Journal of Chromatography, 572 (1991) 302–306
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6065

Short Communication

Trimethylsilyl-O-methyloxime derivatives for the measurement of [6,6-$^2H_2$]-D-glucose-enriched plasma samples by gas chromatography–mass spectrometry

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(First received July 10th, 1990; revised manuscript received July 9th, 1991)

ABSTRACT

A new method for the determination of the enrichment of [6,6-$^2H_2$]-D-glucose in human plasma by gas chromatography–mass spectrometry (GC–MS) is described. (2,3,4,5,6)-Pentakis-O-trimethylsilyl-O-methyloxime-D-glucose is used as a derivative for the GC measurement. Using GC–MS with electron-impact ionization, the enrichment is measured in the single-ion monitoring mode observing the masses *m/z* 319 and 321. In contrast to other methods the use of this glucose derivative reduced the amount of plasma needed from 200 to 10 μl and no chemical ionization equipment is needed for the mass spectrometer.

INTRODUCTION

Stable isotope-labelled glucose tracer infusions, such as [6,6-$^2H_2$]-D-glucose are frequently used to determine glucose turnover rates in humans [1,2]. In these experiments relatively low enrichments of plasma glucose with deuterated glucose have to be measured with high precision. Until recently, such measurements have involved gas chromatography–mass spectrometry (GC–MS) with electron-impact (EI) ionization, and D-glucofuranose cyclic 1,2:3,5-bis(butylboronate)-6-acetate or D-glucopyranose pentaacetate derivatives [2]. Alternatively, chemical ionization (CI) and glucose pentaacetate or glucose penta(trifluoracetate) [1] derivatives have been used. However, the frequently used mass-selective detectors are not equipped with CI, and the previously described methods using EI require relatively large plasma samples [2].

The present study aimed to evaluate the use of D-glucose (2,3,4,5,6)-pentakis-O-trimethylsilyl-O-methyloxime as a derivative for gas chromatography. This derivative has been employed to separate glucose from other monosaccharides and to determine the glucose concentration in serum [3,4]. Its mass spectrum has an ion of high intensity at *m/z* 319. The fragmentation pattern after EI, described by Björkhem *et al.* [3] and Pelletier and Cadieux [4], suggests that, during the

0378-4347/91/$03.50

analysis of [6,6-$^2H_2$]glucose, the glucose fragment m/z 319 still carries the deuterium label. Therefore, the pentakis-trimethylsilyl-O-methyloxime derivative should be suitable for the analysis of [6,6-$^2H_2$]glucose plasma enrichment.

EXPERIMENTAL

Reagents

Glucose, O-methylhydroxylamine hydrochloride, N,O-bis(trimethylsilyl)trifluoracetamide (BSTFA), pyridine, Dowex AG 50W-X8 and Dowex AG 1-X8 were purchased from Fluka (Buchs, Switzerland). [6,6-$^2H_2$]-D-glucose was purchased from Tracer Technologies (Somerville, MA, USA).

Sample preparation

A 10-μl sample of plasma was deproteinized with 100 μl of 5.5% $ZnSO_4$ solution and 100 μl of 4.73% $Ba(OH)_2$ solution. After centrifugation, the supernatant was placed on a ion-exchange column with Dowex AG 50W-X8 (100–200 mesh) cation-exchange resin, together with Dowex AG 1-X8 (100–200 mesh). A 1.5-ml volume of eluent was collected in a 2-ml vial and evaporated to dryness and 100 μl of a solution of 1% (w/v) O-methylhydroxylamine hydrochloride in pyridine were added to the residue. The vial was capped and heated to 80°C for 2 h. After cooling, 100 μl of BSTFA were added to the solution, and the mixture was heated to 80°C for 20 min. The derivatization solution was injected into the gas chromatograph.

Gas chromatography

The analysis was made with a Hewlett-Packard HP 5890/5970 GC–MS system. A fused-silica capillary column (12.5 m × 0.2 mm I.D.) coated with methylsilicone was used (purchased from Hewlett-Packard, Wohlen, Switzerland). The injector temeprature was held at 250°C and the transfer line temperature at 270°C. The carrier gas was helium at a column head-pressure of 75 kPa. A 1-μl volume of the derivatization solution was injected in the splitless injection mode. At injection, the oven temperature was 90°C. After 0.2 min the oven was heated at 20°C/min to 220°C and kept at this temperature for 2 min. The masses 319 and 321 were observed in the selected-ion monitoring (SIM) mode between 7.5 and 8.4 min.

Calculations

Molar percent excess (MPE) values were calculated by comparison of the peak ratios 321/319 with peak ratios of known enrichment.

Turnover studies

Twenty-seven healthy overnight-fasted volunteers gave their consent to participate. After obtaining three blood samples for determination of the background

enrichment in plasma glucose, a constant intravenous infusion of [6,6-$^2H_2$]glucose was started at a rate of 0.2 μmol/kg per min. After 2 h of equilibration, three blood samples were drawn to determine the enrichment (MPE) of deuterated glucose.

RESULTS

The mass spectra of unlabelled and of dideuterated glucose are shown in Fig. 1. The fragments at *m/z* 319 for unlabelled and at *m/z* 321 for dideuterated glucose are in good accordance with the fragmentation pattern reported by Björkhem *et al.* [3]. The fragment corresponds to carbons 3–6.

Fig. 2 shows the ion chromatograms for *m/z* 319 and 321 obtained in the SIM

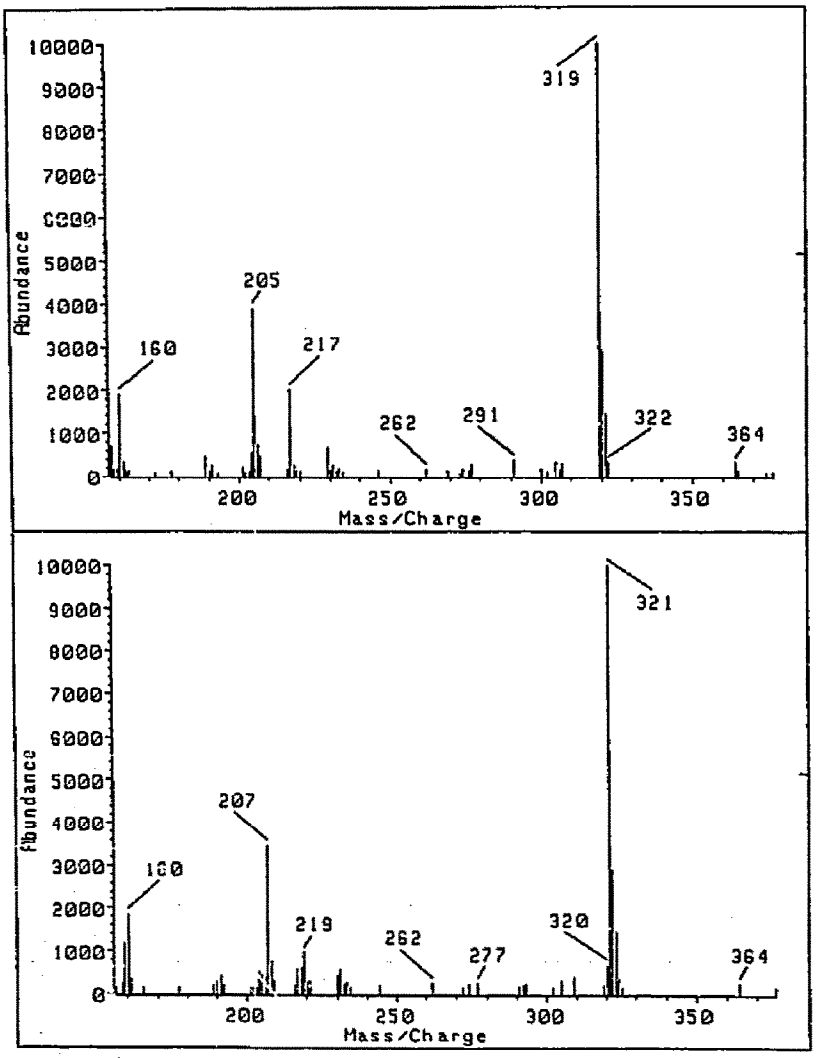

Fig. 1. Mass spectra of the pentakis-trimethylsilyl-O-methyloxime derivative of unlabelled and of 6,6-dideuterated glucose.

