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ANALYSIS OF CHLORMETHIAZOLE, ETHCHLORVYNOL AND TRICHLOROETHANOL IN BIOLOGICAL FLUIDS BY GAS–LIQUID CHROMATOGRAPHY AS AN AID TO THE DIAGNOSIS OF ACUTE POISONING

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SUMMARY

A simple method has been developed whereby chlormethiazole, ethchlorvynol and trichloroethanol can be simultaneously detected and measured in biological fluids. The procedure is based upon the rapid extraction of a small (50-μl) sample volume with an equal volume of chloroform containing an internal standard, followed by the gas–liquid chromatographic analysis of this extract. Specimens of blood plasma or serum, urine and gastric contents can be used, and no interference from either endogenous or exogenous sources has been observed. The method is suitable for the measurement of the plasma concentrations of these compounds attained after overdosage.

INTRODUCTION

Chlormethiazole, ethchlorvynol and trichloroethanol (the pharmacologically active metabolite of chloral hydrate[1,2]) can all cause coma if ingested in sufficient quantity, and thus the detection and identification of these compounds may be of clinical relevance. Ethchlorvynol and trichloroethanol can be detected by simple chromogenic reactions which are applicable essentially to urine and only provide qualitative information[3]. Moreover, in the case of this latter compound, the test[4] is not specific and will detect other trichloro-compounds such as chloroform. Chlormethiazole and its metabolites may be detected by the thin-layer chromatographic analysis of a chloroform extract of alkaline urine, but identification may prove difficult, especially if some other drugs have been ingested.

A gas–liquid chromatographic (GLC) technique for the analysis of ethchlorvynol in specimens of either plasma or urine obtained from poisoned patients has been described[5]. This method is rapid, specific, sensitive and requires only 100 μl of sample to enable a duplicate analysis to be performed. The investigation of possible sources of interference in this assay showed that not only were chlormethiazole and

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trichloroethanol resolved from both ethchlorvynol and the internal standard on the chromatographic system used, but also that they were extracted into chloroform under the same conditions as this latter drug. Subsequently, a modification of this technique has been found to be applicable to the analysis of all three compounds in specimens obtained from poisoned patients.

EXPERIMENTAL

Chemicals and reagents

Chlormethiazole free base (Astra Chemicals, Watford, Great Britain) and 2,2,2-trichloroethanol (Aldrich, Gillingham, Great Britain) were stored at −20° prior to use. The source and purity of the ethchlorvynol used have been discussed previously[5]. The internal standard, 2-methylnaphthalene (Hopkin and Williams, Chadwell Heath, Great Britain) was used as a 20 mg/l solution in chloroform (analytical-reagent grade).

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 1.5 m × 4 mm I.D. coiled glass tube, was packed with 2% (w/w) Carbowax 20 M (Field Instruments, Richmond, Great Britain) and 5% (w/w) KOH on HP Chromosorb W, 80–100 mesh[5]. On this system, trichloroethanol, ethchlorvynol and chlormethiazole had retention times of 0.52, 0.69 and 1.28, respectively, relative to 2-methylnaphthalene.

Extraction procedure

The sample (50 μl) was introduced into a Dreyer tube (Poulten, Selfe and Lee, Wickford, Great Britain) by means of a semi-automatic pipette; specimens of gastric contents containing large amounts of solid material were centrifuged prior to analysis in order to obtain a clear fluid. 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). 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 30 sec. at 9950·g in an Eppendorf centrifuge 5412 (Anderman and Co., East Molesey, Great Britain and modified to accept Dreyer tubes by slight drilling-out of the 0.4 ml test tube centrifuge adaptors). Subsequently, a 3- to 5-μl portion of the chloroform phase was obtained as described[5] and 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.

TABLE I

DRUG STANDARD SOLUTIONS AND CALIBRATION GRADIENTS

Each solution also contained 2-methylnaphthalene at a concentration of 20 mg/l.

