INTERNATIONAL RESEARCH JOURNAL OF PHARMACY **ISSN 2230 – 8407**
Available online http://www.irjponline.com
Research Article

METHOD DEVELOPMENT AND VALIDATION FOR THIRD GENERATION CEPHALOSPORIN BY UV-VIS SPECROPHOTOMETER

Devkhile A. B and Shaikh K. A*
P.G. Department of Chemistry, Yeshwant Mahavidyalaya, Nanded- 431602, (MS), India
Swami Ramanand Thirth Marathwada University Nanded- 431 606, (MS), India

*P.G. Department of Chemistry, Sir Sayyed College, P.B. No. 89, Roshan gate, Aurangabad, 431001(MS), India E. Mail: shaikh_kabeerahmed@rediffmail.com
Article Received on: 17/12/10 Revised on: 15/01/11 Approved for publication: 20/01/11

ABSTRACT
The present study describes a simple, accurate, precise and cost effective UV-Vis Spectrophotometric method for the estimation of ceftazidime, a third generation cephalosporin anti-biotic in dry powder injection and drug substances. The solvent used throughout the experiment was distilled water. The λmax or the absorption maxima of the drug was found at 275 nm. Beer's law was obeyed in the range of 2.0-16.0 µg/ml. The developed method was successfully validated with respect to linearity, accuracy and precision. The sample concentrations are measured on weight basis throughout the experiment. The method was validated and shown linear in the mentioned concentrations. The correlation coefficient for ceftazidime was 0.9999. The recovery values for ceftazidime ranged from 99.9-100.3. The relative standard deviation of six replicates of assay was less than 2 %. The percent relative standard deviation of inter-day precision ranged 1.1-1.4 % and intra-day precision 1.2-1.6 % of ceftazidime. The limit of detection and limit of quantification of ceftazidime was 0.12 µg/ml and 0.46 µg/ml. The developed method was cross checked with high performance liquid chromatography for six replicate assays, the mean assay was 100.1% and %RSD was 0.35%.Hence proposed method was precise, accurate and cost effective. This method can be applicable for quantitative determination of the titled drug with respect to assay from their new commercial formulation of injection in quality control laboratories.

KEYWORDS: UV-Vis Spectrophotometer, Method validation, Ceftazidime

INTRODUCTION

Ceftazidime chemically, pentahydrate of pyridinium, 1-[[7-[[(2-amino-4-thiazolyl)[(1-carboxy-1-methylethoxy)imino]acetyl]amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo [4.2.0]oct-2-en-3- yl]methyl]-, hydroxide, inner salt, [6R-[6α,7β (Z)]]. Ceftazidime, a third generation cephalosporin broad-spectrum beta-lactam antibiotic, used for parental administration. Also it is used to treat the skin and skin structure infections, lower respiratory tract infection, bone and joint infection, central nervous system infection and bacterial septicemia.Ceftazidime is a sterile, dry powder mixture of sterile ceftazidime, sodium carbonate and L-arginine. Ceftazidime dissolves without any evolution of gas. However no UV-Vis Spectrophotometric method was proposed for the estimation of ceftazidime without using hydro tope in bulk and pharmaceutical dosage forms. The aim of the work was to develop and validate an analytical method by using UV-Vis Spectrophotometer for the estimation of ceftazidime in bulk and pharmaceutical dosage forms. The formulation available in Indian market under the trade name of "CEFTAZIM" of different strength like 1 g, 500 mg and 250 mg per vial, manufactured by Aristo Pharmaceuticals Ltd. Literature survey revealed that few analytical methods are available for the individual estimation of ceftazidime in bulk drug and dosage formulations by HPLC in different pharmacopeia[1-3] .Few methods were reported for individual estimation of ceftazidime by HPLC[4-10] and some method in visible region by UV-Vis Spectrophotometer[11-15] . After development, analytical method was validated to ensure their quality and suitability as per ICH guideline[16]. Yet there is no method reported in the literature for the

estimation of ceftazidime in ultraviolet range of pharmaceutical dosage forms. In the present research work a simple, accurate and economical UV-Vis Specrophotometric method has been developed for the estimation of ceftazidime in dry powder injection and bulk drug substances.

