



# **Determination of the $\delta^{15}\text{N}$ of Total Nitrogen in Solids; RSIL Lab Code 2893**

Chapter 11 of  
Section C, Stable Isotope-Ratio Methods  
**Book 10, Methods of the Reston Stable Isotope  
Laboratory**

Techniques and Methods 10–C11

**U.S. Department of the Interior  
U.S. Geological Survey**

**This page left blank intentionally.**

# **Determination of the $\delta^{15}\text{N}$ of Total Nitrogen in Solids; RSIL Lab Code 2893**

By Kinga Révész, Haiping Qi, and Tyler B. Coplen

Chapter 11 of  
Section C, Stable Isotope-Ratio Methods  
**Book 10, Methods of the Reston Stable Isotope  
Laboratory**

Edited by Kinga Révész and Tyler B. Coplen

Techniques and Methods 10–C11

**U.S. Department of the Interior  
U.S. Geological Survey**

**U.S. Department of the Interior**  
KEN SALAZAR, Secretary

**U.S. Geological Survey**  
Marcia K. McNutt, Director

U.S. Geological Survey, Reston, Virginia: 2012  
Version 1.0, 2006  
Version 1.1, 2007  
Version 1.2, 2012

For sale by U.S. Geological Survey, Information Services  
Box 25286, Denver Federal Center  
Denver, CO 80225

For more information about the USGS and its products:  
Telephone: 1-888-ASK-USGS  
World Wide Web: <http://www.usgs.gov/>

Any use of trade, product, or firm names in this publication is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Although this report is in the public domain, permission must be secured from the individual copyright owners to reproduce any copyrighted material contained within this report.

*Suggested citation:*

Révész, Kinga, Qi, Haiping, and Coplen, T.B., 2012, Determination of the  $\delta^{15}\text{N}$  of total nitrogen in solids; RSIL lab code 2893, chap. 11 of *Stable isotope-ratio methods*, sec. C of Révész, Kinga, and Coplen, T.B. eds., *Methods of the Reston Stable Isotope Laboratory* (slightly revised from version 1.1 released in 2007); U.S. Geological Survey Techniques and Methods, book 10, 30 p., available only at <http://pubs.usgs.gov/tm/2006/tm10c11/>. (Supersedes versions 1.0 and 1.1 released in 2006 and 2007, respectively.)

## Foreword

The Reston Stable Isotope Laboratory (RSIL) provides stable isotope analyses on a routine basis for a large user community within the U.S. Geological Survey (USGS) and elsewhere. The RSIL also serves the USGS National Research Program (NRP) through its project on Stable Isotope Fractionation in Hydrologic Processes. The NRP conducts basic and problem-oriented hydrologic research in support of the mission of the USGS. The stable isotope project conducts research on the use of isotope-ratio measurements in studies of water resources and environmental quality. One objective of this project is to develop new techniques for isotopic analysis of hydrogen, nitrogen, oxygen, carbon, and sulfur in environmental samples. New analytical techniques expand the range of tools available for studying the movement of those elements in hydrologic and biogeochemical systems. Another objective of the project is to test new applications of isotope measurements in specific field settings. Field studies of isotope behavior have contributed to understanding water-supply sustainability, groundwater/surface-water interactions, paleoclimate history, biologic cycling of nutrients, groundwater contamination, and natural remediation. This project also contributes to the improvement of measurement science and the development of isotope databases.

Book 10 of the Techniques and Methods Series of the USGS contains detailed descriptions of methods of the RSIL, including routine analytical methods called standard operating procedures (SOPs), along with safety guidelines, maintenance procedures, and other information about the operation of the RSIL. Section C of book 10 contains the SOPs for a variety of methods to measure stable isotope ratios, each of which constitutes a chapter. Each chapter is limited to a narrow field of subject matter to permit flexibility in revision as the need arises.



Pierre Glynn  
Chief, Branch of Regional Research, Eastern Region

**This page left blank intentionally.**

# Contents

Foreword.....	iii
Summary of Procedure.....	1
Reporting Units and Operational Range.....	1
Reference Materials and Documentation.....	2
Reference Materials Used, Storage Requirements, and Shelf Life.....	2
Documentation.....	2
Labware, Instrumentation, and Reagents.....	3
Sample Collection, Preparation, Analysis, Retention Times, and Disposal.....	4
Sample Containers, Preservation, and Handling Requirements.....	4
Sample Preparation and Time Requirements.....	5
Performing the Analysis and Time Requirements.....	6
Problematic Samples.....	6
Interferences.....	7
Troubleshooting and Bench Notes.....	7
Maintenance and Maintenance Records.....	8
Sample Retention Time and Disposal.....	8
Data Acquisition, Processing, Evaluation, Quality Control, and Quality Assurance.....	8
Laboratory Information Management System for Light Stable Isotope (LIMS-LSI).....	8
Quality Control (QC) Samples.....	8
Acceptance Criteria for All QC Samples.....	9
Corrective Action Requirements.....	9
Responsible Parties for All QC/QA Functions for Procedures Covered in RSIL SOPs.....	9
Data Management and Records.....	9
Health, Safety, and Waste-Disposal Information.....	10
Applicable Health and (or) Safety Issues.....	10
Personal Protection.....	10
Electrical Hazards.....	10
Chemical Hazards.....	10
Gas Cylinder Handling.....	10
Specific Waste-Disposal Requirements.....	10
Revision History.....	11
References Cited.....	13
Appendix A. Step-by-Step Procedure to Log-In Samples to LIMS-LSI.....	14
Appendix B. Step-by-Step Procedure to Create an Excel Sample-List Workbook and Print a Sample List.....	15
Excel Sample-List Workbook.....	15
Sample List.....	15
Worksheet 1. Weighing template.....	16
Worksheet 2. Samples to be analyzed.....	17
Appendix C. Step-by-Step Procedure for Weighing and Storing Samples.....	19
Appendix D. Step-by-Step Procedure of Zero Blank Autosampler Operation.....	20
Appendix E. Step-by-Step Procedure to Add Sample Information to Sequence Table.....	21
Appendix F. Step-by-Step Procedure to Retrieve Data from ISODAT 2.0 for LIMS-LSI and for Data Back-Up.....	22
Appendix G. Step-by-Step Procedure to Transfer Data to LIMS-LSI, to Transfer Data to Back-Up Computer, and to Reevaluate Old Data.....	23
Appendix H. Step-by-Step Procedure to Determine and Apply Correction Factors and Evaluate Data.....	24
Appendix I. Step-by-Step Procedure to Check Elemental Analyzer for Leaks.....	25
Appendix J. Daily Checklist.....	26

Appendix K. Changing the Insertion Tube.....	27
Appendix L. Changing the Water Trap.....	28
Appendix M. Changing the Reaction Tubes .....	29
Appendix N. Step-by-Step Procedure to Report Data.....	30

## Figures

1. Diagram of the Carlo Erba Instruments NC 2500 (CE NC 2500) Elemental Analyzer (used with permission of Thermo Scientific). .....	3
2. Diagram of a Finnigan MAT (now Thermo Scientific) ConFlo II Open Split (used with permission of Thermo Scientific). .....	4
3. Schematic of a continuous flow isotope-ratio mass spectrometer (CF-IRMS) (modified from Clark and Fritz, 1997).....	5

## Conversion Factors

Multiply	By	To Obtain
Length		
millimeter (mm)	0.03937	inch
centimeter (cm)	0.3937	inch
meter (m)	3.281	foot
Volume		
micro liter ( $\mu\text{L}$ )	$0.0610210^{-3}$	cubic inch
milliliter (mL)	0.06102	cubic inch
cubic centimeter ( $\text{cm}^3$ )	0.06102	cubic inch
Mass		
nanogram ( $\text{ng}$ ) = $10^{-3}$ $\mu\text{g}$	$3.527 \times 10^{-11}$	ounce
microgram ( $\mu\text{g}$ ) = $10^{-3}$ mg	$3.527 \times 10^{-8}$	ounce
milligram (mg) = $10^{-3}$ g	$3.527 \times 10^{-5}$	ounce
gram (g) = $10^{-3}$ (kg)	$3.527 \times 10^{-2}$	ounce
kilogram = $10^3$ g; $10^6$ mg; $10^9$ $\mu\text{g}$ ; $10^{12}$ ng	2.205	pound
Density		
gram per cubic centimeter ( $\text{g}/\text{cm}^3$ )	0.5780	ounce per cubic inch
Temperature		
Celsius ( $^{\circ}\text{C}$ )	$^{\circ}\text{F} = 9/5 (^{\circ}\text{C}) + 32$	Fahrenheit ( $^{\circ}\text{F}$ )
Pressure (force/area)		
kilopascal (kPa)	$9.869 \times 10^{-3}$	atmosphere, standard (atm)
kilopascal (kPa)	$1.450 \times 10^{-1}$	pound-force/square inch (psi)
kilopascal (kPa)	$1.000 \times 10^{-2}$	bar
kilopascal (kPa)	$2.961 \times 10^{-1}$	inches of mercury at 60 $^{\circ}\text{F}$

## Acronyms and Abbreviations

ANSI	American National Standards Institute
CF-IRMS	continuous flow isotope-ratio mass spectrometer
cm	centimeter
EA	elemental analyzer
GC	gas chromatograph
HDPE	high-density polyethylene
IRMS	isotope-ratio mass spectrometer

kPa	kilopascal
LIMS-LSI	Laboratory Information Management System for Light Stable Isotopes
mg	milligram
mg/g	milligram per gram
min	minute
mL	milliliter
mL/min	milliliter per minute
MSDS	Material Safety Data Sheets
NBS	National Bureau of Standards
NFPA	National Fire Protection Association
NWQL	National Water-Quality Laboratory (USGS)
per mil, ‰	one part in one thousand parts, with value $10^{-3}$
pF	picofarad
QA	quality assurance
QC	quality control
QC/QA	quality control/quality assurance
RSIL	Reston Stable Isotope Laboratory
SOP	standard operating procedure
std	international measurement standard
USGS	U.S. Geological Survey
µg	microgram

## Symbols

Ω	ohm
<	less than
≤	less than or equal

# Determination of the $\delta^{15}\text{N}$ of Total Nitrogen in Solids; RSIL Lab Code 2893

By Kinga Révész, Haiping Qi, and Tyler B. Coplen

## Summary of Procedure

The purpose of the Reston Stable Isotope Laboratory (RSIL) lab code 2893 is to determine the  $\delta(^{15}\text{N}/^{14}\text{N})$ , abbreviated as  $\delta^{15}\text{N}$ , of total nitrogen in solid samples. A Carlo Erba NC 2500 elemental analyzer (EA) is used to convert total N in a solid sample into  $\text{N}_2$  gas. The EA is connected to a continuous flow isotope-ratio mass spectrometer (CF-IRMS), which determines relative difference in the isotope-amount ratios of stable nitrogen isotopes ( $^{15}\text{N}/^{14}\text{N}$ ) of the product  $\text{N}_2$  gas. The combustion is quantitative; no isotopic fractionation is involved. Samples are placed in a tin capsule and loaded into the Costech Zero Blank Autosampler of the EA. Under computer control, samples are dropped into a heated reaction tube that contains an oxidant, where the combustion takes place in a helium atmosphere containing an excess of oxygen gas. Combustion products are transported by a helium carrier through a reduction tube to remove excess oxygen and convert all nitrous oxides into  $\text{N}_2$  and through a drying tube to remove water. The gas-phase products, mainly  $\text{CO}_2$  and  $\text{N}_2$ , are separated by a gas chromatograph (GC). The gas is then introduced into the isotope-ratio mass spectrometer (IRMS) through a Finnigan MAT (now Thermo Scientific) ConFlo II interface, which also is used to inject  $\text{N}_2$  reference gas and helium for sample dilution. The IRMS is a Thermo Scientific Delta V Plus CF-IRMS. It has a universal triple collector, two wide cups with a narrow cup in the middle, capable of measuring mass/charge ( $m/z$ ) 28, 29, 30, simultaneously. The ion beams from  $\text{N}_2$  are as follows:  $m/z$  28 =  $\text{N}_2 = ^{14}\text{N}^{14}\text{N}$ ;  $m/z$  29 =  $\text{N}_2 = ^{14}\text{N}^{15}\text{N}$  primarily;  $m/z$  30 =  $\text{NO} = ^{14}\text{N}^{16}\text{O}$  primarily, which is a sign of contamination or incomplete reduction.

## Reporting Units and Operational Range

Variations in stable isotope ratios typically are small. Stable isotope ratios commonly are measured and expressed as the relative difference in the ratio of the number of the less abundant isotope (usually the heavy isotope) to the number of the more abundant isotope (usually the light isotope) of a sample with respect to the measurement standard, std (Coplen, 2011). This relative difference is designated  $\delta^i\text{E}$ , which is a shortened form of  $\delta(^i\text{E}/^j\text{E})$  or  $\delta(^i\text{E})$ , and is defined according to equation 1 (Coplen, 2011):

$$\delta^i\text{E} = \delta(^i\text{E}) = \delta(^i\text{E}/^j\text{E}) = \frac{N(^i\text{E})_{\text{P}}/N(^j\text{E})_{\text{P}} - N(^i\text{E})_{\text{std}}/N(^j\text{E})_{\text{std}}}{N(^i\text{E})_{\text{std}}/N(^j\text{E})_{\text{std}}} \quad (1)$$

where  $N(^i\text{E})_{\text{P}}$  and  $N(^j\text{E})_{\text{P}}$  are the numbers of the two isotopes  $^i\text{E}$  and  $^j\text{E}$  of element E in specimen P and equivalent parameters follow for the international measurement standard, “std.” A positive  $\delta^i\text{E}$  value indicates that the specimen is enriched in the heavy isotope,  $^i\text{E}$ , relative to the std. A negative  $\delta^i\text{E}$  value indicates that the specimen is depleted in the heavy isotope,  $^i\text{E}$ , relative to the std. For stable nitrogen isotope-amount ratios,  $\delta^{15}\text{N}$  is defined as follows:

$$\delta^{15}\text{N} = \delta(^{15}\text{N}) = \delta(^{15}\text{N}/^{14}\text{N}) = \frac{N(^{15}\text{N})_{\text{P}}/N(^{14}\text{N})_{\text{P}} - N(^{15}\text{N})_{\text{std}}/N(^{14}\text{N})_{\text{std}}}{N(^{15}\text{N})_{\text{std}}/N(^{14}\text{N})_{\text{std}}} \quad (2)$$

The primary reference material for relative nitrogen isotope-ratio measurements ( $\delta^{15}\text{N}$ ) is atmospheric nitrogen gas ( $\text{N}_2$ ), which is widespread and homogeneous and, by convention, has a consensus  $\delta^{15}\text{N}$  value of 0 (Mariotti, 1983). By interspersing internationally distributed isotopic reference materials with accepted  $\delta$  values among unknown samples, the  $\delta$  values can be determined on a normalized  $\delta^{15}\text{N}$  scale. The  $\delta^{15}\text{N}$  measurements are made with a CF-IRMS, which alternately measures the isotope-number ratios of the sample  $\text{N}_2$  gas and one or more injections of the “working reference”  $\text{N}_2$  gas. Values of  $\delta^{15}\text{N}$  typically are reported in parts per thousand (‰).

The system was tested by analyzing pure inorganic samples (potassium nitrate, sodium nitrate, and ammonium sulfate), an array of biological tissues from vertebrates and invertebrates, and geological samples (Révész, 1998), such as suspended organic matter in sediment. The routine analysis requires a minimum of 40  $\mu\text{g}$  of sample as nitrogen in a maximum of 40 milligrams (mg) of solid sample; however, the system is capable of analyzing samples as small as 19  $\mu\text{g}$  of nitrogen in one sample aliquot. The  $\delta^{15}\text{N}$  values of reference materials ranged from approximately  $-5$  to  $+180$  ‰, which covers much of the range of  $\delta^{15}\text{N}$  values of natural samples. The precision and accuracy of the results were acceptable ( $\pm 0.2$  ‰), and no memory effects were observed (Qi and others, 2003).

## Reference Materials and Documentation

### Reference Materials Used, Storage Requirements, and Shelf Life

In this method, internationally distributed isotopic reference materials used when inorganic N-bearing samples are analyzed. These include IAEA-N-1 ( $(\text{NH}_4)_2\text{SO}_4$  with  $\delta^{15}\text{N}$  of  $+0.43$  ‰ (slightly revised from Böhlke and Coplen, 1995; Böhlke and others, 1993), IAEA-N-2 ( $(\text{NH}_4)_2\text{SO}_4$ ) with  $\delta^{15}\text{N}$  of  $+20.41$  ‰, IAEA-NO-3 ( $\text{KNO}_3$ ) with  $\delta^{15}\text{N}$  of  $+4.7$  ‰, USGS25 ( $(\text{NH}_4)_2\text{SO}_4$ ) with  $\delta^{15}\text{N}$  of  $-30.4$  ‰, USGS26 ( $(\text{NH}_4)_2\text{SO}_4$ ) with a  $\delta^{15}\text{N}$  of  $+53.7$  ‰, and USGS32 ( $\text{KNO}_3$ ) with  $\delta^{15}\text{N}$  of  $+180$  ‰. When organic nitrogen samples or mixtures of inorganic and organic nitrogen are analyzed, USGS40 and USGS41 L-glutamic acid ( $\text{HO}_2\text{CCH}_2\text{CH}_2(\text{NH}_2)\text{CO}_2\text{H}$ ) isotopic reference materials with  $\delta^{15}\text{N}$  of  $-4.52$  ‰ and  $+47.57$  ‰, respectively (Qi and others, 2003), are interspersed with samples. These two L-glutamic acids were prepared in the RSIL (Qi and others, 2003). Detailed preparation and calibration procedures for USGS25, USGS26, and USGS32 nitrate salts are given by Böhlke and others (1993).

All of these reference materials are stored in glass bottles capped with Teflon-coated or cone-shaped caps to keep moisture out. Their shelf life is indefinite.

