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Application News

No. M273A

Gas Chromatograph Mass Spectrometry

Analysis of Short-Chain Fatty Acids in Biological Samples Using GC-MS

Short-chain fatty acids, such as acetic acid, are compounds that have been commonplace in our lives from long ago. While they are recently gaining attention as useful components within intestinal flora, they are also known as compounds that prove difficult to quantitatively analyze. Generally, quantitation at low concentrations is not possible by LC-MS since formic acid and acetic acid are used in the mobile phase. With GC-MS, which requires derivatization of hydroxyl groups, there is the problem where most short-chain fatty acids volatize when drying moisture in samples, a process necessary in most derivatization processes.

In this research, we avoided this problem by using the condensing agent 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium Chloride (DMT-MM), which induces condensation reactions between carboxylic acid and amines even in water or methanol, to derivatize short-chain fatty acids with an amine and then performed GC-MS analysis. We also evaluated the quantitative performance of the analysis system when analysis is performed using derivatization.

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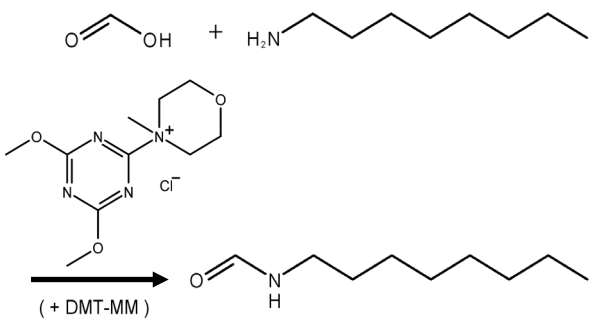

Fig. 1 Condensation Reaction Between Formic Acid and n-Octyl Amine

Sample

Pretreatment was performed on human standard plasma (Kohjin Bio Co., Ltd.: Human Plasma Pooled - EDTA-2Na (12271450)) and the concentration of each fatty acid was measured using the internal standard method.

50 µL of sample
↓
Add 10 µL of short-chain fatty acid mixed aqueous solution to obtain a final concentration of 0 to 100 µmol/L.
↓
Add acetic acid-d4 aqueous solution to obtain a final concentration of 10 µmol/L.
↓
Add 250 µL of methanol. (deproteinization)
↓
Perform high-speed shaking for 30 seconds and then centrifuge for 3 minutes at 25 °C and 16,000 G.
↓
Collect 180 µL of supernatant.
↓
Add 20 µL of methanol solution that contains 100 mmol/L of both DMT-MM and n-octyl amine.
↓
Leave standing at room temperature for 9 hours and then analyze.

Fig. 2 Pretreatment of Human Standard Plasma

Analysis Conditions

Table 1 lists the analysis conditions.

Table 1 Measurement Conditions

GC		
Column	:	BPX-5 (30 m × 0.25 mm I.D. 0.25 µm)
Carrier Gas	:	He
Injection Port Temp.	:	250 °C
Carrier Gas Control	:	Linear Velocity (39.0 cm/sec)
Injection Mode	:	Split (30:1)
Oven Temp.	:	60 °C (2 min) → 15 °C /min → 330 °C (5 min)
MS		
Ion Source Temp.	:	200 °C
Interface Temp.	:	280 °C
Measurement Mode	:	MRM
Loop Time	:	0.3 sec

Results

The calibration curves for the fatty acid derivatives all showed good linearity and we were able to measure the concentration of each type of short-chain fatty acid in the human standard plasma (Table 2).

