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Automated Analysis of Alcohols in Blood

Gas chromatography is a valuable analytical tool for the measurement of volatiles in a variety of different samples. Published methods utilizing this technique for the analysis of ethanol and related volatiles in blood and urine are now too numerous to mention. However, in a recent publication by Jain,[2] a simple, rapid gas chromatographic method for the simultaneous determination of methanol, ethanol, acetone, isopropanol, as well as low boiling hydrocarbons associated with glue sniffing was described. The method involves the direct injection of blood mixed with an internal standard. Jain reported the successful use of his G.C. column for more than two years and several thousand injections.

The recent development of automatic liquid samplers and ultrastable gas chromatographic systems has allowed this analysis to be completely automated. We would like to report here, a blood alcohol analyzer which has the capability of identifying and quantifying methanol, ethanol, isopropanol and acetone in blood or urine at the rate of 8 analyses/h. If the analyst desires to screen for ethanol only, the load can be increased to 20/h, using isopropanol or methanol as the internal standard.

The Hewlett-Packard blood alcohol analyzer is composed of a gas chromatograph, automatic liquid sampler (ALS), electronic integrator, and data processor with special software. The column and analysis conditions are shown in Table 1. In the analyzer, the ALS is controlled by a subroutine in the data processor software which will advance the sampler turntable to each of its 36 positions automatically. The analysis of up to 33 samples can be accomplished as follows.

Vials filled with a rinse solution containing 200 units of heparin/ml in water are placed in positions 1 and 2 of the ALS tray. A calibration mixture containing the alcohols of interest is placed in position 3. The remaining 33 positions are filled with vials containing the prepared blood or urine samples. On start up, the processor advances the ALS tray to position 1, where the syringe is rinsed with the heparin solution and then to position 3 where the calibration mix is sampled and injected. Retention times and response factors relative to *n*-propanol are stored for later reference. If only ethanol is to be analyzed, isopropanol or methanol can be used as the internal standard.

After the calibration has been completed, the sampler tray will index to position 1 where the syringe is immediately rinsed. Subsequently, the syringe will be rinsed each time a sample is analyzed. Once the calibration is complete, the sampler tray indexes to position 4 where the first unknown blood solution is sampled.

In each analysis, the sample is analyzed in duplicate unless it is found to contain less than 0.05 percent of ethanol—in that case, it is analyzed only once. The only deviation

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[2] Jain, N. C., *Clinical Chemistry*, Vol. 17, No. 2, 1971.

TABLE 1—*Operating conditions.*

Column:	
6 ft by 1/8 in. inside diameter, stainless steel, packed with 50% Poropak Q + 50% Poropak R preceded by 10 cm by 3 mm inside diameter glass precolumn packed with glass wool.	
Temperatures, deg C:	
Column	150
Injection Port	150
Flame Detector	300
Flows, cc/min:	
Air	350
Nitrogen	50
Hydrogen	28
Sampler Control Module, min:	
Analysis Cycle	8
Stop Integrate	7

from this analysis scheme occurs if the sample is found to contain between 0.09 and 0.11 percent ethanol. These values bracket 0.10 percent which is considered to be the lower statutory limit for DWI (driving while intoxicated) charges in many states. If the ethanol concentration is between these values, the analyzer is automatically recalibrated and the sample is reanalyzed in duplicate to insure additional accuracy. Also, if the internal standard area varies by ±10 percent from the first run, indicating a drastic change in instrument conditions, the analyzer shuts down. At present, the software does not allow for the determination of methanol although this can be included with no problem.

All calculations are carried out by the data processor using the internal standard method. Muirhead et al[3] have published a specific gravity range for blood of 1.052–1.064. We have chosen 1.058 as a suitable value and this weight is used in all calculations. Thus the results are on a weight per weight basis. Multiplying such results by 1.058 converts the value to percent weight per volume (wt/vol.).

