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IN-PROCESS CONTROLS IN THE MANUFACTURING PROCESS DESIGN AND DEVELOPMENT OF OPHTHALMIC APPLICATIONS

TARAL PRAJAPATI*

Department of Quality Assurance, Saraswati Institute of Pharmaceutical Sciences, Dhanap, Gandhinagar, Gujarat, India

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Corresponding Author

Mr. Taral Prajapati

Department of Quality Assurance,

Saraswati Institute of Pharmaceutical Sciences,

Dhanap, Gujarat.

Abstract

Facilities and processes involved in pharmaceutical production affects the quality of the product. The manufacturing processes should meet the requirements of Good Manufacturing Practices, especially with regard to cross-contamination. Raw material and equipment inspections as well as in-process controls are an integral part of process control. The development of a drug product is a lengthy process involving drug discovery, laboratory testing, animal studies, clinical trials and regulatory registration. Many regulatory agencies across the world require that the drug product should be tested for its identity, strength, quality, purity and stability before it can be released for distribution and use. For this reason, pharmaceutical validation and process controls are important in spite of the problems that may be encountered while performing the same. The following information is intended to provide very broad guidelines concerning the main steps to be followed during production of ophthalmic preparations, indicating those that are the most important. Throughout manufacturing, certain procedures should be validated and monitored by carrying out appropriate in-process controls

INTRODUCTION

What is the need for In-process controls?[1]

It has always been known that facilities and processes involved in pharmaceutical production impact significantly on the quality of the products. The processes include raw material and equipment inspections as well as in-process controls. The development of a drug product is a lengthy process involving drug discovery, laboratory testing, animal studies, clinical trials and regulatory registration. To further enhance the effectiveness and safety of the drug product after approval, many regulatory agencies such as the United States Food and Drug Administration (FDA) also require that the drug product be tested for its identity, strength, quality, purity and stability before it can be released for use. For this reason, pharmaceutical validation and process controls are important in spite of the problems that may be encountered. Process controls include raw materials inspection, in-process controls and targets for final product. The purpose is to monitor the on-line and off-line performance of the manufacturing process and then validate it. Even after the manufacturing process is validated, current good manufacturing practice also requires that a well-written procedure for process controls is established to monitor its performance. Process controls are mandatory in good manufacturing practice (GMP).

Ophthalmic Preparations: Types & In-process Controls[2]

Ophthalmic preparations (eye preparations) are sterile, liquid, semi-solid, or solid preparations that may contain one or more active pharmaceutical ingredient(s) intended for application to the conjunctiva, the conjunctival sac or the eyelids. The choice of base and any excipients used for the preparation of ophthalmic preparations must be proven through product development studies not to affect adversely either the stability of the final product or the availability of the active ingredients at the site of action. The addition of colouring agents is not recommended. The different categories of ophthalmic preparations include drops consisting of emulsions, solutions or suspensions, and ointments.

Types[3]

- Eye drops,
- Eye lotions,
- Powders for eye drops and eye lotions,
- Semi-solid eye preparations,
- Ophthalmic inserts.

Requirements for specific types of ophthalmic preparations[2]

Ophthalmic drops

Ophthalmic drops (eye drops) are sterile aqueous or oily solutions, suspensions, or emulsions intended for instillation into the conjunctival sac. Ophthalmic drops should be clear and practically free from particles when examined under suitable conditions of visibility.

"Water for injections" should be used in the manufacture of aqueous ophthalmic drops. The preparation of aqueous ophthalmic drops requires careful consideration of the need for isotonicity, a certain buffering capacity, the desired pH, the addition of antimicrobial agents and/or antioxidants, the use of viscosity-increasing agents, and the choice of appropriate packaging. Ophthalmic drops are considered isotonic when the tonicity is equal to that of a 0.9% solution of sodium chloride. The eye can usually tolerate solutions equivalent to 0.5–1.8% of sodium chloride.

Ideally, the pH of ophthalmic drops should be equivalent to that of tear fluid, which is 7.4. However, the decision to add a buffering agent should be based on stability considerations. The pH selected should be the optimum for both stability of the active pharmaceutical ingredient and physiological tolerance. If a buffer system is used, it must not cause precipitation or deterioration of the active ingredient. The influence on the lachrymal flow should also be taken into account.

