

Arikaree Environmental Laboratory

Revision 1

Date: April 6, 2020

Anions by Ion Chromatography

TITLE: EPA 300.1

ANALYTES: Br, F, Cl, NO₃, NO₂, PO₄, SO₄

1) Applicable Matrices

- a) This method is applicable to ground, surface, and waste waters.
- b) The method is applicable in the range from 0 to 10.0 mg/L for all anions analysis when using the low range sample loop, and 0-100.0 mg/L when using the high range sample loop.

2) Scope and Application

- a) Anions are separated using Metrohm Metrosep A Supp 5 150/2.0 analytical column and discerned with a conductivity detector.

3) Interferences

- a) Any ion which elutes with the same retention time can interfere with the detection and quantification of target analytes.

4) Equipment and Supplies

- a) Analytical balance capable with 0.0001g sensitivity.
- b) Top loading balance with 0.01g sensitivity.
- c) Adjustable Pipets capable of precisely dispensing 1.0-10.0mL and 0.1-1.0mL
- d) Pipet tips appropriate for each pipet.
- e) 100mL graduated cylinder
- f) Class A volumetric flasks and pipets for reagent and standard preparation.
- g) Metrohm 930 Compact IC Flex
- h) Water purifier for preparation of ASTM Type I water (Ultrapure).
- i) Grey rubber tube stoppers/septa

5) Reagents and Standards

1,000ppm Cl, NO₃(N), SO₄ Stock Standards

- 1.) Use purchased certified stock standards for preparation of calibration and check standards.

Sodium Bicarbonate Stock Solution

- 1) In a 250mL class A volumetric flask, dissolve 2.10g sodium bicarbonate. Dilute to mark and invert to mix. Filter solution through a 0.45µm membrane filter. Store in a clean 250mL Nalgene bottle. Mark with date and store in refrigerator.

Sodium Carbonate Stock Solution

- 1) In a 250mL class A volumetric flask, dissolve 8.48g sodium carbonate. Dilute to mark and invert to mix. Filter solution through a 0.45µm membrane filter. Store in a clean 250mL Nalgene bottle. Mark with date and store in refrigerator.

Eluent Solution

- 1) In a 2L volumetric flask, pipet 20.0mL (2 draws of 10mL is ok) of both the sodium carbonate and sodium bicarbonate stock solutions. Fill flask to the mark, invert to mix, and degas with helium for 5 minutes. Pour eluent into the 2L bottle on top of the IC (this can be done before degassing).

Regenerant Stock Solution

- 1) Fill a 1L volumetric flask with about 500mL of ultrapure water. Then, using a graduated cylinder, add 55.6mL of concentrated sulfuric acid to the flask. Solution will become hot. Fill flask to the 1L mark with ultrapure water. Carefully invert to mix. Allow solution to cool to room temperature. After solution is cool, top off flask with ultrapure water to the 1L mark. Invert to mix. Store in labeled glass bottle.

Regenerant Solution

- 1) Fill a 1L volumetric flask with about 500mL of ultrapure water. Using a graduated cylinder, add 100mL of Regenerant Stock Solution to the flask. Fill flask to 1L mark with ultrapure water. Replace the stopper and invert to mix. Pour solution in regenerant flask on top of the IC. Degas the solution with helium for 5 minutes. Replace regenerant lines in the bottle.

6) Sample Collection, Preservation, Shipment, and Storage

- a) Collect and filter samples in clean plastic bottles. Samples shall be filtered through a 0.45µm membrane filter. Keep samples refrigerated between 0-4°C.

