Water-Soluble Vitamins Capsules

Water-Soluble Vitamins Capsules 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, Dexpanthenol or Panthenol, Niacin or Niacinamide, Pantothenic acid (as Calcium Pantothenate or Racemic Calcium Pantothenate), Pyridoxine Hydrochloride, Riboflavin, and Thiamine Hydrochloride or Thiamine Mononitrate. Capsules contain not less than 90.0 per cent and not more than 150.0 per cent of the labeled amounts of ascorbic acid ($C_6H_8O_6$), biotin ($C_{10}H_6N_2O_3S$), calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$), cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$), folic acid ($C_{19}H_{19}N_7O_6$), dexpanthenol or panthenol ($C_9H_{19}NO_4$), niacin ($C_6H_5NO_2$) or niacinamide ($C_6H_6N_2O$), pyridoxine hydrochloride ($C_8H_{11}NO_3$,HCl), riboflavin ($C_{17}H_{20}N_4O_6$), and thiamine ($C_{12}H_{17}ClN_4OS$) as thiamine hydrochloride or thiamine mononitrate.

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.

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. 1 g is free from *Escherichia coli.* 10 g is free from *salmonella*.

Other tests. Comply with the tests stated under Capsules.

Assay.

Ascorbic Acid, Calcium Ascorbate, and Sodium Ascorbate.
Weigh 20 capsules. Open the capsules without the loss of shell material and transfer the contents to a 100-ml beaker. Remove any content adhering to empty shell by washing, if necessary with several portions of ether. Discard the washing and dry the capsule shell with the aid of current of dry air until the odour of ether in no longer perceptible. By subtracting the weights of dry empty shell from weight of intact capsules, calculate the average net weight per capsules. Transfer a quantity of capsule contents containing 100 mg of ascorbic acid, to a 200-ml volumetric flask. Add 75 ml of *metaphosphoric-acetic acid solution* and shake by mechanical means for 30 minutes, dilute to volume with *water* and centrifuge. Transfer a 4 ml volume of the solution into a 50-ml conical flask, and add 5 ml of *metaphosphoric-acetic acid solution* 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, Carry out a blank titration (A mixture of 5.5 ml of *metaphosphoric-acetic acids solution* and 15 ml of *water*). 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 20 capsules. Open the capsules without the loss of shell material and transfer the contents to a 100-ml beaker. Remove any content adhering to empty shell by washing, if necessary with several portions of ether. Discard the washing and dry the capsule shell with the aid of current of dry air until the odour of ether in no longer perceptible. By subtracting the weights of dry empty shell from weight of intact capsules, calculate the average net weight per capsules.
Transfer a quantity of capsule contents containing 1 mg of Biotin to a 200-ml volumetric flask, add 3 ml *dimethyl sulfoxide* and swirl to wet the contents. Place the flask in a water-bath at 60°to 70° for 5 minutes and with the aid of ultrasound for 5 minutes with intermittent shaking, dilute with *water* to volume and filter.

Reference solution. A 0.0333 per cent w/v solution of *biotin IPRS* in *dimethyl sulfoxide*. 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 chemically bonded to totally porous silica (3 µm),
- mobile phase: a mixture of 85 ml of *acetonitrile*, 1 g of *sodium perchlorate* and 1 ml of *orthophosphoric acid* 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 capsules.

Cyanocobalamin. Determine by liquid chromatography (2.4.14).

NOTE—Use low-actinic glassware throughout this procedure.

Test solution. Weigh 30 capsules. Open the capsules without the loss of shell material and transfer the contents to a 100-ml beaker. Remove any content adhering to empty shell by washing, if necessary with several portions of ether. Discard the washing and dry the capsule shell with the aid of current of dry air until the odour of ether in no longer perceptible. By subtracting the weights of dry empty shell from weight of intact capsules, calculate the average net weight per capsules.
Transfer a quantity of capsule contents 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 (crystalline) 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 volume 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 ($C_{63}H_{88}CoN_{14}O_{14}P$) in the capsules.

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. Transfer 5.0 g of pentetic acid to a 50-ml volumetric flask. Using sonication if necessary dissolve in and dilute with *1 M sodium hydroxide* to volume.

