

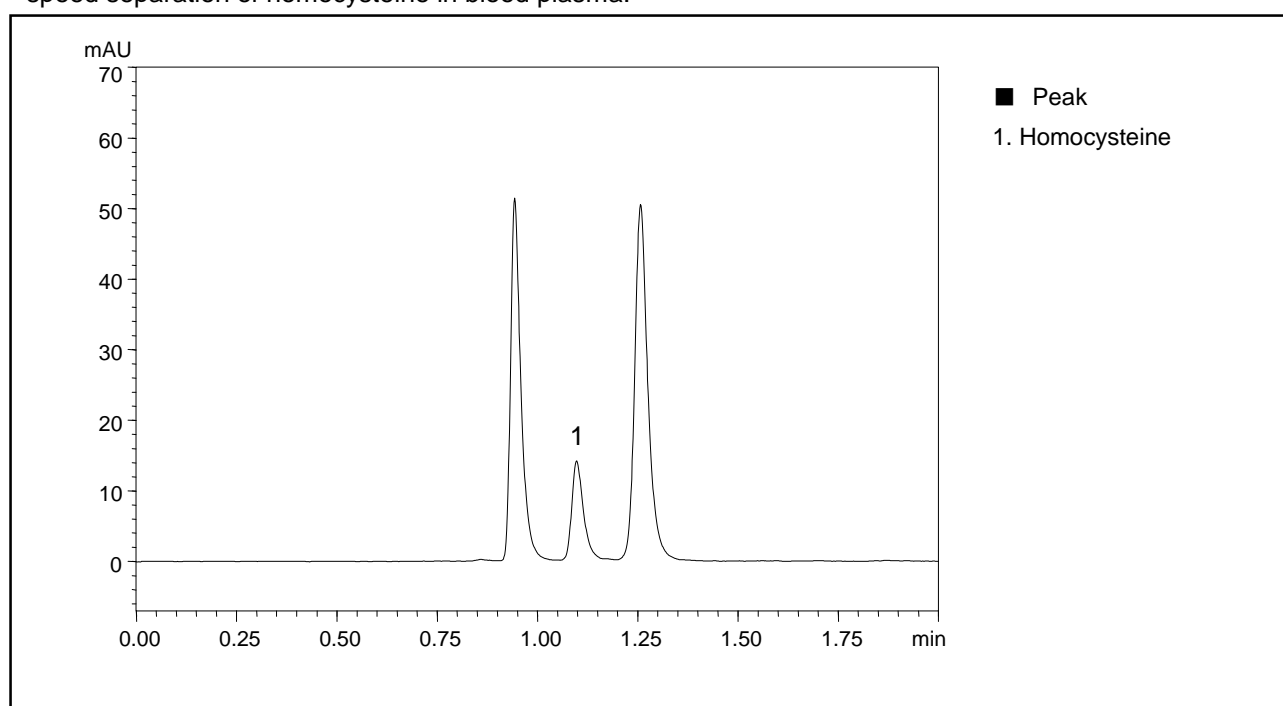
Application Data Sheet

High Performance Liquid Chromatography

No. 65

Fast Analysis of Homocysteine

Homocysteine is an amino acid biosynthesized from methionine in metabolism. Recent reports have linked it to hardening of the arteries and heart attacks. This chromatogram shows an example of a high speed separation of homocysteine in blood plasma.



Analysis of Homocysteine in Blood Plasma [Note]

[Sample Preparation]

- 1) Add 20 μ L tri-n-butylphosphine solution to 150 μ L of a blood plasma sample and let it sit for 30 minutes at 4°C.
- 2) Add 125 μ L of 0.6 mol/L perchloric acid to deproteinize the solution from Step 1.
- 3) Add SBD-F solution, a fluorescent derivatizing agent, and potassium borate solution to the solution from Step 2 and let it react for 60 minutes at 60°C, then cool to inject it into the HPLC system.

Note: This data was provided by BML Inc.

Analytical Conditions

Instrument	: Prominence UFLC system
Column	: Chromolith Performance (100 mm L. \times 4.6 mm <i>i.d.</i>)
Mobile Phase	: 100 mmol/L (Sodium) phosphate buffer / Methanol = 95 / 5 (v / v)
Flow Rate	: 2.0 mL/min
Column Temperature	: 40 °C
Detection	: Fluorescence (Ex: 385nm, Em: 515nm) with semi-micro flow cell
Sample Volume	: 5 μ L

Note: The indicated data was not acquired using a system registered by Japanese Pharmaceutical Affairs Act.