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Research Article

DEVELOPMENT AND VALIDATION OF A UV-VIS SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION AND DEGRADATION MONITORING OF CEFADROXIL IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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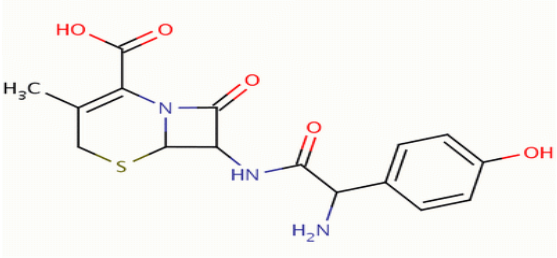
ABSTRACT

The aim of the present work is to develop a simple accurate, precise and cost effective UV-Vis spectrophotometric method for the estimation of cefadroxil, a first generation cephalosporin an anti-biotic drug, in bulk and pharmaceutical dosage form. The solvent used was methanol and distilled water (50:50) and the λmax or the absorption maxima of the drug was found to be 264nm. A linear response was observed in the range of 10-50µg/ml with a regression coefficient of 0.9999. The method was then validated for different parameters as per the I.C.H. (International Conference for Harmonization) guidelines. This method can be used for the determination of cefadroxil in quality control of formulation without interference of the excipients. Cefadroxil was subjected to stress degradation under different conditions recommended by ICH. The samples generated were used for degradation studies using the developed method.

Keywords: Cefadroxil, antibiotic, λmax, ICH, UV-Vis spectroscopy

INTRODUCTION

Cefadroxil chemically a 7-[[2-amino -2-(4-hydroxyphenyl) acetyl] amino]-3-methyl- 8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid[1]



Cefadroxil, a first-generation cephalosporin antibiotic, is used to treat urinary tract infections, skin and skin structure infections, pharyngitis and tonsillitis. Like all beta-lactam antibiotics, cefadroxil binds to specific penicillin-binding proteins (PBPs) located inside the bacterial cell wall, causing the inhibition of the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that cefadroxil interferes with an autolysin inhibitor. Literature survey revealed that cefadroxil was qualitatively assayed in biological fluids either individually or in presence of other antibacterial drugs using liquid chromatography [5], other new methods and using hydrotope are also there for the determination of cefadroxil [2,6,7]. However no UV spectrophotometry method was proposed for the estimation of cefadroxil without using hydrotope[2] in bulk and pharmaceutical dosage forms. The aim of this work is to develop and validate an analytical method by using UV spectrophotometry for the estimation of cefadroxil in bulk and pharmaceutical dosage forms and also perform degradation studies on the drug as per ICH guidelines using the proposed method [3,4]

MATERIALS AND METHODS

The drug, Cefadroxil is a gift of Cipla, Goa branch. The instrument used for the present study was a UV-Vis double beam spectrophotometer (model T60, Analytical Technologicals Ltd.) with 1cm matched pair quartz cell. The solvent used was methanol and water (50:50) which was of AR grade, purchased from SD Fine Chemicals Limited, India and double distilled water.

UV method development

Solubility test

Solubility test for the drug cefadroxil was performed by using various solvents. The solvents include Water, Methanol, Ethanol, Acetonitrile, Hydrochloric Acid (HCl), Sodium Hydroxide (NaOH) and Chloroform.

Determination of λ max

Preparation of stock solution

Standard stock solution of cefadroxil was prepared by dissolving 10mg of cefadroxil in 10ml of methanol and distilled water (50:50) which gives 1000 µg/ml. One ml of this stock solution was taken and was diluted up to 10ml by using methanol and distilled water (50:50) to produce a concentration of 100 µg/ml solution.

Preparation of working solution

From the above stock solution 2ml was transferred into 10ml volumetric flask and volume was made up to the mark with methanol to make 20µg/ml. Then the sample was scanned with UV-Vis Spectrophotometer in the range 200-400nm against methanol and distilled water (50:50) as blank and the wavelength corresponding to maximum absorbance was noted which is its λmax i.e. at 264nm (fig. 1).

