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Targeted Lipidomics of Oxylipins (Oxygenated Fatty Acids)

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APPLICATION BENEFITS

Here, we present a high-throughput approach for profiling bioactive oxylipins (oxidized fatty acids) in plasma. The combination of mixed mode solid-phase extraction (Oasis® MAX SPE) and UPLC®-ESI-MRM mass spectrometry (Xevo® TQ-S) provides a comprehensive analysis of oxylipins in a targeted analytical workflow. Retention times and transitions of 107 oxylipins (including prostaglandins, prostacyclines, thromboxanes, dihydroprostaglandins, and isoprostanes) were annotated for routine high-throughput analysis of plasma samples. Considering the prominent roles played by oxylipins in health and disease (*e.g.*, inflammation), such a UPLC-based assay could become important in nutritional research, clinical research, and drug discovery and development.

WATERS SOLUTIONS

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KEY WORDS

UPLC-MS/MS, fatty acids, metabolomics, lipidomics, triple quadrupole, oxylipins, multiple reaction monitoring, MRM, Xevo TQ-S

INTRODUCTION

Oxylipins are signaling lipids that play prominent roles in the physiological regulation of many key biological processes, such as the relaxation and contraction of smooth muscle tissue, blood coagulation, and most notably inflammation. Alterations in oxylipin pathways have been associated with response to cardiovascular diseases, host defense, tissue injury and surgical intervention. The ability to semi-quantitatively profile a wide range of oxylipin in plasma samples could help our understanding of their roles in health and disease, as well as serve as biomarkers for disease diagnosis or prognosis.

Oxylipins are produced via enzymatic (e.g., mono- or dioxygenase-catalyzed) or non enzymatic oxygenation of an array of both omega-6 polyunsaturated fatty acid substrates (e.g., linoleic acid, dihomo-γ-linolenic acid, adrenic acid and arachidonic acid) and omega-3 polyunsaturated fatty acid substrates (α-linolenic acid, acid, eicosapentaenoic acid, and docosahexaenoic acid) (Figure 1A and 1B). Three major enzymatic pathways are involved in their generation: cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CYP). These pathways are important drug targets for multiple diseases (Figure 1A and 1B).

The main challenge for the measurement of oxylipins is the extremely low endogenous concentration of such lipid species and their limited stability. Furthermore, oxylipins are not stored in tissues but are formed on demand by liberation of precursor fatty acids from esterified forms. Lastly, the same fatty acid can be oxidized in different positions of its acyl chain leading to many isomeric species, each with specific metabolic actions. As a consequence, this requires a rapid, highly-sensitive, and specific analytical method.

Historically, measurements of oxylipins have been performed using radiometric and enzymatic immunoassays, which often lacked specificity and targeted only few compounds. GC-MS methodology has also been used, but this still requires multi-step procedures involving derivatization of the oxylipins to increase their volatility and stability.

Recently, various LC-MS methodologies have been described to monitor a broad range of low abundance oxylipins.[1-5] In particular the method by Strassburg et al.[2] reports on a wide range of oxylipins produced both enzymatically and non-enzymatically in human plasma. Although such methods are both sensitive and specific, there is an increasing demand for a comprehensive and high-throughput screening method to enable wide-ranging lipidomic studies.

Here we report a high-throughput assay for the profiling of over 100 oxylipins, including prostaglandins, prostacyclines, thromboxanes, dihydroprostaglandins, and isoprostanes, in plasma samples.

Internal standard	Cayman #number	MRM transition	RT (min)	Cone voltage (V)	Collision energy (eV)
d4-6-Keto PGF1α	315210	373.20 >167.20	2.28	35	15
d4-TBX2	319030	373.20 >173.10	2.86	35	15
d4-PGF2α	316010	357.30 >197.20	3.12	35	20
d4-PGE2	314010	355.20 >275.20	3.19	40	16
d4-PGD2	312010	355.20 >275.20	3.31	10	16
d5-LTE4	10007858	443.10 >338.00	4.11	35	20
d4-LTB4	320110	339.20 >197.10	4.48	35	15
d4-12,13-DiHOME	10009994	317.30 >185.20	4.56	35	15
d4-9,10-DiHOME	10009993	317.30 >203.20	4.69	35	15
d11-14,15-DiHETrE	10008040	348.30 >207.10	4.77	35	15
d4-15-deoxy-Δ12,14-PGJ2	318570	319.20 >275.30	5.20	35	15
d6-20-HETE	390030	325.20 >281.10	5.24	20	18
d4-9-HODE	338410	299.20 >172.10	5.53	35	20
d8-12-HETE	334570	327.30 >184.20	5.78	35	20
d8-5-HETE	334230	327.30 >116.10	5.97	35	20

Table 1. Internal standards used for profiling natural oxylipins in plasma and optimal UPLC-ESI-MS settings.

EXPERIMENTAL

Sample preparation

Materials

All chemicals were purchased from Sigma-Aldrich (Germany) and were of analytical grade or higher purity. Oxylipins standards were purchased from Cayman Chemicals (Ann Arbor, MI), Biomol (Plymouth Meeting, PA), and Larodan (Malmö, Sweden). For mixed mode solid phase extraction we used Waters Oasis MAX 3 cc Vac Cartridge, 60 mg Sorbent per Cartridge, 30 µm Particle Size (p/n 186000367). An internal standard mixture containing 16 isotopically labeled compounds was used (Table 1).