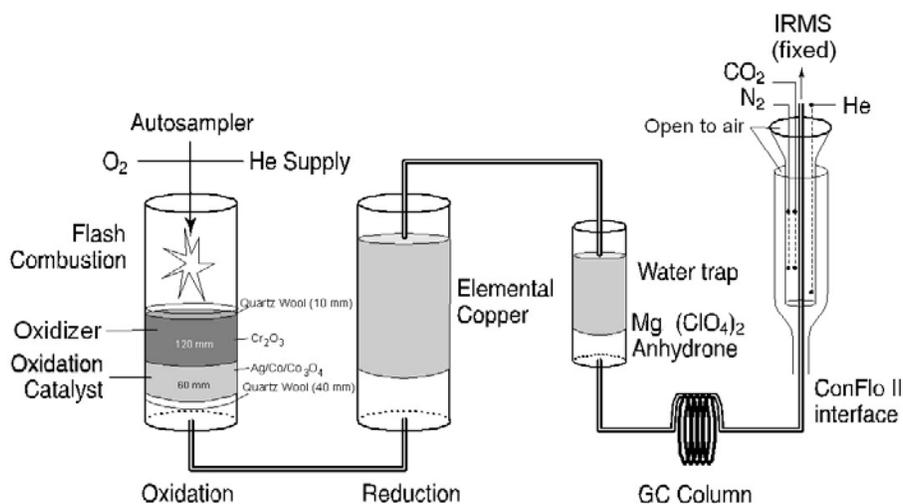
### Documentation

All calibration results are stored in the Laboratory Information Management System for Light Stable Isotopes (LIMS-LSI) (Coplen, 2000) under sample identifiers N-1 (IAEA-N-1), N-2 (IAEA-N-2), N-3 (IAEA-NO-3), N-6 (USGS25), N-7 (USGS26), N-25 (USGS32), G-6740 (USGS40), and G-6741 (USGS41).

## Labware, Instrumentation, and Reagents

Preparatory labware and apparatus include tin capsules and a microbalance capable of measuring samples with 0.001-mg precision. The analytical apparatus consists of four different units: (1) EA, (2) ConFlo II interface, (3) IRMS, and (4) computer software.

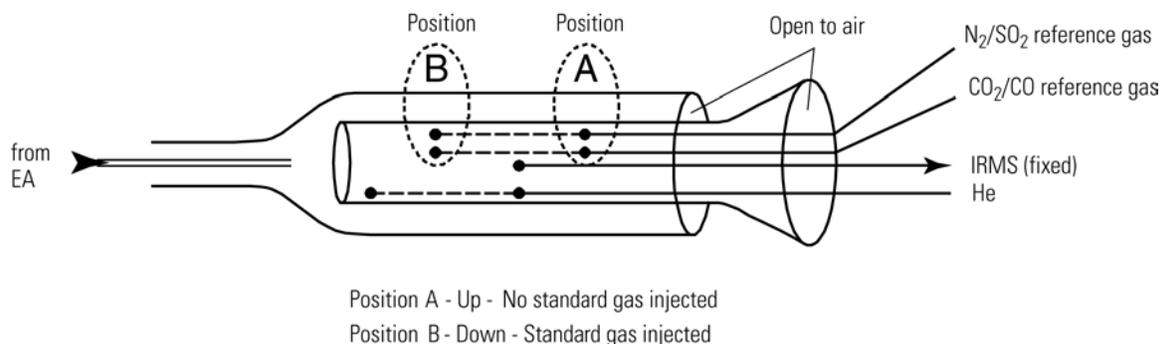
The EA is a Carlo Erba Instruments NC 2500 (CE NC 2500) system, and it is equipped with a Costech “zero blank” autosampler that holds 49 samples enclosed in tin capsules (fig. 1). Each tin capsule falls into the reaction-tube, which is kept at 1020 °C and is under constant helium flow (90 milliliters per minute (mL/min)). The sample immediately reacts with a measured amount of oxygen released from a 5-milliliter (mL) loop purged at 35 mL/min. The reaction of oxygen with the tin capsule is exothermic, which results in localized temperatures of up to 1800 °C, thus ensuring complete and instantaneous sample combustion. This is called “dynamic flash combustion.” The combustion gases are first carried by helium through an oxidative catalyst layer ( $\text{Cr}_2\text{O}_3$ ) in the reaction tube, where oxidation is completed, and then through a reduction agent (Cu) in another reaction tube kept at 650 °C, where any nitrogen oxides are converted into elemental nitrogen and any excess oxygen is retained. At the outlet of the second reaction tube, the gas mixture ( $\text{N}_2$ , usually  $\text{CO}_2$  from carbon compound in the solid, and  $\text{H}_2\text{O}$ ) meets a trap containing Anhydrone, which absorbs water. The gas mixture, now  $\text{N}_2$  and  $\text{CO}_2$ , flows through a GC column (Porapak Q kept at 50 °C), which separates the gases, screens out sampling noise, and ensures that pure nitrogen gas pass separately through the thermal conductivity detector, through the Finnigan MAT ConFlo II open split, and into the IRMS.



**Figure 1.** Diagram of the Carlo Erba Instruments NC 2500 (CE NC 2500) elemental analyzer (used with permission of Thermo Scientific).

The Finnigan MAT ConFlo II interface provides the means for coupling the EA to the CF-IRMS with an open-split arrangement (fig. 2). The principle of an open split is that it reduces the 90 mL/min helium flow needed to operate the EA to a 0.3 mL/min helium flow needed to operate the IRMS. This is achieved by placing the end of the capillary that leads to the IRMS directly in the flow of helium coming from the EA. The narrow diameter and length of this capillary limit the flow of gas into the IRMS. Furthermore, extra capillaries carrying reference gas or helium gas can be added to the open split, thereby making it

possible to inject reference gas into the IRMS or to dilute the sample with extra helium if the sample is too large (or to direct gases away by dilution if they are too large to ensure total separation).



**Figure 2.** Diagram of a Finnigan MAT (now Thermo Scientific) ConFlo II Open Split (used with permission of Thermo Scientific).

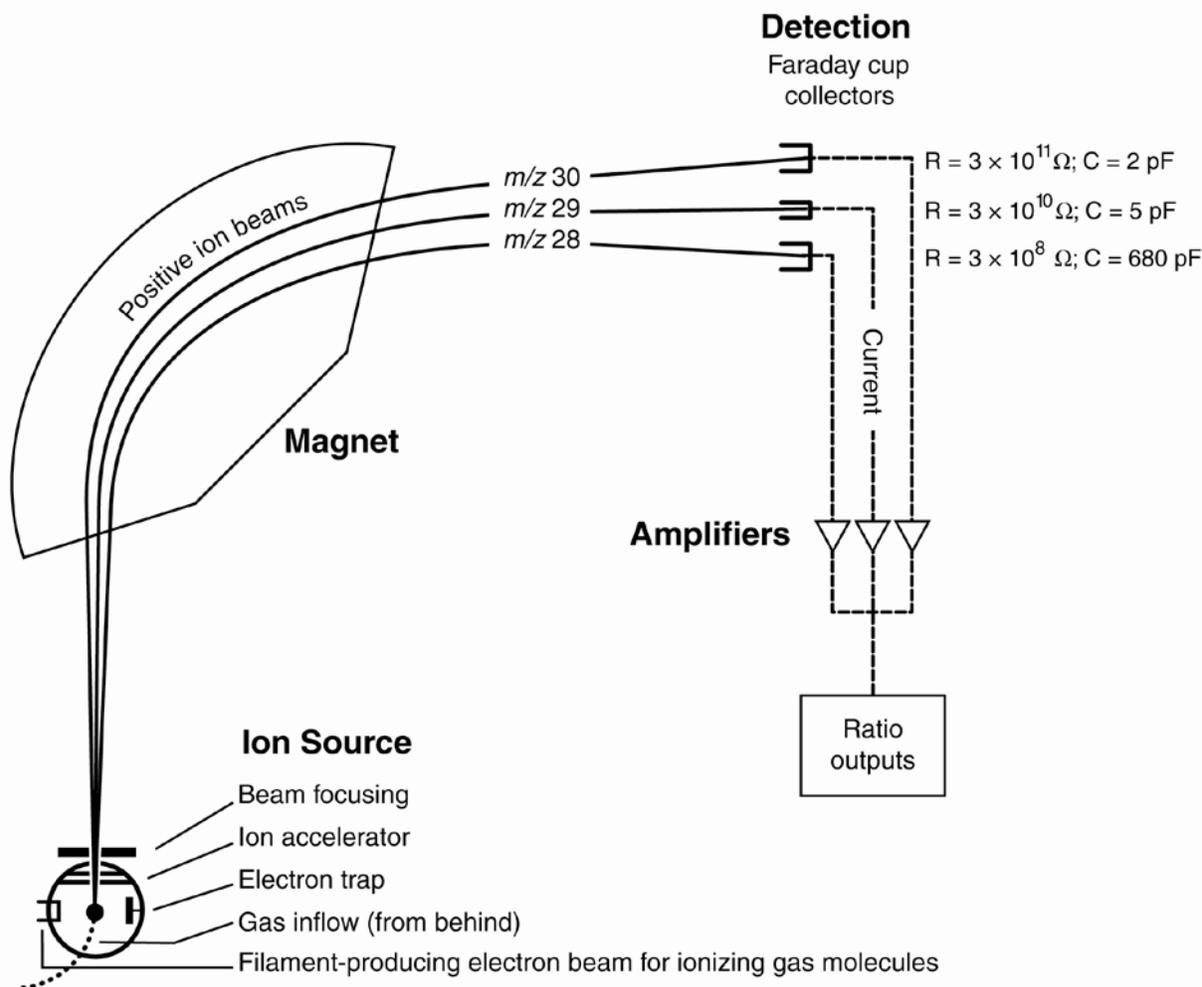
The IRMS is a Thermo Scientific Delta V Plus CF-IRMS (fig. 3). The fundamental principle of the CF-IRMS technique is that a carrier gas transports the analyte through an initial stage of online chemistry for conversion to a form acceptable by the IRMS (Brenna and others, 1997). This is an automated system generating online, high-precision,  $\delta$  values of bulk solid and nonvolatile liquid samples. In the IRMS, gas molecules are ionized in a source by electrons emitted from a hot filament. The ions are accelerated into an analyzer, separated in a magnetic field, and collected in Faraday cup collectors. The ion-beam intensities are measured with electrometers. This IRMS has a universal triple collector with two wide cups and a narrow cup in the middle, capable of measuring  $m/z$  28, 29, and 30, simultaneously. The resistor-capacitor combination on the electrometer used to measure these masses are as follows:  $3 \times 10^8$  ohms ( $\Omega$ ) and 680 picofarads (pF) on cup 1,  $3 \times 10^{10}$   $\Omega$  and 5 pF on cup 2, and  $3 \times 10^{11}$   $\Omega$  and 2 pF on cup 3.

