

⟨571⟩ VITAMIN A ASSAY

ASSAY

Chemical Methods

• PROCEDURE 1

The following procedure is provided for the determination of vitamin A in dietary ingredients or pharmaceutical ingredients. It conforms to the procedure that was adopted in 1956 for international use by the International Union of Pure and Applied Chemistry.

Complete the assay promptly, and exercise care throughout the procedure to keep to a minimum the exposure to actinic light and to atmospheric oxygen and other oxidizing agents, preferably by the use of low-actinic glassware and an atmosphere of an inert gas.

For the test articles that contain tocopherol, an appropriate chromatographic method should be used.

Sample solution: Accurately weigh, count, or measure a portion of the test specimen expected to contain the equivalent of NLT 0.15 mg of retinol but containing NMT 1 g of fat. If in the form of capsules, tablets, or other solid, so that it cannot be saponified efficiently by the ensuing instructions, reflux the portion taken in 10 mL of water on a steam bath for about 10 min, crush the remaining solid with a blunt glass rod, and warm for about 5 min longer.

Transfer to a suitable borosilicate glass flask, and add 30 mL of alcohol, followed by 3 mL of potassium hydroxide solution (9 in 10). Reflux in an all-borosilicate glass apparatus for 30 min. Cool the solution, add 30 mL of water, and transfer to a conical separator. Add 4 g of finely powdered sodium sulfate decahydrate. Extract by shaking with one 150-mL portion of ether for 2 min, and then, if an emulsion forms, with three 25-mL portions of ether. Combine the ether extracts, if necessary, and wash by swirling gently with 50 mL of water. Repeat the washing more vigorously with three additional 50-mL portions of water. Transfer the washed ether extract to a 250-mL volumetric flask, add ether to volume, and mix.

Evaporate a 25.0-mL portion of the ether extract to about 5 mL. *Without applying heat and with the aid of a stream of inert gas or vacuum*, continue the evaporation to about 3 mL. Dissolve the residue in sufficient isopropyl alcohol to give an expected concentration of the equivalent of 3 µg–5 µg of vitamin A per mL or to give an absorbance in the range 0.5–0.8 at 325 nm.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelengths: 310, 325, and 334 nm

Cell: 1 cm

Blank: Isopropyl alcohol

Analysis

Sample: *Sample solution*

Determine the absorbances of the *Sample solution* at 310, 325, and 334 nm. Calculate the vitamin A, as retinol ($C_{20}H_{30}O$) content, in mg, in the portion of sample taken, using one of the following formulas:

$$\text{Result} = (0.549 \times A_{325}) / (L \times C)$$

or

$$\text{Result} = (0.549 \times [A_{325}]) / (L \times C)$$

L = length of the absorption cell (cm)

C = concentration in the final isopropyl alcohol solution of test specimen (g/100 mL) or capsules or tablets (units/100 mL)

$[A_{325}]$ = corrected absorbance at 325 nm, calculated:

$$\text{Result} = (6.815 \times A_{325}) - (2.555 \times A_{310}) - (4.260 \times A_{334})$$

Each mg of vitamin A, as retinol ($C_{20}H_{30}O$), represents 3333 USP Units of vitamin A.

Use the first formula when A_{325} , the observed absorbance at 325 nm, is between $[A_{325}]/1.030$ and $[A_{325}]/0.970$. Use the second formula when $[A_{325}]$ has a value less than $A_{325}/1.030$.

[NOTE—The range of the limits of error for this analytical procedure, indicating the extent of discrepancy to be expected in the results of different laboratories at $P = 0.05$, is approximately $\pm 8\%$.]

• PROCEDURE 2

This procedure is used for dietary ingredients or pharmaceutical ingredients in the form of pure retinyl esters or prepared from pure retinyl esters into a vehicle excipient.

