1.0 INTRODUCTION

Antibiotics originally known as *antibiosis* (which means "against life,") were drugs which acted against bacteria. The term *antibiosis* was introduced by the French bacteriologist Vuillemin as a descriptive name of the phenomenon exhibited by these drugs. (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*. These drugs were later renamed as antibiotics by Selman Waksman, an American microbiologist in 1942.

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 [1].

**Cephalosporins** are beta-lactam compounds in which the beta-lactam ring is fused to a 6-membered dihydrothiazine ring, thus forming the cephem nucleus that inhibits the synthesis of a structural component of the bacterial cell wall. The Cephalosporins were first isolated from cultures of the fungus *Cephalosporium acremonium*. Modifications of the β-lactam ring have resulted in more than 20 derivatives with a range of antibacterial properties. The cephalosporins are often used as an alternative in patients who are sensitive to penicillin.

Cephalosporins are sometimes grouped into "generations" by their antimicrobial properties. The first Cephalosporins were designated first-generation Cephalosporins (*e.g.*, cephalexin and cefazolin), whereas, later, more extended-spectrum Cephalosporins were classified as second-generation Cephalosporins. Each newer generation of Cephalosporins has significantly greater *gram-negative* antimicrobial properties than the preceding generation, in most cases
with decreased activity against gram-positive organisms. Fourth-generation Cephalosporins, however, have true broad-spectrum activity \[^{[2, 3]}\]. The classification of Cephalosporins into "generations" is commonly practiced, although the exact categorization of Cephalosporins is often imprecise.

Most first-generation Cephalosporins were originally spelled "ceph-" in English-speaking countries. This continues to be the preferred spelling in the United States and Australia, while European countries (including the United Kingdom) have adopted the International Nonproprietary names, which are always spelled "cef-". Newer first-generation Cephalosporins and all Cephalosporins of later generations are spelled "cef-", even in the United States.

In 1981, the first third-generation drug, Ceftriaxone was introduced. Ceftriaxone is considered as a third-generation molecule for intravenous or intramuscular administration. Broad-spectrum Cephalosporin antibiotic with a very long half-life and high penetrability to usually inaccessible infections, including those involving the meninges, eyes, inner ears and urinary tract. Ceftriaxone is (6R,7R)-7-[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetamido]-3-\{[(2-methyl-5,6-dioxo-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)sulfanyl]methyl\}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid \[^{[4]}\].
Ceftriaxone for injection is indicated for the treatment of various infections when caused by susceptible organisms like lower respiratory tract infections. Acute bacterial otitis media, skin and skin structure infections, meningitis and surgical prophylaxis \[5, 6\].

**Cefotaxime** is a third-generation cephalosporin. It has a bactericidal action similar to Cefamandole, but a broader spectrum of activity for intravenous or intramuscular administration. It is highly stable to hydrolysis by most beta-lactamases and has greater activity than first- or second-generation Cephalosporins against gram-negative bacteria. Although Cefotaxime is generally considered to have slightly less activity than first-generation Cephalosporins against gram-positive bacteria, many streptococci are very sensitive.

Cefotaxime sodium is \((6R, 7R, Z)-3-(acetoxymethyl)-7-(2-(2-aminothiazol-4-yl)-2-(methoxyimino) acetamido)-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid\).
Despite the development of alternative and promising approaches in antimicrobial therapy; Beta-lactam antibiotics continue to be the important pillars in antibiosis and one of the top therapeutic categories in terms of pharmaceutical sales.

Despite this development, beta-lactams could however increase the share of the world market for systemic antibiotics from 50% in 1998 to now 65%, while their total sales volume increased from US $11 to US $18 billion. Sales leader are the Cephalosporins which garner US $10 billion, representing 30% of the total antibiotic market. The Penicillins sales volume thus ranges at about US $8 billion \[11, 12\].

Campers who have reconstituted dried food and who have mixed powdered baby formula with water to prepare a bottle have applied the technique of reconstitution of dry products. Some medications are supplied in the form of powders or crystals to which a liquid must be added for reconstitution shortly before use. The medications are supplied in dry form because the product can be stored for long time, but becomes unstable and deteriorates in solution within relative short time. Such solutions are said to have a “short shelf life”.

Many new drugs, especially those developed by biopharmaceutical companies, are initially marketed in lyophilized form for two primary reasons: shelf life and time to market. A lyophilized drug maintains its stability and potency over time, extending its shelf life for prolonged storage. Some drugs marketed in lyophilized form may eventually be available as liquids, but lyophilization provides the fastest route to market for many drugs. It is also the only option for those not stable in a liquid form.

These drugs often packaged in powder form in vials require reconstitution prior to administration. With traditional reconstitution, there are two vials and one disposable syringe. One vial contains the lyophilized drug and the other contains the diluent (often water, but
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Occasionally another liquid. The patient or caregiver must use the syringe to insert air into the vial containing diluent, draw the diluent from the vial and into the syringe, inject the diluent into the vial containing the lyophilized drug, mix the solution to create an injectable medication and draw a measured dose back into the syringe for injection. Reconstitution presents several formidable challenges:

Chromatography is a powerful separation technique that finds application to all branches of science. Chromatography is a separation technique whereby the components of a mixture may be separated by allowing the sample to be transported through packed bed of material by fluid mobile phase. Out of almost 700 pharmaceutical formulations that are documented in the USP almost 43% of the procedures are those documented by thin layer chromatography.

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Reversed-phase liquid chromatography (RPLC) is the most popular mode of chromatography for the analytical and preparative separations of compounds of interest in the chemical, biological, pharmaceutical and biomedical sciences. In reversed-phase gradient HPLC, the mobile phase changes composition over a specified time. A steady increase in the percentage
of organic solvent (acetonitrile, methanol, and propanol) leads to an increase in the mobile phase eluotropic strength and this makes it possible for highly retained non-polar analytes to be analyzed within the same run as poorly retained polar analytes. This approach has been extremely successful and has been widely adopted, particularly for drug metabolites \cite{13,14}.

In the present study efforts were made to develop and validate HPLC method for third generation Cephalosporin’s - Ceftriaxone Sodium and Cefotaxime Sodium from injections. Reconstitution stability of both products in different intravenous and intramuscular diluents was studied with the stability indicating HPLC method. Also, evaluation of three lot samples of Ceftriaxone Sodium commercial samples for its safety and stability viz identification and quantification of impurities.

References:


