DETERMINATION OF ^2H_2O AT VERY LOW ENRICHMENT BY GAS CHROMATOGRAPHY MASS SPECTROMETRY FOR MEASURING BODY WATER SPACE

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Deuterated water (^2H_2O) is used to measure total body water space for estimation of the total body protein and fat in sheep. In measurements, a few grams of ^2H_2O is injected intramuscularly and after equilibrium a blood sample is taken to assay ^2H-enrichment. The enrichment in plasma is usually as low as 100 ppm. This is traditionally analysed by isotope ratio of mass spectrometry (IRMS), but the analysis is labour intensive and expensive. A novel and economical method using gas chromatography mass spectrometry (GCMS) has recently been developed to determine ^2H-enrichment as low as 80-500 ppm (Yang *et al*. 1998). The method is based on analysis of ^2H-enrichment in $^{13}C_3$-acetone after isotopic exchange of protons in water with hydrogens adjacent to the carbonyl group of acetone. The approach was modified in our laboratory for analysis of low ^2H-enrichment in sheep plasma.

Blood samples from sheep (about 45 kg) were collected before and 6 h after injection of 3 g ^2H_2O (99.9% atom %. Sigma-Aldrich, USA). ^2H-enrichment in plasma was determined using IRMS as described by Turner and Gailitis (1988). Sample preparation for GCMS analysis followed the method described by Yang *et al*. (1998) except that dichloromethane was used to extract acetone to improve the chromatography of acetone. The analyses were carried out on a Shimadzu QP5050A GCMS system equipped with an Alltech AT-WAX Heliflex capillary column (30 m × 0.25 mm i.d., 0.5 μm film thickness). The carrier gas was helium and the column flow rate was 1.1 ml/min. The GC injector temperature was set at 250°C, and the interface temperature at 240°C. The column temperature program was 2.5 min at 45°C, increased by 60°C/min to 225°C, and followed by 2 min at 225°C. Acetone was eluted at 2.75 min. Samples were analysed at 70 eV under chemical ionization with methane as the reagent gas. The selected ions monitored were *m/z* 61 and 62 with the carrier gas split ratio set at 25, or *m/z* 61 and 63 with the split ratio set at 7. The ratio of *m/z* 61 to 62 was found to be constant at 0.0731 (SE 0.0053) and therefore, it was multiplied with measured *m/z* 63 : 61 ratio to get *m/z* 63 : 62 ratio. The calibration curve was obtained by a linear regression analysis ($r^2 = 0.999$, n = 5) of theoretical ^2H-enrichment in standards (ranged from 50 to 250 ppm) against measured *m/z* 63 : 62 ratios. Samples were injected in triplicates.

Analyses of the samples showed that ^2H-enrichment measured by GCMS was not different from that by IRMS (Table 1. P = 0.3, paired t-test). With chemical ionisation the molecular ion of acetone was obtained and the sensitivity of GCMS analysis was significantly improved. A low enrichment of 50 ppm could be detected with a high reproducibility (CV = 0.7%). The method provides an alternative and less costly approach to determine ^2H-enrichment for animal scientists to measure body water and so estimate whole body protein and fat compositions.

Table 1. The comparison between the GCMS and IRMS assays of the ^2H-enrichment (ppm) in sheep plasma

Sample No.	GCMS	CV (%) of GCMS assay	IRMS
704	105.3	3.3	108.0
125	100.4	2.3	107.0
100	100.8	1.1	100.3
78	100.8	0.5	100.2
68	96.8	0.5	96.4

The author is grateful to Dr Michael Tavendale, AgResearch, New Zealand for suggestions on GCMS analysis.

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