Sample pre-treatment

(dilution, performed in borosilicate glass tubes 13 x 100 mm):

1. Add 200 µL of 10% glycerol in water to a glass tube
2. Add 50 – 250 µL of plasma (maximum sample volume available) sample to the tube and mix thoroughly
3. Add 5 µL of 10 mg/mL BHT in ethanol and mix thoroughly
4. Add 5 µL of internal standard solution (400 ng/mL) and mix
5. Make up the total sample volume to 3 mL with 25% MeCN(aq) and mix thoroughly

MAX mixed mode solid phase extraction

1. Condition Oasis MAX SPE Cartridge with 3 mL of MeCN
2. Condition Oasis MAX SPE Cartridge with 3 mL of 25% MeCN(aq)
3. Load the entire pre-treated sample onto the Oasis MAX SPE Cartridge
4. Wash Oasis MAX SPE Cartridge with 3 mL of 25% MeCN(aq)
5. Wash Oasis MAX SPE Cartridge with 3 mL of MeCN
6. Elute analytes with 1.3 mL of 1% Formic in MeCN8*
7. Transfer eluate to a glass HPLC vial (TruView™ Max Recovery Vial)
8. Evaporate eluate down until only the glycerol remains (under nitrogen at 40 °C)
9. Add 60 µL of 50/50 MeOH/MeCN and mix thoroughly
10. Inject 3 µL onto the UPLC-MS/MS System

**Sample eluted into a glass tube containing 200 µL of 10% glycerol in methanol*

UPLC conditions

System:	ACQUITY UPLC® System in negative ESI mode
Column:	ACQUITY UPLC BEH $C_{18,}$ 1.7 µm, 2.1 x 100 mm
Mobile phase A:	H_2O + 0.1% acetic acid
Mobile phase B:	ACN/IPA (90/10 v/v)
Flow rate:	0.6 mL/min
Column temp.:	40 °C
Volume:	3.0 µL

Elution gradient:

Min	A%	B%	Curve
0.0	75	25	
1.0	75	25	6
8.0	5	95	6
8.50	5	95	6
8.51	75	25	6
10.00	75	25	6

MS conditions

For optimum reproducibility of retention times we recommend the following tubing to connect UPLC analytical column to ESI probe: PEEK Tubing, 1/16 in. (1.6 mm) O.D. X 0.004 in. (0.100 mm) I.D. X 5 ft (1.5 m) length, cut to 400 mm in length.

MS system:	Xevo TQ-S in negative ESI mode
Acquisition mode:	MRM
Capillary voltage:	2.5 kV
Cone voltage:	10-40 V (compound Specific, default = 35 V)
Source temp.:	150 °C
Desolvation gas temp.:	600 °C
Desolvation gas flow:	1000 L/h
Cone gas flow:	150 L/h
Collision energy:	15-20 V (compound Specific, default = 15 V)

Data management

TargetLynx Application Manager

RESULTS AND DISCUSSION

The primary focus of this work was to provide a high-throughput method to profile bioactive oxylipins in plasma samples.

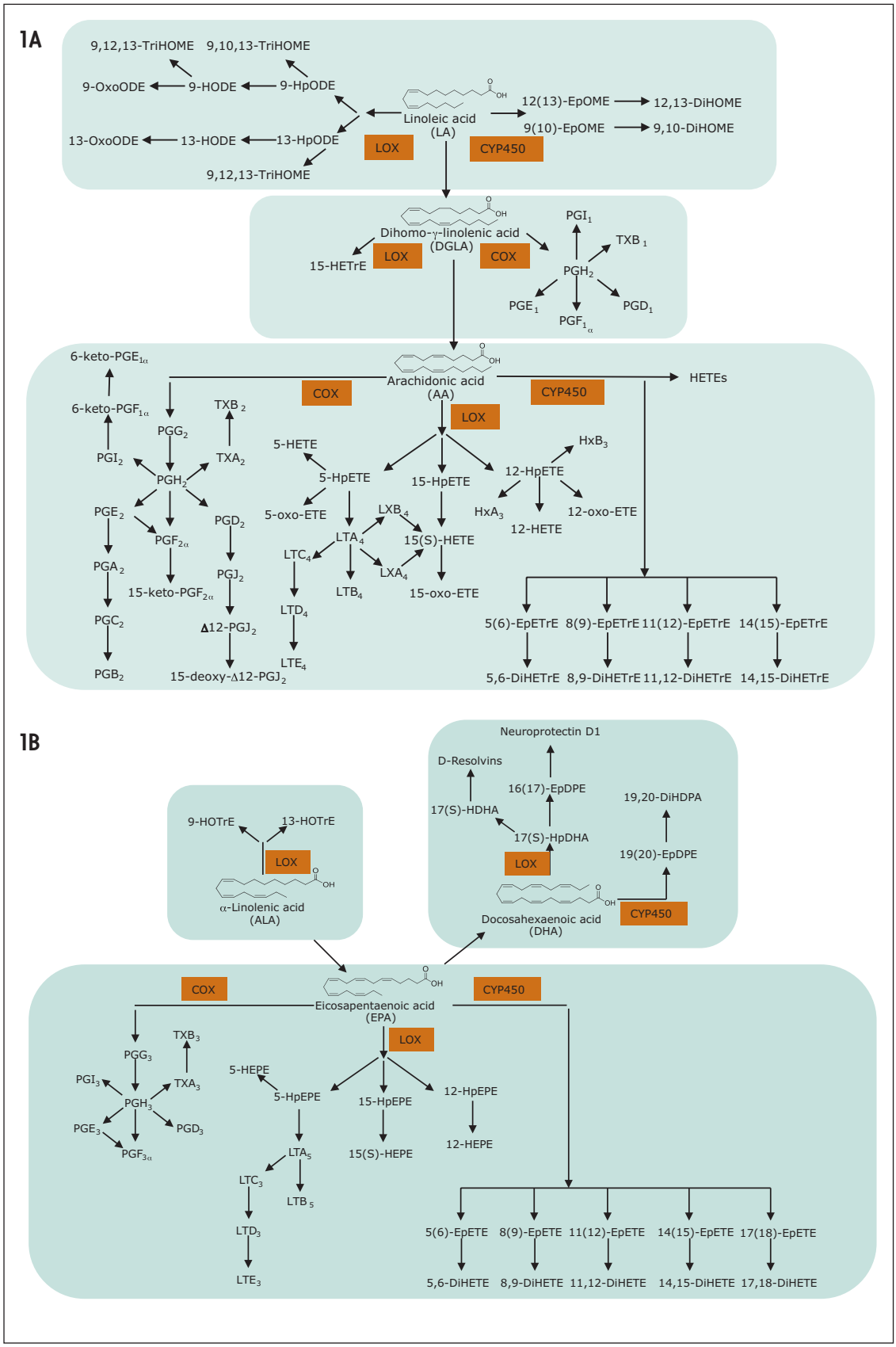

Figure 1. A. Schematic outline of the oxylipins of the omega-6 series produced by linoleic acid C_{18}:2 (LA), dihomo-γ-linoleic acid C20:3 (DHGLA), and arachidonic acid C20:4 (AA), via the cyclooxygenase (COX), lipoxygenase (LOX), CYP-450, or free radical catalyzed pathways.

B. Schematic outline of the oxylipins of the omega-3 series produced by α-linolenic acid C_{18}:3 (ALA), eicosapentaenoic acid C20:5 (EPA), and docosahexaenoic acid C22:6 (DHA), via the COX, LOX, CYP-450, or free radical catalyzed pathways.

Abbreviations: dihydroxyeicosatetraenoic acid (DiHETE), epoxy-octadecenoic acid (EpOME), hydroxy-eicosatrienoic acid (HETrE), hydroxy-eicosatetraenoic acid (HETE), hydroxy-heptadecatrienoic acid (HHTrE), hydroxy-octadecadienoic acid (HODE), hydroxy-eicosapentaenoic acid (HEPE), oxo-eicosatetraenoic acid (KETE), oxo-octadecadienoic acid (KODE), prostaglandin (PG), thromboxane (TX).

	Compound name	M1	M2	RT	I	Precursor	Class	Pathway
1	Tetranor-PGFM	329.2	311.2	0.48	(d4) PGF2α	AA	Prostanoid	COX
2	Tetranor-PGEM	327.1	309.2	0.53	(d4) PGE2	AA	Prostanoid	COX
3	20-hydroxy PGE2	367.2	287.2	1.01	(d4) PGE2	AA	Prostanoid	COX
4	Δ17-6-keto PGF1α	367.2	163.1	1.76	(d4) 6-keto PGF1α	AA	Prostanoid	COX
5	6-keto PGF1α	369.2	163.1	2.27	(d4) 6-keto PGF1α	AA	Prostanoid	COX
6	2,3-dinor-11b PGF2α	325.2	145.1	2.27	(d4) PGF2α	AA	Prostanoid	COX
7	(d4) 6-keto PGF1α	373.2	167.2	2.28	ISTD			
8	20-carboxy LTB4	365.2	347.2	2.35	(d4) LTB4	AA	Leukotriene	LOX
9	6-keto PGE1	367.2	143.1	2.37	(d4) PGE2	AA	Prostanoid	COX
10	20-hydroxy LTB4	351.2	195.1	2.46	(d4) LTB4	AA	Leukotriene	LOX
11	TXB3	367.2	169.1	2.48	(d4) TXB2	EPA	Thromboxane	COX
12	PGF3α	351.2	193.2	2.75	(d4) PGF2α	EPA	Prostanoid	COX
13	TXB1	371.2	171.1	2.79	(d4) TXB2	DGLA	Thromboxane	COX
14	PGE3	349.2	269.2	2.83	(d4) PGE2	EPA	Prostanoid	COX
15	(d4) TXB2	373.2	173.1	2.86	ISTD			
16	8-iso PGF2α	353.2	193.2	2.87	(d4) PGF2α	AA	Isoprostane	non enzymatic
17	TXB2	369.2	169.1	2.88	(d4) TXB2	AA	Thromboxane	COX
18	PGD3	349.2	269.2	2.92	(d4) PGD2	EPA	Prostanoid	COX
19	11β-PGF2α	353.2	193.2	2.93	(d4) PGF2α	AA	Prostanoid	COX
20	(+/-) 5-iPF2α-VI	353.2	115.1	3.04	(d4) PGF2α	AA	Isoprostane	non enzymatic
21	9,12,13-TriHOME	329.2	211.2	3.07	(d4) 9(S)-HODE	LA	Triol	LOX
22	9,10,13-TriHOME	329.2	171.1	3.12	(d4) 9(S)-HODE	LA	Triol	LOX
23	(d4) PGF2α	357.3	197.2	3.12	ISTD			
24	PGF2α	353.2	193.2	3.14	(d4) PGF2α	AA	Prostanoid	COX
25	PGF1α	355.2	293.2	3.14	(d4) PGF2α	DGLA	Prostanoid	COX
26	(d4) PGE2	355.2	275.2	3.19	ISTD			
27	PGE2	351.2	271.2	3.2	(d4) PGE2	AA	Prostanoid	COX
28	11β-PGE2	351.2	271.2	3.25	(d4) PGE2	AA	Prostanoid	COX
29	PGK2	349.2	205.1	3.28	(d4) PGE2	AA	Prostanoid	COX
30	15-keto PGF2α	351.2	219.1	3.28	(d4) PGF2α	AA	Prostanoid	COX
31	5(S),14(R)-Lipoxin B4	351.2	221.2	3.29	(d4) LTB4	AA	Lipoxin	LOX
32	PGE1	353.2	273.2	3.29	(d4) PGE2	DGLA	Prostanoid	COX
33	(d4) PGD2	355.2	275.2	3.31	ISTD			
34	PGD2	351.2	271.2	3.32	(d4) PGD2	AA	Prostanoid	COX
35	PGD1	353.2	273.2	3.32	(d4) PGD2	DGLA	Prostanoid	COX
36	11β-13,14-dihydro-15-keto PGF2α	353.2	113.2	3.35	(d4) PGF2α	AA	Prostanoid	COX
37	15-keto PGF1α	353.2	221.1	3.37	(d4) 6-keto PGF1α	DGLA	Prostanoid	COX
38	13,14-dihydro PGF2α	355.2	275.2	3.39	(d4) PGF2α	AA	Prostanoid	COX
39	13,14-dihydro-15-keto PGE2	351.2	175.2	3.54	(d4) PGE2	AA	Prostanoid	COX
40	13,14-dihydro-15-keto PGF2α	353.2	183.1	3.56	(d4) PGF2α	AA	Prostanoid	COX
41	5(S),6(R)-Lipoxin A4	351.2	115.1	3.58	(d4) LTB4	AA	Lipoxin	LOX
42	5(S),6(S)-Lipoxin A4	351.2	115.1	3.68	(d4) LTB4	AA	Lipoxin	LOX
43	13,14-dihydro-15-keto PGF1α	355.2	193.2	3.72	(d4) PGF2α	AA	Prostanoid	COX
44	13,14-dihydro-15-keto PGD2	351.2	175.2	3.77	(d4) PGD2	AA	Prostanoid	COX

	Compound name	M1	M2	RT	I	Precursor	Class	Pathway
45	1α,1b-dihomo PGF2α	381.3	337.2	3.77	(d4) PGF2α	ADA	Prostanoid	COX
46	14,15-LTE4	438.2	333.2	3.78	(d3) LTE4	AA	Leukotriene	LOX
47	LTD4	495.2	177.1	3.9	(d3) LTE4	AA	Leukotriene	LOX
48	Resolvin D1	375.2	141	3.9	(d11) 14,15-DiHETrE	DHA	rRsolving	LOX
49	Resolvin E1	349.2	195	3.9	(d11) 14,15-DiHETrE	EPA	Resolving	LOX
50	13,14-dihydro-15-keto PGD1	353.2	209.1	3.91	(d4) PGD2	AA	Prostanoid	COX
51	PGA2	333.2	271.2	3.91	(d4) PGE2	AA	Prostanoid	COX
52	Δ12-PGJ2	333.2	233.1	3.97	(d4) 15-deoxy-Δ12,14-PGJ2	AA	Prostanoid	COX
53	PGJ2	333.2	233.1	3.97	(d4) PGD2	AA	Prostanoid	COX
54	LTB5	333.2	195.1	4.03	(d4) LTB4	EPA	Leukotriene	LOX
55	11-trans LTD4	495.2	177.1	4.05	(d3) LTE4	AA	Leukotriene	LOX
56	(d3) LTE4	441.2	336.2	4.12	ISTD			
57	LTE4	438.2	333.2	4.13	(d3) LTE4	AA	Leukotriene	LOX
58	8(S),15(S)-DiHETE	335.2	235.2	4.23	(d4) LTB4	AA	Diol	CYP450
59	12,13-DiHODE	311.2	293	4.23	(d4) 9,10-DiHOME	ALA	Diol	CYP450
60	bicyclo-PGE2	333.2	113.2	4.25	(d4) PGE2	AA	Prostanoid	CYP450
61	11-trans LTE4	438.2	333.2	4.26	(d3) LTE4	AA	Leukotriene	LOX
62	10(S),17(S)-DiHDoHE	359.2	153.2	4.34	(d8) 12(S)-HETE	DHA	Protectin	LOX
63	Neuroprotectin D1	359.2	206	4.34	(d8) 12(S)-HETE	DHA	Protectin	LOX
64	17,18-DiHETE	335.2	247.2	4.34	(d11) 14,15-DiHETrE	EPA	Diol	CYP450
65	5(S),15(S)-DiHETE	335.2	115.2	4.37	(d4) LTB4	AA	Diol	CYP450
66	6-trans-LTB4	335.2	195.1	4.4	(d4) LTB4	AA	Leukotriene	LOX
67	14,15-DiHETE	335.2	207.1	4.46	(d11) 14,15-DiHETrE	EPA	Diol	CYP450
68	(d4) LTB4	339.2	197.1	4.48	ISTD			
69	15-deoxy-Δ12,14-PGD2	333.2	271.2	4.49	(d4) 15-deoxy-Δ12,14-PGJ2	AA	Prostanoid	COX
70	Hepoxilin A3	335.2	273.2	4.5	(d8) 12(S)-HETE	AA	Hepoxilin	LOX
71	LTB4	335.2	195.1	4.5	(d4) LTB4	AA	Leukotriene	LOX
72	(d4)(±)12,13-DiHOME	317.3	185.2	4.56	ISTD			
73	12,13-DiHOME	313.2	183.2	4.58	(d4) 12,13-DiHOME	LA	Diol	CYP450
74	(d4)-(±)9,10-DiHOME	317.3	203.2	4.69	ISTD			
75	9,10-DiHOME	313.2	201.1	4.71	(d4) 9,10-DiHOME	LA	Diol	CYP450
76	(d11) 14,15-DiHETrE	348.3	207.1	4.77	ISTD			
77	19,20-DiHDPA	361.2	273.3	4.79	(d11) 14,15-DiHETrE	DHA	Diol	CYP450
78	14,15-DiHETrE	337.2	207.2	4.8	(d11) 14,15-DiHETrE	AA	Diol	CYP450
79	12S-HHTrE	279.2	179.2	4.84	(d8) 12(S)-HETE	AA	Alcohol	COX
80	11,12-DiHETrE	337.2	167.2	4.98	(d11) 14,15-DiHETrE	AA	Diol	CYP450
81	5,6-DiHETrE	337.2	145.1	4.99	(d11) 14,15-DiHETrE	AA	Diol	CYP450
82	9-HOTrE	293.2	171.1	5.07	(d4) 9(S)-HODE	ALA	Alcohol	LOX
83	17(18)-EpETE	317.2	259.2	5.16	(d11) 14,15-DiHETrE	EPA	Epoxide	CYP450
84	(d4) 15-deoxy-Δ12,14-PGJ2	319.2	275.3	5.2	ISTD			
85	(d6) 20-HETE	325.3	279.2	5.24	ISTD			
86	20-HETE	319.2	289.2	5.25	d6-20-HETE	AA	Alcohol	CYP450
87	15(S)-HEPE	317.2	219.2	5.25	(d8) 5(S)-HETE	EPA	Alcohol	LOX
88	12(S)-HpETE	317.1	153.0	5.34	(d8) 12(S)-HETE	AA	Hydroxyperoxide	LOX

	Compound name	M1	M2	RT	I	Precursor	Class	Pathway
89	8,9-DiHETrE	337.2	127	5.35	(d11) 14,15-DiHETrE	AA	Diol	CYP450
90	5(S),6(S)-DiHETE	335.2	115.1	5.35	(d4) LTB4	AA	Diol	CYP450
91	12(S)-HEPE	317.2	179.1	5.35	(d8) 12(S)-HETE	EPA	Alcohol	LOX
92	13-HODE	295.2	195.2	5.5	(d4) 9(S)-HODE	LA	Alcohol	LOX
93	5(S)-HEPE	317.2	115.1	5.51	(d8) 5(S)-HETE	EPA	Alcohol	LOX
94	(d4) 9(S)-HODE	299.2	172.1	5.53	ISTD			
95	9-HODE	295.2	171.1	5.56	(d4) 9(S)-HODE	LA	Alcohol	LOX
96	15-HETE	319.2	219.2	5.62	(d8) 5(S)-HETE	AA	Alcohol	LOX
97	16(17)-EpDPE	343.2	233.2	5.62	(d11) 14,15-DiHETrE	DHA	Epoxide	CYP450
98	13-HpODE	293.1	113.0	5.63	(d4) 9(S)-HODE	LA	Hydroxyperoxide	LOX
99	13-KODE	293.2	113.1	5.64	(d4) 9(S)-HODE	LA	Ketone	LOX
100	17-HDoHE	343.2	281.3	5.67	(d8) 5(S)-HETE	DHA	Alcohol	LOX
101	9-HpODE	293.1	185.0	5.68	(d4) 9(S)-HODE	LA	Hydroxyperoxide	LOX
102	15-HpETE	317.	113.0	5.71	(d8) 5(S)-HETE	AA	Hydroxyperoxide	LOX
103	15-KETE	317.2	113.2	5.72	(d8) 5(S)-HETE	AA	Ketone	LOX
104	11-HETE	319.2	167.1	5.74	(d8) 12(S)-HETE	AA	Alcohol	COX
105	14(15)-EpETE	317.2	207.1	5.74	(d11) 14,15-DiHETrE	EPA	Epoxide	CYP450
106	9-KODE	293.2	185.2	5.77	(d4) 9(S)-HODE	LA	Ketone	LOX
107	(d8) 12(S)-HETE	327.3	184.2	5.78	ISTD			
108	12-HETE	319.2	179.2	5.81	(d8) 12(S)-HETE	AA	Alcohol	LOX
109	8-HETE	319.2	155.1	5.85	(d8) 5(S)-HETE	AA	Alcohol	LOX
110	15(S)-HETrE	321.2	221.2	5.88	(d8) 5(S)-HETE	DGLA	Alcohol	LOX
111	9-HETE	319.2	167.1	5.91	(d8) 12(S)-HETE	AA	Alcohol	non-enzymatic
112	(d8) 5(S)-HETE	327.3	116.1	5.97	ISTD			
113	5-HETE	319.2	115.1	6.00	(d8) 5(S)-HETE	AA	Alcohol	LOX
114	19(20)-EpDPE	343.2	281.3	6.09	(d11) 14,15-DiHETrE	DHA	Epoxide	CYP450
115	12(13)-EpOME	295.2	195.2	6.09	(d4) 12,13-DiHOME	LA	Epoxide	CYP450
116	14(15)-EpETrE	319.2	219.2	6.11	(d11) 14,15-DiHETrE	AA	Epoxide	CYP450
117	5(S)-HpETE	317.1	203.1	6.11	(d8) 5(S)-HETE	AA	Hydroxyperoxide	LOX
118	9(10)-EpOME	295.2	171.2	6.15	(d4) 9,10-DiHOME	LA	Epoxide	CYP450
119	12-KETE	317.2	273.3	6.25	(d8) 12(S)-HETE	AA	Ketone	LOX
120	5-KETE	317.2	203.2	6.26	(d8) 5(S)-HETE	AA	Ketone	LOX
121	11(12)-EpETrE	319.2	167.1	6.27	(d11) 14,15-DiHETrE	AA	Epoxide	CYP450
122	8(9)-EpETrE	319.2	155.1	6.33	(d11) 14,15-DiHETrE	AA	Epoxide	CYP450
123	5(6)-EpETrE	319.2	191.2	6.42	(d11) 14,15-DiHETrE	AA	Epoxide	CYP450

Table 2. List of MRM transitions (M1=precursor; M2= fragment) and retention times (RT) for oxylipins.

Oxylipins are present at very low abundance in biological samples, and as such the quality of sample preparation is an important factor for successful analyses. To eliminate non-lipid contaminants and highly abundant species like phospholipids, we used mixed mode solid-phase extraction (SPE) prior to UPLC-MS analysis. Normalization of the extraction efficiency was achieved by adding stable isotope labeled compounds (internal standards), prior to the extraction procedure (Table 1 and 2, and Figure 2).

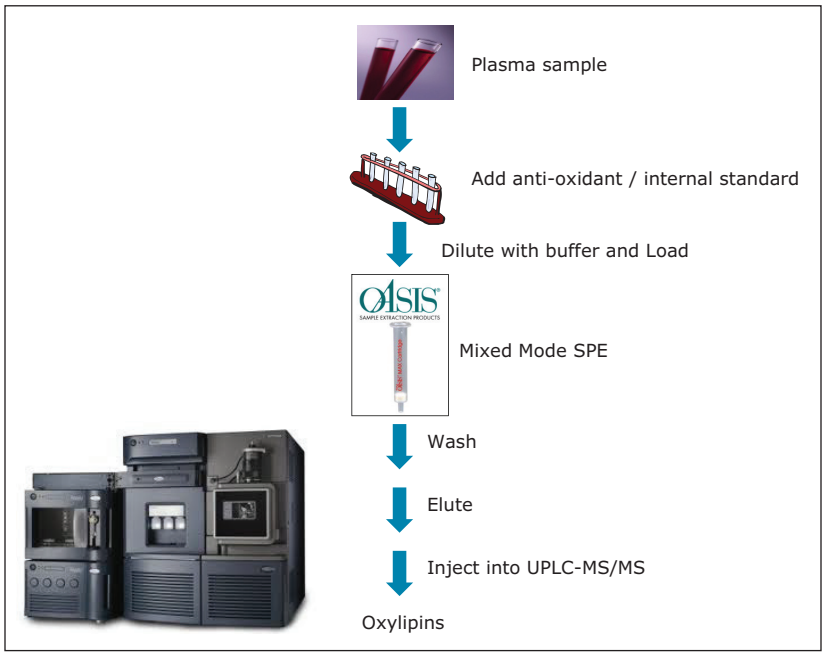

Figure 2. Workflow of the sample preparation for the analysis of oxylipins from plasma.

To optimize the chromatographic separation of our analytes, we used a mixture of a wide chemical variety of commercially available oxylipins. Using reversed-phase UPLC (see Experimental), oxylipins eluted in order of decreasing polarity, numbers of double bonds and increasing acyl chain length, allowing the separation of most isomeric and isobaric species (e.g., PGE2 and PGD2) in less than 10 minutes (Figure 3). Using a Xevo TQ-S in negative ESI-mode, retention times and optimal MRM transitions (compound specific precursor ⟹ product ion transitions) were determined for all individual oxylipins (Table 2).

To enhance the sensitivity of detection, these MRM transitions were monitored in defined retention time windows, maximizing dwell times by reducing overlapping transitions. In the case of co-eluting metabolites, compound specific precursor ions and their corresponding fragment ions allowed selective profiling of those compounds. Calibration curves for the majority of the analytes were produced and displayed a linear coefficient (Pearson's correlation, R^2) higher than 0.99. (Figure 4). Using this UPLC-MS/MS assay, we rapidly profiled 107 oxylipins in human plasma samples (Figure 5).

With minor modifications in the sample preparation protocol, this assay could be extended to the measure of oxylipins in other biological matrices.

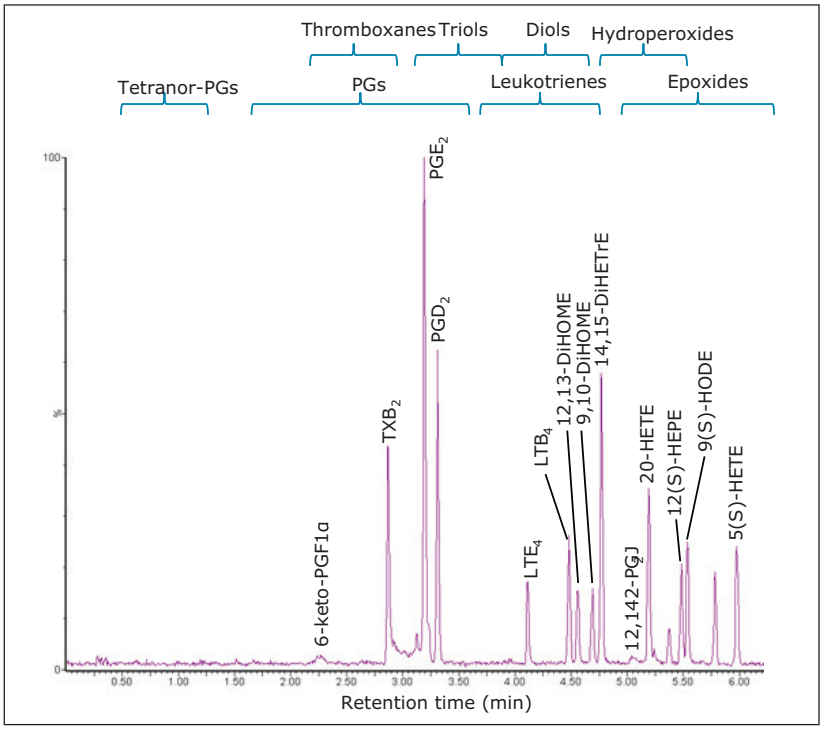

Figure 3. Representative UPLC-MS/MS chromatogram of a wide chemical variety of oxylipin species.

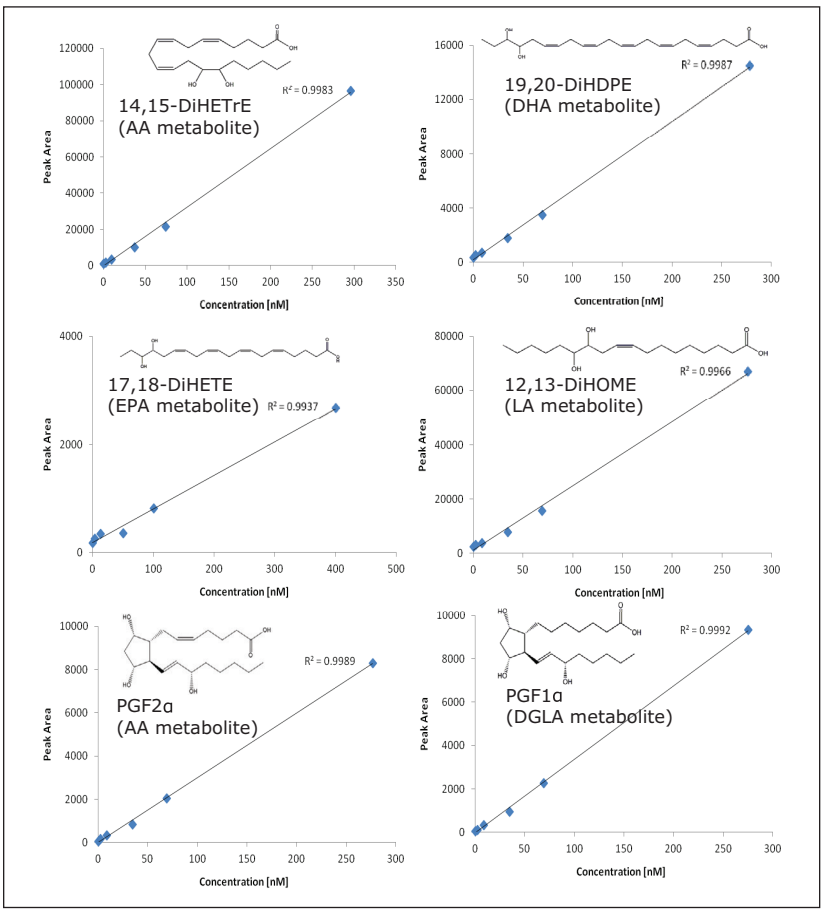

Figure 4. Linearity of response for representative endogenous oxylipin species present in the plasma samples.

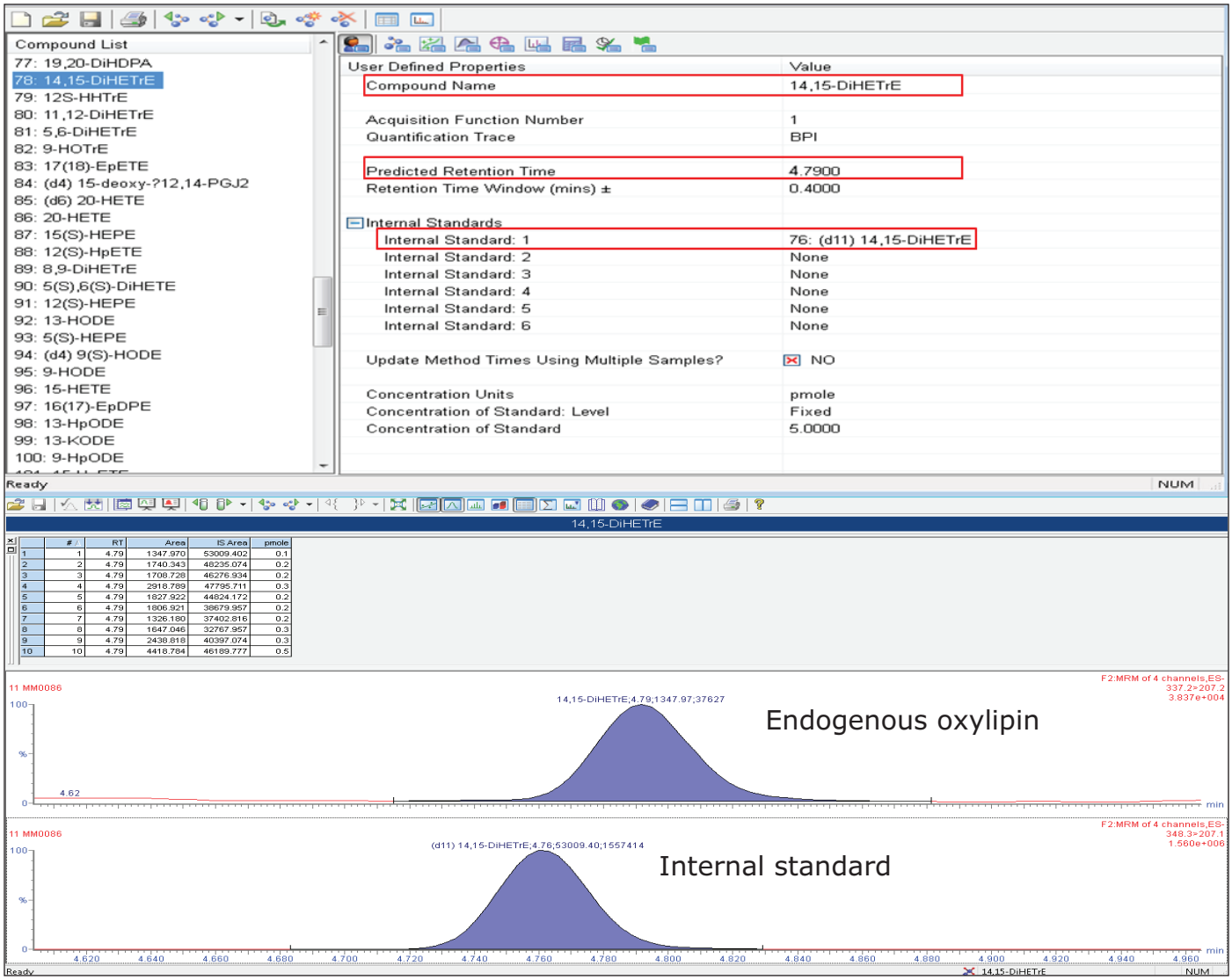

Figure 5. An example of oxylipin quantification in plasma using TargetLynx, showing the use of a specified retention time, MRM transitions and internal standard for the identification and quantification of a selected oxylipin.

CONCLUSIONS

We have presented a routine high-throughput MRM method to profile over 100 oxylipins in plasma. These targets include a wide array of both pro- and anti-inflammatory lipid mediators. This SPE-UPLC-MRM assay could find applications in basic research to facilitate our understanding of the role of these lipid mediators in health and disease, nutritional research, clinical research, and drug discovery and development.

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