

Status: Currently Official on 17-Feb-2025
 Official Date: Official as of 01-May-2013
 Document Type: Dietary Supplement Monographs
 DocId: GUID-ADD8023E-7348-4330-8CAA-06EE3A892AF4_1_en-US
 DOI: https://doi.org/10.31003/USPNF_M7169_01_01
 DOI Ref: acl40

© 2025 USPC
 Do not distribute

Oil-Soluble Vitamins with Minerals Oral Solution

DEFINITION

Oil-Soluble Vitamins with Minerals Oral Solution contains 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 D₂) or cholecalciferol (Vitamin D₃); Vitamin E, as alpha tocopherol, alpha tocopheryl acetate, or alpha tocopheryl acid succinate; Phytonadione (Vitamin K₁); beta carotene; and one or more minerals derived from substances generally recognized as safe, furnishing one or more of the following elements in ionizable form: chromium, fluorine, iodine, iron, magnesium, manganese, molybdenum, and zinc. It contains NLT 90.0% and NMT 150.0% of the labeled amounts of vitamin A, as retinol equivalent (C₂₀H₃₀O); vitamin D, as cholecalciferol (C₂₇H₄₄O) or ergocalciferol (C₂₈H₄₄O); vitamin E, as alpha tocopherol (C₂₉H₅₀O₂), alpha tocopheryl acetate (C₃₁H₅₂O₃), or alpha tocopheryl acid succinate (C₃₃H₅₄O₅); phytonadione (C₃₁H₄₆O₂); and beta carotene (C₄₀H₅₆); NLT 90.0% and NMT 160.0% of the labeled amounts of chromium (Cr), fluorine (F), iodine (I), and molybdenum (Mo); and NLT 90.0% and NMT 125.0% of the labeled amounts of iron (Fe), magnesium (Mg), manganese (Mn), and zinc (Zn).

STRENGTH

• VITAMIN A

[NOTE—Use low-actinic glassware throughout this procedure.]

Mobile phase: *n*-Hexane

Standard solution 1: 13 µg/mL of retinol from [USP Retinyl Acetate RS](#) in *n*-hexane

Standard solution 2: 13 µg/mL of retinol from [USP Retinyl Palmitate RS](#) in *n*-hexane

System suitability solution: Mix equal volumes of *Standard solution 1* and *Standard solution 2*.

Sample solution: Transfer an accurately measured volume of Oral Solution, equivalent to 3.25 mg of retinol, to a separatory funnel containing 10 mL of water and 20 mL of dehydrated alcohol. Add 100 mL of *n*-hexane, insert the stopper, and shake for 1 min. Allow the layers to separate, drain the aqueous layer into another separatory funnel, and repeat the extraction with 100 mL of *n*-hexane. Discard the aqueous layer, and combine the hexane extracts. Wash the combined extracts with 25 mL of water, allow the layers to separate, and discard the aqueous layer. Filter the washed hexane layer through anhydrous sodium sulfate into a 250-mL volumetric flask. Rinse the funnel and sodium sulfate with *n*-hexane, and add the rinsing to the hexane solution in the flask. Dilute the extracts in the volumetric flask with *n*-hexane to volume to obtain a solution with a concentration of 13 µg/mL of vitamin A as retinol (C₂₀H₃₀O).

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

Mode: LC

Detector: UV 325 nm

Column: 4.6-mm × 15-cm; packing L8

Flow rate: 1 mL/min

Injection volume: 40 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 10 between all-*trans*-retinyl acetate and all-*trans*-retinyl palmitate

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution 1* or *Standard solution 2* and *Sample solution*

Calculate the percentage of the labeled amount of vitamin A, as retinol (C₂₀H₃₀O), in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of the all-*trans*-retinyl ester from the *Sample solution*

r_S = peak area of the all-*trans*-retinyl ester from the appropriate *Standard solution*

C_S = concentration of retinol in the appropriate *Standard solution* (µg/mL)

C_U = nominal concentration of vitamin A, as retinol, in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–150.0% of the labeled amount of vitamin A as retinol equivalent ($C_{20}H_{30}O$)

• VITAMIN D

[NOTE—Where vitamin D (cholecalciferol or ergocalciferol) is specified in the following procedure, use the chemical form present in the formulation and the relevant USP Reference Standard. Use low-actinic glassware throughout this procedure.]

