J. Pharm. Pharmac., 1969, **21**, 674–677 *Received April* 18, 1969

Gas chromatographic identification of thioridazine in plasma, and a method for routine assay of the drug

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Thioridazine had a retention time of approximately 4 min on a 3% OV-17 gas chromatographic column at 260°. Using flame or argon ionization detectors samples as low as 0·01 μg could be detected. The drug could be extracted into n-heptane containing 1·5% isoamyl alcohol or 20% toluene. Recovery of drug added to plasma was 100%. Unchanged thioridazine was identified in the plasma of two experimental samples, on the basis of chromatographic and solvent distribution data.

Studies of thioridazine metabolism in man have included determinations of apparent concentrations of the drug in blood, serum or plasma. Eiduson & Geller (1963), Mellinger (1965) and Mellinger, Mellinger & Smith (1965), have implied but not proved that serum concentrations of the drug vary between zero and 0·9 μg/ml during the 24 h after single doses of 100 mg. However, the techniques used by these authors are not known to be specific for any one compound, and it was not clear whether drug or drug and metabolite concentrations were measured. Indeed, Eiduson, Geller & Wallace (1963) found 97% of excreted radioactivity in man after [^{35}S]thioridazine to be extractable in solvents of the polarity of ether and chloroform even though unchanged phenothiazines are seldom detectable in urine. This suggests that the "apparent thioridazine" is at least in part accounted for by metabolites of the drug.

Solvent extracts of the plasma of patients receiving chlorpromazine can contain large quantities of its metabolites (Curry & Marshall, 1968) but phenothiazines and their metabolites show only slight spectroscopic variations (Beckett & Curry, 1963; Beckett, Curry & Bolt, 1964). As part of a wider investigation of plasma levels of psychoactive phenothiazines, an examination of the gas-liquid chromatographic properties of solvent extracts of the plasma of humans receiving thioridazine has been made. We describe the characterization of the gas-chromatographic response of apparent thioridazine in plasma, and a specific method for the routine assay of the drug in biological fluids.

EXPERIMENTAL

Identification of thioridazine

Two samples of blood from patients receiving chronic thioridazine treatment (oral dose unchanged for one month or more), and receiving no other drugs, were collected into Vacutainer Tubes (B-D Products) containing potassium oxalate, and centrifuged immediately. Aliquots of plasma were examined as below. Details of doses and times of sample collection are given in Fig. 2.

A sample of plasma was mixed with 1 ml of 5% NaOH solution and extracted with 10 ml of n-heptane containing 1·5% isoamyl alcohol. An aliquot of the heptane solution was extracted with 2 ml of 0·01N HCl solution and an aliquot of this aqueous solution was made alkaline with 0·3 ml of NaOH solution and extracted with 50 μl of n-heptane containing 1·5% isoamyl alcohol. Samples (1–10 μl) of the final heptane layer were examined by gas-chromatography.

Gas-chromatographic conditions were as follows: instrument, Barber-Colman Model 5000; inlet temperature, 270°; column packing, 3% OV-17 on Gas-Chrom Q (100–120 mesh) (Applied Science Laboratories); column temperature, 260°; carrier gas, nitrogen flowing at 60 ml/min; detector, 10-mCi ^{90}Sr ionization detector operated at 270° and 750 V.

Signals were assessed by measurement of peak height and comparison with a reference curve obtained from standard quantities of thioridazine. The response was linear over the range 0·1 to 10 μg.

Routine assay of thioridazine

A sample of plasma was mixed with 2·5 ml of 5% NaOH solution and extracted with 5 ml of n-heptane containing 20% toluene. An aliquot of the heptane solution was extracted with 2 ml of 0·1N HCl solution and an aliquot of this aqueous solution was made alkaline with 0·2 ml of N NaOH solution and extracted with 50 μl of the heptane-toluene mixture. Samples (1–2 μl) of the final heptane layer were examined by gas-chromatography.

Gas-chromatographic conditions were as above, except for the substitution of a Pye Series 104 gas-chromatograph with a flame ionization detector (H_2 flow rate, 60 ml/min; O_2 flow rate, 600 ml/min).

The method of Brodie, Udenfriend & Baer (1947) was used for specificity checks by the technique of comparative distribution ratios.

Standard thioridazine solutions

Standard solutions of thioridazine were prepared by dissolving known quantities of the hydrochloride in water, adding 1 ml of N NaOH solution, and extracting the thioridazine into known volumes of either the n-heptane-isoamyl alcohol mixture or the n-heptane-toluene mixture. These extracts contained all of the thioridazine originally dissolved in the water.

RESULTS AND DISCUSSION

Fig. 1 shows the chromatogram obtained from a reference sample of thioridazine, and the chromatograms obtained from extracts of "blank" plasma and from an extract of the same plasma when used as the solvent for thioridazine. Fig. 2 shows chromatograms obtained from the two experimental plasma samples. Table 1 shows solvent distribution data of actual and apparent thioridazine between n-heptane containing 1·5% isoamyl alcohol and aqueous solutions of various pH values. Table 2 shows the actual and found values for thioridazine in plasma for a series of synthetic solutions assayed by the routine method. As can be seen from the data, "blanks" were of minimum significance (the signal from a "blank" extract was comparable with the signal from solvent alone), recovery was virtually complete, and the apparent thioridazine had the same retention time and partition coefficients as actual thioridazine. In view of the fact that the OV-17 column effectively separates

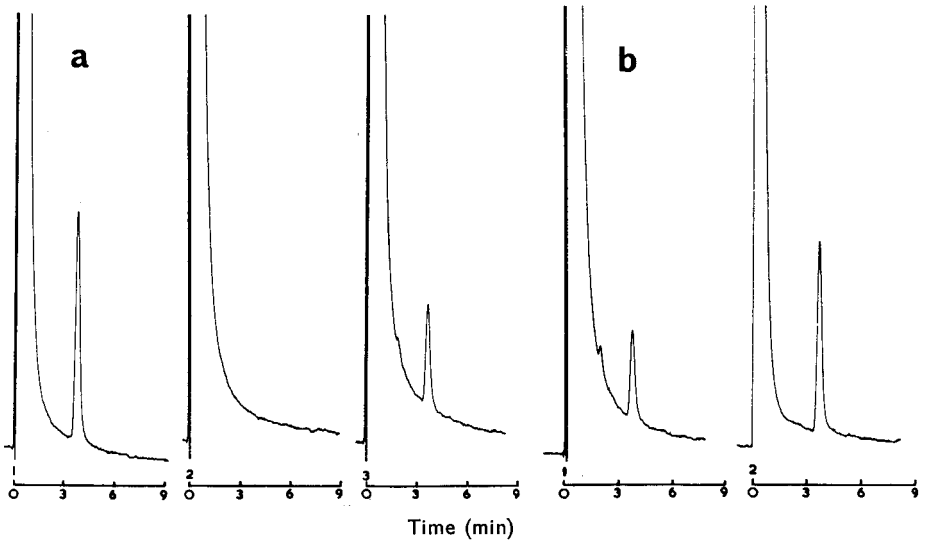

FIG. 1a. Gas-liquid chromatographic traces of: (1) reference thioridazine (0·5 μg) in n-heptane containing isoamyl alcohol; (2) 10 μl of an extract of blank plasma prepared as for "identification of thioridazine"; (3) 10 μl of extract of a second sample of the plasma used in (2), but after addition of 1 μg/ml of thioridazine to the plasma (recovery 89%).

b. Gas-liquid chromatographic traces of experimental extracts from plasma of patients receiving chronic treatment with oral thioridazine: (1) dose 100 mg twice daily; sample 2 h after morning dose; concentration 0·24 μg/ml; (2) dose 300 mg twice daily; sample 2 h after morning dose; concentration 1·80 μg/ml.

the metabolites of the analogous compound chlorpromazine (Curry, 1968), and that solubility of the actual and apparent thioridazine is identical, it is considered that the specificity of the method is proved.

Adsorption of thioridazine on to glass, a major problem in drug extraction (Brodie & others, 1947), could be prevented by the presence in the heptane of either 1·5% isoamyl alcohol or 20% toluene. However, the latter was preferred as the solvent in the later experiments because less emulsion formation occurred in the extractions.

Table 1. *Distribution of thioridazine and apparent thioridazine between water at various pH values and n-heptane containing* 1·5% *isoamyl alcohol*

Aqueous solution or pH value of buffer solution	Fraction of material remaining in organic phases		
	Standard	Recovery solution	Experimental sample
0·05N HCl	0	0	0
4	0·01	0·01	0·02
4·7	0·40	0·41	0·40
5·2	0·89	0·90	—
7	0·96	0·95	0·98
7·3	0·98	0·96	—
10	1·00	1·00	—
0·1N NaOH	1·00	1·00	1·00

Aliquots of the standard solution (1 ml) and of the diluted extracts (50 μl) were shaken with equal volumes of various buffer solutions. The amount of thioridazine remaining in the organic phase after equilibration was determined by GLC and is expressed as a fraction of the amount originally present.

Table 2. *Values of true and found thioridazine in a number of 2·5 ml plasma solutions of the drug. Limits, where given, are ± standard error of the mean*

Thioridazine added to plasma (μg/ml)	No. of Samples	Thioridazine recovered (μg/ml)	Recovery (%)
0·4	4	0·38 ± 0·01	96
1	8	1·05 ± 0·05	105
2	4	1·98 ± 0·04	99
10	6	10·6 ± 0·50	106

Large quantities of either solvent mixture were tolerated by the argon ionization detector, but the response of the flame ionization detector was adversely affected by more than two μl of solvents containing heptane. Evaporation of solvents resulted in high concentrations of undesired contaminants in the extracts, so concentration of extracts to small volumes was achieved by back extractions, using techniques originally described for chlorpromazine (Curry, 1968). The two extraction systems resulted in clean blanks, and adequate extraction of apparently-identical materials. With 5 ml plasma samples, thioridazine could be assayed at concentrations as low as 0·05 μg/ml using the argon ionization detector, and at concentrations as low as 0·2 μg/ml using the hydrogen flame detector.

The analyses of the two plasma samples provide evidence of the adequacy of these techniques for a wider study of thioridazine kinetics and for studies of the relation between kinetics and pharmacological responses. It has recently been shown that plasma concentrations of chlorpromazine vary widely, and that this variation is at least partly responsible for variations in pharmacological effects (Curry & Marshall, 1968).

Acknowledgements

The authors are grateful to Sandoz Pharmaceuticals for the supply of thioridazine; the two experimental plasma samples were kindly supplied by Dr. J. H. L. Marshall of St. Elizabeth's Hospital, Washington, D.C., U.S.A., during an extensive study of kinetics of phenothiazine derivatives in psychiatric patients; preliminary experiments designed to test the suitability of the OV-17 column for reference and extracted thioridazine were made at the Laboratory of Chemical Pharmacology, National Heart Institute, Bethesda, Maryland, U.S.A.

This work was supported (in part) by contract number PH 43-66-1167 with the United States National Institute of General Medical Sciences.

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