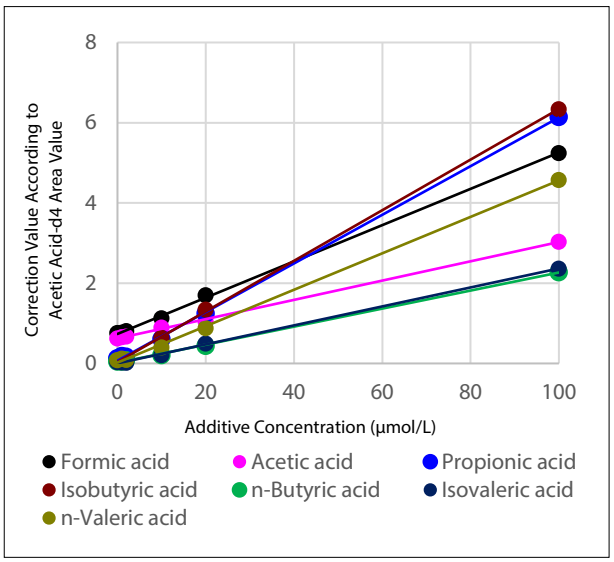

Fig. 3 Fatty Acid Calibration Curves

Table 2 Calibration Curve Linearity and Concentration in Human Standard Plasma

Compound Name	Linearity of Calibration Curve (R^2 Value)	Concentration in Plasma (µmol/L)
Formic acid	0.9994	115.64
Acetic acid	0.9990	184.36
Propionic acid	0.9996	8.02
Isobutyric acid	0.9997	3.97
n-Butyric acid	0.9991	5.89
Isovaleric acid	0.9992	0.82
n-Valeric acid	0.9991	3.25

Conclusion

We were able to quantitatively analyze seven types of short-chain fatty acids, namely formic acid, acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, and isovaleric acid, in a biological sample by GC-MS, by performing the following:

- Performing derivatization with amines using DMT-MM
- Normalizing area values using acetic acid-d4 as the internal standard

References:
The information in this article presents an experiment and verification performed by Shimadzu, based on a technique described in "The TRC News No. 115 (May 2012)" published by Toray Research Center, Inc.

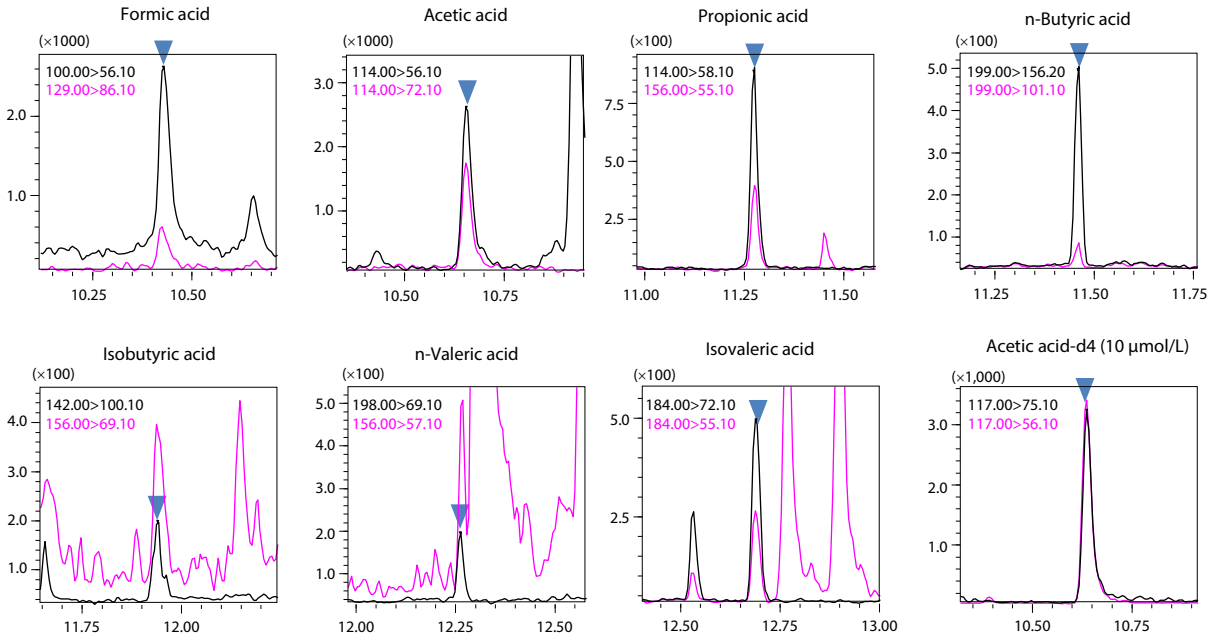

Fig. 4 MRM Chromatograms of Sample Spiked at 1 µmol/L

Note: The product described in this document has not been approved or certified as a medical device under the Pharmaceutical and Medical Device Act of Japan. It cannot be used for the purpose of medical examination and treatment or related procedures.

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