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The Review Article on- Analytical Method Development and Validation of Antibiotic Drugs Cefepime and Enmetazobactam for Urinary Tract Infection

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ABSTRACT:

The presented study's recent literature review of analytical method development and validation of antibiotic drugs chemical nature, and structure for Urinary tract infection. A robust and stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Cefepime and Enmetazobactam in a synthetic mixture. The chromatographic separation was achieved using a suitable C8 column with a mobile phase consisting of a precise ratio of water and organic solvent, optimized for efficient resolution. The method was validated as per ICH guidelines for parameters including specificity, linearity, accuracy, precision, robustness, and forced degradation studies to confirm its stability-indicating nature. The developed method demonstrated excellent linearity within the selected concentration range, with acceptable recovery and precision. The forced degradation studies confirmed that the method effectively separated the degradation products from the analyses, ensuring its suitability for stability assessment. Thus, the proposed RP-HPLC method is simple, reliable, and suitable for routine quality control and stability studies of Cefepime and Enmetazobactam in pharmaceutical formulations.

Keywords: Method development, Validation, RP-HPLC method, Cefepime, Enmetazobactam, Antibiotic drugs.

1. INTRODUCTION[1-2]

Urinary tract infection (UTI): A urinary tract infection (UTI) is an infection in the organs in your urinary tract, which includes the bladder and kidneys. Symptoms depend on the part of the urinary tract affected. Urinary tract infections (UTIs) are caused by a wide range of pathogens, including Gram-negative and Gram-positive bacteria, as well as fungi. The most common causative agent for both uncomplicated and complicated UTIs is uropathogenic Escherichia coli (UPEC). Patients suffering from a symptomatic UTI are commonly treated with antibiotics.Your urinary tract is made up of your: kidneys, ureters, bladder, urethras.

Most UTIs only involve the urethra and bladder, in the lower tract. But UTIs can involve the ureters and kidneys, in the upper tract. Although upper tract UTIs are rarer than lower tract UTIs, they're also usually more severe.

UTIs are categorized as:

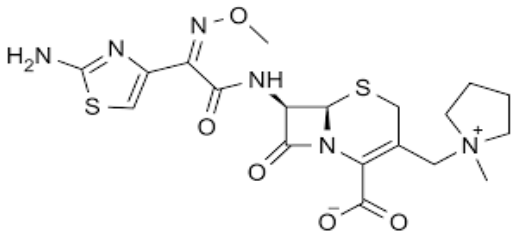
Uncomplicated: Uncomplicated UTIs typically affect individuals who are otherwise healthy and have no structural or neurological urinary tract abnormalities. Uncomplicated UTIs typically affect women, children and elderly patients who are otherwise healthy. Uncomplicated UTIs, UPEC is followed in prevalence by Klebsiella pneumoniae, Staphylococcus saprophyticus, Enterococcus faecalis, group B Streptococcus (GBS), Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus and Candida spp. These infections are differentiated into: Lower UTIs (cystitis) and Upper UTIs (pyelonephritis)

Complicated: Complicated UTIs are defined as UTIs associated with factors that compromise the urinary tract or host defence, including urinary obstruction, urinary retention. Complicated UTIs are usually associated with indwelling catheters, urinary tract abnormalities, immunosuppression or exposure to antibiotics. Complicated UTIs, the order of prevalence for causative agents, following UPEC as most common, is Enterococcus spp., K. pneumoniae, Candida spp., S. aureus, P. mirabilis, P. aeruginosa and GBS.

The literature review disclosed that a Cefepime belongs to class cephalosporin antibiotics & Enmetazobactam. belongs to class Penicillanic acid sulfone extended-spectrum beta (β)-lactamase (ESBL) inhibitor combination therapy of increasing cases of antimicrobial resistance for the treatment of complicated urinary tract infections (cUTI) including pyelonephritis caused by designated susceptible microorganisms. This drugs are Extended-spectrum beta-lactamases (ESBLs) are a group of bacterial serine beta-lactamases that hydrolyse third-generation cephalosporins (3GC), leading to the development of 3GC-resistant bacteria. When used in combination with Cefepime, Enmetazobactam protects Cefepime from degradation by ESBLs and prevents antibiotic resistance.

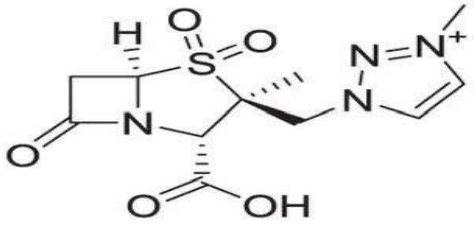
2. DRUG INTRODUCTION

Table 1: Drug profile of Cefepime

Name	**Cefepime**[3,4]
Chemical Structure	
Molecular Formula	$C_{19}H_{24}N_6O_5S_2$
Molecular Weight	480.561 g/mol
IUPAC Name	1-{[(6R,7R)-7-[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetamido]-2-carboxylato-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl}-1-methylpyrrolidin-1-ium
Solubility	Water: Highly soluble, Ethanol: Slightly soluble, Methanol: Easily soluble, Diethyl ether: Easily soluble
Melting Point	$150^{O}C$ to $155^{O}C$
pKa(Strongest Acidic):	2.82
pKa(Strongest Basic):	3.62
Pharmacological Class	Cephalosporin Antibiotics
Dosage Form:	The intravenous solution as Cefepime hydrochloride: 1 g/50 mL (50 mL), 2 g/100 mL (100 mL). Injection powder for reconstitution as Cefepime hydrochloride: 500 mg (each vial), 1 g (each vial), 2 (each vial). Intravenous solution for reconstitution, as Cefepime hydrochloride: 1 g Cefepime per 50 mL (5% w/v) dextrose USP in water for injection.
BCS Class	Class III drug (High Solubility & Low Permeability)
	Pharmacodynamics
Mechanism of Action	Fourth-generation cephalosporin, beta-lactam antibiotic with clinical utility against susceptible gram-negative and gram-positive bacteria Bactericidal action results from inhibition of cell wall synthesis Cefepime penetrates the cell wall of most gram-positive and gram-negative bacteria to bind penicillin-binding protein (PBP) targets Cefepime is stable to hydrolysis by some beta-lactamases, including penicillinases and cephalosporinases produced by gram-negative and gram-positive bacteria, with the exception of extended spectrum beta-lactamases (ESBL), some oxacillinases, and carbapenem hydrolyzing beta-lactamases
Therapeutic indication	Pneumonia Complicated and uncomplicated urinary tract infections Skin and soft tissue infections Complicated intra-abdominal infections (with metronidazole) Empiric treatment for neutropenic fever
Route of Administration	Intravenous
Adverse effects	Dark Urine, Fever, Chills, Nausea or Vomiting

Contraindications	In patients who have had previous hypersensitivity reaction to Cefepime or the cephalosporin class of antibiotics, penicillins or other beta-lactam antibiotics.
	Pharmacokinetics
Bioavailability	Cefepime is completely absorbed after parenteral injection.
Distribution	The volume of distribution is approximately 18 L. Plasma protein binding of Cefepime is low (20%); Cefepime is removed by dialysis in poisoning cases. Like most cephalosporins, Cefepime is widely distributed throughout body tissue and fluids, including pleural fluid, synovial fluid, bones, cerebral spinal fluid, and breast milk.
Metabolism	10 % to 20% of administered Cefepime is metabolized to N-methyl pyrrolidine (NMP) and rapidly converted to NMP-N-oxide.
Excretion	The primary route of elimination is renal excretion by glomerular filtration as an unchanged drug. The half-life is about 2 to 2.3 hours and is longer in patients with renal failure.

Table 2: Drug profile of Enmetazobactam

Name	**Enmetazobactam [5,6]**
Chemical Structure	
Molecular Formula	$C_{11}H_{14}N_4O_5S$
Molecular Weight	314.32 g/mol
IUPAC Name	(2S,3S,5R)-3-methyl-3-[(3-methyltriazol-3-ium-1-yl)methyl]-4,4,7-trioxo-4λ6 thia-1-azabicyclo[3.2.0]heptane-2-carboxylate
Solubility	Enmetazobactam is Freely soluble in water.
pKa(Strongest Acidic):	2.09
pKa(Strongest Basic):	2.83
Pharmacological Class	Penicillanic acid sulfone extended-spectrum beta (β)-lactamase (ESBL) inhibitor
	Pharmacodynamics
Mechanism of Action	Extended-spectrum beta-lactamases (ESBLs) are a group of bacterial serine beta-lactamases that hydrolyse third-generation cephalosporins (3GC), leading to the development of 3GC-resistant bacteria. When used in combination with cefepime, Enmetazobactam protects cefepime from degradation by ESBLs and prevents antibiotic resistance
Therapeutic indication	Treatment of adults with complicated urinary tract infections (cUTI) including pyelonephritis caused by designated susceptible microorganisms.
Route of Administration	Intravenous
Adverse effects	Pain and Inflammation at the infusion site Diarrhea Skin Rash Headache

Contraindications	Enmetazobactam is contraindicated in patients with a history of serious hypersensitivity reactions to the components of the fixed-combination preparation, or other beta-lactam antibacterial drugs
	Pharmacokinetics
Bioavailability	The mean (SD) Cmax is 19.8 (6.3) µg/mL in patients with cUTI and eGFR greater than or equal to 60 mL/min. The mean AUC0-last is 75.3 (30.8) µgxh/m
Distribution	Mean (SD) steady state volume of distribution (Vss) is 25.26 (9.97) L in patients with cUTI and eGFR greater than or equal to 60 mL/min.
Metabolism	Enmetazobactam is minimally metabolized.
Excretion	About 90% of Enmetazobactam is excreted unchanged in urine.

3. LITERATURE REVIEW

Official Methods

Table 3: Official method for Cefepime

Sr. No.	Title	Chromatographic Parameters	References
1.	IP	Mobile Phase: a mixture of 94 volumes of a solution prepared by dissolving 5.76 g of sodium 1-pentanesulphonate in 2000 mL of water, adjusted to pH 3.4 with glacial acetic acid and then pH 4.0 with potassium hydroxide, and 6 volumes of acetonitrile Column: Stainless steel column porous silica (5 µm) Flow Rate: 2 mL/min. Wavelength: 254 nm.	7
2.	USP	Mobile Phase A: 0.68 mg/mL of monobasic potassium phosphate in water Mobile Phase B: Acetonitrile and Mobile Phase A (1:9%v/v) adjusted with 2% phosphoric acid or 2% potassium hydroxide to a pH of 5.0 %v/v Mobile Phase C: Acetonitrile and Solution A (1:1%v/v), adjusted with 2% phosphoric acid or 2% potassium hydroxide to a pH of 5.0 Column: 5-µm packing L1 Flow Rate: 1 mL/min. Wavelength: 254 nm	8
3.	BP	Column: Octadecylsilyl silica gel (5 µm) Mobile phase A: mix 10 volumes of acetonitrile Rand 90 volumes of a 0.68 g/L solution of potassium dihydrogen phosphate R previously adjusted to pH 5.0 with a 0.5 M potassium hydroxide solution prepared from potassium hydroxideR Mobile phase B: mix equal volumes of acetonitrile R and a 0.68 g/L solution of potassium dihydrogen phosphate R previously adjusted to pH 5.0 with a 0.5 M potassium hydroxide solution prepared from potassium hydroxide Flow Rate: 1 mL/min. Wavelength: 254 nm	9

Table 4: Published methods for Cefepime

Sr. No.	Title	Chromatographic Parameters	References
1.	Development and Validation of a Green Analytical Method of RP-HPLC for Quantification of Cefepime Hydrochloride in Pharmaceutical Dosage Form: Simple, Sensitive and	Mobile phase: Ethanol: Water (55:45%v/v) Column: Luna C18 Flow rate: 0.5 mL/min. Wavelength:258 nm.	10

	Economic		
2.	Stability Studies of Cefepime Hydrochloride by Stability Indicating RP-HPLC Method	Mobile phase: ammonium acetate: acetonitrile (92:8%v/v) Column: C18 column Flow rate: 1.5 mL/min Wavelength: 256 nm	11
3.	Validation of an HPLC Method for Determination of Cefepime. Determination in Human Serum, Cerebrospinal Fluid, and Urine. Pharmacokinetic Profiles	Mobile phase: Phosphate buffer (pH 7): methanol (75:25%v/v) Column: 18 column Flow rate: 1.0 mL/min. Wavelength: 256 nm.	12

Table 5: Published methods for Cefepime with other drug

Sr. No.	Title	Chromatographic Parameters	References
1.	Simultaneous quantification of cefepime, meropenem, ciprofloxacin, moxifloxacin, linezolid and piperacillin in human serum using an isotope-dilution HPLC–MS/MS method	Mobile phases A: 10 mM ammonium formate with 0.1% formic acid in water. Mobile phases B: methanol %v/v Flow rate: 0.5 mL/min in a step dilution mode. Column: Fortis C18	13
2.	Stability and Validation of a High-Throughput LC-MS/MS Method for the Quantification of Cefepime, Meropenem, and Piperacillin and Tazobactam in Serum	Mobile phase A: 10 mM ammonium formate in water with 0.1% formic acid. Mobile phase B: 0.1% formic acid in acetonitrile %v/v Column: Agilent Eclipse Plus C18 Flow rate: 0.3 mL/min.	14
3.	RP-HPLC Method for Simultaneous Estimation of Cefepime Hydrochloride and Tazobactam Sodiumin Bulk and Pharmaceuticals	Mobile phase : 25 mm Potassium Dihydrogen phosphate buffer, pH 6.2 and acetonitrile(94 : 6%v/v) Column: PrincetonSPHER-100 C-18column Flow rate: 1 mL/min. Wavelength: 210 nm	15
4.	A Simultaneous, Validated RP-HPLC Method for Determination of Eight Cephalosporins in Pharmaceutical Formulations	Mobile phase: 0.1 M ammonium acetate buffer and acetonitrile in 95:5%v/v Flow rate: 0.8 mL/min Wavelength: 230 nm, 240 nm, 250 nm, 260 nm, 270 nm, and 280 nm.	16
5.	Quantification of Cefepime, Meropenem, Piperacillin, and Tazobactam in Human Plasma Using a Sensitive and Robust Liquid Chromatography-Tandem Mass Spectrometry Method, Part 1: Assay Development and Validation	Mobile phase: water with 0.1 % formic acid (solvent A) and acetonitrile with 0.1 % formic acid 132 (solvent B) %v/v Column: coupled 129 with a Phenomenex Security Guard ULTRA cartridge UPLC Evo C18 Flow rate: 0.25 mL/min.	17
6.	Stability indicating RP-HPLC method development and validation of cefepime and amikacin in pure and pharmaceutical dosage forms	Mobile phase : methanol: acetonitrile: acetate buffer 75:20%v/v ratio and 5% of acetate buffer Column: XTerra© RP- C-18 Wavelength: 210 nm	18
7.	Development and validation of RPHPLC method for simultaneous estimation of cefepime and tazobactam in Injection formulation	Mobile phase: phosphate buffer: methanol: acetonitrile: in the ratio 90:5:5 %v/v Column: C18 column (150 × 4.6 mm, 5 µ particle size) Wavelength: 260 nm	19

		Flow rate: mL/min	

Table 6: Published methods for Cefepime and Enmetazobactam

Sr. No.	Title	Chromatographic Parameters	References
1.	Liquid chromatography–tandem mass spectrometry for the simultaneous quantitation of Enmetazobactam and cefepime in human plasma	Mobile phases A: ammonium formate in water and acetonitrile Column: Acquity BEH HILIC column (50 mm × 2.1 mm, 1.7 m) Flow rate: lower limit of quantification was 0.05 g/mL for Enmetazobactam and 0.5 g/mL for cefepime.	20

5. CONCLUSION

This drug combination is recently approved by Central Drugs Standard Control Organisation (CDSCO). The above study gives the analytical methods for analysis of antibiotics drug in synthetic mixture. Literature survey reveals that various methods are reported for the development and validation of various drugs. At this literature review presence both drug Extended-spectrum beta-lactamases (ESBLs) are a group of bacterial serine beta-lactamases that hydrolyse third-generation cephalosporins (3GC), leading to the development of 3GC-resistant bacteria. When used in combination with Cefepime, Enmetazobactam protects Cefepime from degradation by ESBLs and preven00ts antibiotic resistance. These methods are reported for the development and validation of drugs. Analysis of drug plays a significant role during formulation to identify the drug and its metabolites.

6. RESULT

The presented study's recent literature review of antibiotic drug their chemical nature, structure for Urinary tract infection (UTI) is a disorder this is putting a growing burden on health carrier delivery internationally. Therefore, it has to turn out to be more and more crucial that physicians who deal with such patients have an excellent understanding of antibiotic.

This review geared toward specializing in frequent analytical strategies according for the assay of antibiotic drug. A broad vary of techniques is out there for the estimation of antibiotic drug and Urinary tract infection in biological samples, and pharmaceutical indefinite quantity type. The analysis of revealed information unconcealed that chemical analysis strategies are the straightforward and economical strategies for estimation of antibiotic drug in pharmaceutical formulation. For analysis of antibiotic drug, and Urinary tract infection, HPLC provides correct results and low price compared to advance detection techniques. HPLC with personal organizer detection was extensively used for the event of stability- indicating assay strategies for separation and quantification of oral antibiotic drug within the presence of degradation product. This survey conjointly highlights the combined techniques that incorporate the economical separation of metabolites of antibiotic drug persecution HPLC sensitive detection has become an imperative tool for quantification of antibiotic drug in biological fluids and pharmacokinetic studies

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