Journal of Chromatography, 137 (1977) 119–126

CHROM. 9960

RAPID MICRO-METHOD FOR THE MEASUREMENT OF ETHCHLORVYNOL IN BLOOD PLASMA AND IN URINE BY GAS–LIQUID CHROMATOGRAPHY

R. J. FLANAGAN and T. D. LEE
Poisons Unit, New Cross Hospital, Avonley Road, London SE14 5ER (Great Britain)
(Received January 11th, 1977)

SUMMARY

A rapid gas–liquid chromatographic method has been developed for use in the measurement of the hypnotic drug ethchlorvynol in small (50 μl) volumes of either blood plasma or urine. Neither solvent transfer nor evaporation steps are used in the procedure and sources of interference have proved to be minimal. The method has been applied primarily to the analysis of specimens obtained from patients who had ingested an overdose of this drug. However with slight modification, the technique may be used in the measurement of the plasma concentrations of ethchlorvynol attained during therapy.

INTRODUCTION

Ethchlorvynol (1-chloro-3-ethyl-1-penten-4-yn-3-ol) is used as both a sedative and hypnotic agent. Overdosage with this drug is infrequent[1], but fatal cases of self-poisoning have been reported[2,3] and the treatment of such patients by either haemodialysis or charcoal haemoperfusion has been discussed[1,4,5].

The presence of ethchlorvynol in a urine specimen can be detected easily by means of the chromogenic reaction of this compound with diphenylamine sulphate[6,7]. However, even a urine sample obtained from a volunteer who had ingested a normal therapeutic dose of ethchlorvynol was found to yield a strongly positive reaction in this test[7]. Therefore, in order to establish that overdosage with this drug has occurred and also to assess the value of any treatment applied, an accurate, rapid and reproducible method for use in the measurement of plasma ethchlorvynol concentrations is required.

Some of the techniques already available have been discussed by Cravey and Jain[8]. The spectrophotometric methods, including that of Wallace *et al.*[9], require relatively large sample volumes, involve derivative formation and may measure metabolites in addition to the parent drug. A liquid chromatographic procedure has been advocated[10], but again the formation of a derivative is required. Indeed, the use of this technique in the measurement of ethchlorvynol is difficult to justify since the volatility, ease of detection, pH-independent solubility in organic solvents[9] and concentrations of this compound encountered in overdose facilitate a straight forward anal-

ysis by the more widely available technique of gas–liquid chromatography (GLC). The analysis of ethchlorvynol by this latter method has been described as "unrewarding"[9,10], but this applies only if solvent evaporation procedures are used following the extraction of the drug from the biological fluid since such procedures will often result in the simultaneous loss of ethchlorvynol. The GLC analysis of solvent extracts of plasma without prior concentration has been used for some time in the assay of ethchlorvynol[8,11,12], but large volumes (1–5 ml) of both sample and solvent, together with long extraction times, were employed. Furthermore, only one procedure which has been applied to the analysis of biological specimens incorporated an internal standard[11].

The method described in this paper is based upon the principle of rapid extraction of a small (50 μl) plasma volume with an equal volume of solvent containing an internal standard, followed by the direct GLC analysis of this extract. This principle has been applied previously to the plasma analysis of barbiturates and allied hypnotics[13,14], diazepam and desmethyldiazepam[15] and acetanilide[16]. The use of a sample–solvent ratio of 1:1 enables the measurement of ethchlorvynol concentrations down to 2 mg/l. However, as shown for both diazepam and desmethyldiazepam[15], an increase in this ratio to 4:1 yields a concomitant increase in the sensitivity of the method. Thus the plasma concentrations of ethchlorvynol reported after therapeutic dosage can be measured without resort to electron capture detection[17]. In addition, the method described here has been shown to be applicable to the analysis of ethchlorvynol in urine specimens.

EXPERIMENTAL

Gas–liquid chromatography

A Pye 104 Model 24 dual-column gas chromatograph fitted with flame ionisation detectors was used throughout. The column and detector oven temperatures were 140° and 200°, respectively, and the injection port setting was 2. The carrier gas (nitrogen) flow-rate was 60 ml/min and the hydrogen and oxygen inlet pressures were 15 and 10 p.s.i., respectively, giving flow-rates of approximately 45 and 200 ml/min.

The column, a coiled glass tube 1.5 m × 4 mm I.D., was treated with a solution of 5% dichlorodimethylsilane in toluene for 1 h. It was then washed with methanol, dried at 100° and subsequently packed with 2% (w/w) Carbowax 20M (Field Instruments, Richmond, Great Britain) and 5% (w/w) KOH on HP Chromosorb W (80–100 mesh). The column packing was prepared using the rotating evaporator technique, the KOH loading being obtained initially by the evaporation of a methanolic solution of this compound in the presence of the support. The final loading was then obtained using this pretreated material and a solution of Carbowax 20M in chloroform. The packed column was conditioned at 200° with a nitrogen flow-rate of 60 ml/min for approximately 12 h prior to use.

On this system, ethchlorvynol had a retention time of 0.69 relative to the internal standard, 2-methylnaphthalene. The chromatography of a chloroform solution of both ethchlorvynol and 2-methylnaphthalene is illustrated in Fig. 1.

Extraction procedure

The extraction solvent was a 25 mg/l solution of 2-methylnaphthalene (Hopkin

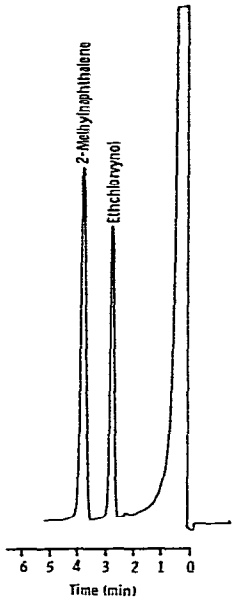

Fig. 1. The analysis of a chloroform solution containing ethchlorvynol (30 mg/l) and 2-methylnaphthalene (25 mg/l) on the Carbowax 20M–KOH column system. Injection, 3 μl.

& Williams, Chadwell Heath, Great Britain) in chloroform (analytical reagent grade). Ethchlorvynol was obtained as a light yellow oil from Abbott Labs., Queenborough, Great Britain; such material is said to be greater than 98% pure[18].

Plasma or urine (50 μl) was introduced into a Dreyer tube (Poulten, Selfe & Lee, Wickford, Great Britain) by means of a 50-μl semi-automatic pipette. Subsequently, 50 μl of the internal standard solution were added via a 2.5-ml Hamilton gas-tight luer-fitting glass syringe fitted with a Hamilton repeating mechanism (both available from Field Instruments, Richmond, Great Britain). An Everett stainless-steel needle (No. II serum) was affixed to this syringe.

The contents of the tube were mixed thoroughly on a vortex mixer for 30 sec and the tube was centrifuged for 2–3 min at approximately 2750 *g*. A 3–5-μl portion of the chloroform phase was subsequently obtained by (1) drawing 5 μl of air into a gas chromatographic syringe, (2) passing the syringe needle through the lipoprotein interface into the chloroform phase, (3) expelling the air and drawing-up the required volume of chloroform and (4) withdrawing the syringe and wiping the needle with a tissue. This extract was then injected onto the column of the gas chromatograph.

The extraction was performed in duplicate and a mean result obtained; if the difference between the duplicates was greater than 10% both the extractions and analysis were repeated.

Instrument calibration and calculation of results

Standard solutions containing ethchlorvynol at concentrations of from 10 to 100 mg/l in increments of 10 mg/l were prepared in chloroform by dilution of a 1-g/l

stock solution in this same solvent. Each standard also contained 2-methylnaphthalene at a concentration of 25 mg/l, obtained from a separate stock source. The ratio of the peak height of ethchlorvynol to the peak height of 2-methylnaphthalene bore a linear relationship to the ethchlorvynol concentration over the range studied; the normal calibration gradient obtained (*i.e.*, peak height ratio/drug concentration) was 0.028 l/mg. The results of plasma analyses were multiplied by a "recovery factor" of 1.08 to compensate for the incomplete extraction of the drug; the factor of 1.03 was used for urine analyses.

