6.17 Insoluble Particulate Matter Test for Therapeutic Protein Injections

6.17 タンパク質医薬品注射剤の不溶性微粒子試験法

Insoluble particulate matters in injections consist of mobile undissolved particles other than gas bubbles in preparations. Extraneous substances, substances derived from manufacturing processes, protein aggregates and the like may be included in therapeutic protein injections. In this chapter, method 1 Light Obscuration Particle Count Test under Insoluble Particulate Matter Test for Injections *<6.07>* is used for the determination of insoluble particulates in therapeutic protein injections. This test is applied to the injections whose active ingredients are peptides, proteins or their derivatives. This test is not applicable when the injections are not appropriate for applying this test because of their properties and so on.

Since this test is a sampling test conducted on a part of samples, the test must be performed under a statistically sound sampling plan in order to estimate correctly the number of particles in the population.

1. Apparatus

Use a suitable apparatus based on the principle of light blockage which allows an automatic determination of the size of particles and the number of particles according to size. The verification of calibration, sample volume accuracy, sample flow rate accuracy and counting accuracy is performed according to Method 1 under Insoluble Particulate Matter Test for Injections *<6.07>*. When one measurement is performed with sample volume less than 1 mL, confirm the sample volume accuracy separately by an appropriate method.

2. General precautions

The test is carried out under conditions limiting particulate contamination, preferably in a laminar-flow cabinet. Very carefully wash the glassware and filtration equipment used, except for the membrane filters, with a warm detergent solution and rinse with abundant amounts of water to remove all traces of detergent. Immediately before use, rinse the equipment from top to bottom, outside and then inside, with *particle-free water*. Take care not to introduce gas bubbles into the preparation to be examined, especially when fractions of the preparation are being transferred to the container in which the determination is to be carried out. In order to check that the test environment is suitable for the test, that the glassware is properly cleaned and that the number of particles in the *particle-free water* to be used is within specifications, the following test is carried out using 5 mL of the *particle-free water*. When one measurement is performed with sample volume less than 1 mL, 1 mL of the *particle-free water* may be used. Determine the particulate contamination of 5 samples. If the number of particles of 10 μm or greater size exceeds 1 for 1 mL, the environment is judged to be insufficient. In this case, remeasure the *particle-free water* to be used and repeat washing the glassware and the filtration equipment used until the environment becomes suitable for the test.

3. Method

Treat a protein solution in an appropriate manner because of its tendency to generate gas bubbles. In the case of an injection to be dissolved before use, use a specified solvent. Confirm that the particles in the solvent used are comparable to those in *particle-free water*. Mix the content of a sample gently and thoroughly by an appropriate procedure such as swirling the container slowly. If necessary, cautiously remove the sealing closure. Clean the outer surfaces of the container opening using *particle-free water* and remove the closure, avoiding any contamination. For elimination of gas bubbles, it is recommended to allow a container to stand under atmospheric pressure or reduced pressure. Other procedures are applicable if confirmed to be appropriate. Sonicating is not appropriate because it may aggregate or denature proteins. After degassing, mix the contents gently and thoroughly by swirling slowly the container so as not to introduce gas bubbles, and use it for the test. The measurement volume is 1 to 5 mL. The volume can be reduced to 0.2 mL when the validity of the reduction is sufficiently confirmed in considering the property of the sample and the tare volume of the apparatus. Set the volume necessary for the test in consideration of counting 4 portions.

In the case of injections where the volume necessary for the test can be obtained from one container, individual containers are tested. For injections with insufficient volume, combine the contents of plural containers in one clean container to obtain the necessary volume after mixing the contents of containers gently and thoroughly. Where justified, the volume necessary for the test may be prepared by diluting the test solution with *particle-free water* or an appropriate solvent comparable to *particle-free water*. The validity of the dilution procedure and the solvent used for the dilution is confirmed by, for example, demonstrating consistent result regardless of the dilution. The number of test specimens must be adequate to provide a statistically sound assessment.

Take 4 portions and count the number of particles equal to or greater than 10 μm and 25 μm. Disregard the result obtained for the first portion, and calculate the mean number of particles for the preparation to be examined.

4. Evaluation

The preparation complies with the test if the average number of particles meets the following requirement.

A ― Solutions for injection supplied in containers with a nominal content of equal to or more than 100 mL

The average number of particles of not less than 10 μm does not exceed 25 per mL and that of particles of not less than 25 μm does not exceed 3 per mL.

B ― Solutions for injection supplied in containers with a nominal content of less than 100 mL.

The average number of particles of not less than 10 μm does not exceed 6000 per container and that of particles of not less than 25 μm does not exceed 600 per container.