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Application News

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Liquid Chromatography Mass Spectrometry

# Measurement of Methylmalonic Acid, 3-OH Propionic Acid and Succinic Acid in DBS (Dried Blood Spot) with LCMS-8040

Compounds such as methylmalonic acid (MMA), propionylcarnitine, 3-OH propionic acid (3-OH PA), and succinic acid (SA) can be used as indicators when analyzing for methylmalonyl-CoA mutase and propionyl-CoA carboxylase activity, which are enzymes involved in amino acid, cholesterol, and fatty acid metabolism (Fig. 1).
Here we describe an example analysis 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 Extraction from DBS and MS Analysis

Filter paper blotted with blood (dried blood spot, DBS) was used to prepare the analytical samples. After cutting a 3.2-mm diameter disk from a DBS, samples were extracted in accordance with the preparation method shown in Fig. 2. Samples extracted from plasma and urine may also be analyzed, and the relevant preparative methods are shown for reference.
LC and MS conditions are shown in Table 1. Multiple reaction monitoring (MRM) was performed with methylmalonic acid, 3-OH propionic acid, and succinic acid as the target compound and using 13C-methylmalonic acid as an internal standard.

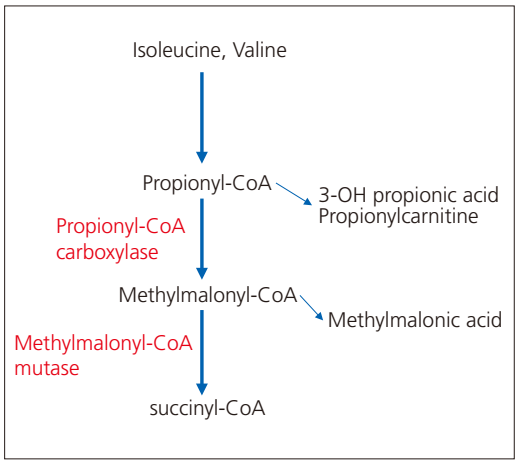


Fig. 1 Metabolic Pathway of Amino Acid

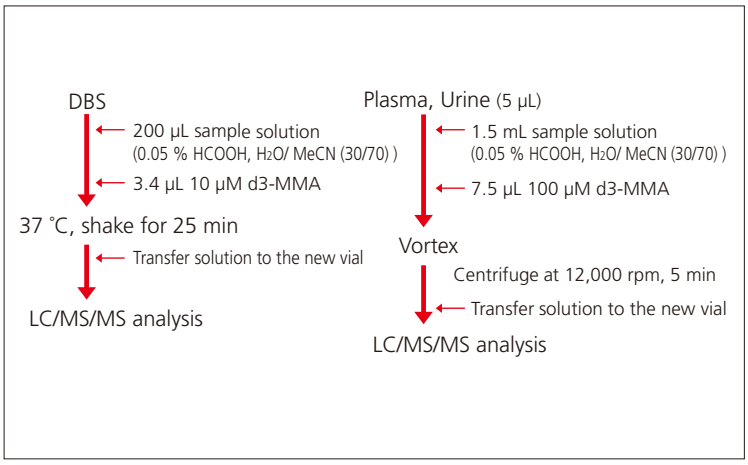


Fig. 2 Preparation Protocol

Table 1 Analytical Conditions

| | | | |
|---|---|---|---|
| Column | : Gemini C6-Phenyl (100 mm L. × 2.0 mm I.D., 3 µm) | Probe Voltage | : +4.5 kV |
| Mobile Phase A | : 0.1 % HCOOH-$H_2O$ | Nebulizing Gas Flow | : 2.5 L/min |
| Mobile Phase B | : 0.1 % HCOOH-$CH_3CN$ | Drying Gas Flow | : 15.0 L/min |
| Ratio | : 60 %B | DL Temperature | : 250 °C |
| Flowrate | : 0.2 mL/min | Block Heater Temperature | : 400 °C |
| Column Temperature | : 30 °C | MRM | : Succinic acid (116.9 > 73.2) |
| Injection Volume | : 5 µL | | MMA (116.9 > 73.1) |
| Analysis Time | : 5 min | | d3-MMA (119.9 > 76.1) |
| Ionization Mode | : ESI (+) | | Lactic acid (89 > 58.9) |
| | | | 3-OH Propionic acid (89 > 58.9) |

## Analysis Results

Results of analysis are shown in Fig. 3. The "Sample A" and "Sample B" plots show when there is no methylmalonyl-CoA mutase activity and no propionyl-CoA carboxylase activity present in the sample, respectively. The "Normal" plot shows when both these enzymes are active and present in the sample. Peaks representative of methylmalonic acid, 3-OH propionic acid, and succinic acid were detected in Sample A, and peaks representative of 3-OH propionic acid and succinic acid were detected in Sample B. Lactic acid (LA) and 3-OH propionic acid have the same molecular weight and the same MRM transition, but on separating the two compounds, a peak specific to 3-OH propionic acid was detected. This analytical system can be used to check for enzyme activity.

