An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

www.jgtps.com

DEVELOPMENT AND VALIDATION OF SPECTROSCOPIC METHOD FOR THE ESTIMATION OF CYPROHEPTADINE HCl IN TABLET DOSAGE FORM

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ARTICLE INFO

Article history:

Received: 22 Feb 2016
Revised: 24 Feb 2016
Accepted: 06 Mar 2016

Keywords:

ICH Guidelines,
Cyproheptadine HCl,
Ethanol,
Dibenzocycloheptenes,
H1 Blocker

ABSTRACT

A Simple, rapid, precise and accurate UV spectrophotometric method was developed and validated for the estimation of Cyproheptadine HCl in tablet dosage form.A seritonine antagonist and H1 blocker used as anti-pruritic,apetite stimulant, anti-allergic and for post gastrectomy dumping syndrome. This compound belongs to the dibenzocycloheptenes. Ethanol was chosen as common solvent for the estimation Cyproheptadine HCl. Different aliquots of Cyproheptadine HCl in ethanol were prepared in the concentration range of 10–60 µg /ml.The absorbance valves of solutions were measured at 286 nm and the calibration curve was plotted using concentration against absorbance.Results of the analysis werevalidated statistically as per the ICH guidelines.Linearity studies were carried out and the range was found to be 10–60 □g/ml for Cyproheptadine HCl in ethanol. The regression coefficient value of Cyproheptadine HCl was found to be 0.99978 which was not less than 0.995. The accuracy of the method was performed by recovery studies. The percentage recovery was found to be in the range of 99.71–100.09% for Cyproheptadine HCl in ethanol.The precision was performed by analyzing standard and sample solutions of Cyproheptadine HCl (40 µg/ml) at working concentration level for 6 times. The % RSD value of system precision and method precision were found to be 0.376601and 0.40156 respectively. The intra-day and inter-day precision studies were carried out.Hence, the proposed method was found to be simple, precise, accurate and rapid for estimation of Cyproheptadine HCl in tablets.

INTRODUCTION

Cyproheptadine HCL is a seritonine antagonist and H1 blocker used as anti-pruritic, apetite stimulant, anti-allergic and or post gastrectomy dumping syndrome (Fig. No.1). This compound belongs to the dibenzocycloheptenes. These are compounds containing a dibenzocycloheptene moiety, which consists of two benzene connected by a cycloheptene ring.Cyproheptadine competes with free histamine for binding at HA-receptor sites. This antagonizes the effects of histamine on HA-receptors, leading to a reduction of the negative symptoms brought on by histamine HA-receptor binding. Cyproheptadine also competes with serotonin at receptor sites in smooth muscle in the intestines and other locations. Antagonism of serotonin on the appetite center of the hypothalamus may account for Cyproheptadine's ability to stimulate appetite.Literature survey revealed that there are few analytical methods have been reported for the determination of Cyproheptadine HCl in pure drug, pharmaceutical dosage forms and biological samples using Titrimetry, Visible Spectrophotometry, High Performance Liquid Chromatography and Mass Spectroscopy.But UV Visible spectroscopic methods are not available for the determination of Cyproheptadine HCl and in bulk as well as in their formulations. Seda G Sagdinc et al., (2014)1 reported "FT-IR and FT-Raman spectra, molecular structure and first-order molecular hyperpolarizabilities of a potential antihistaminic drug, Cyproheptadine HCl". The study reveals that the antihistaminic pharmacological property of CYP HCl has a large β value and, hence, may in general have potential applications in the development of non-linear optical materials. The experimental and calculated results for CYP HCl have also been compared with those for Mianserin HCl.Belal F, et al., (2012)2 developed "Micelle-enhanced spectrofluorimetric method for determination of Cyproheptadine hydrochloride in tablets: application to in-vitro drug release and content uniformity test." A highly sensitive and simple

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spectrofluorimetric method was developed for the determination of Cyproheptadine hydrochloride in its pharmaceutical formulations. Madihalli Srinivas, et al., (2012)3 performed "Sensitive and selective methods for the determination of Cyproheptadine in tablets using n-bromosuccinimide and two dyes". One titrimetric and two spectrophotometric methods are described for the determination of cyproheptadine hydrochloride (CPH) in bulk drug and tablets.A rapid and sensitive liquid chromatographic-tandem mass spectrometric (LC-MS/MS) method was developed and validated for the qualitative and quantitative assay of Cyproheptadine (CP) in pharmaceutical samples by Feas X et al., (2009)4.

