Journal of Chromatography, 107 (1975) 107–114

CHROM. 8094

DETERMINATION OF TRICHLOROETHANOL AT THERAPEUTIC AND OVERDOSE LEVELS IN BLOOD AND URINE BY ELECTRON CAPTURE GAS CHROMATOGRAPHY

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(Received October 23rd, 1974)

SUMMARY

A specific and sensitive gas chromatographic method for the determination of trichloroethanol, the active metabolite of chloral hydrate, in blood and urine is reported. A simple dilution of the sample with an ethanolic solution of internal standard followed by gas chromatography with electron capture detection is described. The method has been used to determine plasma levels after therapeutic dosing with chloral preparations.

INTRODUCTION

Chloral hydrate has been in use medically for more than one hundred years and, in one form or another, is still widely prescribed. It is formulated as the hydrate either as a solution diluted and flavoured to mask the unpleasant taste or, more commonly, it is complexed chemically and incorporated into an acceptable solid-dose form.

In the body, chloral hydrate is rapidly metabolised to trichloroethanol, the pharmacologically active moiety, and to trichloroacetic acid[1,2]. None of the unchanged parent compound appears in human blood, even shortly after administration[3,4], so measurement of trichloroethanol is the best index of ingestion. This metabolite has been determined by a number of different techniques, colorimetry[5] and gas chromatography[4–8] both having been used.

When presented with urine from an unconscious patient, the toxicological analyst may be asked to undertake a rapid screening in order to determine whether drugs are implicated as the cause of the patient's condition. The urine spot test for chloral sedatives, based on the Fujiwara reaction[9], can be performed in a few minutes, but this is extremely sensitive, so a positive reaction is not necessarily indicative of overdose. Since these drugs are commonly prescribed and give a positive urine test at therapeutic doses, it is helpful to perform a rapid measurement of trichloroethanol levels in small samples of blood. By relating this concentration to known therapeutic values, one can assess whether in fact an overdose has been taken.

The present paper describes a rapid method of measuring trichloroethanol in

only 0.1 ml of plasma, or urine, following the ingestion of therapeutic doses, and reports some plasma concentrations after single oral dosing with two different formulations of the drug.

EXPERIMENTAL

Reagents

The following reagents were used: Absolute ethanol; dibromobenzene (Hopkin and Williams, Chadwell Heath, Great Britain); trichloroethanol (Koch-Light, Colnbrook, Bucks, Great Britain).

A diluting solution containing 0.01 mg of dibromobenzene in 100 ml absolute ethanol was prepared.

Gas chromatography

A Pye 104 Model 84 gas chromatograph was used and modified by removing the final column splitter so that all of the eluate passed to one detector. The instrument was fitted with a 7 mCi ^{63}Ni electron capture detector and attached to a 1-mV Hitachi 159 recorder.

The column was a 5-ft. × ¼-in.-I.D. coiled glass tube which had been silanised by filling with 5% dimethyldichlorosilane in toluene for 24 h. Glass wool was silanised in the same solution. After drying, the column was packed with Carbowax 6000 (Applied Science Labs., State College, Pa., U.S.A.) on 85–100 mesh Diatomite "CQ" (J.J., King's Lynn, Great Britain). This packing was prepared as follows: 0.75 g of Carbowax 6000 was dissolved in 200 ml chloroform and 24.25 g of Diatomite "CQ" were added to the flask, which was then left to stand, with occasional swirling, for 2 h. The solvent was removed under vacuum in a rotary evaporator, the final stages of evaporation being completed in a water-bath at 100° for 30 min. The prepared column was then packed with coated support while applying a vacuum. After filling, the end was closed with silanised glass wool and the column conditioned for 24 h at 170° with a nitrogen flow-rate of 55 ml/min. This column has been in constant use for two years and no significant change in behaviour has been observed.

The instrument settings were as follows: column oven temperature, 130°; detector oven temperature, 260°; sensitivity, 5×10^{-10} A; electron capture supply pulse space, 150 μsec; carrier gas (nitrogen) flow-rate, 70 ml/min.

At all times when not in use the detector was purged with a gas mixture of 5% carbon dioxide in nitrogen.

Extraction procedure

To 10 ml of the dibromobenzene diluting solution was added 0.1 ml sample (plasma, urine or aqueous standard) and it was mixed briefly using a vortex mixer. After centrifuging, 1–3 μl of the clear alcoholic solution was injected into the gas chromatograph.

