British Beef Origin Project (Q01123)

STANDARD OPERATING PROCEDURE

Global Isotopic Measurement of Bulk Materials

Parameters measured: $\delta^{2}H$ and $\delta^{18}O$

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Created by: Dr Simon Kelly, Institute of Food Research, Norwich Research Park, Colney, Norwich, NR4 7UA.

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' deuterium/hydrogen ($^{2}H/^{1}H$) and oxygen-18/oxygen-16 ($^{18}O/^{16}O$) ratio of bulk materials.

2. PRINCIPLE

Sufficient organic test material is placed in a tin or silver capsule to provide approximately 100 µg and 100 µg of hydrogen and oxygen respectively. Typically the capsule is sealed and dropped into a high temperature elemental analyser reaction tube, lined with glassy carbon and containg glassy carbon chips as a support material. Depending on the system in use the furnace will be maintained between 1260°C to 1450°C. Depending on the elemental composition the sample is converted into hydrogen, carbon monoxide, nitrogen, nitrogen monoxide and elemental carbon. The pyrolysis products pass a packed GC column that separates hydrogen and carbon monoxide gas. The GC effluent then flows into the stable isotope ratio mass spectrometer via an interface and the ratio of the isotopomers of hydrogen and carbon monoxide are determined against reference materials or gasses of known $^{2}H/^{1}H$ and $^{18}O/^{16}O$ 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	Glassy carbon	*or equivalent*
4.2	Nickelised carbon	*or equivalent*
4.3	Nickel wool	*or equivalent*
4.4	Carbon powder	*or equivalent*
4.5	Carbon wool	*or equivalent*

5. APPARATUS

5.1 General

5.1.1	*Pressed tin solid sample capsule*	Tin or silver, diameter 5 mm, length 8 mm (or equivalent, appropriately sized capsule)
5.1.2	*Solid wall liquid sample capsule*	Tin or silver, 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 helium*

5.3 Stable Isotope Ratio Mass Spectrometry

5.3.1 *Stable Isotope Ratio Mass Spectrometer capable of measuring $^{2}H/^{1}H$ ratios in a helium carrier strea*

6. STANDARDS

6.1. Reference gas

The hydrogen and carbon monoxide reference gasses used by the Stable Isotope Ratio Mass Spectrometer, to measure the hydrogen and oxygen isotope ratio (delta deuterium per mil, δ^2H‰ and delta oxygen-18 per mil, δ^{18}O‰) of samples, is calibrated against accepted international standards, supplied by the International Atomic Energy Agency (IAEA).

6.2 International Atomic Energy Association (IAEA-601)

MBS22 is a mineral oil supplied by the International Atomic Energy Association, with an accepted δ^2H‰ value of -118.5‰ versus Vienna-Standard Mean Ocean Water (V-SMOW).

6.3 International Atomic Energy Association (IAEA) standard NBS 22

IAEA-601 is benzoic acid, supplied by the International Atomic Energy Association, with an accepted δ^{18}O‰ value of +23.3‰ versus Vienna-Standard Mean Ocean Water (V-SMOW).

6.3 Casein In-House-Reference Material (CAS IHR)

This material has been analysed in a previous EU project by gas isotope laboratories participating in TRACE. The material has an assigned δ^2H‰ value of -113.0‰ versus Vienna-Standard Mean Ocean Water (V-SMOW).

6.4 Collagen Inter-Comparison Material (COL ICM)

This is a highly processed porcine collagen material. It has been characterised by the gas isotope laboratories participating in TRACE. After comparative equilibration and nomalisation with the casein IHR (6.3) the material should have a δ^2H‰ value of -62.6 +/- 6.0‰ versus Vienna-Standard Mean Ocean Water (V-SMOW).

