Temperature: 37 ± 2°
Time: 45 min
Acceptance criteria: Meet the requirements
- **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements

SPECIFIC TESTS
- **ABSORBANCE RATIO:** The ratio of the corrected absorbance [A_{325}] to the observed absorbance [A_{325}], determined as directed in *Vitamin A Assay* ⟨571⟩, is NLT 0.85.

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** Label the Capsules to indicate the form in which the vitamin is present, and to indicate the vitamin A activity in terms of the equivalent amount of retinol in mg. The vitamin A activity may be stated also in USP Units per Capsule, on the basis that 1 USP Vitamin A Unit equals the biological activity of 0.3 µg of the all-*trans* isomer of retinol.
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Vitamin A RS

Vitamin A Oral Liquid Preparation

DEFINITION
Vitamin A Oral Liquid Preparation is an emulsion, suspension, or solution that contains 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. PROCEDURE**
  Sample solution: Prepare a solution in methylene chloride containing an amount of Oral Liquid Preparation equivalent to about 6 µg of retinol in 1 mL.
  Analysis: Add 10 mL of antimony trichloride TS to 1 mL of *Sample solution*.
  Acceptance criteria: A transient blue color appears at once.
- **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST ⟨201⟩**
  Standard solution: 0.5 mg/mL of retinol from USP Retinyl Acetate RS or USP Retinyl Palmitate RS in methylene chloride
  Sample solution: Dissolve or extract with methylene chloride a quantity of Oral Liquid Preparation to obtain a solution with a nominal concentration of 0.5 mg/mL of retinol.
  Application volume: 10 µL as an 8-mm band
  Developing solvent system: A mixture of cyclohexane and ether (4:1)
  Spray reagent: 0.2 g/mL of phosphomolybdic acid in alcohol. Filter and use only the clear filtrate.
  Analysis: Apply the *Sample solution* at the starting point of the chromatogram, and proceed as directed for *Chromatography* ⟨621⟩*, Thin-Layer Chromatography*. Allow the solvent front to move 10 cm, remove the plate, and air-dry. Spray with *Spray reagent*.
  Acceptance criteria: The blue-green spot formed is indicative of the presence of retinol, and its R_F value corresponds to that of the *Standard solution*. The approximate R_F values of the predominant spots, corresponding to the different forms of retinol, are 0.1 for the alcohol form, 0.45 for the acetate, and 0.7 for the palmitate.

ASSAY
- **VITAMIN A**
  [NOTE—Use low-actinic glassware.]
  Standard solution 1: 13 µg/mL of retinol from USP Retinyl Acetate RS in *n*-hexane
  Standard solution 2: 13 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
  For Oral Liquid Preparation in oil vehicles packaged in single-unit containers: Deliver the contents of NLT 30 single-unit containers, following the directions for use as stated in the labeling. Weigh directly the individual contents delivered from each single-unit container, and calculate the average. [NOTE—Do not weigh the contents delivered by difference between full containers and empty containers. Capsules intended as single-unit containers are not rinsed after expulsion of the contents.] Mix the contents to obtain a homogeneous sample. Transfer an amount of the composite to a suitable volumetric flask; dissolve it with hexane, and dilute with hexane quantitatively and stepwise, if necessary, to obtain a solution containing the equivalent of about 13 µg/mL of retinol, based on the labeled amount.
  For Oral Liquid Preparation in oil vehicles packaged in multiple-unit containers: Dissolve an accurately measured volume of Oral Liquid Preparation in a suitable volume of hexane, and dilute with hexane, quantitatively and stepwise, if necessary, to obtain a solution containing the equivalent to about 13 µg/mL of retinol, based on the labeled amount.
  For Oral Liquid Preparation in aqueous vehicles: Transfer a mass, or an accurately measured volume of Oral Liquid Preparation, into a separatory funnel, and extract quantitatively with hexane or other suitable solvent. Dilute with hexane, quantitatively and stepwise, if necessary, to obtain a solution containing the equivalent of about 13 µg/mL of retinol, based on the labeled amount.
  Chromatographic system
  (See *Chromatography* ⟨621⟩*, System Suitability*.)
  Mode: LC
  Detector: UV 325 nm
  Column: 4.6-mm × 15-cm; packing L8
  Flow rate: 1 mL/min
  Injection size: 40 µL
  System suitability
  Sample: *System suitability solution*
  Suitability requirements
  Resolution: NLT 10 between all-*trans*-retinyl acetate and all-*trans*-retinyl palmitate
  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, as retinol ($C_{20}H_{30}O$), in each individual container:

$$\text{Result} = (r_U/r_S) \times (C/W) \times (V/D) \times U \times (100/L)$$

r_U	= peak response from the corresponding all-*trans*-retinyl ester in the *Sample solution*
r_S	= peak response from the all-*trans*-retinyl ester in the appropriate *Standard solution*
C	= concentration of retinol ($C_{20}H_{30}O$), in the appropriate *Standard solution* (mg/mL)
W	= mass or volume of the Oral Liquid Preparation composite taken (mg or mL)
V	= volume of the *Sample solution* (mL)
D	= dilution factor (dilution volume/aliquot volume)
U	= for multiple-unit containers: labeled volume of each dosage unit (mL); or for single-unit containers: average mass (mg) of the contents delivered from each individual container, following the directions for use as stated in the labeling
L	= labeled amount of vitamin A, as retinol ($C_{20}H_{30}O$), in each dosage unit (mg)

  Acceptance criteria: 95.0%–120.0%

PERFORMANCE TESTS
- **DELIVERABLE VOLUME ⟨698⟩**
  For Oral Liquid Preparation packaged in multiple-unit containers: Meets the requirements

• **UNIFORMITY OF DOSAGE UNITS** ⟨905⟩
For Oral Liquid Preparation packaged in single-unit containers: Empty the single-unit containers, following the directions for use as stated in the labeling. The contents so delivered, and weighed directly, meet the requirements. [NOTE—Do not weigh the contents delivered by difference between full containers and empty containers. Capsules intended for use as single-unit containers are not rinsed after expulsion of the contents.]

ADDITIONAL REQUIREMENTS
• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. It may be packaged in single-unit containers. [NOTE—Capsules may be suitable as single-unit containers provided they are packaged in critical secondary containers as described in *Good Packaging Practices* ⟨1177⟩.]
• **LABELING:** The label states that the product is Vitamin A Oral Liquid Preparation. Label the Oral Liquid Preparation to indicate the ester form in which the vitamin is present, and to indicate the amount of vitamin A delivered in each dosage unit in terms of the equivalent amount of retinol in mg/dosage unit. The amount of vitamin A delivered may be stated also in USP Units/dosage unit, on the basis that 1 USP Vitamin A Unit equals the biological activity of 0.3 µg of all-*trans*-retinol. Capsules used as single-unit containers may be exempted from the requirements of individual labeling, provided they are packaged in an appropriately labeled secondary container, including directions for use and delivery of each dosage unit of Oral Liquid Preparation. Label the Oral Liquid Preparation packaged in multiple-unit containers to indicate the volume of each dosage unit.
• **USP REFERENCE STANDARDS** ⟨11⟩
USP Retinyl Acetate RS
USP Retinyl Palmitate RS

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. PROCEDURE**
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: A solution containing the equivalent of 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
• **PROCEDURE**
[NOTE—Use low-actinic glassware.]
Mobile phase: *n*-Hexane
Standard solution 1: A solution containing the equivalent of 15 µg/mL of retinol from USP Retinyl Acetate RS in *n*-hexane
Standard solution 2: A solution containing the equivalent of 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, 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 three 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 size: 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, 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 ($C_{20}H_{30}O$)

PERFORMANCE TESTS
• **DISSOLUTION** ⟨711⟩
[NOTE—Perform this test under light conditions that minimize photo degradation.]
Medium: 1% (w/v) sodium ascorbate and 1% (w/v) octoxynol 9 in 0.05 M phosphate buffer pH 6.8; 900 mL
Apparatus 2: 75 rpm
Time: 45 min
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 the concentration similar to that expected in the *Sample solution*. [NOTE—The amount of isopropyl alcohol should be 5%–10%.]
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.