

# Determination of $\delta^{13}\text{C}$ , $\delta^{15}\text{N}$ , or $\delta^{34}\text{S}$ by Isotope-Ratio-Monitoring Mass Spectrometry Using an Elemental Analyzer

Chapter 4 of  
Section D, Geologic Analysis  
**Book 5, Laboratory Analysis**



Techniques and Methods 5-D4

**Cover** Photomicrograph of an ore sample from a zinc-lead-silver mine, an example of the materials analyzed using the methods described in this techniques and methods.

# **Determination of $\delta^{13}\text{C}$ , $\delta^{15}\text{N}$ , or $\delta^{34}\text{S}$ by Isotope-Ratio-Monitoring Mass Spectrometry Using an Elemental Analyzer**

By Craig A. Johnson, Craig A. Stricker, Cayce A. Gulbransen,  
and Matthew P. Emmons

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Techniques and Methods 5–D4

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

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

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## Conversion Factors

International System of Units to U.S. customary units

Multiply	By	To obtain
Length		
meter (m)	3.281	foot (ft)
centimeter (cm)	0.3937	inch (in)
millimeter (mm)	0.03937	inch (in)
micrometer ( $\mu\text{m}$ )	0.00003937	inch (in)
Volume		
liter (L)	33.81402	ounce, fluid (fl oz)
liter (L)	61.02	cubic inch ( $\text{in}^3$ )
milliliter (mL)	0.03382	ounce, fluid (fl oz)
milliliter (mL)	0.06102	cubic inch ( $\text{in}^3$ )
microliter ( $\mu\text{L}$ )	0.0000338	ounce, fluid (fl oz)
microliter ( $\mu\text{L}$ )	0.0000610	cubic inch ( $\text{in}^3$ )
Flow rate		
milliliter per minute (mL/min)	0.03381	ounce, fluid per minute (fl oz/min)
Mass		
milligram (mg)	0.00003527	ounce, avoirdupois (oz)
gram (g)	0.03527	ounce, avoirdupois (oz)
kilogram (kg)	2.205	pound avoirdupois (lb)

Temperature in degrees Celsius ( $^{\circ}\text{C}$ ) may be converted to degrees Fahrenheit ( $^{\circ}\text{F}$ ) as follows:

$$^{\circ}\text{F} = (1.8 \times ^{\circ}\text{C}) + 32.$$

## Abbreviations

$\text{Ag}_2\text{S}$	silver sulfide
$\text{AgNO}_3$	silver nitrate
AVS	acid-volatile sulfur
$\text{BaCl}_2$	barium chloride
$\text{BaCO}_3$	barium carbonate
$\text{BaSO}_4$	barium sulfate
$\text{Cl}^-$	chloride
$\text{CO}_2$	carbon dioxide
$\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$	chromium (III) chloride
CRS	chromium-reducible sulfur
$\delta^{13}\text{C}$	isotopic ratio $^{13}\text{C}/^{12}\text{C}$
$\delta^{15}\text{N}$	isotopic ratio $^{15}\text{N}/^{14}\text{N}$

$\delta^{34}\text{S}$	isotopic ratio $^{34}\text{S}/^{32}\text{S}$
$\delta$ -value	isotopic ratio
DIW	deionized water
EA	elemental analyzer
$\text{Fe}_{0.9}\text{S}$	pyrrhotite
$\text{Fe}_{1.11}\text{S}$	mackinawite
$\text{Fe}_3\text{S}_4$	greigite
$\text{FeS}_2$	pyrite
h	hour
$\text{H}_2\text{S}$	hydrogen sulfide
HCl	hydrogen chloride
$\text{HgCl}_2$	mercury (II) chloride
IAEA	International Atomic Energy Agency
IRMS	isotope-ratio mass spectrometer
kV	kilovolt
<i>M</i>	molar (moles of solute per liter of solution)
<i>m/z</i>	mass-to-charge ratio
min	minute
<i>N</i>	normal (equivalents per liter of solution)
$\text{N}_2$	nitrogen
NaCl	sodium chloride
NBS	National Bureau of Standards
NIST	National Institute of Standards and Technology
$\text{O}_2$	oxygen
PbS	galena
ppt	parts per thousand, permil, or ‰
$R_{\text{SA}}$	isotope abundance ratio in the sample, expressed as numbers of atoms
$R_{\text{ST}}$	isotope abundance ratio in the reference standard, expressed as numbers of atoms
s	second
$\text{SnCl}_2$	tin (II) chloride
$\text{SO}_2$	sulfur dioxide
$\text{SO}_4$	sulfate
TOC	total organic carbon
VCDT	Vienna Cañon Diablo Troilite
VPDB	Vienna Pee Dee Belemnite
wt%	weight percent
ZnS	sphalerite





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## Abstract

This report describes procedures used in the Geology, Geophysics, and Geochemistry Science Center of the U.S. Geological Survey in Denver, Colorado, to determine the stable-isotope ratios  $^{13}\text{C}/^{12}\text{C}$ ,  $^{15}\text{N}/^{14}\text{N}$ , and  $^{34}\text{S}/^{32}\text{S}$  in solid materials. The procedures use elemental analyzers connected directly to gas-source isotope-ratio mass spectrometers. A different elemental-analyzer-mass-spectrometer system is used for  $^{13}\text{C}/^{12}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$  than is used for  $^{34}\text{S}/^{32}\text{S}$  to accommodate differences in reagents, catalysts, and instrument settings.

## Method Summary

This method is used to determine the isotopic ratio  $^{13}\text{C}/^{12}\text{C}$  for total carbon, the isotopic ratio  $^{15}\text{N}/^{14}\text{N}$  for total nitrogen, or the isotopic ratio  $^{34}\text{S}/^{32}\text{S}$  for total sulfur in a wide variety of solid materials. In combination with specific chemical treatments of samples prior to analysis, the method is also used to determine  $^{13}\text{C}/^{12}\text{C}$  of total organic carbon (TOC) in geological or biological materials,  $^{34}\text{S}/^{32}\text{S}$  of dissolved sulfate in aqueous solutions, and  $^{34}\text{S}/^{32}\text{S}$  of acid-extractable sulfides in geological materials. Per convention in the international research community, the results are expressed as  $\delta$ -values as follows:

$$\delta = \left( \frac{R_{SA}}{R_{ST}} - 1 \right) \times 1,000 \quad (1)$$

where  $R_{SA}$  is the atomic abundance ratio  $^{13}\text{C}/^{12}\text{C}$ ,  $^{15}\text{N}/^{14}\text{N}$ , or  $^{34}\text{S}/^{32}\text{S}$  in the sample and  $R_{ST}$  is the atomic abundance ratio  $^{13}\text{C}/^{12}\text{C}$ ,  $^{15}\text{N}/^{14}\text{N}$ , or  $^{34}\text{S}/^{32}\text{S}$  in the reference standard.