- **Visual inspection**

Evidence of physical instability is demonstrated by the cloudiness of aqueous solutions, due to the formation of a precipitate.

- **Containers**

Ophthalmic drops are normally supplied in suitable multidose containers that allow successive drops of the preparation to be administered. The container should be fitted with a tamper-evident device. The maximum volume of the preparation in such a container should be no more than 10 ml, unless otherwise specified and authorized. Multidose ophthalmic drop

preparations may be used for up to 4 weeks after the container is initially opened. Droppers supplied separately should also comply with the "Test for sterility" Ophthalmic drops may also be provided in suitable single-dose containers that will maintain the sterility of the contents and the applicator up to the time of use. It is recommended that single-dose containers for surgical use should not include any antimicrobial agents

Ophthalmic emulsions

Ophthalmic emulsions are generally dispersions of oily droplets in an aqueous phase. There should be no evidence of breaking or coalescence.

Ophthalmic suspensions

Ophthalmic suspensions contain solid particles dispersed in a liquid vehicle; they must be homogeneous when shaken gently and remain sufficiently dispersed to enable the correct dose to be removed from the container. A sediment may occur, but this should disperse readily when the container is shaken, and the size of the dispersed particles should be controlled. The active ingredient and any other suspended material must be reduced to a particle size small enough to prevent irritation and damage to the cornea.

- **Visual inspection**

Evidence of physical instability is demonstrated by the formation of agglomerates or precipitates in aqueous solutions (suspensions) that do not disperse when the solution is shaken gently.

Ophthalmic ointments

Ophthalmic ointments are sterile, homogeneous, semi-solid preparations intended for application to the conjunctiva or the eyelids. They are usually prepared from non-aqueous bases, e.g. soft paraffin (Vaseline), liquid paraffin, and wool fat. They may contain suitable additives, such as antimicrobial agents, antioxidants, and stabilizing agents.

- **Organoleptic inspection**

Evidence of physical instability is demonstrated by: — a noticeable change in consistency, such as excessive "bleeding" (separation of excessive amounts of liquid) or formation of agglomerates or grittiness; discoloration; emulsion breakdown; crystal growth; shrinking due to evaporation of water; or evidence of microbial growth.

➢ **Uniform consistency**

Ophthalmic ointments should be of uniform consistency. When a sample is rubbed on the back of the hand, no solid components should be noticed.

➢ **Containers**

Ophthalmic ointments are normally supplied in small, sterilized, collapsible tubes fitted with a tamper-evident applicator. The containers or the nozzles of the tubes are shaped so that the ointment can be applied without contaminating what remains in the tube. The content of such a container is limited to not more than 5g of the preparation. Suitable single-dose containers may also be used.

B) Manufacture & In-process Controls[2]

The manufacturing processes should meet the requirements of Good Manufacturing Practices, especially with regard to cross-contamination. The following information is intended to provide very broad guidelines concerning the main steps to be followed during production, indicating those that are the most important. Throughout manufacturing, certain procedures should be validated and monitored by carrying out appropriate in-process controls. These should be designed to guarantee the effectiveness of each stage of production.

For the formulation of aqueous ophthalmic solutions, many critical factors must be taken into consideration such as[4]

- Appropriate salt of the drug substance
- Solubility
- Therapeutic concentration required
- Ocular toxicity
- pKa
- Effect of pH on stability and solubility
- Tonicity
- Buffer capacity
- Viscosity
- Compatibility with other formulation ingredients and packaging components
- Choice of preservative (Antimicrobial)
- Ocular comfort
- Ease of manufacturing

In-process controls[1] during production of ophthalmic preparations should include monitoring environmental conditions (especially with respect to particulate and microbial contamination), pyrogens (use of a limulus amoebocyte lysate (LAL) test may be advantageous), pH and clarity of solution, and integrity of container (absence of leakage, etc.). Test for sterility, pyrogen

test, particulate matter test, uniformity of weight, deliverable mass or volume tests are similar to that of parenteral preparations.

Appropriate limits should be set for the particle size of the active ingredient(s). It is essential that ophthalmic preparations are sterile. An aseptic manufacturing process is usually employed when the dosage form does not allow routine sterilization methods to be used. Packaging must be adequate to protect ophthalmic preparations from light, moisture, microbial contamination, and damage due to handling and transportation.

