ANALYSIS OF ENVIRONMENTAL SAMPLES
USING CONTINUOUS FLOW
GAS ISOTOPE RATIO MASS SPECTROMETRY

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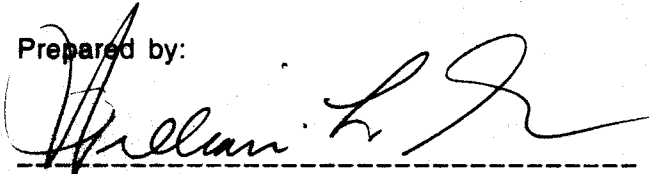
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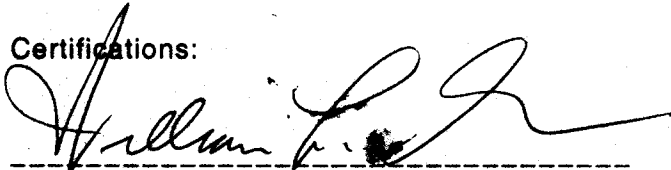Version 1.00
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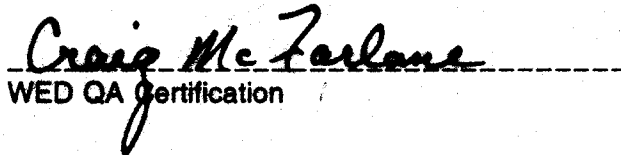Integrated Stable Isotope Research Facility

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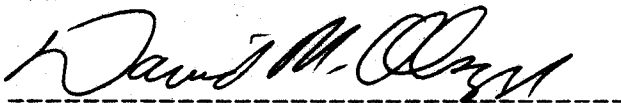Integrated Stable Isotope Research Facility

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. TABLE OF CONTENTS

C. INTRODUCTION

Measurement of the relative abundances of the stable isotopes of carbon and nitrogen provides basic information on soil biological activity and the processing of carbon and nitrogen. Understanding the isotopic composition and the effects of elevated CO_2 and temperature are critical in resolving questions relating to soil biological processes that impact nutrient cycling and supply in forest ecosystems. Stable isotopic analysis can provide a powerful tool for the understanding of biologically mediated decomposition processes and the symbioses between tree roots and some fungi and/or bacteria that affect nutrient availability.

The measurement of stable isotopic abundances will be extremely valuable in other areas of ecological research as well. Virtually all biological and chemical processes involve isotopic fractionation whereby the heavier isotopes of an element are discriminated against, albeit only slightly. Because this fractionation process changes the relative abundance or ratio of heavy and light isotopes in environmental substrates, analysis of stable isotope ratios can provide a powerful tool for understanding biologically mediated processes such as photosynthesis, nitrogen uptake, decomposition, symbiotic transfers and food web dynamics. Therefore the mass spectrometry techniques used to measure stable isotope ratios of carbon and nitrogen in plant tissue, soil and water will provide essential information on the biological and chemical transformations of carbon and nitrogen in physiological and ecological studies conducted at WED NHEERL.

This OP describes the analytical protocols used in the analysis of environmental samples such as plant tissue, soil, insect tissue or fungal tissue using continuous flow gas isotope ratio mass spectrometry. Samples are obtained from terracosms or other source and are collected, processed, preserved and stored according to existing standard operating procedures and experimental procedures. Sample analysis is conducted using a Finnigan Delta plus isotope ratio mass spectrometer which is interfaced to a Carlo Erba elemental analyzer. A small sample of solid material for analysis (a few milligrams) is weighed out and automatically introduced into the Carlo Erba elemental analyzer where the carbon is combusted to carbon dioxide and the nitrogen is ultimately converted to nitrogen gas. These gases are separated by a packed gas chromatography column and sequentially enter the ion source of the mass spectrometer where ionization takes place. Ions are accelerated down a flight tube which is held in a strong magnetic field. Ions are focused into appropriate detectors according to their differing momentums under the conditions of analysis (accelerating voltage and magnetic field strength). Signals generated by the detectors are used to quantify the relative abundances of the stable isotopes of carbon and nitrogen and are also used to calculate the concentrations of carbon and nitrogen in the sample. An IBM compatible personal computer is used for instrument control, data calculation and data storage.

In this analytical protocol, small samples (typically .5-5 milligrams) are weighed out and introduced into the instrument using an autosampler. The analysis is carried out automatically in a little over 8 minutes with results being stored in computer memory and outputted to a hard

copy printer simultaneously.

Data generated by this method will help answer the question:

Will bacterial and fungal populations, soil fauna, nematode and protozoan community structure, and the colonization of tree roots by mycorrhizal fungi, be affected by elevated CO_2 and climate change?

In addition to addressing the above question, information generated may also provide inputs for modeling, integration and inference tasks.

D. OBJECTIVES STATEMENT

This OP is intended to document proper procedures for conducting stable isotopic abundance measurements using continuous flow electron impact gas isotope ratio mass spectrometry. The objective of this analysis is to quantify changes in stable isotopic abundances of carbon and nitrogen as a result of elevated CO_2, elevated temperature or other environmental factor.

Data quality objectives for the measurement of carbon and nitrogen stable isotopic abundances are summarized below in Table D-1. Data quality objectives for carbon and nitrogen concentrations are summarized below in Table D-2. The emphasis is on practices and procedures that insure all measurements of carbon and nitrogen stable isotopic abundances are consistent (high precision), are of known quality (high accuracy) and are comparable or reproducible over the duration of the anticipated research now underway.

Table D-1. Data Quality Objectives for the Measurement of Carbon and Nitrogen Stable Isotopic Abundances

ANALYSIS	AMPLITUDE-VOLTS	ACCURACY	PRECISION	COMPLETENESS
$\partial^{13}C$[1]	1 - 10	$\pm.5$ $^0/_{00}$[2]	$.5$ $^0/_{00}$[3]	***
$\partial^{15}N$[1]	.5 - 10	$\pm.5$ $^0/_{00}$[2]	$.5$ $^0/_{00}$[3]	***

[1] Delta units are described by the equation: ∂ value $= \{(R_{sample} - R_{standard}) / R_{standard}\} * 1000$ where R_{sample} is the isotopic ratio of the sample and $R_{standard}$ is the isotopic ratio of the standard.

[2] Agreement with the predicted or certified value of the reference material of plus or minus .5 $^0/_{00}$.

[3] Based on one standard deviation. Repeated measurements should have a standard deviation of not more than .5 $^0/_{00}$. For practical purposes, the standard deviation of the delta value should be interpreted as a coefficient of variation. For a detailed explanation, see page 58 of the addenda section.

***Completeness is not an issue. All samples submitted and accepted for analysis will be analyzed barring some unexpected mishap in the laboratory during transport, storage or analysis.

Table D-2. Data Quality Objectives for the Measurement of Carbon and Nitrogen Concentrations

ANALYSIS	CONCENTRATION	ACCURACY	PRECISION	COMPLETENESS
Carbon	50%	90-110%[4]	10%[5]	***
Nitrogen	10%	90-110%[4]	10%[5]	***

[4] Percent of predicted or certified value of the reference material.
[5] Coefficient of variation based on one standard deviation.
***Completeness is not an issue. All samples submitted and accepted for analysis will be analyzed barring some unexpected mishap in the laboratory during transport, storage or analysis.

D.1 Precision, Accuracy and Completeness

Only limited data is available on precision and accuracy at this time due to limited experience with the chosen method of analysis. As more information becomes available in this area, the data in Tables D-1 and D-2 will be adjusted as necessary.

For the purposes of instrument evaluation, precision is a measure of the variation between repeated analyses of the same sample. It is assessed by replicate measurements of the same sample or standard. The standard deviation and resultant coefficient of variation are indicative of the precision of a given analysis. To calculate precision values for stable isotopic abundance measurements one would determine the standard deviation of replicate measurements (delta values). The value of the standard deviation should not exceed 0.5 per mil. The standard deviation of the delta replicate delta values should be interpreted as a coefficient of variation as explained above in footnote 3 of table D-1. To calculate precision values for carbon or nitrogen concentration analyses one would determine the standard deviation of replicate analyses. The standard deviation is divided by the mean and multiplied by 100 to obtain the coefficient of variation. The coefficient of variation should not exceed 10% for carbon or nitrogen concentration measurements.

Accuracy is a measure of the difference between the actual determined value of an analysis and the "actual", "true" or reference value. Accuracy may be assessed by the use of "spikes", that is, the addition of known amounts of analyte to a sample and the analysis of the sample with and without the spike to assess "recovery". Accuracy may also be assessed by analyzing reference materials supplied by organizations such as the National Institutes of Standards and Technology. These reference materials have certified values which are documented by exhaustive analysis, usually by several separate methods. Bias, which affects accuracy, is characterized as a

systematic error due tó experimental method or analytical procedure which causes an analytical result to deviate from the "actual" or "true" value in a predictable manner. Accuracy of stable isotopic abundance measurements is best assessed by comparing measured values of a reference material to its certified or true value. Accuracy is acceptable if the measured value agree with the certified value within plus or minus 0.5 per mil. Accuracy of carbon and nitrogen concentration measurements is calculated be measuring the value of a reference material, dividing the measured value by the certified value and multiplying by 100. An accuracy value of 90-110% of the predicted value is acceptable.

Accuracy may also be assessed by the use of "spiked" samples. In this case at least three aliquots of sample material are analyzed. Two aliquots are measured as replicates and a third is measured after the addition of a known amount of spiking material having known isotopic abundance and concentration values for carbon and nitrogen. A predicted value for isotopic abundance or concentration can be calculated using the replicate analyses of the unspiked material and the known values for the spiking material. The measured value for isotopic abundance is compared to the predicted value to obtain a "recovery" or accuracy value. The measured value of the spike should agree with the predicted value of the spike to within plus or minus 0.5 per mil. The measured concentration value of the spiked sample is divided by the predicted value and multiplied by 100 to obtain a "recovery" or accuracy value. An acceptable accuracy or recovery value would be 90-110% of predicted. See section H of this OP for more details.

Completeness is not an issue for this particular analysis protocol. All samples submitted and accepted for analysis will be analyzed barring some unexpected mishap in the laboratory during transport, storage or analysis.***

D.2 Comparability

Continuous flow isotope ratio mass spectrometry (CF-IRMS) has only recently come on the scene (Preston and Owens, 1983). Isotope ratio mass spectrometers without continuous flow capability were first designed in the early 1950s (McKinney, et al, 1950). As one would expect, electronics and data handling capabilities were extremely primitive even on the early continuous flow instruments. Instrument sensitivity on early models would be considered poor by today's standards. Instrument stability and the precision and accuracy of measurements have all improved drastically compared to instruments available only a few years ago.

No other techniques are currently available for the analysis of stable isotopes at natural abundance levels. It should also be noted that the use of this particular technique is quite new in ecological research and therefore no certified environmental reference materials such as plant tissue, animal tissue or soil are available for stable isotope work at natural abundance levels. Therefore, a great deal of effort has gone into careful calibration of our instrumentation against reference materials which are traceable to the National Institute of Standards and Technology.

NIST 8541, graphite; NIST 8542, Sucrose; NIST 8543, Carbonatite; NIST 8547, Ammonium Sulfate and NIST 8548, Ammonium Sulfate were all extensively used in the initial calibration of the new Finnigan Delta Plus system (See the Addenda Section, Pages 34-40). The instrument was also used for the analysis of several materials which have long been used by the University of Utah at the Stable Isotope Ratio Facility for Environmental Research. Isotopic values for carbon and nitrogen on these materials have been carefully established by this facility over the past several years. These materials were cabbage tissue, spinach tissue, graphite and cellulose (See the Addenda Section, Page 57). Isotopic abundance data was obtained on these materials using the new Finnigan Delta Plus. These data were very similar to data obtained by the University of Utah on these same materials.

D.3 Representativeness

Representativeness is not an issue in terms of the successful completion of this analysis protocol. All samples must be collected, processed and stored according to existing, approved SOPs, EPs and OPs. Due to the fact that very small samples are taken for analysis, it may be necessary to implement sample grinding procedures which give exceedingly fine particle sizes. This may require the use of grinding equipment not currently available in our facilities. Sample homogeneity is established by the analysis of replicates from the same sample container. A significant number of all samples submitted and accepted for analysis (usually 4%) will be replicated in this manner.

D.4 Instrument Detection Limits

Instrument detection limits are based on several factors such as the sample type, condition of the Carlo-Erba combustion analyzer and whether the mass spectrometer is tuned for maximum linearity or maximum sensitivity. Detection limits also depend on whether sample combustion gases must be diluted to obtain ideal, onscale signals from the detectors (mass spectrometer Faraday cup detectors saturate at approximately 10 volts of signal). Most samples having natural levels of carbon and nitrogen will have very high amounts of carbon and very low amounts of nitrogen (very high carbon to nitrogen ratios). Therefore, if a single sample is to be analyzed for both carbon and nitrogen in a single pass, nitrogen would not be diluted while carbon in a sample of, for example, plant tissue would need to be diluted about 30 fold to obtain a signal in the optimum range. Samples containing high levels of carbon and low levels of nitrogen may also be run twice using differing sample sizes and lower dilution rates for carbon to achieve on-scale signals. A large sample would be run for nitrogen and a small sample would be run for carbon. If high carbon low nitrogen samples are to be run for carbon and nitrogen in the same pass and carbon is to be heavily diluted, the linear response range for carbon is drastically shortened. A maximum signal for carbon at 30 fold dilution would be on the order of 3.5 volts as opposed to 6-10 volts at 7 fold dilution rate or 10 volts on rare occasions when no dilution at all is required for carbon. Carbon concentration values (as opposed to isotopic

abundance values) may also be affected by high dilution rates which cause degradation of peak shapes and subsequent problems with data acquisition and data processing. All of the factors mentioned above require careful consideration of sample analysis strategies.

As a general rule, the minimum signal level to obtain valid isotopic abundance values for nitrogen is about 0.5-1.0 volts and about 1.0 volts for carbon. These signal levels are equivalent to about 25 micrograms of nitrogen (no dilution) and about 225 micrograms of carbon at 30 fold dilution. 55 micrograms of carbon would be required to give a 1 volt signal at a 7 fold dilution level and about 8 micrograms would be required for a 1 volt signal if no dilution at all were used. These minimum signal levels will usually give good concentration values for carbon and nitrogen as well.

E. LIST OF EQUIPMENT, REAGENTS AND EXPENDABLE SUPPLIES

E.1 Laboratory Instrumentation

1. Finnigan Delta Plus mass spectrometer with dual viscous inlet.

2. Carlo Erba Model NC2500 elemental analyzer configured for carbon and nitrogen and water removal.

3. Hewlett-Packard model 6890 high resolution gas chromatograph.

4. Gateway G6-200 personal computer with 32K RAM and 2.1 gigabyte hard drive. Configured with REAL 32 operating system and ISODAT software.

5. Hewlett-Packard Desk Jet 600 ink jet printer.

6. Mettler AT 21 microbalance. For weighing milligram quantities to 3 decimal places.

E.2 Reagents and Consumable Supplies

Chevron Turbine Oil, GST ISO 68. For small mechanical vacuum pumps.

Chevron Turbine Oil, GST ISO 100. For large mechanical vacuum pump.

Costech #011001 Chromium Oxide or equivalent.

Costech #021022 Magnesium Perchlorate or equivalent.

Costech #011009 Tungsten Oxide on Aluminum or equivalent.

Costech #021025 Quartz turnings or equivalent.

Costech #021020 Carbon Dioxide Absorbent or equivalent.

Costech # 021026 Quartz Wool or equivalent.

Fisons #33821710 Cupric Oxide Wires or equivalent.

Costech #011005 Reduced Copper, Pure or equivalent.

Costech #061105 Opaque Quartz Reaction Tube or equivalent.

Costech #041061 Tin Capsules or equivalent.

Costech #041060 Tin Capsules or equivalent.

Finnigan #M0000-56911 Gasket or equivalent.

Finnigan #M00-1027920 Filament Assembly.

Finnigan #M0000-69322 Gasket or equivalent.

Finnigan #00950-00911 Lubricant Cartridge for Turbo Molecular Drag Pump.

Finnigan #00950-01116 Lubricant Cartridge for Turbo Molecular Drag Pump.

Oxygen, Zero Grade, for Carlo Erba Elemental Analyzer.

Helium, Ultra High Purity, 99.999%, for Carlo Erba Elemental Analyzer.

Nitrogen, Ultra High Purity, 99.999%, Delta Plus Reference Gas.

Carbon Dioxide, Coleman Grade, 99.99%, Delta Plus Reference Gas.

F. SAMPLING PROCEDURES AND SAMPLE CUSTODY

Generally speaking, sampling procedures will be the responsibility of the project personnel submitting samples. Staff personnel of the ISIRF (Integrated Stable Isotope Research Facility) will offer appropriate advice in this area as necessary.

Of greatest concern with sampling procedures (which may include sampling intervals, sample collection, sample preparation such as grinding, sample containment, sample preservation and sample storage) is the prevention of contamination by materials which would change isotopic abundance levels. Also of great concern are any types of processes which could alter isotopic abundances such as exposure to chemical attack or photodegradation due to long term exposure to ambient light levels.

All samples submitted and accepted into the ISIRF for analysis will be documented by hardcopy and/or electronic methods. A complete chain of custody will be maintained on all samples while they reside in the facility. Upon completion of all analyses, samples will either be returned to the primary investigator or will be archived in the facility for future use as necessary.

G. ANALYTICAL PROCEDURES

The Finnigan Delta Plus isotope ratio mass spectrometer as operated in the continuous flow mode is a very complex and sophisticated system. As such, the instrument system has several main sub-systems which will be discussed in detail. These subsystems consist of microbalance and computer interface, Carlo Erba elemental analyzer, Finnigan Conflo II interface which interfaces the Carlo Erba elemental analyzer to the Finnigan Delta Plus mass spectrometer, Finnigan Delta Plus mass spectrometer and instrument control -- data acquisition computer system with REAL 32 operating system and ISODAT software. All operating and service manuals are stored in lab 232 (mass spectrometry lab).

G.1 Mettler AT 21 Microbalance

Reference the Mettler AT 21 operating manual (Mettler Toledo, 1994) for exhaustive details on the operation of this balance.

Turn on the microbalance and allow at least 30 minutes for warm-up. Turn off the air conditioning system in Lab 232 for all weighings. The rather violent air movement in this small lab caused by the air conditioning fan will have a drastic negative effect on precision if the fan is left on. Check the calibration of the balance at least twice monthly using a five milligram ASTM Class I weight. A properly calibrated balance will give a reading of 5.000 milligrams plus or minus .005 milligrams. Record all balance calibration checks in the daily activity log.

Check all samples visually for homogeneity and uniform particle size. Samples having poor homogeneity or non-uniform particle size will give very poor precision due to the small sample weights used for analysis.

Select the proper size tin cups (large or small)(5mm x 7mm or 3.5mm x 5mm) most suited for the analysis to be performed. Tin cups may often be used directly from the container without further cleaning. Be aware, however, that some lots may require cleaning to achieve acceptable blank levels. Sequential washing with methanol, acetone and hexane will usually result in suitably clean tin cups. Tin cup cleanliness should be evaluated by running actual instrument blanks. Tare the tin cup and place the desired amount of sample in the tin cup. Sample sizes will usually range from 0.5 milligrams to 5 milligrams depending on the amount of carbon and nitrogen in the sample. Write the sample weight in the proper box on the sample location list for stable isotope analysis (See the addendum section, pages 55-56, for a copy of this form). Remove the tin cup and sample from the balance and using flat tweezers, carefully fold over the top of the cup to seal the sample inside. Carefully compress the tin cup containing the sample into a small nugget. Cup and sample must be tightly compressed to exclude any air pockets. Place the weighed and compressed sample into the proper well of the sample storage tray. Repeat as necessary.

G.2 Carlo Erba Combustion Analyzer

A Carlo Erba elemental analyzer is used to convert carbon and nitrogen in the sample to carbon dioxide and nitrogen gas for analysis in the Finnigan Delta Plus mass spectrometer. For exhaustive details on the operation of the Carlo Erba elemental analyzer consult the operating manual (CE Instruments, 1996).

The Carlo Erba elemental analyzer is set up to run under the following conditions:

1. Oxygen Pressure = 100 PSI

2. Helium Pressure = 100 PSI

3. Helium Flow Rate = 100 ml/minute

4. Oxidation Furnace Temperature = 1020 Degrees C

5. Reduction Furnace Temperature = 650 Degrees C

6. Actuator Compressed Air Pressure = 70 PSI

Standby conditions for the Carlo Erba combustion analyzer are as follows:

1. Oxygen Pressure = OFF

2. Helium Flow Rate = 20 ml/minute

3. Oxidation Furnace Temperature = 820 Degrees C

4. Reduction Furnace Temperature = 520 Degrees C

Check maintenance logs for the Carlo Erba elemental analyzer and determine if the expected number of analyses can be performed before the next maintenance interval. Ashes must be removed from the oxidation tube approximately every 150 samples analyzed. The oxidation tube must be replaced approximately every 1000 analyses and the reduction tube must be replaced approximately every 500 analyses. The water trap must be replaced approximately every 300 analyses. Counters are available on the instrument to record the numbers of analyses accumulated on each tube or trap. These counters are accessed by keying in the appropriate commands on the instrument control keypad.
Place the elemental analyzer in the operational mode by entering the appropriate commands through the key pad on the front of the instrument. About 30 minutes is required to reach

operating temperatures if the instrument is in stand-by mode. Several hours are required to reach operating temperatures if the instrument is cold.

Place prepared samples in the appropriate stations on the autosampler carousel. Place the lid and dust cover on the carousel.

The Carlo Erba elemental analyzer is now ready for analysis.

G.3 Conflo II Interface

The Conflo II interface serves three discrete purposes in the process of moving sample gases from the Carlo Erba elemental analyzer to the ion source of the Finnigan Delta Plus mass spectrometer (See the Conflo II Interface Operating Manual)(Finnigan MAT, 1997b). The first function of the Conflo II interface is to serve as a flow reduction device for decreasing the relatively high gas flow from the Carlo Erba elemental analyzer (100 ml/min) to the relatively low flow required by the mass spectrometer (0.3 ml/min). A relatively low flow into the mass spectrometer ion source is necessary to maintain the required high vacuum. The second function served by the Conflo II interface is to serve as a device for introducing each of the reference gases (carbon dioxide and nitrogen gas) required for determining the relative isotopic abundances of carbon and nitrogen in the sample gases from the Carlo Erba elemental analyzer. It in essence acts as a programmable injection device for adding standard gases into the mass spectrometer in the required amounts at the specific times required. The third function performed by the Conflo II interface is to serve as a dilution device. In most environmental samples to be analyzed on the Carlo Erba elemental analyzer, the amount of carbon present is extremely high compared to the amount of nitrogen. To facilitate analysis of both carbon and nitrogen in the same pass it is necessary to dilute carbon dioxide to maintain a signal on scale for carbon and still use a sample size large enough to detect nitrogen. The Conflo II interface can be programmed to perform this function at the proper time in a given analytical sequence. The amount of dilution of a given sample type is manually adjusted by increasing or decreasing the pressure of the helium diluent gas using the regulator on the front panel of the interface. As a general rule, a dilution factor of approximately 7 fold is accomplished by a pressure setting for helium of about 1.2 bar. A further 4.3 fold dilution (30 fold total dilution) is accomplished by increasing the helium pressure to 1.6 bar. Pressure settings for the Conflo II interface are therefore set in the following manner:

1. Carbon Dioxide Reference Gas = 1.5 bar

2. Nitrogen Gas Reference Gas = 1.5 bar

3. Helium Diluent Gas, 7 Fold Dilution = 1.2 bar

4. Helium Diluent Gas, 30 Fold Dilution = 1.6 bar

The Conflo II interface also acts as a device for varying the amount of carbon dioxide or nitrogen gas entering the mass spectrometer during evaluation of instrument linearity. Linearity checks of the mass spectrometer are required to insure that isotopic abundance values are the same or nearly the same for highly variable amounts of carbon dioxide or nitrogen gas entering the mass spectrometer. Linearity evaluations will be discussed in detail in the mass spectrometer calibration section of this OP.

G.4 Finnigan Delta Plus Isotope Ratio Mass Spectrometer

The Finnigan Delta Plus is a low resolution electron impact mass spectrometer (See the figures showing the instrument configuration and ion path, Figures Section, Pages 60-61). A gaseous sample from the Carlo Erba elemental analyzer is fed into the mass spectrometer ion source. This sample may contain either carbon dioxide or nitrogen gas mixed with helium. A high energy electron beam in the ion source strips electrons from the parent molecules to form ions which are accelerated out of the ion source under an accelerating voltage of about 2 KV. The ions move into a curved flight tube surrounded by a .75 Tesla electromagnet held at constant field strength for the ions of interest. Ions are focused into the proper Faraday cup detectors on the basis of their momentum under the chosen conditions of accelerating voltage and magnetic field strength. The heavier ions will have more momentum and therefore less curvature in the flight tube. Three Faraday cup detectors are arranged to pick up masses 28, 29 and 30 for nitrogen or masses 44, 45 and 46 for carbon dioxide. Ions striking the Faraday cup collectors generate an electronic signal which is directly proportional to the number of ions. These electronic signals are amplified and then integrated for the purposes of calculating the relative abundances of the carbon and nitrogen stable isotopes. Data may be outputted to a hard copy printer or into computer memory or both.

Refer to the Finnigan Delta Plus operating manual (Finnigan MAT, 1997a) and the ISODAT software operating manual (Finnigan MAT, 1996) for exhaustive details on the operation of the Delta Plus mass spectrometer.

SUMMARY OF OPERATION

Turn on all compressed gas supplies and check all gas pressures at cylinders, at Carlo Erba elemental analyzer pressure gauges and at the Conflo II interface pressure gauges. Take the Carlo Erba elemental analyzer off standby and wait at least 30 minutes for the system to reach operating temperatures. Check the mass spectrometer rough vacuum and high vacuum readouts by clicking on the ISODAT command CNF-C, SUPP-C. Rough vacuum should read about 2.7×10^{-3} mbar. High vacuum should read about 1×10^{-8} mbar. Open the ion source inlet valve and again note both the rough vacuum and high vacuum readings. Rough vacuum should now be about 3.4×10^{-3} mbar and high vacuum now be about 1.4×10^{-6} mbar.

Bring up the Conflo II interface control screen of the ISODAT software by clicking on the command CNF-C, SUPP-C. Turn on the nitrogen reference gas by clicking on the Reference 2 valve icon. Bring up the instrument control screen on the ISODAT software by clicking on the command CNF-D, ACON-D. Do a manual cup configuration to nitrogen by clicking on the cup configuration command and selecting N_2 from the pop-up menu. Wait 60 seconds and then perform a manual peak center for nitrogen by clicking on the peak center command. Record the resulting peak center HVDAC value on the screen in the daily activity log. Go back to the reference gas control screen and turn off the nitrogen reference gas and turn on the carbon dioxide reference gas by clicking on the Reference Gas 1 valve icon. Go back to the instrument control screen. Perform a manual jump to CO_2 by clicking on the appropriate command. Enter the HVDAC value for the CO_2 jump into computer memory and into the daily activity log.

Bring up the sequence editing screen of the ISODAT software by clicking on the command CNF-C, EDIT-C. Click on the sequence editor icon. Select the sequence entitled "MAIN". The ISODAT software is now ready to accept information on a sample run. A "sequence" is the term applied to the sample run set-up and describes all of information necessary for the mass spectrometer to analyze a set of samples, calculate isotopic abundance values for carbon and nitrogen and calculate concentrations of carbon and nitrogen in each sample. A sequence line corresponds directly to a location on the sample location list form and to a location in the sample storage tray (see the addenda section, pages 55-56). Enter the required information on port number, sample identification, method (see the addenda section for printouts of the appropriate methods, pages 43-54), action (identifies the particular analysis as a blank, standard or sample) and sample weight, if any, for each sequence line. When all information has been added, click on the save command to store the new entries.

Bring up the Conflo acquisition screen of the ISODAT software by clicking on the command CNF-C, ACON-C. Click on the sequence acquisition icon. Click on the command for activating the sequence table menu. Select sequence "MAIN". Enter the number of the sequence starting line, the number of the sequence ending line, the DOS file name if data is to be saved to the computer hard drive, the hardcopy printout format type, the DOS file type (a Lotus/Excel format is always requested) and whether the data is to be saved into the ISODAT database by the command "COL".

To activate a sample run, click on the measure command. The system will commence a sample run with the Carlo Erba elemental analyzer and mass spectrometer under control of the computer. No further operator intervention is required until the run ends. Samples will be analyzed automatically according to the designated sequence, at a rate of approximately one sample every 8 minutes and 20 seconds. Data will be calculated and entered into the ISODAT database, onto the computer hard drive and will be printed out at the hard copy printer as requested (See the addenda section, page 42, for an example of a data printout).

