British Beef Origin Project (Q01123)

STANDARD OPERATING PROCEDURE

Global Isotopic Measurement of Bulk Materials

Parameters measured: $\delta^{13}C$ and $\delta^{15}N$

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This version replaces all previous versions of this document. On receipt of this version, all previous versions should be discarded.

1. SCOPE AND FIELD OF APPLICATION

This method can be used to determine the 'global' or 'average' carbon 13/carbon 12 ($^{13}C/^{12}C$) and nitrogen 15/nitrogen 14 ($^{15}N/^{14}N$) ratio of bulk materials.

2. PRINCIPLE

Sufficient organic test material is placed in a tin capsule to provide approximately 100 µg and 100 µg of carbon and nitrogen respectively. The tin capsule is sealed and dropped into an elemental analyser reaction tube containing chromium oxide at 1000°C to 1050°C. The sample is quantitatively converted into carbon dioxide, nitrogen oxides and water. The combustion products pass into a second reactor at 600°C to 650°C that quantitatively coverts the nitrogen oxides to dinitrogen gas. Water is then removed from the carrier stream by a chemical trap containing drying agent and the carbon dioxide and nitrogen are separated on a packed GC column. The GC effluent then flows into the stable isotope ratio mass spectrometer via an interface and the ratio of the isotopomers of nitrogen and carbon dioxide are determined against nitrogen and carbon dioxide reference materials or gasses of known $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios versus accepted international standards.

3. SAFETY ASPECTS ASSOCIATED WITH THIS METHOD

A full assessment of the risk has been made according to the COSHH Regulations and a copy of the assessment can be found as an appendix to this document. All staff performing this method must follow the listed safety procedures at all times.

4. CHEMICALS

4.1	Chromium oxide	*or equivalent*
4.2	Silvered (cobalt or copper) oxide	*or equivalent*
4.3	Copper oxide wires	*or equivalent*
4.4	Copper wires, reduced	*or equivalent*
4.5	Magnesium perchlorate	*or equivalent*

5. APPARATUS

5.1 General

5.1.1	*Pressed tin solid sample capsule*	tin, diameter 5 mm, length 8 mm (or equivalent, appropriately sized capsule)
5.1.2	*Solid wall liquid sample capsule*	tin, diameter 2.0 mm, length 5.0 mm (or equivalent, appropriately sized capsule)
5.1.3	*Syringe*	total volume 0.5µl, plunger-in-needle (or equivalent)

5.2 Elemental analyser

5.2.1 *Elemental analyser*
5.2.2 *Carrier gas*
5.2.3 *Oxidant gas*

5.3 Stable Isotope Ratio Mass Spectrometry

5.3.1 *Stable Isotope Ratio Mass Spectrometer*

6. STANDARDS

6.1. Reference gas

The carbon dioxide and nitrogen reference gasses used by the Stable Isotope Ratio Mass Spectrometer, to measure the nitrogen and carbon isotope ratio (delta nitrogen 15 per mil, δ^{15}N‰ and delta carbon 13 per mil, δ^{13}C‰) of samples, is calibrated against accepted international standards, supplied by the International Atomic Energy Agency.

6.2 International Atomic Energy Association (IAEA) standard USGS 40

USGS 40 is a non-essential amino acid L-glutamic acid, supplied by the International Atomic Energy Association, with an accepted δ^{13}C‰ value of -26.2‰ versus Pee Dee Belemnite and δ^{15}N‰ value of -4.5‰ versus AIR.

6.3 Collagen In-House-Reference Material (COL IHR)

This material has been analysed by TRACE participant stable isotope laboratories and has a nominal δ^{13}C‰ versus Pee Dee Belemnite of XX‰ and a δ^{15}N‰ value of YY‰ versus AIR.

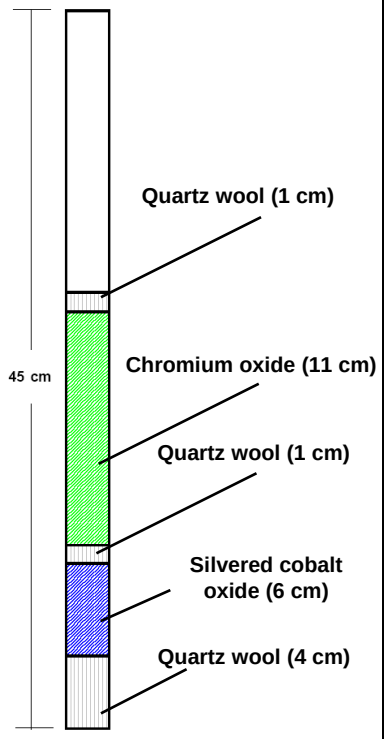
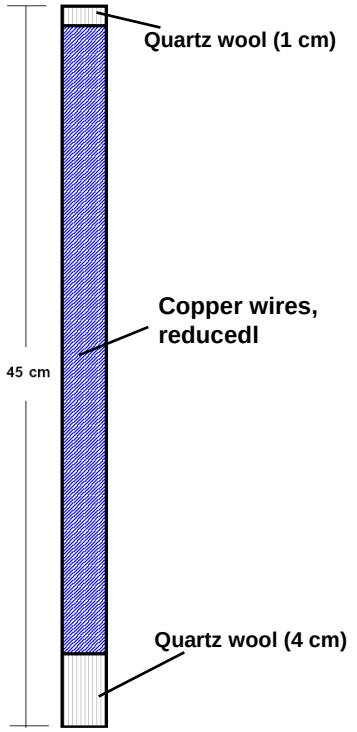
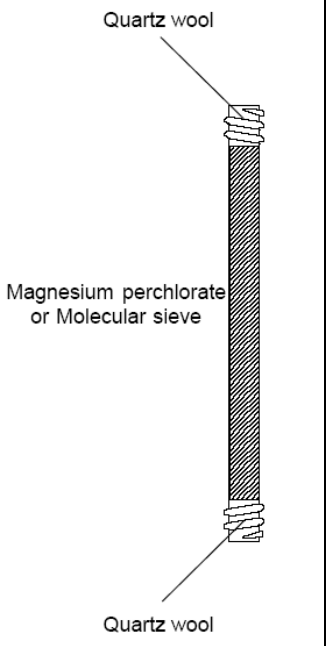
7. PROCEDURE

The information given in this section is for general guidance only. For example, It is accepted that the configuration of specific elemental anlaysers and gas isotope ratio mass spectrometers will vary according to manufacturer's requirements.

Simailarly the quantity of test material required to obtain reliable δ^{13}C‰ and δ^{15}N‰ results will vary according to the instrument manufacturer and the age of the instrument.

Consequently it is recognised that demonstration of and adherence to agreed Quality Assurance is essential.

7.1 Pepraration of combustion and reduction reactors

7.1.1 Combustion reactor	7.1.2 Reduction reactor	7.1.3 Moisture trap
		
See section 6 of the Costech ECS 4010 operation manual for further details		

7.2 Preparation of Reference Materials and samples for $\delta^{13}C$ and $\delta^{15}N$ analysis

Weigh accurately about 1 mg of solid test or reference material ± 0.2 mg, or sufficient to give an equivalent of 100 µg of nitrogen for $\delta^{15}N$ analysis or 100 µg of carbon for $\delta^{13}C$ analysis into a pressed tin capsule (5.1.1). Fold and seal the capsule with a pair of flat nosed tweezers.

7.3 Batch protocol

Blank capsules must be included in the batch of samples for analysis. A duplicate anlysis of the collagen In-house reference material (COL IHR) should be included every 20 samples. This standard is included after every 10th sample pair (as shown below). Sufficient standards should be located in the batch to permit reliable drift correction over the duration of the analysis. Batches consist of multiples of this standard/samples/standard sequence. Each batch must also include at least one duplicate measurement of

International Atomic Energy Association refernce material L-glutamic acid (USGS 40) (6.2).

1	Blank (empty tin capsule)
2	Blank (empty tin capsule)
3	USGS 40 reference material
4	COL In-house reference material
5	COL In-house reference material
6	sample 1a
7	sample 1b
8	sample 2a
9	sample 2b
10	sample 3a
11	sample 3b
12	sample 4a
13	sample 4b
14	Sample 5a
15	Sample 5b
16	Sample 6a
17	Sample 6b
18	Sample 7a
19	Sample 7b
20	Sample 8a
21	Sample 9a
22	Sample 9b
23	Sample 10a
24	Sample 10b
25	COL In-house reference material
26	COL In-house reference material
27	USGS 40 reference material
	etc.

8. DATA PROCESSING

The operating system of the IRMS system in will usually be equipped with the manufacturer's proprietory software for data proceesing (calculation of δ^{13}C‰, δ^{15}N‰,drift correction). Alternatively these parameters can be calculated or normalised manually by the operator.

9. QUALITY ASSURANCE ACCEPTANCE CRITERIA

9.1. Sample and reference material data

The absolute difference between duplicate measurements of the same sample or the same reference material (recorded as the absolute difference) should be less than or equal to 0.3‰ and this will be the action limit for repeating the duplicate analysis of the sample or reference material.

9.2 Collagen In-House-Reference-Material (COL IHR)

COL IHR $\delta^{13}C$ and $\delta^{15}N$ results be set at ± 2 standard deviations of the mean of replicate analyses of this reference material from the initial ring-test.

From the first ring-test analyses of COL:

$\delta^{13}C‰$ = XX, sd = XX
$\delta^{15}N‰$ = YY, sd = YY

Assuming that the COL IHR data will be normally distributed then at the 99.9 % confidence interval only one in one thousand results should fall outside ± 3 standard deviations of the mean value. In other words the COL IHR result should lie within a $\delta^{13}C$ value of xx‰ and XX‰ and a $\delta^{15}N$ value of yy‰ and YY‰. These will be the action limits for repeating samples within the batch.