Mobile phase: *n*-Hexane and isopropyl alcohol (99:1)

Standard solution: 2 µg/mL of [USP Cholecalciferol RS](#) or [USP Ergocalciferol RS](#) in *n*-hexane

System suitability solution: Heat a volume of the *Standard solution* at 60° for 1 h to partially isomerize vitamin D (cholecalciferol or ergocalciferol) to its corresponding precursor.

Sample stock solution: Using an accurately measured volume of Oral Solution, equivalent to 5.0 mg of cholecalciferol or ergocalciferol, proceed as directed for the *Sample solution* in *Vitamin A* to obtain a solution with a concentration of 20 µg/mL of cholecalciferol or ergocalciferol in *n*-hexane.

Sample solution: Transfer 5.0 mL of the *Sample stock solution* to a container having a polytetrafluoroethylene-lined screw cap. Heat, with constant shaking, for 1 h 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 2 µg/mL of cholecalciferol or ergocalciferol.

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

Mode: LC

Detector: UV 265 nm

Column: 4.6-mm × 15-cm; 3-µm packing L8

Flow rate: 1 mL/min

Injection volume: 100 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 10 between the vitamin D form present and its corresponding precursor

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cholecalciferol ($C_{27}H_{44}O$) or ergocalciferol ($C_{28}H_{44}O$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

r_U = peak area of cholecalciferol or ergocalciferol from the *Sample solution*

r_S = peak area of cholecalciferol or ergocalciferol from the *Standard solution*

C_S = concentration of [USP Cholecalciferol RS](#) or [USP Ergocalciferol RS](#) in the *Standard solution* (µg/mL)

C_U = nominal concentration of cholecalciferol or ergocalciferol in the *Sample solution* (µg/mL)

F = correction factor to account for the average amount of pre-vitamin D present in the *Sample solution*, 1.09

Acceptance criteria: 90.0%–150.0% of the labeled amount of vitamin D as cholecalciferol ($C_{27}H_{44}O$) or ergocalciferol ($C_{28}H_{44}O$)

• VITAMIN E

[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 USP Reference Standard. Use low-actinic glassware throughout this procedure.]

Solution A: Phosphoric acid solution (1 in 100) in water

Mobile phase: Methanol and *Solution A* (19:1)

Standard solution: 2 mg/mL of [USP Alpha Tocopherol RS](#), [USP Alpha Tocopheryl Acetate RS](#), or [USP Alpha Tocopheryl Acid Succinate RS](#) in methanol

Sample solution: Transfer NLT 20 mL of the solution prepared as directed for the *Sample solution* in *Vitamin A* to a suitable container, and evaporate in vacuum at room temperature to dryness. Transfer the residue with the aid of methanol to a suitable volumetric flask, and dilute with methanol to volume to obtain a concentration of 2 mg/mL of alpha tocopherol, alpha tocopheryl acetate, or alpha tocopheryl acid succinate.

Chromatographic system

(See [Chromatography \(621\), System Suitability](#).)

Mode: LC

Detector: UV 291 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 50 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of 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 portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of the relevant vitamin E form from the *Sample solution*

r_S = peak area of the relevant vitamin E form from the *Standard solution*

C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)

C_U = nominal concentration of the corresponding form of vitamin E in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–150.0% of the labeled amount of vitamin E

• PHYTONADIONE (VITAMIN K₁)

[NOTE—Use low-actinic glassware throughout this procedure.]

Mobile phase: Methanol and water (19:1)

Standard stock solution: 200 μg/mL of [USP Phytonadione RS](#) in methanol. Dissolve with the aid of sonication if necessary.

Standard solution: 20 μg/mL of [USP Phytonadione RS](#) from the *Standard stock solution* diluted with methanol

System suitability solution: 0.65 mg/mL of [USP Alpha Tocopheryl Acetate RS](#) and 20 μg/mL of [USP Phytonadione RS](#) from the *Standard stock solution* diluted with methanol. [NOTE—Dissolve [USP Alpha Tocopheryl Acetate RS](#) in a portion of methanol, add the *Standard stock solution*, and then dilute with methanol to volume.]

