Supporting Information

Pharmacokinetics and Safety of Mitragynine in Beagle Dogs

Elizabeth A. Maxwell[1], Tamara I. King[2], Shyam H. Kamble[2, 3], Kanumuri Siva Rama Raju[2, 3], Erin C. Berthold[2], Francisco León[4], Bonnie A. Avery[2, 3], Lance R. McMahon[5], Christopher R. McCurdy[3, 4], Abhisheak Sharma[2, 3]

Affiliations

[1]Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA

[2]Department of Pharmaceutics, College of Pharmacy, University of Florida, Gainesville, FL, USA

[3]Translational Drug Development Core, Clinical and Translational Science Institute, University of Florida, Gainesville, FL, USA

[4]Department of Medicinal Chemistry, College of Pharmacy, University of Florida, Gainesville, FL, USA

[5]Department of Pharmacodynamics, College of Pharmacy, University of Florida, Gainesville, FL, USA

Correspondence

Dr. Abhisheak Sharma

Research Assistant Professor of Pharmaceutics and Assistant Director of UF Translational Drug Development Core

Department of Pharmaceutics

University of Florida

1345 Center Drive

Gainesville, FL, 32610

USA

Phone: +1 352 294 8690

Fax: +1 352 273 7854

asharma1@cop.ufl.edu

Dr. Christopher R. McCurdy

Professor of Medicinal Chemistry and Director of UF Translational Drug Development Core

Department of Medicinal Chemistry

University of Florida

1345 Center Drive

Gainesville, FL 32610

USA

Phone: +1 352 294 8691

Fax: +1 352 392 9455

cmccurdy@cop.ufl.edu

Table of Contents

Isolation of mitragynine

Mitragynine was obtained from the commercial product Optimized Plant Mediated Solutions (OPMS) Gold capsules (Choice Organics). Ethanol (300 mL, 190 proof) was added to the brown powder of 100 capsules (~70 gr) and then stirred for aprox. 1 h. Further, the mixture was filtered using a Buchner funnel (600 mL) with fritted disc C, obtaining a brown solution. Additional ethanol (200 mL) was passed through the funnel until a clear white solid remained as a residue. The combined filtrates were subjected to vacuum evaporation through rotovapor to produce a brown solid (22 gr). The solid (18 gr) was fractionated using vacuum silica gel (5″ × 4.2″) column chromatography (CC) with gradients increasing the polarity from hexanes to ethyl acetate (EtOAc) and methanol. The fraction eluted with hexanes/EtOAc (7:3) was subjected to successive medium pressure chromatography columns using a combiflash (Teledyne) instrument in an isocratic mobile phase hexanes-EtOAc (8:2) to yield pure mitragynine as a slight yellow solid (6 gr).

Synthesis of 7-hydroxymitragynine

Mitragynine (500 mg, 1.25 mmol) was dissolved in acetonitrile (50 mL), then Pd/C 10% (1.5 g) was added. The resulting suspension was stirred at room temperature, and hydrogen peroxide (30%, 5 mL) was added slowly dropwise. The reaction mixture was stirred for an additional 10 min and passed over a pad of celite to obtain a pinkish solution. An additional 50 mL of acetonitrile were passed through the celite pad, the filtrates were combined and subjected to a vacuum evaporation through rotovapor to produce a yellow gummy solid. The residue was redissolved in chloroform and by CC using a combiflash (Teledyne) instrument in an isocratic mobile phase hexanes-EtOAc (6:4) to yield pure 7-hydroxy mitragynine as a slight yellow solid (300 mg, 0.72 mmol, 58%).