MATERIAL AND METHOD

Ceftazidime used as working standard kindly provided by Bharati Vidhypeeth, (Pune, Maharashtra State (M.S.), India). The injection formulation available in Indian market under the trade name of "CEFTAZIM" of different strength like 1 g, 500 mg and 250 mg per vial, manufactured by Aristo Pharmaceuticals Ltd, Mumbai, India. Distilled water was used throughout the experiment. Other chemicals were analytical grade.

Instrumental Condition

The instrument used was an UV-Vis double beam spectrophotometer, of Shimazu make (Mode: 2501PC) with matched pair quartz cell for this study.

MEHTOD DEVELOPMENT

Solubility Test

Solubility test of the drug ceftazidime was performed by using various solvents. The solvents include water, methanol, ethanol, acetonitrile, 0.1N hydrochloric acid (HCl), 0.1 N sodium hydroxide (NaOH) and chloroform. However, the drug is freely soluble in water hence water was chosen as a solvent for developing the method and cost of water is low as compare other solvent.

Determination of λ max

Preparation of Stock Solution

Weigh and transfer accurately, equivalent to 100 mg of ceftazidime as working standard into 100 ml volumetric flask, dissolved and diluted up to mark with distilled water. Transfer 10 ml solution from the stock to 100 ml volumetric flask with distilled water to produce a concentration of 100 µg/ml, use this as standard stock solution.

Preparation of Working Solution

From the above stock solution, 10 ml pipetted into a 100 ml volumetric flask and the volume was made up with distilled water to produce a concentration of 10µg/ml. The solution was scanned in UV-Vis Spectrophotometer in the range 400-200 nm using distilled water as a blank. The wavelength corresponding to maximum absorbance (λmax) was found at 275 nm (Figure 2).

Preparation of Calibration Curve

2 ml solution of the 100 µg/ml was diluted to 100 ml to produce 2µg/ml solution. 4ml, 6ml, 8ml, 10ml, 12ml, 14ml, and 16ml of 100µg/ml solution were diluted to 100 ml with distilled water to produce 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml, 10µg/ml, 12µg/ml, 14µg/ml, and 16µg/ml solutions respectively. The calibration curve was construction by taking the solution concentrations ranged from 2-16µg/ml. The calibration curve was plotted by taking concentration on x axis and absorbance on y axis (Figure3). The curve showed linearity in the concentration range of 2-16µg/ml. This straight line obeyed linearity in the concentration range of 2-16µg/ml. The method was validated and shown linear in the mentioned range. The correlation coefficient for ceftazidime was 0.9999 (Figure 3).

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. The method was validated for different parameters like linearity, accuracy, precision, specificity, robustness, ruggedness, limit of detection (LOD) and limit of quantification (LOQ).

Linearity

Various aliquots were prepared form the stock solution (100µg/ml) ranging from 2- 16µg/ml. Linearity of the method for ceftazidime was tested from 20-160 % of the targeted level of the assay concentration in triplicate. The solutions were scanned using distilled water as blank. It was found that the selected drug shows linearity between the 2-16µg/ml. The correlation coefficient (r^2) was found 0.9999 (Figure.3).

Accuracy (Recovery Test)

Accuracy of the method was studied by performing recovery experiments. To perform the recovery experiment, add a known amount of the drugs in the placebo or blank. The solution were prepared in triplicate at three different levels 80 %, 100 % and 120 % of the test concentration using ceftazidime as working standard, and absorbance was measured of each solution. Recovery values ranged from 99.6-100.9 % at 80% recovery, 99.5 %-101.0 % at 100 recovery and 99.4 %-100.4 % for 120 % recovery. (Table 4) The average recoveries at three levels were 100.2 %, 100.3 % and 99.9 % respectively. The recovery results showed that the proposed method had acceptable level of accuracy for ceftazidime.

