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METHOD DEVELOPMENT AND METHOD VALIDATION OF CEFTAZIDIME AND AVIBACTAM BY RP-HPLC

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ABSTRACT

Using a Waters PDA-detected HPLC mode and a quick, sensitive, and precise RP-HPLC approach, Avibactam and Ceftazidime have been identified and quantified. An Inertsil-ODS C18 (250 x 4.6 mm, 5) column was used to separate Avibactam and Ceftazidime at a volumetric rate of 1.0 ml/min. The filtered mobile phase of the process was combined with degassed Methanol and Acetonitrile (70:30), and the wavelength of detection was 275 nm.

KEYWORDS: Ceftazidime, Avibactam, RP-HPLC.

1. INTRODUCTION

1.0 Introduction to HPLC

High Performance Liquid Chromatography (HPLC) was derived from the classical column chromatography and, is one of the most important tools of analytical chemistry today.[1]

In the modern pharmaceutical industry, high-performance liquid chromatography (HPLC) is the major and integral analytical tool applied in all stages of drug discovery, development, and production.[2] HPLC is the method of choice for checking peak purity of new chemical entities, monitoring reaction changes is in synthetic procedures or scale up, evaluating new formulations and carrying out quality control / assurance of the final drug products.[3]

The Goal of HPLC method is to try & separate, quantify the main drug, any reaction impurities, all available synthetic intermediates and any degradants.[4] High Performance Liquid Chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantify the compounds that are present in any sample

that can be dissolved in a liquid. HPLC is the most accurate analytical methods widely used for the quantitative as well as qualitative analysis of drug product and used for determining drug product stability.[5] HPLC principle is the solution of sample is injected into a column of porous material (stationary phase) and liquid phase (mobile phase) is pumped at higher pressure through the column. The principle of separation followed is the adsorption of solute on stationary phase based on its affinity towards stationary phase. (Figure-1) The technique of HPLC has following features.[6]

- High resolution
- Small diameter, Stainless steel, Glass column
- Rapid analysis
- Relatively higher mobile phase pressure
- Controlled flow rate of mobile phase

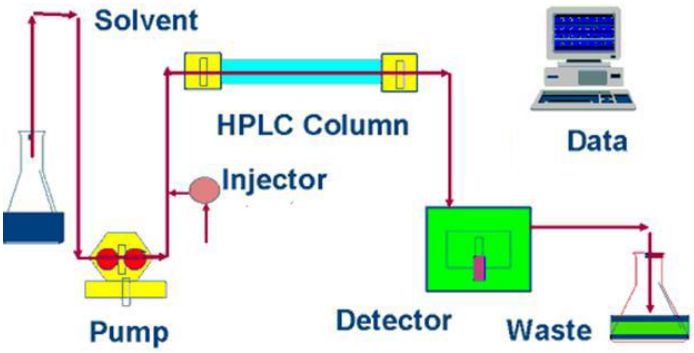


Fig. 1: Flow Diagram of HPLC.

2.0 HPLC Method Development

Methods are developed for new products when no official methods are available. Alternate methods for existing (Non-Pharmacopoeial) products are to reduce the cost and time for better precision and ruggedness. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available. The goal of the HPLC-method is to try & separate, quantify the main active drug, any reaction impurities, all available synthetic inter-mediates and any degradants.[7]

Steps involved in Method development are.[6,7]

- Understanding the Physicochemical properties of drug molecule.
- Selection of chromatographic conditions.
- Developing the approach of analysis.

- Sample preparation
- Method optimization
- Method validation (figure-2)

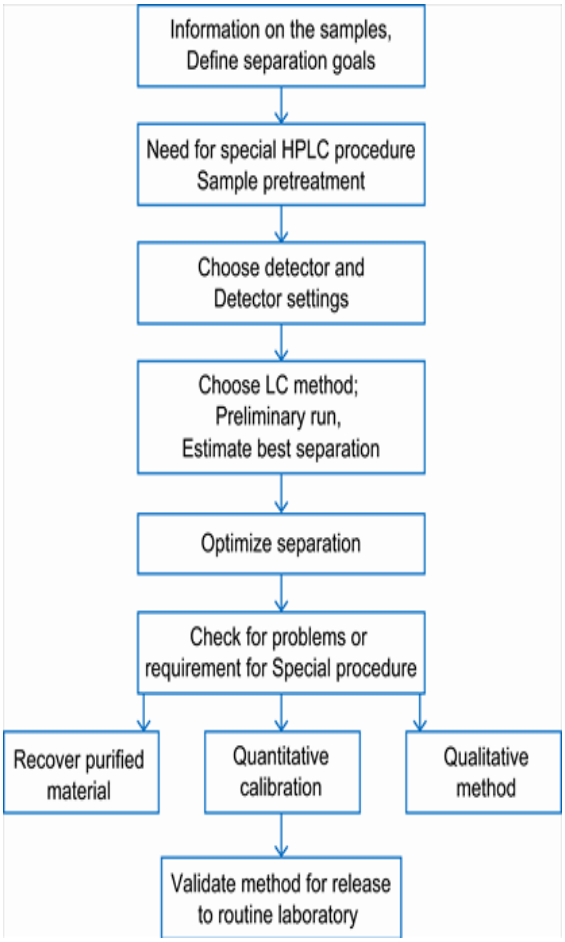


Figure 2: Steps involved in HPLC Method development.

2.1 Understanding the physicochemical properties of drug molecules

Physicochemical properties of a drug molecule play an important role in method development. For Method development one has to study the physical properties like solubility, polarity, pKaand pH of the drug molecule. Polarity is a physical property of a compound. It helps an analyst, to decide the solvent and composition of the mobile phase.[6] The solubility of molecules can be explained on the basis of the polarity of molecules. Polar, e.g. water, and nonpolar, e.g. benzene, solvents do not mix. In general, like dissolves like i.e., materials with similar polarity are soluble in each other. Selection of diluents is based on the solubility of analyte. The acidity or basicity of a substance is defined most typically by the pH value. Selecting a proper pH for ionizable analytes often leads to symmetrical and sharp peaks in HPLC.[7]

2.2 Selection of chromatographic conditions

During initial method development, a set of initial conditions (detector, column, mobile phase) is selected to obtain the first "scouting" chromatograms of the sample. In most cases, these are based on reversed-phase separations on a C18 column with UV detection. A decision on developing either an isocratic or a gradient method should be made at this point.

2.2.1 Selection of Column

A column is of course, the starting and central piece of a chromatograph. An appropriately selected column can produce a good chromatographic separation which provides an accurate and reliable analysis. An improperly used column can often generate confusion, inadequate, and poor separations which can lead to results that are invalid or complex to interpret.[9] The heart of a HPLC system is the column. Changing a column will have the greatest effect on the resolution of analytes during method development. Choosing the best column for application requires consideration of stationary phase chemistry, retention capacity, particle size, and column dimensions. The three main components of an HPLC column are the hardware, the matrix, and the stationary phase.

Fig. 3: Stationary Phases of HPLC.

There are several types of matrices for support of the stationary phase, including silica, polymers, alumina, and zirconium. Silica is the most common matrix for HPLC columns. Silica matrices are robust, easily derivatized, manufactured to consistent sphere size, and does not tend to compress under pressure. Silica is chemically stable to most organic solvents and to low pH systems. One short coming of a silica solid support is that it will dissolve above pH.[7] In recent years, silica supported columns have been developed for use at high pH. The nature, shape and particle size of the silica support effects separation. Smaller particle results in a greater number of theoretical plates, or increased. The nature of the

stationary phase will determine whether a column can be used for normal phase or reverse phase chromatography.

Normal phase chromatography utilizes a polar stationary phase and a non-polar mobile phase. Generally, more polar compounds elute later than non-polar compounds. Commonly used reverse phase columns and their uses are listed below. Propyl (C3), Butyl (C4), and Pentyl (C5) phases are useful for ion-pairing chromatography (C4) and peptides with hydrophobic residues, and other large molecules. C3–C5 columns generally retain non-polar solutes more poorly when compared to C8 or C18 phases. Examples include Zorbax SB-C3, YMC-Pack C4, and Luna C5. These columns are generally less stable to hydrolysis than columns with longer alkyl chains. Octyl (C8, MOS) phases have wide applicability. This phase is less retentive than the C18 phases, but is still quite useful for pharmaceuticals, nucleosides, and steroids.[10] Selection of the stationary phase/column is the first and the most important step in method development. The development of a rugged and reproducible method is impossible without the availability of a stable, high performance column. To avoid problems from irreproducible sample retention during method development, it is important that columns be stable and reproducible. The separation selectivity for certain components vary between the columns of different manufacturer as well as between column production batches from the same manufacturer. Column dimensions, silica substrate properties and bonded stationary phase characteristics are the main ones. The use of silica-based packing is favored in most of the present HPLC columns due to several physical characteristics.[6]

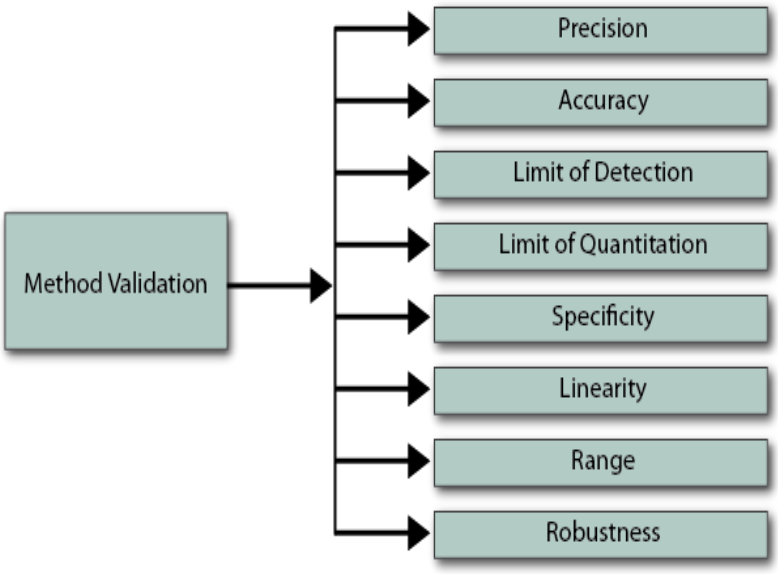


Figure 4: Method validation parameters.

Table 1: Important Information concerning sample composition and properties.

Number of Compounds presents
Chemical Structures (functionality) of compounds
Molecular weight of compounds
PKa values of compounds
UV Spectra of compounds
Concentration range of compound in sample of interest
Sample Solubility

2.2.2 Selection of Chromatographic mode: chromatographic modes based on the analyte's molecular weight and polarity. All case studies will focus on reversed-phase chromatography (RPC), the most common mode for small organic molecules. Ionizable compounds (acids and bases) are often separated by RPC with buffered mobile phases (to keep the analytes in a non-ionized state) or with ion-pairing reagents.[8]

2.2.3 Optimization of Mobile phase

Buffer Selection: Different buffers such as potassium phosphate, sodium phosphate and acetate were evaluated for system suitability parameters and overall chromatographic performance.

Effect of pH.:-If analytes are ionisable, the proper mobile-phase pH must be chosen based on the analyte p*Ka*so the target analyte is in one predominate ionization state, ionized or neutral. Alteration of the mobile-phase pH is one of the greatest tools in the "chromatographer's toolbox" allowing simultaneous change in retention and selectivity between critical pair of components.[12]

- **Effect of organic modifier:** -Selection of the organic modifier type is relatively simple in reverse phase HPLC, The usual choice is between acetonitrile and methanol (rarely THF). Gradient elution is usually employed with complex multicomponent samples since it may not be possible to get all components eluted between k *(*retention factor*)* 1 and 10 using a single solvent strength under isocratic conditions.[12]

2.2.4 Selection of detector and wavelength

After the chromatographic separation, the analyte of interest is detected by using suitable detectors. Some commercial detectors used in LC are: ultraviolet (UV) detectors, fluorescence detectors, electrochemical detectors, refractive index (RI) detectors and mass spectrometry (MS) detectors. The choice of detector depends on the sample and the purpose

of the analysis. In case of multicomponent analysis the absorption spectra may have been shifted to longer or shorter wavelengths compared to the parent compound. Therefore the UV spectra of target analyte and impurities must be taken and overlaid with each other, and the spectra should be normalized due to different amounts present in the mixture. A wavelength must be chosen such that adequate response is for most of the analytes can be obtained.[12,13]

2.3. Developing the approach for analysis

While developing the analytical method on RP-HPLC the first step which is followed, the selections of various chromatographic parameters like selection of mobile phase, selection of column, selection of flow rate of mobile phase, selection of pH of mobile phase. All of these parameters are selected on the basis of trials and followed by considering the system suitability parameters. Typical parameters of system suitability are e.g. retention time should be more than 5 min, the theoretical plates should be more than 2000, the tailing factor should be less than 2, resolution between 2 peaks should be more than 5, % R.S.D. of the area of analyte peaks in standard chromatograms should not be more than 2.0 %.like other. Detection wavelength is usually isobestic point in the case of simultaneous estimation of 2 components.[6]

2.4 Sample preparation

Sample preparation is a critical step of method development that the analyst must investigate. For example, the analyst should investigate if centrifugation (determining the optimal rpm and time) shaking and/or filtration of the sample is needed, especially if there are insoluble components in the sample. The objective is to demonstrate that the sample filtration does not affect the analytical result due to adsorption and/or extraction of leachable. The effectiveness of the syringe filters is largely determined by their ability to remove contaminants/insoluble components without leaching undesirable artifacts (i.e., extractable) into the filtrate. The sample preparation procedure should be adequately described in the respective analytical method that is applied to a real in-process sample or a dosage form for subsequent HPLC analysis. The analytical procedure must specify the manufacturer, type of filter, and pore size of the filter media. 12The purpose of sample preparation is to create a processed sample that leads to better analytical results compared with the initial sample. The prepared sample should be an aliquot relatively free of interferences that is compatible with the HPLC method and that will not damage the column.[13,15]

2.5 Method optimization

Most of the optimization of HPLC method development has been focused on the optimization of HPLC conditions.[14] The mobile phase and stationary phase compositions need to be taken into account. Optimization of mobile phase parameters is always considered first as this is much easier and convenient than stationary phase optimization. To minimize the number of trial chromatograms involved, only the parameters that are likely to have a significant effect on selectivity in the optimization must be examined. Primary control variables in the optimization of liquid chromatography (LC) methods are the different components of the mobile phase determining acidity, solvent, gradient, flow rate, temperature, sample amounts, injection volume, and diluents solvent type. This is used to find the desired balance between resolution and analysis time after satisfactory selectivity has been achieved. The parameters involved include column dimensions, column-packing particle size and flow rate. These parameters may be changed without affecting capacity factor or selectivity.[10]

2.6 Method Validation

Validation of an analytical method is the process by which it is established by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical application. Validation is required for any new or amended method to ensure that it is capable of giving reproducible and reliable results, when used by different operators employing the same equipment in the same or different laboratories. The type of validation program required depends entirely on the particular method and its proposed applications.[13] Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice. Use of equipment that is within specification, working correctly and adequately calibrated is fundamental to the method validation process. Analytical methods need to be validated or revalidated.[16]

- Before their introduction into routine use;
- Whenever the conditions change for which the method has been validated
- Whenever the method is changed

Typical parameters recommended by FDA, USP, and ICH are as follow.[16,18]

1. Specificity
2. Linearity & Range
3. Precision

- Method precision (Repeatability)
- Intermediate precision (Reproducibility)

4. Accuracy (Recovery)
5. Solution stability
6. Limit of Detection (LOD)
7. Limit of Quantification (LOQ)
8. Robustness
9. System suitability

Specificity :selectivity of an analytical method as its ability to measure accurately an analyte in the presence of interference, such as synthetic precursors, excipients, enantiomers, and known (or likely) degradation products that may be expected to be present in the sample matrix.[17]

Linearity and range: The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of analyte in the sample. A linear relationship should be evaluated across the range of the analytical procedure. It is demonstrated directly on the drug substance by dilution of a standard stock solution of the drug product components, using the proposed procedure. Linearity is usually expressed as the confidence limit around the slope of the regression line.[16-18] For the establishment of linearity, minimum of five concentrations are recommended by ICH guideline[19] The range of an analytical method is the interval between the upper and lower levels that have been demonstrated to be determined with precision, accuracy and linearity using the method.[17]

Precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.[19] The precision of an analytical procedure is usually expressed as the standard deviation or relative standard deviation of series of measurements. Precision may be either the degree of reproducibility or of the repeatability of the analytical procedure under normal conditions. Intermediate precision (also known as ruggedness) expresses within laboratories variations, as on different days, or with different analysts or equipment within same laboratory. Precision of an analytical procedure is determined by assaying a sufficient number of aliquots of a

homogeneous sample to be able to calculate statistically valid estimates of standard deviation or relative standard deviation.[20]

Accuracy (Recovery): The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. lt is determined by applying the method to samples to which known amounts of analyte have been added. These should be analysed against standard and blank solutions to ensure that no interference exists. The accuracy is then calculated from the test results as a percentage of the analyte recovered by the assay. It may often be expressed as the recovery by the assay of known, added amounts of analyte.[18,19]

Solution stability: During validation the stability of standards and samples is established under normal conditions, normal storage conditions, and sometimes in the instrument to determine if special storage conditions are necessary, for instance, refrigeration or protection from light.[18]

Limit of Detection (LOD): Limit of detection (LOD) of an individual procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value. In analytical procedures that exhibit baseline noise, the LOD can be based on a signal-to-noise (S/N) ratio (3:1), which is usually expressed as the concentration of analyte in the sample. The signal-to-noise ratio is determined by: s = H/h Where H = height of the peak corresponding to the component. h = absolute value of the largest noise fluctuation from the baseline of the chromatogram of a blank solution.[18-20]

Limit of Quantification (LOQ): The limit of Quantitation (LOQ) or Quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. For analytical procedures such as HPLC that exhibit baseline noise, the LOQ is generally estimated from a determination of S/N ratio (10:1) and is usually confirmed by injecting standards which give this S/N ratio and have an acceptable percent relative standard deviation as well.[19,20]

Robustness: is defined as the measure of the ability of an analytical method to remain unaffected by small but deliberate variations in method parameters (e.g. pH, mobile phase composition, temperature and instrumental settings) and provides an indication of its

reliability during normal usage. Determination of robustness is a systematic process of varying a parameter and measuring the effect on the method by monitoring system suitability and/or the analysis of samples.[18,19]

System Suitability: System suitability tests are an integral part of liquid chromatographic methods. They are used to verify that the detection sensitivity, resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Factors, such as the peak resolution, number of theoretical plates, peak tailing and capacity have been measured to determine the suitability of the used method.[16-20]

System suitability testing originally believed by the industry of pharmaceuticals to decidewhether a chromatographic system is being utilized day today in a routine manner in pharmaceutical laboratories where quality of results is most important which is suitable for adefinite analysis.

The parameters used in the system suitability tests (SST) report are as follows:

1. Number of theoretical plates or Efficiency (N).
2. Capacity factor (K).
3. Separation or Relative retention (α).
4. Resolution (Rs).
5. Tailing factor (T).
6. Relative Standard Deviation (RSD).

1. Number of theoretical plates/Efficiency (N)

In a specified column, efficiency is defined as the measurement of the degree of peak dispersion and it should have the column characteristics. The efficiency is conveyed in terms of number of theoretical plates". The formula of calculation of N is illustrated bellow in the following

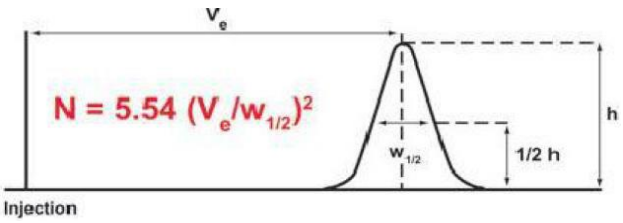


Figure 5: (Half height method).

N = Efficiency / Number of theoretical plates.

Ve = Retention time of analyte.

h = Height of the peak.

w 1/2 = Gaussian function of the peak width at the half- height.

Sigma/tangential method (USP method) With the help of sigma/tangential method N iscalculated which is shown in the following figure 1.2 duly noting the formula for calculation of N.

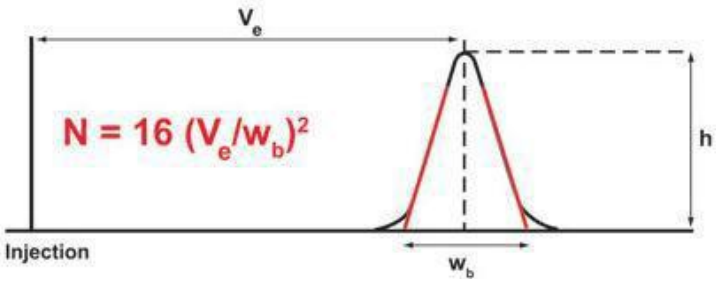


Figure 6: Sigma/tangential method relating to determination of N.

N = Number of theoretical plates.

Ve = elution volume, retention time or retention distance (mL, sec, or cm).

h = peak height.

wb = width of the peak at the base line (mL, sec, or cm).

The plate number depends on column length. Theoretical plate number is the measure of columnefficiency. As stated by plate theory, the analyte will be in instant equilibrium with stationaryphase and column has to be divided into number of hypothetical plates and each plate consists ofa fixed height and analyte spends finite time in the plate. Height equivalent to theoretical plate(HETP) is given by following formula:

$$HETP = L/N,$$

Where, (1) L = length of column. N = plate number

Capacity ratio or Capacity factor (k)

$$K'=tR\text{-}tM/tM$$

The above said capacity factor sometimes is called as a retention factor which has no dimensionand independent from flow rate of mobile phase as well as column dimensions

which is themeasure of extent of retention relating to an analyte relative to an un-retained peak. Where tRimplies retention time of the sample peak and retention time of an un-retained peak is tM. k' = 0means no compound is left in the column. Generally the value of k' is > 2.

Relative retention or separation factor (α)[3]

α = t2-ta/t1-ta

α = Relative retention.

t2= Retention time calculated from point of injection.

ta= Unretained peak time (Retention time (tR) of an inert component not retained by thecolumn).

t1= the retention time from the point of injection of reference peak defined. (Suppose noreference peak is found, value would be zero).

Resolution (Rs) Resolution is the capability of the column to separate 2 drugs in 2 individual peaks or chromatographic zones and it is improved by enhancing column length, reduction of particle size and rising temperature, altering the eluent or stationary phase. It can be told in terms of ratio of separation of the apex of two peaks by the tangential width average of the peaks. By using the following formula resolution is calculated.

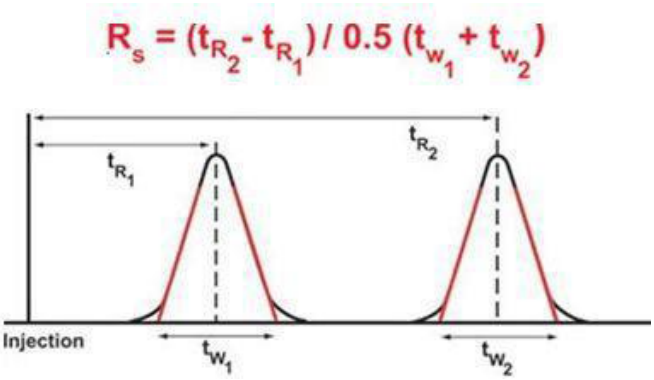


Figure 7: Determination of resolution between two peaks. tR1.

tR1 and tR2 are the retention times for the two peaks of components. tw1 and tw2 = At thebaseline lies between tangents drawn to the sides of the peaks. (Tangents are drawn at 0.6 timesthe peak height). If the peaks are correctly symmetric, provided the valley between the two peaksshould touch the baseline Rs is 1.5. Generally good value of resolution is Rs ≥2 should beadequate and preferred normally.

Resolution factor (R)

Resolution is a function of capacity factor, function of selectivity and a function of efficiency(or) number of theoretical plates (N). In order to separate any two peaks you must have rightcapacity factor ideally between 2 and 10, but appropriate selectivity is required i.e., ideally 1.2 and enough efficiency i.e., number of theoretical plates (more than 2000 theoretical plates).

Resolution should be ≥ 1.5. 1.5 defines baseline resolution.

$R=k'/1+k'(\alpha-1/\alpha)(\sqrt{(N/4)}$

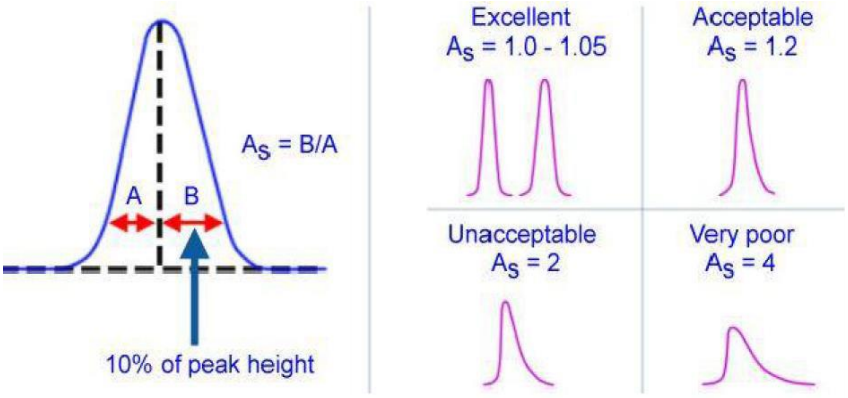
Tailing factor or Asymmetry factor

Chromatographic peak assumed to have a Gaussian shape under ideal conditions. Howeverinpractical conditions, there is always a deviation from normal distribution which indicates non-uniformmigration and non-uniform distribution process. Hence the regulatory organizations like USP and EP have recommended this as one of the system suitability parameter. The asymmetryfactor and tailing factor are roughly same and rarely accurate and equal in most cases. Valuesshould normally between 1.0-1.5 and values greater than 2 are unacceptable. The peakasymmetry is computed by utilizing the following formula.

$$As = B/A \quad (6)$$

Where: As = peak asymmetry factor.

B = distance from the point at peak midpoint to the trailing edge. (Measured at 10 % of peakheight). A = distance from the leading edge of peak to the midpoint. (Measured at 10 % of peakheight). Ideally, peaks should be Gaussian in shape or totally symmetrical. Determination oftailing and asymmetric factor is shown in Figure 1.5.



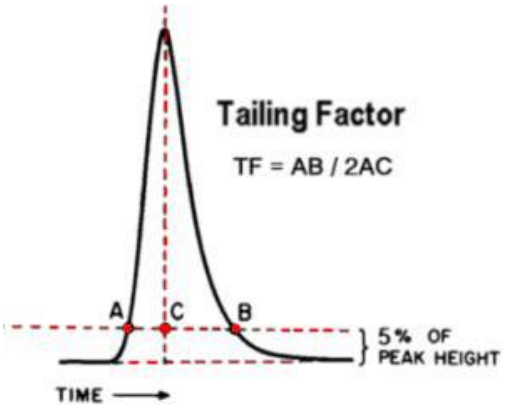


Figure 8: Determination of tailing and asymmetric factor.

2. THE PURPOSE AND SCOPE OF THE CURRENT WORK

1. Ceftazidime and Avibactam dose forms should be measured concurrently using the RP-HPLC method.
2. The procedure needs to pass ICH guidelines-compliant testing.

WORK-PLAN

- The suggested experimental work was as follows:
- The expansion of the novel analytical RP-HPLC procedure, Ceftazidime and Avibactam combination, was created by analysing the compositions on Ceftazidime and Avibactam with regard to their physical and chemical characteristics.

- **DEVELOPMENT OF THE RP- HPLC METHOD**

1. Selection of the mobile phase and the diluent solvent

choosing a stable solvent that allows the substance to dissolve.

Cost-effective, user-friendly, and HPLC-calibrated.

2. Mobile step choice

- The initial step in the mobile process is selecting an eluent type, either aqueous or organic. For the RP-HPLC procedure, an aqueous eluent or an exceptionally polar organic solvent, like methanol or acetonitrile, is required. If the K values are too high for an aqueous solvent, think about employing an organic one.
- A combination of two different kinds of solvents should be used to assess the separation if the organic solvent's K value is extremely low.

- The elution time of non-retained components, or K' capacity factor, assess how long the peak of interest is positioned in the void volume. K' frequently has a number higher than 2.
- If a buffer is employed, its pH and ionic strength can be tested.

3. To choose the wavelength at which the research will be carried out, the drug's ultraviolet absorption spectra will be thoroughly examined.
4. Examination of the drug's physicochemical makeup and structure; selecting the chromatography's operating parameters.
5. Choosing a research methodology for quantitative chromatography. figuring out the scope of attention at work.
6. verification of the approach created in compliance with ICH standards.

3. LITERATURE REVIEW[21,22,23,24,25]

Monika R Dumbre, Aishwarya P Walke, Shreyash P Vyavahare, Pranav S Pawar, Shubham U Gholap Students, Samarth College of Pharmacy, Belhe, Pune, Maharashtra, India.

Ceftazidime-avibactam is an intravenously administered combination of the third-generation cephalosporin ceftazidime and the novel, non-β-lactam β-lactamase inhibitor avibactam. ceftazidime-avibactam is approved for the treatment of adults with complicated urinary tract infections, complicated intra-abdominal infections, hospital-acquired pneumonia (HAP), and other infections caused by aerobic. This article discusses the in vitro activity and pharmacological properties of ceftazidimeavibactam, and reviews data on the agent’s clinical efficacy and tolerability relating to use in these indications. Ceftazidimeavibactam has excellent in vitro activity against many important Gram-negative pathogens, including many extendedspectrum. It is not active against metallo-β-lactamase-producing strains. Ceftazidime-avibactam treatment was associated with high response rates at the test-of-cure visit in patients with infections caused by ceftazidime-susceptible and - nonsusceptible Gram-negative pathogens. Ceftazidime-avibactam was generally well tolerated, with a safety and tolerability profile consistent with that of ceftazidime alone and that was generally typical of the injectable cephalosporins. Thus, ceftazidime-avibactam represents a valuable new treatment option for these serious and difficult-to-treat infections. Objective: Ceftazidime/avibactam resistance due to mutation in the omega loop of KPC-2 has been documented in vitro and in vivo. This study evaluated the mechanism of ceftazidime/avibactam resistance in a KPC-2-expressing Klebsiella pneumoniae isolated from

a patient following ceftazidime/avibactam combination therapy with gentamicin for the treatment of ventilator-associated pneumonia.

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Background:Literature review reveals that there is no analytical method reported for the analysis of Avibactam and ceftazidime by simultaneous estimation by RP-HPLC. Titrimetric analysis, HPLC and HPTLC are the reported analytical methods for compounds either individually or in combination with other dosage form. A simple reverse phase high performance liquid chromatography (RP-HPLC) method has been developed and subsequently validated for simultaneous determination of Avibactam and Ceftazidime in combined dosage form. Ceftazidime and Avibactam is used to treat such gram – negative infection that are multidrug – resistant. Materials and methods: The separation was carried out using mobile phase consisting of potassium dihydrogenortho phosphate buffer of pH 3.0 and Methanol in the ratio of 30:70 v/v. The column used is InertsilODS (5 µm, 4.6 X 150 mm) with flow rate of 1.0 ml/min using PDA detection at 260 nm. The calibration curves were linear over a concentration range of 1-5 µg/mL and 100-500 µg/mL for Avibactam and Ceftazidime. The retention times of were found to be 3.7 min and 2.05 min respectively. Results of analysis were validated statistically and by recovery studies. The LOD and LOQ value for Avibactam and Ceftazidime was found to be 3, 2.9ppm and 10.1, 10.03ppm respectively. Results: The linear regression coefficient, slope and intercept of were found to be 0.997, 0999, 3003, 28847, 37313, 38353 respectively. The results of the study showed that the proposed RP-HPLC method is rapid, specific, precise and accurate and is useful for the routine analysis of Avibactam and Ceftazidime in bulk drug and in its pharmaceutical dosage form. Conclusion: The results obtained on the validation parameters met ICH and USP requirements. it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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A delicate, fast, Accurate, exact and steadiness, showing isocratic RP-UPLC method was developed for the concurrent assurance of the Ceftazidime and Avibactam in bulk and formulation. To optimize, a column HSS C18 100 x 2.1mm, 1.8µm, Mobile phase containing water: acetonitrile choose in the ratio 75:25v/v was pumped through column at a flow rate of 0.3ml/min at 260nm, initiate to be an efficient method for elution of drug with good peak shapes as well as retention times. Rt of Avibactam and Ceftazidime were initiate to be 1.463 min and 2.109 min. %Recovery was obtained as 100.07% and 100.08% for Avibactam and Ceftazidime separately. LOD, LOQ values got from relapse conditions of Avibactam and Ceftazidime were 0.85, 2.56 and 3.53, 10.70 correspondingly. Regression equation of Ceftazidime is y = 7883.2x + 12277, and y = 3279.1x + 1137 of Avibactam.

