Method for Determining Ethchlorvynol in Urine and Serum by Gas Chromatography

By DON W. ROBINSON

A new gas chromatographic technique for the identification and quantitative determination of ethchlorvynol in urine and serum is described. The method was developed because of the need for a rapid analysis of the drug in cases of suspected overdosage. The procedure was tested at concentrations of 3 to 100 mcg./ml.

PREVIOUS METHODS (1, 2) for the determination of ethchlorvynol,[1] a nonbarbiturate hypnotic, in biological fluids have involved distillations and chemical reactions. The procedure described is specific and does not require altering the ethchlorvynol molecule.

In developing a gas chromatographic method, it was noted that the presence of alcohol in urine would interfere with the ethchlorvynol peak. The syringe and injector become clogged when serum was injected directly. These problems were solved by the addition of an extraction step.

Two drugs of similar structure, ethinamate[2] and methylparafynol,[3] were found not to interfere. Kazyak and Knoblock have also shown different retention times for these compounds on a silicone column (3).

Recovery of therapeutic and overdoses of ethchlorvynol using chemical methods have been reported in the literature (1, 2).

EXPERIMENTAL

Method and Materials—*Instrument*—A gas chromatograph equipped with a flame ionization detector. A 5 ft. by $^1/_8$ in. outside diameter stainless steel column packed with Silicone L-46[4] 20% on acid washed Chromosorb W 80 to 100 mesh.[5] The liquid phase was applied by the evaporation technique. The column should be conditioned at 225° for 24 hr. prior to use. A Hamilton microliter syringe equipped with a Chaney adapter set to deliver 5 μl.

Instrument Conditions—Column temperature, 125°; injector temperature, 150°; nitrogen carrier gas, 22 ml./min.; electrometer, maximum sensitivity; and attenuator, 1.

Reagents and Solutions—Carbon disulfide analytical reagent.[6] Urine and serum from a subject who has not recently ingested ethchlorvynol and which does not produce an ethchlorvynol response after extraction and injection into the chromatograph.

Procedure—Weigh accurately 0.1 Gm. of ethchlorvynol into a 100-ml. volumetric flask. Bring to volume with urine or serum which does not contain the drug. This solution contains 1000 mcg./ml. and is used for making further dilutions. The solution may be frozen and used at a later date.

Urine Analysis—Using a separator, extract 50 ml. of urine with two portions of carbon disulfide, 30 and 20 ml. Inject 5 μl. of the carbon disulfide layer and measure the area of the ethchlorvynol peak. Extract a standard of ethchlorvynol in urine at the suspected concentration in exactly the same manner. Inject 5 μl. of the standard extract and measure the peak area.

Serum Analysis—The extraction procedure for serum must be modified because of the usually small volume available. Pipet 5 ml. of serum along with 5 ml. of carbon disulfide into a glass-stoppered centrifuge tube. Shake gently for 2 min. Care must be taken to prevent formation of an emulsion which cannot be broken. Centrifuge at 2000 r.p.m. for 15 min. Inject 5 μl. of the carbon disulfide layer and measure the peak area. Treat a standard of ethychlorvynol in serum at the suspected concentration in exactly the same manner.

Under the conditions described, the ethchlorvynol should be eluted in 3 to 4 min. A chromatogram is shown in Fig. 1. Using toluene as the reference standard, the relative retention time is 3.0:

Calculation—

$$\frac{\text{area sample peak}}{\text{area std. peak}} \times \text{concn. of std., mcg./ml.} = \text{sample concn., mcg./ml.}$$

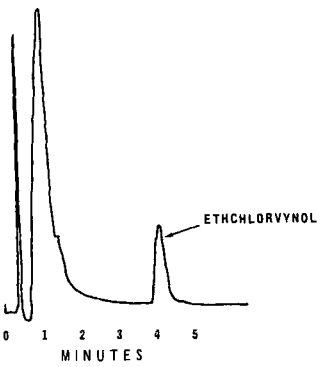

Fig. 1—Chromatogram of carbon disulfide extract from human serum containing 18 mcg./ml. ethchlorvynol. Five foot × $^1/_8$ in. stainless steel column packed with 20% Silicon L-46 on Chromosorb W 80 to 100 mesh. Temperature 125°; nitrogen at 22 ml./min.

DISCUSSION

Carbon disulfide was chosen as the extracting solvent because of its good solvent properties and because of the low response it produces on the flame ionization detector (4).

