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RESEARCH ARTICLE

Process Validation of Sterile Product

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ABSTRACT

Process validation studies examine a process under normal operating conditions to prove that the process is in control. Once the process has been validated, it is expected that it remains in control, provided no changes are made. In the event that modifications to the process are made, or problems occur, or equipment or systems involved in the process are changed, a re-validation of the process would be required. Very often validation studies require more measurements than they are required for the routine process. The validation must prove the consistency of the process and therefore must assess the efficiency and effectiveness of each step to produce its intended outcome**.** The aseptic process validation conducted for the Ondansetron Injection USP, 2 mg/mL, 2ml Single Dose Vial, was found to be complying with the acceptance criteria. Thus documented evidence for the manufacturing process for the Ondansetron Injection USP, 2 mg/mL, 2ml Single Dose Vial, was shown that the process has consistently produced the product within the predetermined specifications. From the review of data recorded during manufacturing process, in process testing and finished product analysis of all the three validation batches, it is concluded that the manufacturing process is consistent and meets the pre-determined specifications and quality attributes. Hence the manufacturing process of Ondansetron Injection USP, 2 mg/mL, 2ml Single Dose Vial, stands validated.

KEYWORDS

Ondansetron Injection USP, Environmental Monitoring, CFU, BET, Bioburden Test

INTRODUCTION

Process Validation

Process knowledge depends on accurate and precise measuring techniques used to test and examine the quality of drug components, in-process materials, and finished products. Validated analytical methods are not necessarily required during product- and process-development activities or when used in characterization studies. Nevertheless, analytical methods should be scientifically sound (e.g., specific, sensitive, and accurate) and provide results that are reliable. There should be assurance of proper equipment function for laboratory experiments. Procedures for analytical method and equipment maintenance, documentation practices, and calibration practices supporting process-development efforts should be documented or described. New analytical technology and modifications to existing technology are continually being developed and can be used to characterize the process or the product. Use of these methods is particularly appropriate when they reduce risk by providing greater understanding or control of product quality. However, analytical methods supporting commercial batch release must follow cGMP in parts 210 and 211. Clinical supply production should follow the cGMP

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appropriate for the particular phase of clinical studies.

Process validation is establishing documented evidence, which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality characteristics[1]. "The collection and evaluation of data, from the process design stage throughout production, which establishes scientific evidence that a process is capable of consistently delivering quality products" is done in process validation. Process validation is the means of ensuring and providing evidence that processes are capable of repeatedly and reliably producing a finished product of the required quality. Process validation will include acceptable release of not less than three batches that meets the processing limits for all critical parameters. The number of process for the validation should depend on the complexity of the process or the magnitude of the process change being considered. Three batches are taken for the purpose of process validation, to demonstrate the Consistency.

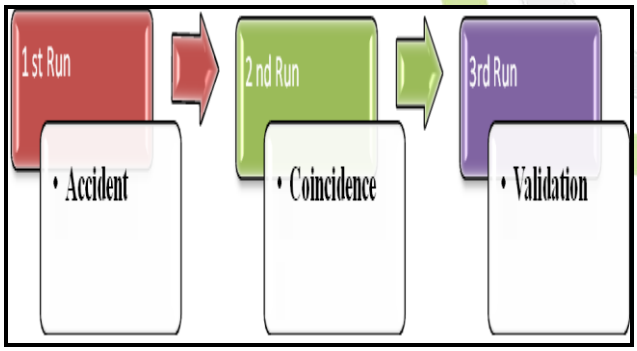

Figure 1: Process Validation Concept

Critical process parameters should be controlled and monitored during the process validation studies.

Process Flow of Parenteral Dosage Form[2]

In the preparation of parenteral dosage form mixing, filtration, filling & sealing are the most critical step. Flow diagram shows the process flow of parenteral dosage form.

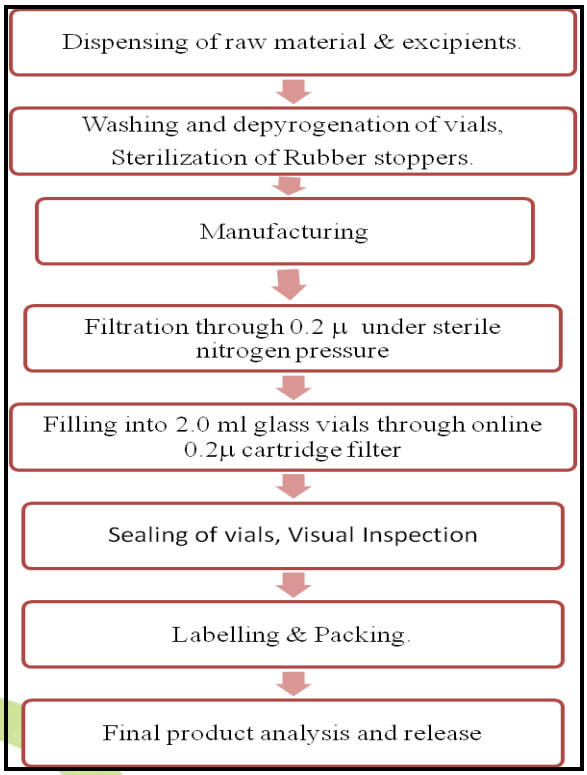

Figure 2: Process flow diagram of parenteral dosage form

Processing Variables in Parenteral[3]

Following are the common variations that may occur during the process of parenteral formulations. These variations can be minimized by proper calibration of instruments and qualification of equipments, materials, utilities, facility, personnel, supporting systems and validating the process as a whole.

Dispensing

Dispensing is one of the critical factor which may lead to product contamination while transferring or dispensing. There are also chances of dispensing more or less quantity of API.

A. Assay

The calculation for the amount to be dispensed is done with respect to the COA of the material, the calculations are done as on dried basis only thus Assay value have great impact in the quantity of material dispensed, which may affect the final product.

B. Approved Vendors

All the materials used for the production should be procured from approved vendors only. The vendors are approved by prior vendor audit. If the materials are procured from approved vendors the standard of the materials should be validated. If materials are procured from unapproved vendors the purity and standard of the materials cannot be assured.

C. LAF∆P and Room Temperature and RH

Pressure differentials in a controlled environment is important to ascertain that the correct degree of over pressure is maintained relative to the adjacent areas of lower classifications in order to minimize the contamination drawn into the controlled environment from its surroundings. The LAF ΔP should be maintained within limits to maintain a controlled environment.

D. Temperature and RH

Temperature and RH of the dispensing area should be within the specified limit since the products can be detoriated at extreme environmental conditions. The temperature and RH are monitored regularly to verify whether the area is in controlled state. The failure of AHU leads to changes in temperature & RH.

E. Balance Calibration

All the balances used for dispensing should be calibrated before starting the days work. Apart from this calibration by external party should be conducted for periodically. If balances are not calibrated there are chances of dispensing wrong quantity of materials which may affect the final product assay.

Sterilization

Sterilization is the process by which a product made free of viable organisms with a specified probability. Sterilization is carried out in a sequence of defined operating parameters such as time, temperature and pressure and conditions required to render an item sterile.

A. Validated Load Pattern

When a multiple products are processed using the same cycle, a minimum lethality to be delivered for product specific loads. By a validated load pattern a safety margin is built into the minimum F_0 requirements. It assures that the lethality requirements are constantly delivered to each load. During the load pattern study the exact physical nature of each product and materials are studied and an appropriate sterilization process are selected.

B. Clean-in-place / Sterilize-in-place

Validation of these systems may be difficult because of the potential incompatibilities in requirements for the design of CIP and SIP facilities.

All systems have dead legs to a greater or lesser extent and the required orientation of the dead legs differ for CIP and SIP. The orientation for CIP dead legs is slightly sloping so that the cleaning solution can enter and also drain away. The dead leg for SIP is vertically up so that steam can downwardly displace the air. The CIP and SIP procedures should be validated as the containers are not supposed to be cleaned manually and the parameters used during process should consistently provide the acceptance limits.

C. Hold Time for Sterilized Goods

The hold time for the sterilized goods should be validated to determine the effectiveness of the sterility process. The time period until which the products remain sterile if not opened from the pack is determined. The products that lapses the hold period must be sterilized and used (provided the packs are not opened).

Manufacturing Process[4]

A. LAF∆P, Temperature and RH in manufacturing area

These parameters should be maintained within the limits throughout the process in all the stages of manufacturing, filling, sealing and packing. To avoid the product failure, the possible defects are mentioned in the earlier section.

B. Subdued Light

The intensity of the light in different areas is qualified during area qualification. In some cases the products which are light sensitive should not be dispensed at normal light. The activity should be carried out in subdued light which are of less intensity than normal light. If the product is not manufactured under subdued light it may leads to product detoriation.

C. *Environmental Monitoring*[5]

Measurement and determination of the number and size of airborne particulate contamination is essential to ensure that a suitable environment is maintained for preparation of asepticaly prepared products. If any changes from the normal acceptance limit may lead to product failure due to product contamination. Acceptable methods for monitoring the microbiological quality of the environment include

a. ***Surface Monitoring:*** Environmental monitoring involves sampling various surfaces for microbiological quality. For example, product contact surfaces, floors, walls, and equipment should be tested on a regular basis. Touch plates, swabs, and contact plates can be used for such tests.

b. ***Active Air Monitoring:*** Assessing microbial quality of air should involve the use of active devices including but not limited to impaction, centrifugal, and membrane (or gelatin) samplers. Each device has certain advantages and disadvantages, although all allow testing of the number of organisms per volume of air sampled.

c. ***Passive Air Monitoring (Settling plates):*** Passive air samplers are such as settling plates (petri dishes containing nutrient growth medium exposed to the environment). Because only microorganisms that settle onto the agar surface are detected, settling plates can be used as qualitative, or semi-quantitative, air monitors. Their value in critical areas will be enhanced by ensuring that plates are positioned in locations posing the greatest risk of product contamination.

D. Mixing Time and RPM[6]

The product processing is done by mixing of the active and excipients together in the solution preparation tank with water for injection. Mixing time is the critical parameter as the dissolution of the materials mainly depends on mixing time.

The mixing time is determined during the validation process. If mixing time is not followed it may lead to improper dissolution of API thus leads to wrong assay results. Mixing is facilitated by using the stirrer. The stirrer speed should be validated so that we can assure that proper mixing has occurred each time. The validation is conducted at different speeds and the optimized.

E. *pH*

One of the main parameter used to check the product quality is by checking the pH of the solution prepared, since the parenterals solutions are being injected directly it should be adjusted to the pH that is more or less equal to that of blood. In some cases due to drug solubility characteristics the pH may be acidic or basic. The pH is checked by using a calibrated pH meter in the manufacturing area. Before starting every day's activity pH meter should be calibrated.

F. Volume Make Up

Volume make up for the solution can be done by two ways such as by weighing or by dip stick. Dipstick method is done by using the calibrated dipstick present along with the manufacturing vessel. The dip stick has a measuring scale. If volume make up is done wrongly it may leads to increase or decrease in the assay values. It should be carried out at ambient temperature (25°±2°C).

G. Hold Time Study

The product manufactured aseptically should be subjected to hold time study to confirm that the product produced remain sterile, without any chemical change. During hold time study the product is frequently sampled to determine any change has occurred in stability and sterility

aspects. This is carried out since there are chances of any of equipment failure during process.

H. Filtration Activity

In aseptic processing the product is sterilized only by filtration, thus the filtration activity should be adequately validated.

The integrity of the sterilization filter should be verified before use and should be confirmed immediately after use by an appropriate method such as a bubble point, or diffusive flow or pressure hold test etc. The integrity of critical gas and air vent filters should be confirmed at appropriate intervals.

Vial Washing and Depyrogenation[7]

A. LAF∆P (across the HEPA filter), Temperature and RH

These parameters should be maintained within the limits throughout the process in all the stages of manufacturing, filling, sealing and packing.

B. Water and Compressed Air Pressure

The vials are washed first by series of water at a high pressure. The pressure of the water should be maintained throughout the process as the pressure is directly proportional to the effectiveness of the washing process.

If the pressure is less than the acceptable value it may lead to improper washing. Compressed air is used for drying the washed vials before it reaches the tunnel. Thus any deviations in the pressure maintained will leads to improper drying which will affect the depyrogenation of the vials.

C. Clarity Check

The washed vials are checked manually for the effectiveness of the washing process. The vials which are broken or containing any dirt can be identified during these check's which prevent the rejections after filling.

D. Depyrogenation Temperature and Conveyor Belt Speed[8]

Vial depyrogenation is another critical factor to be checked. Depyrogenation of vials can be achieved at a temperature between 280˚C to 350˚C. If the temperature is not maintained throughout the process it may affect the depyrogenation of the vials. The tunnel should be qualified before starting the process. During the qualification stage the depyrogenation temperature and the conveyor belt speed for the different vial size are done and established. The validated limits should be followed during the process.

E. ∆P (Across the HEPA Filter and Across the Zones & Rooms)

Pressure differentials in the tunnel is important to ascertain that the correct degree of over pressure is maintained relative to the adjacent areas of lower classifications in order to minimize the contamination drawn into the controlled environment from its surroundings. The ΔP should be maintained within limits to maintain a controlled environment. The difference in ΔP may be due to blocked or partially blocked HEPA filters. The ΔP across various zones of tunnel and the ΔP between the tunnel cooling area and vial receiving area should be maintained.

Vial Filling and Sealing[9]

A. Gowning procedure

The persons entering the sterile area should not contaminate the area by shedding contaminants from own body. The person should follow the gowning procedure strictly. The gowning qualification is done by taking swabs from the gowned persons at the commonly used parts of the body on consecutive three days and incubated for checking the presence of any viable organism in it. The results showing less than the alert level is the criteria for acceptance. Person without proper training may lead to product contamination, thus proper training is given to all personnel entering sterile area.

B. LAF∆P (across the HEPA filter), temperature and RH[10]

These parameters should be maintained within the limits throughout the process in all the stages of manufacturing, filling, sealing and

packing, to avoid the product failure. The possible defects are mentioned in the earlier section.

C. Filling Speed and Fill Volume

The filling speed of the machine should be validated during the machine qualification stage. The filling speed depends upon the size of the vial and volume of the liquid filled. The fill volume may be altered due to increasing or decreasing the speed of the machine and also depends on the product physical nature. The changes from the established limits may leads to reduced extractable volume. Thus it should be frequently monitored by doing fill volume checks by using calibrated syringes or measuring cylinders.

D. *Filter Integrity Testing*[11]

Fiteration is the only process of sterilization in aseptically filled products, thus to confirm the sterilization has achieved filter integrity should be done. The integrity of the sterilization filter should be verified before use and should be confirmed immediately after use by an appropriate method such as a bubble point, or diffusive flow or pressure hold test.

E. Sealing of Vials[12]

The vials filled aseptically should be closed and sealed immediately after filling of the solution. The sealing gives the proper closing of the vials. The sealing activity can be confirmed by doing leak testing for the sealed vials frequently. Sealing prevents the leakage of the containers during the transporting or shipping.

MATERIAL AND METHOD

Product Name

Table 1: Drug product used in process validation

Packaging material	Name of Manufacturer
Ondansetron Injection USP, 2 mg/mL, 2ml Single Dose Vial.	Wockhardt Biotech Park, Waluj, Aurangabad

Equipments Used

Table 2: Equipments used in process validation

Sr. No.	Name of the equipment / Instrument	Manufacturer
1	Balance	Motter Toledo
2	Vial washer	Pyroklenz
3	Tunnel sterilizer	Fedegari
4	Steam sterilizer	Metalchem industries
5	Vial filling and plugging machine	Macofar (Romaco)
6	Vial sealing machine	Macofar(Romaco)
7	Filtration assembly	Adam
8	Integrity tester	Fartorious
9	20 L capacity jacketed manufacturing vessel	Adam
10	30 L capacity jacketed manufacturing vessel	Adam
11	Visual inspection hood / machine	Umasons
12	Online Filter	Adam
13	Nonviable Particle Counter machine	Met-1
14	Ph meter	Mettler Toledo
15	CIP Unit	Adam
16	Leak test Apparatus	Millipore
17	Domino Inkjet Printer	Domino
18	CVC labeling machine	CVC

Method

Processing Controls of Parenteral Dosage Form

Following are the processing control steps which carried out during process validation

Dispensing

- Check and ensure dispensing booth is clean.
- Check and ensure that balance is not due for calibration.
- Check and ensure that the expiry date of raw material is later than that of batch expiry date. Retest date is older than day of dispensing.
- Check and ensure that temperature and humidity of the dispensing room is with in limit.

Washing and Depyrogenation of Vials

- Wash all the vials. All the checks need to be made immediately after beginning the washing and at every one hour. Record the remarks if any.
- Wash & sterilize all the equipment's coming in contact with the product during processing, filtration and filling.
- Inspect each and every vial. Note down the washing time.
- Check the depyrogenation time, conveyor belt speed and ΔP between the different zones and rooms.

Processing

- Check and ensure that processing area is in aseptic condition or not.
- Check and ensure that the processing operations are carried out as per BMR instructions.
- Check and ensure the temperature of the solution processed in the tank is maintained.
- Check the final pH and record it.
- Collect the samples at regular predetermined intervals during stirring form the top and bottom of the vessel using sterile sampling tool.

Filtration

- Filter the solution by 0.22 µ sterilization filter.
- Collect the clear filtrate in a clean, sterilized holding tank.
- Store the solution as per specifications.

Filling and In Process Checking

- Inspect filling and packing lines.
- Check and ensure that the vial washing, drying and depyrogenation, filling, sealing are carried out.
- Filter the solution by 0.22 µ online filter before filling.
- Check and record the fill volume.
- Collect two filled vials at every one hour interval and subject it for fill volume check and sealing (leak test) as per in-house specification.
- Check the pH and Assay of the Solution form vials filled immediately after setting the filling machine, and during initial, middle and end filling and every break.
- Samples shall be collected every hour from all filling heads.

Visual Inspection

- Inspect manually or on the inspection machine each and every vial Inspect all the vials.
- Store the inspected vials with appropriate status label.

Labeling and Packing

- Check and record the temperature at the heating roller and sealing roller.
- Check and record that the over printing instructions on labels and cartons as per instructions of BPR.
- After ensuring the proper labeling of vials, check, for correctness of cartons packing for the same.

Finished Product Analysis and Release

- Finished product needs to be analyzed as per in-house specification. Product needs to be released only after pre-determined specifications and quality attributes are met.

