Ferrous Ascorbate and Folic Acid Suspension

Ferrous Ascorbate and Folic Acid Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of ferrous ascorbate, $C_{12}H_{16}FeO_{12}$, equivalent to elemental iron and not less than 90.0 per cent of folic acid, $C_{19}H_{19}N_7O_6$.

Strength. Ferrous ascorbate equivalent to 30 mg of elemental iron and 550 mcg folic acid per 5 ml.

Identification

A. In the Assay of folic acid, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Dissolve 100 mg of the suspension in 10 ml of *water*, add 1 ml of *0.05 M sulphuric acid*, 1 ml of *2,6-dichlorophenolindophenol solution* and 1 ml of *dinitrophenylhydrazine solution.* Mix the solutions and keep in boiling water-bath for 10 minutes. The solution gives orange-red colour.

C. It gives reaction (A) of ferrous salts (2.3.1).

Tests

pH (2.4.24). 3.5 to 7.0.

Other tests. Comply with the tests stated under Oral Liquids.

Assay.

For Ferrous Iron-

Test solution. Weigh a quantity of the suspension containing 30 mg of elemental iron and disperse in sufficient amount of *water*, add 8 ml of *acetic acid* and dilute to 100.0 ml with *water*. Dilute 10.0 ml of the solution to 100.0 ml with *water*.

Reference solution. Dissolve 250 mg of *ferrous ammonium sulphate RS* in sufficient amount of *water*, add 8 ml of *acetic acid* and dilute to 100.0 ml with *water.* Dilute 10.0 ml of the solution to 100.0 ml with *water.*

Transfer 5.0 ml of the reference solution and the test solution separately into 100.0 ml volumetric flask, add 20 ml of *water* and 3.0 ml of *1,10-phenanthroline solution*. Mix well and dilute to volume with *water*. Measure the absorbance after 1 hour at 515 nm (2.4.7). Calculate the content of ferrous iron.

For Folic Acid –

Determine by liquid chromatography (2.4.14).

Solvent mixture. 80 volumes of 1.36 per cent w/v solution of *sodium acetate*, adjusted to pH 6.5 with *glacial acetic acid* and 20 volumes of *acetonitrile*.

Buffer solution. A solution prepared by dissolving 13.6 g of *sodium acetate trihydrate* in 1000 ml of *water*, adjusted to pH 6.5 with *glacial acetic acid.*

Test solution. Dissolve a quantity of oral suspension containing 1.0 mg of folic acid in 20 ml of *0.1 M sodium hydroxide* with the aid of ultrasound and dilute to 200.0 ml with the solvent mixture.

Reference solution. Weigh a quantity of oral suspension containing 30.0 mg of *folic acid RS* in 10 ml of *0.1 M sodium hydroxide* with the aid of ultrasound and dilute to 100.0 ml with the solvent mixture. Dilute 2.0 ml of the solution and 10 ml of *0.1 M sodium hydroxide* to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm), (Such as Inertsil ODS)
- sampler temperature: 5°,
- mobile phase: A. buffer solution,
  B. a mixture 75 volumes of *acetonitrile* and 25 volumes of buffer solution,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 283 nm,
- injection volume: 20 µl.

Time	Mobile phase A	Mobile phase B

(in min.)	(per cent v/v)	(per cent v/v)
0	100	0
5	100	0
15	70	30
20	30	70
25	100	0
30	100	0

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0.

Inject the reference solution and the test solution.

Determine the weight per ml of the oral suspension (2.4.29) and calculate the content of $C_{19}H_{19}N_7O_6$.

Microbial contamination (2.2.9). Total aerobic viable count is not more than 100 CFU per ml and total fungal count is not more than 10 CFU per ml. 1 ml is free from *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa.* 10 ml is free from *Salmonella.*

Storage. Store at a temperature not exceeding 30º.

Labelling. The label states, the strength in terms of ferrous ascorbate equivalent to elemental iron and folic acid.