ENVIRONMENTAL MONITORING[5, 6]

The environmental monitoring[5] program for the sterile bulk drug substance manufacturer should be similar to the programs employed by the SVP industry. This includes the daily use of surface plates and the monitoring of personnel. As with the SVP industry, alert or action limits should be established and appropriate follow-up action taken when they are reached.

There are some bulk drug substance manufacturers that utilize UV lights in operating areas. Such lights are of limited value. They may mask a contaminant on a settling or aerobic plate. They may even contribute to the generation of a resistant (flora) organism. Thus, the use of Rodac or surface plates will provide more information on levels of contamination.

There are some manufacturers that set alert/action levels on averages of plates. For the sampling of critical surfaces, such as operators' gloves, the average of results on plates is unacceptable. The primary concern is any incidence of objectionable levels of contamination that may result in a non-sterile product.

As previously discussed, it is not unusual to see the highest level of contamination on the surfaces of equipment shortly after systems are steamed. If this occurs, the cause is usually the inadequate removal of condensate.

Since processing of the sterile bulk drug substance usually occurs around the clock, monitoring surfaces and personnel during the second and third shifts should be routine.

In the management of a sterile bulk operation, periodic

(weekly/monthly/quarterly) summary reports of environmental monitoring are generated. Review these reports to obtain those situations in which alert/action limits were exceeded. Review the firm's investigation report and the disposition of batches processed when objectionable environmental conditions existed.

Environmental monitoring should cover three operational shifts by following methods[6]

- Settle Plate method
- Air sampling
- Swab testing
- Personnel monitoring

Cleaning of area must be done by routine cleaning agent and disinfectant solution as per latest SOP. Microbiological Environmental monitoring should be carried out to cover the entire media fill program for manufacturing area by Settle plate, Active Air sampling, Swab testing and Personnel monitoring as per the latest SOP.

pH[7]: Many drugs, notably alkaloidal salts, are most effective at pH levels that favour the undissociated free bases. At such pH levels, however, the drug may be unstable so that compromise levels must be found and held by means of buffers. One purpose of buffering some ophthalmic solutions is to prevent an increase in pH caused by the slow release of hydroxyl ions by glass. Such a rise in pH can affect both the solubility and the stability of the drug. The decision whether or not buffering agents should be added in preparing an ophthalmic solution must be based on several considerations. Normal tears have a pH of about 7.4 and possess some buffer capacity. The application of a solution to the eye stimulates the flow of tears and the rapid neutralization of any excess hydrogen or hydroxyl ions within the buffer capacity of the tears. Many ophthalmic drugs, such as alkaloidal salts, are weakly acidic and have only weak buffer capacity. Where only 1or 2 drops of a solution containing them are added to the eye, the buffering action of the tears is usually adequate to raise the pH and prevent marked discomfort. In some cases pH may vary between 3.5 and 8.5. Some drugs, notably pilocarpine hydrochloride and epinephrine bitartrate, are more acid and overtax the buffer capacity of the lacrimal fluid. Ideally, an ophthalmic solution should have the same pH, as well as the same isotonicity value, as lacrimal fluid. This is not usually possible

since, at pH 7.4, many drugs are not appreciably soluble in water. Most alkaloidal salts precipitate as the free alkaloid at this pH. Additionally, many drugs are chemically unstable at pH levels approaching 7.4. This instability is more marked at the high temperatures employed in heat sterilization. For this reason, the buffer system should be selected that is nearest to the physiological pH of 7.4 and does not cause precipitation of the drug or its rapid deterioration.

An ophthalmic preparation with a buffer system approaching the physiological pH can be obtained by mixing a sterile solution of the drug with a sterile buffer solution using aseptic technique. Even so, the possibility of a shorter shelf-life at the higher pH must be taken into consideration, and attention must be directed toward the attainment and maintenance of sterility throughout the manipulations.

Many drugs, when buffered to a therapeutically acceptable pH, would not be stable in solution for long periods of time. These products are lyophilized and are intended for reconstitution immediately before use (e.g., Acetylcholine Chloride for Ophthalmic Solution).[5]

UNIFORMITY OF VOLUME[3]

Pour completely the contents of each container into calibrated volume measures of the appropriate size and determine the volume of contents of 10 containers. The average net volume of the contents of the 10 containers is not less than the labelled amount, and the net volume of the contents of any single container is not less than 91% and not more than 109 % of the labelled amount where the labelled amount is 50 ml or less or not less than 95.5% and not more than 104.5% of the labelled amount where the labelled amount is more than 50 ml but not more than 200 ml, or not less than 97% and not more than 103% of the labelled amount where the labelled amount is more than 200 ml but not more than 300 ml. If these requirements are not met, determine the net volume of the contents of 10 additional containers. The average net volume of the contents of the 20 containers is not less than the labelled amount, and the net volume of the contents of not more than 1 of the 20 containers is less than 91 % or more than 109% of the labelled amount where the

labelled amount is 50 ml or less or not less than 95.5% and not more than 104.5% of the labelled amount where the labelled amount is more than 50 ml but not more than 200 ml, or not less than 97% and not more than 103% of the labelled amount where the labelled amount is more than 200 ml but not more than 300 ml.

TEST FOR METAL PARTICLES IN OPHTHALMIC OINTMENTS

Take 10 ophthalmic ointments to be tested, and extrude the contents into a Petri dish. Cover the dish and heat between 85°C to 110°C for 2 h to dissolve the bases. Allow the sample to room temperature without agitation to solidify the contents. Invert each dish on the stage of suitable microscope previously adjusted to provide more than 40 times magnifications and equipped with eye piece micrometer disk. Each dish is illuminated from above 45° relative to the plane dish. Examine the entire bottom of each dish for metal particles and record the total number of particles, measuring 50 µm or more in dimensions. No particles should be present.

INSOLUBLE PARTICULATE MATTER TEST FOR OPHTHALMIC SOLUTIONS

Fit the membrane filter on to the membrane filter holder. Filter under reduced pressure 200 ml of the purified water for particulate matter test at the rate of 20 to 30 ml / min. Apply vacuum until the surface of the membrane is free from water and remove the membrane and dry it carefully below 50°C. After the filter is dried, place it under the microscope. Adjust the microscope to get the best view of the particles that are equal to or greater than 150 µm. Ascertain that the number is not more than 1.

Fit another membrane filter and wet it with purified water for particulate matter test. Pour the sample solution into the filter. For viscous solutions, dilute suitably with purified water for particulate matter test and filter. When the amount of solution on the filter becomes small, add 30 ml of water. Repeat the process 3 times with 30 ml of the water. Apply the vacuum gently until the surface of membrane filter is free from water. Dry it and observe under microscope. Count the number of particles which are equal to or larger than 300 µm.

PARTICLE SIZE

Introduce a suitable quantity of the preparation into a counting cell or with a micropipette onto a slide, as appropriate and scan under microscope an area corresponding to 10 µg of the solid phase. For practical reasons, it is recommended that the whole sample is first scanned at low magnification (e.g. 50X) and particles greater than 25 µm are identified. These larger particles can then be measured at a larger magnification (e.g. 200X to 500X). For each 10 µg of solid active substance, particle size and number of particles are given in the Table 1.

UNIFORMITY OF CONTENT

Determine the content of the active ingredient of each of 10 containers taken at random. The preparation under examination complies with the test if the individual values thus obtained are all between 85 and 115 percent of the average value. The preparation under the examination fails to comply with the test if more than one individual value is outside the limits 85 to 115 percent of the average value or if any one individual value is outside the limits 75 to 125 percent of the average value. If one individual value is outside the limits 85 to 115 percent but within the limits 75 to 125 percent of the average value, repeat the determination using another 20 containers taken at random. The preparation under examination complies with the test if in the total sample of 30 containers not more than one individual value is outside the limits 85 to 115 percent and none is outside the limits 75 to 125 percent of the average value.

TEST FOR STERILITY

Culture Media

1. Fluid thioglycollate medium: It is used for anaerobic bacteria. Use fluid thioglycollate medium by incubating it at 30° - 35°C.

2. Soyabean-casein digest medium: It is used for fungi and aerobic bacteria. Use soybean- casein digest medium by incubating it at 20°C - 25°C under aerobic conditions.

3. Alternative thioglycollate medium: For use with turbid and viscid products and for devices having tubes with small Lumina.

