

HOMOCYSTEINE IN PLASMA IN LC/MS – 500 TESTS - CODE LC09015

INTRODUCTION

Homocysteine (HCY) is a sulfurated aminoacid that derives from the demethylation of **Methionine**. (Fig. 1)

HOMOCYSTEINE

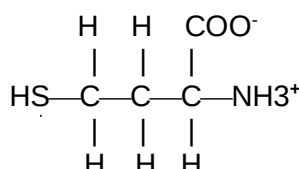


Fig. 1 : 2-Amino-4-Mercaptobutyric acid

It does not take part in the proteins synthesis. It is present in the bloodstream above all as disulfuric compounds such as **HCY-HCY (homocystine)** and **HCY-Cysteine**. These molecules can be free or bound to proteins.

BIOCHEMISTRY

It derives from **Methionine**, which is an essential aminoacid. Methionine is converted to **S-adenosylhomocysteine**, and then hydrolyzed in homocysteine.

Homocysteine can be involved in two different metabolic pathways:

- 1) Homocysteine can be methylated again to **Methionine** by the **5-10 methylene tetrahydrofolate reductase enzyme** (5-10-Metil-THF) This reaction needs **B₁₂ Vitamin** as cofactor and **N-methyl tetrahydrofolate** as donor of -CH₃ groups.
- 2) **Homocysteine** can be linked to **Serine** and then can be converted first in **Cystathionine** and then cysteine by the **Cystathionine β-synthetase** enzyme. This reaction needs **B₆ Vitamin** as cofactor.

Hyperhomocysteinemia can be caused by enzymatic impairment of one of these pathways. The impairment can be:

- **total**, when the enzymatic dysfunction has an **homozygote** heritage
- **partial**, when the enzymatic dysfunction has an **heterozygote** heritage

The incidence of homozygote **hyperhomocysteinemia** is estimated to be around 1:200.000. These patients have markedly increased **HCY** levels markedly increased (sometimes over 400 μM/l). Atherosclerotic cerebrovascular diseases and venous thrombosis are often present in this syndrome.

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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° 004	Homocysteine in plasma by LC/MS	09/2024
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Mudd et al (1985) published the results of an international survey on clinic manifestation and natural evolution of 629 patients affected by deficit of cystathionine β -synthetase enzyme. Thromboembolic complications were present in 158 patients (25%). These patients had 253 thrombotic events as following:

- 51% deep venous thrombosis
- 32% acute cerebrovascular events
- 11% arterial peripheral obstructions
- 4% myocardial infarctions.

Vascular thrombotic diseases are the most common causes of premature death in patients with high HCY. It has been demonstrated that high HCY is an independent factor of early coronary illness in man. Boushey et al. has recently demonstrated that the increase of 5 μ M/l of HCY in plasma levels or the increase of 20 mg/dl of cholesterol has the same effect on cardiovascular risk.

Moderate hyperhomocysteinemia (heterozygote heritage) can determine only a slight increase of the fasting level of HCY. However, this condition can be better identified by an oral methionine load that can evidences a strong increase of homocysteine plasma levels respect to healthy subjects.

Intermediate hyperomocysteinemia effects 5-7% of the Caucasian population and it is defined as an independent cardiovascular risk factor. It is potentially dangerous as few symptoms are present until the thrombotic event.

Folates, B₁₂ and B₆ Vitamins intake can be successfully used in different forms of hyperhomocysteinemia. For this reason an early diagnosis is fundamental to avoid the risk of cardiac and cerebrovascular thrombotic diseases.

This method allows you to analyze the plasma homocysteine by LC/MS/MS.

TECHINICAL FEATURES

Principle of the Method:

Homocysteine is analyzed by treating the plasma with a suitable reducing agent. After deproteinization, the solution is directly injected into the LC / MS.

