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Homocysteine in plasma

Introduction

Homocysteine is a strongly oxidising and toxic product of amino acid metabolic pathways and raised blood levels (homocysteinaemia) are an established risk factor for arteriosclerosis and coronary heart disease. Increases of 10-15 % in the level of homocysteine raise the risk of coronary heart disease and cerebral vascular incidents approximately 3 to 4 fold. The current HPLC methods, relying on derivatisation of homocysteine and fluorescence detection, are laborious and the fluorescence label is quite expensive.
Therefore, a highly sensitive, economical, reproducible and rapid method for the analysis of homocysteine in plasma by isocratic reversed phase LC with electrochemical detection (EC) has been developed. The detection limit with standards is 0.3 nmol/L, RSD in peak heights is better than 1% and homocysteine is eluted within 5 minutes. Recovery from plasma samples is nearly 100%.

Method

About 70 % of homocysteine in plasma is bound to albumine, 30% is present as mono- or disulphide. Therefore, prior to determination of total homocysteine, disulphides are reduced and released from albumine by TBP (tri-n-butylphosphine). The internal standard N-(2-mercapto propionyl)glycine (2MPG, 50 µL of 16 mg/L), and 50 µL of TBP are added to 200 µL of plasma. After 30 min of incubation at 4 ºC 200 µL of trichloroacetic acid (TCA) is added, and vigorously shaken for 2 min. After incubation for 15 min at 4 ºC, the mixture is centrifuged at 10,000 RPM for 10 min. A volume of 100 µL of supernatant is diluted to 5 mL with mobile phase and analysed by LC-EC.
A working potential of +600 mV is applied. Prior to each run a cleaning pulse of +1 V and -1 V both of 1 s is applied, followed by 5 minutes stabilisation. The linearity has been checked using concentrations of 20, 40, 60, 80 and 100 nmol/L. The detector response is linear in this range with a regression coefficient better than 0.998 and with a detection limit of 0.3 nmol/L.

LC-EC conditions

Column	Spherisorb S3 ODS2, 4.6 * 100 mm, 3 µm
Flow rate	1 mL/min
Sample	20 µL
Mobile phase	0.15 mol/L phosphoric acid (1%), 2 mmol/L KCl, 10 mg/L OSA pH 1.75 (with NaOH).
Temperature	30°C
E-cell	600 mV (vs. ISAAC 2 mM KCl)
I-cell	1.90 nA

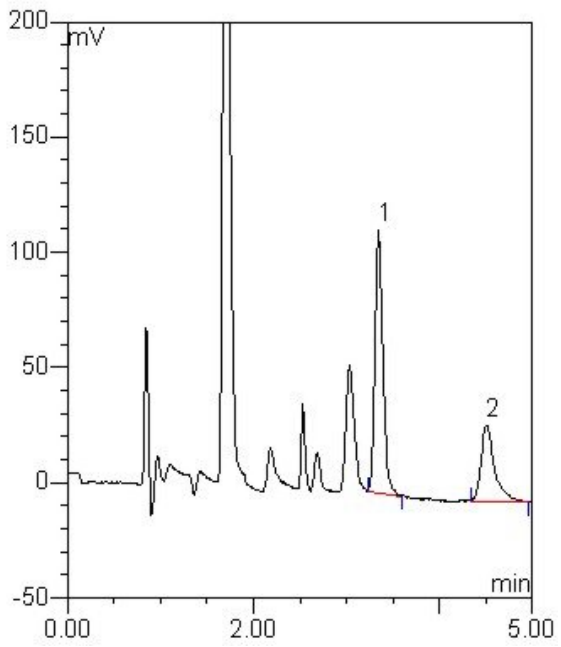

Fig. 1. Chromatogram of 39 µmol/L homocysteine (1) and 98 µmol/L 2MPG (2) in plasma. Range setting is 5 nA/V. Courtesy: J. v.d. Kolk, Bethesda hospital, Hoogeveen [1].

References

1. Determination of homocysteine, Standard operating procedure A.29 HCYS_1, Bethesda Hospital, Hoogeveen, The Netherlands
2. Nicola C. Smith, Mark Dunnet, Paul C. Mills, Journal of Chrom. 673 (1995), 35-41.
3. G.H.J. Boers, Mediator 8(2), March 1997, 9-11

Part numbers and configuration used

120.0035	DECADE EC detector
110.4225	VT-03 flow cell with 3mm Au WE and ISAAC REF

Configuration suggestions & comment

Because of the cleaning pulse required, only the DECADE II (p.n. 171.0035) can be used for this application.