SUPPORTING INFORMATION

Reversed-phase UHPLC/ESI-MS determination of oxylipins in human plasma: case study of female breast cancer

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5 FIGURES, 5 TABLES

Fig. S-1 MS/MS spectra of 63 oxylipins with multiple product ions at optimized collision energy

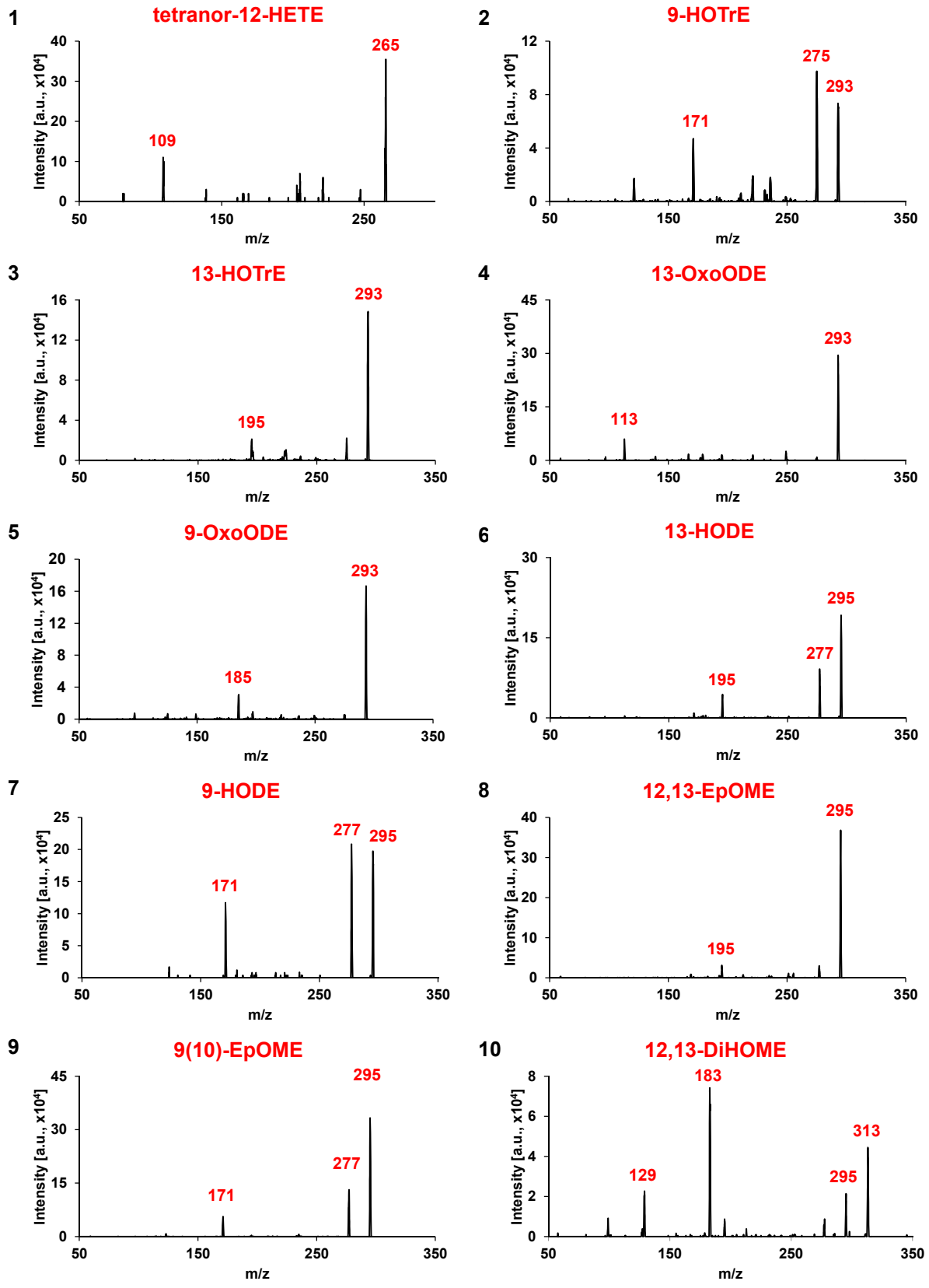

Fig. S-1 (Continuation)

