

# A Liquid Chromatographic Method for the Simultaneous Determination of Promethazine and Three of Its Metabolites in Plasma Using Electrochemical and UV Detectors

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## Abstract

A new assay method has been developed for the quantitation of promethazine (PMZ) with a sensitivity and reproducibility as good as any previously reported method. This method is also capable of quantitatively determining three metabolites of PMZ (monodemethylated, sulphoxidated, and monodemethylated sulphoxidated PMZ), which has not been previously described. The method uses high-performance liquid chromatography with amperometric and UV detection simultaneously and requires only one extraction step from serum with chloroform. The method uses trifluoperazine as the internal standard. The limit of detection level for PMZ is 1.0 ng/mL when a 0.2-mL specimen of plasma is assayed. A validation study is also conducted for evaluating the recovery, precision, linearity of response, sensitivity, and selectivity of the method.

## Introduction

Promethazine (PMZ) is an effective antihistamine and antiemetic drug. It is rapidly metabolized, and few disposition studies involving humans have been recorded because of the current inadequate assay methods that are unable to detect low concentrations of PMZ and its metabolites promethazine sulfoxide (PMZSO), desmonomethyl promethazine (DMPMZ), and desmonomethyl promethazine sulfoxide (DMPMZSO) (Figure 1) simultaneously in the plasma. Methods have been reported for determining promethazine and some of its metabolites using gas chromatography (GC) and high-performance liquid chromatography (HPLC) (1,2). Assay methods developed over the past years have also attempted to detect PMZ only and not its metabolites, which is crucial for drug disposition studies. No assay method has been reported for detecting PMZ and its three major metabo-

lites in plasma. For drug disposition studies, it is necessary that the quantitative determination of PMZ and its metabolites be specific and sensitive enough to measure in the limit of 1 to 5 ng/mL. In many cases, the plasma sample volume is critical, and the detection of a drug in as low as 100  $\mu$ L would be very advantageous. Many methods now available use large plasma samples in the range of 2 to 10 mL. An assay method is described that allows for the simultaneous determination of PMZ and its three major circulating metabolites.

## Experimental

### Apparatus

A Shimadzu (Kyoto, Japan) LC-6A liquid chromatograph (LC) pump, a Model SPD-6A module UV detector (Shimadzu), a Model LC-4A amperometric controller with an LC-17 oxidative flowcell equipped with an RE-1 silver-silver chloride reference electrode, and a TL-5 glassy carbon thin-layer electrode (Bioanalytical Systems, West Lafayette, IN) were used.

### Reagents

The reagents used were all of analytical grade. Ammonium acetate, chloroform (HPLC-grade), sodium hydroxide, and hydrogen peroxide were obtained from Sigma Chemicals (St. Louis, MO). Methanol (HPLC-grade) was obtained from Fisher Scientific (Pittsburgh, PA). Acetic acid was from Mallinckrodt (Paris, KY).  $\alpha$ -Chloroethyl chloroformate was obtained from Aldrich Chemicals (Milwaukee, WI).

### Reference standards

PMZ hydrochloride and trifluoperazine were obtained from Sigma chemicals. PMZSO was prepared in the laboratory by the method described by Taylor et al. (3). DMPMZSO was prepared (4) in the laboratory by adding 4 g of  $\alpha$ -chloroethyl chloroform-

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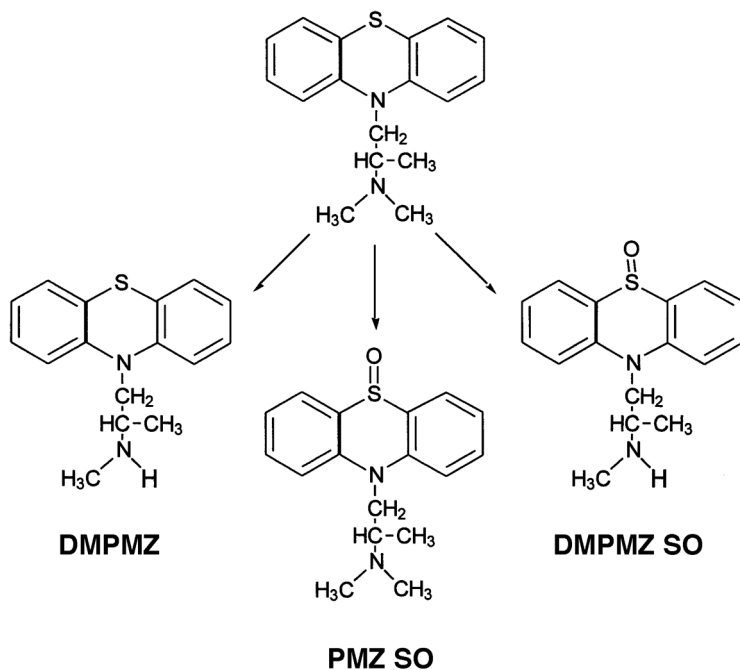


Figure 1. The structure of PMZ and its three metabolites.

