Development and Validation of Ratio Spectra Derivative Spectrophotometric Method for Estimation of Chlorpheniramine Maleate and Diphenhydramine Hydrochloride

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ABSTRACT

Diphenhydramine Hydrochloride and Chlorpheniramine Maleate are the antihistaminic drugs used in allergic conditions, common cold, etc. The study was aimed to develop a simple, accurate and precise method for the estimation of Chlorpheniramine Maleate in presence of Diphenhydramine Hydrochloride using it as a divisor and Diphenhydramine Hydrochloride in presence of Chlorpheniramine Maleate using it as a divisor with Methanol as a solvent throughout the experiment. The ICH guidelines have strictly adhered to the validation of this method. Chlorpheniramine Maleate and Diphenhydramine Hydrochloride give linearity in the concentration range from 4.5-9.5 µg/ml. R^2 for both DPH and CPM used as a divisor was found to be 0.999 and 0.993 respectively. LOD and LOQ calculated were 1.440µg/ml and 4.365µg/ml and system precision for both DPH and CPM used as a divisor was found to be 1.152% and 1.240% respectively.

Key Words: *Chlorpheniramine Maleate; Diphenhydramine Hydrochloride; Ratio Spectra Derivative method; UV-Visible Spectroscopy; ICH Guidelines.*

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INTRODUCTION

Chlorpheniramine Maleate (CPM) is an antihistaminic agent. Chemically it is 3(4-chlorophenyl)-N, N-dimethyl-3-pyridin-2-yl-propan-1-amine. It is used in the management of common cold and other allergic conditions. Chlorpheniramine binds to H1 histamine receptors and blocks the histamine action which leads to relief of negative symptoms. The structure of CPM is shown in Figure- 1 [1].

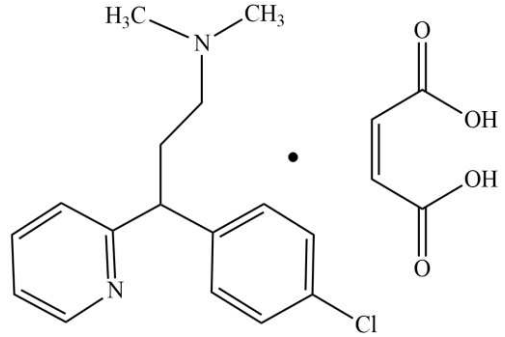

Figure 1: Structure of Chlorpheniramine Maleate

Diphenhydramine Hydrochloride (DPH) is first generation antihistaminic agent. Chemically it is 2-diphenyl methoxy-

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N, N-dimethyl ethylamine hydrochloride. It occurs in a white crystalline powder and is soluble in water, ethanol, methanol, and chloroform. The Structure of DPH is shown in Figure- 2 [2].

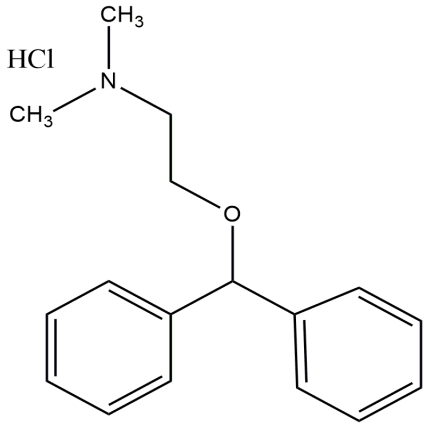

Figure 2: Structure of Diphenhydramine Hydrochloride

Derivative Spectroscopy is an analytical technique for extracting both qualitative and quantitative information from spectra which are composed of unresolved bands. This technique involves the conversion of normal spectra to its first, second and third derivative spectra. The first derivative spectrum is constructed between the rate of change of absorbance and wavelength. [3, 4].

The spectrum of the mixture is divided by the standard spectra of each analyte and further deriving the ratio of the obtained spectrum to the 1st order is the method of procuring a Ratio Spectra Derivative for each analyte that becomes unaffected by the concentration of analyte used as a divisor.

In this work, a new Spectrophotometric method (Ratio Spectra Derivative Spectrophotometry Method) is used for the estimation of CPM and DPH [3, 5]. In this method, the mixture's absorption spectra and the standard spectra are divided and the ratio spectra thus obtained is derivatized to the first order by using a "divisor" measuring either at maximum or minimum wavelengths [6, 7]. The major pro of the presented method is the ease of doing the measurements with respect to its peaks and thus permitting the choice of the wavelength corresponding with the highest value of analytical signal [4, 6, 8, 9].

