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Oil- and Water-Soluble Vitamins Tablets

DEFINITION

Change to read:

Oil- and Water-Soluble Vitamins Tablets contain one or more of the following oil-soluble vitamins: Vitamin A ▲ as retinyl acetate or retinyl palmitate, ▲ (USP 1-May-2020) 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, ▲ (USP 1-May-2020) Phytonadione (vitamin K₁), and Beta Carotene; and one or more of the following water-soluble vitamins: Ascorbic Acid or its equivalent as Calcium Ascorbate or Sodium Ascorbate, Biotin, Cyanocobalamin, Folic Acid, Niacin or Niacinamide, pantothenic acid (as Calcium Pantothenate or Racemic Calcium Pantothenate), Pyridoxine Hydrochloride, Riboflavin, and Thiamine Hydrochloride or Thiamine Mononitrate. Tablets contain NLT 90.0% and NMT 165.0% of the labeled amount of vitamin A ▲ as retinol ▲ (USP 1-May-2020) (C₂₀H₃₀O); vitamin D as cholecalciferol (C₂₇H₄₄O) or ergocalciferol (C₂₈H₄₄O); vitamin E ▲ as 2*R*-alpha-tocopherol ▲ (USP 1-May-2020) (C₂₉H₅₀O₂); phytonadione (C₃₁H₄₆O₂); and beta carotene (C₄₀H₅₆); and NLT 90.0% and NMT 150.0% of the labeled amount of ascorbic acid (C₆H₈O₆); ▲ (USP 1-May-2020) biotin (C₁₀H₁₆N₂O₃S), cyanocobalamin (C₆₃H₈₈CoN₁₄O₁₄P), folic acid (C₁₉H₁₉N₇O₆), niacin (C₆H₅NO₂) or niacinamide (C₆H₆N₂O), calcium pantothenate (C₁₈H₃₂CaN₂O₁₀), pyridoxine ▲ (USP 1-May-2020) (C₈H₁₁NO₃) ▲ (USP 1-May-2020), riboflavin (C₁₇H₂₀N₄O₆), and thiamine ▲ as thiamine ion (C₁₂H₁₇N₄OS⁺) ▲ (USP 1-May-2020)

They do not contain any minerals. They may contain other labeled added substances that are generally recognized as safe, in amounts that are unobjectionable.

IDENTIFICATION

Add the following:

▲ • **A.** The retention times of the vitamin peaks of the *Sample solutions* correspond to those of the corresponding vitamin peaks of the *Standard solutions* as obtained in the tests for *Strength*. ▲ (USP 1-May-2020)

STRENGTH

[NOTE—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.]

Change to read:

• **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*. ▲ (USP 1-May-2020)

Sample stock solution: Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 5 Tablets, 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 3 additional 15-mL portions of *n*-hexane. Dilute the extracts in the volumetric flask with *n*-hexane to volume.

Sample solution: Dilute a 10-mL 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).

▲ ▲ (USP 1-May-2020)

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

Change to read:• **VITAMIN A, Method 2**

▲ Proceed as directed in [Vitamin A Assay \(571\)](#), [Assay, Chromatographic Methods, Procedure 2](#). ▲ (USP 1-May-2020)

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

Change to read:• **VITAMIN A, Method 3**

▲ Proceed as directed in [Vitamin A Assay \(571\)](#), [Assay, Chromatographic Methods, Procedure 3](#). ▲ (USP 1-May-2020)

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

Change to read:• **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*. ▲ (USP 1-May-2020)

Sample solution: Proceed 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.

▲ ▲ (USP 1-May-2020)

Acceptance criteria: 90.0%–165.0% of the labeled amount of vitamin D as cholecalciferol ($C_{27}H_{44}O$) or ergocalciferol ($C_{28}H_{44}O$)

Change to read:• **VITAMIN D (CHOLECALCIFEROL OR ERGOCALCIFEROL), Method 2**

▲ Proceed as directed in [Vitamin D Assay \(581\)](#), [Assay, Chromatographic Methods, Procedure 2](#). ▲ (USP 1-May-2020)

Acceptance criteria: 90.0%–165.0% of the labeled amount of vitamin D as cholecalciferol ($C_{27}H_{44}O$) or ergocalciferol ($C_{28}H_{44}O$)

Change to read:• **VITAMIN D (CHOLECALCIFEROL OR ERGOCALCIFEROL), Method 3**

▲ Proceed as directed in [Vitamin D Assay \(581\)](#), [Assay, Chromatographic Methods, Procedure 3](#), except for the *Analysis*. ▲ (USP 1-May-2020)

Analysis

Samples: *Standard solution* and *Sample solution*

Measure the peak areas for vitamin D.

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 Tablets taken:

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

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)

Acceptance criteria: 90.0%–165.0% of the labeled amount of vitamin D as cholecalciferol ($C_{27}H_{44}O$) or ergocalciferol ($C_{28}H_{44}O$)

Change to read:• **VITAMIN E, Method 1**

▲ Proceed as directed in [Vitamin E Assay \(551\)](#), [Assay, Procedure 1](#), except for the *Sample solution* and *Analysis*. ▲ (USP 1-May-2020)

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 dryness. Transfer the residue to a suitable volumetric flask with the aid of methanol, 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.

▲ ▲ (USP 1-May-2020)

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 ($C_{33}H_{54}O_5$) $\blacktriangle$ as 2R-alpha-tocopherol ($C_{29}H_{50}O_2$) $\blacktriangle$ (USP 1-May-2020) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \blacktriangle (\text{USP 1-May-2020}) \times 100$$

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 $\blacktriangle$ 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](#) $\blacktriangle$ (USP 1-May-2020) in the *Standard solution* (mg/mL)

C_U = nominal concentration of the corresponding form of vitamin E $\blacktriangle$ as 2R-alpha-tocopherol $\blacktriangle$ (USP 1-May-2020) in the *Sample solution* (mg/mL)

F = conversion factor for the content of all-*rac*-alpha-tocopherol to 2R-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) $\blacktriangle$ (USP 1-May-2020)

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 ($C_{33}H_{54}O_5$) $\blacktriangle$ as 2R-alpha-tocopherol $\blacktriangle$ (USP 1-May-2020)

Change to read:

• VITAMIN E, Method 2

$\blacktriangle$ Proceed as directed in [Vitamin E Assay \(551\), Assay Procedure 2](#), except for Analysis.

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 ($C_{33}H_{54}O_5$) as 2R-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 2R-alpha-tocopherol in the *Sample solution* (mg/mL)

F = conversion factor for the content of all-*rac*-alpha-tocopherol to 2R-alpha-tocopherol, 1/2 $\blacktriangle$ ($\blacktriangle$ (ERR 1-May-2020) for products labeled to contain all-*rac* vitamin E sources) and 1 (for products labeled to contain *RRR* vitamin E sources)

$\blacktriangle$ (USP 1-May-2020)

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 ($C_{33}H_{54}O_5$) $\blacktriangle$ as 2R-alpha-tocopherol $\blacktriangle$ (USP 1-May-2020)

Change to read:

• VITAMIN E, Method 3

$\blacktriangle$ Proceed as directed in [Vitamin E Assay \(551\), Assay Procedure 3](#), except for the Analysis.

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 ($C_{33}H_{54}O_5$) as 2R-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)

▲ (USP 1-May-2020)

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 ▲ (USP 1-May-2020)

• **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.

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.]

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

Sample solution: Transfer NLT 20 mL of the solution retained as directed for the *Sample stock solution* in *Vitamin A, Method 1* 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 concentration of 20 µg/mL of phytonadione.

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 8-mm × 10-cm; 5-µm packing L1

Flow rate: 2 mL/min

Injection volume: 100 µL

System suitability

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

[NOTE—The relative retention times for alpha-tocopheryl acetate and phytonadione are 0.68 and 1.0, respectively.]

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*

Measure the peak areas. 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 of phytonadione from the *Sample solution*

r_s = peak area of phytonadione 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* (µg/mL)

Acceptance criteria: 90.0%–165.0% of the labeled amount of phytonadione ($C_{31}H_{46}O_2$)

• **PHYTONADIONE, Method 2**

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

Solvent: Methanol and isopropyl alcohol (19:1)

Mobile phase: Mix 800 mL of methanol, 200 mL of methylene chloride, 0.1 mL of glacial acetic acid, 1.36 g of zinc chloride, and 0.41 g of sodium acetate.

Internal standard solution: 5 µg/mL of menaquinone 4 (vitamin K₂) in *Solvent*. [NOTE—A concentrated stock solution of menaquinone 4 (100 µg/mL) can be stored for 2 months in a refrigerator.]

Standard stock solution: 5 µg/mL of [USP Phytonadione RS](#), prepared by dissolving in methylene chloride with the aid of sonication, and diluting with *Solvent* to final volume.

Standard solution: Transfer 1.0 mL of the *Standard stock solution* and 1.0 mL of the *Internal standard solution* to a suitable flask, and dilute with *Solvent* to 5 mL. Pass through a membrane filter of 0.45-µm or finer pore size.

Sample solution: Finely powder NLT 20 Tablets. To a centrifuge tube fitted with a cap transfer an amount of powder not exceeding 800 mg and equivalent to an amount of phytonadione not exceeding 50 µg. Add 4 mL of water. Insert the stopper, and mix using a vortex mixer until the sample is dispersed. Place the tube in a water bath at 60° for 5 min. Remove from the bath, and again shake or mix using a vortex mixer for 1 min while the preparation is still hot. Add 8 mL of alcohol, and swirl the contents to mix. Place the tube in a water bath at 60° for 5 min. Remove from the bath, and again shake or mix using a vortex mixer for 2 min while the preparation is still hot. Cool to room temperature. Add a volume of the *Internal standard solution*, equivalent to 1.0 mL for each 5 µg of the expected amount of phytonadione in the aliquot taken. Add 20.0 mL of petroleum ether, and cap the tube tightly. Shake or mix using a vortex mixer for 15 min to thoroughly mix the contents. Centrifuge to separate the 2 layers. Transfer a volume of the top layer of petroleum ether, equivalent to 5–50 µg of the nominal amount of phytonadione, to an appropriate flask. Place the flask in a water bath at 35°–45°, and evaporate the solvent under a stream of nitrogen until an oily residue is left. Dissolve the residue in a volume of *Solvent* to obtain a concentration of 1 µg/mL of phytonadione.

Chromatographic system

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

Mode: LC

Detector: Fluorometric detector

Excitation wavelength: 320 nm

Emission wavelength: 420 nm

Column: 4.6-mm × 25-cm; 5-µm, end-capped packing L1, and a postcolumn reactor constituted with a 4.6-mm × 3-cm PEEK column tightly packed with zinc powder. [NOTE—Prepare the postcolumn reactor daily, or as necessary, to meet the *System suitability* requirements.]

Flow rate: 1 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for the internal standard and phytonadione are 1.0 and 1.4, respectively.]

Suitability requirements

Column efficiency: NLT 2500 theoretical plates for the phytonadione peak

Tailing factor: NMT 1.5 for the phytonadione peak

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of phytonadione (C₃₁H₄₆O₂) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak response ratio of phytonadione to that of the internal standard from the *Sample solution*

R_S = peak response ratio of phytonadione to that of the internal standard 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* (µg/mL)

Acceptance criteria: 90.0%–165.0% of labeled amount of phytonadione (C₃₁H₄₆O₂)

Add the following:

▲ • **VITAMIN A, VITAMIN D (CHOLECALCIFEROL OR ERGOCALCIFEROL), VITAMIN E, Method 4; PHYTONADIONE, Method 3**

[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 from [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 to a 50-mL volumetric flask to obtain a solution containing 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 sonicated for 30 min with vigorous intermittent shaking. Cool to room temperature, dilute to volume with [isopropyl alcohol](#) and mixed well. Pass a portion of the solution through a 0.45-µm pore size nylon filter 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 to a 50-mL volumetric flask to obtain a solution containing known nominal concentrations in the ranges of 1.0–3.5 µg/mL of vitamin D and 1.0–3.5 µg/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* to 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 *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 × 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 *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 because of 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 (as 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 the related vitamin in the *Standard solution* (μg/mL)

C_U = nominal concentration of the related vitamin in the *Sample solution* (μg/mL)

Acceptance criteria: 90.0%–165.0% of the labeled amount of each individual vitamin▲ (USP 1-May-2020)

• BETA CAROTENE

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

Potassium hydroxide solution: Dissolve 58.8 g of potassium hydroxide in 50 mL of water.

Iodine solution: 0.01 mg/mL of iodine in cyclohexane. [NOTE—Prepare this solution fresh daily.]

Sample solution: Weigh NLT 20 Tablets. Grind the Tablets to a fine powder, and transfer a quantity of the powder, equivalent to 2 mg of beta carotene, to a 500-mL saponification flask. Add 100 mL of alcohol, 6 mL of *Potassium hydroxide solution*, and a magnetic stirring bar. Attach an air condenser to the flask, and heat under reflux for 45 min with constant stirring. Cool to room temperature. Add 170 mL of solvent hexane, and stir for 30 min. Quantitatively transfer the contents of the flask to a 500-mL separatory funnel with portions of solvent hexane. Allow the layers to separate for 5–10 min, 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 solvent hexane, and stir for an additional 20 min. Quantitatively transfer the contents of the saponification flask to the separatory funnel with the aid of portions of solvent hexane. Allow the layers to separate for 10 min. 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 solvent hexane, and transfer the washings to the volumetric flask. Dilute the hexane extracts with solvent hexane to volume. Add 3 g of anhydrous sodium sulfate, 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 *Iodine solution*, and heat for 15 min in a water bath maintained at 65°. Cool rapidly, and dilute with cyclohexane to volume.

Instrumental conditions

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

Mode: Vis

Analytical wavelength: 452 nm

Blank: Cyclohexane

Analysis

Sample: *Sample solution*

Determine the absorbance against the *Blank*. Calculate the percentage of the labeled amount of beta carotene ($C_{40}H_{56}$) in the portion of Tablets taken:

$$\text{Result} = (A_U/F) \times (100/C_U)$$

A_U = absorbance of the *Sample solution*

F = absorptivity of beta carotene at 452 nm, 223

C_U = nominal concentration of beta carotene in the *Sample solution* (mg/mL)

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

Change to read:

• **ASCORBIC ACID, CALCIUM ASCORBATE, and SODIUM ASCORBATE**

(See [Vitamin C Assay \(580\)](#).)

[NOTE—For labeling purposes, consider [\(580\), Method I—Titrimetric Method](#) as Method 1; ▲ [\(580\), Method II—Chromatographic Method, Procedure 1](#) as Method 2; and [\(580\), Method II—Chromatographic Method, Procedure 2](#) as Method 3. ▲ (USP 1-May-2020)]

Acceptance criteria: 90.0%–150.0% of the labeled amount of ascorbic acid ($C_6H_8O_6$), calcium ascorbate ($C_{12}H_{14}CaO_{12} \cdot 2H_2O$), or sodium ascorbate ($C_6H_7NaO_6$)

• **BIOTIN, Method 1**

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

Mobile phase: Mix 85 mL of acetonitrile, 1 g of sodium perchlorate, 1 mL of phosphoric acid, and dilute with water to 1000 mL.

Standard stock solution: 0.333 mg/mL of [USP Biotin RS](#) in dimethyl sulfoxide

Standard solution: 5 µg/mL of [USP Biotin RS](#) prepared by diluting the *Standard stock solution* in water

Sample solution: Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 1 mg of biotin, to a 200-mL volumetric flask.

Add 3 mL of dimethyl sulfoxide, and swirl to wet the contents. Place the flask in a water bath at 60°–70° for 5 min. Sonicate for 5 min, dilute with water to volume, and filter.

Chromatographic system

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

Mode: LC

Detector: UV 200 nm

Column: 4.6-mm × 15-cm; 3-µm packing [L7](#)

Flow rate: 1.2 mL/min

Injection volume: 100 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Measure the peak areas of biotin. Calculate the percentage of the labeled amount of biotin ($C_{10}H_{16}N_2O_3S$) in the portion of Tablets taken:

