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Research Article

DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF CEFTAZIDIME IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT
The present study was undertaken to develop and validate a simple, accurate, precise, reproducible and cost effective UV-Visible spectrophotometric method for the estimation of ceftazidime in bulk and pharmaceutical formulation. The solvent used throughout the experiment was the mixer of methanol and water. Absorption maximum (λ_{max}) of the drug was found to be 266 nm. The quantitative determination of the drug was carried out at 266 nm and Beer's law was obeyed in the range of 2-20 µg/mL. The method was shown linear in the mentioned concentrations having line equation y = 0.044x with correlation coefficient of 0.999. The recovery values for ceftazidime ranged from 99.83 % - 100.56 %. The relative standard deviation of six replicates of assay was less than 2 %. The percent relative standard deviation (RSD %) of interday precision range was 0.275 – 0.420 % and intraday precision range was 0.222 – 0.418 %. The limit of detection and limit of quantification was 0.079 µg/mL and 0.241 µg/mL. The percent relative standard deviation of robustness and ruggedness of the method was 0.146 - 0.231 %. Hence, proposed method was precise, accurate and cost effective. This method could be applicable for quantitative determination of the bulk drug as well as dosage formulation.
Keywords: UV-Vis Spectrophotometer, Method Validation, Recovery studies.

INTRODUCTION

Ceftazidime is a broad spectrum third generation cephalosporin, active against gram positive and gram negative aerobic bacteria[1]. 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[2]. 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[3-4]. Few methods were reported for individual estimation of ceftazidime by voltammetry[5-6], electrophoresis[7], HPLC[8-11] and some method in visible region by UV-Vis Spectrophotometer[12-18]. But single estimation of this drug with mixture of methanol and distilled water as solvent has not been reported in bulk and in pharmaceutical formulation. Thus, the aim of the present work was to develop a simple, reproducible and economic analytical method to estimate ceftazidime in routine analysis.

MATERIALS AND METHOD

Drug

Pure Standard of ceftazidime was received as a kind gift from Square Pharmaceuticals. Vials of Tazid (cefitazidimide -500 mg) were purchased from the local market.

Reagents and Chemicals

Methanol (Merk, Germany) and distilled water were used as solvent. All other reagents were of analytical grade and were purchased from the local suppliers.

Instruments

A Shimadzu UV-Visible spectrophotometer UV-1800 was used.

Method Development

Solubility Test

Solubility test of ceftazidime was performed by using various solvents. Water, methanol, ethanol, acetonitrile, 0.1N HCl and 0.1N NaOH were used as solvents. However, the drug is slightly soluble in methanol and in water. So, methanol was chosen and the further dilution was done by water.

Preparation of stock solution

The standard stock solution of 100 µg/mL of ceftazidime was prepared by weighing 100 mg of the drug, taken in 100 mL volumetric flask and was dissolved in 50 mL methanol and then make up to the mark with distilled water. 10 mL of the stock solution was taken in 100 mL volumetric flask and was diluted with water up to 100 mL to produce a concentration of 100 µg/mL which was used as standard stock solution. Further dilutions were made with distilled water to obtain concentrations ranging from 02-20 µg/mL.

Determination of λ_{max}

By appropriate dilution of standard solutions with distilled water, solutions containing 10 µg/mL of ceftazidime were scanned in the range of 200-800 nm to determine the wavelength of maximum absorbance for the drugs. Ceftazidime showed absorbance maxima at 266 nm (Figure 1).

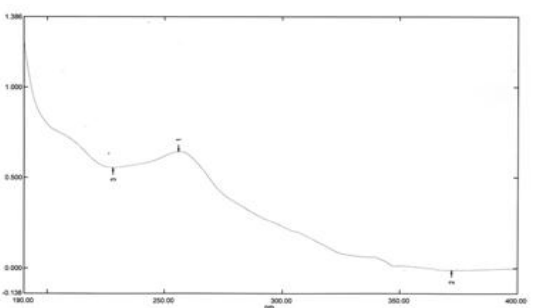

Figure 1: UV spectrum of Ceftazidime (λ_{max} determination)

Method Validation

The proposed method was validated for different parameters like linearity, precision, accuracy, specificity, robustness, LOD, LOQ and assay.

