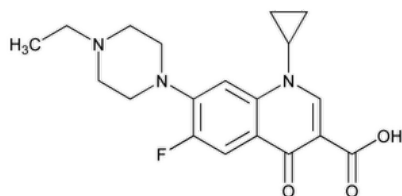


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Enrofloxacin



$C_{19}H_{22}FN_3O_3$ 359.39

3-Quinolonecarboxylic acid, 1-cyclopropyl-7-(4-ethyl-1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo-;

1-Cyclopropyl-7-(4-ethyl-1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid CAS RN®: 93106-60-6; UNII: 3DX3XEK1BN.

» Enrofloxacin contains not less than 98.5 percent and not more than 101.5 percent of $C_{19}H_{22}FN_3O_3$, calculated on the dried basis.

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

Labeling—Label it to indicate that it is for veterinary use only.

USP REFERENCE STANDARDS (11)—

[USP Enrofloxacin RS](#)

[USP Fluoroquinolonic Acid RS](#)

[USP N-Ethylpiperazine RS](#)

[USP Enrofloxacin Related Compound Mixture RS](#)

Contains a mixture of USP Enrofloxacin RS, desfluoro-enrofloxacin, and USP Ciprofloxacin RS.

Desfluoro-enrofloxacin: 1-cyclopropyl-7-(4-ethyl-1-piperazinyl)-1,4-dihydro-4-oxo-3-quinoline-carboxylic acid.

Change to read:

CLARITY OF SOLUTION—

Hydrazine sulfate solution—Transfer 1.0 g of hydrazine sulfate to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Allow to stand for 4 to 6 hours.

Hexamethylenetetramine solution—Transfer 2.5 g of hexamethylenetetramine to a 100-mL volumetric flask, add 25.0 mL of water, insert a glass stopper, and mix to dissolve.

Primary opalescent suspension—[NOTE—This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well-mixed before use.] Transfer 25.0 mL of the *Hydrazine sulfate solution* to the *Hexamethylenetetramine solution* in the 100-mL glass-stoppered flask. Mix, and allow to stand for 24 hours.

Opalescence standard— [NOTE—This suspension should not be used beyond 24 hours after preparation.]

Transfer 15.0 mL of the *Primary opalescent suspension* to a 1000-mL volumetric flask, dilute with water to volume, and mix.

Reference suspension—Transfer 10.0 mL of the *Opalescence standard* to a 100-mL volumetric flask, dilute with water to volume, and mix.

Test solution—To 1.0 g of Enrofloxacin add about 0.25 g of potassium hydroxide and 7 mL of water. Sonicate to dissolve, and dilute with water to 10.0 mL.

Procedure—Transfer a sufficient portion of the *Test solution*, the *Reference suspension*, and water to separate test tubes of colorless, transparent, neutral glass with a flat base and an internal diameter of 15 to 25 mm to obtain a depth of 40 mm. Compare the *Test solution*, the *Reference suspension*, and water in diffused daylight 5 minutes after preparation of the *Reference suspension*, viewing vertically against a black background (see ▲ [Visual Comparison \(630\)](#) ▲ (CN 1-May-2019)). [NOTE—The diffusion of light must be such that the *Reference suspension* can be readily distinguished from water.] The *Test solution* shows the same clarity as that of water or its opalescence is not more pronounced than that of the *Reference suspension*.

Change to read:

COLOR OF SOLUTION—

Standard stock solution—Combine 9.6 mL of ferric chloride CS, 0.2 mL of cobaltous chloride CS, and 0.2 mL of cupric sulfate CS, and mix.

Standard solution—[NOTE—Prepare the *Standard solution* immediately before use.] Transfer 5.0 mL of the *Standard stock solution* to a 100-mL volumetric flask, and dilute with dilute hydrochloric acid (10 g per 1000 mL).

Test solution—To 1.0 g of Enrofloxacin add about 0.25 g of potassium hydroxide and 7 mL of water. Sonicate to dissolve, and dilute with water to 10.0 mL.

Procedure—Transfer a sufficient portion of the *Test solution*, the *Standard solution*, and water to separate test tubes of colorless, transparent, neutral glass with a flat base and an internal diameter of 15 mm to 25 mm to obtain a depth of 40 mm. Compare the *Test solution*, the

Standard solution, and water in diffused daylight, viewing vertically against a white background (see ▲[Visual Comparison \(630\)](#)▲ (CN 1-May-2019)). [NOTE—The diffusion of light must be such that the *Reference suspension* can be readily distinguished from water.] The *Test solution* has the appearance of water or is not more intensely colored than the *Standard solution*.

Identification—

Change to read:

A: ▲[SPECTROSCOPIC IDENTIFICATION TESTS \(197\)](#), [Infrared Spectroscopy: 197K](#)▲ (CN 1-May-2020) ·

B: [THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST \(201\)](#).—

Test solution: Prepare a solution containing 10 mg of Enrofloxacin per mL of 0.05 M hydrochloric acid.

Application volume: 5 µL.

Developing solvent solution: methylene chloride, methanol, 25% ammonia solution, and acetonitrile (2:2:1:0.5).

Procedure—Proceed as directed in the chapter except apply 5-µL portions of the *Test solution* and the *Standard solution* to the starting line of the chromatographic plate. Place the plate in an atmosphere of ammonia for approximately 15 minutes. Develop the chromatogram in an unsaturated chamber with the *Developing solvent solution*.

[LOSS ON DRYING \(731\)](#).—Dry a 2-g sample, accurately weighed, 5 to 7 hours under vacuum at 120° to constant weight: it loses not more than 1.0% of its weight.

[RESIDUE ON IGNITION \(281\)](#): not more than 0.1%, based on a test specimen of about 2 g.

Change to read:

[CHLORIDE \(221\)](#).—Add 58 mL of water to 0.5 g of Enrofloxacin, shake for 5 minutes, and pass through a chloride-free filter paper, add 2 mL of 2 M acetic acid, and mix. Transfer 15.0 mL of the filtrate to a 50-mL color-comparison tube (*Test solution*). To a second matched 50-mL color-comparison tube transfer 10.0 mL of a *Standard solution* of sodium chloride having a concentration of 8.2 µg per mL, corresponding to 5 µg of chloride per mL, add 5.0 mL of water, and mix. To each tube add 1 mL of 2 N nitric acid, mix, add 1 mL of silver nitrate TS, and mix. Allow the solutions to stand for 5 minutes, protected from light. Examine the tubes vertically against a black background (see ▲[Visual Comparison \(630\)](#)▲ (CN 1-May-2019)). Any opalescence in the *Test solution* is not more intense than that in the *Standard solution* (0.04%).

Change to read:

[SULFATE \(221\)](#).—Dissolve 0.5 g of Enrofloxacin in 5.0 mL of 2 N acetic acid and 15.0 mL of water (*Test solution*). To each of two 50-mL matched color-comparison tubes transfer 1.50 mL of a *Standard solution* of potassium sulfate in 30% alcohol having a concentration of 18.1 µg per mL, equivalent to 10 µg of sulfate per mL. To each tube add, successively and with continuous shaking, 1.0 mL of barium chloride solution (1 in 4), and allow to stand for 1 minute. To one of the tubes transfer 15.0 mL of the *Standard solution* and 0.5 mL of 30% acetic acid, and mix. To the second tube add 15.0 mL of the *Test solution* and 0.5 mL of 30% acetic acid, and mix. Allow solutions to stand for 5 minutes. Examine the tubes vertically against a black background (see ▲[Visual Comparison \(630\)](#)▲ (CN 1-May-2019)). Any opalescence in the *Test solution* is not more intense than that in the *Standard solution* (0.04%).

Limit of N-ethylpiperazine—

Internal standard solution—Dissolve an accurately weighed quantity of *n*-decane in chloroform, and dilute quantitatively, and stepwise if necessary, with chloroform to obtain a solution having a known concentration of about 0.1 mg per mL.

Standard stock solution—Dissolve an accurately weighed quantity of USP *N*-Ethylpiperazine RS in chloroform, and dilute quantitatively, and stepwise if necessary, with chloroform to obtain a solution having a known concentration of about 9.0 mg per mL.

Standard solution—To 2.0 mL of the *Internal standard solution* add 20 µL of the *Standard stock solution*, and mix.

Test solution—To 200 mg of Enrofloxacin, accurately weighed, add 2.0 mL of the *Internal standard solution*, and mix.

Chromatographic system (see [CHROMATOGRAPHY \(621\)](#)).—The gas chromatograph is equipped with a flame-ionization detector and a split injector system and contains a 0.32-mm × 50-m column with 100% liquid phase G1 with a film thickness of about 5.0 µm. The carrier gas is hydrogen (helium may be used), flowing at a rate of about 2.9 mL per minute. The auxiliary gas is nitrogen flowing at a rate of about 30 mL per minute. The chromatograph is programmed as follows. Initially the temperature of the column is equilibrated at 80°, then the temperature is increased at a rate of 10° per minute to 240°, and maintained at 240° for 15 minutes. The split injector (25:1 split ratio) temperature is maintained at 200°, and the detector is maintained at 250°. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.90 for *N*-ethylpiperazine and 1.0 for *n*-decane.

Procedure—Inject a volume (about 1.0 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatogram, and measure the responses for the major peaks. Calculate the percentage of the impurity in the portion of Enrofloxacin taken by the formula:

$$100(C_s/C_u)(R_u/R_s)$$

in which C_s is the concentration of the impurity, in mg per mL, in the *Standard solution*; C_u is the concentration, in mg per mL, of Enrofloxacin in the *Test solution*; R_u is the peak response ratio of the impurity peak to the internal standard peak obtained from the *Test solution*; and R_s is the peak response ratio of the impurity peak to the internal standard peak obtained from the *Standard solution*. Not more than 0.1% of the impurity is found.

Related compounds—

TEST 1 (FOR FLUOROQUINOLONIC ACID)—

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

Test solution—Prepare as directed for the *Test solution* in *Identification test B*.

Diluent—Transfer 0.1 mL of 6 M ammonium to a 100-mL volumetric flask, mix, and dilute with water to volume.

Standard stock solution—Dissolve and mix an accurately weighed quantity of USP Fluoroquinolonic Acid RS with *Diluent* to prepare a solution containing about 0.10 mg per mL, and mix.

Standard solution 1 (0.1%)—Transfer 1.0 mL of the *Standard stock solution* to a 10-mL volumetric flask, dilute with water to volume, and mix.

Standard solution 2 (0.2%)—Transfer 2.0 mL of the *Standard stock solution* to a 10-mL volumetric flask, dilute with water to volume, and mix.

Application volume: 5 µL.

Developing solvent system—[NOTE—Carefully follow the mixing order stated below.] Shake butyl acetate, *n*-butanol, water, and glacial acetic acid (50:9:15:25), and allow to settle. Use the upper layer as the mobile phase and discard the lower layer.

Procedure—Proceed as directed for *Thin-Layer Chromatography* under [Chromatography \(621\)](#). Apply separately the *Test solution*, *Standard solution 1*, and *Standard solution 2* to the thin-layer plate, and chromatograph using the *Developing solvent system*. Dry the developed chromatogram in the air under a fume hood for 30 to 60 minutes, then view under short-wavelength UV light. Determine the quantity of fluoroquinolonic acid by comparing the size and intensity of the spots from the *Test solution* to the *Standard solutions*. The intensity of any spot from the *Test solution* at about the same retardation factor, R_F , as that of the *Standard solutions* is not greater than the intensity of the *Standard solution 2 (0.2%)* spot.

TEST 2 (FOR CIPROFLOXACIN, DES-FLUORO COMPOUND AND OTHER UNSPECIFIED IMPURITIES)—

Phosphoric acid buffer—Prepare 25 mM phosphoric acid, and adjust with triethylamine to a pH of 3.0.

Mobile phase—Prepare a solution containing *Phosphoric acid buffer* and acetonitrile (87:13).

Control solution—Dissolve about 5 mg of USP Enrofloxacin Related Compound Mixture RS, accurately weighed, in *Mobile phase* in a 5-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Test solution 1—Dissolve about 50 mg of Enrofloxacin, accurately weighed, in *Mobile phase* in a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Test solution 2—Transfer 1.0 mL of *Test solution 1* into a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 1.0 mL of this solution into a 10-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Chromatographic system (see [CHROMATOGRAPHY \(621\)](#))— The liquid chromatograph is equipped with a 278-nm detector and a 4.6-mm × 25-cm stainless steel column that contains 5-µm packing L1. The column temperature is maintained at 40°, and the flow rate is about 1.5 mL per minute. Chromatograph the *Control solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.58 for the des-fluoro compound, 0.74 for ciprofloxacin, and 1.0 for enrofloxacin. The resolution, R , between the des-fluoro compound and ciprofloxacin is not less than 1.5.

Procedure—Inject a volume (about 25 µL) of *Test solution 1*, *Test solution 2*, and the *Control solution* into the chromatograph, record the chromatogram, and measure the peak responses. Identify the ciprofloxacin and the des-fluoro compound peaks in *Test solution 2* by comparing their retention times with those from the *Control solution*. Calculate the percentage of each related compound in the portion of Enrofloxacin taken by the formula:

$$100C(r_i/r_s)$$

in which C is the concentration of Enrofloxacin in *Test solution 2* as a percentage of *Test solution 1 (0.2%)*; r_i is the individual peak response of each related compound obtained from *Test solution 1*; and r_s is the individual peak area of enrofloxacin obtained from *Test solution 2*: not more than 0.1% of des-fluoro compound, not more than 0.3% of ciprofloxacin, not more than 0.1% of any unspecified impurity, and not more than 0.5% of total impurities are found.

Assay—Transfer about 250 mg of Enrofloxacin, accurately weighed, to a 125-mL flask, dissolve in 100 mL of anhydrous acetic acid, and titrate with 0.1 M perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 M perchloric acid is equivalent to 35.94 mg of $C_{19}H_{22}FN_3O_3$.

Auxiliary Information - Please [check for your question in the FAQs](#) before contacting USP.

Topic/Question	Contact	Expert Committee
ENROFLOXACIN	Documentary Standards Support	SM32020 Small Molecules 3

Chromatographic Database Information: [Chromatographic Database](#)

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