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 30 capsules. Open the capsules without the loss of shell material and transfer the contents to a 100-ml beaker. Remove any content adhering to empty shell by washing, if necessary with several portions of ether. Discard the washing and dry the capsule shell with the aid of current of dry air until the odour of ether in no longer perceptible. By subtracting the weights of dry empty shell from weight of intact capsules, calculate the average net weight per capsules. Transfer a quantity of capsule contents containing 0.0016 per cent w/v solution of Folic acid in 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 30 cm x 3.9 mm, packed octadecylsilane bonded to porous silica (10 µm),
- mobile phase: dissolve 2 g of *monobasic potassium phosphate* in 650 ml of *water* in a 1000-ml volumetric flask, 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 volume 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 (between 1% and 3%).]*
- 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 ($C_{19}H_{19}N_7O_6$) using ratio of the peak area of folic acid to that of peak area of the internal standard in the capsules.

Dexpanthenol or Panthenol.

NOTE-The following procedure is applicable also to the determination of the dextrorotatory component of racemic panthenol in preparations containing panthenol.

Dehydrated mixtures yielding formulations similar to the media described herein may be used provided that, when constituted as directed, they have growth-promoting properties equal to or superior to those obtained with the media prepared as described herein.

Test solution. Weigh 30 capsules. Open the capsules without the loss of shell material and transfer the contents to a 100-ml beaker. Remove any content adhering to empty shell by washing, if necessary with several portions of ether. Discard the washing and dry the capsule shell with the aid of current of dry air until the odour of ether in no longer perceptible. By subtracting the weights of dry empty shell from weight of intact capsules, calculate the average net weight per capsules. Transfer a quantity of capsule contents containing 1.2 mg of Dexpanthenol or 2.4 mg of Panthenol in 100.0 ml of *water*. Dilute the solution with *water* to obtain a nominal concentration of 0.00012 per cent w/v of dexpanthenol or 0.00024 per cent w/v of panthenol.

Reference solution (a). A solution containing 0.08 per cent w/v of *dexpanthenol IPRS* or 0.16 per cent w/v of *racemic panthenol IPRS* in *water*. Store in a refrigerator protected from light, and use within 30 days.

Reference solution (b). On the day of the assay, prepare a dilution of 0.00012 per cent w/v of *dexpanthenol IPRS* or 0.00024 per cent w/v of *panthenol IPRS* from the reference solution (a) diluted with *water*.

Acid-hydrolyzed casein solution. Dissolve 100 g of vitamin-free *casein* with 500 ml of *6 M hydrochloric acid* and reflux the mixture for 8 to 12 hours. Remove the *hydrochloric acid* from the mixture by distillation under reduced pressure until a thick paste remains. Redissolve the resulting paste in about 500 ml of *water*, adjusted to pH 3.5 with *1 M sodium hydroxide solution* and dilute with *water* to 1000 ml. Add 20 g of activated charcoal, stir for 1 hour, and filter. Repeat the treatment with activated charcoal. Store under *toluene* in a cool place at a temperature not less than 10°. Filter the solution if a precipitate forms during storage.

Cystine-tryptophan solution. Suspend 4.0 g of *L-cystine* in a solution of 1.0 g of *L-tryptophan* (or 2.0 g of *D,L-tryptophan*) in 700 to 800 ml of *water*, heat to 75 ± 5° and add *hydrochloric acid solution* (1 in 2) dropwise, with stirring, until the solids are dissolved. Cool and dilute with *water* to 1000 ml. Store under *toluene* in a cool place at a temperature not less than 10°.

Adenine-guanine-uracil solution. Dissolve 0.2 g each of *adenine sulphate*, *guanine hydrochloride*, and *uracil*, with the aid of heat, in 10 ml of *4 M hydrochloric acid.* Cool, and dilute with water to 200 ml. Store under *toluene* in a refrigerator.

Polysorbate 80 solution. A 10 per cent w/v solution of *polysorbate 80* in *ethanol.*

Riboflavin-thiamine hydrochloride-biotin solution. A solution containing 0.002 per cent w/v of riboflavin, 0.001 per cent w/v of thiamine hydrochloride and 0.000004 per cent w/v of biotin in *0.02 M acetic acid*. Store under toluene, protected from light, in a refrigerator.

p-Aminobenzoic acid-niacin-pyridoxine hydrochloride solution. A solution containing 0.001 per cent w/v of p-aminobenzoic acid, 0.005 per cent w/v of niacin and 0.004 per cent w/v of pyridoxine hydrochloride in neutral 25 per cent ethanol. Store in a refrigerator.

Salt solution A. A solution containing 5 per cent w/v each of *monobasic potassium phosphate* and *dibasic potassium phosphate* in *water*. Add 10 drops of *hydrochloric acid* per liter of solution. Store under *toluene.*

Salt solution B. A solution containing 2 per cent w/v of *magnesium sulphate,* 0.1 per cent w/v of *sodium chloride,* 0.1 per cent w/v of *ferrous sulphate,* and 0.1 per cent w/v of *manganese sulphate* in *water.* Add 10 drops of *hydrochloric acid* per liter of the solution. Store under *toluene.*

Pyridoxal-calcium pantothenate solution. A solution containing 0.02 per cent w/v of *pyridoxal hydrochloride* and 0.0001875 per cent w/v of *calcium pantothenate* in 10 per cent *ethanol.* Store in a refrigerator and use within 30 days.

Polysorbate 40-oleic acid solution. A solution containing 5 per cent w/v of *polysorbate 40* and 0.05 per cent w/v of *oleic acid* in 20 per cent *ethanol*. Store in a refrigerator and use within 30 days.

Modified pantothenate medium: Dissolve *anhydrous dextrose* and *sodium acetate* in the solutions previously mixed according to table and adjusted to pH 6.8 with *1 M sodium hydroxide.* Dilute with *water* to 250 ml.

Acid-hydrolyzed casein solution	25 ml
Cystine–tryptophan solution	25 ml
Polysorbate 80 solution	0.25 ml
Dextrose, anhydrous	10 g
Sodium acetate, anhydrous	5 g
Adenine–guanine–uracil solution	5 ml
Riboflavin–thiamine hydrochloride–biotin solution	5 ml
p-Aminobenzoic acid–niacin–pyridoxine hydrochloride solution	5 ml
Salt solution A	5 ml
Salt solution B	5 ml
Pyridoxal–calcium pantothenate solution	5 ml
Polysorbate 40–oleic acid solution	5 ml

Double-strength modified pantothenate medium. Prepare as directed in modified pantothenate medium, but make the final dilution to 125 ml instead of 250 ml. Prepare fresh.

Stock culture of Pediococcus acidilactici. Dissolve 6.0 g of peptone, 4.0 g of pancreatic digest of *casein*, 3.0 g of yeast extract, 1.5 g of beef extract, 1.0 g of *dextrose*, and 15.0 g of agar in 800 ml of *water*, with the aid of heat. Adjusted to pH 6.5 to 6.6 with *0.1 M sodium hydroxide* or *0.1 M hydrochloric acid* and dilute with *water* to 1000 ml. Add 10 ml portions of the solution to culture tubes, place caps on the tubes, and sterilize in an autoclave at 121° for 15 minutes. Cool on a slant, and store in a refrigerator. Prepare a stock culture of *pediococcus acidilactici* on a slant of this medium. Incubate at 35° for 20 to 24 hours, and store in a refrigerator. Maintain the stock culture by monthly transfer onto fresh slants.

Inoculum. Inoculate three 250 ml portions of sterile modified pantothenate medium from a stock culture slant, and incubate at 35° for 20 to 24 hours. Centrifuge the suspension from the combined portions and wash the cells with sterile modified pantothenate medium. Resuspend the cells in sufficient modified pantothenate medium so that a 1-in-50 dilution, when tested in a 13-mm diameter test tube, gives 80 per cent light transmission at 530 nm. Transfer 1.2 ml portions of this stock suspension to sterile glass ampuls, seal, freeze in liquid nitrogen, and store in a freezer. On the day of the assay, allow the ampuls to reach room temperature, mix the contents, and dilute 1 ml of thawed culture with sterile saline to 150 ml.

NOTE—This dilution may be altered when necessary to obtain the desired test response.

Analysis. Prepare in triplicate a series of eight culture tubes by adding the following quantities of *water* to the tubes within a set: 5.0, 4.5, 4.0, 3.5, 3.0, 2.0, 1.0, and 0.0 ml. To these same tubes and in the same order add 0.0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, and 5.0 ml of the reference solution (b).

Prepare in duplicate a series of five culture tubes by adding the following quantities of *water* to the tubes within a set: 4.0, 3.5, 3.0, 2.0, and 1.0 ml. To these same tubes and in the same order add 1.0, 1.5, 2.0, 3.0, and 4.0 ml of the test solution.

