and II (16, 18). The binding constant for I reported from this laboratory was $2 \times 10^{-3} M^{-1}$ and that for II was $3 \times 10^{4} M^{-1}$. The number of binding sites was ~2.6 for I and 0.25 for II (20). Thus, increased lipid solubility of II results in its increased strength of interaction with albumin molecules, but the steric hindrance due to the additional *tert*-butyl group makes the extent of binding only about 10% of that observed for I. Thus, the total exchangeable albumin present in the extravascular compartments provides a large pool for possible storage of I but not for II, which probably is taken up by the body fatty tissues due to its higher lipid solubility (5).

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Human Whole Blood and Parotid Saliva Concentrations of Oral and Intramuscular Promethazine

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Received February 13, 1980, from the *Department of Pharmacology, Hahnemann Medical College, Philadelphia, PA 19102.* Accepted for publication June 20, 1980.

Abstract □ Ten healthy male subjects received 25 mg of promethazine intramuscularly, followed within 3 weeks by oral administration of 25 mg. Whole blood and parotid saliva were collected over a 12-hr period after drug administration. Promethazine was quantitated by high-performance liquid chromatography. After intramuscular administration, the promethazine concentrations were 3.0–22.4 ng/ml in blood and 0.9–2.8 ng/ml in parotid saliva. After oral administration, the promethazine concentrations were 1.4–5.5 ng/ml in blood and 0.2–0.8 ng/ml in parotid saliva. The peak blood promethazine concentration after the intramuscular dose was four times higher than that following the oral dose, which indicates that promethazine possesses an extensive first-pass effect. The mean parotid saliva to whole blood ratio (S/B) was calculated to be 0.24 after the intramuscular route and 0.20 after the oral route over the 12-hr period. The calculated percentage of free drug in the blood was 20–24% of the whole blood concentration determined from the S/B ratio.

Keyphrases □ Promethazine—whole blood and parotid saliva concentrations following oral and intramuscular administration □ High-performance liquid chromatography—analysis, promethazine, whole blood and parotid saliva concentrations following oral and intramuscular administration □ Phenothiazines—promethazine, high-performance liquid chromatographic analysis in whole blood and parotid saliva following oral and intramuscular administration

Several investigators have reported analytical methods for the determination of phenothiazines, including colorimetric (1), spectrofluorometric (2), spectrophotometric (3), GLC (4, 5), and liquid chromatographic (6) procedures. However, little or no information is available on the quantitation of promethazine in therapeutic doses in human whole blood or parotid saliva.

This report presents a sensitive method for the detection and quantitation of promethazine in whole blood and parotid saliva after the administration of 25 mg of promethazine orally and intramuscularly in human volunteers. Parotid saliva to blood promethazine concentration ratios were calculated, and the bioavailability of promethazine was compared for the two routes of administration.

EXPERIMENTAL

Materials—Powdered promethazine[1] and promazine[1], the internal standard, were used without further purification as the analytical standards. Commercially available promethazine tablets[1] and injectable liquid[1] were obtained from a local pharmacy. All solvents and reagents were analytical reagent grade, except for methanol[2], which was high-performance liquid chromatographic (HPLC) grade. All glassware was coated with silicone[3] by the method described on the package insert.

Apparatus—The liquid chromatograph[4] was fitted with a loop injector and a fixed-wavelength UV detector (254 nm). A 1-mv recorder[5] was attached to the chromatograph. The detector was attenuated at 0.005 aufs.

Column—A prepacked, 300 × 3.9-mm i.d., μBondapak CN column[6] was run at ambient temperature.

[1] Wyeth Laboratories, Philadelphia, Pa.
[2] Fischer Chemical Co., King of Prussia, Pa.
[3] Siliclad, Clay Adams, New York, N.Y.
[4] Model 200, Waters Associates, Milford, Mass.
[5] Perkin-Elmer Corp., Norwalk, Conn.
[6] Waters Associates, Milford, Mass.

