Oil-Soluble Vitamins Capsules

Oil-Soluble Vitamins Capsules contain two or more of the following oil-soluble vitamins: Vitamin A, Vitamin D as Ergocalciferol (Vitamin D_2) or Cholecalciferol (Vitamin D_3), Vitamin E, Phytonadione (Vitamin K_1), and Beta Carotene. Capsules contain not less than 90.0 per cent and not more than 165.0 per cent of the labeled amounts of vitamin A ($C_{20}H_{30}O$) as retinol or esters of retinol in the form of retinyl acetate ($C_{22}H_{32}O_2$) or retinyl palmitate ($C_{36}H_{60}O_2$); vitamin D as cholecalciferol ($C_{27}H_{44}O$) or ergocalciferol ($C_{28}H_{44}O$); vitamin E as alpha tocopherol ($C_{29}H_{50}O_2$), alpha tocopheryl acetate ($C_{31}H_{52}O_3$), or alpha tocopheryl acid succinate ($C_{33}H_{54}O_5$); phytonadione ($C_{31}H_{46}O_2$); and beta carotene ($C_{40}H_{56}$). Oil-Soluble Vitamins Capsules contain no other vitamins or any minerals. They may contain other labeled added substances that are generally recognized as safe, in amounts 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.

Vitamin A. Determine by liquid chromatography (2.4.14).
[*Note—Where the use of a vitamin A ester (retinyl acetate or retinyl palmitate) is specified in the following procedure, use the chemical form present in the formulation. Vitamin A IPRS is retinyl acetate. It is to be used where Vitamin A IPRS is specified. Use low-actinic glassware throughout this procedure.]*

Test solution (a). Weigh 20 capsules. Open the capsules without the loss of shell material and transfer the contents to a 100-ml beaker, equivalent to 5 capsules, to a container having a polytef-lined screw-cap. *[Note—For hard gelatin Capsules, remove, as completely as possible, the contents of NLT 20 Capsules by cutting open the Capsule shells, transferring the shells and their contents to a suitable container, and triturating to a homogeneous mass. Transfer a portion of the mass, equivalent to 5 Capsules, to a container having a polytef-lined screw cap.]* Add 10 ml of *dimethyl sulphoxide* and 15 ml of *n-hexane*, and shake for 45 minutes on a wrist-action shaker in a water-bath maintained at 60°. *[Note —Set up the wrist-action shaker to ensure that the contents of the container are mixed vigorously and thoroughly]*. Centrifuge at 3000 rpm for 10 minutes, and transfer the hexane layer by means of a pipet to a 100-ml volumetric flask. Add 15 ml of *n-hexane* to the dimethyl sulphoxide layer, shake thoroughly for 5 minutes, and transfer the n- hexane layer by means of a pipet to the 100-ml volumetric flask. Repeat this extraction with 3 additional 15-ml portions of *n*-hexane. Dilute the extracts in the volumetric flask with *n*-hexane to volume.

Test solution (b). Dilute a suitable volume of test solution (a) with *n*-hexane to obtain a solution having concentration 0.0015 per cent w/v of Vitamin A as Retinol.

*Reference solution (a).*A 0.0015 per cent w/v solution of *retinyl acetate from vitamin A IPRS* in *n-hexane*.

*Reference solution (b).*A 0.0015 per cent w/v solution of *retinyl palmitate IPRS* in *n-hexane*.

Reference solution (c). A mixture of equal volumes of reference solution (a) and reference solution (b) with *n-hexane* to obtain a solution having concentration 0.00075 per cent w/v each of *retinyl acetate* and *retinyl palmitate.*

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with aminopropylsilane bonded to porous silica (3µm)
- mobile phase: *n*-hexane,
- flow rate: 1 ml per minute,
- spectrophotometer set at 325nm,
- injection volume: 40 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to all-*trans*-retinyl acetate and all-*trans*-retinyl palmitate is not less than 10.0 and the relative standard deviation for replicate injections is not more than 3.0 per cent obtained with reference solution (c).

Inject reference solution (a) and test solution (b).
(Note: Measure the peak area of all-trans-retinyl acetate from the Standard solution and the peak area of all-trans-retinyl acetate or all-trans-retinyl palmitate from the Test solution (b).)

Calculate the content of vitamin A, as retinol ($C_{20}H_{30}O$) in the capsules.
[Note—The molar responses of retinyl acetate and retinyl palmitate are equivalent.]

1 mg of retinyl acetate $C_{22}H_{32}O_2$ is equivalent to 0.872 mg of vitamin A, as retinol $C_{20}H_{30}O$.

(Note -One Vitamin A IPRS Unit= 0.3 µg of all-trans-retinol(vitamin A alcohol) or 0.344 µg of all-trans-retinyl acetate (vitamin A acetate) or 0.55 µg of all-trans-retinyl palmitate (vitamin Apalmitate), and 1 µg of retinol (3.3 Vitamin A IPRS Units)= 1 retinol equivalent (RE);)

Vitamin D. Determine by liquid chromatography (2.4.14).
[Note—Where vitamin D (cholecalciferol or ergocalciferol) is specified in the following procedure, use the chemical form present in the formulation and the relevant IPRS. Use low-actinic glassware throughout this procedure.]

