roform, collecting this chloroform extract as before and combining it with the previous three. To another glass-stoppered test tube transfer 20.0 mL of a solution of USP Norgestrel RS, in chloroform, having a known concentration of about 3.75 µg per mL. Evaporate the contents of both tubes in a water bath with the aid of a current of air to dryness. Add 5.0 mL of *Isoniazid reagent* to each tube, insert the stopper in each tube, and swirl occasionally for 1 hour. Concomitantly determine the absorbances of both solutions in 1-cm cells, at the wavelength of maximum absorbance at about 380 nm, using a suitable spectrophotometer, and using *Isoniazid reagent* as the blank. Calculate the quantity, in µg, of $C_{21}H_{28}O_2$ in the portion of Tablets taken by the formula:

$$20C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Norgestrel RS in the Standard solution, and A_U and A_S are the absorbances of the solutions from the Tablets and the Standard solution, respectively.

Norgestrel and Ethinyl Estradiol Tablets

» Norgestrel and Ethinyl Estradiol Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of norgestrel ($C_{21}H_{28}O_2$) and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ethinyl estradiol ($C_{20}H_{24}O_2$).

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩**—**
USP Ethinyl Estradiol RS
USP Norgestrel RS

Identification—The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Dissolution ⟨711⟩**—**

Medium: 0.0005% (w/v) polysorbate 80; 500 mL.

Apparatus 2: 75 rpm.

Time: 60 minutes.

Determine the amount of $C_{21}H_{28}O_2$ and $C_{20}H_{24}O_2$ dissolved by employing the following method. [NOTE—Do not use plastics during the preparation of solutions.]

Mobile phase—Prepare a filtered and degassed mixture of water and acetonitrile (3:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution [NOTE—A volume of alcohol not exceeding 2% of the final volume of the solution may be used to aid in dissolving the USP Reference Standards.]—Dissolve an accurately weighed quantity of USP Norgestrel RS and USP Ethinyl Estradiol RS in *Medium,* and dilute quantitatively, and stepwise if necessary, with *Medium* to obtain a solution having known concentrations similar to those expected in the *Test solution.*

Test solution—Use a portion of the solution under test filtered through 0.7-µm borosilicate microfiber filter.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 247-nm detector (for norgestrel analysis), and a spectrofluorometric detector (for ethinyl estradiol analysis) with an excitation wavelength of about 285 nm and an emission wavelength of 310 nm, and a 4.6-mm × 15-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.7 for ethinyl estradiol and 1.0 for norgestrel; and the relative standard deviation for replicate injections is not more than 3.0% for the ethinyl estradiol and norgestrel peaks.

Procedure—Separately inject equal volumes (about 100 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantities, in mg, of norgestrel ($C_{21}H_{28}O_2$) and ethinyl estradiol ($C_{20}H_{24}O_2$) dissolved by the formula:

$$(500C)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard solution;* and r_U and r_S are the peak responses obtained from the *Test solution* and the *Standard solution,* respectively.

Tolerances—Not less than 75% *(Q)* of the labeled amount of $C_{21}H_{28}O_2$and $C_{20}H_{24}O_2$ is dissolved in 60 minutes.

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

Assay—

Mobile phase—Prepare a degassed mixture of water, acetonitrile, and methanol (45:35:15). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Norgestrel RS and USP Ethinyl Estradiol RS in *Mobile phase,* and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having known concentrations of about 100 µg of norgestrel per mL and 10 µg of ethinyl estradiol per mL.

Assay preparation—Transfer an accurately counted number of Tablets, equivalent to about 10 mg of norgestrel, to a 200-mL volumetric flask. Add 100.0 mL of *Mobile phase,* accurately measured, sonicate for 10 minutes to disintegrate the Tablets, and shake by mechanical means for 20 minutes. Centrifuge the clear portion of the solution at about 2000 rpm for 10 minutes, and filter the clear supernatant.

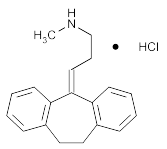
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 1.0 for ethinyl estradiol and 1.5 for norgestrel; the resolution, *R,* between the two major peaks is not less than 2.5; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 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 ethinyl estradiol ($C_{20}H_{24}O_2$) and norgestrel ($C_{21}H_{28}O_2$) in the portion of Tablets taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of the relevant USP Reference Standard in the *Standard preparation;* and r_U and r_S are the peak responses for the relevant analyte obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Nortriptyline Hydrochloride



$C_{19}H_{21}N \cdot HCl$ 299.84
1-Propanamine, 3-(10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)-*N*-methyl-, hydrochloride.

10,11-Dihydro-*N*-methyl-5*H*-dibenzo[*a*,*d*]cycloheptene-Δ^5, γ-propylamine hydrochloride [894-71-3].

» Nortriptyline Hydrochloride contains not less than 97.0 percent and not more than 101.5 percent of $C_{19}H_{21}N \cdot HCl$, calculated on the dried basis.

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

USP Reference standards ⟨11⟩**—**
USP Nortriptyline Hydrochloride RS

Identification—

A: *Infrared Absorption* ⟨197S⟩—

Solution: 50 mg per mL.

