Journal of Chromatography, 111 (1975) 422–425

CHROM. 8391

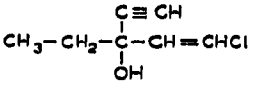
Note

Determination of ethchlorvynol by high-pressure liquid chromatography

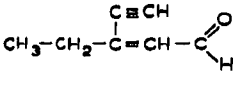
LARRY L. NEEDHAM and MAN M. KOCHHAR
School of Pharmacy, Auburn University, Auburn, Ala. 36830 (U.S.A.)
(Received April 21st, 1975)

1-Chloro-3-ethylpent-1-en-4-yn-3-ol (ethchlorvynol, I), a non-barbiturate sedative, has often been abused by itself or in combination with other drugs[1]. Most of the qualitative and quantitative methods of its detection from biological specimens have disadvantages. Its detection by gas–liquid chromatography is often unrewarding due to the high volatility of the drug. Colorimetric methods[2–4] vary from being non-specific to somewhat specific. Other procedures[5,6] utilize the acid-catalyzed reaction of ethchlorvynol to an unsaturated aldehyde (II), which then is reacted with semicarbazide hydrochloride to form the semicarbazone (III)[7]. This reaction converts ethchlorvynol, a non-UV-absorbing compound, to a semicarbazone, a very strong UV absorber. In our work[8] which was designed to identify metabolites of ethchlorvynol it was observed that the method of Wallace *et al.*[6] sometimes gave UV readings on drug-free serum from rats. Also, Gibson and Wright[1] pointed out that the method of Wallace *et al.*[5] is non-specific; however, Wallace *et al.*[6] disagreed and gave evidence supporting the specificity of their method. Nevertheless, the problems of non-specificity and interference of substances from serum are circumvented by the use of high-speed liquid chromatography (HPLC) which utilizes a UV detector.

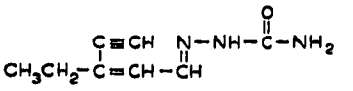
This paper describes a rapid, sensitive and very specific method for the detection of ethchlorvynol using HPLC.



I



II



III

MATERIALS AND METHODS

Apparatus

A GPC/ALC 202/401 Waters liquid chromatograph with a differential UV detector equipped with a 280 nm UV converter was employed. The chromatograph was equipped with a U6K universal injector, which allows injections of up to 2 ml. A 1 ft. × 1/4 in. stainless-steel column packed with C_{18} microbondapak was used in this study. The chromatograph was run at room temperature with a pressure of approximately 2000 p.s.i., which varied slightly due to the different solvent systems.

A Powerstat (Superior Electric) variable temperature heater was used in order to reflux the sample.

Procedure

Serum from rats, which were given 100 mg of ethchlorvynol per kg body weight intraperitoneally, was collected and extracted twice with *n*-heptane (10 and 15 ml). The organic layers were combined and placed in a round bottom flask containing 10 ml of 1 *N* hydrochloric acid. The mixture was refluxed for 25 min while stirring. After cooling the organic layer was separated and shaken with 5 ml of 0.5 *N* semicarbazide hydrochloride buffered to pH 3.5 with sodium acetate for 3 min at room temperature. Amounts of the aqueous phase ranging from 25 μl to 2 ml were injected into the liquid chromatograph. A similar extraction procedure was followed with drug-free human urine to which various amounts of ethchlorvynol and/or other drugs, which are often taken with ethchlorvynol, had been added.

Chromatographic operation

The solvent system for the chromatography consisted of either methanol–water (60:40), methanol–water (45:55), or water–absolute ethanol (70:30) at a flow-rate of 1 ml/min. The column effluent was monitored with a UV detector at 280 nm with various sensitivity ranges. Sample sizes varying from 25 μl to 2 ml were injected through the U6K injector.

RESULTS

For the purpose of quantitation, the semicarbazone was synthesized and isolated on a 1-g scale. From this pure compound, solutions ranging from 1 ng/ml to 10 μg/ml were prepared and analyzed by HPLC. The absorbance of 2 ml of 1 ng/ml of semicarbazone at 0.02 absorbance units full scale (a.u.f.s.) was 0.0004 mm; this was the lowest amount detected. A graph (Fig. 1) of absorbance *versus* concentration was plotted. This graph is a close fit to linearity. It was also shown that a plot from 1 μg/ml

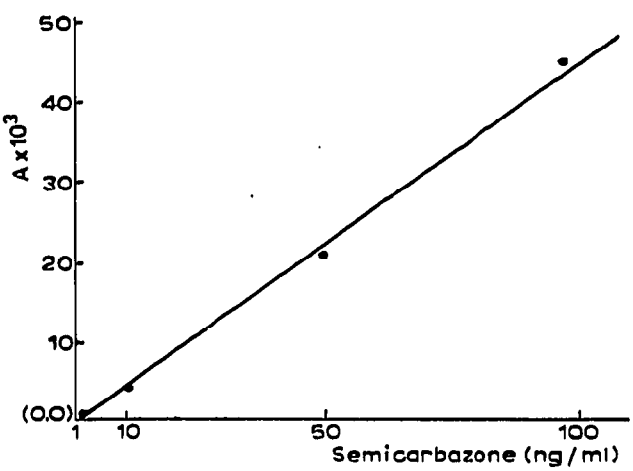


Fig. 1. A plot of absorbance *vs.* concentration (ng/ml) of the semicarbazone. Conditions: sample size, 2 ml; eluent, water–absolute ethanol (70:30); flow-rate, 1 ml/min; range, all at 0.04 a.u.f.s. except 100 ng/ml, which was run at 0.16 a.u.f.s., but for graph purposes proportioned to 0.04 a.u.f.s.

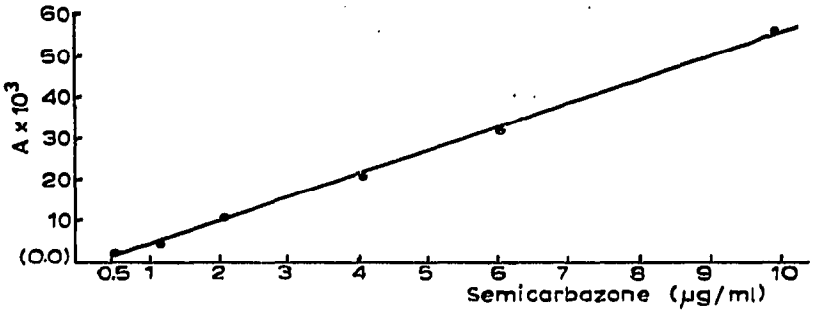


Fig. 2. A plot of absorbance *vs.* concentration (μg/ml) of the semicarbazone. Conditions: sample size, 25 μl; eluent, water–absolute ethanol (70:30); flow-rate, 1 ml/min; range, 0.16 a.u.f.s.

to 10 μg/ml is nearly linear (Fig. 2); however, it was necessary in order to keep the peak on scale to inject less of these higher concentrations of semicarbazone and to increase the sensitivity range. Nevertheless, these higher concentrations are also nearly linear to the lower ones; *e.g.*, 2 ml of 0.01 μg/ml at 0.04 a.u.f.s. gave an absorbance of 0.004 mm; whereas 25 μl/ml at 0.16 a.u.f.s. gave an absorbance of 0.0052 mm. Had 2 ml of the 1 μg/ml solution been injected, the absorbance should have been 0.41. Since a 1 μg/ml sample is one hundred times more concentrated than 0.01 μg/ml, which had an absorbance of 0.004, the graph is linear over a wide range and different sensitivity ranges and sample volumes can be directly compared. The same type of comparative results were obtained with biological specimens.

Since it is known that the extraction of ethchlorvynol from biological specimens is independent of pH and that the formation of the semicarbazone is linear to the concentration of the ethchlorvynol[6], our efforts were directed toward the determination of the sensitivity limits of this method and the linearity at low concentration levels. As seen in Fig. 3 the plot is nearly linear and the lowest level of ethchlorvynol detected as the semicarbazone was 0.05 μg/ml.