RESULTS AND DISCUSSION

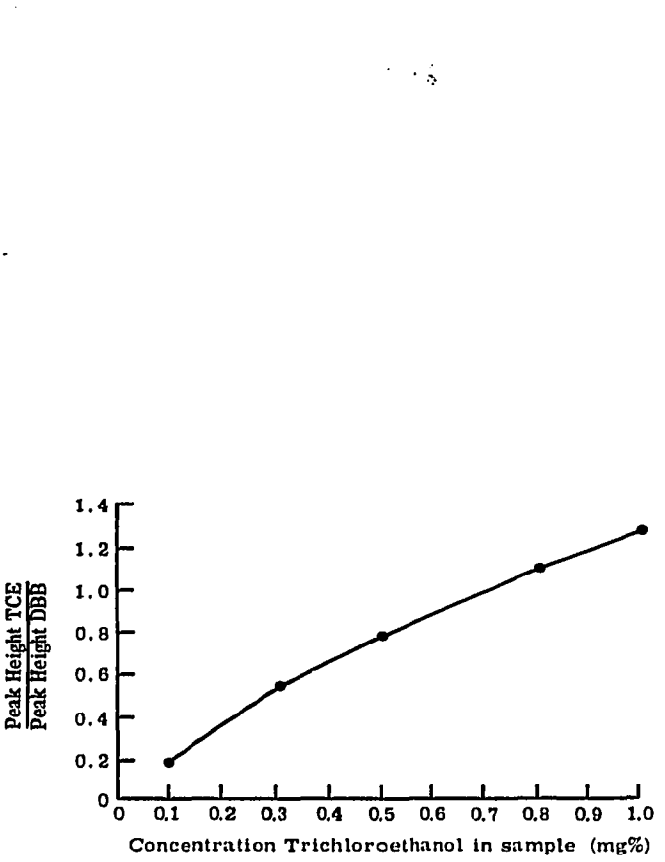
Measurement

Dibromobenzene (DBB) was chosen as internal standard since it eluted imme-

diately after trichloroethanol (TCE) with this column and was readily available in a sufficiently high state of purity.

A range of standard solutions containing 0.2 mg to 1.0 mg/100 ml of trichloroethanol was made in water. A standard curve was prepared by mixing 0.1-ml aliquots of each of these with 10 ml of diluting solution and injecting 1–3 μl into the gas chromatograph prior to measuring the unknown samples. A graph of trichloroethanol concentration *vs.* the ratio (peak height TCE)/(peak height DBB) was plotted from these standards and the peak height ratio for the samples was related to this so as to give a direct measure of the drug concentration. If the ratio fell outside the range of the standard curve, an aliquot of the work-up was further diluted with DBB solution before re-injecting and a dilution factor was then introduced into the final calculation.

The calibration graph was not completely linear over the range employed (Fig. 1), so it was necessary to prepare the standard curve on a daily basis. The relative retention time of trichlorethanol with respect to dibromobenzene was 0.8 (Fig. 2).



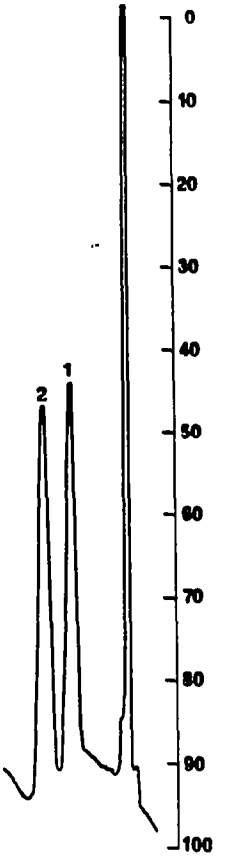


Fig. 1. Standard calibration graph relating the ratio of the peak heights of trichloroethanol and dibromobenzene to the concentration of trichloroethanol in the sample.

Fig. 2. Gas chromatogram of an aqueous standard showing the separation of trichloroethanol (1) and dibromobenzene (2).

Recovery studies and accuracy

A set of standards containing 0.2–1.0 mg/100 ml of trichloroethanol was also made up in plasma and the standard curve prepared using these. This curve did not differ significantly from that prepared with the aqueous standards, so recovery was taken to be essentially quantitative. The standard deviation of the method was ± 4%, being limited very much to the reproducibility of the electron capture detector and the accuracy with which one can take such a small plasma sample. It was found best to perform each analysis in duplicate and to take an average result.

