Science without f(r)iction
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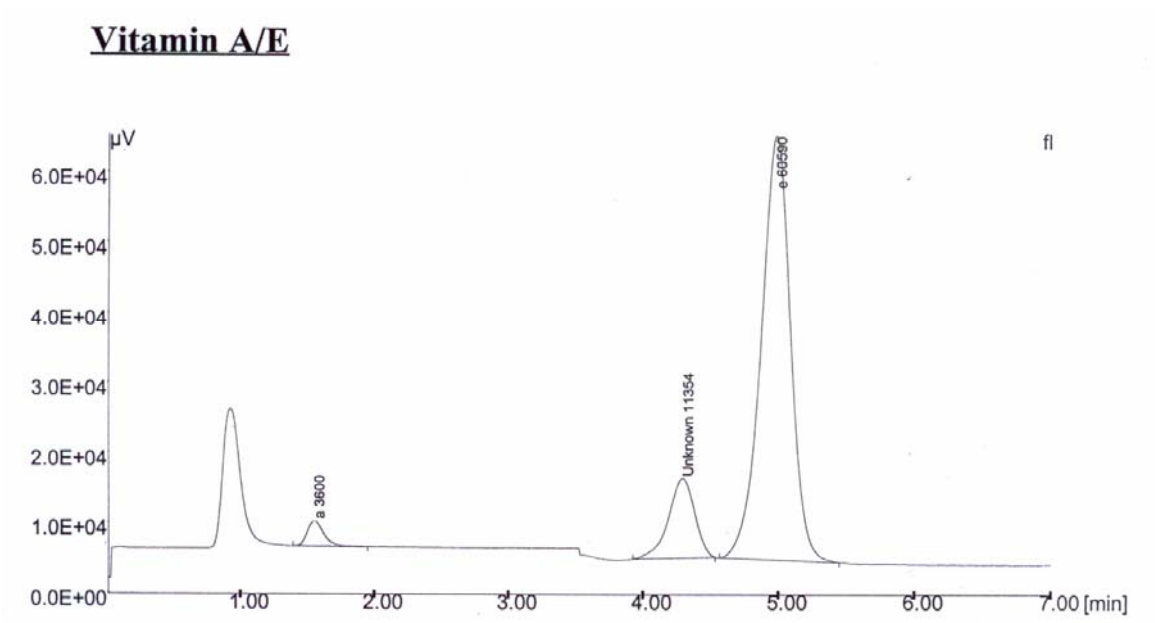
VITAMIN A/E

DETERMINATION OF VITAMIN A AND E IN SERUM OR PLASMA

- *HPLC Fluorescence Method*
- *Determination of Vitamin A and E in one run*
- *Use Serum or Plasma*
- *Runtime 7 minutes*
- *Wavelength 315 Ex, 455 Em and 295 Ex, 340 Em*

Products	Product no.	Quantity
Complete Set (contains according to product insert):		
Vitamin A/E Reagent Set	2795	50 - 70 Determinations
Components:		
Vitamin A/E Calibration Standard	2838	1 x 2 ml
Vitamin A/E Deproteinization Reagent	2843	1 x 60 ml
Vitamin A/E Mobile Phase Reagent	2844	1 x 500 ml
Additional:		
Vitamin A/E Control Low Level	2840	1 x 1 ml
Vitamin A/E Control Normal Level	2841	1 x 1 ml
Vitamin A/E Control High Level	2842	1 x 1 ml
Analytical column Polaris C18-A, 5 µm, 100 x 3 mm OR:	A2000100R030	1 x 1 pcs
Analytical column Pursuit 5-C18, 5 µm, 100 x 3 mm	A3000100R030	1 x 1 pcs
Chromsep Guard column SS, 10 x 2 mm	28141	1 x 5 pcs



SUMMARY

CLINICAL BACKGROUND

Vitamin A

Retinol, retinaldehyde and retinoic acid are collectively known as vitamin A, and it was first identified in 1920 and, being the first vitamin, it was named vitamin A under the alphabetical nomenclature system.
Retinol is not obtained directly from the diet, but is rather generated in the intestine from the enzymatic cleavage of beta-carotene, alpha-carotene and beta-cryptoxanthin, which are obtained from fruits and vegetables. Animal products such as egg yolk, milk, liver and fat contain retinyl esters which are hydrolysed to retinol in the intestine. Retinol is transported in serum to target cells complexed with a specific Retinol-Binding Protein (RBP) and transthyretin, a thyroxin-binding protein. In the target cells, retinol is oxidized to retinal and retinoic acid. Retinal is utilized in the synthesis of rhodopsin in the cones, and is necessary for night vision. The first nutritional deficiency disease to be identified and studied was night blindness.
So symptoms associated with a deficiency of vitamin A are night blindness, changes in the eyes, poor bone growth, weak tooth enamel, slow growth and dry skin.
Vitamin A has been shown to enhance gap junction communication in a dose-dependent manner and may promote normal cell growth. Vitamin A is necessary for maintenance of healthy epithelial tissue and can prevent the inception or progress of skin cancers by stimulating normal cell differentiation. Vitamin A therapy has been useful for inhibiting or suppressing tumour growth in the mouth, breast, bladder, cervix, lung and skin. Other functions include mucous production and normal bone growth.
Its high concentration in the liver is due to the fat-soluble nature of this polyene biochemical, although because of the storage mechanism, excessive doses of vitamin A can be very toxic. Hypervitaminosis A, usually resulting from overmedication leads to diverse symptoms such as headache, skeletal pain, hepatomegaly and haematological abnormalities.

