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DEVELOPMENT AND VALIDATION OF VISIBLE METHOD FOR ESTIMATION OF CEPHALEXIN IN BULK FORMULATION

Ch. Bhargavi, G. Rohit Reddy* and VVS. Rajendra Prasad

Department of Pharmaceutical Analysis and Quality Assurance, Vishnu Institute of Pharmaceutical Education and Research, Vishnupur, Narsapur, Medak, Telangana, India.

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***Corresponding Author**
G. Rohit Reddy
Department of Pharmaceutical Analysis and Quality Assurance, Vishnu Institute of Pharmaceutical Education and Research, Vishnupur, Narsapur, Medak, Telangana, India.

ABSTRACT

A simple, sensitive, accurate, precise and economical visible Spectrophotometric method was developed and validated for the estimation of Cephalexin in Bulk form. The method is based on the reaction of Cephalexin with FC Reagent [Folin Ciocalteu] in the presence of 10% Sodium Carbonate giving greenish blue colour chromogen which shows maximum absorbance at 720nm against reagent blank. The Chromogen obeyed Beer's law in the concentration range of 10-50 µg/ml for Cephalexin. The results of the analysis have been validated statistically and recovery studies.

KEYWORDS: Cephalexin, Folin Ciocalteu(Fc)reagent, Visible Spectrophotometric.

INTRODUCTION

Cephalexin is chemically (6R, 7R) -7-[(2R) – Aminophenylacetyl] amino]-3-methyl-8-oxo-5-thia-1-aza bicyclo [4.2.0] Oct-2-ene-2- Carboxylic acid.[1] Cephalexin is a first generation Cephalosporin antibacterial for the treatment of susceptible infections including those of respiratory tract, urinary tract and skin[2]. Cephalexin is official in IP, USP and BP. Literature survey reveals Septrophotometric[3-4] methods for estimation of Cephalexin in biological fluids and in pharmaceutical formulations. The present communication describes simple, sensitive, accurate, precise and economical visible spectrophotometric method using Folin-ciocalteu Reagent for the estimation of Cephalexin in bulk formulation.

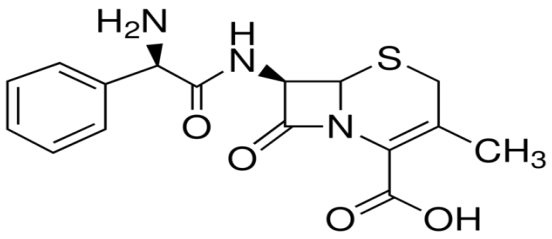

Figure 1: Cephalexin structure.

Mechanism of action

Cephalexin is a beta-lactam antibiotic of the cephalosporin family. It is bactericidal and acts by inhibiting synthesis of the peptidoglycan layer of the bacterial cell wall. Cephalexin closely resembles d-alanyl-d-alanine, an amino acid ending on the peptidoglycan layer of the cell wall, it is able to irreversibly bind to the active site of PBP, which is essential for the synthesis of the cell wall. It is most active against gram-positive cocci, and has moderate activity against some gram-negative bacilli.

MATERIALS AND METHODS

Apparatus

A Shimadzu model T60 double beam UV/Vis Spectrophotometer with spectral width of 2nm wave length accuracy of 0.5 nm and a pair of 10mm matched quartz cells was used to measure absorbance of the resulting solutions. Shimadzu analytical balance, an ultra sonic cleaner were used in the study.

Reagents and Materials

Cephalexin drug was procured as a gift sample from Synthokem Labs Pvt. Ltd. Sanathnagar, Hyderabad, Telangana. Folin-Ciocalteu reagent was prepared and NaOH [A.R.Grade, SD Fine Chemicals Ltd., Mumbai] were used in the study.

Preparation of Reagent and Working standard stock solution

Folin Ciocalteu Reagent: Accurate weigh the 10gm of sodium tungstate and 2.5 gm of sodium molybdate taken in a conical flask to it add 70ml of water, 5ml of 85% phosphoric acid is added and 10ml of conc. HCL is also added. Reflux for 1 Hr. Later add 15gm of Lithium Sulphate, 5ml of Water, 1 drop of bromine, reflux for 15 mins. Make up to 100 ml with water.

10% Sodium Carbonate: The solution was prepared by dissolving 10gm of sodium carbonate in 100ml of water.

Working Standard Stock Solution: Accurate weigh the 100mg of pure drug and transferred in 100 ml of volumetric flask later diluted with distilled water upto100ml gives 1000µg/ml.

Methodology

Different aliquots of working standard solution containing 10-50 µg/ml concentration of Cephalexin was transferred into series of volumetric flask. To it 1.5ml of Fc reagent and 5ml of 10%sodium carbonate was added and volume was made up to 10 ml with distilled water. The contents of the each flask was mixed well and allowed to stand at room temperature for 10 minutes. The absorbance of coloured species was measured at 720nm against reagent blank. The amount of drug present in the sample solution was computed from the calibration curve.

Reaction Mechanism: The results obtained in this method were based on the condensation of Cephalexin with Folin Ciocalteu Reagent in the presence Sodium Carbonate producing blue coloured species which is measured at 720nm shown in the Figure 2.

Method Validation

Linearity: Five points calibration curve were obtained in a concentration range from 10-50 µg/ml for Cephalexin. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation was y = 0.012x+0.010 with correlation coefficient 0.998 results are tabulated in table No.1 & Figure 3.

Precision: Precision of the analytical method is ascertained by carrying out the analysis as per the procedure and as per normal weight taken for analysis. Repeat the analysis six times. Calculate the % assay, mean assay, % Deviation and % relative standard deviation and %RSD. The developed method was found to be precise as the %RSD values for the repeatability and intermediate precision studies were <0.98% and <0.79%, respectively shown 998 results are tabulated in table No.2.

Accuracy: Accuracy of the method is ascertained by standard addition method at 3 levels. Standard quantity equivalent to 50%, 100% and 125% is to be added in sample. The result shown that best recoveries (98.54-99.13%) of the spiked drug were obtained at each added concentration, indicating that the method was accurate and results are tabulated in table No.3.

Robustness: Measure of the capacity of an analytical method to remain unaffected by small intentional variations in the operational parameters and provide an assurance of its reliability

during the normal usage. It may be determined by various parameters like ph, flow rate, temperature etc. Robustness studies are performed during the method development stage. Results are tabulated in table No.4.

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. Results are tabulated in table No.5.

Sensitivity

Limit of Detection and Quantitation (LOD and LOQ)

From the linearity data calculate the limit of detection and quantitation using the following formula.

LOD = 3.3σ / S

σ = Standard deviation of response.

S = Slope of the calibration curve of the analyte.

LOQ = 10σ / S

σ = Standard deviation of response.

S = Slope of the calibration curve of the analyte.

The results are tabulated in Table No.6.

RESULTS AND DISCUSSION

The analytical method was developed by studying different parameters. The method was validated for all validation parameters as per ICH guidelines. The lambda max of Cephalexin was found to be 720nm. Linearity was found with the concentration range 10-50 µg/ml and correlation coefficients found to be 0.998 indicate good linearity between concentration and slope area. Beer's law was obeyed by the fundamental spectrum. This method was found to be simple, sensitive, accurate, precise and economical for routine analysis for the estimation of Cephalexin in Bulk form. Recovery studies were found to be close 99% indicated the accuracy and precision of the above two proposed methods. Values of LOD and LOQ were found to be 2.75 and 8.3 respectively. The accuracy and robustness was calculated to be 99 and 100.9%.

Table No.1: Result For Linearity.

Concentration (µg/ml)	Absorbance
10	0.14
20	0.26
30	0.37
40	0.5
50	0.61
Correlation	0.998
Intercept	0.010
Slope	0.012

