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Research Article

METHOD DEVELOPMENT AND VALIDATION OF CEFIXIME BY USING RP-HPLC IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

Analytical method development and validation are ongoing and interconnected activities that are essential in research and development, quality control, and quality assurance departments. They involve the creation and testing of analytical procedures to assess equivalence and manage risks effectively. These procedures are crucial for establishing specific acceptance criteria for products and ensuring the reliability and consistency of results. Validations are essential in assessing whether an analytical procedure is appropriate and reliable for its intended purpose. Literature survey reveals that the analytical methods based on UV spectrometry, RP-HPLC and HPTLC for the determination of Cefixime individually and combination with others drugs . The methods were validated according to ICH guidelines in terms of accuracy, precision, lod, and other aspects of analytical validation. The developed analytical methods for cefixime have been designed to be straightforward, making them easy to perform. They have also demonstrated high sensitivity, enabling the detection and quantification of cefixime at low concentrations. This sensitivity is crucial for accurately determining the amount of cefixime present in bulk samples or tablet formulations.

INTRODUCTION

Cefixime is an antibiotic belonging to the third-generation cephalosporin class, similar to ceftriaxone and cefotaxime. It possesses high stability even in the presence of beta-lactamase enzymes. This stability allows cefixime to be effective against organisms that are resistant to penicillins and some other cephalosporins due to the presence of beta-lactamases. The antibacterial effect of cefixime results from inhibition of mucopeptide synthesis in the bacterial cell wall used to treat susceptible Gram negative and Gram-positive bacterial infections. Cefixime is an antibiotic medication that is commonly used to treat various bacterial infections. It is effective against a range of infections such as otitis media (middle ear infection), strep throat, pneumonia, urinary tract infections, gonorrhea, and Lyme disease.

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For gonorrhea typically only one dose is required. In the United States it is a second-line treatment to ceftriaxone for gonorrhea. It is taken by mouth. Synonyms: Cefixim, Cefixima, Céfixime, Cefixime, Cefiximum.

IUPAC Name:

(6R,7R)-7-[[(2Z)-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[12]

Molecular Formula: C16H15N5O7S2

Molar Mass: 453.44 g·mol−1

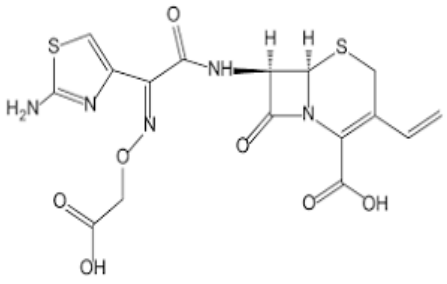

Figure1: Structure of Cefixime

Mechanism of Action

The mode of action of cefixime is bactericidal, meaning it kills bacteria. It achieves this by inhibiting the synthesis of the bacterial cell wall. Specifically, cefixime binds to one of the penicillin binding proteins (PBPs) present in bacteria. These PBPs are responsible for the final step of transpeptidation in the synthesis of peptidoglycan, a vital component of the bacterial cell wall. By binding to the PBPs, cefixime prevents this transpeptidation step from occurring, leading to the inhibition of peptidoglycan synthesis and the arrest of cell wall assembly. As a result, the bacteria are unable to build a proper cell wall, which ultimately leads to their death.. 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 refers to the process by which bacterial cells break down and rupture. This phenomenon is facilitated by autolytic enzymes, such as autolysins, which are responsible for degrading the bacterial cell wall. Autolysins are normally regulated by specific inhibitors to prevent uncontrolled cell lysis.

