1.0 TITLE

Total Kjeldahl Nitrogen and Total Kjeldahl Phosphorus Sample Digestion and Analysis

2.0 LOCATION

Wet Chemistry Instrument Lab

3.0 SCOPE & APPLICATION

This method is used to determine Kjeldahl nitrogen from surface water, saline water, soils and sludge. The Kjeldahl phosphorus is only applicable for soils and sludge. This procedure converts nitrogen components of biological origin such as amino acids, proteins and peptides to ammonia; but may not convert nitrogenous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semicarbazones and some refractory tertiary amines. Kjeldahl nitrogen is defined as the sum of free ammonia and organic nitrogen compounds that are converted to $(NH_4)_2SO_4$ by the digestion procedure described. The applicable range for TKN aqueous analysis is 0.5mg N/L to 20mg N/L. The method detection limit is 0.1mg/L. The phosphorus analysis method is based on reactions that are specific for the orthophosphate ion. The applicable range is 0.5mg P/L to 20.0 mg P/L.

NOTE: ACZ has extended the scope of this procedure to the analysis of saline waters, soils, & sludge. Although ACZ believes this procedure to be technically sound, it should not be used for regulatory compliance monitoring of such matrices in lieu of an approved method.

4.0 SUMMARY

4.1 Sample is heated in the presence of sulfuric acid (H_2SO_4) with a mercuric oxide catalyst for 2-1/2 hours. Potassium sulfate is added to raise the boiling temperature of the digestion and to speed the digestion. The residue is cooled, diluted and analyzed for ammonia and phosphorus.

4.2 Total Kjeldahl nitrogen is the sum of free-ammonia and organic nitrogen compounds that are converted to ammonium sulfate $(NH_4)_2SO_4$ under the conditions of the digestion described. Organic nitrogen equals the difference obtained by subtracting free-ammonia concentration from the total Kjeldahl nitrogen concentration.

4.3 Digested sample is injected into the chemistry manifold where its pH is controlled by raising it to a known, basic pH by neutralization with a concentrated buffer. This in-line neutralization converts the ammonium cation to ammonia, and also prevents undue influence of the sulfuric acid matrix on the pH-sensitive color reaction that follows. The method uses a 660nm absorbance filter.

4.4 The ammonia produced is heated with salicylate and hypochlorite to produce blue color, which is proportional to the ammonia concentration. The color is intensified by adding sodium nitroprusside. The presence of potassium tartrate in the buffer prevents precipitation of calcium and magnesium.

4.5 The orthophosphate ion (PO_4^{3-}) reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex is reduced with ascorbic acid to form a blue complex with absorbs light at 880nm. The absorbance is proportional to the concentration of orthophosphate in the sample.

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4.6 Method Modifications:

4.6.1 EPA351.2 requires the LCR to be verified every 6 months; however, Jim O'Dell of the EPA confirmed that the intent of the LCR is to establish the upper limit of the calibration range. ACZ's procedure requires daily instrument calibration and dilution of samples with a concentration greater than the high calibration standard; therefore, verification of the LCR every 6 months does not provide a quality control measurement pertinent to laboratory activities and is not required.

4.6.2 In this SOP 20 mL sample volumes are used instead of 25 mL volumes specified by Method 351.2, because splattering of samples has been observed with use of a larger sample volume. Samples will boil over if a larger sample volume is placed into the tubes. The Sample: Reagent ratio specified in the method must be maintained.

4.6.3 **Note on modifications in this SOP**: Any discrepancies between this SOP and the attached Lachat QuikChem method are intentional, and what is stated in this SOP supersedes QuikChem

5.0 REFERENCE

5.1 "Nitrogen, Kjeldahl, Total Method 351.2 (Colorimetric, Semi-Automated Block Digester, AAII),"Methods for the Chemical Analysis of Water and Wastes, EPA-600/R-93-100-August, 1993.

5.2 "Total Kjeldahl Nitrogen in Waters", Wendt, K., Lachat Instruments, August 1995.

5.3 “Determination of Total Phosphorus by Flow Injection Analysis,” Ninglan Liao, Lachat Instruments, May 2001.

6.0 SAMPLE COLLECTION, HANDLING AND PRESERVATION

6.1 Aqueous samples are preserved to pH< 2 with H_2SO_4 (**yellow dot**) and are maintained at 0-6.0 °C in the north inorganic walk-in cooler. Hold time is 28 days. Prep and analysis must be completed within the hold time.

6.2 Soil samples are not preserved and should be used from un-prepped sample. The “wet” sample should be used, because inaccurate data may result if dried soils are digested, due to ammonium salts being lost in the drying process. Unprepped soil and sludge samples are stored in the north inorganic walk-in cooler at 0-6.0 °C. Other soils samples can be found downstairs on the sample racks. .

6.3 If the sample to be digested consists of large amounts of plant material, the sample should be prepped by soils first to ensure a representative aliquot.

Table 6.1

Parameter	Matrix	Hold Time starts – ends with
N-TK and P-T	Liquid and Solid without extraction.	28 days from collection date to analysis.
Solid		

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	Solid with extraction	No hold time has been established from sample collection to sample extraction; 28 days from sample extraction to sample's extract analysis including sample's extract preparation.

7.0 APPARATUS & SUPPLIES

7.1 Block Digester capable of maintaining 380°C.

7.2 50mL plastic centrifuge tubes with caps.

7.3 Balance -- analytical, capable of accurately weighing to the nearest 0.0001 g. The calibration of the balance must be verified daily or before use. Refer to SOPAD013 for additional details.

7.4 Glassware – Digestion tubes and Class A volumetric flasks and pipettes.

7.5 Hengar or Teflon Boiling Chips.

7.6 Mechanical pipettes delivery accuracy verified according to SOPAD013.

7.7 Vortex mixer (Fischer Scientific Touch Mixer model 232 or equivalent).

7.8 Flow injection analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

7.9 Sampler

7.9.1 Multi-channel proportioning pump
7.9.2 Reaction unit or manifold
7.9.3 Colorimetric detector
7.9.4 Data system

7.10 Heater Module (Lachat Part No. A85100/A85200)

7.11 Peristaltic pump tubing.

7.12 Centrifuge

7.13 15mL Plastic centrifuge tubes.

7.14 Disposable culture tubes

8.0 REAGENTS & STANDARDS

NOTE: Reagents are prepared using Class A volumetric glassware and Type I H_2O unless otherwise indicated. Store prepared standards and reagents separately from samples.

NOTE: Preparation of reagents is documented in the Wet Chem Inst Reagent Logbook. Documentation includes reagent, prep and expiration dates, analyst's initials, new SCN if applicable, and SCN/PCN(s) used to make the reagent. If different SCN/PCN(s) of chemical solutions or stocks

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are combined to make the new reagent then document the quantity of each SCN/PCN that were combined.

8.1 **Mercuric Sulfate Solution**: In a 100mL volumetric flask add 40mL Type I H_2O, followed by 10mL sulfuric acid. Add 8.0 g mercuric oxide. Stir until dissolved (warming the solution while stirring will help dissolve mercuric oxide). Allow solution to cool and dilute to 100ml mark with Type I H_2O. Store at room temperature in the cyanide lab. Shelf life is one year.

8.2 **Digestion Solution**: In a 1000mL flask add 500mL of Type I H_2O, followed by 200mL sulfuric acid. Next add 133 g potassium sulfate followed by 25mL of the mercuric sulfate solution (§8.1). Dilute to 1000mL with Type I H_2O. Shelf- life = 1 month. Store at room temperature in the cyanide lab.

NOTE: The potassium sulfate may begin to precipitate out over time; if this occurs the solution should be heated and stirred to until everything has been re-dissolved before use.

8.3 **Reagents Used on the Lachat for TKN**

Degassing with helium: To prevent bubble formation, degas all solutions except the standards with helium. Use He at 140kPa (20 lb/in^2) through a degassing tube. Bubble He through the solution for a minimum of one minute.

8.3.1 **TKN Buffer**: In a 2 L flask add 1400 mL Type I H_2O, 100g potassium tartrate (or potassium sodium tartrate, D,L-$NaKC_4H_4O_6 \cdot 4H_2O$), 100 g NaOH, and 53.6 g sodium phosphate dibasic heptahydrate ($Na_2HPO_4 \cdot 7H_2O$). Mix until dissolved and then boil for 10 minutes. Cool to room temperature. Dilute to the 2L mark with Type I H_2O and invert to mix. Store in glass bottle (amber or clear) at room temperature in instrument lab. Reagent shelf-life is one month.[1]

NOTE: The cooling process can be accelerated by placing the flask in a large container and running water around the flask. Be sure no water is able to get into the flask.

8.3.2 **Salicylate Nitroprusside:** In a 1 L flask, dissolve 150 g sodium salicylate [salicylic acid sodium salt, $C_6H_4(OH)(COO)Na$], and 1.0 g sodium nitroprusside [sodium nitroferricyanide dihydrate, $Na_2Fe(CN)5NO \cdot 2H_2O$] in ≈ 800mL Type I H_2O water. Dilute to the mark and dissolve. Shelf-life = 1 month. Store reagent at room temperature in a dark glass bottle in the instrument lab.[2]

8.3.3 **Hypochlorite Solution:** In a 250mL flask, add 15mL of 4—6% sodium hypochlorite (NaOCl), dilute to the mark with Type I H_20. Invert to mix. **Prepare fresh daily**.

8.3.4 **0.8M Sodium Hydroxide**: In a 1 L flask dissolve 32 g NaOH in ≈ 800mL of Type I H_2O water. Dilute to the mark and stir to dissolve. Store in a glass or plastic at room temperature in instrument lab. Shelf-life is one year.[2]

8.3.5 **0.2% H_2SO_4**: Add ~ 900mL of Type I H_2O to a 1L volumetric flask. Carefully add 2mL of concentrated H_2SO_4 into the flask and dilute to the mark with Type I H_2O. Invert to

[1] EPA 351.2 method deviation as per manufacturer's recommendation.
[2] EPA 351.2 method deviation as per manufacturer's recommendation.

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mix. Store in a plastic/glass bottle at room temperature in instrument lab. Shelf-life is one year.

8.3.6 **TKN / P-T soils Carrier:** In a 1 L flask dissolve 31.7g Potassium Sulfate (K_2SO_4) and 48 mL of H_2SO_4 in ≈ 800 mL of Type I H_2O water. Dilute to the mark and stir to mix. Prepare weekly and store in glass bottle (clear or amber) at room temperature in instrument lab.[2]

8.4 **Reagents Used on the Lachat for P-T**

Degassing with helium: To prevent bubble formation, degas all solutions except the standards and Ascorbic Acid Reducing solution with helium. Use He at 140kPa (20 lb/in^2) through a helium degassing tube. Bubble He through the solution for one minute.

8.4.1 **Stock Ammonium Molybdate:** In a 1L flask, dissolve 40.0g ammonium molybdate tetrahydrate $[(NH_4)_6Mo_7O_{24}\cdot 4H_2O]$ in approximately 800mL Type I H_2O. Dilute to the mark and dissolve**.** Store in plastic for up to two months at 0-6.0˚C in the reagent lab cooler.

8.4.2 **Stock Antimony Potassium Tartrate:** In a 1L flask, Dissolve 3.22g antimony potassium tartrate [potassium antimonyl tartrate trihydrate $9C_8H_4O\ 12K_2Sb_2\cdot 3H_2O$)] or dissolve 3.0g antimony potassium tartrate (potassium antimonyl hemihyrate $[K(SbO)C_4H_4O_6\cdot {}^1/_2H_2O]$) in approximately 800mL Type 1 H_2O. Dilute to the mark and dissolve. **Prepare fresh every two months. Store in an amber glass bottle and store at 0-6.0˚C in the reagent lab cooler.**

8.4.3 **Molybdate Color Reagent:** In a 1L flask, add 213mL ammonium molybdate stock solution and 72mL antimony potassium tartrate solution and dilute to the mark with Type 1 H_2O. Invert to mix. **See expiration of stock solutions to determine expiration date.** Store in an amber glass bottle at room temperature in the instrument lab.

8.4.4 **Ascorbic Acid Reducing Solution:** In a 1L flask, dissolve 60.0g ascorbic and 1.0g sodium dodecyl sulfate in about 700mL Type 1 H_2O. Dilute to the mark and mix using a magnetic stir bar. Shelf-life = 1 week. Store in a glass bottle at 0-6.0˚C in the reagent lab cooler.

NOTE: The dodecyl sodium sulfate is a surfactant. **DO NOT** invert to mix or degas.

8.4.5 **Sodium Chloride / Sodium Hydroxide Solution:** In a 1L flask, add 160g sodium chloride and 20g sodium hydroxide in about 600mL Type 1 H_2O. Dilute to the mark and dissolve. Shelf-life = 1 month. Store in a plastic or glass at room temperature in instrument lab.

8.4.6 **P-T soils / TKN Carrier** (same as § 8.3.6): In a 1 L flask dissolve 31.7g Potassium Sulfate (K_2SO_4) and 48 mL of H_2SO_4 in 800 mL of Type I H_2O water. Dilute to the mark and stir to mix. Prepare weekly. Store in glass at room temperature in the instrument lab.

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8.5 **Preparation of Stock and Working standards[3]**

8.5.1 **1000 mg/L Total Kjeldahl Nitrogen Calibration and Spike Stock:** Purchase from a reputable vendor. Manufacture's expiration date applies. Store at 0-6.0˚C in the reagent cooler.

Or prepare 1000 mg/L NH_3 stock from Ammonium Chloride, NH_4Cl: Dissolve 3.8195g NH_4Cl (that has been dried for 2 hours at 110ºC) in a 1L class A volumetric flask containing ~ 500mL Type I H_2O. Preserve using 5 mL 25% H_2SO_4. Dilute to the mark with Type I H_2O. Prepare once a year. Store in glass container at 0-6.0˚C in the reagent cooler.

8.5.2 **1000mg/L Total Kjeldahl Nitrogen ICV Stock:** Purchase from a reputable vendor. Manufactures' expiration date applies. Store at 0-6.0˚C in the reagent cooler.

Or prepare 1000 mg/L NH_3 ICV Stock from Ammonium Sulfate, $(NH_4)_2SO_4$: Dissolve 4.71464g $(NH_4)_2SO_4$ (that has been dried for 2 hours at 110ºC) in a 1L class A volumetric flask containing ~ 500mL Type I H_2O. Preserve using 5 mL 25% H_2SO_4. Dilute to the mark with Type I H_2O. Prepare once a year. Store in glass container at 0-6.0˚C in the reagent cooler.

8.5.3 **1000mg/L Phosphorus Calibration and Spike Stock:** Purchase from a reputable vendor. Manufacture's expiration date applies. Store at 0-6.0˚C in the reagent cooler.

Or prepare 1000mg/L Phosphorus (P) Calibration and Spike Stock: Dissolve 5.624g potassium phosphate (K_2HPO_4) in a 1L volumetric flask into approximately 750mL of Type 1 H_2O. Dilute to the mark and invert or use a magnetic stir bar to dissolve. Prepare once a year and store in glass at 0-6.0˚C in the reagent cooler.

8.5.4 **1000mg/L Phosphorus ICV Stock:** Purchase from a reputable vendor. Manufacturer's expiration date applies. Store at 0-6.0˚C in the reagent cooler.

Or prepare 1000mg/L Phosphorus (P) Calibration and Spike Stock: Dissolve 5.624g potassium phosphate (K_2HPO_4) in a 1L volumetric flask into approximately 750mL of Type 1 H_2O. Dilute to the mark and invert or use a magnetic stir bar to dissolve. Prepare once a year and store in glass at 0-6.0˚C in the reagent cooler. The potassium phosphate must be a different lot number or from a different supplier than the potassium phosphate used to prepare the calibration standards.

