**SOP for AA**

**Revised 11/30/12 (BAH)**

This document covers:

1. Sample preparation for Ca, Mg, Na, and K analyses
2. Standard and Quality Check preparation
3. Machine optimization
4. Running samples

Procedure for extracting cations from soils is described in “Mehlich III SOP PO4-P.doc” in wright.lab/methods/AA

**Sample preparation for Ca, Mg, Na, K**

* Samples should already be filtered Whatman No. 42 filter paper
* Keep samples refrigerated until acidification with LaCl3
* In new 15 mL polypropylene centrifuge tubes, dilute your samples 10-fold in ddi. Standards and qc’s will be prepared in a solution of 10x diluted Mehlich III.
  + If you want to dilute your samples less, be sure the standards and qc’s are prepared in the same matrix. (Does higher concentration of Mehlich interfere with analysis?) Budget at least 1 mL of diluted sample per element analyzed. To analyze four elements, 1 mL original sample + 9 mL ddi, will be plenty.
  + If you need to further dilute your samples so they fall within the range of the standard curve, you don’t need to make all new standards and qc’s. Just make sure you also prepare a sample blank (diluted like samples, + LaCl3).
* Acidify samples in LaCl3 at 10% of the volume (add 1mL LaCl to 10 mL diluted sample) **LaCl3 recipe:**
  + To make 1L, fill a 1L volumetric flask with about 200 mL DI.
  + Add 58 g LaO3 (Lanthanum Oxide) to flask using funnel and DI wash bottle. Note: LaO3 does not dissolve well in water so most of it will settle to the bottom.
  + In fume hood, slowly add 500 ml HCl while swirling. (use goggles, long nitrile gloves & lab coat)
  + Allow the flask to cool to room temperature before diluting to volume with DI and mixing thoroughly.
  + Note: LaCl3 is very stable. Check for precipitates or if it has changed from clear to yellow in color, otherwise the solution is good for several years.

**Standard preparation**

1. Make standards using 10x diluted Mehlich extraction solution (from same batch as samples).
   1. Mixed standard #3: 30 ppm Ca, 12 ppm K, 6 ppm Na, 3 ppm Mg
      1. In 100 ml flask, add:

|  |  |
| --- | --- |
| 3 ml | Ca standard solution (1000 ppm) |
| 1.2 ml | K standard solution (1000 ppm) |
| 0.6 ml | Na standard solution (1000 ppm) |
| 0.3 ml | Mg standard solution (1000 ppm) |

* + 1. Dilute to line with 10x diluted Mehlich
  1. Mixed standard #2: 15 ppm Ca, 6 ppm K, 3 ppm Na, 1.5 ppm Mg
     1. In 100 ml flask, add:

|  |  |
| --- | --- |
| 1.5 ml | Ca standard solution (1000 ppm) |
| 0.6 ml | K standard solution (1000 ppm) |
| 0.3 ml | Na standard solution (1000 ppm) |
| 0.15 ml | Mg standard solution (1000 ppm) |

* + 1. Dilute to line with 10x diluted Mehlich
  1. Mixed standard #3: 5 ppm Ca, 2 ppm K, 1 ppm Na, 0.5 ppm Mg
     1. In 100 ml flask, add:

|  |  |
| --- | --- |
| 0.5 ml | Ca standard solution (1000 ppm) |
| 0.2 ml | K standard solution (1000 ppm) |
| 0.1 ml | Na standard solution (1000 ppm) |
| 0.05 ml | Mg standard solution (1000 ppm) |

* + 1. Dilute to line with 10x diluted Mehlich

1. Sensitivity check: 4 ppm Ca, 2 ppm K, 0.5 ppm Na, 0.3 ppm Mg
   1. In 250 ml flask, add:

|  |  |
| --- | --- |
| 1 ml | Ca standard solution (1000 ppm) |
| 0.5 ml | K standard solution (1000 ppm) |
| 0.125 ml | Na standard solution (1000 ppm) |
| 0.075 ml | Mg standard solution (1000 ppm) |

* 1. Dilute to line with 10x diluted Mehlich

1. QC’s:
   1. Use cations #530 vial from ERA QC box in refrigerator
   2. Determine appropriate values from data sheet in ERA binder
      1. Make dilutions at high, middle, and low end of your standards
      2. Use 10x diluted Mehlich as your matrix!
2. Blank:
   1. measure 100 ml of 10x diluted Mehlich solution into 125 ml Nalgene bottle
3. Acidify standards, sensitivity check, QC’s, blank.
   1. Pour 100 ml of each solution into 125 ml Nalgene bottle
   2. Add 10 ml of LaCl3 to each bottle and mix.

**ANALYSIS**

1. **Turn on AA**. Power switch is on the right panel of the machine below the lamp. The AA needs to be on before opening software
2. **Turn on computer & start software.**
3. Click cancel when asks for user & password (not attached to network).
4. OpenAA WinLab Analyst.
5. Note: The software appears like you can set wavelength, slit, etc. using the computer, but most of setup must be done manually on the AA.
6. Click “Use a custom-designed workspace.” A window will pop up with File name. Select manual.flm. Ok.
7. **Set up run**
   1. **Load a method**. Go to File, Open, Method... choose a method.
      1. Can also create a new method by going to File, New, Method.
      2. Set the standard concentrations, sig figs, decimal places, and number of replicate measurements in calibration tab.
   2. **Set up sample sheet** \*\* note, this is optional; can write down data in notebook (computer isn’t on network; can’t email document)
      1. go to File, New, Sample Info File. The batch ID can only be 8 characters.
      2. Use the table to setup your sample sheet; copy and paste it from excel. Worksheet won’t let you cut, paste, or insert lines.
      3. Click the X to close the window when finished (there is no OK or SAVE).
      4. Then go to File, Save As, Sample Info File. Give the file an 8 character or less file name.
      5. Then on the manual analysis window, click on the Browse… button next to Sample Information File: and navigate to the file you just saved.
8. **Set parameters on AA**.
   1. Use the method in the blue Perkin-Elmer folder to determine the wavelength and appropriate range of standards to use.
   2. Typically use a three point standard curve plus a sensitivity check, blank and QC.
9. **Install lamp**.
   1. Click on the lamp icon to set the lamp type, current, wavelength, and slit. Current should be the ‘continuous’ value on lamp box label. Adjust wavelength and slit values on AA itself.
   2. Turn on the lamp by clicking the green circle. It will change from olive to lime green when the lamp/detector is on.
   3. Maximize lamp energy, use the black knobs on top of the lamp housing, and if necessary, make small adjustments to the wavelength knob,
      1. Green bar will grow & shrink as adjustments are made; want green bar to be as large as possible.
      2. Click on the “set midscale” icon to adjust the x-axis scale so that the green bar reaches the middle of the graph.
      3. The energy number to the right of the bar should be close to the following: Ca 58, K 58, Na 54, Mg 56, Sr 54, Li 53, Fe 60.
10. **Line up burner head**.
    1. Close lamp window.
    2. Click on Tools, Continuous Graphics.
    3. Lower the burner head to its lowest position using the big black knob at the base.
    4. Click the auto zero icon. Raise the burner head until it interferes with the absorbance in the continuous graphics window. Then lower head back to zero absorbance.
11. **Igniting burner**
    1. Turn on acetylene tank on, make sure flow pressure is 12 psi, and open the valve to let fuel flow to the AA. Record the tank psi in the log.

***Important:*** if the tank level is less than 50 psi turn it off immediately. Do not proceed with analysis until you have a new tank. A low acetylene tank can lead to acetone in the fuel line—a hazard!

* 1. Turn on house air (knob on wall). Set oxidant knob on the AA to Air.
  2. If the red ignite button *does not* light up press the button to ignite the burner.
  3. If the red ignite button *does* light up, DO NOT ignite
     1. Turn the oxidant knob back to off.
     2. Most likely there is air in drain line. It needs to be free of bubbles and holding water about 12 inches above the bottle.
     3. Detach the drain tube from the AA & decouple the wires.
     4. Straighten out drain tube and lower so end is slightly above the waste carboy.
     5. Pour water down the tube; when full, slide thumb over end and straighten tube, forcing air bubbles to come up to surface. May need to gently tap the tube to make all the air bubbles emerge.
     6. Re-loop drain tube, quickly attach to AA and reconnect wire coupler.
     7. Turn the oxidant knob on to see if ignite button doesn’t light up; can then proceed with igniting.

1. **Optimize settings**.
   1. Sensitivity check; should come out with an absorbance between 0.15 and 0.25.
      1. Zero the baseline in Continuous Graphics while the capillary tube is in your blank.
      2. Begin aspirating your sensitivity check.
      3. First adjustment is to lateral position of burner head. Move it side to side using the front black knob until the absorbance has been maximized.
      4. Next adjustments are to the oxidant and fuel input levels to optimize absorbance.
      5. Final adjustment is to sample flow rate
         1. unscrew the nebulizer knob until the capillary tube is bubbling into the water blank.
         2. Zero the baseline in continuous graphics.
         3. Put the capillary tube into the sensitivity check and retighten the nebulizer until the absorbance is optimized.
2. **Analyze Blank & Standards**
   1. Allow instrument to warm up for at least 20 minutes after flame is lit before running standards
   2. Put the capillary tube in blank
   3. In the Manual Analysis screen click the Analyze Blank icon.
      1. The results window will display the measurements then the mean when finished.
      2. Remove tube as soon as mean is displayed to conserve sample
   4. Put the tube in lowest standard and click the Analyze Standard button.
   5. Repeat for middle and high standards
   6. Record the final r2 and slope of your standard curve in the log. The readout from the software will give you a slope and r2 value with each standard you analyze.
   7. May need to run a few standard curves in a row to ensure that you are getting similar absorbance values and slopes each time.
3. **Analyze QC’s**
   1. Put the tube in low QC and click Analyze Sample
   2. Repeat for middle & high QC’s
   3. Check values against certified values
   4. Good practice to analyze a QC every 15-20 samples
4. **Analyze Samples**
   1. Continue with analyzing samples…
   2. Run standards every 20 samples to check for drift (change in slope)
   3. If sample has a higher concentration than the highest standard, manually dilute sample and rerun
   4. The curves often have a quadratic fit, so using the value generated by the curve for a sample with a high concentration will give you results that are inaccurate.
   5. If using sample file:
      1. If you want to run a sample out of order of your Sample Info File (you’d like to run a QC or a concentration looks strange) you can navigate through your samples by clicking on the arrows by the sample number
      2. can also update the .sif file and save it.
      3. ***Important:*** The values generated by the software for the standards are absorbances, but it automatically converts sample values to mg/L.
5. **Turn off flame** 
   1. record the acetylene psi in log. If the tank level has dropped to less than 50 psi, order a new tank for next time.
   2. Turn off oxidant knob
   3. Then, let the remaining fuel burn out. The flame should go out.
   4. Turn off acetylene at tank
   5. If the acetylene delivery gauge (on the left) and remaining pressure gauge (on the right) are not both at zero, turn the oxidant knob back to air to let the rest of the acetylene bleed out. Once this is done, turn the oxidant knob back to zero and release the regulator fully (turn the large black knob counter clockwise) and close the valve to the line (turn clockwise). It is important to remember to bleed out the acetylene because acetylene remaining inside the regulator will corrode the inner parts of the regulator.
   6. Turn off house air
6. **Turn off lamps & AA**
   1. click on the lamp icon, and change current to zero.
   2. Return the lamp to its case—it may not be used for a long time and we should avoid getting dust or scratches on the lamps.
   3. Turn off the AA power switch.
7. **Retrieve data** (if using sample file)
   1. click on File, Utilities, Reformat.
   2. The most recent run will be open (or you can navigate to an old run by clicking on the Browse button).
   3. Give the file a name, designate the path to save it to, and choose the extension .TXT.
   4. Click on the tabs Sample, Mean, and Repl. to click/unclick boxes of information to include in the file. Then click Save Results.
   5. open Excel, open the .TXT file (make sure you have selected “all file types” to show in the open window), and the file is comma delimited. Click OK.
8. Make notes of any errors, troubleshooting, or oddities in the log book
9. *Disposal: Samples acidified in LaCl3 can be diluted down the drain. LaCl3 dissociates to form Cl- and La2O3 which are not toxic.*

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Ca | K | Mg | Na | Fe | Sr | Li | Rb | Pb |
| **Mode** | abs | em | abs | em |  | abs | em | em |  |
| **Std 1 (ppm)** | 5 | 2 | 0.5 | 6 |  | 0.4 | 0.1 | 0.2 |  |
| **Std 2** | 15 | 6 | 1.5 | 3 |  | 1 | 0.3 | 0.6 |  |
| **Std 3** | 30 | 12 | 3 | 1 |  | 2 | 0.75 | 1.5 |  |
| **Wavelength (nm)** | 421.8 | 767 | 285.2 | 589.2 |  | 461 | 671 | 780.7 |  |
| **Slit (nm)** | 0.7 | 0.7 | 0.7 | 0.2 | 0.2 | 0.2 | 0.7 | 0.7 | 0.7 |
| **Filter** | none | red | none | none |  | none | red | red |  |
| **Energy** | 58 | 58 | 56 | 54 |  | 54 | 53 | 48 |  |
| **\*Sensitivity check** | 4 | 2 | 0.3 | 0.5 | 5 | 5 | 2 | 50 | 20 |
| **Flame gas** | air-ac | air-ac | air-ac | air-ac | N-ac | N-ac | air-ac | air-ac | air ac |
| **\*\*Impact bead** | no | yes | yes | yes | yes | no | yes | yes | yes |
| **\*\*\*LaCl** | no | yes | no | yes | no | yes | no | yes | no |
|  |  |  |  |  |  |  |  |  |  |
| \*ppm which will give a reading of approx. 0.2 absorbance units. | | | | | | |  |  |  |
| \*\*impact bead will improve sensitivity about 2x. | | | | |  |  |  |  |  |
| \*\*\*Addition of the alkali salt LaCl is recommended to control ionization. | | | | | | |  |  |  |