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

ASSAY

Carry out the assay as rapidly as possible, avoiding exposure to actinic light, air, oxidising agents, oxidation catalysts (e.g. copper, iron), acids and prolonged heat; use freshly prepared solutions. If partial crystallisation has occurred, homogenise the material at a temperature of about 65 °C, but avoid prolonged heating.

Carry out the assay according to Method A. If the assay is not shown to be valid, use Method B.

Method A. Ultraviolet absorption spectrophotometry (2.2.25).

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 10-15 IU/ml.

Verify that the absorption maximum of the solution lies between 325 nm and 327 nm and measure the absorbances at 300 nm, 326 nm, 350 nm and 370 nm. Repeat the readings at each wavelength and take the mean values. Calculate the ratio \( A_{326}/A_{326} \) for each wavelength.

If the ratios do not exceed: 0.60 at 300 nm, 0.54 at 350 nm, 0.14 at 370 nm, calculate the content of vitamin A in International Units per gram using the following expression:

\[
A_{326} \times V \times 1900 \\
100 \times m
\]

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

If one or more of the ratios \( A_{326}/A_{326} \) exceeds the values given, or if the wavelength of the absorption maximum does not lie between 325 nm and 327 nm, use Method B.

Method B. Liquid chromatography (2.2.29).

Test solution (a). Introduce 0.100 g of the preparation to be examined into a 100 ml volumetric flask and dissolve immediately in 5 ml of pentane R. Add 40 ml of 0.1 M tetrabutylammonium hydroxide in 2-propanol. Swirl gently and let the mixture react for 10 minutes at 60-65 °C for hydrolysis, swirling occasionally. Allow to cool to room temperature, dilute to 100.0 ml with 2-propanol R containing 1 g/l butylhydroxytoluene R, and homogenise carefully to avoid air-bubbles.

Test solution (b). Dilute test solution (a) with 2-propanol R to a final concentration of 100 IU/ml.

Reference solution (a). Introduce about 0.100 g of retinol acetate CRS into a 100 ml volumetric flask and proceed as described for test solution (a).

Reference solution (b). Introduce into a 50 ml volumetric flask 5.0 ml of reference solution (a) and dilute to 50.0 ml with 2-propanol R. Homogenise carefully to avoid air-bubbles.

Column:

- size: \( l = 0.125 \) m, \( \phi = 4 \) mm,
- stationary phase: octadecysilyl silica gel for chromatography R (5 µm).


Flow rate: 1 ml/min.

Detection: spectrophotometer at 325 nm.

Injection: 10 µl of test solution (b) and reference solution (b).

Run time: 1.5 times the retention time of retinol.

Retention time: retinol = about 3 min.

Calculate the content of vitamin A in International Units per gram using the following expression:

\[
\frac{A_1 \times C \times m_2}{A_2 \times m_1}
\]

\( A_1 \) = area of the peak due to retinol in the chromatogram obtained with test solution (b),
\( A_2 \) = area of the peak due to retinol in the chromatogram obtained with reference solution (b),
\( C \) = concentration of retinol acetate CRS in International Units per gram, determined by method A; the absorption ratios \( A_{326}/A_{326} \) must conform,
\( m_1 \) = mass of the substance to be examined in test solution (a), in milligrams,
\( m_2 \) = mass of retinol acetate CRS in reference solution (a), in milligrams.

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,
- the name of any added stabilisers,
- the method of restoring the solution if partial crystallisation has occurred.

01/2008:0218

VITAMIN A CONCENTRATE (POWDER FORM), SYNTHETIC

Vitamini synthetici densati A pulvis

DEFINITION

Powder concentrate obtained by dispersing a synthetic retinol ester (0217) in a matrix of Gelatin (0330) or Acacia (0307) or other suitable material.

Content: 95.0 per cent to 115.0 per cent of the vitamin A content stated on the label, which is not less than 250 000 IU/g.

It may contain suitable stabilisers such as antioxidants.

CHARACTERS

Appearance: yellowish powder usually in the form of particles of almost uniform size.

Solubility: practically insoluble in water, swells or forms an emulsion, depending on the formulation.

