

$$\text{Micrograms of chloramphenicol per milligram} = \frac{\text{Absorbance of sample at 276 nanometers} \times \text{micrograms of standard per milliliter} \times \text{potency of chloramphenicol working standard in the micrograms per milligram}}{\text{Absorbance of standard at 278 nanometers} \times \text{micrograms of sample per milliliter}}$$

Calculate the milligrams per milliliter of the reconstituted solution in the dispensing container as follows:

$$\text{Milligrams per milliliter of the reconstituted vial} = \frac{\text{Absorbance of sample at 276 nanometers} \times \text{micrograms of standard per milliliter} \times \text{labeled content of reconstituted vial in milligrams per milliliter}}{\text{Absorbance of standard at 278 nanometers} \times 20}$$

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens*. Proceed as directed in § 436.32(a) of this chapter, using a solution containing 5 milligrams of chloramphenicol per milliliter.

(4)–(5) [Reserved]

(6) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(7) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 250 milligrams of chloramphenicol per milliliter.

(8) *Specific rotation*. Dilute the sample with sufficient distilled water to give a solution containing approximately 50 milligrams per milliliter. Proceed as directed in § 436.210 of this chapter, using a 1.0-decimeter polarimeter tube. Calculate the specific rotation on the anhydrous basis.

[39 FR 19166, May 30, 1974, as amended at 39 FR 37486, Oct. 22, 1974; 45 FR 64568, Sept. 30, 1980; 50 FR 1504, Jan. 11, 1985; 50 FR 19921, May 13, 1985]

#### § 455.15 Clavulanate potassium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Clavulanate potassium is the potassium salt of *Z*-(2*R*,5*R*)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylic acid. It is so purified and dried that:

(i) It is equivalent to not less than 755 micrograms and not more than 920 micrograms of clavulanic acid per milligram on an anhydrous basis.

(ii) Its moisture content is not more than 1.5 percent.

(iii) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 5.5 and not more than 8.0.

(iv) It gives a positive identity test.

(v) Its content of the potassium salt of [3*R*,5*S*]-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-3-carboxylic acid (clavam-2-carboxylate) is satisfactory if it is not greater than .01 percent.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, identity, and clavam-2-carboxylate content.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 12 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Clavulanic acid content*. Proceed as directed in § 436.351 of this chapter, using ambient temperature, an ultraviolet

detection system operating at a wavelength between 220 and 230 nanometers, and a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as octadecyl silane bonded silica. Reagents, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(a) *0.05M Sodium phosphate buffer solution, pH 4.4*. Transfer 7.8 grams of monobasic sodium phosphate to a 1-liter volumetric flask and dissolve in 900 milliliters of distilled water. Adjust the pH to  $4.4 \pm 0.1$  with 18N phosphoric acid or 10N sodium hydroxide. Dilute to volume with distilled water. Mix well.

(b) *Mobile phase*. Mix methanol: 0.05M sodium phosphate buffer, pH 4.4 (5:95 v/v) and stir or ultrasonicate for no less than 2 minutes. Degas by passing through a 0.5-micrometer filter with vacuum. The mobile phase may be sparged with helium through a 2-micrometer metal filter for the duration of the analysis. Adjust the ratio of methanol to aqueous buffer as necessary to obtain satisfactory retention of the peaks.

(ii) *Preparation of clavulanic acid working standard and sample solutions*. Accurately weigh and transfer into volumetric flasks sufficient clavulanic acid working standard or clavulanate potassium sample to obtain a final concentration of 250 micrograms per milliliter. To the clavulanic acid working standard, add sufficient amoxicillin trihydrate to provide a final concentration of 500 micrograms per milliliter. (The amoxicillin serves as an internal marker for system suitability testing.) Dissolve in water by shaking or ultrasonication until solution becomes clear. Dilute the solutions as required to final volume with water. Use within 8 hours.

(iii) *System suitability requirements*—(a) *Tailing factor*. The tailing factor ( $T$ ) is satisfactory if it is not more than 1.5.

(b) *Efficiency of the column*. The efficiency of the column ( $n$ ) is satisfactory if it is greater than 550 theoretical plates.

(c) *Resolution factor*. The resolution factor ( $R$ ) between the clavulanic acid

and amoxicillin peaks is satisfactory if it is not less than 3.5.

(d) *Coefficient of variation*. The coefficient of variation ( $S_R$  in percent) is satisfactory if it is not more than 2.0 percent.

