VALIDATION OF ANALYTICAL METHODS AND PROCESSES IN STERILE PRODUCTS – A REVIEW

Mohd Abdul Hadi*
Assistant Professor, Department of Pharmaceutics, Nizam Institute of Pharmacy, Deshmukhi (V), Pochampally (M), Behind Mount Opera, Yadadri Bhuvanagiri (Dist)-508284, Telangana, India.

ABSTRACT
The process validation is establishing documented evidence which provides high degree on assurance that a specific process concisely produced a product meeting its predetermined specifications and quality characteristic. According to GMP validation studies are essential part of GMP these are required to be done as per predefined protocols, the minimum that should be validated include process, testing and cleaning as a result such control procedures establish to monitor the output and validation of manufacturing processes that may be responsible for variability of drug product. The validation study provides the accuracy, sensitivity, specificity and reproducibility of the test methods employed by the firms, shall be established and documented. This overview examines the need for pharmaceutical validation, the various approaches and steps involved, and other pertinent considerations.

KEYWORD: GMP, Quality Assurance, Pharmaceutical Validation, Pharmaceutical Process Control.

INTRODUCTION
Sterile products have several unique dosage form properties, such as freedom from micro-organisms, freedom from pyrogens, freedom from particulates, and extremely high standards of purity and quality; however, the ultimate goal in the manufacture of a sterile product is absolute absence of microbial contamination. The emphasis of this chapter will be the validation of the sterilization processes responsible for achieving this goal.

The problem of accidental contamination is a serious yet unavoidable limitation of the sterility test. The Food and Drug Administration (FDA) published guidelines pertaining to general principles of process validation. General concepts and key elements of process validation considered acceptable by the FDA were outlined. A major point stressed in the guidelines was the insufficiency of relying solely on end-product sterility testing alone in ascertaining the sterility of a parenteral of a sterile product lot. Greater significance should be placed on process validation of all systems involved in producing the final product. These major limitations demonstrate that reliance on end-product sterility testing alone in ascertaining the sterility of a parenteral product may lead to erroneous results. One purpose of validation in the manufacture of sterile products is to minimize this reliance on end-product testing. Three principles are involved in the validation process for sterile product.

1. To build sterility into a product
2. To demonstrate to a certain maximum level of probability that the processing and sterilization methods have established sterility to all units of a product batch.
3. To provide greater assurance and support of the results of the end product sterility test.

BASIC PRINCIPLES IN THE VALIDATION OF STERILE PRODUCTS
The key to successful validation in sterile product processing, as in any of type of process validation, is being systematic in the theoretical approaches to validation, the performance of the actual validation experiments, and the analysis and documentation of the validation data.

Theoretical Approaches
Generally, five basic steps are necessary to validate any manufacturing process.
1. Written documentation
2. Manufacturing parameters
3. Testing parameters
4. In-process controls
5. Final product testing

1. Select or define the desired attributes of the product.
Example: The product will be sterile.
2. Determine specifications for the desired attributes.
Example: The product will be sterilized by a sterilization process sufficient to produce a
probability of non sterility of one out of 1 million containers.

3. Select the appropriate processes and equipment. Example: Use microbial kinetic equations to determine the probability of non sterility. Select cleaning equipment and container component.

Procedures designed and validated to reduce the product bio burden to the lowest practical level. Select an autoclave that can be validated in terms of correct operation of all mechanical controls. Use the appropriate types of thermocouples, thermal sensing devices, biological indicators, integrated chemical indicators, and culture media to conduct the validation tests.

4. Develop and conduct tests that evaluate and monitor the processes, equipment, and personnel.

Examples
a. Determine microbial load counts prior to container filling.
b. Determine D and Z values of biological indicator organism.
c. Perform heat distribution studies of empty and loaded autoclave.
d. Perform heat penetration studies of product at various locations in the batch.
e. Examine the test procedures themselves to ensure their accuracy and reliability.

