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DETERMINATION OF PERPHENAZINE AND ITSSULPHOXIDE METABOLITE IN HUMAN PLASMA AFTER THERAPEUTIC DOSES BY GAS CHROMATOGRAPHY

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SUMMARY

A gas chromatographic method for the determination of perphenazine (Trilafon) and its main metabolite in human plasma, perphenazine sulphoxide, has been developed. The procedure involves the use of an electron capture detector and permits the determination of the drug and its metabolite at concentrations down to 0.2 μg/l. This is sufficient for analyzing plasma from patients on ordinary treatment with perphenazine. Tests for specificity revealed no interference by nortriptyline or biperidine.

INTRODUCTION

Until recently, the determination of neuroleptic drugs in human plasma has been possible only after high doses. Several methods have been tried in order to quantitate phenothiazines, but gas chromatography seems to be the most suitable[1–4].

Quantitation of perphenazine (PPZ, Trilafon) after normal doses requires an analytical method sensitive enough to measure concentrations in the range 0.2–5.0 μg/l in plasma. In order to achieve this sensitivity, some modifications were made in the method previously reported by us[3] and used in clinical investigations[5]. However, the analytical procedure did not permit the determination of the main metabolite in plasma, perphenazine sulphoxide (PPZSO).

Therapeutic control of plasma concentrations of the parent compound (PPZ) and of the main metabolite (PPZSO) might be important from a clinical point of view if a correlation between these concentrations and the effect could be established.

This paper describes a gas chromatographic method with a sensitivity sufficient to determine the concentrations of PPZ and PPZSO that occur after conventional single doses.

EXPERIMENTAL

Reagents and glassware

Toluene of analytical-reagent grade from E. Merck (Darmstadt, G.F.R.) was distilled once before use. Undiluted borate buffer (Titrisol buffer) of pH 10 (Merck) was used to buffer the solutions. For derivatization, N,O-bis-(trimethylsilyl)acetamide (BTSA) of specially purified grade from Pierce (Rockford, Ill., U.S.A.) was used. Glassware was cleaned with detergent in an ultrasonic bath and rinsed twice with redistilled water and once with methanol.

Reference substances

Structural formulae are shown in Fig. 1.

Stock solutions (1 g/l) in ethanol were prepared of PPZ, PPZSO and of the internal standard, 4-[3-(2,8-dichlorophenothiazin-10-yl)propyl]-1-piperazinethanol (8-chlorinated PPZ, CPPZ). The solutions should be kept in a refrigerator and protected from light. When stored in this way, the solutions are stable for 1 year.

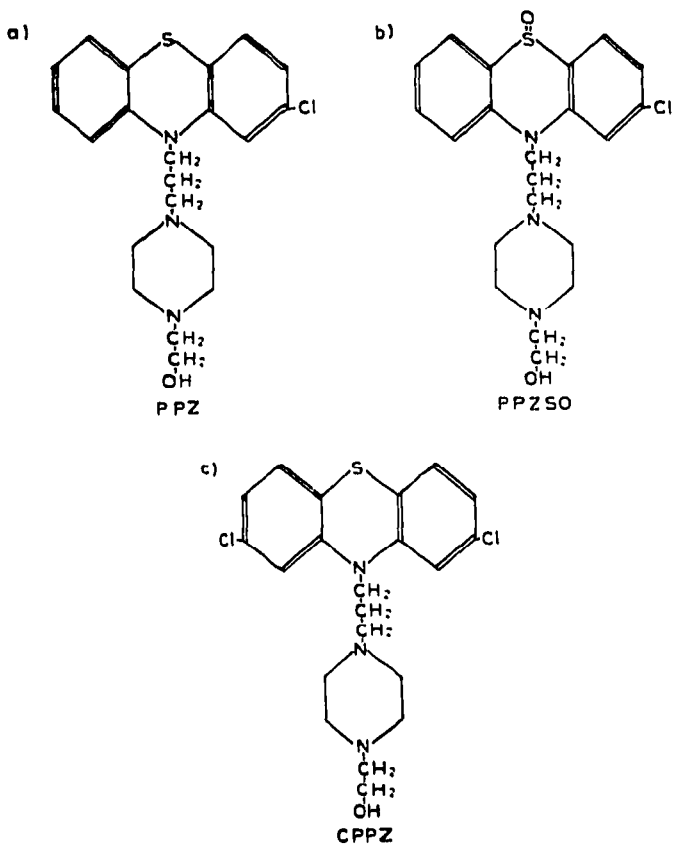

Fig. 1. Structural formulae of a, perphenazine (PPZ), b, perphenazine sulphoxide (PPZSO) and c, the internal standard (CPPZ).

Extraction procedure

To a centrifuge tube containing 2.5 ml of plasma, 5 ng of the internal standard (CPPZ) are added. The sample is buffered to pH 10 by adding 250 μl of borate buffer and extracted with 6 ml of toluene by mixing for 10 min in a rotary mixer (20–30 rpm). After centrifugation for 5 min, the organic phase is transferred into a 10-ml glass-stoppered tube containing 1 ml of 0.1 *N* sulphuric acid. The compounds are extracted into the aqueous phase by mixing for 10 min. After centrifugation, the organic phase is discarded. The buffered plasma sample is extracted once more with 6 ml of toluene, which is washed with the same portion of sulphuric acid. After centrifugation, this organic phase is also discarded. The aqueous phase is washed with 6 ml of fresh toluene. After centrifugation, the organic phase is discarded. The aqueous phase is made alkaline by adding 100 μl of 4 *N* sodium hydroxide solution. The compounds are extracted into 3 ml of toluene by mixing for 10 min in a rotary mixer. After centrifugation, the organic phase is transferred into a tapered tube and evaporated to dryness at 70° under a stream of nitrogen. The alkaline aqueous phase is extracted once more with 3 ml of toluene. This phase is transferred into the same tapered tube and likewise evaporated to dryness. The residue is dissolved in 1.5 ml of toluene and derivatized by adding 50 μl of a mixture consisting of 100 μl of BTSA in 10 ml of toluene. This mixture is made to react at 70° for 10 min. After reaction, the solvents are evaporated at 70° under a stream of nitrogen and the residue is dissolved in 30 μl of toluene. A volume of 1.5 μl of this solution is injected into the gas chromatograph.

Gas chromatography

A Pye Series 104, Model 74, gas chromatograph equipped with an electron capture detector was used. The pre-heater temperature was 310°, the column temperature 305° and the detector temperature 350°. A glass column, 1.5 m × 4 mm I.D. packed with 1% (w/w) OV-17 on Celite JJ CQ, 100–120 mesh, was used. The amount of column material was 12 g. The column was conditioned at 350° for 65 h. The carrier gas (argon–methane, 90:10) flow-rate was 60 ml/min. The pulse interval was 150 μsec and attenuation 5×10^2. The column was deactivated with hexamethyldisilazane and injections of ethyl acetate extracts of blank plasma.

Calculations

The plasma concentrations are read from standard curves constructed from chromatograms of plasma samples containing varying but known amounts of PPZ and of PPZSO (Fig. 2). Both compounds are added in amounts from 2.5 to 12.5 ng, corresponding to concentrations from 1.00 to 5.00 μg/l. The peak height ratios between PPZ and CPPZ, and between PPZSO and CPPZ, are plotted against the concentrations (Fig. 3).

RESULTS AND DISCUSSION

The presence of PPZSO in human urine after long-term treatment with PPZ was demonstrated about 10 years ago[6]. If this metabolite is active, which some preliminary results seem to indicate (unpublished work), it must be of importance to correlate the plasma levels of both this metabolite and the parent compound with the efficacy and side-effects. This is the reason for the development of the present method.

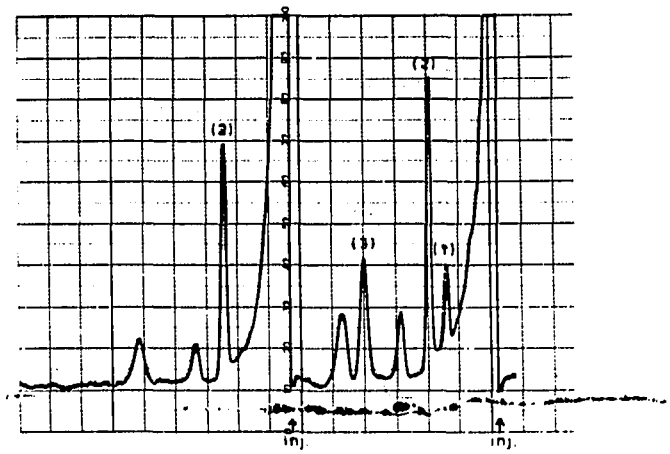

Fig. 2. Chromatograms of two plasma samples. The sample in the right-hand chromatogram contained PPZ (1), PPZSO (3) and the internal standard CPPZ (2). The left-hand chromatogram illustrates a blank plasma with added CPPZ (2).

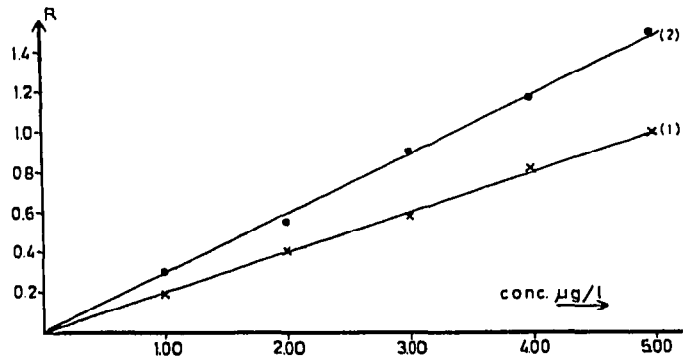

Fig. 3. Calibration graph constructed on the basis of chromatograms from plasma samples containing varying but known amounts of PPZ (1) and of PPZSO (2).

At first, we tried to apply the procedure described by Hansen and Larsen[5] to plasma samples drawn from patients receiving ordinary doses of PPZ. However, this method involves derivatization with acetic anhydride, and the procedure was evidently not suitable for the determination of PPZSO. It subsequently proved impossible to acetylate S-oxidised phenothiazines because of a displacement of the unsaturated electron bonds resulting from degradation of the aromatic ring system (unpublished work). By introducing N,O-bis-(trimethylsilyl)acetamide (BTSA) instead, stable trimethylsilyl derivatives of PPZ and of PPZSO were produced.

PPZ was previously shown to be quantitatively extracted with toluene from aqueous alkaline solutions[5]. Extraction recoveries with toluene carried out as described[5] of PPZ and of PPZSO were found to be 100% only for perphenazine. In order to obtain 100% recovery of all three compounds, it is necessary to repeat all toluene extractions. No loss was recorded on washing the sulphuric acid phases with the organic solvents.

Accuracy tests for PPZ and PPZSO when present in varying concentrations were performed on twenty plasma samples within the therapeutic concentration range and found to be ± 10% for both substances. Details are given in Table I. The lower limit for quantitation (sensitivity) was found to be 0.2 μg/l when a plasma volume of 2.5 ml was used.

TABLE I

ACCURACY TEST FOR PERPHENAZINE (PPZ) AND PERPHENAZINE SULPHOXIDE (PPZSO) FROM PLASMA

The plasma volume extracted was 2.5 ml in each instance.

Concentration added (μg/l)		*No. of samples, n*	*Calculated concentration (μg/l)**	
PPZ	*PPZSO*		*PPZ*	*PPZSO*
1.00	1.00	10	0.98 ± 0.05	1.04 ± 0.10
5.00	5.00	10	5.05 ± 0.07	5.01 ± 0.02

* Mean ± standard deviation.

Schizophrenic patients are often treated with other drugs in addition to PPZ [*e.g.*, nortriptyline and biperidine (Akinetone)]. The specificity of the method was therefore examined in the presence of these drugs added to plasma samples with known therapeutic PPZ concentrations. Details are given in Table II. As clearly demonstrated, none of these drugs obviously interferes with the determination of PPZ or PPZSO.

TABLE II

SPECIFICITY OF THE METHOD DEMONSTRATED IN MIXTURES OF DRUGS IN PLASMA SAMPLES

Results are given as concentrations (μg/l).

Perphenazine		*Perphenazine sulphoxide*		*Nortriptyline*		*Biperidine*	
Added	*Recovered*	*Added*	*Recovered*	*Added*	*Recovered*	*Added*	*Recovered*
1.00	1.05	1.00	1.05	100	—	20	—
2.00	1.90	2.00	2.05	100	—	20	—
3.00	3.10	3.00	2.90	100	—	20	—
4.00	4.05	4.00	3.95	100	—	20	—
5.00	4.90	5.00	5.05	100	—	20	—

In order to ensure that unknown metabolites probably formed *in vivo* are not co-determined, an attempt to check the specificity was made by means of mass fragmentography. However, the sensitivity turned out to be grossly insufficient, as 2.5 ng had to be injected in order to produce a reliable response. Consequently, specificity tests had to be carried out on the gas chromatograph with the use of various derivatives. It is difficult to produce stable compounds related to PPZSO except with BTSA and, furthermore, underivatized PPZSO is unsuitable for gas chromatography. It was therefore necessary to limit the specificity tests to PPZ. In pooled plasma samples

drawn from patients treated with PPZ the concentrations were determined in the following ways: (1) underivatized, (2) acetylated and (3) silylated (BTSA). The determinations turned out to give identical results indicating a high probability for specific PPZ determinations.

To illustrate the application of the method, the concentrations of PPZ and PPZSO were determined in plasma samples from a patient treated with perphenazine enanthate. The concentration curves are shown in Fig. 4.

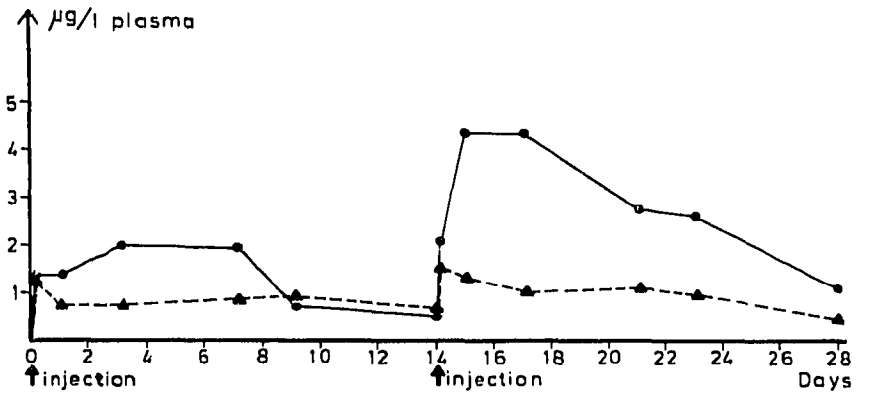

Fig. 4. Fluctuations in PPZ (●) and PPZSO (▲) concentrations in plasma. On each of the days 0 and 14, 100 mg of perphenazine enanthate (Trilafon enanthate) were given intramuscularly.

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