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# A SIMPLE UV AND STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF CEFTAROLINE FOSAMIL IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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**ABSTRACT**

A simple, rapid and precise reverse phase liquid chromatographic (RP-HPLC) method was developed and subsequently validated for estimation of ceftaroline fosamil in bulk drug and pharmaceutical dosage forms. The analysis was carried out using $C_{18}$ - Octa decyl silane -250cm x4.6mm i.d, 5µ. The separation was carried out using a mobile phase containing Buffer: Acetonitrile: Methanol (40:30:30), was pumped at a flow rate of 1.0mL/min with UV-detection at 242nm. The present study was carried out to develop a simple, sensitive, accurate and more precise UV and RP-HPLC methods for the estimation of ceftaroline fosamil in bulk and pharmaceutical dosage forms. The % purity of was calculated by using both methods and it was found to be 100.02% and 99.63%, respectively and correlation coefficient of 0.999. The proposed UV and HPLC methods can be applied for the routine quality control analysis of ceftaroline fosamil in bulk and IV Injection forms.

**KEYWORDS**

$C_{18}$ - Octa decyl silane, Acetonitrile, UV-detection and Ceftaroline fosamil.

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## INTRODUCTION

### Chromatography

The Chromatography is a family of analytical chemistry techniques for the separation of mixtures. A chromatograph takes a chemical mixture carried by liquid or gas and separates it into its component parts as a result of these differential distributions of the solutes as they flow around or over a stationary liquid or solid phases. Various techniques for this separation of complex mixtures on the differential affinities of substances for a gas or liquid mobile

mediums and for a stationary adsorbing medium through which they pass; such as paper, gelatin, or magnesium silicate gel.

## HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

### Instrumentation

The liquid chromatographic system consisted of following components: prominence HPLC containing isocratic LC 20 AT pump with variable programmable UV/VIS detector.

### Procurement of pure drug

Ceftaroline fosamil pure drug was obtained as a gift sample from Fact Pharmaceuticals, U.S.A.

### Selection of wave length

Sensitivity of an HPLC method that uses UV detection depends up on the proper selection of wavelength. UV spectra of ceftroline fosamil in the mobile phase was recorded and detection wave length i.e. 242nm was selected for further analysis.

### Selection of Liquid Chromatographic Method

A proper selection of HPLC method depends upon the nature of the sample, its molecular weight and solubility. The selected drug in the present study is ionisable, so either reversed phase or ion exchange chromatography can be used. In the present study reversed phase chromatography was chosen because of its simplicity and suitability, where ever this separation method proves inadequate the addition of ion pairing reagent to mobile phase was consequired.

## OPTIMIZED CHROMATOGRAPHIC CONDITIONS

| | |
|---|---|
| Stationary phase | : $C_{18}$ - Octa decyl silane - 250cm x4.6mm i.d., 50µ |
| Mobile phase | : Buffer: Acetonitrile : Methanol (40:30:30) |
| Flow rate | : 1.0ml/min |
| Operating temperature | : Ambient |
| Detection wave length | : 242nm. |
| Diluent | : Mobile phase |
| Injection volume | : 20µl |
| Run time | : 10min. |

### Buffer

It was pre pared by mixing of 0.02M potassium dihydrogen phosphate and 0.1M Methanol sulphonic acid and $^{PH}$ was adjusted to $P^{H}$: 7 with 0.1M KOH.

### Mobile Phase

It was prepared by mixing of Buffer: Acetonitrile: Methanol in the ratio of 40:30:30.

### Preparation of Standard

Accurately weighed quantity of 50mg ceftaroilne fosamil was transferred to a 50ml volumetric flask, dissolved in 20ml of mobile phase, sonicated for 15 min and the volume were made up to the mark with mobile phase.

### Preparation of working standard solutions

Working standard solutions of ceftaroilne fosamil were prepared by diluting 2ml, 3ml, 4ml, 5ml and 6ml of stock solution with the mobile phase to get the concentrations of 40µg/ml, 60µg/ml, 80µg/ml, 100µg/ml and 120µg/ml of ceftroline fosamil.

### Preparation of Sample

Accurately weighed the powder equivalent to 50mg of Ceftaroline fosamil was transfered into 50ml volumetric flask and 20ml of mobile phase was added. The mixture were subjected to sonication for 30 min with intermediate shaking for complete extraction of drugs. Filtered and cooled to room temperature and solution was made up to mark with mobile phase. It was further diluted with a mobile phase, to acquire a concentration within the linearity range.

### Method Validation

After the method development, the method is validated in terms of parameters like linearity, LOD, LOQ, precision, accuracy, robustness, specificity and system suitability parameters as per ICH guidelines.

### Specificity

### Blank Interference

Selected mobile phase was prepared and injected into HPLC in triplecate. And analyzed as per the test method. No interference was observed at the retention time of cefaroline fosamil in the chromatograms of diluent.

**Linearity**

To demonstrate the linearity of the method, varying concentrations of working standard solutions was injected separately and the chromatograms was recorded. The calibration graph was plotted with peak area on the Y axis and concentration of standard solutions on the X axis. The degree of linearity were estimated by calculating the correlation coefficient. And also Y- Intercept, slope of the regression line.

**Limit of Detection and Quantification**

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected, but not necessarily quantitated as an exact value.

The LOQ is the concentration that can be quantitated reliably with a specified level of accuracy and precision.

Detection and quantification limit were calculated by the method based on the standard deviation ( $\sigma$) and slope of the calibration plot, using the formula

$$\text{Limit of Detection} = \frac{\sigma \times 4.3}{S}$$

$$\text{Limit of Quantitation} = \frac{\sigma \times 10}{S}$$

Where $\sigma$ = the standard deviation of the response.

S = the slope of the calibration curve (of the analyte).

**Precision**

The precision of the developed method is normally demonstrated by determining the inter day and intraday variations. Intra Day variations were determined by injecting the sample solution six times within a day and calculated the amount of drug present in the sample solution. Inter day variations were determined by estimating the drug present in the IV do sage form on three different days and % RSD was calculated. The system and method precision of test method was performed by injecting six replications of standard and sample solutions into the analytical column and the peak areas were recorded and % RSD was calculated.

**Accuracy**

Accuracy of the method was confirmed by studying recovery at 3 different levels of 80, 100 and 120% in accordance with ICH guidelines, by replicate analysis (n=3). The standard drug solution was added to a pre analyzed sample solution and percentage drug content was measured.

**Robustness**

The experimental conditions was purposely altered and evaluated. The method must be robust enough to withstand such slight changes and allowed routine analysis of the sample. Robustness of method was carried out with a variation of flow rate ± 0.1ml/min of set value i.e. 0.9ml/min and 1.1ml/min, by changing the mobile phase ratio with ± 0.2% and variation of the detection wavelength (± 2nm).

**System Suitability Parameters**

System suitability studies were carried out as specified in the United States of Pharmacopoeia. These parameters include Retention time ($R_t$), column efficiency, peak area, asymmetry factor, correlation coefficient. Although USP requires only two of these criteria for method validation, several parameters like column efficiency (N) and peak asymmetry factor were calculated in the present study.

