Standard Operating Procedure: Receiving NEON KCl Extracts

5/18/2017

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1. Take the cooler to the bench near the NEON freezer.
2. Open the cooler and step away for a minute to allow the carbon dioxide (CO2) to dissipate. CO2 is an asphyxiation hazard so move to fresh air and get help if you feel dizzy or light headed.
3. Remove the sample manifest.
4. Remove enough of the packaging material and dry ice to be able to access the samples. Wear gloves when handling dry ice!
5. Note the temperature state of the samples and write it on the manifest along with the date and your name. The temperature states are: Frozen, Thawed but cold, or Warm.
6. Check the sample labels on the vials against the manifest. Make a note on the manifest of any missing samples, illegible or missing labels, broken or damaged vials or caps, and any signs of leaks.
7. Put the samples in the freezer promptly after inspection.
8. Add the samples to the monthly task log including the temperature state and any problems with the sample condition.
9. Notify NEON if the samples were warm or there were any problems with the sample condition.
10. Put the manifest in the NEON file drawer in room A250.

Standard Operating Procedure: Preparing Reagents for NO_3, and NH_4 analysis

5/26/2017

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Calibration standards and check standards should both be prepared from primary standard grade salts or other certified or traceable materials, but the two should come from entirely different sources to be able to use them to verify each other.

Materials and Cleaning

All solid standard materials (salts) are oven dried at 105° C overnight, then placed in desiccators to cool to room temperature before weighing. All weighing is performed on a 4 place (0.0001 g) balance using a clean spatula and a disposable plastic weighing boat. Balances are calibrated by a certified balance technician annually and performance is verified using check weights periodically by the lab managers.

All solutions are made in Class A glass volumetric flasks and all dilutions are made using Class A glass volumetric pipettes and volumetric flasks. Standards are stored in Nalgene polyethylene or polypropylene bottles. All calibration and check standards for NEON kcl ammonium analyses are prepared using 2 molar KCl. The KCl solution is prepared using DI water with an electrical conductivity of less than 1 micro Siemen. All calibration and check standards for NEON kcl nitrate analyses are prepared using ultrapure DI water as above.

All storage bottles, volumetric flasks and pipettes, funnels and beakers used for standard preparation are acid washed prior to use. The acid washing procedure is: items are individually rinsed 3 times with DI water. Items are then fully immersed in a 1.2 Molar Hydrochloric Acid bath (1 part concentrated HCl to 9 parts DI water) and left overnight. Items are then individually rinsed inside and out 6 times with DI water. Volumetric flasks are stored full of DI water and emptied just before use. Volumetric pipettes are oven dried at 105° C. Beakers and funnels and storage bottles are air dried. All materials are stored in a designated space separate from other lab glassware.

Calibration and check standards are prepared using primary standard grade salts. Ammonium standards are prepare using Ammonium Sulfate, nitrate standards are prepared using Potassium Nitrate and the nitrite check standard is prepared using Potassium Nitrite.

Calibration Standards

A series of calibration standards are prepared and analyzed to create a standard curve which is used to calibrate the instrument. The concentrations of these standards are chosen to cover the expected range of the samples in milligrams nitrogen per liter. 5-9 standards are typically run in a calibration curve. A significant volume (typically 500 ml) of a stock solution with a high concentration (e.g. 1000 mg-N/l) is prepared. This allows for the amount of salt to be weighed to fall in the 2-5 gram range for both analytes. The salt is weighed to +/- 0.0001 grams of the nominal weight on a 4 place analytical balance. This allows for an extremely small weighing error. Stock solutions are prepared in DI water.

Working standards are prepared by diluting the stock solution with 2 M KCl (for ammonium) and DI water (for nitrate) using volumetric pipettes and flasks. Very low concentration standards (less than 0.5

ppm) are prepared by a two-step serial dilution. The stock solution is diluted to 10 or 1 ppm, then this solution is diluted to make the final working standards.

Check Standards

Check standards are prepared using the same techniques specified above for the working standards, but using a separate chemical source.