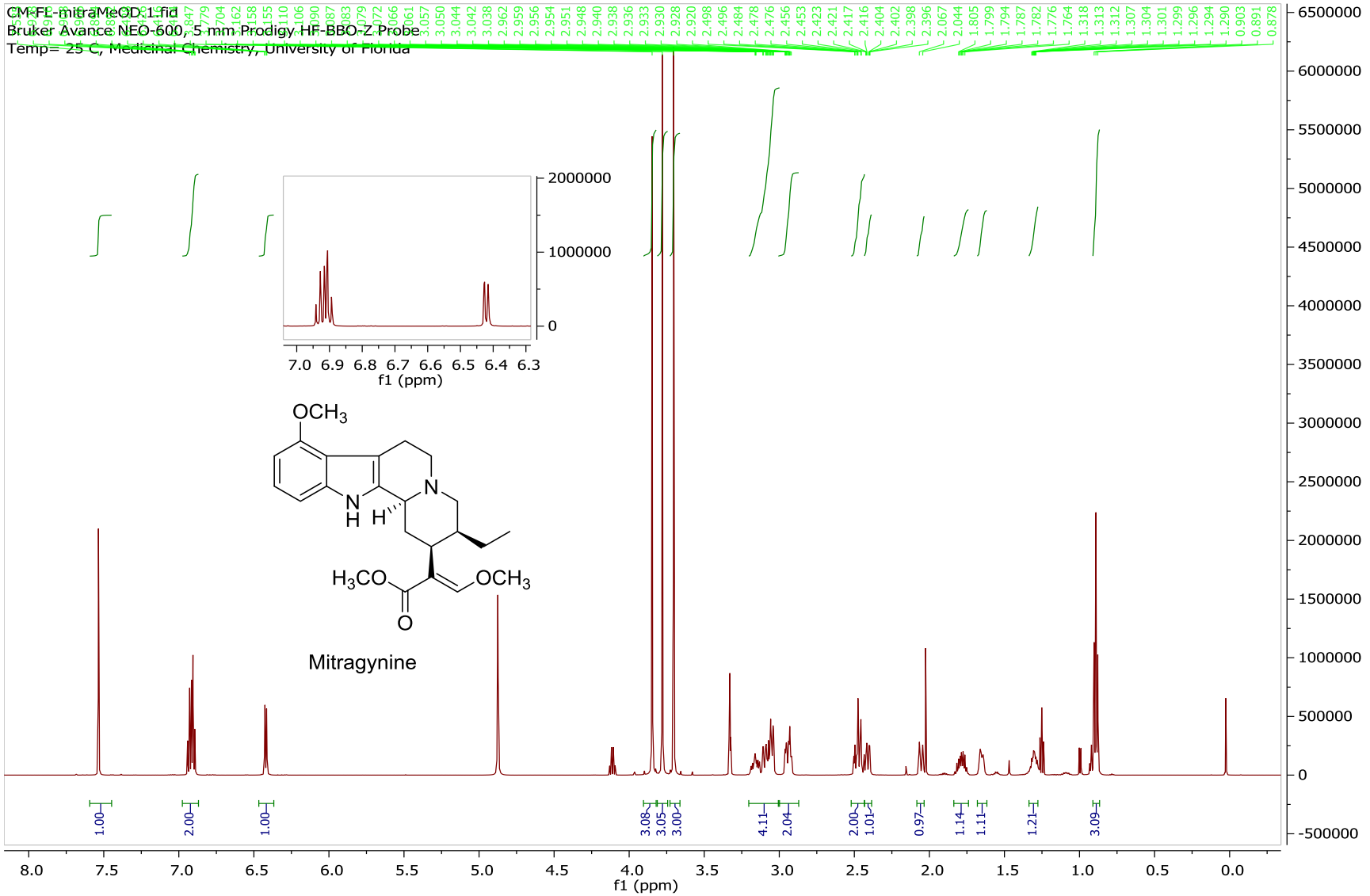

Fig. 1S ^{1}H NMR spectrum mitragynine in CD_3OD, 600 MHz

