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Add the following:

Oil-Soluble Vitamins Preparation

DEFINITION

Oil-Soluble Vitamins Preparation is a mixture of two or more of the following oil-soluble vitamins: Vitamin A as retinyl acetate or retinyl palmitate, Vitamin D as cholecalciferol, Vitamin E as *RRR*- or *all-rac*-alpha-tocopheryl acetate, and Vitamin K as phytonadione with one or more inert substances and suitable antioxidants. It may be in a solid or liquid form. It contains NLT 90.0% and NMT 120.0% of the labeled amount of vitamin A as retinol equivalents ($C_{20}H_{30}O$), vitamin D as cholecalciferol ($C_{27}H_{44}O$), Vitamin E as 2*R*-alpha-tocopherol ($C_{29}H_{50}O_2$), and Vitamin K as phytonadione ($C_{31}H_{46}O_2$).

IDENTIFICATION

• A.

Analysis: Proceed as directed in *Composition*.

Acceptance criteria: The retention times of the main peaks of the *Sample solution* correspond to those of the vitamins contained in the appropriate *Standard solution*.

COMPOSITION

• CONTENT OF VITAMIN A AND VITAMIN E

[NOTE—Where vitamin A (retinyl acetate or retinyl palmitate) is specified in the following procedure, use the chemical form present in the formulation and the relevant USP Reference Standard. Use low-actinic glassware.]

Mobile phase: [Methanol](#)

Standard stock solution 1: [USP Retinyl Acetate RS](#) in [isopropyl alcohol](#) containing 0.2 mg/mL of retinol equivalents

Standard stock solution 2: [USP Retinyl Palmitate RS](#) in [isopropyl alcohol](#) containing 0.6 mg/mL of retinol equivalents

Standard stock solution 3: 2.0 mg/mL of [USP Alpha Tocopheryl Acetate RS](#) in [isopropyl alcohol](#)

Standard solution: 18 µg/mL of retinol equivalents and 0.55 mg/mL of alpha tocopheryl acetate solution in [methanol](#) derived from the appropriate *Standard stock solution*

[NOTE—Concentrations of [USP Retinyl Acetate RS](#) or [USP Retinyl Palmitate RS](#) as well as [USP Alpha Tocopheryl Acetate RS](#) in the *Standard solution* can be adjusted according to the expected concentrations of the respective vitamins in the *Sample solution*.]

Sample solution

For Oil-Soluble Vitamins Preparation in solid form: Accurately weigh NLT 500 mg of Oil-Soluble Vitamins Preparation¹ into a suitable volumetric flask. Add 10 mL of [water](#) and swirl immediately.

[NOTE—An additional 10 mL of [water](#) may be added if the sample size exceeds 1.5 g.]

Add 20 drops of protease from *Aspergillus oryzae* containing NLT 500 units/g² to the bottom of the flask using a plastic pipette and gently swirl the contents to mix. Place the sample in a water bath at 60° for 5 min. Swirl the flask 3–4 times while heating to ensure that the sample is completely dispersed and that it did not form clumps or stick to the walls of the flask. Remove the flask from the water bath, add 80 mL of [isopropyl alcohol](#), and swirl for 1 min. Add 50 mL of [methanol](#) and swirl to mix. Sonicate for 5 min, cool the flask to room temperature, dilute with [methanol](#) to volume, and mix well. If necessary, further dilute with [methanol](#) to obtain a solution with a nominal concentration of retinol equivalents in the range of 1.8–75 µg/mL and alpha tocopheryl acetate in the range of 0.05–2.0 mg/mL and mix well. Pass a portion of the solution through a 0.2-µm polytetrafluoroethylene (PTFE) membrane filter, and discard the first 1 mL of the filtrate.

[NOTE—Add 0.5 g of [sodium lauryl sulfate](#) after adding [water](#) if Oil-Soluble Vitamins Preparation contains NLT 20% (w/w) of maltodextrin.]

For Oil-Soluble Vitamins Preparation containing trisodium phosphate (TSP): Prepare as directed for solid form, except add 10 mL of *Diluted glacial acetic acid* instead of water.

[NOTE—An additional 10 mL of [water](#) may be added if the sample size exceeds 1.5 g.]

Diluted glacial acetic acid: Transfer an accurately measured amount of [glacial acetic acid](#) (10.5 µL for each mg of TSP in Oil-Soluble Vitamins Preparation taken) into a 100-mL volumetric flask, using a gas-tight syringe if necessary. Dilute with [water](#) to volume and mix well.

For Oil-Soluble Vitamins Preparation in liquid form: Transfer 100–250 mg of Oil-Soluble Vitamins Preparation, accurately weighed, to a 200-mL volumetric flask. Add 5 mL of [n-hexane](#) and swirl for 1 min to dissolve. Dilute with [absolute alcohol](#) to volume and mix well. Further dilute with a mixture of [methanol](#) and [alcohol](#) (1:1) to obtain a solution with a nominal concentration of retinol equivalents in the range of 1.8–75 µg/mL and alpha tocopheryl acetate in the range of 0.05–2.0 mg/mL, and mix well. Pass a portion of the solution through a 0.2-µm PTFE membrane filter, and discard the first 1 mL of the filtrate.

Chromatographic system

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

Mode: LC

Detector: UV 325 nm for retinyl acetate and retinyl palmitate; UV 280 nm for alpha-tocopheryl acetate

Column: 2.1-mm × 10-cm; 1.8-µm packing [L1](#)

Temperatures

Sample: 8°

Column: 35°

Flow rate: 0.9 mL/min

Injection volume: 1.0 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time of *all-trans*-retinyl acetate is about 0.5 min, of alpha tocopheryl acetate is about 1.2 min, and of *all-trans*-retinyl palmitate is about 3 min.]

Suitability requirement

Relative standard deviation: NMT 2.0% for each peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of vitamin A as retinol equivalents ($C_{20}H_{30}O$) in the portion of Oil-Soluble Vitamins

Preparation taken:

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

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

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

C_S = concentration of vitamin A as retinol equivalents in the *Standard solution* (µg/mL)

C_U = nominal concentration of vitamin A as retinol equivalents in the *Sample solution* (µg/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 Oil-Soluble Vitamins

Preparation taken:

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

r_U = peak area of the alpha tocopheryl acetate from the *Sample solution*

r_S = peak area of the alpha tocopheryl acetate from the *Standard solution*

C_S = concentration of alpha tocopherol from [USP Alpha Tocopheryl Acetate RS](#) in the *Standard solution* (mg/mL)

C_U = concentration of alpha tocopherol from alpha tocopheryl acetate in the *Sample solution* (mg/mL)

F = conversion factor for the content of alpha tocopherol to 2*R*-alpha-tocopherol equivalent: 1/2 for products labeled to contain *all-rac*-alpha-tocopheryl acetate and 1 for products labeled to contain *RRR*-alpha-tocopheryl acetate

Acceptance criteria: 90.0%–120.0% of the labeled amount of each individual vitamin

• CONTENT OF VITAMIN D₃ AND VITAMIN K₁

[NOTE—Use low-actinic glassware.]

Solution A: 5 mg/mL of [sodium lauryl sulfate](#) and [water](#)

Solution B: 0.02% (w/v) of [butylated hydroxytoluene](#) in [n-hexane](#)

Solution C: Mixture of [dimethyl sulfoxide](#) and [water](#) (10:2)