Chapter – II

DEVELOPMENT AND VALIDATION OF ANALYTICAL PROCEDURE FOR THE ESTIMATION OF CEFTAZIDIME THROUGH NEW RP-HPLC METHOD
1. DRUG PROFILE

Ceftazidime [Figure 2.1] is a third generation cephalosporin antibacterial with enhanced activity against pseudomonas aeruginosa. It is used in the treatment of susceptible infections especially those due to pseudomonas species. They include biliary tract infections, bone and joint infections, cystic fibrosis (respiratory tract infections) endophthalmitis, infections in immuno compromised patients (nutrophenic patients) meliodosis, meningitis, peritonitis, pneumonia, upper respiratory tract infections, septicaemia, skin infections (including burns, ectymagangrenosum, and ulceration) and urinary tract infections. It is also used for surgical infection prophylaxis [1].

Figure 2.1: Molecular structure of Ceftazidime
Molecular formula : \( C_{22}H_{22}N_6O_7S_{2.5}H_2O \)

Molecular weight : 636.7

Chemical name : \((Z)-(7R)-7-[2-(2-Aminothiazol-4-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-(1-pyridiniomethyl)-3-\text{cephem-4-carboxylate pentahydrate}\)

**Table 2.1: List of important brand names of Ceftazidime formulations [2]**

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Formulation</th>
<th>Strength</th>
<th>Name of the manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMCEFT</td>
<td>Tablet</td>
<td>250 mg, 1000 mg</td>
<td>Health Biotech Pvt. Ltd., Chandigarh</td>
</tr>
<tr>
<td>CEFZIDIME</td>
<td>Vial</td>
<td>250 mg, 500 mg, 1000 mg</td>
<td>Chandra Bhagat Pharma Pvt. Ltd., Mumbai</td>
</tr>
<tr>
<td>FORTUM</td>
<td>vial</td>
<td>250 mg, 500 mg, 1000 mg, 2000 mg</td>
<td>Glaxo SmithKline Pharmaceuticals Ltd., Mumbai</td>
</tr>
<tr>
<td>TUFZID</td>
<td>vial</td>
<td>125 mg, 250 mg</td>
<td>Glenmark Pharmaceuticals Ltd., Mumbai</td>
</tr>
<tr>
<td>ZYTAZ</td>
<td>vial</td>
<td>250 mg, 1000 mg</td>
<td>Zydus Cadila Healthcare Ltd., Ahmedabad</td>
</tr>
</tbody>
</table>
2. REVIEW OF THE PAST WORK ON THE ANALYTICAL METHODS FOR CEFTAZIDIME

Literature survey revealed that a few analytical methods have been reported for the determination of Ceftazidime in pure drug, pharmaceutical dosage forms and in biological samples using liquid chromatography [3-16], spectrophotometry [17-23], high performance thin layer chromatography [24] and electrokinetic chromatography [25] either in single or in combined forms.

Jiang et al [4] developed a high performance liquid chromatography method for the determination of ceftazidime and impurities in ceftazidime drug using mobile phase containing Acetonitrile and phosphate buffer on all-time C_{18} column. They separated fourteen impurities with a good linearity of about 0.267-1.069 μg/mL. The limits of quantitation and quantification of Ceftazidime were 3.1 ng and 0.93 ng, respectively.

Xia et al [7] developed a HPLC method for determination of ceftazidime in plasma. The HPLC separation was performed on Kromasil C_{18} column. The mobile phase was methanol-potassium dihydrogen (15:85) at a flow rate of 1.0 ml/mL and results showed recovery of ceftazidime was 94.91-105.95%.

Zhao et al [8] developed a reversed phase-HPLC method for the simultaneous separation and analysis of cephalosporins containing ceftazidime, cefradine, cefalexin, cefotaxime, cefoperazone and cefazolin and these drugs were detected by
using ODS column, acetonitrile-HAC-NaAc Buffer as mobile phase. The detection limits for all six drugs were 0.20 µg/mL.