## RESULTS AND DISCUSSION

Preliminary development trials have been performed under isocratic conditions with mixtures of solvents like methanol, water and acetonitrile with buffers in different combinations and the method was opted under trail 6, which are incorporated in the following table.

After the optimization of chromatographic conditions, estimation of Ceftaroline fosamil was carried out by the developed RP-HPLC method. Standard solutions of drug were injected separately and chromatograms of Ceftaroline fosamil were recorded shown in Figure No.1. Now the sample solution was injected separately and chromatograms of Ceftaroline fosamil were recorded until the reproducibility of the peak areas were satisfactory (Figure No.2). The amount of the drug present in the marketed formulation was calculated and the % purity was found to be 99.63%. The results are incorporated in Table No.2.

After the development of RP-HPLC method for the estimation of Ceftaroline fosamiin injection form, validation of the method was also carried out by checking Linearity, Accuracy, Precision, LOD and LOQ, Specificity, Robustness and System suitability parameters.

Linearity was performed by preparing standard solutions of ceftaroline fosamil at different concentration levels ranging from 40-120µg/ml. Twenty microlitres of each solution was injected into the HPLC system. The peak responses were measured at about 242nm and the corresponding chromatograms were recorded (Figures No.3 to Figure No.7). Calibration graph was plotted by using the values mentioned under Table No.3 with a correlation coefficient value 0.999 shown in Figure No.8 and the results were incorporated in Table No.4.

Precision was performed by injecting six replicates of standard and sample solutions which were prepared and analyzed on same day and on different days by using the proposed method. The resulting chromatogram were recorded and shown in Figure No.9 and Figure No.10. The percent relative standard deviation (% RSD) for peak responses was calculated and the results are presented in Tables No.5 and Table No.6.

Accuracy of the method was determined by standard addition method. The standard addition method was performed at 80%, 100% and 120% level of sample solution. The resulting solutions were analyzed in triplicate at each level as per the ICH guidelines and the resulting chromatograms were shown in Figures No.11 and Figure No.12 and Figure No.13. The percent recovery was calculated and results are presented in Tables No.7 and Table No.8.

Detection (LOD) is defined as lowest concentration of analyte that can be detected, but not necessarily quantified, by the analytical method. Limit of detection is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected and it was found to be 1.64µg/ml of ceftaroline fosamil.

Limit of quantification (LOQ) is the concentration that can be quantitated reliably with a specified level of accuracy and precision. LOQ was found to be 4.85µg/ml of ceftaroline fosamil.

Robustness of the developed method was demonstrated by purposely altering the experimental conditions. Robustness of method was carried out with variation of mobile phase, flow rate ±0.1 ml/min (Figure No.14 and Figure No.15) and detection wave length ± 2nm (Figure No.16 and Figure No.17). The results were incorporated in Tables No.9 and Table No.10.

Specificity was determined by comparing test results obtained from analyses of sample solution containing ingredients with that of test results those obtained from standard drug. Chromatograms for placebo, standard and samples were recorded (Figures No.18 to 20) and they represent no interference.

**Table No.1**

| S.No | Mobile phase | Retention time | Efficiency | Observation |
|---|---|---|---|---|
| Trail 1 | Water: ACN (40: 60) with 1ml/min | 4.81 | 3992 | Symmetry of peak is not so good. |
| Trail 2 | Water: methanol (60: 40) with 1.2ml/min | 3.18 | 4156 | Tailing of peak appears and symmetry is not good. |
| Trail 3 | Potasium dihydrogen Buffer: methanol: ACN (15:50:35) with 1ml/min | 2.78 | 3550 | Tailing of peak appears and symmetry is not good. |
| Trail 4 | Acetonitrile: 0.005M Potassium dihydrogen phosphate buffer (50:50 v/v) with 1.2ml/min | 5.24 | 4152 | Retention time is more |
| Trail 5 | 0.002M potasium dihydrogen phosphate buffer: ACN: Methanol (45:30:30% v/v) with 1.5ml/min | 4.95 | 3939 | Retention time is more |
| Trail 6 | Potasium dihydrogen Buffer: Acetonitrile: Methanol in the ratio 40: 30:30, pH is adjusted to 7 with 0.1M potassium hydroxide with 1.0ml/min | 3.57 | 4939 | Optimized |

**Table No.2: Summary of assay results for ceftaroline fosamil**

| Formulation used | Label claim | Amount found* | % Assay* |
|---|---|---|---|
| Ceftroline fosamil | 400mg | 398.53mg | 99.6 3 |

*Average of Six determinations

**Table No.3: Linearity of ceftaroline fosamil by RP-HPLC**

| S.No | Concentration of Ceftaroline fosamil (µg/mL) | Peak area |
|---|---|---|
| 1 | 0 | 0.0000 |
| 2 | 40 | 2256.85 |
| 3 | 60 | 3320.56 |
| 4 | 80 | 4415.93 |
| 5 | 100 | 5523.17 |
| 6 | 120 | 6609.28 |

**Table No.4: linearity validation results for Ceftaroline fosamil**

| S.No | Parameters | Ceftaroline fosamil |
|---|---|---|
| 1 | Linearity Range | 40-120 (µg/ml) |
| 2 | Regression coefficient | R2 =0.999 |
| 3 | System precision | % RSD = 0.022 |
| 4 | Method precision | % RSD = 0.179 |
| 5 | Accuracy | % Recovery = 98.24-99.26 |
| 6 | LOD | 1.65µg/ml |
| 7 | LOQ | 4.84µg/ml |

**Table No.5: System precision of Ceftaroline fosamil**

| S.No | Area of Ceftaroline fosamil | Rt (min) |
|---|---|---|
| 1 | 6059.452 | 3.507 |
| 2 | 6062.154 | 3.511 |
| 3 | 6057.542 | 3.510 |
| 4 | 6061.254 | 3.517 |
| 5 | 6062.156 | 3.516 |
| 6 | 6060.511 | 3.512 |
| 7 | 1.781 | 0.0037 |
| 8 | 0.022 | 0.10 |

**Table No.6: Method precision of Ceftaroline fosamil**

| S.No | Area of Ceftaroline fosamil | Rt (min) |
|---|---|---|
| 1 | 6140.543 | 3.527 |
| 2 | 6132.642 | 3.543 |
| 3 | 6108.769 | 3.522 |
| 4 | 6134.077 | 3.524 |
| 5 | 6134.354 | 3.534 |
| Mean | 6130.078 | 3.528 |
| S.D | 10.994 | 0.00814 |
| % RSD | 0.1793 | 0.321 |

**Table No.7: Accuracy of ceftaroline fosamil by HPLC**

| S.No | Concentration Level | Spiked Concentration | Obtained Concentration | % recovery |
|---|---|---|---|---|
| 1 | 80% Ceftaroilne fosamil | 32 | 31.18 | 97.4 |
| | | 32 | 31.65 | 98.90 |
| | | 32 | 31.38 | 98.5 |
| 2 | 100% Ceftaroilne fosamil | 40 | 39.5 | 98.7 |
| | | 40 | 39.55 | 98.87 |
| | | 40 | 39.53 | 98.65 |
| 3 | 120% Ceftaroilne fosamil | 48 | 47.55 | 99.05 |
| | | 48 | 47.85 | 99.68 |
| | | 48 | 47.52 | 99.0 |

