

Table 1. Types of samples and SIRFER SOPs

Sample Type	Analyte	Sample Receiving and Handling SOP	Sample prep SOP	Sample Analysis SOP	Data Processing SOP	QC SOP
Algal samples(filters)	Measure concentrations of Total C and N and stable isotope ratios of C, N and S	SIRFER Sample Handling	2.2 Weighing Samples	3.1 CNS-Isolink Instrument method	4.3.1 Isotope data processing IRMS	4.3.1 Isotope data processing IRMS QMA
Surface water particulates (filters)	Measure stable isotope ratios of C and N	SIRFER Sample Handling	2.2 Weighing Samples	3.2 CN-EA solids Instrument Method	4.3.1 Isotope data processing IRMS	4.3.1 Isotope data processing IRMS QMA
Aquatic plants	Measure concentrations of C and N	SIRFER Sample Handling	2.1.1 Grinding samples mortar and pestle 2.2 Weighing samples	3.2 CN-EA solids Instrument Method	4.3.1 Isotope data processing IRMS	4.3.1 Isotope data processing IRMS QMA

S.I.R.F.E.R.	
Stable Isotope Ratio Facility for Environmental Research	
Method for grinding solid samples using a mortar and pestle prior to isotope analysis via CF-IRMS	
Folder Section 2.1.1	Version 1.0
Approved by Steering Committee 12/06/17	

Scope

This method describes the preparation of solid samples as homogeneous powders for isotope ratio analysis via CF-IRMS. Samples are ground using a mortar and pestle. The method applies to easily pulverized samples. Note that some types of samples do not lend themselves to grinding with a mortar and pestle. For example, stems are not easily pulverized. For such samples, the use of a ball mill for grinding is more appropriate.

Training

Users should be trained to use this step-wise method until they are proficient.

Equipment

Mortar and pestle
 Liquid nitrogen (LN₂) and dewar
 Spatula
 Forceps
 Glass vials, typically 1-dram or 3-dram size
 Write-on labeling tape and (optional) clear adhesive tape, to cover label
 Permanent marker
 Laboratory wipes
 95% ethanol
 Non-latex exam gloves (optional)

Method

1. Dry Samples
 - 1.1. Remove all water from samples by drying overnight in an oven (70° C) or freeze-drying.
2. Grind Samples
 - 2.1. Place a dried sample into the mortar using forceps or gloved hands.
 - 2.2. Add enough liquid nitrogen to mortar to just cover the sample.

- 2.3. Allow the liquid nitrogen to evaporate until there is just enough remaining to be visible.
- 2.4. Quickly grind the frozen sample with the pestle. The sample should grind to a very fine powder.
- 2.5. Remove the ground sample from the mortar using the spatula and store in an appropriate container, typically a labeled glass vial.
- 2.6. Clean the mortar, pestle, forceps, and spatula using 95% ethanol and laboratory wipes.

Version number	Date	Created or modified by	Changes implemented
0.1	11/21/17	Lesley Chesson	Creation of method using text from Iso-Camp Lab Manual.
1.0	12/06/17	Lesley Chesson	Addition of Steering Committee approval date and creation of PDF.

S.I.R.F.E.R.	
Stable Isotope Ratio Facility for Environmental Research	
Method for weighing and encapsulating solid samples and reference materials prior to isotope analysis via CF-IRMS	
Folder Section 2.2	Version 1.1
Approved by Steering Committee 12/06/17	

Scope

This method describes the weighing and encapsulation of solid samples and/or reference materials for isotope ratio analysis. Samples/reference materials are carefully weighed into small tin or silver capsules using a microbalance. A microbalance is typically readable to 1 µg and reproducible to ≤ 1 µg. Samples and reference materials are routinely weighed to 1 µg before analysis.

The method applies to dry samples and reference materials that will be analyzed using CF-IRMS. Dry samples and reference materials are typically powdered prior to weighing, although some solid substrates may be weighed “as is” for isotope analysis (e.g., fingernail clippings, hair strands, sampling filters, etc.). See **SOP 2.1.1 Grinding samples_mortar and pestle** and **SOP 2.1.2 Grinding samples_ball mill** for instructions on powdering samples, if required.

For ¹³C, ¹⁵N, and/or ³⁴S analysis, a 0.2 – 20 mg subsample is accurately weighed (0.001 mg) into a tin capsule, which is then carefully closed to prevent loss of material. If weighed capsules are not analyzed immediately, they are placed into a dry storage cabinet.

For ²H and/or ¹⁸O analysis, a 0.10 – 0.15 mg subsample is accurately weighed (0.001 mg) into a silver capsule, which is then carefully closed to prevent loss of material. If weighed capsules are not analyzed immediately, they are placed into a vacuum storage cabinet.

Training

Users should be trained to use this step-wise method until they are proficient.

