"Robust Inhibitory Effects of Conjugated Linolenic Acids on a Cyclooxygenase-related Linoleate 10*S*-Dioxygenase: Comparison with COX-1 and COX-2

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SUPPLEMENTARY DATA

Analyses of commercial linoleic acid

Shipments of linoleic acid were stored at minus 20 °C upon receipt.
UV and HPLC analyses were performed on the day the ampoule or vial was opened in the laboratory (usually within a few weeks of shipment).

The linoleic acid is dissolved at a concentration of 10 or 20 mg/ml in ethanol (that had been bubbled with argon for 15 to 30 min), overlaid with argon, and kept on ice during the UV and HPLC analyses.

UV analysis: The UV spectrum (200 – 350 nm) of linoleic acid is recorded at a concentration of 100 μg/ml in ethanol. This gives a saturated signal for the linoleate below 210 nm (not taken into consideration) and readily detectable impurities, e.g. potential conjugated dienes (around 233 – 235 nm) and conjugated trienes (around 270 nm).

For example (with a linoleic acid concentration of 100 μg/ml):

- an absorbance of 0.1 AU at 235 nm of HPODE (ε = 25,000 for conjugated diene) corresponds to 1.25 μg/ml and therefore just over 1 % impurity in 100 μg/ml linoleic acid.
- an absorbance of 0.1 AU at 270 nm in a conjugated triene fatty acid (ε = 50,000) corresponds to 0.56 μg/ml and therefore about 0.5% impurity in 100 μg/ml linoleic acid.

RP-HPLC/UV analysis: An aliquot of approximately 5 - 10 μg is analyzed on RP-HPLC, sufficient to give a strong signal for linoleate at 205 nm, with the peak absorbance less than 1 AU.

Column: Waters Symmetry C18 column (25 x 0.46 cm) with a solvent of methanol/water/glacial acetic acid in the proportions 90/10/0.01 (by volume) and a flow rate of 1 ml/min.

The retention time of linoleic acid in the 90/10/0.01 solvent is about 16 - 19 min (varying with the column used, different batches of solvent, or the presence or absence of a guard column).

Silver ion HPLC: Fig. S10 illustrates purification of linoleic acid using argentation HPLC

The UV signals are recorded using an Agilent 1100 series diode array detector with all channel wavelengths on the same sensitivity and set to record at:
205 nm - in blue in the figures,
220 nm - in green,
235 nm – in red,
270 nm – in black

Note: Linoleic acid has relatively weak absorbance in the low UV. It is difficult to define a molar extinction coefficient. According to our observations the value is very approximately 7,000 [± 1,500, range] at 205 nM (the lowest wavelength monitored in our HPLC system). Therefore this signal is around 3 - 4 times weaker than a conjugated diene at its λmax (ε = 25,000 at ~235 nm) and 6 - 8 times weaker than a conjugated triene (ε = 50,000 at ~270 nm).

To consider minor impurities in perspective to the large peak of linoleic acid, note that on the Agilent UV diode array detector the peak height of linoleate recorded at 220 nm is approximately 2 % of the peak height at 205 nm.

Figure S1: A suspect batch of linoleic acid is poorly metabolized by *Nostoc* 10*S*-DOX

(A, B) Oxygen uptake assays of *Nostoc* 10*S*-DOX that first pointed to a suspect batch of linoleic acid. There was an unexpectedly slow rate and incomplete reaction with the suspect sample (A), whereas the highly purified linoleic acid reacts faster and is completely consumed (B).

(C, D) In a later analysis, using a higher concentration of linoleic acid (200 μM), the differences are even more apparent.

Suspect batch of Linoleic Acid: Nu-Chek, lot U59A-A3-W (Fig. 1, Fig. S2, S3, S10)
Purified Linoleic Acid: Nu-Chek, lot U59A-S24-S (Fig. S2, S4)

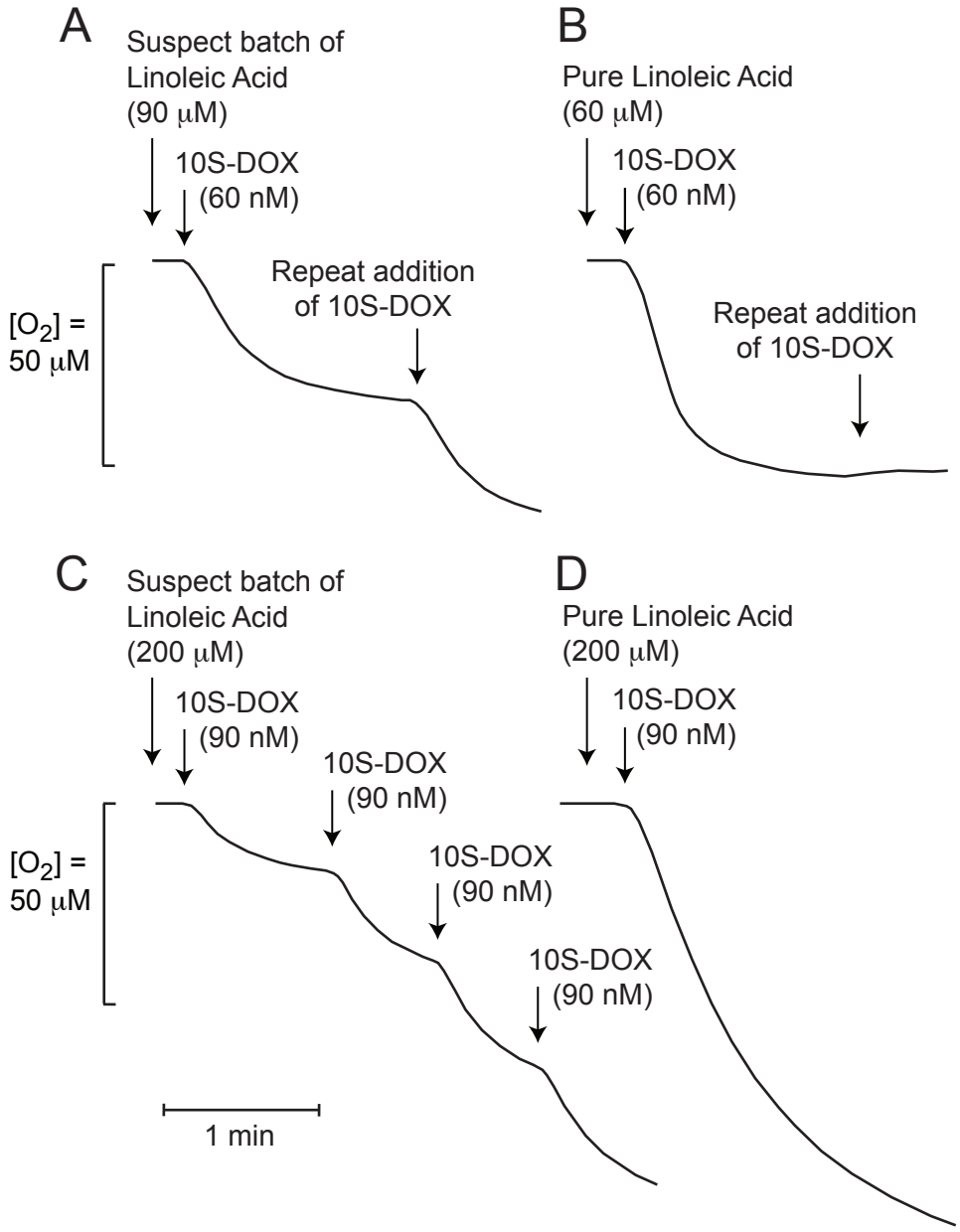


Figure S2:
UV spectra (200-330 nm) of Linoleic Acids (100 μg/ml in ethanol) from freshly opened ampoules

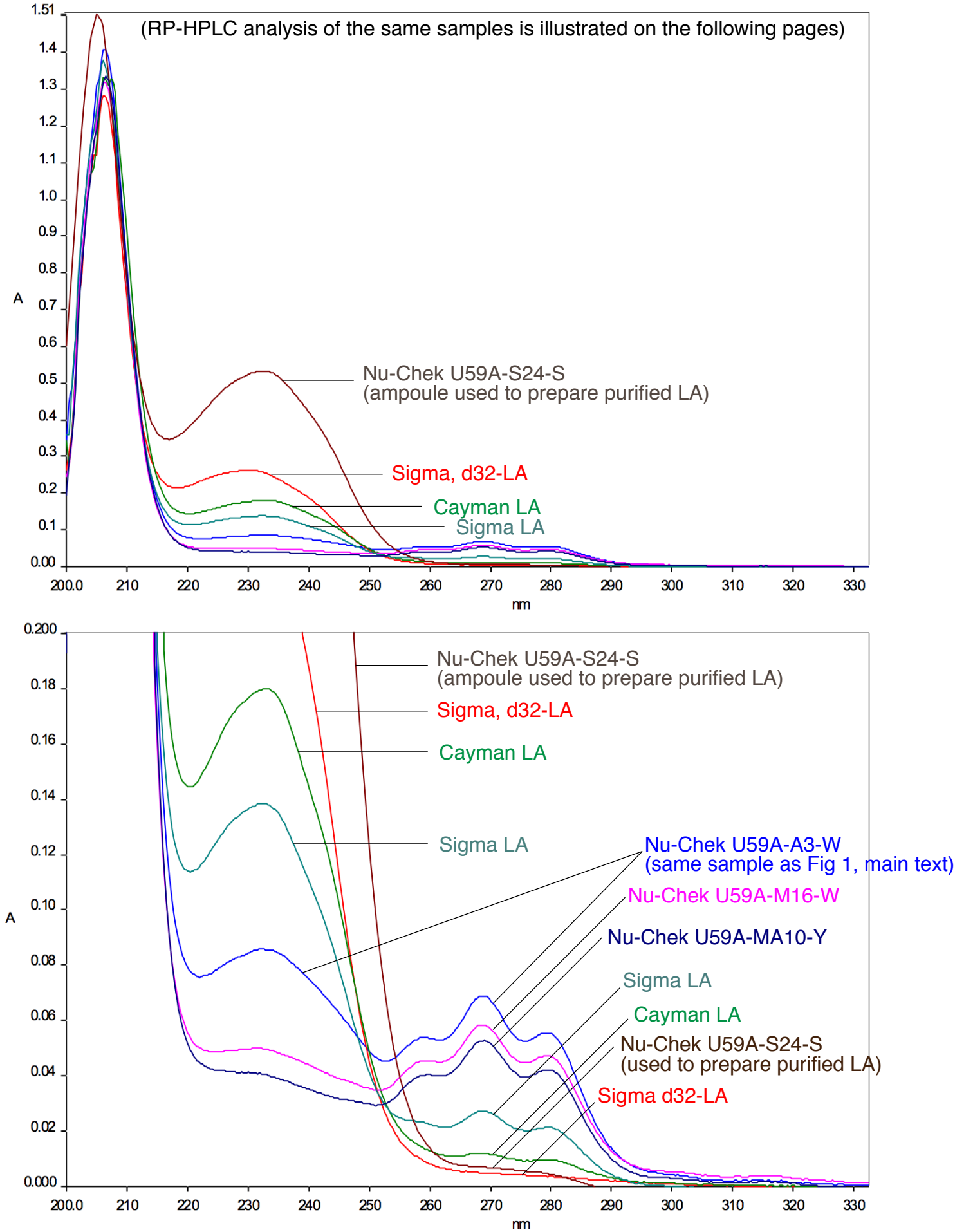
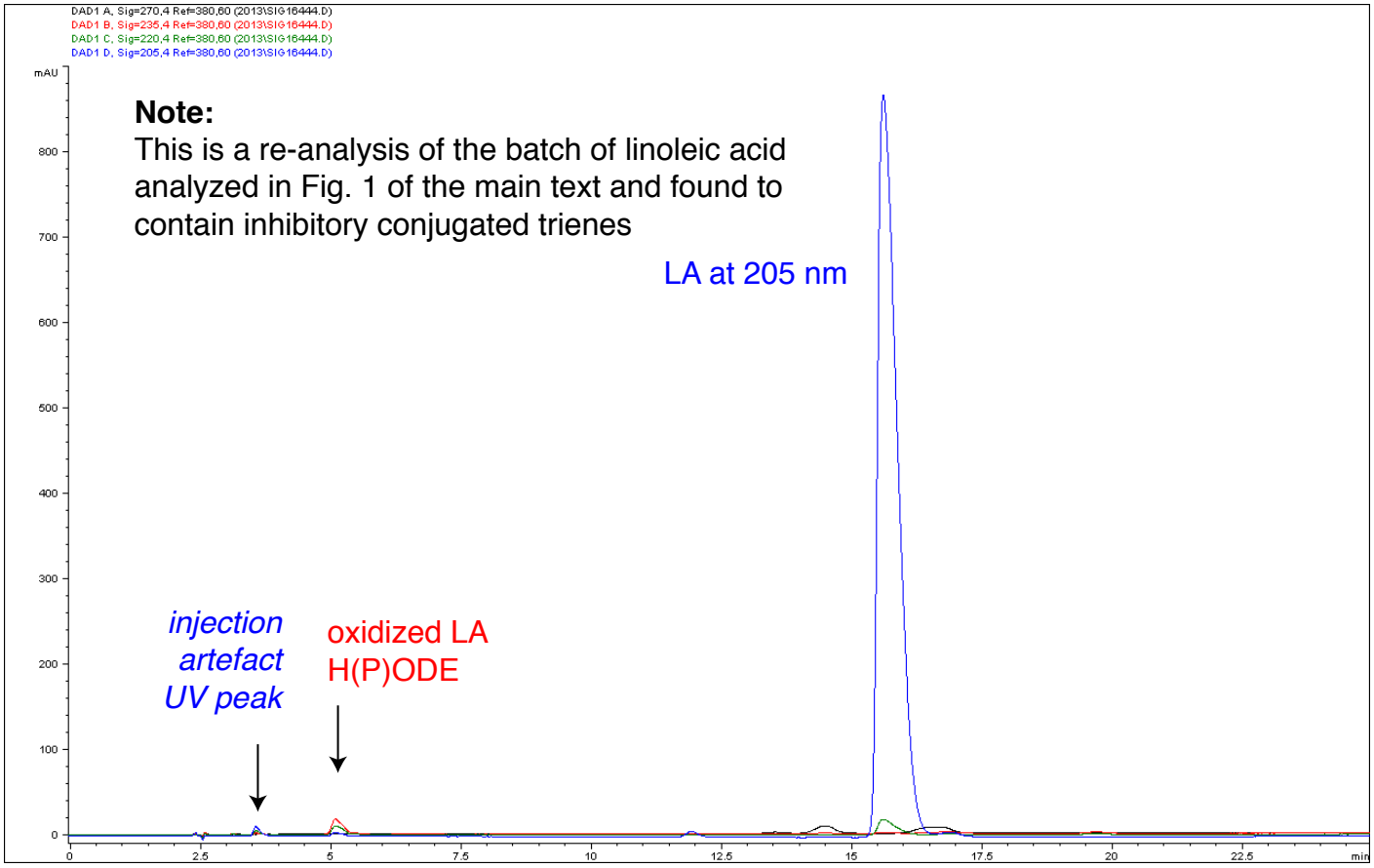


Figure S3:
Nu-Chek Prep, Linoleic Acid (LA), lot U59A-A3-W, supplied as >100 mg in sealed ampoule