If the system suitability requirements have been met, then proceed as described in §436.351(b) of this chapter.

(iv) *Calculations*. Calculate the micrograms of clavulanic acid per milligram of sample as follows:

$$\text{Micrograms of clavulanic acid per milligram} = \frac{A_u \times P_s \times W_s \times 100}{A_s \times W_u \times (100 - m)}$$

where:

$A_u$ =The clavulanic acid peak response in the chromatogram of the sample (at a retention time equal to that observed for the standard);

$A_s$ =The clavulanic acid peak response in the chromatogram of the clavulanic acid working standard);

$P_s$ =Potency of the clavulanic acid working standard in micrograms per milligram;

$W_u$ =Milligrams of sample;

$W_s$ =Milligrams of standard; and

$m$ =Percent moisture content of the sample.

(2) *Moisture*. Proceed as directed in §436.201 of this chapter.

(3) *pH*. Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(4) *Identity*. Proceed as directed in §436.211 of this chapter, using the sample preparation described in paragraph (b)(2) of that section.

(5) *Clavam-2-carboxylate content*. Proceed as directed in §436.352 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 210 nanometers, and a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing materials such as octadecyl silane bonded silica. Mobile phase, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Mobile phase*. *0.1M Sodium phosphate buffer solution, pH 4.0*. Prepare a 0.1M aqueous solution of monobasic sodium phosphate and adjust to pH 4.0 with phosphoric acid.

(ii) *Working standard and sample solutions*—(a) *Preparation of working standard solution*. Accurately weigh and

transfer into a 50-milliliter volumetric flask approximately 16 milligrams of clavam-2-carboxylate authentic sample. Dilute to volume and transfer 10 milliliters into a 100-milliliter flask. Dilute to volume with water.

(b) *Preparation of sample solution.* Accurately weigh 100 milligrams of the sample into a 10-milliliter flask. Dilute to volume with water.

(iii) *System suitability requirements—*

(a) *Tailing factor.* The tailing factor (*T*) for the clavulanate standard peak is satisfactory if it is not more than 1.5.

(b) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory if it is greater than 4,000 theoretical plates.

(c) *Resolution factor.* The resolution factor (*R*) between the clavulanic acid and clavam-2-carboxylic acid peaks is satisfactory if it is greater than 1.0.

(d) *Coefficient of variation (Relative standard deviation).* The coefficient of variation (*S<sub>r</sub>* in percent) is satisfactory if it is not more than 2.0 percent.

If the system suitability requirements have been met, then proceed as described in § 436.352(b) of this chapter.

(iv) *Calculations.* Calculate the percent of clavam-2-carboxylate content as follows:

$$\text{Percent clavam-2-carboxylate content} = \frac{\text{Mean sample height (or area)} \times \text{weight of standard} \times P}{\text{Mean peak height (or area) of standard} \times \text{weight of sample} \times 50}$$

where:

*P* = Percent clavam-2-carboxylic acid in the standard.

[49 FR 39674, Oct. 10, 1984, as amended at 55 FR 11584, Mar. 29, 1990]

#### § 455.15a Sterile clavulanate potassium.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Clavulanate potassium is the potassium salt of *Z*-(2*R*,5*R*)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylic acid. It is so purified and dried that:

(i) It is equivalent to not less than 755 micrograms and not more than 920 micrograms of clavulanic acid per milligram on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) Its moisture content is not more than 1.5 percent.

(v) Its pH of an aqueous solution containing 10 milligrams per milliliter is not less than 5.5 and not more than 8.0.

(vi) It gives a positive identity test.

(vii) Its [3*R*,5*S*]-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-3-carboxylic acid (clavam-2-carboxylate) content is satisfactory if it is not greater than .01 percent.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, identity, and clavam-2-carboxylate content.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 12 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay—(1) Clavulanic acid content.* Proceed as directed in § 455.15(b)(1) of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 10 milligrams per milliliter of clavulanate potassium.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution containing 10 milligrams per milliliter.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using the sample preparation described in paragraph (b)(2) of that section.

(7) *Clavam-2-carboxylate content.* Proceed as directed in § 455.15(b)(5) of this chapter.

[50 FR 33519, Aug. 20, 1985, as amended at 54 FR 11584, Mar. 29, 1990]

#### § 455.20 Cycloserine.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Cycloserine is a white to slightly yellowish compound. It has the