Journal of General Medicine

Research Article

An Over View of Sterile Filtration Validation: A Key Elements for Sterile Drug Product Manufacturing

Prashant Khemariya[1]*, Elango Minnoor[2], and Richa Khemariya[3]

[1]PhD Scholar, Rabindranath Tagore University, India

[2]Department of Chemistry, University of Iowa, India

[3]Pharmaceutical Quality and Regulatory Consultant, India

Abstract

Sterile filter validation is an important process in the pharmaceutical, biotechnology, and healthcare industries to ensure the effectiveness and integrity of sterile filtration systems. The validation process involves testing and verifying the performance of sterile filters to ensure they can effectively remove microorganisms and particles from a fluid stream while maintaining sterility. Selecting a sterilizing grade filter requires consideration of many important issues, such as materials of construction and their compatibility with the product to be filtered. The processes requirements and validation need differ based on the filtration requirement. Validation has always been a key aspect of assuring sterility of the final formulation produced under the aseptic conditions. As sterilizing-grade filters play a major role in obtaining a high sterility assurance level, validation of these filtration processes has become a subject of increasing awareness and regulatory scrutiny. The bacterial challenge test serves on the major functions. the filter manufacturer uses it to classify filters as sterilizing grade if the filter provides a sterile effluent with a minimum of 10^7 cells of *Brevundimonas diminuta* ATCC 19146/cm^2 of effective filter surface area. Therefore, the design, validation and ongoing monitoring of a sterile filtration system are essential for assuring the quality and safety of the pharmaceutical product. Proper understanding and testing of the sterile filtration system according to international regulatory standards is important for both the validation and ongoing monitoring of the system.

Introduction

Conferring to Transparency Market Research, the demand for sterile injectable drugs has been on the rise in recent years and will continue to do so. Expansion of the market for biologic drugs, which can only be injected, is a major driver [1]. The markets have been affected very differently by the COVID-19 pandemic. While vaccines and antiviral drugs against the virus are generating additional revenue, and the development of mRNA drugs has received a boost, most pharmaceutical fields have been negatively impacted, mainly due to fewer new patients and barriers to healthcare access [2].

Sterility is critical and one of most important quality parameters for injections, parenteral formulations, biologics and many others. It involves the removal of microorganisms and particulate matter from liquids or gases to achieve sterility. The primary purpose of sterile filter validation is to demonstrate and document the effectiveness of the filtration process in achieving and maintaining sterility. The validation process encompasses various tests and assessments to ensure the filter's reliability, efficiency, and consistency in removing contaminants.

The regulatory agencies as like USFDA, Eudralex EU GMP and PDA [3-7], have been evolving with increased demands from pharmaceutical and biotech companies to improve patient safety. According to the Food and Drug Administration (FDA, USA) and the United States Pharmacopoeia (USP), sterile filtration is defined as a nominal rating of 0.2 µm and 0.22 µm respectively to produce sterile effluent. Solid pharmaceutical dosage forms have been used since ancient times [8-16,13], where the sterile filteration is not required but maintaining GMP environment is a key parameter.

In the early 1960s, 0.45 µm -rated membranes were considered "sterilizing grade" filters, and were used successfully in the sterilizing filtration of parenteral product. These filters were used to qualified using 0.6 X 1.0 µm *Serratia marcescens* (a species of rod-shaped, Gram-negative bacteria from *Yersiniaceae* family), a standard bacterium for qualifying analytical membranes used for water quality testing. After that in the mid-1960s, Dr. Frances Bowman observed a 0.45 µm "sterile-filtered" culture medium to be contaminated with an organism, subsequently shown to penetrate 0.45 µm-rated membranes repeatedly in small numbers at challenge levels above 10^4 to10^6 per cm^2 also observed that the next finer grade commercial membrane (nominally 0.22 µm) effectively retained this organism at similar challenge levels. This 0.3 X 0.6-0.8 µm contaminant was identified as *Pseudomonas diminuta* (currently re-classified as *Brevundimonas diminuta*), and registered with the American Type Culture Collection (ATCC) as Culture No. 19146. Now a day *Brevundimonas diminuta* is accepted broadly, FDA incorporated