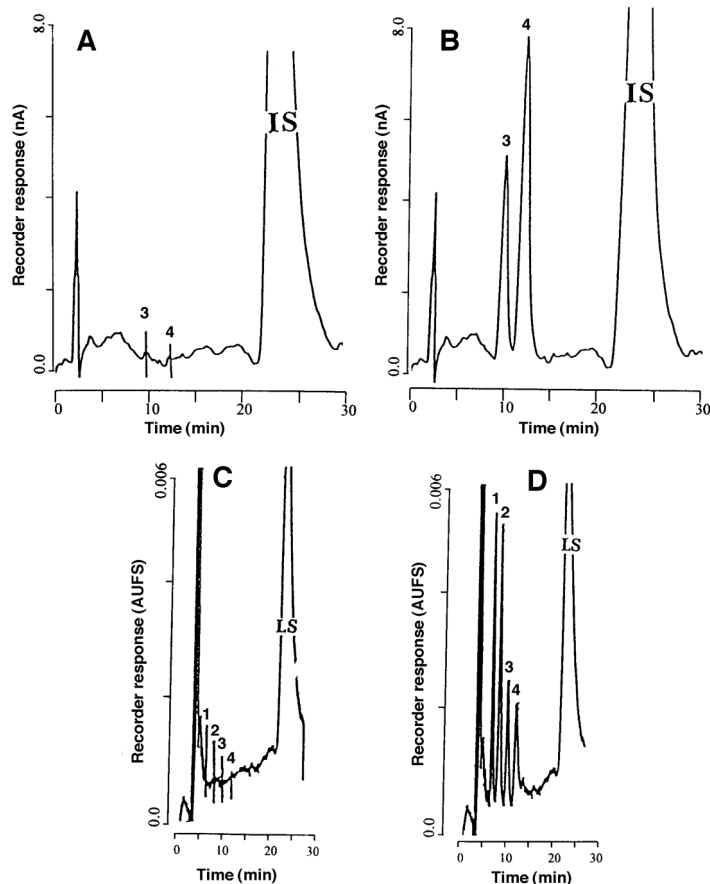


Figure 2. Typical chromatograms for spiked human-plasma standards from an EC detector (A and B) and a UV detector (C and D): DMPMZSO (30 ng/mL), 1; PMZSO (40 ng/mL), 2; DMPMZ (30 ng/mL), 3; and PMZ (30 ng/mL), 4. Trifluoperazine (60 ng/mL) was used as the internal standard and added to each specimen.

mate to an equal or slightly small amount of PMZ in dichloroethane at 0°C for 15 min. The mixture was then refluxed for 1 h. The reaction mixture was allowed to evaporate in vacuum. The residue was heated in methanol (45 min at 50°C to reflux). The methanol mixture was evaporated to dryness, and the crude hydrochloride salt was recrystallized from acetonitrile. A 76% yield of white crystals was produced. Contamination of the product was checked by using mass spectroscopy. The melting point ranged from 235°C to 238°C, and the mass spectrum (12 eV) produced the following results:  $m/z = 269.9$  ( $m+$ , 5.5%), 214 ( $m+$ , 6.4%), 213 ( $m+$ , 100%), 198 ( $m+$ , 3.8%), 72 ( $m+$ , 1.4%), and 58 ( $m+$ , 4.6%).

DMPMZSO was prepared in the laboratory by dissolving 2 g of DMPMZ hydrochloride in 10 mL of distilled water. A 5-mL solution of 30% hydrogen peroxide was then added, and the mixture was incubated at room temperature for 24 h in the darkness. After incubation, the mixture was made alkaline by adding sodium hydroxide and extracted with 20 mL of dichloromethane. The dichloromethane extract was evaporated to dryness and recrystallized from acetonitrile. A 40% yield of a white powder was produced. The melting point ranged from 119°C to 125°C, and the mass spectrum (12 eV) produced the following results:  $m/z = 286$  ( $m+$ , 11.2%), 230 ( $m+$ , 8.9%), 229 ( $m+$ , 43%), 214 ( $m+$ , 2%), 213 ( $m+$ , 6.9%), 212 ( $m+$ , 100%), 198 ( $m+$ , 8.1%), and 58 ( $m+$ , 38%).

### Chromatography

A J.T. Baker (Phillipsburg, NJ) 5- $\mu$ m CN column (250- $\times$  4.6-mm i.d.) was used for the analysis. The mobile phase consisted of a mixture of methanol–0.15M ammonium acetate (pH 5.0)–water (38:50:12) and was maintained at a flow rate of 0.9 mL/min.

