Attachment 2: Standard Operating Procedures (SOPs)

Field Vadose Zone Coring
07_04_02.001
Dates Active:
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1: Field Soil Coring

1 INTRODUCTION

This method describes the coring techniques used to plan, obtain, label, document, and store cores for further analyses. This procedure is used when assisting the DPT and hollow stem auger drilling operator.

2 SCOPE AND APPLICATION

Coring sites are selected based off of a number of criteria. Two cores are to be taken every 5 feet. Two 2.5 foot plastic coring tubes are placed inside a 5 foot core barrel each time the drilling operator drills to a new depth. Once the sample is obtained, the core barrel is opened and the cores are removed. Each core is capped, labeled, and recorded in a field notebook before being stored in a labeled Styrofoam container. Cores are stored in a freezer to preserve field conditions (e.g., moisture content, Nitrogen, and VOC's).

2.1 SAMPLE PRESERVATION

Collected cores should be capped on both ends and preserved in a freezer while in the field and transferred to a freezer once returned from the field.

3 REQUIRED TRAINING

Field workers should be trained on how to keep a field notebook.

4 EQUIPMENT AND MATERIALS

4.1 APPARATUS AND MATERIALS

i. Computer with google earth or GIS
ii. 2.5 foot plastic tubes
iii. Rubber tube caps
iv. Styrofoam coolers
v. Water resistant field notebook
vi. Work gloves
vii. Thick sharpies
viii. Tools and supplies provided by drilling operator:
    1. Drill Rig

2. Hollow-stem augers
3. Core barrels
4. Water level indicator
5. Egg shell stoppers
6. Core holding rack
7. Pipe vice
8. Pipe wrenches
9. Sediment separator
10. Brushes
11. Water bucket
12. Bentonite bore-hole plug
13. GPS unit

4.2 REAGENTS

None

4.3 CHEMICALS

None

5 SAFETY PRECAUTIONS

Use work gloves, hard hat, steel toed boots, and keep a safe distance from the drilling rig.

6 PROTOCOL

6.1 SUBSECTION 1

6.1.1 Site Selection

i. Sites will vary based on project goals and land-owner consent. Examples include:
    1. Areas of high contaminant concentration vs. low concentration
    2. Varied land use
    3. Previously sampled vs. newly sampled

ii. Select sites that are:
    1. Accessible
        a. Agricultural land
        b. Lawns and parks
    2. In low-lying areas with greater leaching potential
    3. Are within the depth constraints of the drilling equipment
    4. Sampled past the rooting zone into the vadose zone, ideally reaching the water table

6.1.2 Field Book Record Keeping

iii. Start a new entry for every site and record the following:

1. Name
2. Site Location
    a. Record latitude/longitude or legal description
3. Date & Time
4. Weather
5. GPS coordinates
6. At what depth visible changes between any sediment horizons occur and
7. Groundwater depth
8. Depth of any missing core intervals

6.1.3 Coring Preparation

iv. Ensure that the two 2.5 foot plastic tubes are aligned with the top and bottom of the open steel core encasement once placed inside.
v. Place top shell of the steel core encasement on top of the loaded bottom shell. To avoid cross threading, ensure the two shells are properly aligned with each other before using the monkey wrenches to assist in screwing on the rear steel cap.
vi. Screw on the core barrel head assembly and then the shoe after securing the core barrel in place with the pipe vice.
    1. **Note:** An egg shell can be placed inside the front steel cap to prevent sample loss when drilling in sandy sediment.

6.1.4 Core Collection and Labeling

vii. The core barrel will be placed on the core holding rack and secured by the pipe vice.
viii. Unscrew the head assembly and the shoe. Clean the thread of any debris with a wet brush.
ix. Lift and gently drop one end of the steel core encasement on the holding rack to open it.
    1. **Note:** if both tubes aren't fully filled with sediment, inform the drilling operator so adjustments can be made.
x. Insert sediment separator tool in-between the two cores to divide them.
xi. Carefully remove the cores and place a rubber tube cap on each side.
xii. Label every core with their respective site location found in the coring locations pdf file. If a site has more than one drilling location, add a "-#" to the end to denote the location (i.e., HC1-1, HC1-2, and HC1-3).
xiii. Label the top cap and highest part of the tube with the depth the sample was taken at. Do the same with the bottom cap and the lowest part of the tube.
xiv. Place the labeled core into a Styrofoam cooler labeled with the site location, date, and range of depths.
xv. Revert back to coring preparation.

6.1.5 Sample Preservation

xvi. Styrofoam coolers should be stored in a freezer as soon as possible to preserve field conditions.

7 QUALITY ASSURANCE

1 Use clean liners and caps to prevent cross contamination

2 Perched water tables can interfere with perceived aquifer depths. If unusually shallow saturated sediments are recovered in the core barrel, drill 5 more feet and attempt to push through the perched table. If sediments are still unsaturated, reassess the situation.
3 If refusal occurs, mark the depth of refusal and discontinue drilling operations.

8 ADDITIONAL INFORMATION

8.1 DEFINITIONS

- ***Core Barrel*** – Encases core liners and prevents sample loss down the bore-hole
- ***DPT*** – Direct Push Technology (e.g., GeoProbe)
- ***GIS*** – Geographical Information Systems used for planning and site selection
- ***Head Assembly***—Head of the core barrel that screws onto the hex rods for sample retrieval
- ***Refusal***—When the drill is unable to penetrate deeper into the sediment
- ***Shoe*** –End of the core barrel where the sample enters the core barrel
- ***VOC's*** – Volatile Organic Compounds

9 PREVIOUS ISSUES AND CHANGES

Document File Name	Issue	Issue Effective Dates	Author
N/A			

10 DIAGRAMS, FIGURES, AND PHOTOGRAPHS

The following pages contain the tables, figures, and photographs referenced by this method.
N/A

2: Soil Core Processing

1 Introduction

Soil core processing is a method used to prepare a soil core subsample for instrumental analysis. Prior to chemical analysis of ammonia and nitrate, soil cores must be thawed, divided into smaller subsamples, homogenized and extracted with a with a potassium chloride solution. After this 1-2 day process is complete, the core subsample will be represented as a liquid, 100 mL potassium chloride solution. Potassium chloride was chosen as the carrier for this process instead of water in order to better match the chemical properties of the soil.

2 Scope and Application

2.1 Overview

This method describes the process of sediment separation, homogenization and extraction for determining moisture content, bulk density, pH, as well as the preparation for ammonia and nitrate analysis.

2.2 Method Detection Limit

A method detection limit is not applicable for this procedure. A method detection limit for nitrate and ammonia on the Lachat can be found on the Lachat SOP (13_02_02).

2.3 Acceptable Ranges

Ranges are not applicable for this procedure. Acceptable ranges for nitrate and ammonia Lachat analysis can be found in the Lachat SOP.

2.4 Training time

1-3 days of training time may be necessary for this procedure.

2.5 Sample Preservation

The final subsample should be preserved with 5 drops of sulfuric acid per 100 mL. If the sample will not be ran on the Lachat the same day the sample must be frozen.

3 REQUIRED TRAINING

Users should receive 3-4 full days of training on the procedure.

4 EQUIPMENT AND MATERIALS

4.1 APPARATUS AND MATERIALS

4.1.1. Aluminum Foil
4.1.2. Knife
4.1.3. Beakers, 250 mL
4.1.4. Top Loader Scale
4.1.5. Drying Oven
4.1.6. 20 Liter Carboy
4.1.7. Graduated Cylinder
4.1.8. Thomas-Wiley Mill
4.1.9. Whirl-Pak Bags
4.1.10. Erlenmeyer Flasks, 250 mL
4.1.11. Wrist-Action Shaker
4.1.12. Buchner Filters
4.1.13. Whatman #42 filter paper, 7cm
4.1.14. Erlenmeyer Vacuum Flasks
4.1.15. Polyethylene Bottles, 150 mL
4.1.16. Pipette
4.1.17. pH Electrode
4.1.18. EC Electrode

4.2 REAGENTS

4.2.1. Reagent Grade 1 N KCl
4.2.2. DDI Water
4.2.3. pH Buffer Solutions of pH= 4.00 and pH=7.00

4.3 CHEMICALS

% Populate this table from the **chemical database**

Chemical	CAS Number	Hazards	Location
Potassium Chloride	7447-40-7		Room 203 Countertop
Sulfuric Acid	7664-93-9		Dropper located in Soil lab, stock solution located in Room 205

5 SAFETY PRECAUTIONS

5.1 SAFETY PRECAUTIONS

5.1.1 Facemasks should be worn to protect against dust particles when using the mill.
5.1.2 Gloves should be worn when handling chemicals.

5.2 WASTE DISPOSAL

5.2.1 KCl can be dumped down the sink.

6 SOLUTIONS AND REAGENTS

6.1 1 M POTASSIUM CHLORIDE

Potassium Chloride	74.55	1491.2 g
Distilled Deionized Water	FW	20 Liters

Protocol: Carefully weigh out 1491.2 g of reagent grade KCl and transfer to a 20-liter carboy. Measure 4 liters of DDI water using a graduated cylinder and add it to the carboy containing the KCl. Shake vigorously to dissolve the KCl. Add an additional 16 liters (again, measured with a graduated cylinder) and swirl to mix thoroughly.
Storage: 1 M KCl may be stored at room temperature.
Disposal: 1 M KCl may be dumped down the sink without any further dilution.

7 STANDARD SOLUTIONS

7.1.1 **Standard 1: Stock Nitrate Standard 100.0 mg N/L as NO_3 in 1 M KCl**
In a 1 L volumetric flask, dissolve 1.444 g potassium nitrate (KNO_3) in about 600 mL 1 M potassium chloride (KCl). Dilute to the mark with 1 M potassium chloride and invert to mix.

7.1.2 **Standard 2: Stock Nitrite Standard 100.0 mg N/L as NO_2 in 1.0 M KCl**
In a 1 L volumetric flask, dissolve 0.986 g sodium nitrite ($NaNO_2$) in approximately 800 mL 1 M potassium chloride. Dilute to the mark and invert to mix. Prepare this standard and all nitrite standards fresh daily.

7.1.3 **Working Stock Standard 3.0 mg N/L as NO_3**
In a 1 L volumetric flask add 100.0 mL Stock Standard 1. Dilute to the mark with 1 M KCl and invert to mix. Prepare fresh weekly.

7.1.3 **Working Stock Standard 3.0 mg N/L as NO_2**
In a 1L volumetric flask, add 100.0 mL Stock Standard 2. Dilute to the mark with 1.0 M KCl and invert to mix.

8 PROTOCOL

8.1 PREPARATION OF SOIL CORE EXTRUSION

8.1.1 Line the workbench with aluminum foil and tape in place.
8.1.2 From the freezer, remove 10-12 soil cores (2.5 feet each) and arrange them in order of depth, marking the aluminum foil with the depths.
8.1.3 Allow to thaw for 2-24 hours.
8.1.4 Cores should not be left out unprocessed for longer than 24 hours.

8.2 CORE EXTRUSION

8.2.1 After the cores have thawed (usually the next day), carefully slide the sediment out of the barrel onto the foil. If the soil does not easily slide out of the barrel, a hammer may be used to carefully break the plastic barrel. Be careful not to break the soil core any more than necessary.
8.2.2 Lay successive cores end to end, matching the depths.
8.2.3 Cut the cores into subsamples where a lithologic change is identified. Each 2.5' core should be broken into at least 2 subsamples. Use a new knife to cut each interval in order to minimize cross contamination.
8.2.4 Record the new depths of subsample ranges on the foil with a sharpie. Depths can be determined by using a ruler and measuring the length of the subsample and adding it to the previous depth.
8.2.5 On the soil lab worksheet, record observations of color, texture, and evidence of organic matter as well as chemical or physical iron. In addition to these observations, use the Soil Texture Pyramid to classify sediment texture.
8.2.6 Use a ruler and a knife to cut a 1" segment (lengthwise) from each sample. This 1" segment will be processed for gravimetric water content and bulk density. Record the weight of the 1" processed sample by first recording the weight of a 250 mL beaker and then adding it to the beaker and recording the total weight of the beaker and the processed sample.
8.2.7 Ensure the beaker is labeled to properly identify which sample it contains and place the beaker into the oven for 24 hours at 105ºC. After 24 hours, record the weight of the dry sample and the beaker. This data will be used to measure bulk density and gravimetric water content. See section 9.1 for calculations.

8.3 NO_3/NH_4 & pH Processing

8.3.1 Cut each subsample in half, lengthwise. Save half in a plastic Ziploc bag and freeze it, maintaining the field moisture content. With the other half, homogenize with the soil separator tool and allow it to sit overnight with a fan blowing air over the samples to air dry.

8.3.2 Once the samples have been given ample time to air dry, collect each sample in a large aluminum weighing dish. Grind each processed interval in the Thomas-Wiley Mill with a 2 mm screen. To minimize cross contamination, clean out the mill between samples by removing all soil particles using a flat-head screwdriver, brushes and the shop vacuum located in the soil lab.

8.3.3 Using a scale, weigh out 10.0 g (±0.1 g) of the ground soil and place into a 125mL Erlenmeyer flask. Weigh out an additional 5 g (±0.1 g) of ground soil for pH testing in a dram vial. Weigh out 15g (±0.1 g) for particle size analysis. For this method, see the particle size analysis SOP (07_05_02). Put the remaining ground soil in a Ziploc bag, labeled with the date and soil sample I.D. and store in the freezer.

8.3.4 For QA/QC purposes, for every 20 samples processed there should be a duplicate, a known soil standard, a LFB and a LRB.

8.3.5 To each 10 g of processed soil in an Erlenmeyer flask, add 100mL of 1 M KCl. The Dispensette® dispenses 50 mL 1 M KCl per pump. The first time you use the Dispensette® each day, dispense the potassium chloride into an empty beaker in order to remove all of the air and ensure that a full 50 mL is being dispensed each time.

8.3.5.1 The Dispensette® should be cleaned at the end of each day it is used as the KCl dries and degrades the pump over time. To clean the pump, remove it from the container of KCl and pump at least 200 mL of DDI water through it. Make sure that all of the water is out of the pump before placing it back onto the container of KCl.

8.3.6 After 100 mL of KCl has been added to the Erlenmeyer flasks containing samples, put the stoppers onto the flasks and make sure they are tight. Shake by hand for one minute to ensure sediments in the bottom are dislodged.

8.3.7 Fasten the samples onto the wrist action shaker and start the machine at a low speed before increasing the speed to 2 or 3. Leave the samples to shake for one hour.

8.3.8 Set up the Buchner filtering assembly using 7cm Whatman #42 filter paper. Rinse the filters before use by wetting them with 1 N KCl into a separate flask and discard the filtrate.

8.3.9 After an hour on the wrist action shaker has passed, remove the samples and filter into clean Erlenmeyer vacuum flasks. If the filtrate appears cloudy, murky, or discolored, filter again until sample is clear.

8.3.10 Transfer the filtrate into a clean, labeled, 125 mL polyethylene bottle, acidify with 5 drops of sulfuric acid, and store in the freezer. Samples may be stored for 2-3 months. Labels on the bottle should include project name, core number, foot interval and date.

8.4 pH

8.4.1 Using buffers pH= 4.00 and pH 7.00, calibrate the pH meter before use. Check the calibration buffer every 20 samples.

8.4.2 Carefully pipette 5mL of deionized distilled water into the dram bottle containing the 5 g of sample. Shake vigorously to mix and then allow it to sit for 10 minutes before inserting the pH electrode.

8.4.3 Insert the pH electrode and gently swirl the sample until the reading is stable. It is important to consistently record the pH value within a few seconds of stabilization.

8.4.4 Thoroughly rinse the pH electrode with deionized distilled water before measuring the pH of the next sample. When finished, clean the electrode thoroughly, using care to remove any sediment from the reference junction, put the cap back on and soak in electrode soaking solution.

9 Data Reduction and Statistics

9.1 Calculations

9.1.1 Bulk density measurements use a sample volume from the 1”
- For large auger cores = 65.22 cm^3
- For smaller GeoProbe cores = 34.41 cm^3

9.1.2 Bulk Density (g/cm^3) = Oven Dried soil weight (g) ÷ sample volume (cm^3)

9.1.3 Sample water content (g) = Field moisture content soil (g) – oven dried soil (g)

9.1.4 Gravimetric Water Content = Oven dried soil weight (g) ÷ Sample water content (g)

10 Additional Information

10.1 References

- Black, C. A., Evans, D. D., Ensminger, L. E., White, J. L., & Clark, F. E. (1965). *Methods of Soil Analysis*. (C. A. Black & R. . Dinauer, Eds.). Madison, Wisconsin: American Society of Agronomy, Inc.
- Bobier, M. W., Frank, K. D., & Spalding, R. F. (1993). Nitrate-N movement in a fine textured vadose zone. Journal of Soil and Water Conservation, 48(4), 350–354.
- Spalding, Roy F., and Lisa A. Kitchen. "Nitrate in the intermediate vadose zone beneath irrigated cropland." *Groundwater Monitoring & Remediation* 8.2 (1988): 89-95.

11 PREVIOUS ISSUES AND CHANGES

Document File Name	Issue	Issue Effective Dates	Author
N/A			

3: Lachat QuikChem 8500

1 INTRODUCTION

Flow injection analysis, or FIA, is a continuous flow method for rapidly processing samples (Lachat Instruments, 2012). The peristaltic pump draws sample from the sampler into the injection valve. Simultaneously, reagents are continuously pumped through the system. The sample is loaded into the sample loop of one or more injection valves. The injection valve is then switched to connect the sample loop in line with the carrier stream. This sweeps the sample out of the sample loop and onto the manifold. Mixing occurs in the narrow bore tubing under laminar flow conditions. For each method, the operating parameters are optimized for sample throughput, precision and accuracy.

2 SCOPE AND APPLICATION

Analysis Methods:	FIA (Flow Injection Analysis)
Channels:	Max. 5
Light Source:	Tungsten Halogen Lamp
Detector:	Dual beam photometer (340-880 nm)
A/D Converter:	24 bit
Heating Unit:	25-160 ¨C
Valve:	High-performance 6 port injection sample valve
Filter Type:	Interference Filter
Reagent Pump:	12 and 16 positions controlled by software
Mixing Coil:	Teflon Tubing
Flow Cell:	10 mm or 20 mm path length
Dimensions: *(w x d x h)*	5 channel unit= 27.60 x 24.03 x 9.90 in.
Peak Measurement:	Area/Height
Tube I.D.:	0.8 mm/0.5 mm
Injected Sample Volume:	2 µL - 250 µL
Sample Throughput:	90 tests/hour/channel
Data Quality Control:	Real time closed-loop control of data quality
Hardware:	PC included with system
Software:	32-bit Omnion 3.0

(Lachat Instruments, 2012)

2.1 OVERVIEW

The components of the QuikChem 8500 FIA are a Cetac ASX-520 Autosampler, a Reagent Pump, a System Unit, and a Windows compatible computer with Omnion FIA software (Seibold, 2010). The System Unit has multiple processing modules (channels) configured for performing flow injection analysis with photometric detection. Analyte methods are stored in the WSL Box cloud (Box/Water Sciences Laboratory/Lab Manual/Part 4 - Analytical SOPs/13 LACHAT).

2.2 METHOD DETECTION LIMIT

Detection limits vary by analyte and are determined by the method's specific instrument detection limit (IDL) (See specific Quikchem method Scope and Application section). A full list of method details have been published by Hach (Lachat Instruments, 2012).

2.3 ACCEPTABLE RANGES

Ranges vary by analyte and are determined by the method's specific instrument detection limit (IDL) (See specific Quikchem method Scope and Application section).

2.4 TRAINING TIME

2.5 SAMPLE PRESERVATION

Sample preservation varies by analyte and are specified in the relevant QuikChem method (See specific Quikchem method Sample Collection, Preservation and Storage section).

3 REQUIRED TRAINING

These must be recorded in staff or student training file. Link to **training table**

4 EQUIPMENT AND MATERIALS

4.1 APPARATUS AND MATERIALS

The components of the QuikChem 8500 FIA are a Cetac ASX-520 Autosampler, a Reagent Pump, a System Unit, and a Windows compatible computer with Omnion FIA software (Seibold, 2010).

5 SAFETY PRECAUTIONS

Nitrile gloves should be worn any time chemicals are handled. They should be changed frequently to eliminate cross contamination.

5.1 SAFETY PRECAUTIONS

The toxicity or carcinogenicity of each reagent used in this method has not been fully Established (Hofer, 2003) (Knepel, 2012). Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Following Occupational Health and Safety Act (OSHA) regulations regarding the safe handling of all chemicals is advised (See specific Quikchem method Safety section).

5.2 WASTE DISPOSAL

Waste should be tagged with the proper Environmental Health and Safety (EHS) documentation. Required documentation includes chemical composition, storage location, and container type. Generated waste must be reported to UNL's EHS department.

6 SOLUTIONS AND REAGENTS

6.1 REAGENTS

Reagent 1. Salicylate/Citrate Mixed Reagent
By Volume: In a **1 L** volumetric flask dissolve **34.0 g sodium salicylate** [salicylic acid, sodium salt, 2-(HO)C6H4CO2Na] and **40.0 g trisodium citrate** (sodium citrate, C6H5Na3O7) in about **950 mL DI water**. Then add **0.400 g sodium nitroprusside** [sodium nitroferricyanide dihydrate, Na2Fe(CN)5NO.2H2O]. Swirl to dissolve the solid then dilute to the mark with **DI water**. Transfer to an amber glass bottle. This reagent is stable for two weeks.

