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The Rapid Determination of Ethanol in Postmortem Brain Samples by Solvent Extraction and Gas Chromatography

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ABSTRACT

Distillation of brain tissue is a commonly used technique in the determination of brain ethanol concentrations. However, distillation suffers the disadvantages of being both time consuming and cumbersome in the forensic laboratory. In this study, we present a new, rapid method for the determination of ethanol content in brain tissue samples. This method employs solvent extraction with n-butanol and subsequent gas chromatographic analysis using n-propanol as an internal standard. The sensitivity of this technique was found to be similar to that obtained by distillation. The solvent extraction method presented has the advantages of rapidity of assay and ease of quantitation.

Key words : Ethanol, Postmortem Brain, Solvent Extraction, Gas Chromatography

INTRODUCTION

Deaths resulting from acute alcohol intoxication are due to centrally mediated respriatory failure (Forney and Harger, 1970). Therefore, forensic analysis of brain alcohol content can be especially important in the medical-legal establishment of cause of death (Baselt and Cravey, 1980). Postmortem blood ethanol concentrations are affected by postmortem diffusion of ethanol from the cadaver stomach and by enzymatic activity in the blood (Bonventre *et al.*, 1982). Brain samples should also be analyzed for alcohol concentration when creditable blood samples can not be obtained. One laboratory has reported as many as 3% of their cases involving situations wherein creditable blood samples were unavailable (Budd, 1982, 1983).

The concentration of ethanol in tissues has been determined by numerous methods including : steam distillation with sequential dichromate analysis (Nicloux, 1906), steam distillation with subsequent chromatographic analysis (Christopoulos *et al.*, 1073), direct chromatographic analysis of protein precipitated supernatant (Budd, 1983), and various enzymatic oxidation methods employing alcohol dehydrogenase (Jatlow and Bailey, 1980). Most of these methods have the disadvantage of being both time consuming and cumbersome; as well as, inaccurate in the hands of the inexperienced. In this study, steam distillation, a method still commonly used in forensic laboratories, is compared to an extraction procedure designed for the rapid and convenient determination of brain ethanol concentrations.

MATERIALS AND METHODS

I. *Reagents and Instrument*

Absolute ethanol was obtained from Abbott Laboratories (North Chicago, Illinois, USA). Reagent grade n-butanol and n-propanol were obtained from the J.T. Baker company (Phillipsburg, New Jersey, USA).

All gas chromatographic analyses were performed utilizing a Hewlett-Packard Model 5720 gas chromatograph equipped with a flame ionization detector (FID). A glass column (6' long with a 1/4" outer diameter), packed with 80 mesh Poropak Q^R was employed. The gas chromatograph was operated under the following conditions: inlet temperature=105°C, column temperature =100°C, detector temperature =300°C, and carrier gas flow=20ml/min. The detector output was recorded on a Hewlett-Packard 3390A integrator. Peak areas produced by ethanol and the n-propanol internal standard were used in the calculation of ethanol concentrations. Brain alcohol concentrations were corrected for recovery rate.

II. *Sample Preparation*

Samples were distilled via an Ace^R mini-lab distillation assembly (distilling adapter, West condenser, short stem adapter, and single neck balloon flask). Samples were placed into the balloon flask and connected by ground glass joints to the water cooled reflux condenser and receiver. Heat was provided by an asbestos heating mantle. The distillate was analyzed according to a modification of the gas chromatographic method of Parker et al., (1962).

Samples were extracted after sonication with a Polytron PT-10 sonicator. One milliliter of n-butanol, containing 0.8 mg of n-propanol, was added to each tissue homogenate. One milliliter of saturated ammonium sulfate was subsequently added to aid separation. The diluted homogenate was shaken thoroughly. Subsequently, the samples were centrifuged for five minutes at high speed (on an IEC^R Clinical Centrifuge). One microliter of the n-butanol layer was injected into the gas chromatograph.

III. *Assay Validation*

"Spiked" brain tissue samples from naive rats, brain tissue from intoxicated rats, and human forensic samples were analyzed by each technique. One milliliter of either 0.149, 0.233, 0.396, 0.494, or 0.699% (w/v) ethanol was added to one gram of brain tissue obtained from naive rats. Groups of three rats each were administered 47.5% (v/v) ethanol in doses of 38, 76, or 150 mg/kg body weight by intraperitoneal injection. Brain samples were obtained upon sacrifice of the animals. Human brain samples were obtained from autopsied victims predetermined to have been intoxicated at death. Methanol, acetone and isopropanol were tested for possible interference.

IV. *Statistics*

The student-t test was utilized to determine statistically significant differences between values obtained by both techniques.