MATERIALS AND METHODS

Table 1: Instrumentation

S.No	INSTRUMENT	MODEL
1	HPLC	WATERS, PEAK HPLC, Isocratic method, ADM
2	UV/VIS spectrophotometer	Autochrome 3000
3	Detector	UV (Water-model 487)
4	Column	C18(ZODIAC COMPANY)
5	Weighing machine	Afcoset ER-1000A
6	Pipettes and Burettes	Borosil
7	Beakers	Borosil

Table 2: Chemicals and Solvents

S.No	Chemical	Company Name
1	Cefixime	Ipca Laboratories ltd
2	Water for HPLC	Thermo Fisher Scientific Indi LTD
3	Methanol for HPLC	Thermo Fisher Scientific Indi LTD

Method Development

Diluent: Methanol and Acetonitrile are taken from HPLC gradient grade of assay 99.8% in ratio 30:70 respectively as mobile phase

Preparation of Standard stock Solutions: Accurately weigh and transfer 0.05mg standard drug of Cefixime into volumetric flask and add 5ml of methanol and dissolve by sonication process for 3 minutes and label it as standard stock solution of 5000µg/ml. From the 5000µg/ml prepare 1000µg/ml concentration by taking the volumes as 1ml of stock solution and 10ml of methanol into a test tube and label it as 1000µg/ml.

Preparation of Standard working solutions from (1000% solution): From 1000µg/ml prepare 100µg/ml concentration by taking the volumes as 1 ml of stock solution and 9ml of methanol into an empty test tube and label it as 100µg/ml. From the stock solution of concentration 1000µg/ml pipette out the required volumes of concentration as 100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml, 500µg/ml and 600µg/ml.

Preparation of 0.1% TFA buffer: Take 1ml of TFA in 1000 ml of HPLC water, pH was adjusted with NaOH up to 3.0. Final solution was filtered through 0.45 µ Membrane filter and sonicate it for 10 mins.

HPLC Optimized Conditions

Column: c18 (zodiac company)

Detector: UV visible

Injection volume: 20 ul

Flow rate: 1.1ml\min

Temperature: Ambient

Run time: 2.9mins

Mobile phase: Methanol:Acetonitrile (30:70)

Method Validation

The developed method was validated as per ICH guidelines for linearity, precision, accuracy, LOD as follows.

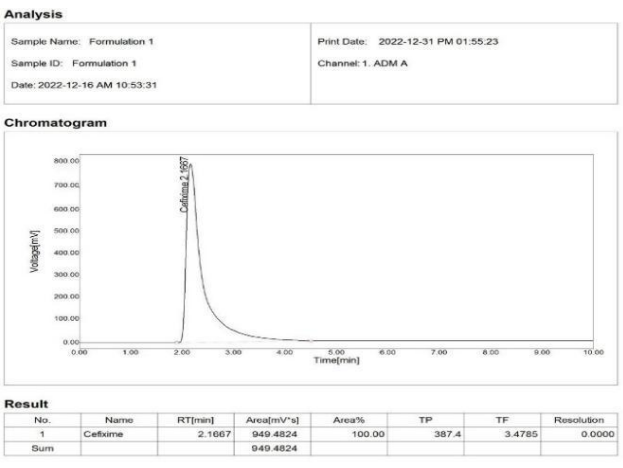

Figure 2: Chromatogram for cefixime tablet

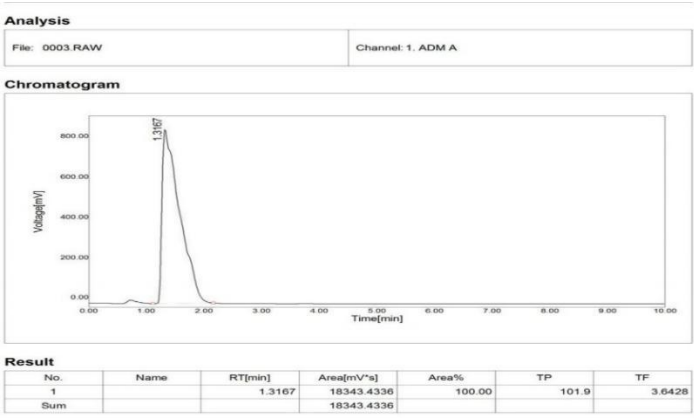

Figure3: Chromatogram for Standard

Linearity

From the prepared stock solution, a series of calibrated standards were prepared at concerntrations of 100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml, 500µg/ml and 600µg/mlusing mobile phase as solvent. Each of these drug solutions (20µl) was injected into the column, the peak area and retention times werw recorded. The calibration curve for cefixime was instructed by plotting the mean peak area against the drug concerntration. Regression equation was found to be y =5.5086.x + 402.67 (r^2=0.993). Linearity results were given in table 3.

