CHROM. 5557

GAS CHROMATOGRAPHIC DETERMINATION OF DOXEPIN IN HUMAN URINE FOLLOWING THERAPEUTIC DOSES

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(Received June 28th, 1971)

SUMMARY

A gas–liquid chromatographic method for the estimation of doxepin in human urine is described. The method allows the estimation of concentrations of the drug as low as 0.1 μg/ml. Results are presented showing urine levels of the drug up to 24 h after a therapeutic dose.

INTRODUCTION

Doxepin hydrochloride (Sinequan) is a new tricyclic antidepressant currently being used in the treatment of patients with psychoneurotic anxiety[1]. It is a dibenzoxepin derivative, being an isomeric mixture of 11-(3-dimethylaminopropylidine)-6H-dibenz(*b*,*e*)oxepin hydrochloride.

The method of HOBBS[2], although resulting in a recovery of 90%, was not suitable for our purpose since no internal standard was used and aqueous solutions were injected into the column. The method described in this article is suitable for the assay of doxepin in urine down to levels of 0.01 mg% and has been used to assay urine samples from subjects up to 24 h after oral administration of 50 mg of doxepin as the hydrochloride.

EXPERIMENTAL

Reagents

The following reagents were used: Analar chloroform (B.D.H.); Analar anhydrous sodium sulphate; phosphate buffer 0.07 *M*, pH 7.4 and 0.5 *N* sodium hydroxide. The internal standard was a 30 mg% solution of diazepam in chloroform.

Gas chromatography

A Packard Model 7400 Series gas chromatograph equipped with a flame ionization detector was used. The column was a 6 ft. × 4 mm I.D. coiled glass tube which had been silanized with a solution of 5% dichlorodimethyl silane in benzene then rinsed with methanol. The packing consisted of 3% SE-30 (Applied Science Labora-

tories) on Chromosorb W H.P. 80–100 mesh support (Johns Manville Products Co.) and was prepared as follows: A known amount of support was weighed into a 500 ml round bottom flask. The solvent, chloroform, was added to the support until the liquid level was about ¼ in. above the surface of the support material. The stationary phase was weighed into a small erlenmeyer flask, dissolved in chloroform, and then transferred to the flask containing the support. The mixture was swirled and left to stand for 10 min. It was then placed in a water bath at 60° and the solvent slowly removed by rotary evaporation under a partial vacuum. This ensured a uniform coating of the stationary phase on the support material. When dry, the support was placed in a wide evaporating basin and left in an oven overnight at 100°. After packing the column was conditioned at 245° for 48 h with a nitrogen flow rate of 30 ml/min. For analysis the instrument settings were as follows: column temperature, 220°; injection port and detector temperature, 230°; carrier gas flow rate, 50 ml/min; column inlet pressure 20 p.s.i. Under these conditions the retention times of doxepin and diazepam were 5.0 and 8.8 min, respectively.

Extraction procedure

A 50 ml sample of urine, adjusted to pH 6–8 with dilute hydrochloric acid or ammonia solution, was extracted with 100 ml chloroform by shaking vigorously for 2 min in a 250 ml separating funnel. The extraction was repeated using 75 ml of chloroform and the organic phases combined. This was washed with 10 ml phosphate buffer followed by 10 ml of 0.5 *N* sodium hydroxide and 10 ml of distilled water. The chloroform was dried by filtering through anhydrous sodium sulphate into a round bottom flask and taken to low volume on a rotary evaporator at 40° under a vacuum of 300 mm of mercury. The contents were transferred to a 10 ml graduated centrifuge tube and gently evaporated just to dryness using a stream of dry nitrogen, the tube being immersed in a water bath at 45°. The residue was redissolved in 200 μl of diazepam internal standard solution and 5 μl of this injected into the gas chromatograph at a sensitivity setting of 1×10^{-9} A.

Quantitation

A range of standard solutions were prepared containing varying amounts of doxepin and diazepam such that the amount of doxepin injected ranged from 0.1 μg to 0.5 μg. Over this range the ratio of the peak height of doxepin to internal standard was linear (Fig. 1).

The relative retention time of doxepin with respect to diazepam was 0.57.

Recovery studies

Amounts ranging from 25 μg to 250 μg of doxepin as the hydrochloride were added to 50 ml samples of blank urine to examine the efficiency of the extraction procedure. The mean recovery achieved was 97 $\pm$ 6% on seventeen spiked samples.