RESULTS AND DISCUSSION

Recovery studies

Standard ethchlorvynol solutions were prepared in 10.0 ml of either heparinised bovine plasma or drug-free human urine by dilution of from 50 to 250 μl of a 2-g/l solution of the drug in ethanol; the range of concentrations thus obtained was from 10 to 50 mg/l, in increments of 10 mg/l. The triplicate analysis of the plasma solutions by the present method revealed a mean ethchlorvynol recovery of 94 $\pm$ 3% (S.D.); this recovery was uniform over the range studied. The duplicate analysis of the urine standards revealed a similar uniform recovery of 97 $\pm$ 2% (S.D.).

Specificity

No interference from either endogenous sample constituents or other drugs has been encountered and extract contamination from other sources has not been found to occur. However, some possible sources of non-drug interference in direct-extract analyses have been discussed[14]. The analysis of a chloroform extract of drug-free human plasma is illustrated in Fig. 2. The chromatography of similar extracts performed in the absence of the internal standard has not revealed the presence of compounds which could elute with 2-methylnaphthalene. The analysis of a chloroform extract of plasma obtained from a patient who had ingested an overdose of ethchlorvynol is illustrated in Fig. 3. Entirely analogous results have been obtained on the analysis of urine specimens.

Two compounds which were found both to extract from plasma and to elute on the column system under the conditions of this assay are trichloroethanol and chlormethiazole. The retention times of these drugs were, respectively, 0.52 and 1.28 relative to 2-methylnaphthalene and thus they did not interfere in the analyses; the chromatography of a chloroform solution containing all of these compounds is illustrated in Fig. 4.

Alternative column systems

Although the Carbowax 20M–KOH column system has proved the system of choice for use in this assay (*cf.*, Figs. 1–4), additional systems were investigated. One was a 0.9-m coiled glass tube packed with 3% (w/w) Poly A 103 (Field Instruments) on HP Chromosorb W (80–100 mesh). The GLC conditions were identical to those described for the Carbowax 20M–KOH system, except that the column oven temperature was 120°. Ethchlorvynol had a retention time of 0.54 relative to 2-methylnaphthalene on this system and the calibration gradient obtained was 0.031 l/mg. Since this same column system formed an integral part of the procedure routinely used

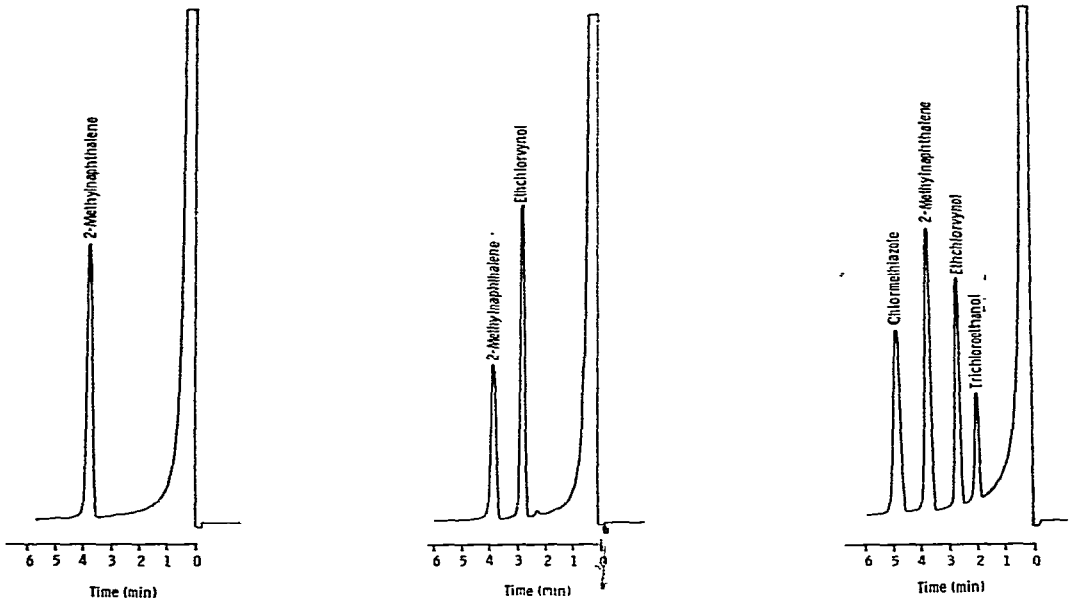

Fig. 2. The analysis of an extract of drug-free human plasma on the Carbowax 20M–KOH column system. The concentration of 2-methylnaphthalene was 25 mg/l; 3-μl injection.