Shaik Mahammad Noorulla and Sadath Ali, Department of Pharmaceutical Analysis and Quality Assurance, Deccan School of Pharmacy, Hyderabad, India.

The purpose of the investigation was to develop a new HPLC method for simultaneous estimation of Ceftazidime and Avibactum in pharmaceutical dosage forms. Chromatography was carried out on an particle size with a isocratic mobile phasemODS 250mm x 4.6 mm, 5 composed of Buffer, Acetonitrile and methanol at a flow rate of 1mL/min. The column temperature was maintained at 30°C and the detection was carried out using a PDA detector at 260 nm. Validation parameters such as system suitability, linearity, precision, accuracy, specificity, limit of detection (LOD), limit of quantification (LOQ), Stability of sample and standard stock solutions and robustness were studied as reported in the International Conference on Harmonization guidelines. The retention times for Ceftazidime and Avibactum and were 2.221 min and 3.725 min respectively. The percentage recoveries of Ceftazidime and Avibactum were 100.51 %and 100.56% respectively. The relative standard deviation for assay found to be less than 2%. The method is fast, accurate, precise and sensitive hence it can be employed for routine quality control of both drugs in quality control laboratories and pharmaceutical industries.

Parag A. Pathade, Amol H. Jogdand*, Bhaskar O. Aher and Vinod A. Bairagi

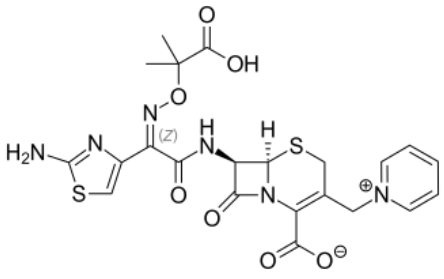
m. The Mobile phase combination used was 0.1% Perchloric acid and Acetonitrile (80:20). Flow rate at 0.5 ml/min and wavelength at 230 nm with run time of 20 minutes. The retention time of Ceftazidime (CEF) and Avibactam (AVB) peaks was at 4.42 and 6.05 minutes, respectively. The developed method was validated according to ICH Q2 (R1) guidelines. The instrument precision for CEFmA precise and robust method was developed method for the

estimation of Ceftazidime and Avibactamin bulk and pharmaceutical dosage form. The Method used Agilent 1260 Infinity II model HPLC with DAD detector and Agilent Zorbax Bonus RP Column with dimension 250 x 4.6 mm, 5 & AVB had a %RSD of 0.24% and 0.12%, respectively. The Intra & Inter day precision for CEF & g/ml for CEFmg/ml and 40-60mAVB had a %RSD of 0.39% and 0.19%, respectively. Method was linear and accurate for concentration range of 160-240 & AVB respectively, with regression coefficient of 0.999 for both CEF & AVB and % RSD for accuracy for CEF at 80%, 100% and 120% was found to be 0.89%, 0.11% and 0.36%, respectively; and for AVB at 80%, 100% and 120% was found to be 0.14%, 0.09% and 0.15% respectively. The LOD & g/ml respectively and the LODmg/ml and 11.19mLOQ for CEF are 3.69 & g/ml respectively.mg/ml and 4.60 mLOQ for AVB are 1.52.

4. DRUG PROFILE[26,27,28,29]

Ceftazidime

Building



Formula Chemical: $C_{22}H_{22}N_6O_7S_2$

Molar mass: 546.57 g·mol^{-1}

Pharmacodynamics

Ceftazidime is a semisynthetic, broad-spectrum, third-generation cephalosporin antibiotic that is bactericidal through inhibition of enzymes responsible for cell-wall synthesis, primarily penicillin-binding protein 3 (PBP3). Among cephalosporins, ceftazidime is notable for its resistance to numerous β-lactamases and its broad spectrum of activity against Gram-negative bacteria, including Pseudomonas aeruginosa. However, it is less active than first- and second-generation cephalosporins against Staphylococcus aureus and other Gram-positive bacteria and also has low activity against anaerobes. Ceftazidime has confirmed activity against clinically relevant Gram-negative bacteria including Citrobacter spp., Enterobacter spp., Klebsiella spp., Proteus spp., Serratia spp., _Escherichia coli, Haemophilus influenzae,

Neisseria meningitidis, Pseudomonas aeruginosa, and some Gram-positive bacteria including Staphylococcus spp. and Streptococcus spp. There are also in vitro data for ceftazidime efficacy against a wide variety of other bacteria, such as Acinetobacter baumannii and Neisseria gonorrhoeae, but no clear clinical studies to support the use of ceftazidime for infections caused by these bacteria.

Although β-lactam antibiotics like ceftazidime are generally well tolerated, there remains a risk of serious acute hypersensitivity reactions, which is higher in patients with a known allergy to ceftazidime or any other β-lactam antibiotic. As with all antibiotics, ceftazidime may result in the overgrowth of non-susceptible organisms and potentially serious effects including Clostridium difficile-associated diarrhea (CDAD); CDAD should be considered in patients who develop diarrhea and, in confirmed cases, supportive care initiated immediately. Ceftazidime is primarily renally excreted such that high and prolonged serum concentrations can occur in patients with renal insufficiency, leading to seizures, nonconvulsive status epilepticus (NCSE), encephalopathy, coma, asterixis, neuromuscular excitability, and myoclonia. Treatment may lead to the development or induction of resistance with a risk of treatment failure. Periodic susceptibility testing should be considered, and monotherapy failure may necessitate the addition of another antibiotic such as an aminoglycoside. Cephalosporin use may decrease prothrombin activity, which may be improved by exogenous vitamin K. Inadvertent intra-arterial administration of ceftazidime may result in distal necrosis.

Mechanism of action

The bacterial cell wall, which is located at the periphery of Gram-positive bacteria and within the periplasm of Gram-negative bacteria, comprises a glycopeptide polymer synthesized through cross-linking of glycans to peptide stems on alternating saccharides, which is known commonly as peptidoglycan. Cell wall formation, recycling, and remodelling require numerous enzymes, including a family of enzymes with similar active site character despite distinct and sometimes overlapping roles as carboxypeptidases, endopeptidases, transpeptidases, and transglycosylases, known as "penicillin-binding proteins" (PBPs). The number of PBPs differs between bacteria, in which some are considered essential and others redundant. In general, inhibition of one or more essential PBPs results in impaired cell wall homeostasis, loss of cell integrity, and is ultimately bactericidal.

Ceftazidime is a semisynthetic third-generation cephalosporin with broad activity against numerous Gram-negative and some Gram-positive bacteria. Like other β-lactam antibiotics, ceftazidime exhibits its bactericidal effect primarily through direct inhibition of specific PBPs in susceptible bacteria. In vitro experiments in Gram-negative bacteria such as Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii, and Klebsiella pneumoniae suggest that ceftazidime primarily binds to PBP3, with weaker binding to PBP1a/1b and PBP2 as well; although binding to other PBPs, such as PBP4, is detectable, the concentrations required are much greater than those achieved clinically.4,5,6,7,8 Similarly, ceftazidime showed binding to Staphylococcus aureus PBP 1, 2, and 3 with a much lower affinity for PBP4.5 Recent data for Mycobacterium abcessus suggest that ceftazidime can inhibit PonA1, PonA2, and PbpA at intermediate concentrations.

Absorption

Ceftazidime administered intravenously in healthy males produced mean Cmax values of between 42 and 170 μg/mL for doses between 500 mg and 2 g, and are reached immediately following the end of the infusion period. The Cmax for 1 g of ceftazidime administered intramuscularly is attained approximately one hour following injection and is between 37 and 43 mg/L.4 Following intramuscular administration of 500 mg and 1 g of ceftazidime, the serum concentration remained above 4 μg/mL for six and eight hours, respectively.

Ceftazidime Cmax and AUC show linear proportionality to the dose over the therapeutic range. In individuals with normal renal function, ceftazidime given intravenously every eight hours for 10 days as either 1 or 2 g doses showed no accumulation.

Half-life

Ceftazidime has an elimination half-life of 1.5-2.8 hours in healthy subjects. As ceftazidime is primarily renally excreted, its half-life is significantly prolonged in patients with renal impairment. In patients with creatinine clearance < 12 mL/min, the half-life is prolonged to between 14 and 30 hours.

Metabolism

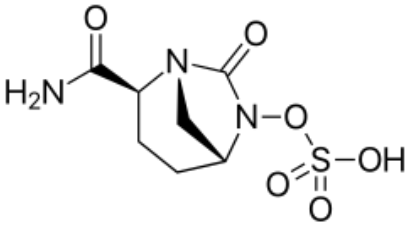
Ceftazidime is not appreciably metabolized.

Route of elimination

Approximately 80% to 90% of an intramuscular or intravenous dose of ceftazidime is excreted unchanged by the kidneys over a 24-hour period. When administered intravenously, 50% of the dose appears in the urine within two hours, with another 32% of the dose appearing by eight hours post-administration.

Avibactam

Building



Chemical formula: $C_7H_{11}N_3O_6S$

Molar mass: 265.24 $g \cdot mol^{-1}$

Mechanism of action

Avibactam is a non-β lactam β-lactamase inhibitor that inactivates some β-lactamases (Ambler class A β-lactamases, including Klebsiella pneumoniae carbapenemases, Ambler class C and some Ambler class D β-lactamases) by a unique covalent and reversible mechanism, and protects ceftazidime from degradation by certain β-lactamases. Avibactam rapidly reaches the periplasm of bacteria at high enough concentrations to restore activity of ceftazidime against ceftazidime-resistant, β-lactamase-producing strains. Avibactam does not decrease the activity of ceftazidime against ceftazidime susceptible organisms.

Volume of distribution

The steady state volumes of distribution of avibactam and ceftazidime is 22.2L and 17L respectively.

Half-life

Ceftazidime-avibactam has a half life of ~2.7-3.0 hours.

Metabolism

No metabolism of avibactam was observed in human liver preparations. Unchanged

avibactam is the major drug-related component in human plasma and urine. 80-90% of ceftazidime is eliminated as unchanged.

Route of elimination

Avibactam and ceftazidime are excreted mainly by the kidneys.

5. MATERIALS AND METHODS

Tools-Instruments

- HPLC – Waters Model NO.2690/5 Compact System consisting of panel with Inertsil-C18 ODS.
- Electronic equilibrium (SARTORIOUS)
- Sonicator(CLEAN FAST)

Chemical Substances

- Methanol grade HPLC.
- HPLC Class buffer (KH2PO4).

Raw Stock

Standards in dealing with Ceftazidime and Avibactam.

5.1 METHOD FOR HPLC DEVELOPMENT

The goal of this endeavor was to enhance the test procedure for the simultaneous estimate of the Avibactam and Ceftazidime literature study. Consequently, the following trials provide an outline of the optimization process.

Trial: 1

Mobile Phase: Water : Degassed Acetonitrile 90:10.

Preparing the Standard Solution

After weighing 10 mg of the medications Avibactam and Ceftazidime, 10 ml of mobile phase was added to each of two distinct 10 ml volumetric flasks. After 20 minutes of sonication to achieve 1000 ppm, 1 ml of each solution was taken and diluted with 10 ml of mobile phase.

Requirements for chromatography

Flow speed: 1.0ml / min

Column: Inertsil-C18, plate ODS

Wave length measuring device:275nm

speed of the column: Ambient

The injection's size: 20µl

Time to leave: 10min

Retaining period : Ceftazidime for 2.801min and Avibactam for 3.190.

Observation: The two summits are partially merged together. Fig. 1 displays the results of the trial 1 chromatogram.

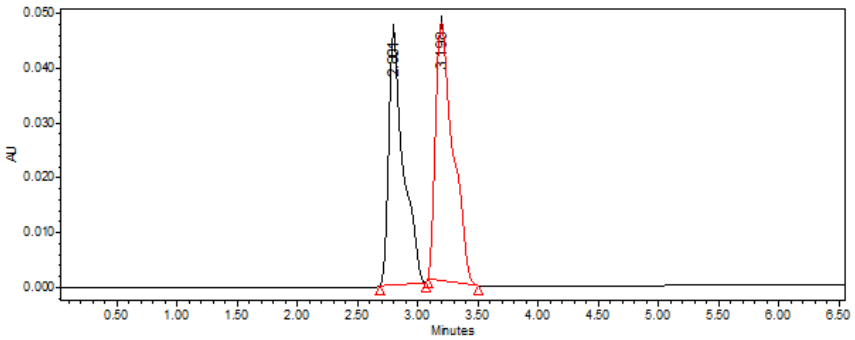


Fig. 1: Chromatography in trial 1.

Inference: Peak forms are undesirable.

S.NO	Peak's Name	Retention time (min)
1	**Ceftazidime**	2.801
2	**Avibactam**	3.190

Trail: 2

cellular phase: Water and methanol were degassed in a 45:55 V/V ratio.

preparing the Standard Solution

Weigh and dissolve 10 mg of Avibactam and Ceftazidime in 10 ml of mobile phase. To obtain 1000 ppm, split the liquid into two 10 ml volumetric flasks and sonicate for 20 minutes. Then, we took 1ml of each solution and diluted it to 10ml with mobile phase.

Requirements for chromatography

Flow speed : 1.0ml / min

Column: Inertsil-C18, plate ODS

Wave length measuring device :275nm

Speed of the column: Ambient

The injection's size : 20µl

Time to leave : 10min

Retaining period : Ceftazidime for 3.865min and Avibactam for 4.290min.

Observation: Despite the perfect separation between the two peaks, the peak kinds are unpleasant. Fig. 2 displays the results of the trial 2 chromatogram.

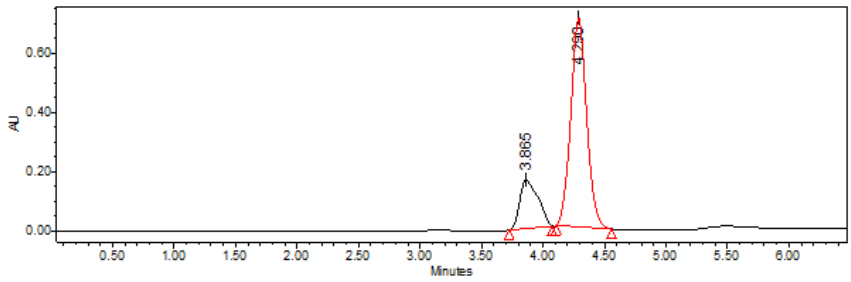


Fig. 2: Chromatography in trial 2.

Inference: Shapes of peaks aren't healthy.

S.NO	Peak's Name	Retention time (min)
1	**Ceftazidime**	3.865
2	**Avibactam**	4.290

Trail: 3

Cellular phase: Acetonitrile and methanol were degassed at a 10:90 V/V ratio.

Standard Solution preparation

To obtain 1000 parts per ml, weigh 10 mg of Avibactam and Ceftazidime, dissolve them in 10 ml of mobile phase, divide the mixture between two 10 ml volumetric flasks, then sonicate the mixture for 20 minutes. Next, we used mobile phase to dilute 1 ml of each solution to 10 ml.

Requirements for chromatography

Flow speed : 1.0ml / min

Column : Inertsil-C18, plate ODS

Wave length measuring device : 275nm

speed of the column : Ambient

The injection's size : 20µl

Time to leave : 10min

Retaining period : Ceftazidime for 2.902 min and Avibactam for 3.618 min.

Observation: Different peaks entirely, but bonus point. The outcomes of the three-chromatogram trial were displayed in Figure 3

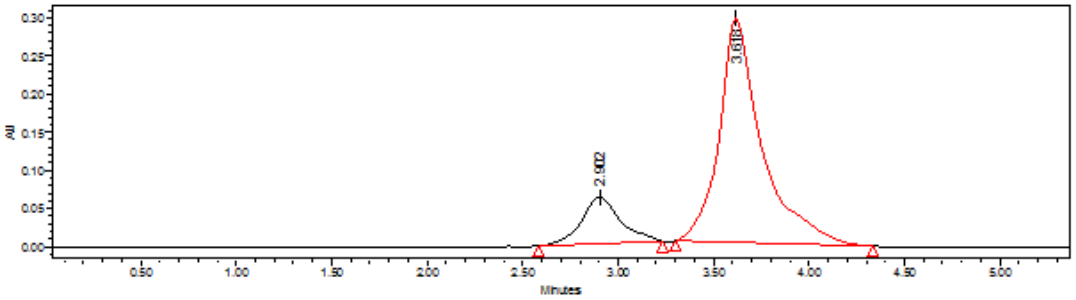


Fig. 3: Chromatography test 3.