Wang et al [12] developed a High performance liquid chromatography method for determination of Ceftazidime in rat plasma. Ceftazidime was separated on a YWG-C_{18} column with a mobile phase of methanol and Ammonium acetate. Liu et al [14] developed a HPLC method for simultaneous analysis of Ceftazidime and its related substances. In this method ceftazidime and pyridine were simultaneously detected by using Shim-pack ODS column with Acetonitrile /methanol- water-MeCN as mobile phase at a flow rate of 1.0 ml/mol. The linear ranges were 50-150 and 0.5-12.5 µg/mL.

Abdellatef et al [17] developed a colorimetric and titrimetric methods for determination of some cephalosporins are cefotaxime sodium (CFT), cefuroxime sodium (CFU) and ceeftazidime pentahydrate. The colorimetric method was based on the reaction of these cited drugs with 4-chloro-7-nitrobenzofran, in the presence of borax, with the formation of stable coloured chromogens with maximim absorbance in the range of 409-414 nm. The method obeys Beer’s law for ceftazidime in the concentration range of 63.66-254.66 µg/mL.

Abdel-hamid et al [19] developed a method for simultaneous multicomponent analysis of two and three components mixture of drugs by spectrometric full spectrum quantitation (FSQ). By using FSQ method the mixture of phenobarbitone -phenytoin...
sodium, aspirin-caffeine-salicylic acid and cefotaxime-ceftazidime-ceftriaxone at variable proportions were detected.

Basavaraj et al [20] developed two spectrophotometric methods were developed and validated for determinations of ceftazidime. The first method based on the reaction of 3-methylbenzthiazolin-2-one hydrazone (MBTH) with ceftazidime in the presence of ferric chloride and produces blue color complex absorbs at $\lambda_{\text{max}}$ 628 nm. The second method describes the reaction between the diazotized drug and N-(1-napthyl)thlenediamine dihydrochloride (NEDA) to yield a purple coloured product with $\lambda_{\text{max}}$ at 567 nm. The two methods obey’s the Beer’s law within the concentration range of 2-10 and 10-50 $\mu$g/mL for MBTH and NEDA respectively.

Moreno et al [21] developed a rapid and selective UV spectrophotometric method for the analysis of ceftazidime. Beer’s law is obeyed in the concentration range of 7.0-14.0 $\mu$g/mL.

Mohammad Farduos et al [24] developed a selective densitometric method for analysis of cephalosorins using Dragendorff’s reagent and validated for analysis of the cephalosporins cefpodoxime proxetil, ceftriaxone sodium, ceftazidime pentahydrate, cefotaxime sodium, cefoperazone sodium, cefazolin sodium, and cefixime in bulk and pharmaceutical formulations. TLC was performed on aluminium sheets precoated with silica gel G 60F$_{254}$ as stationary phase. The R$_f$ values of all the
drugs within the range of 0.43-0.60. Limit of detection and quantitation for the drugs ranged from 0.35 to 2.48 and 1.07 to 7.50 µg per spot respectively.

Hsin-Hua Yeh et al [25] developed a micellar electrokinetic chromatographic method for determination of ceftazidime in plasma and cerebrospinal fluid with UV detection at 254 nm. The separation of ceftazidime from biological matrix was performed at 25°C using a background electrolyte consisting of Tris buffer with sodium dodecylsulphate (SDS) at the electrolyte solution. The linear ranges of the method for the determination of ceftazidime in plasma and in CSF were all over the range of 3-90 µg/mL, and the detection limit of the drug in plasma and CSF was 2.0 µg/mL.

3. EXPERIMENTAL AND RESULTS

MATERIALS AND METHODS

Instrumentation

The author had attempted to develop a liquid chromatographic method for quantitative estimation of Ceftazidime. A Shimadzu HPLC instrument, equipped with a Luna C18 column (250mm x 4.6 mm, 5µ), an LC 20 AD pump and a SPD 20AD UV-Visible detector was employed in this study. Chromatographic analysis and data acquisition was monitored by using Spinchrome software. A 20 µL Hamilton syringe was employed for sample injection. Degassing of the mobile phase was done by using
a Spectra lab DGA 20A3 ultrasonic bath sonicator. A Shimadzu electronic balance was used for weighing the materials.

Drugs

The reference sample of Ceftazidime was supplied by Dr. Reddy’s laboratories, Hyderabad. The branded formulations of Ceftazidime (Amceft Tablets) were purchased from the local market.

Chemicals and solvents

Acetonitrile- HPLC grade (Qualigens)

Disodium hydrogen phosphate- ExcelaR grade (Qualigens)

o-Phosphoric acid - ExcelaR grade (Qualigens)

Water-Triple distilled water prepared by using Borosil Glass Distillation Unit.

Preparation of the 0.01 M buffer solution (pH 5.0)

About 1.42 gm of disodium hydrogen phosphate was transferred into a one liter volumetric flask containing 200 mL of water. The contents were sonicated for 10 min and volume was made up to one liter with water. The solution was filtered through 0.22 μ membrane filter and pH of the solution was adjusted to 5.0 with o-phosphoric acid.
Preparation of the mobile phase

A mobile phase mixture of acetonitrile, water and disodium hydrogen phosphate buffer (pH 5.0) in a ratio of 25:25:50 v/v/v was prepared by diluting 250 mL acetonitrile, 250 mL of water and 500 mL of buffer in a one liter flask. The mixture was also used as diluent for preparing working standard solutions of the drug.

Preparation of stock and working standard 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 a further quantity of mobile phase to get a 1mg/mL solution. This solution was suitably diluted with the mobile phase to get a working standard solution of 100 µg/mL of Ceftazidime.

OPTIMIZATION OF THE CHROMATOGRAPHIC CONDITIONS AND METHOD DEVELOPMENT

For developing the method, a systematic study for optimization of chromatographic conditions was taken up. This was done by varying one parameter at a time and keeping all other conditions constant. The following studies were conducted for this purpose. A non-polar C\textsubscript{18} column was chosen as the stationary phase for this study.
The mobile phase

In order to get sharp peak and base line separation of the components, the author has carried out a number of experiments by varying the commonly used solvents with different compositions and its flow rate.

In order to effect ideal separation of the drug under isocratic conditions, mixtures of commonly used solvents like water, methanol and acetonitrile with or without different buffers in different combinations were tested as mobile phases on a C\textsubscript{18} stationary phase. A mixture of acetonitrile, water and disodium hydrogen phosphate buffer (pH 5.0) in a ratio of 25:25:50 v/v/v was proved to be the most suitable of all the combinations since the chromatographic peaks obtained were better defined and resolved and almost free from tailing. A flow rate of 1.5 ml/min mobile phase was found to be suitable in the studied range of 0.5-1.5 mL/min.

Detection wavelength

The spectra of diluted solutions of the Ceftazidime in methanol were recorded on UV spectrophotometer. The peaks of maximum absorbance wavelengths were observed. The spectra of the Ceftazidime showed that a balanced wavelength was found to be 254 nm.
Retention time of Ceftazidime

Under the above optimized conditions a retention time of 6.447 min was obtained for Ceftazidime. A typical chromatograms showing the separation of Ceftazidime is presented in Fig 2.2 and 2.3.

After a thorough study of the various parameters the following optimized conditions mentioned in Table 2.2 were followed for the determination of Ceftazidime in bulk samples and pharmaceutical formulations.
Fig 2.2: A typical Chromatogram showing the separation of Ceftazidime Standard

Fig 2.3: A typical Chromatogram showing the separation of Ceftazidime Sample
Table 2.2: Optimized Chromatographic conditions

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Column</td>
<td>Luna C$_{18}$ reverse phase column</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(250 mm x 4.6 mm, 5 µ)</td>
</tr>
<tr>
<td>2</td>
<td>Mobile phase</td>
<td>Acetonitrile:water:buffer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(25:25:50 v/v/v)</td>
</tr>
<tr>
<td>3</td>
<td>Flow rate</td>
<td>1.5 mL/min</td>
</tr>
<tr>
<td>4</td>
<td>Run time</td>
<td>9.0 min</td>
</tr>
<tr>
<td>5</td>
<td>Column temperature</td>
<td>Ambient</td>
</tr>
<tr>
<td>6</td>
<td>Volume of injection</td>
<td>20 µL</td>
</tr>
<tr>
<td>7</td>
<td>Detection wavelength</td>
<td>254 nm</td>
</tr>
<tr>
<td>8</td>
<td>Retention time</td>
<td>6.447 min</td>
</tr>
</tbody>
</table>

**Linearity and construction of calibration curve**

The quantitative determination of the drug was accomplished by the external standard method. The mobile phase was filtered through a 0.45µ membrane filter before use. The flow rate of the mobile phase was adjusted to 1.5mL/min. The column was equilibrated with the mobile phase for at least 30 min prior to the injection of the drug solution. The column temperature is maintained at 25±1°C throughout the study. Linearity of the peak area response was determined by taking six replicate
measurements at seven concentration points. Working dilutions of Ceftazidime in the range of 50-150\(\mu\)g/mL were prepared by taking suitable dilutions of the standard solutions in different 10 mL volumetric flasks and diluted up to the mark with the mobile phase. Twenty microlitres of the dilution were injected six times into the column. The drug in the eluents was monitored at 254 nm and the corresponding chromatograms were obtained. From the chromatograms, the mean peak areas were noted and a plot of concentrations over the peak areas was constructed. The regression of the plot was computed by least squares method. The linear relationship was found to be in the range of 50-150 \(\mu\)g/mL between the concentration of Ceftazidime and peak area response. This regression equation was later used to estimate the amount of Ceftazidime in pharmaceutical dosage forms. The linearity plot was shown in the Fig.2.4 and the linearity data and the statistical parameters for the linearity plot are reported in Table 2.3 and 2.4.
Table 2.3: Linearity study of Ceftazidime by proposed HPLC Method

<table>
<thead>
<tr>
<th>Concentration of Ceftazidime (µg/mL)</th>
<th>Mean Peak Area (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>77062</td>
</tr>
<tr>
<td>80</td>
<td>123301</td>
</tr>
<tr>
<td>90</td>
<td>136811</td>
</tr>
<tr>
<td>100</td>
<td>154123</td>
</tr>
<tr>
<td>110</td>
<td>169533</td>
</tr>
<tr>
<td>120</td>
<td>184954</td>
</tr>
<tr>
<td>150</td>
<td>231186</td>
</tr>
</tbody>
</table>

Fig 2.4: Linearity plot for Ceftazidime
Table 2.4: Regression characteristics of the linearity plot of Ceftazidime

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range (µg/mL)</td>
<td>50-150</td>
</tr>
<tr>
<td>Slope (a)</td>
<td>1540.922</td>
</tr>
<tr>
<td>Intercept (b)</td>
<td>-209.458</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9995</td>
</tr>
<tr>
<td>Regression equation</td>
<td>Y = 1540 X - 209.4</td>
</tr>
</tbody>
</table>

VALIDATION OF THE PROPOSED METHOD

The method was validated in compliance with ICH [26] guidelines. The following parameters were determined for validation.

Specificity

The method specificity was assessed by comparing the chromatograms obtained from the drug with the most commonly used excipient mixture with those obtained from the blank solution. The blank solution was prepared by mixing the excipients in the mobile phase without the drug. The drug to excipient ratio used was similar to that in the commercial formulations. The commonly used excipients in formulations like lactose, starch, microcrystalline cellulose, ethyl cellulose, hydroxy propyl methylcellulose, magnesium stearate and colloidal silicon dioxide were used
for the study. The mixtures were filtered through 0.45 μ membrane filter before injection. An observation of the chromatograms indicates absence of excipient peaks near the drug peak in the study run time. This indicates that the method is specific.

