Supporting Information for:

HU-331 and Oxidized Cannabidiol Act as Inhibitors of Human Topoisomerase IIα and β

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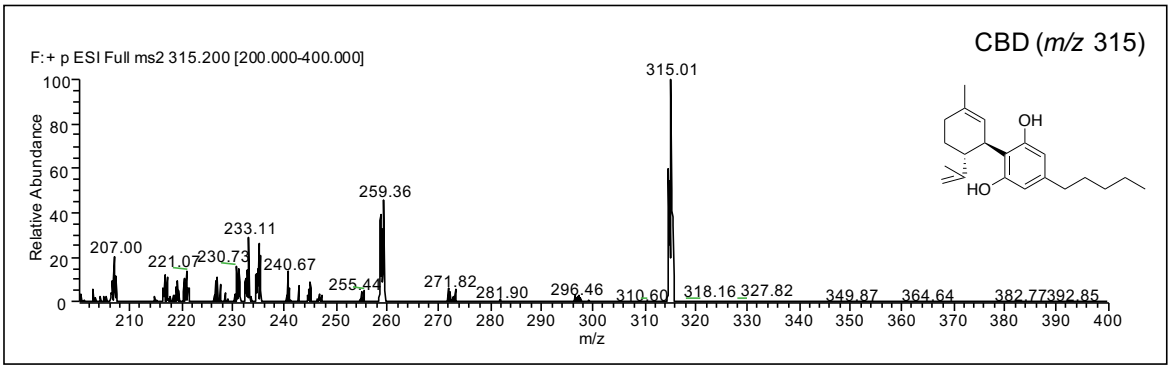
LC-MS/MS Analysis of Cannabidiol, Oxidized Cannabidiol, and HU-331. Cannabidiol and HU-331 were subjected to LC-MS/MS analysis to develop a method for detection and relative quantitation. LC-MS/MS analysis was performed using a Thermo TSQ Quantum Triple Quadrupole mass spectrometer coupled to a Thermo Accela HPLC/UHPLC system. An aliquot (20 μl) was injected into the equilibrated HPLC system using a Thermo PAL autoinjector. The following solvents were used – Mobile Phase A: Optima LC-MS-grade water (Fisher Scientific) with 0.1% (v/v) formic acid, and Mobile Phase B: Optima LC-MS-grade acetonitrile (Fisher Scientific) with 0.1% (v/v) formic acid. Compound separation was achieved using a Kinetex EVO C18 column (2.6 μm, 50 mm x 2.1 mm, 100 Å) (Phenomenex Inc., Torrance, CA) with a flow rate of 0.3 ml/min and column oven temperature 30°C. The LC gradient system was as follows: 30 to 95% B (0 – 2 minutes), 95% B (2 – 4 minutes), 95 to 30% B (4 – 4.5 minutes), and 30% B (4.5 – 5 minutes). The mass spectrometer was operated in positive ion mode using electrospray ionization (ESI+). MS spectral data were analyzed by Thermo Xcalibur software 2.0.

Standard solutions of CBD and HU-331 (20 mM in DMSO) were diluted to 20 μM in acetonitrile for LC-MS/MS analysis. Collision induced dissociation of the precursor ion m/z 315 for CBD and m/z 329 for HU-331 was performed in product ion mode at collision energy 21 V to identify the characteristic fragmentation pattern for each compound (**Figure S1**). Next, a selected reaction monitoring (SRM) method was developed for compound detection using the precursor-to-product ion transitions m/z 315 $\rightarrow$ 259 for CBD and m/z 329 $\rightarrow$ 287 for HU-331. Relative levels of oxidized CBD were measured based on the SRM peak area (m/z 329 $\rightarrow$ 287), similar to HU-331 (**Figures S2 and S4**). Peak areas were determined using the auto-integration function in Thermo Xcalibur software.

A.



B.