Vitamin E

Vitamin E is widely distributed in foodstuffs. Vitamin E is the primary fat soluble antioxidant and it assists the preventing peroxidation of unsaturated fatty acids and the subsequent formation of cell-damaging free radicals. These free radicals increase the fragility of erythrocytes. For this reason, premature newborns whose red blood cells are more fragile than those of adults are often treated with Vitamin E to prevent haemolytic anaemia. Vitamin E therapy, in some cases, may have a favourable effect on moderate and severe cases of the retinopathy of prematurity[6] and the retinopathy of abetalipoproteinemia.
Foodstuffs contains the Vitamin E isomers α, β, γ-tocopherol, where α-tocopherol is the most abundant isomer in normal plasma (> 90%) and is also the most biologically active.
Vitamin E deficiency can produce the following symptoms: cirrhosis of the liver, coeliac disease, cystic fibrosis and low red blood count. Large doses of Vitamin E can be toxic and a physician should supervise large doses.

ASSAY PRINCIPLE

After protein precipitation and extraction retinol and α-tocopherol can be determined fluorometric on an isocratic HPLC system, with separation from interferences of the analytes by using an Analytical column Polaris C18-A or Analytical column Pursuit
5-C18, 5 µm, 100 x 3 mm. This approach is sensitive and simple. As these vitamins are detected at different wavelengths, a detector with programmable wavelength switching is very useful for simultaneous analysis of Vitamin A and Vitamin E in a single HPLC run. In the first run we measure the fluorescence (λ excitation 315 nm; λ emission 455 nm) which is proportional to Vitamin A concentration and after 3.5 minutes, we measure the fluorescence again (λ excitation 295 nm, λ emission 340 nm) which is proportional to Vitamin E concentration in the sample.

ANALYTICAL CONDITIONS A

Analytical Column	: Pursuit C18, 5 µm, 100 x 3 mm, artnr.: A3000100R030
Guard Column	: SS 10 x 2 mm Chromsep, artnr. CP 28141
Flow Rate	: 1.0 ml/min (< 90 kg/cm^2)
Detection	: 0 – 3.5 min: Fluorescence (excitation 315 nm, emission 455 nm)
	: 3.5 – 7 min: Fluorescence (excitation 295 nm, emission 340 nm)
Injection Volume	: 20 µl
Run time	: 7 minutes

ANALYTICAL CONDITIONS B

Analytical Column	: Polaris C18-A, 5 µm, 100 x 3 mm, artnr.: A2000100R030
Guard Column	: SS 10 x 2 mm Chromsep, artnr. CP 28141
Flow Rate	: 0.8 ml/min (< 90 kg/cm^2)
Detection	: 0 – 3.5 min: Fluorescence (excitation 315 nm, emission 455 nm)
	: 3.5 – 7 min: Fluorescence (excitation 295 nm, emission 340 nm)
Injection Volume	: 20 µl
Run time	: 7 minutes

EXPECTED VALUES

Retinol:	1 – 6 years:	0.20 – 0.80	mg/l	0.70 – 2.79	µmol/l
	7 years & older:	0.30 – 1.20	mg/l	1.05 – 4.19	µmol/l
α-Tocopherol:	Premature infants:	0.5 – 3.5	mg/l	1.16 – 8.12	µmol/l
	0 – 1 month:	1.0 – 3.5	mg/l	2.32 – 8.12	µmol/l
	2 – 5 month	2.0 – 6.0	mg/l	4.64 – 13.92	µmol/l
	6 month – 1 year:	3.5 – 8.0	mg/l	8.12 – 18.56	µmol/l
	2 – 12 years	5.5 – 9.0	mg/l	12.76 – 20.88	µmol/l
	13 years & older:	5.5 – 18.0	mg/l	12.76 – 41.76	µmol/l

PERFORMANCE

Linearity:

Vitamin A	Up to 2.8 mg/L (8.8 µmol/l)
Vitamin E	Up to 20 mg/l (46.4 µmol/l)

QUALITY CONTROLS

Lyophilised human serum preparation with analytical results for Vitamin A and Vitamin E by HPLC.

Vitamin A/E Control, Low Level	2840	1 x 1 ml	assayed
Vitamin A/E Control, Normal Level	2841	1 x 1 ml	assayed
Vitamin A/E Control, High Level	2842	1 x 1 ml	assayed

NOTES

1. For in vitro diagnostic use only.
2. For professional use only.
3. Always contact INstruchemie for the complete product insert and latest edition.
4. Printed in the Netherlands, Vitamin A/E-summary-290317-1.FEN