Mechanistic Investigations of Human Reticulocyte 15- and Platelet 12-lipoxygenases with Arachidonic Acid†

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Supporting Information

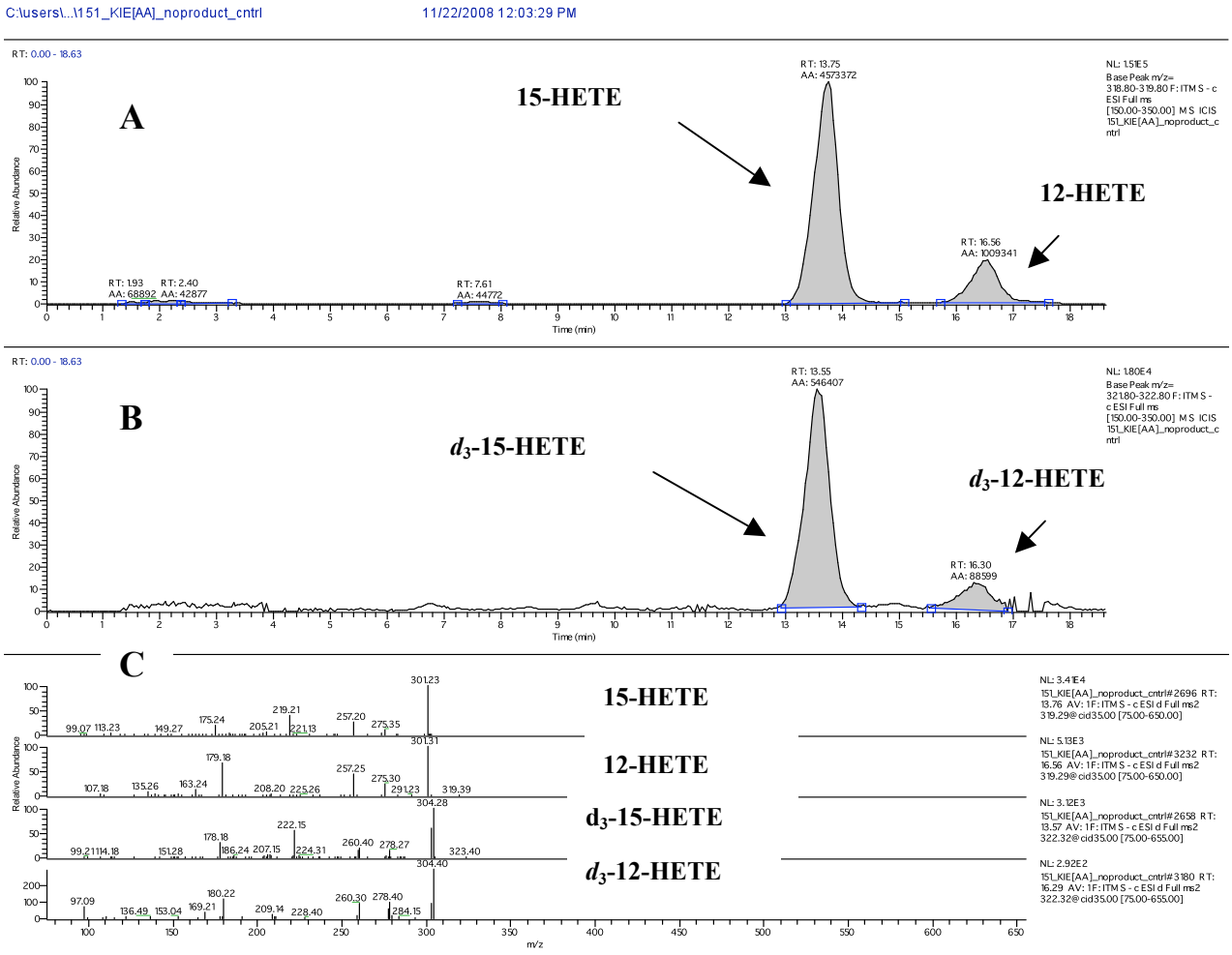

Figure S1. Chromatogram of the competitive KIE[AA] experiment for 15-hLO-1 using LC-MS/MS. Total Ion Count (TIC) chromatogram for protiated 15-HETE (319.3 m/z) and 12-HETE (319.3 m/z) (Panel A) and deuterated 15-HETE (d_3-15-HETE) (322.3 m/z) and 12-HETE (d_3-12-HETE) (322.3 m/z) (Panel B). Products were generated from a (1:1) mixture of d_4-AA:H-AA, and a single deuterium atom was abstracted during catalysis. Fragmentation patterns of all products (Panel C). Note: trace amounts of d_3-deuterated AA can be seen at 303.3 m/z for both deuterated products, however, the total amount of d_3-deuterated AA is less than 1% of total deuterated substrate, and has a negligible affect on the determined KIE. $^{D}k_{cat}/K_m$ is determined using the ratio of the protiated/deuterated calculated areas: C13 KIE = (15-HETE/d_3-15-HETE) and C10 KIE = (12-HETE/d_3-12-HETE). All enzymatic assays were run in at least triplicate, using 25 mM Hepes buffer (pH 7.5).