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ACTIVE PHARMACEUTICAL INGREDIENTS IN STERILE MANUFACTURING: A REVIEW

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ABSTRACT

Active Pharmaceutical Ingredients (API's), used as ingredients in sterile medicinal products, must be sterile unless the final dosage form is terminally sterilized, or produced by a process including a sterilising filtration step. API's intended for use in parenteral products must also comply with relevant specifications on pyrogens or bacterial endotoxins. The manufacture of sterile API's must be strictly controlled in order to minimise the risk of contamination with micro-organisms, endotoxins and particles. If the final dosage form is not to be sterilised by filtration, the API's should be practically free of particles.

KEYWORDS: Active Pharmaceutical Ingredients, Parenteral products etc.

INTRODUCTION

Commonly used API:

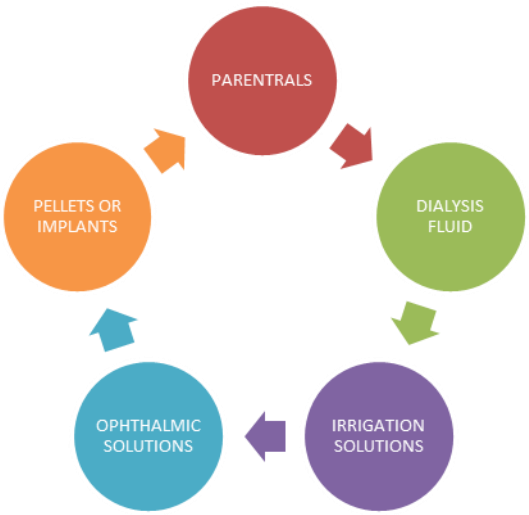
1. **Acyclovir** is an antiviral medicine used predominantly in treating conditions like Herpes Simplex and Herpes Zoster. The chemical falls under the name of acycloguanosine and is marketed through trade names like Zovir, Acivir, Herpex, Cyclovir etc. The drug **Acyclovir** was the precursor to all antiviral treatments, which are available today.
2. Aripiprazole is an FDA approved drug used to treat schizophrenic patients. People with bipolar disorders or manic disorders are given **Aripiprazole** as a suitable treatment.

Severe cases of depression are also treated with the active pharmaceutical ingredients in this drug.

3. Commonly used in the treatment of tonsillitis, sinusitis and pharyngitis cases, **Carithromycin** is the one drug all doctors turn to for such cases. For severe cases of pneumonia or bronchitis the drug **Carithromycin** is prescribed. If there are skin infections or if a person has HIV or AIDS then too **Carithromycin** is prescribed to alleviate the condition. Common trade names under which these active pharmaceutical ingredients are marketed include Clacid, Biaxin, Klabax, Klaricid, Claridar, Claripen, Fromilid, Infex etc.
4. Defination:

- Sterile: Any product / preparations which are free from bacteria or microorganism.
- 2Sterile manufacturing: It means manufacturing of product under hygienic condition which follow the process under strict aseptic condition.
- Parentrals: These are the liquid injectables product should be sterile, non pyrogenic and isoonic with blood plasma and administerd directly in blood system rather than oral route.
- Transfussion fluid: Large volume parentrals is intended by intravenous route are called as transfusion fluid or intravenous infusion.

STERILE DOSAGE FORMS



1. Parentrals

A. Considerations in compounding: Sterile, Pyrogen free, Isotonic, Prepared in hermatic containers, Prepared in environmental controlled area etc.

B. Routes of administration : Intravenous, Intramuscular, Intrarterial, Subcutaneous etc.

C.Official Types of injection

Types	Examples
1.Injection	Insulin injection. USP
2.For injection	Cefuroxime for injection USP
3.Injectable emulsion	Profopol USP
4.Injectable suspension	Methylprednisolone Acetate Suspension USP

D. Vehicles used

Aqueous	Non aqueous
Water for injection	Oil- Arachis oil, Almond oil, Cotton seed oil etc.
Water for injection free from carbondioxide	Alcohols- Ethyl Alcohol
Water for injection free from dissolved air	
Bacteriostatic water for injection	

E. Additives

Name	Example
Antimicrobial Agents	Phenol 0.5%, Cresol 0.3%, Chlorocresol0.2% etc.
Antioxidant	Ascorbic acid, Sodium bisulfite, Thiourea etc.
Buffering Agents	Acetate, Citrate, Phosphate etc.
Suspending Agents	Methyl cellulose, Carboxy methyl cellulose etc.
Emulsifying Agents	Lecithin
Wetting Agents	Sorbitol, Tween 80 etc.
Tonicity Contributor	Sodium Chloride 0.9%, Borax, Sodium Sulphate etc.

