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Novel Analytical Method Development and Validation for Cefotetan New β-Lactam Antibiotics in Bulk and Dosage Form

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ABSTRACT

The aspire and intention of the present study is to expand moreover authenticate a novel as well as rapid reverse phase chromatography separation technique for the estimating Cefotetan in bulk as such active pharmaceutical ingredient and dosage form to justify the presence of drug in the developed dosage forms and give satisfaction towards presence of medicine and its assay estimation. As the drug Cefotetan compendial monograph is not available in Indian Pharmacopoeia and British Pharmacopoeia, but a compendial monograph is available in United Sate Pharmacopoeia i.e. USP-40. USP monograph has a drawback that the standard solution and sample solution must be kept away from the light and to be used within 90 minutes after freshly preparation and which is time consuming, expensive and non eco-friendly method. To overcome these problems a new method is developed and validated in this research.

Keywords: chromatography, Cefotetan, HPLC method

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INTRODUCTION:

Cefotetan is a semi synthetic cephamycin antibiotics basically used to treat various bacterial infections [1]. Cefotetan administered intravenously or intramuscularly, it is highly resistant to a broad spectrum of β- lactamase and show efficacy towards wide range of both aerobic and anaerobic gram-positive and gram-negative microorganisms [1, 10].

Molecular formula of Cefotetan is $C_{17}H_{15}N_7Na_2O_8S_4$ and chemically Cefotetan is (6R,7S)-7-{4-[carbamoyl(carboxy) methylidene]-1,3-dithietane-2-amido}-7-methoxy-3-{[(1-methyl-1H-1,2,3,4-tetrazol-5-yl)sulfanyl]methyl}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid[1, 3].

Molecular weight of Cefotetan is 575.623 g/mol, it is soluble in methanol, extremely soluble in water with pKa value 6.2 [5].

Literature survey indicated that very few analytical methods have been establishes for the qualitative and quantitative analysis of Cefotetan in bulk and dosage form [6,8]. However drug is widely used in pharmaceutical field for the treatment of bacterial infections and drug does not any Pharmacopoeial or compendial analytical method in IP and BP [2,3]. A monograph of drug is present in USP-40 which has a drawback that stock and sample solution prepared for analysis must be kept away from the light and to be used within 90 minutes after freshly preparation which make this method time consuming, not accurate in manner of adequate analysis, expensive and non eco friendly[4].

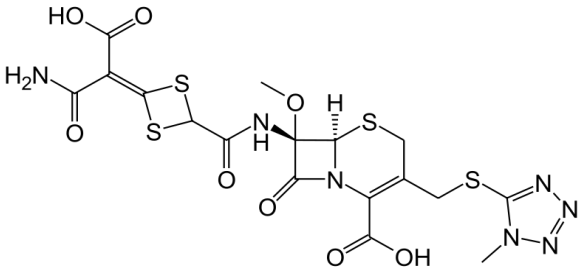

Fig.1: Chemical Structure of Cefotetan.

The objective of this work is to develop a new, simple, economic, rapid, eco friendly, accurate, and precise HPLC method for qualitative and quantitative estimation of Cefotetan in bulk and dosage form.

MATERIALS AND METHODS:

Material & Reagents:

The material used in the research should be collected from the proper sources so that it could be used in research by an assurance for that the material is going to be used have been validated in manner to gives that the material is justified and suitable to use in the research and also compendial fulfillment of substance. Cefotetan pure drug was brought from Natco laboratories which is located in Hyderabad in India. 20ml vials were purchased from Apollo pharmacy. As per the solubility's studies acetonitrile, ortho phosphoric acid was brought from Merck chemicals' which is located in Mumbai. Milli- Q water was used for experiment.

Instrument used:

Analytical balance (Aicoset), **HPLC Instrument** (WATERS) Alliance e2695 EMPOWER- 2, **Column** used [Phenyl (100mm × 2.1 mm, 5µm) & TSS column with acquity column octadecylsilane, acquity column], **Detector** (UV detector), **Sonicator** (SONICA 2200MH), **pH meter** (Metler Toledo), **Vacuum filter** (Model XI 5522050 of Millipore).

Method:

Preparation of Phosphate buffer as mobile phase:

Accurately weigh 7.0 g of Potassium dihydrogen phosphate by using a calibrated weighing instrument and transfer into 1000 ml beaker, caution that milli pore water should be used as the drug is not showing solubility, adjust pH to 4 by using acidic compound phosphoric acid.

Preparation of acetonitrile and buffer mobile phase:

Take 40 ml along with 60 ml of buffer and acetonitrile in the ratio 60:40 and degas this by using sonication and filter the above solution by using 0.45um membrane filter with aid of vacuum.

Cefotetan Standard Solution Preparation:

Take 10.0 mg of drug in to 10ml volumetric flask to this add 7 ml of diluents followed by sonication and volume is made up to mark by suing diluents. (Stock solution) From the above Solution pipette out exactly measured 0.5 ml of solution in to 10 ml of glass I having grade A of volumetric flask it is filtered through 0.45 um membrane filter.

Cefotetan Sample Solution Preparation:

An equivalent 10.0 mg of Cefotetan is pipette out from vial and pour into a 10ml of class I grade of volumetric flask. After that add accurately pipette out 7 ml to make diluents and further sonicate to enhance solubility and filtered through 0.45 um membrane filter.

Chromatographic condition:

Table 1.Selection of Chromatographic condition for Cefotetan

Parameters	Method
Stationary phase i.e. column	Column used is designed by WATERS along with high strength silica with Octadecylsilane column with 100mm length and 20. Mm diameter.
Mobile Phase	KH_2PO_4: C_3H_6O in ratio of (40:60% v/v)
pH	6 ± 0.02
run speed ml per minute	1 ml per minute
scamper instance (minutes)	6 min
article hotness (°C)	Ambient
amount of inoculation loop (mL)	20
recognition wavelength(nm)	243
Drugs having RT (min)	2.754

Chromatogram got for Cefotetan shown in The in **Figure 2.**

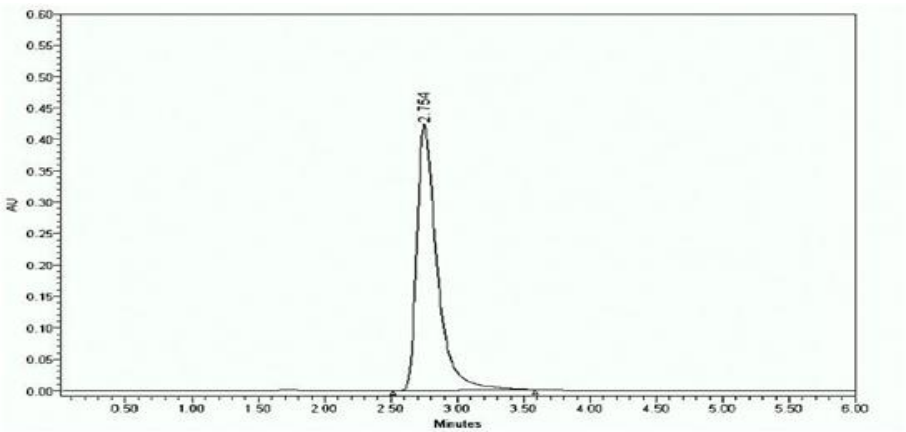

Figure 2.Typical Chromatogram for Cefotetan

RESULT & DISCUSSION:

System acceptance

While using the method for the analysis to be identified on the basis of various parameters like resolution of the peak, retention time of the peak and sharpness too, which include the justification of segment that would give the area under curve in mentioned of the total area for the ratio of standard and sample, which repeatedly mentioned that the method used is validated and accurate to identify the correlation and regression for the complied of straight line equation which indicates that the result obtained is accepted in manner to accuracy and repeatability [8, 11].