Test procedure: Method A (membrane filtration) is to be preferred where the substance under examination is

- An oil

- An ointment that can be put into solution
- A non-bacteriostatic solid not readily soluble in the culture medium and
- A soluble powder or a liquid that have bacteriostatic and /or fungistatic properties.

For liquid products where the volume in a container is 100 ml or more, method A should be used.

• Method A – Membrane filtration

The method calls for the routine use of positive and negative controls.

Apparatus: Cellulose nitrate filters are used for aqueous, oily and weakly alcoholic solutions and cellulose acetate filters are recommended for strongly alcoholic solutions.

Diluting Fluids (IP, BP)

Fluid A: Dissolve 1 g of peptic digest of animal tissue (such as bacteriological peptone) or its equivalent in water to make 1 L, filter or centrifuge to clarify, adjust to pH 7.1 ± 0.2, dispense into flasks in 100 ml quantities and sterilize at 121°C for 20 min.

Fluid B: If the test sample contains lecithin or oil, use fluid A to each liter of which has been added 1 ml of polysorbate 80, adjust to pH 7.1± 0.2, dispense into flasks and sterilize at 121°C for 20 min.

❖ **Quantities of sample to be used**

For ophthalmic preparations: Take an amount within the range prescribed in column (A) of table 3, if necessary, using the contents of more than one container, and mix thoroughly. For each medium use the amount specified in column (B) of Table 3, taken from the mixed sample.

Test method

For aqueous solutions:

Aseptically transfer a small quantity of fluid A on to the membrane and filter it. Transfer aseptically the combined quantities of the preparation under examination prescribed in the two media onto one membrane. If the solution under examination has antimicrobial properties, wash the membrane(s) by filtering through it (them) not less than three successive quantities, each of 100 ml, of sterile fluid A. Do not exceed a washing cycle of 5 times or 200 ml, even if it has been demonstrated during validation that such a cycle does not fully eliminate the antimicrobial activity. The quantities of fluid used should be sufficient to allow growth of a small inoculum of

organisms (approximately 50 CFU) sensitive to the antimicrobial substance in the presence of the residual inhibitory material on the membrane.

After filtration, aseptically remove the membrane(s) from the holder, transfer the whole membrane or cut it aseptically into 2 equal parts. Transfer one half to each of two suitable media. Incubate the media for not less than 14 days.

Observe the containers of media periodically during the 14 days of incubation. If the test specimen is positive before 14 days of incubation, further incubation is not necessary. For products terminally sterilized by a validated moist heat process, incubate the test specimen for not less than 7 days.

For liquids immiscible with aqueous vehicles, and suspensions

Carry out the test described under for aqueous solutions but add a sufficient quantity of fluid A to the pooled sample to achieve rapid filtration. Sterile enzyme preparations such as penicillinase or cellulase may be added to fluid A to aid in dissolving insoluble substances. If the substance being examined contains lecithin, use fluid B for diluting.

For oils and oily solutions

Filter oils or oily solutions of sufficiently low viscosity without dilution through a dry membrane. Dilute viscous oils as necessary with a suitable sterile diluent such as isopropyl myristate that has been shown not to have antimicrobial properties under the conditions of the test. Allow the oil to penetrate the membrane and filter by applying pressure or by suction, gradually. Wash the membrane by filtering through it at least three successive quantities, each of approximately 100 ml of sterile fluid B or any other suitable sterile diluent.

After filtration, aseptically remove the membrane(s) from the holder, transfer the whole membrane or cut it aseptically into 2 equal parts. Transfer one half to each of two suitable media**.** Incubate the media for not less than 14 days.

Observe the containers of media periodically during the 14 days of incubation. If the test specimen is positive before 14 days of incubation, further incubation is not necessary. For products terminally sterilized by a validated moist

heat process, incubate the test specimen for not less than 7 days.

For ointments and creams

Dilute ointments in a fatty base and emulsions of the water-in-oil type to give a fluid concentration of 1 % w/v, by heating, if necessary, to not more than 40°C with a suitable sterile diluent such as isopropyl myristate previously rendered sterile by filtration through a 0.221 µm membrane filter that has been shown not to have antimicrobial properties under the conditions of the test. Filter as rapidly as possible and complete the test as described under for oils and oily solutions. In exceptional cases, it may be necessary to heat the substance to not more than 44°C and to use warm solutions for washing the membrane.

