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Note

Simple and sensitive high-performance liquid chromatographic procedure with electrochemical detection for the determination of plasma concentrations of trimeprazine following single oral doses

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The quantitation of phenothiazine drugs in the plasma of patients under treatment with these agents is difficult, due to the low levels encountered for such reasons as the low therapeutic doses used, a significant first-pass metabolism on oral administration, extensive metabolism to numerous metabolites, and a large volume of distribution partly resulting from extensive binding to multiple sites. This challenge of attaining adequate sensitivity (ng ml^{-1}) is compounded by the instability and adsorptive loss of phenothiazines in all stages of handling for analysis [1]. However, despite these problems there has in recent years been development of specific and sensitive analytical methods, which in some cases have been demonstrated as capable of quantitating the subnanogram plasma levels encountered 24 h and later after low single oral doses of phenothiazine drugs. These latter methods are generally either the more sensitive chemical methods, as for example those based on gas—liquid chromatography—mass spectrometry (GLC—MS) [1,2], and/or biological methods, especially those based on an immune response [1,3].

Trimeprazine is one of the more difficult phenothiazines to quantitate in plasma, being a potent antihistamine and antipruritic, the recommended maximum daily dosage not exceeding 15 mg as the tartrate salt. Indeed, although methods such as GLC with nitrogen—phosphorus dectection have been reported as being suitable for quantitating toxic levels of this drug [4], these authors are unaware of any suitable, sensitive and specific published procedure for quantitating trimeprazine in plasma after administration of the usual oral therapeutic doses. Recently a radioimmunoassay method, capable of quantitating 0.32 ng ml^{-1} of trimeprazine in a 200-μl plasma sample, was developed in these laboratories [5]. Since such methods are doubted for their specifity, a

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specific and sensitive chemical method was required to be developed in order to verify the biological procedure. Such a method, based on high-performance liquid chromatography with electrochemical detection (HPLC—ElCD), which is also simple and easy to adopt, was recently developed in these laboratories for chlorpromazine [6]. This paper reports a similar ultrasensitive (subnanogram) HPLC—ElCD method for trimeprazine, which for the first time allows determination of plasma concentrations after oral administration of therapeutic doses to patients. In fact, plasma concentration—time profiles in healthy volunteers up to 24 h after single 5-mg oral doses of trimeprazine tartrate were demonstrated.

EXPERIMENTAL

Materials

Trimeprazine tartrate (Panectyl®) was obtained commercially from Poulenc, Montreal, Canada. Prochlorperazine mesylate was a gift from Poulenc. HPLC solvents were distilled in glass prior to use; all other chemicals used were of the highest commercial grade available. Double-distilled deionized water was used to make stock solutions of trimeprazine and prochlorperazine. Appropriate dilutions of standard solutions made in distilled deionized water were placed in pooled fresh plasma obtained prior to analysis from blood collected from healthy volunteers.

Instruments

A Waters Model M-45 liquid chromatographic pump (Waters Scientific Co., Mississauga, Canada) fitted with a Model 7125 Rheodyne valve-loop (500-μl loop) injector, (Technical Marketing Associates, Calgary, Canada) was used. A Dupont Zorbax CN, particle size 5 μm, 250 X 4.6 mm column (Fisher Scientific and Co., Edmonton, Canada) was connected to a Bioanalytical Systems electrochemical detector (Technical Marketing Assoc., Calgary, Canada) operated in the oxidation mode at +0.9 V utilizing a 10-nA feed to a Model 56 linear recorder (Perkin-Elmer, Montreal, Canada). The mobile phase consisted of a 10% 0.1 *M* ammonium acetate buffer in acetonitrile. The mobile phase was degassed by Millipore filtration (Millipore Corp., Bedford, NA, U.S.A.) prior to use. The chromatograph was operated at ambient temperature with a flow-rate of 4 ml/min.

Preparation of standard curve

A stock solution of trimeprazine tartrate (100 μg ml^{-1} as the free base) was made monthly in distilled deionized water and stored at 4°C. Prochlorperazine mesylate stock solution (1000 μg ml^{-1} as the free base) was made monthly in distilled deionized water and stored at 4°C.

Appropriate volumes of a standard trimeprazine solution (100 ng ml^{-1}) diluted with distilled deionized water were added to fresh blank plasma (2.0 ml) and to this was added prochlorperazine (1.0 ml from a 100 ng ml^{-1} solution). The standard samples were then extracted in an identical fashion as unknown samples.