Received May 15, 1967, from the Analytical Research Department, Abbott Laboratories, North Chicago, IL 60064
Accepted for publication August 8, 1967.

[1] Placidyl. Abbott Laboratories, North Chicago, Ill.
[2] Marketed as Valmid by Eli Lilly & Co., Indianapolis, Ind.
[3] Marketed as Dormison by Schering Corp., Union, N. J.
[4] Silicone Division, Union Carbide Corp.
[5] Johns-Manville Corp.
[6] Mallinckrodt No. 4352. Purification is not necessary.

The standard extracts are stable and may be tightly sealed and stored at low temperature for use at a later date.

To further confirm the presence of ethchlorvynol, the extract can be chromatographed on a column of different polarity. A 5 ft. by $^1/_8$ in. outside diameter stainless steel column packed with diethylene glycol succinate 20% on Gas-Pack W[7] 60/80 gives good separation. When operated under the same conditions employed with the Silicone L-46 and using benzyl alcohol as the reference standard, the relative retention time for ethchlorvynol is 0.54.

To establish the linearity of the method, standard curves were prepared for both urine and serum. Concentrations of 5, 10, 25, 50, and 100 mcg./ml. were prepared and extracted. The curves are shown in Figs. 2 and 3. The ability of the procedure to recover the drug quantitatively from urine and serum was tested. The results were obtained by comparing the sample to a standard of similar concentration. Two of the urine samples also contained 0.2% ethanol to check for possible interferences. The results are in Tables I and II.

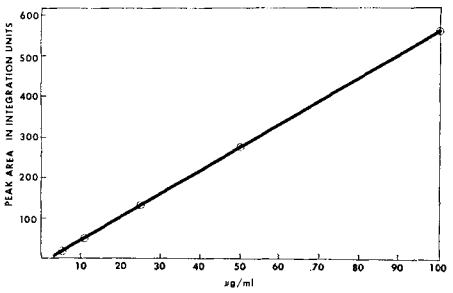

Fig. 2—Standards of 5, 10, 25, 50, and 100 mcg./ml. ethchlorvynol in urine extracted with CS_2.

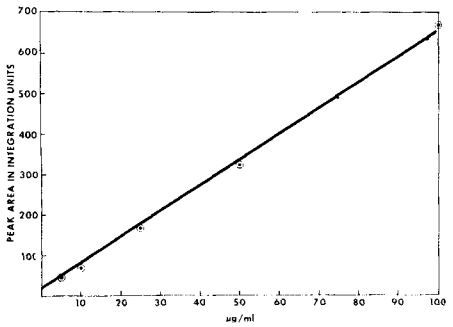

Fig. 3—Standards of 5, 10, 25, 50, and 100 mcg./ml. ethchlorvynol in serum extracted with CS_2.

The method can be performed in approximately 1 hr. and used to confirm suspected misuse of the drug.

[7] Chemical Research Services.

TABLE I—RECOVERY OF ETHCHLORVYNOL FROM HUMAN URINE

Added to Urine, mcg./ml.	Found, mcg./ml.
3[a]	2
3[a]	2
3[a]	2
18	18
18	17
18	19
33	32
33	31
33	32
65[a]	70
65[a]	61
65[a]	63
82	81
82	83
82	87

[a] Also contained 0.2% ethanol.

TABLE II—RECOVERY OF ETHCHLORVYNOL FROM HUMAN SERUM

Added to Serum, mcg./ml.	Found, mcg./ml.
3	3
3	4
3	3
18	19
18	18
18	18
33	33
33	34
33	33
65	65
65	67
65	66
82	82
82	79
82	80

REFERENCES

(1) Algeri, E. J., Katsas, G. G., and Luango, M. A., *Am. Clin. Pathol., J.* **38**, 125(1962).
(2) Wallace, J. E., Wilson, W. J., and Dahl, E. V., *J. Forensic Sci.*, **9**, 342(1964).
(3) Kazyak, L., and Knoblock, E. C., *Anal. Chem.*, **35**, 1448(1963).
(4) Dal Nogare, S., and Juvet, R. S., Jr., "Gas-Liquid Chromatography," Interscience Publishers, New York, N. Y., 1962, p. 222.

Keyphrases

Ethchlorvynol—analysis
Urine—extraction of ethchlorvynol from
Plasma—extraction of ethchlorvynol from
Carbon disulfide reagent
Gas chromatographic analysis