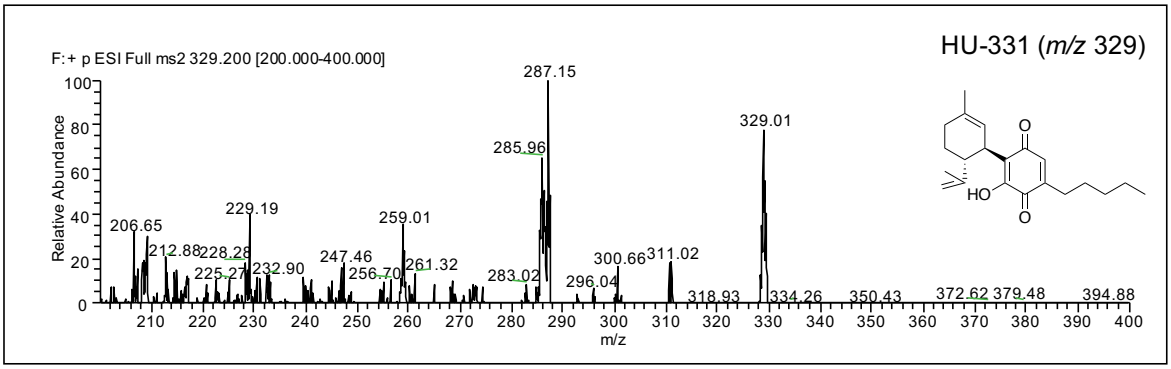


Figure S1: LC-MS/MS Analysis of Cannabidiol (CBD) and HU-331. Standard solutions of 20 μM CBD (A) and HU-331 (B) were subjected to liquid chromatography - tandem mass spectrometry (LC-MS/MS) analysis via electrospray ionization in positive ion mode. CBD has a precursor ion of m/z 315, and HU-331 has a precursor ion of m/z 329. Shown are representative product ion spectra generated from collision induced dissociation of CBD (m/z 315) (A) and HU-331 (m/z 329) (B) at collision energy 21 V.

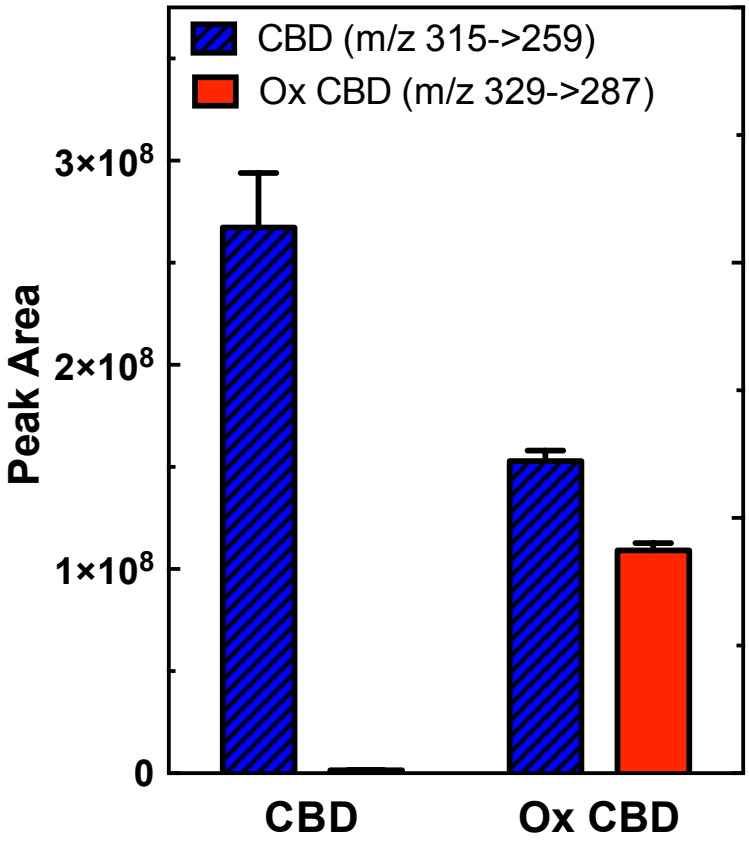


Figure S2: Analysis of Oxidation of Cannabidiol in Solution. Pure cannabidiol has a molecular ion at m/z 315 when analyzed by LC-MS/MS in positive ion mode, while HU-331 is observed at m/z 329. Samples of cannabidiol (CBD) and oxidized cannabidiol (Ox CBD) were subjected to LC-MS/MS analysis at matching concentrations. The precursor-to-product ion transitions for CBD (m/z 315>259) and Ox CBD (m/z 329>287) were used to measure relative levels of each compound by selected reaction monitoring. Results above display the peak areas for CBD (m/z 315>259) and Ox CBD (m/z 329>287) in two samples from an initial concentration of CBD at 200 μM. Cannabidiol appears to oxidize in solution over time. Results represent the mean of two samples and error bars represent the standard error of the mean.

