Supplemental information

Metabolism of a kratom alkaloid metabolite in human plasma increases its opioid potency and efficacy

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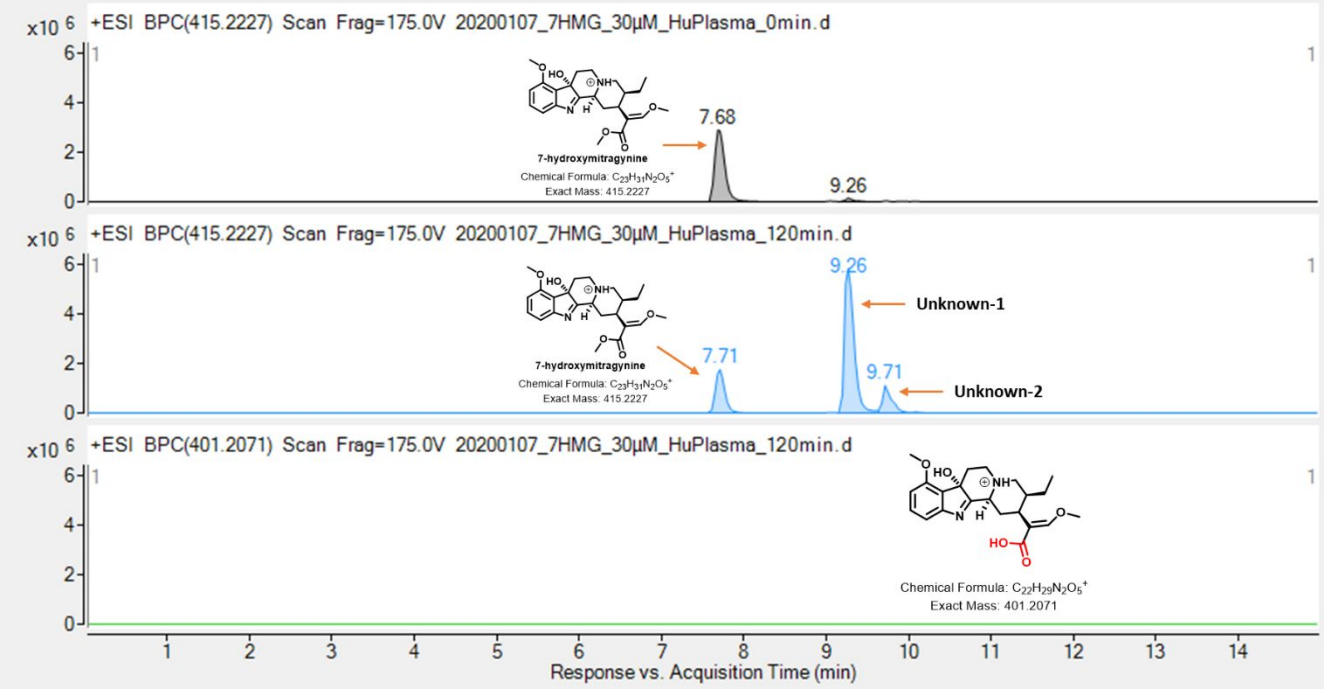

Figure S1: High resolution mass spectrometry analysis of 7-hydroxymitragynine human plasma stability***.*** *Top panel indicates 7-hydroxymitragynine (m/z 415.2227) base peak chromatogram of 0 min incubation sample in human plasma, the middle panel indicates base peak chromatogram of 2 hr human plasma incubated with 7-hydroxymitragynine sample extracted at m/z 415.2227 suggesting unknown peak having same exact mass as of 7-hydroxymitragynine and bottom panel represents the absence of hydrolyzed product of 7-hydroxymitragynine in 2 hr human plasma incubation sample.*

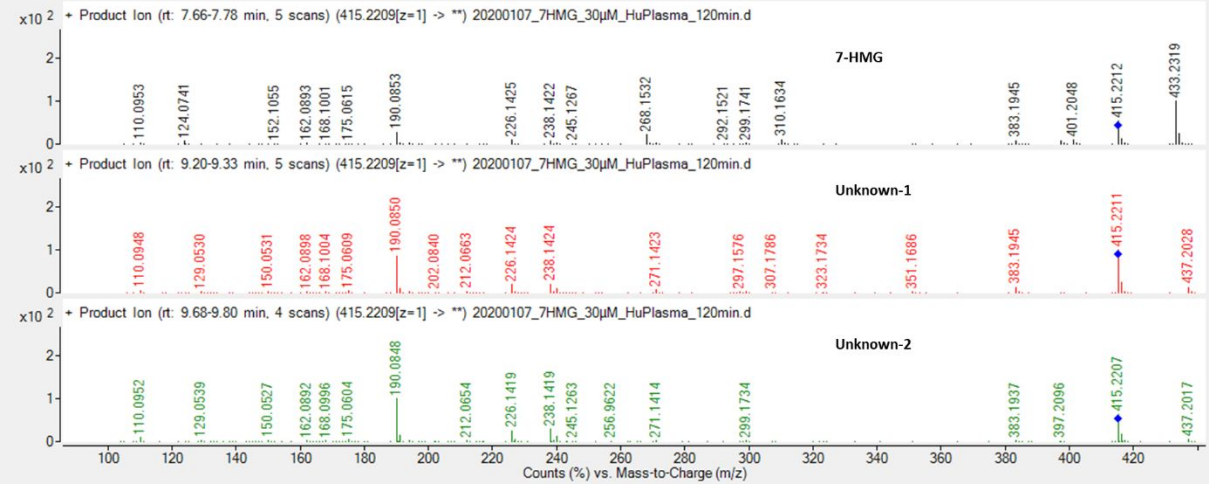

Figure S2: Comparison of MS^2 fragmentation of 7-hydroxymitragynine (top), unknown-1 (middle), and unknown-2 (bottom).

Isolation and purification of unknown-1

The plasma reaction was lyophilized and 20 mg of solid was obtained, the sample was subjected to a partition liquid-liquid with chloroform and water in a separation funnel. Three portions of 10 mL of chloroform were used, the organic layers were combined and 11 mg of a gummy solid was obtained. The sample (11 mg) was then chromatographed in thin layer chromatography (TLC) silica gel G (Sorbtech technologies, UV254, 1000 µm), as a stationary phase, and hexanes-EtOAc (7:3) with 0.1% of ammonium hydroxide as a mobile phase, three major bands were chosen, band at RF: 0.8, 0.7 and 0.4. The bands were extracted and 2, 1, and 3 mg were recovered, respectively. The bands were subjected to high- resolution electrospray ionization mass spectrometry (HR-ESI-MS) analysis the band at 0.8 was identified as the unknown-1, band at 0.7 corresponded to the 7-hydroxymitragynine (7-HMG) and band at 0.4 was a mixture of at least three compounds. ^{1}D and ^{2}D NMR experiments, as well as nuclear Overhauser effect (NoE) selective experiments of the unknown-1 were carried out (Figures S3-S10), careful analysis of all the experiments coincide with those of the known compound, mitragynine pseudoindoxyl.

