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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*

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**