Sample solution: Transfer NLT 20 mL of the solution prepared as directed for the *Sample solution* in *Vitamin A* to a suitable container, and evaporate in vacuum at room temperature to dryness. Transfer the residue with the aid of methanol to a suitable volumetric flask, and dilute with methanol to volume to obtain a concentration of 20 μg/mL of phytonadione.

Chromatographic system

(See [Chromatography \(621\), System Suitability](#).)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 50 μL

System suitability

Samples: *Standard solution* and *System suitability solution*

Suitability requirements

Resolution: NLT 5 between alpha tocopheryl acetate and phytonadione, *System suitability solution*



Documents

Document Sharing Hub

Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of phytonadione ($C_{31}H_{46}O_2$) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area from the *Sample solution*

r_S = peak area from the *Standard solution*

C_S = concentration of [USP Phytonadione RS](#) in the *Standard solution* ($\mu\text{g/mL}$)

C_U = nominal concentration of phytonadione in the *Sample solution* ($\mu\text{g/mL}$)

Acceptance criteria: 90.0%–150.0% of the labeled amount of phytonadione ($C_{31}H_{46}O_2$)

• BETA CAROTENE

[NOTE—Use low-actinic glassware.]

Mobile phase: Transfer 50 mg of butylated hydroxytoluene into a 1-L volumetric flask, and dissolve with 20 mL of 2-propanol. Add 0.2 mL of *N*-ethyl-diisopropylamine, 25 mL of 0.2% 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.

Diluent: 50 $\mu\text{g/mL}$ of butylated hydroxytoluene in alcohol

System suitability solution: Transfer 20 mg of [USP Beta Carotene System Suitability RS](#) to a 50-mL volumetric flask. Add 1 mL of water, 4 mL of tetrahydrofuran, and sonicate for 5 min. Dilute with *Diluent* to volume, and sonicate for 5 min. Cool to room temperature, pass the suspension through a membrane filter of 0.45- μm pore size, and use the clear filtrate.

Standard stock solution: 60 $\mu\text{g/mL}$ of [USP Beta Carotene RS](#) in tetrahydrofuran

Standard solution A: Transfer 5.0 mL of the *Standard stock solution* into a 100-mL volumetric flask, add 5.0 mL of tetrahydrofuran, and dilute with *Diluent* to volume.

Determine the concentration of *Standard solution A* from the concentration of *Standard solution B* as described below.

Standard solution B: Transfer 5.0 mL of the *Standard stock solution* into a 100-mL volumetric flask, and dilute with cyclohexane to volume. Prepare in triplicate.

Instrumental conditions

(See [Ultraviolet-Visible Spectroscopy \(857\)](#).)

Analytical wavelength: 457 nm

Cell path: 1 cm

Blank: Cyclohexane

Analysis

Sample: *Standard solution B*

Calculate the concentration of total beta carotene (mg/mL) as all-*trans*-beta carotene ($C_{40}H_{56}$) in *Standard solution B*. [NOTE—The concentration of *Standard solution B* equals the concentration of *Standard solution A*.]

$$\text{Result} = A/F$$

A = average absorbance of the three preparations of *Standard solution B*

F = absorptivity of pure all-*trans*-beta carotene in cyclohexane, 250

Sample solution: Transfer NLT 20 mL of the solution prepared as directed for the *Sample solution* in *Vitamin A* to a suitable container, and evaporate in vacuum at room temperature to dryness. Dissolve the residue in a mixture of methylene chloride and *Diluent* (1:1), and dilute with the same mixture to obtain a concentration of 3 $\mu\text{g/mL}$ of beta carotene. Pass through a membrane filter of 0.45- μm pore size if necessary.

Chromatographic system

(See [Chromatography \(621\)](#), *System Suitability*.)

Mode: LC

Detector: UV-Vis 448 nm

Column: 4.6-mm $\times$ 25-cm; 5- μm packing L68

Column temperature: 30°

Flow rate: 0.6 mL/min