



NOVEL ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR CEFOXITIN NEW β -LACTAM ANTIBIOTICS IN BULK AND DOSAGE FORM

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ABSTRACT:

The Objective of this work based on for development of a novel method and validation of method used for determination of amount in manner of assay present into Cefoxitin in bulk and Tablet Dosage form. 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 Cefoxitin in bulk 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 Cefoxitin 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 for injection dosage form only. The developed method is new, simple, economic, rapid, eco friendly, accurate, and precise HPLC method for qualitative and quantitative estimation of Cefoxitin in bulk and dosage form like tablet, capsule, injection also.

INTRODUCTION

Cefoxitin is a semi synthetic cephamycin antibiotics basically used to treat various bacterial infections. It is highly resistant to a broad spectrum of β -lactamase and show efficacy towards wide range of both aerobic & anaerobic gram-positive and gram-negative microorganisms^(2, 12).

Molecular formula of Cefoxitin is $C_{16}H_{17}N_3O_7S_2$ and chemically Cefoxitin is (6R,7S)-3-[(carbamoyloxy)methyl]-7-methoxy-8-oxo-7-[2-(thiophen-2-yl)acetamido]-5-thia-1azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. Molecular weight of Cefoxitin is 427.454 g/mol. Cefoxitin is sparingly soluble in water and insoluble in crude with PKa value 3.59^(2, 5).

Literature survey indicated that very few analytical methods have been establishes for the qualitative and quantitative analysis of Cefoxitin in bulk and dosage form^(1, 6). 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^(3, 4).

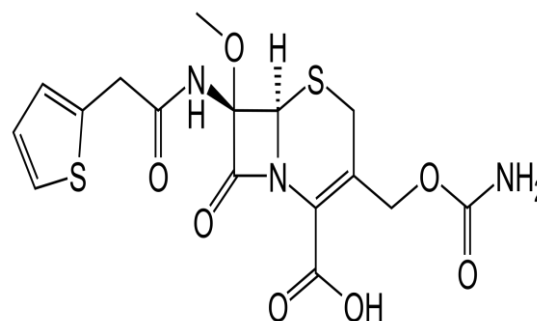


Fig.1: Chemical Structure of Cefoxitin.

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 Cefoxitin in bulk and dosage form.

MATERIALS AND METHOD:

Material and Reagents

Cefoxitin working standard might have been procured starting with Teena laboratories, Hyderabad, India. Economically accessible Cefoxitin bought from nearby drug store. Methanol HPLC evaluation also Ortho phosphoric corrosive are

evaluation were got starting with Merck chemicals, Mumbai.

Instrument used:

Analytical balance (Aicoset), **HPLC Instrument** (WATERS) Alliance e2695 EMPOWER- 2, **Column** used Acquity made high strength silica T-3 C18 Distance End To End 100MM, Width 2.1MM. Acquity ethylene bridged hybrid C18 Phenyl (100mm x2.1mm with pore of 5µm) **Detector** (UV detector), **Sonicator** (SONICA 2200MH), **pH meter** (Metler Toledo), **Vacuum filter** (Model XI 5522050 of Millipore).

METHOD:**Preparation of mobile phase:**

Take an accurately weigh 7g of potassium dihydrogen ortho phosphate in to calibrated 1000 ml/1litter of beaker as this solution is not stable buffer and adjust its pH to 4 by using ortho phosphoric acid and milli-Q water. Filter it through membrane filters through membrane filter.

Take 600 ml of methanol, 400 ml of Potassium dihydrogen phosphate and 600 ml of methanol, sonicate and filter through 0.45µ channel under presence of vacuum filtration now this will become 60:40 mobile phase.

Cefoxitin Standard Preparation:

Perfectly exchanged 10mg claiming Cefoxitin working standard under An 10 ml volumetric cup Also around 7 ml from claiming diluents included that point sonicate to break down it totally and the volume might have been aggravated to level with those solvent considered as Stock solution.

Further pipette out 5 ml of the over stock result under a 50mL volumetric cup Furthermore weakened dependent upon those diluents. Blend great through 0.45µm filter. Supplementary pipette 3 ml of the over stock result under 10ml volumetric cups also weaken up to those mark with diluents. Blend great Furthermore channel through 0.45µm filter.

Cefoxitin Sample Preparation:

Perfectly take test equal to 10 mg about Cefoxitin in 10 ml volumetric cup. Over 7ml from claiming diluents included also sonicate with break down it totally and the volume will be committed

dependent upon the mark with diluents. Blended great Furthermore separated by 0.45µm filter unit. Pipette out exactly 5 ml of the over standard result under a 50mL volumetric cup and weakened dependent upon those mark with diluents. Blend great passed through 0.45µm filter unit. Supplementary pipette out exactly 3 ml of over stock of above solution and filler into the result under a 10ml volumetric celebrated flask and diluted or weaken up to those mark for diluents. Blend great also passes through channel 0.45 µm filter.

Chromatographic Condition:

The chromatographic conditions included **stationary phase** (column: Waters HSS T-3 C₁₈ (100 × 2.1 mm, 5µm), Mobile Phase (Potassium dihydrogen phosphate: methanol in the ratio (60:40v/v), pH (7± 0.02), Flow rate (0.8 ml/min), Run time (5.0 min), Column temperature (°C) Ambient, Volume of injection loop (20µl), Detection wavelength (228nm), Drugs RT (2.433min)

System suitability:

Standard result from claiming Cefoxitin was ready as for every technique and might have been injected six times under the HPLC framework. Those framework suitable parameters were assessed starting with standard Chromatograms acquired Eventually Tom's perusing figuring the % RSD for maintenance times, tailing factor, hypothetical plates Furthermore top regions starting with six reproduce injections^(8,9).

