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LCMS-8060RX High-Performance Liquid Chromatograph Mass Spectrometer

Application News

MRM Based Free Fatty Acids Profiling in Human Plasma and Serum Using LC-MS/MS

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User Benefits

- Simultaneous analysis of 19 free fatty acids in blood was allowed with simple pretreatment.
- The delay column worked to separate the target fatty acids from the undesirable contaminants.
- LCMS-8060RX newly equipped with CoreSpray enables stable and reliable quantitative analysis for trace amount of compounds.

■ Introduction

Free fatty acids, about 5% of the total fatty acids in the body, are known to be important signal transducers that regulate functions such as insulin secretion. It is also attracting attention as a biomarker for various endocrine disorders[1].

Gas chromatography (GC) or gas chromatography-mass spectrometry (GC/MS) generally requires pretreatment such as methyl esterification for long-chain fatty acids and polyunsaturated fatty acids analysis. Multiple reaction monitoring (MRM) using liquid chromatography-mass spectrometry (LC/MS) enables highly sensitive quantitative analysis of fatty acids without derivatization. However, fatty acids widely found in the environment, such as palmitic acid and stearic acid, are introduced as impurities in LC system. It has been reported that there is difficulty in accurate quantification of these fatty acids in real samples analysis[2].

This report presents an example of quantitative analysis of free fatty acids in human plasma and serum using the highly stable LCMS-8060RX system coupled with a delay column.

Fig. 1 LCMS-8060RX and CoreSpray

■ Samples and Preparation Conditions

Chemical standards were purchased from Cayman Chemical (Ann Arbor, MI). Human plasma from human volunteer obtained from Kojin-Bio Co. (Japan). Two types of human plasma were used: EDTA plasma and heparin plasma.

295 µL of acetonitrile containing 1% formic acid and 5 µL of internal standard were added to 100 µL of plasma and serum followed by stirring for about 1 minute. After centrifugation, the supernatant was collected as it was used as an analytical sample (Fig. 2). They were diluted with acetonitrile containing 1% formic acid and subjected to LC/MS/MS analysis. The internal standard added was adjusted to a final concentration of 25 ng/mL according to the dilution ratio.

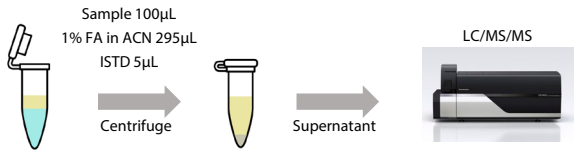

Fig. 2 Preparation flow

■ Analytical Conditions

An LC-40 series Nexera™ UHPLC system and an ultrafast triple quadrupole mass spectrometer LCMS-8060RX were used (Fig. 1). The LCMS-8060RX features CoreSpray technology to provide more uniform nebulizing gas flow. This gives more stable and reliable quantitative results over a long-time period.

The analytical conditions for HPLC as well as MS are shown in Table 1. A delay column has been installed between the gradient mixer and the autosampler to improve the separation of target and environmental contaminant components from mobile phase.

Table 1 Analysis condition

HPLC (Nexera X3 system)	
Column	: Shim-pack Scepter™ Claris C18-120*1 (100 mm × 2.1 mm I.D., 1.9 µm)
Delay column	: Shim-pack™ XR-ODS Ⅱ *2 (50 mm × 2.0 mm I.D., 2.2 µm)
Mobile phase A	: Acetonitrile/Water = 20/80
Mobile phase B	: Acetonitrile/2-propanol = 20/80
Flow rate	: 0.2 ml/min
Gradient program	: B.conc. 50% (0-0.5 min) → 65 % (7 min) → 100% (14-17.5 min) → 50 % (17.6-20 min)
Column temp.	: 55 ˚C
Injection volume	: 1 µL

*1 P/N：227-31210-02 *2 P/N：228-41623-94

MS (LCMS-8060RX)	
Ionization	: ESI (Negative mode)
Mode	: MRM
Nebulizing gas flow	: 5.0 L/min
Drying gas flow	: 10.0 L/min
Heating gas flow	: 10.0 L/min
DL temp.	: 250 ˚C
Heat block temp	: 400 ˚C
Interface temp	: 150 ˚C

■ Chromatographic Separation of matrix peaks by delay column

We developed a simultaneous analytical method for following compounds: Lauric Acid, Stearidonic Acid, Eicosapentaenoic Acid (EPA), α- Linolenic Acid, γ-Linolenic Acid, Myristic Acid, Docosahexaenoic Acid (DHA), Palmitoleic Acid, Arachidonic Acid (AA), Linoleic Acid, Docosapentaenoic Acid (DPA), Dihomo-γ-Linolenic Acid (DGLA), Palmitic Acid, Adrenic Acid, Oleic Acid, Stearic Acid, Arachidic Acid, Nervonic Acid, and Lignoceric Acid. The MRM chromatograms of the free fatty acid standard sample at 500 ng/mL were shown in Fig. 3.

