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DEVELOPMENT AND VALIDATION OF UV-SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF CYPROHEPTADINE HYDROCHLORIDE IN BULK AND TABLET FORMULATION BY CO-SOLVENCY APPROACH

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ABSTRACT

Simple precise accurate UV Spectroscopic method has been developed and validated for estimation of Cyproheptadine HCl in bulk and pharmaceutical dosage form. It is approved for the treatment of allergic rhinitis. It is 5-HT_2 receptor antagonist. UV Spectroscopic method which is based on measurement of absorption of UV light, the spectra of Cyproheptadine HCl in Co-solvent, Methanol:Water (1:4) showed maximum wavelength at 286 nm and calibration curve was plotted over the concentrations ranging from 2-20 µg/ml of Cyproheptadine HCl with correlation coefficient 0.9973, validation was performed as per ICH Q2 (R1) guidelines for linearity, accuracy, precision and recovery. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.2179 and 0.6605 respectively by simple UV Spectroscopy. The proposed method was validated.

KEYWORDS: Cyproheptadine HCl, Co-solvent, spectrophotometry and validation.

INTRODUCTION

Cyproheptadine HCl is chemically 4-(5H-dibenzo[a,d]-(cyclohepten-5-ylidene)-1-methyl piperidine hydrochloride sesquihydrate. A white or slightly yellow, cyrstalline powder Slightly soluble in water, sparingly soluble in alcohol, freely soluble in methyl alcohol. It acts

as antihistaminic agent. Structure of Cyproheptadine HCl is shown in figure – I. Here calibration curve method was employed by using Co-solvent for the estimation of Cyproheptadine HCl in bulk and tablet dosage forms. "Co-solvent is aqueous solution in which mixture of two solvents is used to increase the aqueous solubility of another solute (poorly water soluble drug)". In the present investigation, Co-solvent act as solublizing agent, was employed to solubilize Cyproheptadine HCl fine powder and its tablet dosage form to carryout spectrophotometric analysis.

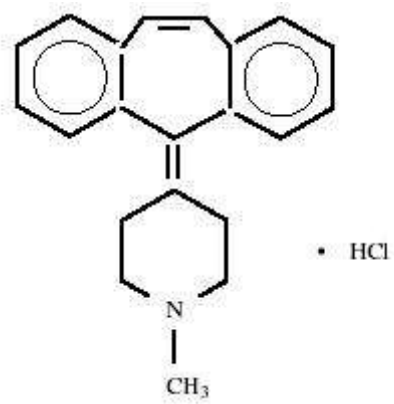

Fig 1: Structure of Cyproheptadine HCl

MATERIALS AND METHODS

Materials: Cyproheptadine HCl working standard drug was obtained from Vamsi Lab Limited, Chincholi, Solapur. Methanol and Distilled water in ratio was used to prepare Co-solvent. Freshly prepared solutions were always employed.

Equipment: The UV-spectrophotometry **(**Jasco V630**)** with data processing system (UV Probe Software 2.31) was used. All analytical grade chemicals and solvents were supplied by Shubham Pharmaceuticals, Mumbai, was recorded in 1 cm quartz cell against solvent blank over the range 200-400 nm. The citizen electronic balance (Schimadzu 220h) was used for weighing the sample. An ultrasonicator bath (PCI Analytics Pvt. Ltd) was used for sonicating the drug sample.

Preparation of standard stock solution

Standard drug solution of Cyproheptadine HCl was prepared by dissolving 5 mg of pure drug Cyproheptadine HCl in 10ml methanol then volume was make up with distilled water upto 50 ml to obtain 100 µg/ml of stock solution from which desired concentrations of solutions were prepared.

Preparation of test solution

10 Tablets were weighed and its average weight was determined. An accurately weighed tablet power equivalent to 10 mg of Cyproheptadine HCl transferred into 100 ml volumetric flask dissolved in 100 ml of Co-solvent in ratio (1:4) and sonicated for 10 min and volume was made upto the mark and solution was filtered using whattman filter paper to obtain 100µg/ml stock solution.

Determination of λmax

10 µg/ml solution of Cyproheptadine HCl was prepared and scanned in UV range of 200-400 nm and spectrum was obtained. The λmax was found to be at 286 nm wavelength where absorbance was maximum at this wavelength. Hence this is considered as absorbance maxima (λmax) shown in figure 2.

Preparation of calibration curve

Standard stock solution was suitably diluted with Co-solvent to obtain concentrations ranging from 2-20 µg/ml. Absorbance of these solutions was measured at 286 nm (λmax Cyproheptadine HCl) using UV, calibration curve was obtained by plotting graph between concentration and absorbance shown in figure 3.

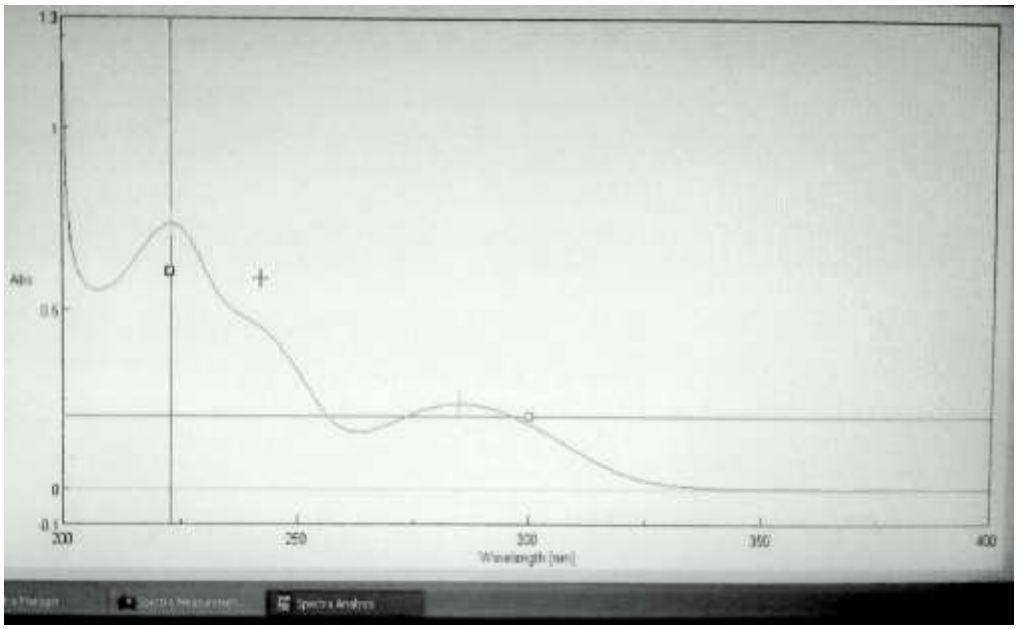

Fig. 2: Determination of λmax

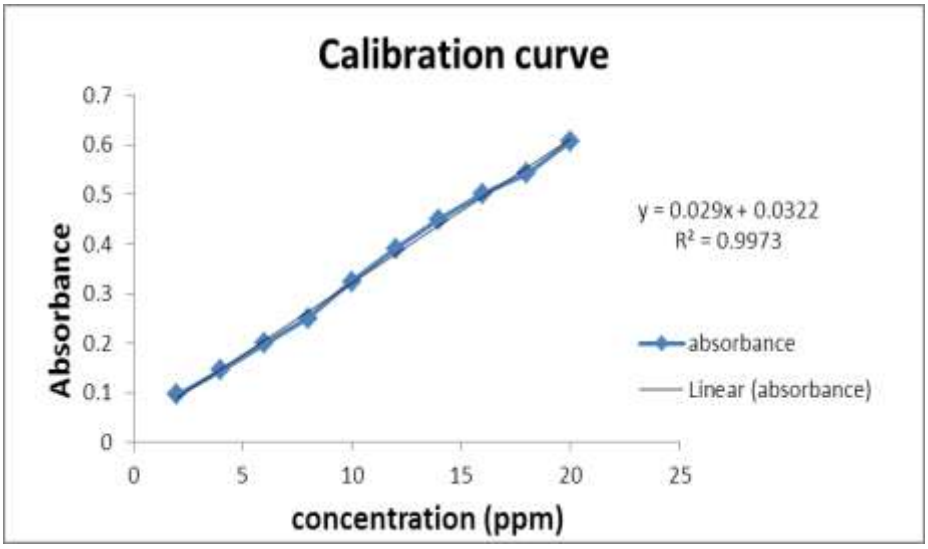

Fig3: Calibration Curve of Cyproheptadine HCl in Co-solvent Methanol : Water

Method Validation

Validation of the Proposed Method

The proposed method was validated according to the (ICH) guidelines.

