

Ethinyl Estradiol Tablets

» Ethinyl Estradiol Tablets contain not less than 90.0 percent and not more than 115.0 percent of the labeled amount of $C_{20}H_{24}O_2$.

Packaging and storage—Preserve in well-closed containers.

Add the following:

• **Labeling**—When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used. • (RB 1-Jul-2009)

Change to read:

USP Reference standards <11>—*USP Ethinyl Estradiol RS*. • *USP Norgestrel RS*. • (RB 1-Jul-2009)

Thin-layer chromatographic identification test

 <201>—

Test solution—Transfer 25 Tablets to a suitable container, add 50 mL of water, and sonicate until the Tablets disintegrate (if needed, remove any coating with water before sonication). Place the sample in a separatory funnel, add 25 mL of ether, and shake well to extract the actives. Using a glass pipet, transfer the ether layer to a clean beaker, and evaporate to about 10 mL.

Standard solution—Dissolve an accurately weighed quantity of USP Ethinyl Estradiol RS in methanol to obtain a solution containing about 0.03 mg per mL.

Application volume: 30 μ L.

Developing solvent system: a mixture of chloroform and alcohol (96 : 4).

Procedure—Proceed as directed in the chapter, and then air-dry. Spray the plate with a mixture of methanol and sulfuric acid (50 : 50), place in an oven at 105° for about 5 minutes, and examine the plate: meet the requirements.

Add the following:

• **Dissolution** <711>—[NOTE—Care must be taken not to expose any of the solutions to plastic or rubber. Fluorescent material will leach into the solutions and interfere with the quantitation of ethinyl estradiol. Also, adsorption may occur.]

TEST 1—

Medium: 0.3% sodium lauryl sulfate in water; 500 mL, degassed.

Apparatus 2: 100 rpm.

Time: 30 minutes.

pH 6.0 phosphate buffer—Transfer about 2.7 g of monobasic potassium phosphate to a 1-L volumetric flask. Dissolve in about 900 mL of water. Adjust with 1 N sodium hydroxide to a pH of 6.0, and dilute with water to volume.

Mobile phase—Prepare a filtered and degassed mixture of pH 6.0 phosphate buffer and acetonitrile (1 : 1). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

Standard stock solution—Dissolve an accurately weighed quantity of USP Ethinyl Estradiol RS in methanol, and dilute quantitatively, and stepwise, if necessary, with methanol to obtain a solution having a concentration of about 0.25 mg per mL. This solution is stable for 14 days.

Working standard solution—Dilute the *Standard stock solution* with *Medium* to obtain a solution having a concentration of about 0.06 μ g per mL. Add 1 or 2 drops of methanol to dissipate the bubbles if necessary. This solution is stable for 24 hours.

Test solution—Centrifuge the solution under test for 10 minutes at 2000 rpm. Use the supernatant.

Chromatographic system—The liquid chromatograph is equipped with a fluorescence detector with excitation at 285 nm and emission at 310 nm, a 4.6-mm \times 1.25-cm guard column containing 5- μ m packing L11, and a 4.6-mm \times 15-cm analytical column containing 5- μ m packing L11. The flow rate is about 2.0 mL per minute. Chromatograph the *Working standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 3.0%.

Procedure—Separately inject equal volumes (about 200 μ L) of the *Working standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of ethinyl estradiol dissolved by the formula:

$$\frac{r_U \times C_S \times 500 \times 100}{r_S \times L}$$

in which r_U and r_S are the peak responses obtained from the *Test solution* and the *Working standard solution*, respectively; C_S is the concentration of the *Working standard solution*, in μ g per mL; 500 is the volume of *Medium*; 100 is the conversion factor to percentage; and L is the Tablet label claim, in μ g.

Tolerances—Not less than 80% (Q) of the labeled amount of ethinyl estradiol is dissolved in 30 minutes.

TEST 2—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 5 ppm of polysorbate 80 in water; 500 mL, deaerated with helium.

Apparatus 2: 75 rpm.

Time: 45 minutes.

Standard stock solution—Accurately transfer about 10 mg of USP Ethinyl Estradiol RS and 50 mg of USP Norgestrel RS to a 500-mL volumetric flask. Add 250 mL of acetonitrile, and sonicate until dissolved. Cool to room temperature, and dilute with water to volume. The final concentration is about 20 μ g per mL of ethinyl estradiol and 100 μ g per mL of norgestrel. This solution is stable for 15 days.

Working standard solution—Dilute the *Standard stock solution* quantitatively, and stepwise, with *Medium* to obtain a solution with a final concentration of about 0.02 μ g per mL of ethinyl estradiol. This solution is stable for 6 days.

Test solution—Centrifuge the solution under test at about 3000 rpm for 20 minutes. Use the supernatant. This solution is stable for 12 hours.

Mobile phase—Prepare a filtered and degassed mixture of water, acetonitrile, and methanol (55 : 40 : 5). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

Chromatographic system—The liquid chromatograph is equipped with a 200-nm detector and a 4.6-mm \times 10-cm column that contains 3- μ m packing L1. The column is maintained at 30°. The flow rate is about 1.2 mL per minute. Chromatograph the *Working standard solution*, and record the peak responses as directed for *Procedure*: the resolution between the ethinyl estradiol and norgestrel peaks is not less than 6.0, and the relative standard deviation for ethinyl estradiol is not more than 3.0%.

