Procedural overview of Lachat QuikChem FIA+® autoanalyzer

Holly Meehan[1]

INTRODUCTION

The Lachat QuikChem FIA+® autoanalyzer is an automated ion analyzer that was obtained through National Science Foundation funds with the intention of streamlining lab work and increasing the sample processing capacity of the lab. The machine also allows for the integration of research and teaching, and permits the Biological Field Station to continue to provide local communities with data and information regarding its natural resources.

The autoanalyzer consists of four components: an autosampler, reagent pump, the analyzer itself, and a computer loaded with Omnion7 software. These components together serve to take raw or pretreated samples through the analysis and give an analyte concentration at a rate of about one minute per sample. Samples are arranged in a rack of test tubes, from which the autosampler draws a certain amount of sample from each test tube and the reagent pump pushes the samples and reagents through the analyzer. Reagents are injected into the lines at certain points according to the method, and the sample and reagents are heated, if appropriate to the analysis. The sample then passes through a flow cell where the voltage is measured and converted to a concentration based on the standard curve established at the beginning of the run.

This report is intended to review the use of the Lachat QuickChem FIA+ for nutrient analysis, to provide suggestions for the management of samples and data files, and also for use as a quick reference guide for the preparation of reagents, standards, checks, and samples. Methods discussed include those for the determination of Total Phosphorus (TP), Total Nitrogen (TN), Nitrate/Nitrite (NO_3/NO_2), and Ammonia (NH_3). The methods used for TP, NO_3/$NO_{2,}$, and NH_3 analyses were developed by Lachat, while the method used for TN digestion was taken from Ebina et al. (1983). Additional manifolds may be purchased for the analysis of other compounds and elements. The suggestions presented here come from the use of the machine over the course of the summer of 2005, with the concurrent analysis of samples from various projects. Many of the suggestions come from personal preference, having proved to streamline the process and reduce error. The user, software, and training manuals provided by Lachat contain more detailed information pertaining to parts, maintenance, and troubleshooting, though they are not ideal for quick reference. Abbreviated, somewhat modified, methods are given in Appendices I-IV.

[1]Rufus J. Thayer Otsego Lake Research Assistant, summer 2005. Present Affiliation: University of Rhode Island, Kingston, RI

Components: General Mode of Operation

Sample Collection & Preservation

As with any sample collection for water quality, collection bottles should be acid washed followed by rinses with hot and then distilled water. At least 20mL of sample should be collected for each analysis to be run by the auto analyzer, in the case that samples need to be rerun; a greater volume will be necessary for additional analyses not performed by the auto analyzer.

Immediately following collection, the portion intended for nutrient analysis should be preserved through acidification. Acid preservation is achieved by adding 0.8mL Strong Acid Solution (5.76M H_2SO_4) per 125mL sample in polyethylene bottles. This brings sample pH to < 2.0, which inhibits microbial action and gives a H_2SO_4 concentration equal to 2 parts per thousand (ppt). Once acidified, exposure to open air should be limited in order to avoid ammonia contamination from the air (acidified samples may take up ammonia from ambient air) (Tucker 2005).

Sample Management

Use of a sample log book is crucial during the summer when many projects are underway, each with different analysis requirements. A system should be estabiloshed to avoid missing or over-acidifying samples. A sheet has been created to help when organizing samples to be prepped for a run (Figure 1). It is also helpful to arrange the sample bottles in the configuration of the rack (Figure 2), as it is easy to refer to the rack order sheet and check to see that they compliment one another.

Run Parameter: ___________ Sites: ________________________

Rack #'s _____ & _____

☐ File Exported
☐ Copied to Desktop folder
☐ 2 copies printed

Cup	Date	Sample	Cup	Date	Sample	Cup	Date	Sample
1A			9A			5A		
B			B			B		
C			C			C		
D			D			D		
E			E			E		
2A			10A			6A		
B			B			B		
C			C			C		
D			D			D		
E			E			E		
3A			11A			7A		
B			B			B		
C			C			C		
D			D			D		
E			E			E		

Figure 1. Sample rack order sheet for organization of samples in analyzer racks.

Figure 2. Arrangement of sample bottles corresponding to rack order sheet

Start-up

Before initiating a run, bring all refrigerated reagents to room temperature for at least 12 hours. Failure to do so will result in degassing, during which bubbles will move through system causing interfering voltage spikes. Conversely, if necessary, warm reagents in a water bath to

above room temperature for a few hours, then allow to return to room temperature (this will help drive off gasses).

Begin with reagent lines in distilled water, using a separate vessel for each manifold. After powering up the autosampler, reagent pump, analyzer, and computer, engage the reagent tube clamps to begin running water through the manifold(s) for a few minutes. Tubes should never be engaged unless the pump is running, as this will flatten them, reducing flow rates. Check all junctions for leaks. If any are detected, shut down pump and detach and reattach faulty junctions (all fittings need to be only barely snug. Excess tightening will collapse tubes and cause leaks. See "switching manifolds" below). If using the nitrate/nitrite manifold, the cadmium column should remain in the "off line" position during this rinse. On the computer, in the Omnion7 software, open the template file for the analyte you will be determining (e.g. TN Template). Check the calibration standards to make sure that the concentrations listed are the same as what you prepared in the rack. If there are differences, you can change the standard concentration in the run template. The reagent lines should be placed in the corresponding reagent vessel, each of which should be covered (not sealed) with Parafilm® to avoid contamination to/from the ambient air (especially those containing ammonia or those used in the ammonia analysis). When using the cadmium column, the column should be put in the "on line" position only after all air has passed through the flow cell. The column should never be on unless buffer solution is moving through the manifold. Distilled water, and even more so air bubbles, will degrade the column. After all air has passed, take out the flow cell, being sure not to touch the top or bottom, and gently tap the side to dislodge any air bubbles that may have been trapped, reinsert it into the slot, and lightly tighten the screw. "Preview" the run (in Omnion7 software) once all bubbles have passed the flow cell to ensure that the baseline is stable and appropriate for the analysis. With the rack in place (A1 at the lower left-hand corner), the machine is ready to start the run. Refer to *Run Set-up* for notes on configuring the run in the Omnion software. "Stop" the preview, and hit "start" to begin your run. As the standards are processed, check to make sure the voltage reading for the baseline and high standard are similar to those listed on the procedure page for each analyte.

Shut-down

1. The run will stop automatically when all samples entered in the run file have been analyzed. The pump speed may remain at 35 or may slow to "rc-3." In the case that it slows, return the speed to 35 by going to the "configuration" pull-down menu, choosing "instruments", "pump", and click "normal." The speed should return to 35.

2. If using the cadmium column, turn to the "off-line" position before removing any reagent lines.

3. Turn off heater(s), if appropriate.

4. Transfer reagent lines to distilled water rinse vessels, let rinse for 3-5 minutes.

5. Remove the lines from water, hang them on a hook, and let the pump run until no liquid remains in the lines. Lines for different procedures should be hung separately, as cross-contamination from reagents could occur.

6. Disengage the pump lines,turn the autosampler, reagent pump, and autoanalyzer off, shut down the Omnion7 software (after saving the run file; see *Data and File Management*).

7. Return the reagent lines in the rinse vessels for storage.

Run Set-up & Running Multiple Racks

When creating the run in Omnion7, you will need to specify the number of samples to be tested. To insert rows between preexisting samples, simply click on the row that you would like to insert above, right click, and choose "insert" or "insert many". To add rows beyond those that already exist, click on the last row to highlight it, right click, and select "append row" or "append many". This function can be done while the run is in progress in the case that you need to rerun a sample or make a dilution. The sampler is able to go back to a cup that has already been drawn from by simply inserting a row with the desired cup number. To automatically assign consecutive cup numbers to each sample, drag down the rows on the "sample no." column, right click and choose "columns", then "auto number cups".

When running multiple racks, adjust the run (in Omnion7) accordingly, with each rack beginning at cup one. For example, if you are running two full racks, and one partial (with 23 samples), you will have 143 rows total, but you will number the cups 1-60 for the first rack and start again at 1 for the second and third racks. During the run you must manually switch the racks after the last sample (in cup 60) has been drawn, taking care to orient the rack so that cup one (A1) is in the bottom left-hand corner of the autosampler tray.

