Add the following:

■Doxycycline Hyclate Delayed-Release Tablets

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

Packaging and storage—Preserve in tight, light-resistant containers. Store at controlled room temperature.

USP Reference standards ⟨11⟩**—***USP Doxycycline Hyclate RS. USP Methacycline Hydrochloride RS. USP Doxycycline Related Compound A RS. USP Oxytetracycline Hydrochloride RS.*

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Change to read:

Dissolution, *Method B* ⟨711⟩**—**

ACID STAGE—

Medium: 0.06 N hydrochloric acid; 900 mL, degassed with helium.

Apparatus 1: 50 rpm.

Time: 20 minutes.

Standard solution—Transfer an accurately weighed quantity of USP Doxycycline Hyclate RS to a suitable volumetric flask, add *Medium* to about 50% of the final volume, sonicate until dissolved, dilute with *Medium* to volume, and mix, to obtain a solution having a known concentration of about 1.28 mg of doxycycline hyclate per mL. Transfer 20.0 mL of this solution to a 200-mL volumetric flask, and dilute with *Medium* to volume. Calculate the concentration, C_S, in mg of doxycycline per mL, using the designated potency, in µg of doxycycline per mg of USP Doxycycline Hyclate RS.

Test solution—Use portions of the solution under test passed through a suitable 0.45-µm PVDF filter.

Procedure—Determine the amount of $C_{22}H_{24}N_2O_8$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 346 nm in the *Test solution* in comparison with the *Standard solution,* using a 0.1-cm quartz cell and *Medium* as the blank. Calculate the amount of $C_{22}H_{24}N_2O_8$ dissolved by the formula:

$$\frac{A_U \times 900 \times 100}{A_S \times L}$$

in which A_U and A_S are the absorbances obtained from the *Test solution* and *Standard solution,* respectively; C_S is the concentration, in mg of doxycycline per mL, of the *Standard solution;* 900 is the volume, in mL, of *Medium;* 100 is the conversion factor to percentage; and L is the Tablet label claim, in mg.

Tolerances—•Level 1 (6 Tablets tested): No individual value is more than 30% of the labeled amount of $C_{22}H_{24}N_2O_8$ dissolved in 20 minutes. Level 2 (6 Tablets tested): not more than 2 individual values of the 12 tested are greater than 30% of the labeled amount of $C_{22}H_{24}N_2O_8$ in 20 minutes.• (RB 1-Aug-2009)

BUFFER STAGE—[NOTE—Conduct this stage of testing on separate Tablets, selecting those that were not previously subjected to the *Acid stage* testing.]

Medium: pH 5.5 neutralized phthalate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL, degassed.

Apparatus 1: 50 rpm.

Time: 30 minutes.

Standard solution—Transfer an accurately weighed quantity of USP Doxycycline Hyclate RS to a suitable volumetric flask, add *Medium* to about 50% of the final volume, sonicate until dissolved, dilute with *Medium* to volume, and mix, to obtain a solution having a known concentration of about 1.28 mg of doxycycline hyclate per mL. Transfer 20.0 mL of this solution to a 200-mL volumetric flask, and dilute with *Medium* to volume. Calculate the concentration, C_S, in mg of doxycycline per mL, using the designated potency, in µg of doxycycline per mg of USP Doxycycline Hyclate RS.

Test solution—Use portions of the solution under test passed through a suitable 0.45-µm PVDF filter.

Procedure—Determine the amount of $C_{22}H_{24}N_2O_8$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 346 nm in the *Test solution* in comparison with the *Standard solution,* using a 0.1-cm quartz cell and *Medium* as the blank. Calculate the amount of $C_{22}H_{24}N_2O_8$ dissolved by the formula:

$$\frac{A_U \times 900 \times 100}{A_S \times L}$$

in which A_U and A_S are the absorbances obtained from the *Test solution* and *Standard solution*, respectively; C_S is the concentration, in mg of doxycycline per mL, of the *Standard solution;* 900 is the volume, in mL, of *Medium*; 100 is the conversion factor to percentage; and L is the Tablet label claim, in mg.

Tolerances—Not less than 85% *(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 Ia* ⟨921⟩**:**not more than 5.0%.

Related compounds—[NOTE—Throughout the following procedure, protect the solution containing doxycycline from light.]

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

Standard stock solution—Transfer an accurately weighed quantity of USP Doxycycline Hyclate RS to a suitable volumetric flask, add methanol to about 10% of the final volume, sonicate for about 5 minutes or until dissolved, dilute with water to volume, and mix, to obtain a solution having a known concentration of about 1.16 mg of doxycycline hyclate per mL. Calculate the concentration, in mg of doxycycline per mL, using the designated potency, in µg of doxycycline per mg of USP Doxycycline Hyclate RS.