Equipment

Microbalance
Glass plate
Small paintbrush
Forceps – flat and angled tip
Spatula or small scoops
Small (typically 3.5×5 mm) pressed tin or silver capsules
Plastic 96-well plate
70% ethanol
Laboratory wipes
Non-latex exam gloves (optional)

Method

1. Using the angled forceps, place an empty capsule onto the microbalance pan. Close draft shield and tare the balance.
2. Remove the capsule from the tared microbalance and place it onto the (clean) glass plate.
3. Add dry, powdered sample or reference material with a small spatula or scoop to the desired mass. Alternatively, use angled or flat forceps to carefully add sample pieces to the desired mass.
 - 3.1. Check mass often to determine if more or less material should be added to capsule.
 - 3.2. If powdered material needs to be removed, hold capsule with angled forceps, tip gently, and tap the “crook” of the forceps lightly on glass plate to dislodge powder. Be sure to turn capsule upright and tap lightly onto glass plate to resettle powder.
 - 3.3. If sample pieces need to be removed, use angled or flat forceps to carefully remove material, piece by piece. A clean razor blade can also be used to carefully trim sample pieces to remove mass.
4. Put the filled capsule back onto the microbalance pan and close draft shield.
5. Record the final mass of the weighed material to the nearest $1 \mu\text{g}$ (0.001 mg).
6. Open the draft shield and remove the weighed, filled capsule; place it onto the (clean) glass plate.
7. With flat forceps, pinch closed the top third of the weighed, filled capsule.
8. Fold the capsule over to close the top. Fold over twice, if possible.
9. Using the angled forceps, push the folded capsule off the flat forceps and continue to collapse and fold the capsule to form a small, dense cube or sphere.
10. Place the encapsulated sample or reference material into the 96-well tray. If some material may have been lost during encapsulation, check mass folded capsule before placing into tray.
11. Clean the forceps, spatula/scoop, and glass plate using 70% ethanol and laboratory wipes between each sample or reference material.

12. If any material is spilled onto microbalance pan, use a clean paintbrush to gently sweep away material.

Notes on tray setup

Trays of samples and reference materials prepared for isotope analysis via CF-IRMS should include *at a minimum*:

- Laboratory reference materials (LRMs) in sets of three – including two primary LRMs, for data normalization, and one secondary LRM, for QA/QC checks, per set.
- One internal laboratory reference materials per 4 samples
- At least three total sets of laboratory reference materials, regardless of total number of samples
- One blank (empty) filter, if samples were collected on a filtering substrate

Version number	Date	Created or modified by	Changes implemented
0.1	11/21/17	Lesley Chesson	Creation of method using text from Iso-Camp Lab Manual.
0.2	12/05/17	Lori Ann Long	Corrected ethanol concentration from 95% to 70%.
1.0	12/06/17	Lesley Chesson	Addition of Steering Committee approval date and creation of PDF.
1.1	12/19/17	Lesley Chesson	Generalization to describe weighing of samples <i>and</i> reference materials.

S.I.R.F.E.R.	
Stable Isotope Ratio Facility for Environmental Research	
Method for analysis of C+N in solids via EA-IRMS Instrument: Mad-Eye Moody or Saltbush Bill	
Folder Section 3.1	Version 1.0
Approved by Steering Committee 12/06/17	

Scope

This method describes the measurement of carbon (C) and nitrogen (N) isotope ratios of organic and inorganic solid materials using an isotope ratio mass spectrometer (IRMS) coupled to an elemental analyzer (EA), operated in continuous flow (CF) mode. The method applies to the instruments known as Mad-Eye Moody and Saltbush Bill.

Materials analyzed using this method must be accurately weighed and encapsulated into tin capsules. Note that additional methods are required prior to this method, including sample preparation and sample weighing. Data generated from this analysis method should be corrected using a data reduction method.

Training

Operators should be trained and proficient with daily maintenance procedures (i.e., leak checking, insert changing) and the Isodat software. Operators should be trained to use this step-wise method until they are proficient. A quick-start checklist is provided as an appendix to this method as a guide for trained users; however, the checklist is not a substitute for this method.

Instrumentation

Finnigan Mat Delta+ Advantage or Finnigan Mat Delta+ IRMS
Carlo Erba CHN EA1110
ThermoFinnigan Conflo III

Method

1. Verify Instrument Set Up
 - 1.1. Check the IRMS front panel for source and pump lights. All should be green, indicating the source and pumps are on.
 - 1.2. Verify the O₂ pressure using the marked gauge on the front of the EA.
 - 1.3. Open the Acquisition software and then open the appropriate sequence table (e.g., i.e. "N₂_CO₂ +He_1.seq").