The retention volume of semicarbazone using absolute ethanol–water (30:70) or methanol–water (60:40) at 1 ml/min was approximately 5 ml. Since the polar

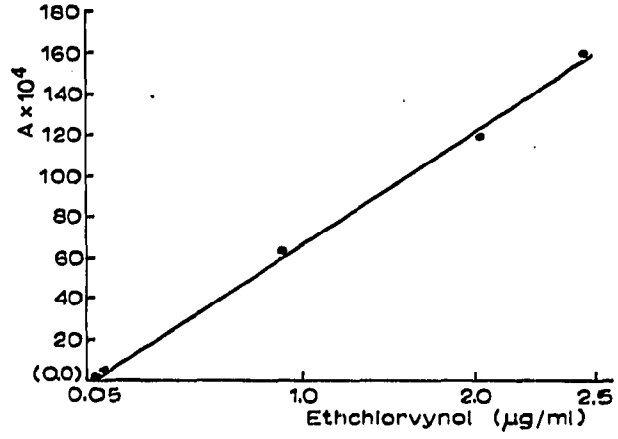


Fig. 3. A plot of absorbance *vs.* concentration of ethchlorvynol. Sample sizes and sensitivity range varied in order to keep peak on scale; however, for graph purposes they are proportioned to 2 ml at 0.04 a.u.f.s. Eluent, methanol–water (45:55). Flow-rate, 1 ml/min.

semicarbazide hydrochloride was only slightly retained on this nonpolar reversed-phase column, separation of peaks was achieved by injecting 25 μl of the samples. When 2 ml of sample was injected, the peaks for the semicarbazide hydrochloride and semicarbazone overlapped. The separation of these compounds was achieved by decreasing the quantity of methanol in the eluent, *e.g.*, methanol–water (45:55). This increased the retention volume of the semicarbazone to 15 ml but did not affect the semicarbazide hydrochloride.

DISCUSSION

Since the molar extinction coefficient of the semicarbazone of 289 nm is 31,000 (ref. 9), UV spectroscopy is a very sensitive method to detect this ethchlorvynol derivative. If interfering substances are present, separation can be achieved using various solvent systems; therefore, the liquid chromatographic method adds specificity. Several drugs which are often taken in conjunction with ethchlorvynol did not interfere at concentrations of 10 μg/ml. These drugs include diazepam, ethinamate, glutethimide, several phenothiazines, methyprylon, several barbiturates, meprobamate, and 5,5-diphenylhydantoin. Another advantage of this method over other methods, such as ionization gas chromatography, is the recovery of the semicarbazone which can be reconfirmed by other analytical techniques.

ACKNOWLEDGEMENTS

This study was supported by grant No. 2275-17-6728 from the National Institute on Drug Abuse. We also acknowledge Ms. Rashmi Bhushan and Mr. Lalit Bavda for preparing the blood samples from rats. Also, Abbott Laboratories is acknowledged for the supply of ethchlorvynol.

REFERENCES

1 P. F. Gibson and N. Wright, *J. Pharm. Sci.*, 61 (1972) 169; and references therein.
2 C. S. Frings and P. S. Cohen, *Amer. J. Clin. Pathol.*, 38 (1970) 833.
3 E. J. Algeri, G. G. Katsas and M. A. Luongo, *Amer. J. Clin. Pathol.*, 28 (1962) 125.
4 S. Andrayanskas, W. Matusiak, J. R. Broich, P. Giaquimta and L. A. Dal Cortivo, *Int. Microfilm J. Leg. Med.*, 2 (1967) 26.
5 J. E. Wallace, W. J. Wilson and E. V. Dahl, *J. Forensic Sci.*, 9 (1964) 342.
6 J. E. Wallace, H. E. Hamilton, J. A. Riloff and K. Blum, *Clin. Chem.*, 20 (1974) 159.
7 L. L. Needham and M. M. Kochhar, *Clin. Chem.*, 21 (1975) 169.
8 M. M. Kochhar and L. L. Needham, in preparation.
9 W. N. French, *J. Pharm. Sci.*, 53 (1964) 842.