Synthesis of mitragynine pseudoindoxyl

7-HMG (200 mg, 0.48 mmol) was dissolved in dry toluene (6 mL), and $Zn(OTf)_2$ (350 mg, 2 molar equiv) was added. The reaction was stirred over argon at reflux for 2 h. The reaction mixture was cooled at room temperature and a solution of $NaHCO_3$ (20 mL) was added. The solution was extracted with EtOAc (50 mL), washed with brine (20 mL) and water (20 mL) and dried over anhydrous Na_2SO4. After evaporation of the solvent under reduced pressure. The residue was redissolved in chloroform and isolated by chromatography column using a combiflash (Teledyne®) instrument in an isocratic mobile phase hexanes-EtOAc (6:4), to yield: 100 mg (50 %) mitragynine pseudoindoxyl as a yellowish solid. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.34 (t, *J* = 8.1 Hz, 1H), 7.30 (s, 1H), 6.42 (d, *J* = 8.1 Hz, 1H), 6.15 (d, *J* = 8.0 Hz, 1H), 5.36 (s, 1H), 3.91 (s, 3H), 3.68 (s, 3H), 3.64 (s, 3H), 3.17 (d, *J* = 10.7 Hz, 2H), 2.84 – 2.76 (m, 1H), 2.36 (dt, *J* = 12.3, 9.3 Hz, 2H), 2.31 – 2.22 (m, 2H), 1.93 (dd, *J* = 14.3, 5.7 Hz, 1H), 1.72 – 1.62 (m, 1H), 1.54 (d, *J* = 11.3 Hz, 1H), 1.22 (ddd, *J* = 13.6, 7.5, 2.8 Hz, 1H), 1.15 (dd, *J* = 11.4, 3.7 Hz, 1H), 0.87 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 199.49, 168.82, 162.14, 160.24, 158.58, 138.72, 111.56, 109.59, 103.78, 98.94, 74.98, 73.34, 61.43, 55.64, 54.76, 53.19, 51.16, 40.02, 38.39, 35.01, 23.73, 19.24, 12.85.

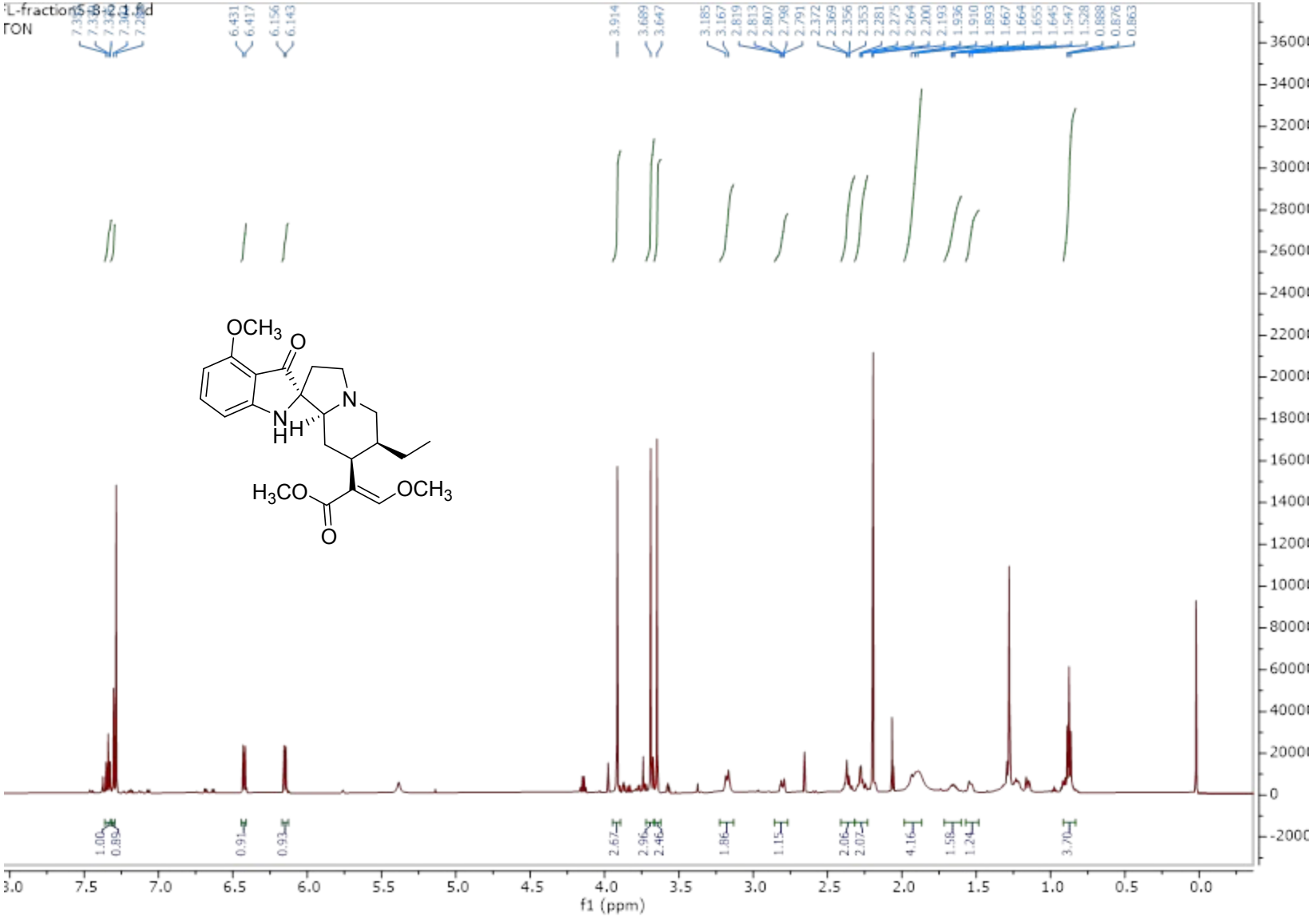

Figure S3. ^{1}H NMR spectrum of plasma isolated unknown-1 (mitragynine pseudoindoxyl) in $CDCl_3$, 600 MHz.

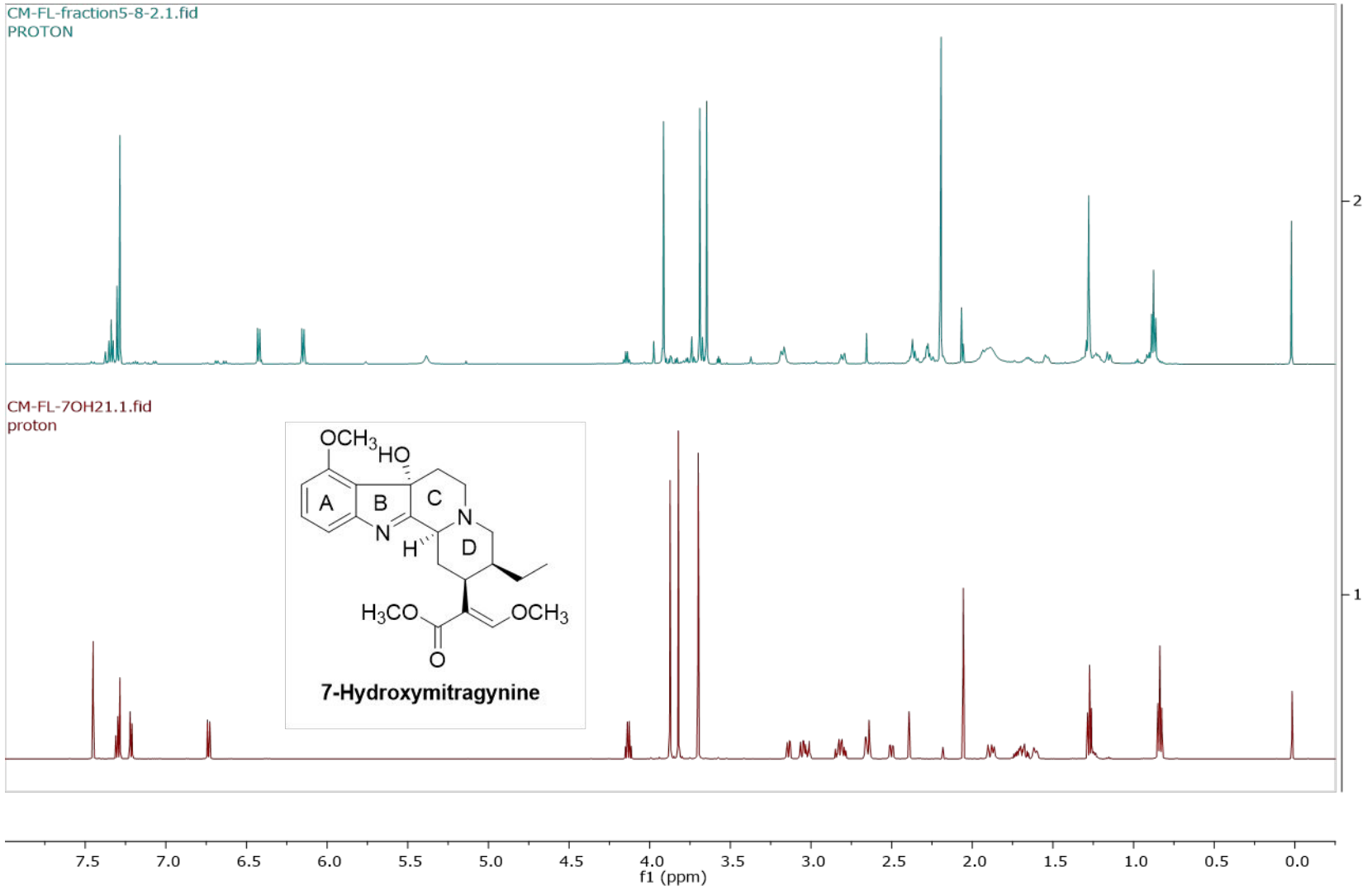

Figure S4. Comparison of ^{1}H NMR spectra of plasma isolated unknown-1 (mitragynine pseudoindoxyl) and 7-HMG in $CDCl_3$, 600 MHz.

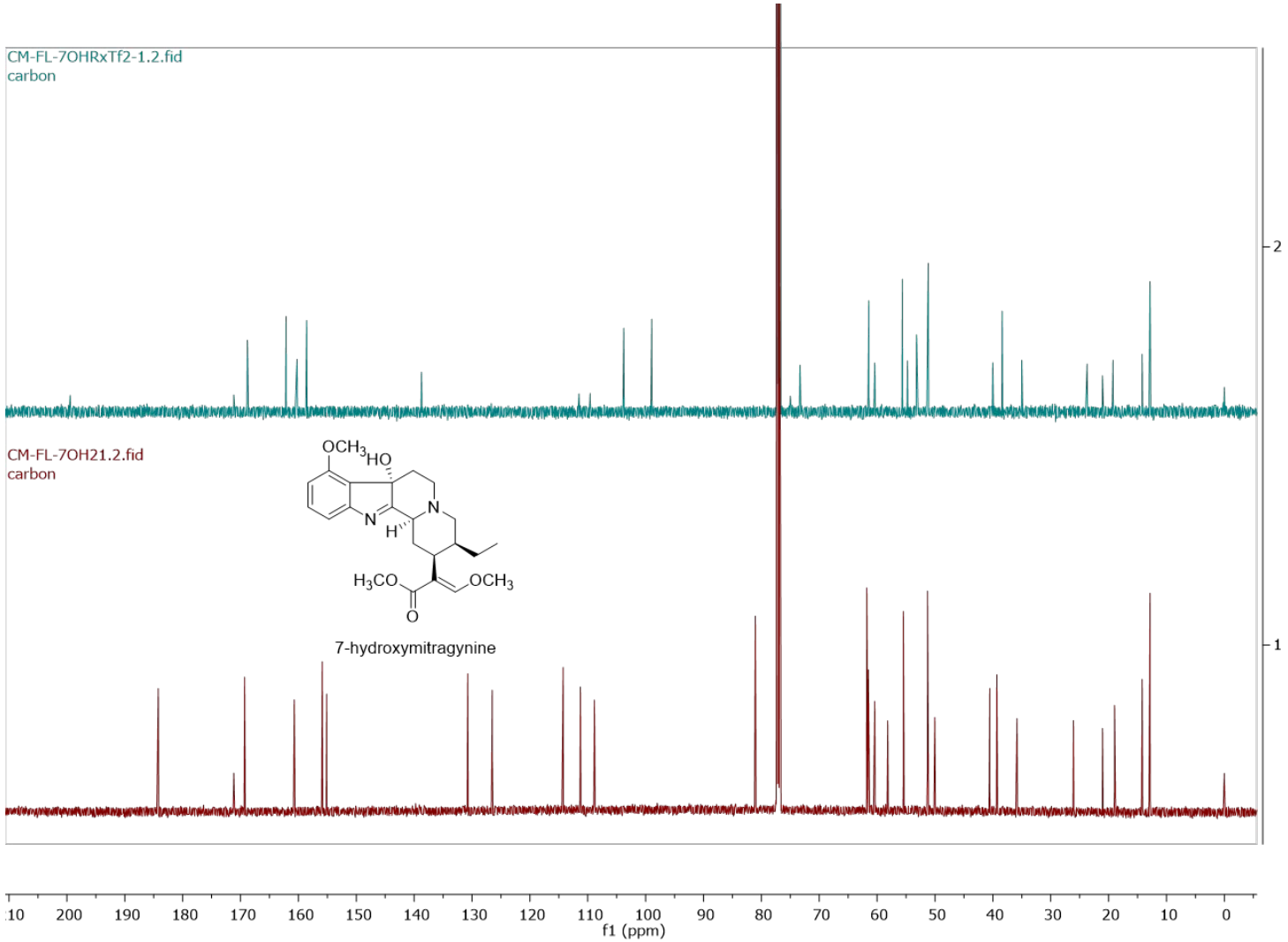

Figure S5. Comparison of ^{13}C NMR spectrum of plasma isolated unknown-1 (mitragynine pseudoindoxyl) and 7-HMG in $CDCl_3$, 150 MHz.

