Supporting information for

Double Derivatization Strategy for High-Sensitivity and High-Coverage Localization of Double Bonds in Free Fatty Acids by Mass Spectrometry

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FA standards, including palmitoleic acid (C16:1(9Z)), heptadecenoic acid (C17:1(10Z)), oleic acid (C18:1(9Z)), vaccenic acid (C18:1(11Z)), linoleic acid (C18:2 (9Z, 12Z)), α-linolenic acid (C18:3(9Z, 12Z, 15Z)), γ-linolenic acid (C18:3 (6Z, 9Z, 12Z)), cis-gondoic acid (C20:1(11Z)), 11,14-eicosadienoic acid (C20:2(11Z, 14Z)), 8, 11, 14- eicosatrienoic acid (C20:3(8Z, 11Z, 14Z)), arachidonic acid (C20:4 (5Z, 8Z, 11Z, 14Z)), eicosapentaenoic acid (EPA, C20:5(5Z, 8Z, 11Z, 14Z, 17Z)), erucic acid (C22:1(13Z)), docosahexenoic acid (DHA, C22:6(4Z, 7Z, 10Z, 13Z, 16Z, 19Z)), nervonic acid (C24:1(15Z)) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 7,7,8,8-palmitic acid-d4 (C16:0-d4) was purchased from Cambridge Isotope Laboratories (Andover, MA). The FA standards were used without further purification.

Mice Models and Collection of Liver Samples

Twenty-four male C57BL/6J mice were used in this study. Five weeks old male mice were purchased from Sino British Sippr/BK Animal Co. Ltd. (Shanghai, China). All mice were kept in Tongji Medical College, Huazhong University of Science and Technology (Wuhan, China). The mice were randomly assigned to two dietary groups (n=12/group) and fed on (1) high-fat diet containing 5.31 kcal/g (60% of energy from fat, nonalcoholic fatty liver disease group (NAFLD)); (2) low-fat diet containing 3.88 kcal/g (10% energy from fat, healthy normal control group (Normal)). Detailed composition of the diets were described in previous reports.[1,2] All mice were housed in a temperature regulated room (21–23 °C), with a 12 h light/dark cycle and allowed free access to water and food. After 10 weeks, the mice were fasted overnight and sacrificed. Liver tissues were harvested and stored at −80 °C prior to analysis. All animal procedures were performed in accordance with “Guiding Principles in the Care and Use Animals” approved by Tongji Medical College Council on Animal Care Committee (SCXK 2014-0004).

Sample Preparation

Liver tissues were ground into fines by a frozen tissue grinder (JXFSTPRP-I, Jingxin Industrial Development Co., Ltd, Shanghai, China). About 20 mg tissue fines were weighed and homogenized in 1mL Dulbecco's Phosphate Buffered Saline (dPBS). A protein assay of the homogenates was performed by using a bicinchoninic acid protein assay kit (Sangon Biotech Co., Ltd, Shanghai, China) with bovine serum albumin as standards. All determined FFAs levels were normalized to the protein content of individual samples.

Ten microlitres (10 μL) of individual homogenate of the liver samples was accurately transferred into a 16mm×100mm glass tube. Twenty-five microlitres (25 μL) internal standard mixture containing 7,7,8,8-palmitic acid-d4 (C16:0-d4) and 10Z-heptadecenoic acid (C17:1(10Z)), both at 4 nmol/L, was added prior to FFAs extraction for quantitation of saturated FFAs and unsaturated FFAs, respectively. FFAs were extracted from liver samples following the extraction protocol from LIPID MAPS (www.lipidmaps.org/protocols/PP0000005301.pdf), with a slight modification:

1) One hundred microlitres (100 μL) dPBS was added into the homogenate of liver samples, then 900 μL methanol was added and acidified with 50 μL (0.5 mol/L) HCl to 25 mM final concentration. Samples were stored at -20 °C for 2 hours to improve protein precipitation.
2) Three milliliters (3 mL) isooctane was added, and the sample was vortexed and centrifuged at 3000 x g for 1 minute to separate layers. The top layer was collected and transferred to another 16mm × 100 mm glass tube.
3) Step 2 was repeated twice.
4) The top layers were combined, and the extract was dried under nitrogen stream.

For each sample, all experiments were carried out independently in triplicate.

DEEA Further Labeling of Acetone-labeled FAs

Dried acetone-labeled sample was dissolved in 880 μL ACN, to which 30 μL of

TEA (20 μmol/mL) and 30 μL of CMPI (20 μmol/mL) were sequentially added, and the sample was mixed through vortexing for 1 min. 60 μL of DEEA (20 μmol/mL) was added to label FFAs. The derivatization reaction was performed with an ultrasonic water bath for 10 min at 40 °C, followed by the evaporation under nitrogen stream and then 1 mL chloroform was added to redissolve the residue. After that, liquid-liquid extraction was performed to remove the excess CMPI and DEEA, which would interfere with the ionization process of MS, resulting in lower peak intensity of analyte and contamination of ion source. Two milliliters (2 mL) formic acid-water (10:90, v/v) was added into the chloroform solution, and then vortexed for 5 min and allowed to stand to separate into layers, after that, the upper formic acid-water phase containing unreacted CMPI and DEEA was removed. This step was repeated for three times to completely get rid of the remaining catalyst and DEEA. Finally, the chloroform phase was evaporated under a nitrogen gas. The residue was redissolved in 1 mL ACN and subjected to MS analysis.

MS Analysis

Neutral loss scan (NLS) 73 Da and enhanced product-ion (EPI) scan in positive-ion mode were used for quantitative and qualitative analysis of double-labeled FAs, respectively. Instrument parameters were optimized to obtain the highest $[M+H]^+$ ion abundance by infusing double-labeled FA standard solutions via a syringe pump into MS with an optimal flow of 10 μL/min. The optimized MS parameters were as follows: collision gas pressure set at high; curtain gas pressure, 30.0 psi; nebulizer pressure, 45 kPa; temperature, 450 °C; ion source gas 1 (nebulizer gas), 20 psi; ion source gas 2, 20 psi; declustering potential (DP), 40 V. The mass resolution of Q1 and Q3 was set to “unit”. The Q1 mass range was set to m/z 50-600. Scan time per cycle was 2.0 s with a pause of 5.0 ms for each scan. For NLS 73 Da, the optimized collision energy (CE) was 45 V. For EPI, CEs of 35-50 V were used. Channel electron multiplier (CEM) (positive), 2400 V.

NLS 58 Da and EPI scan in negative-ion mode were used for analysis of acetone-labeled FAs, respectively. The optimized source/gas parameters and the

compound parameters were as follows: curtain gas pressure, 20.0 psi; collision gas, high; nebulizer pressure, 45 kPa; temperature, 450 °C; ion source gas 1 (nebulizer gas), 20 psi; ion source gas 2, 20 psi; DP, 40 V. For NLS 58 Da, the optimized CE was 35 V. For EPI, CEs of 35-45 V were used. CEM (negative), 2300 V.

Qualitative and Quantitative Analysis

For qualitative analysis of FFAs, firstly, by applying NLS 73Da, carbon number and degree of unsaturation of FFA could be clearly identified. Then, assignment of C=C bond locations was achieved according to diagnostic ions in the MS/MS spectrum. Each pair of diagnostic ions has generic chemical formulas of $C_{(x+2)}H_{(2x-2a+4)}O_2N^+$ (aldehydes) and $C_{(x+5)}H_{(2x-2a+10)}ON^+$ (isopropenes), respectively (x: C=C location(s) according to the Δ-nomenclature, a: the sequence number of C=C bond counted from methyl end). Thus, the C=C bond locations could be deduced according to the m/z value of diagnostic ions. Because this double derivatization strategy is unable to differentiate the cis or trans C=C configuration (E/Z), all FFAs identified in this work are not specified with C=C configuration.

C16:0-d4 and C17:1(10Z) was used for quantitation of saturated FFAs and unsaturated FFAs, respectively. For pure form FFAs, the intensities of labeled FFAs were compared with the intensities of internal standard to obtain the quantitative results. For FFA C=C positional isomers, based on the established calibration curves between abundance ratios of C=C diagnostic ions of the isomers and their isomer concentration ratios, molar ratios of FA isomers in real biological samples could be calculated.

orthoPLS-DA was performed on MetaboAnalyst 4.0 (https://www.metaboanalyst.ca/faces/home.xhtml).

Numbering of the carbon atoms and double bond locations in fatty acids

The location of the carbon atoms in a FA can be indicated from the -COOH end, or from the -CH_3 end. If indicated from the -COOH end, then the C-1, C-2, C-3, …, *etc*. notation is used (where C-1 is the -COOH carbon). If the location is counted from the other, -CH3, end then the position is indicated by the omega (ω)-x (or n-x) notation (where ω-1 refers to the methyl carbon). The locations of the double bonds in a FA chain can, therefore, be indicated in two ways, using the C-x or the ω-x notation. For an instance, in an 18 carbon FA, a double bond between C-11 (or ω-8) and C-12 (or ω-7) is reported either as Δ11 if counted from the -COOH end (indicating only the "beginning" of the double bond), or as ω-7 if counting from the -CH_3 end. IUPAC names employ Δ-counting method, and double bonds are labelled with cis(E)-/trans(Z)- notation.

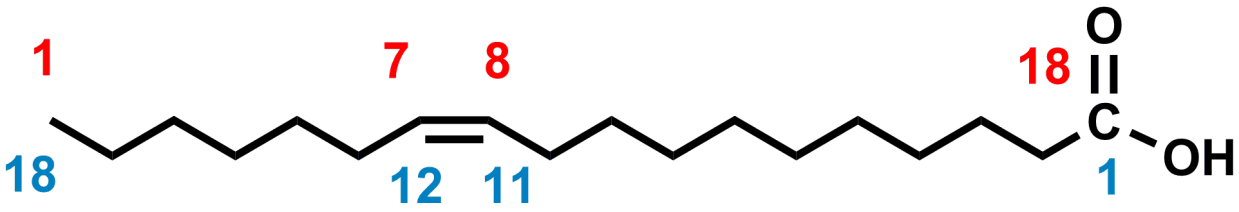

Figure S1. Numbering of the carbon atoms and double bond locations in C18:1(11Z)

Optimization of Derivatization Conditions

To achieve the best derivatization efficiency, six FA standards (C16:1(9Z), C18:1(9Z), C18:2(9Z, 12Z), C20:3(8Z, 11Z, 14Z), C20:5(5Z, 8Z, 11Z, 14Z, 17Z) and C22:6(4Z, 7Z, 10Z, 13Z, 16Z, 19Z)) at 50 nmol/L were used to optimize the reaction conditions. Acetone was used as the PB reagent for labeling of C=C bonds in FAs to form oxetane rings. The volume ratio of acetone/water in the PB reaction system was investigated. The results showed that the MS intensity of FA derivatives increased as the volume ratio of acetone/water increased from 5:5 to 6:4 (v/v), and then decreased (Figure S2a). Therefore, the volume ratio of 6:4 of acetone/water was used for the reaction. However, the PB reaction for PUFAs would lead to a sequential acetone labeling of multiple C=C bonds. This excessive labeling will reduce the degree of singly labeled products, which was useful for structural elucidation. The addition of ethanol (5%, v/v) in reaction solution would decrease competitive sequential PB reactions through photoreduction of electronically excited acetone.[3] In addition, the effects of reaction time and distance between the lamp and the quartz cuvette were also examined. The optimal derivatization efficiency was obtained when the reaction was under UV exposure for 60 min with the distance of 6 cm between the lamp and the quartz cuvette. Furthermore, the offline PB reaction allowed multiple samples to receive UV irradiation simultaneously, therefore contributing to the high throughput sample preparation and analysis. To further perform DEEA derivatization to label carboxyl group of acetone-labeled FAs, the molar ratio of CMPI/DEEA and the molar ratio of DEEA/FAs in the reaction solution were investigated. CMPI was utilized as an activator to selectively activate carboxyl groups to form a reactive ester, and then amines from DEEA attacked the reactive ester quickly to form a stable product with a tertiary amino group.[4] The results showed that the highest intensities of double-labeled FAs could be achieved when the molar ratios of CMPI/DEEA and DEEA/FAs were set at 1:2 and 400, respectively (Figure S2 (b, c)). In addition, the effects of reaction time and temperature were also examined. As shown in Figure S2 (d, e), the highest intensities of maximum double-labeled FAs were obtained when the reaction was performed with an ultrasonic water bath for 10 min at 40 °C.

Taken together, PB reaction was conducted in acetone/water (6:4, v/v) containing 5% ethanol (v/v), and UV exposure for 60 min with the distance of 6 cm between the lamp and the quartz cuvette. Then further DEEA derivatization reaction was performed in an ultrasonic water bath for 10 min at 40 °C, with the molar ratios of CMPI/DEEA and DEEA/FAs set at 1:2 and 400, respectively.

Under optimized reaction conditions, acetone labeling could be achieved with 20-50% yield in PB reaction. The result is consistent with previous reports.[3,5] For further DEEA labeling reaction, almost all of the PB reaction products can react with DEEA to form the corresponding double-labeled FAs (remaining PB reaction products are below the detection limits in negative ion mode). The extremely high yields of conversion for chemical labeling between carboxylic group and other similar derivatization reagents were also reported in many other studies.[6,7] Taken together, yield of double derivatization could be achieved with 20%-50% (e.g., ~42% of C18:2 (9Z, 12Z) (Figure S3)).

As Ma *et al*. reported, PB reaction coupled with MS/MS allowed confident assignment of C=C locations in an unsaturated FA from a complex mixture with a moderate yield (~20−60%).[3,5] In this double labeling strategy, DEEA was employed to further react with carboxyl groups of acetone-labeled FAs, converting primary amines into tertiary amino group, invoking a readily protonated tertiary amine group on acetone-labeled FAs and thereby increasing proton affinity. Thus, a significant advantage of the introduction of DEEA labeling for PB reaction products is that signal enhancement of FFAs in positive mode is combined with the ability to assign double bond positions. Incomplete PB reaction did not hinder the confident aggignment of C=C locations of FFAs due to the high detection sensitivity obtained after further DEEA derivatization. Therefore, this double derivatization strategy enables unambiguous and sensitive determination of C=C locations in an unsaturated FA with a moderate yield (20−50%).

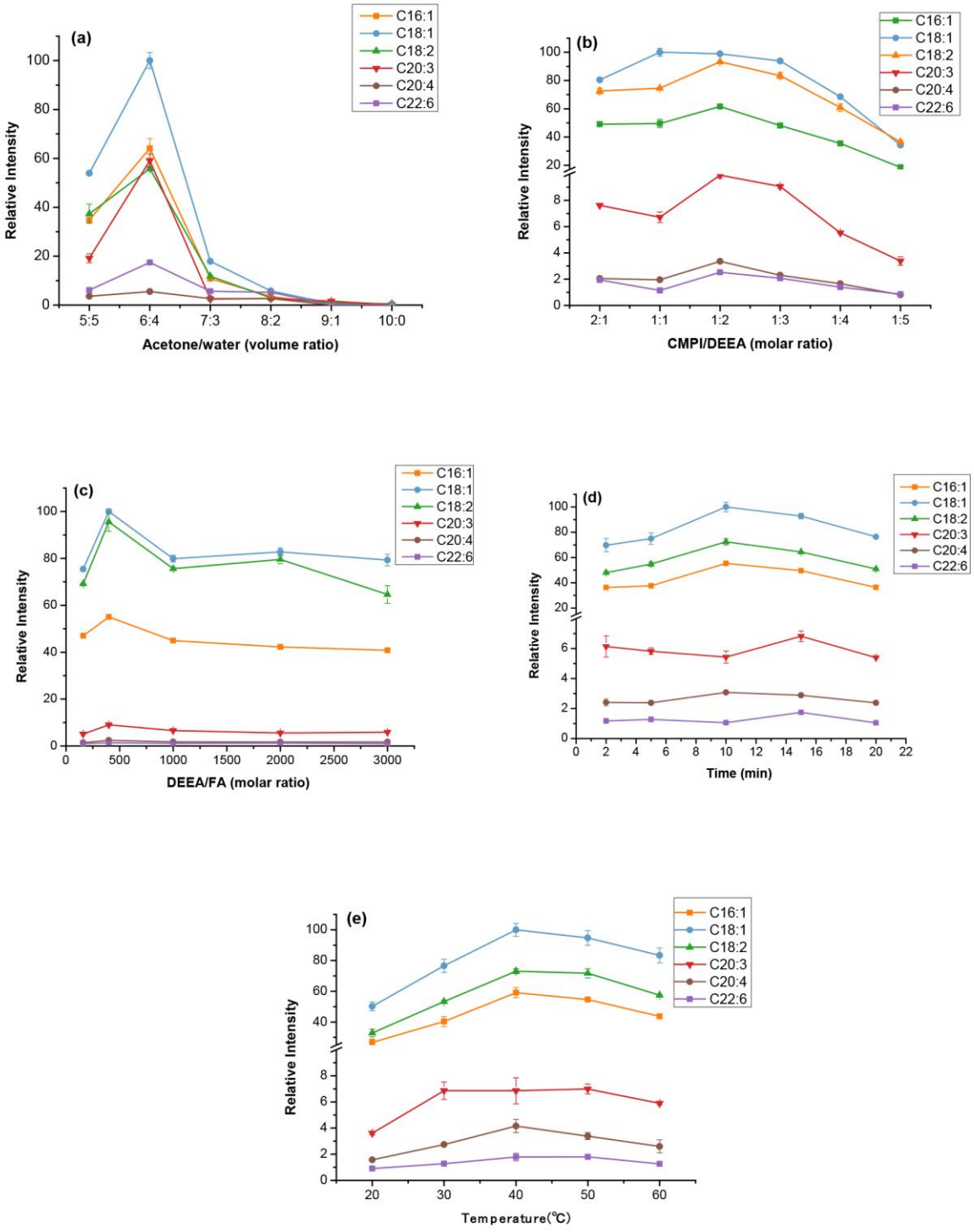

Figure S2. Optimization of derivatization conditions. The effect of the volume ratio of acetone/water for labeling of C=C bonds in FAs (a); the effects of the molar ratio of CMPI/DEEA (b), molar ratios of CMPI/FAs (c), reaction time (d), and reaction temperature (e) for further labeling of carboxyl group of acetone-labeled FAs.

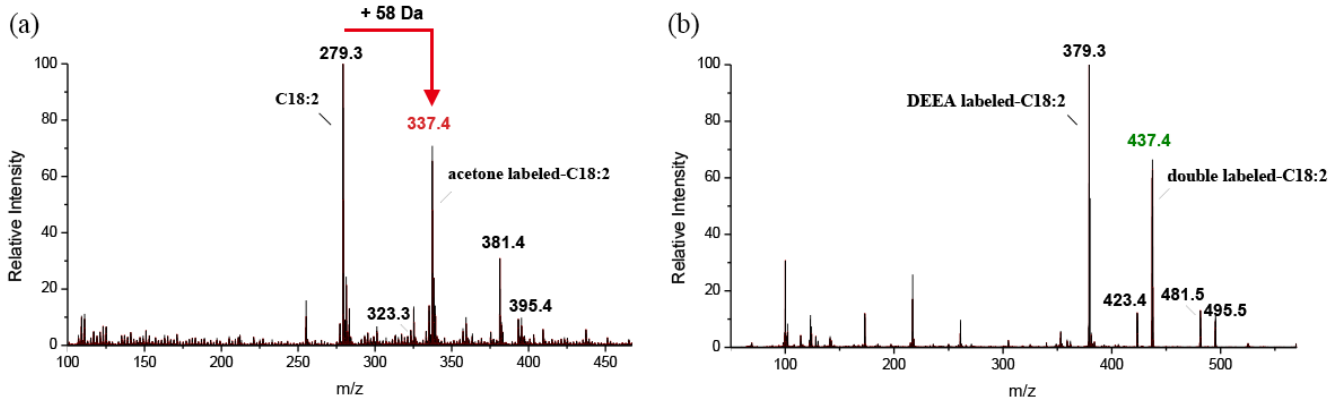

Figure S3. (a) Full scan mass spectrum of FA C18:2 (9Z, 12Z) in negative ion mode after acetone labeling; (b) full scan mass spectrum of FA C18:2 (9Z, 12Z) in positive ion mode after double labeling. Because acetone labeling could be achieved with 20-50% yield in PB reaction, PB reaction products, as well as the remaining FAs can react with DEEA, forming the double-labeled FAs and DEEA labeled FAs, respectively.

A small extent of side reactions e.g., Norrish reactions (forming ions at m/z 323, m/z 381 of C18:2(9Z, 12Z)) were inevitable in PB reactions (Figure S3a), which were also observed in double derivatization (forming ions at m/z 423, m/z 481 of C18:2(9Z, 12Z) (Figure S3b)). As Ma reported, Norrish reactions were found to be competitive for acetone labeling, which interfered with the detection and quantitation of UFAs. An addition of ethanol into the reaction solvent system could reduce this side reaction, for ethanol could be used to slow down PB reactions through photoreduction of electronically excited acetone.[3] Thus, in this double derivatization, 5% ethanol was added in the reaction solution to slow down PB reactions and reduce side reactions. In addition, oxidization would occur when the solution contains trace O_2, which will also significantly impair lipid detection and quantitation. In previous report, a higher PB reaction yield was achieved after purging the lipid solution with N_2 to remove trace O_2.[8] Therefore, in this study the reaction solution was also purged with N_2 to eliminate dissolved trace O_2 to reduce FA oxidation.

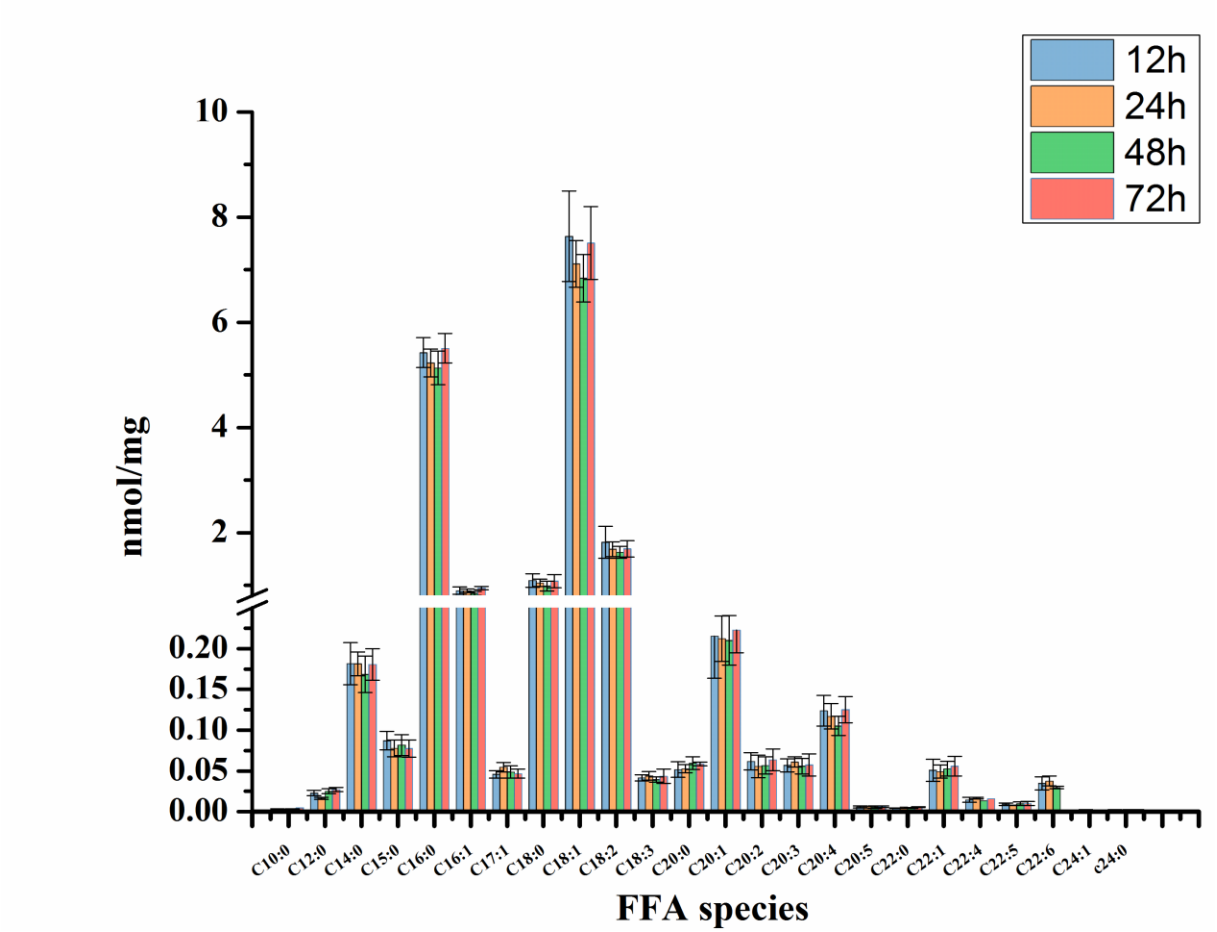

Figure S4. Stability of double-labeled FFAs. Stability of double-labeled FFAs was evaluated by analysis of the quality control (QC) samples over 72 h (4 °C). QC samples were composed of a mixture of each biological sample.

The results showed that the double derivatization products were stable for at least 72 h at 4 °C, which was sufficient for the subsequent analysis.

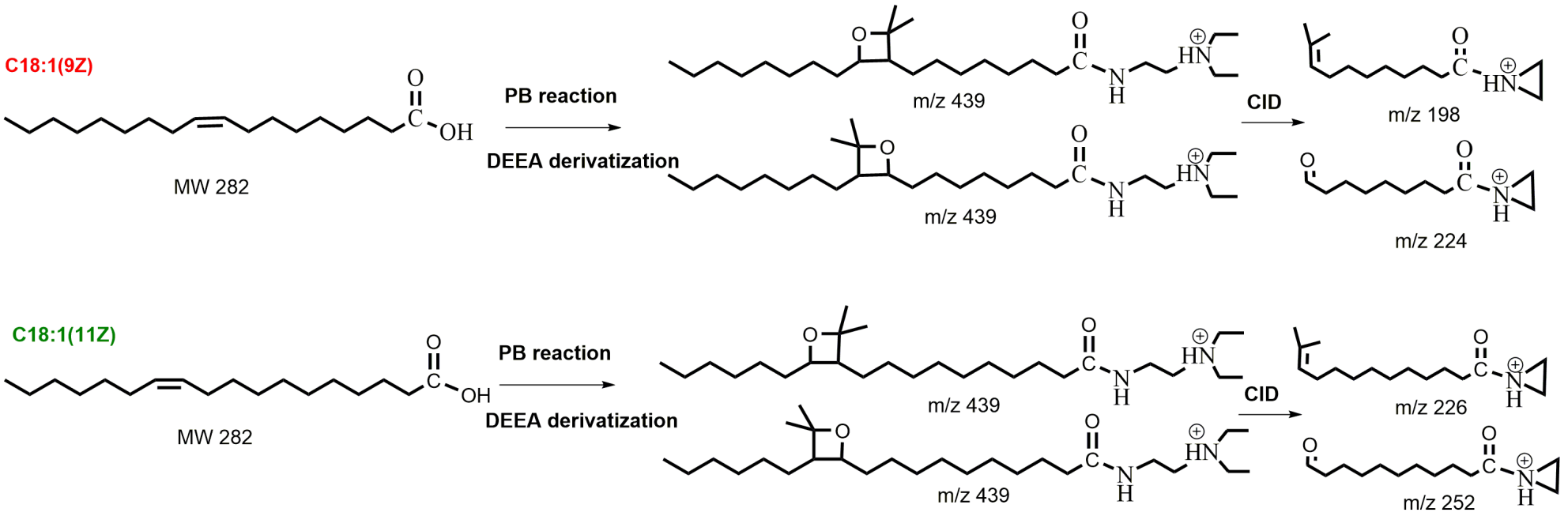

Figure S5. Schemes explaining the generation of double-labeled FA C18:1(9Z) and C18:1(11Z), and the formation of diagnostic ions under CID. In C18:1(9Z), acetone reacted with C=C bond, two position isomers of oxetane ring produced depending on the locations of the carbonyl and the C=C bond, and DEEA further labeled carbonyl group to form tertiary amino group compounds. Two protonated position isomers derivatives (*m/z* 439) were fragmented under CID to produce a pair of diagnostic ions (*m/z* 198/224). The same reaction occurs in C18:1(11Z) and generated another pair of diagnostic ions (*m/z* 226/252).

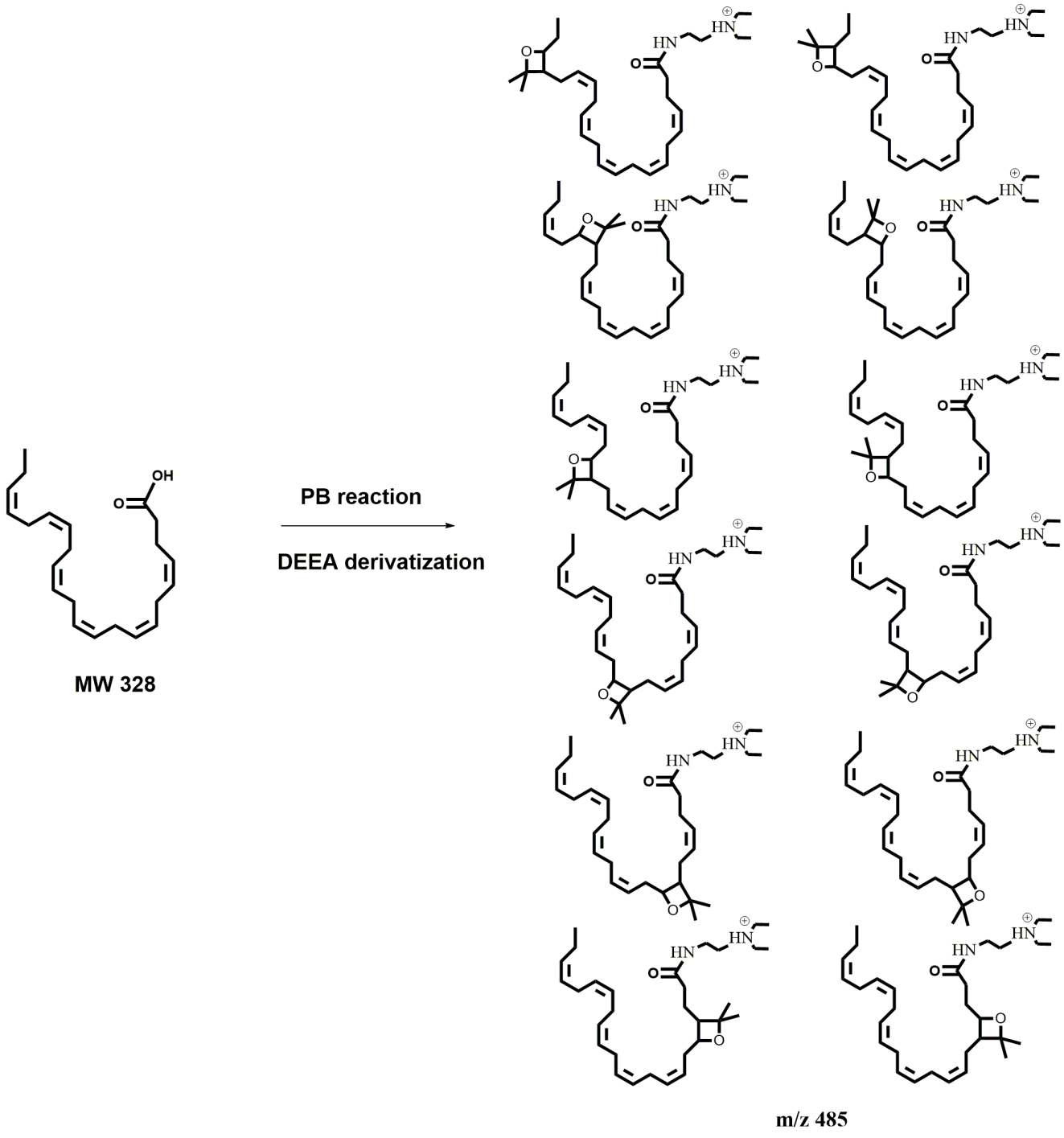

Figure S6. Schemes explaining the generation of double-labeled decosahexaenoic acid (DHA, C22:6(4Z, 7Z, 10Z, 13Z,16Z, 19Z)). Twelve position isomers of oxetane ring were produced depending on the locations of the carbonyl and the C=C bond after double labeling of DHA.

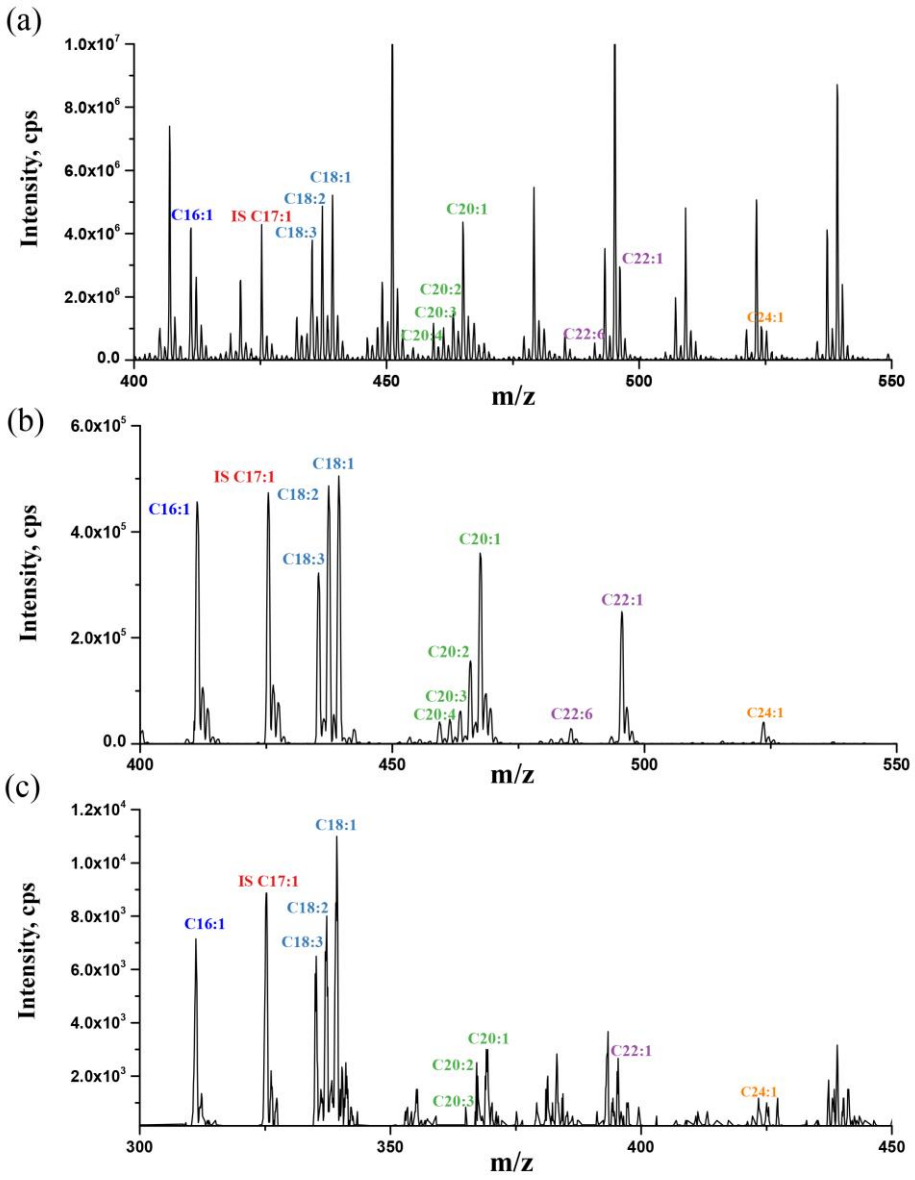

Figure S7. Mass spectra of labeled FA standards mixture. Double-labeled FA standards (50 nmol/L) analyzed by full MS scan in the positive-ion mode (a); double-labeled FA standards (50 nmol/L) analyzed by NLS 73 Da in positive-ion mode (b); and single acetone-labeled FA standards (500 nmol/L) analyzed by NLS 58 Da in negative-ion mode (c).

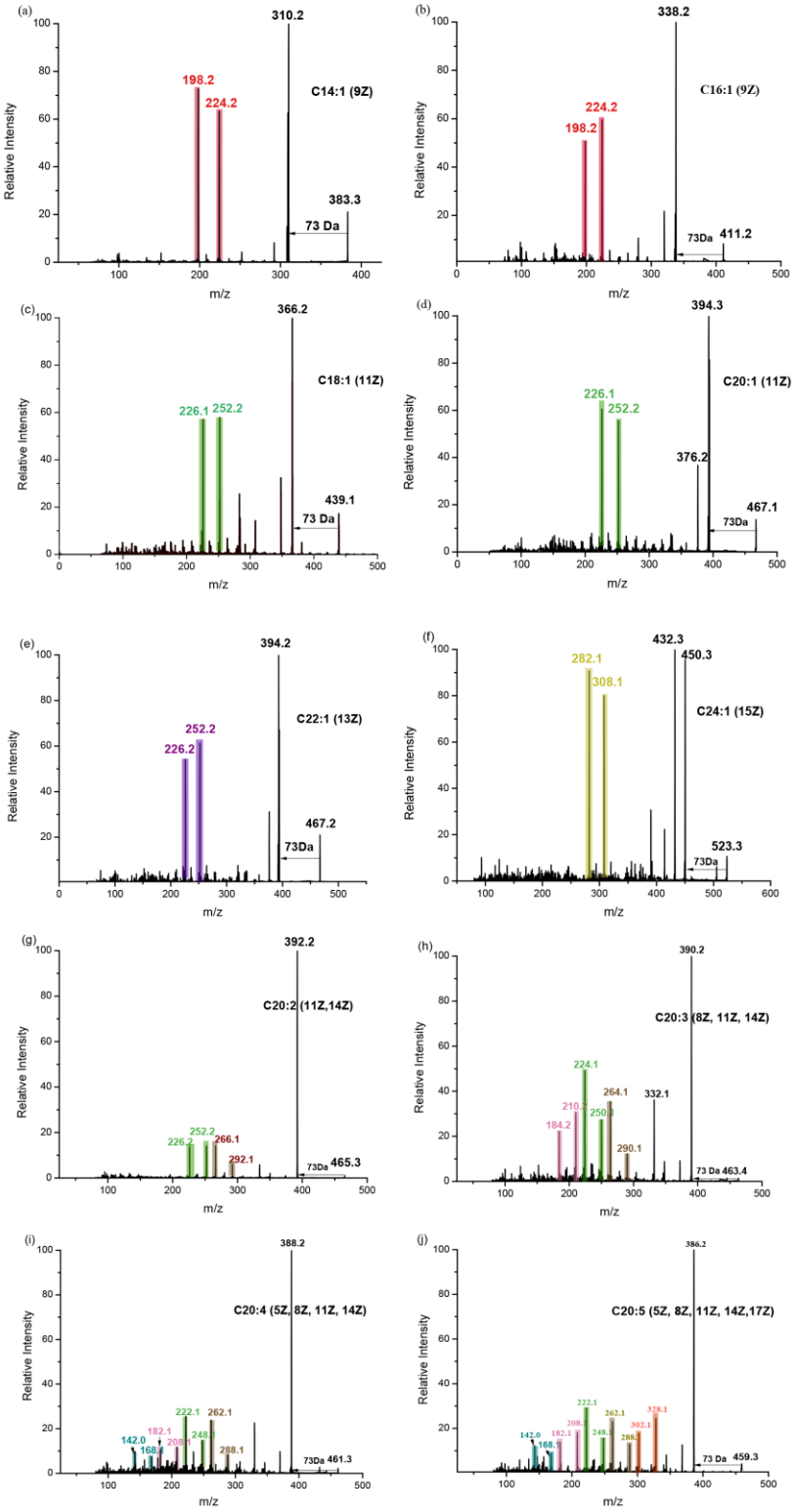

Figure S8. Tandem mass spectra of a series of double-labeled UFAs containing different chain lengths, double bond number and location(s). The product ion mass spectra presented in panels were double-labeled C14:1 (9Z) (a), C16:1(9Z) (b), C18:1 (11Z) (c), C20:1(11Z) (d), C22:1(13Z) (e), C24:1(15Z) (f), C20:2(11Z, 14Z) (g), C20:3(8Z, 11Z, 14Z) (h), C20:4(5Z, 8Z, 11Z, 14Z) (i), C20:5(5Z, 8Z, 11Z, 14Z, 17Z) (j).

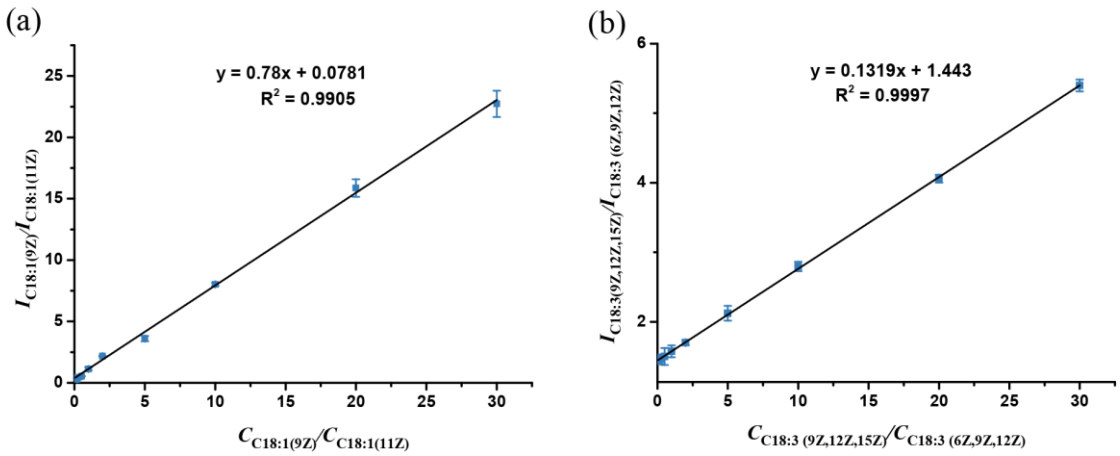

Figure S9. Linear relationship between intensity ratios of diagnostic fragmentation ions and molar ratios of FA isomers: C18:1(9Z) *vs* C18:1(11Z) (a), C18:3(9Z, 12Z, 15Z) *vs* C18:3(6Z, 9Z, 12Z) (b). The error bars were plotted according to standard errors determined in quadruplicates.

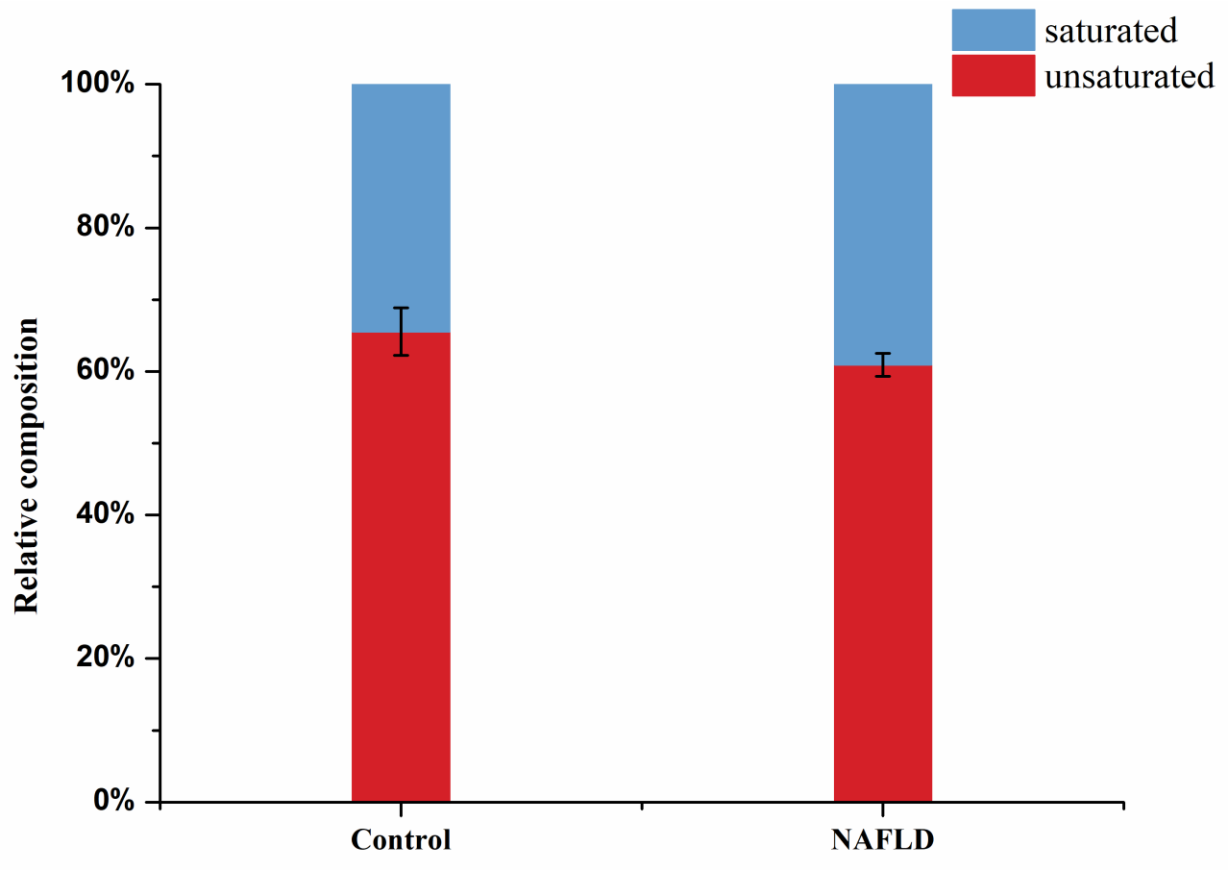

Figure S10. The composition of total saturated FFAs and total unsaturated FFAs in control and NAFLD mouse liver samples.

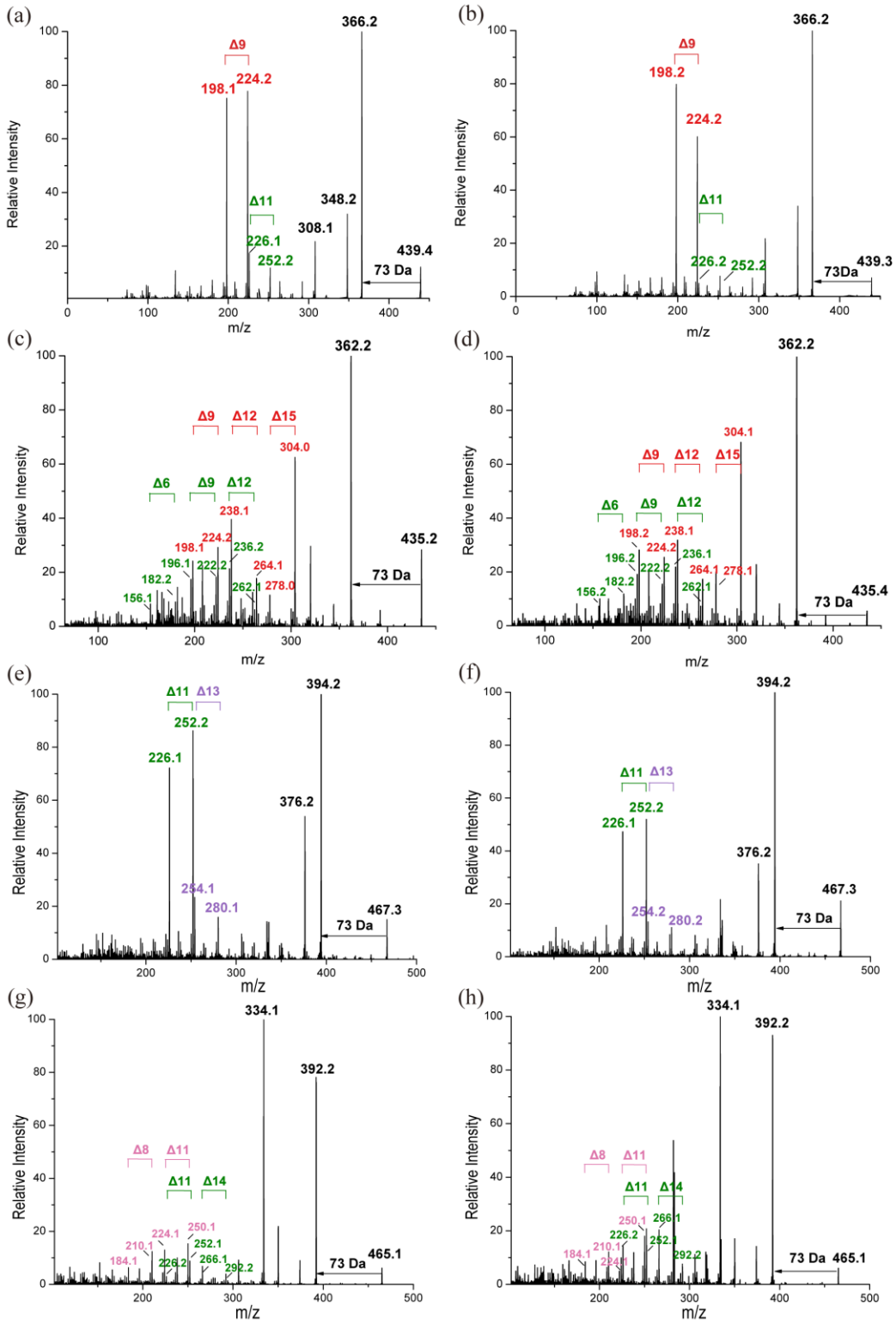

Figure S11. Identification of FFA C=C positional isomers in control and NAFLD groups. The tandem mass spectra of double-labeled FFA positional isomers in control and NAFLD mouse liver samples. C18:1 positional isomers in control (a) and NAFLD group (b); C18:3 positional isomers in control (c) and NAFLD group (d); C20:1 positional isomers in control (e) and NAFLD group (f); C20:2 positional isomers in control (g) and NAFLD group (h) respectively.

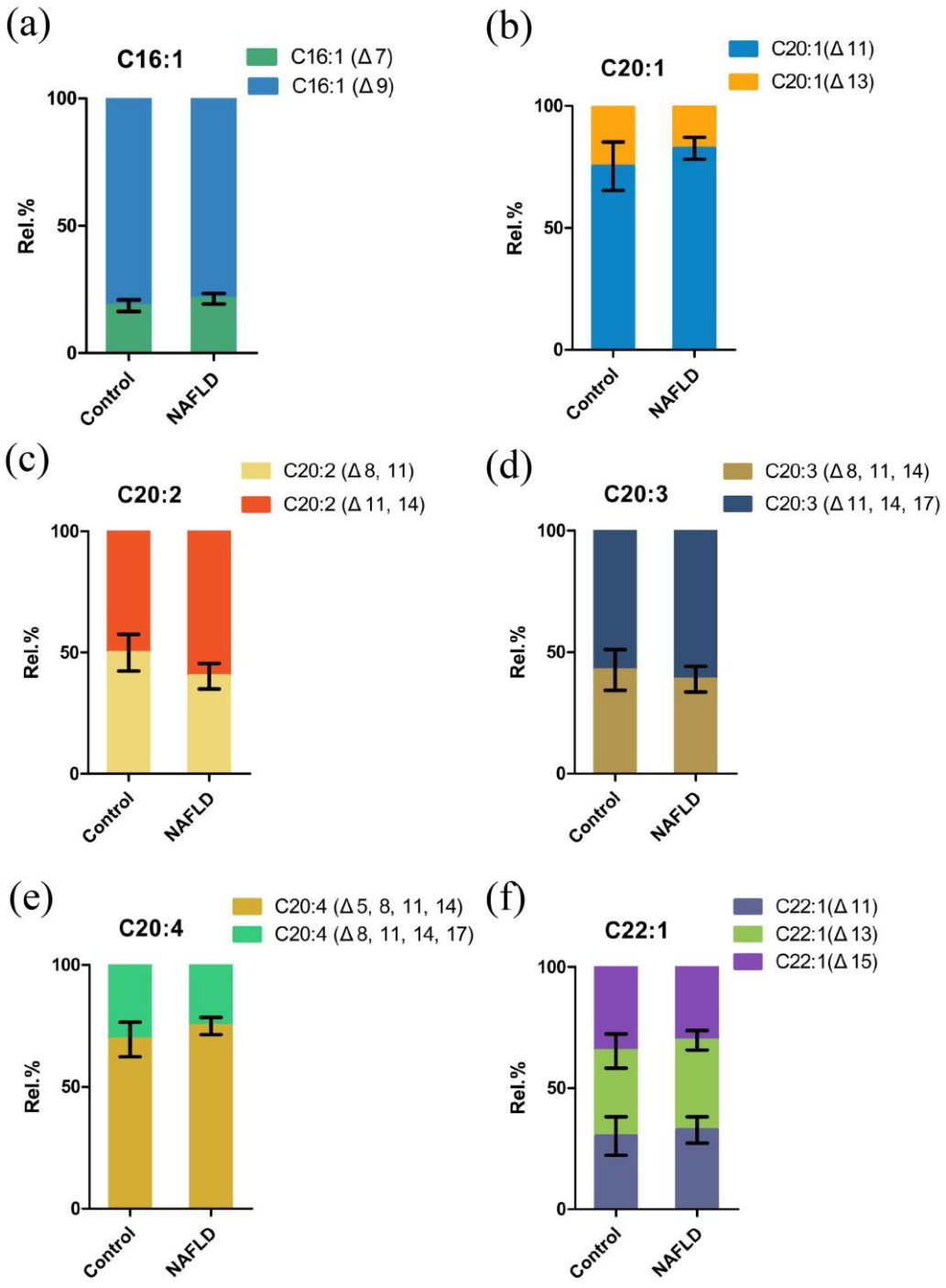

Figure S12. Comparison of the relative compositions of C=C location isomer of FFA C16:1 (a), C20:1 (b), C20:2 (c), C20:3 (d), C20:4 (e) and C22:1 (f) between control and NAFLD mice livers. There was no obvious trend in the changes of the isomer ratios in C16:1(Δ7/Δ9), C20:1(Δ11/Δ13), C22:1 (Δ11/Δ13/Δ15), C20:2 (ω-6/ω-9), C20:3(ω-3/ω-6) and C20:4 (ω-3/ω-6) between control and NAFLD mice. Differences between the two groups of samples were evaluated for statistical significance using the two-tailed student's t test.

Table S1. Calibration curves, LODs and LOQs for the analysis of FA standards by double derivatization method.

FA	Linearity	R^2	LOD (nmol/L)	LOQ (nmol/L)	Dynamic range (nmol/L)
C14:0	y = 0.2784x + 0.1015	0.9995	0.03	0.1	0.1-400
C16:0	y = 0.3332x + 0.2634	0.9963	0.03	0.1	0.1-400
C16:1(9Z)	y = 0.79066x - 0.2521	0.9989	0.03	0.1	0.1-400
C18:0	y = 0.3476 x + 0.2538	0.9943	0.03	0.1	0.1-400
C18:1(9Z)	y = 0.3677x + 0.0921	0.9997	0.03	0.1	0.1-400
C18:1(11Z)	Y=0.3891x+0.0993	0.9981	0.03	0.1	0.1-400
C18:2(9Z, 12Z)	y = 0.520x + 0.247	0.997	0.03	0.1	0.1-400
C18:3(9Z, 12Z, 15Z)	y = 0.3793x + 0.2735	0.9955	0.1	0.5	0.5-400
C18:3(6Z, 9Z, 12Z)	y=0.3273x+0.1177	0.9971	0.1	0.5	0.5-400
C20:1(11Z)	y = 0.530x + 0.304	0.9942	0.1	0.5	0.5-400
C20:2(11Z, 14Z)	y = 0.4268 x + 0.2566	0.994	0.2	0.8	0.8-400
C20:3(8Z, 11Z, 14Z)	y = 0.4845 x + 0.1983	0.9971	0.2	0.8	0.8-400
C20:4(5Z, 8Z, 11Z, 14Z)	y = 0.3181 x - 0.0276	0.9963	0.4	1.5	1.5-400
C20:5(5Z, 8Z, 11Z, 14Z, 17Z)	y = 0.3917 x - 0.0571	0.9977	0.4	1.5	1.5-400

C22:1 (13Z)	y = 0.6077 x + 0.2344	0.9901	0.1	0.5	0.5-400
C22:6(4Z, 7Z, 10Z, 13Z,16Z, 19Z)	y = 0.3924 x + 0.0264	0.9956	0.4	1.5	1.5-400
C24:1(15Z)	y = 0.3692 x - 0.0211	0.9935	0.1	0.5	0.5-400

C16:0-d4 and C17:1(10Z) was used for quantitation of saturated FFAs and unsaturated FFAs, respectively.

Table S2. Predicted diagnostic ions from double-labeled FAs.

FA	Double-labeled FAs	Aldehydic amide cation	Isopropenyl amide cation
C14:1(Δ9)	383	198	224
C16:1(Δ7)	411	170	196
C16:1(Δ9)	411	198	224
C17:1(Δ10)	425	212	238
C18:1(Δ9)	439	198	224
C18:1(Δ11)	439	226	252
C18:2(Δ9, 11)	437	198	224
		224	250
C18:2(Δ9, 12)	437	198	224
		238	264
C18:2(Δ10, 12)	437	212	238
		238	264
C18:3(Δ6, 9, 12)	435	156	182
		196	222
		236	262
C18:3(Δ9, 12, 15)	435	198	224
		238	264
		278	304
C20:1(Δ11)	467	226	252
C20:1(Δ13)	467	254	280
C20:1(Δ14)	467	268	294
C20:2(Δ11, 14)	465	226	252
		266	292
C20:2(Δ13, 16)	465	254	280
		294	320
C20:2(Δ14, 17)	465	268	294
		308	334
C20:3(Δ11, 14, 17)	463	226	252
		266	292
		306	332
C20:3(Δ8, 11, 14)	463	184	210
		224	250
		264	290
C20:4(Δ5, 8, 11, 14)	461	142	168
		182	208
		222	248
		262	288
C20:4(Δ8, 11, 14, 17)	461	184	210
		224	250

		264	290
		304	330
C20:5(Δ5, 8, 11, 14, 17)	459	142	168
		182	208
		222	248
		262	288
		302	328
C22:1(Δ11)	495	226	252
C22:1(Δ13)	495	254	280
C22:1(Δ15)	495	282	308
C22:6(Δ4, 7, 10, 13, 16, 19)	485	128	154
		168	194
		208	234
		248	274
		288	314
		328	354
C24:1(Δ15)	523	282	308

Each pair of diagnostic ions has generic chemical formulas of $C_{(x+2)}H_{(2x-2a+4)}O_2N^+$ (aldehydes) and $C_{(x+5)}H_{(2x-2a+10)}ON^+$ (isopropenes), respectively (x: C=C location(s) according to the Δ-nomenclature, a: the sequence number of C=C bond counted from methyl end). The C=C bond locations could be deduced according to the m/z value of diagnostic ions.

Table S3. Intra- and inter-day precisions.

FA	Intra-day (RSD%) (n=3)			Inter-day (RSD%) (n=3)		
	0.1 nM	5 nM	200 nM	0.1 nM	5 nM	200 nM
C14:0	7.4	5.4	1.2	11.3	4.6	4.8
C16:0	11.5	4.4	2.2	12.0	4.7	4.2
C16:1(9Z)	2.4	7.2	6.2	7.4	7.0	9.7
C18:0	5.0	8.5	6.0	5.9	4.4	10.0
C18:1(9Z)	7.3	10.7	10.8	10.0	11.3	9.7
C18:2(9Z, 12Z)	3.6	9.6	4.3	5.3	4.5	10.3
C18:3 (9Z,12Z,15Z)	3.6	9.9	10.9	0.5	6.0	7.2
C20:0	13.1	8.6	6.8	12.2	6.9	12.1
C20:1(11Z)	7.5	6.0	11.8	15.5	4.3	5.7
C20:2 (11Z, 14Z)	9.0	9.8	14.9	6.8	15.8	8.1
C20:3 ((8Z, 11Z, 14Z))	5.8	7.2	11.0	6.3	12.9	9.2
C20:4 (5Z, 8Z, 11Z, 14Z)	8.0	9.6	8.4	0.4	13.8	11.0
C20:5 (5Z, 8Z, 11Z, 14Z, 17Z)	3.4	2.2	9.1	12.0	12.9	12.9
C22:1 (13Z)	3.8	6.6	7.4	1.7	11.1	7.6

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