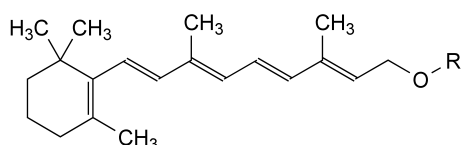


incinerate until all the black particles have disappeared. Allow to cool. Add a few drops of *ammonium carbonate solution R*. Evaporate and incinerate carefully, allow to cool and weigh again. Repeat the incineration for periods of 5 min to constant mass.

**STORAGE**

Store in a dust-proof package in a dry place.

**VITAMIN A****Vitaminum A**

Substance	R	Molecular formula	$M_r$
all-( <i>E</i> )-retinol	H	$C_{20}H_{30}O$	286.5
all-( <i>E</i> )-retinol acetate	CO-CH <sub>3</sub>	$C_{22}H_{32}O_2$	328.5
all-( <i>E</i> )-retinol propionate	CO-C <sub>2</sub> H <sub>5</sub>	$C_{23}H_{34}O_2$	342.5
all-( <i>E</i> )-retinol palmitate	CO-C <sub>15</sub> H <sub>31</sub>	$C_{36}H_{60}O_2$	524.9

**DEFINITION**

*Vitamin A* refers to a number of substances of very similar structure (including (*Z*)-isomers) found in animal tissues and possessing similar activity. The principal and biologically most active substance is all-(*E*)-retinol (all-(*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraen-1-ol;  $C_{20}H_{30}O$ ). Vitamin A is generally used in the form of esters such as the acetate, propionate and palmitate.

*Synthetic retinol ester* refers to an ester (acetate, propionate or palmitate) or a mixture of synthetic retinol esters.

The activity of vitamin A is expressed in retinol equivalents (R.E.). 1 mg R.E. corresponds to the activity of 1 mg of all-(*E*)-retinol. The activity of the other retinol esters is calculated stoichiometrically, so that 1 mg R.E. of vitamin A corresponds to the activity of:

- 1.147 mg of all-(*E*)-retinol acetate,
- 1.195 mg of all-(*E*)-retinol propionate,
- 1.832 mg of all-(*E*)-retinol palmitate.

International Units (IU) are also used to express the activity of vitamin A. 1 IU of vitamin A is equivalent to the activity of 0.300 µg of all-(*E*)-retinol. The activity of the other retinol esters is calculated stoichiometrically, so that 1 IU of vitamin A is equivalent to the activity of:

- 0.344 µg of all-(*E*)-retinol acetate,
- 0.359 µg of all-(*E*)-retinol propionate,
- 0.550 µg of all-(*E*)-retinol palmitate,

1 mg of retinol equivalent is equivalent to 3333 IU.

**CHARACTERS***Appearance:*

Retinol acetate: pale-yellow crystals (mp: about 60 °C). Once melted retinol acetate tends to yield a supercooled melt.

Retinol propionate: reddish-brown oily liquid.

Retinol palmitate: a fat-like, light yellow solid or a yellow oily liquid, if melted (mp: about 26 °C).

*Solubility:* all retinol esters are practically insoluble in water, soluble or partly soluble in anhydrous ethanol and miscible with organic solvents.

Vitamin A and its esters are very sensitive to the action of air, oxidising agents, acids, light and heat.

*Carry out the assay and all tests as rapidly as possible, avoiding exposure to actinic light and air, oxidising agents, oxidation catalysts (e.g. copper, iron), acids and heat; use freshly prepared solutions.*

**01/2008:0217 IDENTIFICATION**

A. Thin-layer chromatography (2.2.27).

*Test solution.* Prepare a solution containing about 3.3 IU of vitamin A per microlitre in *cyclohexane R* containing 1 g/l of *butylhydroxytoluene R*.

*Reference solution.* Prepare a 10 mg/ml solution of *retinol esters CRS* (i.e. 3.3 IU of each ester per microlitre) in *cyclohexane R* containing 1 g/l of *butylhydroxytoluene R*.

*Plate:* TLC silica gel  $F_{254}$  plate *R*.

*Mobile phase:* ether *R*, *cyclohexane R* (20:80 V/V).

*Application:* 3 µl.

*Development:* over 2/3 of the plate.

*Drying:* in air.

*Detection:* examine in ultraviolet light at 254 nm.

*System suitability:* reference solution:

- the chromatogram shows the individual spots of the corresponding esters. The elution order from bottom to top is: retinol acetate, retinol propionate and retinol palmitate.

*Results:* the composition of esters is confirmed by the correspondence of the principal spot or spots of the test solution with those obtained with the reference solution.

B. It complies with the test for related substances.

**TESTS**

**Retinol.** Thin-layer chromatography (2.2.27).

*Test solution.* Prepare a solution in *cyclohexane R*, stabilised with a solution containing 1 g/l of *butylhydroxytoluene R*, containing about 330 IU of vitamin A per microlitre.

*Reference solution.* Shake 1 ml of the test solution with 20 ml of 0.1 M *tetrabutylammonium hydroxide in 2-propanol* for 2 min and dilute to 100 ml with *cyclohexane R*, stabilised with a solution containing 1 g/l of *butylhydroxytoluene R*.

*Plate:* TLC silica gel  $F_{254}$  plate *R*.

*Mobile phase:* ether *R*, *cyclohexane R* (20:80 V/V).

*Application:* 3 µl.

*Development:* over a path of 15 cm.

*Drying:* in air.

*Detection:* examine in ultraviolet light at 254 nm.