Reference standards are Vienna Pee Dee Belemnite (VPDB) for  $^{13}\text{C}/^{12}\text{C}$ , air nitrogen ( $\text{N}_2$ ) for  $^{15}\text{N}/^{14}\text{N}$ , and Vienna Cañon Diablo Troilite (VCDT) for  $^{34}\text{S}/^{32}\text{S}$ .

Two different configurations are described, one dedicated to combined determination of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  and the other dedicated to determination of  $\delta^{34}\text{S}$ . An elemental analyzer (EA) is used to quantitatively convert sample carbon and nitrogen into carbon dioxide ( $\text{CO}_2$ ) and  $\text{N}_2$  gas, respectively, or sample sulfur into sulfur dioxide ( $\text{SO}_2$ ) gas. This is accomplished by weighing an aliquot of powdered material into a tin capsule that is released from a computer-controlled carousel into a heated reaction tube. An injection of oxygen ( $\text{O}_2$ ) as promotes combustion of the sample. Reagents and catalysts control redox conditions, promote quantitative conversion to  $\text{CO}_2$  and  $\text{N}_2$  gases or to  $\text{SO}_2$  gas, and remove excess oxygen and contaminants. The  $\text{CO}_2$  and  $\text{N}_2$  gases or  $\text{SO}_2$  gas are carried in a stream of helium through a water absorbant and then through a chromatographic column where the gases are isolated from contaminants. The gases are then passed to an isotope-ratio mass spectrometer (IRMS) where the isotopic measurement is made by the method of isotope-ratio monitoring. Calibration is accomplished by analyzing reference materials before and after the unknowns. The configurations for  $\text{CO}_2$  and  $\text{N}_2$  and for  $\text{SO}_2$  differ in the reagents and catalysts used, in various instrument settings, and in the standards that are used for calibration.

## Reporting Units and Operational Range

The units of  $\delta$  are parts per thousand, or permil (‰). The VPDB scale for carbon isotopes is formally defined by two reference materials (RM) distributed by the International Atomic Energy Agency (IAEA) and the U.S. Department of Commerce National Institute of Standards and Technology (NIST): National Bureau of Standards (NBS) 19 calcite and NIST RM 8545 lithium carbonate, which have assigned  $\delta^{13}\text{C}$  values of 1.95 ‰ and -46.6 ‰, respectively. The air  $\text{N}_2$  scale for nitrogen isotopes is formally defined by atmospheric  $\text{N}_2$ , which has an assigned  $\delta^{15}\text{N}$  value of 0 ‰. The VCDT scale for sulfur isotopes is formally defined by IAEA-S-1 silver sulfide, which has an assigned  $\delta^{34}\text{S}$  value of -0.3 ‰. For purposes of instrument calibration,  $\delta$ -scales are defined by analyzing

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two or more reference materials upon which  $\delta$ -values have been agreed by international consensus (Brand and others, 2014; Qi and others, 2016; Schimmelmann and others, 2016).

The procedure described here is designed for unknowns within the isotopic ranges commonly observed in nature, which are approximately -130 to 30 ‰ for carbon, -150 to 150 ‰ for nitrogen, and -50 to 130 ‰ for sulfur (Coplen and others, 2002). NIST- and IAEA-distributed standards do not completely encompass these ranges, but they are appropriate for the vast majority of natural compositions. Synthetic materials with more extreme isotopic compositions can also be analyzed, but these require special calibration procedures that are developed on a case-by-case basis.

Although not the principal objective of this procedure, the concentrations of carbon, nitrogen, or sulfur can also be determined from the mass spectrometer results. For the protocols followed in routine isotopic measurements (sample mass  $\leq 50$  milligrams [mg]), the operational ranges for the concentration measurements are 0.8–100 weight percent (wt%) carbon, 0.2–100 wt% nitrogen, and 0.1–100 wt% sulfur.

## Supplies, Instrumentation, and Reagents

### Sample Preparation

To prepare samples for loading into the carousel of the EA, the following supplies are used:

- Tin capsules measuring either 4 millimeters (mm) in diameter by 6 mm in height or 5 mm in diameter by 9 mm in height: these are of high purity so that their contributions to the carbon, nitrogen, and sulfur blanks are insignificant
- Analytical balance: readable to  $\pm 0.00001$  gram (g)
- Vanadium pentoxide powder: used for sulfur isotope analysis, reagent grade so that any sulfur in the powder represents negligible blank
- Spatula, tweezers, powder brush, and wipes: tools for manipulating powders, closing capsules, and cleaning between samples

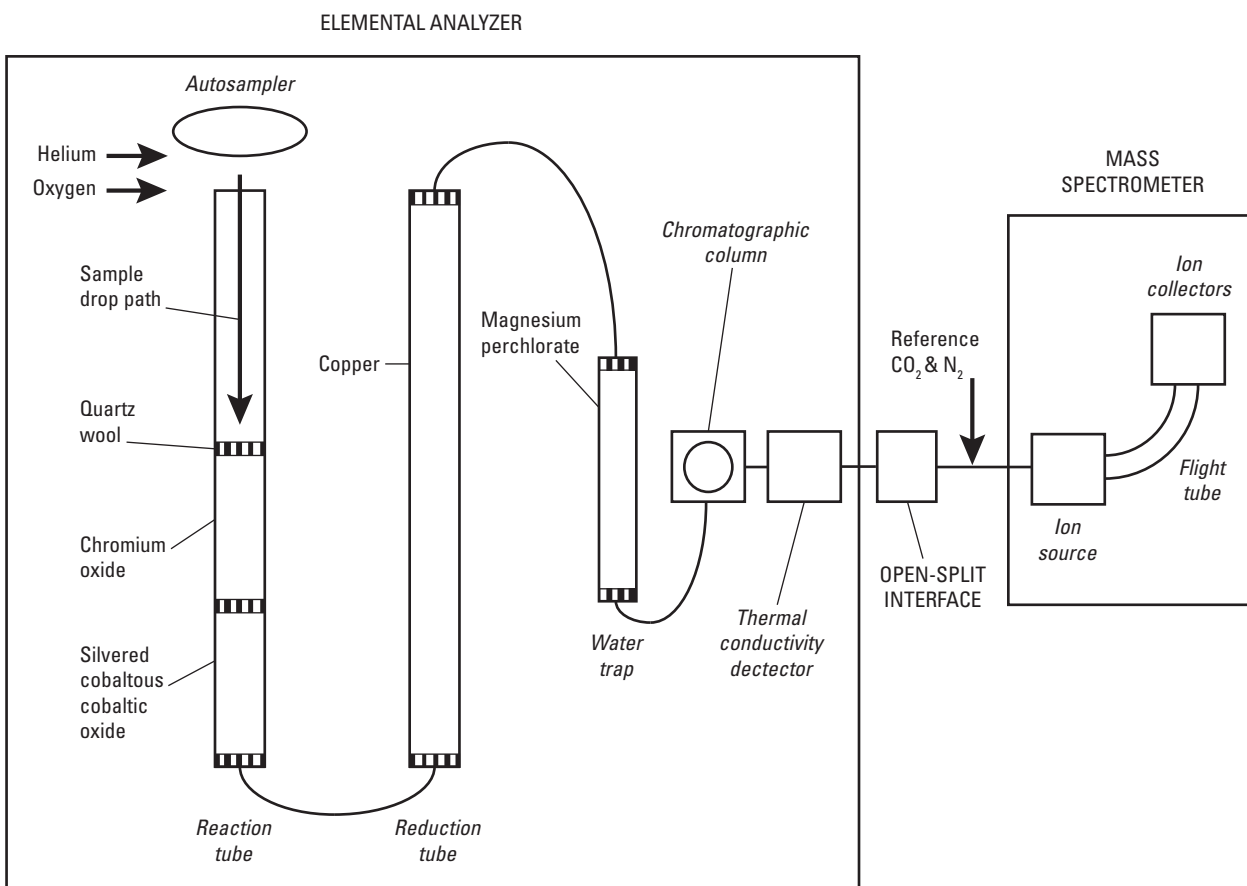
For samples requiring pretreatment, as in the determination of  $\delta^{34}\text{S}$  of aqueous sulfate,  $\delta^{13}\text{C}$  of total organic carbon, or  $\delta^{34}\text{S}$  of acid-extractable sulfides, additional supplies are required, as described in Appendixes 1–4. Appendix 5 gives the procedure that is used to remove residual dirt and oil from feathers, fur, and hair prior to analysis.