- 1.4. Open the Instrument Control software. Select the EA-Conflo configuration and N₂ as the gas type from the dropdown menus at the bottom of the screen.
 - 1.5. Begin a scan on N₂ backgrounds (masses 28, 29, and 30). Record initial voltages and time in the instrument start checklist.
 - 1.5.1. If signal is not present, confirm source and pumps lights are illuminated, close Isodat software, restart the computer, and begin scan.
 - 1.6. Check the combustion, reduction, and GC temperatures on the EA. Record temperatures in the instrument start checklist.
 - 1.7. Open the front panel of the EA and verify that helium flow is set to the black mark on the gauge.
 - 1.8. Check and record the gas tank pressures for the helium tanks as well as the reference gas tanks in the instrument start checklist.
 - 1.8.1. The following five gas tanks must have >400 psi before beginning an analytical sequence. If tanks are low, inform facility manager:
 - Helium Primary (1°) and Secondary (2°) (He inert carrier gas tanks)
 - Oxygen (O₂ combustion aid)
 - Nitrogen (N₂ reference wall gas tank)
 - Carbon Dioxide (CO₂ reference wall gas tank)
 - 1.9. Review and fill out the instrument eLog (.xls files) accessible from the computer desktop. Record the Job Number, analyst, and the number of samples to be analyzed.
 - 1.9.1. Use the eLog to determine whether the oxidation column insert needs to be changed.
 - 1.9.2. Oxidation column inserts are changed following a soil run or following analysis of 100 organic samples.
 - 1.9.3. If the insert should be changed, proceed to Step 2. Otherwise, proceed to Step 3.
 - 1.9.4. Save eLog file and then close Excel.
 - 1.10. Close isolation valve between autosampler and oxidation column.
2. Replace Insert (as needed)
- Smaller diameter quartz inserts protect the oxidation column against samples that attack and weaken the quartz glass. They can be cheaply and quickly changed to preserve the life of an oxidation column.
- 2.1. Turn off the source and then close the mass spectrometer (MS) valve finger tight (approximately 3 turns).
 - 2.2. Loosen and remove the autosampler from the oxidation column. A wrench may be necessary because the oxidation column is hot.
 - 2.3. Use the end of a larger Sharpie marker to remove the old insert while wearing oven mitts. Transfer the 1000 °C insert to an asbestos cooling pad. Note whether there was any quartz wool stuck to the bottom of the old insert.
 - 2.4. Pack a new insert with a small piece of quartz wool (*not glass wool*). If quartz wool was stuck to the bottom of the old insert, also add more directly to combustion column.
 - 2.5. If insert has been pre-cut to the correct size, carefully lower the new insert into combustion column, then reattach the autosampler.

- 2.6. If insert has not been pre-cut to the correct size:
 - 2.6.1. Carefully lower the new insert into the combustion column. Use a Sharpie marker to mark the top of oxidation column then quickly remove the insert.
 - 2.6.2. Use as glasscutter to score the insert below the Sharpie mark by a few millimeters. Caution – insert is hot! Once cut, the insert should sit **BELOW** the lip of the oxidation column.
 - 2.6.3. Carefully lower the cut insert into the combustion column, then reattach the autosampler.
 - 2.7. Check for He leaks using the leak detector:
 - 2.7.1. Turn on leak detector and remove wand from sleeve.
 - 2.7.2. Allow leak detector to auto “zero.” When lights have stopped moving and one green light appears, leak detector is ready.
 - 2.7.3. Move wand over autosampler connection and monitor for helium leaks. Make any necessary corrections/adjustments and verify the autosampler/oxidation column connection is leak-free before proceeding.
 - 2.8. Open MS isolation valve 3 turns. Wait 10 minutes (load samples while waiting; see Step 3) before turning on source.
3. Load Samples
 - 3.1. With autosampler isolation valve **closed**, open vacuum valve for 10-15 seconds to evacuate autosampler.
 - 3.2. Close vacuum, then loosen and flip open screw clamps securing autosampler lid.
 - 3.3. Fill autosampler with helium to “pop” open autosampler lid. Once lid is open, verify there are no samples from previous analytical sequence in wells.
 - 3.4. Check carousel size (i.e., 50 or 100-position carousel). Change carousel, if necessary, depending on sample tray size.
 - 3.5. Verify the appropriate carousel size has been selected on autosampler control box. Use toggle switch to select correct carousel size.
 - 3.6. Check alignment of carousel opening over oxidation column by manually advancing carousel one position using control box.
 - 3.6.1. Adjust alignment as needed using the “fine movement” toggle located on the back of the autosampler control box.
 - 3.6.2. Carousel alignment should be adjusted so that one advance sweeps past the oxidation column opening and just into the next sample well. This helps avoid sample hang-ups.
 - 3.7. Advance carousel so that position 50 (or 100) is over the oxidation column.
 - 3.8. Load samples from tray into the carousel in the order detailed by weigh sheet. One sample should be loaded into each well.
 - 3.9. Close the autosampler lid. Secure lid with screw clamps.
 - 3.10. Verify the auto-sampler valve is still **closed** and then open the vacuum valve. Tighten lid screw clamps again.
 - 3.11. Close vacuum valve, fill autosampler with helium to marked pressure on gauge, then open vacuum valve again and leave open for a minimum of 5 minutes. (Enter sample information into analytical sequence table while waiting; see Step 4).

- 3.12. After 5 minutes, close vacuum valve.
 - 3.13. Fill autosampler with helium to marked pressure on gauge.
 - 3.14. Open autosampler isolation valve. If necessary, turn on source.
 - 3.15. Continue monitoring N₂ backgrounds (masses 28, 29, and 30) for at least 200 seconds after autosampler isolation valve has been opened before proceeding.
4. Enter Sample Information
 - 4.1. Enter sample identifiers into Identifier 1 column of analytical sequence table.
 - 4.2. Note which wells hold reference materials by typing “RM” into Identifier 2 column of analytical sequence table.
 - 4.3. Enter sample masses into Amount column of analytical sequence table.
 - 4.4. Verify the correct analytical method is selected for each line of the analytical sequence table (i.e., “fake” method for wall gas source conditioners, dual gas method for samples).
 - 4.4.1. Tip – Sometimes the Isodat Acquisition software is buggy. If you notice that the analytical sequence table has several grid boxes shaded (selected) with navy blue fill and you are unable to “deselect” them, try this trick: Click on line 1 of the sequence table. Right click and select “insert row above.” Highlight the new row, right click, and delete it. Cells in the sequence table should now be modifiable.
 - 4.5. Save analytical sequence table.
5. Check Instrument Configuration
 - 5.1. Once N₂ backgrounds are flat (i.e., not changing) or slowly decreasing, stop scan in Instrument Control software. Note background voltages and time in instrument start checklist.
 - 5.2. Turn on N₂ gas (Reference 2) and then peak center by selecting the center scan button in the top left corner of the screen.
 - 5.3. Record peak center value and the mass 28 voltage in the instrument start checklist.
 - 5.4. Turn off N₂ gas (Reference 2) and turn on CO₂ gas (Reference 1).
 - 5.5. Check jump from N₂ to CO₂.
 - 5.5.1. Click on the “Jump” button.
 - 5.5.2. Highlight line 1 (N₂ to CO₂) and click on the green check mark.
 - 5.5.3. Click “yes” when asked “Are you sure?” and confirm that CO₂ reference gas (Reference 1) is on.
 - 5.5.4. Record the previous jump value and the new jump value.
 - 5.5.5. If new jump value is the same as previous value, save and continue.
 - 5.5.6. If new jump value is not the same as previous value, switch to N₂ gas configuration and then repeat jump check until the same jump calibration is returned twice. Save and record final jump value in instrument start checklist.
 - 5.6. Peak center on CO₂ by selecting the center scan button in the top left corner of the screen.
 - 5.7. Record peak center value and the mass 44 voltage in the instrument start checklist.

- 5.8. Turn off CO₂ reference gas, then switch back to N₂ gas configuration. Verify that background voltages for masses 28, 29, and 30 are still low.
 - 5.9. Close Instrument Control software. Leave only Acquisition software open.
6. Start Instrument
 - 6.1. Verify that autosampler isolation valve is **open**.
 - 6.2. If necessary, manually advance carousel using autosampler control box so that Well 1 (or the first well with a sample) will be the first well analyzed after instrument start.
 - 6.3. In the Acquisition software, use the mouse to highlight the rows in the analytical sequence table that will be analyzed.
 - 6.3.1. As you do this, verify that the green peak center “check” mark is present for every line.
 - 6.3.2. Verify again that the “fake” analysis method is only selected for wall gas Source Conditioners.
 - 6.4. Select “Start” to begin acquisition.
 - 6.5. Enter job number for File name. Re-enter job number as Folder name.
 - 6.6. Verify that results will be exported as .xls file.
 - 6.7. Locate waving flag and verify check mark for “Measure Only Selection” (i.e., the lines you highlighted) then select OK.
 - 6.8. The first sample analyzed is an EA conditioner that should be used to check the “timing” of the acquisition.
 - 6.8.1. Verify the sample drops from the autosampler into the oxidation column and that flash combustion occurs.
 - 6.8.2. Verify that the Acquisition software records peaks for this sample.
 - 6.8.3. Following the timing sample, wall gas samples will run for the next 2 hours. Thus, this is your last opportunity to identify problems before walking away from the instrument.
7. Complete Post-Acquisition Checks
 - 7.1. Open Work Space software.
 - 7.1.1. Click on “Results” tab.
 - 7.1.2. Find your Job Number and double click.
 - 7.2. You will see a chromatogram for every acquisition (i.e., for every line in the analytical sequence table).
 - 7.2.1. Double click to open each chromatogram and verify the peaks look as expected.
 - 7.2.2. Make notes for any sample acquisition that was unusual (no peak, very large peak, etc.).
 - 7.3. Check the autosampler carousel and verify that every capsule dropped.
 - 7.4. Oxidation columns must be re-built after approximately 600 samples; note how many samples are on the current column and then schedule a column rebuild as needed.

Appendices

I. EA-IRMS Background for Carbon and Nitrogen Isotope Ratio Analysis

Biological and soil samples are analyzed for carbon and nitrogen isotopes using continuous-flow Isotope Ratio Mass Spectrometry (CF-IRMS). Here, samples are analyzed using an elemental analyzer (EA) coupled to an IRMS through an open split interface. A weighed sample is combusted in the presence of O₂ forming H₂O, CO₂, and N₂ gases that are pushed along in a helium (He) stream. Halogen or sulfur-containing gases are chemically removed and incomplete combustion products (i.e., CO_x, CO, etc.) are reduced, followed by the removal of excess O₂ and all water. The final product gasses, N₂ and CO₂, are separated on a gas chromatography column before entering the IRMS. Samples are analyzed alongside pure gases (Ultra High Purity UHP N₂ and CO₂) for calibration purposes.

