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Oil-Soluble Vitamins Capsules

DEFINITION

Oil-Soluble Vitamins Capsules contain two or more of the following oil-soluble vitamins: Vitamin A, Vitamin D as Ergocalciferol (Vitamin D₂) or Cholecalciferol (Vitamin D₃), Vitamin E, Phytonadione (Vitamin K₁), and Beta Carotene. Capsules contain NLT 90.0% and NMT 165.0% of the labeled amounts of vitamin A (C₂₀H₃₀O) as retinol or esters of retinol in the form of retinyl acetate (C₂₂H₃₂O₂) or retinyl palmitate (C₃₆H₆₀O₂); 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).

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.

STRENGTH

[NOTE—In the following assays, where more than one assay method is given for an individual ingredient, the requirements may be met by following any one of the specified methods, the method used being stated in the labeling only if *Method 1* is not used.]

• VITAMIN A, Method 1

[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. [USP Vitamin A RS](#) is retinyl acetate. It is to be used where [USP Vitamin A RS](#) is specified. Use low-actinic glassware throughout this procedure.]

Mobile phase: *n*-Hexane

Standard solution: 15 µg/mL of retinyl acetate from [USP Vitamin A RS](#) in *n*-hexane

System suitability stock solution: 15 µg/mL of retinyl palmitate in *n*-hexane

System suitability solution: Mix equal volumes of the *System suitability stock solution* and the *Standard solution* to obtain concentrations of 7.5 µg/mL each of retinyl acetate and retinyl palmitate.

Sample stock solution: Transfer the contents of NLT 20 Capsules to a suitable container, mix, and weigh. Transfer a portion of the mixture, equivalent to 5 Capsules, to a container having a polytetrafluoroethylene-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 polytetrafluoroethylene-lined screw cap.] Add 10 mL of dimethyl sulfoxide and 15 mL of *n*-hexane, and shake for 45 min 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 min, 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 min, and transfer the hexane layer by means of a pipet to the 100-mL volumetric flask. Repeat this extraction with three additional 15-mL portions of *n*-hexane. Dilute the extracts in the volumetric flask with *n*-hexane to volume.

Sample solution: Dilute a volume of the *Sample stock solution* with *n*-hexane to obtain a solution with a concentration of 15 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; 3-µm packing L8

Flow rate: 1 mL/min

Injection size: 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* and *Sample solution*

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 *Sample solution*.

For products containing vitamin A acetate or vitamin A palmitate, calculate the percentage of the labeled amount of vitamin A, as retinol ($C_{20}H_{30}O$), in the portion of Capsules taken:

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

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 *Standard solution*

C_S = concentration of retinyl acetate ($C_{22}H_{32}O_2$) from [USP Vitamin A RS](#) in the *Standard solution* ($\mu\text{g/mL}$)

C_U = nominal concentration of vitamin A, as retinol ($C_{20}H_{30}O$) in the *Sample solution* ($\mu\text{g/mL}$)

F = factor used to convert retinyl acetate, the ester form present in [USP Vitamin A RS](#), to retinol, 0.872

[NOTE—The molar responses of retinyl acetate and retinyl palmitate are equivalent.]

Acceptance criteria: 90.0%–165.0% of the labeled amount of vitamin A, as retinol ($C_{20}H_{30}O$)

• VITAMIN A, Method 2

[NOTE—Where a vitamin A ester (retinyl acetate or retinyl palmitate) is indicated in the following procedure, use the chemical form present in the formulation. [USP Vitamin A RS](#) is retinyl acetate. It is to be used where [USP Vitamin A RS](#) is specified. Use low-actinic glassware throughout this procedure.]

3 N methanolic sulfuric acid solution: Cautionously add 9 mL of sulfuric acid to 80 mL of methanol in a 100-mL volumetric flask. Cool, and dilute with methanol to volume.

Sodium ascorbate–pyrogallol solution: Transfer 10 g of sodium ascorbate and 5 g of pyrogallol to a 100-mL volumetric flask, and add sufficient water to dissolve. Add 1.7 mL of sulfuric acid, and dilute with water to volume.

Lecithin solution: 5 mg/mL of lecithin in 2,2,4-trimethylpentane

Mobile phase: *n*-Hexane and ethyl acetate (99.7:0.3)

Standard solution: 15 $\mu\text{g/mL}$ of retinyl acetate from [USP Vitamin A RS](#) in 2,2,4-trimethylpentane

System suitability stock solution: 15 $\mu\text{g/mL}$ of retinyl palmitate in 2,2,4-trimethylpentane

System suitability solution: Mix equal volumes of the *System suitability stock solution* and the *Standard solution* to obtain concentrations of 7.5 $\mu\text{g/mL}$ each of retinyl acetate and retinyl palmitate.