Preparing Checks, Standards & Standard Curves

The quality of the standards and the standard curve is dependent on the care taken in their preparation and analysis. Statistical analysis of the standards can be checked as soon as the last standard is analyzed. Acceptable calibration standards are those that have an R^2 value of 0.998 or higher. Standards may be "unused" after the run is complete, but should only be done in certain circumstances, as in the case that a peak was unusually high or low and it can be attributed to a known reason, or if a mishap occurred while the standard was being processed, such as a bubble passing through the flow cell. A standard should never be "unused" simply due to poor linearity. Standards should be treated exactly as the samples to ensure that all exposure to potential contamination is equal.

Checks should be used for all analyses to ensure that laboratory techniques and procedures are working properly. Checks created for Total Phosphorus and Total Nitrogen should be some organic compound containing those nutrients and serve to check the efficiency of

the digestion procedure, and its ability to transform all forms of the respective elements to a detectable (soluble) form. Nitrate/Nitrite and Ammonia checks serve to check the analyzer and the lab, and are important when new reagents are introduced while a run is in progress. Checks run every ten or 20 samples, and should be made from a separate check solution (not that used to make the standards), by a different person, ideally in a different lab, to satisfy quality assurance issues.

Preparation of Standards

This section discusses the test-tube preparation of standards. The methods listed avoid unnecessary switching of volumes on the auto-pipettes, and are only suggestions. Stepwise instructions for the preparation of particular standard solutions are available in the procedures provided in Appendices I through IV.

For 10mL standards:
Add 5ml carrier to each test tube, except for the high standard (gets no carrier)
Add the appropriate volume of standard working solution (see Appendices)
Add the remainder of the carrier to each tube
Nitrate and ammonia standards should be vortexed to adequately mix the carrier and standard solution (for TN and TP, adequate mixing occurs during digestion)

For 5mL TN standards:
Add the appropriate volume of carrier to each test tube, from high to low
Add the appropriate volume of standard working solution
All other additions will be done at the same time as the rest of the samples

Reasons for Stopping a Run

There are many reasons for stopping a run, most of which are noticeable in the first several minutes of the run, while the standards are being processed. Below is a list of reasons to stop, followed by possible solutions. It should be noted that individual circumstances should be examined closely, as these possible solutions come from previous experiences with run failures. Based on the graphs created by the Omnion7 software, the cause of the problem is generally easy to identify. Rerunning or remaking the standards should be done to check the correction before running samples.

- Computer does not produce peaks; analyzer not on, bulb out, or filter wrong
- Standards are not linear; standards not prepared accurately; contamination of equipment
- Baseline unstable (with spikes); reagents degassing
- Baseline creeping upward; contaminated carrier
- Standard curves lower than baseline; contaminated carrier
- Large refractive index (optical density of carrier does not match that of samples); pH/ ionic mismatch
- Voltage is not typical for the analysis; contamination of carrier, standards not prepared accurately

- Timing is off (see Lachat User Manual)

Switching Manifolds

1. Have the manifold boxes out, side-by-side, and have easy access to manifold diagrams
2. Switch the filters, taking care not to touch the filter itself, barely tighten set screw
3. Switch the sample loops (valve locations 1 &4)
4. Disconnect line from valve location 3
5. Disconnect heating loop (connects to the manifold in 2 places)
6. Disconnect the flow cell
7. Disconnect carrier line from valve location 2
8. Remove reagent lines from pump clamps and rinse vessels
9. Pick up the manifold and loosely wrap the reagent lines around it
10. Store in the box with desiccant packets
11. Make sure that the filter and sample loop are in the box before storage

When tubing is switched, care must be taken to avoid bending the tubing. Insert the tubing into the slightly-loosened connector; you should feel the tubing pass through the two o-rings inside. The first few times, you may need to disassemble the connection and put the o-rings on the tubing manually. All connections should be barely finger-tight (a slight tug won't disengage the tube). Anything more will damage tubing.

Installing a new manifold will be very similar to the procedure above, in reverse order, and should be done according to the manifold diagram provided with the equipment. Timing will differ between analyses, and should be checked in the Omnion7 software when creating a template file or beginning a run.