Standard solution—Pipet 5.0 mL of the *Standard stock solution* into a 250-mL volumetric flask, and dilute with water to volume, to obtain a solution having a known concentration of about 0.02 mg of doxycycline per mL.

Sensitivity solution—Pipet 5.0 mL of the *Standard solution* into a 100-mL flask, and dilute with water to volume.

Impurity stock solution—Prepare a solution in water containing about 0.04 mg per mL of each of USP Oxytetracycline Hydrochloride RS, USP Methacycline Hydrochloride RS, and USP Doxycycline Related Compound A RS.

Resolution solution—Transfer 5 mL of the *Standard stock solution* into a 25-mL volumetric flask. Heat on a steam bath for 60 minutes, and gently evaporate to dryness on a hot plate (partial degradation of doxycycline to 4-epidoxycycline). Add 3 mL of the *Impurity stock solution* to the flask, dilute with water to volume, and mix. Pass through a 0.45-µm PVDF filter, and use the clear filtrate.

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 *Standard solution,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 5.0%. Chromatograph the *Resolution solution,* identify the components using their relative retention time in *Table 1,* and record the peak responses as directed for *Procedure:* the resolution between doxycycline and 6-epidoxycycline is not less than 1.5. Chromatograph the *Sensitivity solution,* and record the peak responses as directed for *Procedure:* the signal-to-noise ratio of the doxycycline peak is not less than 10.

Table 1

Name	Relative Retention Time	Relative Response Factor (*F*)	Limit (%)
Oxytetracycline	0.3	1.0	0.5
4-Epidoxycycline[1]	0.4	1.0	1.0
Methacycline	0.6	1.0	2.0
6-Epidoxycycline (doxycycline related compound A)[2]	0.7	0.86	2.0
Doxycycline	1.0	—	—

[1] 4*R*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(Dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide.
[2] 4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide.

Procedure—Separately inject equal volumes (about 15 µL) of the *Standard solution* 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 percentage of each impurity relative to the labeled amount of doxycycline in the portion of the Tablets taken by the formula:

$$100\ (1 / F)(C_S / C_T)(r_i / r_S)$$

in which F is the relative response factor as listed in *Table 1;* C_S is the concentration, in mg of doxycycline per mL, of the *Standard solution;* C_T is the concentration, in mg per mL, of doxycycline in the *Test solution,* based on the label claim; r_i is the peak response for each impurity obtained from the *Test solution;* and r_S is the peak response for the doxycycline peak obtained from the *Standard solution.*

Assay—[NOTE—Throughout the following procedure, protect the *Standard preparation* and the *Assay preparation* from light.]

Mobile phase—Transfer 0.77 g of ammonium acetate, 0.75 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 70 g of tertiary butyl alcohol with the aid of water, dilute with water to volume, and adjust the pH to 9.00 ± 0.05 with acetic acid or ammonium hydroxide. Pass this solution through a 0.45-µm nylon filter, and degas. Make any necessary adjustments (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Transfer an accurately weighed amount of USP Doxycycline Hyclate RS to a suitable volumetric flask, add methanol to about 10% of the final volume, sonicate for about 5 minutes or until dissolved, dilute with water to volume, and mix, to obtain a solution having a known concentration of about 1.16 mg of doxycycline hyclate per mL. Calculate the concentration, C_S, in mg of doxycycline per mL, using the designated potency, in µg of doxycycline per mg of USP Doxycycline Hyclate RS.

Assay preparation—Weigh and crush not fewer than 10 Tablets, placing no more than 2 Tablets at a time in a suitable mortar, and grind to a fine powder with a pestle. Mix the combined powder, and transfer an accurately weighed portion, equivalent to about 100 mg of doxycycline, to a 100-mL volumetric flask. Add 10 mL of methanol, and sonicate for 5 minutes. Add about 50 mL of water, and sonicate for a further 5 minutes. Allow to cool to room temperature, dilute with water to volume, and mix. Pass a portion of this solution through a 0.45-µm PVDF filter, and use the clear filtrate.

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 52 ± 2°. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2.0; and the relative standard deviation for six replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 15 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 percentage of the labeled amount of doxycycline ($C_{22}H_{24}N_2O_8$) in the portion of Tablets taken by the formula:

$$100(C_S / C_T)(r_U / r_S)$$

in which C_S is as defined above; C_T is the concentration, in mg per mL, of doxycycline in the *Assay preparation,* based on the label claim; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.■1S *(USP32)*