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

Folic Acid Injection

General Notices

NOTE: This monograph has been developed to cover unlicensed formulations.

Action and use

Vitamin B component.

DEFINITION

Folic Acid Injection is a sterile solution of Folic Acid Hydrate in a suitable liquid.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements. Where appropriate, the injection also complies with the requirements stated under Unlicensed Medicines.

Content of folic acid, $C_{19}H_{19}N_7O_6$

90.0 to 110.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.
(1) Dilute a quantity of the injection containing the equivalent of 15 mg of folic acid with sufficient of a mixture of 1 volume of 13.5M *ammonia* and 9 volumes of *methanol* to produce 30 mL.
(2) 0.05% w/v of *folic acid BPCRS* in a mixture of 2 volumes of 13.5M *ammonia* and 9 volumes of *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel G*.
(b) Use the mobile phase as described below.
(c) Apply 2 µL of each solution.
(d) Develop the plate to 15 cm.
(e) After removal of the plate, dry in air and examine under *ultraviolet light (365 nm)*.

MOBILE PHASE

20 volumes of 13.5M *ammonia*, 20 volumes of *propan-1-ol* and 60 volumes of *ethanol (96%)*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position, fluorescence and size to that in the chromatogram obtained with solution (2).

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity or alkalinity

pH, 7.0 to 9.0, Appendix V L.

Hydrolysis products

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

(1) Dilute a quantity of the injection with sufficient of the mobile phase to produce a solution containing the equivalent of 0.01% w/v of folic acid.
(2) 0.00005% w/v of *4-aminobenzoic acid* and 0.0002% w/v of *N*-(4-aminobenzoyl)-L-glutamic acid in the mobile phase.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (10 µm) (Spherisorb ODS 1 is suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 2 mL per minute.
(d) Use an ambient column temperature.
(e) Use a detection wavelength of 269 nm.
(f) Inject 20 µL of each solution.

In the chromatogram obtained with solution (2) the substances elute in the following order: *N*-(4-aminobenzoyl)-L-glutamic acid; 4-aminobenzoic acid.

MOBILE PHASE

0.05M *potassium dihydrogen orthophosphate*, adjusted to pH 5.5 with 5M *sodium hydroxide*.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the two principal peaks is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to 4-aminobenzoic acid is not greater than the area of the peak due to 4-aminobenzoic acid in the chromatogram obtained with solution (2) (0.5%);

the area of any peak corresponding to *N*-(4-aminobenzoyl)-L-glutamic acid is not greater than the area of the peak due to *N*-(4-aminobenzoyl)-L-glutamic acid in the chromatogram obtained with solution (2) (2%).

ASSAY

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

(1) Dilute a quantity of the injection containing the equivalent of 7.5 mg of folic acid to 100 mL with a buffer solution prepared by diluting 25 volumes of 0.1M *anhydrous sodium dihydrogen orthophosphate*, adjusted to pH 4.0 with 0.1M *sodium hydroxide*, to 250 volumes with *water*.
(2) 0.0075% w/v of *folic acid BPCRS* in the buffer solution.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Lichrosorb RP-18 is suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 1.2 mL per minute.
(d) Use an ambient column temperature.
(e) Use a detection wavelength of 280 nm.
(f) Inject 20 µL of each solution.

MOBILE PHASE

4 volumes of *acetonitrile*, 6 volumes of *methanol* and 90 volumes of 0.01M *sodium acetate*, adjusted to pH 4.5 with *acetic acid*.

DETERMINATION OF CONTENT

Calculate the content of $C_{19}H_{19}N_7O_6$ in the injection using the declared content of $C_{19}H_{19}N_7O_6$ in *folic acid BPCRS*.