

Application News

No. C182

Liquid Chromatograph Mass Spectrometry

A Method of Simultaneous Analysis for 196 Lipid Mediators and Related Compounds Using Triple Quadrupole LC/MS/MS

Introduction

"Lipid mediators" collectively refer to a class of bioactive lipids that take a role in various physiological functions. Along with the increase in speed and sensitivity of liquid chromatograph mass spectrometers in recent years, a comprehensive analysis method for lipid mediators and related metabolites was developed, which has revealed the causal relationship between lipid mediators and a variety of disorders including allergy diseases and thrombosis.

This article introduces "LC/MS/MS Method Package for Lipid Mediators Ver. 3", a simultaneous analysis system for lipid mediators and related substances using a newly developed high speed triple quadrupole mass spectrometer, and provides an example of application.

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MRM and Retention Time Information for 214 Compounds

This Method Package contains optimized MRM parameters and retention time information for a total of 214 compounds encompassing 196 target compounds and 18 internal standards.

Table 1 lists these compounds, which include 100 arachidonic acid metabolites (including arachidonic acid), 26 EPA metabolites (including EPA), 23 DHA metabolites (including DHA), 11 ethanolamides, and 33 other fatty acid metabolites, platelet activating factor (PAF), Azelaoyl-PAF, and Lyso-PAF. The compounds highlighted in gray are those newly added in version 3 of this Method Package. To facilitate highly reliable peak identification, version 3 is configured with confirmatory MRM transitions (Ch. 2) for 112 of the 214 compounds.

Newly Developed Retention Time Correcting Tool

To identify isomers that cannot be distinguished by MRM, peak assignments based on the retention time difference are indispensable. Version 3 provides a "Retention Time Correcting Tool" for correcting minute shifts between predicted retention times and measured retention times.

Table 1 List of Compounds

(Category Codes: LA: linoleic acid, ALA: α -linolenic acid, EDA: eicosadienoic acid, AA: arachidonic acid, ADA: adrenic acid, DGLA: dihomo- γ -linolenic acid, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, EA: ethanolamide, ISTD: internal standard)
 (Compound Name Codes: DIHOME: dihydroxyoctadecenoic acid, HODE: hydroxyoctadecadienoic acid, Hp: hydroperoxy, KODE: keto-octadecadienoic acid, HETE: hydroxyeicosatetraenoic acid, EET: epoxyeicosatrienoic acid)

