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Fluorescein Injection

DEFINITION

Fluorescein Injection is a sterile solution, in Water for Injection, of Fluorescein prepared with the aid of Sodium Hydroxide. It contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of fluorescein sodium ($C_{20}H_{10}Na_2O_5$). It may contain Sodium Bicarbonate.

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.

Change to read:

• **B.** ▲The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲ (USP 1-May-2019)

ASSAY

Change to read:

• **Procedure**

▲**Buffer:** 0.61 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 2.0.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Buffer (%)	Acetonitrile (%)
0	85	15
20	20	80
29	20	80
30	85	15
35	85	15

Diluent: Acetonitrile and *Buffer* (30:70)

Standard stock solution: [Note—1.1 mg of USP Diacetylfluorescein RS is equivalent to 1 mg of fluorescein sodium.]1.0 mg/mL of fluorescein sodium prepared as follows. Transfer USP Diacetylfluorescein RS to a suitable volumetric flask. Add 2.5 N sodium hydroxide to fill 2% of the final volume and add alcohol to fill 10% of the final volume. Heat on a water bath for 20 min, mixing frequently. Cool and dilute with water to volume.

Standard solution: 0.02 mg/mL of fluorescein sodium in *Diluent* from *Standard stock solution*

Sample stock solution: Nominally 1.0 mg/mL of fluorescein sodium in *Diluent* prepared as follows. Transfer a portion of Injection equivalent to 10.0 mg of fluorescein sodium into a 10-mL volumetric flask. Add 1 mL of alcohol. Dilute with *Diluent* to volume and mix well.

Sample solution: Nominally 0.02 mg/mL of fluorescein sodium in *Diluent* from *Sample stock solution*

Chromatographic system

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

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Column temperature: 35°

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

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Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

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

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

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

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

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

▲ (USP 1-May-2019)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Add the following:

▲• **Organic Impurities**

Buffer, Mobile phase, Diluent, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard stock solution A: Use the *Standard stock solution* from the *Assay*.

Standard stock solution B: 0.05 mg/mL of fluorescein sodium in *Diluent* from *Standard stock solution A*

Standard stock solution C: 0.05 mg/mL each of USP Resorcinol RS, USP Phthalic Acid RS, and USP Fluorescein Related Compound C RS in *Diluent*

Standard solution: 0.001 mg/mL each of fluorescein sodium, USP Resorcinol RS, USP Phthalic Acid RS, and USP Fluorescein Related Compound C RS in *Diluent* from *Standard stock solution B* and *Standard stock solution C*

Sample solution: Use the *Sample stock solution* from the *Assay*.

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between resorcinol and phthalic acid

Relative standard deviation: NMT 5.0% for the resorcinol, phthalic acid, fluorescein related compound C, and fluorescein peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of resorcinol, phthalic acid, and fluorescein related compound C in the portion of Injection taken:

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

r_U = peak response of resorcinol, phthalic acid, or fluorescein related compound C from the *Sample solution*

r_S = peak response of the corresponding Reference Standard from the *Standard solution*

C_S = concentration of USP Resorcinol RS, USP Phthalic Acid RS, or USP Fluorescein Related Compound C RS in the *Standard solution* (mg/mL)

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

Calculate the percentage of any individual unspecified impurity in the portion of Injection taken:

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

r_U = peak response of each unspecified impurity from the *Sample solution*

r_S = peak response of fluorescein sodium from the *Standard solution*

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

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

Acceptance criteria: See *Table 2*.

Table 2

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Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Resorcinol	0.4	0.5
Phthalic acid	0.5	0.5
Fluorescein related compound C	0.9	0.5
Fluorescein	1.0	—
Any individual unspecified impurity	—	0.2
Total unspecified impurities	—	0.5

▲ (USP 1-May-2019)

SPECIFIC TESTS

- **Pyrogen Test ⟨151⟩:** Meets the requirements, the test dose being the equivalent of 250 mg/kg of fluorescein sodium
- **pH ⟨791⟩:** 8.0–9.8
- **Injections and Implanted Drug Products ⟨1⟩:** Meets the requirements

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in single-dose containers, preferably of Type I glass.

Change to read:

- **USP Reference Standards ⟨11⟩**

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

▲ USP Fluorescein Related Compound C RS

2-(2,4-Dihydroxybenzoyl)benzoic acid.

$C_{14}H_{10}O_5$ 258.23

USP Phthalic Acid RS

Phthalic acid.

$C_8H_6O_4$ 166.13

USP Resorcinol RS

Resorcinol.

$C_6H_6O_2$ 110.11

▲ (USP 1-May-2019)

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

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

Chromatographic Database Information: Chromatographic Database

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