8.5.5 **4 mg/L TKN ICV Solution:** In a 500 mL volumetric flask containing ~ 400 mL of Type I H_2O add 1 mL of concentrated H_2SO_4 and 2 mL of 1000 mg/L TKN ICV stock. Q.S. to the 500 mL and invert to mix. Prepare this solution monthly and store at room temperature in the reagent lab.

8.5.6 **4 mg/L P-T Soils ICV Solution:** In a 500 mL volumetric flask containing ~ 400 mL of Type I H_2O add 1 mL of concentrated H_2SO_4 and 2 mL of 1000 mg/L Phosphorous ICV stock. Q.S. to the 500 mL and invert to mix. Prepare this solution monthly and store at room temperature in the reagent lab.

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[3]ACZ has found inconsistency between different manufacturer's storage recommendations of some standards and has decided that storing them at 0-6.0˚C in the reagent cooler is sound practice. Samples that are tested for these parameters are also stored at 0-6.0˚C. (CAR1211)

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8.5.7 **100 mg/L P + TKN Combination Calibration and Spike stock Intermediate:** Add 20mL of the 1000 mg/L Phosphorus (P) Calibration Stock and 20mL of the 1000 mg/L TKN Calibration Stock to a 200mL class A volumetric flask containing ≈100 mL 0.2% H_2SO_4 (2 mL/L). Dilute to the mark with 0.2% H_2SO_4 and invert to mix. Shelf-life is 3 months and store at room temperature in the instrument lab.

Working calibration standards stock for **TKN - H_2O, TKN Soils and P-T Soils** analyses: Use only class A volumetric flasks. All standards must be made in a 0.2% H_2SO_4 (2 mL/L) solution and are stored at room temperature in the reagent lab. **The undigested working standard solution expiration date is the same as the intermediate.** Store undigested standard solutions at room temperature in the reagent lab. After the standards are digested, they are stored at 0-6.0˚C in the reagent cooler. **The shelf-life of digested standards is one month, or expiration date of intermediate, whichever comes first.**

Table 8.1: Standard Concentrations

STD	C1	C2***	C3	C4 **	C5
Total Volume (mL)	200	500	200	*	*
mL of 100 mg/L intermediate	10	12.5	2	*	*
Final Concentration mg/L	5	2.5	1	0.5	0

***C4 is a 10x dilution of C1.** This dilution should be digested or otherwise noted on the data review checklist if dilution was made at the instrument. **C5** (calibration blank) is 0.2% H_2SO_4.

** There must be a calibration standard at the same concentration as the PQL.

*** The event of digesting the standards must be recorded in the Working Calibration Standards Logbook, along with the analyst's initials, date, SCNs/PCNs of reagents used in digestion, and expiration date.

NOTE: Digestion of standards in full batches, utilizing the entire hot block, serves as a quality control check on the hot block; it demonstrates proper hot block functioning and consistent heating in all locations.

HELPFUL HINT: If prepping samples TKN-H_2O, TKN soils and P-T soils analyses, then prep the standards in the P-T soils format. Usually, the client samples prepped for TKN soils are the same as P-T soils, hence, the duel prep. The tray set up on the Lachat reflects this sequence so no adjustment of the standards or QC is necessary between runs.

8.5.8 **Table 8.2: Quality Control Standards**

QC STD	Final Conc	Source	mL of Stock used	Final Vol (mL)
ICV for TKN*	4 mg/L NH_3	1000 mg/L TKN ICV Stock	2	500

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ICV P-T soils*	4 mg/L P	1000 mg/L stock Phosphorus	2	500
CCV (C2)	2.5 mg/L	100 mg/L Intermediate	12.5	500
Spike/LFB	2.5 mg/L	100 mg/L intermediate	0.5	20

*ICB/CCB is 0.2% H_2SO_4.

* The event of digesting the standards must be recorded in the Working Calibration Standards Logbook, along with the analyst's initials, date, SCNs/PCNs of reagents used in digestion, and expiration date. The expiration date of the ICV solution applies to the digested TKN/P-T ICV. In order to track proper amounts of standards are being made, it is helpful to record in the logbook the quantity of each standard prepared to compare to remaining excess standards when new standards will be digested.

9.0 SAFETY

9.1 HAZARDS

9.1.1 All analytical procedures performed at ACZ pose some safety hazards that may be avoided with attention to detail.

9.1.2 Digested samples and TKN/P-T Soils manifold wastes contain high mercury concentrations and are considered as hazardous. Make sure to wear gloves and safety glasses when handling these samples. Analyzed client samples should be disposed into labeled mercury waste containers. When analyzing samples make sure both sample/rinse and manifold waste lines are draining into designated mercury waste containers.

9.2 SAFETY TECHNIQUE

9.2.1 Safety glasses are required and the use of gloves and lab coat is strongly recommended. Shorts and open-toed shoes are not allowed in the lab.

9.2.2 Use care when pouring and pipetting reagents. Always add acid to water. Use the proper method when washing glassware.

9.2.3 Do not eat or use tobacco products in unauthorized areas.

9.2.4 Wipe up ALL spills immediately. Implement the Emergency Response Plan if necessary.

9.2.5 Do not wear gloves or lab coat outside of the laboratory. Remove gloves before using a computer, telephone, etc.

9.2.6 Do not conduct "experiments" unrelated to the analysis.

9.3 PROTECTIVE EQUIPMENT

9.3.1 Use a fume hood when there is a potential for strong fumes.

9.3.2 A fire extinguisher is located near each analytical laboratory.

9.3.3 An emergency shower and eye wash station are located in the metals prep lab.

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10.0 INTERFERENCES

10.1 Samples must not consume more than 10% of the H_2SO_4 during the digestion. The buffer will accommodate a range of 4.5 - 5.0% (v/v) H_2SO_4 in the diluted digestion sample with no change in signal intensity.

10.2 High nitrate concentrations (10X or more than the TKN level) result in low TKN values. If interference is suspected, samples should be diluted and reanalyzed.

10.3 Digestates must be free of turbidity. Boiling chips have been shown to crumble upon vigorous vortexing. Shake the digestates well and centrifuge before analyzing.

10.4 Silica forms a pale blue complex which also absorbs at 880nm. This interference is generally insignificant as a silica concentration of approximately 4000ppm would be required to produce a 1ppm positive error in orthophosphate.

10.5 Glassware contamination is a problem for Kjeldahl digestions. Do not allow the digestion tube to sit for any length of time with the digestion residue in the tube. Rinse the tubes immediately after use (clean with a brush if necessary) and then either soak in 1:1 HCl or fire in the kiln at 400 ºC. Coordinate firing of glassware with Organic department personnel.

11.0 PROCEDURES

11.1 SAMPLE DIGESTION

11.1.1 Clean glassware before use. Either soak in 1:1 HCl for at least 30 minutes or fire in the kiln at 400 ºC. Rinse all clean glassware thoroughly with DI water before use.

11.1.2 <u>All client samples, QC samples, and calibration standards must be digested.</u>

11.1.3 In a 100 mL digestion tube, add 20 mL of sample or standard plus **4 mL** of digestion solution. The diluent used for PBS, LRB, LFB and dilutions is the 0.2% H_2SO_4 solution. (dilutions are performed using calibrated mechanical pipettes and 0.2% H_2SO_4 as the diluent)

11.1.4 For soil and sludge use 0.1 –1.0 g of sample (0.1g for dry soils and 1.0 g for "watery" soil or sludge). Add 20mL of 0.2% H_2SO_4 to the tube with the soil plus 4 mL of the digestion solution. Record mass of sample by opening LIMS and 232 Key on the balance computer located in the cyanide prep lab. Open Workgroup Review in LIMS, enter workgroup number and select sample to be recorded. Click “Start” in 232 Key and weigh sample. Hit “transfer” on the balance when mass of smaple stops fluctuating to transcribe into LIMS. Once completed, print the final benchsheet with all masses recorded and include with final prep data package. Print another copy of the completed benchsheet to include with the samples.

NOTE: The "wet" sample should be used for the digestion when possible. Organic and Kjeldahl nitrogens determinations on dried sludge and soil samples are not accurate, because drying results in the loss of ammonium salts.