**Table No.8: Percentage Mean recovery of Ceftaroilne fosamil Limit of**

| S.No | Spiked level | Mean recovery of Ceftaroilne fosamil (%) |
|---|---|---|
| 1 | 80% | 98.26 |
| 2 | 100% | 98.73 |
| 3 | 120% | 99.24 |

**Table No.9: Effect of flow rate**

| S.No | Flow rate (ml/min) | Retention time of Ceftaroline fosamil (min) |
|---|---|---|
| 1 | 0.9ml/min | 3.533 |
| 2 | 1.1ml/min | 3.527 |

**Table No.10: Effect of detection wave length**

| Drug name | Rt (min) | Peak Area | Asymmetry | Efficiency |
|---|---|---|---|---|
| Ceftaroline fosamil | 3.527 | 5523.172 | 0.993 | 4692 |

| Detection wave length (nm) | Rt of Ceftaroline fosamil (min) |
|---|---|
| 240nm | 3.537 |
| 24 4nm | 3.507 |

| Drug name | $R_t$ (min) | Peak Area | Asymmetry | Efficiency |
|---|---|---|---|---|
| Ceftaroline fosamil | 3.533 | 6609.289 | 1.409 | 3578 |

| Drug | Rt (min) | Area (mV.s) | Asymmetry | Efficiency |
|---|---|---|---|---|
| Ceftaroline fosamil | 3.533 | 4415.937 | 1.100 | 45086 |

| Drug | Rt (min) | Area (mV.s) | Asymmetry | Efficiency |
|---|---|---|---|---|
| Ceftarolin fosamil | 3.507 | 6059.452 | 0.995 | 3511 |

