ThP-362

Method development of high-throughput eicosanoid profiling for micro-sampling plasma

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1. Overview

・To develop high-throughput monitoring method of fatty acid metabolites including eicosanoids.

・LC/MS system equipped with ultra fast triple quadrupole mass spectrometer LCMS-8060 and *Nexera*™ LC-40 series was used.

・200 MRM transitions were set in 10 minutes' chromatography. Over 30 eicosanoids and related fatty acids metabolites were quantitatively detected from 5.6 µL micro-sampling human plasma.

2. Introduction

Eicosanoids or omega-3 fatty acids metabolites are attractive targets for understanding pathophysiological functions and discovering disease biomarker. We reported that we developed multiple reaction monitoring (MRM) method to detect fatty acid metabolites quantitatively in disease model mice serum in ASMS2019. To make it the faster and the more sensitive, the number of MRMs was narrowed down for the detected targets and internal standards. Consequently, we have accomplished 10 minutes' LC-MS/MS method consisted of 200 MRMs for monitoring target 114 eicosanoids and related fatty acid metabolites. The method was applied to 5.6 µL of healthy human serum and plasma corrected by micro-sampling devise (MSW^2) to detect major arachidonic acid metabolites.

MS conditions (LCMS-8060)

Ionization	ESI, Positive/Negative
Nebulizing Gas Flow	2.5 L/min.
Drying Gas Flow	10.0L/min.
Heating Gas Flow	10.0L/min.
DL Temp.	250 ºC
Block Heater Temp.	400 ºC
Interface Temp.	270 ºC
CID Gas Pressure	230 kPa

Ultra Fast Mass Spectrometer
UF Polarity Switch in 5 msec)
UF MRM (Max. 555/sec)

Figure 1 LCMS-8060 triple quadrupole mass spectrometer

3. Methods

Authentic standards were purchased from Cayman Chemical (Ann Arbor, MI). Plasma and serum from healthy human volunteer were obtained from Kojin-Bio Co. Micro-sampling device MSW^2$_{TM}$ (Shimadzu Corporation) was used for correcting exact amount of plasma and serum. STRATA-X 10 mg (Phenomenex, Torrance, CA) cartridge was used for solid phase extraction (SPE). An LC/MS system consisting of LC-40 series *Nexera*™ UHPLC system and ultra-fast triple quadrupole mass spectrometer LCMS-8060 (Shimadzu Corporation) was used. A Shim-Pack GIST-HP C18 column, 2.1 x 100 mm, 1.9 µm (Shimadzu Corporation) was used for 10 minutes' method, where polarity switching time was 5 msec, and dwell time and pause time were set at 10 msec. and 1 msec. for a MRM transition, respectively.

4. Results

4-1. Method development

• Previously we have reported a MRM method consisted of 326 MRM transitions for 196 targets including eicosanoids, other fatty acid metabolites and 18 deuterium labeled analogs for internal standards, ISTD (ASMS 2019). The method enabled us to quantitatively detect 109 targets in the serum from some disease model mice. A chromatogram of 18 ISTD analyzed by previous 20 minutes' method was shown in Figure 2 upper.

• To develop high-throughput and sensitive method, we focused the targets into the 114 species detected in the serum and some other interest species. Consequently, 200 MRM transitions for 114 targets and 18 deuterium labeled analogues were set in 10 minutes' chromatographic condition. Two MRM transitions for each targets were applied to 63 of the 114 targets and one transition for the other 51 targets. ISTD mixture was analyzed (Figure 2 lower). Retention times of prostaglandin E_2-d4 (PGE_2-d4) and PGD_2-d4 in the 10 minutes' method were 5.05 and 5.24 minutes, respectively. Peak resolution (R) between them was 3.0, which was corresponding R value 3.8 in the 20 minutes' method.

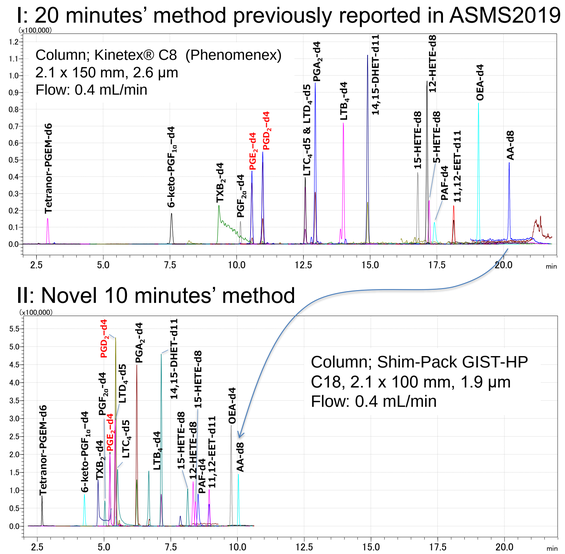
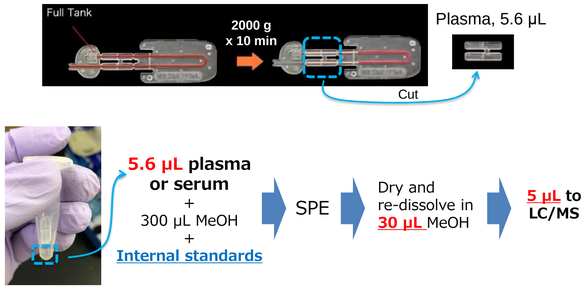