1. That % RSD to those maintenance times about vital crest starting with 6 replication injections about each standard result ought to make NMT 2.0%⁽⁷⁾.
2. The number obtained from claiming hypothetical plate (noted as N) to the Cefoxitin obtained peak if is NLT AUC 2000.
3. The tailing variable (T) for the Cefoxitin peak ought to make NMT 2.0. Framework reasonableness tests are an essential piece of gas and fluid chromatographic strategies.
4. They are basically utilized for identification and check for the various goals and analysis of reproducibility or analysis for the justification of the chromatographic condition into the framework to be found sufficient in manner of determination

for the estimation for the finished dosage form and as well as bulk also.

Assay

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

Where:

AT = Test Absorbance

AS = Sample Absorbance.

WS= Weight of Standard in mg

WT = Weight of Sample in mg

DS = Dilution of standard

DT = Dilution of Test

P = Purity of working standard

Final chromatogram obtained was shown in Fig. 2

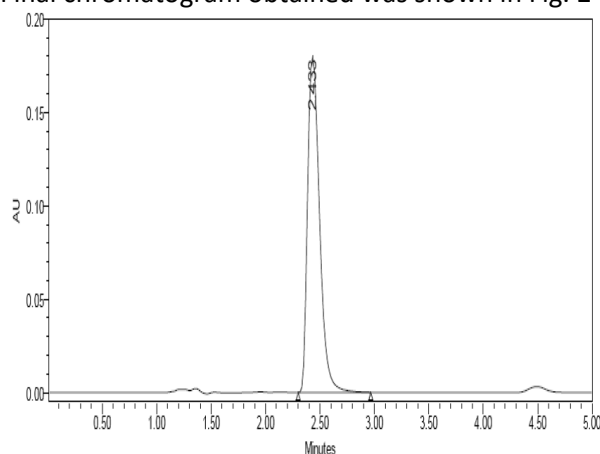


Fig.2: The typical chromatogram for Cefoxitin
Precision

The percentage R.S.D from claiming Cefoxitin test throughout those system precision might have been found should a chance to be 0.15% demonstrating great precision of the technique^(10, 11). Those outcomes was found justified and suitable to said that the developed method gives precisely results and same was concluded into the below table and chromatogram of **Figure 3 to 7**.

Table 1: Results of precision

Injection	Area
1 st Injection	693078
2 nd Injection	693338
3 rd Injection	695080
4 th Injection	694843
5 th Injection	695336
Average	694335
Standard Deviation	1047.5
%RSD	0.15%

