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

Folic Acid Tablets

General Notices

Action and use

Vitamin B component.

DEFINITION

Folic Acid Tablets contain Folic Acid Hydrate.

The tablets comply with the requirements stated under Tablets and with the following requirements.

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

90.0 to 110.0% of the stated amount.

IDENTIFICATION

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

(1) Extract a quantity of the powdered tablets containing the equivalent of 0.5 mg of folic acid with 1 mL of a mixture of 1 volume of 13.5M *ammonia* and 9 volumes of *methanol*, centrifuge and use the supernatant liquid.
(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).

TESTS

Hydrolysis products

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

(1) Shake a quantity of the powdered tablets containing the equivalent of 5.0 mg of folic acid with 50 mL of the mobile phase, centrifuge and use the supernatant liquid.
(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 and 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 peaks is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1):

the areas of the peaks corresponding to 4-aminobenzoic acid and *N*-(4-aminobenzoyl)-L-glutamic acid are not greater than the areas of the peaks corresponding to 4-aminobenzoic acid and *N*-(4-aminobenzoyl)-L-glutamic acid in the chromatogram obtained with solution (2) (0.5% and 2% respectively).

Uniformity of content

Tablets containing the equivalent of less than 2 mg and/or less than 2% w/w of folic acid comply with the requirements stated under Tablets using the following method of analysis. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake one tablet with 5 mL of 0.1M *sodium hydroxide*, add sufficient mobile phase to produce a solution containing the equivalent of 0.001% w/v of folic acid, centrifuge and use the supernatant liquid.
(2) Add 1 mL of 0.5M *hydrochloric acid* to 5 mL of a 0.0020% w/v solution of *folic acid BPCRS* in 0.1M *sodium hydroxide* and dilute to 10 mL with 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 283 nm.
(f) Inject 20 µL of each solution.

MOBILE PHASE

7 volumes of *acetonitrile* and 93 volumes of 0.05M *potassium dihydrogen orthophosphate* adjusted to pH 6.0 with 5M *sodium hydroxide*.

DETERMINATION OF CONTENT

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

ASSAY

For tablets containing the equivalent of less than 2 mg and/or less than 2% w/w of folic acid

Use the average of the individual results determined in the test for Uniformity of content.

For tablets containing the equivalent of 2 mg or more and 2% w/w or more of folic acid

Weigh and powder 20 tablets. Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions protected from light.

(1) Shake a quantity of the powdered tablets containing the equivalent of 20 mg of folic acid with 50 mL of 0.1M *sodium hydroxide*, dilute to 100 mL with the same solvent, centrifuge and dilute 5 mL of the supernatant liquid to 100 mL with the mobile phase.
(2) Dilute 5 mL of a 0.020% w/v solution of *folic acid BPCRS* in 0.1M *sodium hydroxide* to 100 mL with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Uniformity of content may be used.

DETERMINATION OF CONTENT

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

STORAGE

Folic Acid Tablets should be protected from light.