Figure 2 Overlaid MRM chromatograms. Internal standard mixture of 18 deuterium analogues was analysed by 20 minutes' chromatography (I) and by novel 10 minutes' chromatography (II). Formic acid 0.1% in water and acetonitrile was used for elution buffer A and B. Chromatographic conditions were inset in figures.

4-2. Micro-sampling and SPE treatment

• MSW^2 is a unique device for micro-sampling plasma from whole blood (Figure 3). Here we used this device for correcting exact amount 5.6 µL of plasma and serum. Fatty acids extraction was as follows; 5.6 µL of human plasma or serum was mixed with 300 µL methanol including 0.1% formic acid and 10 µL of 18 internal standards and vortexed for 5 minutes. After centrifuged, supernatant was diluted by 0.1% formic acid including water and loaded to SPE column. After SPE treatment, the extract was dried and re-dissolved by 30 µL of methanol including 0.1% fatty acid. The 5 µL aliquot was applied to the 10 minutes' method.



Figrue 3 Micro-sampling device MSW^2 was shown in upper. Plasma or serum was corrected 5.6 µL exactly by MSW^2. Schematic workflow was shown in the lower. 5 µL of re-dissolved sample solution was co-injected with 15 µL of water.

4-3. Reproducibility of micro-sampling

• Reproducibility of "5.6 µL" micro-sampling was evaluated by repeating the procedure from micro-sampling to LC/MS analysis. Three serum "5.6 µL" aliquots were prepared by MSW^2. LC/MS analysis was triplicated for each aliquot. Table 1 showed peak area of 11 targets and their inter-lot variation (CV%) was under 20% in first analysis "n=1". Lyso-PAF (platelet activation factor) showed the highest intensity and the best reproducibility, 2.8%, average. Accuracy of micro-sampling 5.6 µL could be estimated around 3% error.

Table 1 Peak area of 11 targets detected from "5.6 µL" serum. Inter-lot variation (CV%) was calculated for each analysis.

		Area (n=1, analytical)				Area (n=2, analytical)				Area (n=3, analytical)				
	Sample Name	Serum1	Serum2	Serum3	CV%	Serum1	Serum2	Serum3	CV%	Serum1	Serum2	Serum3	CV%	CV% average
LPC	Lyso-PAF	71,763,035	76,927,426	76,913,768	3.2	73,082,655	75,827,634	77,446,151	2.4	73,182,043	77,073,869	78,021,781	2.8	2.8
AA	12-HETE	16,583	13,797	15,443	7.5	13,597	12,595	12,275	4.4	15,453	15,049	16,306	3.4	5.1
EA	OEA	106,536	130,408	119,247	8.2	95,966	109,904	103,930	5.5	92,151	105,356	103,032	5.7	6.5
LA	9,10-DiHOME	44,698	45,398	39,875	5.7	49,313	43,854	38,281	10.3	48,256	43,944	41,444	6.3	7.4
EA	AEA	10,143	9,279	12,068	11.1	8,512	7,872	10,461	12.3	7,977	9,225	9,532	7.5	10.3
LA	13-HODE	29,649	38,642	33,445	10.9	28,785	41,983	31,699	16.6	40,794	38,265	35,807	5.3	10.9
LA	12,13-DiHOME	29,674	29,736	22,450	12.5	31,562	27,039	22,861	13.1	30,740	26,326	22,669	12.4	12.7
LA	13-KODE	10,758	13,636	13,094	10.0	8,663	12,360	10,779	14.3	8,541	14,575	12,303	21.1	15.1
EPA	EPA	47,734	61,950	73,545	17.3	50,794	71,610	71,453	15.1	52,511	70,101	70,522	13.0	15.1
AA	AA	344,711	416,942	544,604	19.0	349,413	422,116	573,940	20.9	353,180	438,799	608,187	22.7	20.8
DHA	DHA	149,723	176,415	236,653	19.4	142,917	173,492	241,217	22.1	143,126	179,218	242,352	21.8	21.1

4-4. Human plasma profiling

• Over 50 targets were detected from 5.6 µL of human plasma including major arachidonic acid metabolites such as 5-, 8-, 11-, 12- and 15-HETEs. LC/MS analysis was triplicated. Peak height ratio (target/corresponding internal standard) and analytical reproducibility of the 36 targets were listed in Table 2.

