Ultraviolet-visible Spectrophotometry, using water as the blank. Separately, pipet 1 mL of the sample solution, add exactly 5 mL of trichloroacetic acid TS A or B to the solution as specified in the monograph, and shake. To this solution add exactly 5 mL of the substrate solution specified in the monograph, shake immediately, and stand it at 37 ± 0.5°C for 30 minutes. Follow the same procedure for the sample solution, and determine the absorbance A_B at 660 nm.

Protein digestive activity (unit/g)

$$= (A_T - A_B) \times F \times \frac{11}{2} \times \frac{1}{10} \times \frac{1}{W}$$

W: Amount (g) of the sample in 1 mL of the sample solution

F: Amount (μg) of tyrosine for absorbance 1 determined from Tyrosine Calibration Curve

(3) Assay for Fat Digestive Activity

The fat digestive activity can be obtained by back titration of the amount of fatty acid produced from the hydrolysis of the ester linkage, when lipase acts on olive oil. One fat digestive activity unit is the amount of enzymes that produces 1 μmole of fatty acid per minute under the conditions described in Procedure.

Preparation of Sample Solution

Dissolve or suspend the sample in an appropriate amount of cold water, or a buffer or salts solution specified in the monograph so that the amount of fatty acid increases in proportion to the concentration of the sample solution, when measuring under the conditions described in Procedure. The concentration is normally 1 to 5 fat digestive activity unit/mL.

Preparation of Substrate Solution

Take 200 to 300 mL of a mixture of emulsifier and olive oil (3:1) in a blender, and emulsify it at 12,000 to 16,000 revolutions per minute for 10 minutes, while cooling the solution to a temperature below 10°C. Stand this solution in a cool place for 1 hour, and make sure before use that the oil does not separate.

Preparation of Emulsifier

Dissolve 20 g of polyvinyl alcohol specified in the monograph in 800 mL of water by heating between 75°C and 80°C for 1 hour while stirring. After cooling, filter the solution if necessary, and add water to make exactly 1000 mL.

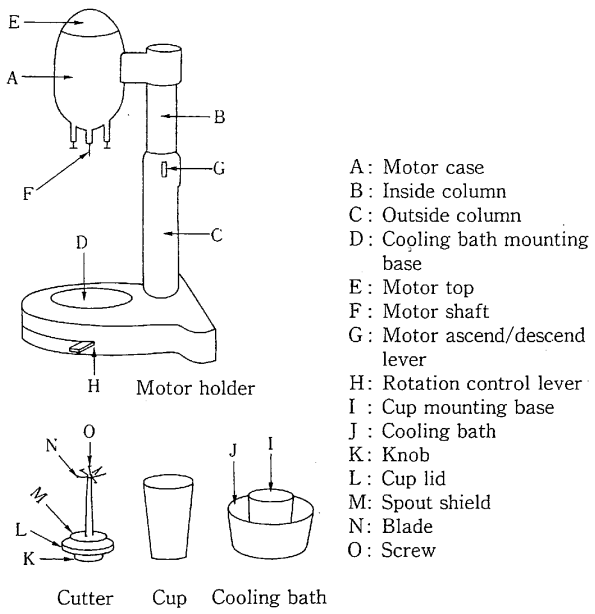
Procedure

Pipet 5 mL of the substrate solution and 4 mL of the buffer solution specified in the monograph, transfer them to a conical flask, and shake. After standing the mixture at 37 ± 0.5°C for 10 minutes, add exactly 1 mL of the sample solution, and shake immediately. Stand this solution at 37 ± 0.5°C for exactly 20 minutes, add 10 mL of a mixture of ethanol (95) and acetone (1:1), and shake. Then add exactly 10 mL of 0.05 mol/L sodium hydroxide VS, add 10 mL of a mixture of ethanol (95) and acetone (1:1), and shake. Titrate the excess sodium hydroxide with 0.05 mol/L hydrochloric acid VS (b mL) (indicator: 2 to 3 drops of phenolphthalein TS). Separately, pipet 5 mL of the substrate solution and 4 mL of buffer solution specified in the monograph, transfer them to a conical flask, and shake. After standing it at 37 ± 0.5°C for 10 minutes, add 10 mL of a mixture of ethanol (95) and acetone (1:1), then add exactly 1 mL of the sample solution, and shake. Add exactly 10 mL of 0.05 mol/L sodium hydroxide VS, and titrate in the same manner (a mL).