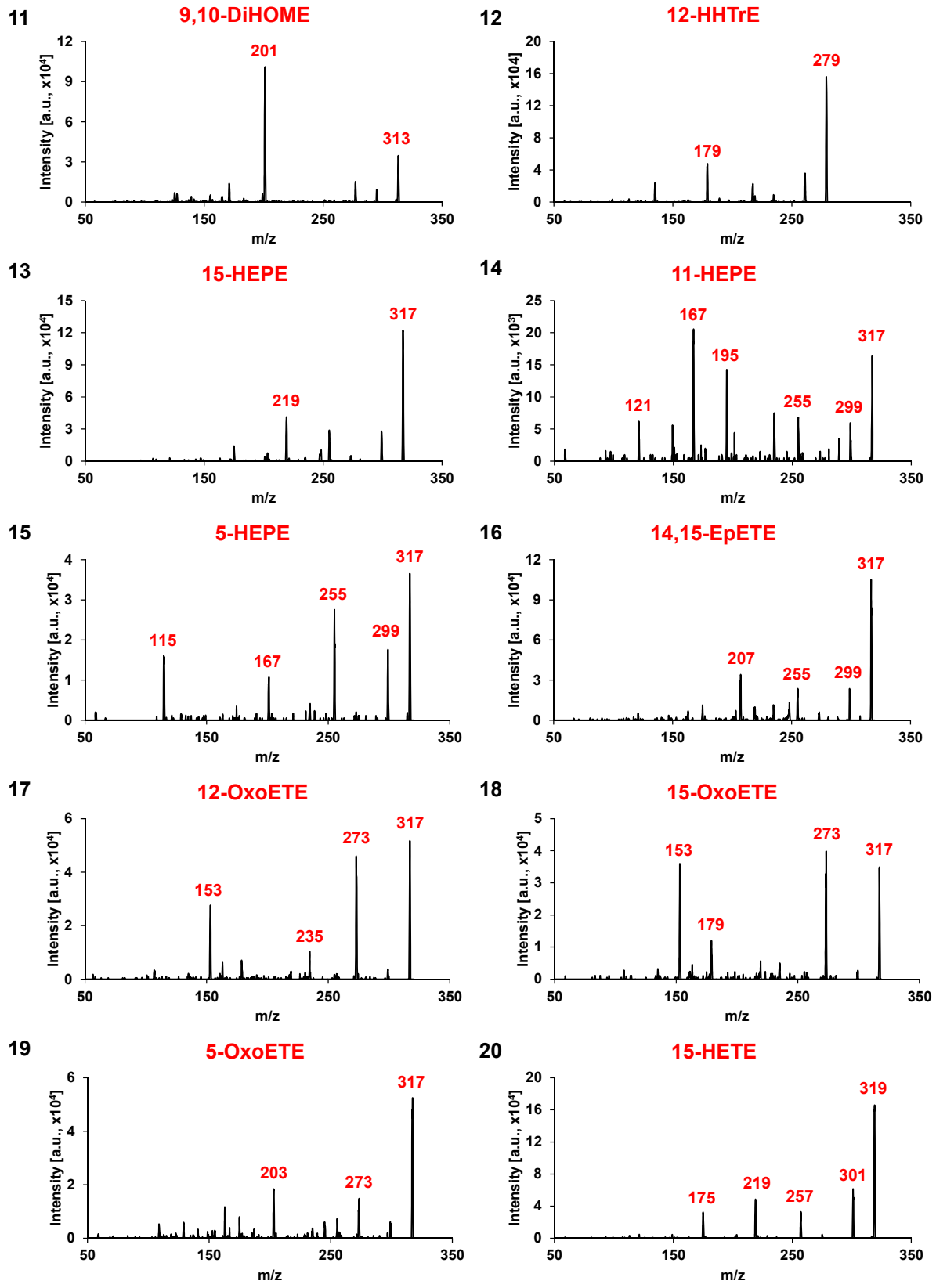

Fig. S-1 (Continuation)

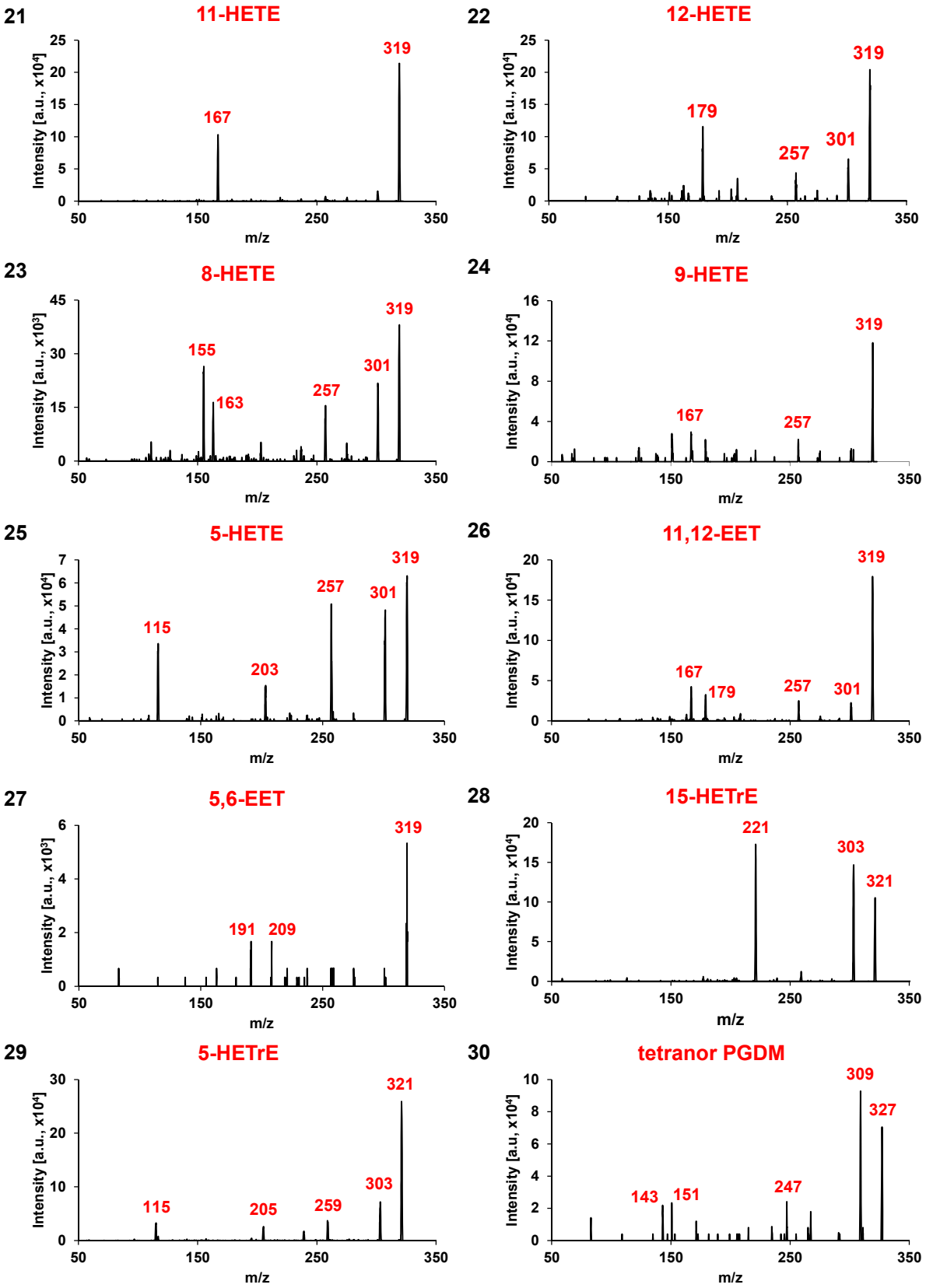

Fig. S-1 (Continuation)

