University of Wyoming
Stable Isotope Facility

Methods and 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
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	10/31/17	CSC	Edited for NEON/Battelle use

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Section 1 The Stable Isotope Facility at the University of Wyoming

1.1 General Description of the Stable Isotope Facility

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, advices 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, O, C and N) 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 Technical and safety training

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

- Introduction to the UWSIF Quality Management Plan.
- General laboratory safety procedures.
- Proper use of instruments.
- Proper handling of reference materials.

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.

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

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

1.5 Abbreviations

Abbreviation	Description
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

1.6 Symbols used

Symbol	Name	Description
δ	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.
ºC	degree Celsius	Unit of measure of temperature.
µL	microliter	Metric system unit of volume. A microliter is 1/1000000 of a liter.
m	meter	Metric system unit of length.
mL	milliliter	Metric system unit of volume. A milliliter is 1/1000 of a liter.
mg	milligram	Metric system unit of mass. A milligram is 1/1000 of a gram.
psi	pounds per square inch	English system unit of pressure.
min	minute	A unit of time equal to 1/60 of an hour.
^2H	hydrogen-2	A stable isotope of hydrogen.
^{13}C	carbon-13	A stable isotope of carbon.
^{15}N	nitrogen-15	A stable isotope of nitrogen.
^{18}O	oxygen-18	A stable isotope of oxygen.

1.7 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.

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 inert gas that carries the sample gas into the IRMS. Connecting the IRMS with various automated sample preparation devices generates the sample gas, including but not limited to elemental analyzers (EA) and gas chromatographs (GC).

Delta. Delta (δ) 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_2, H_2O and N_2.

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, and O.

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.

Section 2 Instrument Inventory

2.2 Laboratory Equipment

2.2.1 Laboratory instruments and equipment

Instruments/Equipment	Quantity
Carlo Erba NC 1110 Elemental Analyzer	1
Costech 4010 Elemental Analyzer	1
Costech Zeroblank autosampler	3
Finnigan ConFlo III open-split interface	2
Finnigan Delta^{plus} XP Isotope Ratio Mass Spectrometer	2
Retsch MM-200 Mixer Mill	1
Sartorius microbalance	3
Thermo Scientific ConFlo IV open-split interface	2
Thermo Scientific Delta V Isotope Ratio Mass Spectrometer	2
Thermo Scientific Flash^{plus} Elemental Analyzer	1
Thermo Scientific IsoLink Elemental Analyzer	1

2.2.2 Mass spectrometers and peripherals

Model	Peripherals	Isotope	Gas form
Finnigan Delta^{plus} XP	Costech 4010 (EA) Costech Zeroblank autosampler Finnigan ConFlo III interface	^{13}C, ^{15}N	CO_2, N_2
Delta V	Thermo Flash (EA) Thermo ConFlo IV interface Costech Zeroblank autosampler	^{13}C, ^{15}N	CO_2, N_2
	Carlo Erba NC 1110 (EA)	^{13}C, ^{15}N	CO_2, N_2
Delta V	Thermo IsoLink (EA) Thermo ConFlo IV interface Costech Zeroblank autosampler	^{13}C, ^{15}N	CO_2, N_2

Section 3 Isotope Ratio Mass Spectrometry

3.1 Isotope Ratio Mass Spectrometry (IRMS) - Principal of Operation

3.1.1 The Electron Impact Ionization Source

The isotope ratio mass spectrometers are operated in continuous flow mode (the sample gas is carried to the instrument in a helium carrier stream). The helium carrier/sample gas enters the source through an open-split interface that directs the gas flow directly into the source ionization chamber (ion box). Attached to the ionization chamber are the filament (cathode), an electron trap (anode), 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^{Plus} XP, ~1 in 1500 gas molecules are ionized, ~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.
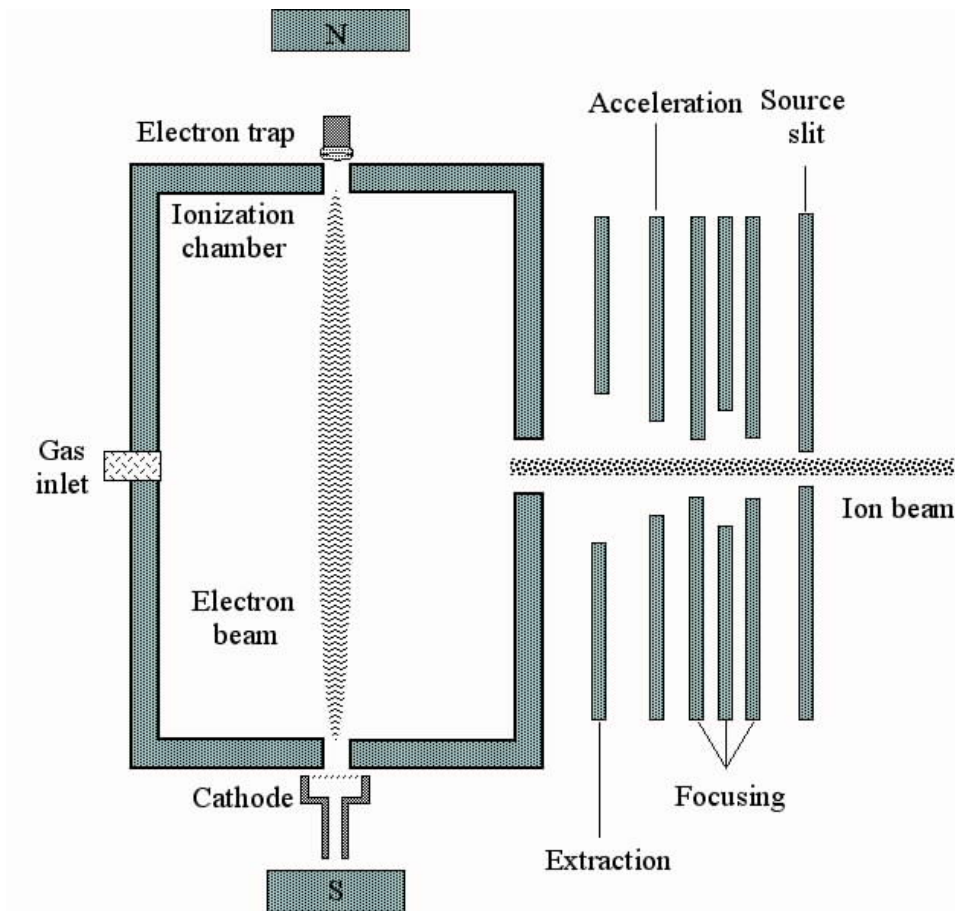
Figure 1. Schematic of the electron impact ionization source.

3.1.2 The Electromagnet and the Flight Tube

The flight tube has a 90-degree deflection path fitted with a permanent magnet with a maximum magnetic field strength of 0.75 Tesla providing mass ranges up to 70 amu (Delta^{Plus}XP) or 96 amu (Delta V) at full accelerating voltage. The radius of the flight tube is fixed and the magnet is variable but constant for any given gas species. At a constant accelerating voltage (V) and magnet strength (B) the lighter masses will be deflected more than the heavier masses. In the case of CO_2, mass 44 is deflected more than mass 45 and mass 46. In order to analyze another gas such as N_2 with a fixed accelerating voltage, the magnetic field strength is varied.

