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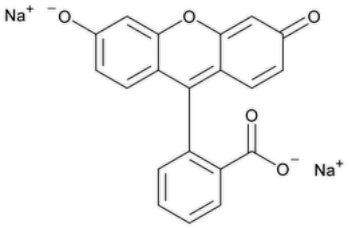
Fluorescein Sodium



$C_{20}H_{10}Na_2O_5$ 376.27

Benzoic acid, 2-(6-hydroxy-3-oxo-3*H*-xanthene-9-yl), sodium salt (1:2);

Fluorescein disodium salt CAS RN®: 518-47-8; UNII: 93X55PE38X.

DEFINITION

Fluorescein Sodium contains NLT 90.0% and NMT 102.0% of fluorescein sodium ($C_{20}H_{10}Na_2O_5$), calculated on the anhydrous basis.

IDENTIFICATION

• **A.** A solution 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.** **Identification Tests—General, *Sodium*⟨191⟩:** The residue remaining after incineration meets the requirements.

• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **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 is 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 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: 1.0 mg/mL of Fluorescein Sodium in *Diluent*

Sample solution: 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 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of fluorescein sodium ($C_{20}H_{10}Na_2O_5$) in the portion of Fluorescein Sodium 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 = concentration of Fluorescein Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–102.0% on the anhydrous basis

IMPURITIES

• **Zinc**

Sample solution: 10 mg/mL of Fluorescein Sodium in a saturated solution of sodium chloride

Analysis: To 10 mL of *Sample solution* add 2 mL of 3 N hydrochloric acid. Shake well, filter, and add 1 mL of potassium ferrocyanide TS to the filtrate.

Acceptance criteria: No turbidity is produced.

• **Acriflavine**

Sample solution: 2 mg/mL of Fluorescein Sodium in water. Swirl the mixture, and filter.

Analysis: To the *Sample solution* add a few drops of 100-mg/mL sodium salicylate solution.

Acceptance criteria: No precipitate is formed.

• **Organic Impurities**

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

Standard stock solution: Proceed as directed in the *Assay* for *Standard solution*.

Standard solution A: 0.005 mg/mL of fluorescein sodium in *Diluent* from *Standard stock solution*

Standard solution B: 0.005 mg/mL each of USP Resorcinol RS, USP Phthalic Acid RS, and USP Fluorescein Related Compound C RS in *Diluent*

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

System suitability

Sample: *Standard solution B*

Suitability requirements

Resolution: NLT 1.5 between resorcinol and phthalic acid

Analysis

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

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

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

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

r_S = peak response of resorcinol or phthalic acid or fluorescein related compound C from *Standard solution B*

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

C_U = concentration of Fluorescein Sodium in the *Sample solution* (mg/mL)

Calculate the percentage of unspecified impurity in the portion of Fluorescein Sodium taken:

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

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

r_S = peak response from *Standard solution A*

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

C_U = concentration of Fluorescein Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Resorcinol	0.42	0.5
Phthalic acid	0.48	0.5
Fluorescein related compound C	0.86	0.5
Fluorescein	1.0	—
Unspecified impurity	—	0.10
Total unspecified impurities	—	0.5

SPECIFIC TESTS

• **Water Determination, *Method I*(921):** NMT 17.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers.

• **USP Reference Standards (11)**

USP Diacetylfluorescein RS $C_{24}H_{16}O_7$ 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 $C_8H_6O_4$ 166.13

USP Resorcinol RS $C_6H_6O_2$ 110.11

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

Topic/Question	Contact	Expert Committee
FLUORESCEIN SODIUM	Documentary Standards Support	SM32020 Small Molecules 3
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SM32020 Small Molecules 3

Chromatographic Database Information: Chromatographic Database

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