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Note

Simple rapid and sensitive method for the simultaneous quantitation of ethanol and acetaldehyde in biological materials using head-space gas chromatography

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Alcoholism represents one of the more serious socioeconomic and health problems. Hence, a need exists for simple rapid methods to quantitate ethanol and its principal metabolite acetaldehyde. Numerous methods have been described using breath sampling devices[1,2], enzymatic[3–7], colorimetric[8], photometric[9] or fluorometric techniques[10]. Of all available methods, gas chromatography is rapidly becoming the method of choice[11–14] since it requires no prior separation, is very sensitive and specific and is relatively simple to perform. This report describes a new gas chromatographic method for the simultaneous quantitation of both ethanol and acetaldehyde with significant improvements and simplifications from existing methods.

EXPERIMENTAL

Reagents and chemicals

The extraction mixture consisted of an aqueous solution containing 40 m*M* thiourea and 0.6 *M* perchloric acid (PCA). Standard solutions were prepared from dehydrated reagent-grade ethanol (U.S. Industrial Chemical Co., Tuscola, Ill., U.S.A.) and acetaldehyde (Mallinckrodt, St. Louis, Mo., U.S.A.). The internal standard was prepared from an aqueous stock solution containing 0.78 μg per ml of reagent-grade isopropanol (Mallinckrodt).

Instrumentation

Gas chromatography was performed on a Hewlett-Packard Model 5830A gas chromatograph using a 1.8 m × 2 mm I.D. coiled glass column. The column was packed with Porapak Q 100–120 mesh (Supelco 2-0332). The injection port was maintained at 100° and the column at 130°. The carrier gas was helium at a flow-rate of 37 ml/min. The flame-ionization detector was operated at 195°.

Extraction procedure

The biological materials selected for analyses were liver and blood from male Holtzman rats and tissue culture medium NCTC 135 with 10% horse serum (Grand Island Biol. Co., Grand Island, N.Y., U.S.A.). The medium had been used to culture

NCTC clone 1469 mouse liver cells treated with ethanol at 10 μmoles per ml. The liver was divided into aliquots of 1–3 g, homogenized in a solution containing 40 m*M* thiourea and 0.6 *M* PCA (1:3, w/v) and centrifuged at 1500 *g* for 10 min. The supernatant fluid was decanted, and 0.6 ml was added to a 25-ml Erlenmeyer flask fitted with an air-tight puncture type rubber stopper. A 0.1-ml portion of the internal standard solution was added and the final volume was adjusted to 0.8 ml with 0.9% saline. The flask was stoppered and incubated in a water-bath with continuous aggitation for 30 min at 65°. A 3-ml gas aliquot was withdrawn through the stopper with a 5-ml gas-tight Hamilton syringe and injected directly into the gas chromatograph.

For whole blood, 0.2 ml was pipetted directly into the 25-ml Erlenmeyer flask containing 0.4 ml of 40 m*M* thiourea–0.6 *M* PCA solution and 0.1 ml of internal standard. The final volume was adjusted to 0.8 ml with saline. Tissue culture media were analyzed in a manner identical to whole blood.

Quantitation

Isopropanol was used in the internal standard since no endogenous isopropanol could be detected in liver or serum. External standards for ethanol were prepared in distilled water in various concentrations from 2.7 to 21.7 μmoles per 1.3 nmoles of isopropanol. Acetaldehyde standards were prepared in the cold by dilution with chilled 0.9% sodium chloride solution in concentrations from 0.023 to 0.182 μmoles per 1.3 nmoles of isopropanol. The retention time of each compound was varified by injecting 3 ml of ethanol, isopropanol and acetaldehyde vapor directly into the gas chromatograph. The areas under the ethanol, acetaldehyde and isopropanol peaks were measured automatically with a Hewlett-Packard programmable GC Terminal (18850 A). Quantitation was determined from the plot of the ethanol/isopropanol and acetaldehyde/isopropanol peak-area ratios *versus* the ethanol and acetaldehyde concentrations, respectively.

RESULTS AND DISCUSSION

Using this technique, the total time from tissue homogenization to completion of the chromatograph is less than an hour. The retention times for acetaldehyde, ethanol and isopropanol were sufficiently different (2.1, 3.5 and 6.9 min, respectively) to allow good baseline resolution. Fig. 1 shows a typical chromatogram.

Standard curves, prepared using 2.7 to 21.7 μmoles of ethanol and 0.023 to 0.182 μmoles of acetaldehyde, yielded a straight line passing through the origin when the peak area ratio was plotted against the ethanol and acetaldehyde concentrations. This represented a 5- to 10-fold increase in sensitivity as compared to previously reported methods[11–14]. As seen in Fig. 2, the recovery of known amounts of ethanol and acetaldehyde added to rat blood yielded apparent recoveries of 100.6 ± 1.1% and 105.8 ± 3.6% (mean ± S.E.M.) for ethanol and acetaldehyde, respectively. These curves were linear with a correlation coefficient (r) of 0.999 and 0.998 ($P < 0.001$).

The precision of the method was measured from eight determinations performed on rat blood after pre-treatment with ethanol. The ethanol content was 16.4 ± 0.17 mmoles/ml (mean ± S.E.M.) with an acetaldehyde content of 134.9 ± 1.7 nmoles/ml (mean ± S.E.M.). This gave a relative standard deviation of 3.04 and 3.48%, respectively.