These parameters are related by the equation:

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

Where: m/e 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
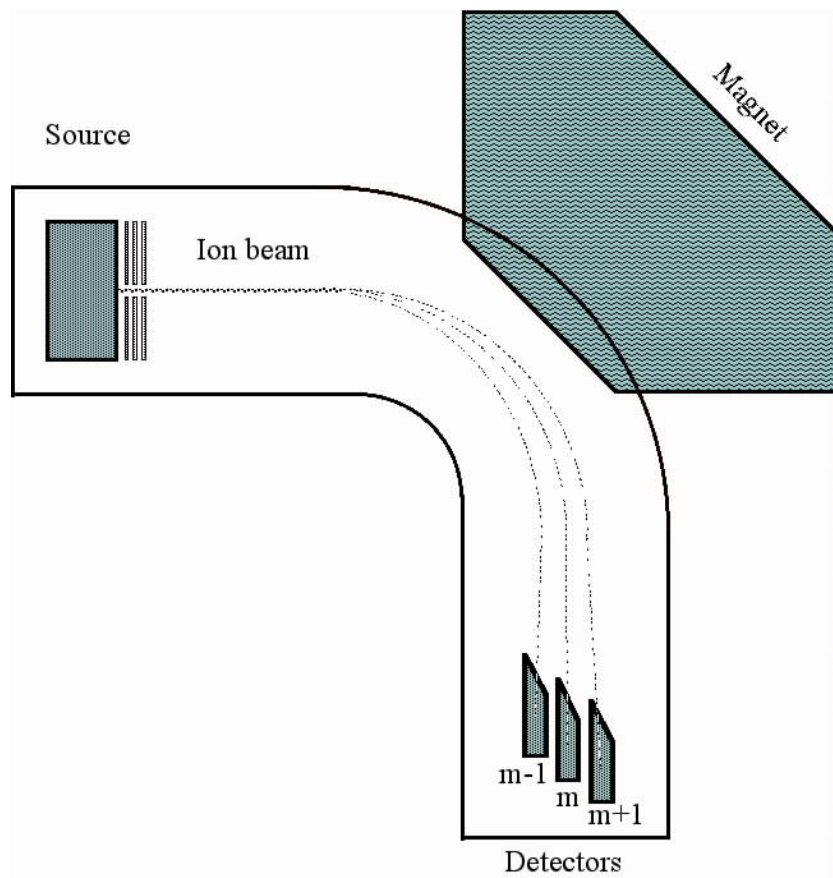
Figure 2. Schematic of the isotope ratio mass spectrometer.

3.1.3 The Faraday Cup Detectors

After leaving the magnet, the separated ion beams are directed to 3 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_2, the resistor on cup 1 = 3 x 10^8 Ω, the resistor on cup 2 = 3 x 10^{10} Ω, and the resistor on cup 3 = 1 x 10^{11} Ω. 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_2, all three masses (44, 45, and 46) are detected at one time, allowing the software to calculate both $\delta^{18}O$ and $\delta^{13}C$ ratios for a given sample.
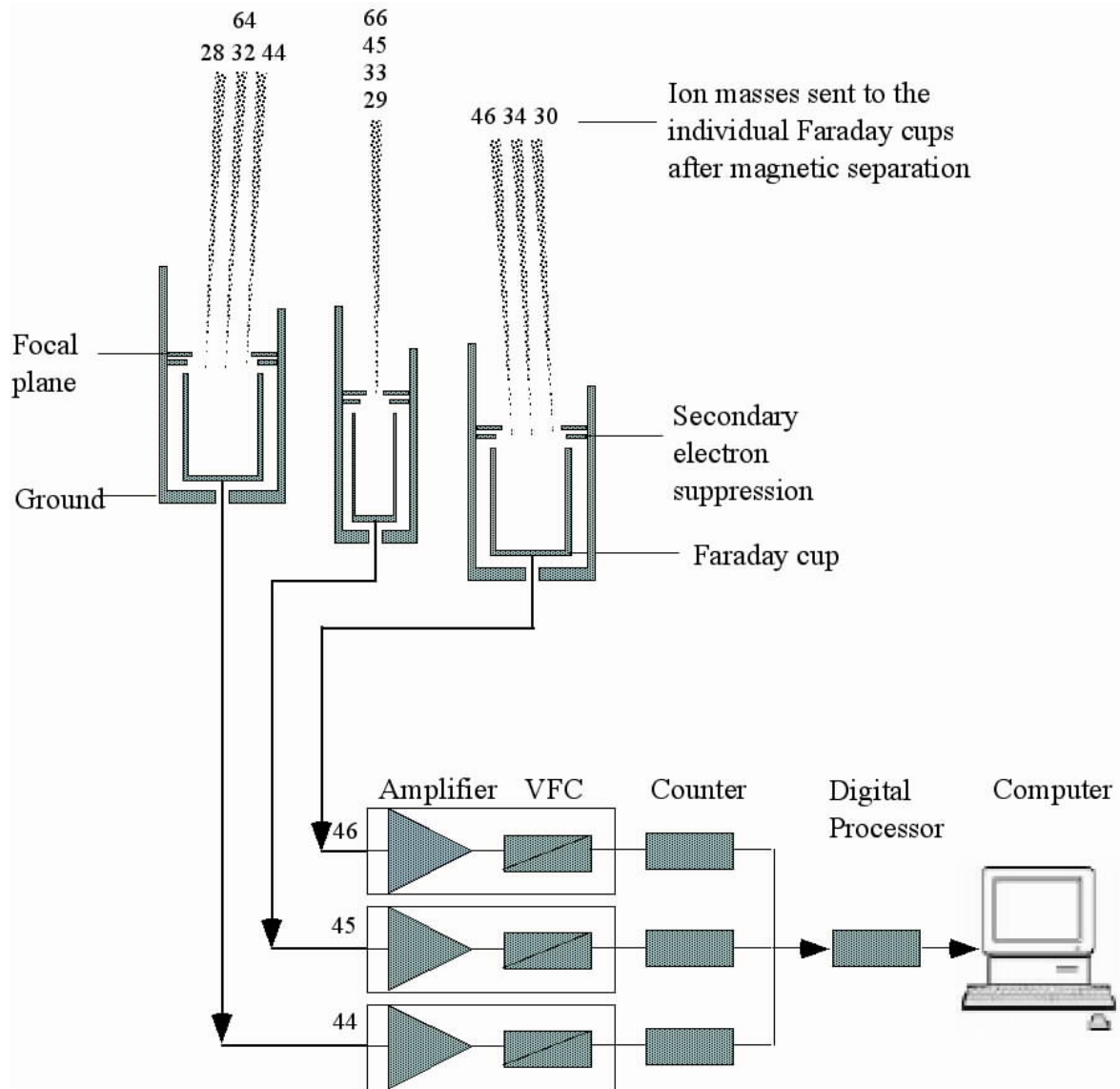
Figure 3. Schematic of the triple Faraday cup detector system.

3.2 The Open-split – Principal of Operation

Prior to analysis in an isotope ratio mass spectrometer, complex samples (solids, liquids, gases) must be converted into simple gases. This is accomplished by oxidation, carbon reduction or high temperature conversion. These simple gases (H_2, N_2, CO and CO_2) 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 the open-split principal. 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 mL•min^{-1}. Since sample gas flows entering the open-split cell range from 2-140 mL•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 mL•min^{-1} and enter the open-split cell. Since the mass spectrometer capillary is so small (0.1mm ID) only a small portion (<10%) of sample and carrier gas can enter the mass spectrometer. This essentially reduces the flow to ~0.3 mL•min^{-1} and allows the ion source to operate at 10^{-6} mbar pressure.

High flow (EA)
(80-140 ml·min^{-1})

Low flow (GC)
(2-4 ml·min^{-1})

Open split cell
(Atmospheric pressure)

Split flow (IRMS)
(0.2-0.3 ml·min^{-1})

Ion source
(10^{-6} mbar pressure)
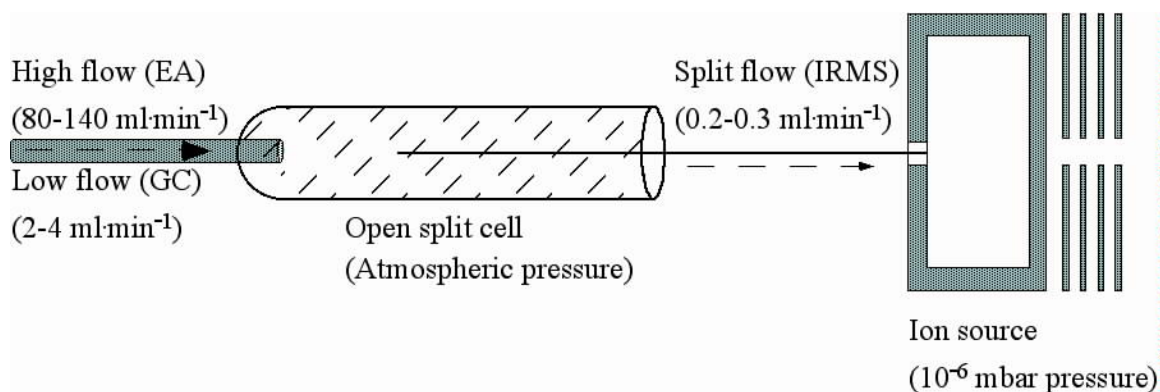
Figure 4. Schematic of the open-split.

3.3 ConFlo III Interface - High Flow - Principal of Operation

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_2 reference gas, one for the CO_2 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. Before the effluent from the elemental analyzer enters the sample open-split cell, it passes a pneumatic valve. With the valve closed, flow to the open-split cell is 8 mL·min^{-1}. The valve opens when helium dilution of the sample gases is required, reducing the helium flow to the sample open-split cell from 80-100 mL·min^{-1} to approximately 0.5 mL·min^{-1}.
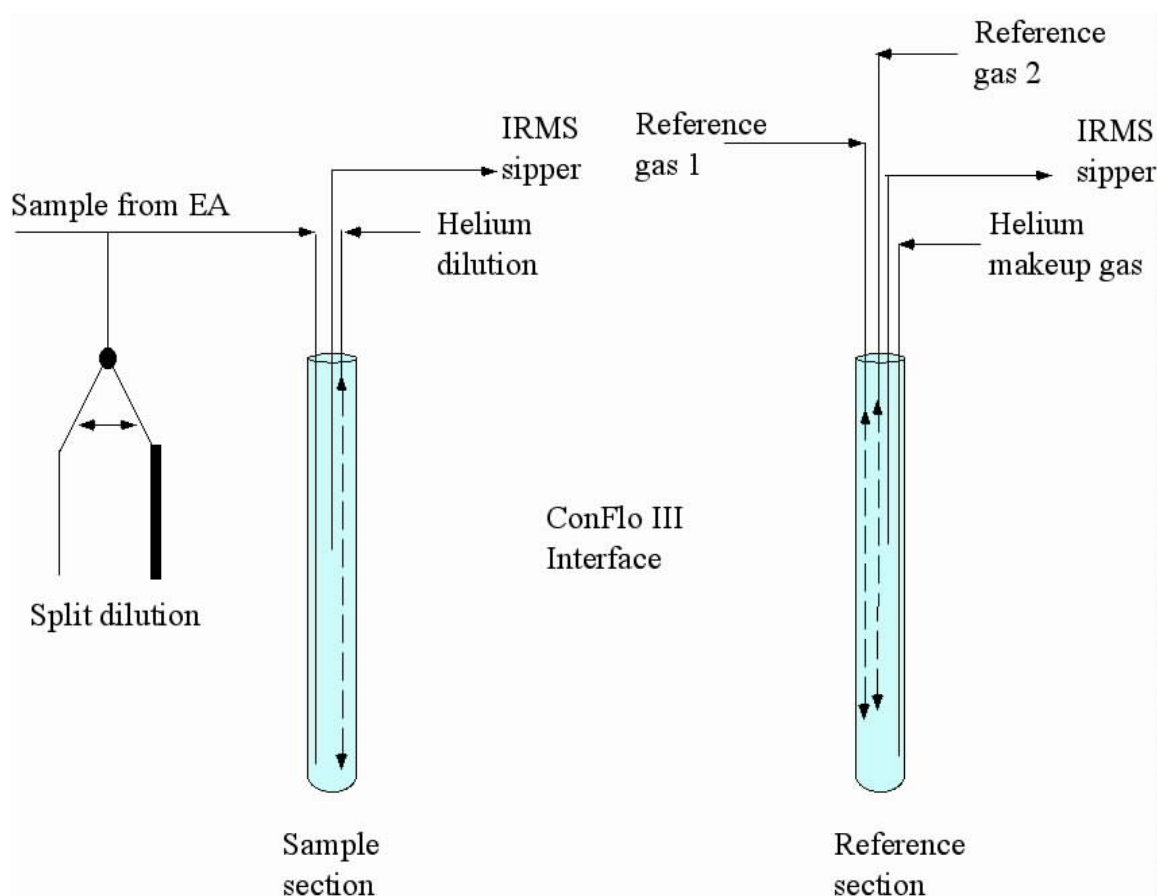
Figure 5. Schematic of the high flow open-split interface.

3.5 Thermo ConFlo IV Interface - Principal of Operation

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).
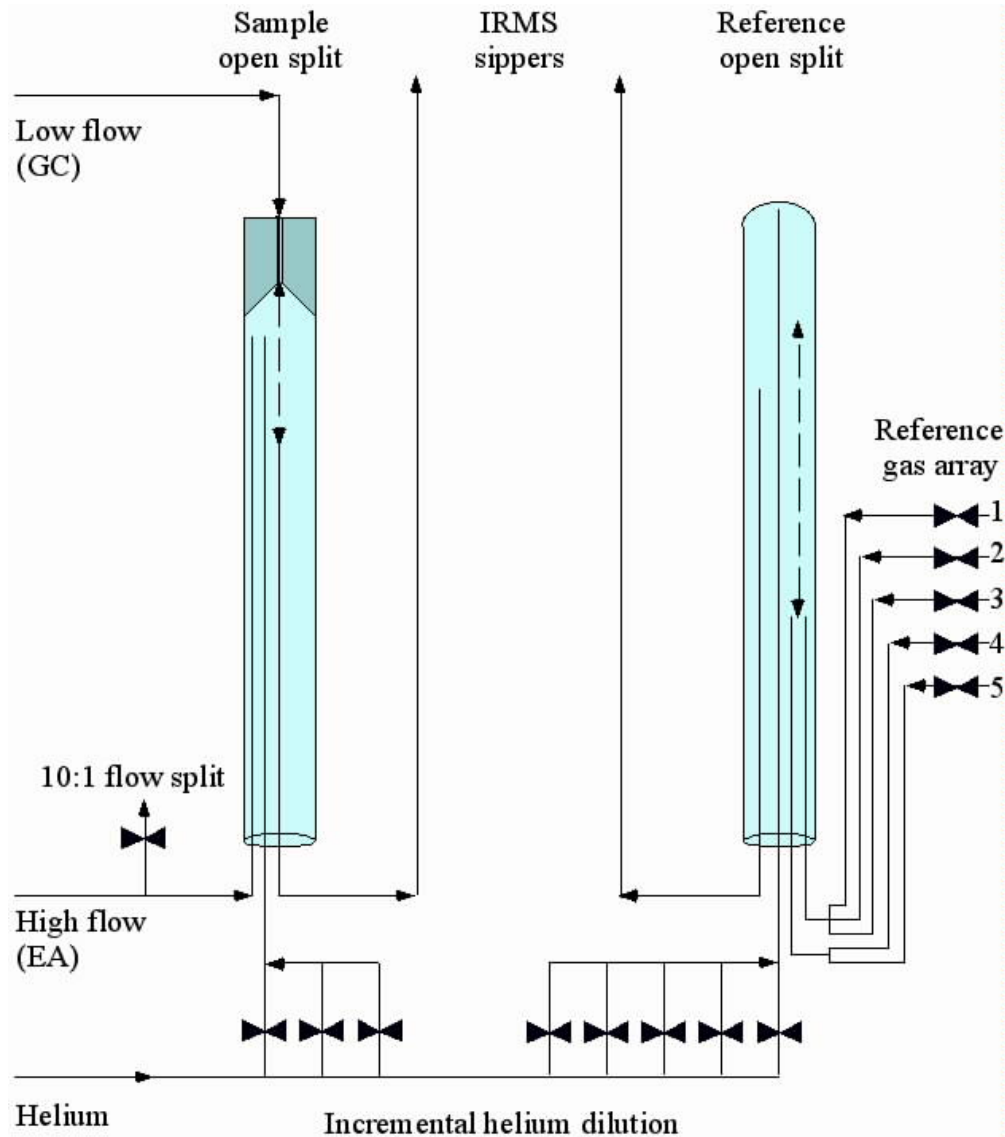

Figure 7. Schematic of the Thermo ConFlo IV open-split interface.