## Elemental Analyzer–Isotope-Ratio Mass Spectrometer Device for Determination of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

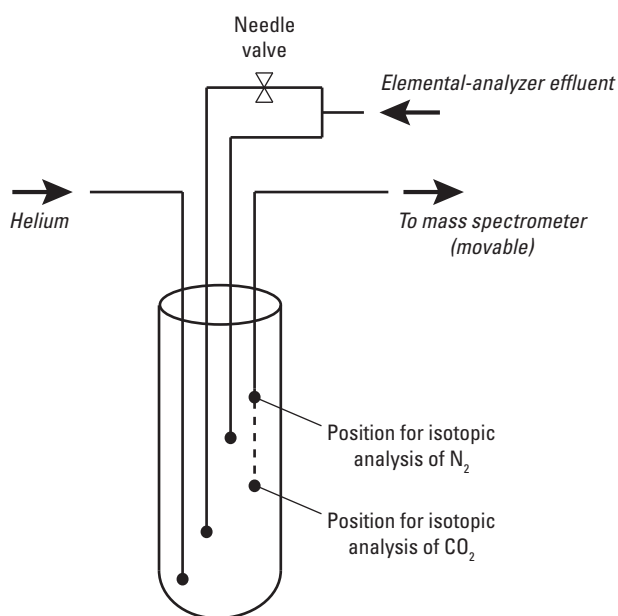
The instrumentation consists of a Carlo Erba NC2500 elemental analyzer coupled to a Micromass Optima IRMS via a custom-built, open-split interface. A schematic drawing of the system is shown in figure 1. The EA is operated at a helium flow rate of 85 milliliters per minute (mL/min). It consists of a 50-position, carousel-type autosampler positioned above a quartz reaction tube (18 mm outer diameter, 2 mm wall). The reaction tube is packed with chromium oxide and silvered cobaltous cobaltic oxide separated by quartz wool and is maintained at a temperature of 1,020 °C. The  $\text{O}_2$  gas that initiates sample combustion is injected upstream of the tube. Downstream of the reaction tube are (1) a reduction tube containing pure copper wires maintained at a temperature of 600 °C, (2) a water trap containing magnesium perchlorate, (3) a chromatographic column (Carbosieve G, 0.5 meters [m] in length) that separates  $\text{CO}_2$  and  $\text{N}_2$  from each other and from contaminant gases, and (4) a thermal conductivity detector.

The effluent from the EA is split into two stainless-steel capillaries that lead to an open-split interface. The interface, which is shown schematically in figure 2, consists of a glass tube sealed at one end into which are inserted (1) the capillaries from the EA, (2) a capillary that emits pure helium gas, and (3) a capillary that leads to the ion source of the mass spectrometer. A needle valve is positioned on the longer of the EA capillaries; closure of this valve diverts a greater proportion of the flow toward the shorter capillary. When the capillary leading to the IRMS is inserted above the shorter EA capillary, the full EA stream is sampled. This configuration is used for isotopic analysis of the  $\text{N}_2$  gases. When the capillary leading to the IRMS is inserted midway between the EA capillaries, a diminished EA stream is sampled. This configuration is used for isotopic analysis of the  $\text{CO}_2$  gases. The  $\text{CO}_2$  stream is diminished because most sample types submitted to the laboratory have high C:N elemental ratios so that an aliquot that produces the optimal amount of  $\text{N}_2$  for mass spectrometry produces too much  $\text{CO}_2$ . Reference  $\text{N}_2$  and  $\text{CO}_2$  gases are introduced to the IRMS downstream of the open-split interface through a Micromass dual-gas injector unit.

The IRMS consists of an electron-impact ion source, a flight tube that passes through a magnetic field, and ion collectors (Faraday cups). Ionized gas molecules are accelerated by a potential gradient of approximately 3 kilovolts (kV) through the magnetic field where the different isotopic species are separated on the basis of mass-to-charge ratio ( $m/z$ ). The  $m/z$  28 and 29 beams are collected to determine



**Figure 1.** Schematic diagram of the elemental analyzer–isotope-ratio mass spectrometer system used to determine  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . [ $\text{CO}_2$ , carbon dioxide;  $\text{N}_2$ , nitrogen]