No	Compound	CAT	No	Compound	CAT	No	Compound	CAT	No	Compound	CAT
1	(±)12,13-DIHOME	LA	55	11 β -13,14-dihydro-15-keto Prostaglandin F _{2a}	AA	109	(±)5,6-DHET-lactone	AA	163	Resolvin D ₁	DHA
2	(±)9,10-DIHOME	LA	56	15-keto Prostaglandin E ₁	AA	110	5(S)-HpETE	AA	164	7(R)-Maresin 1	DHA
3	13(S)-HODE	LA	57	13,14-dihydro Prostaglandin F _{1a}	AA	111	(±)14(15)-EET	AA	165	10(S),17(S)-DIHDHA	DHA
4	9(S)-HODE	LA	58	14,15-LTC ₄ , Eoxin C ₄ , EXC ₄	AA	112	5-OxoETE	AA	166	Resolvin D ₂	DHA
5	(±)9-HpODE	LA	59	13,14-dihydro-15-keto Prostaglandin F _{2a}	AA	113	(±)11(12)-EET	AA	167	7(S),17(S)-hydroxy-docosapentaenoic acid	DHA
6	13-OxoODE	LA	60	5(S),6(R)-Lipoxin A ₄	AA	114	(±)8(9)-EET	AA	168	(±)19(20)-DIHDPA	DHA
7	13(S)-HpODE	LA	61	13,14-dihydro-15-keto Prostaglandin E ₂	AA	115	(±)5(6)-EET	AA	169	(±)20-HDHA	DHA
8	9-OxoODE	LA	62	5(S),6(S)-Lipoxin A ₄	AA	116	Arachidonic Acid (AA)	AA	170	(±)16-HDHA	DHA
9	(±)12(13)-EpOME	LA	63	14,15-LTE ₄ , Eoxin E ₄	AA	117	1a,1b-dihomo-Prostaglandin F _{2a}	ADA	171	(±)17-HDHA	DHA
10	(±)9(10)-EpOME	LA	64	13,14-dihydro-15-keto Prostaglandin D ₂	AA	118	2,3-dimor Thromboxane B ₂	DGLA	172	(±)10-HDHA	DHA
11	9(S)-HOTfE	ALA	65	Leukotriene C ₄	AA	119	6-keto Prostaglandin E ₁	DGLA	173	(±)14-HDHA	DHA
12	13(S)-HOTfE	ALA	66	11-trans LTC ₄	AA	120	2,3-dimor Prostaglandin E ₁	DGLA	174	(±)11-HDHA	DHA
13	13(S)-HpOTfE	ALA	67	Leukotriene D ₄	AA	121	Thromboxane B ₂	DGLA	175	(±)7-HDHA	DHA
14	11(S)-HEDE	EDA	68	Leukotriene E ₄	AA	122	8-iso Prostaglandin F _{2a}	DGLA	176	(±)8-HDHA	DHA
15	(±)15-HEDE	EDA	69	Leukotriene F ₄	AA	123	Prostaglandin F _{1a}	DGLA	177	(±)6-HDHA	DHA
16	15-OxoEDE	EDA	70	8-iso Prostaglandin A ₂	AA	124	8-iso Prostaglandin E ₁	DGLA	178	17(S)-HpDHA	DHA
17	tetranor-PGFM	AA	71	11-trans LTD ₄	AA	125	Prostaglandin E ₁	DGLA	179	(±)4-HDHA	DHA
18	tetranor-PGEM	AA	72	Prostaglandin A ₂	AA	126	Prostaglandin D ₂	DGLA	180	(±)19(20)-EpDPA	DHA
19	tetranor-PGDM	AA	73	Prostaglandin J ₂	AA	127	13,14-dihydro Prostaglandin E ₁	DGLA	181	(±)16(17)-EpDPA	DHA
20	tetranor-PGJM	AA	74	11-trans LTE ₄	AA	128	13,14-dihydro-15-keto Prostaglandin D ₂	DGLA	182	Docosahexaenoic Acid (DHA)	DHA
21	tetranor-PGAM	AA	75	Prostaglandin B ₂	AA	129	8-iso Prostaglandin A ₂	DGLA	183	Prostaglandin F _{2a} Ethanolamide	EA
22	20-hydroxy Prostaglandin F _{2a}	AA	76	8,12-iso- β -F _{2a} -VI 1,5- lactone	AA	130	Prostaglandin A ₁	DGLA	184	Prostaglandin E ₁ Ethanolamide	EA
23	20-hydroxy Prostaglandin E ₁	AA	77	8(S),15(S)-DIHETE	AA	131	15(S)-HETE	DGLA	185	Prostaglandin E ₁ ethanolamide	EA
24	18-carboxy dinor LTB ₄	AA	78	6-trans LTB ₄	AA	132	8(S)-HETE	DGLA	186	Prostaglandin D ₂ Ethanolamide	EA
25	13,14-dihydro-15-keto-tetranor Prostaglandin F _{1a}	AA	79	5(S),15(S)-DIHETE	AA	133	5(S)-HETE	DGLA	187	LTB ₄ ethanolamide	EA
26	2,3-dinor-8-iso Prostaglandin F _{2a}	AA	80	13,14-dihydro-15-keto Prostaglandin A ₁	AA	134	Δ 17-6-keto Prostaglandin F _{1a}	EPA	188	(±)14(15)-EET ethanolamide	EA
27	2,3-dinor Thromboxane B ₂	AA	81	Leukotriene B ₄	AA	135	Resolvin E ₁	EPA	189	(±)11(12)-EET ethanolamide	EA
28	13,14-dihydro-15-keto-tetranor Prostaglandin F _{1a}	AA	82	13,14-dihydro-15-keto Prostaglandin J ₂	AA	136	8-iso Prostaglandin F _{2a}	EPA	190	(±)8(9)-EET ethanolamide	EA
29	2,3-dinor-11 β -Prostaglandin F _{2a}	AA	83	12-oxo LTB ₄	AA	137	Thromboxane B ₂	EPA	191	(±)5(6)-EET ethanolamide	EA
30	6-keto-Prostaglandin F _{1a}	AA	84	tetranor-12(S)-HETE	AA	138	Prostaglandin F _{2a}	EPA	192	Arachidonoyl ethanolamide	EA
31	13,14-dihydro-15-keto-tetranor Prostaglandin D ₂	AA	85	N-acetyl LTE ₄	AA	139	11-dehydro Thromboxane B ₂	EPA	193	OEA (oleoyl ethanolamide)	EA
32	20-carboxy leukotriene B ₄	AA	86	Leukotriene B ₂	AA	140	Prostaglandin E ₂	EPA	194	Lyso-PAF C-16	EA
33	20-hydroxy leukotriene B ₂	AA	87	(±)14(15)-DIHET	AA	141	Prostaglandin D ₂	EPA	195	PAF C-16	EA
34	11-dehydro-2,3-dinor Thromboxane B ₂	AA	88	12(S)-HETE	AA	142	Lipoxin A ₄	EPA	196	Azelaoyl PAF	EA
35	13,14-dihydro-15-keto-tetranor Prostaglandin E ₂	AA	89	(±)11(12)-DIHET	AA	143	Leukotriene B ₄	EPA	197	tetranor-PCEM-d ₄	ISTD
36	6,15-dihydro-13,14-dihydro Prostaglandin F _{1a}	AA	90	(±)8(9)-DIHET	AA	144	(±)17,18-DIHETE	EPA	198	6-keto-Prostaglandin F _{1a} -d ₄	ISTD
37	ipF _{2a} -IV	AA	91	20-carboxy arachidonic acid	AA	145	(±)14(15)-DIHETE	EPA	199	Thromboxane B ₂ -d ₄	ISTD
38	8-iso-15(R)-Prostaglandin F _{2a}	AA	92	(±)5(6)-DIHETE	AA	146	(±)5(6)-DIHETE	EPA	200	Prostaglandin F _{2a} -d ₄	ISTD
39	8-iso Prostaglandin F _{2a}	AA	93	19(S)-HETE	AA	147	(±)18-HEPE	EPA	201	Prostaglandin E ₁ -d ₄	ISTD
40	Thromboxane B ₂	AA	94	15-deoxy-delta12,14-PGJ ₂	AA	148	15(S)-HEPE	EPA	202	Prostaglandin D ₂ -d ₄	ISTD
41	11 β -Prostaglandin F _{2a}	AA	95	20-HETE	AA	149	11(S)-HEPE	EPA	203	Leukotriene C ₄ -d ₄	ISTD
42	(±)5- β -F _{2a} -VI	AA	96	(±)18-HETE	AA	150	8(S)-HEPE	EPA	204	Leukotriene D ₄ -d ₄	ISTD
43	8-iso-15-keto Prostaglandin F _{2a}	AA	97	(±)17-HETE	AA	151	9(S)-HEPE	EPA	205	Prostaglandin A ₂ -d ₄	ISTD
44	Prostaglandin F _{2a}	AA	98	(±)16-HETE	AA	152	12(S)-HEPE	EPA	206	Leukotriene B ₂ -d ₄	ISTD
45	8-iso-13,14-dihydro-15-keto Prostaglandin F _{2a}	AA	99	15(S)-HETE	AA	153	5(S)-HEPE	EPA	207	(±)14(15)-DIHET-d ₄	ISTD
46	8-iso Prostaglandin E ₂	AA	100	11(S)-HETE	AA	154	15(S)-HpEPE	EPA	208	15(S)-HETE-d ₄	ISTD
47	Prostaglandin E ₂	AA	101	8(S)-HETE	AA	155	12(S)-HpEPE	EPA	209	12(S)-HETE-d ₄	ISTD
48	11-dehydro Thromboxane B ₂	AA	102	15-OxoETE	AA	156	5(S)-HpEPE	EPA	210	5(S)-HETE-d ₄	ISTD
49	15-keto Prostaglandin F _{2a}	AA	103	15(S)-HpETE	AA	157	(±)17(18)-EpETE	EPA	211	PAF C-16-d ₄	ISTD
50	11 β -Prostaglandin E ₂	AA	104	12(S)-HETE	AA	158	(±)11(12)-EpETE	EPA	212	(±)11(12)-EET-d ₄	ISTD
51	5(S),14(R)-LXB ₄	AA	105	(±)9-HETE	AA	159	Eicosapentaenoic Acid (EPA)	EPA	213	Oleoyl ethanolamide-d ₄	ISTD
52	Prostaglandin K ₂	AA	106	5(S)-HETE	AA	160	Resolvin D ₂	DHA	214	AA-d ₄	ISTD
53	Prostaglandin D ₂	AA	107	12(S)-HpETE	AA	161	Resolvin D ₁	DHA			
54	15-keto Prostaglandin F _{1a}	AA	108	12-OxoETE	AA	162	Resolvin D ₁	DHA			

Using the Newly Developed Tool to Correct Predicted Retention Times

This Method Package divides the 196 target compounds into groups based on the 18 internal standards. By performing an analysis of mixed internal standard solution and inputting the measured retention time into the retention time correcting tool, correction values of predicted retention times for all 214 compounds are calculated. The upper left of Fig. 1 shows the MRM chromatogram of PGE₂-d₄ when analyzing the mixed internal standard solution. Although the predicted retention time of PGE₂ is 10.700 minutes (registered information), inputting the measured time of 10.584 minutes for PGE₂-d₄ into the retention time correcting tool corrected the predicted retention time to 10.613 minutes. The lower left of Fig. 1 shows the MRM chromatogram of PGE₂ in the mixed target solution acquired in the same batch analysis. The measured retention time of PGE₂ was 10.608 minutes, which only had an error of 0.005 minutes, or 0.3 seconds, compared to the correction value calculated by the retention time correcting tool.

This demonstrates that the analysis of mixed internal standard solution and use of the retention time correcting tool allow highly accurate peak assignment of the 196 target compounds.

Example of Human Plasma Analysis

A sample of human plasma was analyzed in the same batch analysis as the above example. A volume of 30 µL of commercially available human plasma was refined through solid-phase extraction and a 5 µL equivalent was analyzed using the LCMS-8060. Fig. 2 shows the peak intensities of 66 identified compounds. The tolerance width from the predicted retention time in peak identification was set to 0.05 minutes (3 seconds). Lyso-PAF was excluded from the figure because the peak intensity was saturated. This example shows that a large number of lipid mediator metabolites can be accurately identified and quantitatively analyzed from trace amounts of a plasma sample.

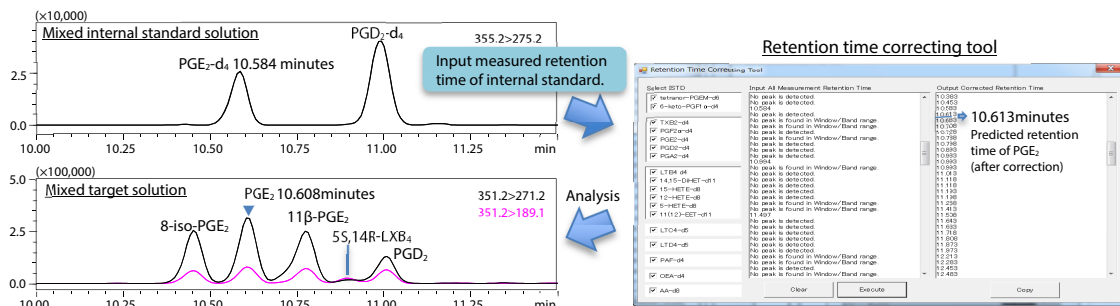


Fig. 1 MRM Chromatogram of PGE₂-d₄ from Analysis of Mixed Internal Standard Solution (Upper Left), GUI Indicating Predicted Retention Time Corrected Using the Retention Time Correcting Tool (Right), and MRM Chromatogram of PGE₂ Acquired from Analysis of Mixed Target Solution (Lower Left)

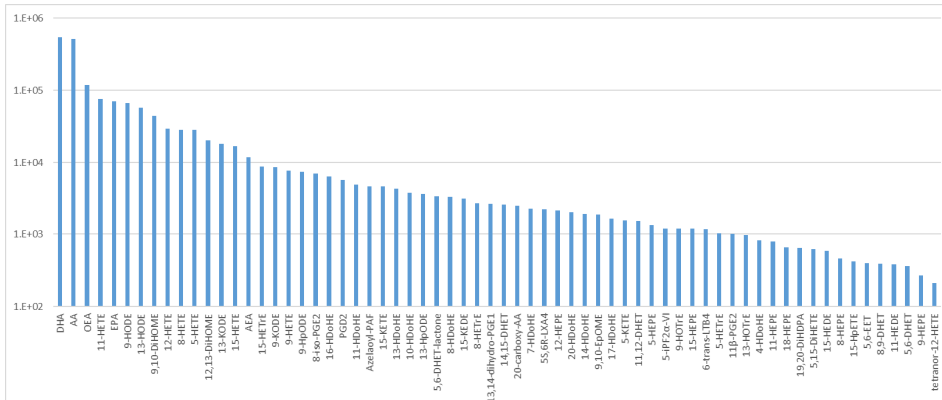


Fig. 2 Peak Intensities (Heights) of 66 Target Compounds Acquired in the Analysis of Commercially Available Human Plasma Using the LCMS-8060

HPLC Conditions

Analytical column : Phenomenex Kinetex™ C8 (2.1 mm I.D. × 150 mm L., 2.6 µm)
 Mobile phase A : 0.1 % formic acid/water
 Mobile phase B : Acetonitrile
 Time program : 10 % B. (0 min) → 25 % B. (5.0 min) → 35 % B. (10.0 min) → 75 % B. (20.0 min) → 95 % B. (20.1 - 25.0 min)
 Flow rate : 0.4 mL/min.
 Injection volume : 5 µL
 Column oven temperature : 40 °C

MS Conditions (LCMS-8060)

Ionization method : ESI (Positive/Negative)
 Nebulizer gas flow rate : 2.5 L/min.
 Drying gas flow rate : 10.0 L/min.
 Heating gas : 10.0 L/min.
 DL temperature : 250 °C
 Heat block temperature : 400 °C
 Interface temperature : 270 °C
 CID gas pressure : 230 kPa

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