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A GAS CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF HALOPERIDOL IN HUMAN PLASMA

INA A. ZINGALES
Research Laboratory, Cleveland State Hospital, Cleveland, Ohio 44105 (U.S.A.)*

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SUMMARY

A gas chromatographic method for the qualitative and quantitative determination of haloperidol in plasma at therapeutic levels has been developed.

The procedure involves extraction of the drug from alkalinized plasma into *n*-heptane, successive concentration into aqueous and organic solvents, and separation on a gas–liquid chromatograph equipped with an electron capture detector.

The method has been applied to plasma samples from patients receiving 9 to 15 mg of haloperidol *pro die*. Plasma levels encountered after oral doses of 3 to 5 mg varied from zero to 10 ng of haloperidol per ml of plasma.

INTRODUCTION

Haloperidol, 4-[4-(*p*-chlorophenyl)-4-hydroxypiperidino]-4'-fluorobutyrophenone, has been used for more than a decade in the treatment of acute and chronic psychoses.

There is an extensive literature concerning the pharmacology and clinical properties of the drug, but little information is available on its excretion, metabolism and distribution in tissues. A number of studies relating to the chemistry of the drug have been published[1–4]. DEMOEN[2] describes the physical properties and analytical procedures for the assay of haloperidol in dosage forms. Colorimetric methods for the determination of the drug in pure forms or in pharmaceutical preparations are based on color reaction with *m*-dinitrobenzene[5] or 3,5-dinitrobenzoic acid[6]. An analytical procedure developed by SOEP[7] involves the extraction of the drug from urine of rat, paper chromatography, and determination of the fluorine content of the spot. The color complex formed with methyl orange is the basis of a method applied by DEMOEN[2] to urine and feces of laboratory animals.

The excretion and metabolism of the drug have been studied in rats after the administration of tritium labeled haloperidol[8]. According to the authors, oxidative N-dealkylation represents the major metabolic pathway. BRAUN *et al.*[9] studied the distribution of haloperidol in tissues. Their results show that, 3 h after its adminis-

* William L. Grover, M.D., Superintendent.

tration, approximately 15% of the tritium labeled dose was present in the tissues. Most of the radioactivity was concentrated in the liver. Concentration in blood varied between an average of 0.38% of the administered dose at 1 h and 0.59% at 5 h.

None of these methods are applicable to the analysis of blood samples following administration of therapeutic doses.

The increasing interest in the use of haloperidol in mental diseases prompted the present investigation. It is confined to the description of an analytical procedure for the determination of unmetabolized haloperidol in the plasma of patients receiving doses ranging between 9 and 15 mg *pro die*.

Gas chromatography utilizing the affinity of the electron capture detector to halogenated substances was, for its sensitivity and selectivity, the technique of choice.

EXPERIMENTAL

Reagents

Only analytical grade reagents were used. Stock solutions were prepared by dissolving 5 mg of haloperidol in 100 ml of *n*-heptane containing 1.5% isoamyl alcohol. Under refrigeration these solutions were stable for several weeks. Working standard solutions contained 1 to 40 ng of haloperidol per microliter.

Material

Blood samples were collected in the morning 1 to 3 h after oral administration of 3 to 5 mg doses. Only samples from patients receiving 9 to 15 mg of the drug *pro die* were analyzed. They had been in haloperidol therapy at various dosages for at least two weeks prior to sampling.

Extraction procedure

A sample of 10 ml of plasma made alkaline by the addition of 1 ml of 2.5 *N* sodium hydroxide, was extracted with 20 ml of *n*-heptane containing 1.5% isoamyl alcohol by shaking mechanically for 15 min. After centrifugation at 3,000 r.p.m., 18 ml of the organic phase were transferred to a 30-ml glass test tube containing 5 ml of 0.1 *N* hydrochloric acid. The tube was mechanically shaken for 10 min. Following centrifugation, the acidic aqueous layer was made alkaline by adding 1 ml of 1 *N* sodium hydroxide and then extracted with 5 ml of the *n*-heptane–isoamyl alcohol mixture. After centrifugation, 4.8 ml of the organic phase were transferred to a 5-ml glass centrifuge test tube and evaporated to dryness under nitrogen. The residue was dissolved in 25–50 μl of *n*-heptane–isoamyl alcohol; 3 to 10 μl of this were injected into the chromatograph.