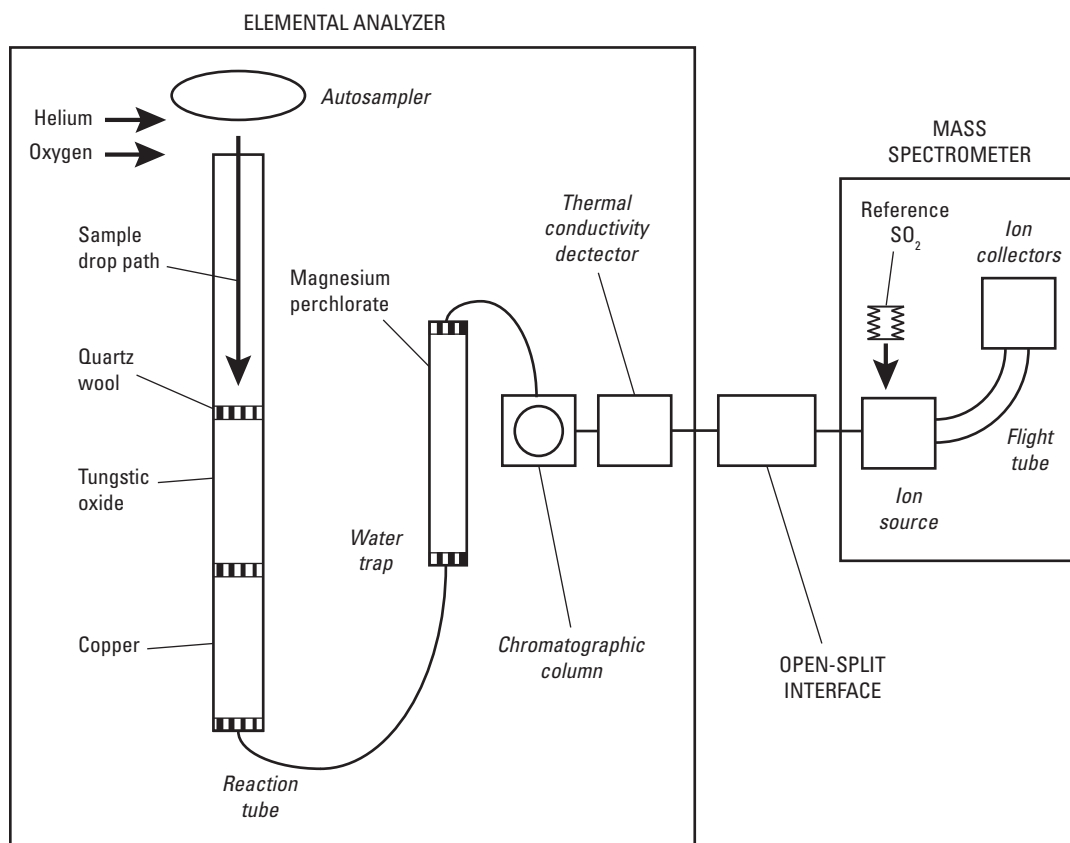


**Figure 2.** Schematic diagram of the open-split interface on the elemental analyzer–isotope-ratio mass spectrometer system used to determine  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . [ $\text{N}_2$ , nitrogen;  $\text{CO}_2$ , carbon dioxide]

the abundances of nitrogen-14 nitrogen-14 ( $^{14}\text{N}^{14}\text{N}^+$ ) and nitrogen-14 nitrogen-15 ( $^{14}\text{N}^{15}\text{N}^+$ ), and the  $m/z$  44, 45, and 46 beams are subsequently collected to determine the abundances of carbon-12 oxygen-16 oxygen-16 ( $^{12}\text{C}^{16}\text{O}^{16}\text{O}^+$ ), carbon-13 oxygen-16 oxygen-16 ( $^{13}\text{C}^{16}\text{O}^{16}\text{O}^+$ ), and carbon-12 oxygen-16 oxygen-18 ( $^{12}\text{C}^{16}\text{O}^{18}\text{O}^+$ ). The raw data consist of time-integrated currents recorded by each of the Faraday cups, corrected for backgrounds.

### Elemental Analyzer–Isotope-Ratio Mass Spectrometer Device for Determination of $\delta^{34}\text{S}$

The instrumentation consists of a Thermo Scientific Flash 2000 elemental analyzer coupled to a Thermo Scientific Delta Plus XP IRMS via a Thermo Scientific ConFlo III open-split interface. A schematic drawing of the system is shown in figure 2. The EA is operated at a helium flow rate of 90 mL/min. It consists of a 32-position, carousel-type autosampler positioned above a quartz reaction tube (18 mm outer diameter, 2 mm wall). The reaction tube is packed with tungstic oxide and copper wire separated by quartz wool and is maintained at a temperature of 980 °C.



**Figure 3.** Schematic diagram of the elemental analyzer–isotope-ratio mass spectrometer system used to determine  $\delta^{34}\text{S}$ . [ $\text{SO}_2$ , sulfur dioxide]

The  $\text{O}_2$  gas that initiates sample combustion is injected upstream of the tube. Downstream of the reaction tube are (1) a water trap containing magnesium perchlorate, (2) a chromatographic column (HayeSep Q, 0.5 m in length) that separates the  $\text{SO}_2$  from contaminant gases, and (3) a thermal conductivity detector.

The effluent from the EA is transmitted to the ConFlo III interface, where it feeds a glass tube into which are inserted (1) a fused-silica capillary that emits pure helium gas and (2) a fused-silica capillary that leads to the ion source of the IRMS. This last capillary moves in and out of the glass tube so that the IRMS samples pure helium, the EA effluent, or a mixture of pure helium and EA effluent. Reference  $\text{SO}_2$  is delivered to the ion source from a variable-volume bellows that is part of the inlet system of the IRMS.

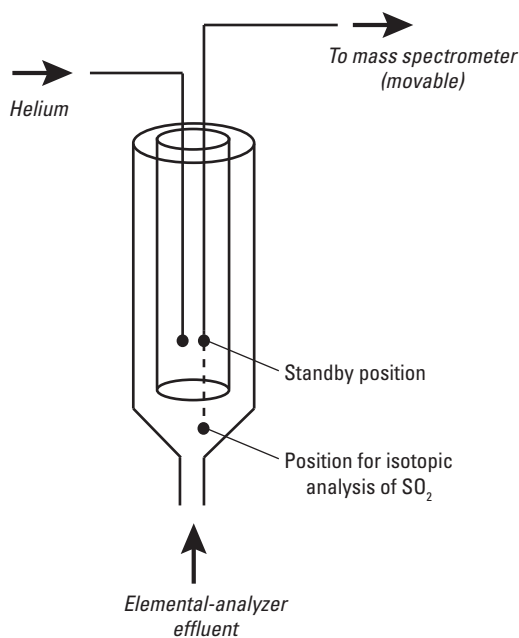
The IRMS consists of an electron-impact ion source, a flight tube that passes through a magnetic field, and ion collectors (Faraday cups). Ionized gas molecules are accelerated by a potential gradient of approximately 3 kV through the magnetic field where the different isotopic species are separated on the basis of  $m/z$ . The  $m/z$  64 and 66 beams are collected to determine the abundances of sulfur-32 oxygen-16 oxygen-16 ( $^{32}\text{S}^{16}\text{O}^{16}\text{O}^+$ ) and sulfur-34 oxygen-16 oxygen-16 ( $^{34}\text{S}^{16}\text{O}^{16}\text{O}^+$ ). The raw data consist of time-integrated currents recorded by each of the Faraday cups, corrected for backgrounds.