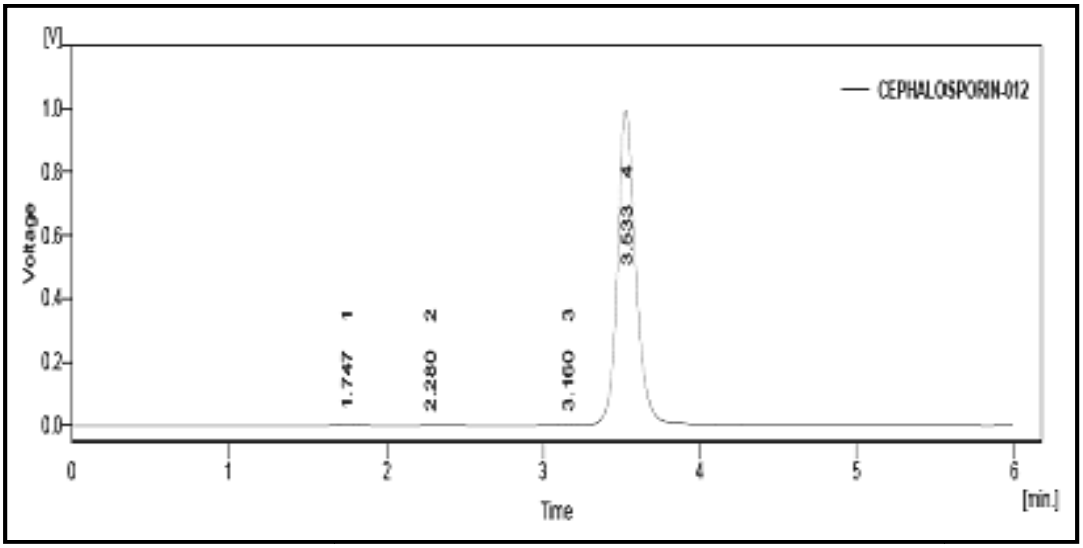


**Figure No.1: Typical chromatogram of ceftarolin fosamil Standard**

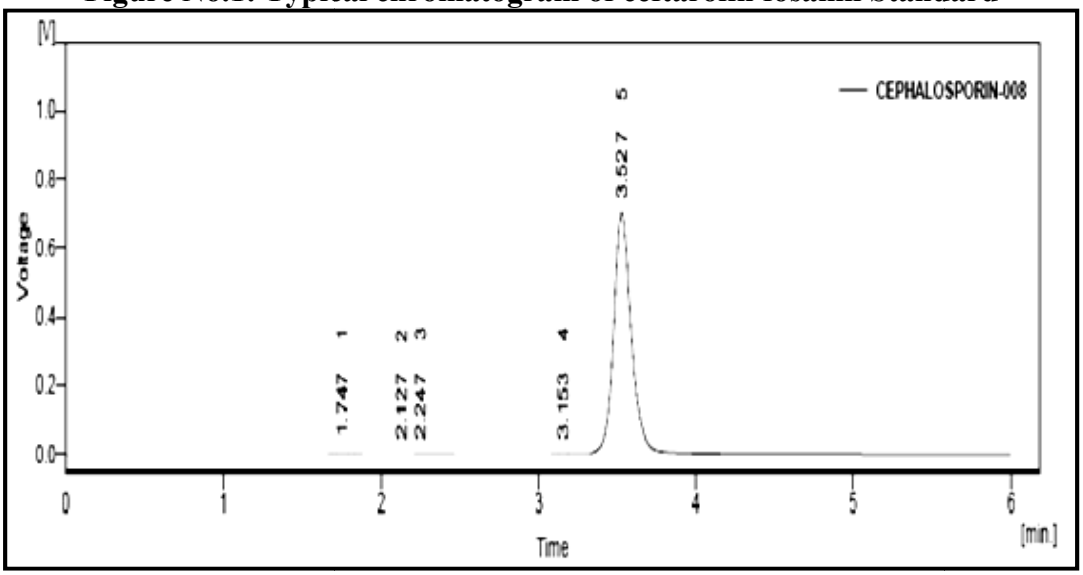


**Figure No.2: Typical chromatogram of ceftaroline fosamil Sample**

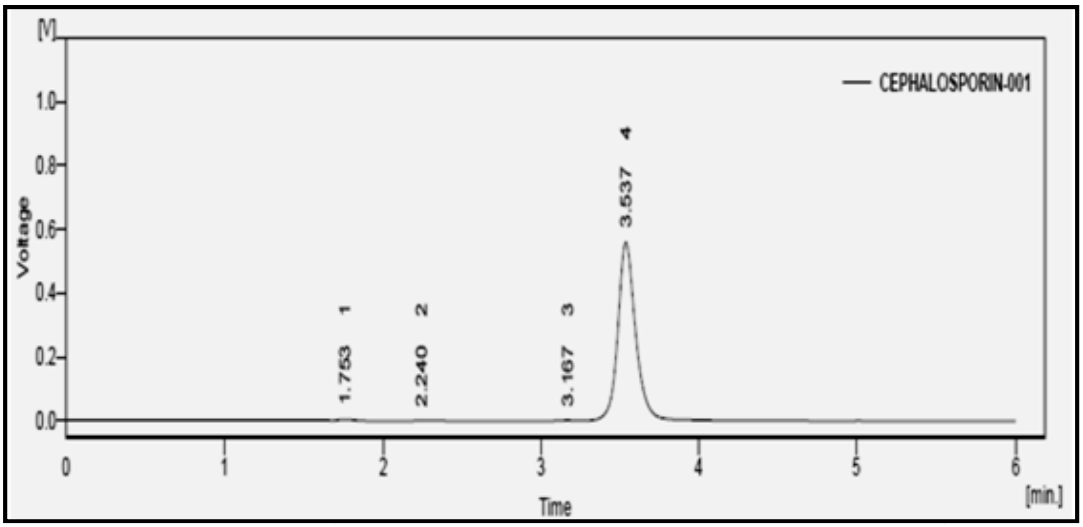


**Figure No.3: Typical Chromatogram of Linearity-1 (40µg/ml)**

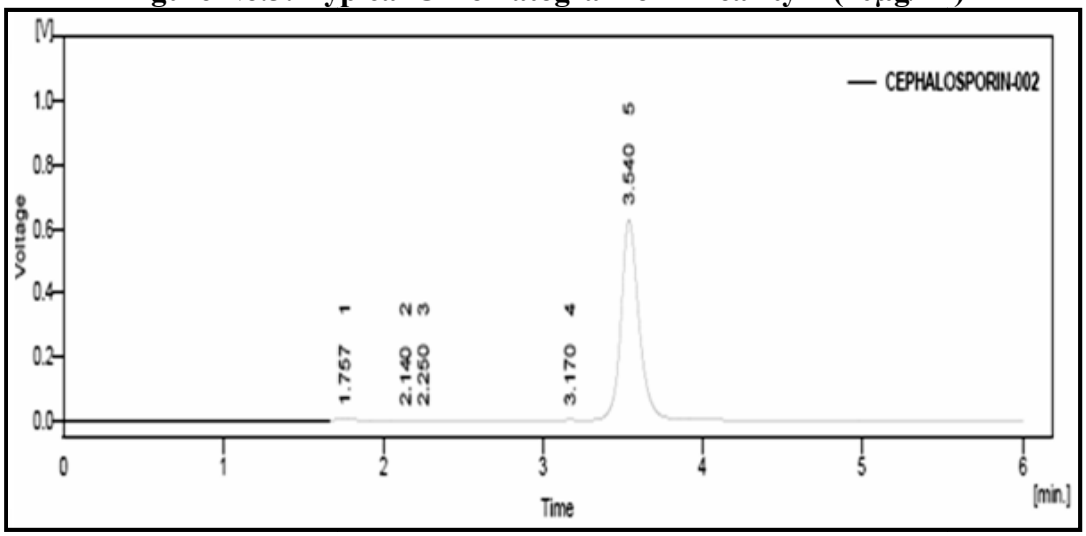


**Figure No.4: Typical Chromatogram of Linearity-2 (60µg/ml)**

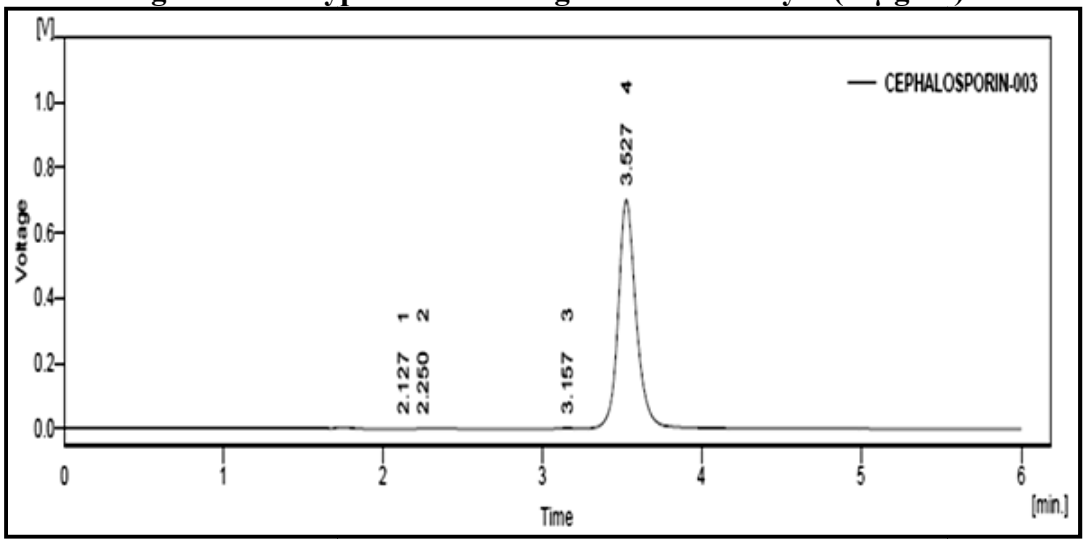


**Figure No.5: Typical Chromatogram of Linearity-3 (80µg/ml)**

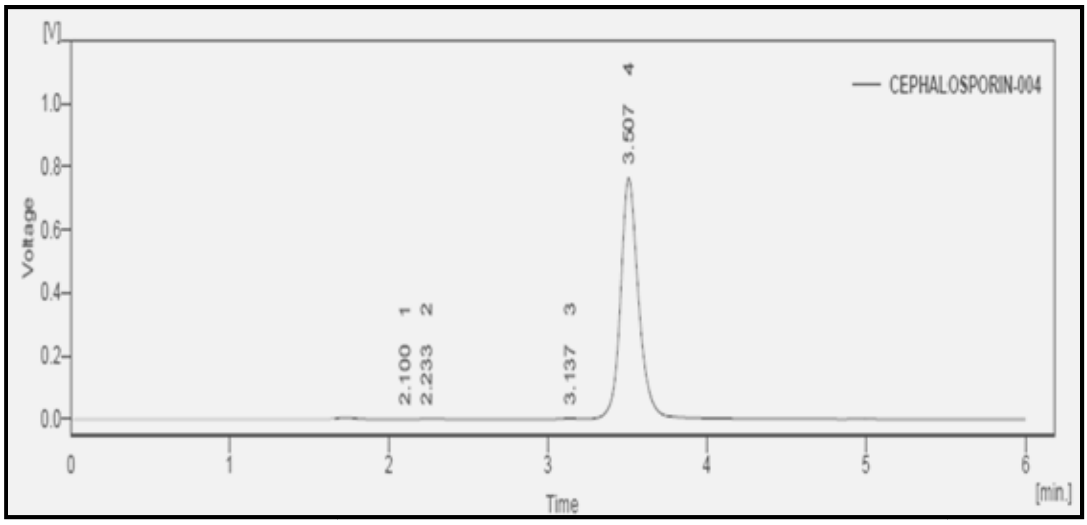


**Figure No.6: Typical Chromatogram of Linearity-4 (100µg/ml)**

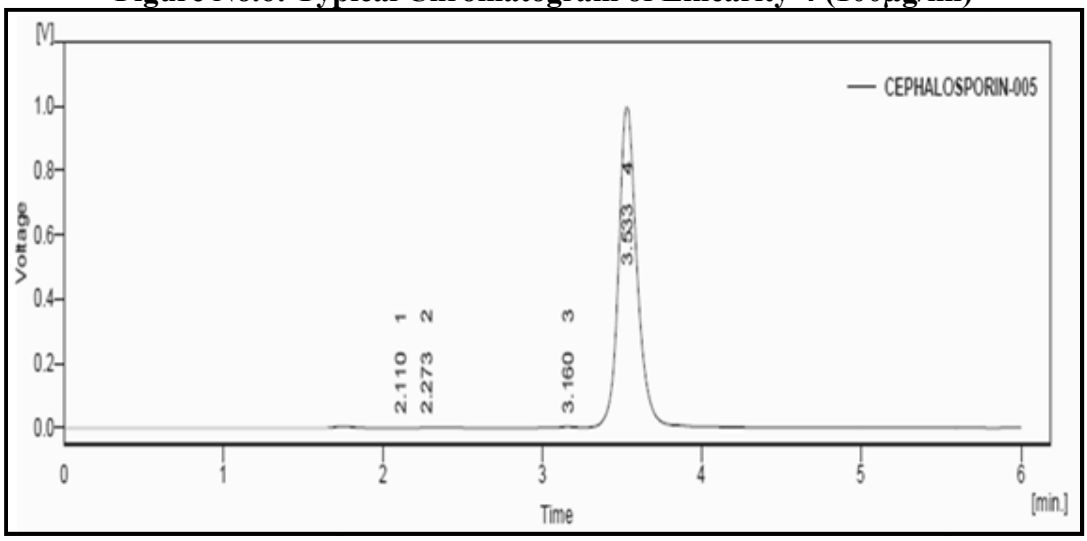


**Figure No.7: Typical Chromatogram of Linearity-5 (120µg/ml)**

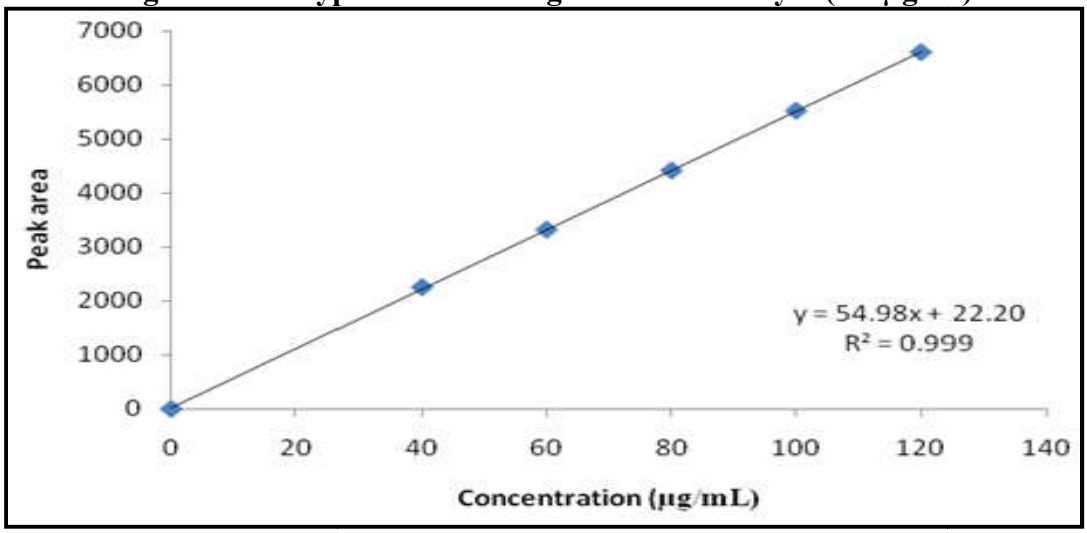


**Figure No.8: Calibration Plot for ceftaroline fosamil**

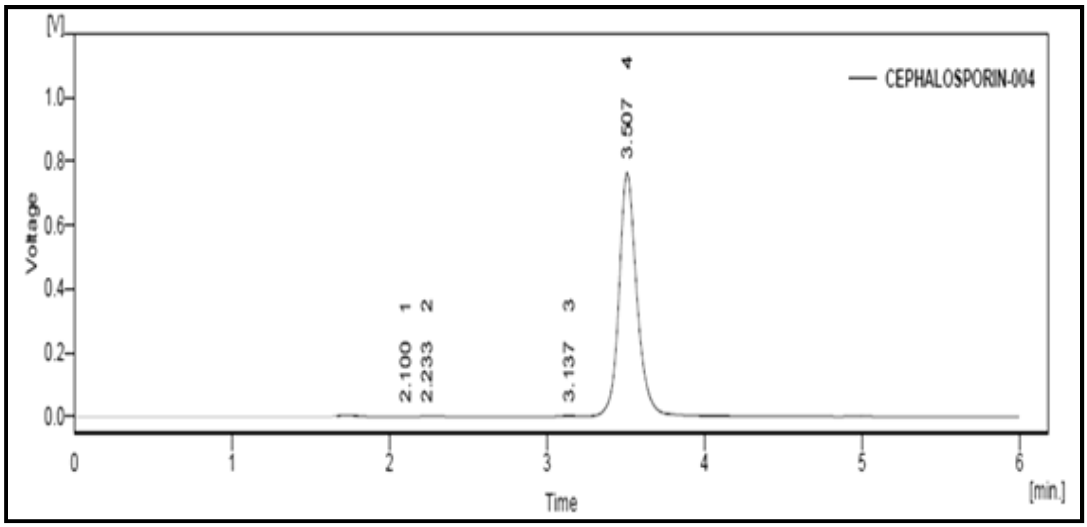


**Figure No.9: Typical Chromatogram of system precision**

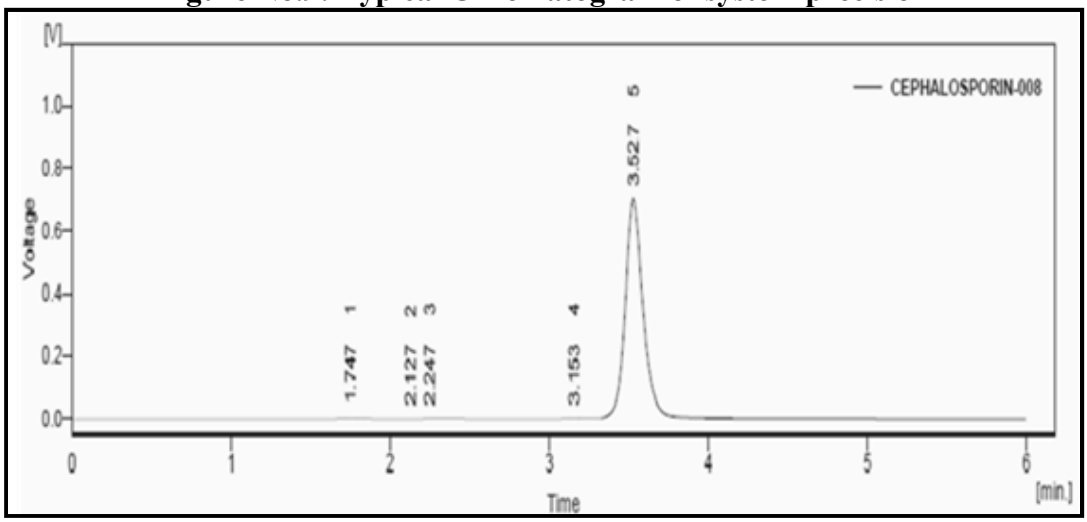


**Figure No.10: Typical Chromatogram of method precision**

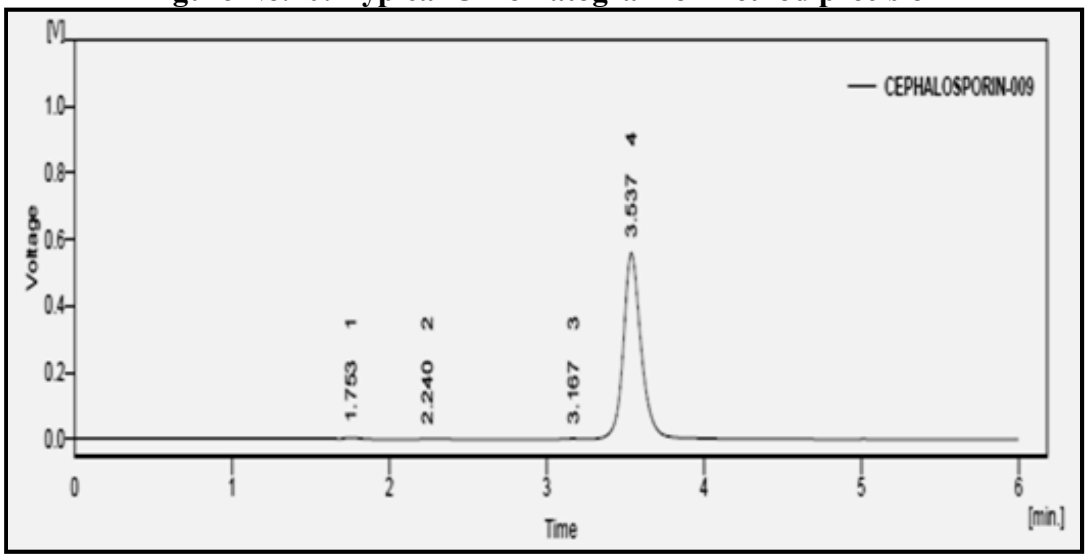


**Figure No.11: Typical Chromatogram of Accuracy 80%**

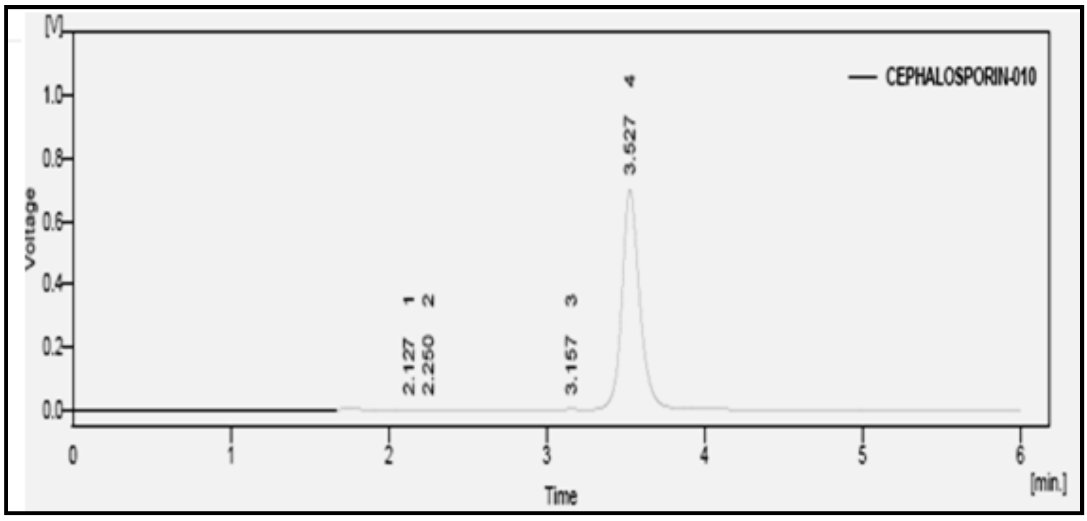


**Figure No.12: Typical Chromatogram of Accuracy 100%**

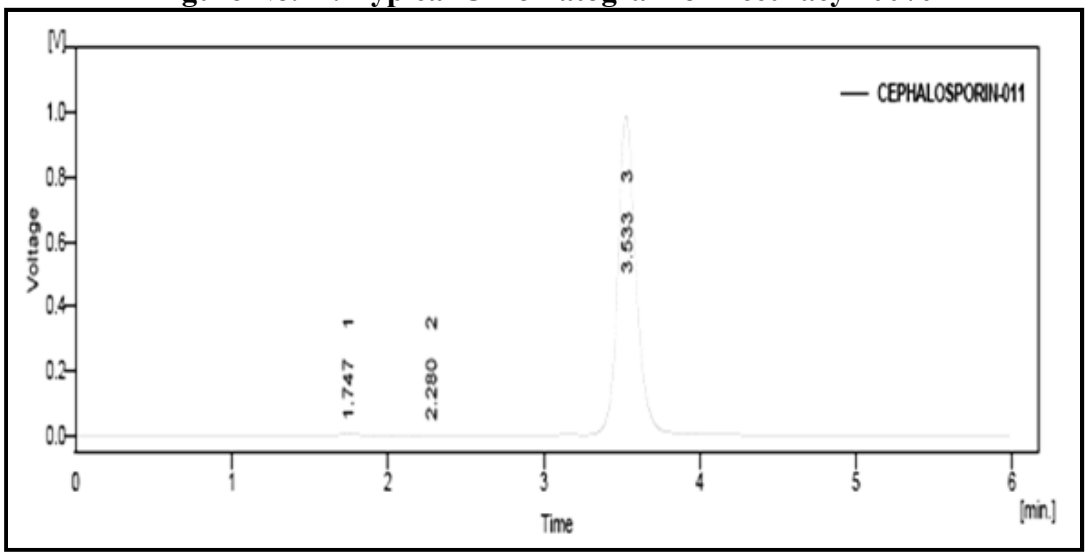


**Figure No.13: Typical Chromatogram of Accuracy 120%**

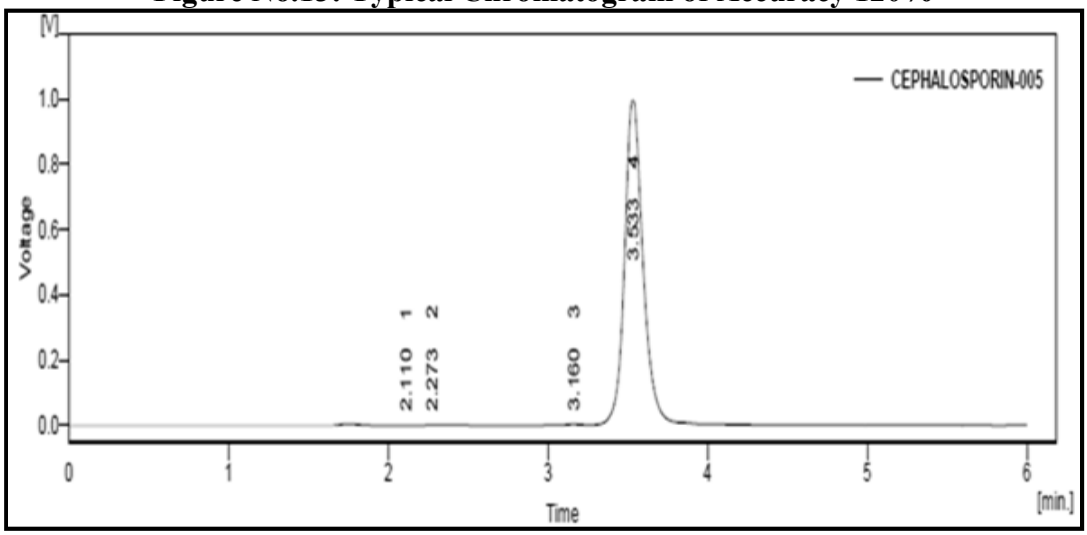


**Figure No.14: Typical Chromatogram of Effect of flow rate- 0.9ml/min**

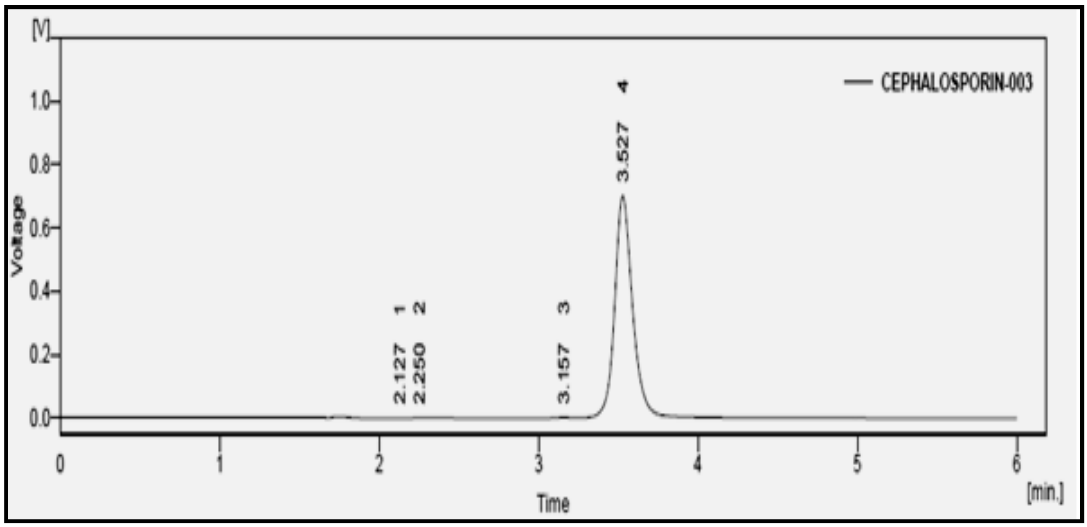


**Figure No.15: Typical Chromatogram of Effect of flow rate- 1.1ml/min**

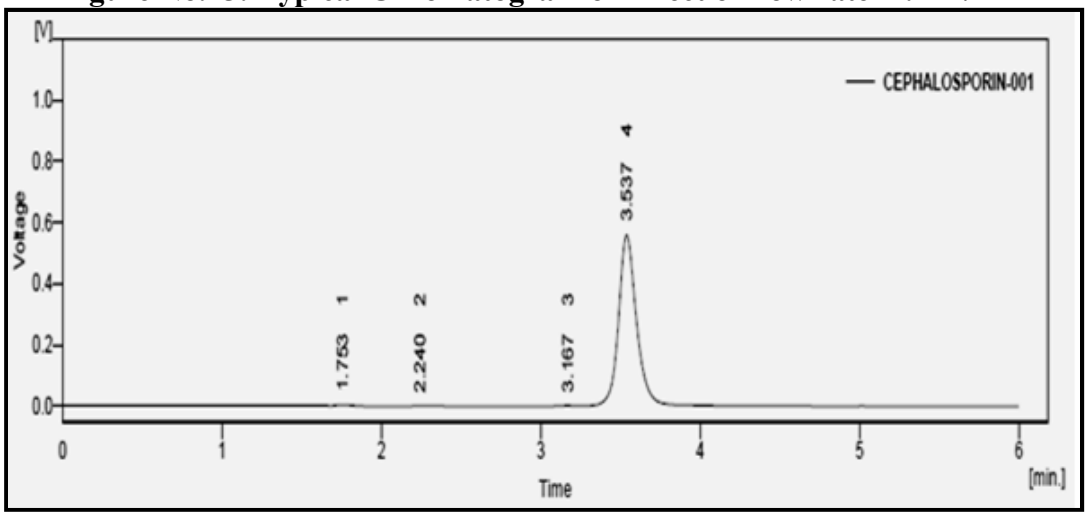


