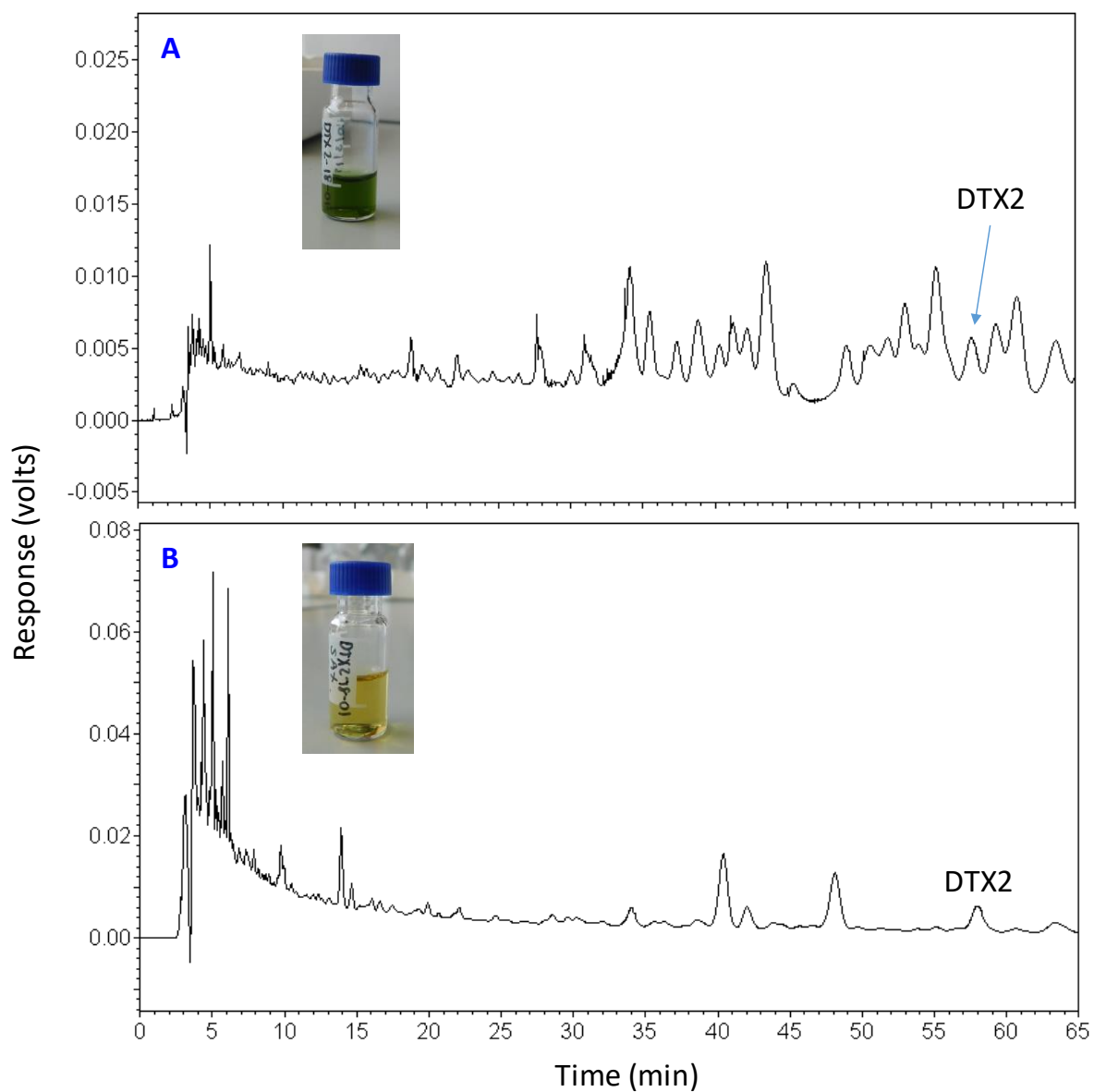


# Improved Isolation Procedures for Okadaic Acid Group Toxins from Shellfish (*Mytilus edulis*) and Microalgae (*Prorocentrum lima*)

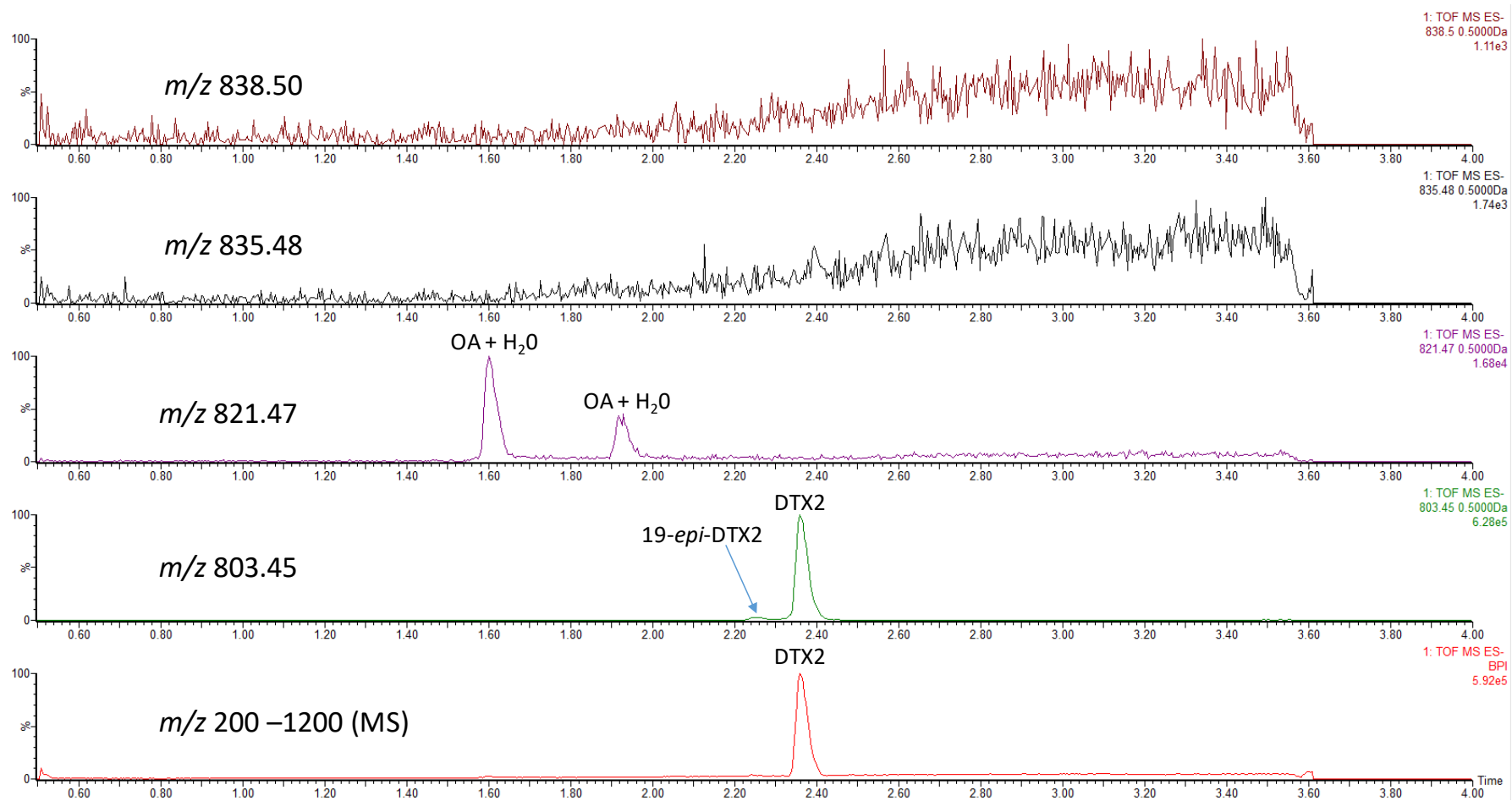
Jane Kilcoyne, Stephen Burrell, Cíara Nulty, Rafael Salas, Elliott J. Wright, Isabelle Rajotte, Christopher O. Miles

