Determination of Homocysteine in plasma using HPLC and Electrochemical Detection

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Introduction

Homocysteinaemia is universally recognised as an independent risk factor for arteriosclerosis and coronary heart disease. Increases of 10-15 % in the level of Homocysteine raises the risk of coronary heart disease and cerebral vascular incident by approximately 3 to 4 fold. Homocysteine is an important sulphur containing amino acid in the metabolism of Methionine and Cysteine. Methionine takes part in the syntheses of adrenaline, amino acids and fosfolipides.

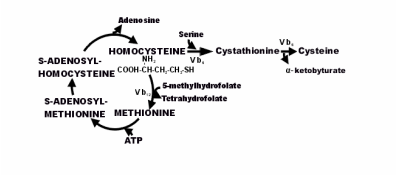

Fig. 1. Methionine metabolism

The aim of the study was to determine Homocysteine in human plasma using reversed phase HPLC with electrochemical detection. Assay parameters such as linearity, detection limit , reproducibility and recovery are determined.

Experimental

HPLC	Gynkotek M 480 pump, Degasys DG-1310 Degasser (Degasys, Japan), Triathlon autosampler (Spark Holland, the Netherlands)
Column	Spherisorb S3 ODS2, 100 x 4.6 mm, 3 µm
Flow rate	1000 µL/min
Mob. phase	0.15 M phosphoric acid (1%), 2 mM KCl, 10 mg/L 1-octanesulfonic acid (OSA), brought to pH 1.75 with 19.2 M NaOH
Injection	20 µL
Detector	DECADE (Antec Leyden)
Temperature	30 °C
Cell	VT-03 wall-jet flow cell with 3 mm gold electrode, spacer thickness 50 µm
E cell	600 mV vs. Ag/AgCl reference electrode (ISAAC)
I cell	8 nA

Standard and sample preparation

All the working standard solutions and plasma samples were diluted in mobile phase. The plasma samples were deproteinized with 10 % TCA Trichloroacetic Acid in 1 mmol/L EDTA. To liberate the conjugated Homocysteine, 10 % Tri-n-butylphosphine in DMF was used as a reducing agent.

Results

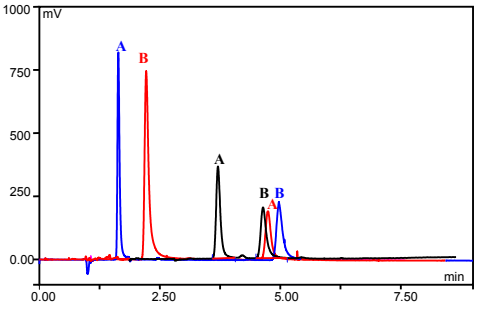

Fig. 2 Analysis of 100 nmol/L (13.5 µg/L) Homocysteine (A), 20 µg/L N-(2-Mercapto -propionyl) Glycine (α-MPG), internal standard (B); using mobile phase with different 1-octanesulfonic acid (OSA) concentrations. No OSA (–); 10 mg/L OSA (–); 500 mg/L OSA(–).

Calibration line 10-100 nmol/L Homocysteine in plasma was Y= 0.11 (±0.02) X + 0.04(±0.0004); R = 0.9985. The detection limit for this method is 5 fmol.

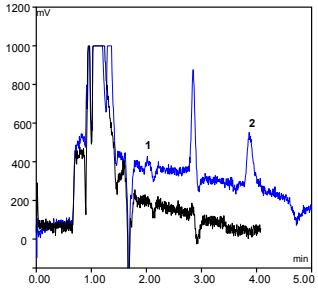

Fig. 3 Analysis of 5 fmol (0.027 µg/L) Homocysteine (1) in plasma, 0.4 µg/L α-MPG (2). Blanco (–), 5 fmol Homocysteine (–).

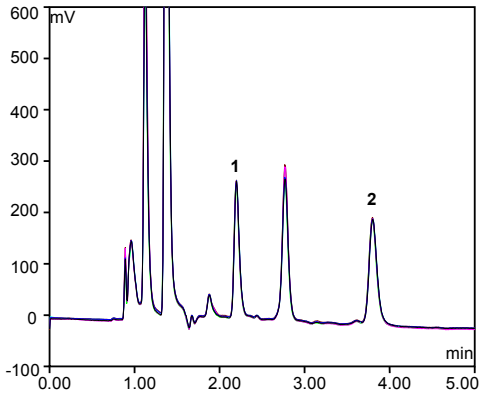

Fig. 4 Analysis of Homocysteine (1) in plasma, (2) α-MPG. Overlay of 6 chromatograms. The RSD of the height (nA) of the Homocysteine peaks is 1 %.

A pulse between 1 volt and -1 volt was introduced 5 minutes before every injection. The reproducibility was tested with four different Homocysteine concentrations, 40 , 60, 80 and 100 nmol/L . The recovery of 20 µmol/L Homocysteine standard in three plasma samples is 100%.

Conclusion

The developed method provides accurate, precise determination of Homocysteine in plasma. The analysis time is less then 12 minutes. A regular cleaning pulse of the gold working electrode before every run improves the performance of the cell. The relative standard deviation of the Homocysteine peak height is 1 %.

The detection limit is 5 fmol. Therefore very small quantities can be analysed. Diluting the plasma samples reduces the risk of contaminating the working electrode and results in a longer column life time.

References

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