Several methods have been used for the determination of Chlorpheniramine Maleate in pharmaceutical preparations and biological fluids including HPLC [10], UV Spectroscopy method [11], Spectrophotometric-Chemometric method [12], and Reverse phase-HPLC [13]. Similarly, several methods also reveal for determination of Diphenhydramine Hydrochloride in pharmaceutical preparations and biological samples includes UV Spectroscopic method [14], Conductometric titration [15], Ion pair formation [16], Reverse phase-HPLC.

Validating an analytical procedure means providing that the proposed method has suitable analytical information. As provided by the ICH Guidelines, Validation is official and organized proof of the method that it shall comply with the defined procedures while testing of the product. The various validation parameters to validate method are:

- Accuracy
- Precision
- Linearity and Range
- Specificity
- Robustness
- Limit of Detection (LOD)
- Limit of Quantitation (LOQ)

- **Accuracy:** Closeness of the test result to the true value is the accuracy of the analytical method. It measures the correctness of the analytical method that is developed. Accuracy can be defined as the % recovery as of the assay of a known amount of the added analyte. It is also needed to establish accuracy in the specified range of the stated analytical course of action.

- **Precision:** The degree of agreement among each test result is known as the precision of an analytical method and it carried on several times in numerous homogenous samples. The precision results are denoted in the form of either standard deviation or relative standard deviation which is also known as the coefficient of variation. It can be also stated that it measures the level of reproducibility of the analytical method under the stated operating conditions. It performs the analysis of the different replicates by either one or more different analysts using the same instrument and method over a short period.
  - **Repeatability:** It is the precision of the procedure when performed by the same analyst under similar operating situations over a short period. The repeatability is performed by using a minimum of six replicates and tabulating the results to get the mean, standard deviation, and the relative standard deviation. These calculations play an important role as they depict the degree of variance in standard situations. (The RSD should be below 1% for bulk drugs and below 2% for the finished product)
  - **Reproducibility:** When the precision is carried out under different conditions such as different labs or different batches it is known as reproducibility. The data provides useful information.
  - **Intermediate precision:**

a) **Intra-day precision:** A variation of results within the same day is called intra-day variation. Repeating the calibration curve 3 times on the same day provides the intraday precision data.
b) **Inter-day precision:** Variation of results amongst day is called inter-day variation. Repeating the calibration curve daily for 3 different days provides the inter-day precision data.

- **Linearity and Range:** Plot of each signal that is a function of analyte concentration and amount evaluated by visual inspection in the linearity. There should be an application of a suitable statistical method like the regression coefficient is the results are linear. It might also be needed to go for the mathematical transformation of the data before subjecting it to regression analysis and such data help in providing the degree of linearity.
- **Limit of Detection (LOD):** The minimum amount of analyte in a sample which can be detected but not necessarily quantified as an accurate value is known as Limit of Detection. The limit of detection (LOD) may be expressed as:

$$LOD = 3.3\ \sigma / S$$

Where, σ = the standard deviation of the response.
S = the slope of the calibration curve.

- **Limit of Quantification:** It is expressed as the minimum amount of analyte that can be quantified with accuracy and precision. The Limit of Quantification (LOQ) may be expressed as:

$$LOQ = 10\ \sigma / S$$

Where, σ = the standard deviation of the response.
S = the slope of the calibration curve.

- **Specificity:** It is the measure of an analyte of interest in the presence of the different components that are expected to be present in the sample.
- **Robustness:** The measure of the capability of an analytical method to remain unaltered by small and premeditated variation in method parameters is known as robustness. It gives a sign of its consistency during normal usage [17].

MATERIAL AND METHOD

Instruments and Apparatus:

The spectrophotometer used for the study was Shimadzu UV-1800 spectrophotometer with a slit width of 2 mm was used. The analytical balance was used for weighing, volumetric flasks, Pipettes were used in the study.

Materials and Reagents:

The drug Chlorpheniramine Maleate and Diphenhydramine Hydrochloride was provided as a gift sample from Sigma Aldrich and Hi-media laboratories and Methanol Loba Chemie Pvt Ltd. from which was used as a solvent in the experiment. The physical mixture of Chlorpheniramine and Diphenhydramine was used in further recovery studies.

Preparation of Standard Stock and Working Solutions:

Chlorpheniramine Maleate Stock Solution: 25mg was weighed accurately and transferred in a beaker containing 25 ml methanol to make 1mg/ml concentration of the solution.