Test solution. Proceed as directed for test solution (a) in Vitamin A. transfer not less than 20.0 ml of the solution retained as specified in the direction for the test solution (b) in Vitamin A, to a suitable container, and evaporate, if necessary, in vacuum at room temperature to obtain a solution with a concentration of 0.0002 per cent w/v of Cholecalciferol or Eergocalciferol.

Reference solution (a). A 0.0002 per cent w/v solution of *cholecalciferol IPRS or ergocalciferol IPRS* in *n*-hexane.

Reference solution (b). Heat a volume of reference solution (a) at 60° for 1 hour to partially isomerize vitamin D (cholecalciferol or ergocalciferol) to its corresponding precursor.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with aminopropylsilane bonded to porous silica (3 µm)
- mobile phase: a mixture of 99 volumes of *n-hexane* and 1 volume of *isopropyl alcohol*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume: 100µl.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to the vitamin D form present and its corresponding precursor is not less than 10.0 in the chromatogram obtained with reference solution (b) and the relative standard deviation for replicate injections is not more than 3.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution.

$$\text{Cholecalciferol or Ergocalciferol (Vitamin D)} = (r_u/r_s) \times (C_s/C_u) \times F \times 100$$

r_u= peak area of cholecalciferol or ergocalciferol from test solution (b).
r_s= peak area of cholecalciferol or ergocalciferol from the reference solution (a).
C_s= concentration of Cholecalciferol IPRS or Ergocalciferol IPRS in the reference solution (a) (µg/ml)
C_u= nominal concentration of cholecalciferol orergocalciferol in the test solution (b). (µg/ml)
F= correction factor to account for the average amount of previtamin D present in the test solution (b), 1.09

Calculate the content of vitamin D, as cholecalciferol ($C_{27}H_{44}O$) or ergocalciferol ($C_{28}H_{44}O$) in the capsules.
(Note- 1 Vitamin D Unit = 0.025 µg of ergocalciferol or cholecalciferol)

Vitamin E. Determine by liquid chromatography (2.4.14).

[Note—Where vitamin E (alpha tocopherol, alpha tocopheryl acetate,or alpha tocopheryl acid succinate) is specified in the following procedure, use the chemical form present in the formulation and the relevant IPRS. Use low-actinic glassware throughout this procedure.]

Solvent mixture. A 1 per cent v/v solution of *orthophosphoric acid* in *water*.

Test solution. Proceed as directed for test solution (a) in Vitamin A. transfer not less than 20.0 ml of the solution retained as specified in the direction for test solution (b) in Vitamin A, to a suitable container, and evaporate if necessary under vacuum at room temperature to dryness. Transfer the contents to a suitable volumetric flask with the aid of *methanol*, and dilute with *methanol* to volume to obtain a solution of 0.2 per cent w/v of Vitamin E (alpha-tocopherol, alpha-tocopheryl acetate, or alpha-tocopheryl acid succinate).

Reference solution (a). A solution containing 0.2 per cent w/v each of *alpha tocopherol IPRS, alpha tocopheryl acetate IPRS, or alpha tocopheryl acid succinate IPRS* in the *methanol*.

Reference solution (b). A 0.065 per cent w/v solution of *ergocalciferol IPRS* in *methanol*. Dilute 1.0 ml of the solution to a 100-ml volumetric flask containing 100 mg of *alpha tocopheryl acetate IPRS.* Dissolve in 30 ml of *methanol*, with the aid of sonication if necessary, and dilute with *methanol* to volume. Store of the solution in a refrigerator.

Chromatographic system
- a stainless steel column 10 cm x 8.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: mixture of 95 volumes of *methanol* and 5 volumes of the solvent mixture,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 100 µl.

The relative retention times with reference to alpha tocopheryl acetate for ergocalciferol is about 0.5.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due ergocalciferol and alpha tocopheryl acetate is not less than 12.0 and the tailing factor is not less than 0.8 and not more than 1.2 in the chromatogram obtained with reference solution (b) and the relative standard deviation for replicate injections is not more than 3.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution.