3.6 Carlo Erba NC-1110 Elemental Analyzer - Principal of Operation

The Carlo Erba NC-1110 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_2, N_xO_x, CO_2, H_2O, and SO_2. The reduction reactor contains reduced copper wires for the reduction of nitrogen oxides to N_2 and the removal of excess O_2. An adsorption trap containing magnesium perchlorate removes the H_2O. The remaining N_2 and CO_2 gases travel through a chromatographic column (Porapak Q) and then move to the ConFlo IV open-split interface. See Section 3.2 for a detailed schematic.

3.7 Costech 4010 Elemental Analyzer - Principal of Operation

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 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_2, N_xO_x, CO_2, and H_2O. The reduction reactor contains reduced copper wires for the reduction of nitrogen oxides to N_2 and the removal of excess O_2. An adsorption trap containing magnesium perchlorate removes the H_2O. The remaining N_2 and CO_2 gases travel through a chromatographic column (Porapak Q) and then move to the ConFlo III open-split interface. See Section 3.2 for a detailed schematic.

Instrument temperatures:
Combustion reactor   1020°C
Reduction reactor   650°C
Chromatographic column   60°C

Flow rates:
Helium   80-100 mL·min^{-1}
Oxygen   25-30 mL·min^{-1}

3.8 Thermo IsoLink Elemental Analyzer - Principal of Operation

The Thermo IsoLink 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_2, N_xO_x, CO_2, and H_2O. The reduction reactor contains reduced copper wires for the reduction of nitrogen oxides to N_2 and the removal of excess O_2. An adsorption trap containing magnesium perchlorate removes the H_2O. The remaining N_2 and CO_2 gases travel through a chromatographic column and then move to the ConFlo IV open-split interface. See Section 3.2 for a detailed schematic.

Instrument temperatures:

Combustion reactor 1020°C

Reduction reactor 650°C

Chromatographic column 50-60°C

Flow rates:

Helium 80-160 mL·min^{-1}

Oxygen 25-30 mL·min^{-1}
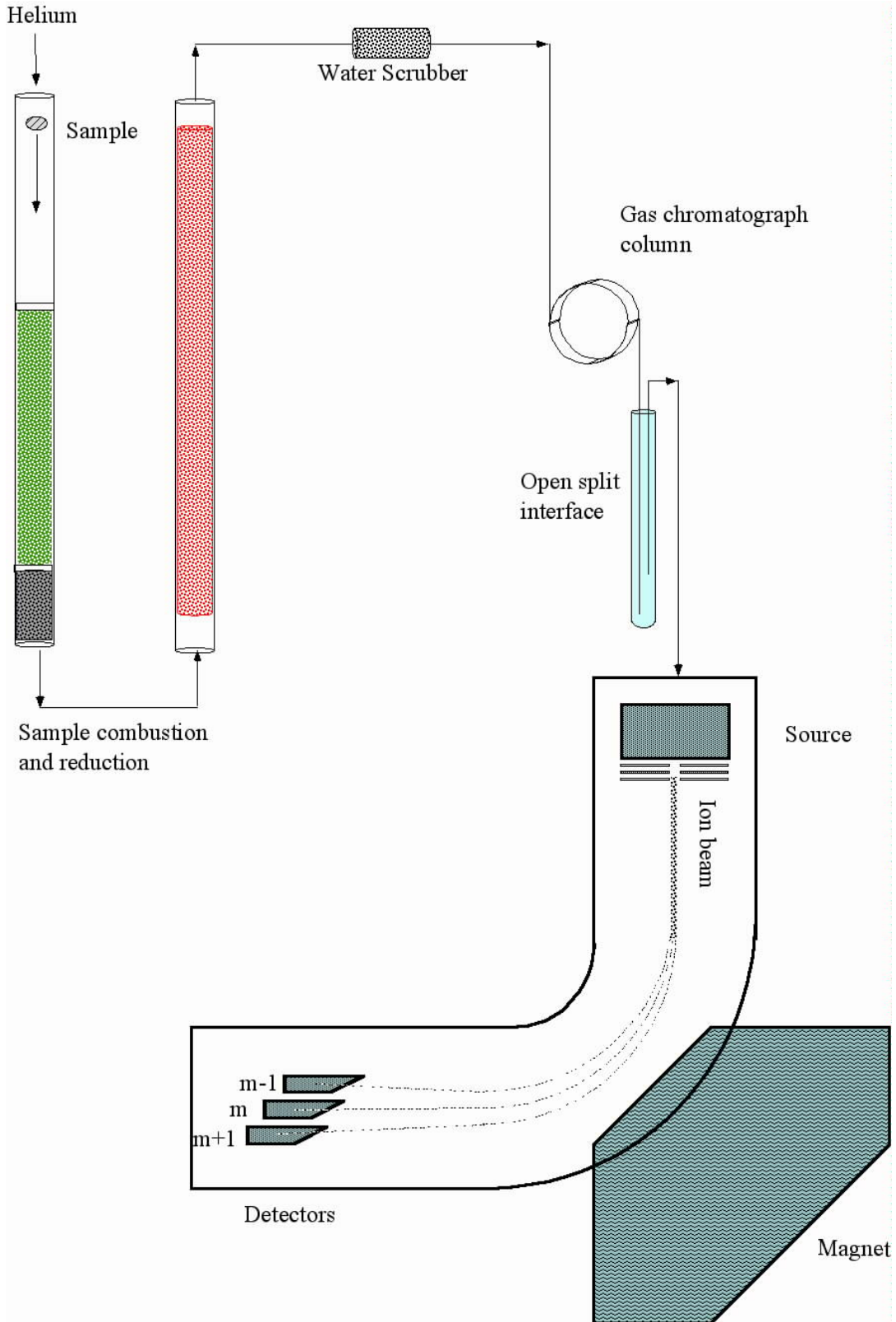
Figure 8. Schematic of the elemental analyzer coupled to an IRMS (solid samples).

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Section 4 Sample Analysis

4.1 Carbon and Nitrogen Analysis of Solids (combustion)

Carbon and nitrogen analysis of solids is performed using either a Finnigan Delta^{Plus} IRMS or a Thermo Delta V IRMS. The Finnigan Delta^{Plus} 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-1110 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_2, N_xO_x, CO_2, and H_2O. The reduction reactor contains reduced copper wires for the reduction of nitrogen oxides to N_2 and the removal of excess O_2. An adsorption trap containing magnesium perchlorate removes the H_2O. The remaining N_2 and CO_2 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^{-1}
Oxygen   25-30 mL·min^{-1}

Section 5 Sample Preparation

5.1 Receiving Samples

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

5.2 Sample Handling

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 follow 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.

5.3. Storage of Samples

Samples are stored in dry, cool, dark cabinets until analysis.

5.4. Sub-sampling Procedures for Isotope Analysis

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. If you sub-sample from a bulk container and transfer 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.

5.5 Sample tracking guidelines

To keep track of samples from the moment they arrive at the laboratory to the time results are reported, the analysis 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 along with normalization parameters is sent to the PI.
6. Finally, an invoice containing details of the job number and analysis type is sent to the PI.

5.11 Grinding Samples: Mortar/Pestle Method

Materials needed:

- Liquid nitrogen (LN)
- Mortar and pestle
- Kimwipes®
- 95% Ethanol
- Storage vials
- Labeling tape
- Markers
- Spatula
- Forceps

Safety:

Liquid nitrogen is very cold and will get on you. You must wear safety glasses during this process. Gloves are also available for additional protection.

Step-by-step procedure:

1. Dry the samples overnight in a drying oven (70ºC).
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 95% 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.

5.12 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 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.

Materials needed:

- Vials
- Labeling tape
- Retsch ball mill
- Stainless steel grinding jars and steel balls

To grind a sample:

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 ball 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.

5.13 Weighing Solid Samples

Weighing of samples involves the use of microbalances. A microbalance is typically readable to 1µg and reproducible (standard deviation) to ≤ 1 µg. 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 µg before analysis.

For $\delta^{13}C$ and $\delta^{15}N$ 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 weighed immediately, the samples are placed into a dry storage cabinet.

Materials needed:

- Kimwipes
- 95% Ethanol
- Spatula
- Forceps
- Tin or silver capsules

The procedure for weighing is as follows:

1. Using only clean forceps, place an empty 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.

5.14 Carbonate Acid Digestion for Soils or Minerals

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.

