Journal of Chromatography, 133 (1977) 167–172

CHROM. 9669

QUANTITATIVE ANALYSIS OF DISULFIRAM AND ITS METABOLITES IN HUMAN BLOOD BY GAS–LIQUID CHROMATOGRAPHY

ANDRÉ M. SAUTER and JEAN P. VON WARTBURG
Medizinisch-chemisches Institut, University of Berne, Bühlstrasse 28, CH-3012 Berne (Switzerland)
(First received May 28th, 1976; revised manuscript received August 13th, 1976)

SUMMARY

A simple method is described that permits the direct quantitative determination of carbon disulphide, free diethyldithiocarbamate and disulphides derived from disulfiram in 1 ml of a patient's blood. It is based on a gas chromatographic determination of carbon disulphide produced from diethyldithiocarbamate and disulfiram using the head-space technique and a flame-photometric detector. The method is compared with a recently described spectrophotometric method.

INTRODUCTION

Disulfiram (tetraethylthiuram disulphide, Antabuse) has frequently been used as an aversively protective drug in the treatment of alcoholism since its introduction in 1948 by Hald *et al.*[1]. It is administered either orally or by implantation. After resorption, disulfiram is known to be reduced immediately to diethyldithiocarbamate (DDC) or to react with free thiol groups of proteins to form mixed disulphides[2,3]. Further degradation leads to carbon disulphide, which is another metabolite found in the blood and breath of patients treated with disulfiram[4,5].

Previous methods for the determination of disulfiram or its metabolites lack the necessary specificity and sensitivity[6–11]. Hence, a spectrophotometric method was recently developed in our laboratory, which permits the separate quantitative analysis of disulfiram and its two main metabolites, carbon disulphide and DDC, with adequate precision in 10 ml of a patient's blood[5]. This method has been used with success in our laboratory for the routine determination of disulfiram and its metabolites in the blood of alcoholic patients receiving the drug orally or by implantation, but it suffers from the following inconveniences: firstly, it is time consuming (1 h per analysis); secondly, 20 ml blood for double analyses are required; and thirdly, patients with implanted disulfiram show blood levels that are at the limit of the sensitivity. Therefore, a more sensitive and rapid gas chromatographic method has been developed, which is based on the determination of free carbon disulphide and carbon disulphide liberated from DDC and mixed disulphides in human blood using the head-space technique.

EXPERIMENTAL

Reagents

All reagents were of the highest available commercial grade from Fluka (Buchs, Switzerland) or Merck (Darmstadt, G.F.R.) and were used without further purification.

Instrumentation

A Hewlett-Packard 5710 A gas chromatograph equipped with a Tracor flame-photometric detector operating at 394 nm for sulphur detection and a Goerz Elektro Servogor S recorder were used. The glass column, 200 × 0.3 cm I.D., was packed with 2.4% QF-1 and 0.5% Carbowax 20 M on 80–100-mesh Gas-Chrom Q. The operating conditions were as follows: column temperature, isothermal at 25°; detector temperature, 200°; injection temperature, 150°; gas flow-rates, carrier gas (nitrogen) at 30 ml/min, hydrogen at 50 ml/min, air at 50 ml/min and oxygen at 10 ml/min.

Preparation of samples

For the determination of free carbon disulphide, a 60-ml vial containing 0.4 g of sodium chloride was sealed under nitrogen with an aluminium-coated rubber septum. For the determination of carbon disulphide liberated from free DDC and disulphides (mixed disulphides and/or disulfiram), a 60-ml vial containing 0.4 g of sodium chloride, 10 mg of cysteine hydrochloride and 0.5 ml of 98–100% formic acid was used. Differentiation between mixed disulphides and disulfiram is not possible. A sample of 1 ml of blood was then injected into each vial by use of a 1-ml syringe and the vials were immediately shaken vigorously for 30 sec using a Vortex mixer.

Injection and calculation

After equilibration at room temperature for at least 2 h, 1 ml of the head-space gas was withdrawn from the vial with a gas-tight Hamilton syringe and injected into the gas chromatograph. The peak height was used for quantitation.

RESULTS AND DISCUSSION

Calibration graphs

A calibration graph for carbon disulphide was obtained by adding various amounts of carbon disulphide to 1-ml samples of human blood. The retention time was 41 sec (Fig. 1). After 80 sec, the analysis was complete and a new sample was injected. Peak heights were plotted against the carbon disulphide concentration. A non-linear graph was obtained owing to the non-linear response of the detector to sulphur (Fig. 2). However, the graph became linear for carbon disulphide concentrations between 0.01 and 0.60 μg/ml when the cube root of the square of the peak heights was plotted against the carbon disulphide concentration (Fig. 3).

