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.

REFERENCES

(1) M. Tarasiewicz, *Acta Pol. Pharm.*, **29**, 578 (1972).

(2) S. L. Tompsett, *Acta Pharmacol. Toxicol.*, **26**, 298 (1968).

(3) J. E. Wallace and J. D. Biggs, *J. Pharm. Sci.*, **60**, 1346 (1971).

(4) A. DeLeenheer, *J. Chromatogr.*, **77**, 339 (1973).

(5) J. Quinn and R. Calvert, *J. Pharm. Pharmacol.*, **28**, 59P (1976).

(6) W. Lindner, R. W. Frei, and W. Santi, *J. Chromatogr.*, **108**, 299 (1975).

(7) G. J. DiGregorio, A. J. Piraino, and E. Ruch, *Drug Alcohol Depend.*, **3**, 43 (1978).

(8) G. J. DiGregorio, A. J. Piraino, and E. Ruch, *Clin. Pharmacol. Ther.*, **6**, 720 (1978).

(9) G. J. DiGregorio, A. J. Piraino, and E. Ruch, *J. Pharm. Sci.*, **68**, 1192 (1979).

ACKNOWLEDGMENTS

Supported by National Aeronautics and Space Administration Contract NAS9-15620.

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

C. J. MARTIN ˣ and S. J. SAXENA

Received May 15, 1980, from *McGaw Laboratories, Irvine, CA 92714.* Accepted for publication June 20, 1980.

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.

0022-3549/80/1200-1459$01.00/0

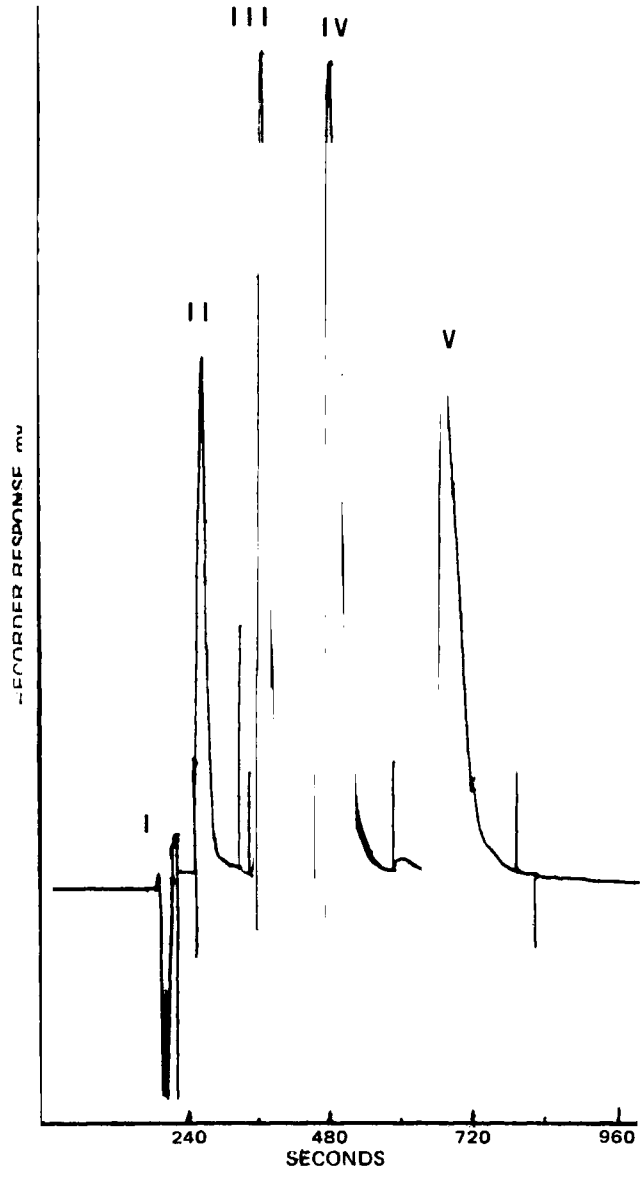

Figure 1—*Typical chromatogram of metaraminol bitartrate using a reversed-phase C_{18} column (room temperature) at a flow rate of 1.0 ml/min. The mobile phase was methanol–distilled water (60:40) with 0.005 M dioctyl sodium sulfosuccinate and 10 ml of acetic acid/liter. Key: I, solvent front; II, methylparaben (attenuation 256); III, propylparaben (attenuation 16); IV, butylparaben (attenuation 16); and V, metaraminol (attenuation 16).*

a polycarbonate membrane filter[5] (0.4-μm pore size, 47-mm diameter).

Internal Standard Solution—A 0.1-mg/ml solution of butylparaben was prepared by dissolving 50 mg in methanol and diluting the solution with water to 500 ml.

Working Standard Solution—A methylparaben stock solution was prepared by dissolving 15 mg of methylparaben USP reference standard in 5 ml of methanol in a 10-ml volumetric flask and diluting to volume with distilled water. A propylparaben stock solution was prepared by dissolving 10 mg of propylparaben USP reference standard in 5 ml of methanol in a 50-ml volumetric flask and diluting to volume with distilled water.

The working standard solution was prepared by dissolving 40 mg of metaraminol bitartrate USP reference standard in 50 ml of distilled water in a 100-ml volumetric flask. Then 2 ml of the methylparaben stock solution, 2 ml of the propylparaben stock solution, and 5 ml of the internal standard solution were added to the 100-ml volumetric flask, and the solution was diluted to volume with distilled water.

Sample Stock Solution—A sample stock solution was prepared by dissolving 75 mg of methylparaben and 10 mg of propylparaben in 10 ml of methanol in a 50-ml volumetric flask. Then 950 mg of metaraminol bitartrate was added to the 50-ml volumetric flask and dissolved in 30 ml of distilled water. The solution then was diluted to 50 ml with distilled water.

Sample Solutions for Linearity, Recovery, and Precision—Seven solutions were prepared from the sample stock solution. Portions of 0.5, 1, 2, 3, 4, and 5 ml of stock solution were added to separate 100-ml volumetric flasks, in which 5 ml of the internal standard solution had been placed. Each solution was diluted to 100 ml with distilled water. These six solutions were used for testing the linearity of the HPLC method. A solution of known concentration was used for determination of recovery and precision.

Determination of Purity of Metaraminol Bitartrate Raw Materials—Three stock solutions of different metaraminol bitartrate raw materials were prepared similar to the sample stock solution used for the linearity study. Then 2 ml of each stock solution was added to separate 100-ml volumetric flasks in which 5 ml of the internal standard solution had been placed. The solutions were diluted to 100 ml with distilled water. These three solutions were run against a USP working standard on the chromatograph to determine the assay values.

The USP XX procedure for metaraminol bitartrate injectables also was performed on these three solutions to show correlation between the USP XX method and the HPLC procedure. The three metaraminol bitartrate raw materials also were assayed by the USP XX procedure for the purity of metaraminol bitartrate raw material.

Conditions for Chromatographic Quantification—The liquid chromatograph[6] was equipped with a variable-wavelength detector[7] and

Table I—Linear Regression Data for Metaraminol Bitartrate, Methylparaben, and Propylparaben

Drug	Theoretical Concentration, % w/v	Observed HPLC Results[a]	Slope	Intercept	r^b
Metaraminol	0.250	0.254 ± 0.004	0.9995	0.0035	1.0000
	0.501	0.502 ± 0.001			
	1.002	1.010 ± 0.006			
	1.503	1.501 ± 0.007			
	2.004	2.007 ± 0.021			
	2.505	2.508 ± 0.008			
Methylparaben	0.0376	0.0379 ± 0.0004	0.9728	0.0033	0.9999
	0.0752	0.0764 ± 0.0004			
	0.1503	0.1507 ± 0.0016			
	0.2254	0.2250 ± 0.0004			
	0.3006	0.2964 ± 0.0010			
	0.3758	0.3666 ± 0.0004			
Propylparaben	0.0053	0.0058 ± 0.0001	0.9583	0.0008	1.0000
	0.0105	0.0108 ± 0.0001			
	0.0211	0.0212 ± 0.0001			
	0.0316	0.0313 ± 0.0002			
	0.0422	0.0422 ± 0.0002			
	0.0528	0.0528 ± 0.0005			

[a] Result is based on three replicate injections. The table value is the mean ± *SD*. [b] Correlation coefficient between the theoretical and observed concentrations as determined by linear regression analysis.

Table II—Recovery and Precision of the HPLC Method

Drug	Theoretical Concentration, % w/v	Observed HPLC Results[a], % w/v	Recovery, %
Metaraminol	0.752	0.747 ± 0.003	99.3
Methylparaben	0.1127	0.1133 ± 0.0009	100.5
Propylparaben	0.0158	0.0158 ± 0.0001	100.0

[a] Result is based on six replicate injections. Confidence limits at $p = 0.05$

[5] Catalog No. N040, Nucleopore Corp., Pleasanton, Calif.

[6] Spectra-Physics model 3500B.

[7] Model SF770 UV-visible detector, Schoeffel Instrument Co.

