Gas Chromatographic Analysis of Alcohol and Certain Other Volatiles in Biological Material for Forensic Purposes.* (23701)

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Gas-liquid partition chromatography affords a general method of analysis for microgram concentrations of volatile mixtures that is expedient in procedure, specific, and quantitative in character. These attributes are lacking in the customary technics utilized for toxicological analysis of volatiles(1).

The previously reported use of glycol columns(2,3) have indicated their usefulness for analysis of water-alcohol mixtures. This report is concerned with the use of glycol columns for quantitative micro determinations of some volatile compounds from biological distillates which are of toxicological interest.

Method. Distillation. One to 5 cc of whole blood (urine or tissue) were pipetted into a 250 cc electrically heated distilling flask containing 25 cc of water, 3 cc of 50% sodium tungstate, and 5 cc of 1 N sulfuric acid. The vapour was passed through a heated fractionating column (Eck & Krebs #3470) for one hour. The column was 55 cm in length, 1 cm in diameter filled with glass helicis. Twenty to 25 cc of distillate were collected in an ice-cooled receiver. *Gas Chromatography*: The gas chromatography apparatus used was a Perkin-Elmer Fractometer model 154B set at full Sensitivity. The recorder was a Leeds and Northrup Speedomax with 5 mv. full scale sensitivity and a chart speed of 12 in. per hour. A 0.1 cc or 0.05 cc aliquot of the distillate was injected into one of 2 glycol columns using a one-quarter cc tuberculin syringe. Column #1 was a 50:30:20 by weight, 30-60 mesh Johns-Manville C22 firebrick, glycerol, tricresyl phosphate (TCP) mixture(3) in a ¼" copper tube 1 meter long. Column #2 was a 60:18:22 firebrick, glycerol, TCP mixture in a ¼" copper tube 1 meter long. The identity of ethanol and other separated components was determined by adding the suspected substance to the distillate and re-running the sample to see if the peak height in question was increased. Separated components were quantitatively assayed by direct calibration of the column.

Results. Fig. 1 shows the direct calibration for ethanol. Blood alcohols were determined to an accuracy of 5 mg % as illustrated in Table I. The concentrations of the "control" blood alcohol solutions never varied more than 5 mg % from a modified Widmark alcohol analysis(4). One microgram of ethanol in 0.10 cc injection volume, that is a 0.001% solution, gave a discernible peak (of 0.10 mv) on the chromatogram. Using 3.00 cc of blood in the distillation this corresponded to a 10 mg % blood alcohol concentration. Column #1 gave the better ethanol peaks with respect to symmetry and sensitivity but failed to separate ethanol from formaldehyde and ethanol from methanol. Column #2 separated the binary mixtures as shown in Fig. 2. The separation of ethanol and methanol eliminates the necessity of separate methanol tests (6).

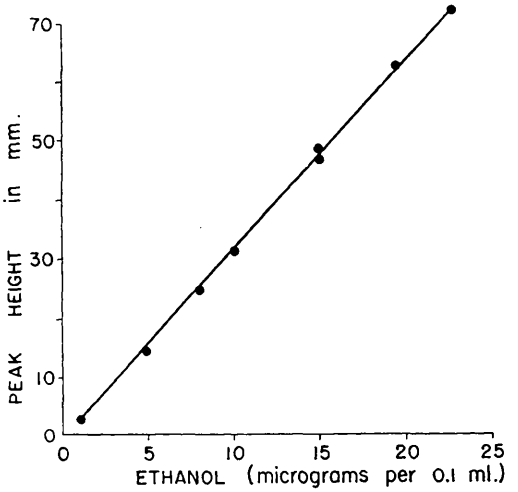

FIG. 1. Direct calibration of ethanol in 0.1 ml of water using column #1. Carrier gas, helium; flow rate, 20 cc per minute; column pressure, 1 psi, and temp., 106°C.

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TABLE I. Recovery of Added Ethanol from Blood by Vapor Phase Chromatography Method Using Column 1.

Ethanol added	Ethanol recovered*	Error	Corrected ethanol error†
	(mg/cc of blood)		
.10	.10	.00	.00
.40	.45	−.05	+.05
.40	.40	.00	.00
.50	.50	.00	+.05
.50	.45	−.05	.00
.65	.60	−.05	.00
1.00	.95	−.05	.00
1.50	1.40	−.10	−.05
1.50	1.45	−.05	.00
1.50	1.55	+.05	+.10
2.00	1.95	−.05	+.05
3.00	2.90	−.10	+.05

* Significant to .05 mg.
† Measured from theoretical 95% recovery from alcohol-water azeotrope.

This method provides a more rapid means of performing blood alcohols on embalmed blood as compared to the existing procedures of distilling with aldehyde and ketone reagents(1,5). Although using 0.1 cc of H_2O overloaded the columns, that is the flow became irregular, no adverse effects on the glycol columns resulted. Gas chromatographic alcohol values and Widmark alcohol values on the same non-alcoholic putrified blood samples were compared. The reducing substances

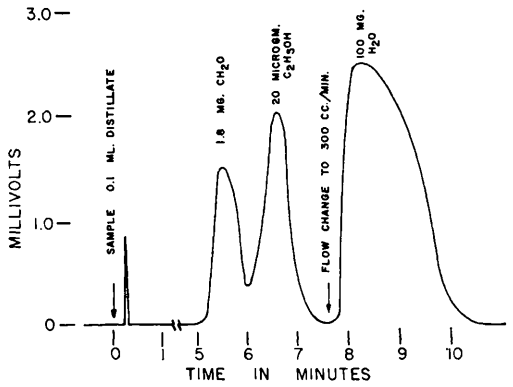

FIG. 2. Chromatogram of ethanol-formaldehyde-water using column #2; carrier gas, helium; initial flow rate, 20 cc per min; temperature, 84°C; sensitivity, 1 for aldehyde and alcohol; sensitivity, 1/128 for water.

(30-60 mg %)(1) as determined by the Widmark oxidation test on the slightly to moderately putrified blood did not add to the gas chromatographic blood alcohol value more than 5 mg %.

The two glycol columns also separated paraldehyde from binary mixtures of 100 mg of water and 20 μg of the following substances: ether, acetone, acetaldehyde. Two micrograms of hydrogen sulfide gas were detected in blood distillates in 1 M 20:80 TCP-firebrick column at 94° and a 10 cc per minute helium flow. By the use of other columns additional volatile compounds may be determined. Preliminary investigation has shown that one microgram of carbon monoxide in air samples gave a millivolt recorder response in a 1 M Linde Molecular Sieve 13 X column at room temperature and 40 cc He/min flow. 10 μg carbon tetrachloride and chloroform (from alkaline distillation of chloral hydrate) in 0.1 cc distillate were detected on a 20:80 triethylene glycol, firebrick column at temperature 78°C and flow of 30 cc He/min.

Summary. By fractional distillation of 1 to 3 cc of blood and gas chromatographic analysis of the distillate in glycol columns, blood ethanol concentrations were determined to an accuracy of 5 mg % and to a sensitivity of 10 mg % in the presence of methanol, formaldehyde and putrified blood. Microgram amounts of hydrogen sulfide, ether, acetone, acetaldehyde, chloroform and carbon tetrachloride were also identified in aqueous solutions.

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