Method Reproducibility (Precision)

The system precision is measured of method variability, by measuring the absorbance of five replicates of the same working solution. The percent relative standard deviation is less than 2. Precision of the method was demonstrated by intra-day and inter-day variation studies. In intra-day variation study three different solutions of same concentration, 10µg/ml was analyzed for three times in a day i.e. zero hours six hours and 12 hours the absorbance is measured. From the absorbance obtained %RSD was calculated. In the inter-day precision, solution of same concentration, 10µg/ml was analyzed for three times, percent relative and standard deviation for inter-day assay was 1.1 % - 1.4 % and for intra-day assay 1.2- 1.6 % 5 (Table 5).

Limit of Detection and Quantitation

For determination of limit of detection (LOD) and limit of quantitation (LOQ) the method is based on the residual standard deviation of a regression line and slope. To determine the LOD and LOQ, a specific calibration curve was studied using samples containing the analyte in the range of detection limit (DL) and quantitation limit (QL). The limit of detection for ceftazidime was 0.12 µg/ ml and limit of quantitation was 0.46 µg/ ml.

Ruggedness and Robustness

Ruggedness and **r**obustness of the method was determined by carrying out the analysis by two analysts at two different temperatures i.e. at 25 °c and at 20°c. The absorbance was measured and assay was calculated for six times. The percent relative standard deviation of six replicates was less than 2 for both the analyst at the mentioned temperature condition (Table 6).

ASAAY OF CEFTAZIDIME (CEFTAZIM 1 g)

Ten injection vials were weighed and mixed properly. A quantity equivalent to 100 mg of Ceftazidime was weighed in to 100 ml volumetric flask. To this flask, 20 ml distilled water was added and sonicate for 5 minutes with continuous shaking, the solution was cooled to ambient temperature and dilute up to mark with the same solvent. The solution was then filtered using whatman filter paper No.41. From the filtrate, appropriate dilutions were made in distilled water to obtain the desired concentration (10µg/ml).

To get better assurance with respect to assay, the test was conducted as per the assay procedure mentioned in US Pharmacopeia (USP33) by HPLC method of injection formulation. All parameters used to perform the assay were as per the mentioned pharmacopeia (Table 8 and Figure 4).The percent relative standard deviation of six replicates assay was less than 2 (Table 7).

SOLUTION STABILITY

The stability of the sample solutions was performed at intervals of zero hour, 6 hours and 12 hours. The stability of solution was determined in terms of the assay of the drugs in sample solutions against the freshly prepared standard solutions. The relative standard deviation for the assay values determined up to 12 hours. The relative standard deviation is less than 2 % up to 12 hour. The results indicate that the solutions were stable to 12 hours at an ambient temperature.

CONCLUSION

The proposed UV-Vis Spectrophotometric method can be used for the determination of ceftazidime in the pharmaceutical dosage form and bulk drug substances. The developed method was validated and shown accurate, precise and cost effective (Table 1). It can be used in the quality control department for the estimation of assay of titled drug in pharmaceutical dosage form and in cleaning validation.

ACKNOWLEDGEMENT

The authors would like to thanks to the Principle, Yeshwant Mahavidylaya, Nanded, (MS), India, Tilawat Ali, Chairman, Sir Sayyad College, Aurangabad (MS), India for providing the necessary facilities and DST New Delhi for financial assistance..