RESULTS

Figure 1 is a typical chromatograph of a n-butanol extract of brain tissue containing ethanol. Ethanol was noted to elute rapidly off the column, followed closely by n-propanol. A clean separation was obtained with well defined integration. Table 1 demonstrates that other alcohols did not interfere with the n-butanol method of ethanol determination. Methanol, isopropanol , and acetone were extracted satisfactorily with n -butanol and presented no problem in the chromatography.

Table 2 demonstrates that both procedures had very similar results. No statistically significant difference was determined between ethanol concentrations arrived at by either technique at any "spike" concentration. Like-wise, Table 3 shows that solvent extraction and subsequent chromatography arrived at concentrations nearly identical to those obtained via distillation follo-

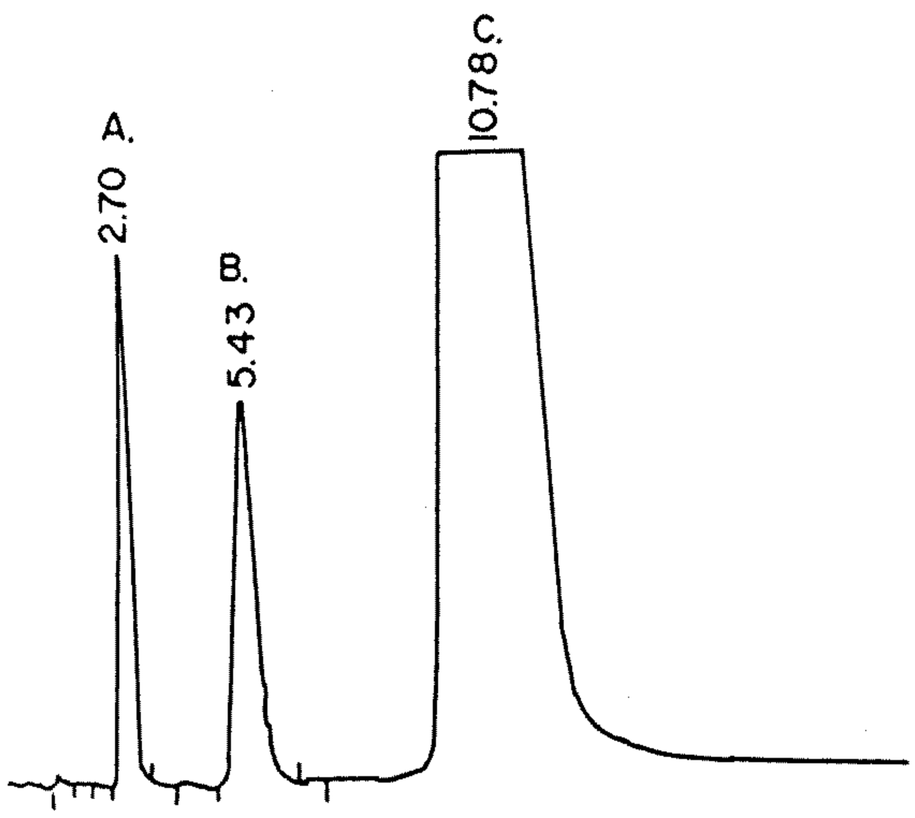

Figure 1. Gas chromatogram of an n-butanol extract of brain tissue containing ethanol.
a. ethanol, b. n-propanol (internal standard), c. n-butanol.

wed by gas chromatographic analysis. Table 4 demonstrates that there was very little difference between the ethanol concentrations determined in human forensic brain samples by either method.

DISCUSSION

Ethanol is a central nervous system depressant and as such produces death via respiratory arrest (Gleason *et al.* 1969). Blood concentrations are often used to predict ethanol concentrations in the brain (Forney et al., 1970). However, it must be recognized that brain ethanol concentrations provide a more accurate measurement of the extent of intoxication at time of death (Bonventre et al., 1982). In addition, creditable blood samples are sometime unobtainable (Budd, 1982).

