JOURNAL OF FORENSIC SCIENCES

J Forensic Sci, January 2010, Vol. 55, No. 1
doi: 10.1111/j.1556-4029.2009.01241.x
Available online at: interscience.wiley.com

TECHNICAL NOTE

CRIMINALISTICS

Sarah J. Benson,[1,2] *Ph.D.; Christopher J. Lennard,*[1,3] *Ph.D.; David M. Hill,*[4] *B.Sc.; Philip Maynard,*[2] *Ph.D.; and Claude Roux,*[2] *Ph.D.*

Forensic Analysis of Explosives Using Isotope Ratio Mass Spectrometry (IRMS)—Part 1: Instrument Validation of the DELTA^{plus}XP IRMS for Bulk Nitrogen Isotope Ratio Measurements

ABSTRACT: A significant amount of research has been conducted into the use of stable isotopes to assist in determining the origin of various materials. The research conducted in the forensic field shows the potential of isotope ratio mass spectrometry (IRMS) to provide a level of discrimination not achievable utilizing traditional forensic techniques. Despite the research there have been few, if any, publications addressing the validation and measurement uncertainty of the technique for forensic applications. This study, the first in a planned series, presents validation data for the measurement of bulk nitrogen isotope ratios in ammonium nitrate (AN) using the DELTA^{plus}XP (Thermo Finnigan) IRMS instrument equipped with a ConFlo III interface and FlashEA™ 1112 elemental analyzer (EA). Appropriate laboratory standards, analytical methods and correction calculations were developed and evaluated. A validation protocol was developed in line with the guidelines provided by the National Association of Testing Authorities, Australia (NATA). Performance characteristics including: accuracy, precision/repeatability, reproducibility/ruggedness, robustness, linear range, and measurement uncertainty were evaluated for the measurement of nitrogen isotope ratios in AN. AN (99.5%) and ammonium thiocyanate (99.99+%) were determined to be the most suitable laboratory standards and were calibrated against international standards (certified reference materials). All performance characteristics were within an acceptable range when potential uncertainties, including the manufacturer's uncertainty of the technique and standards, were taken into account. The experiments described in this article could be used as a model for validation of other instruments for similar purposes. Later studies in this series will address the more general issue of demonstrating that the IRMS technique is scientifically sound and fit-for-purpose in the forensic explosives analysis field.

KEYWORDS: forensic science, isotope ratio mass spectrometry, validation, nitrogen isotope ratios, ammonium nitrate

A significant amount of research has been conducted into the use of stable isotopes to assist in determining the origin/movement of various materials and living things (1–17). The vast majority of this research has been performed by industries in fields such as geology and environmental science, where the provision of evidence to a court is not generally required. Relatively recently, the international forensic community commenced conducting research into the potential use of isotope ratio mass spectrometry (IRMS) to assist in the investigation of complex forensic cases. In the forensic laboratory, it is generally not possible to conclusively identify two or more materials as originating from the same source. Previous studies have shown the potential of IRMS to link two or more samples sharing the same origin, including explosives (1,12). However, few, if any, publications address the scientific validation of the technique for forensic applications.

Demonstrating that method performance characteristics meet the requirements of the intended application is essential in forensic science and is achieved through method validation. Various publications are available that provide detailed descriptions of the performance characteristics that can be evaluated as part of a method validation (18,19). Method validation is an expectation placed on all forensic laboratories, and indeed any laboratory accredited against the international standard ISO 17025, whether the method be one that is recognized, slightly modified, or entirely new. This study details a validation study conducted for the measurement of bulk nitrogen isotope ratios of inorganic solid materials, specifically ammonium nitrate (AN) measurements using the Thermo Finnigan DELTA^{plus}XP instrument (Rydalmere, NSW, Australia). AN is of interest to forensic explosives laboratories due to its potential to be used as an oxidizer in improvised explosive mixtures and its mass production around the world.

The results from this study indicate that the instrument in question and the analytical method utilized is fit for the purpose of the measurement of bulk nitrogen isotope ratios in AN samples. This study provides a model protocol, which can be considered by other

[1]Forensic & Data Centres, Australian Federal Police, GPO Box 401, Canberra, ACT 2601, Australia.
[2]Centre for Forensic Science, University of Technology, Sydney, PO Box 123, Broadway, NSW 2007, Australia.
[3]National Centre for Forensic Studies, University of Canberra, Canberra, ACT 2601, Australia.
[4]Australian Nuclear Science and Technology Organisation, PMB 1, Menai, NSW 2234, Australia.

Received 18 Sept. 2007; and in revised form 5 Feb. 2009; accepted 5 Feb. 2009.

forensic practitioners in relation to the validation of the same or similar instrumentation for forensic case work.

Materials and Methods

Standards and Samples

The following international standards (i.e., certified reference materials; CRM) were utilized throughout the validation: IAEA-N1 (ammonium sulfate), IAEA-N2 (ammonium sulfate), USGS25 (ammonium sulfate), and IAEA-N3 (potassium nitrate). These standards were stored in their original packaging throughout the experiments. The IAEA certified values used for the correction calculations are listed in Table 1 (20). Note that the values as reported by Böhlke and Coplen (21) are the most recent and accepted values for these standards (refer to the certified values in Table 2).

The following were utilized as laboratory (working) standards: AN (99.5% BDH AnalaR [Kilsyth, VIC, Australia] Product Code: 10030-500G Batch: 5635 1), ammonium thiocyanate (99.99+% Sigma Aldrich [St. Louis, MO] Product Code: 431354-50G Batch: 13419JC), potassium nitrate (99.999% Aldrich Product Code: 54204010G Batch: 10014EB), and AN (99.0% Sigma Product Code: A9642-500G Batch: 083K0671).

The following chemicals were also evaluated as potential laboratory standards during the validation: ammonium sulfate, sodium nitrite, ammonium perchlorate, ammonium carbonate, urea, cornflour, self-raising flour, and plain flour.

Samples of explosive grade AN prill from an Australian AN manufacturer were also measured during the validation.

All samples (laboratory standards and AN prills) were stored in 24 mL Wheaton sample vials (clear) (Millville, NJ) with Teflon-lined screw cap lids (Sigma-Aldrich).

The following gases (from BOC Gases, Sydney, NSW, Australia) were used: helium ultra high purity (99.999%), nitrogen ultra high purity (99.999%), and oxygen (99.996%).

Instrumentation and Equipment

A Genius ME5 (Sartorius, Goettingen, Germany) analytical balance was utilized to weigh all samples and standards. Standards and prepared samples were stored in either a Perspex (i.e., acrylic) or glass desiccator with self-indicating orange silica gel (LabServ, Biolab, Tamaki, Auckland, New Zealand). Samples were weighed into 3.3 × 5 mm tin capsules for solids (Santis Analytical, Teufen, Switzerland).

A DELTA^{plus}XP IRMS instrument, with ConFlo III interface and FlashEA™ 1112 elemental analyzer (EA) with an AS2000 auto sampler (all Thermo Finnigan) was utilized for all experiments. The operating software for the IRMS was Isodat NT 2.0 (Thermo Finnigan) and for the EA was Eager 300 Version 2.1 (Thermo Finnigan). Figure 1 outlines the configuration of the instrument and the primary function of each component. In summary, the sample is weighed and subsequently sealed in a tin capsule. The capsules for measurement are placed in a carousel, which automatically rotates dropping the capsules individually into a combustion tube containing an oxidation catalyst and other materials. When dropped, a pulse of oxygen temporarily replaces the helium carrier gas resulting in a flash combustion of the solid sample to N_2, NO_x, CO_2, O_2, and H_2O. This combustion process raises the temperature from *c*. 900°C to 1700°C. The combustion products are swept into a reduction tube (*c*. 600°C) to reduce NO_x to N_2 and remove excess O_2. The samples then pass through a trap to remove the H_2O. The analyte gases (e.g., N_2 and CO_2) are then separated from each other and impurities on a packed gas chromatograph (GC) column. A small fraction of the effluent from the GC column enters the IRMS through an open split interface. The primary role of the interface is to reduce the gas flow from the EA to an appropriate flow for the IRMS (1).

