Manufacturing of Dry Powder injection of Cefoperazone + Sulbactum 1.5gm IP

An internship Report submitted

For the partial fulfilment of the Degree of Master of Science

By

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[M.Sc. Biotechnology]

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2022-23

CERTIFICATE

This is to certify that this training report entitled "**Manufacturing of dry powder injection of Cefoperazone + Sulbactum 1.5gm IP**" was successfully carried out by **Miss. Shrusti Bharadiya** towards the partial fulfilment of requirements for the degree of Master of Science in Biotechnology of Atmiya University, Rajkot. It is an authentic record of her own work, carried out by her under the guidance of **Mr. Soumya Ghosh** for a period of 3 months From 18 Jan 2023 to 18 April 2023 during the academic year of 2022-23. The content of this report, in full or in parts, has not been submitted for the award of any other degree or certificate in this or any other University.

SANOLET LIFECARE PRIVATE LIMITED AHMEDABAD

Head of Department

Signature

19/04/23

Supervisor

Signature

ATMIYA UNIVERSITY
(Established under the Gujarat Private University Act 11, 2018)
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C E R T I F I C A T E

This is to certify that this training report entitled "**MANUFACTURING OF DRY POWDER INJECTION OF CEFOPERAZONE + SULBACTUM IP 1.5gm**" "was successfully carried out by Miss **Shrusti Bharadiya** towards the partial fulfilment of requirements for the degree of Master of Science in Biotechnology of Atmiya University, Rajkot. It is an authentic record of his/her own work, carried out by him/her under the guidance of Mr Soumya Ghosh for a period of three months during the academic year 2022–23. The content of this manuscript, in full or in parts, has not been submitted for the award of any other degree or certificate in this or any other university.

Signature
Dr. Nutanprakash Vishwakarma

Signature
Mr Soumya Ghosh

D E C L A R A T I O N

I hereby declare that the work incorporated in the present Internship report entitled "**Manufacturing of dry powder injection of cefoperazone + Sulbactum 1.5gm IP"** is my own work and is original. This work (in part or in full) has not been submitted to any University for the award of any Degree or a Diploma.

Date Shrusti .K. Bharadiya

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ABSTRACT

The goal of this training programme was to analyse and study the developed manufacturing process for Dry powder injection of cefoperazone + Sulbactum 1.5 gm IP, a 3rd generation combination antibiotic used to treat a variety of bacterial infections including nosocomial pneumonia, intra-abdominal infections, gynaecological infections, sepsis, and others. As noted from the early evidences that cefoperazone, like all beta-lactam antibiotics, binds to specific penicillin-binding proteins (PBPs) located inside the bacterial cell wall, causing the inhibition of the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins. While performing the inhibition mediated treatment, the molecules of cefoperazone are then attacked by enzymes such as anti-beta-lactamases, to stop the activity of cefoperazone. That's where Sulbactum play the role of a protector for the cefoperazone molecules making the treatment more effective. The procedure began with the synthesis and purification of cefoperazone and Sulbactum, which was then combined into a single dosage form. Analytical procedures such as high-performance liquid chromatography were used to confirm the purity and identity of the medicinal component. (HPLC). The final product's physical and chemical qualities, such as dissolution rate, stability, sterility, minimum microbial count, presences of endotoxins, and other microbiological properties, were evaluated. To achieve consistency in drug quality, yield, and purity, the manufacturing process was optimised.

2. INTRODUCTION

2.1. Company information and brief background of different departments,

Sanolet lifcare pvt. Ltd was incorporated on 21st September 2020, and was registered at the registrar of the companies, Ahmedabad. It is a non-govt. company. Sanolet lifecare pvt.ltd. Is a beta-lactam dry powder injection manufacturing company with large volume parental formulations. The plant is newly constructed, yet has the most technologically advanced instruments and procedure running according to the standard guideline provided by the Government and the pharmacopeia together.

In designing the whole process, Special attention was taken to ensure absolute sterile manufacturing conditions. The primary feature of the procedure is that no human physical contact is made with the product at any point. This has been accomplished through a succession of fully automated manufacturing techniques. The vials are packed in an aseptic system. They are washed and dried completely before they are, filled with the powder mixture, and sealed under sterile conditions in a single manufacturing cycle with no environmental exposure or human touch.

The dry Antibiotic powder comes in a professionally constructed glass vial bottle with an extra safe metal seal caps and reusable grey butyl rubber seals. The entire procedure is carried out in a class 100 clean room. The air in this room is cleaned to a particle count of 100 per cubic foot by passing it through HEPA (High Efficiency Particulate Air) filters. Thus, an improved sterile environment is maintained during the manufacturing process to prevent the introduction of bacteria, pyrogen, and inert particles into the products. This provides the greatest quality and purity requirements, as well as the highest level of safety.

Sanolet lifecare pvt.ltd was therefore incorporated with total of 5 departments including production department, quality control unit, Quality assurances unit, packaging and marketing department, and engineering that further sees for the water and air filtration systems. All the departments are calibrated with standard guidelines and sterility and safety procedures. To maintain least of hazards and errors to occur.

There’s a total of 30+ people staff working currently into the plant facility. In different departments and units.

Each department present has a very crucial role in the manufacturing and maintaining the product and its sterility and safety.

2.2. OBJECTIVE OF THE TRAINNING PROJECT

The objective of this project training and of this project report is to give readers a thorough overview of the complete production process for the pharmaceutical product cefoperazone + Sulbactum 1.5gm IP. The report's primary goal is to offer in-depth details on the following topics:

Production process: A thorough description of the production of cefoperazone + Sulbactum 1.5gm IP, including the raw materials used, the machinery used, and the many stages of production, will be provided in the report.

Measures done to guarantee that the finished product fulfils the necessary quality standards and specifications will be covered in the report's section on quality control.

Analytical testing: A thorough description of the analytical testing carried out during the production process, including the procedures used and the outcomes, will be provided in the report.

Compliance with regulations: The report will outline the regulations that must be adhered to throughout the production of cefoperazone + Sulbactum 1.5gm IP and how this compliance is achieved

This project report's overall goal is to give readers a thorough overview of the manufacturing process for cefoperazone + Sulbactum 1.5gm IP, including quality control procedures, analytical testing, regulatory compliance, and environmental and safety concerns.

2.3. LITRATURE REVIEW

1. Cefoperazone

- It is a third-generation antibiotic drug used to treat a variety of bacterial diseases in the body, including endometritis, respiratory tract infections, peritonitis, skin infections, and bacterial septicemia.
- Among other places, such as the respiratory system, abdomen, skin, and female genital tracts, cefoperazone, a broad-spectrum cephalosporin antibiotic, is used to treat bacterial infections. A broad-spectrum cephalosporin that is semi-synthetic and has been linked to Pseudomonas infections is cefoperazone
- While cefoperazone's therapeutic use has been abandoned in the United States, it is nonetheless accessible in a number of European nations, most frequently under the brand name Sulperazon.
- Generic Name: Cefoperazone
- Drug Bank Accession Number : DB01329
- Weight Average: 645.67
- Chemical Formula : $C_{25}H_{27}N_9O_8S_2$
- Synonym: Cefoperazono or Cefoperazonu
- Indication
- Indicated for the treatment of following infections caused by susceptible bacteria:
- Respiratory tract infections caused by *S. pneumoniae, H. influenzae, S. aureus (penicillinase and non-penicillinase producing strains), S. pyogenes P. aeruginosa, Klebsiella pneumoniae, E. coli, Proteus mirabilis, and Enterobacter species.*
- Peritonitis and other intra-abdominal infections caused by *E. coli, P. aeruginosa, and anaerobic gram-negative bacilli.*
- Bacterial septicemia caused by *S. pneumoniae, S. agalactiae, S. aureus, Pseudomonas aeruginosa, E. coli, Klebsiella spp., etc.*
- Pelvic Inflammatory Disease, Endometritis, and Other Infections of the

Female Genital Tract caused by *N. gonorrhoeae, S. epidermidis, S. agalactiae, E. coli, Clostridium spp., Bacteroides species (including Bacteroides fragilis), and anaerobic gram-positive cocci*.

