Supporting Information

Chemicals and reagents

Stearic acid (SA, C18:0), palmitic acid (PA, C16:0), oleic acid (OA, C18:1), palmitoleic acid (PLA, C16:1), docosapentaenoic acid (DPA, C22:5 n-3), docosahexaenoic acid (DHA, C22:6 n-3), eicosapentaenoic acid (EPA, C20:5 n-3), α-linolenic acid (ALA, C18:3 n-3), eicosadienoic acid (EDA, C20:2 n-6), linoleic acid (LA, C18:2 n-6), arachidonic acid (AA, C20:4 n-6), α-eleostearic acid (αESA, C18:3), eicosapentaenoic acid-d5 (EPA-d5), linoleic acid-d4 (LA-d4), arachidonic acid-d8 (AA-d8), palmitoleic acid-d14 (PLA-d14), docosahexaenoic acid-d5 (DHA-d5) and α-parinaric acid (αPA, C18:4 n-3) were purchased from Cayman Chemicals (Ann Arbor, MI). Stearic acid-d3 (SA-d3), myristic acid (MA, C14:0), oxalyl chloride (2M in dichloromethane), N,N-dimethylethanolamine (DMAE), 3-picolylamine (3-PA), 3-pyridylcarbinol (3PE), triphenylphosphine (TPP), 2,2'-dipyridyl disulfide (DPDS) were purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals and solvents were from Fisher (Pittsburgh PA).

Standards preparation

The working stock of fatty acid standards contained 14 biologically relevant fatty acids (Table 1), including saturated FAs: MA, SA and PA; ω-3 and ω-6 polyunsaturated FAs: LA, ALA, AA, DHA, DPA, EPA, and EDA; and mono-unsaturated and conjugated FAs: PLA, OA, α-PA and α-ESA at a concentration of 100 μg/mL. Six calibration working standards were prepared in ethanol by serial dilution covering the expected biological concentration range from 1.5 to 1500 ng/mL after dilution.

An internal standard mixture was prepared in ethanol containing EPA-d5 (10 μg/mL), DHA-d5 (10 μg/mL), PLA-d14 (10 μg/mL), LA-d4 (50 μg/mL), AA-d4 (50 μg/mL), and SA-d3 (100 μg/mL).

Fatty acids extraction from red blood cells

Lipids were extracted from red blood cells (RBCs) according to Rose et al [1] with slight modifications. Briefly, RBCs were separated from whole blood after centrifugation at 2500 rpm for 20 min, and subsequently washed with 1x PBS buffer. 20 μL of the washed RBCs were mixed with 20 μL internal standard mixture and extracted with 1 mL 2-propanol/ dichloromethane (11/7, v/v, containing 2.5% BHT as anti-oxidant). The resulting mixture was vortexed vigorously at high speed for 10 min. After centrifugation at 2500 rpm for 5 min, the organic portion was separated and dried under nitrogen flow. Free fatty acids were subsequently released from the lipids under basic hydrolysis conditions. The lipid extract was dissolved in 850 μL methanol/dichloromethane (8/1, v/v), to which 150 μL of 40% aq. potassium hydroxide was added. The mixture was incubated at 60 ^{o}C for 30 min followed by buffering with 700 μL of aq. phosphate buffer (75 mM, pH7) and acidifying to pH 1~2 with 250 μL of aq. 2.5 M hydrochloride. The fatty acids were extracted with diethyl ether /hexane (1/1, v/v) twice (1 mL x2). The organic portions were combined, dried under nitrogen and subjected to derivatization. For comparison reasons, the QC RBCs were also lyophilized overnight to give powders for analysis, and around 5 mg of RBC powder was weighted for extraction as described above.

Method validation

The accuracy of the FA-PA method was validated using a NIST standard reference material (SRM 3274, FAs in botanical oils) for long-chain FAs including SA, PA, MA, , OA and LA concentrations. The sample preparation was similar to the RBC preparation with slight modifications. In brief, to 150 μL of FA calibration standards or 200 μL SRM oil (10 mg/mL in dichloromethane) and 20 μL of internal standard was added followed by drying under nitrogen. The residue was dissolved in 850 μL MeOH/DCM (v/v 1:1) and hydrolyzed with 150 μL KOH aq. and heated at 60 ^{o}C for 30 min. After the incubation, the reaction mixture was buffered with 700 μL phosphate buffer (pH7) and acidified with 2.5 M HCl. The free fatty acids were extracted with hexanes 1mL x2 and dried. The extracted free FAs underwent FA-PA derivatization as described above. The consistency of the method was evaluated by repeated analysis of FAs in RBC QC samples by orbitrap FA-PA method. The QCs was repeated in triplicate for 5 days.

References

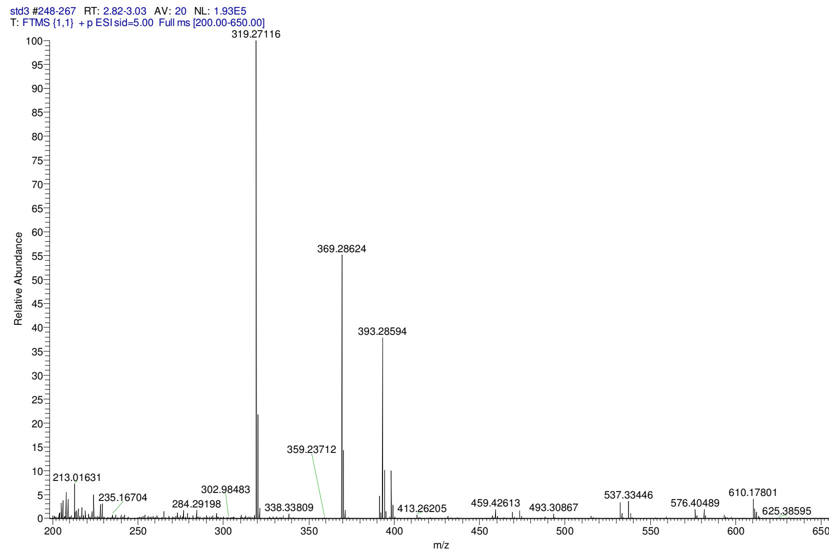
(1) Rose, H. G.; Oklander, M. *J. Lipid Res.* **1965**, *6*, 428-431.

Supporting information 2

Spectrum of FA-PA derivatized fatty acids. Detection was set at 10 ppm within the calculated masses.

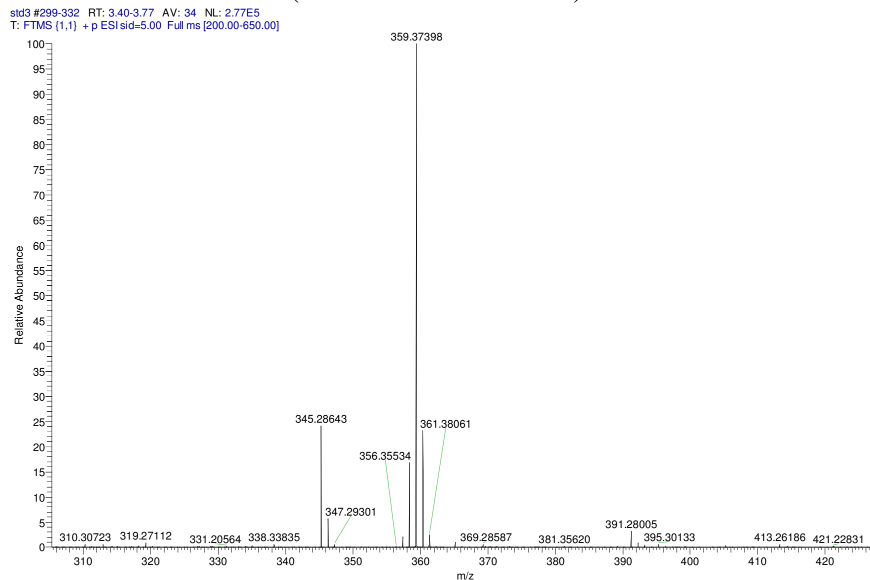
RT 2.82-3.03 min

EPA: 393.28594 (calculated 393.29004)
EPA-d5: 398.31725 (calculated 398.32142)
ALA: 369.28624 (calculated 369.29004)
MA: 319.27439(calculated 319.27439)



RT: 3.40-3.77 min

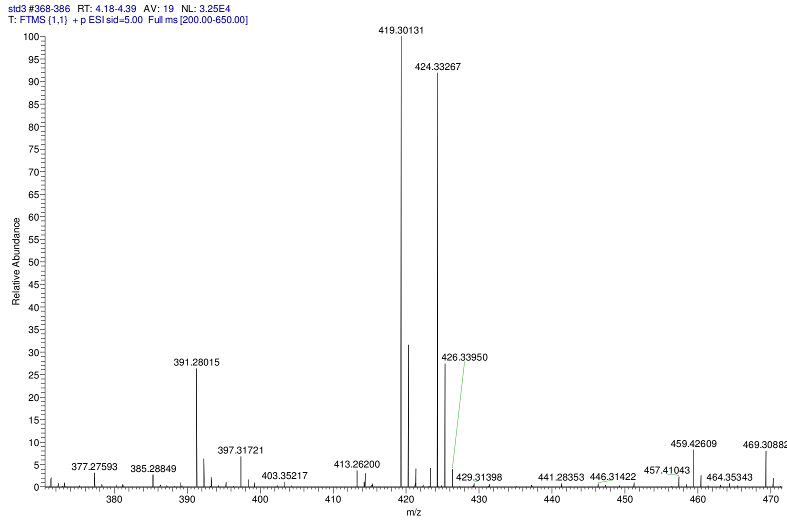
PLA: 345.28643 (calculated 345.29004)
PLA-d14: 359.37398 (calculated 359.37791)



RT: 4.18-4.39 min

DHA: 419.30131 (calculated 419.30569)

DHA-d5: 424.33267 (calculated 424.33707)



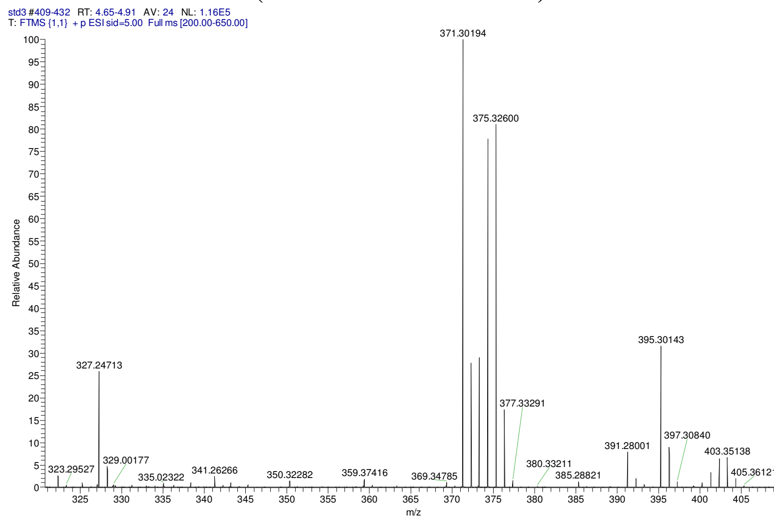
RT: 4.65-4.91 min

AA: 395.30143 (calculated 395.30569)

AA-d8: 403.35138 (calculated 403.35590)

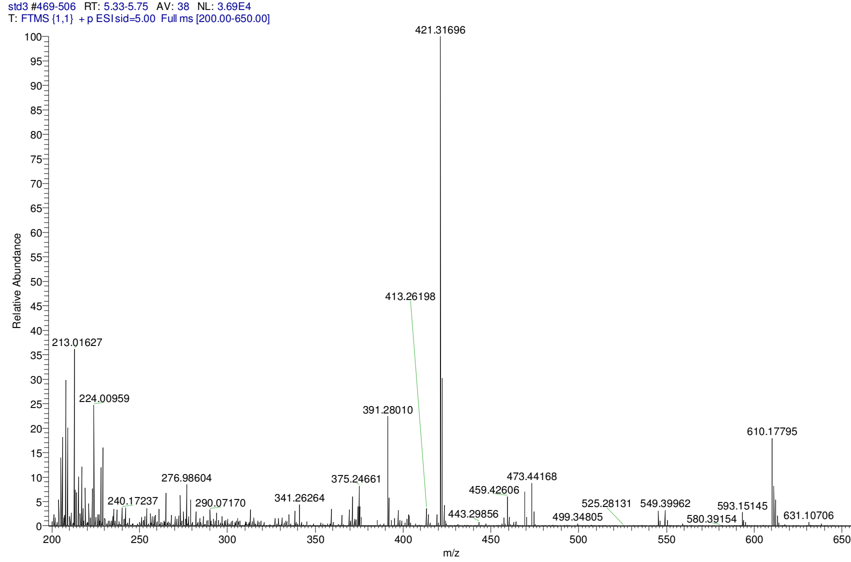
LA: 371.30194 (calculated 371.30569)

LA-d4: 375.32600 (calculated 375.33080)



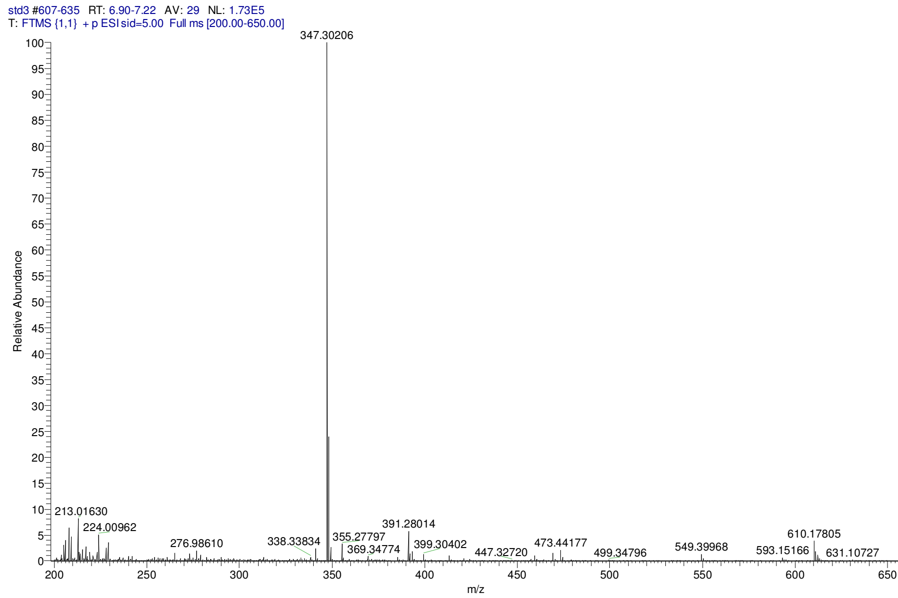
RT: 5.33-5.75 min

DPA: 421.31696 (calculated 421.32143)



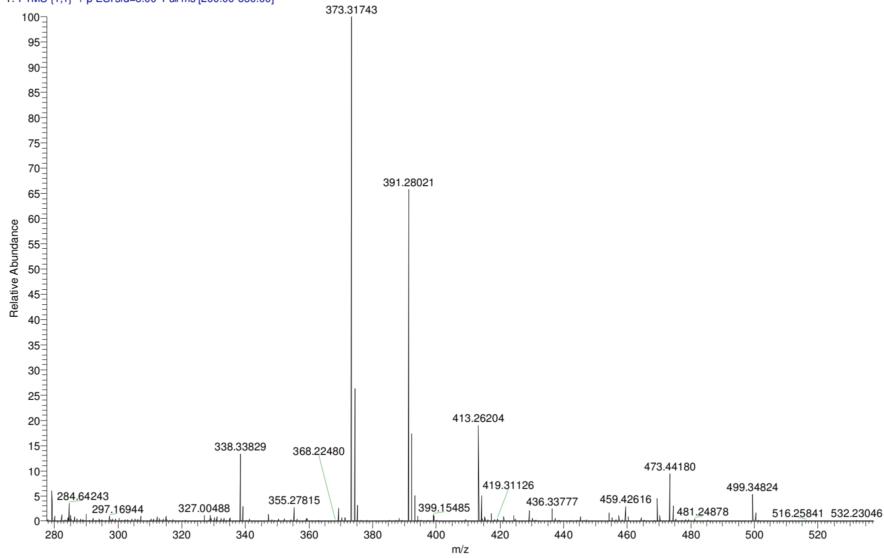
RT: 6.90-7.22 min

PA: 347.30206 (calculated 347.30569)



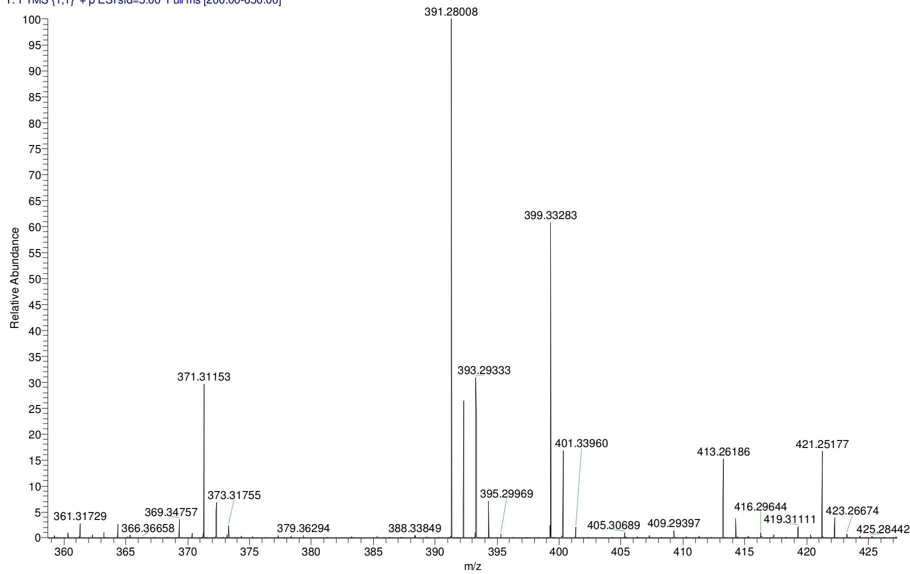
RT: 8.47-8.79 min

OA: 373.31743 (calculated 373.32134)



RT: 9.21-9.58 min

EDA: 399.33283 (calculated 399.33699)



RT: 11.21-11.58 min

SA: 375.33387 (calculated 375.33699)

SA-d3: 378.35233 (calculated 378.35582)