Specificity

The procedure was specific no interfering peaks in the same region, as either trichloroethanol or dibromobenzene have been encountered from constituents of normal plasma or urine (Fig. 3). In addition, no exogenous compounds were found in samples derived from patients receiving a large variety of other drugs.

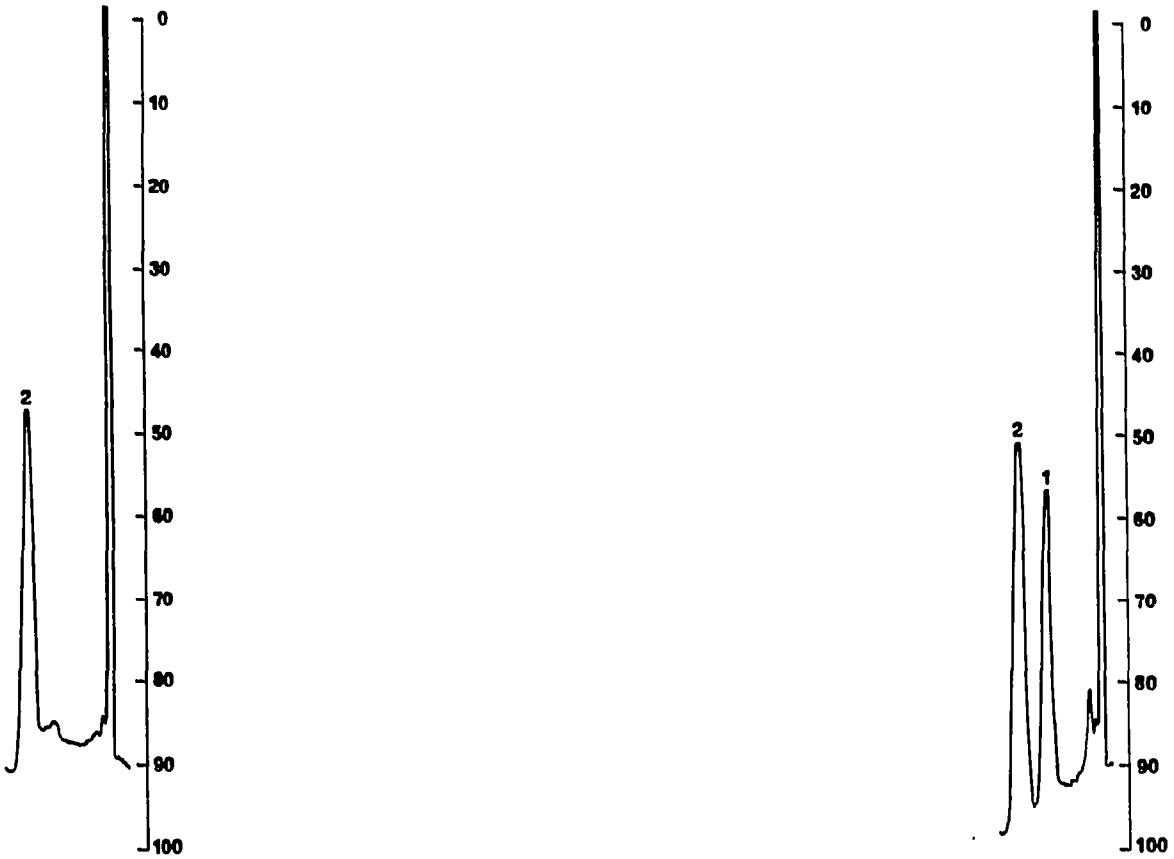


Fig. 3. Gas chromatogram of blank human plasma, with internal standard. For peak numbering, see Fig. 2.

Fig. 4. Gas chromatogram from plasma of an individual after a single 825-mg oral dose of chloral hydrate. For peak numbering, see Fig. 2.

Application

The procedure is extremely quick and sufficiently sensitive to measure plasma and urine levels of trichloroethanol in human subjects following a single therapeutic administration of chloral (Fig. 4). Plasma trichlor ethanol levels were measured following oral administration of chloral in two forms: (a) two Welldorm tablets supplied by Smith and Nephew Pharmaceuticals, Welwyn Garden City, Great Britain[12] (equivalent to 825 mg chloral hydrate in 1300 mg dichloral phenazone); (b) a gelatine capsule containing 825 mg chloral hydrate.