- Urinary tract infections caused by Escherichia coli and Pseudomonas aeruginosa

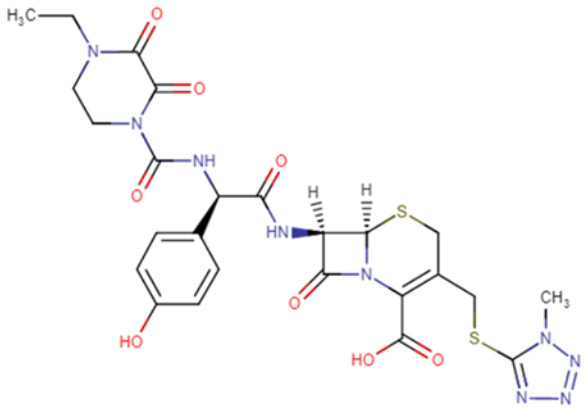
Pharmacodynamics

Cefoperazone exerts its bactericidal effect by inhibiting the bacterial cell wall synthesis.

Mechanism of action

Like all beta-lactam antibiotics, cefoperazone binds to specific penicillinbinding proteins (PBPs) located inside the bacterial cell wall, causing the inhibition of the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins.

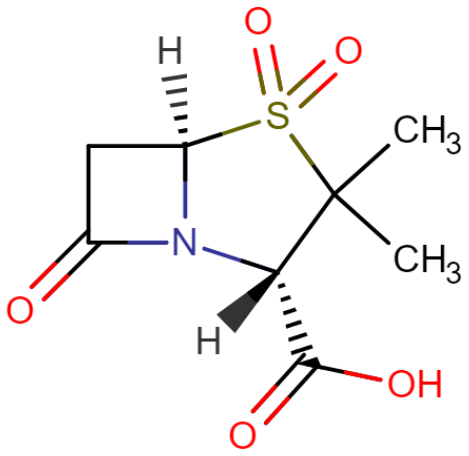
- **Metabolism**: No significant quantity of metabolites have been identified in urine.
- **Route of elimination:** Cefoperazone is excreted mainly in the bile.
- **Half-life**: The mean serum half-life is approximately 2.0 hours, independent of the route of administration.



2. Sulbactum

- Sulbactum is an beta-lactamase inhibitor antibiotic combined with other antibiotics to treat a variety of susceptible bacterial infections.
- Sulbactam is a β-lactamase inhibitor given in combination with β-lactam antibiotics to inhibit β-lactamase, an enzyme produced by bacteria that destroys antibiotic activity.
- Generic Name : Sulbactam
- DrugBank Accession Number : DB09324
- Weight Average: 233.242
- Chemical Formula : $C_8H_{11}NO_5S$
- Synonyms : Penicillanic acid 1,1-dioxide, Penicillanic acid sulfone,Sulbactam,Sulbactamum
- Indication
- Sulbactam is currently available for the treatment of infections due to susceptible strains of the designated microorganisms in the conditions Skin and Skin Structure Infections caused by beta-lactamase producing strains of *Staphylococcus aureus, Escherichia coli, Klebsiella spp. (including K. pneumoniae), Proteus mirabilis, Bacteroides fragilis, Enterobacter spp., and Acinetobacter calcoaceticus*.
- Intra-Abdominal Infections caused by beta-lactamase producing strains of *Escherichia coli, Klebsiella spp. (including K. pneumoniae), Bacteroides spp. (including B. fragilis).*
- Mechanism of action
- Sulbactum is an irreversible inhibitor of beta–lactamase, by attaching to and inhibiting beta-lactamase produced by bacterial cells, Sulbactum is able to prevent it from decreasing antibiotic activity. While whole organism investigations have indicated that Sulbactum restores ampicillin action against beta-lactamase generating organisms.
- Sulbactum alone has only modestly useful antibacterial activity, **except** against the Neisseriaceae.

- Sulbactum, in particular, has considerable inhibitory efficacy against the clinically significant plasmid-mediated beta-lactamases that usually cause transferred drug resistance. Hence, medicines containing ampicillin and Sulbactum have both broad-spectrum antibacterial and beta-lactamase inhibitor characteristics.
- Route of elimination : Approximately 75 to 85% of Sulbactum is excreted unchanged in the urine during the first 8 hours after administration.
- Half-life ~1 hr.
- Structure,



3. Cefoperazone + Sulbactum

- Cefoperazone (as Cefoperazone sodium) 0.5g and Sulbactum (as Sulbactum sodium) 0.5g are the two primary components of the medication Sulbactum. This third generation cephalosporin semi-synthetic antibiotic, which contains the active component Cefoperazone, is renowned for its potent bactericidal capacity by preventing the manufacture of the bacterial cell wall as it divides and grows.
- When used against beta-lactamases produced by the majority of gram-negative bacteria, cefoperazone is a relatively stable antibiotic. Due to this, the component has potent antibacterial effects against a variety of gram-negative bacteria (including strains of *N. gonorrhoeae* that produce penicillinase as well

as *Enterobacter, Citrobacter, Proteus, Salmonella, Shigella,* and *Serratia species.*

- This active component is especially powerful against germs that are resistant to other beta-lactam antibiotics
- With the ingredient Sulbactum, this is a substance similar in structure to beta lactam but has weak antibacterial activity, so it is rarely indicated for clinical use alone. Because Sulbactum has the ability to inactivate beta lactamase enzymes, it will contribute to the protection of beta lactam antibiotics against degradation.
- From here, Sulbactum is often used in combination with the penicillin group to expand the spectrum of action of penicillin against bacteria including enteric bacteria, *E. coli, staphylococcus,* anaerobic bacteria *Bacteroides, Acinetobacter or Branhamella, Klebsiella, Neisseria, Proteus.*
- Indications and contraindications of Cefoperazone Sulbactum:
- It is indicated for the treatment of the following infections when caused by susceptible organisms:
    - Respiratory tract infections (upper and lower) ○ Urinary tract infections (upper and lower) ○ Peritonitis, cholecystitis, cholangitis, and other intra-abdominal infections
    - Septicemia Meningitis ○ Skin and soft tissue infections ○ Bone and joint infections
    - Pelvic inflammatory disease, endometritis, gonorrhoea, and other infections of the genital tract.
- **Chemical Composition**: composition is made up of cefoperazone sodium and Sulbactum sodium, it is characterized in that: described cefoperazone ($C_{25}H_{27}N_9NaO_8S_2$) and described Sulbactum ($C_8H_{10}NNaO_5$The ratio of sign weight S) is 3: 1.

Mechanism of action

- Cefoperazone exerts its bactericidal effect by inhibiting the bacterial cell wall synthesis, and Sulbactum acts as a beta-lactamase inhibitor, to increase the antibacterial activity of cefoperazone against beta-lactamase-producing organisms.
- Distribution: The protein binding of Cefoperazone is 82-93% and that of Sulbactum is 38%.
- Metabolism and Excretion: No significant quantity of metabolites of Cefoperazone has been found in the urine. Cefoperazone is excreted mainly in the bile. About 75-85% of Sulbactum is excreted in the urine during the first eight hours of administration.
- Combination Therapy Because of the broad spectrum of activity of Sulbactum/ Cefoperazone, most infections can be treated adequately with this antibiotic combination alone. However, Sulbactum/Cefoperazone may also be used concomitantly with other antibiotics if such combinations are indicated. If an aminoglycoside is used, renal function should be monitored during the course of therapy.

Dosage,

Cefoperazone Sulbactum - intramuscular injection or intravenous infusion ○

Adults:

- Patients with mild and moderate infections use the drug at a dose of 1-2g (Cefoperazone) every 12 hours.
- Patients with severe infections use the drug at a dose of 2-4g every 12 hours. ○ Children:

○ Use the drug at a dose of 25-100mg/kg every 12 hours. Note, patients with kidney disease do not need to reduce the dose, however, the dose for people with liver disease or biliary obstruction should not exceed 4g/24 hours.

3. TRAINING CONTENT (TIMELINE AND WORKDONE)

3.1. Introduction to different departments at the company

- Sanolet lifecare was made out of 5 the most essential departments along with systems incorporated with advanced technology and Procedures.
- Brief background check of the departments are as follow,

3.1.1. WATER SYSTEM,

- Controls the water system, AHU (Air handling unit), HVAC (heat ventilation and air conditioning system), and PSG (pour steam Generator).
- Generates purified water that is necessary in drug formulations and is used in a variety of processes such as cleaning, rinsing, and sterilization. Therefore, it is essential to ensure that the water used in these processes is of high quality and meets specific standards to prevent contamination of the drugs.
- The water used in the pharmaceutical industry needs to be free of harmful microorganisms, endotoxins, and other impurities that could pose a risk to human health. Any contamination in the water system could compromise the safety of the final drug product.
- Compliance with numerous regulatory agencies, such as the FDA, is vital in the pharmaceutical industry. Compliance with the FDA's tight criteria for the quality and purity of water used in medication manufacturing processes is critical for drug approval and commercialization.
- Water systems that are properly planned and maintained can assist save costs by assuring effective use of water and energy resources, reducing downtime due to equipment failure or maintenance, and reducing the danger of contamination or product loss.