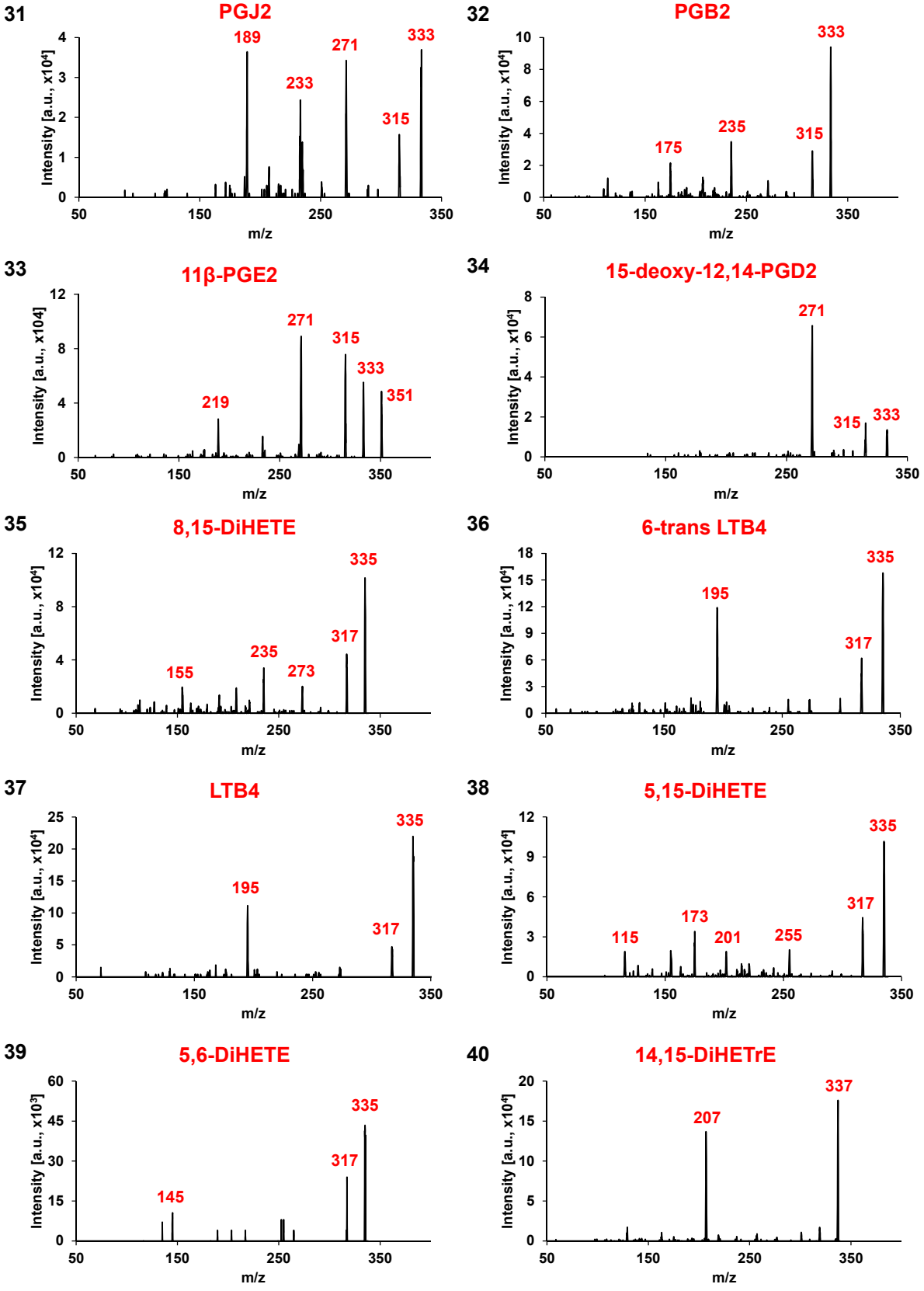

Fig. S-1 (Continuation)

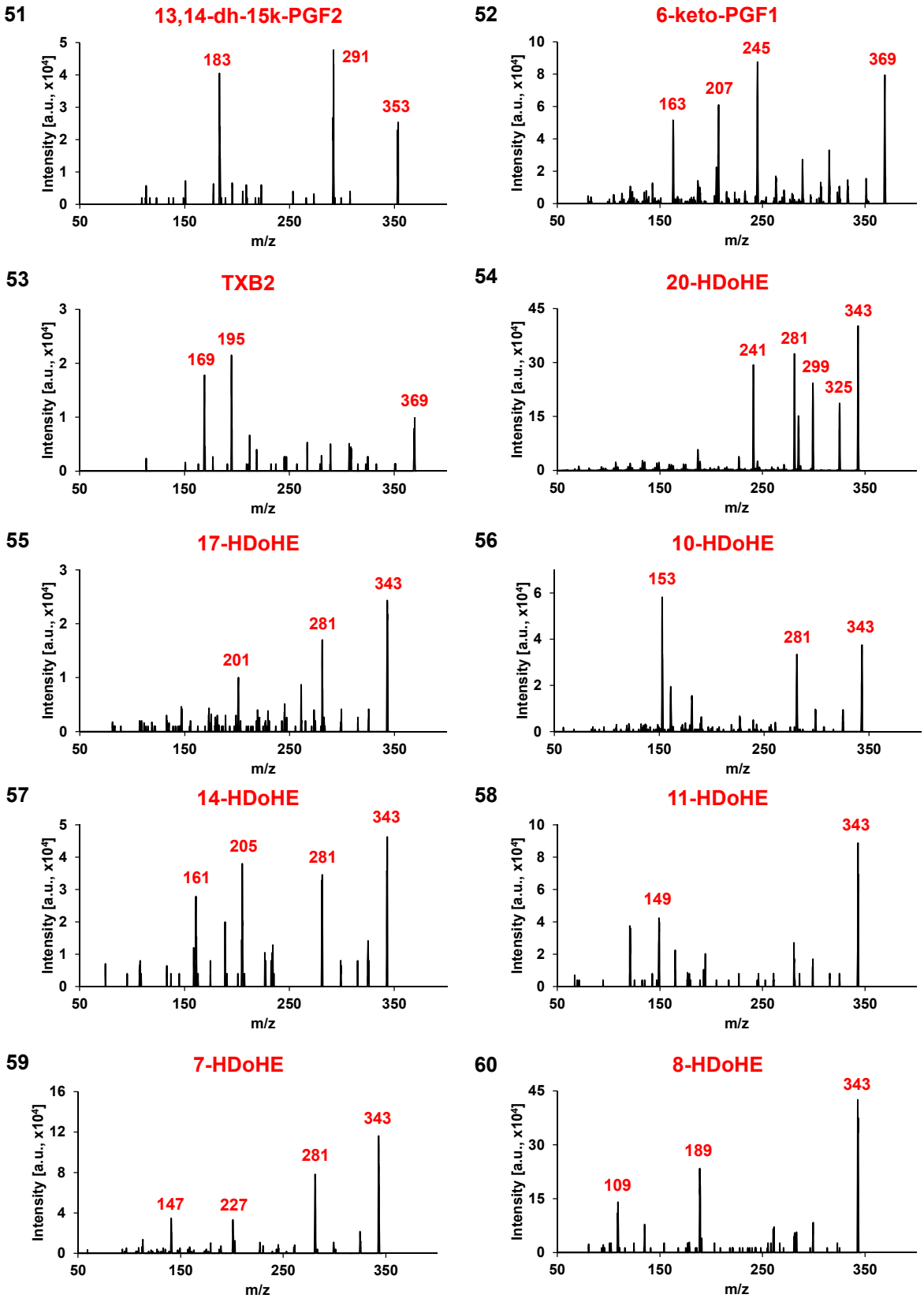

Fig. S-1 (Continuation)