Aliquots of the reference solutions containing 1 to 50 ng of haloperidol per microliter were transferred to a centrifuge test tube. The solvent was evaporated under nitrogen and the residue dissolved in 100 μl of 0.001 *N* hydrochloric acid. Ten milliliters of distilled water, or haloperidol free plasma or urine were added and the extraction continued as described above.

Gas chromatography

A Beckman GC-4 gas chromatograph equipped with an electron capture detector, and a Beckman 10 in. potentiometric recorder were used in this work. The

ordinate scale was expanded for increase in electrometer sensitivity as suggested by the manifacturer. Column, temperatures, flow rates and electrical settings are described below. The column was conditioned at 320° for 48 h with a helium flow rate of 25 ml/min.

Conditions for chromatography

Column: glass, 4 ft., ¼ in. O.D., 2 mm I.D.; 2% OV-1 on Chromosorb W HP 80–100 mesh.

Temperatures: inlet lines 300°; column oven 210°; detector and detector lines 320°.

Flow rates: helium, carrier gas 55 ml/min; helium, discharge gas 100 ml/min; carbon dioxide 3 ml/min.

Detector electrical settings: polarizing voltage 675 duodial, bias voltage 520 duodial, source current 7 mA.

Electrometer settings: range 100, attenuation 1024, suppression current off (background current equivalent to 80% full scale); range 100, attenuation settings from 512 to 128, suppression current on (for scale expansion).

RESULTS AND DISCUSSION

Under the chromatographic conditions described above, haloperidol has a

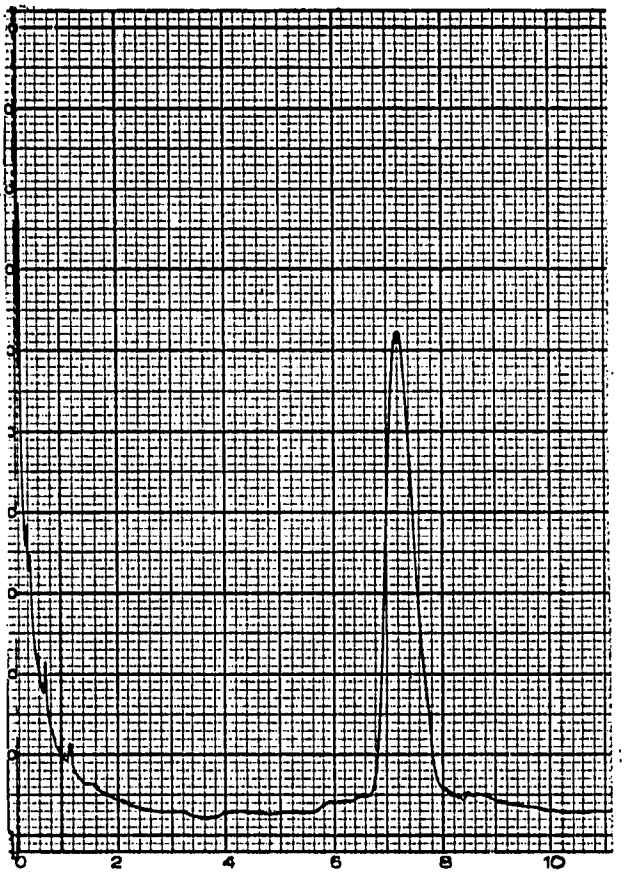

Fig. 1. GLC traces of a standard solution of haloperidol in *n*-heptane–isoamyl alcohol. Injected 20 ng.