F. Methods of Sterilization

1. Dry heat sterilization
2. Steam heat Sterilization
3. Membrane Sterilization
4. GasStrilization

2. Biological Solution

Sterile manufacturing that exerts immunologic effects to develop the immunity from the disease.

Normally packed as small volume parentrals.

B. Storage

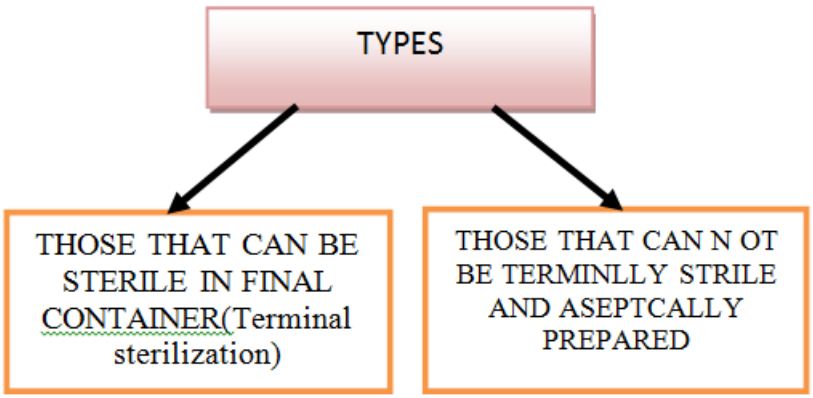
Stored in refrigerator (between temperature 2-8 drgreecelcius).

C. Examples

MMR Virus Vaccine, PolioVirus Vaccine, Tetanus Antitoxoid etc.

Aseptic Process Overview

Two categories of sterile products



Manufacturing Environment

The manufacture of sterile APIs is generally conducted within clean rooms, the grade required (A, B or C) being dependant on the type of processing carried out. Clean rooms should be properly designed and equipped to maintain the relevant air quality both in terms of particulates and microbial levels. Air supplies should be filtered through HEPA (High Efficiency Particulate Air) filters with an appropriate efficiency. The number of air changes should be related to the classification of the room, to the equipment and to the number of persons present in the worst situation. An air pressure differential 10-15 Pascals [higher air change rates may be necessary e.g. when handling API's which generate many particles] is recommended. HEPA filters should be regularly tested for integrity, using a suitable aerosol challenge test. An initial test should be done when the HEPA units are installed, thereafter

integrity testing should be repeated with a suitable frequency. Consideration should be given to the frequency of change of the HEPA filters.

Sanitization of rooms

The sanitization of clean areas is particularly important. They should be cleaned thoroughly in accordance with a written programme. Where disinfectants are used, more than one type should be employed. Microbiological monitoring should be undertaken regularly to check the efficacy of the sanitization and to detect the development of resistant strains. Disinfectants and detergents should be monitored for microbial contamination; dilutions should be kept in previously cleaned containers and should only be stored for defined periods. Disinfectants and detergents used in grades A and B areas should be sterilised prior to use. Fumigation of clean areas may be useful for reducing microbiological contamination.

Class	maximum particles/m^3			
	At Rest	At Rest	In Operation	In Operation
Particle size NMT	**0.5 µm**	**5 µm**	**0.5 µm**	**5 µm**
Grade A	3,520	20	3,520	20
Grade B	3,520	29	352,000	2,900
Grade C	352,000	2,900	3,520,000	29,000
Grade D	3,520,000	29,000	n/a	n/a

Personnel

Only the minimum number of personnel required should be present in clean areas; this is particularly important during aseptic processing. All personnel (including those concerned with cleaning and maintenance) employed in such areas, should receive regular training in disciplines relevant to the manufacture of sterile API's. Training should include reference to hygiene and to the basic elements of microbiology. Staff entering the area who have not received such training (e.g. building or maintenance contractors) must be closely supervised. High standards of personal hygiene and cleanliness are essential. Personnel involved in the manufacture of sterile ingredients should be instructed to report any condition that may cause the shedding of abnormal numbers or types of micro-organisms; periodic health checks for such conditions are necessary. Actions to be taken about personnel who could be introducing undue microbiological hazard should be decided by a designated, competent person. Changing and washing should follow a written procedure designed to minimise contamination of clean area clothing or carry-through of contaminants to the clean areas.

Wristwatches, make-up and jewellery should not be worn in clean areas. The clothing and its quality should be appropriate for the process and the grade of the working area. It should be worn in such a way as to protect the API from contamination, and, if necessary, to protect the operator from exposure to the API.

Clothing required for the individual grades

Grade A/B: Headgear should totally enclose hair and, where relevant, beard and moustache; it should be tucked into the neck of the suit; a face mask should be worn to prevent the shedding of droplets. Appropriate sterilised, non-powdered rubber or plastic gloves and sterilised or disinfected footwear should be worn. Trouser-bottoms should be tucked inside the footwear and garment sleeves into the gloves. The protective clothing should shed virtually no fibres or particulate matter and retain particles shed by the body. Outdoor clothing should not be brought into changing rooms leading to grade A, grade B or grade C rooms. For every worker in a grade A or grade B area, clean sterile protective garments should be provided at each work session, or at least once a day if monitoring results justify this. Gloves should be regularly disinfected during operations. Masks and gloves should be changed at least at every working session. Clean area clothing should be cleaned and handled in such a way that it does not gather additional contaminants that can later be shed. Laundry facilities should not contaminate the garments with particles. These operations should follow written procedures. Separate laundry facilities for such clothing are desirable. Inappropriate treatment of clothing may damage fibres and increase the risk of shedding of particles.

Grade C: Hair and where relevant beard and moustache should be covered. A single or two-piece trouser suit, gathered at the wrists and with high neck and appropriate shoes or overshoes should be worn. They should shed virtually no fibres or particulate matter.

HEPA filters Specifications

HEPA filters remove at least 99.97% of airborne particles 0.3 micrometers (μm) in diameter. The filters maximum resistance to airflow or pressure drop is usually specified around 300 Particals and its nominal flow rate used to prevent the spread of airborne radioactive contaminants. To satisfy the higher and higher demands for air quality in various high technology industries, such as aerospace, pharmaceutical processing, hospitals, health care, nuclear fuels, nuclear power, and electronic microcircuitry (computer chips) Function The common assumption that a HEPA filter acts like a sieve, HEPA filters are designed to target much smaller pollutants and particles. Diffusion predominates below the 0.1 μm diameter

particle size near to the Most Penetrating Particle Size (MPPS) 0.3 μm, both diffusion and interception are comparatively inefficient Biomedical applications of HEPA filters HEPA filters are critical in the prevention of the spread of airborne bacterial and viral organisms and infection. Medical-use HEPA filtration systems also incorporate highenergy ultra-violet light units to kill off the live bacteria and viruses trapped by the filter media.

Five classifications of HEPA filters exist

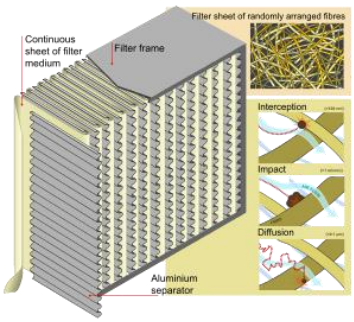
Type A HEPA filters: Also referred to as industrial filters. An efficiency performance of 99.97% retention of particulate matter 0.3 micrometers in size at an airflow of 85 L/minute.

Type B HEPA filters Known as nuclear type are designed to handle nuclear containment. Filters are tested for pinhole leaks, as significant numbers of these leaks lead to an efficiency drop at slower air flows. The test checks for 99.97% retention of particulate matter 0.3 micrometers in size, but at 20% the normal airflow.

Type C HEPA filters Called laminar flow filters due to their mostly exclusive use in biological laminar flow systems, filters are tested for particulate matter of larger sizes. filter has an efficiency of 99.99%.

Type D HEPA filters Known as ultra-low penetration air.an efficiency rating of 99.999% retention of particulate matter 0.3micrometers in size at airflow of 85 L/minute.

Type E HEPA filters Referred to as biological filters. These filters are created with a focus on stopping toxic, nuclear, chemical and biological threats.



Type of HEPA Filter

A. Horizontal Flow (Laminar Flow Hood) 1. Air blows towards worker. 2. Used for non-chemotherapy preparations.

B. Vertical Flow (Biological Safety Cabinet or Chemotherapy Hood)

1. Air blows from top to bottom maintain sterility and protect the worker.
2. Used to make chemotherapy.
3. The HEPA filter is located in the fragile mesh between thin metal strips at the back of the hood behind the HEPA filter screen. 4. Nothing should be permitted to come in contact with the HEPA filter.
5. No cleaning solution, aspirate from syringes, fluids, even if sterile touch HEPA filter, glass from ampoules Good aseptic technique is still required barrier isolators are exempted from placement restrictions of materials within the workspace. Barrier isolator workstations consist of physical structure, internal environment, and interaction technology, monitoring systems.

1. Physical Hard shell or soft shell. Hard shell (plastic, plexiglas, stainless steel) and Soft shell (soft plastic film).
2. Internal Environment Less airflow required to achieve ISO 5 (Class 100) conditions and entering and exiting air is to be HEPA filtered. Isolators for cytotoxic preparations should capture vapour and positive pressure maintained for non-chemotherapy products.
3. Monitoring Systems Gauges to monitor positive pressure environment and surface sampling for contamination. Aseptic Technique Vials and ampules. To prevent contamination. Swab rubber closure with 70% alcohol using firm strokes in the same direction.

LAMINAR AIR FLOW (LAF) SYSTEM High efficiency particle air filtration. "HEPA" filters + Lamination of Air flow. Laminar flow ensures a directional air flow for a distance of 140-200cm Combined by HEPA filters remove particles > 0.3 micron in an efficiency of 99.97% over the aseptic operating field in a uni-direction flow offering. Laminar airflow system should provide a homogenous air speed of 0.45 m/s ±2.0% at the working position. "AN ULTRA CLEAN AIR".

Laminar Flow Clean Air Benches Laminar flow clean air benches used in pharma labs, food (quality control) parenteral feeding. Tissue culture, horticulture, sterile testing, IVF, optics, micromechanics, Electronics industries. Laminar flow benches are specially designed for particulate and bacterial free sterile atmosphere to handle non hazardousnon pathogenic samples, cell & tissue cultures, alimentation controls in microbiology.

Sterilisation of equipment

All process equipment, including pipework, that comes into contact with sterile process materials, should be cleaned and sterilised before use according to validated procedures. This should be done after complete reassembling whenever possible. Sterilisation of equipment by heat is the method of choice. To obtain the highest possible assurance of sterility, sterilisation with steam of assembled equipment ("steam in place") is preferred. A combination of physical measurements and biological indicators should be used to validate the sterilisation process. Equipment sterilisation records, showing that the validation criteria have been met, should be available for each sterilisation run. Results should be recorded (preferably in an equipment log) and the equipment should be status labelled. The validity of the sterilisation procedures should be demonstrated at an established frequency and after significant modification of procedures or equipment.

1 Sterilisation of equipment by moist heat

Each heat sterilisation cycle should be recorded on a time/temperature chart with a suitable scale, or using other appropriate equipment with suitable accuracy and precision. The position of the temperature probes used for controlling and/or recording should have been determined during validation and, where applicable should be checked against a second independent temperature probe located at the same position. All steam sterilisation processes must be validated using a combination of temperature mapping and biological indicator studies. Precautions should be taken against contamination of the sterilised equipment during cooling and prior to use.

Manufacture of sterile active pharmaceutical ingredients

Care should be taken to ensure that steam used for sterilisation is of suitable quality and does not contain additives at a level which could cause contamination of the equipment. The quality of the steam condensate should meet the chemical, biological and particulate standards defined for WFI. Any air admitted to the equipment for cooling etc., must be first passed through a microbiologically retentive filter.

2. Autoclavesterilisation

Both temperature and pressure should be used to monitor the sterilisation process. The autoclave should at least be monitored at the coolest point determined during the validation, normally the condensate drain.

3. Sterilisation using Steam In Place

It is recommended that temperature and if possible pressure are monitored during steam in place sterilisation. Temperature probes should be fitted at representative points in the equipment, normally the coolest points (determined during validation). For equipment fitted with a condensate drain, it may also be necessary to record the temperature at this position, throughout the sterilisation cycle.

4. Sterilisation of equipment by dry heat

Each heat sterilisation cycle should be recorded on a time/temperature chart with a suitable large scale or by other appropriate equipment with suitable accuracy and precision. The position of the temperature probes used for controlling and recording should have been determined during validation, and where applicable, should be checked against a second independent temperature probe located at the same position. The equipment to be sterilised should allow the air in the equipment to circulate and the maintenance of a positive pressure to prevent the entry of non-sterile air. Any air admitted should be passed through a microbiologically retentive filter. Precautions should be taken against contamination of the sterilised equipment during cooling and waiting before use.

Dry heat sterilisation processes are normally validated using a combination of temperature mapping and biological indicator studies. Where the process is intended to sterilise and remove endotoxins, biological indicator studies can be replaced by endotoxin challenge tests.

VALIDATION OF API

Aseptic processes must be validated, and this normally involves the use of process simulation. The setting of acceptance criteria for the validation of an API process is complicated by the small number of product containers filled. Whilst the usual acceptance criteria of <0.1% non sterile units cannot be directly applied, the acceptance criteria set should be based upon sound scientific rationale, and should give an equivalent level of sterility assurance.

- Validation of aseptic processing in closed systems

The sterilisation process of closed systems should be validated using biological indicators and thermocouples. Process simulation may not be necessary for closed systems.

- Validation of aseptic processing in open systems

Validation of aseptic processing in open systems should be validated by means of process simulation tests. Process simulation should imitate, as closely as possible, the routine aseptic manufacturing process and include all critical manufacturing steps. Depending on the design of the equipment, the process can be simulated using the whole equipment in one run or the simulation can be split up into unit operations.

The use of an inert, non inhibiting substance, suitable to be conveyed through the entire installation, is preferred. Process simulation should be repeated at defined intervals and after significant modifications of the equipment and/or the process. Validity criteria for the simulation process should be established on sound statistical grounds, taking into account accepted sterility assurance levels (SAL).

The efficacy of any new procedure should be validated, and the validation verified at scheduled intervals, based on performance history, or when a significant change is made to the process or equipment

PROCESS OF STERILIZATION

1. Terminalsterilisation of API's

Bioburden, endotoxin and particulate levels must be controlled in terminally sterilised APIs. The final steps of processing must be carried out in a grade C environment.

Terminal sterilisation can be accomplished by dry heat, by moist heat and by radiation. Sterilisation procedures and precautions employed should give a "sterility assurance level" (SAL) of 10-6 or better.

Manufacture of sterile active pharmaceutical ingredients.

2. Sterilisation of the API by dry heat

If an API can withstand the lengthy time and high temperature necessary for dry heat sterilisation, this is the method of choice to achieve sterility. The validation of such a sterilisation process should include heat penetration and distribution studies related to cycle times and temperatures. Suitable biological indicators should be used. The effect, if any, of the sterilisation process on the stability and performance of the API must also be established.

3. Moist Heat [steam] sterilisation of the API

Steam sterilisation is an acceptable method of sterilisation for those aqueous API's that can withstand high temperature and high moisture conditions. Clean steam should be used [clean steam has to be made from purified water with a system where the condensate also complies with compendial purified water specifications].

The validation of the sterilisation process should include measurement of heat penetration into the aqueous API solution and temperature distribution. Suitable biological indicators may be used to demonstrate the sterilising properties of the process. The effect, if any, of the steam sterilisation process on the stability and performance of the API must also be established.

4. Sterilisation of the API by radiation

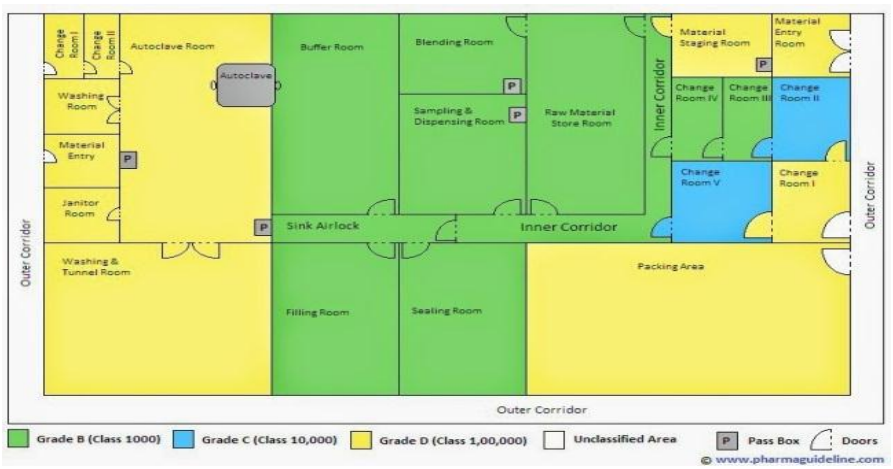
Some heat sensitive ingredients may be resistant to gamma radiation from a suitable radio-isotopic source or a beam of electrons. For this method the reference absorbed dose must be greater than 25 kGy. During sterilisation the radiation absorbed by the ingredient is monitored by means of established dosimetry procedures, independent of the dose rate. When, additionally, a biological assessment is carried out, suitable biological indicators should be used. The radiation procedure must be validated. Validation procedures should include all variations in weights to be used and all types of packaging materials to be used. The effect, if any, of the radiation process on the stability and performance of the API must be established.

5. Sterilisation of the API by gas

Sterilisation by gas e.g. ethylene oxide, or formaldehyde, is not a recognised method of terminal sterilisation. The effect of gas is limited to a treatment of the surface of the active pharmaceutical ingredient. Moreover, there is the possibility of chemical reactions and the risk of residuals.

Sterile API handling

Exposure of sterile API's to the environment (e.g. filling, sampling and dispensing) must be done in a grade A area.



Finishing of sterile active pharmaceutical ingredients

Containers used for sterile APIs, should be sterile, airtight and tamperproof. If the container is intended to be opened on more than one occasion, it must be so designed that it remains airtight after re-closure. Containers for API's should be made of inert, non-shedding, sterilizable, cleanable materials such as glass, plastic, aluminium or stainless steel. The compatibility of each combination of container-closure and ingredient should be demonstrated experimentally. The integrity of the container after filling and during storage should be validated. Such validation should include a microbiological penetration test. The

quality of containers and closures depends on the type of API it will contain and should comply with pharmacopoeial specifications, as appropriate. The cleaning process of the containers and closures should be validated to show a suitable reduction in endotoxins and particulate matter. Aseptically manufactured API's should be filled into their final containers under grade A conditions. Containers should be closed immediately after filling and sampling to avoid contamination and uptake of moisture.

PACKAGING OF PARENTRAL PRODUCTS

Types of containers

AMPULES: OLDEST TYPE OF PARENTRAL PRODUCTS CONTAINERS (MADE UP O F GLASS)

VIALS: GLASS OR PLASTIC CONTAINERS; CLOSED WITH A RUBBER STOPPER SEALED WITH ALUMINIUM CRIP

PRE-FILLED SYRINGES AND CARTRIDGES: FOR QUICKEST ADMINISTRATION

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