REFERENCES

1. British Pharmacopoeia, London, 5th ed, UK; 2008:347-349.
2. United State Pharmacopoeia, USP 32,1, MD, USA; 2010:67.
3. European pharmacopoeia, (EDQM), 6th ed; 2008:455-457.
4. Whinney BC, Wallis SC,Hillisteer T.,Robert JA.,Limpan J., analysis of 12 beta-lactam antibiotics in human plasma by HPLC with ultraviolet detection., Chromatogr B; 2010:917-924,.
5. Salwa R., El-Shaboury,.Gamal A,.Fardous A, Mohamed,. Azza H, Rageh, Analysis of cephalosporin antibiotics by HPLC, J Phar.Bio.Anal, 2007;45:1-19.
6. Masoom RS.,Abu T., Chaudhar M., Reddy D., Negi PS., Development and validation high performance loqiud chromatographic method for simultenius determination ceftazidime and sulbactam in spiked plasma and combined dosage form-zydotam., American Journal of Applied Science 2009;6,(10):1781-1787.
7. Isla A, Arzuaga A, Maynar J, Determination of ceftazidime and cefepime in plasma and dialysate-ultrafiltrate from patients undergoing continuous veno-venous hemodiafiltration by HPLC, J Pharm Biomed Anal 2005; 4, (39):996-1005.
8. Krystyna L. Tyczkowska P., Shemida S., Michael K., Stoskop P., David. A., etermination of ceftazidime in dolphin serum by liquid chromatography with ultraviolet-visible detection and confirmation by thermospray liquid chromatography-mass spectrometry., J Chromatogr B: 1999;(576):2-8.
9. Lalitha N,.Puranik SB., Pai S., Rao PN., Stress degradation studies on ceftazidime and development of a validated stability indicating HPLC assay , The Pharm Review;2009:143.
10. Moreno AH.,Salgado HR., Development of a new high-performance liquid chromatographic method for the determination of ceftazidime, J.AOAC Int., 2008; (4):739-743.
11. Basilio M., Zero-crossing derivative specrophotometric determination of mixture of cephapirin sodium and ceftazidime in pure form and injection, Analyst, (113) 1988:1077-1082.
12. Arun. K., Saravanan C.,. Balachandar R, Kumuthavalli M. V., Jayakar B.,UV- Spectrophotometric determination of ceftazidime in pure and pharmaceutical formulation, J. Chem. Pharm. Res., 2010: (21): 424-431.
13. Rodenas V., García MS., Sanchez P.C, Spectrophotometric methods for the determination of cephradine or ceftazidine in human urine using batch and flow-injection procedures., J Pharm Biomed Anal, 1997;15,(11):1687-1693.
14. Moreno AH,Salgaro HR., Rapid and selective UV spectrophotometric method for the analysis of ceftazidime, JOAC Int,J, 2009;(92):820-823.
15. Andreia HM., Herida RN., Spectrophotometric determination of ceftazidime in pharmaceutical preparations using neocuproin as a complexing agent., Anal letters, 2008;41(12): 2143-2152.
16. ICH draft guidelines on validation of analytical procedures: definitions and terminology, federal register, vol.60, IFPMA, Switzerland, 1995:11260,

Table 1: Summary	
Parameter	Result
Linearity (Correlation coefficient)	0.9999
Precision (% RSD)	0.64
Accuracy (%Recovery)	100.1 %
Limit of Detection (LOD)	0.12 µg/ml
Limit of Quantitation (LOQ)	0.46 µg/ml
Range	2-16 µg/ml
Linear regression equation	0.044x-0.003
Ruggedness & Robustness (% RSD)	0.07-0.28 %
Assay UV-Vis (% recovery)	99.9
Assay HPLC	100.1

Table 2: Linearity of Ceftazidime by UV-Vis Spectrophotometer	
Concentration (µg/ml)	Absorbance
0	0
2	0.0874
4	0.1711
6	0.2589
8	0.3499
10	0.4391
12	0.5217
14	0.6154
16	0.7097

Table 3: Optical Characteristic by UV-Vis Spectrophotometer	
Parameter	Result
Molar extinction coefficient	339
Correlation coefficient	0.9999
Regression equation	0.044x-.0.003
Slope	0.044x
Intercept	0.003