Table III—Purity of Metaraminol Bitartrate Raw Materials by HPLC and USP Methods

Sample[a]	Initial Concentration of Metaraminol, % w/v	Observed Results, % w/v		Purity of Metaraminol Bitartrate Raw Material, %	
		HPLC[b]	USP	HPLC	USP[c]
Raw material 1	1.065	1.037 ± 0.010	1.038	97.4	97.5
Raw material 2	1.019	0.994 ± 0.010	1.014	97.5	99.5
Raw material 3	1.001	0.980 ± 0.009	0.980	98.0	98.0

[a] The same solution of each raw material was used for both methods. [b] Result is based on two replicate injections. The value given is the mean ± *SD*. [c] The purity of each raw material also was determined by the USP XX procedure for metaraminol bitartrate raw material. The results for the three raw materials were 100.2, 100.5, and 99.6%, respectively.

an octadecylsilane column[8]. The degassed mobile phase [methanol–water (60:40) with 0.005 *M* dioctyl sodium sulfosuccinate and 10 ml of acetic acid/liter] was pumped through the column at a flow rate of 1.0 ml/min at ambient temperature until a stable baseline was obtained. Replicate 20-μl injections of the sample and standard solutions were made. The peaks were detected at 254 nm.

RESULTS AND DISCUSSION

Studies on metaraminol bitartrate preserved with methylparaben and propylparaben showed that these three drug components can be separated by HPLC and determined by UV absorption at 254 nm within 15 min. When injected into the chromatograph, methylparaben was eluted first, followed by propylparaben, butylparaben (the internal standard), and metaraminol at retention times of ~4.5, 6.3, 8.2, and 11.4 min, respectively (Fig. 1).

Standard solutions of metaraminol bitartrate, methylparaben, and propylparaben were chromatographed using the reversed-phase C_{18} column. The concentration of each component was determined by a programmable integrator[9]. A linear regression analysis of the data for six concentrations of metaraminol bitartrate, methylparaben, and propylparaben is shown in Table I. The data obtained show that metaraminol bitartrate, methylparaben, and propylparaben are linear to at least 25, 3.8, and 0.53 μg, respectively. A separate solution was run to determine the recovery and precision of the HPLC method. These data are shown in Table II.

The purity of three metaraminol bitartrate raw materials was determined by the HPLC method and the USP XX procedure as shown in Table III. The utility of HPLC in the analysis of metaraminol bitartrate is clearly demonstrated, and the results agree favorably with those obtained by the USP method.

[8] μBondapak C_{18} (<10 μm), 30-cm × 4-mm i.d. column, Waters Associates, Milford, Mass.

[9] Spectra-Physics System I.

REFERENCES

(1) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, p. 309.

(2) J. D. Weber, *J. Assoc. Off. Anal. Chem.*, **61**, 949 (1978).

(3) "The United States Pharmacopeia," 20th rev., United States Pharmacopeial Convention, Rockville, Md., 1980, p. 490.

(4) J. T. Stewart, I. L. Honigberg, J. P. Brant, W. A. Murray, J. L. Webb, and J. B. Smith, *J. Pharm. Sci.*, **65**, 1536 (1976).

(5) S. J. Saxena, J. T. Stewart, I. L. Honigberg, J. G. Washington, and G. R. Keene, *ibid.*, **66**, 813 (1977).

High-Pressure Liquid Chromatographic Assay of Quinestrol Tablets

DIMITRI C. TSILIFONIS and LESTER CHAFETZ ˣ

Received April 30, 1980, from the *Analytical Research Laboratories, Pharmaceutical Research Division, Warner-Lambert Company, Morris Plains, NJ 07950.* Accepted for publication June 19, 1980.

Abstract □ A specific assay for quinestrol was developed using high-pressure liquid chromatography. The estrogen was separated from tablet excipients on a chemically bonded hydrocarbon column utilizing acetonitrile–water as the mobile phase. Linearity studies were carried out using peak height measurements, and the detector response to the concentration of the steroid was confirmed. This procedure was rapid, accurate, precise, and specific for the assay of the synthetic estrogen in the presence of formulation excipients and structurally similar estrogens.

Keyphrases □ Quinestrol—high-pressure liquid chromatographic analysis, tablets □ High-pressure liquid chromatography—analysis, quinestrol tablets

The pink chromophore resulting from the reaction of quinestrol with methanolic sulfuric acid was shown to afford an accurate, precise, and selective colorimetric assay for quinestrol in pharmaceutical dosage forms (1). This method correlated with the GLC assay (2), which proved useful as a reference method but had disadvantages in speed and convenience. High-pressure liquid chromatography (HPLC) affords a simple alternative with advantages in speed, interlaboratory precision, and productivity.

Roos (3) described an HPLC method for ethinyl estradiol, estrone, estradiol, and estradiol esters as their dansyl

Table I—Peak Height *versus* Concentration of Quinestrol

Concentration, μg/ml	Peak Height, mm
6.8	26
13.6	52
20.4	80
34.0	130

0022-3549/80/1200-1461$01.00/0