Human Cytochrome P450, CYP2S1, Metabolizes Cyclooxygenase – and Lipoxygenase – Derived Eicosanoids

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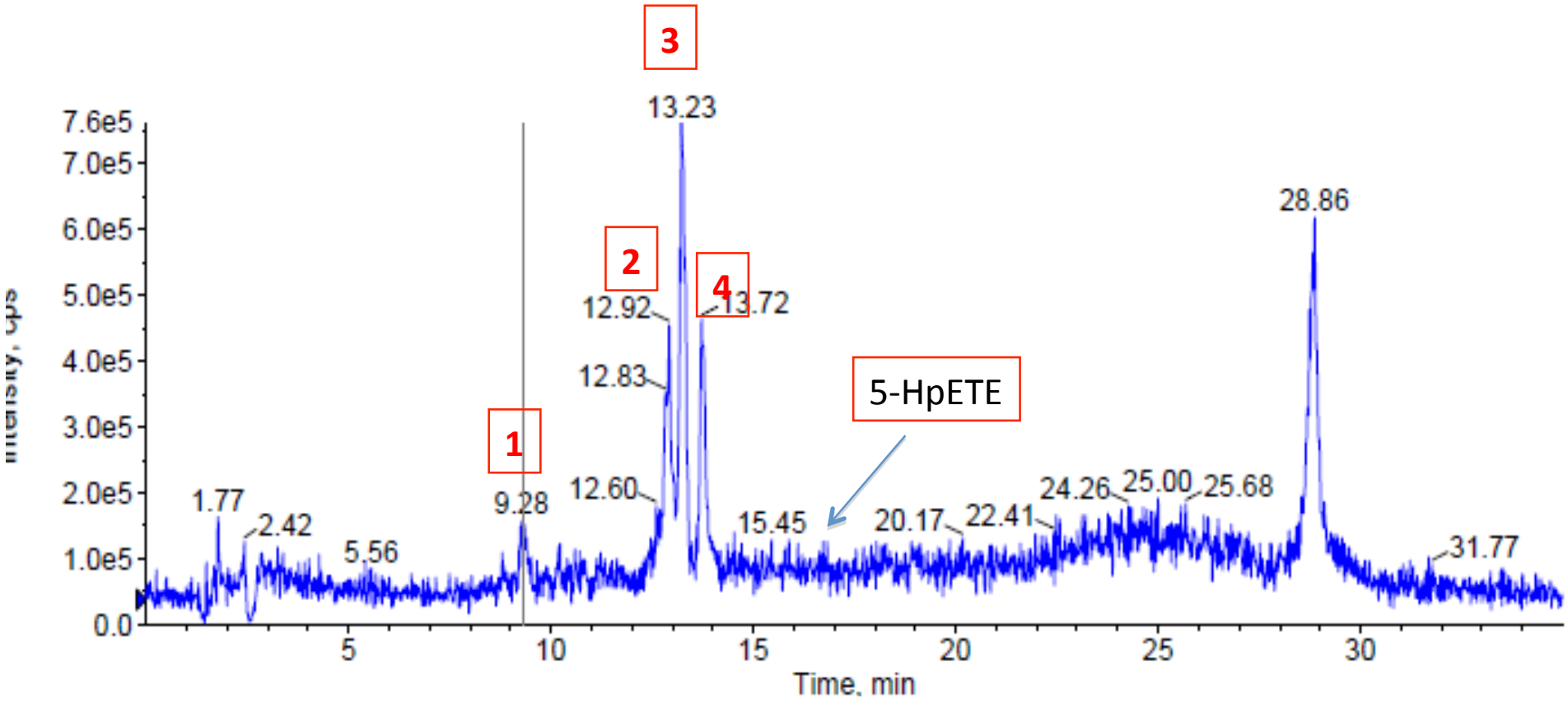
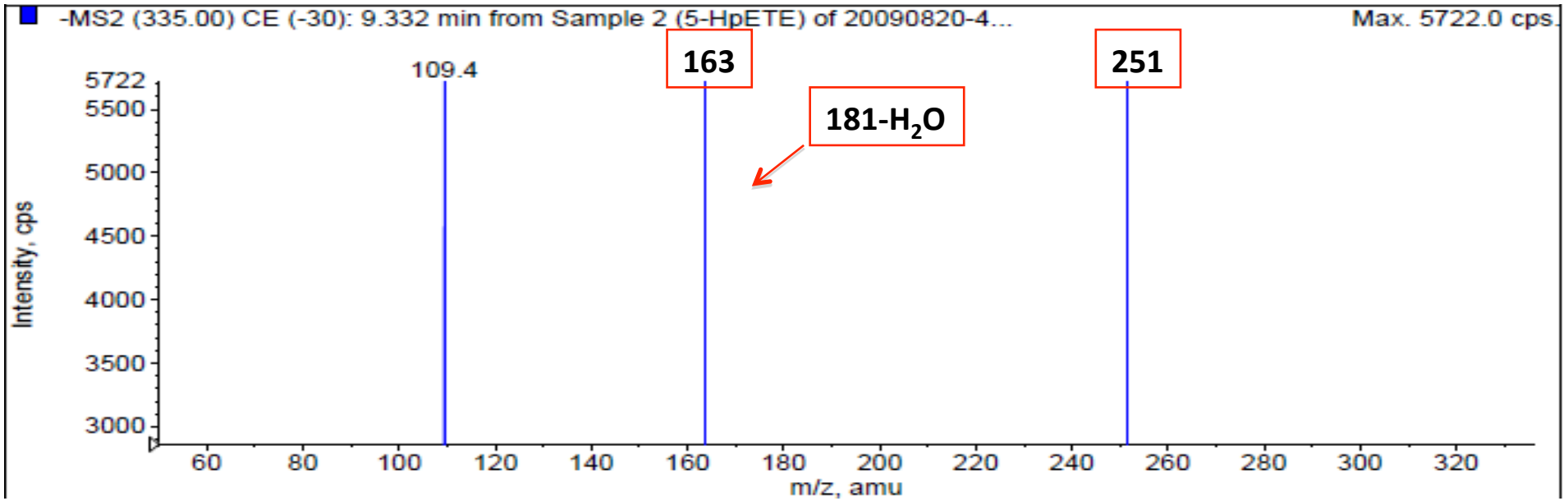
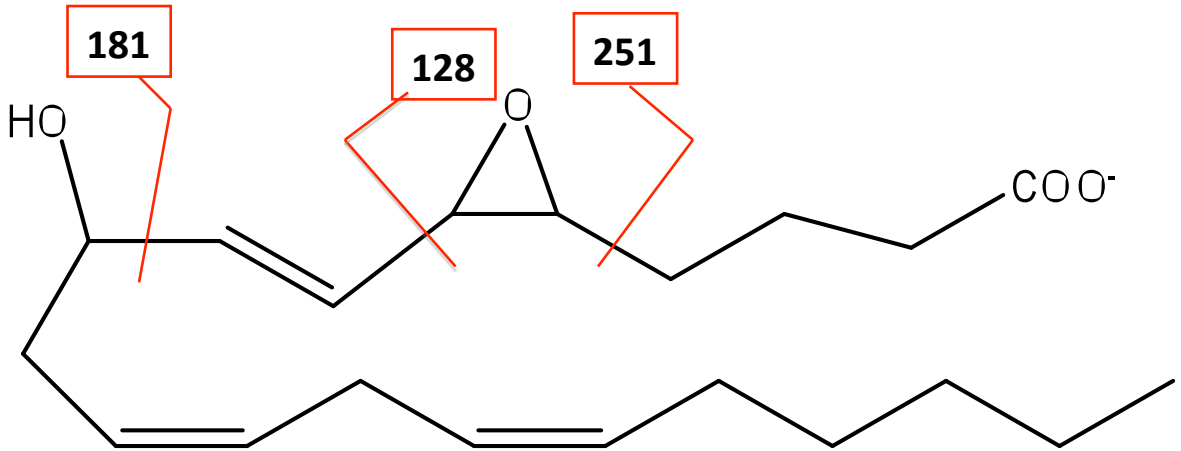
Drug Metabolism & Deposition. Manuscript # 35121

S.Figure 1 A) LC-ESI-MS/MS of 5-HpETE incubated with human CYP2S1. Parent mass [M-H]-, 335

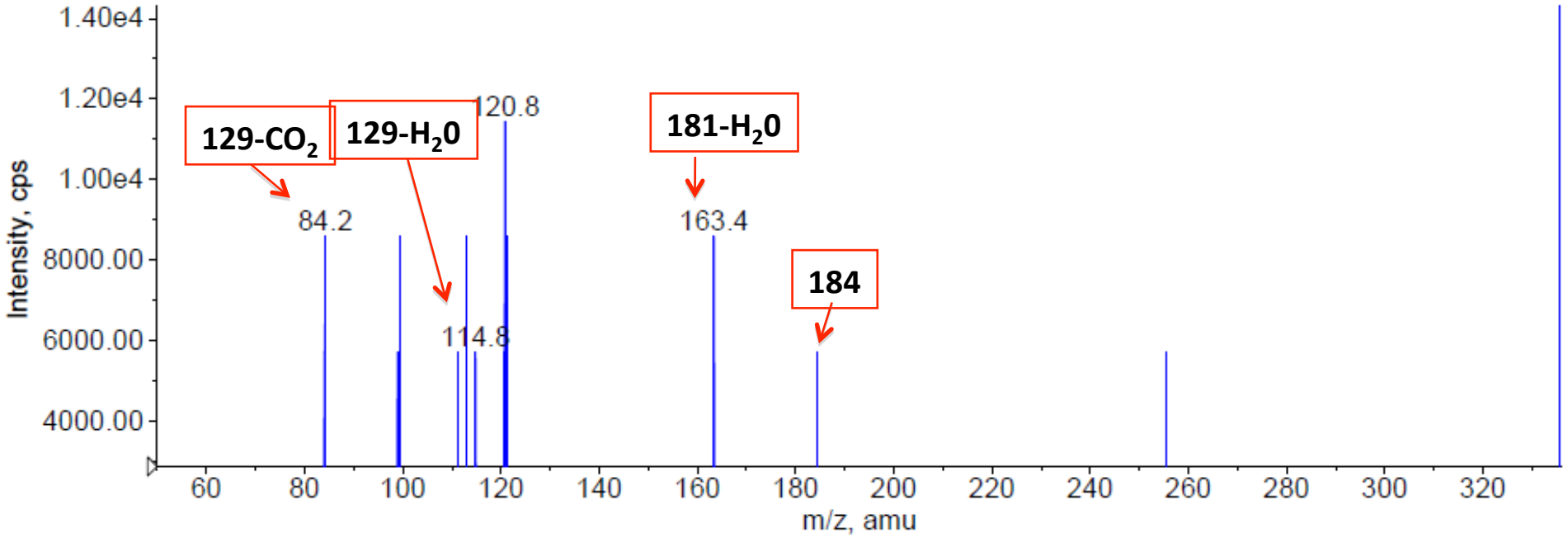


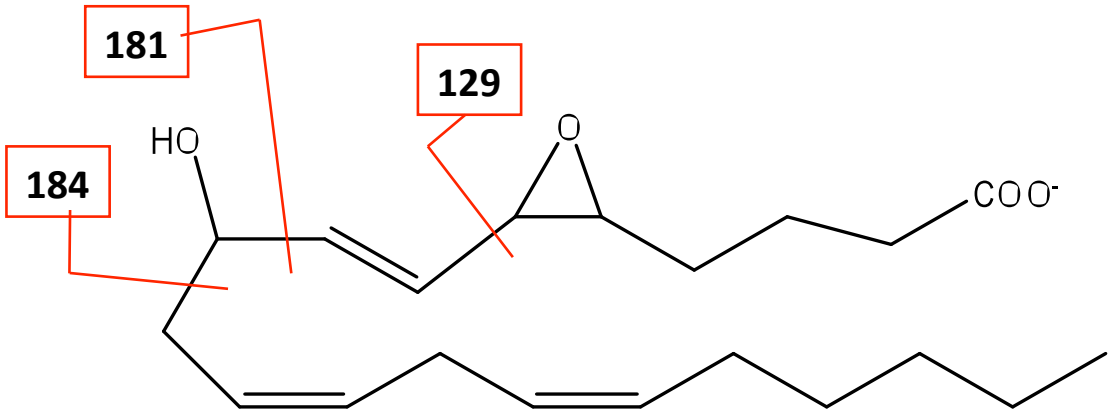
S.Figure 1B) Fragmentation of product 1 = 9.332 min. 9-hydroxy-5,6-epoxy-7,11,14,-eicosatrienoic acid



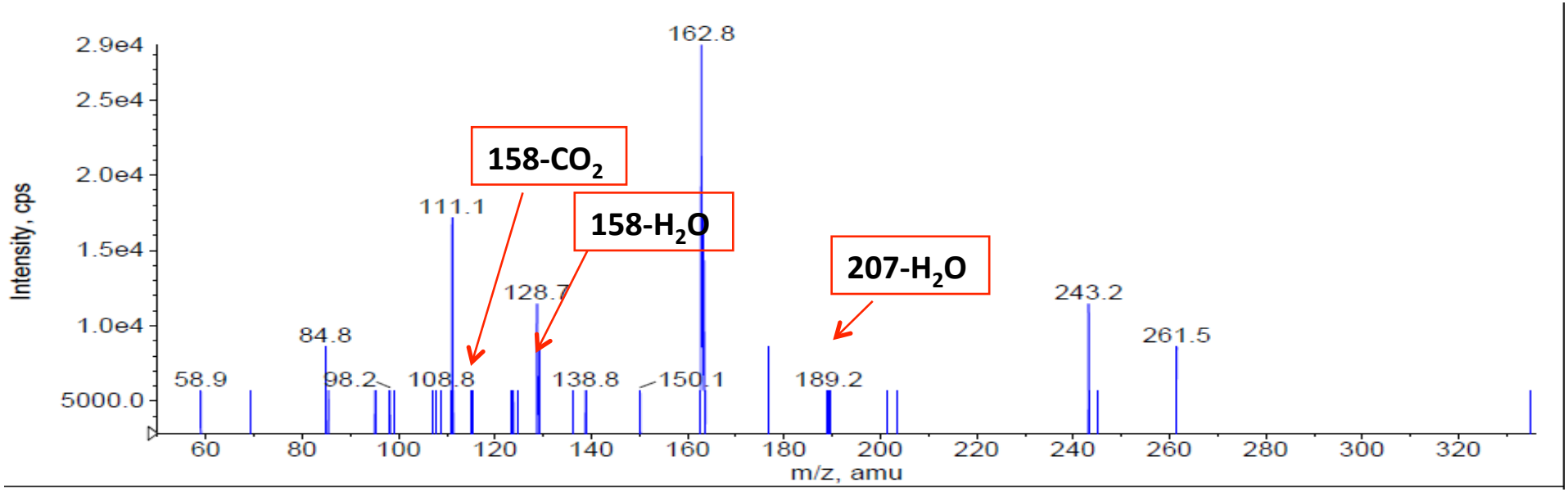


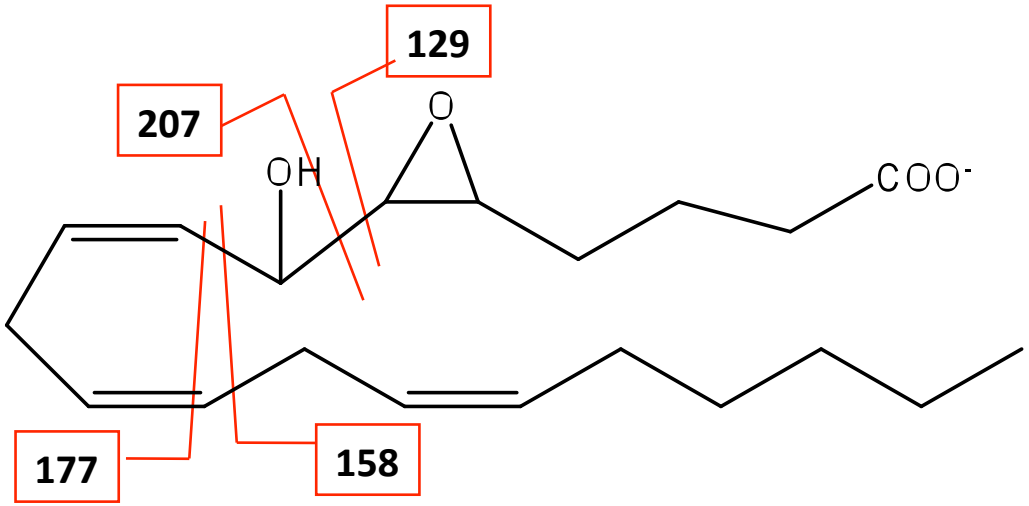
S.Figure 1C) Product 2 = 12.92 min. 9-hydroxy-5,6-epoxy-7,11,14,-eicosatrienoic acid



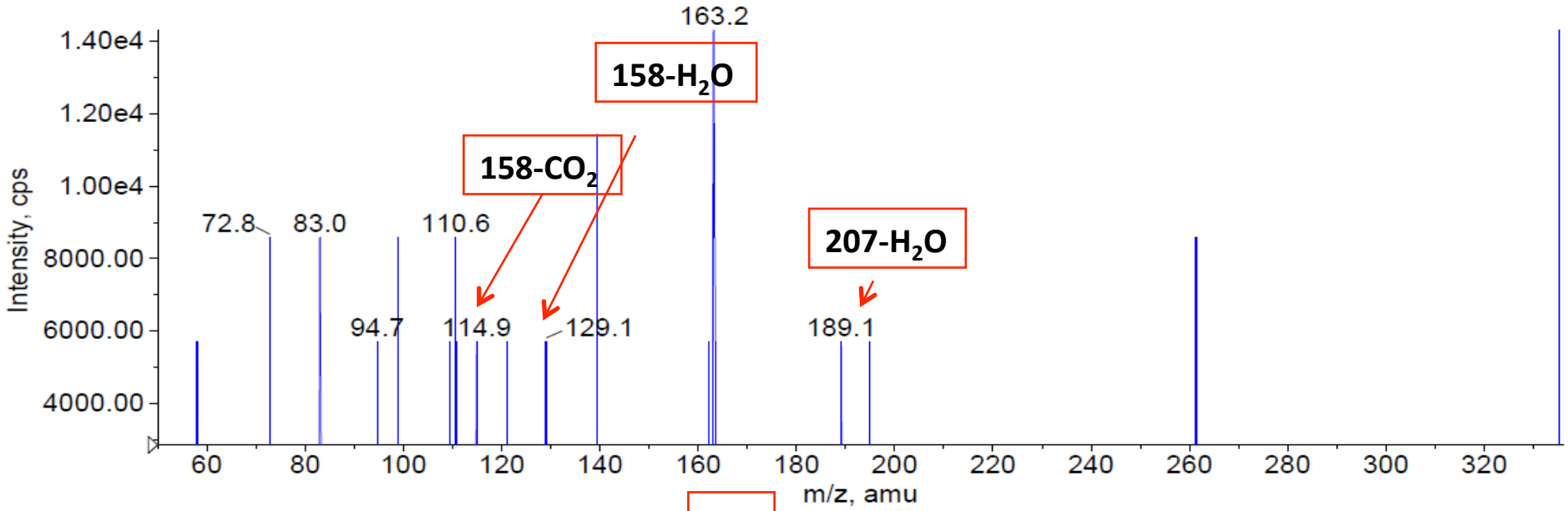


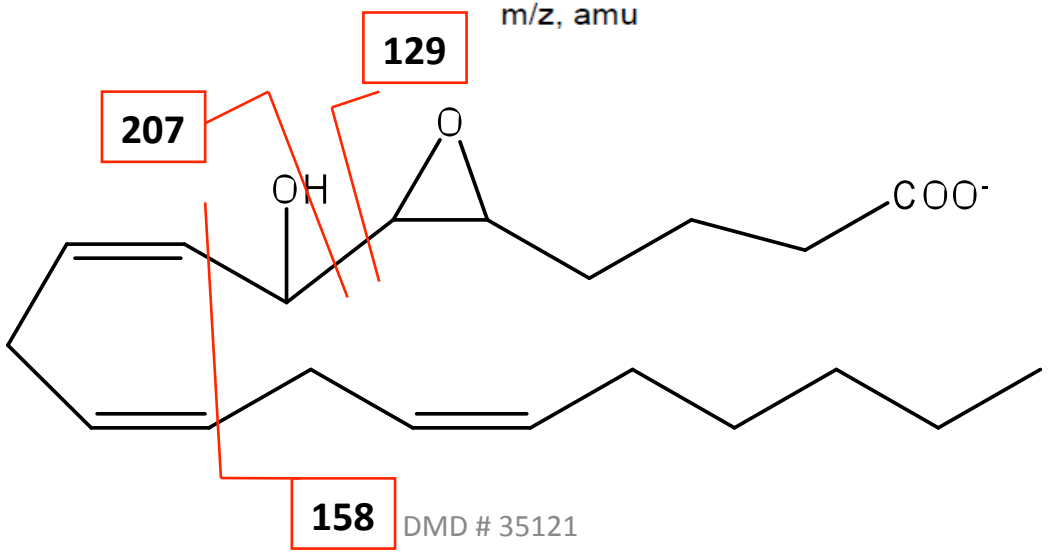
S.Figure 1D) Fragmentation of Product 3 = 13.23 min. 7-hydroxy-5,6-epoxy-8,11,14,-eicosatrienoic acid



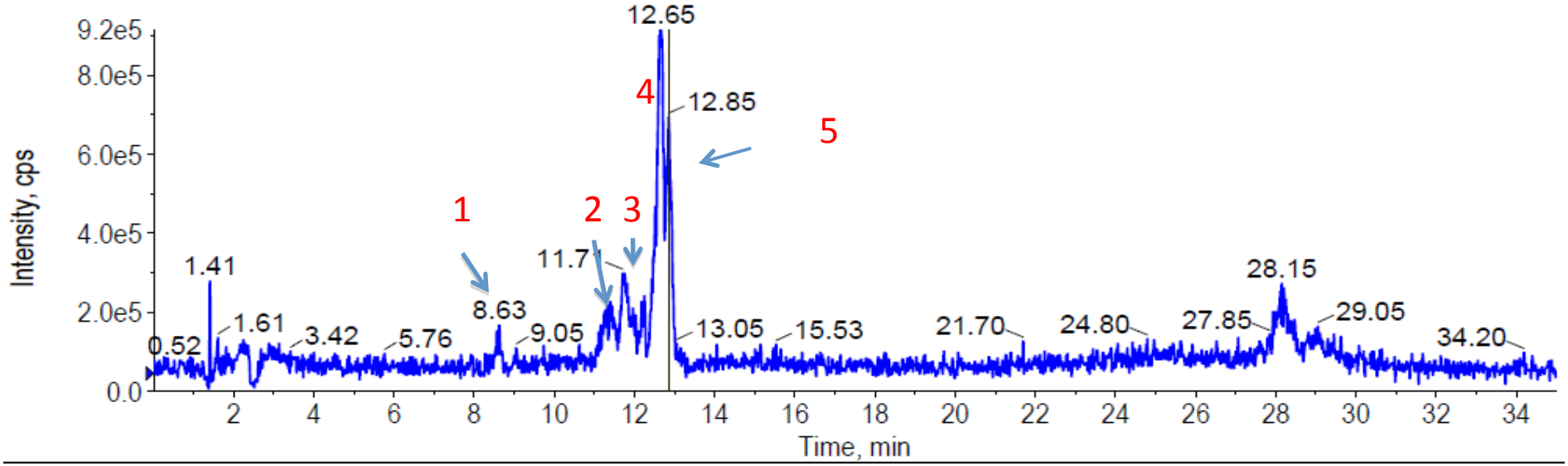


S.Figure 1E) Fragmentation of Product 4 = 13.72 min. 7-hydroxy-5,6-epoxy-8,11,14,-eicosatrienoic acid

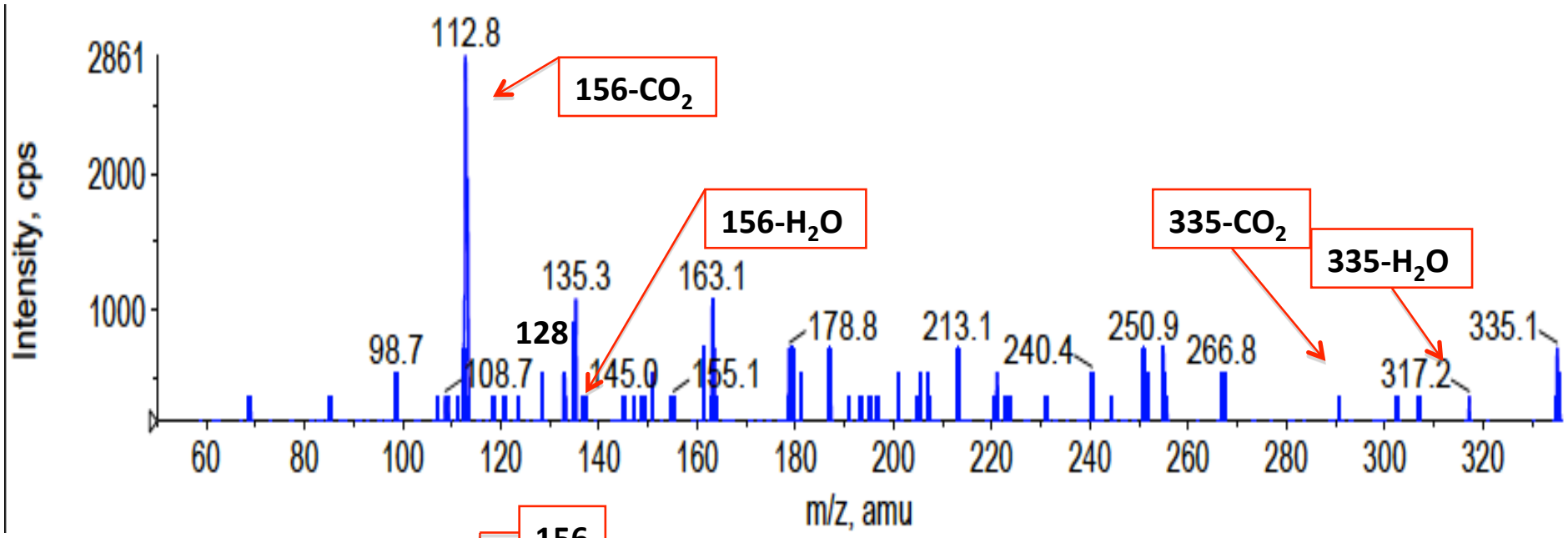


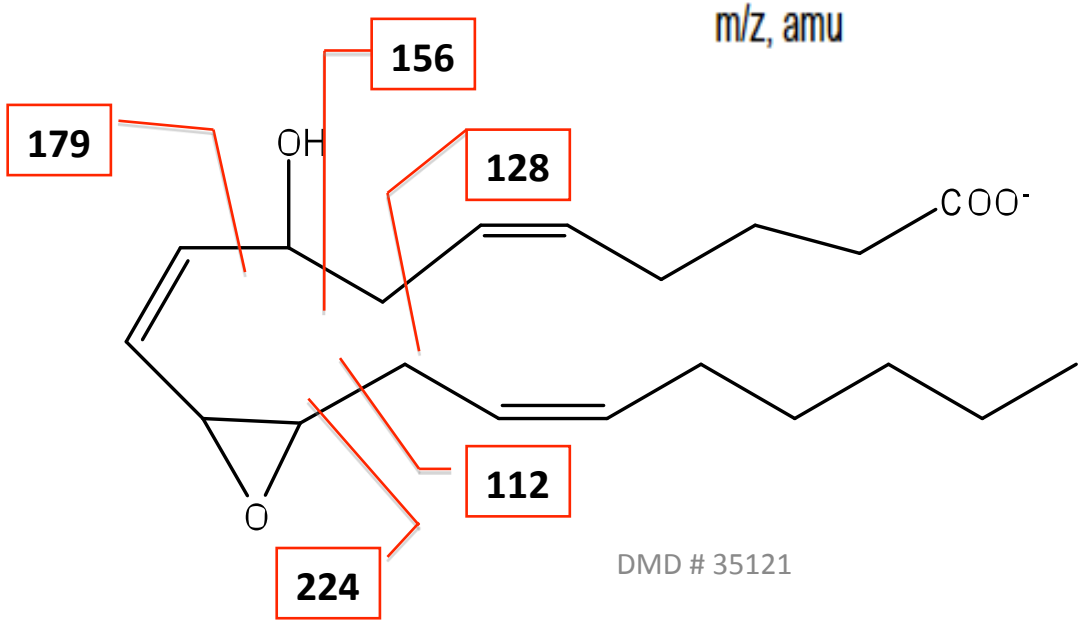


S. Figure 2A) LC-ESI-MS/MS of 12-HpETE incubated with human CYP2S1. Parent mass [M-H]-, 335

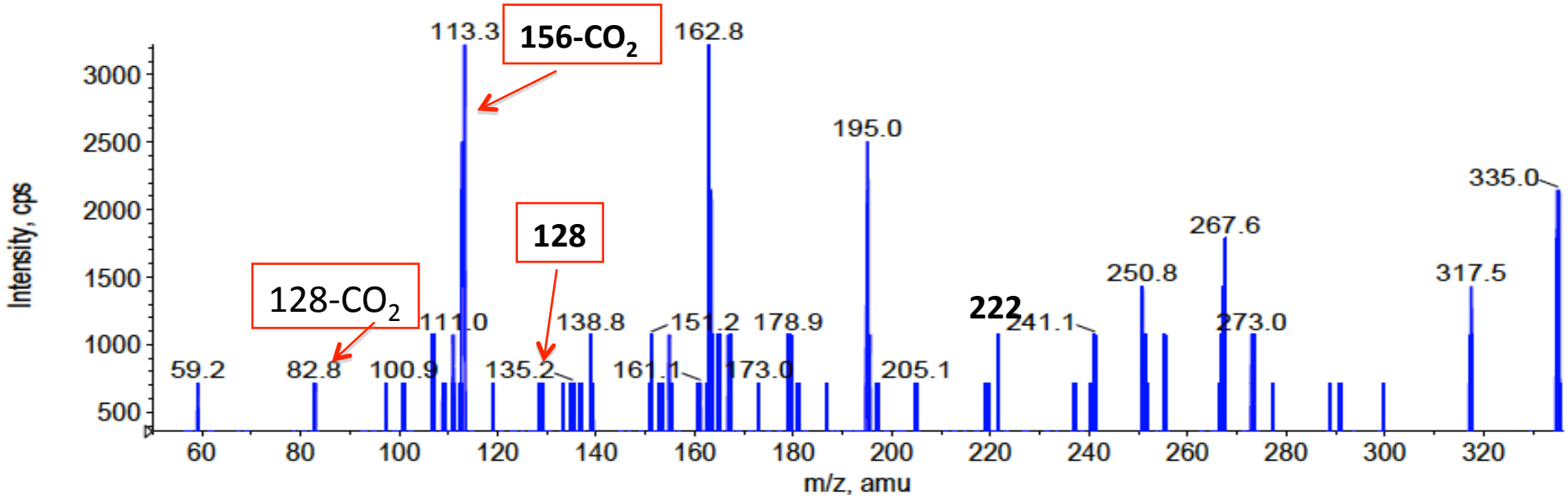


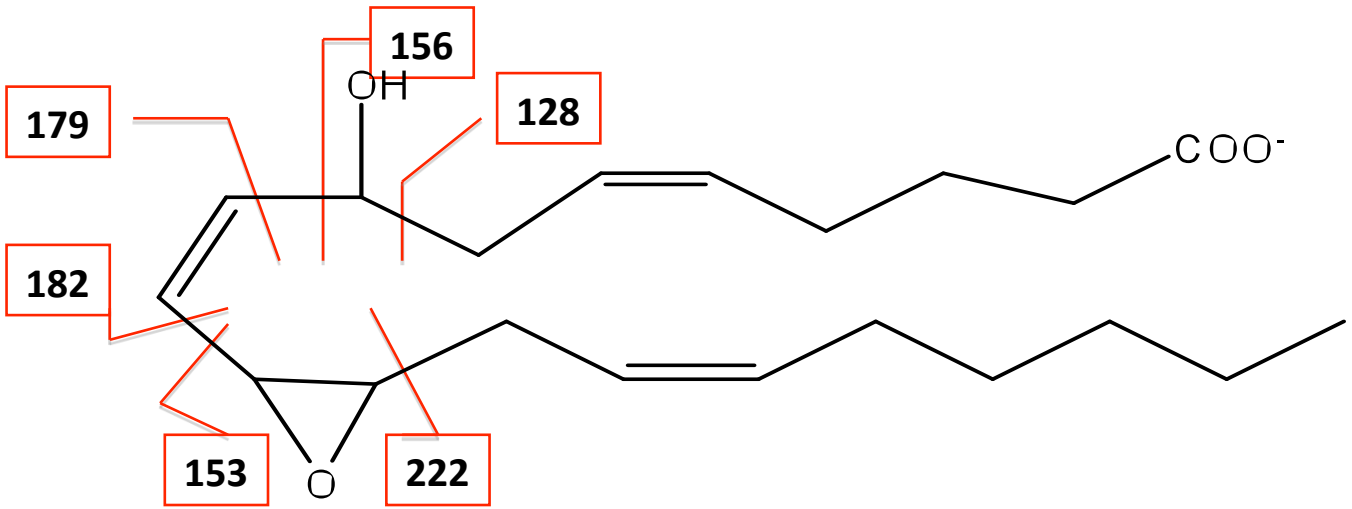
S.Figure 2B) Product 1= 8.63 min. 8-hydroxy-11,12-epoxy-5,8,14-ecosatrienoic acid, Hepoxilin A_3



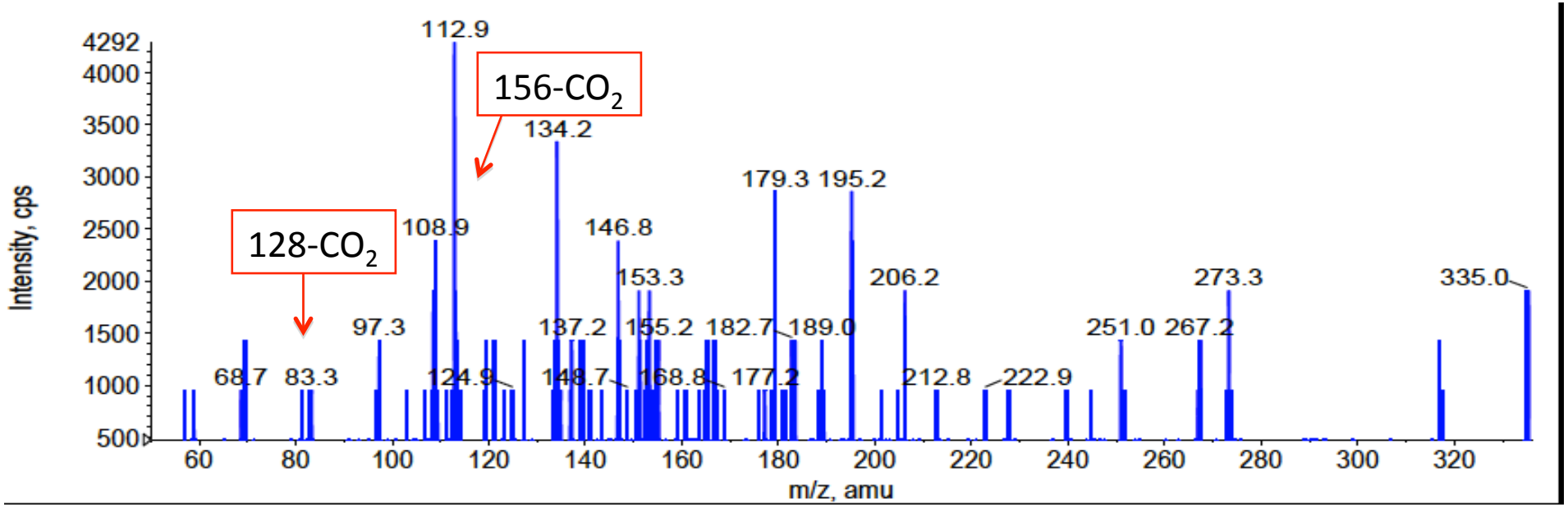


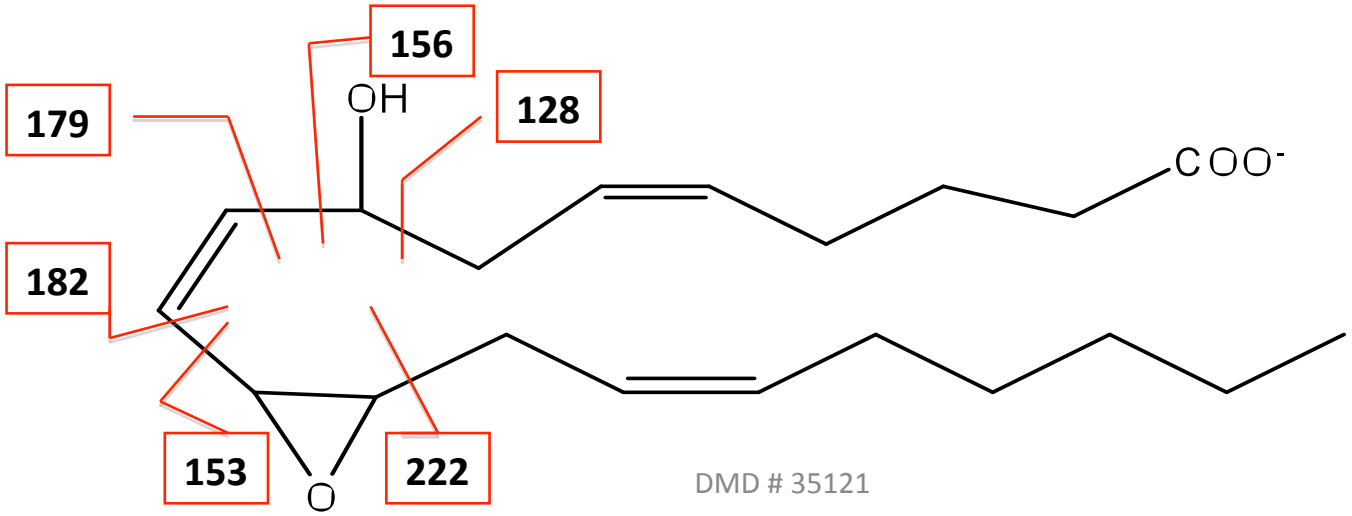
S.Figure 2C) Product 2 = 11.39 min. 8-hydroxy-11,12-epoxy-5,8,14-ecosatrienoic acid, Hepoxilin A_3



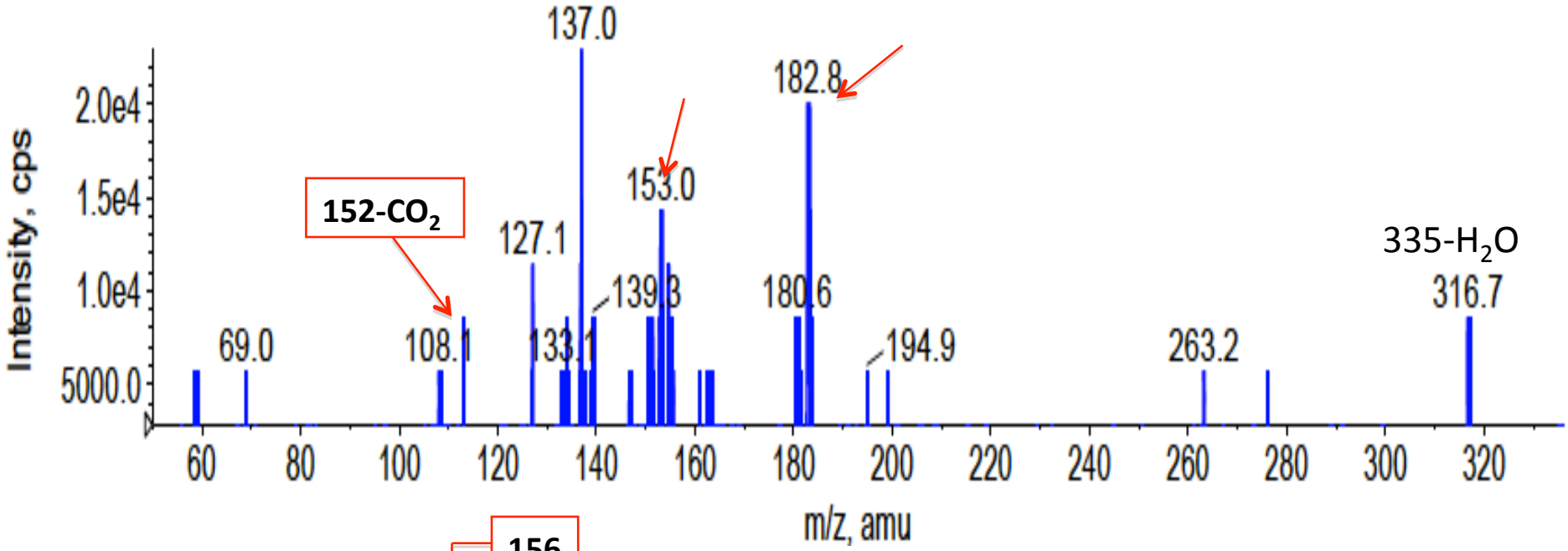


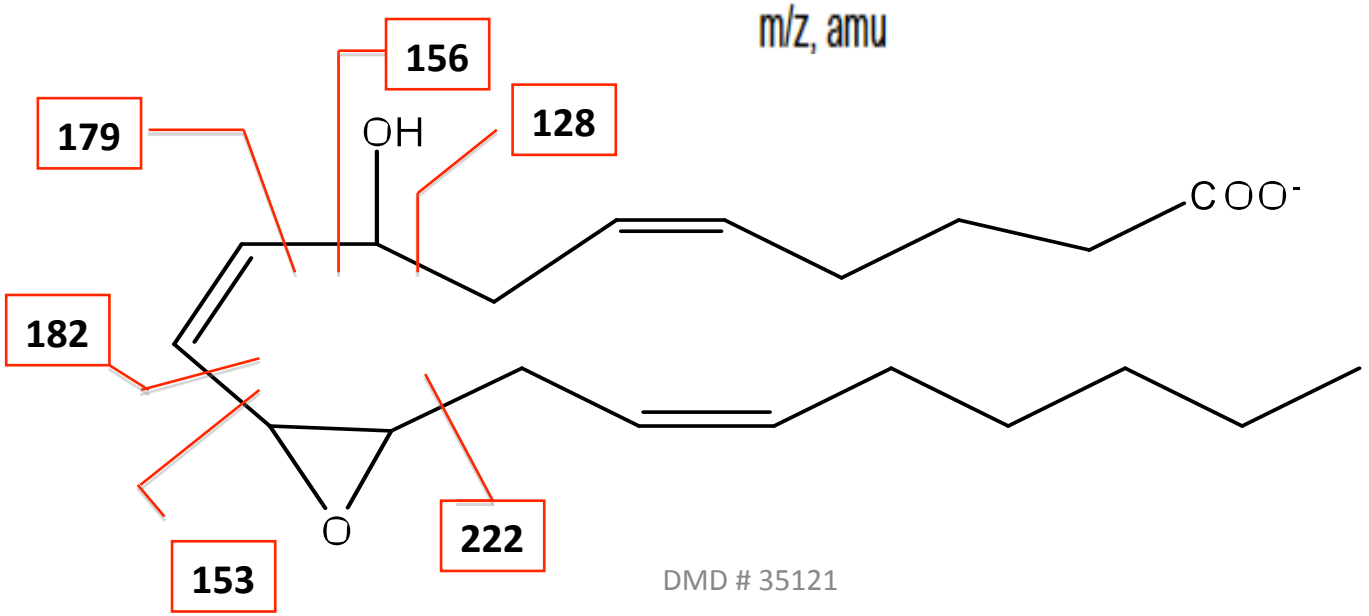
S.Figure 2D) Fragmentation of product 3 = 11.7 min. 8-hydroxy-11,12-epoxy-5,8,14-ecosatrienoic acid, Hepoxilin A_3



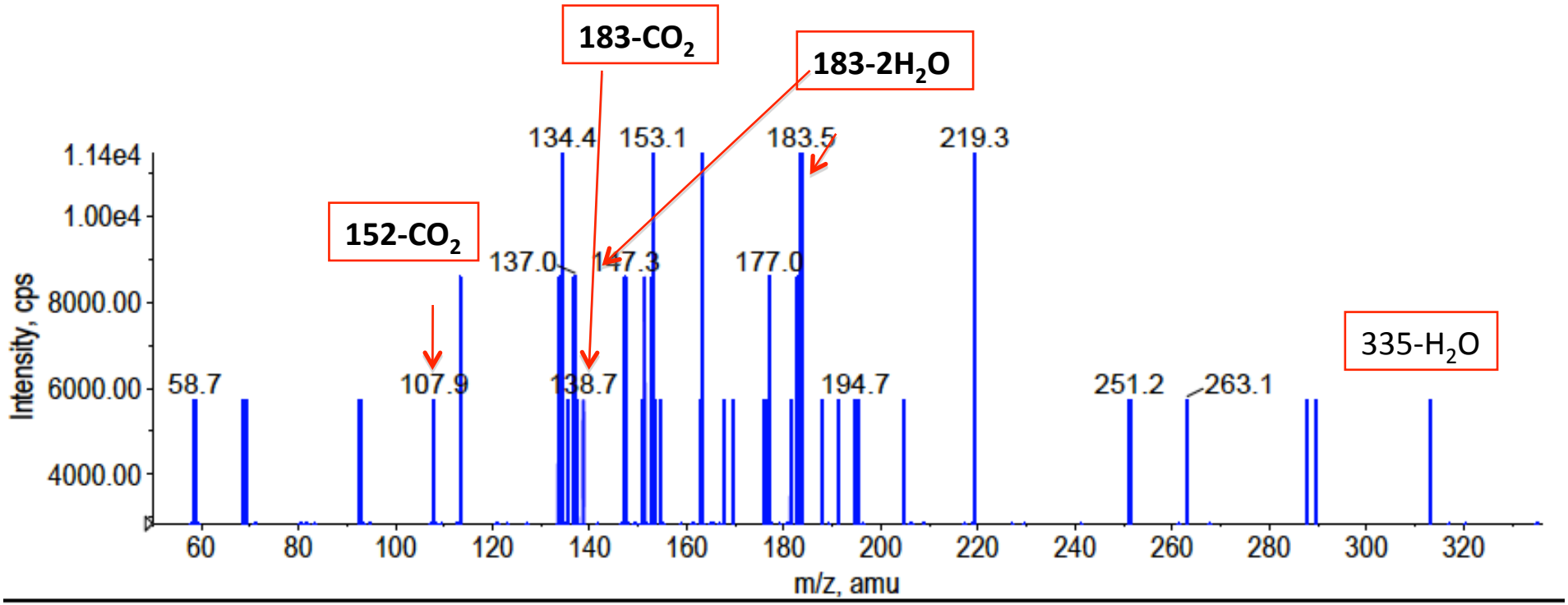


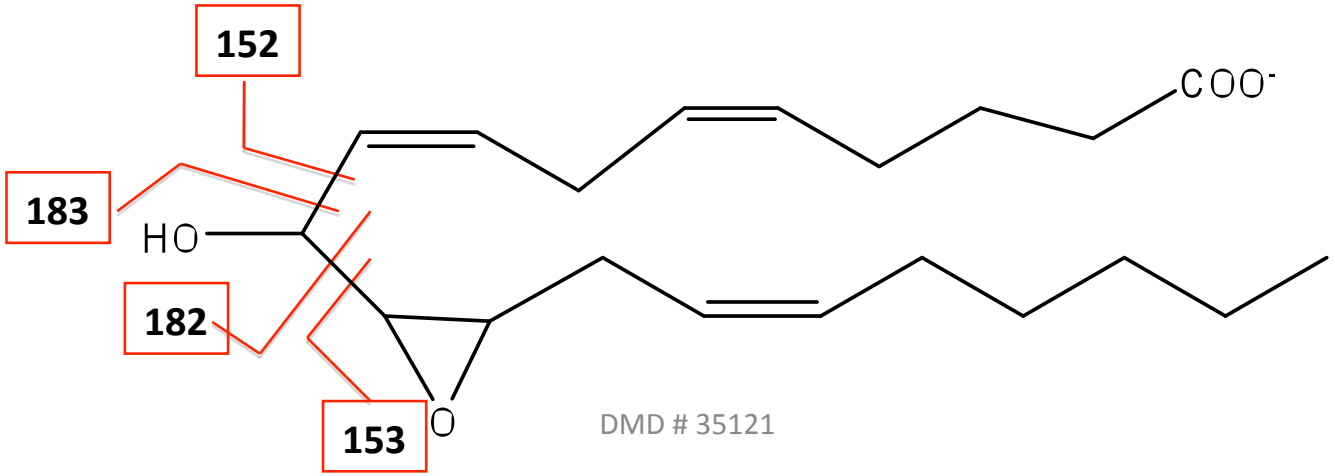
S.Figure 2E) Fragmentation of product 4 = 12.65 min. 8-hydroxy-11,12-epoxy-5,8,14-ecosatrienoic acid, Hepoxilin A_3



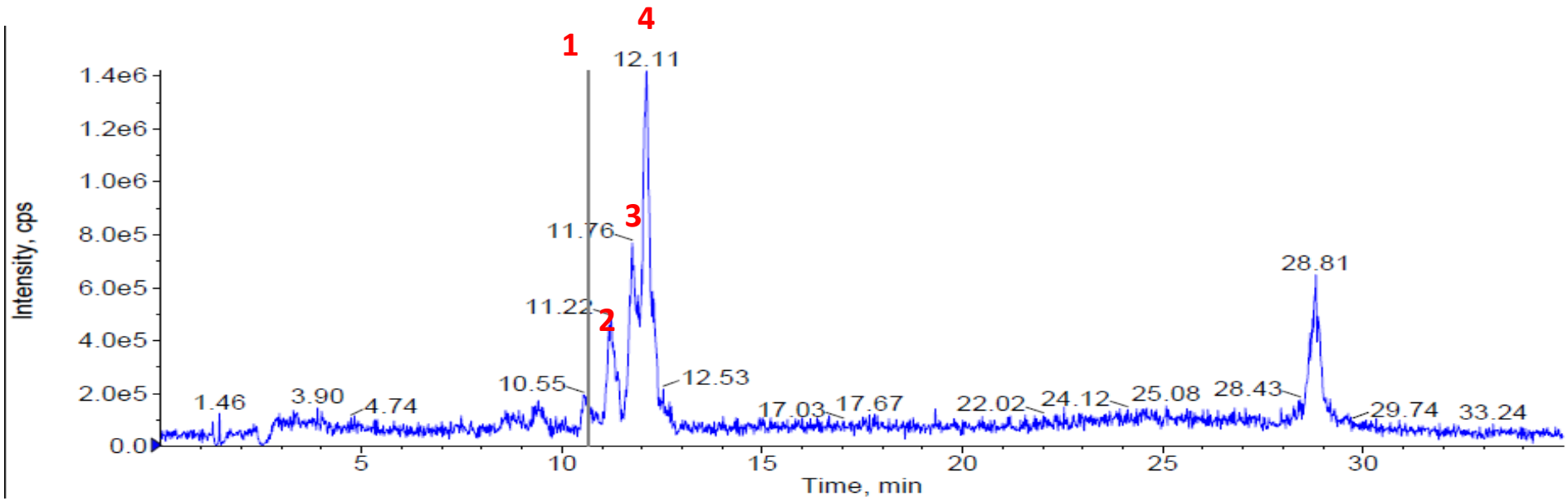


S.Figure 2F) Fragmentation of product 5 = 12.85 min. 10-hydroxy-11,12-epoxy-5,8,14-ecosatrienoic acid), Hepoxilin B_3

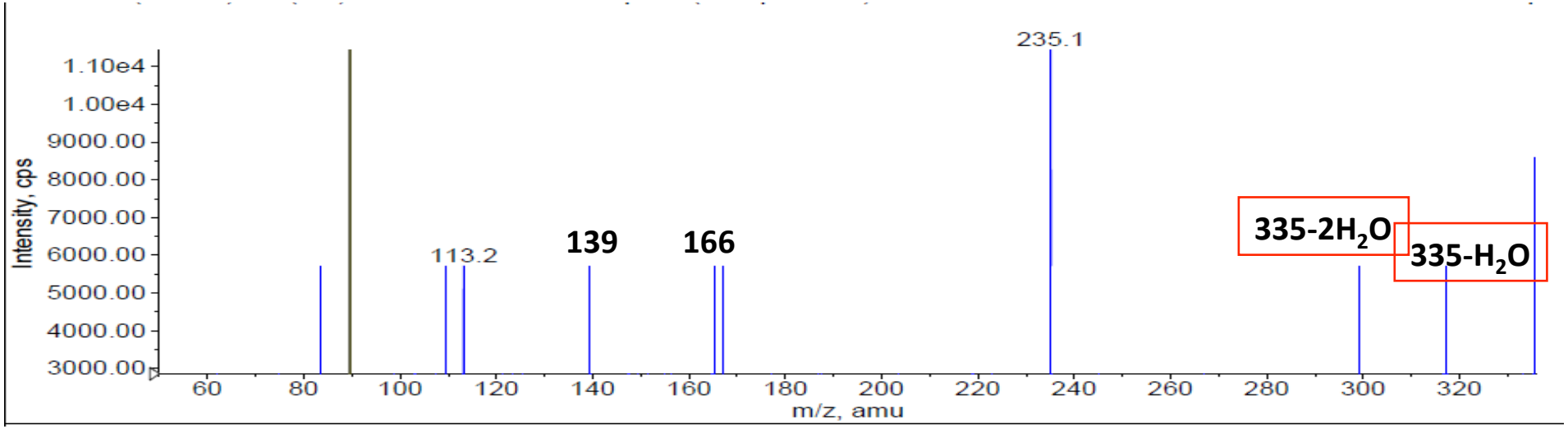


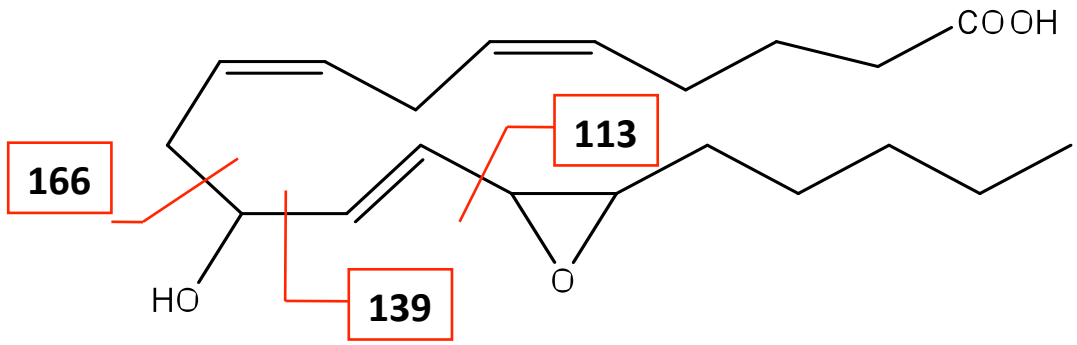


S.Figure 3A) LC-ESI-MS/MS of 15-HpETE incubated with human CYP2S1. Parent mass [M-H]-, 335

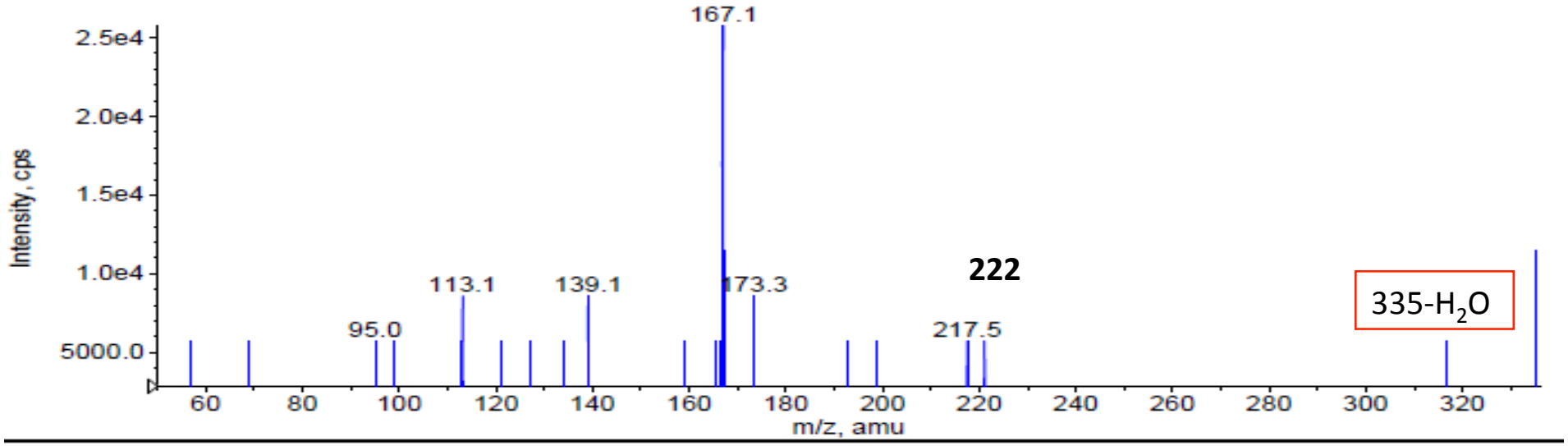


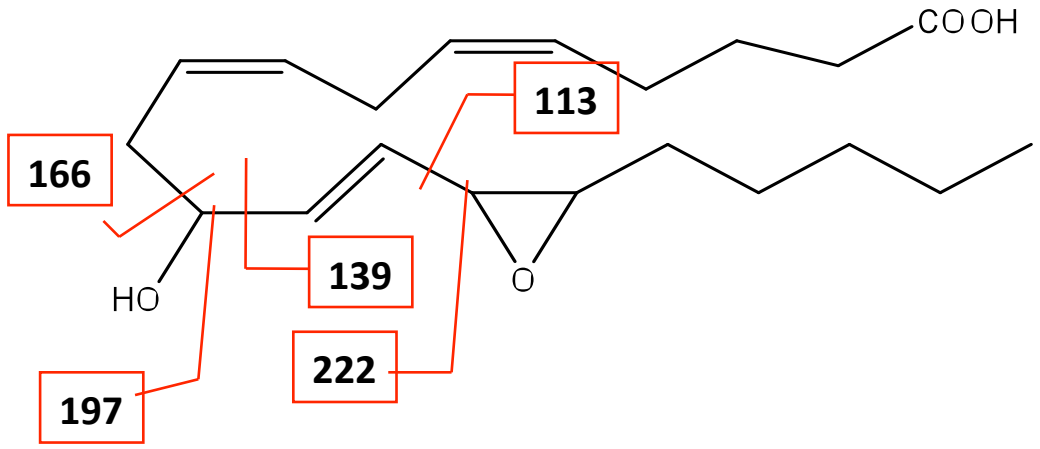
S.Figure 3B) Fragmentation of product 1 = 10.55 min. 11-hydroxy-14.15-epoxy-5,8,12,-eicosatrienoic acid



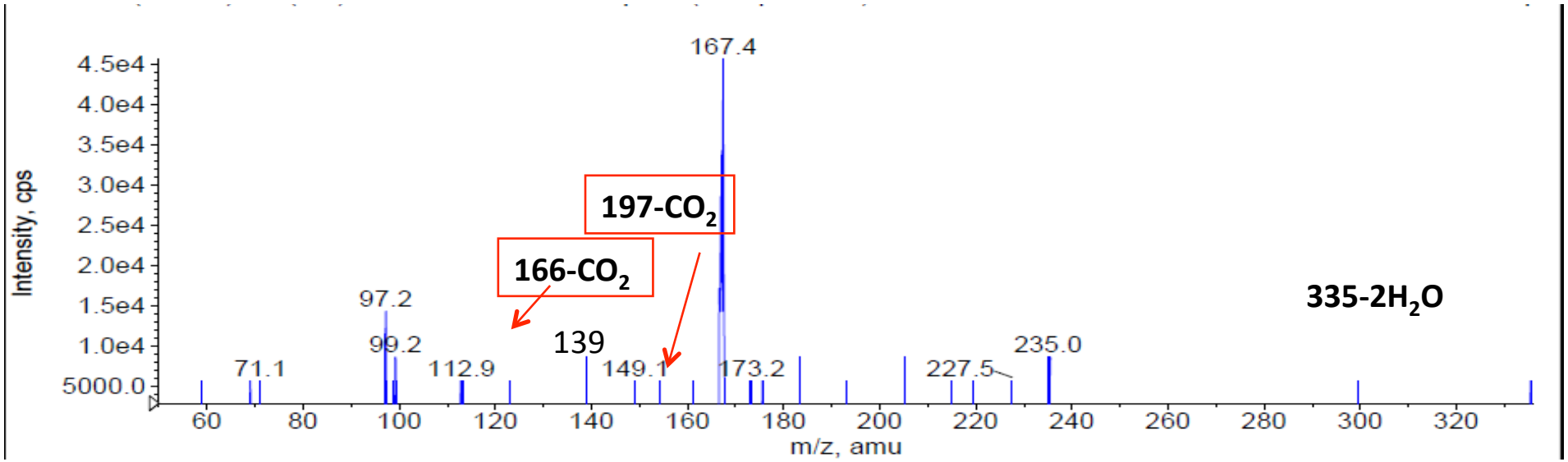


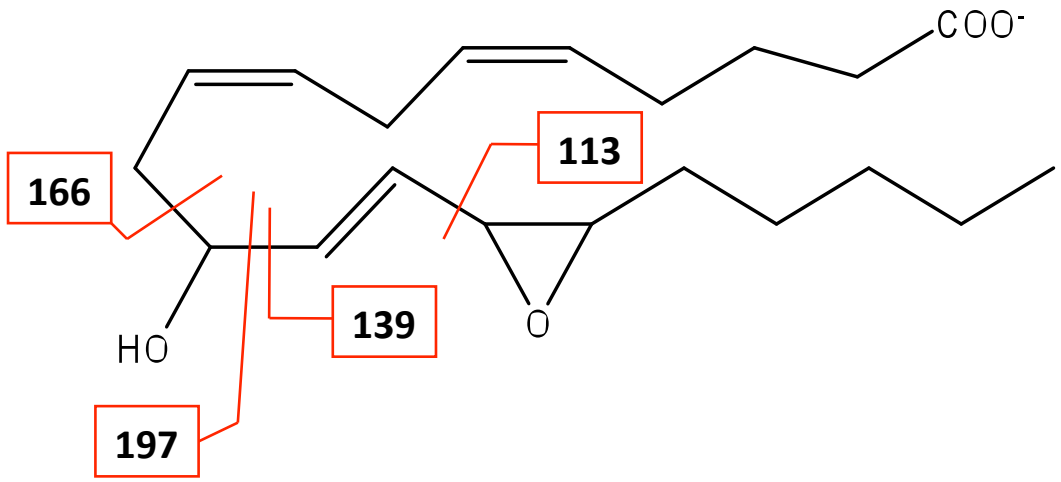
S.Figure 3C) Fragmentation of Product 2: 11.22 min. 11-hydroxy-14.15-epoxy-5,8,12,-eicosatrienoic acid



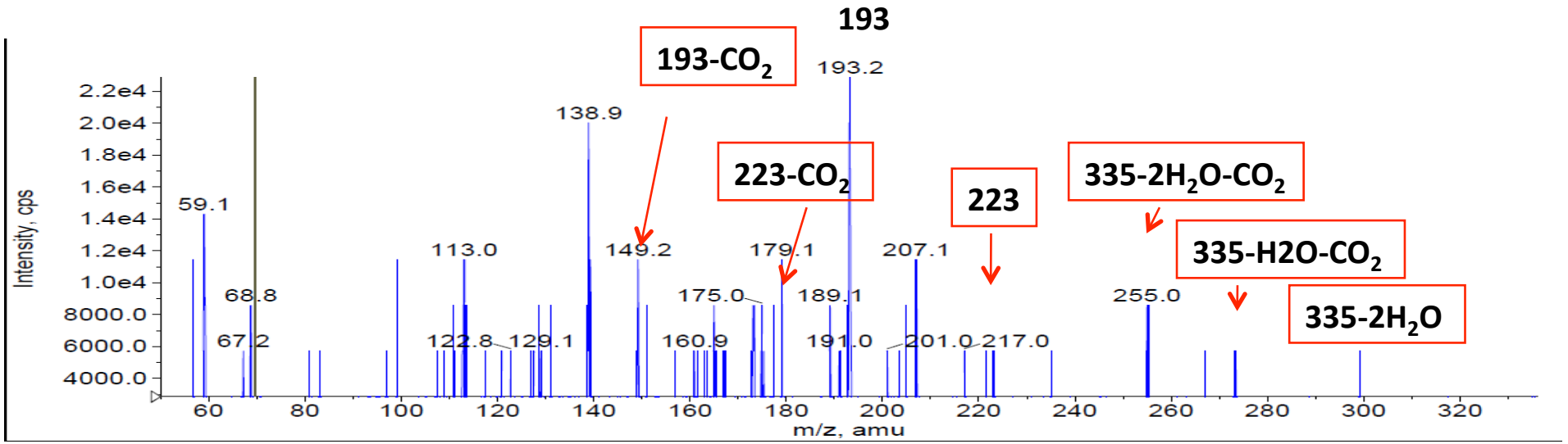


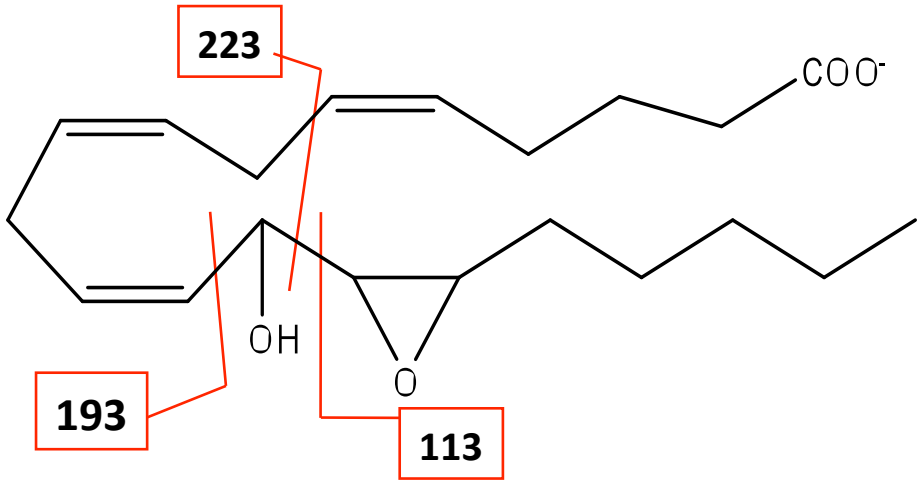
S.Figure 3D) Fragmentation of product 3 = 11.76 min. 11-hydroxy-14.15-epoxy-5,8,12,-eicosatrienoic acid



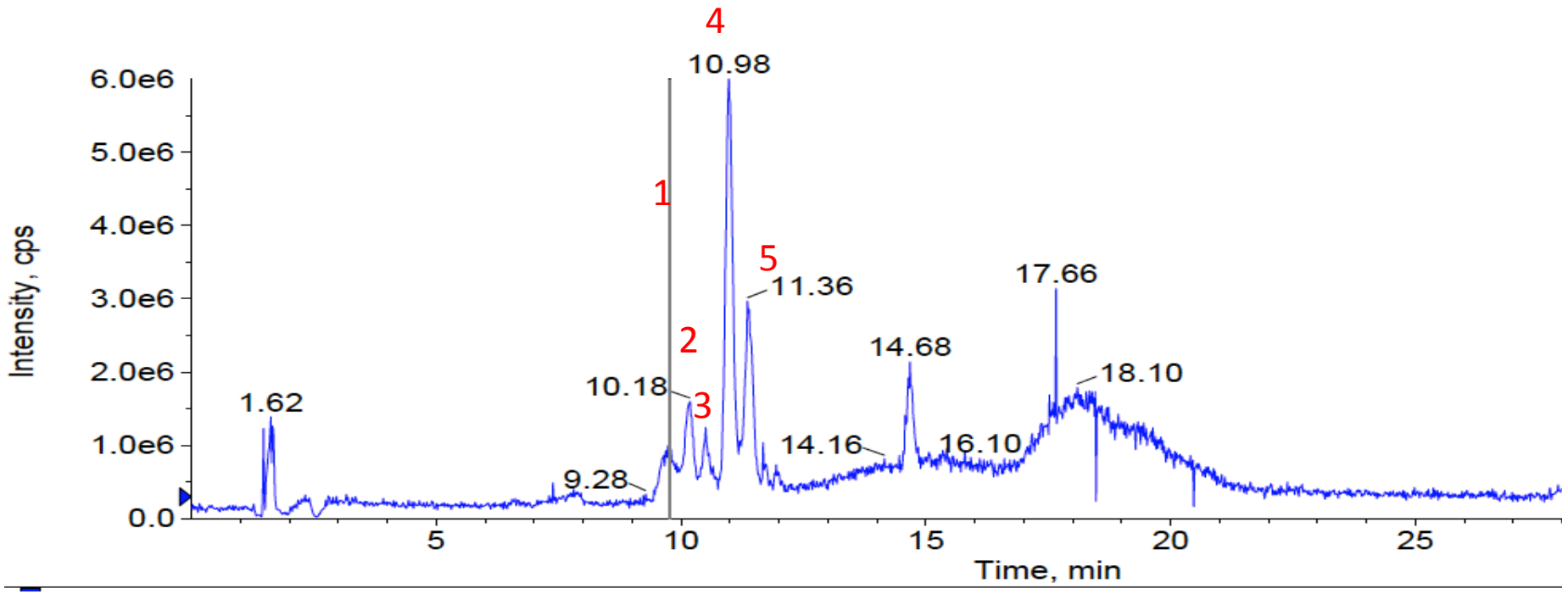


S.Figure 3E) Fragmentation of product 4 = 12.11 min. 13-hydroxy-14.15-epoxy-5,8,11,-eicosatrienoic acid

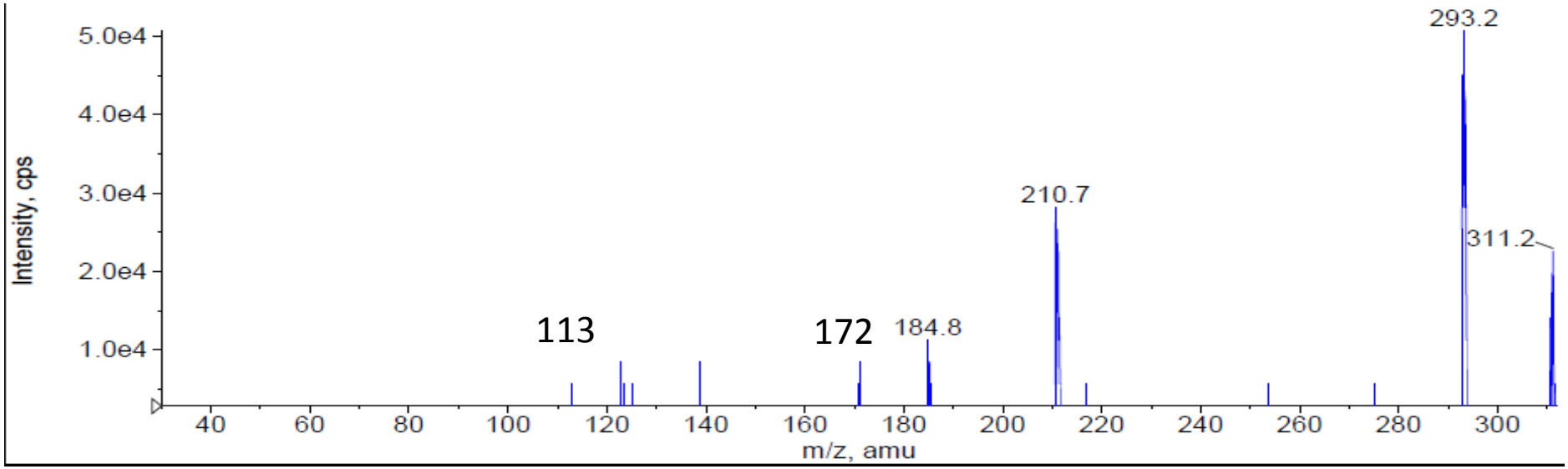


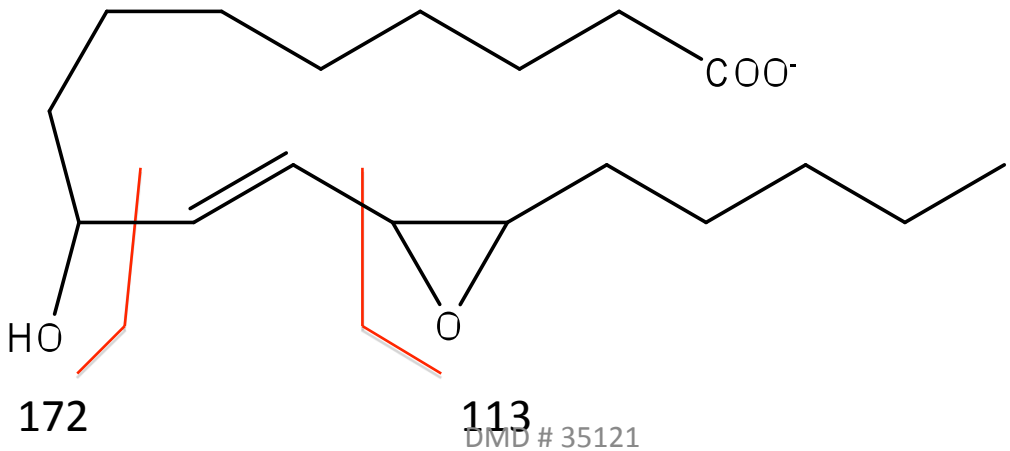


S.Figure 4A) LC-ESI-MS/MS of 13-HpODE incubated with human CYP2S1. Parent mass [M-H]-, 311

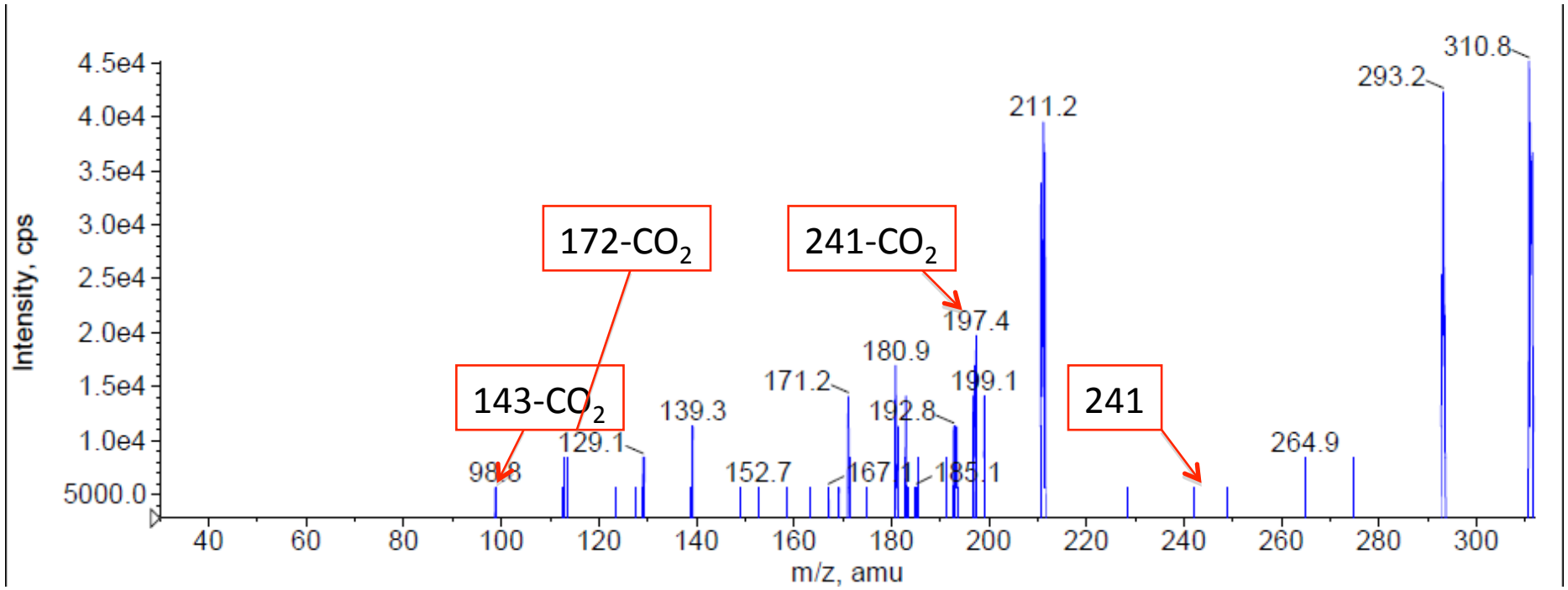


S.Figure 4B) Fragmentation of product 1 = 9.77 min. 9-hydroxy-12,13-epoxy-10-octadecenoic acid





S.Figure 4C) Fragmentation of product 2 = 10.18 min. 9-hydroxy-12,13-epoxy-10-octadecenoic acid



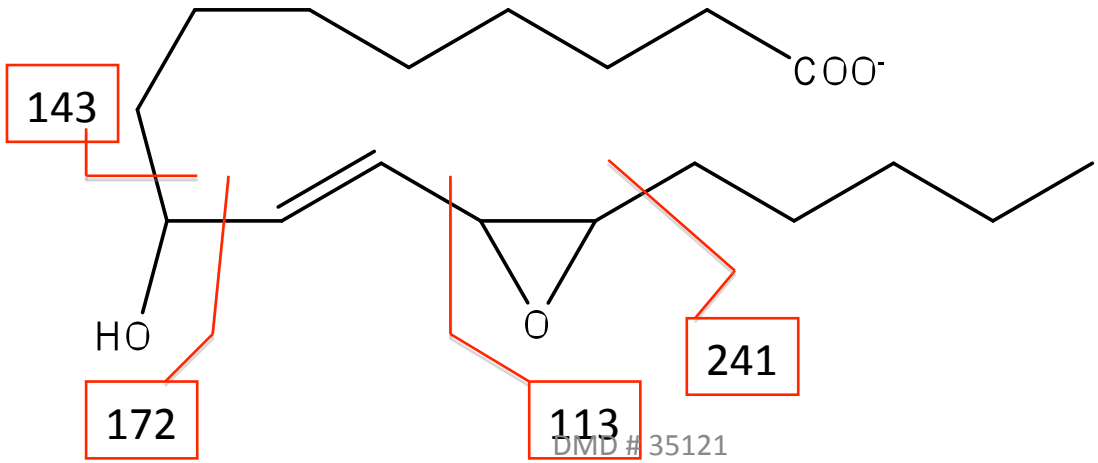
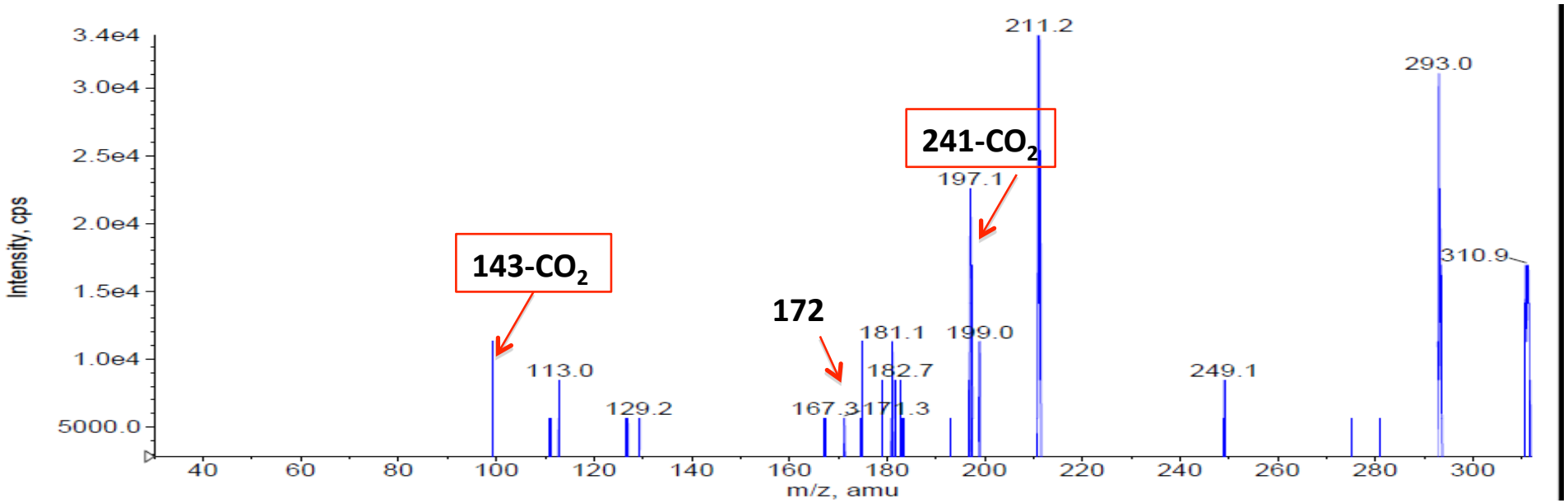


Figure 4D) Fragmentation of product 3 = 10.55 min. 9-hydroxy-12,13-epoxy-10-octadecenoic acid



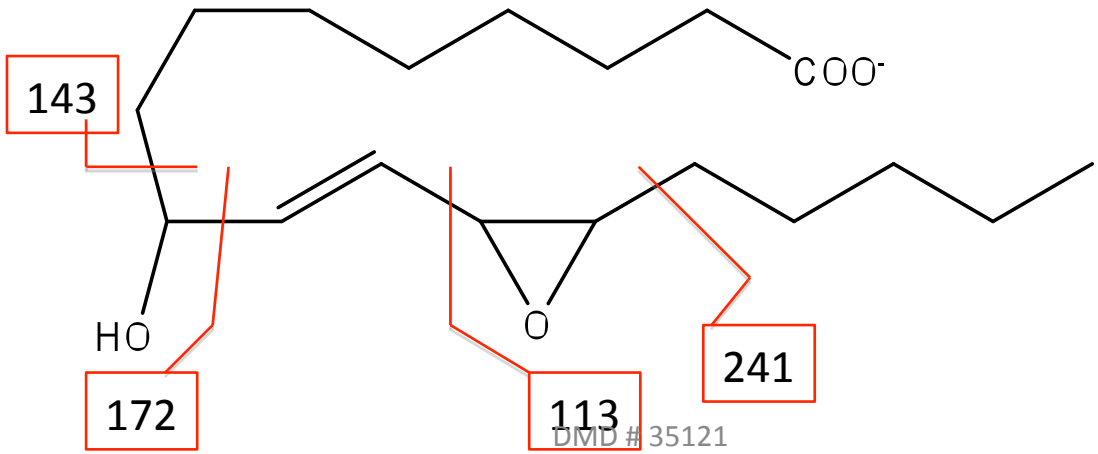
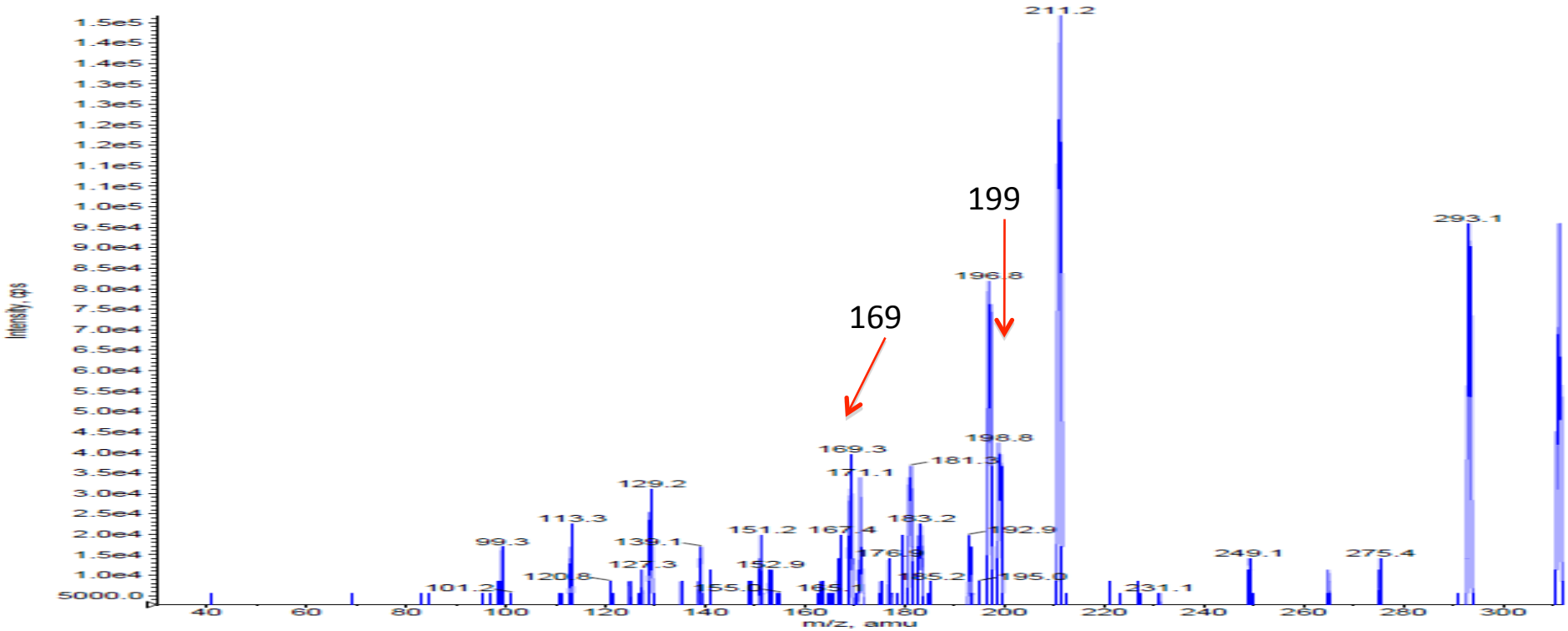
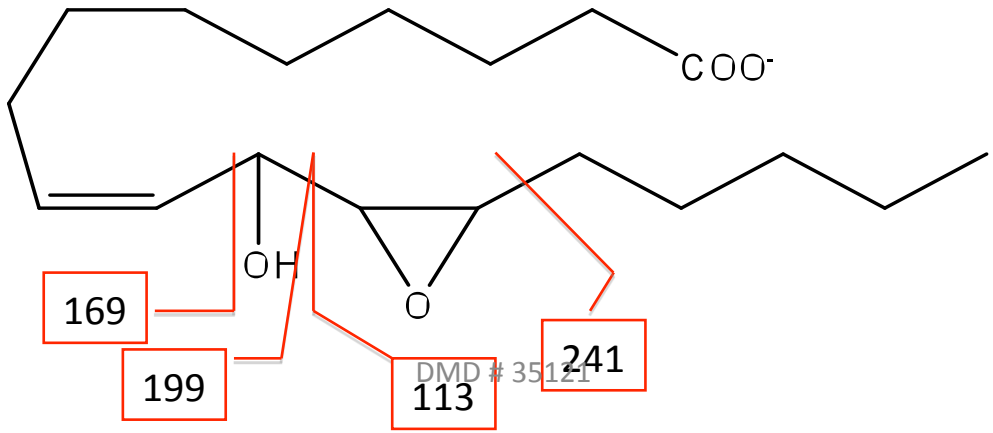
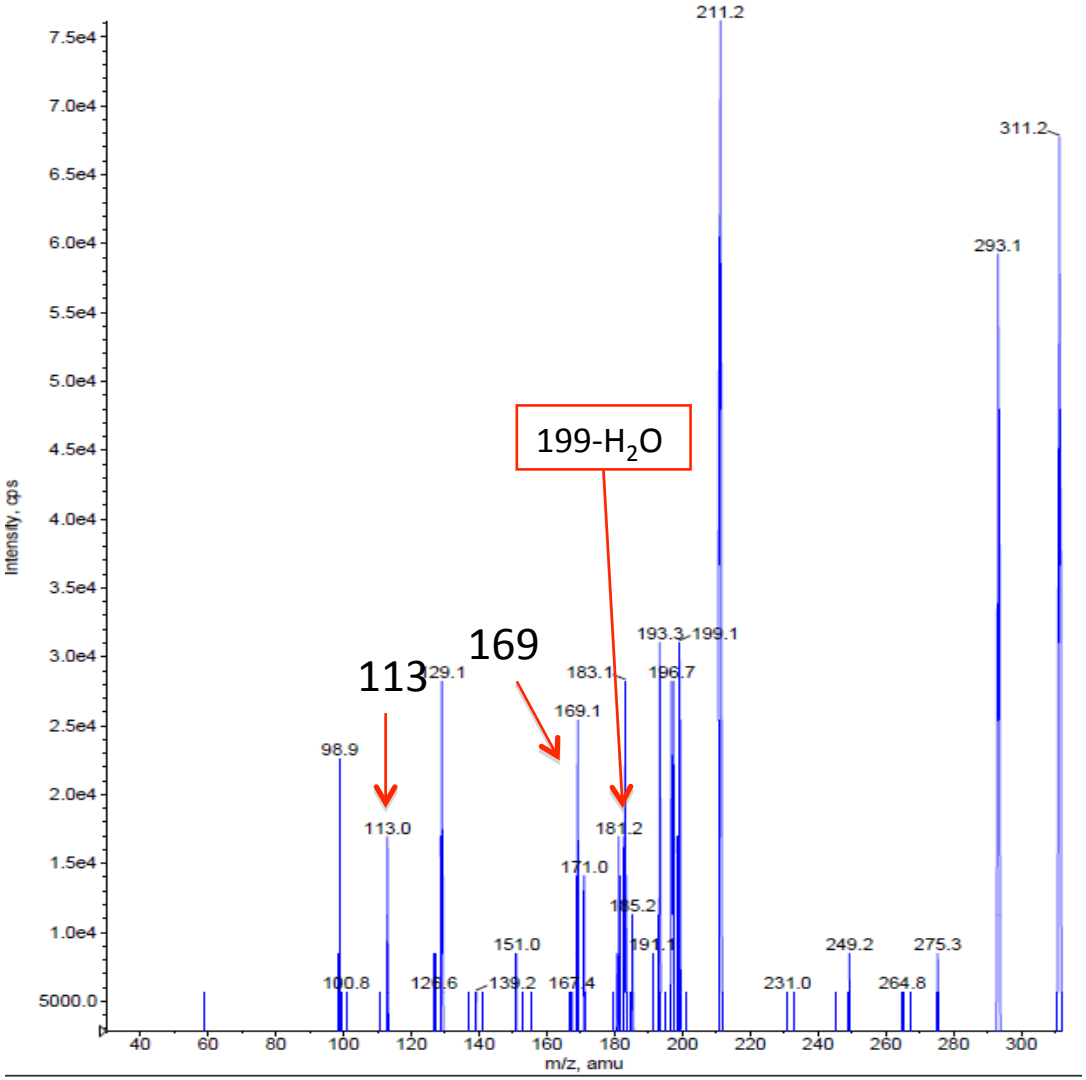


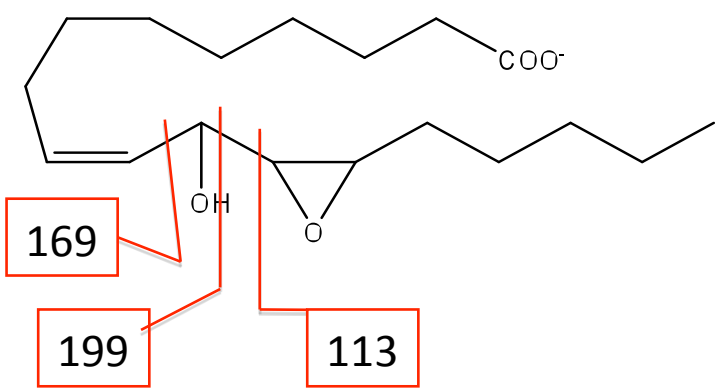
Figure 4E) Fragmentation of product 4 = 10.99 min. 11-hydroxy-12,13-epoxy-9-octadecenoic acid



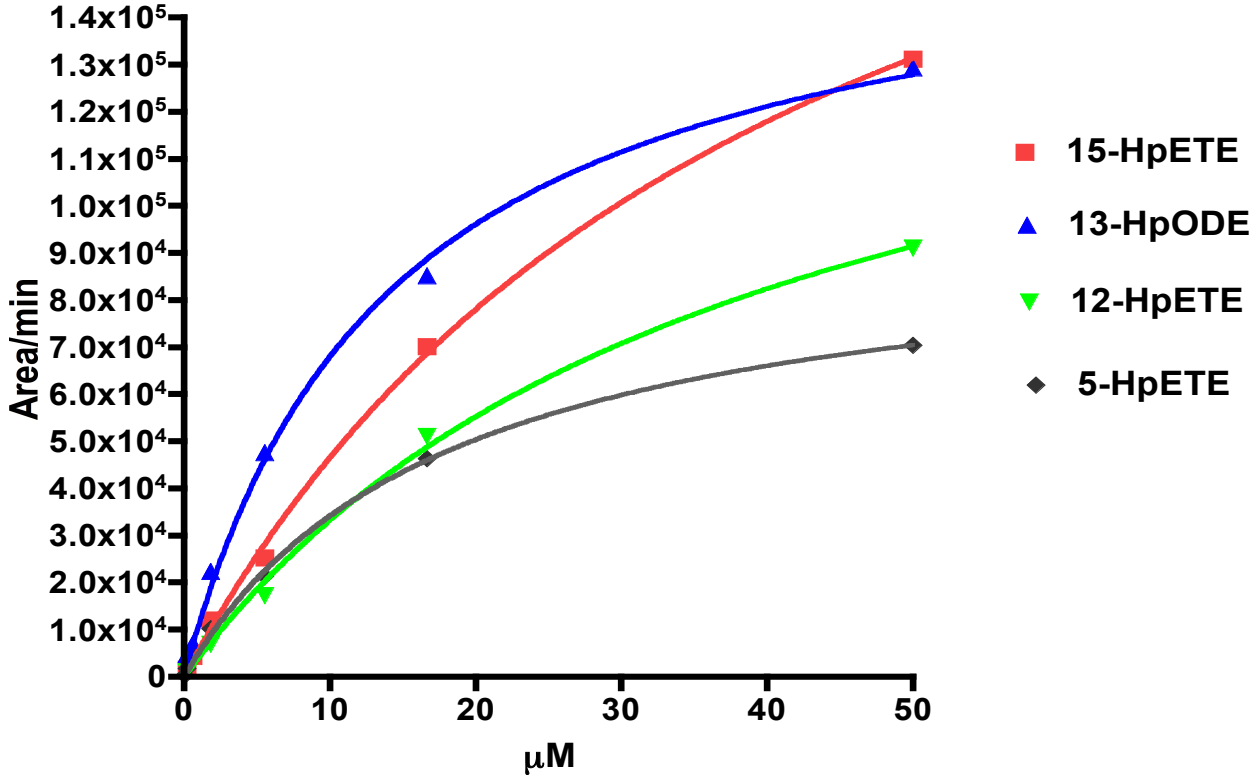


S.Figure 4F) Fragmentation of product 5 = 11.36 min. 11-hydroxy-12,13-epoxy-9-octadecenoic acid





S.Figure 5) Michaelis-Menten plot for Km determinations and the parameters for goodness of fit.



Goodness of Fit	15-HpETE	13-HpODE	12-HpETE	5-HpETE
Degrees of Freedom	4	4	4	5
R squared	0.9988	0.9972	0.9975	0.9987
Absolute Sum of Squares	1.51E+07	3.37E+07	1.57E+07	5.42E+06
95% Confidence Intervals for Km	30.35 to 53.61	10.04 to 18.19	23.63 to 54.06	14.69 to 21.39

S. Figure 6) The analysis of human CYP2S1 and PGG_2 incubation using mass spectrometry. Products were detected using selected ion monitoring (SIM) mode with m/z set at 349 $[M-H]^-$. Red represents human CYP2S1 and PGG_2 incubation. Blue represents heated CYP2S1 and PGG_2 incubation. * are products that have m/z $[M-H]^-$ 349. The m/z $[M-H]^-$ of 15-keto-PGG_2 and 12-oxoHT is 349 and 278 respectively. No product was detected at m/z 278 $[M-H]^-$.