Data and File Management

Each new run should be created using the template for the given analyte, as a new OM.omn file is automatically created each time that a run is started without overwriting the template (Using a date/time signature). Use of the template ensures that the analyzer settings are correct for the analysis to be performed, and that the standard concentrations are correct. At the end of a run, the file should be saved with a descriptive name identifying the parameter analyzed, the sites included in the run, and the date range of samples that were analyzed; e.g. TP Savannah TR4C Moe July 21-31.omn. After saving the run, all OM files that were automatically created can be deleted. This will help to eliminate confusion and reduce clutter in the data folder. Details of a run dealing with standards may be changed after the run is complete. Most times you can save these changes without renaming the file, though occasionally Omnion7 will not allow you to save the file under the same name.

Data from the run can be exported in Excel format after the file has been saved by clicking "export data to file." This also occurs automatically at the end of a run, and those having the date/time signature should be deleted after naming. If the file does not immediately appear in

the “data” folder, you will have to close and reopen the file, and choose to “export data to file” again.

Waste Management

Separate containers should be used for each type of waste that is created: TN/NO_3+NO_2, TP, and NH_3. The wastes created in the TN and NO_3+NO_2 are the same, so a single container may be used to store them. Containers should be clearly labeled as hazardous and with the contents listed according to OSHA standards and campus waste management procedures. Labels should include the date on which filling started. Lines flowing out of the flow cell at the end of the analyzer should be draped into such hazardous waste containers. Ammonia waste contains phenols, which can be hazardous if inhaled, and should be covered lightly. Waste from rinse and wash lines may be collected and disposed of in the sink, as it is composed of distilled water and sample residue.

Methods for Analysis: Quirks and Modifications

Total Phosphorus (TP)

Prep-time: 3 to 4 hours for 2-3 racks, including checks & standards
Approximate TP baseline voltage: 0.17v
Approximate 2.0 μg/L standard voltage: 0.43v
Insure appropriate acidification of checks & standards
(Note: Lachat methods refer to H_2SO_4 strength in molarity, not normality. For this acid, M= $^1/_2$N)

At some point, a blue film might be observed accumulating on the lines, indicating that there may be phosphorus contamination in the reagents. This would be confirmed by the baseline creeping upward during the course of the run. To remove any potential accumulation, a Sodium EDTA rinse solution (65 g sodium hydroxide and 6 g Na_4EDTA per L) can be inserted in a cup during a run. Blue in the lines will remain because the cleaner is injected after this point, though any build-up in the flow cell will be removed. The same cleanser should be run through the ascorbic acid reagent line at the conclusion of the run for 20 to 30 seconds, prior to the distilled water rinse to remove the accumulation.

Total Nitrogen (TN)

Typical Prep time: 3hrs for 2-3 racks, with checks and standards
Approximate baseline voltage: 0.2v
Approximate 2mg/L standard voltage: 1.75v

Completeness of digestion should be evaluated by using a compound such as urea. Also, the efficiency of the oxidation of ammonia to nitrate should be occasionally evaluated by running an ammonia check through the digestion and analysis. The organic fraction of nitrogen can be calculated as the difference of TN and (NH_3+NO_2+NO_3). However, incomplete digestion and/or oxidation of ammonia will artificially accumulate all error into the (derived) organic fraction.

Efficiency of the column should also be checked periodically by running nitrite checks during a run.

Ammonia (NH_3) & Nitrite+Nitrate (NO_2+NO_3)

Samples for neither Ammonia nor Nitrite + Nitrate require preparation (other than acidification for preservation) and, as such, both analyses can be run concurrently. The software is currently set up to analyze ammonia on channel 1 and nitrite+nitrate on channel 2.

Typical Prep time: 20 minutes per rack
Approximate NH_3 baseline voltage: 0.13v
Approximate 2mg/L NH_3 standard voltage: 2.27v

Approximate NO_3 baseline voltage: 0.2v
Approximate 2mg/L NO_3 standard voltage: 1.6v

Avoiding contamination of acidic reagents and samples is crucial, as ammonia is taken out of the air by solutions of acidic nature. Carrier should be made fresh each time the analysis takes place. This carrier should be used to make the standards and checks to avoid ammonia contamination from the air to the acidic solution.

