THE CREATION AND VALIDATION OF A RELIABLE RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF CEFEPIME AND ENMETAZOBACTAM IN PHARMACEUTICAL FORMULATIONS

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Abstract

A reverse-phase high-performance liquid chromatography (RP-HPLC) method was used to concurrently quantify the Exblifep® lyophilized powder for injection. Cefepime and enmetazobactam are the primary medications utilized in this method. Using an Eclipse column (150×4.6mm, 5µm) and a mobile phase of acetonitrile and water (40:60) at a flow rate of 1 millilitre per minute, chromatographic separation was achieved; detection was done at PDA 240 nm, and the run time was set at 15 minutes. The corresponding durations for cefepime and enmetazobactam were 5.3 and 7.1 minutes. The technique was verified for accuracy, precision, linearity, limit of detection (LOD), and robustness. For the simultaneous estimation of Exblifep® lyophilized powder, the developed approach exhibits great accuracy, precision, selectivity, and efficiency. It worked well for measuring both medications in commercially available goods.

Key words:
Cefepime, Enmetazobactum, RP-HPLC, Validation, Simultaneous quantification, Efficiency.

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Introduction

For the treatment of complex urinary tract infections, the Food & Drug Administration permitted cefepime and Enmetazobactam, a new injectable β-lactamase inhibitor combination, in February 2024. Gram-positive as well as gram-negative bacteria can be killed by cefepime, a fourth-generation cephalosporin (1). The new β-lactamase inhibitor cefepime/Enmetazobactam a novel β-lactamase inhibitor, exbit broad activity against both gram-positive and gram-negative bacteria. Clinical evidence shows that Cefepime/Enmetazobactam is superior to piperacillin/tazobactam for the combined clinical and microbiological cure of complex UTIs (2). Orchid Pharma, located in Chennai, India, submitted a product patent in 2006 for the creation of Enmetazobactam, which was recently developed in India. In 2010 the company filed a use patent for the treatment of a bacterial infection that produces carbapenemase(3). In January 2024, the European Medicines

Agency gave cefepime/Enmetazobactam a favourable opinion for the treatment of adult cUTI patients. In February 2024, the US Food and Drug Administration confirmed a marketing authorization for the treatment of cUTIs, ventilator-associated pneumonia, and hospital-acquired pneumonia (4). β- lactams effectiveness is mostly reliant on maintaining sufficient antibiotic concentrations in serum during the course of treatment, rather than on high peak concentrations like aminoglycosides (5). Considering the antibacterial spectrum of the cephalosporin, presumed improved penetrance of porin, and inherent opposition to hydrolyzes by AmpCs and OXA-48-like enzymes, Cefepime was selected as an Enmetazobactam partner. Clinical trials and preclinical research have shown that cefepime/enmetazobactam is effective (6). Research and advancement, and initiative to stop and mange antibiotic resistance are guided by the WHO bacterial priority pathogens list,2024, which identifies bacterial infections that affect public health relevance (7). A zwitterionic oxy-mino β-lactam with an amino-thiazole side chain, cefepime improves its capacity to quickly pierce gram-negative bacteria's outer cell membrane (8). In paediatric intensive care units, cefepime really reduces the colonization of resistant bacteria (9).Up to 50.1 million severe infections caused by both Klebsiella pneumoniae and 3GC-resistant Escherichia coli were estimated to have occurred worldwide in 2014, according to a recent study (10).When combined with cefepime, Enmetazobactam is said to exabit much superior antimicrobial capabilities against bacteria that produce class A ESBLs compared to the popular combination of piperacillin and tazobactam (11).It is crucial to diagnose pneumonia as soon as possible in order to begin antibiotic treatment (12). A crucial study including patients with complex UTIs was just concluded with cefepime-enmetazobactam (13). The chemical name (IUPAC) for cefepime is 1-{[(6R,7R)-7-[(2Z)-2-(2-amino-1, 3-thiazol-4-yl)}-2-(methoxyimino) acetamido]-2carboxylato-8-oxo-5-thia-1-azabicyclo[4.2.0]oct 2en-3-yl]methyl}-1-methylpyrolidin-1-ium. Fig.1. Molecular formula of $C_{19}H_{24}N_6O_5S_2$ Fig.1 (14) .The chemical name (IUPAC) for Enmetazobactam is (2S,3S,5R)-3-methyl-3-[(3-methyltriazol-3-ium-1-yl) methyl]-4,4,7-trioxo-4λ^6-thia-1-azabicyclo [3.2.0] heptane-2-corboxylate.Fig.2 (15).According to literature review, many RP-HPLC,(16–23).Liquid chromatography,(24–28).And UV spectrophotometric techniques have been documented for the quantification of cefepime either alone or in combination with other medications (29–31). Science: There isn't a recognized analytical technique for estimating these medications simultaneously in a combination dosage form. The current paper presents that maintaining the safety and effectiveness of pharmaceutical products containing cefepime and enmetazobactam requires accurate analysis and quality control. Analytical technique development and validation are essential for meeting regulatory standards. Such as those established by the International Council for Harmonisation (ICH). Cefepime and enmetazobactum have different physicochemical properties like solubility, stability, and other spectral characteristics; it can be difficult to measure their presence simultaneously. The most popular of the several analytical techniques is high-performance liquid chromatography (HPLC) is a widely used analytical method because it gives great accuracy, sensitivity, and reproducibility. Throughout a product's life cycle, these techniques are essential for spotting deteriorating products and reducing quality compliance. Developing and validating the reverse phase high-performance liquid chromatography (RP-HPLC) technique for the concurrent measurement of cefepime and enmetazobactam in commercial pharmaceutical products is the primary goal of the project.

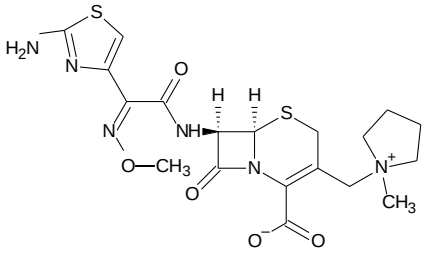


Fig.1 chemical structure of Cefepime

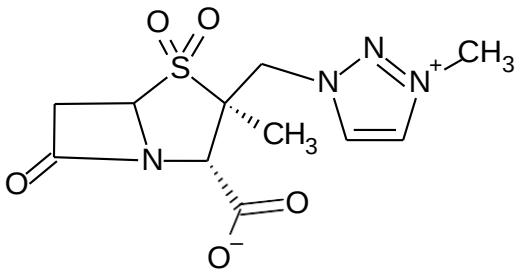


Fig.2 chemical structure of Enmetazobatam

MATERIALS AND METHODS

Instrumentation:

Equipment such as the column eclipse XDB-(Octadecylsilane) C18 (150×4.6mm, 5 micron) was used to modify the chromatographic optimization. A PDA detector was used for detection, and the injection volume was set at 20 microliters. To increase productivity, empower software was used

Reagents and materials

The experiment's ingredients and reagents comprised Enmetazobactum active pharmaceutical ingredients and cefepime active pharmaceutical ingredients.in addition to distilled water for preparation and dilution, HPLC- grade water and HPLC-grade acetonitrile were employed as solvents.

METHOD DEVELOPMENT AND VALIDATION

Preparation of mobile phase:

To create mobile phase A, 0.01M potassium dihydrogen orthophosphate was dissolved in one thousand milli litter of water, and ortho-phosphoric acid was used to bring the pH down to 3.0. The acetonitrile component was part of mobile phase B. To make the diluent, acetonitrile and water were mixed in a 40:60 (v/v) ratio.

Preparation of standard and test solutions:

Standard solution: A100 ml volumetric flask was filled with precisely weighed 750 mg of Cefepime and 1500 mg of Enmetazobactam, which were then dissolved and diluted with the diluent. Five millilitres of the aliquot were placed into a fifty-millilitre volumetric flask and diluted to volume.

Test solution:

In a hundred millilitre volumetric flask, 3750 milligrams of Exblifep® lyophilized powder (corresponding to 750mg of cefepime and 1500 mg of Enmetazobactam) was precisely weighed, and diluted with the diluent. A50 ml volumetric flask was filled with a 5ml aliquot, which was then diluted to volume.

METHOD VALIDATION:

1. Specificity

Excipients, contaminants, and diluents were examined for their ability to interfere with the procedure. The absence of co-eluting or interfering peaks verified the method's specificity.

2. Linearity

Linearity was assessed at 40%, 60%, 80%, 100%, and 120% of the reference concentration. The linearity of the approach was confirmed by calibration curves, which showed a corelation coefficient (R2) of 0.99 (Fig.2-3)

3. Precision

Six separate sample preparations were assayed under the same conditions in order to determine the method's precision. Repeatability was confirmed by the percentage relative Standard deviation (%RSD), which was with in allowable bounds (Table-1)

4. Accuracy

By injecting known volumes of standard solution into the sample at 80%, 100%, and 120% levels, accuracy was evaluated. The rates of recovery fell with in the acceptable range (Table-2).

5. Limits of both detection and quantitation

Lower concentration of the standard solution was gradually injected to determine the LOD and LOQ. Cefepime and Enmetazobactam were found to have a LOD of 0.02% and a LOQ of 0.06%(Table-3).

6. Robustness

Variation in column type and flow rate were introduced in order to evaluate the method's robustness. Under these circumstances, the approach stayed constant, demonstrating its dependability (Table-4).

RESULTS AND DISCUSSION:

According to ICH recommendations, the RP-HPLC technique created for the concurrent measurement of cefepime and Enmetazobactam in injectable formulations was validated. The mobile phase was a 40:60 ratio of acetonitrile to water, as in an Eclipse XDB-C15 column with a flow rate of 1.0 ml/min to accomplish the separation. UV detection was performed at 240

nanometres. Sufficient linearity, specificity, accuracy, precision, and robustness were demonstrated by the validation results.

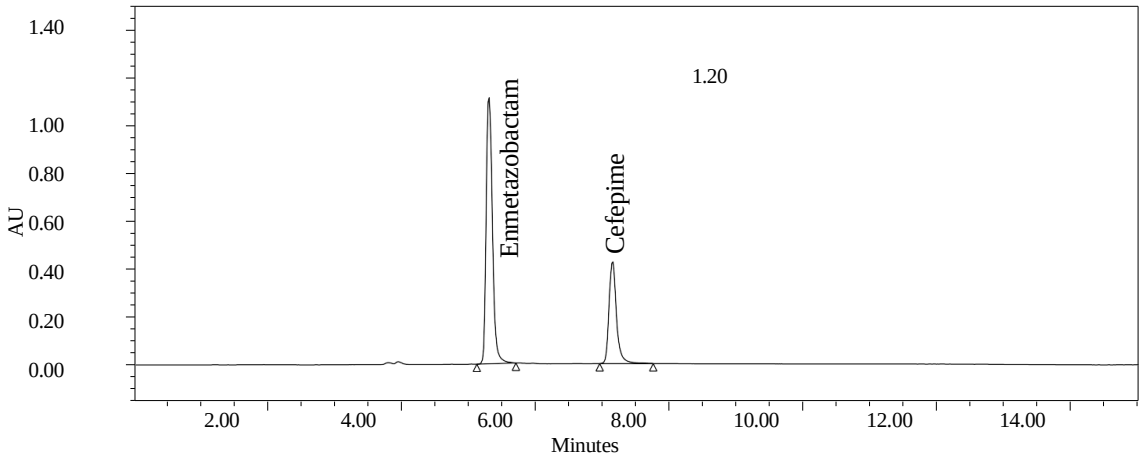


Figure-1 chromatogram for optimization of cefepime and Enmetazobactam

Table-1 data for optimization of cefepime and enmetazobactam

Sno	Peak name	RT	Area	% Area	USP tailing	USP plate count	USP resolution
1	Enmetazobactam	5.313	7264033	68.77	1.27	17640.80	
2	Cefepime	7.158	3298549	31.23	1.29	22515.89	10.25

LINEARTY:

The linearity of the approach was confirmed using calibration curves, which showed a the corelation coefficient (R2) of 0.99.

Figure -3 calibration curve of cefepime 300-900 micrograms/ml

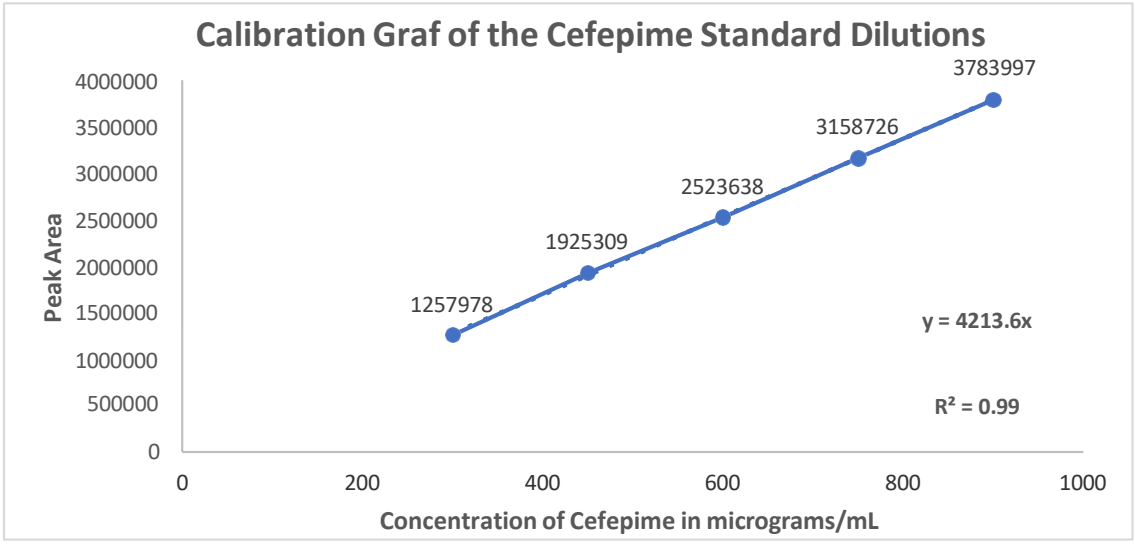
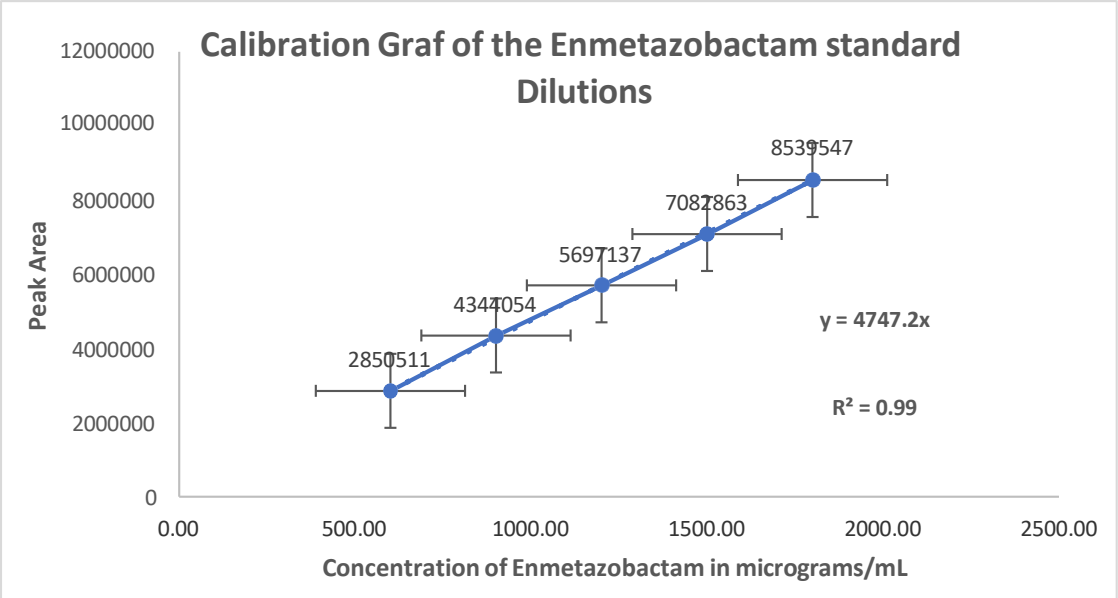


Figure -4 calibration curve of enmetazobactam 600-1800 micrograms/ml



PRECISION:

Six separate sample preparations were assayed under the same conditions in order to determine the method's precision. Repeatability was confirmed by the percentage relative standard deviation (%RSD), which was reasonable bounds.

Table-2 Precision data for Enmetazobactam and Cefepime

Enmetazobactam standard			**Enmetazobactam sample**	**Cefepime standard**	**Cefepime sample**
Sno	**Concentration(inj)**	**The Peak area**	**The Peak area**	**The Peak area**	**The Peak area**
1	1	7251105	7298434	3195882	3212949
2	2	7292812	7249552	3191109	3221922
3	3	7256680	7284832	3186000	3223196
4	4	7244666	7282774	3199952	3235038
5	5	7280844	7258031	3184730	3211061
6	6	7273777	7273377	3194682	3225821
	%RSD	**0.3**	**0.2**	**0.2**	**0.3**
		Assay:99.32%		**Assay:99.88%**	

SPECIFICITY:

Specificity was determined by injected the diluent as a blank and ensured a steady baseline. Injected Cefepime and Enmetazobactam known quantities of standard and sample solutions. Recovery rates were within the acceptance range.

Table-3 Specificity data for Enmetazobactam and Cefepime

Specificity for standard						
Name of the peak	**Retention time**	**Area**	**%Area**	**The USP tail**	**USP plate count**	**USP resolution**
Enmetazobactam	5.313	7264033	68.77	1.27	17640.80	
Cefepime	7.158	3298549	31.23	1.29	22515.89	10.25
Specificity for sample						
Enmetazobactam	5.310	7306035	69.53	1.27	16974.90	
Cefepime	7.155	3201466	30.47	1.25	21454.17	10.21

ACCURACY:

Accuracy was assessed by injecting known amounts of standard solutions into the sample at 80%, 100%, and 120% levels. The rates of recovery fell within the acceptable range.

Table-4 Accuracy data for Enmetazobactam and Cefepime

Cefepime standard				**Cefepime sample**		**Assay % Recovery**	**Enmetazobactam Standard**		**Enmetazobactam Sample**		**Assay % Recovery**
S.no	**Concentration %**	**Peak area**	**%RSD**	**Peak area**	**%RSD**		**Peak area**	**%RSD**	**Peak area**	**%RSD**	
1	80	2512049	0.3	2932309	0.1	**115.00**	5747223	0.3	6509943	0.1	**110.70**
2	100	32735597	1.2	3575424	0.2	**113.90**	7421760	0.6	7925562	0.2	**83.60**
3	120	3887168	0.5	4225466	0.3	**100.80**	8711512	0.9	9387906	0.1	**92.50**
Mean % recovery =109.9%							**Mean % recovery=95.6%**				

ROBUSTNESS:

Method robustness was evaluated by introducing variations in column type and flow rate. The method remained consistent under these conditions, confirming its reliability.

Table-5 Robustness data for Enmetazobactam and Cefepime

Different Column							
Enmetazobactam standard		**Enmetazobactam sample**		**Cefepime standard**		**Cefepime sample**	
The Peak area	**%RSD**	**The Peak area**	**%RSD**	**Peak area**	**%Relative standard deviation**	**The Peak area**	**%%Relative standard deviation**
7307165	0.9	7256230	0.4	3222697	0.3	3247943	0.4
Assay = 99.04%				**Assay = 99.87%**			

Column flow Rate Increase (1ml/min)							
7013084	1.2	6714312	0.5	3171210	1.3	3063153	0.7
Assay = 99.20%				**Assay = 99.69%**			
Column flow Rate Decrease (0.9ml/min)							
8141815	0.5	8114052	0.4	3607430	0.2	3586302	0.5
Assay:99.04%				**Assay:99.29%**			

LOD &LOQ:

Cefepime and Emetazobactam were found to have a LOD of 0.02% and a LOQ of 0.06%. the rates of recovery fell within the acceptance rage.

Table-6 LOQ &LOD data for Enmetazobactam and Cefepime

S.no	Concentration in %	Enmetazobactam peak area	Cefepime peak area
1	20	1453478	650216
2	10	695815	323069
3	5	397562	189293
4	2.0	188555	90914
5	1.0	75521	44479
6	0.5	49395	27225
7	0.2	41940	15744
8	0.1	23799	9752
9	0.05	9123	4760
10	0.02	5489	3079
11	0.01	ND	ND
LOD		**0.02%**	**0.02%**
LOQ		**0.06%**	**0.06%**

CONCLUSION:

The suggested RP-HPLC method provides a quick, easy, affordable, and precise way to quantify Cefepime and Enmetazobctam simultaneously in Exblifep® lyophilized powder. The lack of excipient involvement further validates the method's specificity. It is appropriate for routine quality control tests in pharmaceutical environments due to the direct measurement of both components.

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