Add 5.0 ml of double-strength modified pantothenate medium to each tube. Cover the tubes with metal caps, and sterilize in an autoclave at 121° for 5 minutes. Cool to room temperature in a chilled water bath, and inoculate each tube with 0.5 ml of the Inoculum. Allow to incubate at 37° for 16 hours. Terminate growth by heating to a temperature not less than 80°, such as by steaming at atmospheric pressure in a sterilizer for 5 to10 minutes. Cool, and determine the percentage transmittance of the suspensions, in cells of equal path length, on a suitable spectrophotometer at a wavelength of 530 nm.

Calculation: Draw a dose-response curve on arithmetic graph paper by plotting the average response, in percentage of transmittance, for each set of tubes of the standard curve against the Standard level concentrations. The curve is drawn by connecting each adjacent pair of points with a straight line. From this standard curve, determine by interpolation the potency of each tube containing portions of the test solution. To obtain the individual responses, divide the potency of each tube by the amount of the test solution added to it. Calculate the mean response by averaging the individual responses that vary from their mean by not more than 15 per cent, using not less than half the total number of tubes. Calculate the potency of the portion of the material taken for assay by multiplying the mean response by the appropriate dilution factor.

Calculate the percentage of the labeled amount of dexpanthenol or panthenol ($C_9H_{19}NO_4$) in the portion of Capsules taken:

$$\text{Result} = (P/N) \times 100$$

P = potency of dexpanthenol or panthenol in the portion of Capsules taken (mg)

N = nominal amount of dexpanthenol or panthenol in the portion of Capsules taken (mg)

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 20 capsules. Open the capsules without the loss of shell material and transfer the contents to a 100-ml beaker. Remove any content adhering to empty shell by washing, if necessary with several portions of ether. Discard the washing and dry the capsule shell with the aid of current of dry air until the odour of ether in no longer perceptible. By subtracting the weights of dry empty shell from weight of intact capsules, calculate the average net weight per capsules. Transfer a quantity of capsule contents containing 0.06 per cent w/v solution of Calcium Pantothenate in internal standard solution, to a centrifuge tube.

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 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 ($C_{18}H_{32}CaN_2O_{10}$) using ratio of the peak area of calcium pantothenate to that of peak area of the internal standard in the capsules.

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

Test solution. Weigh 20 capsules. Open the capsules without the loss of shell material and transfer the contents to a 100-ml beaker. Remove any content adhering to empty shell by washing, if necessary with several portions of ether. Discard the washing and dry the capsule shell with the aid of current of dry air until the odour of ether in no longer perceptible. By subtracting the weights of dry empty shell from weight of intact capsules, calculate the average net weight per capsules. Transfer a quantity of capsule contents 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. Use the filtrate within 3 hours.

Reference solution. Transfer 80 mg of *niacinamide IPRS* and 20 mg each of *pyridoxine hydrochloride IPRS, riboflavin IPRS* and *thiamine hydrochloride IPRS* to a 200-ml volumetric flask and add 180 ml of the 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 with solvent mixture to volume. *(Note—Use niacin IPRS in place of niacinamide IPRS for formulations containing niacin.)*

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm, packed octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 73 volumes of *water,* 27 volumes of *methanol* and 1volumes of *glacial acetic acid* containing 0.14 per cent w/v solution of *sodium 1-hexanesulfonate*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume: 10 µl.

The relative retention times with reference to theamine for niacinamide, pyridoxine, riboflavin, are about 0.3, 0.5, 0.8, respectively.

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 niacin ($C_6H_5NO_2$) or niacinamide ($C_6H_6N_2O$), pyridoxine hydrochloride ($C_8H_{11}NO_3$,HCl), riboflavin ($C_{17}H_{20}N_4O_6$), and thiamine as thiamine hydrochloride ($C_{12}H_{17}CIN_4OS$,HCl) or thiamine mononitrate ($C_{12}H_{17}N_5O_4S$) in the capsules.

1 mg of thiamine mononitrate $C_{12}H_{17}N_5O_4S$ is equivalent to 0.9706 mg of thiamine hydrochloride $C_{12}H_{17}CIN_4OS$,HCl.

Storage. Store protected from light and moisture.