Submitted: 24 November 2023 | **Accepted:** 08 December, 2023 | **Published:** 11 December, 2023

***Corresponding author:** Prashant Khemariya, PhD Scholar, Rabindranath Tagore University, Chiklod Road, Bhopal 464993, Madhya Pradesh, India

Citation: Khemariya P, Minnoor E, Khemariya R (2023) An Over View of Sterile Filtration Validation: A Key Elements for Sterile Drug Product Manufacturing. J Gen Med 4: 6.

demonstration of its retention in the definition of a sterilizing filter.

It is widely believed that filters work by permitting fluid passage through their pores, retaining particles too large to fit through these apertures. Figure 1 showing the sterile filter step during the manufacturing process (downstream) of any parenteral product.

Sterilization

There are numerous sterilization methods depend on product, container and closure. Sterilization by filtration is also called as "Cold Sterilization", because it doesn't require temperature or other form of energy to destroy microorganisms. Basically, sterilization by filtration does not kill or destroy the microorganisms but it eliminates microorganism from the product. Sterilization via filtration is the only option if the other processes are not suitable for the specific product or component. USFDA explains in the guidance about Terminal sterilization and an aseptic process [15,17].

Terminal sterilization – this process usually involves filling and sealing of drug product containers under high-quality environmental conditions. Products are filled and sealed in this type of environment to minimize the microbial and particulate content of the in-process product and to help ensure that the subsequent sterilization process is successful. In most cases, the product, container, and closure have low bioburden, but they are not sterile. The product in its final container is then subjected to a sterilization process such as heat or irradiation.

An aseptic process – in the process the drug product, container, and closure are first subjected to sterilization methods separately, as appropriate, and then brought together. Because there is no process to sterilize the product in its final container, it is critical that containers be filled and sealed in an extremely high-quality environment. Aseptic processing involves more variables than terminal sterilization. Before aseptic assembly into a final product, the individual parts of the final product are generally subjected to various sterilization processes. For example, glass containers are subjected to dry heat; rubber closures are subjected to moist heat; and liquid dosage forms are subjected to filtration.

Each of these manufacturing processes requires validation and control because any process step could introduce an error that ultimately could lead to the distribution of a contaminated product. According to the 21 CFR 211.113 (b) 'Appropriate written procedures, designed to prevent microbiological contamination of drug products purporting to be sterile, shall be established and followed. There are eight major elements consideration for the filter validation:

a. **Integrity Testing:** Non-destructive tests prove that the filter is performing bacterial retention adequately. As described in ASTM F838-05, it is well understood that there is a relationship between bacterial retention and a non-destructive integrity test. Sterilizing filters for stream processes can test integrity by bubble point, forward flow or diffusive flow tests or pressure hold tests, depending on the feasibility of each test for the applications [18].

b. **Fit for use:** This is the most important consideration when choosing the sterilising filters, will nonetheless be 'what is the product to be filtered' and 'what processes will the product be going through'. The risk of contamination in the product & process conditions proportionately increases from upstream, downstream

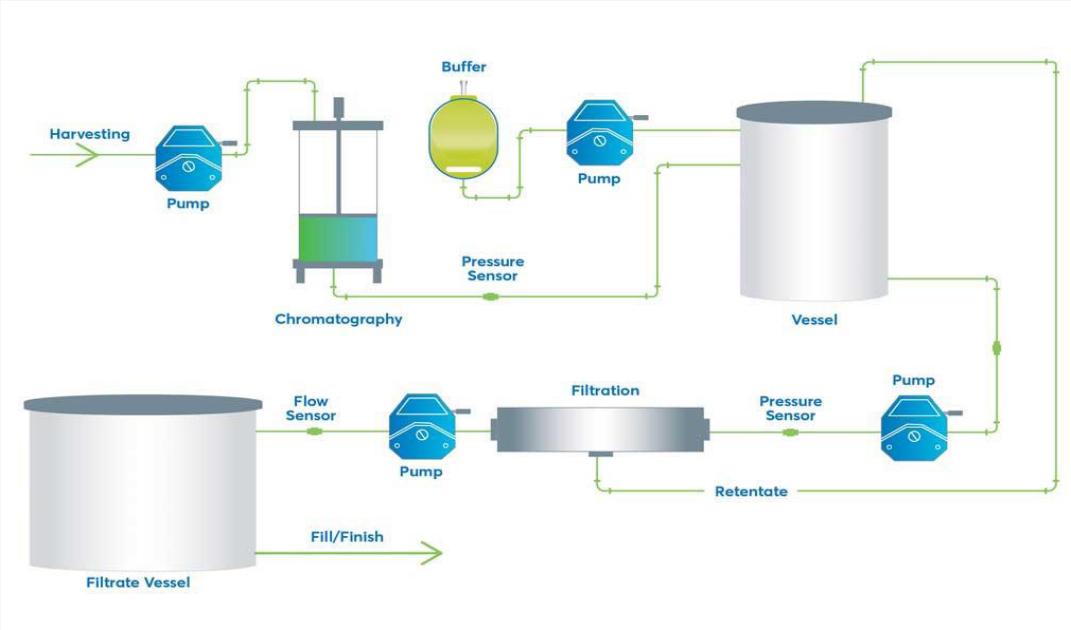

Figure 1 Sterile Filtration in the fill finish process (Avantor, 2023).

Table 1: Sterilization filters of Pore Size.

Pore Size	Organism Used to Validate
0.03 µm	Acholeplasma laidlawii
0.10 µm	Brevundimonas diminuta
0.22 µm	Brevundimonas diminuta
0.45 µm	Serratia marcescens
0.65 µm	Saccharomyces cerevisiae

and eventually the final fill. This has given a good idea on the proper weightage of suitable and sizable filters should be placed in a validation plan. There is also a need for the sterilizing filters to be revalidated when if there are any process changes and product redesign. Over all this step determines if the filter meets the product and process requirements.

c. **Sterilization of the Filter:** Uses standard operating procedures, lab controls, accuracy, and reproducibility tests to determine that the process of sterilizing the product will not compromise the filter. Before pushing the filter into the sterilization stage, it must be claimed that the sterilization process is verified to be effective and it does not compromise the filter. Sterilizing filters can be sterilized in a certain way. Capsule filters can be gamma irradiated or autoclaved. Disc filter holders are autoclaved with wet filters. Cartridge filter installations are often sterilized by Steam in Place (SIP) operation.

The steam used should be free of particulate substance, such as rust and pipe scale that will be removed by the filter to be sterilized and shorten the filter lifespan. The validation of this step is rational if the filter is integrity tested following the mean of an actual filtration process.

d. **Stability of the Filter Used:** It is also important for the validation engineer to take note that the construct of the filter does not unfavourably affecting the product filtration process. Sometimes, the filter construct material can interact with the product, changing the conditions, such as temperature, pH, physical appearance etc. in the stream process.

This can commonly be validated by collecting data from the stream process conditions, then have them analysed using statistical means.

It verifies that the filter does not interfere with or otherwise damage the process stream.

e. **Binding on the Filter:** Binding on the filter is basically referring to the study whereby the product-contact surfaces of a sterilizing filter does not bind to any formulation component causing product loss in the process. The filter should not remove active pharmaceutical ingredients, excipients, carriers, diluents, proteins, preservatives, or any other formulation component.

Binding and adsorption filter characteristics are measured in the qualification phase by using the adsorption analysis, to identify if the product content is partially retained in the filtration mechanism.

In short, this tests that the filter removes only the impurities from the process stream and allows the desired stream components to pass through.

f. **Compatibility of the Filter with the Process:** This element establishes that the filter can meet the physical needs of the production process. For example at any conditions in the process, such as thermal, hydraulic, or chemical clash can be a major cause of adverse deformity in the structure of the sterilizing filter, the filter should fit properly, should not dissolve or break, the filter system must be qualified to demonstrate that all product-contact surfaces of the filter and its structural parts, including membrane support layers, core, cage, o-rings and other related components of the construct, can tolerate challenges of all the conditions of the sterilization and production processes.

Recently, there is an increasing demand for the testing of biocompatibility (biological safety) associated with the filters to be used in the pharmaceutical production, as part of the sterilized filtration validation.

g. **Extractables/Leachable from the Filter membrane:** Extractables/ leachable testing is an important sterile filter validation as it identifies, quantifies and assesses the filter itself acting as a source of physical or chemical contaminants migrating to the process stream. Extractables are usually extracted from plastic or elastomeric materials in solvent under distressed conditions, while leachable refer to compounds that leach from plastic or elastomeric materials into the pharmaceutical product under normal conditions.

The non-volatile residue test (NVR) is normally used to quantify the amount of such contaminants released by a filter into the process stream. Identifies, quantifies, and assesses the impact of compounds after they have been filtered and remain in the process stream. The product should not be adversely impacted after it is filtered.

h. **Bacterial Retention:** Proves that the filter works as it should, removing bacteria from the product. A bacterial challenge test validates the ability of a filter to provide sterile effluent in any pharmaceutical product. Under controlled test conditions, the filter is challenged with a minimum of 10^7 colony forming units (cfu) of *Brevundimonas diminuta* (ATCC 19146) per cm^2 under process conditions and demonstrated by testing to produce a sterile filtrate [19].

This has been considered as most widely accepted approach for sterilizing filter validation because the bacterial challenge concurrently tests the physical-chemical interactions of the liquid product and the filter according to process conditions.

Validation of the bacterial challenge is usually performed by the filter company or an outsourced laboratory, using 47-mm disk

filters to improvise a scaled-down volume of the pharmaceutical product required.

Selection of Sterilizing Grade Filters

Selecting a sterilizing grade filter requires consideration of many important issues, such as materials of construction and their compatibility with the product. The selection also should consider the processing characteristics, including the volume of product filtered, flow rate, pressure differential, temperature and the chemical characteristics of the product.

The obvious objective of a sterile filtration step is the removal of any viable microorganism excluding viruses, that may be present in the bulk product. Materials used to manufacture sterilizing filters must be carefully selected and controlled to make sure they meet applicable quality and regulatory requirements. All materials must be non-toxic and meet USP Class VI and other toxicology requirements. In addition, the filters must not contain extractable substances that could alter or contaminate the fluid being filtered. This includes proper selection of raw materials, manufacturing equipment, and area cleanliness to protect the filters from the surrounding environment.

Choosing the right filter supports to capitalize on yields and ensure efficient and reliable processes. Level of Sterilization is the first and most important factor is what level of sterilization is required. An Ideal sterilizing grade filter must remove all microorganisms present in a fluid stream without adversely affecting product quality [20]. An Ideal sterilizing grade filter should -

a. Removal of only bacteria,

b. Only mycoplasma removal,

c. endotoxin contamination a concern.

Bacteria removal can be achieved with an 0.2 or 0.22 µm (different manufacturers tend to use these ratings interchangeably - the most important factor is that it is validated for bacteria retention). For mycoplasma removal, a smaller pore size is required - 0.1 or even 0.03 µm depending on the manufacturer and validation testing. Where endotoxin removal is also required, a positively charged version of these filters is needed.

After deciding what pore size rating and filter type are required, certain fluid characteristics need to be accounted for to pick the best filtration solution. Whether we are filtering simple solutions, viscous high concentration mAbs or complex LNP formulations, every process has a unique challenge. Some filter manufacturers offer single-layer membrane sterilizing filters. Others may offer filters with two layers of membrane, sometimes with different pore sizes (a larger pore size in front of the final sterilizing filter), or two layers of identical material in the same pore size.