The analytical wavelength for the UV detector was set at 236 nm, and the potential for the amperometric detector was set at +0.8 V.

### Extraction procedure

A 1.0-mL stock methanolic solution of trifluoperazine was evaporated to dryness and made up to 500 mL with chloroform in order to give a final concentration of 200 ng/5 mL for the internal standard.

Exactly 0.2 mL of plasma was pipetted into a test tube, made alkaline with 0.8 mL of 1M sodium hydroxide, and vortexed for 15 s. Then, 5.0 mL of chloroform containing trifluoperazine as the internal standard was added and vortexed for 1.0 min. The mixture was centrifuged at 2000 rpm for 10 min. The lower organic layer (approximately 4.5 mL) was transferred to a test tube and

**Table I. Recovery Percentage, Precision, and LODs for PMZ and Its Three Metabolites**

	%Recovery	%CV between-day*			%CV within-day*			LOD <sup>†</sup> (ng/mL)
		10 ng/mL	30 ng/mL	50 ng/mL	10 ng/mL	30 ng/mL	50 ng/mL	
PMZ	99.3	5.7	3.3	6.1	7.1	12.9	4.1	1.0
DMPMZ	91.6	16	6.2	7.6	11.7	11.8	4.5	1.5
PMZSO	91.8	2.7	4	10.2	4.5	4.6	5.8	2.0
DMPMZSO	97.3	11.3	11.3	8.3	9.3	10	6.3	1.5

\* For each concentration:  $n = 3$ .

† Defined as the peak height equal to twice the baseline noise in the blank plasma.

evaporated to dryness. The residue was redissolved in 50  $\mu$ L of methanol. Aliquots (20  $\mu$ L) of the reconstituted solution were injected onto the HPLC column.

### Preparation of standard curves

Plasma standards were prepared by transferring 0.00, 0.01, 0.02, 0.03, 0.04, and 0.05 mL of a methanolic solution of PMZ (2  $\mu$ g/mL) and its three metabolites into test tubes; gently evaporating the methanol; and adding 2.0 mL of a drug-free plasma and vortexing it for 1 min. The resulting plasma standards provided the concentration range of 0, 10, 20, 30, 40, and 50 ng/mL.

A set of 0.2-mL samples of these standards were extracted and analyzed for their drug and metabolite concentrations. The standard curves consisted of the peak height ratio of PMZ, PMZSO, DMPMZ, and DMPMZSO versus trifluoperazine plotted against the concentrations.

## Results and Discussion

The detection of PMZ and DMPMZ with an electrochemical detector at +0.8V and PMZSO and DMPMZSO with a UV detector at 236 nm was well-observed. PMZ, its three metabolites, and the internal standard were well-resolved eluting at 13.2, 11.1, 8.9, 7.3, and 24.5 min for PMZ, DMPMZ, PMZSO, DMPMZSO, and trifluoperazine, respectively. Specificity of the assay method was determined in order to check if there were any interfering peaks from endogenous components in the plasma. No interferences in the chromatogram at the respective retention times of PMZ and its three metabolites and the internal standard were observed with a blank plasma extract. Typical chromatograms for spiked human-plasma standards are shown in Figure 2.

With a sample volume of 0.2 mL, the limits of detection (LODs) for this assay method were found to be 1.0, 1.5, 2.0, and 1.5 ng/mL for PMZ, DMPMZ, PMZSO, and DMPMZSO, respec-

tively. Standard curves for these four compounds had good linearity in the range of a 10.0- to 100.0-ng/mL concentration and had  $R^2$  values of greater than 0.99. Within-day variation (%CV) ranged from 4.1% to 12.9% for PMZ, 4.5% to 11.8% for DMPMZ, 4.5% to 5.8% for PMZSO, and 6.3% to 10.0% for DMPMZSO. Between-day precision (%CV) values were 3.3% to 6.1%, 6.2% to 16.0%, 2.7% to 10.2%, and 8.3% to 11.3%, respectively, for PMZ and its three metabolites during a three-day validation study (Table I).

The recovery of the extraction procedure was determined by spiking the drug-free plasma samples with known amounts of PMZ and its three

metabolites and extracting them with chloroform containing known amounts of trifluoperazine (used as the internal standard). The peak heights were then compared with the absolute peak heights that were obtained by the direct injection of the methanolic solution having the same concentration of PMZ, its three metabolites, and the internal standard. The recoveries for PMZ, DMPMZ, PMZSO, and DMPMZSO were 99.3%, 91.6%, 91.8%, and 97.3%, respectively (Table I).

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