

VALIDATED SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF CEFTAZIDIME IN DRY POWDER FOR INJECTION

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ABSTRACT

The present manuscript describes simple, sensitive, accurate, precise and economical visible spectrophotometric method for estimation of ceftazidime in pharmaceutical dosage form. Method is based on the reaction of ceftazidime with Folin-Ciocalteu (FC) reagent in presence of 10 % sodium carbonate solution, giving blue colour chromogen, which shows maximum absorbance at 752 nm against reagent blank. Beer's law was obeyed in the concentration range of 2.5-50 µg/ml. The method was successfully applied to pharmaceutical dosage form because no interference from the dosage form excipients was found. The results of analysis have been validated statistically and by recovery studies.

KEYWORDS: Ceftazidime, Visible spectrophotometric method, Folin-Ciocalteu (FC) reagent, Sodium carbonate, Chromogen

INTRODUCTION

Chemically, ceftazidime (CFZ) is 1-[[[(6R,7R)-7-[[[2Z]-(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]pyridinium¹. Ceftazidime is a broad spectrum, third generation, parenteral antibacterial cephalosporin effective against several bacterial infections². Ceftazidime is official in Indian Pharmacopoeia (IP), British Pharmacopoeia (BP) and United States Pharmacopoeia (USP). IP³, BP⁴ and USP⁵ describe liquid chromatographic method for its estimation. Literature survey reveals voltammetry⁶⁻⁷, electrophoresis⁸, HPLC⁹⁻¹⁰ and spectrophotometric¹¹⁻¹⁴ methods for determination of ceftazidime in pharmaceutical formulations and biological fluids. The present communication describes simple and cost effective spectrophotometric method for the estimation of ceftazidime in pharmaceutical dosage form.

MATERIALS AND METHODS

Apparatus

A Shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India).

Reagents and Materials

Ceftazidime bulk powder was kindly gifted by Mann Pharmaceuticals Ltd., Mehsana, Gujarat, India. The pharmaceutical formulation was procured from the local market. Folin-Ciocalteu's (FC) reagent, sodium carbonate (AR Grade, S. D. Fine Chemicals Ltd., Mumbai, India), Whatman filter paper no. 41 (Whatman International Ltd., England) and double glass-distilled water were used in the study.

Preparation of FC reagent and sodium carbonate (10 %) solutions

FC reagent was prepared by diluting 25 ml reagent to 100 ml with distilled water. Sodium carbonate solution (10 %) was prepared by dissolving 10 g sodium carbonate to 100 ml with distilled water.

Preparation of standard stock solution

The standard stock solution of ceftazidime was prepared by dissolving 25 mg of ceftazidime in 100 ml volumetric flask using distilled water to obtain final concentration, 250 µg/ml.

Methodology

Standard stock solution of ceftazidime (1.0 ml) was transferred to a 10 ml coming volumetric flask. To each flask, 3 ml 10 % sodium carbonate solution and 4 ml FC reagent was added. After a thoroughly shaking the flasks were set aside for 10 minutes for the reaction to complete. The volumes of each flask were adjusted to 10 ml with distilled water. The solution was scanned in the range of 400 to 800 nm against reagent blank, prepared similarly in which volume of standard solution was replaced by an equal volume of water. Maximum absorbance was obtained at 752 nm.

Preparation of calibration curve

Aliquots of 0.1 to 2 ml portion of standard stock solution were transferred to a series of 10 ml coming volumetric flasks. To each flask, 3 ml 10 % sodium carbonate solution and 4 ml FC reagent was added. After a thoroughly shaking the flasks were set aside for 10 minutes for the reaction to complete. The volumes of each flask were adjusted to 10 ml with distilled water. The absorbance of solution in each flask was measured at 752 nm against reagent blank and calibration curve was plotted. Similarly the absorbance of sample solution was measured and the amount of ceftazidime was determined by referring to the calibration curve.

Validation of the proposed method

The proposed method was validated for linearity, precision, accuracy, limits of detection and limits of quantification according to the International Conference on Harmonization (ICH) guidelines¹⁵.

Analysis of pharmaceutical preparation (Dry powder for injection)

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 solutions were filtered through Whatman filter paper No.41. The volume was made up to 100 ml with distilled water. From this solution, aliquots containing required concentration of the drug were taken for analysis and the solutions were then analyzed as described under calibration curve procedure. The amount of drug was determined by referring to the calibration curve. The analysis procedure was repeated five times with pharmaceutical formulation.