Compounds	*Calibration gradient (l/mg)*	*Standard drug solutions available (mg/l)*											
		5	*10*	*15*	*20*	*25*	*30*	*40*	*50*	*75*	*100*	*150*	*200*	*250*
Chlormethiazole	0.016	×	×	×	×	×	×	×	×					
Ethchlorvynol	0.035		×		×		×	×	×	×	×	×		
Trichloroethanol	0.013								×		×	×	×	×

Instrument calibration and calculation of results

Standard solutions containing each drug were prepared in chloroform by dilution of a 1 g/l stock solution in this same solvent (Table I). Each standard also contained 2-methylnaphthalene at a concentration of 20 mg/l, obtained from a separate stock source. The ratio of the peak height of each drug to the peak height of 2-methylnaphthalene bore a linear relationship to the drug concentration over the ranges studied. The normal calibration gradients obtained (*i.e.* peak height ratio–drug concentration) are shown in Table I. The results of sample analyses were multiplied by a "recovery factor" to compensate for the incomplete extraction of each drug. The factors used for either plasma or urine analyses are given in Table II.

TABLE II

RECOVERIES OF ADDED DRUG FROM EITHER HEPARINISED BOVINE PLASMA OR DRUG-FREE HUMAN URINE

Compounds	*Standard solutions*		*Plasma*		*Urine*	
	Range (mg/l)	*Increment (mg/l)*	*Mean ± SD (%)*	*Recovery factor*	*Mean ± SD (%)*	*Recovery factor*
Chlormethiazole	10– 50	10	96 ± 4	1.04	100 ± 2	1.00
Ethchlorvynol	20–100	20	95 ± 4	1.05	95 ± 2	1.05
Trichloroethanol	50–250	50	75 ± 4	1.33	78 ± 3	1.28

RESULTS AND DISCUSSION

Recovery studies

Standard solutions were prepared in 10.0 ml of either heparinised bovine plasma or drug-free human urine by dilution of a 2-g/l solution of each drug in ethanol, and the range of concentrations thus obtained is shown in Table II. The quintuplicate and triplicate analyses of the plasma and urine solutions, respectively, revealed the mean drug recoveries given in Table II. Each recovery was uniform over the range studied.

Sources of interference

The method has been applied primarily to the analysis of plasma or serum specimens obtained from poisoned patients and no interference from either endogenous sample constituents or other drugs has been encountered. Examples of the

chromatograms obtained on analysis of drug-free human plasma and of plasma obtained from an ethchlorvynol overdose patient have been given previously[5]. The analysis of a plasma specimen from a patient who had ingested a large amount of dichloralphenazone is illustrated in Fig. 1.

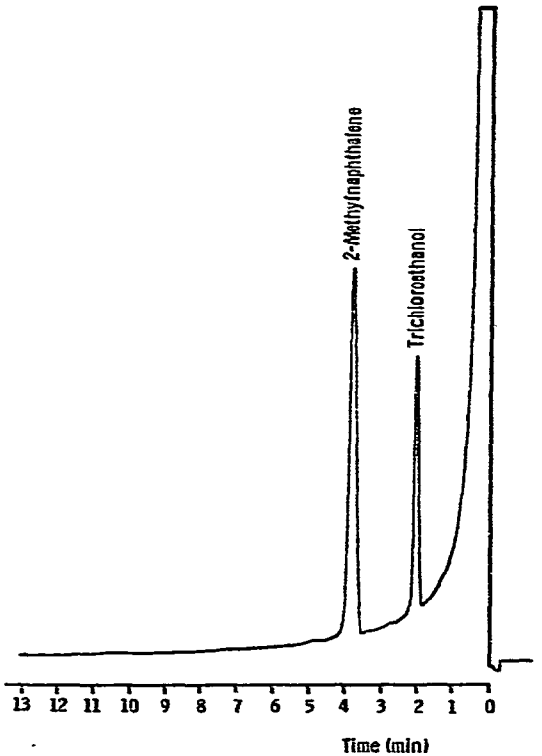

Fig. 1. The analysis of an extract of plasma obtained from a dichloralphenazone overdose patient on the Carbowax 20M–KOH column system; 3-μl injection. The plasma trichloroethanol concentration was found to be 71 mg/l.

A feature of the analyses of urine (and in some cases of plasma) from chlormethiazole overdose patients is the presence of several compounds which elute after chlormethiazole on the Carbowax 20 M–KOH column system (Fig. 2). These compounds are probably chlormethiazole metabolites, but specific identifications have not been attempted. Although up to five metabolites of this drug have been identified in human urine[6,7], all thought to result from oxidation of the 2-chloroethyl moiety of chlormethiazole[7], the fate of only approximately 20% of the dose has been defined. The possibility that metabolites of chlormethiazole might interfere in the assay has been investigated. Analyses on a second GLC column system (2.1 m × 4 mm I.D. glass column packed with 10% Apiezon L–2% KOH on 80–100 mesh Chromosorb W AW (obtained ready-prepared from Chromatography Services Ltd., Hoylake, Great Britain)) at 160° have given identical quantitative results to those obtained from the same extract on the Carbowax 20 M–KOH column system. The retention times relative to 2-methylnaphthalene of the peak corresponding in area to "metab-

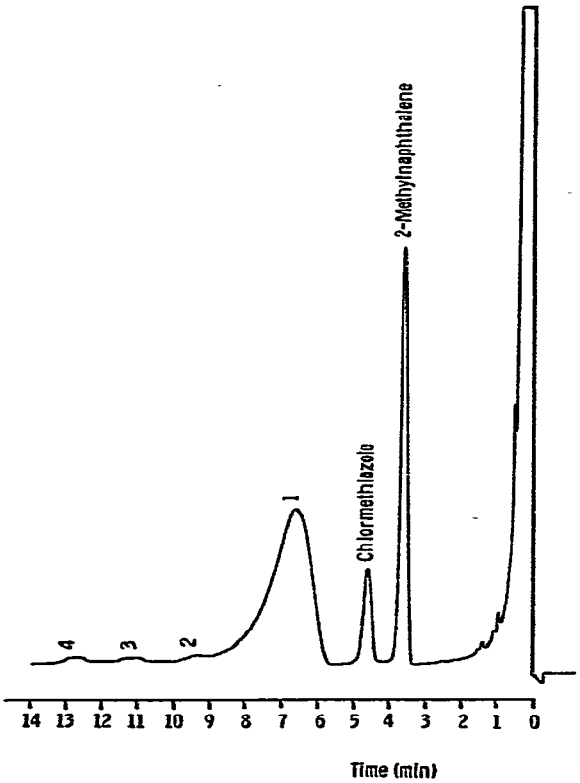

Fig. 2. The analysis of an extract of a urine specimen obtained from a chlormethiazole overdose patient on the Carbowax 20M–KOH column system; 3-μl injection. Compound 1 (and possibly compounds 2–4) are metabolites of chlormethiazole (see text).

olite 1" (*cf.* Fig. 2) and of chlormethiazole were 0.40 and 0.51, respectively, on the Apiezon L–KOH column system. All of these compounds gave rise to sharp, symmetrical peaks, but no other metabolites were observed on this latter system.

It has been found that both chlormethiazole and ethchlorvynol may be readily detected and identified in gastric content specimens by use of this procedure, and an example of such an analysis is given in Fig. 3. However, chloral hydrate was neither extracted[8] nor chromatographed under the conditions of this assay.