Fig. 3. The analysis of an extract of plasma obtained from an ethchlorvynol overdose patient on the Carbowax 20M–KOH column system; 3-μl injection. The plasma ethchlorvynol concentration was found to be 78 mg/l.

Fig. 4. The analysis of a chloroform solution containing ethchlorvynol (30 mg/l), 2-methylnaphthalene (25 mg/l), trichloroethanol and chlormethiazole (both 50 mg/l) on the Carbowax 20M–KOH column system; 3-μl injection.

in our laboratory for the analysis of barbiturates and other hypnotics[14], a chromatograph containing such a system was always available, and was used in some emergency ethchlorvynol analyses. However, trichloroethanol was found to co-chromatograph with ethchlorvynol on this system and thus could interfere; chlormethiazole had a retention time of 0.85 relative to 2-methylnaphthalene and was resolved from the compounds of interest.

Columns based upon the stationary phase CDMS have proved less satisfactory than the systems discussed above since the ethchlorvynol peak was found to "tail". Non-polar phases, such as OV-1, were found to elute ethchlorvynol much more rapidly than 2-methylnaphthalene, *e.g.*, the relative retention time of ethchlorvynol on a 2.1-m 3% OV-1 system was 0.19, and thus a different internal standard would have been required if such columns had been used in the assay. Conversely, ethchlorvynol was found to have a retention time of 0.83 relative to 2-methylnaphthalene upon a 2.1-m 3% Carbowax 20M column system. Therefore, the Carbowax 20M–KOH system was preferred since the resolution of ethchlorvynol from the internal standard was greater. However, the retention times relative to 2-methylnaphthalene of trichloroethanol and chlormethiazole on the Carbowax 20M system were 0.55 and 1.55, respectively, and thus these compounds would not interefere in analyses performed using this sytem.

Limits of sensitivity

The use of a 1:1 sample–solvent ratio in the extraction procedure resulted in a limit of sensitivity of the technique to ethchlorvynol of 2 mg/l. However, it was found that by the use of a sample–solvent ratio of 4:1, plasma drug concentrations down to 0.5 mg/l could be measured easily. The formation of emulsions during the extraction was found to present no more of a problem when using this high sample–solvent ratio than in the normal extraction procedure; similar findings have been reported when using *n*-butyl acetate as an extraction solvent[15].

To illustrate this point, standard ethchlorvynol solutions in the range 2.0–10.0 mg/l were prepared in chloroform, in increments of 2.0 mg/l; each solution also contained 5.0 mg/l of 2-methylnaphthalene. The calibration gradient given upon analysis of these solutions was 0.148 l/mg. A further set of solutions containing from 0.5 to 2.0 mg/l of ethchlorvynol in increments of 0.5 mg/l were prepared in heparinised bovine plasma; the triplicate analysis of these solutions using 200 μl of plasma and 50 μl of internal standard solution (5 mg/l 2-methylnaphthalene in chloroform) revealed a mean ethchlorvynol recovery of 77 $\pm$ 2% (S.D.).

Applicability to urine analyses

Although the present method was designed for use with plasma specimens, it has been found to be equally applicable to urine; the qualitative analysis of urine specimens by the plasma hypnotic assay[13,14] has given similar results (R. J. Flanagan, unpublished results).

The measurement of urinary ethchlorvynol concentrations could prove of use in the measurement of the severity of overdosage if a plasma specimen is not available. As has been emphasised, the diphenylamine sulphate test[7] is not suitable for this purpose. The analysis of a urine specimen which gave a strongly positive reaction in this test following the ingestion of 500 mg of the drug[7] revealed an ethchlorvynol concentration of only 2 mg/l. In contrast, the urinary concentrations measured in six "spot" specimens which were obtained at approximately the same time as plasma samples from ethchlorvynol overdose patients were found to be from 34 to 63% of the plasma values; the lowest urinary concentration observed in these samples, the results from which are summarised in Table I, was 17 mg/l. Thus, although inevitably subject to a large degree of variability when compared to plasma concentrations, the measurement of urinary ethchlorvynol concentrations is likely to give more infor-

