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Technical Report

Development of a Comprehensive Detection Method of Eicosanoids and Platelet Activating Factor Using Ultra-High Performance Liquid Chromatography/Mass Spectrometry

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Abstract:

Using LCMS-8040, simultaneous detection method of eicosanoids and PAF was developed. 54 MRM transitions for 50 eicosanoids and PAF (platelet activation factor) were optimized. Limit of quantitation for several targets was in the subpicogram range.

Keywords: Eicosanoids, Lipid Mediator Molecules, Kinetex C8, Positive/Negative Ion-Switching

1. Introduction

- Comprehensive analysis of eicosanoids and their isomers or metabolites is expected to be important for disease research.
- Selected reaction monitoring (SRM) using liquid chromatography/mass spectrometry (LCMS) has been widely used for detecting those eicosanoids.
- Although SRM is both selective and sensitive, performing a large number of SRMs can cause poor sensitivity.
- To comprehensively analyze eicosanoids, a quantitative method using a UHPLC/MS system was developed.

2. Method

- Fifty eicosanoids, PAF, and PAF-d_4 (total 52 compounds) were purchased from Cayman Chemical Company. All samples were dissolved in methanol.
- The separation was conducted using a Shimadzu *Nexera* UHPLC system equipped with a triple quadrupole mass spectrometer LCMS-8040 (ion optics modified).
- Shim-Pack XR-ODS III (2.2 µm, 2.0 × 150 mm, Shimadzu), CAPCELL PAK C1 B IF-2 (2 µm, 2.1 × 100 mm, Shiseido), and Kinetex CB (2.6 µm, 2.1 × 150 mm, Phenomenex) columns were used.
- 0.1% formic acid (FA) and acetonitrile (ACN) were used as mobile phases A and B, respectively.

3. Results and Discussion

- A mixture of 50 compounds (100 pg each) to include 49 eicosanoids and PAF-d_4 were analyzed using a UHPLC/MS/MS system.
- A simultaneous detection method using fifty optimized SRMs and single ion monitoring (SIM) of *m/z* 319 in negative mode was developed.
- All compounds except for 5,6 DHET lactone appeared in the chromatograms. See Figs. 1 and 2.
- Elution times were identified for all detected compounds.
- The four peaks in a blue-line box in Fig. 1 included two compounds in each peak, such as $PGF_{2\alpha}$ and *ent*-$PGF_{2\alpha}$, that have the same *m/z* pair of precursor and product ion.
- The three peaks with a blue-dotted line box in Fig. 2 also included two compounds but those two compounds have different precursor ions or product ions.
- Peak broadening made it difficult to observe LTC_4 and PAF-d_4 as seen in Fig. 1.
- This broadening was improved using the CAPCELL PAK column as seen in Fig. 2.
- Positive mode SRM optimization was applied to lactone species as seen in Fig. 3.
- Both positive and negative mode SRMs were applied to PAF as seen in Fig. 4.
- Further improvement of chromatographic separation was carried out using the Kinetex C8 column with the results shown in Fig. 5.
- The limits of quantification (LOQ) for 10 compounds are listed Table 1.
- A linear calibration range spanning from 0.5 pg to 1000 pg (R^2 > 0.999) was obtained.
- Sub-picogram sensitivity was obtained for a number of eicosanoids and isomers even with the simultaneous detection of 54 transitions.
- The method will be expanded to encompass more fatty acid metabolites and other mediators without compromising throughput or sensitivity.

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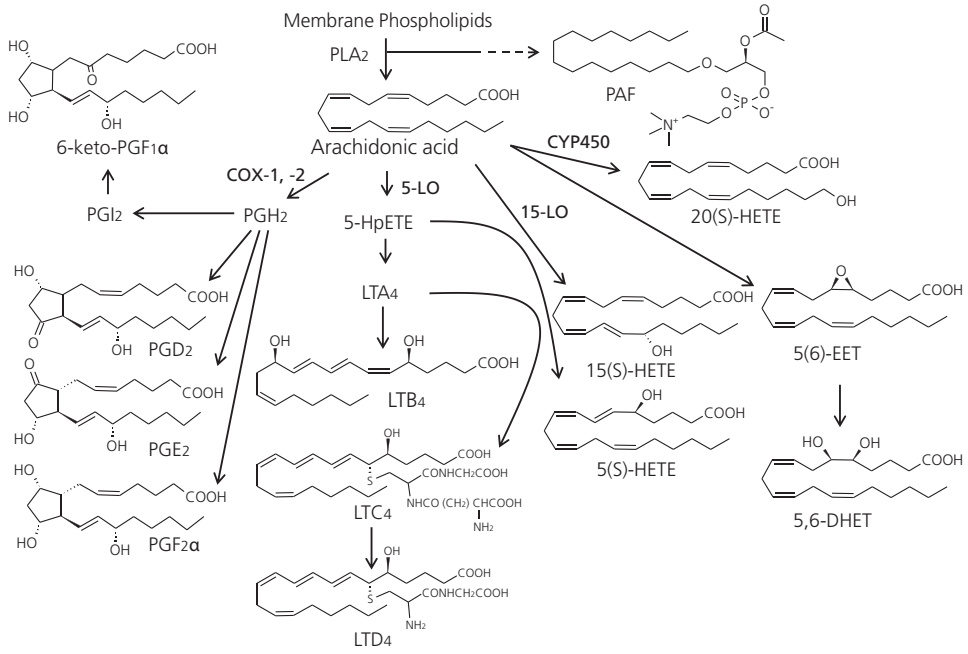
4. Target Compounds

Arachidonic acid cascade



Abbreviations:
PG, Prostaglandin: HETE, Hydroxyeicosatetraenoic acid; LT. Leukotriene: EET, Epoxyeicosanoic acid; DHET, Dihydroxyeicosatrienoic acid; PLA_2, Phospholipase A_2; COX. cyclooxygenase; LO, Lipoxygenase; CYP5, Cytochrome P450; PAF, Platelet activating factor

5. Instrument

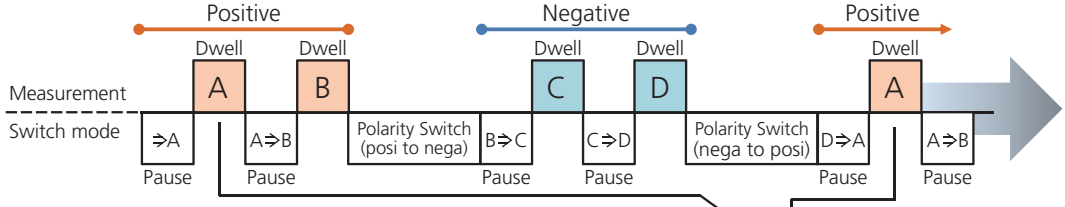
LC MS/MS system

LCMS-8040 with modified ion optics

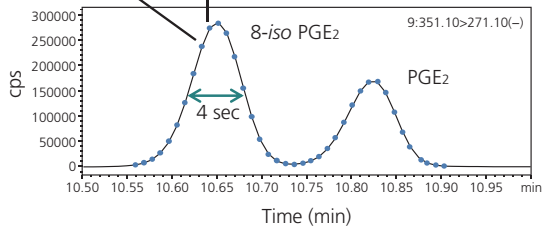
SRM (max) : 500 channels/sec
Polarity Switch: 15 msec
Pause time : 1 msec
Scan speed : 15,000 u/sec

"Nexera" UHPLC system 130 MPa (Max)

SRM Time Schedule



- At least 5 data points above 50 % intensity of the peak are intended.
- 10 msec timing was applied to each SRM transition, including 1 msec pause and 9 msec dwell times.
- 54 transitions for one cycle including 15 msec polarity switching times yielded a loop time of 0.57 seconds.



6. Simultaneous SRM chromatograms of 50 compound mixture (100 pg each)

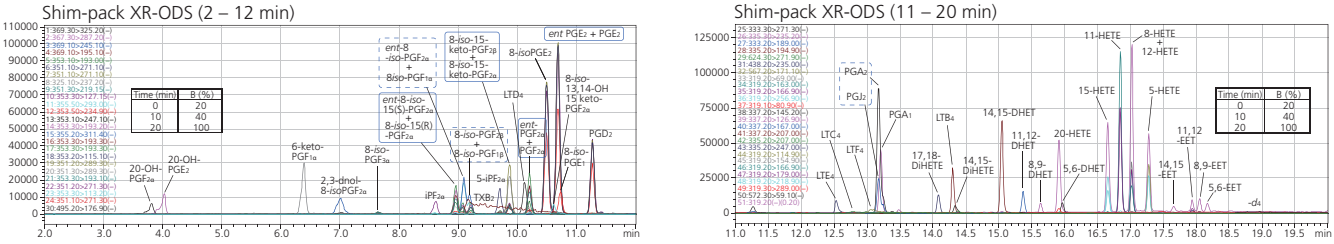


Fig. 1 MRM Chromatogram of 50 Components Measured Using the Shim-pack XR-ODS III

Fig. 1 shows a chromatographic profile of 50 compounds using a Shim-Pack ODS XR-III column (2.2 µm I.D. × 150 mm, Shimadzu). The gradient flow rate was 0.4 mL/min. Simultaneous detection was performed using an SIM at *m/z* 319 and 50 SRM transitions.

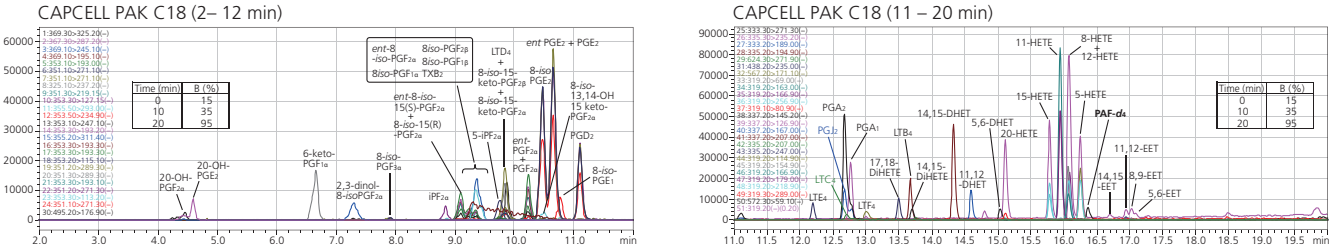


Fig. 2 MRM Chromatogram of 50 Components Measured Using the CAPCELL PAK ODS IF-II

Fig. 2 shows a simultaneous SRM chromatogram of 50 compounds using the CAPCELL PAK ODS IF-II column (2.1 mm I.D. × 100 mm, Shiseido) at a gradient flow rate of 0.5 mL/min. The detection method was the same as in Fig. 1.

7. SRM optimization

Positive mode was used for lactones.

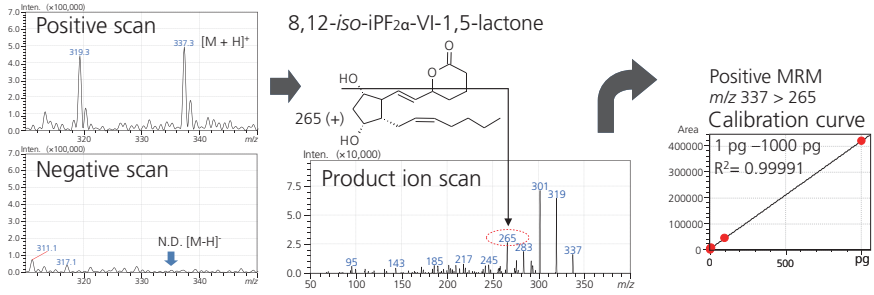
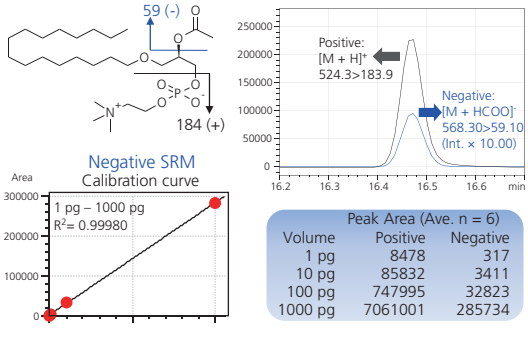


Fig. 3 Optimization of MRM Parameters for 8,12-*iso*-iPF$_{2\alpha}$-VI-1,5-lactone

Simultaneous positive and negative SRM of PAF was used.



	Peak Area (Ave. n = 6)	
Volume	Positive	Negative
1 pg	8478	317
10 pg	85832	3411
100 pg	747995	32823
1000 pg	7061001	285734

Fig. 4 Optimization of MRM Parameters for PAF

Fig. 3 (upper) shows positive SRM optimization for 8,12-*iso*-iPF$_{2\alpha}$-VI-1,5-lactone. Almost all fatty acids were detected in negative ion mode with the exception of the lactone species which were detected in positive mode.

Fig. 4 (below) shows PAF being detected in positive mode with 20 times greater intensity than in negative mode. Both positive and negative modes were used for PAF detection.

8. Quantitation

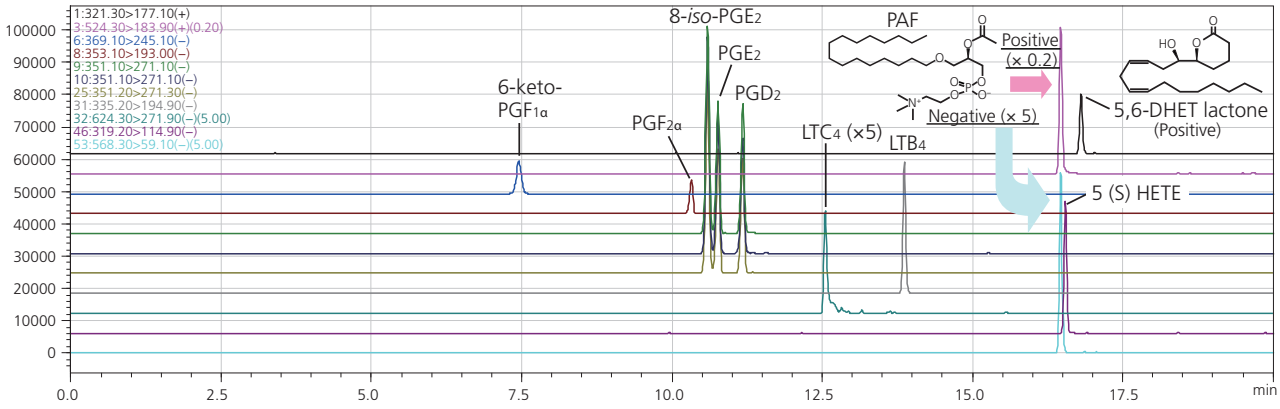


Fig. 5 Simultaneous detection of 10 compounds (100 pg each)

Table 1 Quantitation results of ten compounds (n = 6)

MS condition	
Time	0 - 20 min
SRM	53
SIM	*m/z* 319
Dwell time	9 ms
Pause time	1 ms
PN switch	15 ms
Loop time	0.57 s

Name	RT	Q1/Q3 (*m/z*)	R^2	Dynamic range
6-keto-PGF1α	7.473	369>245	0.99949	1 pg - 1000 pg
PGF2α	10.345	353>193	0.99945	1 pg - 1000 pg
8-*iso*-PGE2	10.614	351>271	0.99952	0.5 pg - 1000 pg
PGE2	10.789	351>271	0.99961	0.5 pg - 1000 pg
PGD2	11.196	351>271	0.99970	0.5 pg - 1000 pg
LTC4	12.561	624>271	0.99970	5 pg - 1000 pg
LTB4	13.869	335>194	0.99976	0.5 pg - 1000 pg
PAF	16.457	568>59	0.99978	1pg - 1000 pg
5 (S)-HETE	16.542	319>115	0.99982	0.5 pg - 1000 pg
5-6 DHET lactone	16.798	321>177 (+)	1.00000	1pg - 1000 pg

Fig. 5 and Table 1 show a chromatographic separation of a 10 compound mixture performed using a KINETEX C8 column for quantitation. 52 SRM transitions, including 49 for eicosanoids and 3 for PAF in both positive and negative modes, PAF-*d*4 in negative mode only, and a single SIM transition were conducted.

The limit of quantitation was 0.5 pg and a linear range from 0.5 pg to 5 pg was established. The data for PAF was obtained in negative mode. Because lyso-phosphatidylcholine will be co-eluted at the same elution time and same transition in positive mode when biological sample was analyzed.

Reference

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