

Supplementary information

Development of an LC-ESI(-)-MS/MS method for the simultaneous quantification of 35 isoprostanes and isofurans derived from the major n3- and n6-PUFAs

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Tab. S1: Parameters of the LC-ESI(-)-MS/MS method for the quantification of oxylipins. Shown are all analytes covered by the method with their mass transitions for quantification in scheduled SRM mode, electronical MS parameters (declustering potential (DP), entrance potential (EP), collision energy (CE), collision exit potential (CXP)), the assigned internal standards (IS), retention time (t_R), full peak width at half maximum (FWHM), limit of detection (LOD) and the calibration range (lower limit of quantification (LLOQ), upper limit of quantification (ULOQ)) and correlation coefficient of the calibration curve (r).

Analyte	Mass transition		MS parameters				Internal standard	t_R ¹⁾ [min]	FWHM ²⁾ [sec]	LOD ³⁾		Calibration range LLOQ ⁴⁾ ULOQ ⁵⁾ [nM]		r ⁶⁾
	Q1	Q3	DP	EP	CE	CXP				[nM]	[pg on column]			
20-OH-PGE ₂	367.2	189.1	-30	-10	-25	-8	² H ₄ -PGE ₂	3.68	3.4	0.10	0.18	0.25	500	0.9986
<i>ent</i> -16(<i>R,S</i>)-13- <i>epi</i> -ST- Δ^{14} -9-PhytoF 1	343.1	200.9	-80	-10	-33	-8	C19-17- <i>epi</i> -17-F _{1t} -PhytoP	4.19	3.4	0.12	0.20	0.24	235	0.9993
<i>ent</i> -16(<i>R,S</i>)-13- <i>epi</i> -ST- Δ^{14} -9-PhytoF 2	343.1	200.9	-80	-10	-33	-8	C19-17- <i>epi</i> -17-F _{1t} -PhytoP	4.30	3.4	0.13	0.23	0.26	265	0.9995
<i>ent</i> -16- <i>epi</i> -16-F _{1t} -PhytoP	327.3	225.0	-70	-10	-34	-8	C19-17- <i>epi</i> -17-F _{1t} -PhytoP	4.72	3.3	0.25	0.41	0.5	500	0.9983
<i>ent</i> -16-F _{1t} -PhytoP	327.3	225.0	-70	-10	-34	-8	C19-17- <i>epi</i> -17-F _{1t} -PhytoP	4.89	3.4	0.50	0.82	1.0	500	0.9997
<i>ent</i> -9-F _{1t} -PhytoP	327.3	170.9	-40	-10	-31	-8	C19-17- <i>epi</i> -17-F _{1t} -PhytoP	4.81	3.3	0.10	0.16	0.25	500	0.9991
<i>ent</i> -9- <i>epi</i> -9-F _{1t} -PhytoP	327.3	170.9	-40	-10	-31	-8	C19-17- <i>epi</i> -17-F _{1t} -PhytoP	4.98	3.3	0.25	0.41	0.5	500	0.9990
Δ^{17} -6-keto-PGF _{1α}	367.2	163.2	-40	-10	-36	-10	² H ₄ -6-keto-PGF _{1α}	4.99	21	0.50	0.92	1.0	500	0.9938
2,3-dinor-TxB ₁	343.0	142.9	-30	-10	-19	-8	² H ₄ -TxB ₂	5.11	4.1	2.0	3.4	5.0	500	0.9961
15(<i>R,S</i>)-2,3-dinor-15-F _{2t} -IsoP	325.2	237.0	-40	-10	-18	-8	C19-17- <i>epi</i> -17-F _{1t} -PhytoP	5.41	3.3	0.25	0.41	0.5	500	0.9995
2,3-dinor-TxB ₂	341.2	167.0	-40	-10	-15	-8	² H ₄ -TxB ₂	5.59	3.8	0.50	0.86	1.0	500	0.9971
6-keto-PGF _{1α}	369.3	163.2	-70	-10	-36	-6	² H ₄ -6-keto-PGF _{1α}	6.10	24	0.90	1.7	1.8	500	0.9937
8-F _{3t} -IsoP	351.1	155.0	-60	-10	-27	-8	C19-17- <i>epi</i> -17-F _{1t} -PhytoP	6.15	3.6	0.50	0.88	1.0	500	0.9980
8- <i>epi</i> -8-F _{3t} -IsoP	351.1	155.0	-60	-10	-27	-8	C19-17- <i>epi</i> -17-F _{1t} -PhytoP	6.51	3.3	0.50	0.88	1.0	500	0.9992
RvE1	349.3	195.0	-65	-10	-22	-10	² H ₄ -TxB ₂	6.21	3.5	0.60	1.1	1.2	480	0.9947
20-COOH-LTB ₄	365.2	347.2	-80	-10	-25	-8	² H ₄ -TxB ₂	6.25	3.8	0.50	0.92	1.0	40	0.9619
TxB ₃	367.3	169.3	-70	-10	-34	-8	² H ₄ -TxB ₂	6.51	3.5	0.10	0.18	0.25	100	0.9976
20-OH-LTB ₄	351.2	195.2	-80	-10	-25	-8	² H ₄ -PGD ₂	6.52	3.3	0.10	0.18	0.25	40	0.9984
5(<i>R,S</i>)-5-F _{3t} -IsoP	351.2	114.9	-50	-10	-27	-8	C19-17- <i>epi</i> -17-F _{1t} -PhytoP	6.53	3.4	1.0	1.8	2.0	500	0.9962
13,14-dihydro-15-keto-tetranor-PGE ₂	296.9	109.0	-30	-10	-19	-8	² H ₄ -PGE ₂	7.29	3.9	0.10	0.15	0.25	500	0.9992
TxB ₁	371.3	171.2	-70	-10	-34	-10	² H ₄ -TxB ₂	7.36	3.9	0.25	0.47	0.5	100	0.9982
15-F _{2t} -IsoP (8- <i>iso</i> -PGF _{2α})	353.1	193.1	-70	-6	-33	-8	² H ₄ -15-F _{2t} -IsoP	7.56	3.7	0.25	0.44	0.5	500	0.9989
TXB ₂	369.2	169.1	-60	-10	-25	-7	² H ₄ -TxB ₂	7.68	3.8	0.25	0.46	1.3	500	0.9949
11-dehydro-TxB ₃	365.3	161.2	-30	-10	-25	-8	² H ₄ -TxB ₂	7.75	3.6	0.50	0.92	1.0	100	0.9969
PGE ₃	349.3	269.2	-60	-10	-22	-6	² H ₄ -PGE ₂	7.76	3.5	0.15	0.26	0.3	120	0.9995

Tab. S1: Continued.

Analyte	Mass transition		MS parameters				Internal standard	t_R ¹⁾	FWHM ²⁾	LOD ³⁾		Calibration range		r^2 ⁶⁾
	Q1	Q3	DP	EP	CE	CXP		[min]	[sec]	[nM]	[pg on column]	LLOQ ⁴⁾	ULOQ ⁵⁾	
11 β -PGF _{2α}	353.3	193.1	-30	-12	-36	-12	² H ₄ -PGE ₂	7.84	3.3	0.25	0.44	0.5	500	0.9952
10-F _{4t} -NeuroP	377.2	153.0	-40	-10	-25	-8	² H ₄ -15-F _{2t} -IsoP	8.04	3.3	0.25	0.47	0.5	500	0.9989
10- <i>epi</i> -10-F _{4t} -NeuroP	377.2	153.0	-40	-10	-25	-8	² H ₄ -15-F _{2t} -IsoP	8.37	3.5	0.50	0.95	1.0	500	0.9990
5(<i>R,S</i>)-5-F _{2t} -IsoP (5-iPF _{2α} -VI)	353.2	114.8	-60	-10	-26	-8	² H ₁₁ -5(<i>R,S</i>)-5-F _{2t} -IsoP	8.07	3.5	0.25	0.44	0.5	500	0.9959
PGD ₃	349.3	269.2	-60	-10	-22	-6	² H ₄ -PGD ₂	8.15	3.4	0.50	0.88	1.0	40	0.9992
16-B ₁ -PhytoP	307.3	235.0	-60	-10	-27	-8	² H ₁₁ -5(<i>R,S</i>)-5-F _{2t} -IsoP	8.26	3.7	0.10	0.15	0.25	500	0.9994
9-L ₁ -PhytoP	307.3	185.1	-60	-10	-27	-8	² H ₁₁ -5(<i>R,S</i>)-5-F _{2t} -IsoP	8.33	3.7	0.10	0.15	0.25	500	0.9986
PGF _{2α}	353.2	309.2	-80	-10	-26	-7	² H ₄ -PGE ₂	8.59	3.5	0.35	0.62	0.70	281	0.9991
14(<i>R,S</i>)-14-F _{4t} -NeuroP	377.2	205.1	-50	-10	-27	-8	C21-15-F _{2t} -IsoP	8.62	6.5	10	19	20	500	0.9998
PGF _{1α}	355.4	293.2	-90	-12	-36	-6	² H ₄ -PGE ₂	8.63	3.5	0.10	0.18	0.25	500	0.9985
PGE ₂	351.2	271.3	-60	-10	-24	-6	² H ₄ -PGE ₂	8.98	3.5	0.10	0.18	0.25	500	0.9982
11-dehydro-TxB ₂	367.0	161.1	-50	-10	-27	-8	² H ₄ -TxB ₂	9.08	3.7	0.25	0.46	0.50	100	0.9988
PGE ₁	353.3	317.2	-60	-10	-20	-6	² H ₄ -PGE ₂	9.27	3.6	0.13	0.23	0.33	260	0.9984
4(<i>R,S</i>)-4-F _{4t} -NeuroP	377.1	101.3	-60	-10	-26	-8	C21-15-F _{2t} -IsoP	9.35	3.8	0.50	0.95	1.0	500	0.9982
PGD ₁	353.3	317.2	-60	-10	-20	-6	² H ₄ -PGD ₂	9.45	3.6	0.25	0.44	0.50	40	0.9956
PGD ₂	351.2	271.3	-60	-10	-24	-6	² H ₄ -PGD ₂	9.46	3.6	0.50	0.88	1.0	500	0.9960
15-keto-PGF _{1α}	353.3	193.1	-40	-12	-38	-6	² H ₄ -PGE ₂	9.54	3.7	0.10	0.18	0.25	500	0.9970
4(<i>R,S</i>)-ST- Δ^5 -8-NeuroF	393.3	187.2	-40	-10	-29	-8	C21-15-F _{2t} -IsoP	9.59	4.2	20	39	40	500	0.9941
17(<i>R,S</i>)-17-F _{2t} -dihomo-IsoP 1	381.3	263.2	-90	-10	-31	-8	C21-15-F _{2t} -IsoP	9.78	3.6	0.63	1.2	1.3	314	0.9967
17(<i>R,S</i>)-17-F _{2t} -dihomo-IsoP 2	381.3	263.2	-90	-10	-31	-8	C21-15-F _{2t} -IsoP	9.95	3.2	0.37	0.71	0.75	186	0.9980
7(<i>R,S</i>)-ST- Δ^8 -11-dihomo-IsoF	397.3	245.1	-50	-10	-31	-8	C21-15-F _{2t} -IsoP	9.81	3.8	1.0	2.0	2.0	500	0.9993
<i>ent</i> -7(<i>R,S</i>)-7-F _{2t} -dihomo-IsoP	381.3	143.0	-50	-10	-31	-8	C21-15-F _{2t} -IsoP	9.86	5.7	0.25	0.48	0.50	500	0.9972
11,12,15-TriHETrE	353.2	167.1	-80	-10	-28	-10	² H ₄ -PGE ₂	10.20	3.4	0.25	0.44	0.50	100	0.9988
LXA ₄	351.2	115.2	-60	-10	-21	-8	² H ₄ -PGE ₂	10.23	3.7	0.088	0.15	0.18	70	0.9985
RvD1	375.3	141.0	-50	-10	-20	-8	² H ₄ -PGE ₂	10.35	3.6	0.10	0.19	0.25	100	0.9993
13,14-dihydro-15-keto-PGF _{2α}	353.3	183.3	-80	-10	-36	-10	² H ₄ -PGE ₂	10.41	3.6	0.25	0.44	0.50	500	0.9990
13,14-dihydro-15-keto-PGE ₁	353.3	221.2	-40	-6	-30	-6	² H ₄ -PGE ₂	10.97	4.5	0.25	0.44	0.50	500	0.9989
dihomo-PGF _{2α}	381.4	221.1	-60	-10	-38	-10	² H ₄ -PGE ₂	11.01	3.8	0.025	0.048	0.10	500	0.9977
4(<i>R,S</i>)-4-F _{3t} -NeuroP _{n6}	379.2	273.0	-50	-10	-27	-8	C21-15-F _{2t} -IsoP	11.03	4.1	0.50	0.95	1.0	500	0.9971

Tab. S1: Continued.

Analyte	Mass transition		MS parameters				Internal standard	t_R ¹⁾	FWHM ²⁾	LOD ³⁾		Calibration range		r^2 ⁶⁾
	Q1	Q3	DP	EP	CE	CXP		[min]	[sec]	[nM]	[pg on column]	LLOQ ⁴⁾	ULOQ ⁵⁾	
17(<i>R,S</i>)-10- <i>epi</i> -SC- Δ^{15} -11-dihomo-IsoF	397.1	221.0	-90	-10	-31	-8	C21-15-F ₂₁ -IsoP	11.26	3.7	0.52	1.0	1.0	260	0.9992
17(<i>R,S</i>)-10- <i>epi</i> -SC- Δ^{15} -11-dihomo-IsoF	397.1	221.0	-90	-10	-31	-8	C21-15-F ₂₁ -IsoP	11.44	3.7	0.48	0.96	0.96	240	0.9982
RvE2	333.2	253.3	-60	-10	-20	-9	² H ₄ -PGE ₂	11.42	3.7	1.0	1.7	2.0	100	0.9991
PGJ ₂	333.3	189.2	-60	-10	-25	-8	² H ₄ -PGE ₂	12.02	3.8	0.80	1.3	1.6	160	0.9957
Δ^{12} -PGJ ₂	333.1	189.0	-40	-10	-22	-8	² H ₄ -PGE ₂	12.09	4.0	0.50	0.84	1.0	500	0.9986
LTB ₅	333.3	195.2	-65	-10	-22	-8	² H ₄ -LTB ₄	12.11	4.0	0.050	0.084	0.10	200	0.9992
PGB ₂	333.3	175.1	-60	-10	-28	-8	² H ₄ -PGE ₂	12.13	4.0	0.20	0.33	0.40	800	0.9982
THF diol	353.2	127.1	-80	-10	-32	-8	² H ₄ -LTB ₄	12.27	4.0	0.13	0.22	0.25	100	0.9987
18(<i>S</i>)-RvE3	333.2	201.3	-60	-10	-20	-9	² H ₄ -PGE ₂	12.78	4.1	0.50	0.84	1.0	100	0.9976
12-OH-17(18)-EpETE	333.1	179.3	-65	-10	-20	-6	² H ₄ -9,10-DiHOME	12.85	4.3	0.25	0.42	0.50	100	0.9992
15,16-DiHODE	311.2	223.2	-80	-10	-29	-10	² H ₄ -9,10-DiHOME	12.99	4.3	0.50	0.78	1.0	80	0.9992
9,10-DiHODE	311.2	201.2	-65	-10	-27	-10	² H ₄ -9,10-DiHOME	13.05	4.2	0.10	0.16	0.20	80	0.9998
12,13-DiHODE	311.2	183.1	-80	-10	-30	-8	² H ₄ -9,10-DiHOME	13.15	4.3	0.50	0.78	1.0	80	0.9990
8,15-DiHETE	335.2	235.2	-65	-10	-22	-4	² H ₁₁ -14,15-DiHETrE	13.17	4.0	0.40	0.67	0.80	80	0.9981
10(<i>S</i>),17(<i>S</i>)diH n3 DPA	361.3	262.9	-40	-10	-23	-8	² H ₄ -9,10-DiHOME	13.36	4.3	0.50	0.91	1.0	500	0.9991
18(<i>R</i>)-RvE3	333.2	201.3	-60	-10	-20	-9	² H ₄ -PGE ₂	13.45	4.2	0.25	0.42	0.50	100	0.9960
NPD1	359.0	153.0	-50	-10	-21	-8	² H ₄ -9,10-DiHOME	13.49	4.2	0.25	0.45	0.50	500	0.9985
6- <i>trans</i> -LTB ₄	335.2	195.1	-65	-10	-23	-9	² H ₄ -LTB ₄	13.58	4.2	0.25	0.42	0.50	200	0.9984
5,15-DiHETE	335.3	173.2	-60	-10	-21	-8	² H ₁₁ -14,15-DiHETrE	13.60	4.0	0.13	0.21	0.25	100	0.9984
17,18-DiHETE	335.3	247.2	-65	-10	-24	-8	² H ₁₁ -14,15-DiHETrE	13.71	4.3	0.13	0.21	0.25	100	0.9978
LTB ₄	335.2	195.1	-65	-10	-23	-9	² H ₄ -LTB ₄	14.07	4.3	0.10	0.17	0.25	200	0.9996
7(<i>S</i>),17(<i>S</i>)-diH n3 DPA	361.5	263.3	-65	-10	-20	-4	² H ₄ -9,10-DiHOME	14.29	3.8	0.30	0.54	0.75	500	0.9989
14,15-DiHETE	335.3	207.2	-65	-10	-25	-10	² H ₁₁ -14,15-DiHETrE	14.34	4.5	0.13	0.21	0.25	100	0.9992
11,12-DiHETE	335.2	167.1	-65	-10	-26	-5	² H ₁₁ -14,15-DiHETrE	14.57	4.6	0.13	0.21	0.25	100	0.9977
12,13-DiHOME	313.2	183.2	-80	-10	-30	-8	² H ₄ -9,10-DiHOME	14.76	4.7	0.25	0.39	0.50	200	0.9986
8,9-DiHETE	335.2	127.1	-65	-10	-26	-5	² H ₁₁ -14,15-DiHETrE	14.98	4.7	0.25	0.42	0.50	100	0.9962
10(<i>S</i>),17(<i>S</i>)diH n6 DPA	361.2	153.1	-50	-10	-21	-8	² H ₁₁ -14,15-DiHETrE	15.14	4.5	0.10	0.18	0.25	500	0.9987
9,10-DiHOME	313.2	201.2	-80	-10	-29	-8	² H ₄ -9,10-DiHOME	15.24	4.7	0.25	0.39	0.50	200	0.9991
10(<i>S</i>),17(<i>S</i>)diH AdA	363.4	263.2	-80	-10	-23	-8	² H ₁₁ -14,15-DiHETrE	15.90	4.6	0.50	0.91	1.0	500	0.9993

Tab. S1: Continued.

Analyte	Mass transition		MS parameters				Internal standard	t_R ¹⁾	FWHM ²⁾	LOD ³⁾		Calibration range		r^2 ⁶⁾
	Q1	Q3	DP	EP	CE	CXP		[min]	[sec]	[nM]	[pg on column]	LLOQ ⁴⁾	ULOQ ⁵⁾	
12(S)-HHTrE	279.1	179.0	-50	-10	-17	-8	² H ₁₁ -14,15-DiHETrE	15.96	4.9	0.25	0.35	0.50	500	0.9977
14,15-DiHETrE	337.2	207.1	-65	-10	-25	-10	² H ₁₁ -14,15-DiHETrE	16.00	4.8	0.050	0.085	0.10	200	0.9985
19,20-DiHDPE	361.2	273.2	-65	-10	-24	-6	² H ₁₁ -14,15-DiHETrE	16.05	4.8	0.25	0.45	0.50	100	0.9972
LTB ₃	337.2	195.2	-65	-10	-22	-8	² H ₄ -LTB ₄	16.27	5.0	0.25	0.42	0.50	200	0.9995
9,10-diH-stearic acid	315.0	170.8	-60	-10	-36	-9	² H ₄ -9,10-DiHOME	16.61	5.1	1.0	1.6	2.0	500	0.9994
16,17-DiHDPE	361.2	233.2	-65	-10	-24	-6	² H ₁₁ -14,15-DiHETrE	16.69	4.7	0.25	0.45	0.50	100	0.9984
11,12-DiHETrE	337.2	167.1	-65	-10	-26	-8	² H ₁₁ -14,15-DiHETrE	16.82	5.0	0.10	0.17	0.25	200	0.9980
19-HEPE	317.2	229.3	-50	-10	-18	-8	² H ₈ -12-HETE	16.98	-	0.30	0.48	0.71		#
13,14-DiHDPE	361.2	193.2	-65	-10	-24	-6	² H ₁₁ -14,15-DiHETrE	16.99	4.8	0.13	0.23	0.25	100	0.9985
20-HEPE	317.2	287.3	-50	-10	-20	-8	² H ₈ -12-HETE	17.11	5.0	0.50	0.80	1.0	100	0.9985
9-HOTrE	293.2	171.2	-65	-10	-22	-8	² H ₄ -9-HODE	17.23	5.2	0.25	0.37	0.50	500	0.9994
10,11-DiHDPE	361.2	153.2	-65	-10	-24	-6	² H ₁₁ -14,15-DiHETrE	17.37	4.8	0.13	0.23	0.25	100	0.9987
8,9-DiHETrE	337.2	127.1	-70	-10	-30	-8	² H ₁₁ -14,15-DiHETrE	17.48	5.1	0.25	0.42	0.50	200	0.9993
13-HOTrE	293.2	195.1	-70	-10	-24	-8	² H ₄ -9-HODE	17.60	4.6	0.30	0.44	0.60	12	0.9961
18-HEPE	317.2	259.2	-55	-10	-17	-7	² H ₈ -12-HETE	17.67	4.9	0.50	0.80	1.0	500	0.9972
15-deoxy-PGJ ₂	315.2	271.2	-65	-10	-20	-6	² H ₁₁ -14,15-DiHETrE	18.09	6.1	0.20	0.32	0.50	400	0.9989
19-HETE	319.3	230.9	-50	-10	-21	-6	² H ₄ -9-HODE	18.15	5.3	5.0	8.0	10	500	0.9981
7,8-DiHDPE	361.2	113.1	-65	-10	-24	-6	² H ₁₁ -14,15-DiHETrE	18.22	5.3	0.50	0.91	1.0	100	0.9988
20-HETE	319.2	289.1	-80	-10	-24	-6	² H ₆ -20-HETE	18.40	5.1	0.50	0.80	1.0	500	0.9983
15-HEPE	317.2	219.2	-60	-10	-20	-10	² H ₈ -12-HETE	18.43	5.1	0.63	0.99	1.3	500	0.9991
5,6-DiHETrE	337.2	145.1	-70	-10	-26	-10	² H ₁₁ -14,15-DiHETrE	18.44	5.4	0.25	0.42	0.50	200	0.9989
11-HEPE	317.0	167.0	-40	-10	-21	-8	² H ₈ -12-HETE	18.52	4.5	0.25	0.40	0.50	500	0.9962
8-HEPE	317.2	155.2	-60	-10	-20	-8	² H ₈ -12-HETE	18.77	5.1	0.25	0.40	0.63	500	0.9986
12-HEPE	317.2	179.2	-65	-10	-20	-8	² H ₈ -12-HETE	18.96	5.0	0.25	0.40	0.63	500	0.9989
9-HEPE	317.2	166.9	-40	-10	-19	-8	² H ₈ -12-HETE	19.11	4.4	0.25	0.40	0.50	500	0.9970
21-HDHA	343.0	255.0	-60	-10	-18	-7	² H ₈ -12-HETE	19.38	-	1.3	2.24	1.65		#
5-HEPE	317.2	115.1	-60	-10	-20	-6	² H ₈ -12-HETE	19.49	5.2	0.20	0.32	0.50	500	0.9988
22-HDHA	343.2	313.2	-65	-10	-20	-7	² H ₈ -12-HETE	19.49	-	1.40	2.41	2.80		#
4,5-DiHDPE	361.2	229.3	-65	-10	-24	-6	² H ₁₁ -14,15-DiHETrE	19.51	4.7	1.0	1.8	2.0	100	0.9940

Tab. S1: Continued.

Analyte	Mass transition		MS parameters				Internal standard	t _R ¹⁾	FWHM ²⁾	LOD ³⁾		Calibration range		r ² ⁶⁾
	Q1	Q3	DP	EP	CE	CXP		[min]	[sec]	[nM]	[pg on column]	LLOQ ⁴⁾	ULOQ ⁵⁾	
13-HODE	295.2	195.2	-80	-10	-26	-9	² H ₄ -9-HODE	19.70	5.3	2.5	3.7	5.0	400	0.9985
9-HODE	295.2	171.1	-80	-10	-26	-7	² H ₄ -9-HODE	19.81	5.2	2.5	3.7	5.0	400	0.9995
20-HDHA	343.2	241.2	-55	-10	-19	-7	² H ₈ -12-HETE	20.07	4.9	0.25	0.43	0.50	20	0.9975
15(16)-EpODE	293.3	235.2	-65	-10	-20	-4	² H ₄ -9(10)-EpOME	20.34	5.5	0.25	0.37	0.50	20	0.9973
15-HETE	319.2	219.2	-60	-10	-20	-8	² H ₈ -12-HETE	20.45	5.0	0.50	0.80	1.3	500	0.9996
9(10)-EpODE	293.3	171.2	-65	-10	-20	-8	² H ₄ -9(10)-EpOME	20.51	5.4	0.20	0.29	0.40	16	0.9953
17(18)-EpETE	317.2	215.2	-65	-10	-20	-6	² H ₁₁ -14(15)-EpETrE	20.58	5.2	0.50	0.80	1.0	20	0.9994
16-HDHA	343.2	233.2	-55	-10	-19	-7	² H ₈ -12-HETE	20.61	4.6	0.10	0.17	0.25	20	0.9993
17-HDHA	343.2	201.2	-60	-10	-20	-6	² H ₈ -12-HETE	20.73	4.7	1.0	1.7	2.0	500	0.9990
13-HDHA	343.2	193.1	-55	-10	-19	-7	² H ₈ -12-HETE	20.91	4.5	0.25	0.43	0.50	20	0.9985
12(13)-EpODE	293.2	183.1	-65	-10	-24	-8	² H ₄ -9(10)-EpOME	20.93	5.1	0.25	0.37	0.50	20	0.9960
13-oxo-ODE	293.2	195.1	-75	-10	-20	-8	² H ₄ -9-HODE	20.93	4.8	0.50	0.74	1.0	20	0.9966
11-HETE	319.2	167.2	-60	-10	-23	-7	² H ₈ -12-HETE	20.98	4.7	0.25	0.40	0.50	500	0.9995
10-HDHA	343.2	153.2	-45	-10	-21	-7	² H ₈ -12-HETE	21.14	4.4	0.25	0.43	0.50	20	0.9994
14-HDHA	343.2	205.2	-50	-10	-19	-7	² H ₈ -12-HETE	21.14	4.2	0.50	0.86	1.0	500	0.9979
9-oxo-ODE	293.2	185.1	-90	-10	-28	-8	² H ₄ -9-HODE	21.17	5.0	0.50	0.74	1.0	20	0.9934
15-oxo-ETE	317.2	113.1	-65	-10	-25	-8	² H ₈ -5-HETE	21.18	4.3	0.25	0.40	0.50	100	0.9981
14(15)-EpETE	317.2	207.2	-65	-10	-20	-6	² H ₁₁ -14(15)-EpETrE	21.25	4.8	0.25	0.40	0.50	20	0.9952
8-HETE	319.2	155.2	-60	-10	-22	-6	² H ₈ -12-HETE	21.33	4.5	0.50	0.80	1.3	500	0.9989
12-HETE	319.2	179.2	-60	-10	-20	-8	² H ₈ -12-HETE	21.36	4.4	0.25	0.40	0.50	500	0.9970
11(12)-EpETE	317.2	167.2	-65	-10	-20	-6	² H ₁₁ -14(15)-EpETrE	21.40	4.3	0.25	0.40	0.50	20	0.9970
11-HDHA	343.2	121.1	-45	-10	-20	-7	² H ₈ -5-HETE	21.41	4.0	0.10	0.17	0.25	20	0.9992
7-HDHA	343.2	141.2	-55	-10	-19	-7	² H ₈ -5-HETE	21.54	4.2	0.50	0.86	1.0	500	0.9984
8(9)-EpETE	317.2	127.2	-65	-10	-20	-6	² H ₁₁ -14(15)-EpETrE	21.55	4.9	0.50	0.80	1.0	20	0.9969
9-HETE	319.2	167.2	-60	-10	-23	-7	² H ₈ -5-HETE	21.64	4.2	1.3	2.0	2.5	1000	0.9987
15(S)-HETrE	321.2	221.2	-70	-10	-23	-10	² H ₈ -5-HETE	21.68	4.3	0.25	0.40	0.50	200	0.9984
8-HDHA	343.2	189.2	-50	-10	-19	-7	² H ₈ -5-HETE	21.73	3.8	0.50	0.86	0.50	20	0.9976
5-HETE	319.2	115.2	-60	-10	-21	-7	² H ₈ -5-HETE	21.88	4.1	0.25	0.40	0.50	1000	0.9990
4-HDHA	343.2	101.1	-55	-10	-19	-7	² H ₈ -5-HETE	22.31	3.7	0.25	0.43	0.25	500	0.9986

Tab. S1: Continued.

Analyte	Mass transition		MS parameters				Internal standard	t_R ¹⁾	FWHM ²⁾	LOD ³⁾		Calibration range		$r^{2\ 6)}$
	Q1	Q3	DP	EP	CE	CXP		[min]	[sec]	[nM]	[pg on column]	LLOQ ⁴⁾	ULOQ ⁵⁾	
19(20)-EpDPE	343.2	241.2	-65	-10	-20	-7	² H ₁₁ -14(15)-EpETrE	22.39	3.3	0.25	0.43	0.50	20	0.9994
12(13)-EpOME	295.3	195.2	-80	-10	-23	-8	² H ₄ -9(10)-EpOME	22.45	3.8	0.10	0.15	0.25	40	0.9980
14(15)-EpETrE	319.2	219.3	-65	-10	-20	-4	² H ₁₁ -14(15)-EpETrE	22.60	3.8	0.25	0.40	0.50	20	0.9985
9(10)-EpOME	295.3	171.1	-80	-10	-23	-8	² H ₄ -9(10)-EpOME	22.64	3.8	0.10	0.15	0.25	40	0.9949
16(17)-EpDPE	343.2	233.2	-65	-10	-20	-7	² H ₁₁ -14(15)-EpETrE	22.80	3.5	0.25	0.43	0.50	20	0.9978
13(14)-EpDPE	343.2	193.2	-65	-10	-20	-7	² H ₁₁ -14(15)-EpETrE	22.88	3.4	0.25	0.43	0.50	20	0.9961
5-oxo-ETE	317.2	273.2	-65	-10	-22	-6	² H ₄ -9(10)-EpOME	22.95	3.5	1.0	1.6	2.0	20	0.9998
10(11)-EpDPE	343.2	153.2	-65	-10	-20	-7	² H ₁₁ -14(15)-EpETrE	22.98	3.4	0.13	0.22	0.25	20	0.9990
11(12)-EpETrE	319.3	167.2	-60	-10	-20	-7	² H ₁₁ -14(15)-EpETrE	23.09	3.3	0.25	0.40	0.50	40	0.9965
8(9)-EpETrE	319.2	155.2	-65	-10	-20	-6	² H ₁₁ -14(15)-EpETrE	23.24	3.3	0.50	0.80	1.0	20	0.9993
5(6)-EpETrE	319.2	191.1	-60	-10	-20	-7	² H ₁₁ -14(15)-EpETrE	23.41	2.9	1.0	1.6	2.0	20	0.9974
5(S)-HETrE	321.2	115.1	-70	-10	-19	-9	² H ₈ -5-HETE	23.55	3.0	0.1	0.2	0.2	500	0.9991
9(10)-ep-stearic acid	297.0	170.8	-100	-10	-28	-11	² H ₄ -9(10)-EpOME	23.97	3.5	1.0	1.5	2.0	500	0.9953

1) Relative standard deviation for t_R within one batch was $\leq 0.18\%$ (± 0.01 min).

2) Full peak width at half maximum (FWHM) was determined as mean width of standards, concentration 1-200 nM.

3) LOD was set to the lowest concentration yielding a signal to noise ratio ≥ 3 .

4) LLOQ was set to the lowest calibration standard yielding a signal to noise ratio ≥ 5 and an accuracy within the calibration curve of $\pm 20\%$.

5) ULOQ concentration does not represent the end of the dynamic range, but is the highest calibration standard injected.

6) Calibration was performed as weighted regression using 1/x² weighting.

#) 19-HEPE and 21- and 22 HDHA are quantified based on a response factor relative to 20-HEPE and 20-HDHA respectively.

Tab. S2: List of all internal standards used for the quantification of oxylipins with their LC-ESI(-)-MS/MS parameters. Shown are the mass transitions for quantification in scheduled SRM mode, electronical MS parameters (declustering potential (DP), entrance potential (EP), collision energy (CE), collision exit potential (CXP)), and retention time (t_R).

Analyte	Mass transition		MS parameters				t_R [min]
	Q1	Q3	DP	EP	CE	CXP	
C19-17- <i>epi</i> -17-F1t-PhytoP	341.3	239	-70	-10	-35	-8	6.02
2H_4 -6-keto-PGF _{1α}	373.3	167.1	-80	-10	-36	-8	6.10
2H_4 -15-F _{2t} -IsoP	357.2	196.8	-50	-6	-33	-8	7.54
2H_4 -TXB ₂	373.3	173.2	-65	-10	-24	-8	7.64
$^2H_{11}$ -5(<i>R,S</i>)-5-F _{2t} -IsoP	364.3	115.2	-40	-10	-29	-10	7.97
2H_4 -PGE ₂	355.2	275.3	-60	-10	-25	-6	8.93
C21-15-F _{2t} -IsoP	367.2	193.1	-60	-10	-35	-8	9.18
2H_4 -PGD ₂	355.2	275.3	-60	-10	-25	-6	9.43
2H_4 -LTB ₄	339.2	197.2	-65	-10	-23	-9	14
2H_4 -9,10-DiHOME	317.2	203.4	-80	-10	-29	-8	15.13
$^2H_{11}$ -14,15-DiHETrE	348.2	207.1	-65	-10	-25	-10	15.83
2H_6 -20-HETE	325.2	295.2	-70	-10	-24	-6	18.31
2H_4 -9-HODE	299.2	172.3	-80	-10	-26	-6	19.69
2H_8 -12-HETE	327.2	184.2	-65	-10	-22	-8	21.2
2H_8 -5-HETE	327.2	116.1	-60	-10	-21	-8	21.75
$^2H_{11}$ -14(15)-EpETrE	330.2	219.3	-65	-10	-20	-4	22.48
2H_4 -9(10)-EpOME	299.2	172.2	-80	-10	-23	-8	22.54

Tab. S3: Total concentrations of IsoP (i.e. free and esterified) in HCT116 cells following incubation with 50 μ M and 200 μ M t-butylhydroperoxide for 30 min, 1 h and 2 h. Shown are the mean \pm SD. For analytes below the limit of quantification the LLOQ is indicated.

	analyte [fmol/ 10^6 cells]	incubation time	control		50 μ M		200 μ M	
			Mean	SD	Mean	SD	Mean	SD
AA	5(<i>R,S</i>)-F _{2t} -IsoP	30 min	12 \pm 3		47 \pm 5		123 \pm 16	
		1h	11 \pm 2		47 \pm 8		165 \pm 19	
		2 h	15 \pm 7		43 \pm 13		232 \pm 115	
	15-F _{2t} -IsoP (8- <i>iso</i> -PGF _{2a})	30 min	< 5.0		15 \pm 2		34 \pm 3	
		1h	< 5.0		14 \pm 2		44 \pm 2	
		2 h	< 5.0		12 \pm 3		54 \pm 25	
	<i>ent</i> -7(<i>R,S</i>)-F _{2t} -dihomo-IsoP	30 min	< 2.5		4 \pm 0		9 \pm 1	
		1h	< 2.5		4 \pm 1		14 \pm 3	
		2 h	< 2.5		4 \pm 1		18 \pm 10	
EPA	17(<i>R,S</i>)-F _{2t} -dihomo-IsoP 2	30 min	< 3.7		< 3.7		4 \pm 0	
		1h	< 3.7		< 3.7		5 \pm 1	
		2 h	< 3.7		< 3.7		5 \pm 1	
	5(<i>R,S</i>)-F _{3t} -IsoP	30 min	40 \pm 11		167 \pm 39		424 \pm 17	
		1h	39 \pm 4		155 \pm 30		625 \pm 113	
		2 h	44 \pm 19		133 \pm 46		737 \pm 300	
DHA	4(<i>R,S</i>)-F _{4t} -NeuroP	30 min	6 \pm 1		26 \pm 5		69 \pm 6	
		1h	6 \pm 1		24 \pm 5		89 \pm 13	
		2 h	7 \pm 2		22 \pm 3		105 \pm 48	
	10-F _{4t} -NeuroP	30 min	< 2.5		< 2.5		6 \pm 1	
		1h	< 2.5		< 2.5		7 \pm 1	
		2 h	< 2.5		< 2.5		9 \pm 4	
	10- <i>epi</i> -F _{4t} -NeuroP	30 min	< 5.0		6 \pm 0		13 \pm 2	
		1h	< 5.0		5 \pm 1		16 \pm 2	
		2 h	< 5.0		6 \pm 1		20 \pm 9	

(A) α -Linolenic acid derived PhytoP and PhytoF

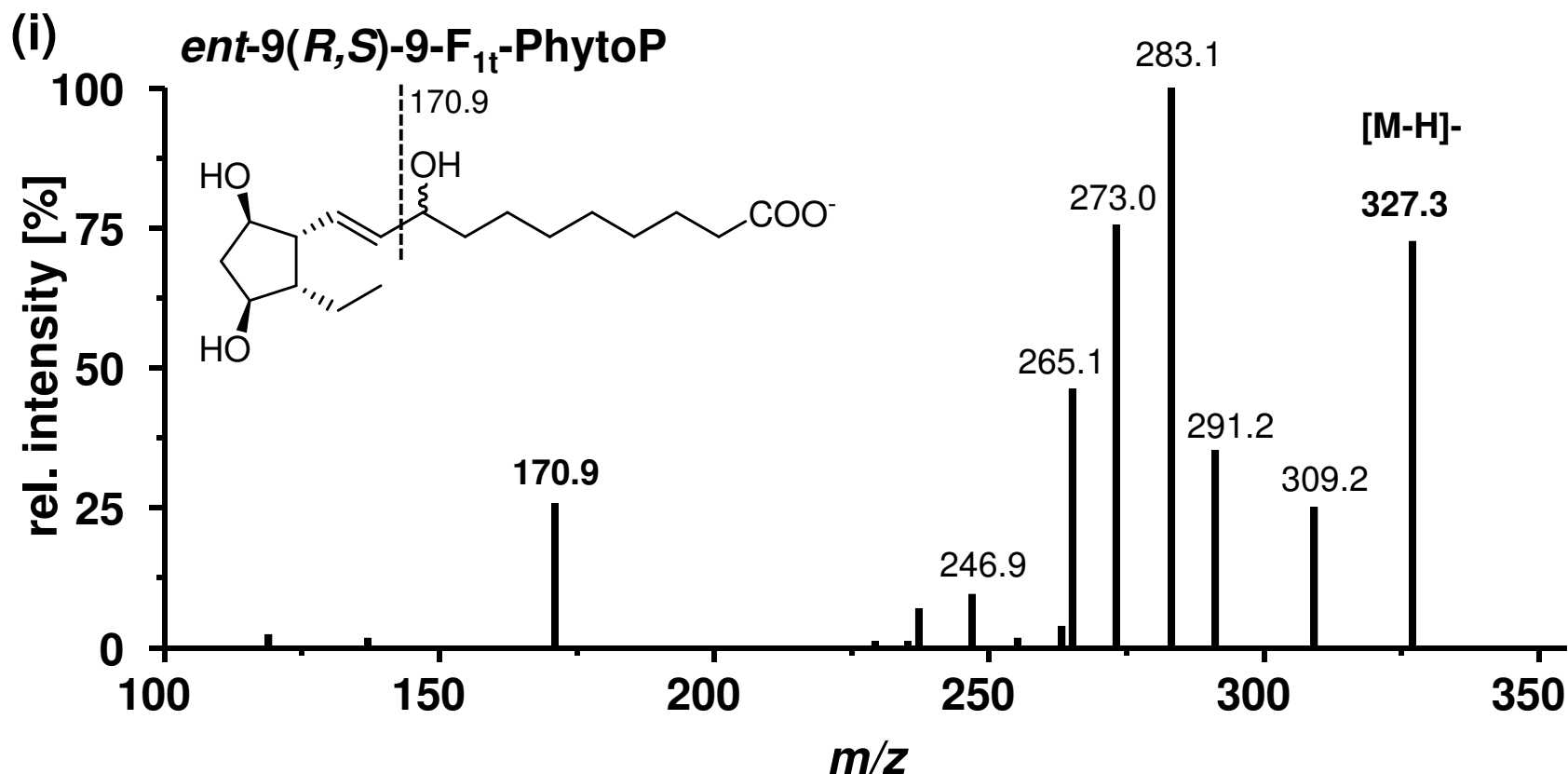


Fig. S1: Product ion spectra of isoprostanooids and isofurans derived from different PUFA. The spectra were acquired via CID of the ESI(-)-generated carboxylate-anion $[M-H]^-$ using a collision energy ramp from -17 to -30.

(A) (i)-(v) α -Linolenic acid derived PhytoP and PhytoF

(B) (i)-(iii) Arachidonic acid derived F_{2t} -IsoP

(C) (i)-(ii) Eicosapentaenoic acid derived F_{3t} -IsoP

(D) (i)-(iv) Docosahexaenoic acid derived F_{4t} -NeuroP and NeuroF

(E) (i)-(iv) Adrenic acid derived F_{2t} -dihomo-IsoP and dihom-IsoF

(F) (i) Docosapentaenoic acid derived NeuroP

(G) (i)-(iv) Internal Standards

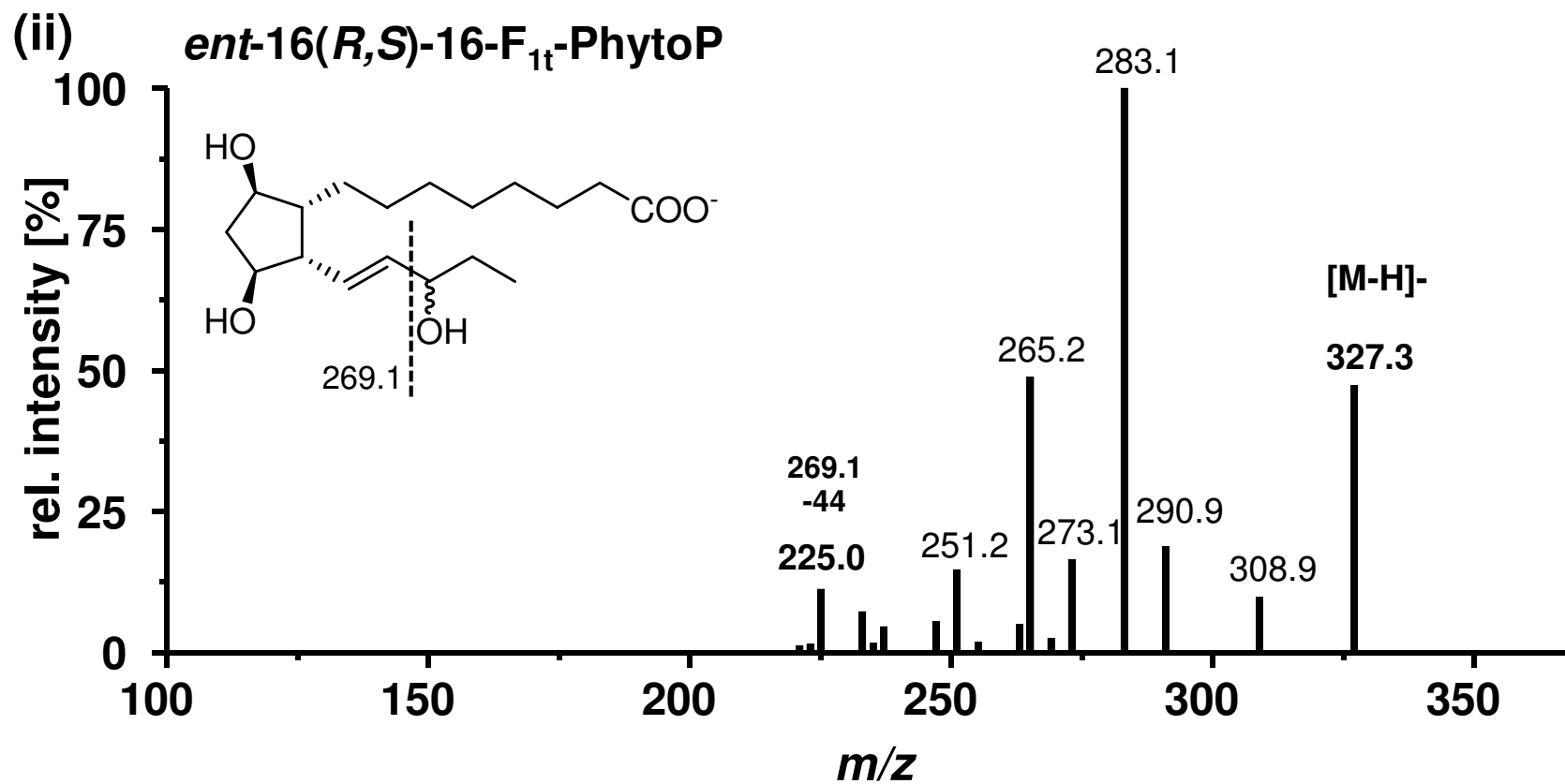


Fig. S1: Continued.

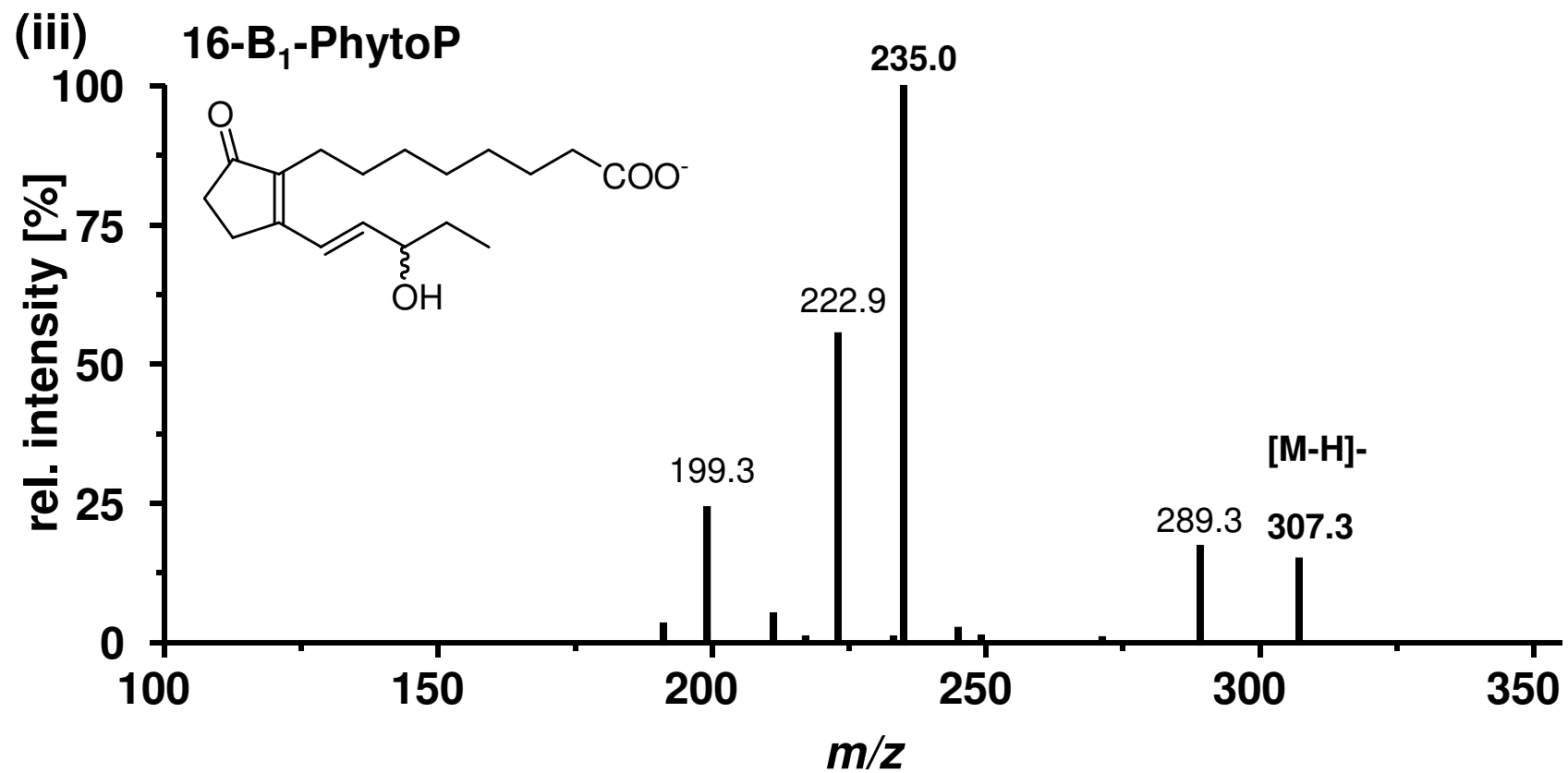


Fig. S1: Continued.

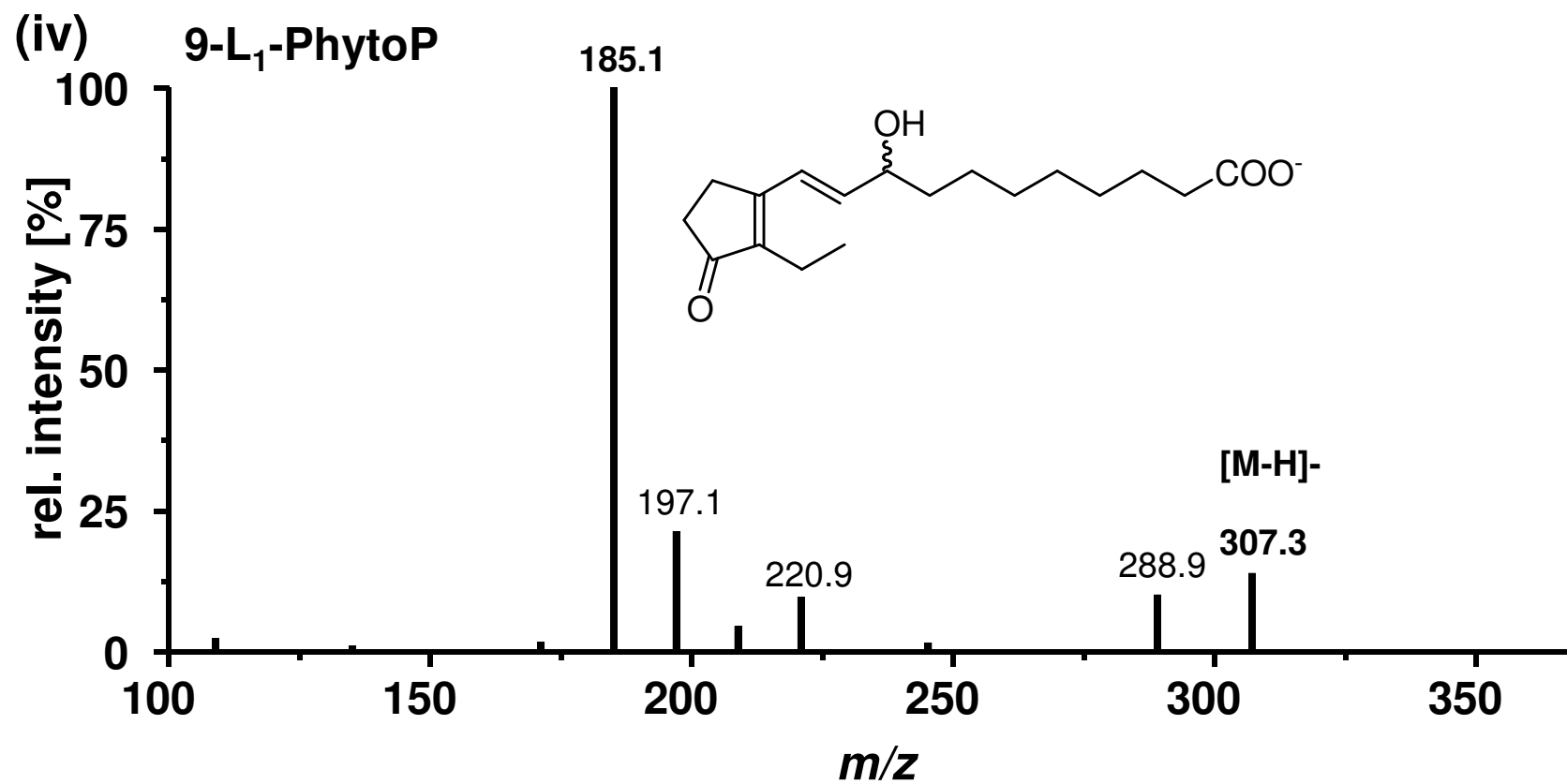


Fig. S1: Continued.

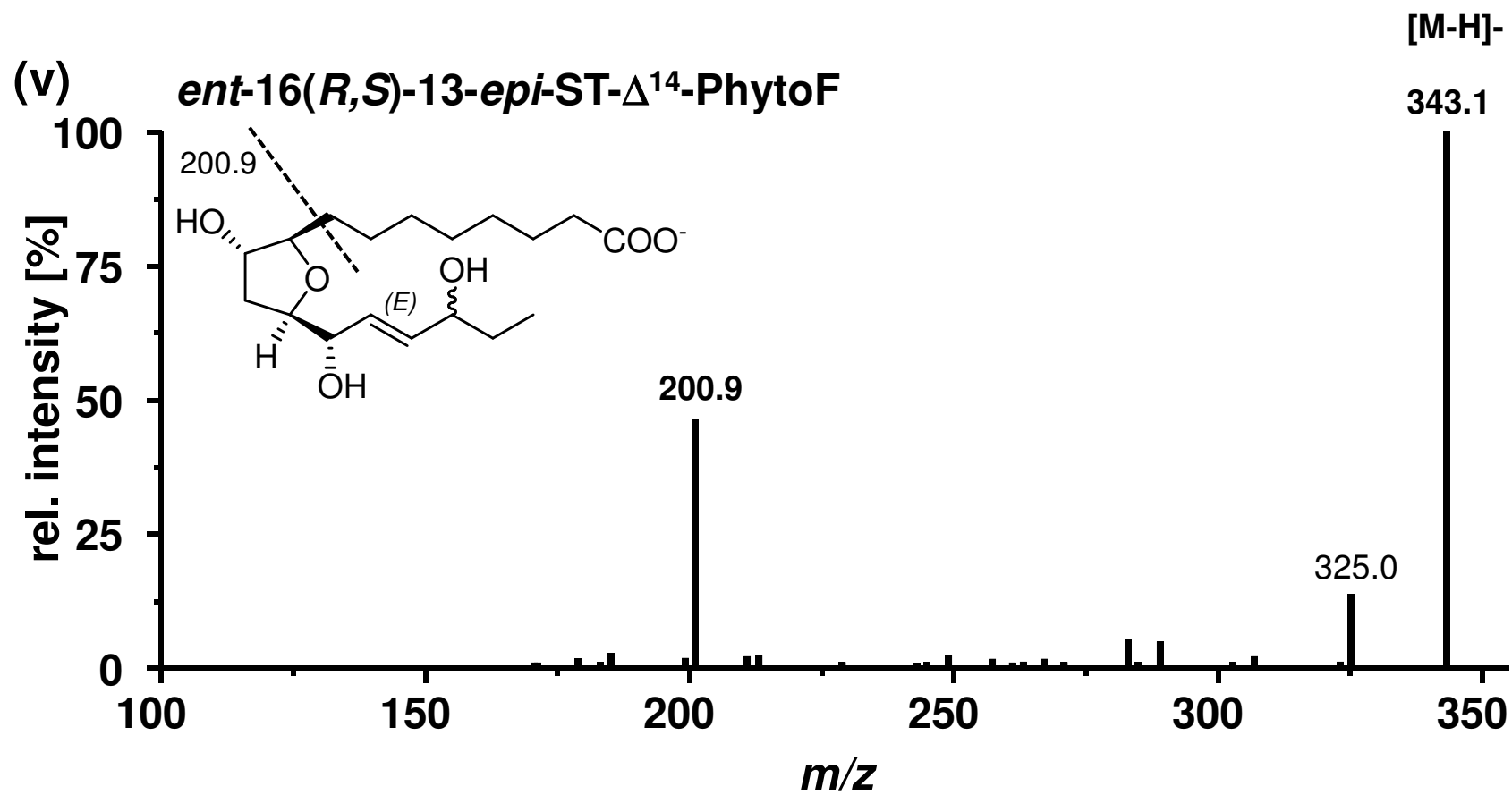


Fig. S1: Continued.

(B) Arachidonic acid derived IsoP

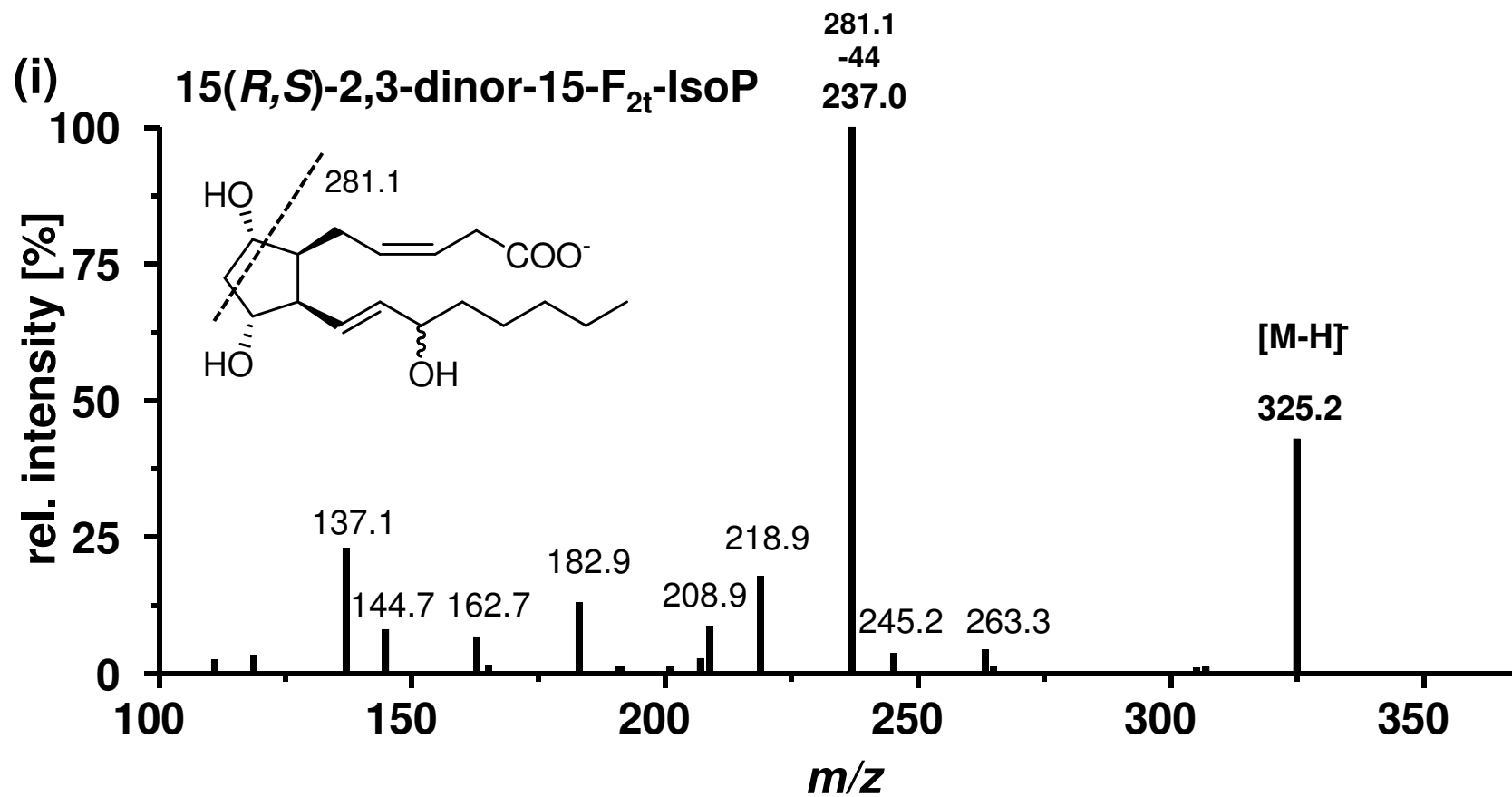


Fig. S1: Continued.

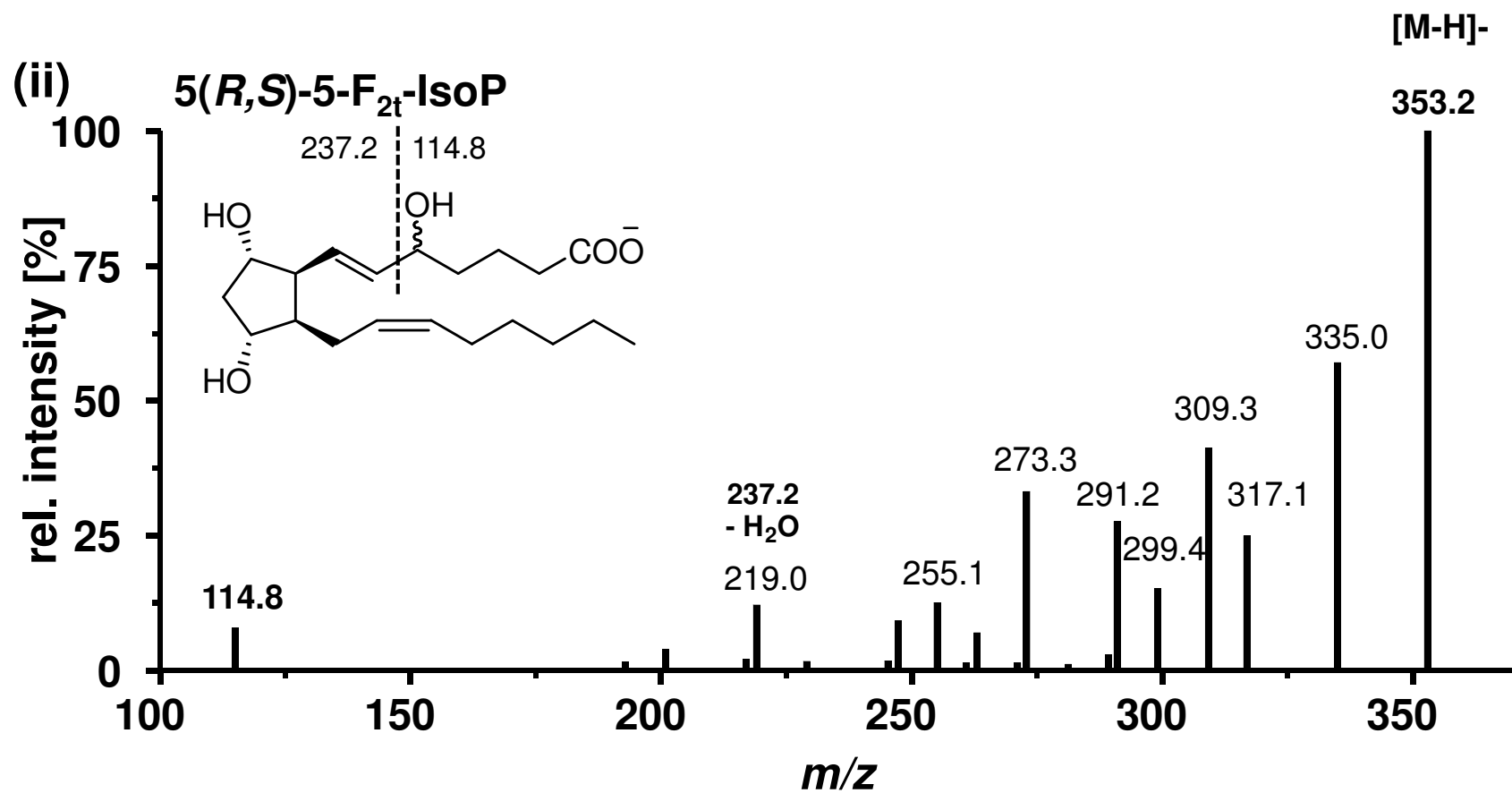


Fig. S1: Continued.

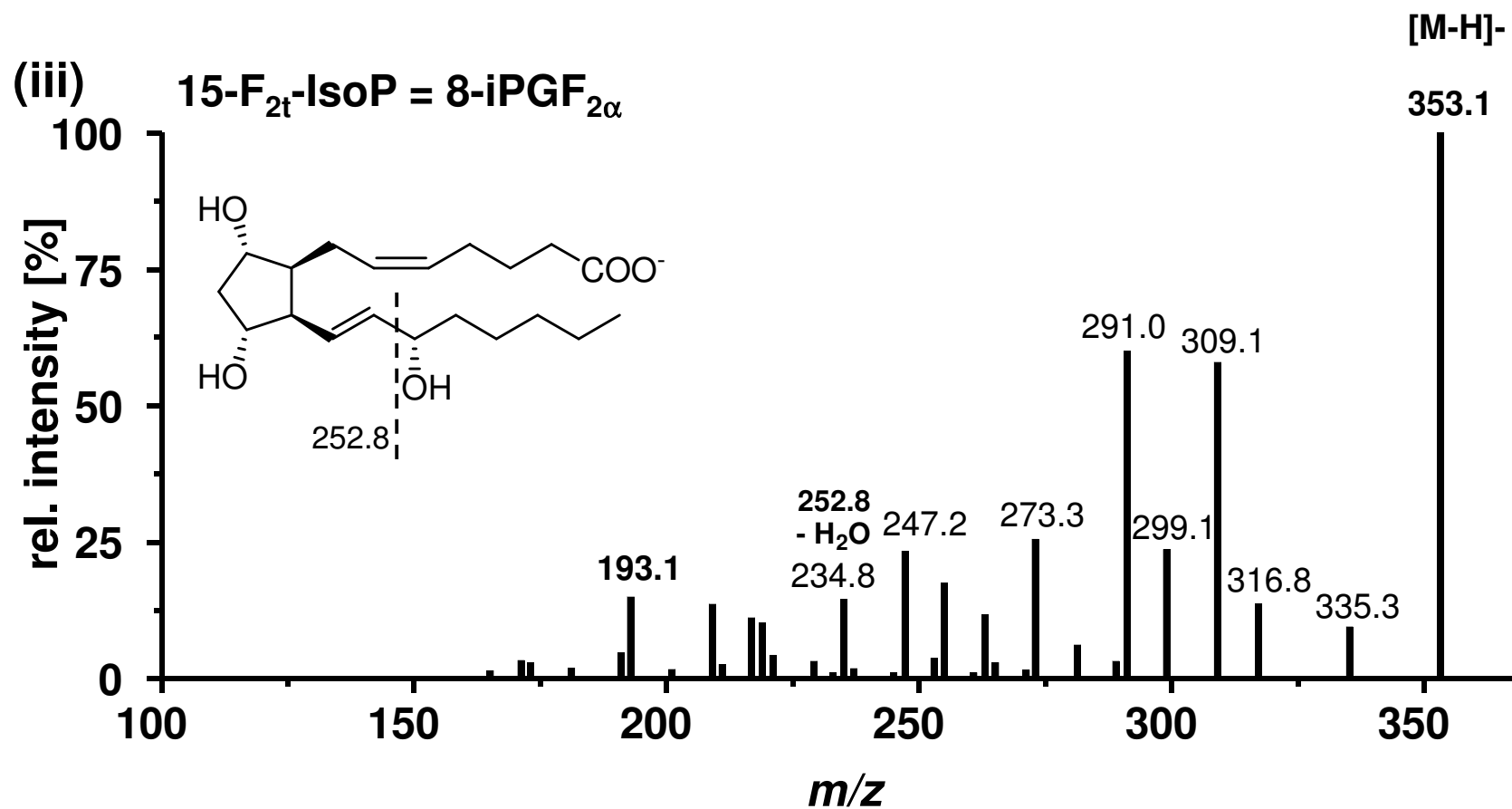


Fig. S1: Continued.

(C) Eicosapentaenoic acid derived IsoP

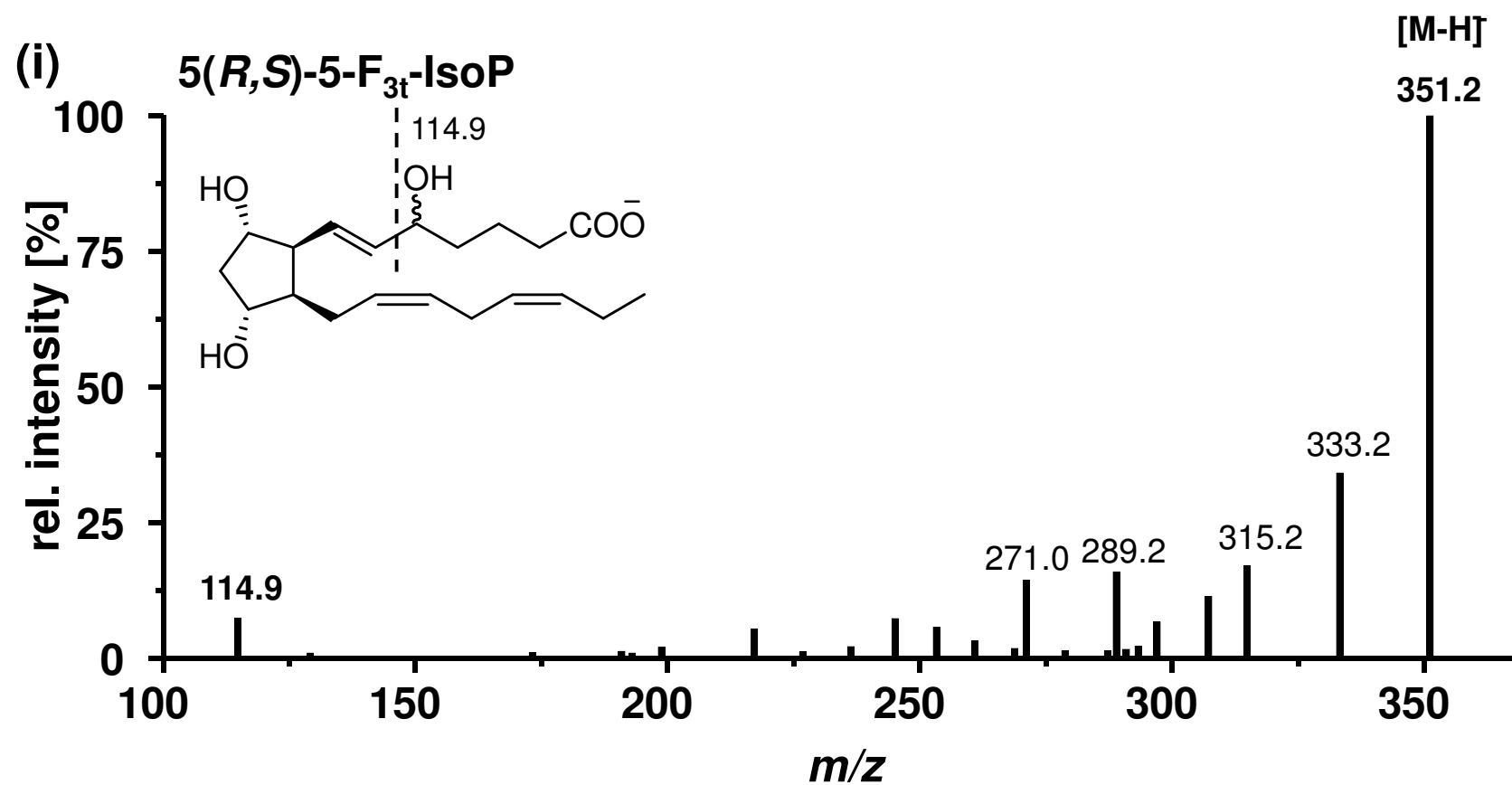


Fig. S1: Continued.

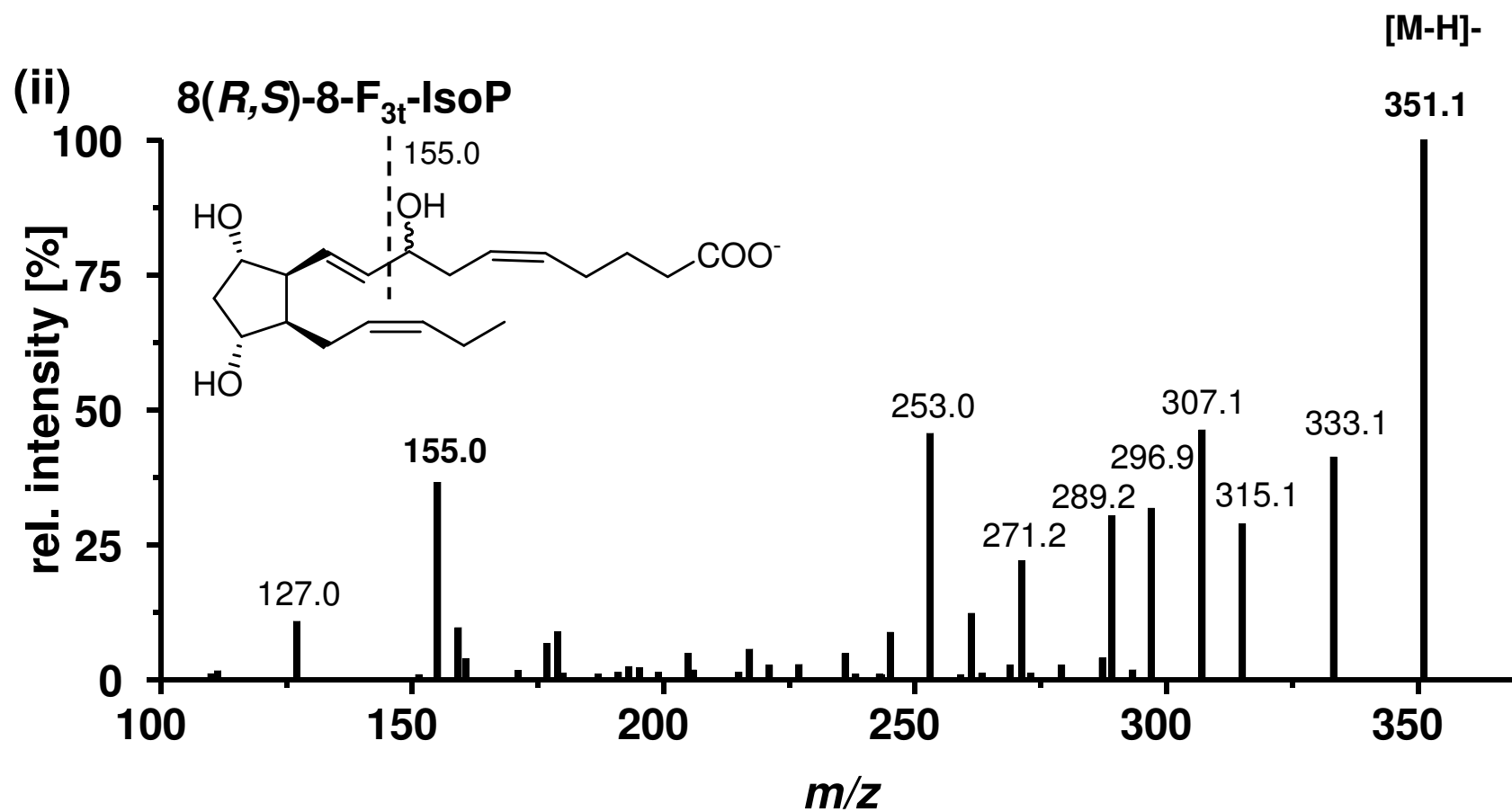


Fig. S1: Continued.

(D) Docosaheptaenoic acid derived NeuroP and NeuroF

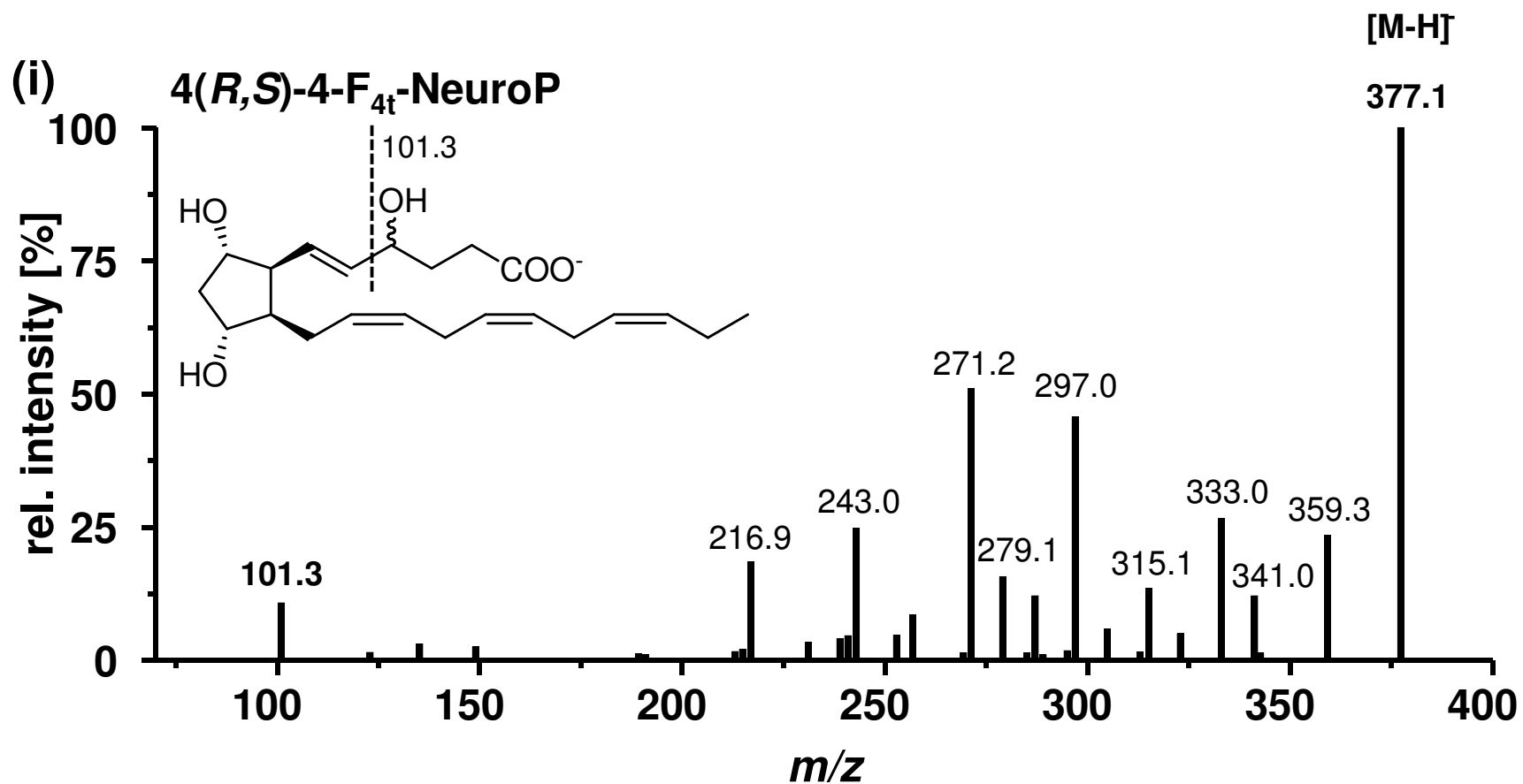


Fig. S1: Continued.

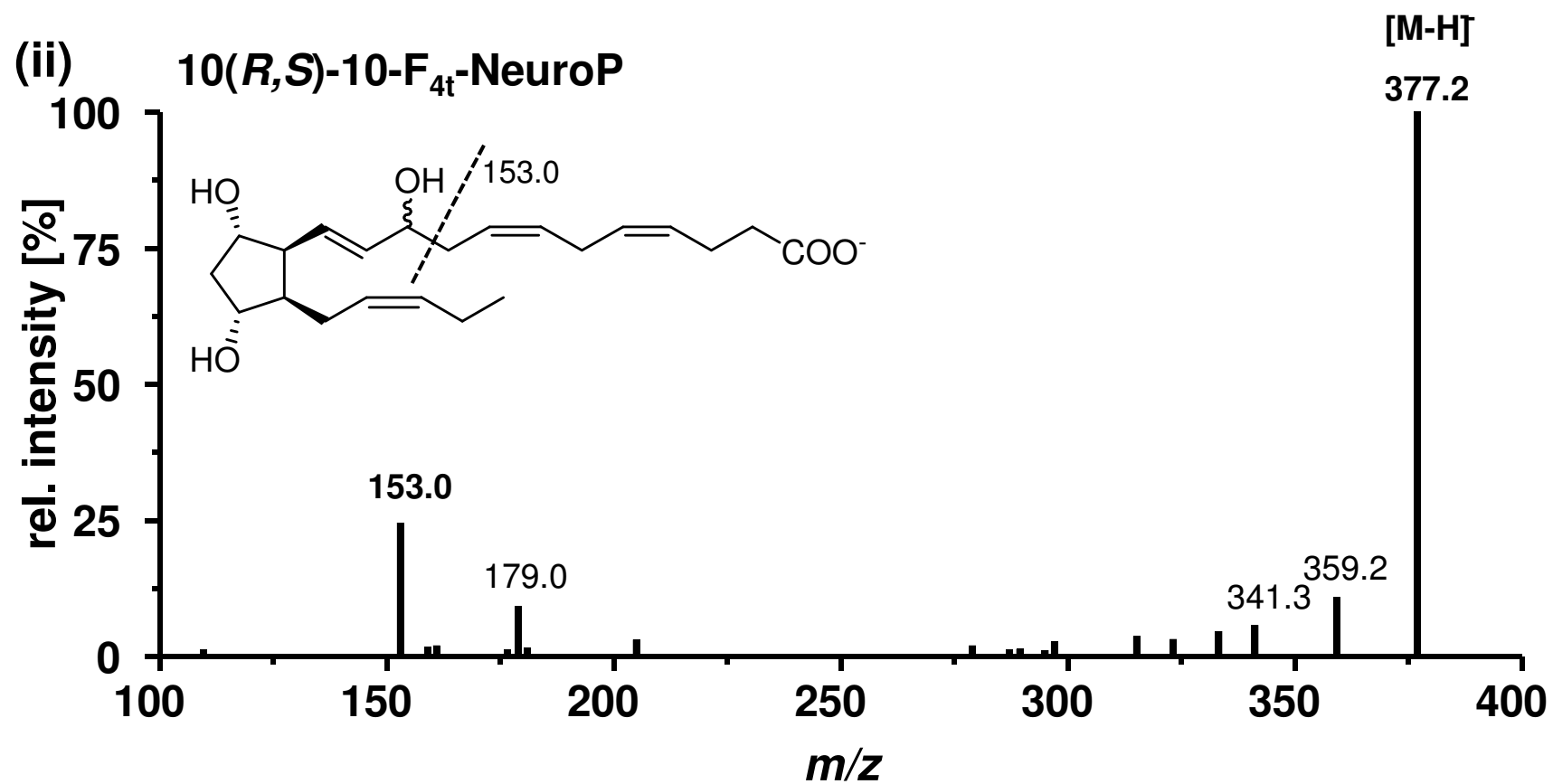


Fig. S1: Continued.

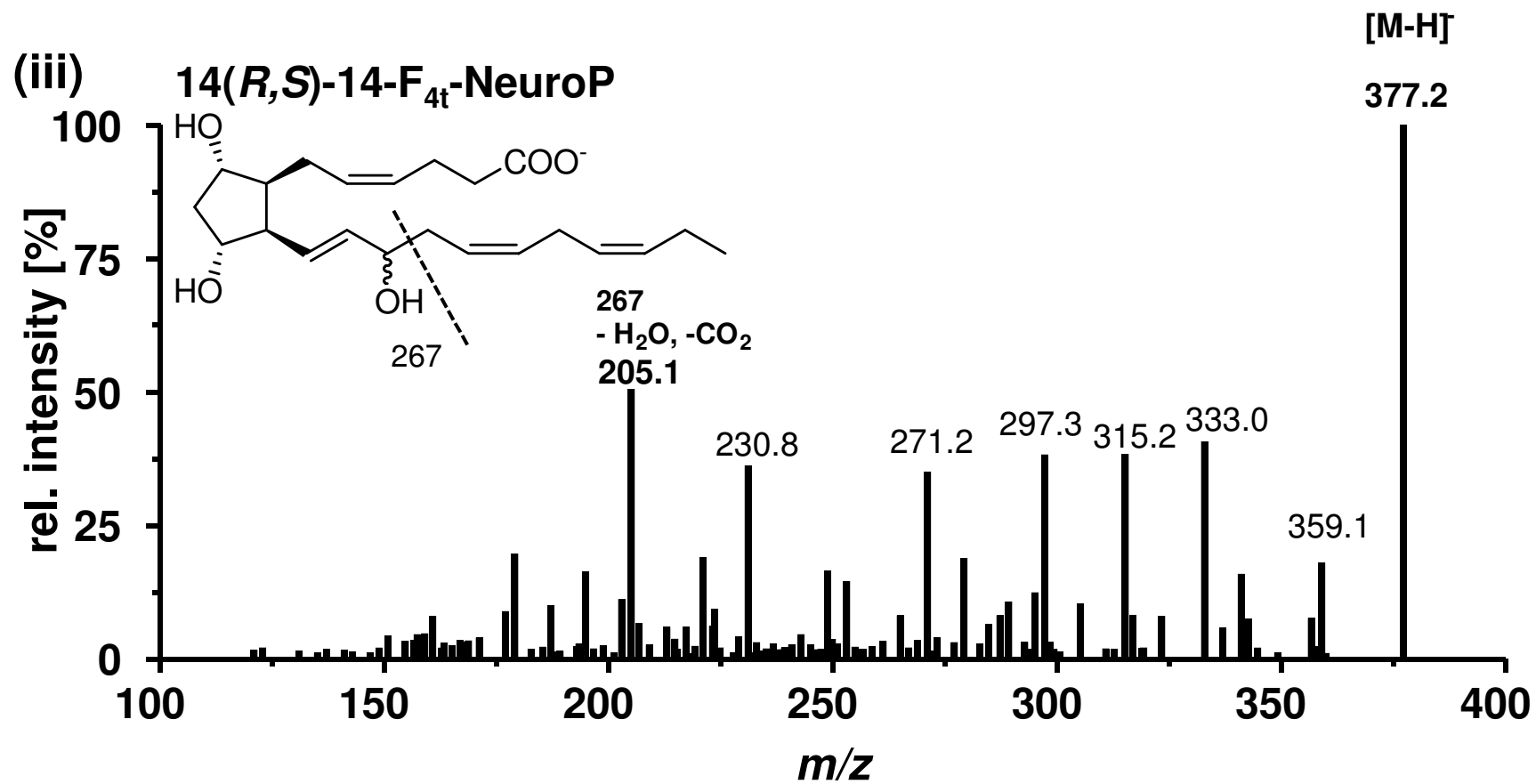


Fig. S1: Continued.

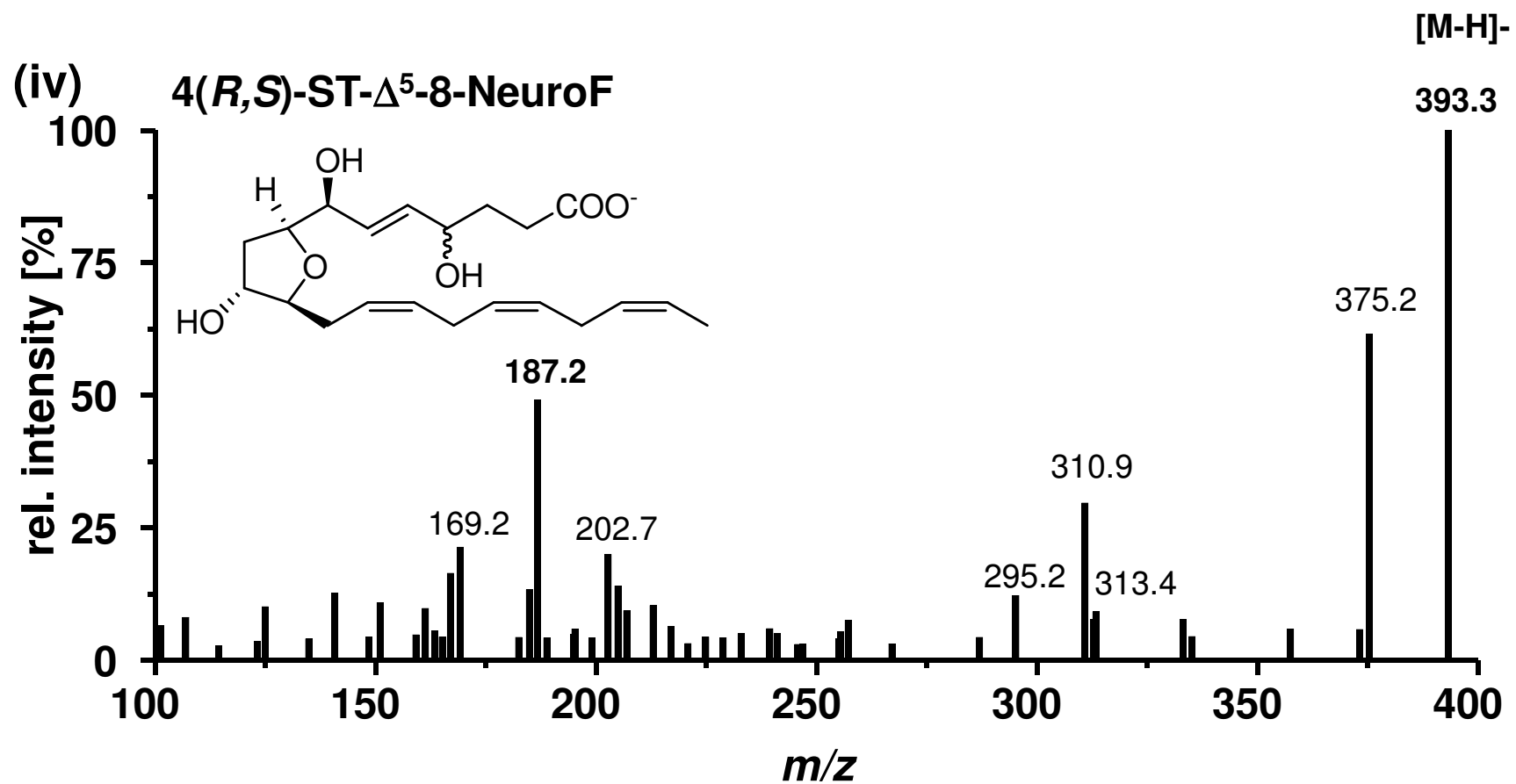


Fig. S1: Continued.

(E) Adrenic acid derived dihomomo-IsoP and dihomomo-IsoF

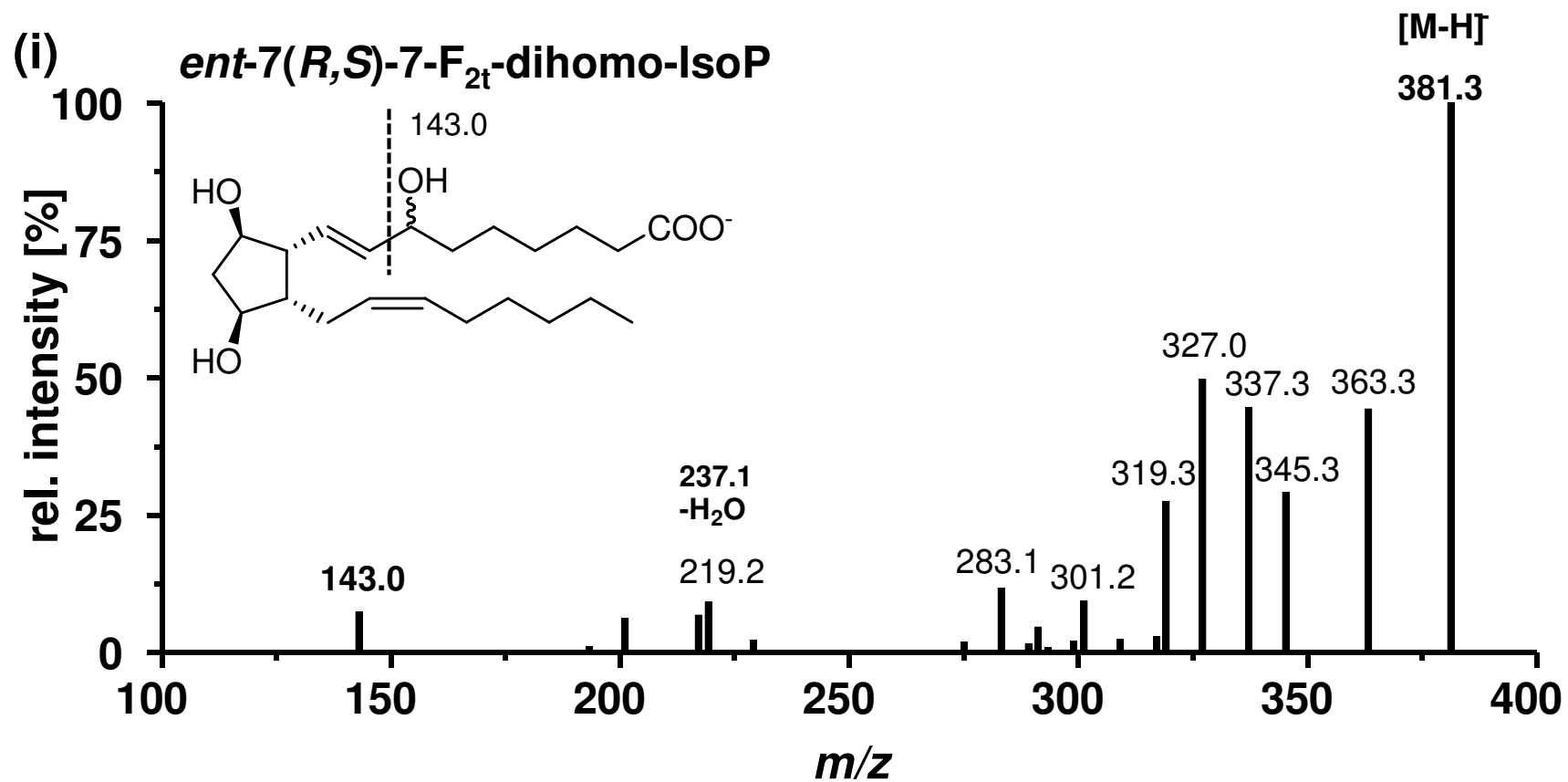


Fig. S1: Continued.

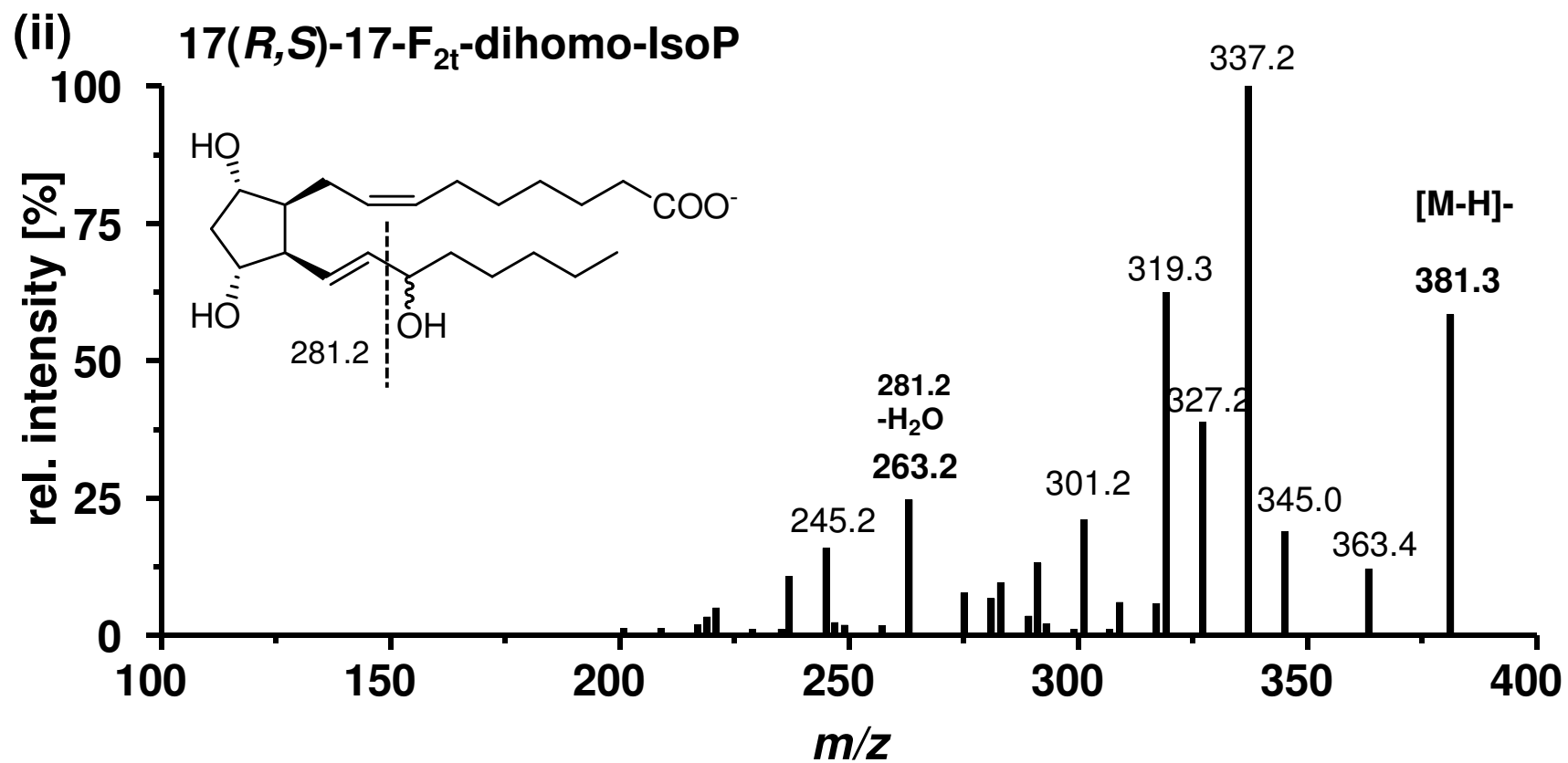


Fig. S1: Continued.

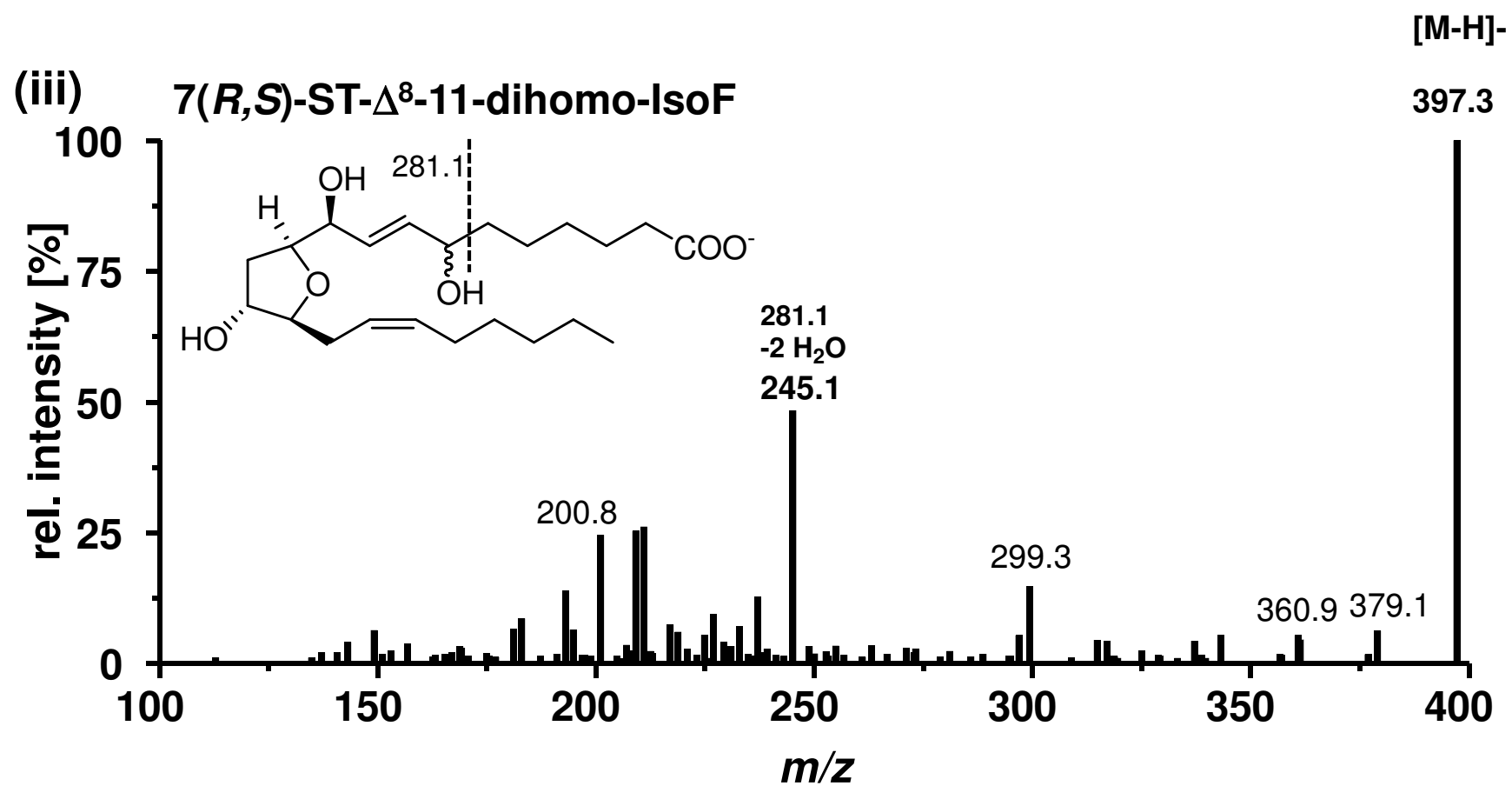


Fig. S1: Continued.

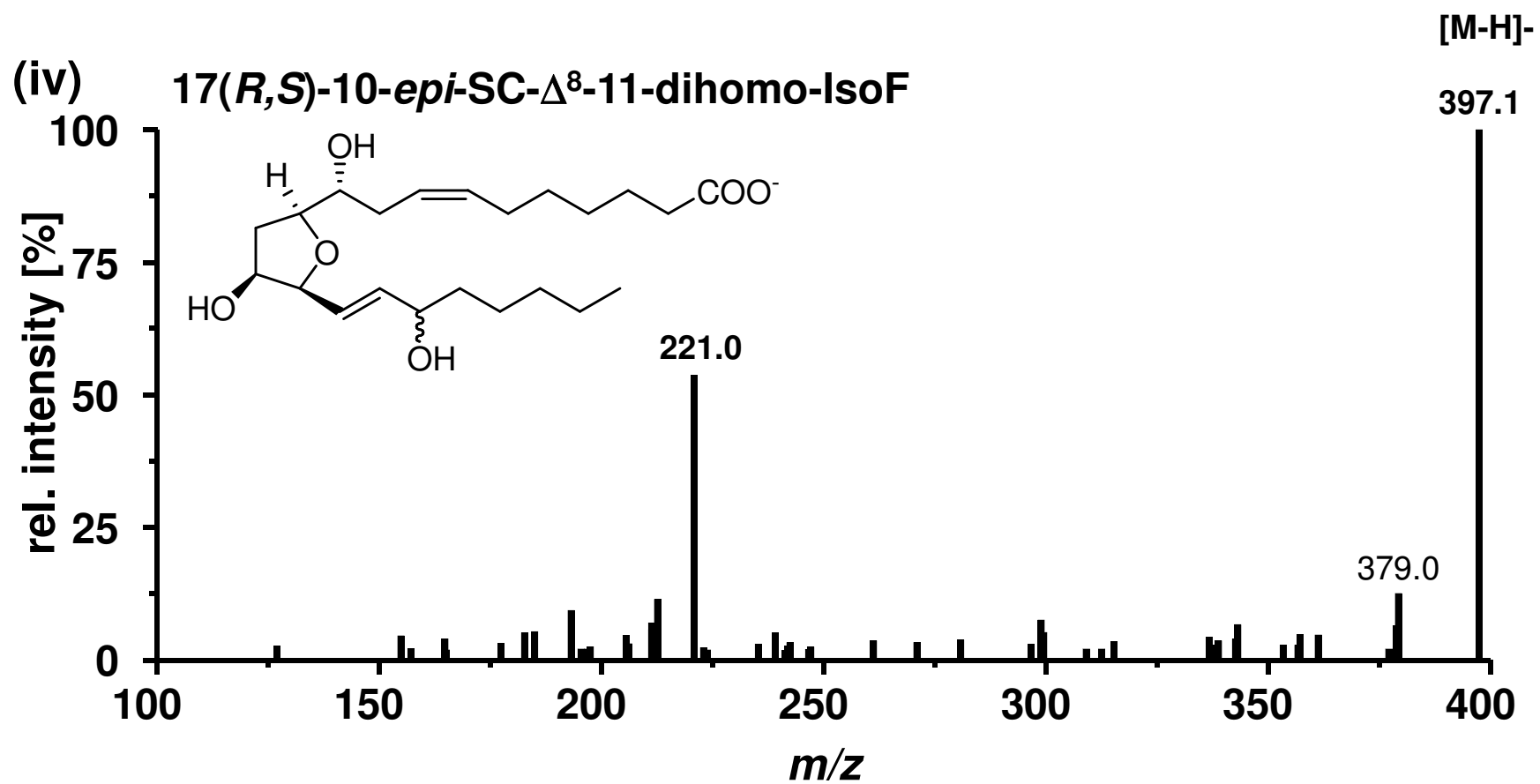


Fig. S1: Continued.

(F) Docosapentaenoic acid derived NeuroP

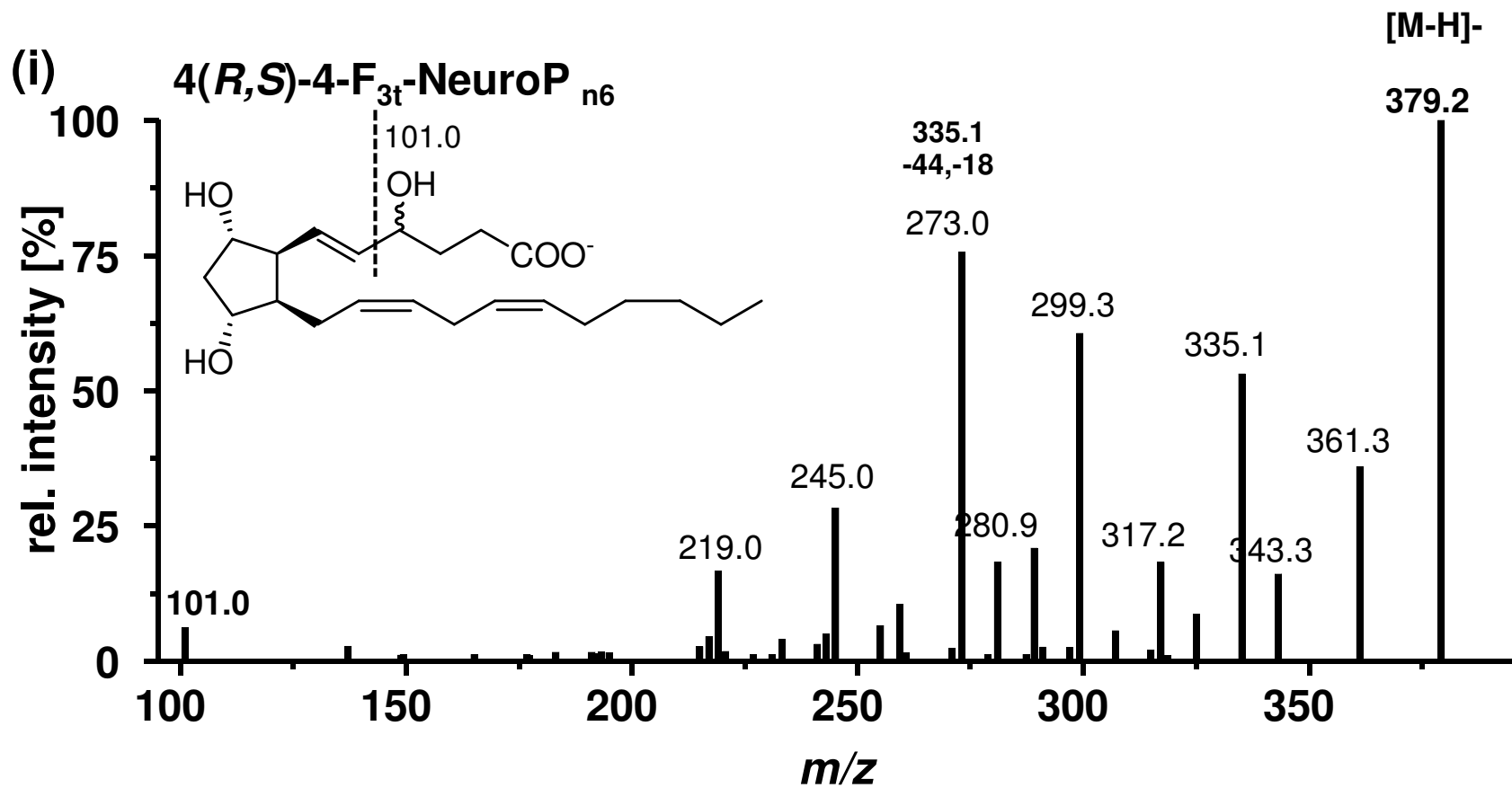


Fig. S1: Continued.

(G) Internal Standards

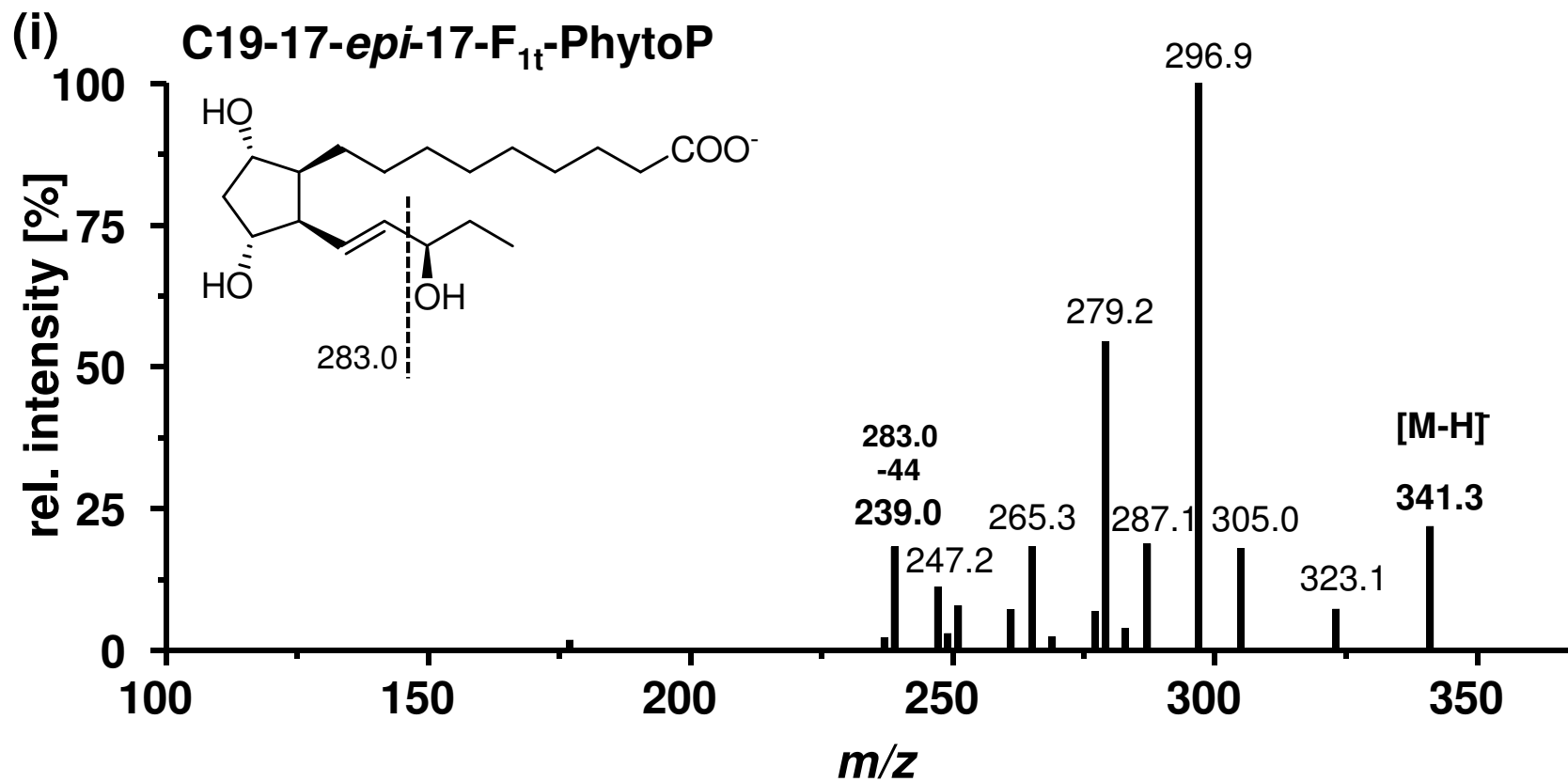


Fig. S1: Continued.

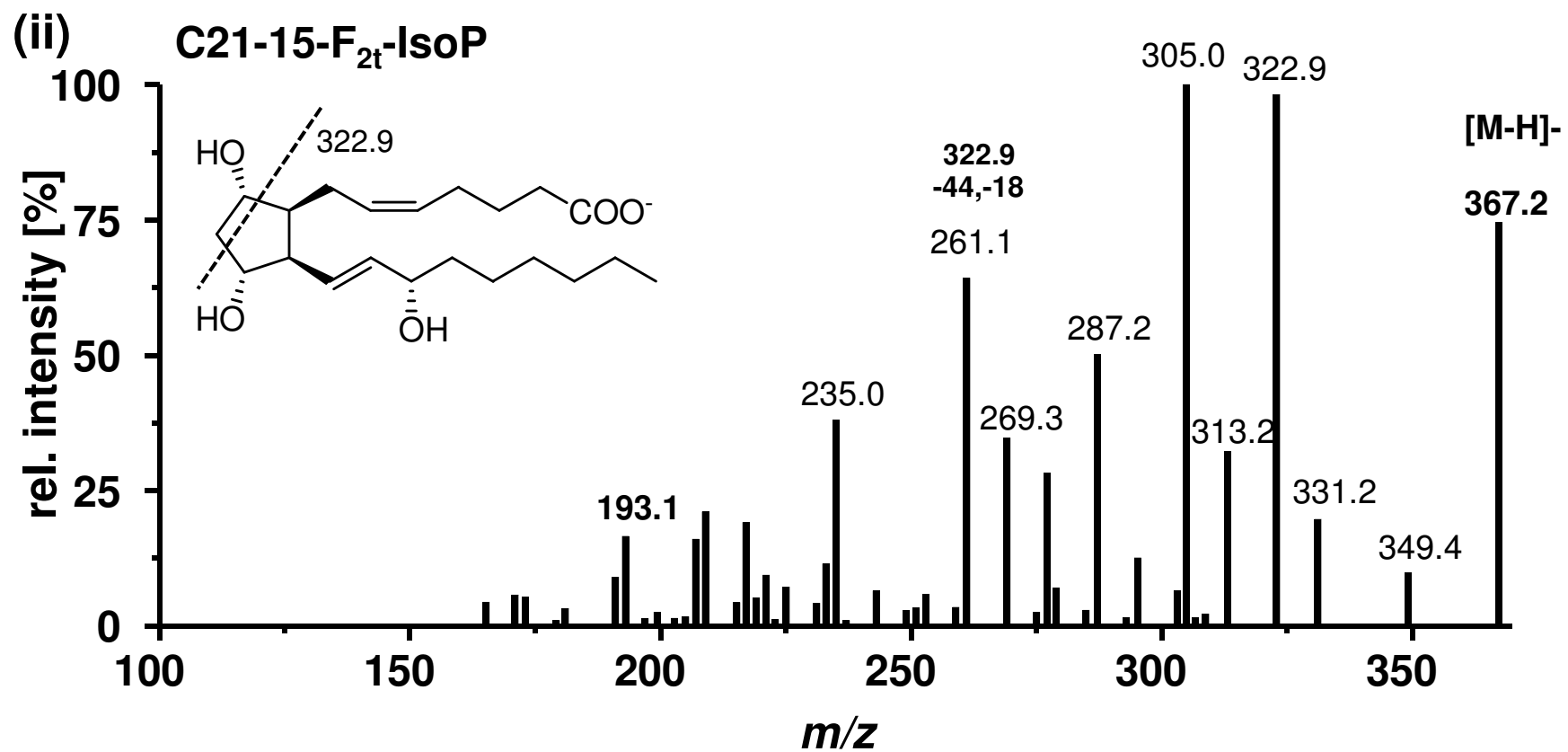


Fig. S1: Continued.

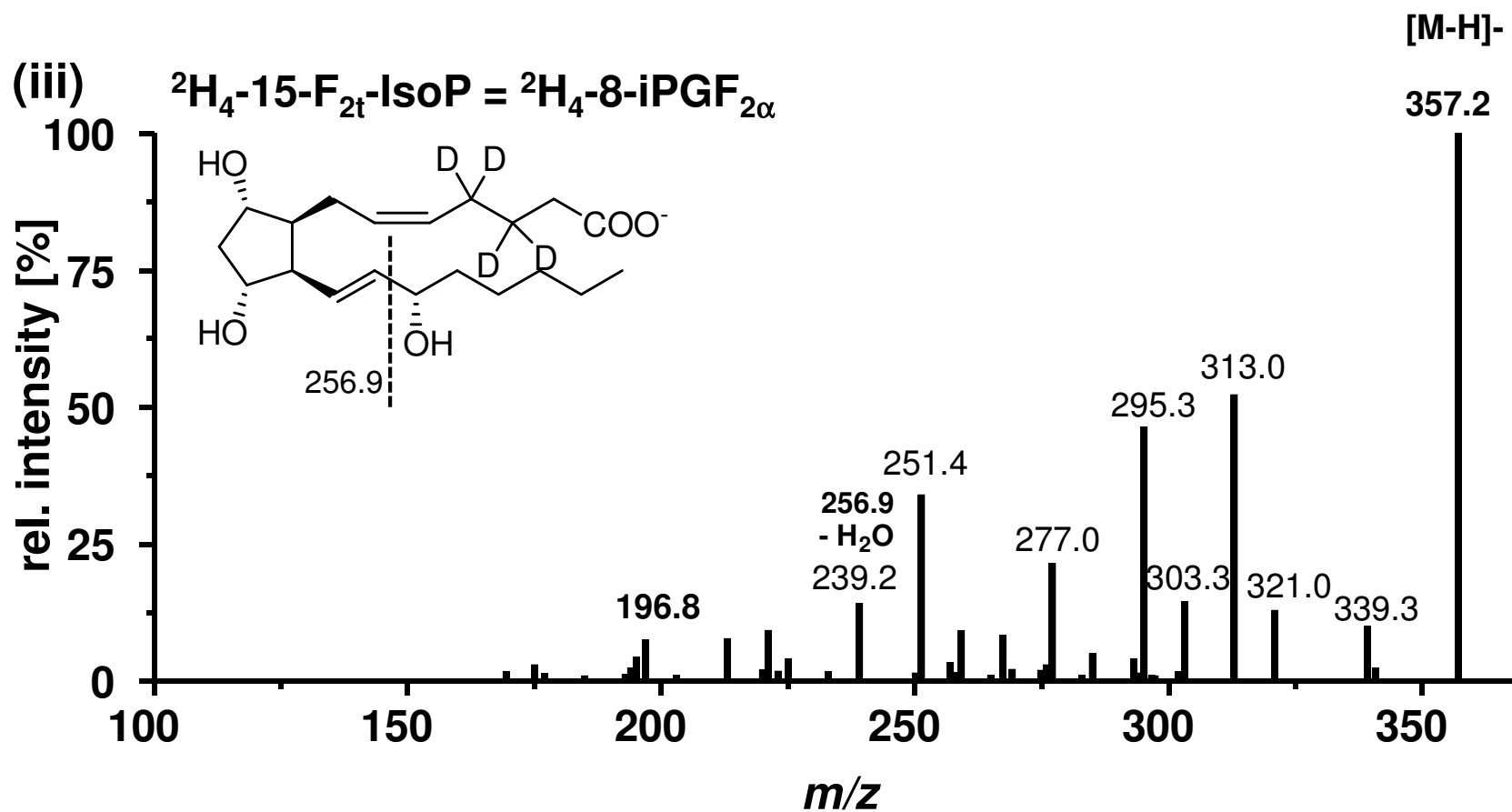


Fig. S1: Continued.

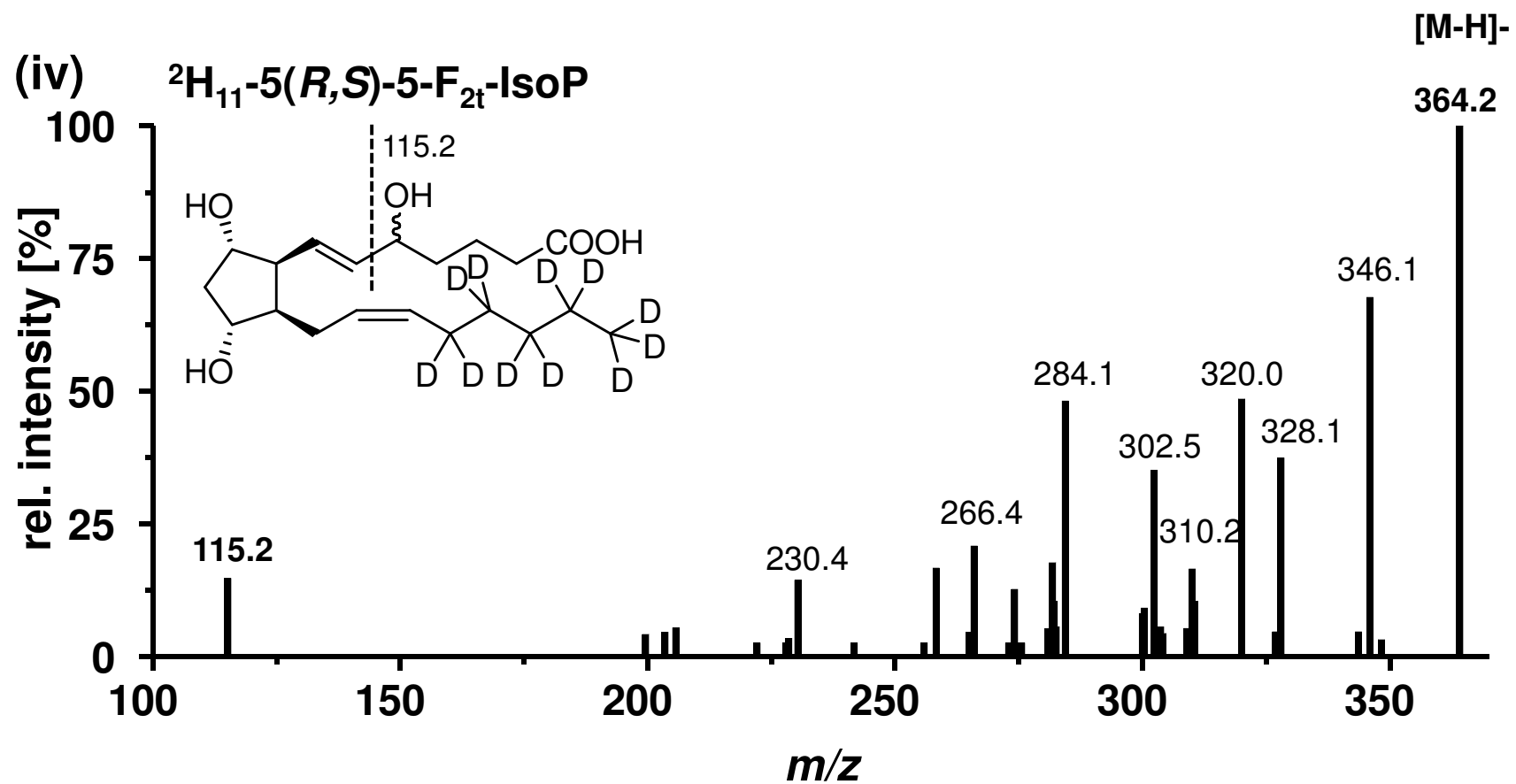


Fig. S1: Continued.

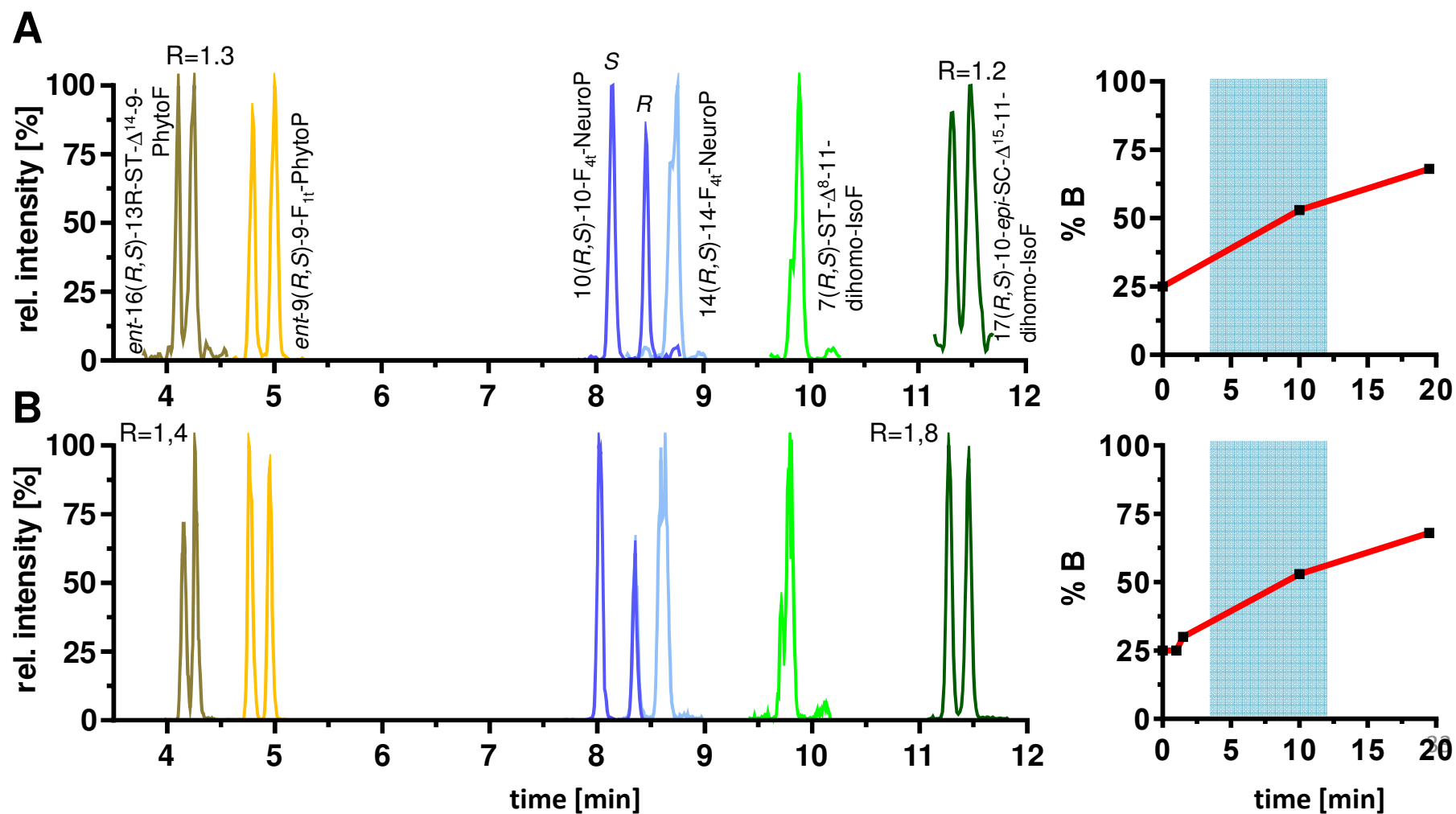


Fig. S2: Chromatographic separation efficiency of selected epimeric pairs using a gradient starting with 25% organic (**A**) without and (**B**) with inclusion of a short isocratic step (1 min) at the beginning of the gradient. The resolution (*R*) indicated for selected epimeric pairs.

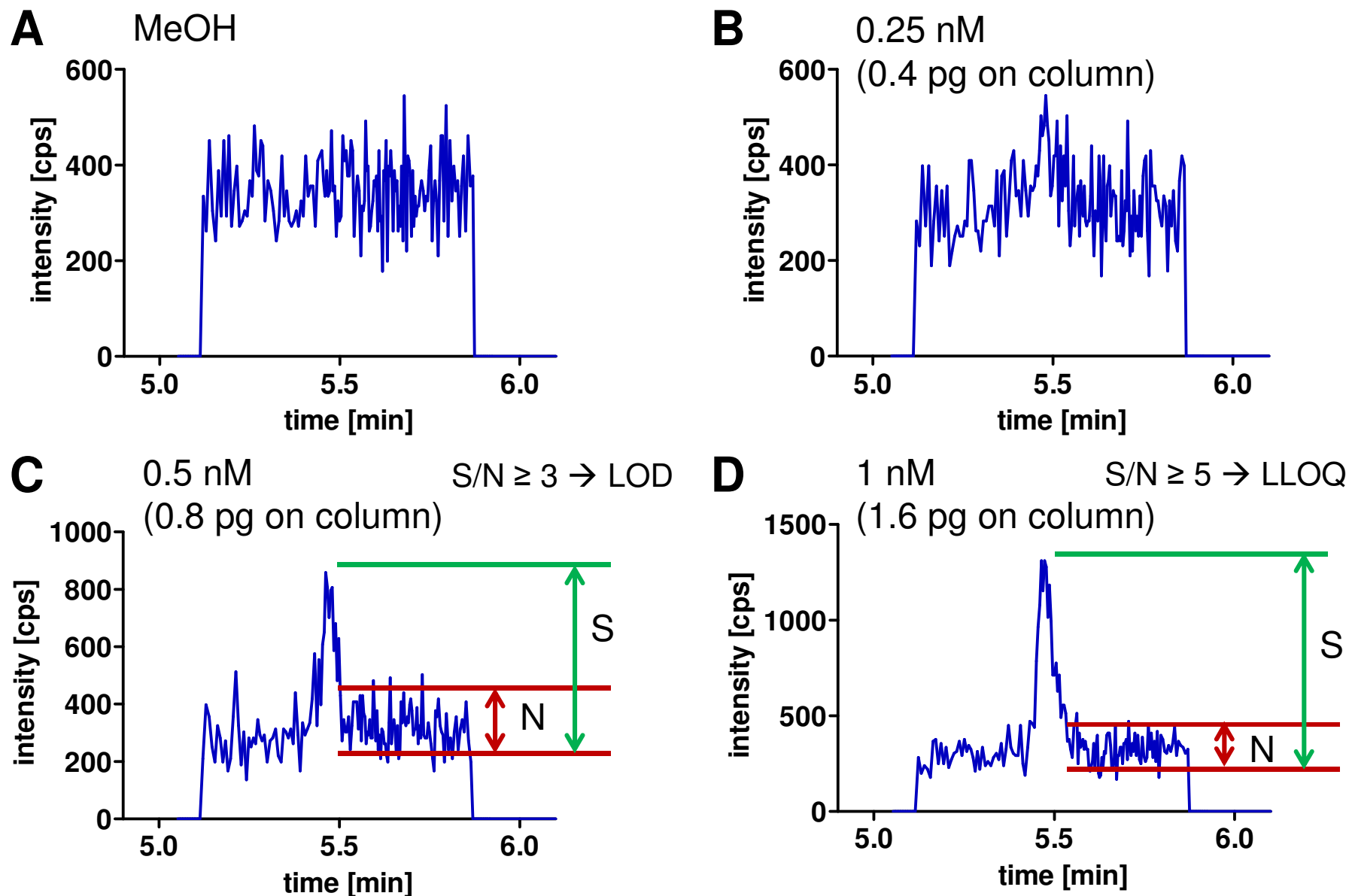


Fig. S3: Determination of the limit of detection (LOD) and lower limit of quantification (LLOQ) for 2,3-dinor-15- F_{2t} -IsoP based on the signal to noise (S/N) ratio. Starting from **(A)** blank (MeOH), successive standards **(B)**, **(C)**, **(D)** with increasing concentration of the analyte were injected. **(C)** The concentration yielding a $S/N \geq 3$ was set as LOD. **(D)** The concentration with a $S/N \geq 5$ and an accuracy within the calibration curve of $\pm 20\%$ was defined as LLOQ.

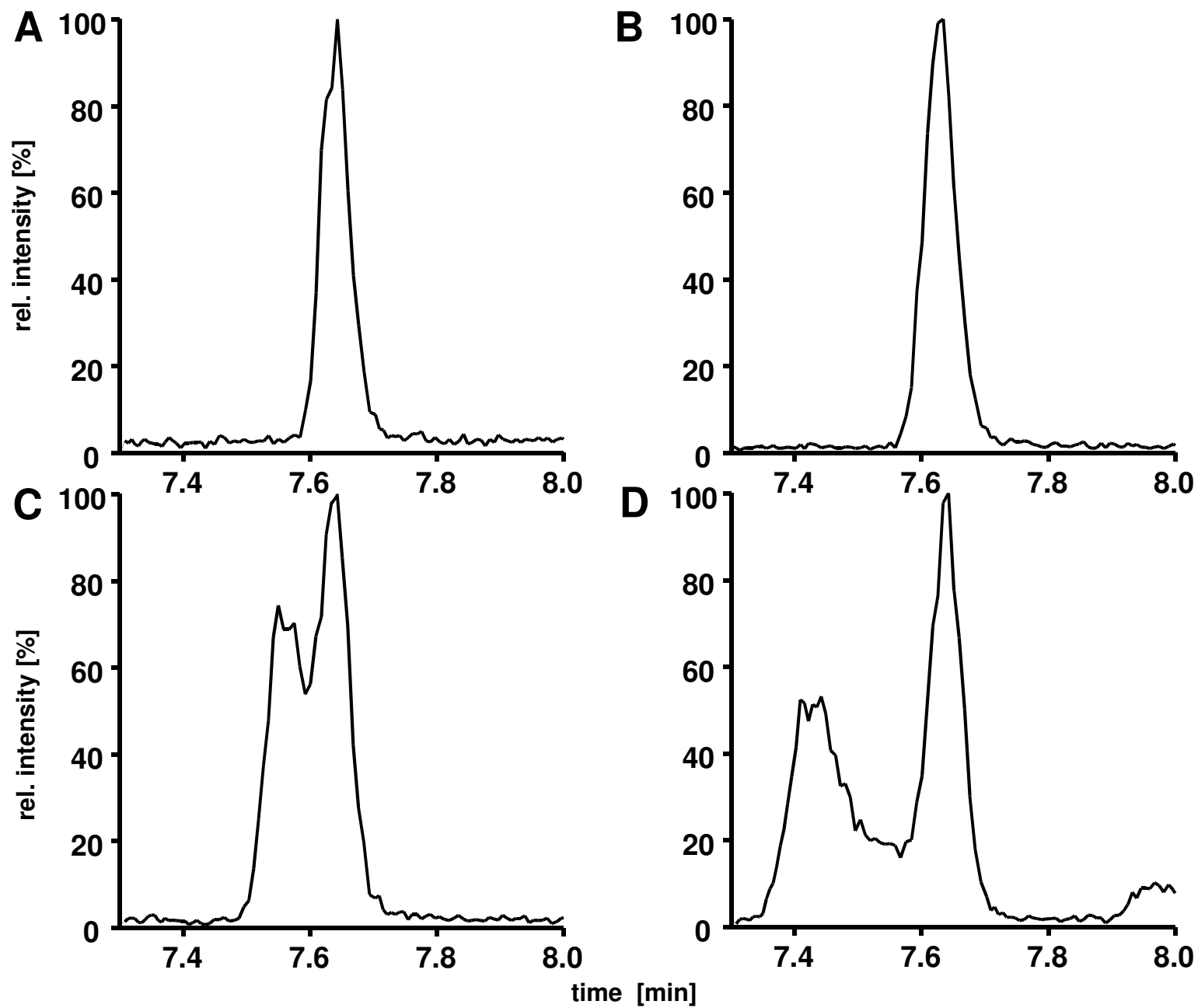


Fig. S4: Peak shape of 15-F_{2t}-IsoP (10 nM in methanol) with increasing injection volumes: **(A)** 5 µL, **(B)** 10 µL, **(C)** 15 µL, **(D)** 20 µL.

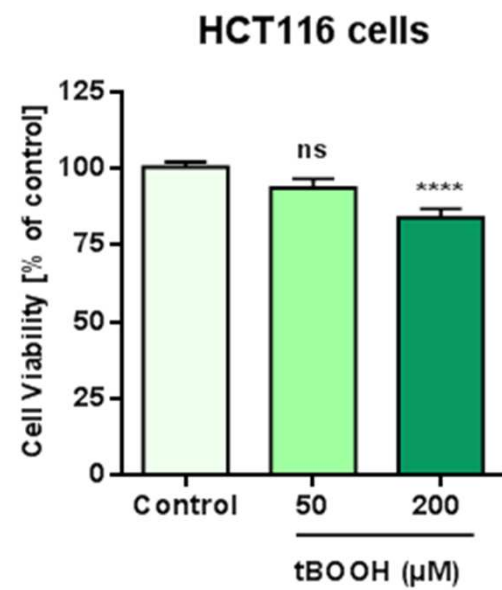


Fig. S5: Cell viability treated with increasing doses of t-BOOH for 2 h determined by MTS assay.