For soluble solids

For each medium, dissolve not less than the quantity of the substance under examination, as prescribed in Table 3, in a suitable sterile solvent such as fluid A and carry out the test described under for aqueous solutions using a membrane appropriate to the chosen solvents.

For solids for injection other than antibiotics

Constitute the test articles as directed on the label, and carry out the test as described under for aqueous solutions or for oils and oily solutions, as applicable.

- **Method B: Direct inoculation method**

The quantity of the substance or preparation under examination to be used for inoculation in the culture media varies according to the quantity in each container.

Test method

For aqueous solutions and suspensions

Remove the liquid from the test containers with a sterile pipette or with a sterile syringe or a needle. Transfer the quantity of the preparation under examination prescribed in Table 3 directly into the culture medium so that the volume of the preparation under examination is not more than 10 % of the volume of the medium, unless otherwise prescribed. When the quantity in a single container is insufficient to carry out the tests, the combined contents of two or more containers are to be used to inoculate the media.

If the preparation under examination has antimicrobial activity, carry out the test

after neutralizing this with a suitable neutralizing substance or by dilution in a sufficient quantity of culture medium. When it is necessary to use a large volume of the product it may be preferable to use a concentrated culture medium prepared in such a way that it takes account of the subsequent dilution. Where appropriate, the concentrated medium may be added directly to the product in its container.

Incubate the inoculated media for not less than 14 days. Observe the cultures several times during the incubation period. Observe the containers of media periodically during the 14 days of incubation. If the test specimen is positive before 14 days of incubation, further incubation is not necessary. For products terminally sterilized by a validated moist heat process, incubate the test specimen for not less than 7 days.

For oils and oily solutions

Use media to which has been added a suitable emulsifying agent at a concentration shown to be appropriate in the validation of the test, for example, polysorbate 80 at a concentration of 10g/L and which has been shown not to have any antimicrobial properties under the conditions of the test. Carry out the test as described under for aqueous solutions and suspensions.

During the incubation period shake the cultures gently each day. However, when thioglycollate medium or other similar medium is used for the detection of anaerobic micro-organisms, keep shaking or mixing to a minimum in order to maintain anaerobic conditions.

For ointments and creams

Prepare by diluting to about 1 in 10 by emulsifying with the chosen emulsifying agent in a suitable sterile diluent such as fluid A. Transfer the diluted product to a medium not containing an emulsifying agent. (Before use, test the emulsifying agent to ascertain that in the concentration used it has no significant antimicrobial effects during the time interval for all transfers). Mix 10 ml of the fluid mixture so obtained with 80 ml of the medium and proceeds as directed under for aqueous solutions and suspensions.

For solids

Transfer the quantity of the preparation under examination to the quantity of medium specified in Table 3 and mix.

Proceed as directed under for aqueous solutions and suspensions.

Observation and Interpretation of Results

At intervals during the incubation period and at its conclusion, examine the media for macroscopic evidence of microbial growth. If the material being tested renders the medium turbid so that the presence or absence of microbial growth cannot be easily determined by visual examination, 14 days after the beginning of incubation, transfer portions (each not less than 1 ml) of the medium to fresh vessels of the same medium and then incubate the original and transfer vessels for not less than 4 days.

If no evidence of microbial growth is found, the preparation under examination complies with the test for sterility. If evidence of microbial growth is found, the preparation under examination does not comply with the test for sterility. Do not repeat the test unless it can be clearly shown that the test was invalid for causes unrelated to the preparation under examination. The test may be considered invalid only when one or more of the following conditions are fulfilled:

- Microbial growth is found in negative controls.
- Data on microbial monitoring of the sterility testing facility show a fault.
- A review of the testing procedure used for the test in question reveals a fault.

After identifying the micro organisms isolated from the containers showing microbial growth may be as described without any doubt to faults with respect to the materials and/or technique

used in conducting the test procedure. If the test is declared to be invalid, repeat with the same number of units as in the original test. If no evidence of microbial growth is found in the repeat test, the preparation under examination complies with the test for sterility. If microbial growth is found in the repeat test and confirmed microscopically, the preparation under examination does not comply with the test for sterility. Table 4 gives guidance on the minimum no. of items recommended to be tested.

TEST FOR DELIVERABLE MASS OR VOLUME OF LIQUID AND SEMI-SOLID PREPARATIONS

The test applies to liquid (solutions, emulsions and suspensions) and semi-solid preparations supplied in single-dose containers where only part of the contents is used.

LIQUID PREPARATIONS

Empty as completely as possible the contents of one container and determine the mass or volume of the contents as appropriate. In the case of emulsions and suspensions, shake the container before the determination. The mass or volume is not less than the amount stated on the label.

SEMI-SOLID PREPARATIONS

Empty as completely as possible the contents of one container. The mass of the contents is not less than that which is stated on the label.

UNIFORMITY OF WEIGHT

Remove labels and wash the container and dry. Weigh the container along with its contents. Empty the containers as completely as possible. Rinse with water and with ethanol and dry at 100°C to a constant weight. Allow to cool in desiccators and weigh. The difference between the weights represents the weight of the contents. Repeat the procedure with further 19 containers and determine the average weight. Not more than two of the individual weights deviate from the average weight by more than 10% and none deviates by more than 20%.

TEST FOR PYROGEN

The test involves measurement of the rise in body temperature of rabbits following the intravenous injection of a sterile solution of the substance under examination. Do not use animals for pyrogen tests more frequently than once every 48 h. After a pyrogen test in the course of which a rabbit's temperature has risen by 0.6°C or more, or after a rabbit has been given a test substance that was adjudged pyrogenic, at least 2 weeks must be allowed to elapse before the animals is used again.

Test animals: Healthy adult rabbit of either sex (1.5 Kg)

Recording of temperature: Use temperature-sensing device such as a clinical thermometer or thermistor or other suitable probes (accuracy of 0.10°C). Insert the thermometer or temperature-sensing probe into the rectum of the test rabbit to a

depth of about 5 cm. (IP, BP) **{7.5 cm –USP, Ph. Eur and JP}**

Preliminary Test (Sham Test)

Injecting intravenously 10 ml/ kg body weight of a pyrogen-free saline solution warmed to about 38.5°C. Record the temperatures of the animals, beginning at least 90 min before injection and continuing for 3 h after injection of the test solution. Any animal showing a temperature variation of 0.6°C or more must not be used in the main test.

Main Test: Carry out the test using a group of three rabbits.

Preparation of the sample: Dissolve the substance with pyrogen-free saline solution. Warm the liquid under examination to approximately 38.5°C before injection.

Procedure: Record the temperature of each animal 90 min before the injection and continue for 3 h after the injection for every 30 min. Record the "initial temperature" of each rabbit and temperature after 30 min. Rabbits showing a temperature variation greater than 0.2°C between two successive readings in the determination of "initial temperature" should not be used for the test. Do not use any rabbit having a temperature higher than 39.8°C and lower than 38°C.

Inject the solution slowly into the marginal vein of the ear of each rabbit over a period not exceeding 4 min. The volume of injection is not less than 0.5 ml/kg and not more than 10 ml/kg of body weight. The difference between the "initial temperature" and the "maximum temperature" which is the highest temperature recorded for a rabbit is taken as its response. When this difference is negative, the result is counted as a zero response.

Interpretation of results: Having carried out the test, first on a group of three rabbits, repeat if necessary on further groups of rabbits given in the Table 6 depending on the results obtained. If the summed response of the first group does not exceed the figure given in the third column of the Table 6 the substance passes the test. If the response exceeds the figure given in the third column of the table but does not exceed the figure given in the fourth column of the table, repeat the test as indicated above. If the summed response exceeds the figure given in the fourth

column of the table, the product fails the test.

Table 1

Limits for Particle number in different Pharmacopoeias.

Limits for particle number as per IP, USP, BP, JP, Ph. Eur Pharmacopoeia	Particle size	No. of particles allowed
IP	25 µm	Not more than 20
	50 µm	Not more than 2
	100 µm	Nil
BP, JP, Ph. Eur	25 µm	Not more than 20
	50 µm	Not more than 10
	90 µm	Nil

Table 2

Strains of the microorganisms used for the test as per IP, USP, BP, JP, Ph. Eur

Medium	Test Microorganism	Incubation		
		Temp. (°C)	Duration in Days	Type of Microorganism
Fluid Thioglycolate	- Bacillus subtilis	30-35		
	- Staphyllococcus aureus	30-35	3 days	Anaerobic
	- Pseudomonas aeruginosa	30-35	3 days	Anaerobic
			3 days	Anaerobic
Alternate Thioglycolate	- Bacterides vulgates	30-35	3 days	Anaerobic
	- Clostridium sporogenes	30-35	3 days	Anaerobic
Soya bean casein digest	- Aspergillus niger	20-25	5 days	Aerobic
	- Candida albicans	20-25	5 days	Aerobic

Table 3

Quantities of samples to be used for ophthalmic preparations

Type of preparation	Quantity to be mixed (A)	Quantity to be used for each culture medium (B)
Ophthalmic solution other than non-parenteral liquid preparations	10 to 100 ml	5 to 10 ml
Other preparations soluble in water or appropriate solvents; insoluble preparations to be suspended or emulsified.	1 to 10 g	0.5 to 1 g
Absorbent cotton	1 to 10 g	Not less than 1 g.

Table 4

Minimum No. of items to be tested

Number of items in the batch	Minimum number of items recommended to be tested
Ophthalmic and other non-Parenteral preparations.	
Not more than 200 containers	5% or containers whichever is greater.
More than 200 containers	10 containers.

Table 5

Limits for uniformity of weight

Pharmaceutical formulation	Average mass	Percentage deviation (%)
Powders for eye drops	Less than 300 mg	10
Powders for eye lotions	300 mg or more	7.5

Table 6

Results according to IP, BP, USP, JP, Ph. Eur.

Pharmacopeia	No. of rabbits in a group	Passes if temp. is not more than (°C)	Fails if temp is more than (°C)
IP	3	1.4	Each rabbit temp raise should not be more than 0.6°C
	8	3.7	
USP	3	----	Each rabbit temp raise should not be more than 0.6°C
	8	3.3	
BP, Ph. Eur	3	1.15	2.65
	6	2.80	4.30
	9	4.45	5.95
	12	6.6	6.6
JP	3	1.3	2.5
	6	3	4.2
	9	5	5

Table 7

Specifications of each test for eye drops and eye lotions according to IP, BP, USP, Ph. Eur. & JP

Tests	IP	BP	USP	Ph. Eur	JP
Uniformity of volume	50 ml or less ±9% 50 to 200 ml ±4.5% 200 to 300 ml ±3%	×	×	×	×
Sterility	No growth in 14 days	No growth in 14 days	No growth in 14 days	No growth in 14 days	No growth in 14 days
Particulate matter	×	×	≥ 25µm -2 can be present	×	≥ 25 µm -2 can be present
Particle size	×	No particle should be ≥ 90µm.	×	No particle should be ≥ 90 µm	No particle should be ≥ 90 µm
Deliverable mass or volume	×	×	×	Not less than 100%	×

Table 8

Specifications of each test for powders for eye drops according to BP, USP & Ph. Eur

Tests	BP	USP	Ph. Eur
Sterility	No growth in 14 days	No growth in 14 days	No growth in 14 days
Uniformity of content	85-115%	85-115%	85-115%
Uniformity of mass	90-110%	90-110%	90-110%

Table 9

Specifications of each test for semisolid eye preparations according to IP, BP, Ph. Eur & JP

Tests	IP	BP	Ph. Eur	JP
Particle size	No particle should be ≥ 100µm	No particle should be ≥ 90µm	No particle should be ≥ 90µm	×
Sterility	No growth in 14 days	No growth in 14 days	No growth in 14 days	No growth in 14 days
Uniformity of weight	90-110 %	×	×	×
Deliverable mass/volume	×	×	Not less than stated amount	×
Test of metal particles	×	×	×	Metal particles should be absent

Table 10

Specifications of each test for ophthalmic inserts according to IP, BP, USP, Ph. Eur & JP

Tests	IP	BP	USP	Ph. Eur	JP
Uniformity of content	85-115%	85-115%	×	85-115%	85-115%
Sterility	No growth in 14 days	No growth in 14 days	No growth in 14 days	No growth in 14 days	No growth in 14 days

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