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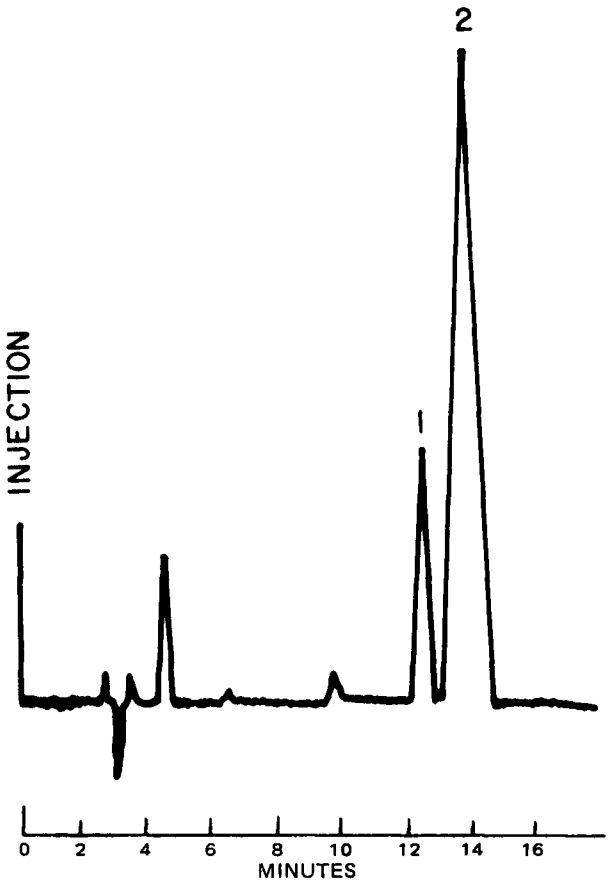

Figure 1—*Typical high-performance liquid chromatogram of (1) promethazine and the internal standard (2), promazine.*

Mobile Phase—A solution of 1% acetic acid in 0.005 *M* heptanesulfonic acid and methanol (50:50) was used. The mixture was pumped isocratically through the column at a flow rate of 2 ml/min.

Method—Ten healthy male volunteers, 25–57 years, participated in the crossover study. Complete histories and physical examinations, including blood and urine chemistries, were performed before and after the experiments. Each subject initially was given 25 mg of promethazine intramuscularly, followed in 3 weeks by 25 mg orally.

The subjects fasted for a minimum of 12 hr prior to each experiment. Baseline parotid saliva and blood samples were taken before each experiment. Parotid saliva samples were collected by means of a modified double-lumen parotid cup (7). Orange-flavored lozenges[7] served as a reflex stimulus to induce salivation, and the resultant parotid secretions were collected in silicone-coated graduated test tubes. At least 5 ml of parotid saliva was collected over a period not exceeding 10 min. Blood samples (10 ml) were collected in silicone-coated vacuum tubes[8] containing 143 IU of heparin sodium.

After intramuscular administration of 25 mg of promethazine, venous blood and parotid saliva samples were taken at 0.5, 1, 2, 4, 6, 8, and 12 hr. To make certain that the subjects returned to baseline levels, 3 weeks was allowed between experiments. At that time, the same 10 subjects were given 25 mg of promethazine orally; blood and parotid saliva samples were collected at the same time intervals.

Whole blood (5 ml) or parotid saliva (5 ml) was added to 1 ml of 1 *N* NaOH. Promazine, which was chosen as the internal standard because of the similarity of its structure and chemical properties to promethazine, was dissolved in the mobile phase, and 25 ng was added to the whole blood or parotid saliva sample before extraction. The sample then was shaken with 25 ml of heptane for 10 min and centrifuged for 5 min.

The heptane layer was removed by aspiration with a siliconized Pasteur pipet and allowed to evaporate under a steady stream of air at 55°. The residue was redissolved in 50 μl of the mobile phase, and 10 μl was injected into the liquid chromatograph. Quantitation was performed using the method of internal standards by measuring the peak heights and comparing the peak height ratios to a calibration curve. The sensitivity of the method was tested by the extraction of various concentrations of promethazine in blood and saliva and determination of the smallest detectable peak on the liquid chromatograph.

[7] Regal Crown Sour Orange, Trebor Sharps Ltd., London, England.
[8] Venojet, Kimble-Terumo, Elkton, Md.

