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Comparative Study of In-Process and Finished Product Quality Control Test's Of IP, BP, USP, EP, JP for Parenterals.

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ABSTRACT

The present study deals with the elaborated overview of comparative study of in-process and finished product quality control tests for parenteral products taking compendia specifications of Indian Pharmacopoeia (IP), British Pharmacopoeia (BP), United States Pharmacopoeia (USP), European Pharmacopoeia (EP) and Japanese Pharmacopoeia (JP) into consideration. When it comes to most sensitive parts of body i.e.; veins, a high degree of precautions should be taken during and after production of product for it to avoid any hitches. The sterility of these parenteral products, as well as accuracy in the calculation and preparation of doses is of great importance and a must in terms of compliance. The high graded quality product always refers to a product which is within the compendia limits. This article focuses on the comparison of Pharmacopoeial specifications of parenteral preparations, the procedures that are employed to maintain the quality and sterility of these ophthalmic products. This covers specifications and limits of different dosage forms according to different Pharmacopeia (like IP, BP, USP, JP etc.) which helps in comparative study of specifications provided in different Pharmacopeia as well as highlighting the differences in standards and also focussing on the specifications of different nations. Different regulatory requirements of the respective countries demand products with different specific limits so this comparative study will help in meeting all the requirements of all the pharmacopoeias and later the regulatory requirements of that particular country.

Keywords: Indian Pharmacopoeia (I.P), British Pharmacopoeia (B.P), United States Pharmacopoeia (USP), European Pharmacopoeia (EP), Japanese Pharmacopoeia (JP) and parenteral.

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INTRODUCTION

1. Sterile products[1]

Sterile products are the dosage forms of therapeutic agents that are free of viable microorganisms. Principally these include parenteral, ophthalmic and irrigational preparations. Of these, parenteral products are unique dosage forms of drugs as they are injected through the skin or mucous membranes into the internal body compartments. As they have circumvented the highly efficient first line of body defense i.e., the skin mucous membranes, they must be free from microbial contamination and from toxic compartments as well as possess an exceptionally high level of purity. All components and processes involved in the preparation of these products must be selected and designed to eliminate, as much as possible, contamination of all types, whether physical, chemical or microbiological origin[1]

Preparations for eye, though not introduced into the internal body cavities, are placed in contact with tissues that are very sensitive to contamination. Therefore similar standards are required for ophthalmic preparations.

Irrigating solutions are also required to meet the same standards as parenteral solutions because during an irrigation procedure, substantial amounts of these solutions can enter the blood stream directly through the blood vessels of wounds or abraded mucous membranes. Therefore similar characteristics and standards apply equally to irrigations also.

Sterile products discussed here includes

Parenteral preparations[1]

Parenteral:

Parenteral preparations are sterile preparations intended for administration by injection, infusion or implantation into the human or animal body. Parenteral preparations may require the use of excipients, for example to make thepreparation isotonic with blood, to adjust the pH, to increase solubility, to prevent deterioration of the active substances or to provide adequate antimicrobial properties but not to adversely affect the intended medicinal action of the preparation or, at the concentrations used, to cause toxicity or undue local irritation.

Containers for parenteral preparations are made as far as possible from materials that are sufficiently transparent to permit the visual inspection of the contents, except for implants and in other justified and authorized cases**.**

Several categories of parenteral preparations may be distinguished:

1.Injections,

2.Infusions,

3.Concentrates for injections or infusions,

4.Powders for injections or infusions,

Thevarious in process and finished product tests to be performed for parenteral preparations and the detailed procedure for performing the tests according to IP, BP, USP, EP and JP are as follows:

SPQC-1UNIFORMITY OF CONTENT[2]

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.

SPQC-2 EXTRACTABLE VOLUME[3]

Suspensions should be shaken before the contents are withdrawn. Oily injections may be warmed but should be cooled to 25^0 C before carrying out the test.

Single dose containers: for IP[1]

Method I: **Where the nominal volume does not exceed 5ml.**

Use 6 containers, 5 for the tests and 1 for rinsing the syringe used. Using a syringe with appropriate capacity, rinse the syringe and withdraw as much as possible the contents of one of the containers reserved for the test and transfer, without emptying the needle, to a dry graduated cylinder of such capacity that the total combined volume to be measured occupies not less than 40% of the nominal volume of the cylinder. Repeat the procedure until the contents of the 5 containers have been transferred and measure the volume. The average content of the 5 containers is not less than the nominal volume and not more than 115% of the nominal volume.

Alternatively the volume of contents in milliliter can be calculated as mass in grams divided by the density.

Method II:Where the nominal volume is more than 5 ml.

Transfer the contents of not less than 3 containers separately to dry graduated cylinders such that the volume to be measured occupies not less than 40% of the nominal volume of the cylinder and measure the volume transferred. The contents of each container are not less than the nominal volume and not more than 110% of the nominal volume.

Multi dose containers: same as single dose containers.

No. of containers to be used for the test according to BP, USP, EP, JP (Table: 1.1)

Table: 1.1 No of containers to be used for the test according to BP, USP, EP, JP

Volume of the solution	No of containers to be used for the test
≥ 10 ml	1
3-10 ml	3
< 3 ml	5

SPQC-3 TEST FOR PYROGEN[3]

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 hours. After a Pyrogen test in the course of which a rabbit's temperature has risen by 0.6 0C 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 0C). 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, EP, and JP}

Preliminary Test (Sham Test)

Injecting intravenously 10 ml per kg body weight of a pyrogen-free saline solutionwarmed to about 38.5 0C. Record the temperatures of the animals, beginning at least 90 minutes before injection and continuing for 3 hours after injection of the test solution. Any animal showing a temperature variation of 0.6 0C 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 minutes before the injection and continue for 3 hours after the injection for every 30 minutes. Record the "initial temperature"

of each rabbit and temperature after 30 minutes. 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 1.2, 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 1.2, 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.

The following table (Table 1.2) gives the results according to IP, BP, USP, EP, JP.

Table1. 2 Results according to IP, BP, USP, EP, JP.

Pharmacopeia	No. of rabbits in a group	Passes if temp. is not more than (^{0}C)	Fails if temp is more than (^{0}C)
IP	3	1.4	Each rabbit temp raise should not be more than 0.6 ^{0}C
	8	3.7	
USP	3	----	Each rabbit temp raise should not be more than 0.6 ^{0}C
	8	3.3	
BP, EP	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

SPQC-4 TEST FOR PARTICULATE CONTAMINATION[1]

For sub-visible particles

Two methods are specified, one involving the counting of particles viewed under the microscope and the other based on the count of particles causing light obscuration. Both methods are applied on small samples.

Method 1.Microscopic particle count test

This method is suitable for revealing the presence of particles where the longest axis or effective linear dimension of which is 10 µm or more.

Method: Invert the container of the preparation 20 times. For large volume parenterals, single units should be tested. For small volume parenterals less than 25 ml in volume, the contents of 10 or more units should be combined in a clean container. Where the volume of liquid in a container is very small, the test solution may be prepared by mixing the contents of a suitable number of containers and diluting to 25 ml with particle free water. Small-volume parenterals having a volume of 25 ml or more may be tested individually. Powders for parenteral use should be constituted with particle free water.

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 the vacuum until the surface of the membrane is free from water, remove the membrane and dry it carefully below 50 ^{0}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 vaccum gently until the surface of membrane filter is free from water. Dry it and observe under microscope. Count the number of particles that are equal to or greater than 10 µm, the number of particles equal to or greater than 25 µm and the particles equal to or greater than 50 µm. Maximum number of particles that can be present are mentioned in the following table 1.3

Table1.3: Limits for particle number as per IP, USP, BP, EP, JP

Volume of solution	Particle size ≥ 10 µm	Particle size ≥25 µm
Small volume injections (< 100 ml)	3000 per container	300 per container
Large volume injections (> 100 ml)	12 per ml	2 per ml

Method 2: Light obscuration particle count test[3]

Use a suitable apparatus based on the principle of light blockage which allows an automatic determination of particle size and the number of particles according to size.

Method: Invert the container of the preparation 20 times. For large volume parenterals, single unit should be tested. For small volume parenterals less than 25 ml in volume, the contents of 10 or more units should be combined in a clean container. Where the volume of liquid in a container is very small, the test solution may be prepared by mixing the contents of a suitable number of containers and diluting to 25 ml with particle free water. Small-volume parenterals having a volume of 25 ml or more may be tested individually. Powders for parenteral use should be constituted with particle free water.

Remove 4 portions, each of not less than 5 ml, count the number of particles greater than 10µm and 25µm. Maximum number particles that can be present are mentioned in the following table 4.

Table 1.4: Limits for particle number as per IP, USP, BP, EP, JP

Volume of solution	Particle size ≥ 10 µm	Particle size ≥25 µm
Small volume injections (< 100 ml)	6000 per container	600 per container
Large volume injections (> 100 ml)	25 per ml	3 per ml

SPQC-5 TEST FOR STERILITY[1]

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° - 25°C under aerobic conditions.

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

Table 1.5: Strains of the Micro-organisms used for the test as per IP, USP, BP, EP, JP

Medium	Test micro organism	Incubation		
		Temp.(˚C)	Duration (Days)	Type of micro organism
Fluid thioglycollate	*Bacillus subtilis*	30-35	3 days	Anaerobic
	Staphylococcus aureus	30-35	3 days	Anaerobic
	Pseudomonas aeruginosa	30-35	3 days	Anaerobic
Alternate thioglycollate	*Bacterides vulgates*	30-35	3 days	Anaerobic
	Clostridium sporogenes	30-35	3 days	Anaerobic
Soya bean casein digest	*Asperigillusniger*	20-25	5 days	Aerobic
	Candida albicans	20-25	5 days	Aerobic

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

A) An oil

B) An ointment that can be put into solution

C) A non bacteriostatic solid not readily soluble in the culture medium and

D) 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 waterto make 1 litre, filter or centrifuge to clarify, adjust to pH 7.1 ± 0.2, dispense into flasks in 100 ml quantities and sterilize at 121 ^{0} C for 20 minutes.

Fluid B: If the test sample contains lecithin or oil, use fluid A to each litre of which has been added 1 ml of polysorbate 80, adjust to pH 7.1± 0.2, dispense into flasks and sterilize at 121 ^{0}C for 20 minutes.

Quantities of sample to be used

For parenteral preparations: Whenever possible, use the whole contents of the container, but in any case not less than the quantities prescribed in Table 1.6, dilute to about 100 ml with a suitable diluent such as fluid A.

For ophthalmic and other non-parenteral preparations: Take an amount within the range prescribed in column (A) of table 1.7, 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 1.7, 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 myristatethat 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 myristatepreviously 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 Tables 1.6 and 1.7, 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 1.6 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, polysorbate80 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.

Table1.6: Minimum quantity to be used for each medium

Quantity in each container of inject able preparation	Minimum quantity to be used for each culture medium
For liquids	Total contents of the container
Less than 1 ml	Half the contents of the container.
1ml or more but less than 40ml	20 ml
40ml or more but less than 100ml	10 % of the contents of container but not less than 20 ml.
100ml or more	
Antibiotic liquids	1ml
Other preparations soluble in water or in isopropyl myristate	The whole contents of each container to provide not less than 200 mg
Insoluble preparations, creams and ointments to be suspended or emulsified.	The whole contents of each container to provide not less than 200 mg
For solids	Total contents of the container
Less than 50 mg	Half the contents of the container.
50 mg or more but less than 300 mg	100 mg
300 mg or more	
For catgut and other surgical sutures for veterinary use	3 sections of the strand
For surgical dressings / cotton / gauge	100 mg per package
For sutures and other individually packed single use materials	The whole device or materials, cut into pieces or disassembled

Table 1.7: Quantities of samples to be used for ophthalmic and non parenteral 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 on 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.

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 proceed 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 1.7 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:

a) Microbial growth is found in negative controls.
b) Data on microbial monitoring of the sterility testing facility show a fault.
c) A review of the testing procedure used for the test in question reveals a fault.
d) After identifying the microorganisms isolated from the containers showing microbial growth may be as scribed 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 1.8 gives guidance on the minimum no. of items recommended to be tested.

