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# A Gas Chromatographic Method for Ethanol Determination in Vapors of Biological Fluids

Rapid identification and quantitation of blood ethanol by gas chromatography (GC) has been especially useful to the clinical and forensic chemist, particularly as court evidence in prosecuting drunken drivers. Certain identification can be made and concentration determined rapidly by GC. A number of GC methods are available for ethanol determination in biological liquid samples. Currently, advances are being made in the area of vapor phase analysis [*1–4*]. Direct vapor injections overcome several disadvantages of liquid sample injection. Liquid samples containing sodium fluoride as a preservative tend to etch the syringe. Clotted samples are no longer a problem with vapor injections and frequent cleaning of the syringe is not required. With direct injection of blood or diluted blood samples, special inlets are necessary and must be changed periodically to remove protein and other nonvolatile deposits. In addition, the life of the columns is increased with vapor samples. According to Henry's law, at a given temperature there is a definite ratio between the concentration of ethanol in the blood phase and the air in direct equilibrium with it. Investigators have verified Henry's law [*5–10*] for both aqueous and blood ethanol solutions. The method described here consists of equilibrating blood with *n*-propanol as internal standard and injecting the vapors. Because of the internal standard, the volume of the injected sample is no longer a critical quantity.

## Method

### *Instrument and Conditions*

A Beckman GC-5 chromatograph with dual hydrogen flame detectors and flash vaporization inlets was used. The columns were 6 ft by ⅛ in. stainless steel, containing Porapak S, 100–120 mesh.

| Area Temperatures: | | Gas Flows: | |
|---|---|---|---|
| Column | 160 C | Helium carrier | 50 cc/min |
| Detector | 200 C | Helium make-up | 70 cc/min |
| Detector Line | 190 C | Hydrogen | 50 cc/min |
| Inlet | 150 C | Air | 275 cc/min |
| Attenuation | 64 | | |
| Recorder | 1 mV, 0.5 in./min | | |

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*Procedure*

*Preparation of Reference Standards*—Standards were prepared from fresh beef blood containing 0.14 percent wt./vol. sodium fluoride as a preservative and 0.50 percent wt./vol. potassium oxalate as anticoagulant. The beef blood was diluted 25 percent with distilled water to approximate human blood consistency. Listed in Table 1 is the volume of 10 percent wt./vol. ethanol stock solution[2] added to 200-ml volumetric flasks containing blood to obtain samples containing from 0.05 to 0.35 percent wt./vol. ethanol.

TABLE 1—*Quantity of 10 percent wt./vol. stock solution required for ethanol standards.*

| Percent Blood Ethanol | Volume Stock Solution, ml |
|---|---|
| 0.050 | 1.00 |
| 0.100 | 2.00 |
| 0.150 | 3.00 |
| 0.200 | 4.00 |
| 0.250 | 5.00 |
| 0.300 | 6.00 |
| 0.350 | 7.00 |

*Preparation of Samples for Injection*—Add to 50-ml volumetric flask or other similar container

1.00 ml standard or sample
1.00 ml 0.10 percent (vol./vol.) *n*-propanol[3]
Mix well and immerse flask to 1 in. from top in a constant water bath at 27 C.
Mix just before injection.

Inject 0.5 $cm^3$ vapor with a gastight syringe of 2.5 $cm^3$ capacity. Injection must be rapid to prevent back pressure and subsequent loss of sample.

## Results

Figure 1 shows a typical chromatogram. Retention times were 3.4 min for ethanol and 4.7 min for *n*-propanol. Concentration was determined by the peak height ratio method.

$$\text{peak height ratio} = \frac{\text{ethanol peak height (mm)}}{n\text{-propanol peak height (mm)}} = E/n - P$$

$$\text{concentration}_{\text{Unknown}} = \frac{\text{concentration}_{\text{Standard}}}{(E/n - P)_{\text{Standard}}} \times (E/n - P)_{\text{Unknown}}$$

The concentrations were linear over the range 0.00 to 0.35 percent wt./vol. blood ethanol as illustrated in Fig. 2. Blood ethanol samples prepared in our laboratory and used in our statewide proficiency program were analyzed by this method and by the Dubowski-Shupe distillation method [*11*]. Correlation of blood ethanol concentration results between the two methods was excellent, as is shown in Table 2.

[2] 10 percent wt./vol. stock solution—Add 127.00 ml absolute ethanol to a 1-liter volumetric flask half filled with distilled water and dilute to the mark. The temperature of absolute ethanol should be about 25 C.

[3] 0.10 percent *n*-propanol—Dilute 0.10 ml *n*-propanol to 100 ml.

