

should have spots of corresponding  $R_f$  values.

[39 FR 34032, Sept. 23, 1974; 48 FR 11427, Mar. 18, 1983, as amended at 49 FR 2242, Jan. 19, 1984]

**§ 436.312 Atomic absorption method for determining the zinc content of zinc bacitracin.**

(a) *Equipment.* An atomic absorbance spectrophotometer equipped with a zinc hollow-cathode discharge lamp, an air-acetylene flame, a nebulizer-burner system for introducing the sample solution into the flame, an optical dispersing device (such as a monochromator) for isolating a resonance line of zinc from others produced by the emission source, and a suitable radiation detector and recorder.

(b) *Preparation of working standard and sample solutions—(1) Workingstandard solutions.* Prepare a standard stock solution containing 10 milligrams of zinc per milliliter as follows: Weigh 3.11 grams of zinc oxide into a 250-milliliter volumetric flask, add 80 milliliters of 1N HCl, warm to dissolve, cool to room temperature, and dilute to volume with water. Dilute aliquots of this standard stock solution with 0.001N HCl to obtain three working standard solutions containing respectively 0.5, 1.5, and 2.5 micrograms of zinc per milliliter.

(2) *Sample solution.* Accurately weigh approximately 200 milligrams of the sample into a 100-milliliter volumetric flask. Dissolve and dilute to volume with 0.01N HCl. Transfer a 2.0-milliliter aliquot of this solution to a 200-milliliter volumetric flask and dilute to volume with 0.001N HCl.

(c) *Procedure.* Using 0.001N HCl as the blank, adjust the absorbance of the instrument to zero at a detection wavelength of 213.8 nanometers. Determine the absorbance of each standard solution and the sample solution at 213.8 nanometers.

(d) *Calculations.* Plot the absorbance versus the concentration of each of the working standard solutions. Draw a straight response line of best fit through these points. Read the concentration of zinc in micrograms per milliliter corresponding to the absorbance of the sample solution. Calculate

the percent zinc in the sample as follows:

$$\text{Percent zinc} = \frac{C \times 100,000}{\text{Milligrams of sample} \times (100 - m)}$$

where:

$C$ =Concentration of zinc in the sample solution in micrograms per milliliter;  
 $m$ =Percent moisture in the sample.

[40 FR 15088, Apr. 4, 1975]

**§ 436.316 Determination of penicillin G content.**

(a) *Reagents.* The reagents are freshly prepared every three days and are of such quality that when used in this procedure with an authentic sample of penicillin G, not less than 97 percent of penicillin G is recovered.

(1) *Amyl acetate (iso-amyl acetate) solution.* Saturate the amyl acetate (boiling range 138.5° C—141.5° C) with the *N*-ethylpiperidine salt of penicillin G by adding 2 milligrams of the salt for each 1.0 milliliter of the solvent. Cool this solution to 0° C—8° C and filter it through a sintered-glass filter immediately before use.

(2) *Acetone solution.* Saturate reagent grade acetone with the *N*-ethylpiperidine salt of penicillin G using 3 milligrams of salt for each 1 milliliter of acetone. Cool this solution to 0° C—8° C and filter it through a sintered-glass filter immediately before use.

(3) *N-ethylpiperidine solution.* *N*-ethylpiperidine (boiling range 129.5° C—131.0° C) should be stored in brown bottles in a refrigerator. Dilute 1.0 milliliter of this reagent with 4.0 milliliters of amyl acetate. Saturate this solution with the *N*-ethylpiperidine salt of penicillin G, using about 3 milligrams of the salt for each 1.0 milliliter of solution. Cool this solution to 0° C—8° C and filter it through a sintered-glass filter immediately before use.

(4) *Phosphoric acid solution.* Prepare by dissolving 1.0 milliliter of reagent grade phosphoric acid (85 percent) in 4.0 milliliters of water. Cool to 0° C—8° C and shake before using.

(5) *Silica gel.* Use dry silica gel (mesh size 6-16, Tyler standard). Place about