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Note

Rapid determination of β-hydroxybutyric acid in blood and milk by gas chromatography*

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β-Hydroxybutyric acid, CH_3–CH(OH)–CH_2–COOH, is one of three "ketone bodies" (acetone and acetoacetate are the others) found in the blood (ketonemia) and urine (ketonuria) of diabetics or in starvation. The term ketosis describes the condition of an abnormally high level of ketone bodies in the blood.

Bovine ketosis usually occurs in high-producing dairy cows at the beginning of lactation. A ketotic cow not only has reduced milk production but also gives low-fat test milk[1,2].

There are various analytical procedures[3] available for quantitative determination of ketone bodies, but most of these suffer from interferences and lack of specificity. Enzymatic assay[4] for D(*l*)-β-hydroxybutyric acid requires a specific enzyme, special care in the control of pH, reduction of NAD^+ co-enzyme, and final determination of NADH. Gas chromatographic (GC) techniques are available for determining β-hydroxybutyric acid in urine[5] and incubator-reject eggs[6].

This paper presents a simple and rapid GC technique for the quantitative analysis of DL-β-hydroxybutyric acid in blood and milk.

EXPERIMENTAL

An Aerograph Hi-Fi Model 600 gas chromatograph equipped with a hydrogen generator (Aerograph Model 650), a gold-plated flame ionization detector and a Minneapolis-Honeywell recorder was used in this study.

DL-β-Hydroxybutyric acid (General Biochemicals, Chagrin Falls, Ohio, U.S.A.), determined as methyl ester derivative, was separated with a 6-ft. stainless-steel coiled column containing 20% diethylene glycol succinate (DEGS) and 5% isophthalic acid on 60–80 mesh, Chromosorb W AW. The injector temperature was 175° and the column temperature 150°. The flow-rate of the carrier gas (high-purity nitrogen) was set at 35 ml/min. The hydrogen flow-rate was 25 ml/min and the air flow-rate, 100 ml/min. The attenuator was set at 128.

Lyophilized samples of cow blood and milk were used as the starting material for this experiment. Samples of 0.1 to 0.5 g were introduced into 175 × 15 mm I.D. glass tubes with PTFE-lined screw-caps (VWR Scientific, Los Angeles, Calif., U.S.A.)

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and 5 ml of a 5% HCl–methanol solution added into each tube. The tubes were then sealed and the samples subjected to ultrasonic mixing for 10 min and thermal incubation at 60° for 2 h. Three-microliter sample aliquots at room temperature were used for injection into the chromatograph.

A series of DL-β-hydroxybutyric acid standards ranging from 20–600 mg% (General Biochemicals) were prepared as described above and injected into the chromatograph.

RESULTS AND DISCUSSION

The GC curves for the solvent, methyl DL-β-hydroxybutyric standards and the selected biological samples are shown in Fig. 1. Methyl esters of DL-β-hydroxybutyrate had a retention time of 7 min under the conditions of this experiment. Co-injections of esterified DL-β-hydroxybutyric acid standard and esterified liver, blood, or milk samples resulted in only one enlarged peak which appeared at the same retention time of the methyl DL-β-hydroxybutyrate standard.

The linear response for increasing concentrations of DL-β-hydroxybutyrate standards is shown in Fig. 2. Quantitative analysis of DL-β-hydroxybutyrate in test samples can be readily achieved by comparing samples against the standards analyzed under the same experimental conditions. An example of actual quantitation is illustrated in Fig. 1a. The instrumental detectability is 0.6 μg or 20 mg% methyl DL-β-hydroxybutyrate at an attenuation of 128. The sensitivity can be further increased by concentrating the sample solution or decreasing the attenuation.

A recovery study was performed by adding increasing amounts of DL-β-hydroxybutyrate (1–5 mg) to blood samples and measuring the increase in response due to these additions as compared to the response of unfortified samples. Recovery values for DL-β-hydroxybutyrate ranged from 96–104%.

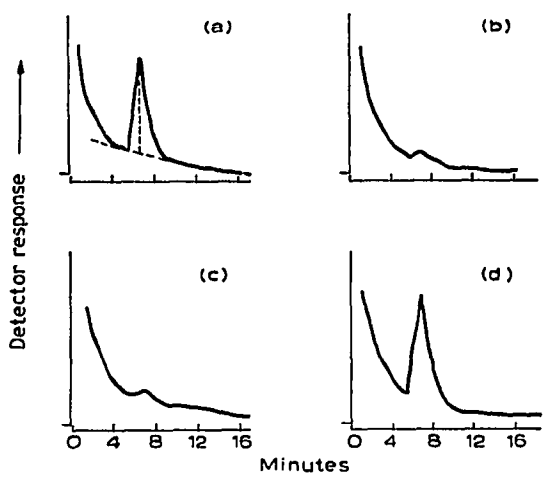

Fig. 1. Gas chromatographic curves obtained from a 1/8 in. × 6 ft. column containing 20% DEGS and 5% isophthalic acid on 60–80 mesh Chromosorb W AW. The inlet temperature was 175° and the column temperature, 150°. (a) Methyl DL-β-hydroxybutyrate, 6 μg; (b) blood, 300 μg; (c) milk, 300 μg; (d) blood, 300 μg + DL-β-hydroxybutyric acid, 6 μg.

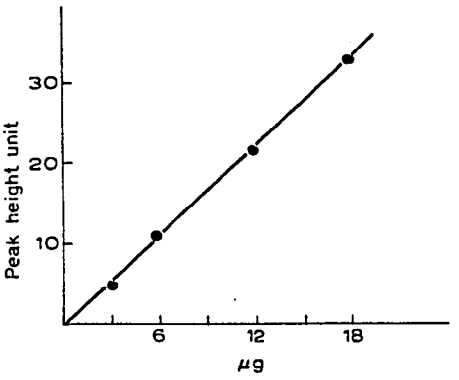

Fig. 2. Response of the flame ionization detector to methyl DL-β-hydroxybutyrate. Column: 1/8 in. × 6 ft. stainless steel, containing 20% DEGS and 5% isophthalic acid on 60–80 mesh Chromosorb W AW; temperature 150°; nitrogen flow-rate, 35 ml/min; attenuation, 128.

The rapidity, simplicity and high rate of recovery of this procedure demonstrates its utility for the specific determination and measurement of DL-β-hydroxybutyric acid in blood and milk samples.

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