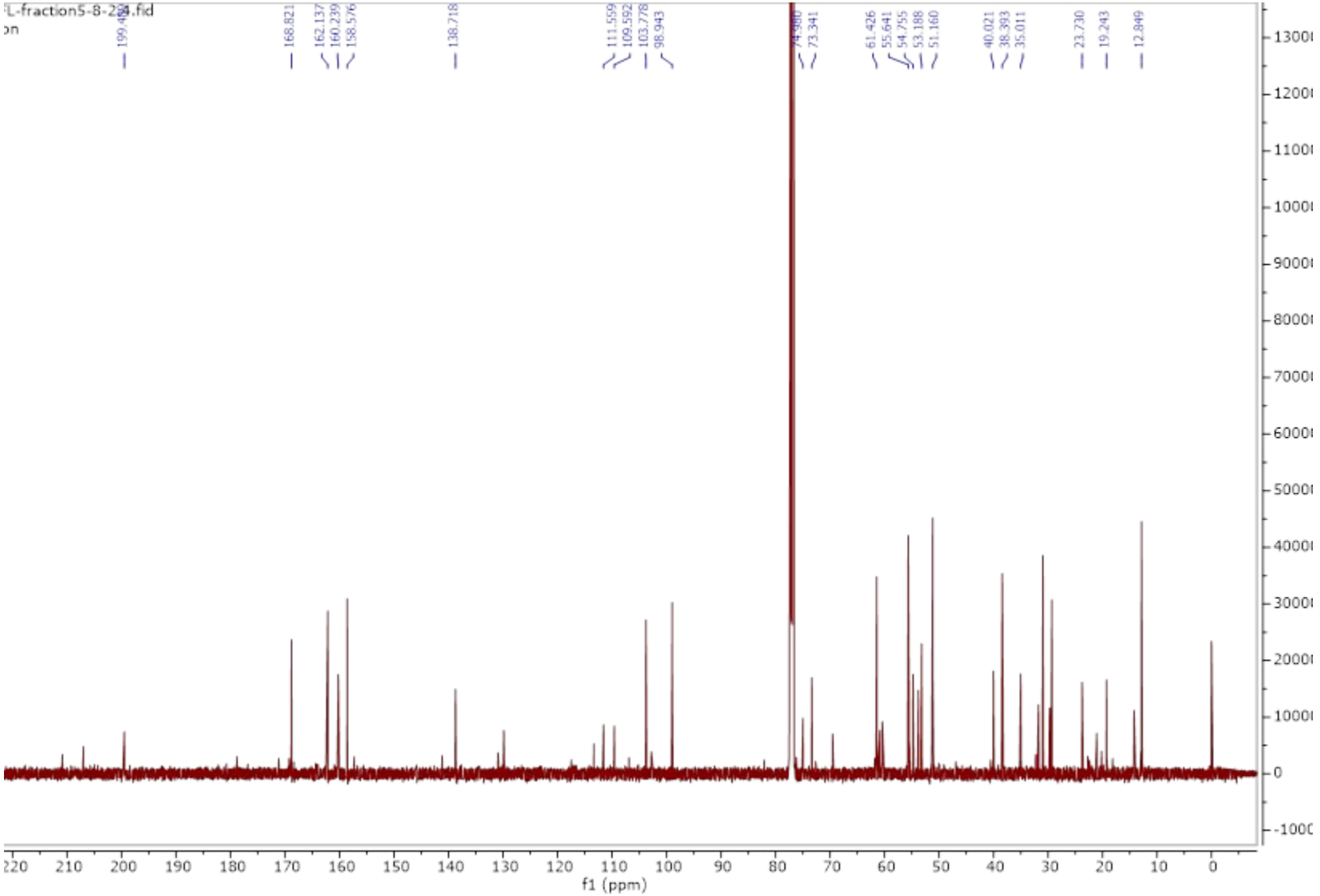

Figure S6. ^{13}C NMR spectrum of plasma isolated unknown-1 (mitragynine pseudoindoxyl) in $CDCl_3$, 150 MHz

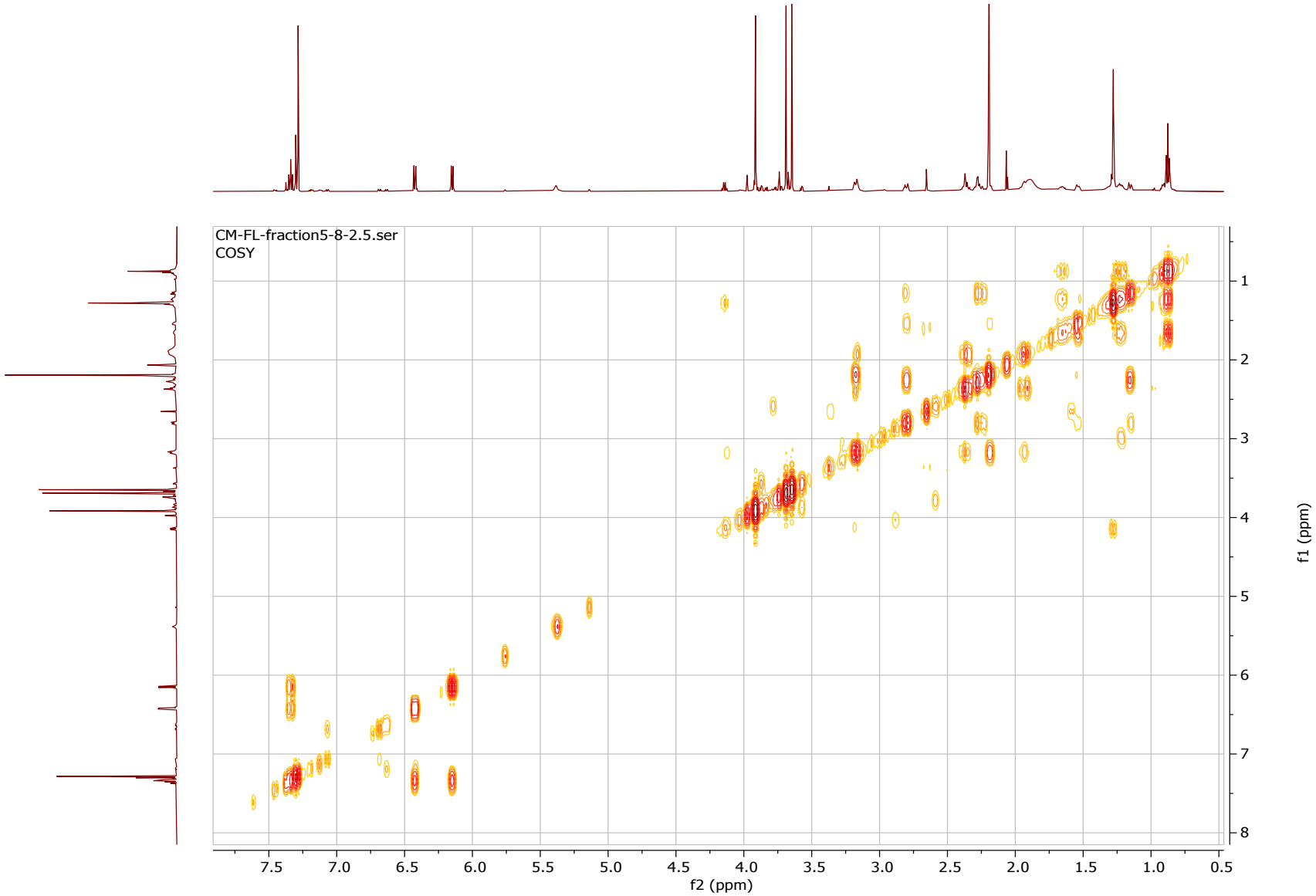

Figure S7. COSY experiment of plasma isolated unknown-1 (mitragynine pseudoindoxyl).