Examples
a. Accuracy of thermocouples as a function of variances in time and temperature.
b. Repeatability of the autoclave cycle in terms of temperature and F value consistency.
c. A challenge of the sterilization cycle with varying levels of bio indicator organisms.
d. Reliability of cleaning processes to produce consistent low-level product bioburden.

Each validation process should have a documented protocol of the steps to follow and the data to collect during the experimentation. As an example, App. I present a protocol for the validation of a steam sterilization process. Upon completion of the experimental phase of validation, the data are compiled and evaluated by qualified scientific personnel. The results may be summarized on a summary sheet, an example of which is shown as follows.

Once a process has been validated, it must be controlled to assure that the process consistently produces a product within the specifications established by the validation studies. Documentation should present original validation records, a schedule of revalidation dates, and data from the revalidation studies. The interval between validation studies strictly depends on the judgment of the validation team based on the experience and history of the consistency of the process.

**The sterilization methods used for sterile products.**
There are five basic methods

1) Heat
1. Moist heat (steam) = saturated steam under pressure = autoclave.
2. Dry heat = oven or tunnel.

2) Gas
1. Ethylene oxide.
2. Per acetic acid.
3. Vapor phase hydrogen peroxide.

3) Radiation
1. Gamma.
2. Beta.
3. Ultraviolet.
4. Microwave.

4) Light
1. Pure Bright.

5) Filtration

**VALIDATION OF STEAM STERILIZATION CYCLES**

**Qualification and Calibration**

A. Mechanically Checking, Upgrading, and Qualifying the Sterilizer Unit.

The main concern with steam sterilization is the complete removal of air from the chamber and replacement with saturated steam. Older autoclaves relied on gravity displacement. Modern autoclaves use cycles of vacuum and steam pulses to increase the efficiency of air removal. Autoclaves can also involve air-steam mixtures for sterilizing flexible packaging systems and syringes. Whatever autoclave system is used, the unit must be installed properly and all operations qualified through installation qualification and operation qualification (IQ/OQ). Utilities servicing the autoclave must be checked for quality, dependability, proper installation, and lack of contamination. The major utility of concern here is steam. All equipment used in studying the steam sterilizer, such as temperature and pressure instrumentation, must be calibrated.
B. Selection and calibration of thermocouples.
Thermocouples obviously must be sufficiently durable for repeated use as temperature indicators in steam sterilization validation and monitoring. Copper constant and wires coated with Teflon are a popular choice as thermocouple monitors, although several other types are available. Accuracy of thermocouples should be 0.5°C. Temperature accuracy is especially important in steam sterilization validation because an error of just 0.1°C in temperature measured by a faulty thermocouple will produce a 2.3% error in the calculated $F_0$ value. Thermocouple accuracy is determined using National Bureau of Standards (NBS) traceable constant temperature calibration instruments such as those shown in Figure 2. Thermocouples should be calibrated before and after a validation experiment at two temperatures: 0°C and 125°C. The newer temperature-recording devices are capable of automatically correcting temperature or slight errors in the thermocouple calibration. Any thermocouple that senses a temperature of more than 0.5°C away from the calibration temperature bath should be discarded. Stricter limits (i.e., <0.5°C) may be imposed according to the user’s experience and expectations. Temperature recorders should be capable of printing temperature data in 0.1°C increments.

C. Heat distribution studies
Heat-distribution studies include two phases.
(1) Heat distribution in an empty autoclave chamber and.
(2) Heat distribution in a loaded autoclave chamber.

