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QUANTITATION OF PROMETHAZINE HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORMS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A stability-indicating HPLC assay method has been developed to quantify promethazine hydrochloride in pharmaceutical dosage forms, injection, oral liquids, suppositories and tablets. The method is accurate and precise with a percent relative standard deviation of 0.4 based on 6 readings. The recoveries from the synthetic mixtures were quantitative. Three new peaks in the chromatogram were detected from a decomposed sample. A number of active and inactive ingredients, colors, preservatives, flavors, antioxidants, phenylephrine and codeine present in the dosage forms did not interfere with the assay procedure.

BACKGROUND

Promethazine hydrochloride is extensively used as an antihistamine and antiemetic agent. There are a number of dosage forms available commercially, injections, suppositories, syrups and tablets. All of these dosage forms are official in the USP-NF (1). The official assay method for injection is based on HPLC(1) with the mobile phase containing a counterion. The assay method for the suppositories and tablets is based on extraction and reaction with palladium chloride (1). The color so produced is measured at 450 nm for suppositories versus 470nm for tablets. The assay method for syrup is based on extraction and spectrophotometric reading at 298 nm (1). The purpose of these investigations was to develop a stability

indicating HPLC assay method for the quantitation of promethazine hydrochloride in all of the official dosage forms and some others which are available commercially.

MATERIALS AND METHODS

Chemical Reagents: All the chemicals and reagents were USP-NF or ACS quality and were used without further purification. Promethazine hydrochloride powder was supplied by Napp Chemicals, Inc. and used as received.

Apparatus: A high-pressure liquid chromatograph (Waters ALC 202) equipped with a universal injector (Rheodyne Model 7125), a multiple wavelength detector (Schoeffel's SF 770, Kratos, Inc.) and a recorder (Omniscribe 5213-12, Houston Instruments, Austin) was used. A micro C_{18} column (Whatman, 25 cm x 3.9 mm i.d.) was the stationary phase.

Chromatographic Conditions: The mobile phase contained 40% v/v of acetonitrile and 0.1% of glacial acetic acid in 0.01M KH_2PO_4 aqueous buffer. The flow rate was 2.0 ml/min., the wavelength was 271 nm (sensitivity 0.1 AUFS), the chart speed was 30.5 cm/hr and the temperature was ambient.

Preparation of Stock and Standard Solutions: The stock solutions of promethazine hydrochloride (50mg/50ml) and of the internal standard dextromethorphan hydrobromide (100mg/50ml) were prepared fresh every day in water. A most commonly used standard solution was prepared by diluting the stock solution of drug and the stock solution of the internal standard, (added to the final dilution) with water to contain 300 μg/ml of promethazine hydrochloride and 200 μg/ml of the internal standard. The solutions of other concentrations were prepared as needed.

Preparation of Assay Solutions From the Dosage Forms:

A. Promethazine Hydrochloride injection, Syrup and Syrup with Phenylephrine hydrochloride: The sample was diluted with water to contain 300 μg/ml of promethazine hydrochloride based on the label claim. Before the final assay solution was brought to volume, an appropriate quantity of the stock solution of the internal standard was added to contain 200 μg/ml.

B. Suppositories: One or two suppositories were crushed in a mortar, 10 ml of ~ 1 N HCl solution and 35ml of water added and the mixture was stirred for 30 minutes in the dark. The supernatant was collected in a 50 ml volumetric flask, the mass was washed with water and used to bring the mixture to volume. The mixture was diluted further with water (after adding the appropriate quantity of the stock solution of the

internal standard) to contain 300 μg/ml of promethazine hydrochloride based on the label claim.

C. Tablets: One or ten tablets (if average assay reading is required) were ground to a fine powder. The powder equivalent to one tablet was transferred to a 50 ml volumetric flask, 5 ml of ~ 1 N HCl solution and enough water was added to bring the mixture to volume. The mixture was shaken for 30 minutes using a mechanical stirrer and filtered. First 15 ml of the filtrate was rejected, then a portion of the clear filtrate was diluted with water (appropriate quantity of the stock solution of the internal standard was added) to volume to contain 300 μg/ml of the drug based on the label claim.

Decomposition of the stock solution: A 7.5 ml quantity of the stock solution of the drug was diluted to about 20 ml with water, boiled for 2-3 minutes, cooled, brought to volume (25.0 ml) with water in a volumetric flask. In a separate experiment, the internal standard was added (final concentration 200 μg/ml) before bringing the solution to volume with water. On heating, the clear solution turned first to blue and then to pink indicating decomposition.

Assay Procedure: A 25 μl quantity of the assay solution was injected into the chromatograph using the conditions described. For comparison, an identical volume of the standard solution was injected after the sample eluted. The standard solution contained identical concentrations of the drug (based on the label claim) and the internal standard.

Calculations: Preliminary investigations indicated that the ratio of peak heights were related to the concentrations of the drug (range tested 100-500 μg/ml of the drug). Therefore, the results were calculated using a simple equation:

$$\frac{(Rph)_a}{(Rph)_s} \times 100 = \text{percent of the label claim found,}$$

where $(Rph)_a$ is the ratio of the peak heights of drug to internal standard of the assay solution and $(Rph)_s$ that of the standard solution.

RESULTS AND DISCUSSION

Assay Method - The developed HPLC assay method is accurate and precise with a relative percent standard deviation of 0.4 based on 6 readings. The recoveries of drug from 2 synthetic mixtures were quantitative (Table 1). The mobile phase contained 40% acetonitrite and the drug was retained for more than 4 minutes

TABLE 1

ASSAY RESULTS

Name of the Sample	Percent of the Label Claim Found	Other Ingredients (if known)
Injection[a] 25 mg/ml	99.3	Per ml. Edetate disodium, 0.1 mg; calcium chloride, 0.04 mg; Sodium metabisulfite, 0.25 mg and phenol 5 mg with acetate buffer.
Suppositories[b] 12.5mg	98.1	Ascorbyl palmitate, silicon di-oxide, white wax and cocoa butter.
Plain syrup[b] 6.25 mg/5 ml	99.6	Colors, flavored syrup, citric acid, glycerin, saccharin sodium, sodium benzoate, sodium citrate and sodium propionate.
Syrup[b]with phenylephrine hydrochloride 5 mg/ 5 ml	98.7	as above
Tablets[c] 25 mg	98.6	not disclosed on the label
Synthetic Mixture 1	100.1	25mg of drug and 250mg of dextrose
Synthetic Mixture 2	100.9	25mg of drug and 250mg of lactose
Decomposed Solution	84.0	

[a] Elkins-Sinn Inc, Cherry Hill, NJ
[b] Phenergan® by Wyeth Laboratories, Philadelphia, PA
[c] Schein Pharmaceuticals, Port Washington, NY

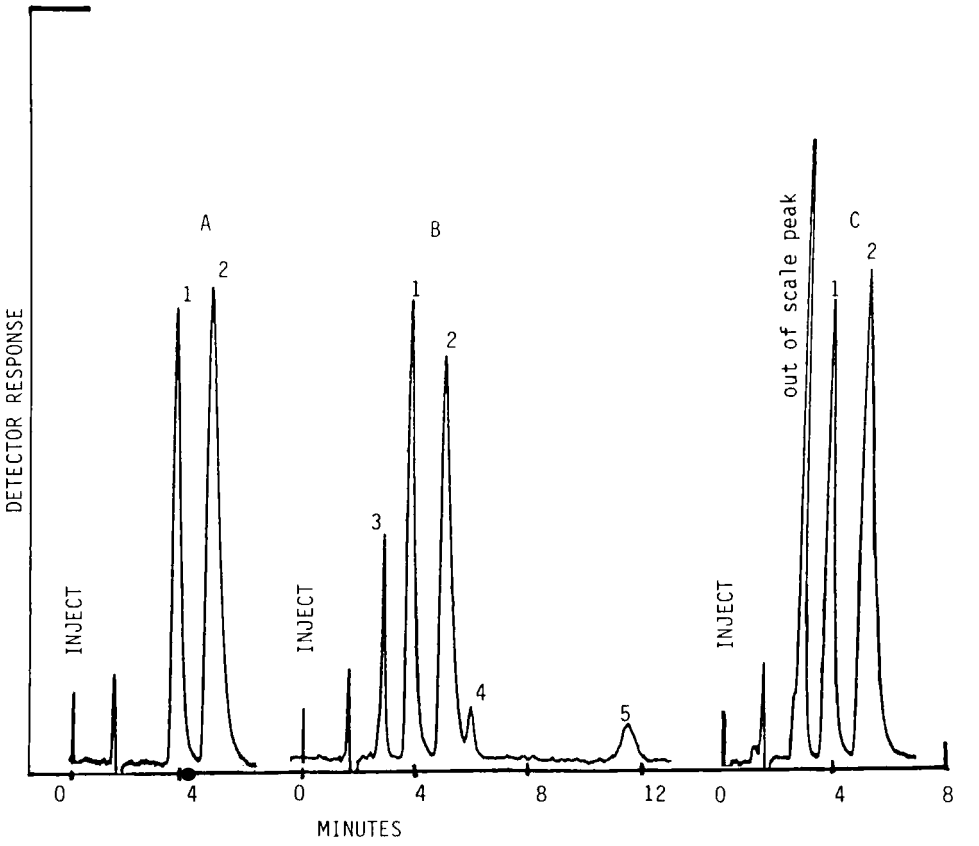

Figure 1 - Sample Chromatograms; Peaks 1-2 are from dextromethorphan (the internal standard) and promethazine, respectively. Peaks 3-5 are from the decomposition products. Chromatogram A is from a standard solution; B from a decomposed solution (see text) and C from an injection. For chromatographic conditions, see text.

without the addition of a counterion (Fig 1., peak 2). The HPLC method for injection given in the USP - NF (1) recommends the addition of a counterion, which can shorten the lives of the columns and detector's cells.

The developed method appear to be stability - indicating since the discolored solution (i.e. the decomposed solution) gave 3 additional peaks in the chromatogram (Figure 1B, peaks 3-5). On injecting a sample of the decomposed solution without the internal standard, no additional peaks were detected in the

chromatogram. The decomposed solution still contained approximately 84% of the intact drug. A light-blue colored solution when assayed after 2 day storage at 25° in amber colored bottle still contained more than 99% of the intact drug.

The wavelengths of maximum absorption for promethazine are 249 and 298 nm. At these wavelengths, the internal standard, dextromethorphan hydrobromide, had very poor absorption. Since sensitivity was not a problem, it was decided to use 271 nm where both the drug and the internal standard had good absorption. Analysis of Dosage Forms: The USP- NF had recommended a different assay method (1) for each dosage form. The authors were able to assay all these dosage forms using the developed HPLC method (Table 1). In addition to these four, promethazine was also quantified in a syrup which contained an additional ingredient, phenylephrine hydrochloride, the most commonly used decongestant. Amongst the excipients present in the syrups were 4 different colors (2) and sodium benzoate (preservative). None of these interfered with the assay procedure (Table 1). It was determined that if the syrup contained codeine, it will elute out with the solvent front.

The various excipients in the injection, especially edetate sodium, sodium metabisulfite and phenol (5 mg/ml) did not interfere with the assay procedure (Figure 1 C and Table 1).

Furthermore, the excipients present in the suppositories, ascorbyl palmitate, silicon dioxide, white wax and cocoa butter did not interfere with the assay method (Table 1). The excipients present in the tablets were not disclosed on the label.

REFERENCES

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