Electronic Supplementary Information for

Quantification and Molecular Imaging of Fatty Acid Isomers from Complex Biological Samples by Mass Spectrometry

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Materials and Reagents

Fatty acid (FA) standards including FA 16:1 (9Z), FA 18:1 (6Z), FA 18:1 (9Z), FA 18:1 (11Z), FA 20:1 (11Z), FA 18:2 (9Z, 12Z), and arachidonic acid FA 18:4 (5Z, 8Z, 11Z, 14Z), and *meta*-Chloroperoxybenzoic acid (*m*-CPBA) were purchased from Sigma-Aldrich (St. Louis, MO). Methanol (MeOH), ethanol (EtOH), acetonitrile (ACN), 2-propanol (IPA), peracetic acid (30% in acetic acid, w/w), distilled water, trichloromethane, ammonia methanol (2 M) and other solvents were all optima grade and purchased from Fisher Scientific (Pittsburgh, PA). 1,5-Diaminonaphthalene (DAN) and Stearoyl-CoA desaturase 1 (SCD1) inhibitor of A939572 (purity > 98%) were purchased from APExBIO Technology (Houston, TX, USA). Indium tin oxide (ITO)-coated glass microscope slides (25 mm × 75 mm × 1 mm) were purchased from Bruker (Billerica, MA, United States).

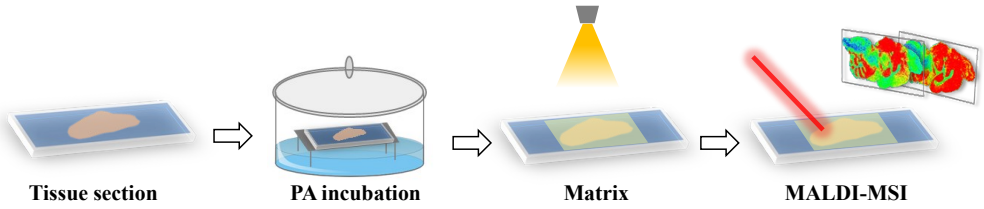

Figure S1 Schematic diagram of the on-tissue epoxidation of unsaturated fatty acids for MALDI-TOF-MS imaging analysis.

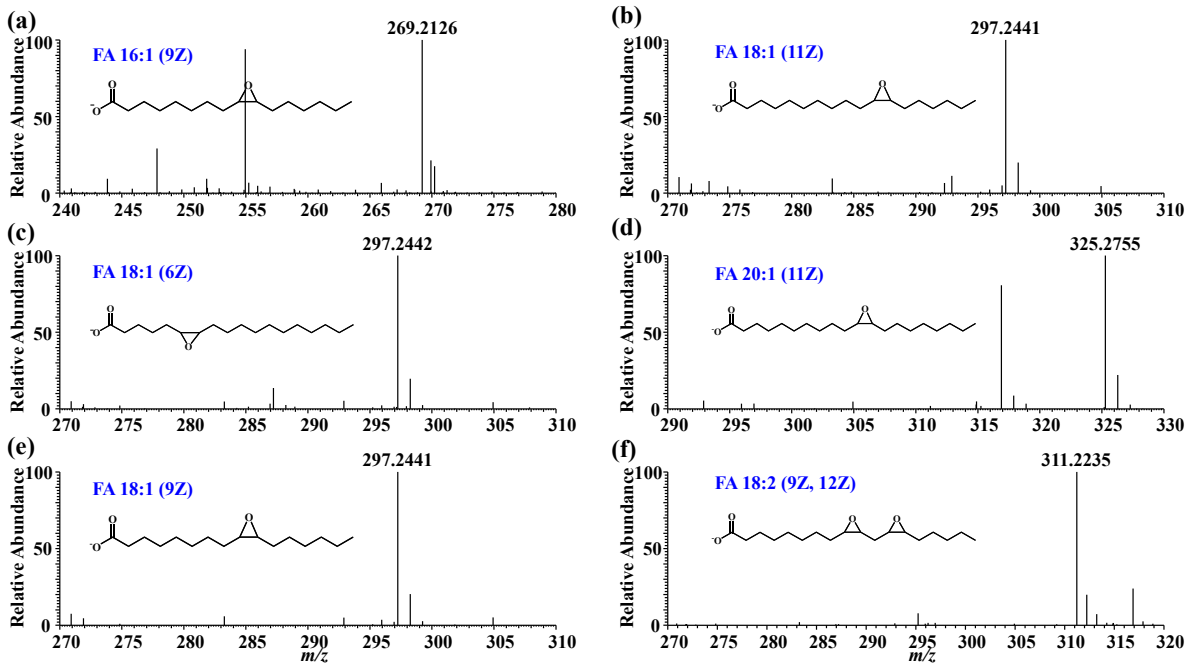

Figure S2. Nano-ESI-MS spectra of epoxide fatty acids after one hour in-solution epoxidation using peracetic acid: (a) FA 16:1 (9Z), (b) FA 18:1 (11Z), (c) FA 18:1 (6Z), (d) FA 20:1 (11Z), (e) FA 18:1 (9Z), (f) FA 18:2 (9Z, 12Z).

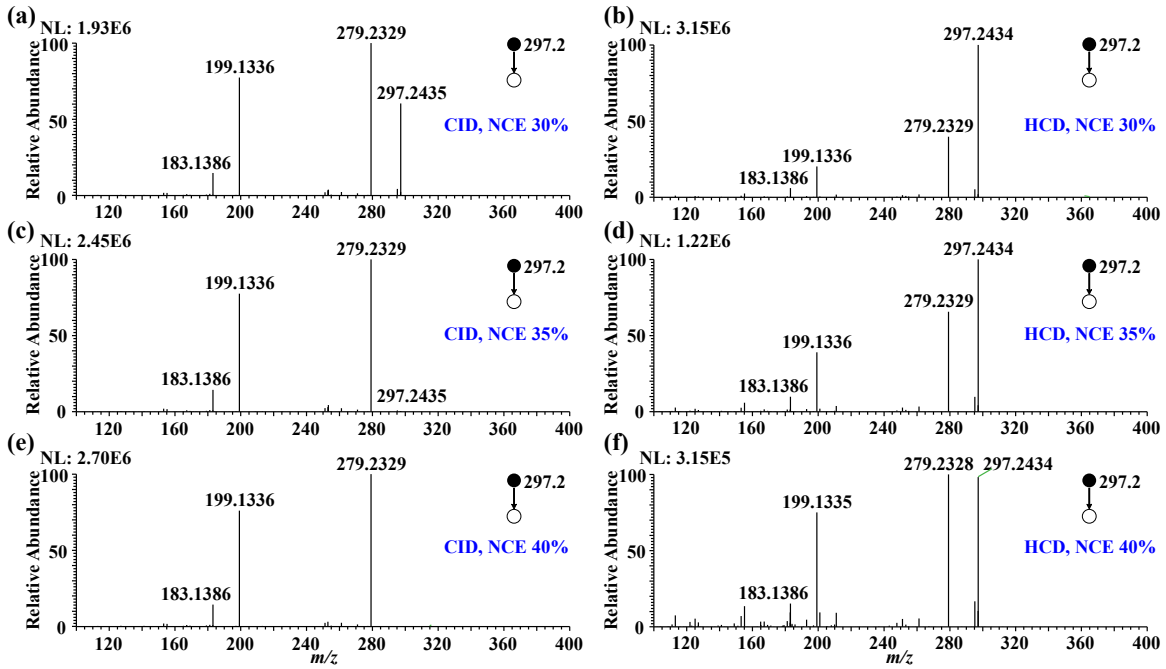

Figure S3. Tandem mass spectra of epoxide FA18:1 (11Z) obtained from collision induced dissociation (CID) fragmentation and high-energy collisional dissociation (HCD) fragmentation under different energy level: (a) fragmentation using CID mode with 30% normalized collision energy (NCE), (b) fragmentation using HCD mode with 30% NCE, (c) fragmentation using CID mode with 35% NCE, (d) fragmentation using HCD mode with 35% NCE, (e) fragmentation using CID mode with 40% NCE, (f) fragmentation using HCD mode with 40% NCE.

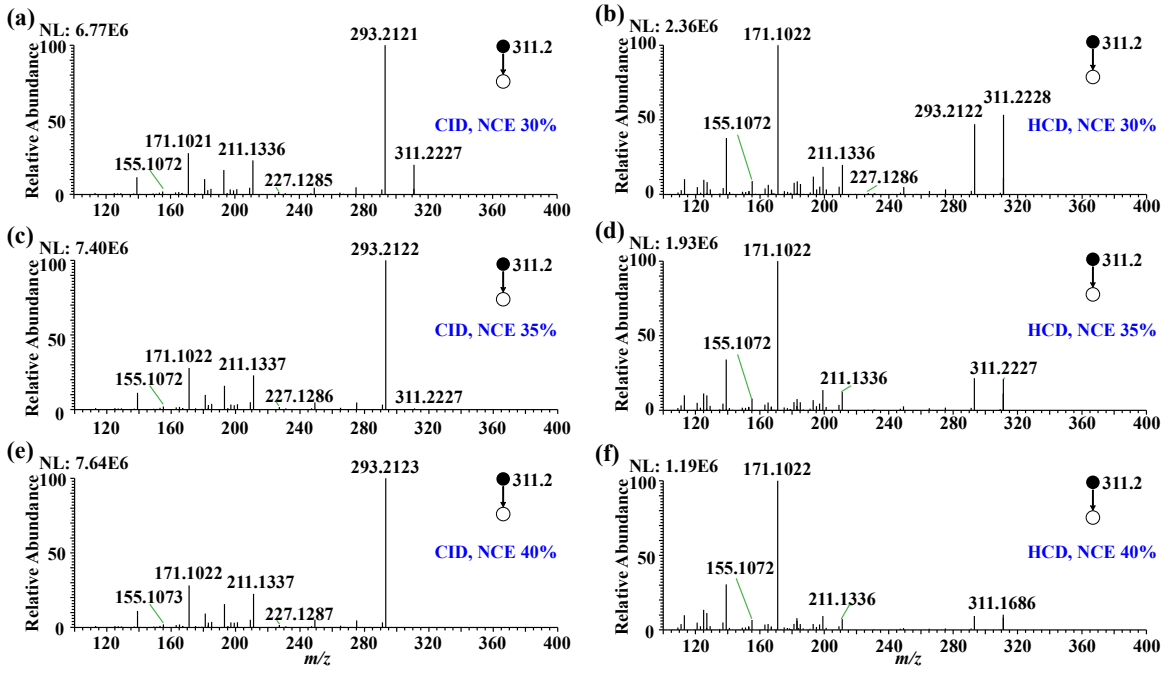

Figure S4. Tandem mass spectra of epoxide FA18:2 (9Z, 12Z) obtained from collision induced dissociation (CID) fragmentation and high-energy collisional dissociation (HCD) fragmentation under different energy level: (a) fragmentation using CID mode with 30% normalized collision energy (NCE), (b) fragmentation using HCD mode with 30% NCE, (c) fragmentation using CID mode with 35% NCE, (d) fragmentation using HCD mode with 35% NCE, (e) fragmentation using CID mode with 40% NCE, (f) fragmentation using HCD mode with 40% NCE.

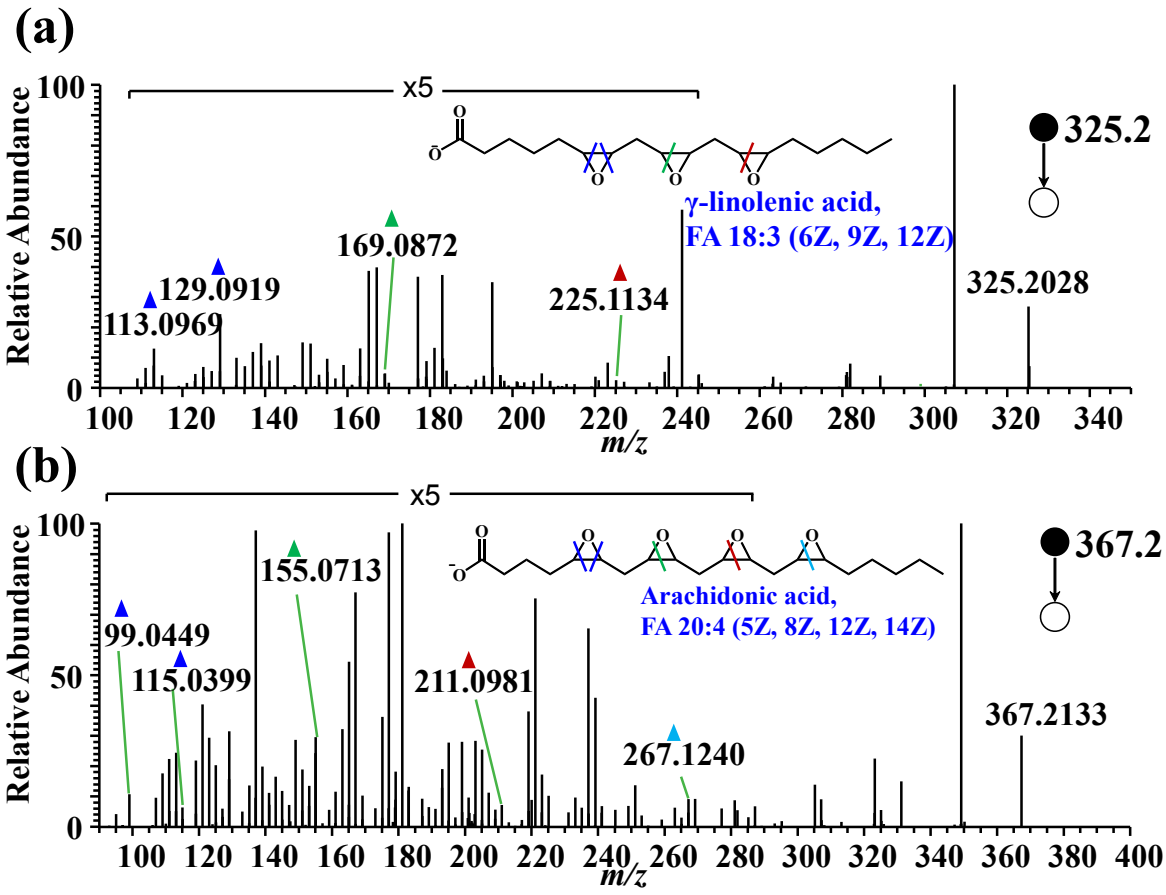

Figure S5. CID-MS/MS spectra of epoxide polyunsaturated fatty acids after the treatment of in-solution epoxidation using peracetic acid: (a) γ-linolenic acid (FA 18:3 (6Z, 9Z, 12Z)) and (b) arachidonic acid (FA 20:4 (5Z, 8Z, 12Z, 14Z)).

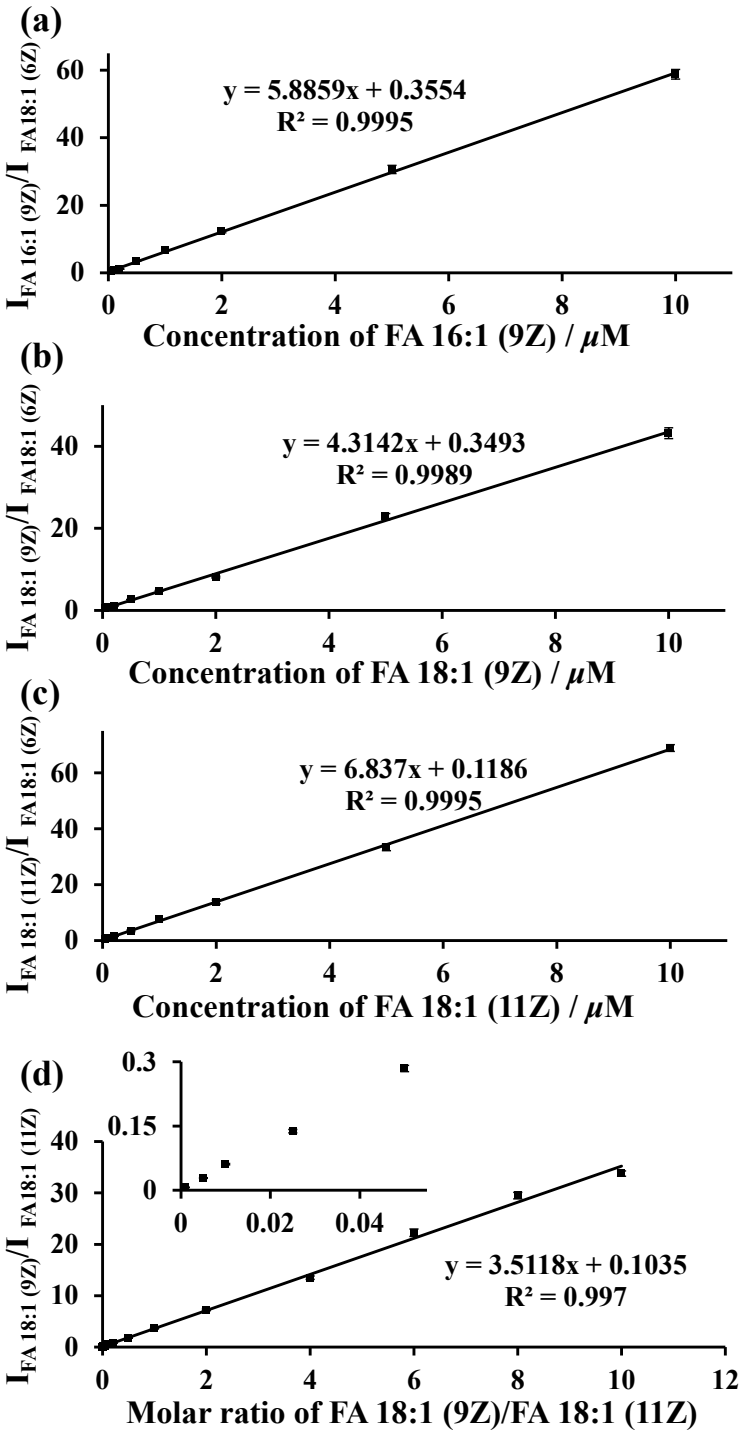

Figure S6. Quantitative analysis of C=C bond positional isomers. (a) Calibration curve for quantitation of (a) FA 16:1 (9Z), (b) FA 18:1 (9Z), (c) FA 18:1 (11Z); (d) relative quantitation of FA 18:1 (9Z) and FA 18:1 (11Z), the inset presents the zoomed-in linear relationship ranging from 0.001:1 to 0.05:1. Data points are shown as mean ± SD, n=3.

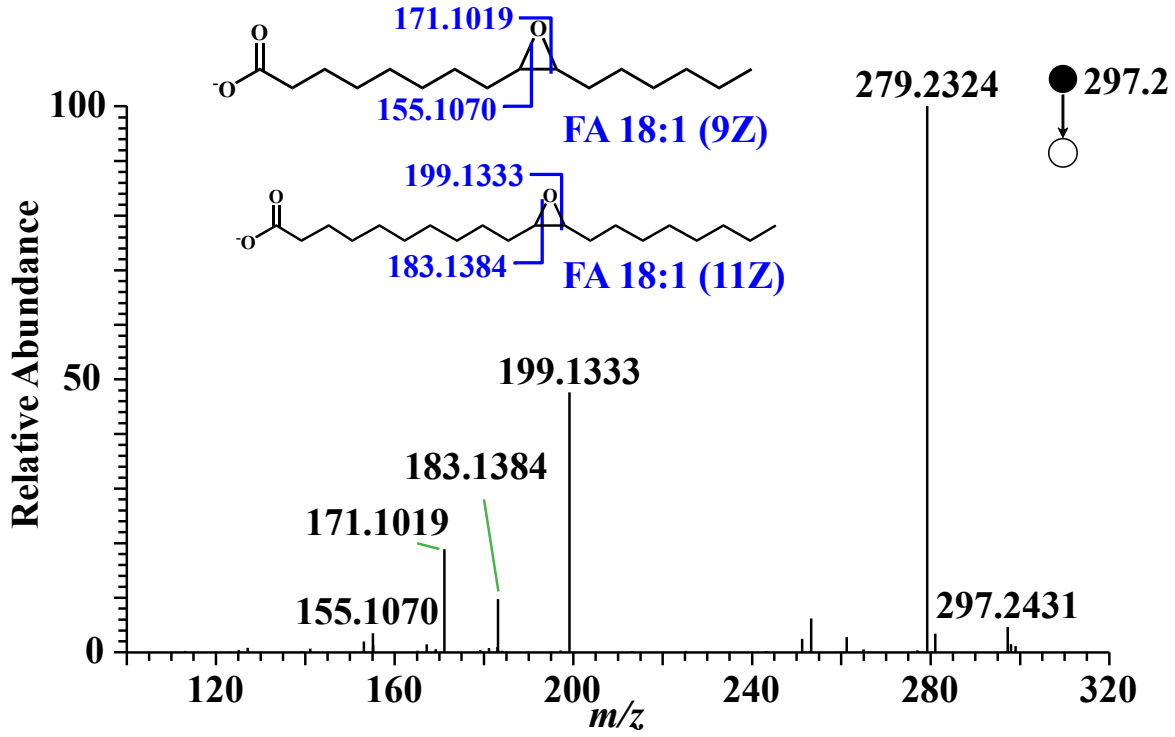

Figure S7. MS/MS spectrum of epoxide fatty acid isomer mixture sample with FA 18:1 (9Z) and FA 18:1 (11Z) at molar ratio of 0.1:1.

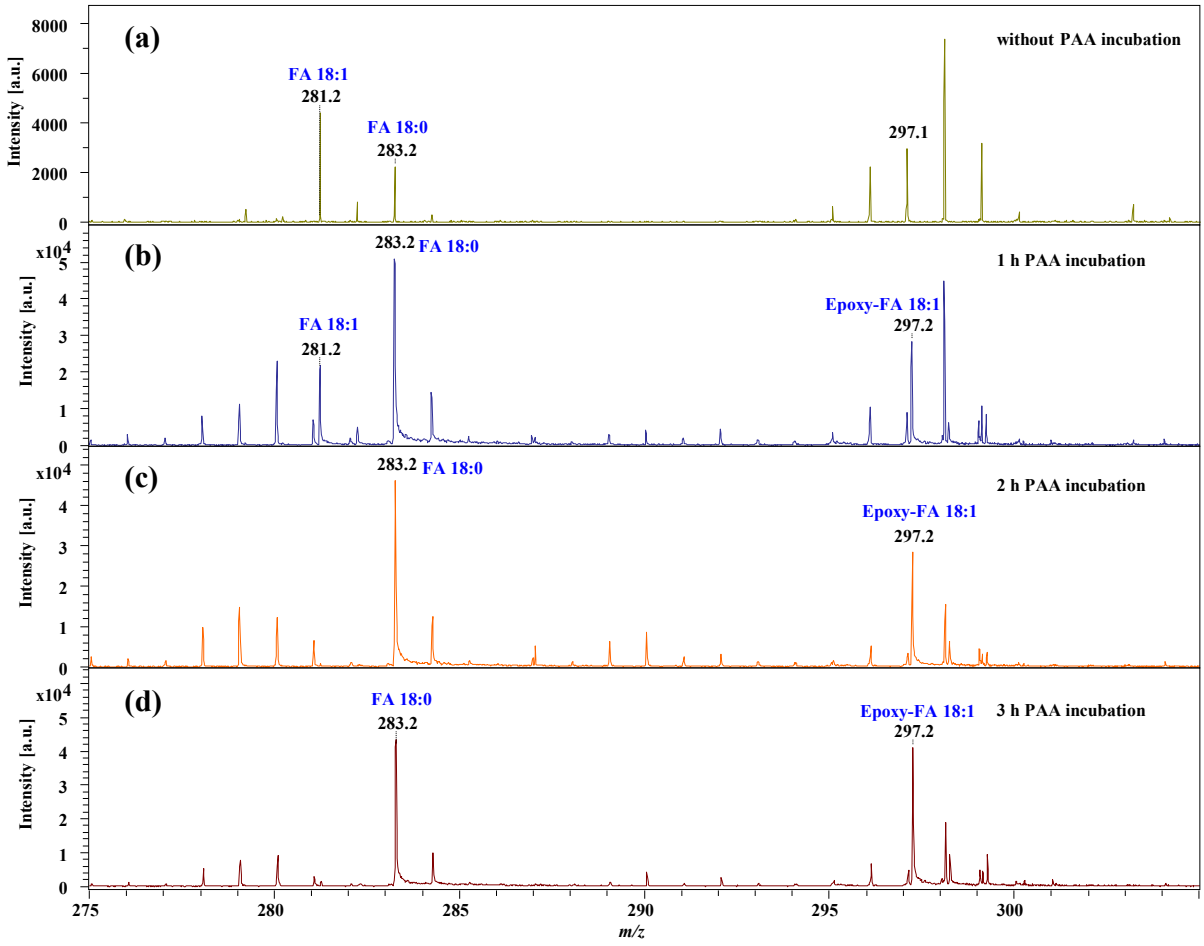

Figure S8. Optimization of PAA incubation time: (a) MALDI-TOF-MS spectrum of fatty acids in cancer tissue without the on-tissue PAA epoxidation treatment, (b) MALDI-TOF-MS spectrum of fatty acids in cancer tissue with 1 h PAA incubation, (c) MALDI-TOF-MS spectrum of fatty acids in cancer tissue with 2 h PAA incubation, (d) MALDI-TOF-MS spectrum of fatty acids in cancer tissue with 3 h PAA incubation. All the mass spectra were obtained by spotting analysis of the same tissue region from sequential tissue section samples.

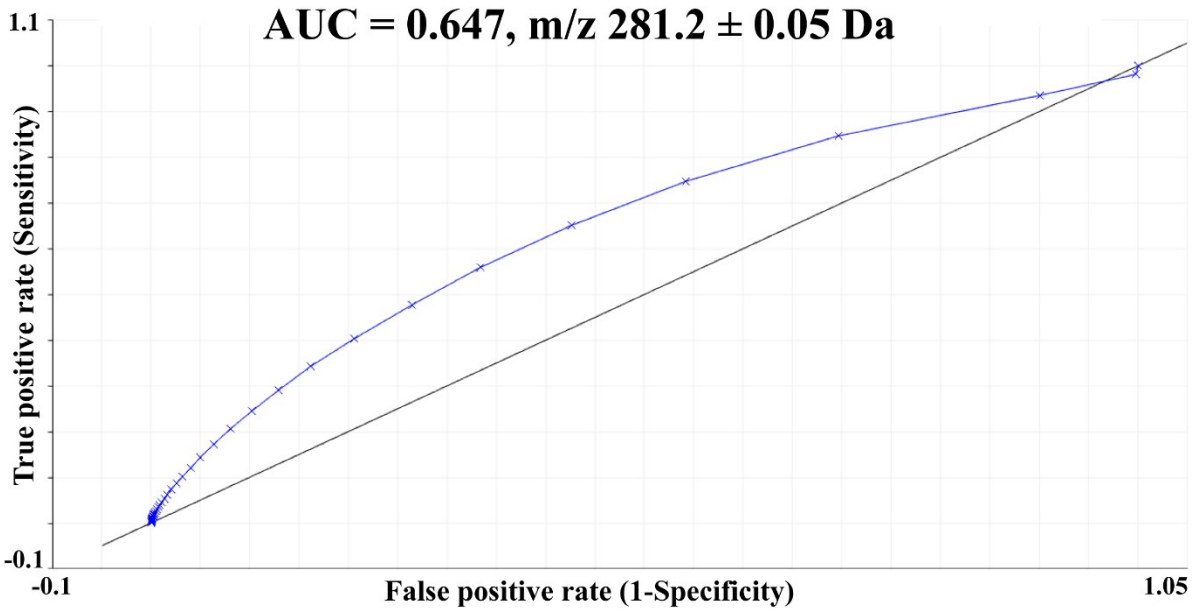

Figure S9. ROC curve of control sample plotted against radiated tumor sample at *m/z* 281.2, representing unepoxidated FA 18:1.

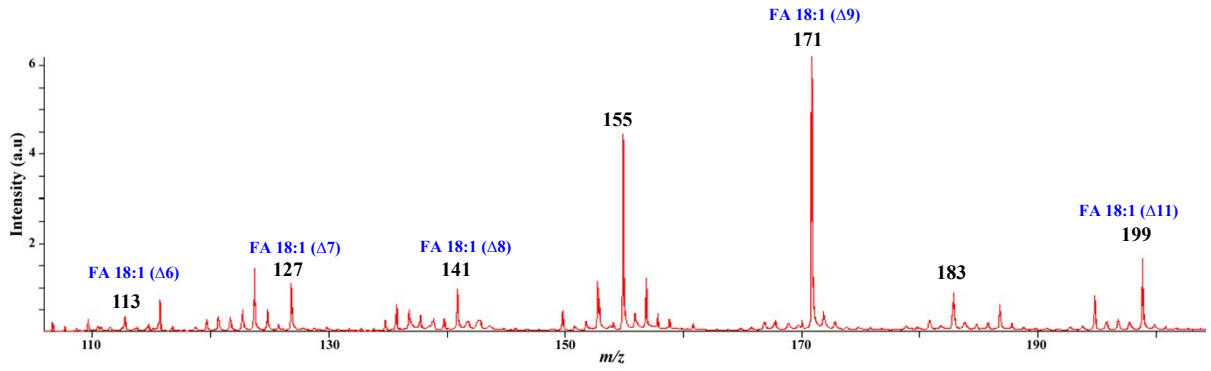

Figure S10. MALDI-TOF/TOF tandem MS of target precursor ion of *m/z* 297.2 from tissue section sample after on-tissue epoxidation derivatization.

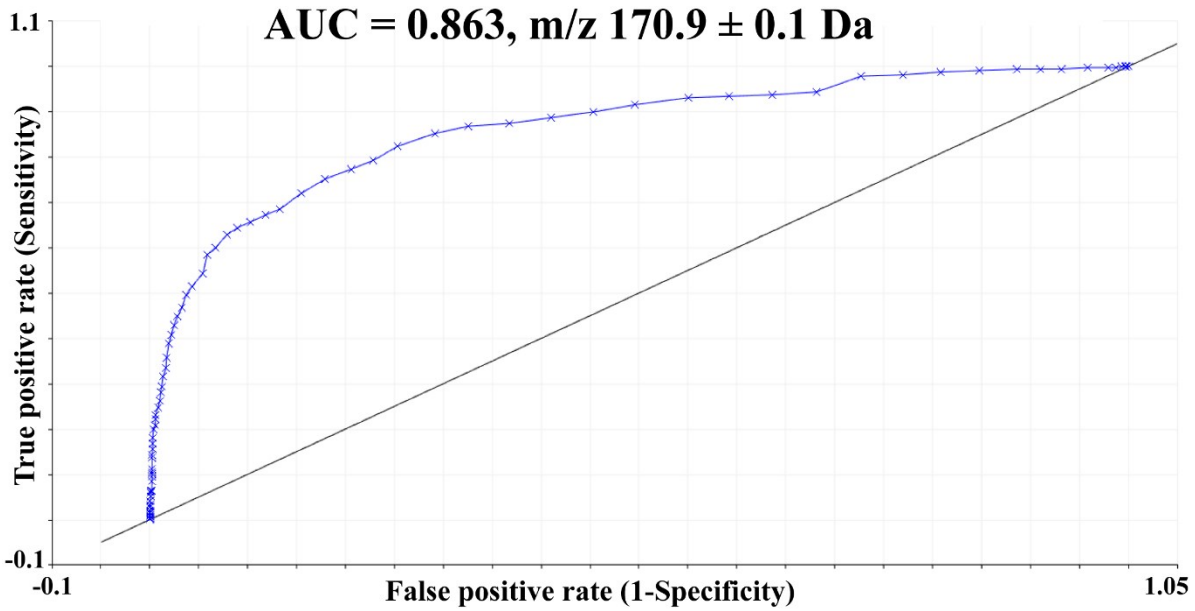

Figure S11. ROC curve of featured cancerous regions from control plotted against that in the radiated tumor sample at *m/z* 170.9, corresponding to FA 18:1 (Δ9). This fragment was obtained from epoxidated FA 18:1 via MALDI-TOF-TOF-MS^2 imaging.

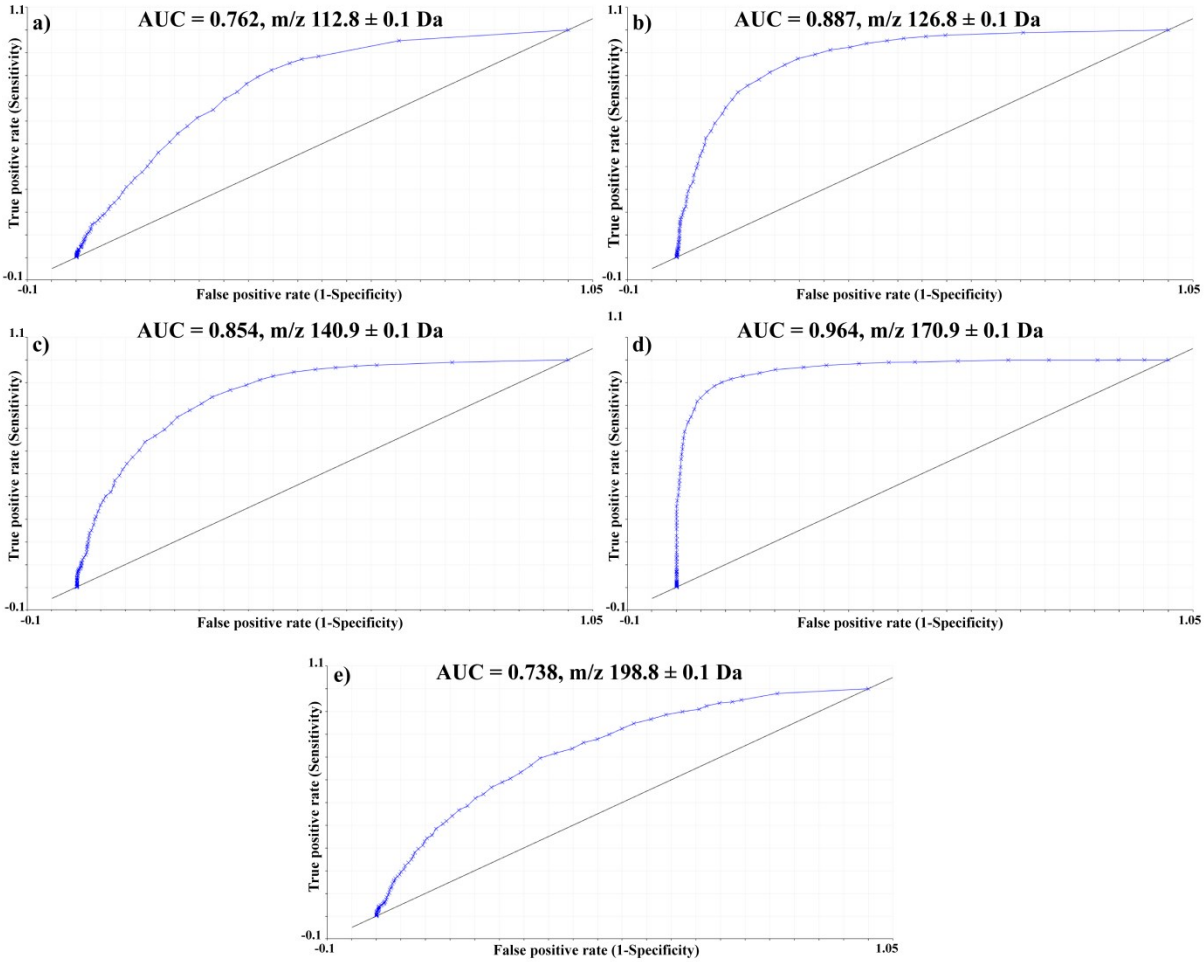

Figure S12. ROC curves of outer cancerous regions plotted against inner necrotic regions from both radiated sample and control at ion peaks representing: (a) FA 18:1 (Δ6), (b) FA 18:1 (Δ7), (c) FA 18:1 (Δ8), (d) FA 18:1 (Δ9), and (e) FA 18:1 (Δ11).

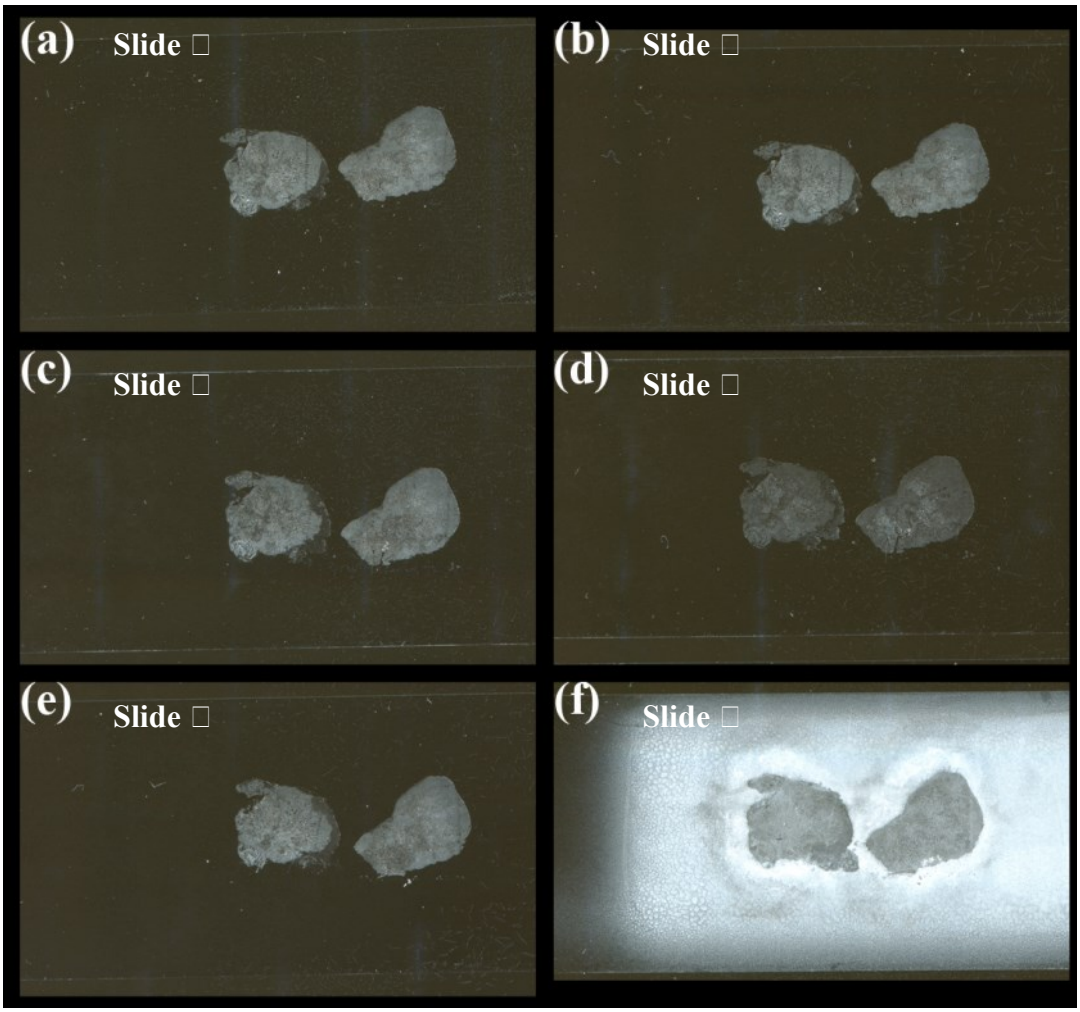

Figure S13. Optical images of tumor tissue section samples before and after on-tissue epoxidation treatments. Slides Ⅰ, Ⅱ, and Ⅲ are consecutive tissue sections. Slide Ⅰ ((a) and (b)) is a control group without any on-tissue epoxidation treatment; (c) and (d) are optical images of Slide Ⅱ taken before and after PAA vapor treatment, respectively; (e) and (f) are optical images of Slide Ⅲ taken before and after *m*-CPBA treatment, respectively.

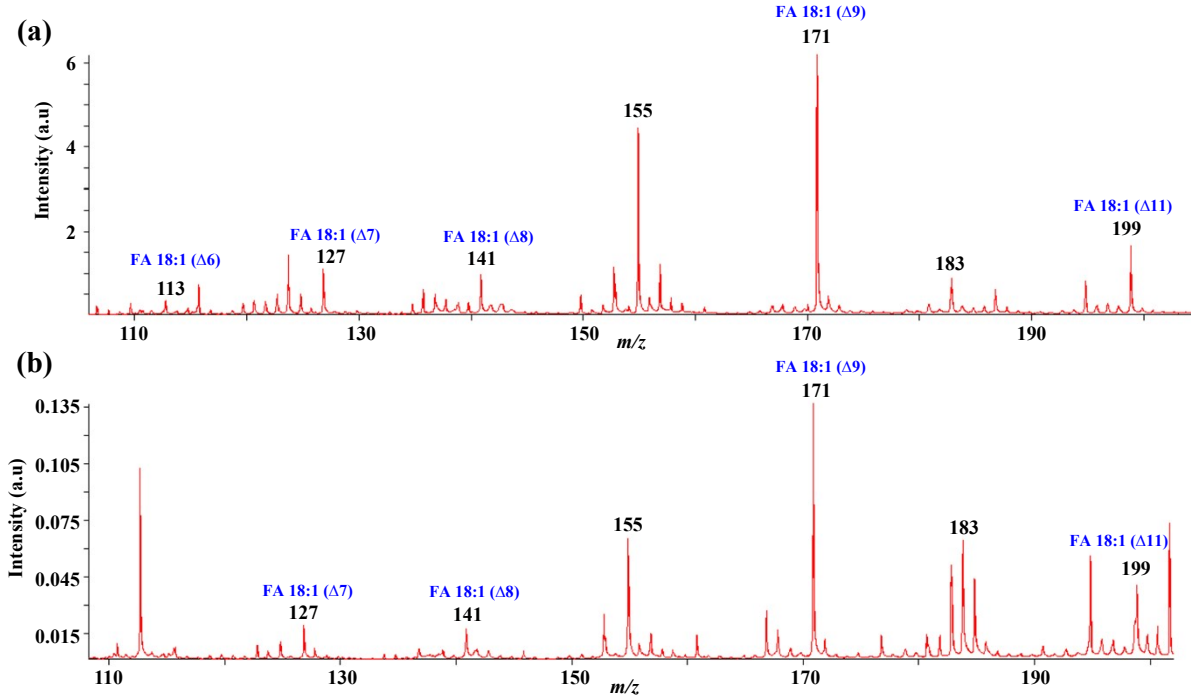

Figure S14. MALDI-TOF/TOF tandem MS of target precursor ion of *m/z* 297.2 from tissue section sample after (a) on-tissue PAA epoxidation derivatization and (b) on-tissue *m*-CPBA epoxidation derivatization.

Table S1. Monounsaturated C=C fatty acid isomers detected from cell samples

Fatty acids	Isomers	Detected mass of diagnostic fragments	Theoretical mass of diagnostic fragments	Error (ppm) [a]
FA 14:1	Δ7	127.07588	127.07590	0.13
		143.07083	143.07081	0.14
	Δ9	155.10703	155.10720	1.06
		171.10210	171.10211	0.06
	Δ10	169.12226	169.12285	3.48
		185.11813	185.11776	2.00
	Δ11	183.13797	183.13850	2.87
		199.13324	199.13341	0.84
FA 16:1	Δ6	113.06012	113.06025	1.11
		129.05499	129.05516	1.29
	Δ7	127.07574	127.07590	1.20
		143.07063	143.07081	1.23
	Δ8	141.09130	141.09155	1.72
		157.08629	157.08646	1.04
	Δ9	155.10701	155.10720	1.17
		171.10182	171.10211	1.64
	Δ10	169.12274	169.12285	0.64
		185.11757	185.11776	0.98
	Δ11	183.13827	183.13850	1.21
		199.13325	199.13341	0.78
	Δ12	197.15397	197.15415	0.88
		213.14896	213.14906	0.44
	Δ13	211.16976	211.16980	0.17
		227.16466	227.16471	0.18
FA 18:1	Δ6	113.06033	113.06025	0.72
		129.05520	129.05516	0.35
	Δ7	127.07600	127.07590	0.86
		143.07080	143.07081	0.03
	Δ8	141.09157	141.09155	0.14
		157.08649	157.08646	0.20
	Δ9	155.10722	155.10720	0.17
		171.10215	171.10211	0.25
	Δ10	169.12297	169.12285	0.75
		185.11791	185.11776	0.84
	Δ11	183.13855	183.13850	0.31
		199.13351	199.13341	0.51
	Δ12	197.15430	197.15415	0.79
		213.14924	213.14906	0.86

	Δ13	211.16989	211.16980	0.44
		227.16485	227.16471	0.63
	Δ14	225.18562	225.18545	0.79
		241.18062	241.18036	1.09
	Δ15	239.20140	239.20110	1.28
		255.19651	255.19601	1.99
FA 20:1	Δ7	127.07596	127.07590	0.48
		143.07072	143.07081	0.57
	Δ8	141.09159	141.09155	0.32
		157.08640	157.08646	0.33
	Δ9	155.10719	155.10720	0.01
		171.10213	171.10211	0.16
	Δ10	169.12289	169.12285	0.24
		185.11785	185.11776	0.52
	Δ11	183.13863	183.13850	0.71
		199.13350	199.13341	0.47
	Δ13	211.16979	211.16980	0.05
		227.16504	227.16471	1.48
FA 22:1	Δ6	113.06024	113.06025	0.05
		129.05530	129.05516	1.14
	Δ7	127.07602	127.07590	0.97
		143.07087	143.07081	0.43
	Δ8	141.09180	141.09155	1.79
		157.08662	157.08646	1.04
	Δ9	155.10743	155.10720	1.49
		171.10229	171.10211	1.10
	Δ10	169.12286	169.12285	0.07
		185.11793	185.11776	0.96
	Δ11	183.13893	183.13850	2.38
		199.13345	199.13341	0.23
	Δ12	197.15442	197.15415	1.39
		213.14933	213.14906	1.27
	Δ13	211.16983	211.16980	0.18
		227.16475	227.16471	0.22
	Δ15	239.20144	239.20110	1.45
		255.19637	255.19601	1.42

[a] Error = (abs(experimental mass - theoretical adduct mass)/ theoretical adduct mass)*1000000