- A well-designed water system can also help to promote sustainable manufacturing practises by lowering water usage, waste creation, and the environmental effect of pharmaceutical manufacturing operations.
- Work flow of the water system present at the Sanolet lifecare pvt. Ltd. Is as follow:

1. Pre-treatment
    - Pre-treatment of water includes treating of ground water that comes from the bore well that has mixed impurities including small solid, organic materials, some microorganisms and bacteria too.
    - The water is further sent to the storage tank where chlorination takes place.
2. Chlorination
    - The storage tank is already infused with chlorine.
    - The impurities are then chlorinated and removed through filtration.
3. De-chlorination
    - The chlorinated water is then fed into micron-cartilage filter by the means of raw water pump.
    - NaClO is added as a de-chlorinating agent to the water. This dis-infects the water from the chlorine.
    - SMBS (Sodium metabisulphate) is used as de-chlorination agent too as it will prevent the chlorine to attack the softeners added later in the process.
4. Filtration
    - Removes the suspended matter and floating matter from the water.
    - Sand filters is used. Which contains pure graded silex Quartz as a filtering media. The filtration vessel is fabricated with fibre-reinforcedplastic.
    - Waste water flows vertically through fine bed of sand and gravel.
    - The particles are removed with the help of Absorption.
    - Filtered Water then moves to the RO plant.

5. RO plant ,
    - Antiscalant Dosing is done to prevent to Scaling and accumulation of minerals as $CaCO_3$, Mg, etc. upon the surface of the membrane.
    - Polyacrylic acid is used as an Antiscalant in the plant's water system.
    - Simultaneously the water is passed through micron cartilage filter (10 micron). The high suspended particles may or may not passes through the membrane, as it might can damage the membrane too.
    - MCF pressure gauge is used to check for the choking of the filters.
6. RO system premated water
    - After the treatment from the RO system, there are 2 types of water flows, premated water and rejected water.
    - The rejected water gets back to storage tank and gets reprocessed.
    - The premated water then moves forward to mix bed unit.
7. Mix bed unit
    - Employed for polishing the water to attain demineralized state of water.
    - Strong acid cation and Strong base Anion exchangers are present that removes the dissolved ions from the water.
8. Filter unit
    - 1 micro meter cartilage is installed to prevent the impurities of water which is greater in size than the pores of the filter cartilage.
9. UV system
    - Wavelength of 254nm is used for inhibit microbial growth and presences in the water. Also, used to lower the TOC (total organic carbon) is the measure for the total amount of carbon present in the water making fit or unfit for the same requirement.
10. Purified water and storage
    - The purified water is stored in the tanks and is supplied to all over the plant.

3.1.2. HVAC SYSTEM,

- Heating, ventilation, and air conditioning system is referred to as the HVAC system.
- The main goal of the HVAC system is to keep the office spaces, microbiology lab, and infusion manufacturing area at a constant temperature and relative humidity (RH).
- The most unique elements of the infusion unit are the most contemporary air conditioning idea.
- Pharmaceuticals require HVAC Because, ○ To keep a certain temperature ○ To keep a particular relative humidity. Less than 40% for some hygroscopic materials, such as ranitidine.
    - To purge the producing area of dust particles.
    - To keep the rooms' ventilation functioning properly while preventing crosscontamination.

3.1.3.PRODUCTION AND MANUFACTURING STEPS,

- The production department conducts the filling, sterilization, filtration, and formulation of the dry powder injection.
- The production department therefore, works as follow,

1. **Static pass box**
    - ➢ Vials arrives from the suppliers and are kept in the static pass box before being received by the worker.
2. **Vial de-carting room**
    - ➢ The vials are taken out from the big packages of plastic seals and are arranged on the conveyor-belt for further washing.
    - ➢ The vials are checked for the presences of crack, de-moulding, black particle, white particle, Air-bubble, etc.
3. Rotary vial washing machine
    - ➢ The vials therefor are washed thoroughly with 3 types of water and compressed air pressure.(< NLT 1.0kg/cm^2), recycled water pressure (=NLT 1.0kg/cm^2), Purified water pressure (=NLT 1.0kg/cm^2), and WFI (water for injection) (=NLT 1.0kg/cm^2) ➢ 240 vials are washed per minute.

4. De-pyrogenation and sterilization tunnel
    - Pyrogens are fever-causing substances that can be introduced into the manufacturing process through various sources such as raw materials, equipment, and personnel.
    - Dehydrogenation tunnels are used to remove Pyrogens from the surfaces of containers, equipment, and other materials before they are used in the manufacturing process.
    - Depyrogenation tunnels are essential in the pharmaceutical industry due to their critical role in Pyrogens control, product quality, regulatory compliance, cost savings, and process efficiency.
    - For the same, different temperatures (NLT 300^{o}C) and pressures are used to create different zone for the same, Dry Zone, Sterilization zone, cooling zone, Stability zone.
5. Powder filling machine
    - Here the actual critical work occurs, where the formulated dry powder of beta-lactam (Cefoperazone + Sulbactum 1.5gm IP) in filled into the glass vial according to the fill weight fed into the machine.
    - At the plant, there present two rotary filling machines which has 6 nozzles, hence was filling 6 vials at a time.
    - Minimum weight to filled was (2% of the given standard weight)
    - Standard weight was 1.180mg
    - Maximum weight to filled per vial = 1.204mg
    - The fill weight is usually given by calculating the Q&Q formula for the specific product.
6. AP sealing machine
    - Sealing of the vials with GBRS and aluminium seal caps help to preserve products against environmental variables including moisture, oxygen, and light, which can deteriorate the drug's quality and potency.
    - Sealed packaging also aids in contamination prevention and guarantees that the product is safe for consumption.

- The surface was sterilized by 70% IPA solution.
- For the sealing of cefoperazone + Sulbactum 1.5gm IP Pista green Seal were used by the company.

7. External vial washing
   - Outside cleaning of the vials after the filling to ensure a sterile outer surface of the product also.
8. Visual inspection booth
   - Ensure the temperature and relative humidity, Cleanliness, and checks for the presence of common ye important rejection such as, presences of black or white particle inside the sealed glass vial along with product, presences of Air-bubble, Broken or cracked seals or seal caps, cracked vials, and moulds effects.
   - To check for the presences of particles the booth is incorporated with halogen lights for 2000 – 3750 lux.
   - Black particles are still can be seen from the naked eye, but for white particle the content of the Accounted vial is dissolved into WFI to check for the presences of the same .

Q&Q formula to take out the fill weight,

The formula is based on the HPLC analysis value of the raw materials that are yet to be mixed to form the product.

The raw materials are always present with sodium, like Cefoperazone Sodium 1000mg + Sulbactum Sodium 500mg will be mixed at a ratio to give out the product weighing 1.5gm. Therefore to remove the sodium from the raw material and get exact weight of the product, following formula is used,

1. Avg. potential mol. Wt. of the product,

Cefoperazone /	=	645.67 g/mol /
Cefoperazone sodium		667.7 g/mol

Sulbactum/	=	233.24g/mol /

Sulbactum sodium 255.23g/mol

2. Assay value of the Product, where factor is taken as 0.9265 **Raw material assay value * Factor**
3. Calculation for fill weight,
   Fill weight = label claim * 100 * 100 / Assay * (100 – Water or LOD)
4. Requirement of Raw material, **Fill weight* Batch size / 1000**

BMR (Batch manufacturing record)

The Batch Manufacturing Record (BMR) is an important document in the pharmaceutical business that offers a complete description of the manufacturing process for a given batch of a medicinal product. It is a detailed record that provides all crucial information about the manufacturing process, such as raw materials, equipment, process parameters, and testing data. Here's a full breakdown of the information normally found in a BMR:

1. The BMR should contain general product information such as the product's name, strength, dosage form, batch size, and production date.
2. All raw materials utilised in the manufacturing process, including their names, origins, lot numbers, and amounts, should be listed in the BMR. This data is essential for assuring traceability and spotting any problems with raw materials.
3. All of the equipment used in the manufacturing process, including their names, identification numbers, and calibration status, should be listed in the BMR. This information assists in ensuring that the equipment used in the process is properly maintained and operating properly.
4. The BMR should include a detailed description of the manufacturing process, including all process parameters such as time, temperature, pressure, and pH. This information is crucial for assuring the manufacturing process's consistency and reproducibility.

