

Application News

No. C92

Liquid Chromatography Mass Spectrometry

Measurement of Homocysteine in Plasma with LCMS-8040

Homocysteine is used as an indicator when analyzing for activity of enzymes involved in the methionine metabolism pathway, such as methionine synthase and methylenetetrahydrofolate reductase (Fig. 1).

Here we describe an example analysis for homocysteine performed using an LCMS-8040 high-performance liquid chromatograph-triple quadrupole mass spectrometer and employing an analytical protocol used by the Mass Spectrometry, Clinical Chemistry and Pharmacology Lab. of Meyer Children's Hospital (Florence, Italy).

Sample Preparation and Analytical Conditions

Samples for analysis were extracted from plasma specimens in accordance with the preparation method shown in Fig. 2. LC and MS conditions are shown in Table 1. Multiple reaction monitoring (MRM) was performed with homocysteine as the target compound and using d8-homocysteine as an internal standard.

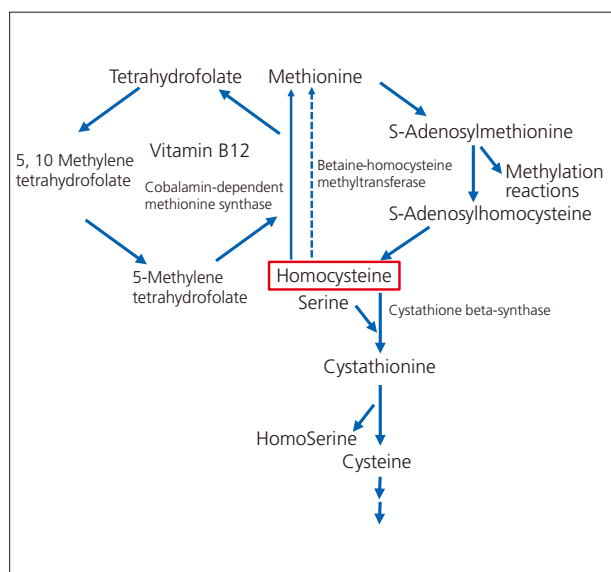


Fig. 1 Metabolic Pathway

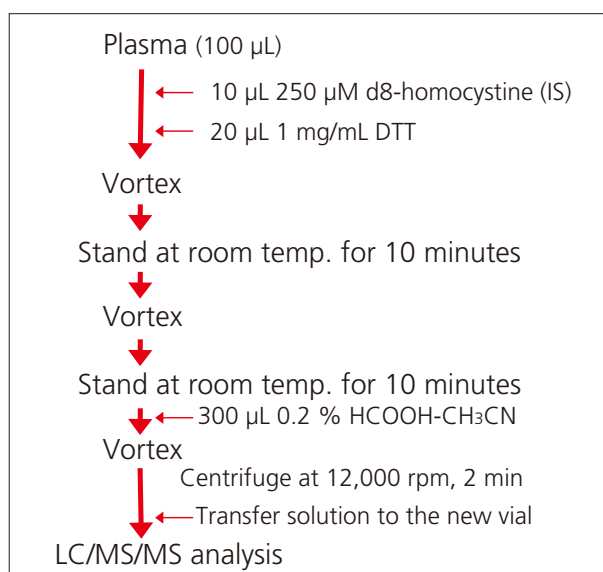


Fig. 2 Preparation Protocol

Table 1 Analytical Conditions

Column	: SUPELCO SIL LC-CN (33 mm L. x 3.0 mm I.D., 3 µm)	Ionization Mode	: ESI (+)
Mobile Phase A	: 0.1 % HCOOH-H ₂ O	Probe Voltage	: +4.5 kV
Mobile Phase B	: CH ₃ CN	Nebulizing Gas Flow	: 3.0 L/min
Ratio	: 70 % B	Drying Gas Flow	: 15.0 L/min
Flowrate	: 0.45 mL/min	DL Temperature	: 200 °C
Column Temperature	: 30 °C	Block Heater Temperature	: 350 °C
Injection Volume	: 1 µL	MRM	: Homocysteine (135.8 > 90.1) d4-Homocysteine (IS) (139.8 > 94.1)
Analysis Time	: 5 min		

■ Analysis Results

Results of analysis are shown in Fig. 3. The "Sample" plot shows when there is no methionine synthase or methylenetetrahydrofolate reductase activity present in the sample. The "Control" plot shows when these enzymes are active and present in the sample. The

"Positive Control" plot shows when homocysteine is added to the plasma specimen in advance. A peak specific to homocysteine was detected in the "Sample" plot results. This analytical system can be used to check for enzyme activity.

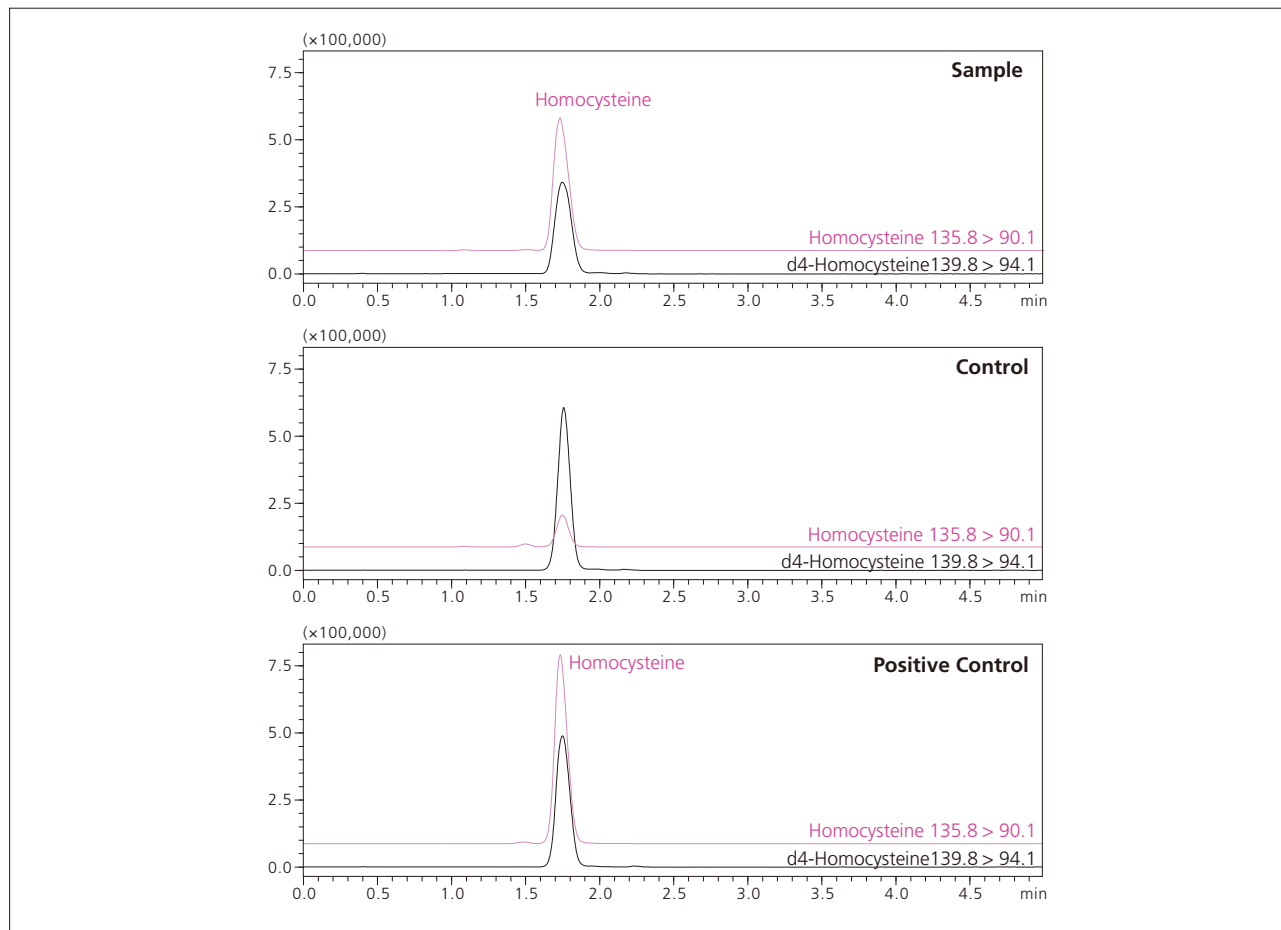


Fig. 3 Extracted-Ion Chromatograms of Target Compounds

[References]

la Marca G, et al. Progress in expanded newborn screening for metabolic conditions by LC-MS/MS in Tuscany: Update on methods to reduce false positive. JIMD Short report #127 (2008)
 J. Magera M, et al, Method for the determination of total homocysteine in plasma and urine by stable isotope dilution and electrospray tandem mass spectrometry. Clinical Chemistry 45:9 (1999) 1517-1522

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Note: This analytical system may only be used for research applications, and may not be used for clinical diagnosis.