Hence an attempt was made to develop and validate simple, rapid and reliable analytical method for estimation of Cyproheptadine HCl.5-14

MATERIALS AND METHOD

Drug Samples

Cyproheptadine HCl was obtained as a gift sample from Ra ChemPharma Pvt. Ltd. Hyderabad.

Figure 1. Structure of cypraheptadine HCl

Reference standards

Cyproheptadine HCl - RA Chem Pharma Pvt. Ltd, Hyderabad, Percentage purity - 99.87

Instruments

The different instruments used to carry out the present work are as follows: Electronic Weighing balance: Single pan balance, Model Axis LC/GC. Sonicator: Ultra Sonicator, Model- Bandelin sonorex. Double Beam UV-Visible spectrophotometer, Shimadzu Model UV-1800 with matched pair of 10mm quartz cells. Data acquisition was performed by UV Probe software.

Experimental Method

Simple, rapid, precise and accurate UV spectrophotometric method was developed and validated for the estimation of Cyproheptadine HCl in tablet dosage form. The following steps were conducted to establish the UV method conditions for drug substances.

The solubility studies are performed to dissolve the drug in polar and non polar solvents. λ max determination. Optimization of concentration of drug.

Method Development

Preparation of standard stock solution

Weigh accurately about 100.0 mg of Cyproheptadine HCl. working standard in a 100 ml volumetric flask. Add 50 ml of ethanol and mix well, then make up to the final volume. Further dilution was made by pipetting 4 ml of mother liquor into 100 ml volumetric flask and make up to the volume with solvent. The optimized conc. of standard was 40 µg/ml. The solution was scanned in UV region in the wavelength range from 200 to 400 nm and λ max was optimized at 286 nm.(Fig. No. 2).

Table 1. Calibration data for Cyproheptadine HCl

Concentration (µg/ml)	Absorbance
10	0.148
20	0.275
30	0.415
40	0.538
50	0.665
60	0.791

Preparation of sample solution

Weigh accurately about 67.18 mg of tablet content in to 50 ml volumetric standard flask and add 40 ml of ethanol and mix well, then make up to the final volume. Further dilution was made by pipetting 4 ml of mother liquor into 100 ml volumetric flask and make up to the volume with solvent. The optimized conc. of Cyproheptadine HCl standard was 40 □g/ml. The solution was scanned in UV region in the wavelength range from 200 to 400 nm and λ max was optimized at 286 nm. (Fig. No. 3)

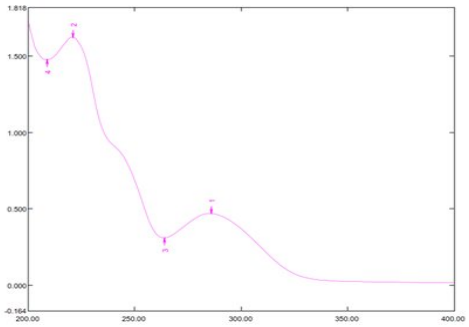

Figure 2. Standard spectra

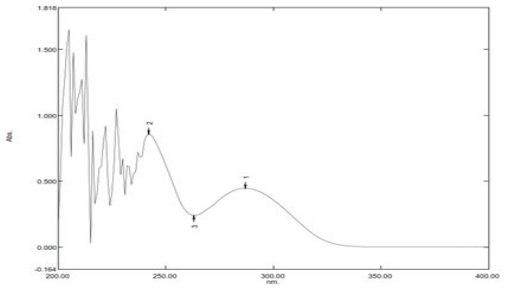

Figure 3. Standard spectra

Method Validation

The proposed method was validated as per ICH guidelines for specificity, accuracy, precision, intermediate precision, linearity and range.

RESULTS AND DISCUSSION

Development of the spectrophotometric method

Proper wavelength selection of the methods depends upon the nature of the sample and its solubility. To develop a rugged and suitable spectrophotometric method for the quantitative determination of Cyproheptadine HCl, the analytical condition were selected after testing the different parameters such as diluents, diluents concentration, diluents pH and other conditions.

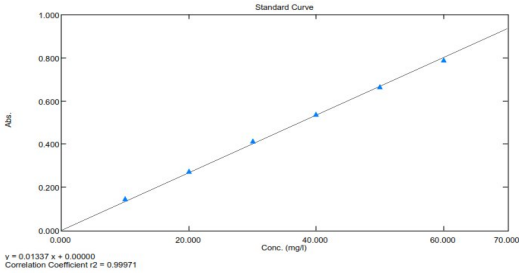

Figure 4. Calibration curve for cypraheptadine HCl

Selection of wavelength

By scanning the standard solution of Cyproheptadine HCl in UV spectrophotometer between 200 nm to 400 nm on spectrum mode, using ethanol as a blank, thewavelength of analysis (λmax), 286 nm was selected. Sample and standard solution absorbance was measured at 286 nm.

Validation of developed method

Specificity

Both placebo and analyte was scanned in UV range, resulting spectra shows the there is no interference between sample and placebo it proves the method specificity.

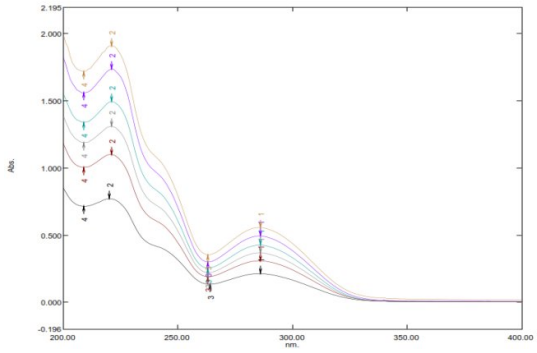

Figure 5. Overlay cypraheptadine standard spectrum

Linearity & Range

The calibration curve constructed was evaluated by using correlation coefficient. The absorbances of Cyproheptadine HCl were linear over the range of 10-60mcg/ml (Fig No. 4, 5 & 6). The average absorbance of each concentration obtained was plotted against the concentration of the analyte. The correlation coefficient for the data was calculated as 0.99978. The regression line were observed to be in the form of y = 0.013 x + 0.011. The results are summarized in Table No.1.The experiments indicated that there was a linear relationship between the amount of analyte and the absorbances within the range studied.

Table 2. Evaluation data of Precision Study

S. No	Cyproheptadine HCl Absorbance at 286 nm	
	Standard	Sample
1	0.476	0.465
2	0.473	0.461
3	0.474	0.464
4	0.475	0.462
5	0.478	0.464
6	0.474	0.466
Mean	0.475	0.463667
SD	0.001789	0.001862
% RSD	0.376601	0.40156

Precision

The precision of the method was calculated from the reproducibility of percentage assay of six Cyproheptadine HClsamples. The results are summarized in Table No 2. The results showed that the precision of the method is good.

Intermediate Precision

Further the precision of the method was confirmed by intra-day and inter-day analysis. The analysis of formulation was carried out for three times in the same day and one time in the three consecutive days. The % RSD values of intraday analysis were shown in Table No 3, 4. The results were well within acceptable limits of % RSD less than 2.0% for all parameters viz., intraday, inter day and analyst to analyst variation. These results indicated that the developed method is rugged.

Accuracy

Accuracy of the method was expressed in terms of recovery of added compound at 80%, 100% and 120% level of sample. Mean % recovery and % RSD were calculated and were summarized in Table No 5. The result shown that best recoveries (99.71 – 100.09%) of the drug were obtained at each added concentration, indicating that the method was accurate.