5. The BMR should include a list of all in-process controls that were executed during the manufacturing process, as well as the outcomes of each check. This data assists in ensuring that the product fulfils the necessary quality requirements.
6. The BMR should include information about the product's packaging and labelling, such as the type of packaging material used, labelling requirements, and the product code or lot number. This information is crucial for ensuring that the product is packaged and labelled accurately.
7. The BMR should include the findings of all finished product tests, such as purity, potency, and stability. This information is crucial for verifying the product's quality and safety.

3.1.4. PACKAGING DEPARTMENT,

- The packaging department plays a critical role in ensuring the quality, safety, and efficacy of pharmaceutical products. Its primary functions include product protection, regulatory compliance, quality control, product identification and traceability, and efficiency and costeffectiveness. Effective packaging is essential for ensuring that pharmaceutical products are safe, effective, and meet regulatory requirements.
- The packaging department at the company included 3 stage packing procedure that is as follow,

  A) Primary packaging

  Include the material that comes directly in contact with the product. Like Vial, seal, GBRSs.

  B) Secondary packaging

  Packaging material that surrounds the primary packaging. Label, carton, and inseit reflox.

  C) Territory packaging

  The final packaging when all the cartons are placed according to their batch no. and production number Seape (box), Shrink.

3.1.5 QUALITY ASSURENCES UNIT,

Quality Assurance (QA) is a vital job in the pharmaceutical industry, ensuring that goods are manufactured in accordance with set quality standards and regulatory criteria. The quality assurance (QA) department is critical in the pharmaceutical sector since it provides oversight, direction, and assistance to all other departments involved in the manufacturing process. Some of the primary responsibilities of QA at a pharmaceutical company are as follows:

a) Establishing and maintaining quality systems: The QA department is in charge of establishing and maintaining quality systems that meet regulatory criteria and ensure that products are manufactured in compliance with set standards. This includes creating and implementing quality rules, processes, and guidelines.

b) Reviewing and approving batch records: The QA department evaluates and approves batch records to ensure that the manufacturing process was carried out in accordance with the approved procedures and that the product fulfils the specified specifications.

c) Auditing: The QA department conducts internal and external audits of all departments engaged in the production process to guarantee compliance with regulatory requirements and internal quality standards.

d) Reviewing and approving change controls: The QA department examines and approves change controls to verify that any modifications made to the manufacturing process or product formulation do not jeopardize product quality or safety.

e) Addressing quality complaints and investigations: The QA department is in charge of addressing quality complaints and investigations relating to product quality issues, as well as ensuring that necessary corrective and preventative steps are done to prevent recurrence.

f) Training and development: The QA department provides training and development to all staff involved in the production process to ensure that they are aware of and understand the quality requirements and processes.

3.2. OVERVIEW OF THE QUALITY CONTROL DEPARTMENT AT SANOLET LIFCARE Pvt. Ltd.

- The role of Quality Control (QC) in Sanolet lifecare is ensuring that products meet the required quality standards, and the manufacturing process is performed in compliance with regulatory requirements. QC is responsible for performing tests and inspections of raw materials, in-process materials, and finished products to verify that they meet the established specifications and are safe for use by patients.
- QC department of the Sanolet lifecare is handled by Mr Soumaya Ghosh Who was at the position of QC manager. Under whom Ms Priyanka was assigned the position of Senior Microbiologist and QC Executive. Who was also appointed for my project training supervision along with Mr.Soumaya Ghosh.
- The Department was packed with advanced instruments to check for the best quality possible in the manufacturing products.
- The whole unit was divide into Labs, HPLC section lab, walk-in incubators, and highly sterile lab area to perform complicate experiments such as sterility analysis of water, Raw material, and product.
- The department along with the performance area, was greatly ventilated by the HVAC system.
- Here are some of the key responsibilities of QC at Sanolet lifecare:
- Testing of raw materials: QC is in charge of examining raw materials to determine their identification, purity, potency, and quality. Excipients, packaging components, and active pharmaceutical ingredients (APIs) are all tested as part of this process.

- In-process testing: QC is in charge of keeping an eye on the production line to make sure that items are made in accordance with approved processes and requirements. Testing of crucial process parameters, intermediate products, and completed goods are all included in this.

- . Finished product testing: QC is in charge of testing finished products to ensure that they fulfil the defined quality standards and requirements. This includes testing for physical, chemical, and microbiological properties.
- Stability testing: QC is in charge of conducting stability testing to assess the shelf life of the product as well as its capacity to maintain quality and efficacy over time.
- Documentation: QC is responsible for keeping accurate and full records of all testing activities, including test results, deviations, and investigations.
- Investigation and troubleshooting: QC is responsible for evaluating any deviations or non-conformances discovered during testing and implementing corrective and preventive actions to prevent recurrence.
- Training and development: Quality control is responsible for providing training and development to testing staff to ensure that they are aware of and understand the quality requirements and processes.

3.3 GOOD MANUFACTURING PRACTICE (GMP) AND ITS IMPORTANCE,

Good Manufacturing Practices (GMP) are a set of principles that ensure pharmaceutical product safety, efficacy, and quality. GMP rules must be followed by pharmaceutical businesses in order to meet regulatory requirements and ensure that their medications are safe for patients to use. Some of the GMPs that should be observed at a pharmaceutical company are as follows:

1. All personnel involved in pharmaceutical product manufacture, testing, packing, and labelling should be suitably trained and qualified. They must adhere to the set procedures and be aware of the quality standards.

2. Manufacturing facilities should be designed, built, and maintained in a way that prevents contamination and assures proper equipment operation.
3. All equipment used in the manufacturing process should be designed, built, and maintained to prevent contamination and to assure precision, dependability, and consistency.
4. All raw materials used in the production process must be of appropriate quality and meet the stipulated criteria.
5. Documentation: All production, testing, and distribution activities should be documented accurately and completely.
6. Quality control: Adequate quality control methods should be performed throughout the production process to ensure that the finished product fulfils the defined specifications and quality standards.
7. Validation: The manufacturing process should be validated to verify that it consistently provides the requisite quality product and that any changes to the process are adequately examined and validated.
8. Complaints and recalls: The Company should have systems in place to manage consumer complaints and product recalls.
9. Storage and distribution: The products should be stored and supplied in proper circumstances to avoid degradation, contamination, or damage.
10. Regulatory compliance: The Corporation should follow all applicable regulatory regulations, including those pertaining to product labelling, advertising, and marketing.

3.4 QUALITY CONTROL TESTS AND PROCEDURES FOR FOLLOWING ANALYSIS,

At Sanolet lifecare, there were following analysis being performed at the QC department,

1. Raw material analysis
2. Packing material Analysis
3. In-process / Finished product analysis
4. Stability analysis of the sample (raw material or product)
5. Microbiological analysis

3.4.1. RAW MATERIAL ANALYSIS,

- Raw material analysis is critical to ensure that the raw materials used in the manufacturing process meet the established quality standards and specifications. Testing typically includes identification, purity, potency, microbial limits, heavy metals, residual solvents, water content, and particle size analysis. The results of these tests help to ensure the safety, efficacy, and quality of pharmaceutical products.
- Identification: To guarantee that raw materials are the correct substance and meet purity and quality criteria, they were identified using proven procedures such as, HPLC analysis.
- Purity: To verify that raw materials fulfil the stated purity criteria, they were evaluated for purity using procedures such as HPLC, GC, or titration.
- Active pharmaceutical ingredients (APIs) were tested for potency using procedures such as HPLC, UV spectrophotometry, or titration to confirm that they fulfil the established potency criteria.
- Microbiological limits: Raw materials was checked for microbiological contamination using MLT analysis.
- Water content: To guarantee that raw materials fulfil the prescribed limitations, water content should be checked using procedures such as Karl Fischer titration. Or LOD was performed for the same.
- Particle size: To verify that raw materials such as excipients satisfy the stated criteria, particle size should be examined using methods such as laser diffraction or microscopy.

3.4.2. PACKAGING MATERIAL ANALYSIS,

The packaging material analysis includes the verification of the materials used to pack and deliver the product to the patient. Verification usually includes visual inspection, Description verification, printing mistake encircling, cracked vial, broken seal or teared up carton, carton without description slips, misprinting or mistake in the description of the chemical content written over the box, etc.

