



**REVISED DRAFT MONOGRAPH FOR *THE*
*INTERNATIONAL PHARMACOPOEIA***

RETINOL CONCENTRATE, OILY FORM

(August 2010)

DRAFT FOR COMMENT

This document was provided by a quality control expert and was discussed at the recent WHO tele-/videoconference on specifications for medicines and quality control laboratory issues held on 25 August 2010. Should you have any comments thereon, please send these to Dr S. Kopp, Manager, Medicines Quality Assurance Programme, Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland; fax: (+41 22) 791 4730 or e-mails: kopps@who.int with a copy to Dr Herbert Schmidt (schmidth@who.int) by 10 October 2010.

In order to speed up the process of receipt of comments, if you do not already receive our documents electronically, please let us have your e-mail address (to bonnyw@who.int) and we will add it to our electronic mailing list.

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SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/10.387
International Pharmacopoeia monograph on Retinol concentrate, oily form

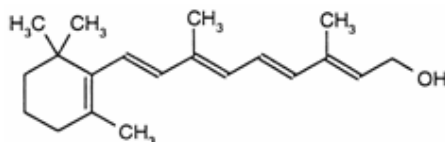
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Any further action as required	...

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Note from the secretariat: The formula will be changed to the ester (-R)



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 ₂ H 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

Chemical name. all-(E)-3,7-Dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraen-1-ol; CAS Reg. No. 68-26-8 [retinol].

Other name. Vitamin A concentrate (oily form).

Description. A yellow to brownish yellow, oily liquid.

Solubility. Practically insoluble in water; soluble or partly soluble in dehydrated ethanol R; miscible with organic solvents.

Category. Vitamin.

Storage. The oily form of Retinol concentrate should be kept in a well-closed and well-filled container, protected from light. Once the container has been opened its contents should 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 designation on the container should state the name of the retinol ester or esters, and their quantities expressed as the content of vitamin A in International Units (IU),

whether any stabilizing agents are added and their quantities, as well as the method of restoring the solution if partial crystallization has occurred.

Additional information. Even in the absence of light, the oily form of Retinol concentrate is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures.

Partial crystallization may occur in concentrated solutions and upon refrigeration.

Requirements

Definition. The oily form of Retinol concentrate consists of an ester or a mixture of esters (acetate, propionate, or palmitate) of retinol, usually prepared by synthesis. It may be diluted in a suitable vegetable oil. It may contain suitable antimicrobial agents and stabilizing agents such as antioxidants.

The declared content of vitamin A is not less than 500 000 IU/g. Retinol concentrate contains not less than 95.0% and not more than 110.0% of the amount of vitamin A stated on the label.

Identity tests

- Either tests A and B or tests A and C or tests A and D may be applied.

A. Carry out test A.1 or, where UV detection is not available, test A.2.

A.1 Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance and a mixture of 12 volumes of cyclohexane R and 1 volume of ether R as the mobile phase. Apply separately to the plate 2 µl of each of 4 solutions in cyclohexane R. For solution (A) dissolve a quantity of the concentrate containing the equivalent of 50,000 IU of vitamin A in 10 ml. For solution (B) prepare a solution of retinol acetate RS equivalent to 5000 IU of vitamin A per ml. For solution (C) prepare a solution of retinol propionate RS equivalent to 5000 IU of vitamin A per ml. For solution (D) prepare a solution of retinol palmitate RS equivalent to 5000 IU of vitamin A per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution (A) corresponds in position and appearance to one or more of the spots obtained with solutions (B), (C), and (D).

A.2. Carry out the test as described under [1.14.1 Thin-layer chromatography](#), using the conditions described above under test A.1 but using silica gel R5 as the coating substance. After removing the plate from the chromatographic chamber, allow it to dry in air, and spray with antimony trichloride TS. Examine the chromatogram in daylight.

The principal spot obtained with solution (A) corresponds in position and appearance to one or more of the spots obtained with solutions (B), (C), and (D).