Diphenhydramine HCl Stock Solution: 25mg was weighed accurately and transferred in a beaker containing 25 ml methanol to make 1mg/ml concentration of the solution.

Chlorpheniramine Maleate Working Solution: From the above stock solution 10 ml was transferred in a beaker and was diluted up to 100 ml with methanol to make 100µg/ml concentration of the solution.

Diphenhydramine HCl Working Solution: From the above stock solution, 10 ml was transferred into a beaker and diluted up to 100 ml with methanol to make 100 µg/ml concentration of the solution.

Selection of Analytical Wavelength:

Solutions of CPM and DPH were prepared in diluents (Methanol) by appropriate dilution and spectrum was recorded between 200-400 nm. The absorption spectra of the solutions prepared at different concentrations of CPM(4.5-9.5µg/ml) as shown in Figure 3**,** DPH (4.5-9.5µg/ml) as shown in Figure 4 were recorded and divided by the absorption spectra of solutions of DPH+CPM, CPM+DPH as a divisor to get their First Derivative Ratio Spectra.

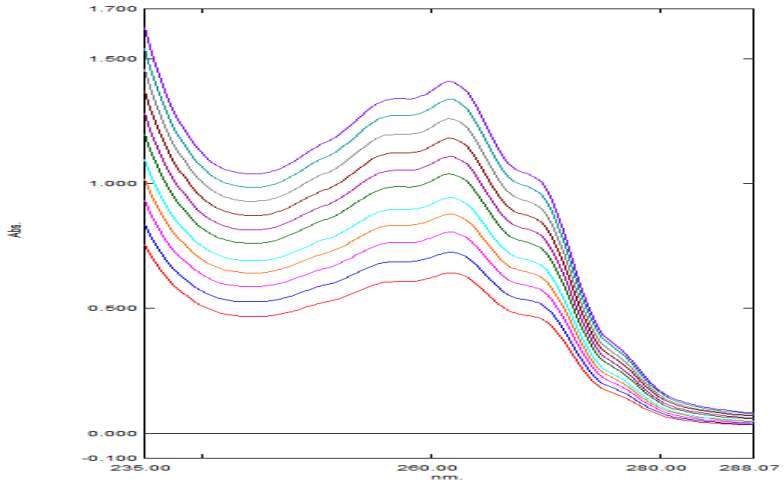

Figure 3: Overlain spectra of Chlorpheniramine Maleate (4.5µg/ml-9.5µg/ml).

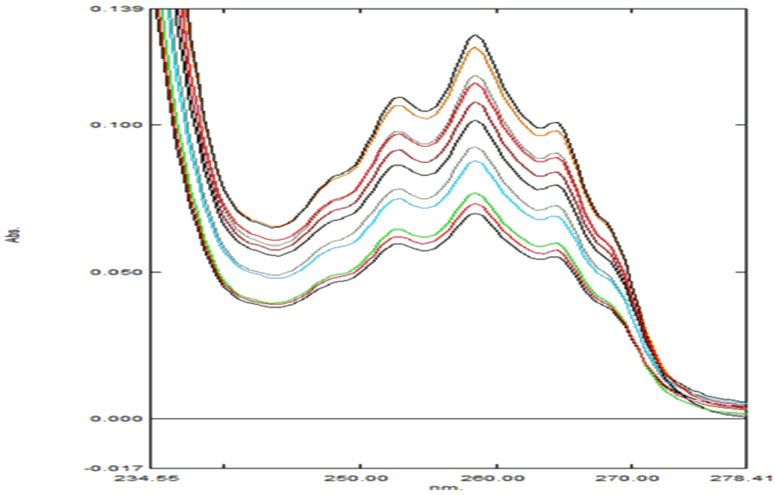

Figure 4: Overlain spectra of Diphenhydramine Hydrochloride (4.5µg/ml-9.5µg/ml).

Determination of Linearity and Range:

For the determination of CPM in presence of DPH, the UV absorption spectra of the solutions were prepared at different concentrations in its binary mixture with DPH were recorded against methanol and then divided by the smoothed spectrum of the standard solution of DPH, the ratio spectra were obtained. The first derivative was calculated for the obtained spectra with Delta Lambda = 10, and Scaling Factor = 4 as shown in Figure 5. The statistical parameters of the calibration graph were calculated. The statistical parameters of the calibration graph were calculated. These gave the best compromise in terms of sensitivity, repeatability, and signal to noise ratio.

