

# Thermal and Photolytic Degradation Studies of Promethazine Hydrochloride: A Stability-Indicating Assay

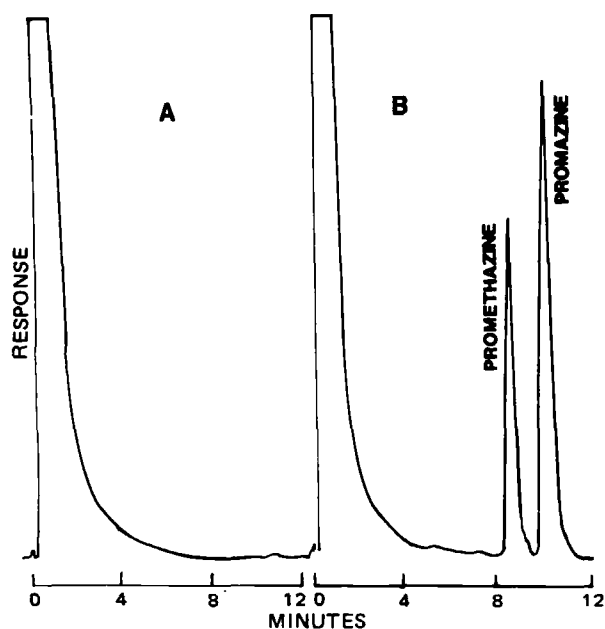
S. STAVCHANSKY \*\*, J. E. WALLACE †, and P. WU \*

Received February 11, 1982, from the \*University of Texas at Austin, College of Pharmacy, Division of Pharmaceutics, Drug Dynamics Institute, Austin, TX 78712, and the †University of Texas Health Science Center, Department of Pathology, San Antonio, TX 78284. Accepted for publication May 25, 1982.

**Abstract** □ A stability-indicating GLC method for the analysis of promethazine hydrochloride in polyethylene glycol delivery systems is reported. This method is capable of distinguishing the intact drug from its thermal and photodegradation products. A linear relationship between peak height ratio (promethazine-promazine) and promethazine concentration is found up to a concentration of 600 µg/ml. Kinetic studies were performed to determine the photolytic and thermal degradation rates of promethazine hydrochloride as a function of pH. The activation energies at pH 2.98, 3.94, and 5.12 were obtained from linear Arrhenius plots and were found to be 6601, 5888, 5570 cal/mole, respectively. The first-order rate constant increased with increasing pH. The photolytic degradation of promethazine hydrochloride does not follow simple first-order kinetics.

**Keyphrases** □ Promethazine hydrochloride—thermal and photolytic degradation studies, stability-indicating GLC assay □ Thermal degradation—promethazine hydrochloride, photolytic degradation, stability-indicating GLC assay □ Photolytic degradation—promethazine hydrochloride, thermal degradation, stability-indicating GLC assay

Evaluation of the stability of promethazine hydrochloride in rectal delivery systems containing polyethylene glycols requires an assay method that is specific for the drug in the presence of degraded products and vehicles. There have been several spectrophotometric methods developed (1-3), but none of them has adequate specificity for stability testing. A direct GLC method without prior extraction has been used (4), but in the presence of poly-



**Figure 1**—Chromatogram of promethazine hydrochloride blank vehicle extract (A), and 300 µg/ml of promethazine hydrochloride in polyethylene glycol aqueous solution in the presence of promazine as internal standard, (B).

ethylene glycol, the flame ionization detectors are adversely affected. In addition, the injection of water and ethanol into the gas chromatographic system causes a rapid column deterioration. In the present investigation, a GLC method with a simple organic phase extraction was developed to specifically determine promethazine hydrochloride in polyethylene glycol suppositories undergoing stability testing.

## EXPERIMENTAL

**Materials**—Promethazine hydrochloride<sup>1</sup> and promazine hydrochloride<sup>2</sup> were used as received. All solvents and chemicals were commercial analytical grade<sup>3</sup>. Precoated silica gel plates<sup>4</sup> were used.