Specificity

In no normal urine or blood sample was any peak found that could correspond to the same position as doxepin. Several other common antidepressants were examined and found to have differing retention times than doxepin. These compounds included: amitriptyline 4.6 min; desipramine 5.4 min; promazine 6.8 min; diazepam 8.8 min;

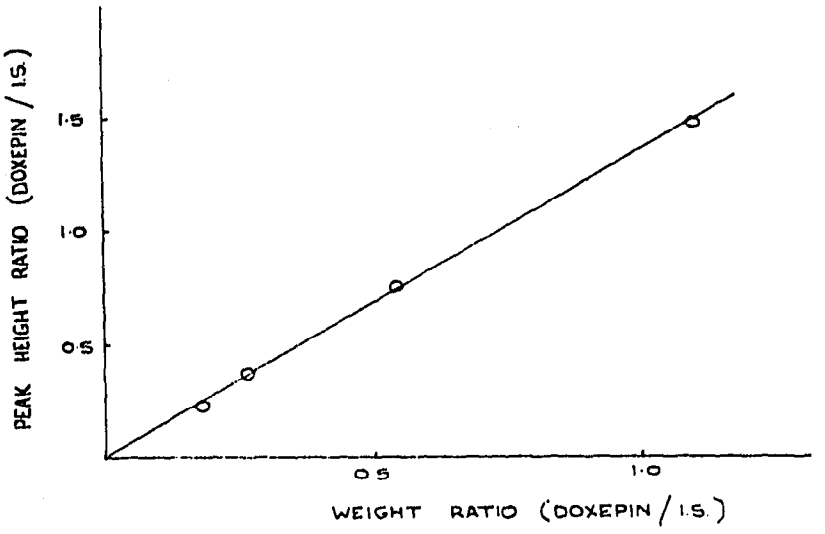

Fig. 1. Relationship of gas–liquid chromatographic peak heights to doxepin concentrations. The peak heights and concentrations of doxepin are expressed relative to the internal standard (I.S.).

dibenzepin 9.3 min; chlorpromazine 14.9 min; nitrazepam 22.6 min; chlordiazepoxide 28.4 min; thoridazine > 30 min.

Application

In the first of two controlled experiments, doses of between 20–50 mg of doxepin as the hydrochloride were administered to twenty subjects and a further ten subjects received a placebo. Samples of urine and blood from all subjects were collected 4 h after ingestion. In the second experiment single oral doses of 50 mg of doxepin as the

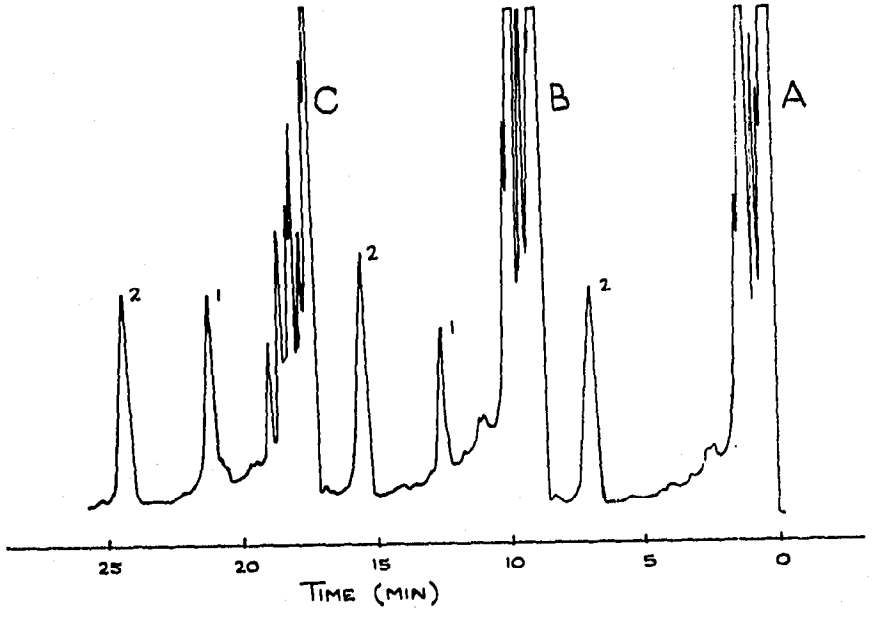

Fig. 2. A, Chromatogram of a blank urine extract; B, Chromatogram of a urine extract containing added doxepin at a concentration of 0.05 mg%; C, Chromatogram of a urine extract from a subject administered 50 mg of doxepin. 1, doxepin; 2, diazepam (internal standard).

TABLE I

THE EXCRETION OF DOXEPIN IN THE URINE OF SIX SUBJECTS FOLLOWING ORAL DOSES OF 50 mg DOXEPIN AS THE HYDROCHLORIDE

Subject	*Time (h)*	*Volume (ml)*	*Doxepin concentration (μg/ml)*	*Excreted doxepin (μg)*	*Total excreted in 24 h (μg)*	*Dose excreted as free doxepin (%)*
M 1	4	500	0.56	280.0		
	8	155	1.12	173.5		
	12	350	0.28	98.0	576.3	1.15
	16	208	0.10	20.8		
	24	200	0.02	4.0		
M 2	4	114	0.22	25.1		
	8	203	0.07	14.2		
	12	250	0.01	2.5	44.5	0.09
	16	270	0.01	2.7		
M 3	4	190	0.31	58.9		
	8	95	0.52	49.4	135.8	0.27
	12	150	0.17	25.5		
	16	200	0.01	2.0		
M 4	4	175	0.07	12.2		
	8	200	0.44	88.0	104.2	0.21
	12	201	0.02	4.0		
F 1	4	340	0.30	102.0		
	8	340	0.01	3.4	107.9	0.22
	12	245	0.01	2.5		
F 2	4	195	0.40	78.0		
	8	90	0.22	19.8	101.0	0.20
	12	160	0.02	3.2		

hydrochloride were given in capsule form to six subjects. Urine samples were collected at regular intervals over a period of 24 h. After measurement of the volume, the samples were stored at 4° prior to analysis.

RESULTS AND DISCUSSION

The results of samples labelled with random code numbers to eliminate bias, from the first experiment, indicated that doxepin was detected in the urine of all the subjects receiving the drug and in none of the subjects receiving the placebo (Fig. 2). The results from the second experiment are tabulated in Table I. In the subjects tested, only an average of 0.4% of the dose ingested appeared in the urine as free doxepin during the first 24 h. Doxepin was not detected in significant amounts in the urine after this period. Acid or enzymatic hydrolysis of these samples gave no increase in the yield of doxepin.

Recoveries using blood samples (5 ml) were also performed following a similar procedure to that of urine. Recoveries ranged from 75% at 0.1 mg% to 96% at levels of 1.0 mg%, the lower limit of detection being 1 μg/ml (Fig. 3).

Doxepin was not detected in the blood of the subjects who were administered

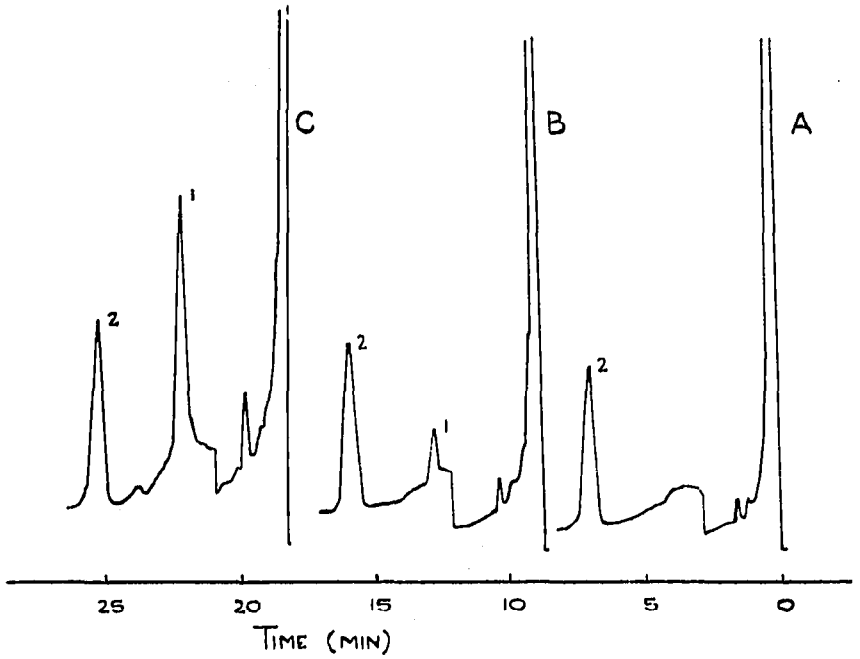

Fig. 3. A, Chromatogram of a blank blood extract; B, Chromatogram of a blood extract spiked with doxepin at a level of 0.1 mg%; C, Chromatogram of a blood extract spiked with doxepin at a level of 0.5 mg%. 1, Doxepin; 2, diazepam (internal standard).

doses of between 20–50 mg of doxepin using the above method. HOBBS[2] found the highest level to be 0.1 mg% in plasma of dogs dosed at levels at least fivefold higher (on a mg/kg basis) than the usual daily human dose. He also found that the concentration of doxepin reached a maximum after 1–3 h and then declined rapidly. From a toxicological viewpoint the presence of ingested doxepin may be established more easily by the analysis of urine rather than blood.

Major routes of metabolism of amitriptyline and imipramine involve hydroxylation on the aromatic rings, hydroxylation on the ethylene bridge, N-demethylation and N-oxidation[3–7]. HOBBS[2] has stated that the metabolism of doxepin in rats and dogs followed many of the above transformations. Thus the breakdown of doxepin resembled that of amitriptyline. HOBBS[2] also reported 50% excretion of doxepin in rats while CASSANO *et al.*[8] reported 50–60% excretion of amitriptyline in mice. The results obtained in this paper, showing a urinary excretion of only 0.4% of the dose of doxepin ingested, parallels those of BRAITHWAITE AND WHATLEY[9] who found 0.15% of an amitriptyline dose excreted in the urine of human subjects.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. M. DICK for his help in the preparation of this paper and the Public Health Department, for allowing the data to be published. We are also indebted to Dr. G. MILNER for his assistance in obtaining urine specimens from clinical subjects.

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