System suitability:

System suitability is the method developed which gives repeatability in manner of accuracy and gives appropriate results. Standard result for Cefotetan might have been readied concerning illustration for every technique also might have been injected six times under those HPLC frameworks [12, 9]. The framework suitable parameters were assessed starting with standard Chromatograms gotten toward ascertaining those percent RSD of maintenance times, tailing factor, hypothetical plates and top territories starting with six reproduce injections [7, 14].

1. The % RSD for the maintenance times for vital top starting with 6 replication injections of each standard result ought further bolstering make not more than 2.0%.

2. The amount of hypothetical plates (N) for those Cefotetan top ought to be NLT 2000.

3. The tailing figure (T) to those Cefotetan crest ought to a chance to be NMT 2.0.

Assay:

$$\text{Assay\%}=\frac{AT}{AS}x\frac{WS}{DS}x\frac{DT}{WT}x\frac{P}{100}x\frac{AVG\ Wt}{Label\ claim}x100$$

Where:

AT = top range of got for test preparation.

AS = crest range about gotten for standard sample concentration preparation.

WS = amount in accurately weight for attempting standard in mg

WT = Weight from claiming example made on mg.

DS = weakening of standard result.

DT = weakening from claiming test result

P = rate purity about working standard.

Precision

The percentage relative standard deviation R.S.D. of Cefotetan examines stated to be 0.4%, representative expert accuracy of technique. The consequences are reviewed in **Table 3.** Remaining chromatogram was placed from **Figure 3 to 7.**

Table 2.Table for Results developed in precision

Injections	Area under curve
Injection(1)	4796667
Injection(2)	4712916
Injection(3)	4721422
Injection(4)	4771493
Injection(5)	4750737
Average of Inj.	4750647
Standard Deviation (SD)	34749.6
%RSD	0.73%

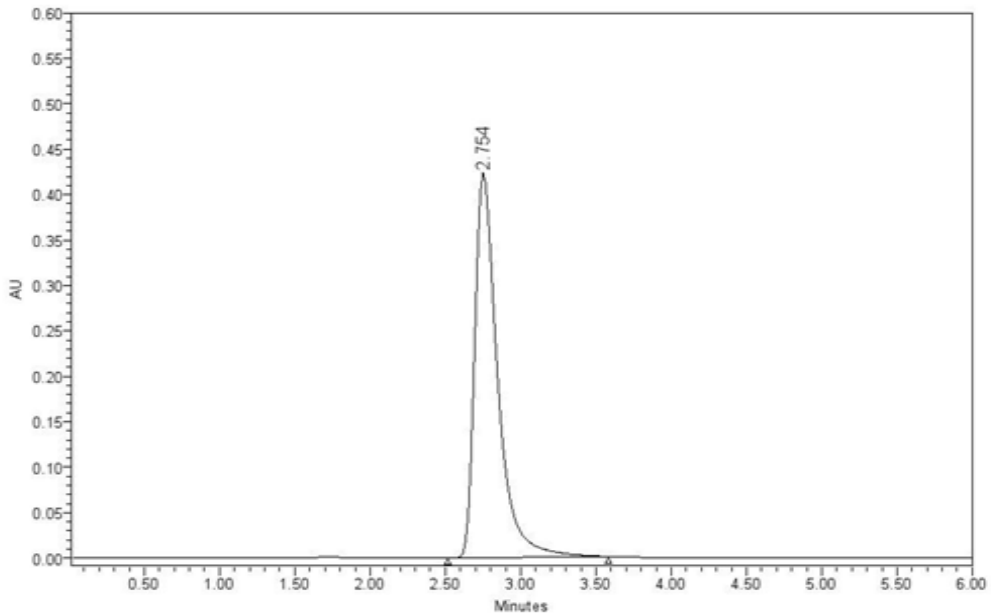

Figure 3.Precision injection-1, chromatogram

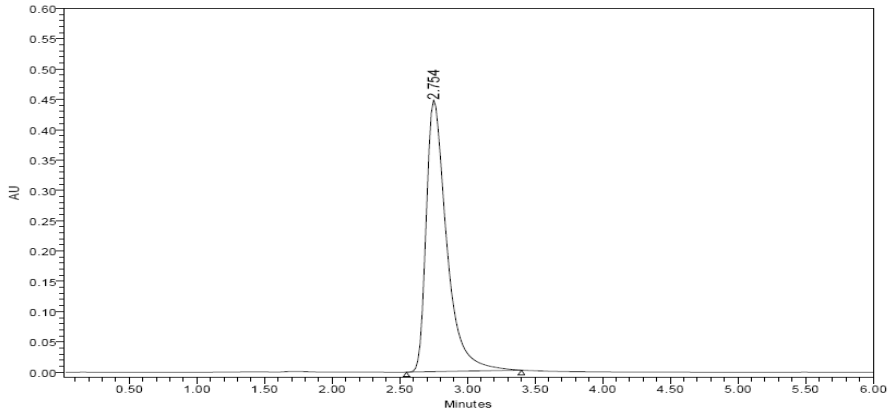

Figure 4.Precision injection-2, chromatogram

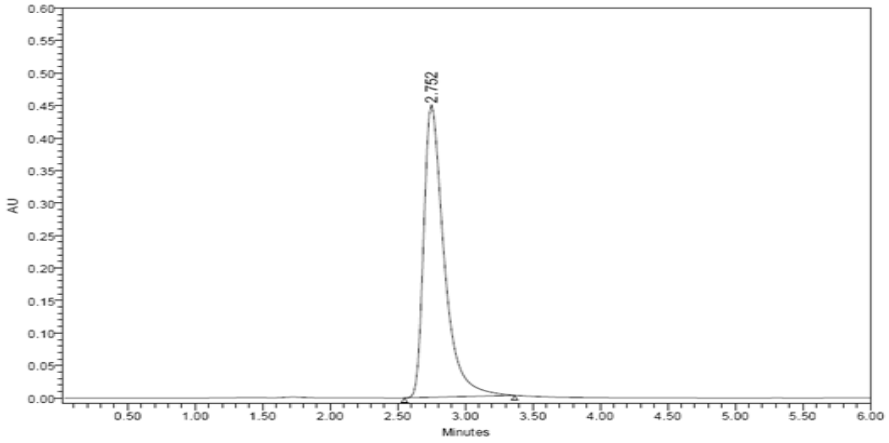

Figure 5.Precision injection-3, chromatogram

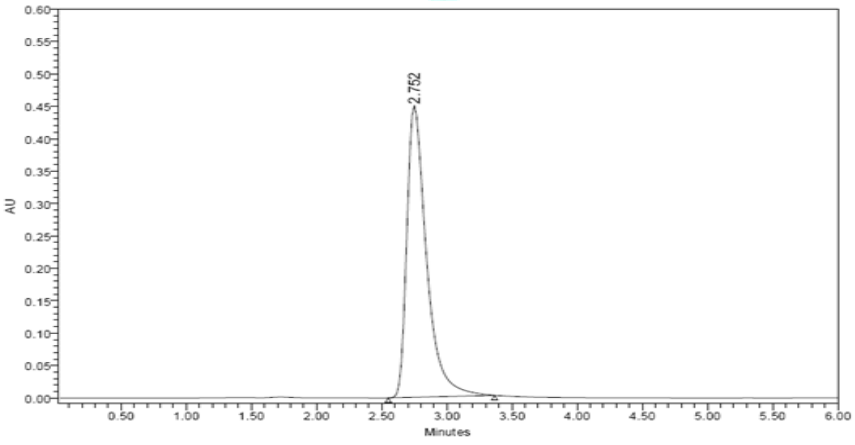

Figure 6.Precision injection-4, chromatogram

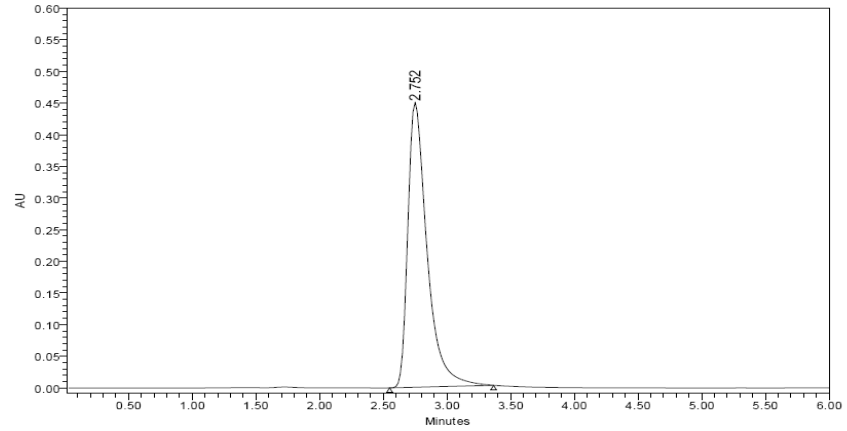

Figure 7.Precision injection-5, chromatogram

Intermediate Precision

The percentage relative standard deviation R.S.D. of Cefotetan for intermediate study was found at 0.08%, value developed during the analysis within the limits and the data of same was interpreted in the table below marked as .

number four in which results obtains as per injection given while analysis and their data in interpreted in to the tables and for the same chromatogram developed also attached herewith for reference and consequence displayed in figure 8-12

Table 4.Results of obtained during intermediate precision

Injections	Area under curve
Injection(1)	4696666
Injection(2)	4616416
Injection(3)	4621626
Injection(4)	4671793
Injection(5)	4656697
Average of inj.	4652640
Standard deviation	33895.5
%RSD	0.728%

