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Fluorescein Sodium and Proparacaine Hydrochloride Ophthalmic Solution

DEFINITION

Fluorescein Sodium and Proparacaine Hydrochloride Ophthalmic Solution is a sterile aqueous solution of Fluorescein Sodium and Proparacaine Hydrochloride. It contains NLT 90.0% and NMT 110.0% of the labeled amounts of fluorescein sodium ($C_{20}H_{10}Na_2O_5$) and proparacaine hydrochloride ($C_{16}H_{26}N_2O_3 \cdot HCl$). 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.**

Solution A: 20 mg/mL of 2-naphthol in 1 N sodium hydroxide

Analysis: To 1 mL of Ophthalmic Solution in a test tube add 5 mL of diluted hydrochloric acid (1 in 100), and cool in an ice bath for 2 min. Add 1 mL of *Solution A*.

Acceptance criteria: A scarlet-red precipitate is formed. Add 5 mL of acetone. The precipitate does not dissolve.

ASSAY

• FLUORESCHEIN SODIUM

Standard stock solution: 1 µg/mL of fluorescein sodium from [USP Diacetylfluorescein RS](#) in water prepared as follows. Dissolve [USP Diacetylfluorescein RS](#) in 10 mL of alcohol contained in a 100-mL volumetric flask, add 2 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. Transfer a suitable aliquot to a volumetric flask, and dilute with water to volume. [NOTE—110.7 mg of anhydrous [USP Diacetylfluorescein RS](#) is equivalent to 100.0 mg of fluorescein sodium.]

Standard solution: 0.03 µg/mL of fluorescein sodium in water prepared as follows. Transfer 3 mL of *Standard stock solution* to a 100-mL volumetric flask containing 20 mL of pH 9.0 alkaline borate buffer (see [Reagents, Indicators, and Solutions—Buffer Solutions](#)), and dilute with water to volume.

Sample stock solution: Nominally equivalent to 1 µg/mL of fluorescein sodium from Ophthalmic Solution in water

Sample solution: Nominally equivalent to 0.03 µg/mL of fluorescein sodium in water prepared as follows. Transfer 3.0 mL of *Sample stock solution* to a 100-mL volumetric flask containing 20 mL of pH 9.0 alkaline borate buffer (see [Reagents, Indicators, and Solutions—Buffer Solutions](#)), and dilute with water to volume.

Instrumental conditions

Mode: Fluorescence

Excitation wavelength: 485 nm

Emission wavelength: 515 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Concomitantly determine the fluorescence intensities, I , of the *Standard solution* and the *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:

$$\text{Result} = (I_U/I_S) \times (C_S/C_U) \times 100$$

I_U = fluorescence value of the *Sample solution*

I_S = fluorescence value of the *Standard solution*

C_S = concentration of fluorescein sodium in the *Standard solution* (µg/mL)

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

Acceptance criteria: 90.0%–110.0%

• **PROPARACAINE HYDROCHLORIDE**

Buffer: 6.8 g/L of monobasic potassium phosphate in 1000 mL of water. Add 5 mL of triethylamine, and adjust with 5 N potassium hydroxide to a pH of 7.5.

Mobile phase: Acetonitrile and *Buffer* (40:60)

Standard solution: 0.1 mg/mL of proparacaine in *Mobile phase* prepared as follows. Prepare 1 mg/mL of [USP Proparacaine Hydrochloride RS](#) in water, and dilute it with *Mobile phase* to 0.1 mg/mL. Use this solution within 6 h.

Sample solution: Nominally equivalent to 0.1 mg/mL of proparacaine hydrochloride in *Mobile phase* from Ophthalmic Solution. Use this solution within 6 h.

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 15-cm; 5-µm packing L10

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 3000 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of proparacaine hydrochloride ($C_{16}H_{26}N_2O_3 \cdot HCl$) in the portion of Ophthalmic Solution taken:

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

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of [USP Proparacaine Hydrochloride RS](#) in the *Standard solution* (mg/mL)

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

Acceptance criteria: 90.0%–110.0%

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):** 4.0–5.2

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, preferably of Type I amber glass, and store in a refrigerator.
- **LABELING:** Label it to state that it is to be stored in a refrigerator before and after the container is opened.
- **USP REFERENCE STANDARDS (11)**
[USP Diacetylfluorescein RS](#)
 Spiro(isobenzofuran-1(3H), 9'-(9H)xanthen)-3-one, 3',6'-bis(acetyloxy)-.
 $C_{24}H_{16}O_7$ 416.39
[USP Proparacaine Hydrochloride RS](#)

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