The results of administering the two different dosage forms, three weeks apart, to five individuals are shown in Tables I and II. Fig. 5 illustrates the plasma drug level curves in one of these subjects following the two types of medication. The red blood

TABLE I

TRICHLOROETHANOL PLASMA LEVELS (mg/100 ml) IN FIVE SUBJECTS FOLLOWING A SINGLE ORAL DOSE OF BOTH DOSAGE FORMS* OF CHLORAL HYDRATE (825 mg)
The sex, age and weight of the subjects were as follows: R.G. —male, 56 years, 188 lbs.; P.M. —female, 33 years, 115 lbs.; J.G. —male, 33 years, 160 lbs.; D.B. male, 25 years, 147 lbs.; R. B. —male, 23 years, 175 lbs.

Subject	*R.G.*		*P.M.*		*J.G.*		*D.B.*		*R.B.*	
Time (h)	*CH*	*DP*	*CH*	*DP*	*CH*	*DP*	*CH*	*DP*	*CH*	*DP*
0.5	0.76	0.39	0.64	0.51	0.88	0.75	0.94	0.86	0.98	1.00
1	0.66	0.69	1.22	1.13	0.96	0.77	0.94	0.95	0.90	1.00
1.5	0.53	0.64	1.15	1.24	0.76	0.76	0.89	0.96	0.88	0.95
2	0.53	0.57	0.93	1.07	0.71	0.66	0.86	0.90	0.85	0.74
3	0.44	0.48	0.76	0.82	0.60	0.66	0.75	0.87		
6	0.34	0.35	0.55	0.55	0.40	0.42	0.60	0.58	0.45	0.41
24	0.17	0.16	0.16	0.16	0.23	0.15	0.12	0.13	0.12	0.07

* Dosage form: CH = chloral hydrate capsule (825 mg); DP = dichloral phenazone (1300 mg).

TABLE II

URINARY OUTPUT OF TRICHLOROETHANOL DURING THE 24 HOURS FOLLOWING INGESTION OF 825 mg CHLORAL IN THE TWO DOSAGE FORMS

Subject	*Form*	*Trichloroethanol (mg excreted)*
R.G.	CH	4.1
	DP	3.5
P.M.	CH	6.1
	DP	5.0
J.G.	CH	5.4
	DP	4.6
D.B.	CH	5.7
	DP	4.1
R.B.	CH	3.3
	DP	2.8

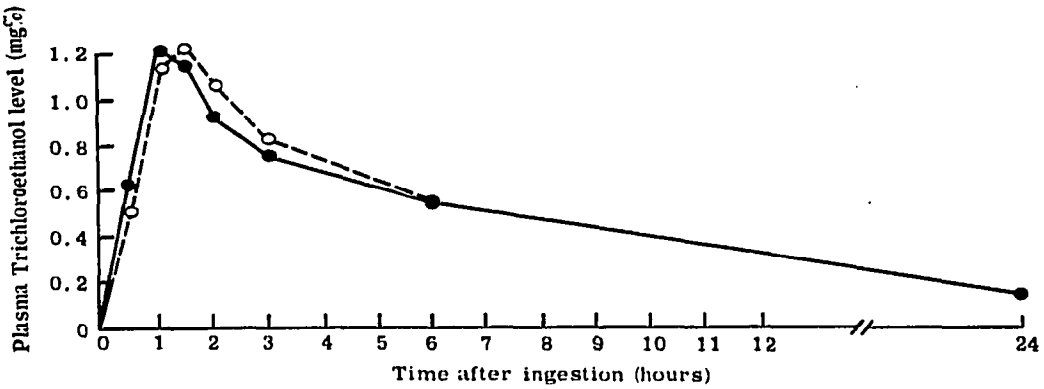


Fig. 5. A typical plasma trichloroethanol concentration *vs.* time curve indicating the levels found in one subject following both chloral preparations. ●—●, Chloral hydrate capsule; ○---○, dichloral phenazone.

cells from some of these experiments were analysed by washing with isotonic saline and haemolysing with cold water before extraction. The distribution of TCE between the red cells and plasma after these therapeutic doses was found to be approximately 50:50.

