

Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Development and application of a UHPLC-MS/MS metabolomics based comprehensive systemic and tissue-specific screening method for inflammatory, oxidative and nitrosative stress

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Table S1 Target list with optimised MRM transitions and retention times for the identified metabolites, putative identified metabolites and ISTDs

Compound class	Compound name	Retention time (min)	Precursor ion> Product ion (m/z)	CE (V)	Dwell time (msec)	MS Polarity	Internal standard	Lipid Maps ID
Identified metabolites based on standards								
Alkyl-lysophosphatidic acid *	aLPA C18:1	3.13	421.20>78.95	26	12	Neg	LPA C17:0	x
Cyclic-lysophosphatidic acid *	cLPA C18:1	3.70	417.20>281.00	17	12	Neg	cLPA C17:0	LMGP00000056
Isoprostane	2,3-dinor-11B-iso-PGF2 α	3.12	325.00>145.20	20	20	Neg	(d4) PGF2 α	LMFA03010011
Isoprostane	2,3-dinor-8-iso-PGF2 α	2.89	325.10>237.20	12	20	Neg	(d4) 8-iso-PGF2 α	LMFA03110010
Isoprostane	5-iPF2 α VI	4.70	353.30>115.05	22	40	Neg	(d11) 5-iPF2 α VI	LMFA03110010
Isoprostane	8,12-iPF2 α VI	6.20	353.30>115.05	22	40	Neg	(d11) 8,12-iPF2 α VI	
Isoprostane	8-iso-13,14-dihydro-PGF2 α	4.98	353.30>183.10	25	20	Neg	(d4) 8-iso-PGF2 α	
Isoprostane	8-iso-15-keto-PGE2	5.19	349.10>287.20	16	20	Neg	(d4) 8-iso-PGE2	LMFA03110009
Isoprostane	8-iso-15-keto-PGF2 α	4.55	351.10>315.15	22	20	Neg	(d4) 8-iso-PGF2 α	LMFA03110005
Isoprostane	8-iso-15-keto-PGF2b	4.40	351.10>315.15	22	20	Neg	(d4) 8-iso-PGF2 α	
Isoprostane	8-iso-15-R-PGF2 α	4.17	353.30>193.20	25	20	Neg	(d4) 8-iso-PGF2 α	
Isoprostane	8-iso-PGA1	6.77	335.10>273.15	19	20	Neg	(d4) PGA2	LMFA03110008
Isoprostane	8-iso-PGA2	6.71	333.10>271.20	15	20	Neg	(d4) PGA2	
Isoprostane	8-iso-PGE1	5.18	353.30>317.20	15	20	Neg	(d4) PGE1	LMFA03110002
Isoprostane	8-iso-PGE2	4.97	351.10>271.15	17	20	Neg	(d4) 8-iso-PGE2	LMFA03110003
Isoprostane	8-iso-PGF1 α	4.39	355.30>311.10	22	20	Neg	(d9) 8-iso-PGF1 α	
Isoprostane	8-iso-PGF2 α (15-F2t-IsoP)	4.37	353.30>193.20	25	20	Neg	(d4) 8-iso-PGF2 α	LMFA03110001
Isoprostane	8-iso-PGF3 α	3.52	351.10>307.15	19	20	Neg	(d4) 8-iso-PGF2 α	LMFA03110007
Isoprostane	iPFa-IV	4.05	353.30>127.10	22	20	Neg	(d4) iPFa-VI	
Lysophosphatidic acid *	LPA C16:0	3.11	409.00>153.05	22	12	Neg	LPA C17:0	LMGP10050006
Lysophosphatidic acid *	LPA C18:0	3.40	437.30>153.05	24	12	Neg	LPA C17:0	LMGP10050005
Lysophosphatidic acid *	LPA C20:4	3.03	456.70>153.05	22	12	Neg	LPA C17:0	LMGP10050013
Sphingoid	Sph C18:1	8.03	300.10>252.30	-18	8	Pos	Sph C17:1	LMSP01010001
Sphingoid	Spha C18:0	8.18	302.50>60.05	-22	8	Pos	Spha C17:0	LMSP01020001
Sphingoid *	Spha1P C18:0	3.24	380.00>79.05	41	12	Neg	Spha1P C17:0	LMSP01050002
Sphingoid *	S1P C18 :1	3.12	378.00>79.05	25	12	Neg	S1P C17:1	LMSP01050001
Nitro-Fatty acid	NO2-LA (C18:2)	8.40	324.30>277.25	12	40	Neg	10-Nitrooleate-d17	LMFA01120001/2
Nitro-Fatty acid	NO2-OA (C18:1)	8.55	326.10>46.00	14	40	Neg	10-Nitrooleate-d17	LMFA01120003/4
Platelet activating factor *	PAF C16:0	4.66	524.20>125.10	-25	15	Pos	PAF C18:0-d4	LMGP01020046
Prostaglandins	13,14-dihydro-PGF2 α	5.99	353.30>183.10	25	20	Neg	(d4) PGF2 α	LMFA03010079
Prostaglandins	PGA1	7.08	335.10>273.15	19	20	Neg	(d4) PGA2	LMFA03010005
Prostaglandins	PGA2	6.82	333.10>271.20	15	20	Neg	(d4) PGA2	LMFA03010035
Prostaglandins	PGD2	5.42	351.10>271.15	17	20	Neg	(d4) PGD2	LMFA03010004
Prostaglandins	PGD3	4.40	349.10>269.20	17	20	Neg	(d4) PGD2	LMFA03010142
Prostaglandins	PGE1	5.55	353.30>317.20	15	20	Neg	(d4) PGE1	LMFA03010134
Prostaglandins	PGE2	5.22	351.10>271.15	17	20	Neg	(d9) PGE2	LMFA03010003
Prostaglandins	PGE3	4.25	349.10>269.20	17	20	Neg	(d9) PGE2	LMFA03010135

Prostaglandins	PGF1 α	5.25	355.30>311.10	22	20	Neg	(d9) 8-iso-PGF1 α	LMFA03010137
Prostaglandins	PGF2 α	5.15	353.30>193.20	25	20	Neg	(d4) PGF2 α	LMFA03010002
Prostaglandins	PGF3 α	4.15	351.10>307.15	19	20	Neg	(d4) PGF2 α	LMFA03010138
Putatively identified metabolites								
Alkyl-lysophosphatidic acid *	aLPA C16:1	3.00	393.20>78.95	25	12	Neg	LPA C17:0	x
Cyclic-lysophosphatidic acid *	cLPA C16:0	3.62	390.90>255.00	20	12	Neg	cLPA C17:0	LMGP00000057
Cyclic-lysophosphatidic acid *	cLPA C18:0	3.93	418.90>283.00	20	12	Neg	cLPA C17:0	LMGP00000055
Cyclic-lysophosphatidic acid *	cLPA C18:2	3.49	414.90>279.00	20	12	Neg	cLPA C17:0	x
Cyclic-lysophosphatidic acid *	cLPA C20:3	3.63	440.90>305.00	20	12	Neg	cLPA C17:0	x
Cyclic-lysophosphatidic acid *	cLPA C20:4	3.48	438.90>303.00	20	12	Neg	cLPA C17:0	x
Lysophosphatidic acid *	LPA C14:0	2.85	381.20>153.05	22	12	Neg	LPA C17:0	LMGP10050007
Lysophosphatidic acid *	LPA C16:1	2.94	407.20>153.05	22	12	Neg	LPA C17:0	LMGP10050016
Lysophosphatidic acid *	LPA C18:1	3.13	435.20>153.05	22	12	Neg	LPA C17:0	LMGP10050008
Lysophosphatidic acid *	LPA C18:2	3.02	433.20>153.05	22	12	Neg	LPA C17:0	LMGP10050017
Lysophosphatidic acid *	LPA C18:3	2.91	431.20>153.05	22	12	Neg	LPA C17:0	LMGP10050023
Lysophosphatidic acid *	LPA C20:0	3.90	465.20>153.05	22	12	Neg	LPA C17:0	LMGP10050018
Lysophosphatidic acid *	LPA C20:1	3.50	463.20>153.05	22	12	Neg	LPA C17:0	LMGP10050026
Lysophosphatidic acid *	LPA C20:2	3.32	461.20>153.05	22	12	Neg	LPA C17:0	LMGP10050027
Lysophosphatidic acid *	LPA C20:3	3.08	459.20>153.05	22	12	Neg	LPA C17:0	LMGP10050028
Lysophosphatidic acid *	LPA C20:5	2.91	455.20>153.05	22	12	Neg	LPA C17:0	LMGP10050033
Lysophosphatidic acid *	LPA C22:4	3.19	485.20>153.05	22	12	Neg	LPA C17:0	LMGP10050020
Lysophosphatidic acid *	LPA C22:5	3.10	483.20>153.05	22	12	Neg	LPA C17:0	x
Lysophosphatidic acid *	LPA C22:6	2.99	481.20>153.05	22	12	Neg	LPA C17:0	LMGP10050019
Nitro-Fatty acid	NO2-aLA (C18:3)	8.25	322.10>46.05	12	12	Neg	10-Nitrooleate-d17	
Internal standards								
Cyclic-lysophosphatidic acid *	cLPA C17:0	3.77	405.20>269.25	20	12	Neg	na	
Isoprostane	5-iPF2 α -VI-d11	4.68	364.20>115.05	22	20	Neg	na	LMFA03110011
Isoprostane	8-iso-PGE2-d4	4.93	355.30>275.25	18	20	Neg	na	LMFA03010008
Isoprostane	8-iso-PGF1 α -d9	4.36	364.20>320.25	23	20	Neg	na	
Isoprostane	8-iso-PGF2 α -d4	4.35	357.30>197.15	20	20	Neg	na	
Isoprostane	iPF2 α -VI-d4	4.70	357.20>114.90	22	20	Neg	na	
Isoprostane	8,12-iso-iPF2 α -VI-d11	6.17	364.20>115.05	23	40	Neg	na	
Lysophosphatidic acid *	LPA C17:0	3.19	423.20>153.05	22	12	Neg	na	LMGP10050036
Sphingoid	Sph C17:1	7.82	285.90>238.30	-14	8	Pos	na	
Sphingoid	Spha C17:0	7.94	288.10>59.80	-18	8	Pos	na	
Sphingoid *	S1P C17:1	2.91	364.00>78.95	30	12	Neg	na	
Sphingoid *	SphaIP C17:0	3.01	366.00>79.05	28	12	Neg	na	
Nitro-Fatty acid	10-Nitrooleate-d17	8.53	343.10>46.05	15	20	Neg	na	
Platelet activating factor *	PAF C16:0-d4	4.65	527.70>125.00	-52	15	Pos	na	
Prostaglandins	PGD2-d4	5.40	355.30>275.25	18	20	Neg	na	LMFA03010007
Prostaglandins	PGF2 α -d4	5.13	357.30>197.15	20	20	Neg	na	LMFA03010006
Prostaglandins	PGE2-d9	5.20	359.90>280.25	18	20	Neg	na	
Prostaglandins	PGE1-d4	5.53	357.30>321.20	15	20	Neg	na	
Prostaglandins	PGA2-d4	6.80	336.90>275.00	11	20	Neg	na	
Reference	Cuda	7.58 / 2.65*	339.30>214.25	24		Neg	na	

* Indicates that the metabolite is measured during the high pH run

Table S2 Provides an overview of the concentrations of the calibration solution and eventual spiked levels obtained during this study

Calibration level	Stock concentration (nM)	dilution factor based on C8 level	Calibration level when 20 μ L is spiked into 150 μ L sample (nM)
C8	1304 [*] /4000 [#]	-	173/533
C7	1004/3080	0.77	133/410
C6 (high)	495/1520	0.38	66/202
C5	247/760	0.19	33/101
C4 (Medium)	104/320	0.08	13/42
C3	10/30.8	0.0077	1.3/4.1
C2 (Low)	2.5/7.6	0.0019	0.3/1.0
C1	0.75/2.3	0.00058	0.09/0.3
C0	0/0	0	0/0

^{*} Isoprostanes, prostanoids and nitro-fatty acids concentrations

[#] Lysophosphatidic acid and sphingoid base standards concentrations

Table S3 Lysophosphatidic acid and cyclic-lysophosphatidic acid species target list

Lysophosphatidic acid targets	Dehydrated parent (M-H) (m/z)	Fragments		Retention time (min)#	Cyclic-lysophosphatidic acid targets	Dehydrated Parent (M-H) (m/z)	Fragments		Retention time (min)#
		Dehydrated glycerol phosphate (m/z)	Phosphate (m/z)				Fatty acid (m/z)	Phosphate (m/z)	
LPA C14:0	381.2	152.9	78.9	2.85	εLPA C14:0	363.2	227.2	78.9	ND
LPA C16:0 **	409.2	152.9	78.9	3.11	εLPA C16:0	391.2	255.2	78.9	3.62
LPA C16:1	407.2	152.9	78.9	2.94	εLPA C16:1	389.2	253.2	78.9	ND
LPA C17:0 **	423.9	152.9	78.9	3.19	εLPA C17:0 **	405.2	269.2	78.9	3.77
LPA C18:0 **	437.2	152.9	78.9	3.4	εLPA C18:0	419.2	283.2	78.9	3.93
LPA C18:1	435.2	152.9	78.9	3.13	εLPA C18:1 **	417.2	281.2	78.9	3.7
LPA C18:2	433.2	152.9	78.9	3.02	εLPA C18:2	415.2	279.2	78.9	3.49
LPA C18:3	431.2	152.9	78.9	2.91	εLPA C18:3	413.2	277.2	78.9	ND
LPA C18:4	429.2	152.9	78.9	ND	εLPA C18:4	411.2	275.2	78.9	ND
LPA C20:0	465.2	152.9	78.9	3.9	εLPA C20:0	447.2	311.2	78.9	ND
LPA C20:1	463.2	152.9	78.9	3.5	εLPA C20:1	445.2	309.2	78.9	ND
LPA C20:2	461.2	152.9	78.9	3.32	εLPA C20:2	443.2	307.2	78.9	ND
LPA C20:3	459.2	152.9	78.9	3.08	εLPA C20:3	441.2	305.2	78.9	3.63
LPA C20:4 **	457.2	152.9	78.9	3.03	εLPA C20:4	439.2	303.2	78.9	3.48
LPA C20:5	455.2	152.9	78.9	2.91	εLPA C20:5	437.2	301.2	78.9	ND
LPA C22:0	493.2	152.9	78.9	ND	εLPA C22:0	475.2	339.2	78.9	ND
LPA C22:1	491.2	152.9	78.9	ND	εLPA C22:1	473.2	337.2	78.9	ND
LPA C22:2	489.2	152.9	78.9	ND	εLPA C22:2	471.2	335.2	78.9	ND
LPA C22:4	485.2	152.9	78.9	3.19	εLPA C22:4	467.2	333.2	78.9	ND
LPA C22:5	483.2	152.9	78.9	3.10	εLPA C22:5	465.2	331.2	78.9	ND
LPA C22:6	481.2	152.9	78.9	2.99	εLPA C22:6	463.2	329.2	78.9	ND

** Available Commercial standards which was used to define column retention and elution sequence.

ND – Not Detected

Retention time based on optimized method.

Table S4 An overview of the conditions tested and each class's response in the selected setup

pH conditions	Low pH (<3.4)		High pH (10.3) (* Addition of salt)	
Mobile phase B	Methanol + 0.1 % AA	Acetonitrile + 0.1 % AA	Methanol + 0.1 % NH ₄ OH	Acetonitrile+ 0.1 % NH ₄ OH
Mobile phase A	H ₂ O + 0.1 % AA	H ₂ O + 0.1 % AA	H ₂ O + 0.0625 % NH ₄ OH * H ₂ O + 5mM NH ₄ CH ₃ COO + 0.0625 % NH ₄ OH	H ₂ O + 0.0625 % NH ₄ OH * H ₂ O + 5mM NH ₄ CH ₃ COO + 0.0625 % NH ₄ OH
Isoprostanes	Isomer separation problems	<i>Good peak shape</i>	Good *Matrix effects	Elutes too early < 20 % B *Elutes too early < 20 % B
Prostaglandins	Isomer separation problems	<i>Good peak shape</i>	Good *Matrix effects	Elutes too early < 20 % B *Elutes too early < 20 % B
Nitro-fatty acids	<i>Good peak shape</i>	<i>Good peak shape</i>	<i>Good peak shape</i> * <i>Good peak shape</i>	<i>Good peak shape</i> * <i>Good peak shape</i>
LPAs & aLPAs	Peak tailing Carry over > 20%	Peak tailing Carry over > 20%	<i>OK peak shape</i> Carry over > 5 % * <i>Carry over < 3%</i>	<i>OK peak shape</i> Carry over > 5 % * <i>Carry over < 3%</i>
cLPAs	<i>Good peak shape</i>	<i>Good peak shape</i>	<i>Good peak shape</i> * <i>Good peak shape</i>	<i>Good peak shape</i> * <i>Good peak shape</i>
S1P & Spha1P	Peak tailing Carry over > 20%	Peak tailing Carry over > 20%	<i>OK peak shape</i> Carry over > 5 % * <i>Carry over < 2%</i>	<i>OK peak shape</i> Carry over > 5 % * <i>Carry over < 2%</i>
Sph & Spha	<i>Good peak shape</i>	Peak tailing	Good peak shape Carry over > 5 % * Carry over > 5 % Bad Ion suppression due to LPC/PAF co-elution	Peak tailing Carry over > 5 % * Carry over > 5 %
PAF	Good peak shape but co-eluting with LPC18:0	Good peak shape but co-eluting with LPC 18:0	*Good peak shape but co-eluting with LPC 18:0	* <i>Good peak & separating from LPC 18:0. The separation is sensitive to the column lifespan, and column overloading.</i>

Green/Italics - Good chromatographic performance; - No shading - Chromatographic issues as indicated; AA – Acetic acid

Table S5 Methodology validation recoveries and matrix effects in serum

		% Recovery (Matrix)					Matrix effect		
		I4 - Day 1	I4 - Day 2	I4 - Day 3	Average	RSD	I2-low	I4-medium	I6-high
<i>Lysophosphatidic acid</i>	LPA C17:0	101.4	78.5	96.5	92.1	13.0	1.03	0.72	0.45
	cLPA C17:0	95.8	112.1	106.9	104.9	7.9	1.53	2.02	0.97
<i>Sphingoids</i>	Sph C17:1	101.1	87.0	98.6	95.6	7.9	0.94	0.65	0.38
	S1P C17:1	111.3	94.8	101.7	102.6	8.0	0.89	0.78	0.90
	Spha C17:0	106.4	92.8	102.1	100.4	6.9	0.73	0.67	0.59
	Spha1P C17:0	105.3	109.7	122.2	112.4	7.8	1.28	1.10	0.86
<i>Nitro Fatty acids</i>	d17 10-O2-OA	94.1	106.2	102.3	100.9	6.1	0.25	0.26	0.34
<i>Prostaglandins</i>	d9-PGE2	91.6	93.7	97.4	94.2	3.1	1.16	1.13	0.81
	d4-PGE1	93.6	95.7	96.7	95.3	1.6	1.39	1.16	0.84
	d4-PGD2	95.1	94.0	95.8	95.0	0.9	1.11	1.13	0.79
	d4-PGF2 α	93.6	97.3	95.5	95.5	1.9	1.00	1.11	0.80
	d4-PGA2	96.0	98.4	96.3	96.9	1.3	0.94	1.08	0.78
<i>Isoprostanes</i>	d9-8-iso-PGF1 α	106.5	100.2	97.7	101.5	4.4	1.30	1.35	0.96
	d4-8-iso-PGF2 α	100.0	97.1	97.5	98.2	1.5	1.26	1.33	0.93
	d4-8-iso-PGE2	95.9	95.8	96.6	96.1	0.4	1.30	1.30	0.91
	d11- + 5-iPF2 α VI	97.5	90.6	93.5	93.9	3.7	1.18	1.16	0.81
	d4-iPF2 α VI	101.1	93.6	94.5	96.4	4.2	0.74	0.74	0.58
	d11-8,12-iPF2 α VI	96.3	92.8	92.9	94.0	2.1	0.75	0.71	0.54

Table S6 Six different tissue validation recoveries and matrix effect results

		% Recoveries						Matrix effect *					
		Kidney	Spleen	Liver	Lung	Heart	Brain	Kidney	Spleen	Liver	Lung	Heart	Brain
<i>Lysophosphatidic acid</i>	LPA C17:0	78.5	97	70.8	75.7	105.2	52.6	0.63	0.78	0.80	0.49	0.65	0.47
	cLPA C17:0	89.2	72.1	100.8	91.4	98.9	60.8	0.54	0.66	0.53	0.42	0.87	0.31
<i>Sphingoids</i>	Sph C17:1	112.5	96.6	103.2	103.5	86.6	81.3	0.95	0.94	1.22	0.64	0.61	0.43
	S1P C17:1	90.5	110.4	83	74.6	109.2	69.5	0.55	0.67	0.67	0.46	0.70	0.40
	Spha C17:0	109.0	102.7	101.2	137.9	121.0	71.5	0.37	0.42	0.40	0.27	0.37	0.14
<i>Nitro Fatty acids</i>	d17 10-NO2-OA	43.0	49.9	53.9	37.7	ND	37.8	0.40	0.29	0.29	0.26	0.43	0.28
<i>Prostaglandins</i>	d9-PGE2	121.3	106.3	80.7	95.9	88.8	61.0	1.04	1.07	0.96	0.68	1.00	0.58
	d4-PGE1	119.6	102.7	85.4	96.5	84.1	59.8	1.01	1.04	0.67	0.66	0.99	0.58
	d4-PGD2	113.0	95.7	75.6	94.1	85.3	57.7	1.04	1.07	0.92	0.73	1.02	0.61
	d4-PGF2 α	113.7	99.9	75.3	96.4	84.4	57.7	1.00	1.02	0.75	0.65	0.98	0.53
	d4-PGA2	60.4	46.2	11.2	30.3	48.3	29.3	0.57	0.48	0.14	0.24	0.56	0.28
<i>Isoprostanes</i>	d9-8-iso-PGF1 α	125.9	121.0	70.4	99.1	95.7	53.9	1.01	1.00	0.79	0.64	0.94	0.48
	d4-8-iso-PGF2 α	118.4	105.5	65.9	102.0	87.9	55.7	0.98	1.00	0.79	0.65	0.98	0.52
	d4-8-iso-PGE2	111.1	97.7	62.4	92.3	83.5	57.8	1.01	1.04	0.56	0.66	0.96	0.56
	d11- + 5-iPF2 α VI	128.6	93.9	80.0	105.7	94.5	54.7	1.23	1.11	0.93	0.78	1.07	0.62
	d4-iPF2 α VI	119.9	97.2	77.1	99.5	94.1	56.5	1.27	1.16	0.94	0.82	1.10	0.66
	d11-8,12-iPF2 α VI	110.0	90.0	94.7	102.2	88.9	58.5	1.23	1.13	1.22	0.80	1.06	0.70