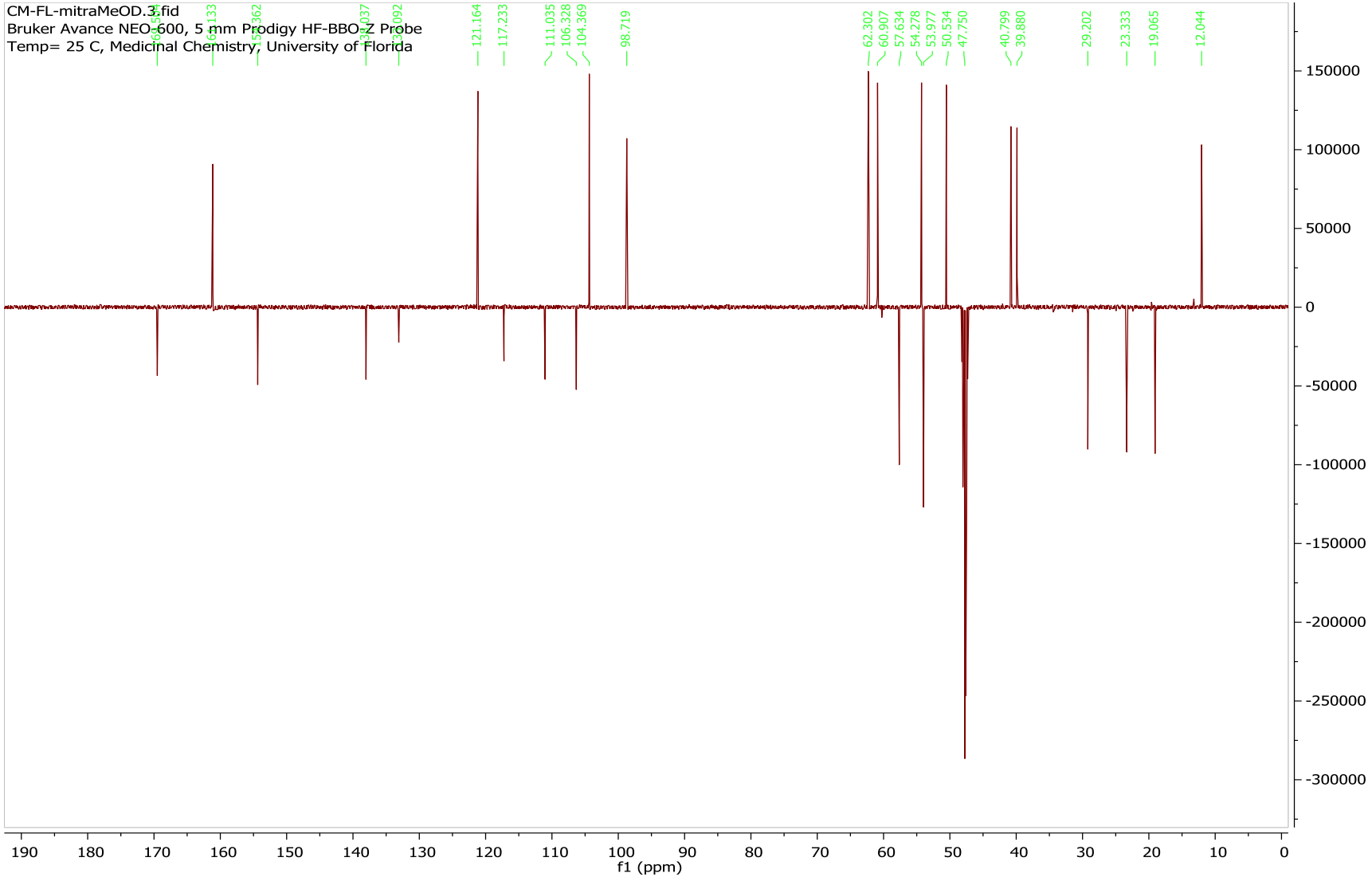

Fig. 2S. ^{13}C NMR APT experiment of mitragynine in CD_3OD, 150 MHz