Robustness

The evaluation of robustness should show the reliability of an analysis with respect to deliberate variations in method parameters.If measurements are susceptible to variation in analytical conditions, the analytical condition should be suitably controlled or a precautionary statement should be included in the procedure. The result of robustness study of the developed assay method was established in Table No 6.The result shown that during all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory; hence the analytical method would be concluded as robust.

Table 3. Intraday & Interday Precision Data

Parameter	Intraday Precision		Intetday Precision			
			Standard		Sample	
	Standard	Sample	Day-1	Day-2	Day-1	Day-2
Absorbance at λ max	0.477	0.462	0.470	0.471	0.461	0.462
	0.474	0.466	0.473	0.474	0.459	0.460
	0.476	0.464	0.471	0.475	0.464	0.464
Mean	0.475	0.464	0.471333	0.473333	0.461333	0.462
SD	0.0015	0.002	0.001528	0.002082	0.002517	0.002
%RSD	0.32113	0.4310	0.324086	0.439789	0.545508	0.4329

Table 4. Ruggedness Data for Analyst to Analyst

Parameter	Cyproheptadine HCl Standard			Cyproheptadine HCl Sample		
	Analyst 1	Analyst 2	Analyst 3	Analyst 1	Analyst 2	Analyst 3
Analyst to Analyst	0.472	0.471	0.470	0.462	0.461	0.465
	0.474	0.474	0.473	0.461	0.463	0.466
	0.475	0.475	0.471	0.459	0.465	0.463
Mean	0.473	0.4733	0.4713	0.460667	0.463	0.46466
SD	0.0015	0.0020	0.0015	0.001528	0.002	0.00152
%RSD	0.3225	0.4397	0.3240	0.33159	0.431965	0.328736

Table 5. Evaluation Data of Accuracy Study

% Recovery Level	% Recovery	Mean % Recovery	SD	% RSD
80%	0.376	99.71	0.001	0.2666
	0.375			
	0.374			
100%	0.471	100.03	0.001528	0.32341
	0.472			
	0.474			
120%	0.543	100.09	0.00208	0.38289
	0.546			
	0.542			

System suitability

A system suitability test of the spectrophotometric system was performed before each validation run. Six replicate reading of standard preparation were taken and % RSD of standard reading were taken for same. Acceptance criteria for system suitability, % RSD of standard reading not more than 2.0%, were full fill during all validation parameter.

Table 6. Robustness Data for Wavelength Variation

Wavelength (nm)	Cyproheptadine HCl in Ethanol	
	Standard	Sample
284	0.4739	0.4643
286	0.4742	0.4645
288	0.4741	0.4644
Mean	0.474067	0.4644
SD	0.000153	0.0001
%RSD	0.032222	0.021533

The optical parameters like molar absorptivity, correlation coefficient, slope, intercept, LOD, LOQ and standard error were calculated and results were shown in Table No 7.

CONCLUSION

The present analytical method was validated as per ICH Q2(R1) guideline and it meets to specific acceptance criteria. It is concluded that the analytical method was specific, precise, linear, accurate, robust and it proves all validation characteristics, hence the present developed analytical method can be used for its intended purpose.

ACKNOWLEDGEMENT

The authors thank Sri. C. Gangi Reddy, Founder, Annamacharya Educational Trust for providing all the facilities for graduate studies and dissertation work. They also thank to RA Chem Pharma, Pvt. Ltd, Hyderabad, India for providing standard Cyproheptadine HCl. They also thank the faculty of Annamacharya College of Pharmacy for their valuable suggestions and constant encouragement during research work.

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Table 7. Validation Dataof Cyproheptadine HCl

Parameters	Cyproheptadine HCl in ethanol
Beers law limit (µg/ml)	05-80
Molar absorptivity (L mol^{-1} $cm^{-1)}$	0.011875
Correlation coefficient (r^2)	0.99978
Regression equation (y = mx+c)	y = 0.013x + 0.011 R^2 = 0.99978
Slope (m)	0.013
Intercept (c)	0.011
LOD (µg/ml)	0.4541
LOQ (µg/ml)	1.3761
Standard Error	0.00073

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