Sample blanks (tin cup only), standards, replicates and spiked samples should be run at regular intervals throughout a sample run and throughout every working day. Samples of known isotopic composition, having the same matrix type as the samples of interest, should also be run on a regular basis during every working day. This will provided regular checks of precision and accuracy from a completely separate source.

Carbon and nitrogen are quite variable in most environmental samples. The most common scenario is to have high levels of carbon and much lower levels of nitrogen. In some soils carbon levels are quite low and nitrogen is almost undetectable. Plant tissues usually have high carbon levels and levels of nitrogen which vary from a few percent to almost nothing. Animal tissues have high carbon levels and levels of nitrogen which are usually much higher than the levels in plant tissues. It is always prudent to evaluate data to make sure that instrument responses are in the ideal detection range. This means that signal amplitudes for nitrogen must not fall below about 0.5 volts and should not be above 10 volts (detector saturation level). Carbon signals should not fall below about I volt and should not be above 10 volts for undiluted samples. These signal ranges should give data of the best quality. Sample signal values outside of these ranges should be rerun using an adjusted sample weight or, if possible, a larger dilution should be used for carbon to insure that both carbon and nitrogen will be on scale.

See section H of this operating plan for complete details of quality assurance/quality control requirements.

H. QUALITY ASSURANCE/QUALITY CONTROL

H.1 Quality Assurance

Quality assurance deals with those aspects of an analytical method which relate to procedures and operations that guarantee data quality and may be defined as the overall system used to establish that the quality control process is operating properly. Method selection, experimental design, sample collection and preservation, determination of and correction for background levels of the analyte of interest (blank), instrument calibration/standardization and determination of interferences, whether chemical or other type, are all aspects of the quality assurance process. Proper quality assurance is necessary to eliminate problems associated with precision, accuracy and bias. The ability to compare data from different methods or sources and from different time periods is enhanced through the implementation of good quality assurance practices.

H.1.1 Blank

Carbon and nitrogen are ubiquitous in nature and in the laboratory. Great care must be taken to insure that carbon and nitrogen contamination have no adverse effect on data quality during carbon and nitrogen stable isotopic abundance analyses. All background levels of carbon and nitrogen (blank) must be consistent and as low as possible. To insure consistent, low level blanks, check blank responses frequently. Every twelfth sample analyzed will normally be a blank. Check all new batches of tin cups very carefully for low consistent blank values. Keep the lab as clean as possible to prevent outside contamination during sample weighing and other sample manipulations.

Sample Collection and Storage: It is imperative that materials used in sample collection and storage equipment be inert and free from contaminating forms of carbon and nitrogen. If possible, glass should be used for sampling equipment and for sample storage containers. Brown borosilicate glass is preferred but brown soda lime glass may also be used. Brown glass containers are especially desirable for sample storage because they decrease the exposure of samples to ambient light which may cause sample degradation. Glass equipment should be baked at 350 degrees C. for 6 hours to insure cleanliness. If glass cannot be used, teflon, polyethylene or other polymeric material may be used if it has been established that these materials have suitable light excluding properties and neither add nor remove carbon or nitrogen from the sample.

Laboratory Operations: Great care should be taken during sample collection, sample preparation and sample storage procedures to insure that carbon and nitrogen containing materials do not contaminate samples. Excessive contamination may drastically effect isotopic abundance measurements. During laboratory manipulation of samples for isotopic abundance measurements, it is important to carefully contain finely divided samples such as

plant tissue or soil. If static electricity becomes a problem, deionizing devices should be used or laboratory humidity should be adjusted, if possible. All spills of sample material should be immediately and scrupulously cleaned up to prevent future contamination and subsequent error in isotopic abundance values for carbon and nitrogen. Equipment such as grinders and spatulas must be carefully cleaned to prevent cross contamination of samples. Avoid plastic equipment or sample containers as they tend to build up static charges which can cause erratic balance performance and can cause materials to "jump" or "fly" out of containers and off of spatulas and into the air or onto flat surfaces in the lab. The air conditioning system in Lab 232 (instrument lab) should always be turned off during sample weighing procedures. This lab is very small and the large fan on the air conditioning system causes rather violent air movement during normal operation. Resulting violent air movement causes balance instability during sample weighing and may contaminate the laboratory with airborne particles of sample material. All flat surfaces in the lab should be frequently cleaned by wet wiping if possible.

Instrument Reagent Blanks: The Finnigan Delta Plus mass spectrometer, as configured for continuous flow measurements, is an extremely sensitive instrument for the measurement of low levels of carbon and nitrogen. However, due to contaminants in the oxygen supply used for combustion on the Carlo Erba elemental analyzer, contaminants in the helium carrier gas, contaminants in the tin cups used for sample containers and contamination from atmospheric nitrogen, minute amounts of carbon and nitrogen exist in the system and appear as background signals or blank values. In order to compensate for these background levels of carbon and nitrogen in the instrument, reagent blanks must be run until levels are acceptably low and stable. It should be noted that some lots of tin cups may need to be sequentially washed with methanol, acetone and hexane to achieve lowest blanks. Acceptable blanks must then be recorded and used for correct computation of stable isotopic abundances for carbon and nitrogen and for correct calculation of carbon and nitrogen concentrations.

As a general rule, a series of four blanks consisting of a tin cup only, are run as the first four sequence lines. The fourth blank is usually acceptably low and stable for both carbon and nitrogen. The ISODAT software uses the last blank run to make corrections on all data. Subsequent new blanks are used for data correction as they are run in the analytical sequence. Instrument reagent blanks are run at every twelfth line of the analytical sequence. This insures that all data for carbon and nitrogen is properly corrected for changes in background over the duration of the sample run.

H.1.2   Instrument Calibration

The Finnigan Delta Plus gas isotope ratio mass spectrometer as configured for continuous flow analysis, is an extremely sophisticated instrument system for the determination of the stable isotopic abundances of carbon and nitrogen in environmental samples at natural levels and is also capable of determining both nitrogen and carbon concentrations in these samples. Since the instrument system makes both stable isotopic abundance measurements as well as

concentration measurements, two separate instrument calibration protocols are necessary.

Instrument Calibration for Stable Isotopic Abundance Measurements: Certified reference materials for stable isotopic abundance measurements on environmental materials such as plant tissue, animal tissue or soil are not currently available. This is due in part to the relatively recent application of stable isotopic abundance measurements to ecological research problems. The National Institutes of Standards and Technology does, however, have several materials which while not of "environmental" origin, do provide a viable means of calibrating the mass spectrometer for precise and accurate stable isotopic abundance measurements. These materials are NIST 8541, graphite; NIST 8542, Sucrose; NIST 8543, Carbonatite; NIST 8547, Ammonium Sulfate and NIST 8548, Ammonium Sulfate (see the addenda section, pages 34-40). Also, refer to section D-2 of this document.

The Finnigan Delta Plus mass spectrometer, Carlo Erba elemental analyzer and the Conflo II interface are used in combination to make continuous flow stable isotopic abundance measurements on environmental samples containing carbon and nitrogen. Carbon dioxide and nitrogen gas are separated gas chromatographically before they enter the mass spectrometer. Just before the nitrogen gas from the sample enters the mass spectrometer, a metered amount of standard reference nitrogen gas from a large compressed gas cylinder is injected through the Conflo II interface into the mass spectrometer as a calibrating standard for the isotopic abundance measurement of nitrogen in the sample. The same process occurs for carbon isotopic abundance measurements except that the standard reference carbon dioxide is injected shortly after the carbon dioxide from the sample has passed through the mass spectrometer.

The two standard reference gases are used for each and every sample analysis that is performed by the mass spectrometer. It is, therefore, of critical importance that these two standard gases be assigned correct stable isotopic abundance values. The two standard reference gases are calibrated and assigned correct isotopic abundance values by analyzing small amounts of the standard reference materials above just as if they were regular samples. The standard reference gas values for nitrogen and carbon are adjusted on the basis of these analyses until the stipulated values for NIST 8541, 8542, 8543, 8547 and 8548 are obtained by actual analysis. The delta ^{15}N value for standard reference nitrogen gas was determined as above to be -.87 per mil. The delta ^{13}C value for standard reference carbon dioxide gas was determined as above to be -37.27 per mil at standard dilution (7 fold). To get a carbon dioxide gas standard reference value for the increased 30 fold dilution, acetanilide (the material used as a concentration calibrating standard) was analyzed at normal dilution and at the increased 30 fold dilution. The difference between the delta ^{13}C value obtained for acetanilide at normal dilution for carbon and the delta ^{13}C value for acetanilide at the 30 fold dilution value was calculated. This difference was used to correct the standard reference gas value for carbon. The delta ^{13}C value for the carbon dioxide standard reference gas at the 30 fold dilution rate was determined to be -36.44 per mil for a signal amplitude of approximately 1-3 volts.

The calibration of the two standard reference gases was carefully done and since both gas supplies are very large, it is expected that this calibration procedure will not need to be repeated for some years. No deterioration of either gas or change in isotopic abundances of either gas is expected to occur for an extended period of time.

It is extremely important to understand the effects of helium dilution on overall mass spectrometer response. Helium dilution is almost always necessary in continuous flow analysis due to a very common and vexing problem. This problem revolves around the fact that most biological materials such as coniferous foliage and many other materials such as some soils have very large carbon to nitrogen ratios -- that is, carbon is very high compared to nitrogen. If one is to derive the maximum amount of information per unit time from a continuous flow analysis it is desirable to obtain valid data for both carbon and nitrogen during the same run. This becomes very difficult due to the high carbon to nitrogen ratios in most materials of interest and because the mass spectrometer is much less sensitive to nitrogen than to carbon. The ultimate goal of this analysis, and all others for that matter, is to obtain data of the highest possible quality. This goal should, of course, be achieved as efficiently as possible. The analyst is therefore left with two possible alternatives for conducting routine analytical operations since relative instrument sensitivity for carbon and nitrogen cannot be changed. In the first case, two separate samples can be analyzed, a smaller sample diluted as little as possible to obtain a valid carbon value and a large sample not diluted at all to obtain a valid nitrogen value. This, of course, takes at least twice as much time as the analysis of a single sample that obtains valid results for both carbon and nitrogen. In the second case, a single sample, of sufficient size to obtain a valid nitrogen value is analyzed. This approach results in a carbon signal which is much too high to give a valid carbon value under normal circumstances. The carbon signal must somehow be reduced to an acceptable level. This is best achieved by helium dilution through the Conflo II interface as the carbon dioxide peak exits the Carlo-Erba combustion analyzer on its way to the mass spectrometer ion source. This approach, while valid, may drastically effect data quality and rigorous steps must be taken to insure that proper reference gas values are utilized to correct for the effects of helium dilution. In general, increasing helium dilution will cause mass spectrometer linearity to degrade to a greater and greater extent. Linearity refers to the ability of the mass spectrometer to obtain the same delta value on a particular sample over a wide range of signal sizes (1-10 volts). A further complication arises in that higher signal levels seem, to a small degree, to also adversely affect instrument linearity, even at lower dilution levels. The most useful technique for correcting the effect of helium dilution is to run a given sample at a given dilution over a wide range of signal amplitudes. One may then clearly understand the effects of dilution and signal strength on data quality. To this end, several aliquots of NIST 8542 Sucrose were weighed out so as to give signal amplitudes ranging from approximately 0.5-9 volts at 30 fold dilution. Delta values for carbon were plotted against signal amplitude. Regression analysis of this data yielded the equation $y = -10.906 + .29X - .015X^2$ and an R^2 value of .995 (See page 63 of the Figures section). It should be noted that the stipulated

delta value for carbon 13 of NIST 8542 is -10.47 $\pm$ .13 . It is apparent from examining the plot of carbon 13 delta values versus signal amplitude that dilution does indeed have a significant effect on instrument linearity. In order to obtain data of the best quality at high dilution, one would therefore select an ideal signal amplitude value of say 4 to 5 volts. Samples, standards and spikes would we weighed out to give this response within narrow limits. The deviation from the stipulated value of NIST 8542 at the selected amplitude would be determined from the plot above. The reference gas value would be adjusted to reflect this deviation. The result would be high quality data properly corrected for the effects of dilution. It should be further noted that other sample matrixes may not respond to helium dilution in the same way as sucrose. It would therefore be necessary run a correction curve for that particular matrix.

Instrument Calibration for Carbon and Nitrogen Concentration Measurements: A single point calibration method is used for all concentration measurements of carbon and nitrogen. During a given analytical sequence the fifth sample run is a concentration calibration standard (the first four samples of the sequence are blanks as mentioned above in section H.1.1). A concentration calibration standard is run every twelfth sequence line during a given run. Each new standard serves as the standard for calculating concentration values for carbon and nitrogen on the next ten samples. See table D-2 for data acceptance criteria.

Instrument calibration for carbon and nitrogen concentration measurements is accomplished by weighing out approximately 0.5 milligrams of high purity acetanilide and analyzing the acetanilide in the same way that samples are analyzed. As mentioned above, standards and blanks are specially identified as such by the information placed in the "action" prompt of each sequence line. Costech acetanilide, #031040, containing 71.09% carbon, 10.36% nitrogen, 6.71% hydrogen and 11.84% oxygen are used for this purpose. The container of Costech acetanilide currently in use in the laboratory has a delta ^{13}C value of -27.97 per mil and a delta ^{15}N value of 1.18 per mil (See the addenda section, page 57, for complete information on reference materials).

As mentioned previously, each sequence line in an analytical run whether blank, standard or sample, generates a complete data listing including stable isotopic values for carbon and nitrogen and concentration values for carbon and nitrogen as well as numerous other data sets. These analytical data are placed in the ISODAT database for future recalculation if necessary, placed onto the hard drive of the computer and are printed out at the hard copy printer if desired. Eventually it is hoped that the ISODAT software may be adapted to continuously update and evaluate QA data on blanks and standards.

Instrument Tuning and Calibration For Linearity: Linearity refers to the ability of the instrument to give nearly the same stable isotopic abundance values and to give nearly the same concentration values for carbon and nitrogen over a specific weight range of carbon or nitrogen. It is especially important that the instrument have good linearity over a fairly large concentration range so that sample sizes can be adjusted to obtain data on both carbon

and nitrogen by analyzing a sample only once.

Refer to the Delta Plus operating manual (Finnigan MAT, 1997a) and the ISODAT operating manual (Finnigan MAT, 1996) for complete details on procedures for tuning the Delta Plus for best linearity.

As a general rule, the instrument should be tuned for linearity on a monthly basis. Note all tuning information in the daily activity log.

When tuning the Delta Plus for linearity, one must take into account that instrument sensitivity and linearity are related. As linearity is increased, sensitivity decreases and conversely, as sensitivity increases linearity decreases. When tuning the instrument for best linearity one must therefore compromise so that both sensitivity and linearity are satisfactory for the analytical task at hand.

Proceed to tune the instrument for linearity as follows: Bring up the Conflo II interface control screen of the ISODAT software by clicking on the command CNF-C, SUPP-C. Turn on the CO_2 standard reference gas by clicking on the Reference 1 valve icon. Bring up the instrument control screen of the ISODAT software by clicking on the command CNF-D, ACON-D. Signal intensity bar graphs for masses 44, 45 and 46 are now displayed. Note the small panel containing nine potentiometers located in the upper right hand corner of the front panel of the Delta Plus console. This is the ion source voltage divider panel (See the figures section, page 62, for an illustration of the voltage divider panel). These nine potentiometers are used to tune the ion source for best sensitivity and linearity.

Proceed to the middle potentiometer of the middle row. Set this pot to a reading of 7. Experience has shown that this pot has the most influence on linearity and sensitivity. A setting of 7 usually provides the best compromise of sensitivity and linearity. Go to the right hand potentiometer of the middle row and tune for maximum signal as displayed on the computer video monitor. Go to the middle potentiometer of the bottom row and tune for maximum signal. Proceed in order to tune the potentiometers located on the left in the top row, on the left in the middle row and on the left in the bottom row. The potentimeters located in the middle of the top row and to the right in the top row seem to have very little effect on signal intensity and seldom need to be tuned. The usual values set on these two pots are about 9.2 and 8.3. The above protocol should prove satisfactory for giving best linearity and sensitivity for most applications.

In order to verify suitable linearity and sensitivity, a special analysis protocol of varying amounts of standard reference carbon dioxide is conducted and archived for QA/QC purposes. This special protocol proceeds as follows: Bring up the Conflo acquisition screen of the ISODAT software by clicking on the command CNF-C, ACON-C. Click on the single acquisition icon. Type in necessary information on sample identification, method, action and on whether the data is to be saved to the ISODAT data base by typing in the command "COL". The method

to be used is entitled "CO2 STD ON OFF". This method injects ten 20 second pulses of standard reference carbon dioxide into the mass spectrometer. The carbon dioxide pulses are separated by 30 second time intervals. The amount of carbon dioxide entering the mass spectrometer for each pulse is controlled by the pressure of the carbon dioxide standard reference gas as adjusted manually at the Conflo II pressure regulator. Set a starting pressure of 0.50 bar on the carbon dioxide pressure regulator on the Conflo II interface front panel. Start data acquisition by clicking on the "measure" command. After the first pulse of carbon dioxide passes through the mass spectrometer, increase the carbon dioxide pressure to 0.75 bar. Wait for this pulse to pass through the mass spectrometer. Repeat this process for the next eight programmed pulses at pressures of 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50 and 2.75 bar. These eight pressure settings should give signal intensities of about 0.6, 1.0, 1.6, 2.2, 2.8, 3.5, 4.4, 5.1, 6.3 and 7.2 volts.

When data acquisition sequence finishes, display the data by clicking on the ISODAT commands CNF-C, DETECT and CNF-C, CAL-C. All data will be displayed (See the addenda section, page 40, for an example of a printout of linearity data). Refer to the displayed data and note the column labeled "delta vs.PDB 44/45". Also note the column labeled "Peak". Peak five has a star. This is the reference peak for the run. Suitable instrument linearity is reflected by delta values that vary by no more than about plus or minus 0.2 per mil from the reference peak. All values would therefore fall into the range of -37.27 plus or minus 0.20 per mil for a properly tuned instrument.

H.1.3   Interferences

Contamination from extraneous carbon and nitrogen sources usually accounts for almost all interference problems associated with this analysis. Some interference problems may also occur if samples are subjected to chemical degradation, are placed in improper storage containers or are subject to improper storage conditions such as exposure to high levels of ambient light or exposure to high temperatures. At this point in time not much is known about sample collection, preparation, containment, preservation and storage conditions as they effect sample stability for stable isotopic abundance measurements. Until more is known, it seems prudent to take a very conservative view on all sample handling and storage matters.

Perhaps the most common contamination problems associated with stable isotopic abundance measurements are those that relate to poor housekeeping. Instrument laboratories should be kept scrupulously clean. Spilled samples should be immediately and thoroughly cleaned up.

Nitrogen contamination from atmospheric nitrogen gas may also adversely affect performance of the Carlo Erba combustion analyzer. Plastic plumbing on this instrument should be replaced with metal plumbing wherever possible to prevent problems with nitrogen diffusion into the instrument. The sealing surfaces and helium purging efficiency of the autosampler should be checked frequently to insure that all systems are functioning

properly.

H.2 Quality Control

Quality control deals with those aspects of an analytical method which define procedures and operations that guarantee data quality during the routine use of an analytical method. The periodic running of replicate analyses to assess precision, the periodic running of spikes to assess accuracy and the periodic analysis of certified reference materials all are aspects of quality control. Participation in intra- and interlaboratory quality assurance studies may also be helpful in maintaining good quality control over a given analytical procedure.

H.2.1 Replicates

Our state of the art isotope ratio mass spectrometer is, of necessity, a highly precise instrument due to its designed capability for detecting very small differences in isotopic abundance ratios. Two replicates are generally sufficient to give a good indication of precision. Careful attention must, however, be given to careful preparation of samples for proper homogeneity. In general, about 4% of all samples will be subject to replicate analysis. This percentage may be adjusted upward or downward depending on the performance of the analytical method and the needs of the research project. These replicates will be used to assess long term precision during the course of routine use of the analytical method. Generally speaking, precision of replicates for natural abundance samples should agree within about 0.5 per mil based on the standard deviation of a large number of replicate pairs of stable isotopic abundance analyses for both carbon and nitrogen. This standard deviation of 0.5 per mil should be interpreted as a coefficient of variation. (See page 63 of the addenda section for further explanation.) Precision of replicates from concentration measurements for carbon and nitrogen should be about 10% based on the coefficients of variation of a large number of replicate pairs.(See the data quality objectives section of this OP and tables D-1 and D-2). If agreement falls outside these limits,instrument drift may be a problem or background levels of carbon and nitrogen may have changed. Sample homogeneity may also be suspect. In rare instances mechanical problems associated with instrument operation (autosampler) may account for poor precision. Steps should be immediately implemented to identify sources of unacceptable precision and corrective action should be taken as soon as possible. It may be necessary to re-run some samples once corrective action has been taken. As stated above, this is a very precise instrument. The precision criteria set down above are probably at the very outer limits of instrument capability. Usual agreement between replicates is almost without fail much better than the stated limits.

H.2.2 Spikes

In general, about 4% of all samples will be subject to spiking for accuracy assessment. The

same sample that is replicated as per H.2.1 above is usually spiked as well. A spike is a third replicate of the same sample which has a known amount of the analyte of interest added directly to it. NIST Corn Stalk (NIST #8412) is suitable for spiking most plant tissue samples. Accuracy (spike recovery) is calculated by taking the average of the results of the two unspiked replicates and adding the amount of spike added. This becomes the "predicted value" for assessing accuracy. It is obvious that a highly precise instrument will be required if good spike recoveries are to be obtained. For stable isotopic abundance measurements of carbon and nitrogen, the following formula is used to calculate a predicted spike recovery:

If:

A = the delta ^{13}C value or the ^{15}N value of the mean of the unspiked replicates
B = the sample weight in milligrams
C = the percent carbon or nitrogen in the sample
D = the delta ^{13}C value or the delta ^{15}N value of the spike
E = the weight of spike in milligrams
F = the percent carbon or nitrogen in the spike

Then:

$$\text{Spike Predicted Value} = \frac{(A \times B \times C) + (D \times E \times F)}{(B \times C) + (E \times F)}$$

And the units of the spike predicted value are per mil

As a general rule, actual mean spike recoveries should fall within about plus or minus 0.5 per mil of the predicted value (See the data quality objectives section of this OP and table D-1). For measurements of carbon and nitrogen concentrations, the approach for calculating spike recoveries is much the same. The weight or concentration of carbon or nitrogen from the mean value of the two replicates is added to the weight or concentration of carbon or nitrogen from the spike and then divided by the total weight of the combined spiked sample and then multiplied by 100. This gives the total possible or predicted amount of carbon or nitrogen in the spiked sample in percent. The actual analyzed value for the spiked sample is divided by the predicted value and multiplied by 100 to give a percent recovery. Accuracy or spike recovery values may be calculated according to the following formula:

If:

A = weight in milligrams of carbon or nitrogen from sample
B = weight in milligrams of carbon or nitrogen from added spike

C = combined weight in milligrams of sample and added spike in spiked sample

Then:

Spike Predicted Value = $\dfrac{A + B}{C}$ X 100

Recovery (accuracy value) = $\dfrac{\text{measured value for spike}}{\text{predicted value for spike}}$ X 100

Units are percent.

The mean percent recovery of a large number of spikes can be expected to be in the range of 90-110% for concentration measurements (See the data quality objectives section of this OP and table D-2).

Unacceptable spike recoveries are usually a result of poor sample homogeneity, unexpected matrix interferences, instrument malfunction or errors made by the analyst in spiking the sample. Reasons for poor accuracy should be determined immediately and corrected as soon as possible. It may be necessary to re-run some samples after corrective action has been taken.

H.2.3   Certified Reference Materials

The use of stable isotopic abundance measurements in ecological research has become common only in recent years. As a result of this relatively new approach, there are no currently known reference ecological or environmental materials such as plant tissue or soil which have been certified for stable isotopic abundance measurements. Our instrument has, however, been calibrated against various materials not ecological or environmental in nature which are traceable to the National Institutes of Standards and Technology (See the addenda section, pages 34-40). Because of this approach, we may proceed to certify our own reference materials with some certainty. This has been done with several NIST reference materials such as plant tissue, soil and animal tissue (See the addenda section, page 57).

It is usually prudent to periodically assess instrument performance by running an instrument accuracy check using a certified reference material. Although instrument drift is usually not a large problem with this mass spectrometer, it is reasonable to check instrument accuracy during the working day by running a number of samples of known carbon and nitrogen stable isotopic abundance and known carbon and nitrogen concentration.

This accuracy check is best accomplished by running at least two samples of NBS #1570, Spinach, NBS 1575, Pine Needles or other suitable material per working day. Certified reference materials should match the matrix of the samples of interest. NBS Spinach has been internally certified in our laboratory to have a delta ^{13}C value of -26.68 and a delta ^{15}N value of 1.07. We have found that NBS spinach contains 39.58% carbon and 5.56% nitrogen by analysis in our laboratory. NBS has certified a value for nitrogen of 5.9% in this material. Many other materials have been internally certified in our laboratory and should be used as necessary (See page 57 of the Addenda Section). NIST corn stalk, NBS spinach or other suitable material will be used routinely for spiking plant tissue samples and for doing daily accuracy checks of overall instrument performance. Suitable instrument performance with a certified reference material is reflected by values as stated above in the replicates and spikes sections (See the data quality objectives section and tables D-1 and D-2 of this OP).

I. PREVENTIVE MAINTENANCE AND CORRECTIVE ACTION

Custody of the Finnigan Delta Plus mass spectrometer system currently resides with the senior chemist in the ISIRF. Necessary parts for common preventive maintenance procedures are currently on hand. An inventory of preventive maintenance parts will be routinely maintained to prevent excessive instrument down time. The Finnigan Delta Plus system seems to require very little routine maintenance. The Carlo Erba elemental analyzer must have oxidation, reduction and water removal tubes replaced at intervals previously stated in this OP. Mechanical vacuum pump oil must be changed at 3-6 month intervals and lubricant in the high vacuum turbo molecular drag pumps must be replaced on a yearly basis. Ion source filament assemblies can also be expected to fail at approximately one year intervals.

Refer to the instrument manufacturer's service manual for troubleshooting, maintenance and repair procedures (Finnigan MAT, 1996, 1997).

All maintenance procedures will be documented in the daily activity log. This daily activity log resides with the senior chemist in the ISIRF.

J. DATA REDUCTION, VALIDATION AND ARCHIVING

All data collection and reduction is performed by the Finnigan Delta Plus mass spectrometer system. Raw data and calculated results are automatically stored on the computer hard drive and/or are printed out on an ink jet hard copy printer.

Data validation is accomplished by a careful evaluation of quality assurance/quality control data obtained at the time of data collection.

All data will be archived for 3 years after the conclusion of the project. Data will then be managed at the discretion of responsible project personnel.

Summarized reports in the form of Excel spread sheets will be made available to each principal investigator as necessary.

K. REFERENCES

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ADDENDA

National Institute of Standards & Technology

Report of Investigation

Reference Materials 8538–8542

8538: NBS30 (biotite)
8539: NBS22 (oil)
8540: PEF1 (polyethylene foil)
8541: USGS24 (graphite)
8542: Sucrose ANU (sucrose)

These Reference Materials (RMs) are intended to provide samples of known isotopic composition with D/H, $^{13}C/^{12}C$ and $^{18}O/^{16}O$ isotope ratios stated in parts per thousand difference (‰) from the Vienna Standard Mean Ocean Water [VSMOW (RM 8535)] or Vienna Pee Dee belemnite (VPDB, [1,2]) isotope-ratio standards. These RMs are not certified, but their use allows comparability of stable hydrogen, carbon, and oxygen isotope-ratio data obtained by investigators in different laboratories. RM 8538 (NBS30) is intended for stable hydrogen and oxygen isotope-ratio calibration of silicates and is issued in units of 2 g. Hydrogen isotope ratios should be determined on the water fraction (3.5%). RM 8539 (NBS22), RM 8540 (PEF1), and RM 8542 (Sucrose ANU) are intended for stable hydrogen and carbon isotope-ratio calibration of organic materials and are issued in units of 1 mL, a few mg, and 1 g, respectively. RM 8541 (USGS24) is intended for stable carbon isotope-ratio analysis and is issued in units of 0.8 g.

These RMs are distributed on behalf of the International Atomic Energy Agency (IAEA), Vienna, Austria.

The overall coordination of preparation for NIST distribution was carried out by T. B. Coplen, U.S. Geological Survey and R. D. Vocke, Jr., NIST Inorganic Analytical Research Division.

The supporting aspects concerning the distribution by NIST of these RMs were coordinated through the Standard Reference Materials Program by J. S. Kane.

Material Preparation

NBS30 was prepared by I. Friedman, J.R. O'Neil, and G. Cebula of the U.S. Geological Survey from a sample of Lakeview tonalite (Southern California batholith) provided by L. Silver, California Institute of Technology, Pasadena. RM 8539 (NBS22) was prepared by S. Silverman, Chevron Oil Company, La Habra, California. RM 8540 (PEF1) was prepared by H. Gerstenberger and M. Herrmann, Zentralinstitut für Isotopen-und Strahlenforschung, Leipzig, Germany [2]. RM 8541 (USGS24) was prepared by T.B. Coplen, U.S. Geological Survey from Baker® technical grade graphite (96%, <44 µm). Prior to splitting with a sample splitter, six spatially separated ~1-mg samples were analyzed to ensure isotopic homogeneity of the material. Peak-to-peak variation was 0.11‰. RM 8542 (Sucrose ANU) was supplied to the IAEA by H. Polach, Australian National University, Canberra, and was originally intended to replace NBS oxalic acid used for ^{14}C standardization [1].

Gaithersburg, MD 20899
June 22, 1992

William P. Reed, Chief
Standard Reference Materials Program

(over)

It is recommended that these RMs be stored in the containers in which they are supplied to the user.

NOTE: Because very limited quantities of these materials exist, distribution is limited to one unit of each per three-year period of time. Users are strongly advised to prepare their own internal standards for daily use and calibrate those standards against these RMs.

Isotope Abundances

The hydrogen isotopic abundance of these RMs relative to VSMOW (RM 8535) is [1]:

RM	δD_{VSMOW}, ‰
RM 8538 (NBS30)	−66.7 ± 0.3
RM 8539 (NBS22)	−118.5 ± 2.8
RM 8540 (PEF1)	−100.3 ± 2.0

The carbon isotopic composition of these RMs relative to VPDB using a $\delta^{13}C_{VPDB}$ value of +1.95 ‰ for RM 8544 (NBS19) is [1]:

RM	$\delta^{13}C_{VPDB}$, ‰
RM 8539 (NBS22)	−29.73 ± 0.09 [1]
RM 8540 (PEF1)	−31.77 ± 0.08 [1]
RM 8541 (USGS24)	−15.9 ± 0.25 [5]
RM 8542 (Sucrose ANU)	−10.47 ± 0.13 [1]

The oxygen isotopic composition of NBS30 is:

RM	$\delta^{18}O_{VSMOW}$, ‰
RM 8538 (NBS30)	−5.10 ± 0.02 [4]
	−5.24 ± 0.24 [1]

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2. Coplen, T.B., Normalization of oxygen and hydrogen data, Chemical Geology (Isotope Geoscience Section), vol. 72, 293-297 (1988).

3. Gerstenberger, H., and Herrmann, M., Report on the intercomparison for the isotope standards Limestone KH2 and Polyethylene Foil PEF 1, ZFI-Mitteilungen, vol. 66, 67-83 (1983).

4. Coplen, T.B., Kendall, C., and Hopple, J., Comparison of stable isotope reference samples, Nature, vol. 302, 236-238 (1983).

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-2-

National Institute of Standards & Technology

Report of Investigation

Reference Materials 8543-8546

8543: NBS18 (carbonatite)
8544: NBS19 (limestone)
8545: LSVEC (lithium carbonate)
8546: NBS28 (silica sand-optical)

These Reference Materials (RMs) are intended to provide samples of known isotopic composition for isotope-ratio analysis of lithium, carbon, oxygen, and silicon. RM 8543 (NBS18) is intended for $^{13}C/^{12}C$ and $^{18}O/^{16}O$ isotope-ratio analysis of carbonates with isotope ratios stated in parts per thousand difference (‰) from Vienna Pee Dee Belemnite (VPDB) or Vienna Standard Mean Ocean Water [VSMOW (RM 8535)], respectively. RM 8544 (NBS19) is intended for $^{13}C/^{12}C$ and $^{18}O/^{16}O$ isotope-ratio analysis of carbonates and is used to define the VPDB scale [1,2]. RM 8545 (LSVEC) is intended for lithium and carbon isotopic abundance calibrations. RM 8546 (NBS28) is the silicon isotope reference for reporting silicon isotope-ratio data, and it is also intended for oxygen isotope-ratio calibration of silicates. These RMs are issued in units of approximately 0.4 g each.

These RMs are distributed on behalf of the International Atomic Energy Agency (IAEA), Vienna, Austria.

The overall coordination of preparation for NIST distribution was performed by T. B. Coplen, U.S. Geological Survey and R.D. Vocke, Jr., NIST Inorganic Analytical Research Division.

The supporting aspects concerning the distribution by NIST of these RMs were coordinated through the Standard Reference Materials Program by J.S. Kane.

Material Preparation

RM 8543 (NBS18) and RM 8544 (NBS19) were prepared by I. Friedman, J.R. O'Neil and G. Cebula [3] of the U.S. Geological Survey. RM 8543 (NBS18), a carbonatite from Fen, Norway, was collected by B. Taylor, University of California, Davis, and crushed by H. Friedrichsen, University of Tübingen, Federal Republic of Germany. The fraction between 88 μm and 440 μm was stored and bottled as NBS18. RM 8544 (NBS19), also known in the literature as TS-Limestone, was obtained from a single slab of white marble of unknown origin. After crushing, the fraction between 177 μm and 325 μm was bottled. RM 8545 (LSVEC) was prepared by H. Svec, Iowa State University [4]. RM 8546 (NBS28) was obtained by I. Friedman, U.S. Geological Survey from Corning Glass Company. It was washed with acid to remove impurities and the fraction between 100 μm and 177 μm was separated and packaged.

Gaithersburg, MD 20899
June 22, 1992

William P. Reed, Chief
Standard Reference Materials Program

(over)

Storage

It is recommended that these RMs be stored in the containers in which they are supplied to the user. To minimize the potential for oxygen isotope exchange of carbonate RMs with atmospheric water vapor, RM 8543 (NBS18) and RM 8544 (NBS19) can be stored in a desiccator.

NOTE: Because very limited quantities of these materials exist, distribution is limited to one unit of each per three-year period of time. Users are strongly advised to prepare their own internal standards for daily use and calibrate those standards against these RMs.

Isotope Abundances

The carbon isotopic abundance of NBS18 and NBS19 have been measured as [5]:

RM 8543 (NBS18)	RM 8544 (NBS19)
98.8998 ± 0.0028 atom percent ^{12}C	98.8922 ± 0.0028 atom percent ^{12}C
1.1002 ± 0.0028 atom percent ^{13}C	1.1078 ± 0.0028 atom percent ^{13}C

The carbon isotopic composition of these carbonate RMs relative to VPDB, using the defined $\delta^{13}C_{VPDB}$ value of +1.95 ‰ for NBS19, is [1,2]:

RM	$\delta^{13}C_{VPDB}$, ‰
RM 8543 (NBS18)	−5.04 ± 0.06
RM 8544 (NBS19)	+1.95
RM 8545 (LSVEC)	−46.7 ± 0.3

The oxygen isotopic composition of these RMs relative to VPDB using the defined $\delta^{18}O$ value of −2.20 ‰ for NBS19, is listed below [1,2]. Also listed is the $\delta^{18}O$ value relative to VSMOW using the relationship [6] that

$$\delta^{18}O_{VSMOW} = 1.03092\,\delta^{18}O_{VPDB} + 30.92$$

RM	$\delta^{18}O_{VPDB}$, ‰	$\delta^{18}O_{VSMOW}$, ‰
RM 8543 (NBS18)	−23.05 ± 0.19	+7.16 ± 0.19
RM 8544 (NBS19)	−2.20	+28.65
RM 8546 (NBS28)		+9.58 ± 0.10

The lithium isotope abundance ratio of LSVEC is [4]:

$$^6Li\,/\,^7Li = 0.0832 \pm 0.0002$$

Although no absolute isotope abundance measurement of RM 8546 (NBS28) has been performed, this material has served as the reference for relative $^{30}Si/^{28}Si$ measurements for more than a decade; thus, $\delta^{30}Si_{NBS28}$ of RM 8546 = 0 ‰.

REFERENCES

1. Hut, G., Consultants' group meeting on stable isotope reference samples for geochemical and hydrological investigations, Report to the Director General, International Atomic Energy Agency, April 1987.

2. Coplen, T.B., Normalization of oxygen and hydrogen isotope data, Chemical Geology (Isotope Geoscience Section), vol. 72, 293-297 (1988).

3. Friedman, I., O'Neil, J.R., and Cebula, G., Two new carbonate stable isotope standards, Geostandards Newsletter, vol. 6, 11-12 (1982).

4. Flesch, G.D., Anderson, A.R., and Svec, H.J., A secondary isotopic standard for $^6Li/^7Li$ determinations, Int. J. Mass Spectrom. Ion Phys., vol. 12, 265-272 (1973).

5. Chang, T.L. and Li, W., A calibrated measurement of the atomic weight of carbon, Chinese Science Bulletin, vol. 35, no. 4, 290-296 (1990).

6. Coplen, T.B., Kendall, C., and Hopple, J., Comparison of stable isotope reference samples, Nature, vol. 302, 236-238 (1983).

National Institute of Standards & Technology

Report of Investigation

Reference Materials 8547-8552

8547:	IAEA-N1	(ammonium sulfate)
8548:	IAEA-N2	(ammonium sulfate)
8549:	IAEA-N3	(potassium nitrate)
8550:	USGS25	(ammonium sulfate)
8551:	USGS26	(ammonium sulfate)
8552:	NSVEC	(gaseous nitrogen)
8558:	USGS32	(potassium nitrate)

These Reference Materials (RMs) are intended to provide samples of known isotopic composition with $^{15}N/^{14}N$ isotope ratios stated in parts per thousand difference (‰) from atmospheric nitrogen in air (AIR). These RMs are not certified, but their use allows comparability of stable nitrogen isotope-ratio data obtained by investigators in different laboratories. RM 8552 (NSVEC) is issued in units of 300 μmol and the other RMs are issued in units ranging from 0.4 to 2 g.

These RMs are distributed on behalf of the International Atomic Energy Agency (IAEA), Vienna, Austria.

The overall coordination of preparation for NIST distribution was carried out by T.B. Coplen, U.S. Geological Survey and R.D. Vocke, Jr., NIST Inorganic Analytical Research Division.

The supporting aspects concerning the distribution by NIST of these RMs were coordinated through the Standard Reference Materials Program by J.S. Kane.

Material Preparation

RM 8547 (IAEA-N1) and RM 8548 (IAEA-N2) were prepared by E. Salati, Centro de Energia Nuclear na Agricultura, Brazil. RM 8549 (IAEA-N3) was prepared by A. Mariotti, Université P. and M. Curie, Tour, Paris, France. RM 8550 (USGS25) was prepared in 1991 by J.K. Böhlke, U.S. Geological Survey from Fisher● ammonium sulfate (A938-500, lot #915021) and Cambridge Isotopes ● ammonium sulfate (99.99 percent ^{14}N; lot BI-1328). RM 8551 (USGS26) was prepared in 1991 by J.K. Böhlke, U.S. Geological Survey from Fisher● ammonium sulfate (A938-500, lot #915021) and Cambridge Isotopes ● ammonium sulfate (10 percent ^{15}N; lot BI-1050). RM 8552 (NSVEC) was originally prepared by G. Junk and H.J. Svec, Iowa State University [1]; C. Kendall, U.S. Geological Survey then split the sample into several hundred aliquots. RM 8558 (USGS32) was prepared in 1992 by J.K. Böhlke, U.S. Geological Survey from Baker Analyzed● potassium nitrate (3190-01, lot #D40117 and 3190-05, lot #19162) and Cambridge Isotopes ● potassium nitrate (5 percent ^{15}N, lot #F-2685).

Gaithersburg, MD 20899
February 3, 1993
(Revision of certificate dated 6-22-92)

William P. Reed, Chief
Standard Reference Materials Program

(over)

<u>Storage</u>

It is recommended that these RMs be stored in the containers in which they are supplied to the user. When RM 8552 (NSVEC, gaseous nitrogen) is opened, it should be used immediately for calibration. It can be stored in a glass container fabricated with an all-glass stopcock coated with Apiezon N● hydrocarbon-based stopcock grease. Because the salts are hygroscopic, they should be stored in a desiccator.

NOTE: Because very limited quantities of these materials exist, distribution is limited to one unit of each per three-year period of time. Users are strongly advised to prepare their own internal standards for daily use and to calibrate those standards against these RMs.

<u>Isotope Abundances</u>

The absolute isotope ratio of nitrogen in air, reported by Junk and Svec is [1]:

$$^{14}N/^{15}N = 272.0 \pm 0.3$$

Reference 2 lists the $\delta^{15}N$ values of several of these RMs relative to AIR, but more precise values are available for many of the materials. By definition, $\delta^{15}N_{AIR}$ of air is 0 ‰.

$$\delta^{15}N_{AIR}, ‰$$

RM 8547 (IAEA-N1)	+0.4	±	0.2	[3]
RM 8548 (IAEA-N2)	+20.3	±	0.2	[3]
RM 8549 (IAEA-N3)	+2	to	+5	[4]
RM 8550 (USGS25)	−30.4	±	0.5	[5]
RM 8551 (USGS26)	+53.5	±	0.5	[5]
RM 8552 (NSVEC)	−2.81			[6]
RM 8558 (USGS32)	+179.9	±	0.5	[5]

REFERENCES

1. Junk, G., and Svec, H.J., The absolute abundance of nitrogen isotopes in the atmosphere and compressed gas from various sources, Geochimica et Cosmochimica Acta, vol. 14, 234–243 (1958).

2. Hut, G., Consultants' group meeting on stable isotope reference samples for geochemical and hydrological investigations, Report to the Director General, International Atomic Energy Agency, April 1987.

3. Böhlke, J.K., U.S. Geological Survey, written communication, 1992.

4. Stichler, W., International Atomic Energy Agency, Vienna, oral communication, 1991, 1993.

5. Böhlke, J.K., U.S. Geological Survey, unpublished data.

6. Kendall, C. and Grim, E., Combustion tube method for measurement of nitrogen isotope ratios using calcium oxide for total removal of carbon dioxide and water, Analytical Chemistry, vol. 62, 526–529 (1990).

-2-

```
Sample Name  :  EPA CORVALLIS /SN 7294  /101498
Time Code    : 981014065840
Spec.-no.    :   5424
Method Name :CO2 STD ON OFGas Type    :  CO2     Background  :  I
Action       :NO
Sample Wt.   :          1.00000[mg]
Comment      :
Peak Detection Criteria:
Start Slope : 0.200[mV/s]  End Slope   : 0.400[mV/s]  Min. Amplit.: 0.050[V]
```

Peak	Start [s]	tR [s]	Width [s]	Ampl. [V]	Background [counts/s] 44	45	46	PRESSURE BA 2
1	33.7	53.7	22.0	0.156	207.5	239.6	271.2	.2
2	83.8	103.7	22.2	0.645	207.5	239.6	271.0	.5
3	133.8	153.8	22.6	1.123	207.6	239.8	271.2	.75
4	183.7	203.8	22.6	1.643	207.7	239.9	271.5	1.00
5*	233.7	253.7	22.9	2.252	207.8	239.9	271.5	1.25
6	283.6	303.7	23.1	2.888	207.9	240.1	271.6	1.50
7	333.7	353.8	23.4	3.635	208.0	240.2	271.9	1.75
8	383.8	403.8	23.4	4.535	208.2	240.4	272.0	2.00
9	433.8	453.8	23.9	6.496	208.3	240.6	272.4	2.50
10	483.8	503.8	24.4	8.714	208.6	240.9	272.7	3.00

```
Sample Name  :  EPA CORVALLIS /SN 7294  /101498
Time Code    : 981014065840
Spec.-no.    :        5424
Method Name :CO2 STD ON OFFGas Type   :  CO2     Background  :  I
Action       :NO
Sample Wt.   :          1.00000[mg]
Comment      :
Peak Detection Criteria:
Start Slope : 0.200[mV/s]  End Slope   : 0.400[mV/s]  Min. Amplit.: 0.050[V]
no slope correction
```

Peak	AREA [Vs]	CONC %C	ratio 45/44	delta 18/16	delta vs.PDB 45/44	delta 17 corr 13/12
1	3.05	1.00	1.135837	-0.07	-36.97	-36.94
2	12.68	4.17	1.135600	0.11	-37.17	-37.16
3	21.67	7.13	1.135562	0.08	-37.20	-37.20
4	31.99	10.52	1.135468	0.06	-37.28	-37.28
5*	43.93	14.45	1.135479	0.00	-37.27	-37.27
6	56.74	18.67	1.135371	-0.10	-37.36	-37.36
7	71.13	23.40	1.135399	-0.08	-37.34	-37.34
8	88.97	29.27	1.135274	-0.11	-37.44	-37.45
9	125.55	41.31	1.135309	-0.16	-37.41	-37.41
10	167.94	55.26	1.135308	-0.13	-37.41	-37.42

```
--------------------------------------------------------------------------------
Sample Name : TOMATO 1//
Time Code    : 971202113137
Spec.-no.    :       1840
Method Name :       N2      Gas Type    :    N2
Sample Wt.   :       0.93900[mg]
Peak Detection Criteria:
Start Slope : 0.200[mV/s]  End Slope   : 0.400[mV/s]  Min. Amplit.: 0.050[V]
--------------------------------------------------------------------------------
```

Peak	Start	tR	Width	Ampl.	Background[counts/s]			AREA
	[s]	[s]	[s]	[V]	28	29	30	[Vs]
1*	33.9	53.7	22.1	1.946	252.5	243.9	860.8	37.17
2	150.5	167.0	57.6	2.047	253.2	244.0	772.5	27.58

Peak	CONC	ratio	ratio	delta vs.at-air	delta vs.at-air	delta blk corr	delta blk corr	
	%N	29/28	30/28	29/28	30/28	29/28	30/28	at%
1*	6.69	0.723276	0.238391	-0.87	0.00	-0.87	0.00	0.365
2	4.97	0.726356	0.289571	3.38	214.69	3.45	223.11	0.367

```
--------------------------------------------------------------------------------
Sample Name : TOMATO 1//
Time Code    : 971202113624
Spec.-no.    :       1841
Method Name :       CO2     Gas Type    :    CO2
Sample Wt.   :       0.93900[mg]
Peak Detection Criteria:
Start Slope : 0.200[mV/s]  End Slope   : 0.400[mV/s]  Min. Amplit.: 0.050[V]
--------------------------------------------------------------------------------
```

Peak	Start	tR	Width	Ampl.	Background[counts/s]			AREA
	[s]	[s]	[s]	[V]	44	45	46	[Vs]
1	31.1	50.1	81.1	6.424	205.2	236.7	268.1	120.82
2*	153.7	173.7	24.7	4.471	209.0	241.0	273.4	87.23

Peak	CONC	ratio	ratio	delta	delta vs.PDB	delta 17 corr	delta blk corr	
	%C	45/44	46/44	18/16	45/44	13/12	13/12	at%
1	37.90	1.150028	1.409622	40.90	-26.08	-26.75	-26.74	1.081
2*	27.37	1.136816	1.354259	0.00	-37.27	-37.27	-37.27	1.070

	Run Summary (all deltas [per mil])							
	----15N----		----13C----		----Wt%----			
SAMPLE	delta	at%	delta	at%	C	N	C/N
TOMATO 1	3.45	0.36751	-26.74	1.08184	37.90	4.97	7.63

METHOD :   CO2

>>> E X P E R I M E N T <<<

COMMENT

	COMMENT	:

GAS

	GASNAME	: CO2
	CUP # 1	: 44.0
	CUP # 2	: 45.0
	CUP # 3	: 46.0
	RATIO 1 X/Y	: 45.0 / 44.0
	RATIO 2 X/Y	: 46.0 / 44.0

CHANGEOVER

	COV PORT #1	: VAR. VOLUME 1
	COV PORT #2	: VAR. VOLUME 2
	COV PORT #3	:
	COV PORT #4	:
	STANDARD PORT #	: 5
	SAMPLE PORT #	: 5

MEASURE

	PEAKCENTER	: YES
	MASS	: 45.0
	STORAGE OFFSET TIME [SEC]	: 0
	ACQ. END TIME [SEC]	: 200
	INTEGRATION TIME [SEC]	: 0.2500

PEAK DETECT

	STARTING SLOPE [mV/SEC]	: 0.200
	ENDING SLOPE [mV/SEC]	: 0.400
	MIN. AMPLITUDE [V]	: 0.050

PEAK CALCULATION

	BACKGROUND TYPE	:	I
	TYPE T STARTTIME:	0
	TYPE T ENDTIME	:	0
	SOURCE CALIBRATION:	NO

STANDARD PEAKS

1	:	160	DELTA	:	-36.440
2	:	0	DELTA	:	0.000
3	:	0	DELTA	:	0.000
4	:	0	DELTA	:	0.000
5	:	0	DELTA	:	0.000
6	:	0	DELTA	:	0.000
7	:	0	DELTA	:	0.000
8	:	0	DELTA	:	0.000

>>> P R O C E S S   <<<   MODE : NORMAL

	STD. MEASUREMENT [SEC]		ELEM. ANALYZER [SEC]		DILUTION [SEC]	
	ON	OFF	ON	OFF	ON	OFF
1:	150	170	1: 0	0	1: 1	200
2:	0	0	2: 0	0	2: 0	0
3:	0	0	3: 0	0	3: 0	0
4:	0	0	4: 0	0	4: 0	0
5:	0	0	5: 0	0	5: 0	0
6:	0	0	6: 0	0	6: 0	0
7:	0	0	7: 0	0	7: 0	0
8:	0	0	8: 0	0	8: 0	0
9:	0	0	9: 0	0	9: 0	0
	STD. MEASUREMENT [SEC]		ELEM. ANALYZER [SEC]		DILUTION [SEC]	

	ON	OFF		ON	OFF		ON	OFF
10:	0	0	10:	0	0	10:	0	0
11:	0	0	11:	0	0	11:	0	0
12:	0	0	12:	0	0	12:	0	0
13:	0	0	13:	0	0	13:	0	0
14:	0	0	14:	0	0	14:	0	0
15:	0	0	15:	0	0	15:	0	0
16:	0	0	16:	0	0	16:	0	0
17:	0	0	17:	0	0	17:	0	0
18:	0	0	18:	0	0	18:	0	0

>>> F O R M A T   <<<   MODE : FORMAT

PRINTER

LIST TYPE   : LONG
GRAPHICS   : NO

METHOD : CO2 BLANK

>>> E X P E R I M E N T <<<

COMMENT

| | COMMENT | : |

GAS

	GASNAME	: CO2
	CUP # 1	: 44.0
	CUP # 2	: 45.0
	CUP # 3	: 46.0
	RATIO 1 X/Y	: 45.0 / 44.0
	RATIO 2 X/Y	: 46.0 / 44.0

CHANGEOVER

	COV PORT #1	: VAR. VOLUME 1
	COV PORT #2	: VAR. VOLUME 2
	COV PORT #3	:
	COV PORT #4	:
	STANDARD PORT #	: 5
	SAMPLE PORT #	: 5

MEASURE

	PEAKCENTER	: YES
	MASS	: 45.0
	STORAGE OFFSET TIME [SEC]	: 0
	ACQ. END TIME [SEC]	: 200
	INTEGRATION TIME [SEC]	: 0.2500

PEAK DETECT

	STARTING SLOPE [mV/SEC]	: 0.100
	ENDING SLOPE [mV/SEC]	: 0.200
	MIN. AMPLITUDE [V]	: 0.001

PEAK CALCULATION

	BACKGROUND TYPE :	I
	TYPE T STARTTIME:	0
	TYPE T ENDTIME :	0
	SOURCE CALIBRATION:	NO

STANDARD PEAKS

1 : 160	DELTA : -36.440
2 : 0	DELTA : 0.000
3 : 0	DELTA : 0.000
4 : 0	DELTA : 0.000
5 : 0	DELTA : 0.000
6 : 0	DELTA : 0.000
7 : 0	DELTA : 0.000
8 : 0	DELTA : 0.000

>>> P R O C E S S   <<< MODE : NORMAL

	STD. MEASUREMENT [SEC]			ELEM. ANALYZER [SEC]			DILUTION [SEC]	
	ON	OFF		ON	OFF		ON	OFF
1:	150	170	1:	0	0	1:	1	200
2:	0	0	2:	0	0	2:	0	0
3:	0	0	3:	0	0	3:	0	0
4:	0	0	4:	0	0	4:	0	0
5:	0	0	5:	0	0	5:	0	0
6:	0	0	6:	0	0	6:	0	0
7:	0	0	7:	0	0	7:	0	0
8:	0	0	8:	0	0	8:	0	0
9:	0	0	9:	0	0	9:	0	0
	STD. MEASUREMENT [SEC]			ELEM. ANALYZER [SEC]			DILUTION [SEC]	

	ON	OFF		ON	OFF		ON	OFF
10:	0	0	10:	0	0	10:	0	0
11:	0	0	11:	0	0	11:	0	0
12:	0	0	12:	0	0	12:	0	0
13:	0	0	13:	0	0	13:	0	0
14:	0	0	14:	0	0	14:	0	0
15:	0	0	15:	0	0	15:	0	0
16:	0	0	16:	0	0	16:	0	0
17:	0	0	17:	0	0	17:	0	0
18:	0	0	18:	0	0	18:	0	0

>>> F O R M A T   <<<   MODE : FORMAT

PRINTER

LIST TYPE   : LONG
GRAPHICS   : NO

METHOD : CO2 STD ON OFF

>>> E X P E R I M E N T <<<

COMMENT
                   COMMENT   : MONTHLY LINEARITY CHECK
GAS
                   GASNAME   : CO2
                   CUP # 1   : 44.0
                   CUP # 2   : 45.0
                   CUP # 3   : 46.0
                   RATIO 1 X/Y   : 45.0   /   44.0
                   RATIO 2 X/Y   : 46.0   /   44.0
CHANGEOVER
                   COV PORT #1   : VAR. VOLUME 1
                   COV PORT #2   : VAR. VOLUME 2
                   COV PORT #3   :
                   COV PORT #4   :
                   STANDARD PORT #   : 5
                   SAMPLE PORT #   : 5
MEASURE
                   PEAKCENTER   : YES
                   MASS   : 45.0
                   STORAGE OFFSET TIME   [SEC]   : 0
                   ACQ. END TIME   [SEC]   : 550
                   INTEGRATION TIME   [SEC]   : 0.2500
PEAK DETECT
                   STARTING SLOPE   [mV/SEC]   : 0.200
                   ENDING SLOPE   [mV/SEC]   : 0.400
                   MIN. AMPLITUDE   [V]   : 0.050
PEAK CALCULATION
                        BACKGROUND TYPE   :   I
                        TYPE T STARTTIME: 0
                        TYPE T ENDTIME :   0
                        SOURCE CALIBRATION: NO
STANDARD PEAKS
                        1 :   240   DELTA   :   -37.270
                        2 :   0   DELTA   :   0.000
                        3 :   0   DELTA   :   0.000
                        4 :   0   DELTA   :   0.000
                        5 :   0   DELTA   :   0.000
                        6 :   0   DELTA   :   0.000
                        7 :   0   DELTA   :   0.000
                        8 :   0   DELTA   :   0.000

>>> P R O C E S S   <<<   MODE : NORMAL

| STD. MEASUREMENT [SEC] | | ELEM. ANALYZER [SEC] | | DILUTION [SEC] | |
ON	OFF	ON	OFF	ON	OFF
1: 30	50	1: 0	0	1: 0	0
2: 80	100	2: 0	0	2: 0	0
3: 130	150	3: 0	0	3: 0	0
4: 180	200	4: 0	0	4: 0	0
5: 230	250	5: 0	0	5: 0	0
6: 280	300	6: 0	0	6: 0	0
7: 330	350	7: 0	0	7: 0	0
8: 380	400	8: 0	0	8: 0	0
9: 430	450	9: 0	0	9: 0	0

       STD. MEASUREMENT [SEC]   ELEM. ANALYZER [SEC]   DILUTION [SEC]

	ON	OFF		ON	OFF		ON	OFF
10:	480	500	10:	0	0	10:	0	0
11:	0	0	11:	0	0	11:	0	0
12:	0	0	12:	0	0	12:	0	0
13:	0	0	13:	0	0	13:	0	0
14:	0	0	14:	0	0	14:	0	0
15:	0	0	15:	0	0	15:	0	0
16:	0	0	16:	0	0	16:	0	0
17:	0	0	17:	0	0	17:	0	0
18:	0	0	18:	0	0	18:	0	0

>>> F O R M A T   <<< MODE : FORMAT

PRINTER

LIST TYPE   : NO
GRAPHICS   : NO

METHOD : N2

>>> E X P E R I M E N T <<<

COMMENT

    COMMENT   :

