Method 2. Analytical Sieving Method

The analytical sieving method is a method to estimate the particle size distribution of powdered pharmaceutical drugs by sieving, which is usually applicable to powdered materials having a particle size of more than about 75 μm. Essentially, this method is to evaluate the two-dimensional size of the samples. The particle size determined by this method is shown as the size of a minimum sieve opening through which the particle passes.

Apparatus and instruments

(1) Sieves

Use the sieves specified in Measuring Instruments, Appliances under General Tests, Processes and Apparatus. Unless otherwise specified, principally use 200 mm-sieves made of stainless steel. To avoid loosing sample and the change in sieve opening caused by distortion of the frame, it is necessary to handle the sieves carefully. Remove the particles from the aperture of sieve, without beating the frame of the sieve or strongly brushing the mesh-wires of the sieve, by using a brush for sieve, an air jet, cleaning agents or organic solvents carefully not to injure the mesh-wires. After cleaning, dry the sieves in a suitable drying chamber at temperatures below 100°C. The sieves treated as described above should be used after checking that they are not disordered.

(2) Balances

Use balances readable to the nearest 0.1 g.

(3) Apparatus

Use either a ro-tap-type or electromagnet-type sieve shaker.

Sampling

(1) Sample should be taken so as to be representative of the test specimen. When the amount of the sample taken is larger, divide it into a suitable amount by an adequate way.

(2) Usually, the amount of sample is between 25 and 100 g, depending on the bulk density of the sample.

(3) The most appropriate sample mass is determined as the following example: Take 25, 50, 75 and 100 g of the sample, and proceed test sieving with them as directed in Procedure tentatively. If the results obtained with 25-, 50- and 75-g samples are similar, but the percentage through the finest sieve for the 100-g sample is lower compared with the cases of 25-, 50- and 75-g samples, 100 g is too large as the sampling size of the specimen.

(4) In the case that the amount of sample is less than 25 g, 75-mm diameter sieves may be used, though the amount of sample should be not less than 10 g.

Pretreatment of sample

The following treatments may be performed, depending on the properties of the sample:

(1) Drying agglomerated samples owing to their hygroscopicity under a condition which does not change the essential qualities of the sample.

(2) Sieving the agglomerated sample through a coarse mesh sieve previously to deagglomerate it.

(3) Addition of adequate additives to adhesive or agglomerated samples due to their electrostatic charge in an amount which does not affect the results to avoid the generation of electrostatic charge.

Procedure

Usually, this method is proceeded under the controlled temperature and humidity conditions, taking into consideration of the physicochemical characteristics such as hygroscopicity or static electricity.

Unless otherwise specified, select sieves which cover the entire particle size range of the sample to be tested. Place the sieves one upon another on a collecting pan in order from small to large opening, place the sample on the top sieve, replace the lid, and fix the nest of sieves on a mechanical shaker. Agitate the nest of sieves for the time period previously obtained by the endpoint determination and then remove each sieve from the nest. If there is some fine powder on the down surface of each sieve, take it off by the brush gently, and combine it with the sieve fraction retained on each next down sieve, then weigh each sieve and the collecting pan. Determine the mass of material on each sieve and in the collecting pan by the following equation to obtain the particle size distribution. The difference between the mass of the sample taken and the total mass of sample on each sieve and in the collecting pan, the total loss, must not exceed 2% of the mass of the original test specimen.

$$\text{Amount of the material on each sieve (\%)} = \frac{W_i}{W_T} \times 100$$

W_i: Mass of the material on each sieve (g)
W_T: Total mass of the material on each sieve and in the collecting pan (g)

Particle size distribution: Cumulate the amount of the material on each sieve in order from smaller to larger opening, and obtain cumulative amount undersize (%) corresponding to the sieve opening (μm).

Endpoint Determination

Unless otherwise specified, it is decided as the endpoint when the change in mass of material on any sieve is not more than either 5% or 0.1 g after repeating the continuous agitating for every 5 minutes.

47. Pyrogen Test

The Pyrogen Test is a method to test the existence of pyrogens by using rabbits.

Test animals

Use healthy mature rabbits each weighing not less than 1.5 kg which have not lost body mass when kept on a constant diet for not less than one week. Do not use the rabbits repeatedly in the same test unless as long a resting period as possible is taken. Animals should be excluded which have been used for a previous test that was decided as pyrogen-positive.

Record the rectal temperature four times at 2-hour intervals during 1 to 3 days prior to the test. House the animals individually during this period in an area free from disturbances likely to excite them, and exercise particular care to avoid disturbances on the day of the test.

Keep the temperature in an area of performing test uniform between 20°C and 27°C and preferably maintain constant humidity for at least 48 hours before the test.

Apparatus

(1) Thermometer—Use a rectal thermometer or any other temperature-recording devices of equal sensitivity for which the time necessary for reading the rectal temperature

is known.

(2) Syringe and injection needle—Render the syinges and needles pyrogen-free by heating at 250°C for not less than 30 minutes.