Because some reagents contain ammonia compounds, which have pH adjusted upward, they should be prepared in the hood to avoid contamination of the ammonia analysis. Reagents containing ammonia should be made in the hood and care should be taken to keep them covered when running an analysis. Nitrate color reagent contains phosphoric acid. Exercise care in keeping lines, rinse solutions, etc. separate from those used in phosphorus analyses to avoid contamination.

REFERENCES

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Appendix I

Total Phosphorus Prep & Procedure

Reagents, Samples, and Standards

(Liao and Marten 2001)

Reagents

Stock Ammonium Molybdate:
35.50 g in ~800mL H_2O, dilute to 1L mark; Stir ~4 hrs; store in plastic & refrigerate
Stock Antimony Potassium Tartrate (trihydrate):
3.312g in ~800 mL H_2O, dilute to 1L mark & mix. Store in dark bottle & refrigerate, good for 2 months.
Color Reagent:
In ~200mL H_2O, add 10.5mL H_2SO_4 & swirl; add 35mL stock antimony potassium tartrate and 120 mL stock ammonium molybdate; dilute to 500mL mark. Refrigerate, prepare weekly.
Ascorbic Acid Solution:
Dissolve 12.0g granular ascorbic acid in ~70mL H_2O, dilute to 200 mL & mix; add 0.2g dodecyl sulfate. Prepare weekly, sooner if becomes yellow.
Carrier (0.13M H_2SO_4):
7.2 ml H_2SO_4 to 1 L
Sample preservative (5.76M H_2SO_4):
320 mL H_2SO_4 to 1L
Digestant (4.7M H_2SO_4):
261 mL H_2SO_4 to 1L

Standards

[Make intermediate and working solutions with 2 parts per thousand (ppt) H_2SO_4- this matches pH of preserved samples]

Stock Solution: 50mg/L-P (0.2195 g anhydrous KH_2PO_4 to 1 L)
Intermediate Solution: 5.0mg/L (1:10 of above)
Working Solution: 200µg/L (4.0mL intermediate solution to 100 mL H_2O)

mL Working Stock	mL 2 ppt H_2SO_4	P (µg/L)
10	---	200
5	5	100
2	8	40
1	9	20
0.5	9.5	10
0.2	9.8	4
---	10	0

Samples and Standards

To 10 mL preserved sample (0.8 mL 5.76 M H2SO4 per 125 mL) and to standards, add 0.2 mL digestant and 0.1 g (1 scoop) potassium persulfate. Cover rack tightly with foil and autoclave 30 minutes at 121^oC, 15 psi.

Appendix II

Total Nitrogen Prep & Procedure
Reagents, Samples, and Standards
(Ebina et al. 1982, Pritzlaff 2003)

Reagents

Oxidizing Solution:

Dissolve 20.0g $K_2S_2O_8$ and 3g NaOH to 1L.

0.36N NaOH:

14.4g NaOH to 1L.

Color reagent:

In ~300mL H_2O, add 50mL 85% phosphoric acid, 20.0g sulfanilamide and 0.5g NED; stir with bar for 30 min, take to 500mL. One month in dark bottle; refrigerate.

Ammonia Chloride Buffer Solution:

In ~700mL, dissolve 85g ammonium chloride and 1g disodium ethylenediamine tetraacetic acid. (Na_2EDTA); adjust pH to 8.5 w/ 15N NaOH; Dilute to 1L.

Carrier (2 ppt H_2SO_4):

2mL H_2SO_4 diluted to 1L

Standards

[Make sub-stock and working solutions with 2 parts per thousand (ppt) H_2SO_4- this matches pH of preserved samples]

Stock Solution: 200mg/L NO3-N (1.444 g KNO_3 to 1 L)
Working Solution: 4mg/L (2.0mL stock to 100 mL H_2O)

mL Working Stock	mL carrier	N (mg/L)
5	---	4
2.5	2.5	2
1	4	0.8
0.5	4.5	0.4
0.2	4.8	0.16
0.1	4.9	0.08
---	5	0.00

Samples and Standards

To 5 mL preserved sample (0.8 mL 5.76 M H2SO4 per 125 mL) and to standards, add 5.0 mL oxidation solution. Cover rack tightly with foil and autoclave 30 minutes at 121 ^{o}C, 15 psi). When cool, add 1.0 mL 0.36 N NaOH, vortex and analyze as nitrate.