II. Instrument start checklist

<p>No. Action</p> <p><input type="checkbox"/> 1 Open sequence table in Acquisition.</p> <p><input type="checkbox"/> 2 Start scan and record N2 backgrounds (masses 28 and 29).</p> <p><input type="checkbox"/> 3 Record gas tank pressures and column temperatures.</p> <p><input type="checkbox"/> 4 Close autosampler isolation valve.</p> <p><input type="checkbox"/> 5 Fill out e-log. New insert required? Follow steps 6-13. Insert ok? Skip to step 14.</p> <p><input type="checkbox"/> 6 Turn off source.</p> <p><input type="checkbox"/> 7 Close mass spec valve.</p> <p><input type="checkbox"/> 8 Remove autosampler from oxidation column.</p> <p><input type="checkbox"/> 9 Replace insert.</p> <p><input type="checkbox"/> 10 Replace autosampler and check for leaks with gas detector.</p> <p><input type="checkbox"/> 11 Open mass spec valve and wait 10 minutes.</p> <p><input type="checkbox"/> 12 While waiting, enter sample information into Acquisition.</p> <p><input type="checkbox"/> 13 After 10 minutes, turn on source.</p> <p><input type="checkbox"/> 14 Open vacuum valve to evacuate autosampler with isolation valve closed.</p> <p><input type="checkbox"/> 15 Close vacuum valve, then loosen clamps and backfill autosampler with He.</p> <p><input type="checkbox"/> 16 Open autosampler lid and load samples.</p> <p><input type="checkbox"/> 17 Close autosampler lid and evacuate autosampler; tighten clamps.</p> <p><input type="checkbox"/> 18 Close vacuum valve, fill autosampler with He, then open vacuum valve and leave open.</p> <p><input type="checkbox"/> 19 Enter sample info into Acquisition or begin correcting data from previous run.</p> <p><input type="checkbox"/> 20 After 5 minutes, close vacuum valve and fill autosampler with He.</p> <p><input type="checkbox"/> 21 Open autosampler isolation valve.</p> <p><input type="checkbox"/> 22 Monitor backgrounds for at least 200 seconds.</p> <p><input type="checkbox"/> 23 Once backgrounds are flat, stop scan. Record background values.</p> <p><input type="checkbox"/> 24 Turn on N2 reference gas and peak center. Record value.</p> <p><input type="checkbox"/> 25 Turn off N2 reference gas and turn on CO2 reference gas.</p> <p><input type="checkbox"/> 26 Check jump from N2 to CO2. If value is the same as previous, save. If value is different from previous, do not save. Repeat steps 24-26.</p> <p><input type="checkbox"/> 27 Record saved jump value in sequence.</p> <p><input type="checkbox"/> 28 Peak center on CO2 reference gas. Record value.</p> <p><input type="checkbox"/> 29 Switch to N2 and check backgrounds again.</p> <p><input type="checkbox"/> 30 Verify that autosampler isolation valve is open.</p> <p><input type="checkbox"/> 31 Close all programs and windows except Acquisition.</p> <p><input type="checkbox"/> 32 Highlight rows and start sequence.</p> <p><input type="checkbox"/> 33 Watch first timing sample to verify all peaks are present.</p>	<p>Date</p> <div style="border: 1px solid black; padding: 2px;"> <p>Job #</p> <p>Analyst</p> <p>Instrument: SBB</p> </div> <div style="border: 1px solid black; padding: 2px; margin-top: 10px;"> <p>Helium tanks</p> <p>Primary:</p> <p>Secondary:</p> <p>N2 tank</p> <p>CO2 tank</p> <p>Ox. Temp</p> <p>Red. Temp</p> <p>GC Temp</p> </div> <p><u>Jump check</u></p> <p>Previous:</p> <p>New:</p>
------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

Peak center		Backgrounds	
mV	Peak return	Mass	@
N ₂		28	@
CO ₂		29	@

START TIME

Comments:

Version number	Date	Created or modified by	Changes implemented
0.1	08/29/13	Janet Barnette	Creation of initial document based on training in August 2013 and SIRFER 2008 manual
0.2	11/27/13	Lesley Chesson	Revision of method to parallel instrument start checklist.
0.3	12/11/13	Lesley Chesson	Addition of steps related to O ₂ valve and monitoring blanks.
0.4	11/07/17	Lesley Chesson	Revision of method to align with formatting of HO-TCEA_water method
0.5	12/05/17	Lori Ann Long & Lesley Chesson	Revision of text to address the use of pre-cut inserts.
1.0	12/07/17	Lesley Chesson	Addition of Steering Committee approval date and creation of PDF.

S.I.R.F.E.R.	
Stable Isotope Ratio Facility for Environmental Research	
Method for analysis of NCS in solids via EA-IsoLink Instrument: Delta V Plus	
Folder Section 3.2	Version 1.0
Approved by Steering Committee 12/06/17	

Scope

This method describes the measurement of nitrogen (N), carbon (C), and/or sulfur (s) isotope ratios of organic and inorganic solid materials using an isotope ratio mass spectrometer (IRMS) coupled to an elemental analyzer (EA)—specifically, the EA IsoLink™ manufactured by Thermo Scientific—operated in continuous flow (CF) mode. The method applies to the unnamed Delta V Plus instrument.

Materials analyzed using this method must be accurately weighed and encapsulated into tin capsules. Note that additional methods are required prior to this method, including sample preparation and sample weighing. Data generated from this analysis method should be corrected using a data reduction method.

Training

Operators should be trained and proficient with daily maintenance procedures (i.e., leak checking, insert changing) and the Isodat software. Operators should be trained to use this step-wise method until they are proficient.

Instrumentation

Delta V Plus isotope ratio mass spectrometer
ConFlo IV
Flash IsoLink™

Method

1. Verify Instrument Set Up
 - 1.1. Check the IRMS front panel for source and pump lights. All should be green, indicating the source and pumps are on.
 - 1.2. Open the Acquisition software and then open the appropriate sequence table (e.g., “CNS1 June 2017.seq”).
 - 1.3. Open the Instrument Control software. Select the EA IsoLink configuration and N₂ as the gas type from the dropdown menus at the bottom of the screen.