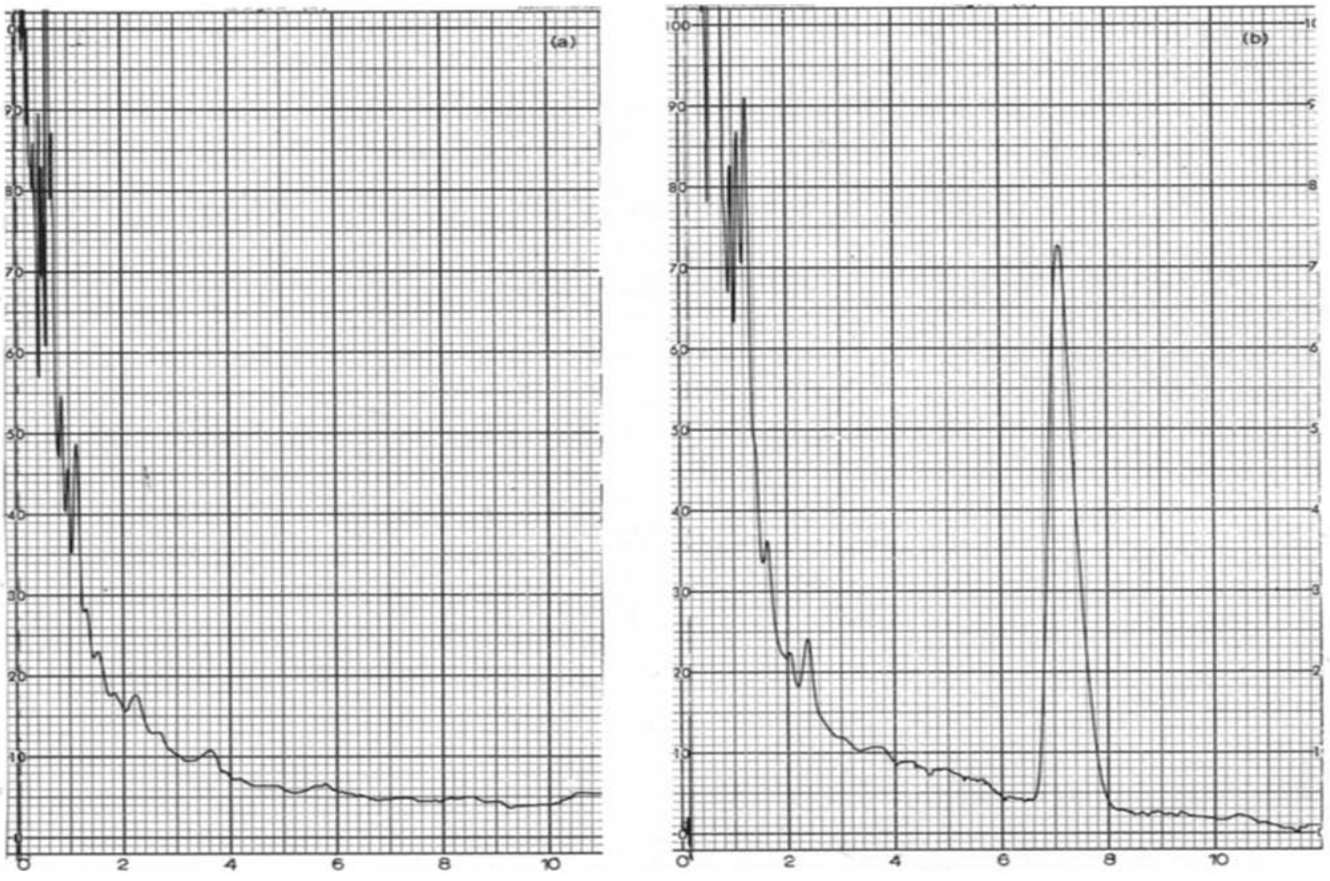

Fig. 2. GLC traces of :(a) an extract from 10 ml of blank plasma and (b) an extract from 10 ml of the same sample to which 250 ng of haloperidol had been added. Injected 30 ng; recovery 86.3%.

TABLE I

RECOVERY OF HALOPERIDOL FROM WATER, URINE AND PLASMA

Sample	*Haloperidol added ng/10 ml*	*No. of determinations*	*Percent recovery ± S.D.*
Water	50	10	98.9 ± 3.1
	100	13	99.8 ± 2.5
	250	5	101.4 ± 2.9
	500	5	100.1 ± 2.3
Urine	50	8	95.4 ± 4.3
	100	10	98.2 ± 4.3
	250	10	99.1 ± 2.9
	500	8	101.3 ± 4.2
Plasma	50	12	85.8 ± 3.9
	100	11	84.9 ± 3.2
	250	7	85.3 ± 2.8
	500	5	88.1 ± 3.8

retention time of approximately 7 min. Fig. 1 shows the chromatogram obtained from a standard solution of haloperidol in *n*-heptane–isoamyl alcohol. Fig. 2 represents chromatograms from an extract of 10 ml of blank plasma and 10 ml of the same sample to which 250 ng of haloperidol had been added. No interfering peaks in the same region of haloperidol have been observed in the chromatograms obtained from several blank plasma samples analyzed.

To verify the adequacy of the extraction procedure, amounts of haloperidol from 50 to 500 ng were added to 10 ml of distilled water or drug free urine or plasma. The results of these determinations are shown in Table I.