**Chromatographic Conditions**—A dual-column GC<sup>5</sup> with a flame ionization detector was used. A silylated, coiled glass column, 183 cm in length, 0.6-cm o.d., 0.4-cm i.d., was packed with 3% OV-17 on 100-120 mesh Gas Chrom Q<sup>6</sup>. The column was conditioned for 36 hr at 280° and was treated with a silylating agent, *N,O*-bis(trimethylsilyl)acetamide<sup>7</sup>. Nitrogen carrier gas flow, hydrogen flow, and air flow were 37, 37, and 350 ml/min, respectively. The column, detector, and sample injection port temperatures were 250, 300, and 260°, respectively.

**Polyethylene Glycol Vehicle Solution**—A stock solution was prepared using the excipients and preservatives employed in the manufacture of the suppositories. Appropriate dilutions were made with deionized water. The resulting solutions reflect the actual amount of each of the ingredients in the formulation.

**Internal Standard Solution**—Fifty milligrams of promazine hydrochloride<sup>2</sup> was placed in a 100-ml volumetric flask and appropriately diluted with deionized water.

**Standard Solutions**—Sixty milligrams of promethazine hydrochloride was placed in a 100-ml volumetric flask and diluted with deionized water. Two, 4, 6, 8, and 10 ml of the above solution were placed in five 10-ml volumetric flasks and diluted with deionized water. The concentration of promethazine hydrochloride was 120, 240, 360, 480, and 600 µg/ml, respectively.

**Standard Curve of Promethazine Hydrochloride in Water**—One milliliter of each standard solution was placed in a 12-ml centrifuge tube and to each tube was added 1.0 ml of the internal standard and 0.3 ml of 5 N NaOH. The centrifuge tube was mixed thoroughly with a vortex mixer for 30 sec. Then 4.0 ml of *n*-hexane was added to the tube and the tube placed on the vortex mixer for 1 min. The mixture was centrifuged at 2000 rpm for 2 min. Three microliters of the hexane layer was injected directly into the GC.

**Standard Curve of Promethazine Hydrochloride in Polyethylene Glycol Vehicle**—The procedures were the same as for the determination of promethazine hydrochloride in water, except for the addition of 0.1 ml of the polyethylene glycol vehicle solution to each centrifuge tube prior to extraction. This volume of solution represents the amount of each of the excipients in each polyethylene glycol suppository after the suppository has been dissolved for analytical purposes. This procedure compares with the extraction of promethazine hydrochloride from a suppository.

<sup>1</sup> Napp Chemicals, Inc., Ladi, N.J.

<sup>2</sup> Wyeth Labs, Inc., Philadelphia, Pa.

<sup>3</sup> Fisher Scientific Co., Fair Lawn, N.J.

<sup>4</sup> Analabs, Inc., North Haven, Conn.

<sup>5</sup> Tracor Instruments Inc., Austin, Tex.

<sup>6</sup> Supelco, Inc., Bellefonte, Pa.

<sup>7</sup> Pierce Chemical Co., Rockford, Ill.

**Table I—Standard Curve of Promethazine Hydrochloride in Water and in Polyethylene Glycol Vehicle**

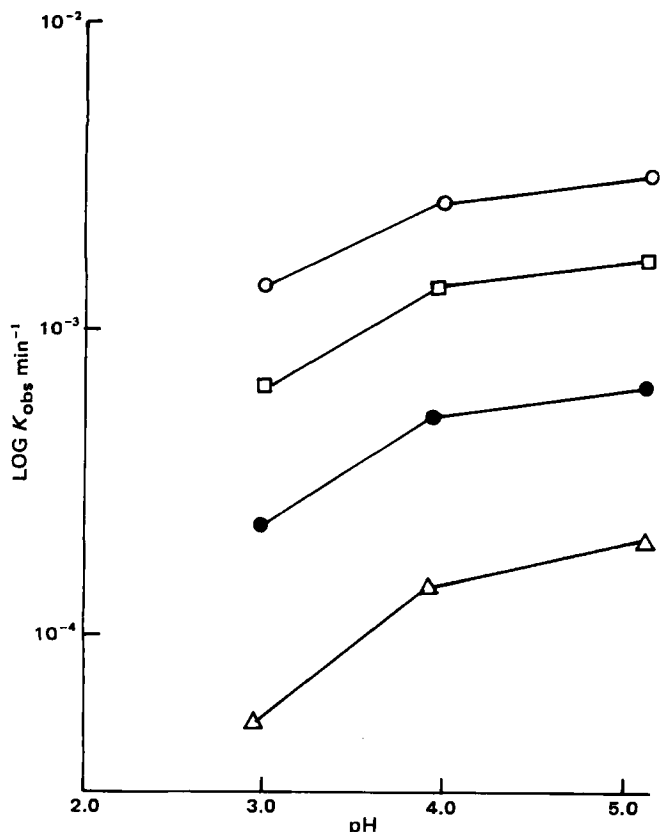
Concentration, $\mu\text{g/ml}$	<i>n</i>	Water		Polyethylene Glycol Vehicle	
		Mean Peak Height Ratio	CV, %	Mean Peak Height Ratio	CV, %
120	3	0.290	4.30	0.280	3.37
240	3	0.580	1.40	0.580	0.81
360	3	0.860	0.97	0.850	1.11
480	3	1.13	0.42	1.17	3.04
600	2	1.38	3.62	—	—

**Thermal Degradation of Promethazine Hydrochloride**—To test the validity of the assay for promethazine in the presence of its degradation products, the thermal degradation of promethazine hydrochloride in aqueous solution was performed. Promethazine hydrochloride, 5 g, was dissolved in 100 ml of water; oxygen was then bubbled through the solution for 30 min. The solution was placed in a screw-capped light-proof amber bottle saturated with oxygen and kept at 65° for 5 days. The mixture was cooled, made alkaline with 1 N NaOH, and extracted with methylene chloride. The aqueous layer was acidified with 1 N H<sub>2</sub>SO<sub>4</sub> and extracted with methylene chloride (5).

Both organic phases were evaporated to dryness in the dark under a nitrogen stream to afford a solid residue. Approximately 100 mg of the residue was dissolved in 2 ml of dichloromethane. A total of 9  $\mu\text{l}$  was applied to a silica gel G.F. TLC plate<sup>4</sup>, (0.25 mm, 5 × 20 cm) and chromatographed in the dark (acetone–6N ammonia; 100:2).

The solvent was evaporated and the plate was placed under a short-wavelength UV light for visualization. The spots were scraped individually and placed in a conical centrifuge tube with a glass stopper. One milliliter of dichloromethane was added to each tube and the mixture was agitated with a vortex mixer for ~1 min. The tube was centrifuged and the supernatant was transferred to a small test tube and evaporated to dryness under a nitrogen stream. The residue was dissolved with 1 ml of methanol. One microliter of this solution was injected into the GC.

To investigate the influence of pH, 40 ml of 0.05% promethazine hydrochloride in Sørensen citrate buffer solutions (pH 2.98, 3.94, and 5.12)



**Figure 2—Influence of pH on the thermal degradation rates of promethazine hydrochloride.** Key: ( $\Delta$ ) 60°, ( $\bullet$ ) 70°, ( $\square$ ) 80°, and ( $\circ$ ) 90°.

