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Pharmaceutical Sciences **Research Article……!!!**

DEVELOPMENT AND VALIDATION OF A RP-HPLC METHOD FOR DETERMINATION OF CEFTAZIDIME IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Ceftazidime, Validation, RP-HPLC, Aacetonitrile and Buffer.

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ABSTRACT

A new, simple, precise and accurate RP-HPLC method was developed and validated for the determination of ceftazidime in pure and tablet dosage form. The separation was carried out using Luna C_{18} (250 x 4.6 mm, 5 µm particle size) column, with a mobile phase consisting of acetonitrile, water and buffer (pH 5.0) in the ratio of 25:25:50 v/v/v. The flow rate was set at 1.5 ml/min and detection was monitored at 254 nm. The retention time of ceftazidime is 6.447 min respectively. The linearity coefficient of ceftazidime was found to be 0.9999 and percentage recoveries for ceftazidime is 99.84.The linearity was found in the concentration range of 50-150 µg/ml for ceftazidime respectively. The liquid chromatography method was extensively validated for linearity, accuracy, precision, and robustness. All these analytical validation parameters were observed and the %RSD was determined which indicates the usefulness of method for determination of ceftazidime in bulk drug and tablet formulation.

INTRODUCTION:

Ceftazidime[1] is chemically known as 1-{[(6R,7R)-7-[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-[(1-carboxy-1-methylethoxy)imino]acetamido]-2-carboxylato-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-en-3-yl]methyl}pyridin-1-ium and its empirical formula is $C_{22}H_{22}N_6O_7S_2$ with a molecular weight of 546.57. It is a semisynthetic, broad-spectrum, beta-lactam antibiotic for parenteral administration. It inhibits the cell wall synthesis via affinity for penicillin-binding proteins (PBPs). It acts on lower respiratory tract infections, skin and skin structure infections, urinary tract infections, bacterial septicemia, bone and joint infections, gynecologic infections, Intra-abdominal infections. The chemical structure was shown in Figure-1. Literature review revealed that very few methods was reported for determining of ceftazidime in bulk and pharmaceutical dosage form by HPLC[1-4] and spectrophotometric methods[5-7]. Hence in the present work an attempt was made to develop simple, precise and accurate analytical method for estimation of ceftazidime in bulk and pharmaceutical dosage form.

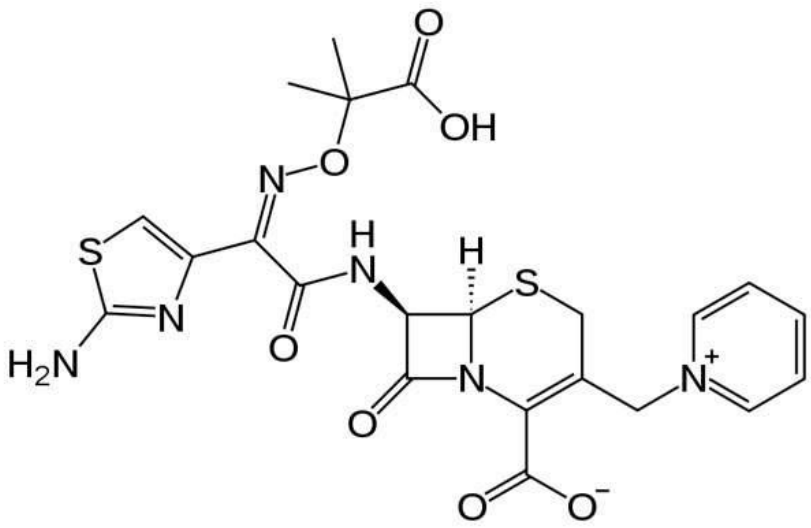

Figure-1: Chemical structure of ceftazidime

EXPERIMENTAL

Materials

Triple distilled water of HPLC grade, Methanol and acetonitrile of HPLC grade, Disodium hydrogen phosphate and o-phosphoric acid, which are of AR grade were used for the analysis. Reference standard sample of ceftazidime is procured from Dr. Reddy's labs, Hyderabad. Commercial Ceftazidime tablets (Amceft tablets) were purchased from local market and used in the analysis.

METHOD

Isocratic RP-HPLC-SHIMADZU LC 20 AD (prominence) and the Column specifications is C_{18} column (2), 250×4.6mm, 5µ particle size, Injector-Rheodyne, UV-Visible Spectrophotometer.

