this test solution and 10 µL each of solutions containing, respectively, about 1 mg per mL of USP Norethindrone RS in alcohol and about 50 µg per mL of USP Mestranol RS in alcohol at equidistant points along a line about 2.5 cm from the bottom of a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel and previously activated by heating at 105° for 30 minutes. Develop the chromatogram in a mixture of equal volumes of ethyl acetate and cyclohexane in a suitable chamber, previously equilibrated with the solvent mixture, until the solvent front has moved about three-fourths of the length of the plate. Remove the plate, air-dry, and observe under short-wavelength UV light: the principal spot from the test solution appears at the same R_F value as the principal spot from USP Norethindrone RS, at about R_F 0.6. Spray the plate with a sulfuric acid and methanol mixture prepared by cautiously adding and mixing sulfuric acid in small increments to 30 mL of chilled anhydrous methanol in a 100-mL volumetric flask. Adjust to room temperature, dilute with sulfuric acid to volume, and mix. Heat the plate at 105° for 10 minutes: the pink spot from the test solution appears at the same R_F value as the pink spot from USP Mestranol RS (about R_F 0.8).

Dissolution ⟨711⟩**—**[NOTE—Exercise care in filtering solutions containing mestranol to prevent adsorptive loss of the drug. Centrifugation may be used instead of filtration with nonadsorptive membrane filters. Withdraw dissolution aliquots with glass or polytef pipets or syringes that have been checked for adsorptive loss. Use glass dissolution vessels and polytef-coated or solid polytef paddles.]

Medium: 0.09% sodium lauryl sulfate in 0.1 N hydrochloric acid; 500 mL.

Apparatus 2: 75 rpm.

Time: 60 minutes.

Determine the amounts of norethindrone ($C_{20}H_{26}O_2$) and mestranol ($C_{21}H_{26}O_2$) dissolved, employing the following method.

Mobile phase—Prepare a degassed and filtered mixture of water and acetonitrile (60:40). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 205-nm detector and a 4.6-mm × 25-cm column that contains packing L10. The flow rate is about 1 mL per minute. Chromatograph replicate injections of a filtered portion of a Standard solution of USP Norethindrone RS and USP Mestranol RS in *Dissolution Medium* having known concentrations similar to those expected in the solution under test, and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 3.0%. The minimum number of theoretical plates for the mestranol peak is 4000, and the tailing factors for the norethindrone and mestranol peaks do not exceed 1.5.

Procedure—Separately inject equal volumes (about 200 µL) of the Standard solution and a filtered portion of the solution under test into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.4 for norethindrone and 1.0 for mestranol. Calculate the quantities of norethindrone and mestranol dissolved by comparison of the corresponding peak responses obtained from the Standard solution and the test solutions.

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{20}H_{26}O_2$ and 75% *(Q)* of the labeled amount of $C_{21}H_{26}O_2$ are dissolved in 60 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements for *Content Uniformity* with respect to norethindrone and to mestranol.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and water (50:50). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Transfer about 80 mg of progesterone into a 100-mL volumetric flask, add 50 mL of acetonitrile, dilute with water to volume, and mix.

Mestranol standard stock solution—Dissolve an accurately weighed quantity of USP Mestranol RS in acetonitrile, and dilute quantitatively and stepwise with acetonitrile to obtain a solution having a known concentration of about 0.05 mg per mL.

Norethindrone standard stock solution—Using an accurately weighed quantity of USP Norethindrone RS, prepare a solution in acetonitrile having a known concentration of about 1 mg per mL.

Standard preparation—Transfer 2.0 mL of *Internal standard solution* into a 100-mL volumetric flask. Add accurately measured volumes of *Mestranol standard stock solution* and *Norethindrone standard stock solution* so that the final known concentrations, in mg per mL, of the Reference Standards correspond numerically to about one-fiftieth of the labeled amounts of the corresponding ingredients in the Tablets. Add 50 mL of water, dilute with acetonitrile to volume, and mix.

Assay preparation—Transfer 10 Tablets to a 250-mL volumetric flask, add 50 mL of water, and shake by mechanical means until the Tablets are completely disintegrated. Add 10.0 mL of *Internal standard solution* and 165 mL of acetonitrile, and mix. Sonicate for about 2 minutes. Dilute with acetonitrile to volume, and mix. Allow solid particles to settle, or centrifuge if necessary, to obtain a slightly turbid solution. Transfer 5.0 mL of this solution to a 10-mL volumetric flask, add 1.0 mL of acetonitrile, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 200-nm detector and a 4.6-mm × 15-cm column that contains packing L7. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the column efficiency determined from the mestranol peak is not less than 6000 theoretical plates, the resolution, *R,* between the progesterone and mestranol peaks is not less than 5.0, and the relative standard deviation for six replicate injections is not more than 2.0% (both peaks).

Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 2.5 for mestranol and 1.0 for norethindrone. Calculate the quantities, in mg, of norethindrone ($C_{20}H_{26}O_2$) and mestranol ($C_{21}H_{26}O_2$) in each Tablet taken by the formula:

$$50C(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*, and R_U and R_S are the peak response ratios, at corresponding retention times, obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Norethindrone Acetate

$C_{22}H_{28}O_3$ 340.46

19-Norpregn-4-en-20-yn-3-one, 17-(acetyloxy)-, (17α).

17-Hydroxy-19-nor-17α-pregn-4-en-20-yn-3-one acetate [51-98-9].

» Norethindrone Acetate contains not less than 97.0 percent and not more than 103.0 percent of $C_{22}H_{28}O_3$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩**—**
USP Norethindrone Acetate RS

Completeness of solution—The solution prepared for the determination of *Specific rotation* is clear and free from undissolved solids.

Identification, *Infrared Absorption* ⟨197K⟩.

Specific rotation ⟨781S⟩: between –32° and –38°.

Test solution: 20 mg per mL, in dioxane.

Loss on drying ⟨731⟩**—**Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

Limit of ethynyl group—Proceed as directed in the test for *Ethynyl group* under *Norethindrone*. Not less than 7.13% and not more than 7.57% of ethynyl group is found.

Chromatographic purity—

TEST 1—

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture.

Test solution—Prepare a solution of Norethindrone Acetate in chloroform having a concentration of 10 mg per mL.

Standard stock solution—Prepare a solution of USP Norethindrone Acetate RS in chloroform having a known concentration of 10 mg per mL.

Standard solutions—Dilute accurately measured volumes of the *Standard stock solution* with chloroform to obtain *Standard solutions A, B, C,* and *D* having known concentrations of 150 µg per mL, 50 µg per mL, 30 µg per mL, and 10 µg per mL, respectively.

Application volume: 10 µL, as two 5-µL portions.

Developing solvent system: a mixture of toluene and ethyl acetate (1:1).

Procedure—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* ⟨621⟩, except to apply the solutions along a line 2.5 cm from the edge of the plate. Spray the plate with a mixture of methanol and sulfuric acid (7:3), and heat at 100° for 5 minutes. The *Test solution* exhibits a principal spot at the same R_F value as the principal spot of *Standard solution A.* Any individual secondary spot is not more intense than the spot in the chromatogram obtained from *Standard solution B:* not more than 0.5% of any individual impurity is found, and the total of impurities found is not more than 1.5%.

TEST 2 —

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and water (6:4). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Resolution solution—Dissolve accurately weighed quantities of desoxycorticosterone acetate and USP Norethindrone Acetate RS in *Mobile phase* to obtain a solution having concentrations of about 80 µg of each per mL.

Test solution—Transfer about 62.5 mg of Norethindrone Acetate, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

Diluted test solution—Transfer 1.0 mL of the *Test solution* to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. 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 0.83 for desoxycorticosterone acetate and 1.0 for norethindrone acetate; and the resolution, *R,* between desoxycorticosterone acetate and norethindrone acetate is not less than 3.5.

Procedure—Separately inject equal volumes (about 20 µL) of the *Diluted test solution* and the *Test solution* into the chromatograph, record the chromatograms for twice the retention time of norethindrone acetate, and measure all of the peak areas. Calculate the percentage of each impurity in the portion of Norethindrone Acetate taken by the formula:

$$r_i / r_s$$

in which r_i is the peak area for each impurity obtained from the *Test solution;* and r_s is the sum of all the peaks obtained from the *Diluted test solution.* [NOTE—Exclude any peak having a response that is less than 0.025%.] Not more than 0.5% of any individual impurity is found; and not more than 1.0% of total impurities is found.

Assay—Transfer about 100 mg of Norethindrone Acetate, accurately weighed, to a 200-mL volumetric flask, add alcohol to volume, and mix. Transfer 5.0 mL of this solution to a 250-mL volumetric flask, dilute with alcohol to volume, and mix. Dissolve an accurately weighed quantity of USP Norethindrone Acetate RS in alcohol, and dilute quantitatively and stepwise with alcohol to obtain a Standard solution having a known concentration of about 10 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 240 nm, with a suitable spectrophotometer, using alcohol as the blank. Calculate the quantity, in mg, of $C_{22}H_{28}O_3$ in the portion of Norethindrone Acetate taken by the formula:

$$10C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Norethindrone Acetate RS in the Standard solution, and A_U and A_S are the absorbances of the solution of Norethindrone Acetate and the Standard solution, respectively.

Norethindrone Acetate Tablets

» Norethindrone Acetate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of norethindrone acetate ($C_{22}H_{28}O_3$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩**—**
USP Norethindrone Acetate RS

Identification—It responds to the *Identification* test under *Norethindrone Tablets*, USP Norethindrone Acetate RS being used to prepare the Standard preparation.

Dissolution ⟨711⟩**—**

Medium: dilute hydrochloric acid (1 in 100) containing 0.02% of sodium lauryl sulfate; 900 mL.

Apparatus 1: 100 rpm.

Time: 60 minutes.

Procedure—Determine the amount of $C_{22}H_{28}O_3$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 248 nm, measured from a baseline drawn from 350 nm through 310 nm and extending beyond the peak maximum, of filtered portions of the solution under test, suitably diluted with *Medium*, in comparison with a Standard solution having a known concentration of USP Norethindrone Acetate RS in the same medium. [NOTE—The Standard solution may be prepared by dissolving the Reference Standard in a volume of methanol, not exceeding 0.5% of the final volume of the solution, and diluting quantitatively with *Dissolution Medium*.]

Tolerances—Not less than 70% *(Q)* of the labeled amount of $C_{22}H_{28}O_3$ is dissolved in 60 minutes.

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

Procedure for content uniformity—Transfer 1 finely powdered Tablet to a 100-mL volumetric flask with the aid of about 75 mL of alcohol. Heat the alcohol to boiling, and allow the mixture to remain at a temperature just below the boiling point for