

should be made in the quantities of materials when dies of other sizes are used. To prepare a 1.0 percent mixture weigh approximately 2 milligrams of the sample and mix thoroughly with 200 milligrams of dried potassium bromide (infrared spectrophotometric quality). For a 0.5 percent potassium bromide mixture, use 1 milligram of sample. For a 0.25 percent potassium bromide mixture, use 0.5 milligram of sample. A mortar and pestle, a ball mill, or other suitable mixing device may be used. Transfer the uniformly milled mixture to the die, evacuate gradually while raising the pressure to 3,000 pounds per square inch until evacuation is complete, then raise the pressure to 16,000 pounds per square inch, and hold that pressure for 2 to 3 minutes. Release the pressure, dismantle the die, and recover the potassium bromide disc. Mount the disc in a suitable holder and proceed as directed in paragraph (c) of this section.

(2) *Mineral oil mull.* Weigh approximately 20 milligrams of the sample into an agate mortar and add 2 drops of mineral oil. Triturate thoroughly with a pestle until a uniform consistency is obtained. Use two rock salt plates as an absorption cell. Place a small drop of the mull in the center of one of the plates. Gently put the other plate on the mull and slowly squeeze the plates together to spread the mull uniformly. Clamp the two plates firmly together in a metal holder. Examine the assembled cell by holding it up to the light. It should appear smooth and free of any air bubbles. Proceed as directed in paragraph (c) of this section.

(3) *1 percent solution.* Prepare a 1 percent solution of the sample in chloroform and use 1.0 millimeter matched absorption cells. Proceed as directed in paragraph (c) of this section.

(c) *Procedure.* Place the sample, prepared as directed in paragraph (b) of this section, in the spectrophotometer. Determine the infrared absorbance spectrum between the wavelengths of 2 to 15 microns. To be suitable the spectrum should have a transmittance of between 20 and 70 percent at most of the wavelengths showing significant absorption. Compare the spectrum to that of an authentic sample of the same antibiotic prepared in an iden-

tical manner. To pass the infrared identity test, the absorption spectrum of the sample should compare qualitatively with that of the authentic sample.

§ 436.212 Disintegration test.

(a) *Apparatus—(1) Basket-rack assembly.* The basket-rack assembly consists of 6 open-ended glass tubes, each 7.75 ± 0.25 centimeters long and having an inside diameter of approximately 21.5 millimeters and a wall approximately 2 millimeters thick; the tubes are held in a vertical position by two plastic plates, each about 9 centimeters in diameter and 6 millimeters in thickness, with six holes, each about 24 millimeters in diameter, equidistant from the center of the plate and equally spaced from one another. Attached by screws to the undersurface of the lower plate is 10-mesh No. 23 (0.025 inch) W. and M. gauge woven stainless steel wire cloth. The glass tubes and the upper plastic plate are secured in position at the top by means of a stainless steel plate, about 9 centimeters in diameter and 1 millimeter in thickness, having six perforations each about 20 millimeters in diameter, which coincide with those of the upper plastic plate and the upper open ends of the glass tubes. A central shaft about 8 centimeters in length, the upper end of which terminates in an eye through which a string or wire may be inserted, is attached to the stainless steel plate. The parts of the apparatus are assembled and rigidly held by means of three bolts passing through the two plastic plates and the steel plate. The design of the basket-rack assembly may be varied somewhat provided the specifications for the glass tubes and the screen mesh size are maintained.

(2) *Disks.* Each tube is provided with a slotted and perforated cylindrical disk 9.5 ± 0.15 millimeters thick and 20.7 ± 0.15 millimeters in diameter. The disk is made of a suitable, transparent plastic material having a specific gravity of between 1.18 and 1.20. Five 2-millimeter holes extend between the ends of the cylinder, one of the holes being through the cylinder axis and the others parallel with it and equally spaced on a 6-millimeter radius from it.

Equally spaced on the sides of the cylinder are four notches that form V-shaped planes perpendicular to the ends of the cylinder. The dimensions of each notch are such that the openings on the bottom of the cylinder are 1.60 millimeters square and those on the top are 9.5 millimeters wide and 2.55 millimeters deep. All surfaces of the disk are smooth.

(3) *Raising and lowering device.* Use a device for raising and lowering the basket in the immersion fluid at a constant rate between 28 and 32 cycles per minute through a distance of not less than 5 centimeters and not more than 6 centimeters.

(b) *Immersion fluids.* During the performance of the tests all immersion fluids are maintained at a temperature of $37^{\circ}\pm 2^{\circ}$ C. by using a thermostatically controlled water bath.

(1) Distilled water.

(2) Simulated gastric fluid: Dissolve 2.0 grams of sodium chloride and 7.0 milliliters of hydrochloric acid in about 500 milliliters of water. Dissolve 3.2 grams of pepsin in this solution and add sufficient water to make 1,000 milliliters. This solution has a pH of about 1.2.

(3) Simulated intestinal fluid: Dissolve 6.8 grams of monobasic potassium phosphate in 250 milliliters of water, mix and add 190 milliliters of 0.2*N* sodium hydroxide and 400 milliliters of water. Add 10.0 grams of pancreatin, mix, and adjust the resulting solution with 0.2*N* sodium hydroxide to a pH of 7.5 ± 0.1 . Dilute to 1,000 milliliters.

(c) *Immersion vessel.* Use a suitable vessel, such as a 1-liter beaker.

(d) *Operation.* Add enough immersion fluid to the immersion vessel so that when the basket-rack assembly is placed on the raising and lowering device at the highest point of the upward stroke, the wire mesh remains at least 2.5 centimeters below the surface of the fluid and descends to not less than 2.5 centimeters from the bottom of the immersion vessel.