SPQC-6 TEST FOR DELIVERABLE MASS OR VOLUME OF LIQUID AND SEMI-SOLID PREPARATIONS[1]

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.

Table 1.8: Minimum No. of items to be tested

Number of items in the batch	Minimum number of items recommended to be tested
1. Parenteral Preparations	
Not more than 100 containers	10% or 4 containers
More than 100 but not more than 500 containers	10 containers
More than 500 containers	2% or 20 containers whichever is less
For large volume parenterals	2% or 20 containers whichever is less
2. Ophthalmic and other non-Parenteral preparations.	
Not more than 200 containers	5% or containers whichever is greater.
More than 200 containers	10 containers.
3. Bulk solids	
Less than 4 containers	Each container
4 containers but not more than 50 containers	20 % or 4 containers whichever is greater.
More than 50 containers.	2% or 10 containers whichever is greater

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.

SPQC-7 TEST FOR BACTERIAL ENDOTOXINS[1]

The test for bacterial endotoxins (BET) measures the concentration of bacterial endotoxins that may be present in the sample or in the articles to which the test is applied using a lysate derived from hemolymph cells or amoebocytes of horse shoe crab, limulus polyphemus.

The endotoxin limit for a given test preparation is calculated from the expression K/M, where M is the maximum dose administered to an adult (taken as 70 Kg for this purpose) per kg hour. The value of K is 5.0 EU/kg for parenteral preparations except those administered intrathecally, and is 0.2 EU/kg for preparations intended to be administered intrathecally.

The following methods can be used to monitor the endotoxin concentration:

Method A - Gel- clot limit test method

Method B -Semi quantitative gel clot method

Method C - Kinetic turbidimetric method

Method D - Kinetic chromogenic method

Method E - End point chromogenic method

Method A. Gel- clot limit test method

Prepare the solutions and dilutions with water BET. If necessary, adjust the pH of the solution to 6.0 to 8.0 using sterile 0.1 M hydrochloric acid BET, 0.1 M sodium hydroxide BET of suitable buffer prepared with water BET.

Prepare the sample solution at any dilution at or below maximum valid dilution (MVD). Use two positive controls, one having the concentration of 2λ and other is spiked to get the concentration of 2 λ. (λ is labelled sensitivity. It is expressed in IU/ml)

Add an appropriate volume of negative control (NC), standard CSE solutions in water BET, test solution and positive control (PPC). At regular intervals add an equal volume of the appropriately constituted lysate unless single vial is used. Mix it and place it in an incubator. Incubation should be done at 37 ±1ºC undisturbed for 60±2 min. Remove and examine the receptacles carefully. A positive reaction is recorded when firm gel is formed that retains the integrity when inverted through 180 ºC in one smooth motion. If no firm gel is formed then it is a negative reaction.

Calculation: Calculate the average of the logarithms of the lowest concentrations of endotoxin in each series of the lowest concentration of endotoxin in each series of dilutions.

Geometric mean end point concentration = antilog (∑e/f)

Where, ∑e = sum of the log end point concentration of the series of dilutions used;

f = number of replicate test- tubes.

The value must be in between 0.5λ and 2.0λ

Interpretation of results:

The product under examination complies with the test if the negative control and test solutions are negative, and if the positive control is positive.

Retests: If a positive control is found for one of the test solution duplicates and a negative result for the other, the test may be repeated as described above. Results of the retest should be interpreted as for initial test.

Method B. Semi quantitative gel clot method:

Preparation of the test solutions: Prepare the test solutions at concentrations of 1 MVD, 0.5MVD, 0.25MVD.

Procedure: same as Method A.

Calculation and interpretation of results: To calculate the endotoxin concentration in the product, determine for the series of test solutions the lowest concentration or the highest dilution giving a

positive (+) reaction. Multiply this dilution with λ to obtain the endotoxin concentration of the product.

For instance, if MVD is equal to 8 and the positive reaction was obtained at 0.25 MVD and 1 was equal to 0.125 EU/ml

Calculate the endotoxin content of the product under examination from endotoxin concentration. The product under examination meets the requirements of test if the endotoxin content of less than endotoxin limit stated in the individual monograph.