Linearity

Standard stock solutions, 100 µg/mL were further diluted with water to obtain 2 µg/mL, 4 µg/mL, 6 µg/mL, 8 µg/mL, 10 µg/mL, 12 µg/mL, 14 µg/mL, 16 µg/mL, 18 µg/mL, 20 µg/mL solutions. The absorbance of the spectra was measured at 266 nm. The calibration curves were constructed by plotting absorbance versus concentration and the regression equations were calculated (Figure 2).

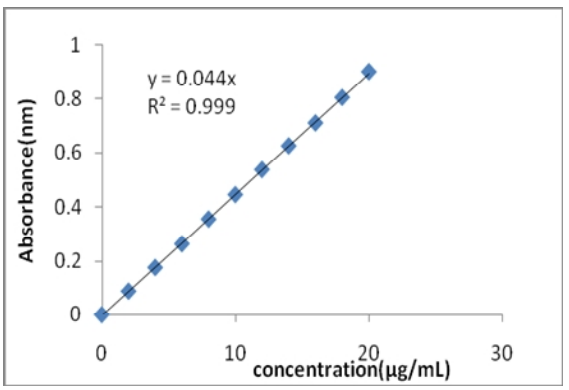

Figure 2: Linearity curve of Ceftazidime

Precision

The system precision is a measure of the method variability. It was determined by performing three replicate analyses of the same working solution. Precision of the method was demonstrated by intraday and interday variation studies. The intraday precision of the developed UV method was determined by preparing the samples of the same batch in nine determinations with three concentrations (5, 10, 20, µg/mL) and three replicate (n = 3) each on same day i.e. zero hour, fourth hour and eighth hour. The percentage RSD of the results was used to evaluate the method precision. The interday precision was determined by assaying the samples in triplicate (n = 3) per day for consecutive 3 days (Table 1).

Accuracy

Accuracy of the method was calculated by recovery studies at three levels (80 %, 100 % and 120 %) by standard addition method. An accurately weighed injection powder equivalent to 10 mg of ceftazidime was transferred in 100 ml volumetric flask. 25 mL methanol was added to dissolve the drugs and then volume was made up to the mark with distilled water and sonicated for 10 minutes. The solution was then filtered through a Whatmann filter paper (No. 41). From the filtrate 1mL was transferred to three 10 mL volumetric flasks and add 0.8 mL (Flask 1), 1 mL (Flask 2), and 1.2 mL (Flask 3) of stock solution of API and then made up to the mark with distilled water to made them 80 %, 100 % and 120 % spiking (Table 2).

Robustness

The robustness of an analytical procedure is the measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It was determined by carrying out the analysis by two analysts at two different temperatures i.e. at 200^0C and 300^0C. The absorbance was measured and assay was calculated for six times. The result was expressed in percent RSD (Table 3).

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ were calculated from the data obtained from the linearity studies. The slope of the linearity plot was determined. For each of the ten replicate determinations of same conc. (10 µg/mL), standard deviation (SD) of the responses was calculated. From these values, the limit of detection and limit of quantitation were determined on the basis of standard deviation and slope of the regression equation (Table 4).

Assay

An accurately weighed injection powder equivalent to 10 mg of ceftazidime was transferred in 100 ml volumetric flask. The content was dissolved in distilled water 50 ml and the volume was made up to 100 ml with methanol. The volumetric flask was sonicated for 20 minutes to effect complete dissolution of ceftazidime; the solution was then filtered. The aliquot of the filtrate was further diluted to get final concentration of 10 µg/mL of ceftazidime. The % assay of the drug was calculated (Table 5).

Statistical analysis

The results were expressed as mean ± SD. Some results were expressed as % RSD.

RESULTS AND DISCUSSION

The method discussed in the present work provides a convenient and accurate way for analysis of ceftazidime. The drug obeys the Beer's law with the concentration range 2– 20 µg/mL with R^2 value 0.999. When absorbance were plotted against concentration levels of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 µg/mL of standard drug, good correlation coefficient (r^2) was obtained as 0.999 which was within the accepted range of guidelines and represented a good linear relationship of the newly developed method (Figure 2). The precision of the proposed method was checked by intra-day and inter-day repeatability of responses after replicate injections of standard solutions of different concentrations thrice times each day for three days where RSD % amongst responses were found as < 2 (Table 1).

Table 1: Intra-day and inter-day precision of assay UV-Visible spectrophotometer

Inter-day precision					
Concentration (µg/mL)	Absorbance			SD	% RSD
	0 hour	4 hour	8 hour		
5	0.239	0.237	0.238	0.001	0.420
10	0.440	0.441	0.443	0.0015	0.346
20	0.912	0.917	0.914	0.0025	0.275
Intra-day precision					
05	0.238	0.240	0.239	0.00058	0.418
10	0.451	0.449	0.450	0.00153	0.222
20	0.907	0.912	0.909	0.001	0.277

The accuracy was evaluated at three different concentrations which were conducted in successive analysis (n = 3) using the proposed method and the value was expressed as percentage of recovery between the mean concentrations of recovered and injected concentration of the drug. The average recoveries were found to be as 99.83 %, 100.56 % and 99.91 % for the concentration levels of 80 %, 100 % and 120 %, respectively (Table 2).

Table 2: Determination of accuracy of ceftazidime by UV-Visible spectrophotometer (n = 3)

% Recovery	Concentration (µg/mL)			% Recovery	Avg. Recovery	% RSD
	Formulation	Drug added	Drug found			
80	10	8	7.97	99.63		
80	10	8	8.01	100.13	99.83 %	0.260
80	10	8	7.98	99.75		
100	10	10	10.10	101.0	100.56 %	
100	10	10	10.09	100.9		0.258
100	10	10	9.98	99.8		
120	10	12	12.02	100.17	99.91 %	
120	10	12	11.98	99.83		0.220
120	10	12	11.97	99.75		

Table 3: Robustness and ruggedness of the method by UV-Visible spectrophotometer (n = 6)

	% Assay (25^0C)	% Assay (20^0 C)		% Assay (25^0C)	% Assay (20^0C)
Analyst 1	99.55	99.67	Analyst 2	99.87	99.64
	99.57	99.76		99.63	99.67
	99.68	99.56		99.57	99.72
	99.91	99.43		100.12	99.56
	100.01	100.11		100.14	99.89
	99.76	99.89		99.65	99.98
Mean	99.74 %	99.73 %	Mean	99.83 %	99.74 %
% RSD	0.169	0.222	% RSD	0.231	0.146

The % RSD was found in the range of 0.146 – 0.231 % for robustness and ruggedness (Table 3). The LOD and LOQ of the developed method were determined by injecting progressively low concentrations of the standard solutions for 6 times and the values of LOD and LOQ were found to be as 0.079 µg/mL and 0.241 µg/mL respectively (Table 4).

Table 4: LOD and LOQ

S. No.	Concentration (µg/mL)	Absorbance	SD	LOD (µg/mL)	LOQ (µg/mL)
1	10	0.441	0.0011	0.079	0.241
2		0.440			
3		0.441			
4		0.440			
5		0.442			
6		0.440			
7		0.443			
8		0.440			
9		0.442			
10		0.441			

Table 5: Assay (n=3)

Concentration	Absorbance	% Assay	Mean	% RSD
10	0.415	99.97 %	99.58 %	0.392
10	0.411	99.19 %		
10	0.413	99.58 %		

All experimental results were within the range of the acceptability, which indicated that the developed method was sensitive enough and accurate for qualitative, quantitative analysis of ceftazidime. Therefore, the method was applied for quantitative analysis of ceftazidime in bulk and pharmaceutical dosage form.

CONCLUSION

This UV-spectrophotometric technique was quite simple, accurate, precise, reproducible and sensitive. The UV method has been developed for quantification of ceftazidime in pharmaceutical dosage forms. The validation procedure confirms that this is a workable method for their quantification in the raw material and also in the formulations.

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