

Automated Determination of the Stable Carbon Isotopic Composition ($\delta^{13}\text{C}$) of Total Dissolved Inorganic Carbon (DIC) and Total Nonpurgeable Dissolved Organic Carbon (DOC) in Aqueous Samples: RSIL Lab Codes 1851 and 1852

Chapter 20 of
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Book 10, Methods of the Reston Stable Isotope Laboratory

Techniques and Methods 10–C20

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By Kinga M. Révész and Daniel H. Doctor

Techniques and Methods 10–C20

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

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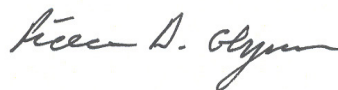
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Foreword

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 Fractionation in Hydrologic Processes 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.



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Conversion Factors

Multiply	By	To Obtain
Length		
micrometer (μm)	0.00003937	inch (in.)
millimeter (mm)	0.03937	inch (in.)
meter (m)	3.281	foot (ft)
Volume		
microliter (μL)	0.0610210^{-3}	cubic inch (in^3)
milliliter (mL)	0.06102	cubic inch (in^3)
liter (L)	61.02	cubic inch (in^3)
Mass		
gram (g)	0.03527	Ounce, avoirdupois (oz)
milligram (mg) = 10^{-3} g	3.527×10^{-5}	ounce (oz)
gram (g) = 10^{-3} (kg)	3.527×10^{-2}	ounce (oz)
Temperature		
Celsius ($^{\circ}\text{C}$)	$^{\circ}\text{F} = 9/5 (^{\circ}\text{C}) + 32$	Fahrenheit ($^{\circ}\text{F}$)
Pressure (force/area)		
kilopascal (kPa)	9.869×10^{-3}	atmosphere, standard (atm)
kilopascal (kPa)	1.450×10^{-1}	pound-force/square inch (psi)
kilopascal (kPa)	1.000×10^{-2}	bar
Temperature in degrees Celsius ($^{\circ}\text{C}$) may be converted to degrees Fahrenheit ($^{\circ}\text{F}$) as follows: $^{\circ}\text{F} = (1.8 \times ^{\circ}\text{C}) + 32$		

Acronyms and Abbreviations

ACS	American Chemical Society
ANSI	American National Safety Institute
CF-IRMS	continuous-flow isotope ratio mass spectrometer
DI	deionized
DIC	dissolved inorganic carbon
DOC	dissolved organic carbon
EA	elemental analyzer
EPA	U.S. Environmental Protection Agency
GC	gas chromatograph
g/L	gram per liter
g/mol	grams per mole
HDPE	high density polyethylene
IRMS	isotope-ratio mass spectrometer
KHP	potassium hydrogen phthalate
LIMS-LSI	Laboratory Information Management System for Light Stable Isotopes
mg/g	milligram per gram
mg/L	milligram per liter
mL/min	milliliter per minute
MSDS	Material Safety Data Sheets
NBS	National Bureau of Standards
NDIR	nondispersive infrared
NFPA	National Fire Protection Association
NWQL	National Water Quality Laboratory (USGS)
per mill, ‰	1/1000
pF	picofarad
ppm	parts per million
pptt	parts per ten thousand
QA	quality assurance
QC	quality control
RSIL	Reston Stable Isotope Laboratory
SOP	standard operating procedure
TIC	total inorganic carbon
TOC	total organic carbon
USGS	U.S. Geological Survey
VOA	volatile organic analysis
VOC	volatile organic compound
VPDB	Vienna Pee Dee Belemnite

Symbols

Ω	ohm
$<$	less than
$>$	greater than
\leq	less than or equal to
\geq	greater than or equal to

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Summary of Procedures

The purposes of the Reston Stable Isotope Laboratory (RSIL) lab codes 1851 and 1852 are to determine the total carbon mass and the ratio of the stable isotopes of carbon ($\delta^{13}\text{C}$) for total dissolved inorganic carbon (DIC, lab code 1851) and total nonpurgeable dissolved organic carbon (DOC, lab code 1852) in aqueous samples. The analyses can be done together in one sample; in that case preservative should not be added to the sample. However, the practice is to collect the samples separately, preserve them differently, and analyze the samples for total DIC and total nonpurgeable DOC separately. The analysis procedure is automated according to a method that utilizes a total carbon analyzer as a peripheral sample preparation device for analysis of carbon dioxide (CO_2) gas by a mass spectrometer. First described by St-Jean (2003), this procedure has been modified for the RSIL. The carbon analyzer used in the RSIL method is an OI Analytical Aurora 1030W, referred to herein as the Aurora. The Aurora is configured in series with, and controlled by, a continuous-flow isotope ratio mass spectrometer (CF-IRMS). This configuration provides a fully automated analysis of total carbon mass and $\delta^{13}\text{C}$ with no operator intervention, additional sample preparation, or other manual analysis. To determine the DIC, the Aurora transfers a specified sample volume to a heated (70°C) reaction vessel with a preprogrammed volume of 10% phosphoric acid (H_3PO_4), which allows the carbonate and bicarbonate species in the sample to dissociate to CO_2 . The CO_2 from the reacted sample is subsequently purged with a flow of helium gas that sweeps the CO_2 through an infrared CO_2 detector and quantifies the CO_2 ; this value is stored by the Aurora software. The CO_2 is then carried through a high-temperature (650°C) scrubber reactor, a series of water traps, and ultimately to the inlet of the mass spectrometer. For the analysis of total dissolved organic carbon, the Aurora analyzer performs a second step on the sample in the heated reaction vessel during which a preprogrammed volume of sodium persulfate ($\text{Na}_2\text{S}_2\text{O}_8$) is added, and the hydroxyl radicals oxidize the organics to CO_2 . The Aurora determines the carbon mass of DIC and DOC in parts per million separately, and the CF-IRMS determines the carbon isotope ratio of CO_2 . The Aurora is a totally automated system. Using heated persulfate oxidation technology, samples containing 2 parts per million (ppm) to 30,000 ppm of carbon are analyzed. The IRMS is a Thermo-Finnigan (Thermo Scientific) Delta^{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) 44, 45, and 46 simultaneously. The ion beams from CO_2 are as follows: m/z 44 = $\text{CO}_2 = {}^{12}\text{C}^{16}\text{O}^{16}\text{O}$; m/z 45 = $\text{CO}_2 = {}^{13}\text{C}^{16}\text{O}^{16}\text{O}$ primarily; m/z 46 = $\text{CO}_2 = {}^{12}\text{C}^{16}\text{O}^{18}\text{O}$ primarily.

Reporting Units and Operational Range

Variations in isotope-amount ratios typically are small. Stable isotope-amount ratios commonly are determined as the relative difference in the ratio of the amount of the less abundant isotope (usually the heavy isotope) to the amount of the more abundant isotope (usually the light isotope) of a sample with respect to a reference. This relative difference is designated δE , which is a shortened form of $\delta(^{\text{E}}/^{\text{E}})$ or $\delta(^{\text{E}}/\text{E})$ and is defined according to equation 1:

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

where $\delta(^i E)$ refers to the delta value of isotope number i and j (heavy and light isotopes, respectively) of element E of sample B relative to an international measurement standard (std); $N_B(^i E)/N_B(^j E)$ and $N_{std}(^i E)/N_{std}(^j E)$ are the ratios of the isotopes in unknown B and the international measurement standard. A positive $\delta(^i E)$ value indicates that the unknown is more enriched in the heavy isotope than the international measurement standard. A negative $\delta(^i E)$ value indicates that the unknown is depleted in the heavy isotope relative to the international measurement standard. The symbol $\delta(^i E)$ is commonly shortened to $\delta^i E$ and has been reported in parts per hundred (percent), parts per thousand (‰ or per mill), and parts per ten thousand (pptt). For stable carbon isotope amount ratios, $\delta^{13}C$ is defined as follows:

$$\delta^{13}C = \delta(^{13}C) = \delta(^{13}C/^{12}C) = \frac{N(^{13}C)_B / N(^{12}C)_B - N(^{13}C)_{std} / N(^{12}C)_{std}}{N(^{13}C)_{std} / N(^{12}C)_{std}} \quad (2)$$

The primary reference materials for relative carbon isotope-ratio measurements ($\delta^{13}C$) are NBS 19 calcium carbonate and L-SVEC lithium carbonate, which have consensus $\delta^{13}C$ values of +1.95 ‰ and -46.6 ‰ on the Vienna Pee Dee Belemnite (VPDB) scale (Hut, 1987; Coplen, 1996; Coplen and others, 2006). By calibrating the “in house” reference materials of sodium bicarbonate ($NaHCO_3$; RSIL lab codes C-1916 and C-11781) for DIC measurement, and/or sucrose and KHP (RSIL lab codes G10212 and G-15728, respectively) for DOC, and interspersing those among unknown samples, the δ values can be determined on normalized $\delta^{13}C$ scales. The $\delta^{13}C$ measurements are made with a CF-IRMS, which alternately measures the isotope ratios of the sample CO_2 gases and one or more injections of the “working reference” CO_2 gas. Values of $\delta^{13}C$ typically are reported in parts per thousand (‰).

The routine analysis for both DIC and DOC requires a minimum of 50 mL of water with a 2 ppm concentration of carbon in a sample; however, the system is capable of analyzing samples with a carbon concentration as small as 1 ppm or less by applying a built-in cryogenic trap. The accuracy is greater than ± 0.5 ‰. The $\delta^{13}C$ values of in-house reference materials range from approximately -5 ‰ to -28 ‰, which covers much of the range of $\delta^{13}C$ values of natural samples. The precision and accuracy of the results were acceptable (± 0.3 ‰), and no memory effects were observed.

Reference Materials and Documentation

Reference Materials Used, Storage Requirements, and Shelf Life

In-house reference materials are used when aqueous carbon-bearing samples are analyzed. These in-house reference materials are C-11781 $NaHCO_3$ with a $\delta^{13}C = -2.7$ ‰ and C-1916 $NaHCO_3$ with a $\delta^{13}C = -20.63$ ‰. When organic carbon samples are analyzed, “in house” isotopic reference materials are used; G-10212 sucrose with a $\delta^{13}C = -10.39$ ‰ and G-15728 KHP, commercially available with approximate $\delta^{13}C = -28.67$ ‰. The isotopic compositions of these reference materials are calibrated as solids against internationally distributed isotopic reference materials using an elemental analyzer (EA). All of these reference materials are diluted in deionized water to create 1, 2.5, 5, 10, 20, 30, 50, 100 (and so forth) ppm as carbon solutions; the concentration depends on the sample concentration ranges, which are interspersed with samples. Organic reference solutions are kept in amber bottles, and they are refrigerated to avoid microbial activity that could alter the isotope and concentration value of the standard. The inorganic reference material is distributed into 40-mL sample vials with no headspace and that have a low-diffusion butyl rubber septum to avoid air contamination by leak or diffusion. The solid reference materials’ shelf lives are indefinite; the diluted ones have approximately 1 year for DIC and 3 months for DOC.

Documentation

All calibration results are stored in the Laboratory Information Management System for Light Stable Isotopes (LIMS-LSI) (Coplen, 2000) under sample identifiers C-1919, C-11781, G-10212 and G-10229 for DIC, and G-10212 and G-15728 for DOC.

Labware, Instrumentation, and Reagents

Preparatory labware, apparatus, and reagents include 1-L or 500-mL volumetric flasks, 40-mL serum bottles with Teflon-lined silicone septa, 0.2-micron polysulfone GD/X syringe filters with 60-mL syringe, 5-percent ACS grade phosphoric acid, ACS grade sodium persulfate, and a balance capable of measuring chemicals with a precision of 0.001 mg. The analytical apparatus consists of three units: (1) total carbon analyzer with computer software, (2) ConFlo interface, and (3) CF-IRMS, with computer software.

The total carbon analyzer is an OI Analytical Aurora 1030W (Wet Oxidation) DOC analyzer equipped with a rotary auto sampler that holds 88 samples in 40-mL serum bottles.

Typically, 5 mL of sample is transferred from each 40-mL vial to a reaction chamber by an automated syringe pump. The volume of sample digested in a single analysis can vary between 1 and 10 mL, depending on the carbon concentration of interest (DIC or DOC) in the sample.

For both DIC and DOC analysis, digestion of the sample begins with an acidification step. In the closed reaction chamber, 10 percent H_3PO_4 is added to the sample—usually between 0.5 and 1.5 mL, depending on the concentration of DIC in the sample—to quantitatively react the DIC to CO_2 . For DIC isotope analysis, the exsolved CO_2 is purged from the liquid and carried through a Nafion permeation tube by helium (He) with a flow rate of 110 mL/min, then through the nondispersive infrared (NDIR) detector for concentration measurement before being sent to the mass spectrometer. For DOC analysis, sodium persulfate solution is added to oxidize the organic compounds in the water after acidifying the sample and removal of the DIC; the CO_2 gas produced from acidification of DIC is vented out. On the other hand, if only the DIC is analyzed, the oxidation process is eliminated. The gases generated from the samples, including the CO_2 of interest, are carried by a flow of He through a reaction tube heated to 650 °C. This scrubber reactor has native copper (Cu) as a reduction agent within the tube so that any nitrogen oxides are converted into elemental nitrogen, and a second scrubbing compound (cobaltous silver) removes corrosive chlorine gas and sulfur-bearing gases (primarily H_2S). At the outlet of the reaction tube the remaining sample gas mixture (dominantly CO_2 , N_2 , and H_2O) passes through a water trap containing magnesium perchlorate and anhydrous calcium sulfate (indicating Drierite) separated by quartz chips. The gas mixture, now dominantly N_2 and CO_2 , flows through a 1.0-m packed gas chromatograph (GC) column kept at room temperature that separates permanent gases (for example, Poraplot PQS). The GC column separates the gases and ensures that pure CO_2 gas passes separately through to the ConFlo II open split and into the IRMS (fig. 1).

The Finnigan (Thermo Scientific) ConFlo II provides the interface for coupling the Aurora analyzer to the CF-IRMS with an open-split arrangement (fig. 2). The principle of an open split is that it reduces the 110 mL/min He flow needed to operate the Aurora analyzer to a 0.3 mL/min He flow needed to operate the IRMS. The lower flow for the IRMS is achieved by placing the end of the capillary that leads to the IRMS directly into the flow of He coming from the Aurora analyzer. The narrow diameter and the length of this capillary limit the flow of gas into the IRMS. Furthermore, extra capillaries carrying reference gas or He 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 He if the sample is too large.

The IRMS is a Thermo-Finnigan (Thermo Scientific) Delta^{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, δ 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 a flight tube, 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 44, 45, and 46, simultaneously. The resistor-capacitor combination on the electrometer used to measure these masses are as follows: $3 \times 10^8 \Omega$ and 680 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 Finnigan (Thermo Scientific) ISODAT 2.0 software is designed to (1) initiate the Aurora analyzer sequence and initiate each sequential sample analysis by remote start signal, (2) control the ConFlo II interface to inject reference gases at the desired time and dilute the sample if desired, (3) operate the IRMS, (4) acquire data from the IRMS, and (5) calculate delta values.

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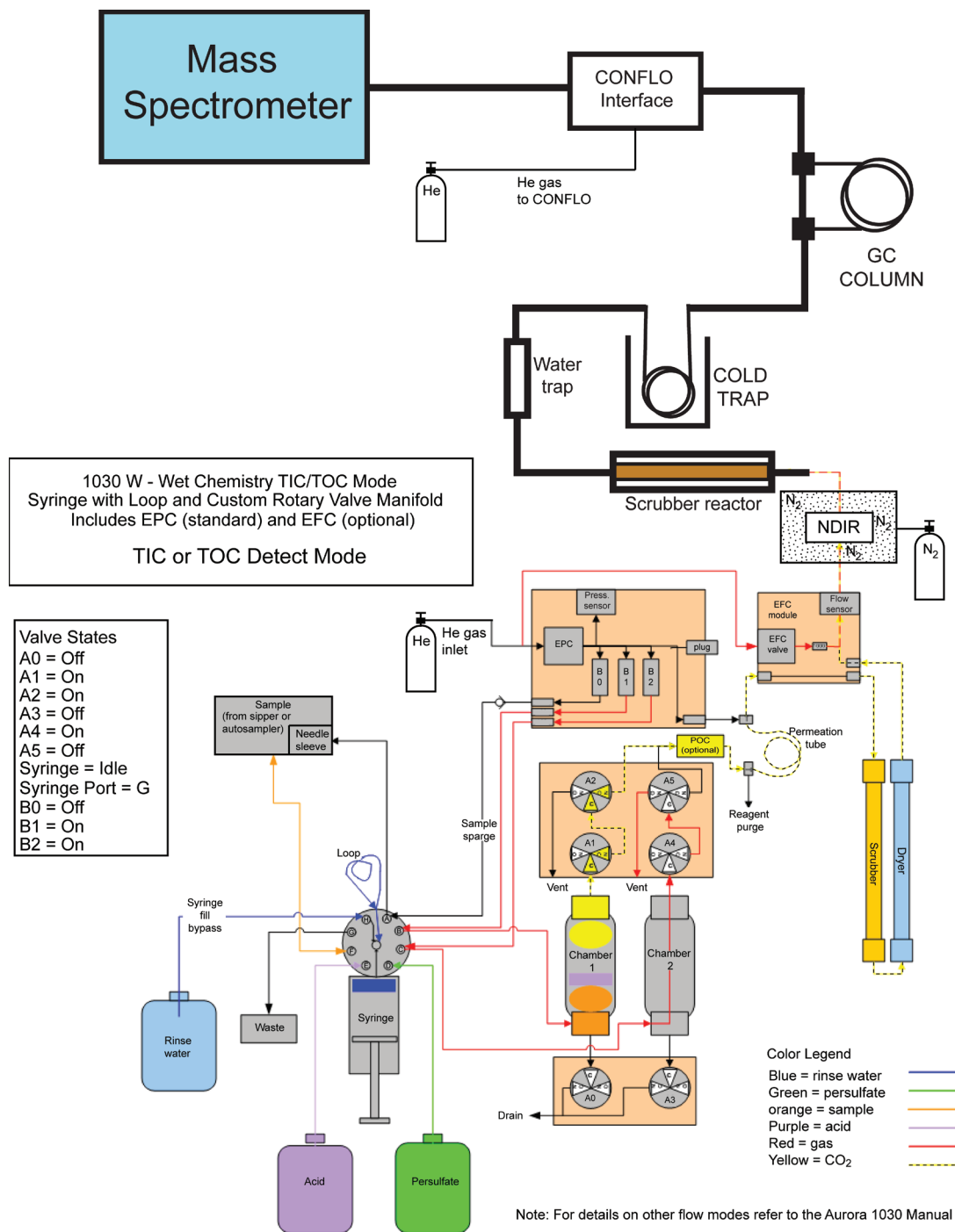


Figure 1. Flow diagram of the Aurora 1030W connected to a mass spectrometer (Aurora flow diagram used with permission of OI Analytical).

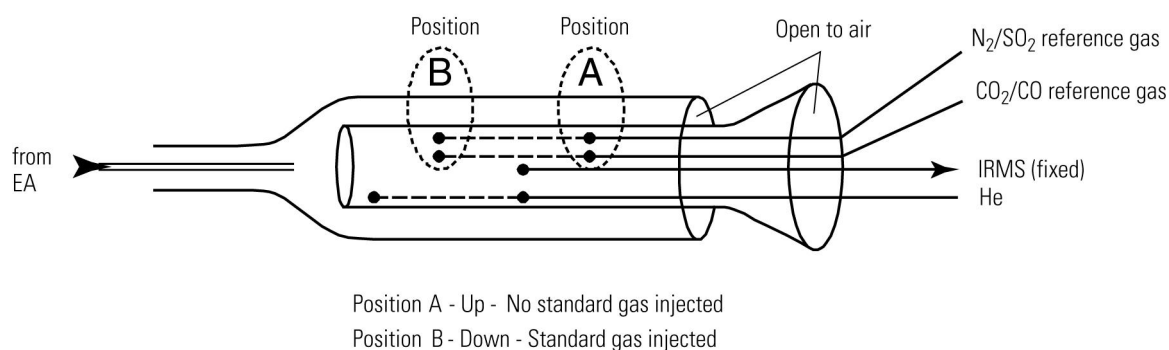


Figure 2. Diagram of a Finnigan (Thermo Scientific) ConFlo II open split (used with permission of Thermo Electron Corp.).

