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Application News

LCMS™-8060 High Performance Liquid Chromatograph Mass Spectrometer

Development of a Fast Eicosanoid Profiling Method Using Plasma Microsamples

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User Benefits

- Eicosanoids and related fatty acids in plasma can be quantified in 10 minutes.
- Nexera™ and the LCMS-8060 ultra fast triple quadrupole mass spectrometer provides high throughput analysis.
- The MSW^2 microsampling device enables the accurate collection of trace quantity plasma samples.

■ Introduction

Eicosanoids and metabolites of omega-3 fatty acids are important targets when investigating pathophysiological functions and identifying disease biomarkers.

The authors have developed a method based on multiple reaction monitoring (MRM) for the quantitative identification of over 100 fatty acid metabolites in disease model mice serum (reported in ASMS 2019).

To increase speed and sensitivity, the number of MRMs was narrowed down to the detection targets and internal standards. As a result, the authors have created a 10-minute LC-MS/MS method consisting of 200 MRMs for the monitoring of 114 target eicosanoids and related fatty acid metabolites. This Application News describes an analysis using this method in which eicosanoids and related metabolites were detected in 5.6 µL of healthy human plasma collected using a microsampling device (MSW^{2TM}).

■ Method

Standard samples were purchased from Cayman Chemical (Ann Arbor, MI). Plasma and serum from healthy human volunteers were obtained from Kojin-Bio Co. The MSW^2$_{TM}$ (Shimadzu Corporation) microsampling device was used to collect a precise amount of plasma and serum. The STRATA^{TM}-X 10 mg cartridge (Phenomenex, Torrance, CA) was used for solid phase extraction (SPE). An LC/MS system consisting of an LC-40 series Nexera UHPLC system and an LCMS-8060 ultra fast triple quadrupole mass spectrometer (Shimadzu Corporation) was used (Fig. 2).

■ MSW^2 Microsampling Device

The MSW^2 is a unique microsampling device for collecting plasma from whole blood. Whole blood collected with the MSW^2 is separated into plasma and blood cells within microchannels by centrifugation (Fig. 1). An exact amount of plasma can then be collected simply by snapping off the constant volume part. Because the MSW^2 is made of a polymer resistant to organic solvents, the chip can be left in the collection tube during deproteinization.

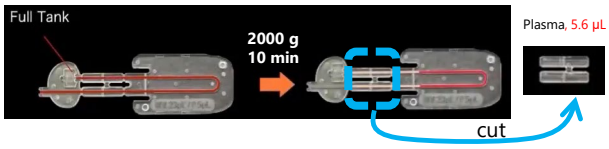


Fig. 1 MSW^{2TM} Microsampling Wing^{TM}

Table 1 Analytical Conditions

[HPLC conditions] (Nexera X3)		
Column	:	Shim-pack™ GIST C18-AQ HP *1 (100 mm × 2.1 mm I.D., 1.9 µm)
Column oven	:	40 °C
Solvent A	:	0.1 % Formic acid – water
Solvent B	:	Acetonitrile
Flow rate	:	0.4 mL/min
Injection volume	:	5 µL (co-injected with 15 µL of water)
[MS conditions] (LCMS-8060)		
Ionization	:	ESI, Positive/Negative
Mode	:	MRM
Nebulizing gas flow	:	2.5 L/min
Drying gas flow	:	10.0 L/min
Heating gas flow	:	10.0 L/min
DL temp.	:	250 °C
Block heater temp.	:	400 °C
Interface temp.	:	270 °C
CID Gas Pressure	:	230 kPa
Dwell time/Pause time	:	10 msec./ 1 msec.

*1 P/N 227-30807-02

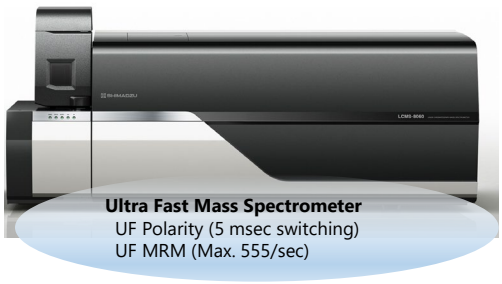


Fig. 2 LCMS^{TM}-8060

■ Pretreatment

Fig. 3 shows the fatty acid extraction procedure. A 5.6 µL sample of human plasma was mixed with 300 µL of methanol containing 0.1 % formic acid and a 10 µL mixture of 18 internal standards, and was vortexed for 5 minutes. After centrifuging, the supernatant was diluted with an aqueous solution of 0.1 % formic acid, and was loaded into a solid phase extraction column. The extract was dried and re-dissolved using 30 µL of methanol. A 5 µL sample was used for the LC/MS analysis.

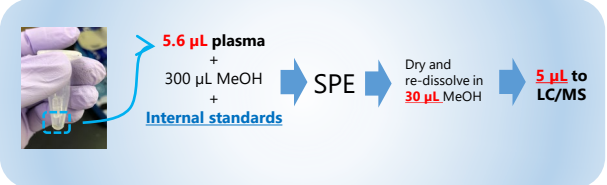


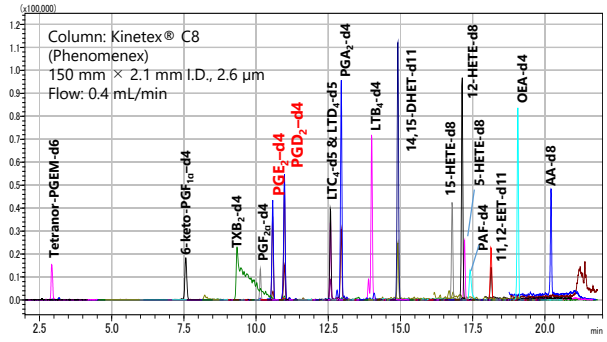
Fig. 3 Pretreatment Workflow

■ Results

<Development of a Fast Profiling Method>

An MRM method for 196 target eicosanoids and 18 internal standards (ISTD) with a 20-minute elution window for all targets was previously reported (Ref. 1). A chromatogram of 18 ISTDs analyzed using the 20-minute method is shown in Fig. 4 (top). This method was capable of quantitatively identifying 109 targets in the serum from disease model mice. To enhance throughput, the targets were narrowed to 114, consisting of the 109 detected in the mouse serum and five other compounds of interest. A total of 200 MRM transitions for the 114 targets and 18 ISTDs were configured within the 10-minute analysis time. The analysis of the ISTD mixture is shown in Fig. 4 (bottom). Retention times for prostaglandin E_2-d4 (PGE_2-d4) and PGD_2-d4 with the 10-minute method were 5.05 and 5.24 minutes respectively. Peak resolution (R) for the two compounds was 3.0, as compared to 3.8 with the 20-minute method. Analysis time has therefore been reduced without compromising separation resolution.

I. 20-minute method



II. New 10-minute method

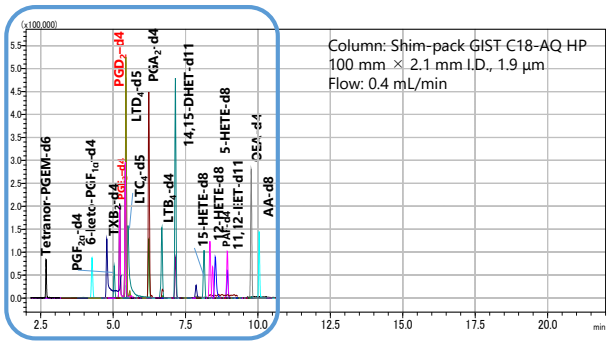


Fig. 4 MRM Chromatograms for 18 Internal Standards
I. 20 minute-method (ASMS 2019)
II. New 10-minute method

<Microsampling Reproducibility>

Using the MSW[2], human serum was microsampled three times, and LC/MS analyses were performed to confirm the repeatability of the 5.6 µL sampling accuracy. The variation for Lyso-PAF (Lyso-platelet activation factor), the most intense peak, was 3.2 %. The 5.6 µL sampling accuracy was estimated at approximately 3 % (CV%).

