Oil-Soluble Vitamins Tablets

Oil-Soluble Vitamins Tablets contain two or more of the following oil-soluble vitamins: Vitamin A (as retinyl acetate or retinyl palmitate), Vitamin D as Ergocalciferol (vitamin D_2) or Cholecalciferol (vitamin D_3), Vitamin E (as RRR-or *all-rac*-alpha-tocopherol, *RRR*-or *all-rac*-alpha-tocopheryl acetate, or RRR-or *all-rac*-alpha-tocopheryl acid succinate), Phytonadione (vitamin K_1), and Beta Carotene.

Oil-Soluble Vitamins Tablets contain not less than 90.0 per cent and not more than 165.0 per cent of the labeled amount of vitamin A as retinol ($C_{20}H_{30}O$), vitamin D as cholecalciferol ($C_{27}H_{44}O$) or ergocalciferol ($C_{28}H_{44}O$), vitamin E as 2*R*-alpha-tocopherol ($C_{29}H_{50}O_2$), phytonadione ($C_{31}H_{46}O_2$), and beta carotene ($C_{40}H_{56}$).

Oil-Soluble Vitamins Tablets 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.

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 tests.

Tests

Microbial contamination (2.2.9). 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* and 10 g is free from *Salmonella*.

Other tests. Comply with the tests stated under Tablets.

Assay.

Vitamin A. Determine by liquid chromatography (2.4.14).

NOTE —Throughout the procedure protect the solutions from the atmosphere and light, preferably by the use of a blanket of inert gas and low-actinic glassware.
Where an ester form of vitamin A (Retinyl acetate or Retinyl palmitate) is specified in the following procedure, use the chemical form present in the formulation and the relevant IPRS.

Test solution (a). Transfer a portion of the powder, equivalent to 5 tablets, to a container having a polytef-lined screw-cap. Add 10 ml of *dimethyl sulphoxide* and 15 ml of *n-hexane*, 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 using a pipet to a 100-ml volumetric flask. Add 15 ml of *n-hexane* to the dimethyl sulphoxide layer, shake for 5 minutes and transfer the hexane layer using 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 10.0 ml of test solution (a) with *n-hexane* to obtain a solution of 0.0015 per cent w/v of Vitamin A as retinol.

Reference solution (a). A 0.0015 per cent w/v solution of retinol from *retinyl acetate[1] IPRS* in *n-hexane*.

Reference solution (b). A 0.0015 per cent w/v solution of retinol from *retinyl palmitate[2] IPRS* in *n-hexane*.

Reference solution (c). Mix equal volumes of reference solution (a) and reference solution (b).

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with aminopropylsilane bonded to porous silica (3 µm) (Such as Zorbax NH_2),
- mobile phase: *n-hexane*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 325 nm,
- injection volume: 40 µl.

Inject reference solution (a) or (b) and (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 in the chromatogram obtained with reference solution

(c) and the relative standard deviation for replicate injections is not more than 3.0 per cent in the chromatogram obtained with reference solution (a) or (b).

Inject reference solution (a) or (b) and the test solution (b).

Calculate the content of vitamin A as retinol ($C_{20}H_{30}O$) in the tablets.

NOTE — This chromatographic system can separate the 13-cis and all-trans-isomers of vitamin A, only the all-trans-isomer peak is used for the quantitation of vitamin A.
[1]Use the value of 0.872 to convert retinyl acetate to its retinol equivalent.
[2]Use the value of 0.546 to convert retinyl palmitate to its retinol equivalent.

Vitamin D. Determine by liquid chromatography (2.4.14).

NOTE — Throughout the procedure protect the solutions from the atmosphere and light, preferably by the use of a blanket of inert gas and low-actinic glassware.
Where vitamin D (cholecalciferol or ergocalciferol) is specified in the following procedure, use the chemical form present in the formulation and the relevant IPRS.

Test solution (a). Transfer a portion of the powder, equivalent to 5 tablets, 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 using a pipet to a 100-ml volumetric flask. Add 15 ml of *n-hexane* to the dimethyl sulfoxide layer, shake thoroughly for 5 minutes, and transfer the hexane layer using 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). Transfer not less than 20.0 ml of test solution (a) to a suitable container, and, if necessary, evaporate under vacuum at room temperature to obtain a solution of 0.0002 per cent w/v of Vitamin D.

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 the 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) (Such as Zorbax NH_2),
- 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: 100 µl.

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 test solution (b).

Calculate the content of vitamin D, as cholecalciferol ($C_{27}H_{44}O$) or ergocalciferol ($C_{28}H_{44}O$) in the tablets.

$$\frac{A_T \times C_R}{A_R \times C_T} \times F \times 100$$

where, A_T = peak response of cholecalciferol or ergocalciferol from the test solution,
A_R = peak response of cholecalciferol or ergocalciferol from the reference solution,
C_R = concentration of cholecalciferol IPRS or ergocalciferol IPRS in the reference solution,
C_T = nominal concentration of cholecalciferol or ergocalciferol in the test solution,
F = correction factor to account for the average amount of previtamin D present in the Sample solution, 1.09.

Vitamin E. Determine by liquid chromatography (2.4.14).

NOTE — Throughout the procedure protect the solutions from the atmosphere and light, preferably by the use of a blanket of inert gas and low-actinic glassware.
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 Reference Standard.

Solvent mixture. A 1.0 per cent v/v solution of *orthophosphoric acid* in *water.*

Test solution (a). Transfer a portion of the powder, equivalent to 5 tablets, to a container having a polytef-lined screw-cap. Add 10 ml of *dimethyl sulphoxide* and 15 ml of *n-hexane*, 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 using a pipet to a 100-ml volumetric flask. Add 15 ml of *n-hexane* to the dimethyl sulphoxide layer, shake for 5 minutes and transfer the hexane layer using 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). Transfer not less than 20.0 ml of test solution (a) to a suitable container, and, if necessary evaporate 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 0.2 per cent w/v of Vitamin E (alpha-tocopherol, alpha-tocopheryl acetate, or alpha-tocopheryl acid succinate).

Reference solution (a). 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 this solution in a refrigerator.

Reference solution (b). 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 *methanol.*

Chromatographic system
- a stainless steel column 10 cm x 8.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a *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 (a) and the relative standard deviation for replicate injections is not more than 3.0 per cent in the chromatogram obtained with reference solution (b).

Inject reference solution (b) and test solution (b).

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 ($C_{33}H_{54}O_5$) as 2*R*-alpha- tocopheryl ($C_{29}H_{50}O_2$) in the tablets.

$$\frac{A_T \times C_R}{A_R \times C_T} \times F \times 100$$

Where, A_T = peak response of relevant vitamin E from the test solution,
A_R = peak response of relevant vitamin E from the reference solution,
C_R = concentration of *all-rac*-alpha-tocopherol from either alpha tocopherol IPRS or alpha tocopheryl acetate IPRS or *RRR*-alpha-tocopherol from alpha tocopheryl acid succinate IPRS in the reference solution b,
C_T = nominal concentration of the corresponding form of vitamin E as 2R-alpha-tocopherol in the test solution,
F = conversion factor for the content of *all-rac*-alpha-tocopherol to 2R-alpha-tocopherol, ½ (for products labeled to contain *all-rac* vitamin E sources) and 1 (for products labeled to contain *RRR* vitamin E sources)

Phytonadione

NOTE—Use low-actinic glassware throughout this procedure.

Test solution (a). Transfer a portion of the powder, equivalent to 5 Tablets, 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 sulfoxide layer, shake thoroughly for 5 minutes, and transfer the 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). Transfer not less than 20.0 ml of test solution (a) to a suitable container, and evaporate 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 tocopheryl acetate IPRS* and 0.002 per cent w/v of *phytonadione IPRS* in *methanol*.

Chromatographic system
- a stainless steel column 10 cm x 8.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a 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 (b).

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

Beta Carotene.

NOTE—Use low-actinic glassware throughout this procedure.

Weigh and powder 20 tablets. 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 ml 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 this solution, equivalent to 100 µg 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 a freshly prepared 0.001 per cent w/v solution of *iodine* in *cyclohexane* 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.

Storage. Store protected from light and moisture.

Labelling. The label states (1) the product is oil-soluble vitamins tablets; (2) the quantity of each vitamin in mg/tablet or µg/tablet and where necessary the chemical form in which it is present; (3) where the products contains vitamin E, indicates whether it is *RRR* or *all-rac* form.

NOTE—One vitamin A 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 A palmitate) and 1 µg of retinol (3.3 vitamin A units)= 1 retinol equivalent (RE); 1 IU of beta carotene = 0.6 µg of all-trans-beta carotene; 1 Vitamin D Unit = 0.025 µg of ergocalciferol or cholecalciferol; and 1 mg of all-rac-alpha-tocopherol = 1.1 Vitamin E Units; 1 mg of all-rac-

alpha-tocopheryl acetate = 1 Vitamin E Unit; 1 mg of all-rac-alpha-tocopheryl acid succinate = 0.89 Vitamin E Units; 1 mg of RRR-alpha-tocopherol = 1.49 Vitamin E Units; 1 mg of RRR-alpha-tocopheryl acetate = 1.36 Vitamin E Units; and 1 mg of RRR-alpha-tocopheryl acid succinate = 1.21 Vitamin E Units. In terms of RRR-alpha-tocopherol equivalents, 1 mg of RRR-alpha-tocopheryl acetate = 0.91; 1 mg of RRR-alpha-tocopheryl acid succinate = 0.81; 1 mg of all-rac-alpha-tocopherol = 0.74; 1 mg of all-rac-alpha-tocopheryl acetate = 0.67; and 1 mg of all-rac-alpha-tocopheryl acid succinate = 0.60. 1 mg of Institute of Medicine (IOM) alpha-tocopherol equivalent = 1 mg of 2R-alpha-tocopherol = 1 mg of RRR-alpha-tocopherol = 2 mg of all-rac-alpha-tocopherol.