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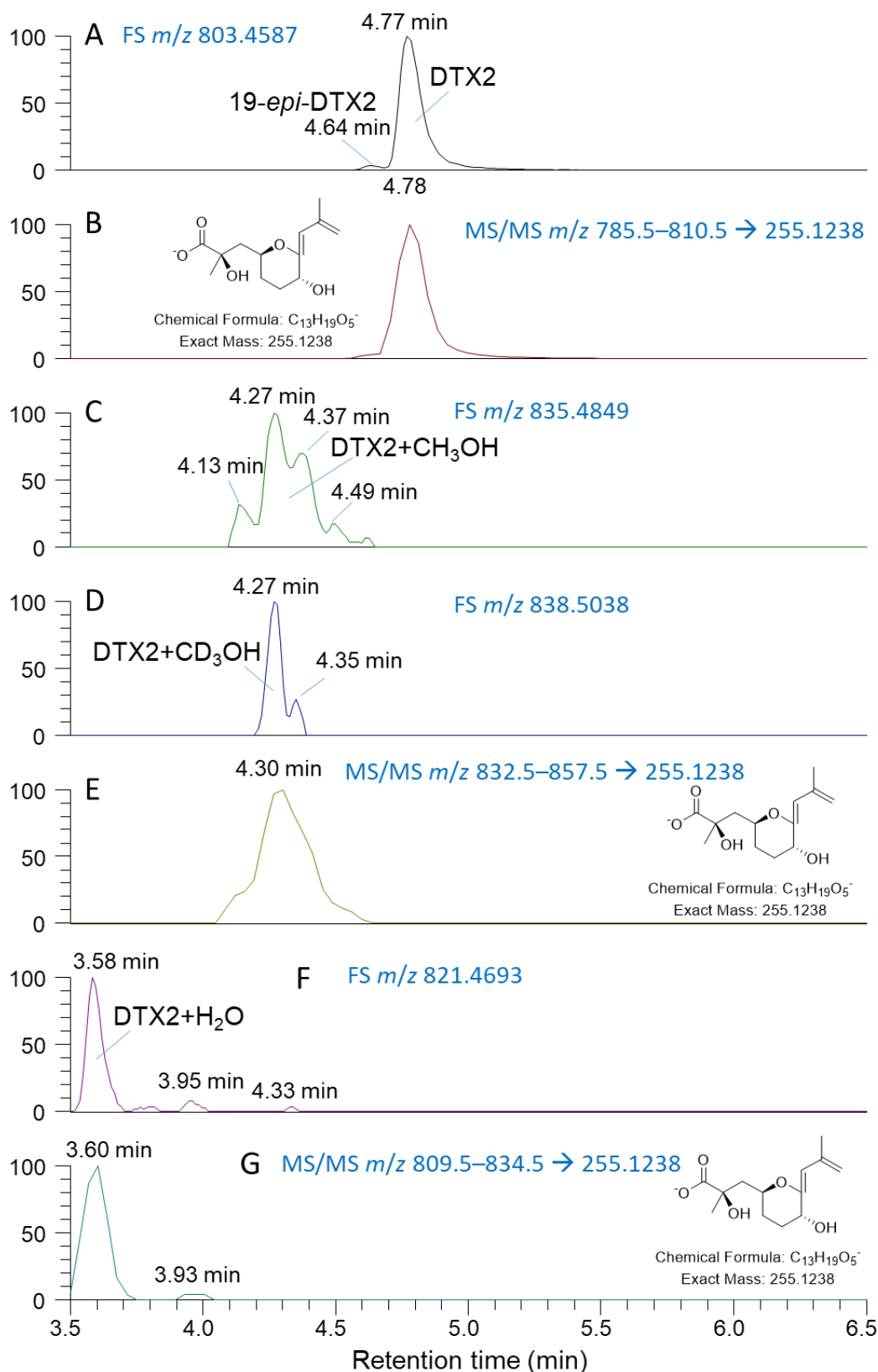
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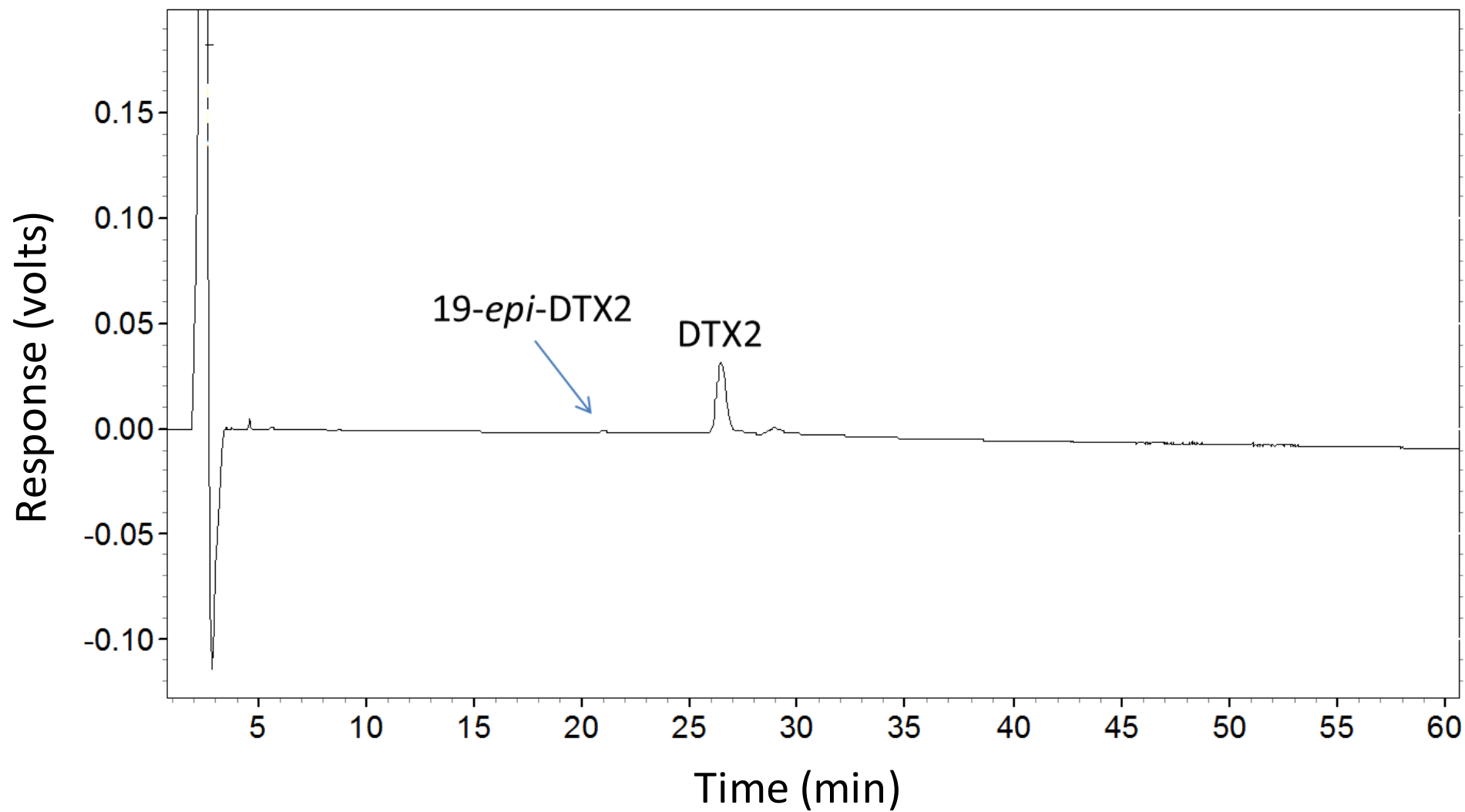
**Figure S1.** LC-UV (210 nm) chromatography showing difference in clean up between A) alumina (1 g loaded onto 30 g alumina) and B) SAX chromatography (3.4 g loaded onto 11.5 g SAX).



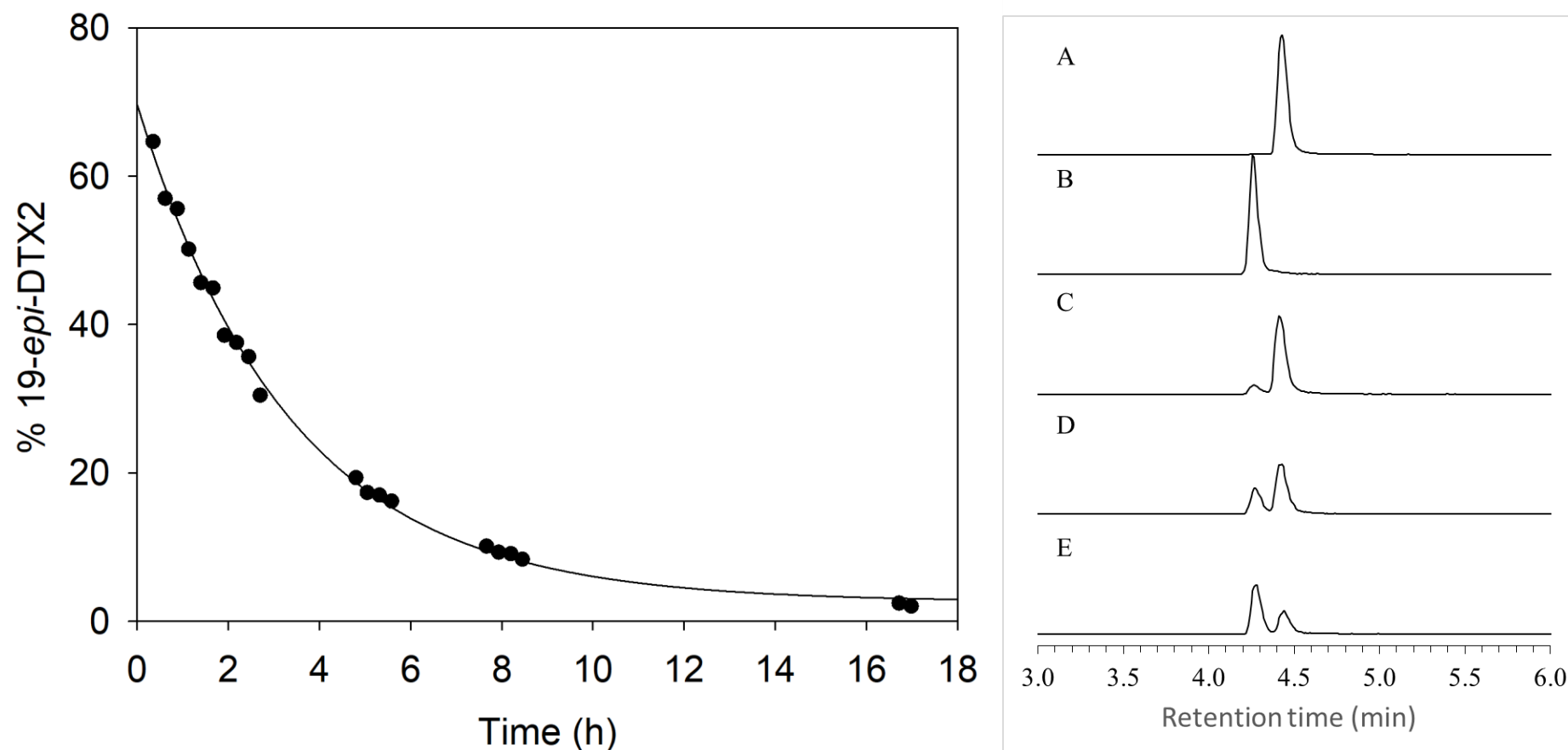
**Figure S2.** Purity analysis of DTX2 by LC-HRMS (acidic mobile phase, section 3.5.1).



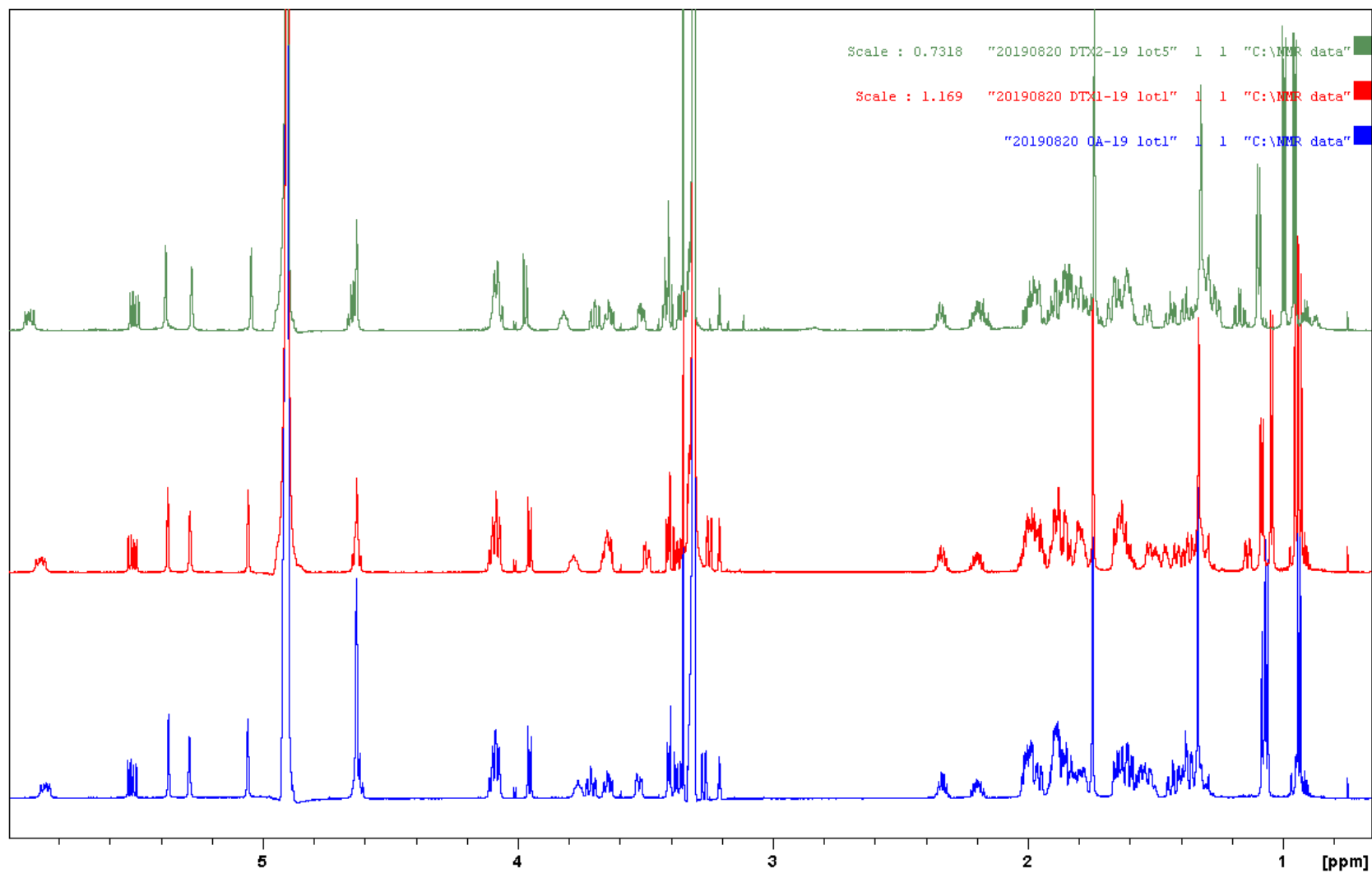
**Figure S3.** LC-HRMS/MS (neutral mobile phase, section 3.5.2) chromatograms of the purified DTX2 after NMR analysis, showing full scan (FS) chromatograms A, C, D and F extracted at the exact  $m/z$  values for DTX2, DTX2 + MeOH, DTX2 + CD<sub>3</sub>OH, and DTX2 + H<sub>2</sub>O ( $\pm 5$  ppm), respectively, above the chromatograms of the corresponding DIA windows B, E and G extracted for product ions at  $m/z$  255.1238 ( $\pm 5$  ppm).