By installing a delay column between the mixer and the autosampler, target compounds derived from the sample was able to be separated from matrix derived from the mobile phase. The wide broad peaks, which are considered to be fatty acids derived from the mobile phase, were detected after the peaks of several free fatty acids (palmitic acid C16:0, stearic acid C18:0 and oleic acid C18:1) in the plasma and serum samples (Fig. 3). Without the delay column, the peak from the blood compound overlapped with the broad peak from the mobile phase, and the accurate peak area could not be obtained.

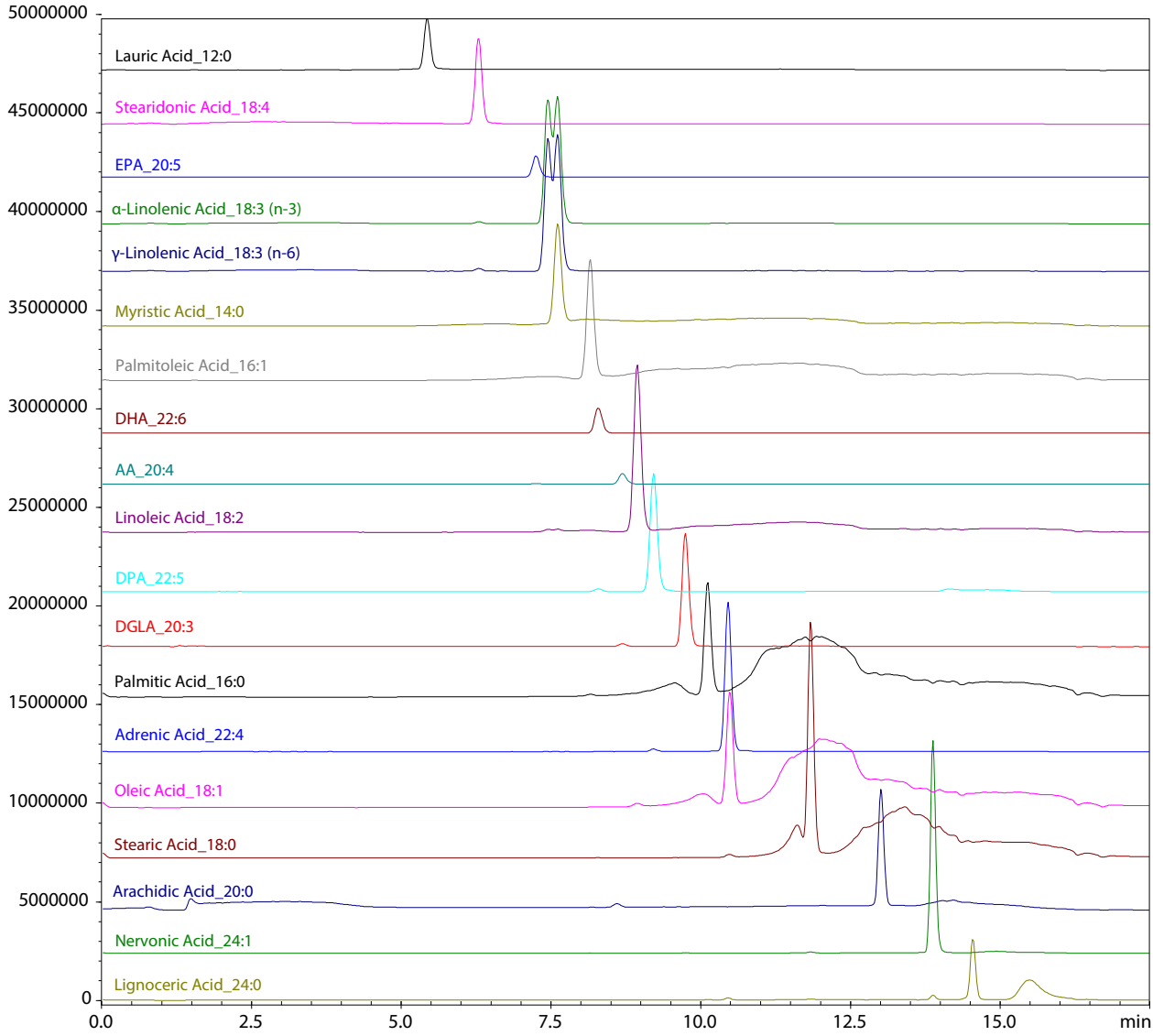

Fig. 3 The MRM chromatogram of 19 free fatty acids

Table 2 Calibration curves and repeatability of 19 free fatty acids

Compound	Transition	Quantification Range (μg/L)	Coefficient of Determination (R^2)	Repeatability (%RSD)
Lauric Acid_12:0	199.20>199.20	10 - 1000	0.997	4.35
Stearidonic Acid_18:4	275.20>275.20	1 – 1000	0.997	4.27
EPA_20:5	301.20>257.10	1 – 1000	0.998	4.77
α-Linolenic Acid_18:3 (n-3)	277.20>277.20	1 – 1000	0.997	8.34
γ-Linolenic Acid_18:3 (n-6)	277.20>277.20	1 – 1000	0.998	7.63
Myristic Acid_14:0	227.20>227.20	10 - 1000	0.999	4.48
