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Note

Gas chromatographic technique for combined measurement of hydrogen and methane using thermal conductivity detector

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Breath hydrogen and methane measurement is a useful diagnostic and research tool for studying bacterial activity in the colon[1]. Since it is believed that a katharometer detector (KD) is not sufficiently sensitive for methane measurement, the methods used for the gas chromatographic analysis of these gases employ different detectors for each gas, *i.e.* katharometer for hydrogen and flame-ionization detector (FID) for methane[2]. As a result of this and presumably due to lack of simultaneous access to both systems, most workers measure only one of the two gases. As a diagnostic tool, the value of measuring methane compared with hydrogen is at present minimal. However, recently one possible and important diagnostic use of methane has been suggested[3] and it is possible that others will emerge. A combined measurement using a single detection system would facilitate application and further investigation of these related gases. In this paper a method of detection of both gases in one breath sample using only a KD is described.

MATERIALS AND METHODS

Samples of alveolar air are collected in syringes via a modified Haldane–Priestly tube[4]. The sample of air is analysed using a gas–solid chromatograph (Pye Series 104) with molecular seive packed glass columns and a KD. The sample is injected by means of a sampling loop, after passage through a tube containing soda lime and silica gel (in series) to remove carbon dioxide and water, respectively. Values are displayed on a 1-mV pen recorder (Philips, PM-8000). Two known concentrations 0.9 μmole/l and 4.5 μmole/l of hydrogen and methane mixtures in nitrogen are used as standard gases (Rank Hilger, Kent, Great Britain). The standard gases are analysed each day during the processing of test samples, to check the sensitivity and stability of the instrument.

The effect of changing each chromatographic variable (*e.g.* detector temperature, bridge current, carrier gas) on sensitivity and peak resolution were determined by keeping other variables constant. Working conditions were chosen which both optimized the resolution and sensitivity for hydrogen and methane measurement and still remained within the limits of the manufacturer's recommendations.

The sensitivity of the system was assessed using standard gases at different concentrations and the recorded deflection plotted against concentration.

The analytical precision was evaluated in two ways. One by analysing 20 samples of each standard gas consecutively, the other by repeated analysis of 30 different breath samples with a wide range of hydrogen and methane concentrations.

RESULTS

Changing the carrier gas from argon to nitrogen (oxygen free) resulted in good resolution of the methane peak (peak resolution between oxygen and methane = 6). Increasing the detector temperature and bridge current, improved detector sensitivity to both gases particularly methane. A finer stationary phase improved the separation between hydrogen and oxygen peaks.

Optimum working conditions and chromatograms of 4.5 μmole/l standard gas and a sample of breath from a person with high hydrogen and methane excretion (1.2 and 1.6 μmole/l, respectively) are shown in Fig. 1. The retention time for hydrogen was about 65 sec and methane about 300 sec. Peak resolution between hydrogen and oxygen was 3.3 and that between oxygen and methane 6.0.

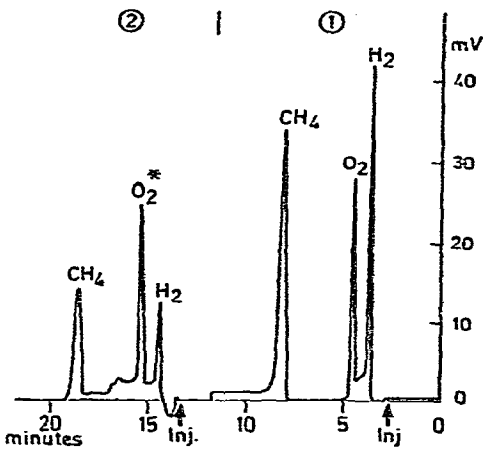

Fig. 1. Chromatograms (1), 4.5 μmole/l standard gas and (2), breath sample. Conditions: column, 2.6 m × 4.0 mm I.D. glass, packed with 60–85 mesh molecular sieve 5A; temperature, 50°; carrier gas, oxygen-free nitrogen at 65 ml/min; detector, katharometer, 140 mA bridge current, temperature 100°; chart speed, 5 mm/min; attenuation, set at 2 (× 500). * set at 50 (× 20).

A linear relationship with a slope of 0.49 for hydrogen and 0.36 for methane was observed when different concentrations of the gases were plotted against recorder deflection. Repeat analysis ($n = 20$) of 0.9 and 4.5 μmole/l hydrogen and methane standards showed a coefficient of variation of 4% for both gases, while different concentrations (range hydrogen, 0–6.8 μmole/l; methane, 0–4.5 μmole/l) of breath samples on different analytical batches ($n = 30$) showed a coefficient of variation of % for hydrogen and 7% for methane.

DISCUSSION

The essential difference in the method used here is the use of nitrogen (oxygen free) as a carrier gas. Most workers use either argon or helium. These gases are ideal for hydrogen measurement and are recommended for such purpose. However, if hydrogen and methane require to be simultaneously measured and if one of these gases is used as carrier, two detector systems (*i.e.* KD and FID) are needed. This is because of the large breath nitrogen peak which obliterates the methane peak. Using nitrogen as carrier gas the breath nitrogen peak is eliminated and good methane peak resolution is achieved. Also, nitrogen can be used at a higher bridge current than argon and this is an advantage for reasons described above.

As the chromatograms in Fig. 1 show, distinct and well resolved peaks are displayed. The elution time of about 6 min is comparable to the time taken to process a single breath sample for hydrogen alone, using the usual carrier gases[2]. The response of the instrument is linear for the range of concentrations usually encountered in practice (*i.e.* 0 to 4.5 μmol/l). The modified attachment to remove carbon dioxide and water from the samples helps to minimise the requirement for purging the stationary phase and gives less noisy peaks.

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