## Sample Containers, Preparation, and Analysis

Samples are provided as dry powders that normally require no special preservation (see Appendixes for exceptions). The minimum sample requirement is the amount of material corresponding to 0.4 mg contained carbon (for example, 1.0 mg of L-glutamic acid [ $\text{C}_5\text{H}_9\text{NO}_4$ ]), 0.1 mg contained nitrogen (for example, 1.0 mg of L-glutamic acid), or 0.06 mg contained sulfur (for example, 0.4 mg barium sulfate [ $\text{BaSO}_4$ ]). In some cases, smaller samples can be analyzed if linearity tests (reproducibility of replicates of varying mass) demonstrate that accuracy and precision are not compromised.

Powders are weighed into tin capsules (see Appendix 3 for an exception that requires silver capsules), and the masses are recorded on a laboratory worksheet. For determinations of  $\delta^{34}\text{S}$ , 1–2 mg of reagent-grade vanadium pentoxide powder are added to the capsule. The capsule is crimped closed using tweezers (the capsules are never touched by fingers), and the crimped capsule is then gently pinched to form a tight ball.

A titer plate or similar holding tray is used to organize unknowns and standards for an automated run (autorun), which normally processes about 50 capsules and requires



**Figure 4.** Schematic diagram of the open-split interface on the elemental analyzer–isotope-ratio mass spectrometer system used to determine  $\delta^{34}\text{S}$ . [ $\text{SO}_2$ , sulfur dioxide]

5–6 hours of instrument time. Two or three warmup samples are placed first in the titer plate. Two different reference standards are then prepared; duplicates of each are placed in plate positions corresponding to the start of the autorun and the end of the autorun. At least one aliquot of one reference standard is also prepared and placed near the middle of the autorun. A minimum of two unknowns are duplicated per autorun. Autoruns can consist of more capsules than positions in the autosampler because emptied carousel positions can be refilled once the autorun has advanced sufficiently.

When determining  $\delta^{34}\text{S}$ , memory effects can be significant when the isotopic composition changes dramatically from one sample to the next (for example, a difference in  $\delta^{34}\text{S}$  values of 50 ‰ or more). The risk of inaccuracy is especially important where standards of widely divergent  $\delta$ -values are analyzed in sequence. As a precaution against this problem, an intermediate composition material is routinely analyzed between the high and low  $\delta$ -value standards. As a precaution against memory in analysis of unknowns, samples with extreme  $\delta^{34}\text{S}$  values are reanalyzed in duplicate or triplicate in subsequent autoruns so that any memory effects can be identified and the affected analyses rejected.

Checks of the EA-IRMS are performed prior to analysis. These include periodic monitoring of background gases in the mass spectrometer ( $m/z$  28, 32, and 40 should be below 0.1, 0.05, and 0.05 nanoamperes, respectively); daily monitoring of the supplies of helium, oxygen, and reference gases; and periodic monitoring of the reproducibility of reference gas injections (stability test) and the

reproducibility of replicates of varying mass (linearity test). In addition, laboratory records are checked to ensure that the reaction and reduction tubes have sufficient remaining capacity to complete the autorun. Reaction tubes are replaced after 150–175 samples for carbon and nitrogen and after 100–120 samples for sulfur. Reduction tubes are replaced after 150–175 samples. Sample ash is removed from reaction tubes after three autoruns or more frequently, depending on the type and size of samples being analyzed.

Sample numbers and masses are typed into the mass spectrometer software, and the autorun is started. Results for the warmup samples are examined in real time to ensure that the EA-IRMS system is operating properly.

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

For each analysis, the mass spectrometer software calculates raw  $\delta$ -values from the time-integrated Faraday-cup currents for the sample gases and reference gases and the isotopic composition of the reference gas (assigned previously on the basis of analyses of NIST or IAEA standards). Autoruns for  $\delta^{34}\text{S}$  require a drift correction to account for progressive oxidation of reaction-tube copper. The drift is normally linear and measures about 1 ‰ over the course of an autorun (Grassineau and others, 2001). Autoruns for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  can also require a small drift correction. Finally, small corrections are made using working curves developed from the reference standards analyzed along with the unknowns. These corrections account for small instrumental fractionations that can vary from one day to the next. The magnitude of the final corrections, which consist of an offset factor and a linear-expansion factor, is normally less than 1 ‰.

The accuracy of  $\delta^{34}\text{S}$  determinations can be reduced for samples with low sulfur contents (less than approximately 1 wt%) due to complications in the combustion reaction and poor chromatography (for example, Kester and others, 2001; Fry and others, 2002; Leckrone and Ricci, 2007). When low-sulfur samples are being analyzed, accuracy is best evaluated by comparing representative results to results obtained in separate experiments in which the sulfur is extracted by acid digestion (Appendix 4), Eschka fusion (Tuttle and others, 1986), or some other technique and then analyzed isotopically as either silver sulfide ( $\text{Ag}_2\text{S}$ ) or  $\text{BaSO}_4$ .

The time-integrated currents can also be used to calculate the concentrations of carbon, nitrogen, or sulfur in the samples. Response factors are developed from the results for isotope reference materials of known chemical composition or results for other materials that have certified elemental concentrations.



Each autorun includes multiple aliquots of reference materials that serve as quality-control samples. Aliquots analyzed at the start and end of the autorun are used for calibration purposes. The results for these are also used as independent checks of reproducibility (see following paragraphs). The results for other aliquots of reference materials, such as those analyzed near the middle of the autorun, are used to check accuracy as well as reproducibility.

In most cases, the  $\delta$ -values of the reference materials used for calibration bracket the  $\delta$ -values of the unknowns so that extrapolation from working curves is avoided. The accepted values for NIST- or IAEA-distributed reference materials are taken from the most recent recommendations (for example, Brand and others, 2014; Qi and others, 2016; Schimmelmann and others, 2016). Where in-house reference materials are used, the accepted values are taken from previous analyses in which the in-house materials were analyzed using NIST- or IAEA-distributed materials as standards.

