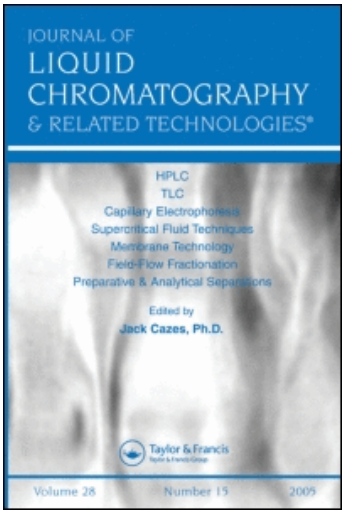

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High Pressure Liquid Chromatographic Determination of Promethazine Hydrochloride in the Presence of its Thermal and Photolytic Degradation Products: A Stability Indicating Assay

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HIGH PRESSURE LIQUID CHROMATOGRAPHIC DETERMINATION OF PROMETHAZINE HYDROCHLORIDE IN THE PRESENCE OF ITS THERMAL AND PHOTOLYTIC DEGRADATION PRODUCTS: A STABILITY INDICATING ASSAY

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ABSTRACT

A stability indicating method has been developed for the quantitation of promethazine hydrochloride in the presence of its photolytic and thermal degradation products. Following a basic extraction with acetonitrile, promethazine is separated from its internal standard, promazine, and vehicle components by direct high performance liquid chromatography using ultraviolet detection (249 nm) and a stainless steel column 25 cm in length, 0.46 cm i.d. packed with octa-decyl silica 5μ in diameter. A linear relationship was obtained between peak height ratio (promethazine/promazine) and promethazine hydrochloride in water over the range 30-600 g/ml. The percent coefficient of variation of the assay is 0.8% and the recovery of promethazine hydrochloride from aqueous solutions is 99.7%. The photolytic degradation of promethazine hydrochloride does not follow simple first order kinetics. Potassium iodide and p-benzoquinone had a significant effect on the degradation rate of promethazine during the first 30 minutes of the photolytic degradation reaction.

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However, after one hour there is no apparent quenching effect on the photolytic degradation rate of promethazine hydrochloride in the presence of these quenchers.

INTRODUCTION

Promethazine hydrochloride is known to undergo thermal and photolytic degradation which is oxidative in character, yielding a wide variety of degradation products including some which are colored. Promethazine hydrochloride has been determined by forming a colored product in various acidic mediuims (1,2) or by forming a colored complex with palladium chloride (3-5). It has been analyzed by the formation of photo-oxidation products which are free radicals characterized by their strong absorption in the visible region (6). Separation of promethazine hydrochloride prior to spectrophotometric determination by partition column chromatography (7,8), and ion exchange chromatography (9,10) has been reported. The USP XX assay for promethazine hydrochloride in injectable solutions is based on the determination of salts or organic nitrogenous bases (11). The method described for syrups and tablets uses ultraviolet spectrophotometry after separation from the inert ingredients by a biphasic extraction or a partition column. These methods are time consuming and may not have the specificity required for the quantitation of promethazine in the presence of its degradation products. In addition, the USP does not describe an assay method for promethazine hydrochloride in suppository dosage forms containing polyethylene glycol. The separation of drugs in proprietary preparations containing phenothiazine by high pressure liquid chromatography (HPLC) has been reported (12-14); however, these methods are not stability indicating. Other HPLC methods have been developed to quantitate promethazine hydrochloride in biological fluids (15-17). In the present investigation, an HPLC method has been developed to

specifically determine promethazine hydrochloride in polyethylene glycol suppositories undergoing stability testing. In addition, the influence of potassium iodide and p-benzoquinone on the photolytic degradation rate of promethazine hydrochloride is reported.

EXPERIMENTAL

Promethazine Hydrochloride (Napp Chemicals, Inc., Ladi, New Jersey) and Promazine Hydrochloride (Wyeth Labs, Inc., Philadelphia, Pennsylvania) were used as received. All solvents and chemicals were commercial analytical grade (Fisher Scientific Co., Fair Lawn, New York).

Chromatographic Conditions

A dual pump high performance liquid chromatographic system with a microprocessor control was used (Beckman Model 100 A Pump, Beckman Instruments, Inc., Berkeley, California). A stainless steel column, 25 cm in length, 4.6 mm i.d., was packed with Octadecyl silica, 5 μ in diamter (Ultrasphere ODS 5 μ . Altex Scientific Inc., Berkeley, California). The mobile phase consisted of 15 mM monobasic potassium phosphate pH 5 and a mixture of 9×10^{-3} mM triethanolamine and acetonitrile in a ratio of 15:85, respectively. The column temperature was maintained at 45° C by means of a water circulator. The flow rate of the mobile phase was 1.6 ml/hr and the variable wavelength detector was set at 249 nm (Hitachi Model 200-40 Spectrophotometer., Altex Scientific Inc., Berkeley, California).

