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Oil-Soluble Vitamins with Minerals Tablets

DEFINITION

Oil-Soluble Vitamins Tablets contain 2 or more of the following oil-soluble vitamins: Vitamin A as retinyl acetate or retinyl palmitate, Vitamin D as Ergocalciferol (vitamin D₂) or Cholecalciferol (vitamin D₃), 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 K₁), and 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: boron, calcium, chromium, copper, fluorine, iodine, iron, magnesium, manganese, molybdenum, nickel, phosphorus, potassium, selenium, tin, vanadium, and zinc. Tablets contain NLT 90.0% and NMT 165.0% of the labeled amount of vitamin A as retinol (C₂₀H₃₀O), vitamin D as cholecalciferol (C₂₇H₄₄O) or ergocalciferol (C₂₈H₄₄O), vitamin E as 2*R*-alpha-tocopherol (C₂₉H₅₀O₂), phytonadione (C₃₁H₄₆O₂), and beta carotene (C₄₀H₅₆); NLT 90.0% and NMT 125.0% of the labeled amount of calcium (Ca), copper (Cu), iron (Fe), manganese (Mn), magnesium (Mg), phosphorus (P), potassium (K), and zinc (Zn); and NLT 90.0% and NMT 160.0% of the labeled amount of boron (B), chromium (Cr), fluorine (F), iodine (I), molybdenum (Mo), nickel (Ni), selenium (Se), tin (Sn), and vanadium (V).

Oil-Soluble Vitamins Tablets may contain other labeled added substances that are generally recognized as safe, in amounts that are unobjectionable.

IDENTIFICATION

- **A.** Characteristic absorption of each mineral as obtained in the tests in *Strength*
- **B.** The retention times of the vitamin peaks of the *Sample solutions* corresponds to those of the corresponding vitamin peaks of the *Standard solutions* as obtained in the tests for *Strength*.

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, with the method used being stated in the labeling only if *Method 1* is not used.]

• VITAMIN A, *Method 1*

Proceed as directed in [Vitamin A Assay \(571\)](#), [Assay, Chromatographic Methods, Procedure 1](#), except for the *Sample stock solution* and *Sample solution*.

Sample stock solution: Finely powder NLT 20 Tablets. To a portion of the powder, equivalent to 5 Tablets, add 15 mL of water, and sonicate for 5 min. Add 15 mL of *n*-hexane, and shake for 15 min in a water bath maintained at 60°. Add 10 mL of dimethyl sulfoxide, and shake for an additional period of 30 min in a water bath maintained at 60°. 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 3 additional 15-mL portions of *n*-hexane.

Sample solution: Dilute the *Sample stock solution* with *n*-hexane to obtain a solution with a concentration equivalent to 13 µg/mL of retinol (C₂₀H₃₀O).

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

• VITAMIN D (CHOLECALCIFEROL OR ERGOCALCIFEROL), *Method 1*

Proceed as directed in [Vitamin D Assay \(581\)](#), [Assay, Chromatographic Methods, Procedure 1](#), except for the *Sample solution*.

Sample solution: Prepare as directed for the *Sample stock solution* in *Vitamin A, Method 1*. Transfer NLT 20 mL of this solution to a suitable container, and, if necessary, evaporate under vacuum at room temperature to obtain a concentration of 2 µg/mL of cholecalciferol or ergocalciferol.

Acceptance criteria: 90.0%–165.0% of the labeled amount of vitamin D as cholecalciferol (C₂₇H₄₄O) or ergocalciferol (C₂₈H₄₄O)

• VITAMIN E, *Method 1*

Proceed as directed in [Vitamin E Assay \(551\)](#), [Assay, Procedure 1](#), except for the *Sample solution* and *Analysis*.

Sample solution: Prepared as directed for the *Sample stock solution* in *Vitamin A, Method 1*. Transfer NLT 20 mL of this solution to a suitable container, and evaporate under vacuum at room temperature to dryness. Transfer the residue with the aid of *n*-hexane 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.

Analysis

Samples: *Standard solution* and *Sample solution*

Measure the peak areas.