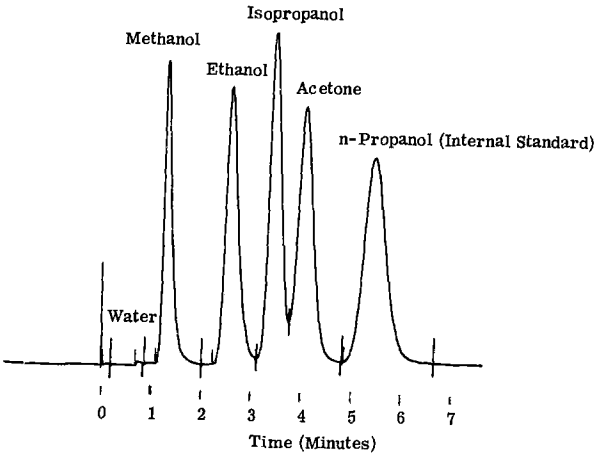
Preparation of the Sample for Analysis

0.5 ml of blood is transferred to a standard Hewlett-Packard sampler vial using a 0.5-ml blowout pipet. The blood is then diluted with 1.0 ml of *n*-propanol internal standard in water (2 ml *n*-propanol/liter). The resulting mixture is then capped. After the samples have been prepared, the capped vials are placed on a Syntron vibrator for 60 s before placing them in the sampler tray.

The calibration mixture is prepared by pipeting 2.0 ml each of methanol, absolute ethanol, isopropanol, acetone and *n*-propanol into a one liter volumetric flask and diluting the mixture to one liter with distilled water. If ethanol is the only volatile to be screened, only ethanol and the internal standard are added to prepare the calibration mixture. Using their respective specific gravities, the final concentrations of the compounds are methanol 0.158, ethanol 0.158, isopropanol 0.157, acetone 0.158, and *n*-propanol 0.161 percent wt/vol.

Figure 1 shows the separation obtained on the mixed Porapak Q and R column. Although the separation of isopropanol and acetone is not complete under the operating conditions shown here, it is adequate for identification and quantitation.

[3] Muirhead, E., Grow, M. H., and Walker, A. T., *Surgery, Gynecology, and Obstetrics*, Vol. 82, 1946, p. 405.



Separation of volatiles	0.128% wt/vol in water
Column	6 ft by 1/8 in. ss; 50% Porapak Q+ 50% Porapak R; 80/100 mesh
Range	10^3
Recorder presentation	20 mV fullscale

FIG. 1—*Separation obtained on mixed Proapak Q and R column.*

Results

The linearity of response between various concentrations of ethanol to a fixed concentration of *n*-propanol internal standard is shown in Fig. 2. The range of linearity is excellent for concentrations ranging between 0.015 and 0.260 percent.

Precision of the method including and excluding the sample preparation step was also found to be quite good. To determine the precision of the analyzer, ethanol and *n*-propanol were added to the blood to give respective concentrations of 0.16 and 0.32 percent. The stock sample was then diluted with water to give the desired 2:1 ratio of water to blood. The sample was thoroughly mixed and aliquots of the solution were then transferred to individual sample vials and analyzed. The precision of the method including the sample preparation was determined in a similar fashion except the 0.5-ml samples of the spiked blood were prepared individually using the sample preparation procedure described earlier. The mean and the percent relative standard deviation from the mean of ETOH area counts/*n*-propanol area counts along with the range are given in Table 2. These results show a percent relative standard deviation of ± 0.63 for the analyzer and a value of ± 1.00 when the sample prep is included. The range between the highest and lowest relative standard deviation values was 3.48 percent for the analyzer and 3.71 percent for the method including the sample prep. Thus one can expect that if the first of two analyses on a sample give a value of 0.100 percent ethanol, the second analysis would not give a value greater than 0.104 percent or less than 0.096 percent.