**Precision**

Precision is the degree of repeatability of an analytical method under normal operational conditions. The precision of the method was studied in terms of repeatability (intra-day assay) and intermediate precision (inter-day assay). Method repeatability was studied by repeating the assay three times in the same day for intra-day precision and intermediate precision was studied by repeating the assay on three different days, three times on each day (inter-day precision). The intra-day and inter-day variation for determination of Ceftazidime was carried out at three different concentration levels. % RSD values are presented in the Table 2.5 shows that the method provides acceptable (<2) intra-day and inter-day variation.
Table 2.5: Precision study

<table>
<thead>
<tr>
<th>Concentration of Ceftazidime (µg/mL)</th>
<th>Intra-day precision</th>
<th>Inter-day precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean amount found (n=3)</td>
<td>% amount found</td>
</tr>
<tr>
<td>25</td>
<td>25.06</td>
<td>100.24</td>
</tr>
<tr>
<td>50</td>
<td>49.92</td>
<td>99.84</td>
</tr>
<tr>
<td>75</td>
<td>74.93</td>
<td>99.90</td>
</tr>
</tbody>
</table>

Accuracy

Accuracy of the method was evaluated by standard addition method. An amount of the pure drug at three different concentration levels in its solution has been added to the pre analysed working standard solution of the drug. The sample solutions were analysed in triplicate at each level as per the proposed method. The percent individual recovery and %RSD for recovery at each level are calculated. The results are tabulated in Table 2.6. A recovery ranged from 99.73 - 99.85% has been obtained by the method, indicates its accuracy.
Table 2.6: Accuracy data (Triplicate values at different concentration levels)

<table>
<thead>
<tr>
<th>Amount taken (µg)</th>
<th>Amount found (µg)</th>
<th>% recovery</th>
<th>Mean % recovery</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>25+20=45</td>
<td>44.91</td>
<td>99.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25+20=45</td>
<td>45.01</td>
<td>100.02</td>
<td>99.85</td>
<td>0.25</td>
</tr>
<tr>
<td>25+20=45</td>
<td>44.89</td>
<td>99.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25+25=50</td>
<td>50.06</td>
<td>100.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25+25=50</td>
<td>49.98</td>
<td>99.96</td>
<td>99.73</td>
<td>0.12</td>
</tr>
<tr>
<td>25+25=50</td>
<td>50.10</td>
<td>100.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25+30=55</td>
<td>54.68</td>
<td>99.42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25+30=55</td>
<td>54.93</td>
<td>99.90</td>
<td>99.78</td>
<td>0.32</td>
</tr>
<tr>
<td>25+30=55</td>
<td>55.01</td>
<td>100.02</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Robustness

A study was conducted to determine the effect of deliberate variations in the optimized chromatographic conditions like composition of the mobile phase, flow rate and pH of the mobile phase. The effect of these changes on the system suitability parameters like tailing factor and number of theoretical plates and on assay was studied. A single condition was varied at a time keeping all other parameters constant.
The results were found to be within the allowed limits which indicate that the method is robust.

i) Variations in composition of the mobile phase

The effect of variation in percent organic content in mobile phase was evaluated by changing the composition of organic component in the mobile phase. The tailing factor and the number of theoretical plates showed a little change with change in mobile phase composition. The values are presented in Table 2.7.

ii) Variations in the pH of the mobile phase

The effect of variation in the pH of the mobile phase was evaluated and the system suitability results were found to be within the limits as shown in the Table 2.7.

iii) Variations in flow rate

A study was conducted to determine the effect of variation in flow rate. The system suitability parameters were evaluated at 1.4 mL/min and 1.6 mL/min. The results were within the acceptance criteria. Hence the allowable variation in flow rate is 1.4 mL/min to 1.6 mL/min.
### Table 2.7: Results of Robustness study

<table>
<thead>
<tr>
<th>Variations</th>
<th>Chromatographic Parameters</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tailing factor</td>
<td>Theoretical plates</td>
<td>% assay</td>
<td></td>
</tr>
<tr>
<td>23% of Acetonitrile in the mobile phase</td>
<td>1.08</td>
<td>7692</td>
<td>99.6</td>
<td></td>
</tr>
<tr>
<td>27% of Acetonitrile in the mobile phase</td>
<td>1.08</td>
<td>7523</td>
<td>98.9</td>
<td></td>
</tr>
<tr>
<td>Flow rate at 1.4 mL/min</td>
<td>1.08</td>
<td>7163</td>
<td>99.6</td>
<td></td>
</tr>
<tr>
<td>Flow rate at 1.6 mL/min</td>
<td>1.08</td>
<td>7189</td>
<td>98.7</td>
<td></td>
</tr>
<tr>
<td>pH of mobile phase at 4.8</td>
<td>1.11</td>
<td>7431</td>
<td>100.1</td>
<td></td>
</tr>
<tr>
<td>pH of mobile phase at 5.2</td>
<td>1.10</td>
<td>7486</td>
<td>100.4</td>
<td></td>
</tr>
</tbody>
</table>

### Stability of the analytical solution

A study to establish bench top stability of the drug solution was conducted. A freshly prepared working standard solution (100 μg/mL of the drug) was analyzed immediately and at different time intervals. The tailing factor, theoretical plates and difference in percent assay at different time intervals were calculated and the results are given in Table 2.8. A maximum difference of 0.8% in the assay at the end of 24 hours was observed. The difference in percent assay meets the acceptance
standard. From the above study it is concluded that the standard drug solution is stable for twenty four hours on bench top.

Table 2.8: Stability of the standard solution

<table>
<thead>
<tr>
<th>Time</th>
<th>Ceftazidime</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Assay</td>
</tr>
<tr>
<td>Initial</td>
<td>98.9</td>
</tr>
<tr>
<td>12th hr</td>
<td>99.6</td>
</tr>
<tr>
<td>18th hr</td>
<td>98.1</td>
</tr>
<tr>
<td>24th hr</td>
<td>99.4</td>
</tr>
</tbody>
</table>

Limit of Detection and Limit of Quantification

Limit of detection (LOD) is defined as the lowest concentration of analyte that gives a measurable response. LOD is determined based on signal to noise ratio (S/N) of three times typically for HPLC methods. The limit of quantification (LOQ) is defined as the lowest concentration that can be quantified reliably with a specified level of accuracy and precision. It is the lowest concentration at which the precision expressed by a RSD of less than 2%. In this study the analyte response is 10 times greater than the noise response. For this study, six replicates of the analyte at lowest concentration in the calibration range were measured and quantified. The LOD and LOQ of Ceftazidime obtained by the proposed method were 0.12 and 0.40 µg/mL respectively (Table 2.9)
Table 2.9: LOD and LOQ of Ceftazidime

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOD</td>
<td>0.12</td>
</tr>
<tr>
<td>LOQ</td>
<td>0.40</td>
</tr>
</tbody>
</table>

System precision and System suitability

System precision and system suitability studies were carried out by injecting six replicates of the working standard solution. The %RSD for the peak areas obtained was calculated. The data presented in Table 2.10 (% RSD<1) establishes reproducible performance of the instrument. The system suitability parameters are given in Table 2.10.
Table 2.10: System precision

<table>
<thead>
<tr>
<th>Injection Number</th>
<th>Peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>154112</td>
</tr>
<tr>
<td>2</td>
<td>154131</td>
</tr>
<tr>
<td>3</td>
<td>153986</td>
</tr>
<tr>
<td>4</td>
<td>154122</td>
</tr>
<tr>
<td>5</td>
<td>154169</td>
</tr>
<tr>
<td>6</td>
<td>154621</td>
</tr>
<tr>
<td>Mean</td>
<td>154190.2</td>
</tr>
<tr>
<td>SD</td>
<td>200.83</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.13</td>
</tr>
</tbody>
</table>

ESTIMATION OF THE DRUG FROM DOSAGE FORMS

Satisfactory results obtained with the method development for the assay of Ceftazidime have prompted the author to attempt its applicability for the estimation of the drug in its formulations.

