

Status: Currently Official on 17-Feb-2025
Official Date: Official as of 01-Nov-2020
Document Type: USP Monographs
DocId: GUID-EA9E5CF6-88AE-49CE-9262-06999560D83D_4_en-US
DOI: https://doi.org/10.31003/USPNF_M88430_04_01
DOI Ref: cw4b6

Vitamin A Tablets

DEFINITION
Vitamin A Tablets contain retinyl acetate or retinyl palmitate in an amount equivalent to NLT 95.0% and NMT 120.0% of the labeled amount of vitamin A as retinol ($C_{20}H_{30}O$).

IDENTIFICATION
[Note—Use low-actinic glassware.]

- **A.**

  Sample solution: A solution containing the equivalent of 6 µg/mL of retinol from powdered Tablets in methylene chloride

  Analysis: To 1 mL of *Sample solution* add 10 mL of antimony trichloride TS.

  Acceptance criteria: A transient blue color appears at once.

- **B. Thin-Layer Chromatographic Identification Test ⟨201⟩**

  Standard solution: Equivalent to 0.5 mg/mL of retinol from USP Retinyl Acetate RS or USP Retinyl Palmitate RS in methylene chloride

  Sample solution: To a portion of finely powdered Tablets, equivalent to 5 mg of retinol, add 15 mL of water, sonicate, and shake vigorously for 2 min. Extract with 10 mL of methylene chloride by shaking for 2 min. Centrifuge, and use the lower layer of methylene chloride extract.

  Application volume: 10 µL, 8-mm band

  Developing solvent system: Cyclohexane and ether (4:1)

  Spray reagent: Phosphomolybdic acid TS

  Analysis: Proceed as directed in the chapter, using the *Developing solvent system*. Locate the spots on the plate using the *Spray reagent*.

  Acceptance criteria: The R_F value of the principal spot from the *Sample solution* corresponds to that from the *Standard solution*.

- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
Add the following:

▲[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 *Procedure 1* is not used.]▲ (USP 1-May-2020)

Change to read:

- **Procedure ▲1**

[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 Reference Standard.▲ (USP 1-May-2020) Use low-actinic glassware.]

Mobile phase: *n*-Hexane

Standard solution 1: 15 µg/mL of retinol from USP Retinyl Acetate RS in *n*-hexane

Standard solution 2: 15 µg/mL of retinol from USP Retinyl Palmitate RS in *n*-hexane

System suitability solution: Mix equal volumes of *Standard solution 1* and *Standard solution 2*.

Sample solution: Finely powder NLT 20 Tablets. To a portion of the powder, equivalent to 5 Tablets, accurately weighed, add 15 mL of water and sonicate for 5 min. Add 15 mL of *n*-hexane, and shake for 15 min on a wrist-action shaker in a water bath maintained at 60°. Add 10 mL of dimethyl sulfoxide, and shake for an additional period of 30 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, and transfer the hexane layer to a 100-mL volumetric flask. Repeat the extraction with 3 additional 15-mL portions of *n*-hexane by thoroughly shaking each for 5 min. Dilute the extracts in the volumetric flask with *n*-hexane to volume. Further dilute this solution with *n*-hexane to obtain a solution with a concentration equivalent to 15 µg/mL of retinol.

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

Mode: LC

Detector: UV 325 nm

Column: 4.6-mm × 15-cm; 3-µm packing L8

Flow rate: 1 mL/min

Injection volume: 40 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 10 between *all-trans*-retinyl palmitate and *all-trans*-retinyl acetate

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution 1* or *Standard solution 2* and *Sample 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 appropriate *Standard solution*

C_S = concentration of retinol in the appropriate *Standard solution* (µg/mL)

C_U = nominal concentration of vitamin A, as retinol, in the *Sample solution* (µg/mL)

Acceptance criteria: 95.0%–120.0% of the labeled amount of vitamin A, as retinol ($C_{20}H_{30}O$)

Change to read:

• **▲Procedure 2**

[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 Reference Standard. Use low-actinic glassware.]

Solution A: Methanol and water (90:10)

Solution B: Methanol and isopropyl alcohol (55:45)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
8	0	100
13	0	100
13.1	100	0
15	100	0

Vitamin A standard stock solution: 0.2 mg/mL of retinol ($C_{20}H_{30}O$) from USP Retinyl Acetate RS or USP Retinyl Palmitate RS in isopropyl alcohol. Dissolve with the aid of sonication if necessary.

Standard solution: 0.04 mg/mL of retinol in methanol from *Vitamin A standard stock solution*

Sample solution: Finely powder NLT 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to 4.0 mg of retinol into a 100-mL volumetric flask. Add 10 mL of water and immediately swirl the sample, add 20 mL of dimethyl sulfoxide, and sonicate for 30 min with vigorous intermittent shaking. Cool to room temperature, add 70 mL of isopropyl alcohol, and swirl for 1 min to dissolve the vitamin. Place the sample in a sonicator for 5 min, and then cool the flask to room temperature. Dilute with methanol 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.

Chromatographic system

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

Mode: LC

Detector: UV 325 nm

Column: 4.6-mm × 10-cm; 3-µm packing L1

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5 for retinyl acetate and NMT 2.0 for retinyl palmitate

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample 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 relevant *all-trans*-retinyl ester from *Sample solution*▲▲ (ERR 1-May-2020)

r_S = peak area of the relevant *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)

Acceptance criteria: 90.0%–120.0%▲ (USP 1-May-2020)

PERFORMANCE TESTS

Change to read:

• **Dissolution** ⟨711⟩

[Note—Perform this test under light conditions that minimize photodegradation.]

Medium: 1% (w/v) sodium ascorbate and 1% (w/v) octoxynol 9 in 0.05 M phosphate buffer with a pH of 6.8; 900 mL

Apparatus 2: 75 rpm

Time: 45 min

▲▲ (USP 1-May-2020)

Sample solution: Withdraw a portion of the solution under test, pass through a suitable filter of 0.45-µm pore size, and use the pooled sample as the test specimen.

Standard solution: Dissolve a suitable amount of USP Retinyl Acetate RS or USP Retinyl Palmitate RS in isopropyl alcohol, and dilute with *Medium* to obtain a concentration similar to that of the *Sample solution*. [Note—The amount of isopropyl alcohol should be 5%–10%.]

▲▲ (USP 1-May-2020)

Analysis

▲Proceed as directed in *Assay, Procedure 2*, making any necessary modifications.▲ (USP 1-May-2020)

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of retinyl acetate or retinyl palmitate dissolved:

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

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 appropriate *Standard solution*

C_S = concentration of retinol in the appropriate *Standard solution* (µg/mL)

V = volume of *Medium*, 900 mL

L = label claim of vitamin A, as retinol (µg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of retinyl acetate or retinyl palmitate is dissolved.

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, and store at room temperature. Protect Tablets from light.

Change to read:

• **Labeling:** Label it to indicate the chemical form of vitamin A present and to indicate the vitamin A activity in terms of the equivalent amount of retinol ▲in mg/Tablet.▲ (USP 1-May-2020)

• **USP Reference Standards** ⟨11⟩

USP Retinyl Acetate RS

USP Retinyl Palmitate RS

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
VITAMIN A TABLETS	Natalia Davydova Scientific Liaison	NBDS2020 Non-botanical Dietary Supplements
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	NBDS2020 Non-botanical Dietary Supplements

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
Pharmacopeial Forum: Volume No. PF 44(6)

Current DocID: GUID-EA9E5CF6-88AE-49CE-9262-06999560D83D_4_en-US

DOI: https://doi.org/10.31003/USPNF_M88430_04_01

DOI ref: cw4b6