The accuracy of the analyzer is shown in Table 3. Various amounts of absolute ethanol were weighed into bottles containing a weighed aliquot of blood. The samples were carried through the dilution steps and analyzed. The theoretical weight percent of ethanol in the

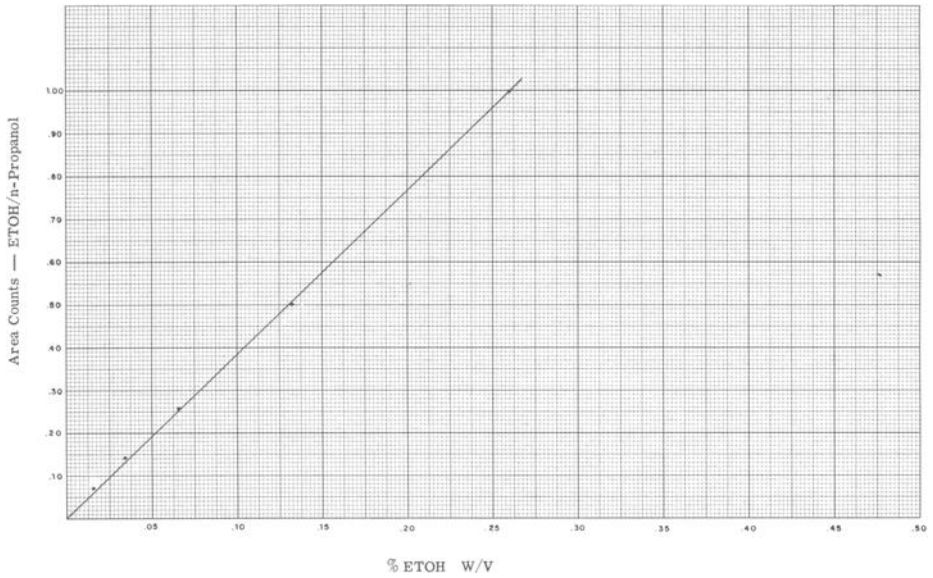


FIG. 2—*Linearity response curve for ethanol/n-propanol peak area ratios versus ethanol concentrations in blood.*

blood compared with the percent measured is shown in the table. All results were within 0.05 percent of the target value.

Sample carryover was also determined and found to be approximately 0.001 percent following injections of blood containing 2 percent ethanol. This carryover would have an insignificant effect on samples within the concentration range encountered in forensic determinations.

A short term study was also conducted to determine the effect of repetitive injections of blood on the column. Up to 1000 injections of blood were made during which time ethanol and isopropanol were observed for changes in peak shape, retention times, and response factors. The relative response of ethanol to isopropanol determined at various points from 0–1000 injections is shown in Fig. 3. As is indicated, the response factor decreased from 0 to 100 injections. However, there was an increase in this value after the glass wool precolumn was replaced. Very little change in the response factor was noted

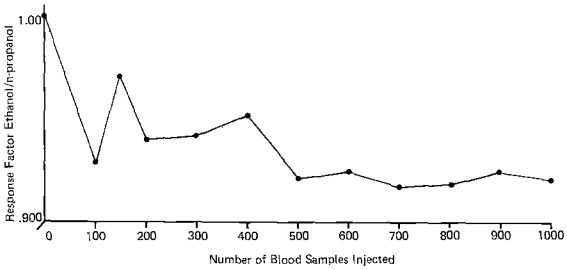


FIG. 3—*Ethanol/n-propanol response factor over range of 0 to 1000 injections of diluted blood.*

TABLE 2—*Precision of analyzer on diluted blood (25 runs).*

Blood contained 0.16% ETOH and 0.32% *n*-propanol.	
Mean $\frac{\text{ETOH area counts}}{n\text{-propanol area counts}}$	0.38954
Relative standard deviation from mean	0.00244
% Relative standard deviation from mean	±0.63
% Range $\frac{\text{ETOH area counts}}{n\text{-propanol area counts}}$	3.48
Precision of method including sample preparation (25 runs).	
Blood contained 0.10% ETOH and 0.32% *n*-propanol.	
Mean $\frac{\text{ETOH area counts}}{n\text{-propanol area counts}}$	0.2405
Relative standard deviation from mean	0.00204
% Relative standard deviation from mean	1.00
% Range $\frac{\text{ETOH area counts}}{n\text{-propanol area counts}}$	3.71

from 200 injections on. It is important to note that this change would not affect the analysis results since the instrument is recalibrated after a full tray load of 33 samples has been analyzed.