Method C- Kinetic turbidimetric method & method D- Kinetic chromogenic method

Using CSE, prepare solutions of not less than 3 endotoxin concentrations to get a standard curve. Carry out the procedure in duplicates, of each standard endotoxin solution in accordance with the instructions of the lysate manufacture.

Preparation of test solutions

Solution A: Solution of the product under examination at the initial dilution (test solution)

Solution B: Test solution spiked with CSE at a concentration at or near the middle of the standard curve(PPC)

Solution C: Standard solutions of CSE in water BET covering the linear part of the standard curve

Solution D: water BET (NC)

Method: Add solution D, followed by solutions C, A, B. Add lysate and carry out the assay solution in accordance with the instructions of the lysate manufacture.

Calculation: Calculate the endotoxin concentration of solutions A and B from the regression equation obtained with solutions of series C. Calculate the mean percentage recovery of the added endotoxin by subtracting the mean endotoxin concentration in solution A from the mean endotoxin concentration in solution B.

Interpretation of results: The assay is valid only if

a) The standard curve is linear for the range of CSE concentrations used

b) The coefficient of correlation r, is not greater than -0.980

c) The mean % recovery of the added endotoxin in the positive product control is between 50 and 150 %

Method E. End point chromogenic method:

Add solution D, followed by solutions C, A, B. The chromogenic substrate and lysate are added to the solution and incubated for the recommended time. Stop the reaction and measure the

absorbance at the specified wavelength in accordance with the instructions of the lysate manufacture.

Interpretation of results:

The assay is valid only if

a) The standard curve is linear for the range of CSE concentrations used

b) The coefficient of correlation r, is not less than 0.980;

c) The mean % recovery of the added endotoxin in the positive product control is between 50 and 150%

SPQC-8 FOREIGN INSOLUBLE MATTER TEST FOR INJECTIONS: (JP) [5]

Clean the exterior of the container and inspect with unaided eyes at a position of light intensity of approximately 1000 lux under incandescent lamp. Injections must be free from readily detectable foreign insoluble matters. As to injections in plastic containers, inspection should be performed with unaided eyes at a position of light intensity of approximately 8000 to 10,000 lux, with an incandescent lamp at approximate distance above and below the container.

SPQC-9 CLARITY OF SOLUTION: IP[1]

Constitute the injection as directed on the label.

a) The solid dissolves completely, leaving no visible residue as undissolved matter.

b) The constituted injection is not significantly less clear than an equal volume of diluents for water for injections contained in a similar container and examined in the same manner.

SPQC-10 UNIFORMITY OF WEIGHT[1]

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 ^{0}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%. The limits for the uniformity of the weight are mentioned in table1.9

Following table 2 gives the specifications for each test for parenterals according to Indian Pharmacopoeia, British Pharmacopoeia, United States Pharmacopoeia

The specifications for the injections according to European Pharmacopoeia &Japanese Pharmacopoeia are mentioned in Table: 3

Table1.9: Limits for uniformity of weight

Pharmaceutical formulation	Average mass	Percentage deviation (%)
Powders for parenteral use	More than 40 mg	10
Powders for eye drops and powders for eye lotions	Less than 300 mg	10
	300 mg or more	7.5

Table 2: Specifications for injections according to IP, BP, & USP

Tests	Reference code	IP	BP	USP
Uniformity of content	SPQC-1	85-115%	85-115%	85-115%
Pyrogen	SPQC-3	Temperature should not increase more than 0.6^0C for each rabbit	Summed temperature of 3 rabbits should not be more than 1.15^0C	Temperature should not increase more than 0.5^0C for each rabbit
Particulate contamination	SPQC-4	≥ 25 µm-2% can be present	≥ 25 µm-2 can be present	≥ 25 µm-2 can be present
Extractable volume	SPQC-2	100-110%	×	100-110%
Bacterial endotoxin	SPQC-7	Should not give positive result	Should not give positive result	Should not give positive result
Sterility	SPQC-5	No growth in 14 days	No growth in 14 days	No growth in 14 days
Foreign insoluble matter	SPQC-8	×	×	×

