

Standard Operating Procedure for:
Vario EL III CHNOS Elemental Analyzer Operation
(CNS R01.doc)

Missouri State University
and
Ozarks Environmental and Water
Resources Institute (OEWRi)

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1 Identification of the method

Elementar vario EL III CHNOS Elemental Analyzer

2 Applicable matrix or matrices

This instrument can be used for natural sediment samples.

3 Detection Limit

The operating ranges for this instrument are: carbon, 0.03 to 20 mg absolute, nitrogen 0.03 to 2 mg absolute, sulfur 0.03 to 6 mg absolute. The sample mass range is 0.2 mg to 200 mg. The method detection limits are: carbon, 0.25%, nitrogen 0.25%, sulfur 0.27%. The method throughput is 15 samples per hour.

4 Scope of the method

This standard operating procedure provides Missouri State University (MSU) laboratory personnel with guidance on the procedure for operation of the Elementar vario EL III CHNOS Elemental Analyzer.

5 Summary of method

Sediment samples are collected with trowels and placed in bags. Sediment samples are stored in Temple Hall in ambient conditions. Information about the sample (for example, collection time, temperature, preservative, etc.) is recorded on the bag and sample collection form. Sediment is transferred from the collection bag to a preformed tin foil boat. The boat is formed into a pellet and loaded into the sample carousel. Each separate sample drops into the instrument and undergoes catalytic tube combustion in an oxygenated, high temperature CO₂ atmosphere. The combustion gases are freed from foreign gases. Helium is used to carry C, N, and S through specific adsorption columns where they are separated from each other. Concentrations of each component are determined by a thermal conductivity detector.

6 Definitions

Analytical batch: The set of samples processed at the same time.

Blank: An empty slot in the carousel that is used to calibrate the instrument at the beginning of the analyses.

Calibration standard: Solids of known concentration and purity prepared from the manufacturer that are used to calibrate the instrument response with respect to analyte concentration. Acetanilide, stearic acid, and sulfanic acid serve as calibration standards for this method.

Chain of Custody (COC): Used to describe the written record of the collection, possession and handling of samples. Chain of custody forms should be completed as described in the Chain of Custody SOP # 1030R01. Chain of custody (COC) forms are located on a board in Temple Hall 125.

Daily factor calibration: Instrument calibration performed before each batch using solids prepared from the manufacturer which illustrate the instrument's response to analyte concentration. Sulfanic acid serves as daily factor calibration standard for this method. Recalibrate when any of the daily factors are outside the range of 0.9 to 1.1. Fact1 is the N-factor, fact2 is the C-factor, and fact3 is the S-factor in the final data sheet. These factors should be recorded with each data sheet to illustrate the quality of the data.

Field duplicate (FD): Two samples taken at the same time and place under identical circumstances which are treated identically throughout field and laboratory procedures. Analysis of field duplicates indicates the precision associated with sample collection, preservation, and storage, as well as with laboratory procedures.

Foil blank: An aluminum boat with additive, but without sample that is used to determine if interferences are present in the laboratory environment or the apparatus and are analyzed after every 15 samples.

Instrument calibration: Extensive calibration of the instrument using various masses of solids prepared from the manufacturer which illustrate the instrument's response to analyte concentration. Over 60 separate calibration standards containing acetanilide, stearic acid, and sulfanic acid are used to calibrate the instrument. The instrument calibration remains stable for at least 6 months

Instrument drift: Gradual change in some operating characteristic of a circuit, tube, or other electronic device in a laboratory instrument. This can be measured during a brief period as an effect of warming up or during a long period as an effect of continued use.

Laboratory duplicate (LD): Two aliquots of the same environmental sample treated identically throughout a laboratory analytical procedure. Analysis of laboratory duplicates indicates precision associated with laboratory procedures but not with sample collection, preservation, or storage procedures.

Method: Defines the oxygen dosing as an O_2 index of 1 through 5 and is determined by the sample matrix and the weight of the sample.

Method detection limit (MDL): The lowest level at which an analyte can be detected with 99 percent confidence that the analyte concentration is greater than zero.

- a. To calculate the MDL:
- b. Prepare triplicates of two sediment samples with low organic matter. The laboratory director or supervisor will choose appropriate samples to use to determine MDL.
- c. Analyze all samples.
- d. Include all sample processing steps in the determination.
- e. Calculate the standard deviation (s).
- f. From a table of the one-sided t distribution select the value of t for $7 - 1 = 6$ degrees of freedom at the 99% level. This value is 3.14
- g. The product 3.14 times s is the desired MDL.

- 6.12 Minimum Quantification Interval: The lowest level that can be quantitated accurately and is generally defined as four times the method detection limit = 4(MDL).
- 6.13 Natural Standards: Standard sediment samples of known concentration and purity that produce consistent concentrations of C, N, and S. These standards are used to check instrument performance.
- 6.14 Relative Percent Difference (RPD): calculated as the difference between a sample and duplicate results, divided by the average of the sample and duplicate results, multiplied by 100%.

7 Interferences

It is possible that fluorine, phosphate, or heavy metals could have a negative influence on the analyses. The instrument is calibrated by the manufacturer and certified by the installer. Tungsten (VI)-oxide is used to remove alkali sulfur.

8 Health and safety

Use protective gloves to handle all of the chemical substances necessary for operation of this instrument. Review the MSDS for additional information and safety concerns regarding the chemical substances used throughout these procedures.

The combustion tube within the instrument is routinely at temperatures exceeding 1150°C. The reduction tube is routinely at temperatures exceeding 850°C. Do not remove the right instrument panel. All maintenance will be performed by authorized personnel only.

Keep all loose clothing away from mechanical moving parts associated with the scale and carousel.

9 Personnel qualifications

Samples will be analyzed by Missouri State University (MSU) laboratory personnel who have received appropriate training from experienced personnel, prior coursework, and laboratory experience regarding the analyses, and who are familiar with all of MSU's sample handling and labeling procedures and appropriate SOPs.

10 Equipment and supplies

Balance: Mettler Toledo microbalance to weigh mass of sample and additive.

Carousel: loads samples into the instrument.

Chain of Custody Forms: used to describe the written record of the collection, possession and handling of samples. Chain of custody (COC) forms are located on a board in Temple Hall 125. Chain of custody forms should be completed as described in the Chain of Custody SOP # 1030R01.

Hand press: forms tin boats into pellets containing sample and additives.

