J Forensic Sci, Apr. 1972, Vol. 17, No. 2

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# Duplicate Analysis of Blood Ethanol by Injection onto Two Parallel Gas Chromotographic Columns in Rapid Succession

Methods for analyzing blood ethanol prior to 1967 have been reviewed by Anders and Mannering [*1*]. Gas chromatographic approaches involving injection of vapors trapped above blood samples in sealed bottles have been presented recently by Biasotti and Bradford [*2*], Glendening and Harvey [*3*], and by Van Stekelenburg and De Bruyn [*4*]. More recently, the Perkin-Elmer Company marketed an automated vapor phase injector to chromatograph large numbers of samples daily.

A method designed to rapidly analyze a moderate load of 10 to 30 samples per day is presented in this paper. Vapor phase or "headspace" injection in duplicate onto two parallel gas chromatographic columns is described, using *n*-propanol as an internal standard.

## Methods

### *Materials and Sample Preparation*

Normal propanol and absolute ethanol, both reagent grades, were distilled in an all glass distillation apparatus after thorough drying with anhydrous sodium sulfate. Both alcohols were shown gas chromatographically to be more than 99.8 percent free of organic contaminants. Absolute alcohol, when chromatographed on Porapak P produces a small water signal with flame detectors with as little as 0.5 percent water present. This signal was not seen with the purified alcohols. Rosin [*5*] has noted that purified, absolute ethanol is better than 99.2 percent pure, and contains less than 0.3 percent water. Sample vials were 8-ml capacity with tight fitting septa.

Whole blood to which citrate and fluoride sodium salts had been added was placed on a flash evaporator to remove all highly volatile compounds and excess water (added with preservative salts) to obtain blood with physiological specific gravity. This blood was used for preparing standard solutions. The preservative salts were added to routine blood samples in the quantities suggested by Smith [*6*]. A thermostatically controlled bath, glass syringes of 1- or 2-ml capacity, Yale 24 1½-in. hypodemric needles, and 1 ml delivery repipets were employed.

Standard solutions of 0.20 percent *n*-propanol in distilled water and 0.20 percent each of ethanol and *n*-propanol in distilled water were prepared from the purified alcohols (W/W basis). Into the vials were weighed 0.98 to 1.02-g amounts of each blood sample, followed by 1.00 ml (considered 1.00 g) amounts of the 0.20 percent *n*-propanol solution. Two

Received for publication 24 Aug. 1971; accepted for publication 10 Dec. 1971.

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calibration standards were prepared with 1.00 g of whole blood and 1.00 ml of the ethanol/*n*-propanol solution. Weighings were made with Pasteur pipets placed on a pan balance. To all vials were added 1.0 ± 0.3 g of sodium chloride with a 1-g scoop spatula. Vials were well mixed and placed in a 30 ± 1 C water bath for 60 min. Samples were mixed at least three times during incubation.

*Gas Chromatography*

The gas chromatograph (Hewlett-Packard model 5751) was able to accept two samples simultaneously, being equipped with dual matched columns, dual flame detectors, dual input-output electrometers and a two pen recorder (Mosely model 7160). Paired 6 ft by ⅛ in.-outside diameter stainless steel columns packed with Hallcomid 18 (3.8 percent) and Carbowax K-600 (0.5 percent) on Teflon 6 HC were employed. Columns were obtained from the Perkin-Elmer Company. Operating parameters were: injection port, 110 C; detector, 180 C; oven, 70 C; helium carrier flow, 33 ml/min; hydrogen, 10 psi; air, 30 psi; attenuation, 10 × 16 or 10 × 8; and chart speed, 0.5 in./min. The columns were conditioned for 2 h at 80 C and ½ h at 70 C. Prior to analysis, one or two injections of the standard solution were made for the purpose of "use conditioning." Critical factors relating to headspace analysis have been treated in previous publications [*2–4*].

Approximately 0.25 ml of sample headspace was injected with a smooth action onto the top column which was linked to the lead (blue) pen on the recorder. After flushing the syringe by hand, a second aliquot of the same sample was injected onto the bottom column which was linked to the second (red) pen. The two columns are henceforth denoted as "blue" and "red." The second *n*-propanol peak emerged from the red column within 3 min after the initial injection. Figure 1 shows the strip chart record of the analysis of 7 routine blood samples with a calibration standard. Ethanol and *n*-propanol peaks were

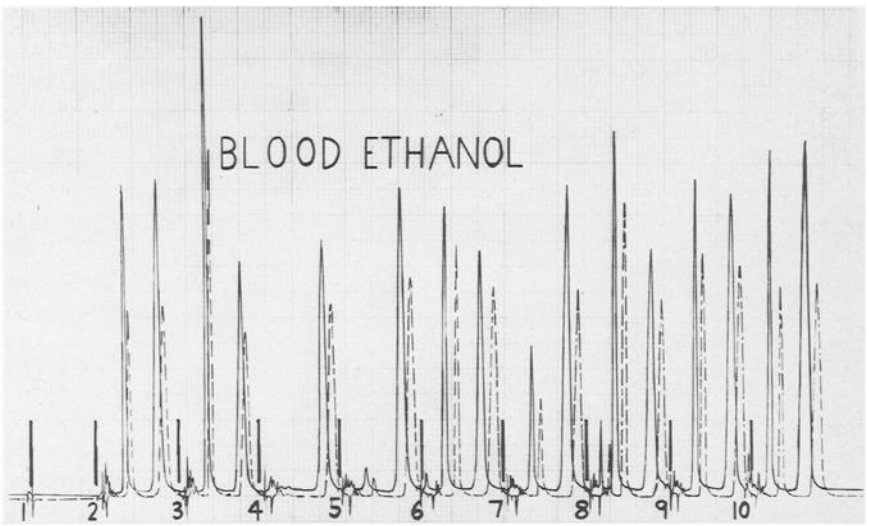


FIG. 1—*Photographic reproduction of strip chart record of the analysis in duplicate of several blood samples. Heavy vertical lines mark point of injection. Solid chromatographic tracings are the "blue" channel and broken tracings are the "red" channel.*

nearly equal in height at equal concentrations, and other volatiles such as, acetone could be distinguished from ethanol. Volatiles of interest emerged in the following order: acetaldehyde, acetone, methanol, ethylacetate, butanone, ethanol, isopropanol, chloroform, and *n*-propanol. Ethylacetate, butanone, and isopropanol were partially resolved from ethanol. A Porapak P column separated compounds which overlapped on Hallcomid 18.

Duplicate values for ethanol peak height/*n*-propanol peak height were obtained for each sample as by Anders and Mannering [*1*] except that weights of internal standard and sample were used instead of volumes.

## Results

### *Precision of Method*

Ethanol concentrations between 0.01 and 0.40 percent were shown to be proportional to the peak height ratio of ethanol/*n*-propanol at constant *n*-propanol concentration. Deviation from linearity above 0.40 percent was not large. Analyses of 10 blood samples of a regular work day are shown in Table 1. The strip chart record of 7 of the 10 analyses of Table 1 is shown in Fig. 1. The minor peaks appearing immediately after the injection spike are normal; the acetone of injection 8 (third highest peak) is higher than normal. Table 2 gives the precision of analysis of 100 routine blood samples by six different analysts over a one month period. Concentrations ranged from 0.004 to 0.378 percent, the majority of samples having concentrations from 0.10 to 0.30 percent.