The Thermo Scientific ISODAT 2.0 software is designed (1) to advance the autosampler carousel, (2) to control the Finnigan MAT ConFlo II interface to inject reference gases at the desired time and dilute the sample if desired, (3) to operate the IRMS, (4) to acquire data from the IRMS, and (5) to calculate delta values.

## Sample Collection, Preparation, Analysis, Retention Times, and Disposal

### Sample Containers, Preservation, and Handling Requirements

Each sample is collected in a high density polyethylene (HDPE) scintillation vial with Polyseal cap (Wheaton 986706), available from RSIL. Sample size should be a maximum of 1 g. The sample should be homogenized, oven dried (90 °C), and powdered by the submitter to 100 to 200  $\mu\text{m}$ . Containers are labeled with isotopes whose concentrations are to be determined and with the respective laboratory code or schedule number. A minimum of 0.15 mg of nitrogen per sample is required. The minimum sample concentration is 1 milligram per gram (mg/g) sample for N. Lower concentrations can be analyzed; contact the laboratory for further information. No treatment, preservation, or special shipping is required.



**Figure 3.** Schematic of a continuous flow isotope-ratio mass spectrometer (CF-IRMS) (modified from Clark and Fritz, 1997).

## Sample Preparation and Time Requirements

Sample preparation involves logging in samples to the LIMS-LSI (see appendix A), creating a sample work-order sheet, and weighing samples into capsules.

The logging-in procedure involves logging samples in batches to LIMS-LSI that could contain from 1 to 100 samples per batch (also called a project), printing labels for each sample, and printing a summary project report. Each sample label contains the Field ID (the identifier by which the sample submitter knows each sample) and the unique RSIL Lab ID assigned by the LIMS-LSI. The summary project report is inserted into a three-ring binder titled “Samples in Progress.”

An Excel sample workbook is created. Step-by-step procedures and examples are shown in the appendix B. Sample weighing includes balance conditioning, weighing appropriate sample into capsule (sufficient to yield 40 micrograms ( $\mu\text{g}$ ) of nitrogen), closing capsules by folding them, and recording weight on template. Step-by-step procedures are shown in appendix C. The time requirement for performing the sample preparation described is approximately 1 to 5 days.

## Performing the Analysis and Time Requirements

All samples are prepared and analyzed in batches of a maximum of 80 per day, and all samples are analyzed in duplicate. Six aliquots of two internationally distributed isotopic reference materials with different  $\delta^{15}\text{N}$  values are analyzed at the beginning of a batch. Four of them are depleted in  $^{15}\text{N}$ , and two are enriched in  $^{15}\text{N}$ . A set of these two reference materials also is interspersed among the samples. No fewer than one reference material set is analyzed for every 10 unknowns. The amount of nitrogen in the reference materials must be in the same range as that of the samples. Therefore, one must preanalyze every sample to obtain the N/C concentration ratio unless it is provided by the customer. If the N/C concentration ratio is unknown and cannot be determined prior to isotopic analysis, a sequence of reference materials containing different amounts of material must be analyzed to determine a mass dependent correction factor. The carbon concentration is necessary to apply the correct dilution with helium to the  $\text{CO}_2$  to avoid its peak interference to the  $\text{N}_2$  peak.

The procedure involves loading the autosampler carousel with capsules containing an appropriate amount of sample in sequence according to the “Template” (appendix D), loading a new sample heading (including sample weights) into the “Sequence Table” in ISODAT 2.0 of the IRMS software, choosing the appropriate “Method,” and starting sequence acquisition (appendix E). After the analyses are completed, data are exported (appendix F) to a removable disk or “memory stick” from the ISODAT 2.0 database and transferred (appendix G) to the LIMS-LSI system and Data Back-Up computer. Final daily correction factors are determined by LIMS-LSI (appendix H), based on the daily analyses of reference materials using equations 3 and 4, which are presented with example numerical values:

$$-4.52 \text{ ‰} = m \times \delta^{15}\text{N}_{\text{USGS40/workingrefgas}} + b \quad (3)$$

$$+47.57 \text{ ‰} = m \times \delta^{15}\text{N}_{\text{USGS41/workingrefgas}} + b \quad (4)$$

The values  $-4.52 \text{ ‰}$  and  $+47.57 \text{ ‰}$  are the assigned values of the two international reference materials (USGS40 and USGS41), respectively. The  $\delta^{15}\text{N}_{\text{ref/workingrefgas}}$  values in the equations 3 and 4 are the mean daily delta values of the respective international reference materials relative to the working reference gas;  $b$  is the additive correction factor and  $m$  is the expansion coefficient correction factor. When two or more reference materials with different isotopic compositions are analyzed, the correction for sample blank does not need to be applied if the magnitude of the blank in the samples and reference materials is identical. However, if only a single reference material is analyzed, only one-point calibration is possible; therefore, a blank correction should be applied.

If replicates do not agree within acceptable tolerances, they are analyzed until acceptable statistics are achieved. The computerized LIMS-LSI will not release data until the statistics are acceptable. The time requirement for performing the analyses described above is a minimum of two days.

The systematic procedure is listed in the “Lab Procedures” binder. The supervisor reviews suggested changes before they are adopted.

## Problematic Samples

Problematic samples include those that do not have nitrogen and carbon concentration ratios prior to isotopic analysis. In that case, the C/N amount ratio needs to be determined by a preliminary analysis to be able to determine the sample amounts that gives an appropriate amount of nitrogen ( $40 \mu\text{g}$ ) and to be able to determine the amount of dilution of  $\text{CO}_2$  with He.

When analyzing samples containing very low nitrogen, a large amount of sample is needed to yield measurable  $\text{N}_2$ . This could cause poor combustion; as a consequence, a poor yield and a poor isotopic

result could be observed. To solve this problem, a larger oxygen loop (10 mL) normally is used. However, this additional oxygen will consume the reactors quickly.

## Interferences

There are no known interferences with this analytical technique. However, it should be pointed out that if samples contained a mixture of different nitrogen sources, reported isotope-ratio values would represent the isotopic composition of the total nitrogen in the sample. The  $\delta^{15}\text{N}$  values of specific N species cannot be provided with this analytical technique.

## Troubleshooting and Bench Notes

The most common problems with this analytical technique are (1) incomplete combustion, (2) leakage in the system, particularly in the EA, and (3) insufficient linearity of the IRMS. The fact that the melting point of the compounds being combusted are usually higher or close to the temperature of the reaction tube (1020 °C) could create insufficient combustion. The “flash point” has a higher temperature, approximately 1800 °C (because the tin oxidation reaction is exothermic); therefore, it is critical to create a sharp, sudden, bright, single flash. This can be done by coordinating (1) helium pressure and flow rate, (2) oxygen pressure and flow rate, and (3) sample “start time.” The system is so sensitive that even a 1-second difference in the sample dropping time can make a difference in the brightness of the flash, peak shape, peak area, and, consequently, the  $\delta$  result. The optimum set-up parameters of the RSIL EA are cycle = 100, oxy = 60 (O<sub>2</sub> flow stops flowing into autosampler), sample start = 10, and sample stop = 40. The helium regulator is set for 150 kilopascals (kPa), and helium flow set up is 95 mL/min, which actually measures 120 mL/min at the vent. The oxygen regulator is set for 150 kPa, and the oxygen flow rate is 54 mL/min at purge. The oxygen loop is 5 mL. The oven temperature of the GC is 50 °C.