Table 3: Specifications for injections according to EP & JP

Tests	Reference code	European Pharmacopoeia	Japanese Pharmacopoeia
Uniformity of content	SPQC-1	85-115%	85-115%
Pyrogen	SPQC-3	Summed temperature of 3 rabbits should not be more than 1.15^0C	Summed temperature of 3 rabbits should not be more than 1.3^0C
Particulate contamination	SPQC-4	≥ 25 µm-2 can be present	≥ 25 µm-2 can be present
Extractable volume	SPQC-2	100-110%	100-110%
Bacterial endotoxin	SPQC-8	Should not give positive result	Should not give positive result
Sterility	SPQC-5	No growth in 14 days	No growth in 14 days
Foreign insoluble matter	SPQC-8	×	Absence of foreign insoluble matter.

The specifications for the powder for injections according to IP, BP, USP are given in the Table: 4

Table 4: Specifications of Powders For Injection's According To IP, BP, USP

Tests	Reference code	IP	BP	USP
Uniformity of content	*SPQC-1*	85-115%	85-115%	85-115%
Pyrogen	*SPQC-3*	Temperature should not increase more than 0.6^0C for each rabbit	Summed temperature of 3 rabbits should not be more than 1.15^0C	Temperature should not increase more than 0.5^0C for each rabbit
Uniformity of weight	*SPQC-10*	100-110%	100-110%	100-110%
Extractable volume	*SPQC-2*	100-110%	×	100-110%
Clarity of solution	*SPQC-9*	The solution should be as clear as water for injection.	×	×
Particulate matter test	*SPQC-4*	≥ 25 µm-2% can be present	≥ 25 µm-2 can be present	≥ 25 µm-2 can be present
Sterility	*SPQC-5*	No growth in 14 days	No growth in 14 days	No growth in 14 days

The specifications for the injections according to the European Pharamcopoeia, & Japanese Pharmacopoeia, are given in the Table : 5

Table 5: Specifications for powders for injections according to EP, & JP

TESTS	Reference code	European Pharamcopoeia	JapanesePharamcopoeia
Uniformity of content	*SPQC-1*	85-115%	------
Pyrogen	*SPQC-3*	Summed temperature of 3 rabbits should not be more than 1.15^0C	------
Uniformity of weight	*SPQC-10*	×	------
Extractable volume	*SPQC-2*	100-110%	------
Clarity of solution	*SPQC-9*	×	------
Sterility	*SPQC-5*	No growth in 14 days	------

The specifications for the concentrates for the injection as per the USP are given in the Table: 6

Table 6: Specifications for concentrates for injections as per USP

Tests	Reference code	United States Pharmacopoeia
Pyrogen test	SPQC-3	Temperature should not increase more than 0.5^0C for each rabbit
Sterility	SPQC-5	No growth in 14 days
Bacterial endotoxin	SPQC-8	Should not give positive result

The specifications for the infusions according to IP, BP & USP are given in the Table : 7

Table 7: Specifications for infusions according to IP, BP &USP

Tests	Reference code	IP	BP	USP
Pyrogen	SPQC-3	Temperature should not increase more than 0.6 ^{0}C for each rabbit	Summed temperature of 3 rabbits should not be more than 1.15 ^{0}C	Temperature should not increase more than 0.5 ^{0}C for each rabbit
Extractable volume	SPQC-2	100-110%	×	100-110%
Sterility	SPQC-5	No growth in 14 days	No growth in 14 days	No growth in 14 days
Particulate contamination	SPQC-4	≥ 25 µm-2 % can be present	≥ 25 µm-2 can be present	≥ 25 µm-2 can be present
Bacterial endotoxin	SPQC-8	Should not give positive result	Should not give positive result	Should not give positive result

Table 8: Specifications for infusions according to EP& JP[4]

Tests	Reference code	EP	JP
Pyrogen	SPQC-3	Summed temperature of 3 rabbits should not be more than 1.15^{0}C	Summed temperature of 3 rabbits should not be more than 1.3^{0}C
Particulate contamination	SPQC-4	×	≥ 25µm-2 can be present
Extractable volume	SPQC-2	100-110%	100-110%
Sterility	SPQC-5	No growth in 14 days	No growth in 14 days
Bacterial endotoxin	SPQC-8	Should not give positive result	Should not give positive result

SUMMARY

The objective of the present work was to compare various in process and finished product QC tests as per IP, BP, USP, JP and EP for sterile products. The formulations for which the comparison was made included are injections, infusions, powders for injections, concentrates for injections, irrigations and ophthalmic preparations. The available QC tests from various pharmacopoeias supplement each other and one pharmacopoeia gives more details on a special issue than the other. Each pharmacopoeia has its own specifications for each test. Sterile products include parenterals, irrigations and eye preparations. Table 9 gives summary of the dosage forms included in IP, BP, USP, JP and EP.