Concentrations of haloperidol in the extracts were measured by disc integration readings. The response of the detector to injections of 5 to 500 ng is a linear function of the concentration (Fig. 3); however, because of variations in the sensitivity of the detector, haloperidol concentrations in the sample analyzed were not calculated from a graph of this relationship. Extracts of blank plasma samples to which appropriate amounts of haloperidol had been added, were prepared each time analyses of unknown samples were performed. Aliquots of these extracts were injected immediately before and after each chromatographic analysis of the unknown. Variations in the sensitivity of the detector were significantly reduced by increasing the source current to 12 mA for 10 min after running 4 or 5 samples from biological material.

The procedure described has been applied to samples of plasma from patients

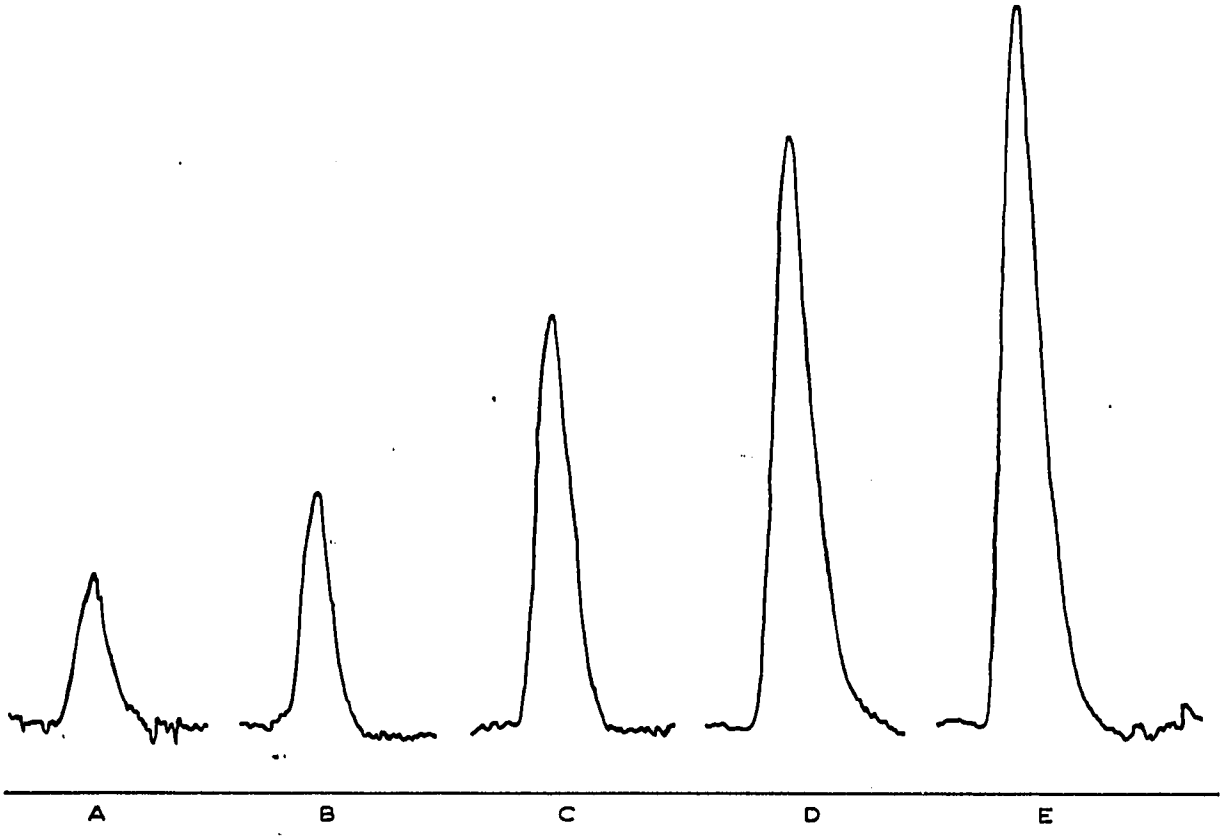

Fig. 3. Peaks obtained from injections of 5 ng (A) to 25 ng (E) of haloperidol in *n*-heptane–isoamyl alcohol. 5 ng can be quantitatively determined with accuracy.

