

a, b, c, d, e—Average absorbance values for each concentration of the standard response line, lowest to the highest, respectively.

(c) *Estimation of potency.* To prepare the standard response line, plot the average absorbance values for each concentration of the standard response line on one-cycle semilogarithmic graph paper with the absorbance values on the arithmetic scale and concentrations on the logarithmic scale. The response line is drawn either through these points by inspection or through points plotted for highest and lowest absorbance values obtained by means of the following equations.

To estimate the potency of the sample, average the absorbance values for the sample and determine the antibiotic concentration from the standard response line. Multiply the concentration by the appropriate dilution factor to obtain the antibiotic content of the sample.

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Subpart E—General Chemical Tests for Antibiotics

§ 436.200 Loss on drying.

Use the method specified in the individual section for each antibiotic.

(a) *Method 1.* In an atmosphere of about 10 percent relative humidity, grind the sample, if necessary, to obtain a fine powder. When tablets, troches, or capsules are to be tested, use four tablets, troches, or capsules in preparing the sample. Transfer about 100 milligrams of the sample to a tared weighing bottle equipped with a ground-glass stopper. Weigh the bottle and place it in a vacuum oven, tilting the stopper on its side so that there is no closure during the drying period. Dry at a temperature of 60° C. and a pressure of 5 millimeters of mercury or less for 3 hours. At the end of the drying period, fill the vacuum oven with air dried by passing it through a drying agent such as sulfuric acid or silica gel.

Replace the stopper and place the weighing bottle in a desiccator over a desiccating agent, such as phosphorous pentoxide or silica gel, allow to cool to room temperature, and reweigh. Calculate the percent of loss.

(b) *Method 2.* Proceed as directed in paragraph (a) of this section, except use a tared weighing bottle or weighing tube equipped with a capillary-tube stopper, the capillary having an inside diameter of 0.20–0.25 millimeter, and place it in a vacuum oven without removing the stopper.

(c) *Method 3.* Proceed as directed in paragraph (a) of this section, except dry the sample at a temperature of 110° C. and a pressure of 5 millimeters of mercury or less for 3 hours.

(d) *Method 4.* Proceed as directed in paragraph (a) of this section, except dry the sample at a temperature of 40° C. and a pressure of 5 millimeters of mercury or less for 2 hours.

(e) *Method 5.* Proceed as directed in paragraph (a) of this section, except dry the sample at a temperature of 100° C. and a pressure of 5 millimeters of mercury or less for 4 hours.

(f) *Method 6.* Proceed as directed in paragraph (a) of this section, except dry the sample at a temperature of 40° C. and a pressure of 5 millimeters of mercury or less for 3 hours.

(g) *Method 7.* Proceed as directed in paragraph (a) of this section, except dry the sample at a temperature of 25° C. and a pressure of 5 millimeters of mercury or less for 4 hours.

(h) *Method 8.* Proceed as directed in paragraph (a) of this section, except transfer approximately 300 milligrams of the sample to a tared weighing bottle equipped with a ground-glass stopper; dry the sample at a temperature of 25 ° C and a pressure of 5 millimeters of mercury or less for 4 hours, and then dry the sample at a temperature of 100 °C and a pressure of 5 millimeters of mercury or less for 3 additional hours.

(i) *Method 9.* Use a suitable thermogravimetric apparatus prepared for vacuum operation. Rapidly and thoroughly grind a portion of the sample and promptly transfer 5 to 10 milligrams to the sample pan. Place the system under vacuum and allow it to come to equilibrium before heating. Obtain an accurate sample weight and

continuously record the weight loss as the sample is heated at a rate of 20° per minute from room temperature to about 200 ° C. The weight loss plateau, or inflection, at about 150 ° C is taken as the total volatile weight loss. Calculate the percent weight loss on drying.

[39 FR 18944, May 30, 1974, as amended at 50 FR 48397, Nov. 25, 1985; 51 FR 11572, Apr. 4, 1986]

§ 436.201 Moisture determination.

(a) *Equipment*—(1) *Apparatus*. Use a closed system consisting of all glass automatic burettes, platinum electrodes, and a magnetic stirrer connected to a suitable electrometric apparatus. This apparatus embodies a simple electrical circuit which serves to pass 5 to 10 microamperes of direct current between a pair of platinum electrodes immersed in the solution to be titrated. At the endpoint of the titration a slight excess of the reagent increases the flow of current to between 50 and 150 microamperes for 30 seconds or longer, depending upon the solution being titrated.

(2) *Titration vessel*. Use a suitable titrating vessel which has been previously dried at 105° C. and cooled in a desiccator.

(b) *Reagents*—(1) *Karl Fischer reagent*. Dissolve 125 grams of iodine in 170 milliliters of pyridine, add 670 milliliters of methanol and cool. To 100 milliliters of pyridine kept in an ice bath, add sulfur dioxide until the volume reaches 200 milliliters. Slowly add this solution to the cooled iodine-methanol-pyridine mixture and shake well. (A commercially prepared Karl Fischer reagent, pyridine containing or pyridine-free, may be used.) Preserve the reagent in glass-stoppered bottles protected from light and from moisture in the air.

(2) *Methanol solution*. Add sufficient water (usually 2 milligrams per milliliter) to methanol so that each milliliter of the resulting methanol solution is equivalent to about 0.5 milliliter of Karl Fischer reagent.

(3) *Solvents*—(i) *Solvent A*. Methanol:chloroform:carbon tetrachloride (1:2:2 by volume).

(ii) *Solvent B*. Chloroform:carbon tetrachloride (1:1 by volume).

(iii) *Solvent C*. Anhydrous methanol.

(c) *Standardization of reagents*—(1) *Water equivalence of Karl Fischer reagent*. Standardize the Karl Fischer reagent no longer than 1 hour before use by one of the following methods.

(i) Accurately weigh 25-35 milligrams of water into a dry titration vessel and add 20 milliliters of solvent A. Start the stirrer and titrate to the endpoint by adding measured quantities of Karl Fischer reagent. Calculate the water equivalence of the Karl Fischer reagent as follows:

$$e = \frac{W}{V_T - V_A}$$

where:

e=Water equivalence of the Karl Fischer reagent in terms of milligrams of water per milliliter;

W=Milligrams of water;

V_T=Milliliters of Karl Fischer reagent used;

V_A=Milliliters of Karl Fischer reagent equivalent to the 20 milliliters of solvent A, determined as directed in paragraph (c)(3) of this section.

(ii) Accurately weigh about 25-35 milligrams of water into a dry titration vessel, add an excess of Karl Fischer reagent, start the stirrer, and titrate to the endpoint with methanol solution. Calculate the water equivalence of the Karl Fischer reagent as follows:

$$e = \frac{W}{V_T - V_A}$$

where:

e=Water equivalence of the Karl Fischer reagent in terms of milligrams of water per milliliter;

W=Milligrams of water;

V_T=Milliliters of Karl Fischer reagent used;

V_m=Milliliters of methanol solution used;

f=Milliliters of Karl Fischer reagent equivalent to each milliliter of methanol solution determined as directed in paragraph (c)(2) of this section.

(2) *Karl Fischer reagent equivalence of methanol solution*. Titrate a known volume of Karl Fischer reagent with methanol solution until the endpoint is reached. Calculate the milliliters of Karl Fischer reagent equivalent to each milliliter of methanol solution as follows: