2.1 LITERATURE REVIEW

2.1.1 Antibiotics

Antibiotics are categorized as narrow, broad, or extended-spectrum agents. Narrow-spectrum agents (e.g., penicillin G) affect primarily gram-positive bacteria. Broad-spectrum antibiotics, such as tetracyclines and chloramphenicol, affect both gram-positive and some gram-negative bacteria. An extended-spectrum antibiotic is one that, as a result of chemical modification, affects additional types of bacteria, usually gram-negative bacteria.

Many treatments for infections prior to the beginning of the twentieth century were based on medicinal folklore. Treatments for infection in ancient Chinese medicine using plants with antimicrobial properties were described over 2,500 years ago [1-5]. Many other ancient cultures, including the ancient Egyptians and ancient Greeks used molds and plants to treat infections [6,7]. The discovery of the natural antibiotics produced by microorganisms stemmed from earlier work on the observation of antibiosis between micro-organisms. Louis Pasteur observed that, "if we could intervene in the antagonism observed between some bacteria, it would offer 'perhaps the greatest hopes for therapeutics'" [8]. Synthetic antibiotic chemotherapy as a science and the story of antibiotic development began in Germany with Paul Ehrlich, a German medical scientist in the late 1880s [9]. Scientific endeavours to understand the science behind what caused these diseases, the development of synthetic antibiotic chemotherapy, the isolation of the natural antibiotics marked milestones in antibiotic development [10].

Originally known as antibiosis, antibiotics were drugs which acted against bacteria. The term antibiosis, which means "against life," was introduced by the French bacteriologist Vuillemin as a descriptive name of the phenomenon exhibited by these drugs [9]. (Antibiosis was first
described in 1877 in bacteria when Louis Pasteur and Robert Koch observed that an airborne bacillus could inhibit the growth of Bacillus anthracis \cite{11}. These drugs were later renamed antibiotics by Selman Waksman, an American microbiologist in 1942 \cite{2,9}.

Bacterial antagonism of Penicillium spp. was first described in England by John Tyndall in 1875 \cite{8}. The significance to antibiotic discovery was not realized until the work of Ehrlich on synthetic antibiotic chemotherapy, which marked the birth of the antibiotic revolution. Ehrlich noted that certain dyes would bind to and color human, animal, or bacterial cells, while others did not. He then extended the idea that it might be possible to make certain dyes or chemicals that would act as a magic bullet or selective drug that would bind to and kill bacteria while not harming the human host. After much experimentation, screening hundreds of dyes against various organisms, he discovered a medicinally useful drug, the man-made antibiotic, Salvarsan \cite{12,13}. In 1928 Fleming made an important observation concerning the antibiosis by penicillin. Fleming postulated that the effect was mediated by a yet-unidentified antibiotic-like compound that could be exploited. Although he initially characterized some of its antibiotic properties, he did not pursue its development \cite{14,15}. In the meantime, another synthetic antibacterial antibiotic Prontosil was developed and manufactured for commercial use by Domagk in 1932. Prontosil, the first commercially available antibacterial antibiotic, was developed by a research team led by Gerhard Domagk (who received the \textbf{1939} Nobel Prize for Medicine for his efforts) at the Bayer Laboratories of the IG Farben conglomerate in Germany. Prontosil had a relatively broad effect against Gram-positive cocci but not against enterobacteria. The discovery and development of this first sulfonamide drug opened the era of antibiotics. In 1939, discovery by Rene Dubos of the first naturally derived antibiotic-like substance named gramicidin from \textit{B. brevis}. It was one of the first commercially manufactured antibiotics in use during World War II to prove highly effective in treating
wounds and ulcers. Florey and Chain succeeded in purifying penicillin. The purified antibiotic displayed antibacterial activity against a wide range of bacteria. It also had low toxicity and could be taken without causing adverse effects. Furthermore, its activity was not inhibited by biological constituents such as pus, unlike the synthetic antibiotic class available at the time, the sulfonamides. The discovery of such a powerful antibiotic was unprecedented. The development of penicillin led to renewed interest in the search for antibiotic compounds with similar capabilities. Because of their discovery of penicillin Ernst Chain, Howard Florey and Alexander Fleming shared the 1945 Nobel Prize in Medicine. Florey credited Dubos with pioneering the approach of deliberately, systematically searching for antibacterial compounds. Such a methodology had led to the discovery of gramicidin, which revived Florey's research in penicillin.

**Facts about the Antibiotic R&D Pipeline: Why Antibiotics Require Special Treatment**

There are several challenges that are unique to antibiotics: 1) Because antibiotics work so well and so fast, in most cases they simply do not have as large a market as drugs that treat chronic, long-term conditions or lifestyle issues. 2) The development of resistant strains of bacteria limits the long-term market potential for an antibiotic. 3) Infectious disease experts often suggest restrictions on the use of new antibiotics in order to preserve the effectiveness of these drugs for those patients who need them most. Although sensible from a public health perspective, such restrictions reduce the incentive for companies to develop new antibiotics. 4) Drugs to treat infectious diseases require an additional burden of proof in the drug approval process. Because antibiotics are used to treat various types of infection, the drug approval process requires clinical trials of each of these indications, enrolment of large numbers of patients to ensure safety and efficacy, and enrolment of large patient numbers to document the drug’s effectiveness against specific bacterial pathogens. Finding patients to enroll in clinical
trials can be difficult because, for many resistant pathogens, there are no rapid diagnostic tests to identify patients who would be eligible for study.

- Drug R&D is expensive, risky, and time-consuming. An aggressive R&D program initiated today would likely require 10 or more years and an investment of $800 million to $1.7 billion to bring a new drug to market.

- A recent analysis published in *Clinical Infectious Diseases (CID)* found only five new antibiotics in the R&D pipeline out of more than 506 drugs in development. By comparison, pharmaceutical companies were developing 67 new drugs for cancer, 33 for inflammation/pain, 34 for metabolic/endocrine disorders, and 32 for pulmonary disease.

- The analysis found that Food and Drug Administration (FDA) approvals of new antibiotics declined 56 percent during the past 20 years (1998-2002 versus 1983-1987).

- Since 1998, only 10 new antibiotics have been approved by FDA, two of which are truly novel (i.e., have a new target of action, with no cross-resistance with other antibiotics).

- In 2002, among 89 new medicines emerging on the market, none was an antibiotic.

- A growing number of companies with track records in antibiotic R&D appear to be withdrawing from this market: Aventis, Abbott Laboratories, Bristol-Myers Squibb, Eli Lilly and Co., Procter & Gamble, Roche and Wyeth.
2.1.2. Cephalosporins

Cephalosporin compounds were first isolated from cultures of Cephalosporium acremonium from a sewer in Sardinia in 1948 by Italian scientist Giuseppe Brotzu[2]. He noticed that these cultures produced substances that were effective against Salmonella typhi, the cause of typhoid fever, which had beta-lactamase. Guy Newton and Edward Abraham at the Sir William Dunn School of Pathology at the University of Oxford isolated cephalosporin C. The cephalosporin nucleus, 7-aminopenicillosporanic acid (7-ACA), was derived from cephalosporin C and proved to be analogous to the penicillin nucleus 6-aminopenicillanic acid, but it was not sufficiently potent for clinical use. Modification of the 7-ACA side-chains resulted in the development of useful antibiotic agents and the first agent cephalothin (cefalotin) was launched by Eli Lilly and Company in 1964.

Cephalosporins are beta-lactam compounds in which the beta-lactam ring is fused to a 6-membered dihydrothiazine ring, thus forming the cepham nucleus. Side chain modifications to the cepham nucleus confers 1) an improved spectrum of antibacterial activity 2) pharmacokinetic advantages and 3) additional side effects. Based on their spectrum of activity, cephalosporins can be broadly categorized into four generations.

FIRST GENERATION CEPHALOSPORINS

**Spectrum of activity:** Gram-positive aerobic cocci: Very active against Streptococci pyogenes (Group A strep), Streptococcus agalactiae (Group B strep), viridans streptococci. Methicillin-resistant Staphylococci, Enterococci, penicillin-resistant Streptococcus pneumoniae are resistant.
**Review of Literature**

**Gram-negative aerobes:** Commonly active against *Escherichia coli*, *Proteus mirabilis* and *Klebsiella pneumoniae*, though susceptibilities may vary. Inadequate activity against *Moraxella catarrhalis* and *Hemophilus influenzae*.

**Anaerobes:** Active against most penicillin-susceptible anaerobes found in the oral cavity, except those belonging to the *Bacteroides fragilis* group.

**General Clinical Uses.** Uncomplicated, community-acquired infections of the skin and soft tissue and urinary tract. Useful for respiratory tract infections caused by penicillin-sensitive *Streptococcus pneumoniae* but not for *Hemophilus influenzae* and *Moraxella catarrhalis*. While effective for these infections, other less expensive alternatives should be used when appropriate because of their efficacy and narrower spectrum of activity (eg: penicillins, trimethoprim/sulfamethoxazole). Parenteral 1st generation agents are used for surgical wound prophylaxis.

**Specific Agents:**

A. **Cefazolin** (*Anacef*, *Kefzol*, *Cephalothin* (*Keflin*, *Vantage*, *Cephpirin* (*Cefadyl*)) IV/IM formulations. Spectrum of cephalothin and cefazolin are similar except that cefazolin is slightly more active against *Escherichia coli* and *Klebsiella* species. The longer half-life of cefazolin allows less frequent dosing.

B. **Cephalexin** (*Keflex*, *Keftab*, *Biocef*), **Cephradine** (*Anspor*, *Velosef*), **Cefadroxil** (*Duricef*, *Ultracef*). Less frequent dosing with cefadroxil.

**SECOND GENERATION CEPHALOSPORINS**

There are 2 groups within the 2nd generation agents that differ in their: 1) spectrum of activity and 2) adverse reaction profile. These groups are the "true" second generation
cephalosporins (cefamandole, cefuroxime) and the cephamycins (cefoxitin, cefotetan, cefmetazole).

**Spectrum of activity.** Gram-positive aerobic cocci: In general, true 2nd generation agents are comparable to 1st generation agents against nonenterococcal streptococci; are less active in-vitro, but still have adequate activity against MSSA. Compared to the 1st generation agents, the cephamycins are less active against gram-positive cocci. Both groups of cephalosporins are inactive against methicillin-resistant Staphylococci and Enterococci.

**Gram-negative aerobes.** The "true" cephalosporins are more active for Hemophilus influenzae, Moraxella catarrhalis, Neisseria meningitidis, and some Enterobacteriaceae. The cephamycins in some instances (eg: cefotetan) have improved activity against Enterobacteriaceae.

**Anaerobes:** Cephamycins are active against most anaerobes found in the mouth as well as colon (eg: Bacteroides species, including Bacteroides fragilis).

**General Clinical Uses.** The "true" 2nd generation agents are useful for community-acquired infections of the respiratory tract (Hemophilus influenzae, Moraxella catarrhalis, Streptococcus pneumonia) and uncomplicated urinary tract infections (Escherichia coli). The cephamycin group is useful for mixed aerobic/anaerobic infections of the skin and soft tissues, intra-abdominal, and gynecologic infections, and surgical prophylaxis.

**Side Effects/Precautions.** The cephamycin agents have a side chain called the methylthiotetrazole (MTT) group which predisposes patients to: 1) Hypoprothrombinemia and bleeding by disturbing synthesis of vitamin-k dependent clotting factors. Risk factors are renal or hepatic disease, poor nutrition, the elderly, and cancer. 2) Alcohol intolerance by causing a disulfiram-like reaction, avoid alcohol products for several days after antibiotics have stopped.
Specific agents:

A. **Cefamandole (Mandol)**. IV/IM formulations. Better activity against selected methicillin-susceptible Staphylococcus aureus than cefazolin. May not be reliable therapy for Hemophilus influenzae. Although not a cephamycin, it contains an NMTT side chain.

B. **Cefuroxime (Zinacef, Kefurox)**. IV/IM/PO formulations. Somewhat less potent against Staphylococcus aureus, but more potent against Streptococcus pneumoniae and Streptococcus pyogenes than 1st generation cephalosporins. Active against Hemophilus influenzae, Moraxella catarrhalis, Escherichia coli, Proteus mirabilis, Klebsiella species. Although cefuroxime has been used for the treatment of bacterial meningitis caused by H. influenzae, it is not recommended because studies show neurologic deficits are more frequent in children treated with cefuroxime versus selected 3rd generation cephalosporins (cefotaxime, ceftriaxone). This finding is related to delayed sterilization of cerebral spinal fluid.

C. **Cefonicid (Monocid)**. IV/IM formulation. Similar to cefamandole and cefuroxime, though less active against gram-positive cocci (methicillin-susceptible Staphylococcus aureus, Group A strep, Streptococcus pneumoniae). Long half-life allows once daily dosing.

D. **Cefoxitin (Mefoxin)**. IV/IM formulations. A cephamycin, which is less active than 1st generation agents against gram-positive bacteria. Active against Neisseria gonorrhea, but less active than "true" second generation cephalosporins against Hemophilus influenzae.

E. **Cefotetan (Cefotan)**. IV/IM formulations. A cephamycin with similar activity to cefoxitin. Compared to second generation cephalosporins and cefoxitin, has improved activity against Enterobacteriaceae including Enterobacter. Also active against Hemophilus influenzae, Neisseria gonorrhea, Neisseria meningitidis. Generally 2-4 fold less active than cefoxitin against gram-positive cocci. For Bacteroides fragilis, is comparable to cefoxitin; but
less active than cefoxitin against non-Bacteroides fragilis species within the Bacteroides fragilis group; the clinical significance of which is unknown. Used in surgical wound prophylaxis when activity against Bacteroides fragilis is needed.

F. **Cefmetazole (ZefazoneR).** IV/IM formulations. A cephemycin, similar to cefoxitin and cefotetan. Similar to cefoxitin and more active than cefotetan against methicillin-susceptible Staphylococcus aureus. 2-4 fold more active than cefoxitin against Enterobacteriaceae (eg: Escherichia coli, Klebsiella sp, Proteus mirabilis). Also active against Hemophilus influenzae and Moraxella catarrhalis. Bacteroides fragilis is similar to cefoxitin, against other Bacteroides species, is similar or slightly less active than cefoxitin. Used in surgical wound prophylaxis when activity against Bacteroides fragilis is needed, repeat dose would be necessary in procedures lasting more than 4 hours.

G. **Cefaclor (Ceclor), Cefprozil (Cefzil), Loracarbef(Lorabid), Cefpodoxime proxetil (Vantin).** PO formulations. Cefaclor is more commonly associated with a serum sickness like illness. Loracarbef is a new category of compounds called the carbacephems, which are analogues of cephalosporins. Loracarbef is the carbacephem analogue of cefaclor.

H. **Cefuroxime axetil (CeftinR).** PO formulation of cefuroxime. Is the oral ester of cefuroxime that is hydrolyzed to cefuroxime during absorption.

**THIRD GENERATION cephalosporins**

Improved activity against Enterobacteriaceae associated with hospital-acquired infections; some agents are also active against Pseudomonas aeruginosa which is a frequent cause of hospital-acquired pneumonia.
**Spectrum of activity.** Gram-positive aerobic cocci: Cefotaxime, ceftriaxone, and ceftizoxime are active against methicillin-susceptible Staphylococcus aureus (though less than 1st and some 2nd generation agents), very active against Groups A and B streptococci and viridans streptococci. Cefotaxime and ceftriaxone are more active than ceftizoxime against Streptococcus pneumonia, particularly intermediately-penicillin resistant Streptococcus pneumoniae. None are active against methicillin-resistant Staphylococci, Enterococci and Listeria monocytogenes.

**Gram-negative aerobes:** Very active against Hemophilus influenzae, Moraxella catarrhalis, Neisseria meningitidis and Enterobacteriaceae (eg: Escherichia coli, Klebsiella species, Proteus mirabilis, Providencia) found in hospital and community-acquired infections. Some Enterobacter species have a tendency to become resistant during cephalosporin therapy, and thus cephalosporins are not the drugs of choice for Enterobacter infections. Only and ceftazidime and cefoperazone are active against Pseudomonas aeruginosa, and ceftazidime is preferred because it is more potent than cefoperazone against gram-negative bacteria.

**Anaerobes:** Cefotaxime, ceftriaxone and ceftizoxime are adequate for oral anaerobes.

**General clinical uses.** For infections involving gram-negative bacteria, particularly hospital-acquired infections or complicated community-acquired infections of the respiratory tract, blood, intra-abdominal, skin and soft tissue and urinary tract. Because of their activity includes the aerobic gram negative bacteria covered by aminoglycosides, they may be an alternative to aminoglycosides in some patients with renal dysfunction.

The clinical situations requiring use of 3rd generation cephalosporins are likely to be encountered in patients who are hospitalized, have recently received antibiotics or are immune compromised.
Specific agents:

A. Cefotaxime (Claforan), Ceftriaxone (Rocephin), Ceftizoxime (cefizox). IV/IM formulations. Activity against Enterobacteriaceae (eg: Escherchia coli, Klebsiella pneumoniae) are similar. None are active against Pseudomonas aeruginosa. Only cefotaxime and ceftriaxone achieve adequate drug levels in the cerebral spinal fluid to constitute reliable empiric therapy for bacterial meningitis. Ceftriaxone is eliminated to a significant degree by the biliary system and as a result, biliary pseudo-lithiasis has been reported as a side effect of this agent.