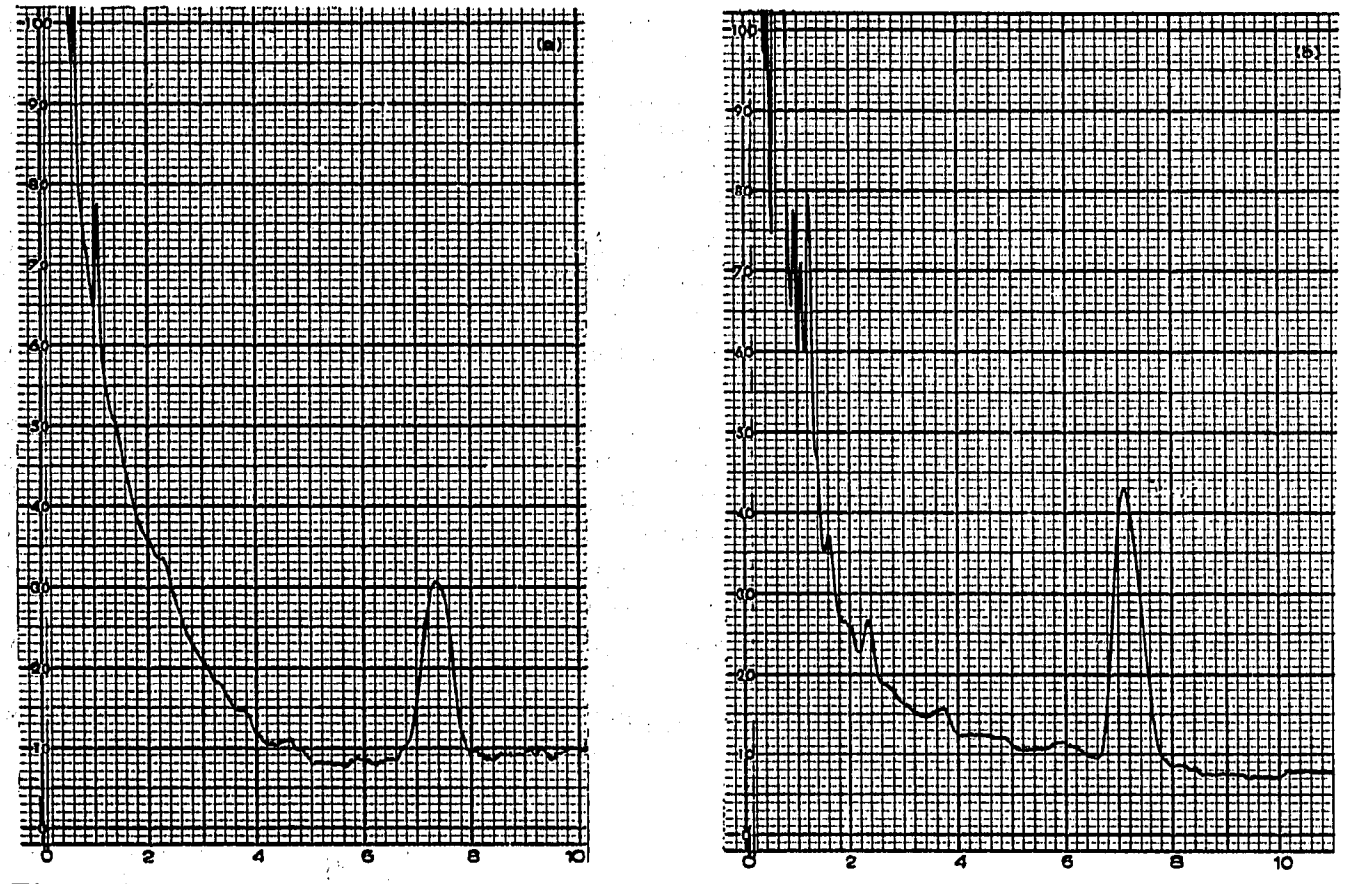

Fig. 4. GLC traces of extracts from plasma samples from two patients receiving: (a) 9 mg and (b) 10 mg of haloperidol *pro die*. No other medication was administered.