Palmitoleic Acid_16:1	253.20>253.20	10 – 1000	0.997	7.10
DHA_22:6	327.20>283.15	10 - 1000	0.998	6.07
AA_20:4	303.20>259.15	10 – 1000	0.998	3.94
Linoleic Acid_18:2	279.20>279.20	1 – 500	0.998	2.75
DPA_22:5	329.30>329.30	1 – 1000	0.998	6.84
DGLA_20:3	305.20>305.20	1 – 1000	0.998	4.67
Palmitic Acid_16:0	255.20>255.20	1 – 1000	0.997	1.11
Adrenic Acid_22:4	331.30>331.30	1 – 1000	0.998	8.59
Oleic Acid_18:1	281.20>281.20	10 – 1000	0.998	6.53
Stearic Acid_18:0	283.30>283.30	10 – 500	0.997	1.67
Arachidic Acid_20:0	311.30>311.30	10 – 1000	0.998	4.15
Nervonic Acid_24:1	365.30>365.30	1 – 500	0.997	1.68
Lignoceric Acid_24:0	367.30>367.30	10 – 1000	0.999	2.40
AA-d8 (ISTD)	311.20>267.20	-	-	-

■ Calibration curves and repeatability

Internal standard method was used for the calibration curve in each compound, with three or more calibration points in the range of 1-1000 ng/mL (Fig. 4). We confirmed that good linearity with the contribution ratio (R^2) >0.999 was obtained for all compounds. Good results were obtained for all compounds, with an accuracy of 70-130% of the mean concentration and repeatability (%RSD) of less than 10% at the lowest calibration point (Table 2).

