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Note

Determination of propiomazine and its N-demethyl metabolite in plasma by gas chromatography with alkali flame ionization detection

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Propiomazine, Fig. 1, is a compound of the phenothiazine type which has found a widespread use as sedative and hypnotic, particularly in geriatric practice. Although the drug has been on the market for more than thirty years, little is known about its disposition in animal or human. For an optimal dosage regimen, it is essential to evaluate the basic pharmacokinetics of the drug after different ways of administration. Lack of sufficiently sensitive and selective analytical procedures has, however, hampered the quantitative determination of the drug and its main metabolites in plasma and urine.

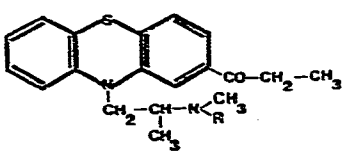

Fig. 1. Structure of propiomazine (R = CH_3) and N-demethylpropiomazine (R = H).

Propiomazine has been positively identified in urine using thin-layer chromatography [1,2]. Spectrophotometric methods have been developed for the urinary analysis of the drug and its sulfoxide metabolite [3,4]. Obviously these methods have an insufficient sensitivity and selectivity for the determination of plasma concentrations of propiomazine.

This paper describes an analytical procedure for the measurement of plasma propiomazine and its N-demethyl metabolite comprising selective extraction, trifluoroacetylation of the secondary amine and determination by gas chromatography with alkali flame ionization detection.

EXPERIMENTAL

Gas chromatography

A Pye series 104B chromatograph equipped with flame ionization and alkali flame ionization detectors was used. The glass column (150 cm × 0.2 cm I.D.) was filled with 3% OV-17 on Gas-Chrom Q(100—120 mesh) and operated at 280°C. Injector and detector temperatures were 280 and 320°C, respectively. The flow-rate of nitrogen carrier gas was 30 ml/min. The flow-rates of hydrogen and air were 30 and 300 ml/min, respectively.

Reagents and chemicals

Propiomazine maleate, N-demethylpropiomazine chloride and propiomazine sulfoxide (base) were kindly supplied by Pharmacia (Uppsala, Sweden). Heptane, trifluoroacetic anhydride and triethylamine were of highest analytical quality and purchased from E. Merck (Darmstadt, G.F.R.). Anhydrous diethyl ether was supplied by Mallinckrodt (St. Louis, MO, U.S.A.). For standardization, propiomazine maleate and N-demethylpropiomazine chloride were dissolved in 0.1 *M* phosphoric acid and diluted with water. Aliquots of this solution were diluted with plasma to contain 20—400 ng/ml of each of the amines. Internal standard was prochloroperazine maleate (Leo, Helsingborg, Sweden). It was diluted with water to 0.5 μg/ml. One ml of this solution was used in the analytical method.

Determination of partition ratio

The amine as base ($2 \cdot 10^{-3}$ *M*) was dissolved in diethyl ether together with an internal standard (octacosane). The peak height ratio of the amine to the internal standard was determined by gas chromatography with flame ionization detection. Five ml of the diethyl ether phase were shaken with an equal volume of phosphate buffer (μ=0.1) pH 4.5—6.0 at 25°C for 1 h. After phase separation, the peak height ratio of amine to internal standard was determined in the diethyl ether phase. The partition ratio, K_D, was determined from the peak height ratio after extraction/(initial peak height ratio minus peak height ratio after extraction).

Determination of propiomazine and N-demethylpropiomazine in plasma

To 2 ml of plasma, 1.0 ml of internal standard solution was added together with 1 ml of 0.5 *M* sodium hydroxide and water to 5 ml. The mixture was shaken with 5 ml of diethyl ether for 1 h at room temperature. After centrifugation for 10 min at 500 *g*, the organic phase was transferred to another tube and shaken for 10 min with 1 ml of 0.1 *M* sulphuric acid.

After removal of the organic layer and alkalinization with 0.5 ml of 0.5 *M* sodium hydroxide, the aqueous phase was shaken with 1 ml of diethyl ether for 10 min. The organic phase was transferred to another tube and evaporated in a stream of nitrogen. Ten microlitres of a mixture of trifluoroacetic anhydride and triethyl amine (1:1) was added to the residue and after 15 min reaction time at room temperature, 0.5 ml of heptane was added. Excess reagent was removed by washing with 1 ml of 0.1 *M* sodium hydroxide. Two to four microlitres of the organic phase were analysed by gas chromatography with alkali flame ionization detection.

A standard curve was prepared in parallel by treating six standard samples in the concentration range 20—400 ng/ml of propiomazine or N-demethylpropiomazine, according to the procedure above.

RESULTS AND DISCUSSION

Extraction conditions

Diethyl ether was chosen for the extraction of the amines from alkalinized plasma samples owing to its good extraction power and for its low tendency for formation of emulsions. An estimation of the extraction degree of propiomazine and its N-demethyl metabolite from buffered aqueous solutions was obtained by determination of partition coefficients. The partition coefficients, K_D, between diethyl ether and buffered aqueous solution are given in Table I, the value of $-\log(K_D \times K_{HA})$ corresponds to the pH at which the amine is present in equal concentration in the phases using equal phase volumes. A quantitative extraction of propiomazine, N-demethylpropiomazine and the internal standard, prochlorperazine, might be obtained at pH > 7.5.

The extraction degree of propiomazine sulfoxide to diethyl ether was shown by gas chromatographic analysis to be low (<1%) even at pH 10. The relative retention of the sulfoxide to propiomazine was 3.1.