Inference: Although peaks vary, their form is not appealing.

S.NO	Peak's Name	Retention time (min)
1	**Ceftazidime**	2.902
2	**Avibactam**	3.618

6. RESULTS AND DISCUSSIONS

6.1 ADVANCED METHOD (OPTIMIZED METHOD)

Cellular phase: Methanol and Acetonitralwere degassed at a 70:30 V/V ratio.

Preparation of a stock solution

Reference remedy

In order to prepare the solution, two volumetric flasks with a volume of 100.0 mL each were filled with 125.0 mg of Avibactam and 120.0 mg of precisely weighed Ceftazidime then sonicated for 20 minutes.Take 10.0 mL of each of the solutions and place them in a 100.0 mL volumetric flask. Next, add mobile phase, and after 10 minutes, sonicate the mixture.

The creation of a Standard Working Solution

Along with the previously mentioned Ceftazidime and Avibactam, For each medically, stock solutions varying in ranging from 20 to 80 ppm were made, sonicated, and filtered through a 0.45 membrane.

Optimised Chromatography Conditions

Parameters	Method
Stage of Stationary (column)	Inertsil -ODS C_{18}(250 x 4.6 mm, 5 µ)
Mobile Phase	Methanol : Acetonitral (70:30)
Flow rate (ml/min)	1.0 ml/min
Duration of operation (minutes)	10 min

Temperature in the column (°C)	Ambient
Volume of injection loop (ml)	20
Wavelength of detection (nm)	275nm
Drug RT (min)	4.250 min for Ceftazidime and 5.994 for Avibactam.

6.1 Method development

OPTIMIZED METHOD

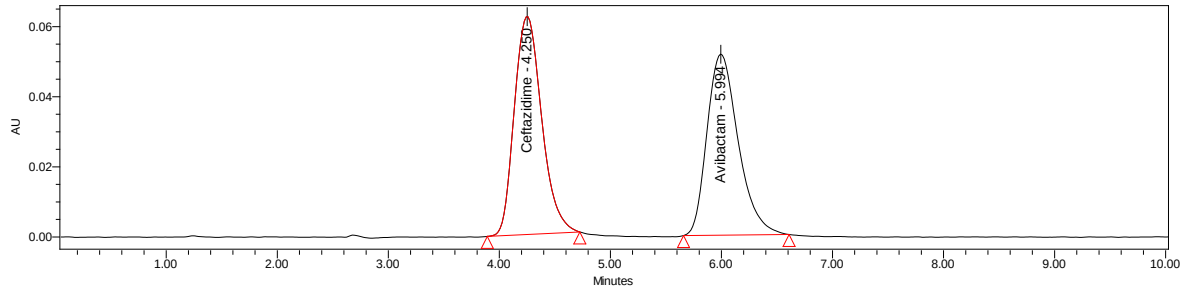


Fig. 4: Conventional Chromatogram.

Inference: With RTs of 4.250 minutes for Ceftazidime and 5.994 minutes for Avibactam, a chromatogram was obtained.

S.NO	Peak's Name	Retention time(min)
1	**Ceftazidime**	4.250
2	**Avibactam**	5.994

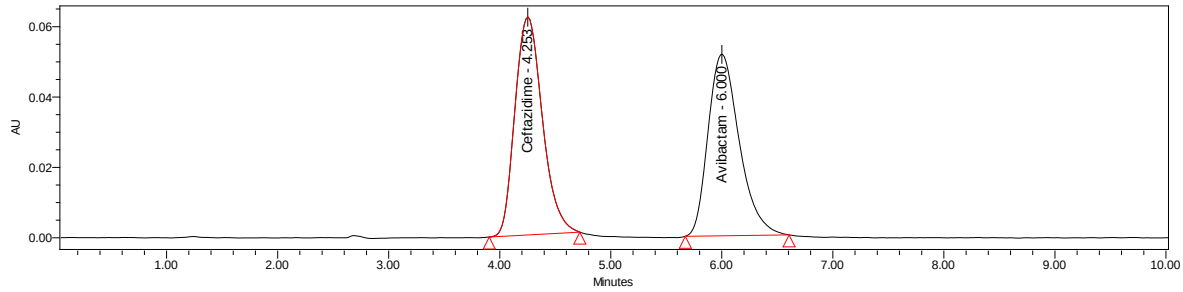


Fig. 5: Chromatogram of standard 2.

Inference: received the same chromatogram and RT values as standard 2.

S.NO	Peak's Name	Retention time(min)
1	**Ceftazidime**	4.253
2	**Avibactam**	6.000

6.3 DATA-VALIDATION(VALIDATION DATA)

6.3.1 SYSTEM ACCOMPANY(SYSTEM SUITABILITY)

Validation Stock Solution Preparation

Solution A: Take 125mg Ceftazidime working standard in 100ml V.F add methanol sonicate it 30minets, (That is 1000ppm solution).

Solution B: Take 120mg Avibactam working standard in 100ml V.F add methanol sonicate it 30minets, (That is 1000ppm solution).

Validation Parameters Solutions Preparation: Take 2ml of each above solutions **A & B** in 100ml V.F add Methanol up to mark sonicate it 10minets (That 20ppm solution).

Take 3ml of each above solutions **A & B** in 100ml V.F add Methanol up to mark sonicate it 10minets (That 30ppm solution).

Take 4ml of each above solutions **A & B** in 100ml V.F add Methanol up to mark sonicate it 10minets (That 40ppm solution).

Take 5ml of each above solutions **A & B** in 100ml V.F add Methanol up to mark sonicate it 10minets (That 50ppm solution).

Take 6ml of each above solutions **A & B** in 100ml V.F add Methanol up to mark sonicate it 10minets (That 60ppm solution).

Take 7ml of each above solutions **A & B** in 100ml V.F add Methanol up to mark sonicate it 10minets (That 70ppm solution).

Take 8ml of each above solutions **A & B** in 100ml V.F add Methanol up to mark sonicate it 10minets (That 80ppm solution).

Table 1(a): Device Eligibility Information for Ceftazidime (Data of System Suitability for Ceftazidime).

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	4.253	7634799	6452.5421	1.28754
2	4.248	7638436	6421.3458	1.24512
3	4.249	7630927	6474.2186	1.22485
4	4.250	7639528	6488.1744	1.27841
5	4.251	7633962	6471.4152	1.25921

Mean	4.2502	763875.564	6420.8742	1.247851
SD	0.001924	8853.254	-------	-------
% RSD	0.045258	0.5478	-------	-------

Table 1(b): Device Eligibility Information for Avibactam (Data of System Suitability for Avibactam).

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	6.000	515623	15231.845	1.15124
2	5.996	515894	15292.721	1.18749
3	5.998	515118	15225.754	1.17877
4	6.002	515236	15742.816	1.12460
5	6.002	515096	15236.789	1.18744
Mean	5.9996	515876.234	15237.897	1.16334
SD	0.002608	2354.8745	-------	-------
% RSD	0.043464	0.7845	-------	-------

Inference: Suitability of the Device Standard Chromatogram-1

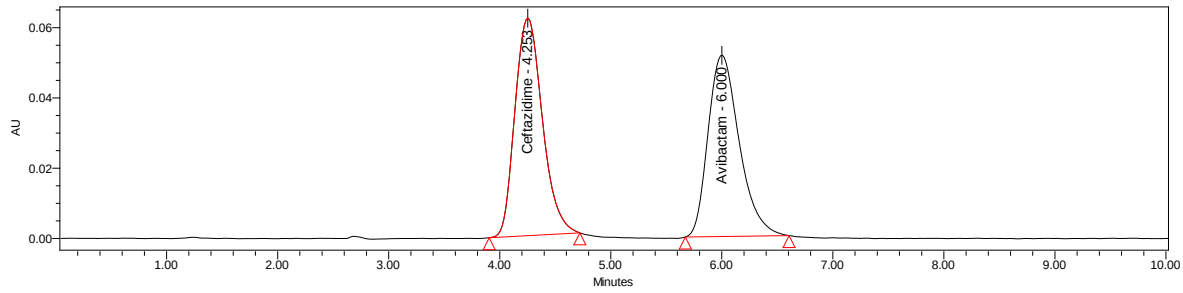
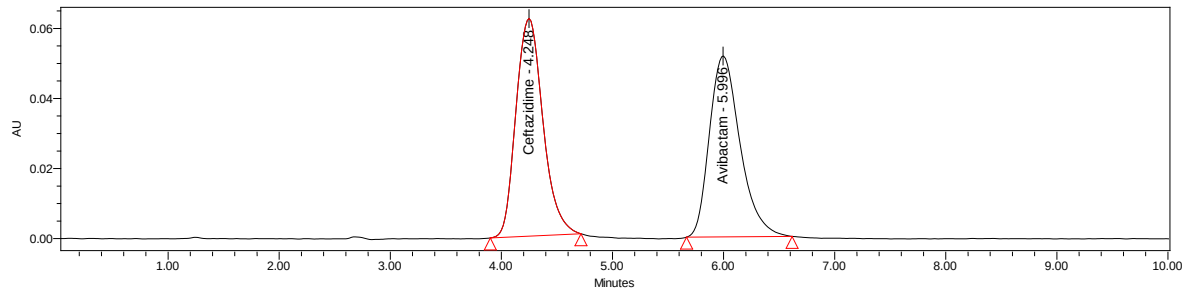
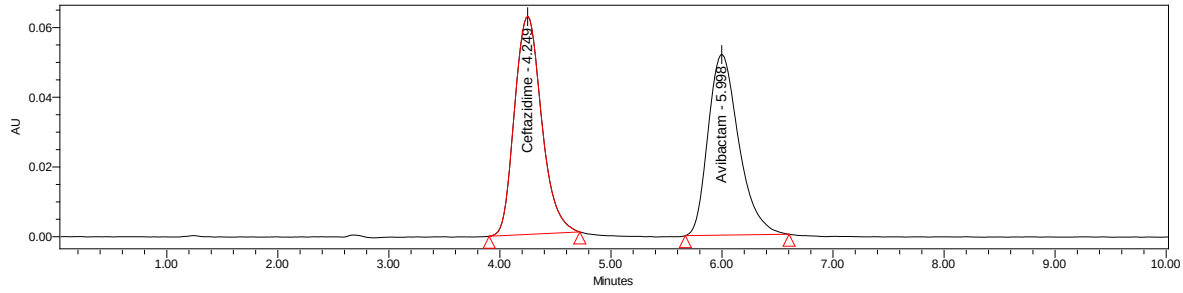


Fig. 6-10: chromatograms compatible with devices (Standards 1–5).

Inference: Suitability of the Device Standard Chromatogram-2

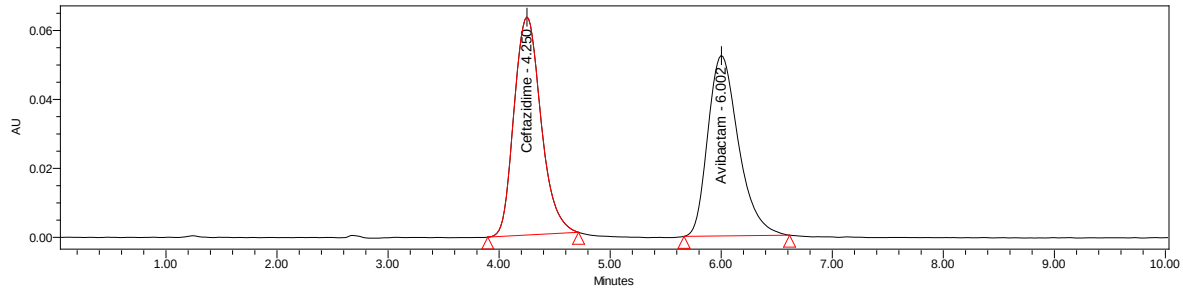


Inference: Suitable apparatus for Chromatogram-3

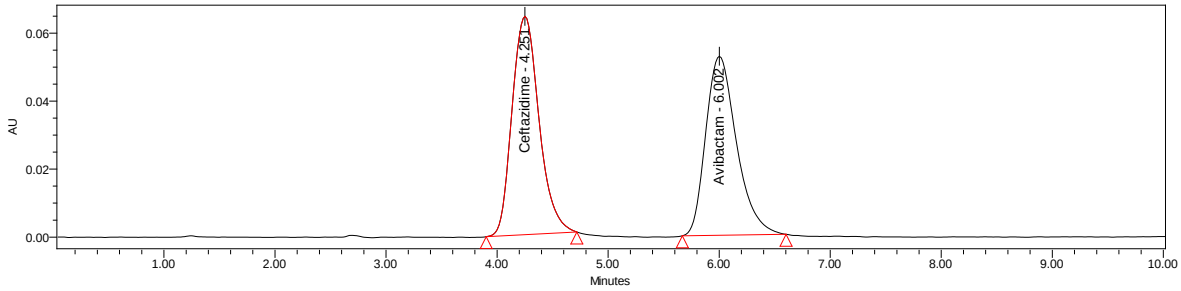


Standards

Inference: Device compatibility with the common Chromatogram-4.



Inference: Suitability of the Device Standard Chromatogram-5



6.3.2: SPECIFICE(SPECIFICITY)

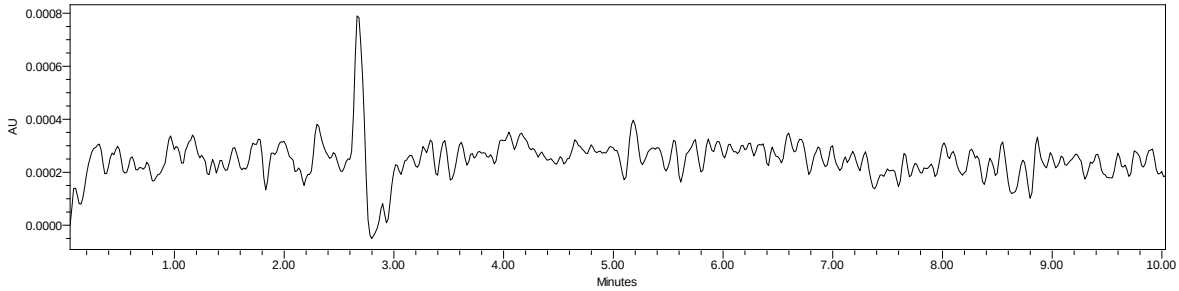


Fig. 11: Chromatogram of Blank.

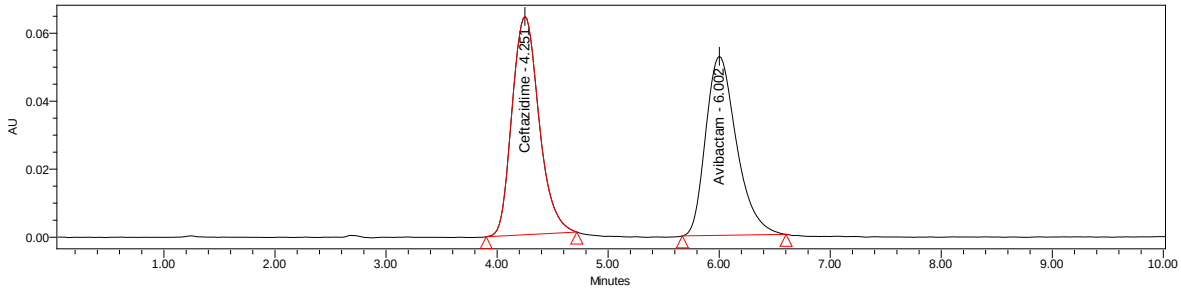


Fig. 12: Std. chromatogram.

Inference: Ceftazidime and Avibactam each had a peak for the sample at a Rt of 4.256 and 6.002 minutes, respectively.

6.3.2: PREQUIRE(PRECISION)

6.3.2.1 Repetition(Repeatability)

(a) System Precise Precise device(System precision)

Table 2(i): Data on repeatability (system precision) for Ceftazidime.

	Injection	Peak Areas of Ceftazidime	%Assay
Concentration 40ppm	1	7632451	99.25
	2	7636845	99.81
	3	7637241	99.25
	4	7638453	100.19
	5	7637128	100.17
Statistical **Analysis**	**Mean**	7637049	99.68
	SD	6546.8751	0.57542
	% RSD	0.9237541	0.561921

Table 2(ii): Avibactam Repeatability Data (System Precise).

