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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 | 11β-13,14-dihydro-15-keto Prostaglandin $F_{2\alpha}$ | AA | 109 | (±)5,6-DHET-lactone | AA | 163 | Resolvin $D_4$ | DHA |
| 2 | (±)9,10-DiHOME | LA | 56 | 15-keto Prostaglandin $E_2$ | AA | 110 | 5(S)-HpETE | AA | 164 | 7(R)-Maresin 1 | DHA |
| 3 | 13(S)-HODE | LA | 57 | 13,14-dihydro Prostaglandin $F_{1\alpha}$ | 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 $D_5$ | DHA |
| 5 | (±)9-HpODE | LA | 59 | 13,14-dihydro-15-keto Prostaglandin $F_{2\alpha}$ | 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 $F_{2\alpha}$ | ADA | 171 | (±)17-HDHA | DHA |
| 10 | (±)9(10)-EpOME | LA | 64 | 13,14-dihydro-15-keto Prostaglandin $D_2$ | AA | 118 | 2,3-dinor Thromboxane $B_1$ | DGLA | 172 | (±)13-HDHA | DHA |
| 11 | 9(S)-HOTrE | ALA | 65 | Leukotriene $C_4$ | AA | 119 | 6-keto Prostaglandin $E_1$ | DGLA | 173 | (±)10-HDHA | DHA |
| 12 | 13(S)-HOTrE | ALA | 66 | 11-trans $LTC_4$ | AA | 120 | 2,3-dinor Prostaglandin $E_1$ | DGLA | 174 | (±)14-HDHA | DHA |
| 13 | 13(S)-HpOTrE | ALA | 67 | Leukotriene $D_4$ | AA | 121 | Thromboxane $B_1$ | DGLA | 175 | (±)11-HDHA | DHA |
| 14 | 11(S)-HEDE | EDA | 68 | Leukotriene $E_4$ | AA | 122 | 8-iso Prostaglandin $F_{1\alpha}$ | DGLA | 176 | (±)7-HDHA | DHA |
| 15 | (±)15-HEDE | EDA | 69 | Leukotriene $F_4$ | AA | 123 | Prostaglandin $F_{1\alpha}$ | DGLA | 177 | (±)8-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 $D_1$ | 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 $D_1$ | DGLA | 182 | Docosahexaenoic Acid (DHA) | DHA |
| 21 | tetranor-PGAM | AA | 75 | Prostaglandin $B_2$ | AA | 129 | 8-iso Prostaglandin $A_1$ | DGLA | 183 | Prostagrandin $F_{2\alpha}$ Ethanolamide | EA |
| 22 | 20-hydroxy Prostaglandin $F_{2\alpha}$ | AA | 76 | 8,12-iso-$iPF_{2\alpha}$-VI 1,5- lactone | AA | 130 | Prostaglandin $A_1$ | DGLA | 184 | Prostagrandin $E_2$ Ethanolamide | EA |
| 23 | 20-hydroxy Prostaglandin $E_2$ | AA | 77 | 8(S),15(S)-DiHETE | AA | 131 | 15(S)-HETrE | DGLA | 185 | Prostagrandin $E_1$ ethanolamide | EA |
| 24 | 18-carboxy dinor $LTB_4$ | AA | 78 | 6-trans $LTB_4$ | AA | 132 | 8(S)-HETrE | DGLA | 186 | Prostagrandin $D_2$ Ethanolamide | EA |
| 25 | 13,14-dihydro-15-keto-tetranor Prostaglandin $F_{1\beta}$ | AA | 79 | 5(S),15(S)-DiHETE | AA | 133 | 5(S)-HETrE | DGLA | 187 | $LTB_4$ ethanolamide | EA |
| 26 | 2,3-dinor-8-iso Prostaglandin $F_{2\alpha}$ | AA | 80 | 13,14-dihydro-15-keto Prostaglandin $A_2$ | AA | 134 | Δ17-6-keto Prostaglandin $F_{1\alpha}$ | 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 $F_{1\alpha}$ | AA | 82 | 13,14-dihydro-15-keto Prostaglandin $J_2$ | AA | 136 | 8-iso Prostaglandin $F_{3\alpha}$ | EPA | 190 | (±)8(9)-EET ethanolamide | EA |
| 29 | 2,3-dinor-11β-Prostaglandin $F_{2\alpha}$ | AA | 83 | 12-oxo $LTB_4$ | AA | 137 | Thromboxane $B_3$ | EPA | 191 | (±)5(6)-EET ethanolamide | EA |
| 30 | 6-keto-Prostaglandin $F_{1\alpha}$ | AA | 84 | tetranor-12(S)-HETE | AA | 138 | Prostaglandin $F_{3\alpha}$ | 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 $B_3$ | EPA | 193 | OEA (oleoyl ethanolamide) | EA |
| 32 | 20-carboxy leukotriene $B_4$ | AA | 86 | Leukotriene $B_5$ | AA | 140 | Prostaglandin $E_3$ | EPA | 194 | Lyso-PAF C-16 | |
| 33 | 20-hydroxy leukotriene $B_4$ | AA | 87 | (±)14(15)-DiHET | AA | 141 | Prostaglandin $D_3$ | EPA | 195 | PAF C-16 | |
| 34 | 11-dehydro-2,3-dinor Thromboxane $B_2$ | AA | 88 | 12(S)-HHTrE | AA | 142 | Lipoxin $A_5$ | EPA | 196 | Azelaoyl PAF | |
| 35 | 13,14-dihydro-15-keto-tetranor Prostaglandin $E_2$ | AA | 89 | (±)11(12)-DiHET | AA | 143 | Leukotriene $B_5$ | EPA | 197 | tetranor-PGEM-$d_4$ | ISTD |
| 36 | 6,15-diketo-13,14-dihydro Prostaglandin $F_{1\alpha}$ | AA | 90 | (±)8(9)-DiHET | AA | 144 | (±)17,18-DiHETE | EPA | 198 | 6-keto-Prostaglandin $F_{1\alpha}$-$d_4$ | ISTD |
| 37 | $iPF_{2\alpha}$-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 $F_{2\alpha}$ | AA | 92 | (±)5(6)-DiHET | AA | 146 | (±)5(6)-DiHETE | EPA | 200 | Prostaglandin $F_{2\alpha}$-$d_4$ | ISTD |
| 39 | 8-iso Prostaglandin $F_{2\alpha}$ | AA | 93 | 19(S)-HETE | AA | 147 | (±)18-HEPE | EPA | 201 | Prostaglandin $E_2$-$d_4$ | 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 | 11β-Prostaglandin $F_{2\alpha}$ | AA | 95 | 20-HETE | AA | 149 | 11(S)-HEPE | EPA | 203 | Leukotriene $C_4$-$d_5$ | ISTD |
| 42 | (±)5-$iPF_{2\alpha}$-VI | AA | 96 | (±)18-HETE | AA | 150 | 8(S)-HEPE | EPA | 204 | Leukotriene $D_4$-$d_5$ | ISTD |
| 43 | 8-iso-15-keto Prostaglandin $F_{2\alpha}$ | AA | 97 | (±)17-HETE | AA | 151 | 9(S)-HEPE | EPA | 205 | Prostaglandin $A_2$-$d_4$ | ISTD |
| 44 | Prostaglandin $F_{2\alpha}$ | AA | 98 | (±)16-HETE | AA | 152 | 12(S)-HEPE | EPA | 206 | Leukotriene $B_4$-$d_4$ | ISTD |
| 45 | 8-iso-13,14-dihydro-15-keto Prostaglandin $F_{2\alpha}$ | AA | 99 | 15(S)-HETE | AA | 153 | 5(S)-HEPE | EPA | 207 | (±)14(15)-DiHET-$d_{11}$ | ISTD |
| 46 | 8-iso Prostaglandin $E_2$ | AA | 100 | 11(S)-HETE | AA | 154 | 15(S)-HpEPE | EPA | 208 | 15(S) HETE-$d_8$ | ISTD |
| 47 | Prostaglandin $E_2$ | AA | 101 | 8(S)-HETE | AA | 155 | 12(S)-HpEPE | EPA | 209 | 12(S)-HETE-$d_8$ | ISTD |
| 48 | 11-dehydro Thromboxane $B_2$ | AA | 102 | 15-OxoETE | AA | 156 | 5(S)-HpEPE | EPA | 210 | 5(S)-HETE-$d_8$ | ISTD |
| 49 | 15-keto Prostaglandin $F_{2\alpha}$ | AA | 103 | 15(S)-HpETE | AA | 157 | (±)17(18)-EpETE | EPA | 211 | PAF C-16-$d_4$ | ISTD |
| 50 | 11β-Prostaglandin $E_2$ | AA | 104 | 12(S)-HETE | AA | 158 | (±)14(15)-EpETE | EPA | 212 | (±)11(12)-EET-$d_{11}$ | 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 $D_3$ | DHA | 214 | AA-$d_8$ | ISTD |
| 53 | Prostaglandin $D_2$ | AA | 107 | 12(S)-HpETE | AA | 161 | Resolvin $D_2$ | DHA | | | |
| 54 | 15-keto Prostaglandin $F_{1\alpha}$ | 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 $PGE_2$-$d_4$ when analyzing the mixed internal standard solution. Although the predicted retention time of $PGE_2$ is 10.700 minutes (registered information), inputting the measured time of 10.584 minutes for $PGE_2$-$d_4$ 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 $PGE_2$ in the mixed target solution acquired in the same batch analysis. The measured retention time of $PGE_2$ 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.

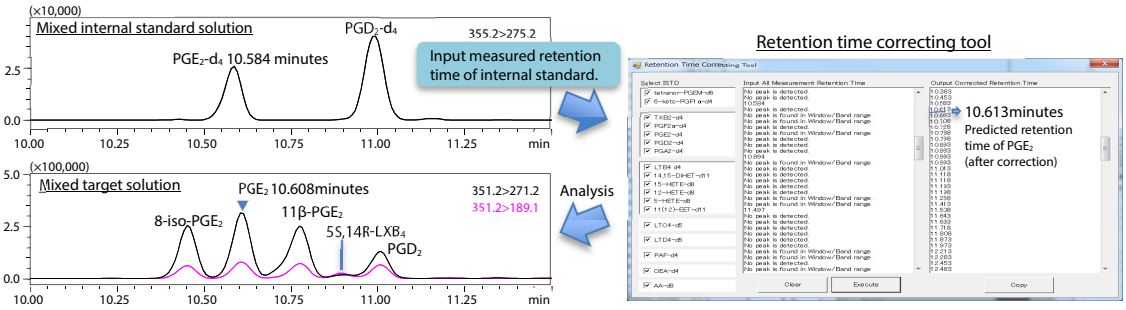


**Fig. 1 MRM Chromatogram of $PGE_2$-$d_4$ from Analysis of Mixed Internal Standard Solution (Upper Left), GUI Indicating Predicted Retention Time Corrected Using the Retention Time Correcting Tool (Right), and MRM Chromatogram of $PGE_2$ Acquired from Analysis of Mixed Target Solution (Lower Left)**

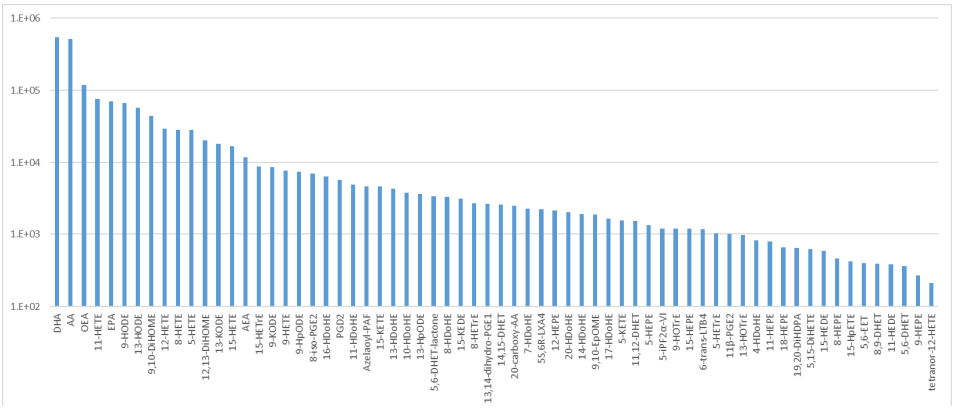

**Fig. 2 Peak Intensities (Heights) of 66 Target Compounds Acquired in the Analysis of Commercially Available Human Plasma Using the LCMS-8060**

**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 |

Acknowledgments:
We would like to thank Ono Pharmaceutical Co., Ltd. for their cooperation in the development of LC/MS/MS Method Package for Lipid Mediators Ver. 3.

Note: The product described in this document has not been approved as a medical device under the Pharmaceutical and Medical Device Act of Japan. This product is for research use only.



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