Water-Soluble Vitamins Tablets

Water-Soluble Vitamins Tablets contain two or more of the following water-soluble vitamins: Ascorbic Acid or its equivalent as Calcium Ascorbate or Sodium Ascorbate, Biotin, Cyanocobalamin, Folic Acid, Niacin or Niacinamide, Pantothenic acid (as Calcium Pantothenate or Racemic Calcium Pantothenate), Pyridoxine Hydrochloride, Riboflavin, and Thiamine Hydrochloride or Thiamine Mononitrate. Tablets contain not less than 90.0 per cent and not more than 150.0 per cent of the labeled amount of ascorbic acid ($C_6H_8O_6$), biotin ($C_{10}H_6N_2\ O_3S$), calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$), cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$), folic acid ($C_{19}H_{19}N_7O_6$), niacin ($C_6H_5NO_2$) or niacinamide ($C_6H_6N_2O$), pyridoxine ($C_8H_{11}NO_3$), riboflavin ($C_{17}H_{20}N_4O_6$), and thiamine as thiamine ion ($C_{12}H_{17}N_4$ OS+).

They do not contain any form of Beta Carotene or Vitamin A, D, E, or K. They do not contain any minerals for which nutritional value is claimed. They may contain other labeled added substances in quantities that are unobjectionable.

Identification

The retention times of the vitamin peaks of the test solutions correspond to those of the corresponding vitamin peaks of the reference solutions as obtained in the test solution.

Tests

Microbial contamination (2.2.9). The total aerobic viable count is not more than 3000 cfu per g, the total combined molds and yeasts count is not more than 300 cfu per g and1 g is free from *Escherichia coli* and 10 g is free from *salmonella* species.
Other tests. Comply with the tests stated under Tablets.

Ascorbic Acid, Calcium Ascorbate, and Sodium Ascorbate. Weigh and powder 20 tablets.Weigh a quantity of the powder containing 100 mg of Ascorbic acid, to a 200-ml volumetric flask, add 75 ml of *metaphosphoric-acetic acids solution* and shake by mechanical means for 30 minutes. Dilute to volume with *water* and centrifuge until a clear supernatant is observed. Transfer a volume of the solution, equivalent to 2 mg of ascorbic acid into a 50-ml conical flask, add 5 ml of *metaphosphoric-acetic acids* and titrate with *standard 2,6-dichlorophenolindo-phenol solution* [Prepared by dissolving 50 mg of *2,6-dichlorophenolindo-phenol sodium* (stored in a desiccators over *soda lime*) in 50 ml of *water* containing 42 mg *sodium bicarbonate,* shake vigorously and when the dye is dissolved, add *water* to make 200 ml. Filter into an amber glass-stoppered bottle. Use within 3 days and standardize immediately before use.] until the rose-pink colour persists for at least 5 seconds. Repeat the operation with a mixture of 5.5 ml of *metaphosphoric acetic acid solution* and 15 ml of *water* omitting the preparation being examined. Correct for the volume of *standard 2,6-dichlorophenolindo-phenol solution* consumed by the blank.

Calculate the percentage of ascorbic acid, $C_6H_8O_6$ by using following expression.

$$\text{Result} = \frac{(V_S - V_B) \times F}{W} \times 100$$

Where,
V_S - Titrant volume consumed by the test solution
V_B -Titrant volume consumed by the Blank
F - Concentration of Titrant in terms of its equivalent of ascorbic acid
W - nominal amount of ascorbic acid taken for analysis

Biotin. Determine by liquid chromatography (2.4.14).
NOTE—Use low-actinic glassware throughout this procedure.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of the powder containing 1 mg of Biotin, to a 200-ml volumetric flask and add 3 ml *dimethyl sulphoxide,* and swirl to wet the contents. Place the flask in a water bath at 60°to 70° for 5 minutes and sonicate for 5 minutes, dilute with *water* to volume and filter.

Reference solution. A 0.0333 per cent w/v solution of *biotin IPRS* in *dimethyl sulphoxide*. Dilute the solution to obtain a concentration of 0.0005 per cent w/v of *biotin IPRS* with *water*.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed octylsilane bonded to porous silica (3 µm),
- mobile phase: a mixture of 85 ml of *acetonitrile,* 1.0 g of *sodium perchlorate,* 1 ml of *orthophosphoric acid* and dilute to 1000 ml with *water,*
- flow rate: 1.2 ml per minute,

- spectrophotometer set at 200 nm,
- injection volume: 100 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 3.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of biotin $C_{10}H_{16}N_2O_3S$ in the tablets.

Cyanocobalamin. Determine by liquid chromatography (2.4.14).

NOTE—Use low-actinic glassware throughout this procedure.

Test solution. Weigh and powder not less than 30 tablets. Disperse a quantity of the powder containing 0.1 mg of Cyanocobalamin, to a 250- ml volumetric flask and add 100.0 ml of *water* and carefully extract for 2 minutes. Filter 10 ml of the extract, and use the filtrate.

Reference solution. A 0.0001 per cent w/v solution of *cyanocobalamin IPRS* in *water.*

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 35 volumes of *methanol* and 65 volumes of *water,*
- flow rate: 0.5 ml per minute,
- spectrophotometer set at 550 nm,
- injection volume: 200 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 3.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of cyanocobalamin $C_{63}H_{88}C_ON_{14}O_{14}P$ in the tablets.

Folic acid. Determine by liquid chromatography (2.4.14).

NOTE—Use low-actinic glassware throughout this procedure.