	Injection	Peak Areas of Avibactam	%Assay
Concentration 40ppm	1	515012	99.68
	2	515874	99.28
	3	515087	99.23
	4	515781	100.66
	5	515567	100.18
Statistical **Analysis**	**Mean**	515764.4	100.12
	SD	3235.274	0.69871
	% RSD	0.928541	0.8754

Inference: Systems precision chromatograph (standard-1)

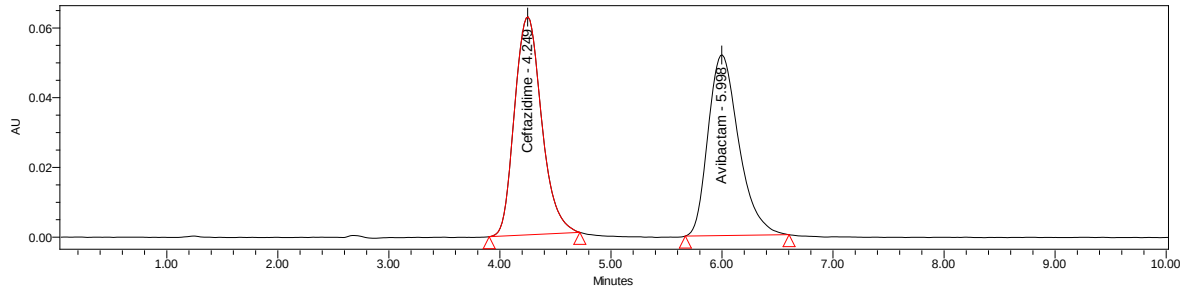
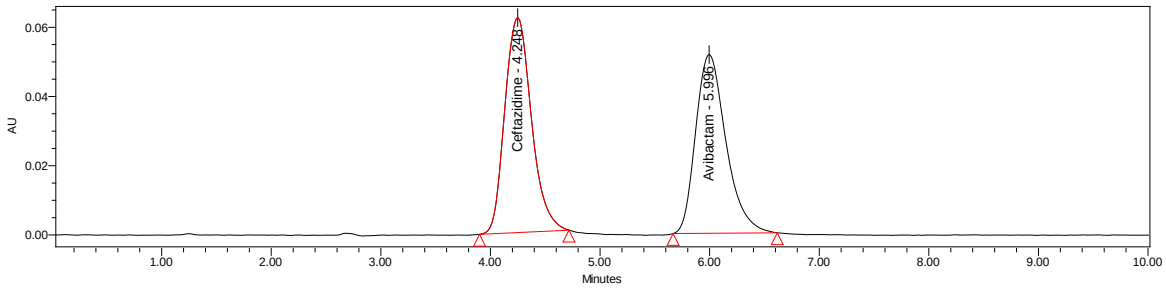
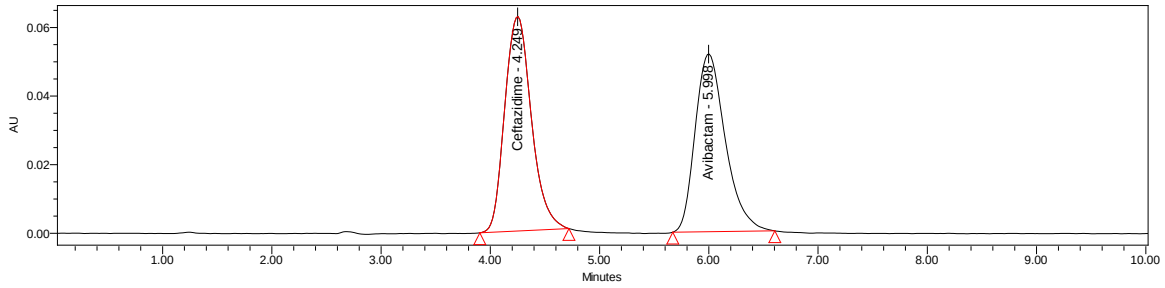


Fig. 13-17: Systems precise chromatograms.

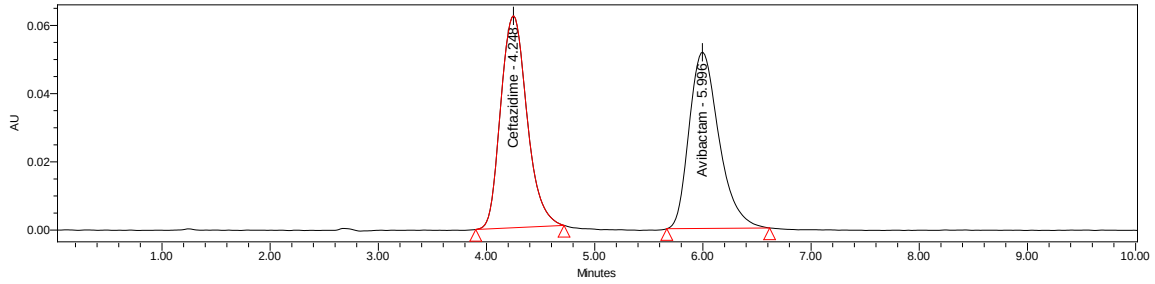
Inference: Devices precision chromatograph (standard-2)



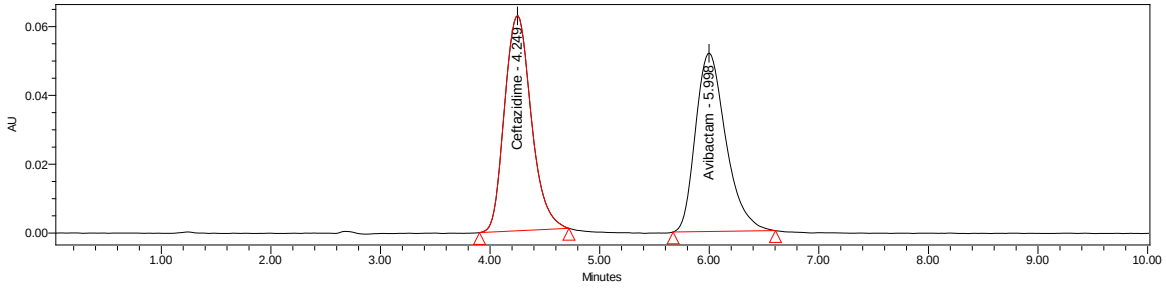
Inference: Devices precision chromatograph (standard-3)



Inference: Systems precision chromatograph (standard-4)



Inference: Devices precision chromatograph (standard-5)



b) Precision method (Method precision)

Table 3(i): Repeatability data (Precision method) for Ceftazidime.

	Injection	Peak Areas of Ceftazidime	%Assay
Concentration 40ppm	1	7637856	99.87
	2	7638921	99.64
	3	7637328	99.87
	4	7639875	99.85
	5	7637591	99.92
	6	7639057	99.66
Statistical **Analysis**	**Mean**	7639571.231	99.85
	SD	2254.321	0.78471
	% RSD	0.87541	0.665421

Table 3(ii): Repeatability data (Precision method) for Avibactam.

	Injection	Peak Areas of Avibactam	%Assay
Concentration 40ppm	1	515547	99.86
	2	515781	99.24
	3	515634	100.45
	4	515781	100.23
	5	515089	100.18
	6	515574	100.23
Statistical **Analysis**	**Mean**	515873.321	100.23
	SD	2684.2314	0.84745
	% RSD	0.8754	0.657421

Inference: Repeatability chromatograph (Standard-1)

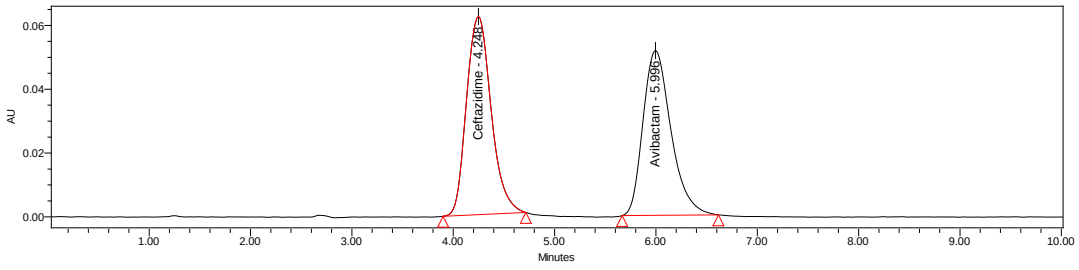
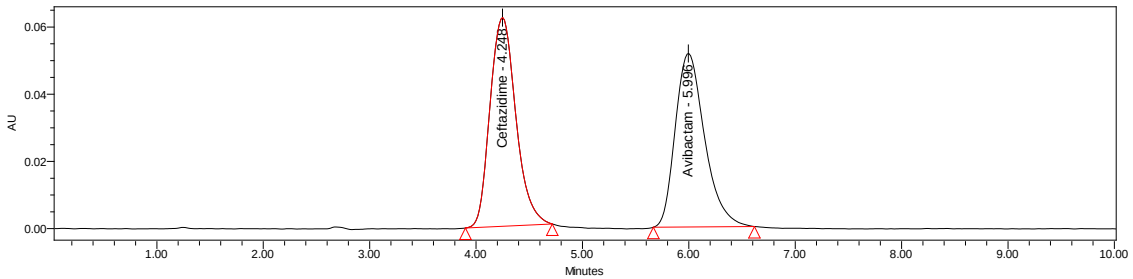
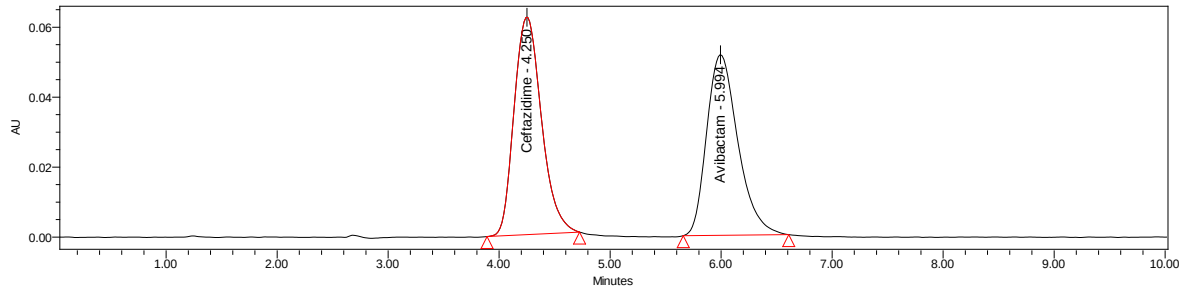


Fig. 18-23: Repeatable Chromatograms.

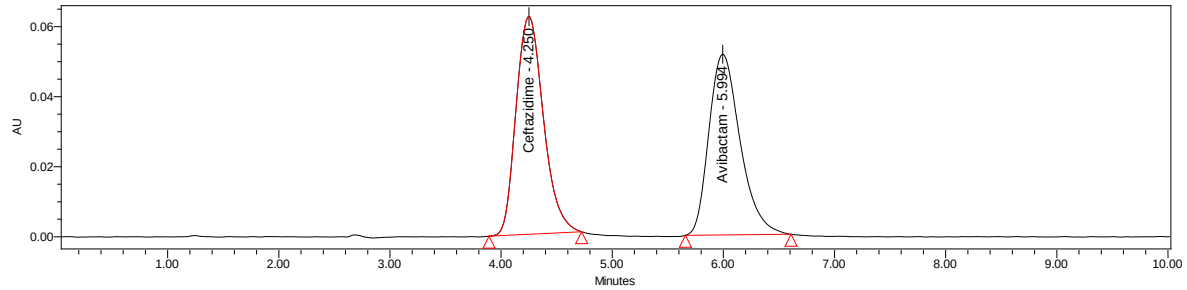
Inference: Repeatability chromatogram (standard -2)



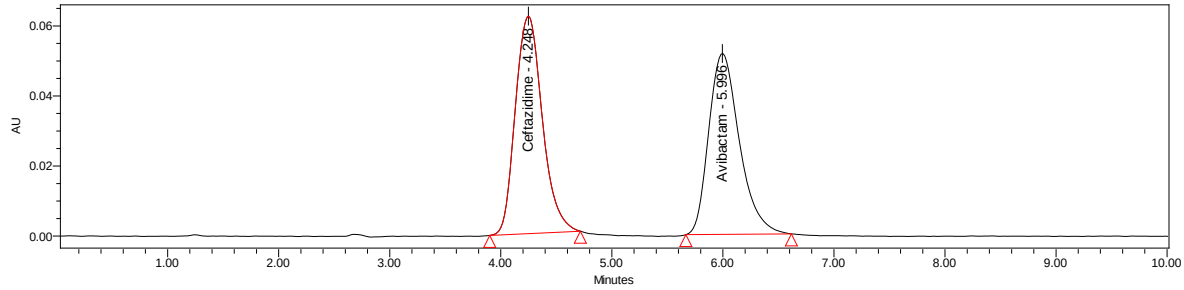
Inference: Repeatability Chromatograph (standard-3)



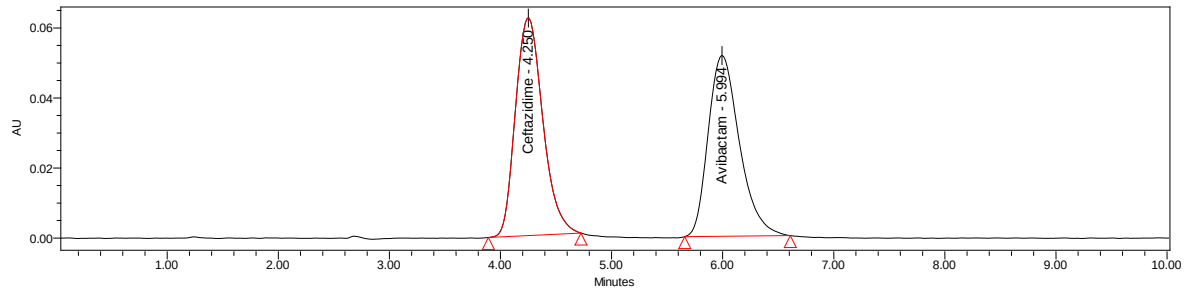
Inference: Repeatability chromatograph (Standard-4)



Inference: Repeatability Chromatograph (standard-5)



Inference: Repeatability chromatogram (Standard-6)



6.3.2.2 In-between precision (Intermediate precision)

For **Analyst 1** ref: Table3.

Table 4: Data of Intermediate precision (Analyst 2) for Ceftazidime.

	Injection	Peak Areas of Ceftazidime	%Assay
Concentration 40ppm	1	7633587	99.85
	2	7630845	99.95
	3	7633845	99.87
	4	7635874	100.07
	5	7633802	101.08
	6	7639278	99.74
Statistical **Analysis**	**Mean**	7633745.234	99.86
	SD	6541.2574	0.567781
	% RSD	0.87441	0.874542

(i) Data of Intermediate precision (Analyst 2) for Avibactam

	Injection	Peak Areas of Avibactam	%Assay
Concentration 40ppm	1	515481	99.91
	2	515951	99.74
	3	515830	100.63
	4	515479	100.75
	5	515087	99.25
	6	515875	100.87
Statistical **Analysis**	**Mean**	515876.541	100.34
	SD	2285.874	0.687732
	% RSD	0.97754	0.87442

Inference: Intermediate-precision chromatograph 1

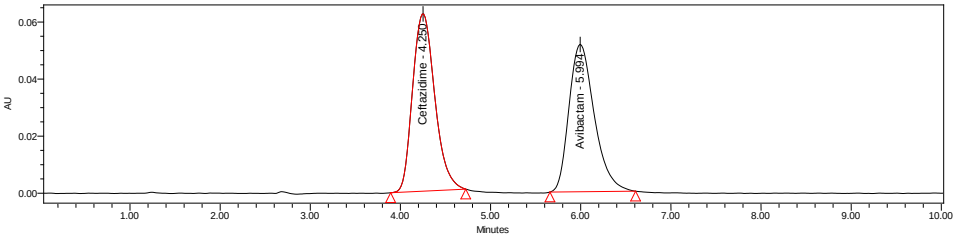
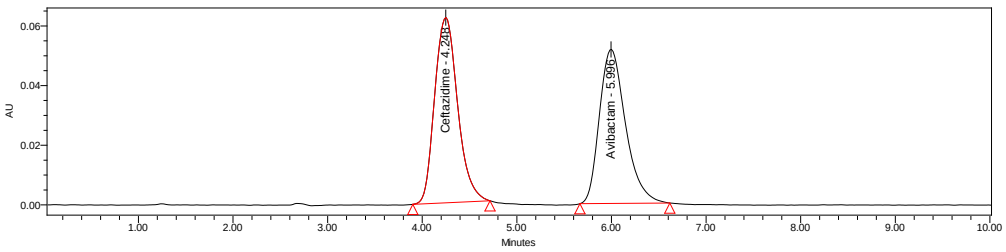
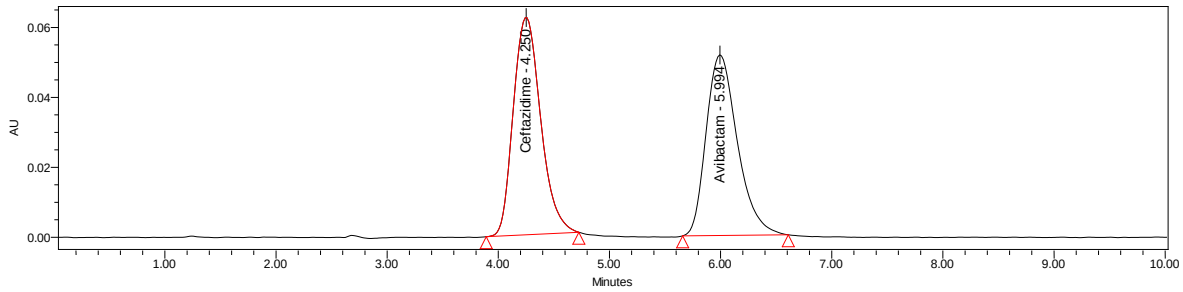


Fig. 24-29: Intermediate precision chromatograms.