7. PROCEDURE

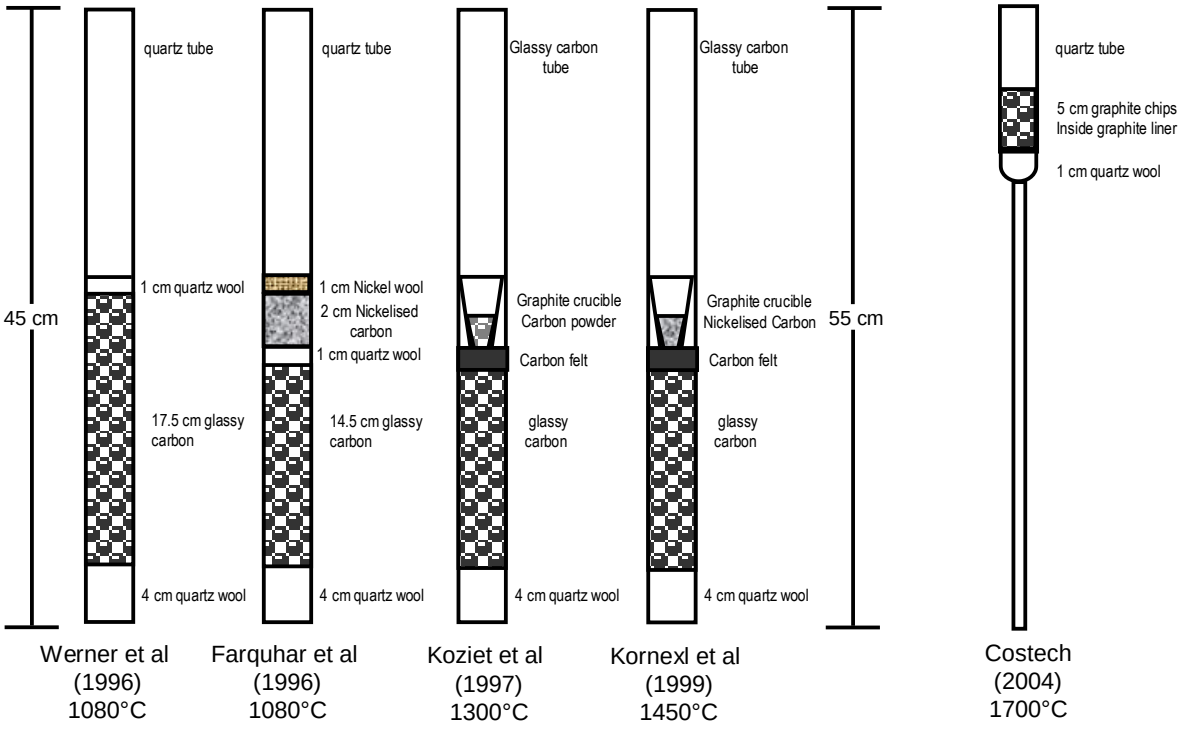
The information given in this section is for general guidance only. For example, It is accepted that the configuration of specific elemental anlaysers and gas isotope ratio mass spectrometers will vary according to manufacturer's requirements.

Simailarly the quantity of test material required to obtain reliable δ^{2}H‰ and δ^{18}O‰ 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 reactors tubes

There are a number of acceptable reactor tube configurations that can be used to pyrolyse organic samples. Tempeartures may also vary depending on instrument manufacturer. It is not possible to specify one set of conditions because there will be temperature and configuration restraints specific to each manufacturer. Some of reactor configurations and temperatures are summarised below with the corresponding literature reference.



7.2 Preparation of Reference Materials and samples for δ^2H and δ^{18}O 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 hydrogen for δ^2H analysis or 100 µg of oxygen for δ^{18}O analysis into a pressed capsule (5.1.1). Fold and seal the capsule with a pair of flat nosed tweezers. Prepare samples and reference materials in triplicate.

7.3 Comparative equilibration of protein samples for δ^2H‰ analysis

Hydrogen stable isotope analysis of proteins has to take into account that about 17 % of the total hydrogen present is exchangeable. This requires

specific methods to account for the variability of hydrogen isotopic ratios from labile positions depending on the hydrogen isotopic ratio of ambient air humidity. This problem can be solved using the recently described "comparative equilibration technique"[1]. After the sample and reference capsules have been weighed (7.2) they are left unsealed in a covered sample tray in ambient lab air for 96 hours. The capsules are then sealed and analysed as normal.

7.4 Batch protocol

The inclusion of specific calibrants and in-house reference materials for each type of sample is provided in the Table at Appendix 1.

For example, the δ^2H‰ analysis of a batch of lamb protein fractions:

Blank capsules must be included. A triplicate casein in-house reference must be included every 15 analyses to normalise the effects exchangeable hydrogen in protein samples. A triplicate anlysis of the collagen inter-comparison material (COL ICM) should be included every 20 samples to ensure the comparative equilibration has worked correctly. 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 (shown below). Each batch must also include at least one triplicate measurement of International Atomic Energy Association refernce material e.g NBS22 (6.3) every 2nd batch..

1	Dummy (conditioning sample)
2	blank (empty tin capsule)
3	blank (empty tin capsule)
4	casein IHR
5	casein IHR
6	casein IHR
7	sample 1a
8	sample 1b
9	sample 1c
10	sample 2a
11	sample 2b
12	sample 2c
13	collagen ICM

[1] Wassenar LI, Hobson KA (2003). Comparative equilibration and online technique for determination of non-exchangeable hydrogen of keratins for use in animal migration studies. Isotopes Environm. Health Stud. 39 (3), 211-217.

14	collagen ICM
15	collagen ICM
16	sample 3a
17	sample 3b
18	sample 3c
19	sample 4a
20	sample 4b
21	sample 4c
22	casein IHR
23	casein IHR
24	casein IHR
25	sample 5a
26	sample 5b
27	sample 5c
	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^2H‰, δ^{18}O‰ and drift correction). Alternatively these parameters can be calculated or normalised manually by the operator.

8.1 H_3^+ correction

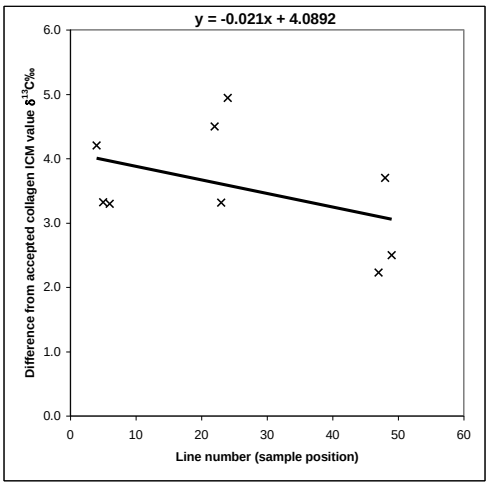
The presence of hydrogen gas in the electron impact source of the mass spectrometer causes the formation of H_3^+ (m/z 3), which is a direct isobaric interference with minor beam measuring species $^2H^1H^+$ (m/z 3). The formation of H_3^+ is dependent upon the pressure of hydrogen in the source and consequently sample size. The IRMS manafacturer's proprietary software will usually be equipped with a facility to calibrate and compensate for the effect of for H_3^+ formation. This correction must be applied to all reported data.

8.2 Correction for protein exchangeable hydrogen

The measured δ^2H‰ of the protein samples should be corrected according to the difference between the measured δ^2H‰ (H3+ corrected) values of the collagen ICM working standard and its accepted values of -113.0‰ V-SMOW. In the example below the accepted δ^2H‰ of the collagen ICM is subtracted from the measured δ^2H‰ values across the analytical batch. These 'correction factors' are then plotted against corresponding positions (line numbers) at which the collagen ICMs were measured in the analytical batch. A correction for each of the 15 samples between consecutive collagen ICMs is then calculated by substituting the sample position (line number) into the equation of the regression line. The mean of the three δ^2H‰ corrected values is then calculated and the sample standard deviation (sn-1) of the results reported.

Line	Identifier 1	H_3^+ corrected $\delta^2H‰_{V\text{-}SMOW}$	Correction factor	Corrected $\delta^2H‰_{V\text{-}SMOW}$	mean $\delta^2H‰_{V\text{-}SMOW}$	standard deviation	n
1	Dummy						
2	Blank						
3	Blank						
4	Casein IHR	-108.8	3.9	-112.7	-113.3	0.5	3
5	Casein IHR	-109.7	3.9	-113.6			
6	Casein IHR	-109.7	3.9	-113.6			
7	sample 1a	-78.3	3.9	-82.1	-82.3	0.5	3
8	sample 1b	-79.0	3.9	-82.8			
9	sample 1c	-78.1	3.8	-81.9			
10	sample 2a	-78.2	3.8	-82.0	-84.5	2.2	3
11	sample 2b	-82.2	3.8	-86.0			
12	sample 2b	-81.8	3.8	-85.6			
13	collagen ICM	-62.1	3.8	-65.9	-63.9	1.8	3
14	collagen ICM	-58.7	3.7	-62.5			
15	collagen ICM	-59.6	3.7	-63.3			
16	sample 3a	-80.3	3.7	-83.9	-85.1	2.2	3
17	sample 3b	-83.9	3.7	-87.6			
18	sample 3c	-80.0	3.6	-83.6			
19	sample 4a	-80.7	3.6	-84.3	-83.3	1.7	3
20	sample 4b	-77.7	3.6	-81.3			
21	sample 4c	-80.7	3.6	-84.3			
22	Casein IHR	-108.5	3.6	-112.1	-112.3	0.8	3
23	Casein IHR	-109.7	3.5	-113.2			
24	Casein IHR	-108.1	3.5	-111.6			

Linear interploation →



9. QUALITY ASSURANCE ACCEPTANCE CRITERIA

9.1. Sample and reference material data

The standard deviation of triplicate $\delta^2H‰$ measurements of the same sample or the same reference material (recorded as $\sigma n\text{-}1$) should be less than or equal to 3.0‰ and this will be the action limit for repeating the triplicate analysis of the sample or reference material.

The standard deviation of triplicate $\delta^{18}O‰$ measurements of the same sample or the same reference material (recorded as $\sigma n\text{-}1$) should be less than or equal to 0.8‰ and this will be the action limit for repeating the triplicate analysis of the sample or reference material.

9.2 Collagen Inter-compaison Material (COL ICM)

COL ICM $\delta^2H‰$ results are set at ± 1 standard deviation of the mean of replicate analyses of this reference material from the initial ring-test.

From the first ring-test analyses of COL ICM:

$\delta^2H‰$ -62.6‰, sd = 6.0‰

After comparartive equilibration (7.3) and normalisation (8.2) with the casein IHR, these will be the action limits for repeating samples within the batch.

9.3 IAEA primary standards

Analysis of NBS22 should give a $\delta^2H‰$ value between -116‰ and -120‰. These will be the action limits for repeating samples within the batch. Analysis of IAEA-601 should give a $\delta^{18}O‰$ value between +22.3‰ and +25.3‰. These will be the action limits for repeating samples within the batch.

APPENDIX 1 – List of calibrants and in-house reference materials for δ^2H‰ & $\delta^{18}O$‰ analysis

Commodity	Fraction	IRMS Measurement	Routine (IHRM) calibrant [interspersed in batch]	Secondary IHRM [at least triplicate per batch]	Intermittent QC [and frequency]
Honey	Protein	δ^2H‰	Calibrated reference gas and/or Casein IHRM	TRACE Collagen ICM	NBS22 [triplicate every 2nd batch at least for the first year]
Wheat	Defatted fraction	$\delta^{34}S$‰	Calibrated reference gas and/or Lab IHRM	Casein IHRM	NBS127 [triplicate every 4th batch]
Olive oil	Bulk	δ^2H‰	Calibrated reference gas and/or Lab IHRM	TRACE olive oil ICM	NBS22 [triplicate every 2nd batch at least for the first year]
Olive oil	Bulk	$\delta^{18}O$‰	Calibrated reference gas and/or Lab IHRM	TRACE olive oil ICM??	IAEA-CH-6 [triplicate every 2nd batch]
Lamb	Protein fraction	δ^2H‰	Calibrated reference gas and/or Casein IHRM	TRACE Collagen ICM & Freeze-dried lamb ICM	NBS22 [triplicate every 2nd batch at least for the first year]
Lamb	Lipid fraction	δ^2H‰	Calibrated reference gas and/or Lab IHRM	TRACE olive oil ICM	NBS22 [triplicate every 2nd batch at least for the first year]