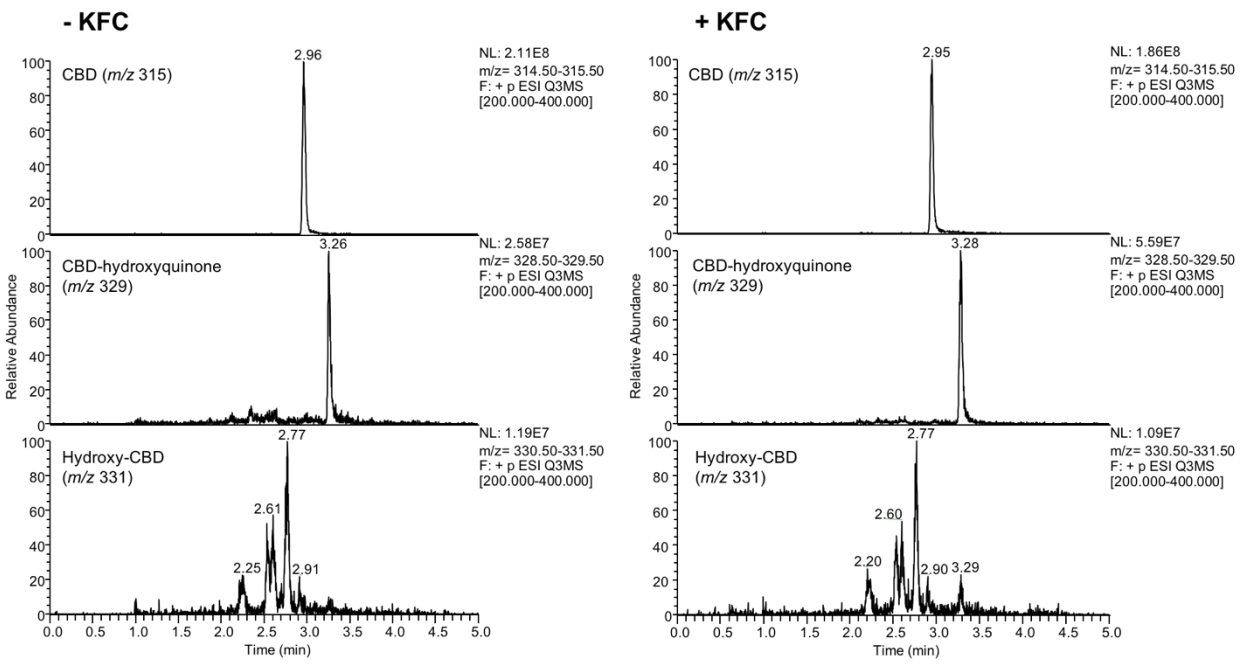


Figure S3: KFC-oxidized CBD. LC-MS/MS analysis of oxidized CBD products with and without KFC treatment. Shown are LC-MS extracted ion chromatograms for CBD (*m/z* 315), CBD-hydroxyquinone (*m/z* 329), and hydroxy-CBD products (*m/z* 331) +/- KFC. It should be noted that the hydroxy-CBD products in the –KFC reaction (left lower chromatogram) are the result autooxidation by air since we have also observed no hydroxy-CBD in spectra of pure CBD (see figure 6).

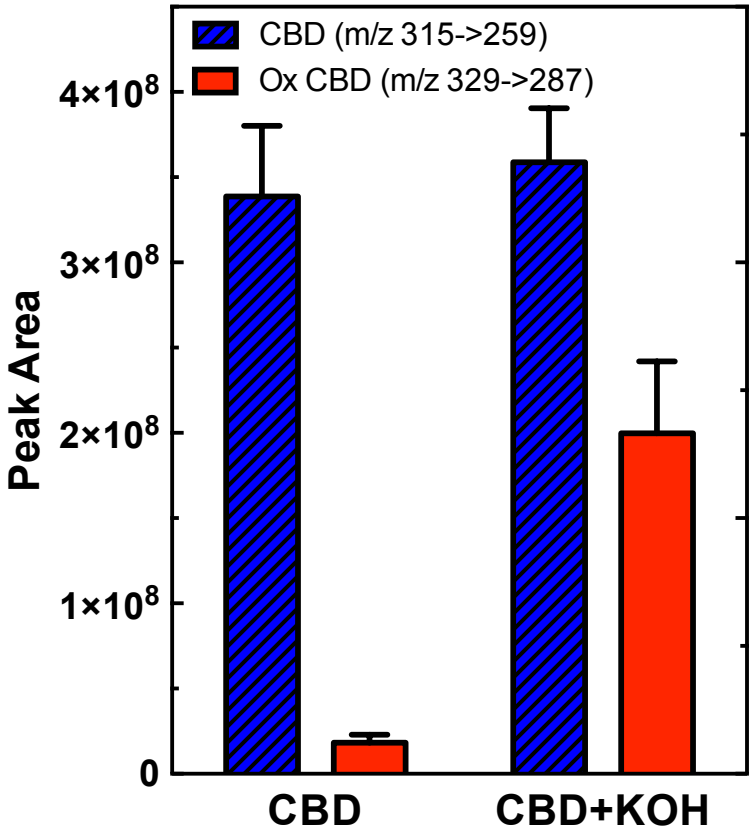


Figure S4: Analysis of KOH-Oxidized Cannabidiol. Samples of cannabidiol (CBD) and KOH-oxidized cannabidiol (Ox CBD) were subjected to LC-MS/MS analysis at matching concentrations. The precursor-to-product ion transitions for CBD (m/z 315>259) and Ox CBD (m/z 329>287) were used to measure relative levels of each compound by selected reaction monitoring. Results above display the peak areas for CBD (m/z 315>259) and Ox CBD (m/z 329>287) in two samples from an initial concentration of CBD at 200 μM. Results are plotted as the mean of four replicates and error bars represent the standard deviation of those samples.

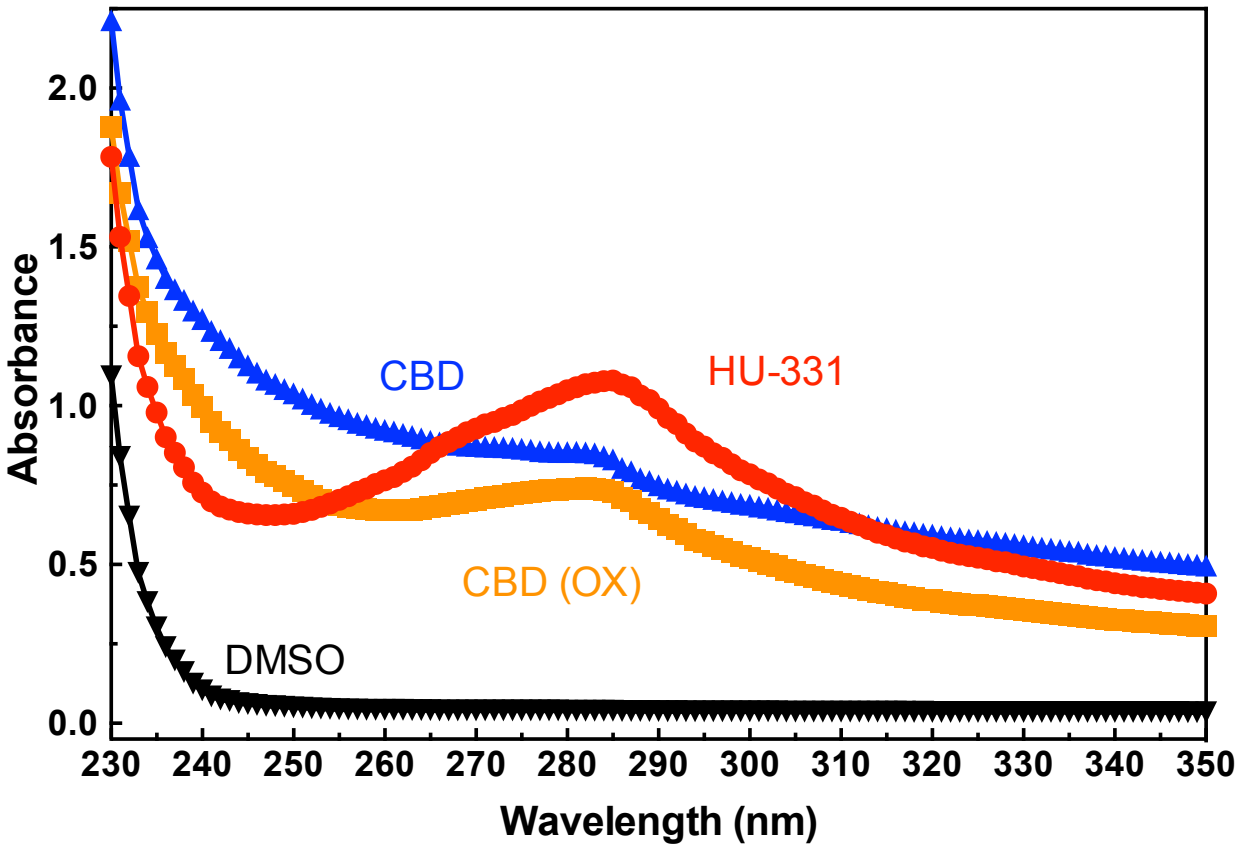


Figure S5: UV Absorbance Spectra for CBD, Oxidized CBD, and HU-331. The absorbance spectra at 1 nm resolution for 20 mM CBD, oxidized CBD (ox CBD), and HU-331 are shown.

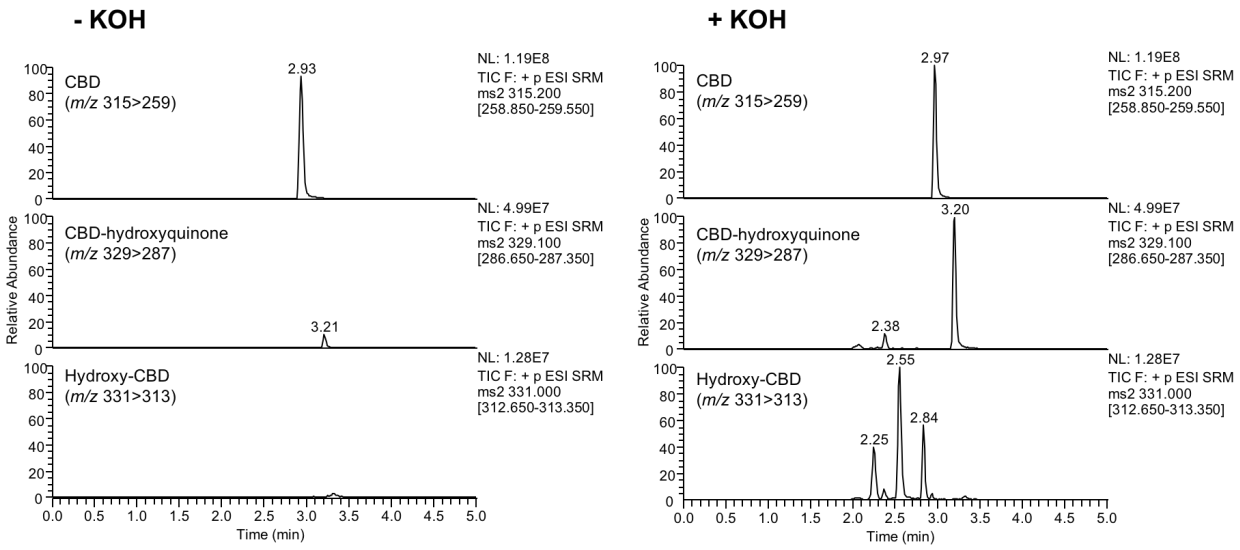


Figure S6: KOH-oxidized CBD. LC-MS/MS analysis of CBD oxidized products with and without KOH treatment. Shown are LC-MS/MS chromatograms from selected reaction monitoring (SRM) of CBD (*m/z* 315>259), CBD-hydroxyquinone (*m/z* 329>287), and hydroxy-CBD products (*m/z* 331>313) +/- KOH. The graphs have been scaled equally for comparison of treatment conditions.