A calibration graph for DDC was obtained by adding various amounts of sodium DDC trihydrate to 1-ml samples of human blood. Taking into account the fact that 1 ml of blood is mixed in the vial with 0.5 ml of formic acid and 10 mg of cysteine hydrochloride in order to liberate carbon disulphide from free DDC and mixed disulphides and that 1 μg of sodium DDC trihydrate (molecular weight 225.3)

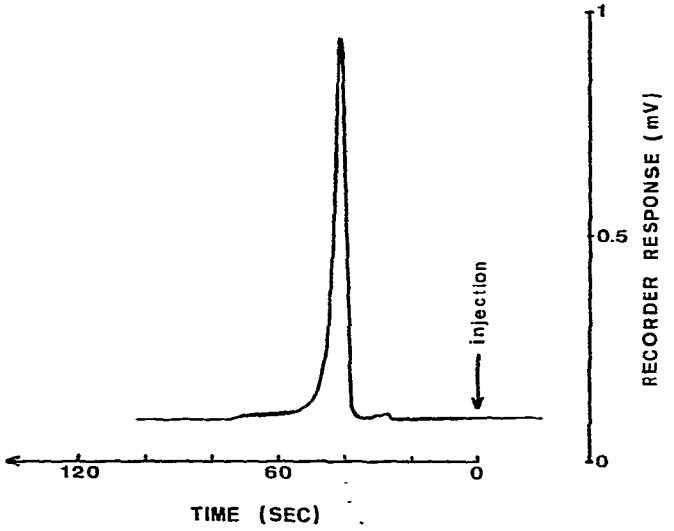

Fig. 1. Chromatogramm of carbon disulphide from a human blood sample. The conditions are described under Experimental.

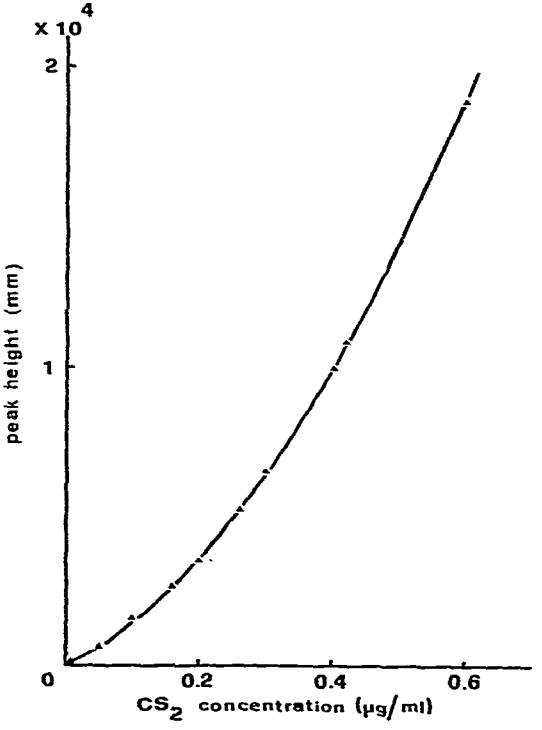

Fig. 2. Calibration graph for carbon disulphide. Various amounts of carbon disulphide added to human blood were analyzed as described under Experimental.

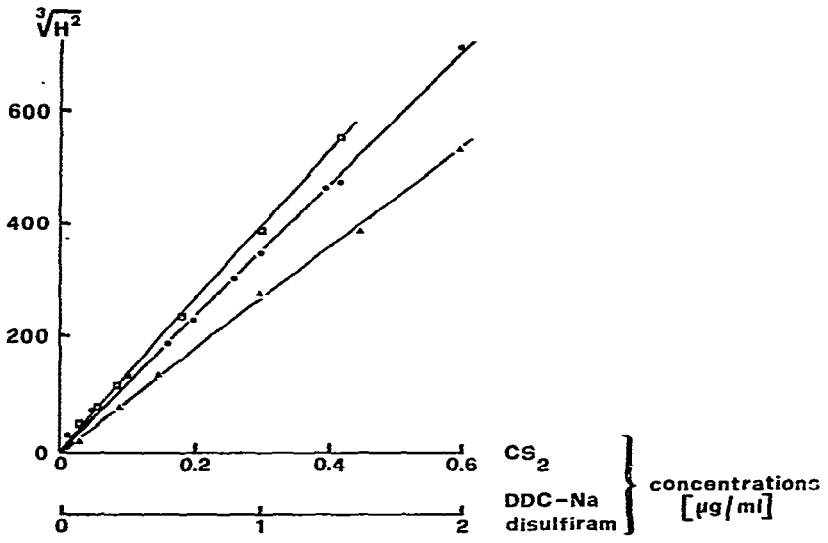

Fig. 3. Calibration graphs for carbon disulphide (●—●), DDC (▲—▲) and disulfiram (□—□). Linearization was obtained by plotting the cube root of the square of the peak height (H mm).

produces 0.30 µg of carbon disulphide (molecular weight 76), the calibration graph for DDC is in good agreement with that for carbon disulphide. Complete recovery of carbon disulphide produced from DDC is obtained with cysteine (Fig. 3). Without reduction of the mixed disulphides by cysteine, only the free fraction of DDC produces carbon disulphide.