The mass spectrometer (MS) generally comprises three main sections: an ion source, a mass analyzer, and an ion collection assembly. Gaseous samples for analysis enter the ionization chamber of the MS. Sample molecules impact with a focused electron beam in a high vacuum environment resulting in the loss of electrons producing positive ions. These ions are accelerated out of the chamber and through a flight tube between the poles of an electromagnet, where they are separated according to their mass-to-charge ratio (*m/z*). The ions are collected by a collector array generally consisting of three (sometimes up to eight) Faraday cup (FC) collectors (1).

The FC are positioned so that the major ion currents simultaneously strike the middle of the entrance slit of the respective cups. The ion currents are continuously monitored, then amplified,

TABLE 1—*Results of accuracy experiments.*

Certified Reference Material		δ^{15}N Measured-Corrected Value (‰)	δ^{15}N Certified Value (‰) (20)	Difference (‰)
IAEA-N1	Mean	0.3	0.4	0.1
	s	0.01	0.2	
IAEA-N2	Mean	20.2	20.3	0.1
	s	0.2	0.2	
IAEA-N3	Mean	4.7	4.7	0.04
	s	0.1	+2 to +5	
USGS25	Mean	−30.4	−30.4	0.01
	s	0.1	0.5	

TABLE 2—*Results of blind trial experiments utilizing certified reference materials as the samples/unknowns.*

Unknown		δ^{15}N Measured-Corrected Value (‰)	δ^{15}N Certified Value (‰) (21)	Difference (‰)
1	Mean	4.8	4.72	0.1
	s	0.1	0.13	
2	Mean	−30.8	−30.41	0.4
	s	0.2	0.27	
3	Mean	20.5	20.41	0.1
	s	0.2	0.12	
4	Mean	0.6	0.43	0.2
	s	0.1	0.07	

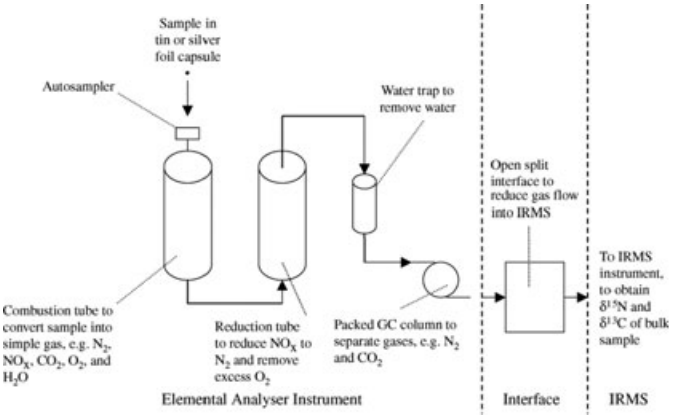

FIG. 1—*Schematic showing a flash combustion elemental analyzer in series with an interface and IRMS for the analysis of bulk nitrogen and carbon isotope ratios (diagram based on a Thermo FlashEA™ 1112 elemental analyzer). Source: Ref. (1) with permission from Elsevier.*

digitized using a voltage-to-frequency converter, and finally transferred to a computer. The computer integrates the peak area for each isotopomer and calculates the corresponding ratios (1). For example, when analyzing N_2, the data consist of three ion traces for the different isotopomers: $^{14}N^{14}N$, $^{14}N^{15}N$, and $^{15}N^{15}N$ with their corresponding masses at *m/z* 28, 29, and 30.

The IRMS instrument produces a delta value. Delta values represent the normalized difference of the isotope concentration ratios (*R*) of the sample and the reference (in this case a reference gas). Delta values are unitless numbers, however because the differences between a sample and reference are normally very small, the delta values are reported in units of per mil difference (parts per thousand = per mil = 10^{-3}), written ‰. Generally, δ-values are quoted relative to an internationally recognized standard (1).

Delta values are calculated using the following formula:

$$\delta = \frac{1000\ (R_{\text{sample}} - R_{\text{standard}})}{R_{\text{standard}}}$$

Method Parameters

Elemental Analyzer—Figure 2 outlines the configuration and composition of the components in the EA.

Elemental Analyzer Parameters—Oven temperature (housing the packed GC column, with a length of 2 m): 35°C; carrier (helium) flow: 140 mL/min; oxygen flow: 250 mL/min; and reference (nitrogen) flow: 300 mL/min.

Interface Parameters—Helium: 1.5 bar and reference nitrogen: 1.5 bar.

IRMS Method Parameters—Acquisition time: 320 sec; pulse of reference gas for 20 sec at 20, 60 (reference peak) and 180 sec; nitrogen peak elution between 95 and 160 sec.

A standard analytical sequence consisted of the measurement of 2× conditioning/blank tin capsules, 3× Standard 1, 3× Standard 2, Samples (up to 18), 3× Standard 1, and 3× Standard 2.

A template was developed in Microsoft® Office Excel (Redmond, WA) to correct the values of the standards and samples in a sequence against CRMs. This ultimately allows not only intra-laboratory comparison, but also inter-laboratory comparisons which

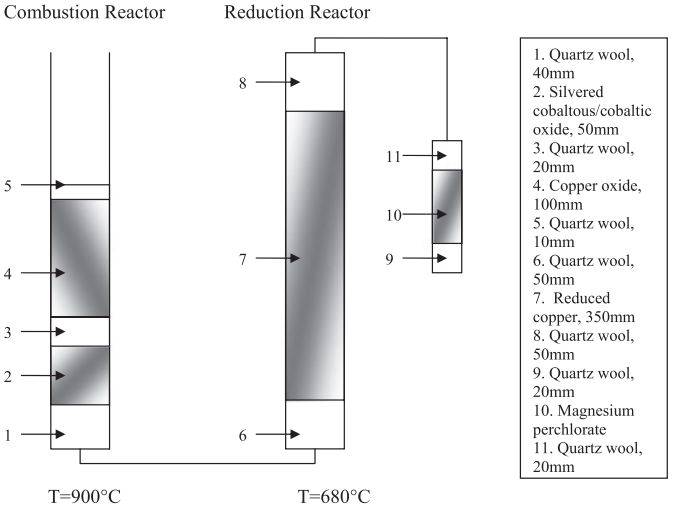

FIG. 2—*Configuration and composition of the combustion and reduction reactors and adsorption filter inside the elemental analyzer (22).*

are of particular importance when considering international databases. The major steps included in the spreadsheet are:

- Enter the measured delta values for the standards and samples as reported by the instrument.
- Exclude outliers using the Grubb's Test. The mean is calculated for the standards and samples and the Grubb's Test is then utilized to exclude outliers. Refer to Hibbert and Gooding (19) for a detailed explanation of the application of the Grubb's Test.
- Compare the mean of Standard 1 measured at the beginning of the sequence to the mean of Standard 1 at the end. Repeat the comparison for Standard 2. Determine whether there has been a significant drift over the course of the sequence (i.e., a drift greater than the reported standard deviation of the measured standard—in the absence of a reported standard deviation, the standard deviation for the instrument). If not, combine the values of the standards (e.g., Standard 1 at start and end) to calculate the measured mean for that standard and repeat for Standard 2, then perform step 4. In each of the cases in this research, there was no significant systematic drift between the standards at the start and end of the sequence. If systematic drift was identified (i.e., each sequential measurement had a greater offset than the previous measurement) then a correction would have been applied. This would have been calculated by determining the total drift (from start to end of sequence) and then dividing this drift by the number of samples measured in the sequence. This value (multiplied by the line number of the sample) would then have been added to the measured values of the samples.
- Calculate the correction factor by plotting the measured delta versus the known delta of the standards (either laboratory or CRM). The plot provides values for: *m* (slope) and *b* (intercept) in the equation: $y = mx + b$. These values are utilized to obtain the true value of the sample, i.e., the corrected value (y) by applying a correction to the measured value of the sample (x).
- Apply this correction factor to all the samples to calculate the true values of the samples versus the reference material.

Sample Preparation

Samples and standards were measured in triplicate unless otherwise specified. Samples and standards were weighed into tin capsules and subsequently sealed and placed in 200 µL centrifuge tubes. If the samples were not measured the same day, they were stored in the centrifuge tubes in a desiccator. Sample and standard amounts were selected so as to produce similar peak heights to the reference gas peaks (*c*. 2000 mV). This equated to *c*. 300 and 260 µg for AN and ammonium thiocyanate, respectively.