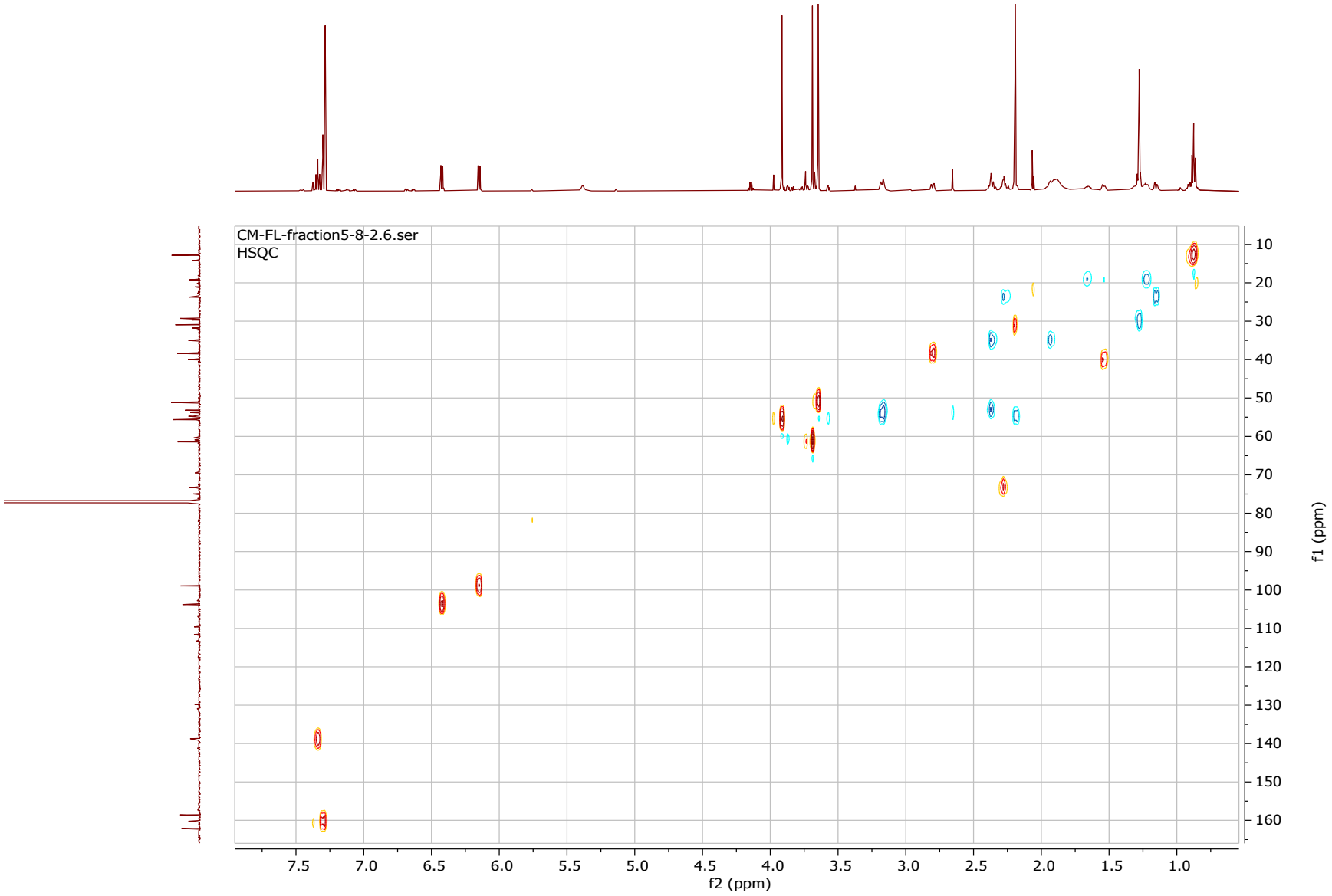

Figure S8. HSQC experiment of plasma isolated unknown-1 (mitragynine pseudoindoxyl).

