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Review Article

Advanced Analytical Strategies for Antibiotic Quantification: A Comparative Study of UV Spectroscopy and HPLC

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ABSTRACT


The development and validation of analytical methods are critical for ensuring the quality, efficacy, and safety of pharmaceuticals, particularly beta-lactam antibiotics. These antibiotics, known for their ability to inhibit bacterial cell wall synthesis, remain indispensable in combating a wide range of infections despite growing concerns about antibiotic resistance. This review focuses on the advancements in analytical methodologies, including UV-Vis spectroscopy and high-performance liquid chromatography (HPLC), which are pivotal in the characterization, quantification, and stability assessment of beta-lactam antibiotics. Key aspects of analytical method development, such as specificity, precision, accuracy, and robustness, are highlighted, alongside validation parameters that ensure regulatory compliance and reproducibility. Innovative approaches, such as derivative UV spectroscopy for enhanced sensitivity and advanced HPLC systems for rapid and precise separations, demonstrate how modern analytical tools address challenges in pharmaceutical quality control. By focusing on widely used antibiotics like cephalexin, amoxicillin, and cefepime, this review underscores the importance of robust analytical methodologies in addressing quality control, regulatory compliance, and resistance issues. Furthermore, it discusses emerging challenges in treating multidrug-resistant infections and emphasizes the critical role of continuous innovation in analytical and pharmaceutical research. This synthesis provides a comprehensive overview, equipping researchers with insights into both the therapeutic significance and analytical intricacies of beta-lactam antibiotics.

INTRODUCTION

Arguably, the most significant medical advancement of the 20th century was the development of antibiotics for clinical use ^[1].

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Apart from curing infectious diseases, antibiotics enabled numerous contemporary medical procedures such as treating cancer, performing organ transplants, and performing open cardiac surgery. The word "antibiotic" refers to a chemical compound that, when diluted to a high concentration, inhibits the growth of germs [2]. Beta-lactam antibiotics are a significant class of medicines used in modern medicine. A significant class of antibiotics known as beta-lactams includes the penicillins (like Piperacillin), carbapenems (like Meropenem), and cephalosporins (like Ceftazidime). For how long the antibiotic concentration is higher than the pathogen's minimum inhibitory concentration (MIC), the beta-lactams' antibacterial efficacy is determined [3]. The β -lactam antibiotics are made up of a β -lactam with four members [4]. The first pure antibiotic that is still in use is penicillin. In 1928, Alexander Fleming made the initial observation of staphylococci inhibition on an agar plate contaminated with *Penicillium* mold. The 1940s saw the start of his isolate's clinical trials, but the necessities of World War II kept the medication off the public market until 1946. Penicillin revolutionized the way infectious disease was treated and sparked a hunt for other antibiotic classes. [5]

Disease

One of the most significant accomplishments of Medicinal Chemistry in the past 70 years has been the development of antibacterial agents to combat bacterial infections. However, the future sustainability of these treatments remains uncertain, as bacteria such as *Staphylococcus aureus* have demonstrated the alarming ability to develop resistance to even the most well-established medications. This constant evolution of resistance ensures that the search for novel antibacterial agents is unending. Although the

widespread availability of antibiotics has led to a sharp decline in death rates from bacterial infections in industrialized nations, bacterial infections remain a leading cause of death in many developing countries. For instance, the World Health Organization (WHO) reported that tuberculosis (TB) was responsible for nearly 2 million deaths in 2002, with a staggering one-third of the global population infected at the time. In the year 2000, WHO also estimated that 1.9 million children were affected by bacterial infections. Despite advancements in antibacterial therapy, these statistics highlight the ongoing global health challenges, particularly in emerging nations, and emphasize the critical need for continued innovation in the fight against bacterial diseases. [6]

1] Tuberculosis (TB):

In 2014, over 480,000 new cases of multidrug-resistant tuberculosis (MDR-TB) and 190,000 related deaths were reported globally, with WHO identifying India, the Russian Federation, and South Africa as the countries with the highest cases. Diagnosing MDR-TB remains a challenge, with only one-third of cases being detected, and treatment availability is even more limited. Around 3.3% of new TB cases and 20% of previously treated cases are classified as MDR-TB, and 10% of these cases meet the criteria for extensively drug-resistant TB (XDR-TB), which is resistant to fluoroquinolones and second-line injectable drugs like amikacin and kanamycin. Countries like Belarus, Latvia, and Georgia report high MDR-TB prevalence. Treating MDR and XDR-TB is complex, requiring long-term, expensive treatment plans with second-line drugs. Newer drugs, such as delamanid and bedaquiline, are being considered, though evidence on their efficacy and safety is still limited. Additionally, carbapenems, such as meropenem and imipenem, are being explored for controlling these drug-



resistant TB cases, though more research is needed to assess their long-term effectiveness. [7]

2]Osteomyelitis:

Osteomyelitis is an infection of the bone, often extending to the bone marrow. Systemic infections such as typhoid, syphilis, tuberculosis, actinomycosis, and brucellosis can spread to bones, but two major conditions leading to significant bone damage are of particular concern. Suppurative osteomyelitis, a form of the disease caused mainly by bacterial infections (and rarely by fungi), presents differently across developing and developed nations. In developing countries, it commonly occurs via hematogenous spread, affecting long bones in children aged 5-15 years, while in developed countries, early use of antibiotics limits this type of spread. Instead, bone infections are often localized, affecting areas like the jaw or skull, usually resulting from direct spread of nearby infections. Osteomyelitis can affect individuals of any age and may complicate conditions such as fractures, surgeries involving prostheses or implants, and cases of gangrene associated with diabetes, malnutrition, or immunosuppression. *Staphylococcus aureus* is the most common cause of osteomyelitis, though other pathogens like *Streptococci*, *Escherichia coli*, *Pseudomonas*, *Klebsiella*, and anaerobes are sometimes involved, especially in mixed infections following trauma. Transient bacteremia may precede the onset of the infection, making blood cultures useful for diagnosis. Children with acute hematogenous osteomyelitis typically present with localized pain, tenderness, fever, and malaise, often accompanied by elevated white blood cell counts. X-rays reveal bone destruction, but sometimes osteomyelitis can progress without early detection. In advanced cases, chronic infection can lead to draining fistulas and, in rare

cases, squamous cell carcinoma can develop at the site of infection. [8]

3] Infections streptococcal:

Streptococci are gram-positive cocci, known not only for their direct infections but also for the autoimmune complications they trigger, especially in underprivileged communities where access to healthcare is limited. Globally, streptococcal infections cause significant health issues, with various subtypes linked to specific illnesses:

1. Group A (*Streptococcus pyogenes*): Responsible for upper respiratory tract infections, skin infections like erysipelas, and urinary tract infections. Additionally, it plays a key role in autoimmune conditions such as rheumatic heart disease (RHD).
2. Group B (*Streptococcus agalactiae*): Common in infants, leading to infections and post-streptococcal complications like acute glomerulonephritis.
3. Groups C and G: These streptococci cause respiratory infections.
4. Group D (Enterococci, *Streptococcus faecalis*): Major contributors to urinary tract infections, septicemia, and endocarditis.
5. Viridans group streptococci: Normally part of the oral flora but can cause bacterial endocarditis when they enter the bloodstream.
6. *Streptococcus pneumoniae* (Pneumococci): The primary cause of bacterial pneumonia and other respiratory infections, especially in vulnerable populations.