Table 4: Accuracy by UV-Vis Spectrophotometer						
% Recovery	Concentration (µg/ml)			% Recovery	Avg. Recovery	% RSD
	Formulation	Drug added	Drug found			
80	10	8	7.97	99.6	100.2	0.53
80	10	8	8.01	100.1		
80	10	8	8.07	100.9		
100	10	10	10.10	101.0	100.3	0.61
100	10	10	9.95	99.5		
100	10	10	10.03	100.3		
120	10	12	11.91	99.4	99.9	0.41
120	10	12	12.05	100.4		
120	10	12	11.99	99.9		

Table 5: Inter-day and intra-day precision of assay UV-Vis Spectrophotometer			
Inter-day precision			
Parameter	0 Hour	6 Hours	12 Hours
Mean concentration (µg/ml)*	10	10	10
% RSD	1.1	1.2	1.4
Intra-day precision			
Mean concentration (µg/ml)*	10	10	10
% RSD	1.2	1.5	1.6
*:n = 3			

Table 6: Robustness and ruggedness of the method by UV-Vis Spectrophotometer					
	% Assay (25 ºC)	% Assay (20 ºC)		% Assay (25 ºC)	% Assay (20 ºC)
Analyst 1	99.7	99.9	Analyst 2	99.5	99.8
	100.1	100.2		100.2	100.1
	100.5	100.4		100.6	100.1
	99.7	99.9		101.1	100.7
	99.1	100.0		100.9	100.5
	100.1	100.2		99.6	99.9
Mean	99.9	100.1	Mean	100.4	100.2
% RSD	0.28	0.21	% RSD	0.07	0.07

Table 7: Assay by HPLC (As per USP33 Method)	
Sr. No.	% Assay
1	100.2
2	99.7
3	100.3
4	99.9
5	99.7
6	100.7
Mean	100.1
% RSD	0.35

Table 8: System Suitability Parameters for assay by HPLC	
Parameters	Result
Theoretical plates[1]	9011
Resolution	3.46
Tailing factor	0.91
% RSD	0.64
[1] :per column length %RSD: percent elative standard deviation	

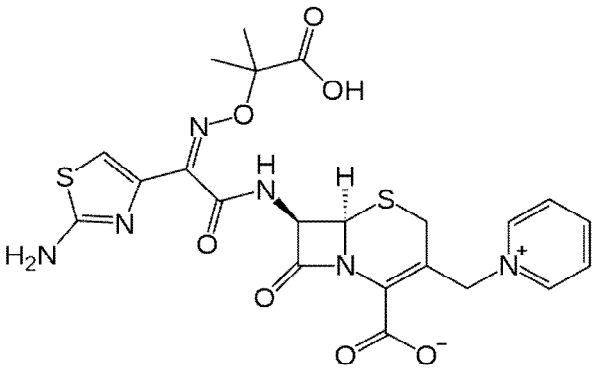

Figure: 1 Chemical Structure of Ceftazidime

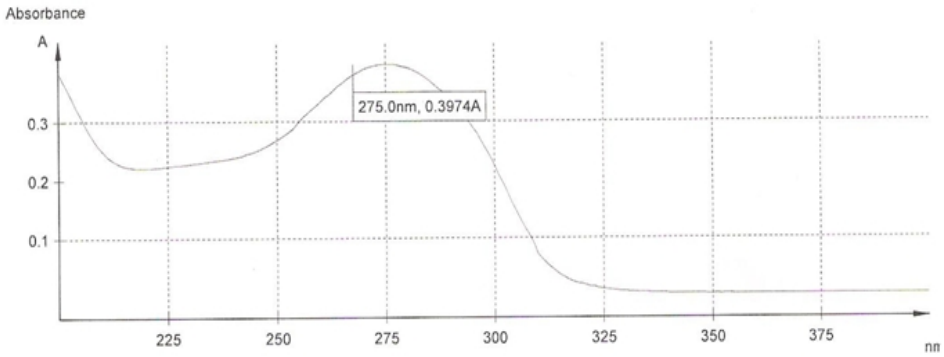

Figure 2: UV spectrum of ceftazidime (λmax determination)

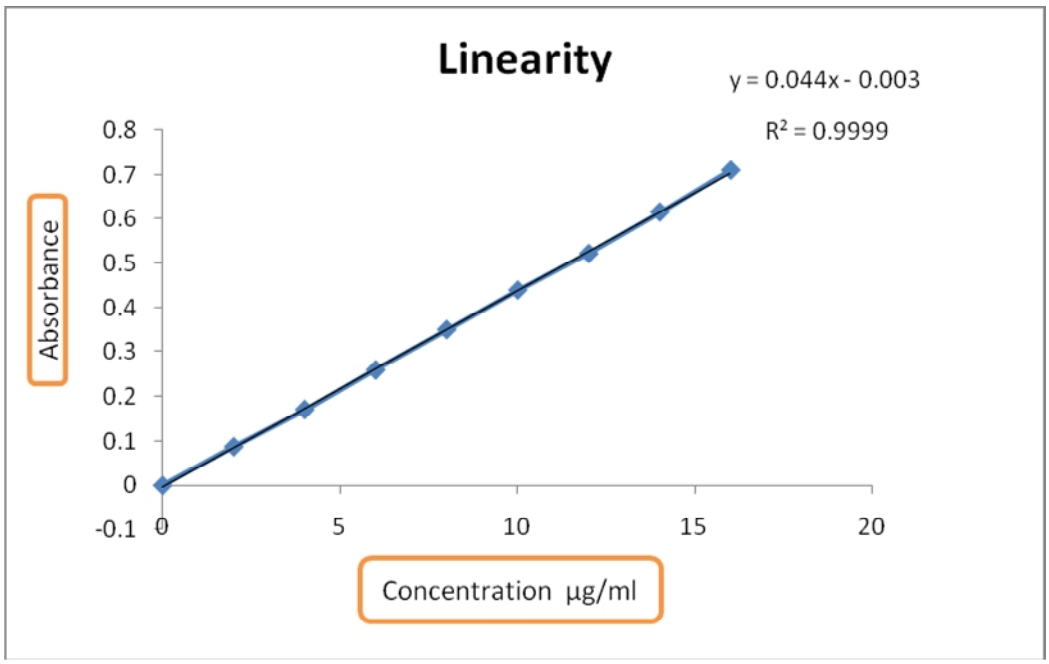

Figure: 3 Calibration curve of ceftazidime

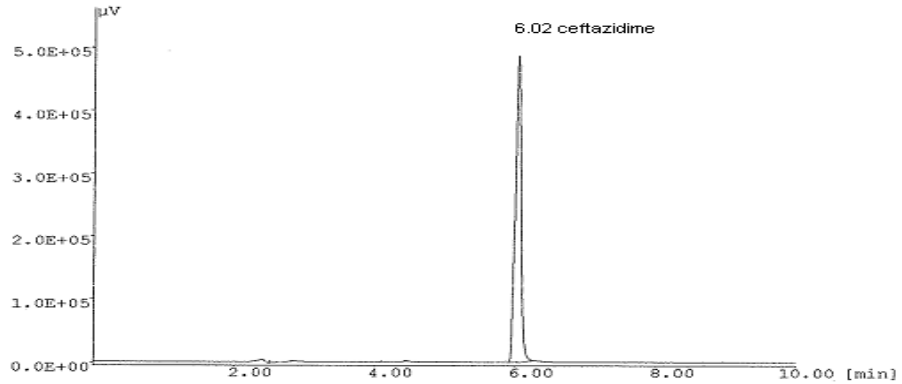

Figure: 4 A typical HPLC chromatogram of the injection containing ceftazidime

Source of support: Nil, Conflict of interest: None Declared