Document:	Code:	Page:
Analytical Method	10001-01	1 of 1

Title:		Valid from:
Determination of vitamin A In Powder, tablet and solution.		Replaces: New Date of revision: September 2008
Prepared by:	Approved by:	Put into force by:
Dennis Eriksen	Dennis Eriksen	Dennis Eriksen

1. Purpose

The purpose of this analytical method is to describe a method for determination of vitamin A in samples containing **no** vitamin E.

2. Index

3. Enclosures

1. Dokumentation, 2 pages

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4. Principle

Vitamin A (retinol) is determined by spectrophotometry after saponification and extraction.
This method is not suitable for samples containing vitamin E.

5. Apparatus

UV/VIS-spectrophotometer, e.g. Perkin Elmer Lambda 20.

Steam bath

6. Reagents

2-Propanol R1, Ph. Eur., gradient grade, e.g. Merck 1040.

2-propanol, Ph.Eur, uvasol, e.g. Merck 0993.

Potassium hydroxide, Ph. Eur., analytical reagent, e.g. Riedel-de Haën 30603.

Ascorbic acid, Ph. Eur., p.a, e.g. Merck 127.

Sodium sulphate, anhydrous, Ph.Eur., analytical reagent, e.g. Riedel-de Haën 31481.

Butylhydroxytoluene, Ph. Eur., e.g. Fluka 34750.

Ether, Ph. Eur., free of peroxides, e.g. Superfos kemi 1416064.

Alcohol, 96%, Ph.Eur, reagent grade, e.g. DDSF.

Ethanol, 99%, Ph. Eur., reagent grade, e.g. DDSF.

Nitrogen, Ph. Eur.

Sodium hydroxide solution, dilute (2N), Ph. Eur.

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Phenolphthalein 0,1%, B&B Lab.

Potassium hydroxide solution (17 M):

Dissolve 180 g of potassium hydroxide in 126 ml of water.
Store for 3 months.

Sodium sulphate solution (3%):

Dissolve 30 g of anhydrous sodium sulphate in water and dilute to 1000 ml with the same solvent.
Store for 3 months.

BHT-solution (0.1%), alcoholic:

Dissolve 1.0 g of butylhydroxytoluene in alcohol and dilute to 1000 ml with the same solvent.
Store for 3 months.

Sodium ascorbate solution (2%):

Dissolve 3.5 g of ascorbic acid in 10 ml of dilute sodium hydroxide solution and dilute to 200 ml with water.
Do not store.

7. Method

7.1 Preparation of solubilized aqueous solutions

Mix the sample and accurately weigh the amount of sample (= p g) stated in table 1 into a 100 ml ground glass stoppered conical flask.
Dilute to 10 ml with sodium ascorbate solution (2%).

7.2 Preparation of tablets

Grind at least 20 tablets in the grinding mill and mix. Accurately weigh an amount of powder (=p g) containing the amount of vitamin A stated in table 1 (max 4,0 g) into a 100 ml ground glass stoppered conical flask.
Add 10 ml of sodium ascorbat solution (2%) and heat on a steam bath for 5 minutes with frequent shaking.

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7.3 Preparation of dry concentrates

Mix the sample and accurately weigh the amount of material (=p g) stated in table 1 into a 100 ml ground glass stoppered conical flask.
Add 10 ml of sodium ascorbate solution (2%) and heat on a steam bath for 5 minutes with frequent shaking.

	Vitamin A conc. in the sample in µg/ml, µg/g or µg/tablet	Weigh an amount of the sample containing about:
A	< 2000	2500 µg vitamin A
B	≥ 2000 and < 10000	10000 µg vitamin A
C	≥ 10000	50000 µg vitamin A

Table 1: Sample amount per analysis

7.4 Saponification

Add 30 ml of alcoholic BHT solution (0.1%) and 3.0 ml of potassium hydroxide solution (17 M). Connect the flask to an air condenser and reflux on a steam bath for 30 minutes with occasional shaking.

Cool and quantitatively transfer to a 500 ml separatory funnel containing 100 ml of ether with the aid of 30 ml of sodium sulphate solution (3%) and 100 ml of ether.

7.5 Extraction

Shake for 2 minutes. Let the layers separate and discard the lower aqueous layer.
If an emulsion is formed transfer the lower phase and the emulsion to another separatory funnel and extract with further 3 x 25 ml of ether. Combine the 4 ether extracts.

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Wash the ether extract with 50 ml portions of water (shake very carefully in the beginning to avoid emulsification) until the washings are no longer coloured red when adding phenolphthalein solution 0.1%.
If an emulsion is formed 2 ml of alcohol may be added to break the emulsion.

Quantitatively transfer the ether layer to a 250 ml volumetric flask with the aid of ether and dilute to volume with the same solvent.

7.6 Measurement

Pipette an amount of the ether extract containing about 200 µg of vitamin A into a 50 ml volumetric flask, i.e. (see table 1):

A: 20.00 ml

B: 5.00 ml or

C: Dilute 5.00 ml of the extract with ether to 25.00 ml and use 5.00 ml of this dilution (= 1.00 ml of the original extract)

Evaporate under a stream of nitrogen until a residue of about 2 ml is left, and dilute to volume with 2-propanol R1.
Dry concentrates: dilute to volume with 2-propanol, uvasol Merck 0993.
(Final concentration about 4 µg of vitamin A/ml)

Measure the absorbance at 310, 325 and 334 nm in a 1 cm cell using 2-propanol R1 as the blank.

The measured absorbance at 325 nm must be between 0.5 - 0.8.

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8. Calculation

Calculate the corrected absorbance at 325 nm (Ac_{325}) from the formula:

$Ac_{325} = 6.815 \times A_{325} - 2.555 \times A_{310} - 4.260 \times A_{334}$

If Ac_{325}/A_{325} is more than 1.030, make a new analysis.

If $0.971 < Ac_{325}/A_{325} \leq 1.030$ do not use Ac_{325}, use A_{325} directly.

If $Ac_{325}/A_{325} \leq 0.970$ use Ac_{325} in the formula.

$$\frac{A_{C325} \cdot 250 \cdot 50 \cdot n \cdot 1000000}{1821.5 \cdot p \cdot N \cdot 100} = \frac{A_{c325} \cdot n \cdot 68625}{p \cdot N} = \mu g \text{ of vitamin A per ml, tablet or gram}$$

n: Density (g/ml) liquid sample
Tablet weight in g

N: Volume of ether extract (ml) used for the final solution
i.e. A:20 B:5 C:1

1821.5 is A (1%, 1cm) of vitamin A (retinol)

9. Reference

USP <571>