Acceptance criteria are as follows. First, the major ion beam for an analysis must attain a peak height within the range of linear behavior of the EA-IRMS. Second, replicate analyses must agree to within  $\pm 0.3\text{‰}$  (one standard deviation) for carbon and nitrogen, and  $\pm 0.4\text{‰}$  (one standard deviation) for sulfur. If a standard deviation exceeds  $0.3\text{‰}$  (for carbon and nitrogen) or  $0.4\text{‰}$  (for sulfur) and the material was analyzed three or more times, the outlier can be censored and the standard deviation reevaluated. If the result is then in compliance, the outlier-rejected mean is deemed acceptable. Third, results for reference material aliquots that were not used for calibration must be within  $\pm 0.6\text{‰}$  for carbon and nitrogen or  $\pm 0.8\text{‰}$  for sulfur of the accepted values. Analyses that fail to meet the acceptance criteria require corrective action.

Corrective action includes consultation with the supervisor to investigate the performance of the EA-IRMS and to evaluate sample-specific questions. Examples of sample-specific questions are (1) whether the mass of material placed into the tin capsule needs to be adjusted, (2) whether the material may be isotopically heterogeneous, and (3) whether the matrix is potentially problematic. Corrective action normally involves reanalysis if sufficient material is available. If satisfactory results cannot be obtained by reanalysis, the results may be recorded and reported, at the discretion of the supervisor, with an appropriate explanatory comment. Unsatisfactory results on reference materials may indicate that equipment maintenance is required. Common maintenance tasks include removal and cleaning of the ion source, replacement of the source filament, and servicing of the vacuum system.

Quality-assurance measures include training of all analysts by either a supervisor or an experienced analyst. Reagents, catalysts, and consumables are either obtained from sources known to be reliable or, if obtained from an unproven source, are tested prior to analyzing unknowns. Schedules that have been established for replacement of reagents and catalysts are strictly maintained. Any irregular performance of the EA-IRMS is noted by the analyst and, if necessary, is discussed with a supervisor to assess possible impact on data quality.

## Data Management and Records

Data are exported from the IRMS software by the analyst in a form that is compatible with Microsoft Excel®. The analyst then uses Excel to make drift corrections, develop working curves, and make final corrections. The results are saved as a separate worksheet in the same Excel file. File names consist of a prefix indicating the element(s) analyzed followed by the consecutive autorun number (for example, “NC056” for the 56th  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  autorun, or “SO2\_099” for the 99th  $\delta^{34}\text{S}$  autorun).

After the corrected results have been saved, the file is passed to a supervisor who checks for errors and verifies that quality-control criteria have been met. Data are then released to the appropriate scientist and the digital file is copied to a U.S. Geological Survey file server for archiving.

## Safety Precautions

With respect to personal protection, safety glasses and gloves are recommended for weighing samples. In addition, a lab coat is recommended when handling reagents and catalysts. Safety data sheets are available for all the chemicals used in the EA-IRMS procedure as part of the ChemSW CISPro chemical-inventory system. Analysts may consult these documents for other precautions.

With respect to electrical hazards, there is no danger of shock during normal operation of the equipment. However, electrical conductors are exposed inside the instruments. Only authorized service personnel, or individuals trained in electronics, are allowed to inspect or repair the electronic components.

With respect to chemical hazards, the catalysts and reagents used in the reaction tubes, reduction tubes, and water traps contain metals to which exposure should be limited. Exposure should also be limited to the vanadium pentoxide that is added to samples for determination of  $\delta^{34}\text{S}$ . This can be accomplished by wearing gloves, safety glasses, and a lab coat whenever these materials are handled. The quartz wool used in tube packing is a potential irritant, so a dust mask is warranted in addition to other personal protective equipment when packing combustion and reduction tubes.

Gas cylinders should be handled only by trained personnel. The cylinders must be secured when in use or in storage and must be capped when in transport.

## Waste Disposal

Analysis of samples creates hazardous wastes that must be transferred to a satellite accumulation area for removal by an individual authorized to perform such work. Hazardous wastes include

1. spent reaction tubes, which contain sample ash, quartz wool, chromium, silver, cobalt, tin, copper, and (or) vanadium;
2. spent reduction tubes, which contain quartz wool and copper; and
3. spent magnesium perchlorate, which is a powerful oxidizer.

## Revision History

This document is the original publication of this standard operating procedure.

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## Appendix 1. Precipitation of Aqueous Sulfate as Barium Sulfate ( $\text{BaSO}_4$ ) for Determination of $\delta^{34}\text{S}$

This procedure is adapted from procedures that have been published by Sakai (1977) and Carmody and others (1998), among others. Caution is warranted for (1) waters containing significant organic sulfur (greater than approximately 1 percent of total dissolved sulfur), (2) waters containing sulfide (ratio of hydrogen sulfide [ $\text{H}_2\text{S}$ ] to sulfate [ $\text{SO}_4$ ] exceeding 0.025 on a weight basis), and (3) waters that are low enough in sulfate to require ion exchange (see step 2) and high in chloride (ratio of chloride [ $\text{Cl}$ ] to sulfate exceeding 300 on a weight basis; chloride interferes with sulfate retention on the column).

1. Pour a volume of water corresponding to between 6 and 21 milligrams (mg) of sulfate into a beaker of appropriate size. This will yield 15–50 mg of barium sulfate ( $\text{BaSO}_4$ ). If the quantity of water exceeds 1 liter (L), which will be the case if the sulfate concentration is less than 6 milligrams per liter, follow the ion exchange procedure in step 2; otherwise skip to step 3.
2. Ion exchange
  - a. Pack a column measuring approximately 1 centimeter (cm) in diameter and 10–20 cm in length with a strong anion-exchange resin (BioRad AG® 1–X8 resin in 50–100 mesh size and in chloride [ $\text{Cl}^-$ ] form, or equivalent resin).
  - b. Rinse column with deionized water (DIW).
  - c. Pass sample water through column at rate of about 5 milliliters (mL) per minute (about 1 drop per second).
  - d. Elute the column into a 250-mL beaker using 100 mL of 0.42 molar ( $M$ ) sodium chloride ( $\text{NaCl}$ ) solution.
  - e. Rinse the column with 25 mL of DIW and collect this with the previous eluent.
  - f. Proceed to step 3; rinse column with 100 mL of DIW in preparation for next sample.
3. Add stir bar and place beaker on hot plate.
4. While solution is heating, acidify with 1 normal ( $N$ ) hydrochloric acid ( $\text{HCl}$ ) to pH 4 (down to 3.5 is acceptable, but  $> 4$  is not acceptable). Add acid slowly so that bubbling over beaker wall is avoided. This step evolves dissolved carbonate as carbon dioxide ( $\text{CO}_2$ ) and avoids coprecipitation of barium carbonate ( $\text{BaCO}_3$ ) with  $\text{BaSO}_4$ .
5. As solution nears boiling temperature, add 0.5  $M$  barium chloride ( $\text{BaCl}_2$ ) solution a few drops at a time. Add about 500 percent excess barium, which corresponds to approximately 2 mL for a yield of 15–50 mg  $\text{BaSO}_4$ .
6. Continue heating and stirring just below boiling for 30 minutes.
7. Allow to cool overnight.
8. Recover the precipitate on a 0.45-micrometer membrane using DIW to rinse.
9. Dry the precipitate at 70 °C for at least 2 hours.
10. Store dried  $\text{BaSO}_4$  in glass vial until analysis by elemental analyzer–isotope-ratio mass spectrometer (EA-IRMS).