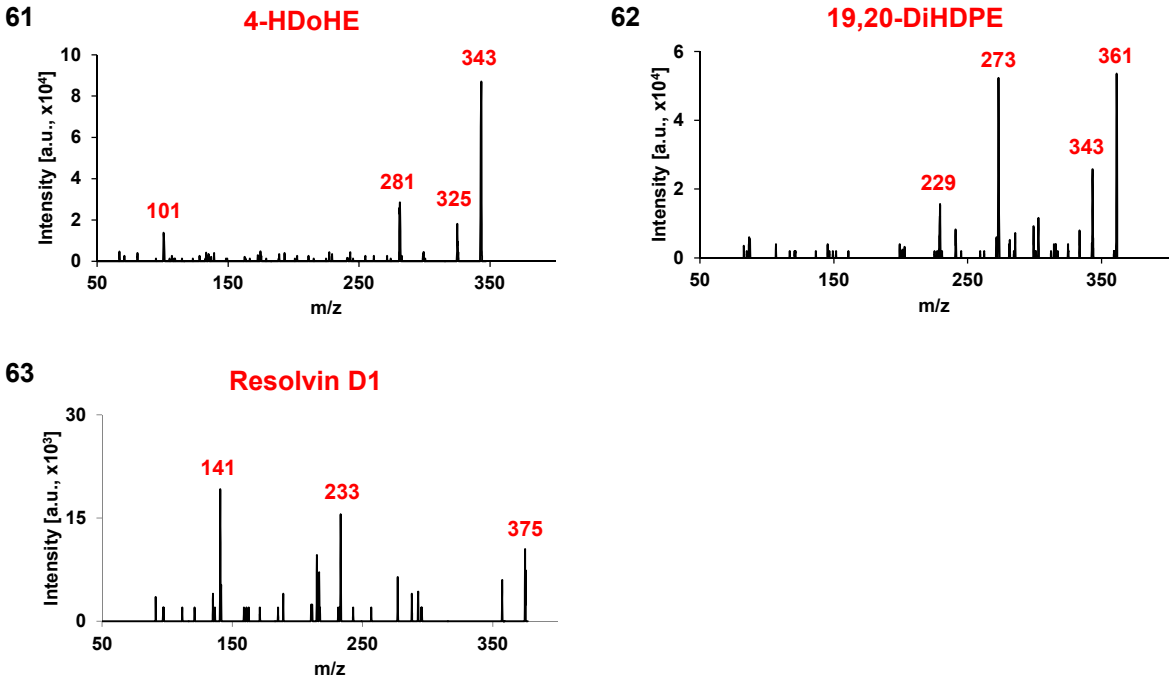

Fig. S-2 MS/MS spectra of selected standard **a** 12-HETE at various collision energies **b** -15V, **c** -18V, **d** -20V, **e** -23V, **f** -25V, **g** -28V, and **h** -30V