11.1.5 For each client sample, verify that the sample Log-in number on the workgroup matches the sample number on the container. This ensures that samples are not inadvertently switched when they are poured off.

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11.1.6 Prepare spike(s) and LFB by pipetting 0.5 mL 100 mg/L P + TKN combination calibration and spike stock intermediate into the digestion tubes.

11.1.7 Mix with vortexer.

11.1.8 Add 2 - 4 boiling chips to each tube. This step is VERY important to avoid sample from "bumping" or violently boiling over.

11.1.9 Turn the switch on the hot block on, then place the samples in a metal rack into the hot block. Place the side panels on the metal rack to help hold more heat, and press green button on the control panel, followed by the up arrow (^) twice, and finally the green button again to start the heating program. This will hold the temperature at 160°C for one and a half hours and then ramp the hot block up to 380°C. The samples must be kept at 380°C for thirty minutes. The hot block set points are verified by the QA/QC department quarterly and may be changed to ensure the heating profile remains consistent with the method.

NOTE: To perform a calibration study for the TKN hot block refer to SOPAD013

11.1.10 Remove the samples from the block digester and allow cooling for ~ 8 to 10 minutes.

Add ~10 mL of Type I H_2O to each digestion tube and vortex for ~ 15 seconds. Transfer digestate to a labeled 50 mL centrifuge tube.

11.1.11 Record centrifuge tube lot # on prep review checklist. Add an additional ~ 5 mL of Type I H_2O and vortex for ~ 15 seconds. Again transfer digestate to the labeled centrifuge tube. QS to a final volume of 20 mL with Type I H_2O.

NOTE: If the samples sit longer than 10 minutes prior to QS they must be vortexed longer (45-60 seconds per sample) to ensure proper mixing. If the boiling chips are stuck to the base of the digestion tube, add a few more fresh boiling chips to the tube to dislodge the stuck ones. The longer the digestates sit after the digestion has been completed, the higher the tendency for the boiling chips to stick to the digestion tube. Samples can also be placed back in the block digester briefly; however, the temperature cannot be too high, and samples cannot be allowed to bump or boil over.

11.1.12 Transfer each digestate one at time to a labeled 50 mL centrifuge tube and cap the tube.

NOTE: If any final digestate volume is greater than 20 mL then update the sample status to redo and re-prep sample.

11.1.13 Shake tubes well. Store the digestates in the north inorganic walk-in cooler until analysis.

11.1.14 If glassware will not be cleaned immediately and filled with 1:1 HCl, fill tubes partially or fully with DI water to prevent boiling chip and sediment residue from sticking to glassware.

11.2 MANUAL DATA ENTRY INTO LIMS

11.2.1 Enter prep data into LIMS for digested samples**.**

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11.2.2 Print two copies of final bench sheet. Include one copy with workgroup data packet and place the other with the prepped samples in the walk-in cooler.

11.2.3 Review the workgroup in LIMS (AREV), Complete a data review checklist (FRMWC022).

11.2.4 Attach all raw data, the data review checklist, and a current standard/reagent form to the bench sheet, and turn in the data package for SREV.

11.3 **INSTRUMENT SET-UP**

NOTE: All dilutions are manually done with calibrated mechanical pipettes, using a digest blank sample as the diluents.

11.3.1 **TKN Analysis Setup**

NOTE: Digestates may be analyzed if they sit for at least 12 hours prior to analysis and are not turbid, or digestates can be shaken and centrifuged at 3000 rpm for 5 minutes prior to analysis.

11.3.1.1 Turn on the instrument and start up the computer and then the Lachat software by clicking on the Omnion 3.0 icon.

11.3.1.2 Refer to Figure 18. 1 for TKN set up. Connect the sample loop (50 cm) to positions 1 and 4 in the six port valve on the FIA (Figure 18.1). Insert the 660 nm interference filter in the FIA (refer FIA 8000 series manual).

11.3.1.3 Check the date the manifold was last inspected. If more than one month has passed since the last inspection, then visually check and verify that all connectors and unions are clean; replace dirty o-rings, pump tube adaptors, and tee fittings; inspect reagent tubing for wear. If the reagent tubing appears flat when twisted then replace the reagent tubing with new tubing. Record inspection and replacements in the logbook. Write the date of inspection and initials on the manifold. **NOTE:** Blue tabbed buffer tubing has a tendency to wear out quickly due to large diameter of tube.

11.3.1.4 Connect the manifold to the unit.

1) Remove the tube from the tee position marked “from valve” (the furthermost left connection on the manifold).

2) Connect this tube to position 2 on the six port valve.

3) Connect the tube from position 3 on the six port valve to the tee position marked “from valve.”

4) Connect the 650 cm heater tubing to the manifold (Refer to Figure 18.1).

11.3.1.5 Fit the pump tubes carefully in the platons, making sure that the tabs are placed in the slots on either side to prevent tubes from twisting.

11.3.1.6 Place pump tubes in Type I H_2O and run H_2O through the manifold for several minutes.

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11.3.1.7 **Connect the FIA and sample rinse waste lines to the mercury waste container. Open the fume cabinet and remove the small cap from the mercury waste container. Place the mercury waste line and sample waste line into the mercury waste container.**

11.3.1.8 Collect all reagents from storage location(s).

11.3.1.9 Stop the peristaltic pump and place reagent tubing in the appropriate reagents **except the salicylate nitroprusside.** Keep this pump tube in H_2O.

11.3.1.10 Turn pump on and continue pumping all other reagents for ~ 5 minutes. Check manifold to ensure reagents are flowing smoothly and there is no back flow.

11.3.1.11 Stop the peristaltic pump and place the salicylate nitroprusside pump tube in the reagent. Turn pump on and continue pumping for ~ 5 minutes. Again, check manifold to ensure reagents are flowing smoothly and no back flow occurring.

11.3.1.12 Continue to §11.4.

11.3.2 **P-T SOIL ANALYSIS SETUP**

NOTE: Digestates may be analyzed if they sit for at least 12 hours prior to analysis and are not turbid, or digestates can be shaken and centrifuged at 3000 rpm for 5 minutes prior to analysis.

11.3.2.1 Turn on the instrument and start up the computer and then the Lachat software (**Omnion**) by clicking on the Omnion 3.0 icon.

11.3.2.2 Refer to Figure 18. 2 for P-T Soils setup. Connect the P-T Soils sample loop (30.5 cm) to positions 1 and 4 in the six port valve on the FIA (Figure 18.2). Insert the 880 nm interference filter in the FIA (refer FIA 8000 series manual).

11.3.2.3 Check the date the manifold was last inspected. If more than one month has passed since the last inspection, then visually check and verify that all connectors and unions are clean; replace dirty o-rings, pump tube adaptors, and tee fittings; inspect reagent tubing for wear. If the reagent tubing appears flat when twisted then replace the reagent tubing with new tubing. Record inspection and replacements in the logbook. Write the date of inspection and initials on the manifold.

11.3.2.4 Connect the manifold to the unit.

1) Remove the tube from the tee position marked “from valve” (the furthermost left connection on the manifold).

2) Connect this tube to position 2 on the six port valve.

3) Connect the tube from position 3 on the six port valve to the tee position marked “from valve.”

4) Connect the 175 cm heater tubing to the manifold (Refer to Figure 18.2).

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11.3.2.5 Fit the pump tubes carefully in the platons, making sure that the tabs are placed in the slots on either side to prevent the tubes from twisting.

11.3.2.6 Place pump tubes in Type I H_2O and run H_2O through the manifold for several minutes.

11.3.2.7 **Connect the FIA and sample rinse waste lines to the mercury waste container. Open the fume cabinet and remove the small cap from the mercury waste container. Place the mercury waste line and sample waste line into the mercury waste container.**

11.3.2.8 Collect all reagents from the storage cabinet in the instrument lab.

11.3.2.9 Stop the peristaltic pump and place reagent tubing in the appropriate reagents. Turn pump on and continue pumping all other reagents for ~ 5 minutes. Check manifold to ensure reagents are following smoothly and no back flow occurs.

11.3.2.10 Continue to §11.4.

11.4 **IMPORTING WORKGROUP**

11.4.1 In the Omnion software under the file heading "Run" click on "Open." Choose "ACZ Methods" and then "TKN" or "Phos."

11.4.2 Open appropriate template: "TKN template," "TKN Soils Template," "TKN Blank Template," "P-T Soils," or "P-T Soils Blank Template."

11.4.3 The Auto Data Quality Management (DQM) should already be set up in the existing template. To modify, highlight the DQM rows and right click on the mouse. Select "Clear DQM Set," "yes." Modifications can then be made to the DQM in the "properties box" to the right. Once changed, set the DQM by highlighting the DQM rows, right click and select "Define DQM set." For ICV/ICB set choose "once" under "Scheduling Options." For CCV/CCB set choose "After every N samples" and type in 10 in Enter # box and "enter." Make sure "End of Run" box is checked to automatically insert a CCV and CCB after every 10 samples and at the end of the run.

11.4.4 **For FIA1/2:** Omnion will display the following prompt "Do you want to change the set points of the relevant heater," select "yes" and this starts the heater.

11.4.5 Open "TKN or P-T Soils Blank Template."

11.4.6 To import the workgroup into the blank template, go under "Run" and choose "Import Worksheet Data." Cut and paste samples into other template.

11.4.7 The templates begin with a calibration followed by ICV and ICB measurement before analyzing the workgroup samples. A CCV and CCB are measured after every ten samples and at the end of each run. This procedure allows the use of a continuing calibration, whereby a calibration curve generated in a previous sample batch is used for subsequent batch analyses so long as instrument QC results remain acceptable. Two consecutive attempts are allowed for a continuing calibration before the instrument must be recalibrated.

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NOTE: If the run uses a continuing calibration, begin the sequence with a CCV. The data set containing the valid calibration curve must be identified by its workgroup # on the data review checklist. The DQM template can be modified by deleting the blue calibration section and the green ICV and ICB DQM sections. The raw data and workgroup bench sheet must indicate which verification standard was used.

11.4.8 Review the workgroup to check for any dilutions changed during prep. Any changes must be reflected on the tray in the Lachat software. In the "Manual Dilution" column find the correct row where the sample is located and type in the correct dilution factor in the MDF column and press "enter." Document the dilution factor on the workgroup sheet and include reason for dilution (reason is not needed if dilution was performed at prep).

11.5 **Sample Organization & Analysis Set-Up**

NOTE: The Autodilutor is not used for TKN or P-T Soil analyses. The carrier is very strong and viscous and is extremely difficult to flush from the auto dilutor. Contamination results in other methods analyzed on the instrument. All dilutions must be prepared manually. Manual dilutions are prepared by using mechanical pipettes and a digested blank sample.

11.5.1 Pour off the samples according to the order listed on the workgroup bench sheet. If desired, print a copy of the samples and their corresponding cup numbers, go to the "Run" heading and choose "export worksheet data."

11.5.2 Set up the cup tray with the samples in their correct locations. Continually check the sample sheet to verify that samples are placed in the correct cup number.

11.5.3 Review current settings in the "Run Properties Display" box click on "Analytes" tab, Analyte name under Channel #. This will display the analyte properties in the box on the right. Make sure "Calibration Fit Type" is first order (if not then select "first order") this will apply a first order (linear) polynomial equation to fit the calibration curve generated. Make sure the weighting is set to "none" (if not then select "none").

11.5.4 Start the analysis by clicking the green **Start** button.

11.6 **Calibration Verification**

Calibrate the instrument by analyzing the standards in order of decreasing concentration, ending with the blank. Check the calibration under the calibration icon. "R" must be ≥ 0.995. If not then try again. Several factors that can lead to a failing calibration. If none of the following apply then remake the standards. Refer also to §11.8.

- Are the correct standards in their corresponding cups?
- Is the autosampler sampling the correct cups?
- Is the tray setup properly for the calibration?
- Are the sample tubes flowing correctly?
- Is the heater set at the correct temperature and functioning properly?
- Reagent contamination?

11.7 **Failing QC**

If QC fails during the analysis then steps must be taken to ensure the data is valid. If $r \geq 0.995$ for the calibration and the ICV or ICB fails (or first CCV or CCB for continuing calibration), then the

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QC pair may be retested once. If the retest fails, then the instrument must be recalibrated and the analysis restarted. If succeeding CCVs or CCBs fail then it is up to the analyst to decide whether to stop the run and run an "R" group or add the associated samples to the end of the tray by cutting, pasting the updated workgroup.

11.8 **Troubleshooting**

Indications of impaired analytical capability should be inspected immediately and resolved prior to continuing analysis. Effective troubleshooting employs a logical, systematic approach. Common problems encountered with this procedure include the following. If problems continue after going through these possible scenarios call LACHAT at (800) 247-7613. **Any general maintenance, troubleshooting, repairs, calls to Lachat technical support, etc. must be documented in the instrument and/or maintenance logbook(s).**

11.8.1 If precipitate starts to form in TKN manifold lines- Stop pump, place all reagent lines in 0.25 N NaOH and turn pump on, visually check to see if precipitate is dissolving in the lines. If precipitate is no longer visible in lines, pump type I H_2O through manifold for approximately 5 minutes then begin pumping reagents through the lines following §11.3.1.9 through §11.3.1.12. If precipitate remains in the lines then replace all tubing in manifold and all manifold connectors with new ones before continuing with the analysis.
11.8.2 Are the platons crimped down on the pump tubes?
11.8.3 Is the peristaltic pump turned on?
11.8.4 Is the sample tray sitting correctly on the autosampler tray?
11.8.5 Are the standards placed correctly on the auto sampler rack (labeled 1-16)?
11.8.6 Are leaks occurring anywhere on the manifold?
11.8.7 Are there cracks or breaks in the manifold tubing?
11.8.8 Do the pump tubes need to be changed?
11.8.9 Is there a sufficient amount of rinse water in the carboy for the auto sampler?
11.8.10 Is the drain carboy for the auto sampler full and needing to be emptied?
11.8.11 Is the heating module set to the correct temperature for the analyte?
11.8.12 Is the correct manifold and filter being used for the analyte?
11.8.13 Does the analyte method require a backpressure loop?
11.8.14 If contamination shows up in non-prepped blanks, check the auto rinse carboy.
11.8.15 If contamination in prepped blanks, check digestion glassware and reagents.
11.8.16 Are the pump tubes in the correct reagents?
11.8.17 Is the correct "Method, DQM, and Channel" defined within the software?
11.8.18 Are clogs anywhere on the manifold or in pump tubes?
11.8.19 Are the drain lines crimped or plugged?
11.8.20 If the baseline is reading perfectly at "0", then the computer is not communicating with the instrument and it must be restarted.
11.8.21 If the software is incorrectly marking peaks then it is probable that the valve timing, threshold and or inject to peak start need to be changed (these can be found within the Analyte table).
11.8.22 If there is no reading, check the bulb and channel.
11.8.23 If the baseline becomes jumpy (huge peaks or just very noisy), check for air bubbles passing through the manifold, check to see that the flow cell is properly set within the detector.
11.8.24 Noisy baselines can also attribute to a bad detector, bad fluidics, etc. If the detector is suspected, exchange with another upon the instrument. Fluidics could be pinpointed to pump tubes, clogs, or a degenerating pump.
11.8.25 If the manifold tubing around the 60 °C heater is discolored orange a 10% percent HCL solution can be pumped through the manifold to clean the stained tubing.

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11.9 **"R" Group**

If any non-conformance occurs and the final CCV/CCB has been sampled then an "R" group must be run. An "R" group includes instrument QC (opening CCV/CCB, and CCV/CCB every 10 samples and end of run). Create an "R" group by editing the current tray delete the calibration and ICV/ICB DQM sets. Deleting any samples that won't be included in the R group and only the desired samples remain and the tray. Save the run by adding an R to the end (i.e. save WG145889 as WG145889R) and start the run by clicking the green arrow.