<Human Plasma Profiling>

Over 50 targets were detected in the 5.6 µL sample of human plasma including major arachidonic acid metabolites such as 5-, 8-, 11-, 12- and 15-HETEs. The LC/MS analysis was repeated three times. Table 2 shows the peak height ratio (target/corresponding internal standard) and reproducibility (CV%, n=3) of the 36 targets detected with a maximum CV of 20 %.

Table 2 36 Targets Detected in 5.6 µL of Human Plasma

	Fatty acid	Name	Retention Time	Height ratio Average, N = 3	CV% N = 3
1	AA	6-trans-LTB4	6.65	0.0378	9.4
2	AA	14,15-DHET	7.229	0.0109	18.2
3	AA	11,12-DHET	7.334	0.0065	19.1
4	AA	15-HETE	8.175	0.4461	7.7
5	AA	11-HETE	8.301	1.1596	1.9
6	AA	8-HETE	8.338	0.4028	14.1
7	AA	12-HETE	8.367	0.3975	14.2
8	AA	15-KETE	8.369	0.0593	12.0
9	AA	9-HETE	8.434	0.1796	13.7
10	AA	5-HETE	8.465	0.7621	3.5
11	AA	12-KETE	8.553	0.0544	9.3
12	AA	AA	10.073	11.6832	11.2
13	ALA	9-HOTrE	7.487	0.0041	4.8
14	DGLA	15-HETrE	8.495	0.2570	14.6
15	DGLA	8-HETrE	8.529	0.1466	14.9
16	DHA	10,17-DiHDHA	6.73	0.0173	13.9
17	DHA	20-HDHA	8.098	0.0423	6.9
18	DHA	16-HDHA	8.265	0.0830	13.1
19	DHA	10-HDHA	8.338	0.0500	10.4
20	DHA	14-HDHA	8.357	0.0510	17.6
21	DHA	7-HDHA	8.431	0.0201	8.1
22	DHA	11-HDHA	8.431	0.1002	19.9
23	DHA	8-HDHA	8.46	0.0850	16.0
24	DHA	DHA	9.993	5.2927	3.8
25	EA	AEA	9.076	0.0256	9.1
26	EA	OEA	9.793	0.2154	2.3
27	EDA	15-HEDE	8.924	0.0239	19.9
28	EPA	EPA	9.584	2.0875	11.0
29	LA	12,13-DiHOME	6.905	0.0844	6.1
30	LA	9,10-DiHOME	6.986	0.1568	5.9
31	LA	9-HODE	8.015	1.8331	6.9
32	LA	13-HODE	8.015	1.2752	7.9
33	LA	9-HpODE	8.206	0.2157	15.1
34	LA	13-KODE	8.209	0.2299	17.8
35	LA	9-KODE	8.299	0.0434	18.7
36	LPC	Lyso-PAF	7.861	575.2240	3.4

Abbreviations
AA: Arachidonic acid, DGLA: Dihomo-gamma-linolenic acid, DHA: Docosahexaenoic acid, EA: Ethanolamide, EDA: Eicosadienoic acid, EPA: Eicosapentaenoic acid, LA: Linoleic acid, LPC: Lyso-phosphatidylcholine

■ Conclusion

A 10-minute high-throughput method capable of monitoring 114 fatty acid metabolites has been developed. Over 30 fatty acid metabolites including eicosanoids, DHA metabolites, and linoleic acid metabolites were quantitatively identified in a 5.6 µL microsample of human plasma. From these results, it is anticipated that this method will be deployed for research in the field of pathophysiology with re-used mice.

Reference

(1) M. Yamada, T. Nakamura, A. Murata and T. Hattori, MP-546, 67th ASMS (2019)

01-00124-EN First Edition: April 2021

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