Recovery:

>98 %

Sensitivity (LLOD) :

1 µmol/l

Minimum Concentration analyzable (LLOQ) :

0,7 µmol/l

Linearity:

1-1007,46 µmol/l

Therapeutic Range in serum :

5 – 15 µmol/l

Accuracy intra serie (relative error %) :

Ci	Cs	
14,5 µmol/l	32,5 µmol/l	
4,9%	1,0%	

Accuracy inter serie (relative error %) :

Ci	Cs	
14,5 µmol/l	32,5 µmol/l	
6,8%	4,6%	

Reproducibility intra serie (relative error %) :

C LLOQ	Cm	Cs
7,5 µmol/l	17,5 µmol/l	37,5 µmol/l
2,4%	3,4%	2,6%

Reproducibility inter serie (coefficient of variation %):

C LLOQ	Cm	Cs	
7,5 µmol/l	17,5 µmol/l	37,5 µmol/l	
4,1%	3,6%	3,1%	

Coefficient of Correlation + DS :

0,9863 + 0,0014

Components of the kit (500 tests):

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

Reagent A – Reducing Solution, 12 x 77 mg

Reagent B – Buffer Solution, 1 x 15 ml

Reagent C – Internal Standard Solution, 5 x 2 ml

Store at – 20 °C

Reagent D – Deproteinization Solution, 1 x 100 ml

Plasma Calibrator – Level 1, 3 x 1 ml

Code LC09016 (packed separately – see data sheet)

Plasma Calibrator – Level 2, 3 x 1 ml

Code LC09016 (packed separately – see data sheet)

Plasma Calibrator – Level 3, 3 x 1 ml

Code LC09016 (packed separately – see data sheet)

Plasma Calibrator – Level 4, 3 x 1 ml

Code LC09016 (packed separately – see data sheet)

Plasma Calibrator – Level 5, 3 x 1 ml

Code LC09016 (packed separately – see data sheet)

Reagent F – Stabilization Solution, 1 x 50 ml

Reagent M1 – Mobile Phase M1, 3 x 500 ml

Reagent M2 – Mobile Phase M2, 5 x 500 ml

Minimum Instrumental equipment required:

LC-MS triple quadrupole
MRM, ESI positive

Optional Equipment:

Autosampler

Plasma Collection Procedure:

Collect 3 ml of blood with sodium citrate.
Centrifuge **immediately** at 4,000 rpm for 5 min.
Plasma samples should be stored at **-20° C** for
no longer than **1 month**.

ANALYTICAL PROCEDURE

Reconstitution of **Reagent A**

- Add to **Reagent A – Reducing Sol.** 1 ml of **Reagent B – Buffer Sol.**

Stable 2 days at 2-8 °C

STEP 1 :

Put in eppendorf.

	Calibrator – Level 1	Calibrator – Level 2	Calibrator – Level 3	Calibrator – Level 4	Calibrator – Level 5	Sample	Controls
Calibrator – Level 1	100 µl						
Calibrator – Level 2		100 µl					
Calibrator – Level 3			100 µl				
Calibrator – Level 4				100 µl			
Calibrator – Level 5					100 µl		
Sample						100 µl	
Controls							100 µl
Reagent C – Internal Std Sol	20 µl	20 µl	20 µl	20 µl	20 µl	20 µl	20 µl
Reagent A – Reducing Sol (after reconstitution)	20 µl	20 µl	20 µl	20 µl	20 µl	20 µl	20 µl

Vortex for 20 sec.

STEP 2: Let rest at Room Temperature (>20°C) for 15 minutes

STEP 3: Add 200 µl of **Reagent D – Deproteinization Solution** directly on vortex for 20 seconds

STEP 4: Centrifuge at 14.000 rpm for 10 minutes

STEP 5: Take 30 µl of supernatant and add 70 µl of **Reagent F – Stabilization Solution**

Vortex for 20 seconds

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

INJECTION :

- Inject 10 µl of solution in LC system

HOMOCYSTEINE IN PLASMA - Warnings

COLUMN CONDITIONING

Install a new analytical SUPELCOSIL LC-CN (33 x 4.6mm, 3um). Disconnect the detector and flush a solution of Acetonitrile : H₂O (95 : 5 v/v) set flow at 0,8 ml / min for 15 minutes. **Don't recycle the washing solutions.** Condition further on the column with a solution of Mobile Phase M2 : H₂O (60 : 40 v/v) set flow at 0,8 ml / min for 15 minutes and discharge first 3 ml. also at recycling phase. Finally inject the Chemical Standard and verify the quality of the HPLC run.

It is NOT possible to make analysis at recycling phase.

If Room Tem. is > 20° C is better to preserve the Mobile Phase at 2 – 8° C among runs.

COLUMN CLEANING

Wash the column with a solution of Mobile Phase M2 : H₂O (95 : 5) at flow of 600 ul / minutes for 15 minutes. Store the column in a solution of Mobile Phase M2 : H₂O (95 : 5).

AUTOSAMPLER SYRINGE CLEANING

Wash the syringe, before each analytical session, with Methanol. Wash the autosampler needle intra-series with Methanol.

FLOW SET

GRADIENT			
Time (min)	% M1 (PUMP A)	% M2 (PUMP B)	FLOW (µl/min)
0	40	60	800
2.00	40	60	800

ANALYTE	PARENT ION
Homocysteine	136.0
Homocystine-D8 (Internal Standard)	140.0

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
LC09016	Calibrator in serum for Homocysteine	5 x 2 x 1 ml
LC09019	Control in serum for Homocysteine – Levels 1 and 2	2 x 5 x 1 ml
S51843550	Clear glass vials with reduced volume 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	1 x 100 Pcs
S58979	SUPELCOSIL LC-CN (33 x 4.6mm, 3um) Analytical Column	1 Pc
SK09010	Starter kit for Homocysteine	2 x 1 ml

HOMOCYSTEINE IN PLASMA (Reference Chromatograms)

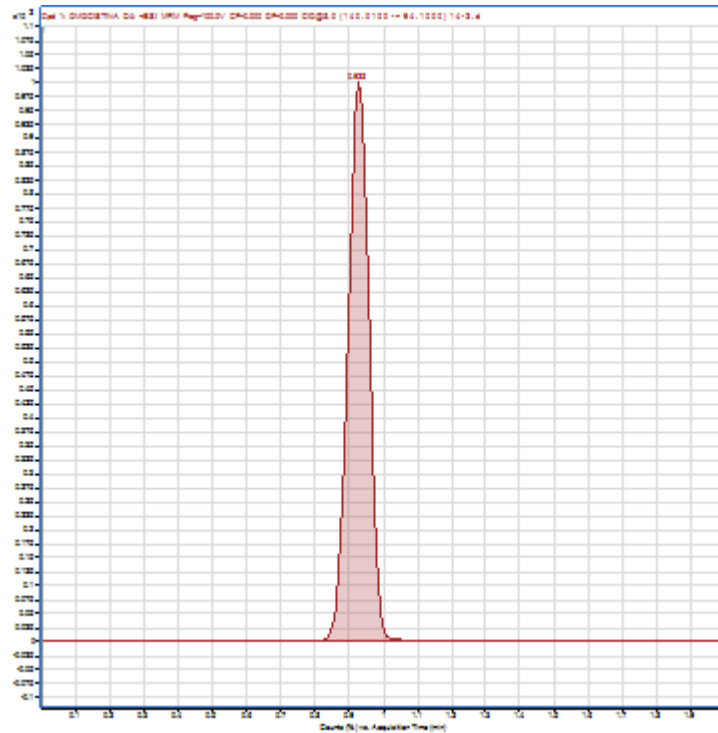


Fig. 1 :	Calibrator Level 1
R.T.	IS

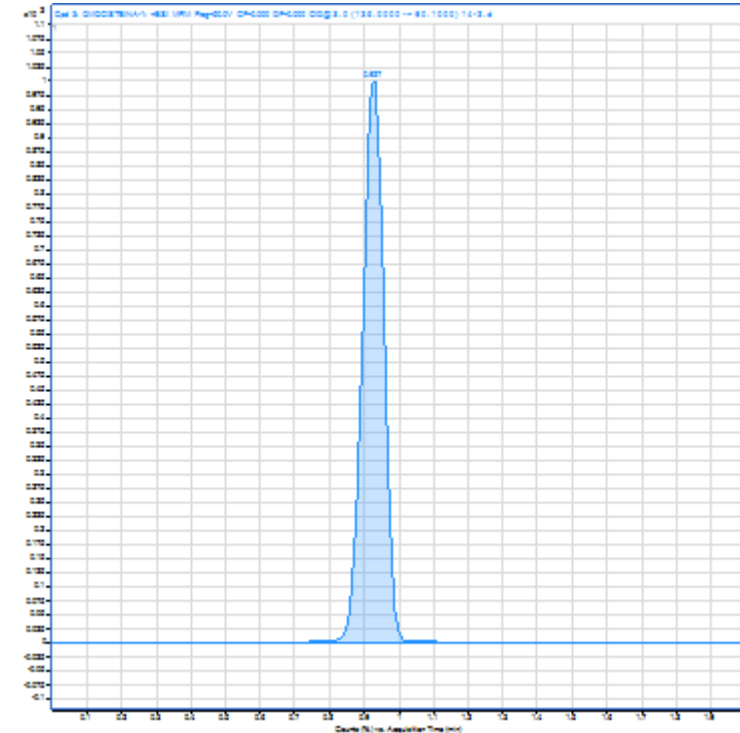


Fig. 2 :	Calibrator
R.T.	Homocysteine 7 umol/L

HOMOCYSTEINE IN PLASMA (Reference Chromatograms)

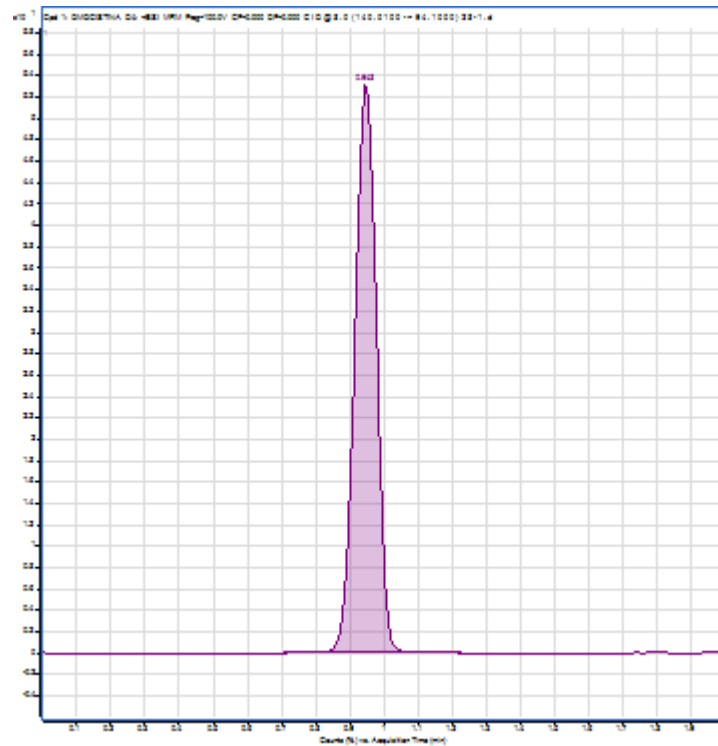


Fig. 3 :	Calibrator Level 3
R.T.	IS

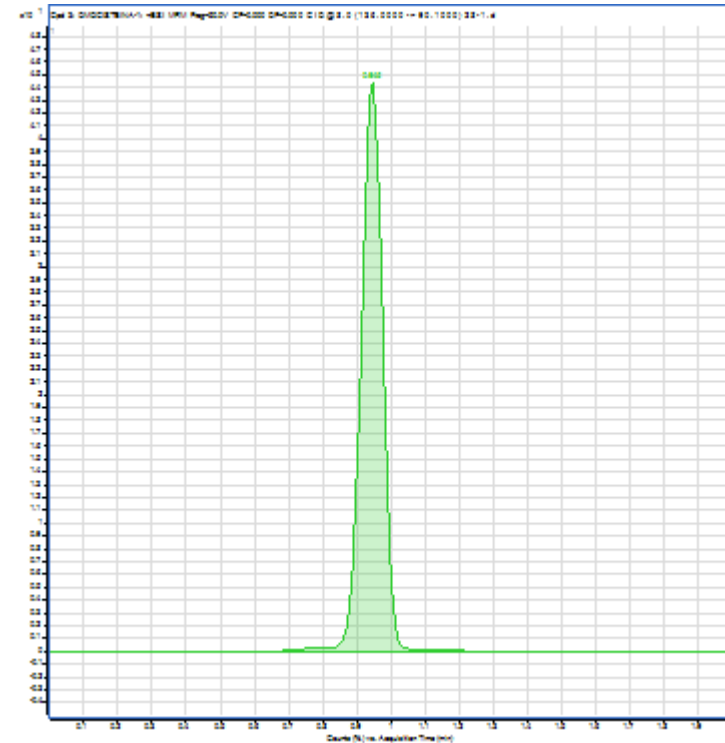


Fig. 4 :	Calibrator
R.T.	Homocysteine 18 umol/L