FINISHED PRODUCT SPECIFICATION

Product:	**Gentamicin Sulfate Ophthalmic Ointment USP 0.3%**
Pack:	5 g Aluminum Collapsible Tubes 3.5 g Aluminum Collapsible Tubes
Label Claim:	Each g contains: Gentamicin Sulfate USP equivalent to Gentamicin base 3 mg
Specification No.:	QA/FPS/143/08
Issue No.:	02
Supersedes:	01
Reason for Revision:	1) This specification is revised as per current BP and current USP. 2) To change limits for the pH & globule size. 3) To add limit for the viscosity.
Pharmacopoeial Reference:	Current BP and current USP.
Date of Issue:	01.08.2009
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Prepared By: Checked By: Authorized By:

FINISHED PRODUCT SPECIFICATION

Product: Gentamicin Sulfate Ophthalmic Ointment USP 0.3%

Reference: Current BP and current USP.

Sr. No.	TESTS	LIMITS	REFERENCE
1	Description	White soft ointment, free from foreign particles.	In-house specification
2	Identification Thin Layer Chromatography	To comply the test as per USP	Current USP
3	Minimum fill	To comply the test as per USP	Current USP
4	pH (10% w/v)	Results to be monitored	In-house specification
5	Particle size	NMT 20 Particles > 25 micron NMT 2 Particle > 50 micron None Particle > 90 micron	Current BP
6	Viscosity	Results to be monitored	In-house specification
7	Metal particles	To comply the test as per USP	Current USP
8	Water	NMT 1.0 %	Current USP
9	Assay for Gentamicin Sulfate eq. to Gentamicin	2.7 mg/g to 4.05 mg/g 0.27 % w/w to 0.405 % w/w 90.0 % L.A. to 135.0 % L.A.	Current USP
10	Leakage Test	To comply the test as per USP	Current USP
11	Sterility	To comply the test as per USP	Current USP

METHOD OF ANALYSIS

Product: Gentamicin Sulfate Ophthalmic Ointment USP 0.3%

Reference: Current USP and current BP.

1) Description: White soft ointment, free from foreign particles

2) Identification: **(Limit: To comply the test as per USP)**

A) Method: Thin layer Chromatography

Adsorbent: Silica gel precoated plate (Merck silica gel 60 plates are suitable)
Mobile Phase: The lower phase of a mixture of chloroform, methanol, and ammonium hydroxide (20:13:10).
Solution (1): Shake a quantity of Ophthalmic Ointment, equivalent to about 5 mg of gentamicin, with a mixture of 200 mL of chloroform and 5 mL of water. Allow to separate, and filter the aqueous layer & use the filtrate.
Solution (2): A solution of Gentamicin Sulphate WS in water containing the equivalent of 0.075 % w/v of Gentamicin.
Application: Apply separately 20 µl each of solution (1) and (2).
Spray reagent: vapors of iodine.
Development of chromatogram: Place the plate in a suitable chromatographic chamber, and develop the chromatogram in a solvent system consisting of the lower phase of a mixture of chloroform, methanol, and ammonium hydroxide (20:13:10) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, air-dry, and expose the plate to vapors of iodine in a detection jar containing iodine crystals.
Assessment of spots: The intensities and R_f values of the three principal spots obtained from the test solution correspond to those obtained from the Standard solution.

3) Minimum fill: **(Limit: To comply the test as per USP)**

Procedure: Select a sample of 10 filled tubes and remove any labeling that might be altered in weight during the removal of the tube contents. Thoroughly cleanse and dry the outside of the tubes by a suitable means and weigh individually. Quantitatively remove the contents from each tube, cutting the latter open and washing with a suitable solvent, if necessary, taking care to retain the closure and other parts of each tube. Dry and again weigh each empty tube together with its corresponding parts. The difference between the two weights is the net weight content of the tube.

The average net content of the 10 tubes is not less than the labeled amount, and net content of any single tube is not less than 90 % of the labeled amount. If this requirement is not met, determine the

content of 20 additional tubes. The average content of the 30 tubes is not less than the labeled amount, and the net content of not more than 1 of the 30 tubes is less than 90% of the labeled amount.

4) pH : (As such) **(Limit: Results to be monitored)**
pH is determined by using glass electrode at 25^0C.

5) Globule Size: **(Limits: NMT 20 Particles > 25 micron**
NMT 2 Particle > 50 micron
None Particle > 90 micron)

List of equipments required:
1) Slides and cover slips
2) Microscope

Procedure: Spread gently a portion of cream as a thin layer. Scan under a microscope the whole area of the sample and measure the globule size.

6) Viscosity: **(Limit: Results to be monitored)**

Viscosity is determined by using Brookfield CAP 200 + Viscometer (model no. CAP 200 +) spindle no. 01 at 100 rpm at temp of 25^0C.

7) Metal Particles: **(Limit: To comply the test as per USP)**

Procedure : Extrude, as completely as practicable, the contents of 10 tubes individually into separate, clear, flat – bottom, 60 mm petri dishes that are free from scratches. Cover the dishes, and heat at 85° for 2 hours increasing the temperature slightly if necessary to ensure that a fully fluid state is obtained. Taking precaution against disturbing the melted sample, allow each to cool to room temperature and to solidify. Remove the covers, and invert each petri dish on the stage of a suitable microscope adjusted to furnish 30 times magnification and equipped with an eyepiece micrometer disk that has been calibrated at the magnification being used. In addition to the usual source of light, direct an illuminator from above the ointment at a 45° angle. Examine the entire bottom of the petri dish for metal particles. Varying the intensity of the illuminator from above allows such metal particles to be recognized by their characteristic reflection of light.

Count the number of metal particles that are 50 um or larger in any dimensions: the requirements are met if the total number of such particles in all 10 tubes does not exceed 50, and if not more than 1 tube is found to contain more than 8 such particles. If these results are not obtained, repeat the test on 20 additional tubes: the requirement are met if the total number of metal particles that are 50 um or larger in any dimension does not exceed 150 in all 30 tubes tested, and if not more than 3 of the tubes are found to contain more than 8 such particles each.

8) Water: **(Limit: NMT 1.0%)**

Reagents required:
1) Karl Fischer Reagent
2) Mixture of toluene and methanol (7:3)

Standardization of the Reagent: Place enough mixture of toluene & methanol (7: 3) in the titration vessel to cover the electrodes, and add sufficient reagent to give characteristic end point color, or 100 ± 50 microampere of direct current at about 200 mV of applied potential.
Quickly add 75 to 125 mg of sodium tartrate ($C_4H_4Na_2O_6.2H_2O$), accurately weighed by difference, and titrate to the end point. The water equivalence factor *F*, in mg of water per mL of reagent, is given by formula:

$$2(18.02/230.08)\ (w/v),$$

in which 18.02 & 230.08 are the molecular weights of water and sodium tartrate dihydrate respectively; w is the weight in mg of sodium tartrate dihydrate; and v is the volume in mL of the reagent consume in the second titration.

Procedure: Transfer 35 to 40 mL of mixture of toluene and methanol (7: 3) to the titration vessel, and titrate with the reagent to the electrometric or visual endpoint to consume any moisture that may be present. (Disregard the volume consume, since it does not enter into the calculations). Quickly add 1g of sample, mix, and again titrate with the reagent to the electrometric or visual end point. Calculate the water content of the specimen in mg taken by the formula:

$$SF,$$

in which *S* is the volume in mL of the reagent consumed in second titration; and *F* is the water equivalence factor of the reagent.

9) Assay for Gentamicin Sulphate: **(Limit: 2.7 mg/g to 4.05 mg/g**
0.27 % w/w to 0.405 % w/w
90.0 % to 135.0 % L.A.)

Method: Cup plate
Test organism : Staphylococcus epidermis ATCC 12228
Medium : Antibiotic Assay No.11 Make : Himedia

Preparation of culture suspension :
Scrape the growth from a slant of 24 hours of old culture in 10 mL sterile saline. Use 0.1 % of culture suspension to prepare assay plates.

Preparation of Assay plates :
Dissolve 3.05 g of assay media in 100 mL of distilled water. Sterilizes at 121°C at 15 psi for 20 minutes. Transfer the flask containing the medium in a water bath and allow it to cool to 40°C. Add 5 mL of cell suspension to the assay medium and mix thoroughly but gently to avoid air bubbles. Distribute the inoculated medium to each of the numbered petri dishes to provide a depth of 3 mm. Allow to set and transfer to the refrigerator until use. Remove the plates 30 minutes before use and bore 4 cups of 8 mm diameter.

Buffer solution : (Buffer B3 USP)
Dissolve 16.73 g of dibasic potassium phosphate and 0.523 g of monobasic potassium phosphate in 1000 mL of water. Adjust the pH with 18N phosphoric acid or 10N potassium hydroxide to 8.0 ± 0.1.

Incubation period and temperature : 16-18 hrs / 32°C - 35°C.

Procedure: Prepare standard and test solution as prescribed in dilution profile chart mentioned below. Apply the standard and test solution to agar plates inoculated with *S. epidermidis* ATCC 12228 using a design which permits full statistical analysis of date including a check for parallelism of standard and test regression lines. Inoculate the plates at 35-37^0 C for 24 hours. Measure the diameters of the zones of inhibition. Calculate the units per g of the Gentamicin content in the cream by standard statistical procedures.

Dilution Profile Chart
Standard Preparation:

124 mg Gentamicin Sulphate WS ---(Buffer B2)---> 100 mL → 5 mL ---(Buffer B2)---> 100 mL (SH)

5 mL ---(Buffer B2)---> 20 mL (SL)

Test Preparation :

1.66 g cream + 30 mL Buffer (warm on water bath to dissolve) ---(Buffer B2)---> 100 mL and filter (TH)

5 mL ---(Buffer B2)---> 20 mL (TL)

Calculation:

$$\% \text{ Potency} = \text{antilog } (2 \pm a \log I)$$

$$\text{Where, } a = \frac{(TH + TL) - (SH + HL)}{(TH - TL) + (SH - HL)}$$

I = dilution ratio

$$\textbf{Content of Gentamicin (\% w/w)} = \frac{\% \text{ Potency / mL} \times \text{Standard factor} \times \text{Dilution factor}}{\text{weight of sample}}$$

10) Leakage test : **(Limit: To comply the test as per USP)**

Procedure: Select 10 tubes of the ointment, with seals applied when specified. Thoroughly clean and dry the exterior surfaces of each tube with an absorbent cloth. Place the tubes in a horizontal position on a sheet of absorbent blotting paper in an oven maintained at a temperature of 60 ± 3^0 for 8 hours. No significant leakage occurs during or at the completion of the test (disregard traces of ointment presumed to originate externally from within the crimp of the tube or from the thread of the cap).

If leakage is observed from one, but not more than one, of the tubes, repeat the test with 20 additional tubes of the ointment. The requirement is met if no leakage is observed from the first 10 tubes tested, or if leakage is observed from not more than one tube of 30 tubes tested.

11) Sterility: **(Limit: To comply the test as per USP)**

Method: Membrane Filtration

Requirement:

List of biological and chemical substances required:

a) Media: Sterile Soyabean Casein Digest Medium

b) Sterile isopropyl myristate obtained by filtration through 0.2 µ membrane filter.

c) 0.1 % w/v sterile peptone water with 0.1 % v/v polysorbate 80 & 0.1 % peptone water.

d) Sterile Fluid thioglycollate Medium

List of apparatus required:

a) Manifold unit for 3 or more folder membrane filter funnel with suitable vacuum flask

b) Sterilized membrane filter of 0.45 micron and 0.2 micron.

c) Sterile forceps and scissors wrapped in parchment paper/ butter paper.

d) Sterile 10 ml pipettes.

e) Sterile empty flasks.

Procedure:

Membrane Filtration Method:

1. Follow the entry procedure in the aseptic area as mentioned in the SOP No. QA/SOP/304/98.
2. Dissolve not less than 0.2 g of ointment from less than 10 tubes/containers in not less than 100 ml of sterile isopropyl myristate, which previously has been rendered sterile by filtration through 0.2-µ membrane filter.

The quantity of the sample taken for sterility tasting from 10 tubes should not be more than .5 g.

3. Warm the sterilized solvent, and if necessary the test material, to not more than 44° C.
4. Swirl the flask to dissolve the ointment taking care to expose a large surface of the material to the solvent.
5. Following dissolution aseptically transfer the mixture in membrane filter funnel and immediately pass the mixture through the membrane filter of 0.45 micron with the aid of vacuum.

6. Keep the filter membrane covered with liquid throughout the filtration for maximum efficiency of the filter. Following filtration of the liquid wash the membrane filter with two 200 ml portion of sterile 0.1 % peptone with 0.1 % polysorbate 80 and then wash with 100 ml of sterile 0.1 % peptone.
7. Aseptically remove the membrane filter from the filter holders, cut the membrane in two halves, immerse on half of the membrane filter with the aid of sterile forceps in 100 ml of sterile soyabean casein digest medium and the other half in 100 ml of sterile fluid thioglycollate medium.
8. Keep the positive control by separately inoculating *Bacilus subtilis ATCC 6633* in sterile fluid thioglycollate medium and *Candida albicans ATCC 10231* in sterile soyabean casein digest medium.
9. Keep the negative control by filtering autoclaved 100 ml of sterile distilled water, cut the membrane filter in two halves and immerse on half of the filter in soyabean casein digest medium and other half in fluid thioglycollate medium.
10. Incubate the tubes at 20 –25 ° C and 30 – 33 ° C for growth of fungus and bacteria respectively for not less than 14 days as per current USP, current BP and current IP.
11. Observe the tubes for growth in terms of turbidity within 14 days as per current USP, current BP and current IP.
12. If none of the tubes shows growth in terms of turbidity within 14 days as per current USP, current BP and current IP then the test passes for sterility.
13. If the material being tested renders the medium turbid so that the presence or absence of microbial growth cannot be readily determined by visual examination, 14 days after the beginning of the incubation transfer the portions [each lot less than 1 ml] of the media to the fresh tube of the same medium & then incubate the original and transfer tube for not less than 4 days.