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🌐 Case - Heavy Water Assays by GC-mass spectrometry

Henri Brunengraber¹

¹Case Western Reserve University

Mouse Metabolic Phenotyping Centers

Metabolomics Protocols & Workflows

Lili Liang

ABSTRACT

Summary:

Heavy water can be used as a tracer for estimating metabolic rates (*in vivo*, *in vitro*) such as total energy expenditure (TEE) and fractional synthesis rates (FSR; eg. protein, lipids, triglycerides). When using both ²H₂O and H₂¹⁸O (DLW), TEE is estimated from the total production of CO₂ as measured by the differences in decay rates of labeled the ¹⁸O and ²H in body water over time following a single bolus of DLW (1). ²H₂O can be used to estimate fractional synthesis rates of metabolic reactions such as those associated with proteins, lipids, triglycerides, and cholesterol (2,4).

References:

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Protocol status: Working

We use this protocol and it's working

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MATERIALS

MATERIALS

Deuterium oxide 2H2O (99 atom % excess); Sigma Aldrich

H2 18O (95% atom excess) Isotec

Reagents and Materials:

Reagent/Material	Quantity Required	Vendor
Deuterium oxide, $^2\text{H}_2\text{O}$ (99 atom % excess);		Sigma-Aldrich
NaOH	2 μL	stock
H_2^{18}O (95% atom excess)		Isotec (Miamisburg, OH)
acetone/acetonitrile solution	4 μL	stock
Chloroform	200- 500 μL	stock
Phosphorus Pentachlorate; TMP*	3 mg	stock
Diethyl ether	120 μl	stock

Analysis of $^2\text{H}_2\text{O}$ and H_2^{18}O : ^2H labeling of body water is determined by exchange with acetone and the ^{18}O labeling of body water is determined by conversion to trimethyl phosphate *(TMP; generated by reacting phosphoric acid with diazomethane).

Note:
Sigma-Aldrich,[RRID:SCR_008988](#)

- 1
1. Quantitation by $^2\text{H}_2\text{O}$ Standard Curve:
 2. Standards are made from deuterium oxide (Aldrich 617385)
 3. Pipette 10 μl of plasma or standard into Eppendorf
 4. $^2\text{H}_2\text{O}$ standards
 - a. Blank (MQ H_2O)
 - b. 0.1% D_2O in (MQ H_2O)
 - c. 0.5% D_2O in (MQ H_2O)
 - d. 0.10% D_2O in (MQ H_2O)
 - e. 1.5% D_2O in (MQ H_2O)
 - f. 2.0% D_2O in (MQ H_2O)

- g. 2.5% D₂O in (MQ H₂O)
- h. 3.0% D₂O in (MQ H₂O)
- i. 3.5% D₂O in (MQ H₂O)
- j. 4.0% D₂O in (MQ H₂O)

5. Add 2µl of a 10N NaOH solution to each sample or standard
6. Add 4µl of acetone/acetonitrile solution (10µl of acetone +200µl of acetonitrile) to each sample
7. Be careful when taking samples out of the centrifuge to make sure that all of the drops are at the bottom of the tube
8. Cap and briefly centrifuge samples (~5sec) to ensure NaOH and acetonitrile react with sample
9. Let samples sit overnight (at least 10hrs)
10. Add 500µl of chloroform to samples
11. Add ~ 50mg Na₂SO₄ salt to each sample
12. Centrifuge sample for 2 minutes
13. Pipette 100µl of chloroform layer into glass insert, place inserts in GC vials and cap, then assay on a GC-MS system, EI mode (see below for parameters and references).

2 For doubly labelled water studies, such as total energy expenditure; TEE (1,4):

1. H₂¹⁸O standards:
 - a. Blank (MQ H₂O)
 - b. 0.01% H₂¹⁸O in (MQ H₂O)
 - c. 0.05% H₂¹⁸O in (MQ H₂O)
 - d. 0.10% H₂¹⁸O in (MQ H₂O)
 - e. 0.15% H₂¹⁸O in (MQ H₂O)
 - f. 0.20% H₂¹⁸O in (MQ H₂O)
 - g. 0.25% H₂¹⁸O in (MQ H₂O)
 - h. 0.30% H₂¹⁸O in (MQ H₂O)
 - i. 0.35% H₂¹⁸O in (MQ H₂O)
 - j. 0.40% H₂¹⁸O in (MQ H₂O)
2. H₂¹⁸O assay: 5 ul of blood or plasma sample or standard into a 12 x 75-mm glass tube

3. Then add ~3 mg of PCl_5 to samples or standards to generate phosphoric acid; let stand for 20 min
4. To generate TMP, then react samples or standards by adding 300 μl of freshly prepared ethereal-diazomethane (to derivatize samples) and allow to stand at room temperature during reaction, until the ether evaporated; may use an additional 120 μl of diethyl ether in hexane solution
5. TMP is extracted by addition of 150 μl of water and 600 μl of chloroform (1:4 ratio) followed by addition of 0.5 g Na_2SO_4
6. Samples are then vigorously mixed, and a small aliquot of the chloroform is transferred to a GC-MS vial and assayed on GC-MS system, EI mode

Gas Chromatography Mass Spectrometry, GC-MS (EI mode): Acetone and the TMP derivatives are analyzed using an Agilent 5973N-MSD equipped with an Agilent 6890 GC system, and a DB-17MS capillary column (30 m x 0.25 mm x 0.25 μm). The mass spectrometer is operated in the electron impact mode (EI; 70 eV), (1,4).

7. Selective ion monitoring of mass-to-charge ratios (m/z)
 - a. For ^2H enrichments monitor acetone (58, 59) and for ^{18}O enrichments monitor TMP (140,142)
 - b. The ^2H enrichments are calculated from the $^2\text{H}_2\text{O}$ standard curve and the ^{18}O enrichments are calculated from the signal ratio $(142)/(142 + 140)$.