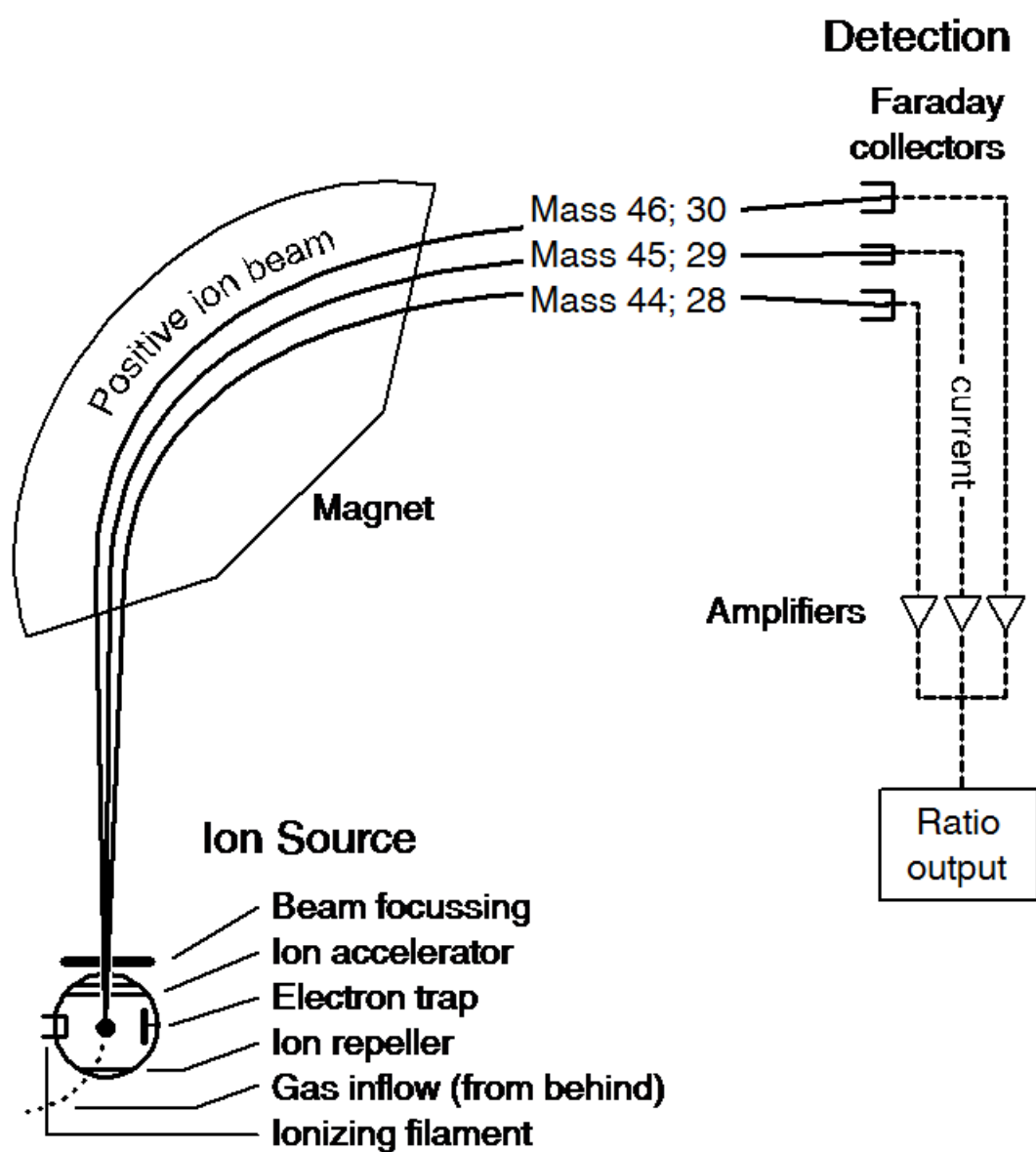


Figure 3. Schematic of a continuous flow isotope-ratio mass spectrometer (CF-IRMS) (modified from Clark and Fritz, 1997).

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

Sample Containers, Preservation, and Handling Requirements

For both DIC and DOC carbon isotope analysis, 40-mL U.S. Environmental Protection Agency (EPA) volatile organic compound (VOC) glass vials are used with open-top septa-lined caps, clear glass for DIC and amber glass for DOC. Teflon-lined silicone septa should be replaced by hand-punched black butyl-rubber septa for DIC samples to prevent gas exchange through the septum. Filter “sterilization” through 0.2- μm pore size is recommended for all samples. Although copper sulfate has previously been used to preserve DIC samples (Winslow and others, 2001), this is no longer recommended due to the possibility of precipitation of a copper carbonate phase at higher DIC concentrations (greater than ~ 30 ppm C), resulting in isotopic fractionation of the DIC remaining in solution.

For DOC samples, 0.1 to 0.2 mL of concentrated phosphoric acid should be added as a preservative. Because acid is added to the DOC samples, the same aliquot cannot be used for DIC analysis. Simultaneous analysis of DIC and DOC on the same samples is not recommended.

A 60-mL syringe and syringe filter cartridges are normally used for sampling. Recommended filter cartridge type is 0.2 μm GD/X Syringe Filters, a low protein binding filter for aqueous samples. Individual cartridge units include two prefilters [graded density Multigrade GMF 150 (10:1 μm) and a polyethersulfone final filter (0.2 μm)] that allow filtration of samples with high suspended or fine sediments that may be difficult to filter otherwise.

For DIC samples, the sample vials must be filled completely without headspace or visible bubble. CO_2 within even a very small headspace in the vial could alter the isotopic composition of the sample. In contrast, DOC samples should be filled to the neck of the sample vial, and not be completely full to avoid spillage of excess acidified sample when the cap is screwed down onto the vial.

Samples should be stored refrigerated and analyzed within 3 months. Detailed instruction for sample containers, sample preservation, and handling requirements are provided in appendix A and appendix B.

Sample Preparation and Time Requirements

Sample preparation involves logging-in samples to the LIMS-LSI, creating a sample work-order sheet, and grouping samples according to similar concentration ranges for isotopic analysis. Given the wide variability of carbon concentrations typically encountered in natural waters, it is necessary to group samples of similar concentration ranges together in order to apply the proper analytical method for isotopic analysis. 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.”

Step-by-step procedures for sample log-in are given in appendix C.

An Excel® sample workbook is created. Step-by-step procedures and examples are shown in appendix D. The time requirement for performing the sample preparation described is approximately 1 to 3 days.

Performing the Analysis and Time Requirements

All samples are prepared and analyzed in batches of 40 per day for a 24-hour run, but the batches can be doubled if desired. All samples are analyzed in replicate. Two in-house isotopic reference materials with different $\delta^{13}\text{C}$ values are analyzed along with the samples. These two reference materials containing different amounts of material are analyzed within the sequence of samples to determine a mass-dependent correction factor. No less than two reference materials at multiple concentrations are analyzed for every 10 unknowns. The amount of carbon in the reference materials must bracket the range of the amount in the samples. Therefore, one must pre-analyze every sample to obtain the carbon concentration unless the customer provides it.

The analytical procedure involves loading the auto sampler carousel with vials in sequence according to the “Template,” loading a new sample heading (including sample volumes to be analyzed) into the “Sequence Table” both in the Aurora software and in the ISODAT 2.0 IRMS software, and choosing the appropriate “Method” (appendix E). Appendix F describes going through the daily checklist and starting the sequence of acquisition. After the analyses are completed, data are exported to an Excel worksheet from the ISODAT 2.0 database and blank correction, which is caused by the possible carbon sources from the deionized (DI) water by which the reference material was dissolved, and mass-dependent corrections are determined (appendix G). Data are exported to a removable disk or “memory stick” and are transferred to the LIMS-LSI system. The original data

from the ISODAT 2.0 database are transferred to the Data Back-Up computer. Final 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:

$$-2.7\text{ ‰} = m \times \delta^{13}\text{C}_{\text{C-11781} - \text{workingrefgas}} + b \quad (3)$$

$$-20.63\text{ ‰} = m \times \delta^{13}\text{C}_{\text{C-1916} - \text{workingrefgas}} + b \quad (4)$$

The values -2.7 ‰ and -20.63 ‰ are the assigned values of the two reference materials (C-11781 and C-1916), respectively, and both are NaHCO_3 . The $\delta^{13}\text{C}_{\text{ref} - \text{workingrefgas}}$ values in equations 3 and 4 are the mean daily delta values of the respective international reference materials relative to the working reference gas after blank and mass-dependent correction; b is the additive delta-value correction factor, and m is the mass-dependent expansion coefficient correction factor (see appendix G). Never analyze a single reference material; one-point calibration is not possible because a blank correction cannot be applied. For DIC samples, the blank correction is only applied to the reference standards to account for the carbon blank in the water used to make the standard solutions; the blank correction is not applied to the samples.

When DOC is analyzed, the reference materials are G-10212 with a -10.44 ‰ $\delta^{13}\text{C}$ value relative to VPDB and G-15728 with an approximate -24 ‰ $\delta^{13}\text{C}$ value relative to VPDB. Blank correction in DOC analysis is similar to that for DIC analysis. The DOC blank is composed of organic carbon from two sources: (1) the water used to mix the DOC reference standards, and (2) the acid reagent added to each aliquot in the first step of the analysis that removes the DIC from the aliquot. The amount of DOC blank in the reference standards is quantified by running a DI water sample at the beginning of the run, and the correction procedure is the same as described above for the DIC blank correction. The amount of DOC blank added from the addition of acid reagent will be the same for each reference and sample analysis, provided that this is specified to be the same amount for every analysis in the Aurora method. Therefore, correction for the acid blank does not need to be applied separately because it will affect every standard and sample equally. The analyst might note that the offset in isotopic composition of the standard reference materials from the known isotopic composition may be greater for DOC analysis than for DIC analysis due to this addition of organic carbon from the acid reagent.

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 laboratory supervisor reports data (appendix I). The time requirement for performing the analyses described above is a minimum of 2 days.

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

Preparing Reagents

Solutions of 10-percent phosphoric acid and 100 g/L sodium persulfate reagents should be prepared as needed. It is recommended to store the reagents in glass containers to reduce the possibility of organic carbon addition to the reagents that may arise from storage in plastic containers. Removal of excess background organic carbon from the acid reagent may be accomplished by adding 1 mL of 100 g/L sodium persulfate reagent to 1 L of 10-percent phosphoric acid, then heating the solution to just below boiling temperature ($\sim 98\text{ }^\circ\text{C}$) within a fume hood and stirring continuously for more than 1 hour to completely break down the oxidative capacity of the added persulfate. Caution is needed in this process due to the highly volatile nature of the heated acid/oxidant mixture, with proper lab safety protections in place. **NOTE: This procedure should not be used if hydrochloric acid is used as an acid reagent; oxidation will result in the production of toxic chlorine gas.**

Problematic Samples

Problematic samples include those of unknown carbon concentration and (or) elevated salinity (above 0.02 molar; Aiken, 1992). In the case of unknown carbon concentrations (DIC or DOC), the C concentration needs to be determined by a preliminary analysis in order to determine the sample amounts that give an appropriate amount of CO_2 gas, and to be able to determine the amount of dilution of CO_2 with He, if needed. For analysis of samples with C concentrations below 10 ppm, please notify the lab ahead of time.

Samples that have any headspace or bubbles within the vials prior to analysis may be problematic for DIC analysis and should be flagged as such. Noting the size of the bubbles or headspace in the vials prior to analysis can be helpful when evaluating the final data. Small bubbles (less than a few millimeters in diameter) are generally not problematic, but larger ones can indicate leakage from the vials, which may cause isotopic fractionation of the DIC.

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Samples of low pH that may contain a large percentage of DIC as $\text{CO}_2(\text{aq})$ are not problematic for DIC analysis given that the analytical system is closed to loss or addition of gases to the atmosphere. However, for such samples, care is needed during sampling to prevent loss of CO_2 from the sample water to the atmosphere, which may fractionate the DIC in solution (Doctor and others, 2008). For samples of high CO_2 concentrations (>10 ppm as $\text{CO}_2(\text{aq})$), this analytical method may not be appropriate due to potential loss of CO_2 during sampling.

Samples containing high salt concentration (halides) are problematic for DOC analysis, but not for DIC analysis. Halides (for example, Cl^- , Br^-) compete with the oxidative capacity of the sodium persulfate (Aiken, 1992; McKenna and Doering, 1995; Osburn and St-Jean, 2007). This may result in incomplete oxidation of the DOC in the sample. The oxidation of these ions also results in the production of corrosive and hazardous halide gases, such as chlorine gas. To ensure complete oxidation of saline samples, a molar ratio of persulfate oxidant to chloride of 50 to 1 is recommended in the sample aliquot to be digested. For example, if running seawater samples an increase in persulfate reagent (100 g/L) to 5 mL/sample, with an increase in the acid to 4 mL/sample, will normally provide enough persulfate so that the organic carbon in a 1-mL sample completely oxidizes. An increase in the reaction time to the maximum allowed by the Aurora software (10 minutes) will also aid in complete oxidation.

Interferences

A potential interference is the nitrogen oxide (NO_x) gases produced and (or) released from samples during purging of CO_2 . These NO_x gases will be reduced to N_2 with the heated scrubbed unit interfaced between the Aurora analyzer and the ConFlo. Poor results may occur if the copper scrubber has passed its capacity. See the “Troubleshooting and Bench Notes” section below for details.

