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Fluorescein Sodium and Benoxinate Hydrochloride Ophthalmic Solution

DEFINITION

Fluorescein Sodium and Benoxinate Hydrochloride Ophthalmic Solution is a sterile aqueous solution of fluorescein sodium and benoxinate hydrochloride. It contains NLT 90.0% and NMT 120.0% of the labeled amounts of fluorescein sodium ($C_{20}H_{10}Na_2O_5$) and benoxinate hydrochloride ($C_{17}H_{28}N_2O_3 \cdot HCl$). It contains a suitable preservative.

IDENTIFICATION

• **A.** A solution of it is strongly fluorescent, even in extreme dilution. The fluorescence disappears when the solution is made acid, and reappears when the solution is again made alkaline.

• **B.** The relative retention times of the major peaks of the *Sample solution* correspond to those of *Standard solution A* and *Standard solution B*, as obtained in the *Assay*.

ASSAY

• **Procedure**

Mobile phase: Dissolve 100 mg of sodium 1-pentanesulfonate in 40 mL of glacial acetic acid in a 2000-mL volumetric flask. Add 600 mL of acetonitrile and 10 mL of triethanolamine, and dilute with water to volume. Adjust with phosphoric acid to a pH of 3.

Standard stock solution A: Transfer 55 mg of USP Diacetylfluorescein RS to a 50-mL volumetric flask containing 5 mL of alcohol. Add 1 mL of 2.5 N sodium hydroxide, and heat on a steam bath at the boiling temperature for 20 min, with frequent swirling. Cool, and dilute with water to volume.

Standard solution A: 0.1 mg/mL of fluorescein sodium in *Mobile phase* prepared as follows. Transfer 10.0 mL of *Standard stock solution A* to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

Standard solution B: 0.1*J* mg/mL of USP Benoxinate Hydrochloride RS in *Mobile phase*, where *J* is the ratio of the labeled amount, in mg, of benoxinate hydrochloride to the labeled amount, in mg, of fluorescein sodium in each mL of Ophthalmic Solution

Sample solution: Nominally equivalent to 0.1 mg/mL of fluorescein sodium in *Mobile phase* from Ophthalmic Solution

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm × 30-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 25 µL

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

Tailing factor: NMT 2.0 for each analyte peak, *Standard solution A* and *Standard solution B*

Relative standard deviation: NMT 2.0% for each analyte peak, *Standard solution A* and *Standard solution B*

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Calculate the percentage of the labeled amount of fluorescein sodium ($C_{20}H_{10}Na_2O_5$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak area of fluorescein from the *Sample solution*

r_S = peak area of fluorescein from *Standard solution A*

C_S = concentration of USP Diacetylfluorescein RS in *Standard solution A* (mg/mL)

C_U = nominal concentration of fluorescein sodium in the *Sample solution* (mg/mL)

M_{r1} = molecular weight of fluorescein sodium, 376.28

M_{r2} = molecular weight of diacetylfluorescein, 416.39

Calculate the percentage of the labeled amount of benoxinate hydrochloride ($C_{17}H_{28}N_2O_3 \cdot HCl$) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of benoxinate from the *Sample solution*

r_S = peak area of benoxinate from *Standard solution B*

C_S = concentration of USP Benoxinate Hydrochloride RS in *Standard solution B* (mg/mL)

C_U = nominal concentration of benoxinate hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria

Fluorescein sodium: 90.0%–120.0%

Benoxinate hydrochloride: 90.0%–120.0%

SPECIFIC TESTS

- **Sterility Tests ⟨71⟩:** It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **pH ⟨791⟩:** 4.3–5.3

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.
- **USP Reference Standards ⟨11⟩**

USP Benoxinate Hydrochloride RS

USP Diacetylfluorescein RS

Spiro(isobenzofuran-1(3*H*), 9'-(9*H*)xanthen)-3-one, 3',6'-bis(acetyloxy)-.

$C_{24}H_{16}O_7$ 416.39

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
FLUORESCEIN SODIUM AND BENOXINATE HYDROCHLORIDE OPHTHALMIC SOLUTION	Documentary Standards Support	SM32020 Small Molecules 3

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
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