Sample solution: Dissolve 25–100 mg, accurately weighed, in 5 mL of pentane, and dilute with isopropyl alcohol to give an expected concentration of the equivalent of 3–4.5 µg/mL of retinol.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: 326 nm

Cell: 1 cm

Blank: Isopropyl alcohol

Analysis**Sample:** *Sample solution*

Calculate the vitamin A, as retinol ($C_{20}H_{30}O$) content, in mg, in the portion of sample taken. Each mg of vitamin A, as retinol ($C_{20}H_{30}O$), represents 3333 USP Units of vitamin A:

$$\text{Result} = (0.570 \times A_{326}) / (L \times C)$$

A_{326} = absorbance at 326 nm

L = length of the absorption cell (cm)

C = concentration of the *Sample solution* (g/100 mL)

Chromatographic Methods

The following liquid chromatographic procedures are provided for the determination of vitamin A as an active pharmaceutical ingredient, a dietary supplement ingredient, or a component in the dietary supplements or pharmaceutical dosage forms.

Throughout these procedures, protect solutions containing and derived from the test specimen and the Reference Standards from the atmosphere and light, preferably by the use of a blanket of inert gas and low-actinic glassware. Where an ester form of vitamin A (retinyl acetate or retinyl palmitate) is specified in the following procedure, use the chemical form present in the formulation and the relevant USP Reference Standard.

• PROCEDURE 1

This is a neutral procedure that involves either simply dissolving the sample directly into hexane and injecting into the liquid chromatograph or sample extraction by first dissolving the sample in dimethyl sulfoxide, followed by a liquid-liquid extraction of the vitamin A with hexane. Although its chromatographic system can separate the 13-*cis* and all-*trans*-isomers of vitamin A, only the all-*trans*-isomer peak is used for the quantitation of vitamin A. The procedure can be used to determine vitamin A in raw material, Oil-Soluble Vitamins Tablets, Oil-Soluble Vitamins Capsules, Oil- and Water-Soluble Vitamins Tablets, Oil- and Water-Soluble Vitamins Capsules, Oil- and Water-Soluble Vitamins with Minerals Tablets, and Oil- and Water-Soluble Vitamins with Minerals Capsules.

Unless specified in the individual monographs, the *Standard solutions*, *Sample solutions*, and *System suitability solution* are prepared as follows.

Mobile phase: *n*-Hexane

Standard solution 1: 15 µg/mL of retinol¹ from USP Retinyl Acetate RS in *n*-hexane

Standard solution 2: 15 µg/mL of retinol² 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 raw materials: Transfer retinyl acetate or retinyl palmitate, accurately weighted, equivalent to 15 mg of retinol, to a 100-mL volumetric flask, dissolve in and dilute with *n*-hexane to volume, and mix. Pipet 5.0 mL of this solution into a 50-mL volumetric flask, dilute with *n*-hexane to volume, and mix.

Sample solution for tablets: Finely powder NLT 20 Tablets. Transfer a portion of the powder, not exceeding 7.5 g, equivalent to NLT 1 mg of vitamin A, as retinol ($C_{20}H_{30}O$), to a centrifuge tube having a polytetrafluoroethylene-lined screw cap. Add about 2 mL of dimethyl sulfoxide and about 3 mL of *n*-hexane per each g of powdered Tablets, and shake for 45 min on a shaker in a water bath maintained at 60°. [NOTE—Set up the shaker to ensure that the contents of the container are mixed vigorously and thoroughly.] Centrifuge at 3000 rpm for 10 min, and transfer the hexane layer by means of a pipet to a volumetric flask. Add 3 mL of *n*-hexane per each g of powdered Tablets to the dimethyl sulfoxide layer, shake thoroughly for 5 min, and transfer the hexane layer by means of a pipet to the same volumetric flask. Repeat this extraction with three additional portions of *n*-hexane. Dilute the extracts in the volumetric flask with *n*-hexane to volume. Dilute a volume of this solution with *n*-hexane to obtain a solution having a nominal concentration of 15 µg/mL of vitamin A, as retinol ($C_{20}H_{30}O$). [NOTE—Dilution may not be necessary.]

Sample solution for capsules: Transfer the contents of NLT 20 Capsules to a suitable container, mix, and weigh. Transfer a portion of the mixture, not exceeding 7.5 g equivalent to NLT 1 mg of vitamin A, as retinol ($C_{20}H_{30}O$), to a centrifuge tube having a polytetrafluoroethylene-lined screw cap. [NOTE—For hard gelatin Capsules, remove, as completely as possible, the contents of NLT 20 Capsules by cutting open the Capsule shells, transferring the shells and their contents to a suitable container, and triturating to a homogeneous mass. Transfer a portion of the mass, equivalent to NLT 1 mg of vitamin A, as retinol ($C_{20}H_{30}O$), to a centrifuge tube having a polytetrafluoroethylene-lined screw cap.] Add about 2 mL of dimethyl sulfoxide and about 3 mL of *n*-hexane per each g of Capsule contents, and shake for 45 min on a shaker in a water bath maintained at 60°. [NOTE—Set up the shaker to ensure that the contents of the container are mixed vigorously and thoroughly.] Centrifuge at 3000 rpm for 10 min, and transfer the hexane layer by means of a pipet to a volumetric flask. Add 3 mL of *n*-hexane per each g of Capsule contents to the dimethyl sulfoxide layer, shake thoroughly for 5 min, and transfer the hexane layer by means of a pipet to the same volumetric flask. Repeat this extraction with three additional portions of *n*-hexane. Dilute the extracts in the volumetric flask with *n*-hexane to volume. Dilute a volume of this solution with *n*-hexane to obtain a solution with a concentration of 15 µg/mL of vitamin A, as retinol ($C_{20}H_{30}O$). [NOTE—Dilution may not be necessary.]

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

¹ Use the value of 0.872 to convert retinyl acetate to its retinol equivalent.

² Use the value of 0.546 to convert retinyl palmitate to its retinol equivalent.

Injection volume: 40 µL

System suitability

Samples: *System suitability solution* and *Standard solution 1* or *Standard solution 2*

Suitability requirements

Resolution: NLT 10 between all-*trans*-retinyl acetate and all-*trans*-retinyl palmitate, *System suitability solution*

Relative standard deviation: NMT 3.0%, *Standard solution 1* or *Standard solution 2*

Analysis

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

Calculate the percentage of the labeled amount of vitamin A, as retinol (C₂₀H₃₀O), in the portion of sample taken:

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

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

r_S = peak response of all-*trans* retinyl ester from *Standard solution 1* or *Standard solution 2*

C_S = concentration of retinol (C₂₀H₃₀O) in *Standard solution 1* or *Standard solution 2* (µg/mL)

C_U = concentration of vitamin A, as retinol (C₂₀H₃₀O), in the *Sample solution* (µg/mL)

• **PROCEDURE 2**

This procedure involves the treatment of sample with methanolic sulfuric acid, followed by extraction with 2,2,4-trimethylpentane. Sample preparation can be used for the formulation containing vitamins A, D, and E.

Application includes Oil-Soluble Vitamins Tablets, Oil-Soluble Vitamins Capsules, Oil- and Water-Soluble Vitamins Tablets, Oil- and Water-Soluble Vitamins Capsules, Oil- and Water-Soluble Vitamins with Minerals Tablets, and Oil- and Water-Soluble Vitamins with Minerals Capsules.

Unless specified in the individual monographs, the *Standard solutions*, *Sample solutions*, *System suitability solution*, and reagent solutions are prepared as follows.

Mobile phase: *n*-Hexane and ethyl acetate (99.7: 0.3)

3 N methanolic sulfuric acid solution: Cautiously add 9 mL of sulfuric acid to 80 mL of methanol in a 100-mL volumetric flask. Cool, and dilute with methanol to volume.

Sodium ascorbate-pyrogallol solution: Transfer 10 g of sodium ascorbate and 5 g of pyrogallol to a 100-mL volumetric flask, and add sufficient water to dissolve. Add 1.7 mL of sulfuric acid, and dilute with water to volume.

Lecithin solution: 5 mg/mL of lecithin in 2,2,4-trimethylpentane

Standard solution 1: 15 µg/mL of retinol¹ from USP Retinyl Acetate RS in 2,2,4-trimethylpentane

Standard solution 2: 15 µg/mL of retinol² from USP Retinyl Palmitate RS in 2,2,4-trimethylpentane

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

Sample solution for tablets: [NOTE—This preparation is suitable for the determination of vitamin A, vitamin D, and vitamin E when present in the formulation. The sample amount may be adjusted depending on the presence or absence of the appropriate vitamins.] Finely powder NLT 20 Tablets. Use a portion of the powder nominally equivalent to an amount between 0.4 mg and 2.5 mg of retinol. Add 0.5 g of sodium bicarbonate, 1.5 mL of *Lecithin solution*, and 12.5 mL of 2,2,4-trimethylpentane, and disperse on a vortex mixer. Add 6 mL of *Sodium ascorbate-pyrogallol solution*, shake slowly, and allow the solution to degas. Continue shaking until the evolution of gas has ceased, and then shake for an additional 12 min. Add 6 mL of dimethyl sulfoxide, mix on a vortex mixer to form a suspension, and shake for 12 min. Add 6 mL of 3 N *methanolic sulfuric acid solution*, mix on a vortex mixer to form a suspension, and shake for 12 min. Add 12.5 mL of 2,2,4-trimethylpentane, mix on a vortex mixer to form a suspension, and shake for 10 min. Centrifuge for 10 min to break up the emulsion and to clarify the supernatant. If necessary, quantitatively dilute a volume of the supernatant with 2,2,4-trimethylpentane to obtain a concentration close to that of the *Standard solution*.

Sample solution for capsules: [NOTE—This preparation is suitable for the determination of vitamin A, vitamin D, and vitamin E when present in the formulation. The sample amount may be adjusted depending on the presence or absence of the appropriate vitamins.] Weigh NLT 20 Capsules in a tared weighing bottle. Using a sharp blade if necessary, carefully open the Capsules, without loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing with several portions of ether. Discard the washings, and dry the Capsule shells with the aid of a current of dry air. Weigh the empty Capsule shells in the tared weighing bottle, and calculate the net weight of the Capsule contents. Transfer a portion of the Capsule contents, equivalent to 2.5 mg of the labeled amount of vitamin A, as retinol. Add 0.5 g of sodium bicarbonate, 1.5 mL of *Lecithin solution*, and 12.5 mL of 2,2,4-trimethylpentane, and disperse on a vortex mixer. Add 6 mL of *Sodium ascorbate-pyrogallol solution*, shake slowly, and allow the solution to degas. Continue shaking until the evolution of gas has ceased, and then shake for an additional 12 min. Add 6 mL of dimethyl sulfoxide, mix on a vortex mixer to form a suspension, and shake for 12 min. Add 6 mL of 3 N *methanolic sulfuric acid solution*, mix on a vortex mixer to form a suspension, and shake for 12 min. Add 12.5 mL of 2,2,4-trimethylpentane, mix on a vortex mixer to form a suspension, and shake for 10 min. Centrifuge for 10 min to break up the emulsion and to clarify the supernatant. If necessary, quantitatively dilute a volume of the supernatant with 2,2,4-trimethylpentane to obtain a concentration close to that of the *Standard solution*.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 325 nm

Column: 4.6-mm × 25-cm; 5-µm packing L24

Flow rate: 1.5 mL/min

Injection volume: 40 µL

System suitability

Sample: *System suitability solution*

Suitability requirements**Resolution:** NLT 8.0 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 the portion of sample taken:

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

 r_U = peak response of all-*trans* retinyl ester from the *Sample solution* r_S = peak response of the all-*trans* retinyl ester from *Standard solution 1* or *Standard solution 2* C_S = concentration of retinol ($C_{20}H_{30}O$) in *Standard solution 1* or *Standard solution 2* ($\mu\text{g/mL}$) C_U = nominal concentration of vitamin A, as retinol ($C_{20}H_{30}O$), in the *Sample solution* ($\mu\text{g/mL}$).[NOTE—Use 26.5 mL as the final volume of the *Sample solution* to calculate the nominal concentration.]**• PROCEDURE 3**

This procedure involves the saponification of both the standard and sample, followed by a liquid-liquid extraction of vitamin A from the sample with a mixture of *n*-hexane and methylene chloride (3:1). The 13-*cis*- and all-*trans*- isomers of vitamin A can be characterized and quantitated. The procedure can be used for Oil-Soluble Vitamins Tablets, Oil-Soluble Vitamins Capsules, Oil- and Water-Soluble Vitamins Tablets, Oil- and Water-Soluble Vitamins Capsules, Oil- and Water-Soluble Vitamins with Minerals Tablets, and Oil- and Water-Soluble Vitamins with Minerals Capsules.

Unless specified in the individual monographs, the *Standard solution*, *Sample solutions*, and reagent solutions are prepared as follows.

Mobile phase: *n*-Hexane and isopropyl alcohol (92:8)**Extraction solvent:** *n*-Hexane and methylene chloride (3:1)**Potassium hydroxide solution:** 800 mg/mL of potassium hydroxide in water. [NOTE—Cautiously add potassium hydroxide in water. Mix, and cool.]**Diluent:** 10 mg/mL of pyrogallol in alcohol**Standard solution:** Dilute USP Retinyl Acetate RS or USP Retinyl Palmitate RS with *Diluent* to obtain a concentration of 8.5 $\mu\text{g/mL}$ of retinol^{1,2} ($C_{20}H_{30}O$). Transfer 10.0 mL of this solution to a stoppered 125-mL flask, and add 5 mL of water, 5 mL of *Diluent*, and 3 mL of *Potassium hydroxide solution*. Insert the stopper tightly, shake for 15 min over a water bath maintained at $60 \pm 5^\circ$, and cool to room temperature. Add 7 mL of water and 25.0 mL of *Extraction solvent*. Insert the stopper tightly, and shake vigorously for 60 s. Rinse the sides of the flask with 60 mL of water, and allow to stand for 10 min until the layers separate. Withdraw a portion of the organic layer for injection into the chromatograph. This *Standard solution* contains 3.4 $\mu\text{g/mL}$ of retinol.**Sample solution for capsules:** Weigh NLT 20 Capsules in a tared weighing bottle. Open the Capsules, without loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing with several portions of ether. Discard the washings, and dry the Capsule shells with the aid of a current of dry air. Weigh the empty Capsule shells in the tared weighing bottle, and calculate the net weight of the Capsule contents. Transfer a portion of the Capsule contents, equivalent to 1.3 mg of retinol, to a stoppered 125-mL flask. Add 5 mL of water, 15 mL of *Diluent*, and 3 mL of *Potassium hydroxide solution*. Insert the stopper tightly, shake for 15 min over a water bath maintained at $60 \pm 5^\circ$, and cool to room temperature. Add 7 mL of water and 25.0 mL of *Extraction solvent*. Insert the stopper tightly, and shake vigorously for 60 s or longer, if necessary, for complete extraction. Rinse the sides of the flask with 60 mL of water, and allow to stand for 10 min until the layers separate. [NOTE—Do not shake, because an emulsion may form.] Withdraw a portion of the organic layer, and dilute quantitatively and stepwise if necessary, with *Extraction solvent*, to obtain a concentration of 3.4 $\mu\text{g/mL}$ of retinol.**Sample solution for tablets:** Finely powder a counted number of Tablets. Transfer a portion of the powder, equivalent to 1.3 mg of retinol, to a stoppered 125-mL flask. Add 5 mL of water, 15 mL of *Diluent*, and 3 mL of *Potassium hydroxide solution*. Insert the stopper tightly, shake for 15 min over a water bath maintained at $60 \pm 5^\circ$, and cool to room temperature. Add 7 mL of water and 25.0 mL of *Extraction solvent*. Insert the stopper tightly, and shake vigorously for 60 s or longer, if necessary, for complete extraction. Rinse the sides of the flask with 60 mL of water, and allow to stand for 10 min until the layers separate. [NOTE—Do not shake, because an emulsion may form.] Withdraw a portion of the organic layer, and dilute with *Extraction solvent* to obtain a concentration of 3.4 $\mu\text{g/mL}$ of retinol.**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 335 nm**Column:** 6.2-mm \times 8-cm; packing L3**Column temperature:** 40° **Flow rate:** 4 mL/min**Injection volume:** 50 μL **System suitability****Sample:** *Standard solution***Suitability requirements****Relative standard deviation:** NMT 3.0%**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 sample taken:

$$\text{Result} = (r_{T1}/r_{T2}) \times (C_S/C_U) \times 100$$

- r_{T1} = sum of peak responses of all-*trans* retinyl ester and 13-*cis*-retinyl ester peaks from the *Sample solution*
 r_{T2} = sum of peak responses of all-*trans* retinyl ester and 13-*cis*-retinyl ester peaks from the *Standard solution*
 C_S = concentration of retinol ($C_{20}H_{30}O$) in the *Standard solution* ($\mu\text{g/mL}$)
 C_U = nominal concentration of vitamin A, as retinol ($C_{20}H_{30}O$), in the *Sample solution* ($\mu\text{g/mL}$)

• PROCEDURE 4

This procedure involves a liquid-liquid extraction of vitamin A from the sample with hexane, followed by the evaporation of hexane and reconstitution of the residue in tetrahydrofuran and acetonitrile mixture (1:1). It can be used for the determination of vitamin A in Oil- and Water-Soluble Vitamins Oral Solution and Oil- and Water-Soluble Vitamins with Minerals Oral Solution.

Unless specified in the individual monographs, the *Standard solution*, *Sample solution*, and *Diluent* are prepared as follows.

Mobile phase: Methanol, acetonitrile, and *n*-hexane (46.5: 46.5: 7.0)

Diluent: Tetrahydrofuran and acetonitrile (1:1)

Standard solution: 0.33 mg/mL of retinol^{1,2} ($C_{20}H_{30}O$) from USP Retinyl Acetate RS or USP Retinyl Palmitate RS in *Diluent*

Sample solution for liquid dosage forms: Transfer an accurately measured volume of Oral Solution, equivalent to 3.3 mg of retinol, to a 500-mL separatory funnel containing 10 mL of water and 20 mL of dehydrated alcohol. Add 150 mL of solvent hexane, insert the stopper, and shake for 1 min. Add another 150 mL of solvent hexane, insert the stopper, shake, and allow the layers to separate. Discard the aqueous layer, and filter the solvent hexane extract through anhydrous sodium sulfate into a 500-mL, round-bottom flask. Evaporate the solution to dryness with the aid of a rotary evaporator over a water bath maintained at about 65°. Immediately add 10.0 mL of *Diluent*, swirl to dissolve the residue, and filter.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 265 nm

Column: 4.6-mm \times 50-cm (prepared from two concatenated 4.6-mm \times 25-cm columns); packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 5.0%

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 sample taken:

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

- r_U = peak response of all-*trans* retinyl ester from the *Sample solution*
 r_S = peak response of all-*trans* retinyl ester from the *Standard solution*
 C_S = concentration of retinol ($C_{20}H_{30}O$) in the *Standard solution* ($\mu\text{g/mL}$)
 C_U = nominal concentration of vitamin A, as retinol ($C_{20}H_{30}O$), in the *Sample solution* ($\mu\text{g/mL}$)

ADDITIONAL REQUIREMENTS

• USP REFERENCE STANDARDS <11>

USP Retinyl Acetate RS

USP Retinyl Palmitate RS