Between 10 and 20 thermocouples should be used per cycle. Thermocouples should be secured inside the chamber according to a definite arrangement (e.g., see Fig. 3): Teflon tape can be used to secure thermocouples.
Studied in heat sterilizers.
The trips where the wires are soldered should not make contact with the autoclave interior walls or any metal surface. One thermocouple each should remain in an ice bath and high temperature oil bath during each cycle for reference when the temperature monitoring equipment has the capability for electronically compensating each temperature measurement against an internal reference. Heat-distribution studies following the initial study may employ fewer thermocouples as the cool spot in the chamber and in the load is identified. The key is to identify on a reproducible basis the location of the cool spot and the effect of the load size and/or configuration on the cool spot location. Most experts suggest the study of the minimum and maximum load size in the proper configuration in elucidating where the cool spot is located.

The difference in temperature between the coolest spot and the mean chamber temperature should be not greater than ± 2.5°C. Greater temperature differences may be indicative of equipment malfunction differences may be indicative of equipment malfunction.

VALIDATION OF STERILIZING FILTERS
A. Introduction to Filtration
The following definitions will be helpful in using this section. When filter is used as a verb (“to filter”) it means to pass a solid–liquid mixture through a permeable medium to cause a separation of the two. Filter when used as a noun refers to a device for carrying out filtration, and it consists of the filter medium and a suitable holder for constraining and supporting it in the fluid path. The permeable material that separates solid particles from the liquid being filtered is called the filter medium. The unit operation of filtration, then, is the separation of solids from a liquid by passage through a filter medium. In many instances, the filter, including the permeable medium, the means for passing liquid through the medium, and the process piping, are all referred to by the term filter system. In general, filtration objectives can be separated into four basic categories: to save solids and reject liquids, to save liquids and reject solids, to save both liquids and solids, and to reject both liquids and solids. As a filtration process proceeds, generally under an applied driving force of pressure, solids are removed by and begin to accumulate on the filter medium. The liquid portion continues to move through the filter medium and out of the filter system. The separated liquid is referred to as the filtrate. The amount of pressure applied to accomplish the filtration depends on the filtration resistance. Filtration resistance is a result of the frictional drag on the filtrate as it passes through the filter medium and the accumulated solids. In equation form,

\[
\text{Filtration rate} = \frac{\text{pressure resistance}}{\text{permeability}}.
\]

Retention \times \text{permeability} = \text{constant}.

B. Sterile Filtration
Production of parenteral drugs requires that the product be sterile. In many cases, terminal sterilization by heat, ethylene oxide gas, or ionizing radiation is used to render a product sterile; however, certain products are not stable when exposed to heat, gas, or radiation and they must be sterilized by other means. Filtrative sterilization is suitable in such cases. Indeed, the practice of sterile filtration is not limited to labile preparations. Unlike the other forms of sterilization, filtration sterilizes by the removal of the bacteria from the product rather than by inducing lethality to the micro-organism. Filtration is straightforward and reliable; it removes particulate matter other than microbiological; it avoids possible pyrogenicity owing to the presence of dead bacteria in the dosage form; it is cost effective and energy efficient; and it allows convenient and flexible manufacturing systems and schedules with low capital investment. Sterile filtration processes are employed to sterile-filter a product prior to filling it aseptically into its final containers. Bulk drug solutions are sterile-filtered prior to aseptic crystallization, thus eliminating the possibility of having organisms within the bulk drug crystals. The bulk drug can then be processed into a dosage form aseptically or further processed to be terminally sterilized. Other filtrative operations reduce the organism content of a final product prior to terminal sterilizations.

C. Filter Qualification
Technical report no. 26 from the Parenteral Drug Association identifies the following factors that should be part of selecting and qualifying a filter for use as a product sterilizing filter:
1. Particle-shedding characteristics
2. Extractables
3. Chemical compatibility
4. Adsorption
5. Thermal stress resistance
6. Hydraulic stress resistance
7. Toxicity testing
8. Bacterial challenge testing
9. Physical integrity testing

Physical integrity testing has already been discussed. Subsequent discussion will focus on extractable and bacterial challenge testing.
D. Bacterial Challenge Test
Microbiological challenging of a filter is the only true means of determining the bacterial retention properties of the system. Such a test is sensitive because of the large number of organisms used and because the organism self-replicate and allow even low numbers of bacteria that might pass through a filter system to make themselves known. Filter media are not repetitive-use items and although used for more than one lot in production, the media are usually discarded after some predetermined number of uses or time. Therefore, it is impossible to test every filter medium individually, since the challenge test is a destructive test. The nondestructive tests, therefore, require a high degree of correlation with a retention test. When such correlated tests are established and controls maintained, filtration users can depend on filtration to produce a sterile parenteral product. The level of sensitivity of the challenged test is dependent on the challenge organism, culture environment of the organism, challenge level of the organism, test volume filtered, challenge rate or the duration of the challenge test, and pressure used during the challenge test.

E. Extractables
Filter validation now includes tests to prove that sterilizing filters do not generate extractable materials when exposed both to water and to the drug product formulation. Tests for filter extractables may be found in the USP, Section <87> Biological Reactivity Tests, in Vitro and Section <88> Biological Reactivity Tests, in Vivo. These tests involve soaking filter material in different solvents, then evaluating them in two animal models and in cell culture. USP Section <661> also describes testing of filters to ensure that no extraneous contaminants are found in the filter material. Filter extracts have been identified as surfactants, wetting agents, additives used in filter manufacture, higher molecular weight polymers of the filter polymer, and general particulates. Extraction procedures with actual drug product may include immersing the filter into the drug product solution, then exposing it to high temperatures and mechanical agitation before taking samples and assaying by various analytical techniques.

F. Retention Efficiency
In the past, several terms have been coined to describe the retention efficiency of the filter system: beta value, microbiological safety index, reduction ratio, and titer reduction ratio. The log reduction value (LRV) is a filter retention efficiency term that is the logarithm to the base of 10 of the ratio of the number of organisms in the challenge suspension to the number of organisms in the filtrate.

\[
\text{LRV} = \log \frac{N_0}{N} \quad (26)
\]

Where,

\[
\text{LRV} = \log \text{reduction value}
\]

\[
N_0 = \text{number of organisms in the challenge}
\]

\[
N = \text{number of organisms in the filtrate}
\]

G. Aseptic Processing
Aseptic fill processes are validated by simulating production conditions and using a bacterial culture medium as the product. This process simulation test is commonly referred to as a “media fill.” Production facilities must be checked to ensure that all installed equipment both satisfies the engineering and quality design criteria (installation qualification) and functions properly (operational qualification). In the performance of a media fill, it is important that everything be conducted just as a normal production run. All equipment normally used should be used. All equipment should be cleaned, sanitized, sterilized, handled, and assembled in a normal manner. All personnel normally involved in an aseptic process must participate in the media fill. Such personnel must have sufficient training in such areas as basic microbiology, personal hygiene, gowning techniques, manipulative techniques, safety, and cleaning procedures.

Table 10 provides a list of considerations for ensuring that every aseptic process is appropriately simulated during a media fill validation exercise. Media fills are conducted to initially qualify a new filling line, a new product, and/or a change in product container configuration.

1) Duration of longest run
2) Multiple runs on separate days
3) Worst-case environmental conditions
4) Number and type of interventions, stoppages, adjustments, transfers; both planned and unplanned (e.g., replacing filling needles, pumps, filters, stopper bowl stopping line, removing all containers, manual stoppering)
5) Aseptic assembly of equipment Maximum number of personnel normally present Number of aseptic additions Shift breaks, changes, multiple gowing
6) Number and type of aseptic equipment disconnections and connections
7) Aseptic sampling Line speed and configurations
8) Manual weight checks
9) Operator fatigue (work time)
10) Container/closure types run on the line
11) Temperature and relative humidity extremes
12) Conditions permitted before line clearance
13) Container/closure surfaces that contact formulation during aseptic process.