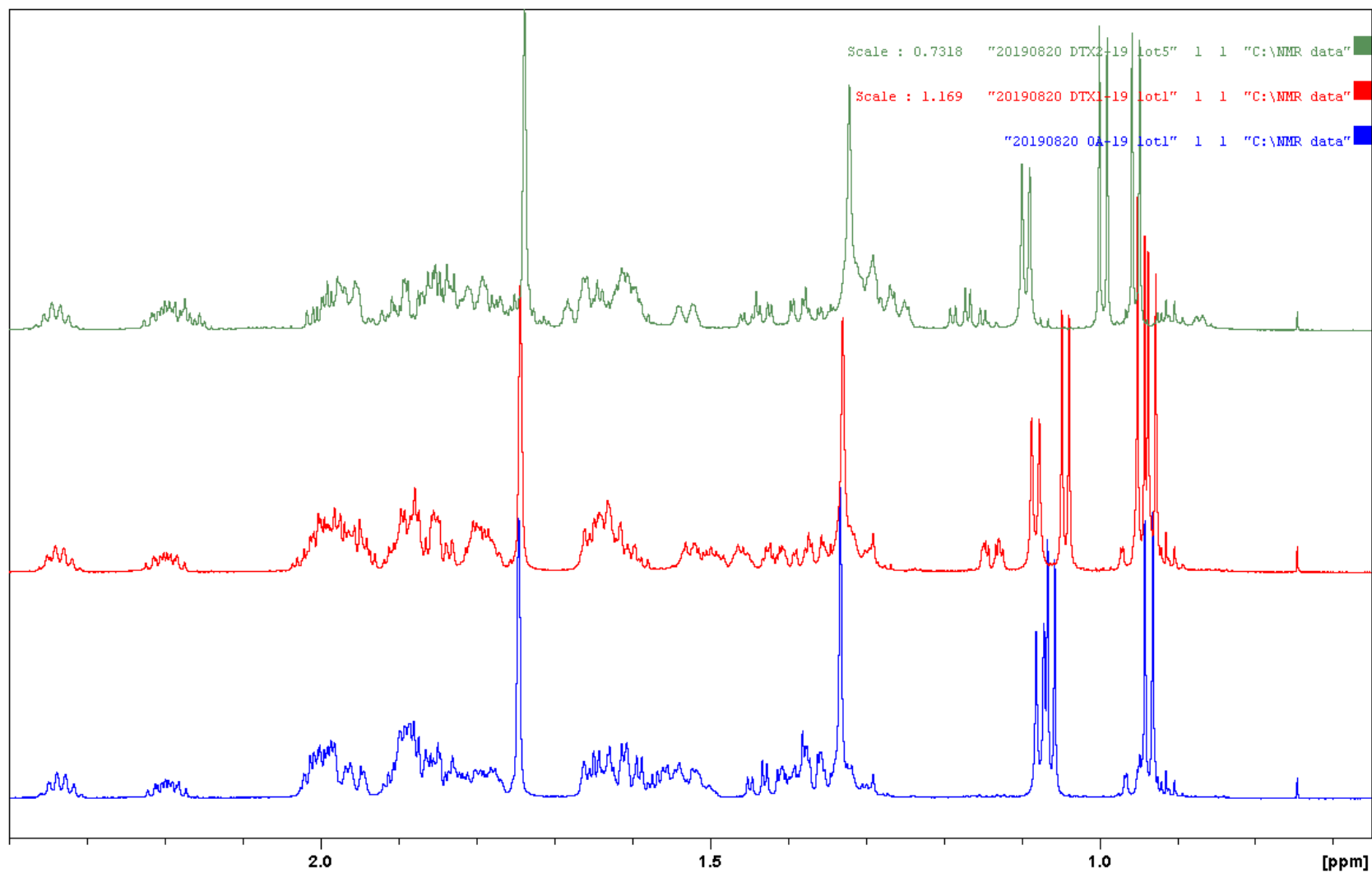
**Figure S4.** Purity analysis of DTX2 by LC-UV (210 nm, section 3.6).



**Figure S5.** Left, percentage of 19-*epi*-DTX2 (as a percentage of the sum of 19-*epi*-DTX2 and DTX2) remaining versus time during incubation at 10 °C in MeOH containing ~1% formic acid. The fitted line is a 3-parameter exponential decay curve with  $t_{1/2}$  2.3 h (SE  $\pm$ 0.1) and final equilibrium concentration 2.6% (SE  $\pm$ 0.7). Right, LC-MS/MS chromatograms ( $m/z$  803.5 $\rightarrow$ 255.1) of: A) NRC-CRM-DTX2b; B) purified 19-*epi*-DTX2, and; C-E) 19-*epi*-DTX2 incubated for 7.68, 2.70, and 0.35 h in MeOH containing ~1% formic acid, which formed part of the kinetic analysis shown in the graph on the left.

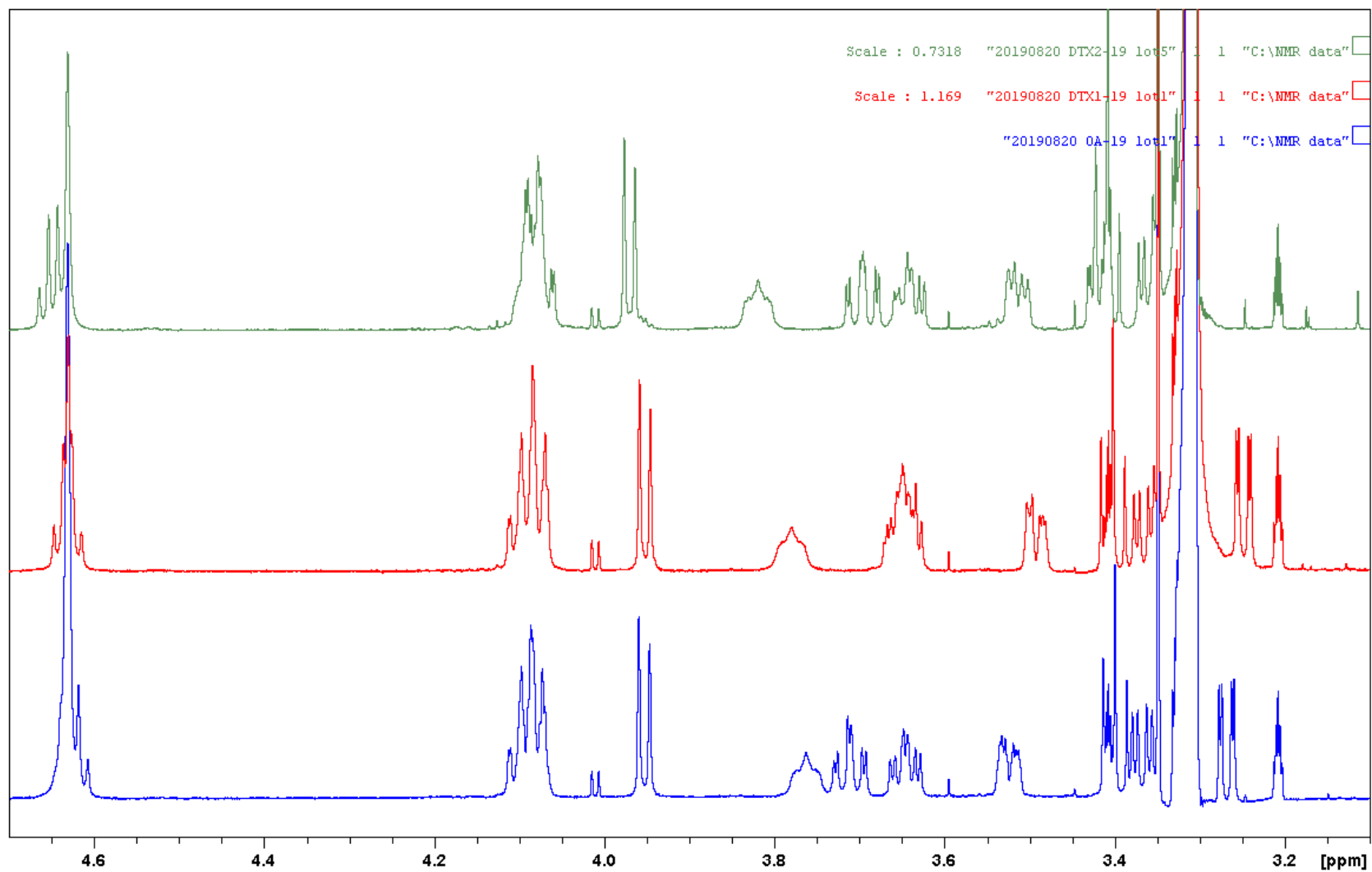


**Figure S6.** <sup>1</sup>H NMR spectra (0.65–6.00 ppm) of: top, DTX2 (green); middle, DTX1 (red), and; OA (blue) in CD<sub>3</sub>OD. Spectral intensities are scaled so that spectra have the same peak heights for the olefinic resonances at 5.0–5.6 ppm.

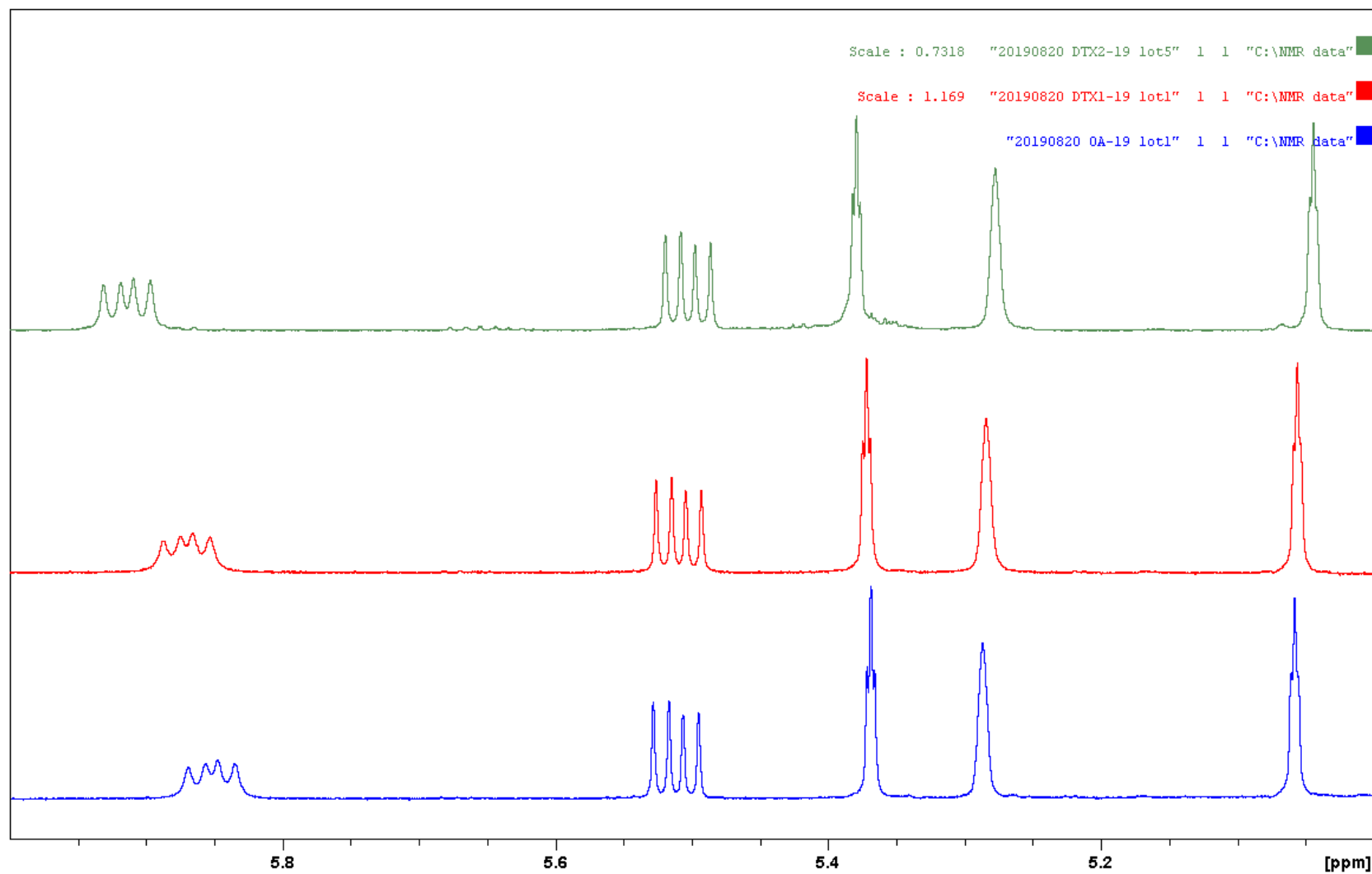


**Figure S7.** Expansion (0.65–2.40 ppm) of the <sup>1</sup>H NMR spectra shown in Figure S6. Top, DTX2 (green); middle, DTX1 (red), and; OA (blue) in CD<sub>3</sub>OD.

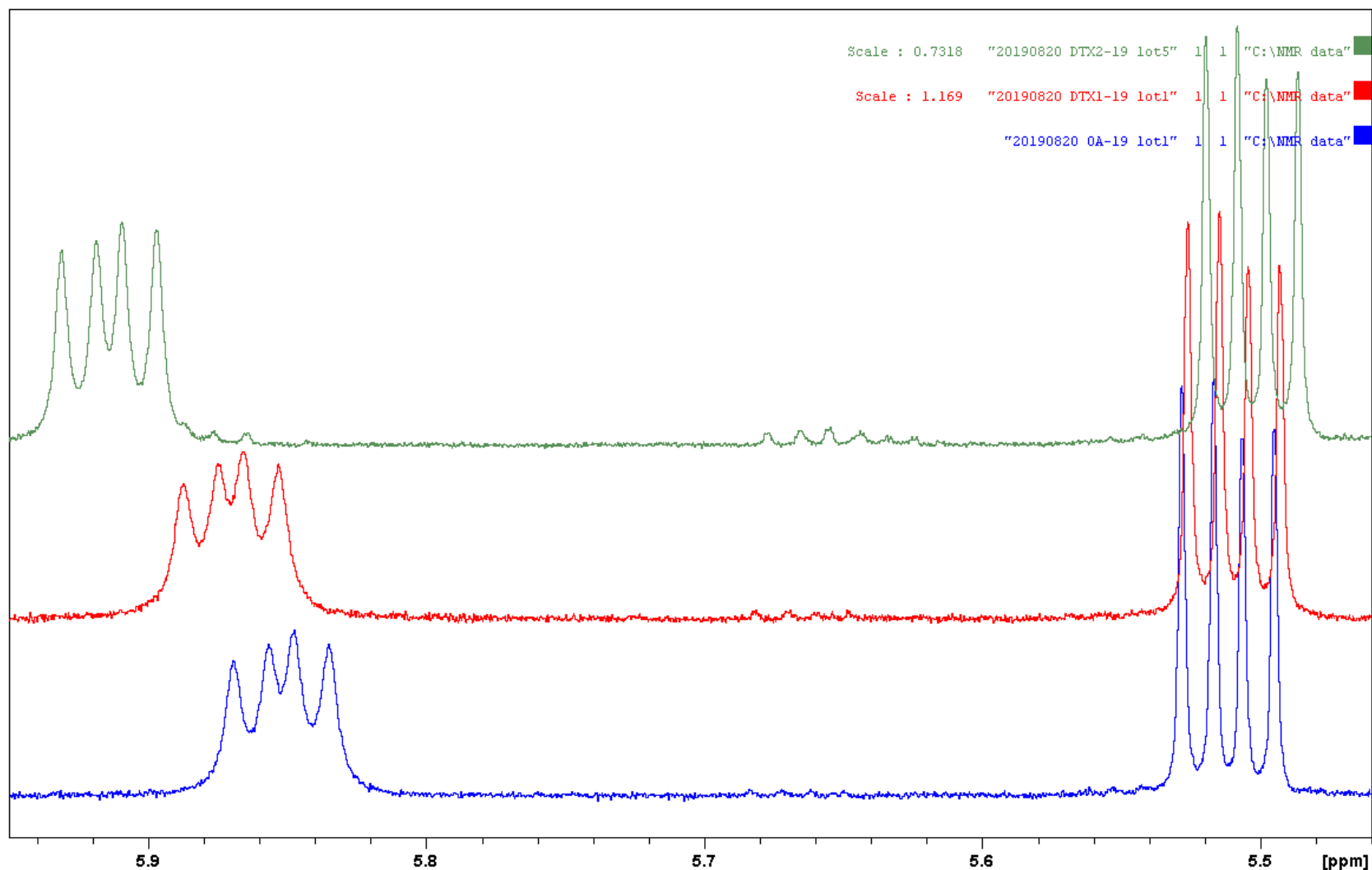




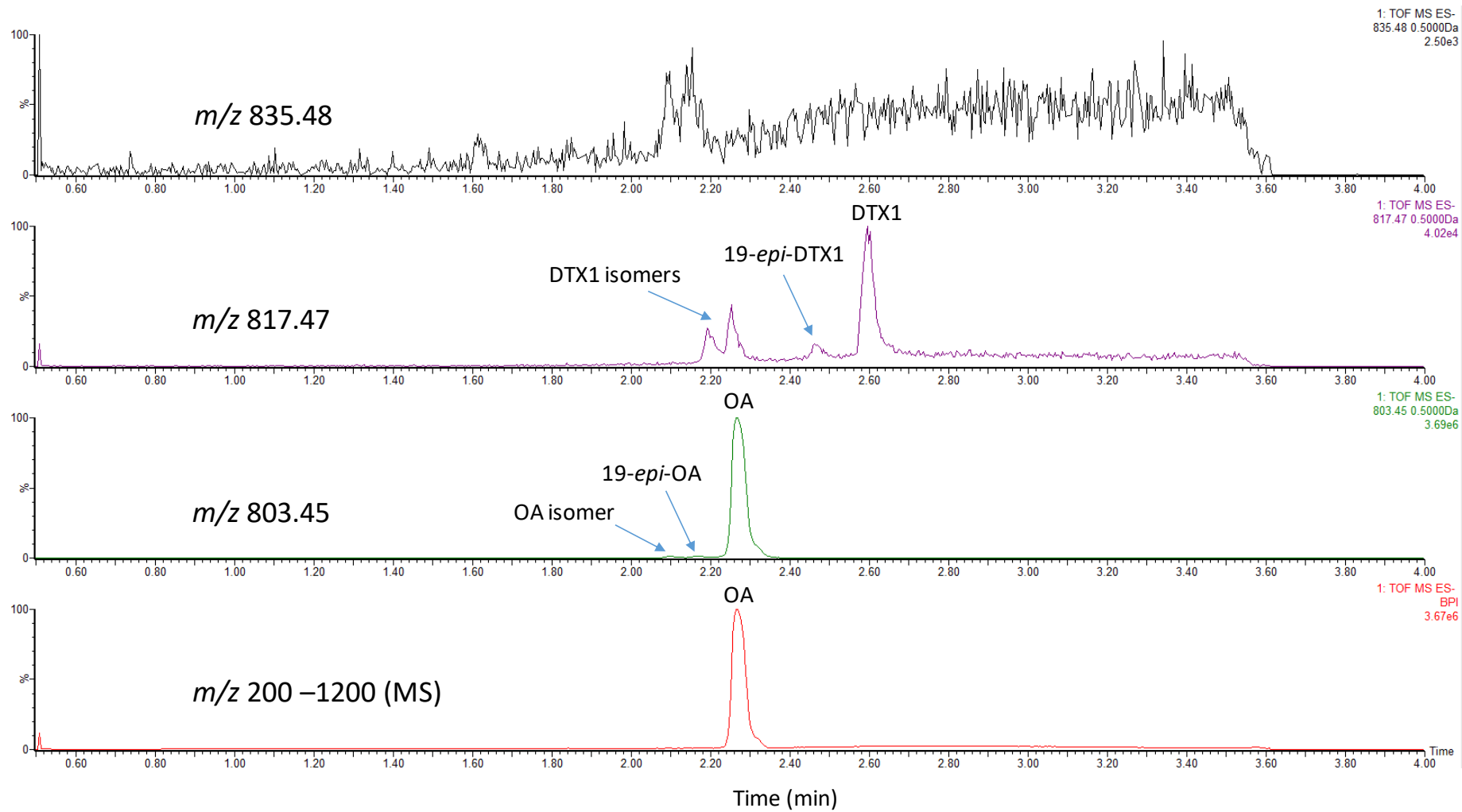
**Figure S8.** Expansion (3.10–4.70 ppm) of the <sup>1</sup>H NMR spectra shown in Figure S6. Top, DTX2 (green); middle, DTX1 (red), and; OA (blue) in CD<sub>3</sub>OD.



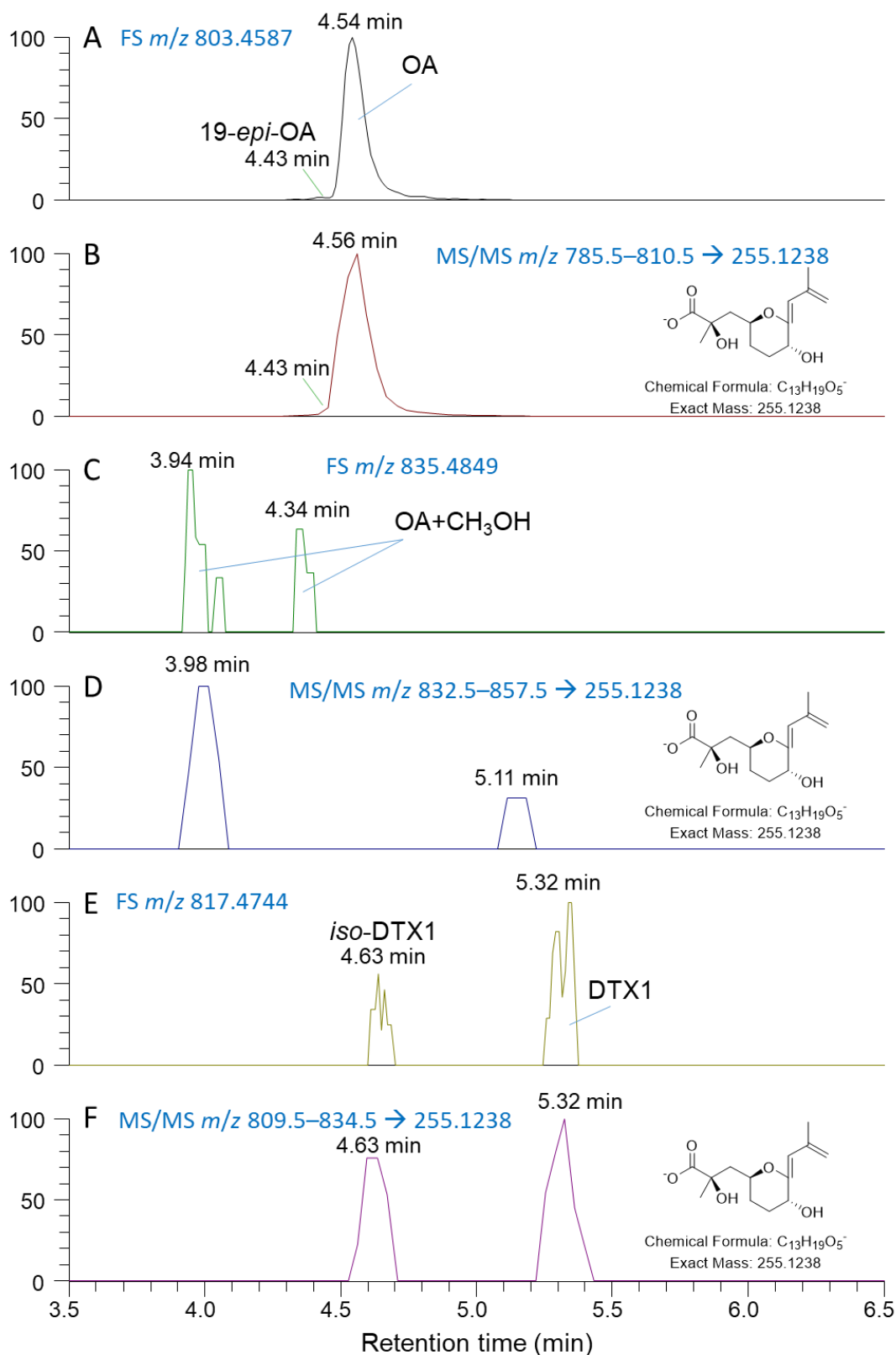
**Figure S9.** Expansion (5.00–6.00 ppm) of the <sup>1</sup>H NMR spectra shown in Figure S6. Top, DTX2 (green); middle, DTX1 (red), and; OA (blue) in CD<sub>3</sub>OD.