Table 2 36 targets detected in 5.6 µL of human plasma.

						Height ratio			
	Category	Sample Name	Ret. Time	MRM1	MRM2	Analysis 1	Analysis 2	Analysis 3	CV% (n=3)
1	AA	6-trans-LTB4	6.65	335.2>195.1		0.041572	0.033025	0.038689	9.4
2	AA	14,15-DHET	7.229	337.2>207.2		0.012245	0.008129	0.012452	18.2
3	AA	11,12-DHET	7.334	337.2>167.1		0.006026	0.005221	0.008153	19.1
4	AA	15-HETE	8.175	319.2>219.2		0.492328	0.409667	0.436362	7.7
5	AA	11-HETE	8.301	319.2>167.1		1.13499	1.15563	1.18817	1.9
6	AA	8-HETE	8.338	319.2>155.1		0.337784	0.394909	0.475839	14.1
7	AA	12-HETE	8.367	319.2>179.1		0.329588	0.395129	0.467863	14.2
8	AA	15-KETE	8.369	317.2>113.1	317.2>139.1	0.04986	0.061057	0.067002	12.0
9	AA	9-HETE	8.434	319.2>123.1	319.2>139.1	0.170129	0.213273	0.155485	13.7
10	AA	5-HETE	8.465	319.2>115.1	319.2>203.2	0.797831	0.755404	0.733057	3.5
11	AA	12-KETE	8.553	317.2>153.1	317.2>179.1	0.049341	0.052432	0.061279	9.3
12	AA	AA	10.073	303.2>259.2	303.2>303.2	10.6887	10.8278	13.5331	11.2
13	ALA	9-HOTrE	7.487	293.2>171.1		0.004136	0.00389	0.004371	4.8
14	DGLA	15-HETrE	8.495	321.2>221.2	321.2>113.1	0.256219	0.303227	0.211577	14.6
15	DGLA	8-HETrE	8.529	321.2>157.2	321.2>163.2	0.177548	0.131154	0.131076	14.9
16	DHA	10,17-DiHDHA	6.73	359.2>153.1	359.2>188.1	0.020379	0.014513	0.016908	13.9
17	DHA	20-HDHA	8.098	343.2>241.2		0.040283	0.040126	0.046427	6.9
18	DHA	16-HDHA	8.265	343.2>233.2	343.2>189.1	0.085992	0.068442	0.094644	13.1
19	DHA	10-HDHA	8.338	343.2>153.1		0.044941	0.05716	0.047977	10.4
20	DHA	14-HDHA	8.357	343.2>205.2		0.06284	0.049079	0.041117	17.6
21	DHA	7-HDHA	8.431	343.2>141.1		0.018353	0.022302	0.019706	8.1
22	DHA	11-HDHA	8.431	343.2>149.1	343.2>121.1	0.103039	0.123105	0.074501	19.9
23	DHA	8-HDHA	8.46	343.2>109.1	343.2>189.1	0.068342	0.101639	0.084926	16.0
24	DHA	DHA	9.993	327.2>283.2		5.01619	5.37379	5.48812	3.8
25	EA	AEA	9.076	348.2>62.1		0.027123	0.02737	0.022302	9.1
26	EA	OEA	9.793	326.2>62.1		0.210939	0.212825	0.222291	2.3
27	EDA	15-HEDE	8.924	323.2>223.2		0.02368	0.0298	0.01818	19.9
28	EPA	EPA	9.584	301.2>257.2		1.9158	1.9338	2.41293	11.0
29	LA	12,13-DiHOME	6.905	313.2>183.1		0.077216	0.087086	0.089005	6.1
30	LA	9,10-DiHOME	6.986	313.2>201.2		0.144074	0.160511	0.165776	5.9
31	LA	9-HODE	8.015	295.2>171.1		1.88808	1.65753	1.95371	6.9
32	LA	13-HODE	8.015	295.2>195.1	295.2>113.1	1.37202	1.13716	1.31632	7.9
33	LA	9-HpODE	8.206	311.2>185.2	311.2>197.2	0.243392	0.170064	0.233564	15.1
34	LA	13-KODE	8.209	293.2>113.1		0.245723	0.173932	0.270153	17.8
35	LA	9-KODE	8.299	293.2>185.1		0.046459	0.051485	0.032295	18.7
36	LPC	Lyso-PAF	7.861	482.3>104.2	482.3>184.1	590.608	547.757	587.307	3.4

5. Conclusions

・10 minutes' high-throughput method which can monitor 114 fatty acid metabolites has been developed.

・Over 30 fatty acid metabolites including eicosanoids, DHA metabolites and linoleic acid metabolites were quantitatively detected from micro-sampled 5.6 µL human plasma.

・We believe that this method will be available to pathophysiological study with re-using mice.

Reference

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