**Figure No.16: Typical Chromatogram of Effect of detection wave length (244nm)**

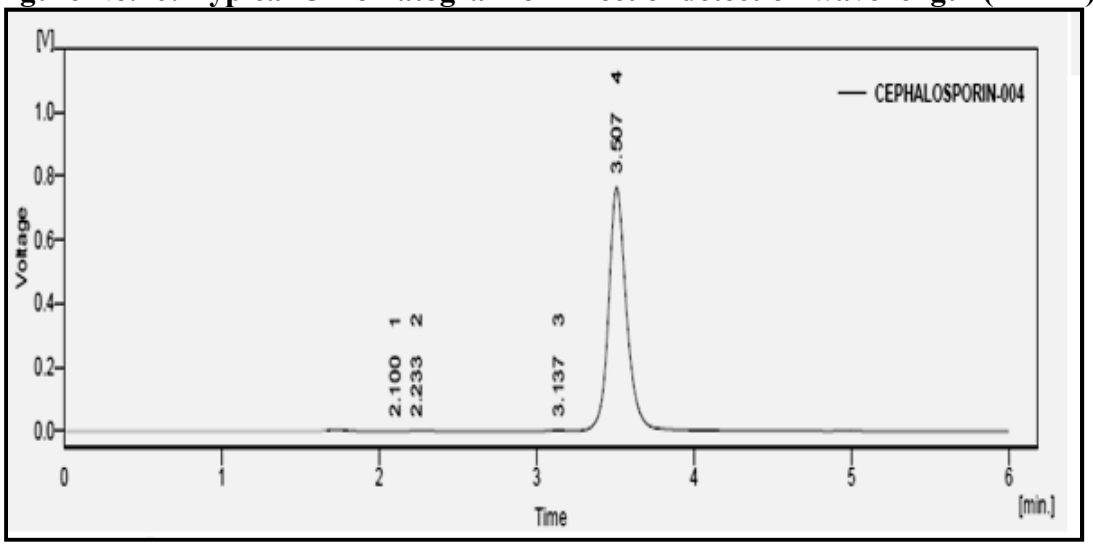


**Figure No.17: Typical Chromatogram of Effect of detection wave length (240n.m)**

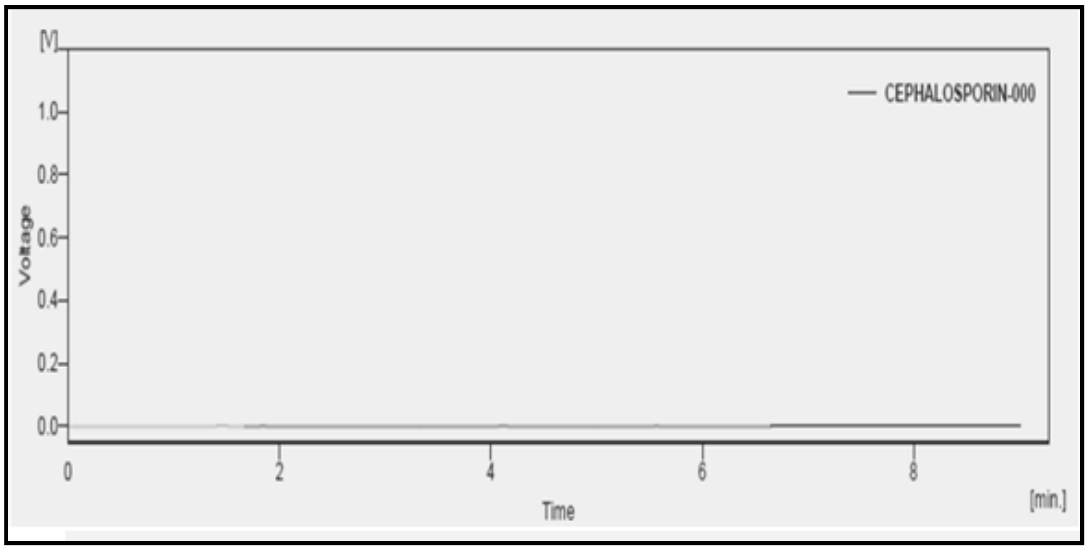


**Figure No.18: Typical blank chromatogram for Specificity**

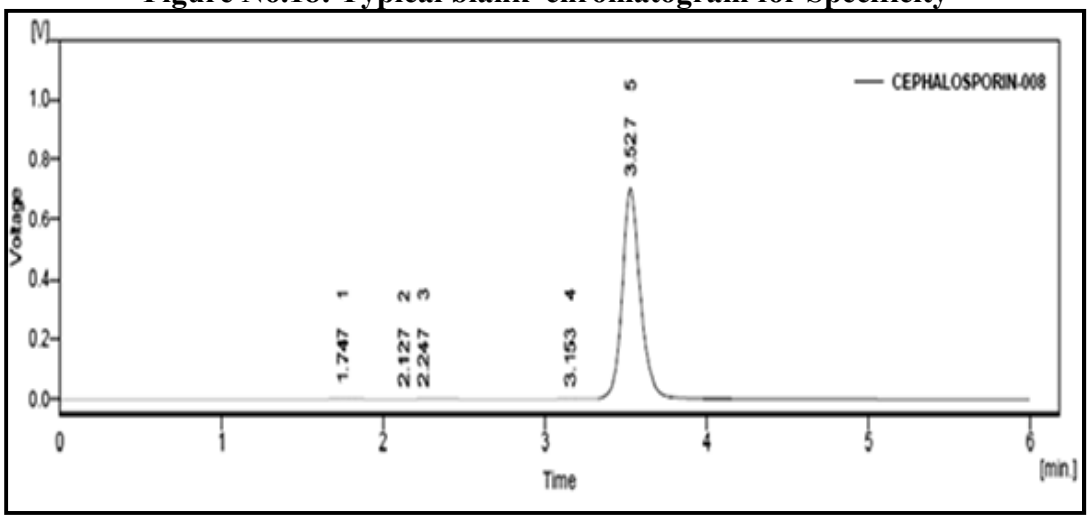


**Figure No.19: Typical Sample Chromatogram for Specificity**

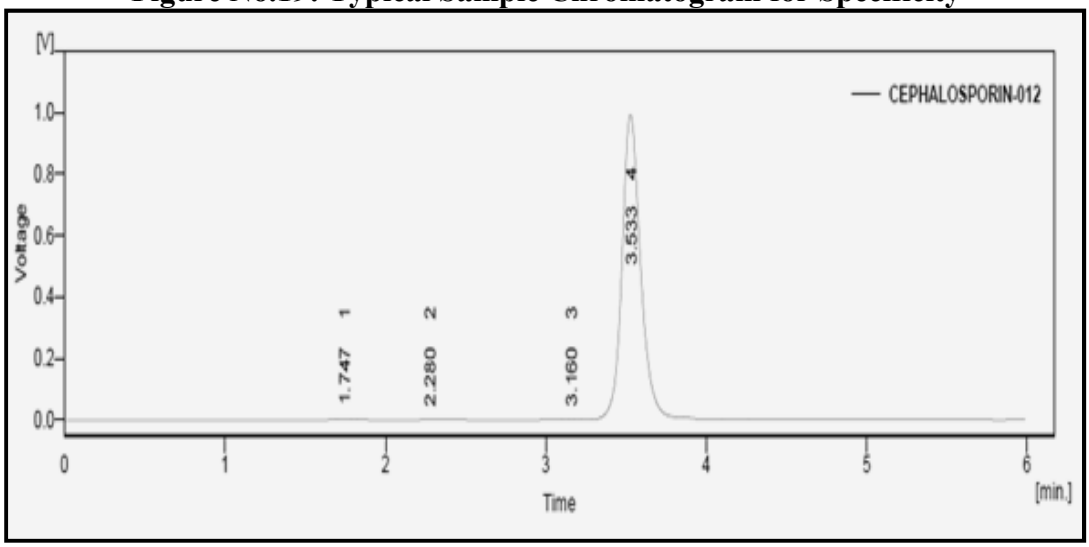


**Figure No.20: Typical Standard chromatogram for Specificity**

## CONCLUSION

The present study was carried out to develop a simple, sensitive, accurate and more precise UV and RP-HPLC methods for the estimation of ceftaroline fosamil in bulk and pharmaceutical dosage forms. The % purity of was calculated by using both methods and it was found to be 100.02% and 99.63%, respectively.