Reagent 2. Sodium dichloroisocyanurate Reagent (D.C.I.C.)
By Volume: In a **1 L** volumetric flask, dissolve **10.0 g sodium hydroxide** (NaOH) in about **500 mL DI water**. Cool the solution to room temperature then add **0.80 g sodium dichloroisocyanurate** [dichloro-s-triazine 2,4,6,(1H,3H,H)-trione sodium salt] to the solution. When dissolution is complete, dilute to the mark with **DI water**. Transfer to an amber glass bottle, and store at 4°C. Prepare fresh weekly.

Reagent 3. Ammonium chloride buffer, pH = 8.5
By Volume: In a **1L** volumetric flask, dissolve **80.0 g ammonium chloride** (NH4Cl) and **1.00 g disodium ethylenediamine tetraacetic acid dihydrate** (Na2EDTA.2H2O) in about **800 mL DI water**. Adjust the pH up to **8.5** with **15 M sodium hydroxide**. Dilute to the mark with **DI water** and invert to mix.

Reagent 4. Sulfanilamide Color Reagent
By Volume: To a **1 L** volumetric flask, add about **600 mL DI water**. Then add **100 mL 85% phosphoric acid** (H3PO4), **40.0 g sulfanilamide**, and **1.0 g N-1-naphthylethylenediamine dihydrochloride** (NED). Shake to wet and stir to dissolve for 20 minutes. Dilute to the mark with **DI water**, and invert to mix. Store in a dark bottle and discard when pink.

6.2 CHEMICALS

Chemical	CAS Number	Hazards	Location
Potassium Chloride	7447-40-7	General/Poison	203 Countertop
Sodium Salicylate	54-21-7	Toxic	203 Countertop
Trisodium Citrate	6132-04-3	General	Cabinet 2 Shelf 3
Sodium Nitroprusside	14402-89-2	General/Poison	Desiccator
Sodium Hydroxide	1310-73-2	Corrosive/irritant	Cabinet 2 Shelf 3
Sodium Dichloroisocyanurate	2893-78-9	Oxidizer/EnvrToxic	Cabinet 3
Ammonium Chloride	12125-02-9	Human Hazard/Irritant	Cabinet 1 Shelf 1
Sodium EDTA salt	6381-92-6	Irritant	Cabinet 1 Shelf 2
Phosphoric Acid	7664-38-2	Acid/Inorganic/Corrosive/Toxic	Room 204
Sulfanilamide	63-74-1	Irritant	Cabinet 2 Shelf 2
N-1-naphthyl ethylenediamine	1465-25-4	General	Dessicator

7 STANDARD SOLUTIONS

Standards								
Concentration								
NH4	(mg/L)	0.00	0.01	0.05	0.10	0.50	1.50	3.00
NO3	(mg/L)	0.00	0.025	0.05	0.10	0.50	1.50	3.00
Amount Added								
NH4		0	10 µL	25 µL	50 µL	250 µL	750 µL	1.5 mL
NO3		0	25 µL	25 µL	50 µL	250 µL	750 µL	1.5 mL
KCl		100 mL	100 mL	50 mL	50 mL	50 mL	50 mL	50 mL

Acidify 2 liters of 1M KCl with 80% H_2SO_4 (at a rate of 5 drops per 100 mL KCl) to make standards. Add NH_4 and NO_3 to volumetric flasks and then fill to the line with acidified KCl

8 PROTOCOL

Specific protocol's vary by the analyte and are specified in the relevant QuikChem method (See specific Quikchem method Procedure section). Below is a general setup protocol for the manifold, instrument, and calibration (Seibold, 2010).

I) Manifold Removal

1) Detach output of manifold from union on flow cell tubing.

2) Detach heating unit tubing from manifold, if necessary.

3) Detach manifold tubing from manifold fitting that is connected to port 3 at the injection module.

4) Disconnect carrier pump tube from port 2 of injection valve. Connect this tubing to the manifold fitting that was connected to port 3.

5) Remove all manifold pump tubes from cartridges.

6) Remove interference filter from the detector module.

7) Remove sample loop from ports 1 and 4 of valve.

8) Remove manifold from System Unit.

9) Carefully wind tubing around manifold and store manifold, sample loop and interference filter in a plastic bubble bag.

II) Manifold Installation

1) Remove manifold, sample loop and interference filter from bubble bag.

2) Insert interference filter into detector.

3) Unwind pump tubes and place manifold on system unit.

4) Attach output of manifold to appropriate length of heated tubing, if heater is to be used.

5) Attach output of heated tubing, if heater is in use, or manifold to union on tubing coming out of the bottom of the flow cell.

6) Connect manifold input to port 3 of injection value.

7) Connect carrier line to port 2 of injection valve.

8) Connect appropriate size sample loop to ports 1 and 4 of injection valve.

9) Mount pump tubes into cartridges according to the direction flow.

III) Instrument Setup

1) Switch ON the autosampler, pump, system unit and computer.

2) Load pump cartridges onto pump rollers, ensuring that the retaining lips are properly engaged. Raise cam adjustment lever to its vertical position.

3) If the method recommends a rinse solution, place all reagent lines into the solution and pump for 5 minutes.

4) Place pump tube lines into deionized water. Pump for 5 minutes.

5) Place pump tube lines into appropriate reagents. Pump for 5 minutes.

6) If a manifold with a column is in use, turn pump off and place the column in line. Pump reagent for 5 minutes.

Note: Never pump air through the cadmium column.

IV) Calibration Procedure

1) Calibrate with standards that bracket the sample concentrations.

2) Review each calibration.

3) Select Calibration Results icon. View graph and r for each analyte. Correlation coefficient should be greater than or equal to 0.990.

4) Delete one or all calibration levels, if necessary.

5) Recalibrate one or all levels, if necessary.

6) Confirm calibration with accuracy and precision controls.

7) Check calibration curve for drift, accuracy and precision with standards and controls every 20 samples.

V) System Shutdown

1) If the manifold in use has a column; turn off the pump, take the column off-line and restart the pump.

2) Remove reagent lines from each reagent and rinse off the lines and glass weights with deionized water.

3) If the method recommends a rinse solution, place all reagent lines into the solution and pump for 5 minutes.

4) Place reagent lines into deionized water and rinse for 5 minutes.

5) Remove reagent lines from the deionized water and pump air for 5 minutes to remove the liquid from the manifold.

6) Power OFF pump and remove all pump tubing cartridges from the pump.

7) Close all files in the Omnion software. Exit Windows. Turn OFF computer.

8) Power OFF autosampler and system unit.

9 DATA REDUCTION AND STATISTICS

9.1 CALCULATIONS

See specific Quikchem method Data Analysis and Calculations section

I) Analysis and Concentration Calculation Procedure

1) Open a previous Data File of the Method to be run. Create a new Data File as Method_YYMMDD (Y=Year, M=Month and D=Day).

2) Modify the worksheet with the standards, controls and samples to be run.

3) Load standard, control and sample vials onto autosampler according to the worksheet information.

4) Select Start icon.

5) View Peaks and Calculated Sample Concentration Values for each channel while samples are processing.

6) Select Stop if tray needs to be Aborted or Paused.

7) The run is complete when the START Icon turns green.

II) Export data

1) Select Tools, Custom Report and Table.

2) Select Rep Number, Cup Number, Peak Height, Peak Area and OK.

3) Select Report and Save as RTF.

4) Close Custom Report.

9.2 STATISTICS

10 QUALITY ASSURANCE

LFB

100mL of 1M KCl
Add 500µL each of 100ppm NO3 and 100ppm NH4 standards
Shake in wrist action shaker for 1 hour
Filter through back lab SPE line with No. 22 Filters
Add 5 Drops of 80% H2SO4

LRB

100 mL of 1M KCl
Shake in wrist action shaker for 1 hour
Filter through back lab SPE line with No. 22 Filters
Add 5 Drops of 80% H2SO4

MDL

8 standards made to test the accuracy of standard made
Must be made in the same manner as LFB

IDL

Make the low standard and run it 8 times to check the instrument accuracy.

11 ADDITIONAL INFORMATION

11.1 REFERENCES

Hofer, S. (2003). *Determination of Ammonia (Salicylate) in 2M KCl Soil Extracts by Flow Injection Analysis*.

Knepel, K. (2012). *Determination of Nitrate in 2M KCl Soil Extracts by Flow Injection Analysis*.

Lachat Instruments. (2008). *QuikChem 8500 Series 2 FIA Automated Ion Analyzer User Manual*.

Lachat Instruments. (2012). Methods List for Automated Ion Analyzers. Retrieved from http://www.lachatinstruments.com/products/quik-chem-flow-injection-analysis/faq.asp

Seibold, C. (2010). Lachat QuikChem 8500 Series 2 Flow Injection Analyzer: Operating Procedure. Retrieved from http://snobear.colorado.edu/cgi-bin/Kiowa/Kiowa.con.pl?lachat8500.doc.html

11.2 INSTRUMENTATION TECHNICAL SUPPORT

Technical support, troubleshooting, and maintenance information can be found in the instrument's user manual (Lachat Instruments, 2008).

Lachat QuikChem 8500
13_02_02.001
Dates Active:
Author: Jordan Shields

Ran 2/27/17	NO3-N IDL Test
Known (ppm)	**Measured (ppm)**
0.025	0.0245
0.025	0.0261
0.025	0.0201
0.025	0.0247
0.025	0.02
0.025	0.029
0.025	0.0246
0.025	0.0241
Average of measured	0.0241
Recovery	96.6

std	0.0030
idl	0.0089

Ran 2/27/17	NO3-N MDL Test				
Known (ppm)	**Measured (ppm)**	**L of extract**	**ug in a mg**	**Soil weight (g)**	**Calculated (ug/g)**
Unknown	0.5107	0.1000	1000	10.1000	5.8210
Unknown	0.5327	0.1000	1000	10.0400	5.3062
Unknown	0.5430	0.1000	1000	10.1000	5.3762
Unknown	0.5190	0.1000	1000	9.9300	5.2266
Unknown	0.5123	0.1000	1000	10.0100	5.6820
Unknown	0.5234	0.1000	1000	9.9300	5.6933
Unknown	0.5247	0.1000	1000	9.9000	5.3000
Unknown	0.5508	0.1000	1000	10.0000	5.7390
			0.0142		
		std		Average of calculated (ug/g):	*5.5180*
			0.0427		
		mdl		Measured/Known:	Unknown

Recovery Test 1 ug NO3-N/g

Known **(ppm)**	Measured (ppm**)**	L of extract	ug **in a mg**	Soil **weight (g)**	Calculated **(ug/g)**
Unknown	**0.6760**	**0.1000**	**1000**	**9.9300**	**6.8077**
Unknown	0.6860	0.1000	1000	9.9600	6.8876
Unknown	0.6601	0.1000	1000	10.0400	6.5747
Unknown	0.6496	0.1000	1000	9.9700	6.5155
		std	0.0162	Average of calculated (ug/g):	6.6964
		mdl	0.0736	Measured/Known:	*Unknown*

Spiking MDL Recovery

1 ug NO3-N/**g of soil spike**		Not **Spiked**
Average of calculated (ug/g):	**Average of** calculated (ug/g):	**Recovery** of spike LFM
6.6964	5.5180	1.1783
	(**recovery of spike LFM/1)*100**	117.83

Ran 2/27/17	NH4-N IDL Test

Known (ppm)	Measured (ppm)
0.01	0.0117
0.01	0.0116
0.01	0.0112
0.01	0.0115
0.01	0.0111
0.01	0.011
0.01	0.012
0.01	0.0115
Average of measured	0.0115
Recovery	114.5

std 0.0003
idl 0.0010

Ran 2/27/17	NH4-N MDL Test				
Known (ppm)	**Measured (ppm)**	**L of extract**	**ug in a mg**	**Soil weight (g)**	**Calculated (ug/g)**
Unknown	0.3740	0.1000	1000	10.0000	3.7400
Unknown	0.3140	0.1000	1000	10.0200	3.1337
Unknown	0.3612	0.1000	1000	10.0000	3.6120
Unknown	0.3190	0.1000	1000	9.9800	3.1964
Unknown	0.3240	0.1000	1000	9.9700	3.2497
Unknown	0.3507	0.1000	1000	10.0600	3.4861
Unknown	0.3970	0.1000	1000	9.9500	3.9899
				Average of calculated (ug/g):	*3.4868*
		std	0.0312	Measured/Known:	Unknown
		mdl	0.0980		

	Recovery Test	1 ug NH4-N/g			
Known (ppm)	**Measured (ppm)**	**L of extract**	**ug in a mg**	**Soil weight (g)**	**Calculated (ug/g)**
Unknown	0.4438	0.1000	1000	10.0000	4.4380
Unknown	0.4401	0.1000	1000	10.0200	4.3922
Unknown	0.4463	0.1000	1000	9.9500	4.4854

Unknown	0.4450	0.1000	1000	9.9900	4.4545
				Average of calculated (ug/g):	*4.4425*
		std	0.0027		
		mdl	0.0121	Measured/Known:	Unknown

Spiking MDL Recovery		
1 ug NH4-N/g of soil spike	**Not Spiked**	
Average of calculated (ug/g):	Average of calculated (ug/g):	Recovery of spike LFM
4.4425	3.4868	0.9557

(recovery of spike LFM/1)*100 95.57

12 PREVIOUS ISSUES AND CHANGES

Document File Name	Issue	Issue Effective Dates	Author
N/A			

4: Particle Fractionation and Particle-Size Analysis

1 INTRODUCTION

Soil particles are the discrete units which comprise the solid phase of the soil. They generally cluster together as aggregates, but can be separated from one another by chemical and mechanical means. The particles have diverse composition and structure, and generally differ from one another in both size and shape. They may be organic or inorganic, crystalline or amorphous. The methods described will apply only to the inorganic particles, the typical ones being single crystalline fragments.

2 SCOPE AND APPLICATION

Particle size is a parameter having the dimension of length, and defined by one or another of several arbitrary criteria, such as:

- The width of the smallest square opening, or the diameter of the smallest circular opening, through which the particle can pass.
- The diameter of a circle having an area equal to the maximum projected area of the particle.
- The diameter of a sphere whose volume is equal to that of the particle.
- The diameter of a sphere whose density and settling velocity are equal to those of the particle.

These criteria all agree for spherical particles, but not for the anisometric particles commonly found in the soil. Hence, the recorded results of a particle-size measurement should always be accompanied by a notion of the method used.

The particle-size distribution of a soil expresses the proportions of the various sizes of particles which it contains. The proportions are commonly represented by the relative numbers of particles within stated size classes, or by the relative weights of such classes. The determination of a particle-size distribution is commonly referred to as particle-size analysis, a term which has largely superseded the older and somewhat ambiguous term “mechanical analysis.”

The term fractionation refers in the present context to any process used to sort the soil particles into distinct classes according to size. Sieving and sedimentation are the most common methods. Fractionation is employed in most methods of particle-size analysis, and is also used independently in the preparing of soil materials for detailed physical, chemical, or mineralogical study. The fractions are often referred to as separates.

Particle-size distribution is one of the most stable soil characteristics, being little modified by cultivation or other practices. Although the usefulness of particle-size analysis in

practical agriculture has sometimes been questioned, its indirect benefits have been extensive. It has been used in many countries as a basis of soil textural classification, particularly in the United States. Particle-size analysis is a valuable research method in problems dealing with weathering, segregation of the soil particles by leaching, soil structure, and sediment transport by water and wind.

The methods of fractionation and particle-size analysis described herein are limited to sieving and sedimentation procedures. The extraction of clay by gravitational sedimentation is the most common method for separating the fraction of the soil. In this procedure, sedimentation and decantation to a fixed depth are repeated a sufficient number of times to achieve almost a complete separation.

The pipette method of particle-size analysis is a sedimentation procedure which utilizes pipette sampling at controlled depths and times. It was developed simultaneously in three different countries, and was later adopted by the International Society of Soil Science. Further developments have put special emphasis on methods of dispersion.

2.1 OVERVIEW

Pre-treatment and Dispersion

The usual methods of fractionation and particle-size analysis require that the particles be dispersed in an aqueous solution, i.e., that they be detached from one another and suspended in the liquid. Shaking the soil in a dilute alkaline solution of sodium metaphosphate is sufficient, in many cases, for the dispersion, of all except the finest colloidal aggregates. However, soils which contain considerable amounts of readily soluble salts, gypsum, or organic matter may not disperse adequately unless these components are removed first. Removal can be accomplished by treating the soil with peroxide to destroy organic matter, followed by filtration and washing with enough water to dissolve the gypsum.

Dispersion usually requires the addition of a reagent, or various reagents, in dilute concentration. The effectiveness of these dispersing agents depends upon the adsorption of sodium in exchange for other cations, and the resultant development of strong electrical repulsion forces between the soil particles. The particles must be separated by shearing action or turbulent mixing, using such devices as mechanical shakers or electric mixers. However, the mixing should not be too vigorous, since the rupture of individual particles should be avoided.

Sieving

Sieving is a convenient procedure for segregating particles coarser than 0.05 mm. Although outwardly simple, it has limitations that are not always recognized. The probability of a particle passing a given sieve in a given time of shaking depends upon the nature of the particle and the properties of the sieve. For example, a particle whose shape permits its passage only in a certain orientation has a limited chance of getting through, except after prolonged shaking. Furthermore, sieve openings are generally unequal in size; requiring extensive shaking before all particles have had the opportunity of approaching the largest openings. In fact, the requirement that sieving be continued to

"completion" can rarely be met in practical times of shaking. Good reproducibility requires careful standardization of procedure.

Sedimentation

Small spherical particles of density and diameter are known to settle through a liquid of density and viscosity at a rate of $v = \frac{X^2 g(p_s - p_L)}{18\eta}$, where g = acceleration of gravity. This relationship between the size of a spherical particle and its settling velocity, known as Stokes' equation, furnishes an arbitrary measure of the sizes of non-spherical particles. Thus, the separation of the clay fraction (0 < X < 0.002 mm) by sedimentation can be accomplished by homogenizing a soil suspension and decanting all that which remains above the plane $z = -h$ after time $t = \frac{18h\eta}{g}(p_s - p_L)X^2$. Quantitative separation by decantation requires that the residue be re-suspended and decanted repeatedly to salvage those particles that had not previously been at the top of the suspension at the start of the sedimentation period.

Theory of the Pipette Sampling Method

The pipette method of analysis depends upon the fact that sedimentation eliminates from the depth *h* in a time *t*, all particles having settling velocities greater than $\frac{h}{t}$. The taking of a small volume element by a pipette at a depth *h,* at time *t,* furnishes a sample from which all particles coarser than X (as determined by Stokes' equation) have been eliminated, and in which all particles finer than that size are present in the same amount as initially. The volume event at depth *h* has, in effect, been "screened" by sedimentation, so that the ratio of the weight *w* of particles present in that volume at time *t*, divided by the weight *w* of particles present in it initially, is equal to $\frac{P}{100}$, where P is the percentage of particles, by weight, smaller than X. Now, the ratio w/w can also be written as the concentration ratio c/c, giving c/c = $\frac{P}{100}$. This equation connects the concentration *c* of the pipette sample, in grams per liter, to the parameter P of the particle-size distribution, *c* being the weight of solids in the entire sample divided by the volume of the suspension.

3 REQUIRED TRAINING

4 EQUIPMENT AND MATERIALS

4.1 APPARATUS FOR SEPARATION OF CLAY, SILT, AND SAND FRACTIONS

4.1.1 Siphon: Prepare a siphon tube from a 30 cm length of glass tubing having an inner diameter of 6 mm. Make a 90° bend in one end (the upper) and a short U-shaped bend in the other, giving an upturned opening to serve as an entry port. Connect a 50 cm length of rubber tubing to the bent end, and close the free end of the rubber tubing with a screw clamp. Using a support rod and adjustable clamp, mount the glass tube in a vertical position to allow its insertion to a measured depth in a suspension.

4.1.2 Suction filtration apparatus: Using rubber tubing with heavy walls, connect a Pasteur-Chamberland filter (fineness F) to a suction flask and to a pressure bulb through a 3-way stopcock (Fig. 43-1). Attach the suction flask to the suction pump.

4.1.3 Sieve, 47, 300-mesh phosphor bronze wire cloth, 0.0015-in wire, in a 5-inch or 8-inch frame.

4.2 APPARATUS FOR PIPETTE METHOD

4.2.1 Suction filtration apparatus.

4.2.2 Pipette sampling apparatus: Construct a special pipette, and determine its volumetric capacity from the weight of mercury that it will hold. Prepare a pipette holder for lowering and positioning the pipette at a controlled depth of 10 to 13 cm below the upper mark of a 1-liter graduated cylinder. Connect the pipette by a 2-foot length of rubber tubing to the suction line through a fine glass capillary and a 2-way stopcock. Try different capillaries until one is found which permits the pipette to fill in about 30 seconds under full suction.

4.2.3 Brass plunger: One the center of a circular brass plate 1/16 inch in thickness and 2 1/8 inches in diameter, fasten a vertical brass rod 20 inches in length and 3/16 inch in diameter.

4.2.4 Sedimentation cabinet or constant-temperature room.

4.2.5 Shaker, horizontal reciprocating type, 2 ½-inch stroke, 120 strokes per minute.

4.2.6 Sieve shaker, ½-inch vertical and lateral movements, 500 strokes per minute, with automatic timer.

4.2.7 Sieves, set of 6, 3-inch diameter frame, with cover and pan.

4.3 REAGENTS FOR BOTH METHODS

4.3.1 Hydrogen peroxide, 30% (H_2O_2).

4.3.2 Calgon (Water softener product).

5 SAFETY PRECAUTIONS

5.1 SAFETY PRECAUTIONS

5.1.1 Wear appropriate hand and eye protection when working with laboratory detergents and hazardous chemicals.

5.2 WASTE DISPOSAL

5.2.1 All acid solutions must be neutralized with sodium bicarbonate before being disposed of in the acid sink in Room 205. Do not allow any un-neutralized acid solution to drain into any sink in the building.