Linearity

The linearity of the proposed UV methods were evaluated by analysing different concentration of standard solution of Cyproheptadine HCl and by plotting Area under curve of analyte against concentration of analyte. Beer's law was obeyed for the method in the concentration range 2-20µg/ml. Graph was plotted for concentration and absorbance. A good linear relationship (R^2=0.9973) was observed between the concentrations of Cyproheptadine HCl and corresponding Area under curve. The regression analysis was made for slope, intercept and correlation coefficient values. The equation of calibration curve obtained was Y = 0.029x + 0.0322.

Precision

Precision is the measure of closeness of values between each concentration under same analytical conditions. It is determined by performing Interday and intraday precision studies. In intraday studies three standard replicates injection of three different concentration were injected on same day and same standard different concentration were injected on three successive days in inter day precision studies. Where, the % RSD was found to be within limit (≤ 2) . Given in table 1.

Table 1: Precision Study.

Concentration%	Absorbance Mean	Standard Deviation	% Relative Standard Deviation
Interday Precision(n=3)			
80	0.3238	0.0009082	0.2805
100	0.3264	0.0019157	0.5869
120	0.3246	0.0021035	0.6480
Intraday Precision(n=3)			
80	0.3245	0.0016522	0.5090
100	0.3247	0.0020049	0.6174
120	0.3280	0.0031320	0.9549

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The accuracy of the method was determined by performing recovery studies at three different levels of standard additions. Accuracy was checked by adding 80, 100 and 120 % amount of Cyproheptadine HCl to pre-analyzed sample. Result are shown in Table 2

Table 2: Accuracy Study.

Recovery	Concentration of Sample	Recovery in (µg/ml)	% Recovery
80 %	8	7.50	99.19
100 %	10	10.19	99.07
120 %	12	12.39	99.87

LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) of the drug were separately determined based on method of the intercept and the average value of slope. (i.e. 3.3 for LOD and 10 for LOQ) ratio using the following equations designated by ICH guideline.

$LOD = 3.3\ \sigma/S$ $LOQ = 10\ \sigma /S$.

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

Table no 3: LOD and LOQ

LOD (µg/ml)	LOQ (µg/ml)
0.2179	0.6605

Table 4: Summary of Validation Parameters

Sr. No.	Parameter	Data
1	λ-max	286 nm
2	Linearity range	2-20 µg/mL
3	Correlation coefficient	0.9973
4	Slope	0.029
5	Intercept	0.0322
6	Interday precision (n=3)	0.2805 - 0.6480
7	Intraday precision (n=3)	0.5090 – 0.9549
8	Accuracy (%)	99.19 - 99.87
9	Limit of detection	0.2179µg/mL
10	Limit of quantitation	0.6605µg/mL

RESULTS AND DISCUSSION

Beer's law is obeyed over the concentration range of 2-20 µg/ml, using regression analysis the linear equation Y = 0.029x + 0.0322 with a correlation coefficient of 0.9973. The limit of detection was found to be 0.2179µg/mL. The limit of quantification was found to be 0.6605µg/ml. Precision was calculated with intra and interday variation. Recovery study was performed on formulations and % RSD was found. The optical parameters such as Beer's law limit, slope, and intercept values were calculated and given in table 3. Method was validated for accuracy and precision. The accuracy of method was proved by performing recovery studies in prepared formulation. The results were given in table 2 and shows relative standard deviation was observed for analysis of three replicate samples, indicating precision and reproducibility.

CONCLUSION

The organic solvents such as ethanol, methanol, acetonitrile used widely in spectrophotometric analysis of poorly water soluble drugs are toxic in nature, costlier and responsible for pollution yet used in large amounts. Inaccuracy in spectrophotometric analysis due to volatility of organic solvents is another drawback. These problems are maximum minimized by development of UV method with Co-solvent i.e. by reducing the amount of organic solvent. It has UV cut off value 286 nm, since it does not interfere above 286 nm. The results concluded that the developed spectrophotometric method for determination of Cyproheptadine HCl in bulk and formulations using Co-solvent is reliable, accurate, precise, sensitive and eco-friendly. This method can be successfully employed in the routine analysis of Cyproheptadine HCl in bulk drug and dosage formulations.

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