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Note

A rapid gas–liquid chromatographic method for determining oxalic acid in biological materials*

A number of gas chromatographic (GC) methods for the analysis of the methyl ester of oxalic acid in biological materials have been reported[1–4]. However, difficulties in sample treatments; *e.g.*, isolation, extraction, esterification and filtration procedures have been encountered. Enzymatic[5,6], fluorometric[7], and colorimetric[8] procedures are sensitive but require extra sample manipulation and care.

This paper presents a simple and rapid GC procedure for quantitative analysis of oxalic acid in forage, blood, feed, and urine.

Experimental

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

Methyl oxalate was separated with a 6-ft., 1/8-in.O.D. stainless-steel coiled column containing 20% diethylene glycol succinate (DEGS) and 5% isophthalic acid on 60–80 mesh, a.w. Chromosorb W. The inlet temperature was 150° and column temperature 105°. The flow-rate of carrier gas (high purity nitrogen) was 40 ml/min. The attenuator was set at 80.

Authentic oxalic acid ($H_2C_2O_4 \cdot 2H_2O$) of 5 to 45 mg and the lyophilized ground samples of 0.1 to 0.3 mg were introduced into a series of 175 × 15 mm I.D. glass tubes with PTFE-lined screw-caps. 5 ml of 5% HCl–methanol solution were then added into each tube. The tubes were sealed with caps followed by ultrasonic mixing for 10 min and thermal incubation at 60° for 2 h. An aliquot of 3-μl sample at room temperature was injected into the chromatograph.

Results and discussion

The gas chromatograph curves of both methylated samples and the oxalic acid standard are shown in Fig. 1. It is noted that the use of ultrasonic mixing–extraction facilitates the subsequent methylation. A linearity for the oxalic acid standard is established as shown in Fig. 2. Quantitative analysis of oxalic acid in biological samples can be readily achieved by comparing samples against the standards run under the same experimental conditions during each trial. An example of actual quantification is illustrated in Figs. 1a and 1b.

The process of esterification requires dry samples. A reduction of esterification yield of 35–55% was noticed when 0.1–0.5 ml of water was introduced into the esterifying samples containing 5–15 mg of oxalic acid. Under the conditions in our laboratory, there was no measurable benefit; instead there was a decreasing

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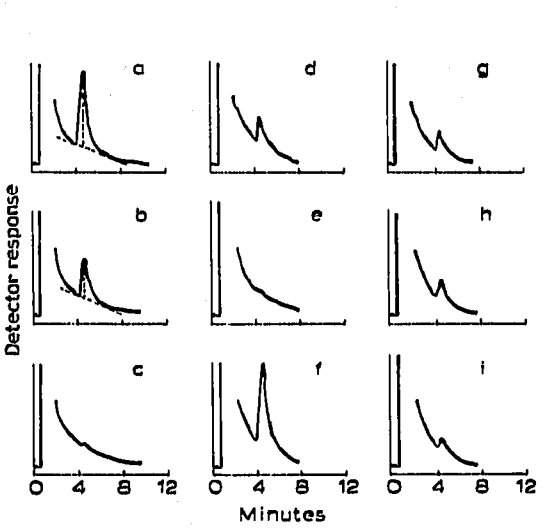

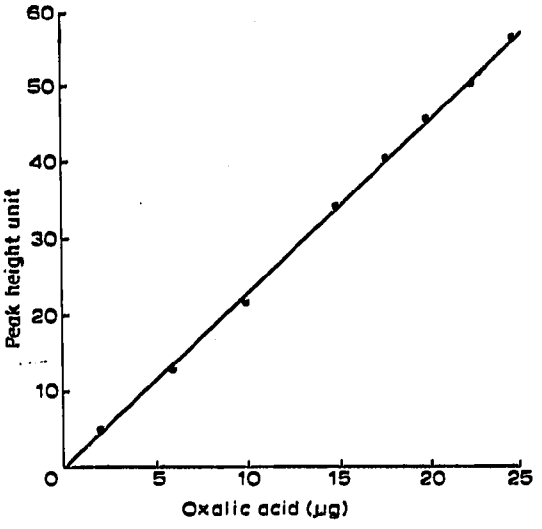

Fig. 1. Gas chromatograph curves obtained from a 1/8-in. × 6-ft. column containing 20% DEGS and 5% isophthalic acid on 60–80 mesh a.w. Chromosorb W. The inlet temperature was 150° and column temperature 105°. (a) methyl oxalate, 4 µg; (b) methyl oxalate, 2 µg + urine, 4 µl; (c) urine, 4 µl; (d) panicum grass, 180 µg; (e), feedstuff, 180 µg; (f) honohono grass, 180 µg; (g) blood, 180 µg; (h) pineapple underflow, 180 µg; (i) pine needle, 180 µg.

Fig. 2. Linearity curve for oxalic acid.

yield of esterification if incubation time was longer than 2½ h. Apparently, the production of water as a result of MeOH + HCl → MeCl + H_2O can adversely affect the esterification process.

The recovery of oxalic acid in urine samples by adding 1 to 10 mg of oxalic acid into 10 ml of urine prior to lyophilization ranged from 92 to 95%. Methyl oxalate was found as a single peak at a retention time of 4.5 min. It is suggested that this method would be useful as regards its specificity and speed to ascertain oxalic acid in biological materials.

Department of Animal Sciences,
University of Hawaii,
Honolulu, Hawaii 96822 (U.S.A.)

JOHN M. L. MEE
RICHARD W. STANLEY

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