7) Quality Control

- a) Calibrate IC once a month or after maintenance or column change. Analyze Instrument Performance Check (IPC)/ from a second source standard every analytical run. Calibration r-value is required to be > 0.995, if not re-calibrate.
- b) Run an initial calibration verification blank (ICVB) at the beginning of each run and then once every 10 samples. ICVB must be <LOD. If not, re-analyze, if still out of control qualify data. Subsequent blanks (CCVB) must be <LOD, or less than 10% of the measured concentration in the samples from the adjacent set.
- c) Analyze an Initial Calibration Verification Standard (ICVS) at the beginning of each run and Continuing Calibration Verification Standard (CCVS) with every 10 samples. Standard must be within 10% of known value. If not, reanalyze. If still out of control, recalibrate.
- d) A duplicate and matrix spike must be analyzed every 10 samples (10%). Prepare a spiked sample by pipetting 0.1mL of 50ppm Cl, 25ppm N, and 50ppm SO₄ matrix spike solution into empty IC sample tube. Pipet 4.9mL of sample into the same tube. Seal tube with rubber cap and invert several times to mix. Place in correct location in autosampler rack.

8) Calibration

- a) Prepare **Low Range** calibration standards as listed below. Use only class A volumetric pipets and flasks. Dilute using ultrapure water.

Standard	mg/L Cl/N/SO ₄	mL 1,000ppm Cl/N/SO ₄ Standard	Total volume
1	10.0/5.0/10.0	10.0/5.0/10.0	1000mL
2	5.0/2.0/5.0	5.0/2.0/5.0	1,000mL
3	1.0/0.5/1.0	50.0 of Standard 1	500mL
4	0.5/0.2/0.5	50.0 of Standard 2	500mL
5	0.1/0.05/0.1	5.0 of Standard 1	500mL
6/BLANK	0.0/0.0/0.0	Fill IC tube with ultrapure water	N/A

- b) Prepare **High Range** calibration standards as listed below. Use only class A volumetric pipets and flasks. Dilute using ultrapure water.

Standard	mg/L Cl/N/SO ₄	mL 1,000ppm Cl/N/SO ₄ Standard	Total volume
1	100.0/50.0/100.0	50.0/25.0/50.0	500mL
2	50.0/20.0/50.0	25.0/10.0/25.0	500mL
3	10.0/5.0/10.0	50.0 of Standard 1	500mL
4	5.0/2.0/5.0	50.0 of Standard 2	500mL
5	1.0/0.5/1.0	5.0 of Standard 1	500mL
6	0.5/0.2/0.5	5.0 of Standard 2	500mL
7/BLANK	0.0/0.0/0.0	Fill IC tube with ultrapure water	N/A

9) Procedure for Metrohm 930 Compact IC Flex Set-up and Operation

- 1) Check if there is enough eluent and regenerant for analysis. If not, prepare solutions as described above in the reagents section.
- 2) Check which analytical column is installed. If it is not a Metrohm Metrosep A Supp 5 150/2.0, disconnect the column and guard column from the IC. Be sure to cap both ends of the guard and analytical columns so they do not dry out. Find the Metrohm Metrosep A Supp 5 150/2.0, but do not install yet. (If correct column is installed skip to step 8)
- 3) Be sure lines are submerged in the eluent and regenerant bottles. If switching methods, disconnect eluent line going into the high-pressure pump (wear nitrile gloves). Have a small beaker ready to catch liquid coming from the line. Allow line to drain into beaker for 5 minutes, then carefully reconnect line to pump.
- 4) Check to see if correct sample loop is installed. For low range anions, it should be very long. For high range anions, it should have a 100uL tag on it. If incorrect, install correct loop.
- 5) Place the end of the guard column inlet line in a small beaker. In the MagIC Net IC software, click on the "Workplace" icon in the upper lefthand corner. In "Run" window, click the "Equilibration" tab. Check to see that the loaded method is "Anions V.2." If not, click on the dotted button to the right of the method field. Choose "Anions V.2" in the pop-up window, then click open.
- 6) Click the "Manual" icon in the bottom left corner of the window. Then click on 930 Compact IC Flex in the list in the left of the pop-up window. The flow should be set to 0.7ml/min. If not, change it. Be sure the line is still in the beaker, and click the "Start" button. Allow eluent to run until no more air bubbles come out of the end of the line. The pump will shut off after a few minutes due to the pressure being too low, but if there are still air bubbles coming from the line, click start again.
- 7) With the pump running and eluent coming out of the line, connect the guard column. Allow eluent to flow through the guard column (into the beaker) for about 30 seconds, then connect the guard to the analytical column. Allow eluent to flow through the analytical column for about a minute, then connect the outlet line to the end of the column.
- 8) In the IC software, in the "workplace" window (icon in upper left corner), click "Start HW" in the upper left corner. If an error window tells you the wrong column is installed, be sure the method in the method field (under the "Start HW" button) is "Anions V.2". If it is not, click the button with the 3 dots (...) to right of the "Method" field. In the pop-up window click "Anions V.2" in the list and then click "Open" on the bottom right of the window. If the method is "Anions V.2," and it still gives you the error, contact the lab manager or someone else who looks like they know what they're doing, or just go home for the day.