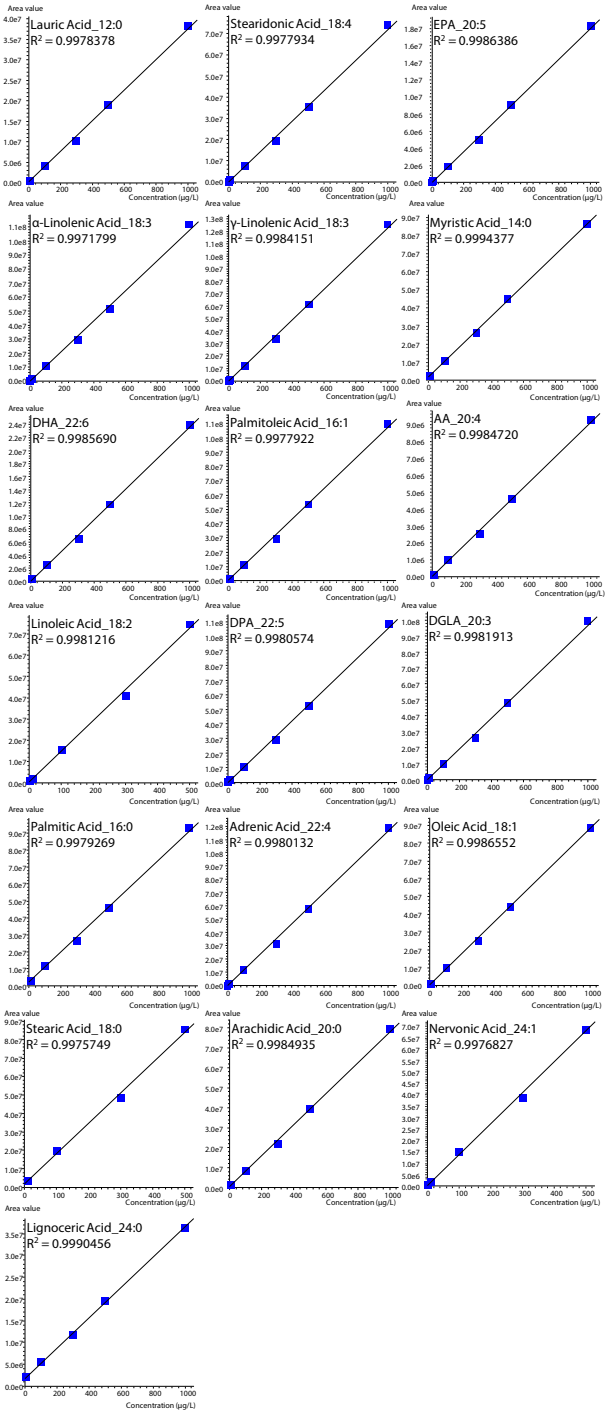

Fig. 4 Calibration curve in acetonitrile solution containing 1% formic acid of 19 free fatty acids

■ Quantitative analysis in human plasma and serum

Pretreated human plasma and human serum were diluted with acetonitrile containing 1% formic acid according to the content and subjected to quantitative analysis. The results of conversion to the concentration in the undiluted solution are summarized in Table 3. All 19 compounds were detected, and good repeatability of the estimated concentrations with RSD < 10% was obtained.

Table 3 Concentration of 19 free fatty acids in human plasma and serum

Compound	EDTA Plasma		Heparin Plasma		Serum	
	Concentration (µg/L)	%RSD	Concentration (µg/L)	%RSD	Concentration (µg/L)	%RSD
Lauric Acid_12:0	374.32	2.52	460.04	4.90	503.16	1.60
Stearidonic Acid_18:4	30.68	3.84	16.6	2.81	23.8	5.66
EPA_20:5	354.12	1.28	97.84	6.61	307.4	2.14
α-Linolenic Acid_18:3 (n-3)	1439.92	2.13	1349.04	3.19	1601.92	1.11
γ-Linolenic Acid_18:3 (n-6)	400.56	7.61	79.28	9.36	118.88	3.39
Myristic Acid_14:0	3363.48	1.25	1915.6	3.27	1683.84	0.89
Palmitoleic Acid_16:1	3368.56	0.88	3708.12	1.88	3313	1.56
DHA_22:6	807.2	0.97	495	3.14	698.6	1.92
AA_20:4	4275.2	2.59	1326.12	1.95	3321.68	0.87
Linoleic Acid_18:2	23459.44	2.43	17523.04	1.32	24026.8	2.69
DPA_22:5	441.28	1.12	196.68	3.61	289.72	2.00
DGLA_20:3	1074.52	0.95	439.6	2.33	785.32	2.03
Palmitic Acid_16:0	42375.52	2.69	29640.64	3.05	35220.72	2.54
Adrenic Acid_22:4	222.84	1.66	170.16	3.27	151.8	2.41
Oleic Acid_18:1	65049.12	1.90	57857.36	0.94	62891.44	1.48
Stearic Acid_18:0	12185.4	0.47	8733.88	2.00	10819.2	1.01
Arachidic Acid_20:0	70.76	5.30	77.8	8.01	113.64	3.14
Nervonic Acid_24:1	23.72	1.84	20.84	2.72	25.44	2.43
Lignoceric Acid_24:0	64.52	6.72	125.76	8.58	57.2	5.86

■ Conclusion

Simultaneous analysis of 19 free fatty acids in human plasma and serum was performed using a high-performance liquid chromatograph mass spectrometer LCMS-8060RX. Good linearity was obtained in the range of 1-1000 for all compounds, and we also confirmed that the repeatability at lowest calibration point was less than 10%.

The use of a delay column allowed removing the matrix peaks derived from the mobile phase. This analytical method provides more stable and accurate quantitative analysis with simple pretreatment.

＜Reference＞

1) Takahashi H, et al., "Long-Chain Free Fatty Acid Profiling Analysis by Liquid Chromatography–Mass Spectrometry in Mouse Treated with Peroxisome Proliferator-Activated Receptor α Agonist", Biosci. Biotechnol. Biochem., 77, 2288–2293 (2013).
2) Okada H., et al., "Development of a liquid chromatography–electrospray ionization tandem mass spectrometric method for the simultaneous analysis of free fatty acids", J. Biochem., 170(3):389-397 (2021).

01-00758-EN First Edition: Oct. 2024

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