Calculate the content of vitamin E, as alpha-tocopherol ($C_{29}H_{50}O_2$), alpha-tocopheryl acetate ($C_{31}H_{52}O_3$), or alpha-tocopheryl acid succinate ($C_{33}H_{54}O_5$) in the capsules.
(NOTE- 1 mg of dl-alpha tocopherol = 1.1 Vitamin E Units, 1 mg of dl-alpha tocopheryl acetate = 1 Vitamin E Unit, 1 mg of dl-alpha tocopheryl acid succinate = 0.89 Vitamin E Unit, 1 mg of d-alpha tocopherol = 1.49 Vitamin E Units, and 1 mg of d-alpha tocopheryl acetate = 1.36 Vitamin E Units, 1 mg of d-alpha tocopheryl acid succinate = 1.21 Vitamin E Units. In terms of d-alphatocopherol equivalents, 1 mg of d-alpha tocopheryl acetate = 0.91, 1 mg of d-alpha tocopheryl acid succinate = 0.81, 1 mg of dl-alphatocopherol = 0.74, 1 mg of dl-alpha tocopheryl acetate = 0.67, and 1 mg of dl-alpha tocopheryl acid succinate = 0.60.)

Phytonadione. Determine by liquid chromatography (2.4.14).

NOTE—Use low-actinic glassware throughout this procedure.

Test solution. Proceed as directed for test solution (a) in Vitamin A. Transfer not less than 20.0 ml of the solution retained as specified in the direction for test solution (b) in Vitamin A, to a suitable container, and evaporate if necessary under vacuum at room temperature to dryness. Transfer the residue to a suitable volumetric flask with the aid of *methanol*, and dilute with *methanol* to volume to obtain a solution of 0.002 per cent w/v of Phytonadione.

Reference solution (a). A 0.002 per cent w/v solution of *phytonadione IPRS* in *methanol*.

Reference solution (b). A solution containing 0.065 per cent w/v of *alpha tocopherol acetate IPRS* and 0.002 per cent w/v of *phytonadione IPRS* in *methanol*.

Chromatographic system
- stainless steel column 10 cm x 8.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: mixture of 95 volumes of *methanol* and 5 volumes of *water*,
- flow rate: 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 100 µl.

The relative retention times with reference to phytonadione for alpha-tocopheryl acetate is about 0.68.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due alpha-tocopheryl acetate and phytonadione is not less than 5.0 in the chromatogram obtained with reference solution (b) and the relative standard deviation for replicate injections is not more than 3.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and test solution.

Calculate the content of phytonadione ($C_{31}H_{46}O_2$) in the capsules.

Beta Carotene.
NOTE—Use low-actinic glassware throughout this procedure.

Solution A. 0.001 per cent w/v solution of *iodine* in *cyclohexane*. *(Note—prepare this solution fresh daily)*

Test solution A (for preparations containing beta carotene in oil solutions): Proceed as directed in Vitamin A except use *cyclohexane* instead of *n-hexane* as the extraction solvent, and dilute the filtered extracts with *cyclohexane* to obtain a solution having concentration 0.0002 per cent w/v of Beta carotene.

Test solution B (for preparations containing beta carotene in dry powder): Remove the contents of not less than 20 Capsules by cutting open the Capsules. Mix, and determine the weight of the contents. Disperse a quantity of the powder containing 2 mg of Beta carotene, to a 500-ml saponification flask. Add 100 ml of *ethanol*, 6 ml of *potassium hydroxide solution* (dissolve 58.8 g of*potassium hydroxide* in 50 of *water*), and a magnetic stirring bar. Attach an air condenser to the flask, and heat under reflux for 45 minutes with constant stirring. Cool to room temperature, add 170 ml of *hexane*, and stir for 30 minutes. Quantitatively transfer the contents of the flask to a 500-ml separatory-funnel with portions of *hexane.* Allow the layers to separate for 5-10 minutes, and transfer the upper organic layer to a 500-ml volumetric flask. Transfer the lower aqueous layer into the saponification flask; add 170 ml of *hexane*, and stir for an additional 20 minutes. Quantitatively transfer the contents of the saponification flask to the separatory-funnel with the aid of portions of *hexane.* Allow the layers to separate for 10 minutes. Drain the lower aqueous layer, and discard. Transfer the organic layer to the volumetric flask containing the previously collected organic layer. Rinse the separatory-funnel with small portions of *hexane*, and transfer the washings to the volumetric flask. Dilute the *hexane* extracts with *hexane* to volume, add 3 g of anhydrous *sodium sulphate*, shake, and allow to settle. Quantitatively transfer a volume of the solution, equivalent to 0.01 per cent w/v of beta carotene, to a 50-ml volumetric flask. Evaporate under a stream of nitrogen to dryness, and immediately add *cyclohexane*. Add 2 ml of *solution A* and heat for 15 minutes in a water-bath maintained at 65°. Cool rapidly, and dilute with *cyclohexane* to volume.

Measure the absorbance of the resulting solution at 452 nm (2.4.7). Calculate the content of $C_{40}H_{56}$, taking 223 as specific absorbance at the maximum at 452 nm. Carry out a blank *cyclohexane*

Storage. Store protected from light and moisture.

Labelling. Label the Capsules to state that the product is Oil-Soluble Vitamins Capsules. The label also states the quantity of each vitamin/dosage unit and, where necessary, the chemical form in which it is present. Where the product contains vitamin E, the label also indicates whether it is the *d*- or *dl*- form.