- 9) Clicking the "Start HW" button starts the suppressor and the detector (as well as the high pressure pump if not already running). Allow the IC to run like this for an hour or two until the baseline equilibrates ("Workplace" window, top right quadrant) to under 2 uS/cm (usually under 1.5 uS/cm).
- 10) Make a list of samples needing anion analysis. Locate and gather these samples, then bring them into the lab and organize them in ascending order based on Lab ID# (this makes it easier when filling out benchsheet and recording data post analysis).
- 11) Prepare an IC benchsheet. One can be printed from the "Benchsheet" folder in the "Arikaree Share" folder.
- 12) List samples to be analyzed on the blank portions of the benchsheet. Record the samples on the sheet in ascending order according to Lab ID#, in the same order the samples should be organized (see step 10). Remember to record a duplicate and a matrix spike every 10 samples.
- 13) Go to the IC autosampler. Remove the wash bottles. If the rack is in a position where removal of wash bottles is impossible, go to the IC computer, click the "Manual" button in the lower left corner of the IC software. In the pop-up window, click on "858 Professional Sample Processor 1" in the list on the left. In the righthand portion of the pop-up window, there is a pull-down menu to the right of "Rack position." Click in the menu and type "35," then click start. The rack should turn to position 35. Remove the wash bottles. Go back and type "151" into the menu field. The rack should move to position 151 (wash bottle well).
- 14) Begin removing the autosampler rack for the autosampler by loosening (turning counterclockwise) the knob in the center until it is completely loose. Gently lift the rack off the autosampler and carry it to the bench where you will be setting up samples. Remove and dump any samples remaining in the rack and place the tubes in a wire rack to be washed. Fill the tubes with RO water and allow to soak (see IC Sample Tube Cleaning Procedure).
- 15) Be sure you have everything needed and organized for setting up IC analysis. Calibrate the pipets (0.1mL and 4.9mL) and record the values on the top of the benchsheet.
- 16) Begin setting up samples by pouring all of the standards and blanks needed and placing them in the rack. Follow the benchsheet and double check that each tube is in the correct well according to the benchsheet. After the standards are in place, continue with the samples. Cover each tube with a grey stopper/septum. Invert the matrix spike samples to mix. Again, double check that each sample is going into the correct well according to the benchsheet. It is better to catch a mistake immediately after it happens, than to realize there was one after the analysis has completed.
- 17) After the samples and standards have been set up in the rack, carry the rack to the autosampler and place it in position. There is only one way it will fit. Tighten the knob in the center of the rack by turning it clockwise.