**Table II—Regression Coefficients for Promethazine Hydrochloride Standard in Water**

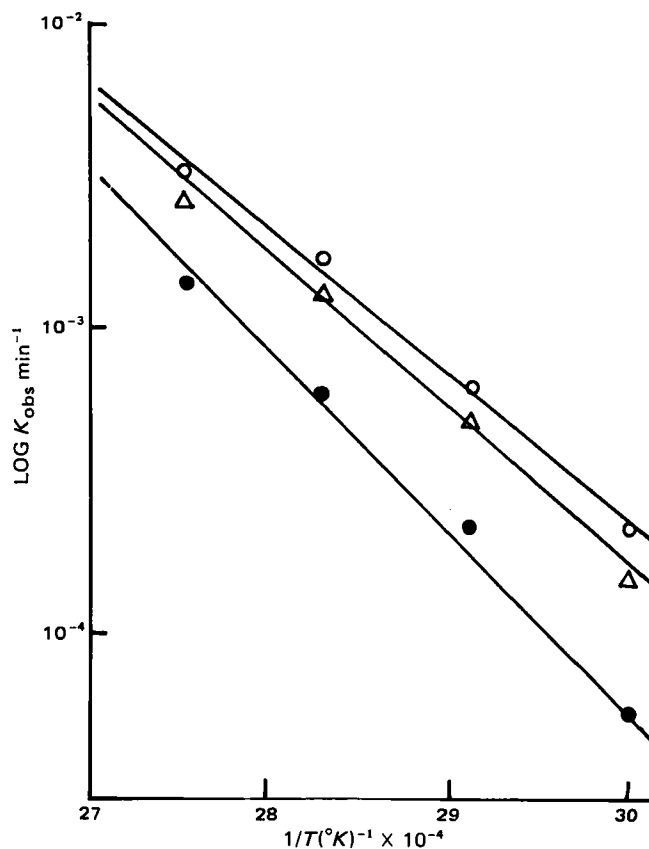
Regression Coefficients	With Polyethylene Glycol	Without Polyethylene Glycol
<i>n</i>	14	12
<i>a</i> (y-intercept)	0.0256	0.0133
<i>b</i> (slope)	0.0022	0.0025
<i>R</i>	0.998	0.998

was placed in 100-ml light-proof amber volumetric flasks and kept at temperatures of 60, 70, 80, and 90°, accurate to within  $\pm 0.2^\circ$ , in a thermostatically controlled oil bath. A solution containing 0.1% of EDTA [tetrasodium(ethylenedinitrilo)tetraacetate] was added to the promethazine buffer solutions before heating. The solutions were adjusted to the same ionic strength ( $\mu = 0.2$ ) with potassium chloride. The pH of the solutions was measured at the beginning and at the end of the heating periods. Then 1 ml of the test solution was withdrawn from the flasks, immediately cooled, and analyzed as previously described. These samples were not chromatographed by TLC. Immediately after the sample was withdrawn from the volumetric flask, the flask was recharged with a stream of oxygen in order to maintain excess oxygen tension.

**Photolytic Degradation of Promethazine Hydrochloride**—Promethazine hydrochloride (0.3% w/v) was dissolved in water. The solution was placed in a quartz reactor tube<sup>9</sup>, length 43 cm, 2.6-cm o.d. and 2.4-cm i.d., which was equipped with a water cooling tube, 1.2-cm o.d. and exposed to UV light (300 nm) by means of a photolytic reactor<sup>8</sup> at 10° for 74 hr.

Following the exposure time, 70 ml of the solution was freeze-dried. The residue was dissolved in 7 ml of methanol. Fifty microliters of the solution was applied to a silica gel G.F. TLC plate (0.25 mm, 10 × 20 cm<sup>2</sup>) and chromatographed using acetone–methanol–6 N NH<sub>3</sub> (140:60:1) as the mobile phase.

The solvent was evaporated and the plate was placed under a short wavelength UV light for visualization. The GC specimen was prepared



**Figure 3—Arrhenius plots of  $\log k_{\text{obs}}$  versus  $1/T$  at different pH values.** Key: ( $\bullet$ ) pH 2.98, ( $\Delta$ ) pH 3.94, ( $\circ$ ) pH 5.12.