H. Facility Design and Construction
The Good Manufacturing Practice (GMP) regulations, FDA, and European Economic Community (EEC) guidelines on aseptic processing, and other documents provide comprehensive details on facility requirements for sterile drug production. The facility must.

1. Use HEPA filters for filtering the air supply to reduce or eliminate particulate contaminants.
2. Maintain higher air pressures (positive pressure) within the critical areas to minimize infiltration of airborne contaminants from outside air.
3. Provide smooth, easily cleanable surfaces on equipment, floors, walls, and ceilings to minimize the opportunity for collection of particulates and growth of micro-organisms.

I. Utility Qualification

Facility design is critical. Likewise, individual utilities require qualification. The most important of these are heating, ventilation and air conditioning (HVAC), water (including clean steam) and compressed gases. Typical programs begin with installation qualification (IQ). The IQ is described in a written protocol that contains the following key elements:

1. Equipment or system specifications
2. Spare parts list
3. As-built drawings
4. Wiring diagrams
5. Piping and installation
6. Installation certification statement

Following completion of the IQ, the equipment or system is subjected to operational qualification (OQ). This is a more rigorous exercise in which the object is to ascertain that the equipment or system being tested performs in accordance with design specifications throughout the full operational range(s).

The OQ protocol contains

1. A full system description
2. Calibration certification documents
3. Testing plans
4. Acceptance criteria
5. Full record of testing results
6. Certification statement

a. Heating, Ventilation and Air Conditioning (HVAC)

Features of the HVAC system that affect product quality (sterility) and therefore require qualification include:

1. HEPA filters integrity
2. Airborne particle control
3. Airflow direction
4. Room air pressure differentials
5. Temperature and humidity control

A popular method for certifying the integrity of the filter installation uses a poly disperse aerosol, created by blowing air through liquid (e.g., poly-alpha-olefin) introduced into the upstream ductwork, followed by scanning the entire downstream side of the filter face and periphery with a probe nozzle of an aerosol photometer. This testing will identify “leaks” caused by damage due to mishandling or faulty construction. Small leaks can be repaired with a suitable silicone-based compound without removing the filter.

b. Water

Water quality is usually defined in terms of chemical and bacteriological purity, particulate matter content and endotoxin levels. Potable water is normally from the municipal water system, which may have been treated with chlorine to control microbiological growth. Soft water and deionized water have undergone ion exchange or similar treatment to eliminate unwanted ionic species, such as Mg2+ and/or Ca2+. Purified water, water for injection, and other types of water meeting compendia specifications are produced by ion exchange, reverse osmosis, distillation, or a combination of such treatments. The validation protocol provides a detailed description of sampling locations and requirements, testing methodology and test limits or specifications. Sampling and testing can be performed daily during qualification and validation. When the system is in routine use, following the validation the testing frequency can be reduced to a weekly schedule for monitoring purposes. An action guideline of not more than 10 CFUs/100 ml for bacteriological purity is suggested. As with the purified water system, the sampling and testing frequency for the water for injection (WFI) system is defined in the protocol and can be reduced after the system is qualified and validated.

c. Compressed Gases

Various kinds of compressed gases (e.g., nitrogen, oxygen, and carbon dioxide) may be found in the sterile drug manufacturing plant; however, as an example only compressed air will be discussed.

Compressed air is one of the utilities that may have director incidental product contact and therefore requires qualification. The types of contaminants found in compressed air, not surprisingly, are the same as those found in the ambient environment. These may include microorganisms (e.g., bacteria, molds and viruses), moisture, particulate matter and possibly pyrogens. Undesirable levels of hydrocarbons from compressor lubricants maybe found if the compressor is not of the oil-free type. A well designed compressed air system eliminates or substantially reduces the levels of these contaminants. Components of such a system include the following:

1. An oil-free compressor — typically a rotary screw, multiple stage design.
2. An oil coalescing filter to trap any liquid hydrocarbons or water.
3. A dryer to remove condensed moisture and reduce levels of gaseous hydrocarbons.
4. A filtration unit to eliminate gross particulate matter, such as fibers and metal particles.
5. A sterilizing filter rated at 0.2µm
6. A sanitary design receiver tank and distribution piping’s loped for proper drainage.
7. Instrumentation suitable for monitoring the temperature, pressure and volume or flow rate in the system.