- 18) Before entering the samples in the software, create a new batch so the data generated data can be easily found after analysis. In the IC software, click on the "Database" icon on the lefthand side of the window. In the menus on the top of the window, click on "Determinations"=>"Batch"=>"New Batch." A pop-up will prompt you to create a new batch name. Type in the date the samples were set up which should be the same date written on the top of the benchsheet by "Prep Date." Click "OK."
- 19) The samples can be entered into the IC software. Looking at the "Workplace" window, click on the tab "Determination Series" in the top left tile. Go to the "Sample Table" pull down menu on the bottom of the tile. Select either "ANIONS TEMPLATE," "LOW RANGE ANIONS NO CAL TAMPLATE," or "HIGH RANGE ANIONS TEMPLATE," depending on the application. Each of these should have the standards already in their correct positions according to the benchsheet. Double-check to make sure the position numbers for the standards in the software match the benchsheet. Click the "Reset Sample Table" button (looks like a blue arrow curving up and to the left with a red dot) above the sample table. This resets the table so it can be edited.
- 20) Double-click the first sample (top most sample). In the window that pops-up, go down to the "Batch name" field and click on it. Scroll through until you find the batch you just created and select it. Click "Apply" and then "Close." Now click the "Filling" button (looks like a blue bar with an arrow pointing down to some blue dashes) above the sample table. The cursor should now be an "F." In the sample table, go to the first row in the "Batch name" column. Click and hold, then drag all the way down to the end of the table. Now all of the samples will go to the same batch you just created.
- 21) Begin entering the samples by double-clicking on the first sample position. This causes an edit window to pop-up. Input the Lab ID# into the "Ident" Field and change the position number if needed. If there are any diluted samples, the dilution factor is input here as well. After the info is put in correctly, press enter to proceed to the next sample.
- 22) If there are multiple samples from the same set (20003-01, 20003-02, etc.), the increment function can be utilized. Click on the "↓+" above the table, then click, hold and pull down on the samples needed to be auto incremented.
- 23) After all of the samples have been entered, check that the position number of the last sample you input matches the benchsheet. If not, figure out where the mistake was made and correct it. Delete the rest of the rows by highlighting them, right clicking and selecting delete, but leave one set of the check standards to close the run (CCVB, CCVS).
- 24) If the IC is fully equilibrated and all of the samples are entered, click the green "Start" button in the upper right corner. Calculate how much eluent you will need and compare the calculated amount to how much is in the bottle ((Total samples in table x 19 min/sample) x 0.7mL/min=mL eluent needed). A full rack will need more than the eluent bottle can hold, so it will have to be topped off the next day regardless.
- 25) Clean up and put away everything from set-up. Wipe down the bench tops and put the samples away in their proper locations.

- 26) Check the progress of the run periodically by looking at a few chromatograms to ensure the instrument is running correctly (click the "Database" button on the left side and select the batch in the pulldown menu in the top right). Top off the eluent if needed.

Data Processing and Quality Control

- 1) After the run is complete, click the "Database" button then select the batch in the batch pulldown menu in the top right.
- 2) (If using a previous run's calibration, skip this step) Highlight all of the calibration standards, right-click them and select reprocess. Go through each standard and make sure each peak is integrated correctly. Correct them manually if they are not. Make sure all peaks are identified (blank should not have peaks, but the places where they should be should be integrated). Click the "Reprocessing" button in the bottom left. Click the tick box in front of "From Standards of Reprocessing table" then click "OK." Click "OK" in the bottom left. This will take you back to the previous database screen.
- 3) With the standards still highlighted, right click and choose "Reprocess..." again. Click on the "CAL BLANK" and click the "Reprocessing" button on the bottom. This time, click the tick box in front of "From selected determination," then click "OK." Click "OK" on the bottom right of the window. Now each standard has every calibration standard applied to its curve.
- 4) Back in the main database window, highlight all of the samples, check standards and the CAL BLANK by clicking and holding the top sample and dragging to the bottom.
- 5) Right click somewhere in the highlighted samples. Select "Reprocess."
- 6) Go through each sample and check that each peak is integrated correctly. If a peak is not, adjust the integration manually. Add peaks to the blanks, as they are used to calculate Method Detection Limits.
- 7) After all the samples have been corrected, select the CAL BLANK. Click "Reprocessing" on the bottom left. Click the tick box in front of "From selected determination," then click "OK." Click "OK" on the bottom right of the window. Now the calibration curve is applied to the whole run and concentrations are calculated. Go through and check that the concentrations of the check standards is within 10% of their known value.
- 8) Go to the "Tools" menu at the top of the window. Select "Export Template." In the pop-up window, select "The Best CSV template," then click "Properties." In the field to right of "Fixed File Name" (be sure tick box next to it is filled), type in the date the samples were prepped. Click "OK" at the bottom of the pop-up window. Close the "Export templates" window.
- 9) Highlight all of the samples in the run you would like to export. Click the "Determinations" menu, then select "Export." The export file is now in the "Processed Data Ready for Transfer" folder on the desktop. Go to it. The data file will be labeled with the setup date. Open it.
- 10) Open the IC reports file on the desktop. Open the correct report form for the results you want to report (High range, Low Range, Etc.)

- 11) Go back to the data file. Right click on the top left cell. Go to "Sort" and select "Sort A to Z." Check that every sample has a value for each analyte concentration and peak area. If not, that analyte does not have a peak integrated for that sample. Go back to the database and reprocess that and all the samples missing values. Delete the export file and re-export after the samples have been corrected. Redo this step until everything is correct.
- 12) Highlight everything in the data file except for the column labels (should be on the bottom). Copy the highlighted data.
- 13) Go back to the IC report. Right click on the top left cell under the column labels. Select paste. Add column labels to the top of each page. Also, add cell borders to all of the cells of the data table.
- 14) Go to "File=> Save As," "Browse," "Desktop=>IC Reports," then choose the correct folder for your analysis (High Range Anions, Low Range Anions). Name the file the set-up date of the run (top of benchsheet).
- 15) Transfer this report to a thumb-drive for printing. Print the report in the lab office. Staple the report behind the benchsheet.
- 16) Calculate the duplicate differences and matrix spike recoveries. Record these data in the QC data in the Anions QC data folder on the Arikaree share folder. Mark any failures for reanalysis and write them on the rerun board on the standards fridge.
- 17) Record sample results on master data sheets and erase X's on board in the lab. Write any samples needing dilutions on the reruns board.
- 18) Three hole punch the run report and place in the correct section of the Ion Chromatography binder, with the newest run on top.
- 19) Clean-up the samples in the autosampler rack (see cleaning procedure below).
Reanalyzing samples is as easy as making sure the instrument has enough eluent and regenerant, turning on the hardware, equilibrating and adding the samples to the end of the determination table and hitting start once equilibrated. These reruns can be added to the same run report (don't reprint the entire report). Examples of samples to rerun: failed duplicate or matrix spike, samples needing dilutions.

Procedure for IC Sample Tube Cleaning

- 1) Remove Autosampler rack (if necessary) according to step 13 in "Procedure for Metrohm 930 Compact IC Flex Set-up and Operation." Carry rack to sink. One-by-one remove tubes from rack, discard rubber stoppers into the trash and dump samples in sink.
- 2) Place tubes in appropriate wire rack.
- 3) Once all tubes have been removed and dumped, carry wire racks with empty tubes into the wash room.
- 4) Fill each tubes with RO water. Allow to soak overnight.

- 5) After soaking, dump water from all tubes at once by holding tubes in the rack with another empty rack on top.
- 6) Rinse tubes at least 3 times with RO water, dumping them as described in step 5.
- 7) Flip the tubes upside down one-by-one in the wire rack to drain. Place on a Kim wipe on the drying bench.
- 8) If tubes still look dirty or have something dried on the bottom, discard tube into the trash.
- 9) Once dry, place rack with other clean, dry IC sample tubes by IC.

10) Corrective Action for Out-of-Control Data

- a) Any CCV blank that exceeds the LOD, the analyst must inspect the concentration of the previous sample. If the blank is greater than 10% of the previous sample, reanalyze the blank immediately following the CCVS. No samples can be run until the blank meets requirements.
- b) CCV and LCS standards must fall within 10% of true value (90%-110% Recovery). If not re-mix and re-analyze, if still out of range re-calibrate.

11) Contingencies for Handling Out-of-Control Data

- a) Samples that fail the CCVB or ICVS will have to be qualified back to the last sample that the quality control met the above conditions.

12) Waste Management

- a) All waste must be collected and disposed of according to CU EHS policies.

13) References

- a) U.S. EPA. 1997. "Method 300.1: Determination of Inorganic Anions in Drinking Water by Ion Chromatography," Revision 1.0. Cincinnati, OH