Solution A. A 25 per cent solution of *tetrabutylammonium hydroxide* in *methanol.*

Solution B. Dissolve 5.0 g of pentetic acid in 50 ml of *1 M sodium hydroxide.*

Internal standard solution. Transfer 40 mg of *methylparaben* to a 1000-ml volumetric flask, and add 220 ml of *methanol* to dissolve. Dissolve 2.0 g of *monobasic potassium phosphate* in 300 ml of *water* in a separate beaker, quantitatively transfer this solution to the flask containing the *methylparaben* solution, and add an additional 300 ml of *water*. Add 19 ml of solution A, 7 ml of *3 M orthophosphoric acid*, and 30 ml of solution B. Adjusted to pH 9.8 with *ammonia*, and bubble nitrogen through the solution for 30 minutes, dilute with *water* to volume and mix.

Test solution. Weigh and powder 30 tablets. Disperse a quantity of the powder containing 0.4 mg of Folic acid, to a 50 ml amber-colored centrifuge tube and add 25.0 ml of the internal standard solution shake by mechanical means for 10 minutes, and centrifuge. Filter a portion of the clear supernatant, and use the filtrate.

Reference solution. A 0.0016 per cent w/v solution of *folic acid IPRS* in internal standard solution.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 2.0 g of *monobasic potassium phosphate* in 650 ml of *water* add 12.0 ml of solution A, 7.0 ml of *3 M orthophosphoric acid*, and 240 ml of *methanol*. Cool to room temperature, adjusted to pH 7.0 with *orthophosphoric acid* or *ammonia*, dilute to 1000 ml with *water*, (Recheck the pH before use by adding *water* or *methanol* to the prepared mobile phase to obtain baseline separation of folic acid and the internal standard. The pH may be increased up to 7.15 to obtain better separation) *[NOTE*: *The methanol and water content may be varied 1 per cent to 3 per cent]*.
- flow rate: 1 ml per minute,
- spectrophotometer set at 280nm,
- injection volume: 15 µl.

The relative retention times with reference to methylparaben for folic acid is about 0.8.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 3.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of folic acid $C_{19}H_{19}N_7O_6$ in the tablets by using the peak area ratio of folic acid to that of peak area of the internal standard.

Calcium Pantothenate. Determine by liquid chromatography (2.4.14).

NOTE—Use low-actinic glassware throughout this procedure.

Internal standard solution. Dissolve 80 mg of *p-hydroxybenzoic acid* in 3 ml of *ethanol,* add 50 ml of *water*, 7.1 g of *dibasic sodium phosphate* and dilute with *water* to 1000 ml, adjusted to pH 6.7 with *orthophosphoric acid*.

Test solution. Weigh and powder not less than 30 tablets. Disperse a quantity of the powder containing 15 mg of Calcium Pantothenate, to a centrifuge tube, add 25.0 ml of the internal standard solution and shake vigorously for 10 minutes. Centrifuge filter, and use the clear filtrate.

Reference solution. A 0.06 per cent w/v solution of *calcium pantothenate IPRS* in internal standard solution.

Chromatographic system
- a stainless steel column 15 cm x 3.9 mm, packed octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 0.1 per cent v/v solution of *orthophosphoric acid* in *water*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

The relative retention times with reference to p-hydroxybenzoic acid for calcium pantothenate is about 0.5.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 3.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of calcium pantothenate $C_{18}H_{32}CaN_2O_{10}$ in the tablets by using the peak area ratio of calcium pantothenate to that of peak of the internal standard.

Niacin or Niacinamide, Pyridoxine Hydrochloride, Riboflavin, and Thiamine. Determine by liquid chromatography (2.4.14).

NOTE—Use low-actinic glassware throughout this procedure.

Solvent mixture. A mixture of 5 volumes of *acetonitrile,* 1 volume of *glacial acetic acid* and 94 volumes of *water*.

Test solution: Weigh and powder not less than 30 tablets. Disperse a quantity of the powder containing 10 mg of Niacinamide and 2.5 mg each of Pyridoxine hydrochloride, Riboflavin and Thiamine hydrochloride, to a 50-ml centrifuge tube. Add 25.0 ml of solvent mixture, and mix using a vortex mixer for 30 seconds to completely suspend the powder Immerse the centrifuge tube in a hot water bath maintained at 65°to70°, heat for 5 minutes, and mix using a vortex mixer for 30 seconds. Return the tube to the hot water bath, heat for another 5 minutes, and mix on a vortex mixer for 30 seconds. Filter a portion of the solution, cool to room temperature, and use the clear filtrate. *[NOTE: Use the filterate with in 3 hour of filtration]*.

*Reference solution. [NOTE: Use Niacin IPRS in place of Niacinamide IPRS for formulations containing Niacin].*Transfer 80 mg of *niacinamide IPRS* and 20 mg each of *pyridoxine hydrochloride IPRS*, *riboflavin IPRS* and *thiamine hydrochloride IPRS* in 180 ml of solvent mixture. Immerse the flask in a hot water bath maintained at 65°to 70° for 10 minutes with regular shaking or using a vortex mixer, until all the solid materials are dissolved. Chill rapidly in a cold water bath for 10 minutes to room temperature and dilute to 200.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 73 volumes of *water* and 27 volume of *methanol* and 1 volume of *glacial acetic acid* containing 0.14 per cent of *sodium 1hexanesulphonate*,

- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 3.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of vitamin B_3 as niacin $C_6H_5NO_2$ or niacinamide $C_6H_6N_2O$, vitamin B_6 as pyridoxine $C_8H_{11}NO_3$, vitamin B_2 as riboflavin $C_{17}H_{20}N_4O_6$, and vitamin B_1 as thiamine ion $C_{12}H_{17}N_4OS^+$ in the tablets.

Storage. Store protected from light and moisture.