RESULTS AND DISCUSSION

As per Lewis acid-base theory, nitrogen-containing group is basic in nature having unshared pair of electrons so it can act as a reducing agent and can reduce tungstate and/or molybdate, which are present in Folin Ciocalteu's (FC) reagent in alkaline medium. Ceftazidime

contain nitrogen in their structure and therefore reduce the FC reagent in alkaline condition forming blue colored chromogen molybdenum blue. Ceftazidime reacts positively with FC reagent and producing blue colored chromogen. Therefore the proposed work is based on the similar reaction principle. In the present work, the quantitative reaction of the drug with FC reagent is proposed. The reaction is based on the reduction of phosphomolybdotungstic acid, the F.C reagent by ceftazidime in presence of 10 % sodium carbonate solution, there by producing reduced species molybdenum blue having characteristic blue colour with maximum absorption at 752 nm.

The blue colored complex formed having wavelength of maximum absorbance at 752 nm (Figure 1). It was found that 4 ml FC reagent with 3 ml 10 % sodium carbonate solution was sufficient for the development of maximum color intensity. Stability study of the developed chromogen was carried out by measuring the absorbance values at a time intervals of 30 minutes for 6 h and it was found to be stable for more than 4 h for the drug at room temperature.

The linearity was found in the concentration range of 2.5 to 50 µg/ml with high value of correlation coefficient ($r^2 > 0.99$) indicates linearity of the method. The reproducibility, repeatability and precision of the methods are very good as shown by the low values of standard deviation and percent relative standard deviation (% RSD). The % recovery values close to 100 % indicates accuracy of the method. The method was successfully applied to estimate ceftazidime from injection powder and the assay results are in good agreement with the label claim of the drug. The results of recovery studies and assay are given in Table 1 and Table 2, respectively. Optical characteristics of method and summary of validation parameters for ceftazidime was given in Table 3.

CONCLUSION

The method described in this paper for the estimation of ceftazidime using Folin Ciocalteu's (FC) reagent in presence of sodium carbonate solution was found to be simple, sensitive, accurate, precise, rapid and economical and can be successfully employed for the routine analysis of ceftazidime in pharmaceutical dosage form.

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TABLE 1: RESULTS OF RECOVERY STUDIES

Formulation	Level	Amount of sample taken (µg/ml)	Amount of standard spiked (%)	Mean % recovery ± S. D.* (n = 3)
Powder	I	10	50	98.83 ± 1.12
	II	10	100	101.3 ± 1.96
	III	10	150	99.23 ± 0.92

S. D. is standard deviation and n is the number of determinations.

TABLE 2: RESULTS OF ANALYSIS OF PHARMACEUTICAL FORMULATION

Formulation	Label claim (mg)	Amount found (mg)	% Label Claim ± S. D.* (n = 5)
Powder	Brand I	500	504.0
	Brand II	1000	992.3

S. D. is standard deviation and n is the number of determinations.

TABLE 3: OPTICAL CHARACTERISTICS AND SUMMARY OF VALIDATION PARAMETERS

Parameters	Results
λ max (nm)	752
Linearity range ($\mu\text{g/ml}$)	2.5 - 50
Sandell's sensitivity ($\mu\text{g/cm}^2/0.001 \text{ A.U.}$)	0.1365
Molar extinction coefficient (l/mol.cm)	3289
Correlation coefficient (r^2)	0.9985
Regression equation ($y^* = b + ac$)	
Slope (a)	0.0271
Intercept (b)	0.0229
Standard deviation (S. D.)	± 0.0053
% Relative standard deviation (% RSD)	± 1.118
Standard error of mean (S.E.M)	± 0.0024
Repeatability (% RSD, n = 6).	1.56
Intermediate Precision (% RSD)	
Interday (n = 3)	0.76-1.89
Intraday (n = 3)	0.93-1.76
Accuracy (% Recovery) (n = 5)	98.83 - 101.3
Limit of detection (LOD) ($\mu\text{g/ml}$)	0.65
Limit of quantification (LOQ) ($\mu\text{g/ml}$)	2.15

$y^* = b + ac$ where 'c' is the concentration in $\mu\text{g/ml}$ and y is absorbance unit. n is the number of determinations, RSD is relative standard deviation and S.E.M is standard error of mean..

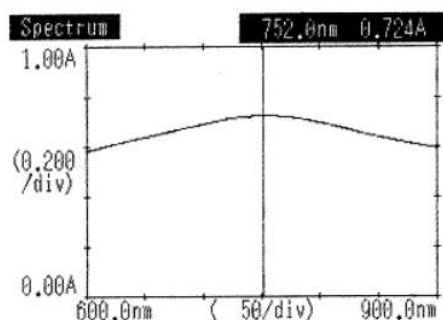


Figure 1: Spectra of ceftazidime with FC reagent in presence of sodium carbonate in distilled water showing maximum absorbance at 752 nm

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