UF Biogeochemistry of Trace Metals Laboratory

Standard Operating Procedure

Procedures Covered:

Digestion of Plant Samples for ICP-MS:
Method # : Not Applicable
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Approval Signatures/Date

Laboratory QA Officer	**Laboratory Supervisor/Manager**
Evandro da Silva, QA Officer	Dr. Lena Ma, Laboratory Director

This document contains all the necessary information to execute this procedure under everyday conditions. Deviations from this SOP will be noted at by the person so deviating with a suitable explanation thereof.

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Section 2: Scope

This SOP is intended for the proper preparation of plant samples for subsequent analysis by Inductively Coupled Plasma Mass Spectrometry.

Section 3: Safety

Appropriate laboratory behavior is expected at all times while performing this procedure. Care is to be taken when dealing with glass and chemicals, and other specific safety precautions should be taken in these cases. Please refer to the UF Lab Safety Manual or the Laboratory Manager for further instructions. This procedure requires the use of hot, concentration mineral acids such as HNO_3, as well as 30% H_2O_2. All of these compounds are extremely dangerous and extra care should be taken to protect the technician's eyes and skin. Required safety materials include eye protection, latex gloves and a lab coat.

Section 4: Digestion of Plant Samples for ICP-MS

For each batch of samples to be prepared via this procedure a minimum of 2 reagent blank samples are to be prepared as well as 2 replicates of plant-matrix appropriate NIST Standard Reference Material (SRM). A minimum of 10% duplicate and spiked samples are to be digested along with the samples. Concentrations of spiked samples should be to levels that are 20% of the highest standard concentration for that analytic technique. For ICP-MS, samples are to be spiked to a final concentration of 20 ug/l. These concentrations correspond to those in the final digestate, not the plant sample's dry weight concentration.

1. Procure Digestion Vessels (50 ml PPH from Environmental Express) and watch glasses for all samples to be prepped, and 18 position racks to hold them. Label the vials with a Sharpie or equivalent permanent marker.
2. Plug in the Hot Block Digester, set the temperature to 105^{o}C, and allow 30 minutes for it to heat up.
3. For all samples to be prepared the initial weight of the sample must be recorded to 3 decimal places. Use the analytic balance (Mettler AE200). **Weigh out approximately 0.2 +\- 0.01 g of dried/ground plant sample into the tared and labeled digestion vial.** Record all sample weights. Keep the vials covered with a watch glass at all times to prevent dust and debris from falling into the vial. Repeat this for all samples.

4. For spiked samples use an appropriate amount of 10 mg/L stock solution from the same lot that is currently being used for calibrating the instrument the samples are to be analyzed on. *Use 1 mL of 10 mg/L std. stock for spiking.* Check with an analyst trained in those techniques for which standard to use.
5. Bring the digestion vials in their racks to the Fume hood in 2176 McCarty Hall. All additions of reagents for this procedure are to be done in the fume hood.
6. Add 10 mls of 1:1 HNO_3 (Trace Metal Grade) to Water solution to each vial and replace the watch glass. Place the racks of samples in the block at 105°C for 2 h.
7. After 2 hours, inspect samples visually. For the samples where there is still excessive coloration to the digestate, add 5 ml of **1:1 HNO_3** continue digesting for 30 min more. *Be sure to not allow the samples to dry out; around 5 ml solution should remain.*
8. Take the samples from the block digestor and allow to cool. Then, add 500 ul of **30% H_2O_2** slowly. The sample will effervesce and its color should lighten. Once effervescence subsides, add another 500 ul aliquot. Repeat 500 ul of **30% H_2O_2 addition** at the analyst's discretion, but no more than 5 mL. *Always remain careful not to let the samples bubble over out of the vials.*
9. Place sample back onto the block digestor for 15 minutes or until the volume has been reduced to approximately 5 mLs.
10. Remove the samples from the block and allow to cool.
11. Dilute the samples with **Double Distilled/DDI H_2O** to the 50ml mark on the side of the digestion tube. Make sure to wash the underside of the watch glass and the sides of the vial as well while doing this.
12. Samples can be capped and allowed to have particulate matter settle to the bottom of the vial. Take a Glass Fiber Filter Cartridge (from Environmental Express) and mate it with a plunger. Insert the Filter Cartridge into the Digestion Tube and press down on the plunger. Sink the Filter Cartridge to the bottom of the tube. Remove the plunger and dispose as necessary.
13. Cap the tube and mix thoroughly. The sample is now ready for metals analysis, or needs further dilution before analysis to match its metal content within the working range of ICP- MS. Please consult laboratory manager, if needed.
14. For calcium analysis, samples will be diluted using 100 mg L^{-1} lanthanum (La) solution.
15. To prepare La stock solution weigh 1.17 g La_2O_3 and transfer to a 100 mL volumetric flask. Add 40 mL of **Double Distilled/DDI H_2O** and, slowly, add 50 mL of HCl (Trace Metal Grade). After cooling down, complete volume to 100 mL with **Double Distilled/DDI H_2O.** To prepare 100 mg L^{-1} La solution, transfer 1 mL of La stock solution to a 100 mL volumetric flask and complete volume to 100 mL with **Double Distilled/DDI H_2O**.
16. Pipette 1 mL of digested sample solution and add 4 mL of 100 mg L^{-1} La solution.
17. Record dilution details.

Section 5: Quality Control Standards and Measurements

To maintain reproducibility throughout a considerable time and over large number of batches (each batch with 15 samples), a large number of quality control samples will be digested and also analyzed (Table 1).

5.1. Sample Digestion Protocol

To check the digestion performance in this study, it will be included digestion blanks, sample duplicates, sample matrix spikes, certified reference materials (CRMs).

Blanks:

Blanks will be used for background correction. At least one reagent blank will be arranged in each 2 digestion batches (36 digestates) to check for possible contamination during the digestion.

Sample Matrix Spikes:

Sample spike will be used prior to digestion to check the matrices effects, at a rate equivalent to at least one spike in each 2 digestion batches (36 digestates). Sample matrix spikes will be prepared at a level of 3-5 times the expected concentration and analyzed at a rate of 1/20 samples.

Certified Reference Materials:

Plant CRMs from National Institute of Standard and Technology (NIST) will be used to determine the accuracy of the selected digestion and analysis method for this study.

Duplicates:

At least 1 unknown plant sample will be duplicated and both samples digested in each of two digestion batches to check precision of the method.

Table 1 - Digestion Protocol in Dr. Lena Ma's Lab

Digestion Sequence (DS#)	Sample/QC
1	Reagent Blank
2	Sample 1
3	Sample 1 – spiked
4	Certified Reference Materials
5	Sample 2
6	Sample 3
7	Sample 4
8	Sample 5
9	Sample 6
10	Sample 7
11	Sample 8
12	Sample 9
13	Sample 10
14	Sample 11
15	Sample 12
16	Sample 13
17	Sample 14
18	Sample 15

5.2. Solution Analytical Protocol

Solution samples will be analyzed for trace metals concentrations in Dr. Lena Ma's Lab. Each SDG will be analyzed concurrently with a series of quality controls which include a minimum of 5 standards, calibration verification standard (CVS), continuing calibration blanks (CCB), and initial/continuing calibration standards (ICS /CCS). An SDG will be analyzed as described in Table 2.

Standards:

At least 5 standard solutions and one blank will be used to establish the calibration curve according to the requirement of the method and/or instrument. The coefficient of determination (R^2) will be calculated to assess the precision and accuracy of the standard curve. Standards will also be used to check the equipment performance, the number and frequency of which is specified in Section 5.3.

Calibration verification standards (CVS):

It is a known value standard, used to verify that the standards and the calibrations are accurate and also confirm the calibration curve. It will be independently prepared standard from a source other than the calibration standards. The value is accepted within 10% deviation from the 100% recovery.

5.3. Calibration Procedure and Frequency

Instrumental calibration in Dr. Lena Ma's Lab will be performed according to procedures and criteria as follows, in addition to following guidelines specified by each method.