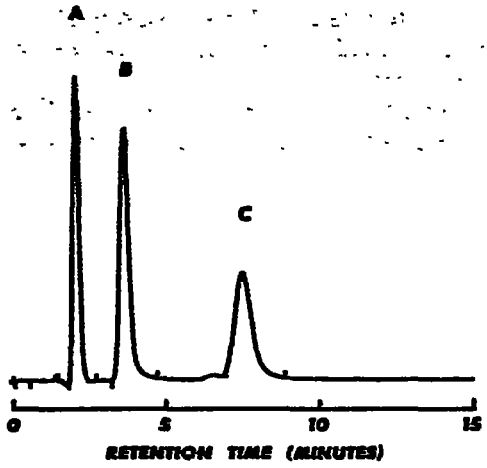

Fig. 1. Head-space gas chromatogram of rat liver after extraction with thiourea and PCA. Coiled glass column 1.8 m × 2 mm I.D., Porapak Q (100–120 mesh). A = Acetaldehyde; B = ethanol; C = isopropanol.

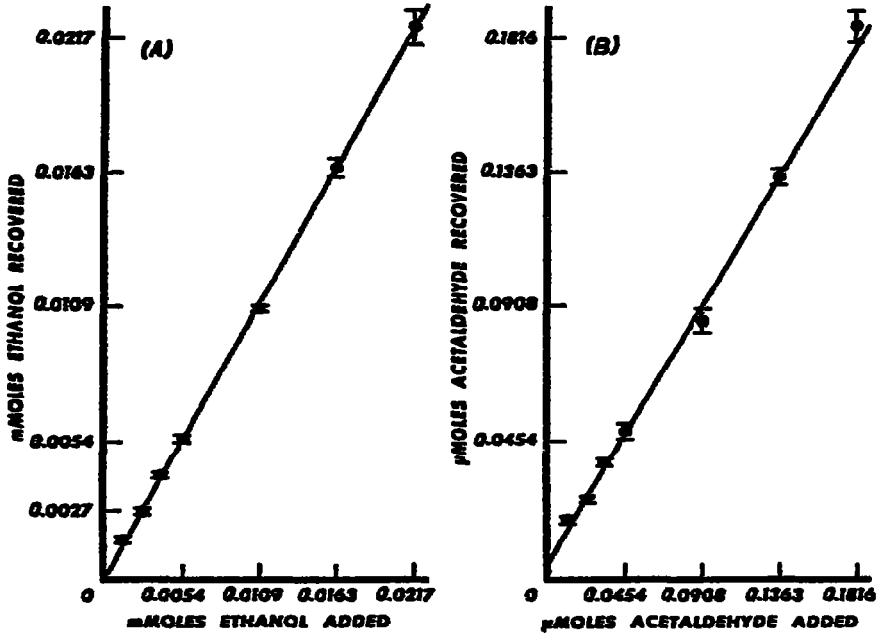

Fig. 2. Linear recovery of known amounts of ethanol (A) and acetaldehyde (B) added to rat blood. Percent recovered 100.6 ± 1.1 and 105.8 ± 3.6, respectively (mean ± SEM). $r = 0.999$ and 0.998, respectively ($P < 0.001$).

Using the technique described in this report, the concentration of ethanol was maintained in NCTC clone 1469 mouse liver cell tissue cultures such that a mean concentration of 9.63 μmoles of ethanol was present per ml of media on days 0, 2, 4 and 6. When the rate of disappearance of ethanol from the liver cell tissue culture

media was monitored, a progressive increase in the rate of ethanol disappearance was observed with chronic exposure. This was in part attributed to intracellular induction of ethanol oxidizing enzymes. Fig. 3 shows this increase.

The advantages of this assay system are that it is simple, rapid, reproducible and accurate —giving simultaneous results for both ethanol and acetaldehyde with sensitivity superior to that previously reported.

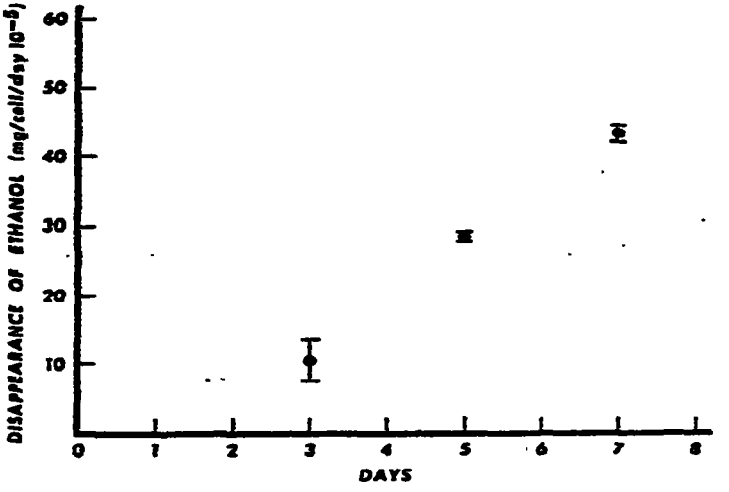

Fig. 3. Rate of ethanol disappearance from NCTC clone 1469 mouse liver cell tissue culture media.

ACKNOWLEDGEMENT

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