ND: Not detected

* with 1 equal to no matrix effect

Table S7 Intraday precision RSD values of the metabolites spiked to the different tissues

Class	Metabolites	Tissue Intraday precision (% RSD, n = 4)					
		Spleen	Lung	Liver	Kidney	Heart	Brain
Isoprostanes	2,3-dinor-8-iso-PGF2 α	5.8	2.9	11.9	5.3	4.5	9.1
	8-iso-PGF3 α	4.7	7.3	13.5	2.0	5.5	8.9
	2,3-dinor-11b-PGF2 α	2.8	4.1	19.2	2.7	1.9	7.5
	8-iso-15-keto-PGF2b	4.2	6.5	10.3	2.4	4.3	5.4
	8-iso-15-keto-PGE2	4.5	9.3	10.9	4.6	2.1	4.9
	8-iso-15-keto-PGF2 α	3.9	9.6	11.2	3.8	4.2	5.2
	iPF2 α	3.2	2.1	5.5	2.02	1.9	6.0
	8-iso-15(R)-PGF2 α	4.6	3.5	18.2	0.6	2.8	5.2
	8-iso-PGF1 α	1.6	3.9	20.6	8.0	2.2	3.3
	8-iso-13,14-dihydro-PGF2 α	2.6	3.7	16.3	5.6	5.5	6.6
	8-iso-PGF2 α	3.8	5.6	11.5	1.8	3.3	7.5
	8-iso-PGE2	3.1	3.5	4.2	2.7	2.2	3.6
	8-iso-PGE1	3.1	6.4	9.3	6.5	0.8	1.7
	5 iPF2 α IV	3.3	2.0	16.2	3.1	3.1	5.3
	8-iso-PGA2	5.8	8.4	27.9	8.6	7.5	16.2
8-iso-PGA1	7.0	5.4	10.7	9.4	6.4	14.8	
8,12-iPF2 α VI	3.3	5.9	24.4	9.8	5.2	35.9	
Lysophosphatidic acids	LPA C20:4	36.2	32.9	57.3	56.8	28.5	42.4
	LPA C16:0	32.7	19.2	16.0	46.7	41.1	33.9
	aLPA C18:1	35.4	35.4	17.9	13.6	21.0	15.7
	LPA C18:0	25.1	14.1	24.8	19.4	22.9	4.4
	cLPA C18:1	5.8	28.1	10.9	15.9	29.7	9.2
Sphingoids	Sph C18:1	12.5	7.4	14.5	10.1	6.2	7.6
	Spha C18:0	25.1	27.2	26.4	29.9	20.6	25.6
	S1P C18:1	17.0	7.9	11.2	16.1	24.2	10.0
Nitro-Fatty acids	NO2-OA	3.7	15.9	4.3	9.3	13.6	7.4
	NO2-LA	10.4	31.5	15.1	14.7	13.3	11.6
Prostaglandins	PGE3	9.5	6.8	4.4	13.3	6.6	9.4
	PGD3	4.5	4.5	27.0	7.2	1.6	6.3
	PGF3 α	3.6	3.0	10.8	3.4	1.0	2.0
	PGE2	6.8	6.2	14.4	6.4	5.4	8.6
	PGE1	2.4	3.6	4.3	6.7	1.2	2.0
	PGD2	6.7	5.2	3.4	2.1	2.8	7.3
	PGF1 α	1.4	5.4	15.0	8.2	2.1	5.1
	PGF2 α	5.2	5.5	3.4	3.0	2.4	4.2
	13,14-dihydro-PGF2 α	2.0	6.5	6.0	2.9	1.2	3.5
	PGA2	1.8	1.9	5.1	2.5	6.3	7.8
PGA1	2.4	2.5	5.5	3.5	5.5	7.7	

Table S8 Comparison of the precision of metabolites (averaged per class) determined for technical replicates and biological replicates. For a simplified overview we averaged the RSDs of the metabolites detected within a class in the different tissues

Metabolite class response	Tissue types											
	Spleen		Lung		Liver		Kidney		Heart		Brain	
	Procedure % RSD (n = 12)	Biological % RSD (n = 8)	Procedure % RSD (n = 12)	Biological % RSD (n = 8)	Procedure % RSD (n = 12)	Biological % RSD (n = 8)	Procedure % RSD (n = 12)	Biological % RSD (n = 8)	Procedure % RSD (n = 12)	Biological % RSD (n = 8)	Procedure % RSD (n = 12)	Biological % RSD (n = 8)
Isoprostanes	11.4	30.9	21.8	29.7	15.4	53.0	22.9	33.1	18.3	34.3	32.1	30.2
Prostaglandins	16.5	36.7	15.5	36.8	17.6	46.1	18.8	76.0	25.9	37.8	22.8	39.3
Nitro-fatty acids	26.7	44.5	45.0	91.4	34.7	19.1	<i>ND</i>	<i>ND</i>	28.9	33.1	<i>ND</i>	<i>ND</i>
Sphingoids	30.7	32.7	19.1	25.0	18.7	41.5	19.8	27.6	18.9	28.7	25.0	41.6
LPA	30.6	41.9	36.5	41.0	40.3	39.1	31.3	33.6	30.2	40.8	31.5	44.2
aLPA	48.0	46.2	15.8	27.7	<i>ND</i>	<i>ND</i>	22.2	25.0	32.3	120.5	18.4	28.0
cLPA	32.3	38.9	28.8	35.1	34.4	47.9	19.2	30.1	36.3	46.8	26.8	64.2

ND - Not detected

LPA - Lysophosphatidic acids, **aLPA** - Alkyl-lysophosphatidic acids; **cLPA** - Cyclic-lysophosphatidic acids