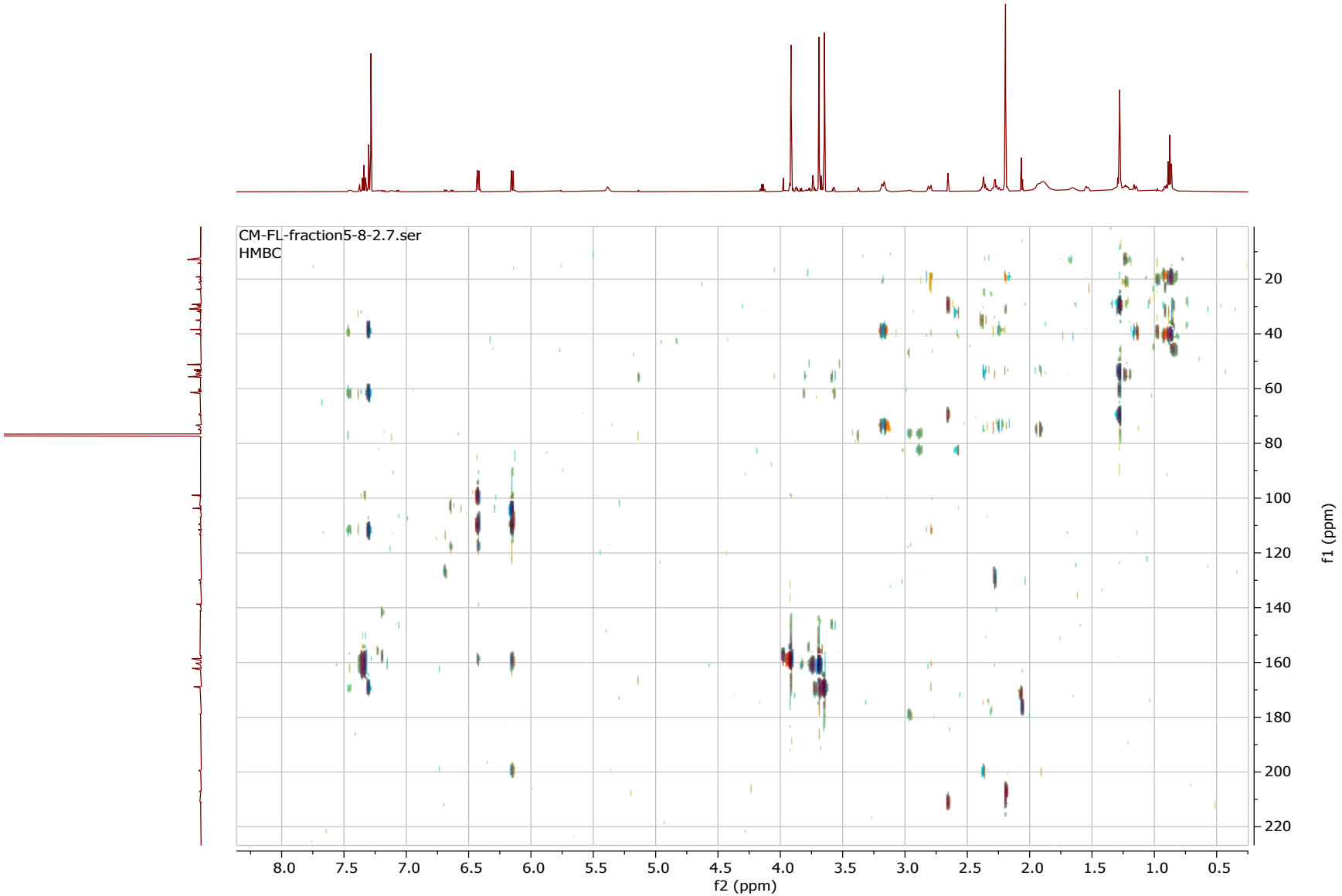

Figure S9. HMBC experiment of plasma isolated unknown-1 (mitragynine pseudoindoxyl).

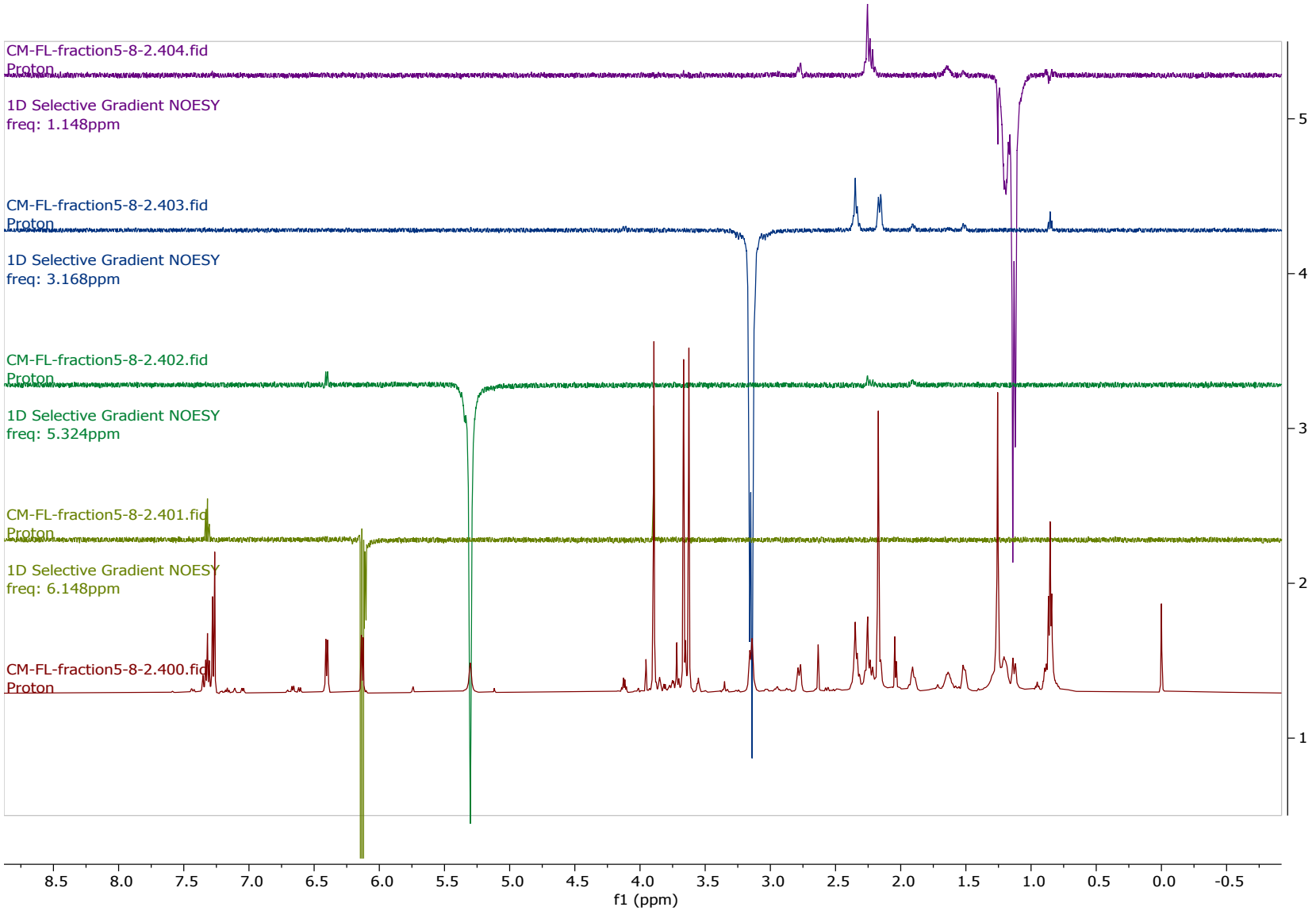

Figure S10. Selected NoE selective experiments of plasma isolated unknown-1 (mitragynine pseudoindoxyl).

