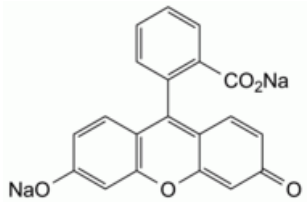
Edition: BP 2025 (Ph. Eur. 11.6 update)

Fluorescein Sodium

General Notices

Soluble Fluorescein

(Ph. Eur. monograph 1213)



$C_{20}H_{10}Na_2O_5$ 376.3 *518-47-8*

Action and use

Detection of corneal lesions, retinal angiography and pancreatic function testing.

Preparations

Fluorescein Eye Drops

Fluorescein Injection

Ph Eur

DEFINITION

Disodium 2-(6-oxido-3-oxo-3*H*-xanthen-9-yl)benzoate.

Content

95.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance

Orange-red, fine powder, hygroscopic.

Solubility

Freely soluble in water, soluble in ethanol (96 per cent), practically insoluble in hexane and in methylene chloride.

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. Dilute 0.1 mL of solution S (see Tests) to 10 mL with *water R*. The solution shows yellowish-green fluorescence. The fluorescence disappears on addition of 0.1 mL of *dilute hydrochloric acid R* and reappears on addition of 0.2 mL of *dilute sodium hydroxide solution R*.
B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation Discs.

Comparison *Ph. Eur. reference spectrum of fluorescein sodium*.

C. The absorption by a piece of filter paper of 0.05 mL of the solution prepared for identification A (before the addition of *dilute hydrochloric acid R*) colours the paper yellow. On exposing the moist paper to bromine vapour for 1 min and then to ammonia vapour, the colour becomes deep pink.
D. Ignite 0.1 g in a porcelain crucible. Dissolve the residue in 5 mL of *water R* and filter. 2 mL of the filtrate gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S

Dissolve 1.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50 mL with the same solvent.

Appearance of solution

Solution S is clear (*2.2.1*) and orange-yellow with yellowish-green fluorescence.

pH (*2.2.3*)

7.0 to 9.0 for solution S.

Related substances

Liquid chromatography (*2.2.29*).

Test solution (a) Dissolve 0.100 g of the substance to be examined in a mixture of 30 volumes of *acetonitrile R* and 70 volumes of mobile phase A and dilute to 100.0 mL with the same mixture of solvents.

Test solution (b) Dilute 5.0 mL of test solution (a) to 250.0 mL with a mixture of 30 volumes of *acetonitrile R* and 70 volumes of mobile phase A.

Reference solution (a) Dissolve 55.0 mg of *diacetylfluorescein CRS* in a mixture of 1 mL of *2.5 M sodium hydroxide* and 5 mL of *ethanol (96 per cent) R*, heat on a water-bath for 20 min mixing frequently, cool and dilute to 50.0 mL with *water R*. Dilute 5.0 mL of the solution to 250.0 mL with a mixture of 30 volumes of *acetonitrile R* and 70 volumes of mobile phase A.

Reference solution (b) Dissolve 10.0 mg of *phthalic acid R* (impurity B) and 10.0 mg of *resorcinol R* (impurity A) in a mixture of 30 volumes of *acetonitrile R* and 70 volumes of mobile phase A and dilute to 100.0 mL with the same mixture of solvents. Dilute 5.0 mL of the solution to 100.0 mL with a mixture of 30 volumes of *acetonitrile R* and 70 volumes of mobile phase A.

Reference solution (c) Dilute 5.0 mL of test solution (b) to 20.0 mL with a mixture of 30 volumes of *acetonitrile R* and 70 volumes of mobile phase A.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 35 °C.

Mobile phase:

— *mobile phase A*: dissolve 0.610 g of *potassium dihydrogen phosphate R* in *water R* and dilute to 1000 mL with the same solvent; adjust to pH 2.0 with *phosphoric acid R*;

— *mobile phase B*: *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	85 → 20	15 → 80
20 - 29	20	80
29 - 30	20 → 85	80 → 15
30 - 35	85	15

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µL of test solution (a) and reference solutions (b) and (c).

Relative retention With reference to fluorescein (retention time = about 15 min): impurity A = about 0.4; impurity B = about 0.5; impurity C = about 0.9.

System suitability Reference solution (b):

— *resolution*: minimum 1.5 between the peaks due to impurity A and impurity B.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity C by 1.6;

— *impurities A, B*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.5 per cent);

— *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);

— *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent);

— *sum of impurities other than A, B, C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent);

— *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Chlorides (*2.4.4*)

Maximum 0.25 per cent.

To 10 mL of solution S add 90 mL of *water R* and 1 mL of *dilute nitric acid R*, wait for at least 10 min and filter. Dilute 10 mL of the filtrate to 15 mL with *water R*.

Sulfates (*2.4.13*)

Maximum 1.0 per cent.

To 5 mL of solution S add 90 mL of *distilled water R*, 2.5 mL of *dilute hydrochloric acid R* and dilute to 100 mL with *distilled water R*. Filter.

Zinc

Dilute 5 mL of solution S to 10 mL with *water R*. Add 2 mL of *hydrochloric acid R1*, filter and add 0.1 mL of *potassium ferrocyanide solution R*. No turbidity or precipitate is formed immediately.

Loss on drying (*2.2.32*)

Maximum 10.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection Test solution (b) and reference solution (a).

Calculate the percentage content of $C_{20}H_{10}Na_2O_5$ using the chromatogram obtained with reference solution (a) and the declared content of *diacetylfluorescein CRS*.

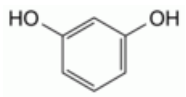
1 mg of *diacetylfluorescein CRS* is equivalent to 0.9037 mg of $C_{20}H_{10}Na_2O_5$.

STORAGE

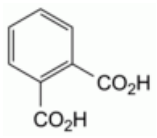
In an airtight container, protected from light.

IMPURITIES

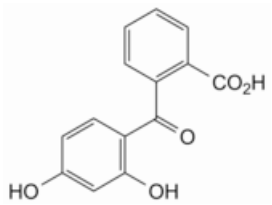
Specified impurities *A, B, C.*



A. benzene-1,3-diol (resorcinol),



B. benzene-1,2-dicarboxylic acid (phthalic acid),



C. 2-(2,4-dihydroxybenzoyl)benzoic acid.