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 ($C_{33}H_{54}O_5$) as 2*R*-alpha-tocopherol ($C_{29}H_{50}O_2$) in the portion of Tablets taken:

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

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 *all-rac*-alpha-tocopherol from either [USP Alpha Tocopherol RS](#) or [USP Alpha Tocopheryl Acetate RS](#) or *RRR*-alpha tocopherol from [USP Alpha Tocopheryl Acid Succinate RS](#) in the *Standard solution* (mg/mL)

C_U = nominal concentration of the corresponding form of vitamin E as 2*R*-alpha-tocopherol in the *Sample solution* (mg/mL)

F = conversion factor for the content of *all-rac* -alpha-tocopherol to 2*R*-alpha-tocopherol, 1/2 (for products labeled to contain *all-rac* vitamin E sources) and 1 (for products labeled to contain *RRR* vitamin E sources)

Acceptance criteria: 90.0%–165.0% 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 ($C_{33}H_{54}O_5$) as 2*R*-alpha tocopherol

• PHYTONADIONE, Method 1

[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: Prepare as directed for the *Sample stock solution* in *Vitamin A, Method 1*. Transfer NLT 20 mL of this solution to a suitable container, and evaporate under 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*

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 Tablets 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* (µg/mL)

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

Acceptance criteria: 90.0%–165.0% of the labeled amount of phytonadione ($\text{C}_{31}\text{H}_{46}\text{O}_2$)

• **VITAMIN A, VITAMIN D, VITAMIN E, and PHYTONADIONE, Method 2**

[NOTE—Where vitamin A (retinyl acetate or retinyl palmitate), vitamin D (cholecalciferol or ergocalciferol), or vitamin E (alpha-tocopherol, alpha-tocopheryl acetate, or alpha-tocopheryl acid succinate) are specified in the following procedure, use the chemical form present in the formulation and the relevant Reference Standard. Use low-actinic glassware.]

Diluent: [Methanol](#) and [water](#) (90:10 v/v)

Solution A: 0.1% (v/v) [trifluoroacetic acid](#) in *Diluent*

Solution B: 0.1% (v/v) [trifluoroacetic acid](#) in [methanol](#)

Mobile phase: See [Table 1](#).

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
19	0	100
27	0	100
28	100	0
30	100	0

Vitamin A standard stock solution: A solution containing the equivalent of 0.2 mg/mL of retinol from [USP Retinyl Acetate RS](#) or [USP Retinyl Palmitate RS](#) in [isopropyl alcohol](#). Dissolve with the aid of sonication if necessary.

Vitamin D standard stock solution: 0.05 mg/mL of [USP Cholecalciferol RS](#) or [USP Ergocalciferol RS](#) in [isopropyl alcohol](#). Dissolve with the aid of sonication if necessary.

Vitamin E standard stock solution: 3.0 mg/mL of [USP Alpha Tocopherol RS](#), [USP Alpha Tocopheryl Acetate RS](#), or [USP Alpha Tocopheryl Acid Succinate RS](#) in [isopropyl alcohol](#). Dissolve with the aid of sonication if necessary.

Phytonadione standard stock solution: 0.04 mg/mL of [USP Phytonadione RS](#) in [isopropyl alcohol](#). Dissolve with the aid of sonication if necessary.

Sample solution 1: Finely powder NLT 20 Tablets. Transfer an accurately weighed portion of the powder into a 50-mL volumetric flask to obtain a solution having known nominal concentrations in the ranges of 0.05–0.1 mg/mL of vitamin A and 0.5–0.15 mg/mL of vitamin E. Add 10 mL of [dimethyl sulfoxide](#), and sonicate for 30 min with vigorous intermittent shaking. Cool to room temperature, dilute with [isopropyl alcohol](#) to volume, and mix well. Pass a portion of the solution through a nylon filter of 0.45- μm pore size, and discard the first milliliter of the filtrate.

Sample solution 2: Transfer an accurately weighed portion of the powder from NLT 20 finely powdered Tablets into a 50-mL volumetric flask to obtain a solution having known nominal concentrations in the ranges of 1.0–3.5 $\mu\text{g/mL}$ of vitamin D and 1.0–3.5 $\mu\text{g/mL}$ of phytonadione. Add 5 mL of water and sonicate for 10 min with intermittent shaking. Add 10 mL of [dimethyl sulfoxide](#) and sonicate for 30 min with vigorous intermittent shaking. Cool to room temperature, dilute with [isopropyl alcohol](#) to volume, and mix well. Pass a portion of the solution through a nylon filter of 0.45- μm pore size, and discard the first milliliter of the filtrate.

Standard solution: Transfer calculated volumes of *Vitamin A standard stock solution*, Vitamin D standard stock solution, Vitamin E standard stock solution, and Phytonadione standard stock solution into a suitable volumetric flask, and dilute with [isopropyl alcohol](#) to volume to obtain a solution with final vitamin concentrations similar to those obtained for the corresponding vitamins in the *Sample solution 1* and *Sample solution 2*.

Chromatographic system

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

Mode: LC

Detectors

For Sample solution 1 containing retinyl acetate: UV 325 nm for 7 min, then switch to 265 nm

For Sample solution 1 containing retinyl palmitate: UV 265 nm for 18 min, then switch to 325 nm

For Sample solution 2: UV 265 nm

Column: 4.6-mm $\times$ 10-cm; 3- μm packing L1

Temperatures**Sample:** 4°**Column:** 35°**Flow rate:** 1.5 mL/min**Injection volume:** 20 µL**System suitability****Samples:** *Sample solution 1, Sample solution 2, and Standard solution***Suitability requirements****Relative standard deviation:** NMT 2.0% for each individual peak, *Standard solution*

Peak purity of vitamin D and vitamin A (as retinyl palmitate): Monitor the chromatograms of the *Sample solution 1* and *Sample solution 2* at 450 nm. The area of any peak at the retention time of retinyl palmitate from *Sample solution 1* and any peak at the retention time of vitamin D (as cholecalciferol or ergocalciferol) from *Sample solution 2* should be NMT 5% of the corresponding peak areas detected at 325 nm and 265 nm, respectively. [NOTE—This test is required due to possible interference with carotenoids (cryptoxanthin and lycopene) that may be present in the formulation. Use another procedure in the monograph for analysis of vitamin D and vitamin A (retinyl palmitate) if the system is not suitable.]

Analysis**Samples:** *Sample solution 1, Sample solution 2, and Standard solution*Calculate the percentage of the labeled amount of vitamin A activity, as retinol ($C_{20}H_{30}O$), in the portion of Tablets 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 *Standard solution* C_S = concentration of retinol in the *Standard solution* (mg/mL) C_U = nominal concentration of vitamin A, as retinol, in the *Sample solution* (mg/mL)Calculate the percentage of the labeled amount of vitamin E, as 2*R*-alpha-tocopherol ($C_{29}H_{50}O_2$), in the portion of Tablets taken:

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

 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 *all-rac*-alpha-tocopherol from either [USP Alpha Tocopherol RS](#) or [USP Alpha Tocopheryl Acetate RS](#) or *RRR*-alpha-tocopherol from [USP Alpha Tocopheryl Acid Succinate RS](#) in the *Standard solution* (mg/mL) C_U = nominal concentration of vitamin E, as 2*R*-alpha-tocopherol, in the *Sample solution* (mg/mL) F = conversion factor for the content of *all-rac*-alpha-tocopherol to *RRR*-alpha-tocopherol equivalent, 1/2 (for products labeled to contain *all-rac* vitamin E sources) and 1 (for products labeled to contain *RRR* vitamin E sources)Calculate the percentage of the labeled amount of vitamin D, as cholecalciferol ($C_{27}H_{44}O$) or ergocalciferol ($C_{28}H_{44}O$), and phytonadione ($C_{31}H_{46}O_2$) in the portion of Tablets taken:

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

 r_U = peak area of the related vitamin from the *Sample solution* r_S = peak area of the related vitamin from the *Standard solution* C_S = concentration of related vitamin in the *Standard solution* (µg/mL) C_U = nominal concentration of related vitamin in the *Sample solution* (µg/mL)**Acceptance criteria:** 90.0%–150.0% of the labeled amount of each individual vitamin• **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 µg/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 µg/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.

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.

Determine the concentration of *Standard solution A* from the concentration of *Standard solution B*.

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 3 preparations of *Standard solution B*

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

Sample solution: Prepare as directed for the *Sample stock solution* in *Vitamin A*. Transfer NLT 20 mL of this solution to a suitable container, and evaporate under 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 µg/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 × 25-cm; 5-µm packing L68

Column temperature: 30°

Flow rate: 0.6 mL/min

Injection volume: 20 µL

System suitability

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

The approximate relative retention times of the components in the *System suitability solution* are listed in [Table 2](#).

