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

Folic Acid Injection

» Folic Acid Injection is a sterile solution of Folic Acid in Water for Injection prepared with the aid of Sodium Hydroxide or Sodium Carbonate. It contains not less than 95.0 percent and not more than 110.0 percent of the labeled amount of folic acid ($C_{19}H_{19}N_7O_6$).

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

USP Reference standards ⟨11⟩—
USP Folic Acid RS
USP Endotoxin RS

Identification—To a volume of the Injection equivalent to about 100 mg of folic acid add water to make about 25 mL. Adjust with hydrochloric acid to a pH of 3.0, cool to 5°, then filter, and wash the precipitate of folic acid with cold water until the last washing shows an absence of chloride. Then wash with acetone, and dry at 80° for 1 hour: the UV absorption spectrum of a 1 in 100,000 solution of the folic acid so obtained in sodium hydroxide solution (1 in 250) exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Folic Acid RS, concomitantly measured. The ratio A_{256}/A_{365} is between 2.80 and 3.00.

Bacterial Endotoxins Test ⟨85⟩—It contains not more than 357.1 USP Endotoxin Units per mg of folic acid.

pH ⟨791⟩: between 8.0 and 11.0.

Other requirements—It meets the requirements under *Injections and Implanted Drug Products* ⟨1⟩.

Assay—

Mobile phase, System suitability solution, Standard preparation, and *Chromatographic system*—Proceed as directed in the *Assay* under *Folic Acid Tablets*.

Assay preparation—Dilute an accurately measured volume of Injection, quantitatively and stepwise, with an aqueous solvent containing 2 mL of ammonium hydroxide and 1 g of sodium perchlorate per 100 mL, to obtain a solution having a concentration close to that of the *Standard preparation* and between 0.20 and 0.80 mg per mL.

Procedure—Proceed as directed in the *Assay* under *Folic Acid Tablets*, and calculate the quantity, in mg, of folic acid ($C_{19}H_{19}N_7O_6$) in each mL of the Injection.

Folic Acid Compounded Oral Solution

DEFINITION

Folic Acid Compounded Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of folic acid ($C_{19}H_{19}N_7O_6$).

Prepare Folic Acid Compounded Oral Solution, 1 mg/mL, as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* ⟨795⟩).

Folic Acid	100 mg
Sodium Bicarbonate	1000 mg
Glycerin	35 mL
Purified Water, a sufficient quantity to make	100 mL