Chloramphenicol and Colistin Sodium Methanesulfonate Ophthalmic Solution

クロラムフェニコール・コリスチンメタンスルホン酸ナトリウム点眼液

Chloramphenicol and Colistin Sodium Methanesulfonate Ophthalmic Solution is an aqueous ophthalmic preparation. It contains not less than 90.0% and not more than 120.0% of the labeled potency of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$: 323.13) and labeled Units of colistin ($C_{53}H_{100}N_{16}O_{13}$: 1169.46).

Method of preparation Prepare as directed under Ophthalmic Liquids and Solutions, with Chloramphenicol and Colistin Sodium Methanesulfonate.

Description Chloramphenicol and Colistin Sodium Methanesulfonate Ophthalmic Solution is a clear, colorless to pale yellow liquid.

Identification **(1)** To a volume of Chloramphenicol and Colistin Sodium Methanesulfonate Ophthalmic Solution, equivalent to about 2.5 mg (potency) of Chloramphenicol, and add water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry *<2.24>*: it exhibits a maximum between 276 nm and 280 nm.

(2) To a volume of Chloramphenicol and Colistin Sodium Methanesulfonate Ophthalmic Solution, equivalent to about 5×10^5 Units of Colistin Sodium Methanesulfonate add 0.5 mL of ninhydrin TS, boil for 1 minute, and cool: a blue color develops.

Osmotic pressure ratio Being specified separately when the drug is granted approval based on the Law.

pH *<2.54>* 6.0 – 8.0

Foreign insoluble matter *<6.11>* It meets the requirement.

Insoluble particulate matter *<6.08>* It meets the requirement.

Sterility *<4.06>* Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics *<4.02>* according to the following conditions.

(1) Chloramphenicol

(i) Test organism－*Kocuria rhizophila* ATCC 9341

(ii) Agar media for base layer, seed layer, and transferring test organisms－Use the medium ii in 3) Media for other organisms under (1) Agar media for seed and base layer of 1.2. Culture media.

(iii) Liquid medium for suspending the test organism－Use the medium (2) Liquid media for suspending test organisms of 3.2. Culture media.

(iv) Standard solutions－Weigh accurately about 20 mg (potency) of Chloramphenicol RS, dissolve in 2.0 mL of ethanol (95), add phosphate buffer solution (pH 6.0) to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 15℃ or below, and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution (pH 6.0) to make solutions so that each mL contains 100 μg (potency) and 25 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(v) Sample solutions－Weigh accurately an amount of Chloramphenicol and Colistin Sodium Methanesulfonate Ophthalmic Solution, equivalent to about 10 mg (potency) of chloramphenicol, add phosphate buffer solution (pH 6.0) to make exactly 100 mL, and filter, if necessary. Take exactly a suitable amount of this solution, add phosphate buffer solution (pH 6.0) to make solutions so that each mL contains 100 μg (potency) and 25 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

(2) Colistin Sodium Methanesulfonate

(i) Test organism－*Bordetella bronchiseptica* ATCC 4617

(ii) Agar medium for base layer－

Casein peptone 17.0 g
Sodium chloride 5.0 g
Glucose 2.5 g
Soybean peptone 3.0 g
Dipotassium hydrogenphosphate 2.0 g
Agar 20.0 g
Water 1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 7.2 to 7.3 with sodium hydroxide TS after sterilization.

(iii) Agar medium for seed layer－

Casein peptone 17.0 g
Glucose 2.5 g
Soybean peptone 3.0 g
Sodium chloride 5.0 g
Polysorbate 80 10.0 g
Dipotassium hydrogenphosphate 2.5 g
Agar 12.0 g
Water 1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 7.2 to 7.3 with sodium hydroxide TS after sterilization.

(iv) Agar medium for transferring test organisms－Use the medium i in 2) Media for other organisms under (2) Agar media for transferring test organisms.

(v) Preparation of test organism and seeded agar layer－Cultivate the test organism on the slant of the agar medium for transferring test organism at 32 to 37℃ for 16 to 24 hours. Subcultures at least three times. Cultivate the grown organism on the slant of the agar medium for transferring test organism at 32 to 37℃ for 16 to 24 hours, add a suitable amount of water to the grown organism, and suspend. Adjust the suspension so that the transmittance at 660 nm is 60% as directed under Ultraviolet-visible Spectrophotometry *<2.24>* using a spectrophotometer or a photoelectric

photometer, and use this suspension as the test organism suspension. Keep the test organism suspension at 15℃, and use within 3 days. Before use, dissolve 0.13 mL of the test organism suspension, add it to 100 mL of agar medium for seed previously cooled at 48℃, mix thoroughly, and use this as the seeded agar layer.

(vi) Standard solutions－Weigh accurately an amount of Colistin Sodium Methanesulfonate RS, equivalent to about 1×10^6 Units, dissolve in phosphate buffer solution (pH 6.0) to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 10℃ or below, and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution (pH 6.0) to make solutions so that each mL contains 1000 Units and 250 Units, and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(vii) Sample solutions－Weigh accurately an amount of Chloramphenicol and Colistin Sodium Methanesulfonate Ophthalmic Solution, equivalent to about 1×10^5 Units of colistin sodium methanesulfonate, add phosphate buffer solution (pH 6.0) to make a solution so that each mL contains 1000 Units, and use this solution as the high concentration sample solution. Separately, pipet 5 mL of the high concentration sample solution, add phosphate buffer solution (pH 6.0) to make a solution so that each mL contains 250 Units, and use this solution as the low concentration sample solution.

Containers and storage Containers－Tight containers.

Storage－At a temperature between 2℃ and 8℃.