Hazardous waste to be collected and transferred to satellite accumulation area for later disposal:

1. Spent solution from filtration step containing barium chloride, hydrochloric acid, and (in the case of ion exchange) sodium chloride.



## Appendix 2. Removal of Carbonate Carbon from Sedimentary Rocks Prior to Determination of $\delta^{13}\text{C}$ of Total Organic Carbon

Sedimentary rocks can contain a variety of different carbonate minerals, including calcite, aragonite, dolomite, ankerite, siderite, or rhodochrosite. These minerals vary widely in their ease of dissolution in acid. The acid-washing procedure described here is aggressive enough that it ensures complete removal of carbonate carbon from finely powdered samples, irrespective of the specific carbonate mineral (Nieuwenhuize and others, 1994; Leventhal, 2001). When applied to sediments or biological materials, the procedure can also remove low-molecular-weight organic compounds and potentially bias the total-organic-carbon isotopic analysis as a consequence. When applied to sedimentary rocks, this is not generally a problem because the organic material is more thermally mature and less susceptible to acid attack.

1. Weigh approximately 200 milligrams (mg) of finely powdered sample into a 50 milliliter (mL) beaker.
2. Add 30 mL of 3 normal (*N*) hydrochloric acid (HCl). Cover with a watch glass.
3. Boil gently for 1 hour, then allow to cool overnight.
4. Recover insoluble residues on 0.45 micrometer membrane, rinsing thoroughly with deionized water to remove HCl residues.
5. Dry the residues at 70 °C for at least 2 hours.
6. Store dried powder in glass vial until analysis by elemental analyzer–isotope-ratio mass spectrometer (EA-IRMS).

Hazardous waste to be collected and transferred to satellite accumulation area for later disposal:

1. Spent hydrochloric acid solution from filtration step, which may contain trace metals if sulfide minerals are present in the sample.



## Appendix 3. Removal of Carbonate Carbon from Soils Prior to Determination of $\delta^{13}\text{C}$ of Total Organic Carbon

Several methods have been developed for removal of carbonate from soil samples prior to isotopic or chemical analysis of total organic carbon (TOC). The most commonly used methods are acid washing and acid fumigation. Acid washing entails digestion of ground samples in dilute hydrochloric acid (HCl) followed by rinsing with deionized water and oven drying. This method has been shown to give inaccurate carbon-isotope results in some cases due most likely to selective removal of low-molecular-weight organic compounds. Acid fumigation is the preferred method for carbon isotope analysis because it does not affect low-molecular-weight organic compounds. Whether acid fumigation affects nitrogen-isotope results has not been thoroughly studied, so it is advisable to split samples prior to fumigation so that untreated material can be used for nitrogen isotope analysis. Our acid-fumigation procedure is patterned after the procedure of Harris and others (2001).

1. Oven-dry samples and homogenize thoroughly with a ball mill or mortar and pestle.
2. Weigh approximately 10 milligrams (mg) of soil into a 5-millimeter by 9-millimeter tin foil capsule. Analyze by elemental analyzer–isotope-ratio mass spectrometer (EA-IRMS) to establish the approximate concentrations of total carbon and total nitrogen. Use these data to calculate the sample mass required for optimal analysis of weight percent (wt%) TOC, wt% nitrogen, carbon-isotope composition of TOC, and nitrogen-isotope composition.
3. Reanalyze the samples by EA-IRMS to obtain definitive values for wt% TOC, wt% N, and nitrogen-isotope composition.
4. Weigh the amount of sample required for the carbon-isotope analysis, plus 10 percent, into a silver foil capsule (5 mm in diameter by 9 mm in height), and transfer to an open well in a microtiter plate. Do not close the capsule.
5. Dispense 50 microliter of deionized water into each capsule.
6. Place the microtiter plate into a vacuum dessicator (5 liters) containing a 100 milliliter (mL) beaker containing 50 mL of concentrated HCl (12 molar).
7. Expose samples to HCl vapors for 6 hours.
8. Remove the microtiter plate from dessicator and oven dry overnight at 60 °C.
9. Carefully remove each silver foil capsule and fold lengthwise, crosswise, and lengthwise again to securely enclose contents. If foil begins to tear or is fragile, place inside a tin foil capsule and seal.
10. Transfer folded capsules to an acid-free microtiter plate. The plate used for acid fumigation can be reused in subsequent fumigations.
11. Analyze acid-fumigated samples by EA-IRMS to determine wt% TOC and carbon-isotope composition of TOC.
 

Hazardous waste to be collected and transferred to satellite accumulation area for later disposal:

  1. Spent hydrochloric acid.



## Appendix 4. Extraction of Acid-Volatile Sulfur and Chromium-Reducible Sulfur from Sediments and Sedimentary Rocks for Determination of $\delta^{34}\text{S}$

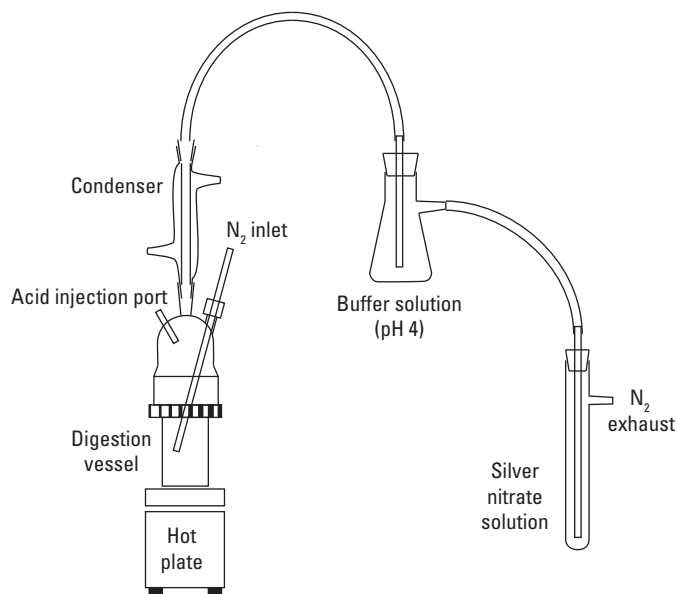
The following procedures extract acid-volatile sulfur (AVS) and chromium-reducible sulfur (CRS) from sediments or sedimentary rocks and convert the sulfur to solid silver sulfide ( $\text{Ag}_2\text{S}$ ) for determination of sulfur-isotope composition. AVS and CRS are operational classifications of reduced-sulfur species. The AVS procedure will recover sulfur from (1) free hydrogen sulfide ( $\text{H}_2\text{S}$ ) in sediment pore waters (if sulfidic pore waters are present); (2) the iron sulfides mackinawite ( $\text{Fe}_{1.11}\text{S}$ ), pyrrhotite ( $\text{Fe}_{0.9}\text{S}$ ), and greigite ( $\text{Fe}_3\text{S}_4$ ); and (3) monosulfides of metals other than iron, including sphalerite ( $\text{ZnS}$ ) and galena ( $\text{PbS}$ ). The CRS procedure will recover sulfur from all sources of AVS in a given sample as well as from pyrite (cubic  $\text{FeS}_2$ ) and marcasite (orthorhombic  $\text{FeS}_2$ ). The AVS and CRS procedures can be performed sequentially to distinguish the isotope compositions of monosulfide minerals (AVS method) and disulfide minerals (CRS method on AVS residues) in a given sample. Organic sulfur and sulfate sulfur are not recovered in either procedure. The procedure described here is adapted from methods used by Canfield and others (1986), Tuttle and others (1986), and Rice and others (1993). The required apparatus is shown schematically in figure A4.1.

1. Weigh an amount of powdered sample corresponding to about 5 milligrams of sulfur into a reaction vessel.
2. Place 25 milliliters (mL) of 5 percent silver nitrate ( $\text{AgNO}_3$ ) solution in a side-arm test tube, cork with glass-tube stopper, and connect to the extraction apparatus.
3. For AVS,
  - a. Add 1 gram tin (II) chloride ( $\text{SnCl}_2$ ) to the vessel if the sample is a sedimentary rock; add nothing if the sample is a modern sediment.
  - b. Add 5 mL ethanol to the reaction vessel and attach to the extraction apparatus.
  - c. Load a 60 mL syringe with 50 mL of 6 normal (*N*) hydrochloric acid ( $\text{HCl}$ ).

For CRS,

- a. Prepare a 1 molar (*M*) solution of chromium (III) chloride ( $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ ), and acidify to 0.5 *N*  $\text{HCl}$ .

- b. Pass the resulting solution through a column of granular zinc that has been amalgamated with 0.25 *M* solution of mercury (II) chloride ( $\text{HgCl}_2$ ) (a Jones reductor, see Mendham and others, 2000).
  - c. Add 5 mL ethanol to the reaction vessel and attach to the extraction apparatus.
  - d. Load a 60 mL syringe with 25 mL of 6 *N*  $\text{HCl}$  and 25 mL of chromium (III) chloride solution.
4. Attach syringe to the entry port above the reaction vessel, start nitrogen ( $\text{N}_2$ ) purge gas, and allow gas to bubble through to the silver nitrate solution for 3–5 minutes.
    - a. Inject the contents of the syringe and turn hot plate to high. Brown flakes of  $\text{Ag}_2\text{S}$  will eventually precipitate in the silver nitrate solution.
    - b. Continue boiling the sample until silver nitrate solution becomes clear again (1.5–3 hours).
    - c. Turn off hot plate and  $\text{N}_2$  gas. Vent the apparatus.
    - d. Recover the  $\text{Ag}_2\text{S}$  on a 0.45 micrometer membrane, rinsing with deionized water.



**Figure A4.1.** Schematic diagram of the apparatus used to extract acid-volatile and chromium-reducible sulfur for determination of  $\delta^{34}\text{S}$ . [ $\text{N}_2$ , nitrogen]

## 16 Determination of $\delta^{13}\text{C}$ , $\delta^{15}\text{N}$ , or $\delta^{34}\text{S}$ by Isotope-Ratio-Monitoring Mass Spectrometry Using an Elemental Analyzer

- e. Dry at 70 °C for a minimum of 2 hours, homogenize by grinding in a mortar and pestle, and store in a glass vial until analysis by elemental analyzer–isotope-ratio mass spectrometer (EA-IRMS). The  $\text{Ag}_2\text{S}$  can be weighed after drying, and the result used to calculate the yield of acid-volatile sulfur or chromium-reducible sulfur for the sample.

Hazardous waste to be collected and transferred to satellite accumulation area for later disposal:

1. Slurry of insoluble mineral powder and acid solution containing chromium, tin (AVS only), chloride, and lesser amounts of metals liberated from the sample (iron, possibly zinc, lead, and others).
2. Spent silver nitrate solution.
3. If renewal of the Jones reductor is required, spent mercuric chloride solution.







## Appendix 5. Procedure for Cleaning Feathers, Fur, and Hair Prior to Determination of $\delta^{13}\text{C}$ , $\delta^{15}\text{N}$ , or $\delta^{34}\text{S}$

Samples of feather, fur, and hair typically contain residual dirt and oil that need to be removed prior to determination of the carbon-, nitrogen-, or sulfur-isotope composition. The procedure described here, which consists of triple washing in a 2:1 (volume-to-volume ratio) chloroform:methanol mixture, is adapted from procedures and recommendations given by Paritte and Kelly (2009). The entire procedure should be performed in a fume hood.

1. Prepare a 2:1 (volume:volume) chloroform:methanol mixture. This can be done conveniently by pouring 600 milliliters chloroform and 300 milliliters methanol into a 1-liter beaker.
2. Half-fill three beakers with the solvent mixture, choosing beaker sizes appropriate for the feather (or fur or hair) samples to be cleaned.
3. Using large forceps, immerse sample in first beaker. Swish for 10 seconds, release and regrip on a different part of the sample, and then swish for 3 seconds more. Lift sample from beaker and gently shake off excess solvent.
4. Repeat step 3 using second beaker.
5. Repeat step 3 using third beaker.
6. Pause a few seconds to allow for evaporation, then place sample into glass scintillation vial. Label the vial.
7. If additional material is needed for this particular sample, repeat steps 3 through 6 with another fragment from the same sample.
8. After 5–15 samples have been cleaned, the solvent in the first beaker will become cloudy. Replace with fresh solvent, and then use this beaker third, as the final wash, for the next sample. This practice ensures that the last immersion of samples is in the cleanest solvent.
9. When all samples have been cleaned, set vials toward the rear of the fume hood, and allow solvent to evaporate for 1 hour.
10. Firmly cap vials and store until analysis by elemental analyzer–isotope-ratio mass spectrometer (EA-IRMS).  
Hazardous waste to be collected and transferred to satellite collection area for later disposal:
  1. Spent 2:1 (volume:volume) chloroform:methanol mixture.



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