

VITAMIN C IN PLASMA BY UV - FAST

Code Z20610





This product fulfills all the requirements of Directive 98/79/EC on in vitro diagnostic medical devices (IVD).

The declaration of conformity (CE) is available upon request.



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N° 002	Vitamin C in plasma by UV - FAST	06/2024
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INTRODUCTION

Vitamin C or L-ascorbate is an essential nutrient for higher primates, and a small number of other species. The presence of ascorbate is required for a range of essential metabolic reactions in all animals and in plants and is made internally by almost all organisms, humans being one notable exception. It is widely known as the vitamin deficiency causes scurvy in human. It is also widely used as a food additive.

The pharmacophore of Vitamin C is the ascorbate ion. In living organisms, ascorbate is an antioxidant, as it protects the body against oxidative stress, and is a cofactor in several vital enzymatic reactions.

Vitamin C is purely the L-enantiomer of ascorbate; the opposite D-enantiomer has no physiological significance. Both forms are mirror images of the same molecular structure. When L-ascorbate, which is a strong reducing agent, carries out its reducing function, it is converted to its oxidized form, L-dehydroascorbate. L-dehydroascorbate can then be reduced back to the active L-ascorbate form in the body by enzymes and glutathione.

L-ascorbate is a weak sugar acid structurally related to glucose which naturally occurs either attached to a hydrogen ion, forming ascorbic acid, or to a metal ion, forming a mineral ascorbate.

In human beings, Vitamin C is a highly effective antioxidant, acting to lessen oxidative stress, a substrate for ascorbate peroxidase, as well as an enzyme cofactor for the biosynthesis of many important biochemicals.

TECHNICAL FEATURES

PRINCIPLE OF THE METHOD

This method determinates the plasmatic concentration of **VITAMIN C**. Deproteinizer is added to an aliquot of plasma (150 µl) which has been treated with a suitable reducing agent and after centrifugation, 10 µl are injected into HPLC.

RECOVERY:	103,4 %		
SENSITIVITY (LLOD):	0,033 mg/l		
MINIMUM CONCENTRATION ANALYZABLE (LLOQ):	0,1 mg/l		
LINEARITY:	0,1 – 200 mg/l		
NORMAL RANGE:	5 – 15 mg/l		
<u>Accuracy intra serie (relative error %) :</u>	Ci	Cs	
	2 mg/l	12 mg/l	
	2,65%	2,73%	
<u>Accuracy inter serie (relative error %) :</u>	Ci	Cs	
	2 mg/l	12 mg/l	
	2,81%	3,01%	
<u>Reproducibility intra serie (coefficient of variation %):</u>	C LLOQ	Cm	CUP
	0,1 mg/l	7 mg/l	20 mg/l
	2,91%	1,54%	0,65%
<u>Reproducibility inter serie (coefficient of variation %):</u>	C LLOQ	Cm	CUP
	0,1 mg/l	7 mg/l	20 mg/l
	4,57%	4,32%	2,3%
<u>Coefficient of Correlation R2 + Dev Std:</u>	0,9991 ± 0,0002		

COMPONENTS OF THE KIT (100 TESTS)

Reagent A – Deproteinization Solution	1 x 50 ml	
Reagent B – Reducing Solution	6 x 100 mg	To reconstitute with 5 ml of Reagent F; reconstituted is stable 20 days at 2-8 °C
Reagent C – Internal Standard Solution	1 x 5 ml	
Reagent D – Test Solution	1 x 10 mg	
Calibrator in plasma	1 x 1 ml	Code Z20016 (Packed separately – see data sheet)
Reagent F – Reconstitution Solution	1 x 30 ml	
Reagent M – Mobile Phase	3 x 500 ml	

All the components are ready-to-use, except for **Reagent B** and **Reagent D**, stable 3 years at 2 -8 °C. As regards the preservation method of the Lyophilized Calibration Standard, it is described in the dedicated technical sheet.

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	CONFEZIONE
Z20016	Calibrator in plasma for Vitamin C	4 x 1 ml
Z20019	Control in plasma for Vitamin C – Levels 1 and 2	2 x 5 x 1 ml
Z20611	Mobile Phase	4 x 500 ml
Z699975902	Poroshell 120 EC C18 (50 x 4,6mm –2,7 u) 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 ul	1 x 100 Pcs
S51820717	Caps for Clear glass vials with reduced volume from 1,5 ml to 15 ul and for amber vials	1 x 100 Pcs

MINIMUM INSTRUMENTAL EQUIPMENT REQUIRED

Isocratic HPLC System with loop of 10 μ l
Spectrophotometric Detector UV $\lambda = 270 \text{ nm}$
Chromatograms Recorder

OPTIONAL EQUIPMENT

Autosampler
Operational Computer

PLASMA COLLECTION PROCEDURE

Tube with anticoagulant Plasma Citrate or Plasma Heparin or Serum
Add at separated plasma an aliquot of **Reagent B**, following this scheme:

- 900 μ l of Plasma
- 100 μ l of reconstitute **Reagent B**

So treated the sample is stable 1 month at -20°C and 2-3 days at $2-8^{\circ}\text{C}$.

PREANALYTICAL PROCEDURE

STEP 1

Reconstitution of Reagent B – Reducing Solution

Add to a bottle of Reagent B – Reducing Solution 5 ml of Reagent F – Reconstitution Solution.

Reagent B – Reducing Solution so reconstituted is stable 20 days at 2-8 °C

STEP 2

Test Solution Preparation at about 100 mg/l

Add to a bottle of Reagent D – Test Solution 9 ml of H₂O HPLC grade and 1 ml of Reagent B –Reducing Solution (after reconstitution).

This Solution is stable about 2 months at – 20 °C

Pipette in a tube :

- 900 µl of H₂O HPLC grade
- 100 µl of Reagent D – Test Solution previously prepared

This is a Test Solution at about 100 mg/l.

Vortex for 10 seconds

INJECTION :

- Inject 10 µl of this solution in the chromatographic system.

Verify that the Test Solution has retention time similar to fig. 1. If the Test is all right you can start with the analytical procedure; if not, check the functionality of the analytical system.

Important : Don't use this solution to calibrate!

ANALYTICAL PROCEDURE

STEP 1

Samples Preparation

N.B.: Calibrator and Controls have to be beforehand treated with Reagent B – Reducing Solution like indicated below

Reconstitution of lyophilized calibrators and controls: add exactly 1 ml of HPLC grade H₂O, put the cap on and mix by inversion. Let it sit for 5-10 minutes. Take a volume of 900 µl and put it into a tube, add 100 µl of Reagent B - Reducing Solution (after reconstitution). Mix well and wait 5 minutes.

Pipette in a tube:

	Calibrator	Control	Sample
Calibrator	150 µl		
Control		150 µl	
Sample			150 µl
Reagent B – Reducing Sol. (reconstituted as below)	10 µl	10 µl	10 µl
Reagent C – Internal Standard Sol.	50 µl	50 µl	50 µl
Reagent A – Deproteinization Sol.	300 µl	300 µl	300 µl

Warning: deproteinize directly on Vortex for at least 10 seconds

STEP 2

Centrifuge at 14.000 rpm for 10 minutes

Take the supernatant and put in a vial

N.B.: at this step, the sample is stable 1 day at 2-8 °C

INJECTION :

- Inject 10 µl of this solution in the chromatographic system.

VITAMIN C IN PLASMA - Warnings

SPECTROFOTOMETRIC DETECTOR PARAMETERS

λ	270 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 Cofilters (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.

And it is possible to make analysis at recycling phase, as long as you have the foresight to filter the same mobile phase with 0.22 μ filter before each analytical run. The signal that the mobile phase must be changed is given by the significant increase of the background noise of the detector Fluorimetric. If room temperature is > 20 °C store the Mobile Phase at 2-8 °C between an analytical session and another.

COLUMN CLEANING

Disconnect the detector. Flux 15 ml of **H₂O : Acetonitrile or Methanol (80 : 20 v / v)** set flow of 1,0 ml/minute and discharge. Flux a solution of **H₂O : Acetonitrile or Methanol (30 : 70 v / v)** for 30 minutes discharge it. **It is recommended to perform the washing of Analytical Column at the end of each analytical run.** Store the column in a solution of **H₂O : Acetonitrile or Methanol (30 : 70 v / v)**.

INJECTION NEEDLE WASHING

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

HPLC PARAMETERS

LOOP	10 μ l
FLOW	0,9 ml/min.
PRESSURE	About 150 bar

VITAMIN C IN PLASMA BY UV

(Reference Chromatograms)

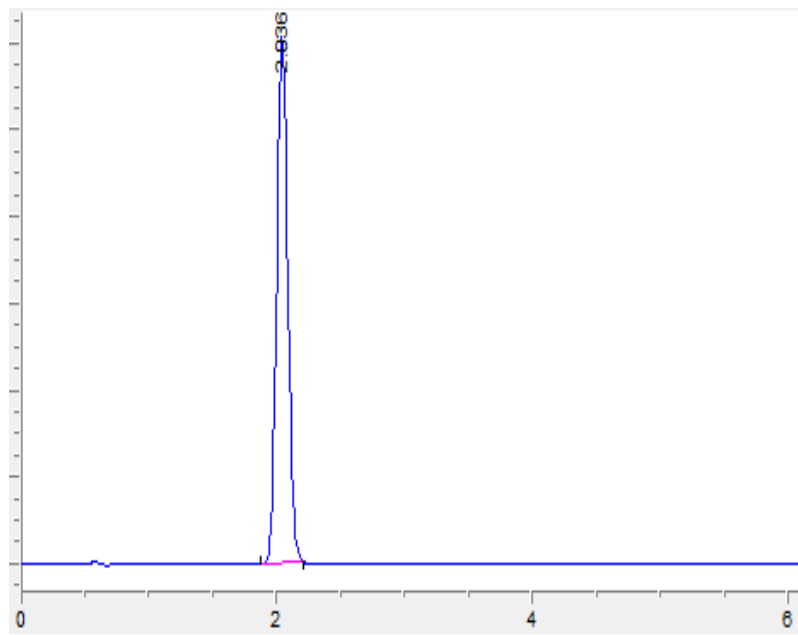


Fig. 1 :	Test Solution
	R.T. 2.036 Vitamin C

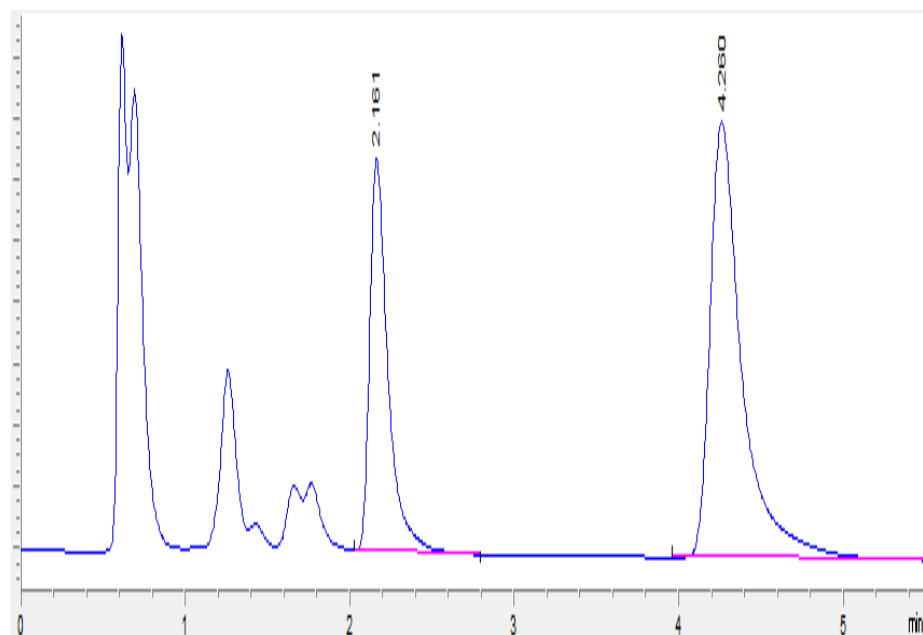


Fig. 2 :	Enriched Plasma sample
	R.T. 2.161 Vitamin C 7 mg/l
	R.T. 4.260 Internal Standard

VITAMIN C IN PLASMA BY UV

(Reference Chromatograms)

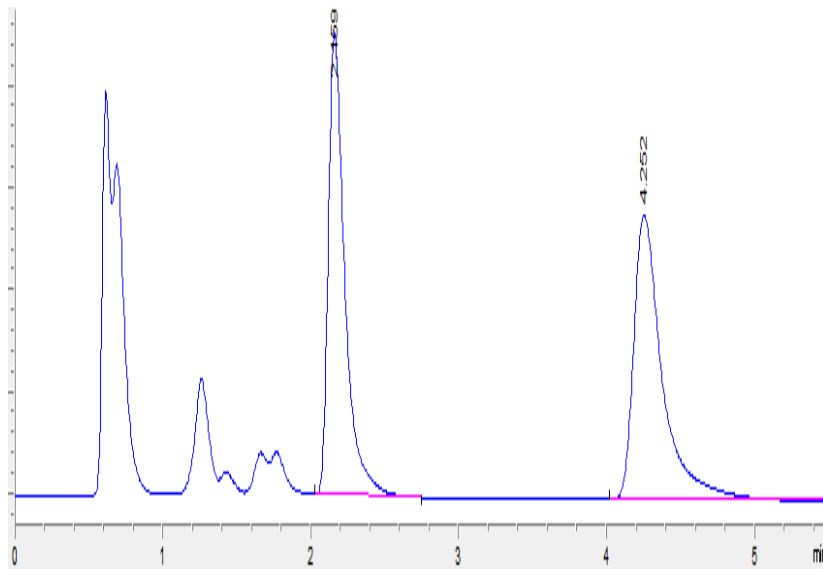


Fig. 3 :	Enriched Plasma sample
	R.T. 2.159 Vitamin C 12 mg/l
	R.T. 4.252 Internal Standard

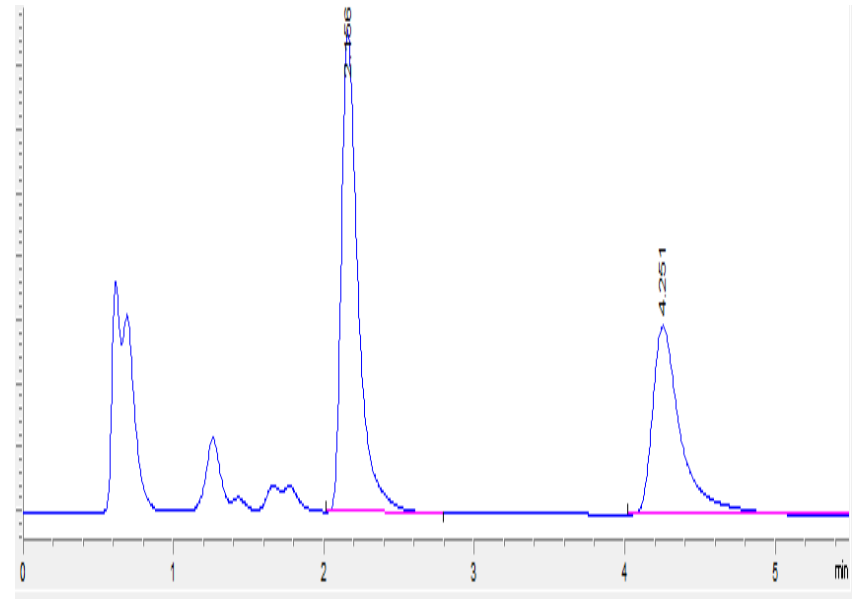


Fig. 4 :	Enriched Plasma sample
	R.T. 2.166 Vitamin C 20 mg/l
	R.T. 4.251 Internal Standard