Estimation of 3-O-methyl-D-glucose in the presence of glucose

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3-O-methyl-D-glucose (3OMG) has been widely used in studies of intestinal absorption (Williams, Mager, and Jacobson, 1963; Winawer, Broitman, Wolochow, Osborne, and Zamcheck, 1966). It is transported by the same mechanism as glucose but is not significantly metabolized. The amount recovered from urine after oral administration is, therefore, an index of intestinal glucose absorption (Fordtran, Clodi, Soergel, and Ingelfinger, 1962). Estimation of 3OMG in urine and body fluids is complicated by the presence of variable amounts of glucose. Previous workers have separately measured glucose using a glucose oxidase technique and total reducing substances or aldohexuronic acids. The difference is then taken to represent 3OMG (Himsworth, 1968). However, this is necessarily inaccurate, for with small concentrations of glucose measurement in urine is difficult, owing to the interfering substances present (Beach and Turner, 1958). With higher concentrations of glucose, these methods involve determination of a small difference between two similar quantities. This paper describes a more accurate method for measurement of 3OMG in the presence of glucose.

Materials

GLUCOSE OXIDASE REAGENT
Three ml Fermcozyme 653AM (Hughes and Hughes Enzymes Ltd) made up to 100 ml with the acetate buffer and stored at 4°C.

TRICHLOROACETIC ACID
Three g/100 ml in water.

O-TOLUIDINE COLOUR REAGENT (Hyvärinen and Nikkilä, 1962)
Sixty ml redistilled o-toluidine is added to a solution of 1·5 g thiourea in 940 ml glacial acetic acid and stored in a dark bottle.

3OMG STANDARD SOLUTIONS
These are of 1·0 g, 0·75 g, 0·5 g, and 0·25 g/100 ml

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3-O-methyl-D-glucopyranose (Koch-Light Laboratories Ltd) in a saturated solution of benzoic acid.

Method

Urine, 0·25 ml, with benzoic acid preservative, is incubated overnight at 37°C with 1·0 ml glucose oxidase reagent. A sample of 0·1 ml is added to 1·0 ml trichloroacetic acid solution. Precipitated protein is removed by centrifugation. O-Toluidine colour reagent, 4·5 ml, is added to 0·5 ml of the supernatant and the mixture placed in a boiling water bath for eight minutes. Optical density is measured at 630 μm after cooling. Standards and a blank of saturated benzoic acid solution are incubated and assayed in the same manner.

Results and Comment

The relationship between optical density and concentration of 3OMG is linear over the range studied (Fig. 1).

The method was evaluated by measuring recovery of 3OMG added to pooled human urine containing various concentrations of added glucose. Figure 2 shows the amount of 3OMG recovered from four concentrations of 3OMG in the presence of five different concentrations of glucose. Control experiments in the absence of added 3OMG or glucose are
Technical methods

LooI also shown. Removal of glucose from concentrations up to 5 g/100 ml is virtually complete. For example, recovery of 3OMG from a 1 g/100 ml solution was 101 ± 1% (mean ± SD) in the absence of glucose and 104 ± 3% in the presence of 5 g/100 ml glucose.

Following oxidation of glucose the procedure is identical with that of Hyvärinen and Nikkilä (1962) for aldosaccharides. Since glucose is the only aldosaccharide normally present in significant amounts in body fluids, the present method should estimate only 3OMG.

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References


Fig. 2 Recovery of 3-O-methyl-D-glucose (3OMG) (mean of 8± SD) in the presence of various concentrations of glucose.

○ No added 3OMG
■ 0.25 g/100 ml 3OMG added
□ 0.50 g/100 ml 3OMG added
▲ 0.75 g/100 ml 3OMG added
× 1.0 g/100 ml 3OMG added