

Application News

LCMS™-8050 High-Performance Liquid Chromatograph Mass Spectrometer

Identification of C5-Acylcarnitines Using Flow Injection Analysis-Tandem Mass Spectrometry

Takanari Hattori, Misa Tanaka¹, Yoshitomo Notsu², Miki Matsui¹, Jun Watanabe, and Hironori Kobayashi²

1. Department of Pediatrics, Shimane University Faculty of Medicine

2. Clinical Laboratory Division, Shimane University Hospital

User Benefits

- ◆ C5-acylcarnitines (isovalerylcarnitine and pivaloylcarnitine) can be distinguished without special reagents or instruments by a few modifications of the analytical method in conventional flow injection analysis-tandem mass spectrometry.

Introduction

In newborn screening, amino acids and acylcarnitines in a dried blood spot (DBS) are analyzed by flow injection analysis-tandem mass spectrometry (FIA-TMS) to find amino acid, organic acid, and fatty acid metabolic disorders. FIA-TMS provides excellent throughput but is normally unable to distinguish isomers because a column is not used. For example, isovalerylcarnitine (i-C5), which is a marker metabolite of isovaleric acidemia (IVA), is isobaric with pivaloylcarnitine (p-C5), 2-methylbutyrylcarnitine, and n-valerylcarnitine. p-C5 can be present in a DBS due to newborn or maternal use of pivalate-containing antibiotics, but i-C5 and p-C5 cannot be distinguished by FIA-TMS. There are many reports of false positives derived from p-C5. An analytical method to distinguish i-C5 and p-C5 is needed to reduce false positives of IVA. This Application News describes a technique (patent pending) to distinguish i-C5 and p-C5 using a reference ion.

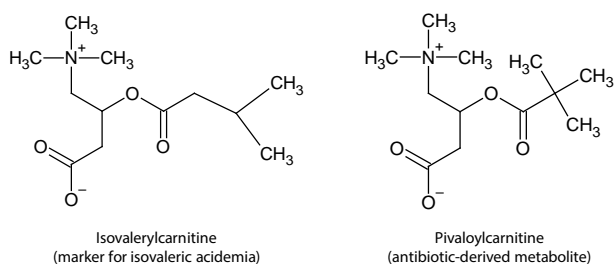


Fig. 1 C5-acylcarnitine Isomers

Analytical Conditions

The analytical instruments used were a Nexera™ UHPLC system and the LCMS-8050 (Fig. 2). The LCMS-8050 is a high-sensitivity and high-speed triple quadrupole mass spectrometer capable of simultaneous analysis of trace compounds. Table 1 shows the analytical conditions used for HPLC and MS. The injection volume is 1 μL and the analysis time is 1 minute in Shimadzu's method for FIA-TMS. This reduces mass spectrometer contamination and allows high throughput analysis of many samples.

Table 1 Analytical Conditions

[HPLC Conditions] (Nexera × 3)	
Mobile Phases:	NeoBase Kit
Flowrate:	0.1 mL/min (0 min)→0.05 mL/min (0.1 min) →0.1 mL/min (0.65 min)→0.5 mL/min (0.66-1 min)
Injection Volume:	1 μL
[MS Conditions] (LCMS-8050)	
Ionization:	ESI (Positive mode)
Mode:	MRM (m/z 246.2 > 85.0, 187.1)
Nebulizing Gas Flow:	3.0 L/min
Drying Gas Flow:	10.0 L/min
DL Temp.:	250 °C
Block Heater Temp.:	400 °C

Samples

A DBS (sample A) with high levels of C5-acylcarnitine derived from an IVA patient and a DBS (sample B) with high levels of C5-acylcarnitine derived from pivalate-containing antibiotics were analyzed. The i-C5 and p-C5 in these samples were also analyzed by LC/MS/MS with a column. In sample A, p-C5 was not detected and only i-C5 was detected, while in sample B i-C5 was not detected and only p-C5 was detected.



Fig. 2 LCMS™-8050

■ Pretreatment

DBS samples were prepared in accordance with the standardized protocol of a non-derivatized method using the NeoBase kit (PerkinElmer). A single 3 mm DBS punch was placed in each well of a 96-well assay plate. 100 μ L of the extraction solution containing an internal standard of acylcarnitines and amino acids was added to each well. The plate was shaken at 700 rpm at 45 $^{\circ}$ C for 45 minutes. After centrifugation for 5 minutes, the supernatant was analyzed.

■ Identification Using a Reference Ion

In the multiple reaction monitoring (MRM) mode, compounds can be distinguished by differences in reference ion ratio (Fig. 3, peak intensity of reference ion (b)/peak intensity of quantitative ion (a) \times 100). m/z 246.2 > 85.0 and m/z 246.2 > 187.1 were used as the quantitative ion and the reference ion of C5-acylcarnitine, respectively. Fig. 3 shows MRM chromatograms of standard solutions of i-C5 and p-C5. The reference ion ratio of i-C5 was lower than that of p-C5, and the reference ion ratio of i-C5 and p-C5 was 20.44% and 28.21%, respectively. To distinguish i-C5 and p-C5, similarities of reference ion ratio of i-C5 and p-C5 were described as i-C5 score and p-C5 score (scored from 0 to 100 where 100 is a perfect match) by the following equation:

- i-C5 score = $100 - |R_{DBS} - R_{i-C5}| / R_{i-C5} \times 100$
- p-C5 score = $100 - |R_{DBS} - R_{p-C5}| / R_{p-C5} \times 100$

where R_{DBS} is the reference ion ratio when the DBS sample is analyzed, R_{i-C5} is the reference ion ratio when the standard solution of i-C5 is analyzed, and R_{p-C5} is the reference ion ratio when the standard solution of p-C5 is analyzed.

The reference ion ratio of sample A and B was 20.48 and 28.54, respectively. The i-C5 score was higher than the p-C5 score in sample A and the p-C5 score was higher than the i-C5 score in sample B (Table 2). Based on the above results, the C5-acylcarnitine in sample A was estimated to be i-C5 and the C5-acylcarnitine in sample B was estimated to be p-C5. The estimations using i-C5 and p-C5 scores were consistent with the identification results using LC/MS/MS with a column.

Table 2 i-C5 and p-C5 Scores in DBS Analyses

DBS	C5 Conc. (μ mol/L)	i-C5 Score	p-C5 Score
A	5.32	98.0	72.6
B	3.72	58.8	97.7

■ Summary

This article shows that i-C5 and p-C5 can be distinguished by FIA-TMS using a reference ion.

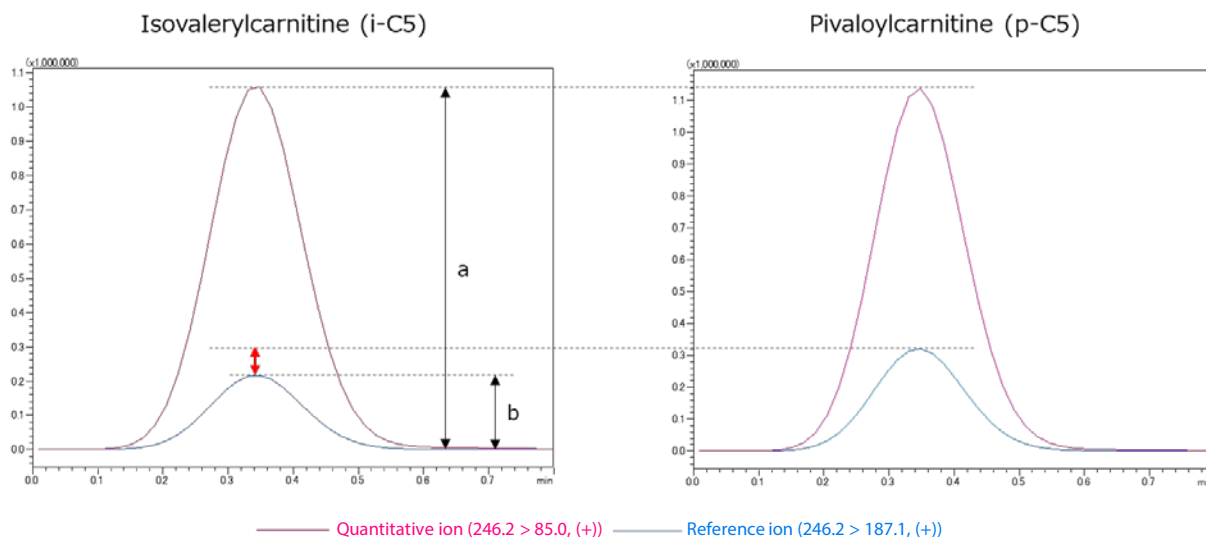


Fig. 3 MRM Chromatograms of Isovalerylcarnitine and Pivaloylcarnitine

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01-00228-EN

First Edition: Oct. 2021