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RESEARCH PAPER OF DETERMINATION AND ANALYSIS OF CEFOTAXIME SODIUM INJ. BY MICROBIOLOGICAL METHOD IN PHARMACEUTICAL INDUSTRY.

KEYWORDS

MLT	Microbial limit test
BET	Bacterial endotoxin test
EM	Environmental monitoring
%W/W	Percentage Weight/Weight
CFU	Colony Forming Units
LAL	Limulus Amoebocyte Lysate
SCDM	Soyabean Casein Digest Medium
FTM	Fluid thioglycolate medium
R2A	Reasoner‘S 2A Agar
MCA	Macconkey’s Agar
CA	Cetrimide Agar
SDA	Sabouraud Dextrose Agar
LAF	Laminar Air Flow

ABSTRACT

- What is the purpose of testing pharmaceutical products? Testing of pharmaceutical product is intended to ensure they meet stringent safty and quality standards and regulations. The purpose of this testing is not only to save cost and time for the procedure but also to help improve public health
- Water designated for use in injectable product required this testing to ensure the source wateris not adding particulate matter into the final product that could be introduced intravenously antimicrobial agents testing demonstrate the affectiveness of antimicrobial protection
- And the environmental monitoring are important test because patient safety matter the manufacture or pharmaceutical product is performed under strictly controlled condition microbial monitoring is an important part of good manufacturing product regulatory complianceused to prove that the manufacturing process is under control , aspecially in aseptic production
- The microbial limit test this tests to determine mesophilic bacteria and fungi that grow under earobic conditions different incubation temprature and media are required for the growth of bacteria and fungi.

1. INTRODUCTION:

- Microbial contamination of products is one of the most serious issues currently facing the pharmaceutical industry. Drugs, which are administered directly into the circulatory system, bypass a number of innate human immune defences associated with the gastrointestinal system. Therefore, to insure the sterility of each of these products prior to patient administration, pharmaceutical companies must adhere to strict government regulations regarding quality control. Maintaining and following a robust quality control program is integral to quality standards and meeting regulatory requirements.

- Pharmaceutical manufacturing companies are licensed facilities that develop, produce, and market drugs. To ensure the sterility of parenteral drugs, severalquality control methods are employed, including operation under current Good Manufacturing Practices (cGMP), sterility testing and product supplementation with antimicrobial preservatives. When appropriately followed, these process prevent product adulteration and microbial contamination.

- cGMP are Food and Drug Associations that govern all pharmaceutical manufacturing companies. They are intended to assure the proper design, monitoring, and control of all manufacturing procedures to confirm the sterility and quality of products. This includes establishing a reputable management system, obtaining high quality raw materials, upholding controlled operating procedures, identifying product deviations, and maintaining reliable laboratories.

REVIEW OF LITERATURE

- Cefotaxime is a third-generation cephalosporin antibiotic. Cephalosporins, cefotaxime is a broad-spectrum antibiotic with activity against numerous Gram positive and Gram-negative bacteria. It is on the world health organization's Listof essential medicines, list of the most important medication needed in a basic health system. (2009)

MEDICAL USE:

- Given its broad spectrum of activity, cefotaxime is used for a variety of injections, including:
- Lower respiratory tract infections e.g. *pneumonia* (Most commonly caused by *S. pneumoniae)*
- Genitourinary system infections- urinary tract infection (e.g. - *E.coli. S.Epidermidis , P. mirabilis) and cervical / urethral gonorrhea.*
- *Bacteriumia* / *septicaemia*-secondary to *streptococcus spp. S. aureus, E.coli. And klebisella spp.*
- Bone and joint infections - *S. aureus , Streptococcus spp.*
- Intra-abdominal infections - e.g. peritonitis
- CNS infections – e.g. *magningitis/ventriculitis* secondary to *N. megnigitidis*, H. *influenzae S. pneuminiae*
- Although cefotaxime had demonstrated efficacy in these infections , it is not necessarily considered to be first – line agent. In meningitis, cefotaxime crossesthe blood – brain barrier than cefuroxime

MECHANISM OF ACTION:

- Cefotaxime β- lactam antibiotic (which refers to the structural components of the drug molecule itself). As a class, β - lactams inhibit bacterial cell wall synthesis by binding toone or more the penicillin - binding site (PBPs). This inhibit the final tras peptidation stepof peptidoglycan synthesis in bacterial cell walls, inhibiting cell wall synthesis.
- Bacteria eventually lyse due to on going activity of cell wall autolytic enzyme inthe absebse of cell wall assembly. Due to the mechanism of there attack on bacterial cell wall synthesis, β- lactams are considered to be bactericidal.

ADMINISTRATION

- Cefotaxime is administered by intramuscular injection or intravenous infusion. As cefotaxime is metabolized to both active and inactive metabolites by the liver andlargely excreted in the urine dose adjustments may be appropriate in people with renal or hepatic

impairment

MATERIALS AND METHODS

- **Antibiotic**

 The antibiotics used for this study is cefotaxime sodium. It can be collected during the filling of vial in the production area for the testing of BET and sterility.

- **Bacteria and Fungi**

 Culture of *bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Clostridia sporogenosa, Aspergillus niger, Candida albicans, And Escherichia coli* provided by company sops. This culture are required for growthpromotion test.

- **Media and Chemicals**

1. **SCDA**

 Soyabean casein digest agar.
 Its used for environmental monitoring.

2. **DENA**

 Dey-Engley Nutralizing agr.
 Its used for contact plate as well as personal monitoring.

3. **SCDM**

 Soyabean casein digest medium.
 It's used for sterility testing.

4. FTM

 Fluid thioglycolate medium.
 Its used for sterility testing.

5. **LYSATE AND ENDOTOXIN**

 Used for BET testing.

METHODS

1. ENVIRONMENTAL MONITORING:

- For environmrntal monitoring we doing a different five tests

1) Settele plate method
    - Indirect sampling
2) Air sampling plate method
    - Direct sampling
3) Personal monitoring method
    - For allowable persons in sterile area persons gowning sampling

4) Contact swabe and Contact plate method
    - For machine door and sterile area room door handle and sterile area wall direct sampling by rodac plate (55 mm)
    - For these testings we used a SCDA (soyabean casein digest agar) media plate (90 mm) for settle plate and air sampling method and DENA (Dey engley neutralizing agar) media plate (90 mm) for finger dab testing in personal monitoring test and DENA (55 mm) media agar plate for persons gowning sampling and swabe used for machine handle and sterile area wall direct sampling.

2. WATER TESTING:

- For the purpose any microorganisms present in water we were doing a water testing by membrane filtration for pw (portable water) and WFI (water for injection) and we useda R2A media agar plate.
- Also used a pour plate method for RO water and RAW water,
- Mainly check availability of pathogen present in water like *E.Coli., Salmonella, S. auruas, Ps. aeruginosa*
- In which we used a media SCDM for to all Bacterial spp.,MCB and MCA for coliforms.,RVSB and XLDA for *salmonella spp*., MSA for *S. aureus* and CA for *Ps. aeroginosa.*

3. MLT TESTING:

- For MLT(microbial limit test) we used a pour plate method.and also used a spread plate method and membrane filtration method
- SCDA (soyabean casein digest agar)media used for Bacterial spp (TAMC). And SDA (sabourauddextrose agar) media used for Fungal spp. (TYMC)

4. BET TESTING:

- The storage and mixing of samples prior to analysis may affect recovery of endotoxin contamination. Sample (product) bottles should be vigorously shaken prior to analysis, preferably on a vertex. A minimum of 30 sec. to 1 min. on the vertex recommended for each product unit.
- Prior to use in the test, the labeled LAL reagent sensitivity must be confirmed. Prepared a control standard endotoxin dilution series having atlist four concentrations equivalent to 2λ, λ,0.5λ and 0.25λ.
- The gel cloth method is qualitative assey that detects Gram-negative bacterial endotoxin based upon a reaction between lysate and endotoxin which results in a firm clot formation. For samples with endotoxin, the endotoxin amount present in a test sample is calculated by dilutingthe sample to determine the assey end point where a clot does not form. If no clot forms in the verified dilution from the inhibition and enhancement testing, the sample does not contain detectable endotoxin.(2008)

5. STERILITY TESTING:

- For sterility testing we used a membrane filtration methods per compony sop (standardoperation method) also we can used a direct inoculation method .
- We used a media for sterility test FTM (fluid thioglycolate medium) and SCDM (soyabean caseindigest medium) media also used a PW (peptone water).
- After doing this procedure we incubate a media like FTM tube at 30-35ċ temperature for 14days and SCDM tube at 20-25ċ temperature for 14 days.

RESULT AND DISCUSSION

1. ENVIRONMENTAL MONITORING:

- For environmental monitoring in settele plate as per standard indian pharmacopeia limit is

GRADE	TAMC (BACTERIA)	TYMC(FUNGI)
A	<1	<1
B	5	<1
C	50	<1
D	100	<1

- For environmental monitoring in air sampling as per standard indian pharmacopia limit is

GRADE	TAMC (BACTERIA)	TYMC(FUNGI)
A	<1	<1
B	10	<1
C	100	<1
D	200	<1

- For environmental monitoring in contact swab and contact plate as per standard indian pharmacopia limit is

GRADE	TAMC (BACTERIA)	TYMC(FUNGI)
A	<1	<1
B	5	<1
C	25	<1

D	50	<1

(Pharmacopeia, 2016a)

- For environmental monitoring in personal monitoring as per indian pharmacopia limit is

GRADE	TAMC (BACTERIA)	TYMC(FUNGI)
A fingerdab	<1	<1
B fingerdab	5	<1
B garment	5	<1
C garment	20	<1

2. WATER TESTING:

- For purified water limit as per indian pharmacopia is TAMC (total aerobic microbial count) is 100cfu/ml and pathogen is totally absent in water
- For RO water, RAW water and potable water limit as per indian pharmacopia is TAMC (total aerobic microbial count) is 500cfu/ml and pathogen is totally absent.(2016b)

3. MLT TESTING:

- As per indian pharmacopia MLT testing performing in raw material and non sterile material so that standard limit is 10 cfu/ml.

4. BET TESTING:

- As per british pharmacopia and indian pharmacopia BET testing performing in WFI (water for injection) and sterile products. And it's standard limit is NMT 0.25 EU/ml.(2008)

5. STERILITY TESTING:

- As per british pharmacopia and indian pharmacopia in sterility test performing only for sterile products so bacteria and fungi is absent.(1958)

CONCLUSION:

- The above result can be conclude that the purity of the cefotaxime is 98%, it can be conclude on basis of the microbial analysis. The quality cefotaxime sodium injection is good, it can be conclude on basis on microbiological analysis the result of raw material analysis of also in the range now this raw material able to manufacturing the drug. It is a green signal of production department. The result of finish product analysis is in the range now this cefotaxime sodium injection is able to inject in the human. It can be now launch in the market.

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