TABLE II

DATA OF CASES STUDIED

Patient No.	*Mg of haloperidol pro die*	*Other drugs administered*	*Times of collection after morning doses*	*Plasma levels (ng/ml)*
1	3 *t.i.d.*	—	120 min	2.3
2	3 *t.i.d.*	Benztropine	125 min	traces
3	3 *t.i.d.*	Chlorpromazine, trifluoperazine	60 min	negative
4	5 *b.i.d.*	—	120 min	3.2
5	4 *t.i.d.*	Chlorpromazine, trifluoperazine, benztropine	80 min	negative
6	4 *t.i.d.*	Imipramine, benztropine	180 min	2.2
7	4 *t.i.d.*	Chlorpromazine, fluphenazine, benztropine	120 min	traces
8	4 *t.i.d.*	Chlorpromazine, diphenhydramine	180 min	2.8
9	4 *t.i.d.*	Diazepam, diphenhydramine, benztropine	150 min	3.3
10	5 *t.i.d.*	Tolbutamide, diphenylhydantoin	120 min	4.1
11	5 *t.i.d.*	Amitriptyline, benztropine	80 min	traces
12	5 *t.i.d.*	Imipramine, benztropine	170 min	2.7
13	5 *t.i.d.*	Chlorpromazine, benztropine	160 min	10.0
14	5 *t.i.d.*	Thioridazine, diphenylhydantoin	90 min	2.9

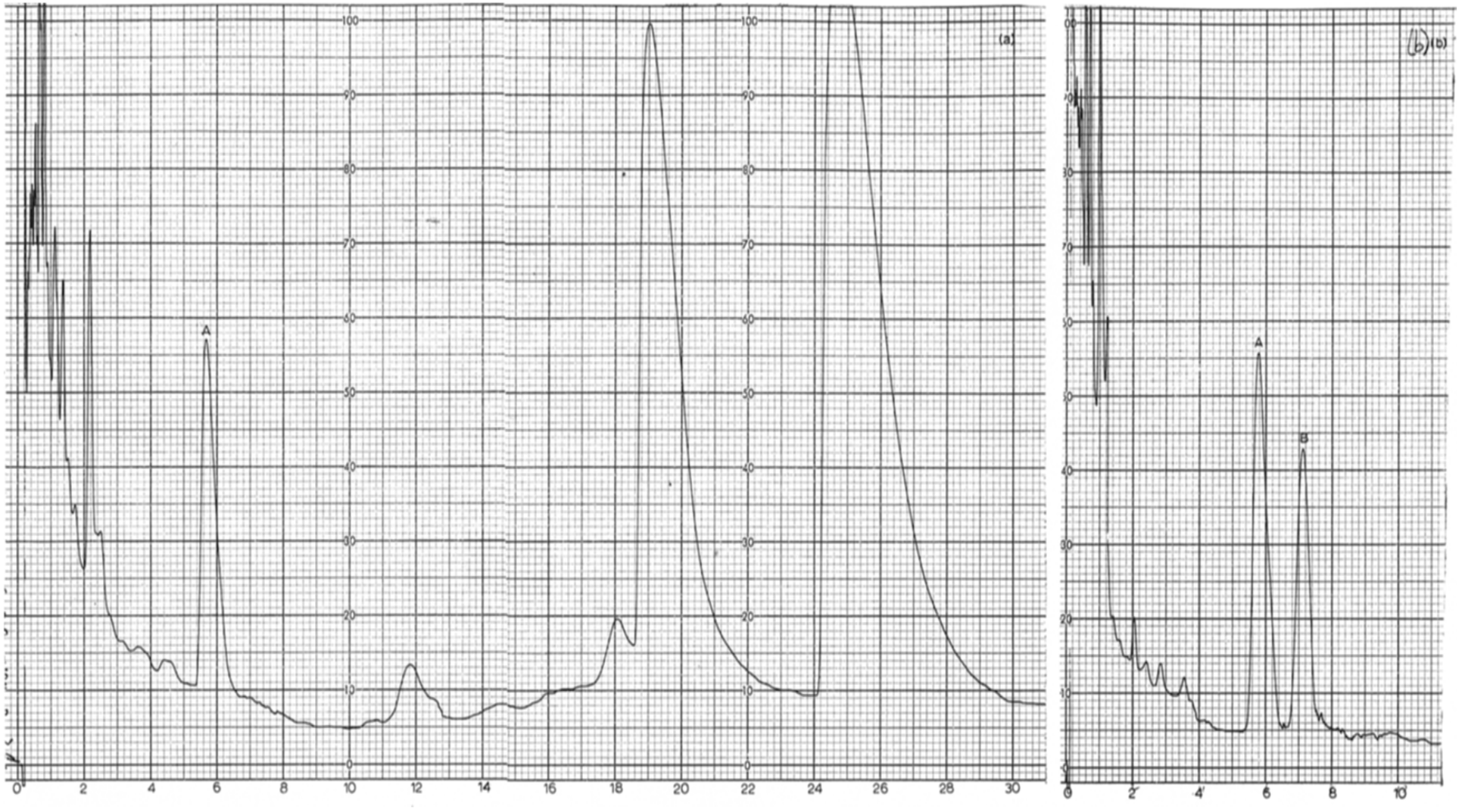

Fig. 5. GLC traces of: (a) an extract from plasma of a patient receiving only chlorpromazine and (b) an extract from another aliquot of the same sample to which 200 ng of haloperidol had been added. Peak A corresponds to a metabolite of chlorpromazine; peak B to haloperidol.

in haloperidol therapy. As mentioned previously, these patients were receiving various dosages of the drug for at least two weeks prior to sampling. At the time of collection of the samples, daily dosages ranged from 9 to 15 mg. Only 2 of the 14 samples analyzed were from patients receiving only haloperidol. Fig. 4 shows typical chromatograms from the extracts of these two samples. The remainder of the patients were receiving, in addition to haloperidol, a variety of other drugs. Doses of haloperidol, drugs administered, times of sample collection and plasma levels detected are given in Table II.

In order to ascertain the specificity of the method for the detection of haloperidol, plasma samples from patients receiving imipramine, amitriptyline, protriptyline, chlordiazepoxide, diazepam, trifluoperazine, chlorpromazine or thioridazine were extracted and chromatographed under the same conditions described for haloperidol. With the exception of chlorpromazine, the chromatograms obtained from these extracts showed no peaks in the haloperidol region. Fig. 5 represents a chromatogram from the extract of a plasma sample from a patient receiving only chlorpromazine (600 mg *pro die*). Under the chromatographic conditions used, chlorpromazine, mono- and di-demethyl chlorpromazine have retention times of approximately 2 min; peak A in Fig. 5 (a and b) has the same retention time as chlorpromazine sulfoxide (approximately 6 min). In order to ascertain conclusively that this

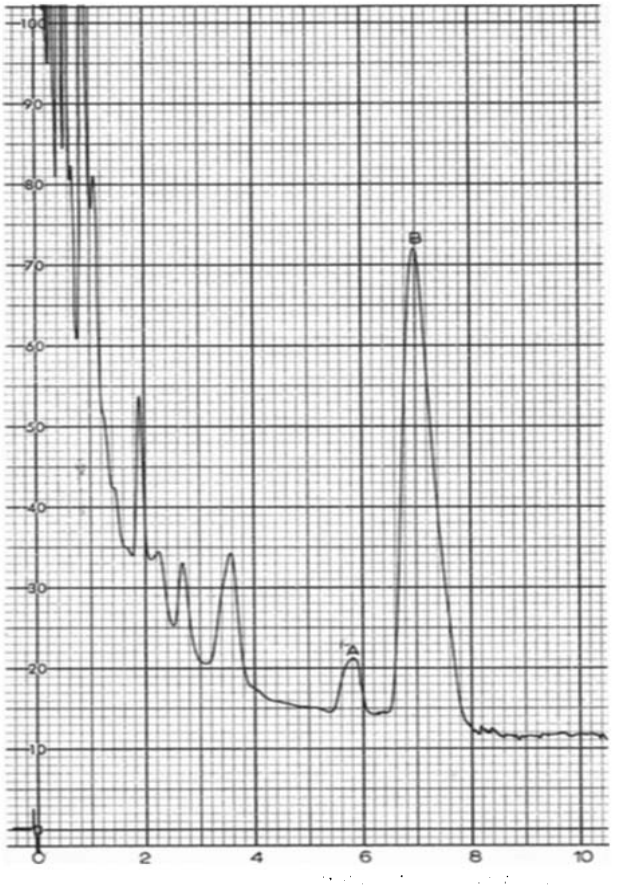

Fig. 6. GLC trace of an extract from plasma of a patient receiving 15 mg of haloperidol and 200 mg of chlorpromazine *pro die*. Peaks A and B as in Fig. 5.

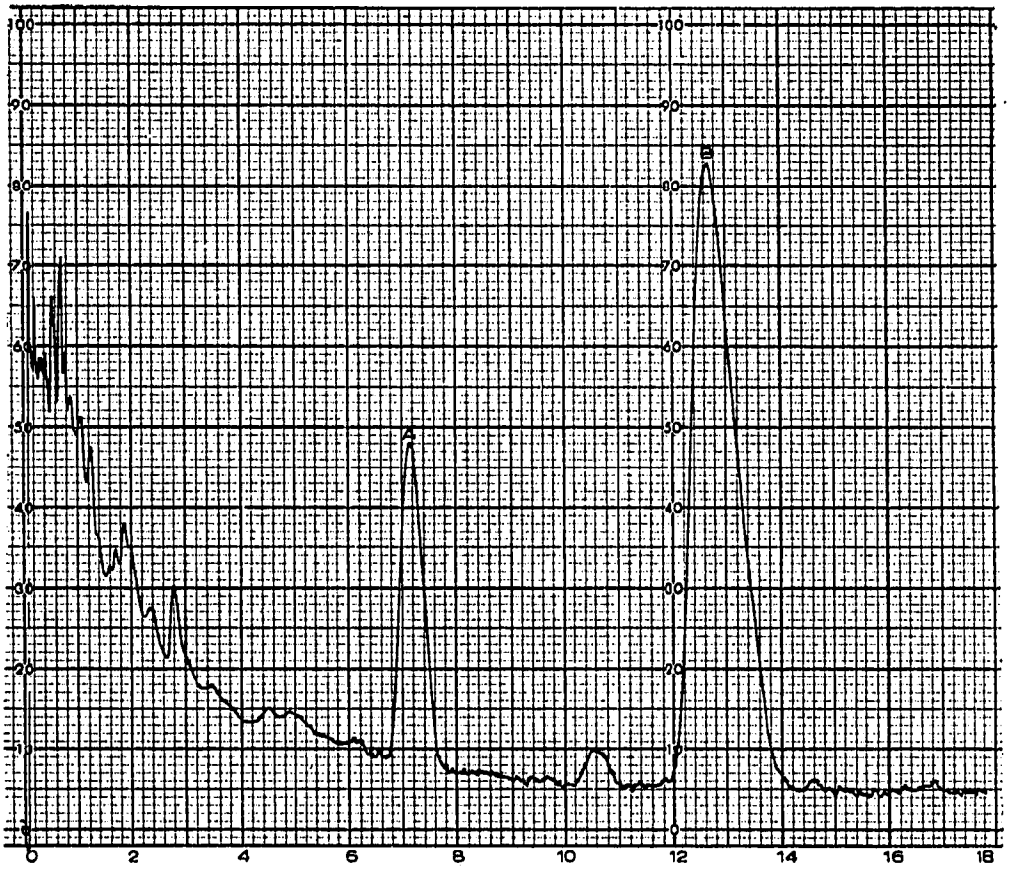

Fig. 7. GLC trace of an extract from urine of a patient receiving only haloperidol. Peak A has the same retention time as haloperidol. Peak B could represent a metabolite of haloperidol.

chlorpromazine metabolite could not interfere with the detection of haloperidol when the two drugs are administered together, 200 ng of haloperidol were added to another portion of the same plasma sample. The sample was then extracted and an aliquot of the extract injected into the chromatograph. Fig. 5 (b) shows the separation of the two peaks. Fig. 6 represents an extract of a plasma sample from a patient receiving 15 mg of haloperidol and 200 mg of chlorpromazine *pro die*.

When the analyses of plasma samples resulted negative for haloperidol, samples of urine from the same subjects were analyzed according to the procedure described, in order to exclude the possibility that the drug had not been ingested. A positive urine analysis indicated that the plasma level in the patient was too low to be detected by the method described. Fig. 7 represents a typical chromatogram from one of these extracts. Peak B could correspond to a haloperidol metabolite, as haloperidol was the only medication administered to the patient during the previous five months (3–9 mg *pro die*). Extracts from blank urine present no interfering peaks in the same region.

As shown by the results obtained, there are considerable variations in the concentration of haloperidol in plasma of patients maintained in similar dosage schedules. The highest plasma level (998 ng %) among the samples analyzed was detected repeatedly in plasma samples from a patient whose response to the therapy was considered by the ward physician to be very poor. Thus, within the limits of the number of samples analyzed, evidence exists that the response of the patient to halo-

peridol therapy is not necessarily related to high plasma levels of the free unmetabolized drug. Preliminary experiments in this laboratory show that the drug is bound to plasma proteins in detectable amounts. The possibility of biotransformation of the drug into pharmacologically active metabolite(s), undetected by the procedure described, must also be considered.

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