GAS

GASNAME	: N2
CUP # 1	: 28.0
CUP # 2	: 29.0
CUP # 3	: 30.0
RATIO 1 X/Y	: 29.0 / 28.0
RATIO 2 X/Y	: 30.0 / 28.0

CHANGEOVER

COV PORT #1	: VAR. VOLUME 1
COV PORT #2	: VAR. VOLUME 2
COV PORT #3	:
COV PORT #4	:
STANDARD PORT #	: 6
SAMPLE PORT #	: 6

MEASURE

PEAKCENTER	: YES
MASS	: 29.0
STORAGE OFFSET TIME [SEC]	: 10
ACQ. END TIME [SEC]	: 240
INTEGRATION TIME [SEC]	: 0.2500

PEAK DETECT

STARTING SLOPE [mV/SEC]	: 0.200
ENDING SLOPE [mV/SEC]	: 0.400
MIN. AMPLITUDE [V]	: 0.010

PEAK CALCULATION

BACKGROUND TYPE :	I
TYPE T STARTTIME:	0
TYPE T ENDTIME :	0
SOURCE CALIBRATION:	NO

STANDARD PEAKS

1	:	40	DELTA :	-0.870
2	:	0	DELTA :	0.000
3	:	0	DELTA :	0.000
4	:	0	DELTA :	0.000
5	:	0	DELTA :	0.000
6	:	0	DELTA :	0.000
7	:	0	DELTA :	0.000
8	:	0	DELTA :	0.000

>>> P R O C E S S   <<<   MODE : NORMAL

	STD. MEASUREMENT [SEC]		ELEM. ANALYZER [SEC]		DILUTION [SEC]	
	ON	OFF	ON	OFF	ON	OFF
1:	30	50	1: 55	56	1: 0	0
2:	0	0	2: 0	0	2: 0	0
3:	0	0	3: 0	0	3: 0	0
4:	0	0	4: 0	0	4: 0	0
5:	0	0	5: 0	0	5: 0	0
6:	0	0	6: 0	0	6: 0	0
7:	0	0	7: 0	0	7: 0	0
8:	0	0	8: 0	0	8: 0	0
9:	0	0	9: 0	0	9: 0	0

    STD. MEASUREMENT [SEC]   ELEM. ANALYZER [SEC]   DILUTION [SEC]

	ON	OFF		ON	OFF		ON	OFF
10:	0	0	10:	0	0	10:	0	0
11:	0	0	11:	0	0	11:	0	0
12:	0	0	12:	0	0	12:	0	0
13:	0	0	13:	0	0	13:	0	0
14:	0	0	14:	0	0	14:	0	0
15:	0	0	15:	0	0	15:	0	0
16:	0	0	16:	0	0	16:	0	0
17:	0	0	17:	0	0	17:	0	0
18:	0	0	18:	0	0	18:	0	0

>>> F O R M A T   <<<   MODE : FORMAT

PRINTER

LIST TYPE   : LONG
GRAPHICS   : NO

METHOD :   N2 BLANK

>>> E X P E R I M E N T <<<

COMMENT

              COMMENT   :

GAS

GASNAME	:	N2
CUP # 1	:	28.0
CUP # 2	:	29.0
CUP # 3	:	30.0
RATIO 1 X/Y	:	29.0 / 28.0
RATIO 2 X/Y	:	30.0 / 28.0

CHANGEOVER

COV PORT #1	:	VAR. VOLUME 1
COV PORT #2	:	VAR. VOLUME 2
COV PORT #3	:	
COV PORT #4	:	
STANDARD PORT #	:	6
SAMPLE PORT #	:	6

MEASURE

PEAKCENTER	:	YES
MASS	:	29.0
STORAGE OFFSET TIME [SEC]	:	10
ACQ. END TIME [SEC]	:	240
INTEGRATION TIME [SEC]	:	0.2500

PEAK DETECT

STARTING SLOPE [mV/SEC]	:	0.200
ENDING SLOPE [mV/SEC]	:	0.400
MIN. AMPLITUDE [V]	:	0.001

PEAK CALCULATION

BACKGROUND TYPE :	I	
TYPE T STARTTIME:	0	
TYPE T ENDTIME :	0	
SOURCE CALIBRATION:	NO	

STANDARD PEAKS

1	:	40	DELTA	:	-0.870
2	:	0	DELTA	:	0.000
3	:	0	DELTA	:	0.000
4	:	0	DELTA	:	0.000
5	:	0	DELTA	:	0.000
6	:	0	DELTA	:	0.000
7	:	0	DELTA	:	0.000
8	:	0	DELTA	:	0.000

>>> P R O C E S S   <<<   MODE : NORMAL

	STD. MEASUREMENT [SEC]		ELEM. ANALYZER [SEC]			DILUTION [SEC]	
	ON	OFF		ON	OFF	ON	OFF
1:	30	50	1:	61	62	1: 0	0
2:	0	0	2:	0	0	2: 0	0
3:	0	0	3:	0	0	3: 0	0
4:	0	0	4:	0	0	4: 0	0
5:	0	0	5:	0	0	5: 0	0
6:	0	0	6:	0	0	6: 0	0
7:	0	0	7:	0	0	7: 0	0
8:	0	0	8:	0	0	8: 0	0
9:	0	0	9:	0	0	9: 0	0

    STD. MEASUREMENT [SEC]   ELEM. ANALYZER [SEC]   DILUTION [SEC]

	ON	OFF		ON	OFF		ON	OFF
10:	0	0	10:	0	0	10:	0	0
11:	0	0	11:	0	0	11:	0	0
12:	0	0	12:	0	0	12:	0	0
13:	0	0	13:	0	0	13:	0	0
14:	0	0	14:	0	0	14:	0	0
15:	0	0	15:	0	0	15:	0	0
16:	0	0	16:	0	0	16:	0	0
17:	0	0	17:	0	0	17:	0	0
18:	0	0	18:	0	0	18:	0	0

>>> F O R M A T   <<< MODE : FORMAT

PRINTER

LIST TYPE   : LONG
GRAPHICS   : NO

METHOD : N2 STD ON OFF

>>> E X P E R I M E N T <<<

COMMENT

| | COMMENT | : MONTHLY LINEARITY CHECK |

GAS

	GASNAME	: N2
	CUP # 1	: 28.0
	CUP # 2	: 29.0
	CUP # 3	: 30.0
	RATIO 1 X/Y	: 29.0 / 28.0
	RATIO 2 X/Y	: 30.0 / 28.0

CHANGEOVER

	COV PORT #1	: VAR. VOLUME 1
	COV PORT #2	: VAR. VOLUME 2
	COV PORT #3	:
	COV PORT #4	:
	STANDARD PORT #	: 6
	SAMPLE PORT #	: 6

MEASURE

	PEAKCENTER	: YES
	MASS	: 29.0
	STORAGE OFFSET TIME [SEC]	: 0
	ACQ. END TIME [SEC]	: 550
	INTEGRATION TIME [SEC]	: 0.2500

PEAK DETECT

	STARTING SLOPE [mV/SEC]	: 0.200
	ENDING SLOPE [mV/SEC]	: 0.400
	MIN. AMPLITUDE [V]	: 0.050

PEAK CALCULATION

	BACKGROUND TYPE :	I
	TYPE T STARTTIME:	0
	TYPE T ENDTIME :	0
	SOURCE CALIBRATION:	NO

STANDARD PEAKS

1 : 240	DELTA : -0.870
2 : 0	DELTA : 0.000
3 : 0	DELTA : 0.000
4 : 0	DELTA : 0.000
5 : 0	DELTA : 0.000
6 : 0	DELTA : 0.000
7 : 0	DELTA : 0.000
8 : 0	DELTA : 0.000

>>> P R O C E S S   <<< MODE : NORMAL

| | STD. MEASUREMENT [SEC] | | | ELEM. ANALYZER [SEC] | | | DILUTION [SEC] | |
	ON	OFF		ON	OFF		ON	OFF
1:	30	50	1:	0	0	1:	0	0
2:	80	100	2:	0	0	2:	0	0
3:	130	150	3:	0	0	3:	0	0
4:	180	200	4:	0	0	4:	0	0
5:	230	250	5:	0	0	5:	0	0
6:	280	300	6:	0	0	6:	0	0
7:	330	350	7:	0	0	7:	0	0
8:	380	400	8:	0	0	8:	0	0
9:	430	450	9:	0	0	9:	0	0

STD. MEASUREMENT [SEC]   ELEM. ANALYZER [SEC]   DILUTION [SEC]

	ON	OFF		ON	OFF		ON	OFF
10:	480	500	10:	0	0	10:	0	0
11:	0	0	11:	0	0	11:	0	0
12:	0	0	12:	0	0	12:	0	0
13:	0	0	13:	0	0	13:	0	0
14:	0	0	14:	0	0	14:	0	0
15:	0	0	15:	0	0	15:	0	0
16:	0	0	16:	0	0	16:	0	0
17:	0	0	17:	0	0	17:	0	0
18:	0	0	18:	0	0	18:	0	0

>>> F O R M A T   <<<   MODE : FORMAT

PRINTER

LIST TYPE   : NO
GRAPHICS   : NO

SAMPLE LOCATION LIST -- STABLE ISOTOPE ANALYSIS

WELL NO.	LAB NUMBER	WEIGHT - MG	SEQ LINE	WELL NO.	LAB NUMBER	WEIGHT - MG	SEQ LINE
A1	BLANK	*****	1	C2			26
A2	BLANK	*****	2	C3			27
A3	BLANK	*****	3	C4	BLANK	*****	28
A4	BLANK	*****	4	C5	ACETAN STD		29
A5	ACETAN STD		5	C6			30
A6			6	C7			31
A7			7	C8			32
A8			8	C9			33
A9			9	C10			34
A10			10	C11			35
A11			11	C12			36
A12			12	D1			37
B1			13	D2			38
B2			14	D3			39
B3			15	D4	BLANK	*****	40
B4	BLANK	*****	16	D5	ACETAN STD		41
B5	ACETAN STD		17	D6			42
B6			18	D7			43
B7			19	D8			44
B8			20	D9			45
B9			21	D10			46
B10			22	D11			47
B11			23	D12			48
B12			24	E1			49
C1			25	E2			50

ANALYST:

DATE:

LOT FILE NAME:

CUP SIZE:

WELL NO.	LAB NUMBER	WEIGHT - MG	SEQ LINE	WELL NO.	LAB NUMBER	WEIGHT - MG	SEQ LINE
E3			51	G2			74
E4	BLANK	*****	52	G3			75
E5	ACETAN STD		53	G4	BLANK	*****	76
E6			54	G5	ACETAN STD		77
E7			55	G6			78
E8			56	G7			79
E9			57	G8			80
E10			58	G9			81
E11			59	G10			82
E12			60	G11			83
F1			61	G12			84
F2			62	H1			85
F3			63	H2			86
F4	BLANK	*****	64	H3			87
F5	ACETAN STD		65	H4	BLANK	*****	88
F6			66	H5	ACETAN STD		89
F7			67	H6			90
F8			68	H7			91
F9			69	H8			92
F10			70	H9			93
F11			71	H10			94
F12			72	H11			95
G1			73	H12			96

Sheet1

PRELIMINARY RESULTS OF STABLE ISOTOPE ANALYSIS OF SELECTED MATERIALS – SUBJECT TO REVISION

ALL UNITS ARE PER MIL

SAMPLE	NIST OR NBS REF NUMBER	DELTA C13	DELTA C13 STD DEV	DELTA N15	DELTA N15 STD DEV	ACTUAL DELTA C13	ACTUAL DELTA N15	PERCENT CARBON	PERCENT NITROGEN	ACTUAL % CARBON	ACTUAL % NITROGEN	NUMBER OF REPLICATES
UTAH CABBAGE		-26.57	0.18	2.70	0.28	-26.40	2.80	44.64	3.06		3.0	5
UTAH SPINACH		-27.35	0.06	-0.69	0.06	-27.59	-0.57	41.63	6.10	40.53	5.90	5
UTAH GRAPHITE		-26.19	0.19			-25.96						5
UTAH CELLULOSE		-24.77	0.05			-24.4						5
NBS SPINACH	1570	-26.68	0.05	1.07	0.07			39.58	5.56		5.9	5
NBS TOMATO LEAVES	1573	-26.73	0.16	3.45	0.17			37.76	4.81			5
NBS PINE NEEDLES	1575	-25.96	0.24	0.45	0.05			49.63	1.19		1.2	5
CANADIAN SOIL SO-4		-25.61	0.07	6.02	0.11			4.20	0.40	4.4	0.4	5
NBS OYSTER TISSUE	1566	-21.66	0.25	9.16	0.08			45.46	6.71		6.81	5
NIST PEACH LEAVES	1547	-25.98	0.08	1.6	0.19			45.81	2.82		2.94	5
NIST APPLE LEAVES	1515	-27.01	0.13	0.1	0.15			46.57	2.19		2.25	5
NIST CORN STALK	8412	-11.59	0.07	11.90	0.15			45.46	0.69		0.7	5
NIST RICE FLOUR	1568	-25.90	0.14	6.74	0.38			39.22	1.35			5
NBS CITRUS LEAVES*	1572	-27.40	0.22	4.53	0.18	-27.15	4.70	41.11	2.54			5
NBS BOVINE LIVER*	1577	-22.38	0.13	7.51	0.08	-21.51	7.48	47.12	9.91			5
CANADIAN SOIL SO-2		-24.99	0.12	6.49	0.17			4.73	0.22	4.8	0.22	10-C, 5-N
NIST BUFFALO RIVER SED	2704	-20.03	0.12	3.92	0.25			3.32	0.2	3.35		10-C, 5-N
NIST MONTANA SOIL	2710	-25.14	0.06	4.99	0.26			3.03	0.3	3		9-C, 10-N
NIST MONTANA SOIL	2711	-16.73	0.05	7.47	0.10			1.77	0.14	2		5
CANADIAN SOIL SO-3		1.51	0.11	***				6.56	***	6.6	0.02	5
COSTECH ACETANILIDE	031040**	-27.97	0.12	1.18	0.07			71.72	10.46	71.09	10.36	5
POTASSIUM NITRATE	FISH. P-263			4.17	0.06				13.49		13.86	5
AMMONIUM NITRATE	JTB 1-0729			-2.21	0.03				34.49		35.00	5

*NOTE: ACTUAL DELTA VALUES FOR NBS CITRUS LEAVES AND NBS BOVINE LIVER TAKEN FROM FRY ET AL, ANALYTICAL CHEMISTRY, 1992, VOL 64, PAGES 288-291.
ACTUAL VALUES FOR CITRUS LEAVES ARE FOR NBS 1524 WHICH APPEARS TO BE THE SAME BATCH AS NBS 1572 WHICH WE ANALYZED.
ACTUAL DELTA 13C VALUE FOR BOVINE LIVER IS QUESTIONABLE AND SUBJECT TO CONFIRMATION.

**COSTECH PART NUMBER

***NO DATA AVAILABLE DUE TO EXTREMELY LOW NITROGEN IN THIS SAMPLE.

LAST REVISED ON 01-27-99

By definition, the accepted index for stable isotope ratios is represented as the relative difference to a standard for that element

$$\delta^{13}C = [(R_{sample} - R_{standard}) / R_{standard}] * 1000$$

Note that $R_{standard}$ is a constant so that $Var(\delta^{13}C) = Var(R_{sample})*(100/R_{standard})^2$ or

$$SD(\delta^{13}C) = SD(R_{sample})*(100/R_{standard}). \tag{1}$$

It is reasonable to assume that $E(R_{sample}) = R_{standard} > 0$ in which case, equation (1) states that $SD(\delta^{13}C) = CV(R_{sample})$. That is, the standard deviation of the δ index is equal to the coefficient of variation of the sample isotope ratio, R_{sample}. The coefficient of variation of the δ index is meaningless because $E(\delta^{13}C) = 0$. Your suggestion to alter the definition of the index as the ratio of the two ratios is irrelevant and does not alter the calculation of precision. Consider

$$I = R_{sample} / R_{standard} * 1000$$

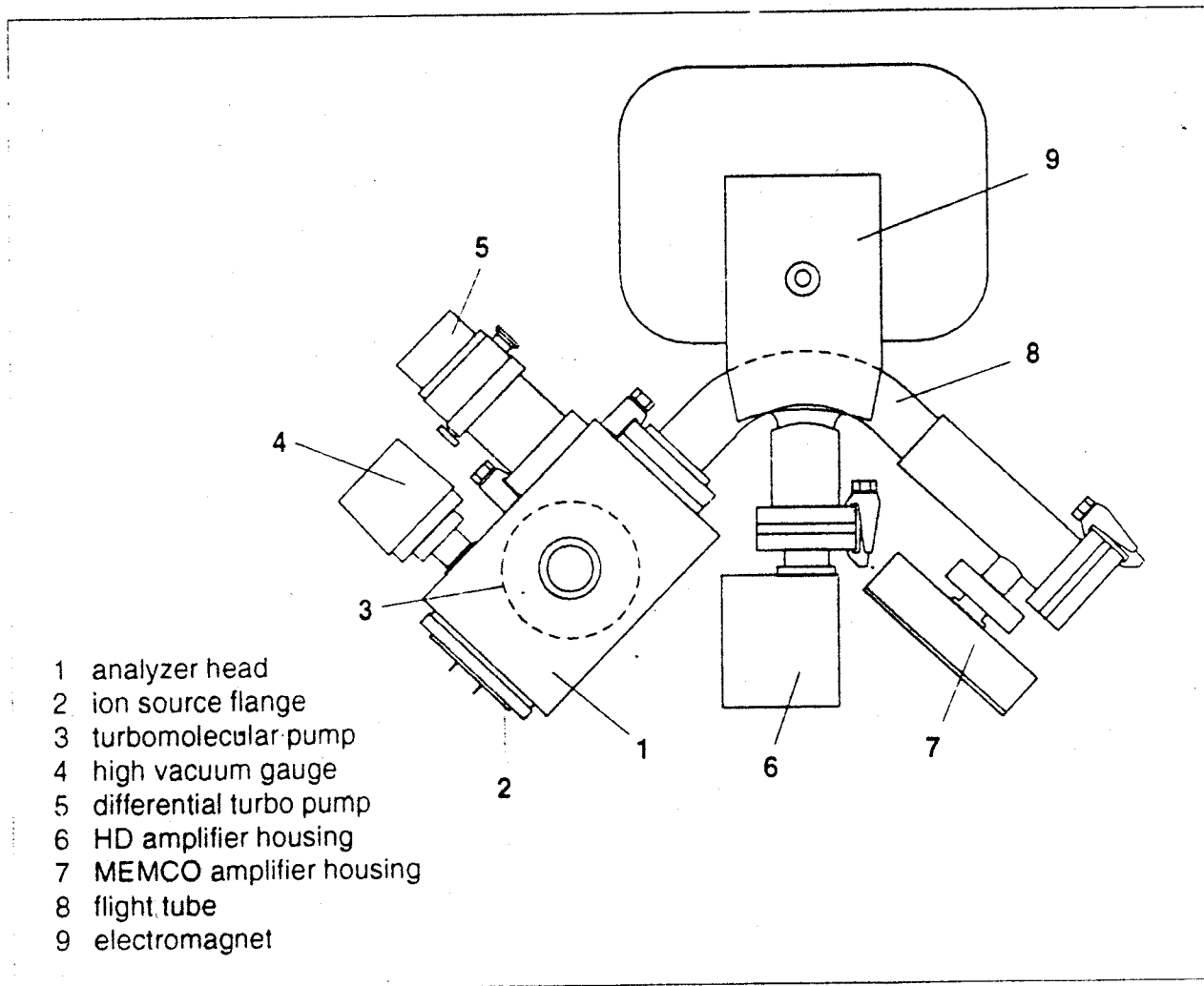The first two moments are $E(I)=100\%$ and $Var(I) = Var(\delta^{13}C)$ which, restating equation (1), gives

$$SD(I) = SD(\delta^{13}C) = SD(R_{sample})*(100/R_{standard}) \tag{2}$$

and $\quad CV(I) = SD(I).$ $\hfill (3)$
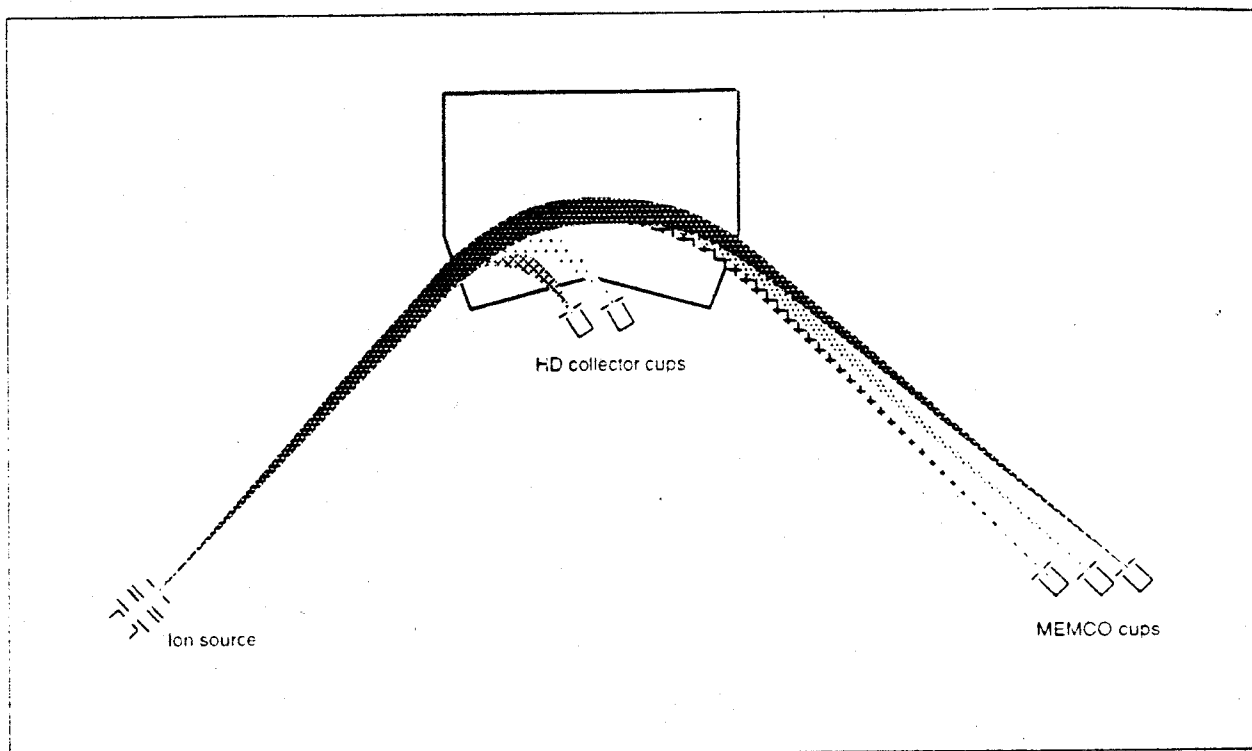
Whether one considers I or $\delta^{13}C$ as the index for stable isotope ratios, the standard deviation of the index is the coefficient of variation of the numerator ratio, R_{sample}, as well as the coefficient of variation of I. Rather than modify the index, the δ index is meaningful and the $SD(\delta^{13}C)$ should be interpreted as a coefficient of variation.

FIGURES

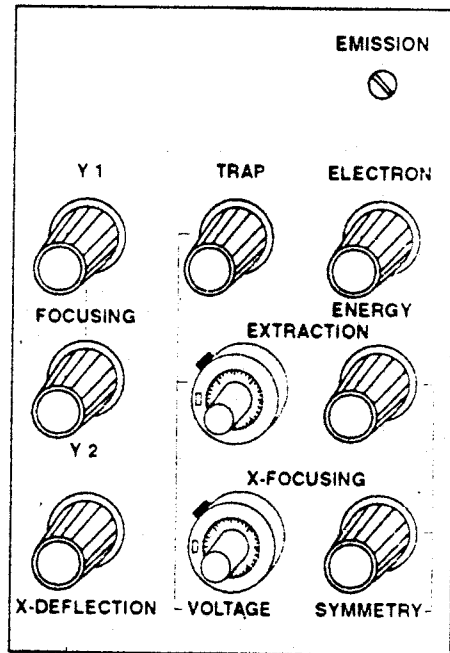Partial view of the instrument showing the arrangement of the analyzer

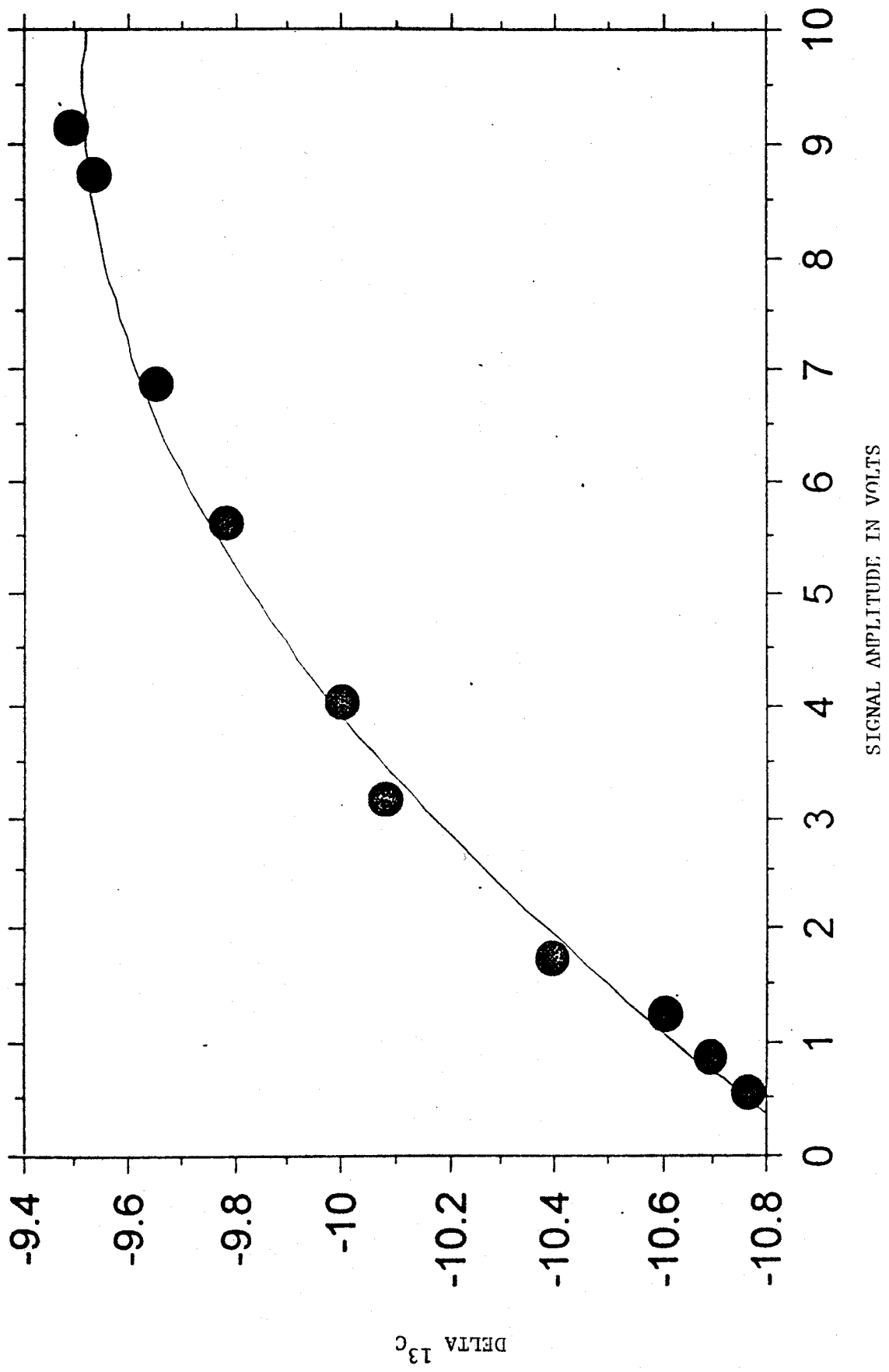1 analyzer head
2 ion source flange
3 turbomolecular pump
4 high vacuum gauge
5 differential turbo pump
6 HD amplifier housing
7 MEMCO amplifier housing
8 flight tube
9 electromagnet

Schematic of the ion path

HD collector cups

Ion source

MEMCO cups

Panel of the ion source control unit

VARIATION OF DELTA ^{13}C VALUES WITH SIGNAL AMPLITUDE -- 30 FOLD DILUTION

SIGNAL AMPLITUDE IN VOLTS

DELTA ^{13}C