

**University of Wyoming  
Stable Isotope Facility**

**Standard Operating Procedures  
(Abbreviated version)**

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## Document Change History

Version	Date	Author	Description of changes
0	8/25/15	csc	Initial draft
1.0	10/25/15	csc	Procedure updates
1.1	11/11/15	csc	Add water extraction
1.2	12/21/16	csc	Add nitrate, new figures
1.3	2/2/17	csc	Add formatting
1.4	9/21/17	csc	Long-term QS reference material SOP
1.4	10/31/17	csc	Edited for NEON/Battelle use
1.4	8/29/19	csc	Update SOP, instruments
1.4	10/9/19	csc	NEON recommends

## Table of Contents

Abbreviations .....	6
Symbols .....	6
Important Terms .....	7
<b>Section 1 The Stable Isotope Facility at the University of Wyoming .....</b>	<b>9</b>
<b>1.1 General Description .....</b>	<b>10</b>
<b>1.2 Oversight and Management .....</b>	<b>10</b>
<b>1.3 Financial Management .....</b>	<b>11</b>
<b>1.4 Training.....</b>	<b>12</b>
1.4.1 Laboratory orientation and safety requirements .....	12
1.4.2 Technical training .....	12
1.4.3 Annual training .....	12
<b>Section 2 Instrument Inventory .....</b>	<b>13</b>
<b>2.1 Laboratory Equipment.....</b>	<b>13</b>
2.1.1 Laboratory instruments and equipment .....	13
2.1.2 Mass spectrometers and peripherals .....	13
<b>Section 3 Isotope Ratio Mass Spectrometry .....</b>	<b>14</b>
<b>3.1 Isotope Ratio Mass Spectrometer (IRMS) - Principal of Operation .....</b>	<b>15</b>
3.1.1 Electron Impact Ionization Source .....	15
3.1.2 Electromagnet .....	16
3.1.3 Faraday Cup Detectors .....	17
<b>3.2 The Open Split Interface - Principal of Operation .....</b>	<b>18</b>
3.2.1 Finnigan ConFlo III Interface .....	19
3.2.2 Thermo ConFlo IV Interface .....	20
<b>3.3 The Elemental Analyzer - Principal of Operation .....</b>	<b>21</b>
3.3.1 Carlo Erba NC-2500 Elemental Analyzer .....	21
3.3.2 Costech 4010 Elemental Analyzer.....	22
3.3.3 Thermo Scientific IsoLink Elemental Analyzer .....	23
<b>Section 4 Sample Handling .....</b>	<b>26</b>
<b>4.1 Shipping Documents .....</b>	<b>27</b>
<b>4.2 Sample Check-in .....</b>	<b>27</b>
<b>4.3 Sample Tracking .....</b>	<b>27</b>
<b>4.4 Storage of Samples .....</b>	<b>27</b>
<b>Section 5 Sample Preparation.....</b>	<b>28</b>
<b>5.1 Drying of Samples .....</b>	<b>29</b>
<b>5.2 Sample Labeling.....</b>	<b>29</b>
<b>5.3 Weighing Solid Samples .....</b>	<b>30</b>
<b>5.4 Grinding Samples: Mortar/Pestle Method .....</b>	<b>31</b>
<b>5.5 Grinding Samples: Mixing Mill Method.....</b>	<b>32</b>
<b>5.6 Grinding <i>Toxicodendron</i> Samples: Mortar/Pestle Method .....</b>	<b>33</b>
<b>5.7 Grinding <i>Toxicodendron</i> Samples: Mixing Mill Method.....</b>	<b>34</b>
5.7.1 Grinding soft material (leaves) .....	34
5.7.2 Grinding hard material (stems and roots) .....	35
<b>5.8 Grinding <i>Toxicodendron</i> Samples: Coffee Grinder Method .....</b>	<b>36</b>
<b>5.9 Carbonate Removal from Soils - Acidification Method .....</b>	<b>37</b>
<b>Section 6 Instrument Operation .....</b>	<b>39</b>
<b>6.1 Delta V.....</b>	<b>40</b>

6.1.1 Startup and status .....	40
6.1.2 Initial instrument checks .....	41
<b>6.2 Delta<sup>Plus</sup>XP.....</b>	<b>43</b>
6.2.1 Startup and status .....	43
6.2.2 Initial instrument checks .....	44
<b>6.3 Zero Blank Autosampler .....</b>	<b>46</b>
<b>6.4 ConFlo III .....</b>	<b>47</b>
<b>6.5 ConFlo IV.....</b>	<b>48</b>
<b>6.6 Instrument On/Offs.....</b>	<b>49</b>
<b>Section 7 Mass Spectrometer Analysis.....</b>	<b>50</b>
7.1 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ Analysis of Solids.....	51
7.2 $\delta^{15}\text{N}$ Analysis of Solids - Low Nitrogen Content.....	52
<b>Section 8 Data Collection.....</b>	<b>53</b>
8.1 Data Processing (Isodat 2.0) .....	54
8.2 Setting Up a Sample Table (Isodat 2.0) .....	56
8.3 Retrieving Data (Isodat 2.0) .....	56
<b>Section 9 Quality Assurance .....</b>	<b>57</b>
9.1 Laboratory Reference Materials .....	58
9.2 Handling of Reference Materials.....	59
9.2.1 Control and distribution of reference materials .....	59
9.2.2 Storage of reference materials .....	59
9.3 UWSIF QAQC Reference Material Certification .....	59
9.4 Long-term Reference Material Data Collection Procedures.....	60
9.5 Carbon and Nitrogen Quality Control Criteria .....	61
9.5.1 Carbon isotope analysis quality control.....	61
9.5.2 Nitrogen isotope analysis quality control .....	61
9.6 Quality Assessment Procedures.....	61
9.6.1 Data reduction.....	62
9.6.2 Acceptance criteria for QC samples .....	62
9.6.3 Corrective action.....	62
<b>Section 10 Calculations and Corrections .....</b>	<b>63</b>
10.1 Calculations .....	64
10.1.1 Delta notation.....	64
10.2 Common Corrections.....	65
10.2.1 Drift.....	65
10.2.2 Memory.....	67
10.2.3 Linearity.....	68
10.2.4 Normalization .....	70
10.2.5 Weight percent.....	71
10.2.6 Relative error .....	72
10.2.7 Blank.....	72
10.2.8 Outlier test .....	73
<b>Section 11 Elemental Analyzer Maintenance .....</b>	<b>74</b>
11.1 Building a Combustion Reactor.....	75
11.2 Building a Reduction Reactor .....	76
11.3 Building/Preparing Quartz Inserts.....	77
11.4 Leak Checking an Elemental Analyzer .....	77
11.5 Replacing the Elemental Analyzer Reactors .....	78

11.6 Replacing the Quartz Insert.....	79
11.7 Replacement of the Water Trap .....	80
<b>Section 12 Troubleshooting.....</b>	<b>81</b>
12.1 Troubleshooting the Elemental Analyzer .....	82
12.2 Troubleshooting: Helium and Oxygen Gases .....	83

## List of Figures

Figure 1. Schematic of the electron impact ionization source.....	15
Figure 2. Schematic of the isotope ratio mass spectrometer. ....	16
Figure 3. Schematic of the triple Faraday cup detector system.....	17
Figure 4. Schematic of the open split interface. ....	18
Figure 5. Schematic of the Finnigan ConFlo III open split interface.....	19
Figure 6. Schematic of the Thermo ConFlo IV open split interface.....	20
Figure 7. Schematic of the elemental analyzer coupled to an IRMS.....	24
Figure 8. Delta V control panel (from the Thermo user manual). ....	40
Figure 9. Computer screenshot peak center icon (from Thermo user manual).....	41
Figure 10. Computer screenshot peak centering (from Thermo user manual).....	42
Figure 11. Delta Plus control panel (from Thermo user manual) .....	43
Figure 12. Computer screenshot peak center icon (from Thermo user manual) .....	44
Figure 13. Computer screenshot peak centering (from Thermo user manual) .....	45
Figure 14. Computer screenshot ConFlo IV readings (from Thermo user manual) .....	48
Figure 15. Drift correction.....	66
Figure 16. Injection memory vs. time. ....	67
Figure 17. Linearization.....	68

## List of Tables

Table 1. Abbreviation used .....	6
Table 2. Symbols used .....	6
Table 3. Important terms .....	8
Table 4. Laboratory instruments and equipment.....	13
Table 5. Mass spectrometers and peripherals.....	13
Table 6 UWSIF laboratory reference materials .....	58
Table 7 UWSIF QAQC reference materials.....	58
Table 8 International reference materials .....	60
Table 9 Dixon Q Test critical values .....	73
Table 10 Troubleshooting guide .....	82

## Abbreviations

Abbreviation	Description
AMU	Atomic mass unit
CF	Continuous flow
CF-IRMS	Continuous flow-isotope ratio mass spectrometer
EA	Elemental analyzer
EA-IRMS	Elemental analyzer-isotope ratio mass spectrometer
IRMS	Isotope ratio mass spectrometer
SOP	Standard operating procedure
UW	University of Wyoming
UWSIF	University of Wyoming Stable Isotope Facility

*Table 1. Abbreviation used*

## Symbols

Symbol	Name	Description
$\delta$	delta	The symbol for stable isotope ratios relative to a standard.
‰	per mil	The unit of stable isotope data. One per mil represents one ‘part per thousand’ enrichment or depletion from a standard.
%	percent	One part in a hundred.
°C	degree Celsius	SI unit of measure of temperature.
$\mu$ l	microliter	SI unit of volume. A microliter is 1/1000000 of a liter.
m	meter	SI unit of length.
ml	milliliter	SI unit of volume. A milliliter is 1/1000 of a liter.
psi	pounds per square inch	A common non-SI unit used to express pressure.
min	minute	An acceptable non-SI unit of time equal to 60 seconds.
<sup>13</sup> C	carbon-13	A stable isotope of carbon.
<sup>15</sup> N	nitrogen-15	A stable isotope of nitrogen.

*Table 2. Symbols used*

## Important Terms

Abundance	Abundance is the relative number of atoms of the different isotopes of one chemical element. Abundance is usually expressed as a percentage of the total of all isotopes of a given element.
Atomic Mass Unit	Atomic mass unit or amu, is defined as exactly 1/12 the mass of an atom of carbon-12. One amu is equal to approximately $1.66 \times 10^{-24}$ grams.
Continuous Flow (CF)	Continuous flow is a term for isotope ratio mass spectrometers that are coupled on-line to preparation devices. These instruments represent a “marriage” of chromatography and mass spectrometry, and are similar to conventional organic mass spectrometers in that gas samples are introduced to the IRMS within a stream of an inert gas (helium).
Continuous Flow-Isotope Ratio Mass Spectrometer (CF-IRMS)	In CF-IRMS a sample is introduced into the IRMS using an inert gas that carries the sample gas into the IRMS. Various automated sample preparation devices generate the sample gas, including but not limited to elemental analyzers (EA) and gas chromatographs (GC).
Delta	Delta ( $\delta$ ) is a special unit used to describe the relative deviation of the isotopic ratio of a sample from the known isotopic ratio of a standard. Delta is expressed in the unit of per mil (‰). One per mil represents a one-part-per-thousand difference from a standard.
Elemental Analyzer (EA)	An EA is an instrument that consists of an autosampler, high temperature furnace, chemical trap and a gas chromatograph column. An EA is used primarily to combust material in an oxygen-enriched helium atmosphere producing some or all of the following products: CO <sub>2</sub> , H <sub>2</sub> O, and N <sub>2</sub> .
Elemental Analyzer-Isotope Ratio Mass Spectrometer (EA-IRMS)	An EA-IRMS is an elemental analyzer coupled to an IRMS used to prepare samples before isotopic analysis.

Isotope Ratio Mass Spectrometer (IRMS)	Isotope ratio mass spectrometers are specialized magnetic sector mass spectrometers that produce precise and accurate measurements of variations in the natural isotope abundance of the light elements H, C, N, O, and S.
Open split interface	The open split interface allows the IRMS to sample the inert gas carrier stream as the stream exits a sample preparation device.
Standard Operating Procedure (SOP)	Standard operating procedures are detailed written procedures designed to standardize an analytical method or procedure.

*Table 3. Important terms*



# **Section 1 The Stable Isotope Facility at the University of Wyoming**

## 1.1 General Description

The University of Wyoming Stable Isotope Facility (UWSIF) is a core research facility located in the Berry Biodiversity and Conservation Center at the University of Wyoming. The UWSIF provides quality isotopic analyses for the research community at the University of Wyoming (UW) and other universities and labs nation-wide. The facility is engaged in development of novel analytical techniques, advises researchers on sampling and analysis and serves as a hands-on teaching facility for UW students, post-doctoral associates and faculty. The UWSIF is dedicated to ecological and environmental applications of light stable isotopes (H, C, N, O, and S) and is fully equipped and staffed for a broad suite of applications. The UWSIF plays a prominent role in helping on-campus researchers address environmental management and natural resource issues in Wyoming.

The facility is equipped with isotope ratio mass spectrometers, and optical isotope instruments. These instruments are connected to different peripheral devices for automated sample processing, helping to meet the growing demands of researchers and students on campus. The UWSIF has vacuum extraction lines for off-line preparation and purification of sample gases, sample storage freezers, ovens, fume hoods, centrifuges, grinding mills, and other miscellaneous sample preparation equipment.

## 1.2 Oversight and Management

The UWSIF is administered by a Faculty Director, a Facility Director, and a Laboratory Manager. The Faculty Director sets the vision and broad objectives for the lab and provides guidance, leadership and oversight for the operation and long-term growth of the facility. The Facility Director helps to shape the long-term objectives for the facility, provides isotope consultation to researchers across campus, prepares annual reports for University Administration, provides technical guidance to all technicians working in the facility and conducts tours of the facility for visitors. The Laboratory Manager is responsible for maintaining the instruments, scheduling sample analyses, and overseeing the routine day-to-day operations of the facility.

Broader oversight of the UWSIF is provided by a steering committee of 6 faculty members from different departments on campus (i.e., Botany, Zoology and Physiology, Geology and Geophysics, Archaeology and Anthropology and Ecosystem Science and Management). The role of the steering committee is to help provide guidance on overall strategic direction of the UWSIF and to provide feedback and oversight on technical challenges, policy issues and financial management of the lab. The steering committee meets with the UWSIF Faculty Director and Facility Director during Spring and Fall semesters. These meetings provide the opportunity to discuss future strategies to be adopted by UWSIF to keep pace with the upcoming technologies. The committee composition reflects balance among participating academic units across campus and allows other new faculty to participate in the evolution of the facility. A major goal is to have participation from faculty with diverse technical requirements and disciplinary

backgrounds. Membership is on a two-year rotation, but each member has the opportunity to serve on a continuing basis as necessary to meet the goals for the facility.

### **1.3 Financial Management**

The UWSIF is a non-profit analytical research facility serving the academic community and researchers at the University of Wyoming. Analysis jobs, client billing, monthly, quarterly and annual accounting reports are performed using software by Zoho. Analysis jobs are also managed using a Zoho database system. The UWSIF accounting system is maintained in parallel with the University of Wyoming Department of Ecosystem Science and Management accounting system that is the primary financial accounting system for the UWSIF. The Department of Ecosystem Science and Management keeps track of expenditure amounts and income received to maintain a cash balance. The Department of Ecosystem Science and Management also makes the actual deposits and all payments and monitors paid invoices. The monthly report generated by the Department of Ecosystem Science and Management enables the UWSIF to further analyze the expenditures. By generating the invoices directly, the UWSIF monitors details of sample analyses and jobs to produce a comprehensive picture of the financial and analytical activities in the lab. The UWSIF reconciles the Zoho cash balance with that determined monthly by the Department of Ecosystem Science and Management to ensure all expenses and income have been included in all UWSIF reports.

The income is also reviewed in relationship to expenses incurred by the UWSIF to provide a complete picture of all aspects of facility efficiency. Our system can analyze income by month, quarter or year and provide details for these periods based on specific analyses performed. We can review not only income, but number of samples and standards demanded by each instrument to evaluate maintenance costs and productivity. We are constantly striving to improve the details needed to build our database in order to establish more useful feedback from the accounting system.