Twenty tablets of Ceftazidime were weighed and powdered into uniform size in a mortar. From this the average weight of a tablet was calculated. An accurately weighed portion from this powder equivalent to 100 mg of Ceftazidime was transferred to a 100mL volumetric flask containing 20 mL of the methanol.
The contents of the flask were sonicated for about 20 min for complete solubility of the drug and the volume was made up to 100 mL with mobile phase. Then the mixture was filtered through 0.45μ membrane filter. From the above solution a five mL of aliquot was taken into a separate 50 mL volumetric flask and made up to the volume with mobile phase and mixed well. The above solution (20 μL) was then injected eight times into the column. The mean peak area of the drug was calculated and the drug content in the formulation was calculated by the regression equation of the method. The results of the recovery are tabulated. The percent recovery was reported in Table 2.11.

Table 2.11: Estimation of Ceftazidime from its formulation

<table>
<thead>
<tr>
<th>Sample</th>
<th>Labeled amount (mg)</th>
<th>Amount found*± S.D.</th>
<th>% Recovery*± R.S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amcef Tablets 250 mg</td>
<td>249.6 ± 0.11</td>
<td>99.84 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>Amcef Tablets 1000 mg</td>
<td>999.8 ± 0.23</td>
<td>99.98 ± 1.8</td>
<td></td>
</tr>
</tbody>
</table>

*Average ± standard deviation of eight determinations

4. SUMMARY OF THE RESULTS AND CONCLUSION

The present study was aimed at developing a sensitive, precise and accurate HPLC method for the analysis of Ceftazidime in bulk drug and in pharmaceutical dosage forms. In order to affect analysis of the component peaks, mixture of acetonitrile with phosphate buffer in different combinations were tested as mobile phase on a C₁₈ stationary phase. A mixture of acetonitrile, Water and buffer (pH 5.0)
in a proportion of 25:25:50 v/v/v was proved to be the most suitable of all combinations since the chromatographic peaks were better defined and resolved and almost free from tailing. The retention time obtained for Ceftazidime was 6.447 min.

Each of the samples was injected six times and the same retention times were observed in all cases. The peak areas of Ceftazidime were reproducible as indicated by low % RSD. A good linear relationship (r=0.9995) was observed between the concentration of Ceftazidime and the respective peak areas. The regression curve was constructed by linear regression fitting and its mathematical expression was \( Y = 1540X - 209.4 \) (where \( Y \) gives peak area and \( X \) is the concentration of the drug). The regression characteristics are given in Table 2.4. When Ceftazidime solutions containing 25, 50 and 75 \( \mu \)g/mL was analysed by the proposed method for finding out intra and inter-day variations, low % RSD was observed. High recovery values obtained from the dosage form by the proposed method indicates the method is accurate. The absence of additional peaks indicates non-interference of common excipients used in the tablets.

The drug content in tablets was quantified using the proposed analytical method. The tablets were found to contain an average of 99.84-99.98% of the labeled amount of the drug. The deliberate changes in the method have not much affected the peak tailing, theoretical plates and the percent assay. This indicates that the present method is robust. The lowest values of LOD and LOQ as obtained by the proposed
method indicate the method is sensitive. The standard solution of the drug was stable up to 24 hours as the difference in percent assay is within, the acceptable limit.

System suitability parameters were studied with six replicate standard solution of the drug and the calculated parameters are within the acceptance criteria. The tailing factor and the number theoretical plates are in the acceptable limits. Hence the author concludes that the proposed HPLC method is sensitive and reproducible for the analysis of Ceftazidime in pharmaceutical dosage forms with short analysis time.
5. REFERENCES