Even a tiny leak can interfere with the result; it can create double peaks, an unstable analysis, and so forth. The high helium flow rate makes the system more sensitive to leaks (Bernoulli principle). Leaks can cause uncontrollable changes in the flow rate, which could prevent quantitative combustion, affecting the peak shape and peak area, and consequently, the  $\delta$  value. Ash accumulation is also critical. Although tin oxide serves as a catalyst in the reaction, it also partially “clogs” the system, interfering with the flow rate, although not necessarily in a measurable manner. However, this assumes a nonrandom interference that causes the shift in  $\delta$  value. By frequently analyzing reference materials, this shift can be corrected. However, after approximately 50 samples (one carousel), it is advisable to remove the ashes (Révész, 1998). The leak-checking procedure is given in appendix I. A leak is indicated in the IRMS when  $m/z$  28 and 29 are high and the ratios of  $m/z$  28, 32, and 40 (N<sub>2</sub>, O<sub>2</sub>, and argon) reflect atmospheric compositions. Acceptable levels for  $m/z$  28 and 29 are approximately 0.15 and 0.015 V, respectively, with typical IRMS settings. (See “Daily Checklist” in appendix J.)

Adjustment of the emission current is critical. The higher it is, the higher the signal will be, but the poorer the linearity will be. The IRMS is temperature sensitive; therefore, it must be operated in a temperature-controlled environment with all of the side panels on. The resistor-capacitor combinations on the electrometers are as follows:  $3 \times 10^8 \Omega$  and 680 pF for cup 1,  $3 \times 10^{10} \Omega$  and 5 pF for cup 2, and  $3 \times 10^{11} \Omega$  and 2 pF for cup 3. The instrument is capable of measuring  $m/z$  28 and 29, and  $m/z$  30 = NO = <sup>14</sup>N<sup>16</sup>O primarily, which is a sign of contamination or incomplete reduction.

An internal precision check needs to be conducted using the “Zero Enrichment” method (standard gas on/off 10 times), and it must give a standard deviation of 0.1 ‰ or better. The “Ratio Linearity” test must give 0.12 ‰/V or better linearity. An external precision check needs to be performed as needed, approximately every week or so; samples of the same amount need to be analyzed within  $\pm 0.010$  mg. The 1- $\sigma$  standard deviation must be 0.2 ‰ or better for  $n = 10$ . If either of these criteria has not been met, the IRMS cannot be used and must be refocused.

## **Maintenance and Maintenance Records**

Routine maintenance is necessary for the upkeep of the IRMS vacuum system, including (1) checking the pump performance from time to time, (2) changing the pump oil, and (3) changing the oil cartridge in each turbomolecular pump at 6-month intervals. Pump conditions, including date of oil changes, problems, and repairs to the pump, are listed in the pump database (file path: LIMS C:\RSIL\vacuum pumps). Maintenance of the EA includes (1) changing the insertion tube every day (appendix K), (2) changing the water trap approximately every 500 samples (appendix L), (3) changing reaction tubes approximately every 200 to 250 analyses (appendix M), and (4) cleaning the sample carousel every week. The IRMS also requires a daily check (appendix J). A logbook is kept for each IRMS, where notes of maintenance checks, history of normal settings, problems, and repairs are listed.

Maintenance of ISODAT 2.0 includes importing (transferring) results from the ISODAT 2.0 database to the LIMS-LSI computer hard disk and the Data Back-Up computer hard disk (appendix G).

## **Sample Retention Time and Disposal**

Samples are retained in the RSIL for at least 6 months after reporting data. Samples are then discarded unless the submitter has requested that the samples be returned.

The ISODAT sample files from the IRMS computer are kept indefinitely on two different hard disks of the Data Back-Up computer. Paper reports are kept for a few weeks. Analytical results from the IRMS are transmitted to LIMS-LSI and kept indefinitely. No data are entered by hand, and no manual calculations are performed on the data.

## **Data Acquisition, Processing, Evaluation, Quality Control, and Quality Assurance**

### **Laboratory Information Management System for Light Stable Isotope (LIMS-LSI)**

In the RSIL, the LIMS-LSI (Coplen, 2000) is used for data acquisition, processing, and evaluation. This system is a database program capable of (1) storing information about samples, (2) storing the results of mass spectrometric delta values of samples after importing from the ISODAT database, (3) calculating analytical results using standardized algorithms stored in a database, (4) normalizing delta values using isotopic reference materials, and (5) generating templates for convenient sample placement to facilitate loading of automated IRMS sample-preparation manifolds. With this system, the following are ensured: (1) quality assurance (QA), (2) laboratory efficiency, (3) reduction of workload and errors owing to the elimination of retyping of data by laboratory personnel, and (4) a decrease of errors in data reported to sample submitters. This database provides a complete record of information on how laboratory reference materials have been analyzed and provides a record of what correction factors have been used as an audit trail for the RSIL.

### **Quality Control (QC) Samples**

Samples are prepared and analyzed in batches—a maximum of 80 daily. Each batch contains approximately six reference materials at the beginning, four depleted in  $^{15}\text{N}$  and two enriched in  $^{15}\text{N}$ , and later in the batch one depleted in  $^{15}\text{N}$  for every ten samples. The last analysis of a batch is that of a reference material depleted in  $^{15}\text{N}$ . All of these reference materials serve as QC samples.

Daily, the analyst (1) examines the computer printouts for indications of analytical problems, (2) determines the daily additive and expansion correction factors by the LIMS-LSI, and (3) applies corrections factors to isotopic data. The values of these corrections factors, along with the date and range of analysis numbers, are recorded manually in the laboratory “EABook” even though the data are already in the LIMS-LSI. After printing the list of isotopic results from the “Table of Samples in Progress,” the analyst reviews the results and determines which samples need to be analyzed a third time to achieve acceptable results (i.e.,  $\Delta\delta \leq 0.2 \text{ ‰}$ ).

### **Acceptance Criteria for All QC Samples**

Acceptance criteria for QC samples are the same as acceptance criteria for the other samples. The rules are as follows:

- If standard deviation is  $\leq 0.2 \text{ ‰}$ , use mean delta.
- If there are three or more analyses, delete the outlier and recalculate.
- If standard deviation of this recalculation is  $\leq 0.2 \text{ ‰}$ , use mean from this recalculation.
- If none of the above is the case, the result is not accepted, and corrective action is required.

The RSIL estimates the expanded uncertainty ( $U = 2\mu_c$ ) of  $\delta^{15}\text{N}$  measurement results. The expanded uncertainty provides an envelope that represents a 95-percent probability of encompassing the true value that has been determined from the aggregation of measurement results over a period of time. The expanded uncertainty can be determined using the guide to the expression of uncertainty (Joint Committee for Guides in Metrology, Working Group 1 (JCGM/WG1), 2010). The application of expanded uncertainty to the reporting of stable isotope measurements is discussed by Coplen and others (2006). The estimated expanded uncertainty of  $\delta^{15}\text{N}$  measurement results is  $\pm 0.5 \text{ ‰}$  unless otherwise specified, and this value is conservative. If any given sample were resubmitted to the RSIL for nitrogen isotopic analysis, the measured value would fall within the uncertainty bounds of the previous result more than 95 percent of the time.

### **Corrective Action Requirements**

If the analyst finds any problem with the daily reference-sample data, the analyst contacts the supervisor. The trouble-shooting process will require an evaluation and reanalysis of certain samples to ascertain the origin of the problem.

If samples do not give satisfactory results after three or more separate analyses, the analyst averages all the data and reports the mean value. Such analytical results are indicated with a comment, and the customer will be advised by e-mail or other means.

### **Responsible Parties for All QC/QA Functions for Procedures Covered in RSIL SOPs**

The analyst, with supervisory approval, is responsible for qualifying data and notifying customers.

### **Data Management and Records**

In addition to evaluating daily sample analyses, every week an analyst evaluates the data project by project, reports results to the customers, and files final project data reports in the laboratory “Correspondence” binder (appendix N).

## Health, Safety, and Waste-Disposal Information

### Applicable Health and (or) Safety Issues

#### Personal Protection

Safety glasses and protective gloves are recommended whenever samples are handled, especially when the samples are of biological origin. For other precautions and safety procedures, consult Material Safety Data Sheets (MSDSs), which are on file in the laboratory and at the URL <http://www.ilpi.com/msds/#Manufacturers>. This URL provides links to the MSDSs of most chemical companies.

#### Electrical Hazards

Electrical systems must conform to the National Electric Code, National Fire Protection Association Code (NFPA 70–1971), and the American National Standards Institute (ANSI) Code (C1–1971). For more information, consult the U.S. Geological Survey's *Safety and Environmental Health Handbook* (U.S. Geological Survey, 2002, sec. 4–4.1).

Shock hazards exist inside the instruments. Only an authorized service representative or an individual with training in electronic repair must remove panels or circuit boards where voltages are greater than 20 volts. The instruments require a third-wire protective grounding conductor. Three-to-two wire adapters are unsafe for these instruments.

#### Chemical Hazards

The hazardous chemicals used in the process are  $\text{Cr}_2\text{O}_3$  and  $\text{Co}_3\text{O}_4$ , which are used in the oxidation reaction tube. Sometimes the reaction tube cracks, and the packing material leaks out. When this happens, protective gloves and a full-face mask are required during the cleanup process. All the materials, including gloves and any cleaning towels, need to be collected in a plastic bag and disposed of as hazardous waste. Upon receipt, all samples must be carefully inspected for indications of hazards.