Tissue ethanol levels are usually determined by steam distillation followed either by chemical reduction via acid dichromate, oxidation by alcohol dehydrogenase, or gas-liquid chromatography. Steam distillation is a burdensome method that requires a certain degree of exper-

Table 1 Alcohol and Acetone Retention Times

Substance	Retention time (min)
Methanol	1.60
Ethanol	2.70
Acetone	4.18
n-Popanol	5.43
n-Butanol	10.78

Table 2 Determination of Ethanol Concentrations in "Spiked" Brain Tissue (n=5)

Ethanol (%w/w)	Distillation (%w/w)	n-Butanol Extraction (%w/w)
0.149	0.102±0.006	0.117±0.008
0.233	0.299±0.038	0.262±0.020
0.396	0.330±0.010	0.378±0.018
0.494	0.453±0.020	0.470±0.014
0.699	0.562±0.028	0.550±0.040
Linear Coefficient Correlation	0.94	0.97

Table 3 Determination of Ethanol Concentrations in Brain Tissue and Blood Obtained from Intoxicated Rats (n=3)

Dose of Ethanol[1]	Blood[2]	Brain[2] Distillation	Brain[2] n-Butanol Extraction
0	0	0	0
38	0.028±0.006	0.020±0.002	0.024±0.002
76	0.061±0.004	0.043±0.008	0.048±0.006
150	0.103±0.023	0.094±0.002	0.102±0.016
1-Doses are calculated in mg/kg body weight			
2-Values are expressed as % w/w			
Linear Correlation Coefficient	0.98	0.99	0.99

Table 4 Determinatin of Ethanol Concentrations in Brain Tissue Obtained from Autopsied Humans

Case Number	Distillation[1]	n-Butanol Extraction[1]
1	0.162	0.142
2	0.092	0.086
3	0.130	0.126
4	0.132	0.135

1-Values are expressed as % w/w.

tise for obtaining consistency in results. Variability in the length of distillation time, or in the source of calefaction, can lead to erroneous results, as can the lack of complete drying of the distillation apparatus between use (Christopoulos *et al.*, 1973).

The extraction procedure presented in this paper is much quicker than distillation and affords similar sensitivity. This method appears to render less opportunities for introducing variability due to lack of experience by personnel, or simple carelessness. It is an uncumbersome method which requires a minimum of laboratory space and clean up. The extraction method provides a quick and accurate means by which ethanol content of the brain can be determined directly.

REFERENCES

1. Baselt, R.C. and Cravey, R.H. 1980. Forensic Toxicology. In: DOULL,J., KLAASSEN, C. D. and AMDUR, M.O. (Eds) Casarett and Doull's Toxicology: The Basic Sciences of Poisons (2nd Edition), New York: Macmillan Publishing Co., Inc., pp. 661-676.
2. Bonventre, J., Valanju, S. and Bastos, M.L. 1982. Evaluation of ethanol analysis on brain and liver by head-space gas chromatography. Forensic Science International. 19 : 75-83.
3. Budd, R.D. 1982. Ethanol levels in postmortem body fluids. J. Chromatography. 252 : 315-318.
4. Budd, R.D. 1983. Postmortem brain alcohol levels. J. Chromatography 259 : 353-355.
5. Christopoulos, G. Kirch, E.R. and Gearien, J. E. 1973. Determination of ethanol in fresh and putrefied postmortem tissues. J. Chromatography. 87 : 455-472.
6. Forney, R.B. and Harger, R.N. 1970. The Alcohols. In: DIPALMA, J.R. (Ed.) Drill's Pharmacology in Medicine (4th Edition), New York: McGraw-Hill Book Co.,, pp. 295-302.
7. Gleason, M.N., Gosselin, R.E., Hodge, H.C. and Smith, R.P. Ethyl Alcohol. 1969. In: Clinical Toxicology of Commercial Products (3rd Edition), Baltimore: The Williams & Wilkins Co., pp. 97-100.
8. Jatlow, P.I. and Bailey D.N. 1980. Analytical Toxicology. In: SONNENWIRTH, A.C. AND JARETT, L. (Eds) Gradwohl's Clinical Laboratory Methods and Diagnosis (8th edition), St. Louis: C.V. Mosby Co., pp. 387.434.
9. Nicloux, M. 1906. Simplification de la methode de dosage de l'alcol dans le sang et dans les tissue, Compt. rend. Soc. de biol. 60 : 1034 -1037.
10. Parker, K.D., Fontan, C.R., Yee, J.L. and Kirk, P.L. 1962. Gas chromatographic determination of ethyl alcohol in blood or aqueous solution. Anal. Chem. 10 : 1234-1236.

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以溶劑萃取及氣相層析法快速測定屍體大腦中之乙醇含量

何 英 剛

摘 要

測定大腦中乙醇濃度常使用大腦組織蒸餾法，然而在法醫實驗室所使用的蒸餾法既費時又累贅，本研究係發展一套新穎又快速的方法以測定大腦組織中乙醇之含量。本方法是以n-butanol爲萃取溶劑，繼之以n-propanol爲內部對照標準品作氣相層析，結果發現此法之靈敏度與蒸餾法之靈敏度相近，並具有快速測定與容易定量之優點。