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Research Article

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Determination of Antimicrobial Activity of Cefixime by Chemical and Biological Methods

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Abstract The aim of this research is that determination of antimicrobial strength of antibiotics against bacteria because most of bacteria have ability multiple drug resistance. There are multi drug resistance bacteria and opportunistic pathogen upon human being. It is resistant mainly narrow spectrum drugs and sensitive to broad spectrum antibiotic. Pathogens are treated with antibiotics from years; bacterial pathogens are either resistant or sensitive to antibiotics. Regular administration of antibiotic to pathogen produces drug resistance strains. Most of the bacterial pathogen already acquires multi-drug resistance characteristics. the antimicrobial study of the antibiotics shows the concentration at which these pathogens can be inhibited. The present study determines the antimicrobial activity of cefixime tablet against different type of bacterial strains E. coli, S. aureus, S. faecalis, and Proteus mirabilis and salmonella typhi. The antibiotics show 99.999% antimicrobial efficacy against microbes both gram positive and gram-negative strains.

Keywords Drug Resistant, Cefixime, Antimicrobial Strength, Microbes

Introduction

Cefixime is used to treat bacterial infections in many different parts of the body. It belongs to the class of medicines known as cephalosporin antibiotics. It works by killing bacteria or preventing their growth. However, this medicine will not work for colds, flu, or other virus infections.

About Antibiotics

An antibiotic was originally defined as a substance produced by one microorganism, which inhibited the growth of other microorganisms.

Types of antibiotics

Broad-spectrum antibiotic

The term broad-spectrum antibiotic refers to an antibiotic that acts against a wide range of disease-causing bacteria. A broad-spectrum antibiotic acts against both Gram-positive and Gram-negative bacteria, in contrast to a narrow-spectrum antibiotic, which is effective against specific families of bacteria. An example of a commonly used broad-spectrum antibiotic is ampicillin.

Narrow Spectrum Antibiotics

Antibiotics may be defined as the sub-group of anti-infective that are derived from bacterial sources and are used to treat bacterial infections.

An antibiotic may be classified basically as "narrow-spectrum" or "broad-spectrum" depending on the range of bacterial types that it affects. Narrow-spectrum antibiotics are active against a selected group of bacterial types.

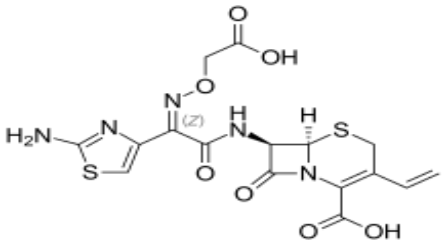
About Cefixime

Cefixime, an antibiotic, is a third-generation cephalosporin like ceftriaxone and cefotaxime. Cefixime is highly stable in the presence of beta-lactamase enzymes.

Mechanism of action

Like all beta-lactam antibiotics, Cefixime binds to specific penicillin-binding proteins (PBPs) located inside the bacterial cell wall, causing the inhibition of the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that Cefixime interferes with an autolysin inhibitor.

Drug Profile



Chemical and physical data:

a) Drug Class: Third Generation Cephalosporins
b) Formula: C16H15N5O7S2
c) IUPAC name: (6R,7R)-7-{[2-(2-Amino-1,3-thiazol-4-yl)-2-(carboxymethoxyimino) acetyl]amino}-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid
d) Molar mass : 453.44 g·mol−
e) Protein binding65% (concentration independent)
a. Protein binding: Approximately 60%
b. AHFS/Drugs.com:Monograph
c. ATC code: J01DD08 (WHO)
d. Bioavailability: 30 to 50%
e. Elimination half-life: Variable; Average 3 to 4 hours
f. Excretion: Kidney and biliary

Pharmacodynamics

Cefixime, an antibiotic, is a third-generation cephalosporin like ceftriaxone and cefotaxime. Cefixime is highly stable in the presence of beta-lactamase enzymes. As a result, many organisms resistant to penicillins and some cephalosporins due to the presence of beta-lactamases, may be susceptible to cefixime. The antibacterial effect of cefixime results from inhibition of mucopeptide synthesis in the bacterial cell wall.

Absorption

About 40%-50% absorbed orally whether administered with or without food, however, time to maximal absorption is increased approximately 0.8 hours when administered with food.

Metabolism

Hepatic. Approximately 50% of the absorbed dose is excreted unchanged in the urine in 24 hours.

Half-life

3-4 hours (may range up to 9 hours). In severe renal impairment (5 to 20 mL/min creatinine clearance), the half-life increased to an average of 11.5 hours.

