Spectrophotometric Determination of Promethaizine Hydrochloride using Sulphanilamide

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An extractive spectrophotometric method for the determination of promethazine Hydeochloride in either pure form or in pharmaceutical formulations is described.The method is based on the formation of a red coloured product with sulphanilamide in the presence of N-bromosuccinimide. The reaction presumably proceeds via preliminary oxidation of the phenothiazine nucleus to a phenothiazinyl radical followed by coupling of the sulphanilamide in C-3 or C-7 position.The chromogen produced is stable for more than 3 days at room temperature. Beer's law is obeyed in the range of concentration of 5-25 µg/ml at the maximum absorption of 510 nm. Optimum analytical conditions were determined. Analytical data for determination of the pure compound is presented together with the application of the proposed method to the analysis of some pharmaceutical preparations. The results compare favourably with those of official methods. Furthermore, the method is specific for C-2 unsubstiuted promethazine hydrochloride.

Phenothiazine derivatives are biologically active heterocyclic compounds that find extensive applications in the field of medicine and chemicals analysis. In clinical practice , they are widely used as tranquillisers, psychotherapeutics, antiemetics, antihistamines and sedative drugs[1-2]. One of the important derivatives is promethazine hydrochloride (PMH). Chemically it is known as 10-(2-dimethylaminopyropyl) phenothiazine. In literature, various analytical methods are available for the assay of PMH, very important being titrimetry[3,4], spectrophotometry[5-8], polarography[9] high-performance liquid chromatography[10] and fluorimetry[11]. In several methods, phenothiazine derivatives were oxidised by different oxidants including N-bromosuccinimide (NBS) and then determined spectrophotometrically[2,12-14].

In this paper, a study of the determination of PMH based on the development of a red product with sulphanilamide (SPA) in presence of NBS. The proposed method offers the advantage of simplicity, sensitivity, stability and high specificity. It has an added advantage that the reaction occurs at room temperature.

A Shimadzu Double-Beam spectrophotometer UV-150-02 with 1.0 cm matched cells was used for the spectral measurements. The purity of promethazine hydrochloride (PMH, Sigma, USA) was checked by non-aqueous titration method[15]. Stock solution of PMH was prepared by dissolving requisite amount of the sample in deionized water. Solution was stored in an amber coloured bottle in a refrigerator. A working solution containing 50 µg/ml was made by suitable dilution of the stock solution as and when required. A 0.02 % w/v aqueous solution of NBS was freshly prepared, a 0.01% w/v aqueous solution of sulphanilamide were used.

Suitable aliquots of standard PMH solution (50-250 µg/ml) was taken into a series of 100 ml separating flasks. To each flask, 1.5 ml of NBS, 2.0 ml of SPA and 6.0 ml of chloroform were added. The contents were shaken for 1.0 min and then allowed to separate. The organic layer was transferred to 50 ml beakers. The aqueous layers were once again extracted with 3.0 ml of solvent. The extracted organic

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Table - 1: Determination of promethazine hydrochloride in pharmaceutical preparations

Formulations	Label claim (mg)	% Recovery of PMH ± RSD* Proposed method	B.P. method
Tablets			
A	25	99.32 ± 1.25	99.71 ± 1.07
B	25	99.70 ± 1.00	98.40 ± 0.52
C	25	98.90 ± 1.27	99.00 ± 1.23
D	25	99.06 ± 0.44	99.06 ± 0.52
Injection			
E	25	100.33 ± 0.88	100.05 ± 0.67
Syrup			
F	1.5	100.0 ± 1.31	98.09 ± 1.20
Elixir			
G	5.0	98.05 ± 1.26	99.10 ± 1.05

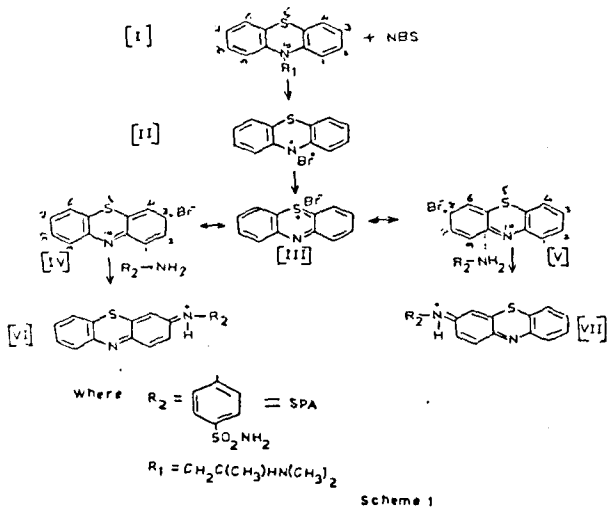
*Average of five determinations. A,C,E,F and G Marketed by Rhone-Poulenc Ltd. [B] Marketed by Swift. [D] Marketed by Sun Pharma.

layers were mixed, dried with anhydrous sodium sulphate and transferred to 10 ml calibrated flasks. The absorbances of the organic layers were measured at 510 nm against a reagent blank prepared under identical condition. The drug concentration was computed using their respective calibration graph.

Twenty tablets were weighed and finely powdered. A weighed amount of the powder (for the injection and elixir, an appropriate volume of the sample was taken) equivalent to 25 mg of PMH was extracted into water and filtered. The filtrate was diluted upto the mark with water in a 100 ml calibrated flask. An appropriate volume of stock solution was transferred into a 25 ml calibrated flask and the assay was done as per the assay procedure described above for the determination of pure PMH.

NBS oxidised the PMH to an unstable light red coloured product. When Sulphanitamide (SPA) added to the oxidised product of PMH, an intense green coloured product in aqueous medium was obtained. Its colour intensity gradually decreased within 10 min. It exhibited λmax at 580 nm. However, when it was extracted into chloroform it gave a stable red colour with SPA (λmax. 510 nm). It was observed that 1.5 ml of 0.02% NBS and 2.0 ml of 0.01% SPA were necessary for achieving of maximum colour intensity. The order of addition of reagents was not critical.

The proposed method obeys the Beer's law in the concentration range of 5.25 μg/ml. The optimum photometric range from Ringbom's plot is found to be between 7.5-24.5 μg/ml. Molar absorptivity, specific absorptivity and Sandell's sensitivity of the reaction as calculated from the Beer's law data is 5.43 $x10^3$ 1/mol/cm, 0.0169 ml/g/cm and 0.0592 μg/cm^2 respectively. The reproducibility of the method was assessed by carrying out ten replicate analyses of a solution containing 125 μg of PMH in a final volume of 10 ml. The relative error and relative standard deviation were found to be ±0.97 and 1.5, respectively. Using the linear least square treatment, the values of slope (a), intercept (b) and correlation coefficient (r) were found be 0.0179, -0.0113 and 0.9979, respectively. The concentration of PMH can be calculated from the regression equation $y = ax + b$, were 'x' is the concentration of PMH μg/ml.



Scheme 1

The reaction between PMH and SPA in presence of NBS was found to be instantaneous. The effect of temperature on the product was studied at different temperatures. The coloured product was stable upto 35°. At higher temperatures the drug's concentration was increased on prolonged heating due to volatile nature of chloroform. As a result, the absorbance values of the coloured products were increased. However, resultant product was stable for more than three days at 25 ± 3°.

Examining the effect of the reagents on C-2 substituted phenothiazine derivatives that include chlorpromazine hydrochloride, methiomeprazine hydrochloride, fluphenazine hydrochloride, mepazine hydrochloride, trifluoperazine hydrochloride and thioproperazine mesylate gave an indication of the specificity of the method. These compounds did not yield any green coloured product (before extraction), red coloured product upon extraction. This may indicate the high specificity for C-2 unsubstiuted phenothiazines.

Based on the Job's method of continuous variation, it was found that PMH reacted with SPA in the ratio 1:1. This lends support to the assumption that a methylene blue-like structure is formed [2,14]. Accordingly,the following sequence of reaction could be suggested. PMH (I) reacts first with NBS to form phenothiazinyl free radical (II)[2,14]. Following this, II is oxidised to the PMH cation, which has the resonating forms III, IV and V. Thus the lowest electron density on the PMH cation was found at positions C-3, C-7 and S-5, which permits the nucleophilic attack at positions 3 or 7 by SPA to form methylene blue-like dyestuff VI or VII (Scheme 1).

The effects of the presence of excipients associated with the drugs on the determination of PMH, in pure and dosage forms were investigated using the developed method. These results indicate that dextrose, talc, starch, stearic acid, sodium alginate and gelatin do not interfere in pharmaceutical formulations in amounts far in excess of their normal occurrence. While other phenothiazines like chlorpromazine hydrochloride, trifluoperazine hydrochloride, thioperazine mesylate, methiomeprazine hydrochloride, mepazine hydrochloride and fluphenazine hydrochloride did not interfere upto the concentration limit of 50 µg/ml, compounds such as amino acids, vitamin-C and iodides interfere in the determination.

The validity of the method for the assay of some pharmaceutical preparations was determined. The Table 1 shows the results obtained on the assay of PMH in dosage forms. These results are compared with the official method[16] for the determination of PMH. The results obtained were found to be in good agreement with proposed method.

The proposed method has the advantages of simplicity, rapidity and selectivity. The assay methods involve less stringent control of experimental parameters, such as time of analysis, the stability of the coloured species, and the concentration of the reagent. The utility of the proposed method for the PMH in dosage forms has been demonstrated. The lower value of relative standard deviation reflects reproducibility of the proposed method for PMH studied from the pharmaceutical preparations. Thus, it can be concluded that this method could be considered for the assay of both bulk and formulations.

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