Note that for DOC samples, only the isotopic composition of the total (bulk) dissolved, nonpurgeable organic carbon in the sample is reported. This analytical procedure does not provide compound-specific results unless the sample is only in a pure, non-volatile, soluble phase. As mentioned above, incomplete oxidation due to halides may result in erroneous carbon isotopic composition of DOC. DIC is not affected by halides; however, samples that have not been properly filtered may be affected by particulate carbonate solid phases contributing to the overall DIC measured.

Troubleshooting and Bench Notes

The most common problems with this analytical technique are listed in appendix J. The system is complicated because it consists of two separate analytical instruments connected together, the OI Analytical Aurora analyzer and the CF-IRMS. Both systems require a thorough check of their parameters, such as helium flow, temperature, leaks, and linearity.

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 of the RSIL (file path: LIMS C:\RSIL\vacuum pumps).

Maintenance of the Aurora analyzer includes (1) changing the sampling loop as needed, (2) changing the Nafion water trap, (3) changing the Cu and Drierite moisture indicator connected to the reaction chamber on the front side of the Aurora, and (4) changing the contents of the scrubber reactor in the furnace as needed. For procedures regarding maintenance of the Aurora analyzer, please see the Aurora User Manual. A PDF copy of the manual is stored in the RSIL on the computer running ISODAT in the Desktop folder, via the Shortcut named “User Manual”. The procedure for changing the scrubber reactor in the furnace is provided in appendix K.

A list of some basic troubleshooting issues is given in appendix J. This list and possible solutions are taken from the Aurora 1030 User Manual, chapter 7.

Changing the Cu scrubber in the reaction furnace as needed is described in appendix K.

The IRMS also requires a daily check (appendix F). 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 will be analyzed within 3 months after the samples are collected. Any remaining sample is discarded after analysis because samples are not stable after analysis. The ISODAT sample files from the IRMS computer are kept indefinitely on two separate 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 can be achieved: (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. The database provides an audit trail for the RSIL.

Quality Control Samples

Samples are prepared and analyzed in batches—a maximum of 60 per load in two aliquots each. Each batch contains approximately six reference solutions at the beginning, three depleted in ^{13}C and three enriched in ^{13}C in different concentrations, and later in the batch every 10 samples. The last analyses of a batch are of two reference solutions, one depleted in ^{13}C and one enriched in ^{13}C ; see appendix D for a template of a batch analysis. All of these solid reference materials are calibrated to one or more international reference materials, and the solutions are prepared in different concentrations (appendix L). They serve as quality control (QC) samples. The preparation and analytical cost of these isotopic reference materials is the responsibility of RSIL. Quarterly, RSIL prepares a summary of results of these isotopic reference material solutions.

At the end of the analyses, the analyst (1) examines the computer printouts for indications of analytical problems, (2) determines the blank and mass-dependent or linearity correction based on the results of the reference solutions, and (3) applies the correction to the samples before the data are transferred to LIMS-LSI. For blank correction, the analyst applies an isotope mass balance calculation originated from the DI water that the standards were prepared with. In the calculation, the RSIL uses the DI water values (peak area and delta values) analyzed in the sequence to correct all reference materials to their known values. For linearity correction after the blank correction, the RSIL plots the reference standards delta value versus the concentration (peak area) for both reference standards separately. The best linear fit will be the linearity of the mass spectrometer. The slope of the two reference standards should be the same or close to each other. If it is not the same, the RSIL (1) averages the two slopes and applies the average to all samples, (2) determines the additive and expansion correction factors by the LIMS-LSI, and (3) applies correction factors to the isotopic data. The values of these correction factors, along with the date and range of analysis numbers, are recorded manually in the laboratory notebook 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 (for example $\Delta\delta \leq 0.3$ for DIC or ≤ 0.5 ‰ for DOC).

Acceptance Criteria for All Quality Control 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 ≤ 0.3 ‰ for DIC, ≤ 0.5 ‰ for DOC, then use the mean delta value of replicate analyses.
- If there are three or more analyses, delete the outlier and recalculate.

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- If standard deviation of this recalculation is ≤ 0.3 ‰ for DIC, ≤ 0.5 ‰ for DOC, use the mean from this recalculation.
- If none of the above is the case, the result is not accepted and corrective action is required.

Corrective Action Requirements

If the analyst finds any problem with the reference-sample data, the analyst contacts the supervisor. The troubleshooting 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 Quality Assurance/Quality Control Functions for Procedures Covered in RSIL Standard Operating Procedures

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

Data Management and Records

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

Health, Safety, and Waste-Disposal Information

Applicable Health and Safety Issues

Personal Protection

Laboratory safety glasses and protective nitrile gloves are recommended whenever samples are handled, especially when the samples are of biological origin. For other precautions and safety procedures, consult the Material Safety Data Sheets (MSDSs), which are on file in the laboratory, and at [http://www.ilpi.com/msds/# Manufacturers](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). Consult the U.S. Geological Survey’s Safety and Environmental Health Handbook 445-2-H (U.S. Geological Survey, 2002).

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 DIC-DOC analytical process are native copper, copper oxide, sodium persulfate, phosphoric acid, and cobaltous silver, which are used in the DIC-DOC system, and phosphoric acid, which is used as a preservative for DOC during the sample collection. All the materials, including gloves and any cleaning towels, need to be collected in a plastic bag and disposed of as hazardous waste. All samples must be carefully inspected upon receipt for obvious indications of hazards. All analyzed samples must be discarded as hazardous material.

Gas Cylinder Handling

Compressed gas cylinders must be handled and stored according to the U.S. Geological Survey Manual, Chapter 6, Hazardous Materials Management (<http://www.usgs.gov/usgs-manual/handbook/hb/445-1-h/ch6.html>; accessed Feb. 6, 2014). 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.

Waste Disposal Procedures

The automated analytical procedure described within this document generates a large amount of liquid waste, on the order of 20 mL of waste per sample analysis. This waste will contain whatever is in the dissolved phase of the sample itself, as well as the phosphoric acid and sodium persulfate reagents, and any chemicals that may have been used as a preservative in the samples. This liquid waste is collected in a 20 L vessel during routine analyses. The contents of this vessel must be checked before each analytical run to be certain that the waste volume generated by the run can be collected without spillage. Periodic emptying of this vessel into 5 L or less high-density polyethylene (HDPE) or glass containers for waste disposal is necessary. The contents of the waste disposal containers must be clearly marked with the chemical composition (as known), approximate concentrations, and clearly labeled as hazardous waste before being placed in the assigned satellite accumulation area.

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Appendix A. Step-by-Step Procedure to Collect Water Samples for Determination of the $\delta^{13}\text{C}$ of Dissolved Inorganic Carbon (DIC) Within the Samples (RSIL Lab Code 1851)

Collect three samples per site or location.

Materials needed for DIC collection

1. 40 mL **clear** EPA VOC vials with open-top septum-lined caps (Teflon-lined silicone septa should be REPLACED by hand-punched black butyl-rubber septa).
2. 60 mL syringe and syringe filter cartridges. Recommended filter cartridge type: 0.2 μm Polysulfone GD/X Syringe Filters, manufactured by Whatman. This is a low protein binding filter for aqueous samples. Units include two prefilters [graded density Multigrade GMF 150 (10:1 μm) and Grade GF/F (0.7 μm)] and a 0.2 μm polysulfone final filter. Prefilters allow filtration of even the most difficult samples with minimal hand force. Choose the 13 mm (0.51 in.) size for samples up to 10 mL or the 25 mm (0.98 in.) size for samples greater than 10 mL.

Preparation of Vials

1. Vials should be triple rinsed in deionized water and dried in a particle-free atmosphere. (Pre-cleaned EPA-VOA vials are available from numerous suppliers.)

Collecting the Dic Samples

1. Pull sample water into a 60 mL syringe and empty 2 to 3 times to **rinse syringe** with fresh sample.
2. SLOWLY pull sample for collection into the syringe (try to avoid entraining excess sediment and air bubbles).
3. Remove bubbles/headspace from syringe by tilting syringe upwards and expelling excess air.
4. Attach syringe filter Flush filter with approximately 10 mL (to rinse filter).
5. SLOWLY push sample water into the vial. Vials need to be COMPLETELY FULL—fill until a positive (convex upward) meniscus forms over the vial opening.
6. Screw the cap on firmly (some spillage will occur) until the septa slightly pops up into the cap opening, but DO NOT OVERTIGHTEN CAP! If the septum appears to be creased, or if it is off-center, the vial may leak and the sample will be compromised.
7. Keep the vials chilled until analysis. DO NOT FREEZE VIALS! Freezing will cause the water to expand and crack the vials!

Note:

- When collecting both DIC and DOC samples, it is better to collect the DIC samples first since these vials must be filled completely with sample. The filter will become clogged as more water is filtered, and thus will require more and more pressure to obtain less and less sample. It is not important for the DOC vials to be filled completely; however, at least 40 mL are required for analysis.

1. Supplies information:

a. Vials:

1. 40 mL clear glass, 27 x 95 mm

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- b. Supplies for black butyl rubber septa:
 - 1. Septum Punch for vials: 3427A22 Arch Punch 7/8-in.-hole diameter.
 - 2. Black Butyl Rubber Sheets: 8609K67 Air-Tight Butyl Rubber Plain Back, 1/8 in. thick, 36-in. width, 60A Durometer.
- c. Syringe filters for DIC/DOC samples:
 - 1. Polysulfone GD/X Syringe Filters, Whatman low protein binding filter for aqueous samples. Units include two pre-filters [graded density Multigrade GMF 150 (10:1 μm) and Grade GF/F (0.7 μm)] and a 0.2 μm polyethersulfone final filter. Prefilters allow filtration of even the most difficult samples with minimal hand force. Choose the 13-mm (0.51-in.) size for samples up to 10 mL or the 25-mm (0.98-in.) size for samples greater than 10 mL.

Appendix B. Step-by-Step Procedure to Collect Water Samples for Determination of the $\delta^{13}\text{C}$ of Dissolved Organic Carbon (DOC) Within the Samples (RSIL Lab Code 1852)

Collect three samples per site or location.

Materials Needed

1. 40 mL amber EPA VOC vials with open-top septum-lined caps (septa are made of Teflon-lined silicone).
2. 60 mL syringe.
3. Syringe filter cartridges. Recommended type:
Polysulfone GD/X Syringe Filters, Whatman: Low protein binding filter for aqueous samples. Units include two prefilters [graded density Multigrade GMF 150 (10:1 μm) and Grade GF/F (0.7 μm)] and a 0.2 μm polysulfone final filter. Prefilters allow filtration of even the most difficult samples with minimal hand force. Choose the 13-mm (0.51 in.) size for samples up to 10 mL or the 25-mm (0.98 in.) size for samples greater than 10 mL.
4. ASC grade 85-percent phosphoric acid (H_3PO_4).

Preparation of Vials

1. Wrap pre-cleaned vials in aluminum foil and heat vials in a muffle furnace at 450 °C for at least 4 hours in order to remove organic residues.
2. Add a small droplet (20 μL) of 85-percent phosphoric acid to the vial prior to filling.