Table I—Whole Blood and Parotid Saliva Concentrations of Promethazine Administered Intramuscularly

Hours	Saliva[a], ng/ml	Blood[a], ng/ml
0.5	1.9 ± 0.36	6.4 ± 0.86
1	2.5 ± 0.33	8.3 ± 1.96
2	2.8 ± 0.52	14.2 ± 3.38
4	2.2 ± 0.25	22.4 ± 5.90
6	2.4 ± 0.36	8.3 ± 0.91
8	1.5 ± 0.31	8.5 ± 2.50
12	0.9 ± 0.40	3.0 ± 0.84

[a] Mean concentration (±*SE*) of promethazine in 10 human volunteers after the intramuscular administration of 25 mg.

RESULTS AND DISCUSSION

The separation and identification of promethazine by HPLC are illustrated in Fig. 1. The retention time for promethazine was 12.6 min; the internal standard, promazine, had a retention time of 13.8 min. The separation was reproducible and sensitive in both extracted whole blood and parotid saliva. The sensitivity of promethazine was 0.2 ng on-column by this procedure. Standard concentration curves for spiked whole blood and parotid saliva were linear between 2.5 and 50 ng/ml (blood) and between 0.5 and 10 ng/ml (parotid saliva).

The mean (±*SE*) whole blood and parotid saliva promethazine concentrations in the 10 subjects receiving 25 mg intramuscularly are presented in Table I. The range of promethazine concentrations determined in whole blood and parotid saliva was 0.9–22.4 ng/ml over the 12-hr period. The blood promethazine concentration peaked within 4 hr (22.4 ± 5.90 ng/ml) but fell rapidly over the next 2 hr to 8.3 ± 0.91 ng/ml and then declined slowly over the next 6 hr to 3.0 ± 0.84 ng/ml. The parotid saliva concentrations peaked within 2 hr (2.8 ± 0.52 ng/ml) and declined more slowly than the plasma to 0.9 ± 0.40 ng/ml over the next 10 hr. The calculated saliva to blood (*S/B*) ratio ranged between 0.10 and 0.30, with a mean of 0.24, over the 12-hr period.

The mean (±*SE*) whole blood and parotid saliva promethazine concentrations in the 10 subjects receiving 25 mg orally are listed in Table II. The range of promethazine concentrations determined in whole blood and parotid saliva was 0.2–5.5 ng/ml over the 12 hr. The blood promethazine concentrations peaked within 2 hr (5.5 ± 1.4 ng/ml) and declined to 1.4 ± 0.2 ng/ml within the next 6 hr. The parotid saliva concentration was elevated consistently between 1 and 6 hr after drug administration. Only at the 8-hr period did it fall from 0.8 to 0.2 ng/ml. The calculated saliva to blood ratio ranged between 0.13 and 0.29, with a mean of 0.20, over the 8-hr period.

The results indicate that the bioavailability of orally or intramuscularly administered promethazine in therapeutic doses can be determined by HPLC. This analytical method was sensitive enough to detect and quantitate parotid saliva concentrations of promethazine during the corresponding times in which whole blood concentrations were determined. The sensitivity of the assay was suitable for detection of pro-

Table II—Whole Blood and Parotid Saliva Concentrations of Promethazine Administered Orally

Hours	Saliva[a], ng/ml	Blood[a], ng/ml
0.5	0.4 ± 0.2	2.5 ± 0.5
1	0.8 ± 0.2	2.9 ± 0.5
2	0.7 ± 0.1	5.5 ± 1.4
4	0.8 ± 0.2	3.8 ± 0.7
6	0.8 ± 0.2	2.8 ± 0.8
8	0.2 ± 0.1[b]	1.4 ± 0.2[b]
12	0	0

[a] Mean concentration (±*SE*) of promethazine in 10 human volunteers after the oral administration of 25 mg. [b] Estimated values from standard concentration curves.

methazine concentrations as low as 0.2 ng/ml of saliva or blood, a 50-fold increase in sensitivity over that reported previously for a GLC procedure (5).