6 SOLUTIONS AND REAGENTS

6.1 CALGON SOLUTION

Calgon	50 g
Water	Enough to create a volume of 1 L

Protocol: Dissolve 50 g of Calgon in water, and dilute the solution to a volume of 1 L.
Storage: Excess Calgon solution can be stored in the non-flammable liquids cupboard in Room 203.
Disposal: Excess Calgon solution can be disposed of down any sink in the lab.

7 STANDARD SOLUTIONS

8 PROTOCOL

8.1 METHOD FOR SEPARATION OF CLAY, SILT, AND SAND FRACTION

8.1.1 Prepare a 7% aqueous suspension of soil in a straight-sided jar. Add 250 mL of Calgon solution per 100 g of soil, allow the soil to slake for a few minutes, and stir the suspension for 3 minutes with an electric mixer. Cover the jar, and store it in a locker to reduce temperature fluctuations and to avoid direct sunlight.

8.1.2 Record the temperature. Determine from Stokes' equation the time required for a spherical particle 0.002 mm in diameter to fall through a definite distance *h*, equal to about 80% of the depth of the suspension. Move the jar to the bench at the proper time, insert the siphon tube to the predetermined depth *h*, and siphon the suspension to another jar. Continue siphoning until the supernatant liquid has been removed to the depth *h*.

8.1.3 Remove the siphon, add distilled water again to the original level, mix the suspension thoroughly as before, and repeat the sedimentation and siphoning.

8.1.4 While the second period of sedimentation is in progress, use filtration apparatus to concentrate the clay that has already collected in the receiver. When a coherent layer of clay has formed on the surface of the filter candle, disconnect the suction and apply air pressure to the interior of the filter by squeezing the rubber bulb. Touch the filter candle to the side of the receiver to initiate the expulsion of the clay, which will slide off as an intact mass. Continue the filtration until no free water remains.

8.1.5 Collect the siphonings in the same jar, and repeat the operations until the yields become negligible. Save the clay fraction as a concentrated paste if needed in that form, or dry it in air and store it in the dry state.

8.1.6 Separate the silt and sand fractions by wet-sieving the residue on a 47μ sieve. Dry the fractions as needed.

8.2 PIPETTE METHOD OF PARTICLE-SIZE ANALYSIS

8.2.1 Sampling and Pre-treatment

8.2.1.1 Dry and crumble the field sample. Separate the "fine earth" fraction by hand-sieving through a 2-mm sieve. Use a mortar and rubber-tipped pestle to break up those aggregates which are larger than 2 mm. When all the fine earth has passed the sieve, leaving only the gravel, weigh both fractions and discard the gravel.

8.2.1.2 Mix the soil on a flexible plastic sheet by raising one corner at a time, causing particles to lift, turn, and tumble over one another until thoroughly intermingled. When subsamples are required, take small, representative portions from several different parts of the sample, and combine them to obtain the desired weight.

8.2.1.3 Place a subsample weighing approximately 10 g in a tared 250-ml beaker, and weigh it to the nearest 0.01 g. Dry the sample overnight at 105°C, cool it in a desiccator, and reweigh it. Add 30 ml of water, cover the beaker with a watch glass, and stir the contents by swirling the beaker. Cautiously add a few ml of 30% H_2O_2, and swirl the suspension if necessary to reduce foaming. When the reaction subsides, add additional amounts of H_2O_2 in the same manner; and complete the digestion by heating the beaker for 1 hour or more at 90°C on a hotplate. Repeat the treatment if visual inspection indicates that the reaction has not been completed.

8.2.2 Filtration

8.2.2.1 Remove the excess liquid by the filtration apparatus. When the filter candle has become coated with soil to a thickness of 1 or 2 mm, reverse the stopcock, and apply pressure with the rubber bulb, touching the candle lightly against the inner wall of the beaker to dislodge the soil from the filter candle. Resume the filtration process, removing the coatings frequently to reduce the impedance to flow.

8.2.2.2 After the free water has been removed, add additional water, and mix the suspension by a jet of water. Filter the suspension again, and repeat the mixing and filtering several times. Dislodge as much of the soil as possible from the filter candle by applying pressure as before, and wash the last traces of soil back into the beaker from the filter by rubbing the latter with a rubber stopper fitted on the end of a glass rod, using a jet of water when needed. Finally, remove the filter, dry the beaker and contents in the oven at 105°C, cool the beaker in a desiccator, and weigh the dry sample and beaker.

8.2.3 Dispersion and Wet-Sieving

8.2.3.1 Add exactly 10 ml of Calgon solution, and swirl the beaker to bring the contents into suspension. Transfer a 10-ml sample of Calgon solution to a tared weighing bottle, dry the bottle overnight at 105°C, cool it in a desiccator, and weigh the bottle and contents to the nearest milligram. Transfer the suspension through a funnel into a 250-ml shaker bottle, add water to bring the volume of suspension to 180 ml, stopper the bottle firmly, and shake the bottle and suspension overnight in a horizontal reciprocating shaker. Remove the bottle from the shaker, place it in an upright position, and let the suspension settle for a few minutes.

8.2.3.2 Put a wide-mouth funnel in a 1-liter graduated cylinder, using the rim of the latter as a support. Inspect the 47μ sieve for cleanliness and mechanical condition. Moisten it on both sides with water, and place it in the funnel. Then, without shaking or swirling the mixture, pour the suspended portion into the sieve. Add more water to the residue in the bottle, stir the mixture, and allow it to settle for 1 or 2 minutes, and decant the suspended portion into the sieve as before, Repeat the mixing and decanting several times until most of the fine material has been transferred.

8.2.3.3 Tilt the bottle, neck downward, over the sieve. Direct a jet of water upward into the bottle, sweeping the soil particles downward into the sieve by the force of the effluent stream. Do not rub the screen at any time. When the transfer has been completed, agitate the residue on the sieve with a jet of water, and obtain as complete a separation of the coarse and fine fractions as possible. Add distilled water to bring the volume of suspension to 1 liter, insert a rubber stopper, and transfer the cylinder to the sedimentation cabinet or constant temperature room.

8.2.4 Dry Sieve Analysis

8.2.4.1 Put the screen and contents in a tared evaporating dish, and dry them in the oven. Cool the screen, and transfer the coarse fraction from the screen to the dish, using a soft brush to complete the transfer. Dry the dish and contents for 2 hours at 105°C, cool them in a desiccator, and weigh the dish and sand to the nearest 0.01 g.

8.2.4.2 Arrange the sieves on the pan in the same sequence, 1,000, 500, 250, 177, 105, and 47μ, reading from top to bottom. Transfer the coarse fraction from the dish into the top screen, using a brush to complete the transfer. Put the cover in place, and fasten the nest of sieves firmly in the shaker. Shake the sieves for 3 minutes.

8.2.4.3 Transfer the separates, one at a time, into the tared evaporating dish, starting at the top and working downward, and weighing the dish and cumulative contents after each addition. Include the weight of “residual silt” which collects in the pan, since this will be reported as a part of the coarse silt fraction. Compare the cumulative weight with the total weight of the course fractions previously determined.

8.2.5 Sedimentation Analysis with Pipette Sampling

8.2.5.1 Record the temperature of the suspension when it has become. Insert the plunger in the suspension, and move it up and down to mix the contents thoroughly. Move the plunger cautiously near the top of the suspension during both upstroke and downstroke to avoid spilling the contents. Use strong upward strokes of the plunger near the bottom to lift into suspension any particles that may have lodged there. Dislodge any sediment that remains in the lower corners by inclining the rod slightly and rotating it to impart a spinning motion to the disk. Finish with two or three slow, smooth strokes and remove the plunger, tipping it slightly to remove adhering drops. Record the time immediately.

8.2.5.2 Move the cylinder into position in the pipette stand, clamp the clean, dry, pipette in its holder, attach the tubing, and make the adjustments required to immerse the pipette 10 cm in the suspension when the proper time has arrived. Record the volume of the particular pipette used. Note the temperature, and determine the sedimentation time required for a 20-micron sampling. Lower the pipette to its proper depth 30 seconds before sampling time.

8.2.5.3 At the chosen time, open the suction stopcock, and be ready to close the pipette stopcock at the instant the liquid has filled the pipette. After closing the stopcock, raise the pipette, wipe the stem with a soft towel, and by reversing the stopcock run the suspension into a tared weighing bottle. Rinse the pipette and add the washings to the bottle.

8.2.5.4 Close the suction stopcock, and detach the pipette from the tubing.

8.2.5.5 Clean the pipette, and dry it by attaching it to a suction line.

8.2.5.6 Place the weighing bottle in an oven at 105°C for 12 hours, cool it in a desiccator, and weigh the bottle and contents to the nearest milligram.

8.2.5.7 Take 5η and/or 2η samples in the same manner, re-stirring the suspension each time or not as desired, but always counting time from the moment of the most recent stirring. Always measure the sampling depth from the existing surface of the suspension, and not from the level used for an earlier sampling.

9 DATA REDUCTION AND STATISTICS

9.1 CALCULATIONS

9.1.1 Let w = dry weight of the pipette sample, v = volume of the pipette, and V = total volume of the suspension. Calculate the apparent weight of particles in the given size range from the formula $\frac{wV}{v}$. Deduct the weight of dispersing agent per liter of suspension, using the data obtained by drying a 10-ml sample of the 0.5 N reagent. The result will be the cumulative weight ΣW. Determine by difference the weights W of the individual fractions. Determine the weights of the sand separates. Calculate solution loss and sieving loss. Calculate the weight of coarse silt.

10 QUALITY ASSURANCE

It is the responsibility of all laboratory personnel to read and understand this and all other necessary SOPs. Any questions should be discussed with the author or the Laboratory Director. All laboratory personnel must document that they read and understood this procedure and all other necessary SOPs as per the Training Schedule. It is the responsibility of the Laboratory Director to keep a record of all personnel who have read this and other SOPs.

11 ADDITIONAL INFORMATION

References

1. Black, C. A. “Particle Fractionation and Particle-Size Analysis.” Methods of Soil Analysis. 1st ed. Vol. Agronomy. Madison, Wis.: American Society of Agronomy, 1965. 1-770. Print.

5: Microwave Assisted Acid Digestion for Elemental Analysis by ICP-MS

1 INTRODUCTION

This procedure is used to analyze aqueous samples for dissolved trace elements in aqueous solution using inductively coupled plasma mass spectrometer (ICP-MS).

2 SCOPE AND APPLICATION

2.1 OVERVIEW

2.1.1 Microwave extraction is designed to mimic extraction using conventional heating with nitric acid (HNO3) or, alternatively, nitric acid and hydrochloric acid (HCl). Variations of the method (such as the addition of HCl or hydrogen peroxide) may be employed to improve the performance for certain analytes or sample types. Specific acid ratios and volumes are not given in this method and must be determined on a case-by-case basis in order to maximize performance.

2.2 METHOD DETECTION LIMIT

2.2.1 New MDLs should be derived for every run to account for variations in the method. A table which includes the most recent MDLs can be found in Section 10 of this document.

2.3 ACCEPTABLE RANGES

2.3.1 The acceptable range for this method is 0 to 5000 µg/L.

2.4 TRAINING TIME

2.4.1 The training time required for this method is 2-3 days.

2.5 SAMPLE PRESERVATION

2.5.1 All samples should be stored in the freezer until the day of analysis. Samples should be thawed completely before weighing.

3 REQUIRED TRAINING

3.1.1 Basic lab and instrument training as detailed in the Standardized Laboratory Training Plan (07_01_09.001).

4 EQUIPMENT AND MATERIALS

4.1 APPARATUS AND MATERIALS

4.1.1 50 mL centrifuge tubes (Fisherbrand, 06-443-20).
4.1.2 Top-loading analytical balance, precision to 1x10-2 g (Mettler PJ360 or equivalent).
4.1.3 Syringe filter, 25mm with 0.45 µm polyethersulfone membrane (VWR 28145-503 or equivalent).
4.1.4 Disposable syringe with polypropylene plunger, 20 mL (Fisher).
4.1.5 Adjustable micropipettes with reference tips (Eppendorf, Thermo Scientific).
4.1.6 Santoprene tubing (Spectron Inc., SIT-SANSD-049-3).
4.1.7 Peristaltic pump tubing, 0.508mm (Thermo, 1320050).
4.1.8 Sample cone, 4450 (Thermo, 3600812).
4.1.9 Ni skimmer cone 0.5, insert version (Thermo, 1311870).
4.1.10 Skimmer cone insert, 3.5 (Thermo, 1318480).
4.1.11 Sample cone gasket (Thermo, 1310900).
4.1.12 Teflon tube, 5.0M (Thermo, 1041071).
4.1.13 Teflon tube, 1/16 O.D .02ID, 10 feet (Thermo, 1600061).
4.1.14 iCap Q quartz torch (Thermo, 1230790).
4.1.15 Quartz injector, 2.5mm ID (Thermo, 1305600).
4.1.16 Ni Sample cone 4450, 1.1 mm diameter orifice (Thermo, 3600812).
4.1.17 Sampling cone gasket (Thermo, 1310900).
4.1.18 Skimmer cone insert, 3.5 (Thermo, 1318480).
4.1.19 Skimmer iCap Q/Qnova Ni for insert, 0.5mm orifice (Thermo, 1311870).
4.1.20 Cyclonic quartz spray chamber iCap Q (Thermo, 1317080).
4.1.21 MicroMist nebulizer, 0.4 mL/min (Thermo, BRE0009386).
4.1.22 Torch socket iCap Q/Qnova (Thermo, 1231000).
4.1.23 iCap Q/Qnova Quartz Torch (Thermo, 1230790).
4.1.24 Quartz injector, 2.0mm ID iCap Q/Qnova (Thermo, 1305640).
4.1.25 Balance
4.1.26 Microwave solvent extraction apparatus, MARS Xpress System (CEM Microwave Technology).
4.1.27 Microwave extraction Teflon vessels, 50 mL (Large size, CEM Microwave Technology).
4.1.28 Microwave extraction Teflon vessel plugs, MARS Xpress System (CEM Microwave Technology, 212020)
4.1.29 Manual torque tool, MARS Xpress System (CEM Microwave Technology, 185245).

4.2 REAGENTS AND STANDARDS

4.2.1 Tube B Working Solution (Thermo, THERMO-4AREV).
4.2.2 Working Setup Solution (Thermo, THERMO-5A).
4.2.3 Hydrogen peroxide 30% in water, unstabilized, ACS grade (Acros Organics, AC41188-1000 or equivalent).
4.2.4 Nitric acid, trace metal grade (Fisher, A509-P212).
4.2.5 Hydrochloric acid, trace metal grade (Fisher, A508-P212).
4.2.6 Distilled deionized water (DDW).
4.2.7 Barium standard solution, 1000 µg/mL, 125 mL (SPEX Certiprep™, PLBA2-2Y).
4.2.8 Copper standard solution, 1000 µg/mL, 125 mL (SPEX Certiprep™, CLCU2-2Y).
4.2.9 Manganese standard solution, 1000 µg/mL, 125 mL (SPEX Certiprep™, PLMN2-2Y).
4.2.10 Strontium standard solution, 1000 µg/mL, 125 mL (SPEX Certiprep™, PLSR2-2Y).
4.2.11 Arsenic standard solution, 1000 µg/mL, 125 mL (SPEX Certiprep™, CLAS2-2Y).
4.2.12 Yttrium standard solution, 1000 µg/mL, 125 mL (SPEX Certiprep™, PLY2-2Y).
4.2.13 Uranium standard solution, 1000 µg/mL, 125 mL (SPEX Certiprep™, PLU2-2Y).
4.2.14 Iron standard solution, 1000 µg/mL, 125 mL (SPEX Certiprep™, PLFE2-2Y).
4.2.15 Selenium standard solution, 1000 µg/mL, 125 mL (SPEX Certiprep™, CLSE2-2Y).

5 SAFETY PRECAUTIONS

5.1 SAFETY PRECAUTIONS

5.1.1 Always wear a lab coat and gloves while handling acids.
5.1.2 The minimum volume for the MARS vessel is 10mL of acid or 50mL of water.
5.1.3 Never attempt to digest samples larger than 0.5 grams if the organic content and composition of the sample is unknown; when working with an unknown sample, perform all pre-digestion steps in an unsealed, open vessel, allowing a minimum of 15 minutes time for the reaction of volatile or easily oxidized compounds to subside before sealing the vessel and microwave heating.
5.1.4 All solvents and chemicals should be treated as a potential health hazard, and exposure to these chemicals should be minimized. Material Safety Data Sheets (MSDS) are available for all materials used in this procedure, and should be referred to regarding specific handling procedures and toxicity.

5.2 WASTE DISPOSAL

5.2.1 ALL acidic solutions must be neutralized with sodium bicarbonate before disposal into the sanitary sewer. Do not allow any un-neutralized acids or acidic solutions to drain into any sink in the building.
5.2.2 Check the Sewer Disposal List in Room 203 for the list of substances that can be disposed of down the sanitary sewer.

6 SOLUTIONS AND REAGENTS

6.1 0.5% Nitric Acid Solution

Nitric Acid (HNO_3)	63.01 g/mol	40 mL
Distilled Deionized Water	18.015 g/mol	

Protocol: There is a container on the work bench of the ICP-MS Lab (Room 204A) designated for the creation and storage of 0.5% Nitric Acid for the ICP-MS. When this container is empty, simply add 40 mL of concentrated nitric acid to the container, and fill the container to the established line using distilled deionized water from the dispenser on the wall. Remember to add the water first, and then add the acid.
Storage: Excess solution can be stored in the designated plastic containers in the ICP-MS lab.
Disposal: Acids should be neutralized using sodium bicarbonate before disposal in any of the lab drains.

7 Standard Solutions

7.1 Blanks

7.1.1 For metal analysis, blanks and calibration standards will be prepared with 0.5% nitric acid (v/v) in distilled deionized water. For some other samples, distilled deionized water is used instead. Refer to training for specific elements (like anions).

7.1.2 Two types of blanks are required for analysis. The calibration blank is used in establishing the calibration curve. The rinse blank is used to flush the system between samples and standards.

7.1.2.1 The calibration blank ought to consist of the same stock of 0.5% nitric acid or distilled deionized water used to prepare the final dilutions of the calibration standard solutions. This 50 mL centrifuge tube should be kept as full as possible throughout the standard preparation process to ensure the process is as uniform as possible.

7.2 Calibration Standards

7.2.1 For metal analysis, blanks and calibration standards will be prepared with 0.5% nitric acid (v/v) in distilled deionized water. For some other samples, distilled deionized water is used instead. Refer to training for specific elements (like anions).

7.2.2 Fresh multi-element calibration standards should be prepared every two weeks, or as needed.

7.2.3 Dilute each of the stock multi-element standard solutions to levels appropriate to the operating range of the instrument using the calibration blank, which is composed of either 0.5% nitric acid or distilled deionized water. Calibration standards should be prepared at a minimum of 3 concentration levels. The equation for dilution is as follows:

$$M_1V_1 = M_2V_2$$

7.2.4 All calibration standards, including the calibration blank, ought to be treated with the same protocol as the samples in order to maintain the same matrix.

8 PROTOCOL

8.1 VESSEL PREPARATION

8.1.1 Remove vessels from 20% nitric acid bath and rinse twice using DDI water. Allow to air dry overnight.

8.2 DETERMINE THE DIGESTION METHOD

8.2.1 Determine the appropriate EPA Method reference by sample: Use EPA Standard Method 3015A if samples are aqueous; Use EPA Method 3051A if samples are sediments, sludges, soils, or oils; Use EPA Standard Method 3052 if samples are siliceous or organically based matrices.

8.2.2 Outside references may be appropriate to develop methods for specific types of samples and/or analytes.

8.2.3 Determine sample size, acid, acid volume, and standard solution concentration.

- 8.2.3.1 Each EPA Standard Methods lists applicable elements and describes which elements may be aided by the addition of HCl.
- 8.2.3.2 H_2O_2 may aid in the oxidation of organic matter.
- 8.2.3.3 DDI water may be added to increase sample volume without increasing acid concentration.

8.3 SAMPLE PREPARATION (DIGESTION)

8.3.1 Weigh samples to the nearest ±0.001 and record sample weights in a laboratory notebook.

8.3.2 Add each weighted sample and calibration standard into vessels.

8.3.3 Add acids and immediately cover with vent plugs. Allow a minimum of 15 minutes time for the reaction of volatile or easily oxidized compounds before screwing caps onto vessels. Use the MARS Xpress manual torque tool to tighten the cap until hearing a click sound.

8.3.4 Place vessels into the 40-place turntable, distributing the weight of samples as evenly as possible. Record the location of the samples as tape/labels should not be used in the microwave. Place the turntable into the MARS Xpress Microwave. Samples are now ready to run.

8.4 RUN DIGESTION

8.4.1 After powering on instrument, “Edit/Create Method” and “Load Method” are displayed on LCD screen.

8.4.1.1 To load a previously used digestion program, select “Load Method” and choose intended method from the CEM or User Directory;

8.4.1.2 Or select “Edit/Create Method”

8.4.1.2.1 To edit an existing method, select the method from the CEM or User Directory.

8.4.1.2.2 To create a new method, select “New Method” in the User Directory

8.4.1.3 Consult the method reference materials and the CEM Operation Manual for assistance in editing/creating new microwave programs.

8.4.2 To run selected digestion program, push the green start button.

8.5 EXTRACTION

8.5.1 When digestion is completed the MARS Xpress Microwave automatically cools samples for 20 minutes. After this time, remove vessels from the turntable and allow them to further cool in under the hood until they reach room temperature. Turn off the Microwave.

8.5.2 Carefully uncap the vessels under the hood. Make sure to keep the vessel opening directed away from face to avoid exposure to vapors potentially released upon unsealing the vessels.

8.5.3 Transfer vessel contents into 50 mL centrifuge tubes and use DDI water to rinse any residual sample from the vessels into the centrifuge tubes. Dilute samples using DDI water.

8.5.3.1 Samples, especially sediments, may not have completely dissolved during digestion. If this is the case, try to minimize the transfer of any remaining solids from the vessel into the centrifuge tubes. The presence of particulates will decrease accuracy of volume measurements and dilutions.

8.5.3.2 If the digested sample contains particulates, the samples must be allowed to settle or filtered before analysis to avoid clogging the ICP-MS tubings and nebulizer. Filtration is preferred.

8.5.3.2.1 Settling: Allow the sample to stand until the supernatant is clear. Generally, allowing the sample to stand overnight is sufficient for this. If not, the sample must be filtered.

8.5.3.2.2 Filtering: If necessary, utilize a thoroughly cleaned and rinsed filtering apparatus. Filter samples through filter paper into ICP-MS autosampler tubes before analysis.

8.5.4 Store all digested samples in the refrigerator if not being run immediately. Some element specific analyses will require the samples are run on the same day as digestion.

8.5.5 Cleaning

8.5.5.1 After digestion, soak vessels, caps, and plugs in 2% Citranox solution for a minimum of 30 minutes. Thoroughly clean all pieces with a brush and rinse with DDI water.

8.5.5.2 Allow vessels and plugs to soak in 10% HCl bath for a minimum of 30 minutes and then rinse with DDI water. Store clean plugs in ICP-MS lab to minimize contamination for future digestions.

8.5.5.3 Place vessels in 20% nitric acid bath and allow to soak overnight.

8.6 Sample Preparation (Analysis)

8.6.1 Samples which are absent of particulate matter can be transferred directly into autosampler tubes for analysis. There should be approximately an inch of sample in each tube to ensure flow into the autosampler probe.

8.6.2 If particulate matter is present in the sample solutions, they must be filtered to avoid clogging the ICP-MS tubes and nebulizer. If necessary, utilize a polyethersulfone filter. Filter samples through filter paper into ICP-MS autosampler tubes before analysis.

8.7 Sample Re-Analysis

8.7.1 Dilute and re-analyze samples that are more concentrated than the linear range for a particular analyte.

8.7.2 This should be indicated in Qtegra under Evaluation Results → Concentrations, where samples outside the linear range will be highlighted in red.

8.8 ICP-MS Start-Up Guide

8.8.1 Note: Always wear gloves when handling any part of the instrument including pumps, tubing, and any samples/standards. The computer/desk area and the chiller are no gloves access.

8.8.2 Turn on the chiller by pushing the power button (no gloves access).

8.8.2.1 Check water level on the front of the chiller – add DI water from carboy under desk if necessary.

8.8.3 Check levels of spent waste and 0.5% HNO3 containers located on the desk to the right of the auto sampler.

8.8.3.1 Fill HNO3 if necessary from the 0.5% HNO3 container located next to the DDI water dispenser.

8.8.3.2 Empty waste container in the Acids lab sink.

8.8.4 Go to the computer. This area is no gloves access. Open programs Qtegra, Instrument Control (IC), and Camera. Maximize the IC window.

8.8.5 Turn on gases to allow gas temperature to stabilize (especially important in cold weather).

8.8.5.1 From IC -> Main (in left-bottom side bar) -> Nebulizer gas.

8.8.5.1.1 Click on small arrow (∆) below the bar to turn on the gas to appropriate level.

8.8.5.2 From IC -> Plasma (in left-bottom side bar) -> Auxiliary gas.

8.8.5.2.1 Click on small arrow (∆) below bar to turn on gas to appropriate level.

8.8.5.3 From IC -> Plasma (in left-bottom side bar) -> Cool gas.

8.8.5.3.1 Click on small arrow (∆) below bar to turn on gas to appropriate level.

8.8.6 The peristaltic (peri) pump is located on the right side of the iCAP RQ.

8.8.6.1 There are 2 tubes: yellow (outlet) and clear (inlet). Both tubes should be replaced daily or after 8 hours of continuous use. When in doubt, change the tubing!!

8.8.6.2 Attach the outlet tube on the last groove at the back of the peri-pump, first securing one side and then the other in the plastic grooves underneath the pump. Close the plastic flaps. **DO NOT SECURE THE TUBING WITH NOTCHES AT THIS STAGE!** The peri pump will spin in a clockwise direction. It is a common error to place the tubes in reverse of flow path. Avoid this by tracing the intended flow path of the tubes to ensure they are going through the pump in the correct direction.

8.8.6.3 Turn on the peristaltic pump. From IC -> Inlet (in side bar) -> Peristaltic Pump On. Peristaltic Pump Turbo -> High.

8.8.6.4 Confirm the tubes are stable (not shaking/moving/bumping a lot). If stable, lock tubes into place **WITH THE NOTCHES** after turning off the pump. If not stable, call for help!

8.8.6.5 Check pump flow. Before that turn on the peristaltic pump (left menu).

8.8.6.5.1 From IC's ASX-560 tab, direct autosampler probe to go to a Blank sample containing 0.5% HNO3 (generally located in Standard Rack, Vial 1. Click on 1 in row R in the ASX-560 software to direct the probe to position 1).

8.8.6.5.2 Watch the intake tubes going out of the auto sampler probe – you will be able to see when liquid begins to flow through the line as well as any air bubbles that may be present.

8.8.6.5.3 Allow the pump to stabilize for ~5 minutes.

8.8.6.5.4 Confirm intake flow.

8.8.6.5.4.1 Intake flow should appear to be a steady, unbroken stream of solution. This can be checked by introducing an air gap in the auto sampler.

8.8.6.5.4.2 This can be done by manually lifting the sampling probe from the blank solution for a few seconds to introduce air bubble. Lower probe back into solution.

8.8.6.5.4.2.1 Confirm that air bubble moves steadily through the inflow tube.

8.8.6.5.4.2.2 If air bubble is not present, or if it is not moving steadily, there is an error in the tubing/pumping. Do Not Proceed!! Call for Help!!

8.8.6.5.4.3 Check output tube to ensure there is outflow.

8.8.6.5.4.3.1 NOTE: The outflow will be a broken stream of solution because this tube is slightly larger. However, flow should still be steady and fast. Check for any liquid build up at the elbow of the spray chamber. If you see any drops of liquid inside the chamber, turn off the peri pump, and call for help.

8.8.7 Warm-Up

8.8.7.1 From Qtegra -> ‘Get Ready’ button -> Select ‘Warm Up’ for 15 minutes -> OK

8.8.7.1.1 Do not select ‘Perform Validation Tests’ – this will be done later.

8.8.7.2 See camera to ensure that plasma is ignited and monitor Log View Tab for any errors until you see the “Operate” log.

8.8.7.3 Allow warm up to finish, software will notify when it is ready.

8.8.8 Performance Report (PR)

8.8.8.1 In IC, use ASX-560 tab to command probe to Tune solution (generally located in Standard Row, Vial 10). Make sure the solution is filled to 50 mL.

8.8.8.1.1 Allow the pump to take up tuning solution for 120 seconds by clicking the High mode of peri pump, one can monitor the flow by clicking Run (in ‘Control’ tab) in IC located at Top Left Corner. After the tuning solution has reached plasma a rise in peak will be seen, after that click Normal mode in peri pump allow to stabilize the speed for ~40 seconds before running PR. NOTE: Not allowing pump to stabilize generally will lead to Mass Calibration errors)

8.8.8.2 Performance Reports (PR) should be run for STD and KED mode.

8.8.8.2.1 From IC -> Performance Report (in ‘Wizards’ tab)

8.8.8.2.1.1 To run a report in STD: ‘Run an Existing Report’ -> STD -> Next -> Next

8.8.8.2.1.2 To run a report in KED:

8.8.8.2.1.2.1 Change measurement mode to KED (from ‘Measurement mode’ tab in IC)

8.8.8.2.1.2.2 ‘Run a Report in the Active Measurement Mode’ -> Next -> Next

8.8.8.2.1.3 Pay careful attention to the parameters which are passing/failing in the PR results.

8.8.9 Troubleshooting Performance Report Failure **(NOTE: For advanced troubleshooting, refer to the Instrument Manual)**

8.8.9.1 If a single sensitivity parameter is failing the PR, allow the instrument to run for a few minutes and retry the PR – the machine often just needs time to settle itself. If this does not work, the instrument needs to be tuned.

8.8.9.2 AutoTune

8.8.9.2.1 From IC -> AutoTune (in ‘Wizards’ tab)

8.8.9.2.2 Ensure probe is in TuneB solution (generally located in Standard Row, Vial 10) and Run.

8.8.9.2.3 Pay attention to which AutoTune adjustments affect which sensitivity parameters. For example, you might notice that adjustments to the extract lens affect one parameter, while adjustments to the nebulizer gas affect another. Know which adjustments affect the parameters that caused the PR failure.

8.8.9.2.4 Save the AutoTune values and re-run Performance Report.

8.8.9.3 If the Performance Report fails again, reference old tune settings from successful runs to manually adjust the instrument.

8.8.9.3.1 From IC -> Tune Settings (in 'Measurement Mode' tab) -> History

8.8.9.3.2 CTRL + Click on two AutoTune reports, your most recent (failed) run and a previous (successful) run

8.8.9.3.3 Click "Compare" to see which instrument tune settings are different. Use this information to make minor adjustments to instrument settings before running the PR again.

8.8.9.4 A Troubleshooting AutoTune can also be run if PR fails after tuning.

8.8.9.4.1 From IC -> AutoTune Wizard in AutoTune dropdown menu (in 'Wizards' tab)

8.8.9.4.2 Select High Matrix AutoTune Troubleshoot

8.8.9.5 In extreme cases, there are two other AutoTune methods, which can be used to adjust the instrument settings. These two methods must be used consecutively **and under supervision**.

8.8.9.5.1 From IC -> AutoTune Wizard in AutoTune dropdown menu (in 'Wizards' tab)

8.8.9.5.1.1 Select CaliTune STDS

8.8.9.5.2 From IC -> AutoTune Wizard in AutoTune dropdown menu (in 'Wizards' tab)

8.8.9.5.2.1 Select CaliTune STDS 100V. **For Advanced troubleshooting, look in the manual!**

8.8.10 Wash all tuning solution from the probe.

8.8.10.1 From IC ASX-560 tab, command the probe to a Rinse solution containing 0.5% HNO_3.

8.8.10.2 'Run' while viewing the analyte levels; the levels will be high at first (due to presence of tuning solution) but will eventually decrease to near zero. ICP-MS is verified for any measurement of choice, like dissolved elements, total digested elements or speciation (all have separate SOP).

8.8.11 Creating a LabBook.

8.8.11.1 In Qtegra -> LabBooks (in left side bar)

8.8.11.2 Create a LabBook using an existing Template or LabBook or from a blank Template.

8.8.11.3 Analytes

8.8.11.3.1 Select desired element analytes, taking care to select the isotopes with the least amount of interference.

8.8.11.4 Acquisition parameters

8.8.11.4.1 Dwell time should always be set to ~0.1s for all analytes.

8.8.11.5 Standards

8.8.11.5.1 Include an entry for each standard you will be including in your calibration.

8.8.11.5.2 When creating a new entry, select 'Create from Analyte List' to ensure that all the analytes you are testing are accounted for in your standards.

8.8.11.6 Manual Sample Control

8.8.11.6.1 Turn off manual sample control.

8.8.11.7 Sample List

8.8.11.7.1 Adjust so that the only columns shown are “Label” “Status” “Evaluate” “Sample Type” “Standard” “Main Runs” “Rack” and “Vial” This can be done by right clicking the top row of the sample list and then selecting appropriate columns.

8.8.11.7.2 Enter each blank, standard, and sample you will run.

8.8.11.7.3 Input 3 main runs for each sample/standard.

8.8.11.7.4 Between the standards (which have high concentration) and the samples (which require detection of low concentrations), run the blank a couple of times to ensure minimal contamination.

8.8.11.7.4.1 For this purpose, do not select “BLK” as the sample type as this will include these runs as part of the calibration. Choose “unknown” instead.

8.8.11.8 Automatic Export

8.8.11.8.1 Select all boxes to automatically export all data. (Note: If **Bold** in Qtegra, there are unsaved changes.)

8.8.12 Samples

8.8.12.1 Samples in the Standard rack must have a volume of at least 20 mL or more in order for the probe to be able to detect them.

8.9 ICP-MS SHUTDOWN GUIDE

8.9.1 Qtegra → ‘Get Ready’ button → Shutdown

8.9.2 Remove tubing from the peristaltic pump to maintain their flexibility.

8.9.3 Before turning off chiller:

8.9.3.1 Ensure that IC → Advanced (in side bar) → Sampling Depth is equal to 15.

8.9.3.2 Log View should show log message ‘Standby’.

8.9.4 Turn off chiller.

9 DATA REDUCTION AND STATISTICS

9.1 CALCULATIONS

Final concentration (µg/g) = amount of digested sample (unknown) (µg) / weight of the sample (g)

The unknown amount of digested sample is quantified using a calibration curve by the Qtegra software

10 QUALITY ASSURANCE

10.1 MDLs

10.1.1 New MDLs should be calculated for every run to account for variations in method. Some example MDLs are listed below.

Replicate no.	Elemental Standard (ng/g)	Calculated Concentration (ng **U**/g soil)	Calculated Concentration (ng **As**/g soil)
1	10	10.949	10.782
2	10	11.166	10.602
3	10	11.132	10.177
4	10	11.124	10.491
5	10	10.663	10.239
6	10	10.855	9.372
7	10	10.594	9.781
8	10	10.386	9.516
STATISTICS			
Average		10.858625	10.12
Standard deviation		0.287722057	0.516147819
Student's t test		2.998	2.998
MDL		0.862590727	1.547411162
% RSD		1.085862	1.012

11 ADDITIONAL INFORMATION

11.1 REFERENCES

11.1.1 EPA Method 3015A – Microwave Assisted Acid Digestion of Aqueous Samples and Extracts

11.1.2 EPA Method 3051A – Microwave Assisted Acid Digestion of Sediments, Sludges, Soils, and Oils

11.1.3 EPA Method 3052 – Microwave Assisted Digestion of Siliceous and Organically Based Matrices

11.1.4 CEM Operation Manual

11.2 ADDITIONAL READING

11.2.1 Water Sciences Laboratory Analytical SOP, Title: ICP-MS Start Up Guide, Staff, March 2, 2018 – Present, University of Nebraska, Lincoln, NE.

12 PREVIOUS ISSUES AND CHANGES

Document File Name	Issue	Issue Effective Dates	Author
19_01_02.001 Acid Digestion	001	March 2, 2018 – December 14, 2018	Suzanne Polzkill
19_01_02.002 Acid Digestion	002	December 14, 2018 – Present	Suzanne Polzkill

12.1 ISSUE CHANGES

12.1.1 Issue 001:

- Original Issue

12.1.2 Issue 002:

- Updated information on equipment and materials
- Updated information on data reduction and statistics
- Added information about ICP-MS instrumentation
- Fixed formatting issues

6: Analysis of Dissolved Trace Elements in Aqueous Solutions by ICP-MS

1 INTRODUCTION

This procedure is used to analyze aqueous samples for dissolved trace elements in aqueous solution using inductively coupled plasma mass spectrometer (ICP-MS).

2 SCOPE AND APPLICATION

2.1 OVERVIEW

2.1.1 Available elements are measured with minimal intervention by the analyst, though certain samples may require dilution or filtration. No digestion is required prior to analysis of dissolved elements (for samples requiring digestion, see protocol 19_01_02). Standards are prepared using 0.5% nitric acid, and elemental standards for desired analytes. Specific ratios and volumes are not given in this method and must be determined on a case-by-case basis in order to maximize performance.

2.2 METHOD DETECTION LIMIT

2.2.1 New MDLs should be derived for every run to account for variations in the method. A table which includes the most recent MDLs can be found in Section 10 of this document.

2.3 ACCEPTABLE RANGES

2.3.1 The acceptable range for this method is 0 to 500 µg/L.

2.4 TRAINING TIME

2.4.1 The training time required for this method is 2-3 days.

2.5 SAMPLE PRESERVATION

2.5.1 For metal analysis, samples should be acidified (HCl). No preservation is required for anion samples. All samples should be stored in the refrigerator until analysis can be performed.

3 REQUIRED TRAINING

3.1.1 Basic lab and instrument training as detailed in the Standardized Laboratory Training Plan (07_01_09.001).

4 EQUIPMENT AND MATERIALS

4.1 APPARATUS AND MATERIALS

4.1.1 50 mL centrifuge tubes (Fisherbrand, 06-443-20).
4.1.2 Syringe filter, 25mm with 0.45 µm polyethersulfone membrane (VWR 28145-503 or equivalent).
4.1.3 Disposable syringe with polypropylene plunger, 20 mL (Fisher).
4.1.4 Adjustable micropipettes with reference tips (Eppendorf, Thermo Scientific).
4.1.5 Santoprene tubing (Spectron Inc., SIT-SANSD-049-3).
4.1.6 Peristaltic pump tubing, 0.508mm (Thermo, 1320050).
4.1.7 Sample cone, 4450 (Thermo, 3600812).
4.1.8 Ni skimmer cone 0.5, insert version (Thermo, 1311870).
4.1.9 Skimmer cone insert, 3.5 (Thermo, 1318480).
4.1.10 Sample cone gasket (Thermo, 1310900).
4.1.11 Teflon tube, 5.0M (Thermo, 1041071).
4.1.12 Teflon tube, 1/16 O.D .02ID, 10 feet (Thermo, 1600061).
4.1.13 iCap Q quartz torch (Thermo, 1230790).
4.1.14 Quartz injector, 2.5mm ID (Thermo, 1305600).
4.1.15 Ni Sample cone 4450, 1.1 mm diameter orifice (Thermo, 3600812).
4.1.16 Sampling cone gasket (Thermo, 1310900).
4.1.17 Skimmer cone insert, 3.5 (Thermo, 1318480).
4.1.18 Skimmer iCap Q/Qnova Ni for insert, 0.5mm orifice (Thermo, 1311870).
4.1.19 Cyclonic quartz spray chamber iCap Q (Thermo, 1317080).
4.1.20 MicroMist nebulizer, 0.4 mL/min (Thermo, BRE0009386).
4.1.21 Torch socket iCap Q/Qnova (Thermo, 1231000).
4.1.22 iCap Q/Qnova Quartz Torch (Thermo, 1230790).
4.1.23 Quartz injector, 2.0mm ID iCap Q/Qnova (Thermo, 1305640).

4.2 REAGENTS AND STANDARDS

4.2.1 Tube B working solution (Thermo, THERMO-4AREV).
4.2.2 Working setup solution (Thermo, THERMO-5A).
4.2.3 Nitric acid, trace metal grade (Fisher, A509-P212).
4.2.4 Hydrochloric acid, trace metal grade (Fisher, A508-P212).
4.2.5 Distilled deionized water (DDW).
4.2.6 Barium standard solution, 1000 µg/mL, 125 mL (SPEX Certiprep™, PLBA2-2Y).

4.2.7 Copper standard solution, 1000 µg/mL, 125 mL (SPEX Certiprep™, CLCU2-2Y).
4.2.8 Manganese standard solution, 1000 µg/mL, 125 mL (SPEX Certiprep™, PLMN2-2Y).
4.2.9 Strontium standard solution, 1000 µg/mL, 125 mL (SPEX Certiprep™, PLSR2-2Y).
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4.2.12 Uranium standard solution, 1000 µg/mL, 125 mL (SPEX Certiprep™, PLU2-2Y).
4.2.13 Iron standard solution, 1000 µg/mL, 125 mL (SPEX Certiprep™, PLFE2-2Y).
4.2.14 Selenium standard solution, 1000 µg/mL, 125 mL (SPEX Certiprep™, CLSE2-2Y).

5 SAFETY PRECAUTIONS

5.1 SAFETY PRECAUTIONS

5.1.1 Always wear a lab coat and gloves while handling acids.
5.1.2 All solvents and chemicals should be treated as a potential health hazard, and exposure to these chemicals should be minimized. Material Safety Data Sheets (MSDS) are available for all materials used in this procedure, and should be referred to regarding specific handling procedures and toxicity.

5.2 WASTE DISPOSAL

5.2.1 ALL acidic solutions must be neutralized with sodium bicarbonate before disposal into the sanitary sewer. Do not allow any un-neutralized acids or acidic solutions to drain into any sink in the building.
5.2.2 Check the Sewer Disposal List in Room 203 for the list of substances that can be disposed of down the sanitary sewer.

6 SOLUTIONS AND REAGENTS

6.1 0.5% NITRIC ACID SOLUTION

Nitric Acid (HNO_3)	63.01 g/mol	40 mL
Distilled Deionized Water	18.015 g/mol	

Protocol: There is a container on the work bench of the ICP-MS Lab (Room 204A) designated for the creation and storage of 0.5% Nitric Acid for the ICP-MS. When this container is empty, simply add 40 mL of concentrated nitric acid to the container, and fill the container to the established line using distilled deionized water from the dispenser on the wall. Remember to add the water first, and then add the acid.
Storage: Excess solution can be stored in the designated plastic containers in the ICP-MS lab.

Disposal: Acids should be neutralized using sodium bicarbonate before disposal in any of the lab drains.

7 STANDARD SOLUTIONS

7.1 BLANKS

7.1.1 For metal analysis, blanks and calibration standards will be prepared with 0.5% nitric acid (v/v) in distilled deionized water. For some other samples, distilled deionized water is used instead. Refer to training for specific elements (like anions).

7.1.2 Two types of blanks are required for analysis. The calibration blank is used in establishing the calibration curve. The rinse blank is used to flush the system between samples and standards.

7.1.2.1 The calibration blank ought to consist of the same stock of 0.5% nitric acid or distilled deionized water used to prepare the final dilutions of the calibration standard solutions. This 50 mL centrifuge tube should be kept as full as possible throughout the standard preparation process to ensure the process is as uniform as possible.

7.2 CALIBRATION STANDARDS

7.2.1 For metal analysis, blanks and calibration standards will be prepared with 0.5% nitric acid (v/v) in distilled deionized water. For some other samples, distilled deionized water is used instead. Refer to training for specific elements (like anions).

7.2.2 Fresh multi-element calibration standards should be prepared every two weeks, or as needed.

7.2.3 Dilute each of the stock multi-element standard solutions to levels appropriate to the operating range of the instrument using the calibration blank, which is composed of either 0.5% nitric acid or distilled deionized water. Calibration standards should be prepared at a minimum of 3 concentration levels. The equation for dilution is as follows:

$$M_1V_1 = M_2V_2$$

8 PROTOCOL

8.1 SAMPLE PREPARATION

8.1.1 Samples which are absent of particulate matter can be transferred directly into autosampler tubes for analysis. There should be approximately an inch of sample in each tube to ensure flow into the autosampler probe.

8.1.2 If particulate matter is present in the sample solutions, they must be filtered to avoid clogging the ICP-MS tubes and nebulizer. If necessary, utilize a polyethersulfone filter. Filter samples through filter paper into ICP-MS autosampler tubes before analysis.

8.2 SAMPLE RE-ANALYSIS

8.2.1 Dilute and re-analyze samples that are more concentrated than the linear range for a particular analyte.
8.2.2 This should be indicated in Qtegra under Evaluation Results → Concentrations, where samples outside the linear range will be highlighted in red.

8.3 ICP-MS START-UP GUIDE

8.3.1 Note: Always wear gloves when handling any part of the instrument including pumps, tubing, and any samples/standards. The computer/desk area and the chiller are no gloves access.
8.3.2 Turn on the chiller by pushing the power button (no gloves access).
    8.3.2.1 Check water level on the front of the chiller – add DI water from carboy under desk if necessary.
8.3.3 Check levels of spent waste and 0.5% HNO3 containers located on the desk to the right of the auto sampler.
    8.3.3.1 Fill HNO3 if necessary from the 0.5% HNO3 container located next to the DDI water dispenser.
    8.3.3.2 Empty waste container in the Acids lab sink.
8.3.4 Go to the computer. This area is no gloves access. Open programs Qtegra, Instrument Control (IC), and Camera. Maximize the IC window.
8.3.5 Turn on gases to allow gas temperature to stabilize (especially important in cold weather).
    8.3.5.1 From IC -> Main (in left-bottom side bar) -> Nebulizer gas.
        8.3.5.1.1 Click on small arrow (∆) below the bar to turn on the gas to appropriate level.
    8.3.5.2 From IC -> Plasma (in left-bottom side bar) -> Auxiliary gas.
        8.3.5.2.1 Click on small arrow (∆) below bar to turn on gas to appropriate level.
    8.3.5.3 From IC -> Plasma (in left-bottom side bar) -> Cool gas.
        8.3.5.3.1 Click on small arrow (∆) below bar to turn on gas to appropriate level.
8.3.6 The peristaltic (peri) pump is located on the right side of the iCAP RQ.
    8.3.6.1 There are 2 tubes: yellow (outlet) and clear (inlet). Both tubes should be replaced daily or after 8 hours of continuous use. When in doubt, change the tubing!!
    8.3.6.2 Attach the outlet tube on the last groove at the back of the peri-pump, first securing one side and then the other in the plastic grooves underneath the pump. Close the plastic flaps. **DO NOT SECURE THE TUBING WITH NOTCHES AT THIS STAGE!** The peri pump will spin in

a clockwise direction. It is a common error to place the tubes in reverse of flow path. Avoid this by tracing the intended flow path of the tubes to ensure they are going through the pump in the correct direction.

8.3.6.3 Turn on the peristaltic pump. From IC -> Inlet (in side bar) -> Peristaltic Pump On. Peristaltic Pump Turbo -> High.

8.3.6.4 Confirm the tubes are stable (not shaking/moving/bumping a lot). If stable, lock tubes into place **WITH THE NOTCHES** after turning off the pump. If not stable, call for help!

8.3.6.5 Check pump flow. Before that turn on the peristaltic pump (left menu).

8.3.6.5.1 From IC's ASX-560 tab, direct autosampler probe to go to a Blank sample containing 0.5% HNO3 (generally located in Standard Rack, Vial 1. Click on 1 in row R in the ASX-560 software to direct the probe to position 1).

8.3.6.5.2 Watch the intake tubes going out of the auto sampler probe – you will be able to see when liquid begins to flow through the line as well as any air bubbles that may be present.

8.3.6.5.3 Allow the pump to stabilize for ~5 minutes.

8.3.6.5.4 Confirm intake flow.

8.3.6.5.4.1 Intake flow should appear to be a steady, unbroken stream of solution. This can be checked by introducing an air gap in the auto sampler.

8.3.6.5.4.2 This can be done by manually lifting the sampling probe from the blank solution for a few seconds to introduce air bubble. Lower probe back into solution.

8.3.6.5.4.2.1 Confirm that air bubble moves steadily through the inflow tube.

8.3.6.5.4.2.2 If air bubble is not present, or if it is not moving steadily, there is an error in the tubing/pumping. Do Not Proceed!! Call for Help!!

8.3.6.5.4.3 Check output tube to ensure there is outflow.

8.3.6.5.4.3.1 NOTE: The outflow will be a broken stream of solution because this tube is slightly larger. However, flow should still be steady and fast. Check for any liquid build up at the elbow of the spray chamber. If you see any drops of liquid inside the chamber, turn off the peri pump, and call for help.

8.3.7 Warm-Up

8.3.7.1 From Qtegra -> 'Get Ready' button -> Select 'Warm Up' for 15 minutes -> OK

8.3.7.1.1 Do not select 'Perform Validation Tests' – this will be done later.

8.3.7.2 See camera to ensure that plasma is ignited and monitor Log View Tab for any errors until you see the "Operate" log.

8.3.7.3 Allow warm up to finish, software will notify when it is ready.

8.3.8 Performance Report (PR)

8.3.8.1 In IC, use ASX-560 tab to command probe to Tune solution (generally located in Standard Row, Vial 10). Make sure the solution is filled to 50 mL.

8.3.8.1.1 Allow the pump to take up tuning solution for 120 seconds by clicking the High mode of peri pump, one can monitor the flow by clicking Run (in 'Control' tab) in IC located at Top Left Corner. After the tuning solution has reached plasma a rise in peak will be seen, after that click Normal mode in peri pump allow to stabilize the speed for ~40 seconds before running PR. NOTE: Not allowing pump to stabilize generally will lead to Mass Calibration errors)

8.3.8.2 Performance Reports (PR) should be run for STD and KED mode.

8.3.8.2.1 From IC -> Performance Report (in 'Wizards' tab)

8.3.8.2.1.1 To run a report in STD: 'Run an Existing Report' -> STD -> Next -> Next

8.3.8.2.1.2 To run a report in KED:

8.3.8.2.1.2.1 Change measurement mode to KED (from 'Measurement mode' tab in IC)

8.3.8.2.1.2.2 'Run a Report in the Active Measurement Mode' -> Next -> Next

8.3.8.2.1.3 Pay careful attention to the parameters which are passing/failing in the PR results.

8.3.9 Troubleshooting Performance Report Failure **(NOTE: For advanced troubleshooting, refer to the Instrument Manual)**

8.3.9.1 If a single sensitivity parameter is failing the PR, allow the instrument to run for a few minutes and retry the PR – the machine often just needs time to settle itself. If this does not work, the instrument needs to be tuned.

8.3.9.2 AutoTune

8.3.9.2.1 From IC -> AutoTune (in 'Wizards' tab)

8.3.9.2.2 Ensure probe is in TuneB solution (generally located in Standard Row, Vial 10) and Run.

8.3.9.2.3 Pay attention to which AutoTune adjustments affect which sensitivity parameters. For example, you might notice that adjustments to the extract lens affect one parameter, while adjustments to the nebulizer gas affect another. Know which adjustments affect the parameters that caused the PR failure.

8.3.9.2.4 Save the AutoTune values and re-run Performance Report.

8.3.9.3 If the Performance Report fails again, reference old tune settings from successful runs to manually adjust the instrument.

8.3.9.3.1 From IC -> Tune Settings (in 'Measurement Mode' tab) -> History

8.3.9.3.2 CTRL + Click on two AutoTune reports, your most recent (failed) run and a previous (successful) run

8.3.9.3.3 Click "Compare" to see which instrument tune settings are different. Use this information to make minor adjustments to instrument settings before running the PR again.

8.3.9.4 A Troubleshooting AutoTune can also be run if PR fails after tuning.
8.3.9.4.1 From IC -> AutoTune Wizard in AutoTune dropdown menu (in 'Wizards' tab)
8.3.9.4.2 Select High Matrix AutoTune Troubleshoot
8.3.9.5 In extreme cases, there are two other AutoTune methods, which can be used to adjust the instrument settings. These two methods must be used consecutively **and under supervision**.
8.3.9.5.1 From IC -> AutoTune Wizard in AutoTune dropdown menu (in 'Wizards' tab)
8.3.9.5.1.1 Select CaliTune STDS
8.3.9.5.2 From IC -> AutoTune Wizard in AutoTune dropdown menu (in 'Wizards' tab)
8.3.9.5.2.1 Select CaliTune STDS 100V. **For Advanced troubleshooting, look in the manual!**

8.3.10 Wash all tuning solution from the probe.
8.3.10.1 From IC ASX-560 tab, command the probe to a Rinse solution containing 0.5% HNO_3.
8.3.10.2 'Run' while viewing the analyte levels; the levels will be high at first (due to presence of tuning solution) but will eventually decrease to near zero. ICP-MS is verified for any measurement of choice, like dissolved elements, total digested elements or speciation (all have separate SOP).

8.3.11 Creating a LabBook.
8.3.11.1 In Qtegra -> LabBooks (in left side bar)
8.3.11.2 Create a LabBook using an existing Template or LabBook or from a blank Template.
8.3.11.3 Analytes
8.3.11.3.1 Select desired element analytes, taking care to select the isotopes with the least amount of interference.
8.3.11.4 Acquisition parameters
8.3.11.4.1 Dwell time should always be set to ~0.1s for all analytes.
8.3.11.5 Standards
8.3.11.5.1 Include an entry for each standard you will be including in your calibration.
8.3.11.5.2 When creating a new entry, select 'Create from Analyte List' to ensure that all the analytes you are testing are accounted for in your standards.
8.3.11.6 Manual Sample Control
8.3.11.6.1 Turn off manual sample control.
8.3.11.7 Sample List
8.3.11.7.1 Adjust so that the only columns shown are "Label" "Status" "Evaluate" "Sample Type" "Standard" "Main Runs" "Rack" and "Vial" This can be done by right clicking the top row of the sample list and then selecting appropriate columns.
8.3.11.7.2 Enter each blank, standard, and sample you will run.
8.3.11.7.3 Input 3 main runs for each sample/standard.

8.3.11.7.4 Between the standards (which have high concentration) and the samples (which require detection of low concentrations), run the blank a couple of times to ensure minimal contamination.

8.3.11.7.4.1 For this purpose, do not select “BLK” as the sample type as this will include these runs as part of the calibration. Choose “unknown” instead.

8.3.11.8 Automatic Export

8.3.11.8.1 Select all boxes to automatically export all data. (Note: If **Bold** in Qtegra, there are unsaved changes.)

8.3.12 Samples

8.3.12.1 Samples in the Standard rack must have a volume of at least 20 mL or more in order for the probe to be able to detect them.

8.4 ICP-MS SHUTDOWN GUIDE

8.4.1 Qtegra → ‘Get Ready’ button → Shutdown

8.4.2 Remove tubing from the peristaltic pump to maintain their flexibility.

8.4.3 Before turning off chiller:

8.4.3.1 Ensure that IC → Advanced (in side bar) → Sampling Depth is equal to 15.

8.4.3.2 Log View should show log message ‘Standby’.

8.4.4 Turn off chiller.

9 DATA REDUCTION AND STATISTICS

9.1.1 Qtegra software generates the calibration curves and performs the quantitation

10 QUALITY ASSURANCE

10.1 MDLs

10.1.1 New MDLs should be calculated for every run to account for variations in method. Some example MDLs are listed below.

Replicate no.	Elemental Standard (ng/mL)	Calculated Concentration (ng **Ba**/mL)	Calculated Concentration (ng **Sr**/mL)	Calculated Concentration (ng **Zn**/mL)
1	0.5	0.525	0.530	0.765
2	0.5	0.525	0.523	0.750
3	0.5	0.530	0.525	0.777
4	0.5	0.518	0.524	0.768
5	0.5	0.527	0.516	0.799
6	0.5	0.528	0.522	0.772
7	0.5	0.508	0.516	0.771
8	0.5	0.509	0.525	0.755

STATISTICS			
Average	0.52125	0.522625	0.769625
Standard deviation	0.008615	0.004719	0.01487
Student's t test	2.998	2.998	2.998
MDL	0.025827	0.014147	0.044581
% RSD	1.6528	0.90294	1.9321

Replicate Number	Elemental Standard (ng/mL)	Calculated Concentration (ng U/mL)
1	1.0	1.280
2	1.0	1.224
3	1.0	1.189
4	1.0	1.118
5	1.0	1.151
6	1.0	1.167
7	1.0	1.143
8	1.0	1.135
9	1.0	1.120
10	1.0	1.130
11	1.0	1.130
12	1.0	1.094

STATSITICS	
Average	1.15675
Standard deviation	0.052026
Student's t test	2.718
MDL	0.003722064
% RSD	1.4008

Replicate Number	Elemental Standard (ng/mL)	Calculated Concentration (ng As/mL)
1	0.5	0.482
2	0.5	0.480
3	0.5	0.485
4	0.5	0.487
5	0.5	0.487
6	0.5	0.486
7	0.5	0.469
8	0.5	0.477
9	0.5	0.461
10	0.5	0.483
11	0.5	0.465
12	0.5	0.461
13	0.5	0.470

STATSITICS	
Average	0.476
Standard deviation	0.010
Student's t test	2.681

MDL	0.027
% RSD	21.01

11 ADDITIONAL INFORMATION

11.1 REFERENCES

11.1.1 EPA Method 6020 – Inductively Coupled Plasma Mass Spectrometry

11.2 ADDITIONAL READING

11.2.1 Water Sciences Laboratory Analytical SOP, Title: ICP-MS Start Up Guide, Staff, March 2, 2018 – Present, University of Nebraska, Lincoln, NE.

11.3 ATTACHMENTS

This section should be used to reference any outside documents, such as excel sheets, which are associated with this method. Any files referenced in this section should be placed on Box under Lab Manual – Part 5 Supporting Files.

12 PREVIOUS ISSUES AND CHANGES

Document File Name	Issue	Issue Effective Dates	Author
19_01_01.001 Trace Elements in Aqueous Solutions	001	March 14th, 2018 – December 12th, 2018	Suzanne Polzkill and Tania Biswas
19_01_01.002 Trace Elements in Aqueous Solutions	002	December 12th, 2018 – Present	Tania Biswas

12.1 ISSUE CHANGES

12.1.1 Issue 001:
- Original Issue

12.1.2 Issue 002:
- Updated information on equipment and materials
- Updated information on data reduction and statistics

- Fixed formatting issues

7: Analysis of herbicides in solid samples (i.e. soils, manure, etc) by microwave assisted solvent extraction (MASE) and gas chromatography mass spectrometry (GC/MS)

1 INTRODUCTION

This method is to be used for the extraction and elution of compounds from solid mediums (soils, manure, etc.) for gas chromatography mass spectrometry (GC/MS). MASE (microwave-assisted solvent extraction) is used. MASE is a procedure for extracting water insoluble or slightly water soluble organic compounds from soils, clays, sediments, sludges, and solid wastes. This procedure uses microwave energy to produce elevated temperature and pressure conditions in a closed vessel containing the sample and organic solvent(s) to achieve analyte recoveries equivalent to those from traditional extraction. The benefits of this method are enhanced extraction times, low solvent consumption, and improved extraction efficiencies.

2 SCOPE AND APPLICATION

2.1 OVERVIEW

2.1.1 Two to five grams of wet sediment or soil is weighed into a 10 milliliter (mL) Teflon microwave digestion vessel, spiked with surrogate compounds, and thoroughly mixed with 6 mL of extraction solvent (acetonitrile). Batches of up to 40 samples are subjected to microwave irradiation (400W) for 10 minutes at 90°C using a MARS Xpress microwave digestion system. Sediment is washed a second time with acetonitrile, combined with the first portion, and volume reduced to near 1-2 mL under dry nitrogen. The sample is spiked with internal standard, diluted in 100 mL of water, and extracted onto a C_{18} solid phase extraction (SPE) cartridge under vacuum. Absorbed compounds are then eluted with ethyl acetate. Sodium sulfate is used to capture water residue. Extracts evaporated to dryness and brought up with 200 µl ethyl acetate before injection onto a gas chromatograph (GC) equipped with a mass selective detector (MSD).

2.2 METHOD DETECTION LIMIT

2.2.1 Detection limits are in the low nanogram per gram levels depending on

compound.

2.3 TRAINING TIME

2.3.1 Training time will be extended across two to three days, as the preparation process is lengthy.

2.4 SAMPLE PRESERVATION

2.4.1 Samples should be preserved by being stored in a freezer. After extraction onto C18 cartridges, samples can be stored in the freezer for 8 weeks.

3 REQUIRED TRAINING

3.1.1 07_02_11 Laboratory Waste Disposal and Management
3.1.2 07_02_05 Balances
3.1.3 07_02_10 Micropipette
3.1.4 Use of the following equipment: RapidVap, MARS microwave, Vacuum Extraction Manifold, Elution Manifold, and Nitrogen Blowdown Manifold.

4 EQUIPMENT AND MATERIALS

4.1 APPARATUS AND MATERIALS

4.1.1 Disposable filter device, 25mm 0.2µm PTFE GD/X filter (GE Healthcare 6874-2502 or equivalent).
4.1.2 Top-loading analytical balance, precision to 1x10-2 g (Mettler PJ360 or equivalent).
4.1.3 Laboratory gloves.
4.1.4 Kimwipe tissues.
4.1.5 Autosampler vials with crimp cap (National Scientific #C4012-1 & C4011-1A or equivalent).
4.1.6 Conical vial inserts with spring type bottom (National Scientific #C4010-S630 or equivalent).
4.1.7 Disposable Pasteur pipette, 225mm (Fisher Scientific, 13-678-20D).
4.1.8 Disposable glass culture tube, 10 x 75mm (Fisher Scientific, 14-961-25).
4.1.9 Disposable glass culture tube, 15 x 85mm (Fisher Scientific, 14-961-28).
4.1.10 Disposable liners for Visiprep DL (Supelco, 57059).
4.1.11 Dry evaporation apparatus (Labconco RapidVap N2 system or equivalent).
4.1.12 Flat-bottomed glass evaporation tubes, 170 mL (Labconco 9727000).
4.1.13 Benchtop Centrifuge (Beckman Coulter, Allegra 6, or equivalent).
4.1.14 Disposable syringe with polypropylene plunger and Luer Lock tip, 6 mL (Fisher).
4.1.15 Microwave extraction Teflon vessels, 20 mL (Medium size, CEM Microwave Technology).
4.1.16 Microwave solvent extraction apparatus, MARS Xpress System (CEM Microwave

Technology).