#### Gas Cylinder Handling

Compressed gas cylinders must be handled and stored according to the U.S. Geological Survey's *Safety and Environmental Health Handbook* (U.S. Geological Survey, 2002, sec. 4–4.5.1). Each cylinder must be (1) carefully inspected when received; (2) securely fastened at all times with an approved chain assembly or belt; (3) capped at all times when not in use; (4) capped when transported; (5) transported only by a properly designed vehicle (hand truck); and (6) stored separately with other full, empty, flammable, or oxidizing tanks of gas, as appropriate.

### Specific Waste-Disposal Requirements

Used reaction tubes containing  $\text{Cr}_2\text{O}_3$  and  $\text{CO}_3\text{O}_4$  must be collected in a closed container and must be given to the safety, health, and environment officer for disposal.

## Revision History

Publication Series and Series Number: Techniques and Methods 10–C11 (Book 10, Section C, Chapter 11)

Publication Title: Determination of the  $\delta^{15}\text{N}$  of Total Nitrogen in Solids; RSIL Lab Code 2893

Publication Authorship: Révész, Kinga, Qi, Haiping, and Coplen, Tyler B.,

Version 1.0, 2006

Version 1.1, 2007

Version 1.2, 2012

### Summary of Revised Product Components

Component	Description	Last Revised In Publication Version	Date of Last Revision
Title	Replaced $\delta(^{15}\text{N}/^{14}\text{N})$ by $\delta^{15}\text{N}$ to be in accordance with recommendations of the Commission on Isotopic Abundances and Atomic Weights of the International Union of Pure and Applied Chemistry.	1.2	July 2012
Authorship	Tyler B. Coplen added as a coauthor.	1.2	July 2012
Summary of procedure	Updated text to reflect new instrumentation.	1.2	July 2012
Reporting units and operational range	Updated text to reflect expression of delta values with number ratios.	1.2	July 2012
Labware, instrumentation, and reagents	Update text to reflect new instrumentation.	1.2	July 2012
Sample collection, preparation, analysis, retention times, and disposal	Updated text to reflect that samples are no longer submitted through the National Water Quality Laboratory (NWQL) and improved analytical techniques.	1.2	July 2012

Data acquisition, processing, evaluation, quality control, and quality assurance	Updated text to reflect that samples are no longer submitted through the National Water Quality Laboratory (NWQL).	1.2	July 2012
References cited	Added three references.	1.2	July 2012
Appendix A	Updated text to reflect that samples are no longer submitted through the National Water Quality Laboratory (NWQL).	1.2	July 2012
Appendix N	Updated text to reflect that samples are no longer submitted through the National Water Quality Laboratory (NWQL).	1.2	July 2012
Main text footer	Removed from document.	1.2	July 2012

## References Cited

- Böhlke, J. K., and Coplen, T. B., 1995, Interlaboratory comparison of reference materials for nitrogen-isotope-ratio measurements, in Reference and intercomparison materials for stable isotopes of light elements: Vienna, Austria, International Atomic Energy Agency, IAEA-TECDOC-825, p. 51–66.
- Böhlke, J. K., Gwinn, C. J. and Coplen, T. B., 1993, New reference materials for nitrogen-isotope-ratio measurements: *Geostandards Newsletter*, v. 17, p. 159–164.
- Brenna, J.T., Corso, T.N., Tobias, H.J., and Caimi, R.J., 1997, High-precision continuous-flow isotope-ratio mass spectrometry: *Mass Spectrometry Reviews*, v. 16, p. 227–258.
- Clark, Ian, and Fritz, Peter, 1997, *Environmental isotopes in hydrogeology*: Boca Raton, Fla., Lewis Publishers, 328 p.
- Coplen, T.B., 2000, A guide for the laboratory information management system (LIMS) for light stable isotopes—Version 7 and 8: U.S. Geological Survey Open-File Report 00–345, 121 p., accessed April 27, 2012, at <http://water.usgs.gov/software/code/geochemical/lims/doc/ofr00345.pdf>.
- Coplen, T.B., 2011, Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results: *Rapid Communications in Mass Spectrometry*, v. 25, p. 2538–2560, accessed April 27, 2012, at <http://onlinelibrary.wiley.com/doi/10.1002/rcm.5129/abstract>.
- Coplen, T.B., Brand, W.A., Gehre, Matthias, Gröning, Manfred, Meijer, H.A.J., Toman, Blaza, and Verkouteren, R.M., 2006, New guidelines for  $\delta^{13}\text{C}$  measurements: *Analytical Chemistry*, v. 78, no. 7, p. 2439–2441.
- Joint Committee for Guides in Metrology, Working Group 1 (JCGM/WG1), 2010, Evaluation of measurement data—Guide to the expression of uncertainty in measurement (known as the GUM) (2010 corrected version of the first edition of 2008): Joint Committee for Guides in Metrology [publication] JCGM 100:2008, 120 p., accessed July 16, 2012, at <http://www.bipm.org/en/publications/guides/>.
- Mariotti, A., 1983, Atmospheric nitrogen is a reliable standard for natural  $^{15}\text{N}$  abundance measurements: *Nature*, v. 303, p. 685–687.
- Qi, H. P., Coplen, T. B., Geilmann, H., Brand, W. A. and Böhlke, J. K., 2003: Two new organic reference materials for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  measurements and a new value for the  $\delta^{13}\text{C}$  of NBS 22 oil: *Rapid Communications in Mass Spectrometry*, v. 17, p. 2483–2487.
- Révész, Kinga, 1998, Carbon and nitrogen isotope ratios of organic and inorganic bulk samples; Instrument performance and intercalibration [abs.], in *The 5th Canadian Continuous-Flow Isotope Ratio Mass Spectrometry Workshop*, August 16–19, 1998: Ottawa, Ontario, Canada, University of Ottawa, not paged.
- U.S. Geological Survey, 2002, USGS handbook 445–3–H, Safety and environmental health handbook, 435 p.

## **Appendix A. Step-by-Step Procedure to Log-In Samples to LIMS-LSI**

1. For sample submitter:
  - a. Download “Standard Submission Excel Form” from the RSIL Web site at <http://isotopes.usgs.gov/>.
  - b. Fill out the requested sample information.
  - c. Send a diskette or CD and a hard copy along with the samples or e-mail the completed RSIL Excel worksheet to [isotopes@usgs.gov](mailto:isotopes@usgs.gov).
2. For RSIL personnel:
  - a. Match up information on sample bottles with the submitted “Standard Submission Excel Form.”
  - b. Enter all Standard Submission Excel Form information into LIMS-LSI by loading media. Submission date is the date samples are logged in.
  - c. Use “Import a New Project From Excel Submission” in LIMS-LSI to assign G#s.
  - d. Print out one project report and container labels (one for each sample).
  - e. Put a label on each sample bottle and cross-check Field IDs between bottles and Excel worksheet forms.
  - f. Punch holes in the original Excel worksheet and all the project information you have and put in “Samples-in-Progress” binder.

## **Appendix B. Step-by-Step Procedure to Create an Excel Sample-List Workbook and Print a Sample List**

### **Excel Sample-List Workbook**

1. Use default worksheet-weighing template and add samples to be analyzed.
2. Fill the appropriate amount in the cells of the rows labeled "Set weight mg."
3. Print first worksheet in workbook.
4. Write the tray ID and the date on both the diskette and paper template.
5. Put them near the balance.

### **Sample List**

1. Use "Create a Sample List -->" in LIMS-LSI.
2. Select appropriate template for EA and mass spectrometer.
3. Select "Create Sample List for Mass Spec" (dialog box informs user how many samples are waiting to be analyzed).
4. Click "OK."
5. Click "Save and Print."
6. Insert diskette to receive sample headings.
7. Click "OK."
8. Write the day of the week that these samples should be analyzed on both the diskette and paper template.
9. Put them near the balance.
10. Exit LIMS-LSI.

Worksheet 1. Weighing template.

[International reference materials are in bold font.]

		1	2	3	4	5	6	7	8	9	10	11	12
	Our Lab ID	G-6740	G-6740	G-6740	G-6740	G-6741	G-6741	G-9031	G-9031	G-9032	G-9032	G-9033	G-9033
A	Set weight mg	0.75	0.75	0.75	0.75	0.75	0.75	0.8	0.8	0.8	0.8	0.8	0.8
	Actual weight, mg	0.75	0.69	0.73	0.766	0.751	0.764	0.804	0.798	0.799	0.802	0.805	0.803
	Comment	USGS40	USGS40	USGS40	USGS40	USGS41	USGS41						
	Our Lab ID	G-9034	G-9034	G-9035	G-9035	G-6741	G-6741	G-9036	G-9037	G-9037	G-9038	G-9036	G-9039
B	Set weight mg	0.8	0.8	0.8	0.8	0.75	0.8	0.8	0.8	0.8	0.8	0.8	0.8
	Actual weight, mg	0.801	0.802	0.795	0.803	0.759	0.795	0.801	0.799	0.793	0.804	0.801	0.795
	Comment					USGS40	USGS41						
	Our Lab ID	G-9039	G-9040	G-9040	G-6740	G-6741	G-9041	G-9042	G-9042	G-9043	G-9043	G-9044	G-9044
C	Set weight mg	0.8	0.8	0.8	0.75	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
	Actual weight, mg	0.804	0.804	0.795	0.766	0.797	0.802	0.8	0.805	0.804	0.797	0.804	0.801
	Comment				USGS40	USGS41							
	Our Lab ID	G-9045	G-9045	G-6740	G-6741	G-9046	G-9047	G-9047	G-9048	G-9048	G-9049	G-9049	G-9050
D	Set weight mg	0.8	0.8	0.75	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
	Actual weight, mg	0.795	0.803	0.766	0.799	0.798	0.796	0.803	0.805	0.805	0.797	0.802	0.796
	Comment			USGS40	USGS41								
	Our Lab ID	G-9051	G-6740	G-6741	G-9052	G-9052	G-9053	G-9053	G-9054	G-9054	G-9055	G-6740	G-6741
E	Set weight mg	0.8	0.75	0.8	1.7	1.7	1.4	1.4	1.1	1.1	0.74	0.74	0.75
	Actual weight, mg	0.796	0.756	0.795	1.703	1.705	1.405	1.399	1.102	1.097	0.739	0.737	0.795
	Comment		USGS40	USGS41								USGS40	USGS41

Worksheet 2. Samples to be analyzed.