Limits of sensitivity

When using a sample–solvent ratio of 1:1, the minimum sensitivities of the technique to chlormethiazole, ethchlorvynol and trichloroethanol were 2, 2 and 10 mg/l, respectively, at the amplifier attenuation normally used ($5 \cdot 10^{-10}$ A). The plasma concentrations of both ethchlorvynol and trichloroethanol attained in overdose are above these limits[5,9], and the available data suggested that this also applied for chlormethiazole; the intravenous infusion to six volunteer subjects of from 1.20 to 2.25 g of chlormethiazole ethanedisulphonate at rates varying from 11.9 to 25.0 mg/min gave rise to plasma drug concentrations between 3 and 40 mg/l at the cessation of infusion[10]. The results from nine patients who had ingested an overdose of chlor-

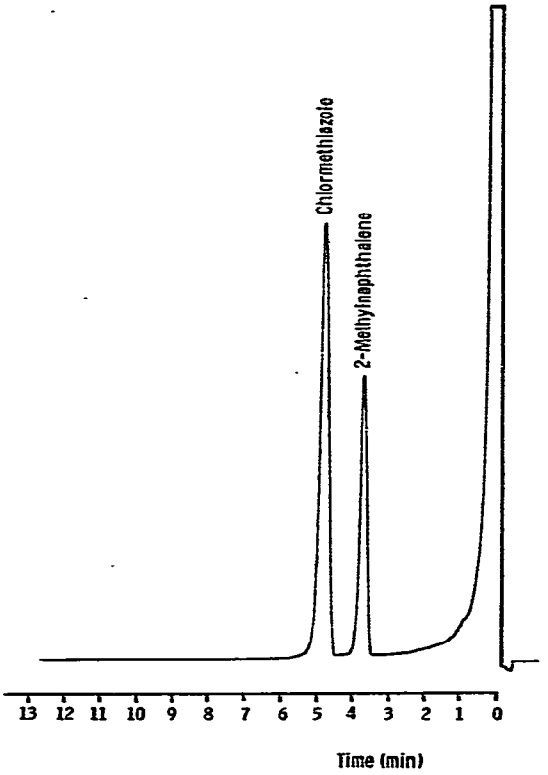

Fig. 3. The analysis of an extract of a 1:20 aqueous dilution of a specimen of gastric contents obtained from a chlormethiazole overdose patient on the Carbowax 20M–KOH column system; 2-μl injection.

TABLE III

PLASMA CONCENTRATIONS AND ADDITIONAL DATA FROM NINE PATIENTS WHO HAD INGESTED AN OVERDOSE OF CHLORMETHIAZOLE

Patient No.	*Age (yr)*	*Sex*	*Plasma chlormethiazole (mg/l)*	*Grade of coma at time of sampling*	*Other drugs detected*
1	63	F	16	IV	Diazepam
2	38	F	9	II	Nil
3	26	F	66	IV	Dextropropoxyphene, Nitrazepam
4	40	M	11	III	Nil
5	61	F	8	III	Nil
6	34	M	8	II	Nil
7	47	M	37	IV	Chlorimipramine, Diazepam
8	48	F	14	IV	Nitrazepam
9	77	F	12	III	Nil

methiazole either alone or together with other drugs supported this view (Table III). All of these patients recovered uneventfully.

The use of electron-capture detection has been advocated recently[9] for the measurement of the plasma trichloroethanol concentrations attained in overdose. However, the results presented here clearly show that flame-ionisation detection is satisfactory even though only 50 μl of specimen are required. Indeed, smaller volumes of both sample and solvent can be used with no decrease in sensitivity. On the other hand, greater sensitivity to all of the drugs studied with the present technique was attainable if required. The use of a higher instrument sensitivity together with a less concentrated internal standard solution served to increase the minimum sensitivities of the method 10-fold without a concomitant increase in the interference observed. In addition, a higher sample–solvent ratio in the cases of both ethchlorvynol[5] and chlormethiazole also increased the minimum sensitivities attainable.

CONCLUSIONS

Analytical techniques which are specific, sensitive, rapid and provide not only qualitative but also quantitative information are advantageous in clinical toxicology. The method described here has been used in the assay of several hundred specimens obtained from poisoned patients during the course of our 24-h drug analysis service over a period of approximately one year. Even quantitative analyses were completed within 20 min and with the use of a very small sample volume. The GLC column system has proved to be extremely stable under the conditions used, and the method represents a considerable improvement over methods used previously in our laboratory for the analysis of chlormethiazole, ethchlorvynol and trichloroethanol in biological fluids.

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