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Add the following:

▲Vitamins with Minerals Oral Powder

DEFINITION

Vitamins with Minerals Oral Powder contains one or more of the following oil-soluble vitamins: Vitamin A as retinyl acetate or retinyl palmitate, Vitamin D as cholecalciferol, Vitamin E as *RRR*-alpha-tocopheryl acetate or *all-rac*-alpha-tocopheryl acetate; one or more of the following water-soluble vitamins: Vitamin C as ascorbic acid, sodium ascorbate or calcium ascorbate, Vitamin B1 as thiamine mononitrate, Vitamin B2 as riboflavin, Vitamin B3 as niacin or niacinamide, Vitamin B6 as pyridoxine hydrochloride, Vitamin B12 as cyanocobalamin, and Folic Acid; and one or more minerals: copper, iodine, iron, selenium, and zinc, derived from substances generally recognized as safe and furnishing the elements in ionizable form. [Note—NMT 25% of elemental iron from the labeled amount may derive from edetate iron (III) sodium when used as a source of iron.] Vitamins with Minerals Oral Powder contains NLT 90.0% and NMT 165.0% of the labeled amount of Vitamin A as retinol ($C_{20}H_{30}O$); NLT 90.0% and NMT 150.0% of the labeled amount of Vitamin D as cholecalciferol ($C_{27}H_{44}O$), and Vitamin E as 2*R*-alpha-tocopherol ($C_{29}H_{50}O_2$); NLT 90.0% and NMT 150.0% of the labeled amount of Vitamin C as ascorbic acid ($C_6H_8O_6$), Vitamin B1 as thiamine ion ($C_{12}H_{17}N_4OS^+$), Vitamin B2 as riboflavin ($C_{17}H_{20}N_4O_6$), Vitamin B3 as niacin ($C_6H_5NO_2$) or niacinamide ($C_6H_6N_2O$), Vitamin B6 as pyridoxine ($C_8H_{11}NO_3$), Vitamin B12 as cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$), and folic acid ($C_{19}H_{19}N_7O_6$); NLT 90.0% and NMT 125.0% of the labeled amounts of copper (Cu), iron (Fe), and zinc (Zn); and NLT 90.0% and NMT 160.0% of the labeled amounts of iodine (I) and selenium (Se).

IDENTIFICATION

• **A.** The retention times of the main peaks of the *Sample solutions* as obtained in *Strength*, correspond to those of the vitamins claimed in their respective *Standard solutions*.

• **B.** The *Sample solution* presents significant emission at the characteristic wavelengths or characteristic absorption as obtained in *Strength* for each mineral claimed.

• **C.** When iodine is claimed: the *Sample solution* turns blue upon the addition of starch TS in the test for *Content of Iodine*.

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.]

• **Content of Vitamin A and Vitamin E**

[Note—Use low-actinic glassware.]

For Oral Powders containing retinyl acetate

Mobile phase: Methanol

Standard stock solution: A solution containing the equivalent of 0.15 mg/mL of retinol from USP Retinyl Acetate RS in isopropyl alcohol

Standard solution: A solution in methanol of USP Retinyl Acetate RS from *Standard stock solution* and USP Alpha Tocopheryl Acetate RS at concentrations similar to those obtained for the corresponding vitamins in the *Sample solution*

Sample solution: Empty 20 containers and combine and mix their contents to obtain a homogenous composite. Weigh the composite and calculate the average fill weight per container. Transfer a calculated amount of accurately weighed composite into a 200- or 250-mL volumetric flask to obtain a solution with a known nominal concentration of vitamins in the range of 2.5–56 µg/mL of retinol from retinyl acetate and 0.2–2.0 mg/mL of alpha tocopheryl acetate. Add 20 mL of water and immediately swirl the sample to slurry it and prevent clumping. Add about 500 units of leucine amino peptidase from a suitable enzyme preparation from *Aspergillus oryzae* containing NLT 500 units of LAP/g[1] to the bottom of the flask using a plastic pipette and gently swirl the contents to mix. Place the sample in a water bath set to 60°–65° for 5 min. Swirl the flask 3–4 times during the 5 min to ensure the sample is completely dispersed in the water and that it has not formed clumps or stuck to the walls of the flask. Remove the flask from the water bath, add 80 mL of isopropyl alcohol, and swirl for 1 min to dissolve the vitamins. Place the sample in a sonicator for 5 min, then cool the flask to room temperature. Add 50 mL of methanol and swirl to mix. Dilute with methanol to volume and mix well. Pass a portion of the solution through a 0.45-µm PTFE membrane filter, and discard the first 1 mL of the filtrate.

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Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 325 nm for 11 min, then switch to 280 nm

Column: 4.6-mm × 25-cm; 4-µm packing L1

Temperatures

Sample: 8°. [NOTE—Use a thermostatic autosampler or a cooled bath.]

Column: 30°

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for retinyl acetate and alpha tocopheryl acetate are 1.0 and 2.2, respectively.]

Suitability requirements

Relative standard deviation: NMT 2.0% for each peak

For Oral Powders containing retinyl palmitate

Mobile phase: Methanol

Standard stock solution: A solution containing the equivalent of 0.15 mg/mL of retinol from USP Retinyl Palmitate RS in isopropyl alcohol

Standard solution: A solution in methanol of USP Retinyl Palmitate RS from *Standard stock solution* and USP Alpha Tocopheryl Acetate RS at concentrations similar to those obtained for the corresponding vitamins in the *Sample solution*

Sample solution: Prepare as directed in *For Oral Powders containing retinyl acetate*, except replace retinyl acetate with retinyl palmitate throughout the text.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 280 nm for 10 min, then switch to 325 nm

Column: 4.6-mm × 15-cm; 4-µm packing L1

Temperatures

Sample: 8°. [NOTE—Use a thermostatic autosampler or a cooled bath.]

Column: 30°

Flow rate: 2.0 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for alpha tocopheryl acetate and retinyl palmitate are 1.0 and 2.6, respectively.]

Suitability requirements

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 ($C_{20}H_{30}O$), in the portion of Oral Powder 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* (µg/mL)

C_U = nominal concentration of vitamin A, as retinol, 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 the Oral Powder 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*

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

C_S = concentration of *all-rac*-alpha-tocopherol from USP Alpha Tocopheryl Acetate 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 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%–165.0% of the labeled amount of vitamin A as retinol and 90.0%–150.0% of the labeled amount of vitamin E as 2*R*-alpha-tocopherol ($C_{29}H_{50}O_2$)

• **Content of Cholecalciferol (Vitamin D)**

[Note—Use amber, low-actinic glassware.]

For Oral Powders containing less than 10 µg/g of vitamin D

Solution A: Dimethyl sulfoxide in water (10:2)

Extraction solvent: 0.02% butylated hydroxytoluene (BHT) in *n*-hexane

Mobile phase: Methanol and water (84:16)

[Note—Wash the column periodically with 100% methanol to eliminate retained substances and avoid carryover interferences.]

Standard stock solution: 0.04 mg/mL of cholecalciferol from USP Cholecalciferol RS in methanol

Standard solution: Dilute the *Standard stock solution* with methanol to obtain a solution with an accurate vitamin concentration as close as possible to that in the *Sample solution*.

System suitability solution: Heat a volume of the *Standard stock solution* at 60° for 1 h to partially isomerize vitamin D to pre-vitamin D.

Sample solution: Empty 20 containers and combine and mix their contents to obtain a homogenous composite. Weigh the composite and calculate the average fill weight per container. Transfer a calculated amount of accurately weighed composite (NMT 3 g) into a 50-mL centrifuge tube. Add 15 mL of *Solution A*, insert the stopper, and shake the sample on a horizontal shaker for 60 min. Add 15 mL of *Extraction solvent* into the tube and shake on a horizontal shaker for 30 min. Centrifuge the sample for 10 min at about 1500 rpm. Accurately transfer 10 mL of the upper hexane layer into centrifuge tube. Gently evaporate the hexane to dryness using a stream of nitrogen. [Note—Some samples may result in an oil residue after the hexane evaporates.] Redissolve the residue in an accurately pipetted volume of absolute alcohol to obtain a solution with a known nominal concentration in the range of 1.0–8.0 µg/mL of cholecalciferol 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.

For Oral Powders containing 10 µg/g or more of vitamin D

Solution A: 50 mg/mL of sodium dodecyl sulfate in water

Mobile phase, Standard stock solution, Standard solution, and **System suitability solution:** Prepare as directed in *For Oral Powders containing less than 10 µg/g of vitamin D*.

Sample solution: Empty 20 containers and combine and mix their contents to obtain a homogenous composite. Weigh the composite and calculate the average fill weight per container. Transfer a calculated amount of accurately weighed composite (NMT 5 g) into a 50-mL volumetric flask. [Note—A larger flask may be used in order to eliminate additional dilutions. The final content of water in the *Sample solution* is NMT 20%.] Add 10 mL of *Solution A* and immediately swirl the flask to mix the contents, and sonicate for 15 min swirling occasionally. Add 5 mL of methyl tert-butyl ether, swirl to mix, add 10 mL of absolute alcohol, and swirl to mix. Continue to add absolute alcohol in 10-mL aliquots, with swirling, almost to volume. Cool to room temperature, and dilute with absolute alcohol to volume to obtain a solution with a known nominal concentration in the range of 1.0–8.0 µg/mL of cholecalciferol. Mix well. [Note—Excessive amounts of dissolved solids may be precipitated by placing the flask in a freezer for 15 min, if necessary.] 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: 265 nm

Column: 2.1-mm × 10-cm; 1.7-µm packing L1

Sample temperature: 8°

Flow rate: 0.4 mL/min

Injection volume: 1 µL

System suitability

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

[Note—The relative retention times for pre-cholecalciferol and cholecalciferol are 1.0 and 1.1, respectively.]

Suitability requirements

Column efficiency: NLT 14,000 theoretical plates, *Standard solution*

Resolution: NLT 3 between pre-cholecalciferol and cholecalciferol, *System suitability solution*

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Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of vitamin D, as cholecalciferol ($C_{27}H_{44}O$), in the portion of Oral Powder taken:

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

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

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

C_S = concentration of cholecalciferol from USP Cholecalciferol RS in the *Standard solution* (µg/mL)

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

Acceptance criteria: 90.0%–150.0%

• **Content of Vitamins B1, B2, B3, B6, and Folic Acid**

[Note—Use low-actinic glassware.]

Solution A: 10 mg/mL of ascorbic acid in water. Prepare fresh at the time of use.

Solution B: Phosphoric acid and water (50:50)

Solution C: 1% sodium bicarbonate in water

Solution D: 0.1 M edetate disodium in water

Mobile phase A: 0.1% trifluoroacetic acid in water

Mobile phase B: 0.1% trifluoroacetic acid in mixture of water and acetonitrile (88:12)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Flow Rate (mL/min)	Mobile Phase A (%)	Mobile Phase B (%)
0	1	100	0
8	1	100	0
20	1	0	100
26	1.5	0	100
40	1.5	0	100
41	1.5	100	0
50	1.5	100	0

[Note—For preparation of the following *Standard stock solutions*, dissolve the related Reference Standard in water first. The solution can be swirled or placed on a steam bath for about 5–10 min with frequent swirling to assist in dissolution. When the vitamin has completely dissolved, cool the solution to room temperature, add *Solution B,* and dilute stepwise with water to final volume. *Solution B* should be added in an amount resulting in a concentration of 0.5% *Solution B* in the final volume of stock solution.]

Thiamine standard stock solution: 150 µg/mL of thiamine from USP Thiamine Hydrochloride RS in water containing 0.5% *Solution B*

Riboflavin standard stock solution: 120 µg/mL of USP Riboflavin RS in water containing 0.5% *Solution B*

Niacin (or Niacinamide) standard stock solution: 1.5 mg/mL of USP Niacin RS or USP Niacinamide RS in water containing 0.5% *Solution B*

Pyridoxine standard stock solution: 150 µg/mL of pyridoxine from USP Pyridoxine Hydrochloride RS in water containing 0.5% *Solution B*

Folic acid standard stock solution: 55 µg/mL of USP Folic Acid RS in *Solution C*

Standard solution: Accurately transfer calculated volumes of *Thiamine standard stock solution*, *Riboflavin standard stock solution*, *Niacin (or Niacinamide) standard stock solution*, *Pyridoxine standard stock solution*, and *Folic acid standard stock solution* into a 200-mL volumetric flask to obtain a solution with final vitamin concentrations similar to the nominal concentrations in the *Sample solution* for the corresponding vitamins. Add 1.0 mL of *Solution B* and 1.0 mL of *Solution A*, and dilute with water almost to volume. Add 2–3 drops of

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