IDENTIFICATION

Thin-layer chromatography (2.2.27).
Test solution. Introduce a quantity of the preparation to be examined containing about the equivalent of 17000 IU of vitamin A into a 20 ml glass-stoppered test tube. Add about 20 mg of bromelains R; 2 ml of water R and about 150 µl of 2-propanol R, swirling gently for 2.5 min in a water-bath at 60-65 °C. Cool to below 30 °C and add 5 ml of 2-propanol R containing 1 g/l of butylhydroxytoluene R. Shake vigorously for 1 min, allow to stand for a few minutes and use the supernatant solution.

Reference solution. Prepare a 10 mg/ml solution of retinol esters CRS (i.e. 3.3 IU of each ester per microlitre) in 2-propanol R containing 1 g/l of butylhydroxytoluene R.


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:
- 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

Related substances. The thresholds indicated under Related substances (Table 2034.1) in the general monograph Substances for pharmaceutical use (2034) do not apply.

ASSAY

Carry out the assay as rapidly as possible, avoiding exposure to actinic light, air, oxidising agents, oxidation catalysts (e.g. copper, iron), acids and prolonged heat.

Liquid chromatography (2.2.29).

Test solution (a). Introduce 0.200 g of the preparation to be examined into a 100 ml volumetric flask. Add 20-30 mg of bromelains R, 5.0 ml of water R and 0.15 ml of 2-propanol R. Heat gently in a water-bath at 60 °C for about 5 min, swirling occasionally. Add 40 ml of 0.1 M tetrabutylammonium hydroxide in 2-propanol. Swirl gently and let the mixture react for 10 min at 60-65 °C for hydrolysis, swirling occasionally. Ensure that all sample material is wetted. Allow to cool to room temperature, dilute to 100.0 ml with 2-propanol R containing 1 g/l of butylhydroxytoluene R, and homogenise carefully to avoid air-bubbles. The solution may be turbid.

Test solution (b). Dilute test solution (a) with 2-propanol R to a final concentration of 100 IU/ml. Filter before injection.

Reference solution (a). Introduce about 0.100 g of retinol acetate CRS into a 100 ml volumetric flask and dissolve immediately in 5 ml of pentane R. Add 40 ml of 0.1 M tetrabutylammonium hydroxide in 2-propanol. Swirl gently and let the mixture react for 10 min at 60-65 °C for hydrolysis, swirling occasionally. Allow to cool to room temperature, dilute to 100.0 ml with 2-propanol R containing 1 g/l of butylhydroxytoluene R, and homogenise carefully to avoid air-bubbles.

Reference solution (b). Introduce into a 50 ml volumetric flask 5.0 ml of reference solution (a) and dilute to 50.0 ml with 2-propanol R. Homogenise carefully to avoid air-bubbles.

Column: size: l = 0.125 m, ø = 4 mm,
- stationary phase: octadecysilyl silica gel for chromatography R (5 µm).


Flow rate: 1 ml/min.

Detection: spectrophotometer at 325 nm.

Injection: 10 µl of test solution (b) and reference solution (b).

Run time: 1.5 times the retention time of retinol.

Retention time: retinol = about 3 min.

Calculate the content of vitamin A using the following expression:

\[
\frac{A_1 \times C \times m_2}{A_2 \times m_1}
\]

- \(A_1\) = area of the peak due to retinol in the chromatogram obtained with test solution (b),
- \(A_2\) = area of the peak due to retinol in the chromatogram obtained with reference solution (b),
- \(C\) = concentration of retinol acetate CRS in International Units per gram, determined by the method below,
- \(m_1\) = mass of the substance to be examined in test solution (a), in milligrams,
- \(m_2\) = mass of retinol acetate CRS in reference solution (a), in milligrams.

The exact concentration of retinol acetate CRS is assessed by ultraviolet absorption spectrophotometry (2.2.25). Dissolve 25-100 mg of retinol acetate CRS, 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 10-15 IU/ml. Verify that the absorption maximum of the solution lies between 325 nm and 327 nm and measure the absorbances at 300 nm, 326 nm, 350 nm and 370 nm. Repeat the readings at each wavelength and take the mean values. Calculate the ratio \(A_{326}/A_{326}\) for each wavelength.

If the ratios do not exceed: 0.60 at 300 nm, 0.54 at 350 nm, 0.14 at 370 nm, calculate the content of vitamin A in International Units per gram using the following expression:

\[
\frac{A_{326} \times V \times 1900}{100 \times m}
\]

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

The absorbance ratios \(A_{326}/A_{326}\) must conform.

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,
- the name of the principal excipient or excipients used and the name of any added stabilisers.