4.1.17 Top-loading analytical balance, precision to $1x10^{-2}$ g (Mettler PJ360 or equivalent).

4.1.18 Stainless steel spatula.

4.1.19 Adjustable micropipettes with reference tips, 10 mL and 100 µL (Eppendorf).

4.1.20 Plastic syringe, 20 cc (Norm-Ject all-polypropylene design).

4.1.21 Vortex mixer.

4.1.22 Adapters for sample reservoir (Supelco).

4.1.23 Flow control valve (Supelco).

4.1.24 Vacuum elution manifold with drying attachment (Supelco Visiprep DL).

4.1.25 Flow control liner (Gast. DOA-P135-AA).

4.1.26 Vacuum pump (GAST, DOA-P135-AA).

4.1.27 Sep-Pak Plus tC18 Environmental Cartridges (Waters, WAT036800).

4.2 REAGENTS

4.2.1 Methanol (Optima, Fisher Scientific).

4.2.2 Acetonitrile (Optima, Fisher Scientific).

4.2.3 Ethyl Acetate (Optima, Fisher Scientific).

4.2.4 Distilled Deionized Water (DDW).

4.2.5 Compressed nitrogen gas.

4.2.6 Isotopically labeled internal standards: *Deethylatrazine-13C, Deisopropylatrazine-13C, Atrazine-13C.*

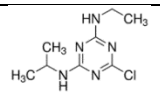
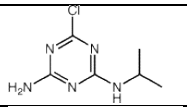
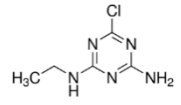
4.2.7 Chemically pure unlabeled analytes: *Acetochlor, Alachlor, Metolachlor, Propachlor, Atrazine, Butylate, Chlorothalonil, Cyanazine, Deethylatrazine (DEA), Deisopropylatrazine (DIA), Dimethenamide, S-ethyl-N,N-dipropylthiocarbamate (EPTC), Metribuzin, Pendamethalin, Permethrin, Propazine, Simazine, Triflurarin, Prometon, Norflorazon, Tefluthrin.*

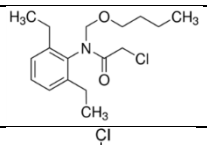
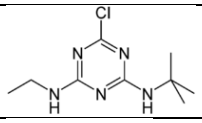
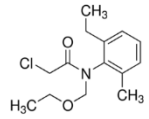
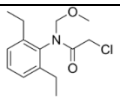
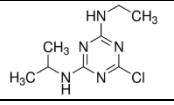
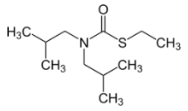
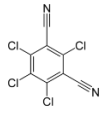
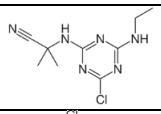
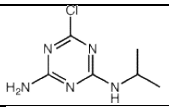
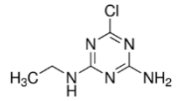
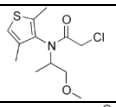
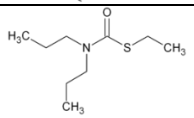
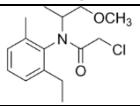
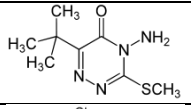
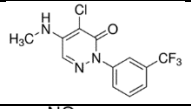
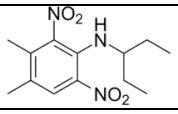
4.2.8 Surrogate compounds: *Butachlor, Terbutylazine.*

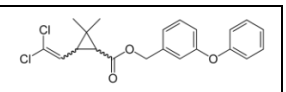
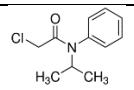
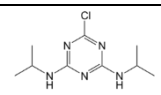
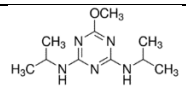
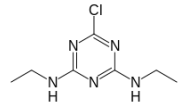
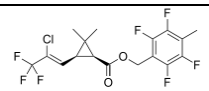
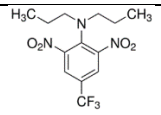
4.3 CHEMICALS

Chemical	CAS Number	Hazards	Location
Sodium Sulfate	7757-82-6	Irritant	Desiccator Room 203

Table 1. Analyte Physical Parameters

Analyte	CAS Number	Mol. Formula	Mol. Weight (g/mol)	Quantitative Ion (m/z)	Structure
$^{13}C_3$-Atrazine (IS)	1443685-80-0	$^{13}C_3C_5H_{14}ClN_5$	218.70	203	
^{13}C-DEA (IS)	111-42-2	$^{13}C_3C_3H_{10}ClN_5$	190.83	175	
^{13}C-DIA (IS)		$^{13}C_3C_2H_8ClN_5$	176.80	176	

Butachlor (Surrogate)	23184-66-9	$C_{17}H_{26}ClNO_2$	311.85	176	
Terbutylazine (Surrogate)	5915-41-3	$C_9H_{16}ClN_5$	229.71	214	
Acetochlor	34256-82-1	$C_{14}H_{20}ClNO_2$	269.77	146	
Alachlor	15972-60-8	$C_{14}H_{20}ClNO_2$	269.767	160	
Atrazine	1912-24-9	$C_8H_{14}ClN_5$	215.68	200	
Butylate	2008-41-5	$C_{11}H_{23}NOS$	217.37	146	
Chlorthalonil	1897-45-6	$C_8Cl_4N_2$	265.90	266	
Cyanazine	21725-46-2	$C_9H_{13}ClN_6$	240.69	212	
DEA	6190-65-4	$C_6H_{10}ClN_5$	187.63	172	
DIA	1007-28-9	$C_5H_8ClN_5$	173.60	158	
Dimethenamid	87674-68-8	$C_{12}H_{18}ClNO_2S$	275.79	154	
EPTC	759-94-4	$C_9H_{19}NOS$	189.32	128	
Metolachlor	51218-45-2	$C_{15}H_{22}ClNO_2$	283.80	162	
Metribuzin	21087-64-9	$C_8H_{14}N_4OS$	214.29	198	
Norflorazon	27314-13-2	$C_{12}H_9ClF_3N_3O$	303.67	303	
Pendimethalin	40487-42-1	$C_{13}H_{19}N_3O_4$	281.31	252	

Permethrin	52645-53-1	$C_{21}H_{20}Cl_2O_3$	391.29	183	
Propachlor	1918-16-7	$C_{11}H_{14}ClNO$	211.69	120	
Propazine	139-40-2	$C_9H_{16}ClN_5$	229.71	214	
Prometon	1610-18-0	$C_{10}H_{19}N_5O$	225.29	210	
Simazine	122-34-9	$C_7H_{12}ClN_5$	201.66	201	
Tefluthrin	79538-32-2	$C_{17}H_{14}ClF_7O_2$	418.74	177	
Trifluralin	1582-09-8	$C_{13}H_{16}F_3N_3O_4$	335.28	306	

All neat analytes are stored in Freezer 4.  GHS06,  GHS07,  GHS08,  GHS09

5 SAFETY PRECAUTIONS

5.1 SAFETY PRECAUTIONS

5.1.1 All solvents and chemicals should be treated as a potential health hazard, and exposure to these chemicals should be minimized. Material Safety Data Sheets (MSDS) are available for all materials used in this procedure and should be referred to regarding specific handling procedures and toxicity.

5.2 WASTE DISPOSAL

5.2.1 All organic solvents should be disposed of in the proper waste container in Room 203 for collection by UNL Environmental Health and Safety. Do not allow any organic solvents to enter the sanitary sewer system.

5.2.2 ALL acidic solutions *must* be neutralized with sodium bicarbonate before disposal into the sanitary sewer. Do not allow any un-neutralized acids or acidic solutions to drain into any sink in the building.

5.2.3 Check the Sewer Disposal List in Room 203 for the list of substances that can be disposed of down the sanitary sewer system.

6 SOLUTIONS AND REAGENTS

6.1 INDIVIDUAL STOCK SOLUTIONS OF ANALYTES (PREPARED IN METHANOL)

Analyte	Concentration	Volume
Simazine	0.5 µg/µL	40 mL
All other Analytes, Surrogates and IS	5.0 µg/µL	4 mL

Protocol: (**Note**: Neat analytes which are already in a solution of known concentration do not need this step.) Accurately weigh approximately 20 mg of each neat analyte into a separate 4 mL silanized amber vial. Add 4.0 mL (3.1656 g) of methanol by weight (d_{20}=0.7914 g/mL) to dissolve the analyte. Volume (weight) of methanol can be adjusted to obtain concentration values near 5.0 µg/µL. (Simazine is not soluble in methanol at 5 µg/µL. Use a 40 mL silanized amber vial and add 40 mL of methanol to obtain a stock concentration of 0.5 µg/µL.) Sonication may be required to dissolve certain analytes. Individual stock solutions are utilized for both calibration and spiking solutions.
Storage: Solutions can be stored at -20°C for up to 6 months.
Disposal: EHS waste disposal protocols are followed.

6.2 INTERNAL STANDARD SPIKING MIX (PREPARED IN METHANOL, FINAL CONCENTRATION = 50 NG/µL)

Internal Standard	Concentration	Volume
$^{13}C_3$-DIA	5 µg/µL	500 µL
$^{13}C_3$-DEA	5 µg/µL	500 µL
$^{13}C_3$-Atrazine	5 µg/µL	500 µL

Protocol: Remove individual stock solutions of $^{13}C_3$-atrazine, $^{13}C_3$-DIA, and $^{13}C_3$-DEA from the freezer. Sonicate the solutions for 30 minutes or until all solids are dissolved. Accurately pipet 500 µL of each individual internal standard stock solution into a clean 50 mL volumetric flask. Dilute to 50.0 mL with methanol and mix completely. Store in a silanized amber 60 mL vial.
Storage: Solution can be stored at -20°C for up to 6 months.
Disposal: EHS waste disposal protocols are followed.

6.3 SURROGATE SPIKING MIX (PREPARED IN METHANOL, FINAL CONCENTRATION = 20 NG/µL)

Surrogate	Concentration	Volume
Butachlor	5 µg/µL	200 µL
Terbutylazine	5 µg/µL	200 µL

Protocol: Remove the individual stock solutions of terbutylazine and butachlor from the freezer. Sonicate these solutions for 30 minutes or until all solids are dissolved. Accurately pipet 200 µL of each individual surrogate stock solution into a clean 50 mL volumetric flask. Dilute to 50.0 mL with methanol and mix completely. Store in a silanized amber 60 mL vial.
Storage: Solution can be stored at -20°C for up to 6 months.

Disposal: EHS waste disposal protocols are followed.

6.4 Analyte Spiking Mix (prepared in methanol, final concentration = 10 ng/µL, (20 ng/µL for selected analytes))

Analyte	Concentration	Volume
Simazine	0.5 µg/µL	1000 µL
DIA, Cyanazine, Permethrin	5 µg/µL	200 µL
Other analytes	5 µg/µL	100 µL

Protocol: Remove the individual stock solutions of analytes from the freezer. Sonicate these solutions for 30 minutes or until all solids are dissolved. Accurately pipet the desired volume of each individual analyte stock solution into a clean 50 mL volumetric flask. Dilute to 50.0 mL with methanol and mix completely. Store in a silanized amber 60 mL vial.
Storage: Solution can be stored at -20°C for up to 6 months.
Disposal: EHS waste disposal protocols are followed.

6.5 Internal Standard Calibration Mix (prepared in ethyl acetate, final concentration = 500 ng/µL)

Internal Standard	Concentration	Volume
$^{13}C_3$-DIA	5 µg/µL	1000 µL
$^{13}C_3$-DEA	5 µg/µL	1000 µL
$^{13}C_3$-Atrazine	5 µg/µL	1000 µL

Protocol: Remove individual stock solutions of $^{13}C_3$-atrazine, $^{13}C_3$-DIA, and $^{13}C_3$-DEA from the freezer. Sonicate the solutions for 30 minutes or until all solids are dissolved. Accurately pipet 1000 µL of each individual internal standard stock solution into a clean 10 mL volumetric flask. Dilute to 10.0 mL with ethyl acetate and mix completely. Store in a silanized amber 12 mL vial.
Storage: Solution can be stored at -20°C for up to 6 months.
Disposal: EHS waste disposal protocols are followed.

6.6 Analyte Mix A (prepared in ethyl acetate, final concentration = 50 ng/µL (100 ng/µL for Cyanazine))

Analyte	Concentration	Volume
Cyanazine	5 µg/µL	1000 µL
Acetochlor, Alachlor, Atrazine, DEA, Metolachlor	5 µg/µL	500 µL

Protocol: Remove the individual stock solutions of analytes from the freezer. Sonicate these solutions for 30 minutes or until all solids are dissolved. Accurately pipet 500 µL (1000 µL for cyanazine) of each individual surrogate stock solution into a clean 50 mL

volumetric flask. Dilute to 50.0 mL with ethyl acetate and mix completely. Store in a silanized amber 60 mL vial.
Storage: Solution can be stored at -20°C for up to 6 months.
Disposal: EHS waste disposal protocols are followed.

6.7 Analyte Mix B (prepared in ethyl acetate, final concentration = 50 ng/µL (100 ng/µL for DIA and permethrin))

Analyte	Concentration	Volume
Simazine	0.5 µg/µL	5000 µL
DIA and Permethrin	5 µg/µL	1000 µL
Surrogates (Butachlor and Terbutylazine)	5 µg/µL	500 µL
All other Analytes (except those in Analyte Mix A)	5 µg/µL	500 µL

Protocol: Remove the individual stock solutions of analytes from the freezer. Sonicate these solutions for 30 minutes or until all solids are dissolved. Accurately pipet 500 µL (5000 µL for simazine and 1000 µL for DIA and permethrin) of each individual stock solution into a clean 50 mL volumetric flask. Dilute to 50.0 mL with ethyl acetate and mix completely. Store in a silanized amber 60 mL vial.
Storage: Solution can be stored at -20°C for up to 6 months.
Disposal: EHS waste disposal protocols are followed.

6.8 Dilute Analyte Spiking Solution (prepared in methanol, final concentration = 1 ng/µL)

Internal Standard	Concentration	Volume
Analyte Spiking Mix	10 ng/µL	1000 µL
Methanol	-	9000 µL

Protocol: Remove 10 ng/µL Internal Standard spiking mix from the freezer. Sonicate the solution for 5 minutes or until all solids are dissolved. Accurately pipet 1000 µL of the analyte spiking mix into a clean 10 mL volumetric flask. Dilute to 10.0 mL with methanol and mix completely. Store in a silanized amber 12 mL vial.

Storage: Solution can be stored at -20°C for up to 6 months.
Disposal: EHS waste disposal protocols are followed.

6.9 Dilute Internal Standard Spiking Solution (prepared in methanol, final concentration = 5 ng/µL)

Internal Standard	Concentration	Volume
Internal Standard Spiking Mix	50 ng/µL	1000 µL
Methanol	-	9000 µL

Protocol: Remove 50 ng/µL Internal Standard spiking mix from the freezer. Sonicate the solution for 5 minutes or until all solids are dissolved. Accurately pipet 1000 µL of the internal standard spiking mix into a clean 10 mL volumetric flask. Dilute to 10.0 mL with methanol and mix completely. Store in a silanized amber 12 mL vial.
Storage: Solution can be stored at -20°C for up to 6 months.
Disposal: EHS waste disposal protocols are followed.

6.10 DILUTE SURROGATE SPIKING SOLUTION (PREPARED IN METHANOL, FINAL CONCENTRATION = 2 NG/µL)

Internal Standard	Concentration	Volume
Surrogate Spiking Mix	20 ng/µL	1000 µL
Methanol	-	9000 µL

Protocol: Remove 20 ng/µL Internal Standard spiking mix from the freezer. Sonicate the solution for 5 minutes or until all solids are dissolved. Accurately pipet 1000 µL of the surrogate spiking mix into a clean 10 mL volumetric flask. Dilute to 10.0 mL with methanol and mix completely. Store in a silanized amber 12 mL vial.
Storage: Solution can be stored at -20°C for up to 6 months.
Disposal: EHS waste disposal protocols are followed.

7 STANDARD SOLUTIONS

7.1.1 Remove the Standard Mix and Analyte Mixes A and B from the freezer and allow them to equilibrate to room temperature. Deliver the amounts listed in Table 1 of Standard Mix, Analyte Mix A, and Analyte Mix B into a clean 10 mL volumetric flask for each solution.

Table 1. Volumes of Standard Mix, Analyte Mix A, and Analyte Mix B needed to prepare GC/MSD Calibration standards.

Calibration standard level	Standard Mix volume (µL)	Analyte Mix A volume (µL)	Analyte Mix B volume (µL)
5	500	50	50
4	500	800	400
3	500	2700	1350
2	500	4600	2300
1	500	6500	3000

7.1.2 Dilute each of the above solutions to 10 mL using ethyl acetate, cap the flask and mix well for at least one minute. Transfer the contents of each to a separate 12 mL silanized amber glass vial. Label each calibration solution in the following format:

WSL Book # – Page # – Calibration Standard Level

7.1.3 Note: If only one volumetric flask is used to make the standards, start with the lowest concentration and proceed to the largest while cleaning the flask between each using the procedure described in the Glassware Cleaning SOP.

The approximate concentrations of analytes in each calibration standard are listed in Table 2.

Table 2. Approximate concentrations of analytes contained in GC/MSD Calibration standards.

Calibration standard level	$^{13}C_3$-IS (ng/µL)	DIA Permethrin (ng/µL)	Cyanazine (ng/µL)	DEA Atrazine Acetochlor Alachlor Metolachlor (ng/µL)	Other Analytes (ng/µL)
5	25.0	0.5	0.25	0.25	0.25
4	25.0	3.0	6.0	3.0	1.5
3	25.0	8.0	15.0	7.5	4.0
2	25.0	20.0	40.0	20.0	10.0
1	25.0	30.0	65.0	32.5	15.0

8 PROTOCOL

8.1 EXTRACTION PROCEDURE

8.1.1 Weighing samples:
- 8.1.1.1 Remove samples from a freezer and allow them to thaw at room temperature.
- 8.1.1.2 Place a 10 mL Teflon tube on balance and tare.
- 8.1.1.3 Using a spatula, weigh 5.0 g wet sediment into the Teflon tube.
- 8.1.1.4 Add 6 mL acetonitrile to each sample.

8.1.2 Surrogate and Analyte Spikes:
- 8.1.2.1 Sonicate spikes for at least 30 min prior to use.
- 8.1.2.2 Spike all samples with 100 µL of 2.0 ng/µL surrogate spike for a total mass of 200 ng.
- 8.1.2.3 Spike LFM and LFB samples with 100 µL of 1.0 ng/µL analyte spike for a total mass of 100 ng. If performing MDLs, spike MDL samples with 15 µL of 1.0 ng/µL analyte spike for a total mass of 15 ng.

8.1.3 Vortex and equilibrate for 30 min.

8.1.4 Mars Xpress parameters:
- 8.1.4.1 Place tubes in the MARS microwave

8.1.4.2 Extract samples using the following parameters (protocol should be saved under EPA 3546 solids_Xpress)

Ramp time:	10 min
Watts:	400 W
Power:	100%
Temperature:	90°C
Hold time:	10 min

8.1.4.3 After microwave cool down, remove samples from microwave.

8.1.5 Following MASE:

8.1.5.1 Centrifuge samples in Teflon tubes for 10 min at 2500 rpm.

8.1.5.2 Decent solvent into RapidVap tube.

8.1.5.3 Rinse sample with 6 mL acetonitrile, vortex, and repeat previous steps.

8.1.6 RapidVap Parameters:

8.1.6.1 Concentrate sample to ~1-2 mL in RapidVap using the parameters below. If sample goes to dryness, add 1 mL acetonitrile to dissolve extracted pesticides.

Temp:	35°C
Speed:	50
Time:	~15-20 min

8.1.6.2 Following RapidVap, spike all samples with 100 µL of 5.0 ng/µL internal standard spike for a total mass of 500 ng.

8.1.6.3 Fill RapidVap tube with ~100 mL of DDW

8.1.7 SPE clean-up of extract:

8.1.7.1 Place C-18 cartridge on the flow control valve attached to the vacuum flask.

8.1.7.2 Install a clean, empty 10cc syringe barrel onto the luer fitting of each cartridge.

8.1.7.3 Pre-condition C-18 SPE cartridges with 6 mL Ethyl Acetate, followed by 6 mL MeOH, and 6 mL DDW. Do not let cartridge run dry between subsequent solvent washes. After the final water wash, do not allow the cartridge to drain completely so that the solid phase remains wet until use.

8.1.7.4 Insert a pre-combusted 1 µm glass filter into the PFA inline filter holder.

8.1.7.5 Turn on the vacuum and flush approximately 10 ml of sample through the sample delivery tube.

8.1.7.6 Insert the SPE cartridge on the sample line and extract the sample through the SPE cartridge.

8.1.7.7 After the extraction is complete, remove the sample delivery tube from the cartridge. Allow the cartridge to dry under vacuum for 10 min or more.

8.1.7.8 Store the cartridge in a clean zip-lock bag labeled with the batch number and extraction date in the refrigerator until the elution of the analyte can be performed (up to 3 months).

8.1.8 Elution of SPE cartridge:

8.1.8.1 Place labeled disposable test tubes (15 x 85 mm) in the elution rack.

8.1.8.2 Replace the liner on the underside of the Supelco VisiPrep elution manifold cover with a clean disposable liner. Replace the manifold cover by inserting the Teflon tubing into the test tubes.

8.1.8.3 Place the SPE cartridges in order on the VisiPrep Teflon holder. Install a clean, empty 10cc syringe barrel onto the luer fitting of each cartridge.

8.1.8.4 Measure 5 ml of ethyl acetate into each syringe barrel reservoir. A slight vacuum will be needed to begin the elution process which can then be continued by gravity.

8.1.8.5 After elution, turn on the vacuum and open the valve at full vacuum for 2-3 minutes to ensure all liquid is eluted.

8.1.8.6 Once elution is complete, remove cartridges and place back in storage zip-lock in freezer until the batch is released. Label the bag with the elution date.

8.1.9 Removal of water layer:

8.1.9.1 Blowdown the sample to ~2 mL under nitrogen gas.

8.1.9.2 Remove the water layer, if visible, from the bottom of the tube with a clean disposable glass Pasteur pipet.

8.1.9.3 Add a small spatula-full of cleaned and dried anhydrous sodium sulfate. Vortex the test tube for 15 seconds to mix and promote drying. Add more sodium sulfate and repeat vortexing if excess powdered sodium sulfate not visible.

8.1.9.4 Let the sample settle and then transfer the remaining sample to a clean test tube (10 x 75 mm) via a clean disposable Pasteur pipet to blow-down the sample

8.1.10 Blowdown of transfer of extract:

8.1.10.1 Evaporate the extract to near dryness under a dry stream of Nitrogen gas.

8.1.10.2 Add 300µL of ethyl acetate and vortex.

8.1.10.3 Pipet the concentrated eluent into a GC vial fitted with a 300 µl conical spring insert. Cap the vial with the crimping tool.

8.1.10.4 Include a spike check for analysis of the spiking solutions. Add 100 uL of each spike (analyte, surrogate, and internal standard) into a 300 µl conical spring insert. Mix the solution by pipetting. Cap the vial with a crimping tool.

8.2 GCMS Analysis

8.2.1 Analyze a 1 µL aliquot on the GC/MS system as described in PESTCAL adjusting calibration concentrations to lower values required.

8.2.2 At the conclusion of data acquisition, use same software settings used for calibration to identify the peaks of interest base on retention time windows of 5-10%. Examine confirmation ion abundances for each compound to determine if the abundances produce approximately the same amount of analyte as the primary quantification ions.

9 Data Reduction and Statistics

9.1 CALCULATIONS

9.1.1 Separation of analyte peaks is unnecessary for quantitation, as long as there are no interfering ions between the co-eluting compounds. Calculation of the amount (ng) of analyte and surrogate compounds in the extract solution is performed at the time of analysis by the instrument manufacturer's software using:

$$Amount(unknown) = \frac{[Area(unknown)\ x\ Amount(IS)]}{[Area(IS) * RF]}$$

Amount (unknown) = Amount of analyte relative to internal standard (ng)
Amount (IS) = Amount of internal standard added to sample (ng)
Area (unknown) = Integrated abundance of quantitation ion of analyte
Area (IS) = Integrated abundance of internal standard quantitation ion
RF = Response factor determined from most recent calibration curve

This amount is then converted to a concentration using the total weight (g) of the sample:

$$Concentration\ (ppb) = Amt(unknown)/Sample\ weight(g)$$

Concentration (ppb) = Concentration of analyte in sample in ng/g (=µg/L)
Sample weight= Total weight of spiked sample (g) - Weight of container (g)

9.2 STATISTICS

9.2.1 Method detection limits are checked at least annually by analyzing seven aliquots of reagent water containing ~0.2 µg/L of each analyte. The results are used to determine the current method detection limits (MDL) with:

$$MDL = S\ x\ t(n-1)$$

S = Sample standard deviation of the replicate analyses
t(n-1) = Student's "t" for the 99% confidence level with (n-1) degrees of freedom (Table 3.)

Table 3. Student t-test values

Samples analyzed (n)	(critical value = 0.99)
2	31.82
3	6.965
4	4.541
5	3.747
6	3.365
7	3.143
8	2.998
9	2.896
10	2.821
11	2.764

Method accuracy and precision are monitored continuously using the results of quality control samples.

10 QUALITY ASSURANCE

Table 3. IDL and MDL

Analyte	Approx. Retention Time (min)	IDL (pg)	MDL (ng/mL)
Butachlor (Surr)	18.72	8.188	0.459
Terbutylazine (Surr)	9.93	6.594	0.307
Acetochlor	12.36	16.467	0.008
Alachlor	12.77	25.494	0.011
Atrazine	9.40	5.903	0.005
Butylate	5.70	12.806	0.092
Chlorthalonil	10.46	29.287	0.018
Cyanazine	13.83	26.680	0.227
DEA	7.98	2.883	0.038
DIA	7.79	15.884	0.132
Dimethenamid	12.09	22.010	0.013
EPTC	5.09	9.177	0.099
Metolachlor	14.52	9.590	0.009
Metribuzin	11.62	28.517	0.018
Norflorazon	23.21	80.551	0.013
Pendimethalin	16.20	8.383	0.014
Permethrin	31.88	91.057	0.042
Propachlor	7.48	7.667	0.036
Propazine	9.56	3.854	0.023
Prometon	9.30	5.363	0.007
Simazine	9.16	25.527	0.007
Tefluthrin	11.21	3.960	0.034
Trifluralin	8.56	5.772	0.016

IDL Date: 03/26/2018 MDL Date: 09/24/2015

11 ADDITIONAL INFORMATION

11.1 REFERENCES

11.1.1 Cassada, D.A., Spalding, R.F., Cai, Z., and Gross, M.L. (1994) Determination of Atrazine, Deethylatrazine, and Deisopropylatrazine in Water and Sediment by Isotope dilution GC/MS. J. Chrom. Acta, v.287, p7.

11.1.2 Eichelberger, J.W., Behymer, T.D., and Budde, W.L. (1988) Method 525,

Determination of organic compounds in drinking water by liquid-solid extraction and capillary column gas chromatography/mass spectrometry. Revision 2.1. In: Methods for the Determination of Organic Compounds in Drinking Water. Environmental Monitoring Systems Laboratory, U.S. EPA, Cincinnati, OH.

11.2 Additional Reading

11.3 Instrumentation Technical Support

11.4 Comments

11.5 Attachments

12 Previous Issues and Changes

Document File Name	Issue	Issue Effective Dates	Author
Analyte-Pesticide_Solids-001	001	May 2010 – June 2014	Unknown
Analyte-Pesticide_Solids-002	002	June 2014 – Feb 2017	Unknown
SOP_06_01_02_Herbicide Solids_GCMS	003	Feb 2017 – December 2018	Lindsey Knight
06_01_02.004 Herbicides in Soils	004	December 2018 – Present	Dave Cassada

12.1 Issue Changes

12.1.1 Issue 001:
- Original Issue

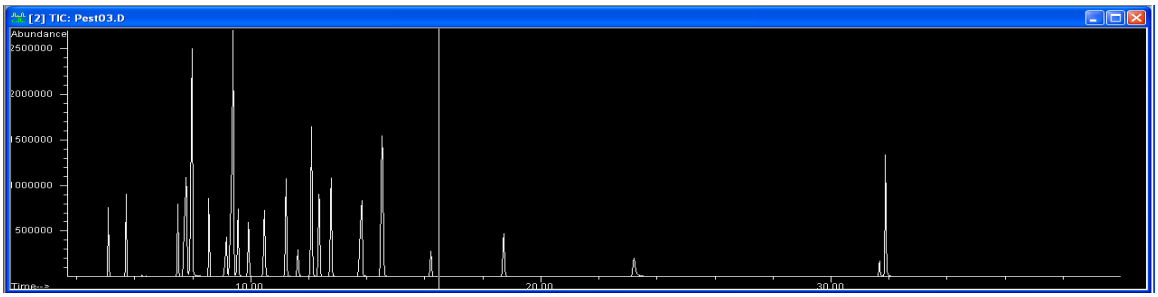
12.1.2 Issue 002:
- Unknown

12.1.3 Issue 003:
- Unknown

12.1.4 Issue 004:
- Added charts, diagrams, photos, etc.
- Added additional details on spiking and calibration solutions.
- Transferred to new format.

13 Diagrams, Figures, and Photographs

13.1.1 Typical total ion chromatograph (TIC) obtained for Calibration Standard 3.


[2] TIC: Pest03.D
Abundance
2500000
2000000
1500000
1000000
500000
Time-->
10.00
20.00
30.00

8: Soil Particle Size Analysis

1 INTRODUCTION

Texture analysis with many samples can be time consuming and expensive. Kettler and others (2001) developed a rapid soil texture analysis method to make the process faster and cheaper. This method involves weighing ~15g of a soil sample into 50mL centrifuge tubes, shaking samples in a 3% sodium hexametaphosphate deflocculating solution, pouring the solution through a sieve to isolate the sand particles, and then decanting off the clay particles in solution after the silt particles have settled out.

2 SCOPE AND APPLICATION

This method describes a rapid technique used to determine soil particle size using the Beaker Method.

3 REQUIRED TRAINING

Users should be given a minimum of one day of training.

4 EQUIPMENT AND MATERIALS

4.1 APPARATUS AND MATERIALS

3.1.1. 50mL centrifuge tubes
3.1.2. 3% by weight solution of Sodium Hexametaphosphate (HMP)
3.1.3. 600-800mL beakers
3.1.4. Soil splitter
3.1.5. Aluminum weighing tins
3.1.6. Wrist action shaker
3.1.7. Oven
3.1.8. 53 micron (#270) sieve
3.1.9. Squirt bottle with DI water
3.1.10. Burette stand with a clamp
3.1.11. Plastic funnel
3.1.12. Soil Textural Class Triangle

4.2 REAGENTS

3.2.1. Sodium HMP

3.2.1.1. Use 30g Na-HMP for every 1L of solution that you are making. Below is for 1L of solution.

3.2.1.2. Weigh out 30g Na-HMP into a 1L beaker and fill to the 800mL mark. Add stir bar and set on stir plate.

3.2.1.3. Slowly add Na-HMP and allow it to stir until dissolved.

3.2.1.4. Fill solution to the 1L mark and transfer to a container with a lid that screws on tight.

4.3 CHEMICALS

Chemical	CAS Number	Hazards	Location
Sodium Hexametaphosphate (HMP)	10124-56-8	Skin, Eye, and Respiratory irritant	Cabinet 2 Shelf 1

5 SAFETY PRECAUTIONS

5.1 SAFETY PRECAUTIONS

Wear nitrile gloves

6 PROTOCOL

6.1 SAMPLE PREPARATION

6.1.1 Analyze sample for texture after the bulk density protocol is complete
Note: If the bulk density procedure has not been done prior to texture analysis, then weight entire sample. First tare the aluminum weigh tin, add sample, and record entire sample weight.

6.1.2 Put sample through a 2mm x 2mm sieve. Record weight of the >2mm fraction and discard the rocks.

6.1.3 Using a soil splitter, partition out ~15g of sieved soil into a labeled and weighed 50mL centrifuge tube. Record tube weight and tube+sample weight to the nearest hundredth of a gram (0.01).

6.2 TEXTURE ANALYSIS

6.2.1 Deflocculating Sample:

6.2.1.1 Shake 3% Na-HMP bottle well to displace any crystallization. Add 3:1 HMP:soil (e.g. 45mL of HMP for 15g of soil sample)

6.2.1.2 Cap Centrifuge tubes and shake well by hand to dislodge any dry soil at the bottom of the tube.

6.2.1.3 Place sample either in tumble or wrist action shaker for a minimum of 2 hours. Samples tend to shake better if placed in a horizontal position.

6.2.2 Wet Sieving Sample:

6.2.2.1 Record the masses of a beaker and aluminum weigh tin for each sample. This process is easiest with a 600mL beaker.

6.2.2.2 Place the plastic funnel into the clamp on the burette stand with just enough distance under the funnel to place one of the 600mL beakers.

6.2.2.3 Place the 53 micron sieve in the top of the funnel.

6.2.2.4 Again, shake the centrifuge tube by hand to make sure that none of the sample has settled and sticks to the bottom.

6.2.2.5 Remove the centrifuge cap and pour solution and sample through sieve and into the beaker.

6.2.2.6 Using a small squirt bottle, rinse any sample remaining in the tube and/or cap and into the sieve. Be careful not to over pressurize squirt bottle, thus loosing sample.

6.2.2.7 Once all of the sample is in the sieve, gently squirt water to wash any silts and clays through the sieve. If the sieve is not draining, very gently rub your gloved finger around on the screen of the sieve to help the liquid pass through. Rinse finger into sieve to make sure all of the sample is accounted for and not lost.

6.2.2.8 When water passing through the sieve is clear (depends on textural class, but usually around 600mL silt beaker has 300mL of water in it) the sample washing is complete.

6.2.2.9 Remove sieve, which now is left with only the sand fraction, and use the squirt bottle to wash all of the sand from the sieve and into the aluminum tin.

6.2.2.10 Place the aluminum tin in the oven at 105°C overnight or until dry.

6.2.2.11 Stir the contents of the 600mL silt beaker to suspend all particles.

6.2.2.12 Let beaker stand for 1.5-6 hours to allow silt particles to settle, leaving the clay particles suspended in solution.

6.2.2.13 After allowing the silt to settle out, carefully decant the clay solution from the 600mL silt beaker and place in the oven at 105°C overnight or until dry.

6.2.2.14 When dry, take samples out of the oven and allow them to cool. Then record mass of aluminum tin+sample and beaker+sample.

5.2.2.15 Once finished with calculations on texture fraction percentages, use a soil textural class triangle to for textural class for each sample.

7 DATA REDUCTION AND STATISTICS

7.1 CALCULATIONS

7.1.1 Clay Fraction(g) = Oven dried weighing tin with sand(g) - Weighing tin weight(g)

7.1.2 Silt Fraction(g) = Oven dried beaker with silt(g) – Beaker weight

7.1.3 Clay Fraction(g) = Sample weight(~13g) – Sand fraction(g) – Silt fraction(g)

7.1.4 Percent Clay = (Clay fraction(g)/Total sample weight(g)) x 100
7.1.5 Percent Silt = (Silt fraction(g)/Total sample weight(g)) x 100
7.1.6 Percent Clay = (Clay fraction(g)/Total sample weight(g)) x 100

8 QUALITY ASSURANCE

An ASTM standard test method for particle size distribution of soils using sieve analysis will be utilized to ensure proper quality assurance practices. This method outlines sieving techniques and reagent preparation.

9 ADDITIONAL INFORMATION

9.1 REFERENCES

- Kettler, T.A., Doran, J.W., Gilbert, T.L. *Simplified method for soil particle-size determination to accompany soil-quality analyses*. Soil Science Society of America Journal.

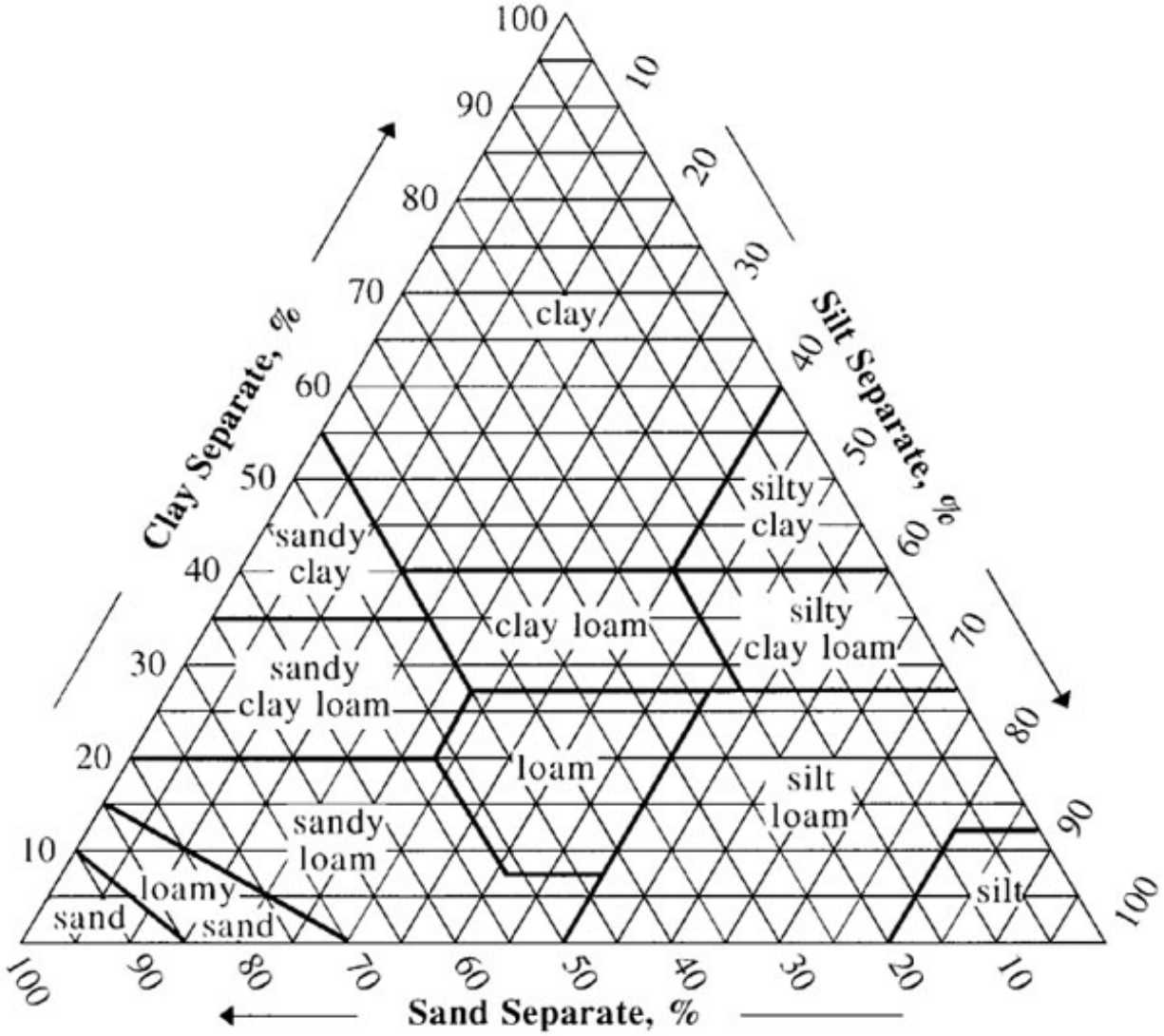
9.2 ADDITIONAL READING

- American Society for Testing and Materials. 2004. "Standard Test Methods for Particle-Size Distribution (Gradation) of Soils Using Sieve Analysis." ASTM. D6913-0

10 PREVIOUS ISSUES AND CHANGES

Document File Name	Issue	Issue Effective Dates	Author
Not applicable for this version			

11 DIAGRAMS, FIGURES, AND PHOTOGRAPHS


Clay Separate, %
Silt Separate, %
Sand Separate, %
clay
silty clay
sandy clay
clay loam
silty clay loam
sandy clay loam
loam
silt loam
sandy loam
loamy sand
sand
silt

General
07_01_04.004
Dates Active: June 6th, 2018 – Present
Author: David A. Cassada

9: Method Validation

INTRODUCTION

This document outlines the set of experimental procedures needed to validate a method that measures specific constituent(s) or characteristic(s) in a specific matrix or specific matrices. Method development generally requires three steps: 1) Determination of the Method Detection Limit (MDL), 2) Determination of the Recovery by analyzing independently prepared unknown samples, and 2) A Holding Time Study. Depending on the constituent or characteristic of interest, the matrix, or the necessary instrumentation, other tests and analyses may be needed. This document does not give guidelines about how to develop the actual steps of the method, but how to validate a method once it has been developed. Methods developed at the Water Sciences Laboratory need to be both robust and accurate. Following this general method validation procedure will assist in ensuring that new methods meet the rigorous standards required at the WSL. The actual steps in the method should be determined by the analyst, and are usually based on previous experience, other similar methods, and peer-reviewed scientific papers.

INSTRUMENT DETECTION LIMIT (IDL)

The Instrument Detection Limit (IDL) is defined as the minimum mass amount (e.g. ng or pg quantities) of a substance that can be measured on the instrument and reported with 99% confidence that the constituent amount is greater than zero. It is determined by analyzing a low-level standard containing the constituents(s) of interest at least 8 times, calculating the standard deviation of the measured concentration, and then multiplying by the appropriate student t value. The injected volume is used to calculate the mass of constituent(s) injected from the known concentration of the standard. Typically, the lowest standard of the instrument calibration curve is used to determine the IDL.

- Analyze eight or more injections of the low calibration standard solution.
- From the determined values, calculate the standard deviation of the eight or more samples. See the Data Reduction section for statistical formulas.
- Multiply the standard deviation of the eight or more samples by the appropriate student t value. See Data Reduction section for the relevant formula. See Student t Values Table for t values. Select the value of t for n – 1 degrees of freedom at the 99% confidence level. If eight replicates have been run, the student t value is 2.998.

METHOD DETECTION LIMIT (MDL)

The Method Detection Limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the constituent concentration is greater than zero. It is determined by taking samples containing the constituent(s) of interest and processing them through the

complete proposed analytical method, calculating the standard deviation of the values, and then multiplying by the appropriate student t value.

- Fortify a suitable quantity of matrix (reagent water, sand, etc.) with target compound(s) as to be able to run eight or more samples of known concentration at a level near the estimated MDL. (This can be estimated by consulting the MDL values listed in other similar methods, or by multiplying the blank/noise for the method by three to five.)
- Analyze eight or more portions of this solution by processing it through all the steps of the proposed method over a period of at least three days. (Processing over three or more days ensures the MDL determination is more representative than measurements performed sequentially.)
- From the determined values, calculate the standard deviation of the eight or more samples. See the Data Reduction section for statistical formulas.
- Multiply the standard deviation of the eight or more samples by the appropriate student t value. See Data Reduction section for relevant formulas. See Student t Values Table for t values. Select the value of t for n – 1 degrees of freedom at the 99% confidence level. If eight replicates have been run, the student t value is 2.998.

RECOVERY

The recovery of the target compound(s) in a matrix is the measured value obtained in a fortified sample divided by the calculated concentration. Recovery pertains to the extraction efficiency of an analytical method within the limits of variability. Recovery should be consistent, precise, and reproducible. Recovery experiments should be performed by comparing the analytical results for the extracted samples at a concentration representative of the concentration of the unknown samples. Known samples can be prepared in the analysts laboratory using either purchased analytical grade reagents, or standards available from the National Institute of Standards and Technology (NIST).

- Prepare or obtain (i.e. from NIST) a sample(s) containing a known amount of the constituent(s) of interest in the appropriate matrix.
- Process the sample(s) through the proposed method.
- Compare the results with the initial known concentration of the sample by dividing the average of the determined results by the initial known concentration, and multiplying by one-hundred. See Data Reduction section for the formula.

HOLDING TIME STUDY

Short Term Stability

Three samples of known concentration should be warmed to room temperature and kept at this temperature from 4 to 24 hours (based on the expected duration that samples will be maintained at room temperature in the intended study) and analyzed. The recovery should then be determined as stated in the Recovery section.

Long Term Stability

The storage time in a long-term stability evaluation should exceed the time between the date of first sample collection, and the date of last sample analysis. Long-term stability should be determined by storing at least three samples of known concentration under the same conditions as the study samples. The volume of samples should be sufficient for analysis on three separate occasions. Test the stability of samples on three separate occasions spaced equally apart, for the expected duration of the study. The recovery of the stability samples should then be determined as stated in the Recovery section.

CALIBRATION CURVE/STANDARD CURVE/CONCENTRATION-RESPONSE (IF APPLICABLE)

A calibration curve of a blank sample (matrix sample processed without internal standard, if applicable), a zero sample (matrix sample processed with internal standard, if applicable), and three to eight non-zero samples covering the expected range. The simplest model that adequately describes the concentration-response relationship should be used. Selection of weighting and use of a complex regression equation should be justified. The following conditions should be met in developing a calibration curve:

- 20% deviation of the low standard from nominal concentration
- 15% deviation of standards other than LLOQ from nominal concentration

At least two-thirds of the non-zero standards should meet the above criteria, including the low standard and the calibration standard at the highest concentration. Excluding the standards should not change the model used.

SPECIFICITY (FOR COLUMN CHROMATOGRAPHY)

Specificity is the ability to measure accurately and specifically the constituent(s) of interest in the presence of other compounds that may be expected to be present in the same matrix. It is a measure of the degree of interference from such things as other active ingredients, excipients, impurities, and degradation products, ensuring that a peak response is due only to a single component; that is, that no co-elutions exist. Specificity is divided into two separate categories:

- **Identification Tests:** For identification purposes, specificity is demonstrated by the ability to discriminate between compounds of closely related structures, or by comparison to known reference materials.
- **Assay/Impurity Tests:** For assay and impurity tests, specificity is demonstrated by the resolution of the two closest eluting compounds. These compounds are usually the major component or active ingredient, and an impurity. If impurities are available, it must be demonstrated that the assay is unaffected by the presence of spiked materials (impurities and/or excipients). If impurities are not available, the tests results are compared to a second well-characterized procedure. For assay tests, the two results are compared; for impurity tests, the impurity profiles are compared head to head. See Data Reduction section for resolution equation. Baseline resolution is achieved when $R_S = 1.5$.

DATA REDUCTION

Method Detection Limit Calculations

$$StandardDeviation(S) = \sqrt{\frac{1}{n-1} * \left[\frac{\sum_{i=1}^{n} X_i^2 - \left(\sum_{i=1}^{n} X_i\right)^2}{n}\right]}$$

$$MethodDetectionLimit(MDL) = t_{99\%} * S$$

Recovery Calculations

$$Recovery(\%) = \left(\frac{\left(\frac{\sum_{i=1}^{n} X_i}{n}\right)}{X_{Actual}}\right) * 100$$

Where:
X_i is the method determined concentration for the known sample.
n is the number of replicates of the known sample.
X_{Actual} is the calculated concentration of the fortified sample.

Resolution

$$R_S = \frac{2\left(t_{R,B} - t_{R,A}\right)}{W_A + W_B}$$

Where:
$t_{R,A}$ and $t_{R,B}$ are the retention times of species A and B.
W_A and W_B are the peak widths of species A and B.
R_S is the resolution, and baseline resolution is achieved when R_S =1.5.

Attachment 3: Standard Laboratory Training Plan

Standardized Laboratory Training Plan

OVERVIEW

This laboratory training plan is intended to be used by any interested party who would like to gain a greater understanding of one or more of our analytical methods listed on our website, https://watersciences.unl.edu. Currently, training is provided on every method, though some advanced methods require sufficient background in mass spectrometry (e.g. – liquid chromatography-mass spectrometry (LC/MS) and stable isotopes instrumentation). Because training requires a substantial investment of both time and effort by the WSL staff, and the trainee, it is important that procedures are in place and understood by everyone participating in the training process.

There are two ways to gain knowledge about the analytical methods performed in the laboratory:

1. Job Shadowing
2. Proficiency Training

During job shadowing, the user will silently observe the analyses being performed by the WSL staff. The technician will answer general questions, but will not be able to engage with the user about the details of the procedure. Observation cannot distract the technician. If the user is interested in learning the procedure, or would like to analyze samples on their own, they would need to move on to proficiency training. During proficiency training, the prospective students and researchers will learn how to become proficient in sample processing and instrumental methods at the Water Sciences Laboratory.

Interested scholars and students will first be invited to observe the procedure that they would like to learn by scheduling a time with the Laboratory Manager. After reviewing the procedures, the individual will have the opportunity to participate in the formal training that first requires the completion of online safety training and general laboratory procedures. All WSL users must first complete online and general laboratory proficiency tests before moving on to more advanced tasks. Every effort will be made to accommodate potential user's schedules, but the training cannot interfere with regular staff work schedules.

This plan has been laid out so that students and researchers can be successfully trained in order to enable them to perform chemical analyses of their samples with minimum error. WSL lab staff will make every effort to give each trainee individual and personalized attention during their training period.

TRAINING COST

The training consists of two parts, the basic lab training that is common for all trainees, and then specific training on analytical instruments. The basic lab training will include using different types of micropipettes, the operation of various analytical balances, good laboratory practices (GLP), and general

procedures for making chemical standards and reagents. This will be followed by a written exam and in-lab demonstration. The basic exam will be graded, and the answer key will be provided to the trainee along with the graded exam. This part of the training has no costs, but is a requirement to move forward to the next part of the training process.

Once basic training is complete, the trainee can then proceed with training on an analytical method. This will be followed by a proficiency test, where users will be tested by independently analyzing unknown laboratory-prepared samples. The results of these 8 test samples will be used to demonstrate proficiency, documented in our Laboratory Information Management System (LIMS), and are reported to the supervisor.

There are two ways this proficiency test can be completed and billed:

1) The first option is that the proficiency test will be billed at 50% of the normal client user rate (see https://watersciences.unl.edu/price-list-0 for the client user rate for all methods). Only after a successful proficiency test will individuals be able to schedule instrument time on QReserve, bring in their samples, and begin independent sample analysis.
2) The second option of the proficiency test will cost nothing, if the trainee gets trained using some of their own samples, run at the client rate (no discounts) by the lab technician. This involves watching and helping the lab technician throughout the entire process. The trainee must work around the staff schedule during this time. When the trainee is ready, they can then complete the proficiency test at no additional cost. This option is good for supervisors without a separate training budget, users on a deadline, or who would like to practice the method multiple times, on multiple batches of samples. It may not be possible to offer this option for certain complex techniques that will require full attention of the lab technician.

It is expected that users will complete their proficiency tests within two months of their training, thereby showing competence on the instrument, before they are permitted to independently analyze their own samples at the WSL. If the user is unable to analyze their samples within two months of their proficiency test, they will have to perform another refresher proficiency test (at no extra cost) before analyzing their own samples. If the user fails this test, they will have the opportunity to repeat the training again, at the same price as before.

Samples analyzed by a trained user will get a 50% discount on the full client rate. Please note the WSL staff cannot guarantee the quality of results on any samples run by trained users. If the results are undesirable, they will be permitted to repeat the analyses one time at no additional expense. If the tests need to be repeated more than once, then the samples will be charged again at the discounted user rate.

TIME COMMITMENT

Training in the laboratory requires a substantial investment of both time and effort by the trainee. During the training period, users are expected to spend up to 5-10 hours per week in the laboratory, or on training material. The length of time required to complete the training varies, and depends on the method, as well as the user. In order to fully master the materials presented, time may need to be spent in the laboratory after hours, and this time can count towards the hours expected per week.

BASIC TRAINING CHECKLIST

This checklist outlines the training sequence for new users at the Water Sciences Laboratory. All training must be signed off on, dated, and finished **prior to working in the laboratory**, and will be completed in order. The entire training should be completed within the span of two months. The trainee is responsible for contacting the lab to schedule any in-lab trainings or demonstrations.

☐ _____ **Pre-Training online questionnaire**, available at: https://watersciences.unl.edu/pre-training-questionnaire.

☐ _____ Read through the **WSL User Guide**, available at: https://watersciences.unl.edu/lab-user-guide, or from the Laboratory Manager.

☐ _____ Complete the required **EHS Core Safety Training modules**, available through in-person instruction by EHS, or online at: http://ehs.unl.edu/training/online. The required modules are:

1. Core – Injury and Illness Prevention Plan (IIPP)
2. Core – Emergency Preparedness Training
3. Core – Bloodborne Pathogens
4. Core – Chemical Safety Training Unit 1
5. Core – Chemical Safety Training Unit 2
6. Core – Chemical Safety Training Unit 3
7. Core – Chemical Safety Training Unit 4
8. Personal Protective Equipment (PPE)

Please e-mail the certificates of completion, given to you at the end of each module, to the Laboratory Manager.

☐ _____ Read through the **Equipment Reservation SOP**, available at: https://watersciences.unl.edu/basic-lab-training. Follow the instructions to become a member of the Water Sciences Laboratory QReserve page.

☐ _____ Read through **General WSL Standard Operating Procedures (WSLSOP) and EHS Safe Operating Procedures (EHSSOP)**. The documents that you are required to read are as follows:

1. **Laboratory Safety** – These documents can be found at: https://watersciences.unl.edu/laboratory-safety
    - Injury Prevention and Response
    - On-The-Job Injury Flowchart
    - EHS Emergency Eyewash and Shower Equipment SOP
    - EHS Laboratory Hood and Cabinet Identification and Use
    - On-The-Job and Student Injuries
2. **Standard Operating Procedures –** These documents can be found at: https://watersciences.unl.edu/basic-lab-training
    - Laboratory Waste Disposal and Management
    - Balances
    - Water

- Micropipette
- Glassware Cleaning
- Sample Bottle Cleaning

☐ _____ Watch the following **Basic Training Videos**, available at: https://watersciences.unl.edu/lab-training-videos

- Using a Micropipette
- Micropipette: Common Mistakes
- Pipette Calibration and Proficiency Test
- Using an Analytical Balance
- Analytical Balance: Common Mistakes
- Calibrating and Leveling a Balance

☐ _____ Complete the **In-Lab Training**. This will take 30 minutes, and allow a technician to physically demonstrate and explain the concepts that have been introduced previously. This is also a time to have any questions answered by a technician.

☐ _____ Complete the **Micropipette Proficiency Test**. The documents needed to complete this proficiency test will be provided during the In-Lab Training. Additional information regarding how to complete the proficiency test can be found in the next section, as well as in the Micropipette SOP. This information will also be reviewed during the In-Lab Training. Once you have completed the Micropipette Proficiency Test, fill out the provided excel sheet. If all pipetted volumes are within the acceptable range, a completed copy of the excel sheet must be e-mailed to the Laboratory Manager before proceeding.

☐ _____ Complete the **Written Exam**. This exam is given out by the Laboratory Manager, and includes an in-lab demonstration portion. This demonstration portion will take 15-20 minutes, and must be scheduled with a technician at the laboratory.

MICROPIPETTE PROFICIENCY TEST INSTRUCTIONS

Micropipettes are one of the most important tools in the analytical laboratory, and operating them properly is key to getting accurate and precise results. A user can determine whether they are using a micropipette correctly by weighing the amount of distilled deionized water dispensed with the pipette, as 1 g of water equals 1 mL.

Please notify the Laboratory Manager, or lab staff, if any micropipettes continue to dispense inaccurate volumes after 3 attempts, or are inoperable for any other reason.

Steps to complete the micropipette proficiency test:

☐ Reserve Balance #1 on Qreserve for the duration of time required. This reservation on QReserve is part of the requirement for passing the Micropipette Proficiency Test, and the completion of this step will be checked by the Laboratory Manager.

☐ Fill a 50 mL beaker up to a height of 3mm with distilled deionized water. Place it on a sufficiently sensitive analytical balance (three decimal places). Fill up a 200 mL beaker with distilled deionized water. This will serve as a water reservoir.

☐ Complete 10 repetitions with the minimum volume, and 10 with the maximum volume of each pipette. Pipette water from the reservoir to the 50 mL beaker, taring the balance between repetitions. The 50 mL beaker can be emptied out if necessary.

- [ ] Compare the results to the limits provided by the manufacturer. These can be found in the excel file given to you by the Laboratory Manager. The average of all 10 dispenses is taken and compared to the maximum permissible error provided by the manufacturer. The column titled 'Acceptance' will indicate whether this value falls within the permissible error by showing either a green 'Accept' or a red 'Reject'.
- [ ] If there are any 'Reject' outcomes, repeat the procedure for those micropipettes until an 'Accept' outcome is reached. Once complete, the excel file should be sent back to the Laboratory Manager, to be stored as proof of proficiency.

ANALYTICAL METHOD TRAINING CHECKLIST

This checklist outlines the steps that a prospective analyst will need to complete in order to work in the Water Sciences Laboratory and generate results and data. This checklist is meant to follow successful completion of the basic training. Training of individuals on specific instrumentation depends on the level of use. Any individual using WSL equipment is responsible for its calibration and general maintenance. It is the user's responsibility to learn and understand the proper procedures to be followed when using equipment, and to notify the Laboratory Director or Manager of any needed repairs.

Analysts are required to pass a validation test for a particular laboratory method before they are allowed to use that method to generate results and data.

- [ ] Read the **Standard Operating Procedure (SOP)** document for the intended method. This is available from the Laboratory Manager, or the member of WSL staff who is in charge of the particular instrument and/or analytical method. Make note of any vocabulary that is not understood, and write down any questions you have while reading through the document. These can be addressed by lab staff during the In-Lab Method Training.
- [ ] Complete the **In-Lab Method Training** for that particular method. This involves scheduling time with the relevant member of staff, who will physically and verbally walk through the process. Depending on the analytical method, this make take more than one visit.
- [ ] Complete a **Proficiency Test**. This test is made up of 8 test samples, and constitutes a test called the Method Detection Limit (MDL). The MDL is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the constituent concentration is greater than zero.
    a. Fortify a suitable quantity of matrix (reagent water, sand, etc.) with target compound(s) as to be able to run eight or more samples of known concentration at a level near the estimated MDL. (This can be estimated by consulting the MDL values listed in other similar methods, or by multiplying the blank/noise for the method by three to five. MDL values can also be obtained from the technician who runs the instrument in question.)
    b. Analyze eight samples (or portions of the solution) by processing them through all of the steps of the proposed method.
    c. From the determined values, calculate the standard deviation of the eight or more samples.
    d. Multiply the standard deviation of the eight or more samples by the appropriate student t value. Select the value of t for n - 1 degrees of freedom at the 99% confidence level. If eight replicates have been run, the student t value is 2.998. The MDL obtained by the

perspective analyst should not be greater than two standard deviations from the mean MDL obtained by other analysts in the laboratory performing the same method.

PROBATIONARY PERIOD

After the successful completion of the proficiency test, new users will be put on a 2 month probationary period. During this time, if certain standards of lab use are not met, or the lab policies are not followed, lab privileges for the user will be revoked. During this period, users must complete at least 5-10 hours of work per week, for both months. After-hours access, or permission to complete scheduled work on the weekends, will be granted to users during this time, based on the sole discretion of the Laboratory Director or Manager.

Attachment 4: Checklists, Forms & Calculations

Nebraska Water Center
Daugherty Water for Food Global Institute

Chain of Custody Record

Ship Samples To:

202 Water Sciences Laboratory
University of Nebraska - Lincoln
Lincoln, NE 68583-0844
(402 472-7539 Fax (402) 472-9599

Project Name/No.

Sampler (Signature)

(Lab Use) Lab Number	Sample ID Number	Date	Time	(Comp)osite or (Grab)?	Sample Matrix	Number & Type of Containers	Requested Analyses				Preservation	Remarks

Relinquished By: (Signature)	Date/Time		Relinquished By: (Signature)	Date/Time
(Print)	Company		(Print)	Company
Received By: (Signature)	Date/Time		Received for Laboratory By: (Signature)	Date/Time
(Print)	Company		(Print)	Company
Shipped by (Carrier):	Airbill No.			

Notes on Receipt:

Samples Intact? (Y/N)
Samples chilled? (Y/N)

Water Sciences Laboratory

University of Nebraska-Lincoln
watersciences.unl.edu

Nebraska Water Center
Daugherty Water *for* Food Global Institute

Sample Submittal Form

WSL Batch Number(s)_____

Date _____

Matrix Type: ☐ **Water** ☐ **Wastewater** ☐ **Sediments**

(Use a separate form for each matrix)

Contact Name_____

Company_____

Number of Samples_____

Address_____

City_____ **State**____**Zip**_____

Tests Desired **(Please refer to protocol code if possible)**

Email Address_____

Telephone_____

Extended sample storage (Y / N) _____
See website under 'Client Services' for storage policy

Project Code_____

To be billed? (Y/N) Acct._____

Billing Address_____

	Sample Field ID#	**Collection Date/Time**	**Sampled By**	**Preservative?**	**Lab ID#** (WSL use only)
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					

Comments_____
Date Completed_____

Mail or deliver samples and completed form to
202 Water Sciences Laboratory – 1840 N 37th St.
University of Nebraska-Lincoln, Lincoln, NE 68583-0844
dsnow1@unl.edu; 402-472-7539

Analyzed By:
Date:

Soil Moisture Content

Batch:

LIMS #	Sample ID	BATCH	Pan #	Tare Wt (t) (in g)	Wet Soil + Tare Wt ($x+t$) (in g)	Dry Soil + Tare Wt ($y+t$) (in g)	Grav Moisture Content (Θ) ($gH_2O/gSoil$)	Ave of Duplicate Samples	Standard Deviation of Duplicate Samples	Relative Stand. Dev.
			1							
			2							
			3							
			4							
			5							
			6							
			7							
			8							
			9							
			10							
			11							
			12							

Quality Control Samples				Tare Wt (t) (in g)	Dried Diatomaceous Earth Wt (e) (in g)	DDW (w) (in g)	Wet Soil + Tare Wt ($x+t$) (in g)	Dry Soil + Tare Wt ($y+t$) (in g)	Grav Water Content (gH2O/gSoil)	Correlation Coefficient / LOD
	LFB		13							
	LRB		14							

Form: Core Breakdown Template (5 pages)

									Core Processing			
												ρb and Θg
Site ID	ID String	Bag Sample ID	Date Processed	Interval (ft)	Start (ft)	Stop (ft)	Total Below Surface (ft)	Total (ft)	Beaker*	Sample (1x2.1in.) + Beaker*	Sample (3.46in.^3)*	ρb (g/ml)
							0				0.00	0.00

Project: Vadose Zone Study Name: Date:

OD Sample + Beaker*	OD Sample Weight (g)	Water (g)	**Θg**	**pH Analysis** pH (~5g)	**KCl Extraction** Sample* (~10g)	**NO3-N (ug/g)**	**NH4-N (ug/g)**	**Pore Water NO3-N (mg/L)**	**lbs-N/Acre**	**lbs-N/Acre in cored interval**	**Description** Color/Texture/OC
	0.00	0.00	#DIV/0!					#DIV/0!	0.00	0.00	

Soil Particle Size																				
				Project:_____		Name:_____		Date:												
WSL ID	**Tube***	**Sample+Tube***	**Sample****	**OD Sample+Tube***	**OD Sample****	**Beaker***	**Tin***	**OD beaker+Sample***	**OD tin+sample***	**WSL ID**	**Sand (g)**	**Silt (g)**	**Clay (g)**	**-**	**Sand %**		**Silt %**		**Clay %**
	CP1																		
	CP2																		
	CP3																		
	CP4																		
	CP5																		
	CP6																		
	CP7																		
	CP8																		
	CP9																		
	CP10																		

Core Processing Project:_____ Name:_____ Date:

Site ID	Sample ID	Date Processed	Interval (ft)	ρb and Θv			pH Analysis	KCl Extraction	Description
				Beaker*	Sample (1in.) + Beaker*	OD Sample + Beaker*	pH (~5g)	Sample* (~10g)	Color/Texture/OC

Soil Particle Size					Project:_____	Name:_____	Date:		
WSL ID	**Tube***	**Sample+Tube***	**Sample****	**OD Sample+Tube***	**OD Sample****	**Beaker***	**Tin***	**OD beaker+Sample***	**OD tin+sample***
	10.51	22.57	12.06	21.94	11.43	159.84	1.00	166.85	1.96
	10.50	22.56	12.06	21.74	11.24	170.46	1.00	177.75	1.84
	10.45	23.23	12.78	22.54	12.09	221.89	1.00	229.56	1.89
	10.49	22.55	12.06	21.98	11.49	170.53	1.01	178.00	1.98

WSL ID	**Sand (g)**	**Silt (g)**	**Clay (g)**	**-**	**Sand %**	**Silt %**	**Clay %**
	0.96	7.01	3.46		8.40	61.33	30.27
	0.84	7.29	3.11		7.47	64.86	27.67
	0.89	7.67	3.53		7.36	63.44	29.20
	0.97	7.47	3.05		8.44	65.01	26.54

Note: *OD = Oven Dried* *% = OD Sample / Particle amount * 100* **Weighed to nearest 0.01 g* ***Calculated*