- 1.4. Begin a scan on N₂ backgrounds (masses 28, 29, and 30). Record initial voltages and time in the instrument start checklist.
 - 1.4.1. If signal is not present, confirm source and pumps lights are illuminated, close Isodat software, restart the computer, and begin scan.
 - 1.5. Right click in the Flash IRMS window on the left side of the screen, then select "Flash Status." (Click "no" to prevent saving changes.) Record information about the EA status – i.e., column temperatures, etc.
 - 1.6. Check and record the gas tank pressures for the helium tanks as well as the reference gas tanks in the instrument start checklist.
 - 1.6.1. The following five gas tanks must have >400 psi before beginning an analytical sequence. *Note*: due to the unavoidable degradation of the tank regulator, it may be impossible to determine the status of the sulfur dioxide (SO₂) reference wall gas tank. If any of the other tanks are low, inform facility manager:
 - Helium Primary (1°) and Secondary (2°) (He inert carrier gas tanks)
 - Oxygen (O₂ combustion aid)
 - Nitrogen (N₂ reference wall gas tank)
 - Carbon Dioxide (CO₂ reference wall gas tank)
 - 1.7. Review and fill out the instrument eLog (.xls files) accessible from the computer desktop. Record the Job Number, analyst, and the number of samples to be analyzed.
 - 1.8. Close isolation valve between autosampler and oxidation column.
2. Replace Insert
- Smaller diameter quartz inserts protect the oxidation column against samples that attack and weaken the quartz glass. They can be cheaply and quickly changed to preserve the life of an oxidation column. The insert should be changed before every "run" on the instrument.
- 2.1. In the ConFlo IV Diagnosis window on the left side of the screen, raise ("close") the high-flow capillary, HF Cap, by clicking the green button once.
 - 2.2. Loosen and remove the autosampler from the oxidation column. A wrench may be necessary because the oxidation column is hot.
 - 2.3. Use the end of a larger Sharpie marker to remove the old insert while wearing oven mitts. Transfer the 1000 °C insert to an asbestos cooling pad. Note whether there was any quartz wool stuck to the bottom of the old insert.
 - 2.4. Pack a new insert with a small piece of quartz wool (*not glass wool*). If quartz wool was stuck to the bottom of the old insert, also add more directly to combustion column.
 - 2.5. If insert has been pre-cut to the correct size, carefully lower the new insert into combustion column, then reattach the autosampler.
 - 2.6. If insert has not been pre-cut to the correct size:
 - 2.6.1. Carefully lower the new insert into the combustion column. Use a Sharpie marker to mark the top of oxidation column then quickly remove the insert.
 - 2.6.2. Use a glasscutter to score the insert below the Sharpie mark by a few millimeters. Caution – insert is hot! Once cut, the insert should sit **BELOW** the lip of the oxidation column.

- 2.6.3. Carefully lower the cut insert into the combustion column, and then reattach the autosampler.
 - 2.7. Check for He leaks using the leak detector:
 - 2.7.1. Turn on leak detector and remove wand from sleeve.
 - 2.7.2. Allow leak detector to auto “zero.” When lights have stopped moving and one green light appears, leak detector is ready.
 - 2.7.3. Move wand over autosampler connection and monitor for helium leaks. Make any necessary corrections/adjustments and verify the autosampler/oxidation column connection is leak-free before proceeding.
 - 2.8. Using the “FLASH IRMS Display Method” window, increase flow rate from standby status of 10 ml/min to XXX ml/min. Wait at least 10 minutes (load samples while waiting; see Step 3) before opening HF Cap.
3. Load Samples
- 3.1. With autosampler isolation valve **closed**, loosen center vent valve on autosampler lid until pressure is released; leave vent valve loosened.
 - 3.2. Loosen and flip open screw clamps securing autosampler lid.
 - 3.3. Fill autosampler with helium to “pop” open autosampler lid. Once lid is open, verify there are no samples from previous analytical sequence in wells.
 - 3.4. Check carousel size (i.e., 50 or 100-position carousel). Change carousel, if necessary, depending on sample tray size.
 - 3.5. Verify the appropriate carousel size has been selected on autosampler control box. Use toggle switch to select correct carousel size.
 - 3.6. Check alignment of carousel opening over oxidation column by manually advancing carousel one position using control box.
 - 3.6.1. Adjust alignment as needed using the “fine movement” toggle located on the back of the autosampler control box.
 - 3.6.2. Carousel alignment should be adjusted so that one advance sweeps past the oxidation column opening and just into the next sample well. This helps avoid sample hang-ups.
 - 3.7. Advance carousel so that position 50 (or 100) is over the oxidation column.
 - 3.8. Load samples from tray into the carousel in the order detailed by weigh sheet. One sample should be loaded into each well.
 - 3.9. Close the autosampler lid. Secure lid with screw clamps.
 - 3.10. Verify the autosampler valve is still **closed** and autosampler vent valve is still **loosened** then open the helium valve. Confirm that helium is flowing out of autosampler vent valve – you should hear/feel the flow.
 - 3.11. Allow helium to flush autosampler for a minimum of 5 minutes. (Enter sample information into analytical sequence table while waiting; see Step 4).
 - 3.12. After 5 minutes, close autosampler vent valve until finger tight. Do not over-tighten, as this may damage the o-ring.
 - 3.13. Count to 5, then shutoff the flow of helium to the autosampler.
 - 3.14. Open autosampler isolation valve.
 - 3.15. If not already done, click the HF Cap button in the ConFlo IV Diagnosis window once more to lower (“open”) high-flow capillary.

