petri dish with the cover slightly ajar, and dry the filter fully at a temperature not exceeding 50°C. After the filter has been dried, place the petri dish on the stage of the microscope. Under a down-light from an illuminating device, adjust the grid of the membrane filter to the coordinate axes of the microscope, adjust the microscope so as to get the best view of the insoluble particulate matter, then count the number of particles that are equal to or greater than 10 μm within the effective filtering area of the filter, moving the mobile stage, and ascertain that the number is not more than 20. In this case the particle is sized on the longest axis.

Fit another membrane filter to the filtration device, and fix them with the clip, then wet the inside of the filter holder with several mL of purified water for particulate matter test. Clean the exterior of the container, and mix the sample solution gently by inverting the container several times. Remove the closures carefully and pour out approximately 50 mL of the solution with gentle mixing in such manner as to wash the opening of the container. Immediately after that, measure 40 mL of the solution using a measuring cylinder, which has been rinsed well with purified water for particulate matter test, and pour it into the filter holder along the inner walls. Apply the vacuum and filter mildly so as to keep the solution always on the filter. As for viscous sample solutions, dilute previously the solution with an appropriate diluent or purified water for particulate matter test, and then filter as described above. When the amount of the solution on the filter becomes small, add 30 mL of purified water for particulate matter test in such manner as to wash the inner walls of the filter holder. Repeat the process 3 times with 30 mL of the water. Apply the vacuum gently until the surface of the membrane filter is free from water. Place the filter in a petri dish, and dry the filter fully at a temperature below 50°C with the cover slightly ajar. After the filter has been dried, place the petri dish on the stage of the microscope, and count the number of particles which are equal to or greater than 10 μm and equal to or greater than 25 μm within the effective filtering area of the filter according to the same procedure of the microscope as described above. In this case the particle is sized on the longest axis.

Small-volume injections

Proceed the test in the manner for large-volume injections, except for using a membrane filter of 13 mm diameter and a filter holder of 4 mm in diameter of particle-retaining mouth.

Aqueous injections

Clean the exterior of container of aqueous injections, mix the content by inverting slowly, then, open carefully, and withdraw the whole content using such a plastics syringe or the like that has not been contaminated with foreign matter. In getting along the inside wall of the filter, pour the sample slowly, and suction slowly for keeping the sample on the filter at any time. For viscous sample, dilute previously with an appropriate diluent or purified water for particulate matter test, and filter similarly. When the sample amount on the membrane filter is reduced, add 30 mL of purified water for particulate matter test or diluent in the manner to wash the inside wall of the filter holder. Moreover, repeat this washing 3 times with each 30 mL, whereas, in the use of the diluent, finally wash with the purified water for particulate matter test, then, suction slowly from the top of membrane filter until water runs out, and proceed in conformity with the operation for the above mentioned large-volume injections.

Injectable sterile solid or lyophilized injections, or injectable sterile solids injections with constituted solution

Prepare sample solution according to the sample preparation procedure for the light obscuration particle count test, and perform the test as directed in the method for aqueous injections.

25. Insoluble Particulate Matter Test for Ophthalmic Solutions

The Test is to examine for the size and the number of insoluble particulate matter in Ophthalmic Solutions.

Apparatus

Use a microscope, filter assembly for retaining insoluble particulate matter and membrane filter for determination.

Microscope: The microscope is equipped with a micrometer system, a mobile stage and an illuminator, and is adjusted to 100 magnifications.

Filter assembly for retaining insoluble particulate matter: The filter assembly for retaining insoluble particulate matter consists of a filter holder made of glass or a proper material uncapable of causing any trouble in testing, and a clip. The unit is capable of fitting with a membrane filter 25 mm or 13 mm in diameter and can be used under reduced pressure.

Membrane filter for testing: The membrane filter is white in color, 25 mm or 13 mm in diameter, not more than 10 μm in nominal pore size and is imprinted with about 3 mm grid marks. Upon preliminary testing, the insoluble particulate matter equal to or greater than 25 μm in size should not be found on the filter. When necessary, wash the filter with purified water for particulate matter test.

Reagents

Purified water for particulate matter test: Purified water which contains not more than 10 particles of 10 μm or greater size in 100 mL. Prepare before use by filtering through a membrane filter with a nominal pore size of 0.5 μm or less.

Procedure

Aqueous ophthalmic solutions

Carry out all operations carefully in clean equipment and facilities which are low in dust. Fit the membrane filter onto the membrane filter holder, and fix them with the clip. Thoroughly rinse the holder inside with the purified water for particulate matter test, and filter under reduced pressure with 200 mL of the purified water for particulate matter test at a rate of 20 to 30 mL per minute. Apply the vacuum until the surface of the membrane filter is free from water, and remove the membrane filter. Place the filter in a flat-bottomed petri dish with the cover slightly ajar, and dry the filter fully at a temperature not exceeding 50°C. After the filter has been dried, place the petri dish on the stage of the microscope. Under a down-light from an illuminating device, adjust the grid of the membrane filter to the coordinate axes of the microscope, adjust the microscope so as to get the best view of the insoluble particulate matter, then count the number of particles that are equal to or greater than 150 μm within the effective filtering area of the filter, moving the mobile stage, and ascertain that the number is not more than 1. In this case the particle is sized on the longest axis.

Fit another membrane filter to the filtration device, and fix

them with the clip, then wet the inside of the filter holder with several mL of purified water for particulate matter test. Clean the outer surface of the container, and mix the sample solution gently by inverting the container several times. Remove the cap, clean the outer surface of the nozzle, and pour the sample solution into a measuring cylinder which has been rinsed well with purified water for particulate matter test. Repeat the process to prepare 25 mL of the test solution. Pour the test solution into the filter holder along the inner wall of the holder. Apply the vacuum and filter mildly so as to keep the solution always on the filter. As for viscous sample solution, dilute suitably with purified water for particulate matter test or suitable diluent and then filter as described above. When the amount of the solution on the filter becomes small, add 30 mL of purified water for particulate matter test or suitable diluent in such manner as to wash the inner wall of the filter holder. Repeat the process 3 times with 30 mL of the water. Apply the vacuum gently until the surface of the membrane filter is free from water. Place the filter in a petri dish, and dry the filter at a temperature below 50°C with the cover slightly ajar. After the filter has been dried, place the petri dish on the stage of the microscope, and count the number of particles which are equal to or larger than 300 μm within the effective filtering area of the filter according to the same procedure of the microscope as described above. In this case the particle is sized on the longest axis.

Ophthalmic solutions which are dissolved before use

Proceed as directed in Aqueous Ophthalmic Solutions after dissolving the sample with the constituted solution.

Suspension type ophthalmic solutions

Proceed as directed in Aqueous Ophthalmic Solutions. Take 25 mL of the sample in a vessel, which has been rinsed well with purified water for particulate matter test, add a suitable amount of a suspension-solubilizing solvent or an adequate solvent, shake to dissolve the suspending particles, and use this solution as the sample solution. Use a membrane filter which is not affected by the solvent to be used.

Ophthalmic solutions contained in a single-dose container

Proceed as directed in Aqueous Ophthalmic Solutions, using 10 samples for the test. A 13-mm diameter membrane filter and a 4-mm diameter filter holder for retaining insoluble particulate matter are used.

26. Iron Limit Test

The Iron Limit Test is a limit test for iron contained in drugs. The limit is expressed in term of iron (Fe).

In each monograph, the permissible limit for iron (as Fe) is described in terms of ppm in parentheses.

Preparation of test solutions and control solutions

Unless otherwise specified, test solutions and control solutions are prepared as follows:

(1) Method 1

Weigh the amount of sample specified in indivisual monograph, add 30 mL of acetic acid-sodium acetate buffer solution for iron limit test, pH 4.5, dissolve by warming if necessary, and designate this solution as the test solution.

Prepare the control solution as follows: To the amount of Standard Iron Solution specified in individual monograph add 30 mL of acetic acid-sodium acetate buffer solution for iron limit test, pH 4.5.

(2) Method 2

Weigh the amount of sample specified in individual monograph, add 10 mL of dilute hydrochloric acid, and dissolve by warming if necessary. Dissolve 0.5 g of L-tartaric acid, and add one drop of phenolphthalein TS. Add ammonia TS dropwise untill the solution develops a pale red color. Add 20 mL of acetic acid-sodium acetate buffer solution for iron limit test, pH 4.5, and designate this solution as the test solution.

Prepare the control solution as follows: To the amount of Standard Iron Solution specified in individual monograph add 10 mL of dilute hydrochloric acid, and proceed as directed for the test solution.

(3) Method 3

Place the amount of sample specified in individual monograph in a crucible, moisten with a small amount of sulfuric acid, heat cautiously and gently at first, and then incinerate by ignition. After cooling, add 1 mL of diluted hydrochloric acid (2 in 3) and 0.5 mL of diluted nitric acid (1 in 3), evaporate on a water bath to dryness, and to the residue add 0.5 mL of diluted hydrochloric acid (2 in 3) and 10 mL of water. After dissolving by warming, add 30 mL of acetic acid-sodium acetate buffer solution for iron limit test, pH 4.5, and designate this solution as the test solution.

Prepare the control solution as follows: Transfer the amount of Standard Iron Solution specified in indivisual monograph to a crucible, and add 1 mL of diluted hydrochloric acid (2 in 3) and 0.5 mL of diluted nitric acid (1 in 3), evaporate on a water bath to dryness, and proceed as directed for the test solution.

In this procedure, use a quartz or porcelain crucible, which is immersed in boiling dilute hydrochloric acid for 1 hour and washed throughly with water and dried.

Procedure

Unless otherwise specified, proceed as follows:

(1) Method A

Transfer the test solution and the control solution to separate Nessler tubes, to each add 2 mL of a solution of L-ascorbic acid (1 in 100), mix well, and allow to stand for 30 minutes. Add 1 mL of a solution of α, α'-dipyridyl in ethanol (95) (1 in 200), add water to make 50 mL, and allow to stand for 30 minutes. Then compare the colors developed in both solutions against a white background. The test solution has no more color than the control solution.

(2) Method B

Dissolve 0.2 g of L-ascorbic acid in the test solution and the control solution, and allow to stand for 30 minutes. Add 1 mL of a solution of α, α'-dipyridyl in ethanol (95) (1 in 200), and allow to stand for 30 minutes. Then add 2 mL of a solution of 2,4,6-trinitrophenol (3 in 1000) and 20 mL of 1,2-dichloroethane, shake vigorously, collect the 1,2-dichloroethane layer, and filter through a pledget of absorbent cotton in a funnel on which 5 g of anhydrous sodium sulfate is placed if necessary. Then compare the colors developed in both solutions against a white background. The test solution has no more color than the control solution.