Fat digestive activity (unit/g)

$$= 50 \times (a - b) \times \frac{1}{20} \times \frac{1}{W}$$

W: Amount (g) of the sample in 1 mL of the sample solution



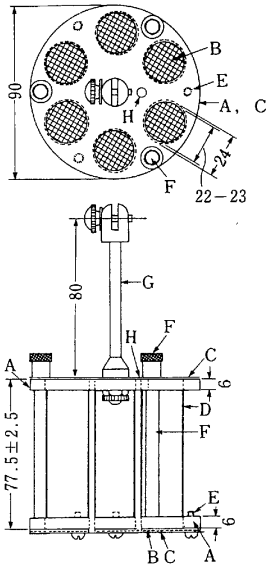
14. Disintegration Test

The Disintegration Test is a method to determine the resistance or disintegration of solid preparations for internal use in the test fluids. Unless otherwise specified, tablets, tablets coated with suitable coating agents, pills, capsules, granules or enteric coated preparations comply with the test described below. This test method, however, is not applied for preparations exceeding 20.0 mm in diameter, for sustained release preparations, or for preparations which are subject to the Dissolution Test.

Apparatus

The apparatus consists of a basket-rack assembly, a beaker about 110 mm in inside diameter and about 155 mm in height, a suitable thermostatic arrangement for heating, and a motor. Auxiliary disks or auxiliary tubes are used as directed in the Procedure.

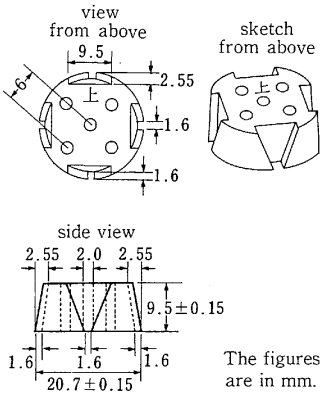
Basket-rack assembly: The basket-rack assembly, as illustrated in Fig. 1, consists of 6 open-ended glass tubes D, each 77.5 ± 2.5 mm long, 21.5 ± 0.5 mm in inside diameter and 23.5 mm in outside diameter; the tubes are held in a vertical position by two plastic plates A, each about 90 mm in diameter and 6 mm in thickness, with 6 holes, each 24 mm in diameter, equidistant from the center of the plate and equally spaced from one another. Attached by screws to the under surface of the lower plate is an acid-resistant wire gauze B, having openings of 2.0 mm and wire diameter of 0.6 mm.



The figures are in mm.

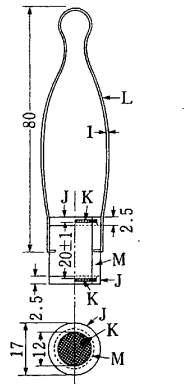
Fig. 1

A: Plastic plate
B: Acid-resistant wire gauze; openings: 2.0 mm; wire diameter: 0.6 mm
C: Acid-resistant metal plate
D: Glass tube
E: Nut
F: Bolt and nut
G: Central shaft
H: Hole for thermometer
J and M: Plastic tube
K: Acid-resistant wire gauze; openings: 0.42 mm; wire diameter: 0.29 mm
L: Acid-resistant wire handle



The figures are in mm.

Fig. 2



The figures are in mm.

Fig. 3

The glass tubes and the upper and the lower plastic plates are secured in position at the top or the bottom by means of an acid-resistant metal plate C, 90 mm in diameter and 1 mm in thickness, having 6 perforations, each about 22 to 23 mm in diameter, which coincide with those of the upper plastic plate and upper open ends of the glass tubes. A central shaft G, about 80 mm in length, the upper end of which terminates in bearing connected with the motor, is attached to the metal plate. The parts of the apparatus are assembled and rigidly held by means of three bolts passing through the two plastic plates and the metal plate C. The design of the basket-rack assembly may be modified slightly excepting the requirements for the glass tubes and the wire gauze.

Auxiliary disk: The auxiliary disk, as illustrated in Fig. 2, is a transparent, smooth plastic cylinder, 9.50 ± 0.15 mm in height, 20.70 ± 0.15 mm in diameter and having a specific gravity of 1.18 to 1.20. Five holes, 2 mm in diameter, extend between the ends of the cylinder, one of the holes being through the cylinder axis and the others parallel with it equally spaced on a 6-mm radius from it. Equally spaced on the sides of the cylinder are four notches that form V-shaped planes that are perpendicular to the ends of the cylinder. The dimensions of each notch are such that the openings on the bottom of the cylinder are 1.6 mm square and those on the top are 9.5 mm wide and 2.55 mm deep.