TABLE 1—*Duplicate analysis of 10 blood samples for ethanol with dual channel gas chromatograph.*

| Injection Number[a] | Alcohol Peak Height Ratios | | Ethanol Concentration, (W/W %) | |
|---|---|---|---|---|
| | Blue | Red | Blue | Red |
| 2 | 0.972 | 0.983 | (0.20 (known)[b] | |
| 3 | 2.05 | 2.09 | 0.422 | 0.426 |
| 4 | 0.016 | 0.008 | 0.003 | 0.002[c] |
| 5 | 0.071 | 0.065 | 0.015 | 0.013 |
| 6 | 1.18 | 1.19 | 0.243 | 0.242 |
| 7 | 0.474 | 0.470 | 0.098 | 0.096 |
| 8 | 1.49 | 1.49 | 0.306 | 0.304 |
| 9 | 1.05 | 1.05 | 0.216 | 0.214 |
| 10 | 0.972 | 0.981 | 0.20 (known)[b] | |
| — | 1.32 | 1.32 | 0.272 | 0.269 |
| — | 0.007 | 0.005 | 0.002 | 0.001[c] |
| — | 0.143 | 0.142 | 0.029 | 0.029 |

[a] Numbers correspond to those in Fig. 1.
[b] Calibration standard containing 0.20% ethanol and 0.20% *n*-propanol.
[c] Reported as negative ethanol, as were all samples less than 0.01%.

The precision of analysis at 0.20 percent ethanol concentration is shown in Table 3. One blue and one red injection of each of 10 individual samples of one blood specimen demonstrated precision better than 1.7 percent on both channels, as seen in the first two rows of Table 3. Repeated injection of a single sample was shown to be a more precise operation than duplicate analysis of one blood sample weighed out several times (0.5 percent versus 1.3 percent). Even though repeated withdrawal noticeably decreased the vapor pressure inside the vial sampled, the peak height ratios did not change appreciably for the first

five or six injections. Occasional wide variations between duplicate answers were usually due to faulty injection technique and could be corrected immediately by reinjecting the sample in question.

TABLE 2—*Precision of 100 consecutive samples analyzed in duplicate on two parallel gas chromatographic systems.*

| Variation Between Duplicates,[a] % W/W | Number of Samples | Samples Showing Variation or Less |
|---|---|---|
| 0.000 | 17 | 17 |
| 0.001 | 22 | 39 |
| 0.002 | 20 | 59 |
| 0.003 | 8 | 67 |
| 0.004 | 12 | 79 |
| 0.005 | 9 | 88 |
| 0.006 | 2 | 90 |
| 0.007 | 3 | 93 |
| 0.008 | 5 | 98 |
| 0.009 | 2 | 100 |
| Avg ± 0.0027 | | |

[a] Absolute difference between duplicate answers covering concentrations from 0.004 to 0.378% W/W (for example, variation between 0.154 and 0.151% is 0.003%). Six analysts working in rotation in a one month period provided data for this table.

TABLE 3—*Precision of analysis with dual channel gas Chromatograph.*[a]

| Samples Prepared | Injections On Each Channel | Absolute Standard Deviation, % | |
|---|---|---|---|
| | | Blue Channel | Red Channel |
| 10 | 10 | 1.4 | 1.5 |
| 10 | 10 | 1.6 | 0.7 |
| 1 | 5[b] | 0.5 | 0.5 |
| 6 | 6 | 1.3[c] | |

[a] Concentration of ethanol was 0.20% in first 3 rows and 0.23% in last row.
[b] Five consecutive injections of a single sample.
[c] Standard deviation of six pair of blue-red answers.

*Accuracy of Method*

Table 4 compares analytical results of this method with results of laboratories of other states. Laboratories 1 and 3 used a headspace gas chromatographic method, injecting 1 ml of sample from a 35-ml container; laboratory 2 used distillation, oxidation, and indirect titration. Results of the method presented here are compared with the results of gravimetric and oxidative analyses. Although the accuracy of the method presented cannot be stated in absolute terms, general agreement with other methods is apparent.