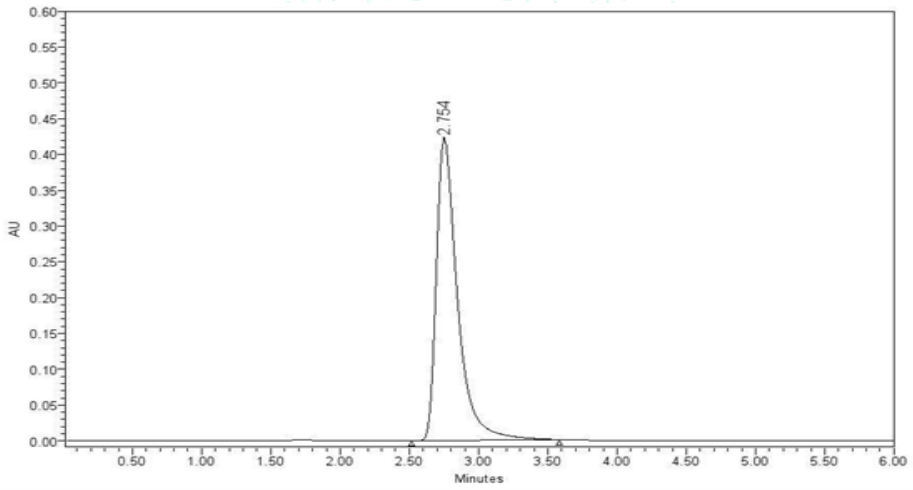

Figure 8.Intermediate Precision injection-1, chromatogram

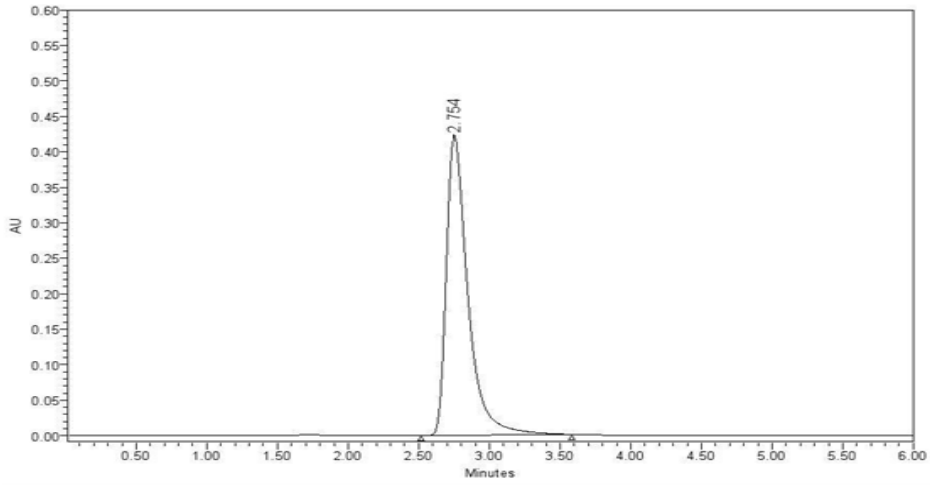

Figure 9.Intermediate Precision injection-2, chromatogram

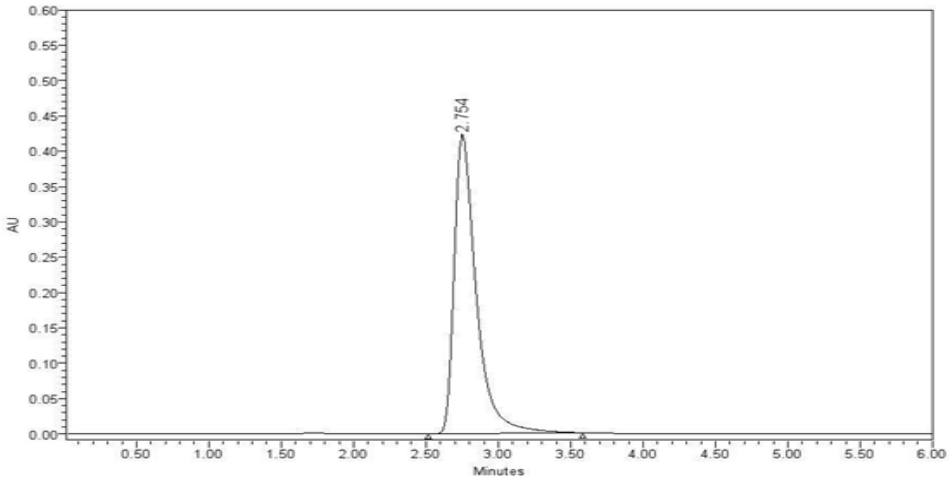

Figure 10.Intermediate Precision injection-3, chromatogram

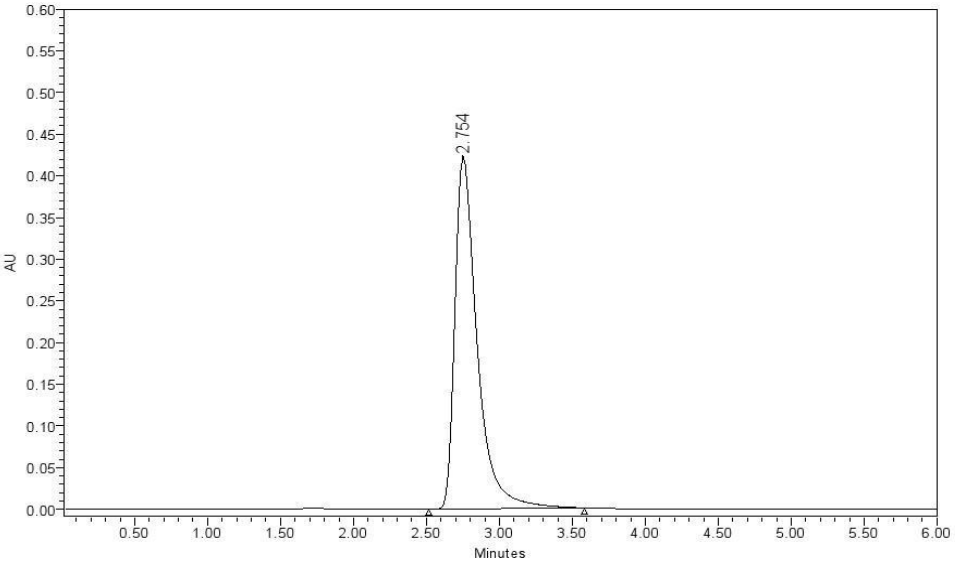

Figure 11.Intermediate Precision injection-4, chromatogram

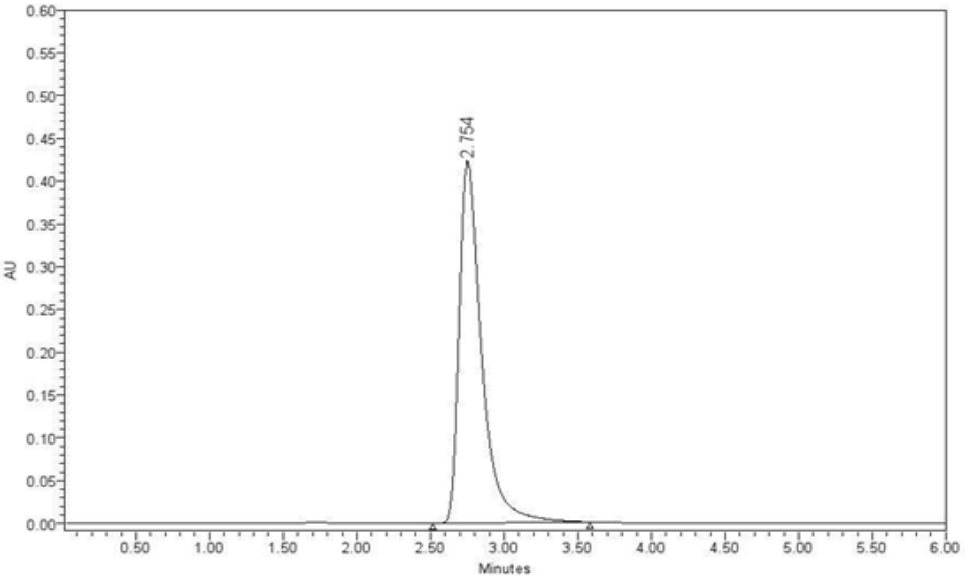

Figure 12.Intermediate Precision injection-5, chromatogram

Accuracy

In accuracy various injection are injected for the development of chromatogram of drug Cefotetan was within 98.8% to 101.4%, revival studies was at 99.8%, which shows high-quality results, which are shown in **Table 5.**

The chromatograms were shown in figures from **Figure 13 to 21.**

Table 5.Results of Accuracy obtained:

%age Conc. at specific level	Mean Peak Area (n=3)	Qty. added (mg)	Qty. found (mg)	Average % Recovery	Mean Recovery
50%	4838809	5.0	5.07	101.4%	100.7%
100%	9462459	10.0	9.91	99.1%	
150%	14149612	15.0	14.8	98.8%	

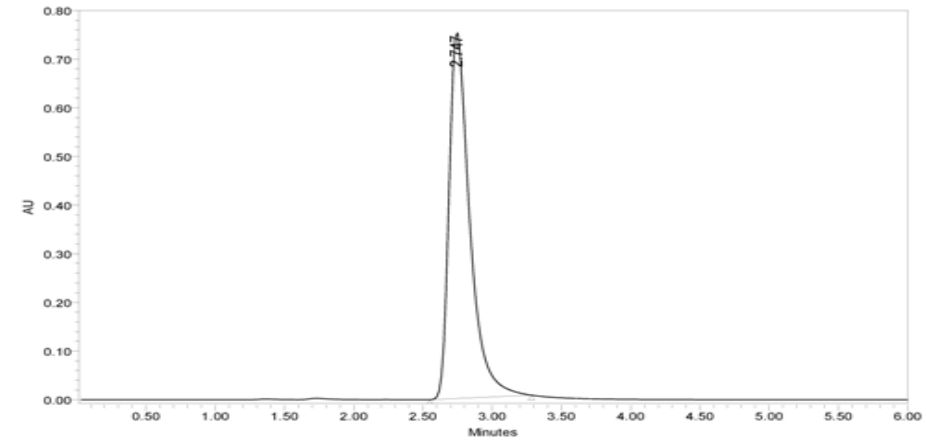

Figure 13.Accuracy 50% injection-1, chromatogram

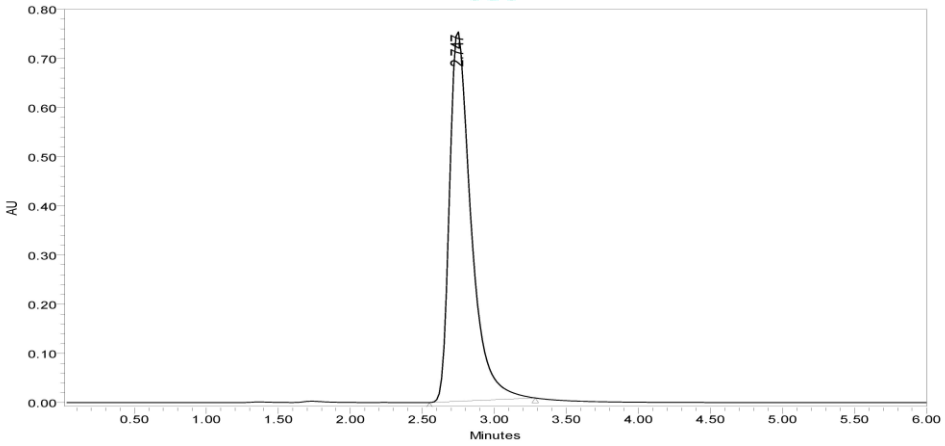

Figure 14.Accuracy 50% injection-2, chromatogram

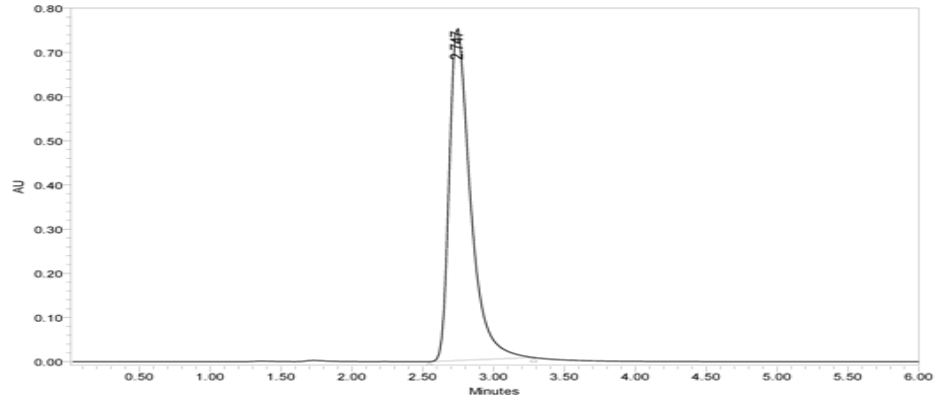

Figure 15.Accuracy 50% injection-3, chromatogram

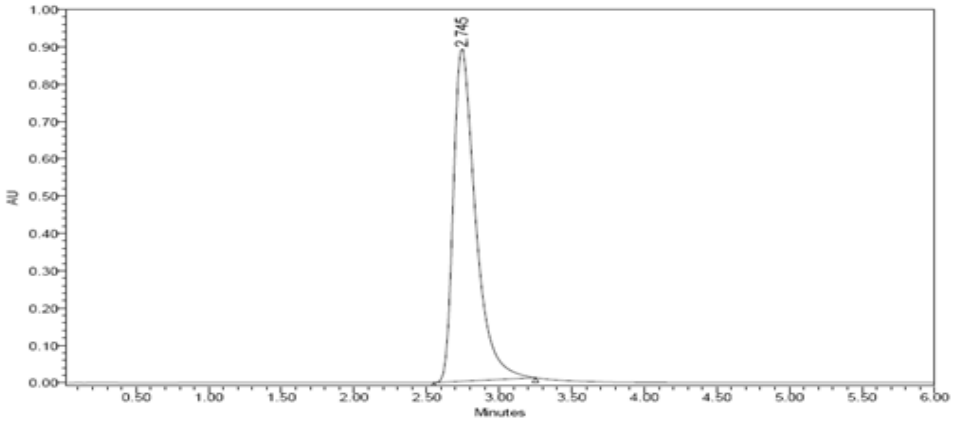

Figure 16.Accuracy 100% injection-1, chromatogram

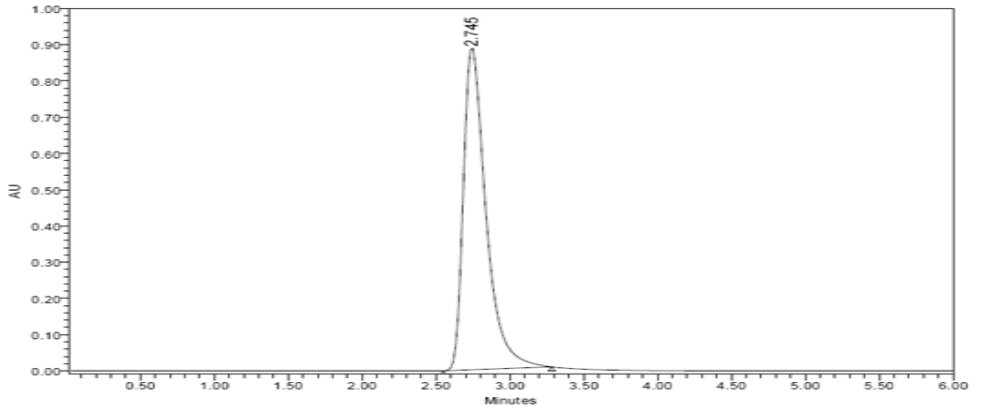

Figure 17.Accuracy 100% injection-2, chromatogram

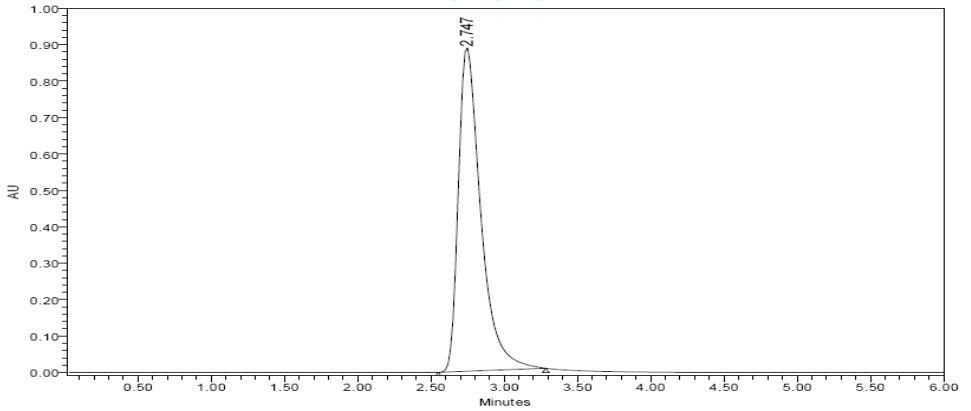

Figure 18.Accuracy 100% injection-3, chromatogram

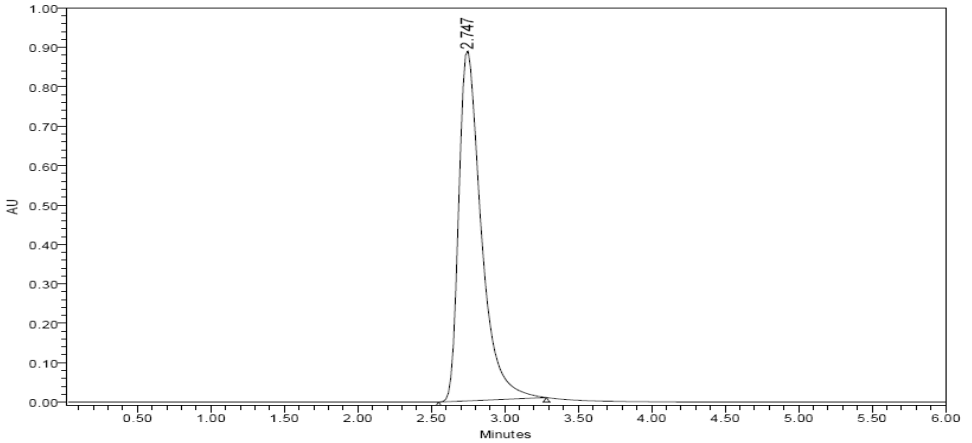

Figure 19.Obtained Chromatogram for Accuracy 150% injection (1)

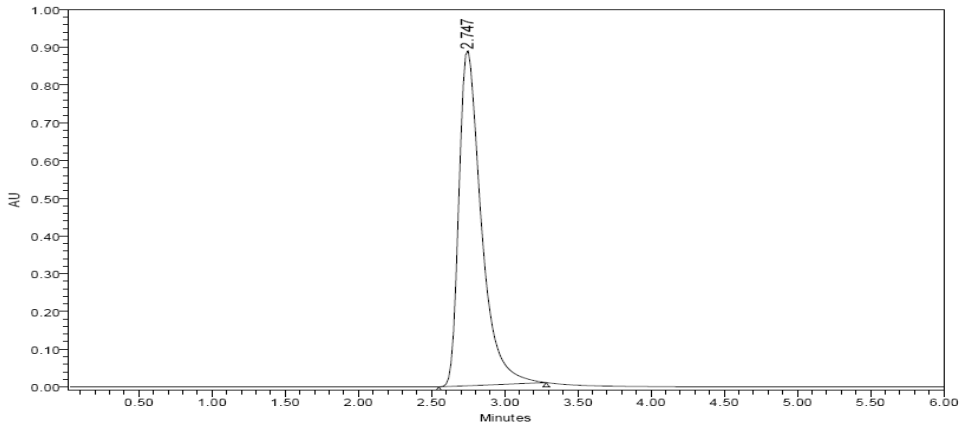

Figure 20.Obtained Chromatogram for Accuracy 150% injection (2)

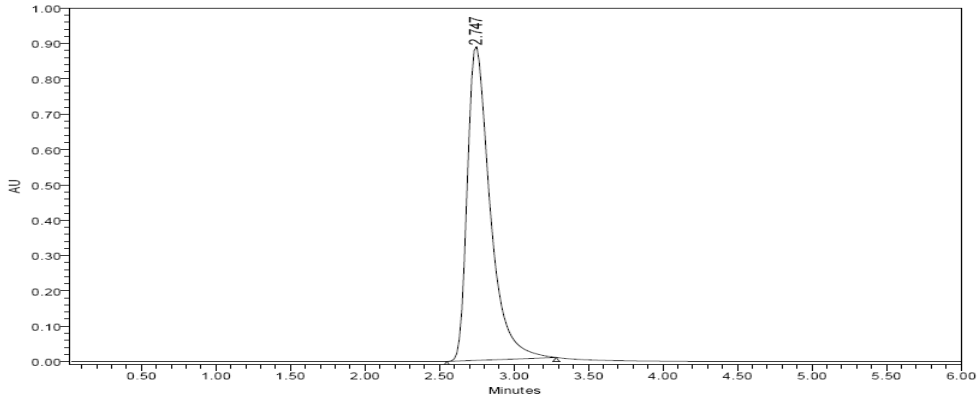

Figure 21.Obtained Chromatogram for Accuracy 150% injection (3)

Linearity

The linearity of the alignment plot to the strategy might have been got again the alignment ranges tested, i.e. 30 - 70µg/ml to three times, and the relationship coefficient acquired might have been 0. 999, therefore demonstrating phenomenal connection between top regions What's more focus of the analyte [13, 15]. The results were summarized in **Table 6** and the linearity curve for Cefotetan was illustrated in **Figure 22.**The corresponding chromatograms were illustrated in figures from **Figure 23 to 27.**

Table 6.Tabulation for Linearity:

S.No	Concentration	Area Under Curve
1	30µg/mL	2939828
2	40µg/mL	3978576
3	50µg/mL	5125552
4	60µg/mL	6168913
5	70 µg/mL	7047599
Correlation Coefficient	0.999	