Micro spatula: used to transfer sample additives into tin boats.

Protective Gloves: for protection against chemicals and from contaminants.

Tin boats: used to form pellets of sample and additives.

11 Reagents and standards

- 11.1 Acetanilide (C_8H_9NO): Elementar 03 679 942.
- 11.2 Ceriumdioxide (CeO_2): Elementar 03 679 915.
- 11.3 Corundum balls (aluminum oxide Al_2O_3): Elementar 50 008 467.
- 11.4 Phosphorpentoxide (Sicapent): Elementar 03 960 352. Must wear safety goggles and protective gloves. Caution will occur if contact occurs.
- 11.5 Quartz wool: Elementar 03 679 908.
- 11.6 Sodium hydroxide granulated (NAOH): Elementar 03 679 900. Must wear safety goggles and protective gloves. Caution will occur if contact occurs.
- 11.7 Sulfanic acid: Serves as a calibration standard. Elementar 03 001 118.
- 11.8 Tungsten (VI)-oxide (oxytetrachloride $WOCl_4$): Elementar 11.02-0008 (granulate for tubes) and 11.02-0017 (powder for sample additive).

12 Quality control

- 12.1 Quality control program: The minimum requirements of the quality control program for this analysis consist of an initial demonstration of laboratory capability and the periodic analysis of laboratory reagent blanks and other laboratory solutions as a continuing check on performance. The laboratory must maintain performance records that define the quality of the data that are generated.
 - a. Analyses of laboratory blanks are required to demonstrate freedom from contamination.
 - b. The laboratory shall, on an ongoing basis, demonstrate through calibration verification and analysis of the ongoing precision and recovery sample that the analysis system is in control.
 - c. The laboratory should maintain records to define the quality of data that is generated.
- 12.1 Initial demonstration of performance. The following must be satisfied before the analytical procedure may be used for samples and before a new analyst may analyze samples.
 - a. Method Detection Limit (MDL) – To establish the ability to detect the analyte, the analyst shall determine the MDL by carrying through 7 or

more separately prepared blanks through the analytical procedures. The average value, \bar{X} , and the standard deviation of the values, s , shall be calculated. The MDL is equal to $3s$ (3 x standard deviation). The MDL should both be less than or equal to: carbon, 0.25%, nitrogen 0.25%, sulfur 0.27%.

- b. Initial Precision and Recovery – To establish the ability to generate acceptably precise and accurate results, the manufacturer performed an extensive calibration of the instrument using various masses of solids which illustrate the instrument’s response to analyte concentration. Over 60 separate calibration standards containing acetanilide, stearic acid, and sulfanic acid are used to calibrate the instrument. The measured values are within 10% of the true value of the standards. The instrument calibration remains stable for at least 6 months.

12.2 The LRB is measured along with the standards at the start of the analytical cycle. The LRB must be less than or equal to the required method detection limit to pass the quality control program. Two laboratory duplicates (LD) will be analyzed with each carousel. A check standard (Nat Stnd) will occupy the last sample port in each carousel. The criteria for these checks are noted in Table 1.

Table 1. Quality control samples and acceptance criteria.

Check	Acceptance Criteria
LRB	\leq MDL
LD	\pm 20% of each element
Nat Stnd	\pm 20% of each element

13 Calibration and standardization

- 13.1 Sulfanic acid is used a calibration standard to calibrate the instrument and is defined in the Standards-Menu as “Calibration Sample”. The sulfanic acid standard contains 8.09% nitrogen, 41.61% carbon, and 18.50% sulfur. The permitted deviation range of each element is defined as 5.0%.
- 13.2 Natural standards are standard sediment samples that produce consistent concentrations of C, N, and S. These natural standards are made from sediment taken from representative soils in the region and are used to condition the instrument and check instrument performance.
- 13.3 The vario EL software automatically computes the calibration function which is a non-linear fourth order polynomial equation. The calibration curve and resulting equations are used to compute element concentration percentage.

- 13.4 The manufacturer indicates that the instrument calibration remains stable for at least 6 months and suggests recalibration when changes are made to the detector or adsorption columns.
- 13.5 Calibration standards and natural standards are analyzed with each batch to monitor instrument shift and combustion tube and reduction tube performance.

14 Procedure

- 14.1 Record ambient laboratory conditions such as temperature and humidity in a laboratory notebook as dictated by the specific project QAPP.
- 14.2 Remove carousel from the instrument.
- 14.3 Balance Operation Overview
 - a. Initial Setup
 - 1. Turn on balance by pressing the On/Off key on the bottom of the unit. Ensure that the screen reads Home in the upper left corner. If it does not, press the Home Key. The Home Key is located on the left side of the unit and looks like a house.
 - 2. If necessary, adjust the units of measure to the appropriate setting (usually mg) by pressing the touch screen. Press on the current unit being displayed and select the new unit of measure.
 - 3. If necessary, adjust the number of decimal places to be measured by pressing on one of the three touch screen buttons labeled "1/10d", "1/100d", or "1/1000d".
 - 4. Calibrate the system using the balance's Internal Calibration. Press the touch screen button to the far right titled "Adjust.int." The scale will make noise and a dialog box will open. The dialog box will indicate when the balance is calibrated.
 - b. Weighing a Sample
 - 1. Use the "SmartSens" sensor on either the upper left or upper right side of the unit to open the glass draft shield. Close the draft shield by using the "SmartSens" sensors. Similarly, you can use the double arrow buttons on the lower left and lower right sides of the unit to open and close the draft shield. Do not press the lever to manually slide the draft shield open, only use the sensors or double arrow buttons. The lever is not used for normal operation of the balance.
 - 2. When weighing a sample, place all materials directly in the middle of the balance making sure that nothing touches the bottom collection plate. The "SmartTrac" graphical weighing aid located below the measured weight readout on the screen indicates where the current sample weight is in the scale's weighing range. An error is produced if the current sample weight exceeds the weighing range.
 - 3. To tare the scale, press the 0/T button on the bottom of the unit. The draft shield will close, the balance will tare, and the draft shield will

open when the balanced has zeroed.

c. Sending Data

The Name and Method columns in the database setup screen must be complete for the balance to automatically send the weight of each sample to the software program. To automatically send the sample weight to the software program press the Send button. The Send button looks like a printer and is located on the right side of the unit's face. The computer will beep which indicates that the information was received.

d. Turning Off the Balance

1. Remove all weight from the weighting plate and remove any spilled materials on the collection plate.
2. Close the glass draft shield.
3. Tare the unit.
4. Press and hold the On/Off button until the balance display is clear.