A calibration graph for disulfiram was obtained in the same way as for DDC. As 1 mole of disulfiram produces 2 moles of carbon disulphide, 1 µg of disulfiram (molecular weight 296.5) corresponds to 0.51 µg of carbon disulphide (Fig. 3). Complete recovery of carbon disulphide is obtained.

Sensitivity and precision

In order to determine the sensitivity and precision of the method, several blood samples containing low and high concentrations of carbon disulphide, DDC or disulfiram were analyzed as described under Experimental. The mean values of the peak heights, standard deviations and coefficients of variation are given in Table I.

As described previously[5], the concentration ranges of disulfiram metabolites found during the daily administration of a standard dose of 200 mg of disulfiram to humans are 0–0.6 µg/ml of carbon disulphide and 0.2–1.0 µg/ml of DDC. A large individual and daily variability is observed. After the usual dose given before a drinking test (800 mg/day for 3 days), carbon disulphide concentrations were found to range between 0.02 and 0.31 µg/ml and DDC concentrations between 0.27 and 1.43 µg/ml (ref. 12).

Analysis of patients' blood and comparison with a spectrophotometric method

In order to test the practicability of this method and to compare it with a

TABLE I

WITHIN-DAY PRECISION OF THE METHOD

All analyses were carried out as described under Experimental. The mean values given are peak heights.

	CS_2 Concentration (μg/ml)			*Na DDC concentration (μg/ml)*			*Disulfiram concentration (μg/ml)*		
	0.01	*0.10*	*0.60*	*0.10*	*0.30*	*2.00*	*0.10*	*0.30*	*1.40*
Mean ($n = 6$)	152	1500	18,800	101	640	12,050	352	700	12,800
Standard deviation	13	63	249	6	41	220	16	18	167
Coefficient of variation (%)	8.6	4.2	1.3	5.9	6.4	1.8	4.5	2.6	1.3

recently published spectrophotometric method[5], we determined the levels of carbon disulphide and total DDC in blood from two individuals taking disulfiram *per os*. For 3 days the daily dose was 800 mg, taken between 8 and 10 p.m.. Blood was taken for analysis on the fourth day at 8 a.m. As shown in Table II, the results obtained with the gas chromatographic and the spectrophotometric methods are in good accordance.

TABLE II

DETERMINATION OF CS_2 AND TOTAL DDC BY GAS CHROMATOGRAPHIC AND SPECTROPHOTOMETRIC METHODS

CS_2 and total DDC were determined in the blood of two subjects pre-treated with a daily disulfiram dose of 800 mg for 3 days by the gas chromatographic method and a recently published spectrophotometric method[5]. Results are means of six determinations in each instance.

Substance determined	*Subject*	*Gas chromatographic method**		*Spectrophotometric method***
		*Peak height****	*Concentration (μg/ml)*	*Concentration (μg/ml)*
CS_2	1	$\bar{X}$ = 1610 S.D. = 68 C.V. = 4.2	$\bar{X}$ = 0.11	$\bar{X}$ = 0.10
	2	$\bar{X}$ = 5190 S.D. = 33 C.V. = 0.6	$\bar{X}$ = 0.25	$\bar{X}$ = 0.24
DDC	1	$\bar{X}$ = 6190 S.D. = 86 C.V. = 1.4	$\bar{X}$ = 0.85	$\bar{X}$ = 0.85
	2	$\bar{X}$ = 8790 S.D. = 94 C.V. = 1.1	$\bar{X}$ = 1.06	$\bar{X}$ = 1.05

* All analyses were carried out as described under Experimental. Mean CS_2 and DDC concentrations were calculated from mean peak heights using the calibration graphs.

** All analyses were carried out as described in ref. 5.

*** S.D. = standard deviation; C.V. = coefficient of variation (%).

The advantages of the gas chromatographic method are evident and characteristic of gas chromatography in general: as a result of the high sensitivity, 10 times less blood (1 ml) is required; the time required is also considerably reduced, which is important if analyses are to be carried out routinely in large numbers.

ACKNOWLEDGEMENTS

We thank Messrs. Ch. Schäfer and P. Trachsler, GRD Laboratory Wimmis, and Mrs. U. Schneider for excellent technical assistance. This work was supported by Deutsche Forschungsgemeinschaft grant No. Wa 302 and NIMH grant No. AA00233.

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