High-throughput lipidomic analysis of arachidonic acid metabolites in biological fluids by liquid chromatography/mass spectrometry

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NOVEL ASPECT:

A novel LC-MS/MS procedure was developed for lipidomic applications to quantify over 40 arachidonic acid's metabolites simultaneously in human plasma.

INTRODUCTION:

Arachidonic acid metabolites are biologically active lipid molecules that regulate many functions and play critical roles in a variety of physiological and pathophysiological processes. Some of the eicosanoids are used as a potential biomarkers related to inflammation, immune reactions and side-effects of drugs. Eicosanoids are derived from arachidonic acid and then from other metabolites by enzymatic and non-enzymatic reactions and have very similar structure and physical properties. Pattern of intermediates and final eicosanoids production is determined in a stimulus and cell-specific fashion. Fast and simultaneous qualitative and quantitative profiling of eicosanoids (lipidomics) in biological samples is possible because of high sensitivity and selectivity of liquid chromatography/mass spectrometry methods and simplicity of sample preparation.

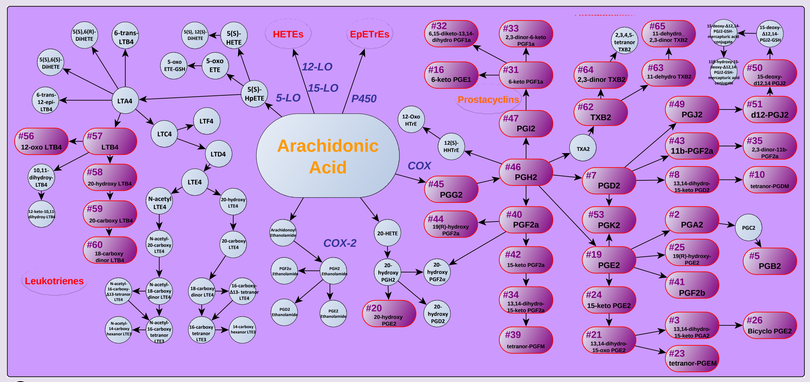
METHODS:

Plasma samples were spiked by eicosanoids mixture and processed by two independent ways: solid phase (SPE) and liquid-liquid extraction. The LC-MS/MS analysis was performed on a Nexera UHPLC system (Shimadzu) coupled to an AB Sciex 5500. Samples were injected onto a Kinetex C18 1.7µm 50 mm (Phenomenex) column maintained at 30°C, flow rate 0.3 mL/min with solvent A: 0.02% formic acid in water and solvent B: acetonitrile/isopropanol 50:50 v/v (gradient and isocratic mode), total run 8 min. Mass spectrometer was operated in negative mode with DP, CE and CPX optimized individually (2 MRM transition for each analyte) and CUR, TEM, IS, CAD and EP same for all analytes.

ABSTRACT:

More than 60 target analytes (prostaglandines A, D, E, F and J series, leukotrienes, prostacyclines and thromboxanes) and internal standards (PGA2-d_4, PGB2-d_4, PGD2-d_4, PGE1-d_4, PGE2-d_4, PGB2-d_4, 15-deoxy-Δ12,14-PGJ2-d_4) were adequately separated within 8 min run. LC program consist of isocratic and gradient elution mode for optimal peak separation. Blank and spiked plasma samples were extracted by liquid-liquid or solid phase extraction. There was no carry-over observed in the analyte-free plasma injected after the spiked plasma sample. Calibration curves were linear over the selected concentration ranges for each analyte, with calculated coefficients of determination (R^2) of greater than 0.99. The limit of quantification (signal-to-noise ratio of 10) for all analytes were under 0.8 ng/mL in plasma with majority fallen into range under 0.04 ng/mL. The total extraction recovery was calculated by comparison of the peak area ratio of quantification ion to IS quantification ion for extracted samples with unextracted reference standard samples. The total extraction recovery was similar for SPE and liquid-liquid extraction with few exceptions of tetranor-species, whose recovery was low for SPE method. For liquid-liquid extraction from plasma, the mean percent recovery was >80% with exception for several leukotrienes.