B. Ceftazidime (Fortaz, Tazidine, Tazicef), Cefoperazone (Cefobid). IV/IM formulations. Spectrum includes Pseudomonas aeruginosa (against which ceftazidime is more active) and Enterobacteriaceae covered by the 3rd generation agents in item A above. Disadvantages of cefoperazone are: 1) the least active 3rd generation agent against Enterobacteriaceae and 2) contains MTT side chain.

C. Cefixime (Suprax), Ceftibuten (Cedax). PO formulations administered once or twice daily. Inactive against methicillin-susceptible Staphylococcus aureus, thus not good choices for skin and soft tissue infections. Generally very active against gram-negative bacteria causing community-acquired infections (Hemophilus influenzae, Moraxella catarrhalis). Cefixime is effective as a single dose therapy for uncomplicated Neisseria gonorrhea infection. While used in otitis media, cefixime may not routinely eradicate Streptococcus pneumoniae.

FOURTH GENERATION CEPHALOSPORIN

Has the excellent activity against Enterobacteriaceae and Pseudomonas aeruginosa which is similar to ceftazidime. In addition, it also has better gram-positive activity than ceftazidime.
**Spectrum of activity.** Gram-positive aerobic cocci: Active against Streptococcus pneumoniae, and Groups A and B streptococci. Though active against methicillin-susceptible Staphylococcus aureus, it is less potent than the 1st and 2nd generation agents.

**Gram-negative aerobes:** Similar to ceftazidime.

**Anaerobes:** Not active against Bacteroides fragilis.

**General Clinical Uses:** Similar to 3rd generation agents.

**Specific agent:**

A. *Cefepime (MaxipimeR)*. IV/IM formulations.

2.1.3 **Literatures about the cefotaxime sodium and ceftriaxone sodium:**

Gupta used HPLC to investigate the stability of cefotaxime sodium in 0.9% sodium chloride injection when packaged in polypropylene syringes and stored at 5 and 25 ºC. The liquid chromatographic system consisted of a Waters model M45 pump, a model 484 multiple-wavelength detector, a Rheodyne model 7125 injector, and an Omniscribe recorder. The stationary phase was a Supelco C8 column (150 × 4.6 mm, 5-µm particle size). The mobile phase consisted of 9.5% (vol/vol) acetonitrile in water containing 0.02 M ammonium acetate buffer (pH 6.9). The flow rate was 1.3 mL/min. UV detection was performed at 290 nm and 0.7 AUFS. Samples were diluted with water. The injection volume was 80 µL. Under these conditions, the retention time for cefotaxime was about 4.7 minutes. The method was demonstrated to be stability indicating by thermal degradation of cefotaxime. A cefotaxime sodium solution was boiled on a hot plate for 40 seconds. The chromatogram showed that the intact cefotaxime was well resolved from its degradation product peaks.[21]

Belliveau et al. evaluated the stability of cefotaxime sodium and metronidazole in 0.9% sodium chloride injection or in ready-to-use metronidazole bags by HPLC. The HPLC system
consisted of a Waters model 510 pump, a Waters model 710A WISP autosampler, a Waters model M440 UV detector, and a Hewlett-Packard model 3396 series II integrator. The stationary phase was a Waters Resolve C$_{18}$ column (150 × 3.9 mm, 5-µm particle size). The mobile phase consisted of an acetate buffer and acetonitrile (86:16, vol/vol). The buffer contained 2.46 g of anhydrous sodium acetate, 8 mL of acetic acid, and 0.2 g of tetrabutylammonium hydrogen sulfate per 1000 mL with the pH adjusted to 3.0. The flow rate was 1.2 mL/min. UV detection was performed at 254 nm. Cefoxitin was used as the internal standard. Samples were diluted 1:1 with internal standard and then further diluted with water. The injection volume was 20 µL. Under these conditions, retention times for metronidazole, cefotaxime, and cefoxitin were 1.8, 2.3, and 3.0 minutes, respectively. The assay was determined to be stability indicating. A solution of cefotaxime, cefoxitin, and metronidazole was heated at 80 °C for 26 hours. This heating caused complete decomposition of cefotaxime; its decomposition products eluted before 1.8 minutes. Standard curves for cefotaxime were constructed from 4 to 12 µg/mL and were linear with a correlation coefficient of 0.9971. The interday coefficient of variation was 1.45% [22].

Rivers et al., examined the stability of cefotaxime sodium and metronidazole in an intravenous admixture. An HPLC system consisted of a Waters 510 pump, a Waters model 712 refrigerated autosampler, and a Waters model 481 variable-wavelength UV detector. The analytical column was a Waters µBondapak column (150 × 3.9 mm, 4-µm particle size). The mobile phase was 7% acetonitrile and 93% 0.02 M potassium dihydrogen phosphate with 0.005 M triethylamine adjusted to pH 4.8 with sodium hydroxide. The flow rate was 1.5 mL/min. UV detection was performed at 270 nm. The injection volume was 20 µL. The run time was 13.5 minutes. Cefotaxime retention time was 8.44 minutes. The analytical method was determined to be stability indicating by intentional degradation of cefotaxime. Calibrator
solutions containing both cefotaxime sodium and metronidazole were mixed with 0.1 M sodium hydroxide or 0.05 M sulfuric acid and heated under reflux conditions for 1 hour. Subsequent chromatograms showed there was no interference with the intact cefotaxime peak by degradation product peaks.\textsuperscript{[23]}

Paap and Nahata used an HPLC method to study the stability of cefotaxime in aqueous solutions at various pH values, temperatures, and ionic strengths as well as in commonly used intravenous admixtures. The HPLC system consisted of a Beckman model 110A pump, an Isco variable-wavelength UV detector, an Anspec D-2000 chromato-integrator, a Waters model 712 WISP autosampler, and a Waters µBondapak 10-µm C\textsubscript{18} analytical column. The mobile phase consisted of 20% acetonitrile and 80% 0.01 M acetate buffer containing 0.007 M tetrabutylammonium hydrogen sulfate. The pH of the mobile phase was adjusted to 5.2 with sodium hydroxide. The flow rate was 2.0 mL/min. UV detection was performed at 254 nm. Under these conditions, the retention times for cefotaxime and the internal standard (cefoxitin) were about 4.5 and 7.5 minutes, respectively. The analytical method was determined to be stability indicating. The accelerated degradation with acid and with heat produced a major metabolite, desacetylcefotaxime, and unknown degradation product peaks that did not interfere with the intact cefotaxime peak. Standard curves were determined daily from 500 to 1500 mg/L. Intraday and interday variations (\(n=6\)) were 2.8 and 5.5%, respectively.\textsuperscript{[24]}

Gupta investigated the stability of cefotaxime sodium at various pH values and in intravenous admixtures by using a stability-indicating HPLC method. A Waters model ALC 202 liquid chromatograph was equipped with a U6K universal injector, a Spectroflow SF 770 multiple-wavelength detector, an Omniscribe recorder, and a Spectra-Physics Autolab minigrator-integrator. The stationary phase was a Waters µBondapak phenyl column (300 \(\times\) 4 mm). The mobile phase contained 0.02 M ammonium acetate and 18% (vol/vol) of methanol in water.
The flow rate was 2.5 mL/min. UV detection was performed at 254 nm and 0.1 AUFS. Samples were diluted with water. The injection volume was 20 µL. The retention time for cefotaxime was about 4.3 minutes. The HPLC method was stability indicating. Degradation products in a solution of pH 1.5 did not interfere with the intact cefotaxime peak [25].

Eric-Jovanovic et al. developed a HPTLC method for the determination of ceftriaxone, cefixime and cefotaxime, cephalosporins widely used in clinical practice. High performance TLC of cephalosporins was performed on pre-coated silica gel HPTLC plates with concentrating zone (2.5×10 cm) by development in mobile phase ethyl acetate-acetone-methanol-water (5:2.5:2.5:1.5 v/v/v/v). A TLC scanner set at 270 nm was used for direct evaluation of the chromatograms in reflectance/absorbance mode. The calibration curves were established as dependence of peak height (linear and polynomial regression) and peak area (polynomial regression) versus ng level (125–500 ng for all cephalosporins investigated). Relative standard deviations obtained from calibration curves was compared. Precision (RSD: 1.12–2.91% (peak height versus ng) and RSD: 1.05–2.75% (peak area versus ng)) and detection limits (ng level) was validated and found to be satisfactory. The method was found to be reproducible and convenient for quantitative analysis of ceftriaxone, cefixime and cefotaxime in their raw materials and their dosage forms [26].

François Jehl et al worked on high-performance liquid chromatographic procedure for the measurement of fifteen β-lactam antibiotics in body fluids is described, with special reference to high-speed techniques. The procedure involves a unique sample preparation before analysis for all the following fifteen compounds: benzyl-penicillin, ampicillin, cloxacillin, ticarcillin, mezlocillin, azlocillin, piperacillin, cefotaxine and its desacetyl metabolite, cefsulofin, cefoperazone, cefmenoxime, ceftazidime, ceftriaxone and the monobactam aztreonam; thus all
biological samples arriving at the laboratory can be treated in batch. Of these fifteen antibiotics, eleven can be chromatographed with the same type of mobile phase, which consists of a mixture of ammonium acetate and acetonitrile in various ratios. Three others need ion-pairing chromatography because of their polarity and ticarcillin requires citric acid. High-speed high-performance liquid chromatography seems to be particularly suitable for the routine analysis of β-lactam antibiotics because columns equilibrate more rapidly, retention times are much shorter, detection limits are lower and the longer lifetime of columns reduces analysis costs\textsuperscript{[27]}. 

Chan et al., developed a simple isocratic HPLC method for the rapid analysis of cephalosporins in body fluids. Sample preparation by protein precipitation takes only five minutes; HPLC analysis is completed within two to ten minutes, using one of two simple solvent mixtures eluted on a single C18 reversed phase column. Nine cephalosporins and nine types of body fluid were formally analysed, but the system was also found to be suitable for the assay of benzyl penicillin, ampicillin, cloxacillin, carbenicillin and chloramphenicol. It is likely that this method, with only minor modifications, would be suitable for the analysis of most β-lactam antibiotics in most clinical specimens. The method is therefore particularly recommended for use in clinical laboratories\textsuperscript{[28]}. 

Mohammed E. Abdel-Hamid et al., performed accurate and precise spectrophotometric full spectrum quantitation (FSQ) and high-performance liquid chromatography (HPLC) procedures for the quantitation of some selected cephalosporins, namely, cefotaxime, ceftazidime and ceftriaxone in the presence of their alkali-induced degradation products and in commercial injections have been described. The degradation products or formulation excipients did not interfere in the analysis. The spectrophotometric method was based on the
use of FSQ software of multi component analysis for simultaneous determination of the examined antibiotics in the presence of their alkali-induced degradation products. The HPLC procedure was based on resolution of cefotaxime or ceftazidime or ceftriaxone from the alkali-induced degradation products using an ODS column and mobile phase composed of acetonitrile-ammonium acetate buffer solution (0.1 M) in a ratio 10:90 (pH 7.5) with peak detection at 270 nm using a diode array detector. The collected data proved that both procedures were of comparable accuracy and precision, however, the HPLC method was of higher sensitivity (1 µg/ml) compared to the FSQ method. Accelerated stability studies of cefotaxime, ceftazidime and ceftriaxone in aqueous solutions (pH 2–10) using the HPLC method indicated that the degradation of the antibiotics followed pseudo-first-order kinetics. The rate constant-pH profiles showed that the antibiotics were relatively stable over the pH range 4–6 with optimum stability at pH 5. The extrapolated shelf-life (t_{90}) values as determined from Arrhenius plots at pH 5 and 25°C were 6.56, 2.14 and 0.88 h for cefotaxime, ceftazidime and ceftriaxone, whereas these values were found to be 15.0, 3.62 and 2.14 h, respectively at 4°C[29].

Hakim et al., worked on a simple and selective high-performance liquid chromatographic method is described for the analysis of the cephalosporins cefotaxime (CXM), desacetylcefotaxime (DACXM) and ceftriaxone (CFX) in rat plasma. Plasma was deproteinized with methanol, and the supernatant was directly injected into the chromatograph and monitored at 254 nm. For determination of the unbound drugs, a centrifugal ultrafiltration method was employed. The calibration curves were linear (r=0.999) from 2.5 to 500 µg/ml; the detection limits were 100 ng/ml for DACXM and 250 ng/ml for CXM and CFX. The method was not interfered with by other plasma components, nor by barbital sodium or caffeine, and has been applied to study the pharmacokinetics of the cephalosporins in rats[30].
Salwa R. El-Shaboury et al., done a comprehensive review with 276 references for the analysis of members of an important class of drugs, cephalosporin antibiotics, is presented. The review covers most of the methods described for the analysis of these drugs in pure forms, in different pharmaceutical dosage forms and in biological fluids [31].

Alvin K. H. Kwok et al., investigated the precipitation process of a mixture of vancomycin and ceftazidime by equilibrium dialysis and determine its subsequent effect on the level of free antibiotics for treatment of endophthalmitis. Concentrations of vancomycin and ceftazidime in an equilibrium dialysis chamber were measured during the equilibrium process by high-performance liquid chromatography. Normal saline (NS), balanced salt solution (BSS), and vitreous were used separately as the medium of dialysis. Precipitation of ceftazidime occurred at 37°C but not at room temperature and did not affect the pH of the medium. It formed precipitate on its own or when mixed with vancomycin in all the three media of NS, BSS, and vitreous. More precipitation was formed if ceftazidime was initially prepared in BSS than in NS. After 168 hours in the dialysis chambers, ceftazidime prepared in NS precipitated to 54% of that in vitreous, compared with 88% if prepared in BSS. At 48 hours, ceftazidime prepared in NS decreased from an initial concentration of 137.5 to 73.4 µg/mL in vitreous medium and to 6.3 µg/mL if prepared in BSS. Precipitation of vancomycin was negligible [32].

Scaglione et al., worked on concentrations of cefaclor (CFC) or amoxicillin-clavulanic acid (AMX/CA) in middle-ear fluid collected preserving the stability and clearing the cell contents has been compared to those obtained using the traditional method. Sixty-seven children with effusive otitis media were treated orally with CFC (20 mg/kg of body weight) or AMX/CA (20 mg/kg) (4:1 ratio). The concentrations in cell-free fluid (C-) appear higher than those in the total fluid (C+) (as assayed traditionally) [33].
Hary et al., compared in two separate studies the kinetics of ceftriaxone and cefotaxime in 8 cirrhotic patients with ascites and 8 control subjects after a single 20 min intravenous infusion of 1 g of each drug. The apparent volumes of distribution ($V_d$) were found to be significantly higher in cirrhotics than in control subjects (0.87, versus 0.49, l·kg$^{-1}$, for cefotaxime and 0.23 versus 0.13 for ceftriaxone). The elimination kinetics of ceftriaxone were similar in the two groups. In contrast, the total and non-renal clearances of cefotaxime were reduced in cirrhotic patients. The two drugs rapidly entered the ascitic fluid. Peritoneal concentrations of ceftriaxone were higher than 7 µg·ml$^{-1}$ from the second hour after the infusion and were 8.9 µg·ml$^{-1}$ at 24 h. Peritoneal concentrations of cefotaxime were higher than 4 µg·ml$^{-1}$ from 0.5 to 8 h after the infusion.$^{[34]}$

Jolanta J. Bafeltowska et al., developed a high-performance liquid chromatographic procedure for the measurement of cefotaxime and desacetylcefotaxime in cerebrospinal fluid. Both compounds were isolated from cerebrospinal fluid samples using solid-phase extraction (SPE). LiChrolut RP-18 (200 mg; 3 ml) columns and a mixture of methanol–phosphate buffer pH 7 (1:1) were applied to elute cefotaxime and its desacetyl metabolite. The separation was performed on a LiChrospher 100 RP-18 (5 µm; 250×4 mm I.D.) column. The mobile phase consisted of 0.01 M acetate buffer pH 4.8–methanol (85:15), flow-rate was 1.5 ml/min. Cefotaxime and desacetylcefotaxime were detected at a wavelength of 254 nm by UV–Vis detector. The range of concentrations for method calibration and for analytical studies was 1.56–100 µg/ml. The quantitation limit in cerebrospinal fluid was 0.39 µg/ml for cefotaxime and 0.78 µg/ml for desacetylcefotaxime. The extraction recovery from cerebrospinal fluid spiked with cefotaxime and desacetylcefotaxime was 90.4–100.1% and 97.4–102.9%, respectively. The RSDs were below 10.7% for cefotaxime and 6.8% for desacetylcefotaxime. The developed SPE–HPLC method was applied for cefotaxime and desacetylcefotaxime
determination in cerebrospinal fluid of children with hydrocephalus after intraventricular administration\textsuperscript{[35]}.\n
Bompadre et al., studied using a single, on-line solid-phase sample clean-up procedure, serum concentrations of ten cephalosporins (cefaloridine, cefalotin, cefamandole, cefazolin, cefodizime, cefoperazone, cefoxitin, ceftriaxone, and cefuroxime) were assayed on a C\textsubscript{18} column, using UV detection. Serum samples diluted with water were directly injected, without any sample preparation, into the extraction column (alkylamino packing) where the drugs were separated from the serum components. Using an on-line column-switching system, cephalosporins were transferred and separated on the analytical column by ion-pair high-performance liquid chromatography. The proposed method was rapid and efficient: it requires a small serum volume (10 µl) and it can be automated. Recovery was nearly complete. Detection limits varied from 0.5 to 2 µg/ml from extraction of 10 µl of serum. The method was highly specific even in the presence of other drugs commonly found in clinical samples and provides a rapid, simple technique suitable for use in routine microbiological practice\textsuperscript{[36]}.\n
Sue J. Kohlhepp et al., worked on the influence of assay methodology on the measurement of the active free fraction of ceftriaxone in plasma. The free fraction was measured by three methods: agar diffusion bioassay, precipitation of plasma protein with methanol followed by high-performance liquid chromatography (HPLC) of the supernatant and ultrafiltration of plasma followed by HPLC of the filtrate. In human serum, the free ceftriaxone levels were significantly lower (P = 0.03) when measured on ultrafiltrates compared to the other two methods. This difference disappeared when dolphin serum was studied. After ultrafiltration, human serum was shown, by Scatchard plot analysis, to have two ceftriaxone binding sites.
Species differences were also demonstrated. Hence, in humans, determination of free plasma ceftriaxone varies with the assay method employed [37].