Collecting the Doc Samples

1. Pull sample water into a 60 mL syringe and empty 2 to 3 times to rinse syringe with fresh sample.
2. SLOWLY pull sample for collection into the syringe (try to avoid entraining excess sediment).
3. Remove bubbles/headspace from syringe by tilting syringe upwards and expelling excess air.
4. Attach syringe filter.
5. Flush filter with approximately 10 mL (to rinse filter).
6. SLOWLY push sample water into the amber vial. Vials need not be completely full—filling to the neck of the vial is okay.
7. Screw the cap on firmly, but DO NOT OVERTIGHTEN CAP! If the septum appears to be creased, or if it is off-center, the vial may leak.
8. Keep the vials chilled until analysis. DO NOT FREEZE VIALS! Freezing will cause the water to expand and crack the vials.

Note:

- When collecting both DIC and DOC samples, it is better to collect the DIC samples first since these vials must be filled completely with sample. The filter will become clogged as more water is filtered, and thus will require more and more pressure to obtain less and less sample. It is not important for the DOC vials to be filled completely; however, at least 40 mL are required for analysis.

Appendix C. Step-by-Step Procedure to Log Samples Into LIMS-LSI

Direct Submittal Formats

1. Download “Sample Submittal Form” or “Alternative Sample Submittal Form” from the RSIL Web site at <http://isotopes.usgs.gov/>. Both types can be submitted via a CD, diskette, or e-mail.
2. DIC and DOC have to be logged in separately. Choose Media Code 1851 for dissolved total inorganic carbon (TIC); it will give you a C #. Choose Media Code 1852 for dissolved total organic carbon (TOC); it will give you a G #.

Verifying Information

1. Place the submitted disk into the appropriate drive and print out the “Sample Submittal File” if the submitter has not already provided one.
2. Ensure that the information provided matches the information on the sample bottle labels.
3. Change the submission date on the submittal file to the date that the samples are actually being logged in.
4. Save any changes before exiting.

Logging Samples Into LIMS-LSI Database

1. Open the LIMS-LSI program.
2. From the LIMS-LSI main page, select “Import New Project.”
3. In the “Import Project” window, select the file being submitted.
4. In the “How Many Samples in Project” window, click “OK.”
5. In “LIMS” window click “OK.”

Getting a Password

1. A password is required if latitudes and longitudes are not provided in the submittal file.
2. See the laboratory supervisor for a password.
3. If some other information is missing and LIMS-LSI does not allow you to log in the samples, send the diskette back to the customer to add the missing information.

Printing Report and Putting Samples into the Template

1. On main LIMS-LSI page, select “Projects.”
2. Select and double click the project to edit.
3. In the “Projects” window, click “Print Report” once.
4. In the “Projects” window, click “Template List—Add & Delete Samples.” Choose the appropriate template such as “DIC for TIC-TOC” or “DOC for TIC-TOC.” Under “Number of non-consecutive sample repeats” enter “1”, and under “Number of consecutive sample repeats” enter “2”. Under “Low Procedure” leave as

the default value. Under “Add at” select “End of queue.” Click “Add” and click OK in the message boxes that follow. Close the “Projects” window.

Printing Labels

1. In the “Projects” window click “Print Label” and follow the instructions of “print sample range,” except when only one set of labels needs to be printed choose label type.

Filing Samples in Progress Binder

1. Take the printed copy of the Submission Report and Submittal File printout along with any other documentation and place in the “Samples in Progress” binder.
2. The projects are in order by date.
3. Arrange in the binder with Project Report first, along with any other documentation received from the submitter.
4. Place a divider after each project as it is placed in the binder.

Placing Labels

1. Match the label to the sample bottle.
2. Ensure all information is correct using the submittal form.

Appendix D. Step-by-Step Procedure to Print a Template (with an example template showing a list of samples to be analyzed)

1. In the main LIMS-LSI window, click “Create Sample List” and then click “List.”
2. Select the appropriate template for Delta Plus IRMS analysis of DIC or DOC; for example, “DIC on TIC-TOC” or “DOC on TIC-TOC.” The list will be populated with all the samples currently in the queue; make sure the list only includes the samples you wish to analyze. Note that any samples with “0” in the “Repeats” column will not be written to the list for the run.
3. The template is designed for an analysis sequence with 40 vials (including standards) to be run over 24 hours. The autosampler capacity is 88 vials, so this list may be doubled for an extended run.
4. Add amount of liquid sample to be analyzed (from 2 to 9 mL) in the column “Sample/Extraction Info.” This amount is determined based on the sample concentration to achieve consistent CO₂ peak heights.
5. Click “Save and Print.” Choose the location where to save the Excel file with the sample list, and rename the file with the date, C-number range, and some description of the project using the format “yyyy-mm-dd_C-####_project”. This list will automatically print when “Save and Print” is clicked.
6. Open the Excel file and make sure it contains the samples and references to be analyzed in their proper sequence. Sort the list on the column “Port” if it is out of order; this is a common occurrence due to a software bug. Exit LIMS-LSI.

Example of a template:

Line	Identifier 1	Identifier 2	Amount ppm	Port
1	Dummy			1
2	Dummy			2
3	DIC blank			3
4	DIC blank			4
5	C-1916 / / 301//	Ref. Stand.	2.5	05
6	C-1916 / / 301//	Ref. Stand.	5.0	06
7	C-1916 / / 301//	Ref. Stand.	10.0	07
8	C-1916 / / 301//	Ref. Stand.	50.0	08
9	C-1916 / / 301//	Ref. Stand.	100.0	09
10	C-1916 / / 301//	Ref. Stand.	200.0	10
11	C-12609 / / 301//	80 BR-D		11
12	C-12644 / / 301//	36BR-A 1		12
13	C-12644 / / 301//	36BR-A 1		13
14	C-12645 / / 301//	80BR-B 1		14
15	C-12645 / / 301//	80BR-B 1		15
16	C-12646 / / 301//	80BR-D 1		16
17	C-12646 / / 301//	80BR-D 1		17
18	C-12647 / / 301//	73BR-E 1		18
19	C-12647 / / 301//	73BR-E 1		19
20	C-12648 / / 301//	71BR-E 1		20
21	C-12648 / / 301//	71BR-E 1		21
22	C-12649 / / 301//	73BR-D2 1		22
23	C-1916 / / 301//	Ref. Stand.	5	23
24	C-1916 / / 301//	Ref. Stand.	10	24
25	C-1916 / / 301//	Ref. Stand.	50	25
26	C-1916 / / 301//	Ref. Stand.	100	26
27	C-12649 / / 301//	73BR-D2 1		27
28	C-12650 / / 301//	71BR-B 1		28
29	C-12650 / / 301//	71BR-B 1		29
30	C-12651 / / 301//	80BR-C 1		30
31	C-12651 / / 301//	80BR-C 1		31
32	C-12652 / / 301//	70BR-72 1		32
33	C-12652 / / 301//	70BR-72 1		33
34	C-12653 / / 301//	80BR-A 1		34
35	C-12653 / / 301//	80BR-A 1		35
36	C-12610 / / 301//	73BR-D1 1		36
37	C-12610 / / 301//	73BR-D1 1		37
38	C-12611 / / 301//	71BR-C-DUP 1		38
39	C-12611 / / 301//	71BR-C-DUP 1		39
40	C-12612 / / 301//	73BR-BC 2		40
41	C-11781 / / 301//	Ref. Stand.	2.5	41
42	C-11781 / / 301//	Ref. Stand.	5.0	42
43	C-11781 / / 301//	Ref. Stand.	10.0	43
44	C-11781 / / 301//	Ref. Stand.	50.0	44

Example of a template:

Line	Identifier 1	Identifier 2	Amount ppm	Port
45	C-11781 / / 301//	Ref. Stand.	100.0	45
46	C-11781 / / 301//	Ref. Stand.	200.0	46
47	C-12612 / / 301//	73BR-BC 2		47
48	C-12613 / / 301//	15BR 1		48
49	C-12613 / / 301//	15BR 1		49
50	C-12615 / / 301//	70BR-BLK 1		50
51	C-12615 / / 301//	70BR-BLK 1		51
52	C-12618 / / 301//	73BR-BC 3		52
53	C-12618 / / 301//	73BR-BC 3		53
54	C-12619 / / 301//	15BR 3		54
55	C-12619 / / 301//	15BR 3		55
56	C-12620 / / 301//	71BR-D 2		56
57	C-12620 / / 301//	71BR-D 2		57
58	C-12621 / / 301//	70BR-BLK 2		58
59	C-11781 / / 301//	Ref. Stand.	2.5	59
60	C-11781 / / 301//	Ref. Stand.	5.0	60
61	C-11781 / / 301//	Ref. Stand.	10.0	61
62	C-11781 / / 301//	Ref. Stand.	50.0	62
63	C-11781 / / 301//	Ref. Stand.	100.0	63
64	C-11781 / / 301//	Ref. Stand.	200.0	64
65	C-12621 / / 301//	70BR-BLK 2		65
66	C-12622 / / 301//	80BR-A 3		66
67	C-12622 / / 301//	80BR-A 3		67
68	C-12623 / / 301//	71BR-C 3		68
69	C-11781 / / 301//	Ref. Stand.	10.0	69
70	C-11781 / / 301//	Ref. Stand.	50.0	70
71	C-1916 / / 301//	Ref. Stand.	10.0	71
72	C-1916 / / 301//	Ref. Stand.	50.0	72

Appendix E. Step-by-Step Procedure to Add Sample Information to ISODAT and DIC-DOC—Sequence Table

Enter a Sample Sequence in the Isodat Software:

1. Insert diskette or memory stick with the Excel “Sample List” that was created by LIMS on it into the computer that has the ISODAT software.
2. Open ISODAT by going to Start>All Programs>ISODAT NT 2.0>Acquisition. This will open the Acquisition window, which will show the current status of the mass spectrometer on the lefthand side. The last sequence file that was run may also be open.
3. Create a new “Sequence.” Go to File>New>Sequence>and enter the number of samples, including all dummy and reference standards. **Note: If you choose to analyze two sequential aliquots of one sample, ISODAT handles them as two different samples. Thus, there will be twice the number of rows in the ISODAT sequence as compared to the Aurora sequence.**
4. Open the “Sample List” in Excel that was created by LIMS.
5. Highlight and “Copy” the columns of sample identifier and comments from the Sample List, then highlight the corresponding empty columns in the ISODAT sequence table and “Paste” the information into the table. Copy over any information for the columns for “Identifier1”, “Identifier2”, and “Port”. Use the “Amount” column in the sample list to fill the column “Preparation” in the ISODAT sequence.
6. Select the appropriate “Method” file for each sample using the drop-down menu in the ISODAT sequence (e.g., ‘DIC no trap new.met’). After selecting the method for one sample, copy to the other samples by selecting the column at the top, right click the mouse, and select “Fill Grid with Data.” This will populate the rest of the column with the same method file.

Enter the Sample Sequence in the Aurora Software:

1. Go back to the Excel file and select every second aliquot from the sample list using the Ctrl-select keyboard command, then copy the selected (alternating) rows over to a separate sheet in the same Excel workbook. Name the sheet “OI sequence.”
2. Open the Aurora TOC software on the same computer as the ISODAT software using the OIC TOC 1030 Launchpad. Double click on the Aurora instrument icon under the “Device Connections” tab. To log in, use the UserID “toc” and password “toc” and click “OK.” The monitor should now display the operating window of the TOC software (see the Aurora user’s manual for further details).
3. In the TOC software, click on the Editor button at the top of the window.
4. Click “New”; then click “Add/Insert.”
5. Under “Sample Type”, choose “Sample.”
6. Under “# Reps”, enter “2”.
7. Under “# of Samples”, enter the number of actual vials to be run, including all dummy, blanks, reference standards, and samples. Create sequence rows for as many samples as you will have in the Aurora sequence, and check this against the number of vials that will be in the sample tray.
8. Under “Methods>Primary” choose the appropriate method. Be sure that the selected method has “Remote Start” enabled and has the correct settings for the run (see appendix F).
9. Under “Sample Description>Sample ID”, leave this field blank, but check the box that says “Use Incrementor”, with a start value of “1”. This will temporarily give each row a numeric sample ID, which will be replaced in the following steps. Leave “Cust ID” at “00000000” and the “Comments” field blank.
10. Click “OK.”