Each group plays a unique role in streptococcal diseases, with their impact varying depending on

access to timely treatment and preventive measures.^[9]

There Are Five Primary Mechanisms Through Which Antibacterial Agents Function:

- 1. Inhibition of cell metabolism:** Antibacterial agents that inhibit cellular metabolism are known as antimetabolites. These compounds disrupt the metabolism of microorganisms without affecting the host's metabolism. This is achieved by blocking enzyme-catalyzed reactions that occur in bacterial cells but not in animal cells. A well-known class of agents that work this way is the sulphonamides. Additionally, antibacterial agents can selectively target enzymes present in both bacterial and mammalian cells if there are significant structural differences between the two.
- 2. Inhibition of bacterial cell wall synthesis:** This mechanism results in bacterial cell lysis and death, as the cell wall is crucial for bacterial survival. Agents that function through this pathway include penicillins,

cephalosporins, and glycopeptides like vancomycin. Since animal cells lack a cell wall, they are not affected by these agents.

- 3. Interaction with the plasma membrane:** Some antibacterial agents interact directly with the bacterial plasma membrane, affecting its permeability and leading to cell death. Examples of agents that act in this manner are polymyxins and tyrothricin.
- 4. Disruption of protein synthesis:** By interfering with protein synthesis, essential proteins and enzymes necessary for the bacterial cell's survival are no longer produced. This mechanism is employed by antibacterial agents such as rifamycins, aminoglycosides, tetracyclines, and chloramphenicol.
- 5. Inhibition of nucleic acid transcription and replication:** This process prevents cell division or the synthesis of vital proteins, leading to the inhibition of bacterial growth. Agents like nalidixic acid and proflavine work through this mechanism^[10]

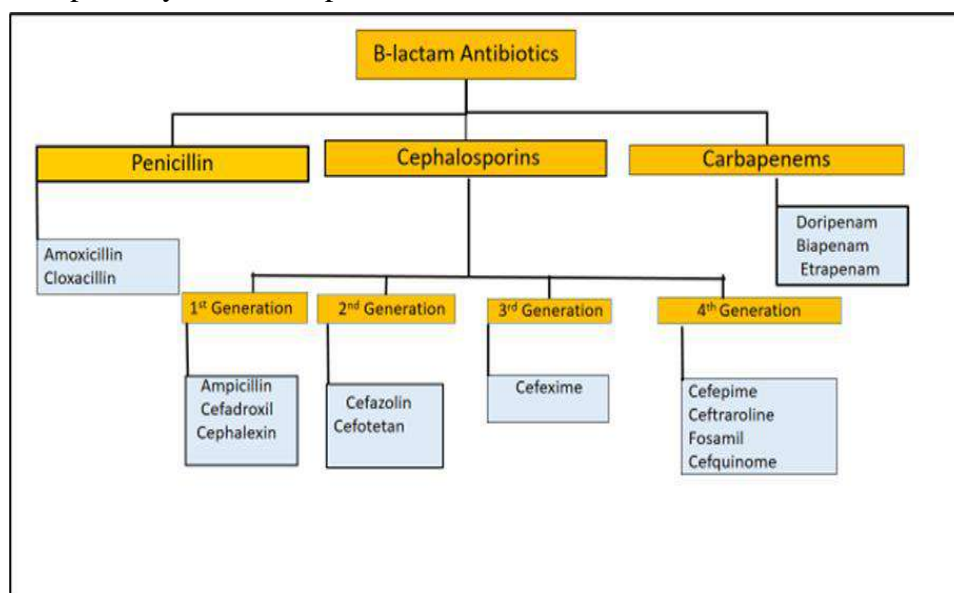


Fig No. 1 Beta-Lactam Antibiotics Classification

Analytical Method Development



Analytical method development is the process of designing and validating procedures to identify, quantify, and study the properties of chemical substances. It is a critical component in pharmaceutical research, quality control, and regulatory compliance. The goal is to ensure that the method is reliable, reproducible, and suitable for its intended purpose. ^[11]

Key Steps in Analytical Method Development:

1. Understanding the Objective:

- Define the purpose of the analysis (e.g., quantification, identification, impurity profiling).
- Identify the target analyte(s) and sample matrix.

2. Selection of Technique:

- Choose appropriate analytical techniques such as chromatography (HPLC, GC), spectroscopy (UV, IR, NMR), titration, or others based on the analyte properties.

3. Method Design:

- Establish conditions such as solvents, pH, temperature, and mobile phase composition (for chromatographic methods).
- Develop an initial procedure based on literature or empirical knowledge.

4. Optimization:

- Adjust method parameters to enhance sensitivity, selectivity, accuracy, and precision.
- Use tools like design of experiments (DoE) to systematically study the effects of various factors.

5. Validation:

- Validate the method according to regulatory guidelines (e.g., ICH Q2(R1)) to ensure reliability.
- Key validation parameters include:
 - **Accuracy:** Closeness of the measured value to the true value.
 - **Precision:** Reproducibility of the method.
 - **Specificity:** Ability to analyze the analyte without interference.
 - **Linearity:** Proportional response over a range.
 - **Limit of Detection (LOD) and Quantitation (LOQ):** Sensitivity of the method.
 - **Robustness:** Method's resilience to small changes in conditions.

6. Documentation:

- Record all steps, data, and results for regulatory submission and reproducibility.

Applications:

- **Pharmaceutical Industry:** Quality control, drug development, stability testing.
- **Environmental Analysis:** Monitoring pollutants and contaminants.
- **Food Industry:** Ensuring food safety and quality.

Importance:

A well-developed analytical method ensures:

- Consistent product quality.
- Regulatory compliance.
- Cost-effective production.
- Reliable data for decision-making.^[45]



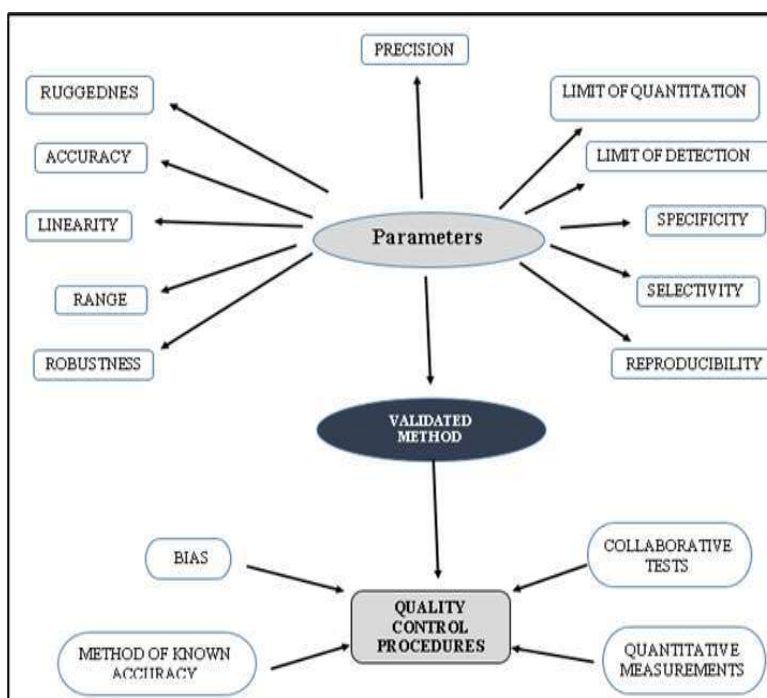


Fig no. 2 Validation Parameters

UV-Vis Spectrophotometric Methods

Ultraviolet visible, or UV-Vis, spectroscopy is widely used to furnish characterization information for a range of materials. UV-visible spectroscopy can be used to observe inorganic or organic, solid or liquid groups, such as organic molecules and functional groups. It can also be used to measure reflectance for coatings, paints, textiles, biochemical analysis, dissolution kinetics, band gap measurements, etc. The UV-Vis provides these details based on the different responses of samples and the degree of transmittance or absorbance of a different wavelength of beam light.^[12] UV-Vis spectrophotometric analysis is a widely used, practical, cost-effective, and rapid technique for analyzing pharmaceutical formulations.

2.1. Spectrophotometric Analysis

In pharmaceutical and biological research, derivative UV spectroscopy is a common tool for quality control, characterization, quantitative analysis, and product management. Compared to conventional absorbance spectra, derivative spectroscopy provides more detailed spectral fingerprints. Its enhanced sensitivity stems from precise electrical or numerical signal amplification, which minimizes noise. The sensitivity is influenced by factors like the band shapes in the primary spectrum, particularly the half-width of the bands. The derivative of the primary peak's curve reflects the changes in slope across the curve, with the intensity of the output spectrum inversely proportional to absorption. Derivative UV spectra enhance sensitivity and selectivity compared to traditional methods and enable precise determination of λ_{max} values, narrow bands, and inflection points.^[13]

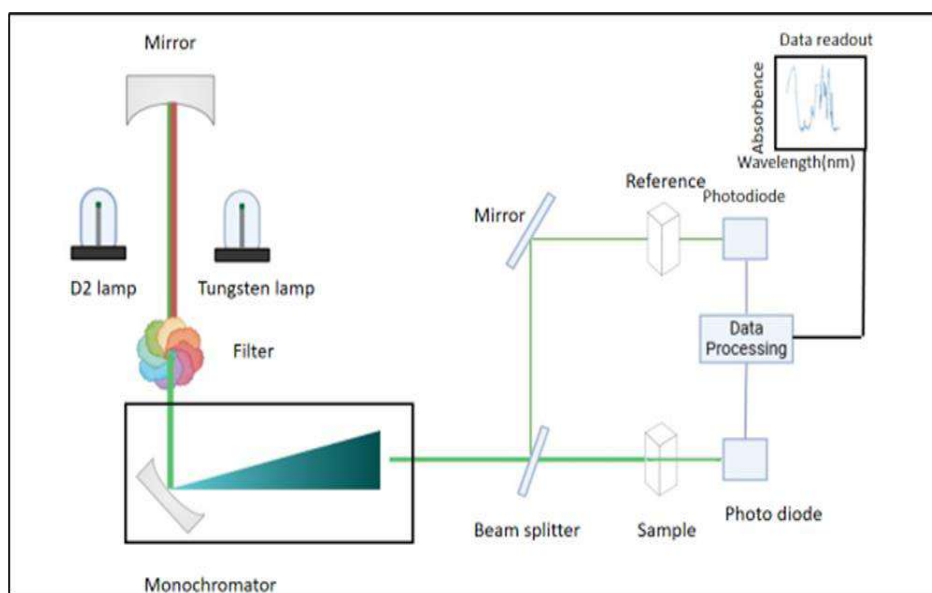


Fig No.3 Instrumentation of UV-visible Spectrophotometer

Principle

Radiation can trigger electronic transitions within a molecule or ion, resulting in absorption in the visible or ultraviolet area. Thus, the absorption of light by a sample in the ultraviolet or visible area causes a change in the electronic state of the molecules in the sample. Light energy moves electrons from the ground state to the excited state or anti-bonding orbital. There are three types of ground state orbital and it involved

1. σ (Bonding) molecular
2. π (Bonding) molecular orbital
3. n (non-Bonding) atomic orbital.

Additionally, the transition may involve two types of anti-bonding orbitals:

- i) σ^* (sigma star).
- ii) π^* (pi star) orbit.

There is no such thing as a n^* anti-bonding orbital since n electrons do not form bonds. Thus, the absorption of UV light can cause the following electronic transitions:

- σ to σ^*

- n to σ^*
- n to π^*
- π to π^* [14]

2.2. High-Performance Liquid Chromatography (HPLC)

Since its introduction over 30 years ago, HPLC has become an established analytical technique. Advanced HPLC systems, including ultra-performance liquid chromatography (UPLC), employ columns with 1.7 μm particle-sized adsorbents. These systems offer significantly improved resolution, faster run times, and greater sensitivity, enabling the analysis of various compounds efficiently.^{[11],[13]} High Performance Liquid Chromatography (HPLC) is an advanced technique derived from classical column chromatography and is now a key instrument in analytical chemistry. In the pharmaceutical sector, HPLC is essential across all stages of drug discovery, development, and production. It is commonly used to evaluate the purity of new chemical compounds, monitor changes in synthetic reactions, assess new formulations, and ensure the quality control and assurance of the final drug products. Known for its high precision,

HPLC is widely utilized for both the qualitative and quantitative analysis of drug products, as well as for testing their stability.^[15]