Initial Calibration:

Instrument will be initially calibrated each time it is set up or upon failure of any quality control calibration checks. The number of standards to be used for initial calibration of any ICP-MS instrument is at least 5. Any standard curves with linear regression correlation coefficients less than 0.995 will be rejected and standard curve reconstructed by using fresh aliquots of stock standard solutions.

A quality control check standard (CVS) at a mid-range concentration will be analyzed prior to sample analyses to verity initial calibration. This quality control check standard will be prepared independent the calibration standards. Recoveries for this check standard should be between 90 and 110%. Any value that is found to deviate by more than +/-10% in the check standard will be rejected and a new standard curve will be prepared from the beginning. For Ca analysis, a separate calibration solution and CVS will be prepared using 100 mg L^{-1} La solution and analyzed in a new run.

Table 2 Analytical Protocol for Laboratory Determinations in Lena Ma's Lab.

Analytical Sequence	DS # / QC:
1	Analytical blank
2	Standard 1
3	Standard 2
4	Standard 3
5	Standard 4
6	Standard 5
7	CCS (Continuing calibration standard)
8	CVS (Calibration verification standard)
9	DS# 1 (Digestion blank)
10	DS# 2 (Sample # 1)
11	DS# 3 (Sample # 1 - digestion spiked)
12	DS# 4 (Sample # 2)
13	DS# 5 (Sample # 3)
14	DS# 6 (Sample # 4)
15	DS# 7 (Sample # 5)
16	DS# 8 (Sample # 6)
17	DS# 9 (Sample # 7)
18	DS# 10 (Sample # 8)
19	DS# 11 (Sample # 9)
20	DS# 12 (Sample # 10)
21	DS# 15 (Sample # 11)
22	DS# 16 (Sample # 12)
23	DS# 17 (Sample # 13)
24	DS# 18 (Sample # 14)
25	DS# 19 (Sample # 15)
26	DS# 20 (Sample # 16)
27	DS# 21 (Sample # 17)
28	DS# 22 (Sample # 18)
29	DS# 23 (Sample # 19)
30	DS# 24 (Sample # 20)
31	CCS
32	CCB
23	DS# 25 (Certified reference materials)
24	DS# 26 (Sample # 20 - digestion duplicate)

Continuing Calibration:

Continuing calibrations (CCS/CCB) will be arranged for each group of 20 samples analyzed to assure calibration accuracy during each analytical run. At least one standard in any given sample analytical run must be at a concentration of 1-2 times of Practical Quantification Limit. Recovery of the continuing calibration check standard should be between 95 and 105% for ICP-MS. Any value which is found to deviate by more than +/- 5% in the continuing calibration check will be rerun using a new standard curve. For the sample matrix spikes and certified reference materials, recovery should be between 80 and 120%. In addition, the calibration/instrument blank, which is analyzed every 20 samples, must exhibit a response below MDL. For Ca analysis, a separate continuing calibration solution and CCB will be prepared using 100 mg L^{-1} La solution.

Support Equipment Calibration:

Temperature of ovens will be checked daily. Any digestion with temperature out of acceptable method range will be rejected. Electric balances used by this project will be checked with weights on the same day of use.

5.4. Equipment Maintenance

Routine Maintenance Activities:

Routine maintenance for all analytical and related equipment in Dr. Lena Ma's Lab will be performed as indicated by respective operation manual, with strict adherence to maintenance schedules. If a major problem arises with laboratory analytical equipment, efforts will be made to have the samples analyzed in another laboratory facility as soon as possible. Notification of such changes will be sent to the NEON Project Officer.