14.4 Pre-analysis Procedures

a. Software Startup

1. Turn on computer and log on with user name and password.
2. Click on the vario EL desktop icon to start the program. Click enter on the password screen without providing a password.
3. The program may take a while to display and when it finds that the instrument is connected, a second window will indicate that the modules are being initialized.
4. Click on Options, and then click on Sleep/Wake-Up. If the Wake Up button is not activated (is light in color), then the system is waking up. This heats up the instrument and flushes the instrument with gas. The lower right hand corner of screen will change from "sleeping" to "heating up", then to "stand by". When it reaches "stand by", it's ready to use.

b. Instrumentation Checks

1. Check the gas cylinder regulators. There are 2 regulators on each cylinder. The regulators on the left indicate gas flow. The regulators on the right indicate actual tank pressure. The left regulator on the helium tank should read 2 bar, look at the number inside the circle. The left regulator on the oxygen tank should read 2½ bar, look at the number inside the circle. Adjust accordingly and only to these flows. If the right regulator on either tank reads below 200 psi, look at the number on the outside of the circle, contact the laboratory manager or supervisor immediately and do not continue with the analyses.
2. The "Furnaces:" indicator located at the lower left hand corner of screen should read: 1150 850 and some smaller number indicating residual heat (these temps can vary by 20°).
3. The "Press.:" indicator located at the lower right hand corner of the screen should read 1.21 bar. "Flow" will vary.

4. Open the left panel on the instrument. Check all tubes for discoloration. If $\frac{3}{4}$ of the tube has changed color (white to blue or grey to light grey) inform the laboratory manager or supervisor. The flow meters are located on the left side. The meter on the right indicates helium flow and should read 2.5 ± 0.5 cm. The meter on the left indicates oxygen flow and should read 1 ± 0.5 cm.
5. The Total Carbon Detector (TCD) signal located in the lower right hand corner of the screen should remain stable; if it varies significantly contact the laboratory supervisor.

14.5 Sample Preparation:


- a. Prepare your blanks, standards, and samples based on the analysis sequence. Make sure that the blanks are followed by the natural standards, the calibration standards, and then the samples.
- b. Organize the project sample bags to minimize confusion when preparing samples and entering sample information into the database setup screen of the template.
- c. Open the standard template by clicking on the open folder hot button, scrolling down to “template”, and clicking ok. The standard template has the correct analysis sequence and number of blanks and standards required of the analyses. The template will look like this:

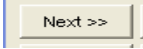
No	Sample name	Wgh. [mg]	N [%]	C [%]	S [%]	N Area	C Area	S Area	N Factor	C Factor	S Factor	Method	Info	Date	Time	N Blank	C Blank	S Blank	
1	Blank	1.0000	0.000	0.000	0.000	0	0	0	1.0000	1.0000	1.0000	Index 1				0	0	0	
2	Blank	1.0000	0.000	0.000	0.000	0	0	0	1.0000	1.0000	1.0000	Index 2				0	0	0	
3	Blank	1.0000	0.000	0.000	0.000	0	0	0	1.0000	1.0000	1.0000	Index 2				0	0	0	
4	Nat Stnd	5.0000	0.000	0.000	0.000	0	0	0	1.0000	1.0000	1.0000	Index 1				0	0	0	
5	Nat Stnd	5.0000	0.000	0.000	0.000	0	0	0	1.0000	1.0000	1.0000	Index 1				0	0	0	
6	Nat Stnd	5.0000	0.000	0.000	0.000	0	0	0	1.0000	1.0000	1.0000	Index 1				0	0	0	
7	Sulfanilic acid	5.0000	0.000	0.000	0.000	0	0	0	1.0000	1.0000	1.0000	Index 1				0	0	0	
8	Sulfanilic acid	5.0000	0.000	0.000	0.000	0	0	0	1.0000	1.0000	1.0000	Index 1				0	0	0	
9	Sulfanilic acid	5.0000	0.000	0.000	0.000	0	0	0	1.0000	1.0000	1.0000	Index 1				0	0	0	
10	Sample ____	50.0000	0.000	0.000	0.000	0	0	0	1.0000	1.0000	1.0000	Index 4				0	0	0	
11		0.0000	0.000	0.000	0.000	0	0	0	1.0000	1.0000	1.0000					0	0	0	
12																			
13																			
14																			
15																			

Other specific project templates may be used.

- d. Double click on the line labeled 10 Sample ____ to bring the CNS Data Input screen in to view.

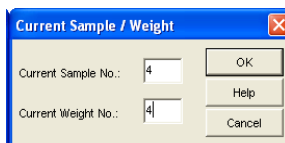
Remove the “Sample ___” and replace it by typing the appropriate

sample name. Enter the O₂ Index by clicking on the  button. The correct O₂ Index is 3 for most samples weighing 20mg. Check with the project supervisor and laboratory supervisor during project setup to determine the correct O₂ Index for samples being analyzed.

- e. Click the next button  to proceed to sample 11 and continue to enter each sample name and O₂ Index until either all 80 slots of the carousel are full or all project samples have been accounted for. Use the Previous button to go back to previous sample data screens. Click on the Close button to end the data input session.
- f. Prepare blanks, natural standards, standards, and samples as follows:

Sample Type	Sulfanic Acid Mass (mg)	Natural Standard Mass (mg)	Sample Mass (mg)	Tungsten Mass (mg)
Blank	---	---	---	---
Natural Standards	---	5	---	15
Standards	5	---	---	---
Samples	---	---	20	60

- g. Turn the metal hole plate on the bottom of the carousel until the hole is located at the sample port without a number, sample port 1 is above the port without a number.
- h. Leave ports 1, 2, and 3 of the carousel empty. A sample weight of 1mg has been given to each blank in the template to satisfy the software setup. The weight of the blanks is not significant therefore the blanks do not need to be weighed. The first blank has an O₂ index of 1 and the second and third blanks have an O₂ index of 2.
- i. Click Edit and then Pointer. Enter 4 in the Current Sample No: field and in the Current Weight No: field. Line 4 will turn green.