- 3.16. Continue monitoring N₂ backgrounds (masses 28, 29, and 30) for at least 200 seconds after autosampler isolation valve and HF Cap have been opened before proceeding. Backgrounds *will* rise with the autosampler connected to the IRMS and HF Cap open, but they should reach a steady, reasonably low plateau.
4. Enter Sample Information
 - 4.1. Enter sample identifiers into Identifier 1 column of analytical sequence table.
 - 4.2. Note which wells hold reference materials by typing “RM” into Identifier 2 column of analytical sequence table.
 - 4.3. Enter sample masses into Amount column of analytical sequence table.
 - 4.4. Under EA Method column, enter “CNS EA isolink BovineM.eam” for each line of the analytical sequence table.
 - 4.5. Verify the correct analytical method is selected for each line of the analytical sequence table (i.e., “fake” method for wall gas source conditioners, dual gas method for samples).
 - 4.5.1. Tip – Sometimes the Isodat Acquisition software is buggy. If you notice that the analytical sequence table has several grid boxes shaded (selected) with navy blue fill and you are unable to “deselect” them, try this trick: Click on line 1 of the sequence table. Right click and select “insert row above.” Highlight the new row, right click, and delete it. Cells in the sequence table should now be modifiable.
 - 4.6. Save analytical sequence table.
5. Check Instrument Configuration
 - 5.1. Once N₂ backgrounds are flat (i.e., not changing) or slowly decreasing, stop scan in Instrument Control software. Note background voltages and time in instrument start checklist.
 - 5.2. Turn on N₂ gas (Reference 1) and then peak center by selecting the center scan button in the top left corner of the screen.
 - 5.3. Record peak center value and the mass 28 voltage in the instrument start checklist.
 - 5.4. Turn off N₂ gas (Reference 1) and turn on CO₂ gas (Reference 2).
 - 5.5. Check jump from N₂ to CO₂.
 - 5.5.1. Click on the “Jump” button.
 - 5.5.2. Highlight line 1 (N₂ to CO₂) and click on the green check mark.
 - 5.5.3. Click “yes” when asked “Are you sure?” and confirm that CO₂ reference gas (Reference 2) is on.
 - 5.5.4. Record the previous jump value and the new jump value.
 - 5.5.5. If new jump value is the same as previous value, save and continue.
 - 5.5.6. If new jump value is not the same as previous value, switch to N₂ gas configuration and then repeat jump check until the same jump calibration is returned twice. Save and record final jump value in instrument start checklist.
 - 5.6. Peak center on CO₂ by selecting the center scan button in the top left corner of the screen.

- 5.7. Record peak center value and the mass 44 voltage in the instrument start checklist.
 - 5.8. Turn off CO₂ reference gas, then switch back to N₂ gas configuration. Verify that background voltages for masses 28, 29, and 30 are still low.
 - 5.9. Close Instrument Control software. Leave only Acquisition software open.
6. Start Instrument
 - 6.1. Verify that autosampler isolation valve is **open**.
 - 6.2. If necessary, manually advance carousel using autosampler control box so that Well 1 (or the first well with a sample) will be the first well analyzed after instrument start.
 - 6.3. In the Acquisition software, use the mouse to highlight the rows in the analytical sequence table that will be analyzed.
 - 6.3.1. As you do this, verify that the green peak center “check” mark is present for every line.
 - 6.3.2. Verify again that the “fake” analysis method is only selected for wall gas Source Conditioners.
 - 6.4. Select “Start” to begin acquisition.
 - 6.5. Enter job number for File name. Re-enter job number as Folder name.
 - 6.6. Verify that results will be exported as .xls file.
 - 6.7. Locate waving flag and verify check mark for “Measure Only Selection” (i.e., the lines you highlighted) then select OK.
 - 6.8. The first sample analyzed is an EA conditioner that should be used to check the “timing” of the acquisition.
 - 6.8.1. Verify the sample drops from the autosampler into the oxidation column and that flash combustion occurs.
 - 6.8.2. Verify that the Acquisition software records peaks for this sample.
 - 6.8.3. Following the timing sample, wall gas samples will run for the next 2 hours. Thus, this is your last opportunity to identify problems before walking away from the instrument.
7. Complete Post-Acquisition Checks
 - 7.1. Open Work Space software.
 - 7.1.1. Click on “Results” tab.
 - 7.1.2. Find your Job Number and double click.
 - 7.2. You will see a chromatogram for every acquisition (i.e., for every line in the analytical sequence table).
 - 7.2.1. Double click to open each chromatogram and verify the peaks look as expected.
 - 7.2.2. Make notes for any sample acquisition that was unusual (no peak, very large peak, etc.).
 - 7.3. Check the autosampler carousel and verify that every capsule dropped.
 - 7.4. Oxidation columns must be re-built after approximately 600 samples; note how many samples are on the current column and then schedule a column rebuild as needed.

Version number	Date	Created or modified by	Changes implemented
0.1	11/15/17	Lesley Chesson	Creation of initial method from CN-EA_solids method
0.2	12/05/17	Lori Ann Long, Suvankar Chakraborty, & Lesley Chesson	Revision of text to address the use of pre-cut inserts; addition of sequence and analysis method details.
1.0	12/07/17	Lesley Chesson	Addition of Steering Committee approval date and creation of PDF.

S.I.R.F.E.R.	
Stable Isotope Ratio Facility for Environmental Research	
Method for processing “raw” isotope ratio data generated during CF-IRMS analysis	
Folder Section 4.3.1	Version 1.0
Approved by Steering Committee 12/06/17	

Scope

This method describes the processing of “raw” data collected during isotope ratio analysis of samples via CF-IRMS. The method applies to all CF-IRMS instruments running the Isodat software.

Method

1. Collect Data from Instrument
 - 1.1. After a run, find the shortcut folder on the computer desktop containing results from the analysis method used.
 - 1.2. Find your Job Number and double click.
 - 1.3. Move the “raw” data file in the Excel folder to a flash drive for transfer to your computer.

2. Process Data using LIMS
 - 2.1. Save a copy of the “raw” Excel data file from the flash drive to your computer. Save another copy of the “raw” data to Working Files.
 - 2.2. Open CHNOS.xlt file. This is an Excel template that uses a visual basic macro script to process the “raw” IRMS data.
 - 2.3. Select the correct mode (e.g., “Gasbench,” “water,” or “solids”) on the Template Gas 1 worksheet.
 - 2.4. Copy and paste the sample data to first gas and second gas spreadsheets, as appropriate
 - 2.4.1. For EA-IRMS analysis, N is the first gas while C is the second gas. For IsoLink-IRMS analysis, the template will need to be used twice – once with N as the first gas, and once with S as the first gas; C is the second gas.
 - 2.4.2. For TC/EA-IRMS analysis, H is the first gas while O is the second gas.
 - 2.4.3. For GasBench-IRMS analysis, O is the first gas while C is the second gas.
 - 2.5. Check for missing lines of data (i.e., missing peaks) in both gas spreadsheets.
 - 2.5.1. Missing lines of data are almost always sample gas, which may indicate “blanks” or skipped samples.
 - 2.5.2. Consult notes made while reviewing chromatograms at the end of a run for help identifying missing lines of data.
 - 2.5.3. Insert lines for missing peaks and label with correct peak number.

- 2.5.4. Adjust peak numbers of other lines of data for that sample acquisition as needed so reference gas and sample gas peaks are labeled properly.
- 2.6. Sort the first gas worksheet to separate lines of reference and sample gas data.
 - 2.6.1. Sort so that reference gas data are sorted to the top of the worksheet.
 - 2.6.2. For gases with more than one reference peak, delete lines with “dummy” reference gas data (e.g., for N data, peak 1 = dummy, peak 2 = reference). “Is Ref” should be marked with “1” for reference gas peaks.
 - 2.6.3. Insert five blank rows between reference gas data and the sample gas data.
- 2.7. Review missing lines of sample gas data and the peak areas for all other lines of sample gas data to identify any issues.
 - 2.7.1. Add short labels to “Identifier 2” column to note likely issues – e.g., “skipped sample,” “small peak area,” “large peak area,” “double drop,” etc.
- 2.8. Repeat steps 2.5 and 2.6 to sort and review data in the second gas worksheet.
- 2.9. Following the instructions in the Template Gas worksheets, process the data.
 - 2.9.1. Indicate the start and end row (line) of sample gas data; this should be the same for Gas 1 and Gas 2 if data have been sorted properly.
 - 2.9.2. Corrections that may be electively applied to sample gas data include memory, drift (time), and area. Check for memory correction using values between 0.01 and 0.04 to lower overall RM standard deviation, is possible. Do not apply a memory correction >4%. Use the R² values and the slope directions of the RMs to determine whether drift and area corrections should be applied.
 - 2.9.3. Data normalization using two points (“slope-intercept” or “shift-stretch” correction) must always be applied to the sample gas data. PLRMs included in the run are used for this correction.
3. Examine Quality Control and Final Sample Data
 - 3.1. Quality control criteria for the run are stored in Excel template. If any of the quality metrics have been violated, as indicated by the ‘Ignore_Run’ flag in “Summary” worksheet, troubleshooting will be necessary to determine root cause.
 - 3.1.1. In this case, all of the samples should be reanalyzed.
 - 3.1.2. Examine the memory correction terms; the raw and corrected values for the PLRMs; and the raw and corrected values for the SLRMs to determine what may have caused the quality parameters to be violated. Correct for this before analyzing samples again.
 - 3.2. Raw data for any individual samples that have violated the quality parameters, as indicated by an “Ignore” flag, should be carefully reviewed.
 - 3.2.1. For samples analyzed via GasBench-IRMS, check for outlier(s) in the 5 injections (> 3 SD different from the other injections in terms of gas concentration or measured δ -value). Results from outlier injection(s) can be deleted from the data file. No more than 2 injections can be deleted for any given sample. If there is no clear outlier(s) in the injections or there are more than 4 lines of data to be deleted, the sample should be flagged.
 - 3.2.2. Flagged samples should be reanalyzed.
 - 3.3. Submit corrected, final sample data for review by another analyst or the Facility

Manager.

- 3.4. Save a copy of the corrected, reviewed data file on Working Files.
- 3.5. Import final SLRM data into the database for long-term QC monitoring.
- 3.6. Use corrected, reviewed data file to generate data report for customer.

Version number	Date	Created or modified by	Changes implemented
0.1	10/18/17	Lesley Chesson	Creation of method using text from SIRFER.
0.2	12/05/17	Suvankar Chakraborty	Updates to some IsoLink-IRMS and Gasbench-IRMS directions.
1.0	12/07/17	Lesley Chesson	Addition of Steering Committee approval date and creation of PDF.