Preparation of mobile phase

Buffer preparation pH (5.0):

To take 1.42 gm of disodium hydrogen phosphate in 1L volumetric flask containing 200 ml of water. The contents were sonicated for 10 min and volume was made up of 1L with water. The pH was adjusted to 5.0 with o-phosphoric acid. The solution was filtered through 0.22 µ membrane filter.

Preparation of stock and working standard drug solutions of ceftazidime

About 100 mg of ceftazidime was weighed accurately and transferred into a 100 mL volumetric flask containing 20 mL of methanol. The solution was sonicated for 5 min and then the volume was made up with further quantity of mobile phase to get a 1 mg/mL solution. This solution was suitably diluted with the mobile phase to get a working standard solution of 100 µg/mL of ceftazidime.

Estimation of the drugs from tablet dosage forms

Twenty tablets of AMCEFT, containing ceftazidime (250 mg) was weighed and finely powdered. A quantity of the powder equivalent to 250 mg of ceftazidime was weighed, transferred in to 100 ml volumetric flask and dissolved in the mobile phase by sonication for about 15 min. This solution was filtered through 0.22µ filter paper. From the filtrate different aliquots were taken in separate 10ml volumetric flasks. The contents of the flask were made up to the volume with methanol and mixed well. Then these samples were injected and peaks were recorded.

Calibration curve

Separate standard calibration curves were prepared for each drug. Different volumes of stock solutions were accurately transferred to a 10ml volumetric flask to 50-150 µg/mL concentration range for ceftazidime respectively. Seven replicate solutions in the above range were prepared for each concentration. The calibration curve was constructed by plotting the analyte peak area against concentration.

RESULTS AND DISCUSSION

Method optimization

The suitable parameters were chosen after several trails with buffers of different pH values and various compositions of acetonitrile, water and buffer. However the final concentration was adjusted to achieve good resolution. The trails revealed that with the decrease in acetonitrile concentration, the peak obtained was broad and showed severe tailing. The peak obtained with a composition of acetonitrile, water and buffer 25:25:50 v/v/v was proved to be most suitable of all the combinations since the peaks obtained were better defined and resolved and free from tailing. To determine the effect of flow rate, the method was performed at different flow rates 0.7ml/min, 0.9 ml/min, 1.1ml/min, 1.4 ml/min and 1.7ml/min. The optimum flow rate 1.5 ml/min was chosen finally. The retention time obtained for

ceftazidime is at 6.447 min and the chromatogram was shown in Figure 2. Validation was carried out and validation summary was tabulated in Table 3.

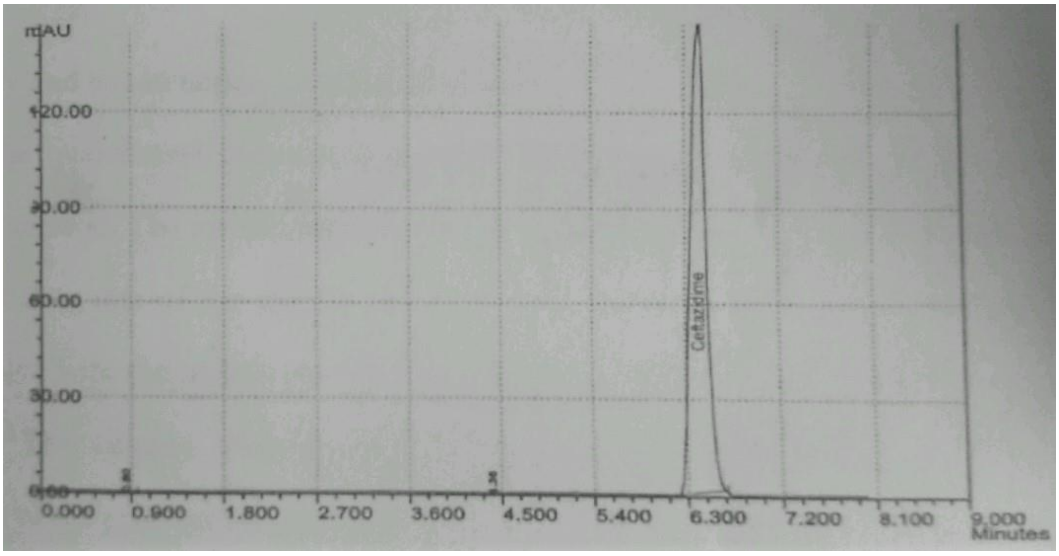

Figure-2: Chromatogram of standard solution

Table-3: Validation summary

Validation parameters	Results
Theoretical plates(N)	7256
Linearity range, mcg/ml	50-150
Tailing factor	1.08
R_t (min)	6.447
LOD, µg/ml	0.12
LOQ, µg/ml	0.40

