DEPARTMENT OF DRUG ADMINISTRATION

National Medicines Laboratory

ANALYTICAL METHOD VALIDATION COMMITTEE

Folic acid & Ferrous ascorbate Tablets

Analytical Profile No.: FFT 074/075/ AP022

Folic acid & Ferrous ascorbate Tablets contains not less than 90 % of the stated amount of folic acid and not less than 90 % and not more than 110 % of the stated amount of elemental iron.

1. Identification:

1.1. Folic acid: The retention time of the test and reference solution of assay preparation should resemble each other.

1.2 Ferrous Ascorbate: Dissolve a quantity of the powdered tablets containing 10 mg of Iron in 2 ml of water, add 1 ml of potassium ferricyanide solution; a blue precipitate is formed that does not dissolve on addition of 5 ml of dilute hydrochloric acid.

Tests:

2. Uniformity of content (Folic acid): *Determine by liquid chromatography*

2.1 Solvent mixture: Prepare a mixture of 80 volumes of 0.57 % w/v solution of dipotassium hydrogen orthophosphate and 13.5 volumes of methanol.

2.2 Test Solution: Weigh individually 10 tablets. Transfer one tablet individually into ten 100 ml amber volumetric flask. Disperse the tablet with solvent mixture, add about 70 ml of solvent mixture, sonicate for about 15 minutes and make up the volume to 100 ml with solvent mixture. Centrifuge the resulting solution and dilute 2 ml of the solution to 20 ml with solvent mixture. Filter the final solution through 0.2 µm membrane filter.

2.3 Reference Solution: Weigh accurately about 25 mg folic acid reference standard and transfer into 100 ml amber volumetric flask. Dissolve with solvent mixture and make up the volume to 100 ml with solvent mixture. Dilute 5 ml of the resulting solution to 50 ml with solvent mixture. Again dilute 5 ml of the resulting solution to 100 ml with solvent mixture. Filter the final solution through 0.2 µm membrane filter.

2.4 Chromatographic system

Column: C18, (250*4.6 mm), 5 µm

Flow rate: 1.0 ml/min

Wavelength: 277 nm

Injection volume: 50 µl

Column Temperature: Ambient

Detector: UV Detector

Buffer: Solution containing 0.938 % w/v of sodium perchlorate and 0.0075 % w/v of potassium dihydrogen orthophosphate.

Mobile Phase: A mixture of 135 volumes of methanol and 800 volumes of buffer, adjust the pH to 7.2 and make up the volume to 1000 ml with HPLC grade water.

2.5 Procedure: Inject 50 µl of reference solution of folic acid as per above mentioned chromatographic condition. In the chromatogram obtained from the standard preparation, the column efficiency determined from the major peak should not be less than 2000 theoretical plates, the tailing factor should be not more than 2.0 and the relative standard deviation of replicate injections should not be more than 2.0 %. Inject 50 µl of the sample preparation and chromatograph as per above mentioned chromatographic condition. Calculate the content of folic acid in the tablet.

2.6 Limit: 85-115% of the average content.

3. Assay:

3.1 Folic acid: *Determine by liquid chromatography, as described in the Uniformity of Content.*

3.2 Ferrous ascorbate equivalent to elemental iron: Determine by UV Spectroscopy

3.2.1 Test Solution: Weigh 20 tablets individually and crush the tablet in the fine powder. Weigh powder eq. to 100 mg of elemental iron and transfer into 100 ml volumetric flask. Add about 10 ml of dilute sulphuric acid and heat in water bath until dissolves. Cool and make up the volume to 100 ml with water. Filter the solution and dilute 2 ml of the resulting solution to 50 ml with water.

3.2.2 Reference Solution: Weigh accurately about 100 mg ferrous ascorbate reference standard and transfer in 100 ml of volumetric flask. Dissolve in 10 ml of dilute sulphuric acid and heat in water bath until it dissolves. Cool and make up the volume to 100 ml with water. Dilute 5 ml of the filtrate to 50 ml with water.

3.2.3 Procedure: Pipette 5 ml of the test and reference solution, add 2 ml of 0.1 % w/v sodium acetate solution, 4 ml of 0.25 % w/v hydroquinone solution and add 4 ml of 0.25 % w/v of 1, 10 phenanthroline, allow to stand for 1 hour. Prepare blank in the similar manner except sample solution. Make up the volume to 50 ml with water. Measure the absorbance of the solution at 515 nm. Calculate the content of elemental iron per tablet.

4. Other tests: As per pharmacopoeial requirement.