Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩**—**
USP Doxycycline Hyclate RS

Identification—To an accurately measured volume of Oral Suspension, equivalent to about 50 mg of doxycycline, add 50 mL of methanol, shake, and allow to settle. Using the clear supernatant as the *Test Solution*, proceed as directed for *Method II* under *Identification—Tetracyclines* ⟨193⟩.

Uniformity of dosage units ⟨905⟩**—**

FOR SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume ⟨698⟩: meets the requirements.

pH ⟨791⟩: between 6.5 and 8.0.

Assay—

Mobile phase, *Resolution solution*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Doxycycline Hyclate*.

Edetate diluent—Transfer 5.2 g of edetate disodium to a 500-mL volumetric flask, add about 400 mL of *Mobile phase*, sonicate for about 10 minutes or until dissolved, dilute with *Mobile phase* to volume, and mix.

Assay preparation—Constitute Oral Suspension as directed in the labeling. Transfer an accurately measured portion of the constituted oral suspension, freshly mixed and free from air bubbles, equivalent to about 100 mg of doxycycline, to a 100-mL volumetric flask, add 50 mL of *Edetate diluent*, sonicate for 15 minutes, and then shake by mechanical means for about 15 minutes. Dilute with *Edetate diluent* to volume, and mix. Pass through filter paper, discarding the first 10 mL of the filtrate, then pass through a filter having a 0.5- µm or finer porosity.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Doxycycline Hyclate*. Calculate the quantity, in mg, of doxycycline ($C_{22}H_{24}N_2O_8$) in each mL of the Oral Suspension taken by the formula:

$$0.1(CP / V)(r_U / r_S)$$

in which V is the volume, in mL, of Oral Suspension taken to prepare the *Assay preparation;* and the other terms are as defined therein.

Doxycycline Hyclate

$(C_{22}H_{24}N_2O_8 \cdot HCl)_2 \cdot C_2H_6O \cdot H_2O$ 1025.89

2-Naphthacenecarboxamide, 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-, monohydrochloride, compd. with ethanol (2:1), monohydrate, [4 *S*-(4α,4aα,5α,5aα,6α,12aα)]-.

4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride, compound with ethyl alcohol (2:1), monohydrate [24390-14-5].

» Doxycycline Hyclate has a potency equivalent to not less than 800 µg and not more than 920 µg of doxycycline ($C_{22}H_{24}N_2O_8$) per mg.

Packaging and storage—Preserve in tight containers, protected from light.

Labeling—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

USP Reference standards ⟨11⟩**—**
USP Doxycycline Hyclate RS
USP Endotoxin RS
USP Methacycline Hydrochloride RS

Identification, *Infrared Absorption* ⟨197K⟩

Crystallinity ⟨695⟩**:** meets the requirements.

pH ⟨791⟩**:** between 2.0 and 3.0, in a solution containing 10 mg of doxycycline per mL.

Water, *Method I* ⟨921⟩**:** between 1.4% and 2.8%.

Related compounds—

Mobile phase and *Diluent*—Prepare as directed in the *Assay*.

System suitability solution—Prepare as directed for *Resolution solution* in the *Assay*.

Methacycline standard stock solution—Dissolve an accurately weighed quantity of USP Methacycline Hydrochloride RS in *Diluent*, and dilute quantitatively, and stepwise if necessar y, to obtain a solution having a known concentration of about 1.2 mg per mL.

Standard solution 1—Prepare as directed for the *Standard preparation* in the *Assay*.

Standard solution 2—Transfer 2.0 mL of *Standard solution 1* and 2.0 mL of the *Methacycline standard stock solution* to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. This solution contains about 0.024 mg each of USP Doxycycline Hyclate RS and USP Methacycline Hydrochloride RS per mL.

Test solution—Use the *Assay preparation*, prepared as directed in the *Assay*.

Chromatographic system (see *Chromatography* ⟨621⟩)— Prepare as directed in the *Assay*. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.4 for 4-epidoxycycline (the main degradation product), 0.6 for methacycline, 0.7 for 6-epidoxycycline, and 1.0 for doxycycline; the resolution, *R*, between 4-epidoxycycline and doxycycline is not less than 3.0; and the tailing factor is not more than 2.0. Chromatograph *Standard solution 1*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of *Standard solution 2* and the *Test solution* into the chromatograph, record the chromatograms for a period of time that is 1.7 times the retention time of doxycycline, and measure the peak areas. Calculate the per centage of methacycline in the portion of Doxycycline Hyclate taken by the formula:

$$10{,}000(C_M / W)(r_U / r_M)$$

in which C_M is the concentration, in mg per mL, of USP Methacycline Hydrochloride RS in *Standard solution 2; W* is the weight, in mg, of Doxycycline Hyclate taken to prepare the *Test solution;* and r_U and r_M are the methacycline peak responses obtained from the *Test solution* and *Standard solution 2*, respectively. Not more than 2% of methacycline is found. Calculate the percentage of each related compound, other than methacycline, in the portion of Doxycycline Hyclate taken by the formula:

$$10{,}000(C_S / W)(r_i / r_S)$$

in which C_S is the concentration, in mg per mL, of USP Doxycycline Hyclate RS in *Standard solution 2; W* is the weight, in mg, of Doxycycline Hyclate taken to prepare the *Test solution;* r_i is the peak response for each impurity obtained from the *Test solution;* and r_S is the doxycycline peak response obtained from *Standard solution 2*. Not more than 0.5% of any impurity eluting before methacycline is found; not more than 2% of 6-epidoxycycline is found; and not more than 0.5% of any impurity eluting after the main doxycycline peak is found.

