

Cation Analysis – Ca, K, Na, Mg, Fe, Mn

Adapted from WSEL protocols.

Revised by: Peter Conowall, Jimmy Sustachek, and Emily Ledin (July 2023)

Purpose: This procedure describes the steps to analyze major cations for filtered Core LTER water samples preserved with concentrated HCl to 1% v/v. These cations include calcium, potassium, sodium, magnesium, iron, and manganese. The units for results of this analysis are ppm ($\mu\text{g/L}$).

Sample Holding Time: ≤ 1 year @ room temperature, preserved with concentrated Optima HCl to a concentration of 1% HCl v/v.

Materials Required:

For analysis/prep:	For standard/ICV prep:
<ul style="list-style-type: none">• Samples (S-bottle)• Plastic Falcon centrifuge tubes•	<ul style="list-style-type: none">•

Personal Protective Equipment / Waste Disposal: Nitrile gloves and safety glasses are always required during this procedure. This is not only for your protection, but also to prevent contamination of samples. Always use chemical resistant gloves (not latex).

Quality Assurance/Quality Control:

The validity of the data collected by the instrument is checked with the following quality control samples:

- Reagent blank, plain Milli-Q water
- Initial Calibration Verification (ICV) sample(s) are prepared from a different commercial stock solution than the calibration curve.
- Every 10th sample is duplicated.

Waste Disposal: Excess sample and standard can be disposed of down the drain. Falcon tubes can be thrown in the garbage.

Note: The ICP-OES is owned and maintained by the Water Science and Engineering Laboratory (WSEL). To run samples, you will need to create a FOM account to reserve instrument time and schedule initial training. After two training sessions, you will be able to reserve instrument time and run samples independently.

Create a FOM account here: <https://wcam-fom.doit.wisc.edu/fom/> and contact James Lazarcik (lazarcik@wisc.edu) for help with account creation and to schedule initial trainings.

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Sample Preparation

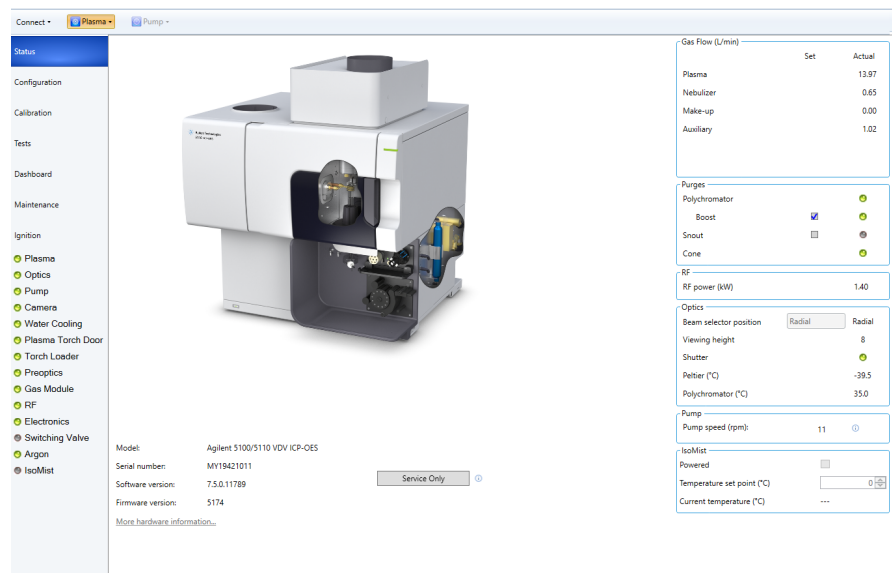
- 1.0 Label small, blue plastic Falcon centrifuge tubes with sample IDs.
- 2.0 Pour 12-14mL of corresponding filtered acidified samples (filtered S-bottles, -3) into the Falcon tubes.
 - 2.1 If possible, let tubes sit out a few days to let any particulates settle out.
- 3.0 Pour 40-50mL of prepared standard and ICV solutions into labeled green, larger standard centrifuge tubes.

Sample and Standards Analysis

Autosampler and Instrument Preparation

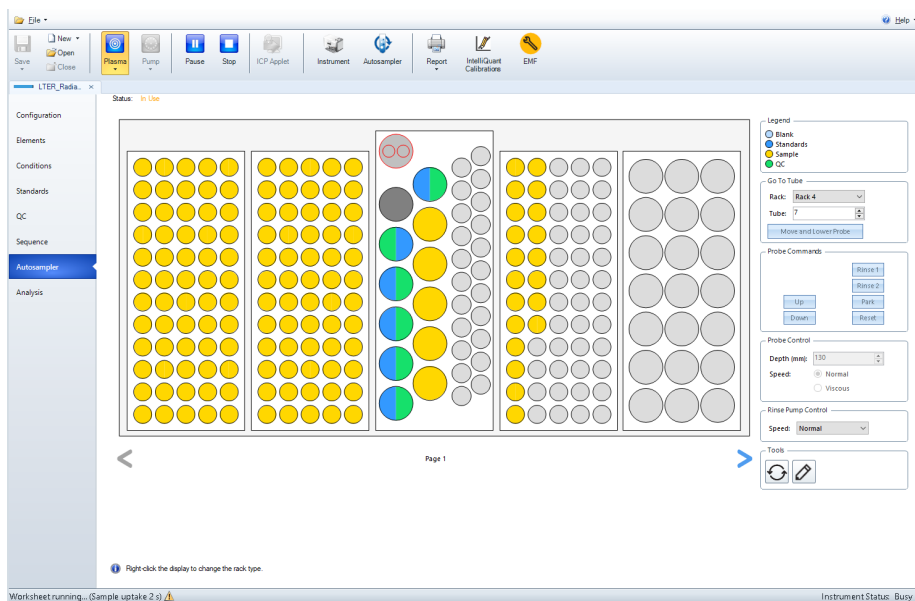
- 4.0 Turn on computer monitor and log into the WSEL FOM System
- 5.0 Check the Argon cylinder pressure and manually check there is enough liquid argon by physically tipping the tank. A tank with adequate volume will have some weight to it. If you are unsure ask WSEL Staff before proceeding.
 - 5.1 Notify James if argon is low.
- 6.0 Check the waste container to make sure there is enough room for your waste. **If you aren't sure - ask.**
 - 6.1 Notify James if the waste container is full.
- 7.0 Check the rinse reservoir to ensure there is enough rinse solution for analysis. **If you aren't sure - ask.**
 - 7.1 Notify James if the rinse reservoir is low.
- 8.0 Turn on the chiller unit below the ICP-OES by pressing the power button once.
 - 8.1 Do not adjust the temperature knob.
- 9.0 Check the alignment of the ignition unit - make sure the notch on torch lines up with notch behind it on the instrument.
- 10.0 Inspect the torch to make sure it is relatively clean, and that the outlet is free of obstructions.
- 11.0 Open ICP Expert via the Desktop.
 - 11.1 Select "New From" and open the LTER_Radial template.
 - 11.2 Make sure "Enable QC" and "IntelliQuant" are checked in configuration.
- 12.0 Via the ICP Expert main page, select 'Instrument' from the upper ribbon and ensure that all status indicators on the left side are green.
 - 12.1 Plasma may remain yellow until ignited.
 - 12.2 Verify there are no flashing red modules in the instrument graphic.

