CAPSULES
notes and applications from Bioanalytical Systems, Inc.
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Plasma Homocysteine

Purpose

Homocysteine (F1) is a naturally occurring amino acid, high levels of which have been associated with coronary artery disease [1,2]. It occurs as the free thiol (homocysteine), its disulfide (homocystine), and conjugated to other thiols and proteins through disulfide linkages.

$$HSCH_2CH_2CH(NH_2)COOH$$

Figure 1. Structure of homocysteine.

The bulk of plasma homocysteine (85%) occurs in conjugated form, rendering it inaccessible to common analytical techniques. Samples must therefore be treated with a reducing agent before analysis, to liberate homocysteine as the free thiol.

BAS has produced a kit for the determination of total plasma homocysteine. In contrast to existing procedures, the BAS kit uses a safe reducing agent that is effective at room temperature and does not cause annoying foaming of the samples. This proprietary chemistry results in samples that are stable at room temperature. Moreover, the BAS kit uses a maintenance-free gold electrode for LCEC determination of homocysteine.

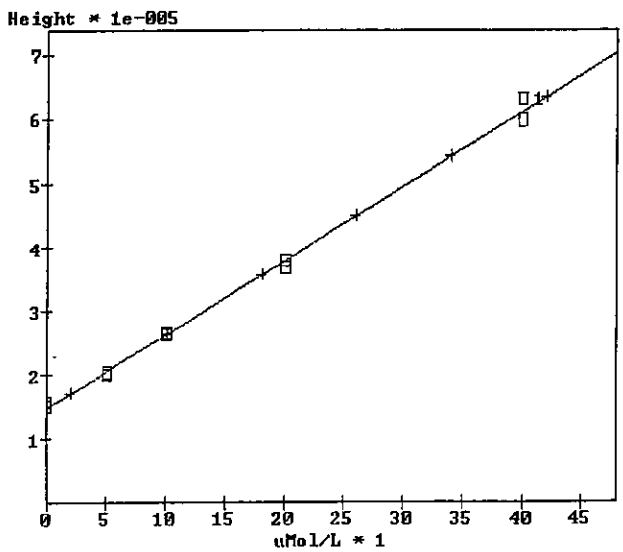

Figure 2. Linear regression of duplicate spiked plasma samples.

Existing Methods

Reduction of plasma with sodium borohydride or tri-*n*-butylphosphine. The first has to be heated to 50 °C, which causes foaming of the sample. The second can be explosive in it's concentrated form.

Detection at mercury/gold amalgam electrodes. These require intensive polishing and reamalgamation at scheduled intervals.

Derivatization with a fluorogenic reagent also is common, but many of the homocysteine derivatives are unstable.

Conditions

System: BAS-200B or BAS 480 Liquid Chromatograph with an EC detector and ChromGraph™ Control Software
Electrode: BAS Dual Gold
Potential: + 700 mV
Column: BAS 100 x 2 mm, C_{18}, MF-8957
Mobile Phase: BAS MP-4, CF-1301
Flow Rate: 0.4 mL/min
Autosampler: BAS Sample Sentinel
Linear Range: At least to 80 µmol/L

Sample Preparation

Human plasma samples are incubated for 30 minutes with the kit reagents. A protein-precipitating agent is added and the samples clarified by centrifugation. Aliquots (10 µL) of the supernatants are injected into the chromatograph.

Notes

Both LC systems are also used with our proprietary line of clinical chemistry kits (Plasma and Urinary Catecholamines and Urinary Metanephrines).

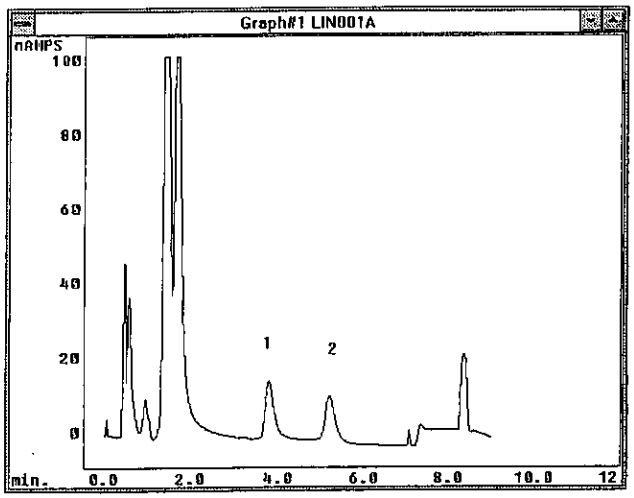

Figure 3. Separation of an unspiked plasma sample. Peak 1 = homocysteine. Peak 2 = cys-gly.

A calibration curve is presented in F2. Determination of homocysteine in biological materials requires the standard-addition method of calibration.

A typical chromatogram of an unspiked plasma sample is shown in F3.

Intra-day precisions of 1.5% are shown in T1. Inter-day precision was 2.1%

References

1. *Malinow, M.R., S.S. Kang, L.M. Taylor, P.W.K. Wong, B. Coull, T. Inahara, D. Mukerjee, G. Sexton, B. Upson, Circulation 79 (1989) 1180-1188.*
2. *Stampfer, M.J., M.R. Malinow, New Engl. J. Med. 332 (1995) 328-329.*

Table 1. Replicate samples on 5 days. Results in µmol/L plasma.

Date	*12 Jun*	*18 Jun*	*19 Jun*	*20 Jun*	*16 Jul*
	11.4	11.6	11.0	12.2	11.8
	11.5	11.7	11.6	11.8	11.8
	11.4	11.7	11.4	11.9	11.5
	11.7	11.7	11.5	12.3	11.8
	11.6	11.5	11.5	12.1	12.0
	11.6	11.7	11.2	11.9	11.6
	11.2	11.6	11.6	12.0	12.2
Mean	11.5	11.6	11.4	12.0	11.8
% RSD	1.5	0.6	2.1	1.4	1.8