Selection and Characterization of Laboratory (Working) Standards

The evaluation of potential laboratory standards focused on: similarity in chemical composition; whether the isotope ratios of the potential materials bracketed the expected isotope ratios of the target materials; homogeneity; availability; similarity of decomposition products in pyrolysis cycles and ease of use.

Samples with similar chemical composition/structure to AN were selected and measured first to determine whether they bracketed the samples of interest and also whether the results indicated sample homogeneity. Different brands and grades of the chemicals were measured.

The calibration of the laboratory standards was performed on multiple occasions as represented by the dates in Table 3. The

TABLE 3—*Results of laboratory standards calibration.*

Laboratory Standard		August 2005	December 2005	February 2006	May 2007	August 2007	Calibration Average	Inter-laboratory Trial
Ammonium nitrate min. 99%	δ^{15}N Mean (‰)	−0.01	−0.2	−0.3			−0.1	−0.2
	s (‰)	0.03	0.03	0.1			0.1	0.6
Ammonium nitrate min. 99.5%	δ^{15}N Mean (‰)	−2.9			−3.1	−2.9	−2.9	
	s (‰)	0.03			0.2	0.1	0.1	
Ammonium thiocyanate 99.99+%	δ^{15}N Mean (‰)	2.8	2.8	2.9	3.0		2.9	3.1
	s (‰)	0.1	0.01	0.1	0.1		0.1	0.3
Potassium nitrate 99.999%	δ^{15}N Mean (‰)	−0.3	−0.4	−0.4			−0.3	−0.4
	s (‰)	0.04	0.1	0.03			0.04	0.2

Results are reported as $\delta^{15}N_{AIR}$ (‰) and calibrated with certified reference materials. The results from the inter-laboratory trial are also reported for comparison.

laboratory standards were calibrated against the CRMs: IAEA-N1 (470 μg), IAEA-N2 (470 μg), USGS25 (470 μg), and IAEA-N3 (720 μg) using the certified values listed in Table 1 (these weights were utilized throughout the experiments where the CRMs were measured). The CRMs were used as standards and the laboratory standards under evaluation were measured as unknown samples. For each sequence, the corrected values of the laboratory standards were determined utilizing the correction template detailed in the Method Parameters section of this study. The mean of the individual corrected values from each sequence yield the calibrated values for the laboratory standards. These values are given in Table 3, together with results from an inter-laboratory trial coordinated by the Australian Federal Police, Forensic Operations laboratory in March 2006. During this trial, samples of AN (99%), ammonium thiocyanate (99.99+%), and potassium nitrate (99.999%) were distributed to seven IRMS laboratories in Australia and New Zealand for independent verification of their true/agreed values. The seven laboratories were representative of the IRMS laboratories in Australia and New Zealand. The laboratories measured the distributed samples according to their standard procedures and the results were compiled and evaluated in accordance with International Standard ISO 13528:2005(E) *Statistical methods for use in proficiency testing by inter-laboratory comparisons* (23). The results of the trial will be the subject of a future publication, however mean delta values are reported in this study.

Two of the standards were selected for further evaluation (i.e., stability over time in different storage locations and suitability for the intended application). The stability of AN (99%) and ammonium thiocyanate (99.99+%) stored under different conditions over a 380-day (54-week) period was evaluated. Three replicates of each sample were measured on each occasion. The storage locations included: glass desiccator, Perspex desiccator, laboratory cool room (0–3°C), and laboratory bench (ambient room temperature). Each sample was stored in a 24 mL Wheaton sample vial (clear) with a Teflon-lined screw cap lid (Sigma-Aldrich). USGS25 and IAEA-N3 were utilized as standards.

Validation Protocol

The validation protocol was developed in line with the guidelines provided by the National Association of Testing Authorities, Australia (NATA) (18), while taking into consideration relevance of each performance characteristic to the IRMS technique.

Measurement Uncertainty—The entire procedure, from the preparation of laboratory standards to correction calculations, was evaluated for potential sources of uncertainty. These uncertainties were divided into sources of method bias/accuracy and method precision which were combined to determine an estimate of the overall measurement uncertainty (i.e., expanded uncertainty) for this specific method/procedure.

Estimation of Measurement Uncertainty—The following series of equations were utilized to provide an estimate of the measurement uncertainty (i.e., expanded uncertainty) for the measurement of nitrogen isotope values in AN prill samples. These equations were utilized as they are recommended by NATA for performing an estimate of the combined standard uncertainty (24). The guidelines published by NATA utilize the key principles and definitions outlined in the *ISO Guide to the Expression of Uncertainty in Measurement* (GUM) (25) and *Eurachem Quantifying Uncertainty in Analytical Measurement* (26); however, they are adapted specifically for chemical testing laboratories. According to the guidelines, a reasonable estimate of measurement uncertainty may be gained from the bias and precision associated with a test result.

Combined Uncertainty—

$$u_C(y)^2 = s_L^2 + u_b^2 \quad (1)$$

$u_C(y)$ = combined standard uncertainty of y; s_L = standard deviation of results obtained from precision experiments; u_b = standard uncertainty associated with the measurement of bias (results from accuracy experiments).

Standard Uncertainty Associated with Bias—

$$u_b^2 = u(\tilde{y})^2 + u(y_{exp})^2 \quad (2)$$

$u(\tilde{y})$ = standard uncertainty of observed (i.e., measured) result from accuracy experiment; $u(y_{exp})$ = standard uncertainty of expected result, i.e., certified value of standard.

Standard Uncertainty Associated with Observed/Measured Result—

$$u(\tilde{y}) = s/\sqrt{n} \quad (3)$$

s = standard deviation of observed (measured) results; n = number of measurements.

Standard Uncertainty Associated with Expected/Certified Result—

$$u(y_{exp}) = \text{range}/\sqrt{3} \quad (4)$$

Range, ± value provided on the CRM certificate; $\sqrt{3}$, utilized as a rectangular distribution best represents this uncertainty data.

Estimation of Bias—

$$b = \tilde{y} - y_{\text{exp}} \quad (5)$$

If $|b| > t(0.05, n-1)u_{\text{b}}$, where t is the Student's t value at $n-1$ degrees of freedom, then the bias is significant.

Determination of Expanded Uncertainty if Bias is Significant—

$$U = ku_C(y) + |b| \quad (6)$$

k = coverage factor.

Determination of Expanded Uncertainty if Bias is Not Significant—

$$U = ku_C(y) \quad (7)$$

A coverage factor (k) of 2 was utilized to determine the expanded uncertainty (U). A coverage factor is a numerical factor used as a multiplier of the combined standard uncertainty to obtain an expanded uncertainty. An expanded uncertainty refers to a quantity defining an interval about the result of a measurement that may be expected to encompass a large fraction of the distribution of values that could reasonably be attributed to the measurement (24). A coverage factor of 2 provides an approximate level of confidence of 95%, i.e., one can be 95% confident that the reported range includes the true value (24).

The following experiments were conducted to provide estimates of uncertainty for a number of key performance characteristics; however, only the results from the AN prill precision and accuracy experiments were utilized in the final estimation of the measurement uncertainty:

- Values for laboratory and international standards obtained over at least a 12-month period were plotted to determine the expected variation of reported results over an extended period of time. These values represent the corrected values for these standards when they were measured as standards in a sequence. The standards were corrected either to the certified value if it was a CRM or the calibrated value if it was a laboratory standard. The resulting uncertainty represents the expected variation/uncertainty when preparing and measuring these sample types, including performing the correction calculations.
- Method Precision/Repeatability (99% AN)—seven replicate measurements of 300 μg AN (99%) were measured together with laboratory standards (AN 99.5% and ammonium thiocyanate 99.99+%) to evaluate the precision (variability) of the instrument over a 125-day period (18 weeks).
- Method Precision/Repeatability (AN prill)—the precision was determined over a 222-day period, using three measurements from three different prills from the same sample on two occasions and seven replicate measurements from a number of crushed prills from the same sample on the third occasion.
- Method Bias/Accuracy—four international standards (IAEA-N1, IAEA-N2, USGS25, and IAEA-N3) were prepared (three replicates of each) and measured as samples/unknowns using IAEA-N2 and USGS25 as the standards.
- Suitability of Laboratory (Working) Standards, Sequence Template, and Correction Calculations—results of the calibration and precision/repeatability experiments were evaluated to determine whether fractionation was occurring within or between sequences.
- International standards were measured as unknowns in the middle of seven sequences to determine whether significant drift was occurring throughout the course of each sequence and whether the Excel correction template was fit-for-purpose.

Robustness (Different Operators)—Three different operators prepared and measured AN (99%), ammonium thiocyanate (99.99+%) and potassium nitrate (99.999%) on different days using international standards (IAEA-N1, IAEA-N2, USGS25, and IAEA-N3) as the standards. Seven replicates of each sample were measured.

Reproducibility/Ruggedness—The effect of the variation of the following environmental conditions on delta values was evaluated: room temperature, night time versus day time, and room door opening and closing. These environmental conditions were identified for evaluation as they were the ones that would vary over the course of the research. It was necessary to ensure that variations in these environmental conditions did not have a significant effect on the measured delta values (i.e., outside the acceptable range in measurements of 99% AN). Seven to 10 replicates of AN (99%) samples were utilized during each evaluation, with AN (99.5%) and ammonium thiocyanate (99.99+%) utilized as the laboratory standards.

Linear Range—Seven replicate measurements of each of the following weights of AN (99%) were collected on 1 day: 15, 37.5, 75, 150, 300, 600, 1050, and 1500 μg. The results obtained from the experiments were utilized to determine linear range and also assist in determining at what amount of nitrogen the measurements displayed unacceptable precision. AN (99.5%) and ammonium thiocyanate (99.99+%) were measured as the laboratory standards. The linear range for prill samples was determined using seven replicates of the following weights of AN prill: 75, 150, 300, 600, and 1200 μg. AN (99.5%) and ammonium thiocyanate (99.99+%) were utilized as the standards.

The prill evaluation was smaller than that conducted using the analytical grade AN, however the study was sufficient to determine whether the major performance characteristics of the technique were acceptable when the analyte of interest was measured (in this case, AN prill samples).

Blind Trial—Samples of four international standards (IAEA-N1, IAEA-N2, USGS25, and IAEA-N3) were prepared by a research assistant (three replicates of each). These were measured as unknown samples by a second person using AN (99.5%) and ammonium thiocyanate (99.99+%) as the standards.

Software Validation—The Isodat software was manually verified/validated using a Microsoft® Office Excel spreadsheet (developed by Thermo Fisher Scientific, Bremen, Germany) which consisted of the calculations performed on the raw peak areas as measured by the instrument to obtain the final reported delta values (i.e., software algorithm for calculation of delta values based on peak area). As the spreadsheet was provided by the instrument manufacturer, the functions, cell values, and order of calculations were checked. Once the basis for the calculations was understood and verified, the spreadsheet was used to compare the results obtained from the spreadsheet to the results obtained from the Isodat software. This was achieved by selecting seven delta values which were high (20.3‰), low (−30.4‰), and in the middle range (0‰) and subsequently using the spreadsheet to independently calculate the delta values using the raw peak areas from the instrument. The final calculated delta values were then compared with the reported delta values from the Isodat software.

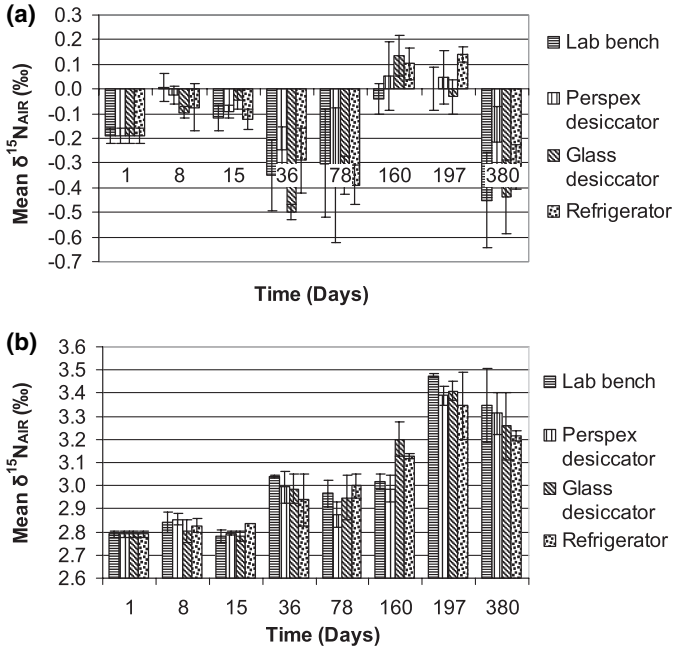

FIG. 3—*(a) Ammonium nitrate stability evaluation over 380 days under different storage conditions. (b) Ammonium thiocyanate stability evaluation over 380 days under different storage conditions.*

Results and Discussion

Results are reported as $\delta^{15}N_{AIR}$ (‰) unless otherwise specified. Error bars in all plots represent ±1 standard deviation (*s*).

Selection and Characterization of Laboratory Standards

Fourteen materials were measured for an initial evaluation as to whether their nitrogen isotope compositions bracketed the samples of interest and for isotopic homogeneity. From these 14 materials, four were selected to perform detailed calibration studies. Table 3 displays the mean corrected values from the calibrations and the final calculated values for the laboratory standards (i.e., calibration average).

Figure 3*a* displays a plot of mean nitrogen delta values measured for AN over a 380-day period (54 weeks). Over this time, samples of AN stored in the following four locations were measured: Location 1—laboratory bench (ambient room temperature); Location 2—Perspex desiccator; Location 3—glass desiccator; Location 4—laboratory refrigerator (0–3°C). Figure 3*b* displays a plot of mean nitrogen delta values measured for ammonium thiocyanate over a 380-day period (54 weeks) in the same storage locations as above.

Despite the apparent significant differences between the different locations over time, the overall mean and standard deviation of the individual measurements of the samples in each location over the time period (as summarized in Table 4) are not significantly different from the mean and standard deviations of the samples as determined during the calibration (refer to Table 3). The standard deviations are also not significantly different from the overall measurement uncertainty estimated for this procedure (see Estimation of Measurement Uncertainty results). Hence, the observed standard deviations cannot be specifically attributed to the effect of the different storage location, the effect of the storage container (vial) or storage over time. Despite this, preference would be given to storage in a desiccated environment to minimize the potential for water absorption. However, it is important to acknowledge that if samples were stored in a refrigerator or on a laboratory bench at ambient temperature, then the bulk nitrogen isotope values would not be compromised.

TABLE 4—*Overall mean and standard deviation of ammonium nitrate (99%) and ammonium thiocyanate (99.99+%) stored in each location during the stability evaluation.*

Location	Ammonium Nitrate Mean $\delta^{15}N$ (‰) and *s*	Ammonium Thiocyanate Mean $\delta^{15}N$ (‰) and *s*
Location 1—laboratory bench	−0.2 ± 0.2	3.03 ± 0.2
Location 2—Perspex desiccator	−0.1 ± 0.2	3.00 ± 0.2
Location 3—glass desiccator	−0.2 ± 0.2	3.02 ± 0.2
Location 4—refrigerator	−0.1 ± 0.2	3.03 ± 0.2

Validation Protocol

Measurement Uncertainty—Examination of the technique from sample preparation to data correction permitted the identification of several potential sources of uncertainty. The sources included, but were not limited to: the uncertainties in the electronic balance and the CRMs, matrix effects, and fractionation. These potential sources may affect (to varying extents) the accuracy through method bias. The precision of the electronic balance and the IRMS, and the inhomogeneity of the test materials and the laboratory standards, all potentially affect the method precision.

The Genius ME5 analytical balance was calibrated by an external NATA accredited body and the limit of performance was determined to be ±0.000148 g. This value equates to *c.* ±0.03‰ (based on the Linear Range experiments conducted utilizing 99% AN).

The standard deviations (*s*) applicable to the CRMs used in this study are given in Table 1. The instrument standard deviation is provided in Table 5.