TABLE I

THE PLASMA AND URINARY CONCENTRATIONS OF ETHCHLORVYNOL MEASURED IN SPECIMENS FROM OVERDOSE PATIENTS

Specimen No.	*Ethchlorvynol (mg/l)*		*Urine–plasma ratio (%)*
	Plasma	*Urine*	
1	47	18	38
2	45	19	42
3	27	17	63
4	44	25	57
5	38	21	55
6	50	17	34

mation than that available from the diphenylamine sulphate test, which takes almost as long to complete. It should be noted that no advantage would be gained from an attempt to relate urinary ethchlorvynol and creatinine concentrations; the utility of this type of measurement has been both generally criticised[19] and shown not to be of use in the measurement of the urinary excretion of either α-amino nitrogen[19] or D-glucarate (R. J. Flanagan, unpublished results).

CONCLUSIONS

The results described in this paper provide further evidence of the advantages of micro-extraction techniques over bulk-extraction methods in drug analysis. Such considerations, *i.e.* the small sample requirement, speed of performance and enhanced accuracy and reproducibility, together with the wide applicability of these techniques, have been discussed in detail elsewhere[14,15].

The procedure described in the present paper represents an improvement over previously published methods for the measurement of both plasma and urinary ethchlorvynol concentrations and has been in routine use for over three years. The duplicate analysis of sample extracts can be accomplished within 15 min and both the qualitative and the quantitative measurements are performed by reference to an internal standard. The investigation of potential sources of interference in this assay has shown not only that both trichloroethanol and chlormethiazole do not interfere in this procedure, but also that this same technique may be of use in the simultaneous measurement of these drugs at the plasma concentrations attained in overdose.

ACKNOWLEDGEMENTS

We should like to thank Mr. D. J. Berry, Mr. D. M. Rutherford and Dr. R. Goulding for their criticism of this manuscript.

REFERENCES

1 B. P. Teehan, J. F. Maher, J. J. H. Carey, P. D. Flynn and G. E. Schreiner, *Ann. Intern. Med.*, 72 (1970) 875.
2 E. J. Algeri, G. G. Katsas and M. A. Luongo, *Amer. J. Clin. Pathol.*, 38 (1962) 125.
3 R. Maes, N. Hodnett, H. Landesman, G. Kananen, B. Finkle and I. Sunshine, *J. Forensic Sci.*, 14 (1969) 235.
4 T. N. Tozer, L. D. Witt, L. Gee, T. G. Tong and J. Gambertoglio, *Amer. J. Hosp. Pharm.*, 31 (1974) 986.
5 B. Widdop, R. K. Medd, R. A. Braithwaite, A. J. Rees and R. Goulding, *Arch. Toxicol.*, 34 (1975) 27.
6 S. Andryanskas, W. Matusiak, J. R. Broich, P. Giaguinta and L. A. Dal Cortivo, *Int. Microfilm J. Legal Med.*, 2 (1967).
7 D. J. Berry and J. Grove, *J. Chromatogr.*, 80 (1973) 205.
8 R. H. Cravey and N. C. Jain, *J. Chromatogr. Sci.*, 12 (1974) 237.
9 J. E. Wallace, H. E. Hamilton, J. A. Riloff and K. Blum, *Clin. Chem.*, 20 (1974) 159.
10 L. L. Needham and M. M. Kochhar, *J. Chromatogr.*, 111 (1975) 422.
11 P. F. Gibson and N. Wright, *J. Pharm. Sci.*, 61 (1972) 169.
12 J. Hedley-Whyte and L. H. Laasberg, *Anesthesiology*, 30 (1969) 107.
13 R. J. Flanagan and G. Withers, *J. Clin. Pathol.*, 25 (1972) 899.

14 R. J. Flanagan and D. J. Berry, *J. Chromatogr.*, 131 (1977) 131.
15 D. M. Rutherford, *J. Chromatogr.*, 137 (1977) 439.
16 F. Harvey, *Ph. D. Thesis*, University of London, London, 1976.
17 L. M. Cummins, Y. C. Martin and E. E. Scherfling, *J. Pharm. Sci.*, 60 (1971) 261.
18 A. W. Davidson, *J. Ass. Offic. Anal. Chem.*, 53 (1970) 834.
19 W. H. Peters, *Clin. Chim. Acta*, 45 (1973) 387.