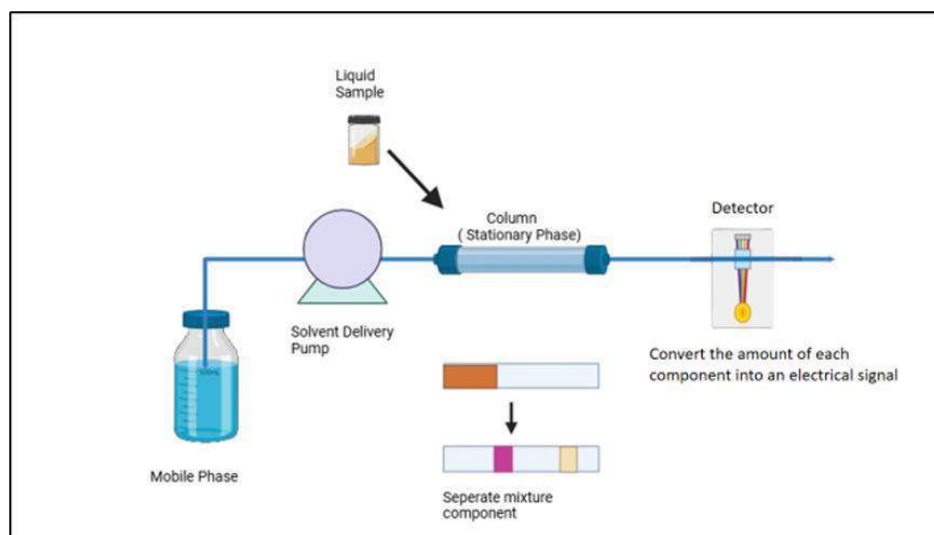


Fig No.4 High-Performance Liquid Chromatography

Principle

HPLC is a type of liquid chromatography in which the separation process occurs between two phases: the mobile phase (solvent) and the stationary phase (column packing). The efficiency of separation depends on how the sample components interact and distribute between these two phases. There are four main modes of separation based on the nature of the stationary phase:

1. Adsorption chromatography: Separation is based on repeated adsorption and desorption interactions between the sample and the stationary phase.

2. Partition Chromatography: Separation occurs due to the partitioning of the sample between the mobile and stationary phases.

3. Ion-Exchange Chromatography: In this mode, the stationary phase consists of an ionic surface with an opposite charge to that of the sample.

4. Size exclusion chromatography: Here, separation happens based on the molecular size of the sample, using a column with a material that has precisely controlled pore sizes. Among these, adsorption chromatography is the most commonly used. It can be carried out in two distinct modes depending on the polarity of the phases:

Normal phase chromatography: The stationary phase is polar (e.g., silica or alumina) and the mobile phase is non-polar (e.g., hexane). In this mode, polar compounds are more strongly retained by the column, allowing non-polar compounds to elute first.

Reversed-phase chromatography: The stationary phase is non-polar (e.g., hydrocarbon), while the mobile phase is polar (e.g., water or methanol). This is the reverse of normal phase chromatography, where non-polar compounds are retained longer on the column.^[16]

Antibiotic Drugs General Details

Cephalexin

IUPAC NAME:

(6R,7R)-7-[[[(2R)-2-amino-2-phenylacetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

Structure

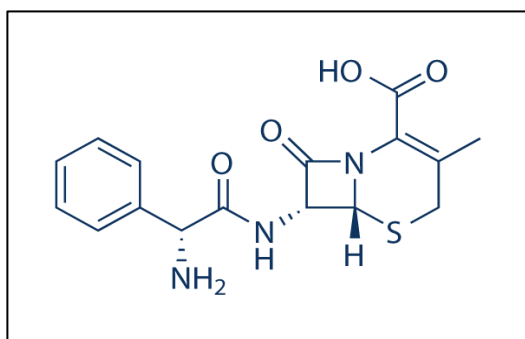


Fig No.5: Chemical structure of Cephalexin

Cephalexin, also known as Cefalexin, is a widely used cephalosporin antibiotic effective against a variety of bacterial infections. It is commonly prescribed for treating skin infections, cuts, and other mild injuries, as well as respiratory, urinary, bone, joint, and ear infections. By disrupting the bacterial cell wall, Cephalexin targets mainly gram-positive bacteria, leading to cell destruction. However, it is ineffective against viral infections such as the flu or the common cold. Developed in 1967 and marketed under brand names like Keflex and Ceporex, Cephalexin has been a staple in antibiotic treatment since the late 1960s.

Mechanism of action:

It's one of the most extensively specified antibiotics, frequently used for the treatment of superficial infections that affect as complications of minor injuries or incisions. It's effective against utmost gram-positive bacteria through its inhibition of the cross linking response between

N- acetyl muramic acid and N- acetylglucosamine in the cell wall, leading to cell lysis.^[17]

Amoxicillin

IUPAC NAME :

(2S,5R,6R)-6-[[[(2R)-2-amino-2-(4-hydroxyphenyl)acetyl].]

Structure

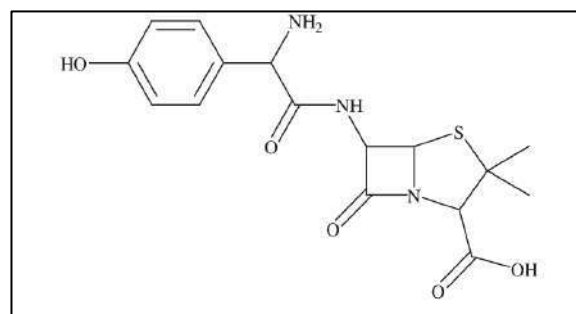


Fig No.6 Chemical structure of Amoxicillin

Amoxicillin, first launched in the 1970s for oral use in the UK, is now widely used as a broad-spectrum antibiotic to treat a variety of disorders. Amoxicillin is more efficient against gram-positive than gram-negative microorganisms and outperforms penicillin and penicillin V. Additionally, it has been proven equivalent to other antibiotics. Amoxicillin has been used to treat infections of the middle ear (otitis media), tonsils (tonsillitis & tonsillopharyngitis), throat, larynx (laryngitis), and pharynx (pharyngitis) for over a decade. Recent research indicates that it can be used as a preventative measure against bacterial endocarditis, in patients with prosthetic joint replacements and in dentistry.

Mechanism of action:

Amoxicillin works by competitively binding to penicillin-binding proteins, which triggers the activation of autolytic enzymes and disrupts cell wall synthesis. It has an extended effect since it is typically administered twice a day. Additionally,

amoxicillin has a broad therapeutic window, with mild overdoses generally not causing major toxic effects. ^[18]

Cefadroxil

Iupac Name:

(6R,7R)-7-[[[(2R)-2-amino-2-(4-hydroxyphenyl)acetyl] amino]-3-methyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid.

Structure

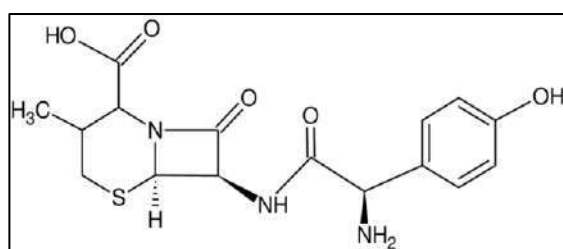


Fig No.7 Chemical structure of Cefadroxil

Cefadroxil (CFD) is a first-generation cephalosporin that works well against both Gram-

positive and Gram-negative bacteria. It is particularly useful for treating bronchitis, tonsillitis, skin infections, infections of soft tissues and ears, gonorrhea, urinary tract infections, and infections of the ligaments and tendons. It additionally offers a high potential for chemotherapeutic oral administration.