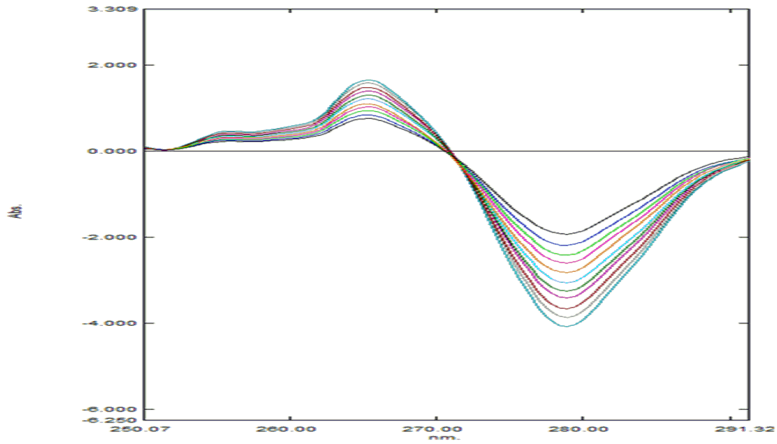

Figure 5: First Derivative ratio spectra of Chlorpheniramine Maleate (4.5µg/ml-9.5µg/ml) using DPH 5.5µg/ml as a divisor.

For the determination of DPH in presence of CPM, the UV absorption spectra of the solutions were prepared at different concentrations in its binary mixture with CPM were recorded against methanol and then divided by the smoothed spectrum of the standard solution of CPM, the ratio spectra were obtained. The first derivative was calculated for the obtained spectra with Delta Lambda = 10, and Scaling Factor = 4 as shown in Figure 6. The statistical parameters of the calibration graph were calculated. These gave the best compromise in terms of sensitivity, repeatability, and signal to noise ratio.

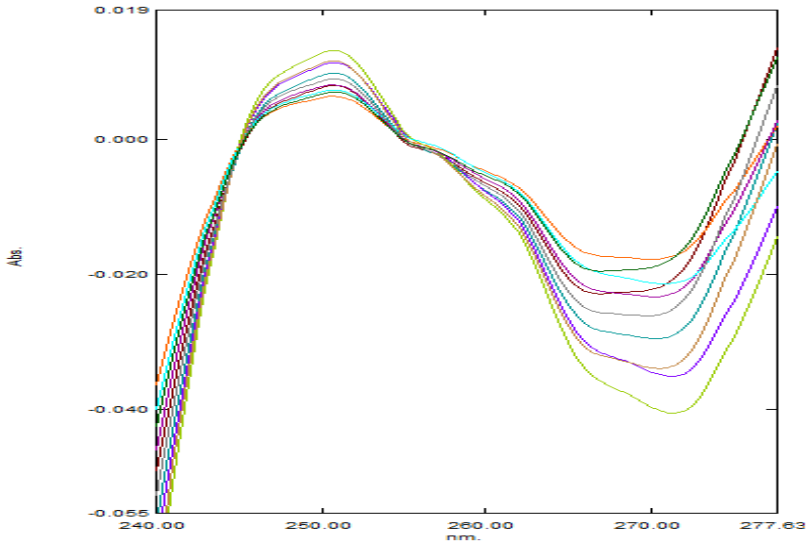

Figure 6: First derivative spectra of Diphenhydramine hydrochloride (4.5-9.5µg/ml) using CPM 5µg/ml as a divisor.

RESULT AND DISCUSSION:

The linearity for both CPM and DPH used as a divisor was observed in the range of 4.5-9.5 µg/ml and the coefficient regression for CPM and DPH divisor was found to be R^2= 0.999 and R^2= 0.993 respectively as shown in Figure 7 & 8. Precision was found to be below 2% as shown in Table No 1 & 2. Robustness was observed below 2% respectively as shown in Table No 3 & 4. LOD and LOQ of CPM in the presence of DPH were found to be 1.4407µg/ml and 4.3657µg/ml respectively. Recovery studies were assessed at different concentration i.e. 80%, 100% and 120% as shown in Table No 5.