In the procedure, a purification step was included by re-extraction of the amines to acidic aqueous phase followed by alkalinization and extraction to a small volume of diethyl ether. It was verified that the yield of the amines through the extraction steps was the same with and without the presence of plasma.

TABLE I

PARTITION COEFFICIENTS OF PROPIOMAZINE, METABOLITES AND THE INTERNAL STANDARD

$K_D = A_{org}/A_{aq}$ = partition coefficient of the amine. K_{HA} = acid dissociation constant of the amine. Aqueous phase: phosphate buffer pH 4.5—6.0 (μ=0.1); organic phase: diethyl ether; equal phase volumes.

Compound	$\log(K_D \times K_{HA})$	pH for 99% extraction	pH for 1% extraction
Propiomazine	—4.4	>6.4	<2.4
N-Demethylpropiomazine	—5.5	>7.5	<3.5
Propiomazine sulfoxide	—	— *	
Prochlorperazine, internal standard	—3.9	>5.9	<1.9

*Less than 1% extraction at pH 8—10.

Reaction conditions

The polar character of the N-demethyl metabolite and its poor gas chromatographic resolution from propiomazine made it necessary to prepare the trifluoroacetyl derivative from the secondary amine. A quantitative acylation was achieved within 5 min in the presence of base. The relative retention of the derivative to propiomazine was 1.25.

Selectivity of the method

The metabolism of propiomazine is poorly investigated. The in vitro biotransformation has been studied using the microsomal fraction of rat liver, and only a ring-hydroxylated metabolite could be identified [5]. An extensive metabolism must, however, be anticipated in accordance with other drugs of the phenothiazine class. Possible routes of metabolism beside ring hydroxylation are sulfoxidation and N-demethylation. Owing to the polar properties of the sulfoxide of propiomazine, this metabolite was excluded in the extraction procedure. Phenolic metabolites were also probably excluded in the alkaline extraction of the plasma sample. Interference from unknown metabolites of propiomazine has not been observed. The selectivity of the method was verified for the following phenothiazine drugs: promazine, promethazine, thioridazine, methopromazine and levomepromazine.

Sensitivity, yield and precision

The detection limit of the method, defined as three times the background noise level, was 10—20 ng/ml of each of propiomazine and the N-demethyl metabolite. Rectilinear standard curves through the origin in the concentration range 20—400 ng/ml were obtained for propiomazine (r = 0.996) and for N-

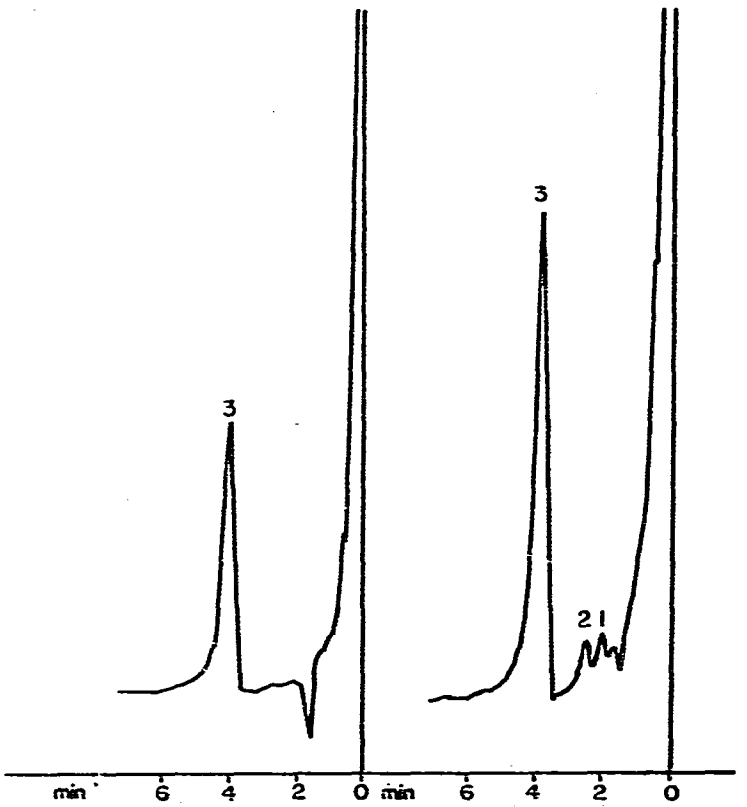

Fig. 2. Gas chromatogram of plasma sample containing propiomazine (25 ng/ml) (1) and N-demethylpropiomazine (25 ng/ml) (2) run through the method (right panel) and of a blank plasma sample containing internal standard prochlorperazine (3) (left panel).

demethylpropiomazine (r = 0.995). Usually five or six standard samples at different concentration levels were processed.

The relative recovery and precision of the method with spiked plasma samples at the 100 ng/ml level were 100 ± 7.6% and 98 ± 4.6% (n = 3) for propiomazine and the N-demethyl metabolite, respectively. The absolute recovery in the method was determined by comparison to pure compound injected in the gas chromatograph with alkali flame ionization detector. The yield was 89% for both compounds.

The amines chromatographed without indication of adsorption losses. A chromatogram of a plasma sample run through the method is shown in Fig. 2. Prochlorperazine was used as internal standard owing to its similar extraction properties and suitable gas chromatographic retention.

Application to biological samples

The method was applied to the determination of propiomazine in plasma samples after therapeutic doses of the drug. Peak plasma concentrations in the range 150—300 ng/ml occurred 2—4 h after oral administration. The plasma concentrations 24 h after administration were still in the range 40—80 ng/ml. Minute amounts of N-demethylpropiomazine were seen.

A full description of the pharmacokinetics of propiomazine will be given.

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