Test procedures

(1) Quantity of injection—Unless otherwise specified, 10 mL of the sample per kg of body mass of the animals is used.

(2) Procedure—Perform the test at an environmental temperature similar to that of the room wherein the animals were housed. The test animals are usually fixed in a suitable type of holder. Insert the thermometer or other temperature-recording device into the rectum of the test animal to a constant depth in the range of 60 to 90 mm, and read the temperature after a sufficient period of time. Withhold food from the test animals beginning several hours before the first temperature recording and until the test is completed. Determine the temperature of the test animals three times at 1-hour intervals before the injection of the sample. When the second and third temperatures show little difference, the latter is taken as the "control temperature." Do not use animals whose second and third temperatures are not in accord or exceed 39.8°C even if these two values are similar. Warm the sample to 37°C before injection, and administer intravenously through an ear vein within 15 minutes after the third temperature recording. Hypotonic solution other than Water for Injection may be made isotonic by the addition of pyrogen-free sodium chloride before the test. Read the temperatures three times at 1-hour intervals after injection.

The difference between the control temperature and the highest temperature is taken to be the rise in body temperature.

Interpretation of results

The test is carried out on a group of three rabbits. If two or three rabbits show an individual rise of 0.6°C or more above the respective control temperature, the test shall be considered as positive. If only one animal shows a temperature rise of 0.6°C or more, or if the sum of the temperature rises of the three animals exceeds 1.4°C, repeat the test on a group of five other rabbits. The test shall be considered positive, if two or more of the five rabbits show an individual temperature rise of 0.6°C or more.

When the pyrogen test is positive, the sample is considered to be rejected.

48. Qualitative Tests

The Qualitative Tests are applied to the identification of drugs and are done generally with quantities of 2 to 5 mL of the test solution.

Acetate

(1) When warmed with diluted sulfric acid (1 in 2), acetates evolve the odor of acetic acid.

(2) When an acetate is warmed with sulfuric acid and a small quantity of ethanol (95), the odor of ethyl acetate is evolved.

(3) Neutral solutions of acetates produce a red-brown color with iron (III) chloride TS, and a red-brown precipitate when boiled. The precipitate dissolves and the color of the solution changes to yellow upon addition of hydrochloric acid.

Aluminum salt

(1) Solutions of aluminum salts, when treated with ammonium chloride TS and ammonia TS, yield a gelatinous, white precipitate which does not dissolve in an excess of ammonia TS.

(2) Solutions of aluminum salts, when treated with sodium hydroxide TS, yield a gelatinous, white precipitate which dissolves in an excess of the reagent.

(3) Solutions of aluminum salts, when treated with sodium sulfide TS, yield a gelatinous, white precipitate which dissolves in an excess of the reagent.

(4) Add ammonia TS to solutions of aluminum salts until a gelatinous, white precipitate is produced. The color of the precipitate changes to red upon addition of 5 drops of alizarin red S TS.

Ammonium salt

When heated with an excess of sodium hydroxide TS, ammonium salts evolve the odor of ammonia. This gas changes moistened red litmus paper to blue.

Antimony salt, primary

(1) When primary antimony salts are dissolved in a slight excess of hydrochloric acid for the test and then diluted with water, a white turbidity is produced. The mixture produces an orange precipitate upon addition of 1 to 2 drops of sodium sulfide TS. When the precipitate is separated, and sodium sulfide TS is added to one portion of the precipitate and sodium hydroxide TS is added to another portion, it dissolves in either of these reagents.

(2) Add water to acidic solutions of primary antimony salts in hydrochloric acid until a small quantity of precipitate is produced, and then add sodium thiosulfate TS: the precipitate dissolves. A red precipitate is reproduced when the solution is heated.

Aromatic amines, primary

Acidic solutions of primary aromatic amines, when cooled in ice, mixed with 3 drops of sodium nitrite TS under agitation, allowed to stand for 2 minutes, mixed well with 1 mL of ammonium amidosulfate TS, allowed to stand for 1 minute, and then mixed with 1 mL of *N*-(1-naphthyl)-*N'*-diethyl-ethylenediamine oxalate TS, exhibit a red-purple color.

Arsenate

(1) Neutral solutions of arsenates produce no precipitate with 1 to 2 drops of sodium sulfide TS, but produce a yellow precipitate with hydrochloric acid subsequently added. The separated precipitate dissolves in ammonium carbonate TS.

(2) Neutral solutions of arsenates produce a dark red-brown precipitate with silver nitrate TS. When dilute nitric acid is added to one portion of the suspension, and ammonia TS is add to another portion, the precipitate dissolves in either of these reagents.

(3) Neutral or ammonia alkaline solutions of arsenates produce with magnesia TS a white, crystalline precipitate, which dissolves in dilute hydrochloric acid subsequently added.

Arsenite

(1) Acidic solutions of arsenites in hydrochloric acid