Actions and Spectrum

- Based on spectrum of activity, classified as a third generation cephalosporin. Expanded spectrum of activity against gram-negative bacteria compared with first and second generation cephalosporins; less active against Enterobacteriaceae than some other third-generation cephalosporins.
- Usually bactericidal.
- Like other β-lactam antibiotics, antibacterial activity results from inhibition of bacterial cell wall synthesis.

Oral drug delivery has been known for decades as the most widely utilized route of administration among all the routes that have been explored for the systemic delivery of drug via various pharmaceutical products of different dosage form. The reason that the oral route achieved such popularity may be attributed to its ease of administration as well as the traditional belief that by oral administration the drug is well absorbed as the food stuff ingested daily1.

The oral route of drug administration has been the one used most for both conventional as well as novel drug delivery. The reasons for this preference are obvious because of the ease of administration and widespread acceptance by patients. The common oral dosage forms include: liquid mixtures like solutions, suspensions, solid dosage forms like tablets, capsules and liquid filled capsules etc. Compared to other oral dosage forms, tablets are the manufacturer's dosage form of choice because of their relatively low cost of manufacture, package and shipment; increased stability and virtual tamper resistance.

Materials and Methods

Strategy: -

Implementation Verification & Validation:

Implementation verification aims to demonstrate the competence and perform the validated method. This is achieved by its ability to obtain the expected results on tested Drug

Procedure Implementation Verification Chemical and instrumentation analysis

Selection of Drug

- Cefixime taken for analysis.
- Product of Material test: 100mg Tablet Cefixime contain not less than 95.0% and not more than 101.0% of $C_6H_{15}N_5O_7S_2$.

Tests

Identification

In the Assay, The Principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Description:

White color, circular, biconvex, film coated tablet

Average Weight: 313.31 mg

Uniformity of Weight: -1.19 to 1.26%

Disintegration: 10 to 13 Minute

Dissolution:

Dissolution Apparatus No. 1

Medium- 900ml of 0.05 M potassium phosphate buffer pH 7.2, prepared by dissolving 6.8 g of monobasic potassium phosphate in 1000 ml of water, adjust to pH 7.2 with 1M Sodium hydroxide.

Speed- 100 rpm and 45 minutes. Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered Solution, suitably diluted with the medium, if necessary, at the maximum at 288nm Calculated the content of C6H15N5O7S2 in the medium from the absorbance obtained from a solution known concentration of cefixime.

Note- A small amount of methanol not exceed 0.1% of total volume used of dissolution of cefixime.

D-1 = 88.63
D-2 = 88.94
D-3 = 91.51
D-4 = 91.98
D-5 = 97.21
D-6 = 97.26
Average- 92.59%

Assay By HPLC

Buffer: Phosphate buffer pH 7.0

7.1 g dibasic sodium phosphate in water and dilute to 500ml with water. Adjust the pH of the solution 7.0 with monobasic potassium phosphate solution.

Monobasic potassium phosphate solution- Dissolve 6.8g of Monobasic potassium phosphate in water and dilute to 500ml with water.

Test Solution:

Weigh and powder 20 tablets. Disperse a quantity of the powder containing about 0.4g of cefixime, disperse in 100ml of phosphate buffer pH 7.0, mix with the aid of ultrasound and centrifuge. Dilute 5 ml of the clear supernatant to 100ml with phosphate buffer pH 7.0.

Reference Solution A:

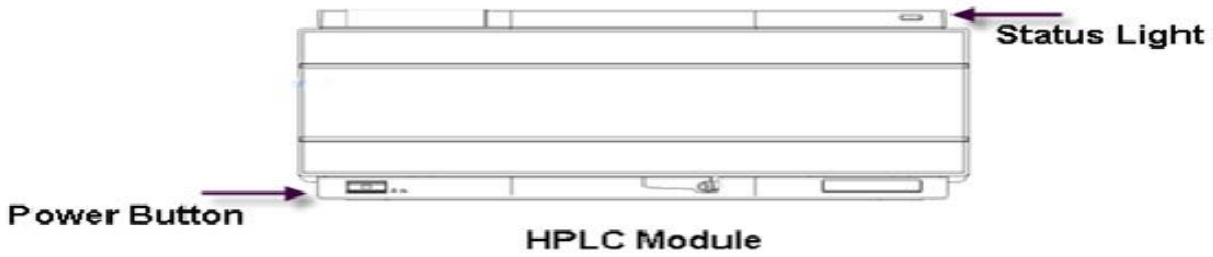
A 0.02% w/v solution of cefixime IPRS in phosphate buffer pH 7.0.

Reference Solution B: Dissolve 10mg of cefixime IPRS in 10ml water. Heat the solution at 95 C for 45 minute. Cool and inject immediately.

Operation:

Switch ON mains power supply and the components of the HPLC module by the push power buttons situated at the left bottom side (It gives green/yellow light indication by switching 'ON' the modules).