Auxiliary tube: The auxiliary tube, as illustrated in Fig. 3, consists of a plastic tube M, 12 mm in inside diameter, 17 mm in outside diameter, 20 mm in length, having both outside ends screw-cut, and two plastic rings J, each 12 mm in inside diameter, 17 mm in outside diameter, 2.5 mm in length, having one inside end screw-cut. Acid-resistant woven wire gauze having 0.42-mm openings and 0.29-mm wire diameter is placed in each plastic ring and the rings are attached by screws to each end of the plastic tube. The distance between two wire gauzes is 20 ± 1 mm. A handle of an acid-resistant wire, 1 mm in diameter and 80 mm in length, is attached to the mid portion of the plastic tube.

Test fluids

(1) 1st fluid—Dissolve 2.0 g of sodium chloride in 7.0 mL of hydrochloric acid, and add water to make 1000 mL. This solution is clear and colorless, and its pH is about 1.2.

(2) 2nd fluid—To 250 mL of 0.2 mol/L potassium dihydrogenphosphate TS add 118 mL of 0.2 mol/L sodium hydroxide TS and water to make 1000 mL. This solution is clear and colorless, and its pH is about 6.8.

(3) Water

Procedure

Attach the basket-rack assembly to the bearing, immerse in the fluid in a beaker, and adjust the apparatus so as to raise and lower the basket smoothly at a constant frequency of 29 to 32 cycles per minute through a distance of 53 to 57 mm. At the lowest point of the downward stroke, the wire mesh must be 25 mm distant from the bottom of the beaker and the volume of the fluid in the beaker is such that, at the lowest point of the downward stroke, the top of the basket is on a level with the surface of the fluid. Maintain the temperature of the fluid at 37 ± 2°C during the test.

Place one sample, except for granules, to be tested in each of the 6 tubes of the basket, immerse the basket in a suitable volume of test fluid, maintained in a beaker at the desired temperature, and operate the apparatus for the directed period of time. At the end of this period, lift the basket gently from the fluid to permit the observation of the samples in the glass tubes. When it is directed to put auxiliary disks in the tubes, place a sample in each tube, then put the disk gently in each tube with the upper side up, and proceed as directed above. If the determination is difficult, the auxiliary disk may be omitted.

(1) Tablets—Carry out the test using water as the test fluid. Observe the tablets after 30 minutes of operation with auxiliary disks: the tablets comply with the test, if no residue, only a spongy substance, or only a small amount of a soft residue or muddy residue remains in the glass tube. If only one tablet remains intact or fragments of the samples are observed in one tube, repeat the test on an additional 6 tablets: the tablets comply with the test if no residue, spongy substance, or only a small amount of soft residue or a muddy substance remains in the glass tube.

(2) Tablets coated with suitable coating agents—Carry out the test using water as the test fluid. Observe the tablets after 60 minutes of operation with auxiliary disks: the tablets comply with the test, if no residue, only the film of the samples or a spongy substance, or only a small amount of a soft residue or a muddy substance remains in the glass tube. If only one sample remains intact or shows no evidence of leakage of its content even though the film is dissolved, ruptured or ripped off, repeat the test on 6 additional samples; the samples comply with the test, when no residue, only the film of the tablets or a spongy substance, or only a small amount of

a soft residue or a muddy substance remains in the glass tube.

(3) Pills—Apply the test for tablets. However, the test is performed with the 1st fluid for 60 minutes. If a residue is observed in a glass tube, the test should be continued for 60 minutes with the 2nd fluid.

(4) Capsules—Carry out the test using water as the test fluid. Observe the capsules after 20 minutes of operation with auxiliary disks: the capsules comply with the test, if no residue of the capsules or only the film of the capsules, or only a small amount of soft residue or a muddy substance remains in the glass tube. If only one capsule remains intact or shows no evidence of leakage of its content even though the film is dissolved, ruptured or ripped off, repeat the test on 6 additional capsules; the capsules comply with the test, if no residue or only the film of the capsules or a small amount of soft residue or a muddy substance remains in the glass tube.

(5) Granules—Shake granules on a No.30 (500 μm) sieve as directed in (1) Granules under the Particle Size Distribution Test for Preparations, transfer 0.10 g of the residue on the sieve to each of the 6 auxiliary tubes, secure the 6 tubes to the bottom of the basket tightly, and carry out the test using water as the test fluid, unless otherwise specified. Observe the granules after 30 minutes of operation: the granules comply with the test, if no residue remains in any of the auxiliary tubes or only one sample remains intact in only one auxiliary tube, or if the residue is the film of the granules or only a small amount of a soft residue or a muddy substance is present. In case of coated granules, apply the test with water, and operate for 60 minutes.