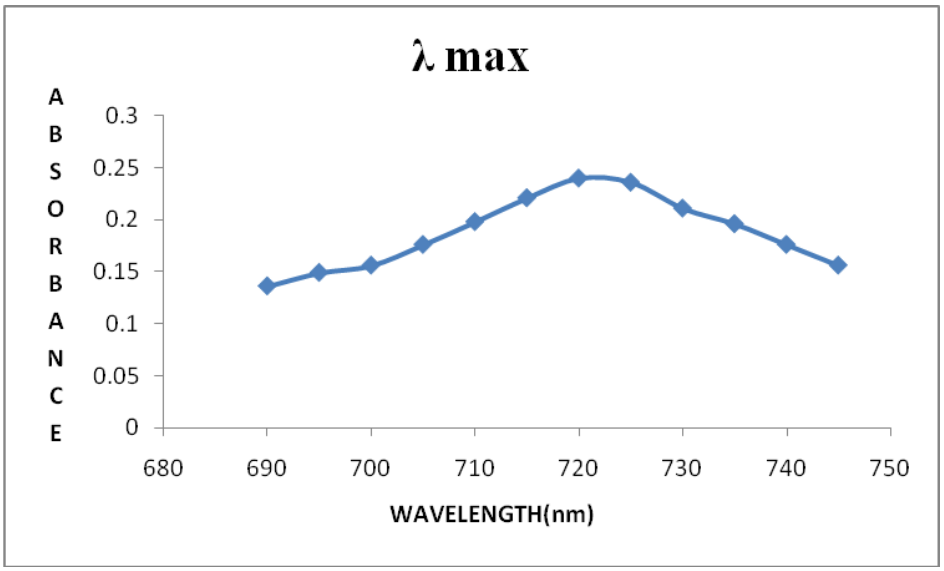

Figure 2: Reaction mechanism (λ max)

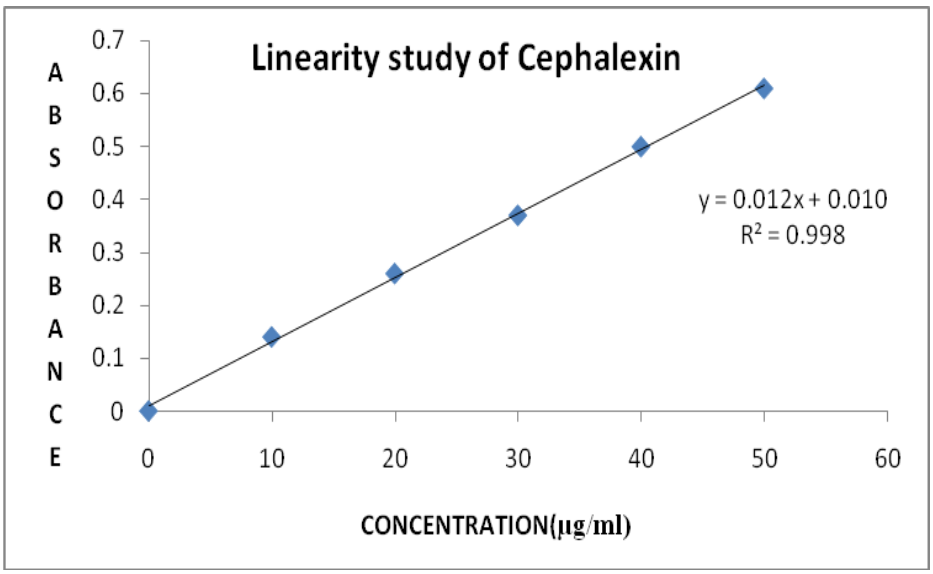

Figure 3: Linearity graph

Table No.2: Result For Precision.

Sample No.	% Assay	
	Intra day	Inter day
1	101.8	99.3
2	101.2	98.2
3	101.3	99
4	101.5	99
5	100.6	98.6
6	99.1	100.5
Mean	101.1	99.1
SD	0.99	0.78
% RSD	0.98	0.79

Table No.3 – Result For Accuracy.

% Recovery Level	% Recovery	Mean	SD	%RSD
50%	98.52	98.55	0.0402	0.0408
	98.53			
	98.61			
100%	98.56	98.54	0.0124	0.0126
	98.57			
	98.54			
150%	99.11	98.12	0.0124	0.0125
	99.14			
	99.13			

Table No. 4 – Result For Robustness.

Parameter	Amount of Cephalexin(µg/ml)		%Recovery	SD	%RSD
	Taken	Found			
1 ml of Volume of FC Reagent and 4ml of 10%sodium carbonate	20	18.3	91.6	0.83	0.90
	50	46.6	93.4		

Table No. 5 – Result for System Suitability Study.

Sample No.	Absorbance
1	0.66
2	0.661
3	0.66
4	0.66
5	0.66
Average	0.66
SD	0.0004078
% RSD	0.061

Table No 6: Result For Lod and Loq.

LOD ((µg/ml)	2.75
LOQ ((µg/ml)	8.3

CONCLUSION

The proposed visible spectrophotometric method was found to be simple, sensitive, accurate, precise and economic for determination of Cephalexin in bulk formulation. Hence it can be conveniently adopted for routine quality analysis of drug in pharmaceutical dosage form.

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