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11. In the Excel file of the sample list from LIMS, highlight and “Copy” the block of cells under the column “Identifier 1” in the “OI sequence” sheet; these should be the C-number sample IDs. In the Aurora TOC software, highlight the exact block of cells in the sequence and paste the C-number samples IDs into sequence. You must highlight the exact block of cells in both the Excel sheet and the Aurora software in order for the Copy-Paste command to work.
12. Give the sequence file a name, starting with the date in the format “yyyy-mm-dd_project” and then click “Save.”
13. Click the “Monitor” button at the top of the screen, and select the “Sequence” tab. Click “Load Active Sequence” and choose the file name of the sequence that was just created. This sequence is now ready to be run by the Aurora TOC software.
14. Turn on the autosampler (switch at right rear). A green LED light should illuminate at the front of the autosampler and a tray rotation check will occur. Click the “Config” button at the top of the Aurora screen and under the “General” tab be sure the icon “1088 remote start” is labeled “Active.” This enables the autosampler.
15. Load the sample vials into the autosampler tray according to the Aurora sequence. The last number in the sequence should match the number of the position of the last vial in the tray. Load the sample tray into the autosampler and continue to the run the procedure in the appendix F checklist below.

Appendix F. Recommended Settings, Checklist Before Each Run, and “Start Run” Procedure

Recommended Aurora method settings for TIC or TOC analyses.

Setting	TIC Analysis	TOC Analysis	Notes
Mode	TIC Only	TIC & NPOC	
Pre-Acid Volume (mL)	n/a	0	
Spurge Time (min:sec)	n/a	0	
Sample Volume (mL)	5.0	5.0	Volume may vary according to sample concentration. May require a new calibration if changed in the method
Use modified oxidant	unchecked	unchecked	
Outlier Removal Criteria	unchecked	unchecked	
EPC System Pressure (psi)	20	20	
Drain Time (sec)	15	15	
Acid Reagent Vol. (mL)	1.0	1.0	Volume may vary according to sample concentration, but may require a new calibration if changed in the method
Persulfate Reagent Vol. (mL)	0	3.0	Volume may vary according to sample concentration and salinity, May require a new calibration if changed in the method
Dilution	unchecked	unchecked	
Sample Stirring	unchecked	unchecked	
Rinse Volume (mL)	10	15	
Rinses Per Sample	1	1	
Rinses Per Replicate	0	0	
TIC React time (min:sec)	2:30	2:30	These times may vary, but may require a new calibration if changed in the method
TOC/TC React time	0:00	6:00	
TIC Max Detect time	10:00	10:00	
TOC/TC Max Detect time	10:00	10:00	
TIC React temp (°C)	70	70	
TOC/TC React temp (°C)	98	98	
TIC Detect temp (°C)	70	70	
TOC/TC Detect temp (°C)	98	98	

Analyst in charge: _____ Date: _____

Weekly Checklist:

- [] Change working reference gas, N₂ gas, and He gas tanks if the pressure is <500 psi. Order new tanks for spares; reference gases are research-grade; He carrier is zero-grade. Turn on He gas first before N₂ to stabilize the NDIR detector.
- [] Check phosphoric acid and sodium persulfate (oxidant) reagent volumes and add some if the bottles are less than ¼ full. See section “Preparing reagents” above for instructions.
- [] Check the volume in the waste accumulation container. Empty as needed into 5 L (or less) hazardous waste containers, label as hazardous waste with the chemical contents indicated, and store in the assigned satellite accumulation area with the start date of satellite accumulation.

Checklist Before Each Run:**Check the MS performance in the ISODAT software:**

- [] Check background masses.

Typical Background at N2 Configuration**Emission Current: 0.54 mA/0.48 mA**

Cups	Mass	Intensity (V)	Mass	Intensity (V)	Resistor (Ω)	Capacity (pF)
1	28	0.010		0	3×10^8	680
2	29	0.007	18	1.256	3×10^{10}	5
3	30	0.140	18	6.818	1×10^{11}	2

- [] Perform “Ref on/off” method three times to stabilize CF-IRMS.
- [] Check the peak center on mass 45. If it is off default value by 50, do mass calibration.
- [] Analyze “Ref on/off” method 10 times to stabilize IRMS (standard deviation should be <0.1 ‰).

ConFlo Pressure Setting CO ₂ ,	CO ₂ signal (V) Mass 45 on Cup 2
0.25	0.173
0.50	0.450
0.75	0.773
1.00	1.180
1.25	1.560
1.50 Optimal setting CO₂	2.074
1.75	2.634
2.00	3.199

- [] Make sure the cryogenic cold trap loop is raised when starting the sequence; the “Trap Valve” icon will be grey and vertically oriented when raised. If the icon is blue and in the horizontal orientation, click it once to change its position. If the cold trap loop is not raised, check to be sure the pneumatic arm is not stuck.

Check the supply of gases and liquids:

- [] Make sure N₂ gas valves are open to provide the required “jacket” flow to the NDIR-CO₂ detector on the Aurora.
- [] Check that the He gas flow is supplied to the Aurora at 110 mL/min. The flow rate can be checked by clicking on the digital pressure/flow gage dial in the lower right corner on the Aurora screen to toggle between Pressure and Flow.
- [] Check that there is enough DI water in the rinse reservoir, and enough acid and oxidant reagents for the run. Each sample analysis will consume approximately 30 mL of DI water. Each of these solution reservoirs should have proper He purge flow (a steady stream of helium bubbles should be evident through the solutions).
- [] Check that there is enough free volume in the waste container to collect the waste from the run. Use 20 mL of liquid waste per sample to estimate the volume that will be generated.

Check the Aurora configuration:

- [] Check the reaction chamber and the scrubber furnace temperatures (they should be ~70 °C and ~650 °C, respectively). These can be seen in the digital temperature dial in the lower left corner of the Aurora screen. “Cmb 1” is the reaction chamber temperature. Click or touch on it to toggle the display to “POC Temp”, which will display the current temperature of the scrubber furnace.

- [] Check the background CO₂ detector value (it should be around 2,000 counts).
- [] Check that the Electronic Pressure Control (EPC) is set at the appropriate value on the Aurora (20 psi).
- [] Check that the Electronic Flow Control (EFC) is set to appropriate value on the Aurora (110 mL/min).
- [] Check the Configuration (Config) of the Aurora so that the appropriate configuration is selected, with the proper autosampler enabled (1088 Autosampler with Remote Start).
- [] Check the Method in sequence of the Aurora, so that appropriate method is selected with “Remote Start” enabled.
- [] Compare the sequences between the Aurora and ISODAT. They should agree, but with two rows in the ISODAT sequence for every one row (coded for two replicate analyses) in the Aurora sequence.
- [] Check communication between the Aurora and ISODAT. On the Aurora screen, there should be two small heads showing on the left corner of the bottom of the screen to indicate communication between the Aurora and ISODAT computers, and the little computer on the right hand corner at the bottom of the screen should be green to indicate the Data Gathering Service (DGS) is active and functioning. If this computer icon is red, then you must restart the Data Gathering Service.
 1. On the ISODAT computer, open the OIC TOC 1030 Launchpad → PC Application → DGS → Restart service → OK
 2. The little computer icon in the lower right of the Aurora screen should turn green.
 3. If this does not work, it is possible that multiple instances of the Data Gathering Service are running at once on the ISODAT computer and are interfering with each other. Using the Windows Task Manager, close all instances of the Data Gathering Service and then re-try steps 1 and 2 above.

Start the run on the Aurora monitor:

- [] Always start the run FIRST in the Aurora software (click the green ‘play’ triangle icon in the lower left corner of the screen). The Aurora will begin its Startup procedure.
- [] Wait until the Aurora screen is indicating “Waiting”; when the run is started in ISODAT, it will send a start signal to the Aurora.

Start the run in ISODAT:

- [] Check paper in printer.
- [] Start ISODAT sequence (click the green ‘play’ triangle icon in the upper left of the ISODAT sequence window).
- [] Enter appropriate folder name and select pre: “Date.”
- [] Give File Name: Select pre: “Analysis #.” Select post: “Identifier 1” and “Identifier 2”.
- [] Select Method as “Excel.”
- [] Select: “Print result.”
- [] Click OK. Wait for first sample to be analyzed.
 - From this point on the system will run automatically. The methods in the Aurora and ISODAT sequences are designed in such a way that the Aurora method is shorter than the ISODAT method, so it always waits for the signal from the ISODAT to continue.
 - Wait for the first sample to be finished.
 - If you have to abort, abort both instruments and start over. If you abort in the middle of the run you have to reload the tray because the Aurora always starts at the first port. There is no such command in its software to start the sequence at a different port.

Appendix G. Step-by-Step Procedure to Retrieve Data from ISODAT and Apply a Blank Correction and Mass-Dependent Correction

1. For LIMS-LSI:
 - a. Go to Windows Explorer in ISODAT computer.
 - b. Find the drive where the data are.
 - c. Choose “Finnigan.”
 - d. Choose “Global.”
 - e. Choose “User.”
 - f. Choose the inlet system where you have data (Conflo II).
 - g. Choose “Results” folder.
2. Select the results folder from which you wish to export the data.
3. Find the file of the run results, and open it in Excel.
4. Save the file as My documents/TIC-TOC result/TIC/; give date and some name to the file.
5. Define all reference peaks by adding “1” under column with the heading “Is Ref”. Manually sort the reference peaks from the sample peaks, and enter the value “1” for each reference peak. Next, sort out the reference standards from the results spreadsheet by selecting only those isotopic value results of the reference standards. Using the Autofilter in Excel, sort on column “Identifier 1,” choosing only the standards (for example, C-11781 and C-1916), and then sort on column “PeakNr” to select only with the sample gas peaks. Highlight and copy these cells over into a new sheet.
6. Add a row that allows estimation of a theoretical carbon blank area value in column “AreaAll,” and an isotopic value in column “d 45CO₂/44CO₂”. The theoretical values represent the contribution of inorganic carbon in the DI water used to create the reference standard solutions used for DIC analyses, or the contribution of organic carbon in the DI water and acid reagent used for DOC analyses.
7. Add a column “Corrected d13C” to shows the results of the calculation using the equation:

$$\text{Corrected d13C} = ((A_{\text{ref}} * \delta_{\text{ref}}) - (A_{\text{blank}} * \delta_{\text{blank}})) / (A_{\text{ref}} - A_{\text{blank}}),$$

where:

A_{ref} = area of the reference standard from the column “AreaAll”,

δ_{ref} = delta value of the reference standard from the column “d 45CO₂/44CO₂”,

A_{blank} = theoretical (estimated) area of the carbon blank in the reference standard, and

δ_{blank} = theoretical (estimated) delta value of the carbon blank in the reference standard.