Our financial policy is based on the following criterion:

- The sample costs for on-campus academic clients are intended to cover costs of expendable materials and supplies associated with analysis, routine instrument calibration and maintenance, instrument repair and replacement over the long run, new methods development and wages for undergraduate technicians who conduct routine tasks in the facility.
- Sample costs are evaluated on an annual basis to ensure the lowest feasible per sample charge.

## **1.4 Training**

### **1.4.1 Laboratory orientation and safety requirements**

Prior to beginning technical work at UWSIF, each researcher, analyst or technician attends a laboratory orientation training session, which includes the following:

- UWSIF safety procedures
- University of Wyoming Environmental Safety laboratory rules
- Facility safety equipment

### **1.4.2 Technical training**

Full-time and part-time technicians are fully trained for their specific tasks by the Facility Director and/or Laboratory Manager. New personnel must show competence in the use of the instruments (as determined by the Laboratory Manager) before they are allowed independent operation of the instruments. All technicians are trained in appropriate data reduction methods, which includes raw data evaluation, instrument stability tests, and the use of reference materials for data corrections.

### **1.4.3 Annual training**

The UWSIF requires that all technical personnel participate in annual training, which includes:

- A review of the UWSIF Quality Management Plan and the UWSIF Standard Operating Procedure Manual, including new additions or deletions.
- New analytical methods (where appropriate).
- New facility instrumentation (where appropriate).
- A review of all appropriate SOPs.

The Laboratory Manager maintains a training log for all training.

## Section 2 Instrument Inventory

### 2.1 Laboratory Equipment

#### 2.1.1 Laboratory instruments and equipment

Instruments/Equipment	Quantity
Carlo Erba NC 2500 elemental analyzer	1
Costech 4010 elemental analyzer	1
Costech Zero Blank autosampler	3
Finnigan ConFlo III open split interface	1
Finnigan Delta <sup>Plus</sup> XP isotope ratio mass spectrometer	1
Retsch MM-400 Mixer Mill	1
Sartorius SE2 ultra-microbalance	3
Thermo Scientific ConFlo IV open split interface	3
Thermo Scientific Delta V isotope ratio mass spectrometer	3
Thermo Scientific IsoLink elemental analyzer	1

*Table 4. Laboratory instruments and equipment*

#### 2.1.2 Mass spectrometers and peripherals

IRMS	Peripherals	Isotopes	Gas form
Finnigan Delta <sup>Plus</sup> XP	Costech 4010 elemental analyzer Thermo ConFlo III interface Costech Zero Blank autosampler	<sup>13</sup> C, <sup>15</sup> N	CO <sub>2</sub> , N <sub>2</sub>
Thermo Delta V Plus	Carlo Erba NC 2500 elemental analyzer Thermo ConFlo IV interface Costech Zero Blank autosampler	<sup>13</sup> C, <sup>15</sup> N	CO <sub>2</sub> , N <sub>2</sub>
Thermo Delta V Advantage	Thermo IsoLink elemental analyzer Thermo ConFlo IV interface Costech Zero Blank autosampler	<sup>13</sup> C, <sup>15</sup> N	CO <sub>2</sub> , N <sub>2</sub>

*Table 5. Mass spectrometers and peripherals*

## **Section 3 Isotope Ratio Mass Spectrometry**

### 3.1 Isotope Ratio Mass Spectrometer (IRMS) - Principal of Operation

An isotope ratio mass spectrometer consists of three main components: an ion source, an electromagnetic, and a detector.

#### 3.1.1 Electron Impact Ionization Source

Attached to the ionization chamber are the filament (cathode), an electron trap (anode), and ion extraction and focusing plates. The filament emits electrons that travel across the ionization chamber where some of the sample gas molecules collide with the electron beam making a positive ion. In the Delta<sup>Plus</sup> XP,  $\approx 1$  in 1500 gas molecules are ionized,  $\approx 1$  in 1100 gas molecules for the Delta V. The electron trap collects all the electrons not involved collisions with the sample gas. The extraction plates accelerate the positive ions out of the ionization chamber and direct the ions toward the exit slits and focusing plates. The accelerating voltage is 3kV. The different focusing plates are designed to focus the ion beam as it leaves the source. The ion exit plate defines the maximum beam width prior to passage down the flight tube.

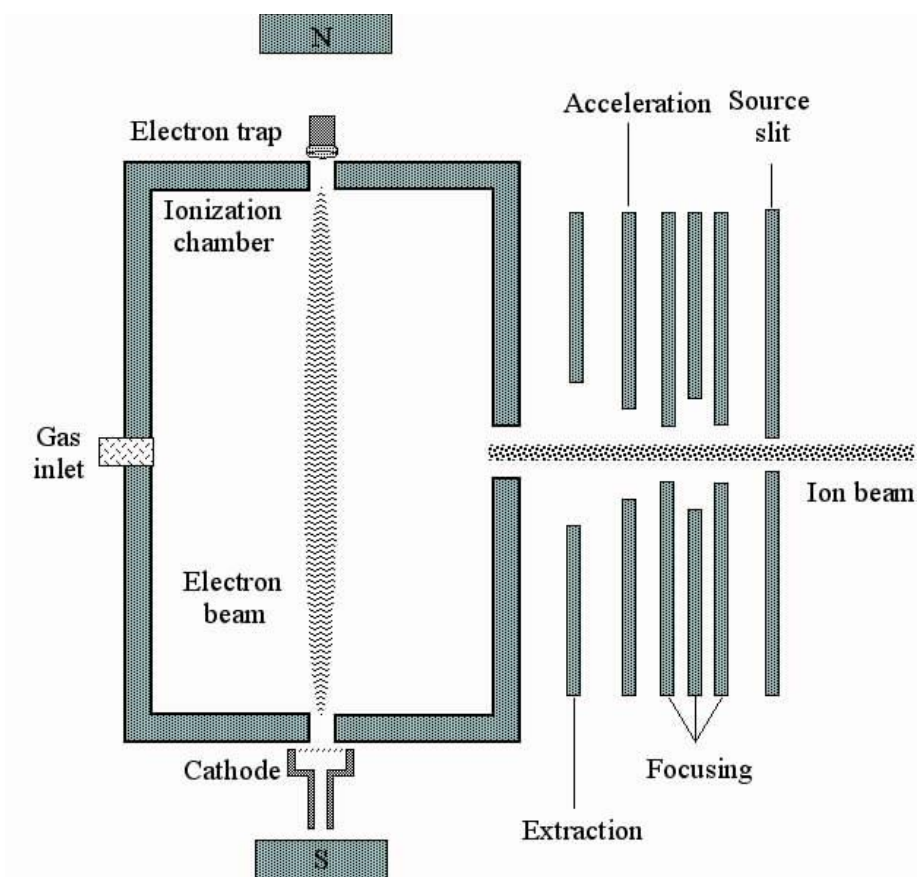


Figure 1. Schematic of the electron impact ionization source.

### 3.1.2 Electromagnet

The flight tube has a 90-degree fixed deflection path fitted with a permanent magnet having a maximum magnetic field strength of 0.75 Tesla, allowing the measurement of mass ranges up to 70 amu (Delta<sup>Plus</sup>XP) or 96 amu (Delta V). For any given gas species the electromagnet is held constant. At a constant accelerating voltage ( $V$ ) and magnet strength ( $B$ ), the lighter ions will be deflected more than the heavier ions. In the case of  $\text{CO}_2$ , mass 44 is deflected more than mass 45 and mass 46. In order to analyze another gas such as  $\text{N}_2$ , the accelerating voltage remains fixed and the magnetic field strength is varied.

These parameters are related by the equation:

$$m/z = r^2 * [B^2/2V]$$

Where:  
 $m/z$  is the mass to charge ratio  
 $B$  is the magnet field strength  
 $r$  is the radius of the ion circular path  
 $V$  is the acceleration voltage

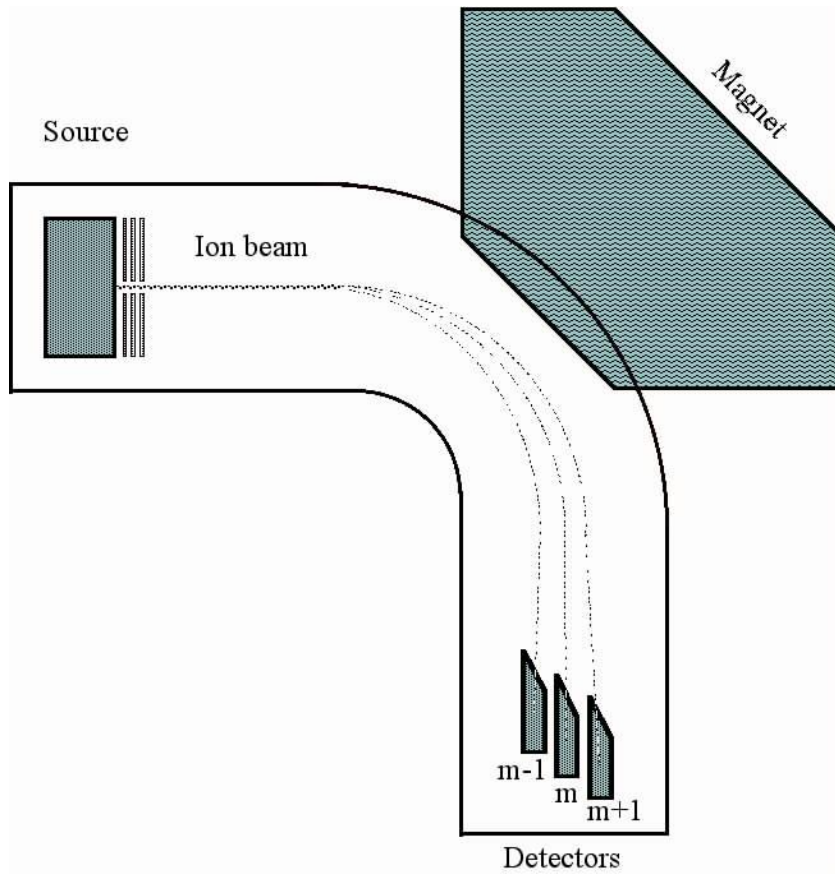


Figure 2. Schematic of the isotope ratio mass spectrometer.



### 3.1.3 Faraday Cup Detectors

After leaving the magnet, the separated ion beams are directed to three collectors called Faraday cups. The three collectors consist of one narrow collector (cup 2) and two wide collectors (cups 1 and 3). These cups are grounded through high ohm resistors. When configured for CO<sub>2</sub> and N<sub>2</sub>, the resistor on cup 1 =  $3 \times 10^8 \Omega$ , the resistor on cup 2 =  $3 \times 10^{10} \Omega$ , and the resistor on cup 3 =  $1 \times 10^{11} \Omega$ . When the positive ions are detected on a collector, an electron returns to the ion, causing a voltage drop in the resistor that acts as a measure of the ion current. The signals received by the collectors are amplified and transferred to voltage-to-frequency converters. This digitizes the analog signal for processing by the computer. In the case of CO<sub>2</sub>, all three masses (44, 45, and 46) are detected at one time, allowing the software to calculate both  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  ratios for a given sample.

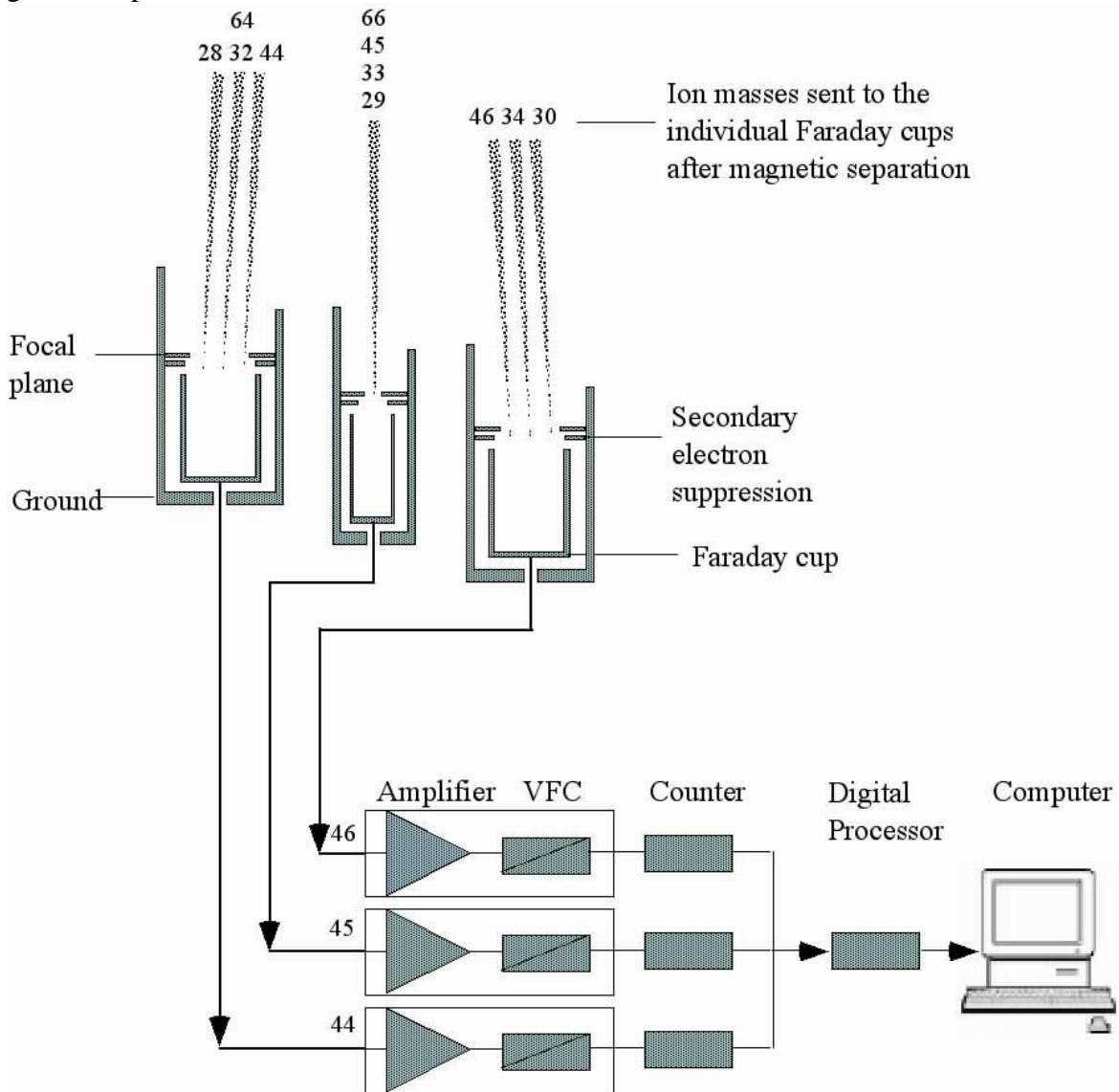


Figure 3. Schematic of the triple Faraday cup detector system.

### 3.2 The Open Split Interface - Principal of Operation

Prior to isotopic analysis, complex samples (solids, liquids, gases) are converted into simple gases (i.e.  $N_2$ , and  $CO_2$ ) through combustion or carbon reduction. When using continuous flow mode, these simple gases are carried to the isotope ratio mass spectrometer by an inert carrier gas (helium). The gas pressure in the ionization source effects the precision and accuracy of the isotope measurement, so it is very important to keep the pressure in the ion source constant at all times. Continuous flow isotope ratio mass spectrometers achieve this using an open split. An open split uses a small capillary to “sniff” the carrier gas/sample gas from an open cell, which is at atmospheric pressure. Because the ionization source is at a very low vacuum, the “sniffed” gas is pulled into the ion source at a constant rate.

The ion source of the mass spectrometer works best with helium flows of about  $0.2 - 0.3 \text{ ml}\cdot\text{min}^{-1}$ . Since sample gas flows entering the open split cell range from  $2-140 \text{ ml}\cdot\text{min}^{-1}$ , this gas flow needs to be reduced to avoid too much gas entering the ion source. This sample gas “splitting” is one of the main purposes of the open split interface.

Mass spectrometers requires molecular flow. This is accomplished in the open split cell. Gases produced in the EA are transported by helium carrier gas flowing at  $80-100 \text{ ml}\cdot\text{min}^{-1}$  and enter the open split cell. Since the mass spectrometer capillary is so small ( $0.1\text{mm ID}$ ) only a small portion ( $<10\%$ ) of sample and carrier gas can enter the mass spectrometer. This essentially reduces the flow to  $\sim 0.3 \text{ ml}\cdot\text{min}^{-1}$  and allows the ion source to operate at  $10^{-6}$  mbar pressure.

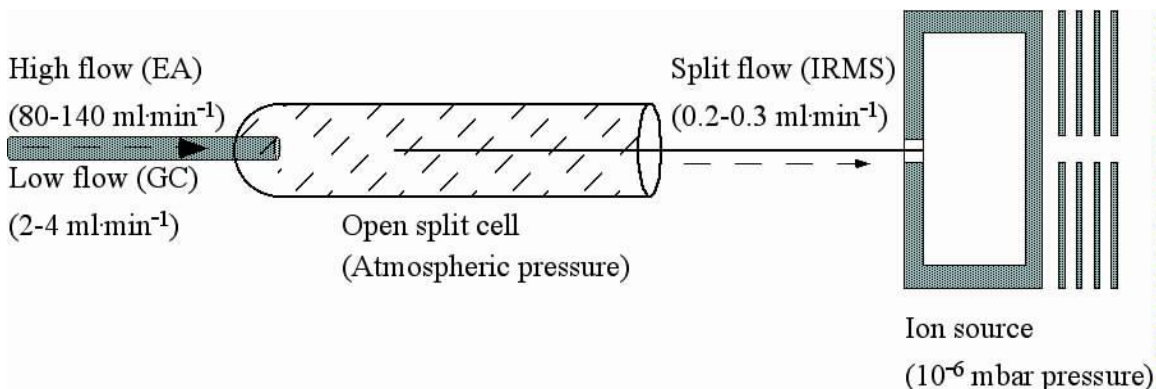


Figure 4. Schematic of the open split interface.

### 3.2.1 Finnigan ConFlo III Interface

The ConFlo III interface consists of two open split cells, one dedicated to reference gases and the other to sample gases. Inside the reference open split cell are four fused silica capillaries, one for the N<sub>2</sub> reference gas, one for the CO<sub>2</sub> reference gas, one for helium make-up gas, and one connected directly to the mass spectrometer source. The reference gas capillaries are attached to pneumatic pistons that allow moving of the capillaries in and out of the helium. The helium make-up and mass spectrometer capillaries are fixed. The sample gas open split cell consists of three fused silica capillaries, one coming from the elemental analyzer, one for helium dilution, and one connected directly to the mass spectrometer source. The helium dilution capillary is attached to a pneumatic piston that allows computer selected sample dilution. The capillary from the elemental analyzer and the capillary connected directly to the mass spectrometer source are fixed. If bulk dilution of the sample is required, the split dilution valve opens and flow to the open split cell is reduced from 80-100 ml•min<sup>-1</sup> to 8-10 ml•min<sup>-1</sup>.

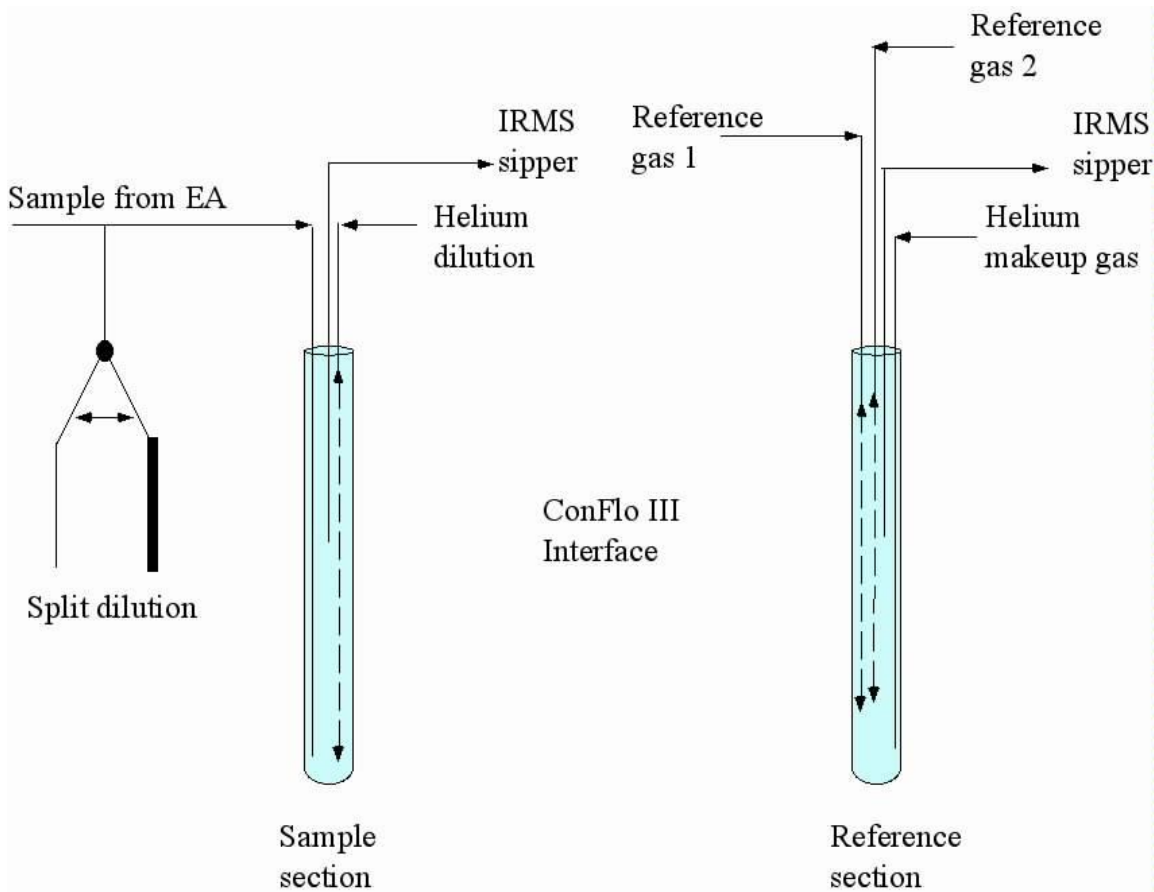


Figure 5. Schematic of the Finnigan ConFlo III open split interface.

### 3.2.2 Thermo ConFlo IV Interface

The ConFlo IV open split interface has five permanently-connected reference gases, low and high carrier flow inputs, and incremental helium dilution. Incremental dilution allows the selection of a wide range of helium dilution options (sample or reference).

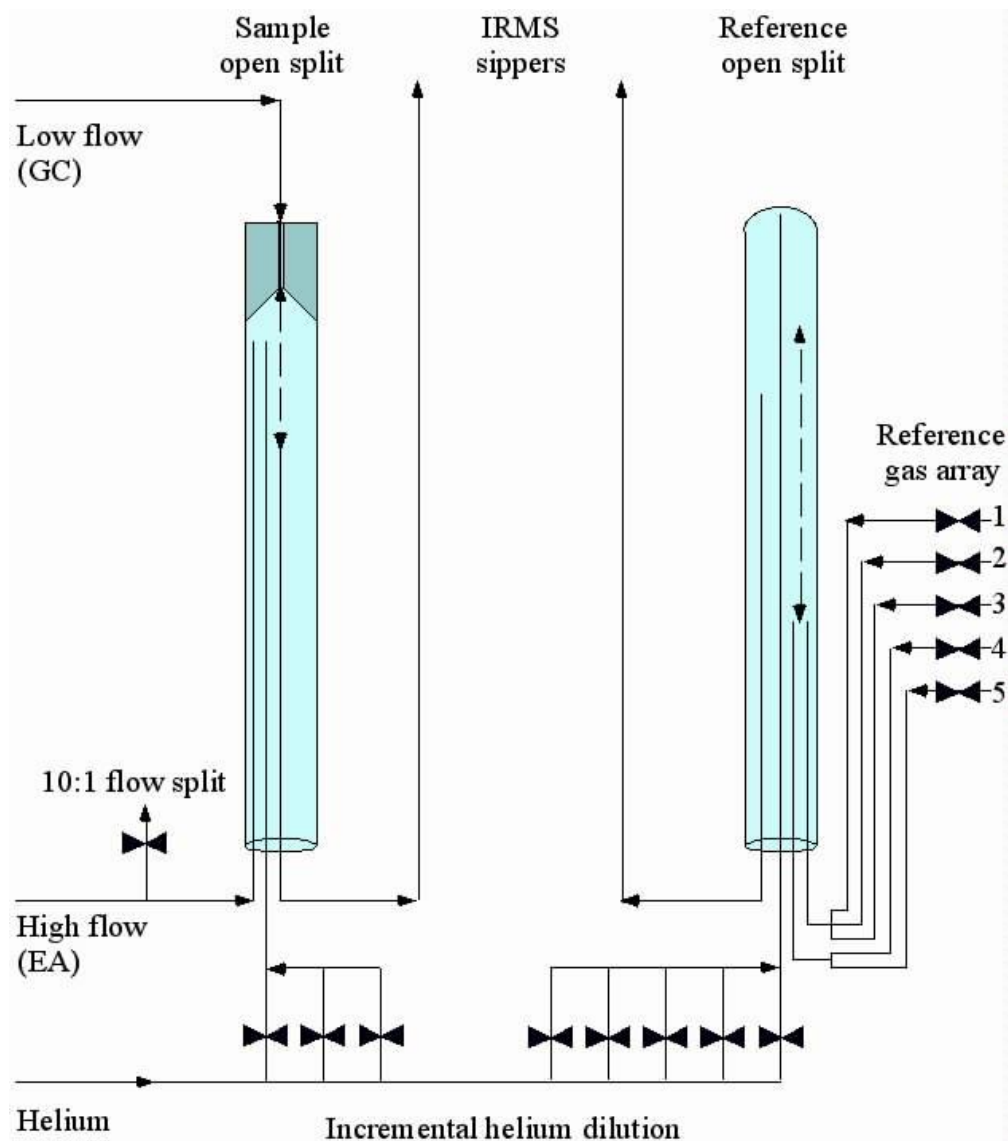


Figure 6. Schematic of the Thermo ConFlo IV open split interface.

### 3.3 The Elemental Analyzer - Principal of Operation

An elemental analyzer is a scientific instrument which determines the elemental composition of a sample. The basic principle of an elemental analyzer is as follows:

The sample is weighed into a tin capsule, which is dropped into an oxygen enriched high temperature combustion reactor. The resulting combustion gases pass through reagents, producing CO<sub>2</sub>, H<sub>2</sub>O, N<sub>2</sub>, and oxides of nitrogen. These reagents also remove halogens, sulfur, and phosphorus. The combustion gases then pass through a reducing reactor to remove excess oxygen and reduce oxides of nitrogen to elemental nitrogen. Water is then chemically removed. The resulting gases enter a gas chromatograph where the N<sub>2</sub> and CO<sub>2</sub> are separated. In stand-alone instruments, the separated gases are then sent to a thermal conductivity detector (TCD) for elemental analysis. If the elemental analyzer is attached to an IRMS, the gases go from the TCD to the IRMS via an open split interface for isotopic analysis.

#### 3.3.1 Carlo Erba NC-2500 Elemental Analyzer

The Carlo Erba NC-2500 elemental analyzer operates based on the principal of flash combustion in which a sample contained within a tin capsule is dropped into a combustion reactor held at 1020 °C. For nitrogen and carbon analysis, the combustion reactor contains chromium oxide as an inorganic oxygen source and silvered cobaltous/cobaltic oxide for removal of halogens and sulfur. When the tin capsule is dropped into helium temporarily enriched with oxygen, the tin ignites and flash combustion occurs. Flash combustion raises the temperature of the sample to >1700 °C. The encapsulated sample, depending on its composition, combusts, generating one or more of these gases: N<sub>2</sub>, N<sub>x</sub>O<sub>x</sub>, CO<sub>2</sub>, and H<sub>2</sub>O. The reduction reactor contains reduced copper wires for the reduction of nitrogen oxides to N<sub>2</sub> and the removal of excess O<sub>2</sub>. An adsorption trap containing magnesium perchlorate removes the H<sub>2</sub>O. The remaining N<sub>2</sub> and CO<sub>2</sub> gases travel through a chromatographic column (Porapak Q) and then move to the ConFlo IV open split interface. See Figure 7 for a detailed schematic of the elemental analyzer. See Section 3.2.2 for a detailed description of the interface.

Instrument temperatures:

Combustion reactor	1020 °C
Reduction reactor	650 °C
Chromatographic column	60 °C

Flow rates:

Helium	80-100 ml•min <sup>-1</sup>
Oxygen	25-30 ml•min <sup>-1</sup>

### 3.3.2 Costech 4010 Elemental Analyzer

The Costech 4010 elemental analyzer operates based on the principal of flash combustion in which a sample contained within a tin capsule is dropped into a combustion reactor held at 1020 °C. For nitrogen and carbon analysis, the combustion reactor contains chromium oxide as an inorganic oxygen source and silvered cobaltous/cobaltic oxide for removal of halogens and sulfur. When the tin capsule is dropped into helium temporarily enriched with oxygen, the tin ignites and flash combustion occurs. Flash combustion raises the temperature of the sample to >1700 °C. The encapsulated sample, depending on its composition, combusts, generating one or more of these gases: N<sub>2</sub>, N<sub>x</sub>O<sub>x</sub>, CO<sub>2</sub>, and H<sub>2</sub>O. The reduction reactor contains reduced copper wires for the reduction of nitrogen oxides to N<sub>2</sub> and the removal of excess O<sub>2</sub>. An adsorption trap containing magnesium perchlorate removes the H<sub>2</sub>O. The remaining N<sub>2</sub> and CO<sub>2</sub> gases travel through a chromatographic column (Porapak Q) and then move to the ConFlo III open split interface. See Figure 7 for a detailed schematic of the elemental analyzer. See Section 3.2.1 for a detailed description of the interface.

Instrument temperatures:

Combustion reactor	1020 °C
Reduction reactor	650 °C
Chromatographic column	60 °C

Flow rates:

Helium	80-100 ml•min <sup>-1</sup>
Oxygen	25-30 ml•min <sup>-1</sup>

### 3.3.3 Thermo Scientific IsoLink Elemental Analyzer

The Thermo Scientific IsoLink elemental analyzer operates based on the principle of flash combustion in which a sample contained within a tin capsule is dropped into a combustion reactor held at 1020 °C. For nitrogen and carbon analysis, the combustion reactor contains chromium oxide as an inorganic oxygen source and silvered cobaltous/cobaltic oxide for removal of halogens and sulfur. When the tin capsule is dropped into helium temporarily enriched with oxygen, the tin ignites and flash combustion occurs. Flash combustion raises the temperature of the sample to >1700°C. The encapsulated sample, depending on its composition, combusts, generating one or more of these gases: N<sub>2</sub>, N<sub>x</sub>O<sub>x</sub>, CO<sub>2</sub>, and H<sub>2</sub>O. The reduction reactor contains reduced copper wires for the reduction of nitrogen oxides to N<sub>2</sub> and the removal of excess O<sub>2</sub>. An adsorption trap containing magnesium perchlorate removes the H<sub>2</sub>O. The remaining N<sub>2</sub> and CO<sub>2</sub> gases travel through a chromatographic column and then move to the ConFlo IV open split interface. See Figure 7 for a detailed schematic of the elemental analyzer. See Section 3.2.2 for a detailed description of the ConFlo IV interface.