- j. Place a tin foil sheet in the sample press or in a pelletizer to form.
- k. Place the formed foil sheet on the scale, tare the scale, and transfer the appropriate amount of natural standard to the foil using a micro scupala.
- l. Send the natural standard weight to the weight column of line 4 by pressing the “send” button on the balance. The send button looks like a printer and is on the right side of the unit. The sample line changes color based on the information that is being transferred to the line.
- m. After sending the natural standard weight, tare the scale, and transfer the appropriate amount of tungsten to the foil using a micro scupala. Always send the weight of the standards and the samples, before taring and adding the additional weight of the tungsten. The tungsten weight does not have to be precise, but should be as close to either the 15mg for natural standards or 60 mg for the samples as possible. Tungsten is used to remove alkali sulfur.
- n. Remove the natural standard from the balance, press with the pelletizer, and insert it into port 4 of the carousel.
- o. Continue forming foil sheets, weighing standards and samples, sending their weights to coinciding lines, adding tungsten when necessary, pressing with the pelletizer, and inserting them into the carousel.
- p. Load the carousel with each standard and sample pellet. Make sure that the each sample is in the correct sample port of the carousel as dictated by the sample numbers and names in the setup screen sequence:

No.	Sample name	Wgh. [mg]
1	Blank	1.0000
2	Blank	1.0000
3	Blank	1.0000
4	Nat Stnd	5.0000
5	Nat Stnd	5.0000
6	Nat Stnd	5.0000
7	Sulfanilic acid	5.0000
8	Sulfanilic acid	5.0000
9	Sulfanilic acid	5.0000
10	Sample ___	50.0000
11		0.0000
12		
13		

Port numbers 1, 2, and 3 should be empty. Port number 4 should have a natural standard in it and so on. Load the samples as dictated by the sample sequence on the setup screen.

- p. Add a blank, foil blank, natural standard or standard, and a laboratory duplicate to the analysis sequence after every 15 samples to determine instrument drift and precision.

14.6 Seating Carousel

- a. The 80-slot carousel is what the instrument is set to use. Contact the laboratory supervisor and request setup alterations if the 40-slot carousel is desired.
- b. Never have the carousel on the drive plate while resetting the drive plate to home. The carousel should remain loaded and ready to go on the lab bench while all drive plate operations occur.
- c. Click Options, then Misc, then Sample Feeder. Enter Hole = 0. It defaults to 1 but you always enter zero. The drive plate will rotate so that the pins line up.
- d. Check to make sure that the hole in the metal hole plate is located at the slot without a number. You can see through that sample hole and the home pin. Sample port 1 is located above the plot without a number.
- e. Seat the carousel on the drive plate making sure that it is flush and level. The handle should be parallel to the longest side of the analyzer

14.7 Sample Analysis

- a. Make sure that the active line is on sample 1 by clicking Edit, then Pointer, and enter "1" into the current sample. Click "OK". Sample line 1 will turn yellow.
- b. Click on System and then Auto to begin analyses.
- c. When the instrument is analyzing a sample the sample line in the database setup screen will turn yellow.
- d. Click on the text hot button near the top of the screen to view the data as it becomes available. Check daily factor calibration by viewing the sulfanic acid standards and samples. Recalibrate and reanalyze when any of the daily factors are outside the range of 0.9 to 1.1. The N, C, and S factors will be the same for the samples and are based on the sulfanic acid standards analyzed prior to the samples. The N, C, and S factors of the samples should be recorded with each data sheet to illustrate the quality of the data.

14.8 Post Sample Analysis Shut Down

- a. After reviewing all data for acceptable daily factor calibration values, click on Options and then Sleep/Wake-Up-Function. If the values are outside of acceptable limits, reanalyze, and shut down afterwards.
- b. Click on Sleep Now button to bring the instrument to shut-down conditions.
- c. The Mode of the Sleep/Wake-Up function can be changed to bring the instrument to shut down conditions after all samples in the carousel have been analyzed or after a specific sample. Those options can be used if the laboratory technician will not be in the lab during analyses.

14.9 Export and Save Data

- a. Save the data as an mbd file by clicking File and Save As. The software automatically names the file based on OEWR format. Click Ok to save the mbd file that contains the sample data, parameter setup information, and graphical peak information.
- b. Save the data for project management use as an Excel file by clicking Export. Click Ok to keep the automatically formatted file name or edit the file name and click Ok. The Save As box will appear and should be set to save to the All Analyses file. If the All Analyses file is not in the window, navigate to the correct file by clicking the "Up One Level" button to My Computer and then clicking on the Shared Documents file. Click on the All Analyses file, change the file type to an MS Excel Files (.xls) within "Save As Type:" field, and click save. A box will appear that reminds the user that no graphical data will be saved with the Excel file, click Ok to acknowledge. Back up data to a removable drive by clicking Export, navigating to the removable drive, and saving the file.
- c. When the data is viewed in any other software program the column titles change and all available columns are included during the export. No figures are exported to Excel. The Stored column contains when the data was exported. SDateTime includes the date when the sample was analyzed. Weight is the weight of the sample in milligrams. Fact1 is the N-factor, fact2 is the C-factor, and fact3 is the S-factor in the final data sheet as shown below.

Stored	Nummer	Name	Info	SDateTime	Weight	Weight1	Dim	Grp	Fact1	Fact2	Fact3
4/18/2007	1	blank		3/30/2007	1	0		0	1	1	1
4/18/2007	2	blank		3/30/2007	1	0		0	1	1	1
4/18/2007	3	blank		3/30/2007	1	0		0	1	1	1
4/18/2007	4	runin		3/30/2007	5.7	0		0	1	1	1
4/18/2007	5	runin		3/30/2007	5.3	0		0	1	1	1
4/18/2007	6	runin		3/30/2007	5.2	0		0	1	1	1
4/18/2007	7	sulf		3/30/2007	5.7	0		0	0.932883	0.946457	0.940992
4/18/2007	8	sulf		3/30/2007	5.5	0		0	0.95891	0.973926	0.968699

4/18/2007	9	sulf	3/30/2007	6	0	0	1.039386	1.054479	1.04804
4/18/2007	10	SDS-1-A	3/30/2007	59.7	0	0	0.97706	0.991621	0.98591
4/18/2007	11	SDS-3-A	3/30/2007	60.6	0	0	0.97706	0.991621	0.98591

Abs1 is the absolute content of the elements in the samples. The absolute values are used in combination with the mass and daily factors to determine percent element content of the samples. Percent1 is the percent nitrogen content of the sample. Percent2 is the percent carbon of the sample and Percent3 is the sulfur content of the sample. Area1, 2 and 3 are the areas under the peaks of nitrogen, carbon, and sulfur. User1, 2, and 3 are user defined calculations. The User1 column is defined as the N test for each sample. The N test determines the amount of nitrogen liberated from a soil sample when it is heated. CompErr provides information about the elemental content compared to the detection range. The "u" after the element indicates that the sample was under range for that element, i.e. Nu = under range nitrogen. The "o" after the element indicates that the sample was over range for that element, i.e. Co = over range carbon. The CNRatio column is the ratio of percent carbon to percent nitrogen. ParamStr illustrates which oxygen index was used. NumOfScans indicates the number of times the thermal conductivity detector scanned the elemental gases within the C, N, and S adsorption columns. The Info and Comment columns will only have content if the user typed additional information into those columns. It is normal for columns titled Version, Weight2, Weight3, Dim, Grp, Blank1, Blank2, Blank3, Blank4, User2, User3, ProcErr, Protein, Humidity, ProtFact, Param, Tagged, WeightAttrib, and Sample Attrib to not include any information in them or be full of zeros. Any title with "4" included within it represents hydrogen attributes and this version of the instrument does not include hydrogen, so any information in those columns is invalid.

15 Data acquisition, calculations, and reporting

15.1 The data from each batch will be saved to the CNS computer and the same data will be exported as an Excel file for project management use.

15.2 The vario EL does not perform absolute measurements (CNS mg absolute) but determines the value by calculations. The vario EL software performs the C, N, and S concentration calculations using a fourth power equation and instrument calibration as well as daily factor calibration. Standards of varying mass and with known element content are used for both instrument and factor calibration. Peak area and absolute content in milligrams are plotted and the differences between theoretical and actual values are determined. The calibration is configured using the non-linear, fourth power equation:

$$y = a + bx + cx^2 + dx^3 + ex^4.$$

The permitted relative tolerance which is the deviation of the results for each element is defined as 5.0%. The instrument calibration remains stable for at least 6 months and factor calibrations occur with each batch. Recalibrate when

the daily factor is outside the range of 0.9 to 1.1 or when adsorption columns have been changed.

The quality of the degree of adaptation is calculated using the polynomial computation:

$$Q = \sqrt{\frac{SFQ}{(n-1)}}$$

$$SFQ = \sum_{i=1}^n \left[\frac{(act. - theo) \cdot 100}{theo_i} \right]^2$$

Where:

- Q = quality
- n = quantity of calibration samples
- SFQ = sum of error squares
- act. = computed absolute content of the respective sample
- theo_i = theoretical absolute content of the respective sample

The calibration coefficients are included in the data file and are printed with a standard report.

- 15.3 There are no dilutions associated with this method.
- 15.4 Results should be reported to 0.01%.
- 15.5 The evaluation of MDL and precision require calculation of standard deviation. Standard deviations should be calculated as indicated below, where n = number of samples, x = concentration in each sample. Note: This is the sample standard deviation calculated by the STDEV function in Microsoft Excel.

$$s = \left(\frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n-1} \right)^{1/2}$$

- 15.6 The Method Detection Limit (MDL) is the lowest level at which an analyte can be detected with 99 percent confidence that the analyte concentration is greater than zero and is 3.14(standard deviation) = 3.14(s).

15.7 Minimum Quantification Interval is the lowest level that can be quantitated accurately and is generally defined as four times the method detection limit = 4(MDL).

15.9 All OEWR project data will be transferred into an OEWR Project Results template and additional QA/QC data will be added from the original data set.

16 Computer hardware and software

16.1 Microsoft Word: this document is prepared using Word.

16.2 The Word document file name for this SOP is: CNS-R01.doc

16.3 The vario EL software controls all aspects of the instrument and sample analysis calculations.

17 Method performance

17.1 The desired performance criteria for this measurement are:

- a. Detection limit: carbon = 0.25%, nitrogen = 0.25%, sulfur = 0.27%.
- b. Precision: $\pm 20\%$
- c. Accuracy: $\pm 20\%$
- d. Minimum Quantification Interval: 0.01%

17.2 The operating ranges for this instrument are: carbon, 0.03 to 20 mg absolute, nitrogen 0.03 to 2 mg absolute, sulfur 0.03 to 6 mg absolute. The sample mass range is 0.2 mg to 200 mg.

17.3 The instrument will be recalibrated when the daily factors for each element are outside the range of 0.9 to 1.1. Fact1 is the N-factor, fact2 is the C-factor, and fact3 is the S-factor in the final exported data sheet. These factors should be recorded with each data sheet to illustrate the quality of the data.

18 Pollution prevention

All wastes from these procedures shall be collected and disposed of according to existing waste policies within the MSU College of Natural and Applied Sciences. Volumes of reagents made should mirror the number of samples being analyzed. These adjustments should be made to reduce waste.

19 Data assessment and acceptable criteria for quality control measures

19.1 The analyst should review all data for correctness (e.g., calculations).

19.2 Precision values are calculated for pairs of duplicate analyses and are recorded as a percent. The desired precision is $\pm 20\%$.

19.3 The desired detection limit is: carbon = 0.25%, nitrogen = 0.25%, sulfur = 0.27%.

19.4 All data is reviewed by the analyst's supervisor or the OEWR QA coordinator.

20 Corrective actions for out-of-control or unacceptable data

- 20.1 The results for precision and blank data are compared to the acceptable values for this analysis; $\pm 20\%$ and 0 for all analytes, respectively.
- 20.2 If a precision value exceeds $\pm 20\%$ then the analyst should note that the data are associated with an out-of-control duplicate analysis in the data report given to the QA/QC manager. The upper control limit (UCL) = 20%.
- 20.3 If a LRB value exceeds the MDL then the analyst should note that the data are associated with a LRB value that exceeds the detection limits in the data report given to the QA/QC manager.
- 20.4 If data are unacceptable for any reason, the analyst should review their analytical technique prior to conducting this analysis again.
- 20.5 If there is sufficient volume of sample remaining for all samples, the batch may be re-analyzed. Quality control data from both batches is recorded.
- 20.6 The instrument may require trouble shooting techniques if the data are unacceptable
- a. Check furnace temperatures and gas flow at time of analyses.
 - b. Check carousel for samples that may not have dropped into the combustion tube.
 - c. Check each gas tank gauge for correct flow.
 - d. Check the ash finger and change if full.
 - e. Check the oxygen lance to ensure through-flow of oxygen.
 - f. Check the combustion and reduction tubes for correct material layering.
 - g. Check drying tubes for discoloration. If entire tube is blue request maintenance from the laboratory supervisor.
 - h. Check all seals and tubes for cracks or wear.
 - i. Contact the laboratory supervisor to request manufacturer service for the instrument.

21 Waste management

The wastes generated in this method are not hazardous. The quantities are very small and can be discarded in the laboratory trash can.

22 References

- 22.1 vario EI III CHNOS Elemental Analyzer Operating Instructions for instruments with serial No. starting with 11054041. May 2005. Elementar Analysensysteme GmbH, Hanau-Germany.

23 Tables, diagrams and flowcharts

All tables, diagrams, or flowcharts for this method are in the body of this SOP.

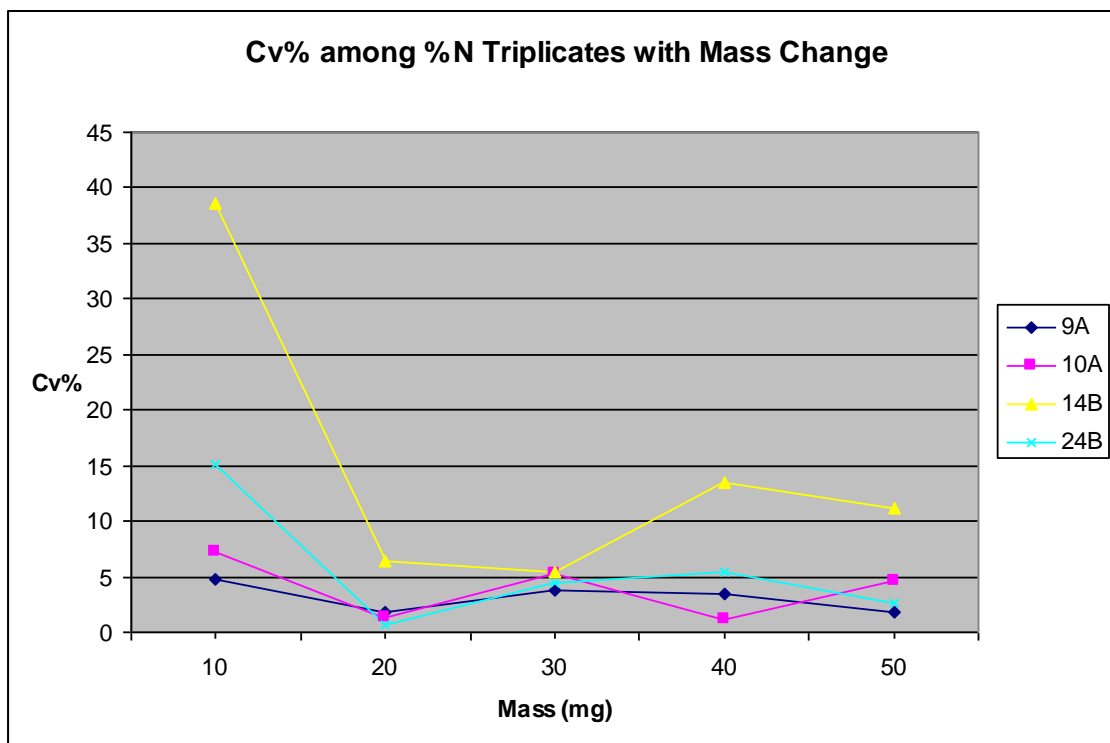
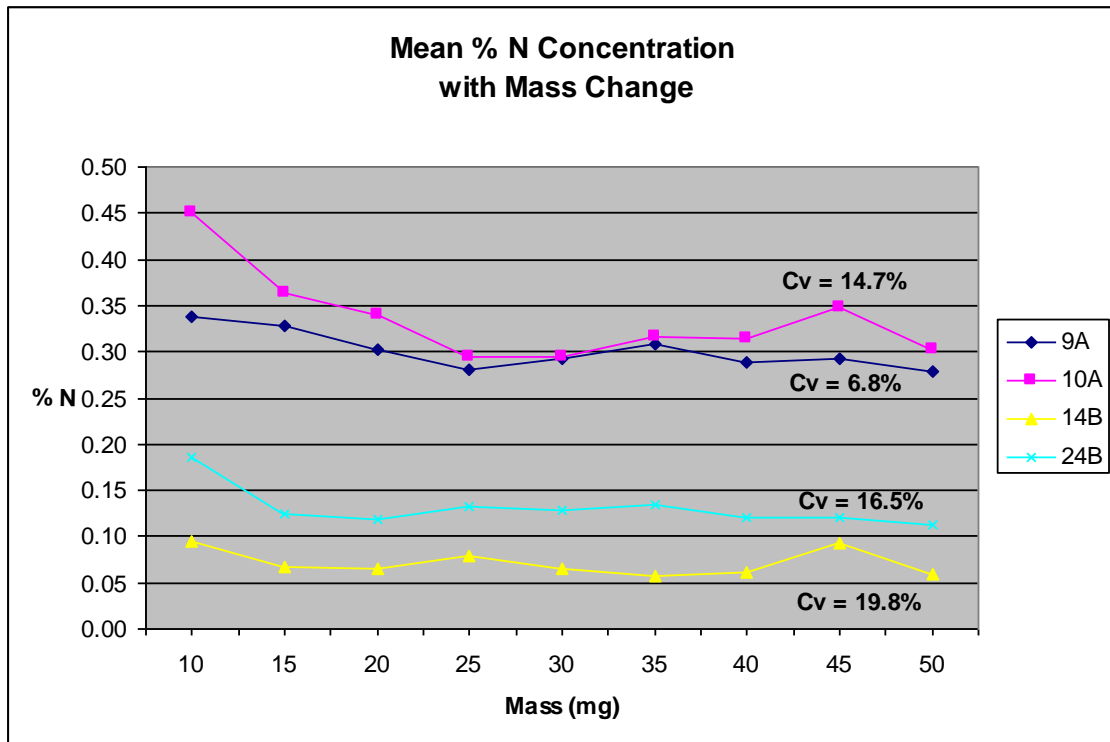
24 APPENDIX A: Appropriate Sample Mass Determination

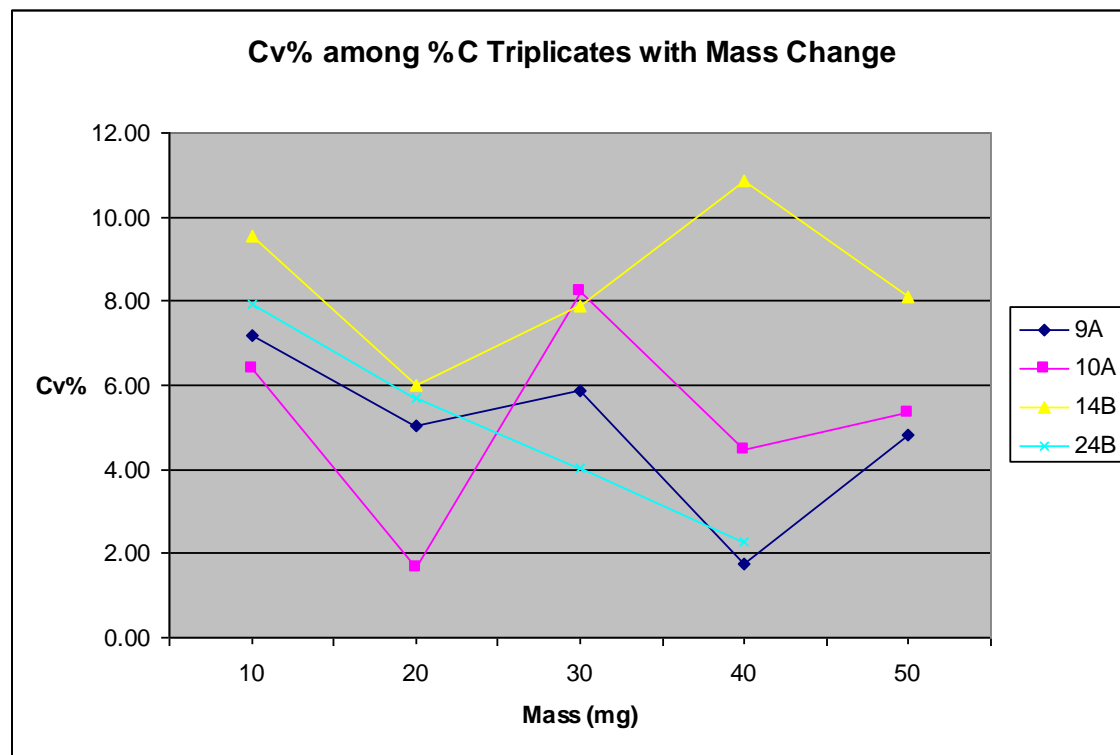
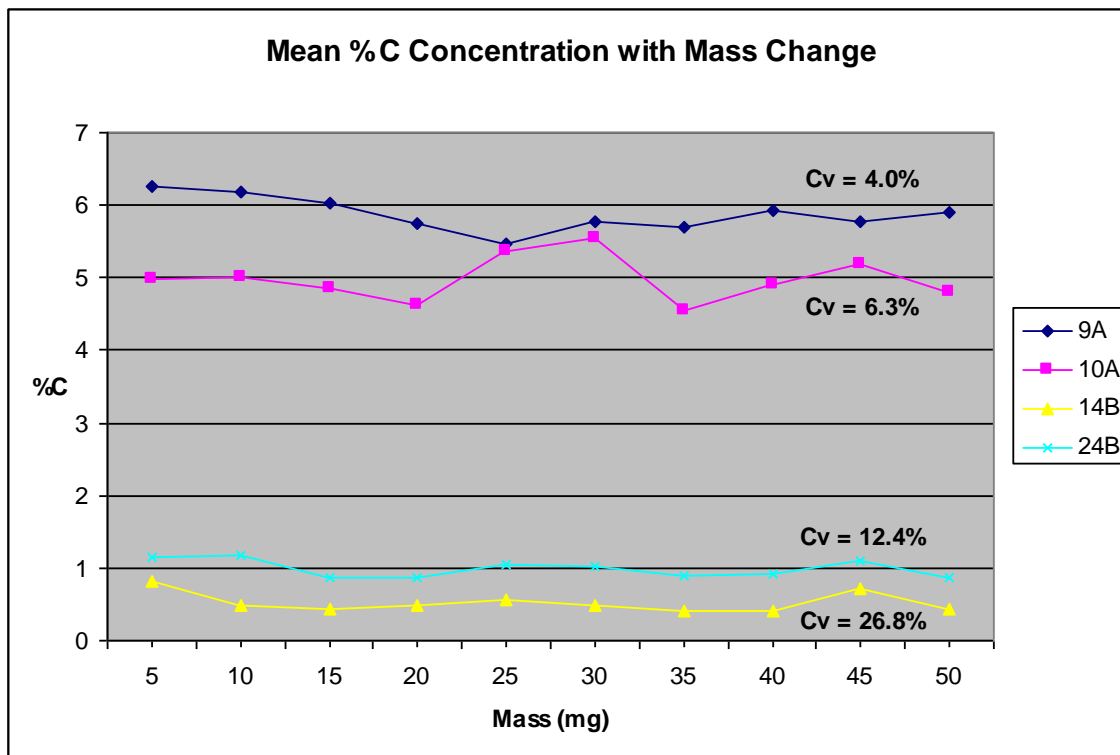
Two organic samples and two inorganic samples were chosen based on visual examination of sediment samples. Various masses of each sample were analyzed to

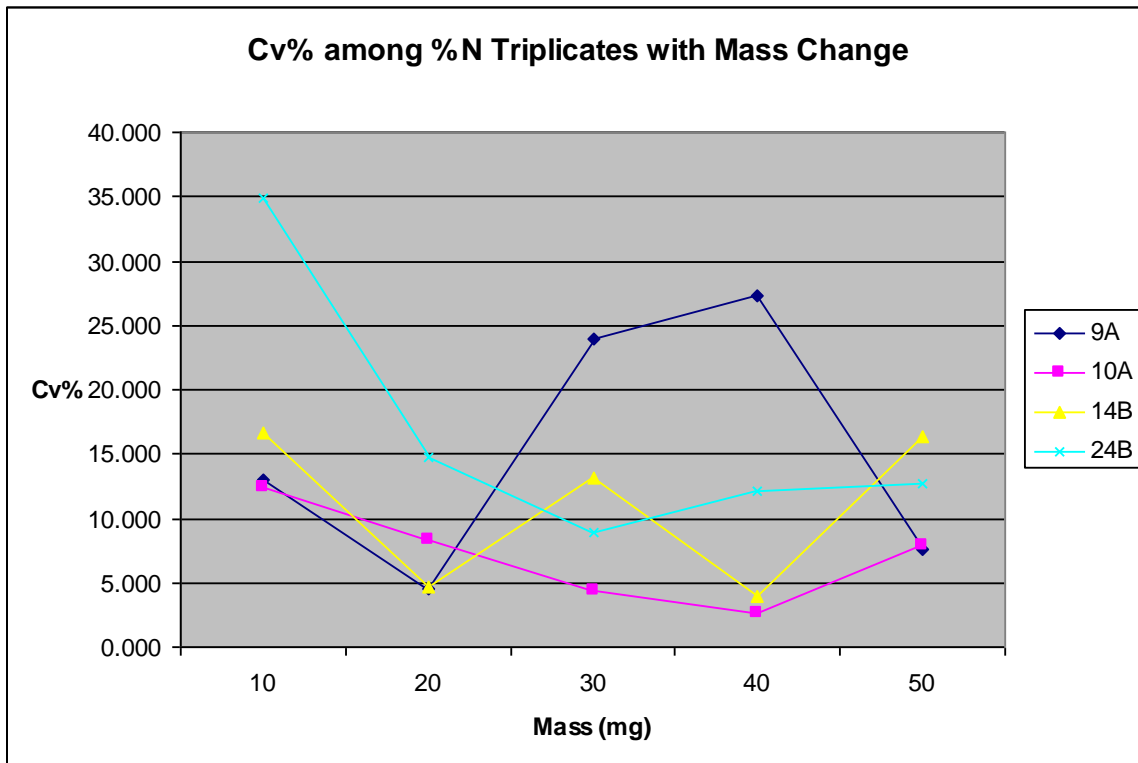
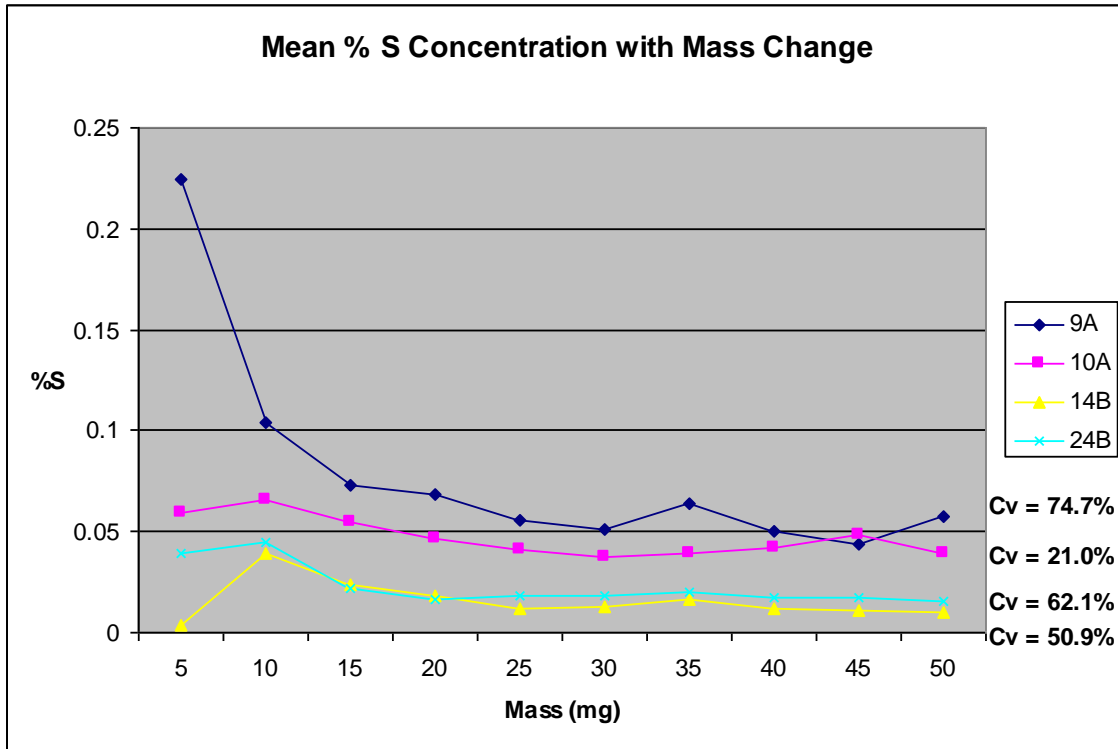
determine the appropriate sample mass and corresponding O₂ dosing associated with this method. Five milligram increments of each sample starting at 5mg and ending with 50mg were used. Triplicates were analyzed for the even masses of each sample. Samples SDS-9-A, SDS-10-A, SDS-14-B, and SDS-24-B collected from the South Dry Sac River basin located north of Springfield, Missouri were used. The oxygen indexes were different and were based on the mass of the sample. Tungsten was added to each sample at three times the sample mass as directed by the manufacturer to remove alkali sulfur. Samples were prepared as illustrated below.

SDS Sample ID	Sample Mass (mg)	Tungsten Mass (mg)	Total Mass (mg)	Method
9-A-5	5	15	20	Index 1
9-A-10-1	10	30	40	Index 1
9-A-10-2	10	30	40	Index 1
9-A-10-3	10	30	40	Index 1
9-A-15	15	45	60	Index 3
9-A-20-1	20	60	80	Index 3
9-A-20-2	20	60	80	Index 3
9-A-20-3	20	60	80	Index 3
9-A-25	25	75	100	Index 4
9-A-30-1	30	90	120	Index 4
9-A-30-2	30	90	120	Index 4
9-A-30-3	30	90	120	Index 4
9-A-35	35	105	140	Index 4
Remainder	Of samples	Have	An Index =	Index 4

Blanks, natural standards, and standards were analyzed prior to the samples to mirror this SOP. The analyses provided the data for the graphs below.







A standard mass of 20mg was determined to be the most appropriate sample mass for N, C, and S analyses. Significant variation among element concentrations decreased when sample mass ≥ 20 mg. Element concentration remained fairly consistent as sample mass increased. Therefore 20mg is the ideal sample mass to produce precise element concentrations and reduce sample and consumable use. Sixty milligrams of tungsten will be used for each 20mg of sample. The oxygen index will be 3 for most samples weighting 20mg.