<sup>8</sup> The South New England Ultraviolet Co.

**Table III—The Apparent Pseudo First-Order Rate Constants of Thermal Degradation of Promethazine Hydrochloride at Different pH and Temperatures**

Temperature	pH	Rate Constants <sup>a</sup> , min <sup>-1</sup> × 10 <sup>5</sup>	Half-Life, hr
60°	2.98	5.38 (0.41) <sup>b</sup>	214.7
	3.94	14.8 (0.66)	78.3
	5.12	21.1 (0.49)	54.7
70°	2.98	22.6 (0.67)	51.1
	3.94	52.9 (2.4)	21.8
	5.12	65.4 (4.1)	17.7
80°	2.98	63.1 (1.7)	18.3
	3.94	142.9 (3.8)	8.1
	5.12	172.7 (3.5)	6.7
90°	2.98	140.6 (5.1)	8.2
	3.94	263.8 (4.9)	4.4
	5.12	320.9 (23.7)	3.6

<sup>a</sup>  $k_{obs}$ . <sup>b</sup> Data in the parentheses represent the standard deviations.

as described under *Thermal Degradation of Promethazine HCl*. One microliter of solution was injected into the GC.

One hundred milliliters of 0.05% promethazine hydrochloride-Sørensen buffer solutions (pH 2.93, 3.94, and 5.12) was placed in quartz reactor tubes as described above and exposed to UV light (330 nm) by means of a photolytic reactor at 30 ± 5°. EDTA 0.1% was added to the promethazine-buffer solutions and the ionic strength adjusted to  $\mu = 0.2$  with potassium chloride. The pH of the solutions was measured at the beginning and at the end of the exposure periods. Periodically, 1 ml of test solution was withdrawn from the flasks, immediately cooled, and analyzed as previously described.

## RESULTS AND DISCUSSION

Promethazine and promazine gave retention times of 8.5 and 10.0 min, respectively (see Fig. 1). Blank vehicle extracts yielded no interferences as illustrated in the same figure.

Quantitation of promethazine hydrochloride in water was obtained from a standard curve in which the peak height ratio (promethazine hydrochloride-promazine hydrochloride) was plotted against the promethazine hydrochloride concentration. The results are shown in Table I. There is a linear relationship between peak height ratio of promethazine hydrochloride to promazine hydrochloride and the concentration of promethazine hydrochloride in water over the range of 120–600  $\mu\text{g}/\text{ml}$ . The least-squares regression equation for the curve is  $y = 0.0023x + 0.0256$  and the correlation coefficient is 0.998. The results of nine replicate assays carried out over several days indicate that the assay method has adequate precision. The coefficients of variation of the assays are 1.4 and 1.1% in the presence and absence of polyethylene glycol vehicle.

The results of the quantitation of promethazine hydrochloride in water in the presence of polyethylene glycol vehicles are shown in Table I. There is a linear relationship between peak height ratio of promethazine hydrochloride to promazine hydrochloride and the concentration of promethazine hydrochloride with polyethylene glycol vehicle over the range of 120–480  $\mu\text{g}/\text{ml}$ . The least-squares regression equation is  $y = 0.0025x - 0.0133$ , and the correlation coefficient is 0.998.

The regression fits of promethazine hydrochloride standard in water with and without polyethylene glycol vehicles are shown in Table II. The slope and intercept of the standard curves with and without polyethylene glycol vehicles were not significantly different ( $F$  test,  $p > 0.05$ ).