Figures

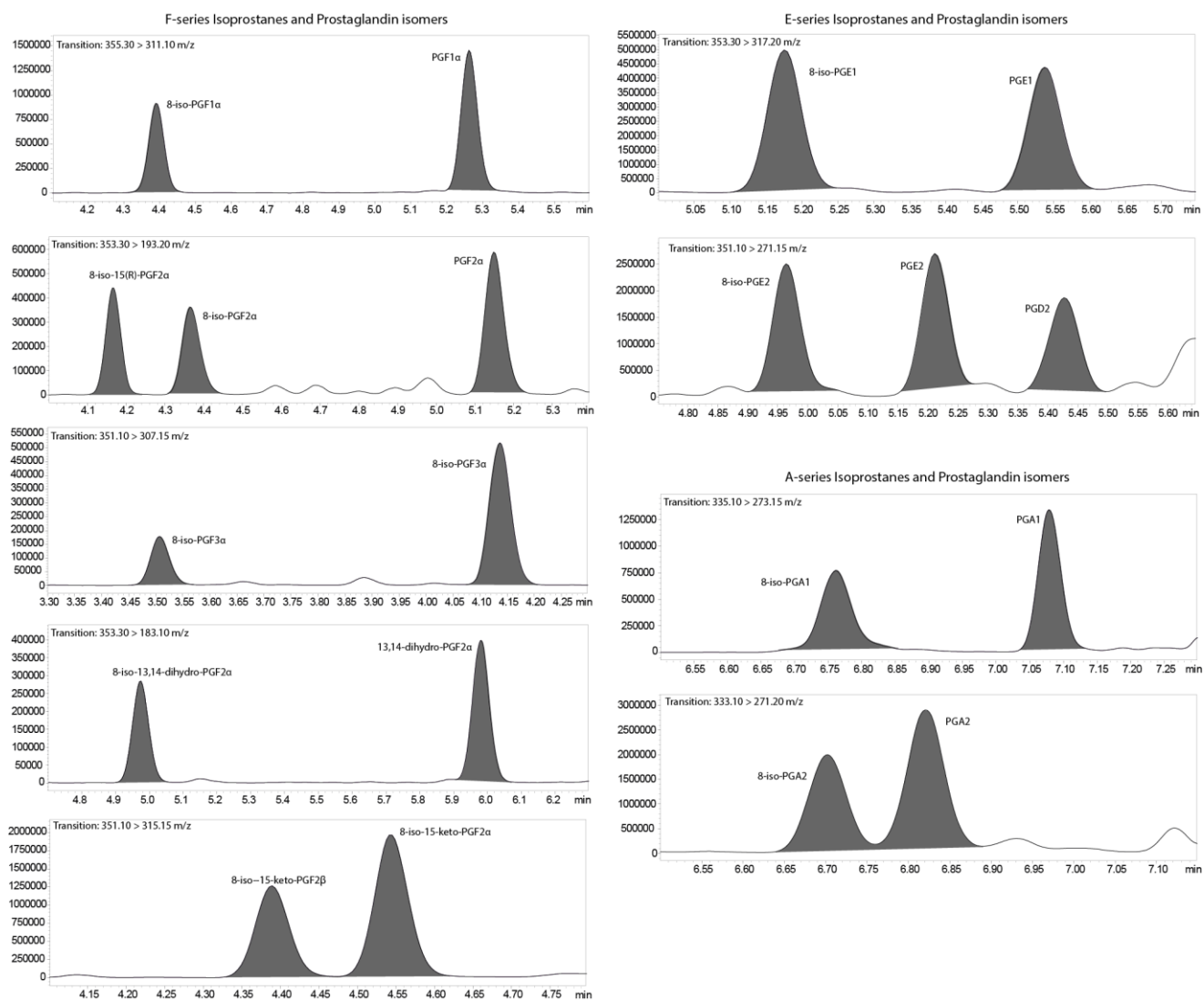


Fig. S1 Chromatographic resolution of the different prostaglandins and isoprostanes isomers

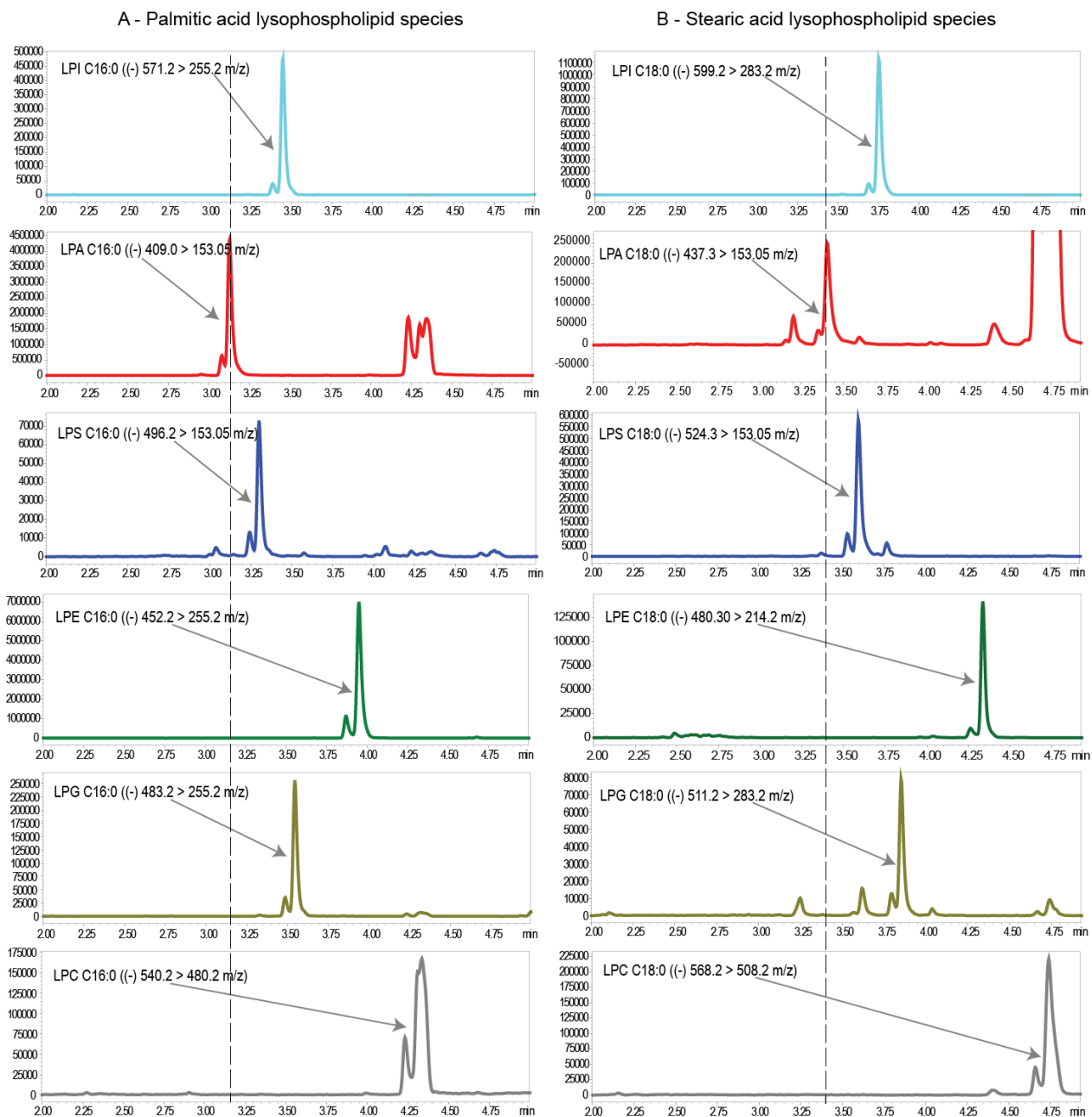


Fig. S2 A chromatographic overview of (a), palmitic acid (C16:0) and (b), stearic acid (C18:0) lysophospholipid species eluting during the high pH chromatographic run. LPA elutes first for both acyl lengths followed by LPS, LPI, LPG, LPE and lastly LPC. The dashed lines indicate the retention time of the two LPA species, and no co-elution is observed for the different lysophospholipid species of the same length. LPI – Lysophosphatidylinositol; LPA – Lysophosphatidic acid; LPS – Lysophosphatidylserine; LPE – Lysophosphatidylethanolamine; LPG – Lysophosphatidylglycerol; LPC – Lysophosphatidylcholine

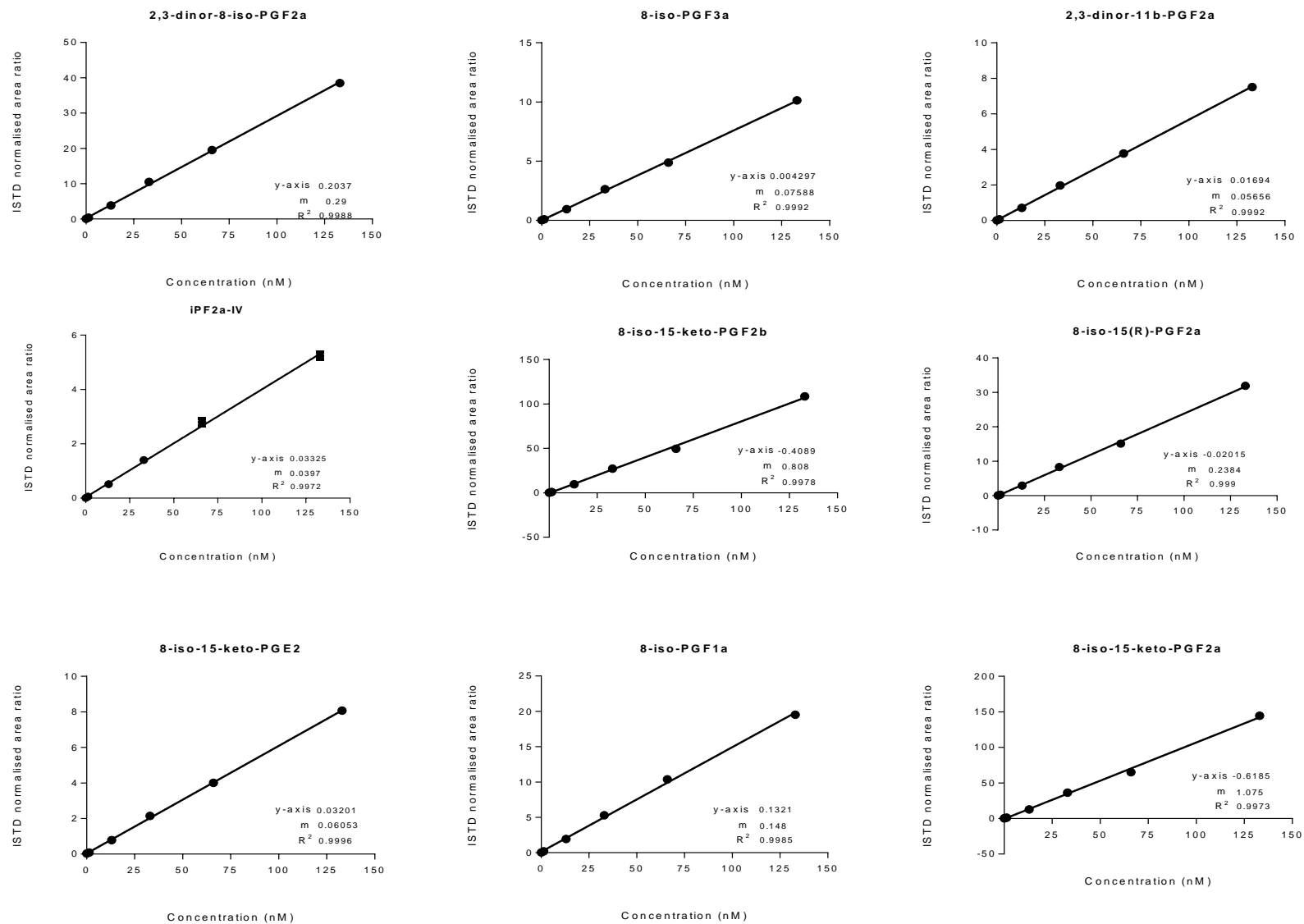


Fig. S3a Academic calibration curves for the isoprostanes, prostaglandin and nitro fatty acid standards

