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Microwave-Assisted Derivatization of Glucose and Galactose for Gas Chromatographic Determination in Human Plasma

To the Editor:

Monosaccharides are usually analyzed by use of automatic monitors or enzymatic immunoassays. However, gas chromatography (GC) is an accurate and precise technique for galactose quantification (1), and it is regarded as a reference method for glucose (2). GC procedures require a long derivatization time in 2 consecutive reactions of 60–90 min to generate the aldonitrile pentaacetate derivative. Fast derivatization techniques are often requested today because the bottleneck for sample

throughput has moved from analysis to sample preparation. Recently, we dramatically decreased the derivatization time for sugars (mono- and disaccharides) in GC analysis (3) by using trimethylsilyl-oxime derivatives. This derivative, however, gives 2 peaks in the chromatogram, which is acceptable for the glucose–fructose pair but not for the glucose–galactose pair.

In this study, we optimized the aldonitrile pentaacetate derivatization step, using microwave-assisted conditions to obtain single-peak derivatives for each sugar. Plasma from healthy adults was used as a model matrix for the recovery experiments.

Glucose and galactose were from Sigma. Plasma samples were prepared as follows: we deproteinized 200 μ L of plasma with 500 μ L of methanol. After centrifugation at 5000g, the supernatant was withdrawn and evaporated to dryness under a stream of nitrogen. We added 100 μ L of hydroxylamine hydrochloride (20 g/L in pyridine) to the vial, vortex-mixed it for 30 s, and then reacted the mixture for 2 min in a microwave oven (200 W, 25% of total exit power). The final step was to add 100 μ L of acetic anhydride and allow the reaction to proceed for 6 min. We directly injected 1 μ L (split ratio 1:25) into a chromatograph (Varian 3380) equipped with a BP-10 Column (SGE). The column temperature started at 120 $^{\circ}$ C for 1 min, then was increased to 280 at 10 $^{\circ}$ C/min rate. The injector and detector (flame ionization detection) were at 280 $^{\circ}$ C.

Optimal reaction times were 2 min for the oxime reaction and 6 min for the acetylation and aldonitrile formation.

Although galactose and glucose are enantiomers differing in only one optical center, the BP-10 column easily achieved the desired baseline separation (Fig. 1). Calibration curves constructed either with aqueous solutions or by standard additions to plasma showed no differences in slope.

Recovery, calculated with the aqueous calibrators, was 97%–101%

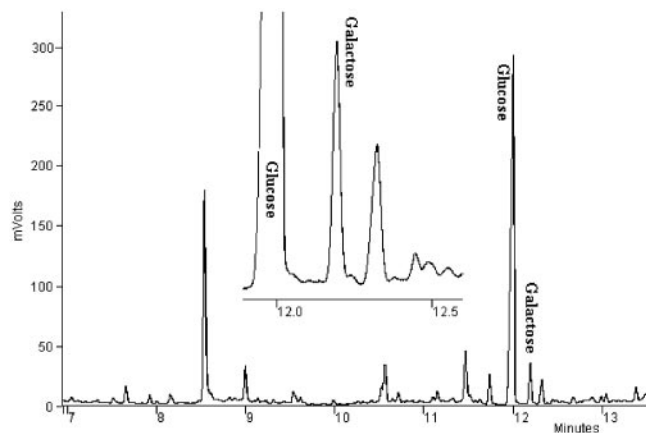


Fig. 1. Chromatogram of a plasma sample with 1 mmol/L glucose and 100 μ mol/L galactose added.

for glucose and 92%–104% for galactose. The CV of 5% ($n = 3$) for both sugars is attributable mainly to the irradiation step because of a lack of homogeneity in domestic ovens. The detection limit was 5 μ mol/L (signal-to-noise ratio = 3) for both sugars without modification of the split ratio.

This study shows that a simple microwave oven can be used to accelerate sample preparation in GC analysis of monosaccharides in plasma. Flame ionization detection enables easy measurement of glucose and can achieve the detection limits necessary for galactose. The present procedure is an alternative for laboratories that lack automated glucose analyzers and for others that do galactose screening with methods such as thin-layer chromatography (4, 5).

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Indirect Methods for Reference Intervals Based on Current Data

To the Editor:

Grossi et al. (1) recently reported an interesting project that used a sophisticated algorithm for the formulation of reference intervals based on ~15 000 000 records related to 197 350 individuals. We noted an important difference between their reference interval calculated for thyrotropin (TSH) based on results obtained with the Architect (Abbott) analyzer in women (0.28–4.45 mIU/L) and that recently reported by Kratsch et al. (2). Kratsch et al. selected a group of 870 blood donors with negative thyroid ultrasonography and thyroid autoantibodies, as recommended by criteria of the National Academy of Clinical Biochemistry, and found a reference interval of 0.4–3.77 mIU/L (2). The optimal serum TSH reference interval is strongly debated, and a lowering of

the upper reference limit is advocated by some authors (3). Furthermore, the algorithm used by Grossi et al. (1) cannot be implemented easily in most institutions because it requires considerable hardware and software resources and statistical expertise that are not commonly available. In our opinion, indirect methods are much simpler and more practical tools for the calculation of reference values or health-related limits (HRLs), especially when the fraction of pathologic values is not too high (4–6).

We retrieved the results of thyroid panels (which included measurement of anti-thyroid peroxidase antibodies) from the records of 15 359 female and 3862 male patients stored in our laboratory information system (LIS) over a 30-month period (January 1, 2003, to June 26, 2005). We calculated the Advia Centaur (Bayer) TSH HRL, using the program GraphROCTM (Fig. 1A), and obtained an upper limit of 3.7 mIU/L. As shown in Fig. 1B, the upper limit of the HRL did not change substantially after we removed the repeat tests (2893) and the results obtained in individuals positive for thyroid antibodies (7995).

This limit confirms a previous study carried out in 2000 with the same analyzer and the same software in 40 095 and 26 001 results retrieved from the LIS of the laboratories of Vicenza and Verona hospitals, without any selection criteria; the HRLs were 0.28–3.5 and 0.22–3.6 mIU/L, respectively, and the test result distribution appeared unimodal (7).

The 3.7 mIU/L limit is also consistent with those reported in 2 multicenter studies carried out in Spain [144 reference individuals (8)] and in the United Kingdom [303 individuals (9)] with the same analyzer; the reference intervals obtained in those studies were 0.43–3.69 (8) and 0.48–3.63 mIU/L (9), respectively. Finally, the 97.5th centile of TSH concentration reported by the National Health and Nutrition Examination Survey (NHANES) III in the decades between 20 and 60 years was between 3.56 and 3.82 mIU/L (10). In conclusion, the retrieval of results from the