

Application News

No. C91

Liquid Chromatography Mass Spectrometry

Measurement of Fumarylacetoacetate Activity in DBS (Dried Blood Spot) with LCMS-8040

The intermediate metabolites fumarylacetoacetate and succinylacetone (SuAC) are used as indicators when analyzing for fumarylacetoacetate hydrolase activity, which is an enzyme involved in amino acid metabolism (Fig. 1).

Here we describe an example analysis for succinylacetone 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 sample. After cutting a 3.2-mm diameter disk from a DBS, samples were extracted in accordance with the protocol described in Fig. 2. Samples extracted from plasma and urine can 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 succinylacetone as the target compound and using ¹³C₄-succinylacetone as an internal standard.

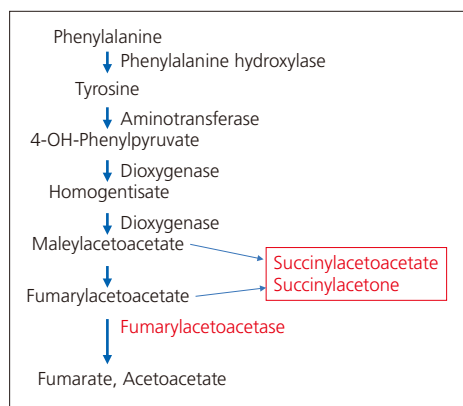


Fig. 1 Metabolic Pathway

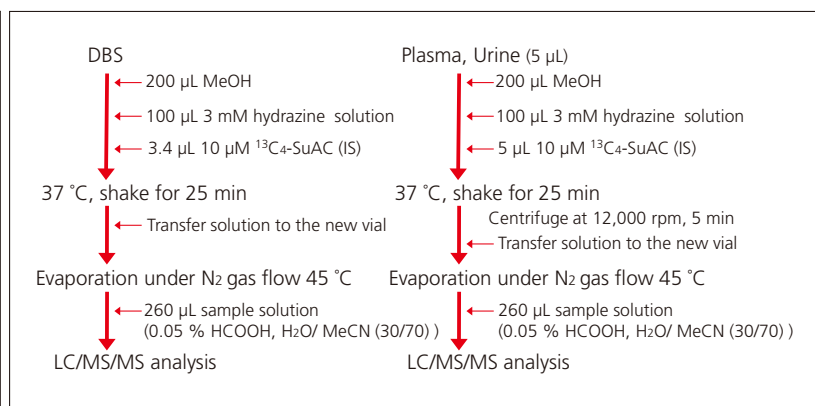


Fig. 2 Preparation Protocol

Table 1 Analytical Conditions

Column	: Synergi POLAR-RP (150 mm L. x 2.0 mm I.D., 4 µm)	Ionization Mode	: ESI (+)
Mobile Phase A	: 0.1 % HCOOH-H ₂ O	Probe Voltage	: +4.5 kV
Mobile Phase B	: CH ₃ OH	Nebulizing Gas Flow	: 2.5 L/min
Ratio	: 80 %B	Drying Gas Flow	: 15.0 L/min
Flowrate	: 0.2 mL/min	DL Temperature	: 250 °C
Column Temperature	: 30 °C	Block Heater Temperature	: 400 °C
Injection Volume	: 5 µL	MRM	: Succinylacetone (154.8 > 136.9) ¹³ C ₄ -Succinylacetone (158.9 > 141)
Analysis Time	: 4 min		

■ Analysis Results

Results of analysis are shown in Fig. 3. The "Sample" plot shows when there is no fumarylacetoacetate hydrolase activity present in the sample, and the "Control" plot shows when fumarylacetoacetate hydrolase activity is present in the sample. A DBS where

the filter paper was blotted with blood spiked with succinylacetone was used as a positive control. A peak representative of succinylacetone was detected in the "Sample" plot results. This analytical system can be used to check for enzyme activity.