Mechanism of action:

Cefadroxil inhibits the synthesis of the bacterial cell wall, which results in bactericidal action^[19]

Ampicillin

IUPAC NAME:

(2S,5R,6R)-6-([(2R)-2-amino-2-phenylacetyl] amino)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2- carboxylic acid

Structure

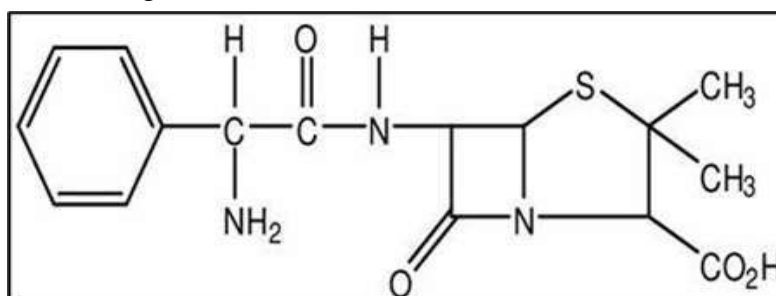


Fig No.8 Chemical structure of Ampicillin

Ampicillin has a greater antimicrobial range than penicillin G. It is effective against the same Gram-positive organisms that are sensitive to other penicillins. Furthermore, it has been shown to be more effective against specific Gram-negative pathogens including enterococci than other penicillins.

Mechanism of action:

The 'drug' works against the same species of Gram-positive organisms that are vulnerable to other penicillins, but it is also more effective against certain Gram-negative organisms and enterococci than other penicillins. The α -amino functional moiety is crucial for the protein's activity, but its specific mechanism is unknown. The α amino moiety of penicillin may allow it to penetrate cell wall barriers that other penicillins cannot ^[20].

Cloxacillin

Iupac Name:

(2S,5R,6R)-6-[[3-(2-chlorophenyl)-5-methyl-1,2-oxazole-4-carbonyl] amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid

Structure

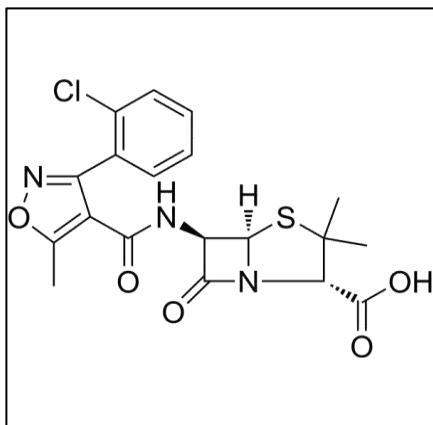


Fig No.9 Chemical structure of Cloxacillin

Cloxacillin, with the chemical formula 3-(2-chlorophenyl)-5-methyl-4-isoxazolyl penicillin, differs from oxacillin by the presence of an extra chlorine atom. It is available in oral capsule doses of 250 mg and 500 mg, as well as injectable forms of 250 mg, 500 mg, and 2 g. Additionally, it is offered as an oral solution containing 120 mg per 5 ml. It serves as both an antibacterial agent and a medication for bacterial infections. Its structure and function are closely related to oxacillin.

Mechanism of action :

Cloxacillin kills bacteria by interfering with the synthesis of their cell walls through binding to one or more penicillin-binding proteins (PBPs). It also promotes bacterial autolysis by inhibiting specific PBPs, which triggers the activation of the bacterial autolytic process.^[21]

CEFEPIME

IUPAC NAME:

(6R,7R)-7-[[[(2E)-2-(2-amino-1,3-thiazol-4-yl)-2-methoxyiminoacetyl]amino]-3-[(1-methylpyrrolidin-1-ium-1-yl)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid

Structure

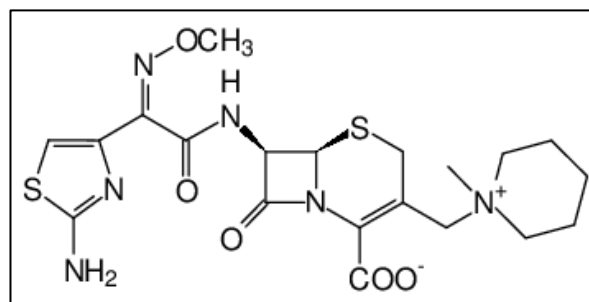


Fig No.10 Chemical structure of Cefepime

Cefepime is a fourth-generation cephalosporin, categorized as an oxymino beta-lactam with an amino thiazoline side chain. It offers a broader spectrum and enhanced potency compared to third-generation cephalosporins. Additionally, cefepime is notably stable against certain beta-lactamases, particularly the AmpC enzymes, whether chromosomal or plasmid-mediated, produced by Gram-negative bacteria.

Mechanism of action:

Cefepime is a bactericidal cephalosporin antibiotic that works similarly to other beta-lactam antibiotics. It targets bacterial cell walls by binding to and inhibiting transpeptidases, also called penicillin-binding proteins (PBPs), which are enzymes crucial for the final steps in the synthesis of the peptidoglycan layer.^[22]

Cefazolin

IUPAC NAME:

(6R,7R)-3-[(5-methyl-1,3,4-thiadiazol-2-yl)sulfanylmethyl]-8-oxo-7-[[2-(tetrazol-1-yl)acetyl]amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid

Structure

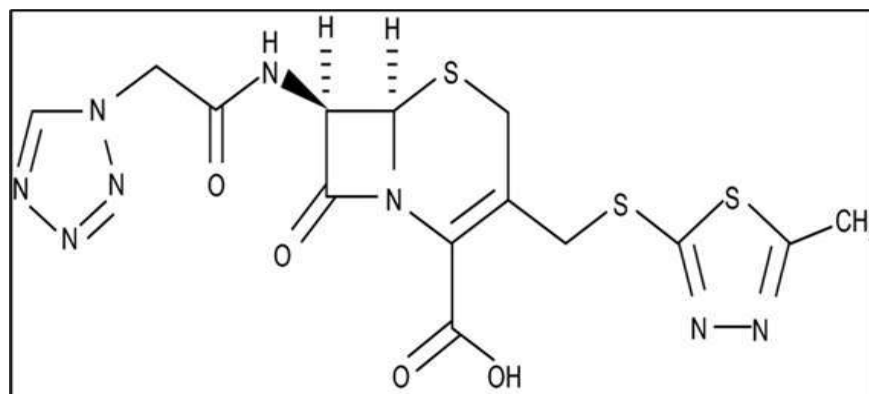


Fig no.11 Chemical structure of Cefazolin

Every year, more than 17 million surgical procedures are performed in the United States. According to the Centers for Disease Control and Prevention's health care-associated infection prevalence survey, approximately 110,800 surgical site infections occurred during inpatient operations. Cefazolin, a first-generation cephalosporin, is the recommended antibiotic for most surgical procedures. Cefazolin is the first-line treatment because it has been extensively studied, has an appropriate spectrum of activity against organisms commonly found in surgical site infections, is well tolerated, and has a low acquisition cost. However, the 10% of patients who report a penicillin allergy are less likely to receive cefazolin and more likely to receive clindamycin or vancomycin, increasing the risk of developing a surgical site infection.^[23]

Mechanism of action:

Cefazolin hinders the formation of bacterial cell walls. attaching to one or multiple of the penicillin-binding Proteins hinder the last transpeptidation stage of peptidoglycan production in bacteria. cell walls are disrupted, therefore preventing the synthesis of cell walls.

Bacteria eventually break down because of their continuous activity. autolytic enzymes responsible for breaking down the cell wall autolysins and Cell-wall assembly occurs concurrently with the action of murein hydrolases.^[24]

CEFIXIME

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

Structure

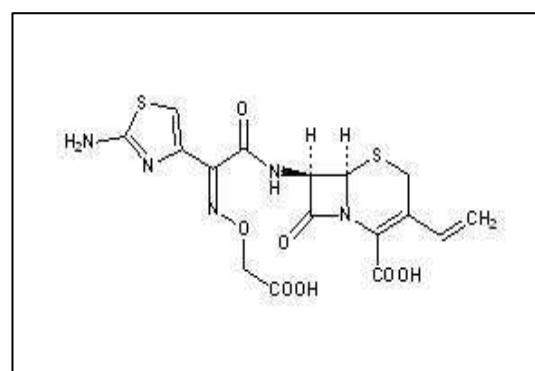


Fig no. 12 chemical structure of Cefixime

In addition to β -lactamase-producing organisms, this well-known oral active third-generation cephalosporin has an outstanding treatment profile against a wide range of *E. coli*, *Klebsiella*, *H. influenzae*, *Branhametlacatarrhalis*, *N. gonorrhoeae*, and meningitidis. Staphylococci are actually resistant to it, but it is effective against several common streptococci species. Although it is always advised for otitis media, respiratory infections, and simple UTIs, its precise therapeutic function is still unclear.

Mechanism of action:

The 'drug' is absorbed gradually and incompletely from the gastrointestinal tract, with a bioavailability of 40-50%. Importantly, the apparent good oral absorption of this medicinal material is attributable to its facilitated and

enhanced transport via the intestinal brush-border membranes, which basically implicate the resulting carrier system for the 'dipeptides'.* The prevailing 'drug' lacks the ionizable α -amino moiety found in 'dipeptides' and ' β -lactams', which were previously known to be carried by the aforementioned carrier system.^{[20],[25]}

Cefotetan

IUPAC NAME:

(6R,7S)-7-[[4-(2-amino-1-carboxy-2-oxoethylidene)-1,3-dithietane-2-carbonyl]amino]-7-methoxy-3-[(1-methyltetrazol-5-yl)sulfanylmethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid

Structure

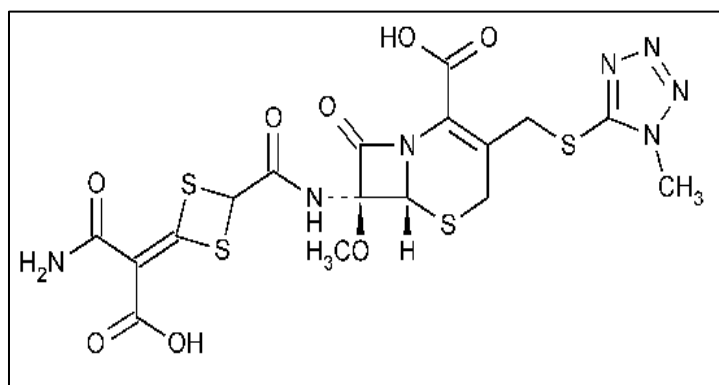


Fig No.13 Chemical structure of Cefotetan

Cefotetan is a semi-synthetic cephamycin antibiotic that is given either intravenously or intramuscularly. It is highly resistant to a wide range of beta-lactamases and is effective against various aerobic and anaerobic bacteria, including both Gram-positive and Gram-negative strains. As a second-generation cephalosporin derived from *Cephalosporium*, cefotetan has broad-spectrum activity and is designed to resist beta-lactamase enzymes.

Mechanism of action :

Cefotetan disodium works as a bactericidal agent by interfering with the synthesis of the bacterial cell wall, particularly by inhibiting the cross-linking of peptidoglycans. This disruption destabilizes the cell wall, leading to cell destruction. When compared to first-generation cephalosporins, cefotetan is more effective against Gram-negative bacteria and less effective against Gram-positive bacteria. This semisynthetic cephamycin antibiotic is typically administered through intravenous or intramuscular routes. Cefotetan is highly resistant to a broad array

of beta-lactamases and is active against a wide range of both aerobic and anaerobic bacteria, covering many Gram-positive and Gram-negative organisms.^[26]

Ceftaroline

IUPAC NAME:

(6R,7R)-7-[[[(2Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-ethoxyiminoacetyl]amino]-3-[[4-(1-methylpyridin-1-ium-4-yl)-1,3-thiazol-2-yl]sulfanyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate

Structure

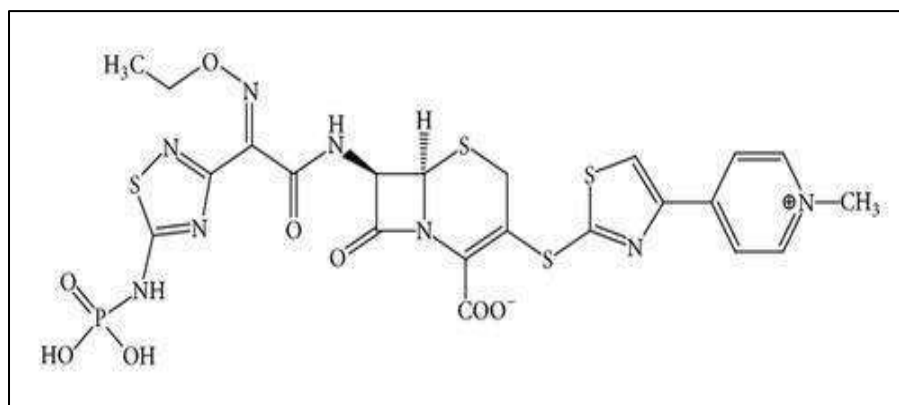


Fig No. 14 Chemical Structure of Ceftaroline

A new cephalosporin, ceftaroline exhibits a good tolerability profile and extensive in vitro activity against a variety of common Gram-negative and resistant Gram-positive pathogens. Adults with acute bacterial skin and soft tissue infections, particularly those brought on by methicillin-resistant *Staphylococcus aureus* (MRSA), can be treated with ceftaroline fosamil, the first cephalosporin authorized by the US Food and Drug Administration (FDA).

Mechanism of action :

Ceftaroline, a fifth-generation cephalosporin, shows in vitro bactericidal activity against MRSA and other bacteria like *Moraxella catarrhalis*, *Haemophilus influenzae*, *S. pneumoniae*, and *S. pyogenes*, commonly causing pediatric infections. It works by inhibiting cell wall formation through binding to penicillin-binding proteins (PBPs). Ceftaroline's unique ability to bind more strongly to mutant PBPs, such as PBP2a in MRSA and altered PBPs in *S. pneumoniae*, enables it to

combat strains resistant to other beta-lactams. It is also effective against penicillin-resistant *S. pneumoniae* and MRSA, but not against carbapenemase or ESBL-producing Gram-negative bacteria.^[27]

Doripenem

IUPAC NAME:

(4R,5S,6S)-6-[(1R)-1-hydroxyethyl]-4-methyl-7-oxo-3-[(3S,5S)-5-[(sulfamoylamino)methyl]pyrrolidin-3-yl]sulfanyl-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

Structure

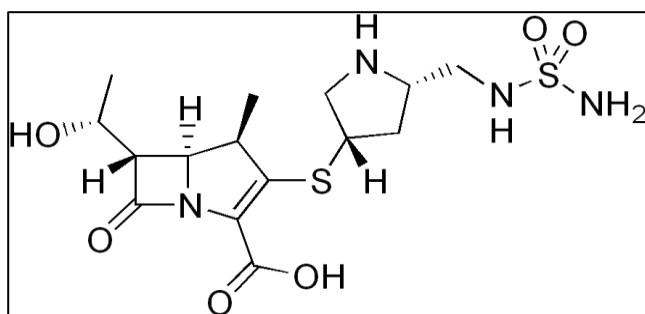


Fig No.15 Chemical structure of Doripenem

Doripenem is a synthetic 1-beta-methylcarbapenem developed by Shionogi & Co. Ltd. It shares a similar structure with meropenem, but features a distinct modification: the dimethylcarbamoyl side chain in meropenem is substituted with a sulfamoylaminomethyl group in doripenem. It was approved by the FDA in 2007 for the treatment of complicated urinary tract and intra-abdominal infections. This antibiotic is effective against a wide variety of Gram-positive and Gram-negative bacteria, encompassing both aerobic and anaerobic organisms.

Mechanism of action :

Doripenem, similar to other beta-lactam antibiotics, disrupts bacterial cell wall formation by binding to and deactivating penicillin-binding proteins (PBPs), which are key enzymes involved in the construction of the cell wall. This disruption compromises the cell wall's integrity, causing it to break apart under osmotic pressure, leading to the eventual death of the bacterial cell.^[28]

Biapenem

IUPAC NAME:

4R,5S,6S)-3-[(6,7-dihydro-5H-pyrazolo[1,2-a]triazol-4-ium-6-ylsulfanyl)-6-[(1R)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate

Structure

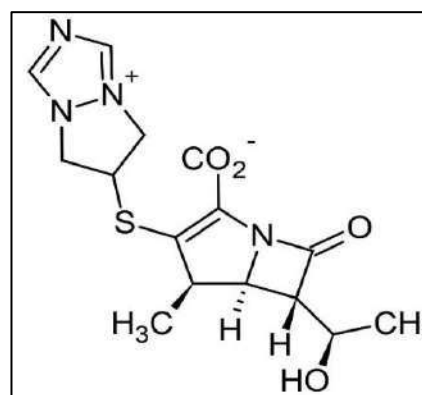


Fig No.16 Chemical structure of Biapenem

Biapenem is a carbapenem antibiotic administered parenterally, showing activity against a wide range of both Gram-positive and Gram-negative bacteria. The introduction of a methyl group at the 1-beta position of its carbapenem structure helps protect it from hydrolysis by renal dehydropeptidase-1 (DHP-1). While biapenem has not been approved for use, its clinical advantages and comparison to other carbapenems, such as imipenem and meropenem, are still unclear.

Mechanism of action :

Biapenem work by targeting bacterial cell walls by binding to and inhibiting transpeptidases, also called penicillin-binding proteins (PBPs), which are enzymes crucial for the final steps in the synthesis of the peptidoglycan layer.^[29]

Ertapenem

IUPAC NAME:

(4R,5S,6S)-3-[(3S,5S)-5-[(3-carboxyphenyl)carbamoyl]pyrrolidin-3-yl]sulfanyl-6-[(1R)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid.

Structure

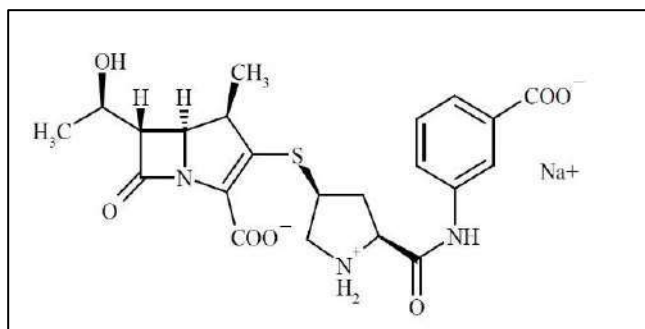


Fig No.17 Chemical structure of Ertapenem

Many gram-positive and gram-negative bacteria, as well as a number of anaerobic organisms, are susceptible to the broad-spectrum antibacterial action of ertapenem. In vitro and in clinical infections, ertapenem is effective against the majority of isolates of the following bacteria. In addition to having a significant post-antibiotic effect on Gram-positive bacteria, it is quickly bactericidal.

Mechanism of action:

Ertapenem works by binding to specific penicillin-binding proteins (PBPs), inhibiting the synthesis of bacterial cell walls. It remains effective against most β -lactamases, including AmpC and extended-spectrum β -lactamases, though it is vulnerable to metallo- β -lactamases. Bacterial resistance to carbapenems can occur through structural alterations in PBPs, the acquisition of metallo- β -lactamases that rapidly degrade carbapenems, or changes in membrane permeability caused by the loss of certain outer membrane porins.

Cefquinome

IUPAC NAME:

(6R,7R)-7-[[[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-methoxyiminoacetyl]amino]-8-oxo-3-(5,6,7,8-tetrahydroquinolin-1-ium-1-ylmethyl)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate

Structure.

