CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1493520

Available online at: http://www.iajps.com Research Article

QUANTITATIVE ANALYSIS FOR CHLORPROMAZINE HYDROCHLORIDE IN TABLET FORMULATION BY DIFFERENCE SPECTROPHOTOMETRY

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Abstract:

Assay for chlorpromazine hydrochloride in tablets by direct spectrophotometric method is subjected to interference from the excipients and its decomposition product chlorpromazine-5-sulphoxide that may present as a result of oxidation during storage. An indirect spectrophotometric method has been developed and validated to estimate chlorpromazine hydrochloride by oxidation to chlorpromazine sulphoxide by Peroxyacetic acid. The difference in absorbance at 343 nm is linear to the concentration of 15-150 µg/ml of chlorpromazine hydrochloride with correlation coefficient 0.9991 and the precision of the assay is about 0.52 %. The proposed method was successfully applied to the analysis of dosage forms; the mean % recovery of the drug when determined by spiking the same in a pre-analyzed sample was found as 99.94 ± 0.60 to 100.29 ± 0.13. The developed method had high sensitivity with LOD and LOQ were found as 0.683 µg/ml and of 2.052 µg/ml respectively.

Key words: *Chlorpromazine hydrochloride, Difference spectrophotometry, Validation, Peroxyacetic acid.*

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Please cite this article in press Sowjanya Swarna et al., ***Quantitative Analysis for Chlorpromazine Hydrochloride in Tablet Formulation by Difference Spectrophotometry.,*** *Indo Am. J. P. Sci, 2018; 05(11).*

1. INTRODUCTION

Chlorpromazine Hydrochloride [1-3] is the hydrochloride salt form of chlorpromazine, a phenothiazine and traditional antipsychotic agent with anti-emetic activity. Chlorpromazine hydrochloride exerts its antipsychotic effect by blocking postsynaptic dopamine receptors in cortical and limbic areas of the brain, thereby preventing the excess of dopamine in the brain. This leads to a reduction in psychotic symptoms, such as hallucinations and delusions. Chlorpromazine hydrochloride appears to exert its anti-emetic activity by blocking the dopamine receptors in the chemical trigger zone (CTZ) in the brain, thereby relieving nausea and vomiting. Chemically it is 3-(2-chloro-10H-phenothiazin-10-yl)-N,N-dimethyl-propan-1-amine; hydrochloride and its chemical structure is as shown in Figure 1. It is white or almost white crystalline powder. It is very soluble in water, freely soluble in ethanol (96 percent). It decomposes on exposure to air and light. Its melting point is at about 196 °C. The acid dissociation constant [4] of CLP is p*Ka* = 9.30. The partition coefficient of CLP in octanol/water system[4] is Log P = 5.35.

A thorough literature survey has been made to get the analytical methods developed for the drug candidate in pure, dosages and in biological samples. Few spectrophotometric methods such as colorimetric [5-8], extractive spectrophotometric [9], study of analytical properties [10], have been developed for determination of Chlorpromazine in pharmaceutical products and in human blood and urine [11]. Some of the advanced analytical methods including electron spin resonance spectroscopy [12], Nuclear Magnetic Resonance spectroscopy [13-14], LC-MS; Positive- and negative-ion mass spectrometry and rapid clean-up [15], analysis of Phenothiazines and Its Derivatives Using LC/Electrochemistry/MS and LC/Electrochemistry/Fluorescence [16], determination of Chlorpromazine and Its Major Metabolites by Gas Chromatography/Mass Spectrometry (GC-MS) [17], few developed methods were of electro analytical [18-20], Atomic Absorption Spectrometric Determination of Chlorpromazine with other Phenothiazines [21], Gas chromatography with electron capture detector [22], HPLC with Electrochemical detection [23], HPLC Method for Simultaneous quantification of chlorpromazine with other analytes [24-25].

Methods that are based on generation of chromogenic derivative are suffering from use of costly reagents and tedious procedures have been done right from the sample preparation to analysis of the drug in formulations. Most advanced methods are able to determine the drug in nano to sub-nanogram level and yet it is a costly affaire and which may not be suitable for routine quality control analysis. Keeping in view, an indirect spectrophotometric method has been developed which is suitable for regular quantification of the analyte in dosage forms. The present study was aimed at developing a precise, sensitive, rapid and accurate method for the analysis of chlorpromazine hydrochloride in pure drug and in pharmaceutical dosage forms. An indirect spectrophotometric method has been developed to estimate chlorpromazine hydrochloride by oxidation to chlorpromazine sulphoxide in the presence of peroxyacetic acid.

2. MATERIALS AND METHODS

All the materials used were of analytical grade and procured from different manufacturers. chlorpromazine hydrochloride pure sample of chlorpromazine hydrochloride was a gift sample from Sun Pharmaceutical Industries Ltd, Mumbai. Tablet formulations were procured from local pharmacy. A *Systronics* Double beam UV- visible spectrophotometer 2203 with 1 cm matched quartz cells was used for all spectral and absorbance measurements and solutions were prepared in double distilled water.

2.1) Oxidizing agent

About 5 ml of hydrogen peroxide 100 volumes was diluted to 500 ml with glacial acetic acid and the solution was heated at 70 °C for 1 h.

2.2) Standard chlorpromazine hydrochloride solution

Accurately weighed about 60 mg of chlorpromazine hydrochloride into a 100 ml volumetric flask, dissolved in water and diluted to volume with water.

2.3) Method for estimation of chlorpromazine hydrochloride

An aliquot (10 ml) of the standard chlorpromazine hydrochloride solution was transferred into two 100 ml volumetric flasks. to one flask, about 5 ml of peroxyacetic acid oxidizing reagent was added and the contents of both the flasks were diluted to 100 ml with water. The absorption spectrum of each solution in 1 cm cells was recorded using water in the reference cell i.e. the difference spectrum of the oxidized solution using the unoxidized solution in the reference cell. From the difference spectrum the difference in absorbance was measured at the λ_{max} at about 343 nm and the concentration of chlorpromazine hydrochloride was calculated using

the below equation; modified Beer-Lambert's equation.

$$\Delta A = \Delta A^{1\%}_{1cm}\, b\, c$$

where ΔA is the difference in absorbance, c is the concentration of chlorpromazine hydrochloride in the reference cell in g/100 ml and b is the path length (1 cm).

2.4) Analysis of chlorpromazine hydrochloride in tablets

Twenty tablets were randomly selected and crushed them to a fine powder. A quantity of tablet powder equivalent to 60 mg of chlorpromazine hydrochloride was transferred to a 100 ml volumetric flask and diluted to volume with water. The procedure described under "method for estimation of chlorpromazine hydrochloride" was followed. The amount of chlorpromazine hydrochloride in sample was calculated from the difference in absorbance; ΔA at 343 nm.

2.5) Validation of the Developed Method

System suitability was ascertained by six replicate analyses of the drugs at concentrations of 60 μg/mL of chlorpromazine hydrochloride. The percentage of RSD of the standard drug solutions of chlorpromazine hydrochloride in terms of their absorption maximum and absorbance. The effect of wide range of other constituents and other additives usually present in the sample was investigated to know the specificity of the method and it shows no interference from other compounds. For linearity, aliquots of primary working standard solutions containing chlorpromazine hydrochloride were diluted in a way such that the final concentrations of chlorpromazine hydrochloride are in the range of 15-150 μg/mL. A calibration curve was plotted between concentration and ΔA and statistical analysis of the calibration curve was performed. Method of least square analysis was carried out for getting the slope, intercept and correlation coefficient, and regression data values. Precision was determined by intraday and inter-day study.

Precision of the method was evaluated by carrying out the assay and analyzing corresponding responses 6 times on the same day and on different days for the sample solution. Accuracy studies were performed for chlorpromazine hydrochloride at three different levels (25%, 50%, and 100%) and the mixtures were analyzed in triplicate by the proposed method. A known amount of standard chlorpromazine hydrochloride at 25%, 50%, and 100% of sample (which was previously analyzed) was added and it was reanalyzed by the proposed method. And the percentage recovery was evaluated. The effect of these variables on the developed method was determined. Limit of detection and limit of quantification were calculated using the following formula LOD = 3.3 (σ)/S and LOQ = 10 (σ)/S, where (σ) = standard deviation of response (peak area) and S = slope of the calibration curve.