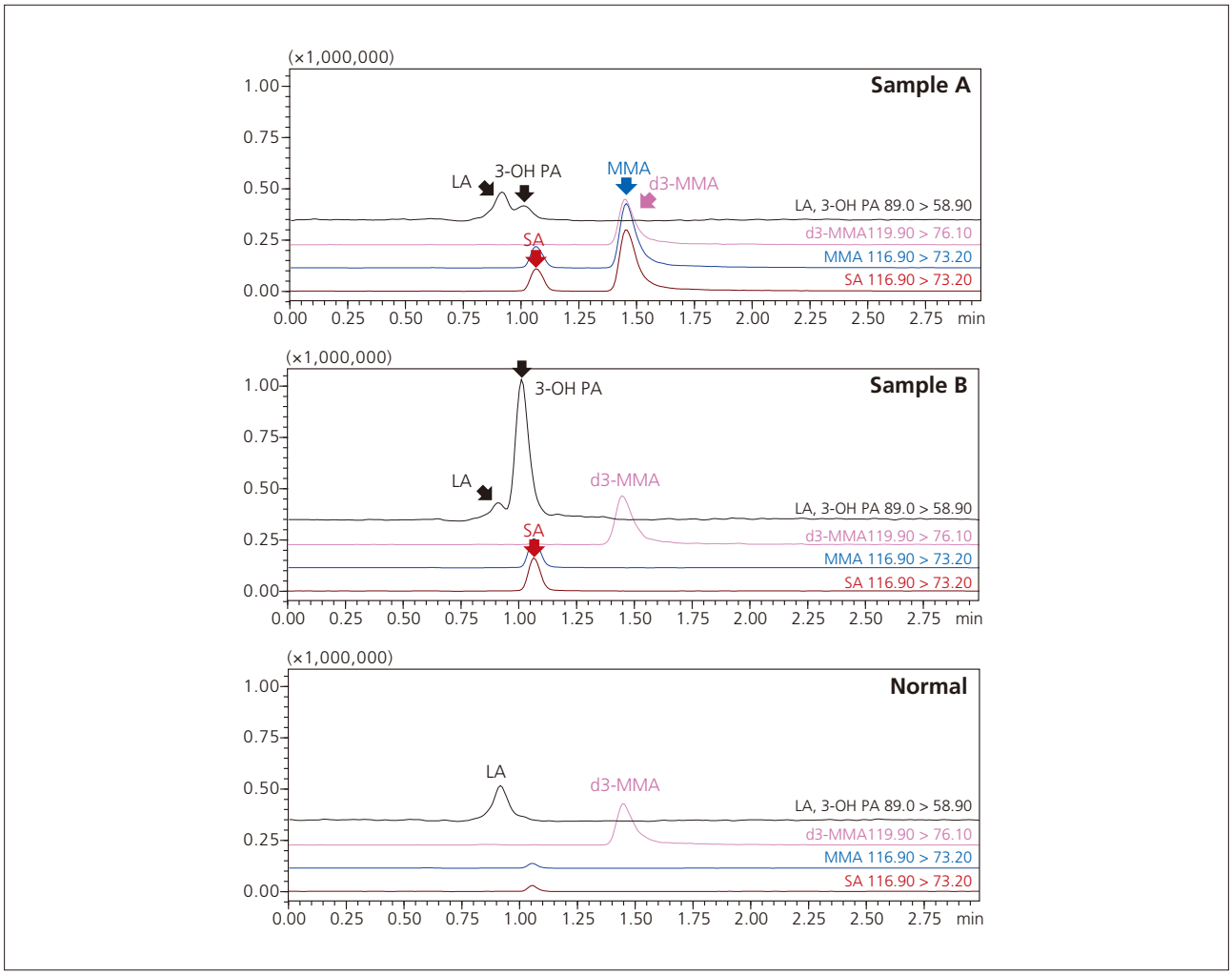


**Fig. 3 Extracted-Ion Chromatograms of Target Compounds**

[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)
la Marca G, et al. Rapid 2nd-Tier Test for Measurement of 3-OH-Propionic and Methylmalonic Acids on Dried Blood Spots: Reducing the False-Positive Rate for Propionylcarnitine during Expanded Newborn Screening by Liquid Chromatography–Tandem Mass Spectrometry. Clinical Chemistry 53:1364–1369 (2007)

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

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