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.*

Microbial enumeration tests ⟨61⟩ **and Tests for specified microorganisms** ⟨62⟩**—**It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Minimum fill ⟨755⟩: meets the requirements.

pH ⟨791⟩: between 5.5 and 7.5.

Content of alcohol—

Internal standard solution—Transfer 10.0 mL of *n*-propyl alcohol to a 200-mL volumetric flask, dilute with water to volume, and mix.

Standard solution—Prepare a mixture containing an accurately weighed quantity of alcohol in water having a known concentration of about 10.0 mg of alcohol per mL. Transfer 3.0 mL of *Internal standard solution* to a 10-mL volumetric flask, dilute with the alcohol solution, and mix.

Test solution—Transfer about 250 mg of Gel, accurately weighed, to a suitable container. Add 14.0 mL of water and 6.0 mL of *Internal standard solution*, and shake for 15 minutes.

Chromatographic system (see *Chromatography* ⟨621⟩)—The gas chromatograph is equipped with a flame-ionization detector and a 3.2-mm × 1.5-m column packed with 80- to 100-mesh support S3. The column temperature is maintained at 170°, and the injection port and detector are maintained at 200°. Nitrogen is used as the carrier gas, flowing at a rate of 45 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed under *Procedure:* the resolution, *R,* between alcohol and the internal standard is not less than 2.0, the capacity factor, *k'*, is between 2.0 and 3.5 for alcohol and between 6.0 and 8.0 for the internal standard, the tailing factor is not more than 2.5, and the relative standard deviation for replicate injections is not more than 2.5%.

Procedure—Inject equal volumes (about 1 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 quantity, in mg, of C_2H_5OH in the portion of Gel taken by the formula:

$$5.6C(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of C_2H_5OH in the *Standard solution*, and R_U and R_S are the ratios of the peak responses for alcohol to those of the internal standard obtained from the *Test solution* and the *Standard solution*, respectively: the content of C_2H_5OH is between 40% and 45%.

Assay—

Mobile phase, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Naftifine Hydrochloride.*

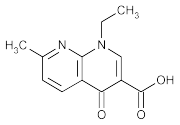
Assay preparation—Transfer about 1000 mg of Gel, accurately weighed, to a 100-mL volumetric flask, dissolve in 60 mL of methanol, mix vigorously for 2 minutes, and dilute with methanol to volume. Heat at 45° for 5 minutes, and cool to room temperature.

Procedure—Separately inject equal volumes (about 20 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 $C_{21}H_{21}N \cdot HCl$ in the portion of Gel taken by the formula:

$$100C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Naftifine Hydrochloride RS in the *Standard preparation,* and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Nalidixic Acid



$C_{12}H_{12}N_2O_3$ 232.24

1,8-Naphthyridine-3-carboxylic acid, 1-ethyl-1,4-dihydro-7-methyl-4-oxo-.

1-Ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid [389-08-2].

» Nalidixic Acid contains not less than 99.0 percent and not more than 101.0 percent of $C_{12}H_{12}N_2O_3$, calculated on the dried basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩**—**
USP Nalidixic Acid RS

Identification—

A: *Infrared Absorption* ⟨197K⟩.

B: *Ultraviolet Absorption* ⟨197U⟩—

Solution: 5 µg per mL.

Medium: 0.01 N sodium hydroxide.

Absorptivities at 258 nm, calculated on the dried basis, do not differ by more than 3.0%.

Melting range ⟨741⟩**:** between 225° and 231°.

Loss on drying ⟨731⟩**—**Dry it at 105° for 2 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⟩**:** 0.002%.

Chromatographic purity—

Standard solutions—Prepare a solution of USP Nalidixic Acid RS in chloroform containing 1.0 mg per mL. Dilute quantitatively with chloroform to obtain *Standard solutions* having the following composition:

Standard solution	Dilution	Concentration (mg RS per mL)	Percentage (%, for comparison with test specimen)
A	1 in 10	0.1	0.5
B	1 in 25	0.04	0.2
C	1 in 50	0.02	0.1

Test solution—Dissolve an accurately weighed quantity of Nalidixic Acid in chloroform to obtain a solution containing 20 mg per mL.

Procedure—Apply separately 10 µL of the *Test solution* and 10 µL of each *Standard solution* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Position the plate in a chromatographic chamber, and develop the chromatograms in a solvent system consisting of a mixture of alcohol, chloroform, and 5 M ammonium hydroxide (70:20:10) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate with the aid of warm circulating air. Examine the plate under short-wavelength UV light. Compare the intensities of any secondary spots observed in the chromatogram of the *Test solution* with those of the principal spots in the chromatograms of the *Standard solutions:* no secondary spot is more intense than the principal spot obtained from *Standard solution A* (0.5%), and the sum of the intensities of all secondary spots obtained from the *Test solution* does not exceed 1.0%.

Assay—Dissolve about 250 mg of Nalidixic Acid, accurately weighed, in 30 mL of dimethylformamide that previously has been neutralized to thymolphthalein TS, and titrate with 0.1 N lithium methoxide VS in methanol, using a magnetic stirrer and taking precautions against absorption of atmospheric carbon dioxide. Each mL of 0.1 N lithium methoxide is equivalent to 23.22 mg of $C_{12}H_{12}N_2O_3$.

Nalidixic Acid Oral Suspension

» Nalidixic Acid Oral Suspension contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of nalidixic acid $C_{12}H_{12}N_2O_3$ in a suitable aqueous vehicle.

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Nalidixic Acid RS

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

Uniformity of dosage units ⟨905⟩—

FOR ORAL SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

Deliverable volume ⟨698⟩—

FOR ORAL SUSPENSION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

Assay—

Mobile phase—Prepare a solution of 784 mg of dibasic potassium phosphate in 325 mL of water. To this solution add a solution of 2.62 g of hexadecyltrimethylammonium bromide in 350 mL of methanol. To the combined solution add 325 mL of methanol, mix, filter, and degas. This solution has an apparent pH of about 10. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Internal standard solution—Prepare a solution of sulfanilic acid in *Mobile phase* containing about 0.8 mg per mL.

Standard preparation—Prepare a solution having a known concentration of about 0.18 mg per mL of USP Nalidixic Acid RS in methanol. Transfer 5.0 mL of this solution and 1.0 mL of *Internal standard solution* to a 25-mL volumetric flask, dilute with methanol to volume, and mix.

Assay preparation—Transfer an accurately measured volume of freshly mixed Oral Suspension, equivalent to about 150 mg of nalidixic acid, to a 500-mL volumetric flask, add about 400 mL of methanol, and sonicate for about 30 minutes. Shake by mechanical means for about 30 minutes, sonicate again for about 30 minutes, dilute with methanol to volume, mix, and filter. Transfer 3.0 mL of the clear filtrate and 1.0 mL of *Internal standard solution* to a 25-mL volumetric flask, dilute with methanol to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.7 for sulfanilic acid and 1.0 for nalidixic acid; the resolution, *R,* between sulfanilic acid and nalidixic acid is not less than 1; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of nalidixic acid ($C_{12}H_{12}N_2O_3$) in each mL of the Oral Suspension taken by the formula:

$$(12{,}500/3)(C/V)(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Nalidixic Acid RS in the *Standard preparation; V* is the volume, in mL, of Oral Suspension taken to prepare the *Assay preparation;* and R_U and R_S are the ratios of the peak areas for nalidixic acid and sulfanilic acid in the chromatograms obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Nalidixic Acid Tablets

» Nalidixic Acid Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of nalidixic acid ($C_{12}H_{12}N_2O_3$).

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Nalidixic Acid RS

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

Dissolution ⟨711⟩—

Medium: pH 8.60 buffer, prepared by mixing 2.3 volumes of 0.2 M sodium hydroxide with 2.5 volumes of 0.2 M monobasic potassium phosphate and 2.0 volumes of methanol, cooling, mixing with water to obtain 10 volumes of solution, and adjusting, if necessary, by the addition of 1 N sodium hydroxide to a pH of 8.60 ± 0.05. The initial volume for the test is 900 mL.

Apparatus 2: 60 rpm.

Time: 30 minutes.

Procedure—Determine the amount of $C_{12}H_{12}N_2O_3$ dissolved from UV absorbances at the wavelength of maximum absorbance at about 258 nm of filtered portions of the solution under test, suitably diluted with 0.01 N sodium hydroxide, if necessary, in comparison with a Standard solution of known concentration of USP Nalidixic Acid RS in 0.01 N sodium hydroxide, using as the blank a mixture of *Medium* and 0.01 N sodium hydroxide in the same proportions as present in the test solution.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{12}H_{12}N_2O_3$ is dissolved in 30 minutes.

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

Assay—

Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Nalidixic Acid Oral Suspension*.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 150 mg of nalidixic acid, to a 500-mL volumetric flask, add about 400 mL of methanol, and sonicate for about 30 minutes. Shake by mechanical means for about 30 minutes, sonicate again for about 30 minutes, dilute with methanol to volume, mix, and filter. Transfer 3.0 mL of the clear filtrate and 1.0 mL of *Internal standard solution* to a 25-mL volumetric flask, dilute with methanol to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Nalidixic Acid Oral Suspension*. Calculate the quantity, in