[International reference materials are in bold font]

Line	Identifier	Port	Comment	Amount	Amt Unit
1	<b>G-6740</b>	A1	USGS40	0.75	mg
2	<b>G-6740</b>	A2	USGS40	0.769	mg
3	<b>G-6740</b>	A3	USGS40	0.763	mg
4	<b>G-6740</b>	A4	USGS40	0.766	mg
5	<b>G-6741</b>	A5	USGS41	0.751	mg
6	<b>G-6741</b>	A6	USGS41	0.764	mg
7	G-9031	A7		0.804	mg
8	G-9031	A8		0.798	mg
9	G-9032	A9		0.799	mg
10	G-9032	A10		0.802	mg
11	G-9033	A11		0.805	mg
12	G-9033	A12		0.803	mg
13	G-9034	B1		0.801	mg
14	G-9034	B2		0.802	mg
15	G-9035	B3		0.795	mg
16	G-9035	B4		0.803	mg
17	<b>G-6740</b>	B5	USGS40	0.757	mg
18	<b>G-6741</b>	B6	USGS41	0.795	mg
19	G-9036	B7		0.801	mg
20	G-9037	B8		0.799	mg
21	G-9037	B9		0.793	mg
22	G-9038	B10		0.804	mg
23	G-9038	B11		0.801	mg
24	G-9039	B12		0.795	mg
25	G-9039	C1		0.804	mg
26	G-9040	C2		0.804	mg
27	G-9040	C3		0.795	mg
28	<b>G-6740</b>	C4	USGS40	0.766	mg
29	<b>G-6741</b>	C5	USGS40	0.797	mg
30	G-9041	C6		0.802	mg
31	G-9042	C7		0.8	mg
32	G-9042	C8		0.805	mg
33	G-9043	C9		0.804	mg
34	G-9043	C10		0.797	mg
35	G-9044	C11		0.804	mg
36	G-9044	C12		0.801	mg
37	G-9045	D1		0.795	mg
38	G-9045	D2		0.803	mg
39	<b>G-6740</b>	D3	USGS40	0.766	mg
40	<b>G-6741</b>	D4	USGS41	0.799	mg
41	G-9046	D5		0.798	mg

Line	Identifier	Port	Comment	Amount	Amt Unit
42	G-9047	D6		0.796	mg
43	G-9047	D7		0.803	mg
44	G-9048	D8		0.805	mg
45	G-9048	D9		0.805	mg
46	G-9049	D10		0.797	mg
47	G-9049	D11		0.802	mg
48	G-9050	D12		0.796	mg
49	G-9051	E1		0.796	mg
50	<b>G-6740</b>	E2	USGS40	0.756	mg
51	<b>G-6741</b>	E3	USGS41	0.795	mg
52	G-9052	E4		1.703	mg
53	G-9052	E5		1.705	mg
54	G-9053	E6		1.405	mg
55	G-9053	E7		1.399	mg
56	G-9054	E8		1.102	mg
57	G-9054	E9		1.097	mg
58	G-9055	E10		0.739	mg
59	<b>G-6740</b>	E11	USGS40	0.737	mg
60	<b>G-6741</b>	E12	USGS41	0.761	mg

## Appendix C. Step-by-Step Procedure for Weighing and Storing Samples

1. Samples should be homogenized and dried.
2. Insert template diskette in the computer connected to the microbalance.
3. Condition balance (this step should be done once a day):
  - a. Place empty capsule on balance and close door. Wait for reading to stabilize (the “mg” on the display appears). Tare the balance.
  - b. Remove and replace capsule and make sure the stabilized weight is 0.000 mg.
  - c. Repeat these steps until the balance is stable.
4. Remove capsule from balance, add sample, and weigh the filled capsule. Repeat until you have the desired weight (which gives 0.15  $\mu\text{g}$  of nitrogen).
5. Enter sample weight on the template.
6. Fold cup; secure sample in it.
7. Repeat steps 4 to 6 for every sample.
8. Note:
  - a. Do not cross-contaminate samples.
  - b. Make sure the spatula and sample area are cleaned using Kimwipes between each sample.
  - c. Always allow balance to stabilize before removing capsule.

## Appendix D. Step-by-Step Procedure of Zero Blank Autosampler Operation

1. Before opening the lid, make sure the isolation valve (between sample chamber and reaction tube) is closed (arrow towards you) and make sure the helium purge isolation valve (on the left) is closed.
2. Open the purge vent on the top of the autosampler to vent the sample chamber.
3. Release the three fittings holding the lid closed and open the lid.
4. Place your samples in the autosampler and close the lid. Load 49 samples for a 50-position carousel, leaving the first hole empty.
5. Secure the lid with three bolts. Screw in all three bolts finger tight, and then lightly tighten two bolts at a time, moving around the lid until they are all completely tight to minimize stress and warping of the lid.
6. Open the helium purge isolation valve (screw down), purge the sample chamber for 5 minutes (min) at a helium flow rate of 298 mL/min. Make sure the purge vent valve (on top) is open. Press your finger on top of the vent valve for 2 s, then release; you should hear the sound of a pressure release.
7. Close the helium purge vent valve (screw down), wait for 2 min for gas pressure at sample chamber and a helium flow rate to stabilize. Close the helium purge isolation valve.
8. Open the isolation valve (arrow toward up). Wait for 2 min for stabilized baseline. With a helium carrier flow rate of 90 mL/min, one should observe  $m/z$  28 on cup 1 at about 50 millivolts,  $m/z$  29 on cup 2 at about 50 to 60 millivolts and  $m/z$  30 on cup 3 at about 140 millivolts to 1 volt (appendix J).

## **Appendix E. Step-by-Step Procedure to Add Sample Information to Sequence Table**

1. Insert diskette with “Weighing Template” on it.
2. Open “Sequence File” under “EA configuration.”
3. Open “Weighing Template” in Excel.
4. “Copy” and “Paste” the list of Sample IDs and sample weight from the template to sequence table.
5. Select appropriate “method” for each sample.
6. Define the reference line and blank line.
7. Start.
8. Give Folder Name: Comment
  - a. Select pre: “Date.”
9. Give File Name:
  - a. Select pre: “Analyzes #.”
  - b. Select post: “Identifier 1.”
  - c. Select: “Print Result.”
10. Click “OK.”
11. Wait for first sample to be analyzed.

## **Appendix F. Step-by-Step Procedure to Retrieve Data from ISODAT 2.0 for LIMS-LSI and for Data Back-Up**

1. For LIMS-LSI:
  - a. Select “Result” in ISODAT 2.0.
  - b. Select the result folder you wish to export data from.
  - c. Select all the individual analyses you want to retrieve by right clicking the selected file.
  - d. Select “Reprocess.”
  - e. Give a file name.
  - f. Add export template previously designed.
  - g. Open.
  - h. Click “OK.” (Reprocessing takes 1–2 min)
  - i. Open Excel.
  - j. Find the file you reprocessed (E-drive, Finnigan, ISODAT 2.0, Global, User, CONFLO II, Interface, Result: File name).
  - k. Open it in Excel.
    1. Save as: “A” drive. Keep in Lotus format.
  - m. Define all reference peaks by adding #1 under column “Is Ref.?”
  - n. Now the file can be imported to LIMS-LSI.
2. For Data Back-Up computer:
  - a. Go to Windows Explorer in ISODAT 2.0 computer.
  - b. Find the drive where the data are (D).
  - c. Choose: “Finnigan.”
  - d. Choose: “User.”
  - e. Choose the inlet system where you have data (Gas Bench or EA).
  - f. Choose “Result folder.”
  - g. Transfer data to memory stick.