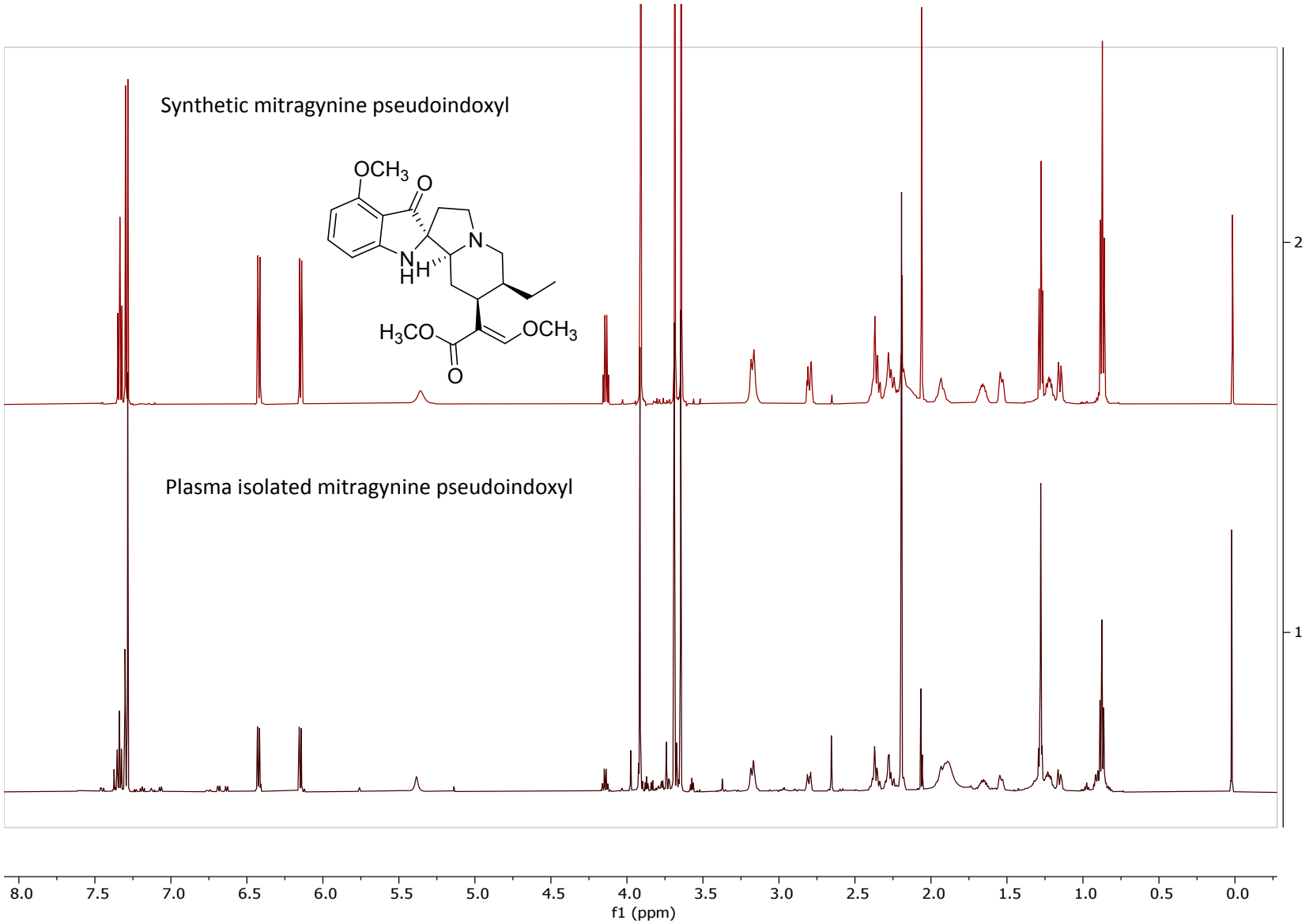

Figure S11. Comparison of ^{1}H NMR spectra of synthetic and plasma isolated mitragynine pseudoindoxyl.

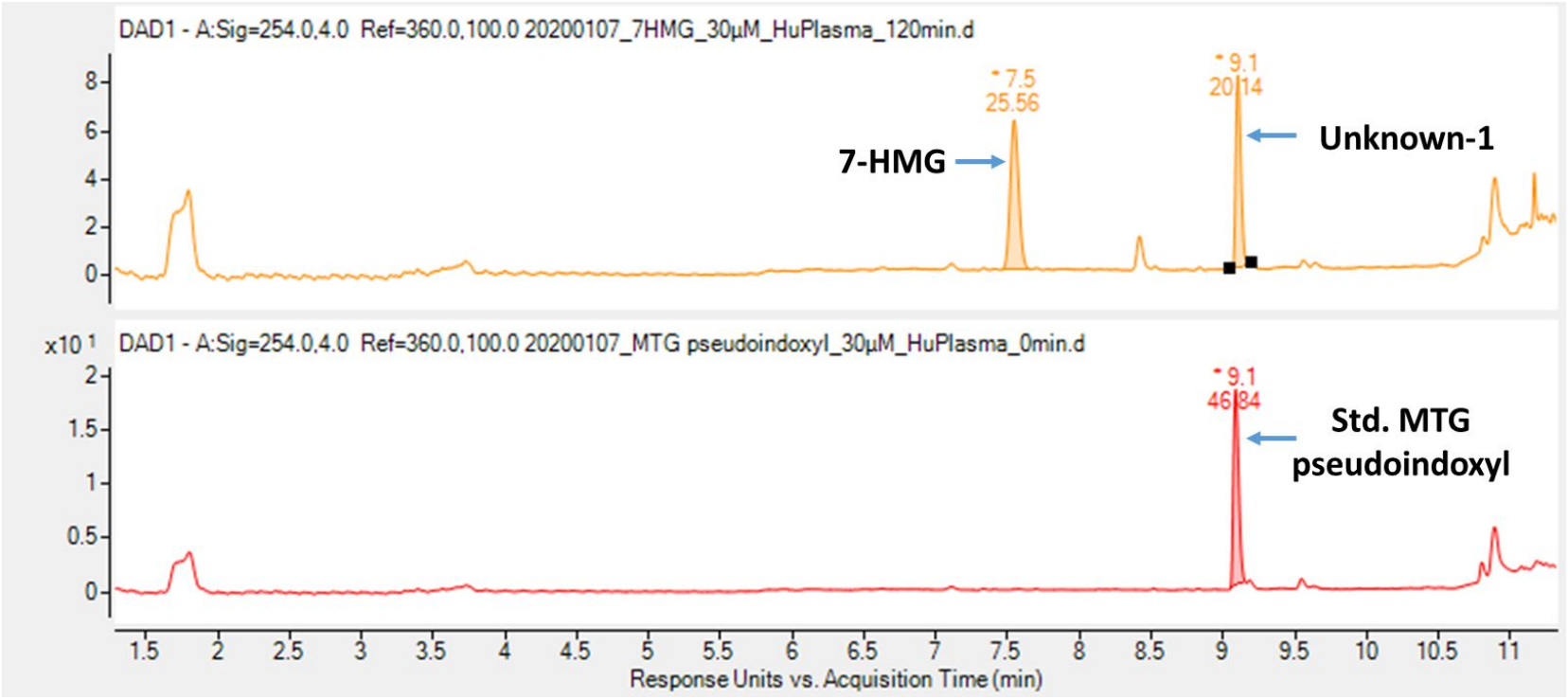

Figure S12. Comparison of UPLC-UV chromatogram of unknown-1 formed upon 7-HMG incubation in human plasma and synthetic standard of mitragynine pseudoindoxyl spiked in human plasma.

