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 Rf value as the principal spot from USP Norethindrone RS, at about Rf 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 Rf value as the pink spot from USP Mestranol RS (about Rf 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 polytetrafluoroethylene syringes that have been checked for adsorptive loss. Use glass dissolution vessels and polytetrafluoroethylene-coated or solid polytetrafluoroethylene 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 (C₂₀H₂₆O₂) and mestranol (C₂₁H₂₆O₂) dissolved, employing the following method.

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

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. Chromatogram the Standard solution and the test solution under the conditions described above, 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 25 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 C₂₀H₂₆O₂ and 75% (Q) of the labeled amount of C₂₁H₂₆O₂ 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 acetone 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 1 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 50 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 2.5, 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 mestranol (C₂₁H₂₆O₂) and mestranol (C₂₁H₂₆O₂) in each Tablet taken by the formula:

\[50C(R_f / R_i)\]

in which C is the concentration, in mg per mL, of the appropriate USP Reference Standard in the Standard preparation, and \(R_f\) and \(R_i\) are the peak response ratios, at corresponding retention times, obtained from the Assay preparation and the Standard preparation, respectively.

Norethindrone Acetate

C₁₂H₂₇O₄ 340.46
19-Norpregn-4-en-20-yn-3-one, (17α)-acetylxyloxy-17α). 17-Hydroxy-19-nor-17α-pregnen-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 C₁₂H₂₇O₄, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards (11)—USP Norethindrone Acetate RS
Uniformity of dosage units

100-mL volumetric flask, dilute with Medium:

etonitrile and water (6:4). Make adjustments if necessary (see the chromatogram obtained from 100 mg with a mixture of methanol and sulfuric acid (7:3), and heat at 60 minutes. In which C is the concentration, in μg per mL, of USP Norethindrone Acetate RS in the Standard solution, and A_w and A_i 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 Dilution 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