Definition  Vitamin A in Oil is a fatty oil obtained from the fresh liver, pyloric appendage, other parts of marine animals, its vitamin A (retinol) concentrate, its dissolution in edible fats and oils, or Vitamin A Esters of Fatty Acids (retinol fatty acid ester), or such substances dissolved in edible fats and oils.

Content  1 g of Vitamin A in Oil contains not less than 30 mg of vitamin A, and the content of vitamin A is in a range of 90 - 120% of the labeled content. Three hundred mg of vitamin A is equivalent to 1,000,000 international units.

Description  Vitamin A in Oil occurs as a light yellow to reddish-light yellow oily substance having a slight, characteristic odor.

Identification  Proceed as directed under Identification for Vitamin A Esters of Fatty Acids.

Purity  (1) Acid value  Proceed as directed under Purity (1) for Vitamin A Esters of Fatty Acids.

(2) Chloroform-insoluble substances  Proceed as directed under Purity (2) for Vitamin A Esters of Fatty Acids.

(3) Absorbance ratio  Proceed as directed under Purity test (3) for Vitamin A Esters of Fatty Acids.

Assay  Weigh accurately an amount of Vitamin A in Oil equivalent to not less than 0.15 mg of vitamin A, which contains not more than 1 g of fats and oils, transfer into a flask, and add 30 ml of aldehyde-free ethanol and 1 ml of a solution of pyrogallol in ethanol (1:10). Add 3 ml of potassium hydroxide solution (9:10), equip with a reflux condenser, and heat on a water bath for 30 minutes to saponify. Cool quickly to ordinary temperature, add 30 ml of water, transfer into separating funnel A. Wash the flask with 10 ml of water and then 40 ml of ether for vitamin A determination, transfer the washings into separating funnel A, shake well, and allow to stand. Transfer the water layer into separating funnel B, wash the flask with 30 ml of ether for vitamin A determination, transfer the washings into separating funnel B, and shake to extract. Transfer the water layer into the flask, transfer the ether layer into separating funnel A, transfer the water layer from the flask above into separating funnel B, add 30 ml of ether for vitamin A determination, and shake to extract. Transfer the ether layer into separating funnel A, add 10 ml of water, invert the separating funnel gently 2 or 3
times, allow to stand, and remove the separated water layer. Wash three times with 50 ml of water each time, shaking stronger. Wash repeatedly with 50 ml of water each time until the washings no longer shows a color with phenolphthalein TS, and allow to stand for 10 minutes. Remove water as much as possible, transfer the ether layer into an Erlenmeyer flask, wash the separating funnel twice with 10 ml of ether for vitamin A determination each time, transfer the washings to the Erlenmeyer flask above. Add 5 g of anhydrous sodium sulfate, shake, and transfer the ether extract into an eggplant-type flask by decantation. Wash the remaining sodium sulfate more than twice with 10 ml of ether for vitamin A determination each time, and transfer the washings into the eggplant-type flask above. Concentrate the ether extract to about 1 ml, using an aspirator while shaking in a water bath at 45°C, immediately dissolve in isopropyl alcohol for vitamin A determination, diluting exactly to obtain the solution containing about 3 mg of vitamin A per ml. Use this solution as the test solution. Measure the absorbances (A1, A2, and A3) of the test solution at wavelengths of 310 nm, 325 nm, and 334 nm, respectively, and calculate the content by the formula

\[
\text{Content of vitamin A} = E \frac{1\%}{1\text{cm}} (325 \text{ nm}) \times 0.549 \text{ (mg/g)},
\]

\[
E \frac{1\%}{1\text{cm}} (325\text{nm}) = \frac{A_2}{W} \times \frac{V}{100} \times f,
\]

\[
f = 6.815 - 2.555 \frac{A_1}{A_2} - 4.260 \times \frac{A_3}{A_2},
\]

where \( f \) = correction factor

\( V \) = total volume (ml) of the test solution,

\( W \) = weight (g) of the sample in \( V \) ml of the test solution.

When the sample contains vitamin A esters of fatty acids, proceed as directed under the Assay for Vitamin A Esters of Fatty Acids.

Storage Standards Place in a light-resistant hermetic container and replace the air with an inert gas.