Extraction of samples

The extraction procedure was similar to that described earlier for the extraction of chlorpromazine [6]. To a 10.0-ml PTFE-lined screw-capped centrifuge tube (13 × 100 mm) were added 1.0 ml of sample plasma, 1.0 ml of fresh control plasma, 1.0 ml of prochlorperazine solution (concentration 100 ng ml^{-1}) and 0.5 ml of saturated sodium carbonate solution. The tube was mixed by Vortex for 5 sec and 5.0 ml of extraction solvent (3% isopropanol in pentane) were added. The tube was tightly capped and mixed for 15 min on a SMI multitube shaker (Canlab, Edmonton, Canada) followed by cnetrifugation for 10 min at 1725 *g* at ambient temperature. The upper organic layer was transferred to a clean test tube and the aqueous phase extracted as above with a further 5.0 ml of 3% isopropanol in pentane. The combined organic extracts were evaporated to dryness in a dry bath at 65°C. The residue was reconstituted in 200 μl of acetonitrile and 100 μl were injected into the chromatograph.

Plasma level study

Two healthy male volunteers weighing 61 kg and 85 kg were fasted overnight and then administered 5 mg of trimeprazine with 200 ml of water. Blood samples were collected in heparinized tubes (Venoject®, Becton Dickinson, through Canlab, Edmonton, Canada) avoiding contact of the blood with the rubber stopper. Samples (8.0 ml) were drawn at 0, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0 and 24.0 h following drug administration. The blood samples were centrifuged and the plasma stored at −20°C until the time of analysis.

Quantitation and recovery study

Standard curves for trimeprazine were constructed by chromatographing spiked plasma samples and plotting peak height ratios versus concentration of the drug. Standard curves were established at the same time as analysis of samples from volunteers.

For the determination of recovery of trimeprazine and prochlorperazine at least five replicates at levels of 5.0 ng ml^{-1} and 1.0 ng ml^{-1} were carried out for trimeprazine and seven replicates at 50 ng ml^{-1} were carried out for prochlorperazine. The absolute peak heights obtained for the extracted recovery samples were compared with peak heights obtained for fresh standards of trimeprazine and prochlorperazine made in mobile phase.

RESULTS AND DISCUSSION

As shown in Fig. 1, trimeprazine gave a sharp symmetrical peak with a retention time of 2.74 min, while prochlorperazine gave a peak at 5.56 min. The peak for trimeprazine shown in Fig. 1b represents a spiked sample of plasma containing 0.5 ng ml^{-1}. Also shown in Fig. 1 is a typical chromatogram obtained from a plasma sample taken from a healthy volunteer (61 kg) 6 h after the administration of an oral dose of trimeprazine (5-mg tablet). The lower limit of sensitivity of the assay as described in the experimental section was 0.25 ng ml^{-1} with a detection limit of 0.125 ng ml^{-1}.

Table I gives an estimate of the accuracy of the described procedure. The

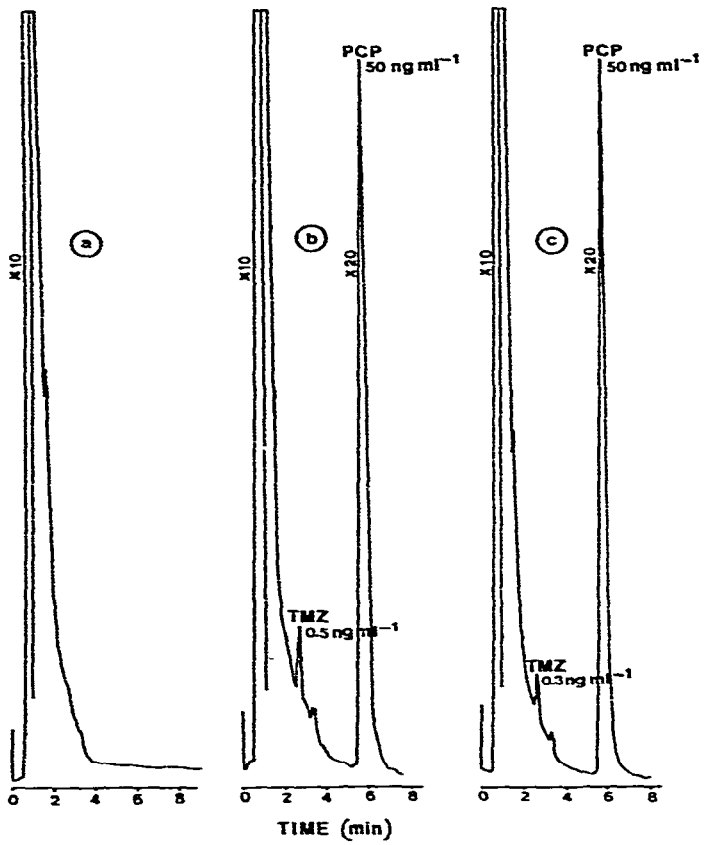

Fig. 1. Chromatograms of trimeprazine (TMZ) and prochlorperazine (PCP) obtained using electrochemical detection. (a) Blank plasma; (b) plasma (2.0 ml) spiked with 0.5 ng ml^{-1} trimeprazine; (c) plasma (2.0 ml) obtained from a healthy volunteer (61 kg) 6 h after an oral dose of trimeprazine tartrate. Chromatographic conditions and sample handling were as described in the Experimental section.

TABLE I

HPLC ESTIMATION OF TRIMEPRAZINE ADDED TO PLASMA

$y = mx + b$, where $m = 0.0507$, $b = 0.0027$. Mean = 3.42, $r^2 = 0.9995$.

Amount added (ng)	n	Mean peak height ratio	S.D.	C.V. (%)
0.25	6	0.0130	0.007	5.40
0.50	6	0.0262	0.0012	4.40
1.0	6	0.0544	0.0019	3.51
2.5	6	0.1277	0.0051	3.98
5.0	7	0.2573	0.0047	1.83
10.0	7	0.5072	0.0070	1.38

standard curve determined from these data had a slope value of 0.0507 and y-intecept of 0.0027. The correlation coefficient for the linear regression line drawn through these points was 0.9995 with a mean coefficient of variation of 3.42%. These results are in line with the accuracy obtained in a similar procedure for the HPLC—ElCD analysis of chlorpromazine reported earlier from these laboratories [6].

The mean percentage recoveries of trimeprazine at 5 ng ml^{-1} and 1 ng ml^{-1} were found to be equivalent with an overall value of 81.6 ± 1.3% (Table II).

TABLE II

RECOVERY OF TRIMEPRAZINE AND PROCHLORPERAZINE FROM PLASMA

Drug	Amount added (ng/ml) plasma)	n	Mean amount recovered (ng)	Percentage recovery mean ± S.D.)
Trimperazine	5.0	7	4.08	81.61±1.15
	1.0	5	0.82	81.64±1.98
Prochlorperazine	50.0	7	43.16	86.31±1.33

When this method of analysis was applied to the determination of the pharmacokinetics of trimeprazine after oral administration of low doses (5 mg) to healthy volunteers, profiles such as that shown in Fig. 2 were obtained. The peak plasma concentration of trimeprazine in this volunteer (85 kg) was 1.75 ng ml^{-1}, while the corresponding peak value for the other volunteer was 0.75 ng ml^{-1}. As can be seen in Fig. 2, the method described permits the determination of plasma trimeprazine concentrations up to 24 h after a single 5-mg oral dose.

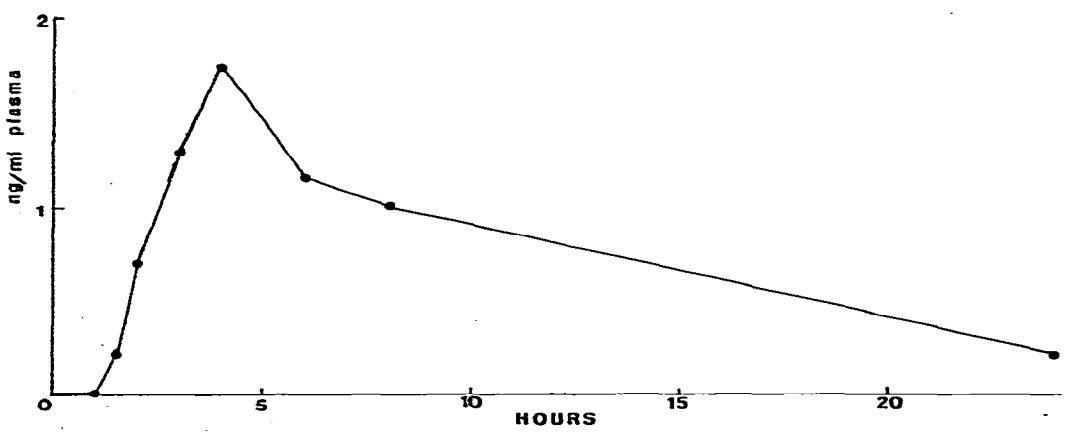

Fig. 2. Plasma concentration—time profile for a healthy volunteer (85 kg) after receiving an oral dose of trimeprazine tartrate (5-mg Panectyl tablet).

The described HPLC—ElCD method allows for the first time the determination of plasma concentrations of trimeprazine following a single therapeutic oral dose of this phenothiazine. In addition, plasma levels can be quantitated as late as 24 h post administration with sufficient sensitivity and specificity to

permit pharmacokinetic analysis of this drug. It is felt that this method of analysis will be of use in the study of pharmacokinetic and bioavailability parameters of this phenothiazine in healthy volunteers either at the steady-state level or even after low (5 mg) single oral doses. Studies of this type are currently being investigated in these laboratories.

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