Change the theoretical A_{blank} and δ_{blank} values iteratively until you obtain the minimum standard deviation of both sets of reference standards.

8. Determine the mass (that is, peak area) dependent correction factor and apply this correction to both the samples and reference standards. Plot the areas of all analyzed peaks of both sets of isotopic reference standards (for example, C-11781 and C-1916 for DIC) against the blank corrected isotope values of the reference standards. To determine the average slope, fit a linear trend to both data sets, and calculate the average of the slopes in the linear equations, as shown in the graph example below.

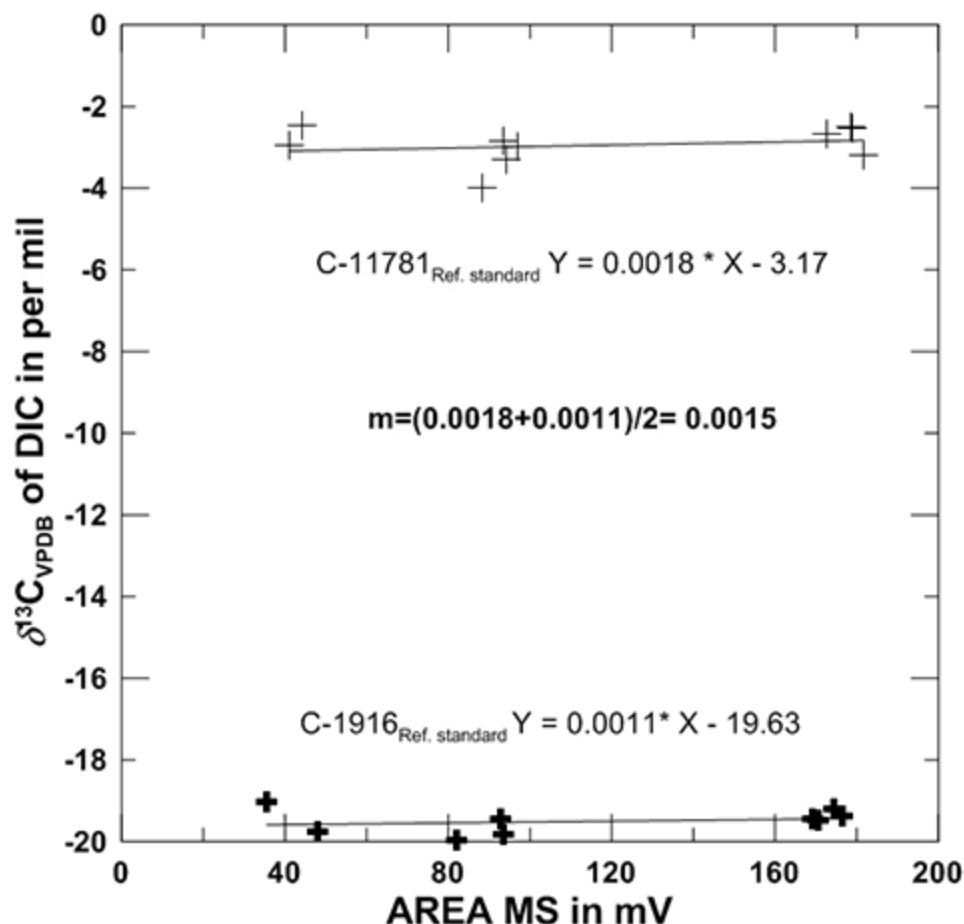


Figure G1. Graph showing determination of the mass-dependent correction factor (m) obtained from linear regression of the area versus the blank-corrected delta values of the isotopic reference standards.

9. This average slope is the mass-dependent correction factor (m) you have to apply to the samples in the run, determined from the equation:

$$\delta_{corr} = m * Area_{sample} + \delta_{sample}$$

where

δ_{corr} = mass-corrected delta value of the sample,

A_{sample} = area of the sample peak from the column "AreaAll",

δ_{sample} = delta value of the sample peak from the column "d 45CO2/44CO2".

10. Change all reference standard values to the blank corrected values, and apply the correction factors to the sample values (samples are mass-corrected only for DIC, but are both mass-corrected and blank-corrected for DOC). Create a separate Excel file which contains all the corrected data (reference standards and samples), and import the corrected data into LIMS.
11. Transfer the corrected data to a USB memory stick.
12. Import the data to LIMS-LSI.
13. For Data Back-up computer:
 - a. Go to Windows Explorer in the ISODAT computer.
 - b. Find the drive where the data are.
 - c. Choose "Finnigan."

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- d. Choose “Global.”
 - e. Choose “User.”
 - f. Choose the inlet system where you have data (Conflo II).
 - g. Choose “Results” folder.
14. Select the results folder you wish to export data from.
 15. Transfer data to a USB memory stick.
 16. Import the data to the Data Back-up computer.

Appendix H. Step-by-Step Procedure to Determine and Apply Isotopic Correction Factors and Evaluate Data in LIMS-LSI

1. Open “Correction Factors and Normalization Equations” in LIMS-LSI.
2. Select the IRMS and isotope, such as “Delta Plus for ^{13}C .”
3. Select “Query.”
4. Double click on the last sample analyzed on that day. Choose separately for C# or for G#. Do not combine the two types of samples in one range.
5. Evaluate data of the reference materials. Ignore outliers. There should not be more than one or two outliers.
6. Choose “Normalize With All References.”
7. Accept “Expansion Correction and Additive Correction factors.”
8. Print out correction factor sheet.
9. Report daily reference values and correction factors along with date and range of analysis number to the “DIC-DOC” binder.
10. Go back to LIMS-LSI main menu by closing open windows.
11. Choose “Print Samples in Progress”:
 - a. Open “Sample in Progress.”
 - b. Choose appropriate isotope.
 - c. Choose appropriate prefix (C for DIC; G for DOC).
 - d. Put in sample ID range from “Samples-to-Be-Analyzed” sheet. Click Print.
12. Review results; determine repeats.
13. Put repeats back to “Table of Samples to Be Analyzed.”
 - a. Go back to LIMS-LSI main menu.
 - b. Open “Print Templates.”
 - c. Select appropriate template name for the IRMS with which the samples are analyzed.
 - d. Find the sample in the “List of Samples.”
 - e. Change “Repeats” from 0 to 1.
 - f. Close windows and exit LIMS-LSI.

Appendix I. 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” printout.
4. Store data.
5. Go back to the LIMS-LSI main menu.
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 other documents in the “Samples In Progress” binder that are related to this project.
10. If the samples were submitted through the National Water Quality Laboratory (NWQL), click “NWQL Export” and transfer data in NWQL format to a disc; report these data to the NWQL by e-mail. Print a copy of the NWQL export file and save in the “Correspondence” binder with the project report printed in step 9.

Appendix J. Troubleshooting

For procedures regarding maintenance of the Aurora 1030 TOC analyzer, please see the User Manual. A PDF copy of the manual is stored on the computer running ISODAT in the Desktop folder as Shortcut. A list of some basic troubleshooting issues is given below. This list and possible solutions are taken from the Aurora 1030 User Manual, chapter 7.

Symptom	Probable Cause	Corrective Action
Sample does not properly aspirate into the Aurora from the sample bottle or autosampler	Incorrect sample loop size entered	Enter corrective values.
Leak in sample line		Check sampling tubing for leaks.
Sample loop not properly tightened		Check sample loop connections for finger tightness.
W-shaped or negative inflection on CO ₂ peak (DIC, DOC, or POC)	Gross CO ₂ contamination in gas	Change the gas cylinder. Use gas with greater than 99.98 percent purity.
NDIR baseline zero too high (greater than 8,000 with no adjustment)	Contaminated NDIR sample cell	Contact OI the Analytical Customer Support Center at (800) 336-1911.
Digestion vessel heater too hot		Check that sample temperature is within 92–99 °C in DOC reaction.
Error light flashing and touchscreen does not respond	Internal memory overfilled due to a long-term error condition	Turn off power and restart. Look in the log and correct the error condition.
Poor peak shape or low flow from vent or detector	Flow leak	Perform a leak check on the system.
Poor combustion TOC peak shape or poor results	Depleted combustion catalyst	Replace the catalyst. Replace the combustor injector inlet. Look for plugged lines.
Poor reproducibility		Verify standards are made correctly. Make new standards.
Catalyst issue		Clean catalyst. Run a cleanup.
Syringe problem or bubble formation		Replace the syringe and or plunger.
Poor gas flow		Perform a flow check and leak check.
Plugged line or injector		Check for obstructed lines and replace.
If Ar peak is >50 mV		Wake up Aurora.
If Ar peak is >50 mV and Aurora is in Standby mode	Leak in the system	Check connection top of the furnace at the red check valve; check the tank if you changed it recently.
Peak comes later than it should be, and it is tailing	1. Gray valve on EPC could be bad. 2. Clog in the system, combustion tube material is old.	1. Check flow rate after the gray check valve—should be 110 mL/min. 2. Cool down furnace and put new tube in with new reagents in it. See Appendix K.
Second replicate peak is smaller than the first one	Not enough sample	Check the vial if there was enough sample in it.
Replicates are not matching	Bubble in the injection loop	After several run it will disappear.

Appendix K. Step-by-Step Procedure for Changing the Reaction Furnace as Needed

NOTE: This procedure will open the flow path from the Aurora to the mass spectrometer to the atmosphere. Take necessary precautions to isolate any sensitive portions of the analytical flow path from air:

1. Close the source to the mass spectrometer, or otherwise isolate from the atmosphere using the ConFlo. The flow of helium from the Aurora will be interrupted.
2. Isolate the gas chromatography (GC) column using the black Whitey valves (fig. K1)—this will prevent air from getting into the column.

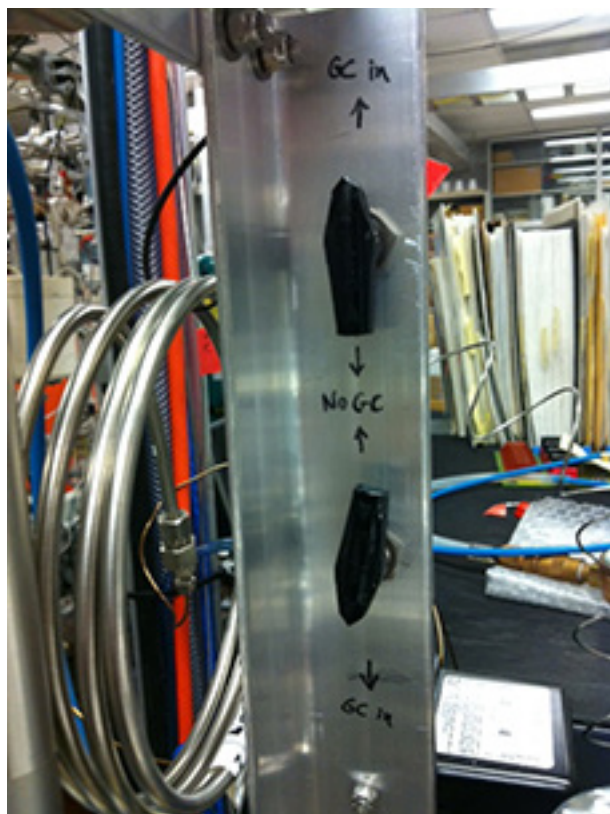


Figure K1. Valve used to isolate gas chromatograph column.

