Packaging and storage—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

USP Reference standards ⟨11⟩**—**
USP Naloxone RS
USP Noroxymorphone Hydrochloride RS

Identification, *Infrared Absorption* ⟨197K⟩**—**

Test specimen—Dissolve about 150 mg in 25 mL of water in a small separator, add a few drops of 6 N ammonium hydroxide slowly until no more white precipitate is formed. Extract with three 5-mL portions of chloroform, pass the extracts through a dry filter, collecting the filtrate in a small flask. Evaporate the filtrate on a steam bath to dryness, and dry the residue at 105° for one hour.

Specific rotation ⟨781S⟩**:** between –170° and –181°.

Test solution: 25 mg per mL, in water.

Loss on drying ⟨731⟩**—**Dry it at 105° to constant weight: the anhydrous form loses not more than 0.5% of its weight, and the hydrous form loses not more than 11.0% of its weight.

Noroxymorphone hydrochloride [(–)-4,5α-epoxy-3,14-dihydroxymorphinan-6-one hydrochloride] and other impurities—Transfer about 40 mg, accurately weighed, to a 5-mL volumetric flask, dissolve completely in 2.0 mL of water, add methanol to volume, and mix, to obtain the test solution. Prepare a solution of USP Naloxone RS in chloroform containing about 7.6 mg per mL. Prepare a solution of USP Noroxymorphone Hydrochloride RS in dilute methanol (3 in 5) containing 0.084 mg per mL. Apply 5 µL each of the test solution and the two Standard solutions to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel that previously has been activated by heating for 15 minutes at 105°. Immediately place the plate in a suitable chromatographic chamber containing a 1 in 20 solution of methanol in ammoniacal butanol previously prepared by shaking 100 mL of butyl alcohol with 60 mL of ammonium hydroxide solution (1 in 100) and discarding the lower layer. Develop the chromatogram, protected from light, until the solvent front has moved about 10 cm from the point of application. Remove the plate, dry thoroughly, and spray with ferric chloride-potassium ferricyanide reagent prepared, immediately prior to use, by dissolving 100 mg of potassium ferricyanide in 20 mL of ferric chloride solution (1 in 10). Other than the principal spot corresponding in R_F value to that of USP Naloxone RS and the spot at the origin (ammonium chloride), no other spot is more intense than the spot corresponding to that of USP Noroxymorphone Hydrochloride RS (1.0%).

Chloride content—Dissolve about 300 mg, accurately weighed, in 50 mL of methanol contained in a 125-mL conical flask, add 5 mL of glacial acetic acid and 2 drops of eosin Y TS, and titrate with 0.1 N silver nitrate VS to a pink endpoint. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride. Not less than 9.54% and not more than 9.94%, calculated on the dried basis, is found.

Assay—Dissolve about 300 mg of Naloxone Hydrochloride, previously dried and accurately weighed, in a mixture of 40 mL of glacial acetic acid and 10 mL of acetic anhydride; add 10 mL of mercuric acetate TS and 1 drop of methyl violet TS; and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 36.38 mg of $C_{19}H_{21}NO_4 \cdot HCl$.

Naloxone Hydrochloride Injection

» Naloxone Hydrochloride Injection is a sterile, isotonic solution of Naloxone Hydrochloride in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of naloxone hydrochloride ($C_{19}H_{21}NO_4 \cdot HCl$). It may contain suitable preservatives.

Packaging and storage—Preserve in single-dose or in multiple-dose containers of Type I glass, protected from light.

USP Reference standards ⟨11⟩**—**
USP Endotoxin RS
USP Naloxone RS

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

Bacterial endotoxins ⟨85⟩**—**It contains not more than 500 USP Endotoxin Units per mg of Naloxone Hydrochloride.

pH ⟨791⟩: between 3.0 and 6.5.

Limit of 2,2′-bisnaloxone —

Mobile phase, Diluting solvent, System suitability preparation, and *Chromatographic system*—Prepare as directed in the *Assay*.

Ferric chloride solution—Transfer 4 mL of ferric chloride TS to a 100-mL volumetric flask, dilute with water to volume, and mix.