9.3 IAEA primary standard USGS 40

Analysis of this material should give a value between a $\delta^{13}C$ value of xx‰ and XX‰ and a $\delta^{15}N$ value of yy‰ and YY‰. These will be the action limits for repeating samples within the batch.

APPENDIX 1 – Stable Isotope Reference Materials

Analyte: ^{2}H

Water	VSMOW	0 [$d^{2}H$]	1978	20 ml	490 (set-price)
	GISP	-189.5 [$d^{2}H$]	-	20 ml	
	SLAP	-428 [$d^{2}H$]	1978	20 ml	
Polyethylene	IAEA-CH-7	-100.3 [$d^{2}H$]	September 1995	3.6 g	150
Oil	NBS 22	-118.5 [$d^{2}H$]	September 1995	1 ml	150
Biotite	NBS 30	-65.7 [$d^{2}H$]	September 1995	1.8 g	150

Analyte: ^{18}O

Matrix	Product	Value	Date of Release	Size	Price [US$]
Water	VSMOW	0 [$d^{18}O$]	1978	20 ml	490 (set-price)
	GISP	-24.8 [$d^{18}O$]	-	20 ml	
	SLAP	-55.5 [$d^{18}O$]	1978	20 ml	
Benzoic Acid	IAEA-601	+23.3 [$d^{18}O$]	April 2004	0.5 g	150
Benzoic Acid	IAEA-602	+71.4 [$d^{18}O$]	April 2004	0.5 g	150

Analyte: ^{13}C

Matrix	Product	Value	Date of Release	Size	Price [US$]
Sucrose	IAEA-CH-6	-10.4 [$d^{13}C$]	September 1995	1.0 g	150
Polyethylene	IAEA-CH-7	-31.8 [$d^{13}C$]	September 1995	3.6 g	150
Oil	NBS 22	-29.7 [$d^{13}C$]	September 1995	1 ml	150
Cellulose	IAEA-C3	-24.9 [$d^{13}C$]	1991	50 g	150
Wood	IAEA-C4	-24 [$d^{13}C$]	1991	50 g	150
Wood	IAEA-C5	-25.5 [$d^{13}C$]	1991	50 g	150
Sucrose	IAEA-C6	-10.8 [$d^{13}C$]	1991	50 g	150
Oxalic Acid	IAEA-C7	-14.5 [$d^{13}C$]	June 1997	50 g	150
Oxalic Acid	IAEA-C8	-18.3 [$d^{13}C$]	June 1997	50 g	150
Caffeine	IAEA-600	-27.5 [$d^{13}C$]	2004	0.5 g	150
L-glutamic acid	USGS 40	-26.2 [$d^{13}C$]	February 2004	1 g	150
L-glutamic acid	USGS 41	+37.8 [$d^{13}C$]	February 2004	0.5 g	150
Cellulose	IAEA-CH-3	-24.5 [$d^{13}C$]	February 2004	0.5 g	150
Wood	IAEA-C9	-23.9 [$d^{13}C$]	-	50 g	150
BCR 657	Sugar	-10.76 ±0.04 [$d^{13}C$]		1g	

Analyte: ^{15}N

Matrix	Product	Value	Date of Release	Size	Price [US$]
Caffeine	IAEA-600	+1 [d^{15}N]	2004	0.5 g	150
L-glutamic acid	USGS 40	-4.5 [d^{15}N]	February 2004	1 g	150
L-glutamic acid	USGS 41	+47.6 [d^{15}N]	February 2004	0.5 g	150

APPENDIX 2 – Official isotopic methods

References	Samples	Fractions	Techniques	Isotopes
EC regulation 2676/90, annex 8		Ethanol	SNIF-NMR	$(D/H)_I$, $(D/H)_{II}$,R
EC regulation 440/ 2003, annex 2	Wine	Ethanol		C13
OIV resolution ENO 2/96 (EC regulation 822/97)		Water	IRMS	O18
AOAC Official method 995.17	Residual sugars	Ethanol (from fermentation)	SNIF-NMR	$(D/H)_I$, $(D/H)_{II}$,R
OIV resolution ENO 17/2001			IRMS	C13
ENV 12140 (CEN/TC174 N108)		Sugar (& ethanol)		C13
ENV 12141 (CEN/TC174 N109)	Fruit juice	Water	IRMS	O18
Analytica Chimica Acta 340 (1997) 21-29		Pulp		C13
AOAC method 991.41	Honey	Honey & proteins	IRMS	C13
J Sci Food Agric 1991, 56, 167-185	Amino acid		IRMS	C13, N15
Arrêté du 19/02/2001, JORF (general principles for GC-IRMS analysis) Ann. Fals. Exp. Chim., 92, N°946, pp 11-16 (method for vanilla)	Vanilla extract	Vanilla, pHB, Vanillic acid, pHB acid	GC-IRMS	C13