

Application News

No. C102

Liquid Chromatography Mass Spectrometry

Comprehensive Monitoring Method for Analyzing 158 Lipid Mediator Species Using the Ultra-Fast LCMS-8050

Lipid mediator is a generic term for bioactive lipids, which are produced in the body and play a role in many biological functions. Simultaneous analysis of over 100 lipid mediator-related compounds has recently been developed by achievement of high speed and high sensitivity on a comprehensive LC/MS system. Comprehensive monitoring of the lipid mediators resulted in showing a causal relationship between lipid mediators and various disorders. A lot of isomers of lipid mediator species show the same molecular weight and MS/MS spectrum, therefore chromatographic separation is needed for identification.

"LC/MS/MS Method Package for Lipid Mediator Ver. 2"

was developed to simultaneously analyze 158 lipid mediator-related compounds by ultra-fast triple quadrupole mass spectrometry. This application data sheet presents a list of targets and an example for measuring lipid mediators in various biological tissues. Furthermore, 87 arachidonic acids and its metabolites, 18 EPA and its metabolites, 16 DHA and its metabolites, 11 ethanolamids, 23 metabolites of other fatty acids, Azelaoyl-PAF, PAF and Lyso-PAF are all included as registered compounds in the method in order to analyze a total of 158 components with optimized MRM transitions and retention times.

- A list of compounds (Classification LA: linoleic acid, ALA: α -linolenic acid, EPA: eicosapentaenoic acid, ADA: adrenic acid, DGLA: dihomo- γ -linolenic acid, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, EA: ethanolamide. Abbreviation of compounds PG: prostaglandin, LT: leukotrien, DiHOME: dihydroxyoctadecenoic acid, HODE: hydroxyoctadecadienoic acid, Hp: hydroperoxy, KODE: keto-octadecadienoic acid, HETE: hydroxyeicosatetraenoic acid, EET: epoxyeicosatrienoic acid)

No.	Category	Compound
1	LA	(\pm)12, 13-DiHOME
2	LA	(\pm)9, 10-DiHOME
3	LA	13(S)-HODE
4	LA	9(S)-HODE
5	LA	(\pm)9-HpODE
6	LA	13-KODE
7	LA	13(S)-HpODE
8	LA	9-KODE
9	LA	12(13)-EpOME
10	LA	9(10)-EpOME
11	ALA	9(S)-HOTrE
12	ALA	13(S)-HOTrE
13	EDA	(\pm)15-HEDE
14	EDA	15-KEDE
15	AA	tetranor-PGFM
16	AA	tetranor-PGEM
17	AA	tetranor-PGDM
18	AA	20-hydroxy-PGF _{2α}
19	AA	20-hydroxy-PGE ₂
20	AA	18-carboxy dinor-LTB ₄
21	AA	13, 14-dihydro-15-keto-tetranor-PGF _{1β}
22	AA	2, 3-dinor-8-iso Prostaglandin F _{2α}
23	AA	13, 14-dihydro-15-keto-tetranor-PGF _{1α}
24	AA	6-keto-Prostaglandin F _{1α}
25	AA	13, 14-dihydro-15-keto-tetranor-PGD ₂
26	AA	20-carboxy-LTB ₄
27	AA	20-hydroxy-LTB ₄
28	AA	13, 14-dihydro-15-keto-tetranor-PGE ₂
29	AA	6, 15-diketo-13, 14-dihydro-PGF _{1α}
30	AA	iPF _{2α} -IV
31	AA	8-iso-15(R)-PGF _{2α}
32	AA	8-iso-PGF _{2α}
33	AA	Thromboxane B ₂
34	AA	11 β -PGF _{2α}
35	AA	5-iPF _{2α} -VI
36	AA	8-iso-15-keto PGF _{2α}
37	AA	PGF _{2α}
38	AA	8-iso-13, 14-dihydro-15-keto-PGF _{2α}
39	AA	8-iso-PGE ₂
40	AA	PGE ₂
41	AA	11-dehydro Thromboxane B ₂
42	AA	15-keto PGF _{2α}
43	AA	5(S), 14(R)-LXB ₄
44	AA	PGK ₂

No.	Category	Compound
45	AA	Prostaglandin D ₂
46	AA	11 β -13, 14-dihydro-15-keto-PGF _{2α}
47	AA	15-keto Prostaglandin E ₂
48	AA	14, 15-LTC ₄
49	AA	13, 14-dihydro-15-keto PGF _{2α}
50	AA	5(S), 6(R)-Lipoxin A ₄
51	AA	13, 14-dihydro-15-keto PGE ₂
52	AA	LTD ₄
53	AA	5(S), 6(S)-Lipoxin A ₄
54	AA	14, 15-LTE ₄
55	AA	13, 14-dihydro-15-keto-PGD ₂
56	AA	LTC ₄
57	AA	LTE ₄
58	AA	LTF ₄
59	AA	8-iso-PGA ₂
60	AA	PGA ₂
61	AA	11-trans-LTC ₄
62	AA	11-trans-LTE ₄
63	AA	PGJ ₂
64	AA	PGB ₂
65	AA	8, 12-iso-iPF _{2α} -VI 1, 5-lactone
66	AA	8(S), 15(S)-DihETE
67	AA	6-trans-LTB ₄
68	AA	5(S), 15(S)-DihETE
69	AA	LTB ₄
70	AA	13, 14-dihydro-15-keto PGJ ₂
71	AA	12-keto-LTB ₄
72	AA	N-acetyl-LTE ₄
73	AA	(\pm)14, 15-DHET
74	AA	12(S)-HHT
75	AA	(\pm)11, 12-DHET
76	AA	(\pm)8, 9-DHET
77	AA	20-carboxy arachidonic acid
78	AA	(\pm)5, 6-DHET
79	AA	19(S)-HETE
80	AA	15-deoxy-delta12, 14-PGJ ₂
81	AA	20-HETE
82	AA	(\pm)18-HETE
83	AA	(\pm)17-HETE
84	AA	(\pm)16-HETE
85	AA	15(S)-HETE
86	AA	11(S)-HETE
87	AA	8(S)-HETE
88	AA	15(S)-HpETE

No.	Category	Compound
89	AA	12(S)-HETE
90	AA	(\pm)9-HETE
91	AA	5(S)-HETE
92	AA	12(S)-HpETE
93	AA	12-KETE
94	AA	(\pm)5, 6-DHET-lactone
95	AA	5(S)-HpETE
96	AA	(\pm)14(15)-EET
97	AA	5-KETE
98	AA	(\pm)11(12)-EET
99	AA	(\pm)8(9)-EET
100	AA	(\pm)5(6)-EET
101	AA	Arachidonic Acid (AA)
102	ADA	1a, 1b-dihomo-PGF _{2α}
103	DGLA	Thromboxane B ₁
104	DGLA	8-iso-PGF _{1α}
105	DGLA	8-iso PGE ₁
106	DGLA	PGE ₁
107	DGLA	PGD ₁
108	DGLA	8-iso-PGA ₁
109	DGLA	PGA ₁
110	DGLA	15(S)-HETrE
111	EPA	8-iso-PGF _{3α}
112	EPA	Thromboxane B ₃
113	EPA	PGF _{3α}
114	EPA	PGE ₃
115	EPA	PGD ₃
116	EPA	Lipoxin A ₅
117	EPA	(\pm)17, 18-DihETE
118	EPA	(\pm)14, 15-DihETE
119	EPA	5, 6-DihETE
120	EPA	18(S)-HEPE
121	EPA	15(S)-HEPE
122	EPA	12(S)-HEPE
123	EPA	5(S)-HEPE
124	EPA	15(S)-HpEPE
125	EPA	12(S)-HpEPE
126	EPA	5(S)-HpEPE
127	EPA	17(18)-EpETE
128	EPA	EPA
129	DHA	Resolvin D ₂
130	DHA	Resolvin D ₁
131	DHA	7(R)-Maresin 1
132	DHA	10(S), 17(S)-DiHDoHE

No.	Category	Compound
133	DHA	7(S), 17(S)-hydroxy-DPA
134	DHA	(\pm)20-HDoHE
135	DHA	(\pm)16-HDoHE
136	DHA	(\pm)17-HDoHE
137	DHA	(\pm)13-HDoHE
138	DHA	(\pm)10-HDoHE
139	DHA	(\pm)14-HDoHE
140	DHA	(\pm)11-HDoHE
141	DHA	(\pm)7-HDoHE
142	DHA	(\pm)8-HDoHE
143	DHA	(\pm)4-HDoHE
144	DHA	DHA
145	EA	PGF _{2α} Ethanolamide
146	EA	PGE ₂ Ethanolamide
147	EA	PGE ₁ ethanolamide
148	EA	PGD ₂ Ethanolamide
149	EA	LTB ₄ ethanolamide
150	EA	(\pm)14(15)-EET ethanolamide
151	EA	(\pm)11(12)-EET ethanolamide
152	EA	(\pm)8(9)-EET ethanolamide
153	EA	(\pm)5(6)-EET ethanolamide
154	EA	AEA (arachidonoyl ethanolamide)
155	EA	OEA (oleoyl ethanolamide)
156		Lyso-PAF C-16
157		PAF C-16
158		Azelaoyl-PAF
159	IS	tetranor-PGEM-d ₈
160	IS	6-keto-PGF _{1α} -d ₄
161	IS	Thromboxane B ₂ -d ₄
162	IS	PGF _{2α} -d ₄
163	IS	PGE ₂ -d ₄
164	IS	PGD ₂ -d ₄
165	IS	LTC ₄ -d ₅
166	IS	LTB ₄ -d ₄
167	IS	5(S) HETE-d ₈
168	IS	12(S) HETE-d ₈
169	IS	15(S) HETE-d ₈
170	IS	PAF C-16-d ₄
171	IS	OEA-d ₄
172	IS	EPA-d ₅
173	IS	DHA-d ₅
174	IS	AA-d ₈

HPLC Conditions

Column	: Phenomenex Kinetex C8 (150 mm L. x 2.1 mm I.D., 2.6 μ m)
Mobile Phase A	: 0.1 % Formic acid - Water
Mobile Phase B	: Acetonitrile
Gradient Program	: 10 % B. (0 min) \rightarrow 25 % B. (5.0 min) \rightarrow 35 % B. (10.0 min) \rightarrow 75 % B. (20.0 min) \rightarrow 95 % B. (20.1 - 25.0 min)
Flowrate	: 0.4 mL / min.
Injection Volume	: 5 μ L
Column Oven Temp.	: 40 °C

MS Conditions (LCMS-8050)

Ionization	: ESI (Positive/Negative)
Nebulizing Gas Flowrate	: 3.0 L / min.
Drying Gas Flowrate	: 10.0 L / min.
Heating Gas Flowrate	: 10.0 L / min.
DL Temp.	: 250 °C
Block Heater Temp.	: 400 °C
Interface Temp.	: 300 °C
CID Gas Pressure	: 230 kPa

During comprehensive analysis, 158 lipid mediator-related compounds were divided into 16 groups by the chemical property similar to the 16 internal standard. Furthermore, this method package supports retention time estimation by using a peak from the proximate internal standard that enables reliable peak identification with high accuracy. Fig. 1 shows the chromatograms of 8-iso-PGF_{2α} and 8-iso-15(R)-PGF_{2α},

PDE₁ and PGD₁ detected from murine brain tissue extracts. As a comparison, the chromatograms of standard mixture are also shown (upper panels). This result indicates high reliability for peak identification because the differences between the retention times of internal standard and that of biological sample were almost same without having to prepare all of the standard compounds.