Procedure—Separately inject equal volumes (about 200 μ L) of the *Working standard solution* and *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of ethinyl estradiol dissolved by the formula:

$$\frac{r_U \times C_S \times 500 \times 100}{r_S \times L}$$

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in which r_U and r_S are the peak responses obtained from the *Test solution* and the *Working standard solution*, respectively; C_S is the concentration of the *Working standard solution*, in μg per mL; 500 is the volume of *Medium*; 100 is the conversion factor to percentage; and L is the Tablet label claim, in μg .

Tolerances—Not less than 80% (Q) of the labeled amount of ethinyl estradiol is dissolved in 45 minutes. (RB 1-Jul-2009)

Related compounds—

Solution A: acetonitrile and 20 mM potassium phosphate buffer, pH 6.0 (50 : 50).

Solution B: acetonitrile and 20 mM potassium phosphate buffer, pH 6.0 (80 : 20).

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Diluent—Prepare a mixture of acetonitrile and water (50 : 50).

Standard stock solution—Dissolve an accurately weighed quantity of USP Ethinyl Estradiol RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.3 mg per mL.

Standard solution—Quantitatively dilute portions of *Standard stock solution* with *Diluent* to obtain a solution containing about 0.12 μg per mL of USP Ethinyl Estradiol RS.

Test solution 1—Transfer 20 Tablets into a 200-mL volumetric flask. Add about 120 mL of *Diluent*, and shake for about 30 minutes. Dilute with *Diluent* to volume, and mix. Centrifuge a portion of the dissolution sample, and use the clear supernatant.

Test solution 2—Dilute a portion of *Test solution 1* with *Diluent* to obtain a solution containing about 0.6 μg per mL of ethinyl estradiol.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 210-nm UV detector and a spectrofluorometric detector with an excitation wavelength of 285 nm and an emission wavelength of 310 nm, a 4.6-mm \times 15-cm column that contains packing L11, and a 4.6-mm \times 12.5-mm guard column that contains packing L11. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution	Flow rate (mL/min)
0–20	100	0	equilibration/ isocratic	2
20–25	100→0	0→100	linear gradient	2.5
25–30	0	100	isocratic	3
30–32	0→100	100→0	linear gradient	2
32–35	100	0	re-equilibration	2

Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.0 for ethinyl estradiol; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Inject a volume (about 200 μL) of *Test solution 2* into the chromatograph, record the chromatograms, and measure the peak heights for the major peaks obtained within 20 minutes. Calculate the percentage of 17 β -ethinyl estradiol in the portion of Tablets taken by the formula:

$$100(r_U / r_S)$$

in which r_U is the height of any peak at the relative retention time of 1.16; and r_S is the peak height of ethinyl estradiol obtained with the spectrofluorometric detector. Inject a volume (about 200 μL) of

Test solution 1 into the chromatograph, record the chromatograms, and measure the peak heights for the major peaks obtained within 20 minutes. Calculate the percentage of estrone in the portion of Tablets taken by the formula:

$$100(r_U / r_S) - E$$

in which r_U is the height of any peak at the relative retention time of 1.2; r_S is the peak height of ethinyl estradiol obtained with the UV detector at 210 nm; and E is the percentage of 17 β -ethinyl estradiol obtained in the Tablets. Calculate the percentage of any other impurity taken by the formula:

$$100(r_U / r_S)$$

in which r_U is the height of any peak other than those mentioned above; and r_S is the peak height of ethinyl estradiol obtained with the UV detector. Not more than 0.5% of 17 β -ethinyl estradiol is found; not more than 0.5% of estrone is found; not more than 0.5% of any unknown impurity is found; and not more than 2.0% of total impurities is found.

Uniformity of dosage units (905): meet the requirements.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and 20 mM potassium phosphate buffer, pH 6.0 (50 : 50). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Diluent—Prepare a mixture of acetonitrile and water (50 : 50).

Standard stock solution—Dissolve an accurately weighed quantity of USP Ethinyl Estradiol RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.3 mg per mL.

Standard preparation—Dilute an appropriate aliquot of the *Standard stock solution* with *Diluent* to obtain a solution having a known concentration of about 0.12 μg per mL of USP Ethinyl Estradiol RS.

Assay preparation—Transfer 20 Tablets into a 200-mL volumetric flask. Add about 120 mL of *Diluent*, and shake for about 30 minutes. Dilute with *Diluent* to volume, and mix. Centrifuge a portion of the solution so obtained, and transfer to a 50-mL volumetric flask an accurately measured volume having a final concentration of about 0.12 μg per mL of ethinyl estradiol. Dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a spectrofluorometric detector with an excitation wavelength of 285 nm and an emission wavelength of 310 nm, a 4.6-mm \times 15-cm column that contains packing L11, and a 4.6-mm \times 1.25-mm guard column that contains packing L11. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for ethinyl estradiol is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 200 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $\text{C}_{20}\text{H}_{24}\text{O}_2$ in the portion of Tablets taken by the formula:

$$(1000C / V)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Ethinyl Estradiol RS in the *Standard preparation*; V is the volume of the aliquot of solution taken for the *Assay preparation*; and r_U and r_S are the peak responses obtained for ethinyl estradiol from the *Assay preparation* and the *Standard preparation*, respectively.