The replicate measurements of CRMs (USGS25 and IAEA-N3) and laboratory standards (AN 99.5% and ammonium thiocyanate 99.99+%) were plotted for a 12-month period (see Figs. 4–7). The mean of the measurements and ±1 standard deviation were plotted on the same figures. After plotting all individual measurements, outliers were excluded using the Grubb's test (19). A number of points suspected of being outliers were excluded based on the fact

TABLE 5—*Summary of the analytical results obtained for the laboratory and international standards over a 12- to 17-month period and comparison with the reported standard deviations for the instrument and certified reference materials.*

Standard	Range (‰)	Mean (‰)	*s* (‰)	95% CI	No.	Instrument *s* (‰) (27)	Certified Value (‰) and *s* (20)	Certified Value (‰) and *s* (21)
USGS25	0.7	−30.4	0.1	0.03	115	±0.15	−30.4 ± 0.5	−30.41 ± 0.27
IAEA-N3	0.5	4.7	0.1	0.02	105	±0.15	4.7	4.72 ± 0.18
Ammonium nitrate	0.6	−2.9	0.1	0.01	290	±0.15		
Ammonium thiocyanate	0.5	2.9	0.1	0.01	301	±0.15		

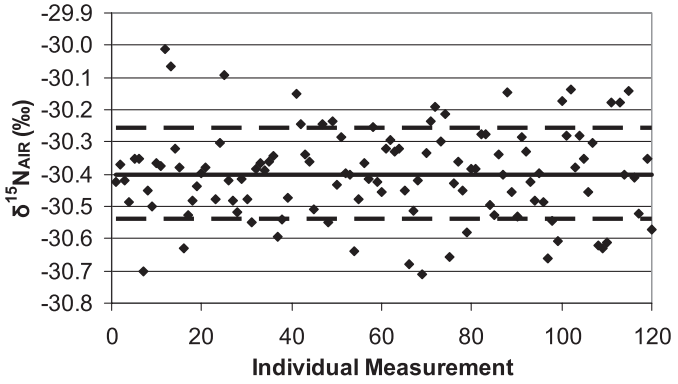

FIG. 4—*Plot of corrected measurements of USGS25 over 17-month period. Mean value and ±1 standard deviation also represented.*

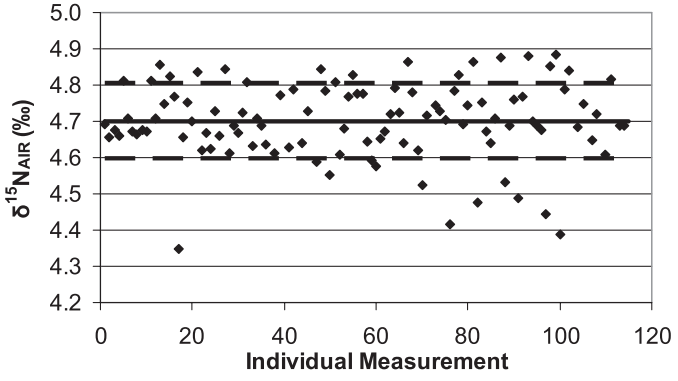

FIG. 5—*Plot of corrected measurements of IAEA-N3 over 17-month period. Mean value and ±1 standard deviation also represented.*

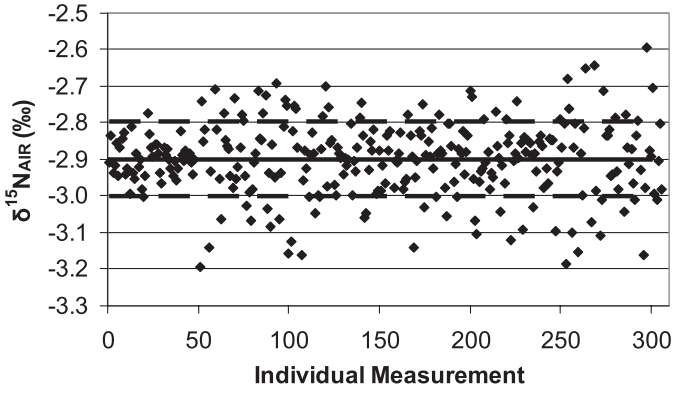

FIG. 6—*Plot of corrected measurements of ammonium nitrate (99.5%) over 12-month period. Mean value and ±1 standard deviation also represented.*

that the measurements were from the first sample in a sequence, hence were considered to be conditioning samples (these values are not included in the figures).

A summary of the range in individual measurements, mean, standard deviation, 95% confidence interval, and number of measurements (No.) relating to the individual corrected values of the measurements over the 12-month period are represented in Table 5.

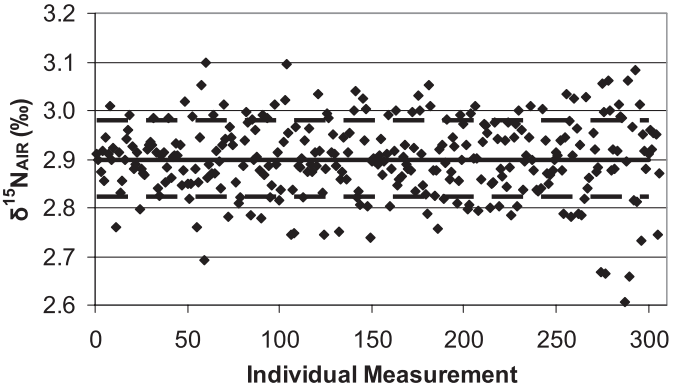

FIG. 7—*Plot of corrected measurements of ammonium thiocyanate (99.99+%) over 12-month period. Mean value and ±1 standard deviation also represented.*

Method Precision/Repeatability (99% AN)—Figure 8 displays a plot of the precision/repeatability experiment results over 125 days for the AN (99%) samples. The range observed in the mean delta values over the 125-day period was 0.2‰ and the mean and standard deviation of all of the individual measurements combined was $\delta^{15}N_{AIR}$ −0.1 ± 0.1‰.

It is worth noting that the necessary maintenance (refer to Table 6) conducted over this period did not have a significant effect on the corrected results; however, once again this is dependant on the number of conditioning samples measured prior to the standards/samples of interest.

Method Precision/Repeatability (AN Prill)—Figure 9 displays a plot of the precision/repeatability experiment results over 222 days for the AN prill samples. The range observed in the mean delta values was 0.2‰ and the mean and standard deviation of all of the individual measurements combined was $\delta^{15}N_{AIR}$ −0.7 ± 0.1‰.

Method Bias/Accuracy—Table 1 details the results of the method bias/accuracy experiments. The difference between the certified and measured values is equivalent to the sum of the method bias and random errors (18). Therefore, within the scope of these accuracy experiments, the method bias and random errors can account for a variation of ±0.1‰. Note that these results are only applicable for the range of standards used in this evaluation. The method will be comprehensively evaluated for bias in a future inter-laboratory study.

Suitability of Laboratory Standards, Sequence Template, and Correction Calculations—The laboratory standards measured during the calibration and precision/repeatability experiments were plotted and evaluated for potential trends, including potential fractionation. There did not appear to be any significant variation between the values of the standards at the beginning and end of each sequence, indicating no apparent drift within an analytical sequence.

One trend observed was that the first AN laboratory standard measured was generally significantly more negative than the other five measurements. This is likely due to the need to run conditioning samples prior to measuring the standards or samples of interest. This leads to the recommendation that at least one conditioning sample of the same or similar composition to the target material should be measured prior to the first set of standards (with additional conditioning samples required if instrument maintenance has

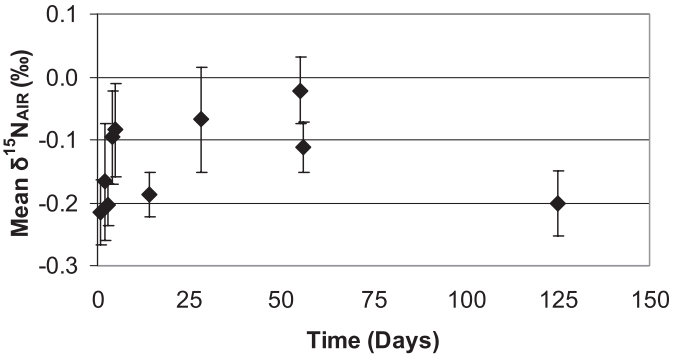

FIG. 8—*Precision/repeatability experiment results utilizing ammonium nitrate (99%).*

TABLE 6—*Maintenance conducted over 125-day period during precision/repeatability experiments.*

Day	Maintenance	Number of samples analyzed
3	Cleaned ash top of combustion reactor	4 blank tin capsules
5	Changed helium cylinder	3 blank tin capsules
28	Cleaned ash top of combustion reactor	71 samples
55	Cleaned ash and changed He cylinder	46 samples
125	1. Replaced reduction reactor	1. 318 samples
	2. Replaced combustion reactor/changed He cylinder	2. 23 samples

The number of samples analyzed refers to the number of samples between the maintenance and repeatability sequences.