3.4.3. IN- PROCESS / FINISHED PRODUCT ANALYSIS,

The analysis for the same includes, verification of the description , Average weight, pH, Sterility Assay, HPLC assay, and other microbiological analysis.

3.4.4. STABILTY ANALYSIS OF PRODUCT AND RAW MATERIAL,

Stability analysis is a critical aspect of pharmaceutical product development and manufacturing. It is used to evaluate the stability of a drug product under various storage conditions over time. Stability analysis is important because it helps to ensure the safety, efficacy, and quality of a pharmaceutical product throughout its shelf life.

The Value of Stability Analysis:

- Stability analysis establishes a pharmaceutical product's shelf life, which is the period during which the product remains within its approved standards.
- Stability analysis assures that the product's quality qualities, such as purity, potency, and physical properties, are maintained during its shelf life.
- Compliance with Regulatory bodies: Regulatory bodies such as the FDA and EMA demand stability studies to guarantee that the product meets the defined stability requirements.
- Stability analysis aids in the prevention of product recalls by finding any stabilityrelated issues before the product is distributed to the market.

3.4.5. MICROBIOLOGICAL ANALYSIS,

In a pharmaceutical industry, microbiological examination of a sample in QC (Quality Control) entails many tests and procedures to guarantee that the product is free of hazardous germs. The following tests and processes are often used in microbiological analysis:

1. **Sterility testing** is used to verify whether a product is free of live germs. This is normally accomplished by incubating the product in a culture medium for a set amount of time and then watching for signs of growth.

   The sterility testing is done to check for the presences of aerobic, anaerobic, yeast and fungal spores.

The procedure at the Sanolet lifecare was as such, a)

Entry procedure

- Remove the regular clothing
- Primary and secondary gowning
- Entry in the sterility lab
- Switch on the LAF 30 mins prior
- Cleaning and sterilization of LAF with 70% IPA spray.
- Sanitize the tube rack and label the media tubes with name of product, Batch no. and date of performance.

b) Test procedure

- Spray the vials with IPA
- Remove the Flip off seal and again spray with IPA
- Dilute the sample with peptone water or sterile WFI

  [1ml beta-lactam + 100ml Peptone water + product vials (20 Vials in each repetition) → Dissolve and pour in the arranged filtration unit.
- Set the filtration assembly and connect with vacuum pump.
- Rinse 3 times with the peptone water.
- Remove the filter membrane with sterile forceps and cut it into half.
- Dip one halve in SCDM media and other half in FTM media
- Incubate for 7 days to check for the presences of turbidity.

2. **Bio-burden testing** is used to determine the total number of live microorganisms in a given product or raw material.

3. Bacterial endotoxin test (LAL test)

- Endotoxins are a type of Pyrogens that can cause fever and other unpleasant effects in individuals. To guarantee that the product is devoid of endotoxins, endotoxin testing is performed.
- The test is known as LAL test, The Limulus Amebocyte Lysate (LAL) test is a sensitive and specific technique for detecting and quantifying bacterial endotoxins (lipopolysaccharides). (LPS). The clotting response that occurs between the LAL reagent and LPS provides the basis for the LAL test.

- The LAL test works on the idea of using horseshoe crab blood, which includes an enzyme called LAL. This enzyme can recognise and bind to the LPS found in gramnegative bacteria cell walls. When LAL comes into contact with LPS, it sets off a chain of enzymatic events that leads to the creation of a gel-like clot.
- The LAL test is a sensitive and specific method for identifying endotoxins in trace levels. The test's sensitivity is owing to LAL's strong affinity for LPS, and even small levels of endotoxins can cause the clotting reaction. Furthermore, the LAL test is endotoxin specific and does not react with other forms of microbial pollutants.
- In the pharmaceutical sector, the LAL test is commonly used for quality control testing of items such as injectable medications, medical equipment, and dialysis fluids. It is also used in research laboratories to identify and quantify endotoxins in a variety of biological samples.

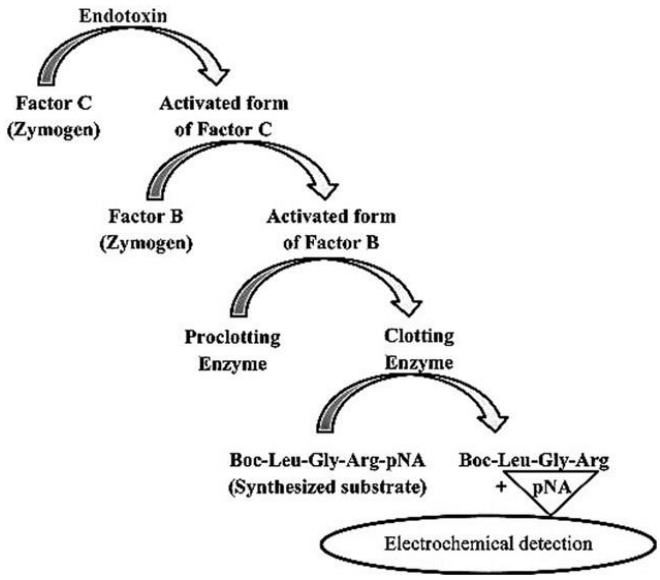


Fig: biochemical pathway of BET test principle.

https://www.researchgate.net/publication/293825120/figure/fig4/AS:748214865113092@1555399733386/The-principle-of-Limulus-amebocyte-lysate-LAL-based-electrochemical-endotoxin-assay.ppm

Results,

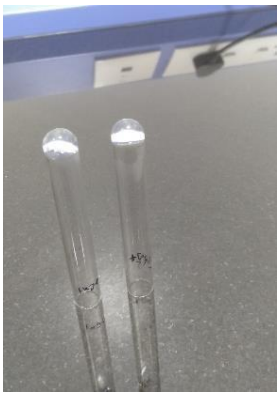



Fig: Results of the Standard tubes of the LAL test, which were positive as gel was formed


Fig: Results of sample (cefoperazone + Sulbactum 1.3gm IP), were negative as no gel was formed

4. Environmental monitoring is routinely inspecting the industrial environment to verify that it is clear of microorganisms that could taint the product. a) Settle Plate Method b) Air Sampler
5. Microorganism identification entails using several ways to determine the type of microorganisms present in a product or raw material. To test whether a product conforms to a set specification for microbiological presences the microbial limits test is performed.

6. raw materials to the finished product and is intended to determine the number of viable aerobic microorganisms present in a product

7. **HPLC ANALYSIS OF THE RAW MATERIAL AND PRODUCT**.

HPLC (High-Performance Liquid Chromatography) is a frequently utilised analytical technique in a pharmaceutical company's QC (Quality Control) unit for the analysis of raw materials and final products. The following are the steps involved in the QC unit's HPLC analysis of raw materials and products:

A) Sample Preparation: The first step in HPLC analysis is sample preparation. The material is weighed and dissolved in a suitable solvent to create a known concentration solution.

B) Preparation of the Mobile Phase: The mobile phase is the solvent that is utilised to transport the sample through the chromatography column. The mobile phase is created by combining two or more solvents in a predetermined ratio.

C) HPLC System Calibration: The HPLC system is calibrated using standard reference substances. This validates that the system is running within the parameters set.

D) Injection of the Sample: Using an auto sampler, the sample solution is injected into the HPLC system.

E) Separation of the Components: As the material passes through the chromatography column, the components are separated. Their chemical features, such as polarity and size, are used to separate them.

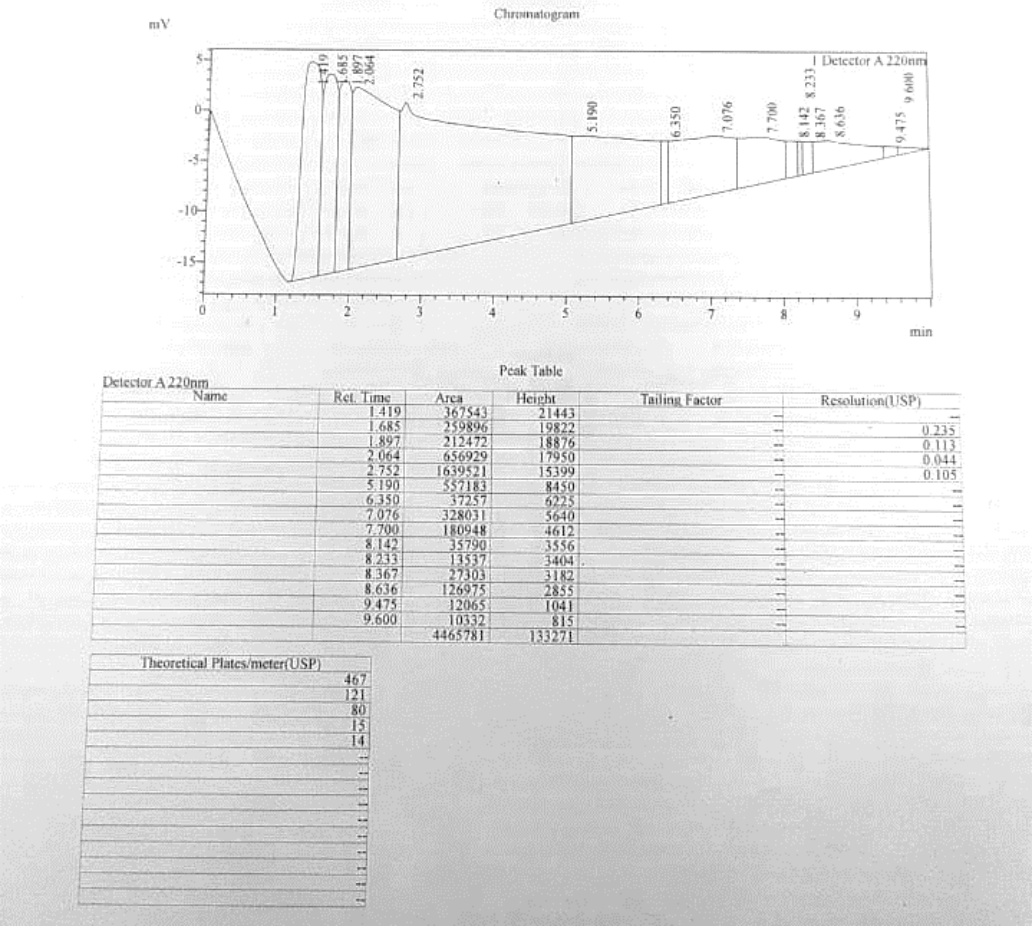
F) Component Detection: The separated components are identified by a detector, such as a UV detector, which measures their absorbance as they move through the column.

G) Data Analysis: The HPLC system's data is analysed to determine the amount of each component contained in the sample. This is accomplished by comparing each component's peak area to that of the standard reference compounds.

Results,

The following Assay graphs were generated from the HPLC chromatographic Analysis of finished product of cefoperazone + Sulbactum Dry powder. Total 6 standard, 2 sample and 1 blank were injected and analysed.

- The HPLC used at Sanolet lifecare was reverse phase HPLC, where mobile phase used was ,4gm tetradecyl ammonium bromide + 4gm tetraheptyal ammonium bromide make up to 100ml with solution A (phosphate buffer + citric acid)

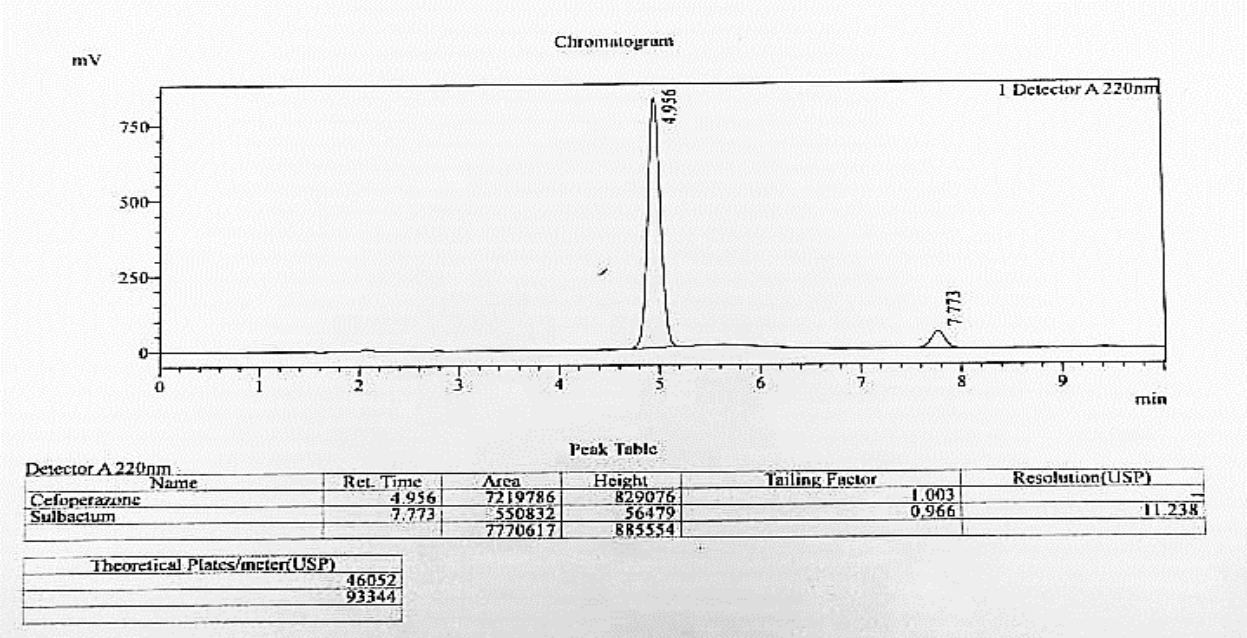


Peak Table

Detector A 220nm

Name	Ret. Time	Area	Height	Tailing Factor	Resolution(USP)	Theoretical Plates/meter(USP)
	1.419	367543	21443			467
	1.685	259896	19822		0.235	121
	1.897	212472	18876		0.113	80
	2.064	656929	17950		0.044	15
	2.752	1639521	15399		0.105	14
	5.190	557183	8450			
	6.350	37257	6225			
	7.076	328031	5640			
	7.700	180948	4612			
	8.142	35790	3556			
	8.233	13537	3404			
	8.367	27303	3182			
	8.636	126975	2855			
	9.475	12065	1041			
	9.600	10332	815			
		4465781	133271			

Fig: Chromatogram of blank for sample (cefoperazone + Sulbactum 1.5gm IP) with Peak table.



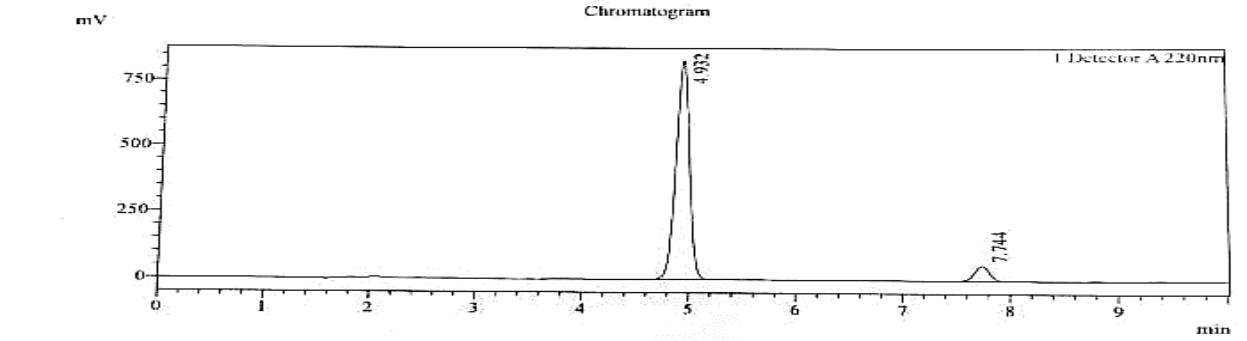
Peak Table

Detector A 220nm

Name	Ret. Time	Area	Height	Tailing Factor	Resolution(USP)
Cefoperazone	4.956	7219786	829076	1.003	
Sulbactum	7.773	550832	56479	0.966	11.238
		7770617	885554		