Storage

All calibration standards, check standards and stock solutions are stored in a refrigerator from day to day and frozen if storage is for more than a week. Stock solutions may be stored up to one year if frozen. Working standards may be stored up to 6 months refrigerated.

Ammonium Reagents

NH4 Buffer

Dissolve 24 grams sodium hydroxide, 58 grams sodium citrate, and 50 grams disodium EDTA in approximately 800 ml DI water in a 1 liter volumetric flask. Add DI water to the 1 liter volume. Filter through a 10 micron filter. May be stored at room temperature for up to 4 months. Add 6 drops Briij -36 solution to 500 ml buffer before use on the instrument. This working buffer is good for up to 2 weeks.

Salicylate- Nitroferricyanide

Dissolve 300 grams Sodium Salicylate and 0.6 grams sodium nitroferricyanide in approximately 400 ml DI water in a 1 liter volumetric flask. Add DI water to the 1 liter volume. Filter through a 10 micron filter. Store refrigerated for up to 2 months.

Sodium Hypochlorite

Add 12 ml household bleach (5.25% sodium Hypochlorite) to 188 ml. DI water. Make fresh daily.

Nitrate Reagents

Ammonium Chloride Buffer

Dissolve 638 grams of ammonium chloride and 0.75 grams of disodium EDTA in approximately 6 liters of DI water. Add DI water to 7.5 liters total volume. Adjust to pH 8.5 +/- 0.1 using ammonium hydroxide. Store at room temperature for up to 3 months.

NO3 Color Reagent

Slowly add 100 ml concentrated phosphoric acid (Caution: severe contact hazard) to approximately 800 ml DI water in a 1 liter volumetric flask. Dissolve 40 grams of sulfanilamide and 2 grams N-1-naphthlyethylenediamine in the diluted phosphoric acid and add DI water to the 1 liter volume. Filter through a 10 micron filter. Store refrigerated for up to 2 months.

2% Cupric Sulfate

Dissolve 2 grams cupric sulfate in 100 ml DI water. Store at room temperature for up to 5 years.

0.2% Cupric Sulfate

Add 10 ml of 2% Cupric Sulfate to 90mls. DI water. Store at room temp

Standard Operating Procedure: Setting up and running NH_4/NO_3 analyses on the Alpkem

2/21/15
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Initial Set-up

1. Check that the instrument is configured for NH_4/NO_3: The line from the sampler should be connected to the "T" where the NH_4 sample line and the NO_3 pull through line meet. The debubbler on the channel 3 detector (NH_4/ PO_4) should be connected to one line labeled "NH_4 from debubbler" and another line labeled "NH_4 to spec". If not, see the section: Switching the Alpkem from PO_4 to NH_4/NO_3.

2. Remove NO_3 color reagent and NH_4 salicylate-nitroferricyanide bottles from the fridge. Place the NO_3 color reagent in a warm water bath to warm up to room temperature.

3. Check sampler wash waste reservoir under bench to right of instrument. Empty into sink if more than half full.

4. Turn on compressed air at the red handled ball on the bottom of the tank regulator.

5. Turn on main power switch and make sure NH_4 heater is on.

6. Open Winflow 4.0 software. If software is already open, exit and reopen. This causes the sample probe to go into the wash reservoir.

7. Lock down the pump platens and pull the engaging levers straight up for all the pump tubes labeled NH_4 and NO_3 plus "to sampler wash" and "from sampler wash".

8. Press the "run stop" button on the pump, the display should read –50.0.

9. Empty, rinse and refill the large DI water bottle for the sampler wash and NO3 carrier.

10. Connect the sampler wash and NO_3 carrier lines to the large DI wash bottle (be sure to connect to the ports as labeled: the sampler wash will not flow properly if connected to the port labeled NO_3 carrier).

11. Connect the NH_4 lines: buffer, hypochlorite, and salicylate, to the NH_4 manifold startup solution.

12. Connect the NO_3 lines: color reagent and buffer, to the NO_3 rinse bottle (DI water).

13. Let pump for 10 minutes and check for leaks.

14. Discard old hypochlorite solution and make fresh. 12 mls bleach and 178 mls DI water.

15. Move the NO_3 buffer line to the buffer bottle.

Condition the cadmium column

1. Place the column, syringe with tube to fit column, open specimen cup of NO_3 buffer, and open cup of 0.2% $CuSO_4$ on the bench within easy reach.

2. Draw about 1 ml of buffer into the syringe and remove all air bubbles from the tube.
3. Break the tubing connection on the column and elevate the connector end slightly until a drop of liquid appears at the tube end.

4. Hold the syringe plunger end up and depress the plunger until a drop of buffer appears at the connector end.

5. Connect the syringe to the column without introducing any air.

6. Place the free end of the column into the buffer cup and slowly depress the plunger to expel the air from the free end of the column.

7. Transfer the free end to the cup of $CuSO_4$ and draw up about 0.5 ml.

8. Depress the plunger slightly to release any tension on the liquid in the column and transfer the end to the cup of buffer.

9. Draw up 3-5 mls of buffer, and then depress the plunger slightly to release any tension on the liquid in the column.

10. Remove the end from the buffer, place the column, tubing and syringe flat on the bench, then disconnect the syringe and connect the two ends of the column together.

11. Take the column and syringe to the sink and rinse the outside with DI water. Put the buffer and $CuSO_4$ cup away and wipe the bench with a wet sponge. The NO_3 buffer contains NH_4, which can contaminate your samples!

12. Connect the cadmium column to the instrument

13. Make sure there are no bubbles in the nitrate cartridge (about 10 minutes after moving the NO3 buffer line to the buffer bottle).

14. Press the “run stop” button on the pump, press the “mode” button 4 times, the display should read “2.0”, press the “run stop” button again, this puts the pump speed to minimum.

15. Break the connection in the line between the “to column” and “from column” ports on the NO3 cartridge.

16. Break the tubing connection on the column and note which end will connect to the line from the “to column” port on the cartridge. Elevate the other end of the tubing until a drop of liquid forms at the end. Wait until a drop forms on the end of the “to column” line and connect it to the column without introducing any air.

17. Connect the other end of the column to the “from column” line on the cartridge.

18. Press the “run stop” button on the pump, press the “mode” button 3 times, the display should read “-50.0”, press the “run stop” button again, this puts the pump speed back to normal.

19. Move the NO3 color reagent line to the color reagent bottle.

20. Move the NH4 reagent lines to their respective reagent bottles.

21. Make sure the line from the sampler is completely clear of all other lines and the reagent bottles so the sample probe can move freely.

22. Let the system pump at least 15 minutes before beginning a sample run.

Set up the sample table

1. Press the sample table button on the main tool bar in Winflow4.

2. Type in “Cup #”, “Name” and “Type” for each sample and standard to be analyzed. Refer to *SOP: Setting up the sample table for NEON KCl.*

Starting a sample run

1. Click the collect data button on the main toolbar. The software will ask for an operator name and ID, type in your initials for both. The software will then ask for a sample table name and a method name. The method name is NH4NO3.mth. Select the correct file names and press OK. The software will ask for a filename for the results with the default being the same as the sample table with an .rst extension. Select or type in a file name and press OK. The software will not allow you to overwrite or append and existing file.

2. The data collection window will appear on the screen. Press the “play” button to start the data collection. At this point, the software will monitor the baselines for 60 minutes and then start sampling.

3. Debubble both flow cells by pinching and releasing the outflow tubing. Repeat until no bubbles appear.

4. Pour standards and samples into the cups and place in the sampler. Get at least 20 cups poured before starting the sampler.

5. Check baselines for stability and drift, should be less than 500 micro-absorbance units.

6. If there are any peaks or jumps in the baseline traces, Press the “stop” button. The software will ask if you want to stop, press yes. Press the “rewind” button and then the “play” button.

7. Press the “fast forward” button to begin sampling.

8. The first NO_3 peak will appear about 1 minute after the first sample is drawn. The first NH_4 peak will appear in about 4 minutes.

Instrument shutdown

1. Press the "run stop" button on the pump to stop the reagent flow.

2. Disconnect the cadmium column from the NO_3 cartridge and connect the two ends together. Be careful to minimize the amount of air let into the column.

3. Connect the "to column" and "from column" lines together.

4. Press the "run stop" button on the pump to restart the flow.

5. Move the NO_3 lines to the NO_3 rinse bottle and the NH_4 lines to the NH_4 manifold startup solution bottle. Let pump about 10 minutes.

6. Disconnect all the lines and let the system pump air for about 15 minutes or until all the liquid is out of the instrument.

7. Press the "run stop" button to stop the pump and turn off the main power switch to the instrument.

8. Push the engaging levers on all the pump platens to horizontal and disconnect one end of each platen from the pump.

9. Empty the NH_4 waste into the container on the floor next to the instrument.

10. Pour the NO_3 waste into the container on the floor next to the instrument.

11. Empty and discard the sample cups and tubes. Rinse the sampler racks with DI water and set on the bench to dry.

12. Return the NO_3 color reagent and NH_4 salycilate-nitroferricyanide bottles to the fridge.

13. Refrigerate the standards if the instrument will be used the next day or freeze them if it will be longer.

14. Wipe down the instrument and benches with a moist sponge.

15. Turn off the compressed air.

Switching the Alpkem from PO_4 to NH_4/NO_3

1. Make sure the PO_4 waste container is empty. The waste goes in the bottle on the bottom shelf of the acid cabinet in room A258.

2. Disconnect the "PO_4 waste from spec" line from the outflow tube of the channel 3 (PO_4/NH_4) detector and connect the "NH_4 waste from spec" line in its place.

3. Disconnect the "PO_4 to spec" line from the bottom connection on the debubbler on the channel 3 detector and replace it with the "NH_4 to spec" line.

4. Disconnect the "PO_4 from debubbler" line from the top connection on the debubbler and replace it with the "NH_4 from debubbler" line.

5. Turn the PO_4 heater off and the NH_4 heater on.

6. Disconnect the sampler line from pump tube labeled "PO_4 sample" and connect it to the "T" where the "NH_4 sample" line and the "NO3 sample pull thru" line meet.

Standard Operating Procedure: Setting up the sample table for NEON KCl

5/12/2017
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This protocol covers Sample Table setup using the Alpkem autoanalyzer and Winflow4 software in order to ensure proper QA/QC for NEON KCl samples. Instrument set-up directions and data processing are covered in separate standard operating procedure (SOP) documents.

Prepare the Alpkem for NH_4/NO_3 analysis according to the instrument set-up SOP.

Creating the Sample Table

To set up the sample table, press the sample table button on the main tool bar in Winflow4. Type in "Cup #", "Name" and "Type" for each sample and standard to be analyzed following the template below.

Definitions - Types

S (SYNC)-This is always the first cup in a run, it is a high standard (usually top standard) which will yield a large peak to let the software know the sample peaks have started.

C- indicates a calibration standard to be included in the calibration curve. Do not use this for internal standards or checks unless you want them included in the calibration curve.

RB- indicates a baseline (wash). The instrument will use these peaks to correct for baseline drift so they should always be a 0 ppm standard.

U- unknown. Should be used for all samples and check standards.

Definitions - Names

The following 7 names are for the example sample table below only. Actual standard names used will be different. See the explanation of standard names below the example table.

Wd- Wash DI water: used as a baseline for the nitrate analysis.

Wk- Wash KCl: used as a baseline for the ammonium analysis.

NHCalX-Calibration standards for ammonium.

NOCalX- Calibration standards for nitrate.

NHCheck- Check standard for ammonium.

NOCheck – Check standard for nitrate.

NO2Check- Nitrite check standard: used to verify the cadmium reduction column efficiency.

Example Sample Table

Row	Cup #	Name	Type
1	1	SYNC	S
2	2	Wk	U
3	2	Wk	RB
4	3	“NHCAL1”	C
5	4	“NHCAL2”	C
6	5	“NHCAL3”	C
7	6	“NHCAL4”	C
8	7	“NHCAL5”	C
9	8	“NHCAL6”	C
10	9	“NHCAL7”	C
11	2	Wk	U
12	2	Wk	RB
13	10	“NHCHECK”	U
14	11	Wd	U
15	11	Wd	U
16	12	“NOCAL1”	U
17	13	“NOCAL2”	U
18	14	“NOCAL3”	U
19	15	“NOCAL4”	U
20	16	“NOCAL5”	U
21	17	“NOCAL6”	U
22	18	“NOCAL7”	U
23	11	Wd	U
24	11	Wd	U
25	19	NOCHECK	U
26	20	NO2CHECK	U
27	101	“SAM 1”	U
28	102	“SAM 2”	U

Samples are loaded into the instrument in the pattern of 10 samples, then an NHCHECK, an NOCHECK, a Wd, and a Wk. Repeat this pattern until the setup is complete.

Cup Numbers

Positions 1-20 are the large tubes at the back of the sampler and are used for standards, checks and baselines (washes). The instrument can sample from these cups up to 6 times each.

There are 3 racks for the 2 ml sample cups. The one on the left (closest to the sampler wash cup) holds cup numbers 101-190. The middle rack holds cup numbers 201-290 and the rack on the right holds number 301-390. These cups should only be sampled one time each.

Because the instrument has some sensitivity drift, runs should not be longer than 90 samples. Larger sets of samples should be split into multiple runs.

Sample Names

Ideally, sample names should match the labels on the bottles to allow easier tracking of the samples from receipt to final data delivery. If the client sample names are too long or cumbersome, Lab assigned ID's may be used. Make sure there is a clear record in the network directory relating the lab ID's to the client ID's.

The standards used in the calibration curve (anything labeled "C" in the "type" column) must be named exactly the same as they are named in the calibrant table in the instrument method file.

To view the calibrant table, click the "edit method" button on the main toolbar, click file, open, and select nh4no3.mth. The calibrant table is the last page of the method file.

The format for naming calibration standards is: X / Y, where X is the concentration of NH_4 and Y is the concentration of NO_3. No leading zeros on values less than 1 and no decimal points except for fractional concentrations.

Quality Assurance

The range of the calibrant concentrations is chosen to cover the range of the samples. At least 5 calibrants are used with 7 or more being preferred.

Washes are DI water or KCL. Those with an "RB" in the type column are used by the instrument software to account for baseline drift of the instrument. These should be run immediately before and after the calibration curve, at least every 10 samples during the run, and as the last sample of the run. When the run is set up, the "Wk"'s should be labeled as RB and the "Wd"'s as U.

Other washes are frequently run to clear any potential carryover after standards or samples with high concentrations. These will have a "U" in the type column so the software will not use them for baseline corrections.

Checks are standards prepared from a different source than the calibration standards. Checks for both ammonium and nitrate are run after the calibration curve and about every 10 samples throughout the run. They are used to verify the calibration and to check for sensitivity drift of the instrument. Their concentrations should be between 20% and 100% of the highest concentration standard used for the run. Their sample name should include the nominal concentration. Calculated values will be used to assess whether the run meets agreed-upon QA thresholds for NEON KCl analyses.

NO2 checks are standards prepared with sodium or potassium nitrite. They are run after the calibration to determine the efficiency of the cadmium reduction column. They should have nitrogen concentrations at least 50% of the highest calibration standard with 100% being the preferred value. Their label in the sample name column should include the nominal concentration.

Other columns in the sample table

R- Number of replicate samples to be taken from the cup. Should be set to 1.

Dil- dilution factor of the sample, usually 1 unless the sample has been diluted so that it will fall within the range of the standard curve. If the sample has been diluted, the dilution factor should be entered in this column (e.g. 1 part sample to 9 parts water would be a dilution factor of 10).

Wt- weight of the sample, should be set to one.

Standard Operating Procedure: Processing NEON KCl Data

5/26/2017

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Exporting results from the Alpkem software

1. In the Alpkem Software, load the result file for the sample run and select the NH4 results.
2. Check that all of NH4 Calibrants have a sample type "C". Check that all the Wk cups have a sample type of "RB". The exception is immediately after the calibration curve, there are 2 Wk cups, the first should have a type "U" and the second should be "RB".
3. Check that all of the NO3 calibrants have a sample type "U". Check that all the Wd cups have a sample type "U".
4. If any of the cups have different samples types, change them to the correct type. The software will automatically update the calculations.
5. Export a detailed report of the NH_4 results. This file will have the correct NH_4 results. The NO_3 results are not valid.
6. Select the NO3 results.
7. Change the sample type for all the NO3 calibrants to "C" and all the NH4 calibrants to "U".
8. Change the sample type for all of the Wd cups to "RB". The exception is immediately after the calibration curve, there are 2 Wd cups, the first should have a type "U" and the second should be "RB".
9. Change the sample type for all of the Wk cups to "U".
10. The calculated results will automatically update after each change.
11. Export a detailed report of the NO3 results. This file will have the correct NO3 results. The NH4 results are not valid.
12. Close the results file and use a USB drive to copy the exported results to a networked computer.
13. Import the results into Excel. Use separate pages for the NH4 and NO3 results and label the pages "NH4 raw" and "NO3 raw". Leave these pages unaltered to serve as record of the results as they came from the instrument.

Processing the ammonium results

1. Copy the "NH4 raw" page onto a new page labeled "NH4 calc".
2. Move the lines containing the NO3 calibrants, NO3 checks, the NO2 check, and the NO3 baseline (Wd) samples down to the bottom of the file.

3. Move the lines containing the NH4 calibrants and the regression information down to about 30 lines below the last sample entry. Verify that the regression correlation is at least 0.995. *If it is not, the results are not valid and the samples will have to be rerun.*
4. Collect all of the lines containing the NH4 check standards into a group below the sample results. Calculate a recovery for each check standard by dividing the measured value by the nominal value. The mean recovery should be between .95 and 1.05. If not, *the results are not valid and the samples will have to be rerun*.
5. Collect all of the baseline samples (type=RB) into a group below the check standards.
6. Check that all sample values are less than the highest standard. Flag any samples that are higher to be diluted and rerun.

Processing the nitrate results.

1. Copy the "NO3 raw" page onto a new page labeled "NO3 calc".
2. Move the lines containing the NH4calibrants, NH4 checks, and the NH4 baseline (Wk) samples down to the bottom of the file.
3. Move the lines containing the NO3 calibrants and the regression information down to about 30 lines below the last sample entry. Verify that the regression correlation is at least 0.995. *If it is not, the results are not valid and the samples will have to be rerun.*
4. Move the NO2 check result line below the sample results. Copy the line with the corresponding NO3 calibrant result onto the line below the NO2 check. Calculate the recovery by dividing the NO3 calibrant result by the NO2 check result. The recovery must be 0.9 or greater; *if not, the results are not valid and the samples will have to be rerun*.
5. Collect all of the lines containing the NO3 check standards into a group below the sample results. Calculate a recovery for each check standard by dividing the measured value by the nominal value. The mean recovery should be between .95 and 1.05. *If not, the results are not valid and the samples will have to be rerun*.
6. Collect all of the baseline samples (type=RB) into a group below the check standards. Average the results. The average must be less than 0.02 or the MDL (whichever is greater). *If not, the results are not valid and the samples will have to be rerun*.
7. Check that all sample values are less than the highest standard. Flag any samples that are higher to be diluted and rerun.

Preparing data for submission to NEON

1. Copy sample names, measured results, and relevant run metadata for both ammonium and nitrate onto a summary page that matches exactly the NEON ingest datasheet template. *Do not include samples that have been flagged for exceeding the top standard as those must be re-run.*
2. Ensure all list-of-value fields are filled in using appropriate choices and any necessary quality flags are indicated using the QF fields.

3. Prepare an additional sheet with the batch QA (check standard) results for each run using the NEON batch QA ingest datasheet template. Ensure that runIDs match those in the sample data.
4. Save both the summary page and the batch QA page as separate .csv files and submit to NEON.