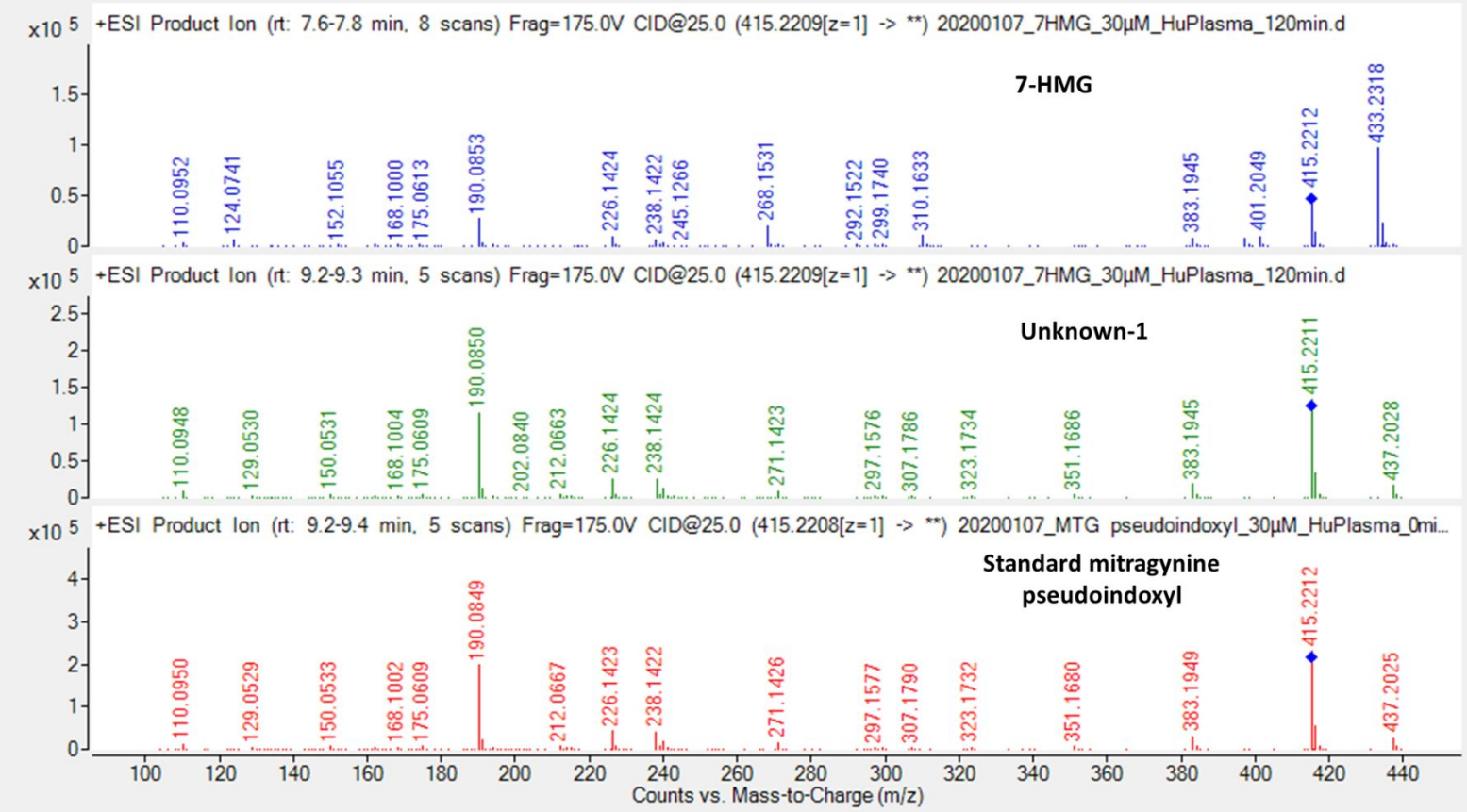

Figure S13. Comparison of MS2 spectra of 7-HMG, unknown-1 formed upon 7-HMG incubation in human plasma and synthetic standard of mitragynine pseudoindoxyl.

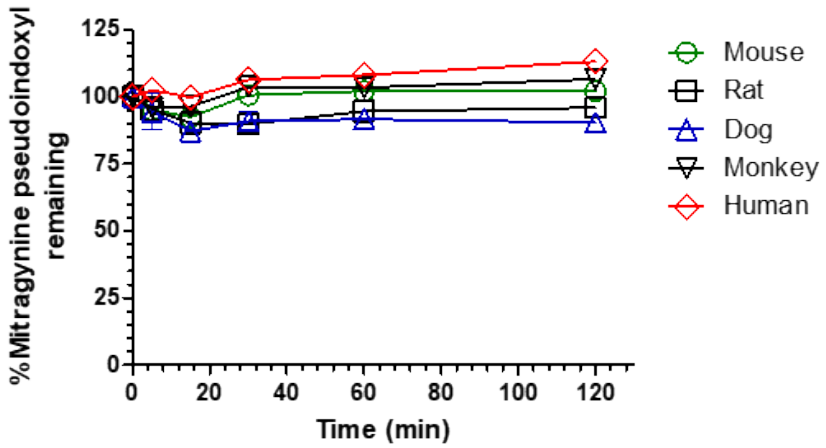

Figure S14. Stability of mitragynine pseudoindoxyl in mouse, rat, dog, cynomolgus monkey, and human plasma. *Mitragynine pseudoindoxyl at 1 μM concentration incubated mouse, rat, dog, cynomolgus monkey, and human plasma for 120 minutes. The data is represented as the percentage of mitragynine pseudoindoxyl remaining (mean ± SD, n=3) versus time.*

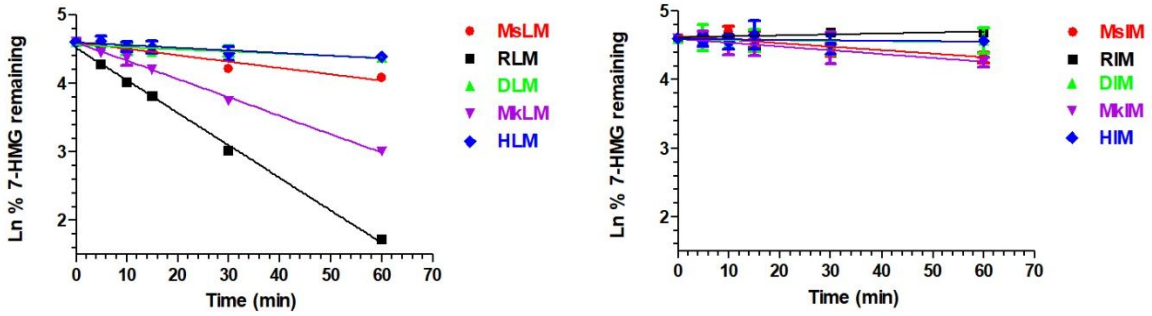

Figure S15. *In vitro* metabolism of 7-HMG in mouse, rat, dog, cynomolgus monkey, and human liver (left) and intestinal (right) microsomes*. 7-HMG at 1 µM concentration incubated with either liver or intestinal microsomes of mouse (MsLM or MsIM) or rat (RLM or RIM) or dog (DLM or DIM) or cynomolgus monkey (MkLM or MkIM) or human (HLM or HIM) supplemented with NADPH. The data is represented as the natural logarithm of the percentage of 7-HMG remaining (mean ± SD, n=3) versus time. 7-HMG was stable in intestinal microsomal incubations of all tested species and in liver microsomal incubations of human, dog, and mouse with a microsomal half-life ($T_{1/2}$)> 60 min, while in RLM and MkLM the $T_{1/2}$ was found to be 14.6 and 25.8 min.*