Peak shape did change after approximately 100 injections but returned to normal after the glass wool precolumn was replaced. Thus the precolumn should be replaced regularly. No significant change in the retention times of ethanol and *n*-propanol were observed. It appears then, that the injection of blood has little effect on the column.

To test the analyzer from a functional standpoint, a series of analyses were made on blood containing various concentrations of ETOH. The printout is shown in Fig. 4. The first set of results are from the calibration run—the first column giving the retention time in hundredths of a minute and the second column the relative response factor. In the results for samples T1–T4, column 1 lists the compounds for which the samples are being screened, column 2 the retention times in hundredths of a minute and column 3 the percent wt/wt of volatile. As we go down the column, sample T1 was found to contain less than 0.05 percent and was only analyzed once. T2 was more than 0.05 percent and was analyzed in duplicate. Sample T3 was found to contain a value between the critical 0.09 to 0.11 percent range and thus the analyzer was recalibrated and the sample analysis repeated in duplicate. T4 had

TABLE 3—*Accuracy of the Hewlett-Packard blood alcohol analyzer on ethanol in blood.*

SAMPLE	% ETHANOL W/W	
	THEORETICAL	MEASURED
1	0.040	0.039
2	0.064	0.061
3	0.099	0.095
4	0.119	0.117
5	0.213	0.211

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CALIBRATION COMPLETE
TIDC 2)= .2630E+03      RFC 2)= .3792E+00
TIDC 3)= .3810E+03      RFC 3)= .3515E+00
TIDC 4)= .4730E+03      RFC 4)= .3458E+00
TIDC 5)= .6770E+03      RFC 5)=1.0000E+00

SAMPLE* T1

ETHANOL          .2710E+03       .3984E-01
I-PROPANOL       .3810E+03       .0000E+00
ACETONE          .4730E+03       .0000E+00

SAMPLE* T2

ETHANOL          .2710E+03       .6115E-01
I-PROPANOL       .3810E+03       .0000E+00
ACETONE          .4730E+03       .0000E+00

SAMPLE* T2

ETHANOL          .2710E+03       .6209E-01
I-PROPANOL       .3810E+03       .0000E+00
ACETONE          .4730E+03       .0000E+00

SAMPLE* T3

ETHANOL          .2700E+03       .9505E-01
I-PROPANOL       .3810E+03       .0000E+00
ACETONE          .4730E+03       .0000E+00
CALIBRATION COMPLETE
TIDC 2)= .2730E+03      RFC 2)= .3787E+00
TIDC 3)= .3830E+03      RFC 3)= .3528E+00
TIDC 4)= .4750E+03      RFC 4)= .3487E+00
TIDC 5)= .5800E+03      RFC 5)=1.0000E+00

SAMPLE* T3

ETHANOL          .2710E+03       .9686E-01
I-PROPANOL       .3830E+03       .0000E+00
ACETONE          .4750E+03       .0000E+00

SAMPLE* T3

ETHANOL          .2710E+03       .9582E-01
I-PROPANOL       .3830E+03       .0000E+00
ACETONE          .4750E+03       .0000E+00

SAMPLE* T4

ETHANOL          .2700E+03       .1178E+00
I-PROPANOL       .3830E+03       .0000E+00
ACETONE          .4750E+03       .0000E+00

SAMPLE* T4

ETHANOL          .2710E+03       .1176E+00
I-PROPANOL       .3850E+03       .2653E-02
ACETONE          .4750E+03       .0000E+00
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FIG. 4—*Test run of blood alcohol analyzer.*

greater than 0.11 percent ETOH and thus analyzed in duplicate. As an example, reading across the first line of sample T1 gives ethanol with a retention time of 271 hundredths of a minute or 2.71 min and a percent of blood wt/wt of 0.03984. This is still a preliminary format and it will be simplified when the final version of the software is written.

Although the analyzer described here contains a data processor, the analyst can still operate in automated fashion without it. However, in this case, all the decision steps concerning critical concentrations, special sampler control, as well as an output of a final report giving concentrations are lost. In this case, the samples to be analyzed are placed in all odd numbered positions and a rinse solution is placed in every even position. Following sample injection, the tray advances to the next position where the syringe is washed.

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