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- 13.0 Hook up the instrument tubing and install the peristaltic pump.
 - 13.1 Follow the rotation indicator on the peristaltic pump for direction of flow. There are three tubing lines. The arrow will point in the direction of the flow for each. Waste should be directed towards the waste container and internal standard and autosampler should be directed towards the nebulizer.
 - 13.2 From **back to front** the order and color of the tubes is as follows:
 - 13.2.1 Waste (blue/blue – thickest of the three)
 - 13.2.2 Internal Standard (orange/blue – skinniest)
 - 13.2.3 Sample Line (white/white)
 - 13.3 Place the internal standard tubing into the Yt internal standard solution.
 - 13.4 Tubing should be hooked into place on both ends. Stretching may be required.
 - 13.4.1 Stretch the internal standard tube to the farthest stops on the peri-pump
- 14.0 Select 'Pump' from the top ribbon and run on fast for ~15 seconds to auto-align tubing.
 - 14.1 Carefully manually adjust the tube position if necessary, making sure they are parallel to one another.
 - 14.2 Once aligned, turn the pump off.
- 15.0 Clamp the tubing into place.
- 16.0 Go to the 'Autosampler' tab and send the autosampler probe to a spare blank (50 mL Falcon tube) by double clicking on the appropriate autosampler location.
 - 16.1 Sample rack type can be changed, if necessary, by right clicking on the sample rack and selecting the appropriate size.

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- 17.0 Select 'Plasma' from the top ribbon and turn plasma on. (Record the time you started the plasma; you will be asked how long plasma was on at FOM Log-out).
- 17.1 When asked if peristaltic pump is installed correctly, double check your installation.
- 17.2 The pump will start automatically, and you should be able to see the plasma ignite through the small window on the ICP-OES after a few moments.
- 17.3 The plasma needs 15 minutes to equilibrate prior to analysis.
- 17.4 Verify that the waste is being drawn out at a consistent rate and the sample and internal standard lines are flowing at a consistent rate. Check internal standard tubing integrity by removing and replacing tubing from bottle quickly to introduce a small amount of air. Watch the bubble move through the system, watching for a smooth and consistent flow.
- 17.5 Verify the waste line has equal segments of liquid and gas flowing steadily out of the bottom of the spray chamber.
- 17.6 Check that a cloud/mist has formed in the spray chamber.
- 18.0 While plasma is equilibrating, select the 'Standards' tab and enter standard concentrations you calculated in the Cations Standards Template during preparation. Make sure to match the date on the Excel spreadsheet with the prep date on the standard bottles.

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Standards

Solution Label (nm)	Mg-r (285.213 nm)	Mn-r (257.610 nm)	Mn-r (259.372 nm)	Na-r (568.821 nm)	Na-r (588.995 nm)	Na-r (589.592 nm)
Blank	0.000	0.000	0.000	0.000	0.000	0.000
A	0.567	0.050	0.050	0.532	0.532	0.532
B	0.986	0.111	0.111	1.010	1.010	1.010
C	5.020	0.204	0.204	5.145	5.145	5.145
D	9.898	0.393	0.393	9.786	9.786	9.786
E	22.461	1.198	1.198	25.941	25.941	25.941
F	74.320	7.202	7.202	74.714	74.714	74.714

Calibration Fit

Label (Wavelength nm)	Unit	Calibration Fit	Weighted Fit	Force Through	Minimum Concentration	Maximum Concentration	Calibration Error	%RSE Limit
Fe-r (238.204 nm)	ppm	Linear	<input checked="" type="checkbox"/>	None	0.000	22.554	20%	25%
Fe-r (239.563 nm)	ppm	Linear	<input checked="" type="checkbox"/>	None	0.000	22.554	20%	25%
K-r (766.491 nm)	ppm	Linear	<input checked="" type="checkbox"/>	None	0.000	18.514	20%	25%
K-r (769.897 nm)	ppm	Linear	<input type="checkbox"/>	None	0.000	18.514	20%	25%
Mg-r (280.270 nm)	ppm	Linear	<input checked="" type="checkbox"/>	None	0.000	81.752	20%	25%
Mg-r (285.213 nm)	ppm	Linear	<input checked="" type="checkbox"/>	None	0.000	81.752	20%	25%
Mn-r (257.610 nm)	ppm	Linear	<input checked="" type="checkbox"/>	None	0.000	7.922	20%	25%
Mn-r (259.372 nm)	ppm	Linear	<input checked="" type="checkbox"/>	None	0.000	7.922	20%	25%
Na-r (568.821 nm)	ppm	Linear	<input type="checkbox"/>	None	0.000	82.185	20%	25%
Na-r (588.995 nm)	ppm	Linear	<input type="checkbox"/>	None	0.000	82.185	20%	25%
Na-r (589.592 nm)	ppm	Linear	<input checked="" type="checkbox"/>	None	0.000	82.185	20%	25%

- 19.0 Go to the 'Sequence' tab and enter sample IDs and proper Rack/Tube locations.
- 19.1 Number of samples can be selected under 'Samples and Calibration'.
- 19.2 A blank check and a standard check should be performed every 10 samples.
- 19.3 A duplicate should be performed every 10 samples.
- 19.4 Ensure that "Park autosampler" is checked.
- 19.5 Ensure "Rinse system" is checked and for 3 minutes.

Sequence

Number of samples: 167

Calibration every (samples): 20

Enable sample information:

QC Tests:

QC Name	QC Type	Count as sample when manually inserted
Blank	CCB	<input type="checkbox"/>
A	CCV	<input type="checkbox"/>
B	CCV	<input type="checkbox"/>
C	CCV	<input type="checkbox"/>
D	CCV	<input type="checkbox"/>
E	CCV	<input type="checkbox"/>
F	CCV	<input type="checkbox"/>

End of Run Actions:

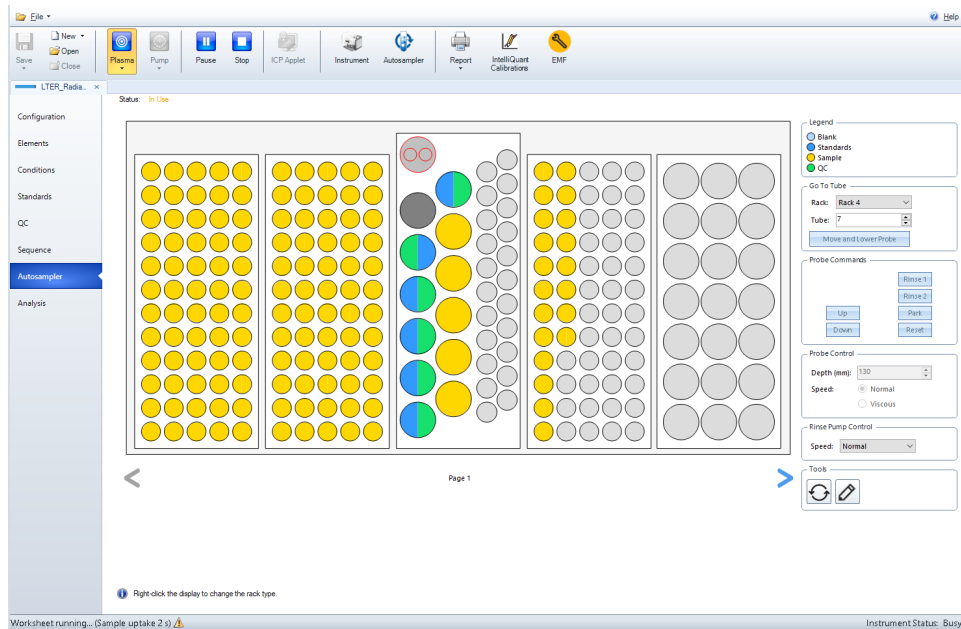
- Set pump speed (rpm) 0.75
- Turn plasma and pump off
- Turn plasma off and reduce pump speed
- Disable polychromator boost purge after run
- Disable snout purge after run
- Rinse system (min) 3.00
- Park autosampler

Rack/Tube	Solution Label	Solution Type	Weight (g)	Volume (mL)	Dilution
S1:1	Blank	Calibration			
S1:2	A	Calibration			
S1:3	B	Calibration			
S1:4	C	Calibration			
S1:5	D	Calibration			
S1:6	E	Calibration			
S1:7	F	Calibration			
S1:8	LOQ	Sample	1.0	1.0	1.0
S1:9	ICV_L	Sample	1.0	1.0	1.0
S1:10	ICV_H	Sample	1.0	1.0	1.0
S1:11	06.19 A Rerun	Sample	1.0	1.0	1.0
S1:12	06.19 F Rerun	Sample	1.0	1.0	1.0
1:1	245913	Sample	1.0	1.0	1.0
1:2	245923	Sample	1.0	1.0	1.0
1:3	245943	Sample	1.0	1.0	1.0
1:4	245953	Sample	1.0	1.0	1.0
1:4	245953 Dup	Sample	1.0	1.0	1.0
S1:1	Blank	Blank			
S1:2	A	A			
1:5	245963	Sample	1.0	1.0	1.0
1:6	245973	Sample	1.0	1.0	1.0
1:7	245993	Sample	1.0	1.0	1.0
1:8	246003	Sample	1.0	1.0	1.0
1:9	246033	Sample	1.0	1.0	1.0
1:10	246043	Sample	1.0	1.0	1.0
1:11	246073	Sample	1.0	1.0	1.0
1:12	246083	Sample	1.0	1.0	1.0

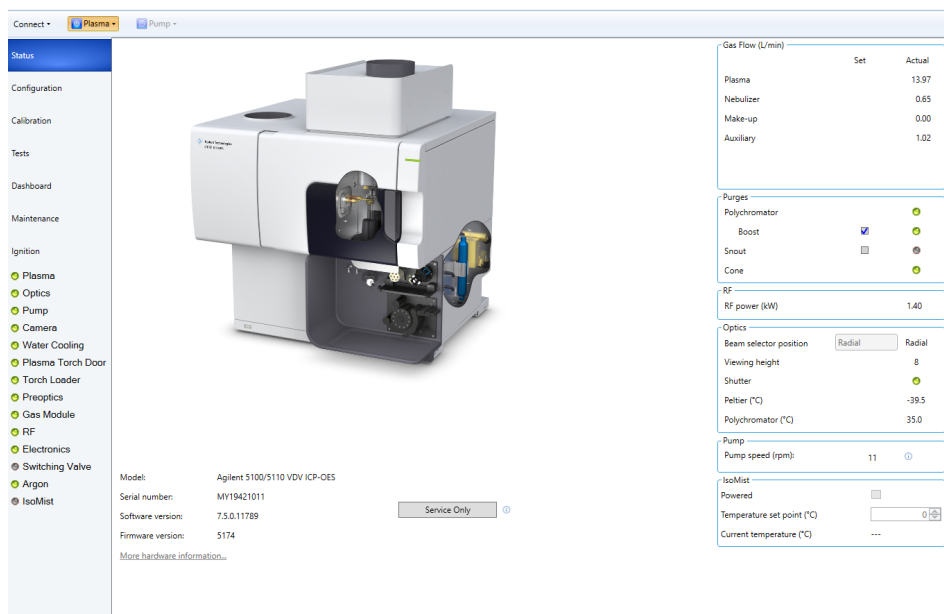
Worksheet running... (Rising 12) Instrument Status: Busy

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- 20.0 Place samples, standards and ICVs into their appropriate autosampler locations.
- 20.1 Go to 'Autosampler' tab and check that the correct autosampler locations are highlighted with the proper sample/standard type.



- 21.0 Return to the 'Instrument' tab to check that all status indicators are green, and no modules are flashing red on the diagram. The Peltier should read -40C and the Polychromator 35C.
- 21.1 Make sure Snout is checked for Radial analysis.
- 21.2 Make sure "Beam selector position" is set to Radial.



- 22.0 Start your run and remain present to check that the run starts properly and ICV checks are accurate.

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- 22.1 Once ICVs have been run and values are acceptable, the instrument can be left to run.
- 22.2 Check periodically to make sure blank and standard checks are good and that argon gas pressure is sufficient.
- 22.3 **RERUN ANY SAMPLES THAT HAVE AN INTERNAL STANDARD RATIO GREATER THAN 1.10 OR LESS THAN 0.90**

Exporting Data and Shutdown

- 23.0 When the analysis is done, cap samples and standards as soon as possible to avoid evaporation loss.
- 24.0 Release the sample and waste tubing from the peristaltic pump and remove tubing from and cap the internal standard.
- 25.0 Turn off the chiller.
- 26.0 In the 'Analysis' tab, click the upper left most cell to select the whole table and right click to export the results table as a csv file.
- 27.0 Log-off from the FOM and record the number of hours that the instrument was in use for.
 - 27.1 Time of use should be determined by the time in which the plasma was on and samples were being analyzed.

Data Entry

28.0 Each analyte has an optimal wavelength response or average of responses from multiple wavelengths for best results. Enter values from the column headers described below.

28.1 It is easiest visually for entry to Chemlab to clean up the raw data spreadsheet by removing extraneous columns and save as a new "cleaned up" version.

Ca: 317.933 nm only

Fe: Average of both wavelengths in method (238.204 nm and 239.563 nm)

K: 766.491 nm only

Mg: Average of both wavelengths in method (280.270 nm and 285.213 nm)

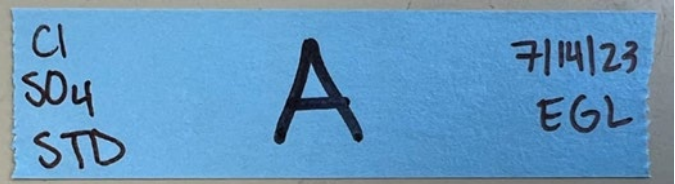
Mn: Average of both wavelengths in method (257.610 nm and 259.372 nm)

Na: 589.592 nm only

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Calibration Standard and ICV Preparation

- 1.0 Prepare 1% Optima HCL
 - 1.1 Use 1L Volumetric Flask – add ~700mL Type I water, pipette 10mL Concentrated Optima HCL, top off flask to 1L. Invert carefully to mix 3x.
 - 2.0 Go to standard calc sheet folder in share drive.
 - 2.1 *LTER Water Chem Lab* → *SOPs and Project Descriptions* → *STANDARD CALC SHEETS* → *CATIONS*
 - 3.0 Make a copy of the file titled **CATIONS-TEMPLATE-Blank**
 - 4.0 Rename the file with the date that you plan to make the standards.
 - 4.1 Ex) *CATIONS_07_14_2023*
 - 4.2 Put the file in the folder titled with the appropriate year.
 - 5.0 Open the sheet and fill out your name and the date of preparation in the top right corner of the sheet, as well as all stock solution information.
 - 6.0 Print a copy of the calculation sheet.
 - 7.0 Obtain eleven clean 250 mL plastic Nalgene bottles.
 - 7.1 Brand new bottles are ideal, but freshly acid washed bottles are also okay. (See acid washing SOP)
 - 8.0 Label the bottles with label tape:
 - 8.1 K-Fe-Mn INT, A, B, C, D, E, F, ICV INT, LOQ, ICV-L, ICV-H,
 - 8.2 On the left side of the tape write “CATION STD” and on the right side write the day of preparation and your initials

Ex) 
 - 8.3 *Tip: color coding the calibration standards vs. ICVs may also be helpful*
 - 8.4 Ex) all calibration standards (A-F) are blue and all ICVs (LOQ, ICV-L, ICV-H) are pink.
- 9.0 Use the file titled CATIONS-TEMPLATE (located in the CATIONS standard calc sheet folder) to find the concentrations of the stock solution you must use to make the standard and ICV solutions.

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9.1 There should be a copy of it near the analytical scale in the River Ecology lab. If not, print one out to reference.

10.0 Use an analytical scale and the template to make the standards and ICVs:

NOTE: The amount of stock solution and MQ water on the calculation sheet is cumulative.

Example: For standard A, add 0.200g of Ca stock, then add 0.200g of Mg stock (so that the total weight is now 0.200g), then add 0.200g of Na stock (so that the total weight is now 0.600g), then add 2.500g of the K-Fe-Mn INT (so that the total weight is now 3.100g). Finally add 196.900g of 1% Optima HCl so that the final weight is 200.000g.

Ca, Mg, Na, K, Fe, Mn Standard Calculations (ICP)
 ALL Stocks are 1,000 ppm (1,000,000 ppb)

Date: _____
 Prepared by: _____

Calibration Standards				ICV Standards		
Brand:	Lot #	Brand:	Lot #			
Ca:						
Mg:						
Na:						
K:						
Fe:						
Mn:						

K-Fe-Mn INT
K Stock (g) 1.000 **Fe Stock (g)** 3.000 **Mn Stock (g)** 3.200 **1% HCl (g)** 50.000

[K] ppm [Fe] ppm [Mn] ppm
 20.000 40.000 4.000

Std	Ca Stock (g)	Mg Stock (g)	Na Stock (g)	K-Fe-Mn INT (g)	1% HCl (g)	[Ca] ppm	[Mg] ppm	[Na] ppm	[K] ppm	[Fe] ppm	[Mn] ppm
A	0.200	0.400	0.600	3.100	200.000	1.000	1.000	1.000	0.250	0.500	0.050
B	0.400	0.800	1.200	6.200	200.000	2.000	2.000	2.000	0.500	1.000	0.100
C	1.000	2.000	3.000	13.000	200.000	5.000	5.000	5.000	1.000	2.000	0.200
D	2.000	4.000	6.000	26.000	200.000	10.000	10.000	10.000	2.000	4.000	0.400

	Ca Stock (g)	Mg Stock (g)	Na Stock (g)	K Stock (g)	Fe Stock (g)	Mn Stock (g)	1% HCl (g)	[Ca] ppm	[Mg] ppm	[Na] ppm	[K] ppm	[Fe] ppm	[Mn] ppm
E	4.000	8.000	12.000	13.000	15.000	15.200	200.000	20.000	20.000	20.000	5.000	10.000	1.000
F	10.000	20.000	30.000	32.000	36.000	37.500	200.000	50.000	50.000	50.000	10.000	20.000	7.500

ICV	Ca Stock (g)	Mg Stock (g)	Na Stock (g)	K Stock (g)	Fe Stock (g)	Mn Stock (g)	1% HCl (g)	[Ca] ppm	[Mg] ppm	[Na] ppm	[K] ppm	[Fe] ppm	[Mn] ppm
ICV INT	5.000	10.000	15.000	16.000	18.000	18.250	50.000	100.000	100.000	100.000	20.000	40.000	5.000
H	6.000	12.000	18.000	19.500	22.500	23.000	200.000	30.000	30.000	30.000	7.500	15.000	2.500
	ICV INT (g)						1% HCl (g)						
LOQ	1.000						200.000	0.500	0.500	0.500	0.100	0.200	0.025
L	6.000						200.000	3.000	3.000	3.000	0.600	1.200	0.150

10.1 Place a 250mL Nalgene bottle on the scale, close all the doors on the scale, and tare.

10.2 Pour the stock solution into a disposable 10mL beaker.

10.2.1 DO NOT USE SQUIRT BOTTLES FOR ANY SOLUTIONS –

Disposable transfer pipettes and disposable 10-20mL beakers should be used to transfer all commercial stock solutions. Great care must be taken to prevent contamination of Calibrants and Check Standards.

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- 10.3 For the low end calibrants, very small volumes of stock solution are needed. Use a 1mL disposable pipette.
 - 10.3.1 One droplet is ~0.01g
- 10.4 For high-range amounts of stock you can pour the stock straight from the bottle, until you get close to the desired weight, then switch back to using the disposable pipettes and beakers.
- 10.5 **Write down the exact amount of stock solution on the blank calculation sheet** you printed out, making sure to close the doors of the analytical scale before recording your measurement.
- 10.6 To add MQ using a clean glass beaker and disposable pipettes.
- 11.0 Enter your stock solution amounts into your standard calculation sheet on a computer to find the concentration values.
- 12.0 Print out your newly filled out sheet.