Table 2

Name	Relative Retention Time	Relative Response Factor
<i>all-trans</i> -Alpha carotene	0.93	1.1
<i>all-trans</i> -Beta carotene	1.00	1
9- <i>cis</i> -Beta carotene	1.07	1
13- <i>cis</i> -Beta carotene	1.17	1



Name	Relative Retention Time	Relative Response Factor
15- <i>cis</i> -Beta carotene	1.21	1.4

Suitability requirements

Chromatogram similarity: The chromatogram from the *System suitability solution* is similar to the reference chromatogram provided with the [USP Beta Carotene System Suitability RS](#) being used.

Resolution: NLT 1.5 between beta carotene and alpha carotene and between beta carotene and 9-*cis*-beta carotene, *System suitability solution*

Tailing factor: NMT 2.0 for the beta carotene peak, *Standard solution A*

Relative standard deviation: NMT 2.0% for the beta carotene peak from replicate injections, *Standard solution A*

Analysis

Samples: *Standard solution A* and *Sample solution*

Calculate the percentage of *all-trans*-beta carotene in the portion of Tablets taken:

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

r_U = peak area of *all-trans*-beta carotene in the *Sample solution*

r_S = peak area of *all-trans*-beta carotene in *Standard solution A*

C_S = concentration of *all-trans*-beta carotene in *Standard solution A* as determined by spectrometric procedure

C_U = nominal concentration of beta carotene in the *Sample solution*

Acceptance criteria: 90.0%–165.0% of the labeled amount of beta carotene ($C_{40}H_{56}$)

• CALCIUM, Method 1

Lanthanum chloride solution: 267 mg/mL of lanthanum chloride heptahydrate in 0.125 N hydrochloric acid

Calcium standard solution: 400 µg/mL of calcium. Dissolve 1.001 g of calcium carbonate, previously dried at 300° for 3 h and cooled in a desiccator for 2 h, in 25 mL of 1 N hydrochloric acid. Boil to expel carbon dioxide, and dilute with water to 1000 mL.

Standard stock solution: 100 µg/mL of calcium from the *Calcium standard solution* diluted with 0.125 N hydrochloric acid

Standard solutions: Into separate 100-mL volumetric flasks pipet 1.0, 1.5, 2.0, 2.5, and 3.0 mL of the *Standard stock solution*. To each flask add 1.0 mL of *Lanthanum chloride solution*, and dilute with 0.125 N hydrochloric acid to volume to obtain concentrations of 1.0, 1.5, 2.0, 2.5, and 3.0 µg/mL of calcium.

Sample solution: Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 5 Tablets, to a porcelain crucible. Heat the crucible in a muffle furnace maintained at 550° for 6–12 h, and cool. Add 60 mL of hydrochloric acid, and boil gently on a hot plate or steam bath for 30 min, intermittently rinsing the inner surface of the crucible with 6 N hydrochloric acid. Cool, and quantitatively transfer the contents of the crucible to a 100-mL volumetric flask. Rinse the crucible with small portions of 6 N hydrochloric acid, and add the rinsings to the flask. Dilute with water to volume, and filter, discarding the first 5 mL of the filtrate. Dilute this solution quantitatively, with 0.125 N hydrochloric acid, to obtain a concentration of 2 µg/mL of calcium, adding 1 mL of *Lanthanum chloride solution* per 100 mL of the final volume.

Instrumental conditions

(See [Atomic Absorption Spectroscopy \(852\)](#).)

Mode: Atomic absorption spectroscopy

Analytical wavelength: Calcium emission line at 422.7 nm

Lamp: Calcium hollow-cathode

Flame: Nitrous oxide–acetylene

Blank: 0.125 N hydrochloric acid containing 1 mL of *Lanthanum chloride solution* per 100 mL

Analysis

Samples: *Standard solutions* and *Sample solution*

Determine the absorbances of the solutions, against the *Blank*. Plot the absorbances of the *Standard solutions* versus the concentration, in µg/mL, of calcium, and draw the straight line best fitting the 5 plotted points. From the graph, determine the concentration (C), in µg/mL, of calcium in the *Sample solution*.

Calculate the percentage of the labeled amount of calcium (Ca) in the portion of Tablets taken: