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Add the following:

Water-Soluble Vitamins Preparation

DEFINITION

Water-Soluble Vitamins Preparation is a mixture of two 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 Niacin or Niacinamide; vitamin B6 as Pyridoxine Hydrochloride; vitamin B12 as Cyanocobalamin; Biotin; Folic Acid; and pantothenic acid as Calcium Pantothenate or Racemic Calcium Pantothenate with one or more inert substances and suitable antioxidants. It contains NLT 90.0% and NMT 125.0% of the labeled amounts of vitamin C as ascorbic acid ($C_6H_8O_6$), vitamin B3 as niacin ($C_6H_5NO_2$) or niacinamide ($C_6H_6N_2O$), vitamin B6 as pyridoxine ($C_8H_{11}NO_3$), folic acid ($C_{19}H_{19}N_7O_6$), pantothenic acid ($C_9H_{17}NO_5$), vitamin B2 as riboflavin ($C_{17}H_{20}N_4O_6$), thiamine as thiamine ion ($C_{12}H_{17}N_4OS^+$), vitamin B12 as cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$), and biotin ($C_{10}H_{16}N_2O_3S$).

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 claimed in each respective *Standard solution*.

COMPOSITION

[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.]

• **CONTENT OF VITAMINS B1, B2, B3, AND B6, FOLIC ACID, AND PANTOTHENIC ACID, Method 1**

[NOTE—Where niacin or niacinamide, thiamine, pyridoxine, riboflavin, calcium pantothenate, and folic acid are specified in the following procedure, use the vitamins present in the formulation and the relevant USP Reference Standards. 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 phases: For gradient, see [Table 1](#).

Mobile phase A: 0.1% [trifluoroacetic acid](#) in [water](#)

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

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

Time (min)	Flow Rate (mL/min)	Mobile Phase A (%)	Mobile Phase B (%)
41	1.5	100	0
50	1.5	100	0

[NOTE—For preparation of the following *Standard stock solutions*, dissolve the related USP 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 standard stock solution* (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. [NOTE—When calcium pantothenate presents in the formulation, accurately weigh a calculated amount of [USP Calcium Pantothenate RS](#) into a 200-mL volumetric flask to obtain a solution with a final vitamin concentration similar to that in the *Sample solution*. Add 30 mL of water, vortex until dissolved, and then transfer the calculated volumes of the other vitamin *Standard stock solutions*.] 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 [polysorbate 80](#) and add [water](#) to final volume. Mix well by completely inverting the flask at least 20 times. [NOTE—After inverting the flask 10–12 times, carefully remove the stopper to release the built-up pressure.]

Sample solution: Transfer a calculated amount of accurately weighed Water-Soluble Vitamins Preparation into a suitable volumetric flask to obtain a solution containing known nominal concentrations of vitamins in the range of 2–130 µg/mL for thiamine, 1.5–110 µg/mL for riboflavin, 10–800 µg/mL for niacin, 10–500 µg/mL for niacinamide, 2–120 µg/mL for pyridoxine, 0.5–2.0 µg/mL for folic acid, and 10–250 µg/mL for pantothenic acid. Add about half volume of [water](#) and an aliquot of *Solution D* equivalent to 2% of the volume of the flask, and swirl to mix thoroughly. Place the flask on a steam bath for 2–3 min, swirl frequently, and avoid overheating. Cool the flask to room temperature and add an aliquot of *Solution C* equivalent to 10% of the volume of the flask, mix well, sonicate for 1 min. Cool to room temperature, add an aliquot of *Solution A* equivalent to 0.5% of the volume of the flask and an aliquot of *Solution B* equivalent to 0.3% of the volume of the flask, swirl to mix, and dilute with [water](#) almost to volume. Add 2–3 drops of [polysorbate 80](#) and add [water](#) to final volume. [NOTE—If more than one dilution is necessary to obtain the required concentrations of vitamins, make the dilutions with [water](#) first and add *Solution A*, *Solution B*, and [polysorbate 80](#) to the last dilution.] Mix well by completely inverting the flask at least 20 times with caution to release the built-up pressure. Pass a portion of the solution through a suitable filter of 0.45-µm pore size, and discard the first milliliter of the filtrate.

Chromatographic system

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

Mode: LC

Detector

Channel 1: UV 254 nm for 15 min, switch to 280 nm for 33 min, then return to 254 nm

Channel 2: UV 210 nm

Column: 4.6-mm × 25-cm; 5-µm packing [L1](#)

Flow rate: See [Table 1](#).

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for ascorbic acid, niacinamide, thiamine, pyridoxine, pantothenic acid, folic acid, and riboflavin are 0.8, 1.0, 1.8, 3.3, 4.0, 5.0, and 6.2, respectively.]

Suitability requirements

Resolution: NLT 1.5 between the ascorbic acid and niacinamide peaks

Relative standard deviation: NMT 2.0% for each individual peak of niacin or niacinamide, thiamine, pyridoxine, riboflavin, folic acid, and pantothenic acid

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate separately the percentages of the labeled amount of 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 ($C_8H_{11}NO_3$), folic acid ($C_{19}H_{19}N_7O_6$), and pantothenic acid ($C_9H_{17}NO_5$) in the portion of Water-Soluble Vitamins Preparation taken:

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

r_U = peak area of the relevant vitamin from the *Sample solution*

r_S = peak area of the relevant vitamin from the *Standard solution*

C_S = concentration of the relevant vitamin Reference Standard in the *Standard solution* ($\mu\text{g/mL}$)

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

Analysis: 90.0%–125.0% of the labeled amount of each individual vitamin

• **CONTENT OF VITAMINS B1, B2, B3, AND B6, AND PANTOTHENIC ACID, Method 2**

[NOTE—Where niacin or niacinamide, thiamine, pyridoxine, riboflavin, and calcium pantothenate are specified in the following procedure, use the vitamins present in the formulation and the relevant USP Reference Standards. Use low-actinic glassware.]

Solution A: 100 mM [potassium dihydrogen phosphate](#), adjusted with 10 N [phosphoric acid](#) to a pH of 3.0

Solution B: 5% [edetate disodium](#) in [water](#). Heat the solution while stirring for complete dissolution.

Extraction solution: *Solution A* and *Solution B* (4:1)

Mobile phases: For gradient, see [Table 2](#).

Mobile phase A: 45 mM [hexane sulphonic acid sodium salt](#), 75 mM [potassium dihydrogen phosphate](#), 2.5% [acetonitrile](#) in [water](#), adjusted with 10 N [phosphoric acid](#) to a pH of 3.5

Mobile phase B: *Mobile phase A* and [acetonitrile](#) (2:1)

Table 2

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0.0	100	0
6.0	100	0
15.0	72	28
19.0	72	28
20.0	30	70
23.0	20	80
23.2	0	100
24.8	100	0
32.0	100	0

Niacin (or Niacinamide) standard stock solution: 1.5 mg/mL of [USP Niacin RS](#) or [USP Niacinamide RS](#) in *Solution A*

Pyridoxine standard stock solution: 350 $\mu\text{g/mL}$ of pyridoxine from [USP Pyridoxine Hydrochloride RS](#) in *Solution A*

Thiamine standard stock solution: 275 $\mu\text{g/mL}$ of thiamine from [USP Thiamine Hydrochloride RS](#) in *Solution A*

Riboflavin standard stock solution: 120 $\mu\text{g/mL}$ of [USP Riboflavin RS](#) in *Solution A*. Heat the solution while stirring for complete dissolution.

Calcium pantothenate standard stock solution: 825 $\mu\text{g/mL}$ of pantothenic acid from [USP Calcium Pantothenate RS](#) in [water](#)

Standard solution: Accurately transfer calculated volumes of *Thiamine standard stock solution*, *Riboflavin standard stock solution*, *Niacin standard stock solution* (or *Niacinamide standard stock solution*), *Pyridoxine standard stock solution*, and *Calcium pantothenate 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. Dilute with *Solution A* to volume, and mix well.