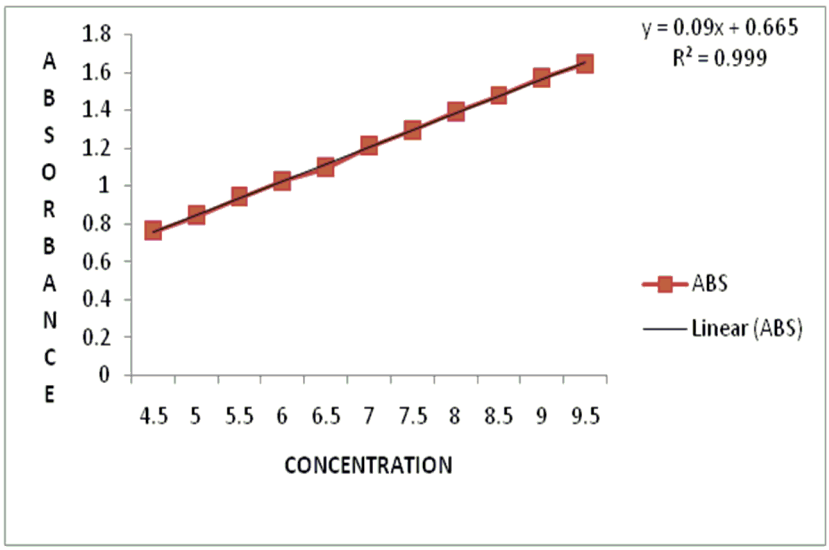

Figure 7: Linear graph of Chlorpheniramine Maleate (4.5-9.5µg/ml) at 265nm.

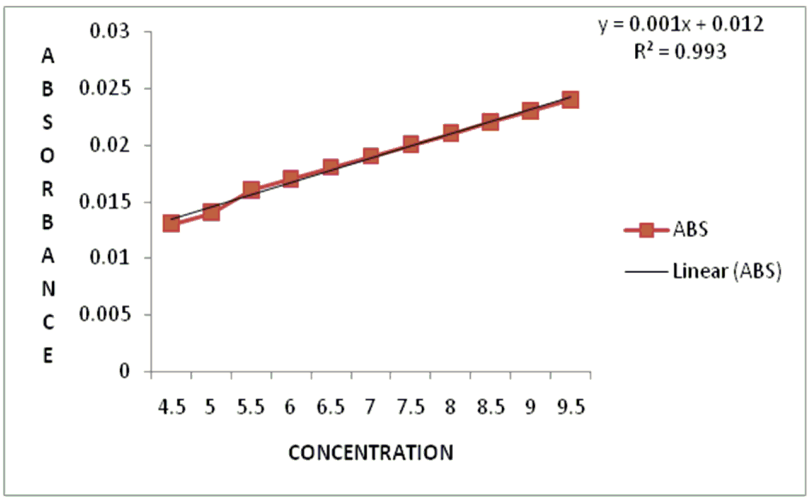

Figure 8: Linear graph of Diphenhydramine hydrochloride (4.5-9.5µg/ml) at 251 nm.

Table No -1: Results of a Precision study using DPH as a divisor.

Inter-day Precision	Up to 3 days (**% RSD < 2%**)
Intra-day Precision	Up to 5 hours (**% RSD < 2%**)
System Precision	1.152 %

Table No -2: Results of a Precision study using CPM as a divisor.

Inter-day Precision	Up to 3 days (**% RSD < 2%**)
Intra-day Precision	Up to 5 hours (**% RSD < 2%**)
System Precision	1.240 %

Table No-3: Result for Robustness using DPH as a divisor.

Different Temperature	
Room Temperature = 1.021%	Cold Temperature = 1.956%
Different Analysts	
Analysts 1 = 1.030%	Analysts 2 = 1.123%
Different Pipette	
1 ml = 1.652%	2 ml = 1.502%

Table No -4: Results for Robustness using CPM as a divisor.

Different Temperature	
Room Temperature = 1.031%	Cold Temperature = 1.125%
Different Analysts	
Analysts 1 = 1.141%	Analysts 2 = 1.083%
Different Pipette	
1 ml = 1.153%	2 ml = 1.915%

Table No -5: Results for Recovery study

Level of recovery	**Amount of sample (µg/ml)**	**Amount obtained (µg/ml)**	**% Recovery**
80%	4.8	4.981	103.7%
100%	6	5.965	99.41%
120%	7.2	7.113	98.79%

CONCLUSION:

The proposed ratio derivative Spectrophotometric method was developed and quantitatively evaluated in following terms viz. linearity, accuracy, precision, robustness, limit of detection, limit of Quantification and recovery studies and was found to be simple, accurate, precise and reliable for the estimation of Chlorpheniramine and Diphenhydramine using methanol as a solvent. The major pro of the presented method is the ease of doing the measurements for its peaks which permits the use of the wavelength of maximum value. The % RSD for all parameters was found to be less than 2 %.

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