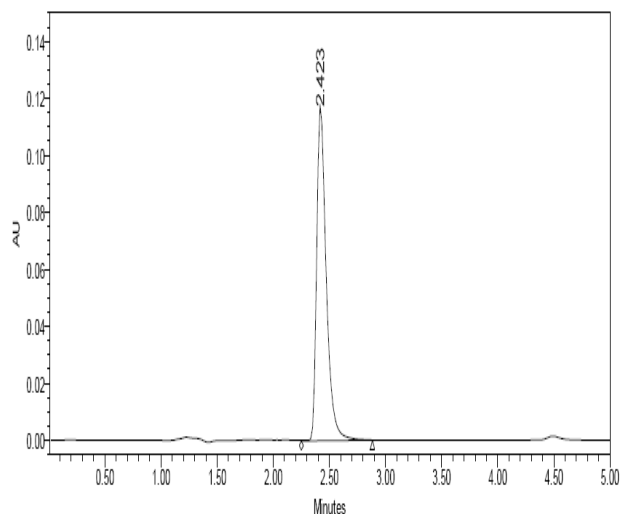


Figure 3: Precision chromatogram for 1st Injection

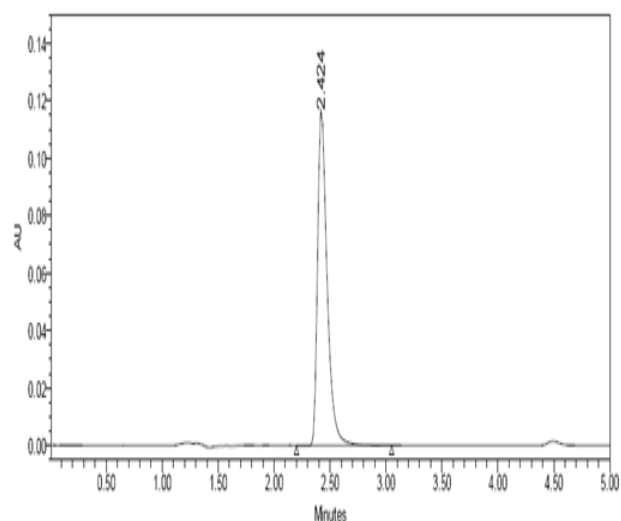


Figure 4: Precision chromatogram for 2nd Injection

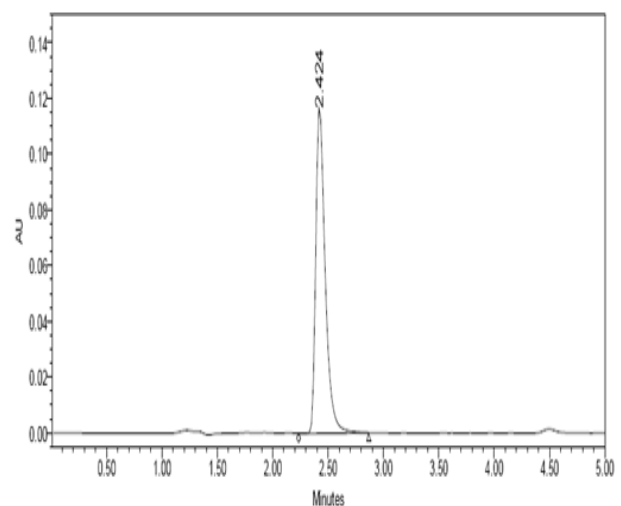


Figure 5: Precision chromatogram for 3rd Injection

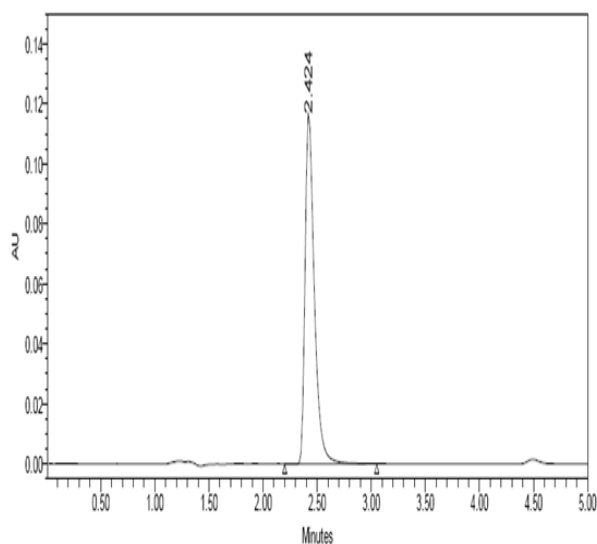


Figure 16: Precision chromatogram for 4th Injection

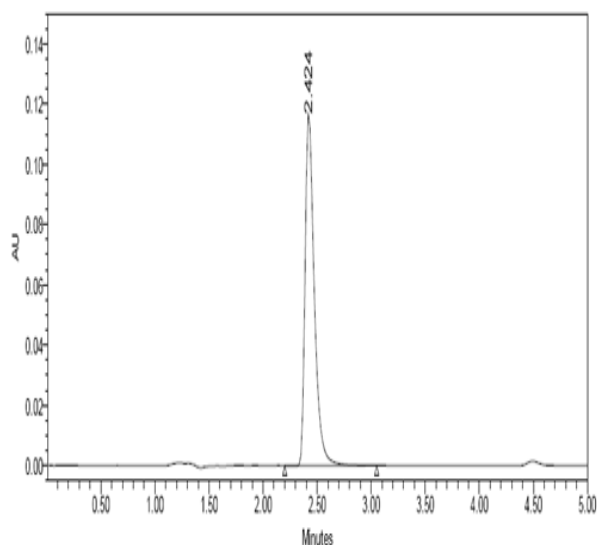


Figure 7: Precision chromatogram for 5th Injection

Intermediate Precision:

Intermediate precision would be calculated on the basis of consecutively five injections and as per their area of peak and their relative standard deviation % RSD is measured and calculated. D. for Cefoxitin test throughout that middle of the road precision might have been discovered to a chance to be 1.1%, the worth is great inside the for the most part satisfactory breaking points ^(12, 14). The effects would summarize for table 6 and the chromatograms were delineated for figures from **Figure 8 to 12.**

Table 2: Tabular interpretation of Results for intermediate precision

Injection	Area
1 st Injection	696977
2 nd Injection	696531
3 rd Injection	696214
4 th Injection	695535
5 th Injection	696708
Average	696393
Standard deviation	8624.7
%RSD	1.1%