Materials needed:

- 0.5 N HCl
- Deionized water
- Hardened glass filter paper
- Buchner funnel
- Erlenmeyer flask
- Drying oven
- Vacuum system

Procedure for the acidification:

1. Put a sub sample of the soil (finely ground) in a 50 mL Erlenmeyer flask. Add enough 0.5 N HCl to make a slurry.
2. Thoroughly mix the soil solution. Note the formation of bubbles. The presence of bubbling will indicate that carbonates are being digested, forming CO_2.
3. Test the solution with pH paper. If the solution is basic, add more acid. If the pH paper indicates that the solution is acidic, proceed to step 5.
4. Let the solutions sit for 30 minutes and retest with the pH paper. If the pH paper indicates a basic solution, repeat steps 2-4. If the solution is acidic, go to step 5.
5. Transfer the acidified soil to glass filter paper in a Buchner funnel and rinse multiple times with deionized water.
6. Put the filter paper with the acidified soil sample into a drying oven overnight.

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

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 on, contact the SIF staff.

Figure from 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_2, N_2, CO, H_2) (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).
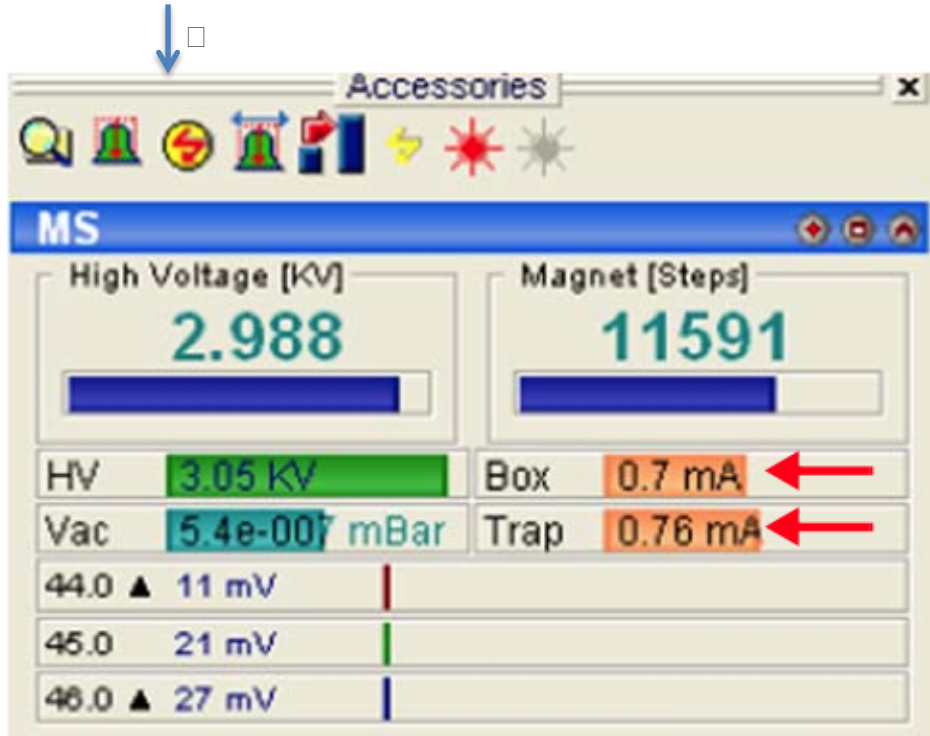
3. Wait for the instrument to finish the peak center routine.
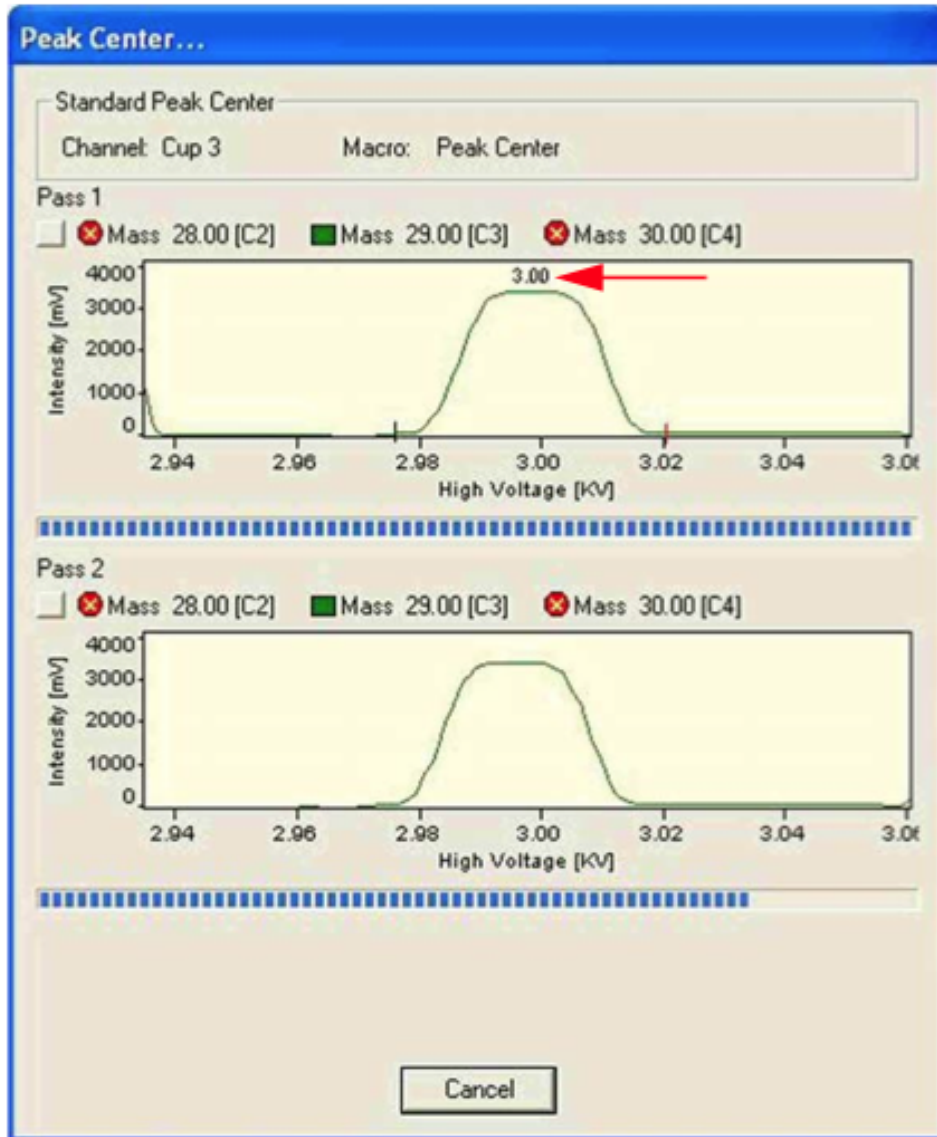
Figure from Thermo user manual

You should see a signal for the appropriate mass of 2000-4000 mV.
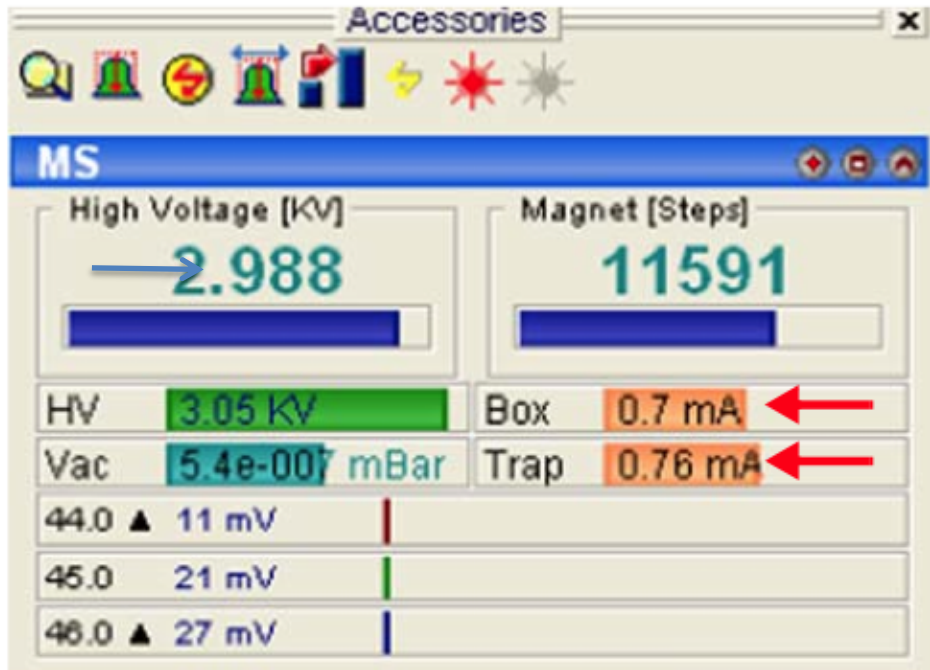
Figure from Thermo user manual

6.2 Delta^{Plus}XP

6.2.1 Initial Instrument Checks

Go to the following screen on the computer:

Screen: Instrument Control.
1. Select the proper gas (CO_2, N_2,) (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 is located at the upper left of the screen).
3. Wait for the instrument to finish the peak center routine.
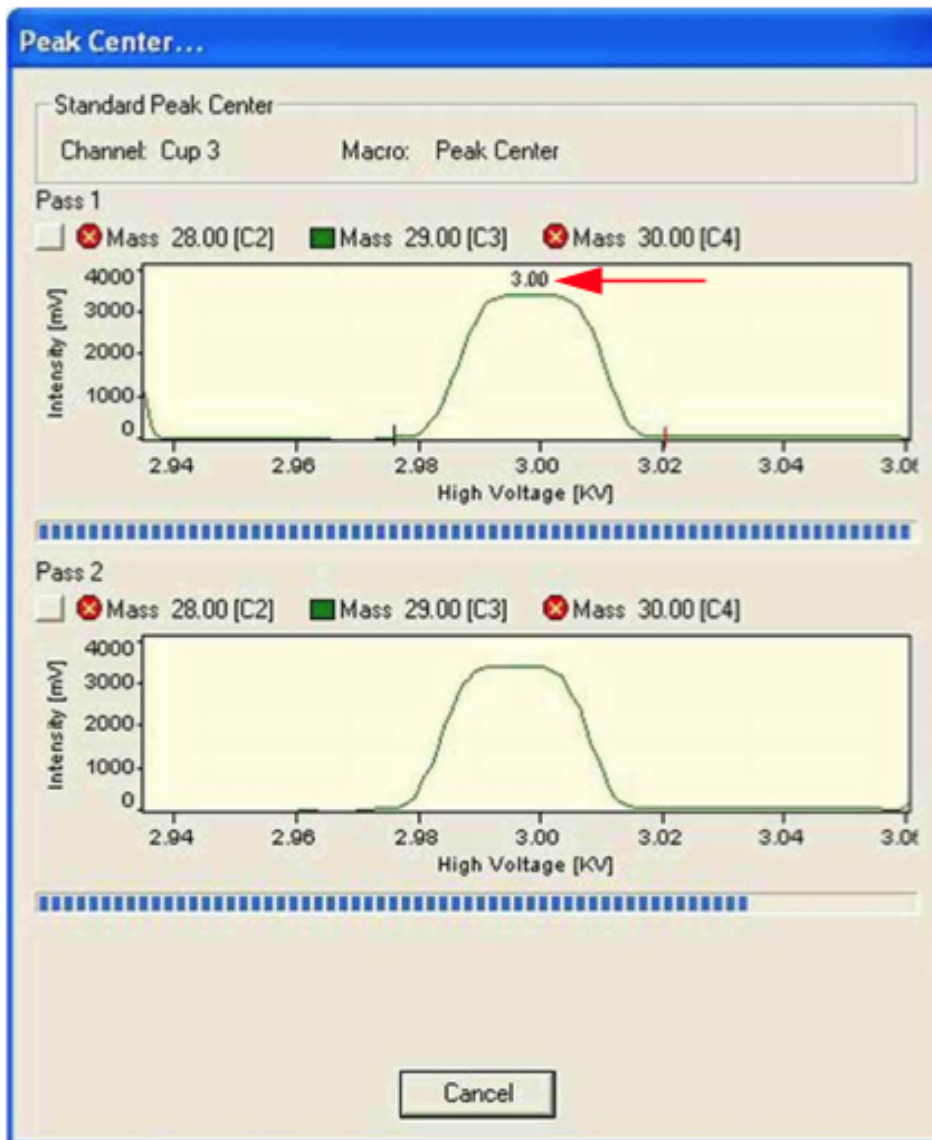
Figure from Thermo user manual

You should see a signal for the appropriate mass of 2000-4000 mV.
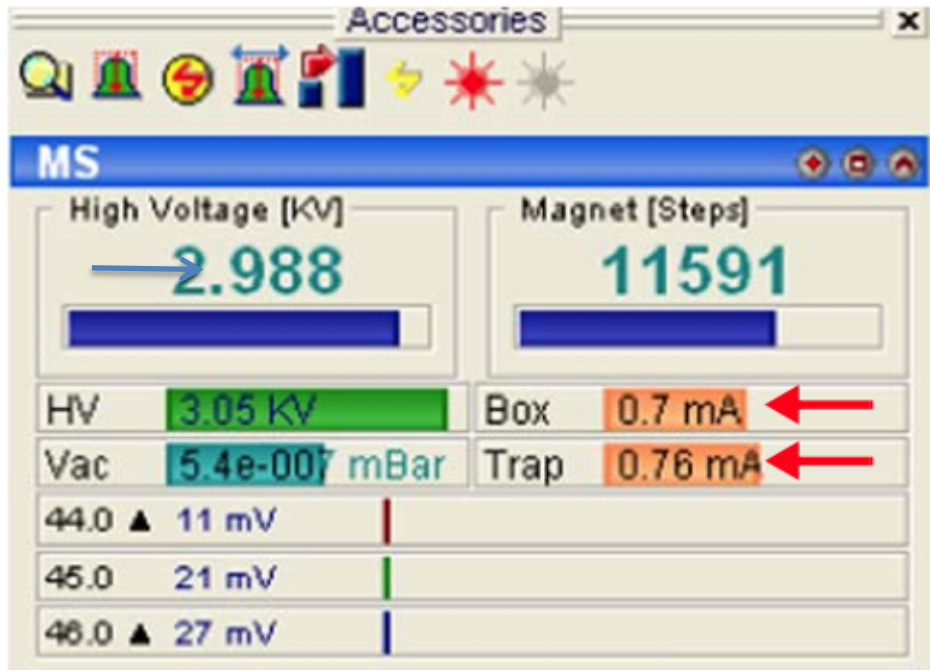
Figure from Thermo user manual

6.3 Zero Blank Autosampler

6.3.1 Sample Loading and Preparation

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.
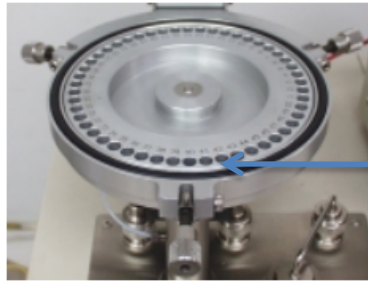
1st sample position

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

Purge valve

Thumbscrew

9.
10. 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 curser to turn on or off):

Screen: ConFlo III

You should select only one reference (Ref I or Ref II).
- Select the reference gas (CO_2).
- Select the reference gas (N_2).

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 curser 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 1 for samples coming from the TC/EA.
- Select sample port LF for samples coming from the GC.

Figure from Thermo user manual

6.7 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}C or ^{15}N) and then right click the highlighted area and choose the CALCULATE option. If the standard deviation of the last On/Off test preformed 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 Data Collection and Calculations

7.1 Data Processing

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

Isodat 2.0

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}C and ^{15}N analyses delete the reference peaks 1, 2, 5, and 6. For ^{13}C only delete peaks 1, 2, and 4.
12. Move the data from peak 4 (under the columns amp 44 and d^{13}C/^{12}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.
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

7.2 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 N2+CO2+He sequence. If only carbon or only nitrogen analysis is needed double-click the CO2 only or N2 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.

7.3 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.