- Ensure that the instrument is in calibrated state.
- Switch ON the computer, monitor and printer which are connected to respective HPLC.
- Wait for instrument modules to start and then launch online application of Chromatography Data acquisition Software. Use Switch on button in Software to make ready all available modules. Module Status light indicates its status whether; it is "Ready", "Not ready" or in "Error" state.



Status Light Color	Module Status
No light (and Module Power switch light is ON)	Ready
Yellow	Not Ready condition
Green	Run mode/ Analysis running
Red	Error

- Prepare mobile phase as described in the analytical method and fill in the reservoir. Always use freshly prepared solvent; especially use demineralize water which was filtered through about 0.2 µm filters.
- Fill the reservoirs for applicable channels A, B, C and D with mobile phase / solvent. Insert respective inlet tubing having suction filter into applicable channel reservoirs placed in the solvent cabinet on top of the quaternary pump/Isocratic/Binary pump.
- Purging of lines: Open the purge valve of pump (by turning it anticlockwise (do not open fully otherwise black knob will come out)) and gradually increase and set the flow rate to 5 ml/min in toolbar, which appears by clicking pump symbol on the online screen. Flush all the four lines one by one ensuring there is no bubble in the same.
- Now gradually decrease and stop the flow. Now remove the front cover of Column compartment to access the heating area. Place the column on the heat exchanger assembly and connect the tubing in such direction that the flow is from pump to detector.
- Fix the column with the column clip.
- Now in software; create new method as per your application or load existing method.
- Set the flow rate and the required composition value of your application and close the purge valve (by turning it clockwise, (do not over tight Purge Valve)).
- Equilibrate the system for at least 30 minutes with mobile phase. Check baseline in software. If found stable, you can run the sample. Equilibration time depends on System, Column and Mobile Phase.
- Ensure that during the analysis the front cover of the column compartment is closed.
- Prepare required standard and test solutions as described in the analytical method and fill the sample vials and keep in the sample tray.
- Run the samples from Software.
- After the completion of Run/Sequence, integrate chromatogram and take print outs in the appropriate report formats.
- After completing analysis, column washing is required with appropriate solvent for approx. 45 minutes.

Method Validation-

procedure was evaluated with method validation parameters such as precision, linearity, specificity, accuracy, ruggedness and robustness. % RSD for replicate standard solutions and replicate test solutions were calculated, linearity correlation coefficient was evaluated, recovery %RSD was evaluated.

System suitability

System suitability was evaluated with freshly prepared standard solutions. Five replicate standard solution injections were performed and calculated the %RSD for retention time and peak area. Other parameters theoretical plates and tailing factor were measured. Peak purity of three components was checked. System suitability results were Blank, placebo and standard solution chromatograms were represented. %RSD values were within the limit 2.0%.

Precision

Precision also called as repeatability. Precision parameter was performed with six replicate test solutions preparations. Six replicate solutions were injected in to the HPLC system. Peak area, %RSD results were calculated. Test solution of cefixime were represented. Precision results were satisfactory and %RSD values were below 2.0%.

Specificity

Specificity parameter is used to evaluate the interference from blank, placebo, known and stress study un-known impurities. Stress studies acid, base, peroxide, thermal and UV light conditions were evaluated and represented the all-stress studies chromatograms for cefixime test samples. Results were satisfactory and all unknown impurities were separated and have no interference with products.

Linearity

Linearity parameter was evaluated with standard solution by preparing five different concentrations. Linearity levels are 50%, 75%, 100%, 125% and 150% concentrations. All five linearity solutions were injected into the HPLC system and calculated the correlation coefficient values. Correlation coefficient was calculated for concentration versus peak area. Results were tabulated and linearity solutions overlay chromatogram was represented and linearity graphs were represented. Results were satisfactory, correlation coefficient values were above 0.999.

Accuracy

Accuracy was evaluated to establish the recovery of the components. Different concentration of active components was added to the placebo (constant concentration for all accuracy levels). Accuracy levels 50%, 75%, 100%, 125% and 150% were evaluated. 50% and 150% were performed with six replicate preparations and remaining concentration levels were three replications. Accuracy recovery and %RSD were calculated and tabulated. % recovery results were between 97% to 103% and %RSD values were below 2.0%.