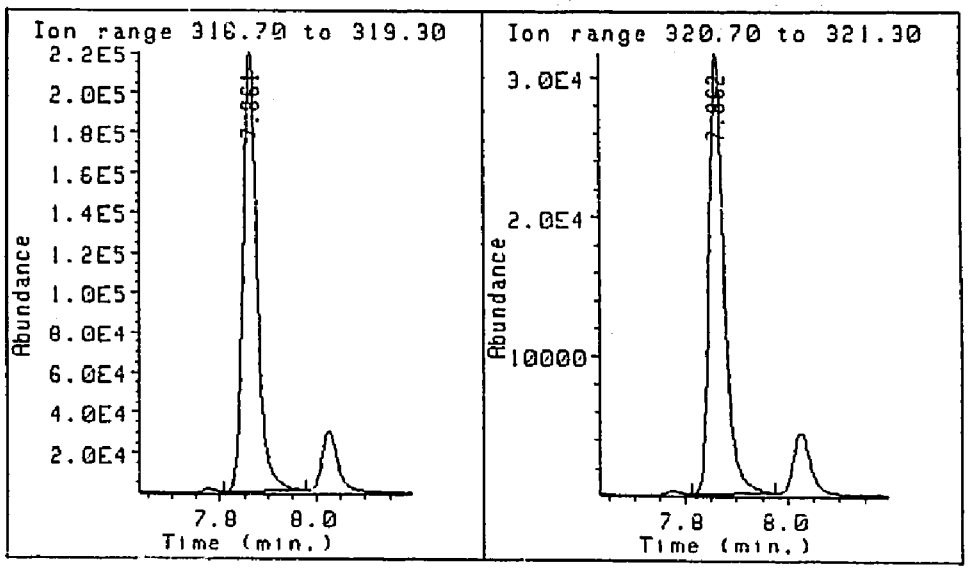

Fig. 2. Ion chromatograms at *m/z* 319 and 321 of the pentakis-trimethylsilyl-O-methyloxime derivative.

mode. The two peaks correspond to the *syn* and *anti* forms of the O-methyloxime derivative [3]. Only the first major peak was used in the analysis. A typical standard curve for the calculation of the MPE from the peak ratios is shown in Fig. 3. The mean (± S.D.) enrichment of dideuterated glucose in 27 volunteers receiving infusions of [6,6-$^2H_2$]glucose was 1.28 + 0.05% MPE. The coefficient of variation (S.D./mean × 100%) of the enrichment after ten measurements of the same sample was 8%.

DISCUSSION

The O-methyloxime derivative is a significant improvement in the determina-

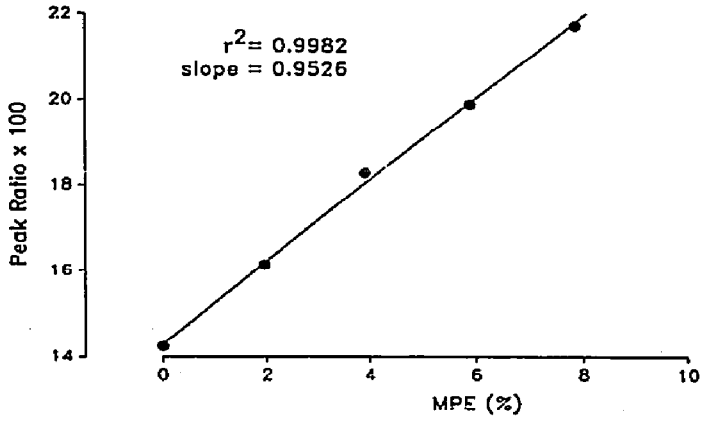

Fig. 3. Standard curve for the [6,6-$^2H_2$]glucose pentakis-trimethylsilyl-O-methyloxime derivative. The peak ratio 321/319 is plotted against the molar percent enrichment.

tion of the enrichment of deuterated plasma glucose. In the EI mode, the mass spectrum of the pentaacetoxy derivative [5] has a fragment at m/z 200 of low intensity. In contrast, the intensity of the fragment at m/z 319 of the methyloxime derivative is high. As a result, the amount of plasma can be reduced from 200 to 10 μl when the latter derivative is used. The formation of this derivative is no more difficult or time-consuming than that of the pentaacetoxy derivative, although it requires two steps compared with one step for the acetoxy derivative. Since the reaction mixture needs no working up between the two steps, and can be injected directly into the gas chromatograph, the synthesis of this derivative is not more laborious.

Other monosaccharides that may be present in the plasma have no influence on the measurements since the mass spectrum of the fructose derivative shows no fragment with m/z 319 [6] and the concentration of galactose in the plasma of healthy volunteers, determined gas chromatographically with this derivative, is *ca.* 0.1% of the glucose concentration [4].

Thus the methyloxime derivative is a suitable derivative for the determination of the enrichment of dideuterated glucose by GC–MS, particularly when the CI mode is not available.

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