## Discussion

Several precautions were necessary for reliable and consistent results. The 60-min sample incubation time and the thorough mixing of the samples two or three times during this period were needed to produce a constant peak height ratio of ethanol to *n*-propanol

TABLE 4—*Comparison of results with results obtained by other methods in various laboratories in the U.S.*

| Concentration of Ethanol in Blood in Weight /Volume Percent[a] | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Lab 1 | Lab 2 | Lab 3 | | Lab 1 | Gas Chrom | Other Method |
| 0.137 | 0.14 | 0.15 | 0.133 | 0.213 | 0.20 | 0.139 | 0.144 oxd.[b] |
| 0.333 | 0.33 | 0.33 | 0.303 | 0.328 | 0.32 | 0.347 | 0.349 |
| 0.227 | 0.23 | 0.22 | 0.218 | 0.245 | 0.24 | 0.079 | 0.077 |
| 0.066 | 0.07 | 0.075 | 0.063 | 0.081 | 0.08 | 0.142 | 0.142 |
| 0.046 | 0.05 | 0.05 | 0.045 | 0.040 | 0.04 | 0.158 | 0.157 |
| 0.395 | 0.37 | 0.37 | 0.333 | 0.414 | 0.40 | 0.011 | 0.010 grav.[c] |
| 0.188 | 0.19 | 0.19 | 0.186 | 0.158 | 0.16 | 0.197 | 0.195 oxd., 0.201 grav. |
| 0.253 | 0.26 | 0.26 | 0.243 | 0.124 | 0.12 | 0.038 | 0.040 grav. |
| | | | | | | 0.020 | 0.020 |

[a] Where compared with results of other laboratories, our results were converted from weight/weight to weight/volume % assuming a specific gravity of 1.06 for this sample.
[b] Method involved steam distillation, oxidation with acid dichromate, and titration.
[c] Purified ethanol, weighed into fresh, whole blood.

with a given sample. The mechanical placement of the sample into the injection port required a relatively unhurried syringe plunger action by the analyst. Hard, sudden injections caused strong negative injection peaks and erratic results.

The ethanol/*n*-propanol peak height ratio of the calibration standard should be measured twice with separately prepared standards. This ratio varied as much as ±5 percent from day to day, the value apparently depending upon the degree of column conditioning and use prior to the blood ethanol analysis. Injections of more than 25 consecutive samples sometimes produced "memory" peaks [7] and the standard ratio began to vary after sustained column use of more than 100 min.

The precision attained here is within the range usually reported for quantitative gas chromatography [*1–3*]. Close agreement of gas chromatographic, oxidative, and gravimetric methods is seen in Table 4. The accuracy of this method theoretically attainable depends on several factors not involving gas chromatography: measurement of the purity of the alcohols used, preferably with NBS standardized potassium dichromate; keeping the alcohols anhydrous prior to preparing solutions; and preparation of accurate standard solutions.

The method described here has been found suitable for the analysis of 10 to 30 samples daily, and conforms to standards of practice suggested by the California Association of Criminalists [*8*].

## Summary

A gas chromatograph having matched dual columns, dual flame ionization detectors, dual input-output electrometers, and a two pen recorder was used for analysis, in duplicate, of blood ethanol samples. Because duplicate injections were only seconds apart, analysis of a set of samples required little more than half the time needed ordinarily.

One-gram samples of each blood to be analyzed were weighed into 8-ml capacity vials and 1.00 ml of *n*-propanol solution (0.20 percent) was added. After the addition of 1 g of sodium chloride, the sample vials were stoppered, mixed, and placed in a 30 C water bath for 60 min. Injections of approximately 0.25 ml of headspace vapor were made. Ethanol and *n*-propanol peaks emerged from both columns within 3 min. Two 6 ft by ⅛ in. outside diameter stainless steel columns packed with Hallcomid 18-Carbowax K-600 on Teflon 6 HC were used. Methanol, acetone, isopropanol, and other volatiles which might be found in blood were separated from ethanol.

The concentration of ethanol was calculated from peak height ratios of ethanol/*n*-propanol. Variation between duplicates averaged ±0.0027 percent ethanol over a wide concentration range. Standard deviations near 0.20 percent ethanol for a large number of replicates were between 1 and 2 percent. Results of this method were in agreement with other methods.

*Acknowledgments*

I wish to thank Abraham Stolman and the chemists of his staff for valuable suggestions and technical assistance provided in developing this method. The Arizona State Department of Health Laboratory Improvement Program collected the data from other laboratories shown in Table 4.

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