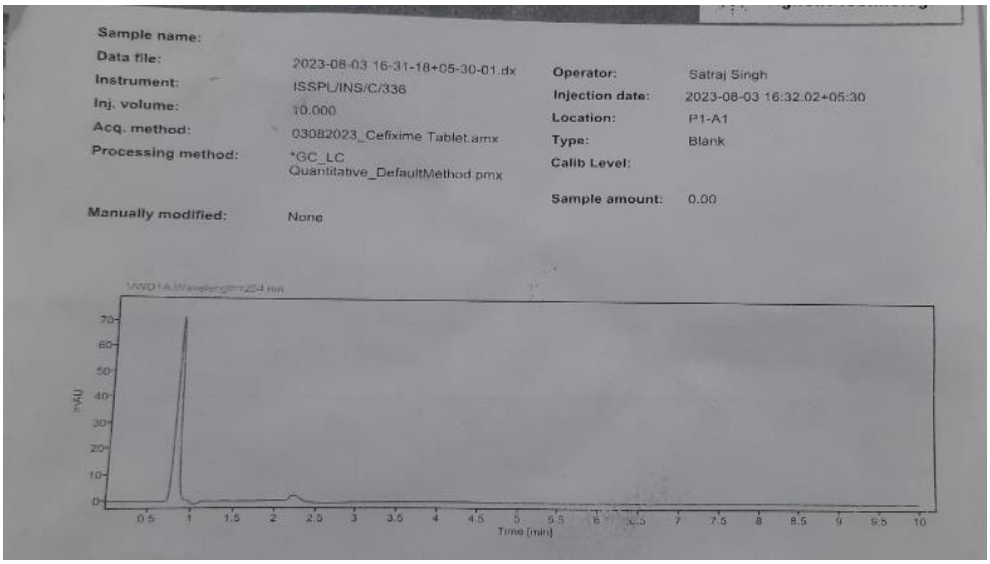
Ruggedness

Sample solutions were used to perform ruggedness of the HPLC method. Precision test samples 1 and 2 were used to perform solution stability at room temperature and refrigerator storage conditions. Post analysis of precision 1 and 2 samples was kept at room temperature and refrigerator conditions. Analysis was performed at day-1 and day 3. Samples assay values were calculated and % assay difference found below 2.0%.

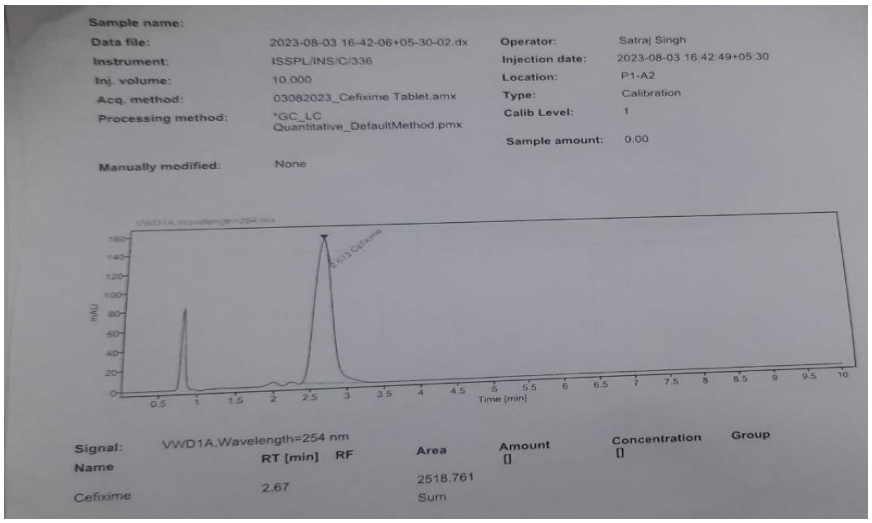
Robustness

Robustness of the method was evaluated by changing the chromatographic conditions like mobile phase flow rate, column oven temperature. System suitability was conducted to check the variation changes and results were satisfactory. Retention time, area %RSD, theoretical plates and tailing factor results.

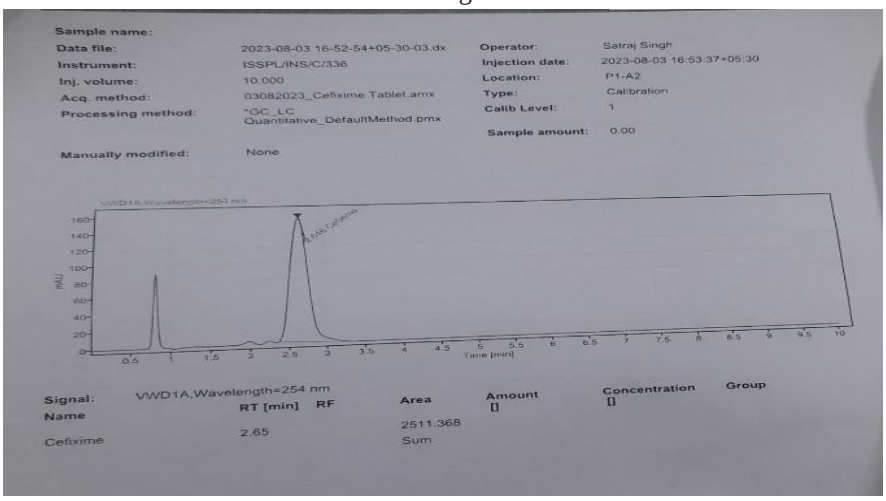
Results and Discussion



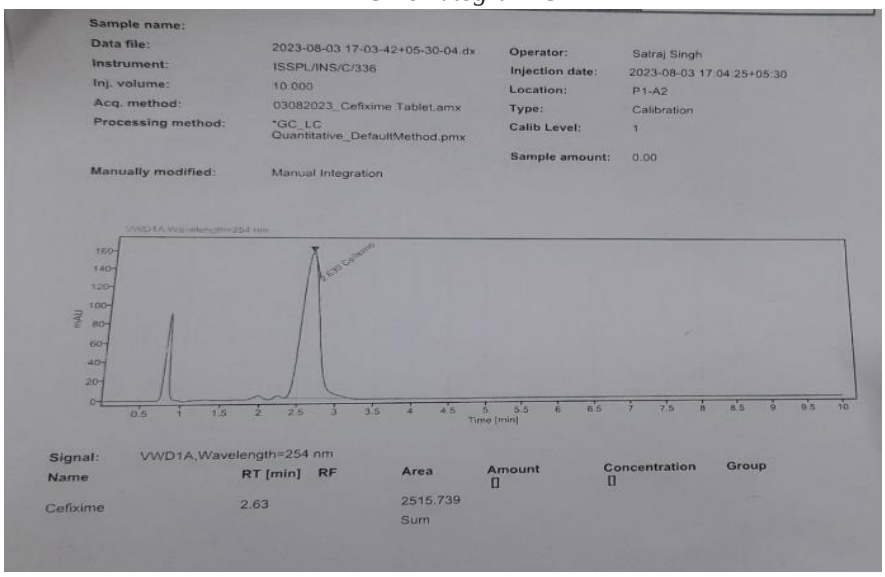
Chromatogram 1



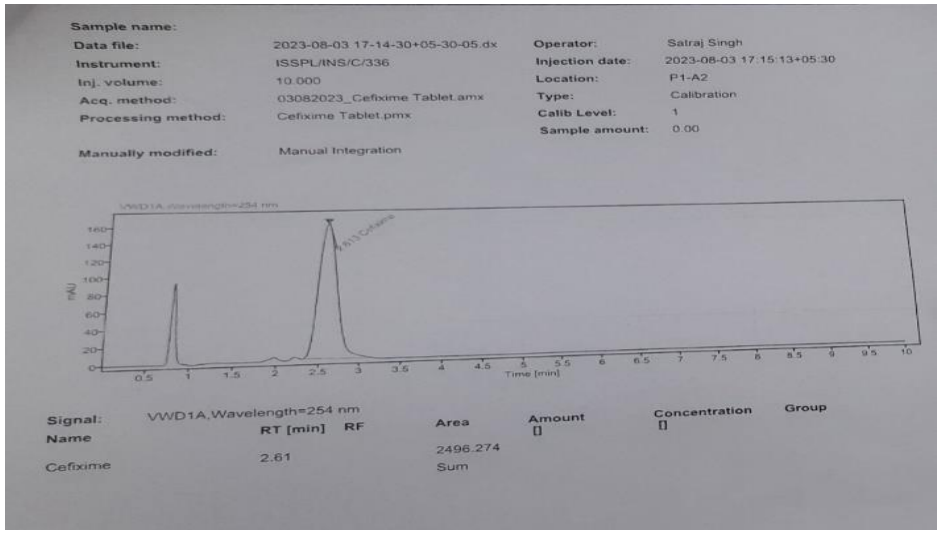
Chromatogram 2



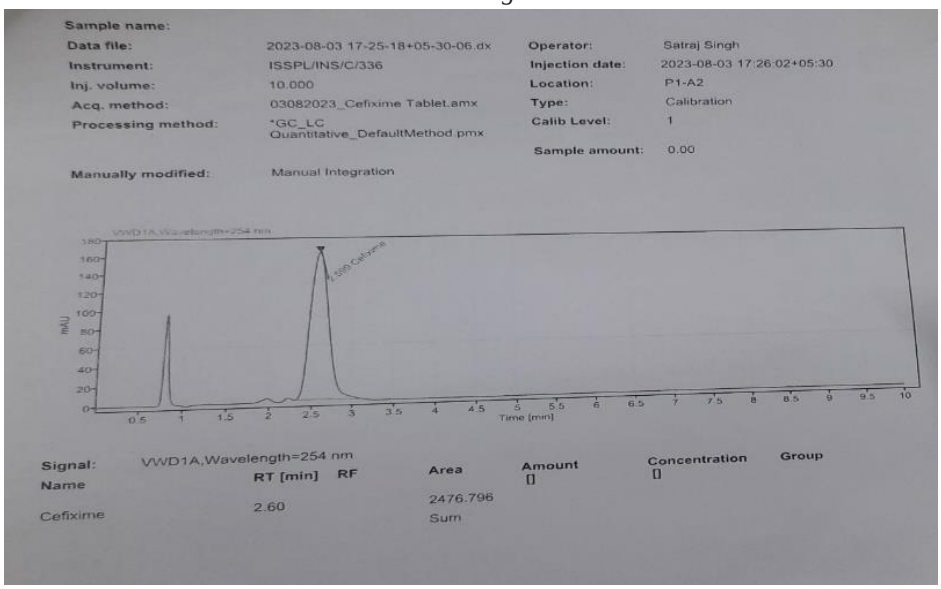
Chromatogram- 3



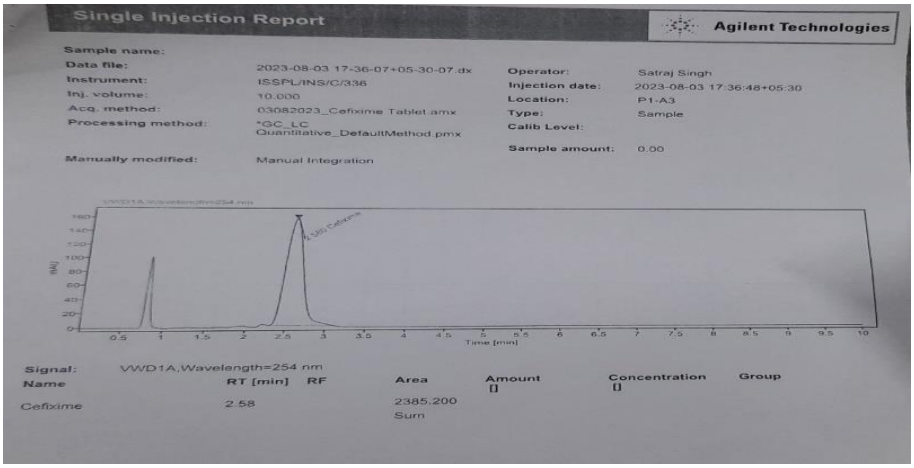
Chromatogram 4



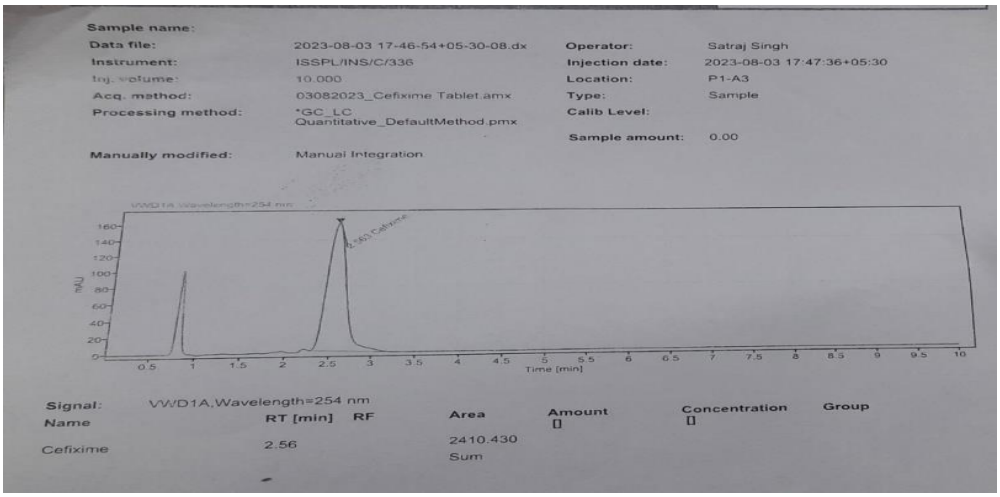
Chromatogram -5



Chromatogram 6



Chromatogram 7



Chromatogram -8

Final Calculation and Result:

2397.815 X 11.75 X 100 X 10 X 87.4 X 313.31 = 199.08
2563.786 50 302.31 2 100

199.08 X 100
200

Result: 99.54

Conclusion

Stable and rugged HPLC method was developed for the quantitative determination of Cefixime in solid dosage form. Cefixime is available in tablet dosage form

Optimized method was evaluated with precision, linearity, specificity, ruggedness and robustness validation parameters. %RSD for area (not more than 2.0%), % recovery (between 97% - 103%), % of degradation, Correlation coefficient (not less than 0.999) and variation change difference (mobile phase flow rate, column oven temperature) were evaluated and results were satisfactory

Microbiological Analysis

- **Selection of the strain**: *Salmonella, E. coli, S. aureus, S. faecalis, P. Mirabilis*
  Preparation of inoculum: Culture was inoculated in soya bean casein digest medium and incubates at 37 °C for overnight.
  Perform Analysis-As per IP/BP/USP/Validated method

- **Name of Test: Measurement of Antibacterial Activity of Cefixime Tablet**
  Test Method: IP- 2022

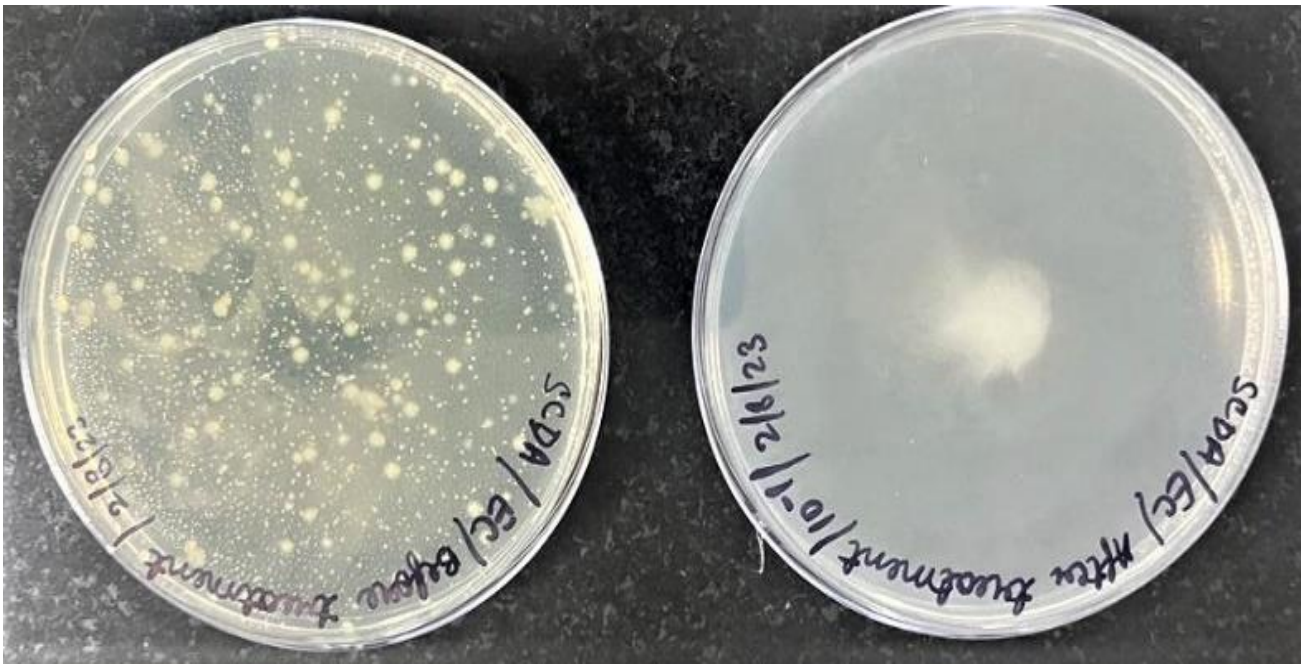
- **Dilution Medium Used**: Buffured Sodium Chloride Petpone Salt Solution/Soyabean Casein Digest Broth with Lecithin

- **Test Medium**: Soyabean Casein Digest Agar/Antibiotic Assay Agar
- **Contact Time** : 30 Minute
- **Test Organisms Used**:
  1. Escherichia coli ATCC 8739
  2. Staphylococcus aureus ATCC 6538