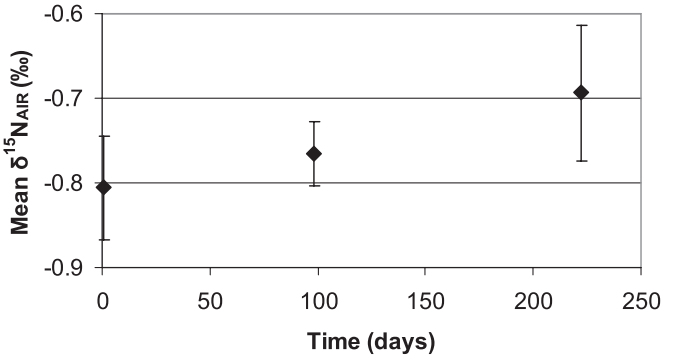

FIG. 9—*Plot of ammonium nitrate prill precision/repeatability results.*

TABLE 7—*Results of robustness evaluation experiments.*

	Ammonium Nitrate		Ammonium Thiocyanate	
Operator	Mean δ^{15}N (‰)	*s* (‰)	Mean δ^{15}N (‰)	*s* (‰)
1	−0.3	0.1	2.9	0.1
2	0.002	0.1	3.02	0.1
3	−0.1	0.1	3.1	0.1
Range in mean (‰)	0.3		0.2	

been conducted). For example, the standard sequence would change to: 2× blank tin capsules; 4× Standard 1; 3× Standard 2; Samples; 3× Standard 1; 3× Standard 2. The first measurement of the first set of standards could then be considered as a conditioning sample only.

The chromatograms of ammonium thiocyanate displayed an extra peak between 210 and 250 sec. This peak was attributed to the carbon in thiocyanate, which is converted to CO_2 in the EA; CO_2 elutes after N_2, but is cleaved to CO in the ion source of the MS. As CO is isobaric with N_2 the carbon in the thiocyanate appears as a second peak in the chromatogram. During method development, the acquisition time was extended to allow the complete elution of this peak so that it would not interfere with subsequent measurements. An alternative would be to include a trap (e.g., ascarite) to remove the carbon prior to entry into the IRMS. This additional peak did not appear to have an effect on subsequent measurements.

The mean and standard deviation of the corrected values for the IAEA-N1 samples measured as unknowns (mid sequence for 7 sequences) were not significantly different from the reported certified values for this international standard. The mean and standard deviation of all the measurements from the sequences were $\delta^{15}N_{AIR}$ $0.4 \pm 0.1‰$, compared with the reported value of $\delta^{15}N_{AIR}$ $0.43 \pm 0.07‰$ (21). This indicates that there was no significant drift occurring throughout a sequence containing up to 18 samples and that there were also no significant sources of uncertainty with the Excel correction calculation spreadsheet that was developed and employed in this study.

Estimation of Measurement Uncertainty—The expanded uncertainty for the measurement of nitrogen isotope values in AN prills was determined to be 0.2‰ (coverage factor of 2) utilizing the equations in the Materials and Methods (Estimation of Measurement Uncertainty) section of this study. The bias was determined to be insignificant.

The systematic bias that may be introduced when measuring AN prill samples as opposed to the CRMs was not evaluated as the AN prill samples do not have certified values. Consideration will be given in the future to the potential bias that may be introduced as a result of the coating agent or other additives in the prill samples.

Robustness (Different Operators)—Delta values and associated standard deviations obtained from the three different operators during the robustness experiments are detailed in Table 7. The mean and standard deviations and the range in mean reported by the three operators for AN and ammonium thiocyanate are not significantly different from the calibrated values of the standards (Table 3) or the estimated measurement uncertainty. The results indicate that the measurements do not vary significantly as a result of different competent operators.

Reproducibility/Ruggedness (Environmental Conditions Varied)—The results of the experiments where the environmental conditions were varied are plotted in Fig. 10. The range observed in the mean throughout these experiments was 0.1‰. This range is within an acceptable measurement uncertainty of the technique. The mean and standard deviation for all of the combined measurements was $\delta^{15}N_{AIR}$ $-0.1 \pm 0.1‰$. Overall there was no significant effect on the mean that could be attributed to the varying environmental conditions (i.e., room temperature, door position, and day vs. night measurements).

Linear Range—Figure 11*a* contains a plot of the mean instrument response for the mass 28 peak amplitude (mV) at a range of increasing weights of nitrogen (in 99% AN samples).

Examination of the relative standard deviation (RSD) (which provides an indication of the imprecision of the measurements [19]

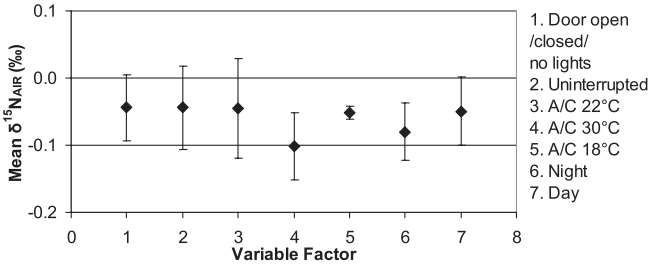

FIG. 10—*Reproducibility evaluation experiment results utilizing ammonium nitrate (99%).*

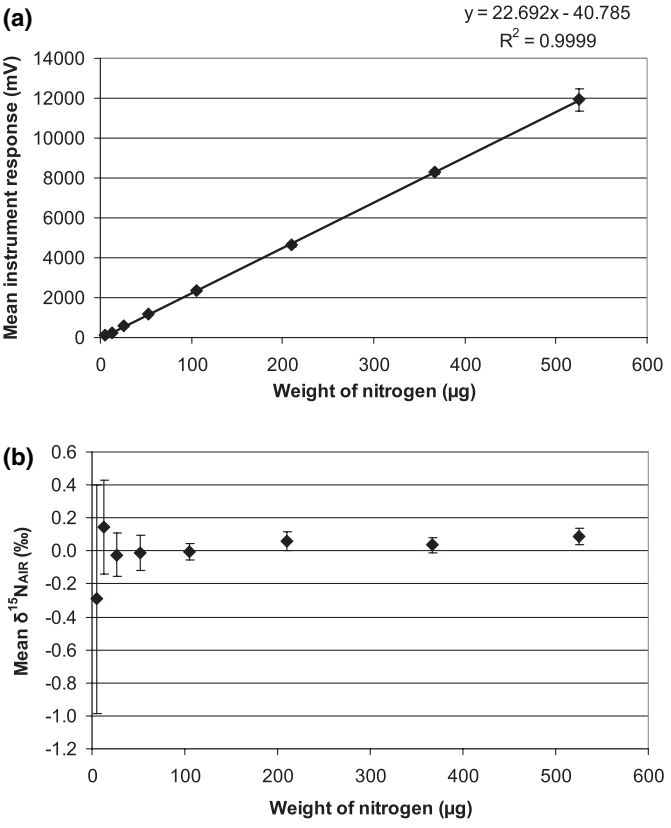

FIG. 11—*(a) Plot of linear range experiments with respect to mean instrument response utilizing ammonium nitrate (99%). (b) Plot of linear range experiments with respect to mean delta utilizing ammonium nitrate (99%).*

detailed in Table 8), indicates that the results are precise, accurate, and linear between the approximate range 52.5–525 µg of nitrogen (i.e., 150–1500 µg of AN). A RSD value of less than 1% is generally viewed as very good; however, routine measurements generally fall between 1% and 5% range (19). The instrument response versus weight (mV/µg) data in Table 8 also demonstrates that the instrument response stabilizes when samples contain *c*. 52.50 µg of nitrogen and above. Based on the experimental results, the instrument appears to have significantly worse precision when measuring samples containing less than 52.50 µg of nitrogen (i.e., 150 µg of AN).

Figure 11*b* contains a plot of the mean delta at a range of increasing weights of nitrogen (in 99% AN samples).

If the unreliable data (i.e., less than 52.50 µg of nitrogen) is excluded, there appears to be a slight, however insignificant, systematic pattern (i.e., 0.0002‰/µg) with regards to the mean delta values with increasing sample size. This linearity pattern may be due to the linearity of the mass spectrometer. The weight of the standards and the reference gas voltage remained constant while the weights of the samples varied which may also have contributed to the observed pattern.

To minimize the bias introduced through variation in sample size it is recommended that samples of AN be measured in the range 300 ± 50 µg which will result in a bias of *c*. ±0.01‰. If measurements are not made in this range, then consideration should be given to the bias introduced through variation in sample size during interpretation.

The limit of detection was not determined as it is not relevant for IRMS measurements. The critical performance characteristic to evaluate was the range in which the response was reliably linear. Figure 12*a* contains a plot of the mean instrument response (mV) at a range of increasing weights of nitrogen in AN prill samples.

The RSD values in Table 9 indicate that the results are precise, accurate, and linear within the range that was evaluated, i.e., 26.25–420 µg of nitrogen (i.e., 75–1200 µg of AN). Weights beyond 420 µg of nitrogen were not tested; however, the low RSD for 420 µg of nitrogen indicates that the linear range could be extended further incorporating larger weights of nitrogen. The results for 105–420 µg of nitrogen (i.e., 300–1200 µg AN) were very good, with RSD values <1%.

Figure 12*b* contains a plot of the mean delta at a range of increasing weights of nitrogen using AN prill samples. When the first two values are excluded (as potential unreliable data), there is no apparent bias in the measured mass range (i.e., 105–420 µg). The instrument response versus weight data in Table 9 indicates that the instrument response starts to stabilize when measuring samples containing 210 µg of nitrogen and above. Measurements of samples containing more than 420 µg of nitrogen should be made in the future to confirm this apparent trend.

Blind Trial—The results of the blind trial (as summarized in Table 2) indicate that the technique, methods, and laboratory standards are fit-for-purpose, i.e., are suitable/capable of determining

TABLE 8—*Summary of linear range results with respect to mean instrument response, mean delta, and standard deviation utilizing ammonium nitrate (99%).*

Weight Nitrogen (µg)	Mean Instrument Response (mV)	*s* (mV)	RSD	δ^{15}N Mean (‰)	*s* (‰)	Range (‰)	mV/µg
5.25 (15 µg NH_4NO_3)	94.9	26.4	27.8	−0.3	0.7	2.0	18.1
13.13 (37.5 µg NH_4NO_3)	254.3	19.6	7.7	0.2	0.3	0.8	19.4
26.25 (75 µg NH_4NO_3)	563.1	29.7	5.3	−0.02	0.1	0.4	21.5
52.50 (150 µg NH_4NO_3)	1198.4	41.8	3.5	−0.01	0.1	0.3	22.8
105.00 (300 µg NH_4NO_3)	2332.7	53.5	2.3	0.0	0.1	0.1	22.2
210.00 (600 µg NH_4NO_3)	4635.9	107.3	2.3	0.1	0.1	0.2	22.1
367.50 (1050 µg NH_4NO_3)	8285.0	112.1	1.4	0.04	0.04	0.1	22.5
525.00 (1500 µg NH_4NO_3)	11,914.0	545.4	4.6	0.1	0.1	0.1	22.7

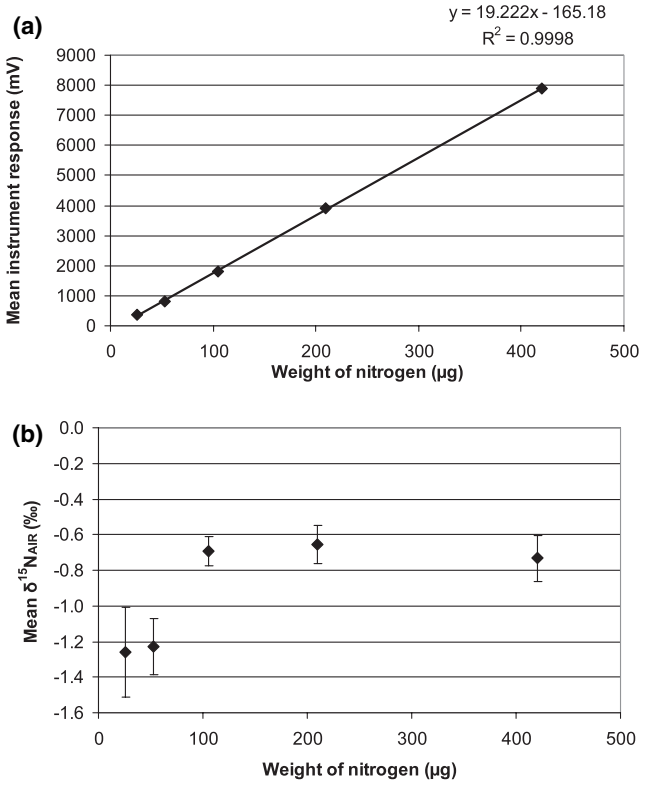

FIG. 12—*(a) Plot of ammonium nitrate prill linear range experimental results with respect to mean instrument response. (b) Plot of linear range experiments with respect to mean delta utilizing ammonium nitrate prill.*

the true/agreed values of a range of samples within an acceptable measurement uncertainty.

Software Validation—The software algorithm for the calculation of delta values based on peak area is summarized from the Excel spreadsheet (created by Thermo Fisher Scientific Bremen) in the Appendix.

The delta values calculated using this Excel spreadsheet were compared with the corresponding reported delta values from the instrument. The values obtained by the two methods were the same. This validation shows that the series of calculations making up the algorithm are logical and that the spreadsheet produces the same values as the instrument software.

Conclusions

The experiments described in the article have demonstrated that the instrument and the analytical method utilized is fit for the purpose of measurement of bulk nitrogen stable isotopes in AN samples, including prill samples. Suitable laboratory standards were evaluated and calibrated against CRMs. The mean values and standard deviations associated with a number of CRMs and laboratory standards were determined to be as follows:

- USGS25: $\delta^{15}N_{AIR}$ −30.4 ± 0.1‰;
- IAEA-N3: $\delta^{15}N_{AIR}$ 4.7 ± 0.1‰;
- AN: $\delta^{15}N_{AIR}$ −2.9 ± 0.1‰; and
- Ammonium thiocyanate: $\delta^{15}N_{AIR}$ 2.9 ± 0.1‰.

The overall measurement uncertainty (i.e., expanded uncertainty) for the measurement of nitrogen isotope values in AN prill samples utilizing the instrumentation, methods, and procedures detailed in this study was estimated as 0.2‰ (coverage factor of 2).

All evaluated method performance characteristics (including: accuracy, precision/repeatability, reproducibility/ruggedness, robustness, and linear range) were within the determined measurement uncertainty range. Measurements also within an acceptable measurement uncertainty range were reported during the blind trial conducted.

Further research is required with respect to the measurement of AN, including: the evaluation of the within sample variation from one manufacturer and between sample variation from different manufacturers; evaluation of potential differences between the nitrogen originating from the ammonium ion and the nitrate ion; evaluation of the manufacturing process for potential fractionation; evaluation of the potential to perform pre- versus post-blast comparisons; and the establishment of suitable databases containing data for each of the isotopes to be used for the potential differentiation of AN samples (i.e., nitrogen, oxygen, and hydrogen). The significance of finding AN samples that cannot be differentiated based on stable isotope values also needs further investigation.