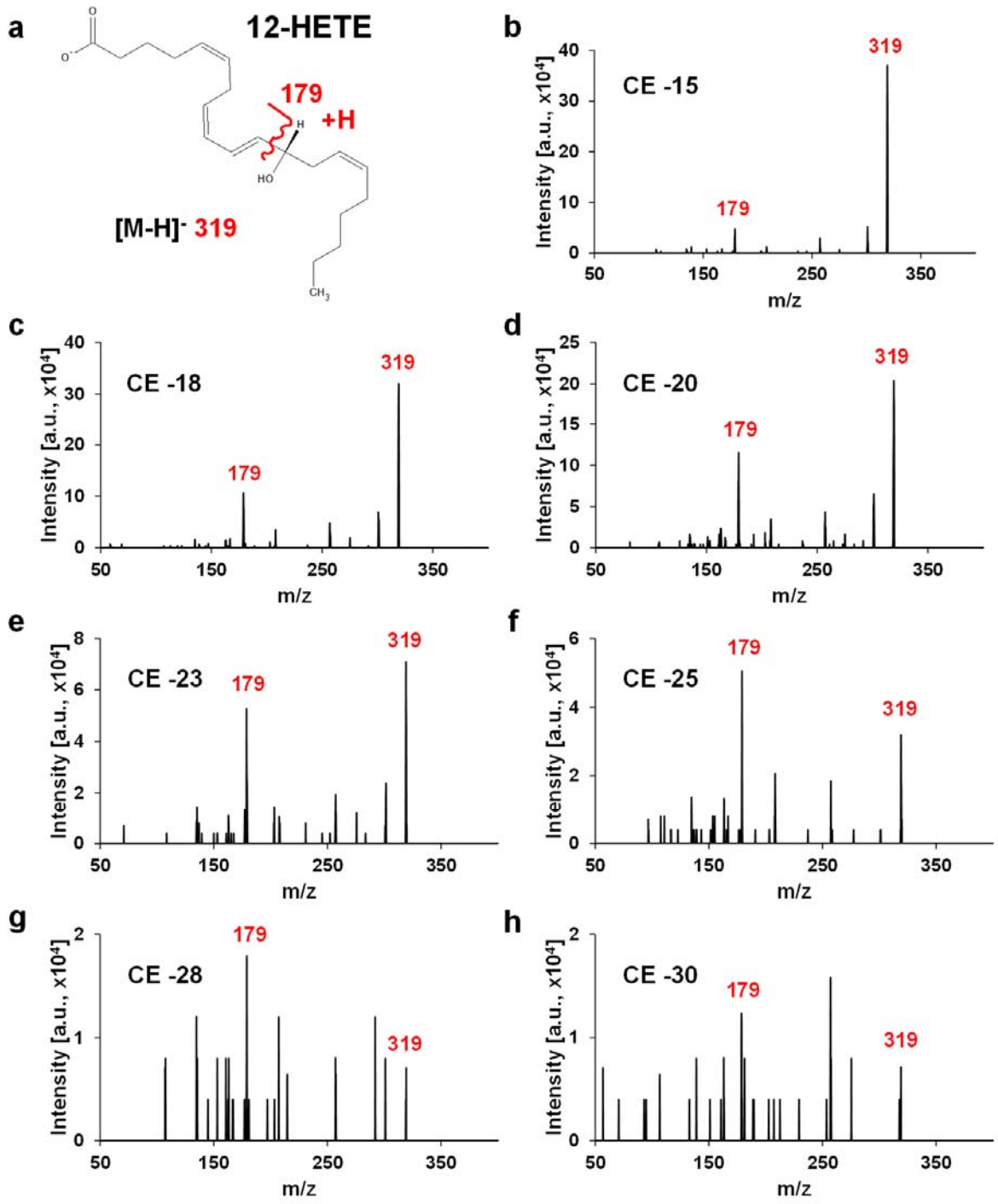

Fig. S-3 Monitoring QC peak areas during the method validation

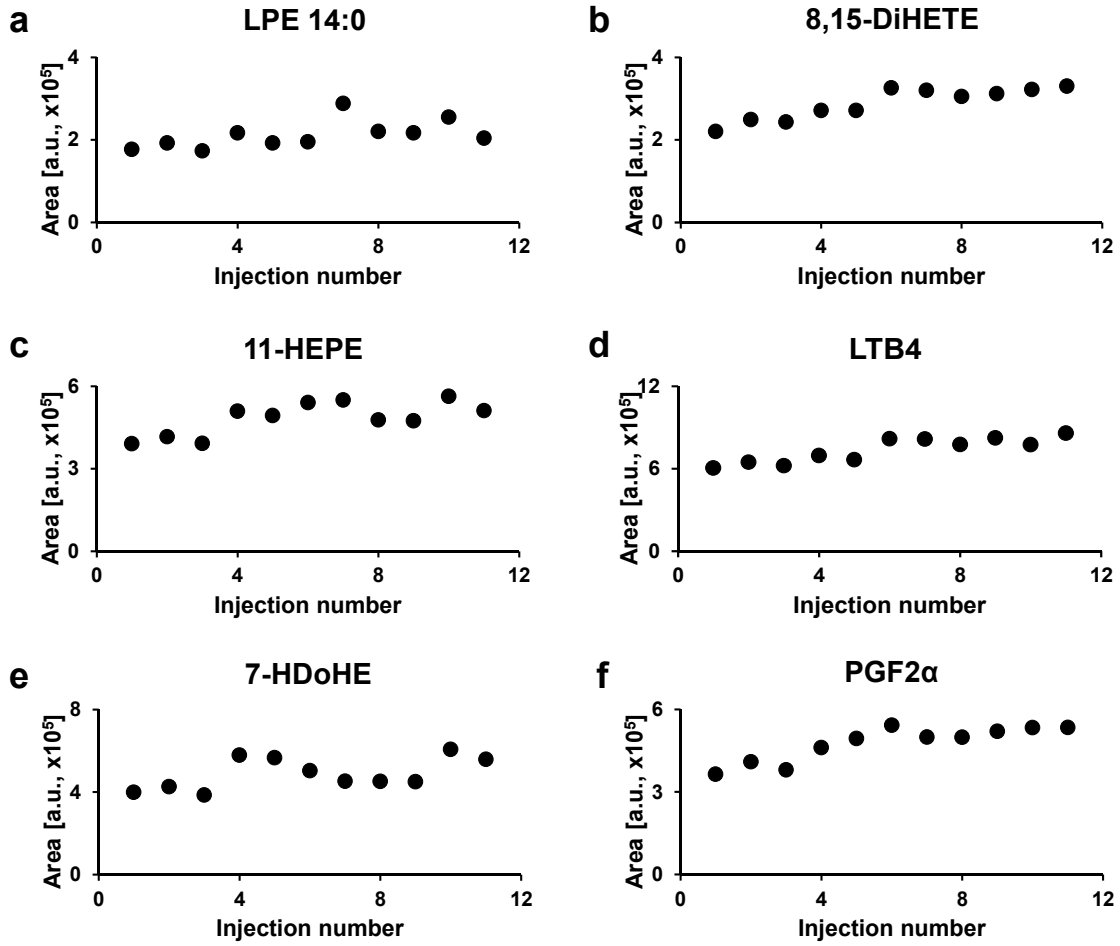

Fig. S-4 Receiver operating characteristic (ROC) and area under the curve (AUC) values

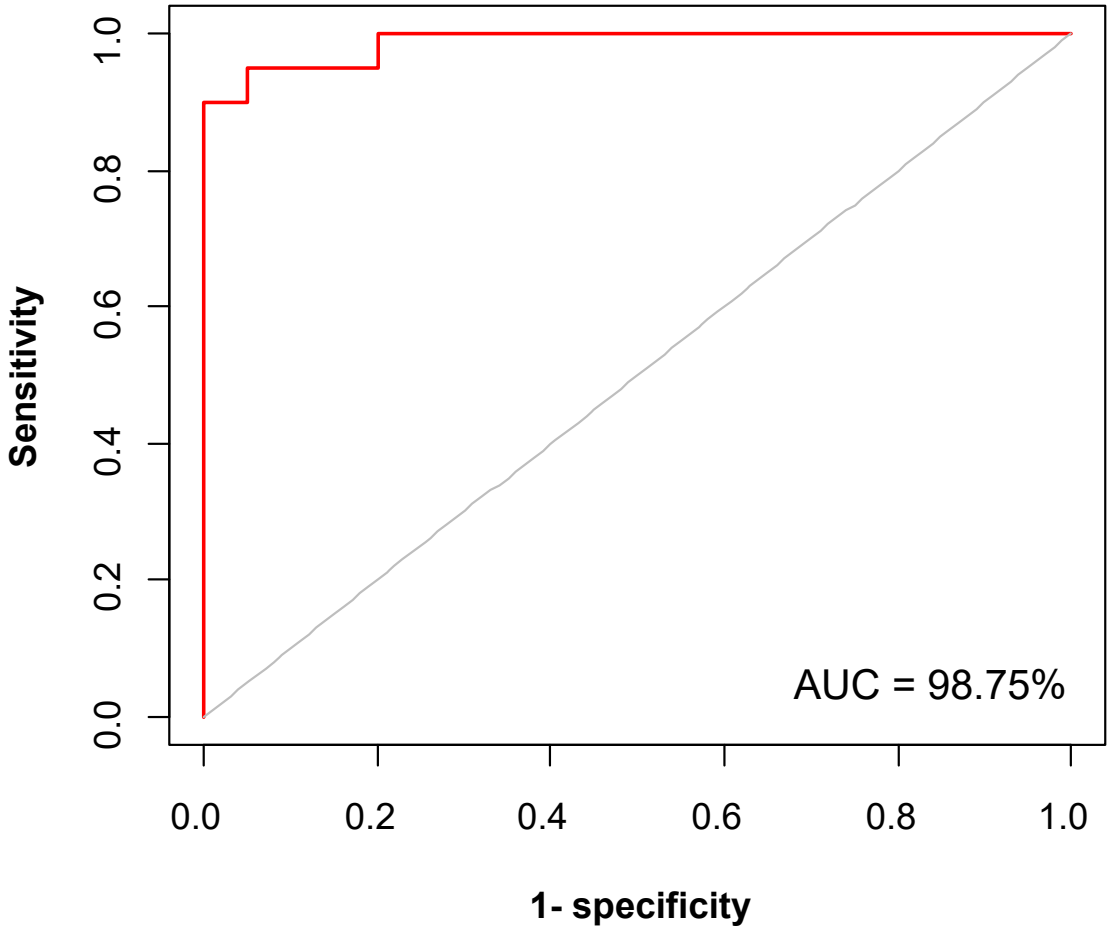

Fig. S-5 S-plot of 21 quantified oxylipins in plasma samples of breast cancer patients, where the most up-regulated lipid species are in the upper right corner

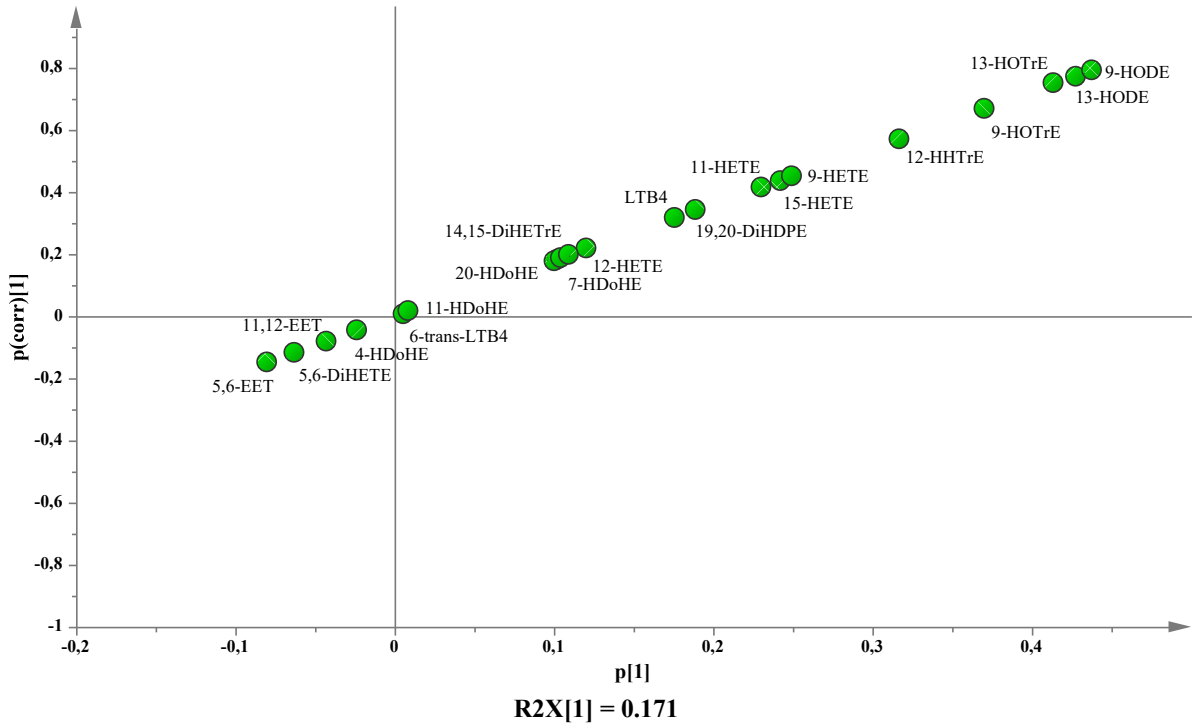

Table S-1 Oxylipin standards with their systematic names used for UHPLC/MS method development

Lipid class	$[M-H]^-$	Oxylipin species	Systematic name
FA and conjugates	265	tetranor-12-HETE	8R-hydroxy-4Z,6E,10Z-hexadecatrienoic acid
Octadecanoids	293	9-HOTrE	9S-hydroxy-10E,12Z,15Z-octadecatrienoic acid
	293	13-HOTrE	13S-hydroxy-9Z,11E,15Z-octadecatrienoic acid
	293	13-OxoODE	13-keto-9Z,11E-octadecadienoic acid
	293	9-OxoODE	9-keto-10E,12Z-octadecadienoic acid
	295	13-HODE	13S-hydroxy-9Z,11E-octadecadienoic acid
	295	9-HODE	9S-hydroxy-10E,12Z-octadecadienoic acid
	295	12(13)-EpOME	(±)-12(13)-epoxy-9Z-octadecenoic acid
	295	9(10)-EpOME	9,10-epoxy-12Z-octadecenoic acid
	313	12,13-DiHOME	12,13-dihydroxy-9Z-octadecenoic acid
	313	9,10-DiHOME	9,10-dihydroxy-12Z-octadecenoic acid
Eicosanoids	279	12-HHTrE	12S-hydroxy-5Z,8E,10E-heptadecatrienoic acid
	317	15-HEPE	(±)-15-hydroxy-5Z,8Z,11Z,13E,17Z-eicosapentaenoic acid
	317	11-HEPE	(±)-11-hydroxy-5Z,8Z,12E,14Z,17Z-eicosapentaenoic acid
	317	5-HEPE	(±)-5-hydroxy-6E,8Z,11Z,14Z,17Z-eicosapentaenoic acid
	317	14(15)-EpETE	(±)-14(15)-epoxy-5Z,8Z,11Z,17Z-eicosatetraenoic acid
	317	12-OxoETE	12-oxo-5Z,8Z,10E,14Z-eicosatetraenoic acid
	317	15-OxoETE	15-oxo-5Z,8Z,11Z,13E-eicosatetraenoic acid
	317	5-OxoETE	5-oxo-6E,8Z,11Z,14Z-eicosatetraenoic acid
	319	15-HETE	15S-hydroxy-5Z,8Z,11Z,13E-eicosatetraenoic acid
	319	11-HETE	11S-hydroxy-5Z,8Z,12E,14Z-eicosatetraenoic acid
	319	12-HETE	12S-hydroxy-5Z,8Z,10E,14Z-eicosatetraenoic acid
	319	8-HETE	8S-hydroxy-5Z,9E,11Z,14Z-eicosatetraenoic acid
	319	9-HETE	9-hydroxy-5Z,7E,11Z,14Z-eicosatetraenoic acid
	319	5-HETE	5S-hydroxy-6E,8Z,11Z,14Z-eicosatetraenoic acid
	319	11,12-EET	11,12-epoxy-5Z,8Z,14Z-eicosatrienoic acid
	319	5,6-EET	5,6-epoxy-8Z,11Z,14Z-eicosatrienoic acid
	321	15-HETrE	15S-hydroxy-8Z,11Z,13E-eicosatrienoic acid
	321	5-HETrE	5S-hydroxy-6E,8Z,11Z-eicosatrienoic acid
	327	tetranor-PGDM	9S-hydroxy-11,15-dioxo-2,3,4,5–tetranor-prostan-1,20-dioic acid
	333	PGJ2	11-oxo-15S-hydroxy-5Z,9,13E-prostatrienoic acid
	333	PGB2	15S-hydroxy-9-oxo-5Z,8(12),13E-prostatrienoic acid
	333	PGA2	9-oxo-15S-hydroxy-5Z,10Z,13E-prostatrienoic acid
	333	15-deoxy-δ-12,14 PGD2	9S-hydroxy-11-oxo-5Z,12E,14E-prostatrienoic acid
	335	8,15 DiHETE	8S,15S-dihydroxy-5Z,9E,11Z,13E-eicosatetraenoic acid
	335	6-trans LTB4	5S,12R-dihydroxy-6E,8E,10E,14Z-eicosatetraenoic acid
	335	LTB4	5S,12R-dihydroxy-6Z,8E,10E,14Z-eicosatetraenoic acid
	335	5,15-DiHETE	5S,15S-dihydroxy-6E,8Z,10Z,13E-eicosatetraenoic acid
	335	5,6- DiHETE	5S,6S-dihydroxy-8Z,11Z,14Z,17Z-eicosatetraenoic acid