Identification solution—Dissolve 10 mg of naloxone in 100 mL of 0.1 N hydrochloric acid. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, and add 0.5 mL of *Ferric chloride solution*. Heat on a steam bath for 10 minutes, cool, dilute with water to volume, and mix.

Standard solution—Transfer 2.0 mL of the *Standard preparation* prepared as directed in the *Assay* to a 100-mL volumetric flask, dilute with *Diluting solvent* to volume, and mix.

Test solution—Use the *Assay preparation*.

Procedure—Separately inject equal volumes (about 100 µL) of the *Identification solution*, the *Standard solution*, and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas of the peak responses for naloxone and 2,2′-bisnaloxone. The relative retention times are about 2.8 for the naloxone dimer and 1.0 for naloxone. Calculate the percentage of 2,2′-bisnaloxone in the volume of Injection taken by the formula:

$$(100 / L)(363.84 / 327.38)(C / 1.8)(V_b / V)(r_U / r_S)$$

in which L is the labeled quantity, in µg per mL, of naloxone hydrochloride ($C_{19}H_{21}NO_4 \cdot HCl$) in the Injection taken, 363.84 and 327.38 are the molecular weights of anhydrous naloxone hydrochloride and naloxone, respectively, C is the concentration, in µg per mL, of USP Naloxone RS in the *Standard solution*, 1.8 is the ratio of UV absorptivity of 2,2′-bisnaloxone to that of naloxone hydrochloride, V_b is the volume, in mL, of the *Test solution*, V is the volume, in mL, of Injection taken, r_U is the peak response for 2,2′-bisnaloxone obtained from the *Test solution*, and r_S is the peak response for naloxone obtained from the *Standard solution*. Not more than 4.0% is found.

Other requirements—It meets the requirements under *Injections* ⟨1⟩.

Assay—

Mobile phase—Prepare a filtered and degassed mixture of 1.36 g of sodium 1-octanesulfonate, 1.0 g of sodium chloride, 580 mL of water, 420 mL of methanol, and 1.0 mL of phosphoric acid. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluting solvent—Transfer 150 mg of edetate disodium to a 2000-mL volumetric flask, and add 0.9 mL of hydrochloric acid. Dilute with water to volume, and mix.

Standard preparation—Dissolve an accurately weighed quantity of USP Naloxone RS in *Diluting solvent*, and dilute quantitatively, and stepwise if necessary, with *Diluting solvent* to obtain a solution having a known concentration of about 10 µg per mL.

Assay preparation 1 (for Injection labeled to contain not more than 100 µg of naloxone hydrochloride per mL)—Transfer an accurately measured volume of Injection, equivalent to

about 100 µg of naloxone hydrochloride, to a 10-mL volumetric flask, add *Diluting solvent* to volume, and mix.

Assay preparation 2 (for Injection labeled to contain more than 100 µg of naloxone hydrochloride per mL)—Transfer an accurately measured volume of Injection, equivalent to about 2000 µg of naloxone hydrochloride, to a 200-mL volumetric flask, add *Diluting solvent* to volume, and mix.

System suitability preparation—Prepare a solution in *Diluting solvent* containing about 20 µg of USP Naloxone RS and about 2.5 µg of acetaminophen per mL.

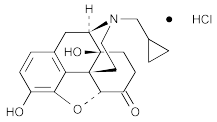
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 229-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation* (about 100 µL) and the *System suitability preparation* (about 20 µL), and record the peak responses as directed under *Procedure:* the resolution, *R,* between the acetaminophen and naloxone peaks is not less than 8, and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 1.5%.

Procedure—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 100 µL) of the *Standard preparation* and the appropriate *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.5 for acetaminophen and 1.0 for naloxone. Calculate the quantity, in µg, of $C_{19}H_{21}NO_4 \cdot HCl$ in each mL of the Injection taken by the formula:

$$(363.84 / 327.38)V_a(C / V)(r_U / r_S)$$

in which 363.84 and 327.38 are the molecular weights of anhydrous naloxone hydrochloride and naloxone, respectively; V_a is the volume, in mL, of the *Assay preparation; C* is the concentration, in µg per mL, of USP Naloxone RS in the *Standard preparation; V* is the volume, in mL, of Injection taken; and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Naltrexone Hydrochloride



$C_{20}H_{23}NO_4 \cdot HCl$ 377.86

Morphinan-6-one, 17-(cyclopropylmethyl)-4,5-epoxy-3,14-dihydroxy-, hydrochloride, (5α)-.

17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan-6-one hydrochloride [16676-29-2].

» Naltrexone Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of $C_{20}H_{23}NO_4 \cdot HCl$, calculated on the anhydrous, solvent-free basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩**—**
USP Naltrexone RS
USP Naltrexone Related Compound A RS
N-(3-Butenyl)-noroxymorphone hydrochloride.
$C_{20}H_{23}NO_4 \cdot HCl$ 377.87

Completeness of solution ⟨641⟩**—**A 650-mg portion dissolves in 10 mL of water to yield a clear solution.

Identification, *Infrared Absorption* ⟨197K⟩—

Test specimen—Dissolve about 150 mg in 25 mL of water in a small separator, add a few drops of 6 N ammonium hydroxide slowly until no more white precipitate is formed. Extract with three 5-mL portions of chloroform, filter the extracts through a dry filter, collecting the filtrate in a small flask. Evaporate the filtrate on a steam bath to dryness, and dry the residue at 105° for one hour.

Specific rotation ⟨781S⟩**:** between –187° and –197°, calculated on the anhydrous, solvent-free basis.

Test solution: 25 mg per mL, in water.

Water, *Method I* ⟨921⟩**—**Determine the water content as directed. [NOTE—The result of this test is used in the calculation of *Limit of total solvents*.]

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

Heavy metals, *Method II* ⟨231⟩**:** not more than 0.002%.

Limit of total solvents—

Internal standard stock solution—Transfer 6.0 mL of isopropyl alcohol to a 500-mL volumetric flask, dilute with water to volume, and mix. [NOTE—The isopropyl alcohol must be free of alcohol impurities.]

Internal standard solution—Transfer 5.0 mL of the *Internal standard stock solution* to a 100-mL volumetric flask, dilute with water to volume, and mix.

Standard solution—Prepare a solution of methanol and alcohol (C_2H_5OH) in water to obtain a solution having a known concentration of about 16 mg of each per mL. Transfer 3.0 mL of this solution and 5.0 mL of *Internal standard stock solution* to a 100-mL volumetric flask, dilute with water to volume, and mix.

Test solution—Transfer about 75 mg of Naltrexone Hydrochloride, accurately weighed, to a suitable container, add 5.0 mL of *Internal standard solution,* and shake to dissolve.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 4-mm × 1.8-m glass column packed with 80- to 100-mesh support S3. The column temperature is maintained at 150°, and the injection port and detector temperatures are maintained at 170°. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.24 for methanol, 0.53 for alcohol, and 1.0 for isopropyl alcohol.

Procedure—Separately inject equal volumes (about 5 µL) of the *Standard solution* and the *Test solution* into the gas chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentages of methanol and alcohol in the portion of Naltrexone Hydrochloride taken by the formula:

$$100(C_S / C_U)(R_U / R_S)$$

in which C_S is the concentration, in mg per mL, of methanol or alcohol (C_2H_5OH) in the *Standard solution; C_U* is the concentration, in mg per mL, of Naltrexone Hydrochloride in the *Test solution;* and R_U and R_S are the peak response ratios of methanol or alcohol to isopropyl alcohol obtained from the *Test solution* and the *Standard solution,* respectively. To the sum of the percentages of methanol and alcohol, add the percentage of water as determined in the test for *Water:* the sum of water and alcoholic solvents is not more than 5.0% for the anhydrous form, and not more than 11.0% for the dihydrate form.

Related compounds—Proceed as directed in the *Assay*. From the chromatogram of the *Assay preparation,* calculate the percentage of each related compound in Naltrexone Hydrochloride taken by the formula:

$$10F(C/W)(r_U / r_S)$$

in which *F* is the relative response factor for each impurity; *C* is the concentration, in mg per mL, of USP Naltrexone RS in the *Standard preparation; W* is the weight, in mg, of Naltrexone Hydrochloride taken for the *Assay preparation;* r_U is the peak response of the relevant related compound obtained from the *Assay preparation;* and r_S is peak response of naltrexone obtained from the *Standard preparation*. [NOTE—The relative re-