

VITAMIN A ASSAY

The following pressurized liquid chromatographic procedure is provided for the determination of Vitamin A. Where the use of vitamin A ester (retinyl acetate or retinyl palmitate) is specified in the following procedure, use the chemical form present in the raw material. Use low-actinic glassware throughout this procedure.

Mobile Phase— Use n-hexane.

System Suitability Preparation— Dissolve an accurately weighed quantity of retinyl palmitate and [USP Vitamin A RS](#) in n-hexane to obtain a solution containing about 7.5 µg per mL of each.

Standard Preparation— Dissolve an accurately weighed quantity of [USP Vitamin A RS](#) in n-hexane, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 15 µg of retinyl acetate per mL.

Assay Preparation— Transfer about 15 mg of vitamin A ester (retinyl acetate or retinyl palmitate), accurately weighed, 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.

Chromatographic System—The liquid chromatograph is equipped with a 325-nm detector and a 4.6-mm × 15-cm column that contains packing L8. The flow rate is about 1 mL per minute.

Chromatograph the System Suitability Preparation, and record the peak responses as directed for **Procedure**: the resolution, R, between retinyl acetate and retinyl palmitate is not less than 10; and the relative standard deviation for replicate injections is not more than 3.0%.

Procedure— Separately inject equal volumes (about 40 µL) of the Standard Preparation and the Assay Preparation into the chromatograph, record the chromatograms, and measure the responses for retinyl acetate obtained from the Standard Preparation and the peak area for retinyl acetate or retinyl palmitate in the chromatogram of the Assay Preparation. Calculate the quantity, in mg, of vitamin A as the retinol equivalent (C₂₀H₃₀O) in the portion of vitamin A taken by the formula:

$$0.872CD(rU / rS)$$

in which 0.872 is the factor used to convert retinyl acetate, obtained from [USP Vitamin A RS](#) to its retinol equivalent; C is the concentration, in mg per mL, of [USP Vitamin A RS](#) in the Standard Preparation; D is the dilution factor, in mL, for the Assay Preparation; and rU and rS are the peak

responses of the retinyl ester obtained from the Assay Preparation and the Standard Preparation, respectively. [note—The molar responses of retinyl acetate and retinyl palmitate are equivalent.]