Instrument temperatures:

Combustion reactor	1020 °C
Reduction reactor	650 °C
Chromatographic column	variable

Flow rates:

Helium	100-120 ml•min <sup>-1</sup>
Oxygen	25-30 ml•min <sup>-1</sup>

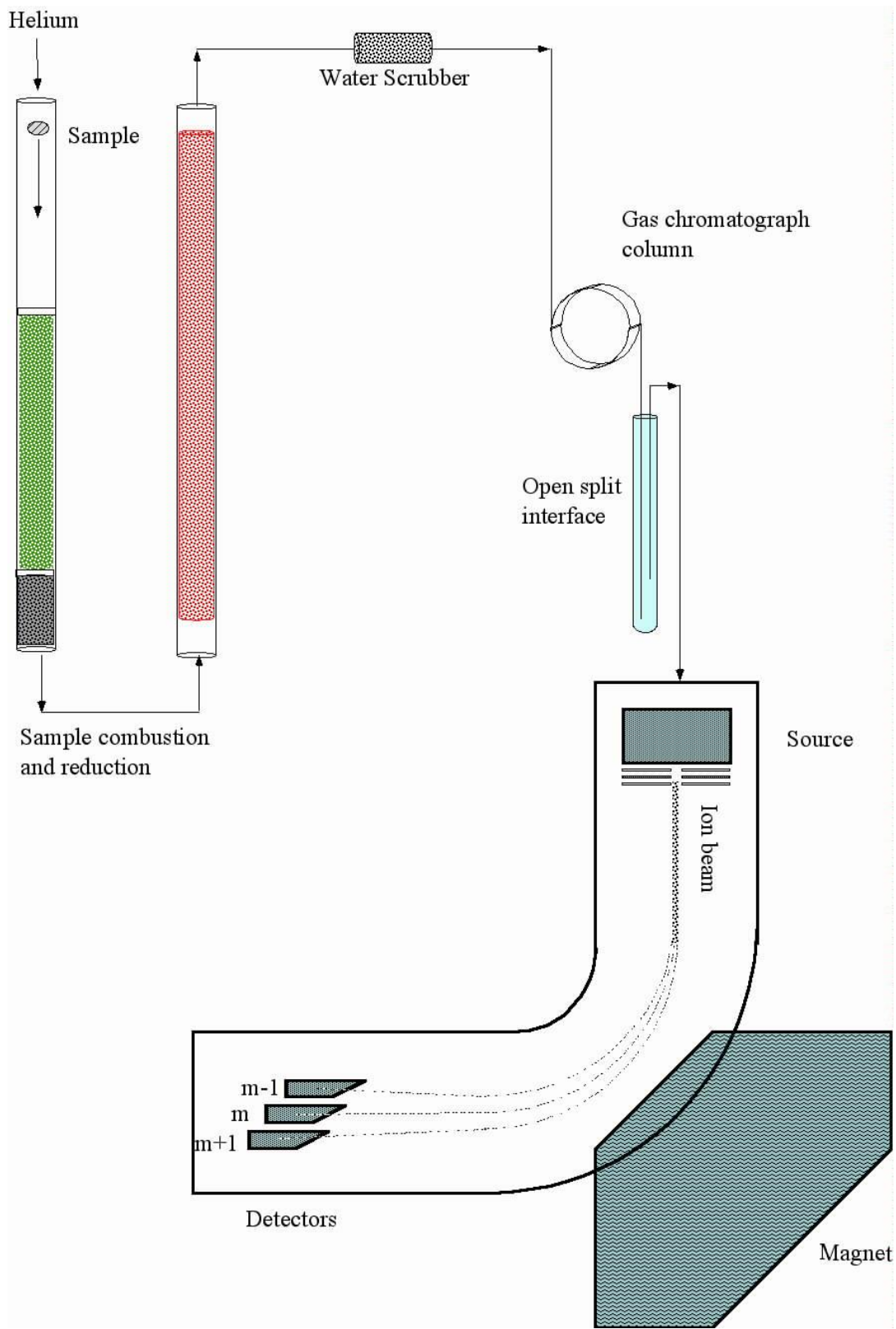


Figure 7. Schematic of the elemental analyzer coupled to an IRMS.



References:

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- Verado, D.J., P.N. Froelich, and A. McIntyre, 1990. Determination of organic carbon and nitrogen in marine sediments using the Carlo Erba NA-1500 analyzer, *Deep-Sea Res.*, 37, 157-165.

## **Section 4 Sample Handling**

## **4.1 Shipping Documents**

Upon receipt of samples, UWSIF files copies of the shipping documents. The shipping documents must show the date of delivery and tracking numbers.

## **4.2 Sample Check-in**

When samples arrive at UWSIF, all sample shipping containers are opened and carefully checked for any sample spillage. In the event there is sample spillage, the following procedures will be followed:

1. Broken sample containers are carefully removed.
2. If any sample can be saved, it is placed into a new container.
3. The missing sample is noted in the sample log.

## **4.3 Sample Tracking**

To keep track of samples from the moment they arrive at the laboratory to the time results are reported, the jobs are managed using a Zoho database system. The database lists the specific analyses to be performed, the cost for the service, the number and the type of samples for analysis, and pertinent contact and billing information for the client. Progress during analysis for each job is tracked continuously, and all jobs in progress are managed and organized daily and weekly by the lab manager and lab technicians. The sample tracking system works as follows:

1. New samples are submitted to the lab via a Routing Sheet. Each routing sheet is assigned a new job number e.g. 2007-31, 32 etc.
2. The samples retain their unique id assigned by the PI when they are entered in the sample sequence table of the Finnigan software.
3. For sample traceability the File name assigned to each run is the same as the job number. If one job has several runs they are named sequentially e.g. 2007-31 run 1 will be followed by 2007-31 run 2 and so on.
4. The results are sorted in different folders according to analysis type and PI's last name.
5. When analysis is done a complete report is sent to the PI.
6. Finally, an invoice containing details of the job number and analysis type is sent to the PI.

## **4.4 Storage of Samples**

Samples are stored in dry, cool, dark cabinets until analysis. After analysis, all remaining sample material is returned to the client. UWSIF will store unused sample material for a maximum of six months.

## **Section 5 Sample Preparation**

## 5.1 Drying of Samples

If the samples require drying, the samples are placed into a 70 °C drying oven overnight. A drying oven logbook is maintained which contains the following information:

- Starting date/time
- Sample type
- Ending date/time
- Monthly temperature checks

The drying oven temperature accuracy is checked monthly using a certified temperature meter (Omega HH22 Digital Thermometer). The monthly checks are recorded in the drying oven logbook.

## 5.2 Sample Labeling

When a sample is removed from bulk containers for analysis and placed in another container, it is not necessary to mark the individual sample, but it is necessary to label the container used to transport the sample for final isotope analysis. When sub-sampling from a bulk container and transferring the sub-sample to a tin capsule for isotope analysis, the tin capsule need not be labeled, but the 96-well tray holding the tin capsule must be labeled with the appropriate UWSIF identification information.

### 5.3 Weighing Solid Samples

Weighing of samples involves the use of microbalances. A microbalance is typically readable to  $1\ \mu\text{g}$  and reproducible (standard deviation) to  $\leq 1\ \mu\text{g}$ . Calibration of the balance is verified by the use of certified calibration weights. Henry Troemner, LLC. is a supplier. The calibration weights are directly traceable to the National Institute of Standards and Technology. Samples are routinely weighed to  $1\ \mu\text{g}$  before analysis.

For  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  analysis of solid samples, a 0.2-20 mg subsample of the unknown is accurately weighed (0.001 mg) into a tin sample container. If the samples are not analyzed immediately, the weighed samples are placed into a dry storage cabinet.

Materials needed:

- 70 % ethanol
- Forceps
- Laboratory wipes
- Spatula
- Tin capsules

Procedure: Weighing samples using the microbalance

1. Using only clean forceps, place an empty tin capsule onto the balance pan and tare the balance.
2. After taring, remove the capsule from the balance and place it onto a clean glass plate. Add dried ground sample with a small spatula to the desired weight.
3. Put the filled capsule back onto the balance pan and weigh the sample.
4. Write down the final weight to the microgram (0.001 mg).
5. Again, remove the capsule from the balance.
6. With flat-jawed forceps, pinch the top third of the capsule.
7. Fold the capsule over to close the top. Do this step twice if possible.
8. Push the folded capsule off the forceps and continue to collapse and fold the capsule until you form a small, dense cube.
9. Place the cube into a 96-well sample tray.
10. Clean the tools and glass plate.

When the weighing session is complete, store the tray in a vacuum desiccator until EA-IRMS analysis.

## 5.4 Grinding Samples: Mortar/Pestle Method

The mortar is a bowl, typically made of hard wood, metal, ceramic, or hard stone. The pestle is a heavy and blunt club-shaped object. The substance to be ground is placed in the mortar, where the pestle is pressed and rotated onto it until the desired texture is achieved.

Materials needed:

- 70 % ethanol
- Forceps
- Labeling tape
- Laboratory wipes
- Liquid nitrogen
- Markers
- Mortar and pestle
- Spatula
- Storage vials

**Safety: Liquid nitrogen is very cold. You must wear safety glasses during this process. Gloves are also available for additional protection.**

Procedure: Grinding with a mortar and pestle

1. Dry the samples overnight in a drying oven (70 °C).
2. Place a dried sample into the mortar.
3. Add enough liquid nitrogen to cover the sample.
4. Allow the liquid nitrogen to evaporate until there is just enough left to be visible.
5. Quickly grind the sample with the pestle. If done correctly, the sample will be a very fine powder.
6. Remove the ground sample and store in an appropriate container.
7. Clean the mortar and pestle with 70 % ethanol.

Note: some samples do not lend themselves to using this method. For example, stems are very hard to grind using a mortar and pestle. For such samples, the use of a ball mill is more appropriate and much easier.

## 5.5 Grinding Samples: Mixing Mill Method

Mixing mills operate on the principle of exposing a sample to a moving ball, which crushes the sample to a fine powder. Samples are placed into stainless steel grinding jars along with a steel ball. The mixing mill rapidly shakes the grinding jar. The inertia of the grinding balls causes them to impact the sample within the grinding jars with high energy and pulverizes it. The movement of the grinding jars combined with the movement of the balls result in the intensive mixing of the sample.

Materials needed:

- 70 % ethanol
- Labeling tape
- Laboratory wipes
- Retsch mixer mill
- Stainless steel grinding jars and stainless steel balls
- Vials

Procedure: Grinding a sample with a mixing mill

1. Place the sample (or an appropriately sized subsample) into the bottom of a Retsch grinding jar. Drop a stainless steel ball in with the sample and fit the top on tightly.
2. Use labeling tape to label each jar.
3. Grind the sample using the Retsch mixer mill for 60 seconds at a frequency of 30 oscillations per second.
4. Check to make sure the sample has been sufficiently ground. If not, grind again.
5. Carefully transfer the ground sample to a vial.
6. Label the vial and store until needed.
7. Clean the grinding jar with ethanol after each use.



## 5.6 Grinding *Toxicodendron* Samples: Mortar/Pestle Method

*Toxicodendron* is a genus of flowering plants in the sumac family, *Anacardiaceae*. It contains trees, shrubs and woody vines, including **poison ivy**, **poison oak**, and the **lacquer tree**. All members of the genus produce the skin-irritating oil urushiol, which can cause a severe allergic reaction.

**NOTE: All grinding must be done in a fume hood. Gloves must be worn for additional protection.**

Materials needed:

- 70 % ethanol
- Labeling tape
- Laboratory wipes
- Liquid nitrogen
- Markers
- Mortar and pestle
- Nitrile gloves
- Spatula
- Storage vials

**NOTE: Liquid nitrogen is very cold. You must wear safety glasses during this process.**

Procedure: Grinding *Toxicodendron* samples with a mortar and pestle

1. Dry the samples overnight in a drying oven (70 °C).
2. Place a dried sample into the mortar.
3. Add enough liquid nitrogen to cover the sample.
4. Allow the liquid nitrogen to evaporate until there is just enough left to be visible.
5. Quickly grind the sample with the pestle. If done correctly, the sample will be a very fine powder.
6. Remove the ground sample and store in an appropriate container.
7. Clean the mortar and pestle with 70 % ethanol.

Note: some samples do not lend themselves to using this method. For example, stems are very hard to grind using a mortar and pestle. For such samples, the use of a mixing mill or coffee grinder is more appropriate and much easier.

To clean the fume hood:

1. Wipe down the fume hood work surface the 70 % ethanol. Take care that all loose material is collected and disposed of properly.

## 5.7 Grinding *Toxicodendron* Samples: Mixing Mill Method

*Toxicodendron* is a genus of flowering plants in the sumac family, *Anacardiaceae*. It contains trees, shrubs and woody vines, including **poison ivy**, **poison oak**, and the **lacquer tree**. All members of the genus produce the skin-irritating oil urushiol, which can cause a severe allergic reaction.

Mixing mills operate on the principle of exposing a sample to a moving ball, which crushes the sample to a fine powder. Samples are placed into stainless steel grinding jars along with a steel ball. The mixing mill rapidly shakes the grinding jar. The inertia of the grinding balls causes them to impact sample within the grinding jars with high energy and pulverizes it. Also, the movement of the grinding jars combined with the movement of the balls result in the intensive mixing of the sample.

### 5.7.1 Grinding soft material (leaves)

Materials needed:

- 70 % ethanol
- Face mask
- Lab coat
- Labeling tape
- Laboratory wipes
- Nitrile gloves
- Retsch mixer mill
- Stainless steel grinding jars and stainless steel balls
- Storage vials

Procedure: Grinding *Toxicodendron* soft tissues with a mixing mill

1. Place the sample (or an appropriately sized subsample) into the bottom of a Retsch grinding jar. Drop a stainless steel ball in with the sample and fit the top on tightly.
2. Use labeling tape to label each jar.
3. Grind the sample using the Retsch mixer mill for 60 seconds at a frequency of 30 oscillations per second.
4. Check to make sure the sample has been sufficiently ground. If not, grind again.
5. Carefully transfer the ground sample to a vial.
6. Label the vial and store until needed.
7. Clean the grinding jar with 70 % ethanol and laboratory wipes.

After all grinding is complete, clean the countertop as follows:

1. Wipe down the countertop work surface the ethanol. Take care that all loose material is collected and disposed of properly.

## 5.7.2 Grinding hard material (stems and roots)

Materials needed:

- 70 % ethanol
- Face mask
- Lab coat
- Labeling tape
- Laboratory wipes
- Liquid nitrogen
- Nitrile gloves
- Retsch mixer mill
- Stainless steel grinding jars and stainless steel balls
- Storage vials

Procedure: Grinding *Toxicodendron* hard tissues using a mixing mill

1. Place the sample (or an appropriately sized subsample) into the bottom of a grinding jar. Drop a stainless steel ball in with the sample and fit the top on tightly.
2. Place the grinding jar into liquid nitrogen for 5 minutes.
3. Grind the sample using the Retsch mixing mill for 60 seconds at a frequency of 30 oscillations per second.
4. Check to make sure the sample has been sufficiently ground. If not, re-freeze in liquid nitrogen and grind again.
5. Carefully transfer the ground sample to a vial.
6. Label the vial and store until needed.
7. Clean the grinding jar with 70 % ethanol and laboratory wipes.

After all grinding is complete, clean the countertop as follows:

1. Wipe down the countertop work surface the ethanol. Take care that all loose material is collected and disposed of properly.

## 5.8 Grinding *Toxicodendron* Samples: Coffee Grinder Method

*Toxicodendron* is a genus of flowering plants in the sumac family, *Anacardiaceae*. It contains trees, shrubs and woody vines, including **poison ivy**, **poison oak**, and the **lacquer tree**. All members of the genus produce the skin-irritating oil urushiol, which can cause a severe allergic reaction.

**NOTE: All grinding must be done in a fume hood. Gloves must be worn for additional protection.**

Coffee grinders have a blade similar to that of a propeller that chops the sample. The fineness of the grind is determined by how long you let the grinder operate.

Materials needed:

- 70 % ethanol
- Coffee grinder
- Forceps
- Labeling tape
- Laboratory wipes
- Markers
- Nitrile gloves
- Spatula
- Storage vials

Procedure: Grinding *Toxicodendron* hard tissues using a coffee grinder

1. Place the sample (or an appropriately sized subsample) into a coffee grinder.
2. Grind the sample for 60 seconds.
3. Check to make sure the sample has been sufficiently ground. If not, grind again.
4. Carefully transfer the ground sample to a vial.
5. Label the vial and store until needed.
6. Carefully clean the coffee grinder with 70 % ethanol and laboratory wipes.

After all grinding is complete, clean the fume hood counter as follows:

1. Wipe down the fume hood work surface the ethanol. Take care that all loose material is collected and disposed of properly.

## 5.9 Carbonate Removal from Soils - Acidification Method

Precaution: loss of nitrogen. When you acidify soil, inorganic nitrogen compounds in the soil such as nitrate and ammonium will likely be broken down and lost. If you desire nitrogen isotope analysis of the soil without losing the inorganic portion, you will need to analyze the soil for nitrogen before acidification.

Note: you might find it necessary to allow the soil acidification procedure to sit overnight to achieve complete carbonate removal. Plan accordingly.

Materials needed:

- 2 ml Eppendorf tubes
- 3N phosphoric acid
- Buchner funnel
- Deionized water
- Drying oven
- Erlenmeyer flask
- Hardened glass filter paper
- pH paper
- Vortex mixer

Procedure: Acid washing of soil samples for isotopic analysis

1. Weigh out  $\approx 100$  mg of finely ground soil into clearly labeled 2 ml Eppendorf tubes.
2. Cautiously add 100  $\mu$ l of 3N phosphoric acid to each Eppendorf tube. Do not close the Eppendorf tube. Note: the sample will bubble as carbonate is converted to  $\text{CO}_2$ .
3. Close the lid when bubbling stops.
4. Using a vortex mixer, mix each tube for 10 seconds. Open the lid after mixing.
5. Cover with foil and place into the fume hood overnight.
6. Measure the pH of the supernatant with pH paper.
7. If the pH is  $< 4$ , skip to step 10.

In the next step, excess supernatant is removed and the sample is rinsed. If the supernatant can be successfully decanted, proceed to step 8. If decanting the supernatant will result in unacceptable sample loss, filtering must be done. If filtering is appropriate, proceed to step 18.

8. Carefully decant/filter the supernatant into a waste beaker.
9. Repeat steps 2-7 until the supernatant is  $< \text{pH } 4$ .
10. Carefully decant the supernatant into a waste beaker.
11. Rinse the sample with distilled water.
12. Centrifuge the tube for 1 minute.
13. Carefully decant/filter the supernatant into a waste beaker.

14. Repeat steps 11-13,  $\approx$  6 times.
15. After the final rinse, test the pH to confirm that all the acid has been removed ( $\approx$ pH 6-7).
16. Carefully decant the remaining supernatant into a waste beaker.
17. Place the samples into a drying oven at 65 °C for 24 hours.

Procedure: Vacuum filtration

18. Pour the supernatant into a 30 ml vacuum Buchner funnel, fitted with a glass fiber filter paper disc. Rinse the sample Eppendorf tube with deionized water and pour into the Buchner funnel.
19. After filtration, carefully remove the collected sample from the filter paper disc and place into a clean Eppendorf tube. Avoid sample loss if possible.
20. Suspend the sample in deionized water and test the pH with pH paper.
21. If the pH is  $>7$ , go to step 23.
22. If the pH is  $<7$ , go to step 28.
23. Cautiously add 100  $\mu$ l of 3N phosphoric acid to each Eppendorf tube. Do not close the Eppendorf tube. Note: the sample will bubble as carbonate is converted to CO<sub>2</sub>.
24. Using a vortex mixer, mix each tube for 10 seconds. Open the lid after mixing.
25. Cover with foil and place into the fume hood overnight.
26. Measure the pH of the supernatant with pH paper.
27. If the pH is  $>7$ , repeat steps 23-27.
28. Pour the supernatant into a 30 ml vacuum Buchner funnel, fitted with a glass fiber filter paper disc. Rinse the sample Eppendorf tube with deionized water and pour into the Buchner funnel.
29. After filtration, place the filter paper disc into a drying oven at 65 °C for 24 hours.
30. Carefully remove the sample from the filter paper disc and store in a cool dry cabinet until analysis.

Reference:

Connin, S.L., R.A. Virginia, and C.P. Chamberlain. 1997. Carbon isotopes reveal soil organic matter dynamics following arid land shrub expansion. *Oecologia* 110:374–386.

## **Section 6 Instrument Operation**

## 6.1 Delta V

### 6.1.1 Startup and status

Make sure the following lights are illuminated on the Delta V control panel:

Connection (5)

Vacuum (4)

Secondary (3)

Main (2)

Voltage (10)

Emission (9)

If any of the above lights are not illuminated or are ORANGE, contact the UWSIF staff.



Figure 8. Delta V control panel (from the Thermo user manual).



### 6.1.2 Initial instrument checks

Go to the following screen on the computer:

Screen: Instrument Control.

1. Select the proper gas (CO<sub>2</sub>, N<sub>2</sub>) (located in a small box on the lower left on the screen). Wait for the instrument to adjust the source and magnet.
2. Perform a Peak Center (click on the icon located at the upper left of the screen).

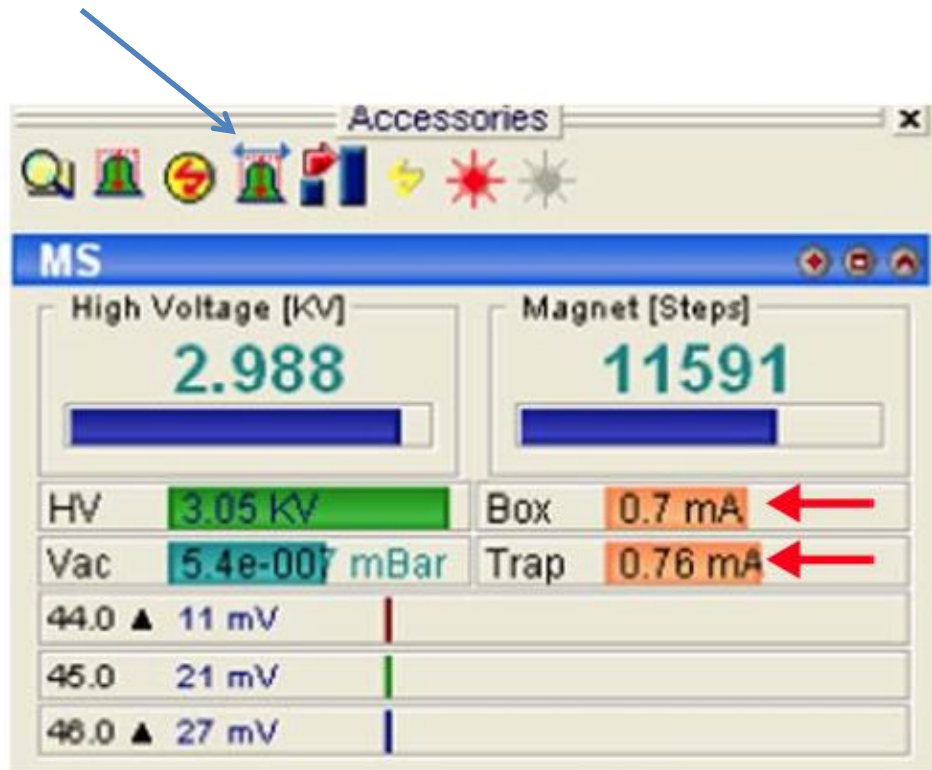


Figure 9. Computer screenshot peak center icon (from Thermo user manual)

3. Wait for the instrument to finish the peak center routine.

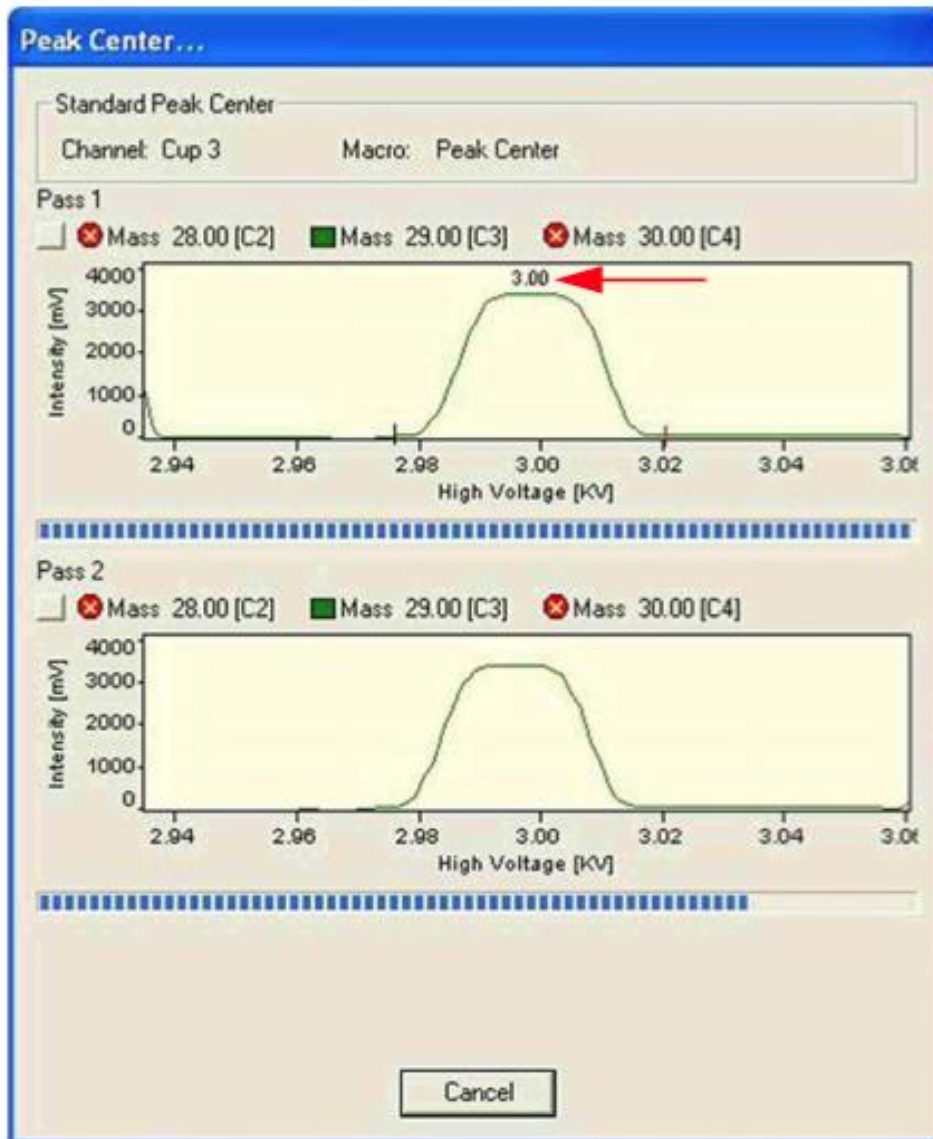


Figure 10. Computer screenshot peak centering (from Thermo user manual)

## 6.2 Delta<sup>Plus</sup>XP

### 6.2.1 Startup and status

Make sure the following GREEN lights are illuminated on the Delta Plus control panel:

POWER  
HOST CONNECTION  
TURBO PUMPS > 80%  
HIGH VACUUM OK  
ACCEL VOLTAGE  
EMISSION

If any of the above lights are not illuminated or are RED, contact the UWSIF staff.

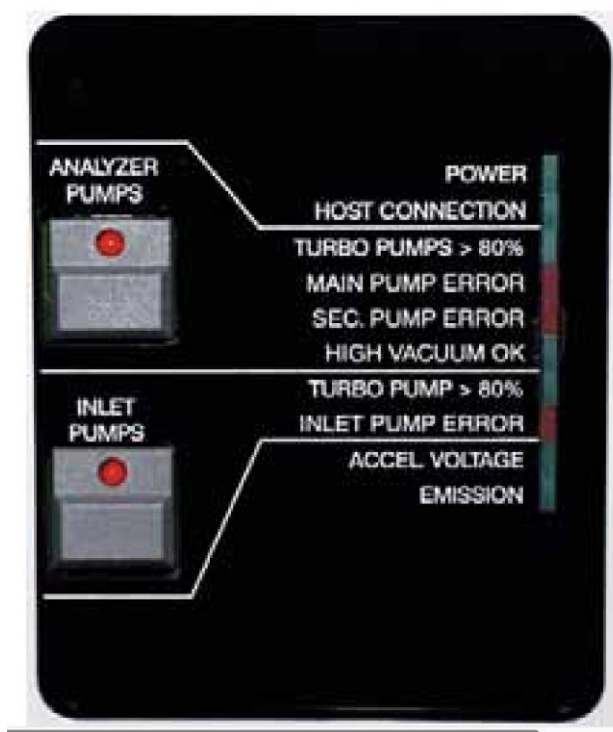


Figure 11. Delta Plus control panel (from Thermo user manual)

## 6.2.2 Initial instrument checks

Go to the following screen on the computer:

Screen: Instrument Control.

1. Select the proper gas (CO<sub>2</sub>, N<sub>2</sub>) (located in a small box on the lower left on the screen). Wait for the instrument to adjust the source and magnet.
2. Perform a Peak Center (click on the icon located at the upper left of the screen).

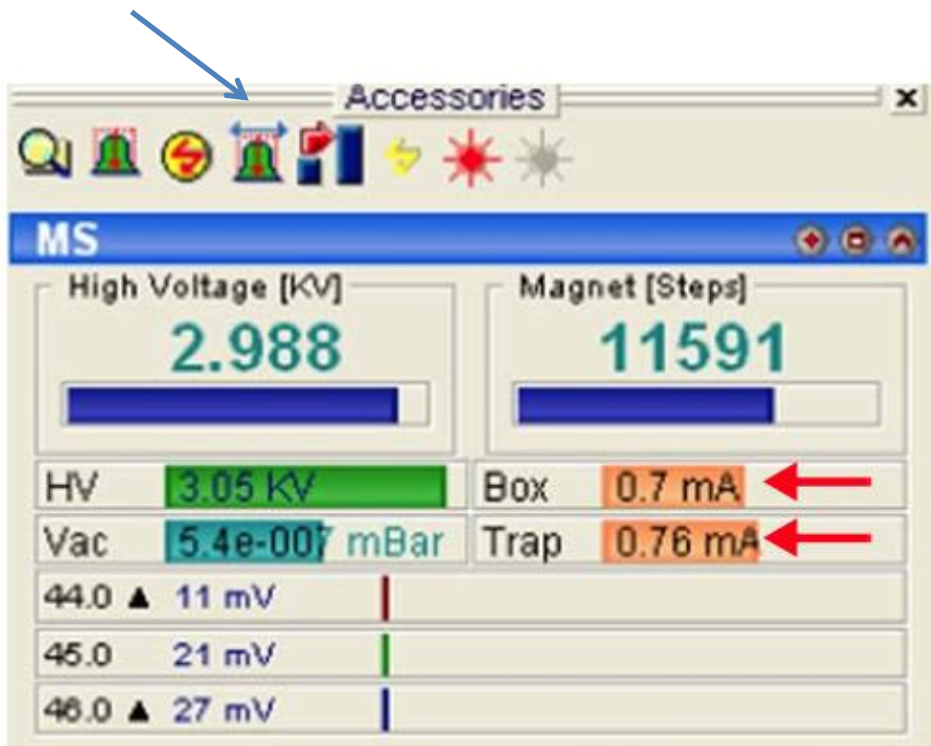


Figure 12. Computer screenshot peak center icon (from Thermo user manual)

1. Wait for the instrument to finish the peak center routine.

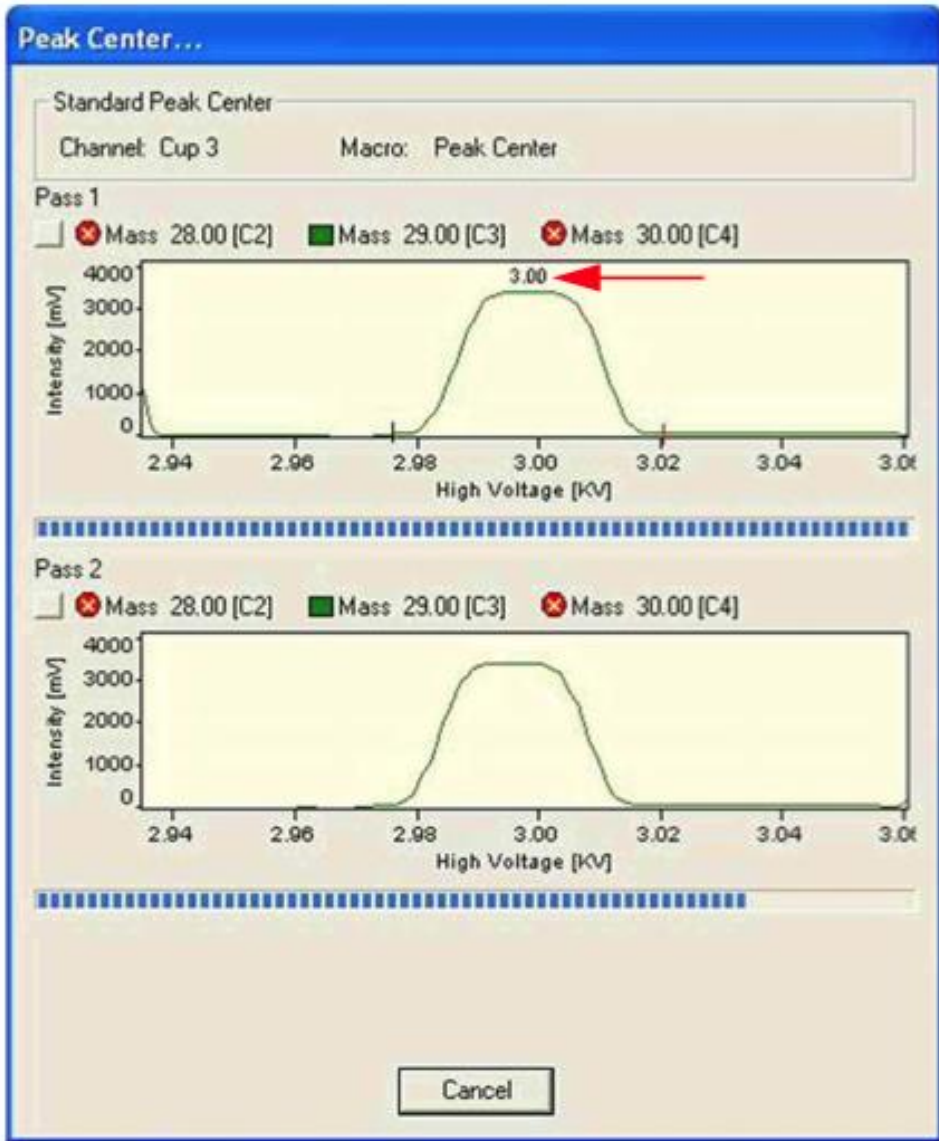


Figure 13. Computer screenshot peak centering (from Thermo user manual)

### 6.3 Zero Blank Autosampler

The samples must not be too large for the carousel and they must not be flat. The proper weight of the sample substance should yield amplitudes between approximately 2000 and 3000 mV.

1. Release the pressure from the sample carousel. There is a purge valve located on the top of the autosampler lid in the center.
2. After the pressure has been released open the lid by loosening the three thumbscrews. Due to the sealed nature of the lid, always tighten and/or loosen the thumbscrews in a random manner (as you would with lug nuts on a car tire).
3. You may need to change the carousel to 50 or 100 holes depending on the size and or number of the samples. Be sure to change the carousel size on the EA Panel (Costech EA only).
4. Line up the carousel if necessary.
5. Load the samples. The first sample should be located to the right of the combustion chamber.



1<sup>st</sup> sample position

6. Close the autosampler lid.
7. Leave the purge valve open for 10 minutes to allow for the evacuation of any remaining atmosphere within the sample carousel.



Purge valve

Thumbscrew

8. After the 10-minute, purge close the purge valve and perform a helium leak check.

## 6.4 ConFlo III

Go to the following screens on the computer and make sure that the listed lights are active (click on the button with the cursor to turn on or off):

Screen: ConFlo III

You should select only one reference (Ref I or Ref II).

- Select the reference gas (CO<sub>2</sub>).
- Select the reference gas (N<sub>2</sub>).

## 6.5 ConFlo IV

Go to the following screens on the computer and make sure that the listed lights are active (click on the button with the cursor to turn on or off):

Screen: ConFlo IV Diagnosis

- MS Cap. light is green.

Screen: ConFlo IV Interface

- Ref I Status On/Off is green.
- Ref II Status On/Off is green.

Screen: ConFlo IV Interface

- Select sample port HF II



Figure 14. Computer screenshot ConFlo IV readings (from Thermo user manual)



## 6.6 Instrument On/Offs

- Check the background of mass 28 by right clicking on the center bar within the MS panel located on the left side of the computers display screen and clicking on JUMP TO MASS and entering 28.
- After the instrument changes have taken place, right click the center bar again and perform a PEAK CENTER check. If the peak center is erratic wait for stabilization then retest.
- Run an On/Off test. An On/Off test consists of multiple injections (10) of a reference gas through the ConFlo III or ConFlo IV interface. After the On/Off test has been completed, check the standard deviation of the On/Off peaks. Skip the first two peaks and highlight the rest of the appropriate column (for example  $^{13}\text{C}$  or  $^{15}\text{N}$ ) and then right click the highlighted area and choose the CALCULATE option. If the standard deviation of the last On/Off test performed is below 0.03 then you are able to move on to the next step. If the value is higher than 0.03, but the standard deviations are decreasing it is possible to start the run. It is important to check the first few reference materials to make sure the values are reasonable. Normally three On/Off sets are more than sufficient.

## **Section 7 Mass Spectrometer Analysis**

## 7.1 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ Analysis of Solids

Organic carbon and total nitrogen isotopic analysis of solids is performed using either a Finnigan Delta<sup>Plus</sup> XP IRMS or a Thermo Delta V IRMS. The Finnigan Delta<sup>Plus</sup> XP is run in continuous flow mode and is connected to a Costech 4010 elemental analyzer via a Finnigan ConFlo III open split interface. The Thermo Delta V is run in continuous flow mode and is connected to a Carlo Erba NC-2500 elemental analyzer or a Thermo Scientific IsoLink elemental analyzer via a Thermo ConFlo IV open split interface.

The elemental analyzer operates based on the principal of flash combustion in which a sample contained within a tin capsule is dropped into a combustion reactor held at 1020 °C. For nitrogen and carbon analysis, the combustion reactor contains chromium oxide for oxidation and silvered cobaltous/cobaltic oxide for removal of halogens and sulfur. When the tin capsule is dropped into helium temporarily enriched with oxygen, the tin ignites and flash combustion occurs. Flash combustion raises the temperature of the sample to >1700 °C. The encapsulated sample, depending on its composition, combusts generating one or more of these gases: N<sub>2</sub>, N<sub>x</sub>O<sub>x</sub>, CO<sub>2</sub>, and H<sub>2</sub>O. The reduction reactor contains reduced copper wires for the reduction of nitrogen oxides to N<sub>2</sub> and the removal of excess O<sub>2</sub>. An adsorption trap containing magnesium perchlorate removes the H<sub>2</sub>O. The remaining N<sub>2</sub> and CO<sub>2</sub> gases travel through a chromatographic column (Porapak Q) for separation and then move to the ConFlo III or ConFlo IV open split interface.

Instrument temperatures:

Combustion reactor	1020 °C
Reduction reactor	650 °C
Chromatographic column	60 °C

Flow rates:

Helium	80-100 ml•min <sup>-1</sup>
Oxygen	25-30 ml•min <sup>-1</sup>

## 7.2 $\delta^{15}\text{N}$ Analysis of Solids - Low Nitrogen Content

Total nitrogen isotopic analysis of solids with low nitrogen content is performed using a Finnigan Delta<sup>Plus</sup> XP IRMS. The Finnigan Delta<sup>Plus</sup> XP is run in continuous flow mode and is connected to a Costech 4010 elemental analyzer via a Finnigan ConFlo III open split interface.

The elemental analyzer operates based on the principal of flash combustion in which a sample contained within a tin capsule is dropped into a combustion reactor held at 1020 °C. For nitrogen analysis, the combustion reactor contains chromium oxide for oxidation and silvered cobaltous/cobaltic oxide for removal of halogens and sulfur. When the tin capsule is dropped into helium temporarily enriched with oxygen, the tin ignites and flash combustion occurs. Flash combustion raises the temperature of the sample to >1700 °C. The encapsulated sample, depending on its composition, combusts generating one or more of these gases:  $\text{N}_2$ ,  $\text{N}_x\text{O}_x$ ,  $\text{CO}_2$ , and  $\text{H}_2\text{O}$ . The reduction reactor contains reduced copper wires for the reduction of nitrogen oxides to  $\text{N}_2$  and the removal of excess  $\text{O}_2$ . An adsorption trap containing soda lime and magnesium perchlorate removes the  $\text{CO}_2$  and  $\text{H}_2\text{O}$ . The remaining  $\text{N}_2$  travels through a chromatographic column (Porapak Q) for separation and then move to the ConFlo III open split interface.

Instrument temperatures:

Combustion reactor	1020 °C
Reduction reactor	650 °C
Chromatographic column	60 °C

Flow rates:

Helium	80-100 $\text{ml}\cdot\text{min}^{-1}$
Oxygen	25-30 $\text{ml}\cdot\text{min}^{-1}$

## Section 8 Data Collection

## 8.1 Data Processing (Isodat 2.0)

If appropriate, check the results from the previous run to ensure that no problems were encountered.

1. Open Excel.
2. Go to: Open, Results, and select the name the run was saved under.
3. You will find a number of generic file folders named “Excel” followed by a numeric number. Check the date of the Excel file to ensure that is the appropriate file (the file and therefore the date is automatically generated when the run is complete). It is usually the last Excel file at the end.
4. Open this folder and then its lone Excel file (again with an automatically generated name).
5. Move and copy the contents of the Excel file to a new Excel document by right clicking on the worksheet tab and choosing the “move or copy”. Check the box that says make copy and press “ok”.
6. Close the original file.
7. Save the new Excel file on the desktop under EA Results in the folder containing the clients name with a file name including the project code and the run number if applicable (i.e. 2008-8 run 1 Client Name).
8. Copy the data from sheet 1 to sheet 2
9. Name sheet 1 “Original”, sheet 2 “Sorted”. Insert EA MS worksheet and rename run 1 or the appropriate run number.
10. Sort the data in “Sorted” sheet by peak number (Select Peak number column, DATA, SORT, PEAK #).
11. For  $^{13}\text{C}$  and  $^{15}\text{N}$  analyses delete the reference peaks 1, 2, 5, and 6. For  $^{13}\text{C}$  only delete peaks 1, 2, and 4.
12. Move the data from peak 4 (under the columns amp 44 and  $d^{13}\text{C}/^{12}\text{C}$ ) sequentially into the spaces above.
13. Delete all of the remaining data from peak 4.
14. Delete the Peak Nr column.
15. Copy this data to sheet 3 (EAMS RUN) and if you are including EAS data do so now. If you are not including EAS (%) data you will need to delete the unnecessary cells.
16. Sequentially (from the beginning to the end of the run) copy all of the laboratory reference materials to the bottom of the run into their appropriate position.
17. Check to make sure that the values are correct and that there are no problems with the run. It is important to check the standard deviation of all laboratory reference materials. The standard deviation for the isotope values should be less than 0.1 and for the EAS data, if included, should be below 0.5. If not, you may need to look at the individual values. If necessary, you can delete a laboratory reference material that has a value that is far off from the rest. These outliers are typically found in the first few laboratory reference materials. It is also important to look for any drift, represented by values getting sequentially higher or lower throughout the run.

18. If a drift or linearity correction is needed, then do the appropriate corrections (see Section 10).
19. Check that all the amplitudes of the laboratory reference materials and samples are above 500 mV. If not, this means that the sample amount is insufficient to produce reliable isotope ratio values and should be reported to the PI.
20. Copy the line equation from the normalization graph into the appropriate column (carbon graph into carbon column, nitrogen graph into nitrogen column.) replace the x in the formula to \* and click on the matching row and column for  $D^{13}C/^{12}C$  for carbon and  $D^{15}N/^{14}N$  for nitrogen. Copy and paste that number into remaining cells that need a correction.
21. Repeat for the nitrogen correction.
22. To check the run, copy the average corrected value for your check reference materials in the Lab QC Check box matching the appropriate check reference materials. The corrected value and the actual value must be close in value.
23. In the Lab QC check box delete any laboratory reference materials not used in the run.
24. Delete laboratory reference materials at the top of the page that are dispersed among the samples.
25. Save

## 8.2 Setting Up a Sample Table (Isodat 2.0)

1. In Excel, open the sample sheet folder on the desktop.
2. Find the clients folder and open the correct sample sheet for the run.
3. Open Isodat Workspace. If the samples need to be analyzed for carbon and nitrogen open the sequence tab and double-click the N<sub>2</sub>+CO<sub>2</sub>+He sequence. If only carbon or only nitrogen analysis is needed double-click the CO<sub>2</sub> only or N<sub>2</sub> only sequence.
4. Copy and paste, from Excel, the sample ID's into the identifier column.
5. Copy and paste, from Excel, the sample weights in the amount column.
6. Double check to ensure that the method column is consistent throughout all samples.
7. Check that you have all the samples that were in your sample sheet.
8. Check that the method column has the correct method and all cells are filled.
9. Highlight the whole sample table by clicking on the box in the upper left-hand corner of the sample table and save it, no file name is needed. The saved data will automatically be carried over to Isodat acquisition when the corresponding sequence is opened. Sometimes the sequence must be closed, and then opened again for the new data to register.

## 8.3 Retrieving Data (Isodat 2.0)

To view the sample chromatogram, open the RESULTS tab under the file browser on the left-hand side of the Isodat screen and navigate to the place where your sample's data was saved.



## **Section 9 Quality Assurance**

## 9.1 Laboratory Reference Materials

A set of UWSIF laboratory reference materials have been developed and calibrated against IAEA, NIST, and USGS primary reference materials. Calibration on these internal reference materials is conducted annually.

UWSIF laboratory reference materials are:

Quality assurance	The quality assurance reference materials are calibrated with international standards using a three-point calibration. These laboratory reference materials are then used in routine analyses distributed at the beginning, middle and end of each run. Besides providing a two-point normalization, they also help to keep a check on issues like machine drift, non-linearity related to sample size, and column degradation.
Quality control	These reference materials provide a check on the two-point normalization produced by the normalization reference materials. The reference materials have similar chemical composition to the unknown samples being analyzed. They also provide a means to check for consistency between runs.

*Table 6 UWSIF laboratory reference materials*

Every analytical run at UWSIF contains multiple quality assurance and quality control reference materials. For bulk nitrogen and/or carbon analyses, UWSIF routinely uses a select group of reference materials. About 20 % of all samples analyzed in the lab are quality assurance and quality control reference materials.

The following table lists the UWSIF laboratory reference materials commonly used for quality assurance and quality control of nitrogen and/or carbon isotope analysis of plants and soil:

### UWSIF Quality Assurance and Quality Control Reference Materials with known $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values

UWSIF reference material	Analytical use	Description	$\delta^{13}\text{C}_{\text{VPDB}}$	$\delta^{15}\text{N}_{\text{AIR-N}_2}$
05-UWSIF-Alfalfa	quality control	plant	-27.48	+0.19
36-UWSIF-Glut 1	quality assurance	glutamic acid	-28.17	-4.69
39-UWSIF-Glut 2	quality assurance	glutamic acid	+24.21	+28.07
46-UWSIF-Soil 3	quality control	soil	-23.53	+6.12

*Table 7 UWSIF QAQC reference materials*

## 9.2 Handling of Reference Materials

### 9.2.1 Control and distribution of reference materials

The daily use of all UWSIF reference materials is strictly controlled. The following rules apply:

- International reference materials are distributed only by the Laboratory Manager and are used only for the standardization of UWSIF quality assurance and quality control reference materials.
- Only one reference material (International, quality assurance or quality control) may be in use at any time. At no time should multiple reference material containers be open.

### 9.2.2 Storage of reference materials

All reference materials (International, UWSIF) are stored in a desiccant cabinet. Indicating Drierite is the hygroscopic compound used to maintain a dry atmosphere inside the desiccant cabinet. Indicating Drierite is blue when dry and changes to pink upon absorption of moisture. The color change is pronounced and clearly visible. When the bulk of the Drierite in the desiccant cabinet has turned pink, the drying agent is replaced. The replacement date of the Drierite is recorded on the door of the desiccator.

## 9.3 UWSIF QAQC Reference Material Certification

On an annual basis, all UWSIF quality assurance and quality control reference materials are compared to appropriate international reference materials to confirm that their accepted isotope values have not changed. Long-term records of these comparisons are maintained for quality assurance purposes. The acceptable thresholds of the UWSIF quality assurance and quality control reference materials during calibration are based on the average standard deviations of all the annual calibrations of the appropriate international reference materials plus the long-term standard deviations of a variety of UWSIF quality assurance and quality control materials. The acceptable tolerances are defined as:

Standard uncertainty (1-sigma) for carbon isotopes is:        +/- 0.15 per mil  
Standard uncertainty (1-sigma) for nitrogen isotopes is:       +/- 0.20 per mil

If the annual calibration of the quality assurance and quality control reference materials is not within the stated tolerance, a second calibration is performed. If the materials are still outside tolerance levels, new unused aliquots of the reference materials are taken from the bulk supply and a new calibration is performed. In the event that the new aliquot does not meet the stated tolerance, the reference material is retired and a new reference material is generated. The calibration of this new reference material is done through round-robin analyses performed by other analytical facilities.

The following table list the international reference materials used for calibration of UWSIF laboratory reference materials:

Reference Materials with known $\delta^{13}\text{C}$ values (solid)				
NIST code	Name	Description	$\delta^{13}\text{C}_{\text{VPDB}}$	
RM 8542	IAEA-CH-6 (ANU)	sucrose	-10.45	
RM 8543	NBS 18	carbonatite	-5.01	
RM 8544	NBS 19	calcite	+1.95	
RM 8545	LSVEC	lithium carbonate	-46.6	
Reference Materials with known $\delta^{15}\text{N}$ values (solid)				
NIST code	Name	Description	$\delta^{15}\text{N}_{\text{AIR-N}_2}$	
RM 8549	IAEA-NO-3	potassium nitrate	+4.7	
Reference Materials with known $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (solid)				
NIST code	Name	Description	$\delta^{13}\text{C}_{\text{VPDB}}$	$\delta^{15}\text{N}_{\text{AIR-N}_2}$
RM 8573	USGS40	glutamic acid	-26.39	-4.52
RM 8574	USGS41	glutamic acid	+37.63	+47.57

*Table 8* International reference materials

## 9.4 Long-term Reference Material Data Collection Procedures

The isotope and elemental composition data (if applicable) for all reference materials are collected during the data reduction process. The collected data for each reference material in an individual analytical run is appended to an existing long-term reference material data file. The UWSIF maintains an individual file for each reference material. Individual run data (isotopic and elemental composition), long-term averages and standard deviations of isotopic and elemental composition, and sample counts are stored for each reference material.

## **9.5 Carbon and Nitrogen Quality Control Criteria**

### **9.5.1 Carbon isotope analysis quality control**

The quality assurance of carbon isotope analysis is based on the standard uncertainty of the known value of the quality control reference materials analyzed during the analytical run. The standard uncertainty is calculated from multiple analyses of the quality control reference materials. If the standard uncertainty is greater than 0.15 ‰, the unknowns are re-analyzed until the 2-sigma expanded standard uncertainty of the result is less than 0.3 ‰. The isotopic composition is reported in per mil relative to VPDB on a scale such that USGS40 and USGS41 are -26.24 ‰ and +37.76 ‰, respectively.

### **9.5.2 Nitrogen isotope analysis quality control**

The quality assurance of nitrogen isotope analysis is based on the standard uncertainty of the known value of the quality control reference materials analyzed during the analytical run. The standard uncertainty is calculated on multiple analyses of the quality control reference materials. If the standard uncertainty is greater than 0.2 ‰, the unknowns are re-analyzed until the 2-sigma expanded standard uncertainty of the result is less than 0.4 ‰. The isotopic composition is reported in per mil relative to AIR-N<sub>2</sub> on a scale such that USGS40 and USGS41 are -4.52 ‰ and +47.57 ‰, respectively.

## **9.6 Quality Assessment Procedures**

In order to ensure that all QC criteria are met for every analytical run, the following information is generated and stored:

- Sample metadata (i.e. type of sample, user information, etc.).
- The raw results of the analytical run, including all mass spectrometric data.
- Reduced analytical results including normalized delta values using the quality assurance and quality control reference materials.
- An assessment of the quality of the analytical run through an analysis of the quality assurance and quality control reference materials.

The final analytical report contains a complete record of how the unknown samples and the QA and QC laboratory reference materials were analyzed and reduced. The report also provides a record of what correction factors were used for data reduction, and a quality assurance evaluation.

### 9.6.1 Data reduction

Samples are analyzed in batches with each batch containing quality assurance and quality control reference materials. These reference materials are located at the beginning, middle, and end of the analytical run.

After each analytical run, the analyst does the following:

1. Examines the raw data for indications of analytical problems.
2. Determines the daily correction factors.
3. Applies correction factors to the isotopic data.
4. Reviews the results and determines which samples need to be re-analyzed to achieve acceptable results.

### 9.6.2 Acceptance criteria for QC samples

The statistical procedure listed below is followed to ensure that the QC samples meet all stated criteria:

1. If the standard deviation of the QC samples is less than or equal to the accepted tolerance for  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  (see sections 9.5.1 and 9.5.2), the mean delta value of the QC samples is used.
2. If the standard deviation of the QC samples is greater than the accepted tolerance for  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$ , an outlier check is performed. If any point is determined to be an outlier, delete the point and recalculate the mean delta value of the QC samples.
3. If the standard deviation of this recalculation is less than the accepted tolerance for  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$ , use the mean delta value from this recalculation.
4. If no outlier is detected, the raw data from each QC sample is examined for abnormalities (i.e. poor chromatography, high background, etc.). QC samples found to have abnormalities are looked at closely to determine whether or not the sample can be removed. If a sample is removed, the mean delta value is recalculated.
5. If none of the above apply, the results are rejected and corrective action is required.

### 9.6.3 Corrective action

If the analyst is unable to solve a problem with the QC reference material data, the analyst contacts the Laboratory Manager. The Laboratory Manager re-evaluates the data to determine whether or not the re-analysis of some of the samples or the re-analysis of the entire sample set is appropriate.

If a re-analysis of the samples does not give satisfactory results, the analyst averages all the QC data and reports the mean value. The analyst documents the problem on the final report, thus advising the client of the problem.

## **Section 10 Calculations and Corrections**

## 10.1 Calculations

*Actual* isotope ratios are very hard to measure accurately. Researchers have found that the measurement of an *actual* isotope ratio can vary between instruments or laboratories or even on different days on the same instrument. To avoid this problem, isotope ratios are generally expressed as *relative* ratios rather than *actual* ratios. A *relative* isotope ratio is found by measuring the isotopic abundance of a sample **and** the isotopic abundance of a known reference material on the same instrument at the same time. Because the final data are expressed as a ratio, any change in the instrument such as changing sensitivity will appear in the analysis of both the sample and the reference material and will cancel in the final data. Analyzing samples in this way makes it possible to compare the isotopic data between instruments and between other laboratories.

### 10.1.1 Delta notation

The *relative* ratio is normally expressed in the terms of the delta unit as follows:

$$\text{Delta } (\delta): \quad \delta_{\text{Sa}} = [R_{\text{Sa}} / R_{\text{Rm}} - 1] \times 1000$$

where:  $R_{\text{Sa}}$  = the measured isotope ratio of the sample  
 $R_{\text{Rm}}$  = the measured isotope ratio of the reference material  
 $\delta_{\text{Sa}}$  = the delta of the sample

Using the above formula, the units of delta would be in per mil, which are parts per thousand differences from a standard.



## 10.2 Common Corrections

### 10.2.1 Drift

Instrument drift is the change in instrument sensitivity over the course of an analytical run. To check for drift and to make a drift correction, the follow steps apply:

1. Calculate residuals of the reference material:

equation:  $\delta_{Rm}^R = \delta_{Rm}^T - \delta_{Rm}^M$

where:  $\delta_{Rm}^T =$  the accepted isotope value of the reference material

$\delta_{Rm}^M =$  the measured isotope value of the reference material

$\delta_{Rm}^R =$  residual for a given analyzed reference material sample

2. Derive a regression trendline of the time of analysis of the reference material ( $T_{Rm}$ ) vs. the reference material residuals.

where:  $m =$  the slope of the residual regression trendline

$b =$  the intercept of the residual regression trendline

At UWSIF, if the  $R^2$  of the residual trendline is  $<0.7$ , no correction is applied. If the  $R^2$  is  $>0.7$ , the following calculation is performed:

- 2.3. Calculate the residual corrected isotope values of the reference material and unknowns: \_\_\_\_\_

equation:  $\delta_{Sa}^T = \delta_{Sa}^M + (T_{Sa} * m + b)$

where:  $T_{Sa} =$  the analysis time of the sample (sequence line number)

$\delta_{Sa}^M =$  the measured isotope value of the sample

$\delta_{Sa}^T =$  the residual corrected isotope value of the sample

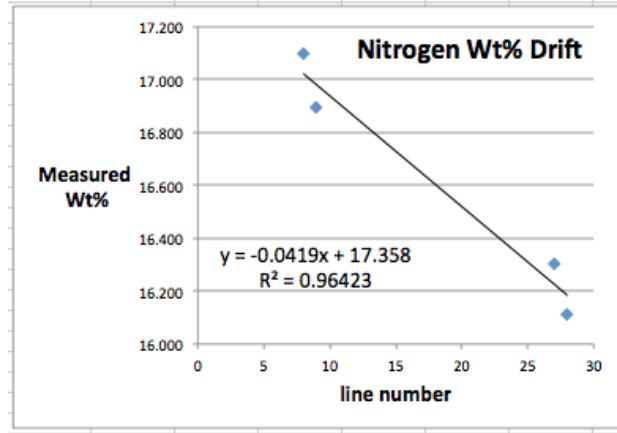


Figure 15. Drift correction

### 10.2.2 Memory

Memory is the mixing of the previously analyzed sample as background with the current sample. When a sample has been contaminated by contribution from another sample as background, the isotopic composition actually determined during the mass spectrometric measurement is that of the sample plus the background. The memory effect is most noticeable during the analysis of water samples using IRIS or when analyzing enriched samples. When measuring water isotopes using IRIS, the memory effect increases with the increase of the difference between the isotopic compositions of the past and current sample. The easiest method to minimize or eliminate this memory effect is to process several consecutive injections of the second sample: the memory effect will be decreased with the increase in the number of injections

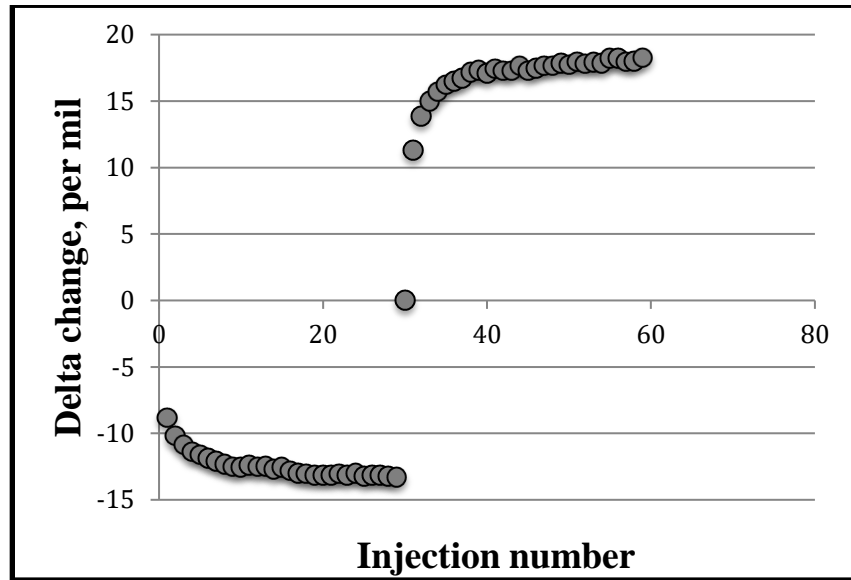


Figure 16. Injection memory vs. time.

### 10.2.3 Linearity

If there exists a relationship between the measured peak area of the laboratory reference material and its known isotopic value, then the results should be linearized to remove this trend. Corrections for instrument non-linearity are normally more applicable to CF-IRMS data. The corrections are based on analyzing a reference material of known isotopic composition using various weights that span the expected weight percent range of the samples. To correct the data:

1. Calculate residuals of the known isotopic values vs. the measured values of the reference material.
2. Make a scatterplot and derive a regression trend line linear equation of the reference material peak area (x-axis) vs. the reference material residuals (y-axis).

Note: The slope and  $R^2$  of the equation can provide guidance as to whether or not one should apply this correction to the data. For example: too shallow of a slope ( $<0.1$ ) indicates no real trend and the correction should not be applied. An  $R^2$  value of  $<0.7$  points toward the data being noisy without a trend and the correction should be reconsidered.

3. Using the equation of step 2, calculate linearity corrected isotope values of the reference material and unknowns

Example of a linearization correction:

Sample	Area 44	$\delta^{13}\text{C}$	Residual $\delta^{13}\text{C}$	Corrected $\delta^{13}\text{C}$
NBS 120C	2.16	+8.23	-14.5	-6.25
NBS 120C	11.13	+7.95	-14.2	-6.37
NBS 120C	16.33	+7.89	-14.2	-6.32
NBS 120C	24.77	+7.81	-14.1	-6.25
NBS 120C	30.90	+7.63	-13.9	-6.31

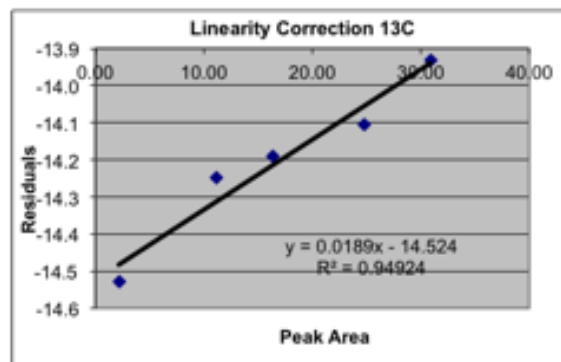


Figure 17. Linearization.

To correct the data:

1. Calculate residuals for **each** measured reference material:

equation:  $\delta_{Rm}^R \equiv \delta_{Rm}^T - \delta_{Rm}^M$

where:  $\delta_{Rm}^T$  = the accepted isotope value of the reference material  
 $\delta_{Rm}^M$  = the measured isotope value of the reference material  
 $\delta_{Rm}^R$  = the residuals of the reference material

2. Derive a regression trendline of the reference material peak areas (x) vs. the reference material residuals (y).

where:  $m$  = the slope of the residual regression trendline  
 $b$  = the 'y intercept' of the residual regression trendline

At UWSIF, if the  $R^2$  of the residual trendline is  $<0.7$ , not correction is applied. If the  $R^2$  is  $>0.7$ , the following calculation is performed:

- 2.3. Calculate the residual corrected isotope values of the samples:

equation:  $\delta_{Sa}^T \equiv \delta_{Sa}^M + (A_{Sa} * m + b)$

where:  $A_{Sa}$  = the measured peak area of the sample  
 $\delta_{Sa}^M$  = the measured isotope value of the sample  
 $\delta_{Sa}^T$  = the residual corrected isotope value of the sample

## 10.2.4 Normalization

Generally, isotope data are placed onto an internationally accepted scale such as VPDB or AIR-N2. The method most commonly used is to compare the data from a sample to the data from calibrated laboratory reference materials. These reference materials are placed onto the appropriate international scale by calibrating the materials using internationally accepted reference materials.

Laboratory reference materials are chosen to span or bracket the expected isotopic range of the unknown samples. For example, if the analysis performed is the carbon isotope composition of plant material (average range -10 to -30 per mil), appropriate laboratory reference materials would have carbon isotope compositions similar to USGS40 and USGS41. USGS40 and USGS41 are internationally accepted reference materials with carbon isotope values of -26.4 and +37.6 per mil versus VPDB and nitrogen isotope values of -4.5 and +47.6 per mil versus AIR-N2.

At UWSIF, we use two 'bracketing' reference materials for normalizing. These reference materials ideally are similar in chemical complexity to the unknowns but have very different isotopic compositions. Using two such reference materials, we generate a linear normalizing equation to corrected the unknowns:

equation:  $\delta_{Sa}^T = m * \delta_{Sa}^M + b$

where:  $m$  = the slope of the linear equation  
 $b$  = the 'y intercept' of the linear equation  
 $\delta_{Sa}^M$  = the measured delta value of sample  
 $\delta_{Sa}^T$  = the corrected delta value of sample

The slope and intercept of the equation are calculated as follows:

$$m = [\delta_{Rm1}^T - \delta_{Rm2}^T] / [\delta_{Rm1}^M - \delta_{Rm2}^M]$$
$$b = \delta_{Rm1}^T - [m * \delta_{Rm1}^M]$$

where:  $m$  = the slope of the linear equation  
 $b$  = the 'y intercept' of the linear equation  
 $\delta_{Rm1}^T$  = the accepted delta value of reference material 1  
 $\delta_{Rm2}^T$  = the accepted delta value of reference material 2  
 $\delta_{Rm1}^M$  = the measured delta value of reference material 1  
 $\delta_{Rm2}^M$  = the measured delta value of reference material 2

### 10.2.5 Weight percent

Weight percent can be corrected by generating a correction factor (kfactor). Kfactors relate the known weight percent of a standard with the measured weight of the standard and the measured area of the eluted peak. A kfactor is calculated as follows:

equation:  $kf = \text{Wt}\%_{\text{Std}}^T * \text{Wt}_{\text{Std}}^M / A_{\text{Std}}$

where:  $kf$  = the weight percent correction factor  
 $\text{Wt}\%_{\text{Std}}^T$  = the known weight percent of the standard  
 $\text{Wt}_{\text{Std}}^M$  = the actual weight of the standard sample  
 $A_{\text{Std}}$  = the measured area of the eluted standard peak

Typically, individual kfactors are calculated for each replicate of a weight percent standard. The final kfactor is the mean value of all the kfactors of the replicate weight percent standard.

To correct sample data using a kfactor, the following calculation applies:

equation:  $\text{Wt}\%_{\text{Sa}}^T = kf * A_{\text{Sa}} / \text{Wt}_{\text{Sa}}^M$

where:  $\text{Wt}\%_{\text{Sa}}^T$  = the true weight percent of the sample  
 $\text{Wt}_{\text{Sa}}^M$  = the actual weight of the sample  
 $A_{\text{Sa}}$  = the measured area of the eluted sample peak

### 10.2.6 Relative error

Uncertainty in weight percent measurements is expressed as the relative error. Calculation of the relative error first requires the determination of the absolute error. The absolute error is defined as the sum of the absolute difference between the known value of a weight percent standard and the individual measurements divided by the number of measurements:

equation: 
$$E_{Std}^{Abs} = \frac{[\sum |Wt\%_{Std1}^M, Wt\%_{Std2}^M, \dots, Wt\%_{Stdn}^M - Wt\%_{Std}^T|]}{n}$$

where:  
 $Wt\%_{Std}^T$  = the known weight percent value of the standard  
 $Wt\%_{Std}^M$  = the measured weight percent values of the standard  
 $n$  = the number of measured weight percent values of the standard  
 $E_{Std}^{Abs}$  = the absolute error of the standard.

The relative error associated with the weight percent standard is defined as the absolute error divided by the known weight percent value for the measured weight percent standard expressed as a percent:

equation: 
$$E_{Std}^{Rel} = (E_{Std}^{Abs} / Wt\%_{Std}^T) \times 100$$

where:  $E_{Std}^{Rel}$  = the relative error of the weight percent standard expressed as a percent.

### 10.2.7 Blank

Mass balance can be used to correct the isotopic composition of the sample for the presence of a blank:

equation: 
$$\delta_{Sa}^T = (\delta_{Sa}^M - \delta_{Blk}^M) / (A_{Sa}^M - A_{Blk}^M)$$

where:  
 $\delta_{Sa}^T$  = the true isotopic value of the sample  
 $\delta_{Sa}^M$  = the measured isotopic value of the sample  
 $\delta_{Blk}^M$  = the measured isotopic value of the blank  
 $A_{Sa}^M$  = the measured area of the sample peak  
 $A_{Blk}^M$  = the measured area of the blank peak



### 10.2.8 Outlier test

The UWSIF uses the Dixon Q test to test for outliers. This test is primarily used for small data sets (between 3 and 30 data points). It can be used to test whether the minimum value is an outlier, the maximum value is an outlier, or either the minimum or maximum value is an outlier. To apply the Dixon Q test, the data are arranged in order of increasing values.  $Q_{cal}$  is calculated as follows:

equation:  $Q_{cal} = gap/range$

where:  $gap$  = the absolute difference between the data point in question and the closest data point to it.  
 $range$  = the absolute difference between the largest and smallest data points.

Compare  $Q_{cal}$  to  $Q_{crit}$  for a chosen confidence ( $\alpha$ ) interval (see Table 9).

If  $Q_{cal}$  is larger than  $Q_{crit}$ , the data point is considered to be an outlier.

Only one point may be rejected from a data set using the Dixon Q test.

Sample size	3	4	5	6	7	8	9	10
$\alpha = .10$	0.941	0.765	0.642	0.560	0.507	0.468	0.437	0.412
$\alpha = .05$	0.970	0.829	0.710	0.625	0.568	0.526	0.493	0.466
$\alpha = .01$	0.994	0.926	0.821	0.740	0.680	0.634	0.598	0.568

Table 9 Dixon Q Test critical values

## **Section 11 Elemental Analyzer Maintenance**

## 11.1 Building a Combustion Reactor

The combustion reactor should be replaced every 1000-1200 samples, or as needed.

Materials needed:

- 1.8 cm x 45 cm quartz tube
- 70 % ethanol
- Chromium oxide
- Funnel
- Laboratory wipes
- Push rod
- Quartz wool
- Ruler
- Silvered cobaltous/cobaltic oxide
- Tray

Procedure:

1. Mark the outside of the combustion tube at the following distances (from the bottom): 4 cm, 10 cm, 11 cm, 21 cm, 22 cm.
2. Insert the column plug.
3. Insert 1 cm of quartz wool into the bottom of the quartz tube. Using the push rod, push the quartz wool into the quartz tube until the quartz wool hits the 11 cm mark (the reactor plug).
4. Using a funnel add silvered cobaltous/cobaltic oxide through the bottom of the quartz tube until the reagent reaches the 4 cm mark. Gently tap the tube to compact the reagent. Add more reagent if necessary.
5. Add additional quartz wool to fill the bottom of the quartz tube.
6. Through the top of the quartz tube, add 10 cm of chromium oxide. As you add the reagent to the tube gently tamp the tube to compact the reagent to avoid separation when the reactor is heated to 1020 °C.
7. Add approximately 1.0 to 1.5 cm of quartz wool on top of the chromium oxide.
8. Prepare a quartz insert by placing 1 cm of quartz wool into the bottom of the tube, followed by 2-3 mm of chromium oxide.
9. Slide the insert into the top of the combustion reactor. The insert should extend past the top of the combustion reactor by 4 to 5 cm.
10. With a marker, draw a line around the quartz insert where it extends above the combustion reactor.
11. Remove the quartz insert and cut it to the proper length.
12. Insert the cut quartz insert into the combustion reactor.
13. Install a black sealing ring at the top with the flat portion down.
14. Clean the outside of the combustion tube with laboratory wipes and 70 % ethanol.

## 11.2 Building a Reduction Reactor

The reduction reactor should be replaced every 1000-1200 samples, or as needed.

Materials needed:

- 1.8 cm x 45 cm quartz tube
- 70 % ethanol
- Analytical grade pure copper (0.3mm mesh wire)
- Funnel
- Laboratory wipes
- Quartz wool
- Tray

Procedure:

1. Insert 1.5 to 2.0 cm of quartz wool into the bottom of the quartz tube. Place the bottom of tube against the tray and use the push rod to tamp down the quartz wool.
2. Using a funnel add 35 to 40 cm of pure Cu wires to the quartz tube. As you add the copper wire to the tube, gently tamp the tube to compact the copper to avoid separation when the reactor is heated to 650 °C. Note: a vortex mixer can also be used to compact the copper wire. As wire is added to the quartz tube, a 2-3 second vortex mixing will easily compact the copper wires in the quartz tube.
3. Add quartz wool to fill the rest of the open tube.
4. Install a black sealing ring at the top with the flat portion down.
5. Clean the outside of the reduction tube with a laboratory wipe and 70 % ethanol and set the tube aside.

## 11.3 Building/Preparing Quartz Inserts

Be sure to wear safety glasses and quartz glassblower glasses while making new inserts.

Materials needed:

- Glass saw
- Natural gas/oxygen torch
- Standard wall 15 mm OD quartz tubing
- Tube scorer

Procedure:

1. Cut the quartz tubing to 12-inch lengths.
2. Using the glass saw, cut notches at 1.5 inch and 2.5 inch from the bottom on one side of the tube. Cut a notch at 2 inches from the bottom on the opposite side of the tube.
3. Fire polish the notches with the torch.
4. Using the natural gas/oxygen torch, heat the bottom of the insert and collapse the opening to roughly  $\frac{1}{2}$  the original diameter. Closing the opening helps keep the contents of the quartz insert within the insert.
5. Heat the completed insert to 850 °C.
6. Insert 1 cm of quartz wool into the bottom of the insert.
7. Add 2-3 mm of chromium oxide to the top of the quartz wool.

## 11.4 Leak Checking an Elemental Analyzer

Whenever consumables are replaced in an elemental analyzer, a leak test is required to ensure that the instrument is not leaking.

Procedure:

1. Place a plug over the Vent-M, release pressure on the helium regulator on the EA (turn black/silver knob counterclockwise).
2. If there is a leak the pressure on the helium gauge will decrease.
3. The most common source of leaks will be at the carousel connection to the combustion reactor, seals in water trap, or the carousel lid seal. Make sure to restore helium pressure before checking for leaks.
4. If there is no leak, remove plug on Vent-M and restore helium pressure.

If it is determined that a leak exists, a helium leak detector is used to isolate the leak. Once the leak is fixed, repeat the leak test procedure.

## 11.5 Replacing the Elemental Analyzer Reactors

The combustion and reduction tubes should be replaced every 1000-1200 samples or as needed.

Materials needed:

- Prepared combustion reactor (with quartz insert)
- Prepared reduction reactor

Be sure to wear leather gloves and safety glasses while changing reactors.

Procedure:

1. Turn off the source by clicking on the gray symbol at the top left side of the Isodat screen.
2. Close the mass spectrometer by turning the source SGE valve carefully until you see the vacuum pressure in Isodat drop to  $10^{-9}$  mbars. Do not over-tighten.
3. Put the EA into "Stand-by" mode.
4. Set both reactor ovens to 500 °C. Wait for both ovens have reached 500 °C or below.
5. Take out the old reactors by unscrewing the bottom and then the top.
6. Insert new reactors. Make sure all O-rings are still in good shape before reusing. (O-rings should be flexible and cracks should not be visible.)
7. Make sure the reactor braces are secure.
8. Put the EA into "Work" mode.
9. With a helium leak detector, check all seals and openings including the carousel. You may have to retighten or replace O rings in order for there not to be leaks. (Be sure to turn off the helium before retightening anything. You may need to wait about ten minutes for the pressure to release before opening anything again.)
10. Set the two oven temperatures to their original temperatures (1020 °C, 650 °C). Wait for the ovens to reach their working temperatures.
11. Open the mass spectrometer by turning the source SGE valve carefully until you see the vacuum pressure rise to approximately  $10^{-6}$  mbars.
12. Turn on the source by clicking on the red sun symbol at the top left of the Isodat screen.
13. Ensure that the nitrogen background is below 400 mV by performing a jump to mass 28 and a peak center.

## 11.6 Replacing the Quartz Insert

The quartz insert is a disposable tube placed into the top of the combustion reactor to catch residue from sample combustion. The insert must be replaced periodically.

Materials needed:

- Forceps
- Prepared quartz insert
- Sharpie felt pen
- Tube scorer

Procedure:

1. Turn off the Delta<sup>Plus</sup> XP or Delta V source.
2. Loosen the purge valve on top of carousel.
3. Loosen the fittings connecting the carousel to the combustion reactor. Move the carousel to the side so the old insert can be removed without obstruction.
4. Carefully remove the used insert from the reactor. Care should be taken when handling the insert because it is at 1020 °C.
5. Slide the quartz insert into the top of the combustion reactor. The insert should extend above the top of the combustion reactor by 4 to 5 cm. With a felt pen marker, draw a line around the quartz insert where it extends above the combustion reactor. Remove the quartz insert and cut it to the proper length. Insert the shortened quartz insert into the combustion reactor.
6. Reposition the carousel onto the combustion reactor and secure the connection. Let the carousel purge for approximately 5 minutes to remove the atmosphere from the carousel headspace. Then close the purge valve.
7. Allow about 7 minutes for the air to purge the EA plumbing.
8. Perform a leak check.
9. Turn on the Delta<sup>Plus</sup> XP or Delta V source.

## 11.7 Replacement of the Water Trap

The entire procedure should be performed over a secondary container, such as a tray, to capture any spilled material. Safety glasses, rubber gloves, and a lab coat/apron are required.

Materials needed:

- Analytical grade magnesium perchlorate
- Forceps
- Funnel
- Quartz wool
- Spatula
- Tray

Procedure:

1. Turn off the Delta<sup>Plus</sup> XP or Delta V source.
2. Remove the magnesium perchlorate water trap. Hold the glass tube and loosen the red caps on both ends of trap and remove. Check the silicon seals for cracks. Replace the seals if necessary.
3. Clean the water trap by removing the quartz wool from each end of the tube. Push all the used magnesium perchlorate out of the tube into a waste container. Rinse the tube with deionized water, followed by a rinse with 70 % ethanol, and finally blow the tube dry with compressed air.
4. Add 5 to 7 mm of quartz wool to one end of the clean glass tube.
5. Using a funnel, fill the glass tube with magnesium perchlorate granules and gently tap the tube on the bench top to compact the granules. Be sure to leave approximately 5 to 7 mm of space in order to insert quartz wool into the open end.
6. Add 5 to 7 mm of quartz wool to fill the glass tube.
7. Install the newly prepared water trap in reverse. Remember the Teflon face of the seal goes against the steel seat inside the red cap and the silicon face goes against the glass water trap tube.
8. Perform a leak check.
9. Turn on the Delta<sup>Plus</sup> XP or Delta V source.



## **Section 12 Troubleshooting**

## 12.1 Troubleshooting the Elemental Analyzer

Observation	Potential problem	Solution
High nitrogen blank	Air leak	Leak test the instrument
	Oxygen contaminated	Replace the cylinder
Nitrogen peak tailing	Bad combustion	Smaller sample size
		Increase the oxygen Remove residues
Carbon peak tailing	Bad combustion	Remove residues
		Smaller sample size
		Increase the oxygen
Bad peak separation	Copper exhausted	Replace reduction reactor
	GC column temperature	Decrease temperature
Peak between nitrogen and carbon peaks	Oxygen contaminated	Check the oxygen blank
Peaks elute late	Leak in system	Perform a leak test
	Water trap exhausted	Replace the magnesium perchlorate

*Table 10* Troubleshooting guide

## 12.2 Troubleshooting: Helium and Oxygen Gases

The nominal pressure of gas supplies should be: 100 kPa for helium and 100 or 125 kPa for oxygen.

Symptom:

- A negative peak between the nitrogen and carbon peaks.

Solution:

- Set both pressures to the same value to eliminate the negative peak and to have good separation between the nitrogen and carbon peaks.

Symptom:

- Tailing on the nitrogen peak.
- Bad separation between the nitrogen and carbon peaks.
- Split or tailing on the carbon peak.

Solution:

- Decrease the weight of the sample.
- Increase the oxygen quantity.
- Remove the residue.
- Change the combustion reactor.

There are two parameters that can be modified to optimize flash combustion:

- The oxygen flow.
- The oxygen injection time.

The normal values are  $100 \text{ ml}\cdot\text{min}^{-1}$  and 4 seconds respectively.

Optimizing the oxygen flow rate:

1. Run 1: oxygen flow rate  $80 \text{ ml}\cdot\text{min}^{-1}$ , injection time 4 seconds.
2. Run 2: oxygen flow rate  $120 \text{ ml}\cdot\text{min}^{-1}$ , injection time 4 seconds.
3. Set the oxygen flow rate to the lowest level that still allows flash combustion.

Optimizing the oxygen injection time:

1. Run 1: oxygen flow  $100 \text{ ml}\cdot\text{min}^{-1}$ , injection time 4 seconds.
2. Run 2: oxygen flow  $100 \text{ ml}\cdot\text{min}^{-1}$ , injection time 13 seconds.
3. Set the oxygen injection time to the lowest level that still allows flash combustion.