3. RESULTS AND DISCUSSION

Assay for chlorpromazine hydrochloride in tablets by direct spectrophotometric method is subjected to interference from the excipients and its decomposition product chlorpromazine-5-sulphoxide, that may present as a result of oxidation during storage. The present study was aimed at developing a precise, sensitive, rapid and accurate method for the analysis of chlorpromazine hydrochloride in bulk drug and in pharmaceutical dosage forms. An indirect spectrophotometric method has been developed to estimate chlorpromazine hydrochloride by oxidation to chlorpromazine sulphoxide by peroxyacetic acid (Fig. 1).

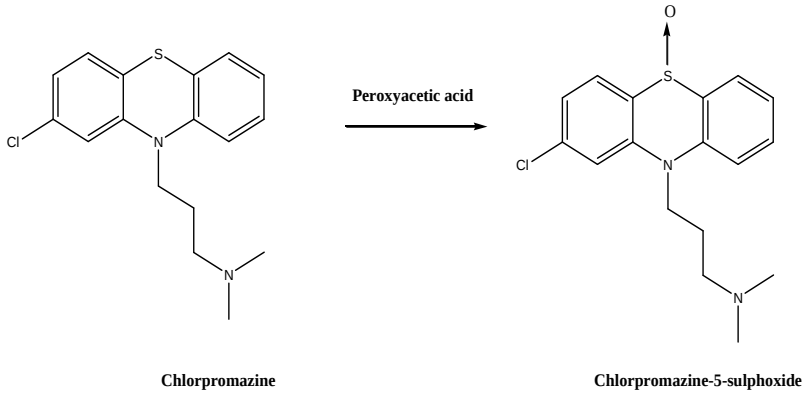

Fig. 1: Oxidation of Chlorpromazine

3.1) Optimum conditions for spectrometric measurements:

In developing the proposed method, the nature of solvent was selected based on the solubility of the absorbing substance and by the absorption of the solvent at the analytical wavelength. water was selected as an ideal solvent for the entire analysis due to less absorbance at the wavelength selected for assay and it provides no chemical interference. The absorption spectrum of chlorpromazine hydrochloride standard was presented in Fig. 2 and the wavelength selected for spectral measurements is at 343 nm. Peroxyacetic acid reagent, which is prepared by adding hydrogen peroxide solution to glacial acetic acid and it provided the extensive decomposition (greater than 10 % of the intact drug) of the chlorpromazine hydrochloride to the corresponding sulphoxide which may be seen in the spectrum of the un oxidized solution (Fig. 3) of a sample of chlorpromazine hydrochloride as a shoulder around 343 nm due to the sulphoxide. The overlain UV absorption spectra was given in Fig. 4.

$$H_2O_2 + CH_3COOH \longrightarrow CH_3COOOH + H_2O$$

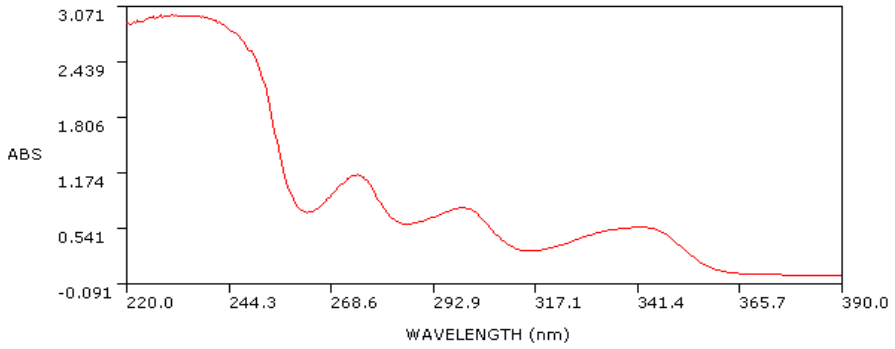

Fig. 2: UV spectra of Chlorpromazine hydrochloride standard
(without oxidant; Absorbance at 343 nm 0.553)

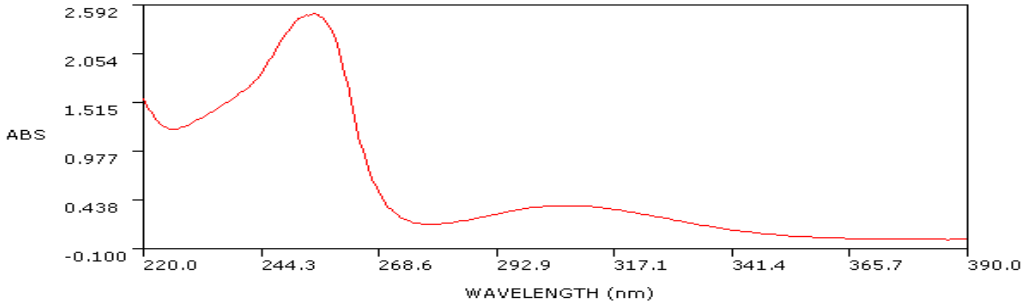

Fig. 3: UV spectrum of chlorpromazine hydrochloride
(treated with Peroxyacetic acid; Absorbance at 343 nm 0.098)

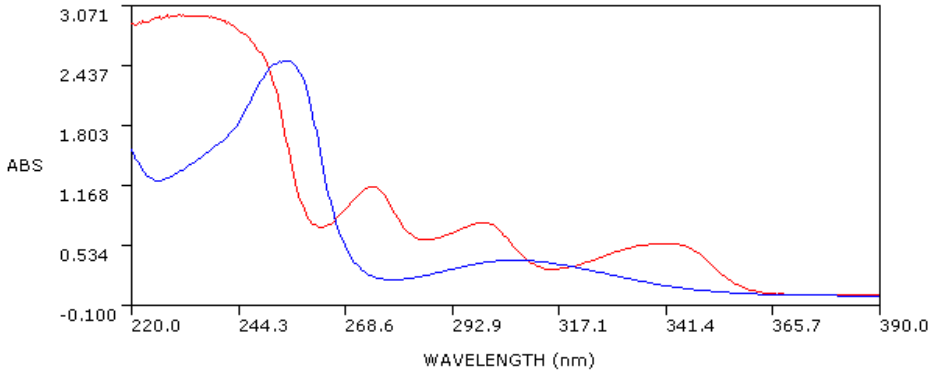

Fig. 4: Overlain UV spectra of chlorpromazine hydrochloride (red color)

and chlorpromazine-5 sulphoxide (blue color)

3.2) Method validation

An integral part of analytical method development is validation. Once the method has been devised, it is necessary to evaluate under the conditions expected for real samples before being used for a specific purpose. The following parameters were evaluated.

a) Specificity:

The effect of wide range of excipients and other additives usually present in the formulations of chlorpromazine hydrochloride in the determinations under optimum conditions were investigated. The common excipients such as lactose anhydrous, microcrystalline cellulose, crosscaramellose sodium and magnesium stearate have been added to the sample solution and analyzed. In fact many have no absorption at this UV maximum.

b) Linearity:

Method of least squares analysis was carried out for obtaining the slope, intercept and correlation coefficient values and the results of optical characteristics were presented in TABLE I. The proposed method was found linear in the concentration range of 15-150 µg/ml which states that the method was linear and the linearity curve was given in Fig. 5. The correlation coefficient was found as 0.9991.

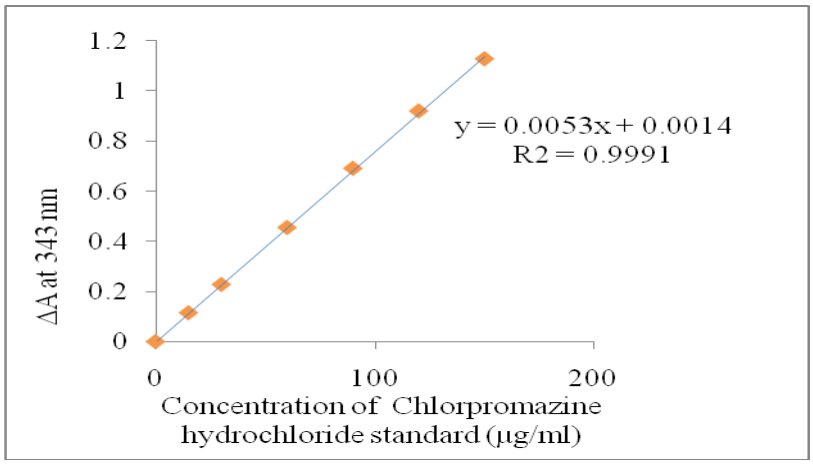

Fig. 5: Calibration curve for Chlorpromazine hydrochloride

Table 1: Optical characteristics, regression data, Precision and accuracy of the proposed method for Chlorpromazine

Parameter	**Method** (Difference spectrometry; Zero order spectra)
ΔA λ_{max} (nm)	343
Beer's law limits (µg / ml)	15-150
Molar absorptivity (L. mole^{-1} cm^{-1})	2.93×10^3
Detection limits (µg/ml)	0.683
Limit of quantitation (µg/ml)	2.052
Sandell's sensitivity (µg /cm ^{2}/0.001 absorbance unit)	0.108
Optimum photometric range (µg/ml)	30-150
Regression equation (Y = a + bc):	y = 0.0053 x + 0.0014
Standard deviation of slope (S_b)	0.0000236
Standard deviation of intercept (S_a)	0.00159
Correlation coefficient (r)	0.9991
% Relative standard deviation* (parts per hundred)	0.52

**Average of six determinations.*

Table 2: Assay and recovery of Chlorpromazine hydrochloride in Tablets

Pharmaceutical Formulation	Labeled Amount (mg)	Proposed Method			% Recovery Found by reference method [24] ± S.D	% Recovery by proposed method** ± S.D
		Amount found* (mg) ± S.D	t (Value)	F (Value)		
Brand -I	50	49.80 ± 0.009	0.672	1.249	100.9	99.66 ± 0.13
Brand –I	100	99.81 ±0.016	0.984	3.129	100.1	99.81 ± 0.24
Brand -II	50	49.86 ± 0.012	1.098	2.456	101.3	99.72 ± 0.60
Brand –II	100	100.51 ± 0.08	1.564	1.439	101.2	100.51 ± 0.29

** Average ± standard deviation of six determinants; the t and F- values refer to comparison of the proposed method. Theoretical values at 95 % confidence limits t = 2.571 and F = 5.05.*

***Average of six determinations*

c) Precision:

The precision of the method was ascertained from the ΔA obtained by determination of six replicates of tablet samples of chlorpromazine hydrochloride. The percent relative standard deviation was found to be 5.2 parts per thousand (ppt) or 0.52 %.

d) Accuracy:

In order to ascertain the accuracy of the method recovery studies were conducted by analyzing each pharmaceutical formulation in the first instance for the active ingredient by the proposed method. A known amount of pure drug was then added to each of the previously analyzed formulation and the total amount of drug was once again determined by the proposed method. the mean % recovery of the drug when determined by spiking the same in a pre-analyzed sample was found between 99.66 ± 0.13 and 100.51 ± 0.29 and suggests that the proposed method has high degree of accuracy and can compete with the existing method. The results were presented in TABLE II.

e) Robustness:

Robustness of the proposed methods was evaluated by making small changes in that deliberately effect the method such as wavelength of spectral measurements ± 2 nm and nature of oxidant were found to be not affected by these small alterations.

4. CONCLUSION

The proposed method is simple, accurate, precise, robust, and specific and has the ability to estimate, Chlorpromazine including its sulphoxide in the tablets. Further short span of time for analysis reveals the time saving. The simplicity of the method allows for application in laboratory for routine quality check also it may be utilized for the determination of content uniformity and dissolution profiling of this product. Overall, the method provides solution for determination of Chlorpromazine in the tablets with excellent selectivity, precision and accuracy in a cost effective manner.

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