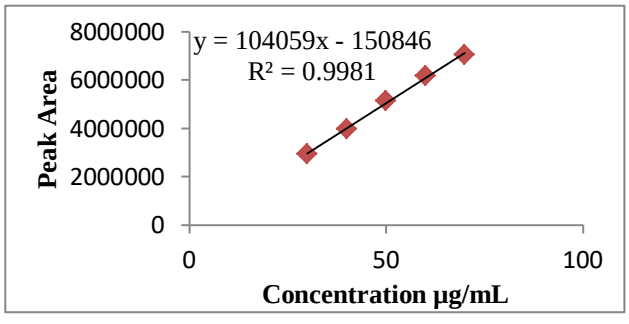

Figure 22.Linearity curve for Cefotetan

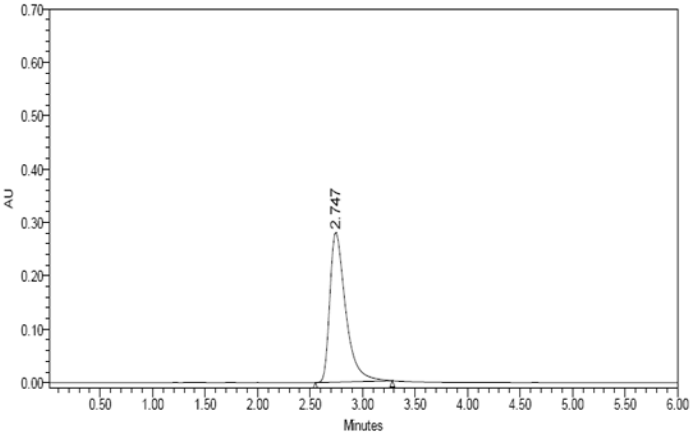

Figure 23.Linearity injection-1, chromatogram

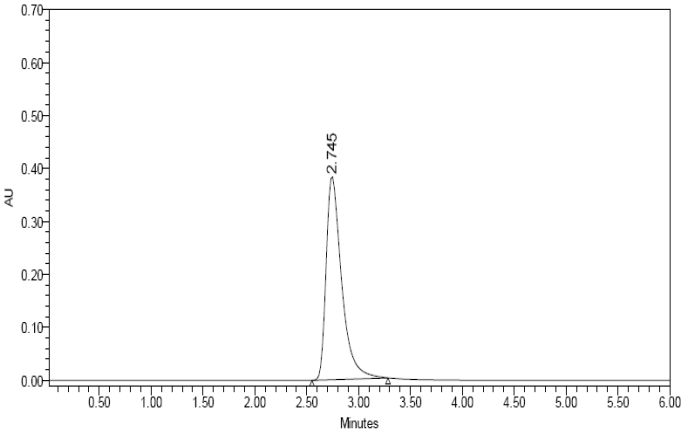

Figure 24.Linearity injection-2, chromatogram

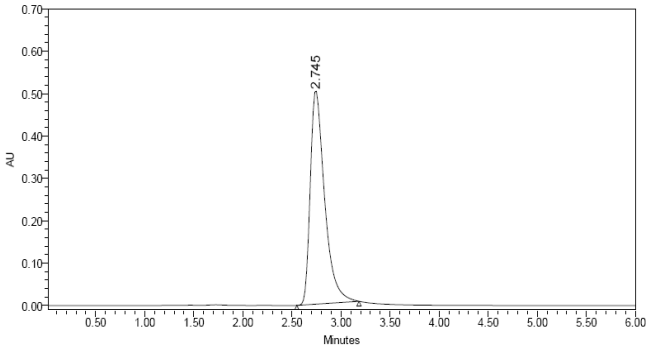

Figure 25.Linearity injection-3, chromatogram

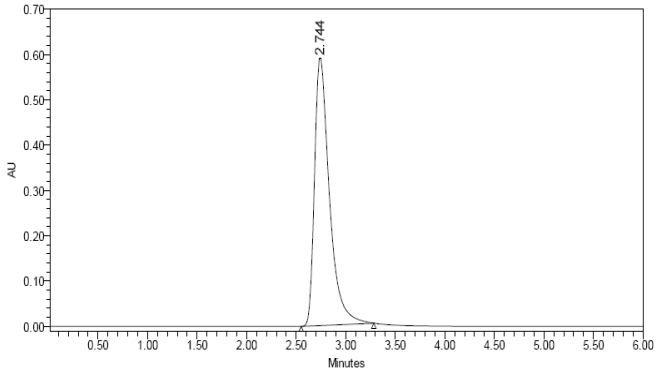

Figure 26.Linearity injection-4, chromatogram

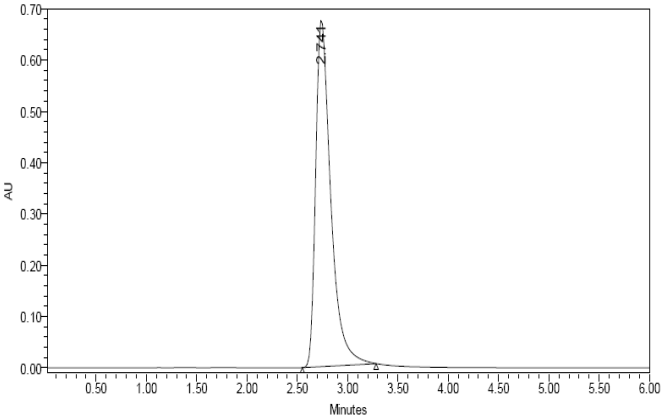

Figure 27.Linearity injection-5, chromatogram

Robustness

Altogether those deliberately shifted chromatographic states in the centralization reach to those assessment for heartiness will be 10 -50 µg/ml, (n=3). It could a chance to be closed that the variety over stream rate and the variety done 10% natural creation don't influence the technique fundamentally [14, 16]. Consequently it demonstrates that those techniques will be strong indeed by progress in the stream rate ±10% what more change in the versatile stage ±10%. Those effects are summarized in **Table 7.**

Table 7.Consequences of Robustness

Chromatographic changes	*United states plate count*	*USP Tailing*
Flow rate(ml/min)		
0.6	2679.7	1.7
0.8×	2736.7	1.6
1.0	2597.1	1.7
adjust in untreated concerto in the movable stage		
10% less	2311.0	1.5
60:40(Buffer: methanol)×	2736.7	1.6
10% more	2218.0	1.5
UV wavelength(nm)		
226	2617.0	1.5
228×	2986.7	1.6
230	2529.0	1.54

× optimized parameters

LOD&LOQ obtained in method developed

Limit of detection and quantification comprise that either the minute to minute concentration in nano gram also can be identified in manner very sensitivity or sharp chromatogram will be generating as per the high concentration of chromatogram [9, 14]. The word quantification indicates that the developed method will not depend upon the vary of concentration but its specificity and sensitivity will be same either the concentration analyte will be any it give same angle of chromatogram and same sensitivity will be noted. Limit of Detection and Limit of Quantification to Cefotetan were 0.01µg/ml and 0.05µg/ml separately [16]. Since those LOQ and LOD qualities from claiming Cefotetan are attained toward a low level, this system could make suitableness for cleaning acceptance in the pharmaceutical industry. The relating chromatograms were provided for to figure28 and also figure 29 to LOD and LOQ separately.

