

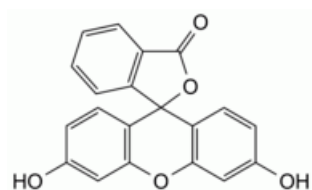


Edition: BP 2025 (Ph. Eur. 11.6 update)

Fluorescein

[General Notices](#)

(Ph. Eur. monograph 2348)



$C_{20}H_{12}O_5$ 332.3 2321-07-5

Action and use

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

Ph Eur

DEFINITION

3',6'-Dihydroxy-3*H*-spiro[isobenzofuran-1,9'-xanthen]-3-one.

Content

97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

Orange-red, fine powder.

Solubility

Practically insoluble in water, soluble in hot ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification: A, D.

Second identification: B, C, D.



A. Infrared absorption spectrophotometry ([2.2.24](#)).

Comparison [fluorescein CRS](#).

Dissolve the substance to be examined and the reference substance separately in the minimum volume of [ethanol \(96 per cent\) R](#), evaporate to dryness and record the spectra using the residues.

B. Dilute 0.1 mL of solution S (see Tests) to 10 mL with [water R](#). The solution shows a 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](#).

C. The absorption by a piece of filter paper of 0.05 mL of the solution prepared for identification B (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. Suspend 0.5 g in 50 mL of [water R](#) and shake for 10 min. The substance does not completely dissolve.

TESTS

Solution S

Suspend 1.0 g in 35.0 mL of [water R](#) and add dropwise with shaking 4.5 mL of [1 M sodium hydroxide](#). Adjust to pH 8.5-9.0 with [1 M sodium hydroxide](#) and dilute to 50.0 mL with [water R](#) to obtain a clear solution.

Appearance of solution

Solution S is clear ([2.2.1](#)) and orange-red with yellowish-green fluorescence.

Related substances

Liquid chromatography ([2.2.29](#)).

Solvent mixture [acetonitrile for chromatography R](#), mobile phase A (30:70 V/V).

Test solution (a) Disperse 50.0 mg of the substance to be examined in 15.0 mL of [ethanol \(96 per cent\) R](#). Sonicate and dilute to 50.0 mL with the solvent mixture.

Test solution (b) Dilute 5.0 mL of test solution (a) to 250.0 mL with the solvent mixture.

Reference solution (a) Disperse 50.0 mg of [fluorescein CRS](#) in 15.0 mL of [ethanol \(96 per cent\) R](#). Sonicate and dilute to 50.0 mL with the solvent mixture. Dilute 5.0 mL of this solution to 250.0 mL with the solvent mixture.

Reference solution (b) Dissolve 10.0 mg of [phthalic acid CRS](#) (impurity B) and 10.0 mg of [resorcinol CRS](#) (impurity A) in the solvent mixture and dilute to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 100.0 mL with the solvent mixture.

Reference solution (c) Dilute 5.0 mL of test solution (b) to 20.0 mL with the solvent mixture.

Reference solution (d) Dilute 10.0 mL of reference solution (c) to 100.0 mL with the solvent mixture.

Reference solution (e) Dissolve the contents of a vial of [fluorescein impurity C CRS](#) in 1 mL of the solvent mixture.

Column:

— size: $l = 0.25$ m, $\varnothing = 4.6$ mm;

— stationary phase: [octylsilyl silica gel for chromatography R3](#) (5 μ m);

— temperature: 35 °C.

Mobile phase:

— mobile phase A: dissolve 0.610 g of [potassium dihydrogen phosphate R](#) in [water for chromatography R](#), adjust to pH 2.0 with [phosphoric acid R](#) and dilute to 1000.0 mL with [water for chromatography 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

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 220 nm.

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

Identification of impurity C Use the chromatogram obtained with reference solution (e) to identify the peak due to impurity C.

Relative retention With reference to fluorescein (retention time = about 15 min): impurity A = about 0.42; impurity B = about 0.48; impurity C = about 0.86.

System suitability Reference solution (b):

— *resolution*: minimum 2.0 between the peaks due to impurities A and B.

Limits:

— *correction factor*: for the calculation of content, multiply the peak area of impurity C by 1.9;

— *impurity C*: not more than 1.2 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.6 per cent);

— *impurities A, B*: for each impurity, not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 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 and C*: not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);

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

Chlorides (2.4.4)

Maximum 0.25 per cent.

To 10.0 mL of solution S add 90.0 mL of [water R](#) and 3.0 mL of [dilute nitric acid R](#), wait for at least 30 min and filter. Dilute 10.0 mL of the filtrate to 15.0 mL with [water R](#).

Loss on drying (2.2.32)

Maximum 1.0 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).

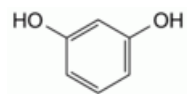
Calculate the percentage content of C₂₀H₁₂O₅ taking into account the assigned content of [fluorescein CRS](#).

STORAGE

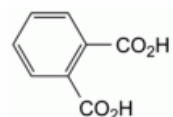
Protected from light.

IMPURITIES

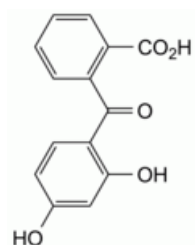
Specified impurities A, B, C.



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



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



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

Ph Eur