Oil-Soluble Vitamins Oral Solution

Oil-Soluble Vitamins Oral Solution contain, two or more of the following oil-soluble vitamins: Vitamin A, as retinol or esters of retinol in the form of retinyl acetate or retinyl palmitate, Vitamin D as Ergocalciferol (Vitamin D_2) or Cholecalciferol (Vitamin D_3), Vitamin E as alpha tocopherol, alpha tocopheryl acetate, or alpha tocopheryl acid succinate, Phytonadione (Vitamin K_1), and Beta Carotene. Oral Solution contain not less than 90.0 per cent and not more than 150.0 per cent of the labeled amounts of vitamin A, as retinol equivalent ($C_{20}H_{30}O$); 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 ($C_{40}H_{56}$).

Tests

Ethanol (2.3.45). Not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of ethanol, determined by method III.

Microbial contamination (2.2.9). The total aerobic viable count is not more than 3000 cfu per ml, the total combined molds and yeasts count is not more than 300 cfu per ml. 1 g is free from *Escherichia coli.* 10 ml is free from *salmonella.*

Other tests. Comply with the tests stated under Oral Solution.

Assay.

Vitamin A. Determine by liquid chromatography (2.4.14).

NOTE—Use low-actinic glassware throughout this procedure.

Test solution. Dissolve a suitable quantity of the oral solution in *n-hexane* to obtain a solution having concentration 0.0013 per cent w/v solution of Retinol.

Reference solution (a). A 0.0013 per cent w/v solution of *retinol from retinyl acetate IPRS* in *n-hexane*.

Reference solution (b). A 0.0013 per cent w/v solution of *retinol from retinyl palmitate IPRS* in *n-hexane*.

Reference solution (c). A mixture of, 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 (3µm) (Such as Zorbax NH2),
- 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) or (b) and the test solution.

Calculate the content of vitamin A, as retinol ($C_{20}H_{30}O$) in the oral solution.
(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-betacarotene)

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 (a). Dissolve a suitable quantity of the oral solution in *n-hexane* to obtain a solution having concentration 0.002 per cent w/v solution of Cholecalciferol or Ergocalciferol.

*Test solution (b).*Transfer 5.0 ml of test solution (a) to a container having a polytef-lined screw cap and heat, with constant shaking, for 1 hour in a water bath maintained at 60° to obtain a solution containing vitamin D (cholecalciferol

or ergocalciferol) and its corresponding precursor. Cool, and dilute with *n-hexane* to obtain a solution containing 0.0002 per cent w/v of Cholecalciferol or Ergocalciferol.

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) (Such as Zorbax NH2),
- 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 (b). The test is not valid unless the resolution between the peaks due to vitamin D form present and its corresponding precursor 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 (b).

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

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

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 oral solution.
(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. Dissolve a suitable quantity of the oral solution in *methanol* to obtain a solution having concentration 0.2 per cent w/v solution of Alpha Tocopherol, Alpha Tocopheryl Acetate or Alpha Tocopheryl Acid Succinate.

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

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm) (Such as Sunfire C18),
- mobile phase: mixture of 95 volumes of *methanol* and 5 volumes of solvent mixture,
- flow rate: 1 ml per minute,
- spectrophotometer set at 291 nm,
- injection volume: 50 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5 and 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 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 oral solution.

(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-alpha tocopherol equivalents, 1 mg of d-alpha tocopheryl acetate = 0.91, 1 mg of d-alpha tocopheryl acid succinate = 0.81, 1 mg of dl-alpha tocopherol = 0.74, 1 mg of dl-alpha tocopheryl acetate = 0.67, and 1 mg of dl-alpha tocopheryl acid succinate = 0.60.)

Phytonadione (Vitamin K_1). Determine by liquid chromatography (2.4.14).

NOTE—Use low-actinic glassware throughout this procedure.

Test solution. Dissolve a suitable quantity of the oral solution in *methanol* to obtain a solution having concentration 0.002 per cent w/v solution 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 15 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm) (Such as Sunfire C18),
- mobile phase: mixture of 95 volumes of *methanol* and 5 volumes of *water,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 50 µl.

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 the test solution.

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

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

Solvent mixture. A 0.005 per cent v/v solution of *butylated hydroxytoluene* in *ethanol.*

Test solution (a). Dissolve a quantity of the oral solution containing 20.0 mg of Beta Caroteneto a 250-ml volumetric flask. Add 250 mg of *butylated hydroxytoluene*, 120 ml of *methylene chloride*, and 100 ml of *ethanol.* Shake the flask until the sample is completely dissolved or suspended. Let the mixture stand in the dark until it reaches room temperature (about 2 hours). Add *methylene chloride* to volume, and shake again vigorously.

Test solution (b). Dilute a volume of test solution (a) with a mixture of, equal volume of *methylene chloride* and solvent mixture to obtain the final concentration 0.0003 per cent w/v of Beta Carotene. Pass through a membrane filter of 0.45-µm pore size.

Reference solution (a). A 0.006 per cent w/v solution of *beta carotene IPRS* in *tetrahydrofuran.*

Reference solution (b). Transfer 5.0 ml of reference solution (a) into a 100-ml volumetric flask, add 5 ml of *tetrahydrofuran,* and dilute with solvent mixture to volume.

NOTE- Determine the concentration of reference solution (b) from the concentration of reference solution (c) as described below.

Reference solution (c). Transfer 5.0 ml of reference solution (a) into a 100-ml volumetric flask and dilute with *cyclohexane* to volume. Prepare in triplicate.

Measure the absorbance of the resulting solution at the maximum at about 457 nm (2.4.7). Calculate the concentration of total beta carotene (mg per ml) as all-trans-beta carotene ($C_{40}H_{56}$) in reference solution (c) taking 250 as the specific absorbance at 457 nm. Blank as a *cyclohexane.*

NOTE—The concentration of reference solution (c) equals the concentration of reference solution (b).

Result = A/F

A = average absorbance of the three preparations of reference solution (c),
F = absorptivity of pure all-trans-beta carotene in cyclohexane, 250.

Reference solution (d). Transfer 20 mg of *beta carotene IPRS* to a 50-ml volumetric flask, add 1 ml of *water*, 4 ml of *tetrahydrofuran*, and sonicate for 5 minutes. Dilute with solvent mixture to volume and sonicate for 5 minutes. Cool to room temperature, filter the suspension through a membrane filter of 0.45-µm pore size, and use the clear filtrate

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with spherical porous silica the surface of withch has been covalently modified with alkyl amide group and not endcapped (5 µm) (Such as Suplex pKb-100),
- column temperature: 30˚,
- mobile phase: dissolve 50 mg of *butylated hydroxytoluene* into a 1000-ml volumetric flask, and dissolve with 20 ml of *2-propanol*. Add 0.2 ml of *N-ethyldiisopropylamine*, 25 ml of 0.2 per cent *ammonium acetate solution*, 455 ml of *acetonitrile* and about 450 ml of *methanol*. Allow the solution to reach room temperature, and dilute with *methanol* to volume,
- flow rate: 0.6 ml per minute,
- spectrophotometer set at 448 nm,
- injection volume: 20 µl.

Name	Relative retention time	Correction factor
All-trans-alpha carotene	0.93	0.91
All-trans-beta carotene	1.00	-
9-cis-Beta carotene	1.07	-
13-cis-Beta carotene	1.17	0.83
15-cis-Beta carotene	1.21	0.71

Inject reference solution (b) and (d). The test is not valid unless the resolution between the peaks due to beta carotene and alpha carotene, between beta carotene and 9-cis-beta carotene is not less than 1.5 in the chromatogram obtained with reference solution (d), the tailing factor of beta carotene peak is not more than 2.0 and the relative standard deviation for replicate injections of beta carotene peak is not more than 2.0 per cent in the chromatogram obtained with reference solution (b).

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

Calculate the content of beta carotene ($C_{40}H_{56}$) in the oral solution.
[Note—use concentration of all-trans-beta carotene in reference solution (c) as determined by the spectrometric procedure.]

Storage. Store protected from light and tightly-closed containers, under an inert gas or with a minimum of headspace.

Labelling. The label states that the product is Oil-Soluble Vitamins Oral Solution. The label states the quantity of each vitamin present in a given volume of Oral Solution and, where necessary, the chemical form in which a vitamin is present. Where the product contains vitamin E, the label indicates whether it is the d- or dl- form.