Medium: chloroform.

B: *Ultraviolet Absorption* ⟨197U⟩—

Solution: 10 µg per mL.

Medium: methanol.

Absorptivities at 239 nm, calculated on the dried basis, do not differ by more than 3.0%.

C: It responds to the tests for *Chloride* ⟨191⟩ when tested as specified for alkaloidal hydrochlorides.

Melting range, *Class I* ⟨741⟩: between 215° and 220°, but the range between beginning and end of melting does not exceed 3°.

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

Residue on ignition ⟨281⟩: not more than 0.1%.

Heavy metals, *Method II* ⟨231⟩: 0.001%.

Chromatographic purity—

Adsorbent: chromatographic silica gel mixture.

Test solution—Transfer about 250 mg of Nortriptyline Hydrochloride, accurately weighed, to a 10-mL volumetric flask. Dissolve in and dilute with methanol to volume, and mix.

Standard solutions—Dissolve an accurately weighed quantity of USP Nortriptyline Hydrochloride RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 25.0 mg per mL (*Standard solution A*). Dilute appropriate portions of this solution with methanol to obtain *Standard solutions B*, *C*, *D*, *E*, and *F* having known concentrations of 125, 75, 50, 25, and 12.5 µg per mL, respectively. The final concentrations of *Standard solutions B*, *C*, *D*, *E*, and *F* represent 0.5%, 0.3%, 0.2%, 0.1%, and 0.05% of *Standard solution A* concentration, respectively.

Application volume: 5 µL.

Developing solvent system: a mixture of acetonitrile, methanol, and ammonium hydroxide (10:1:1).

Procedure—Apply equal volumes of the *Test solution* and *Standard solutions A*, *B*, *C*, *D*, *E*, and *F* as directed in *Ordinary Impurities* ⟨466⟩. Examine the plate under short-wavelength UV light, then spray the plate with Dragendorff's TS, dry the plate with a stream of nitrogen, and then spray with hydrogen peroxide TS: any secondary spot at an R_F value of 0.78 relative to the nortriptyline spot in the *Test solution* is not greater than the principal spot for *Standard solution D;* any other secondary spot in the *Test solution* is not more than 0.1%; and the sum of all secondary spots is not more than 0.5%.

Assay—Dissolve about 600 mg of Nortriptyline Hydrochloride, accurately weighed, in 50 mL of glacial acetic acid, add 10 mL of mercuric acetate TS, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 29.98 mg of $C_{19}H_{21}N \cdot HCl$.

Nortriptyline Hydrochloride Capsules

» Nortriptyline Hydrochloride Capsules contain nortriptyline hydrochloride equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of nortriptyline ($C_{19}H_{21}N$).

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩**—**
USP Nortriptyline Hydrochloride RS

Identification—

A: Transfer the contents of Capsules, equivalent to about 50 mg of nortriptyline hydrochloride, to a suitable flask. Add 15 mL of chloroform, insert the stopper in the flask, and shake for 15 minutes. Transfer the mixture to a suitable centrifuge tube, and centrifuge at about 2900 rpm for about 5 minutes. Pass through a suitable filter paper containing a small amount of anhydrous sodium sulfate. Evaporate the filtrate to dryness, and dissolve the residue in 0.5 mL of chloroform: the IR absorption spectrum of this solution exhibits maxima only at the same wavelengths as that of a Standard solution prepared by dissolving 50 mg of USP Nortriptyline Hydrochloride RS in 0.5 mL of chloroform.

B: A filtered solution in water of the contents of Capsules, equivalent to nortriptyline hydrochloride solution (1 in 20), responds to the tests for *Chloride* ⟨191⟩, when tested as specified for alkaloidal hydrochlorides.

Dissolution ⟨711⟩**—**

Medium: water; 500 mL.

Apparatus 1: 100 rpm.

Time: 30 minutes.

Procedure—Determine the amount of $C_{19}H_{21}N$ dissolved, employing the procedure set forth in the *Assay*, making any necessary modifications.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{19}H_{21}N$ is dissolved in 30 minutes.

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

Assay—

Phosphate buffer—Dissolve 1.63 g of monobasic potassium phosphate in 1 L of water, and adjust with 1 N potassium hydroxide to a pH of 6.7.

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, methanol, and *Phosphate buffer* (40:43:17). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Nortriptyline Hydrochloride RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.38 mg per mL.

Assay preparation—Weigh, empty, and combine the contents of not less than 20 Capsules. Transfer an accurately weighed portion of the powder, equivalent to about 76 mg of nortriptyline hydrochloride, to a 200-mL volumetric flask, and dissolve in about 150 mL of methanol. Shake by mechanical means for 15 minutes, dilute with methanol to volume, mix, and filter.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 239-nm detector and a 4.6-mm × 25-cm column that contains packing L10. The flow rate is about 2.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure:* the column efficiency is not less than 500 theoretical plates, the tailing factor is not more than 3.0, and the relative standard deviation for replicate injections is not more than 2.0%.