- B. Dissolve a small drop in about 1 ml of dichloromethane R and add 5 ml of antimony trichloride TS; a blue colour is immediately produced which turns gradually to violet-red.
- C. See the test described below under Assay method B. The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).
- D. To a quantity of the Retinol concentrate containing the equivalent of 50,000 IU of vitamin A, add 100 ml of ethanol (~750 g/l) TS. Dilute 1 ml of the resulting solution to 50 ml with a mixture of 100 volumes of ethanol (~750 g/l) TS and 1 volume of hydrochloric acid (~420 g/l) TS. Immediately after preparation measure the absorbance (1.6) in the range 300 to 400 nm. The solution exhibits a single maximum at 326 nm. Heat the solution in a water bath for 30 seconds and cool rapidly. The absorption spectrum of the resulting solution, when observed between 300 and 400 nm, exhibits a shoulder at 332 nm and maxima at 348, 367 and 389 nm.

Acid value. Not more than 2.0.

Peroxides. For solution (A) dissolve 0.30 g in 25 ml of a mixture of 4 volumes of methanol R and 6 volumes of toluene R. For solution (B) prepare a solution containing 0.27 g of ferric chloride R per ml, and add 1.0 ml to 99 ml of a mixture of 4 volumes of methanol R and 6 volumes of toluene R. Dilute 2.0 ml to 100ml with the same solvent mixture.

Place in 2 separate test-tubes in the following order, mixing after each addition, 3ml of a solution containing 18 mg of ammonium thiocyanate R per ml, 10ml of methanol R, 0.3 ml of ferrous sulfate/hydrochloric acid TS, and 15 ml of toluene R. Then add 1.0 ml of solution A into one tube and 1.0 ml of solution B into the other, shake, and allow to stand for 5 minutes. The colour produced with solution A is not more intense than that produced with solution B.

Assay. Carry out the assay as rapidly as possible, avoiding exposure to actinic light and oxidizing agents, and maintaining whenever possible an atmosphere of nitrogen above the solution.

- Either method A, where valid, or B may be applied.

A. Carry out each determination in duplicate, using separately weighed amounts of Retinol concentrate. Dissolve a quantity of Retinol concentrate containing the equivalent of about 200,000 IU of vitamin A, accurately weighed, in 5 ml of *n*-pentane R and dilute with 2-propanol R to a presumed concentration of 10-15 IU per ml. Verify that the absorption maximum of the solution to be examined, measured against a solvent cell containing 2-propanol, lies between 325nm and 327nm. Measure the absorbances at 300nm, 326nm, 350nm, and 370 nm. Repeat the readings at each wavelength and take the mean values. Calculate the ratio A_{λ}/A_{326} for each wavelength. If the ratios do not exceed ~~0.592~~ 0.60 at 300 nm, ~~0.537~~ 0.54 at 350 nm, and ~~0.142~~ 0.14 at 370 nm, calculate the content of vitamin A in International Units per gram from the expression: $A_{326} \times V \times 1900/100m$, where A_{326} is the absorbance at 326 nm, V is the total volume used for the dilution to give 10-15 IU per ml, m is the mass of sample used in g and 1900 is the factor to convert the specific absorbances of esters of retinol into IU per g.

If one or more of the ratios A_{λ}/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.

- B. Carry out the test as described under [1.14.4 High-performance liquid chromatography](#), using a stainless steel column (15 cm x 4.6 mm) packed with particles of silica gel, the surface of which has been modified with octadecylsilyl groups (5 μ m). As the mobile phase, use a mixture of 95 volumes of methanol R and 5 volumes of water R.

Prepare the following solutions. For solution (1) Transfer a quantity of Retinol concentrate containing the equivalent of about 100 000 IU of vitamin A, accurately weighed, into a 100 ml volumetric flask. Dissolve immediately in 5 ml of n-pentane R. Add 40 ml of 0.1 M tetrabutylammonium hydroxide TS in 2-propanol R. 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 volume with 2-propanol R containing 1 g/L butylated hydroxytoluene R, and homogenise carefully to avoid air-bubbles. Dilute 5 ml of the resulting solution to 50 ml with 2-propanol R. For solution (2) transfer an amount of retinol acetate RS or retinol palmitate RS containing the equivalent of about 100,000 IU of vitamin A, accurately weighed, into a 100 ml volumetric flask. Proceed as described for solution (1).

Operate with a flow rate of 1 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 325 nm.

Inject 10 μ l of solution (2) in six replicate injections into the chromatographic system. The assay is not valid unless the relative standard deviation for the peak area of retinol is less than 2.0%.

Inject alternatively 10 μ l each of solutions (1) and (2) and record the chromatograms for 1.5 times the retention time of retinol.

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2) and calculate the content of vitamin A.
