Supplementary Information

Novel n-3 Immunoresolvents: Structures and Actions

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Supplementary Figure 1: MS-MS spectra employed for identification of n-3 DPA monohydroxy products in mouse plasma. Mice were subjected to ischemia reperfusion injury (see Methods for details). Two h into reperfusion, blood was collected via cardiac puncture and the plasma was obtained by centrifugation, products were extracted and n-3 DPA monohydroxy products were assessed by lipid mediator metabololipidomics. Representative MS-MS spectra used for identification of (a) 17-HDPA, (b) 14-HDPA, and (c) 7-HDPA. Results are representative of n=4.

Supplementary Figure 2: Chiral lipid mediator metabololipidomics of isobaric monohydroxy-containing acids from n-3 DPA in murine plasma with ischemia reperfusion. In order to detect and quantify each positional isomer without ambiguity, an MRM method was established. Signature daughter ions for each monohydroxy acid (parent m/z, 345) were as follows: 17-HDPA – m/z 245, 14-HDPA – m/z 207 and 7-HDPA – m/z 143. For each enantiomer pair, the R isomer was eluted before S isomers. The signature ion for each species was unique; only two (R and S isomers) peaks are present on each extracted ion chromatogram. Results are representative of n=3.

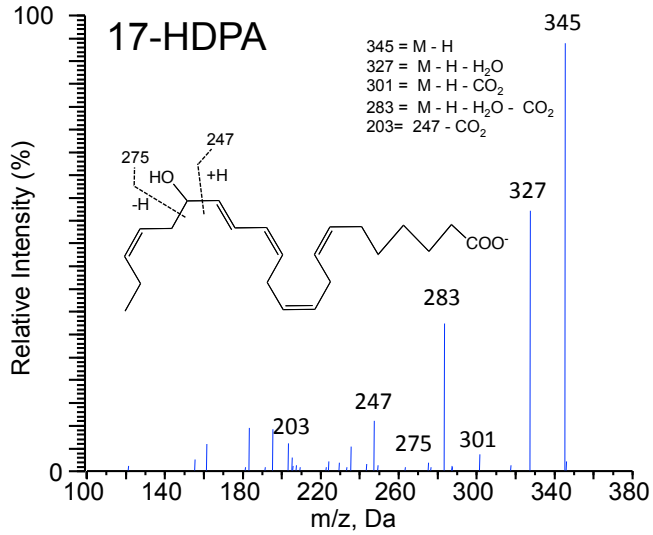
Supplementary Figure 3: n-3 DPA resolvins: physical properties. (a-f) HPLC retention times, online UV, fragment assignments shown in inset, and tandem mass spectra for (a,b) $RvD2_{n\text{-}3\ DPA}$, (c,d) $RvD1_{n\text{-}3\ DPA}$, and (e,f) $RvD5_{n\text{-}3\ DPA}$.

Supplementary Figure 4: n-3 DPA protectins: physical properties. (a–f) HPLC retention times, online UV, fragment assignments (inset), and tandem mass spectra for (a,b) $PD1_{n\text{-}3\ DPA}$ and (c,d) $PD2_{n\text{-}3\ DPA}$.

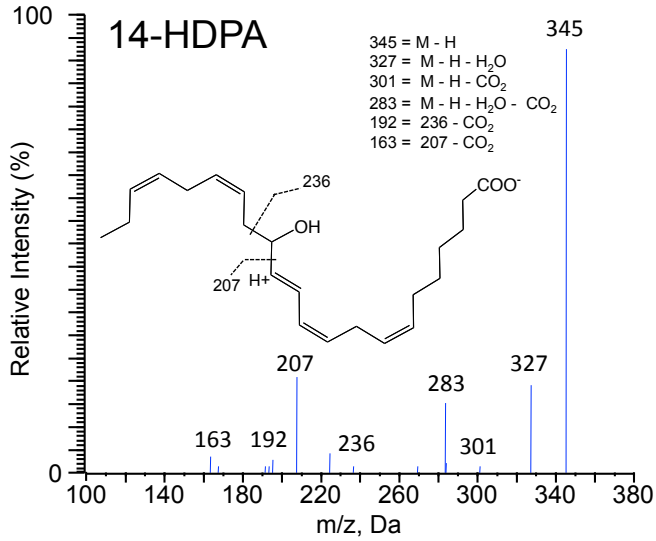
Supplementary Figure 5: n-3 DPA maresins: physical properties. (a-f) HPLC retention times, online UV, fragment assignments (inset), and tandem mass spectra for (a,b) $MaR1_{n\text{-}3\ DPA}$, (c,d) $MaR2_{n\text{-}3\ DPA}$, and (e,f) $MaR3_{n\text{-}3\ DPA}$.

Supplementary Figure 6: n-3 DPA specialized pro-resolving mediators regulate endothelial ICAM-1 expression. HUVEC were incubated with vehicle (0.1% EtOH in PBS) or n-3 DPA products (1nM, 15min, 37^{o}C, pH7.45) and then incubated with TNF-α (10ng/ml, 4h, 37^{o}C, 0.1% FSC). ICAM-1 levels were then assessed by flow cytometry using a fluorescently labeled mouse anti-human ICAM-1 antibody. The ratio of $RvD1_{n\text{-}3\ DPA}$ to $RvD2_{n\text{-}3\ DPA}$ (A) was ~ 3:1; the ratio of $RvD5_{n\text{-}3\ DPA}$ to $PD1_{n\text{-}3\ DPA}$ (B) was ~9:1; the ratio of $PD1_{n\text{-}3\ DPA}$ to $PD2_{n\text{-}3\ DPA}$ (C) was ~1:5. Results are mean ± SEM. n = 4 independent neutrophil and endothelial cell preparations (*P <0.05; **P <0.05 vs. vehicle incubated cells).

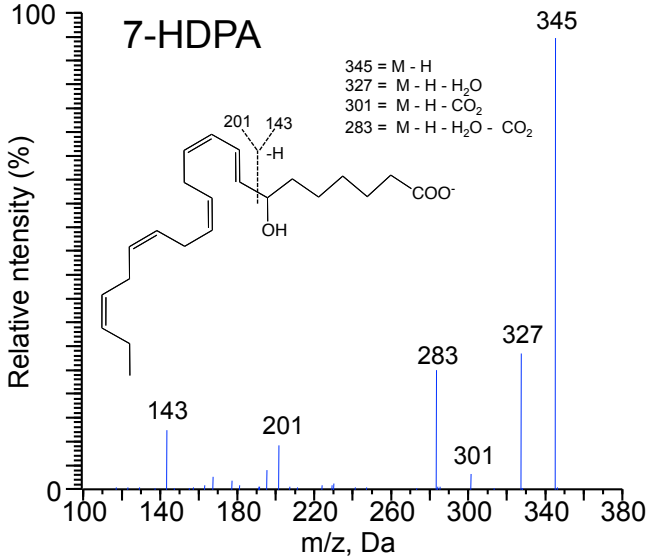
a


17-HDPA
345 = M - H
327 = M - H - H2O
301 = M - H - CO2
283 = M - H - H2O - CO2
203= 247 - CO2
275
247
HO
+H
-H
COO-
345
327
283
247
203
275
301
Relative Intensity (%)
m/z, Da

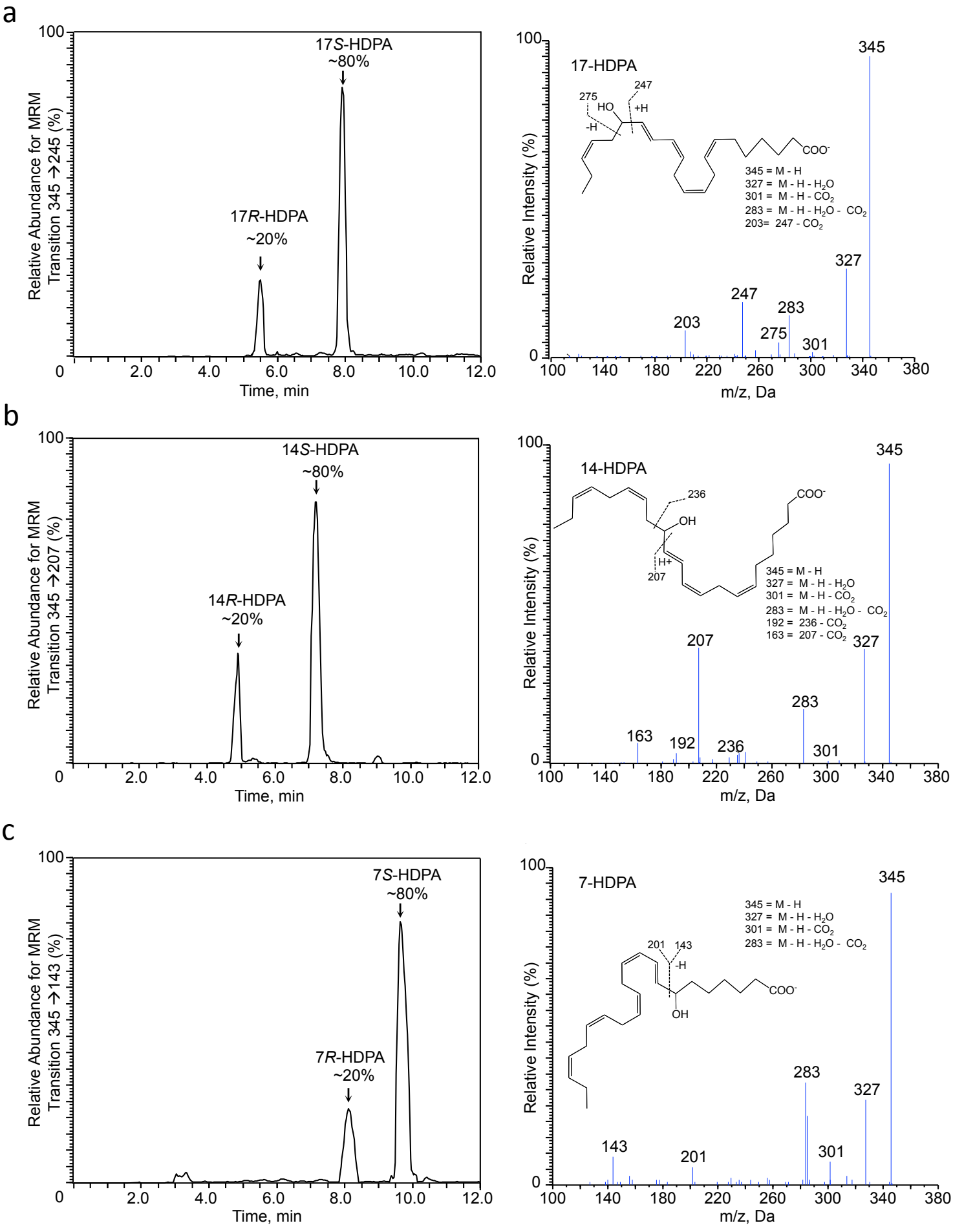
b


14-HDPA
345 = M - H
327 = M - H - H2O
301 = M - H - CO2
283 = M - H - H2O - CO2
192 = 236 - CO2
163 = 207 - CO2
236
OH
207
H+
COO-
345
207
283
327
163
192
236
301
Relative Intensity (%)
m/z, Da

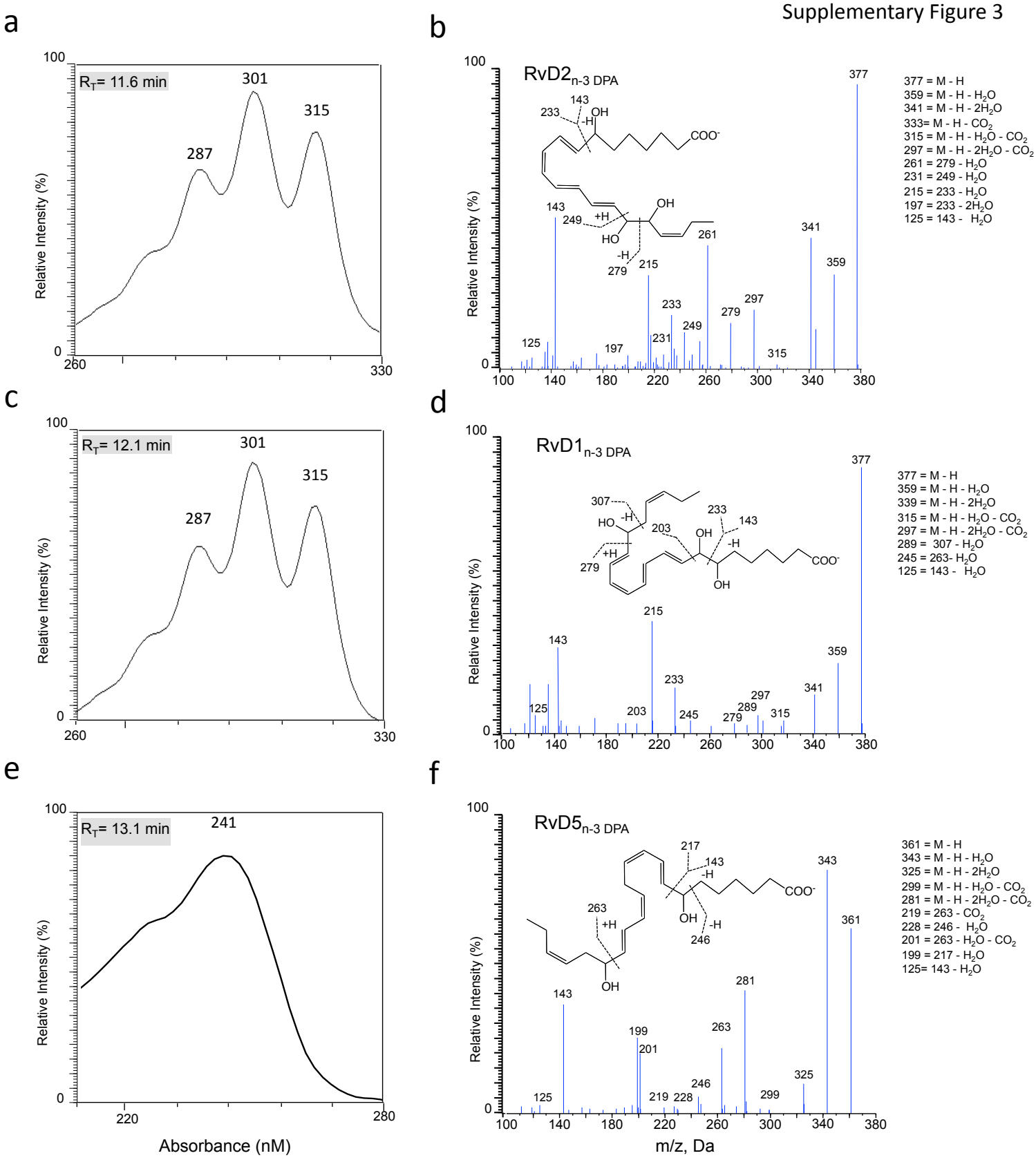
c


7-HDPA
345 = M - H
327 = M - H - H2O
301 = M - H - CO2
283 = M - H - H2O - CO2
201
143
-H
COO-
OH
345
327
283
143
201
301
Relative ntensity (%)
m/z, Da

Supplementary Figure 2

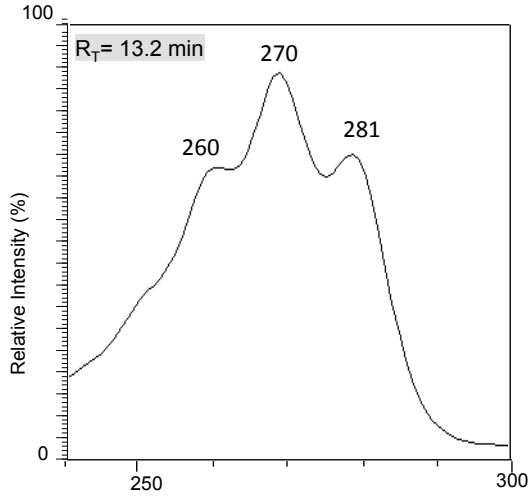


Supplementary Figure 3

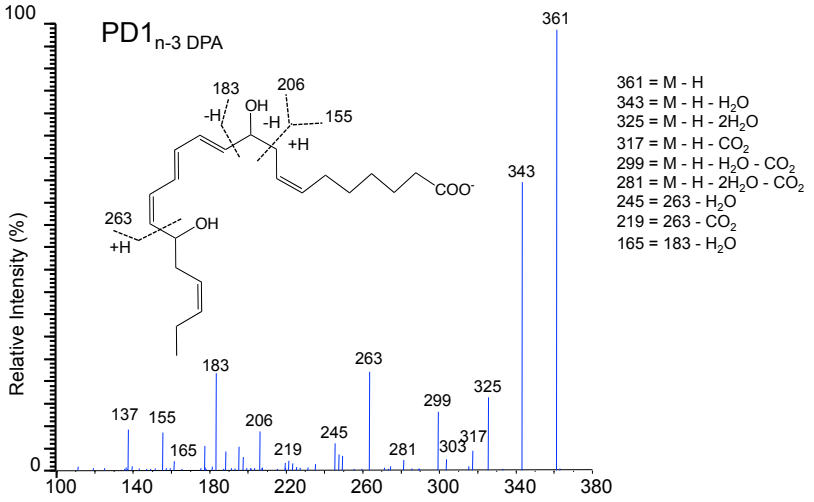


Supplementary Figure 4

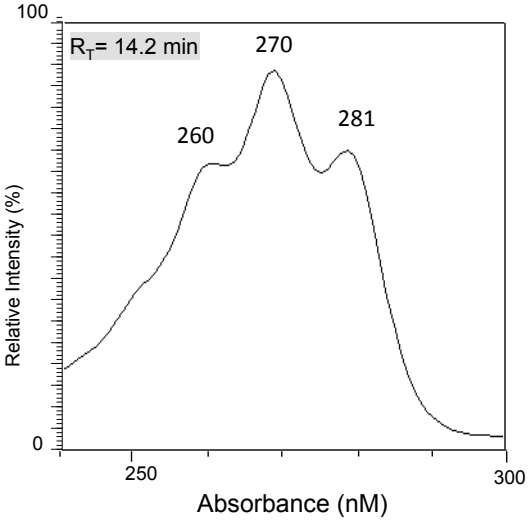
a



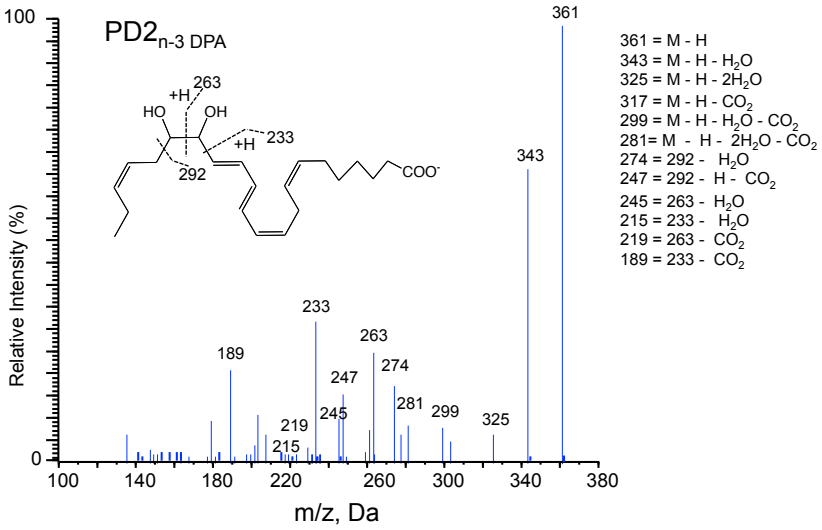
b



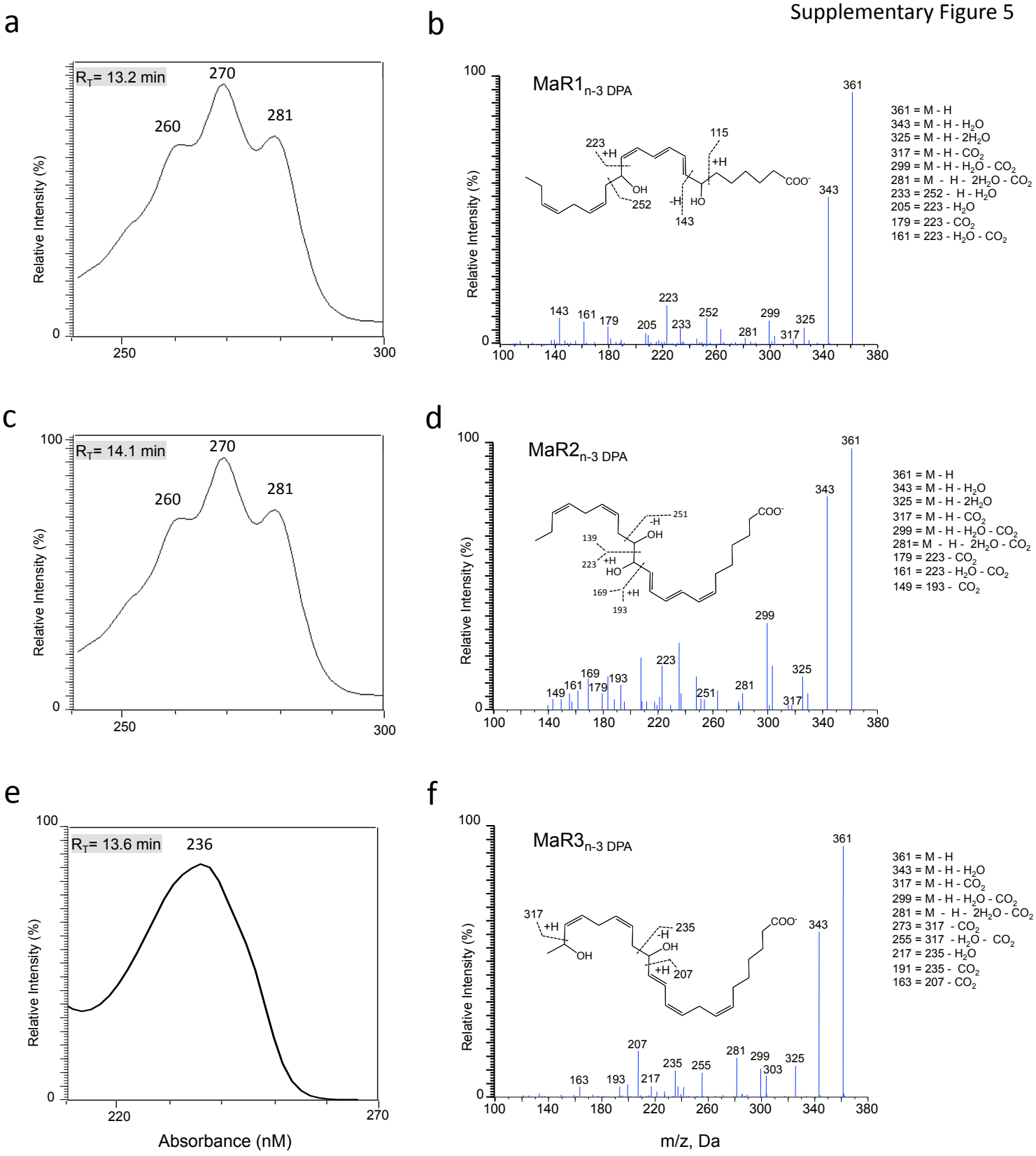
d



d



Supplementary Figure 5



Supplementary Figure 6