TABLE 10—*Notation utilized by Thermo Fisher in explaining the algorithm to calculate isotope ratio values and terminology that may be used by others.*

Thermo Fisher Terminology	Other Terminology	Symbol
Element ratio	Isotope ratio	R or $R_{15/14}$
Molecular ratio	Molecular mass ratio	$R_{29/28}$ or $R_{30/28}$
Atom (abundance) ratio or atom ratio	Isotope mole fraction	X_{15} or X_{14}
Molecular (abundance) ratio		X_{28}, X_{29}, or X_{29}

Acknowledgments

The authors thank Dr. Oliver Kracht (Application Specialist Gas Isotope Ratio MS, Thermo Fisher Scientific Bremen) for the software algorithm for the determination of delta values and his assistance in understanding the algorithm; Australian ammonium nitrate manufacturers for the provision of samples and detailed information; and reviewers for the time taken to review and recommend changes to assist in formulating a useful scientific paper.

TABLE 9—*Summary of linear range results with respect to mean instrument response, mean delta, and standard deviation using ammonium nitrate prills.*

Weight Nitrogen (µg)	Mean Instrument Response (mV)	s (mV)	RSD	δ^{15}N Mean (‰)	s (‰)	Range (‰)	mV/µg
26.25	373.3	10.5	2.8	−1.3	0.3	0.7	14.2
52.5	809.7	9.1	1.1	−1.2	0.2	0.4	15.4
105	1816.3	9.1	0.5	−0.7	0.1	0.2	17.3
210	3922.8	18.9	0.5	−0.7	0.1	0.3	18.7
420	7893.5	31.7	0.4	−0.7	0.1	0.4	18.8

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Additional information and reprint requests:
Sarah Jane Benson, Ph.D.
Australian Federal Police
GPO Box 401
Canberra
ACT 2601
Australia
E-mail: sarah.benson@afp.gov.au

Appendix

Explanation of the Isodat Software Algorithm

This appendix outlines the process that the Isodat software utilizes to calculate isotope ratio values (specifically bulk nitrogen isotope ratios). The terminology referred to in this appendix is utilized by Thermo Fisher Scientific Bremen.

The notation in Table 10 can be utilized to assist in cross-referencing Thermo Fisher terminology with other terminology that may be utilized among IRMS users.

In general, the isotope ratios of the standard are known and are converted to molecular mass ratios. The molecular mass ratios of the sample are measured. The sample molecular mass ratios are compared with the standard molecular mass ratios and the sample ratios are subsequently converted to isotope ratios.

In continuous flow techniques, such as the setup employed in this research for the measurement of bulk nitrogen isotope ratios, the isotope ratios are defined by peak areas within each mass trace. The raw area values in each trace are corrected through amplification, i.e., the mass trace 29 area value is multiplied by 100, the mass trace 30 area value is multiplied by 333 when compared with the area value of mass trace 28 and all three trace area values (i.e., 28, 29, and 30 for nitrogen) are multiplied by 1000 to get mV sec.

The software algorithm for the calculation of nitrogen delta values based on peak area can be summarized from the Excel spreadsheet (created by Thermo Fisher Scientific Bremen) as follows:

1. The delta of the reference gas versus AIR (e.g., $\delta = -2.537‰$) and isotope ratio of the standard AIR are known (i.e., $R_{reference} = 0.00367820$) and are utilized in Eq. A1 to calculate the isotope ratio of the reference gas (R_{sample}).

$$\delta(‰) = (R_{sample}/R_{reference} - 1) \times 1000‰ \qquad (A1)$$

2. Use the isotope ratio of the reference gas (R_{sample} calculated in step 1) to calculate the absolute atom abundance ratio for ^{15}N and ^{14}N ($R_{sample} = {}^{15}N/{}^{14}N$) in the reference gas.

$$\text{Atom ratio for } {}^{15}N = ({}^{15}N/{}^{14}N)/({}^{15}N/{}^{14}N + 1) \qquad (A2)$$

$$\text{Atom ratio for } {}^{14}N = 1 - \text{Atom ratio for } {}^{15}N \qquad (A3)$$

3. Use the atom abundance ratios of the reference gas (calculated in step 2) to calculate the expected molecular abundance ratios of raw areas. As this step involves the conversion from elemental to molecular ratios, the fact that the probability of ^{15}N in the N_2 molecule is twice needs to be accounted for (i.e., $^{15}N^{14}N$ or $^{14}N^{15}N$). Calculate for each molecule, i.e., 29/28, 30/28, and 30/29.

$$\text{Molecular abundance ratio, e.g., for } N_2\ 29/28 \text{ molecule} = (2 \times {}^{15}N \times {}^{14}N)/({}^{14}N \times {}^{14}N) \quad (A4)$$

4. Compare the expected molecular mass ratio of the reference gas (as calculated in step 3) to the measured molecular mass ratio (reference gas peak areas as measured by the instrument) to calculate the correction factor to be applied to the sample peaks corresponding to each molecule, i.e., 29/28, 30/28, and 30/29. The (element) delta of the reference gas versus AIR is defined (as in step 1) and the expected molecular mass ratio is subsequently calculated (as in step 3). The measured ratio of the reference gas must agree with the defined ratio as calculated from the given delta value. This allows stability and independency from system uncertainties thus giving higher precision.

$$\text{Correction factor for each molecular mass ratio} = \text{calculated molecular mass ratio of reference gas} / \text{measured molecular mass ratio of reference gas} \quad (A5)$$

5. Apply the correction factor (as calculated in step 4) to the sample and reference gas molecular mass ratios measured and calculated by the instrument from the molecular mass raw area (conduct for each molecule 29/28, 30/28, and 30/29). This value will be used in step 8.

$$\text{True molecular mass ratio (area)} = \text{measured molecular mass ratio (area)} \times \text{correction factor} \quad (A6)$$

6. Convert measured molecular mass ratio to raw molecular delta (vs. reference gas) using Eq. A1. Conduct for both sample and reference gas peaks for each molecule. R_{sample} = sample molecular mass ratio raw area and $R_{reference}$ = reference gas molecular mass ratio raw area.
7. Convert raw molecular delta of sample versus reference gas to sample molecular delta versus AIR.

$$\delta_3 = \delta_1 + \delta_2 + (\delta_1 \times \delta_2)/1000 \quad (A7)$$

$\delta_1 = \delta_{sample}$ = molecular delta of sample versus reference gas (calculated in step 6); $\delta_2 = \delta_{reference}$ = molecular delta of reference gas versus AIR. Use Eq. A1 to calculate the expected reference gas molecular delta versus AIR. In Eq. (A1), R_{sample} = expected molecular mass ratio of reference gas calculated in step 3 and $R_{reference}$ = raw molecular mass ratio of AIR (known data from AIR standard); δ_3 = unknown sample molecular delta versus AIR.

8. Convert true molecular mass ratio of sample (as calculated in step 5) to sample atom abundance for ^{15}N and ^{14}N using Eq. A8. NB: +2 due to conversion from a molecular ratio to elemental ratio.

$$\text{Atom } {}^{15}N = R_{29/28}/(R_{29/28} + 2) \quad (A8)$$

9. Convert atom abundance of sample (calculated in step 8) to isotope (element) ratio.

$$\text{Isotope ratio} = \text{Atom}{}^{15}N/\text{Atom}{}^{14}N \quad (A9)$$

10. Convert isotope ratio (calculated in step 9) to element delta versus AIR using Eq. A1. In Eq. (A1), R_{sample} = calculated in step 9 and $R_{reference}$ = ratio of primary AIR standard (i.e., 0.00367820).
11. Convert element delta (calculated in step 10) to atom % (using Eqs. A1 and A2).

$$\delta(‰) = (R_{sample}/\ R_{reference} - 1) \times 1000‰ \quad (A1)$$

Eq. A1 rearranged, $R_{sample} = R_{reference}\ (\delta/1000 + 1)$ for substitution into Eq. A2.

$$\text{Atom \% for } {}^{15}N = ({}^{15}N/{}^{14}N)/({}^{15}N/{}^{14}N + 1) \times 100\% \quad (A2)$$

$R_{sample} = {}^{15}N/{}^{14}N = R_{reference}\ \ (\delta/1000 + 1)$; δ = element delta of sample calculated in step 10; $R_{reference}$ = ratio of primary AIR standard (i.e., 0.00367820).