Status: Currently Official on 17-Feb-2025
Official Date: Official as of 01-May-2021
Document Type: Dietary Supplement Monographs
DocId: GUID-8042A3FC-11BB-4DD8-BCAA-AB62DBF8A459_3_en-US
DOI: https://doi.org/10.31003/USPNF_M12898_03_01
DOI Ref: g7tdz

© 2025 USPC
Do not distribute

Add the following:

▲Oil- and Water-Soluble Vitamins with Minerals Chewable Gels

DEFINITION

Oil- and Water-Soluble Vitamins with Minerals Chewable Gels contain 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-* 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 Hydrochloride or Thiamine Mononitrate, vitamin B2 as Riboflavin, vitamin B3 as Niacinamide, vitamin B6 as Pyridoxine Hydrochloride, vitamin B12 as Cyanocobalamin, Biotin, and Folic Acid; and one or more minerals derived from substances generally recognized as safe, furnishing one or more of the following elements in ionizable form: calcium, chromium, iodine, magnesium, molybdenum, phosphorus, selenium, and zinc. Chewable Gels contain NLT 90.0% and NMT 170.0% of the labeled amounts of vitamin A as retinol ($C_{20}H_{30}O$), vitamin D as cholecalciferol ($C_{27}H_{44}O$), and vitamin E as *RRR*-alpha-tocopherol ($C_{29}H_{50}O_2$); NLT 90.0% and NMT 250.0% of the labeled amount of vitamin C as ascorbic acid ($C_6H_8O_6$); NLT 90.0% and NMT 150.0% of the labeled amounts of vitamin B3 as niacinamide ($C_6H_6N_2O$), vitamin B6 as pyridoxine ($C_8H_{11}NO_3$), riboflavin ($C_{17}H_{20}N_4O_6$), and thiamine as thiamine ion ($C_{12}H_{17}N_4OS^+$); NLT 90.0% and NMT 170.0% of the labeled amounts of vitamin B12 as cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$) and biotin ($C_{10}H_{16}N_2O_3S$); NLT 90.0% and NMT 245.0% of the labeled amount of folic acid ($C_{19}H_{19}N_7O_6$) [Note—Any overage should not exceed the tolerable upper intake level.]; NLT 90.0% and NMT 130.0% of the labeled amounts of calcium (Ca), magnesium (Mg), phosphorus (P), and zinc (Zn); and NLT 90.0% and NMT 160.0% of the labeled amounts of chromium (Cr), iodine (I), molybdenum (Mo), and selenium (Se). They do not contain any other vitamins or minerals for which nutritional value is claimed. They may contain other labeled added substances that are generally recognized as safe, in amounts that are unobjectionable.

IDENTIFICATION

• **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*.

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

• **C.** When iodine is claimed: the retention time of the iodine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the tests for *Strength*.

Change to read:

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. Use amber, low-actinic glassware. Use cryogenic gloves when handling liquid nitrogen.]

• **Vitamin A (as Retinyl Acetate),** *Method 1*

Mobile phase: Acetonitrile, methanol, and water (48:48:4)

Standard stock solution: 0.25 mg/mL of retinyl acetate, as retinol, from USP Retinyl Acetate RS in 2,2,4-trimethylpentane. Shake on a mechanical shaker if necessary. [Note—The *Standard stock solution* is good for 1 month when stored in the freezer.]

Standard solution: 50 µg/mL of retinyl acetate, as retinol, in 2,2,4-trimethylpentane from the *Standard stock solution*, prepared accurately

Sample solution: Immerse 25–30 Chewable Gels in liquid nitrogen in a cryogenic vessel for 10 min. Cool a blender jar by swirling liquid nitrogen for about 1 min and discard the contents. Add frozen Chewable Gels to the cooled blender jar and grind to a fine powder. Transfer a portion of the powder, nominally equivalent to 500 µg of retinyl acetate, as retinol, into a 50-mL centrifuge tube. [Note—Proceed to this step immediately or keep the powdered Chewable Gels frozen until use.] Add 300 mg of pancreatin and 5 mL of water, and shake on water bath shaker at 55° until completely dissolved (about 20 min). Vortex the solution to suspend any undissolved solids, add 20 ml of dimethyl sulfoxide, and vortex again until well mixed. Pipet 10 mL of 2,2,4-trimethylpentane, vortex until the phases are well mixed, then shake the

OFFICIAL

AVA Documents
Document Sharing Hub

tube on a horizontal shaker for 20 min, and centrifuge the sample at 3000 rpm for 10 min. Pass a portion of the 2,2,4-trimethylpentane layer through a glass microfiber filter of 0.45-µm pore size, discarding the first 2 mL of the filtrate.

Chromatographic system

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

Mode: LC

Detector: UV 325 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 1.0 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of retinyl acetate, as retinol ($C_{20}H_{30}O$), in the portion of Chewable Gels taken:

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

r_U = peak area of retinyl acetate from the *Sample solution*

r_S = peak area of retinyl acetate from the *Standard solution*

C_S = concentration of retinyl acetate as retinol from USP Retinyl Acetate RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of retinyl acetate as retinol in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–170.0%

- **Vitamin D (as Cholecalciferol),** *Method 1*

Solution A: 1.2% isopropyl alcohol in hexane

Solution B: 5.0% isopropyl alcohol in hexane

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15.0	100	0
15.5	0	100
23.0	0	100
23.5	100	0
30.0	100	0

Standard stock solution: 0.3 mg/mL of cholecalciferol from USP Cholecalciferol RS in 2,2,4-trimethylpentane. Shake on a mechanical shaker if necessary.

Standard solution: 1 µg/mL of cholecalciferol in 2,2,4-trimethylpentane from the *Standard stock solution*, prepared accurately

Sample solution: Immerse 25–30 Chewable Gels in liquid nitrogen in a cryogenic vessel for 10 min. Cool a blender jar by swirling liquid nitrogen for about 1 min and discard the contents. Add frozen Chewable Gels to the cooled blender jar and grind to a fine powder. Transfer a portion of the powder, nominally equivalent to 10 µg of cholecalciferol, into a 50-mL centrifuge tube. [Note—Proceed to this step immediately or keep the powdered Chewable Gels frozen until use.] Add 300 mg of pancreatin and 5 mL of water, and shake on a water bath shaker at 55° until completely dissolved (about 20 min). Vortex the solution to suspend any undissolved solids, add 20 ml of dimethyl

OFFICIAL

AVA Documents Document Sharing Hub

sulfoxide, and vortex again until well mixed. Pipet 10 mL of 2,2,4-trimethylpentane, vortex until the phases are well mixed, then shake the tube on a horizontal shaker for 20 min, and centrifuge the sample at 2500 rpm for 10 min. Pass a portion of the 2,2,4-trimethylpentane layer through a glass microfiber filter of 0.45-µm pore size, discarding the first 2 mL of the filtrate.

Chromatographic system

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

Mode: LC

Detector: UV 265 nm

Column: 4.6-mm × 25-cm; 5-µm packing L3

Flow rate: 1.3 mL/min

Injection volume: 80 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cholecalciferol ($C_{27}H_{44}O$) in the portion of Chewable Gels 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%–170.0%

- **Vitamin E (as Alpha Tocopheryl Acetate),** *Method 1*

Mobile phase: Acetonitrile and alcohol (80:20)

Standard stock solution: 1.0 mg/mL of alpha tocopheryl acetate from USP α-Tocopheryl Acetate RS in hexane. Shake on a mechanical shaker if necessary.

[Note—The *Standard stock solution* is good for 1 month when stored in the refrigerator.]

Standard solution: 0.02 mg/mL of alpha tocopheryl acetate in alcohol from the *Standard stock solution*, prepared accurately

Sample solution: Immerse 25–30 Chewable Gels in liquid nitrogen in a cryogenic vessel for 10 min. Cool a blender jar by swirling liquid nitrogen for about 1 min and discard the contents. Add frozen Chewable Gels to the cooled blender jar and grind to a fine powder. Transfer a portion of the powder, nominally equivalent to 2 mg of alpha tocopheryl acetate, into a 100-mL volumetric flask. [Note—Proceed to this step immediately or keep the powdered Chewable Gels frozen until use.] Add 300 mg of pancreatin and 10 mL of water, and shake on a water bath shaker at 55° until completely dissolved (about 20 min). Vortex the solution to suspend any undissolved solids, add 30 ml of alcohol, and sonicate for 30 min at 40°. Cool the sample, dilute with alcohol to volume, and mix well. Pass a portion of the sample through a nylon filter of 0.45-µm pore size, discarding the first 2 mL of the filtrate. [Note—Analyze the sample within 4 h.]

Chromatographic system

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

Mode: LC

Detector: UV 285 nm

Column: 3.9-mm × 15-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of vitamin E, as *RRR*-alpha-tocopherol ($C_{29}H_{50}O_2$), in the portion of Chewable Gels taken:

OFFICIAL

AVA Documents Document Sharing Hub

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

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

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

C_S = concentration of *all-rac*-alpha-tocopherol from USP α-Tocopheryl Acetate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of vitamin E, as *RRR*-alpha tocopherol, in the *Sample solution* (mg/mL)

F = conversion factor for the content of alpha tocopherol to *RRR*-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%–170.0% of the labeled amount of vitamin E, as *RRR*-alpha-tocopherol equivalent

- **Vitamin A (as Retinyl Acetate or Retinyl Palmitate), Vitamin D (as Cholecalciferol), and Vitamin E (as Alpha Tocopheryl Acetate),** *Method 2*

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 2*.

Table 2

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 cholecalciferol from USP Cholecalciferol RS in isopropyl alcohol. Dissolve with the aid of sonication if necessary.

Vitamin E standard stock solution: 3.0 mg/mL of alpha tocopheryl acetate from USP α-Tocopheryl Acetate RS in isopropyl alcohol. Dissolve with the aid of sonication if necessary.

Standard solution: Accurately transfer calculated volumes of *Vitamin A standard stock solution*, *Vitamin D standard stock solution*, and *Vitamin E 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*.

Sample solution: Weigh 25–30 Chewable Gels and calculate the average weight/Chewable Gel. Immerse the Chewable Gels in liquid nitrogen in a cryogenic vessel for 10 min. Cool a blender jar by swirling liquid nitrogen for about 1 min and discard the contents. Add frozen Chewable Gels to the cooled blender jar and grind to a fine powder. Transfer a portion of the powder, equivalent to 1 Chewable Gel into a 50-mL volumetric flask. [Note—Proceed to this step immediately or keep the powdered Chewable Gels frozen until use.] Add 5 mL of water and shake on a water bath shaker at 45° for 15 min. Vortex the solution to suspend any undissolved solids, add 5 ml of dimethyl sulfoxide, vortex until well mixed. Shake on a water bath shaker at 45° for 15 min or until completely dissolved, then vortex for 2–3 min. Add about 30 mL of isopropyl alcohol, place on a shaker and shake at 350 rpm for 30 min, then sonicate for an additional 30 min. Cool the sample, dilute with isopropyl alcohol to volume, and mix well. Pass a portion of the sample through a nylon filter of 0.45-µm pore size, discarding the first 2 mL of the filtrate.

Chromatographic system

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

Mode: LC

Detector

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

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

OFFICIAL

Documents
Document Sharing Hub
Full document details: Oil- And Water-Soluble Vitamins with Minerals Chewable Gels

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

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0% for each individual vitamin

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 Chewable Gels taken:

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

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

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

C_S = concentration of retinol from USP Retinyl Acetate RS or USP Retinyl Palmitate RS 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 *RRR*-alpha-tocopherol ($C_{29}H_{50}O_2$), in the portion of Chewable Gels taken:

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

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

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

C_S = concentration of *all-rac*-alpha-tocopherol from USP α-Tocopheryl Acetate RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of vitamin E, as *RRR*-alpha tocopherol, in the *Sample solution* (mg/mL)

F = conversion factor for the content of alpha tocopherol to *RRR*-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

Calculate the percentage of the labeled amount of vitamin D, as cholecalciferol ($C_{27}H_{44}O$), in the portion of Chewable Gels 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%–170.0% of the labeled amount of each individual vitamin

- **Vitamin C,** *Method 1*

Solution A: Dissolve 73 g of metaphosphoric acid in 800 mL of water, add 84 mL of glacial acetic acid, and dilute with water to 1000 mL.

Solution B: 7.8 g/L of sodium phosphate monobasic dihydrate in water. Adjust with phosphoric acid to a pH of 2.5.

Mobile phase: See *Table 3*.

Table 3

OFFICIAL

Documents
Document Sharing Hub
Full document details: Oil- And Water-Soluble Vitamins with Minerals Chewable Gels

Time (min)	Solution B (%)	Methanol (%)
0	100	0
3.0	100	0
5.0	0	100
6.0	50	50
7.0	100	0
10.0	100	0

[Note—Wash the column periodically with methanol and water using appropriate gradients to eliminate retained substances and avoid carry-over interferences.]

Standard stock solution: 1 mg/mL of ascorbic acid from USP Ascorbic Acid RS in *Solution A*. [Note—Sonicate with intermittent shaking to help dissolve, if necessary. Prepare fresh every time.]

Standard solution: Dilute the *Standard stock solution* with *Solution A* to obtain a solution containing 0.05 mg/mL of USP Ascorbic Acid RS.

Sample solution: Immerse 25–30 Chewable Gels in liquid nitrogen in a cryogenic vessel for 10 min. Cool a blender jar by swirling liquid nitrogen for about 1 min and discard the contents. Add frozen Chewable Gels to the cooled blender jar and grind to a fine powder. Transfer a portion of the powder, nominally equivalent to 25 mg of ascorbic acid, into a 25-mL volumetric flask. [Note—Proceed to this step immediately or keep the powdered Chewable Gels frozen until use.] Add 15 mL of *Solution A*, vortex until well mixed, sonicate at 35° for 10 min or until the sample has completely dissolved. Cool the solution to room temperature, dilute with *Solution A* to volume, and mix well. Quantitatively dilute a portion of the solution with *Solution A* to obtain a solution containing 0.05 mg/mL of ascorbic acid. Mix and pass through a glass microfiber filter of 0.45-µm pore size, discarding the first few milliliters of the filtrate.

Chromatographic system

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

Mode: LC

Detector: UV 245 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L7

Flow rate: 0.8 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of vitamin C, as ascorbic acid ($C_6H_8O_6$), in the portion of Chewable Gels taken:

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

r_U = peak area of ascorbic acid from the *Sample solution*

r_S = peak area of ascorbic acid from the *Standard solution*

C_S = concentration of USP Ascorbic Acid RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of ascorbic acid in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–250.0%

Change to read:

• **Vitamins B3 (as Niacinamide), B6, and Folic Acid,** *Method 1*

Solution A: 1% acetic acid, glacial in water

Solution B: 0.1 N sodium hydroxide in water

OFFICIAL

Documents
Document Sharing Hub
Full document details: Oil- And Water-Soluble Vitamins with Minerals Chewable Gels