**Lachat QuickChem 8500 Operating Instructions**

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**Methods**

* Ammonia (method 10-107-06-1-J) 0.01 to 2.0 mg N L-1 as NH3
* Nitrate (method 10-107-04-2-D) 0.05 to 7.0 mg N L-1
* Orthophosphate (method 10-115-01-1-A) 0.01 to 2.0 mg P L-1
* Total P (method 10-115-01-1-F) 3 to 200 ug P L-1 (pre-digested using persulfate digestion method)

**Quick Start-Up Check List**

1. Get correct manifolds in place and waste lines going to the appropriate bottles.
2. Check to see that reagents are properly mixed and are fresh (not past proper holding time).
3. Turn on the autodilutor, the autosampler, and the Lachat module.
4. Open Omnion and set up your run in the software. Be sure to get the heating block to the correct temperature.
5. Put reagent lines in reagent bottles and secure the tubing into the cassettes on the pump.
6. Turn on & start the pump.
7. Begin to acquire a baseline in Omnion.
8. Be careful to make sure the sample tube racks and rack 4 (empty tubes for dilutions) are set in the right place. Jiggle them to make sure they are locked into place.
9. When the baseline looks good, stop acquiring a baseline and start your run.
10. Watch Standards and QCs to make sure things are going smoothly.
11. Keep track of reagent levels, waste water levels, and waste accumulation in the bottles.
12. When the run is done, clean the lines appropriately, then rinse with water, then dry the tubes by running air through the lines.
13. Dispose of your samples, Standard Stock, and QCs.

**Record-keeping**

* Notebook: Lachat log and schedule. Use the google calendar to sign up for the Lachat and record your run in the blue Lachat log binder.
* Identify the fund code you will charge. Also be sure to indicate the manifolds used and whether they were on the front or the back. Include the baseline values in the notes section.

**Setting up**

*Manifolds*

1. Check which manifolds are currently installed on instrument
2. If you need to change out, locate empty box of manifold you need to remove
   1. Carefully remove tubing connections to the instrument and leave fittings in place
      1. At heater coil(s)
      2. At flow cell
      3. At valve (port # 3)
   2. Put dangling pump tubing in bag (in manifold box)
   3. Remove sample loop
   4. Remove interference filter and put in small bag to keep clean (in manifold box)
3. Install new manifold
   1. Locate manifold box and read flow diagram located in Lachat Methods Manual (last page of the method)
   2. Follow diagram carefully! Any error in the manifold set up will result in incorrect flow. Regent lines stay with each manifold to prevent contamination.
   3. Connections on the manifold have 2 small O-rings inside of them and should be screwed on until they are finger tight. Over or under tightening the connections will result in flow problems. Connections at the 6-port sampler don’t have O-rings, but the tubing should extend slightly beyond the end of the brown screw and the screws should also be finger tight.
   4. When connecting the heating coils observe that the top heating coil is 175 cm long and the bottom heating coil is 650 cm long. It doesn’t matter which way the sample flows through the coil.
   5. Once manifold is in place, install
      1. Sample loop
      2. Interference filter
      3. Backpressure loop if needed (nitrate and ammonia methods) NOTE: orthophosphate and TP methods indicate backpressure loop, but this actually creates too much backpressure
   6. If you need to check manifold setup for leaks, run DI through each of the lines to make sure each line is pulling sample.
      1. Common to see leaks around 6-port valve- just tighten fitting a bit
      2. To test if line is pulling reagent, lift line out of DI for 5 seconds, and then re-submerge. Follow the bubble to check flow along path.

*Reagents*

1. Best to prepare reagents the day before sample analysis
2. Locate reagent bottles in refrigerator (sodium hydroxide and the stocks for the copper sulfate/hydrazine solution are under Lachat computer)
   1. Check if any are usable; hold times are in reagent directions in methods manual
   2. If expired, empty reagent into appropriate waste bottle
3. Prepare enough reagent to run samples; halve or quarter recipe as appropriate.

*Guidelines*:

NH4 manifold

Hypochlorite: 1L ~ 360 samples \*\*must be made day of run

Sodium nitroprusside: 1L ~ 400 samples

Buffer: 1L ~ 550 samples

Sodium phenolate: 1L ~ 300 samples

**NOTE: Phenol causes burns and is a known carcinogen**. Wear goggles, face shield, lab coat, neoprene gloves over nitrile gloves. Measure on dedicated balance in fume hood & keep covered when mixing in hood.

NO3 manifold

Sodium hydroxide: 1L ~ 600 samples

Sulfanilamide: 1L ~ 300 samples

Copper sulfate/ hydrazine: 1L ~ 600 samples

PO4& TP manifolds

Molybdate:

Ascorbic acid:

DI + H2SO4 carrier (TP):

1. When filling reagent containers, put a small amount of fresh reagent into the bottle, re-lid, shake gently, and empty into appropriate waste container. Then fill bottle with fresh reagent.
2. Before run, allow reagents to warm up at least an hour prior to starting run.
3. Degas reagents with helium for at least 10 minutes prior to run;
   1. Note: there are several reagents that the method manual specifically says to not degas: ascorbic acid, sodium phenolate, sodium hypochlorite
   2. Attach appropriate glass pipet to tubing; can also set up fitting array to degas several reagents at the same time (be careful not to tip over bottles!)
   3. Turn on and adjust regulator to gently bubble gas through reagent
   4. When finished, turn off regulator at tank, remove pipets and rinse well with DI.
   5. Return pipets to correct container.
4. Reagents, recipes, and holding times are listed in the Lachat methods manual. For NO3‑, use the hydrazine method (marked in red). Also, note there are separate (but similar) methods and manifolds for orthophosphate and for total P.

*Standards, QCs, Carrier*

1. Prepare 5ppm mixed standards for the manifolds you are running
   1. NO3- and NH4+ using 1000 ppm NO3-N and NH4-N stock solutions
      1. 0.5 ml of each stock in a 100 ml flask
      2. Fill to line with DDI, invert to mix
   2. NH4+ and PO4- using 1000 ppm NH4-N and PO4-P stock solutions
      1. 0.5 ml of each stock in a 100 ml flask
      2. Fill to line with DDI, invert to mix
   3. Note: While some people make their standards and QCs in the same matrix as their samples (i.e. 0.4M KCl), most people have begun to run the standards in nanopure and running a few KCl blanks. Error caused by difference in optical densities between water and KCL can be corrected by subtracting the values of the KCl blanks from the sample values after the run.
      1. If you do prepare standards in KCl, must make each point in standard curve in KCl (don’t use autodilutor- it will be damaged!)
2. Prepare QC check using certified standard
   1. ERA Simple Nutrients- in ERA box in door of refrigerator
   2. Locate corresponding ERA datasheet in QC binder and determine concentration you want to analyze
   3. Prepare appropriate dilution using instructions on datasheet
3. Fill 50 ml centrifuge tubes and put on S rack behind the sample racks
   1. Standard
   2. QC
   3. Blank DDI

*Samples*

1. Pour samples into disposable borosilicate tubes (needs about 5 ml in each tube) and put in sample rack
   1. If running replicates, need to fill tube ¾ full
   2. If running 2N KCl samples, dilute 5x with DDI (1 ml sample + 4 ml DDI) and vortex before putting in sample rack
   3. Keep track of sample IDs in lab notebook or datasheet
2. Place sample racks on autosampler with cup # 1 in front left corner.
3. Make sure racks are seated securely! If not properly aligned, sampling needle will miss tube and may be bent
4. Fill cups #1-20 of rack 4 with empty tubes. This is where the standard curve will be diluted, as well as any samples that exceed the standard curve by 10%

*Machinery & Physical Set-up*

1. Turn on instrument components
   1. Autoanalyzer
   2. Autosampler
   3. Pump
   4. Autodilutor
2. Turn on computer and open Omnion software
3. Open a file from an existing run that uses the same manifolds
   1. Choose ‘accept’ when asked if it should set the temperature
   2. Go to ‘File’- ‘save as’ to save as new file name.
4. Install pump tubing on pump cassettes
   1. Cassettes are installed with levers on top facing towards the reagents
   2. Make sure pump tubing isn’t twisted as it is stretched across cassette
   3. Snap cassettes into place on pump and flip up lever on top of cassette 1 or 2 notches/clicks to put tension on line. All tubing must have same amount of tension
5. Check that waste lines are going to appropriate waste bottles.
   1. Note waste level in bottle and change bottle if necessary
6. Check for leaks
   1. Put all reagent lines from each manifold in the DI rinse vessel labeled for each compound.
   2. Press ‘start’ on pump and watch lines for leaks
   3. To check that line is pulling reagent, briefly remove and then return reagent line to DI vessel. This will create an air bubble, which can be followed along the line.
      1. If air bubble moves back and forth, line is not pulling correctly; check pump tubing
   4. Stop pumping once checks are complete
7. Put each reagent line in appropriate bottle and cover with parafilm

*Omnion Software*

1. Set up data file to match your samples
   1. Check standard dilution series at beginning
      1. Ensure sample table is referencing the correct cups for Standard, blank and QC
   2. Add a blank and at least 2 QC samples following standard curve
   3. Add rows for each sample and autonumber the cup and sample name
      1. Must use ‘enter’ instead of ‘tab’ or changes will revert back
      2. Can copy & paste sample list into Omnion if data table has correct number of sample lines
      3. Possible to add & remove samples while run is in progress, but can’t paste from Excel or autonumber at that point
   4. Add blanks, QC and replicates as desired throughout run
2. To set software to automatically dilute samples over highest standard, make sure that autodilution trigger box is checked in sample table
   1. DO NOT autodilute KCl samples; must do manual dilution

* When you set up the data file for your analysis: Check the dilution series. Add rows for each sample; auto-number the cup and sample name. Add QCs and blanks approx. every 30 samples, and put a 2nd standard curve on the end of your run. Make sure that under the “racks” tab, all three racks are active. (Otherwise the Lachat may not recognize the second or third tray.)
* It is possible to type up your sample list in Excel and copy and paste it to Omnion, but only before the run has started. The auto-number function also becomes inaccessible one the run has begun.
* It is possible to add or subtract samples to the run while it is in progress, but it is best to get the majority of the run set up before hand.
* Be sure to start each run with a standard curve and a couple of QCs. Give yourself a little space after the QCs before starting to run samples in order to check if the values are correct. I suggest adding 2 blanks after the QCs. This will give you time to stop the run if something doesn’t look right before you waste any sample.
* Some users run duplicate samples occasionally to ensure that the values are consistent. To do this you can tell the autosampler to sample twice from the same cup. Just make sure you have enough sample to do this.
* You can set the software to automatically dilute any sample that is over the highest standard. To do this, click the analytes tab while you have highlighted the analyte (as opposed to the channel). In the analytes tab you will see a box to check for the autodilution trigger. If you click this and input a percentage in the box below, the software will automatically append diluted samples to the end of your run. Usually people ask that samples higher than 110% of the highest standard be diluted by a DF of 10. Be sure to put extra culture tubes in rack 4 for the dilutions. If you are running KCl extracts, it is still possible to use the automatic dilution function because the sample never enters the autodilutor. Be wary of this however, because your samples will be diluted in water, not KCl. (As mentioned before, it is NEVER ok to run KCl as the diluent through the autodilutor.)
* After you have everything ready to go, press the button in the software to preview the baseline. It usually takes 10+ minutes after making sure the heating columns are up to temperature and reagents are running through the lines for the baseline to level off. Go get a cup of coffee and come back. When it has a good baseline, stop the preview and start your run.

**During the run**

* Each sample takes 67 seconds to run. Because of autodilution, standards take about 1.75 minutes.
* Monitor carrier, autodilutor, reagent, and waste levels.
* If waste jugs are full:
  + Water lines: Prop the waste lines over a temporary container and quickly empty the jug into the sink.
  + NH4+, NO3-, and PO4-3 waste: Get a new waste jug from beneath the sink in the analytical lab. Complete and apply waste accumulation label. Switch waste lines and tightly seal with Parafilm to keep down fumes. Leave cap in the bin. Make sure that waste lines are positioned properly. Cap old waste bottle, fill in end date, and place in cabinet under hood labeled “Organics: Waste accumulation area”. The Orthophosphate and TP waste can go in the same bottle.
* As carrier draws down: Keep a jug of carrier (nanopure or other) on the bench behind you; refill as needed.
* As reagents draw down and it looks like you may run out of one before the completion of your run, it is ok to make more reagent. To do this, try to make the reagent in enough time that there is enough of the original to mix the two batches together. Don’t stop your run, but do insert a bunch (at least 7) blank samples to the list while you are adding the freshly mixed reagent. Watch the baseline. Does it shift? If the answer is no, it is ok to continue on with your run. I would suggest adding a couple of QCs after the blanks in order to verify that things truly ok. If the baseline does shift, don’t stop your run, but do run another standard curve before you proceed with your sample run. You can correct for this change post run. Running out of sodium phenolate, sulfanilamide, or molybdate blue reagent should be avoided. Having to add a new batch of any of these will probably result in a shifted baseline.

**After a run**

*Mechanics and Clean-Up*

* Cleaning solutions are located on the shelf in the cabinet beneath the lachat. Run 10% HCl solution through the NH4+ lines for 30 min to reduce colorful solid build-up in the tubing. If you don’t clean the tubing well after running the phenolate method, the tubing around the heating coil will need to be rewrapped, so don’t try to cut corners by skipping the clean-up step. Make sure that the temperature setpoints are those for the run. (The Omnion software will likely lose the setpoints once it has completed the run, so you will need to switch to a different run or open the file again.) For the phosphate method, run the NaOH/EDTA cleaning solution through the manifold for 5-10 minutes then rinse with nanopure. There is no cleaning solution for the NO3- , just rinse for 15 minutes with nanopure.
* Rinse all lines with DI (about 5 minutes); put all lines in open beakers (keep lines from each compound separate); run air through the manifold until you don’t see any more moisture in the lines.
* Empty the culture tubes and dispose of them in the waste glass container. Most of the samples we run can be washed down the sink, but be sure before you dump.
* Refrigerate remaining reagents.
* Pop all cassettes on the reagent pump and release one end of the pump tubing to loosen each line. This prevents permanent deformation in the tubing on the reagent pump.
* Turn off all 4 pieces of equipment: autosampler, reagent pump, manifold, autodilutor.

*Software*

* If you zoom in on your sample curves and want to change the way curves are integrated you can do this any time after the run.
* If you right click and choose “Adjust Expectation Windows,” this will allow you to reintegrate all of the sample windows at the same time. After you change the windows, right click again and click “Rerun Peak Detection.”
* Often you only need to change the way individual peaks are integrated. To do this you can right click, choose “Adjust Baseline Window for Manual Detection” or “Adjust Integration Window for Manual Detection” depending on the change you want to make. Use the four-way arrow to move the whole window or the ↔ to extend/shrink the window. After you are satisfied, right click again and choose “Run Manual Interation.”
* If you don’t like the way the integration has changed, under the right click menu there is an option for clearing either all reintegrations or individual peak reintigrations.
* When you are done making changes, save the file. To get your new data, go to the Run tab and click the “Export Data to File” button.
* Your data will show up as a CSV Excel file in the “Shortcut to Data Output Files” folder on the desktop. If there is no folder on the desktop, your data can be found at C:\ProgramFiles\Lachat\Omnion\Data output files.
* Lachat computer is Win XP and no longer on network (backed up on external hard drive), so use USB drive to save data files.

**Troubleshooting:**

**Leaks** – Turn off the pump and disconnect and reconnect leaking junctions. Try making things a bit tighter. Perhaps the O-rings are old and cracked and need to be replaced?

**Spikey Baseline and Peaks –** You’re looking at an air bubble issue. Try degassing your reagents and start again.

**A Reagent Line that Isn’t Sucking –** The first thing to check is the pump tubing configuration on the pump. Sometimes the tubes get crossed under the cassettes. If you checked this, and the lines are straight across the pump through each cassette, perhaps the pump tubing is worn out. If you have looked through the whole manifold and there are no leaks, it is possible that you need to replace the pump tubing line for that reagent. Check the log book. If the tubing for that reagent has been replaced recently, then that is probably not the issue. See below.

**Backpressure** – This can manifest itself in a couple of ways. If you have a reagent line that isn’t sucking, or is sucking poorly (bubbles that go back and forth jerkily as they move down the tube), you probably have a clog somewhere creating excess backpressure. Try using the diagnostic dye. Run dye through the tubes and see if the dye gets hung up anywhere. On occasion dye has been sucked up tubes that are functional into the manifold, then pushed back out another reagent tube. If you have a clog, you’re going to have to take each junction apart. Look at the tubing closely and look at the junction boxes (take them off an hold them up to the light to see if you can see through them). Sometimes the ends of the tubing get crimped causing excess backpressure. If the end looks squashed, use a razor blade to cut that end off.

The other way that backpressure be diagnosed is if you are not getting any outflow of waste. If this is the case, check for clogs somewhere near the end of the manifold (i.e. into or out of the flow cell or before or after the backpressure loop.)

**Baseline continuously going down –** If you are waiting for the baseline to be stable before you start a run and it seems like it is continuously decreasing, check temperature settings. You may find that the heating coil has turned off.

**Negative peaks on lowest standards -** Your baseline is probably artificially high due to contamination in the carrier or the reagents. Try changing out the carrier first before you remake reagents. Look at baseline values from previous runs. Is yours similar?

**Negative peaks on samples –** If your standards are in a different matrix and they can out ok, then it is probably a matrix issue. Can you dilute your samples in nanopure and still get usable data?

**Very Elevated Baseline –** First check for contamination as mentioned previously, but if that doesn’t solve it, take out and examine the flow cell. If the flow cell is leaking there may be a puddle of reagent at the base of the manifold. The flow cells can be refurbished by changing the O-rings and flare tubing. If this doesn’t fix the issue, there is a spare flow cell in the cabinet under the lachat.

**Omnion Suddenly Shuts Down –** If you are trying to run other programs while Omnion is running it can have glitches and suddenly shut down. If this happens it should save the data from your run up the point at which it stopped. You’ll have to start a new run with a new standard curve. It’s a total bummer.

**Blue Build-Up in the TP Manifold** – This has been happening for some reason. It doesn’t seem to be related to backpressure. To alleviate this issue, try putting a culture tube of the NaOH/EDTA cleaning solution in your sample run. The build-up seems to happen after about 40 or 50 samples, so maybe every 40 samples you run and EDTA clean-up followed by a couple of blanks. This is a new idea and hasn’t been tested yet, so if it works/doesn’t work, please update the procedure.