

FOLIC ACID ASSAY

The following procedure is provided for the estimation of folic acid as an ingredient of Pharmacopeial preparations containing other active constituents.

Mobile Phase— Place 2.0 g of monobasic potassium phosphate in a 1-liter volumetric flask, and dissolve in about 650 mL of water. Add 12.0 mL of a 1 in 4 solution of tetrabutylammonium hydroxide in methanol, 7.0 mL of 3 N phosphoric acid, and 240 mL of methanol. Cool to room temperature, adjust with either 3 N phosphoric acid or 6 N ammonium hydroxide to a pH of 7.0, dilute with water to volume, and mix. Pass through a 0.45- μ m filter, and recheck the pH before use. [note—The methanol-to-water ratio may be varied by up to 3 percent and the pH may be increased up to 7.15 to achieve better separation.]

Diluting Solvent— Prepare as directed under Mobile Phase. Adjust to a pH of 7.0, and bubble nitrogen through the solution for 30 minutes before use.

Internal Standard Solution— Dissolve about 25 mg of methylparaben in 2.0 mL of methanol, dilute with Diluting Solvent to 50 mL, and mix.

Standard Folic Acid Solution— Transfer about 12 mg of [USP Folic Acid RS](#), accurately weighed, to a low-actinic, 50-mL volumetric flask, dissolve in 2 mL of ammonium hydroxide, dilute with Diluting Solvent to volume, and mix.

Standard Preparation— Transfer 2.0 mL of Standard Folic Acid Solution to a low-actinic, 25-mL volumetric flask, add 2.0 mL of Internal Standard Solution, add Diluting Solvent to volume, and mix.

Assay Preparation— Transfer an accurately weighed or measured portion of the preparation to be assayed, containing about 1 mg of folic acid, to a low-actinic, 50-mL volumetric flask, add 4.0 mL of Internal Standard Solution, add Diluting Solvent to volume, and mix.

Chromatographic System— The liquid chromatograph is equipped with a 280-nm detector and a 15-cm \times 3.9-mm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the Standard Preparation, and record the peak responses as directed for Procedure: there is baseline separation of folic acid and methylparaben.

Procedure— Separately inject equal volumes (about 10 μ L) of Standard Preparation and Assay Preparation into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.8 for folic acid and 1.0 for methylparaben.

Calculate the quantity, in μg , of $\text{C}_{19}\text{H}_{19}\text{N}_7\text{O}_6$ in the portion of the preparation taken by the formula:

$$50C(\text{RU} / \text{RS})$$

in which C is the concentration, in μg per mL, of USP Folic Acid RS in the Standard Preparation; and RU and RS are the ratios of the response of the folic acid peak to that of the methylparaben peak obtained from the Assay Preparation and the Standard Preparation, respectively.