3. Turn off the Aurora TOC analyzer (Aurora).
4. Turn off the helium flow to the Aurora at the tank.
5. Turn off the electrical supply to the scrubber furnace located on the back of the Aurora by switching off and unplugging the Variac. Allow the furnace to cool to room temperature before servicing.
6. Disconnect the small knurled plastic luer nut from the top of the reaction tube—this connection carries the flow from the Aurora into the scrubber furnace (fig. K2).
7. Disconnect the stainless steel 1/16-in. tubing from the glass sidearm port extending off of the reaction tube. This connection carries the flow out of the scrubber reaction chamber (fig. K2).

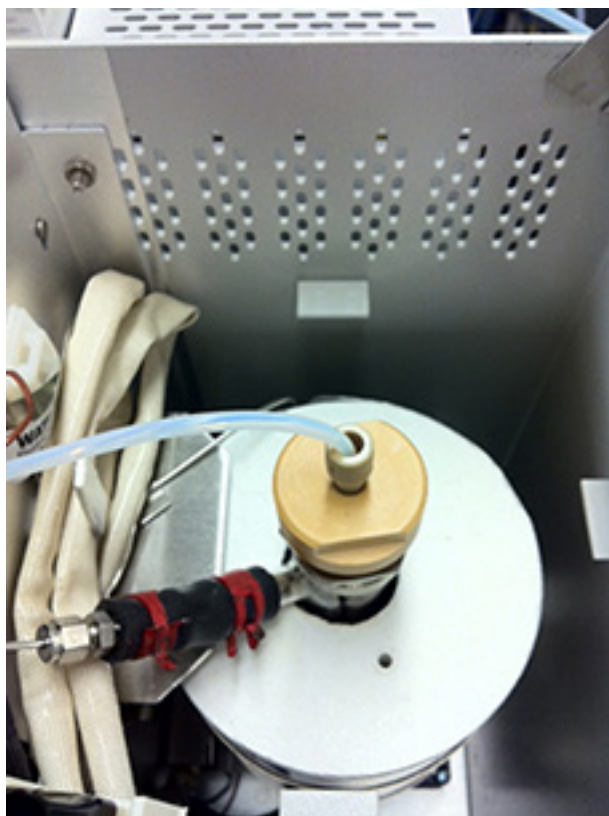


Figure K2. Connections to the scrubber reaction chamber.

8. Lift the reaction tube from the furnace well.
9. Inspect the glass of the reaction tube to see if it may be reused. If spent copper oxide or quartz chips form a solid mass (fig. K3), discard the tube as hazardous waste and replace with a fresh tube (order from OI Analytical).



Figure K3. Spent reaction tube from scrubber furnace.

10. Remove upper plastic connector and molded nut from the top of the chamber but DO NOT DISCARD.
11. Pinch red metal clamps using a pliers and remove black rubber tube connector from side arm but DO NOT DISCARD.
12. Pack the reaction tube as shown in figure K4, replace and test all connections for leaks.

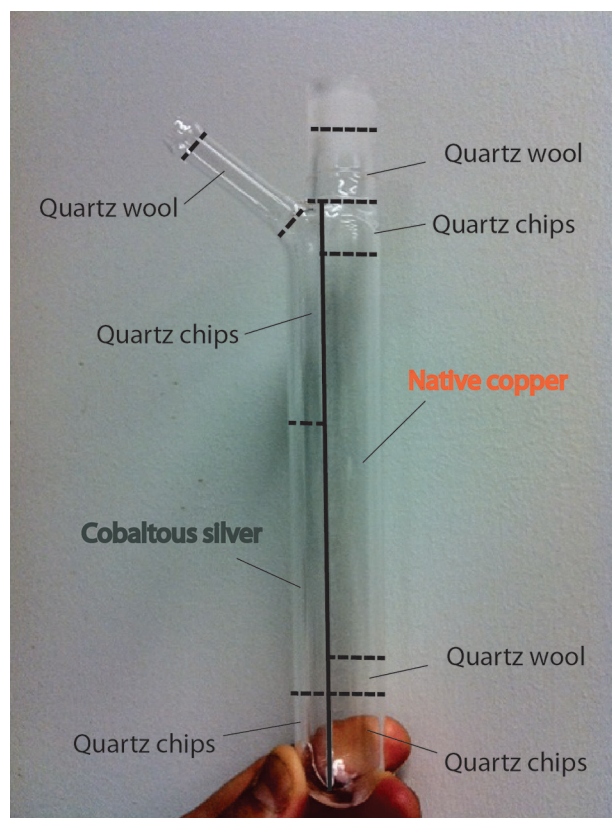


Figure K4. Diagram illustrating packing of reaction tube for scrubber furnace.

13. Turn on helium flow to Aurora.
14. Turn on Aurora 1030 (Aurora); be sure instrument is in Standby mode.
15. Plug in Variac and bring furnace temperature up to $\sim 650^\circ\text{C}$. Monitor the furnace temperature by tapping on the reaction vessel temperature on the Aurora monitor.
16. Allow the reaction furnace to “cook off” organics and to vent to the atmosphere for at least 3 hours.
17. Open GC column to flow from Aurora, and monitor background levels on the mass spectrometer.

Appendix L. Step-by-Step Procedure to Make Reference Standards

DIC

Part 1: Prepare a stock solution of NaHCO₃ isotopic standard (C-1916 or C-11781) at a concentration of 1,000 mg/L DIC:

1. Obtain a clean and dry 1,000-mL volumetric flask.
2. Fill the flask with Milli-Q water up to the base of the neck (leave approximately 50 mL of space).
3. Set the flask to purge with He from the DI-water reservoir purge from the Aurora. Obtain a fresh wash bottle of Milli-Q DI water, and purge this with He as well. Leave these for about 20 minutes to 1 hour, and in the meantime follow the next steps.
4. Carefully measure out **6.993 g** of NaHCO₃ isotope reference standard solid (the solid should be dry and kept in a desiccator—if lumpy, dry in a desiccator, and weigh out once dried). For weighing, follow these steps:
 - a. Obtain a small, 100-mL beaker.
 - b. Take a large square of weighing paper and fold into a small square one-quarter of the original size. Cut off one corner of the folded square with a bit of curvature and open the resulting paper into a closed cone. Using a spatula, carefully open any crease in the bottom pit of the cone.
 - c. Place the dry beaker on the balance, and then place the paper cone into the beaker.
 - d. Zero (tare) the balance.
 - e. Weigh out the NaHCO₃ into the cone.
5. Obtain a clean, dry plastic funnel and place into the 1,000-mL flask that now has purged DI water.
6. Carefully add the NaHCO₃ solid into the 1,000-mL flask through the funnel. Rinse the weigh paper and plastic funnel into the flask with the purged DI water from the DI wash bottle. Open the weigh paper slowly and rinse thoroughly, but **DO NOT OVERFILL THE VOLUMETRIC FLASK** past the scored line.
7. Once the solid has been completely rinsed into the flask, fill the remaining volume of the flask with water from the DI wash bottle.
8. Top off the headspace of the flask with N₂ from the DI-water reservoir purge tube from the Aurora. This is to flush CO₂ in the headspace out with nitrogen. Put the ground glass stopper onto the flask and wrap the top with parafilm.

Part 2: Make a series of diluted standard solutions from the 1,000 mg/L DIC stock solution:

1. Obtain several clean 1,000-mL volumetric flasks (at least three).
2. Fill the flasks nearly to the scored line with Millipore DI water, leaving approximately 10 to 20 mL of volume below the scored line.
3. Flush the DI water with He or N₂ gas for at least 20 minutes to remove any ambient CO₂.
4. Decant about 50 mL of the stock solution into a small, clean and dry beaker for transfer.
5. Using a calibrated volumetric pipette, add the volumes of 1,000 mg/L standard solution shown in the table below to degassed, DI water in 1-L volumetric flask(s) to create solutions of the desired concentrations:

Desired concentration:	1 ppm	5 ppm	15 ppm	25 ppm
mL of 1,000 mg/L DIC solution:	1 mL	5 mL	15 mL	25 mL

- Pour the solution into 40-mL EPA VOA vials with **black butyl septa** (replace the Teflon-coated silicone septa as needed—see appendix A) as soon as possible. Be sure not to leave any bubbles or headspace in the vials. The solution in these vials can serve as reference standards for up to 6 months as long as no bubbles or headspace forms in the vials.

DOC

Part 1: Prepare a stock solution of potassium bi-phthalate (KHP, G-15728) or sucrose (G-10212) at a concentration of 1,000 mg/L as C:

- Obtain a clean and dry 1,000-mL volumetric flask.
- Carefully measure out **2.128 g** of KHP standard ($\text{KHC}_8\text{H}_4\text{O}_4$) (G-15728); the molar mass is approximately 204.2 g/mol, with the molar mass of carbon in KHP being 96.08 g/mol. When labeling, the bottles should be labeled with G-15728, the concentration, and the date. If using the sucrose standard (G-10212), measure out **0.421 g** of sucrose; the molar mass of sucrose ($\text{C}_{12}\text{H}_{22}\text{O}_{11}$) is 342.30 g/mol, and the molar mass of carbon in sucrose is 144.13 g/mol. The solid isotopic standard materials should be kept dry and stored in a desiccator—if lumpy, dry in desiccator, and weigh out once dried. For weighing, follow these steps:
 - Obtain a small, 100-mL beaker.
 - Take a large square of weighing paper and fold into a small square one-quarter of the original size. Cut off one corner of the folded square with a bit of curvature, and open the resulting paper into a closed cone. Using a spatula, carefully open any crease in the bottom pit of the cone.
 - Place the dry beaker on the balance, and then place the paper cone into the beaker.
 - Zero (tare) the balance.
 - Weigh out the KHP into the cone.
- Obtain a clean, dry plastic funnel and place into the 1,000-mL flask.
- Carefully dump the KHP (or sucrose) solid into the 1,000-mL flask. Rinse the weigh paper and plastic funnel into the flask with DI water from the DI wash bottle. Open the weigh paper slowly, and rinse thoroughly into the flask.
- Once the solid has been completely rinsed into the flask, fill the remaining volume of the flask with Millipore DI water.
- Top off the headspace of the flask with N_2 gas from the tank on the back wall. This is to flush CO_2 in the headspace out with nitrogen. Put the ground glass stopper onto the flask and wrap the top with parafilm.

Part 2: Make a series of diluted standard solutions from the 1,000-mg/L DOC stock solution:

- Obtain several clean 1,000-mL volumetric flasks (at least three).
- Fill the flasks nearly to the scored line with Millipore DI water, leaving approximately 10 to 20 mL of volume below the scored line.
- Decant about 50 mL of the stock solution into a small, clean and dry beaker for transfer.

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4. Using a calibrated volumetric pipette, add the following volumes of 1,000-mg/L standard solution to degassed deionized water in 1-L volumetric flask(s) to create solutions of the following concentrations:

Desired concentration:	1 ppm	5 ppm	15 ppm	25 ppm
mL of 1,000-mg/L DOC solution:	1 mL	5 mL	15 mL	25 mL

5. Pour the solution into 40-mL EPA VOA vials with silicone septa as soon as possible. Be sure to flip the septa over so that the silicone side is facing the glass, and the Teflon side is facing up into the cap (this helps the vials maintain a seal and prevents evaporation). A small headspace may be left in the DOC standard vials. The solution in these vials can serve as reference standards for up to 6 months as long as they are refrigerated and properly sealed.