ARACHIDONIC ACID CASCADE

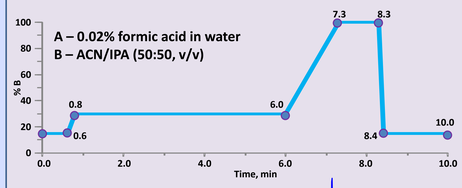


Intermediate and final products not studied under current study.

Intermediate and final products studied under this study. # indicates ID in the table below.

Note: Only 41 of Arachidonic Acid methabolites (out of 68 studied under current study) are shown on schema.
,*- LOQ and Signal-to-Noise (S/N) ratio was not determined for PGG2, PGH2 and PGI2. due to inconsistency of data.
** - r^2 was not determined for PGG2, PGH2 and PGI2 due to lack of sufficient data.
*** - recovery by solid phase extraction was performed not for all compounds.

LC METHOD



SAMPLE PREPARATION

SPE procedure:

Lipid mixture was evaporated in glass tube under N_2 stream and 50 µL plasma was added to it; mixed. Sample was diluted with water (950 µL) and 176 µL of methanol was added, so final concentration of methanol is 15%. Sample was mixed gently and left on ice for 15 min. Men time STRATA SPE cartridges (C18-E, 100 mg, Phenomenex) were preconditioned with 5 mL of methanol and 5 mL of water. Samples were centrifuged at 1000g, 5 min 4°C, supernatant was acidified with acetic acid to pH~3.0 and loaded to SPE cartridges. After applying samples, cartridges were washed with 5 mL of 15% methanol/water (v/v), 5 mL water and 2.5 mL hexane. Analytes were eluted with 2.5 mL of methylformate. Solvent was evaporated under N_2 stream and reconstituted with 100 µL ethanol. Tubes were centrifuged 20 sec at 1000g to collect all ethanol; samples were transferred into HPLC vials (glass insert) and flashed with N_2.

Liquid-liquid extraction procedure:

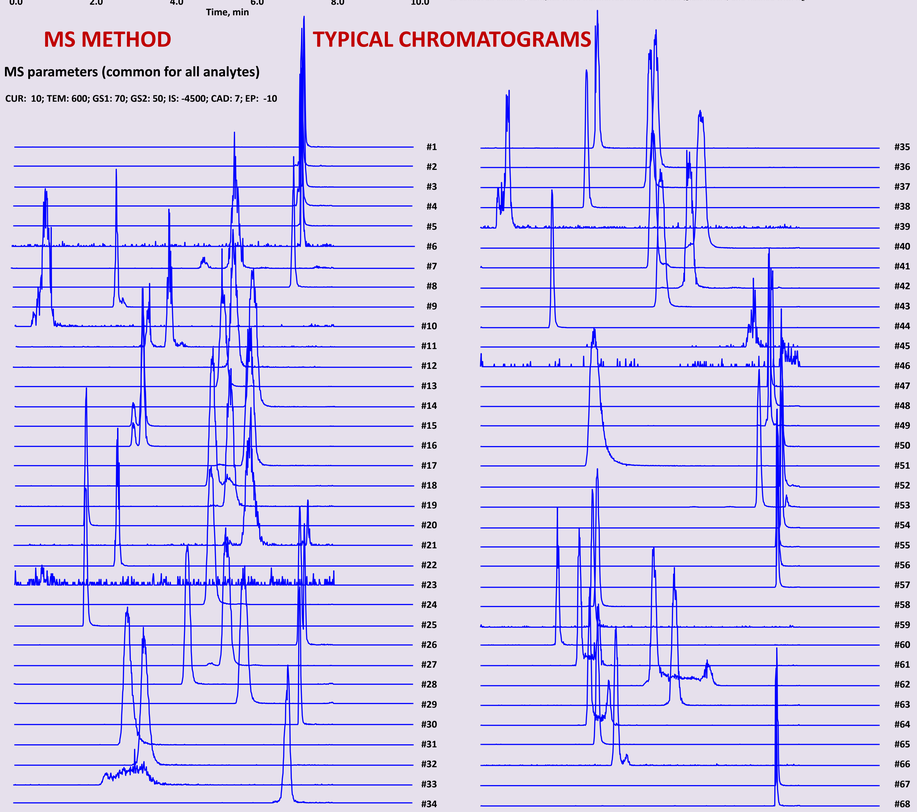
250 µL of plasma was spiked with lipid mixture (25 µL) and 725 µL of 10 µM indomethacin was added. Sample was acidified with acetic acid to pH ~3.0 and extracted twice with 5 mL of diethyl ether and once with 5mL of ethyl acetate. Organic phase was combined, evaporated under N_2 stream and reconstituted with 100 µL ethanol. Tubes were centrifuged 20 sec at 1000g to collect all ethanol; samples were transferred into HPLC vials (glass insert) and flashed with N_2.