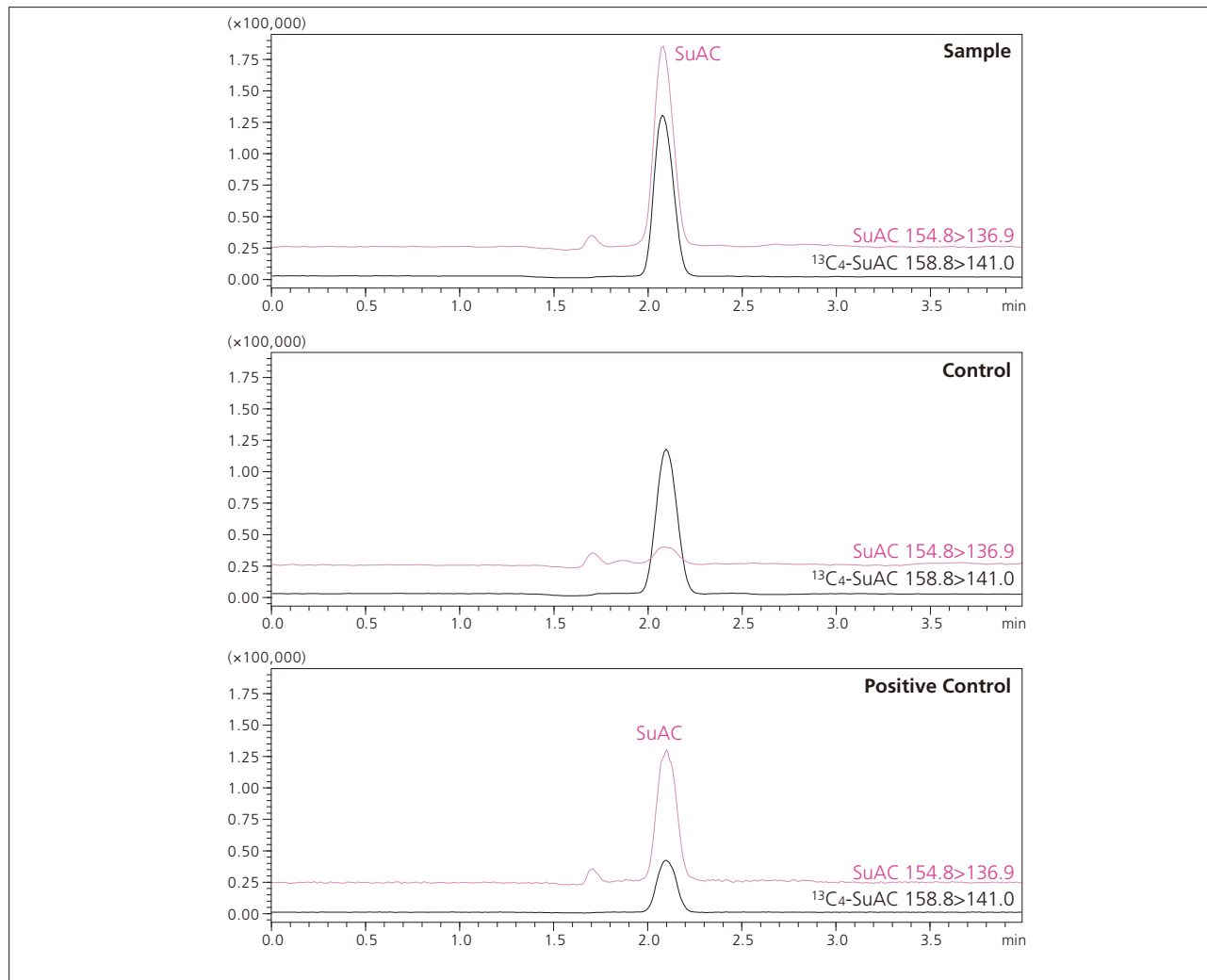


Fig. 3 Extracted-Ion Chromatograms of Target Compounds

[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)
- la Marca G, et al. The inclusion of succinylacetone as marker for tyrosinemia type I in expanded newborn screening programs. Rapid Commun. Mass Spectrom. 22 (2008) 812-818
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Note: This analytical system may only be used for research applications, and may not be used for clinical diagnosis.