7.4 Basic 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.

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

Delta (δ): $\delta_{Sa} = [R_{Sa} / R_{Std} - 1] \times 1000$

where: R_{Sa} = the measured isotope ratio of the sample
            R_{Std} = the measured isotope ratio of the reference material
            δ_{Sa} = the delta of the sample

The units of delta are per mil, which are parts per thousand differences from a standard.

7.4.1 Isotope data corrections

The following corrections may or may not be required correct the data:

- Drift. Drift occurs when there is a slow change in instrument sensitivity over the course of an analytical run.
- Memory effects. Memory occurs when a previous sample affects the readings of the next sample.
- Linearity. Non-linearity occurs when the instrument sensitivity varies with the size of the sample.
- Normalization. Normalization is the process of placing the data onto internationally accepted scales.

7.4.2 Drift correction

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:

Step 1) Calculate residuals of the reference material:

equation: $\delta^R_{Rm} = \delta^T_{Rm} - \delta^M_{Rm}$

where: δ^T_{Rm} = the accepted isotope value of the reference material
δ^M_{Rm} = the measured isotope value of the reference material
δ^R_{Rm} = residual for a given analyzed reference material sample

Step 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 coefficient of determination (R-squared) of the residual trendline is <0.7, no correction is applied. If the coefficient of determination is >0.7, the following calculation is performed:

Step 3) Calculate the residual corrected isotope values of the reference material and unknowns:

equation: $\delta^T_{Sa} = \delta^M_{Sa} + (T_{Sa} * m + b)$

where: T_{Sa} = the analysis time of the sample (sequence line number)
δ^M_{Sa} = the measured isotope value of the sample
δ^T_{Sa} = the residual corrected isotope value of the sample
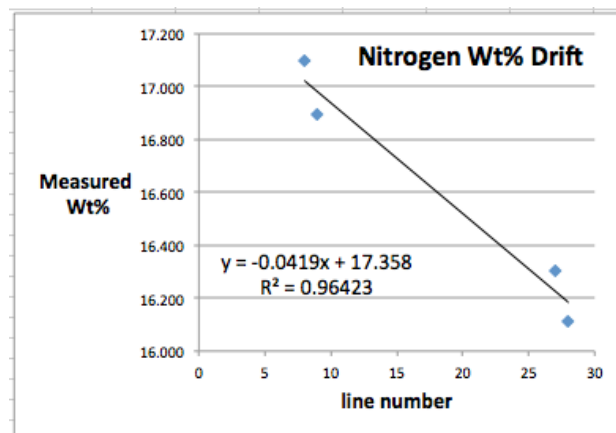
7.4.3 Memory

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
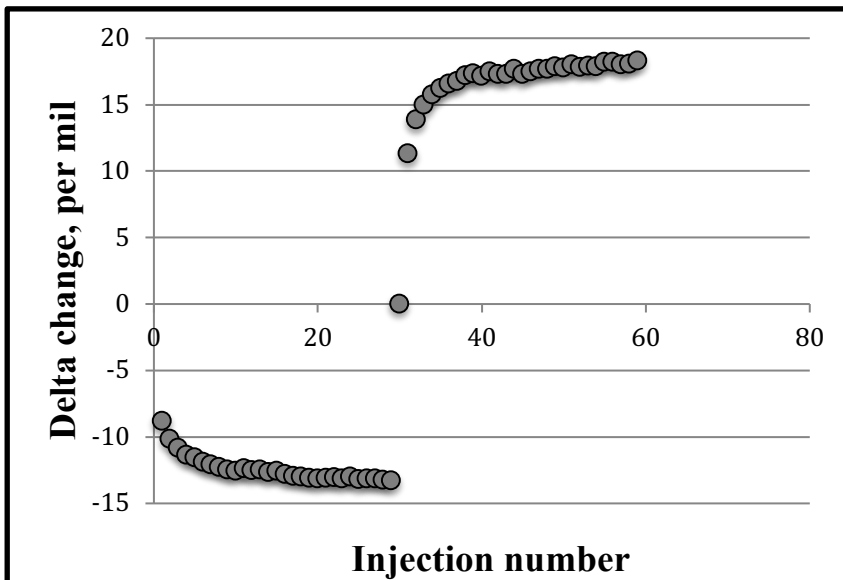
7.4.4 Linearity correction

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	$\delta^{13}C$	Residual $\delta^{13}C$	Corrected $\delta^{13}C$
NBS120C	2.16	8.23	-14.5	-6.25
NBS120C	11.13	7.95	-14.2	-6.37
NBS120C	16.33	7.89	-14.2	-6.32
NBS120C	24.77	7.81	-14.1	-6.25
NBS120C	30.90	7.63	-13.9	-6.31
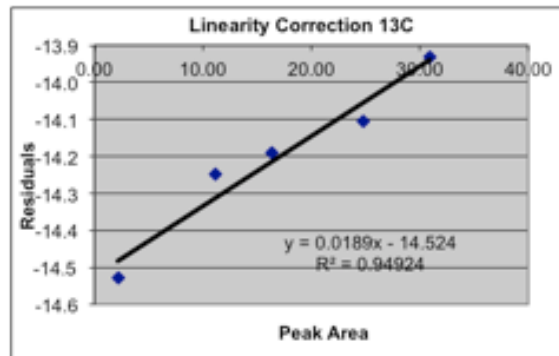
To correct the data:

Step 1) Calculate residuals for **each** measured reference material:

equation: $\qquad \delta^R{}_{Rm} = \delta^T{}_{Rm} - \delta^M{}_{Rm}$

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

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

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

At UWSIF, if the coefficient of determination (R-squared) of the residual trendline is <0.7, not correction is applied. If the coefficient of determination is >0.7, the following calculation is performed:

Step 3) Calculate the residual corrected isotope values of the samples:

equation: $\qquad \delta^T{}_{Sa} = \delta^M{}_{Sa} + (A_{Sa} * m + b)$

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

7.4.5 Normalization

Generally, final isotope data are placed onto an internationally accepted scale such as VPDB or AIR. 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 $\delta^{13}C$ 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, respectively. USGS40 and USGS41 are internationally accepted reference materials with nitrogen isotope values of -4.5 and +47.6 per mil versus AIR, respectively.

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: $\delta^T_{Sa} = m * \delta^M_{Sa} + b$

where: m = the slope of the linear equation
b = the 'y intercept' of the linear equation
δ^M_{Sa} = the measured delta value of sample
δ^T_{Sa} = the corrected delta value of sample

The slope and intercept of the equation are calculated as follows:
$$m = [\delta^T_{Rm1} - \delta^T_{Rm2}] / [\delta^M_{Rm1} - \delta^M_{Rm2}]$$
$$b = \delta^T_{Rm1} - [m * \delta^M_{Rm1}]$$

where: m = the slope of the linear equation
b = the 'y intercept' of the linear equation
δ^T_{Rm1} = the accepted delta value of reference material 1
δ^T_{Rm2} = the accepted delta value of reference material 2
δ^M_{Rm1} = the measured delta value of reference material 1
δ^M_{Rm2} = the measured delta value of reference material 2

7.4.6 Weight percent correction

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: $kf = Wt\%^T_{Std} * Wt^M_{Std} / A_{Std}$

where: kf = the weight percent correction factor
$Wt\%^T_{Std}$ = the known weight percent of the standard
Wt^M_{Std} = the actual weight of the standard sample
A_{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: $Wt\%^T_{Sa} = kf * A_{Sa} / Wt^M_{Sa}$

where: $Wt\%^T_{Sa}$ = the true weight percent of the sample
Wt^M_{Sa} = the actual weight of the sample
A_{Sa} = the measured area of the eluted sample peak

7.4.8 Relative error calculation

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: $E^{Abs}_{Std} = [\sum |Wt\%^M_{Std1}, Wt\%^M_{Std2}, \ldots Wt\%^M_{Stdn} - Wt\%^T_{Std}|] / n$

where: $Wt\%^T_{Std}$ = the known weight percent value of the standard
$Wt\%^M_{Std}$ = the measured weight percent values of the standard
n = the number of measured weight percent values of the standard
E^{Abs}_{Std} = 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: $E^{Rel}_{Std} = (E^{Abs}_{Std} / Wt\%^T_{Std}) \times 100$

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

7.4.9 Blank correction

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

equation: $\delta^T_{Sa} = (\delta^M_{Sa} - \delta^M_{Blk}) / (A^M_{Sa} - A^M_{Blk})$

where: δ^T_{Sa} = the true isotopic value of the sample
δ^M_{Sa} = the measured isotopic value of the sample
δ^M_{Blk} = the measured isotopic value of the blank
A^M_{Sa} = the measured area of the sample peak
A^M_{Blk} = the measured area of the blank peak

Section 8 Reference Materials

8.1 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. About 20% of all samples analyzed in the lab are reference materials, including reference materials for data normalization, and quality assessment reference materials.