J. Equipment Qualification/Validation

1. Container Preparation

Parenteral drug containers are typically fabricated from glass (bottles, vials, syringes, or ampoules) or plastic (bottles, bags, vials, or syringes). Regardless of the
nature of the container, contaminating substances such as paper fibers, glass fragments, viable microbes and pyrogenic materials must be eliminated from the containers before they are used in the filling operation. The suitability of the design and utility services is established during the IQ and OQ phases of qualification discussed earlier in this chapter. Important criteria for a typical washer include the following. Water: quality, temperature, pressure, and Flow rate Steam: quality and pressure Compressed air: quality and pressure

The duration of the prewash, washing, final rinse and flush cycles must be established during validation and maintained within suitably narrow ranges to ensure repeatability.

2. Closure Preparation
The most common type of primary closure used in conjunction with glass containers for parenteral drugs is the elastomeric closure. As with the container itself, the closure must be sterile, pyrogen-free and free from contaminants that could adulterate the drug substance, because the closure is likely to be in direct contact with the drug at some time during the storage, handling, or use of the dosage unit.

The validation of any cleaning procedure must therefore include testing for residual endotoxin, particulate matter, and any adventitious contaminant determined during the pretreatment examination. Achieving sterility during the cleaning cycle is not an absolute requirement; however, the bioburden remaining should not present a significant challenge to the subsequent sterilization process and should be considered in the development of those treatments.

3. Filling Equipment
Validation protocols for filling accuracy should specify the number and duration of filling runs for each size and fill configuration, the filling rates and the limits for filling variability considered acceptable to the manufacturer. The purpose of the validation work is to determine a filling configuration (i.e., line speed, fill quantity, and container size combination) that will provide the optimum line speed while maintaining acceptable filling variability. Generally, the higher the filling rate, the poorer the filling accuracy.

4. Sealing/Capping Equipment
Adequacy of the container-closure system is determined through stability studies during the development work and is not the subject of the validation project for the equipment. It is the objective of this phase to demonstrate that the sealing/ capping equipment will consistently apply the over cap in such a manner that the integrity of the unit is ensured. Container-closure integrity studies also can be conducted to validate the sealing efficiency of the capping equipment.

5. Lyophilization
1) During the OQ the following specialized checks should be conducted.
2) Maximum chamber vacuum under no load.
3) Chamber leak rates under vacuum and pressure.
4) Shelf temperature control (i.e., temperature variation).
5) Vacuum pumping rate.
6) Chamber heating and cooling rates under no-load conditions to establish a reference point for future study.
7) Condenser cooling rate Refrigerant integrity test to verify that coolant does not leak into the chamber.
8) Condenser drying rate to establish the maximum drying rate of which the unit is capable.

K. Environmental Qualification
The effort spent in qualification and validation of the utilities, equipment and processes that make up a sterile product manufacturing operation is wasted unless the manufacturing environment is maintained under control at all times during production. The environment of an aseptic filling operation must be monitored and controlled. Environmental control begins with valid cleaning and sanitization procedures, then proceeds with adequacy of certified HEPA filtration and clean room procedures by personnel within the clean room and is verified by environ- mental monitoring techniques. Such techniques include nonviable particulate monitoring of the air (electronic particle counters), surface sampling of equipment and personnel (Rodac plates primarily; sometimes swab samples) and airborne viable particulate monitoring (fallout or settling plates, and quantitative air samplers such as rotary centrifugal samplers or slit-to-air samplers).

5. REFERENCES