

paper. Use this solution to determine the resolution factor. The resolution (R) between the peaks for minocycline and epi-minocycline is satisfactory if it is not less than 2.0.

(d) *Coefficient of variation (relative standard deviation)*. The coefficient of variation (S_R in percent) of 5 replicate injections is satisfactory if it is not more than 2.0 percent.

(e) *Capacity factor (k)*. Calculate the capacity factor (k) for minocycline as follows:

$$k' = \frac{t_r - t_o}{t_o}$$

where:

t_r =Retention time of minocycline in minutes; and

t_o =Column dead time in minutes, which is estimated from the following equation:

$$t_o = \frac{(3.1416)(D^2)(L)(0.75)}{4F}$$

where:

D =Column diameter in centimeters;

L =Column length in centimeters;

0.75=Average total column porosity; and

F =Flow rate in milliliters per minute.

The capacity factor (k) for minocycline is satisfactory if it is not less than 6.2 and not more than 11.5.

If the system suitability requirements have been met, then proceed as described in §436.216(b) of this chapter. Alternate chromatographic conditions are acceptable provided reproducibility and resolution are comparable to the system. However, the sample preparation described in paragraph (b)(1)(ii)(b) of this section should not be changed.

(iv) *Calculations*—Calculate the micrograms of minocycline per milligram of sample as follows:

$$\frac{\text{Micrograms of minocycline}}{\text{per milligram}} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_u =Area of the minocycline peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s =Area of the minocycline peak in the chromatogram of the minocycline working standard;

P_s =Minocycline activity in the minocycline

working standard solution in micrograms per milliliter;
 C_u =Milligrams of minocycline sample per milliliter of sample solution; and
 m =Percent moisture content of the sample.

(2) [Reserved]

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

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

(5) *Epi-minocycline content*. Proceed as directed in paragraph (b)(1) of this section. Calculate the epi-minocycline content as follows:

$$\text{Percent Epi-minocycline} = \frac{(A_{epi}) \times 100}{(A_{total})}$$

where:

A_{epi} =Area of the epi-minocycline peak in the chromatogram of the sample; and

A_{total} =The sum of the areas of all the peaks eluting after the solvent front.

(6) *Identity*. Proceed as directed in §436.211 of this chapter, using a 0.5 percent potassium bromide disc prepared as described in paragraph (b)(1) of that section.

(7) *Crystallinity*. Proceed as directed in §436.203(a) of this chapter.

(8) *Residue on ignition*. Proceed as directed in §436.207(b) of this chapter.

(9) *Absorptivity*. Accurately weigh about 1 gram of sample into a 100-milliliter volumetric flask, dissolve, and dilute to mark with deionized water. Determine the absorbance of this solution on a suitable spectrophotometer at 560 nanometers (nm) using 5-centimeter cells with water in the reference cell. Calculate the absorptivity as follows:

$$\frac{\text{Absorptivity at 560 nm}}{\text{nm}} = \frac{(A_{560})(100)}{(\text{grams of sample})(1,000)(5)}$$

[39 FR 19076, May 30, 1974, as amended at 43 FR 11156, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978; 44 FR 22058, Apr. 13, 1979; 50 FR 19920, May 13, 1985; 53 FR 32607, Aug. 26, 1988; 53 FR 39839, Oct. 12, 1988; 54 FR 47205, Nov. 13, 1989]

§446.65 Oxytetracycline.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Oxytetracycline is [4S-(4 α ,4 α ,5 α ,5 α ,6 β ,12 α)]-4-(dimeth-

ylamino)-1,4,4a,5,5a,6,11,12a-octa-
hydro-3,5,6,10,12,12a-hexahydroxy-6-
methyl-1,11-dioxo-2-naphthacenecar-
boxamide dihydrate. Oxytetracycline is
produced by the growth of *Streptomyces*
rimosus. It is so purified and dried that:

(i) Its potency is not less than 832
micrograms of oxytetracycline per mil-
ligram on an "as is" basis.

(ii) [Reserved]

(iii) Its moisture content is not less
than 6 percent and not more than 9 per-
cent.

(iv) Its pH in an aqueous suspension
containing 10 milligrams per milliliter
is not less than 4.5 and not more than
7.0.

(v) When calculated on an anhydrous
basis its absorptivity at 353
nanometers relative to that of the oxy-
tetracycline working standard simi-
larly treated is 100±4 percent.

(vi) It gives a positive result to an
identity test for oxytetracycline.

(vii) It is crystalline.

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

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

(i) Results of tests and assays on the
batch for potency, moisture, pH, ab-
sorptivity, identity, and crystallinity.

(ii) Samples required: 10 packages,
each containing approximately 300 mil-
ligrams.

(b) *Tests and methods of assay*—(1) *Po-
tency*. Assay for potency by either of
the following methods; however, the re-

sults obtained from the micro-
biological turbidimetric assay shall be
conclusive.

(i) *Microbiological turbidimetric assay*.
Proceed as directed in § 436.106 of this
chapter, preparing the sample for assay
as follows: Dissolve an accurately
weighed sample in sufficient 0.1*N* hy-
drochloric acid to obtain a concentra-
tion of 1,000 micrograms of oxytetra-
cycline per milliliter (estimated). Fur-
ther dilute an aliquot of the stock solu-
tion with sterile distilled water to the
reference concentration of 0.24
microgram of oxytetracycline per mil-
liliter (estimated).

(ii) *Chemical assay*. Proceed as di-
rected in § 436.320 of this chapter.

(2) [Reserved]

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

(4) *pH*. Proceed as directed in § 436.202
of this chapter, using an aqueous sus-
pension containing 10 milligrams per
milliliter.

(5) *Absorptivity*. Determine the ab-
sorbance of the sample and standard
solutions in the following manner: Dis-
solve approximately 50 milligrams each
of the sample and standard in 250 milli-
liters of 0.1*N* hydrochloric acid. Trans-
fer a 10-milliliter aliquot to a 100-milli-
liter volumetric flask and dilute to vol-
ume with 0.1*N* hydrochloric acid. Using
a suitable spectrophotometer and 0.1*N*
hydrochloric acid as the blank, deter-
mine the absorbance of each solution
at 353 nanometers. Determine the per-
cent absorptivity of the sample rela-
tive to the absorptivity of the stand-
ard using the following calculations:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{Milligrams of standard}}{\text{Absorbance of standard} \times \text{Milligrams of sample}} \times \frac{\text{Potency of standard in micrograms per milligram}}{100 - m} \times \frac{10}{100 - m}$$

where: *m* = Percent moisture in the sample.

(6) *Identity*. To about 1 milligram of
sample, add 2 milliliters of sulfuric
acid; a light-red color is produced when
oxytetracycline is present.

(7) *Crystallinity*. Proceed as directed
in § 436.203(a) of this chapter.

[43 FR 11156, Mar. 17, 1978, as amended at 50
FR 19920, May 13, 1985]

§ 446.65a Sterile oxytetracycline.

(a) *Requirements for certification*—(1)
Standards of identity, strength, quality,