*System suitability:* reference solution:

- in the chromatogram obtained no or only traces of the retinol esters are seen.

*Limit:* any spot corresponding to retinol in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (1.0 per cent).

**Related substances.** Ultraviolet and visible absorption spectrophotometry (2.2.25).

*Test solution.* The solution described under Activity.

*Absorption maximum:* at 325 nm to 327 nm.

*Absorbance ratios:*

- $A_{300}/A_{326}$  = maximum 0.60;

- $A_{350}/A_{326}$  = maximum 0.54;
- $A_{370}/A_{326}$  = maximum 0.14.

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

#### ACTIVITY

The activity of the substance is determined in order to be taken into account for the production of concentrates.

Dissolve 25-100 mg, weighed with an accuracy of 0.1 per cent, in 5 ml of *pentane R* and dilute with *2-propanol R1* to a presumed concentration of 10 IU/ml to 15 IU/ml. Measure the absorbance (2.2.25) at the absorption maximum at 326 nm. Calculate the activity of vitamin A in International Units per gram from the expression:

$$\frac{A_{326} \times V \times 1900}{100 \times m}$$

- $A_{326}$  = absorbance at 326 nm,  
 $m$  = mass of the substance to be examined, in grams,  
 $V$  = total volume to which the substance to be examined is diluted to give 10 IU/ml to 15 IU/ml,  
 1900 = factor to convert the specific absorbance of esters of retinol into International Units per gram.

#### STORAGE

In an airtight container, protected from light.

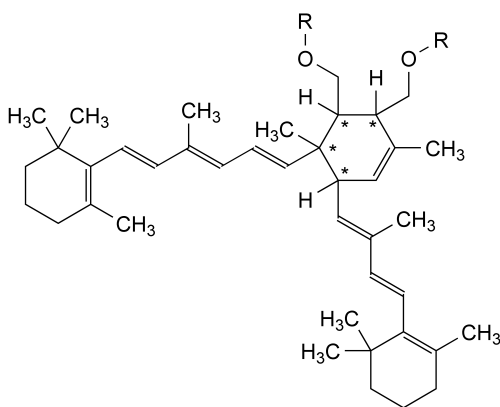
Once the container has been opened, its contents are to be used as soon as possible; any part of the contents not used at once should be protected by an atmosphere of inert gas.

#### LABELLING

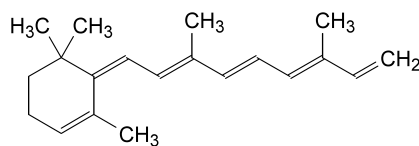
The label states:

- the number of International Units per gram,
- the name of the ester or esters.

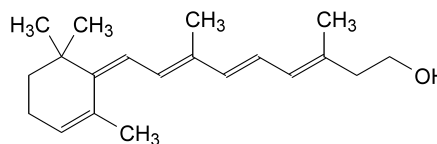
#### IMPURITIES



A. R = H, CO-CH<sub>3</sub>: kitols (Diels-Alder dimers of vitamin A),



B. (3*E*,5*E*,7*E*)-3,7-dimethyl-9-[(1*Z*)-2,6,6-trimethylcyclohex-2-enylidene]nona-1,3,5,7-tetraene (anhydro-vitamin A),



- C. (3*E*,5*E*,7*E*)-3,7-dimethyl-9-[(1*Z*)-2,6,6-trimethylcyclohex-2-enylidene]nona-3,5,7-trien-1-ol (*retro*-vitamin A),  
 D. oxidation products of vitamin A.

01/2008:0219

## VITAMIN A CONCENTRATE (OILY FORM), SYNTHETIC

### Vitaminum A syntheticum densatum oleosum

#### DEFINITION

Oily concentrate prepared from synthetic retinol ester (0217) as is or by dilution with a suitable vegetable fatty oil.

*Content*: 95.0 per cent to 110.0 per cent of the vitamin A content stated on the label, which is not less than 500 000 IU/g.

It may contain suitable stabilisers such as antioxidants.

#### CHARACTERS

*Appearance*: yellow or brownish-yellow, oily liquid.

*Solubility*: practically insoluble in water, soluble or partly soluble in anhydrous ethanol, miscible with organic solvents.

Partial crystallisation may occur in highly concentrated solutions.

#### IDENTIFICATION

Thin-layer chromatography (2.2.27).

*Test solution*. Prepare a solution containing about 3.3 IU of vitamin A per microlitre in *cyclohexane R* containing 1 g/l of *butylhydroxytoluene R*.

*Reference solution*. Prepare a 10 mg/ml solution of *retinol esters CRS* (i.e. 3.3 IU of each ester per microlitre) in *cyclohexane R* containing 1 g/l of *butylhydroxytoluene R*.

*Plate*: TLC silica gel *F<sub>254</sub>* plate *R*.

*Mobile phase*: ether *R*, *cyclohexane R* (20:80 *V/V*).

*Application*: 3 µl.

*Development*: immediately, over a path of 15 cm.

*Drying*: in air.

*Detection*: examine in ultraviolet light at 254 nm.

*System suitability*: reference solution:

- the chromatogram shows the individual spots of the corresponding esters. The elution order from bottom to top is: retinol acetate, retinol propionate and retinol palmitate.

*Results*: the composition of the test solution is confirmed by the correspondence of the principal spot or spots with those obtained with the reference solution.

#### TESTS

**Acid value (2.5.1)**: maximum 2.0, determined on 2.0 g.

**Peroxide value (2.5.5, Method A)**: maximum 10.0.