Appendix III

Nitrate+Nitrite Prep & Procedure

Reagents, Samples, and Standards

(Pritzlaff 2003)

Reagents

Color reagent:
In ~300mL H_2O, add 50mL 85% phosphoric acid, 20.0g sulfanilamide and 0.5g NED; stir w/bar for 30min, take to 500mL. Prepare monthly, store in dark bottle; refrigerate**.**

15N NaOH:
Dissolve 150g NaOH in 250mL H_2O. Store in plastic bottle.

Ammonia Chloride Buffer Solution:
In ~700mL, dissolve 85g ammonium chloride and 1.0g disodium ethylenediamine tetraacetic acid (Na_2EDTA); adjust pH to 8.5 w/ 15N NaOH. Dilute to 1L.

Carrier (2 ppt H_2SO_4):
2mL H_2SO_4 diluted to 1L. If being used to make standards for ammonia, prepare fresh to avoid contamination from air.

100 mg N/L Nitrite check: (to test column efficiency)
Dissolve 0.607g KNO_2 in ~60mL H_2O; dilute to 100mL. Only stable for 2-3 days.

Standards Make working solution simultaneously with ammonia standards.
[Make sub-stock and working solutions with 2 parts per thousand (ppt) H_2SO_4- this matches pH of preserved samples]

Stock Solution: 200mg/L NO3-N (1.444 g KNO_3 to 1 L)
Working Solution: 2mg/L (1.0mL stock to 100 mL H_2O)

mL Working Stock	mL carrier	NO_3-N (mg/L)
10	---	2
5	5	1
2	8	0.4
1	9	0.2
0.5	9.5	0.1
0.2	9.8	0.04
---	10	0.00

Samples and Standards
No sample preparation is necessary. Analyze samples preserved with 0.8 mL 5.76 M H_2SO_4 per 125 mL.
Note: If the column efficiency falls below 90% (i.e., if the value of NO_3 in standard or check falls <90% of the corresponding NO2 check), the column should be replaced or re-charged. Refer to “Emergency procedure for regenerating Cd columns”.

Appendix IV

Ammonia Prep & Procedure
Reagents, Samples, and Standards
(Liao 2001)

Reagents

Sodium Phenolate:
Dissolve 17.6mL of 88% liquefied phenol in ~120mL H_2O. While stirring, slowly add 6.4g sodium hydroxide. Cool, dilute to 200mL, invert to mix. Good for 3-5 days; discard when reagent turns brown.

Sodium Hypochlorite: make fresh daily
In a 200 mL flask, dilute 100 mL 5.25% sodium hypochlorite (regular bleach) to the mark; invert
to mix. Store bleach in refrigerator. Make daily.

Sodium Nitroprusside:
In a 200mL flask, dissolve 0.7g sodium nitroprusside (sodium nitroferricyanide); dilute to mark. Prepare fresh every 1-2 weeks.

1M Sodium Hydroxide:
In a 1L flask, dissolve 40.0g sodium hydroxide in ~900mL H_2O. Dilute to mark, stir w/magnetic stirrer until dissolved.

Buffer Solution for acid preserved samples:
In ~700mL H_2O, dissolve 50.0g disodium ethylenediamine tetraacetic acid (Na_2EDTA) and 254mL 1M sodium hydroxide. Dilute to 1L and mix with magnetic stirrer until dissolved.

Carrier (2 ppt H_2SO_4):
2mL H_2SO_4 diluted to 1L. Prepare fresh to avoid ammonia contamination from air.

Standards Make working solution simultaneously with nitrate+nitrite standards.
[Make sub-stock and working solutions with 2 parts per thousand (ppt) H_2SO_4- this matches pH of preserved samples]

Stock Solution: 200mg/L (0.7638 g anhydrous NH_4CL to 1 L)
Working Solution: 2mg/L (1.0mL intermediate stock to 100 mL H_2O)

mL Working Stock	mL 2 ppt H_2SO_4	NH_3-N (mg/L)
10	---	2
5	5	1
2	8	0.4
1	9	0.2
0.5	9.5	0.1
0.2	9.8	0.04
---	10	0.00

Samples and Standards
No sample preparation is necessary. Analyze samples preserved with 0.8 mL 5.76 M H_2SO_4 per 125 mL.