## **Appendix G. Step-by-Step Procedure to Transfer Data to LIMS-LSI, to Transfer Data to Back-Up Computer, and to Reevaluate Old Data**

1. Transfer data to LIMS-LSI:
  - a. Start LIMS.
  - b. Choose "Import Data From Instruments."
  - c. Choose mass spectrometer, for example "H --> Hercules."
  - d. Click "Import."
  - e. Select the file that will be imported.
  - f. Depending upon the mass spectrometer, the user may be given the opportunity to select the columns containing the isotope data. Enter column names as appropriate for the isotope or isotopes to be imported.
  - g. Click "Import." Note: Sample ID, sample weight, Peak Area, Analyses #s are automatically imported to LIMS-LSI.
2. Transfer data to Data Back-Up computer:
  - a. Go to Windows Explorer in the Data Back-Up computer.
  - b. Find the drive where your back ups are stored.
  - c. Choose: "RSIL."
  - d. Choose: "Mass. Spec. Analysis Back Up."
  - e. Choose the mass spectrometer. For example, "H," which stands for "Hercules," a Thermo Scientific Delta V Plus IRMS.
  - f. Under that folder create a new folder. The name of the folder should be the date range of the analyses that one wishes to back up.
  - g. Transfer data to that folder.
  - h. Make back ups every 2 weeks or so, as needed.
3. Reevaluate old data:
  - a. Find your samples in the Data Back-Up computer.
  - b. Transfer data by a memory stick to the computer where a virtual version of ISODAT 2.0 is installed.
  - c. Reevaluate your data.

## **Appendix H. Step-by-Step Procedure to Determine and Apply Correction Factors and Evaluate Data**

1. Open “Apply Data Normalization” in LIMS-LSI.
2. Select instrument and isotope, for example “H (Hercules) for 34S.”
3. Select “Query.”
4. Double click on the last sample analyzed on that day.
5. Evaluate data of the reference materials.
6. Choose “Normalize with all References.”
7. Click “Apply Normalization -->.”
8. Print out correction factor sheet.
9. Report daily reference values and correction factors along with date and range of analysis number to the “EA” binder.
10. Go back to LIMS-LSI main menu by closing open windows.
11. Choose “Print/Save Analyses in Progress”:
  - a. Choose appropriate isotope.
  - b. Choose appropriate prefix (W for water, N for nitrogen, S for sulfur).
  - c. Enter sample ID range from “Samples-to-Be-Analyzed” sheet.
  - d. Click Print.
12. Review results and identify samples that need to be re-analyzed.
13. Add the samples needing analyses to the Samples to Be Analyzed:
  - a. Go back to LIMS-LSI main menu.
  - b. Open “Create a Sample List -->.”
  - c. Select appropriate sample list for the mass spectrometer and the samples that are to be analyzed.
  - d. Find each sample in the “List of samples.”
  - e. Change entry in “Repeats” column from 0 to 1.
  - f. Close Windows and exit LIMS-LSI.

## **Appendix I. Step-by-Step Procedure to Check Elemental Analyzer for Leaks**

1. Close the VENT carrier output using the proper cap (provided).
2. Adjust the helium pressure to 150 kPa (regulator is on the front central panel), wait 3 min to equilibrate gas in the system.
3. Close the helium inlet valve by turning the above regulator counter clockwise.
4. If the gauge needle does not move, there is no leak.
5. If the gauge needle moves, indicating the lost of pressure, there is a leak in the system. The decreasing pressure rate accounts for the degree of leakage.
6. Locate the leak by separating and testing the system segment by segment, using the helium detector.

## Appendix J. Daily Checklist

Analyst in charge: \_\_\_\_\_ Date: \_\_\_\_\_

Weekly:

1. Change the working reference gas tank and helium gas tank if the pressure is < 500 psi. Order new ones for a spare; reference gas is SO<sub>2</sub> ANHY by Matheson, helium carrier is zero grade, O<sub>2</sub> is research grade.
2. Change the water and CO<sub>2</sub> trap after approximately 200 samples, or when it is necessary.
3. Check and clean the autosampler carousel.
4. Change the reaction tube after approximately 600 samples, or when it is necessary.

THESE ITEMS ARE TO BE CHECKED OFF AS YOU CHECK THEM DAILY!

1. Check the helium, O<sub>2</sub>, and reference gas flow. [ ]
2. Check the EA reaction-tube temperature (1020 °C). [ ]
3. Change the insertion tube (ash-collector liner) in the reaction tube. [ ]
4. Check the background masses. [ ]

Typical background at N<sub>2</sub>/CO<sub>2</sub> configuration

At emission current: 0.54 mA/0.48 mA

Cups	Mass	Intensity (V)	Mass	Intensity (V)	Resistor (Ω)	Capacity (pF)
1	28	0.010		0	3 × 10 <sup>9</sup>	680
2	29	0.007	18	1.256	3 × 10 <sup>10</sup>	5
3	30	0.140	18	6.818	3 × 10 <sup>11</sup>	2

5. Perform the Ref on/off method three times to stabilize the IRMS. [ ]
6. Check the peak center. If it varies from the default value by more than 50, do a mass calibration. [ ]
7. Analyze Ref on/off method 10 times to stabilize the IRMS (standard deviation should be better than 0.1 ‰). [ ]

Optimal ConFlo II Pressure Setting N <sub>2</sub> (bar)	N <sub>2</sub> signal (V) Mass 29 on Cup 2
0.25	0.173
0.50	0.450
0.75	0.773
1.00	1.180
1.25	1.560
<b>1.50 Optimal setting</b>	<b>2.074</b>
1.75	2.634
2.00	3.199

8. Check the paper in printer. [ ]

## Appendix K. Changing the Insertion Tube

The insertion tube needs to be changed before a template is run.

1. Manually close needle valve near the ion source.
2. On the menu of the Model 2500 EA under “Sp. Fun.,” choose “Std By” and press “enter.” The O<sub>2</sub> and helium gases should be turned off.
3. Unscrew the metal seals under the sample carousel to access the reaction tube.
4. Using the heat protective glove and the wire tool, remove the insertion tube from the reaction tube and place in the metal can. Be aware that the tube will be very hot!
5. Insert clean tube that has 0.5 centimeter (cm) of quartz wool packed at the bottom and tap it down with the wire tool.
6. Replace the carousel and tighten the metal seals with a wrench.
7. Turn the O<sub>2</sub> and the helium back on by pressing “enter” and return the screen to the temperature readout by pressing “Sp. Fun.”
8. Open the needle valve.

## Appendix L. Changing the Water Trap

Change the water trap after every 200 samples.

1. Retrieve quartz turnings, quartz wool, magnesium perchlorate, and a clean water trap from Delta V Plus supplies.
2. Under a hood, pack one end of the clean water trap with 1 cm of quartz wool.
3. On a piece of wax paper, create a mix by volume of approximately 70-percent magnesium perchlorate and 30-percent quartz turnings. Pack the mix into the water trap tube, leaving 1 cm of empty space.
4. Pack the open end of the water trap with quartz wool.
5. Unscrew the red plastic ends from the used water trap and replace it with the clean water trap; be sure to replace the rubber O-rings on the ends of the clean water trap and tighten the red ends.
6. Insert the plug into the vent and watch for any decrease in pressure to check for a leak in the system. If everything looks acceptable after 5 min, replace the vent line.
7. Under the “Sp. Fun.” menu of the model 2500 EA, choose “CNT” (counter) and zero “D” to set back the water-trap counter.
8. Clean the water trap by pushing all the mixture out of the tube into the trash can and use a clean cotton swab to wipe out the inside of the tube. Rinse the tube with DIW, and dry it in the oven. Return this tube to the water trap bag in the drawer.

## Appendix M. Changing the Reaction Tubes

The reaction tubes should be changed approximately every 500 to 600 samples. (The new reaction tubes will need to equilibrate over night before they can provide accurate results; thus, change the reaction tubes at the end of the day.)

1. Manually close the needle valve near the ion source.
2. Turn the Model 2500 EA to “Std By” with both gases off.
3. Lower the oxidation-tube temperature to less than 900 °C; leave the reduction-tube temperature as it is.
4. Retrieve the reaction tubes from the cabinet.
5. Insert the insertion tube into the top of the oxidation tube; there will already be a plastic tube in the reaction tube that should be removed.
6. Place black O-rings on the top of the reaction tubes. Make sure that all O-ring connections are clean and free of any dust.
7. Loosen the top metal seal and remove the bottom metal seal. Go back and completely remove the top seal. It will be very hot! Use oven gloves!
8. Remove quartz reaction tubes and place them in a metal can, place clean reaction tubes in column. Make sure the O-rings are clean; then replace the top.
9. On the bottom, put on the metal pieces with metal O-rings first and then add the clean rubber O-rings. Raise the bottom portion and screw together. Then, tighten the connections with wrenches, and put the metal supports back in place.
10. Turn the gases back on; then, go to “CNT” in the menu under “Spc. Fun.” Set to zero the counter of the oxidation-reaction tube, “B,” as well as that of the reduction-reaction tube “C.”
11. Insert the plug into the vent and close the helium supply. Watch for any decrease in the pressure to check for a leak in the system. If everything looks okay after 3 min, open the helium supply and replace the vent line.
12. Open the needle valve.

## **Appendix N. Step-by-Step Procedure to Report Data**

1. Open “Store Samples in Progress” in LIMS-LSI.
2. Choose the appropriate isotope.
3. Choose sample ID range from “Sample in Progress” print out.
4. Store data.
5. Go back to the main menu of LIMS-LSI.
6. Open “Project” and find the appropriate project in the list.
7. Select “Print Report” and check whether the project report contains all the results. If not, search for the missing results in the database.
8. Select “Results,” transfer data in Excel format or (and) text format to a diskette, and report data to customer through e-mail.
9. Click “Print Report” to print a project report and put it in the “Correspondence” binder along with all the other documents in the “Samples In Progress” binder that are related to this project.