S. No	Cefixime	Area
1.	100µg/ml	1020.10
2.	200µg/ml	1448.64
3.	300µg/ml	1971.45
4.	400µg/ml	2656.83
5.	500µg/ml	3043.71
6.	600µg/ml	3806.34

Table 3: Linearity Parameters for Cefixime

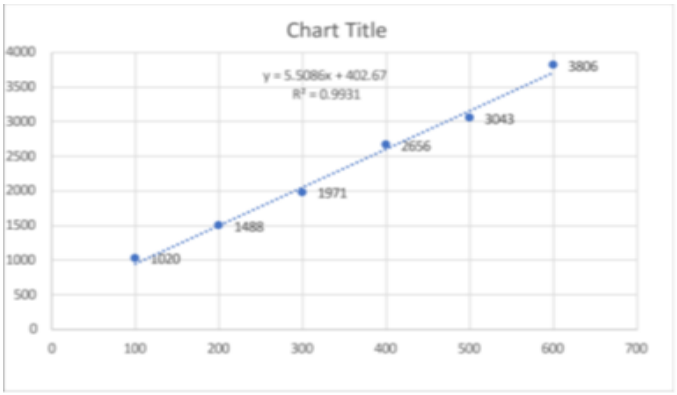

Figure 4: Calibration Curve for Cefixime

Parameters	Cefixime
Slope (m)	5.5086
Intercept (c)	402.67
Correlation coefficient (R2)	0.9931

Table 4: Analytical performance parameters of Cefixime

Precision study: Six replicate analysis of 400µg/ml stock solution of Cefixime was analyzed. The % RSD was found to be 0.16 for intraday precision. The % RSD was found to be less than 2 hence the method was found to be precised. Results were given in table 5.

S. No	Area of Cefixime
1.	2656
2.	2756
3.	2856
4.	2556
5.	2456
6.	2356
Mean	2606
%RSD	0.16%

Table 5: Intraday Precision of Cefixime

Acceptance Criteria

- % RSD for sample should be NMT 2
- The % RSD for the standard solution is below 1, which is within the limits hence method

is precise.

Limit of Detection

Molecule	LOD
Cefixime	0.1ug/ml

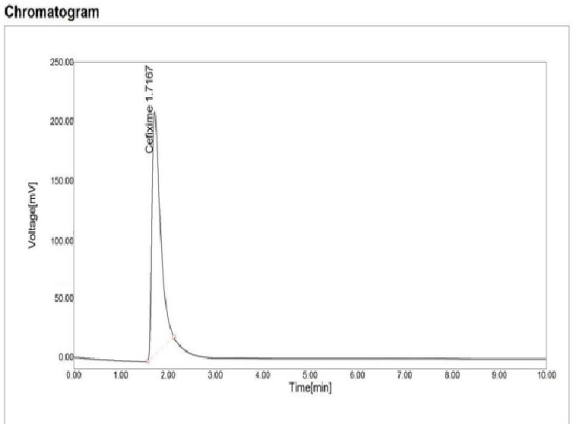

Figure 5: Chromatogram for LOD

RESULT AND DISCUSSION

Table 6: Summary of Results

Parameters	Cefixime	LIMIT
Linearity Range(µg/ml)	100-600µg/ml	R< 1
Regression coefficient	0.993	
Slope(m)	5.5086	
Intercept(c)	402.67	
Regression equation (Y=mx+c)	Y=5.5086x-402.67	98-103%
Assay (% mean assay)	99.17%	No interference of any peak
LOD	0.1µg/ml	
System precision %RSD	0.16%	NMT 2.0%

CONCLUSION

The estimation of Cefixime was done by RP-HPLC. The assay of Cefixime was performed with tablets and the % assay was found to be 99.17% which shows that the method is useful for routine analysis. The linearity of Cefixime was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is0.16 RSD should be not more than 2.0% and the method show precision Cefixime which shows that the method is precise. The acceptance criteria of intermediate precision is 0.16 RSD should be not more than 2.0% and the method show precision.

Cefixime which shows that the method is repeatable when performed in different days also. The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 99.17% for Cefixime. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility.

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