Table 3. Summary of Preventative Maintenance in Dr. Lena Ma's Lab

Instrument	Activity responsible	Frequency	Person
Electric balance	Weight check	Annually	Mettler-Toledo
	Weight check	Before use	Dr. Evandro da Silva
Refrigerators, drying ovens	Clean interior	Monthly	Dr. Evandro da Silva
	Temperature check	Daily	Dr. Evandro da Silva
Hot block digestion	Temperature check	Before use	Dr. Evandro da Silva
ICP-MS	Clean cones, check tubing, pump, gases	Daily	Dr. Evandro da Silva
	Optimization check	Daily	Dr. Evandro da Silva
	Annually maintenance	Annually	Perkin Elmer Corporation
Data System	Computer network	Daily	Dr. Evandro da Silva

5.5. Calibration Standards

Standards Preparation Protocol:

Multi-level standard solutions of 10 ppm trace metals with HNO_3 medium used in Dr. Ma's Lab will be purchased from PerkinElmer or prepared from ultra-high purity grade chemicals or metals (99.99% or greater purity), following EPA-846-6010A.
Those source standard solutions will be stored in a refrigerator. Working stocks will be prepared daily from the multi-element solutions by stepwise dilution with 5% HNO_3 acid and stored in a refrigerator, and used to check the performance of the equipment along with standardization procedures. For Ca, working stocks will be prepared daily from the multi-element solutions by stepwise dilution with 100 mg L^{-1} La solution.

5.6. Corrective Actions

Method Evaluation Criteria:

The quality assurance officers will be responsible for day-to-day audits and corrective action. All samples not meeting evaluation plan criteria will be reanalyzed. Corrective action will be taken whenever the following criteria are not met:

Initial calibration - recoveries of all elements in check standards should be between 90 and 110%, or as specified by the method.

Calibration verification standards (CVS) - the accepted curve should be certified again with the CVS, If the result is within 10% of the true value, continue the measurement of the samples.

Blanks - theoretically they should be non-detect. The detected value for any element must be less than 3-5 times the MDL and /or < 20% of samples from the same digestion batch.

Duplicates - all percentage differences between each pair of repeats must be less than 10%.

Spike samples, standard additions and standard reference materials - all percentage differences from the certified mean values for each element should be less than 20%.

Continuing calibration standards (CCS) – percent recoveries of all elements in check standards should be between 95 and 105%.

Specified Corrective Action:

Once a problem is found, the QA Officer will immediately notify the PI and all analysts involved in the project. No new samples will be analyzed until the problem has been identified and steps taken to correct it. Calculations of precision and accuracy will be done daily and compared with statistically guaranteed acceptance limits derived from other

samples. If deviations are noted, a systems audit will be performed and efforts made to rectify the problems. If the analyst continues to experience QC problems, it will be brought to the attention of the principal Investigator who will review the situation and recommend corrective action.

Analysis of samples will be repeated when necessary if parameters calculated to assess precision and accuracy do not follow historical trends from the laboratory. If multiple re-runs do not result in improved QA results, sample data will be reported to NEON with the appropriate flag while longer-term corrective actions will be investigated. The results of any internal or external audits that indicate problems in the analytical process will likewise initiate corrective action. If the NEON QA Officer should find if necessary to assert corrective actions to the laboratory, laboratory personnel will comply with the corrective action suggested.

Table 4. Problems and Corrective Actions in Dr. Lena Ma's Laboratory

Problems	Actions to be Taken
Trace metals detected in method labware, blank concentrations measured at levels > 3-5 times of MDL.	Identify sources of contamination, re-clean labware, and reanalyze affected samples. If problem continues, concentration of the blank and MDL will be provided for data reduction and result explanation.
Trace metal concentrations detected in method blank are over 20% of those in samples from the same batch.	Reanalyze affected samples. If problem continues, concentration of the blank will be provided for data reduction and result explanation
Spike recoveries and certified reference materials exceed 20% deviation from the expected value.	Check transfer pipettes for accuracy, recheck standards, and rerun affected samples. If results do not improve, report data to NEON with appropriate flag and take steps toward further corrective action to resolve problem
Liner regression coefficient for any standard curve < 0.995.	Rerun samples using new set of standards, replace standards with freshly prepared ones if necessary
Calibration verification standards fall > 10% outside the range as reported by the supplier.	Rerun check samples, rerun affected samples, run new standard curve
Continuing calibration standards are not within the acceptable range of 5% for ICP-MS.	Recheck samples and standards, rerun those samples with new standards that fail to reproduce initial readings within 5%.
ICP-MS fail preventative maintenance checks as described in owner's manual.	Call for repairs, rerun all affected samples using other available instruments