The two types of UWSIF reference materials are:

Quality Control (normalization) reference materials. The quality control reference materials are calibrated with primary standards using a three-point calibration curve. These working reference materials are then used in routine analyses distributed at the beginning, middle and end of each run. Besides providing the normalization curve they also help to keep a check on issues like machine drift, non-linearity related to sample size, and column degradation.

Quality Assessment reference materials. These reference materials provide a check on the two-point normalization curve produced by the normalization reference materials. One sample is placed in the beginning and end of each run. These reference materials have similar matrix to the unknown samples being analyzed for the clients. They also provide a means to check for consistency between runs. The following tables list the international reference materials at UWSIF:

IAEA Reference Materials with known $\delta^{13}C$ delta values (solid)			
NIST code	Name	Substance	$\delta^{13}C_{VPDB}$
RM 8542	IAEA-CH-6	sucrose	-10.45
RM 8543	NBS 18	carbonatite	-5.01
RM 8544	NBS 19	calcite	1.95
RM 8545	LSVEC	lithium carbonate	-46.6

IAEA Reference Materials with known $\delta^{15}N$ delta values (solid)				
NIST code	Name	Substance	$\delta^{15}N_{AIR-N2}$	
RM 8549	IAEA-NO-3	potassium nitrate	4.7	
IAEA Reference Materials with known $\delta^{13}C$ and $\delta^{15}N$ delta values (solid)				Shaded cells not applicable
NIST code	Name	Substance	$\delta^{13}C_{VPDB}$	$\delta^{15}N_{AIR-N2}$
RM 8573	USGS40	glutamic acid	-26.39	-4.52
RM 8574	USGS41	glutamic acid	37.63	47.57
	IAEA-600	caffeine	-27.77	1.0

8.2 Quality Control, Quality Assurance, Quality Assessment

Raw and corrected data are provided to the clients along with analysis data for all reference materials and standards used in each analysis run. That way clients can verify that proper QA/QC procedures were followed. All analysis data are archived electronically using two separate master back-up hard drive systems within the UWSIF for long-term storage and future retrieval. We maintain long-term record of our quality control and quality assessment materials, which serves as an "Early Warning Signal" to detect any minor/major isotopic or sensitivity shifts in our instruments. When possible, we participate in inter-lab comparison exercises to evaluate the robustness of our QA/QC guidelines.

8.3 Long-term reference material data collection procedures

The reduced isotope and elemental composition data (if applicable) for all quality assessment materials in individual runs are collected during the data reduction process. The collected data for each individual quality assessment reference material in an individual analytical run is appended to an existing long-term quality assessment reference material data file. The UWSIF maintains an individual file for each quality assessment reference material. The following table lists UWSIF reference materials used for quality control and quality assessment:

UWSIF Quality Control and Quality Assessment Reference Materials with known $\delta^{13}C$ and $\delta^{15}N$ delta values (solid)			
UWSIF code	Substance	$\delta^{13}C_{VPDB}$	$\delta^{15}N_{AIR-N2}$
36-UWSIF-UT Glut 1	glutamic acid	-28.17	-4.69
39-UWSIF-UW Glut 2	glutamic acid	24.62	27.86
46-UWSIF-Soil3	soil	-23.53	6.12
05-UWSIF-Alfalfa	plant	-27.48	0.19

Section 9 General Maintenance

9.1 Leak Checking an Elemental Analyzer

Place a plug over the Vent-M, release pressure on the helium regulator on the EA (turn black/silver knob counterclockwise).
If there is a leak the pressure on the helium gauge will decrease.
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.
If there is no leak, remove plug on Vent-M and restore helium pressure.

9.2 Building a Combustion Reactor

Materials: Chromium oxide, silvered cobaltous/cobaltic oxide, quartz wool, and one 1.8 cm x 45 cm quartz tube.

Equipment: Funnel, ruler, push rod, and tray.

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.
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 a Kimwipe® and 95% ethanol and set the tube aside.

9.3 Replacing the Quartz Insert

Materials: Prepared quartz insert.

Equipment: Sharpie felt pen and forceps.

1. Turn off the Delta^{Plus} 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 1020ºC.
5. Prepare a new quartz insert by placing 1 cm of quartz wool in the bottom of the tube. Slide the 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^{Plus} XP or Delta V source.

9.4 Building a Reduction Reactor

Materials: Analytical grade pure copper (Cu) at 0.3mm mesh wire, quartz wool, and one 1.8 cm x 45 cm quartz tube.

Equipment: Pliers, funnel, and tray.

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 Cu to the tube gently tamp the tube to compact the Cu to avoid separation when the reactor is heated to 650°C.
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 Kimwipe® and 95% ethanol and set the tube aside.

9.5 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: Analytical grade magnesium perchlorate and quartz wool.

Equipment: Forceps, funnel, spatula, and tray.

1. Turn off the Delta^{Plus} 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. Add 5 to 7mm of quartz wool to one end of a glass tube.
4. 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 7mm of space in order to insert quartz wool into the open end.
5. Add 5 to 7mm of quartz wool to fill the glass tube.
6. 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.
7. Perform a leak check.
8. Turn on the Delta^{Plus} XP or Delta V source.

9.6 Changing Elemental Analyzer Reactors

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

1. Turn the source off before closing the SGE valve. If the valve is closed when the source is on the filament will be blown and the instrument will be down for a considerable amount of time.
2. Turn off the source by clicking on the gray symbol at the top left side of the Isodat screen.
3. Close the mass spectrometer by turning the SGE valve carefully until you see the vacuum pressure in Isodat drop to xxe-009. Do not over tighten.

On the EA instrument control panel:
1. Select stand-by mode button, then enter.
2. Change left oven to 500°C.

Wait for both ovens have reached 500°C or below.

1. Take out the old reactors by unscrewing the bottom and then the top.
2. Insert new reactors and water trap. Make sure all O-rings are still in good shape before reusing. (O-rings should be flexible and cracks should not be visible.)
Make sure the stainless steel reactor braces are secure and put the EA in work mode.

1. Perform a helium leak check to 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.)

Once there are no leaks the left oven temperature can be returned to the original temperature (1020°C).
Once ovens have reached their working temperatures, open the mass spectrometer by turning the SGE valve carefully until you see the vacuum pressure rise to approximately 10^{-6} mbars.
Ensure the vacuum pressure is stable and then turn on the source by clicking on the red sun symbol at the top left of the Isodat screen.
Ensure that the nitrogen background is below 400 mV by performing a jump to mass 28 and a peak center.

9.7 Building Quartz Inserts

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

1. Cut 18 mm id 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. Using the natural gas/oxygen torch, heat the bottom of the insert and collapse the opening to roughly ½ the original diameter. Closing the opening helps keep the contents of the quartz insert within the insert.
4. Heat the completed insert to 850°C.

Section 10 Troubleshooting

10.1 Troubleshooting the Elemental Analyzer

Observation	Potential problem	Solution
High nitrogen blank.	Air leak. Oxygen contaminated.	Leak test the instrument. 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. GC column temperature.	Replace reduction reactor. Decrease temperature.
Peak between nitrogen and carbon peaks.	Oxygen contaminated.	Check the oxygen blank.
Peaks elute late.	Leak in system. Water trap exhausted.	Perform a leak test. Replace the magnesium perchlorate.

10.2 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 mL·min^{-1} and 4 seconds respectively.

Optimizing the oxygen flow rate:
1. Run 1: oxygen flow rate 80 mL·min^{-1}, injection time 4 seconds.
2. Run 2: oxygen flow rate 120 mL·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 mL·min^{-1}, injection time 4 seconds.
2. Run 2: oxygen flow 100 mL·min^{-1}, injection time 13 seconds.
3. Set the oxygen injection time to the lowest level that still allows flash combustion.