Process over Flow for the Process Validation

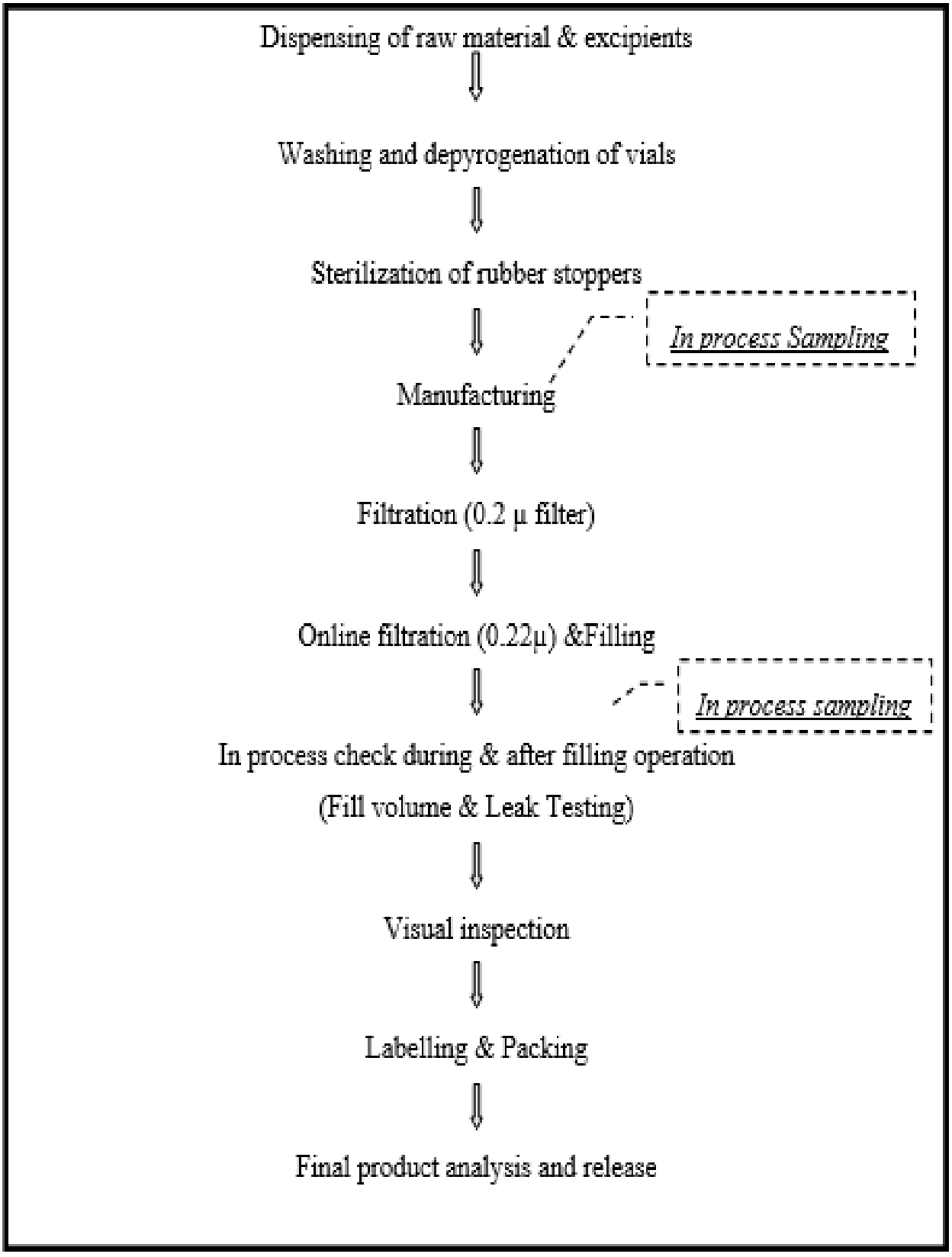

Figure 3: Process over flow for the process validation

Determination of Critical / Non critical Process Parameters

Table 3: Critical / Non critical process parameters

Process Steps	Process Parameter Setting	Rationale	Critical/ Non-Critical	Assessed by testing
Raw Materials	Approved vendors used for the process	All the materials received will be tested against approved specification and released before use.	Non-critical	All the materials are tested as per approved specification before use.
Dispensing	Validated and calibrated balance &LAF used for dispensing.	Balance calibrated regularly as per procedure. Process does not have variable effect on quality as dispensing	Non-critical	Weighing is done by Warehouse / stores and in presence of Production (Checker).On receipt
Water for Injection	Approved water for injection is used at all stages.	Water for injection system is qualified	Critical	Daily testing of WFI is done as per sampling plan and specification.
Mixing	1. Mixing speed (65±5) rpm Mixing Time Nitrogen bubbling	Mixing is required for complete dissolution of the ingredients, to get homogeneous solution and clarity of the solution. Nitrogen bubbling is required for reduction of oxygen.	Critical	Mixing time and speed of stirrer shall be monitored and recorded based on complete dissolution of ingredients.
	2. Mixing speed (65±5) rpm Mixing time	After addition of drug mixture is required to get homogeneous solution and uniform distribution of drug content and clarity of the solution.	critical	Mixing time with the nitrogen bubbling and speed of the stirrer shall be monitored and based on complete dissolution of drug and clarity of the solution.
Final mixing	Mixing speed (65±5) rpm Mixing time 15min.	Volume make up is necessary to get desired drug content per 0.5ml. Mixing is required to get homogeneous	Critical	Tested for 1.Appearance and Clarity 2. pH (3.3 – 4) 3..Assay (95 % - 105 %)

		solution and uniform distribution of drug content and ingredient.		5..Bioburden
Filtration	Filter type and filter size, filter integrity, filtration time, pressure	0.2µ PVDF cartridge using for filtration of the product filtration is most critical step to ensure sterile solution check the integrity of product filter by using bubble point 2070mbar.	Critical	Filter integrity check by Bubble point test before and after filtration Appearance and clarity pH Assay Sterility
Online – filtration	Filtration through 0.2 µfilters.	Online filter before and after filling step.0.2µ PVDF cartridge filter using for online filtration.	Critical	Bubble point test performed before and after filtration.
Filling	Filling speed, Fill volume	Filling speed is critical to the volume variation Fill volume checked at minimum machine speed 206 vial/min. Max. speed 120 vials and optimum 117 vials/min.	Critical	Fill volume shall be checked from each nozzle at minimum maximum & optimum speed at the start of filling. Fill volume checked after 1 hr ±15min by production and 2 hr ±15 min by QA.
Sealing	Seal integrity	Critical with the reference to integrity of the filled vials , so check the closer integrity	Critical	Leak test at the beginning and every 1hr ± 15min including initial and at the end of filling.
Leak testing	On-line checking for integrity of vials	leak test at every 1hour interval including initial and at the end of filling	Critical	Checking leaked vials by vacuum method.
Visual inspection	Suspended particles, cosmetic defects	Removal of defective vials and vials with suspended particles	Critical	200% visual inspection against Black and white background with sufficient light.

Packing	Sticker labelling, cartooning and box / shipper packing.	- Part of the packing automatic and controlled. - Regular online checks available in force.	Critical	Inspection.

Sampling Plan and Acceptance Criteria[13]

Table 4: Sampling plan and acceptance criteria

Process Stage	Sample ID	Sampling details	Testing method	Acceptance Criteria
Solution preparation				
Time for solution preparation	--	--	Time for solution preparation as per BMR	Not more than 5 hours If exceeds 5 hours, solutions need to be tested for pH & estimate bio burden
Time for mixing	T1,B1 –05 min T2,B2 –20 min T3–15 min	25 mL each sample solution from Top and bottom	In-process Specification	1. pH 2. Assay – 95% to 105 %
Unfiltered Bulk solution	Pooled sample	(200mL) sampled to be collected from the bottom of the tank.	In-process Specification	1. pH – 3.3 to 4 2. Assay - 95 to 205 % 3..BET (NMT 5.8 EU/mg) 4. Bioburden (NMT 20CFUml)
Filtration				
Filtration	--	Approximately 250ml of solution be collected 50 ml for chemical analysis and 200 ml sterility testing after filtration process	In-process specification	

Process Stage	Sample ID	Sampling details	Testing method	Acceptance Criteria
Online filtration	--	Approximately 25ml of filtered bulk solution is to be collected from filling nozzles after priming and submitted for analysis.	In-process specification	1.pH 2.Assay
Filling and stoppering	–	20 Vials from each nozzle to be collected at minimum, maximum and optimum speed of filling machine.	In-process specification	0.65 to 0.75mL per vial
During filling and stoppering	--	1.Initial, middle, End of filling 2.pooled sample collected from initial middle and at the end of filling	In-process specification	1.pH 2.Assay 3.BET 4.Sterility 5.Complete Analysis of chemical and microbial
Leak testing				
Sealing	--	5 vials after 1hr 15min by production and every 2hr 15min by QA	In-process specification	Not a single vial should found leaked.

RESULTS AND DISCUSSION

Dispensing

All the raw materials used for production were procured from approved vendor only. All the materials were in approved status and the materials were dispensed in right quantity by the warehouse person which were checked by production person and approved by QA.

Equipment Qualification

All the equipment and instruments used during production activity were qualified and calibrated within the due period. All the instruments had their calibration status label.

Environmental Monitoring

Environmental monitoring was carried out for the process validation batches as per the standard procedures. Environmental monitoring included continuous non-viable particle count in all the predetermined locations and viable particle monitoring of all the critical areas and also done by surface swabs and active air sampling. Personnel monitoring to the persons entering the sterile area was carried out. Personnel monitoring included collection of swab samples from the parts of the body which had the chances of reaching the product filling or sealing area. Swabs were collected from the fingers and arm from the persons.

Table 5: Environmental monitoring reports

Sr. No	Control Stage	Acceptance Criteria	Results/ Remark		
			Batch 1	Batch 2	Batch 3
1	Active Air Sampling (Volumetric)	**Class100 :** Action <1CFU/m^3	Nil	Nil	Nil
		Class 1000 : Alert – 2 CFU /m^3 Action -5 CFU/m^3	Nil	Nil	Nil
2	Settle Plate Exposure	All limits as per the SOP	All counts are within the alert level	All counts are within the alert level	All counts are within the alert level
3	Personnel Monitoring	**Gloves :** Action – <1 CFU/5 fingers	Nil	Nil	Nil
		Gown : Alert-1 CFU/ Plate Action-3CFU/Plate	Nil	Nil	Nil
4	Surface Monitoring (Swab method)	Monitoring locations and limits as per the in-house specifications.	Nil	Nil	Nil

All results obtained from environment monitoring complied with in-house specification.

Mixing Samples Analysis Results

The mixing samples were collected as per sampling plan at regular intervals such as 10, 15, 20 mins from top and bottom of the vessel. All the samples collected during mixing were tested as per the specifications and the reports are tabulated as follows:

Mixing Samples pH

Table 6: Mixing samples pH results

Mixing samples	Location	Batch 1	Batch 2	Batch 3
pH (3.3-4.0)	Top 1 (5 min)	3.6	3.5	3.6
	Top 2 (10 min)	3.6	3.5	3.6
	Top 3 (15 min)	3.6	3.5	3.6
	Bottom 1 (5 min)	3.6	3.5	3.6
	Bottom 2 (10 min)	3.6	3.5	3.6

pH of mixing sample was found within acceptable limit.

Mixing Samples Assay Results

Table 7: Mixing samples assay results

Mixing sample	Location	Batch 1	Batch 2	Batch 3
Assay 95.0% - 105.0%	Top 1(05 min)	102.7	100.8	101.0
	Top 2 (10 min)	105.3	101.1	100.7
	Top 3 (15 min)	105.6	100.8	100.7
	Bottom1 (05min)	102.7	101.0	100.9
	Bottom 2 (10 min)	105.4	101.2	100.5

Assay of mixing sample was found to be within acceptable limit.

Unfiltered Sample Analysis Results

The unfiltered samples were collected as a part of production process sampling to estimate the bio burden level in the prepared solution.

Table 8: Unfiltered sample analysis results

Tests	Acceptance criteria	Batch 1	Batch 2	Batch 3
pH	3.3-4	3.6	3.4	3.5
Assay	NLT 95.0% - NMT 105.0%	99.9%	99.7%	99.8%
BET	NMT 5.8 EU/mL	< 2.9 EU/mL	< 2.9 EU/mL	< 2.9 EU/mL
Bio burden	Complies	Nil CFU/100 mL	Nil CFU/100 mL	Nil CFU/100 mL

All the result of unfiltered sample complied with in house specification.

Filtered Sample Analysis Results

The filtered solutions were collected to estimate the effectiveness of the filtration process. The reports are tabulated as follows,

Table 9: Filtered sample analysis results

Tests	Acceptance criteria	Batch 1	Batch 2	Batch 3
pH	3.3 to 4.0	3.5	3.5	3.5
Assay	NLT 95.0% - NMT 105.0%	102.2%	99.7%	100.3%
Sterility	To Comply as per USP	Complies	Complies	Complies

All the result of filtered sample complied with in house specification

Fill Volume Determination Results

To determine the quantity of solution dispensed by the filling heads fill volume check was done for every one hour during the filling activity. The results of fill volume are tabulated as follows.

Table 10: Fill volume results

Sr. No	Batch	Limit 2.15mL to 2.15mL	
		Minimum	Maximum
1	Batch 1	2.15mL	2.20mL
2	Batch 2	2.15mL	2.20mL
3	Batch 3	2.15mL	2.20mL

Fill volume results for all the batches were found to be within specification limit.

Leak Test results

To determine the effective sealing activity, vials were sampled at regular frequency and leak test was carried out. The results of leak tests are tabulated as follows,

Table 11: Leak testing results

Sr. No	Batches	Leak testing	
		Passes	Fails
1	Batch 1	√	X
2	Batch 2	√	X
3	Batch 3	√	X

Filling Sample Analysis Results

The filled vials were collected during the filling activity at initial, middle and end filling stage, to evaluate any change that had occurred during filling stage and also the effectiveness of online filter.

Table 12: Filling sample analysis results

Sample	Tests	Acceptance criteria	Batch 1	Batch 2	Batch 3
Initial of Filling	pH	3.3 to 4.0	3.7	3.7	3.7
	Assay	NLT 95.0% - NMT 105.0%	100.6%	99.8%	100.8%
	BET	NMT 5.8 EU/mL	< 2.9 EU/mL	< 2.9 EU/mL	< 2.9 EU/mL
	Sterility	Should comply as per USP	Complies	Complies	Complies
Middle of Filling	pH	3.3 to 4.0	3.7	3.7	3.7
	Assay	NLT 95.0% - NMT 105.0%	100.7%	99.6%	99.8%
	BET	NMT 5.8 EU/mL	< 2.9 EU/mL	< 2.9 EU/mL	< 2.9 EU/mL
	Sterility	Should comply as per USP	Complies	Complies	Complies

End of Filling	pH	3.3 to 4.0	3.7	3.7	3.7
	Assay	NLT 95.0% - NMT 105.0%	100.6%	99.5%	99.9%
	BET	NMT 5.8 EU/mL	< 2.9 EU/mL	< 2.9 EU/mL	< 2.9 EU/mL
	Sterility	Should comply as per USP	Complies	Complies	Complies

The result of all the batches were found to be within acceptance limit.