Theoretical Plates/meter(USP)
46052
93344

Fig : Chromatogram and peak table for Standard 1



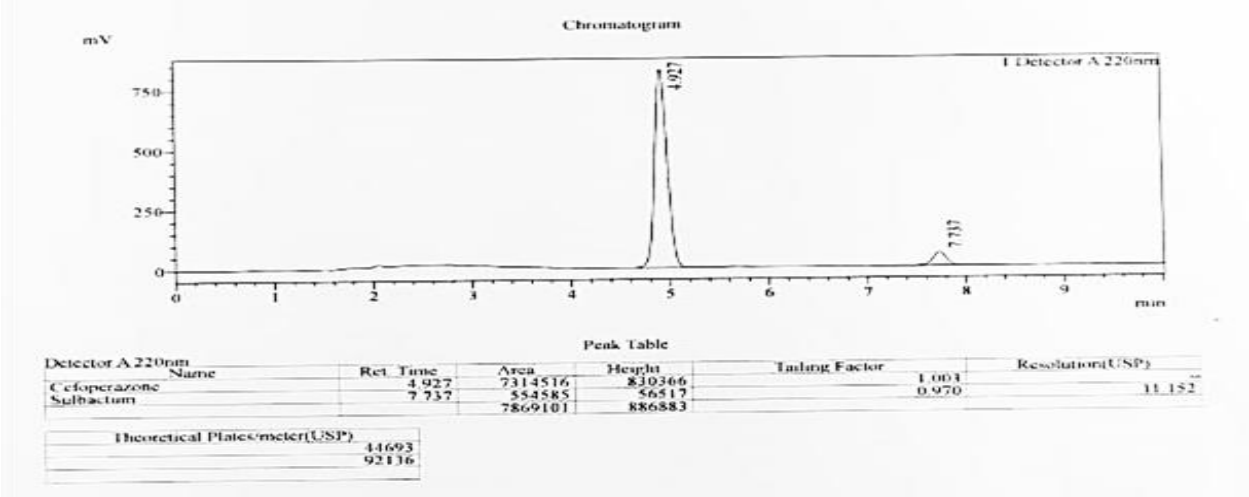
Peak Table

Detector A 220nm

Name	Ret. Time	Area	Height	Tailing Factor	Resolution(USP)
Cefoperazone	4.932	7271235	831116	1.003	
Sulbactum	7.744	562033	56740	0.970	11.15
		7833268	887856		

Theoretical Plates/meter(USP)
45226
91527

Fig: Chromatogram and peak table for Standard 2



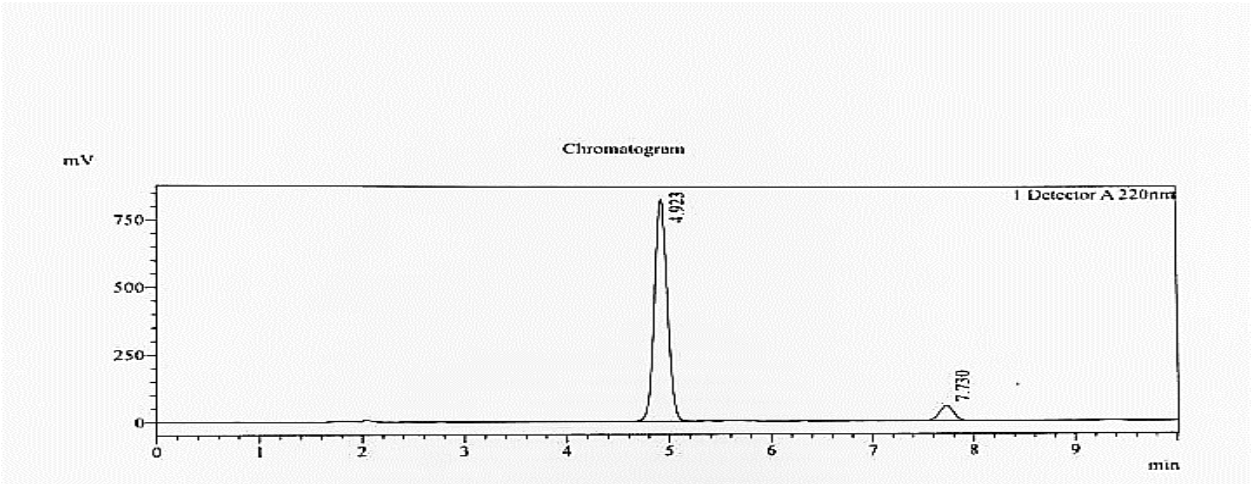
Peak Table

Detector A 220nm

Name	Ret. Time	Area	Height	Tailing Factor	Resolution(USP)
Cefoperazone	4.927	7314516	830366	1.003	--
Sulbactum	7.737	554585	56517	0.970	11.152
		7869101	886883		

Theoretical Plates/meter(USP)
44693
92136

Fig : Chromatogram and peak table for Standard 3



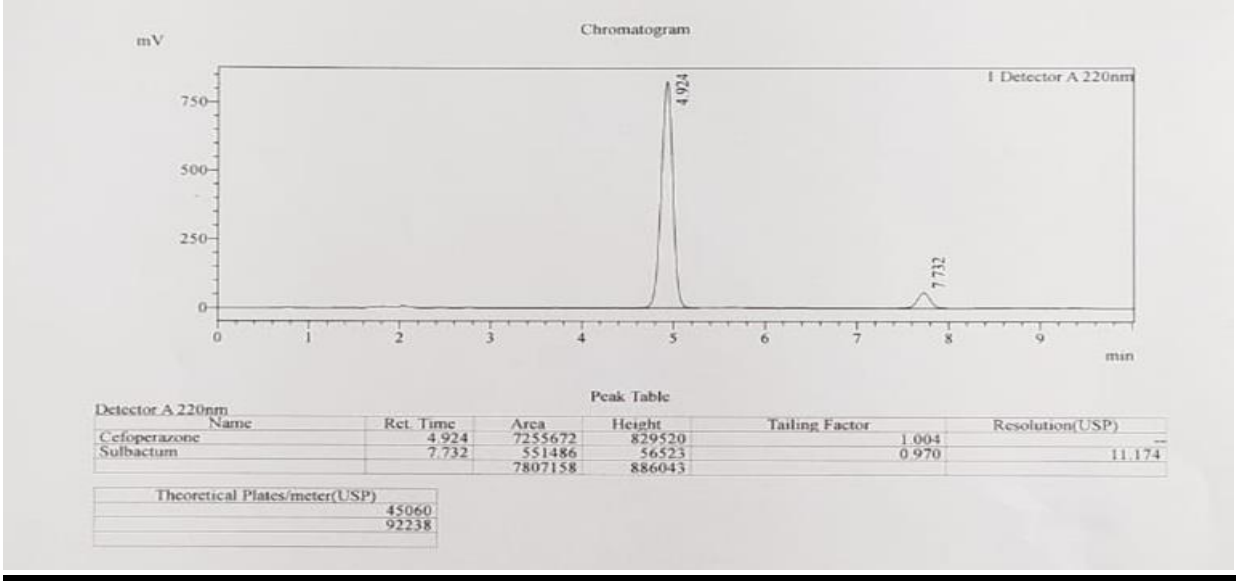
Peak Table

Detector A 220nm

Name	Ret. Time	Area	Height	Tailing Factor	Resolution(USP)
Cefoperazone	4.923	7257042	830190	1.003	--
Sulbactum	7.730	561336	56758	0.970	11.169
		7818377	886948		

Theoretical Plates/meter(USP)
45197
91793

Fig: Chromatogram and peak table of Standard 4



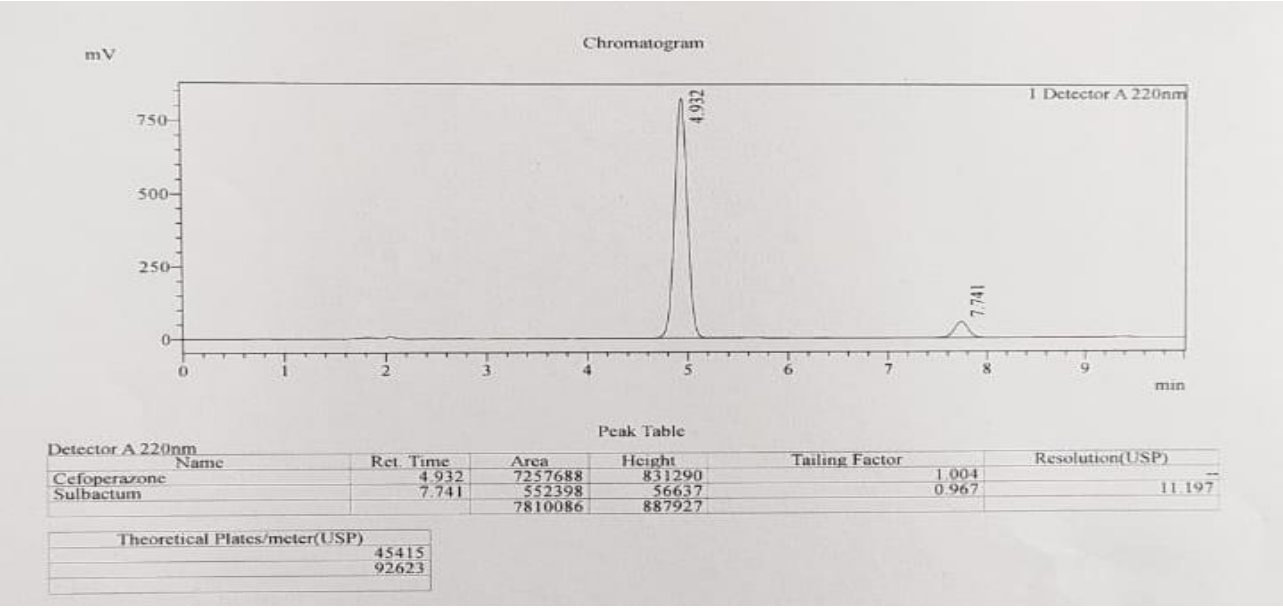
Peak Table

Detector A 220nm

Name	Ret. Time	Area	Height	Tailing Factor	Resolution(USP)
Cefoperazone	4.924	7255672	829520	1.004	--
Sulbactum	7.732	551486	56523	0.970	11.174
		7807158	886043		

Theoretical Plates/meter(USP)
45060
92238

Fig: Chromatogram and peak table of Standard 5



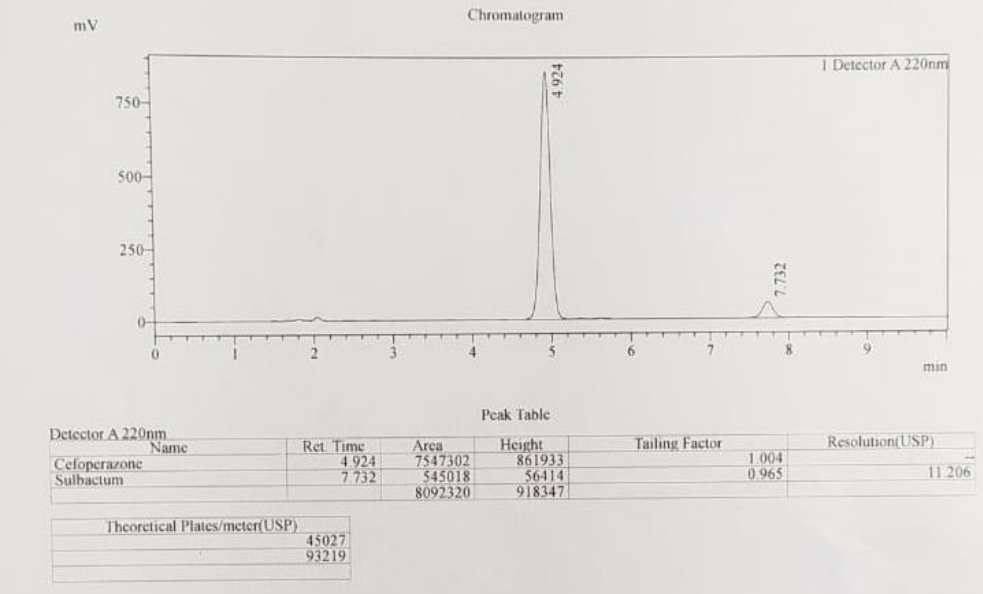
Peak Table

Detector A 220nm

Name	Ret. Time	Area	Height	Tailing Factor	Resolution(USP)
Cefoperazone	4.932	7257688	831290	1.004	--
Sulbactum	7.741	552398	56637	0.967	11.197
		7810086	887927		

Theoretical Plates/meter(USP)
45415
92623

Fig: Chromatogram and peak table of standard 6



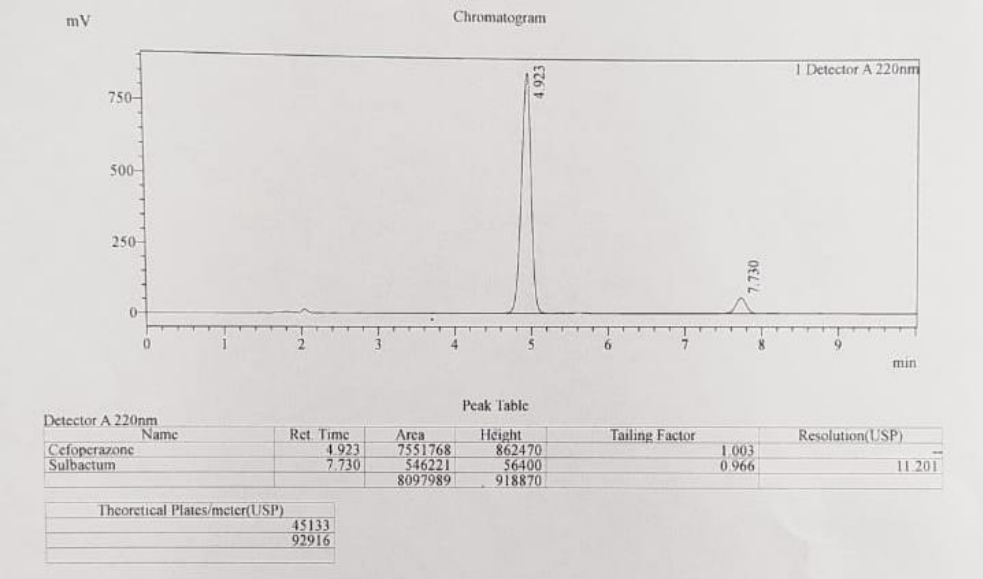
Peak Table

Detector A 220nm

Name	Ret. Time	Area	Height	Tailing Factor	Resolution(USP)
Cefoperazone	4.924	7547302	861933	1.004	
Sulbactum	7.732	545018	56414	0.965	11.206
		8092320	918347		

Theoretical Plates/meter(USP)
45027
93219

Fig: Chromatogram and peak table of sample 1



Peak Table

Detector A 220nm

Name	Ret. Time	Area	Height	Tailing Factor	Resolution(USP)
Cefoperazone	4.923	7551768	862470	1.003	
Sulbactum	7.730	546221	56400	0.966	11.201
		8097989	918870		

Theoretical Plates/meter(USP)
45133
92916

Fig: Chromatogram and peak table of sample 2

TRAINING TIMELINE

- **18th Jan 2023**: joined the Internship program and got a Basic overview of the company.
- **20th Jan to 28th Jan 2023:** worked on literature review read papers and guidelines related to each department.
- **4th Feb 2023:** Introduction to QC, and QA and project allotment.
- **5th Feb to 18th Feb 2023:** Worked in the Production department, learned about production work flow, working of the instruments and management of BMR and BPR.
- **19th Feb to 15th march 2023:** Worked in Quality control unit, performed and learned about different microbiological tests and analysis.
- **16th march to 21th march 2023:** Got a brief introduction to Quality Assurance department.
- **28th mar 2023:** Final presentation submission in the university.
- **30th mar to 16th Apr 2023 :** Worked and learned at the HPLC unit of the QC department
- **18th Apr 2023:** Certification and completion of the internship period.

CONCLUSION

In conclusion, the project report on the manufacturing of Cefoperazone + Sulbactum IP 1.5gm provides a comprehensive understanding of the manufacturing process for this important antibiotic drug combination. The report covers various aspects, including the raw materials used, the manufacturing process, quality control measures, and stability testing.

The production of Cefoperazone + Sulbactum IP 1.5gm involves a complex manufacturing process that requires strict adherence to GMP guidelines to ensure that the drug product meets the required specifications. The use of modern equipment and technology, such as dehydrogenation tunnels, sealing machines, and HPLC systems, is essential in maintaining the quality of the drug product.

The QC measures employed during the manufacturing process, including raw material testing, inprocess testing, and final product testing, ensure that the drug product is safe and effective for human use. Stability testing plays a critical role in ensuring that the drug product remains stable and retains its potency throughout its shelf life.

In conclusion, the manufacturing of Cefoperazone + Sulbactum IP 1.5gm is a complex process that requires strict adherence to GMP guidelines and quality control measures. The successful manufacture of this important antibiotic drug combination ensures that it is readily available to patients in need of treatment for various infections.

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