Other requirements—Where the label states that Doxycycline Hyclate is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Doxycycline for Injection*. Where the label states that Doxycycline Hyclate must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Doxycycline for Injection*.

Assay—

Mobile phase—Transfer 2.72 g of monobasic potassium phosphate, 0.74 g of sodium hydroxide, 0.50 g of tetrabutylammonium hydrogen sulfate, and 0.40 g of edetate disodium to a 1000-mL volumetric flask. Add about 850 mL of water, and stir to dissolve. Add 60 g of tertiary butyl alcohol with the aid of water, dilute with water to volume, and adjust with 1 N sodium hydroxide to a pH of 8.0 ± 0.1. Pass this solution through a filter having a porosity of 0.5 µm or finer, and degas before using. Make any necessary adjustments (see *System Suitability* under *Chromatography* ⟨621⟩). Decreasing the proportion of tertiary butyl alcohol results in a longer retention time of doxycycline and improved separation of doxycycline from the related compounds.

Diluent—Use 0.01 N hydrochloric acid.

Resolution solution—Prepare a solution of USP Doxycycline Hyclate RS in *Diluent* containing about 6 mg of doxycycline per mL. Transfer 5 mL of this solution to a 25-mL volumetric flask, heat on a steam bath for 60 minutes, and evaporate to dryness on a hot plate, taking care not to char the residue. Dissolve the residue in 0.01 N hydrochloric acid, dilute with *Diluent* to volume, and mix. Pass a portion of this solution through a filter having a porosity of 0.5 µm or finer, and use the filtrate as the *Resolution solution*. This solution contains a mixture of 4-epidoxycycline, 6-epidoxycycline, and doxycycline. When stored in a refrigerator, this solution may be used for 14 days. [NOTE—Throughout the following sections, protect the *Standard preparation* and the *Assay preparation* from light.]

Standard preparation—Transfer about 12 mg of USP Doxycycline Hyclate RS, accurately weighed, to a 10-mL volumetric flask, add about 6 mL of *Diluent*, sonicate for about 5 minutes or until dissolved, dilute with *Diluent* to volume, and mix.

Assay preparation—Transfer about 120 mg of Doxycycline Hyclate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix. Pass through a membrane filter having a porosity of 0.5 µm or finer.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 270 nm detector, and a 4.6-mm × 25-cm column that contains packing L21 and is maintained at 60 ± 1°. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.4 for 4-epidoxycycline (the main degradation product), 0.7 for 6-epidoxycycline, and 1.0 for doxycycline, the resolution, *R,* between the 4-epidoxycycline peak and the doxycycline peak is not less than 3.0, and the tailing factor for the doxycycline peak is not more than 2.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms for a period of time that is 1.7 times the retention time of doxycycline, and measure the responses for the major peaks. Calculate the potency, in µg of doxycycline ($C_{22}H_{24}N_2O_8$) per mg, of the Doxycycline Hyclate taken by the formula:

$$100(CP / W)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Doxycycline Hyclate RS in the *Standard preparation; P* is the designated potency, in µg of doxycycline ($C_{22}H_{24}N_2O_8$) per mg, of USP Doxycycline Hyclate RS; W is the quantity, in mg, of Doxycycline Hyclate taken to prepare the *Assay preparation;* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Doxycycline Hyclate Capsules

» Doxycycline Hyclate Capsules contain the equivalent of not less than 90.0 per cent and not more than 120.0 per cent of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$).

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩**—**
USP Doxycycline Hyclate RS

Identification—Shake a suitable quantity of Capsule contents with methanol to obtain a solution containing the equivalent of 1 mg of doxycycline per mL, and filter. Using the filtrate as the *Test Solution*, proceed as directed for *Method II* under *Identification—Tetracyclines* ⟨193⟩.

Dissolution ⟨711⟩**—**

Medium: water; 900 mL.

Apparatus 2: 75 rpm, the distance between the blade and the inside bottom of the flask being maintained at 4.5 ± 0.5 cm during the test.

Time: 30 minutes.

Procedure—Determine the amount of $C_{22}H_{24}N_2O_8$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 276 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Doxycycline Hyclate RS in the same medium.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{22}H_{24}N_2O_8$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Water, *Method I* ⟨921⟩: not more than 8.5%.

Assay—

Mobile phase, *Diluent*, *Resolution solution*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Doxycycline Hyclate*.

Assay preparation—Remove, as completely as possible, the contents of not less than 20 Capsules, and weigh accurately. Mix the combined contents, and transfer an accurately weighed portion of the powder, equivalent to about 100 mg of doxycycline, to a 100-mL volumetric flask, add about 75 mL of *Diluent*, sonicate for 5 minutes, shake for 15 minutes, dilute with *Diluent* to volume, and mix. Filter through a membrane filter of 0.5 µm or finer porosity.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Doxycycline Hyclate*. Calculate the quantity, in mg, of doxycycline ($C_{22}H_{24}N_2O_8$) in the portion of Capsules taken by the formula:

$$0.1CP(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Doxycycline Hyclate RS in the *Standard preparation, P* is the designated potency, in µg of doxycycline per mg, of USP Doxycycline Hyclate RS, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Doxycycline Hyclate Delayed-Release Capsules

» Doxycycline Hyclate Delayed-Release Capsules contain the equivalent of not less than 90.0 percent and not more than 120.0 per cent of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$).