	337	14,15-DiHETrE	14,15-dihydroxy-5Z,8Z,11Z-eicosatrienoic acid
	337	5,6 -DiHETrE	5,6-dihydroxy-8Z,11Z,14Z-eicosatrienoic acid
	351	PGH2	9S,11R-epidioxy-15S-hydroxy-5Z,13E-prostadienoic acid
	351	PGE2	9-oxo-11R,15S-dihydroxy-5Z,13E-prostadienoic acid
	351	11β-PGE2	9-oxo-11S,15S-dihydroxy-5Z,13E-prostadienoic acid
	351	15-keto-PGF2α	9S,11R-dihydroxy-15-oxo-5Z,13E-prostadienoic acid
	351	PGD2	9S,15S-dihydroxy-11-oxo-5Z,13E-prostadienoic acid
	351	13,14-dh-15-k-PGE2	9,15-dioxo-11R-hydroxy-5Z-prostenoic acid
	353	8-iso-PGF2a	9S,11R,15S-trihydroxy-5Z,13E-prostadienoic acid-cyclo[8S,12R]
	353	(+/-) 5-iPF2α-VI	5,9S,11R-trihydroxy-6E,14Z-prostadienoic acid-cyclo[8S,12R]
	353	PGF2α	9S,11R,15S-trihydroxy-5Z,13E-prostadienoic acid
	353	13,14-dh-15-k-PGF2α	9S,11S-dihydroxy-15-oxo-5Z-prostenoic acid
	369	6-keto-PGF1α	6-oxo-9S,11R,15S-trihydroxy-13E-prostenoic acid
	369	TXB2	9S,11,15S-trihydroxy-thromboxa-5Z,13E-dien-1-oic acid
Docosanoids	343	20-HDoHE	(±)-20-hydroxy-4Z,7Z,10Z,13Z,16Z,18E-docosahexaenoic acid
	343	17-HDoHE	(±)-17-hydroxy-4Z,7Z,10Z,13Z,15E,19Z-docosahexaenoic acid
	343	10-HDoHE	(±)-10-hydroxy-4Z,7Z,11E,13Z,16Z,19Z-docosahexaenoic acid
	343	14-HDoHE	(±)-14-hydroxy-4Z,7Z,10Z,12E,16Z,19Z-docosahexaenoic acid
	343	11-HDoHE	(±)-11-hydroxy-4Z,7Z,9E,13Z,16Z,19Z-docosahexaenoic acid
	343	7-HDoHE	(±)-7-hydroxy-4Z,8E,10Z,13Z,16Z,19Z-docosahexaenoic acid
	343	8-HDoHE	(±)-8-hydroxy-4Z,6E,10Z,13Z,16Z,19Z-docosahexaenoic acid
	343	4-HDoHE	(±)4-hydroxy-5E,7Z,10Z,13Z,16Z,19Z-docosahexaenoic acid
	361	19,20-DiHDPE	(±)-19,20-dihydroxy-4Z,7Z,10Z,13Z,16Z-docosapentaenoic acid
	375	Resolvin D1	7S,8R,17S-trihydroxy-4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid
Deuterated IS	299	D_4-13-HODE	(±)-13-hydroxyl-9Z,11E–octadecadienoic–9,10,12,13–d_4 acid
	299	D_4-9-HODE	(±)9-hydroxyl-10E,12Z–octadecadienoic–9,10,12,13–d_4 acid
	317	D_4-9,10-DiHOME	9,10-dihydroxy-12Z-octadecenoic-9,10,12,13–$d_4$acid
	317	D_4-12,13-DiHOME	(±)-12,1-dihydroxy-9Z–octadecenoic–9,10,12,13–d_4 acid
	323	D_7-5-oxoETE	5-oxo-6E,8Z,11Z,14Z–eicosatetraenoic–6,8,9,11,12,14,15–d_7 acid
	325	D_6-20-HETE	(±)-20-hydroxy–5Z,8Z,11Z,14Z–eicosatetraenoic–16,16,17,17,18,18–d_6 acid
	327	D_8-15-HETE	(±)-15-hydroxy-5Z,8Z,11Z,13E–eicosatetraenoic–5,6,8,9,11,12,14,15–d_8 acid
	327	D_8-12-HETE	(±)-12-hydroxy-5Z,8Z,10E,14Z–eicosatetraenoic–5,6,8,9,11,12,14,15–d_8 acid
	327	D_8-5-HETE	(±)-5-hydroxy - 6E,8Z,11Z,14Z-eicosatetraenoic–5,6,8,9,11,12,14,15–d_8 acid
	330	D_{11}-14,15-EET	(±)-14(15)-epoxy–5Z,8Z,11Z-eicosatrienoic-16,16,17,17,18,18,19,19,20,20,20-d_{11} acid
	330	D_{11}-8,9-EET	(±)-8(9)-epoxy–5Z,8Z,14Z–eicosatrienoic–16,16,17,17,18,18,19,19,20,20,20–d_{11} acid
	330	D_{11}-11,12-EET	(±)-11(12)-epoxy-5Z,8Z,14Z–eicosatrienoic–16,16,17,17,18,18,19,19,20,20,20–d_{11} acid
	339	D_4-LTB_4	5S,12R-dihydroxy-6Z,8E,10E,14Z–eicosatetraenoic–6,7,14,15–d_4 acid
	357	D_4-PGF2α	9S,11R,15S-trihydroxy-5Z,13E-prostadienoic-3,3,4,4-$d_4$acid

Table S-2 Information table about control and breast cancer patients. BMI = body mass index, cancer stages: pTis = tumor in situ, pT1 = tumors 1 mm or less, pT2 = tumors from 1.01 – 2 mm, and pTx = not assessed

Healthy volunteers	**Age**	**Breast cancer patients**	**Age**	**BMI**	**Smoker**	**Stage of cancer**
1	63	21	60	30.12	no	pTis
2	51	22	54	25.31	no	pT1b
3	50	23	56	23.05	yes	pT1c
4	59	24	59	22.06	yes	pT1b
5	62	25	65	32.05	yes	pTis
6	55	26	50	-	yes	pTis
7	60	27	65	37.34	yes	pT1c
8	57	28	59	27.45	no	pT1a
9	49	29	48	20.20	yes	pT1c
10	55	30	53	33.86	yes	pT1c
11	59	31	57	-	-	pTis
12	57	32	62	26.37	no	pT1b
13	56	33	50	23.72	-	pT2
14	57	34	52	25.39	no	pT1a
15	61	35	55	25.33	yes	pT1c
16	58	36	53	24.53	no	pTx
17	56	37	54	21.56	yes	pT1c
18	48	38	61	24.68	no	pT1c
19	53	39	52	22.49	no	pT1b
20	56	40	63	-	-	pT1c
Median[1]	**56.5 ± 4.10**	**Median[1]**	**55.5 ± 4.99**			

[1]Median ± standard deviation

Table S-3 Parameters of validation for 14 deuterated oxylipin internal standards for high level (HL) and low level (LL) concentrations

Internal standards	LOD [ng/ml]	LOQ [ng/ml]	Slope	Intercept	Correlation coefficient	Matrix effect[a] [%]	Recovery rate HL [%]	Recovery rate LL [%]
D_4-PGF2α	1.50	5	3406	5205	0.9992	67.9 ± 4.8	87.5	83.3
D_4-LTB_4	0.30	1	3863	3133	0.9995	110 ± 9.3	89.5	79.8
D_4-9-HODE	0.15	0.5	4801	6209	0.9996	127.3 ± 9.8	94.4	77.7
D_4-13-HODE	0.15	0.5	5529	8057	0.9995	109.3 ± 13.9	98.3	79.2
D_4-9,10-DiHOME	0.15	0.5	3071	4324	0.9994	130.5 ± 16.4	97.8	91.1
D_4-12,13-DiHOME	0.60	2	3406	5205	0.9994	120.6 ± 19.5	93.7	102.8
D_7-5-oxoETE	0.60	2	225.3	162.2	0.9996	110.5 ± 18.3	89.0	78.6
D_6-20-HETE	0.60	2	1515	1253	0.9994	145.5 ± 10.8	95.4	71.2
D_8-15-HETE	0.15	0.5	4697	3212	0.9991	152.8 ± 15.8	96.0	92.7
D_8-12-HETE	0.15	0.5	1171	1661	0.9995	159 ± 15.8	88.1	83.9
D_8-5-HETE	0.60	2	2297	2307	0.9993	162.1 ± 11	94.6	81.1
D_{11}-8,9-EET	0.15	0.5	290.6	280.2	0.9995	159.1 ± 12.6	84.3	95.9
D_{11}-11,12-EET	0.15	0.5	1117	2608	0.9995	107 ± 18.7	89.5	94.0
D_{11}-14,15-EET	0.15	0.5	473.6	558.4	0.9994	120 ± 19.1	89.4	86.2

[a]Mean ± standard deviation

Table S-4 Precision and accuracy for 14 deuterated IS for high level (HL) and low level (LL) concentrations

Internal standards	Within-run precision HL [%]	Between-run precision HL [%]	Within-run precision LL [%]	Between-run precision LL [%]	Within-run accuracy HL [%]	Between-run accuracy HL [%]	Within-run accuracy LL [%]	Between-run accuracy LL [%]
D_4-PGF2α	9.5	12.8	5.3	8.3	91.0	93.6	80.1	79.5
D_4-LTB_4	7.7	8.7	9.2	10.9	101.6	100.2	103.7	108.6
D_4-9-HODE	7.7	11.6	11.2	11.7	103.1	111.8	111.4	113.0
D_4-13-HODE	6.0	9.4	12.7	8.3	101.1	110.7	92.6	103.7
D_4-9,10-DiHOME	6.2	13.5	6.6	8.3	94.6	88.1	95.2	106.6
D_4-12,13-DiHOME	5.4	9.2	5.6	6.7	99.4	98.4	107.3	111.7
D_7-5-oxoETE	5.2	6.8	15.0	10.1	96.5	104.2	100.8	104.7
D_6-20-HETE	8.2	12.6	24.8	25.3	112.5	117.0	115.6	123.0
D_8-15-HETE	9.2	10.4	10.5	11.5	99.3	101.9	104.6	92.8
D_8-12-HETE	6.1	7.6	8.9	5.72	101.7	98.6	94.7	104.1
D_8-5-HETE	5.9	8.7	11.5	9.3	89.3	88.9	98.2	86.0
D_{11}-8,9-EET	6.8	7.5	8.9	9.7	109.9	110.2	104.9	110.5
D_{11}-11,12-EET	6.1	7.3	5.5	10.5	102.2	105.2	97.8	114.9
D_{11}-14,15-EET	6.3	7.1	5.8	5.0	112.6	86.1	112.4	113.1

Table S-6 Average concentrations (pmol/mL) of oxylipins in human plasma of healthy volunteers (normal) and breast cancer patients (tumor) with statistical parameters; VIP – variable importance in projection

Oxylipin species	Normal[a]	Tumor[a]	Fold change	p-value[b]	T-value	VIP-value[c]
9-HODE	18.13 ± 7.16	30.85 ± 15.89	1.70	3.75E-03	-3.26	1.77
13-HOTrE	2.04 ± 1.11	3.10 ± 1.14	1.52	6.16E-03	-2.98	1.76
13-HODE	16.50 ± 5.97	22.40 ± 8.57	1.36	1.91E-02	-2.53	1.40
19,20-DiHDPE	1.79 ± 1.03	2.58 ± 1.02	1.44	2.28E-02	-2.44	1.26
9-HOTrE	4.00 ± 2.12	5.96 ± 3.48	1.53	2.61E-02	-2.39	1.18
12-HHTrE	0.51 ± 0.60	0.96 ± 0.66	1.90	3.36E-02	-2.26	1.59
14,15-DiHETrE	3.17 ± 1.35	4.04 ± 1.58	1.28	7.62E-02	-1.87	1.18
15-HETE	0.93 ± 0.50	1.36 ± 0.93	1.46	8.73E-02	-1.82	1.11
12-HETE	13.96 ± 13.47	27.32 ± 31.88	1.73	1.37E-01	-1.57	0.67
LTB4	0.24 ± 0.20	0.33 ± 0.22	1.38	1.89E-01	-1.37	1.14
9-HETE	1.18 ± 0.36	1.91 ± 2.67	1.39	2.01E-01	-1.34	0.80
11-HETE	0.88 ± 0.45	1.54 ± 2.32	1.74	2.42E-01	-1.24	0.84
14-HDoHE	2.81 ± 3.53	4.38 ± 4.54	1.56	2.42E-01	-1.22	0.65
7-HDoHE	9.03 ± 10.82	13.21 ± 12.35	1.46	2.75E-01	-1.14	0.78
20-HDoHE	1.28 ± 0.65	1.53 ± 0.77	1.20	2.80E-01	-1.12	0.52
4-HDoHE	3.34 ± 2.03	3.12 ± 1.65	0.91	6.32E-01	0.50	0.16
5,6-EET	1.35 ± 0.82	1.25 ± 0.74	0.93	6.92E-01	0.41	0.27
6-trans LTB4	0.26 ± 0.08	0.25 ± 0.07	0.96	6.94E-01	0.41	0.23
11,12-EET	3.47 ± 2.84	3.31 ± 2.45	0.92	7.35E-01	0.35	0.07
11-HDoHE	0.48 ± 0.47	0.50 ± 0.36	1.04	9.01E-01	-0.13	0.32
5,6-DiHETrE	0.52 ± 0.28	0.51 ± 0.25	0.99	9.35E-01	0.08	0.09

[a] Mean ± standard deviation

[b] Calculated using T-test

[c] Generated from OPLS-DA model.