After the administration of equal doses of promethazine (25 mg), the peak blood concentrations were significantly different after oral and intramuscular administration. The peak whole blood concentration (22.4 ng/ml) after intramuscular administration was approximately four times higher than that (5.5 ng/ml) after oral administration. The results indicate that promethazine possesses a significant first-pass effect through the liver. Other investigators have demonstrated that promethazine has an extensive first-pass effect, accounting for lower blood levels after oral administration compared to parenteral administration (5). After oral administration of 25 mg, the elimination half-life of promethazine was 3.3 hr as determined from plasma levels measured over the 8-hr period.

The present results indicate that the mean parotid saliva to whole blood promethazine ratios after oral (0.20) and intramuscular (0.24) administration did not significantly differ from each other. Previous studies involving saliva to blood ratios of other drugs consistently showed that saliva drug concentrations correlated with the blood free drug concentrations and that percentages of free drug in blood could be determined from the calculated saliva to blood ratios (7–9). In this study, the ratios of 0.20 and 0.24 indicate that the free form of promethazine in blood ranged between 20 and 24%, meaning that it was bound 80 and 76% to whole blood.

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High-Performance Liquid Chromatographic Determination of Metaraminol Bitartrate in the Presence of Parabens

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Abstract □ A high-performance liquid chromatographic (HPLC) procedure was developed for the determination of metaraminol bitartrate in the presence of methylparaben and propylparaben. Reversed-phase ion-pair chromatography was employed using dioctyl sodium sulfosuccinate as the counterion. The current official USP procedure for the determination of metaraminol requires dilution, extraction, and measurement of UV absorption. The HPLC procedure is rapid and precise and correlates well with the USP XX procedure. It can be used for metaraminol injectables as well as the raw material.

Keyphrases □ Metaraminol bitartrate—high-performance liquid chromatographic analysis in the presence of methylparaben and propylparaben □ High-performance liquid chromatography—analysis, metaraminol bitartrate in the presence of methylparaben and propylparaben □ Methylparaben—high-performance liquid chromatographic analysis with metaraminol bitartrate and propylparaben □ Propylparaben—high-performance liquid chromatographic analysis with metaraminol bitartrate and propylparaben

Metaraminol bitartrate, an adrenergic vasopressor drug, may be administered intravenously or intramuscularly to raise the blood pressure in acute hypotensive states and shock. Some commercially available injectables contain both methylparaben and propylparaben as preservatives. Spectrophotometric (1) and spectrophotofluorometric (2) analyses are currently used for the analysis of metaraminol bitartrate injectables. The USP XX spectrophotometric procedure requires extraction before analysis to remove parabens. While the spectrophotofluorometric procedure needs no extraction, it requires reaction with *O*-phthalaldehyde for 1 hr (60 ± 1 min) before analysis. For both procedures, a separate GLC (3) procedure must be used for the determination of parabens. This GLC procedure for parabens involves extraction and derivatization before analysis.

This paper describes the parameters for the simultaneous qualitative and quantitative determination of metaraminol, methylparaben, and propylparaben by high-performance liquid chromatography (HPLC). The HPLC procedure involves the formation of an ion-pair with dioctyl sodium sulfosuccinate on an octadecylsilane column. The use of this counterion previously was reported for the HPLC determination of some other basic organic drug molecules (4, 5) and was successful for the separation of metaraminol, methylparaben, and propylparaben. The total analysis time to run standards and a sample is ~1 hr. This method is simple, accurate, and precise and compares favorably with the USP XX procedure.

EXPERIMENTAL

Reagents and Chemicals—USP reference standards were used for standard solutions of metaraminol, methylparaben, and propylparaben. Powdered samples of metaraminol bitartrate[1], methylparaben[2], propylparaben[2], and butylparaben[3] were used in the analytical procedure. HPLC grade methanol and reagent grade acetic acid and dioctyl sodium sulfosuccinate[4] were used in the mobile phase.

Mobile Phase—The mobile phase was prepared by mixing 600 ml of methanol with 2.2 g of dioctyl sodium sulfosuccinate. After dissolution, 400 ml of deionized, distilled water and 10 ml of acetic acid were added (pH 4.0). The mobile phase was degassed by vacuum filtration through

[1] Winthrop Laboratories, Rensselaer, N.Y.
[2] Aceto Chemical Co., Flushing, N.Y.
[3] Eastman Kodak Co., Rochester, N.Y.
[4] Aldrich Chemical Co., Milwaukee, Wis.

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