

metry. Determine the absorbances,  $A_T$  and  $A_S$ , of subsequent solutions of the sample solution and the standard solution at 550 nm: the content of free amines is not more than 1.0%.

$$\text{Content (\% of free amines)} = \frac{A_T}{A_S} \times \frac{W'}{W}$$

$W$ : Weighed amount (mg) of Folic Acid, calculated on the anhydrous basis.

$W'$ : Weighed amount (mg) of *p*-Aminobenzoylglutamic Acid Reference Standard.

**Water** Take 5 mL of pyridine for water determination and 20 mL of methanol for Karl Fischer method in a dried titration flask, and titrate with Karl Fischer TS until the solution reaches the end point. Weigh accurately about 0.2 g of Folic Acid, immediately place in the titration flask, and add a known excess volume of Karl Fischer TS. Mix well for 30 minutes, and perform the test: the water content is not more than 8.5%.

**Residue on ignition** Not more than 0.5% (1 g).

**Assay** Weigh accurately about 0.05 g each of Folic Acid and Folic Acid Reference Standard. To each add 50 mL of dilute sodium hydroxide TS, mix well to dissolve, add dilute sodium hydroxide TS to make exactly 100 mL, and use these solutions as the sample solution and the standard solution. To 30 mL each of these solutions, accurately measured, add 20 mL of dilute hydrochloric acid and water to make exactly 100 mL. To 60 mL each of these solutions add 0.5 g of zinc powder, and allow to stand with frequent shaking for 20 minutes. Filter each mixture through a dry filter paper, and discard the first 10 mL of the filtrate. Pipet 10 mL each of the subsequent filtrate, and add water to make exactly 100 mL. To 4 mL each of solutions, accurately measured, add 1 mL of water, 1 mL of dilute hydrochloric acid and 1 mL of a solution of sodium nitrite (1 in 1000), mix well, and allow to stand for 2 minutes. To each solution add 1 mL of a solution of ammonium amidosulfate (1 in 200), mix thoroughly, and allow to stand for 2 minutes. To each of these solutions, add 1 mL of a solution of *N*-(1-naphthyl)-*N'*-diethylethylenediamine oxalate (1 in 1000), shake, allow to stand for 10 minutes, and add water to make exactly 20 mL. Separately, to 30 mL of the sample solution, accurately measured, add 20 mL of dilute hydrochloric acid and water to make exactly 100 mL. Pipet 10 mL of this solution, add 18 mL of dilute hydrochloric acid and water to make exactly 100 mL. Pipet 4 mL of this solution, and prepare the blank solution in the same manner as the sample solution. Perform the test with these solutions as directed under the Ultraviolet-visible Spectrophotometry, using a solution prepared with 4 mL of water in the same manner as a blank. Determine the absorbances,  $A_T$ ,  $A_S$  and  $A_C$ , of the subsequent solution of the sample solution, the standard solution and the blank solution at 550 nm.

$$\begin{aligned} \text{Amount (mg) of } C_{19}H_{19}N_7O_6 \\ = \text{amount (mg) of Folic Acid Reference Standard,} \\ \text{calculated on the anhydrous basis} \\ \times \frac{A_T - A_C}{A_S} \end{aligned}$$

**Containers and storage** Containers—Tight containers. Storage—Light-resistant.

## Folic Acid Injection

葉酸注射液

Folic Acid Injection is an aqueous solution for injection. It contains not less than 95% and not more than 115% of the labeled amount of folic acid ( $C_{19}H_{19}N_7O_6$ ; 441.40).

**Method of preparation** Dissolve Folic Acid in water with the aid of Sodium Hydroxide or Sodium Carbonate, and prepare as directed under Injections.

**Description** Folic Acid Injection is a yellow to orange-yellow, clear liquid.

pH: 8.0 – 11.0

**Identification (1)** To a volume of Folic Acid Injection, equivalent to 1.5 mg of Folic Acid according to the labeled amount, add dilute sodium hydroxide TS to make 100 mL. Proceed as directed in the Identification (2) under Folic Acid, using this solution as the sample solution.

(2) Determine the absorption spectrum of the sample solution obtained in (1) as directed under the Ultraviolet-visible Spectrophotometry: it exhibits maxima between 255 nm and 257 nm, between 281 nm and 285 nm and between 361 nm and 369 nm. Separately, determine the maximal absorbances of the sample solution,  $A_1$  and  $A_2$ , between 255 nm and 257 nm and between 361 nm and 369 nm, respectively: the ratio of  $A_1/A_2$  is between 2.80 and 3.00.

(3) Folic Acid Injection responds to the Qualitative Test (1) for sodium salt.

**Assay** To an exactly measured volume of Folic Acid Injection, equivalent to about 0.05 g of folic acid ( $C_{19}H_{19}N_7O_6$ ) add dilute sodium hydroxide TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.05 g of Folic Acid Reference Standard, dissolve in dilute sodium hydroxide TS to make exactly 100 mL, and use this solution as the standard solution. Proceed with 30 mL each of the sample solution and the standard solution, exactly measured, as directed in the Assay under Folic Acid.

$$\begin{aligned} \text{Amount (mg) of folic acid } (C_{19}H_{19}N_7O_6) \\ = \text{amount (mg) of Folic Acid Reference Standard,} \\ \text{calculated on the anhydrous basis} \\ \times \frac{A_T - A_C}{A_S} \end{aligned}$$

**Containers and storage** Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

## Folic Acid Tablets

葉酸錠

Folic Acid Tablets contain not less than 90% and not more than 115% of the labeled amount of folic acid ( $C_{19}H_{19}N_7O_6$ ; 441.40).