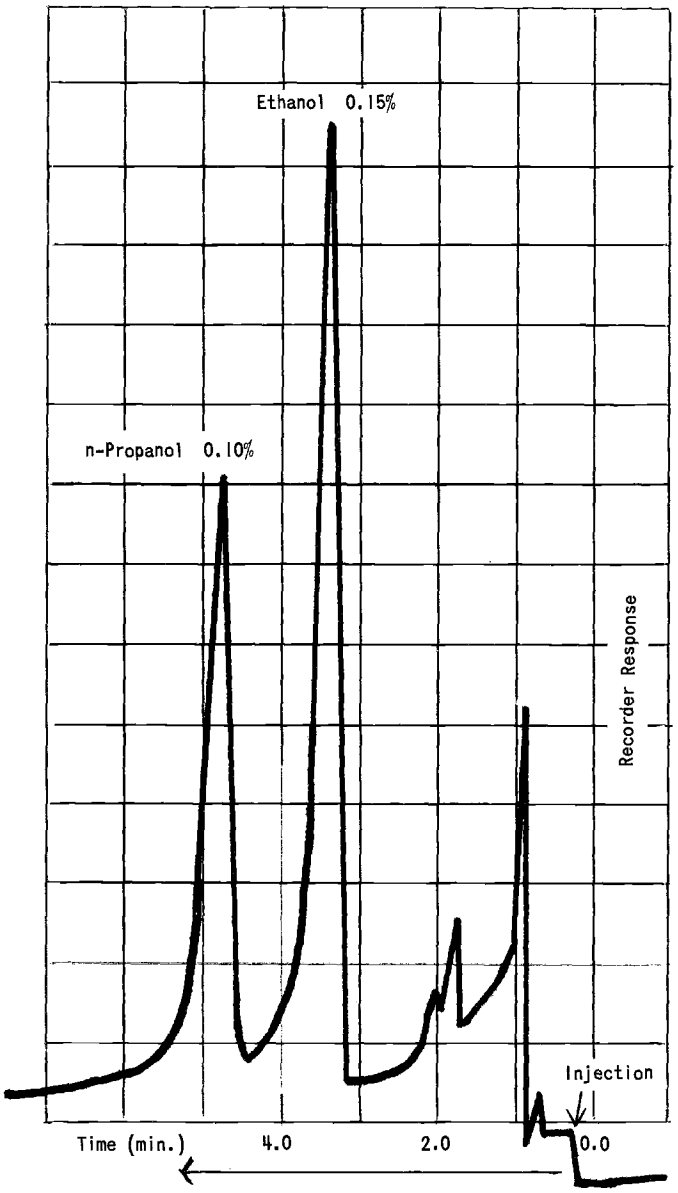


FIG. 1—*Ethanol and* n-*propanol elution peaks.*

**Summary**

Ethanol determination by gas chromatography in headspace vapors is beneficial to direct injection of biological fluids. Syringe life is prolonged and clotting in the syringe is eliminated. Injections are clean, leaving no residue in the inlets. The vapor method described in this paper is rapid, accurate, and precise. Blood ethanol is equilibrated with *n*-propanol, an internal standard, eliminating the problem of duplicating vapor sample volume

TABLE 2—*Correlation of percent wt./vol. blood ethanol on blood proficiency standards by GC vapor phase method and Dubowski–Shupe distillation method.*

| Test Number | Actual | GC | Dubowski–Shupe |
|---|---|---|---|
| 1 | 0.100 | 0.101 | 0.100 |
| 2 | 0.150 | 0.147 | 0.150 |
| 3 | 0.160 | 0.153 | 0.164 |
| 4 | 0.170 | 0.175 | 0.177 |
| 5 | 0.180 | 0.177 | 0.195 |
| 6 | 0.190 | 0.190 | 0.186 |
| 7 | 0.210 | 0.209 | 0.220 |
| 8 | 0.220 | 0.207 | 0.210 |
| 9 | 0.230 | 0.236 | 0.228 |
| 10 | 0.250 | 0.255 | 0.250 |

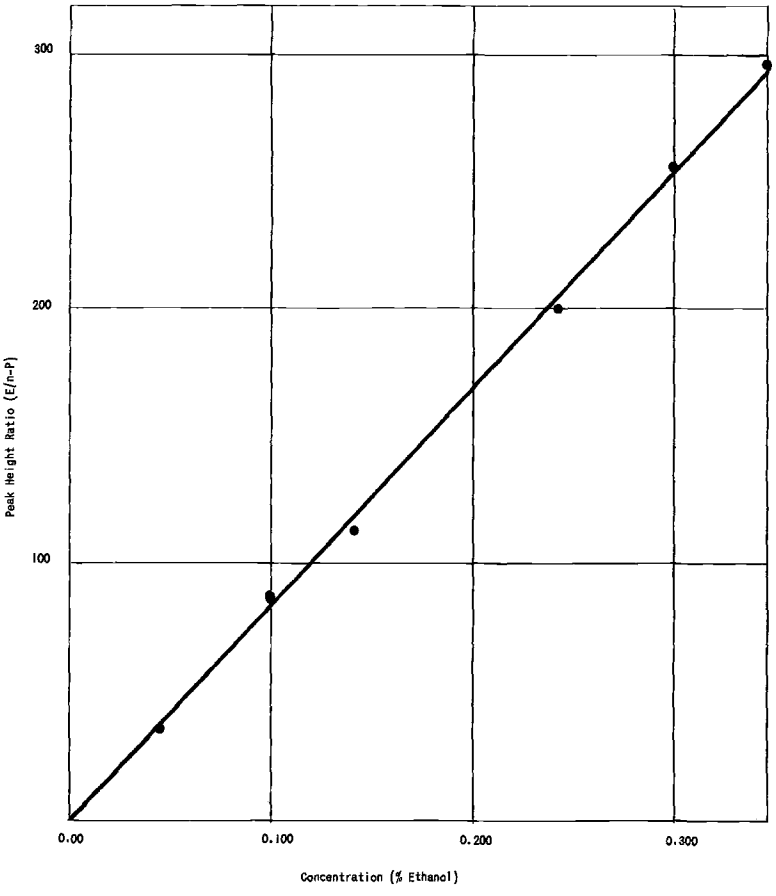


FIG. 2—*Linearity of vapor phase method. Peak height ratio versus concentration (percent blood ethanol).*

injected. Concentrations were linear over the range 0.00 to 0.35 percent wt./vol. blood ethanol. Excellent correlation was obtained between the vapor method and the Dubowski–Shupe distillation method. The average deviation from the correct value was 0.007 percent. An average deviation of 0.002 percent wt./vol. was obtained between duplicate samples.

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