

VITAMINS A / E IN PLASMA BY UV - FAST – CODE Z18610

BIOCHEMISTRY

Vitamins A and E are two fat-soluble vitamins indispensable to animal life for correct growth and development.

Vitamin A exists in three oxidation states, retinol, retinal and retinoic acid. As a group, the various forms of vitamin A are called retinoids which are important to the retina. Additionally, retinoids are necessary for the differentiation of epidermoid and glandular cells. Thus, retinoids might act to suppress the malignant phenotype in transformed cells by promoting the expression of normal epithelial genes.

Vitamin E (tocoferol) functions as a chain-breaking antioxidant, inhibiting the propagation of lipid peroxidation, and thus protecting membranes or lipoproteins from oxidative damage. This constitutes an important biological function of vitamin E, since the deterioration of cellular membranes is associated with cellular dysfunction and oxidative modification of lipoproteins plays a role in the formation of the atherosclerotic plaque. In biological systems, vitamin E is thought to be regenerated by other antioxidants such as ascorbate and glutathione. In addition to antioxidant and atherosclerotic activities, vitamin E exhibits a number of other biological activities, including impact on cellular signalling and prevention of infertility in animals.



EUREKA S.R.L. LAB DIVISION
Via M. D'Antona 28
60033 Chiaravalle (AN) - Italy
Tel. +39 071 7450790
eureka-support@sentinel.it
www.eurekakit.com



This product fulfills all the requirements of Directive 98/79/EC on in vitro diagnostic medical devices (IVD). The declaration of conformity is available upon request.

N° 007	Vitamins A/E in plasma by UV - FAST	06/2024
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TECHNICAL FEATURES

Principle of the Method :

VITAMINS A and E are separated treating the plasma with a suitable precipitant. After this, 50 µl of the eluate is directly injected into HPLC.

Recovery : > 98%

Sensitivity (LLOD) : Vitamin A : 0,003 µg/ml
Vitamin E : 0,08 µg/ml

Minimum concentration analyzable (LLOQ) : Vitamin A : 0,014 µg/ml
Vitamin E : 0,4 µg/ml

Linearity : 0,014 – 10 µg/ml Vitamin A
0,4 – 100 µg/ml Vitamin E

Accuracy intra serie (relative error %) VITAMIN A:

C1	C2
1,2 µg/ml	6,7 µg/ml
4,9%	3,7%

Accuracy inter serie (relative error %) VITAMIN A:

C1	C2
1,2 µg/ml	6,7 µg/ml
6,0%	3,8%

Reproducibility intra serie (variation coefficient %) VITAMIN A:

C LLOQ	Cm	CUP
0,4 µg/ml	0,7 µg/ml	1,0 µg/ml
5,2%	2,6%	1,5%

Reproducibility inter serie (variation coefficient %) VITAMIN A :

C LLOQ	Cm	CUP
0,4 µg/ml	0,7 µg/ml	1,0 µg/ml
6,3%	5,6%	2,2%

Accuracy intra serie (relative error %) VITAMIN E :

C1	C2
4,6 µg/ml	42,2 µg/ml
4,3%	2,1%

Accuracy inter serie (relative error %) VITAMIN E :

C1	C2
4,6 µg/ml	42,2 µg/ml
4,6%	3,5%

Reproducibility intra serie (variation coefficient %) VITAMIN E :

C LLOQ	Cm	CUP
6,53 µg/ml	13,7 µg/ml	16,4 µg/ml
6,6%	2,2%	0,8%

Reproducibility inter serie (variation coefficient %) VITAMIN E :

C LLOQ	Cm	CUP
6,53 µg/ml	13,7 µg/ml	16,4 µg/ml
6,7%	4,8%	2,1%

Coefficient of Correlation + DS :

0,9931 ± 0,0037 Vitamin A
0,9954 ± 0,0025 Vitamin E

Normal Range :

Vitamin A : 0,20 – 0,80 µg/ml
Vitamin E : 5,50 – 17,00 µg/ml

1. Ball GFM: Vitamins: their role in the human body. Oxford, Blackwell Publishing, 2004, pp 234-255
2. Traber MG: Vitamin E. In Modern Nutrition in Health and Disease. 10th edition. Edited by ME Shils, M Shike, AC Ross, et al. Philadelphia, Lippincott Williams and Wilkins, 2006, pp 434-441

Components of the kit :

All the components are ready-to-use and stable 3 years at 2-8 °C.

Reagent A – Deproteinization Sol. with Internal Standard, **2 x 10 ml**

Plasma Calibrator lyophil., **1 x 1 ml**

Code Z18016 (packed separately – see data sheets)

Reagent M – Mobile Phase, **2 x 500 ml**

Minimum Instrumental equipment required:

Isocratic HPLC System with loop of 20 µl or 50 µl
Spectrophotometric Detector UV/VIS $\lambda=298$ nm
Chromatograms Recorder

Optional Equipment:

Sample Automatic Preparator
Autosampler
Operational Computer

Whole Blood Collection Procedure :

Test tube with EDTA

Pre-analytical Phase :

Delayed analyses require sample freezing at –20°C or less. Stable over 3 months.

PREANALYTICAL PHASE

Dilute 100 µl of Deproteinization Sol. with Internal Standard with 100 µl of H₂O. Inject 50 µl of this solution and verify that the Internal Standard peak has a retention time (RT) between 1,5 and 2,5 minutes (see Fig. 1).

ANALYTICAL PHASE

STEP 1 : Samples Preparation

Pipette in a tube:

	Calibrator	Sample
Calibrator	100 µl	
Sample		100 µl
Reagent A – Deproteinization Sol.	200 µl	200 µl

Warning: deproteinize directly on Vortex for at least 30 sec.

STEP 2 : Centrifuge at 14.000 rpm for 5 min.

STEP 3 : Pipette in a glass vial 200 µl of clear supernatant.

N.B.: at this step, the sample is stable 3 days at 2-8 °C

INJECTION :

- Inject 20 or 50 µl of this solution in the chromatographic system.

VITAMINS A / E in PLASMA – Warnings

SPECTROPHOTOMETRIC DETECTOR PARAMETERS

λ	298 nm
GAIN	0,001 AUFS
INTEGRATION TIME	10 sec.

HPLC COLUMN PROTECTION

To save the analytical column Reverse Phase POROSHELL 120 EC C-18 4,6 x 50 mm, 2,7 μ m, the use of Javelin Prefilters (1 x 10 pcs.) cod. S90199511 is obligatory.

HPLC COLUMN CONDITIONING

Install a new analytical C₁₈ Reversed-Phase column POROSHELL 120 EC-C18 (50 x 4,6 mm, 2,7 μ m).
Disconnect the detector and filter 30 ml of **H₂O : Methanol (20 : 80 v / v)** set flow of 1,0 ml/minute. **Don't recycle the washing solutions.** Flux 30 ml of a solution of **H₂O : Methanol (80 : 20 v / v)** at flow of 1,0 ml/minute. **Don't recycle the washing solutions.** Condition the column with the mobile phase at a flow of 1,0 ml/minute and discharge the first 20 ml. Finally inject the Chemical Standard and verify the quality of the HPLC run.
It's NOT possible to make analysis at recycling phase. If room temperature is > 20 °C store the Mobile Phase at 2-8 °C between an analytical session and another.

COLUMN CLEANING

Flux a solution of **H₂O : Methanol (20 : 80 v / v)**.

INJECTION NEEDLE WASHING

Wash with a solution of **Methanol or Acetonitrile/ H₂O (80 : 20 v/v)**.

HPLC PARAMETERS

LOOPS	20 or 50 μ l
FLOW	1,0 ml/min.
PRESSURE	About 70 bar

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z18016	Calibrator for Vitamins A/E in plasma	4 x 1 ml
Z18019	Control for Vitamins A/E in plasma -Levels 1 and 2	2 x 5 x 1 ml
Z18611	Mobile Phase	4 x 500 ml
Z699975902	POROSHELL 120 EC-C18 (4,6 x 50 mm, 2.7 μ m) Analytical Column	1 Pc
S90199511	Javelin Prefilters	1 x 10 Pcs
S51843550	Clear glass vials with reduced volume (globet form) from 1,5 ml to 15 μ l	1 x 100 Pcs
S51820717	Caps for Clear glass vials with reduced volume from 1,5 ml to 15 μ l and for amber vials	1 x 100 Pcs
L-30-210	Eppendorf Tubes by 1,5 ml with caps	1 x 1000 PZ

VITAMINS A / E IN PLASMA

(Reference Chromatograms)

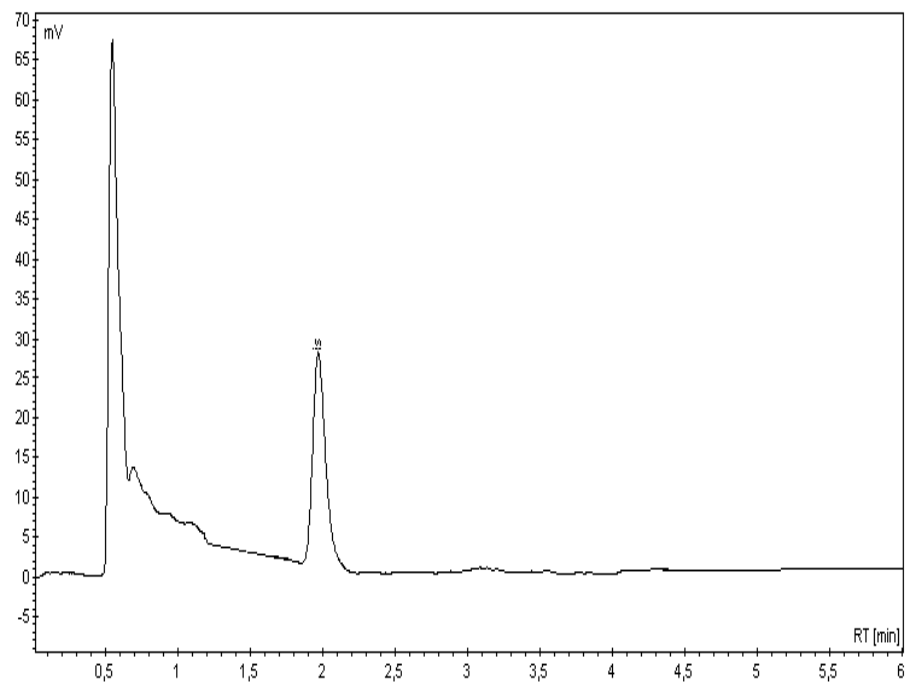


Fig. 1 :	Internal Standard Solution
	R.T. 2.0 Internal Standard

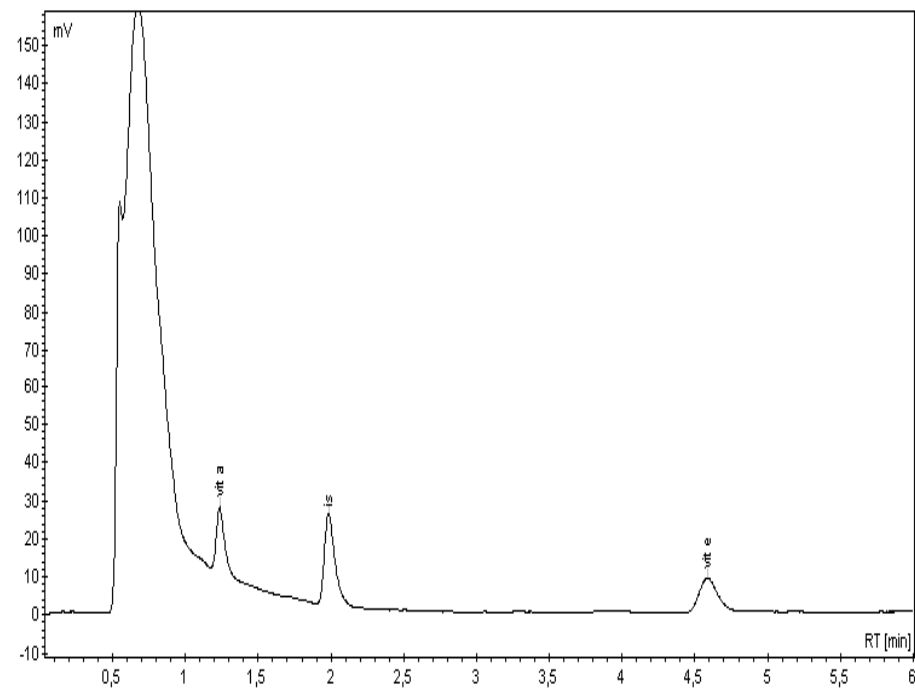


Fig. 2 :	Plasma Calibrator
R.T. 1.2	Vitamin A 0,8 µg/ml
R.T. 2.0	Internal Standard
R.T. 4.7	Vitamin E 16,72 µg/ml

VITAMINS A / E IN PLASMA (Reference Chromatograms)

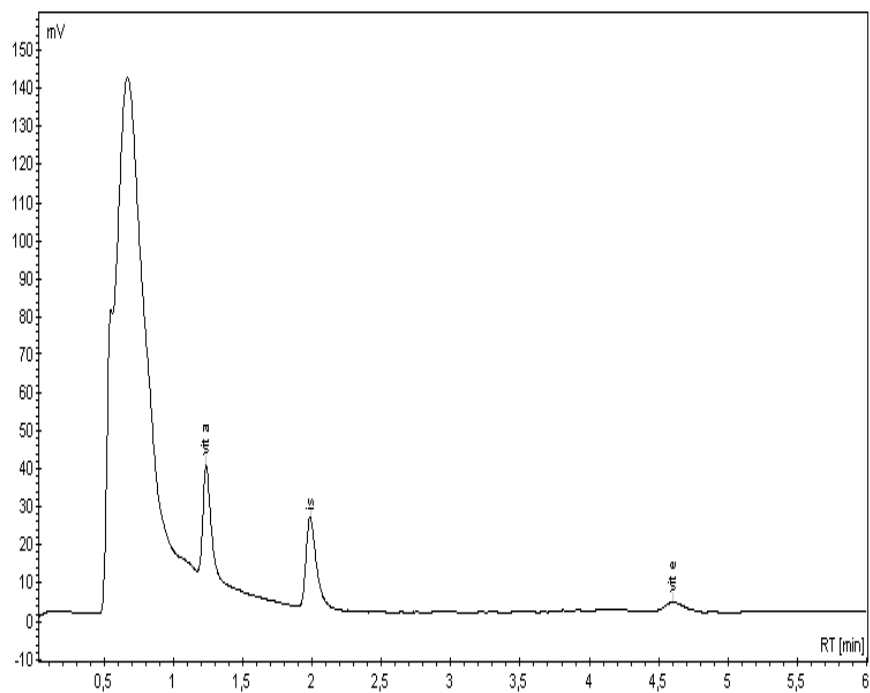


Fig. 3 :	Plasma Control Level 1		
	R.T. 1.2	Vitamin A	1,23 µg/ml
	R.T. 2.0	Internal Standard	
	R.T. 4.7	Vitamin E	4,64 µg/ml

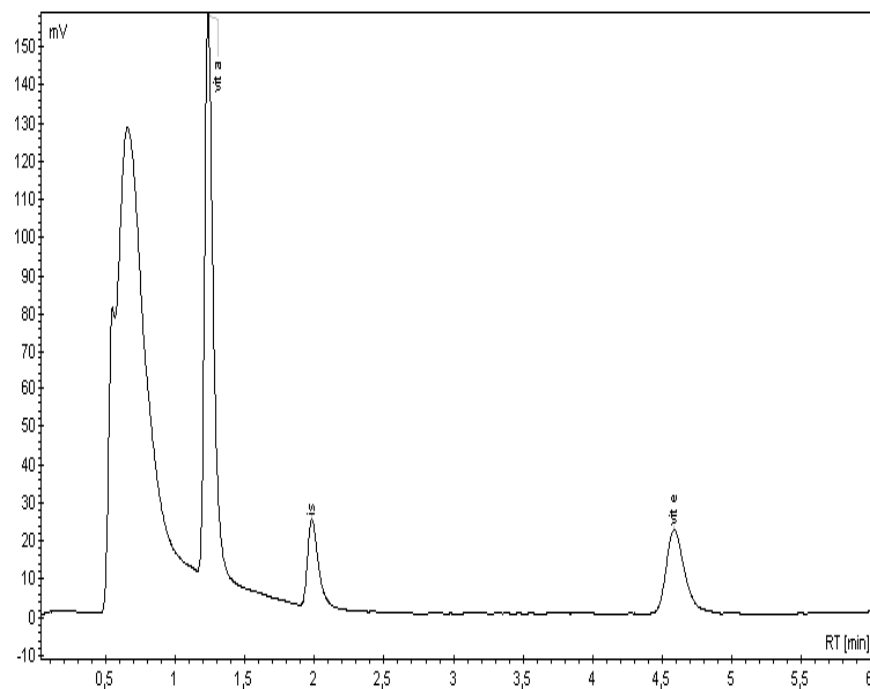


Fig. 4 :	Plasma Control Level 2		
	R.T. 1.2	Vitamin A	6,7 µg/ml
	R.T. 2.0	Internal Standard	
	R.T. 4.7	Vitamin E	42,2 µg/ml