Jean Péduzzi et al., worked with serratia fonticola CUV produces two isoenzymes (forms I and II) with β-lactamase activity which were purified by a five-step procedure. The isoenzymes had identical kinetic parameters and isoelectric point. They were characterized by a specific activity towards benzylpenicillin of 1650 U/mg. The β-lactamase hydrolyzed benzylpenicillin, amoxycillin, ureidopenicillins, first- and second-generation cephalosporins. Carboxypenicillins and isoxazolylpenicillins were hydrolyzed to a lesser extent. Towards cefotaxime and ceftriaxone (third-generation cephalosporins), the S. fonticola enzyme exhibited catalytic efficiencies much higher than those of MEN-1 and extended-spectrum TEM derivative β-lactamases. The β-lactamase from S. fonticola was markedly inhibited by β-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam. The purified isoenzymes were digested by trypsin, endoproteinase Asp-N and chymotrypsin. Amino acid sequence determinations of the resulting peptides allowed the alignment of 267 amino acid residues (Swiss-Prot, accession number P 80545) for form I β-lactamase. Form II is five residues shorter than form I at its N-terminus. From amino acid sequence comparisons, S. fonticola CUV β-lactamase was found to share more than 69.3% identity with the chromosomally encoded β-lactamases of Klebsiella oxytoca, Proteus vulgaris, Citrobacter diversus and the plasmid-mediated enzymes MEN-1 and Toho-1. Therefore, the oxyimino cephalosporin-hydrolyzing β-lactamase of S. fonticola belongs to Ambler's class A. Contribution of the serine at ABL 237 in the broad-spectrum activity of these β-lactamases is discussed [38].

Bozidar Ogorevc et al., done a review on electroanalysis methods for the determination of cephalosporin antibiotics in either simple solutions or biological fluids. Some general data
about the electroactivity of cephalosporins are given and the analytical utilization of those data together with the corresponding analytical parameters, compiled from different literature sources \cite{39}.

Bellido et al., studied the ability of five new beta-lactams to permeate the outer membrane of intact Enterobacter cloacae beta-lactamase-overproducing cells was measured by using a high-pressure liquid chromatography (HPLC)-based technique that avoided certain possible artifacts of the traditional methods. Low concentrations of antibiotics were mixed with bacterial suspensions, and at different times, the cells were removed from the medium by filtration. Residual beta-lactam concentrations in the medium were then assessed by HPLC and UV detection. The assay was performed under conditions in which no beta-lactamase activity was detected in the filtrate and the number of viable cells remained constant during the experiment. Outer membrane permeability was assessed with the Zimmermann-Rosselet equation, in which outer membrane permeability was rate limiting for hydrolysis of the beta-lactam by periplasmic beta-lactamase. Thus, the rate of disappearance of beta-lactam was equal to the rate of outer membrane permeation. Preincubation of bacterial suspensions with 300 micrograms of cloxacillin per ml inhibited the hydrolysis of beta-lactams by intact cells, demonstrating that beta-lactam hydrolysis by periplasmic beta-lactamase was essential in order to allow measurement of outer membrane permeability by this method. Permeability coefficients (P) were calculated from the Zimmermann-Rosselet equation and were independent of the external concentration of antibiotic over a 100-fold concentration range. Cefepime and cefpirome exhibited rates of outer membrane permeation 5- to 20-fold higher than those of carumonam, ceftriaxone, and cefotaxime. Thus, the presence of a positive charge in the 3-lateral chain increased the permeation ability of beta-lactam molecules considerably \cite{40}.
Patel et al., developed a simple, sensitive, accurate and rapid spectrophotometric method for the estimation of cefotaxime and ceftriaxone using Folin-Ciocalteu reagent in presence of 20% sodium carbonate solution. The blue colour chromogen formed is measured at wavelength of maximum absorption 752 nm and 750 nm for cefotaxime and ceftriaxone respectively against reagent blank. The chromogen obeyed linearity over 5.0 to 60 µg/ml for cefotaxime, and 2.0 to 36 µg/ml for ceftriaxone. The results of analysis have been validated statistically and by recovery studies[^41].

Toshihiro Kitahashi et al. developed a method for determining the concentration of cefozopran, a cephem anti-microbial agent which has a broad spectrum, in human serum using micellar electrokinetic capillary chromatography by serum direct injection is developed and the validation of the assays of this method is performed. A borate buffer (25 mM; pH 10.0) containing sodium dodecyl sulfate (SDS) (50 mM) is used as a run buffer. The electrophoresis of serum samples is carried out at 25 kV and the detection of cefozopran at 244 nm as its absorption maximum at the cathodic side of the capillary. The migration time of cefozopran is 6.5 min. Linearity (0–200 mg/l) is good and limit of quantification is 0.5 mg/l at a signal-to-noise ratio of 3. Coefficient of variation (CV) of intra-day precision and that of inter-day precision are 2.4–4.0% (7.3–92.0 mg/l) and 2.9–7.7% (22.5–71.4 mg/l), respectively, and the recovery rate is 92–109%. The detection results of 12 other cephem anti-microbial agents under the analytical conditions of this method show that the migration time of cefmetazol is identical with that of cefozopran, making it impossible to separate these two anti-microbial agents. This method is characterized by the fact that simple and economic determination can be achieved by directly injecting the serum samples of micro-quantities into the capillary[^42].
Ian R. Friedland et al., worked with a single intravenous dose of cefpirome, 50mg/kg, administered to 15 children with bacterial meningitis 24 to 48h after initiation of standard antibiotic and steroid therapy. Cefpirome concentrations in serum and cerebrospinal fluid were determined at selected time intervals. The mean (standard deviation) peak concentration in cerebrospinal fluid (n=5) was 10.8(7.8) µg/ml. Drug concentrations in cerebrospinal fluid above the MIC for Streptococcus pneumoniae at which 90% of the isolates were inhibited were found 2,4, and 8h after the dose of cefpirome was given. The penetration of cefpirome into cerebrospinal fluid compares favorably with that of other extended-spectrum cephalosporins and suggests that this agent would be useful in the therapy of childhood meningitis, including cases caused by drug-resistant S.pneumoniae [43].

Genowefa Pajchel et al., studied the migration behaviour of cephalozin, cefuroxime sodium, ceftriaxone sodium, cefoperazone sodium and ceftazidime in a mixture. Phosphate–borate buffer pH 5–8 alone and with addition of sodium dodecylsulfate (SDS) was used. In capillary zone electrophoresis of all research compounds separation was not achieved. It was observed that supplementation buffer pH 6.5 with SDS (10 g/l) improved resolution of cephalosporins, but addition of pentanesulfonic acid (17.4 g/l) to the running buffer at pH 6.5 results in separation of each cephalosporin. In this condition good repeatability of migration times as well as repeatability of peak area were confirmed [44].

Lensmeyer et al., described an extraction and an isocratic "high-performance" liquid-chromatographic (HPLC) separation of cyclosporine (CsA) and nine metabolites (M1, M8, M17, M18, M21, M25, M26, M203-218, and MUNDF1) from whole blood. Metabolites (for standards) were purified from human bile with liquid-liquid and solid-phase extractions, chromatographed on a cyanopropyl (CN) semipreparative HPLC column, and further purified
on octyl, CN, and silica columns. The identity of each metabolite was verified with authentic standards on three chemically different HPLC columns and on the basis of cross-reactivity data from radioimmunoassay. For the routine analytical method, 1 mL of whole blood is diluted, hemolyzed, and applied to a Bond Elut CN (500 mg) cartridge to extract CsA, metabolites, and cyclosporin C, the internal standard. Interferences are removed by using four wash solutions and an additional cartridge of octyldecyl sorbent introduced prior to elution. Analytes are separated on a Zorbax CN analytical column maintained at 58 degrees C, with detection at 214 nm. Analytical recovery, as tested with three lots of CN sorbent, ranged from 47% to 95% for the 10 cyclosporines. Between-run CVs are less than 10% at 200 micrograms/L (concentration of each compound) and the standard curves are linear to 1500 micrograms/L. We also report a study of the separation mechanisms \cite{45}.

Demotes-Mainard et al., developed a sensitive and rapid high-performance liquid chromatographic method for the determination of ceftriaxone in human plasma and urine. A C18 reversed phase column is used; the mobile phase comprises water-methanol-triethylamine (750:250:4v/v/v) adjusted to pH 3 with orthophosphoric acid. Quantitation is performed at 270 nm with cefazolin as the internal standard. This method involves precipitation of proteins from fluids with acetonitrile followed by extraction of endogenous compounds with chloroform and injection of the upper aqueous phase on to the chromatograph. Relative standard deviations for between-day and within-day assays are $\leq 6.2\%$. The detection limit is 0.5 $\mu g^{-1}$ in plasma and urine. Studies of drug stability during sample storage, sample pretreatment and chromatography showed no degradation of ceftriaxone or of the internal standard. The method is convenient for clinical monitoring and for pharmacokinetic studies \cite{46}.
Gohil et al., studied the pharmacokinetics of ceftriaxone in buffalo calves (Bubalus bubalis) after single intravenous and intramuscular administration of 10 mg/kg body weight. The drug concentrations in plasma samples were measured by high performance liquid chromatography with UV detection. Following intravenous administration, the drug was rapidly distributed (Cpo: 106.5 ± 9.64 µg/ml; t1/2α: 0.09 ± 0.01 h; Vdarea: 0.48 ±0.05 L/kg) and eliminated (t1/2β: 1.27 ± 0.04 h) from the body with a clearance rate of 4.40 ± 0.44 ml/min.kg. Following intramuscular administration, the peak plasma concentration of the drug was 15.8 ± 2.4 µg/ml at 0.5 h and the drug was detected up to 12 h. The drug was rapidly absorbed from the site of injection (t1/2ka: 0.35 ± 0.01 h), widely distributed and slowly eliminated from the body. The bioavailability of ceftriaxone was 70.2 ± 2.0% following intramuscular injection. Intramuscular injection of ceftriaxone has favourable pharmacokinetics and moderate bioavailability in buffalo calves and can be used for susceptible infections in calves [47].

Shingo Horimoto et al., identified the novel method for a heat-unstable antibiotic, FC/TA-891 and its active metabolite (FCE22101) by high-performance liquid chromatography (HPLC)–atmospheric pressure chemical ionization mass spectrometry (APCI-MS) employing bromoform as an ionization acceleration solvent, was applied to eight penicillins and 13 cephalosporins which are groups of β-lactam antibiotics. The conditions of HPLC–APCI-MS were examined with ampicillin. Bromoform or chloroform was added to the mobile phase in HPLC to compare the difference between bromine and chlorine adducted ions. For all penicillins except sulbenicillin, both chlorine adducted and bromine adducted ions were observed with a flow injection method. The results indicated that the relative sensitivity ratios of bromine adducted ions to [M−H]− were higher than those of chlorine adducted ions. These bromine adduct ions could be clearly distinguished from other ions due to its isotopical ratio (1:1), leading to an easy identification of the compounds. For 13 cephalosporins, bromine
adducted ions were detected in nine compounds, and chlorine adducted ions were detected in four compounds. The separation of four antibiotics was investigated with an HPLC column to apply this technique to the actual analysis. The capability was equal as in the flow injection method and it found that this technique, i.e. APCI-MS with bromoform could be applicable in the separation analysis \cite{48}.

Robert E.W. Hancock et al., studied the fourth generation cephalosporins, cefpirome and cefepime, demonstrated better activity against strains of *Enterobacter cloacae* with derepressed $\beta$-lactamase than the third generation compounds cefotaxime and ceftriaxone. Several methodological refinements were used to measure the parameters, predicted by the Zimmermann-Rosselet equation to be important in the efficacy of $\beta$-lactams. Outer membrane permeability was measured by a novel HPLC method. The kinetics of interaction of purified $\beta$-lactamase with $\beta$-lactams were estimated to calculate the inhibition and catalytic constants. The periplasmic concentration of $\beta$-lactams leading to growth inhibition of cells was determined by substituting the above parameters into the Zimmermann-Rosselet equation. Consideration of these three factors allowed accurate prediction of MICs in isogenic *E. cloacae* strains with differing porin or $\beta$-lactamase contents. The fourth generation cephalosporins had markedly reduced affinity for $\beta$-lactamase and increased outer membrane permeability when compared to the third generation cephalosporins. Such advantages were only partly offset by a lower stability of complexes with $\beta$-lactamase and reduced affinity for their targets \cite{49}.

De Juana et al., worked on the stability of the cephalosporins - ceftazidime, ceftriaxone, ceftizoxime and cefotaxime in vitro, at therapeutic concentrations, infused together with a parenteral nutrition mixture with polyols, enriched in branched chained amino acids, and
without lipids. A microbiological stability analysis was carried out on the antibiotics in the parenteral nutrition, and an HPLC aminogram was done to determine the concentration of amino acids in the infusion together with the antibiotic. As well, pH, osmolarity and colour change were measured in the antibiotics, in the parenteral nutrition used and in the joint infusion mixtures. It is concluded that parenteral nutrition can be jointly infused with cefotaxime and ceftazidime, at the concentrations studied, given the stability results obtained both with HPLC (antibiotics and amino acids) and microbiologically (antibiotics). At the same time, the microbiological analysis of ceftriaxone with the nutrition showed its stability in the study conditions. Its joint infusion with parenteral nutrition, studied by HPLC, confirmed the stability of the amino acids. The ceftizoxime analysed by HPLC remained stable during joint infusion with the parenteral nutrition [50].

Thangadurai et al., worked on X-ray powder diffraction (XRD) data for eight β-lactam viz., ampicillin sodium, ampicillin trihydrate, penicillin G procaine, benzathine penicillin, benzyl penicillin sodium, cefalexin, cefotaxime sodium and ceftriaxone sodium; three tetracyclines viz., doxycycline hydrochloride, oxytetracycline dihydrate and tetracycline hydrochloride; and two macrolide viz., azithromycin and erythromycin estolate antibiotic drugs were obtained using a powder diffractometer. The drugs were scanned from Bragg angles (2θ) of 10° to 70°. The obtained data were tabulated in terms of the lattice spacing (Å) and relative line intensities (I/I₀). This new information may be useful for identifying these drugs from confiscated materials, which has been frequently encountered in forensic laboratories [51].

Sanjay Mohan Shrivastava et al., developed an isocratic liquid chromatographic method with UV detection at 220 nm is for simultaneous determination of ceftriaxone sodium and sulbactam sodium in Sulbactomax. Chromatographic separation of two drugs was achieved on a Hypersil ODS C-18 column using a mobile phase consisting of a binary mixture of
acetonitrile and tetrabutyl ammonium hydroxide adjusted to pH7.0 with orthophosphoric acid in ratio 70:30. The developed Liquid Chromatographic method offers symmetric peak shape, good resolution and reasonable retention time for both drugs. Linearity, accuracy and precision were found to be acceptable over the concentration range of 125-750 ppm for ceftriaxone sodium and 62.5-375 ppm for sulbactam sodium. The LC method can be used for the quality control of formulated products containing ceftriaxone and sulbactam \[52\].

Gnann Jr et al., studied the in vitro activity of ceftriaxone against 437 clinical isolates of gram-negative bacilli. Ceftriaxone was found to have high in vitro activity against Enterobacteriaceae, with the exception of Enterobacter cloacae. Ceftriaxone was only minimally active against Pseudomonas aeruginosa and Acinetobacter calcoaceticus. We evaluated the clinical efficacy and toxicity of ceftriaxone in 55 adult patients. Bacterial infection was confirmed by the isolation of etiological bacteria in 30 patients. Infectious disorders treated included 10 pneumonias, 13 urinary tract infections, and 7 soft tissue or bone infections. Pathogens identified were 25 isolates of gram-negative bacilli, 5 isolates of Staphylococcus aureus, 5 isolates of pneumococci, and 4 isolates of other streptococci. The overall efficacy of ceftriaxone was excellent. The clinical cure rate was 93%, and the bacteriological cure rate was 93%. A total of 30 adverse reactions were noted in 22 of 55 patients receiving ceftriaxone, but only one necessitated discontinuation of treatment. Adverse effects frequently noted were elevated hepatic enzymes (16%), thrombocytosis (16%), and eosinophilia (8%). Ceftriaxone is an effective and well-tolerated antimicrobial agent that appears promising for the treatment of serious gram-negative bacillary infections \[53\].

Brett C. McWhinney et al., developed a simple and economical high performance liquid chromatography method and validated for routine analysis of 12 Penicillin, Cephalosporin and Carbapenem antibiotics in 200µL of human plasma. Antibiotics determined were
Ceftazidime, Meropenem, Ceftriaxone, Ampicillin, Cefazolin, Ertapenem, Cephalothin, Benzylpenicillin, Flucloxacillin, Dicloxacillin, Piperacillin and Ticarcillin. There was a common sample preparation approach involving precipitation of proteins with acetonitrile and removal of lipid-soluble components by a chloroform wash. Separations were performed on a Waters X-bridge C18 column with, depending on analytes, one of three acetonitrile–phosphate buffer mobile phases. Detection was by UV at 210, 260 and 304 nm. Validation has demonstrated the method to be linear, accurate and precise. The method has been used in a pathology laboratory for therapeutic drug monitoring (TDM) of beta-lactams in critically ill patients \[54\].

Abdel-Hamid et al., developed a rapid, simple, accurate and specific spectrophotometric procedure for analysis of multi-component mixtures and for assessment of drug stability in pharmaceutical products. The proposed method relies on the application of the spectrophotometric full spectrum quantification (FSQ) software to overcome severe spectral overlap and to permit measurement of the individual components in mixtures with satisfactory degree of accuracy and precision. The potential of FSQ software for multicomponent analysis was elucidated by analyzing complex mixtures of highly overlapping spectra (aspirin/caffeine/salicylic acid) and mixtures of structurally similar compounds (cephalexin/cephradine) and (ceftriaxone/ceftazidime/cefotaxime). Furthermore, the robustness of the FSQ for stability studies was assessed by analyzing chloramphenicol in presence of its alkali-induced degradation products and famotidine in presence of its acid-induced degradation products. The validity of FSQ software for multicomponent analysis and stability studies was evaluated by comparing the results with those of HPLC methods. Statistical analysis indicated that the FSQ and HPLC data were consistent, however the FSQ method is faster, easier and more reliable \[55\].
Nkeoma N. Okoye et al., developed a simple, sensitive and accurate spectrophotometric method of analysis of ceftriaxone, cefotaxime and cefuroxime in pharmaceutical dosage forms and validated. The method is based on the formation of Prussian Blue (PB) complex. The reaction between the acidic hydrolysis product of the antibiotics (T = 70_\text{C}) with the mixture of Fe3+ and hexacyanoferate (III) ions was evaluated for the spectrophotometric determination of the antibiotics. The maximum absorbance of the coloured complex occurred at _ 700 nm and the molar absorptivity is 3.0 \times 10^4 \text{ L.mol}^{-1} \text{ cm}^{-1}. Reaction conditions have been optimized to obtain PB complex of high sensitivity and longer stability. Under optimum conditions the absorbance of the PB complex were found to increase linearly with increase in concentrations of ceftriaxone, cefotaxime and cefuroxime, which corroborated with the correlation coefficient values. The linear range of the calibration graph was 2 - 20 \text{ _g/ml} for ceftriaxone and cefotaxime and 2 - 18 \text{ _g/ml} for cefuroxime. The proposed method was successfully applied to the determination of the selected antibiotics in bulk drugs and pharmaceutical formulations and the results obtained agree well with the labeled contents\textsuperscript{[56]}.

Nanda et al., developed a three simple, accurate and reproducible spectrophotometric methods for the simultaneous estimation of Cefotaxime Sodium and Sulbactam Sodium in pharmaceutical dosage forms. The first method involves determination using the simultaneous equation method, the sampling wavelengths selected are 233.5nm and 264 nm over the concentration ranges of 5-35 mcg ml\textsuperscript{-1} and 2.5-17.5 mcg ml\textsuperscript{-1} for Cefotaxime Sodium and Sulbactam Sodium respectively. The second method is the Area Under Curve method (AUC), the sampling wavelength ranges selected are 238.5-228.5nm and 269-259nm with linearity in the concentration ranges of 5-35 mcg ml\textsuperscript{-1} and 2.5-17.5 mcg ml\textsuperscript{-1} for Cefotaxime Sodium and Sulbactam Sodium respectively. The third method involves determination using the Multicomponent Mode Method, the sampling wavelengths selected are 233.5 nm and 264 nm.
over the concentration ranges of 5-35 mcg ml-1 and 2.5-17.5 mcg ml-1 for Cefotaxime Sodium and Sulbactam Sodium respectively. The results of the analysis were validated statistically and recovery studies were carried out as per ICH guidelines [57].

Hafiz Muhammad Arshad et al., developed a simple, selective and rapid reversed phase High Performance Liquid Chromatographic (HPLC) method for the analysis of cefixime in bulk material and capsule and validated. The chromatographic system consisted of a LC-10 AT VP pump, SPD-10 AVP UV/visible detector. The Separation was achieved from Bondapak C18 column at ambient temperature with a mobile phase consisting of methanol: buffer solution (sodium dihydrogen phosphate) [35: 65 v/v, pH=2.75 adjusted with phosphoric acid] at a flow rate of 1ml/min and the retention time was about 6 minutes. The method is selective to cefixime and able to resolve the drug peak from formulation excipients. The system suitability with retention time was (Mean + %CV) 5.819 + 0.51. The calibration curve was linear over the concentration range of 0.039-20µg/ml (r2 = 0.9998). The proposed method is accurate and precise (Intra day and Inter day variation, RSD were 0.53-1.64) and linear within the desired range. The LOD and LOQ was detected as 0.0195µg/ml and 0.039µg/ml respectively with r2 = 0.9996. The accuracy result of seventy percent drug (70%) was 99.82%, hundred percent (100%) was 99.89%, and one thirty percent (130%) was 100.12%. Therefore, this method could be used as a more convenient and efficient option for the analysis of cefixime in raw material and capsule dosage form [58].

Pefanis et al., used a rat model of intraperitoneal abscess due to Bacteroides fragilis, we evaluated therapy with the combination of ceftriaxone plus the β-lactamase inhibitor tazobactam in comparison with ceftriaxone or cefotaxime alone. When treatment was begun five hours after bacterial challenge, final bacterial counts within abscesses at 3-5 days of
treatment were as follows (mean ± S.D., log\textsubscript{10} cfu/g): ceftriaxone plus tazobactam, 4·15±1·25; cefotaxime, 4·77±1·80; ceftriaxone alone, 5·68±1·04; untreated controls, 9·14±1·13. In spite of pharmacokinetic differences between the two drugs, coadministration of tazobactam significantly enhanced activity of ceftriaxone in this model\textsuperscript{[59]}.

Philippe Cottagnoud et al., studied on linezolid, a new oxazolidinone antibiotic, showed good penetration (38 ± 4\%) into the meninges of rabbits with levels in the CSF ranging from 9.5 to 1.8 mg/L after two iv injections (20 mg/kg). Linezolid was clearly less effective than ceftriaxone against a penicillin-sensitive pneumococcal strain. Against a penicillin-resistant strain, linezolid had slightly inferior killing rates compared with the standard regimen (ceftriaxone combined with vancomycin). \textit{In vitro}, linezolid was marginally bactericidal at concentrations above the MIC (5 × and 10 × MIC)\textsuperscript{[60]}.

Sánchez-Sancho, studied the lack of knowledge of the exact chemical structure of cephalosporin antigenic determinants has hindered clinical interpretation of adverse reactions to these drugs and delayed understanding of the mechanisms involved in the specific recognition and binding of IgE molecules to these antigenic determinants. We further resolve the relationship between structure and activity of proposed antigenic chemicals, including the rational design and synthesis of these haptenic structures. Comparative RAST inhibition studies of the synthesized molecules revealed that they were recognized by IgE antibodies induced by cephalosporin antibiotics. Thus, these data indicate that recognition is mainly directed to the acyl side chain and to the β-lactam fragment that remains linked to the carrier protein in the cephalosporin conjugation course\textsuperscript{[61]}.

Jean-Daniel Hecq et al., worked on intravenous solutions of cefuroxime sodium prepared by a Centralized Intravenous Additive Service (CIVAS). The aim of this study was to investigate how freezing, long-term storage and microwave thawing can affect their stability. Five
polyvinyl chloride (PVC) bags of solution containing 1500 mg of cefuroxime sodium per 100 ml of 5 % glucose were frozen for 3 months at –30°C followed by thawing in a microwave oven with a validated cycle and final storage at 4°C. Concentration was measured by high performance liquid chromatography. Visual inspection and pH measurement were also carried out. No colour change or precipitation was observed. The infusion solutions were stable for 15 days (90 % of the initial concentration) at final storage at 4°C. The pH value increased slowly (+ 0.93) without any effect on the analytical parameters. Within these limits, cefuroxime sodium infusions may be prepared and frozen in advance by a CIVAS then thawed before use.\[62\]

Azoulay-Dupuis et al., worked with BAL5788 is a water-soluble prodrug of BAL9141, a new broad-spectrum cephalosporin with high levels of in vitro activity against methicillin- and vancomycin-resistant staphylococci and penicillin-resistant streptococci. In plasma BAL5788 is rapidly converted to BAL9141. We studied the activity of BAL5788 in a mouse model of acute pneumococcal pneumonia. Leukopenic female Swiss albino mice were challenged intratracheally with \(10^7\) CFU of clinical Streptococcus pneumoniae strains P-52181 (Pen\(^s\) Cro\(^s\) Ctx\(^s\)), P-15986 (Pen\(^t\) Cro\(^o\) Ctx\(^o\)), P-40422 (Pen\(^t\) Cro\(^o\) Ctx\(^o\)), and P-40984 (Pen\(^t\) Cro\(^o\) Ctx\(^o\)). Infected mice received subcutaneous (s.c.) injections of BAL5788 or ceftriaxone starting 3 h after pneumococcal challenge. Uninfected nonleukopenic mice received single s.c. doses of BAL5788 to determine the BAL9141 concentration-time profiles in serum and lungs. Untreated control mice died within 5 days postinfection. Ten-day cumulative survival rates for infected mice receiving BAL5788 (total daily doses of BAL9141 equivalents, 2.1 to 75 mg/kg of body weight) ranged from 57 to 100%, whereas with ceftriaxone (total daily doses, 10 to 400 mg/kg), the survival rates varied between 13 and 100%. In mice infected with P-15986, the survival rates achieved with BAL5788 (BAL9141 equivalent, 8.4 mg/kg) and those
achieved with ceftriaxone (50 mg/kg) were significantly different (93 versus 13%; P < 0.0001) in favor of BAL5788; the outcomes of the trials with all other strains were not significantly different between the two antibiotics, but markedly lower doses of BAL5788 than ceftriaxone were required to obtain similar survival rates. Pharmacokinetic data showed that BAL9141 was effective against the four pneumococcal strains tested at very low values of the time above the MIC (T > MIC), which ranged from 9 to 18% of the dosing interval, whereas the values of T > MICs for ceftriaxone ranged from 30 to 50% of the dosing interval \[63\].

Tomasz Tuzimski et al., studied the correlation of R \(_F\) values for pairs of chromatographic systems has been used for practical separation of a mixture of eight cephalosporins by two-dimensional thin-layer chromatography on silica gel layers. Plates were scanned and videoscanned to show the real picture of separation \[64\].

Choma et al., worked on Cephalosporins, relatively new antibiotics related to penicillins, are widely used in the treatment of both human and veterinary diseases because of their broad spectrum of antibacterial activity and good pharmacokinetic properties. Cefacetril, belonging to cephalosporins, is commonly used in treating mastitis in cows. In the present paper, cefacetril excretion with milk was examined by means of the TLC-DB and HPLC method. Thin-layer chromatography-direct bioautography is the technique which combines TLC with microbiological detection. Semi-quantitative determination of cefacetril in milk by TLC-DB was compared with quantitative HPLC analysis. An exponential relationship was proposed for calibration curves in bioautography \[65\].

Sharir et al., worked with seventeen patients who underwent vitreal surgery received ceftriaxone (Rocephin) 1-2 g intramuscularly at various time intervals before surgery.
Specimens of serum and vitreous were assayed for ceftriaxone concentrations both by bioassay and high pressure liquid chromatography. All patients had detectable vitreous (and serum) ceftriaxone concentrations at all time periods. Vitreous ceftriaxone levels at the first 4.5 hr following the administration of the antibiotic ranged from 1.4-19.4 micrograms/ml and averaged 5.9 micrograms/ml. At 12-13 hr following ceftriaxone administration vitreous concentrations were 11.5 (±/− 9.0) micrograms/ml. Ceftriaxone in intramuscular administration could be used as prophylaxis against ceftriaxone-susceptible microorganisms in vitreal surgery. Ceftriaxone is the first antibiotic for which reliable penetration into the vitreous is demonstrable following intramuscular administration\[^{66}\].

Granich et al., developed a high-performance liquid chromatographic assay to measure ceftriaxone in serum, urine, and cerebrospinal fluid. Ion pairing was used because ceftriaxone is a relatively polar compound which is poorly retained on C18 columns in standard reverse-phase high-performance liquid chromatography and which produces trailing peaks in the absence of ion-pairing agents. The mobile phase was a combination of acetonitrile and water (46:54), adjusted to pH 9.0 with 10 mM K2HPO4, which contained 10 mM hexadecyltrimethylammonium bromide as the ion-pairing agent. Moxalactam (200 micrograms/ml) was used as the internal standard. A silica-packed precolumn (3 cm long) was used to prevent rapid deterioration of the analytical column (30 by 0.4 cm) by the alkaline pH of the mobile phase, and it significantly extended the life of the analytical column. The assay was linear with ceftriaxone concentrations of 1 to 250 micrograms/ml (r = 0.999) and correlated well with an agar diffusion bioassay (r = 0.990). Reproducibility was good, with intrarun coefficients of variation from 2.3 to 6.4% and interrun coefficients of variation from 3.2 to 21.4%. The absolute recoveries of ceftriaxone and moxalactam were 91 to 97 and 96 to 98%, respectively. No interferences were observed with more than 40 commonly prescribed...
drugs, including 10 cephalosporins (cefotaxime, cefoperazone, ceftazidime, ceftriaxone, cefoxitin, cefamandole, cephalothin, cefazolin, cepapirin, and cephalexin), or with sera from patients with renal or hepatic disease \[67\].

Zhao and Zhang worked on a rapid and simple method for the indirect spectrophotometric determination of sodium Ceftriaxone and reported that Sodium ceftriaxone was degraded completely in the presence of 0.20 mol/l sodium hydroxide in boiling water bath for 20 min. The thiol group (-SH) of the degradation product (I) of sodium ceftriaxone could reduce cupric to cuprous ions, and the resulting which was precipitated with the thiol group (-SH) of the degradation product (II) at pH 4.0. By determining the residual amount of copper (II), the indirect determination of sodium ceftriaxone can be achieved. Reported that standard curve of sodium ceftriaxone versus the flotation yield of copper(II) showed that sodium ceftriaxone could be determined in low concentrations. The linear range of sodium ceftriaxone was 0.70-32 mcg/ml and the detection limit evaluated by calibration curve (3sigma/k) was found to be 0.60 mcg/ml \[68\].

Altinoz et al., used a differential-pulse adsorptive stripping voltammetry to determine sub-micromolar concentrations of ceftriaxone in plasma. A hanging mercury drop electrode was chosen as the working electrode. A simple clean-up procedure was developed in which ceftriaxone was extracted from blood plasma with the non-ionic resin Amberlite XAD-2 and eluted with methanol. The recovery from plasma was 97.6% using a 1.52 x 10\((-4)\) M stock ceftriaxone solution. The method was applied to caesarean cases, and total ceftriaxone levels were measured in the maternal and umbilical cord blood. The amount of ceftriaxone transmitted to the baby on administration of the drug to the mother before the caesarean operation was found to be in the range 0.067-0.17\% \[69\].
Zhang et al., developed a novel determination method for Ceftriaxone sodium by using a flow-injection technique. The calibration range was between 0.05 and 100 mcg/ml with a detection limit (3sigma) of 25 ng/ml, and a relative standard deviation (RSD) of 0.6% for eleven replicate determinations of 5.0 mcg/ml ceftriaxone sodium. The proposed method has been successfully utilized for the determination of ceftriaxone sodium in pharmaceutical formulations, while the chemiluminescence reaction mechanisms were investigated [70].

Joshi did a review column and mobile phase conditions for the various classes of antibiotics viz. penicillins, cephalosporins, macrolides, tetracyclines, aminoglycosides, quinolones, rifamycins etc. have been presented from April 1998 to November 2000. A brief discussion on chemical structure, spectrum of activity and action mechanism of each class has also been given [71].

Al-Rawithi et al., developed a rapid, specific and very sensitive liquid chromatographic assay using standard ultraviolet detection has been developed to measure cefazolin (CFZ) or ceftriaxone (CFX) in small samples (200 microl) of plasma using either drug as the internal standard for measurement of the other [72].

Marini and Balestrieri developed a simple and sensitive method for the determination of Ceftriaxone by reverse phase high performance liquid chromatography with ultraviolet detection [73].

Jungbluth and Jusko developed a rapid, sensitive and specific ion-paired reversed-phase HPLC assay for ceftriaxone in human plasma and urine. Small volumes (50 microL) of sample are deproteinized with acetonitrile and are directly injected on a C18 analytical column. The UV absorbance is monitored at 280 nm. The assay was linear between 1 and 125 mcg/ml of ceftriaxone, with less than 10% coefficient of both intra and inter day variation. Chromatography was specific for ceftriaxone as endogenous compounds and 30 common
drugs did not interfere. The assay was used in open heart surgery patients where potential interference from corticosteroids was overcome\textsuperscript{[74]}. Eric et al., developed a HPTLC method for the determination of ceftriaxone, cefixime and cefotaxime, cephalosporins. Solvent composition employed was ethyl acetate-acetone-methanol-water (5:2.5:2.5:1.5 v/v/v/v). A TLC scanner set at 270 nm was used for direct evaluation of the chromatograms in reflectance/absorbance mode. The linearity range was 125-500 ng for all cephalosporins investigated. Precision (RSD: 1.12-2.91% (peak height versus ng) and RSD: 1.05-2.75% (peak area versus ng) and detection limits (ng level) was validated\textsuperscript{[75]}.

2.1.4 References

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