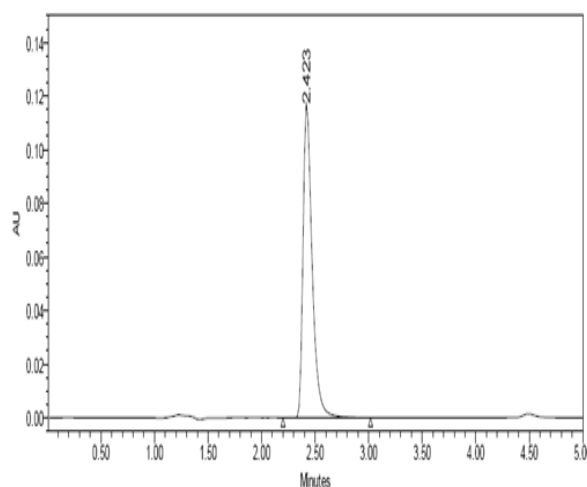


Figure 8: Intermediate Precision of 1st injection Chromatogram

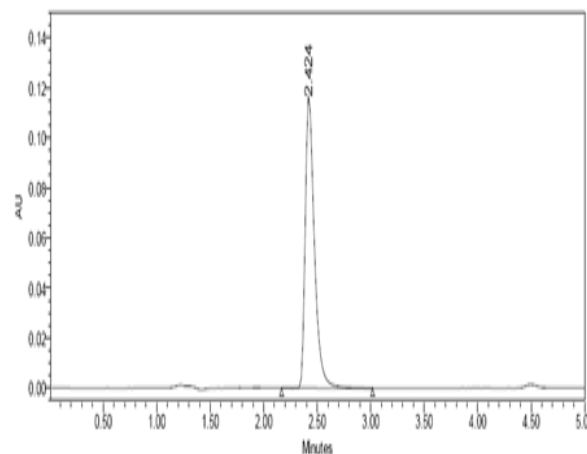


Figure 9: Intermediate Precision of 2nd injection Chromatogram

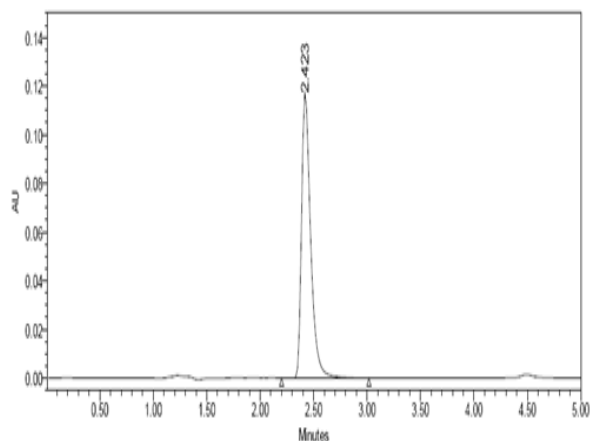


Figure 10: Intermediate Precision of 3rd injection Chromatogram

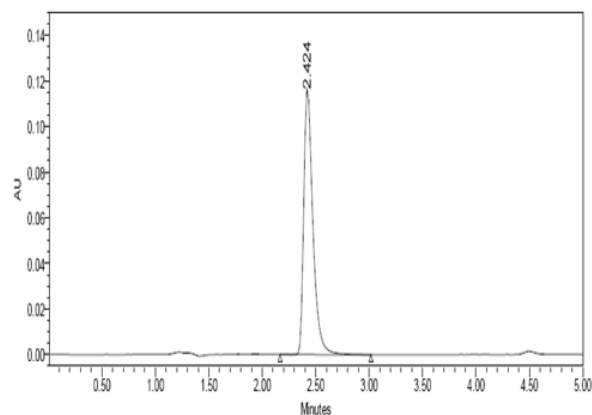


Figure 11: Intermediate Precision of 4th injection Chromatogram

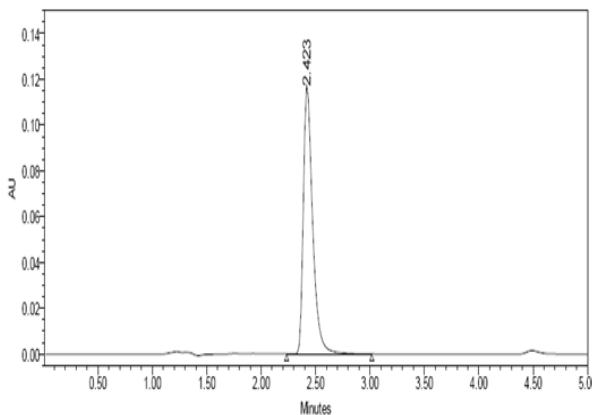


Figure 12: Intermediate Precision of 5th injection Chromatogram

Accuracy

Accuracy depends upon the result developed while performance of analysis that result obtained as per analysis as the injection given and every chromatogram developed is accurately similar in manner of AUC and trailing factor ^(12, 13). Extent

restoration about Cefoxitin test extent starting with 98.2% with 100.9% and recuperation will be 100.7%, demonstrating the great exactness of the strategy. The chromatogram in this condition will be generated as per injection given on various conc. for 50%, 100% and 150% and their three injections were place in method and developed AUC can be calculated and mean of the same was inserted in table and as per their mean results ^(13, 16). Those come about will be demonstrated in table 3. The chromatograms were demonstrated for figures from figure 13 with 20.

Table 3: Tabular demonstration for Accuracy

Conc. %age	Mean of 3 Peak Area (n=3)	Qty. Add. (in mg)	Qty. Estab. (in mg)	An Avg. of %age Recovery	% age Mean Recovery
50%	688287	5.68	5.78	100.9%	100.7%
100%	1378200	10.0	9.97	99.7%	
150%	2065480	14.05	13.93	98.2%	