3. Streptococcus Faecalis ATCC 29212
4. Proteus Mirabilis ATCC 12453
5. Salmonella Typhi NCTC 786

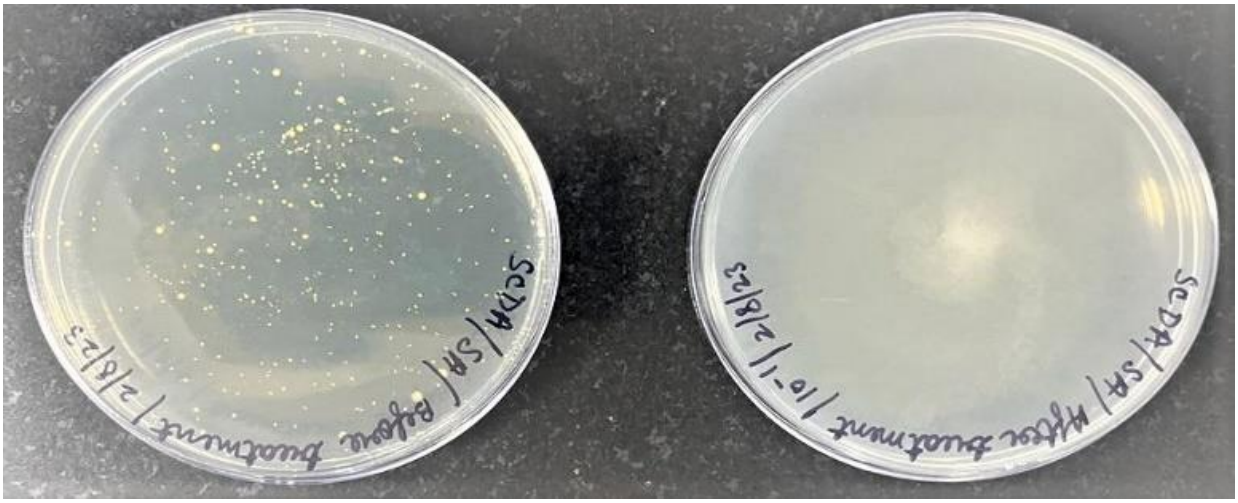
- **Observation:** after antimicrobial efficacy test and antimicrobial sensitivity test

OBSERVATION- 1. *E. coli*



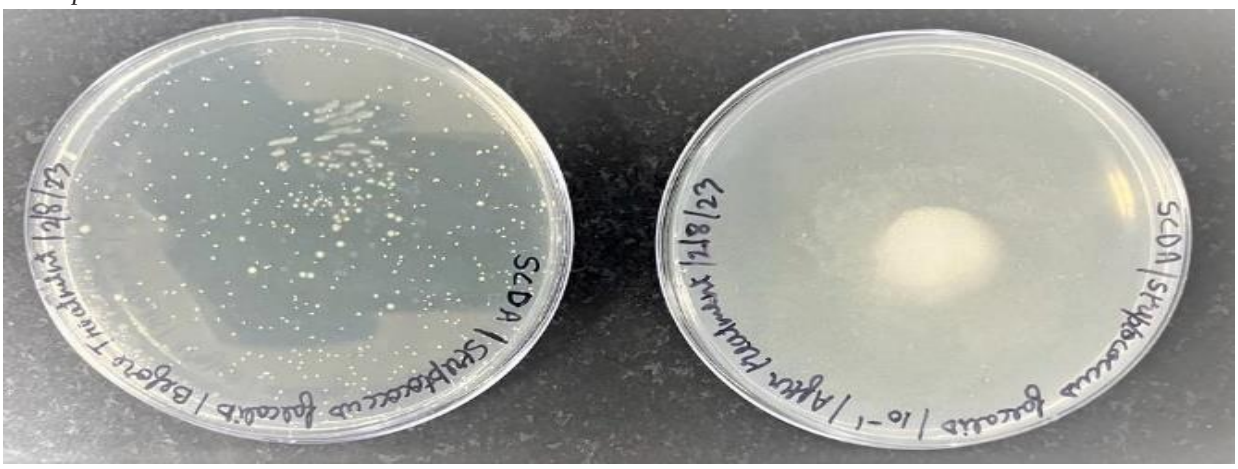
E.coli: (a) Before Treatment, (b) After Treatment E.coli

2. *Staphylococcus aureus*



S. aureus: (a) Before Treatment, (b) S. aureus: After Treatment

3. *Streptococcus Faecalis*



S. faecalis: (a) Before Treatment (b) S. faecalis: After Treatment

4. *Proteus Mirabilis*

Proteus mirabilis: (a) Before Treatment Proteus (b) After Treatment

5. *Salmonella Typhi*

Salmonella Typhi: (a) Before Treatment Proteus (b) After Treatment

Sr. No.	Name of Test Organisms	Size of Sample (ml)	Observation & result			
			Number of bacteria inoculated at 0 min (cfu) (B)	Number of bacteria observed after 30 min. contact duration (cfu) (A)	Bacterial Reduction	
					Log	%
01.	*E. coli*	01	920000	Nil	5	99.999
02.	*S. aureus*	01	950000	Nil	5	99.999
03.	*S. faecalis*	01	850000	Nil	5	99.999
04.	*P .mirabilis*	01	700000	Nil	5	99.999
05	*S. typhi*	01	900000	Nil	5	99.999

Interpretation: Sample showing the antimicrobial activity against *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Streptococcus faecalis and Proteus mirabilis* when tested as per the Validated Test Method
Conclusion : The above tested product having antibacterial efficacy against gram-positive & gram-negative bacteria
Remark:1.CFU- Colony Forming Unit

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