Following are the tables specifying the tests included for various dosage forms in IP, BP, USP, EP and JP.

The in-process and finished product quality control tests for the injections are given in the Table: 10.

Table: 9 Summary of dosage forms available in Pharmacopoeias

Dosage form Parenterals	IP	BP	USP	EP	JP
Injections	✓	✓	✓	✓	✓
Infusions	✓	✓	✓	✓	✓
Powders for injections	✓	✓	✓	✓	NM
Concentrates for injections	NM	NM	✓	NM	NM
Irrigations	NM	✓	NM	✓	NM

Table: 10. In-process and finished product quality control tests for the injections

Tests	IP	BP	USP	EP	JP
Uniformity of content	✓	✓	✓	✓	✓
Pyrogen	✓	✓	✓	✓	✓
Particulate contamination	✓	✓	✓	✓	✓
Extractable volume	✓	×	✓	✓	✓
Bacterial endotoxin	✓	✓	✓	✓	✓
Sterility	✓	✓	✓	✓	✓
Foreign insoluble matter	×	×	×	×	✓

Extractable volume test for injections is not available in British Pharmacopoeia, Foreign insoluble matter test is specified only in Japanese PharmacopoeiaThe in-process and finished process quality control tests for the powders for the injection are given in the Table: 11

Table 11: In process and finished product quality control tests - powders for injection

Tests	IP	BP	USP	EP	JP
Uniformity of content	✓	✓	✓	✓	------
Uniformity of weight	✓	✓	✓	✓	------
Clarity of solution	✓	×	×	×	------
Pyrogen test	×	✓	✓	✓	------
Particulate matter	✓	✓	✓	✓	------
sterility	✓	✓	✓	✓	------

IPC and FPC tests for powders for injections are official in all the pharmacopoeias except for JP. Clarity of solution test is available only in IP. Extractable volume test is not available in BP.

IPC and FPC tests for concentrates for injections are official only in USP. Tests included under them are pyrogen test, test for sterility and bacterial endotoxin test.

The in-process and finished product quality control tests for the concentrates for the injection are given in the Table: 12

Table 12: In process and finished product quality control tests for concentrates for injections as per IP, BP, USP, EP, JP

Tests	IP	BP	USP	EP	JP
Pyrogen test	------	------	✓	------	-----
Sterility	------	------	✓	------	-----
Bacterial endotoxin	------	------	✓	------	-----

The in-process and finished product Quality control Tests for the infusions are mentioned in the Table: 13

Table 13: In process and finished product quality control tests for infusions

Tests	IP	BP	USP	EP	JP
Particulate contamination	✓	✓	✓	✓	✓
Sterility	✓	✓	✓	✓	✓
Pyrogen	✓	✓	✓	✓	✓
Extractable volume	✓	×	✓	✓	✓
Bacterial endotoxin	✓	✓	✓	✓	✓

IPC and FPC tests for powders for injections are available in only BP, USP and JP

CONCLUSION

From the above review it can be concluded that though Indian pharmacopoeia (IP), British Pharmacopoeia (BP), European Pharmacopoeia (EP), Japaneese Pharmacopoeia (JP) and United States Pharamcopoeia (USP) included most of the in process and finished products QC tests for various sterile dosage forms, there were some significant difference observed. Some of the tests are available only in some Pharmacopoeias. The differences in the tests and their limits as specified in the different pharmacopoeias needs to be harmonized and streamlined in such a way that if the test meets the specified limit as per harmonized one, it meets all the requirements of all the pharmacopoeias and later the regulatory requirements of that particular country. This is important for the products which are marketed globally and can also same lot of time, money and man power.

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