DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 9(6), 989-998 (1983)

GAS LIQUID CHROMATOGRAPHIC DETERMINATION OF PROMETHAZINE HYDROCHLORIDE IN COCOA BUTTER-WHITE WAX SUPPOSITORIES

S. Stavchansky, P. Wu and J.E. Wallace*

College of Pharmacy, Drug Dynamics Institute
The University of Texas, Austin, Texas 78712.

* Department of Pathology
University of Texas Health Science Center
San Antonio, Texas 78284

ABSTRACT

A gas liquid chromatographic method is described for the determination of promethazine hydrochloride in cocoa butter-white wax suppositories. This assay method is capable of distinguishing promethazine hydrochloride from its thermal and photolytic degradation products. Promethazine and promazine were eluted in 15 minutes with retention times of 8.6 and 10.1 minutes respectively. A linear relationship between peak height ratio (promethazine/promazine) and promethazine hydrochloride concentration was found up to 600 μg/ml. The coefficient of variation of the assay method over several days were found

 0363-9045/83/0906-0989$3.50/0

to be 2.07%. Finally, the recovery of promethazine ranged from 72 - 76% in the presence and absence of cocoa butter-white wax vehicles.

INTRODUCTION

The USP XX assay for promethazine hydrochloride in injectable solutions is based on the determination of salts of organic nitrogenous bases (1). The method described for syrups and tablets usues ultraviolet spectrophotometry. These two methods are time consuming and may not have the specificity required for the quantitation of promethazine in the presence of its thermal and photolytic degradation products. Other spectrophotometric methods have been reported (2,3,4) but none of them have been designed for stability testing. The USP does not describe an assay method for promethazine hydrochloride in suppository dosage forms. Stability indicating assay methods for the quantitation of promethazine hydrochloride in polyethylene glycol suppositories have been recently reported by our laboratories. These methods are based on gas liquid chromatography with flame ionization or electrlytic conductivity detection (5,6). In the present investigation, a gas liquid chromatographic method with a single organic phase extraction is reported to specifically determine promethazine hydrochloride in cocoa butter-white wax suppositories.

EXPERIMENTAL

Materials - Promethazine hydrochloride (Napp Chemicals, Inc., Lodi, New Jersey), Cocoa butter and white wax (City Chemical Co., New York, New York) were obtained commercially. Promazine hydrochloride was a gift of Wyeth Laboratories. Cocoa butter-white wax promethazine hydrochloride suppositories were obtained commercially. The identity and purity of promethazine hydrochloride and promazine hydrochloride were checked by gas liquid chromatography and mass spectrometry. All other chemicals were analytical grade (Fisher Scientific Co., Fair Lawn, New Jersey). Swinnex adaptors for filters were obtained commercially (Millipore Co., Bedford, Maine). Deionized water was used throughout the study.

Stock Solutions - Solution A - 300 mg of promethazine hydrochloride accurately weighed were placed in a 50 ml volumetric flask dissolved and diluted with chloroform to volume. Solution B - 50 mg of promazine hydrochloride accurately weighed were placed in a 70 ml volumetric flask dissolved and diluted with deionized water to volume.

Solution C - 1.35 g of cocoa butter and 1.35 g of white wax were placed in a 10 ml volumetric flask and dissolved and diluted to volume with chloroform.

Standard Solutions - Two, 4, 6, 8 and 10 ml of solution A were placed in a 10 ml volumetric flask and taken to volume with chloroform. The resulting concentration of promethazine

hydrochloride was 1.2, 2.4, 3.6, 4.8, and 6.0 mg/ml respectively.

Gas Chromatographic Conditions - A model 560 Tracor dual column gas chromatograph (Tracor Instruments, Austin, Texas) equipped 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 or 100-120 mesh Gas Chrom Q (Supelco, Inc., Bellefonte, Pennsylvania). The column was conditioned for 36 hours at 280°C and was treated with a silylating agent, N,O-Bis(trimethylsilyl-acetamide) (Pierce Chemical Co., Rockford, Illinois). Nitrogen carrier gas flow, hydrogen flow, and air flow were 36.6 ml/min, 36.6 ml/min and 350 ml/min. The column, detector, and sample injection port temperatures were 250, 300 and 260°C, respectively.

Standard Curve of Promethazine Hydrochloride in Chloroform - One tenth ml of each standard solution, 0.1 ml of solution B (internal standard) and 3 ml of 0.1N HCl were placed in a 12 ml conical centrifuge tube capped with a teflon lined screw cap and mixed thoroughly with a vortex mixer for one minute. The mixture was centrifuged for one minute at 2000 rpm. The aqueous phase was filtered through a Swinnex filtration system using a 0.45 mμ pore size filter. The filtrate was transferred to a 12 ml centrifuge tube and 4 ml of hexane was added. The mixture was mixed with a vortex mixer for one minute. After centrifugation for 1 minute at 2000 rpm the hexane layer

was discarded. The aqueous phase was basified with 0.3 ml of 5N NaOH and extracted with 4 ml of n-hexane. The mixture was mixed thoroughly with a vortex mixer for one minute. After centrifugation for one minute at 2000 rpm, 3 µl of the hexane phase were injected into the gas chromatograph.

Standard Curve of Promethazine Hydrochloride in the Presence of Cocoa Butter-White Wax Vehicle - The procedure was the same as the one described under the standard curve for promethazine hydrochloride in chloroform except for the addition of 0.1 ml of solution C to each centrifuge tube prior to the first extraction with n-hexane. This volume of solution C is representative of a cocoa butter-white wax suppository. This procedure compares to the extraction of promethazine hydrochloride from a cocoa butter-white wax suppository.

Interference of Thermal and Photolytic Degradation Products- Promethazine hydrochloride, 5 g, was dissolved in 100 ml of water and oxygen was bubbled through the solution for 30 minutes. The solution was placed in a screw-capped light proof amber bottle saturated with oxygen and kept at 65°C for five days. The photolytic degradation studies were conducted using a 0.3% (W/V) promethazine hydrochloride solution in water. The solution was placed in a quartz reactor tube length 43 cm, o.d. 2.6 cm, i.d. 2.4 cm, which was equipped with a water cooling tube, o.d. 1.2 cm and exposed to ultra-violet light (300 nm.) by means of a photolytic reactor (The South New England Ultraviolet Co., Connecticut) at 10°C for 74 hours.

After the degradation, both thermal and photolytic degradation products underwent the same extraction and separation procedures developed by Underberg (7,8). The degradation products were extracted with dichloromethane and then separated by thin layer chromatogrpahy using silica gel plates with fluorescence indicator (Analabs, Inc., North Haven, Conn.). Each spot was scraped individually under UV light and placed in a conical centrifuge tube with a glass stopper. One ml of dichloromethane was added to each tube and mixed for one minute with a vortex mixer. The tube was centrifuged and the supernatant transferred to a small test tube and evaporated to dryness under a stream of nitrogen. The residue was dissolved with 100 µl of methanol. One to 2 µl of this solution were injected into the gas chromatograph.

Determination of Promethazine Hydrochloride in Cocoa Butter - White Wax Suppositories - One commercially available cocoa butter-white wax suppository containing 50 mg of promethazine hydrochloride was placed in a volumetric flask and dissolved with 10 ml of chloroform. Then, 0.1 ml of the resulting solution was placed in a 12 ml conical centrifuge tube. The sample was extracted using the same procedure described under the standard curve of promethazine hydrochloride in chloroform.

RESULTS AND DISCUSSION

Separation - Figure 1 shows a typical chromatogram for the quantitation of promethazine hydrochloride in the presence of

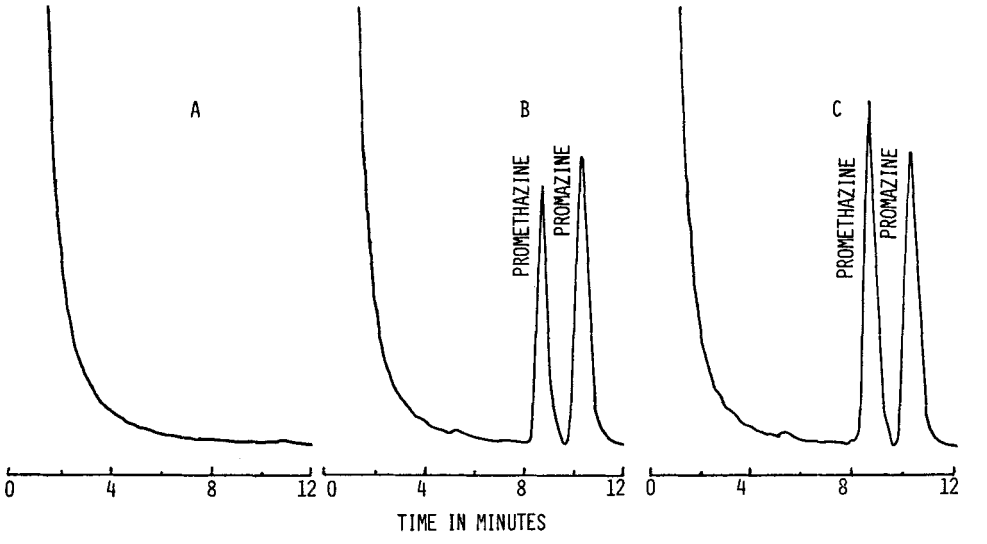

Figure 1. (A). Chromatograms of extracts of a blank cocoa butter-white wax vehicle. (B). Chromatogram obtained after 3.6 mg/ml of promethazine hydrochloride was extracted in the presence of cocoa butter-white wax vehicle and (C). represents the chromatogram obtained after extraction of a comercially available cocoa butter-white wax suppository.

promazine hydrochloride as internal standard. Promethazine and promazine were eluted in 15 minutes with retention time of 8.6 and 10.1 minutes respectively. Figure 1a represents the chromatogram of extracts of a blank cocoa butter-white wax vehicle sample demonstrating no troublesome interferences in the promethazine and promazine region. Figure 1b represents the chromatogram obtained after 3.6 mg/ml of promethazine hydrochloride were extracted using the described procedure in the presence of Cocoa butter-White wax vehicle. Finally, Figure 1c represents the chromatogram obtained after a commercially cocoa butter-white wax suppository was submitted to the procedure.

Standard Curve - Quantitation of promethazine hydrochloride in chloroform was obtained from a standard curve in which the peak height ratio (promethazine/promazine) was plotted against the promethazine hydrochloride concentration. There is a linear relationship over the range of 1.2 - 6.0 mg/ml. The least square regression equation for the curve is $y = 0.245\ X + 0.0166$ and the correlation coefficient is 0.999. The least square regression equation for the standard curve in the presence of cocoa butter-white wax vehicle over the range of 1.2 - 6.0 mg/ml is $y = 0.241\ X + 0.0244$ and the correlation coefficient is 0.999.

In order to test the equality of the two regression lines an F-test was performed (9). No statistical significant differences were observed between the slopes and intercepts of the lines at an α level of $p = 0.05$. Therefore, it can be concluded from the data that the standard curve in chloroform can be used for the quantitation of promethazine hydrochloride suppositories containing cocoa butter and white wax. In order to confirm the effectiveness of this assay method, a commercially available cocoa butter-white wax suppository containing 50 mg of promethazine hydrochloride was investigated. The resulting chromatogram is illustrated in Figure 1c. The amount of promethazine hydrochloride estimated from the standard curve was found to be 96% of the labelled amount. The precision of the assay method was carried out by nine replicate

assays of 3.6 mg/ml of promethazine hydrochloride in chloroform in the presence of cocoa butter-white wax vehicle over several days. The results show a percent coefficient of variation of 2.07%. The recovery of promethazine hydrochloride using this assay method was found to be 72-76%.

Interference of Thermal and Photolytic Degradation Products - Troublesome interferences were not found in the promethazine and promazine chromatographic region after injection of the thermal and photolytic degradation products into the gas chromatograph. These results are in good agreement with those reported previously for polyethylene glycol suppositories by our laboratories (5,6).

In summary, data was presented to support the development of a reliable, specific and sensitive gas liquid chromatographic assay method for the quantitation of promethazine hydrochloride suppositories containing a cocoa butter-white wax base undergoing stability testing.

ACKNOWLEDGMENTS

This work was partially supported by a grant from Alcon Laboratories, Inc., Fort Worth, Texas.

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