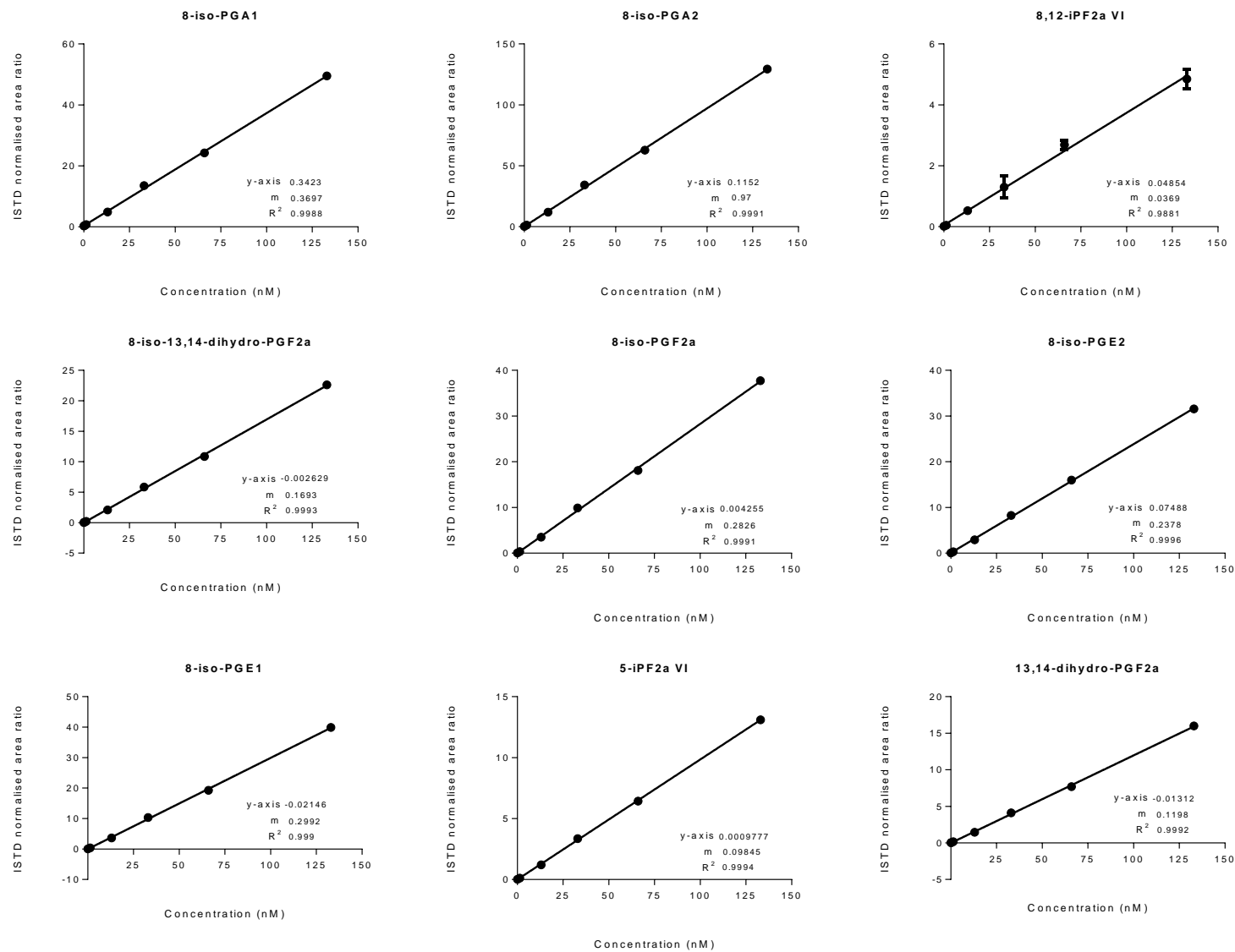


Fig. S3b Academic calibration curves for the isoprostanes, prostaglandin and nitro fatty acid standards

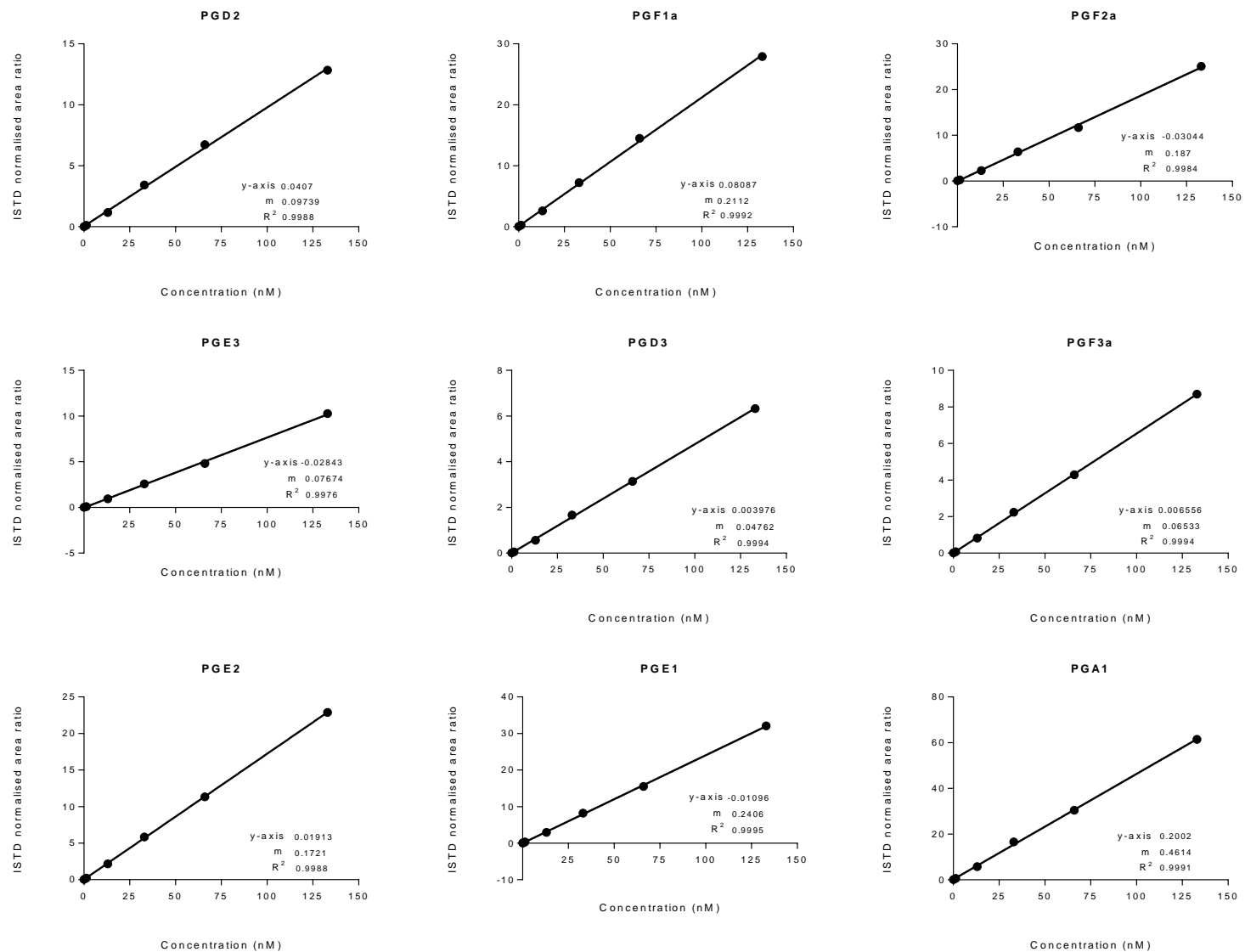


Fig. S3c Academic calibration curves for the isoprostanes, prostaglandin and nitro fatty acid standards

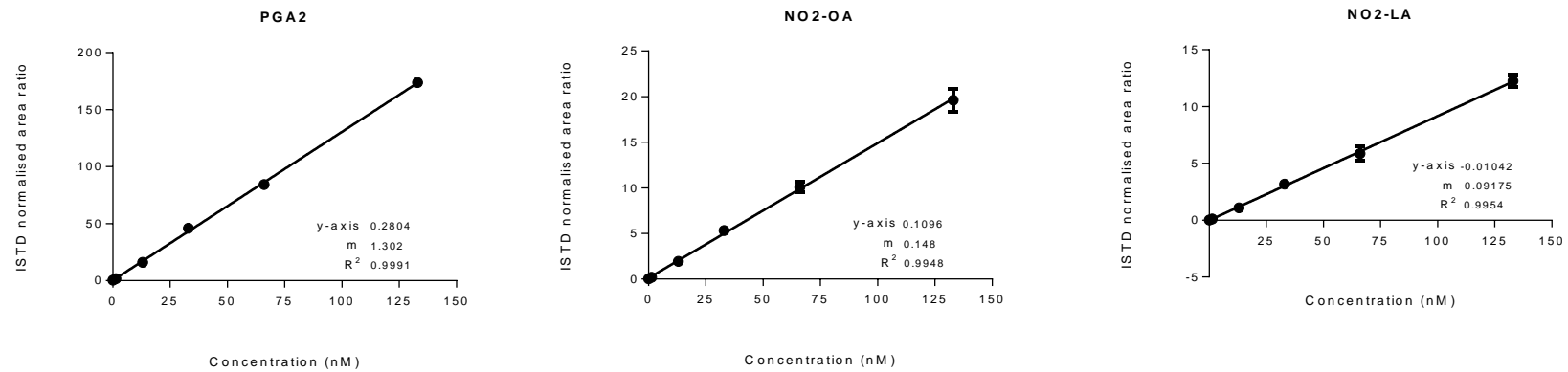


Fig. S3d Academic calibration curves for the isoprostanes, prostaglandin and nitro fatty acid standards