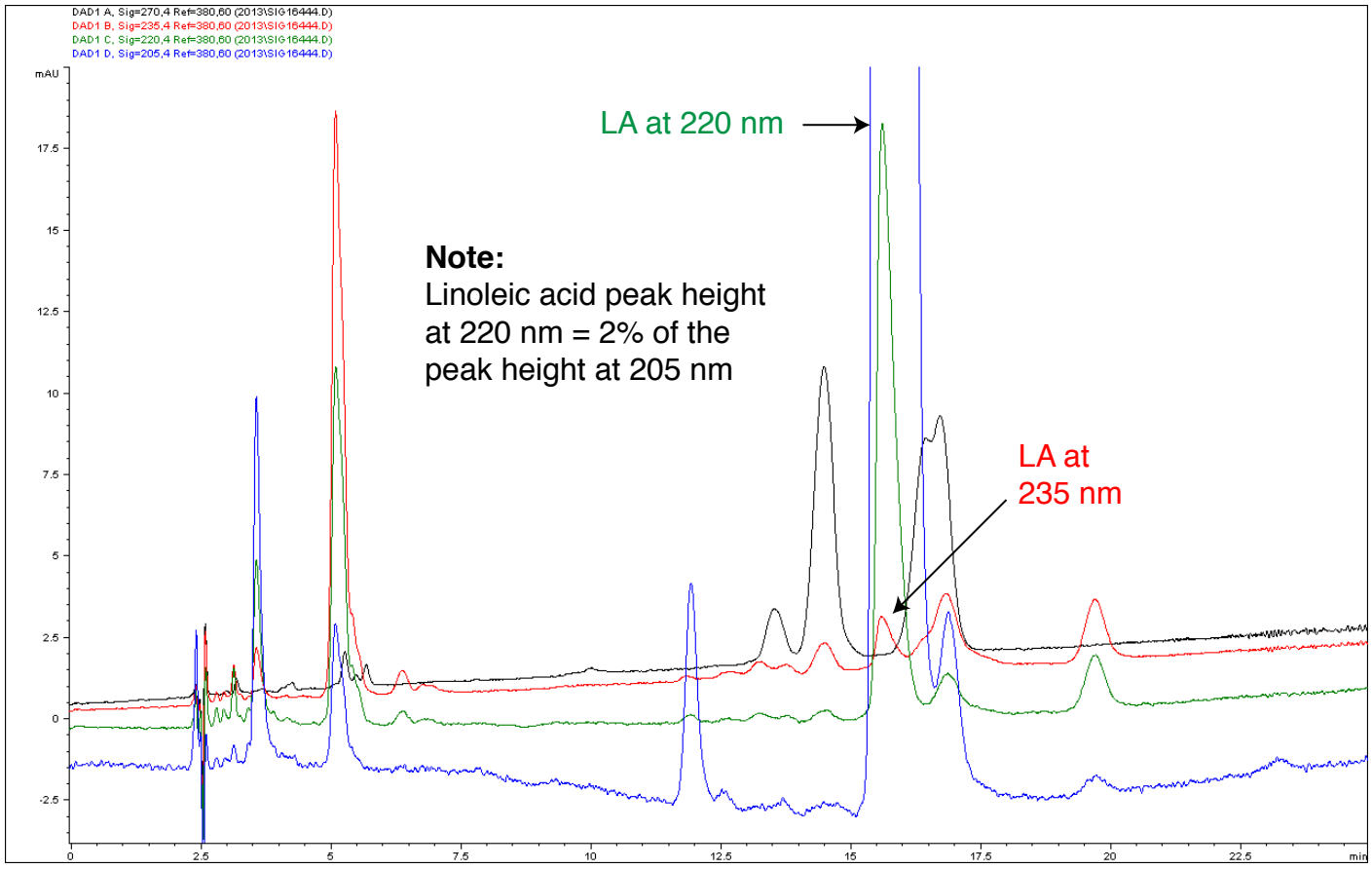
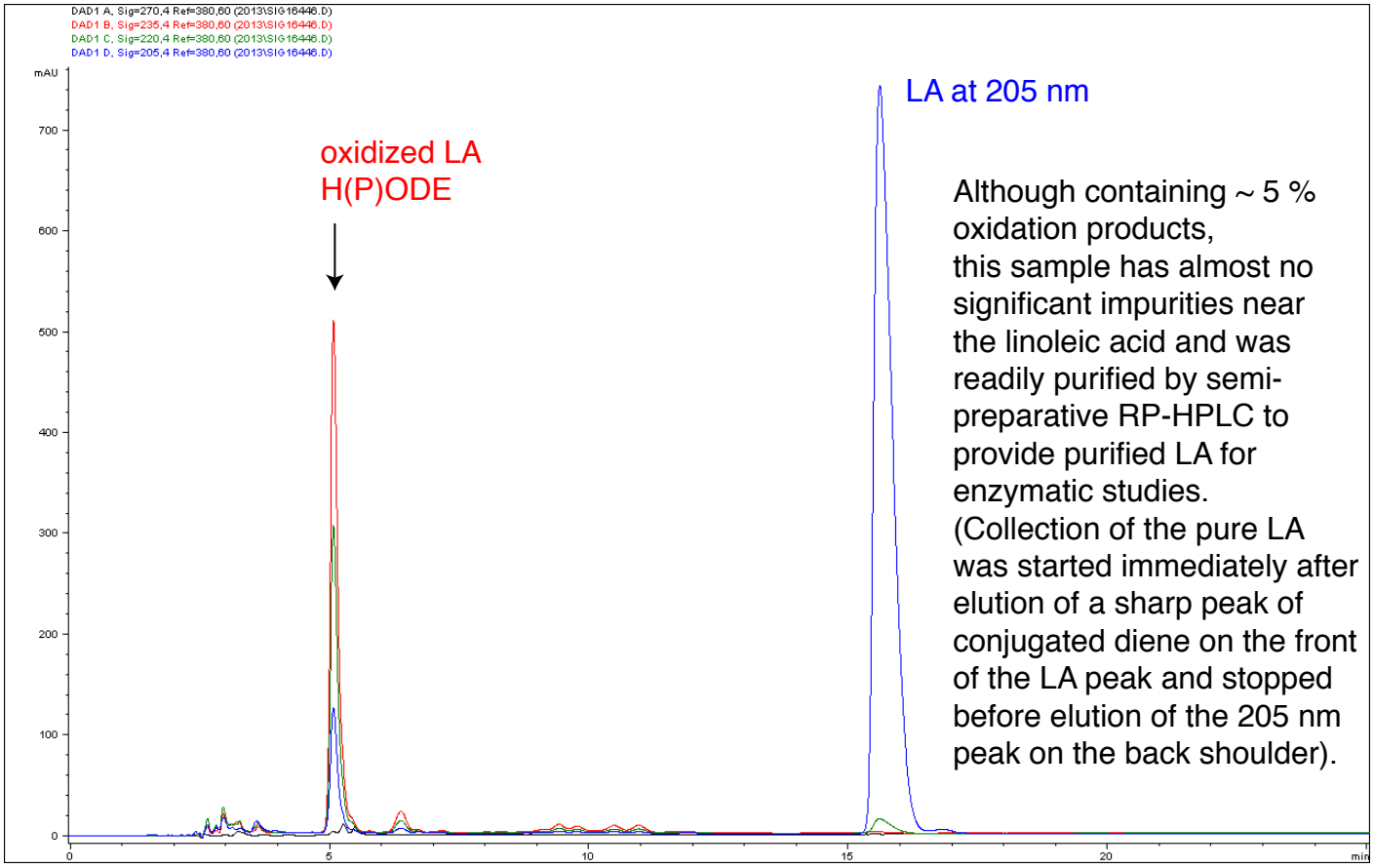


Figure S4:
Nu-Chek Prep, Linoleic Acid (LA), lot U59A-S24-S, supplied as >100 mg in sealed ampoule



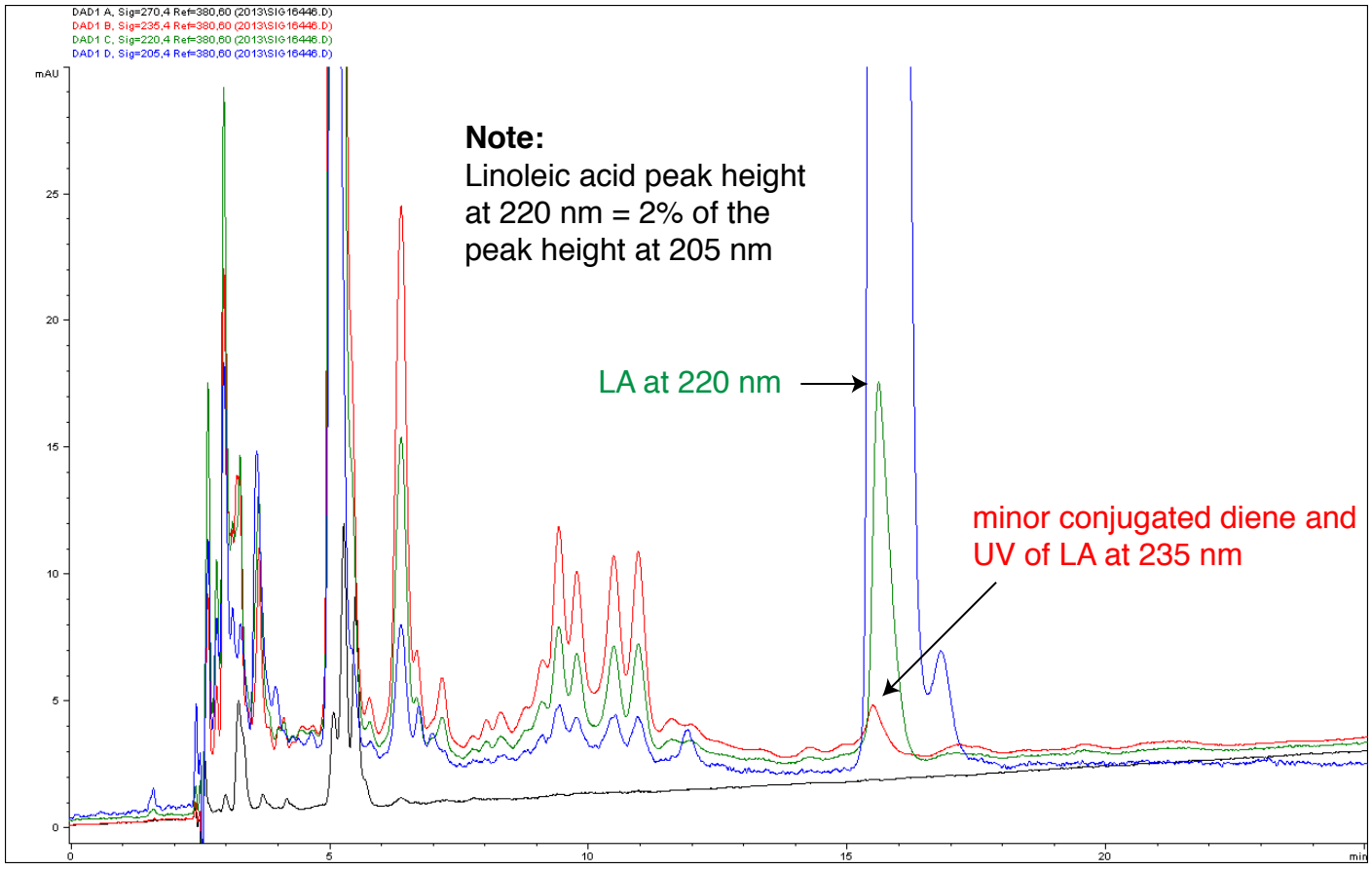
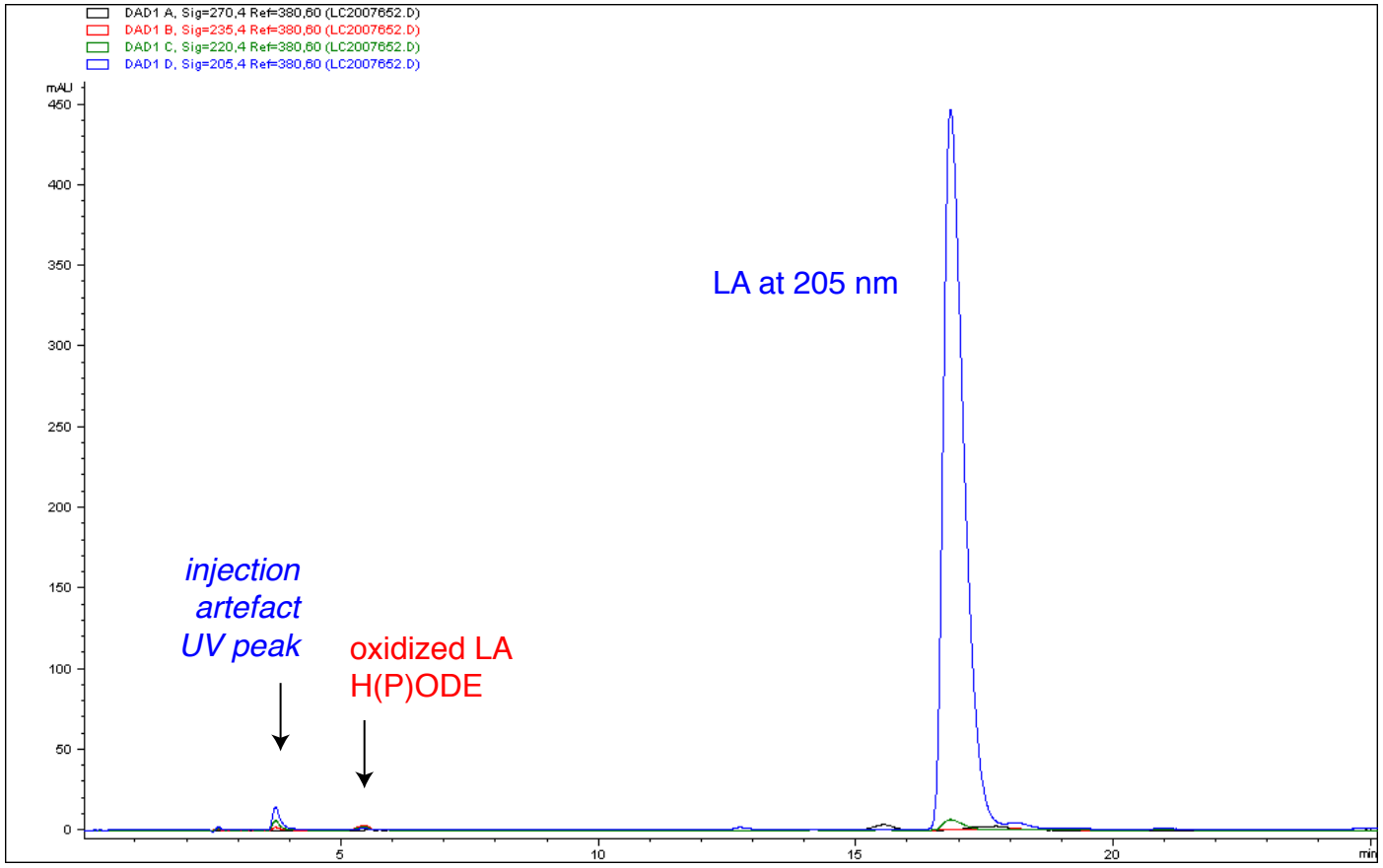


Figure S5:
Nu-Chek Prep, Linoleic Acid (LA), lot U59A-M16-W, supplied as >100 mg in sealed ampoule



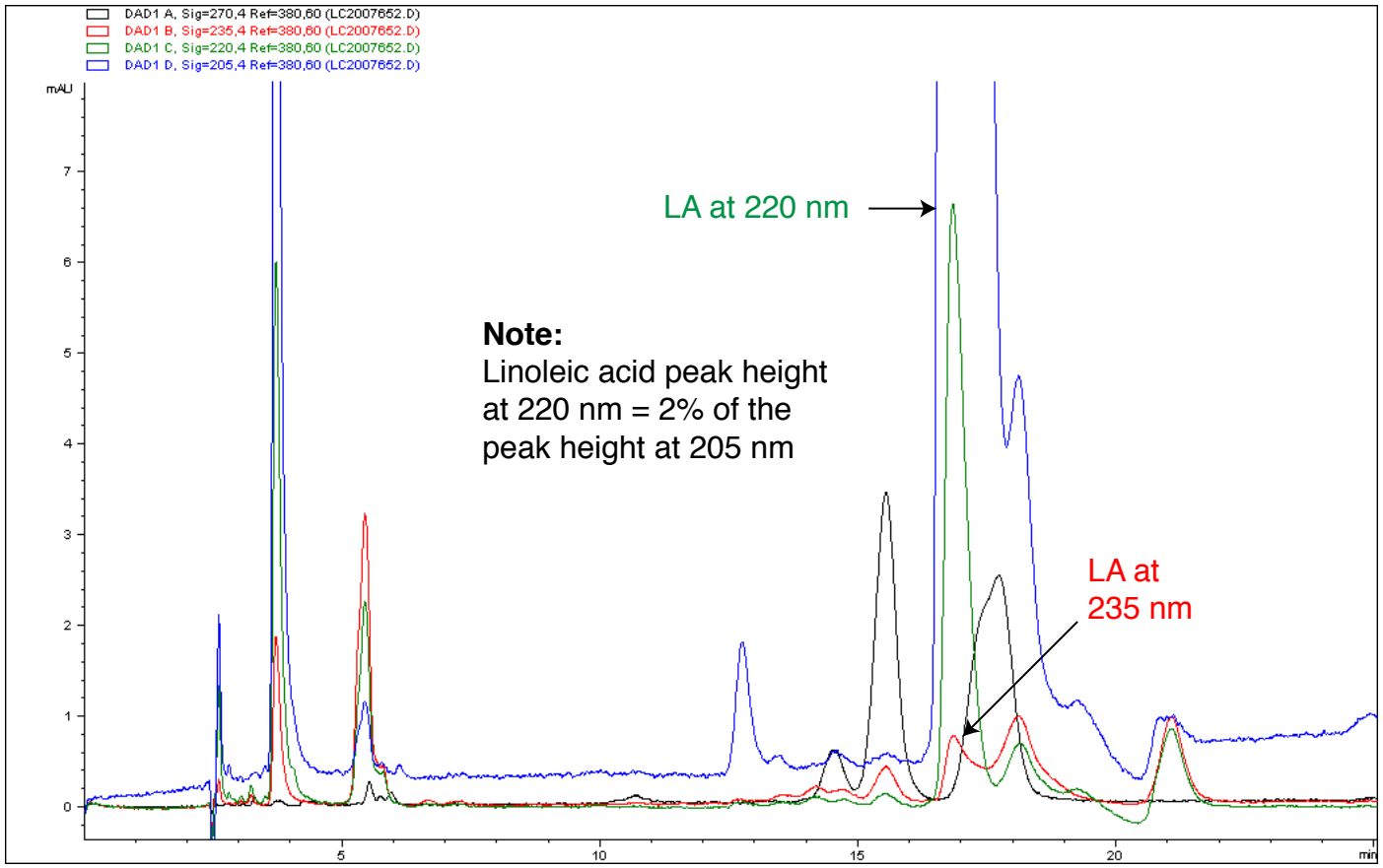
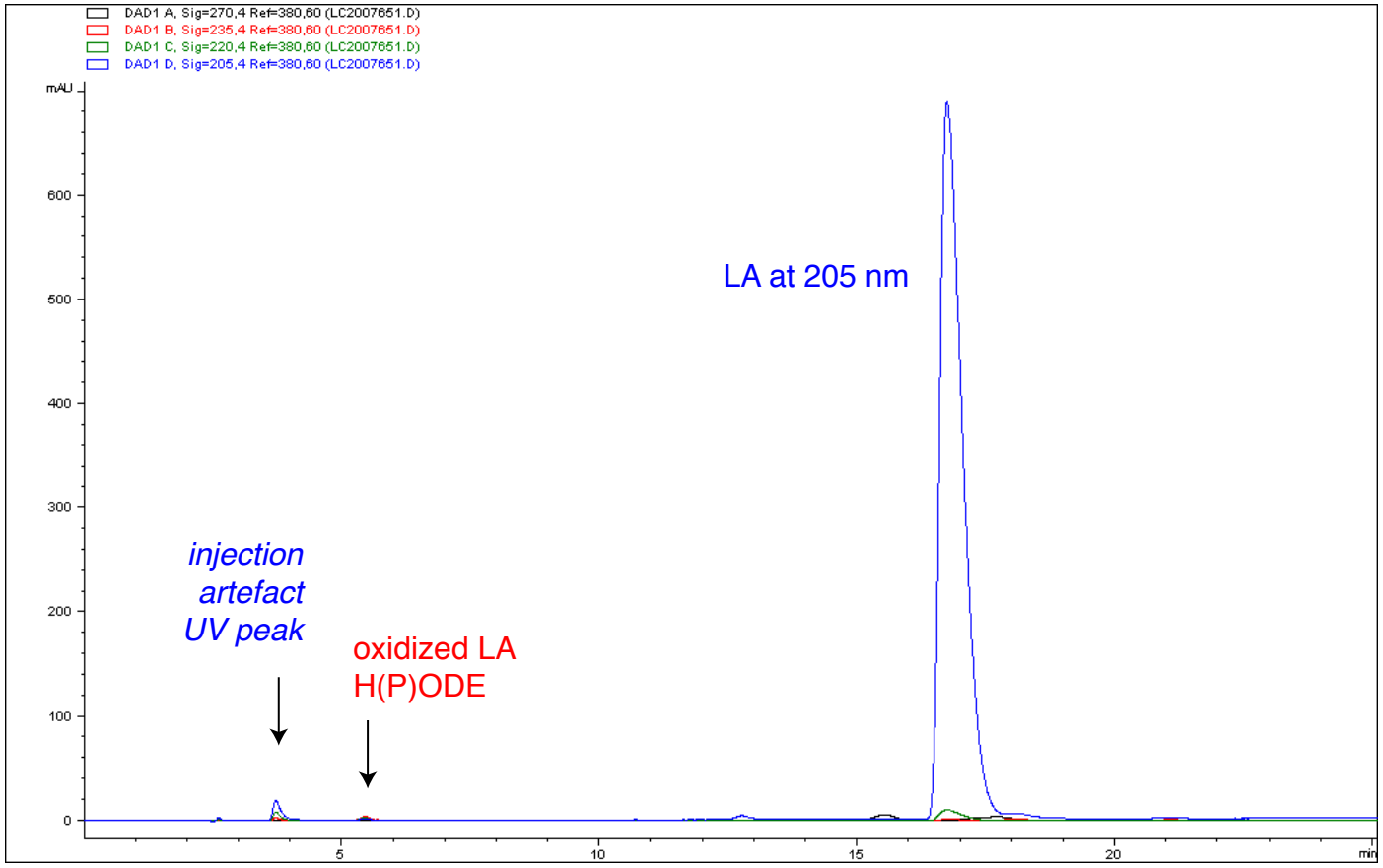


Figure S6:
Nu-Chek Prep, Linoleic Acid (LA), lot U59A-MA10-Y, supplied as >100 mg in sealed ampoule



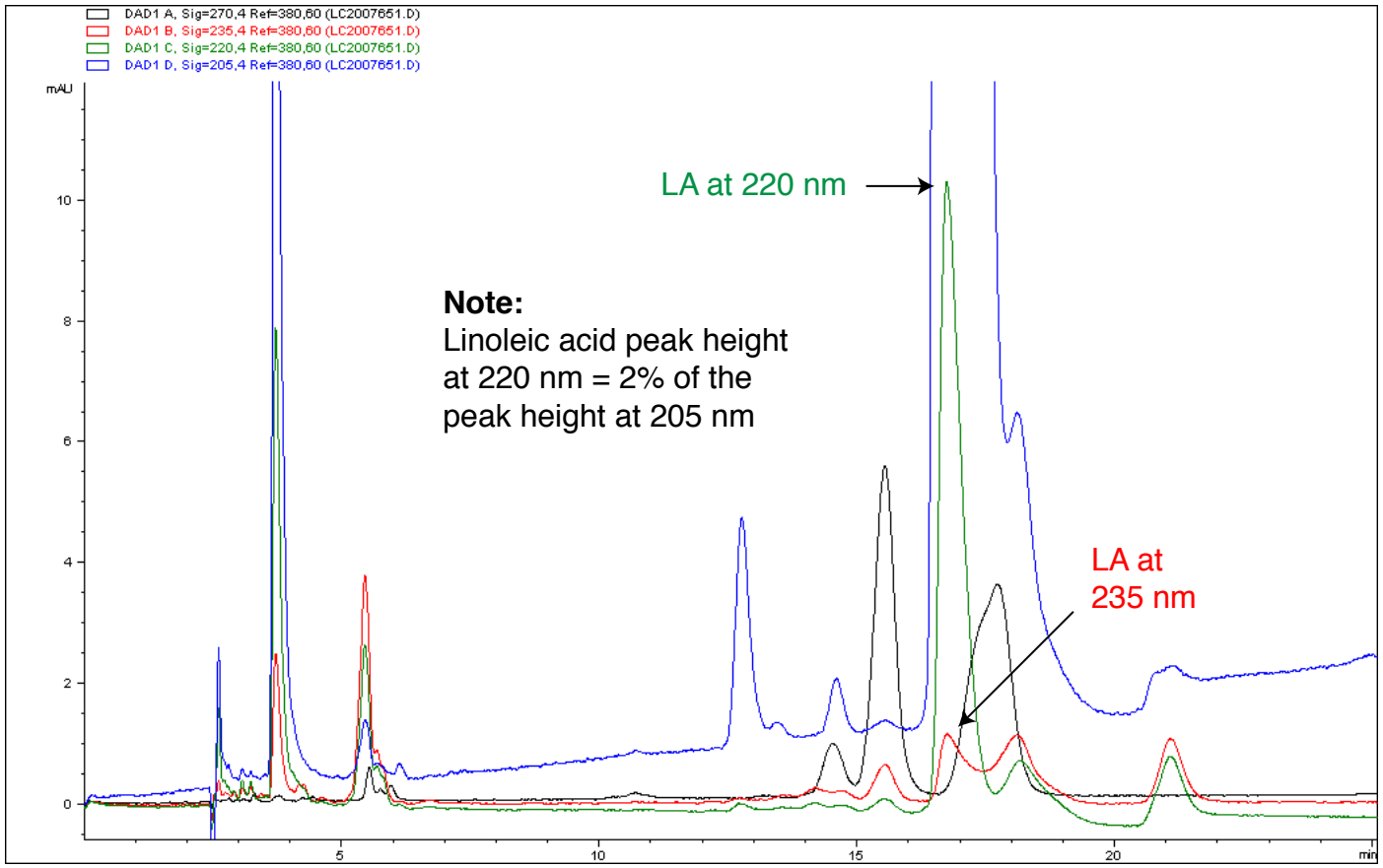
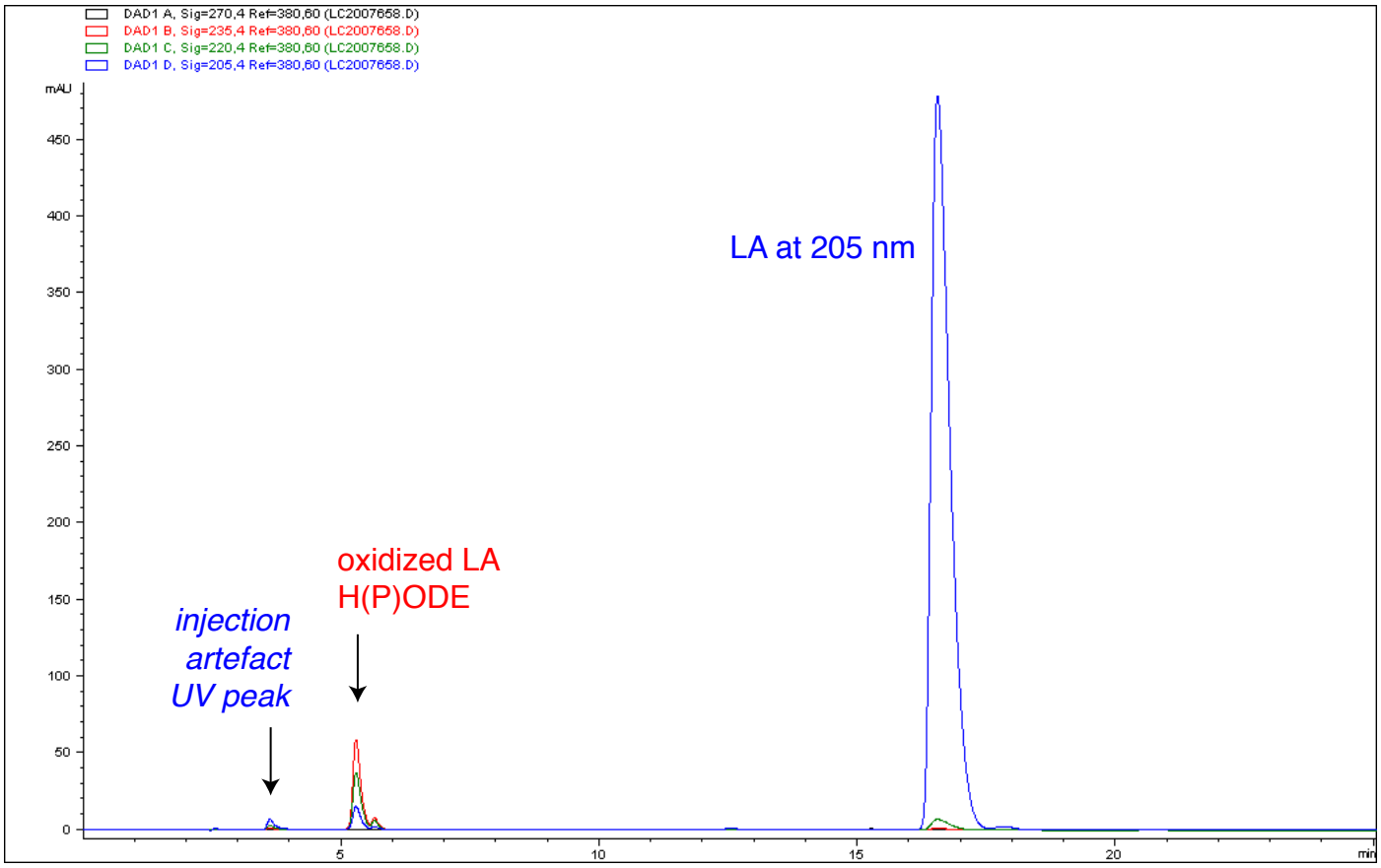


Figure S7:
Cayman Chemical, Linoleic Acid (LA), supplied as 500 mg in 1 ml Ethanol



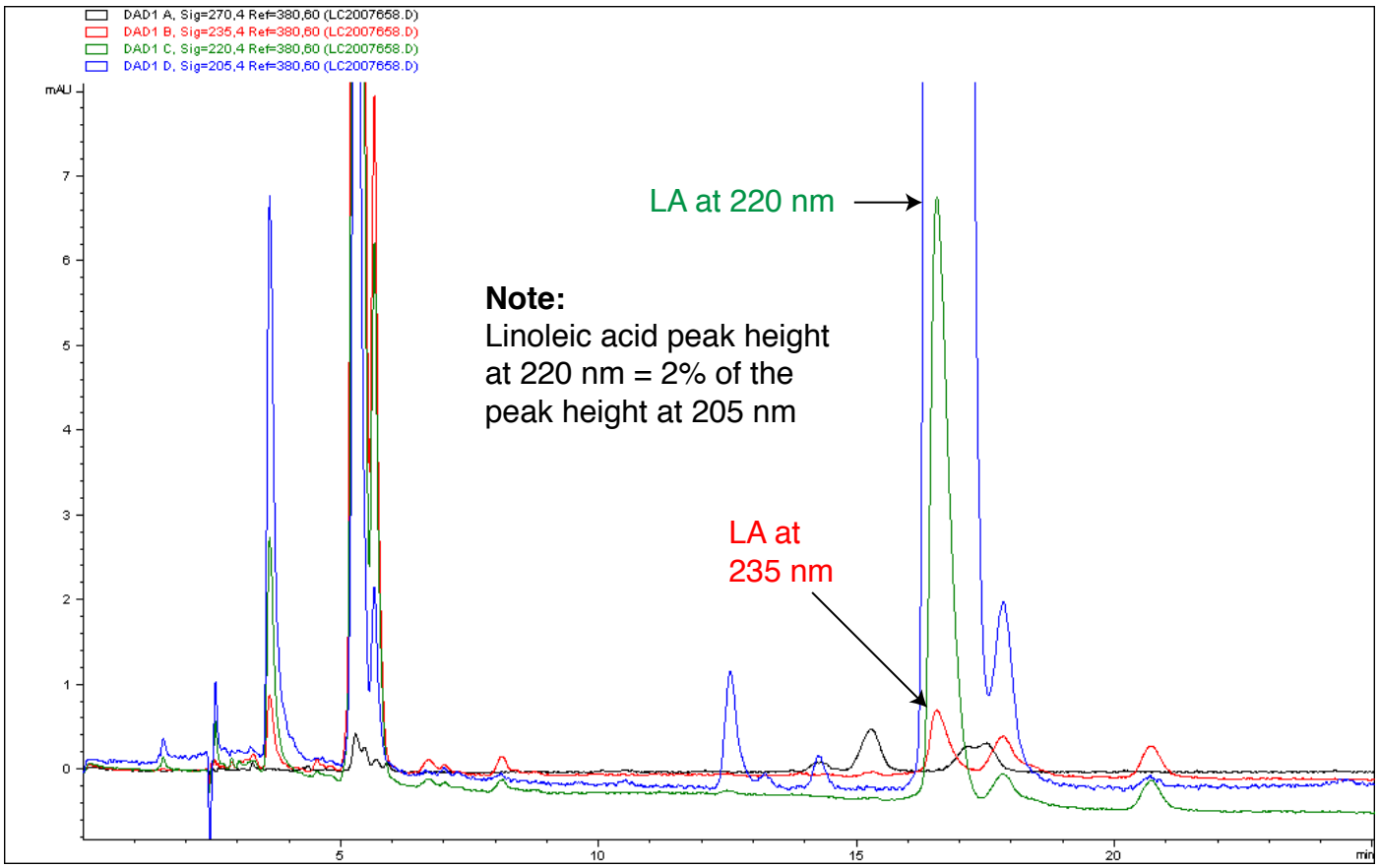
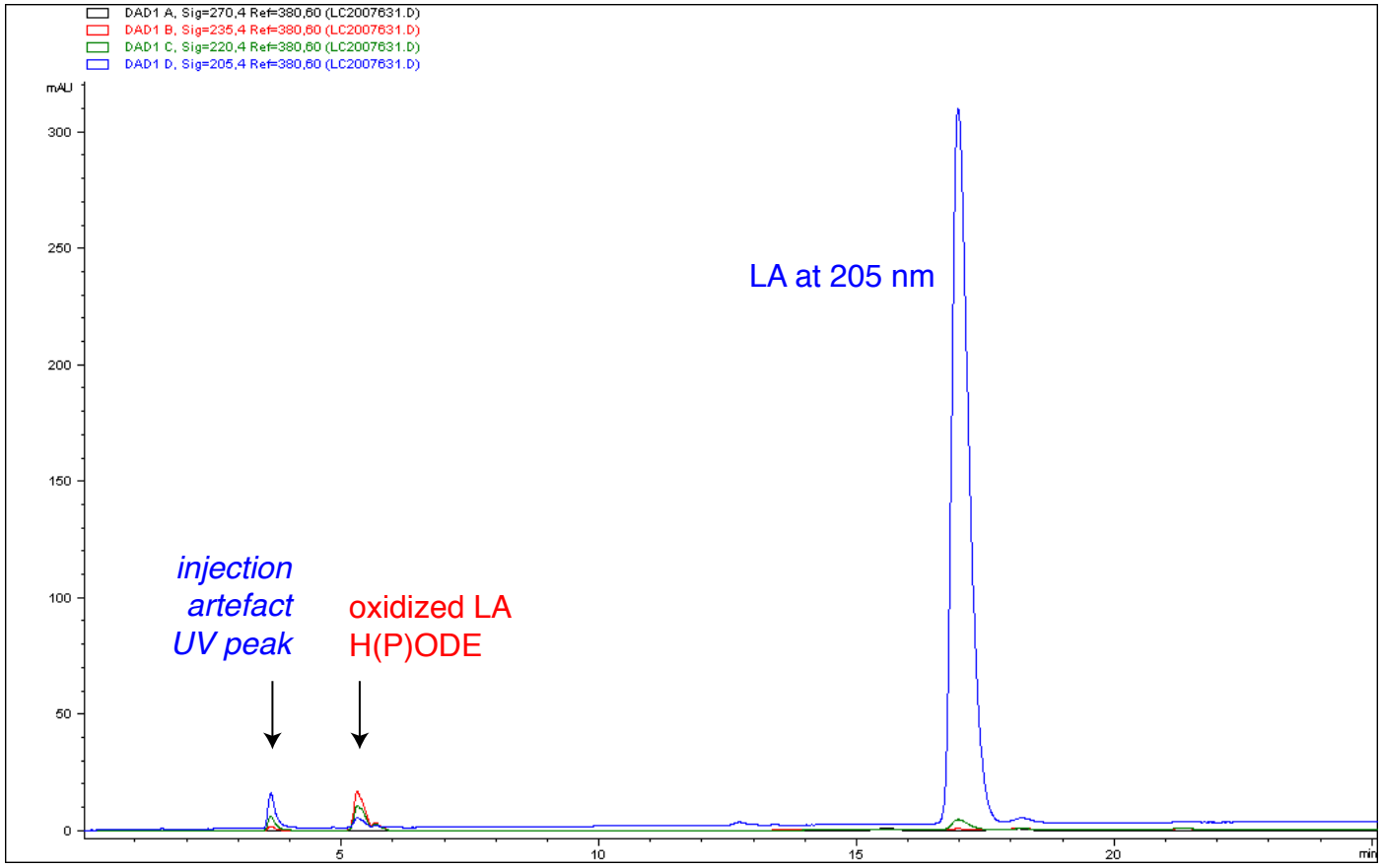


Figure S8:
Sigma Linoleic Acid (LA), supplied as 500 mg in sealed ampoule



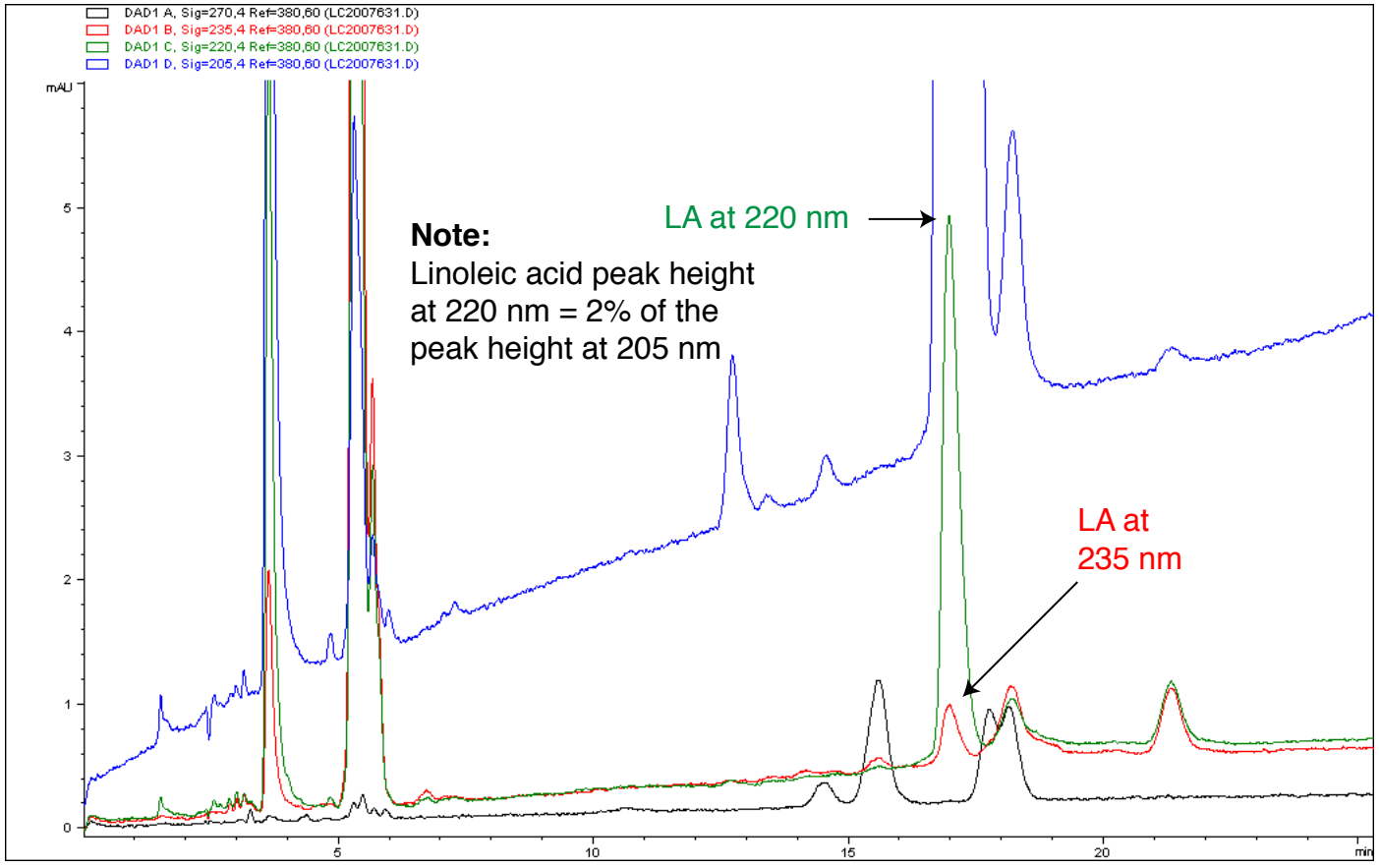
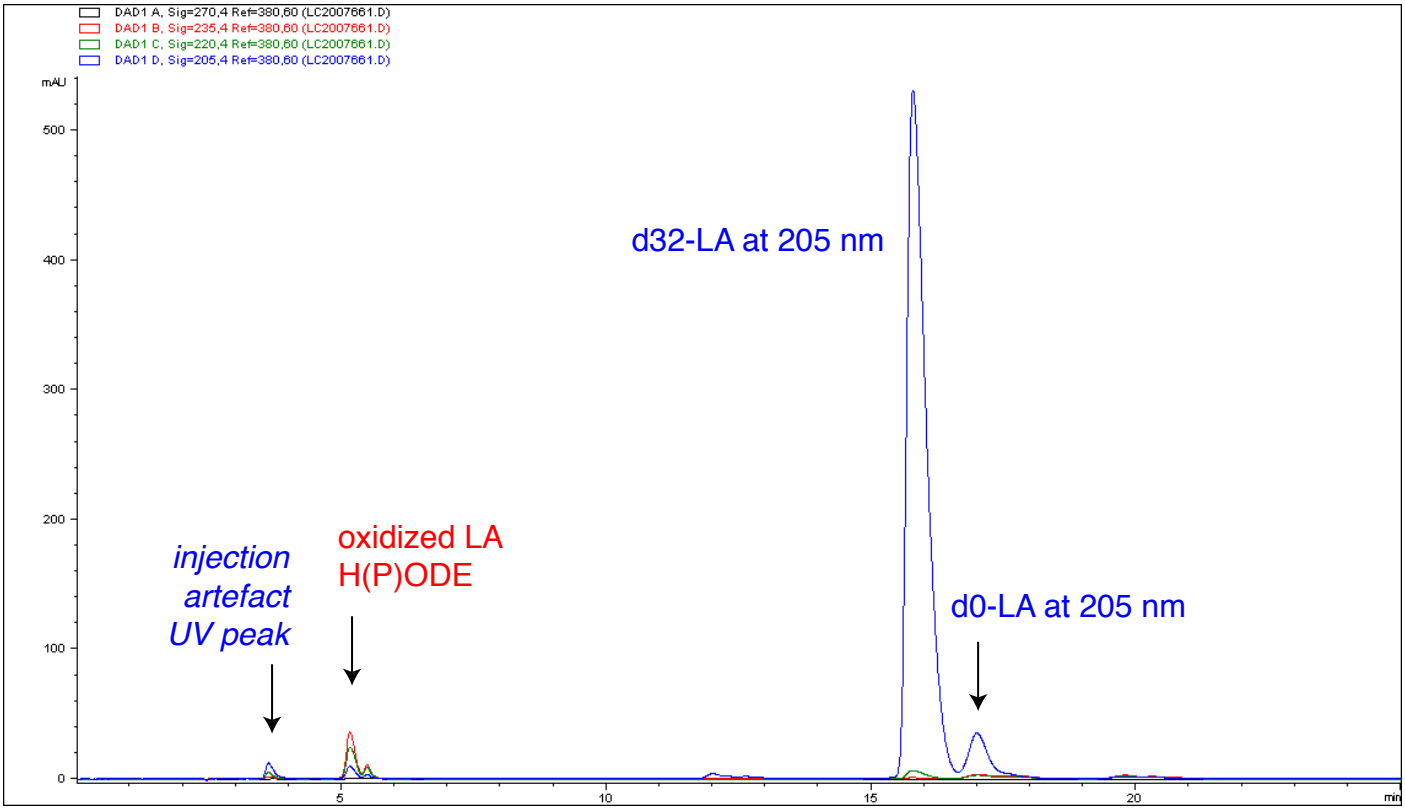


Figure S9:
Sigma/Isotec d32-Linoleic Acid (d32-LA), supplied as 10 mg in sealed ampoule



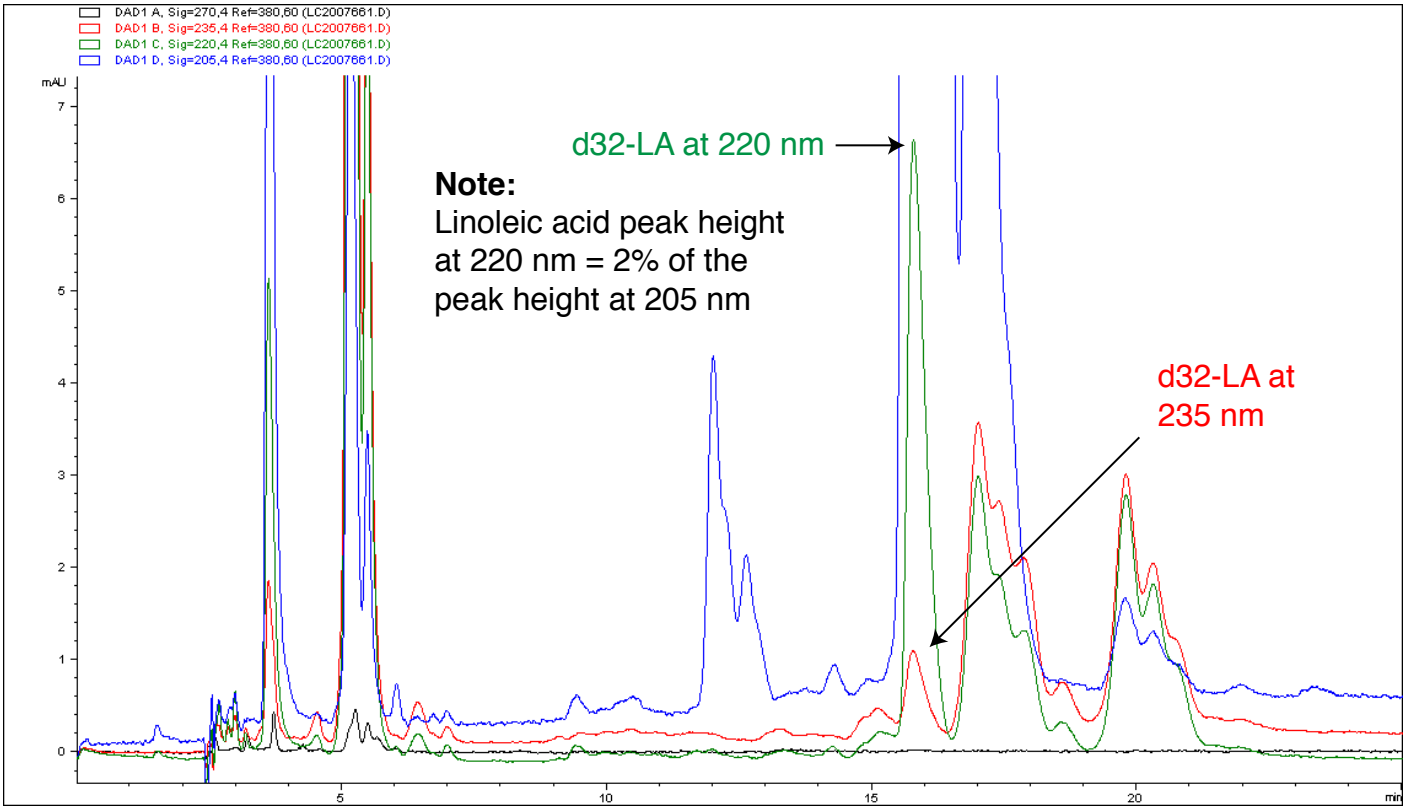


Figure S10: Silver Ion Chromatography
Nu-Chek Prep, Linoleic Acid (LA), lot U59A-A3-W (also analyzed in Fig 1, and Fig S1 - S3)