Finished Product Analysis Results

The finished product samples were collected after the filling of the batch is completed and the results are tabulated as follows,

Table 13: Finished product analysis results

Sr. No	Tests	Acceptance criteria	Batch 1	Batch 2	Batch 3
1	Appearance and Clarity	Clear colorless to slight yellow solution	Clear slight yellow solution	Clear slight yellow solution	Clear slight yellow solution
2	Identification by IR	Complies with specification.	Complies	Complies	Complies
3	Identification by HPLC	Complies with specification.	Complies	Complies	Complies
4	pH	3.3 to 4.0	3.7	3.7	3.7
5	Assay (by HPLC)	NLT 95.0% - NMT 105.0% w/v of labelled claim.	100.3%	100%	99.8%
6	BET	NMT 5.8 EU/mL	< 2.9 EU/mL	< 2.9 EU/mL	< 2.9 EU/mL
7	Sterility	Comply as per USP	Complies	Complies	Complies

Result of all the batches was complying with in house specification limit.

DISCUSSION

From the above results it was found that no growth observed for any single bacteria or fungi in the growth media, except growth promotion test and thus the media fill conducted for aseptic validation of the aseptic process was found to be complying. The result of media fill confirmed that the area for manufacturing and aseptic filling is in controlled state. Hence the capability of aseptic processing by simulating the entire aseptic formulation and filling process by microbial growth medium is evaluated and qualified.

From the review of data recorded during manufacturing process, in process testing and finished product analysis of all the three validation batches, it is concluded that the manufacturing process is consistent and meets the pre-determined specifications and quality attributes. Hence the manufacturing process of Injection A, stands validated, so the acceptability of the manufacturing process of sterile liquid product is evaluated and qualified.

CONCLUSION

The Process validation of the product Injection A, was carried out and the results were compiled. The results of aseptic media fill confirmed that the area for manufacturing and aseptic filling is in controlled state. The environmental monitoring of the area and personnel revealed that, all the personnel involved during aseptic activity did not contaminate the area during production process. Environmental monitoring results confirmed that HVAC system for aseptic manufacturing area is functioning as per the predetermined specifications. If the conditions will be maintained as such during the routine production process, the product will have consistent quality.

The aseptic process validation conducted for the product Injection A, was found to be complying with the acceptance criteria. Thus documented evidence for the manufacturing process for the product Injection A, was shown that the process has consistently produced the product within the predetermined specifications.

From the review of data recorded during manufacturing process, in process testing and finished product analysis of all the three validation batches, it is concluded that the manufacturing process is consistent and meets the pre-determined specifications and quality attributes. Hence the manufacturing process of Injection A, stands validated.

REFERENCES

1. Syed, I. H. pharmaceutical Master Validation plan, 1st ed.; St. Lucie Press, 2006, 2-27.
2. Work group of Scottish QA specialist interest group: Guidelines on test method for environmental monitoring for Aseptic dispensing, 2004.
3. Parenteral Drug Association. (2007). Validation of moist heat sterilization processes: cycle design, development, qualification and ongoing control. *PDA Journal of Pharmaceutical Science and Technology*, 61(1 Suppl TR1), 2.
4. Scott, B. Process Validation of Oral Solids Dosage form, Part-1 General principles (Available on http://www.ikev.org/haber/bozzonejune1.pdf Accessed on 17 June 2010)
5. http://www.answer.com/topic/validation-drug-manufacture. (Assessed on June 25, 2014)
6. Watler. P., Rathore A. P., F. Edward, R. Arling, G. S., Rhona, L. (2002). Process Validation-How much to Do and When to Do It. *Biopharm*., 18-28.
7. Woodcock, J. The concept of Pharmaceutical Quality. Am. Pharm. Rev. 2004, 1-3. (Available on http://americanpharmaceuticalreview.com/ViewArtical.aspxContentID Accessed on 16 July 2010)

8. http://www.fda.gov/ICECI/EnforcementAction/WarningLetter (Accessed on 19 July 2010)
9. http://www.fda.gov/cber/faq/sanofiqa.html (Accessed on 19 July 2010)
10. Mc Burnie, L., Bardo, B. (2004). Validation of Sterile Filtration. Pharma. Tech., S13-S23.
11. Spurgeon, T. (2006). Aseptic Process Validation is a new FDA Guidance Imminent? Contact Pharma.
12. Brett, M., Belongia, S. (2006). Characterization, Qualification and Validation of a Disposable Final Filling Process for Parenteral and Ophthalmic Drug. *Pharma. Tech.*
13. USFDA Guidelines on process validation: General principle and practice, 2011.

