Rapid Analysis of Homocysteine Levels

Aurélie Lolia and Sally Bee
Biochrom Ltd, Cambridge, UK

Using the Biochrom 30 Amino Acid Analyser, homocysteine levels can be measured using a rapid accurate programme.

Elevated plasma total homocysteine has emerged as an important risk factor in the development of vascular disease, so a defined, accurate method to monitor the levels of homocysteine is important for evaluating those patients at risk.

Many recent studies have established homocysteine as a risk factor for disease of the coronary, cerebral, and peripheral arteries, and for arterial and venous thromboemolism. Analysis of prospective studies indicates that elevated homocysteine levels account for a large fraction of coronary heart disease and stroke in the general population.

Using the Biochrom 30 Amino Acid Analyser, a program that reduces analysis time to 24 min injection to injection enables 60 samples to be analysed during a full day, thus allowing screening for homocysteine. The analysis is performed using a 20 cm x 4.6 mm physiological High Performance column and the standard lithium buffers.

The regeneration step of lithium hydroxide 0.3 M ensures that there is no carry-over between injections and therefore enables accurate quantification of homocysteine. Homocysteine is well resolved from methionine which is important when methionine loading tests are performed.

Norvaline, which elutes after methionine, is used as the internal standard as it doesn't interfere with any of the other amino acids occurring in the physiological standard.

Chromatogram: Physiological standard including Homocysteine (10 nmol/20 µL)

References:
www.homocysteine.net
Applications notes 44, 60 and 61

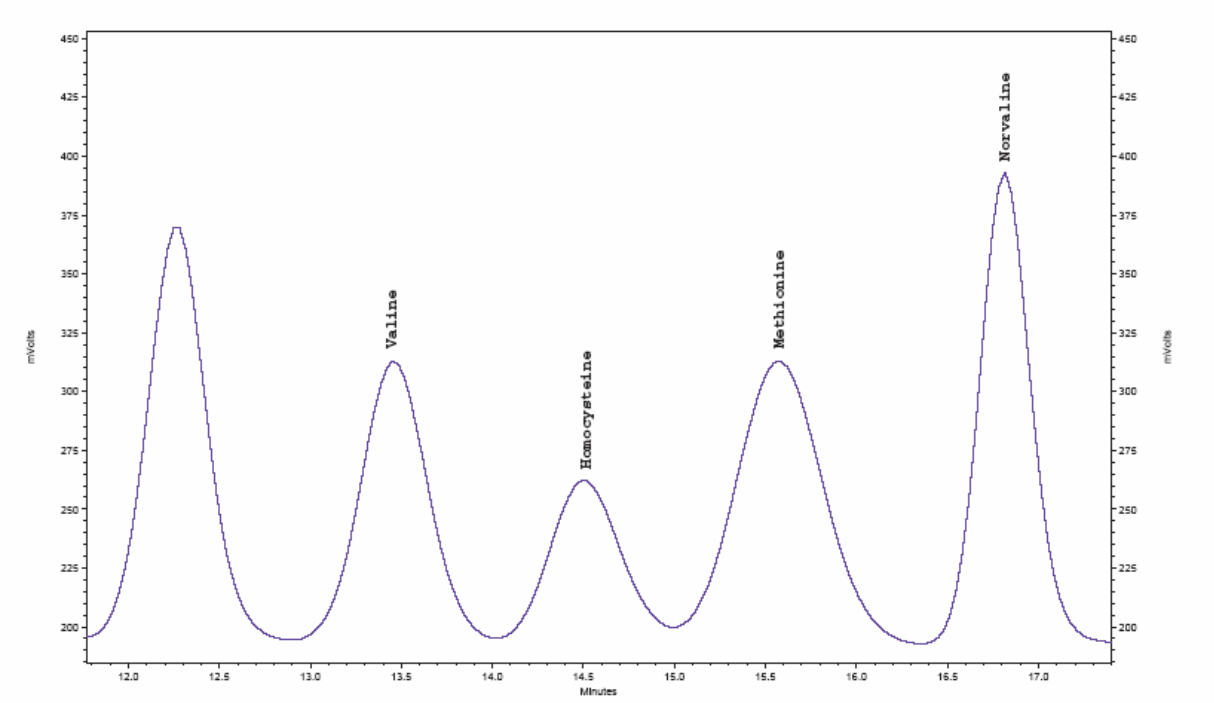

Figure 1. Short Program for the Separation of Homocysteine

BioSys

Sample: Physiological Fluid std

Amount Loaded: 10 nmol

Column Type: Peek

Column Number: U-1889

Resin Batch: 11956

Bed Length (mm): 200

Diameter (mm): 4.6

Instrument Serial Number: 93739

	Buffer	Nin
Flow Rate (ml/h):	30	20

	Buffer	**Molarity**	**pH**	**Batch No.**
Buffer 3 -	Lithium Citrate Buffer CII	0.50	3.15	12251
Buffer 4 -	Lithium Citrate Buffer DII	0.90	3.50	12263
Buffer 6 -	Lithium hydroxide Solution	0.30		11728
Reagent	Ninhydrin			12296
	Ultrosolve			12307

Title: **Homocysteine**

Nin Flow Rate: 20.0 ml/h

No.	**Time**	**Temp**	**Buffer**	**Pump**	**Nin**	**Rec**	**Commands**
1	01:00	65°C	3	30.0ml/h	ON	OFF	
2	00:00	65°C	3	30.0ml/h	ON	OFF	Reset
3	01:00	65°C	3	30.0ml/h	ON	OFF	Load
4	03:00	65°C	3	30.0ml/h	ON	ON	
5	07:00	65°C	4	30.0ml/h	ON	ON	
6	03:00	75°C	6	30.0ml/h	ON	ON	
7	12:00	65°C	3	35.0ml/h	ON	ON	
8	02:00	65°C	3	30.0ml/h	ON	ON	