(e) *Procedure—(1) Uncoated or filmcoated tablets.* Place one tablet into each of the six tubes of the basket, add a disk to each tube, and operate the apparatus, using simulated gastric fluid as the immersion fluid. At the end of the time limit specified in the individ-

ual section for the particular antibiotic tablet being tested, lift the basket from the fluid and observe the tablets.

(2) *Plain-coated tablets.* Place one tablet in each of the six tubes of the basket, add a disk to each tube, and operate the apparatus, using simulated gastric fluids as the immersion fluid. After 30 minutes, lift the basket from the fluid and observe the tablets. If the tablets have not disintegrated completely, substitute simulated intestinal fluid as the immersion fluid and continue the test for a total period of time (including previous immersion in simulated gastric fluid) equal to the time limit specified in the individual section for the particular antibiotic tablet being tested. Lift the basket and observe the tablets.

(3) *Enteric-coated tablets.* Place one tablet in each of the six tubes of the basket and operate the apparatus, using simulated gastric fluid as the immersion fluid. One hour later, lift the basket from the fluid and observe the tablets. If the tablets show no distinct evidence of dissolution or disintegration, add a disk to each tube and operate the apparatus, using simulated intestinal fluid as the immersion fluid, for a total period of time (including the previous immersion in simulated gastric fluid) equal to the time limit specified in the individual section for the particular antibiotic tablet being tested. Lift the basket and observe the tablets.

(4) *Pastilles.* Place one pastille into each of the six tubes of the basket, add a disk to each tube, and operate the apparatus, using distilled water as the immersion fluid. At the end of the time limit specified in the individual section for the particular antibiotic pastille being tested, lift the basket from the fluid and observe the pastilles.

(5) *Capsules.* Place one capsule into each of the six tubes of the basket, add a disk to each tube, and operate the apparatus, using distilled water as the immersion fluid. At the end of the time limit specified in the individual section for the capsules being tested, lift the basket from the fluid and observe the capsules.

(f) *Evaluation.* Complete disintegration is defined as the state in which any residue of the tablet, pastille, or

capsule (except fragments of the insoluble coating) remaining on the screen is a soft mass having no palpably firm core. The tablets, pastilles, or capsules pass the disintegration test if all of the units tested disintegrate completely under the conditions and time specified in the individual section for the antibiotic tablet, pastille, or capsule being tested. If one or two tablets, pastilles, or capsules fail to disintegrate completely, repeat the test on 12 additional tablets, pastilles, or capsules. The tablets, pastilles, or capsules pass the disintegration test if not less than 16 of the total 18 tested disintegrate completely. Enteric coated tablets fail the disintegration test if they show any distinct evidence of dissolution or disintegration after 1 hour immersion in simulated gastric fluid.

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§ 436.213 Nonaqueous titrations.

(a) *Equipment*—(1) *Apparatus*. Use a closed system consisting of a suitable titrimeter equipped with a potentiometer, an automatic burette, a chart recorder, and a glass calomel combination electrode (with saturated methanolic potassium chloride as the electrolyte).

(2) *Titration vessel*. Use a 100-milliliter tall form beaker without a spout.

(b) *Reagents*—(1) Methyl alcohol, reagent grade, anhydrous.

(2) Dimethylsulfoxide, A.C.S., reagent grade.

(3) Glacial acetic acid, A.C.S., reagent grade.

(4) Lithium methoxide reagent: 0.02*N* lithium methoxide in methyl alcohol, standardized against primary grade benzoic acid.

(5) Perchloric acid reagent: 0.02*N* perchloric acid in glacial acetic acid, standardized against primary grade potassium acid phthalate.

(c) *Preparation of sample solutions*. Select the weight of the sample and the solvent listed for each antibiotic. Transfer the accurately weighed sample to a titration vessel. Add the appropriate solvent, cover, and stir magnetically until the sample is dissolved. Proceed as directed in paragraph (e) of

this section, using the procedure or procedures specified in the individual section for each antibiotic.

Antibiotic	Weight in milligrams of sample	Solvent
Amoxicillin-acid titration	100	20 milliliters dimethylsulfoxide and 30 milliliters methyl alcohol.*
Amoxicillin-base titration.	100	50 milliliters glacial acetic acid.
Ampicillin-acid titration	100	20 milliliters dimethylsulfoxide and 30 milliliters methyl alcohol.*
Ampicillin-base titration	100	50 milliliters glacial acetic acid.
Ampicillin sodium-base titration.	50	Do.
Cephaloglycin-base titration.	50	Do.
Cephapirin sodium-base titration.	50	50 milliliters glacial acetic acid.
Cyclacillin-acid titration	100	20 milliliters dimethylsulfoxide and 30 milliliters methyl alcohol.*
Cyclacillin-base titration	100	50 milliliters glacial acetic acid.
Tobramycin-base titration.	30	50 ml glacial acetic acid.

*The methyl alcohol is added after the sample has dissolved in dimethylsulfoxide.

(d) *Blank determination*. Place the same volume of solvent used to prepare the sample solution into a titration vessel and proceed as directed in paragraph (e) of this section, using the procedure or procedures specified in the individual section for each antibiotic.

(e) *Titration procedures*—(1) *Acid titration*. Equilibrate the electrode by soaking it overnight in the solvent used for preparing the sample solution. Start the magnetic stirrer and titrate the sample solution with the lithium methoxide reagent. Record the change in potential of the solution with the addition of the titrant. Determine the number of milliliters of reagent consumed at neutralization (the inflection point of the titration curve). Calculate the antibiotic content as directed in the individual section.

(2) *Base titration*. Proceed as directed in paragraph (e)(1) of this section, except use the perchloric acid reagent as the titrant and calculate the antibiotic