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Note

Simultaneous determination of promethazine and two of its circulating metabolites by high-performance liquid chromatography

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Promethazine (PMZ) has attracted widespread use over a number of years for its antihistaminic and sedative properties. Its disposition in man however, has been paid sparse attention, largely as a result of the inadequacy of the available assay procedures. Recently, methods have been reported for the determination of PMZ at therapeutic concentrations using gas chromatography [1] and high-performance liquid chromatography (HPLC) [2, 3]. An assay which permits the simultaneous determination of circulating metabolites however, has not been previously described.

In this report an HPLC procedure for the assay in PMZ is described, the precision and sensitivity of which compares favourably with any previously reported method. In addition, the concurrent determination of the monodemethylated and sulphoxidated metabolites of PMZ (Fig. 1) is demonstrated, and the applicability of the procedure to human pharmacokinetic studies is illustrated.

EXPERIMENTAL

Reference standards

PMZ, as a reference substance and also as a solution for administration (Phenergan injection), was kindly supplied by May and Baker (Dagenham, Great Britain). Monodesmethylpromethazine (Nor_1PMZ) and imipramine were gifts from Kabi Pharmaceuticals (Stockholm, Sweden) and Berk Pharmaceuti-

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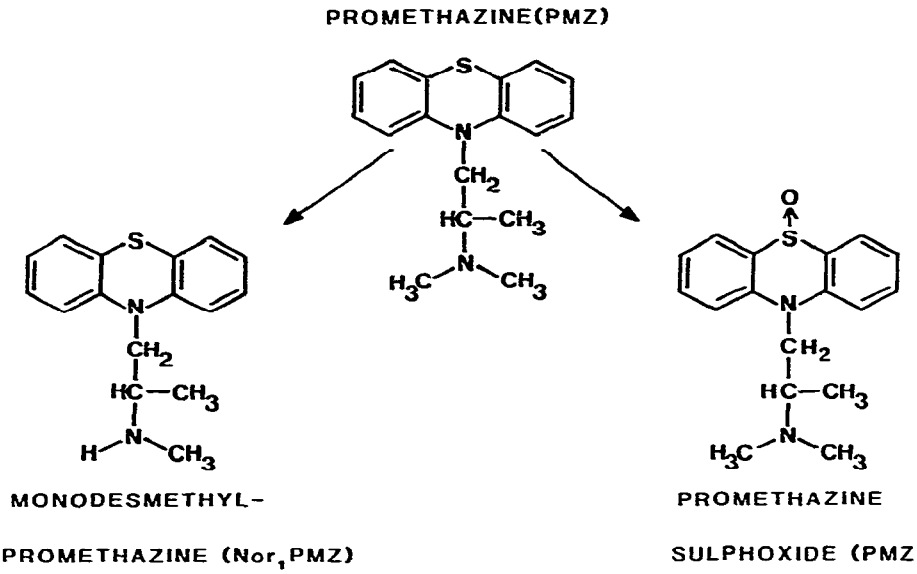

Fig. 1. Structures of promethazine and its monodesmethyl- and sulphoxide metabolites.

cals (Guildford, Great Britain), respectively. Promethazine sulphoxide (PMZSO) was prepared in the laboratory by the following method. Promethazine hydrochloride (1 g) was dissolved in 5 ml of distilled water, 0.6 ml of 27.5% hydrogen peroxide added, and the mixture kept in a dark place at room temperature overnight. It was then made alkaline with sodium hydroxide and extracted with successive 5-ml aliquots of *n*-heptane, diethyl ether, and dichloromethane. The dichloromethane extract was evaporated to dryness and the product recrystallised from dichloromethane. The results of elemental analysis were consistent with the theoretical values for PMZSO. Contamination of the product with the N-oxide of PMZ was checked by mass spectrometry (MS); using a probe temperature of 50°C, phenothiazine N-oxides produce characteristic ion peaks at *m/e* 60 and 61 [4], such peaks were not seen in the MS chromatogram of the synthesised product.

Reagents

All reagents used were of AnalaR grade obtained from BDH Chemicals (Poole, Great Britain) with the exception of dichlorodimethylsilane, propan-2-ol, 1,1,1-trichloroethane and *n*-heptane, which were of reagent grade. Isoamylalcohol was obtained from Aldrich Chemicals (Gillingham, Great Britain).

Chromatography

A Pye Unicam Model LC3 pump was equipped with a variable-wavelength UV detector (Model LC3 Pye Unicam), and a Philips Model 8251 chart recorder. A stainless-steel column (100 mm × 4.8 mm I.D.) containing Hypersil 5-SAS (Shandon Southern Products, Runcorn, Great Britain) was prepared in the laboratory using a propan-2-ol slurry and a packing pressure of 400 bars. The column was fitted with a septum injection system. The eluent, consisting

of methanol containing 30% v/v of 0.05 *M* Sørensen's phosphate buffer, pH 7.4, was maintained at a flow-rate of 0.7 ml/min. The analytical wavelength used was 248 nm, with a band width of 8 nm.

Extraction

All glass tubes used in the extraction procedure were cleansed by overnight immersion in a solution of chromosulphuric acid. After rinsing and drying, the tubes were silanised using 2% dichlorodimethylsilane in 1,1,1-trichloroethane, rinsed with methanol, then distilled water, and dried. Screw-cap tops and PTFE liners for the extraction tubes were cleansed by overnight immersion in a 5% solution of Decon 90 (Decon Labs., Brighton, Great Britain).

Blood samples

Each whole blood sample (10.0 ml) contained in a 16-ml tube, was spiked with imipramine hydrochloride (0.05 ml, 10 μg/ml) as internal standard, made alkaline with 1.0 ml of 1 *M* sodium hydroxide, and extracted with 4.0 ml of *n*-heptane containing 10% dichloromethane and 1.5% of isoamylalcohol for 15 min using an inversion mixer. After centrifugation (3000 *g*, 10 min) the organic layer was removed to a nipple tube and was replenished by a further 4.0 ml of the extraction solvent. After mixing and centrifuging as before, the combined extracts were extracted with 50 μl of 0.1 *M* hydrochloric acid for 2 min using a vortex mixer. Aliquots (10—25 μl) of the acidic phase were then injected immediately onto the HPLC column. It is recommended that blood samples be frozen on collection and extracted immediately on thawing.

Quantitation

Standard curves were prepared by the addition of known amounts of PMZ, Nor_1PMZ and PMZSO to blank blood, and analysing a set of standards with each batch of samples. An unweighted least-squares regression was employed to fit plots of peak height ratios (drug/metabolite:internal standard) versus blood concentration.

RESULTS AND DISCUSSION

The extraction of PMZ and its metabolites using *n*-heptane containing 10% dichloromethane and 1.5% isoamyl alcohol, was reproducible (Table I). The extraction efficiencies for PMZ and Nor_1PMZ were high, whilst that of PMZSO was considerably lower. Inclusion of dichloromethane in the extraction solvent increased the efficiency of, and reduced the variability in, the extraction of PMZSO. Increasing the proportion of dichloromethane above 10%, resulted in higher extraction efficiencies of PMZSO, but led to decreased efficiencies for both PMZ and Nor_1PMZ.

The differences in λ_{max} values for PMZ and Nor_1PMZ (252 nm) and PMZSO (236 nm) were taken into consideration in the choice of detector conditions. Under those conditions used (analytical wavelength, 248 nm; band width, 8 nm) the extinction coefficients for both PMZ and Nor_1PMZ were approximately equal to those measured at 252 nm, whilst for PMZSO, the extinction coefficient was equal to 70% of that measured at 236 nm.

TABLE I

RECOVERY, PRECISION OF THE ANALYSIS AND MINIMUM DETECTION LIMITS FOR PROMETHAZINE AND METABOLITES

Compound	Recovery (% ± S.D.)	C.V. (%)*			MDC** (ng/ml)
		a	b	c	
PMZ	75 ± 4	3.9	8.4	—	0.2
Nor_1PMZ	76 ± 8	4.5	8.9	—	0.2
PMZSO	26 ± 2	—	—	7.0	1.0

*Within-run coefficient of variation (n = 5) for: (a) 8 ng/ml, (b) 1.5 ng/ml, (c) 21 ng/ml.
**MDC = minimum detectable concentration, defined as peak height ≡ twice baseline noise of blank blood.

Fig. 2 shows chromatograms of extracts of whole blood samples taken from a human volunteer subject, immediately prior to, and 11 h following, the oral administration of 25 mg promethazine hydrochloride. Fig. 2a indicates that no interfering substances are coextracted by the procedure. In Fig. 2b the separation of the two metabolites and the parent compound is demonstrated. The retention times for each compound were as follows: PMZSO (6.4 min), Nor_1PMZ (7.5 min), PMZ (9.3 min) and internal standard (14.4 min).

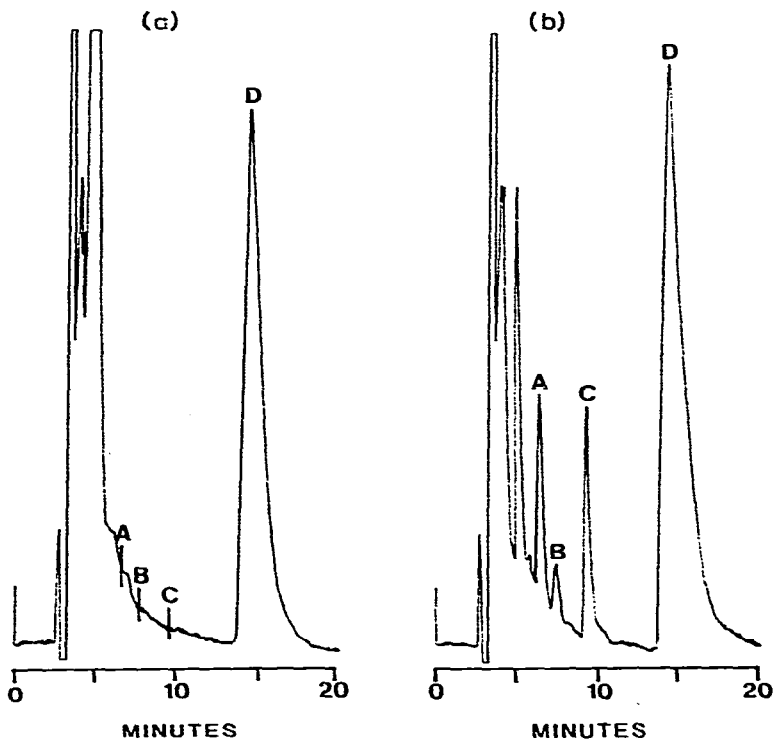

Fig. 2. HPLC traces resulting from extracts of blood obtained prior to (a) and 11 h following (b) the oral administration of 25 mg promethazine hydrochloride to a human volunteer subject. Peaks in (b): A = PMZSO (11.9 ng/ml blood), B = Nor_1PMZ (0.8 ng/ml), C = PMZ (2.8 ng/ml) and D = internal standard (imipramine).

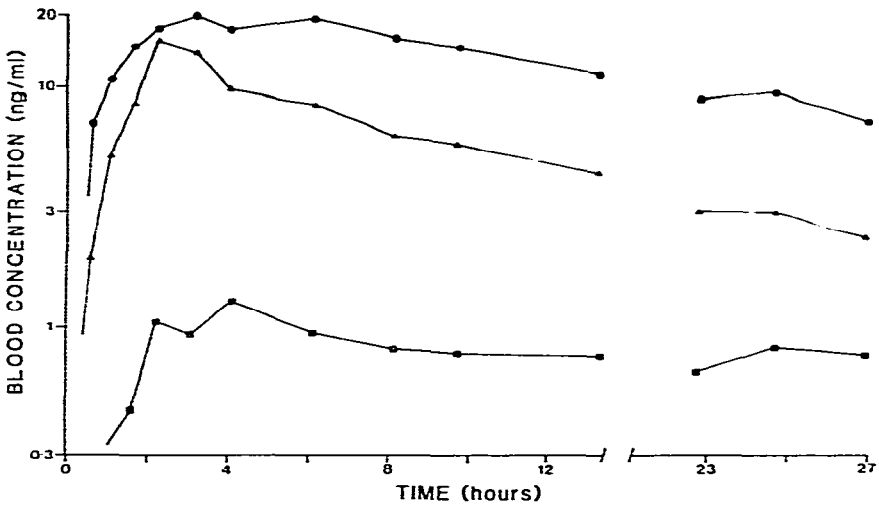

Fig. 3. Blood concentrations of PMZ (▲——▲), PMZSO (●——●) and Nor_1PMZ (■——■) following the oral administration of promethazine hydrochloride (25 mg) to a human volunteer.

Imipramine appears to be a suitable choice for internal standard, being well separated from the other compounds and also chemically similar to PMZ. The column efficiency was found to be 2500 plates (25,000 plates per metre) for PMZ.

Standard curves over the concentration ranges tested of 2—50 ng PMZ and Nor_1PMZ, and 5—50 ng PMZSO, per ml of blood were found to be linear, with intercepts which were not significantly different from zero. Within-run precision was determined using five spiked blood samples. The within-run coefficients of variation, together with minimum detectable concentrations and extraction recoveries are presented in Table I.

The applicability of the method to human pharmacokinetic studies was investigated. Fig. 3 shows blood concentration—time profiles for PMZ, Nor_1PMZ and PMZSO following the oral administration of 25 mg promethazine hydrochloride to a human volunteer. The peak time and elimination half-life for PMZ are similar to those reported previously [1]. Concentrations of PMZSO are comparable to those of PMZ. It is estimated that blood concentrations of these two compounds will be measurable over a time period equivalent to three to four times their blood half-lives (40 h). In contrast, concentrations of Nor_1PMZ were consistently lower than PMZ and PMZSO yet measurable using our specified conditions.

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