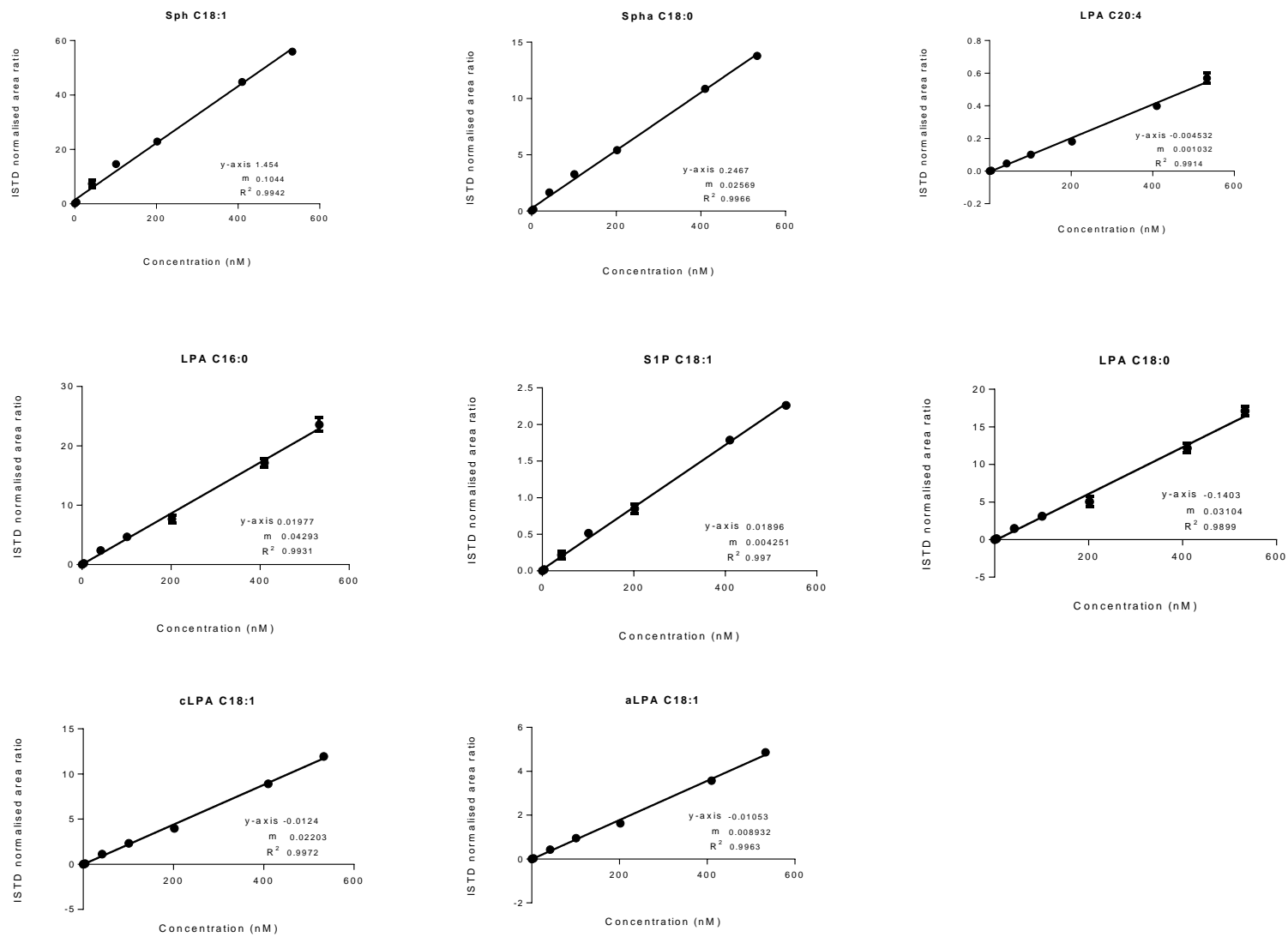


Fig. S3e Academic calibration curves for the sphingoid bases and the different lysophosphatidic acid

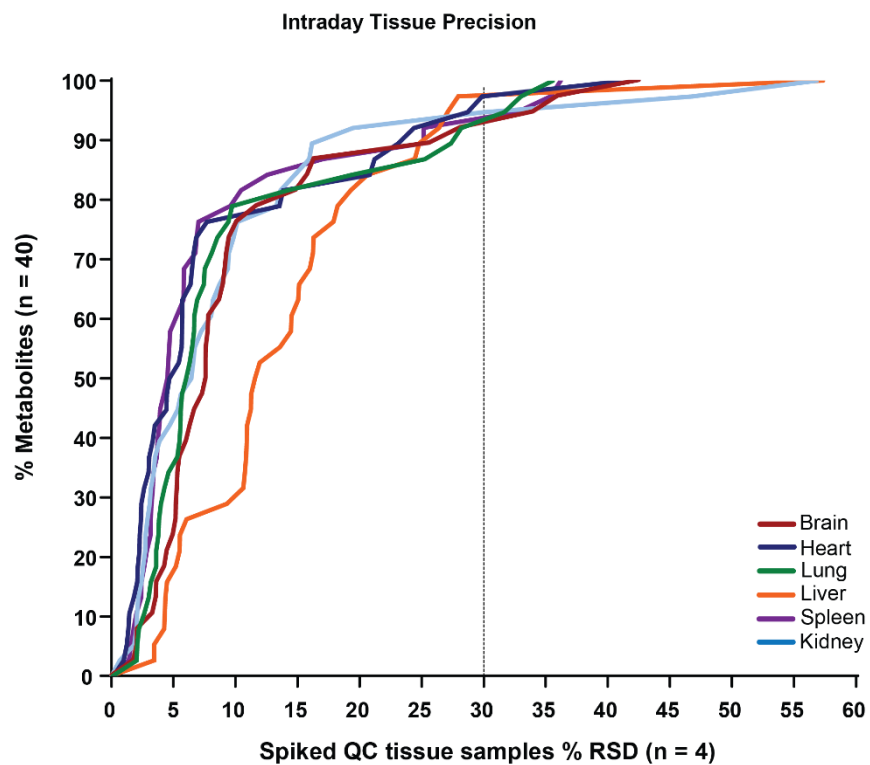


Fig. S4 Intraday precision in six different tissues. Precision was determined using the RSD (n=4) of C4 spiked standards into the different tissue matrixes

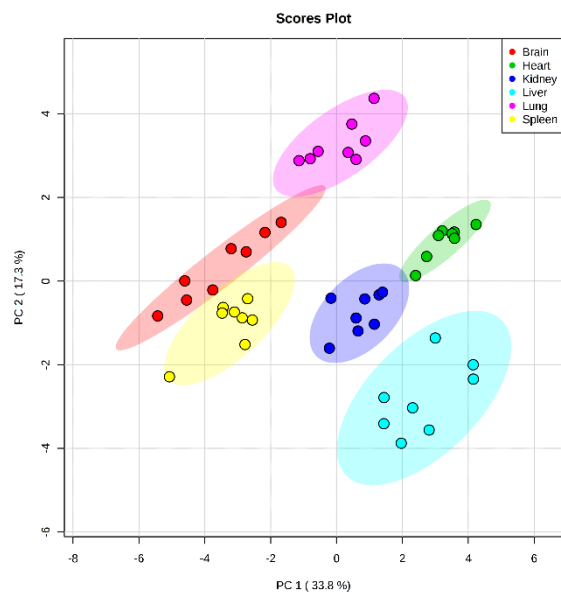


Fig. S5 Principal component analyses (PCA) Scores plot of the six different tissues investigated, using a reduced dataset containing only uniformly detected metabolites with all tissues.