(6) Enteric coated preparations

(i) Enteric coated preparations other than those falling under (ii)

Perform the following two tests, (a) the test with the 1st fluid and (b) the test with the 2nd fluid.

(a) The test with the 1st fluid

Carry out the test using the 1st fluid as the test fluid. Observe the sample after 120 minutes of operation: the samples comply with the 1st fluid test, if the enteric coat of one or none of six samples is disintegrated, the enteric coating film is ruptured, peeled off, or otherwise broken, and active substance has leaked. If two of six samples are disintegrated, repeat the test with 6 additional samples. The samples comply with the test, when all six samples show no distinct evidence of disintegration.

(b) The test with the 2nd fluid

Carry out the test with new 6 samples using the 2nd fluid as the test fluid, and observe the samples after 60 minutes of operation with auxiliary disks: the samples comply with the test, if no residue, only the film of the preparation or spongy substances, or only a small amount of a soft residue or a muddy substance remains in the glass tube.

(ii) Granules and capsules enclosing drugs in granular form

Perform the following two tests, (a) the test with the 1st fluid and (b) the test with the 2nd fluid.

(a) The test with the 1st fluid

Shake granules or contents taken out from capsules on a No.30 (500 μm) sieve as directed in (1) Granules under the Particle Size Distribution Test for Preparations, transfer 0.10 g of the residue on the sieve to each of the 6 auxiliary tubes, secure the 6 tubes to the bottom of the basket tightly, and carry out the test using the 1st fluid as the test fluid. Observe the residue after 60 minutes of operation: the samples comply with the test, if particles fallen from the openings of the wire gauze number not more than 15.

(b) The test with the 2nd fluid

Transfer 0.10 g of the residue, obtained by the same way as directed in the test with the 1st fluid, to each of the 6 auxiliary tubes, secure the 6 tubes to the bottom of the basket tightly, and operate the apparatus using the 2nd fluid as the test fluid for 30 minutes, and observe the samples: the samples comply with the test, if no residue remains in any of the auxiliary tubes or only one sample remains intact, or if the residue is the film of the samples, or is only a small amount of a soft residue or a muddy substance.

15. Dissolution Test

The Dissolution Test is a method to test the dissolution of active ingredients from solid preparations for internal use, and aims at confirming the quality of solid preparations for internal use in relation to a fixed standard and also at preventing significant bioinequivalence.

Apparatus

All parts of the apparatus that may come into contact with the preparation or the dissolution medium must be chemically inert and do not adsorb or react or interfere with the test substance. Usually, all metal parts of the apparatus that may come into contact with the dissolution medium are made from a suitable stainless steel or coated with an inert material. A vessel for the sample and the dissolution medium that permits observation of the inside during the test is preferable.

(1) Method 1 (Rotatory basket method)

The testing assembly consists of a vessel with a hemispherical bottom as shown in Fig. 1, a cylindrical basket and a rotatory shaft as shown in Fig. 2, an electric motor and a thermostat. Capacity of the vessel is about 1000 mL. The lower end of the rotatory shaft is connected to a coupling disk with three clasps which are fitted on top of the basket. The side wall and the lower part of the cylindrical basket are formed by fixing a No. 36 (425 μm) sieve to two rings at the upper and the lower end. The basket is fitted in the coupling disk and the upper end of the rotatory shaft is terminated in a shaft bearing which is devised to transmit the rotatory movement of the motor.

(2) Method 2 (Paddle method)

The testing assembly consists of a round bottom vessel shown in Fig. 1, a paddle shown in Fig. 3, an electric motor and a water bath with a thermostat. The paddle conforms to the specification shown in Fig. 3. The blade is positioned horizontally at the end of the shaft so that the 42.0 mm-edge is on the same level as the lower end of the shaft and the blade passes through the center of the shaft. The upper end of the paddle shaft is terminated in a shaft bearing which is devised to transmit the rotatory movement of the motor. A sinker with 12.0 ± 0.2 mm in inside diameter and 25 – 26 mm in length shown in Fig. 4 may be used when the sample floats in the dissolution medium. The trunk of the sinker consists of a spiral 3.0 – 3.5 mm pitch coil made with an acid-resistant 1 mm thick wire. The spiral is supported on the outside with 10 wires which are fixed almost in parallel and with an equal distance. The sides of the sinker are fixed with two