Folic acid (Acidum folicum) Molecular formula. C₁₉H₁₉N₇O₆

19.19.7-6

Relative molecular mass. 441.4

Graphic formula.



Chemical name. *N*-[*p*-[[(2-Amino-4-hydroxy-6-pteridinyl)methyl]amino]-benzoyl]-L-glutamic acid; *N*-[4-[[(2-amino-1,4-dihydro-4-oxo-6-pteridinyl)-methyl]amino]benzoyl]-L-glutamic acid; CAS Reg. No. 59-30-3.

Description. A yellow or yellowish orange, crystalline powder; odourless or almost odourless.

Solubility. Very slightly soluble in water; practically insoluble in ethanol (~750 g/l) TS, acetone R and ether R.

Category. Haemopoietic.

Storage. Folic acid should be kept in a well-closed container, protected from light.

Requirements

Definition. Folic acid contains not less than 96.0% and not more than 102.0% of $C_{19}H_{19}N_7O_6$, calculated with reference to the anhydrous substance.

Identity tests

A. The absorption spectrum of a 15 μ /mL solution in sodium hydroxide (0.1 mol/l) VS, when observed between 230 nm and 380 nm, exhibits 3 maxima at about 256 nm, 283 nm, and 365 nm. The absorbances at these wavelengths are about 0.82, 0.80 and 0.28, respectively (preferably use 2-cm cells for the measurements and calculate the absorbances of 1-cm layers). The ratio of the absorbance of a 1-cm layer at 256 nm to that at 365 nm is between 2.80 and 3.00.

B. Carry out the test as described under <u>1.14.1 Chromatography</u>, Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of 2 volumes of 1-propanol R, 1 volume of ethanol (~750 g/l) TS, and 2 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 2 μ l of each of 2 solutions in a mixture of 9 volumes of methanol R and 1 volume of ammonia (~260 g/l) TS containing (A) 0.50 mg of the test substance per mL and (B) 0.50 mg of folic acid RS per mL. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (365 nm). The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

Sulfated ash. Not more than 2.0 mg/g.

Water. Determine as described under <u>2.8 Determination of water by the Karl Fischer method</u>, Method A, using about 0.15 g of the substance; the water content is not less than 70 mg/g and not more than 90 mg/g.

Free amines. The ratio of the absorbance A_T of the test solution T_2 to the absorbance A_B of the blank solution B_1 , as measured in the assay, should be larger than 6.

Assay. Prepare the test solution T by dissolving about 0.050 g, accurately weighed, in 50 mL of sodium hydroxide (~80 g/l) TS, mixing and diluting with sodium hydroxide (~80 g/l) TS to 100 mL.

Transfer 30.0 mL of the test solution to a 100-mL volumetric flask (test solution T_1), and a second aliquot of 30.0 mL of the test solution to a second 100-mL volumetric flask (blank B_1). To both solutions, T_1 and blank B_1 add 20 mL of hydrochloric acid (~70 g/l) TS and dilute them both with water to volume. Retain blank solution B_1 . To 60 mL of the test solution T_1 add 0.5 g of zinc R powder, and allow to stand, shaking frequently, for 20 minutes. Filter the mixture through a dry filter paper, discard the first 10 mL of the filtrate, and dilute 10 mL of the subsequent filtrate with water to 100 mL (test solution T_2).

Into three separate 25-mL volumetric flasks place 5.0 mL each of test solution T_2 , of blank solution B_1 and of water (solution B_2), add to each of them 1 mL of water, 1 mL of hydrochloric acid (~70 g/l) TS, and 1 mL of sodium nitrite (1 g/l) TS, mix well and allow to stand for 2 minutes. Then add to each of them 1 mL of ammonium sulfamate (5 g/l) TS, mix thoroughly, allow to stand for 2 minutes, add 1 mL of *N*-(1-naphthyl)ethylenediamine hydrochloride (1 g/l) TS, shake, allow to stand for 10 minutes, and dilute

with water to volume.

Measure the absorbance of the test solution T_2 and of the blank solution B_1 against a solvent cell containing solution B_2 at the maximum of about 550 nm; designate these as A_T and A_B , respectively.

Carry out a similar procedure using folic acid RS and designate the respective absorbances as A_S and A_{BS} .

Calculate the content of $C_{19}H_{19}N_7O_6$ in terms of the percentage of anhydrous substance in the test substance, using the formula: 100(10 A_T - A_B)/(10 A_S - A_{BS}), if necessary multiplying the result by the declared content (%) of $C_{19}H_{19}N_7O_6$ in the chemical reference substance.