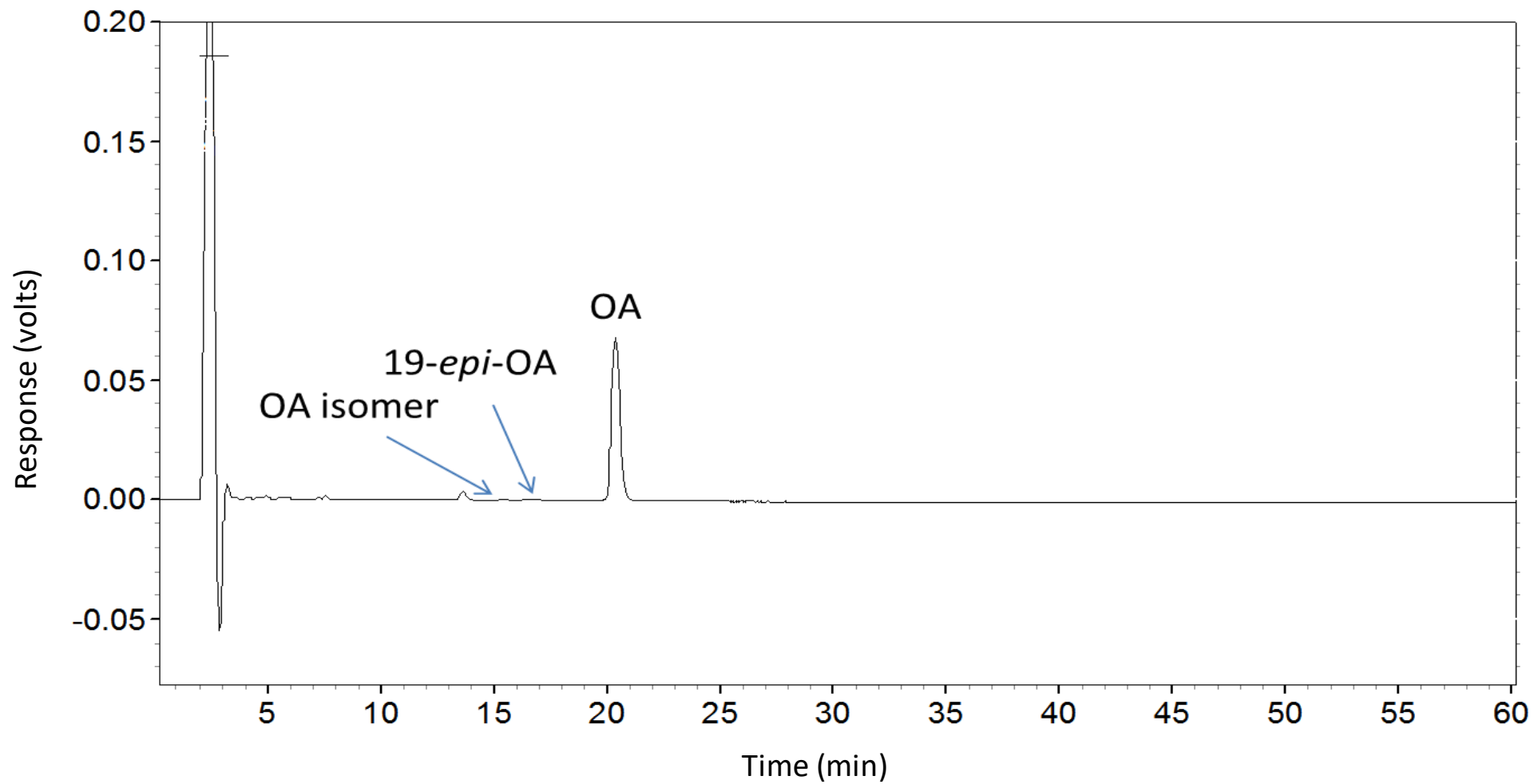
**Figure S10.** Expansion (5.40–5.95 ppm) of the <sup>1</sup>H NMR spectra shown in Figure S6. Top, DTX2 (green); middle, DTX1 (red), and; OA (blue) in CD<sub>3</sub>OD. Signs of signals from possible isomers and analogues are visible at ~5.66 ppm, particularly in DTX2 (~5%). Other minor resonances at 5.88 ppm (DTX2) and at 5.55 may also be due to structurally-related impurities.



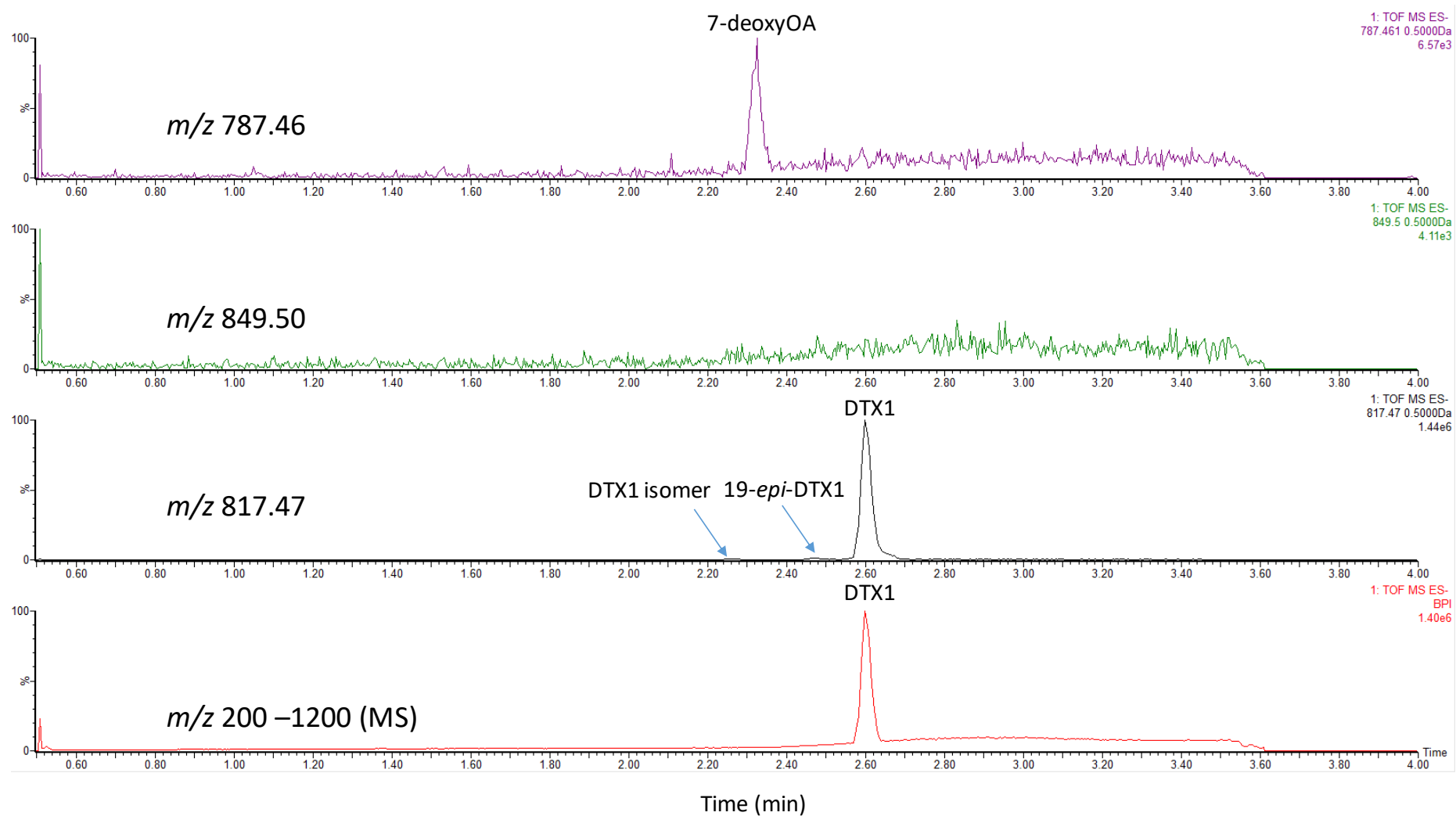
**Figure S11.** Purity analysis of OA by LC-HRMS (acidic mobile phase, section 3.5.1).



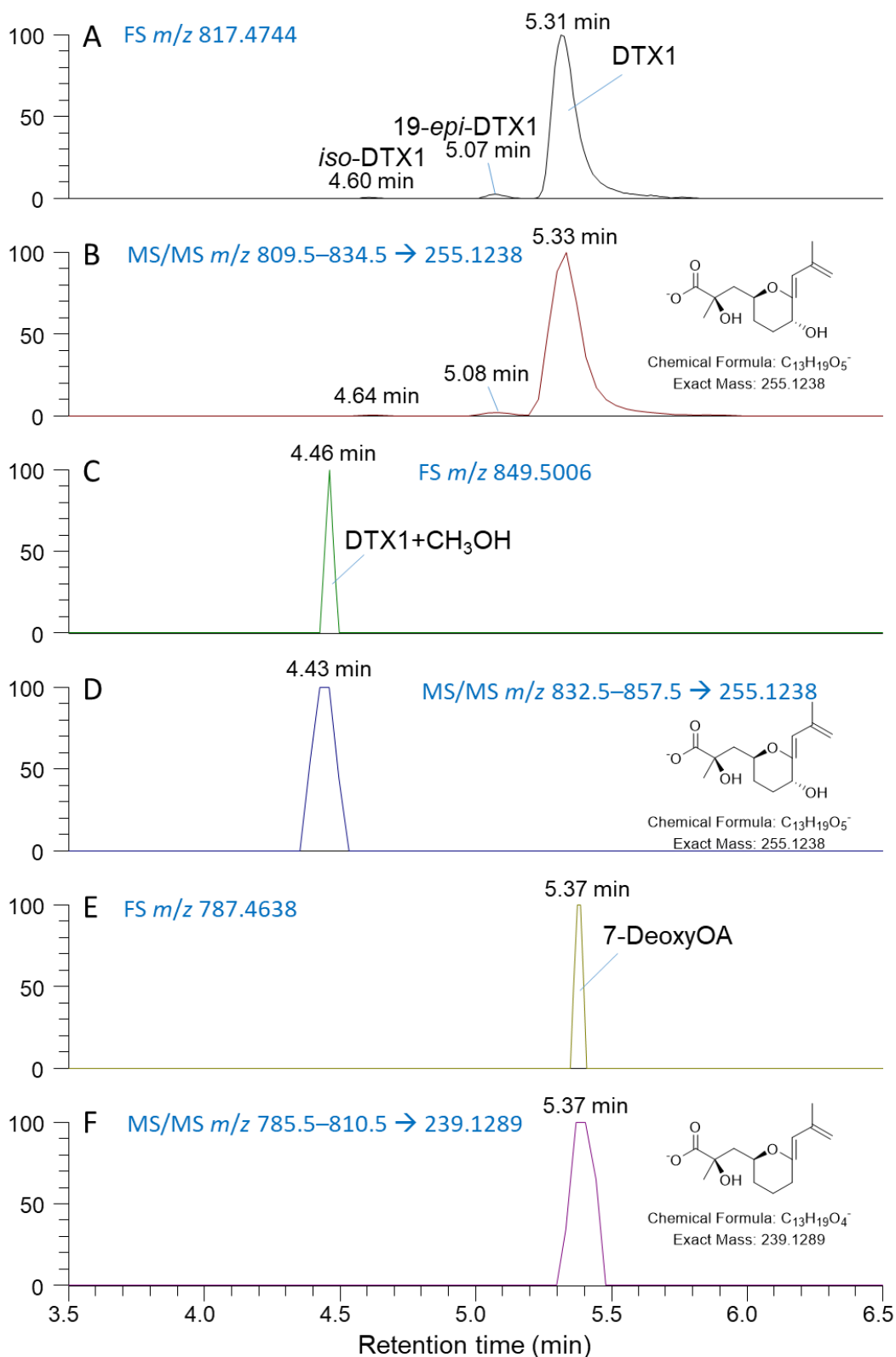
**Figure S12.** LC-HRMS/MS (using neutral mobile phase, section 3.5.2) chromatograms of the purified OA after NMR analysis, showing full scan (FS) chromatograms A, C, and E extracted at the exact  $m/z$  values for OA, OA + MeOH, and DTX1/*iso*-DTX1 ( $\pm 5$  ppm), respectively, above the chromatograms of the corresponding DIA windows B, D and F extracted for product ions at  $m/z$  255.1238 ( $\pm 5$  ppm).



**Figure S13.** Purity analysis of OA by LC-UV (210 nm, section 3.6).

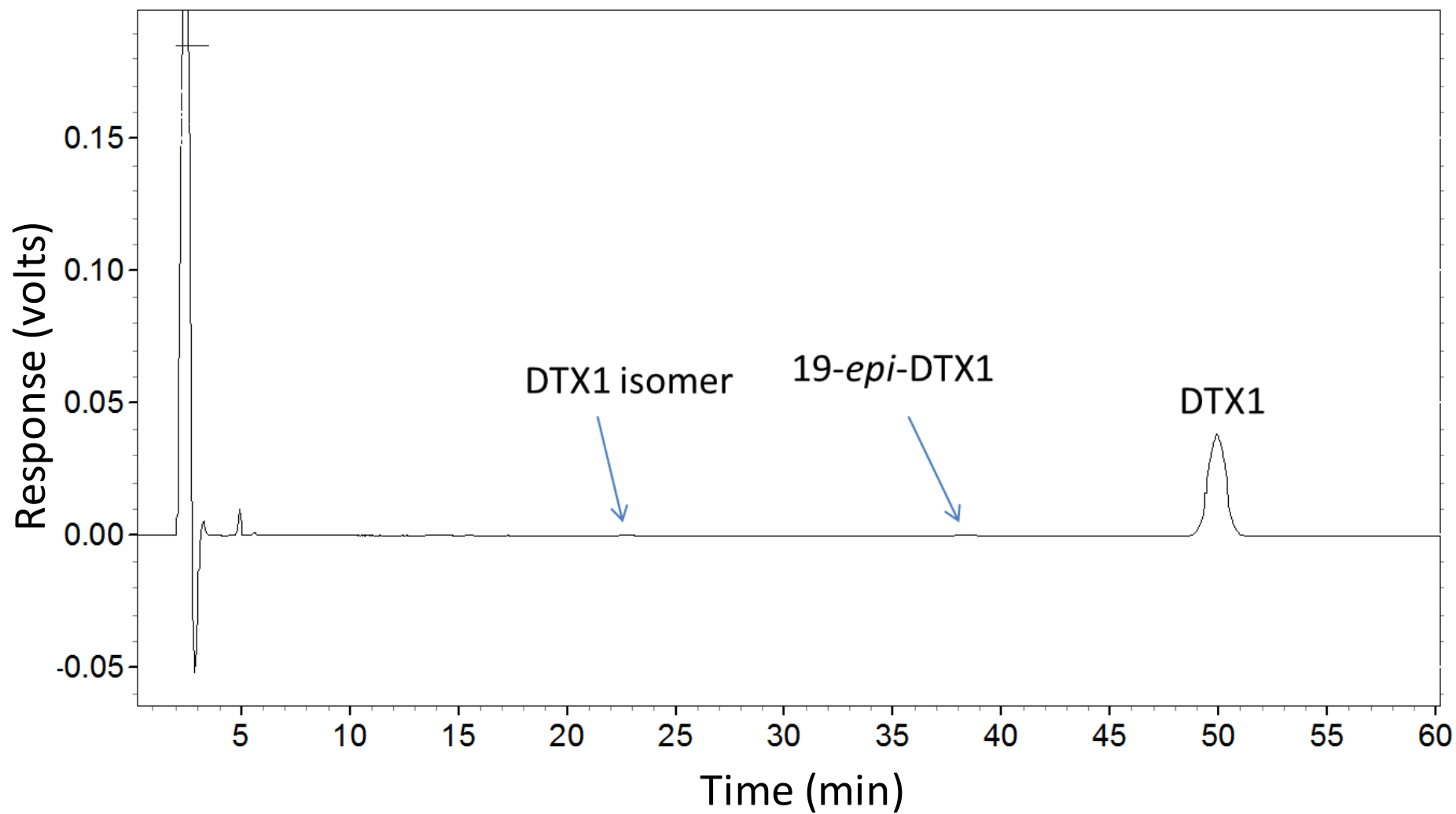


**Figure S14.** Purity analysis of DTX1 by LC-HRMS (acidic mobile phase, section 3.5.1).



**Figure S15.** LC-HRMS/MS (neutral mobile phase, section 3.5.2) chromatograms of the purified DTX1 after NMR analysis, showing full scan (FS) chromatograms A, C, and E extracted at the exact  $m/z$  values for DTX1, DTX1 + MeOH, and 7-deoxyOA ( $\pm 5$  ppm), respectively, above the chromatograms of the corresponding DIA windows B, and D extracted for product ions at  $m/z$  255.1238 and F extracted for  $m/z$  239.1289 ( $\pm 5$  ppm).





**Figure S16.** Purity analysis of DTX1 by LC-UV (210 nm, section 3.6).