INTRODUCTION:

Antibiotic is the term used to describe any antibacterial agent derived from microorganisms, though most of them are now prepared synthetically. Such agents destroy or inhibit the growth of other microorganisms. Examples are penicillin, cephalosporin, aminoglycosides, streptomycin, and tetracycline. The first member of the newer series of beta-lactams was isolated in 1956 from extracts of Cephalosporium acremonium, a sewer fungus. Like penicillin, cephalosporins are valuable because of their low toxicity and their broad spectrum of action against various diseases. In this way, cephalosporin is very similar to penicillin. Cephalosporins are one of the most widely used antibiotics, and, have about 29% of the antibiotic market. The cephalosporins are possibly the single most important group of antibiotics today and are equal in importance to penicillin.

The structure and mode of action of the cephalosporins are similar to that of penicillin. They affect bacterial growth by inhibiting cell wall synthesis, in Gram-positive and negative bacteria.

1.1 Chemistry:

The cephalosporins are derived from the compound Cephalosporin C which is obtained by fermentation of the mould Cephalosporium. Cephalosporins C contains a side chain derived from D-α-aminoadipic acid, which is condensed with a dihydrothiazin 3-lactum ring system (7-aminocephalosporinic acid). Compounds containing 7-aminocephalosporinic acid are relatively stable in dilute acid and highly resistant to penicillinase, regardless of the nature of their side chains and their affinity for the enzyme.

Cephalosporins C can be hydrolyzed by acid to 7-aminocephalosporinic acids. This compound subsequently has been modified by the addition of different side chains to create a whole family of cephalosporins antibiotics. It appears that modification at position 7 of the p-lactum ring are associated with alternation in antibacterial activity and that substitutions at position 3 of the dihydrothiazine ring are associated with changes in the metabolism and the pharmacokinetic properties of the drugs. The cephalosporin ring structure is derived from 7-aminocephalosporanic acid (7-ACA) while the penicillin are derived from 6-aminopenicillanic acid (6-APA). Both structures contain the basic beta-lactam ring but the cephalosporin structure
allows for more gram negative activity than the penicillins and aminocillins. Substitution at the "R" sites (different side chains) allows for variation in the spectrum of activity and duration of action.\cite{5}

The first semi-synthetic cephalosporin, cephalothin, appeared in 1962; it was followed by cephaloridine in 1964. The original cephalosporins had to be given by injection but more recent preparations can be given by mouth. The newer preparations are less readily destroyed by beta lactamases and so they have a much broader spectrum of anti-bacterial activity. The newer cephalosporins include cephalexin, cefazolin, cephacetrile, cephamiprin, cefamandole, cefuroxine, cephradine, cefodroxil and cefotaxime. Inactivation of beta lactamase is the basis of bacterial resistance to both the penicillins and the cephalosporin so that attempts to prepare these antibiotics with resistance to beta lactamase is very important. A synthetic inhibitor of beta lactamase called clavulanic acid has recently been synthesized. This is used in combination with the penicillins and cephalosporins to prevent resistance. The cephamycins are a new addition to the beta lactam antibiotics. They are similar in structure to the cephalosporins but are produced, not by fungi, but by actinomycetes.

1.2 Classification:

Some fifty different cephalosporins are in clinical use or at an advanced stage of development\cite{6,7,8,9} and many attempts have been made to classify these based upon stability to \(\beta\)-lactamases, potency, antibacterial spectrum and pharmacological properties. The most common approach has been to divide the group into various "generations" based primarily on their antibacterial spectrum, as first generation while later, more extended spectrum cephalosporins were classified as second-generation cephalosporins. Currently, three generations of cephalosporins are recognized and a fourth has been proposed. Significantly, each newer generation of cephalosporins has greater gram-negative antimicrobial properties than the preceding generation. Conversely, the "older" generations of cephalosporins have greater Gram-positive (staphylococcus and streptococcus) coverage than the "newer" generations.\cite{10,11}
**First generation**

First generation cephalosporins are moderate spectrum agents. They are effective alternatives for treating staphylococcal and streptococcal infections and therefore are alternatives for skin and soft-tissue infections, as well as for streptococcal pharyngitis.

The first generation cephalosporins are:

- Cefadroxil
- Cephalexin
- Cephaloridine
- Cephalothin
- Cephapirin
- Cefazolin
- Cephradine

Cefazolin is the most commonly used first generation cephalosporin. The other first generation cephalosporins have similar efficacy to Cephalexin, but must be dosed more often, and are therefore not as commonly prescribed.

**Second generation**

The second generation cephalosporins have a greater gram-negative spectrum while retaining some activity against gram-positive bacteria. They are also more resistant to beta-lactamase. They are useful agents for treating upper and lower respiratory tract infections, sinusitis and otitis media. These agents are also active against E. coli, Klebsiella and Proteus, which makes them potential alternatives for treating urinary tract infections caused by these organisms. Cefoxitin is a second generation cephalosporin with anaerobic activity, and although seldom used as a therapeutic agent, it may be useful for prophylaxis in gastrointestinal surgery.

The second generation cephalosporins are:

- Cefaclor
- Cefoxitin
- Cefprozil
- Cefuroxime

**Third generation**

Third generation cephalosporins have a broad spectrum of activity and further increased activity against gram-negative organisms. Some members of this group (particularly those available in an oral formulation) have decreased activity against gram-positive organisms.
The parenteral third generation cephalosporins (ceftriaxone and cefotaxime) have excellent activity against most strains of Streptococcus pneumoniae, including the vast majority of those with intermediate and high level resistance to penicillin. These agents also have activity against N. gonorrhoeae. Ceftazidime has useful antipseudomonal activity.

The third generation cephalosporins are:

- Cefdinir
- Cefixime
- Cefpodoxime
- Ceftibuten
- Ceftriaxone
- Cefotaxime

**Fourth generation**

Fourth generation cephalosporins are extended spectrum agents with similar activity against gram-positive organisms as first generation cephalosporins. They also have a greater resistance to beta-lactamases than the third generation cephalosporins. Many can cross blood brain barrier and are effective in meningitis.

Cefepime has broad gram-negative coverage with somewhat enhanced activity against pseudomonas but slightly lesser activity against pneumococci. Cefpirome is more active against pneumococci and has somewhat lesser activity against pseudomonas. Cefepime and cefpirome are highly active against nosocomial pathogens such as Enterobacter and Acinetobacter and their use should therefore be restricted to the setting of nosocomial sepsis (Tumah H et al., 2005)

The fourth generation cephalosporins are:

- Cefepime
- Cefpirome
- Cefluprenam
- Cefquinome
- Cefozopran

**1.3 Need for analytical method**

The number of drugs introduced into the market is increasing every year. These drugs may be either new entities or partial structural modification of the existing one. Very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, reports of new toxicities (resulting in their withdrawal from the market),
development of patient resistance and introduction of better drugs by competitors. Under these conditions, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore to develop newer analytical methods for such drugs.

In brief, the reasons for the development of newer methods of drug analysis are:

- A proper analytical procedure for the drug may not be available in the literature due to patent regulations,
- Analytical methods for the quantitation of the drug in biological fluids may not be available,
- The drug or drug combination may not be official in any pharmacopoeias,
- Analytical methods may not be available for the drug in the form of a formulation due to the interference caused by the formulation excipients,
- Analytical methods for a drug in combination with other drugs may not be available,
- The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable.

Analytical techniques that are generally used for drug analysis are biological and microbiological methods, radioactive methods, physical methods and miscellaneous techniques like conventional titrimetric, gravimetric and polarimetric methods.