Absorbency

Apparatus. A dry cylindrical copper-wire basket 8.0 cm high and 5.0 cm in diameter. The wire of which the basket is constructed is about 0.4 mm in diameter, the mesh is 1.5 cm to 2.0 cm wide and the mass of the basket is 2.7 ± 0.3 g.

Sinking time. Not more than 10 s. Weigh the basket to the nearest centigram (m_1). Take a total of 5.00 g in approximately equal quantities from 5 different places in the product to be examined, place loosely in the basket and weigh the filled basket to the nearest centigram (m_2). Fill a beaker 11 cm to 12 cm in diameter to a depth of 10 cm with water at about 20 °C. Hold the basket horizontally and drop it from a height of about 10 mm into the water. Measure with a stopwatch the time taken for the basket to sink below the surface of the water. Calculate the result as the average of 3 tests.

Water-holding capacity. Not less than 18.0 g of water per gram. After the sinking time has been measured, remove the basket from the water, allow it to drain for exactly 30 s suspended in a horizontal position over the beaker, transfer it to a tared beaker (m_3) and weigh to the nearest centigram (m_4). Calculate the water-holding capacity per gram of absorbent viscose wadding using the following expression:

$$\frac{m_4 - (m_2 + m_3)}{m_2 - m_1}$$

Calculate the result as the average of 3 tests.

Ether-soluble substances. Not more than 0.30 per cent. In an extraction apparatus, extract 5.00 g with *ether R* for 4 h at a rate of at least 4 extractions per hour. Evaporate the ether extract and dry the residue to constant mass at 100 °C to 105 °C.

Extractable colouring matter. In a narrow percolator, slowly extract 10.0 g with *alcohol R* until 50 mL of extract is obtained. The liquid obtained is not more intensely coloured (*2.2.2, Method II*) than reference solution Y_5, GY_6 or a reference solution prepared as follows: to 3.0 mL of blue primary solution add 7.0 mL of hydrochloric acid (10 g/L HCl) and dilute 0.5 mL of this solution to 10.0 mL with hydrochloric acid (10 g/L HCl).

Surface-active substances. Introduce the 10 mL portion of solution S reserved before filtration into a 25 mL graduated ground-glass-stoppered cylinder with an external diameter of 20 mm and a wall thickness of not greater than 1.5 mm, previously rinsed 3 times with *sulfuric acid R* and then with *water R*. Shake vigorously 30 times in 10 s, allow to stand for 1 min and repeat the shaking. After 5 min, any foam present does not cover the entire surface of the liquid.

Water-soluble substances. Not more than 0.70 per cent. Boil 5.00 g in 500 mL of *water R* for 30 min, stirring frequently. Replace the water lost by evaporation. Decant the liquid, squeeze the residual liquid carefully from the sample with a glass rod and mix. Filter the liquid whilst hot. Evaporate 400 mL of the filtrate (corresponding to 4/5 of the mass of the sample taken) and dry the residue to constant mass at 100 °C to 105 °C.

Hydrogen sulfide. To 10 mL of solution S add 1.9 mL of *water R*, 0.15 mL of *dilute acetic acid R* and 1 mL of *lead acetate solution R*. After 2 min, the solution is not more intensely coloured than a reference solution prepared at the same time using 0.15 mL of *dilute acetic acid R*, 1.2 mL of *thioacetamide reagent R*, 1.7 mL of *lead standard solution (10 ppm Pb) R* and 10 mL of solution S.

Loss on drying (*2.2.32*). Not more than 13.0 per cent, determined on 5.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*). Not more than 0.45 per cent for the lustrous variety and not more than 1.7 per cent for the matt variety. Introduce 5.00 g into a previously heated and cooled, tared crucible. Heat cautiously over a naked flame and then carefully to dull redness at 600 °C. Allow to cool, add a few drops of *dilute sulfuric acid R*, then heat and 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.

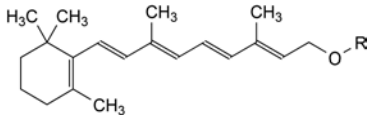
STORAGE

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

01/2008:0217

VITAMIN A

Vitaminum A



Substance	R	Molecular formula	M_r
all-(*E*)-retinol	H	$C_{20}H_{30}O$	286.5
all-(*E*)-retinol acetate	CO-CH_3	$C_{22}H_{32}O_2$	328.5
all-(*E*)-retinol propionate	CO-C_2H_5	$C_{23}H_{34}O_2$	342.5
all-(*E*)-retinol palmitate	CO-$C_{15}H_{31}$	$C_{36}H_{60}O_2$	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.

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. Related substances (see Tests).

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 containg 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.

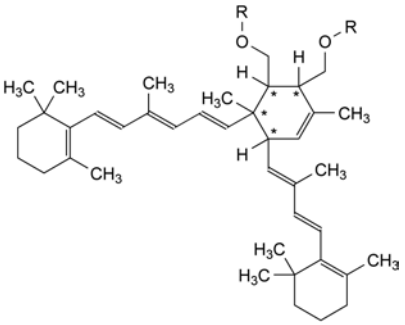
Once the container has been opened, its contents are to be used as soon as possible; any part of the contents not used at once should be protected by an atmosphere of inert gas.

LABELLING

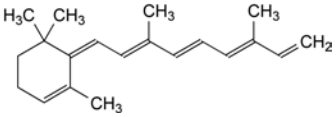
The label states:

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

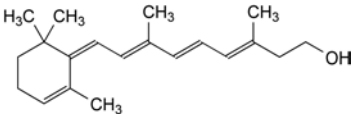
IMPURITIES



A. R = H, CO-CH_3: 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.