- ✓ The chemical composition of process fluid may limit the choice of membrane materials. For aqueous solutions, Polyethersulfone is usually the best choice due to its high flow rate and dirt-holding capacity. For other fluids that may negatively affect PES, Nylon 6,6 membrane would be a better choice.
- ✓ If the fluid is known to be low in particulate contamination and has a fairly low bacteria count a single-layer membrane filter will probably sufficient. In critical applications requiring extra security, a double layer of the same pore size rating could be a better choice.
- ✓ For fluids with low particulates but higher bacteria count, a dual-layer membrane filter with a larger pore size up front (for example 0.45 µm followed by 0.22 µm) could help extend the life of the sterilizing layer.
- ✓ For fluids with high particulate load, a higher capacity prefilter can be incorporated in front of the final sterilizing layer.

Other factors are required flow rate, batch size, and expected filter life. For low flow or low volume requirements, the choice may not matter very much, but where these are important considerations, the choice can make a huge difference. A single-layer filter will generally provide the highest flow rate per cartridge, but depending on the fluid characteristics discussed above, could result in premature fouling and shortened service life.

Filter Validation

Similar to the other processes in the pharmaceutical industry, the filtration process needs to be validated. Filter validation is the procedure of verifying that a filter used to sterilize a pharmaceutical drug product does so sufficiently by efficiently eliminating microorganisms. It is a significant stage in the development and manufacture of pharmaceuticals that use final filtration as a method of sterilization.

ASTM F 838-15(ae1)

Sterilizing filters should be validated using test procedures that comply with ASTM F 838-15(ae1) protocols for the determination of bacterial retention in filters used for liquid filtration. The challenge level is a minimum of 10^7 organisms per cm^2 of filter media.

ASTM F838-20

Standard Test Method for Determining Bacterial Retention of Membrane Filters Utilized for Liquid Filtration. This test method determines the bacterial retention characteristics of membrane filters for liquid filtration using *Brevundimonas diminuta* as the challenge organism. This test method can be used to evaluate any membrane filter system used for liquid sterilization. This standard test the performance of a filter at 0.2 µm.

The Parenteral Drug Association (PDA)

PDA has published the authoritative summary of best practices in sterile filtration and validation of sterile filtration in its 1998. The technical report 26 describes the basic requirements for the validation of product filters for sterile filtration of liquids. The

determination of sterile filtration validation is to demonstrate that a specific filtration method generates a sterile filtrate. This can be achieved by selecting a sterilizing grade filter that is well-suited with the process, nontoxic, integrity testable, sterilizable, that does not adsorb formula components or add extractables to the process and can remove the bioburden associated with the product. The filter then is challenged with 10^7colony forming units (cfu) of *Brevundimonas diminuta* (ATCC 19146) per cm^2 under process conditions and demonstrated by testing to produce a sterile filtrate [21].

In process-specific filter validation studies, consideration must be given to use worst-case test parameters for the specific application (e.g., maximum filtration time and batch size). Below table shows process risk assessment factors, but this offers guidance only, and worst-case parameters should be evaluated for each specific application.

In validating and performing sterile filtration, it is essential to identify the bioburden or endemic microorganism in a given process, to use the grade of filter that quantitatively removes the microorganism and to demonstrate quantitative removal by test before using the filter in production. This is the essence of filter validation.

There are four major elements of the filtration validation process:

a) physical/chemical compatibility, usually established during the qualification phase before validation, is confirmed during the validation process.

b) binding and adsorption filter characteristics are measured in the qualification phase.

c) bacteria retention capability of the filter, which is established by challenging the filter with *B. diminuta.*

d) integrity of the process filtration installation, as verified by the filter integrity test.

Bacterial Retention or Challenge Test

The bacterial retention test is a parameter of filter validation that has to be evaluated according to the requirements of the PDA 26 report and the guidance of the FDA. The test, also known as bacterial challenge test / assay (BCT / BCA), examines if the filter is able to retain a certain number of bacteria of a defined size. Bacterial challenge tests also are required to validate the sterilizing filtration process of a specific product. The filter challenge test must be performed with actual product or, where justified, suitable surrogate fluid.

Bacterial retention testing for process fluids is a critical step in filter validation required by all regulatory bodies worldwide. USFDA says that microbial retention testing be conducted using the candidate pharmaceutical preparation under simulated processing conditions; this validates sterilizing-grade filter performance.

Test procedure

The ASTM F838-20 method is the standardized procedure used to conduct the bacteria retention test (bacterial challenge test) and determines the filter's bacterial retention rate. Testing to be conducted using worst-case client processing conditions. The filter is mounted in a specified device and a defined bacteria solvent is pushed through the filter, to determine the ability of a sterilizing-grade filter to retain a minimum challenge of 10^7 cells of *Brevundimonas diminuta* (B. diminuta) per cm^2 of filter area. The aim is to determine how many log-levels of bacteria the filter can reduce.

The bacterial retention test is a parameter of filter validation that has to be evaluated according to the requirements of the PDA 26 report and the guidance of the FDA. The test, also known as bacterial challenge test / assay (BCT / BCA), examines if the filter is able to retain a certain number of bacteria of a defined size.

Necessary materials

We need certain materials to conduct the test. Primarily, the bacterial strain *Brevundimonas diminuta* (ATCC 19146) is necessary, because it's needed to generate the bacterial suspension later. Furthermore, a testing device with a pressure connection, pressure vessel, three valves and the test filter housing must be available. Moreover, buffer solutions and nutrient media (like for example SLB – saline lactose broth or TSB - tryptic soy broth). The laboratory must also have a sterile workbench as well as an autoclave and an incubator.

Preparing the bacterial suspension

There are multiple steps necessary to generate a bacterial suspension with a suitable number of cells (depending on the type of cultivation, 10^7 to 10^8 or 1-2 x 10^{10} cells/mL).

a. First, a microbial strain from the ATCC culture needs to be cultivated.

b. These bacteria are subsequently put into a nutrient solution and later in a buffer solution and incubated for one day each at 30 ± 2°C.

c. The suspension created this way can then be used for the test, but is usable only for a maximum of eight hours stored in the fridge.

d. The cultivation of *B. diminuta* according to the requirements of the ASTM method guarantees bacteria with a diameter of 0.3 - 0.4 µm and a length of 0.6 - 1.0 µm.

e. Bacteria of this size are considered to be very small and should be retained by a sterile filter with a pore size of 0.2 µm.

Determining the viable cell concentration of bacteria

Since only living bacteria will later be growing on the control filter or medium, it's important to determine the viable cell concentration of the utilized bacteria culture on agar plates. At first the cells are counted under the microscope. Since this includes dead bacteria as well, it's necessary to cultivate additional bacteria cultures on agar plates. For this purpose, different dilutions (10^{-3} – 10^{-5}) of bacterial suspension have to be prepared and plated as a defined quantity (0.1 mL) on

the agar plates. After incubating the plates for 48 hours, the grown colonies are counted and the viable cell concentration is calculated. The concentration of viable cells should be not lower than 25 % the total cell count (dead and living).

Conclusion

Author emphasizes the significance of filter validation in ensuring the reliability of filters and its impact on various real-world applications. Additionally, author highlight areas that require further research and development to address existing challenges and propel the field forward.

Overall, this review article aims to serve as a comprehensive guide for researchers and practitioners interested in filter validation methods. By offering an in-depth understanding of the theoretical aspects, practical techniques, and applications of filter validation, we hope to promote the adoption of robust and validated filters in diverse fields.

References

1. Sterile Injectable Drugs Market to Reach US $901.3 Billion by 2025, Globally: Transparency Market Research. Transparency Market Research. 2018.
2. Patel P, Sharma J, Kharoliwal S, Khemariya P. The Effects of Nobel Corona Virus (Covid-19) in the Tourism Industry in India. Int J Engineering Res Technology. 2020; 9: 780-788.
3. United State Food and Drug Administration (USFDA). Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice. 2004.
4. United States Pharmacopeia (USP) *32-NF. General Chapter <1222> Terminally Sterilized Pharmaceutical Products*-Parametric Release. 2009.
5. WHO good manufacturing practices for sterile pharmaceutical products (Annex 6) WHO Technical Report Series, No. 961. 2011.
6. The Parenteral Drug Association Technical Report no. 26. Sterilizing filtration of liquids. 1998.
7. PDA Journal of Pharmaceutical Science and Technology. Technical Report No. 30: Parametric Release of Pharmaceuticals Terminally Sterilized by Moist Heat. Supplement. 1999; 53: 217-22.
8. Khemariya P, Jadon SR, Nayak S, Amlan A, Sumbhate S. Taste masking of Lornoxicam by polymer carrier system and formulation of oral disintegrating tablets. Int J Drug Delivery. 2009; 1: 27-31.
9. Khemariya P, Gajbhiye KR, Vaidya VD, Jadon RS, Mishra S, Shukla A, et al. Preparation and evaluation of mouth dissolving tablets of meloxicam' Int J Drug Delivery. 2010a; 2: 76-80.
10. Khemariya P, Jain AK, Bhargava M, Singhai SK, Goswami S, Goswami R. Preparation and in-vitro evaluation of sustained-release matrix tablets of Diltiazem'. Int J Adv Pharmaceutical Sci. 2010b; 1: 22-29.
11. Khemariya P, Mishra S, Shukla A, Bhargava M, Singhai S, Goswami S . An emerging trend in tablet technology: Floating tablets of ranitidine HCl. Int J Drug Delivery. 2010c; 2: 154-158.
12. Khemariya P, Rawal S, Mavila A, Rai P. A Pioneering Approach to Enhance Dissolution and Bioavailability of Multiple Drugs in a Single Dosage Form: Speedy Disintegrating Tablet of Cefpodoxime Proxetil and Potassium Clavulanate. J Advanced Sci Res. 2012; 3: 51-57.
13. Khemariya R, Khemariya P. New-fangled approach in the management of Alzheimer by formulation of polysorbate 80 coated chitosan nanoparticles of rivastigmine for brain delivery and there in-vivo evaluation. Int J Sci Engineering Invention (IJSEI). 2016; 2: 1-13.
14. Khemariya P, Dubey K, Khemariya R. Innovative Approach to Sustain the Release of the Drug from Conventional Dosage Form Nifedipine Sustained Release Tablet. Nanomed Nanosci Res. 2017; 2: 1-7.
15. Khemariya P, Agrawal A, Minnoor E. Filter Integrity Test for Aseptic Processing. Global Academic Journal of Pharmacy and Drug Research. 2022a ; 4**:** 89-93.
16. Khemariya P, Agrawal A, Kumar K, Tiwari AK. Quantitative Analysis of Eugenol in Different Parts of Clove. Int J Biotechnol. 2022b: 11: 12-23.
17. ICH. Q10 Pharmaceutical Quality System, Guidance for Industry, Federal Register. 2009; 74.
18. American Society for Testing and Materials. ASTM F838-20. Standard Test Method for Determining Bacterial Retention of Membrane Filters Utilized for Liquid Filtration. 2020.
19. Folmsbee M, Roche Lentine K, Wright C, Haake G, Mcburnie L, Ashtekar D. The Development of a Microbial Challenge Test with Acholeplasma laidlawii To Rate Mycoplasma-Retentive Filters by Filter Manufacturers. PDA J Pharm Sci Technol. 2014; 68: 281-296.
20. The European Agency for the Evaluation of Medicinal Product (EMEA). Committee for Proprietary Medicinal Products: Note for Guidance on Parametric Release. 2001.
21. Sasaki T. Parametric Release for Moist Heated Pharmaceutical Products in Japan. PDA J GMP and Validation in Japan. 2002; 4.