Linearity and calibration

A calibration curve was determined by plotting the peak areas obtained against concentrations. There exists a linear relationship showing concentrations ranging for ceftazidime from 50µg/mL to 150µg/mL. From the data obtained, correlation coefficient for the ceftazidime was found to be 0.999. Linear regression data for calibration curves was shown in the table 1. The resulting linearity plot was shown in the Figure 3.

Table-1: Linear regression data for calibration curves

Drug	Ceftazidime
Concentration range (µg/mL)	50-150
Slope (m)	1544.4
Intercept (c)	-588.48
Correlation coefficient (R^2)	0.9998

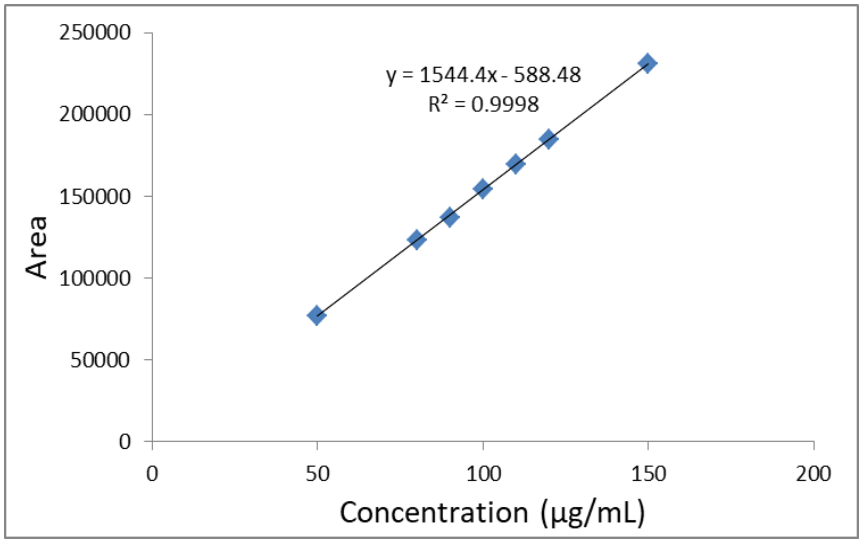

Figure-3: Linearity plot of ceftazidime

The number of theoretical plates was 7618 and tailing factor was 0.8. The retention time was 6.447 min for the developed RP-HPLC method. The number of the theoretical plates was high indicating the efficient performance of the column.

Precision:

Repeatability expresses the precision under the same operating conditions. The % RSD in the present experimentation was found to be 0.955%. The low RSD indicates that the method is precise and accurate.

Recovery studies:

Determination of accuracy by direct comparison to reference standard is a preferred technique. Recovery studies were performed by spiking the blank matrix of the sample at different levels (80%, 100%, and 120%) of the known level in the sample. Average recovery of the analyte was found to be in the range of 99.4-100.8 at different levels of spiking.

The developed RP-HPLC method utilizes acetonitrile, water & buffer (pH-5.0) in the ratio of (25:25:50) as a mobile phase and Luna C_{18} column as a stationary phase. The method precision and

system precision were performed and found to be within the limits. The recovery study reveals the accuracy and precision of the method employed for the present studies and the results are shown in Table 2.

Table-2: Results of assay and recovery studies

Sample	Amount claim(mg / tablet)	Amount found(mg/tablet)	% Recovery*
1.	250	249.6±0.11	99.84±1.2
2.	1000	999.8±0.23	99.98±1.8

**Average of two different concentration levels.*

CONCLUSION:

It is clear from the present study that the prescribed method of analysis is simple, accurate, specific and precise in operation and can be employed for routine batch analysis of ceftazidime in tablets.

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