MS METHOD

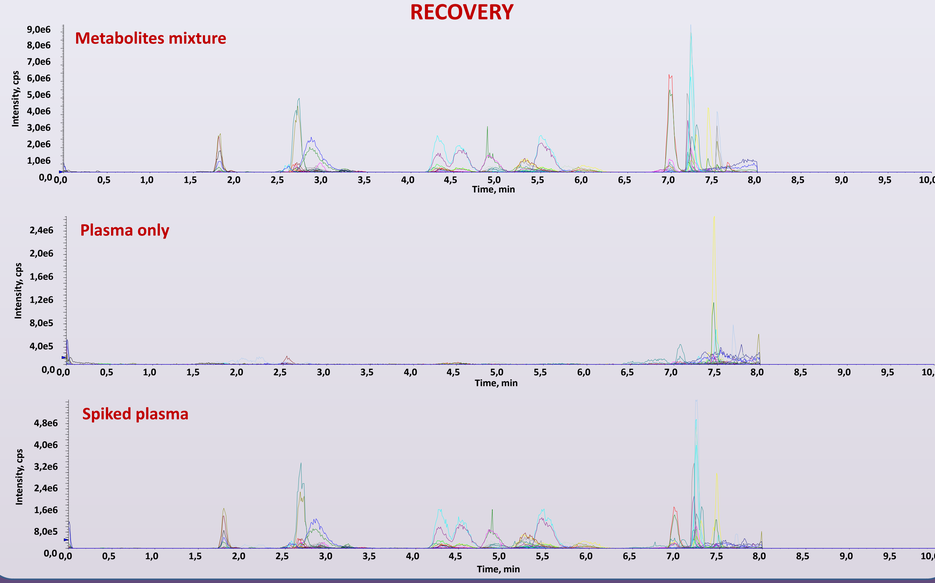
MS parameters (common for all analytes)

CUR: 10; TEM: 600; GS1: 70; GS2: 50; IS: -4500; CAD: 7; EP: -10

TYPICAL CHROMATOGRAMS



RECOVERY



#	Abbreviation	Series	Compound Name	Mass Spec Acquisition Parameters								RT, min	Limit (pg)		Linearity			Recovery, %	
				[M-H]⁻	MRM₁				MRM₂				LOQ	S/N	Range (pg)		r^2	LLE	SPE
					MRM_1	DP	CE	CXP	MRM_2	DP	CE	CXP			Min	Max			
#1	PGA1	A	9-oxo-15S-hydroxy-prosta-10,13E-dien-1-oic acid	335.136	235.095	-45	-20	-13	273.147	-30	-26	-13	7.25	0.4	17.8	0.2	20	0.9999	90.0 ± 3.4	109.5 ± 2.8
#2	PGA2	A	9-oxo-15S-hydroxy-prosta-5Z,10,13E-trien-1-oic acid	333.130	271.134	-55	-20	-13	189.134	-105	-23	-11	7.21	2.0	11.24	0.2	2000	0.9996	87.9 ± 1.6	***
#3	13,14-dihydro-15-keto-PGA2	A	9,15-dioxo-prosta-5Z,10-dien-1-oic acid	333.100	175.000	-90	-24	-15	234.970	-110	-27	-15	7.26	2.0	10.22	2.0	2000	0.9998	76.0 ± 2.4	***
#4	PGA2-d4	A	9-oxo-15S-hydroxy-prosta-5Z,10,13E-trien-1-oic-3,3,4,4-d4 acid	337.134	275.232	-95	-22	-20	193.019	-95	-24	-12	7.21	10	25.57	1.0	1000	0.9998	72.3 ± 2.1	***
#5	PGB2	B	9-oxo-15S-hydroxy-prosta-5Z,8(12),13E-trien-1-oic acid	333.100	175.000	-90	-24	-15	234.970	-110	-27	-15	7.22	2.0	8.85	2.0	2000	0.9998	86.4 ± 1.3	***
#6	PGB2-d4	B	9-oxo-15S-hydroxy-prosta-5Z,8(12),13E-trien-1-oic-3,3,4,4-d4 acid	337.141	179.203	-70	-28	-9	239.110	-65	-27	-17	7.21	1.0	33.9	0.2	200	0.9997	75.0 ± 1.8	112.7 ± 4.0
#7	PGD2	D	9α,15S-dihydroxy-11-oxo-prosta-5Z,13E-dien-1-oic acid	351.133	271.100	-55	-25	-19	315.130	-60	-18	-18	5.54	0.4	20.0	0.4	2000	1.0000	123.0 ± 10.6	107.8 ± 16.0
#8	13,14-dihydro-15-keto PGD2	D	9α-hydroxy-11,15-dioxo-prost-5Z-en-1-oic acid	351.100	175.100	-55	-30	-17	207.050	-75	-24	-25	7.00	0.2	34.1	0.2	20	0.9998	97.1 ± 9.8	117.9 ± 2.1
#9	13,14-dihydro-15-keto-tetranor PGD2	D	9α-hydroxy-11,15-dioxo-2,3,4,5-tetranor-prostanoic acid	297.086	121.010	-30	-29	-11	109.008	-30	-29	-11	2.59	0.4	15.0	0.2	20	0.9999	106.3 ± 2.9	109.7 ± 3.6
#10	Tetranor-PGDM	D	9α-hydroxy-11,15-dioxo-13,14-dihydro-2,3,4,5-tetranor-prostan-1,20-dioic acid	327.100	143.047	-40	-26	-15	154.974	-30	-30	-13	0.76	2.0	27.3	0.2	200	0.9999	92.5 ± 10.0	60.7 ± 7.3
#11	PGD3	D	9α,15S-dihydroxy-11-oxo-prosta-5Z,13E,17Z-trien-1-oic acid	349.100	269.119	-30	-20	-13	189.056	-60	-24	-11	3.84	1.0	35.1	1.0	2000	0.9999	128.2 ± 15.4	117.5 ± 12.8
#12	PGD2-d4	D	9α,15S-dihydroxy-11-oxo-prosta-5Z,13E-dien-1-oic-3,3,4,4-d4 acid	355.150	275.202	-110	-24	-23	319.186	-120	-16	-25	5.52	10	41.27	2.0	2000	1.0000	74.4 ± 1.5	***
#13	8-iso PGE1	E	9-oxo-11α,15S-dihydroxy-(8β)-prost-13E-en-1-oic acid	353.150	317.158	-55	-19	-15	235.067	-55	-22	-16	5.19	0.1	10.2	0.04	20	0.9998	108.4 ± 6.7	124.2 ± 3.7
#14	PGE1	E	9-oxo-11α,15S-dihydroxy-prost-13E-en-1-oic acid	353.150	317.158	-55	-19	-15	235.067	-55	-22	-16	5.98	0.2	15.1	0.2	20	0.9998	104.2 ± 6.8	112.5 ± 7.0
#15	2,3-dinor PGE1	E	9-oxo-11α,15S-dihydroxy-2,3-dinor-prost-13E-en-1-oic acid	325.100	289.050	-75	-16	-7	163.074	-70	-22	-7	3.22	0.4	12.4	0.2	20	0.9998	109.5 ± 12.8	119.3 ± 6.0
#16	6-keto-PGE1	E	6,9-dioxo-11α,15S-dihydroxy-prost-13E-en-1-oic acid	367.100	143.088	-90	-25	-15	331.100	-65	-18	-17	2.67	4.0	126.29	4.0	400	1.0000	73.4 ± 5.6	***
#17	PGE1-d4	E	9-oxo-11α,15S-dihydroxy-prost-13E-en-1-oic-3,3,4,4-d4 acid	357.160	239.129	-110	-22	-17	321.204	-150	-30	-20	5.95	2.0	38.2	10.0	10000	0.9988	74.0 ± 1.8	***
#18	8-iso PGE2	E	9-oxo-11α,15S-dihydroxy-(8β)-prosta-5Z,13E-dien-1-oic acid	351.133	271.100	-55	-25	-19	315.130	-60	-18	-18	4.96	0.2	33.7	0.2	20	0.9999	130.2 ± 23.5	119.0 ± 2.1
#19	PGE2	E	Dinoprostone; 9-oxo-11α,15S-dihydroxy-prosta-5Z,13E-dien-1-oic acid	351.133	271.100	-55	-25	-19	315.130	-60	-18	-18	5.40	0.2	30.0	0.2	20	0.9999	125.8 ± 21.6	137.2 ± 5.3
#20	20-hydroxy PGE2	E	9-oxo-11α,15S,20-trihydroxy-prosta-5Z,13E-dien-1-oic acid	367.100	331.100	-65	-18	-17	287.150	-55	-24	-15	1.79	0.2	30.0	0.2	200	0.9999	102.8 ± 1.3	104.9 ± 9.3
#21	13,14-dihydro-15-oxo-PGE2	E	9,15-dioxo-11α-hydroxy-prost-5Z-en-1-oic acid	351.100	175.100	-55	-30	-17	235.072	-45	-30	-17	5.94	2.0	16.8	0.1	20	0.9990	96.5 ± 8.5	96.7 ± 26.4
#22	13,14-dihydro-15-keto-tetranor PGE2	E	9,15-dioxo-11α-hydroxy-2,3,4,5-tetranor-prostanoic acid	297.086	121.010	-30	-29	-11	109.008	-30	-29	-11	2.60	0.02	17.6	0.02	20	0.9997	106.3 ± 2.9	109.7 ± 3.6
#23	Tetranor-PGEM	E	9,15-dioxo-11α-hydroxy-2,3,4,5-tetranor-prostan-1,20-dioic acid	327.033	291.011	-40	-26	-15	184.900	-26	-46	-17	0.69	100.0	11.3	40	2000	1.0000	118.4 ± 45.5	70.6 ± 20.4
#24	15-keto-PGE2	E	9,15-dioxo-11α-hydroxy-prosta-5Z,13E-dien-1-oic acid	349.104	287.229	-50	-22	-17	235.067	-65	-20	-12	4.94	20	31.38	2.0	200	0.9995	79.9 ± 1.2	***
#25	19(R)-hydroxy PGE2	E	9-oxo-11α,15S,19R-trihydroxy-prosta-5Z,13E-dien-1-oic acid	367.100	331.100	-65	-18	-17	287.150	-55	-24	-15	1.79	10	40.0	1.0	1000	0.9998	72.6 ± 2.5	***
#26	Bicyclo PGE2	E	11-deoxy-13,14-dihydro-15-keto-11β,16ζ-cycloprostaglandin E2	333.100	175.000	-90	-24	-15	234.970	-110	-27	-15	7.17	20	17.17	2.0	2000	0.9999	85.1 ± 5.1	***
#27	PGE2-d4	E	9-oxo-11α,15S-dihydroxy-prosta-5Z,13E-dien-1-oic-3,3,4,4-d4 acid	355.150	275.202	-110	-24	-23	319.186	-120	-16	-25	5.31	2.0	48.01	1.0	1000	0.9997	80.0 ± 2.1	***
#28	8-iso PGF1α	F	9α,11α,15S-trihydroxy-(8β)-prost-13E-en-1-oic acid	355.154	311.178	-105	-29	-15	293.150	-105	-32	-17	4.36	0.4	16.8	0.2	20	0.9995	116.3 ± 7.8	114.7 ± 9.3
#29	PGF1α	F	9α,11α,15S-trihydroxy-prost-13E-en-1-oic acid	355.154	311.178	-105	-29	-15	293.150	-105	-32	-17	5.78	0.2	11.0	0.2	20	0.9998	99.6 ± 2.9	109.3 ± 3.4
#30	13,14-dihydro-PGF1α	F	9α,11α,15S-trihydroxy-prostan-1-oic acid	357.175	113.038	-200	-42	-7	321.204	-150	-30	-20	7.15	0.2	21.5	0.2	200	0.9998	91.3 ± 3.3	96.4 ± 5.6
#31	6-keto-PGF1α	F	6-oxo-9α,11α,15S-trihydroxy-prost-13E-en-1-oic acid	369.146	163.035	-60	-36	-15	245.131	-60	-34	-11	2.83	0.2	15.4	0.1	200	0.9999	66.7 ± 12.0	117.8 ± 1.4
#32	6,15-diketo-13,14-dihydro PGF1α	F	6,15-dioxo-9α,11α-dihydroxy-prostan-1-oic acid	369.133	113.025	-130	-36	-7	82.980	-130	-34	-13	3.23	0.2	17.9	0.2	200	0.9999	19.8 ± 0.9	128.1 ± 3.9
#33	2,3-dinor-6-keto PGF1α	F	6-oxo-9α,11α,15S-trihydroxy-2,3-dinor-prost-13E-en-1-oic acid	341.080	135.024	-65	-30	-11	161.083	-65	-30	-29	3.11	4.0	11.2	2.0	2000	1.0000	226.0 ± 28.0	1474.0 ± 95.0
#34	13,14-dihydro-15-keto PGF2α	F	9α,11α-dihydroxy-15-oxo-prost-5Z-en-1-oic acid	353.143	113.031	-170	-34	-11	183.029	-170	-34	-19	6.88	0.02	14.1	0.02	20	1.0000	86.8 ± 3.9	105.2 ± 3.1
#35	2,3-dinor-11β-PGF2α	F	9α,11β,15S-trihydroxy-2,3-dinor-prosta-5Z,13E-dien-1-oic acid	325.100	144.957	-70	-26	-11	163.074	-70	-22	-7	2.93	2.0	29.8	0.2	200	0.9998	103.8 ± 5.3	90.3 ± 35.3
#36	8-iso-PGF2a	F	9α,11α,15S-trihydroxy-(8β)-prosta-5Z,13E-dien-1-oic acid	353.149	193.058	-100	-32	-19	309.164	-100	-28	-17	4.40	0.2	15.9	0.2	200	0.9995	119.9 ± 7.1	118.9 ± 15.3
#37	8-iso-15-keto PGF2α	F	9α,11α-dihydroxy-15-oxo-(8β)-prosta-5Z,13E-dien-1-oic acid	351.100	315.130	-60	-18	-18	289.128	-80	-26	-7	4.25	0.2	16.6	0.2	20	1.0000	116.4 ± 2.4	104.0 ± 8.9
#38	2,3-dinor-8-iso PGF2α	F	9α,11α,15S-trihydroxy-2,3-dinor-(8β)-prosta-5Z,13E-dien-1-oic acid	325.117	237.104	-65	-18	-27	137.009	-65	-24	-15	2.66	0.1	11.5	0.1	20	0.9993	109.0 ± 5.2	109.8 ± 5.7
#39	Tetranor-PGFM	F	9α,11α-dihydroxy-15-oxo-2,3,4,5-tetranor-prostan-1,20-dioic acid	329.144	293.109	-30	-26	-15	267.248	-30	-28	-13	0.72	2.0	9.7	2.0	200	0.9997	64.1 ± 1.3	31.5 ± 5.4
#40	PGF2α	F	9α,11α,15S-trihydroxy-prosta-5Z,13E-dien-1-oic acid	353.149	309.164	-100	-28	-17	193.058	-100	-32	-19	5.50	4.0	13.22	4.0	400	1.0000	6.0± ± 3.9	***
#41	PGF2β	F	9β,11α,15S-trihydroxy-prosta-5Z,13E-dien-1-oic acid	353.149	193.058	-100	-32	-19	309.164	-100	-28	-17	4.31	40	44.64	4.0	400	0.9995	86.3 ± 0.5	***
#42	15-keto PGF2α	F	9α,11α-dihydroxy-15-oxo-prosta-5Z,13E-dien-1-oic acid	351.100	315.130	-60	-18	-18	289.128	-80	-26	-7	5.17	2.0	15.68	0.2	2000	0.9995	86.3 ± 0.5	***
#43	11β-PGF2α	F	9α,11β,15S-trihydroxy-prosta-5Z,13E-dien-1-oic acid	353.149	193.058	-100	-32	-19	309.164	-100	-28	-17	4.51	4.0	12.89	0.4	400	0.9999	74.6 ± 6.0	***
#44	19(R)-hydroxy PGF2α	F	9α,11α,15S,19R-tetrahydroxy-prosta-5Z,13E-dien-1-oic acid	369.126	325.113	-145	-30	-11	192.971	-145	-38	-13	1.80	1.0	11.31	0.01	1000	0.9999	45.9 ± 4.7	***
#45	PGG2	G	9α,11α-epidioxy-15S-hydroperoxy-prosta-5Z,13E-dien-1-oic acid	367.170	235.400	-85	-12	-15	112.990	-80	-18	-11	6.84	*	*	20	2000	N/A	81.2 ± 5.4	***
#46	PGH2	H	9α,11α-epidioxy-15S-hydroxy-prosta-5Z,13E-dien-1-oic acid	351.100	271.100	-55	-25	-19	307.094	-70	-24	-21	7.54	*	*	20	200	N/A	**	***
#47	PGI2	I	6,9α-epoxy-11α,15S-dihydroxy-prosta-5Z,13E-dien-1-oic acid	351.100	315.130	-60	-18	-18	215.014	-160	-26	-17	7.23	*	*	500	1000	N/A	**	***
#48	13,14-dihydro-15-keto PGJ2	J	11,15-dioxo-prosta-5Z,9-en-1-oic acid	333.100	175.000	-90	-24	-15	207.059	-80	-24	-19	7.28	0.2	20.7	0.04	200	0.9996	85.0 ± 1.9	103.5 ± 6.3
#49	PGJ2	J	11-oxo-15S-hydroxy-prosta-5Z,9,13E-trien-1-oic acid	333.130	271.134	-55	-20	-13	233.061	-55	-14	-20	7.23	0.2	24.5	0.2	20	0.9987	95.2 ± 8.2	59.1 ± 37.6
#50	15-deoxy-Δ12,14-PGJ2	J	11-oxo-prosta-5Z,9,12E,14E-tetraen-1-oic acid	315.124	271.138	-60	-18	-33	203.073	-60	-28	-21	7.54	0.04	13.7	0.02	200	0.9992	27.3 ± 9.5	9.5 ± 5.5
#51	Δ12-PGJ2	J	11-oxo-15S-hydroxy-prosta-5Z,9,12E-trien-1-oic acid	333.130	233.061	-55	-14	-20	271.134	-55	-20	-13	7.55	2.0	68.17	2.0	200	1.0000	69.5 ± 3.3	***
#52	15-deoxy-Δ12,14-PGJ2-d4	J	11-oxo-prosta-5Z,9,12E,14E-tetraen-1-oic-3,3,4,4-d4 acid	319.117	275.182	-120	-20	-14	203.080	-120	-28	-16	7.54	2.0	38.76	0.2	2000	0.9980	24.5 ± 0.9	***
#53	PGK2	K	9,11-dioxo-15S-hydroxy-prosta-5Z,13E-dien-1-oic acid	349.104	249.129	-110	-22	-19	287.229	-50	-22	-17	6.98	2.0	13.45	0.2	200	0.9998	11.1 ± 1.1	***
#54	LTB3	LB	5S,12R-dihydroxy-6Z,8E,10E-eicosatrienoic acid	337.100	195.016	-5	-22	-11	58.987	-25	-46	-9	7.53	0.04	16.1	0.02	200	0.9993	37.9 ± 5.5	34.5 ± 7.0
#55	12-epi LTB4	LB	5S,12S-dihydroxy-6Z,8E,10E,14Z-eicosatetraenoic acid	335.146	195.077	-90	-22	-19	129.007	-90	-26	-12	7.43	0.04	21.3	0.02	200	0.9999	322.1 ± 11.1	244.1 ± 6.4
#56	12-oxo LTB4	LB	5S-hydroxy-12-oxo-6Z,8E,10E,14Z-eicosatetraenoic acid	333.117	179.006	-20	-18	-11	128.992	-20	-18	-11	7.42	0.2	12.5	0.2	20	0.9998	88.4 ± 1.2	105.3 ± 4.4
#57	LTB4	LB	5S,12R-dihydroxy-6Z,8E,10E,14Z-eicosatetraenoic acid	335.146	195.077	-90	-22	-19	129.007	-90	-26	-12	7.43	0.02	31.0	0.004	200	0.9995	322.1 ± 11.1	244.1 ± 6.4
#58	20-hydroxy LTB4	LB	5S,12R,20-trihydroxy-6Z,8E,10E,14Z-eicosatetraenoic acid	351.135	195.100	-100	-24	-17	129.027	-100	-26	-9	2.93	0.1	11.9	0.04	20	0.9994	103.3 ± 4.6	118.1 ± 1.8
#59	20-carboxy LTB4	LB	5S,12R-dihydroxy-6Z,8E,10E,14Z-eicosatetraene-1,20-dioic acid	365.113	303.158	-83	-24	-26	59.010	-35	-54	-9	2.81	4.0	17.6	4.0	200	0.9993	115.5 ± 6.4	119.9 ± 8.6
#60	18-carboxy dinor LTB4	LB	7R,14S-dihydroxy-4Z,8E,10E,12Z-octadecatetraenedioic acid	337.100	58.987	-25	-46	-9	195.016	-5	-22	-11	1.93	1.0	14.5	0.4	200	0.9998	118.1 ± 12.8	115.8 ± 4.0
#61	2,3-dinor TXB1	TB	9α,11,15S-trihydroxy-2,3-dinor-thromba-13E-en-1-oic acid	343.134	143.055	-60	-24	-15	169.054	-60	-20	-17	2.47	1.0	23.6	1.0	200	0.9999	113.7 ± 5.2	128.3 ± 15.1
#62	TXB2	TB	9α,11,15S-trihydroxythromba-5Z,13E-dien-1-oic acid	369.145	169.026	-70	-24	-9	195.035	-70	-20	-23	4.34	0.2	35.6	0.2	20	0.9998	109.9 ± 7.6	126.5 ± 12.4
#63	11-dehydro-TXB2	TB	11-keto TXB2; 9α,15S-dihydroxy-11-oxothromba-5Z,13E-dien-1-oic acid	367.142	305.138	-115	-22	-29	161.011	-115	-26	-15	4.86	1.0	15.3	1.0	200	1.0000	113.0 ± 4.3	102.6 ± 5.0
#64	2,3-dinor TXB2	TB	9α,11,15S-trihydroxy-2,3-dinor-thromba-5Z,13E-dien-1-oic acid	341.053	123.028	-95	-22	-11	141.005	-95	-18	-13	2.74	1.0	20.7	1.0	200	1.0000	110.8 ± 15.5	107.9 ± 6.0
#65	11-dehydro-2,3-dinor TXB2	TB	9α,15S-dihydroxy-11-oxo-2,3-dinor-thromba-5Z,13E-dien-1-oic acid	339.104	277.113	-55	-14	-13	58.998	-65	-34	-1	2.93	0.2	11.4	0.2	200	0.9998	117.1 ± 3.7	114.1 ± 4.7
#66	11-dehydro TXB3	TB	9α,15S-dihydroxy-11-oxo-thromba-5Z,13E,17Z-trien-1-oic acid	365.113	303.158	-83	-24	-26	161.050	-130	-26	-15	3.40	1.0	12.1	1.0	200	0.9999	119.5 ± 9.1	113.7 ± 4.3
#67	14,15-diHETE	AA	14,15-dihydroxy-5Z,8Z,11Z,17Z-eicosatetraenoic acid	335.109	207.077	-20	-24	-17	57.982	-45	-44	-9	7.43	1.0	34.1	0.2	20	0.9982	88.1 ± 2.0	109.1 ± 12.1
#68	17,18-diHETE	AA	17,18-dihydroxy-5Z,8Z,11Z,14Z-eicosatetraenoic acid	335.141	247.090	-50	-22	-21	57.029	-90	-48	-9	7.40	0.2	23.0	1.0	20	0.9990	100.2 ± 21.8	87.2 ± 21.6