Preparation of calibration curve

One ml of this 100µg/ml solution was further diluted and the volume was made up to 10ml by using method to produce 10µg/ml solution. 2ml, 3ml, 4ml and 5ml of 100µg/ml solution were diluted and the volume was made up to 10ml using methanol to produce 20µg/ml, 30µg/ml, 40µg/ml, 50µg/ml solutions respectively. Then the construction of calibration curve was done by taking the above prepared solutions of different concentration ranging from 10-50µg/ml. Then taking the absorbance calibration curve was plotted taking concentration on x-axis and absorbance on y-axis which showed a straight line (in fig. 2).This straight line obeyed linearity in the concentration range of 10-50µg/ml. The correlation coefficient was found to be 0.9999.

Method validation

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics [3].

The validation for UV method development was performed using parameters like Linearity, Accuracy, Precision, Robustness, Ruggedness, and Limit of detection (LOD), Limit of quantification (LOQ).

Linearity

Various aliquots were prepared from the secondary stock solution (100 µg/ml) ranging from 10-50 µg/ml. The samples were scanned in UV-Vis Spectrophotometer against methanol and distilled water (50:50) as blank. It was found that the selected drug shows linearity between the ranges of 10-50 µg/ml (table 1 & 5).

Accuracy

Solutions were prepared in triplicate at levels 80%, 100% and 120% of test concentration using cefadroxil working Standard as per the test method and taken absorbance of each solution in triplicate.

The recovery results showed that the proposed method has an acceptable level of accuracy for cefadroxil which is from 80% - 120% of test concentration is form 99.51 % - 100.01 %(table 1 & 5).

Precision

Precision of the method was demonstrated by intraday and interday variation studies. In intraday variation study nine different solutions of same concentration 20µg/ml were analyzed three times in a day i.e. from morning, afternoon and evening and the absorbance is noted. From the absorbance result mean, standard deviation and %RSD was calculated and given in table 6 & 7.

In the interday variation studies, solution of same concentration 20µg/ml were analyzed three times for the three consecutive days and the absorbance result mean, standard deviation and %RSD was calculated and given in table 8.

Specificity

10mg of Cefadroxil was spiked with 50% (5 mg), 100% (10 mg), and 150% (15 mg) of excipient mix (Magnesium Stearate) and the sample was analysed for % recovery of Cefadroxil (table no.9).

Robustness

Robustness of the method was determined by carrying out the analysis under different temperature condition i.e. at room temperature and at 18^0c. The respective absorbances of 20µg/ml were noted and the result was indicated as %RSD and given in table 10.

Ruggedness

Ruggedness of the method was determined by carrying out the analysis by different analyst and the respective absorbance of 20 µg/ml was noted. The result was indicated as %RSD and given in table 10.

Limit of Detection (LOD)

The limit of detection (LOD) was determined by preparing solutions of different concentrations ranging from 0.1-0.5 µg/ml. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantification as an exact value (table no.1).

Limit of Quantification (LOQ)

The LOQ is the concentration that can be quantification reliably with a specified level of accuracy and precision. The LOQ was calculated using the formula involving standard deviation of response and slope of calibration curve (table no.1).

Assay of Cefadroxil tablet (DROXIL-250mg)

A quantity of powder equivalent to 50mg of cefadroxil was taken in a 50ml volumetric flask and it was dissolved and diluted upto the mark with methanol and water. The resultant solution was ultra-sonicated for 5 minutes. The solution was then filtered using Whatmann filter paper No. 40. From the filtrate, appropriate dilutions were made in methanol and water (50:50) to obtain the desired concentration (50 µg/ml). This solution was then analysed in UV and the result was indicated by % recovery given in table 1.

Degradation studies

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the cefadroxil using the proposed method [4].

Hydrolytic degradation under acidic condition

To 2 ml of stock solution (1000 µg/ml) of Cefadroxil, 1 ml of 3 N HCl was added in 10 ml of volumetric flask and the volume was made up to the mark with methanol and water (50:50). Then, the volumetric flask was kept at normal condition for 90 minutes. After 90 min. time interval, 1 ml of solution was pipetted out from this flask, neutralized and diluted with methanol and water (50:50) in order to make the volume up to 10 ml and the dilution was carried out to achieve the appropriate concentration (20 µg/ml). This solution was taken in cuvette. For the blank, 0.5 ml solution of 3N HCl and 0.5 ml solution of 3N NaOH were diluted with methanol in 10 ml of volumetric flask was repeated (table no.2 & fig.no.3).

Hydrolytic degradation under alkaline condition

To 2 ml of stock solution of cefadroxil 1 ml of 0.1 N NaOH was added in 10 ml of volumetric flask and made up the volume to the mark with methanol and water (50:50). Volumetric flask was kept at normal condition for 90 min. After 90 min time interval, 1 ml of solution was pipetted out from this flask, neutralized and diluted with methanol and water (50:50) in order to make the volume up to 10 ml and the dilutions were carried out to achieve the appropriate concentration (20 µg/ml). The solution was then taken in cuvette. For the blank, 0.5 ml solution of 0.1N HCl and 0.5 ml solution of 0.1N NaOH diluted with methanol in 10 ml of volumetric flask (table no.2 & fig.no.4).

Dry heat induced degradation

Cefadroxil sample was taken in a petriplate and exposed to a temperature of 70°c for 48 hours in an oven. After 48 hours, 10 mg of the sample was diluted with methanol and water (50:50) in order to make the volume up to 10 ml. From this solution, dilutions were carried out to achieve the appropriate concentration (20 µg/ml) and the solution was taken in cuvette for the UV-Vis Analysis (table no.2 & fig.no.5).

Oxidative degradation

To 1.5 ml of the stock solution of cefadroxil (1000 µg/ml), 1 ml of 30 % w/v of hydrogen peroxide added in 10 ml of volumetric flask and the volume was made up to the mark with methanol and water (50:50). The volumetric flask was then kept at room temperature for 15 min. For the blank, 1 ml of the 30 % w/v of hydrogen peroxide was kept at normal condition for overnight in 10 ml of volumetric flask. Both solutions were heated on boiling water bath to remove the excess of hydrogen peroxide. Finally after 15 minutes dilutions were made from the stock solution to achieve the required concentration (30 µg/ml). The solution was then taken in a cuvette and analyzed (table no.2 & fig.no.6).

Photolytic degradation

Sample of cefadroxil was exposed to near ultra violet lamp in photostablity chamber providing illumination of not less than 1.2 million lux hours. Ten milligrams sample was dissolved methanol

and water (50:50) and volume made up to 10 ml. From this solution appropriate dilution (20µg/ml) was made using methanol and water (50:50) and taken in cuvette for the UV analysis (table no.2 & fig.no.7)

RESULTS AND DISCUSSION

The developed method was found to be precise as the %RSD values for intraday and inter-day were found to be less than 2%. Good recoveries (99.97 % to 101.4 %) of the drug were obtained at each added concentration, indicating that the method was accurate. The method was also found to be specific indicated by the % recoveries ranging from 98.2 % - 101.2 %. The LOD and LOQ were found to be in sub-microgram level indicating the sensitivity of the method. The method was also found to be robust and rugged as indicated by the % RSD values which are less than 2 %. The results of assay show that the amount of drug was in good agreement with the label claim of the formulation as indicated by % recovery (101.8 %). Summary of validation parameters of proposed spectrophotometric method is shown in table 1. The stress degradation studies showed that Cefadroxil undergoes degradation in acidic, oxidation and alkaline conditions whereas it is relatively stable when exposed to dry heat and photolytic conditions. Summary of the results of stress degradation studies of Cefadroxil are shown in the table 2.

Table 1: Summary of validation

Parameter	Result
Linearity indicated by correlation coefficient	0.9999
Precision indicated by %RSD	0.26%
Accuracy indicated by % recovery	99.9044%
Specificity indicated by % recovery	100%
Limit of Detection	0.25µg/ml
Limit of Quantification	0.825µg/ml
Range	10-50µg/ml
Linear regression equation	0.033x-0.004
Robustness indicated by %RSD	0.162%
Assay indicated by % recovery	99.98%

Table 2: Summary of result of stress degradation studies

R4b	Time	%Degradation
Acidic Hydrolysis	90min	λmax shifted
Alkaline Hydrolysis	90min	λmax shifted
30% Hydrogen Peroxide(1ml)	15min	λmax shifted
Dry Heat 70°	48hr	8.9%
Photolytic	3hr	5.15%

Table 3: Linearity Table of Cefadroxil in Working Standard

Concentration (µg/ml)	Absorbance
10	0.338
20	0.663
30	0.989
40	1.378
50	1.672

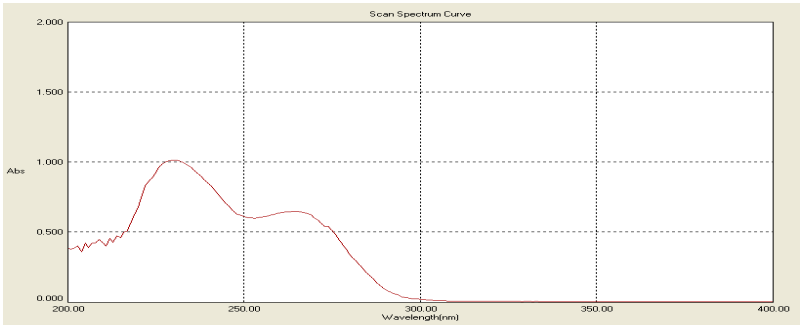


Fig. 1: λmax of Cefadroxil showing at 264nm, Peak 1- 264.00- 0.660

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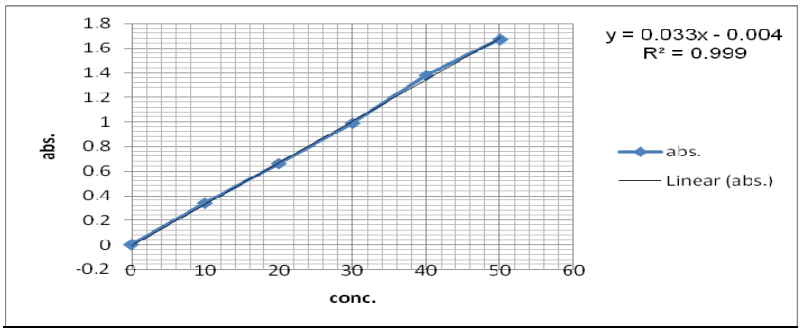

Fig. 2: Calibration curve of Cefadroxil

Table 4: Optical characteristics

Beer's law limit (µg/mL)	**10-50µg/ml**
Molar extinction coefficient (1 mole-1 c.m-1)	338
Correlation coefficient	0.9999
Regression equation (Y*)	0.033x-0.004
Slope (a)	0.033x
Intercept (b)	0.004

Table 5: Accuracy readings of Cefadroxil

No.of preparations	**Concentration (µg/lml)**		**% Recovery**	**Statistical results**		
	Formulation	**Pure drug**		**Mean**	**SD**	**%RSD**
S_1 : 80 %	10	8	99.24			
S_2 : 80 %	10	8	98.75	99.58	1.04	1.04
S_3 : 80 %	10	8	100.75			
S_4 : 100 %	10	10	100.9			
S_5 : 100 %	10	10	99.93	100.67	0.66	0.65
S_6 : 100 %	10	10	101.18			
S_7 : 120 %	10	12	98.98			
S_8 : 120 %	10	12	98.91	99.46	0.90	0.90
S_9 : 120 %	10	12	100.5			

Table 6: Precision results showing repeatability of Cefadroxil

Concentrations (µg/ml)	**Absorbance**	**Statistical analysis**
20	0.660	
20	0.661	
20	0.662	
20	0.660	Mean = 0.6608
20	0.661	SD = 0.000632
20	0.661	%RSD = 0.095
20	0.661	
20	0.661	
20	0.660	
20	0.661	

Table 7: Intra-assay precision

Concentrations (µg/ml)	**Absorbance 1**	**Absorbance 2**	**Absorbance 3**	**Average %RSD**
20	0.660	0.661	0.661	
20	0.658	0.661	0.661	
20	0.661	0.660	0.663	
20	0.660	0.658	0.658	
20	0.663	0.658	0.661	
20	0.661	0.662	0.660	
20	0.661	0.660	0.660	
20	0.663	0.660	0.661	
20	0.659	0.659	0.661	
%RSD	**0.25**	**0.20**	**0.20**	**0.21%**

Table 8: Inter-assay precision

Concentrations (µg/ml)	%RSD			Average %RSD
	Day 1	Day2	Day3	
20	0.165%	0.28%	0.32%	0.255%

Table 9: Test for specificity showing no effect of excipient.

Sample No.	Excipient conc. (%)	Cefadroxil input (mg)	Cefadroxil recovered (mg)	Cefadroxil recovered (%)	Mean recovered (%)	S.D.	% R.S.D.
1	100%	10	9.8	98.4			
2	50%	10	10.4	100.4	100%	1.44	1.44
3	150%	10	10.12	101.2			

Table 10: Results showing robustness & ruggedness of method for Cefadroxil

Analyst-1				**Analyst-2**		
Conc. (µg/ml)	**Abs.**	**Statistical Analysis**		**Conc. (µg/ml)**	**Abs.**	**Statistical Analysis**
20	0.661	Mean = 0.661		20	0.661	Mean = 0.6617
20	0.661			20	0.661	
20	0.660	SD = 0.0011		20	0.662	SD = 0.0008
20	0.660			20	0.662	
20	0.663			20	0.661	
20	0.661	%RSD = 0.16		20	0.663	%RSD = 0.12
Room Temperature				**Temp. 18°C**		
Conc. (µg/ml)	Abs.	Statistical Analysis		Conc. (µg/ml)	Abs.	Statistical Analysis
20	0.661	Mean = 0.6598		20	0.661	Mean = 0.6608
20	0.661			20	0.663	
20	0.660	SD = 0.0012		20	0.661	SD = 0.0013
20	0.658			20	0.659	
20	0.659			20	0.661	
20	0.660	%RSD = 0.17		20	0.660	%RSD = 0.20

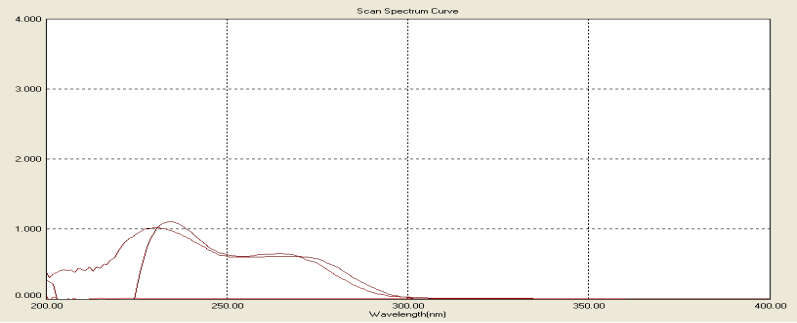

Fig. 3: Comparison between standard Cefadroxil (20 µg/ml) & acid Degraded sample of Cefadroxil (20µg/ml) after 90 minutes. Drug got degraded and its λmax shifted

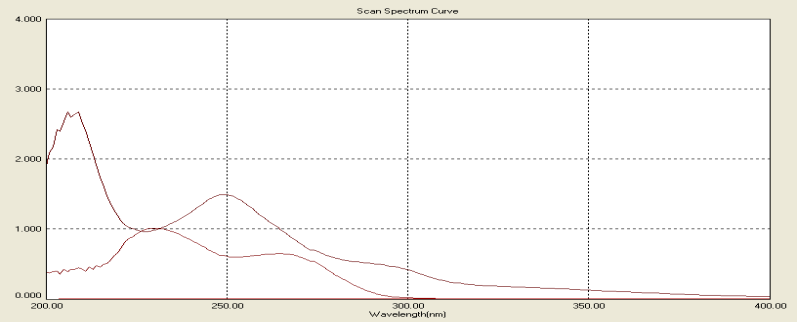

Fig. 4: Comparison between standard Cefadroxil (20 µg/ml) & alkali degraded sample of Cefadroxil (20µg/ml) after 90 minutes. Drug got degraded and its λmax shifted.