Internal Standard Solution

50 mg of Promazine hydrochloride was accurately weighted and placed in a 200 ml volumetric flask and appropriately diluted with deionized water.

Polyethylene Glycol Vehicle Solutions

A stock solution was prepared using the excipients and preservatives employed in the manufacture of the suppositories. Appropriate dilutions were made with deionized water. Ten ml of this solution is approximately equivalent to the vehicle content of a 2.5 g PEG suppository.

Standard Solution

60 mg of promethazine hydrochloride was accurately weighted and placed in a 100 ml volumetric flask and brought to volume with deionized water. Two, 4, 6, 8, and 10 ml of the above solution were pipetted into five separate 10 ml volumetric flasks, and each flask brought to volume with deionized water. The concentration of the resultant promethazine hydrochloride solutions were 120, 240, 360, 480, and 600 μg/ml, respectively.

Standard Curve of Promethazine Hydrochloride in Water

One ml of each standard solution was placed in a 12 ml centrifuge tube and to each tube was added 0.5 ml of internal standard solution and 0.2 ml of 5 N NaOH. The centrifuge tube was thoroughly mixed for 30 seconds. Then, 4.0 ml of acetonitrile was added to each tube, mixed thoroughly, and centrifuged at 2000 rpm for 5 minutes. Ten μl of supernatant were directly injected into the HPLC.

Photolytic Degradation of Promethazine Hydrochloride

One hundred mls of a 1.56×10^{-3} M solution of promethazine hydrochloride in a Sörensen citrate buffer (pH 5) were placed in a quartz reaction tube (The South New England Ultraviolet Co., Connecticut), length 43 cm, o.d. 2.6 cm and i.d. 2.4 cm, equipped with a water cooling tube, o.d., 1.2 cm and exposed to ultraviolet light (350 nm) by means of a photolytic reactor (The South New England Ultraviolet Co., Connecticut). The reaction temperature was maintained at 22°± 0.2°C.

One ml samples were withdrawn at zero time and at 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 12.0, 24.0, 36.0, 48.0, and 60.0 hours. One half ml of each sample was analyzed as described in the section under the standard curve of promethazine hydrochloride in water. The influence of radical quenchers on the photolytic degradation rate of promethazine hydrochloride was studied by adding 2.34 x 10^{-4} moles of potassium iodide, or 3.12 x 10^{-4} moles of p-benzoquinone. The promethazine concentrations were determined as previously described.

Thermal Degradation of Promethazine Hydrochloride

Promethazine hydrochloride, 0.3 g, was dissolved in 10 ml of water. Forty-nine mls of oxygen saturated Sorensen citrate buffer solution (pH 5) was placed in a separate 100 ml light proof amber volumetric flask wrapped with aluminum foil and kept at a temperature of 70°C ± 0.2°C, in a thermostatically controlled oil bath. One ml of the stock solution was added and the flask agitated for 5 seconds. Samples of 1.5 ml were taken at zero time and at appropriate time intervals up to 60 hours. One ml of each sample was analyzed as described previously. After each sample was withdrawn from the volumetric flask, the flask was immediately recharged with a stream of oxygen in order to maintain an oxygen atmosphere.

RESULTS AND DISCUSSION

Figure 1 shows a typical chromatogram for the analysis of promethazine hydrochloride in water. Using the appropriate mobile phase, as described previously, the retention times for promethazine and promazine were 5.9 and 9.0 minutes respectively. Quantitation of promethazine hydrochloride in water was obtained from a standard curve in which the peak height ratio (promethazine/promazine) was plotted against the promethazine

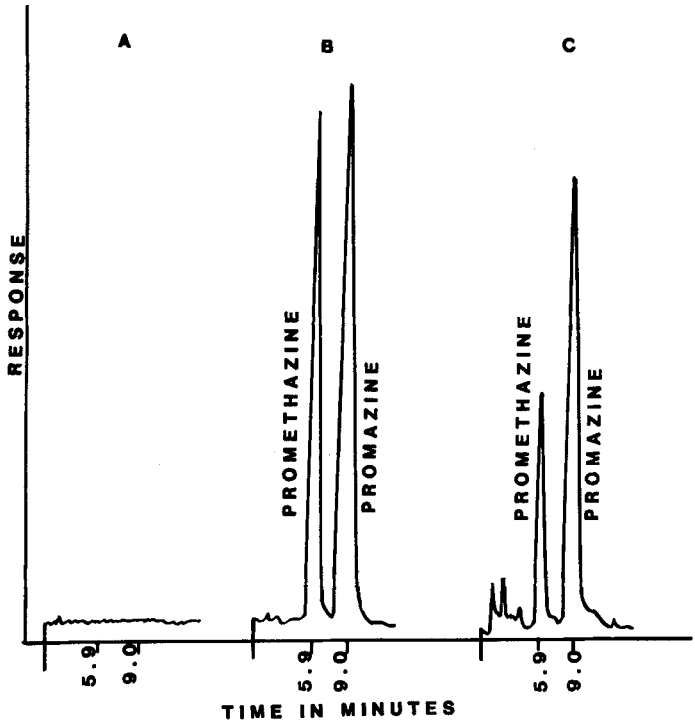

Figure 1. Chromatogram of promethazine hydrochloride blank vehicle extract, (A); 360 mcg/ml of promethazine hydrochloride extracted from an aqueous solution, (B); and chromatogram obtained after 35 hours of photolytic degradation at 340 nm of a 0.5% solution of promethazine hydrochloride aqueous solution.

hydrochloride concentration. There is a linear relationship between peak height ratios of promethazine to promazine and the concentration of promethazine hydrochloride in water over the range of 30-600 μg/ml. The least-square regression equation for the curve is $y = 0.00179X + 0.0262$, and the correlation coefficient is 0.999. The results of ten replicate assays carried out over several days indicate that the assay method has adequate precision. The percent coefficient of variation of the assays is 0.8%. The presence of the polyethylene glycol vehicle did not

have an effect on the assay. The recovery of promethazine hydrochloride from aqueous solution is 99.7%.

The method described by Underberg was used to extract the thermodegradation products (21,22). Nine spots were visualized under ultraviolet light after thin layer chromatography on silica gel G.F. and using acetone:6N NH_3 (100:2) as the mobile phase. The spots were extracted and reconstituted in methanol and injected into the chromatograph. The thermal degradation products did not interfere with the determination of promethazine hydrochloride using the HPLC procedure. In our laboratories, gas chromatographic methods (GC) using electrolytic conductivity or flame ionization detectors have been used to quantitate promethazine hydrochloride in the presence of its thermal degrada-

TABLE 1

Comparison of the Analysis of Promethazine Hydrochloride in Aqueous Solution by a Gas Chromatographic Method and a High Performance Liquid Chromatographic Method

Parameter	Method	
	GC[a]	HPLC[b]
Sensitivity in Nanograms	7.5	1.2
Correlation Coefficient of Standard Curves in Water	0.999	0.999
Promethazine Retention Time in Minutes	10.8	5.9
Percent Coefficient of Variation	0.7	0.8
Percent Recovery	100.0	99.7

a. Gas chromatography with electroconductivity detection.

b. High pressure liquid chromatography with U.V. detection at 249 nm.

tion products (18). Thus, a comparison between the HPLC method and the GC method with electrolytic conductivity detection was performed for the purpose of determining the efficiency of the methods. Table 1 illustrates a comparison of sensitivity, coefficient of correlation of promethazine hydrochloride standard curves in water, retention times, percent coefficient of variation, and percent recovery. The retention time by the HPLC method is estimated to be 1.83 times less than by the GC method. In addition, the HPLC method is more sensitive.

TABLE 2

Thermal Degradation of 600 µg/ml of Promethazine Hydrochloride in pH 5.0 Sörensen Citrate Buffer Solution at 70°C

Time in Hours	Percent Promethazine Hydrochloride Remaining in Solution	
	GC[a]	HPLC[b]
0	100.0	100.0
1	88.2	90.0
2	84.3	85.7
3	82.8	--
4	80.2	78.2
6	75.5	71.5
8	69.5	65.8
12	59.3	56.4
24	35.7	35.2
36	19.2	21.3
48	13.7	13.2
60	7.8	9.8

a. Gas chromatography with electroconductivity detection.

b. High pressure liquid chromatography with U.V. detection at 249 nm.

Table 2 illustrates the results of a thermal degradation study at pH 5 and at 70°C. Three replicate assays using the GC and HPLC methods were performed at each time interval. In order to determine if a statistical significant difference existed between the results obtained by these methods an F-test was performed ($p = 0.05$). The results indicate that there is no significant difference between the degradation kinetics of promethazine hydrochloride as determined by GC or HPLC. The results of this study suggest that both the GC and HPLC methods are suitable for the analysis of promethazine hydrochloride in the presence of its thermal degradation products. The HPLC method is more sensitive and faster than the GC method, therefore, it is recommended as the method of choice for the quantitation of promethazine hydrochloride in polyethylene glycol solid delivery systems. This method may also be extended to cocoa butter-wax solid delivery systems.

The photolytic degradation products were separated by thin layer chromatography using acetone:methanol:6N NH_3 (140:60:1) as mobile phase. Five spots were visualized under ultraviolet light. The spots were extracted with methanol and injected into the chromatograph. No interferences were found in the chromatographic region of promethazine. Figure 2 shows the results obtained from the photolytic degradation studies. In addition, it illustrates the effect of potassium iodide and p-benzoquinone on the degradation rate of promethazine hydrochloride in aqueous solution. Each point in the graph represents the mean of three determinations. It was interesting to note that during the first 30 minutes the promethazine hydrochloride concentration decreased rapidly. No linear relationship could be found from the logarithmic percent of promethazine versus time plots. This result is consistent with the report by Cox (19). However, as shown in figure 2, the degradation rates of promethazine hydrochloride from 1 hour to 60 hours followed a pseudo first-order process. The observed mag-

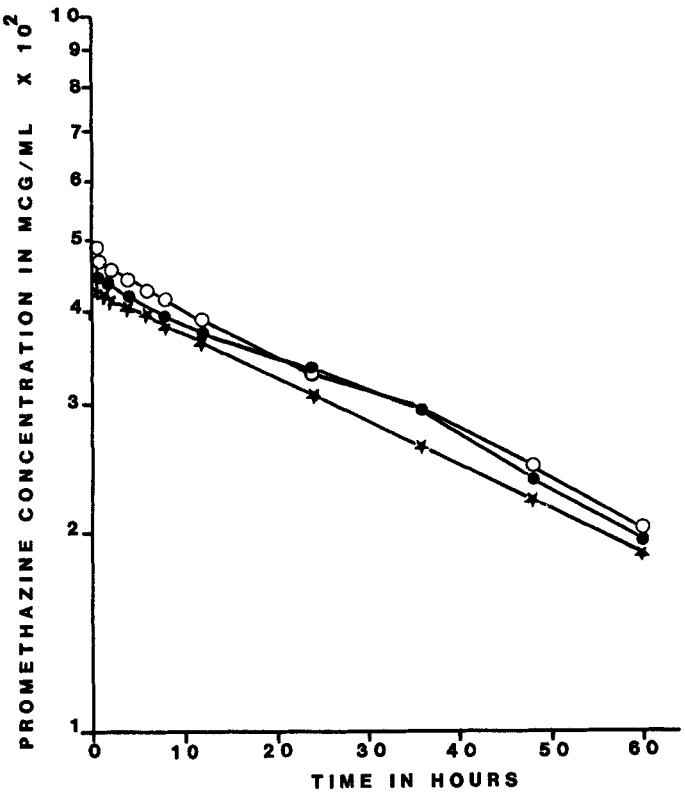

Figure 2. Influence of radical quenchers on the photolytic degradation rate of promethazine hydrochloride in aqueous solution. ★, 1.56 x 10^{-3} M promethazine hydrochloride aqueous solution; in the presence of: ○, 3.12 x 10^{-3} M of p-benzoquinone; ●, 2.34 x 10^{-3} N if potassium iodide.

nitude of the rate constants (Kobs ± standard deviation) were found to be 1.33 x 10^{-2} ± 0.18 hrs^{-1}, 1.53 x 10^{-2} ± 0.25 hrs^{-1}, and 1.65 x 10^{-2} ± 0.2 hrs^{-1} in the absence and presence of potassium iodide and p-benzoquinone, respectively. In order to determine if a significant difference existed among the photolytic degradation rate constants obtained in the presence and absence of the radical quenchers (potassium iodide and p-benzoquinone) a t-test was performed (p=0.05).

The results indicate that there was no statistical difference between the photolytic degradation rates. There was 12.7 and 5.6 percent more promethazine hydrochloride remaining in solution in the presence of p-benzoquinone and potassium iodide during the initial 30 minutes of the reaction, respectively. After one hours, there is no apparent quenching effect on the photolytic degradation rate of promethazine hydrochloride in the presence of these quenchers. The results of this study suggest that the mechanism of decomposition of promethazine is not just a simple free radical mechanism. Potassium iodide is known to be a quencher of triplet state of photo sensitive drugs (20). However, the results of this study suggest that the mechanism of photolytic oxidation of promethazine after 30 minutes may not proceed through a triplet state step or that potassium iodide may not act as a quencher of choice for the promethazine triplet state.

In summary, a sensitive and specific high pressure liquid chromatographic method with a simple organic phase extraction was developed with the purpose of determining promethazine hydrochloride in polyethylene glycol suppositories.

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