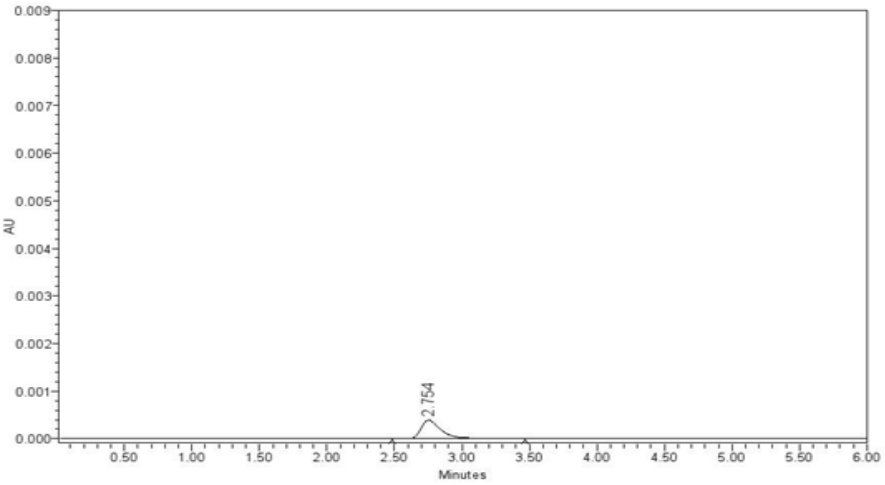

Figure 28.Chromatogram for LOD

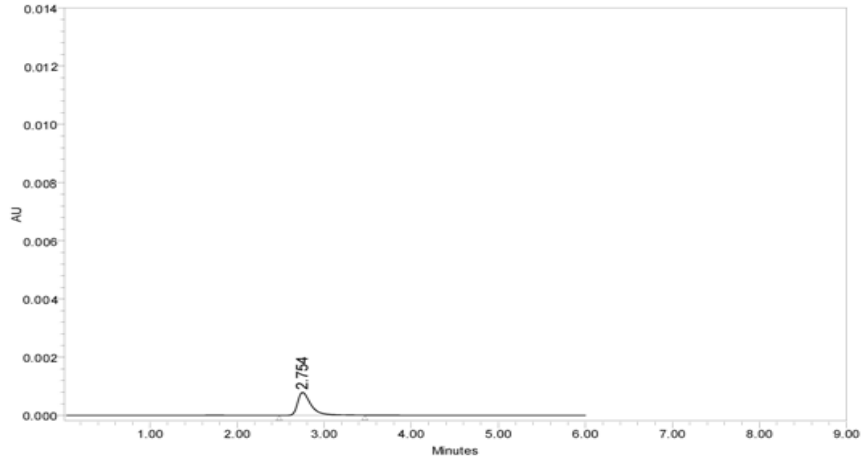

Figure 29.Chromatogram for LOQ

Application of the developed method to commercial Cefotetan dosage forms

As the developed method is a unique method for the estimation of Cefotetan in bulk and dosage form. This method can directly be used in quality control department as in-house testing method for the determination of assay in pharmaceutical developed product for human use.

When the created technique might have been used to dissect a business mark of Cefotetan tablet formulation, intend recuperation of triplicates was at 99.8 % with relative standard deviation of 0.09%.That % recuperation quality demonstrates non-interference starting with those excipient displays in the measurement structure. Those chromatograms were portrayed for figures starting with **Figure 30 to 32.**

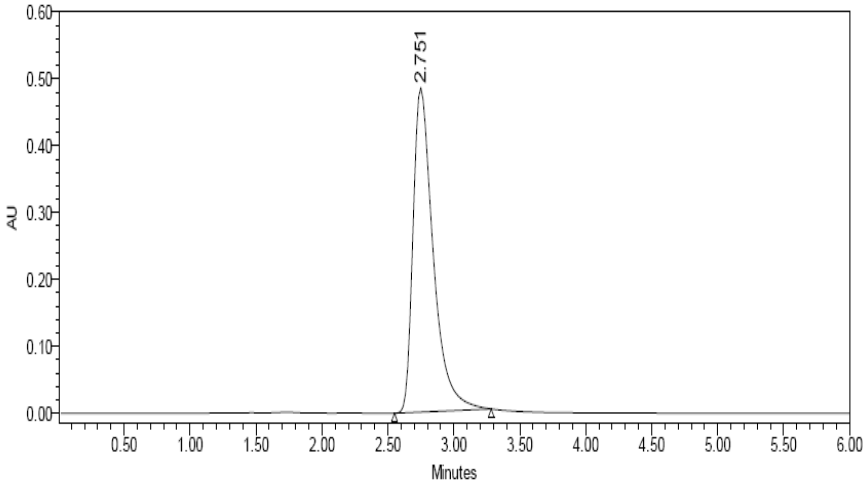

Figure 30.Chromatogram for Sample injection-1

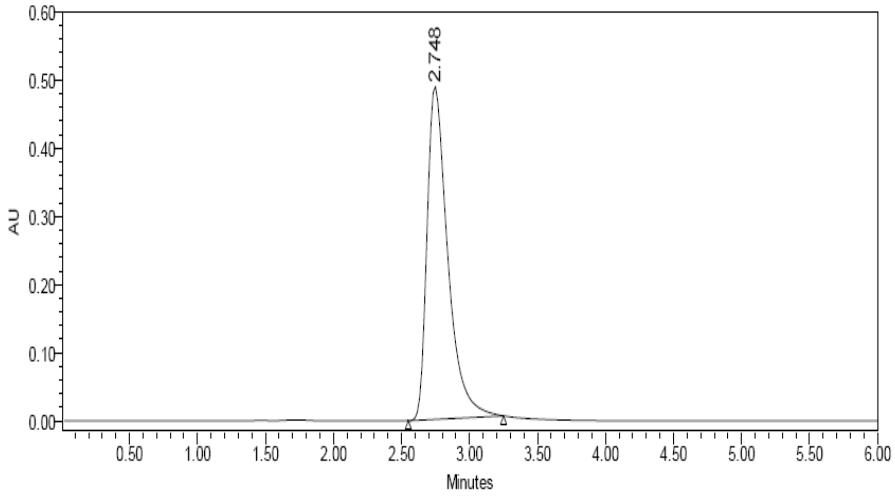

Figure 31.Chromatogram for Sample injection-2

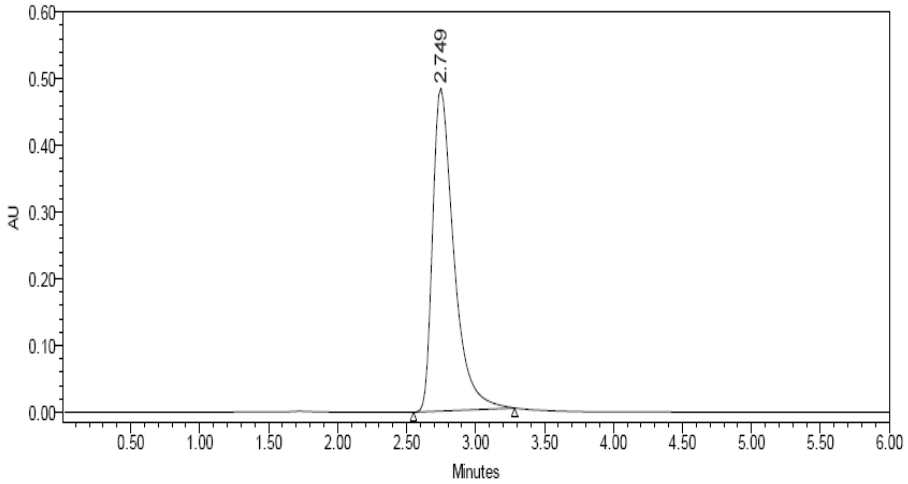

Figure 32.Chromatogram for Sample injection-3

ASSAY:

Weight of Test sample : 0.8285 grams

Average Weight : 0.1971 grams

$$\frac{5165251}{5168473} \times \frac{10}{10} \times \frac{0.5}{10} \times \frac{10}{197.13} \times \frac{10}{0.5} \times \frac{99.9}{100} \times \frac{197.13}{10} \times 100 = 99.8\%$$

CONCLUSION:

- The novel developed method for an isocratic /RP-HPLC technique which shows it's authenticate method to be easy, linear, correct, correct, strong, rough and fast. The urbanized technique had shown quicker elution; with good resolutions when compared to the other techniques my method has shown good resolution parameters with retention time of 2.754 minutes, which is good for analyzing bulk drugs in short time.
- Therefore my method is error free meeting all quality attributes in API and unit dosage forms.
- The urbanized technique was performed as per authorized body (ICH) rule with admiration to linearity, correctness, exactness, specificity and sturdiness.
- Present process was linear for Cefotetan starting 30, 70µg 1/ml and the linearity was 0.999. Correctness, examined via out- and within -day assays and relative standard deviation (R.S.D) principles was within 1.5%.

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