Sample solution: [NOTE—This preparation is suitable for the determination of vitamin A, vitamin D, and vitamin E, when present in the formulation.] Weigh NLT 20 Capsules in a tared weighing bottle. Using a sharp blade if necessary, carefully open the Capsules, without loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing with several portions of ether. Discard the washings, and dry the Capsule shells with the aid of a current of dry air. Weigh the empty Capsule shells in the tared weighing bottle, and calculate the net weight of the Capsule contents. Transfer a portion of the Capsule contents, equivalent to 30 μg of the labeled amount of cholecalciferol or ergocalciferol (vitamin D), to a container with a polytetrafluoroethylene-lined screw cap. If vitamin D is not present in the formulation, use a portion, equivalent to 90 mg of the labeled amount of vitamin E. If vitamin E is not present in the formulation, use a portion, equivalent to 2.5 mg of the labeled amount of vitamin A, as retinol. Add 0.5 g of sodium bicarbonate, 1.5 mL of *Lecithin solution*, and 12.5 mL of 2,2,4-trimethylpentane, and disperse on a vortex mixer. Add 6 mL of *Sodium ascorbate–pyrogallol solution*, shake slowly, and allow the solution to degas. Continue shaking until the evolution of gas has ceased, and then shake for an additional 12 min. Add 6 mL of dimethyl sulfoxide, mix on a vortex mixer to form a suspension, and shake for 12 min. Add 6 mL of 3 N *methanolic sulfuric acid solution*, mix on a vortex mixer to form a suspension, and shake for 12 min. Add 12.5 mL of 2,2,4-trimethylpentane, mix on a vortex mixer to form a suspension, and shake for 10 min. Centrifuge for 10 min to break up the emulsion and to clarify the supernatant. [NOTE—The supernatant is used for the determination of vitamin A, and also vitamin D and vitamin E, if present in the formulation.] If necessary, quantitatively dilute a volume of the supernatant with 2,2,4-trimethylpentane to obtain a concentration close to that of the *Standard solution*.

Chromatographic system

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

Mode: LC

Detector: UV 325 nm

Column: 4.6-mm $\times$ 25-cm; 5- μm packing L24

Flow rate: 1.5 mL/min

Injection size: 40 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 8.0 between all-*trans*-retinyl acetate and all-*trans*-retinyl palmitate

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

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 *Sample solution*.

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

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

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 *Standard solution*

C_S = concentration of retinyl acetate (C₂₂H₃₂O₂) from [USP Vitamin A RS](#) in the *Standard solution* (µg/mL)

C_U = nominal concentration of vitamin A, as retinol (C₂₀H₃₀O) in the *Sample solution* (µg/mL)

F = factor used to convert retinyl acetate, the ester form present in [USP Vitamin A RS](#), to retinol, 0.872

[NOTE—Account for the initial extraction volume of 26.5 mL of 2,2,4-trimethylpentane to calculate the nominal concentration. The molar responses of retinyl acetate and retinyl palmitate are equivalent.]

Acceptance criteria: 90.0%–165.0% of the labeled amount of vitamin A, as retinol (C₂₀H₃₀O)

Change to read:

• **VITAMIN A, Method 3**

[NOTE—Where a vitamin A ester (retinyl acetate or retinyl palmitate) is indicated in the following procedure, use the chemical form present in the formulation. [USP Vitamin A RS](#) is retinyl acetate. It is to be used where [USP Vitamin A RS](#) is specified. Use low-actinic glassware throughout this procedure.]

Extraction solvent: *n*-Hexane and methylene chloride (3:1)

Potassium hydroxide solution: 800 mg/mL of potassium hydroxide in water. [NOTE—Cautiously add the potassium hydroxide to the water. Mix, and cool.]

Diluent: 10 mg/mL of pyrogallol in alcohol

Mobile phase: *n*-Hexane and isopropyl alcohol (92:8)

Standard stock solution: 30 µg/mL of retinyl acetate from [USP Vitamin A RS](#) in *Diluent*. [NOTE—This solution may be stored in a refrigerator for 1 week.]