The thermally degraded promethazine hydrochloride solution was separated with the TLC system previously described. Seven spots with  $R_f$  values of 0.0, 0.10, 0.16, 0.41, 0.57, 0.64, and 0.73, visualized under UV light, were obtained. The  $R_f$  value of promethazine was 0.4 which was confirmed with pure promethazine applied on the same TLC plate. The extracts of each spot were injected into the GC. None of the spots present in the TLC system interfered with the GLC procedure for promethazine

**Table IV—Photolytic Degradation of Promethazine Hydrochloride at Different pH, at 30°**

Degradation, hr	% of Promethazine Hydrochloride Remaining					
	pH 2.98		pH 3.94		pH 5.12	
0	100.0	100.0	100.0	100.0	100.0	100.0
0.5	82.4	84.3	85.0	—	85.9	87.5
1.0	81.1	81.2	83.7	—	81.4	85.0
2.0	78.2	79.2	—	—	79.5	81.8
3.0	—	—	74.9	69.8	—	—
4.0	72.7	75.5	—	—	73.8	74.5
7.0	63.8	66.4	67.3	54.8	65.2	63.5
12.0	51.7	54.2	53.8	42.4	55.3	53.3
24.0	27.7	32.7	30.7	27.9	36.1	31.8
36.0	10.7	15.2	13.0	6.4	20.2	14.6
48.0	1.6	4.0	3.1	0.73	8.8	4.1

and promazine. Only spots with  $R_f$  values of 0.73 and 0.41 showed significant peaks in the GC with retention times of 5.99 and 7.39 min, respectively.

The degradation of promethazine hydrochloride from pH 2.98 to 5.12 followed pseudo first-order kinetics at constant pH, temperature, and ionic strength. Linear semilogarithmic plots of percent of promethazine hydrochloride remaining versus time in three different pH-value buffer solutions at 60, 70, 80, and 90° were obtained. The pseudo first-order rate constants,  $k_{obs}$ , for each pH value at four different temperatures are given in Table III.

Arrhenius plots were constructed and found to be straight lines as illustrated in Fig. 2. From the slopes of the lines, the apparent activation energies of the thermal degradation reactions at pH 2.98, 3.94, and 5.12 were calculated to be 6601, 5888, 5570 cal/mole, respectively.

The influence of pH on degradation rate of promethazine hydrochloride is shown in Fig. 3. The rate constant increased with increasing pH in this experimental range.

The photolytically degraded promethazine hydrochloride was separated by the TLC system previously described. Five spots,  $R_f$  0.81, 0.58, 0.52, 0.44, and 0.00, were obtained. Only the spot with  $R_f$  0.44, which represents promethazine, gave a good response by GC. None of the other spots interfered with the GLC procedure for promethazine and promazine.

The photolytic degradation rate of promethazine hydrochloride was studied in three buffer solutions (pH 2.98, 3.94, and 5.12). The results are shown in Table IV. No linear relationship could be found from the logarithmic percent of promethazine remaining versus time plots at three different pH values at 30°. The photolytic degradation of promethazine hydrochloride under intense UV light did not follow simple kinetics. These results are in agreement with a previous report (6).

In summary, a GLC method with an organic phase extraction was developed and is being used to specifically determine promethazine hydrochloride in polyethylene glycol suppositories undergoing stability testing.

## REFERENCES

- (1) A. Felemeister and C. A. Discher, *J. Pharm. Sci.*, **53**, 756 (1964).
- (2) A. Felemeister, R. Schaubman, and H. Howe, *ibid.*, **54**, 1589 (1965).
- (3) J. A. Ryan, *J. Am. Pharm. Assoc., Sci. Ed.*, **48**, 240 (1959).
- (4) B. J. Meakin, D. J. G. Davies, N. Cox, and J. Stevens, *Analyst (London)*, **101**, 720 (1976).
- (5) W. J. M. Underberg, *J. Pharm. Sci.*, **67**, 1128 (1978).
- (6) N. Cox, B. J. Meakin, and D. J. G. Davies, *J. Pharm. Pharmacol.*, **28**, suppl. 45 (1976).

## ACKNOWLEDGMENTS

Supported by a grant from Alcon Laboratories, Inc., Fort Worth, Texas.