Do not start the run before the pump has stabilized!*
        1. *If the pump has not stabilized within 5 hours see troubleshooting section and report to lab manager or PI.*

1.6 Turn on gas flow

Nitrogen gas is used to pressurize the regenerant bottle, forcing regenerant at a constant flow rate through the suppressor. Regenerant must be flowing during a run to ensure consistent baselines.
NOTE: When the gas is turned on, there must be enough regenerant to ensure the flow remains consistent. <u>If the bottle is allowed to empty, air will be forced through the suppressor,</u> drying the membrane and damaging it.

a. After ensuring that the regenerant bottle is filled, turn on the gas. Ensure both the valve on the tank and the valve on the regulator are open.
b. Verify that the regulator pressure is set to 5psi, and that the regenerant bottle is sealed.
c. After several minutes, check the regenerant flow rate by looking at the regenerant waste line in the sink. There should be droplets forming and dripping a little slower than 1 drop/sec. Adjust pressure as necessary.
d. Allow pump to run for at least one hour with the gas on before initiating a run.

2. Preparing a run

2.1 Take things out of the fridge

Take samples and standards out of the fridge to allow them to warm up to room temperature for 1.5 hours. This reduces possible drift caused by temperature variations.

Storage of NEON IC samples

NEON IC samples are stored in the walk-in fridge

1.) On the left side of the room, there is a set of shelves that should have samples to be run
   a. Samples should be sorted according to date collected
   b. Run oldest samples first
2.) The samples will be in a single Ziplock bag with all samples from collection date and site stored together with a maximum of 25 samples per bag.

2.2 Preparing a Sequence Table

A new sequence table can be made by copying one from a previous run.

a. In the “Data” tab, open a previous run’s data
b. Use **SAVE AS** to create a new sequence table with a descriptor and today’s date.
   i. For NEON samples, the title uses the format “SITE_Date collected_Date run”
c. Enter sample names and add in standards, checks, blanks, and a stop as needed. Details on all of these are listed below.
   ii. For standards, select “calibration standard” in the sample type column.
d. After all samples, standards, checks, blanks, and a stop have been entered into the sequence table, ensure that the position column is correct (meaning that the numbers go in order from 1-50, etc). The number in the position column corresponds to the position of the vial in the auto sampler, so ensuring that the position column has the correct numbers is important.

Standards Determination

a. IC samples from NEON will either contain Chloride (Cl) or Bromide (Br). This information should be on the sample bottle.
b. On Wet Lab computer’s desktop, open the excel file “Expected conc. By site”
c. Verify whether you are running samples for Cl or Br
d. Select a minimum of 4 standards for the site you are running samples for:
   i. One below expected “low/background” concentration

ii. Two in between “background” and “high”
iii. One above the expected “high” concentration

Checks

a. For checks, use a standard in the center of your standard curve.
b. For NEON, checks should be run about every 6 samples and prior to the injectate sample.
c. In the sequence table, label the checks as “check 1”, “check 2”, “check 3”, and so on. Also include the concentration of the checks in their label (for example, 1 ppm).

Blanks and Stops

Blanks of ultrapure water may be added before a run to help stabilize conductivity, or after a run to flush out remaining sample. In general, add 1 blank at the beginning of a run, one blank after the standards, and one blank at the end of the run.

A STOP tells the pump to reduce eluent flow to 0.1ml/min, effectively turning off the instrument after a run is finished.

a. To add a stop into the sequence table, insert a blank injection at the end of a run and change the method from “initial setup” to “stop”.
b. The stop vial also contains ultrapure water.

2.3 Preparing standards and checks

Previously prepared standard solutions can be found in the door of the reagent fridge. If you need to make more solutions, follow the directions below. Standard solutions expire 180 days following preparation. Remake standard solutions if the solutions are expired.

For NEON, we use 1000ppm Cl/Br stock made from salt. This will be labeled in the fridge as 3 anion stock.

d. Dilute the stock to 100ppm
    i. Carefully pour out a small amount of stock into a beaker
    ii. Using a clean glass 10ml pipette, pipette 10ml of stock into a clean 100ml volumetric flask
    iii. Dilute to the line with Ultrapure water.
    iv. Store in the fridge in a clean Nalgene container with a label that includes the solution name, concentration, preparation date, and initials of preparer.
e. From this stock, individual standards can be made

v. Prepare standards needed (ranging from 0.1ppm to 50ppm) using pipettes and 100ml volumetric flasks.

Notes on preparing standards:

- Never pipette directly from the bottle
- Clean all glassware beforehand. Glass pipettes and other glassware can be rinsed with a small amount of the solution they will be used to measure.
- For measuring volumes over 1ml (1000ul), use glass pipettes for greatest accuracy.
- For volumes 1ml (1000ul) and under, micropipettes may be used
    - When using micropipettes, always use the smallest micropipette possible- they are most accurate at the top of their range
    - If you are unsure how to use a micropipette, consult the micropipette protocol before proceeding
- Make check standards to be analyzed every at least 20 but no less than 3 check standards per run
    - Selecting a standard check to repeat from the middle of the standard curve and based off of previous runs from the stream.

2.4 Filling Vials

Autosampler vials and caps are washed with ultrapure water and reused. For sensitive analyses, use new vials and caps.

a. Based on the “position” order designated by your sequence table, fill vials with standard and sample to the line marked on the vial.
b. Cap the vials.
    i. Insert caps, widest part down, and push until the top of the cap aligns with the top of the vial.

    NOTE: Before capping, thoroughly inspect caps for defects. Dirty filters, filter frits pushing out or loose, or damaged threads can interfere with the autosampler mechanism.

c. Once filled, place the vials into a loading tray.
    NOTE: Throughout filling and loading the vials, *continuously check to ensure that the vials are going in the correct order* as corresponds with the sequence table you created. Use the laminated ‘cheat sheet’ that

corresponds to the loading tray to keep track of the order for the vials. If necessary, label the vials.

2.5 Diluting the "00" Injectate Sample

The injectate sample (labelled with .00.) cannot be run full-strength without destroying the column. In general, use a 1000x dilution for Chloride samples, and 100x for Bromide.

a. Example of 1000x dilution: Using a volumetric flask, dilute 100 µl of injectate sample to 100 ml with ultrapure water.
    i. Ensure that the dilution is thoroughly mixed
    ii. Fill 2 vials with the diluted injectate
    iii. Record the dilution factor used in the IC notebook (see below).

2.6 Loading the autosampler

a. Under the plastic cover of the autosampler, press the "carousel release" button. This will raise the needle and allow the carousel to be turned.
b. The numbers on the carousel correspond to the "position" column in your sequence table. Place vials into their correct positions.
c. Once all vials have been inserted into their correct positions, close the plastic cover.

2.7 Starting the run

a. Log your run in the IC notebook. Include:
    i. the date
    ii. your initials
    iii. the pressure and conductivity upon starting the run
    iv. what samples you are running
    v. the dilution (1000x, etc.) of your injectate sample.
    vi. Any relevant notes (i.e. cleaned out eluent reservoir, autosampler making loud noise, etc.)
b. At the top of your sequence table, select the start button, and click "execute" at any dialog boxes that occur.

2.8 Turning the gas off

Once the IC is finished running, don't forget to TURN OFF THE GAS, and vent the bottle. Failure to turn off the gas before the regenerant runs out will force air through the suppressor membrane, drying it out and dramatically reducing its lifespan. Do not run the IC unless you will be able to turn the gas off the following morning.

3. Obtaining data

3.1 Retrieving raw data from Chromeleon Console

a. Once a run has completed, navigate to the “Data” tab located in the bottom left corner of the Chromeleon Console software.
b. In the window located in the upper left corner of the software, select the file corresponding with your run. This will open the run sequence table and chromatographs.
c. Double click on any chromatograph. In the window that opens, click on the “Data Processing Home” tab, and select the “Results” button in the “Presets” section.
    i. This will open a screen showing a single chromatograph and a spreadsheet. The chromatograph corresponds with the sample selected in the spreadsheet.
    ii. To view the chromatograph of a different sample, double click on the sample within the spreadsheet.
d. In the chromatograph window, use the grey tabs at the top of the chromatograph to select the peak for the relevant anion (Bromide or Chloride). This will populate the spreadsheet with values for this anion.
e. On the spreadsheet, select and copy all of Column A through Column H. Paste the columns into an Excel spreadsheet.

3.2 Setting up Excel spreadsheet

a. Save the Excel spreadsheet with a file name in following format: SITE-(Collection)YEARMONTHDAY-(Run)DAYMONTHYEAR
    i. Example: Samples collected at WLOU on April 6, 2019 and ran on the IC on May 1, 2019 would be labelled WLOU-20190406-1MAY2019
b. Label columns as follows:
   Column I: Known Concentrations
   Column J: Parameters
   Column K: Calculated Concentrations
c. Label the tab as “raw data”, add a new tab, and label the new tab as “final”.
d. In the final tab, label the columns as follows:
   Column A: Sample ID
   Column B: Injection Number
   Column C: Concentration
   Column D: Analyte
   Column E: QA_tech
   Column F: QA_PI

Column G: rerun_conc
Column H: rerun_date

3.3 Calculating standard curve

When calculating a standard curve for a run, the equation for the trendline of the standard is used:

$$y = mx+b$$

where y = area under the curve of the chromatograph peak
x= concentration of the standard
m = slope of the trendline
b = y intercept of the trendline

a. In the Known Concentrations column on the “raw data” tab, input the concentrations of the standards.
b. In Row 2 of the Parameters column, enter the equation “=SLOPE(y,x)” where y = standard curve values in the Area column and x = standard values in the Known Concentration column. This provides the “m” value.
c. In Row 3 of the Parameters column, enter the equation “=INTERCEPT(y,x)” using the same y and x values as in step 15b. This provides the “b” value.
d. In Row 4 of the Parameters column, enter the equation “=RSQ(y,x)” using the same y and x values as in step 15b.

3.4 Calculating sample concentrations

Sample concentrations are calculated by using the equation of the trendline for the standards to solve for “x” (concentration):

$$x = (y-b)/m$$

a. In the Calculated Concentrations column, enter the equation “=(y-J$3)/J$2” into the row corresponding with the first standard, where y = value in the Area column.
b. Copy this formula for all remaining standards, blanks, checks, and samples.
c. For the injectate samples, modify the formula to “=(y-J$3)/J$2*1000” for Chloride samples and “=(y-J$3)/J$2*100” for Bromide samples.

3.5 Preparing final data

a. Copy the sample names, injection number, and calculated concentration values into the “final” tab.
b. Run the technical review R code.

i. Navigate to the following OneDrive folder: KU Water and Gas Lab Data and Protocols\protocols\data protocols\NEON
ii. Open the “NEON” R project
iii. Open the icTechCheck.R script
iv. Run the script. Once the script is run, you will be notified whether tech check has been passed or failed for each run.

c. Fill out the analyte (“Chloride” or “Bromide”), and indicate whether each sample has passed technical review. “Y” denotes a sample has passed technical review, and “N” denotes a sample needs to be rerun.
   i. r^2 for standard curve must be ≥ 0.9950
   ii. concentrations for standard checks throughout run must be consistent and within expected range. R script tech check must be passed (script found “KU IC data processing protocol”)
   iii. values for samples should be positive and within the expected range
d. Once samples that failed the initial technical review have been rerun, enter in the rerun concentration and date into the corresponding columns in the “final” tab.
e. Close the document and move it to the “needs checked by PI” folder on Dropbox.

4. IC Run Checklist to double check the above has been completed.

4.1 Prepare Instrument

- ☐ Make and fill eluent and regenerant
- ☐ Prime the pump for at least 30 minutes
- ☐ Turn pump on and allow to stabilize >1 hour
- ☐ Turn on gas and allow to be on for >1 hour
- ☐ Take samples and standards out of fridge for > 1 hour
- ☐ Verify standard solutions are within expiration date.
- ☐ Verify regenerant flow rate

4.2 Prepare Samples

- ☐ Create sequence table
- ☐ Prepare standards and checks
- ☐ Dilute NEON injectate sample
- ☐ Fill vials
- ☐ Add blanks and stop
- ☐ Load autosampler

4.3 Run

- ☐ Record run in log book
- ☐ Start sequence

4.4 After run

- ☐ Turn off gas and vent bottle
- ☐ Check for retention time shifts or other irregularities

Appendix

Preparing 20x Concentrated Eluent Solution

NOTE: purity of the eluent solution is essential to the successful operation of the IC. To ensure purity,

- Only freshly distilled water from the UP machine
- Rinse your glassware thoroughly before using (acid-washing optional)
- Use clean tins, and transfer immediately from the oven to the desiccator

a. Drying the Sodium Carbonate and Sodium Bicarbonate
   i. Eluent chemicals must be dried before weighing
   ii. Fill two aluminum drying tins: one with approximately 24g of Sodium Carbonate and the other one with approximately 9g of Sodium Bicarbonate
   iii. Once the oven is preheated to 550 degrees Celsius, place the two containers into the oven and bake for at least 1 hour.
   iv. After drying, allow chemicals to cool in the oven for at least one hour and in the desiccator for 1 hour or until the tins are cool enough to handle.

b. Preparing the 20x Concentrated Eluent Solution
   i. From the dry chemicals, measure 19.078g of Sodium Carbonate and 4.704g of Sodium Bicarbonate
   ii. Dilute the two chemicals to 2L utilizing a 2L volumetric flask with ultrapure water.
   iii. Store the solution in two 1L Nalgene bottles, labelled with “20x concentrated eluent,” your initials, and date made.
   iv. This makes 2L, enough to last for several months. Store in the reagents refrigerator.