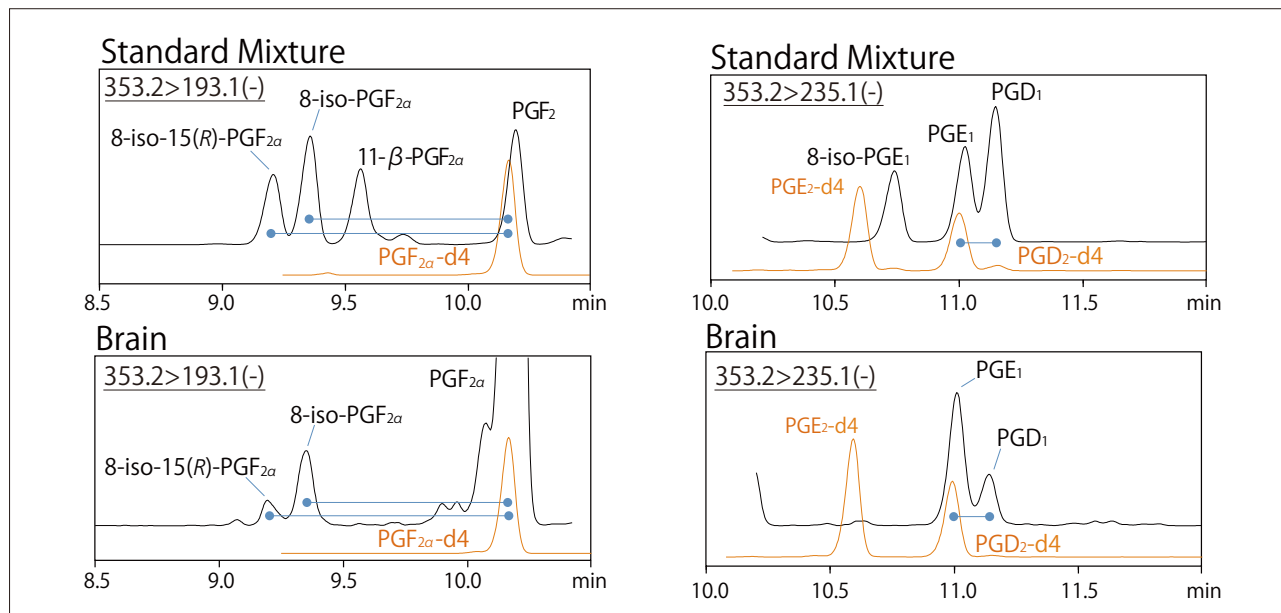


Fig. 1 MRM Chromatograms of 8-iso-PGF_{2α} (left) and PGD₁ (right) in the Analysis for Standard Mixture and Murine Brain Tissue Extracts

Brain, liver and spleen tissue were isolated from a mouse and rapidly frozen in liquid nitrogen. The frozen tissue was then weighed and homogenized. Lipid compounds were extracted from the homogenized tissue by the addition of 1 mL of methanol, including 10 μL of the internal standard mixture. Next, the extracted lipid mediators were purified by a solid phase extraction. Comprehensive analysis for three kinds of

tissue showed quantitative profiling of 78 lipid mediators species, as shown in Fig. 2. The vertical axis displays the concentration per tissue by weight (fg/mg tissue) in logarithmic scale estimated by internal standard method. PGE₂ in liver and PGD₂ in brain were 0.1 pg/mg tissue and 143 pg/mg tissue, respectively. The Method Package can provide such a profiling with wide dynamic range at low concentration region.

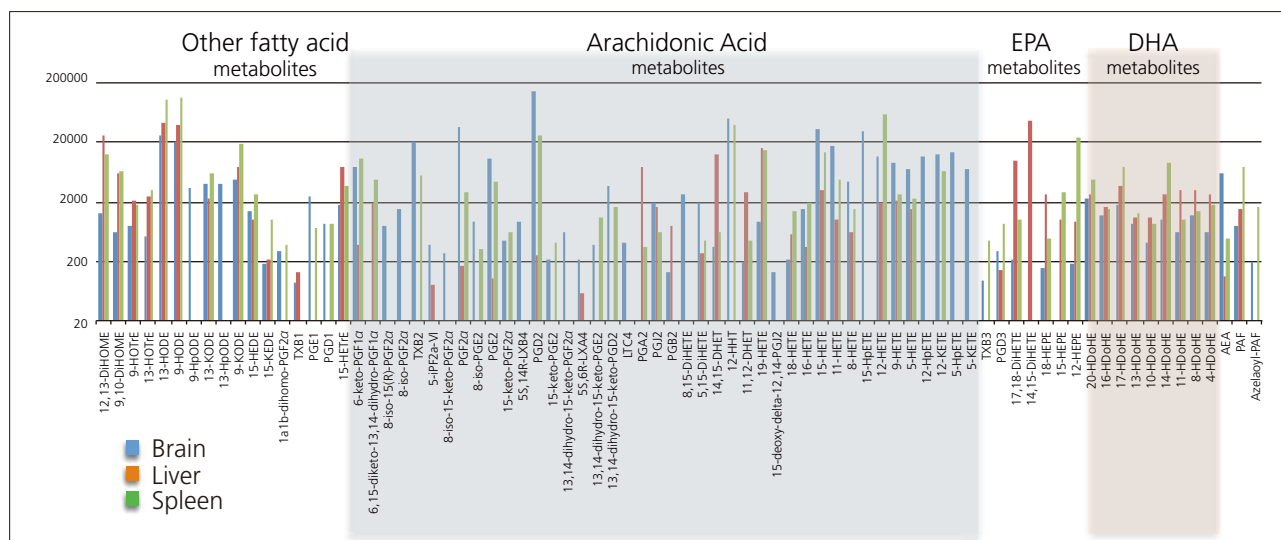


Fig. 2 Lipid Mediator Profiling for a Brain, Liver and Spleen Tissue from a Mouse