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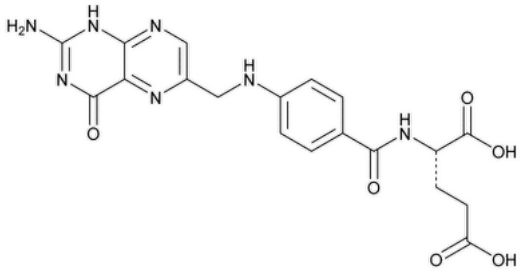
# Folic Acid



$C_{19}H_{19}N_7O_6$ 441.40

L-Glutamic acid, *N*-[4-[[(2-amino-1,4-dihydro-4-oxo-6-pteridinyl)methyl]amino]benzoyl]-;

*N*-[*p*-[[(2-Amino-4-hydroxy-6-pteridinyl)methyl]amino]benzoyl]-L-glutamic acid CAS RN®: 59-30-3; UNII: 935E97BOY8.

## DEFINITION

Folic Acid contains NLT 97.0% and NMT 102.0% of folic acid ($C_{19}H_{19}N_7O_6$), calculated on the anhydrous basis.

## IDENTIFICATION

***Change to read:***

• **A.** ▲**Spectroscopic Identification Tests ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: 197U**▲ (CN 1-May-2020)

**Sample solution:** 10 µg/mL in 0.1 N sodium hydroxide solution

**Acceptance criteria:** Meets the requirements. The ratio $A_{256}/A_{365}$ is 2.80–3.00.

## ASSAY

• **Procedure**

[Note—Use low-actinic glassware throughout the following procedure.]

**3 N phosphoric acid:** 98 g/L of phosphoric acid in water

**6 N ammonium hydroxide:** Dilute 40 mL of ammonium hydroxide with water to 100 mL.

**Mobile phase:** Transfer 2.0 g of monobasic potassium phosphate into a 1000-mL volumetric flask, and dissolve in 650 mL of water. Add 15.0 mL of a solution of 0.5 M tetrabutylammonium hydroxide in methanol, 7.0 mL of *3 N phosphoric acid*, and 270 mL of methanol. Cool to room temperature, adjust with *3 N phosphoric acid* or *6 N ammonium hydroxide* to a pH of 5.0, and dilute with water to volume. Recheck the pH before use.

**Internal standard solution:** 2 mg/mL of methylparaben in *Mobile phase*. Dissolve the methylparaben first with methanol (about 4% of the final volume), and dilute with *Mobile phase* to volume.

**Standard stock solution:** 1 mg/mL of USP Folic Acid RS in *Mobile phase*. Dissolve the folic acid with the aid of 10% ammonium hydroxide (about 1% of the final volume), and dilute with *Mobile phase* to volume.

**Standard solution:** Transfer 4.0 mL of *Standard stock solution* and 4.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

**Sample stock solution:** Transfer 100 mg of Folic Acid to a 100-mL volumetric flask, and dissolve in 40 mL of *Mobile phase* and 1 mL of 10% ammonium hydroxide. Dilute with *Mobile phase* to volume.

**Sample solution:** Transfer 4.0 mL of *Sample stock solution* and 4.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

**Chromatographic system**

(See *Chromatography ⟨621⟩, System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.0-mm × 25-cm; packing L1

**Flow rate:** 1.2 mL/min

**Injection size:** 10 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Resolution:** NLT 3.6 between methylparaben and folic acid

**Relative standard deviation:** NMT 2.0% for the ratios of the folic acid peak area to the internal standard peak area

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of folic acid ($C_{19}H_{19}N_7O_6$) in the sample taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$ = internal standard ratio (peak response of folic acid/peak response of the internal standard) from the *Sample solution*

$R_S$ = internal standard ratio (peak response of folic acid/peak response of the internal standard) from the *Standard solution*

$C_S$ = concentration of USP Folic Acid RS in the *Standard stock solution* (mg/mL)

$C_U$ = concentration of Folic Acid in the *Sample stock solution* (mg/mL)

**Acceptance criteria:** 97.0%–102.0% on the anhydrous basis

## IMPURITIES

• **Residue on Ignition ⟨281⟩:** NMT 0.3%

• **Related Compounds**

**3 N phosphoric acid, 6 N ammonium hydroxide, Internal standard solution, Standard stock solution, Standard solution,** and

**Chromatographic system:** Proceed as directed in the *Assay.*

**Sample solution:** Use the *Sample stock solution*, prepared as directed in the *Assay.*

**Analysis**

**Sample:** *Sample solution*

Allow the *Sample solution* to elute for NLT 2 times the retention time of folic acid. Record the chromatogram, and measure the areas of all the peaks.

Calculate the percentage of total secondary peaks in the portion of Folic Acid taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$ = sum of the areas of all the peaks except that of the folic acid peak

$r_T$ = sum of the areas of all the peaks

**Acceptance criteria:** NMT 2.0%

## SPECIFIC TESTS

• **Water Determination, *Method I*⟨921⟩**

**Analysis:** Proceed as directed in the chapter, except stir the methanol solvent before and during the addition of the test specimen and during the titration.

**Acceptance criteria:** NMT 8.5%

## ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed, light-resistant containers.

• **USP Reference Standards ⟨11⟩**

USP Folic Acid RS



**Auxiliary Information** - Please check for your question in the FAQs before contacting USP.

| Topic/Question | Contact | Expert Committee |
|---|---|---|
| FOLIC ACID | Natalia Davydova<br>Scientific Liaison | NBDS2020 Non-botanical Dietary Supplements |
| REFERENCE STANDARD SUPPORT | RS Technical Services<br>RSTECH@usp.org | NBDS2020 Non-botanical Dietary Supplements |

**Chromatographic Database Information:** Chromatographic Database

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