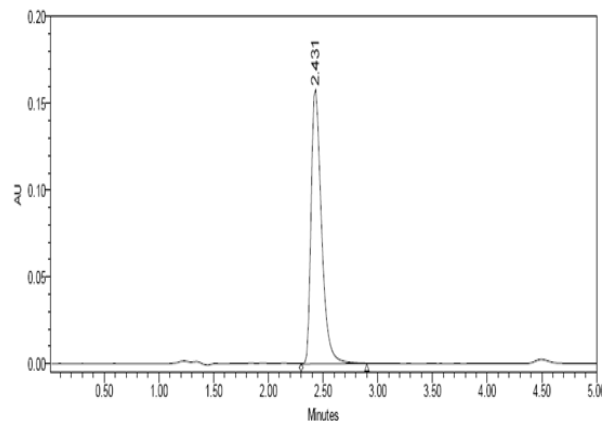


Figure 13: 1st injection chromatogram for Accuracy 50%

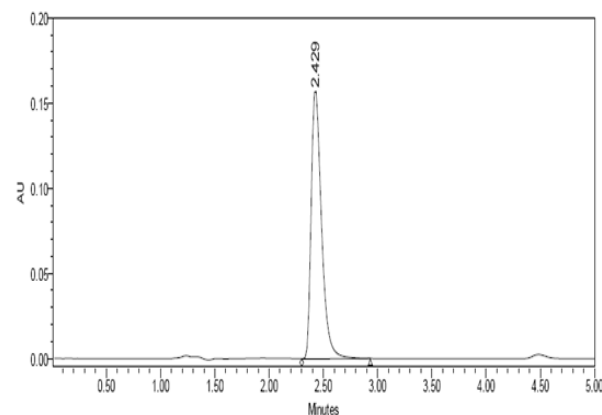


Figure 14: 2nd injection chromatogram for Accuracy 50%

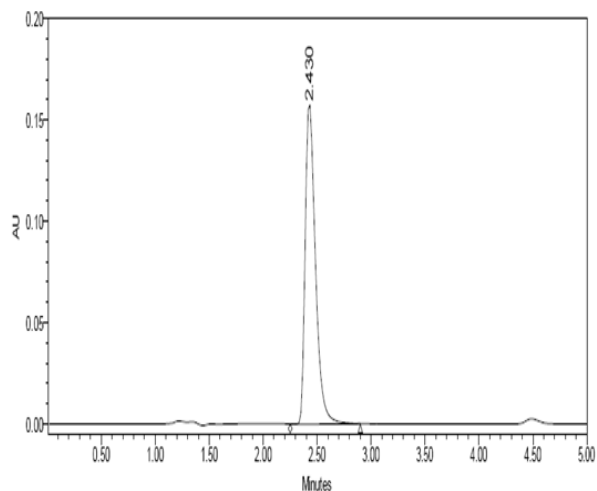


Figure 15: 3rd injection chromatogram for Accuracy 50%

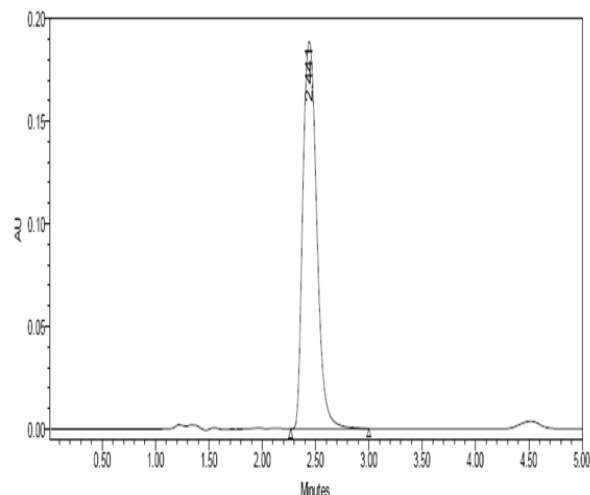


Figure 18: 3rd injection chromatogram for Accuracy 100%

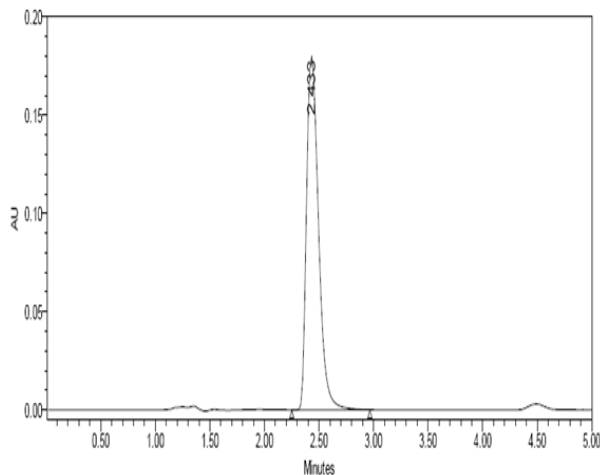


Figure 16: 1st injection chromatogram for Accuracy 100%

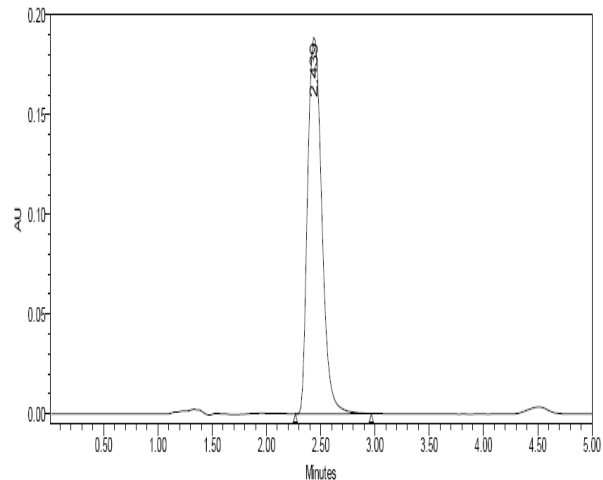


Figure 19: 1st injection chromatogram for Accuracy 150%

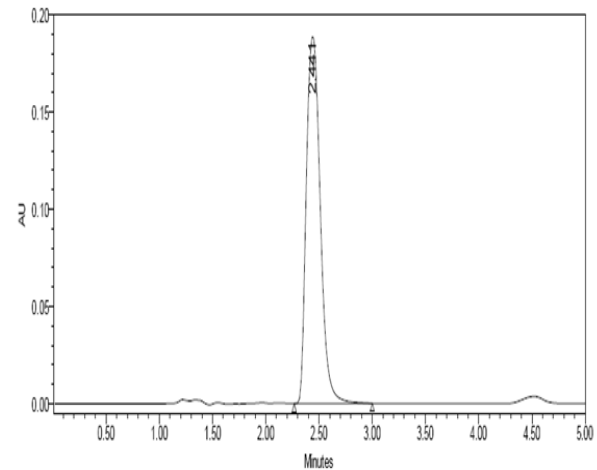


Figure 17: 2nd injection chromatogram for Accuracy 100%

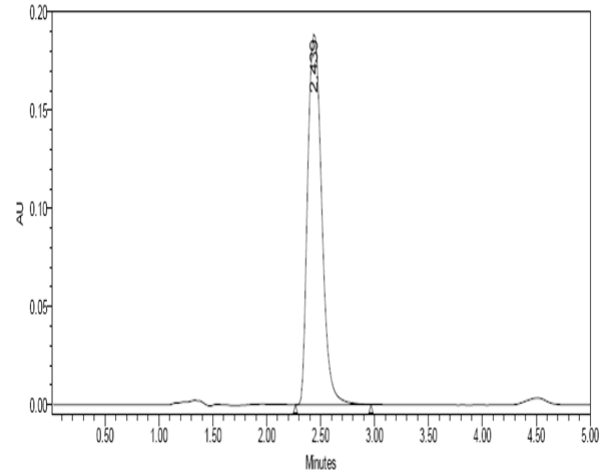


Figure 20: 2nd injection chromatogram for Accuracy 150%

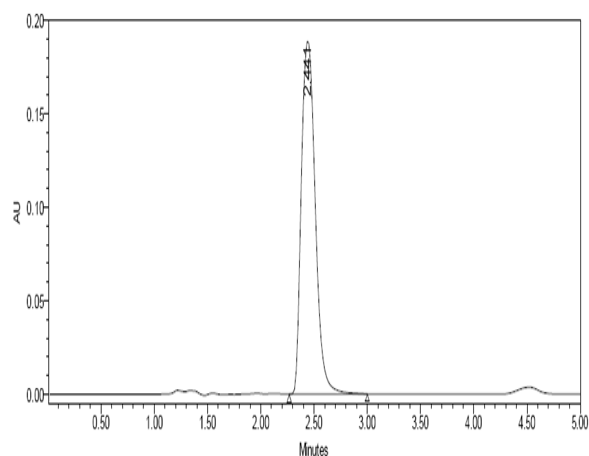


Figure 21: 3rd injection chromatogram for Accuracy 150%

Linearity

The linearity consequences would be calculated as per the mathematical expression of straight line equation in which regression coefficient and trend line will be incorporated as per the computer excel program of ms office and same will be detected by putting a possible line for the most touching points interpreted by the absorbance ⁽¹⁷⁾. The gotten results might have been 1.000, along these lines demonstrating phenomenal connection between crest territories What's more focuses of the analyte. Those comes about were summarized previously, table 8 and the linearity bend for Cefoxitin might have been illustrated over figure 32. Those corresponding chromatograms were illustrated done figures from figure 33 should 37.

Table 4: Linearity tabulation for results

S. No	Concentration	Area
1	10µg/ml	264840
2	20µg/ml	491451
3	30µg/ml	690307
4	40µg/ml	873311
5	50 µg/ml	1065958
Correlation Coefficient	0.9984	

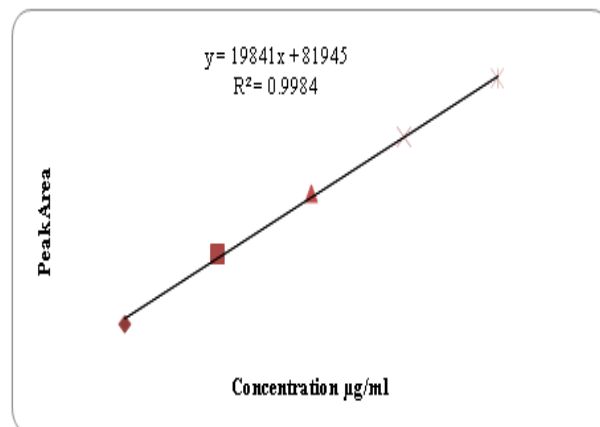


Figure 22: Demonstration of Linearity curve for Cefoxitin

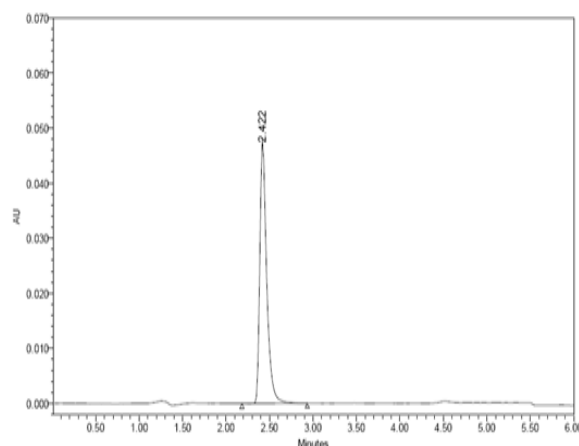


Figure 23: 1st injection chromatogram for linearity

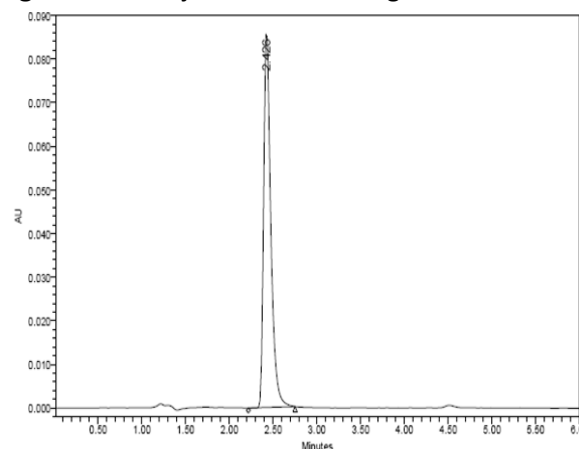


Figure 24: 2nd injection chromatogram for linearity

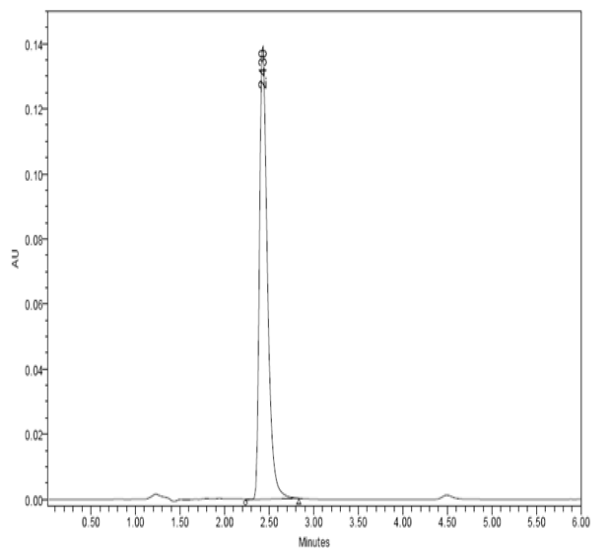


Figure 25: 3rd injection chromatogram for linearity

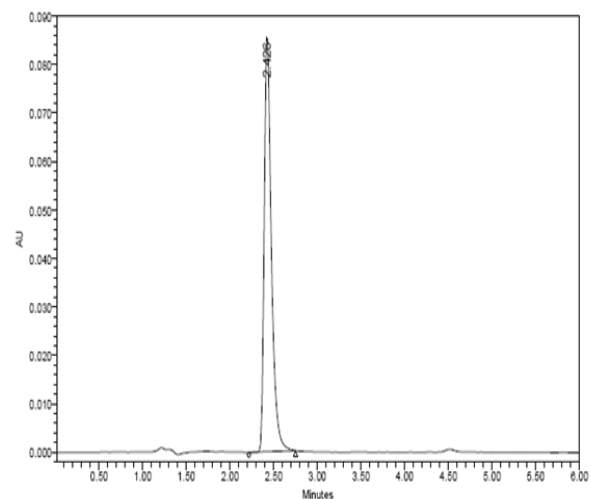


Figure 26: 4th injection chromatogram for linearity

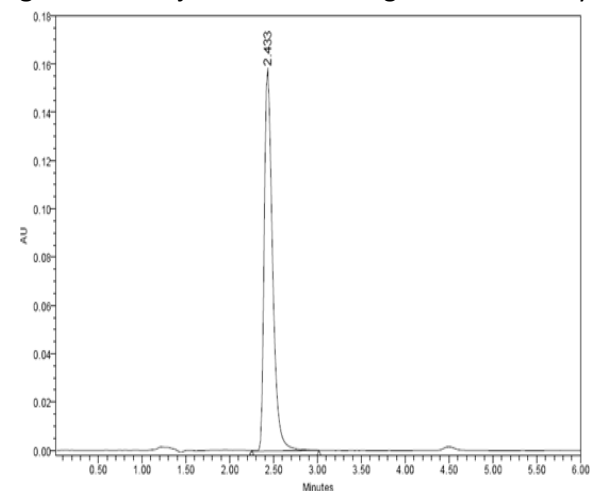


Figure 27: 5th injection chromatogram for linearity
Robustness

It demonstrates that the technique is hearty
Indeed going toward change in the stream rate

$\pm 10\%$ and progress in the portable period $\pm 10\%$ ^(15, 12 & 16). Those comes about are summarized done table 9.

Table 5: Results of Robustness

Chromatographic changes	USP Count	Plate USP Tailing
Flow rate(ml/min)		
0.6	4187.6	1.5
0.8*	4184.7	1.3
1.0	4084.7	1.4
Change in organic composition in the mobile phase		
10% less	4194.5	1.5
60:40(Buffer: methanol)*	3156.0	1.3
10% more	3097.0	1.4
UV wavelength(nm)		
226	4205.0	1.5
228*	3648.3	1.3
230	3354.2	1.4

* optimized parameters

Determination of LOD & LOQ

Limit of detectification and limit of quantification is basically define the specificity of method in manner of determination of result obtained during the analysis by any method in which the reported result will be identical at various stages and various concentration at the minute range also ^(12, 11). The instrument and condition is sensitive to provide a justify value at each & even every injection. LOD and LOQ of Cefoxitin were 0.024 and 0.07 $\mu\text{g}/\text{ml}$ separately. Since those LOQ Furthermore LOD values about Cefoxitin are attained at a low level, this system might make suitability to cleaning acceptance in the pharmaceutical business ^(8, 12). The relating chromatograms were provided in figure 38 and 39 for LOD what's more LOQ separately.

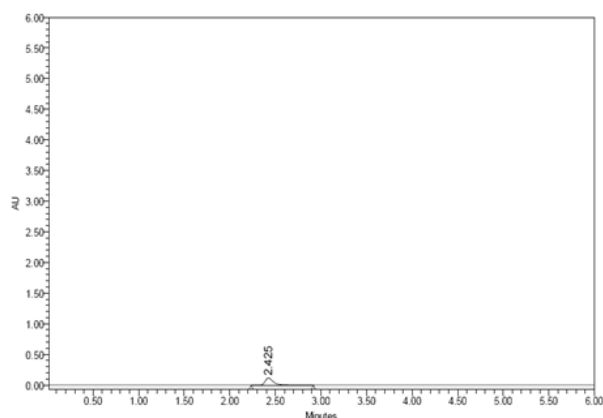


Figure 28: Chromatogram for LOD

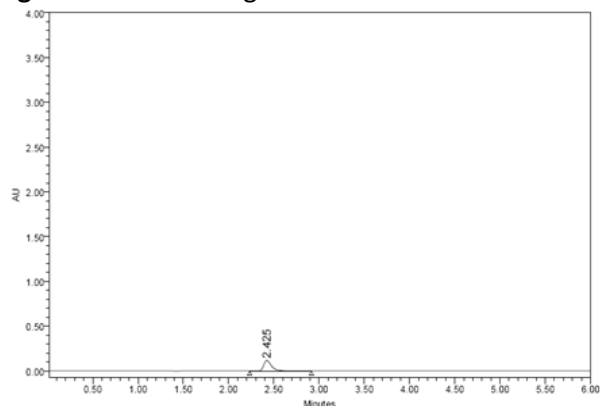


Figure 29: Chromatogram for LOQ

Application of the developed method to commercial Cefoxitin tablets

The point when the produced technique might have been used to dissect a business mark about Cefoxitin tablet formulation, the imply recuperation about triplicates might have been 99.69%. The %age recuperation worth demonstrates non-interference from the excipient introduce in the measurement type. Those chromatograms were delineated over fig. starting with figure 30-32.

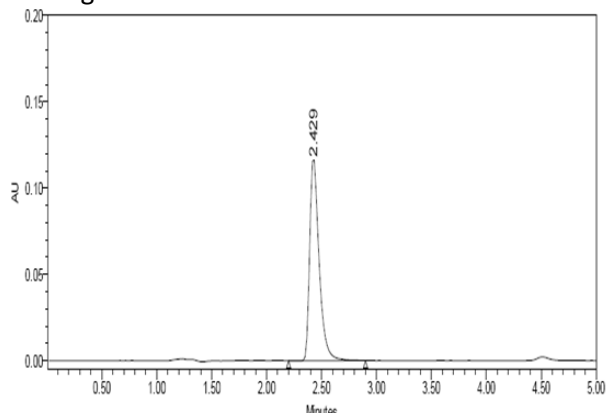


Figure 30: 1st injection chromatogram for Sample

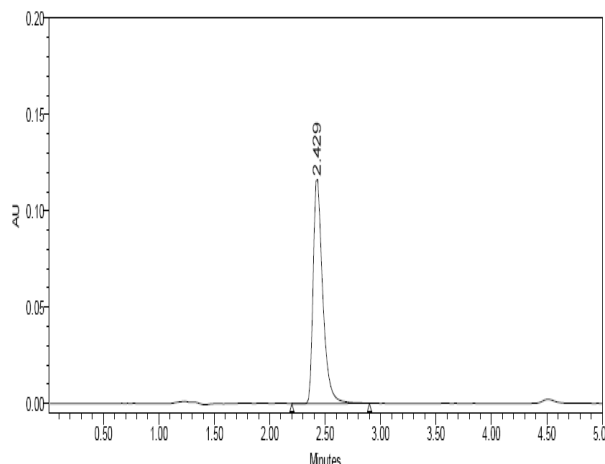


Figure 41: 2nd injection chromatogram for Sample

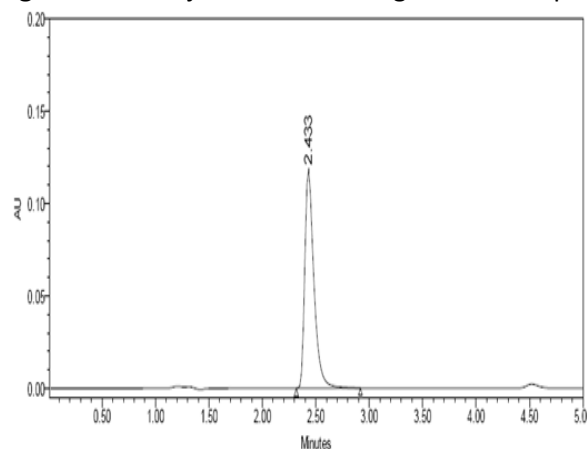


Figure 42: 3rd injection chromatogram for Sample

Weight of 5 tablets: 0.8285 grams

Average Weight: 0.1657 grams

$$\frac{694667}{699483} * \frac{10}{10} * \frac{5}{50} * \frac{3}{10} * \frac{10}{331.4} * \frac{50}{5} * \frac{3}{10} * \frac{99.8}{100} * \frac{165.7}{5} * 100 = 101.1\%$$

CONCLUSION:

In selection of method for HPLC it would be considered as the mobile phase must have a high pH, its constancy at the higher pH is in use into thought toward protecting the extended existence of the article. Most commercially available non polar columns are not steady at elevated pH for long term use, thus limitation their existence period. Waters high strength silica T₃ C18 bearing length 100 mm, 2.1 mm diameter, particle size of 1.8 columns was originate to be further fit and lasting at this pH. The chromatogram was pointed with satisfactory. Flow rate is reduced from 2 ml

per minute to 0.8 ml per minute. When this operating procedure was useful to the method, an excellent chromatogram with sharp trailing factor was obtained and Cefoxitin estimation is every time found similar at a run time of approximately 2.43 min charitable an elution time of 5min.

The present technique was appropriate for Cefoxitin starting 10 - 50µg /ml and the linear found at 0.999. Exactness came out by later- and within- day assays had relative Standard deviation (R.S.D) values within 1.5%. Revival information was in the variety 99.3-103.2% with relative standard deviation 1.5. The original, isocratic reverse phase high performance liquid chromatography technique proved to be easy, linear, exact, exact, strong, hardy plus fast. The urbanized technique was proficient of charitable sooner elution, maintaining high quality division efficient and proved that the method developed is efficient to active results for assay is accurate. The small run time i.e. 2.433min permits the investigation lager samples in less time and it's most suitable in quality control labs and for pill dosage form.

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