Further, some levels found in comatose patients following overdosage with chloral hydrate, together with any other relevant information are shown in Table III.

The method has also been used to measure levels of trichloroethanol in blood and milk from nursing mothers[10], since chloral is favoured as a night hypnotic in many maternity units. Also we have measured trichloroethanol plasma concentrations following trichloroethylene anaesthesia.

TABLE III

TRICHLOROETHANOL PLASMA CONCENTRATIONS IN SOME SELF-PIOSONING CASES

Patient	*Plasma trichloroethanol (mg%)*	*Comments*
A.W.	55	Post mortem specimen
L.J.	40	Post mortem specimen
K.C.	48	Post mortem specimen
C.W.	57	Grade 4 coma, no other drugs detected
I.K.	45	Grade 4 coma, no other drugs detected
I.K.	34	Grade 4 coma, no other drugs detected
R.G.	13	Grade 3 coma, no other drugs detected
L.E.	12	Grade 3 coma, benzodiazepines also present
A.B.	11	Grade 2 coma, alcohol also present
B.B.	9	Grade 2 coma, no other drugs detected
I.K.	7	Grade 2 coma, no other drugs detected
I.S.	4	Grade 3 coma, barbiturates also present
L.D.	4	Grade 1 coma, no other drugs detected
S.R.	3	Grade 3 coma, benzodiazepines and antidepressants also present
L.O.	2	Grade 3 coma, barbiturates and alcohol also present

DISCUSSION

The main criticism against using an electron capture detector for routine estimations is that contamination occurs very easily if proper steps are not taken to avoid it. In this context, the present method, which uses a one hundredfold dilution of plasma or urine with ethanol, is an improvement on some previous procedures, which dilute to a lesser extent and so inject more contaminants onto the column[3]. Also, use of a greater dilution leads to a good column life, the performance of the packing having remained unchanged for two years. Carbowax 6000, moreover, behaved as an excellent column. It yielded distinct peaks and, by using relatively low loadings, it could be operated well below its maximum recommended temperature, resulting in excellent stability. Under these conditions detector contamination by column bleed was negligible. The high-loading FFAP* column used by Jain *et al.*[3] was, in our hands, less stable and more liable to contaminate the detector. Other workers have undertaken either solvent extraction or head space analysis to overcome contamination problems, but the presently reported method is much more useful for emergency work since it is so rapid and also avoids detector contamination by massive dilution of the sample before injection into the gas chromatograph.

The practice of purging the detector was obligatory. This was done at all times when the instrument was not in use by passing a mixture of 5% carbon dioxide in nitrogen into the purge gas line at 25 p.s.i., a measure that proved extremely effective in maintaining detector performance. To operate the instrument, the purge gas was turned off 30 min before use to give the carrier gas time to flush all traces of carbon dioxide from the detector.

The absolute ethanol was occasionally found to contain impurities which interfered in the chromatographic step. No attempt was made to purify this and each batch was pre-tested before use to ensure that it was suitable, being discarded if extraneous peaks occurred.

A set of aqueous trichloroethanol standards was prepared and stored in a refrigerator at 4°. These were diluted and analysed daily to ensure that the detector was performing satisfactorily. When stored at 4° these solutions did not deteriorate significantly.

An internal standard was incorporated to allow for both any injection errors and any slight intra-analysis detector response variations. The chlorbutol recommended by Jain *et al.*[3] on his FFAP did not separate from trichloroethanol on our Carbowax column. Dibromobenzene was found to be quite satisfactory, however, since it eluted close to, but separated well, from trichloroethanol.

Because of the narrow linear response range of the detector it is necessary to successively dilute overdose samples until they fall within the calibration curve. This is done by diluting 1 ml of supernatant from the initial extract with 9.0 ml of dibromobenzene solution before re-injection. If necessary, further successive dilutions are done until a peak ratio is accomplished within the range of the calibration graph and then a dilution factor is applied in the final calculation.

This procedure has been most useful with patients in coma, suspected as being caused by chloral hydrate, and has diagnosed whether in fact this was the case.

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* FFAP = Free fatty acid phase.

ACKNOWLEDGEMENTS

I wish to thank Dr. R. Goulding, Director of the Poisons Unit, for valuable advice and encouragement. Thanks are also due to Miss G. L. Thornley for valuable technical assistance.

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