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

Fluorescein Eye Drops

General Notices

Action and use

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

DEFINITION

Fluorescein Eye Drops are a sterile solution of Fluorescein Sodium in Purified Water.

The eye drops comply with the requirements stated under Eye Preparations and with the following requirements.

Content of fluorescein sodium, $C_{20}H_{10}Na_2O_5$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Evaporate a volume containing 20 mg of fluorescein sodium and dry at 105° for 30 minutes. The *infrared absorption spectrum* of the residue, Appendix II A, is concordant with the *reference spectrum* of fluorescein sodium *(RS 151)*.
B. The eye drops are strongly fluorescent, even in extreme dilution; the fluorescence disappears when the solution is made acidic and reappears when it is made alkaline.
C. Dilute with *water* to produce a solution containing 0.05% w/v of fluorescein sodium. One drop of the solution, absorbed by a piece of filter paper, colours the paper yellow. On exposing the moist paper to bromine vapour for 1 minute and then to ammonia vapour the yellow colour becomes deep pink.

TESTS

Acidity or alkalinity

pH, 7.0 to 9.0, Appendix V L.

Chloroform-soluble matter

To a volume containing 0.1 g of fluorescein sodium add 1 mL of 2M *sodium hydroxide*, extract with 10 mL of *chloroform* and dry the chloroform layer with *anhydrous sodium sulfate*. The *absorbance* of the resulting solution at 480 nm is not more than 0.05, Appendix II B, using *chloroform* in the reference cell.

Related substances and resorcinol

Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

(1) Dilute the eye drops with 0.1M *methanolic hydrochloric acid* to contain 0.5% w/v of fluorescein sodium.

(2) Add to a volume of the eye drops containing 25 mg of fluorescein sodium 2 mL of *phosphate buffer pH 8.0*, 3 mL of *water* and 2.5 g of *sodium chloride*, shake to dissolve the sodium chloride and extract with two 25-mL quantities of *peroxide-free ether*. Dry the combined extracts over *anhydrous sodium sulfate*, evaporate to dryness under reduced pressure and dissolve the residue in 1 mL of 0.1M *methanolic hydrochloric acid*.
(3) Dilute 1 volume of solution (1) to 100 volumes with 0.1M *methanolic hydrochloric acid*.
(4) Dilute 2 volumes of solution (3) to 5 volumes with 0.1M *methanolic hydrochloric acid*.
(5) 0.0125% w/v of *resorcinol* in 0.1M *methanolic hydrochloric acid*.
(6) Mix 5 volumes of a 0.025% w/v solution of *resorcinol* in 0.1M *methanolic hydrochloric acid* with 2 volumes of solution (1) and add sufficient *methanol* to produce 10 mL.

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel 60 F_{254} precoated plate (Merck plates are suitable).
(b) Use the mobile phase as described below.
(c) Apply 10 µL of solution (1) and 5 µL of solutions (2) to (6).
(d) Develop the plate to 15 cm.
(e) After removal of the plate, dry in air, expose the plate to iodine vapour for 30 minutes and examine in daylight and under *ultraviolet light (254 nm)*.

MOBILE PHASE

10 volumes of *methanol* and 90 volumes of *dichloromethane*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (6) shows two clearly separated spots in daylight.

LIMITS

In the chromatogram obtained with solution (1), under *ultraviolet light (254 nm)*:

any *secondary spot*, other than any spot corresponding to resorcinol, is not more intense than the spot in the chromatogram obtained with solution (3) (0.5%);

not more than one such spot is more intense than the spot in the chromatogram obtained with solution (4) (0.2%).

In the chromatogram obtained with solution (2), in daylight:

any spot corresponding to resorcinol in the chromatogram obtained with solution (2) is not more intense than the spot in the chromatogram obtained with solution (5) (0.5%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute the eye drops with the mobile phase to produce a solution containing 0.005% w/v of fluorescein sodium.
(2) Dissolve 55 mg of *diacetylfluorescein BPCRS* in a mixture of 5 mL of *ethanol (96%)* and 1 mL of 2.5M *sodium hydroxide*, heat on a water bath for 20 minutes, mixing frequently, cool and add sufficient *water* to produce 50 mL. Dilute 5 volumes of this solution to 100 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (5 µm) (Spherisorb ODS 2 is suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 1.5 ml per minute.
(d) Use an ambient column temperature.
(e) Use a detection wavelength of 254 nm.
(f) Inject 20 µL of each solution.

MOBILE PHASE

5 volumes of *triethylamine*, 400 volumes of *acetonitrile* and 595 volumes of *water*, adjust the pH to 3.0 with *orthophosphoric acid*.

DETERMINATION OF CONTENT

Calculate the content of $C_{20}H_{10}Na_2O_5$ in the eye drops using the declared content of anhydrous diacetylfluorescein in *diacetylfluorescein BPCRS*. Each mg of anhydrous diacetylfluorescein is equivalent to 0.9037 mg of $C_{20}H_{10}Na_2O_5$.

STORAGE

Fluorescein Eye Drops should be protected from light.