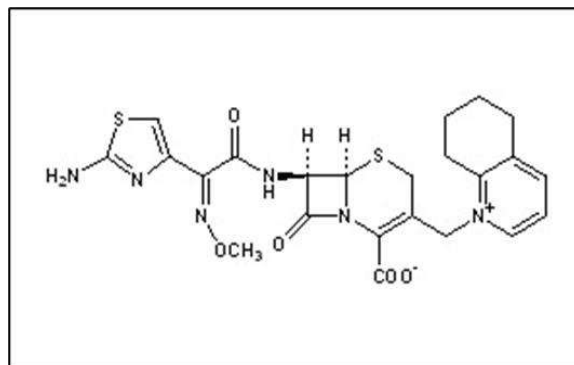


Fig No. 18 Chemical Structure Of Cefquinome

Cefquinome is a novel injectable aminothiazolyl cephalosporin derivative. It is resistant to chromosomal and plasmid-encoded beta-lactamases and has a broad antibacterial range. *Staphylococcus aureus*, streptococci, *Pseudomonas aeruginosa*, and members of the Enterobacteriaceae family (*Escherichia coli*, *Salmonella* spp., *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., and *Serratiamarcescens*) are all suppressed at low doses.

Mechanism of action:

Cefquinome is effective against certain methicillin-resistant staphylococci and enterococci. Its in vitro effectiveness against gram-negative anaerobes is extremely limited. Cefquinome exhibits high in vitro activity and in vivo efficacy against septicemia caused by various gram-positive and gram-negative bacteria.[31]

Analytical Review of Drugs

Method Drug	Method Conditions	Detection	Form of drug	Linearity Ng	LOD	LOQ	Ref

HPTLC, Cephalexin	Mobile phase: Ethyl Acetate : Methanol : Ammonia (6:4:1, v/v/v) Run time – 15 min Plate: TLC plate pre-coated with silica gel 60 F254	Detection wavelength of 260 nm	tablet	0.9991	51.03 ng	154.64 ng	32
HPLC Amoxicillin	Mobile phase: 1:99 Acetonitrile and Phosphate buffer of pH-5 Column -Hypersil ODS, C18 column Flow rate : 1 ml/minute Run time : 20 min	Detection wavelength of 254nm	tablet	0.9919	-	-	33
HPLC Cefadroxil	Mobile phase: Methanol: phosphate buffer (10:90) Column :C18 column (5µm,150mm×4.5mm, water corporation) Run time : 1.5 ml/min	Detection wavelength of 260nm	tablet	0.9935	0.5	1.0	34
HPLC Ampicillin	Mobile phase: 35:36 Acetonitrile and Phosphate buffer Column : Hypersil , C18 Flow rate : 1.0 ml/min	Detection wavelength of 254nm	Capsule	–	0.0165	0.05	35
HPLC Cloxacillin	Mobile phase: 35:36 Acetonitrile and Phosphate Column : Hypersil , C18 Flow rate : 1.0 ml/min	Detection wavelength of 254nm	Capsule	–	0.00341	0.010	35
UV-visible Cefepime	Solvent : water	Detection wavelength of 257.40nm	Injection	20 – 40 µg/ml	0.4609	0.248	36
UV-visible Cefazolin	Solvent : Water; HCl acid 0.1M; NaOH 0.1 M; Methanol; 1% phosphate buffer pH 6 and 1% phosphate buffer pH 8	Detection wavelength of 271nm	Powder	0.9999	0.2229	0.6754	37
HPLC Cefixime	Mobile phase: Sodium dihydrogen phosphate (0.1M aq) pH 2.5 with diluted	Detection wavelength of 254nm	Cefiximetry drate tablet	0.9999	0.61	2.03	38

	orthophosphate : methanol (3:1) Column : C18 waters Flow rate: 1.0 ml/min Run time : 9.25 mins						
HPLC Cefotetan	Mobile phase: KH ₂ PO ₄ : C ₃ H ₆ O in ratio of (40:60% v/v) Column : high strength silica with Octadecylsilane column Flow rate: 1 ml/minute Run time: 6 minutes	Detection wavelength of 243nm	Injection (vials)	0.999	0.01µg/m l	0.05µg/ m	39
HPLC Ceftaroline Fosamil	Mobile phase: Methanol : water : acetonitrile (40:30:30) column : C-18 X- terra column Flow rate: 1.0 ml/min	Detection wavelength of 242nm	Injection	> 0.9998	64 µg/ml	4.85 µg/ml	40
HPTLC Doripenem	Mobile phase: Butyl alcohol : glacial acetic acid : water (4:2:2, v/v/v) Plate : pre-coated silica gel aluminium plates 60 F ₂₅₄	Detection wavelength of 294nm	Injection	1-5 µg/ml	0.031 µg/ml	0.095 µg/ml	41
HPLC Biapenem	Mobile phase: ammonium acetate– acetonitrile (96:4 v/v) Column : RP-18 (250 mm × 4.6 mm, 5 µm) Flow rate: 1ml/min	Detection wavelength of 295 nm	Injection	0.9980	9.60 µg/ml	24.90 µg/ml	42
HPLC Ertapenem	Mobile phase: phosphate buffer 25 mmol/L (pH = 6.5) and methanol (85:15 v/v) Column:LiChrospher RP18 Flow rate: 1.2 mL/min	Detection wavelength of 298 nm	Injection INVANZ	0.012 - 0.1 mg/ml	2.80 × 10 ⁻² mg/ml	8.44 × 10 ⁻³ mg/ml	43
HPLC Cefquinom e	Mobile phase: Acetonitrile : 0.02M phosphate buffer (10:90, v/v) pH 7 Column: LiChroCART 125 mm 0 4 mm (5 µm)	Detection wavelength of 268 nm	Injection	0.2026	–	–	44

Flow Rate: 1.0 mL/min	Run Time: 20 min						
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CONCLUSION:

The development and validation of analytical methods are indispensable in the pharmaceutical field, ensuring the consistent quality, efficacy, and safety of beta-lactam antibiotics. These antibiotics, critical for treating a wide range of infections, are increasingly scrutinized due to the growing threat of antibiotic resistance. By employing robust techniques such as UV-Vis spectroscopy and high-performance liquid chromatography (HPLC), researchers can accurately identify, quantify, and monitor the stability of these compounds, ensuring compliance with stringent regulatory standards. This review emphasizes the vital role of parameters like precision, accuracy, specificity, and robustness in developing reliable analytical methods. Furthermore, advancements in analytical technologies have not only enhanced the sensitivity and efficiency of drug analysis but also contributed to addressing global health challenges associated with resistant bacterial strains. The continued evolution of analytical methods will play a pivotal role in enabling pharmaceutical innovation, particularly in the discovery and optimization of new antibiotic formulations. By integrating advanced techniques with regulatory frameworks, the pharmaceutical industry can effectively combat emerging resistance trends and uphold the therapeutic relevance of beta-lactam antibiotics in modern medicine.

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