11.10 **Run Completion and Data Export**

11.10.1 After the analysis has ended save the data file by selecting "Run" (upper left hand corner of display) "Save File As," type the workgroup number for the file name. Print a copy of the data by clicking on the "Tools," "Custom Report," and printer icon. Close the run file by selecting "Run" and "Close."

11.10.2 Reopen the file and click on the "Run" tab in the "Run Properties" display box. Click on "Export Data to File" box.

11.10.3 Under "Tools" choose "Custom Report." Click on the arrows to ensure all required information is included. Make sure all samples appear on the chromatogram. To alter the format go under "Report" and choose "Format." The # of peaks per chart can be changed under "Charts." To zoom in or out on the chromatogram, alter the view under the channel view on the main page. This will change the view in the Custom Report.

11.10.4 If the workgroup is ready for review, send it directly to U:\Waitlod\FIA1 or … \FIA2 then copy workgroup into U:\Autoload\FIA1 or …\FIA2 directory.

11.10.5 If a workgroup needs additional editing ("R" group, dilution factors must be entered, or soil data need to be entered) then send the workgroup to U:\Waitload\FIA1 or …\FIA2.

11.10.6 If the file does not export check the software export to the Waitload directory. Click on "Configuration," "Options," "Data Export," and "Data Items." Verify data is being exported to either U:\Autoload\FIA1 or U:\Waitload\FIA2. If not then change the directory. Click the "Ominion 2.0 format," "Accept" "Ominion 2.0 format" and "Close." Then try exporting the file again.

11.10.7 If the workgroup was sent to Waitload, go to the data drive on the network and locate U:\Waitload\FIA1 or U:\Waitload\FIA2.

11.10.7.1 Find the appropriate workgroup and the "R" group, if applicable.

11.10.7.2 Double click on the correct file to open and confirm the correct dilution factors will upload (change if necessary).

11.10.7.3 If an "R" group was analyzed, copy the data from the "R" group, open the original workgroup and paste the "R" group data to the end of the original workgroup data.

11.10.7.4 Save the file and send the workgroup to U:\Autoload\FIA1 or U:\Autoload\FIA2.

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11.10.8 Review the workgroup in LIMS (AREV), completing a data review checklist (FRMWC006). Attach all raw data, the data review form/checklist, and a current standard/reagent form to the bench sheet, and turn the data in for secondary review (SREV).

11.10.9 Record workgroup analysis information in the Lachat instrument logbook.

11.11 **Lachat Cleaning Procedure**

11.11.1 Turn off pump and remove salicylate nitroprusside reagent tube and place into Type I water. Turn on pump and let reagents and Type I water pump through manifold for approximately 5 minutes. Then take remaining pump tubes out of reagents and place them in Type I water. Allow the instrument to pump water through for at least 5 minutes. Take pump tubes out of water and allow air to pump through the entire manifold. After air has passed completely through the manifold, unclamp the platons. Reseal all reagents and return them to proper storage location(s).

11.11.2 **Open the fume cabinet and remove the mercury waste lines from the mercury l waste container and place tubes in secondary container in case of any dripping. Replace cap on mercury waste container.**

11.11.3 Routine maintenance must be performed weekly, monthly, and semiannually as recommended by LACHAT FIA User Manual. Refer to the manual for maintenance procedures. Record routine maintenance in instrument maintenance logs.

12.0 QUALITY CONTROL

To ensure data validity and quality, a series of QC samples are analyzed with each analytical run. These are required by the methods published by EPA and by additional regulatory agencies. QC types and associated limits are outlined in Table 12.1 along with the required corrective action to take if the QC fails.

12.1 Calibration Standards. The instrument must be calibrated at the beginning of each working day using at least three standards and one calibration blank. The curve must be linear and $r \geq 0.995$.

12.2 Initial Calibration Verification Standard (ICV). A solution of analyte(s) analyzed immediately after the calibration of the instrument and prepared from a source independent of the calibration standards, preferably purchased from a different manufacturer. The ICV is equivalent to the QCS.

12.3 Initial Calibration Blank (ICB). This solution is the same as the calibration blank and is used to confirm that no background contamination is present in the calibration blank.

12.4 Continuing Calibration Verification Standard (CCV). A solution of method analyte(s) of known concentration(s) used to confirm the continued calibration of the instrument. The CCV is equivalent to the IPC.

12.5 Continuing Calibration Blank (CCB). This is the same solution as the calibration blank. It is intended to detect baseline drift in the calibration of the instrument

12.6 Laboratory Reagent Blank or Preparation Blank Soil (LRB or PBS). This is a reagent blank carried through the entire sample preparation scheme with the samples. It is intended to confirm the absence of contamination in the prep stages.

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12.7 Laboratory Fortified Blank (LFB). This solution is the same as the calibration blank, but has been spiked with a known concentration of the pertinent analytes. The LFB is treated exactly like a sample and confirms that the spiking procedure is accurate.

12.8 Matrix Spike (MS). A solid sample spiked with a known amount of analyte and carried through the sample preparation steps along with the samples. The spike is intended to confirm the appropriateness of the sample prep scheme and to estimate the accuracy of the method.

12.9 Laboratory Fortified Matrix (LFM). An aliquot of aqueous client sample spiked with a known concentration of method analyte. Analysis of the LFM is intended to demonstrate the absence or presence of interference in the sample matrix.

12.10 Sample Duplicate (DUP). A duplicate aliquot of sample processed and analyzed in the same manner as all client samples. The duplicate is designed to demonstrate the precision of the method.

12.11 Relative Percent Difference (RPD). The difference between two replicates (a sample and its duplicate) divided by the average of those two replicates and then multiplied by 100.

12.12 MDL (Method Detection Limit) –The minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero.

12.13 PQL-Practical Quantitation Limit— The lowest level that can be reliably achieved within methods specified limits during routine operations.

Table 12.1: QC Frequency, Limits, and Corrective Action

Standard	Frequency	Limits	Corrective action if QC fails
Calibration	First workgroup each day	$r \geq 0.995$	Recalibrate. If r will not pass then REDO all.
ICV	After calibration	90-110%	1 retest OK then recalibrate. Redo all samples associated with a failed ICV.
CCV	After 10 samples and end	90-110%	Re-analyze all associated samples. 1 retest OK then recalibrate if continuing calibration.
ICB	After ICV	3X - MDL ≤ sx < MDL	1 retest OK then recalibrate. REDO all samples < 10X ICB value.
CCB	After CCV	3X - MDL ≤ sx < MDL	Re-analyze associated samples. Client samples < MDL can be accepted and reported with the appropriate qualifier if bracketing CCB fails high.
LFB (H_2O)	One for every 20 samples	90-110%	1 retest OK then re-prep and reanalyze all samples.
LFB (soils)	One for every 20 samples	85-115%	1 retest OK then re-prep and reanalyze all samples.
LFM (H_2O)	One every 10 samples	90-110%	1 retest OK then re-prep and reanalyze all samples.
MS (soils)*	One every 20 samples	75-125%	If instrument QC passes, accept and qualify data.

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DUP	One every 10 (aqueous) or one every (20 soil)	RPD ≤20	Same as for H_2O. 1 retest OK then REDO associated samples (H_2O) if [sx] > 10x MDL. Flag data for soils.
LRB (H_2O)	One every 20 samples	3X - MDL ≤ sx < MDL	REDO all samples < 10X the LRB. Client samples < MDL can be accepted and reported with the appropriate qualifier if the LRB fails high.
PBS (soils)	One every 20 samples	3X - MDL ≤ sx < MDL	REDO all samples < 10X the PBS. Client samples < MDL can be accepted and reported with the appropriate qualifier if the PBS fails high.

*For solid analyses, the spike recovery must be manually calculated and compared to the LIMS spike recovery. See §13.9 for the recovery equation and acceptance criteria. Document this activity on the benchsheet.

13.0 CALCULATIONS, DATA REPORTING & ARCHIVING

13.1 *Linear Least Square Regression Calibration Model With No Curve Weighting*

$y=mx+b$

where:

m=slope
x= peak area (Vs), A_x
b=y-intercept

Slope (m)

$$m = \frac{\left(\sum_i^n w_i x_i y_i \times \sum_i^n w_i\right) - \left(\sum_i^n w_i x_i \times \sum_i^n w_i y_i\right)}{\left(\sum_i^n w_i \times \sum_i^n w_i {x_i}^2\right) - \left(\sum_i^n w_i x_i\right)^2}$$

where:
w_i = weighting factor (for non-weighted calibration curves wi=1for all standards)
x_i= peak area for the i^{th}calibration standard
y_i=expected concentration for the i^{th} calibration standard
i→n = calibration points in order of increasing concentration

y-intercept (b)

$$b = \frac{\left(\sum_i^n w_i {x_i}^2 \times \sum_i^n w_i y_i\right) - \left(\sum_i^n w_i x_i \times \sum_i^n w_i x_i y_i\right)}{\left(\sum_i^n w_i \times \sum_i^n w_i {x_i}^2\right) - \left(\sum_i^n w_i x_i\right)^2}$$

Calibration Coefficient (R)

$$R = \frac{\left(\sum_i^n w_i \times \sum_i^n w_i x_i y_i\right) - \left(\sum_i^n w_i x_i \times \sum_i^n w_i y_i\right)}{\sqrt{\left(\left(\sum_i^n w_i \times \sum_i^n w_i {x_i}^2\right) - \left(\sum_i^n w_i x_i\right)^2\right) \times \left(\left(\sum_i^n w_i \times \sum_i^n w_i {y_i}^2\right) - \left(\sum_i^n w_i y_i\right)^2\right)}}$$

Note: FRMQA049 may be used to calculated slope, intercept, calibration coefficient and concentrations. Use the worksheet titled "ESTD LIN EW 10". Lachat software calculated the linear equation from plotting the sample concentration, C_x versus peak area A_x. Enter the calibration levels, C1 through C5 in fields A20 through A24. Enter peak areas measured for each standard in fields C20 through C24. Enter standard concentrations in fields B20 through B24. Make sure all weighting factors are set to 1 (fields D20 through D24). The calculated linear calibration curve values are displayed in fields D30 (slope), D31 (y-intercept), D32 (correlation coefficient). Sample concentrations can be calculated in the lower table by entering sample IDs starting with column D45 and computed using equation y=mx+b.

13.2 Samples exceeding the highest standard must be diluted and reanalyzed. Dilute high samples using a digested blank standard. LIMS will automatically qualify all data with a concentration less than the MDL ("U") or less than the PQL ("B").

13.3 Samples analyzed on a dilution that upload with a "U" or "B" qualifier must be reanalyzed on a smaller dilution unless the dilution is necessitated by the sample matrix or volume. The reason for all dilutions must be documented on the bench sheet.

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13.4 Report results in mg/L (water) or % mass (soil/sludge).

13.5 The MDL for TKN (water) is 0.2 mg/L and the PQL is 0.5 mg/L. Reported detection and quantitation limits for soils depend on dilution factors. The MDL for TKN and P-T (solid matrices) is 0.1 percent and the PQL is 0.5percent.

13.6 Relative Percent Difference (RPD) is determined using the following equation:

$$RPD = \frac{(S - D)}{[(S + D) / 2]} \times 100$$ Where: S = Sample Value and D = Duplicate Value

13.7 Equation for calculating the prep dilution factor for soil/sludge samples:

$$Dilution = \frac{TV}{(w)(\%solids)(10000)}$$

Where: TV = total volume (must be equal to the initial volume of standard used before prep)
w = sample weight in grams
solids = %solid of the sample expressed as a decimal **
10000 = Conversion factor (mg/L to %mass) and 1g/kg = 0.1 percent.

NOTE: If the sample is a sludge (unprepped by soils), then use the total solids value from seedpak. If the sample used is a soil (prepped by the soils dept.) then use the air dry value available from the soils dept. If the soils sample is unprepped by the soils dept., use the percent solids value. **Make sure to use the proper dry weight value for the type of sample used.**

13.8 $$\%\text{recovery} = \frac{\text{observed concentration}}{\text{theoretical concentration}} \times 100$$

13.9 Solid spike recovery:
%recovery for TKN or Phosphorus soils MS =

$$\%Recovery\ for\ TKN\ or\ Phos\ soils\ MS = \left(\frac{MS - \left(sx \times \left(\frac{sx\ DF}{MS\ DF} \right) \right)}{MS\ TV} \right) \times 100$$

Where:
MS = instrument matrix spike value (no DF's applied)
sx = instrument sample value (no DF's applied)
sx DF = final sx dilution factor (prep dilution x instrument dilution)
MS DF = final matrix spike dilution factor (prep dilution x instrument dilution)
MS TV = True value of matrix spike as entered in LIMS by SCN (i.e. spike TV adjusted for post-spike dilution; typically 2.5 mg/L ÷ Instrument DF)

NOTE: This equation has been simplified. $sx \times \frac{sx\ DF}{MS\ DF}$ portion of equation is necessary to adjust subtracted [sx] when weight of sample used differs between sx & MS.

NOTE: %recoveries for solid matrices must be hand calculated using the above equation.

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(This equation has not yet been implemented in ACZ's LIMS.) If the LIMS recovery differs by more than 5% from the manually calculated recovery, or if the difference moves the recovery into or out of acceptance limits, the spike recovery must be updated to the manual value in SEEDPAK or ATTACHED TABLES.

13.10 Retrieval of archived data files:

13.10.1 Go to Labweb.
13.10.2 Change the internet address to 'http;//saloon'.
13.10.3 Log onto the internet by typing user name and password.
13.10.4 Click on 'Instrument', 'FIA1' or 'FIA2', 'Program Files', 'Lachat', 'Ominon', 'Data', 'ACZ Methods', 'N-TK'.
13.10.5 Search directory for desired workgroup to retrieve.
13.10.6 Right click on workgroup file name (WGnumber), select 'Save Target As…' and save file to local desk top computer in C;//Program File/Lachat/Ominon/Data/ACZ Methods/ N-TK directory.
13.10.7 Click 'Close' after saving file.
13.10.8 Open Ominon software and open the saved archive file in the N-TK directory.

14.0 METHOD PERFORMANCE/ DETECTION LIMITS

14.1 Method Detection Limit: A MDL study is required for water only and must be performed for initial method development and whenever, in the judgment of the analyst, a change in analytical performance caused by either a change in instrument hardware or operating conditions would dictate the MDL be re-determined.

Spike at least seven (7) aliquots of reagent blank at 2-5x MDL (at the PQL is preferred) and process as described in this SOP over the course of three separate days. Additionally, prepare and analyze seven (7) aliquots of a reagent blank, with no spike added, over the course of three separate days. Include a passing calibration, ICV/ICB, CCV/CCB. Enter the data into form FRMAD031 in LabWeb (non-detect or "0" values cannot be used). Print the form, sign it and submit it to the department supervisor for review. Include raw data and other supporting documentation. Refer to SOPAD001 for more detail.

Quarterly QA MDL Samples: Each quarter, two QA samples will be logged in for prep and analysis. Prepare each sample by spiking reagent blank at the PQL and prepping and analyzing according to the method. These samples need to be prepared and analyzed in separate batches however, they can be brought into workgroups containing other client samples. Refer to SOPAD001 for additional information.

14.2 Demonstration of Capability (DOC): A successful Initial DOC must be completed and approved by the QA/QC department for each analyst prior to independent generation of client data. A Continuing DOC is required annually for each analyst.

Demonstration of Capability: Prepare and analyze four individual aliquots of the ICV. Include a passing calibration, ICV/ICB, and CCV/CCB. Enter all data on form FRMAD023 in LabWeb. Print the completed form, sign it and submit it with all raw data, a current standard/reagent form, and any other supporting documentation to the department supervisor for review. Refer to SOPAD001 for more detail.

14.3 The linear calibration range (LCR) must be determined as part of initial method development and whenever the calibration range is extended.

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Calibrate the instrument. R must be ≥ 0.995 or recalibrate before proceeding. Analyze each calibration standard as a sample, bracketing them with a passing ICV/ICB and CCV/CCB. Compare the observed concentration of each to the actual concentration. The values must agree within +/- 10%. If this is not the case, determine the cause of the discrepancy and perform another study. Enter results on FRMQA029; attach all raw data and supporting documentation; and turn in to the department supervisor for review. Refer to SOPAD013 for instructions on extending the calibration range.

NOTE: EPA351.2 requires the LCR to be verified every 6 months; however, Jim O'Dell of the EPA confirmed that the intent of the LCR is to establish the upper limit of the calibration range. ACZ's procedure requires daily instrument calibration and dilution of samples with a concentration greater than the high calibration standard; therefore, verification of the LCR every 6 months does not provide a quality control measurement pertinent to laboratory activities and is not required.

15.0 DOCUMENTATION

15.1 Record the pertinent information for all prepared standards and reagents in LIMS or the Wet Chem Standards/Reagent Logbook. Include analyst's initials, prep date, expiration date, and the PCN or SCN of any stock standard or reagent used.

15.1.1 For working standards, documentation of preparation of C2 (CCV) shall indicate that all calibration standards were prepared in tandem.

15.2 The event of digesting the standards must be recorded in the Working Calibration Standards Logbook, along with the analyst's initials, date, SCNs/PCNs of reagents used in digestion, and expiration date. The shelf-life of digested standard is one month or expiration date of intermediate, whichever comes first.

15.3 Document all pertinent information regarding the analysis in the instrument logbook.

15.4 Label each standard or reagent prepared in §8.0 with the following information:

- standard name
- SCN (or other unique ID)
- preparer's initials
- expiration date
- prep date

15.5 Make sure the following information is included on the Workgroup:

- Instrument ID
- Dilution factors.
- Analysis date, start time and completion time.
- Lot # of disposable vessels
- Analyst's initials.
- Any remarks about analysis or samples.
- Calibration Workgroup (if using a continuing calibration).
- Reagent Sheet.
- Standard Sheet.

15.6 Attach a completed data review form and a current standard/reagent form to each workgroup.

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16.0 WASTE MANAGEMENT/POLLUTION PREVENTION

16.1 Auto-sampler rinse, as well as the reagent waste, are collected in receptacles next to the instrument. They should be emptied into a sink connected to the neutralization tank.

16.2 Waste from the instrument is collected as hazardous waste. See ACZ's CHO for disposal.

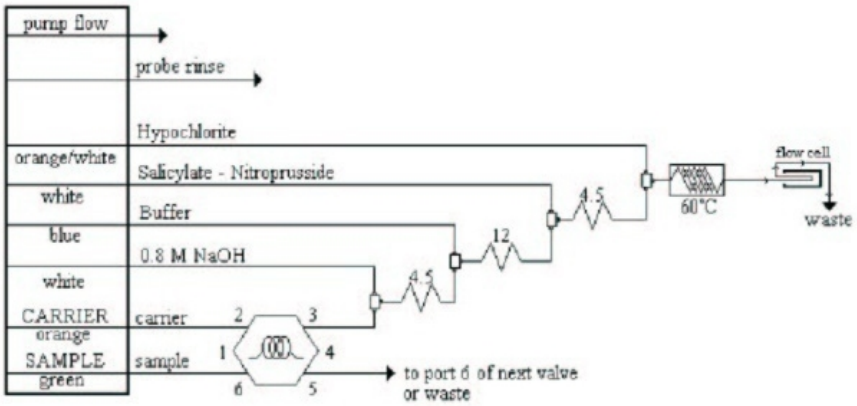
16.3 Refer to ACZ's Waste Management Plan for further disposal details for this method.

17.0 DEFINITIONS

17.1 Refer to §12.0 for QC definitions.

18.0 TABLES & DIAGRAMS

Table 6.1: Hold Times
Table 8.1: Standard Concentrations
Table 8.2: Quality Control Standards
Table 12.1: QC Frequency, Limits, and Corrective Action
Figure 18.1: N/TKN-Soils Manifold Diagram
Figure 18.2: P-T Soils Manifold Diagram



Carrier: Diluent (Reagent 7)
Manifold Tubing: 0.8 mm (0.032 in) i.d. This is 5.2µL/cm.
AE Sample Loop: 44.5 cm
QC8000 Sample Loop: 50 cm
Interference Filter: 660 nm

Apparatus: An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required. The  shows 650 cm of tubing wrapped around the heater block at the specified temperature.

4.5: **70** cm of tubing on a 4.5 cm coil support
12: **255** cm of tubing on a 12 cm coil support

Note 1: PVC PUMP TUBES MUST BE USED FOR THIS METHOD

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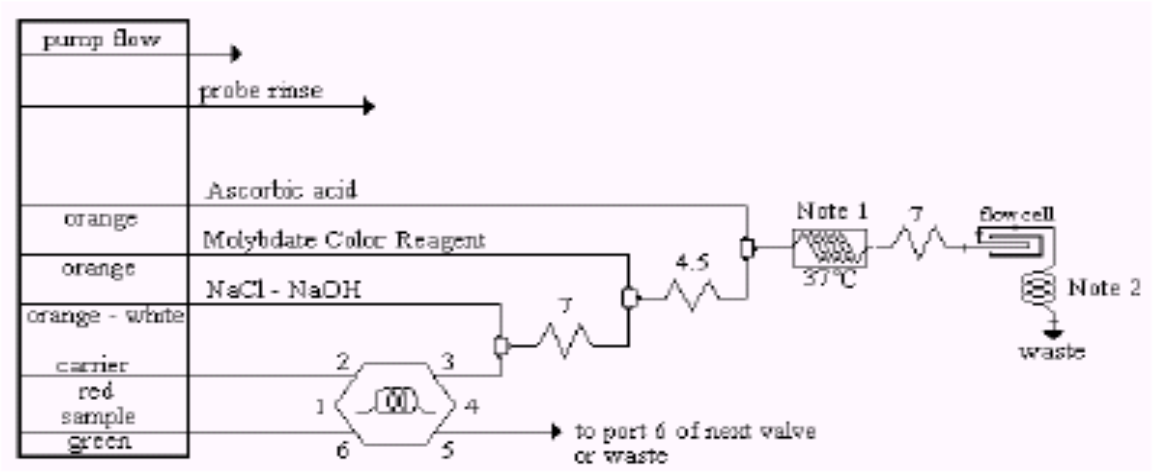
Figure 18.2: P-T Soils Manifold Diagram



Carrier: **Reagent 9**
Manifold Tubing: **0.8 mm (0.032 in) i.d.** This is **5.2 µL/cm**.
AE Sample Loop: 25 cm
QC8000 Sample Loop: 30.5 cm
Interference Filter: 880 nm

Apparatus: An injection valve, a 10 mm path length flow cell , and a colorimetric detector module is required.

4.5: **70** cm of tubing on a 4.5 cm coil support
7: **135** cm of tubing on a 7 cm coil support

Note 1: 175 cm of tubing on the heater.
Note 2: 200 cm restrictor coil, 0.52 mm (0.022 in.) i.d.

19.0 CORRECTIVE ACTION

19.1 For QC samples that do not meet the method acceptance criteria, refer to Table 12.1. For retests that will occur past the method hold time, check first with the department supervisor to determine if the reanalysis should be conducted.

19.2 For any SOP/method deviation fill out section 1 of a corrective action report (FRMQA001). If necessary, the department supervisor and/or project manager may provide additional information in the appropriate sections; however, QA/QC does not need to close a minor corrective action. Attach a copy of the minor corrective action report to all workgroups affected. A minor corrective action is for documentation purposes –any SOP or method deviation may be noted on the data review checklist or on the workgroup bench sheet in lieu of using FRMQA001.

19.3 For any system failure a major corrective must be opened and the problem investigated. A department supervisor or the QA/QC Officer can open a major corrective action. The corrective action will be assigned a unique tracking number by the QA/QC Officer and will be closed by the QA/QC Officer once the failure has been resolved. Use FRMQA001 (in LabWeb).

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