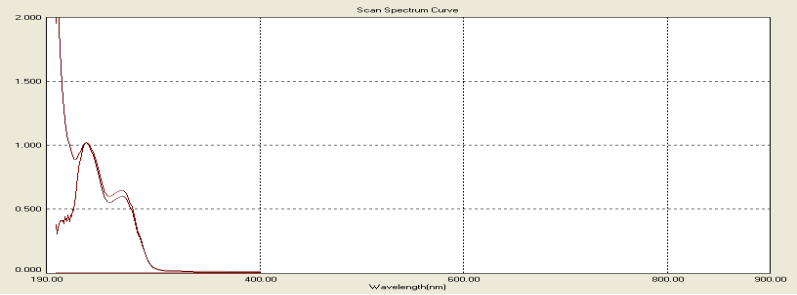

Fig. 5: Comparison between standard Cefadroxil (20 µg/ml) & temperature degraded sample of Cefadroxil (20µg/ml). Drug got degraded by 8.9% when exposed to a temp of 70°c for 48 hours

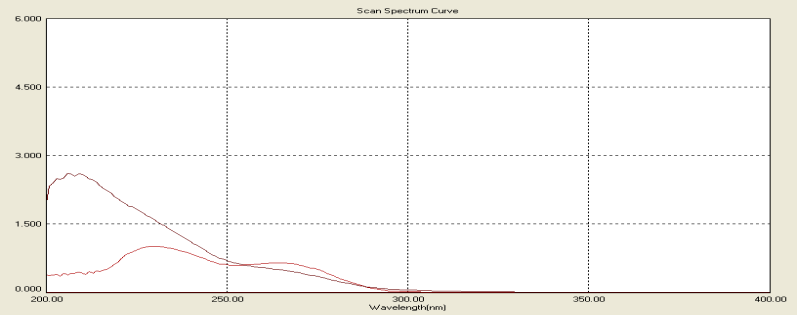

Fig. 6: Comparison between standard Cefadroxil (20µg/ml) & Oxidised sample of Cefadroxil (20µg/ml). Drug got totally degraded and its λmax shifted

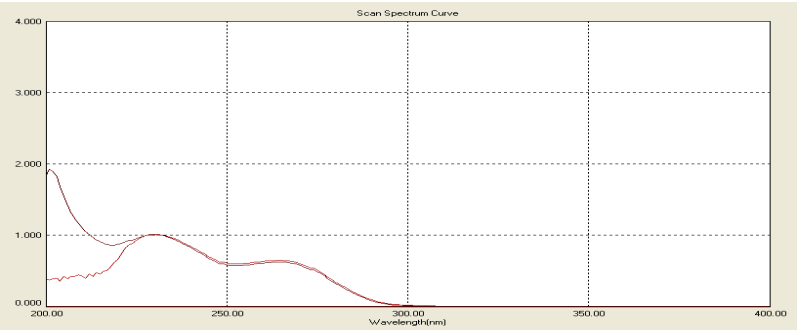

Fig. 7: Comparison between standard Cefadroxil (20 µg/ml) & UV degraded sample of Cefadroxil (20µg/ml) after 3 hours Drug when exposed to UV light for 3hrs, got degraded by 5.15%

CONCLUSION

All the above factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive, robust and cost effective and can be applied successfully for the estimation of cefadroxil in bulk and pharmaceutical formulation. The proposed method is also useful for determination of cefadroxil stability in sample of pharmaceutical dosage forms.

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