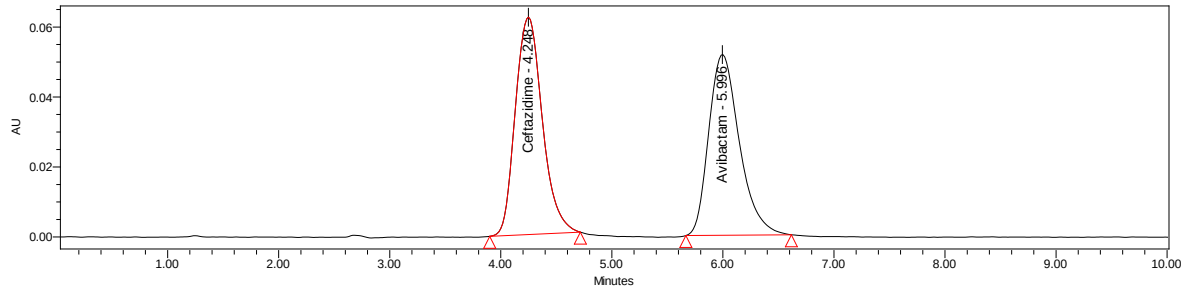
Inference: Intermediate-precision chromatograph2



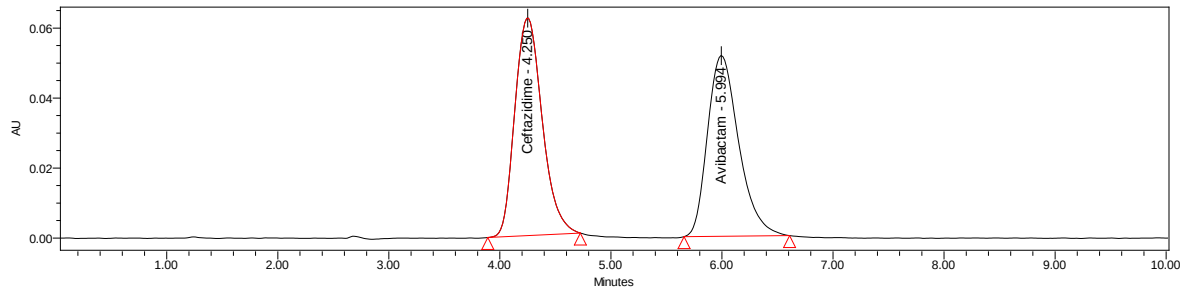
Inference: Intermediate-precision chromatograph 3



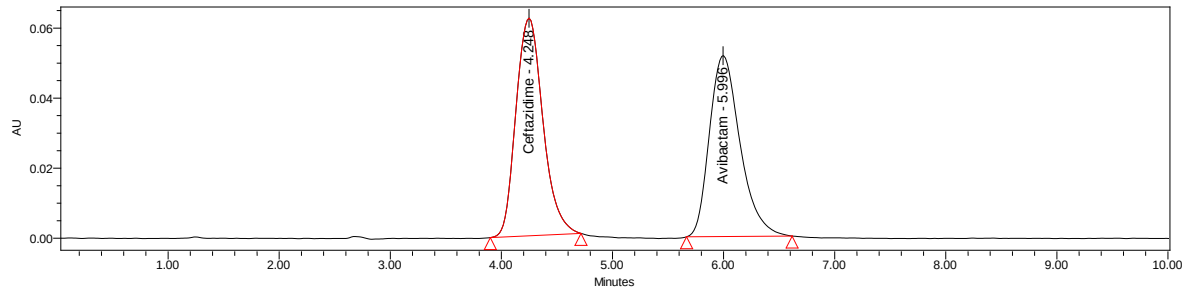
Inference: Intermediate precision chromatograms 4



Inference: Intermediate precision chromatograms 5



Inference: Intermediate precision chromatograms 6



6.3.4 ACCURACY (RECOVERY)

Table 5(i): Precision Data for Ceftazidime.

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% Injection 1	20	19.92	99.78	**MEAN**	99.87
50% Injection 2	20	19.96	99.89		
50% Injection 3	20	19.88	99.78	**%RSD**	0.68
100 % Injection 1	40	39.94	98.92	**MEAN**	99.84
100 % Injection 2	40	39.86	99.75		
100% Injection 3	40	39.76	99.08	**%RSD**	0.657
150% Injection 1	60	59.97	99.96	**MEAN**	100.07
150% Injection 2	60	60.02	100.08		
150% Injection 3	60	60.01	100.01	**%RSD**	0.345

(ii) Precision Data for Avibactam

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% Injection 1	20	19.89	99.87	**MEAN**	99.97
50% Injection 2	20	19.72	99.87		
50% Injection 3	20	20.08	100.03	**%RSD**	0.874
100 % Injection 1	40	39.92	99.88	**MEAN**	99.97
100 % Injection 2	40	40.01	100.07		
100% Injection 3	40	40.05	100.08	**%RSD**	0.687
150% Injection 1	60	59.95	98.87	**MEAN**	99.94
150% Injection 2	60	59.97	99.88		
150% Injection 3	60	59.98	99.98	**%RSD**	0.97

Inference: standard chromatograms 1

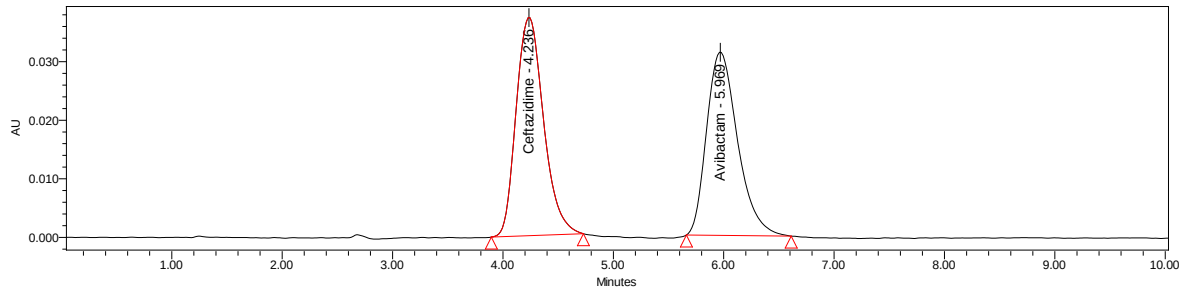


Fig. 30-31: Precision chromatograms (50 percent).

Inference: standard chromatograms 2

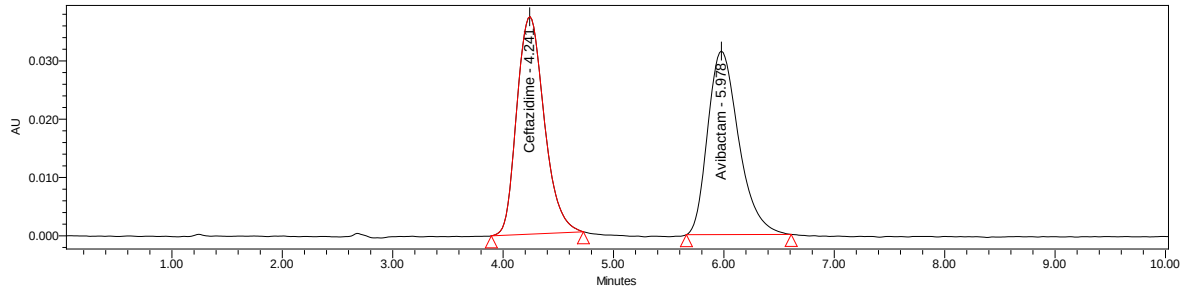
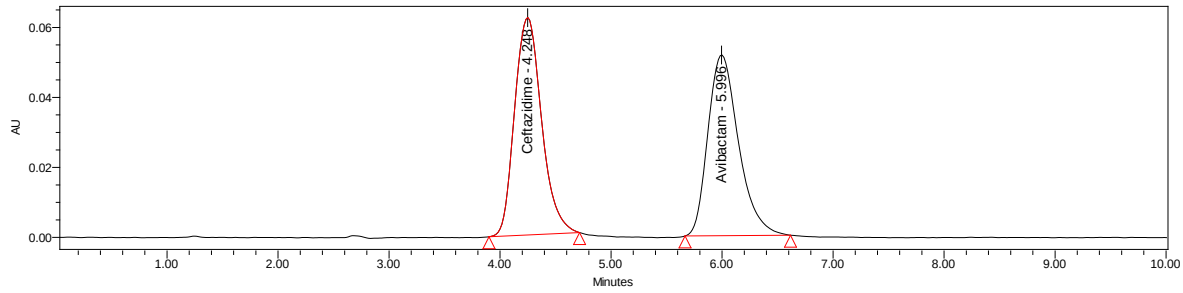


Figure 32-33: precision chromatograms (100 per cent).

Inference: standard chromatograms 1.



Inference: standard chromatograms 2

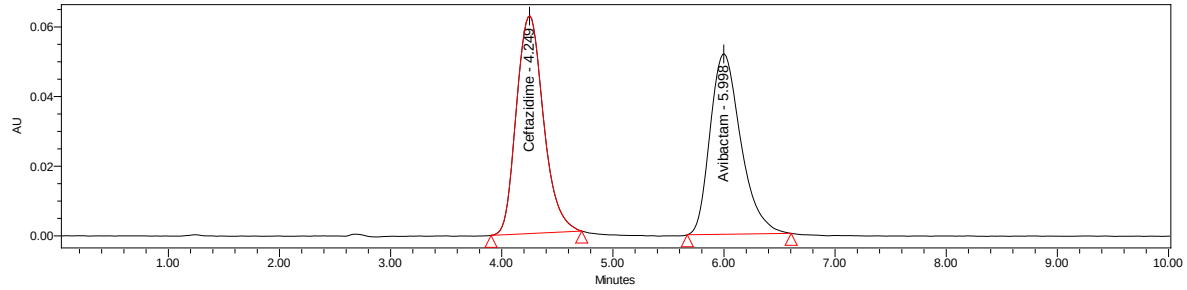
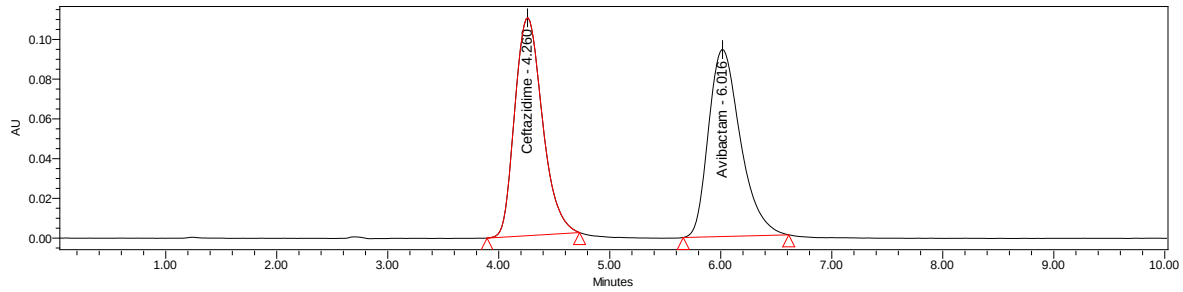
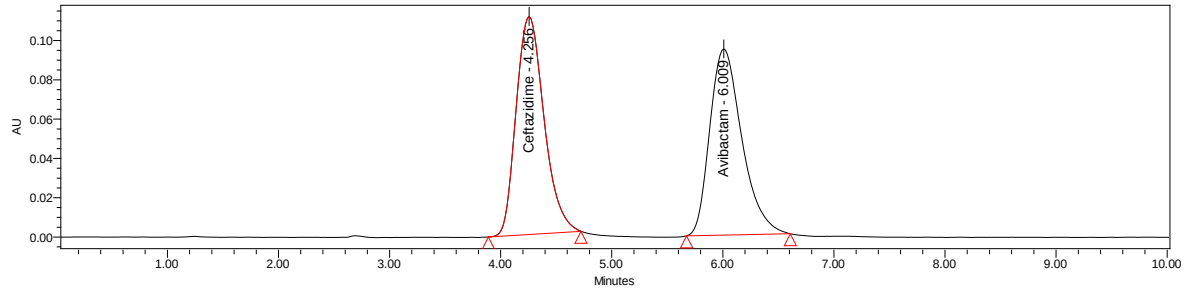


Figure 34-35: For precision chromatograms (150 per cent).

Inference: standard chromatograms 1



Inference: standard chromatograms 2



6.3.5 LINEARITY

Table 6(i): Data of Linearity (Ceftazidime).

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	18951
20	3819379	y-Intercept	38597
30	5729069	Correlation Coefficient	0.999
40	7638757		
50	9548447		
60	11458136		
70	13367826		
80	15077515		

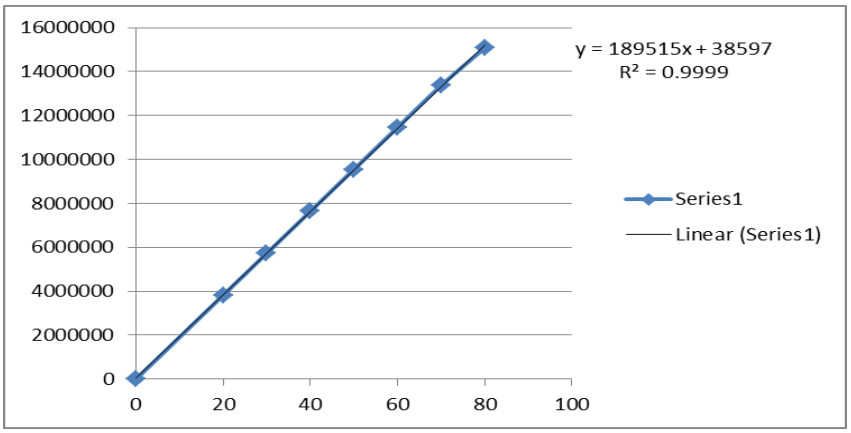


Fig. 42(a): Linearity Plot of Ceftazidime (Concentration Vs response).

Data of Linearity (Avibactam)

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	12825
20	257949	y-Intercept	1929
30	386924	Correlation Coefficient	0.999
40	515898		
50	644873		
60	773847		
70	902822		
80	1021796		

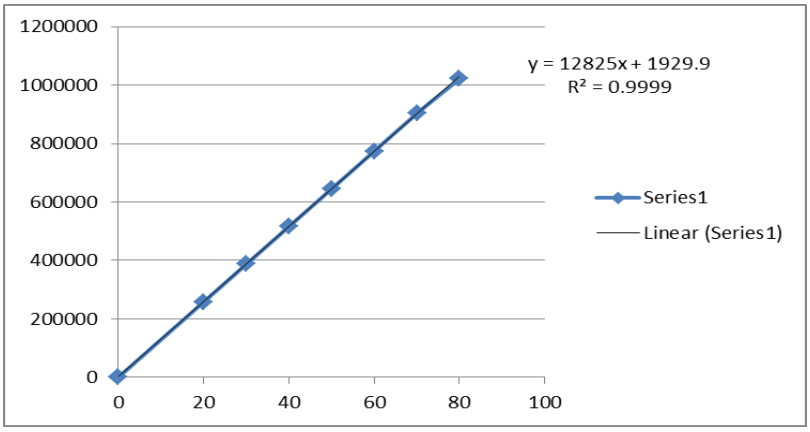


Fig. 42(b): Avibactam linearity plot (Concentration Vs response).

Inference: The std 20 ppm chromatogram.

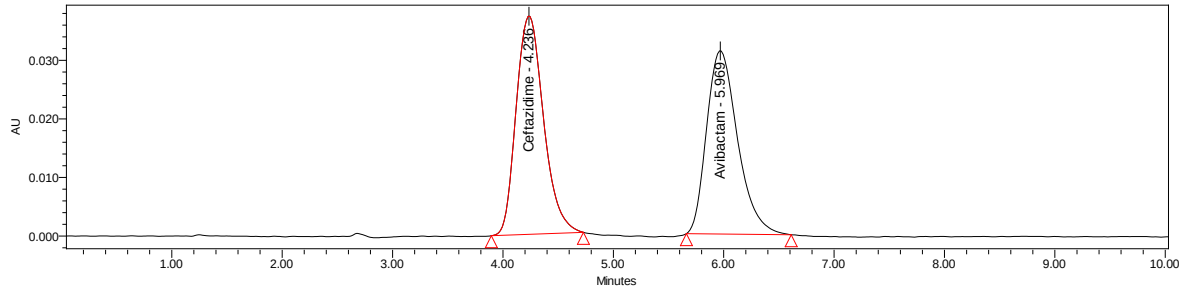


Fig. 36: The 20 ppm chromatograms

Inference: std chromatogram 30 ppm

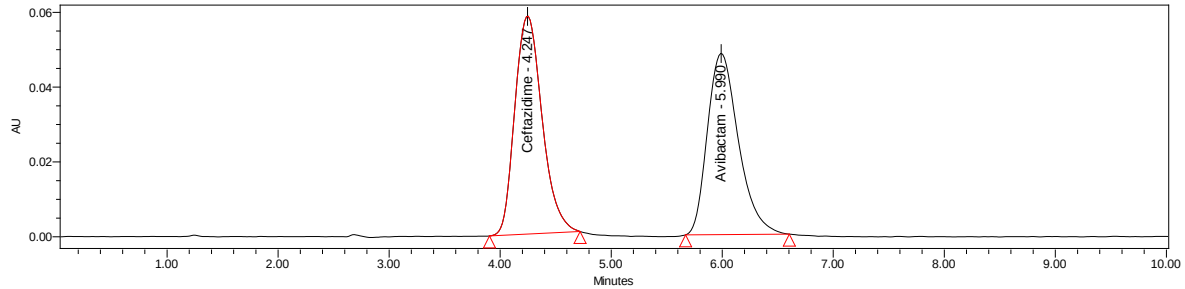


Fig. 37-38: for30ppm chromatograms, for 40ppm.

Inference: std chromatogram 40 ppm

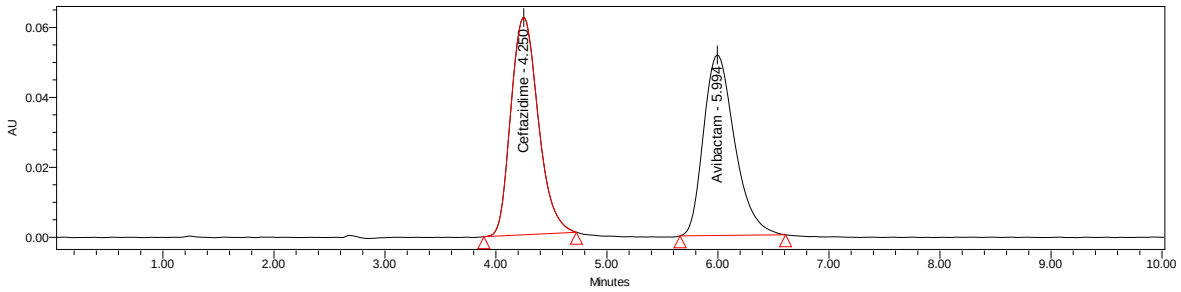
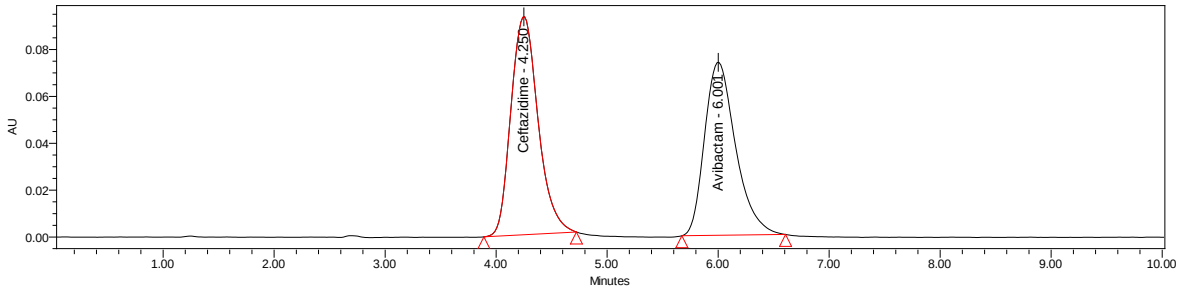


Fig. 39-40: 50 ppm Chromatograms, 60 ppm.

Inference: std chromatogram 50 ppm.



Inference: std chromatogram 60 ppm.

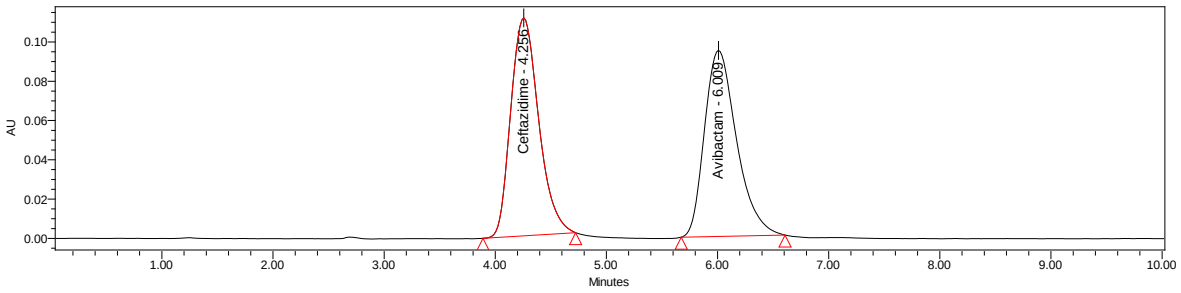
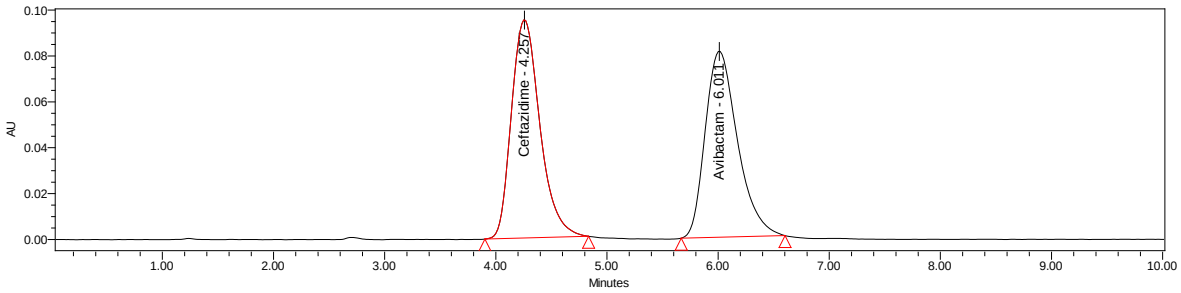
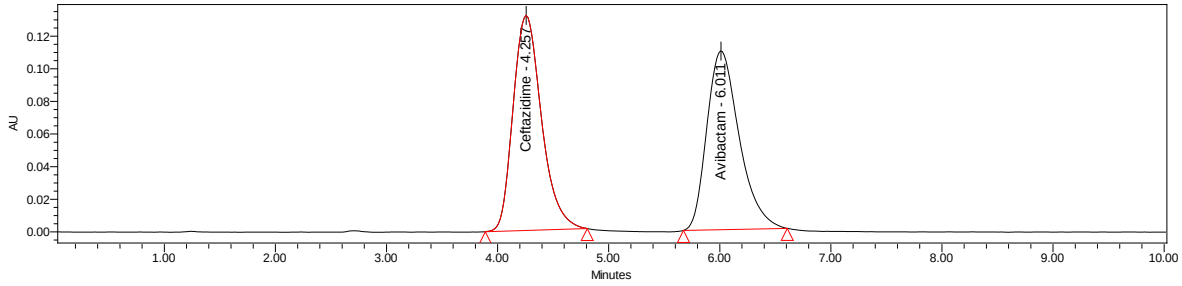


Fig. 41-42: 70 ppm chromatography, 80ppm.

Inference: std chromatogram 70 ppm



Inference: The std 80 ppm chromatogram.



6.3.6 Ruggedly (Ruggedness)

Process Variability of Program(System to System variability):

For **system 1** Refer: Table3

TABLE 7(i): System Variability data (Ceftazidime).

System-2

S.NO:	Peak area	Assay % of Ceftazidime
1	7632746	99.95
2	7631875	99.78
3	7630784	99.98
4	7632170	100.97
5	7633784	100.02
6	7630874	99.87
Mean	763571.5472	99.95
%RSD	1.17452	0.657214

(ii) System Variation data (Avibactam)

System-2

S.NO:	Peak area	Assay % of Avibactam
1	515874	99.75
2	515784	99.87
3	515654	100.65
4	515786	100.75
5	515721	100.87
6	515786	100.22
Mean	515684.27	100.2411
%RSD	0.874	0.78541

Inference: std- 1 for System variation to system chromatogram

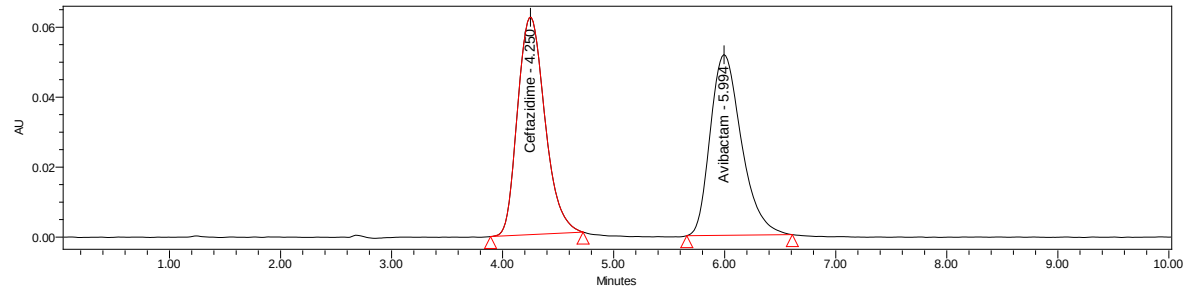
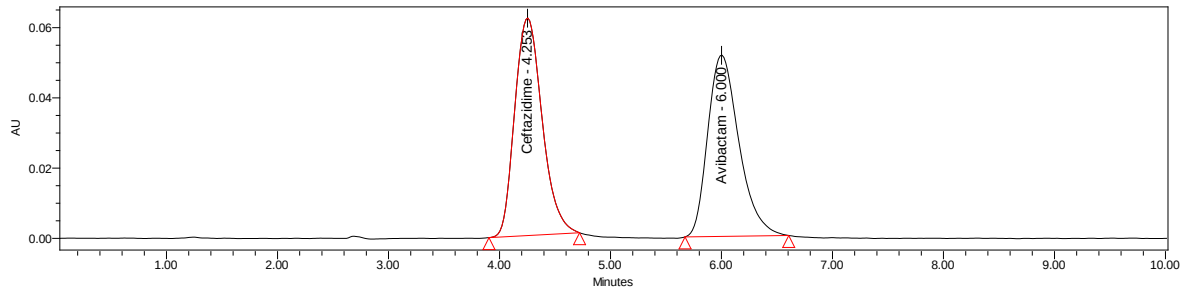
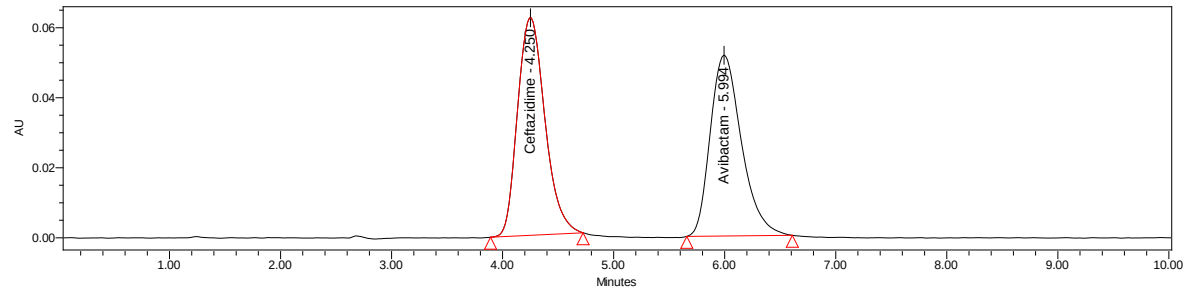


Fig43-48 Machine Chromatograms to ProcessVariability

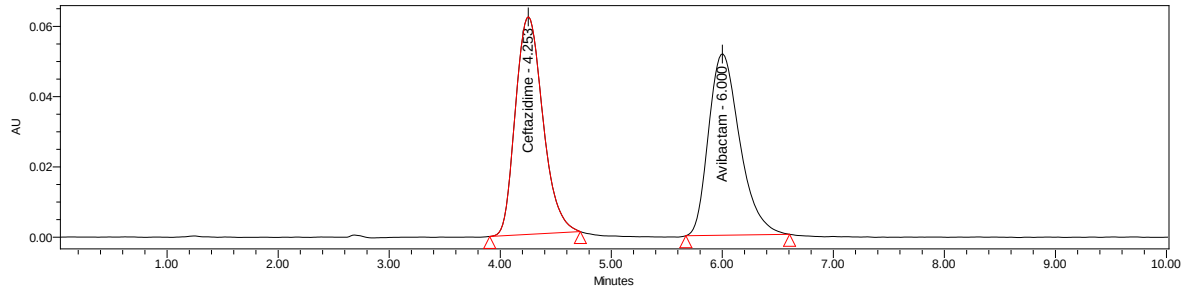
Inference: System chromatogram to system standard deviation



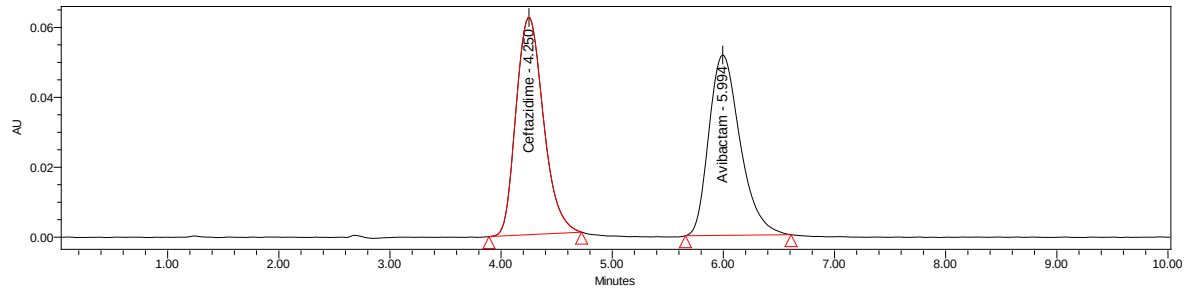
Inference: System variation to system chromatogram standard deviation: 3



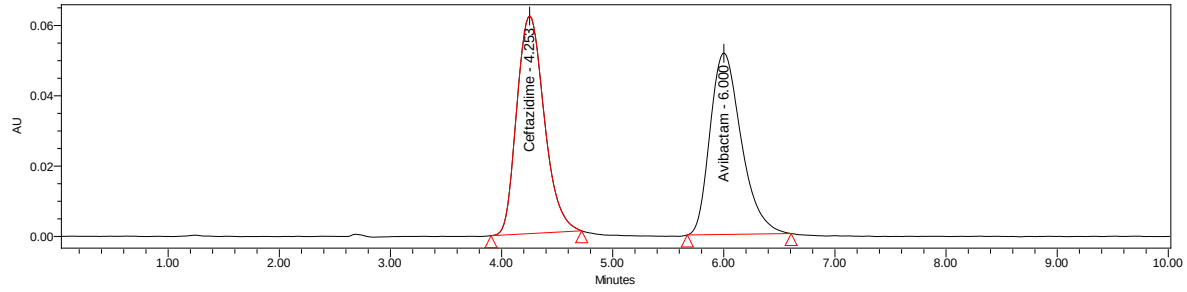
Inference: System chromatogram to system standard deviation is 4.



Inference: System chromatogram to system variability standard deviation: 5



Inference: System chromatogram to system heterogeneity, standard deviation: 6.



6.3.7 Robust Durability(Robustness)

Table 10(i): Information on Ceftazidime's effects on flow rate variability.

Flow 0.8 ml	Std Area	Tailing factor	Flow 1.0 ml	Std Area	Tailing factor	Flow 1.2 ml	Std Area	Tailing factor
	7625687	1.238915		7633684	1.251658		7642137	1.262276
	7620875	1.230637		7635674	1.245435		7647837	1.251581
	7623761	1.240858		7639846	1.262464		7647812	1.237875
	7629612	1.238995		7633761	1.237018		7640217	1.239824
	7620874	1.241073		7630157	1.239010		7647628	1.238257
Avg	7621218.05	1.236496	**Avg**	7633027.58	1.247117	**Avg**	7640789.54	1.245547
SD	32171.254	0.005254	**SD**	2544.2541	0.010328	**SD**	8786.021	0.024570
%RSD	0.87841	0.424907	**%RSD**	0.8100472	0.007845	**%RSD**	0.87741	0.002874

Table 10(ii): Information on the effects of flux rate variation (Avibactam).

Flow 0.8 ml	Std Area	Tailing factor	Flow 1.0 ml	Std Area	Tailing factor	Flow 1.2 ml	Std Area	Tailing factor
	514284	1.099372		515128	1.128451		516215	1.121875
	514852	1.103587		515827	1.112257		516127	1.122254
	514085	1.111587		515028	1.121287		516875	1.124357
	514148	1.117861		515127	1.124752		516257	1.123895
	514982	1.119547		515745	1.123874		516127	1.099157
Avg	514089	1.110875	**Avg**	515781.25	1.120887	**Avg**	516847	1.118248
SD	8741.054	0.008754	**SD**	5623.217	0.00874	**SD**	3745.25	0.07845
%RSD	0.985401	0.98754	**%RSD**	0.95782	0.009874	**%RSD**	1.18754	0.875412

(a) Effects of flow rate variations (0.8 ml/min range)

Inference: The chromatogram for average toughness is 1.

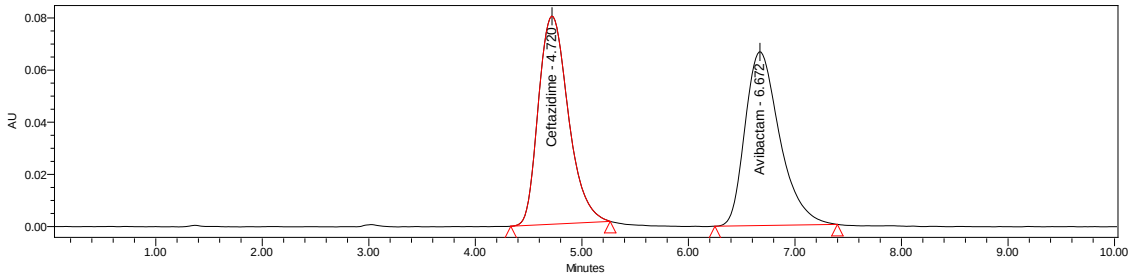
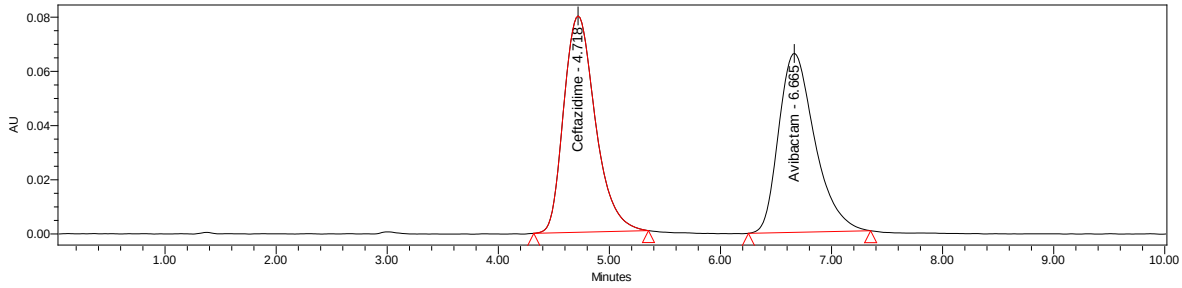


Fig. 49-50: Robust Chromatograms

Inference: The chromatogram of average robustness-2



Inference: The chromatogram for average toughness is 1.

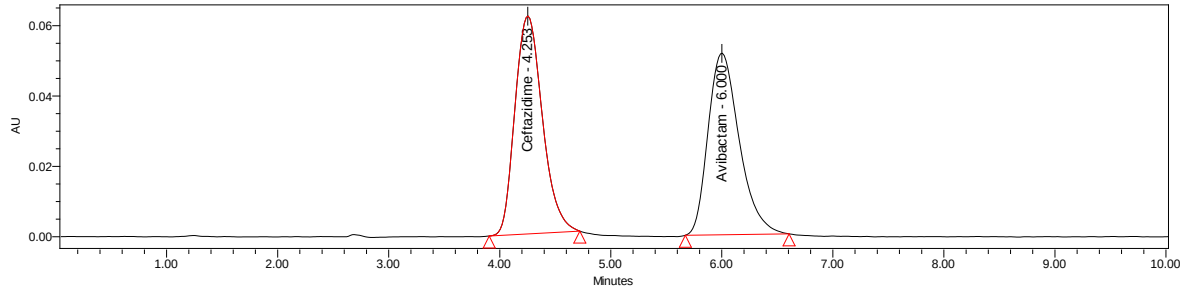


Fig. 51-52: 1ml / min of chromatograms.

Inference: The chromatogram of average robustness-2.

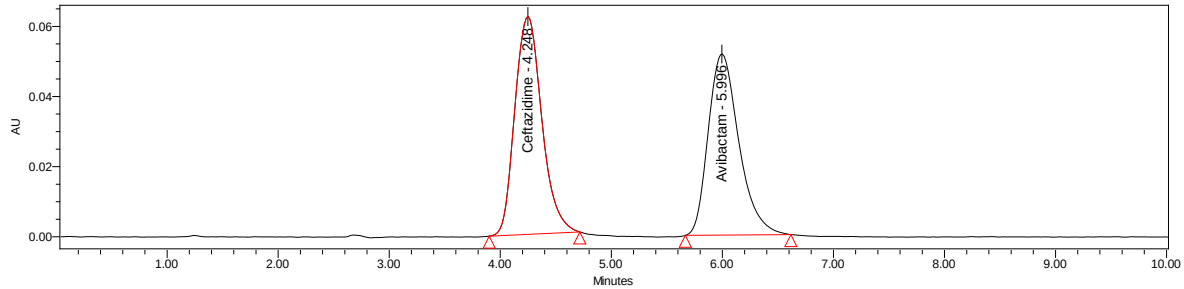
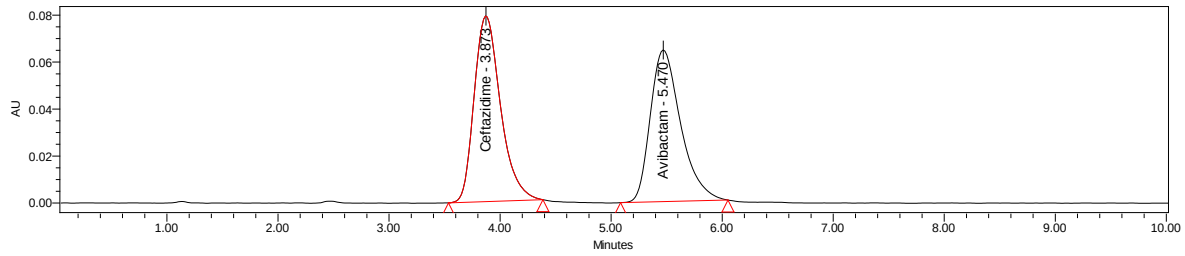
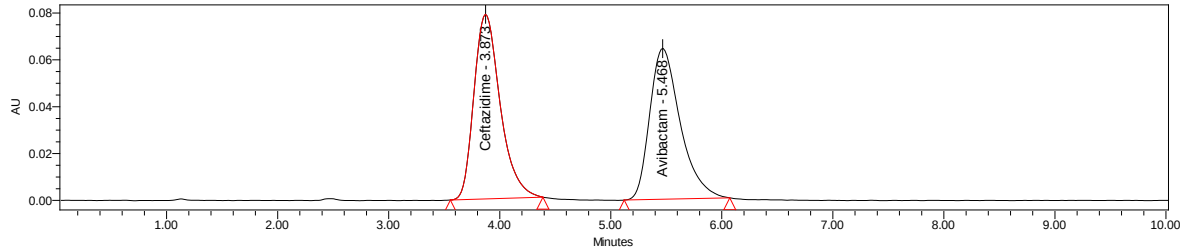


Fig. 53-54: 1.2ml / min Chromatograms.

Inference: The chromatogram for average toughness is 1.



Inference: The chromatogram of average robustness-2



6.3.8 LOD AND LOQ (LIMIT OF DETECTION AND LIMIT OF QUANTITATION):

Ceftazidime

The LOD and LOQ are determined from the linearity plot

$$LOD = \frac{3.3\,\sigma}{S}$$

$$= \frac{3.3\times 2124.413}{20193} = 0.34$$

$$LOQ = \frac{10\,\sigma}{S}$$

$$= \frac{10\times 2124.413}{20193} = 1.05$$

Avibactam

$$LOD = \frac{3.3\,\sigma}{S}$$

$$= \frac{3.3\times 2431.578}{31282} = 0.25$$

$$LOQ = \frac{10\ \sigma}{S}$$

$$= \frac{10 \times 2431.578}{31282} = 0.77$$

7. Analysis and Closing(Summary and Conclusion)

The analytical technique was developed by the analysis of several parameters. Initially, the highest absorbance for Ceftazidime and Avibactam was recorded at 254 and 284 nm, respectively. The typical wavelength was 275 nm, and the peaks had outstanding purity. The injection volume of 20µl was used since it produced a decent peak area. ODS selected Inertsil C18, a strong peak form, as the column for analysis. It has been discovered that the medicinal solution's nature is best served by ambient temperature. The flow rate was set at 1.0 ml/min because of the good peak area, sufficient retention time, and good resolution.After testing a variety of mobile phase ratios, the 65:35 ratio of methanol to acetonitral was chosen because it produced well-symmetrical peaks and acceptable resolution. Instead, this mobile technique was employed for the suggested analysis.

The present recovery was found to be accurate and linear within the same 98.0–101.50 range. It has been determined that the procedure's and the device's precision are both dependable and within acceptable bounds. Avibactam's detection limit was 0.25, while Ceftazidime's was 0.34. The results of the linearity investigation included the coefficient of correlation and curve fitting. The analytical method showed linearity for both medications across the target concentration range of 20–80 ppm.Both the resilience and robustness tests have been passed by the analysis. The relative standard deviation was very good in both situations.

8. BIBLIOGRAPHY

1. V. Gupta, A.D. K. Jain, N.S. Gill, K. Gupta, Development and validation of HPLC method - a review , Int. Res J Pharm. App Sci., 2012; 2(4): 17-25.
2. Y. Kazakevich, R. Lobrutto, HPLC for Pharmaceutical Scientists, John Wiley & Sons, New Jersey, 2007.
3. S. Ahuja, H. Rasmussen, Development for Pharmaceuticals, Separation Science and Technology, Elsevier, New York [2007]; Vol. 8.
4. M.S. Azim, M. Mitra, P.S. Bhasin, HPLC method development and validation: A review, Int. Res. J. Pharm., 2013; 4(4): 39-46.

5. B.V. Rao, G.N. Sowjanya1, A. Ajitha, V.U.M. Rao, Review on stability indicating hplc method development, World Journal of Pharmacy and Pharmaceutical Sciences, 2015; 4(8): 405-423.
6. M.S. Charde, A.S. Welankiwar, J. Kumar, Method development by liquid chromatography with validation, International Journal of Pharmaceutical Chemistry, 2014; 04(02): 57-61.
7. S. Sood, R. Bala, N.S. Gill, Method development and validation using HPLC technique – A review, Journal of Drug Discovery and Therapeutics, 2014; 2(22): 18-24.
8. M.W. Dong, Modern Hplc for practicing scientists, John Wiley & Sons, New Jersey, 2006.
9. P.K. Singh, M. Pande, L.K. Singh , R.B. Tripathi, steps to be considered during method development and validation for analysis of residual solvents by gas chromatography, Int. Res J Pharm. App Sci., 2013; 3(5): 74-80.
10. B. Prathap, G.H.S. Rao, G. Devdass, A. Dey, N. Harikrishnan , Review on Stability Indicating HPLC Method Development, International Journal of Innovative Pharmaceutical Research, 2012; 3(3): 229-237.
11. B. Sriguru, N.P. Nandha, A.S.Vairale, A.V. Sherikar, V. Nalamothu, Development and validation of stability indicating HPLC method for the estimation of 5-Fluorouracil and related substances in topical formulation, Int. J. Res. Pharm. Sci., 2010; 1(2): 78-85.
12. C.K. Kaushal, B. Srivastava, A process of method development: A chromatographic approach, J. Chem. Pharm. Res., 2010; 2(2): 519-545.
13. N.Toomula, A. Kumar, S.D.Kumar, V.S. Bheemidi, Development and Validation of Analytical Methods for Pharmaceuticals, J Anal Bioanal Techniques, 2011; 2(5): 1-4.
14. K. Kardani, N. Gurav, B. Solanki, P. Patel, B. Patel, RP-HPLC Method Development and Validation of Gallic acid inPolyherbal Tablet Formulation, Journal of Applied Pharmaceutical Science, 2013; 3(5): 37-42.
15. B. Nigovic, A. Mornar, M. Sertic, Chromatography – The Most Versatile Method of Chemical Analysis, Intech, 2012; 385-425.
16. T. Bhagyasree, N. Injeti, A. Azhakesan, U.M.V. Rao, A review on analytical method development and validation, International Journal of Pharmaceutical Research & Analysis, 2014; 4(8): 444-448.
17. A. Shrivastava, V.B. Gupta, HPLC: Isocratic or Gradient Elution and Assessment of Linearity in Analytical Methods, J Adv Scient Res., 2012; 3(2): 12-20.

18. V. Kumar, R. Bharadwaj, G.G., S. Kumar, An Overview on HPLC Method Development, Optimization and Validation process for drug analysis, The Pharmaceutical and Chemical Journal, 2015; 2(2): 30-40.
19. Validation of Analytical Procedures: Text and Methodology, International Conferences on Harmonization, Draft Revised, 2005; Q2 (R1).
20. Validation of Compendial Procedures, United State Pharmacopeia, USP 36 NF, 2010; 27(2).
21. https://www.pharmacyjournal.in/assets/archives/2023/vol8issue2/8015-1683607267082.pdf
22. http://iosrphr.org/papers/vol10-issue3/F1003015285.pdf
23. https://rjptonline.org/AbstractView.aspx?PID=2021-14-5-16
24. https://www.wisdomlib.org/uploads/journals/wjpr/volume-5,-march-issue-3_4843.pdf
25. https://www.afjbs.com/issue-content/development-and-validation-of-rp-hplc-method-for-estimation-of-ceftazidime-and-avibactam-in-bulk-drug-and-formulation-4615
26. https://en.wikipedia.org/wiki/Ceftazidime
27. https://go.drugbank.com/drugs/DB00438
28. https://en.wikipedia.org/wiki/Avibactam
29. https://go.drugbank.com/drugs/DB09060