Standard solution: Dilute a volume of *Standard stock solution* with *Diluent* to obtain a concentration of 1 µg/mL of retinyl acetate from [USP Vitamin A RS](#). Transfer 10.0 mL of this solution to a stoppered 125-mL flask, and add 5 mL of water, 5 mL of *Diluent*, and 3 mL of *Potassium hydroxide solution*. Insert the stopper tightly, shake for 15 min over a water bath maintained at 60 ± 5°, and cool to room temperature. Add 7 mL of water and 25.0 mL of *Extraction solvent*. Insert the stopper tightly, and shake vigorously for 60 s. Rinse the sides of the flask with 60 mL of water, and allow to stand for 10 min until the layers separate. Withdraw a portion of the organic layer for injection into the chromatograph. This *Standard solution* contains 0.34 µg/mL of retinol.

Sample solution: Weigh NLT 20 Capsules in a tared weighing bottle. Open the Capsules, without loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing with several portions of ether. Discard the washings, and dry the Capsule shells with the aid of a current of dry air. Weigh the empty Capsule shells in the tared weighing bottle, and calculate the net weight of the Capsule contents. Transfer a portion of the Capsule contents, equivalent to 1.5 mg of retinyl acetate, to a stoppered 125-mL flask. Add 5 mL of water, 15 mL of *Diluent*, and 3 mL of *Potassium hydroxide solution*. Insert the stopper tightly, shake for 15 min over a water bath maintained at 60 ± 5°, and cool to room temperature. Add 7 mL of water and 25.0 mL of *Extraction solvent*. Insert the stopper tightly, and shake vigorously for 60 s or longer, if necessary, for complete extraction. Rinse the sides of the flask with 60 mL of water, and allow to stand for 10 min until the layers separate. [NOTE—Do not shake, because an emulsion may form.] Withdraw a portion of the organic layer, and dilute quantitatively, and stepwise if necessary, with *Extraction solvent*, to obtain a concentration of 0.34 µg/mL of retinol.

Chromatographic system

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

Mode: LC**Detector:** UV 335 nm**Column:** 6.2-mm × 8-cm; packing L3**Column temperature:** 40°**Flow rate:** 4 mL/min**Injection size:** 50 µL**System suitability****Sample:** *Standard solution*[NOTE—The relative retention times for 13-*cis*-retinol and all-*trans*-retinol are about 0.92 and 1.0, respectively.]**Suitability requirements****Relative standard deviation:** NMT 5.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Measure the peak areas for all-*trans*-retinol and 13-*cis*-retinol.Calculate the percentage of the labeled amount of vitamin A, as retinol (C₂₀H₃₀O), in the portion of Capsules taken:

$$\text{Result} = (r_{T1}/r_{T2}) \times (C_S/C_U) \times F \times 100$$

 r_{T1} = sum of the areas of the all-*trans*-retinol and 13-*cis*-retinol peaks from the *Sample solution* r_{T2} = sum of the areas of all-*trans*-retinol and 13-*cis*-retinol peaks from the *Standard solution* C_S = concentration of retinyl acetate (▲C₂₂H₃₂O₂▲ (ERR 1-Nov-2023)) from [USP Vitamin A RS](#) in the *Standard solution* (µg/mL) C_U = nominal concentration of vitamin A, as retinol (C₂₀H₃₀O) in the *Sample solution* (µg/mL) F = factor used to convert retinyl acetate, the ester form present in [USP Vitamin A RS](#), to retinol, 0.872**Acceptance criteria:** 90.0%–165.0% of the labeled amount of vitamin A, as retinol (C₂₀H₃₀O)**• CHOLECALCIFEROL OR ERGOCALCIFEROL (VITAMIN D), Method 1**

[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 solution:** Proceed as directed for the *Sample stock solution* in *Vitamin A, Method 1*. Transfer NLT 20 mL of this solution retained as specified in the directions for the *Sample solution* in *Vitamin A, Method 1* to a suitable container, and evaporate, if necessary, in vacuum at room temperature to obtain a solution with a concentration of 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 size:** 100 µL**System suitability****Samples:** *Standard solution* and *System suitability solution***Suitability requirements****Resolution:** NLT 10 between the vitamin D form present and its corresponding precursor, *System suitability solution***Relative standard deviation:** NMT 3.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*

Measure the peak areas for vitamin D.

Calculate the percentage of the labeled amount of cholecalciferol (C₂₇H₄₄O) or ergocalciferol (C₂₈H₄₄O) in the portion of Capsules taken:

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