A good linear ship was observed between concentration of standard solution and the respective ratio of peak areas in the range of 40-120µg/ml. The drug obeys Beer's law with a correlation coefficient of 0.999.

The ceftaroline fosamil sample solution was analyzed by proposed RP-HPLC method for finding out intra and inter day variations showed a low coefficient of variation. It indicates that the present RP-HPLC method is highly precise.

By spiking various concentrations ranging about 80%, 100%, 120% into previously analyzed sample, the amount of drug recovered was calculated and it was in the range 98.26% - 99.24%. It indicates that the proposed method was highly accurate.

Based on the standard deviation, slope, limit of detection and limit of quantification values for ceftaroline fosamil were calculated and found to be within the acceptance limit. The lowest possible concentrations can be determined by the proposed methods.

Based on the changes in the operating conditions in robustness studies, the % RSD of ceftaroline fosamil was found to be within the limits statistically and method was considered as robust.

Forced degradation studies revealed that the drug was degraded under sun light, oven and oxidative conditions only and the drug was not degraded under acid, alkali and freezer conditions.

System suitability parameters were also checked by using proposed HPLC method.

Thus the Proposed UV and HPLC methods can be successfully applied for the routine quality control analysis of ceftaroline fosamil in bulk and IV Injection forms.

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## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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