

3-NITROTYROSINE IN PLASMA BY UV/VIS – FAST – Code Z61010

BIOCHEMISTRY

3-nitrotyrosine is a product of tyrosine nitration mediated by reactive nitrogen species such as peroxyxynitrite anion and nitrogen dioxide. It is found in many pathological conditions and is considered as a marker of oxidative stress dependent on Nitric Oxide. Nitrotyrosine is found in many tissues affected by diseases, such as the cornea and keratoconus. It is most likely involved in the process of pathogenesis of diabetes.

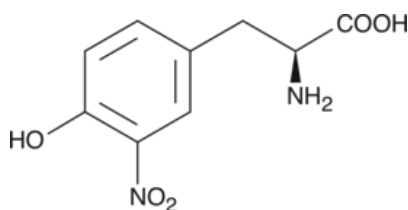


Fig. 1 3-Nitrotyrosine

Is then identified as an indicator of cell damage and inflammation, as well as an index of NO production. It is believed that measuring the concentration of nitrotyrosine serves as a marker for the damage caused by NO in the cell. Previous studies have shown that nitrotyrosine is found in inflammatory conditions such as atherosclerotic plaques and rheumatoid arthritis.

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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° 006	3-Nitrotyrosine in plasma - FAST	10/2024
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TECHNICAL FEATURES

Principle of the Method :

This method is used to determine plasma concentrations of **3-Nitrotyrosine**. To an aliquot of plasma is added a reagent Precipitation and after the addition of a reducing agent, the sample is transferred into the test tube and injected into HPLC.

Recovery : >98%

Analytical Sensitivity (LLOD): 30 µg/L

Minimum concentration analyzable (LLOQ) : 50 µg/L

Linearity : 50 – 7.500 µg/L

Reference Values : 50 – 100 µg/L

Accuracy intra serie (relative error %):

Ci	Cs
80 µg/L	500 µg/L
11,7%	1,1%

Accuracy inter serie (relative error %):

Ci	Cs
80 µg/L	500 µg/L
13,1%	3,5%

Reproducibility intra serie (coefficient of variation %):

CI	Cm	Cs
50 µg/L	250 µg/L	1000 µg/L
14,7%	4,2%	3,0%

Reproducibility inter serie (coefficient of variation %):

CI	Cm	Cs
50 µg/L	250 µg/L	1000 µg/L
15,0%	11,3%	6,7%

Coefficient of correlation R² + Dev Std 0,9963 ± 0,0011

Components of the kit :

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

Reagent A – Deproteinization Solution with Internal Standard, **1 x 30 ml**

Plasma Calibrator, **5 x 1 ml**

Code Z61016 (packed separately – see data sheets)

Reagent C – Test Solution, **1 x 5 ml**

See Warnings

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

Minimum Instrumental equipment required:

Isocratic HPLC System with loop of 10 μ l
Spectrophotometric Detector UV/VIS $\lambda = 232 \text{ nm}$

Blood Collection Procedure :

Add to sample an antioxidant, preferably BHT 4mM
in quantities 1:10.

Pre-analytical Phase :

If you are not analyzed immediately, freeze the
sample at -20 °C. Stable for 1 month.
Stable 1 week at 2-8 °C.

PREANALYTICAL PROCEDURE

Pipette in a tube :

- 280 µl of **Reagent A – Deproteinization Solution with Internal Standard**
- 200 µl of **H₂O HPLC grade**
- 200 µl of **Reagent C – Test Solution**

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

Dispense in a tube:

	Calibrator	Sample
Calibrator	200 µl	
Sample		200 µl
Reagent A – Deproteinization Sol. with Internal Standard	280 µl	280 µl

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

Centrifuge at 10000 rpm for 5 minutes

Vortex for 20 sec.

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

INJECTION :

- Inject 10 µl of supernatant in the chromatographic system.

3-NITROTYROSINE in PLASMA - FAST – Warnings

REAGENT C : TEST SOLUTION

3-Nitrotyrosine	About 1000 µg/L
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SPECTROPHOTOMETRIC DETECTOR PARAMETERS

λ	232 nm
GAIN	0,001 AUFS

HPLC COLUMN PROTECTION

To save the analytical column Reverse Phase POROSHELL EC C18 (50 x 4,6 mm – 2,7 µm), the use of Javelin Prefilters (1 x 10 pcs), code S90199511 is obligatory.
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HPLC COLUMN CONDITIONING

Install a <u>new</u> analytical column Reverse Phase POROSHELL EC C18 (50 x 4,6 mm – 2,7 µm). Disconnect the detector and flux 30 ml of a solution of H₂O : Methanol (20 : 80 v/v) set flow at 1,0 ml / min. Don't recycle the washing solutions. Flux 30 ml of a solution of H₂O : Methanol (80 : 20 v/v) set flow at 1,0 ml / min. Condition the column with the mobile phase at a flow of 1ml / min. discharge first 20 ml. Condition further on the column for 30 min. also at recycling phase. Finally inject the derivatized Chemical Standard and verify the quality of the HPLC run. It is 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

Disconnect the detector. Flush 15 ml of H ₂ O: methanol or Acetonitrile (80: 20 v/v) and set flow at 1,0 ml / min. Flush a solution of H ₂ O: methanol or Acetonitrile (70: 30 v/v) for 30 minutes for download. When the column will be used again, pass 15 ml of H ₂ O: methanol (20: 80 v/v) before made conditional with the Mobile phase. It is recommended to wash the analytical column at the end of each session. The column is stored in a solution of H₂O : Acetonitrile or Methanol (30 : 70 v / v)
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INJECTION NEEDLE WASH

Wash in a solution of H ₂ O / Ethanol or Methanol or Acetonitrile (80 : 20 v/v).

HPLC PARAMETERS

LOOP	10 µl
RECOMMENDED FLOW	1,0 ml/min
PRESSURE	About 120 bar

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z61016	Calibrator for 3-Nitrotyrosine in plasma	4 x 1 ml
Z61019	Control for 3-Nitrotyrosine in plasma – Levels 1 and 2	2 x 5 x 1 ml
Z699975902	Poroshell 120, EC-C18 (50 x 4,6 mm - 2,7 µm) Analytical Column	1 Pc
S90199511	Javelin Colfilters	1 x 10 Pcs
S51843550	Clear glass vials with reduced volume from 1,5 ml to 15 µl	1 x 100 Pcs
S51820717	Caps for Clear glass vials with reduced volume from 1,5 ml to 15 µl	1 x 100 Pcs

3-NITROTYROSINE IN PLASMA (Reference Chromatograms)

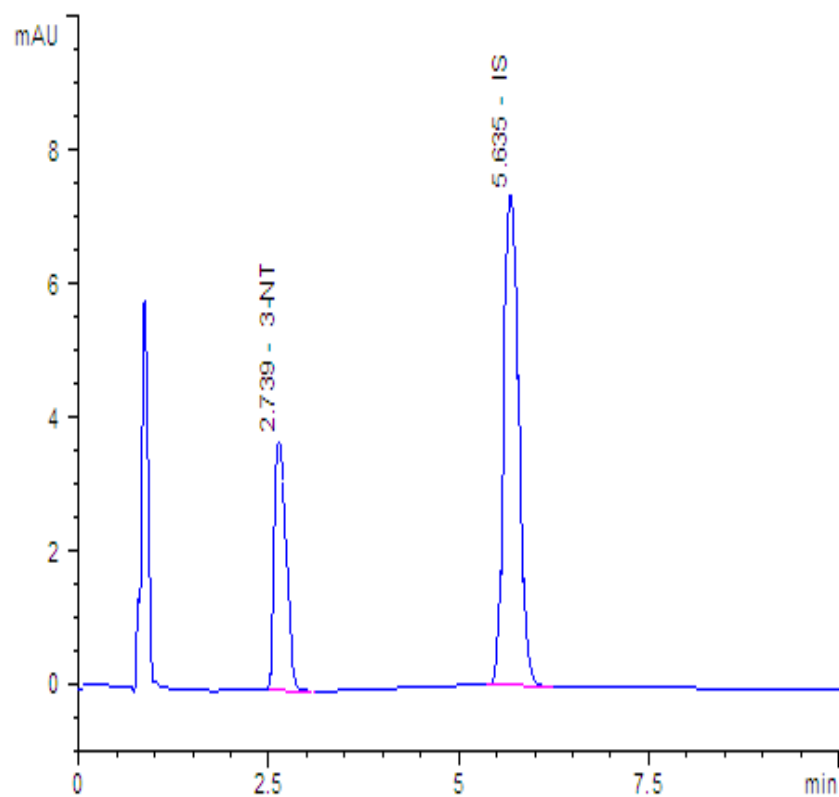


Fig. 1 :	Test Solution
	R.T. 2.739 3-Nitrotyrosine
	R.T. 5.635 Internal Standard

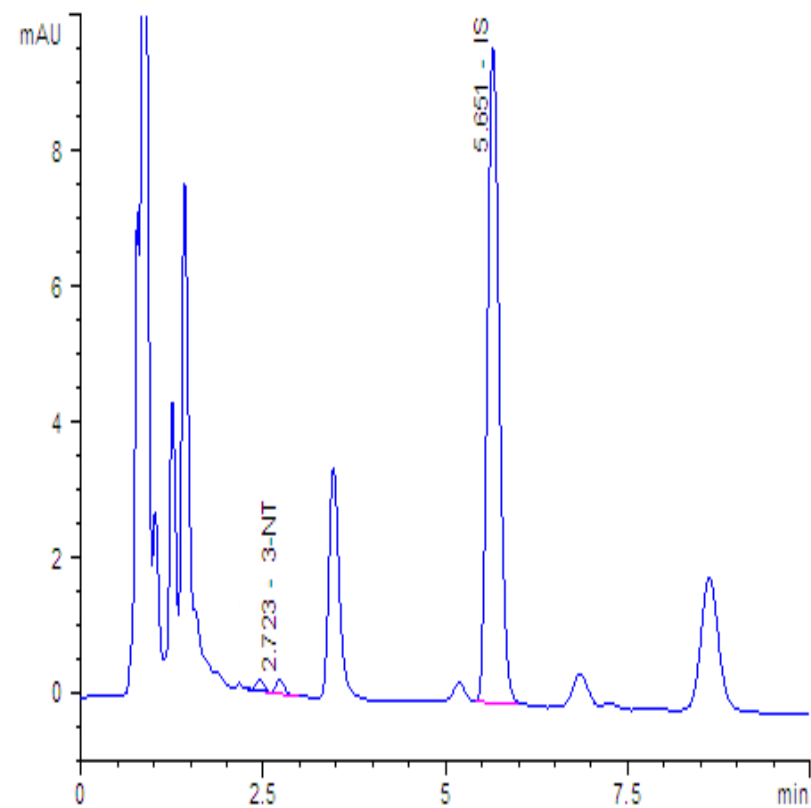


Fig. 2 :	Control Level 1
	R.T. 2.723 3-Nitrotyrosine 100 µg/L
	R.T. 5.651 Internal Standard