**Valett Lab**

**Standard Operating Procedure**

**AP2 Data Management**

(Last modified 4/10/19 by RE)

**Purpose:** This protocol serves to prepare, archive, and process data generated by the Astoria Pacific 2 Segmented Flow Analyzer (AP2). In the Valett lab, the AP2 is used to generate data for the nutrients: N-NO3, N-NH3, P-PO4. Each run of the instrument generates two files on the instrument- a “Run” file of the actual data accumulated by the AP2 and a “Results” file showing the raw peak heights and concentrations obtained via the calibration curve generated during the run. This protocol covers five distinct steps, from preparing the Sample Table to Archiving the final results. The procedure can be applied to all three analytes, but is illustrated here for N-NO3. For runs involving N-NH3 and PO4 simultaneously, the steps in Section 4, Process the Data, need to be done twice, once for each analyte.

Step 5: Store the final results

Step 4: Process the data

Step 3: Archive the data

Step 2: Conduct the analysis

Step 1: Preparing the Sample Table

**File Naming Convention:** For a given set of samples, a standard file naming convention will be used, in order to track the samples all the way through the process. The naming convention is based upon the date the samples are analyzed, not the date they were sampled. The general format for naming is:

Project code\_property code\_instrument code\_date stamp\_stage of processing.file extension.

Definitions:

Project code: Designator of the project under which this analysis falls, e.g. LETREB, REU, etc.

Property code: The analyte, e.g. NO3, PO4, NH4, etc.

Instrument code: AP2, etc.

Date stamp: The date the analytical run was made (not the sampling date), using the format year, month, day.

Stage of processing: There are 5 possibilities:

Sample- this is for the sample table set-up.

Exported Run- this is the file that comes from FASPac to the archive folder.

Exported Results- the results file that comes from FASPac to archives.

Process- the designation for a file that includes the curve-fitting routine

Final- final results for samples.

File Extension: Either xlsx or Fpx

Example names for the various stages of Data Management:

LTREB\_NO3\_AP2\_2018-09-28\_Sample.xlsx

LTREB\_NO3\_AP2\_2018-09-28.fpx

LTREB\_NO3\_AP2\_2018-09-28\_Export-results.xlsx

LTREB\_NO3\_AP2\_2018-09-28\_Process.xlsx

LTREB\_NO3\_AP2\_2018-09-28\_Final.xlsx

**Step 1: Prepare and archive the Sample Table**

1. In UM Box, open the Sample Table Template, located in Valett Lab\Analytical Laboratory Output\AP2\AP2 Data Processing. The template is an excel file.
2. Insert into the template the following information for the samples to be run:
   1. Sample date
   2. Sample location
   3. Replicate designation
3. Add calibrant data, Quality Contol checks: (CC1, etc) and cadmium column efficiency checks, (NO3/NOX%), and make sure the samples themselves begin at sample location 1:25.
4. Save the Sample Table in the AP2 Archive folder, using the “Save As” feature and the naming convention: Example name: LTREB\_NO3\_AP2\_2018-09-28\_Sample.xlsx

**Step 2: Conduct the analysis on the AP2 instrument**

1. Transfer the information from your Sample Table into FASPac to create the Run Table. Fill the racks with calibrants, wash, QC checks, Efficiency checks, and samples according to the Sample Table.
2. Conduct the run. Make whatever “in-run” adjustments to correct baseline, peak positions, etc.
3. After the run is finished, be sure to Save Run.

**Step 3: Archive the data**

Immediately upon completion of a run, the data should be archived to Box to ensure its protection. Alternatively, open the file you wish to archive if not executed immediately following completion of a run. The steps to archive are:

1. In FASPac, go to File- Export- Run File. Assign the location for the file to be C:\users\public\documents\FASPac2\export\ (There is a shortcut on the FASPac computer desktop called FASPac2 export.)

2. Assign the file name using this protocol:

Example name: LTREB\_NO3\_AP2\_2018-09-28\_exported-run.fpx

3. Execute export

4. Repeat for Results file. Go to File-Export- Results file. The file name should reflect exported-results with an xlsx file extension.

Example name: LTREB\_NO3\_AP2\_2018-09-28\_exported-results.xlsx

5. Open UM Box on the FASPac computer using the users account. Open:

Valett Lab\Analytical Laboratory Output\AP2\AP2 Archive

6. Upload the files from the desktop shortcut into the AP2 Archive Box folder by selecting both files from the FASPac2 folder on the machine and executing Upload.

7. Insert the Calibrant data into the archived Results Export file. Add Calibrant identifier ( e.g. C1, C2, etc.) along with concentration and units.

8. Verify that both files transferred into AP2 Archive folder in Box. The data is now in the archive folder. Verify that the Results file includes the values of the calibrants.

**Step 4: Process the data**

This is the process for processing nitrate data. The following section describes processing for ammonia and phosphate.

Part 1: Populating the processing template with relevant information

1. Open the AP2 NO3 Template 2019 Version, (or the NH3-PO4 Template) found in Valett Lab\Analytical Laboratory Output\AP2\AP2 Data Processing\template folder.
2. Open the desired archived Results file from AP2 Archive folder.
3. Open the corresponding Sample Table from AP2 Archive folder.
4. Choose and Move into the AP2 NO3 Processing Template (first Tab “Results and Sample ID”) each of the following using copy and paste:
   1. Results table into location E:7.
   2. Calibrant information into P:6 through P:13, as needed. Close out the archived Results file.
   3. Sample Table, Columns E, F, G, and H (Row, Sample Date, Sample Site, and Sample Replicate) into Data Processing Template, into location A:7.
   4. Edit out of the Processing template the data associated with nitrate column efficiency checks, quality control checks, and autowashes.
   5. IMPORTANT: Check to make sure that the Sample number and the Sample identifying information line up with Results!
   6. Save the Processing Template, using Save As and the naming convention, Example: Example name: LTREB\_NO3\_AP2\_2018-09-28\_Process.xlsx

Part 2: Calibration Curve construction and calculation of results.

1. Copy and move Calibrant information from the Results and Sample ID tab to Columns A, B, and C of the second tab, Standard Curve Construction.
2. Linear fit: Run a linear regression on all calibrants using the Data Analysis package in Excel (be sure to designate the output of the regression into cell M8. Using the intercept and slope values from the regression, apply them to calculating the calibrant concentrations in column E of the template. The F column should populate with the % error (“y-hat”) between the nominal calibrant concentration and the curve-fitted value. Assess error. If the % recovery on all calibrants is less than 10%, the curve can be applied to results (See Step 5). If % Recoveries are greater than 10%, then proceed to the next step.
3. Quadratic Fit- All Data: Using the graphing function built into the Template, construct a graph with the calibrant data (All Data Non-linear Fit). The graph will provide the best-fit quadratic equation of the results. Copy the equation parameters to the cells below the graph (P, Q, R50). The spreadsheet will again calculate curve-fitted results and percent error in Columns G and H. Again, assess the results to determine if %errors are less than 10%. If so, apply the curve-fit parameters to the results (See Step 5). If not, proceed to the next step.
4. Split Curve Fit: The split-curve approach makes use of the quadratic graphing function as in Step 3 above, except the standards are split into a low-range and a high-range. The template includes a graph for both, so for the low range, select the range of calibrans to be used as “low,” and construct the low-range graph. The quadratic equation appears on the graph, so copy the intercept, x coefficient, and x2 coefficient into the cell below the graph. Repeat the procedure with the remaining calibrants, overlapping by one calibrant with the low-range graph. Again, copy the quadratic parameters into the cells below the graph. Apply the equations to the calibrants in columns I-J (you will need to write these equations into the cells) and find the % difference. Apply the parameters to the sample results (See Step 5).
5. Apply the regression to results. Using either the linear fit, the all-data quadratic fit, or the split-curve quadratic fits, transfer the appropriate parameters to the next tab, Apply Std Curve. The parameters that you select to use should be copied into the appropriate cells in Columns O, P, or Q. You will need to make sure that the equation for calculating results uses the correct parameters. Use the Raw Heights from Column H and apply your equation to calculate the concentration of the samples in Column J.

In the case of a linear fit or an all-data quadratic fit, the curve-fit parameters can be applied directly to the sample raw heights and the concentrations calculated. However, if you use the split-curve approach, the data need to be reordered from low to high raw heights. Use the Data Sort function in Excel to sort all of the data based on the Raw Height (Column H) from low to high. Apply the low-range curve parameters to those samples with raw heights that fall in the range of your low-range calibrants. Write a separate equation to apply the high-range parameters to the appropriate samples. Once all of the calculations are made, again sort the data back to its original form by sorting on Peak Number (Column D).

1. After the results have been calculated, use the copy-paste function to add the final results to the last tab, Final. The data processing is finished at this point.

**Step 5: Archive Final Results**

Using Save As, store the final tab of the Process file into a new spreadsheet for final results. Use the naming convention shown here: Example name: LTREB\_NO3\_AP2\_2018-09-28\_Final.xlsx. Save the Final spreadsheet in the Box folder: Valett Lab\Analytical Laboratory Output\AP2\AP2 Finalized Data folder.