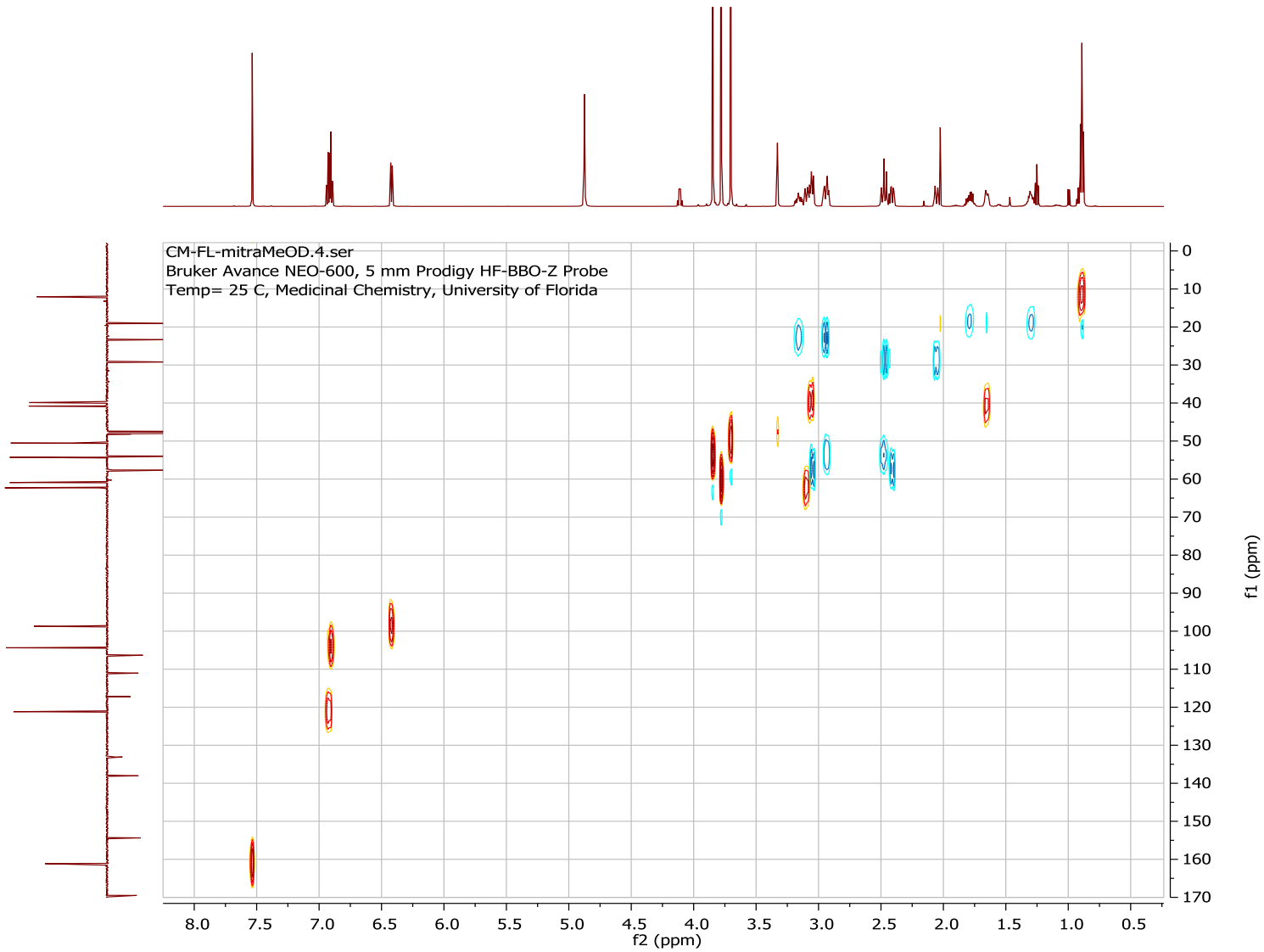

Fig. 3S 2D HSQC experiment of mitragynine in CD_3OD

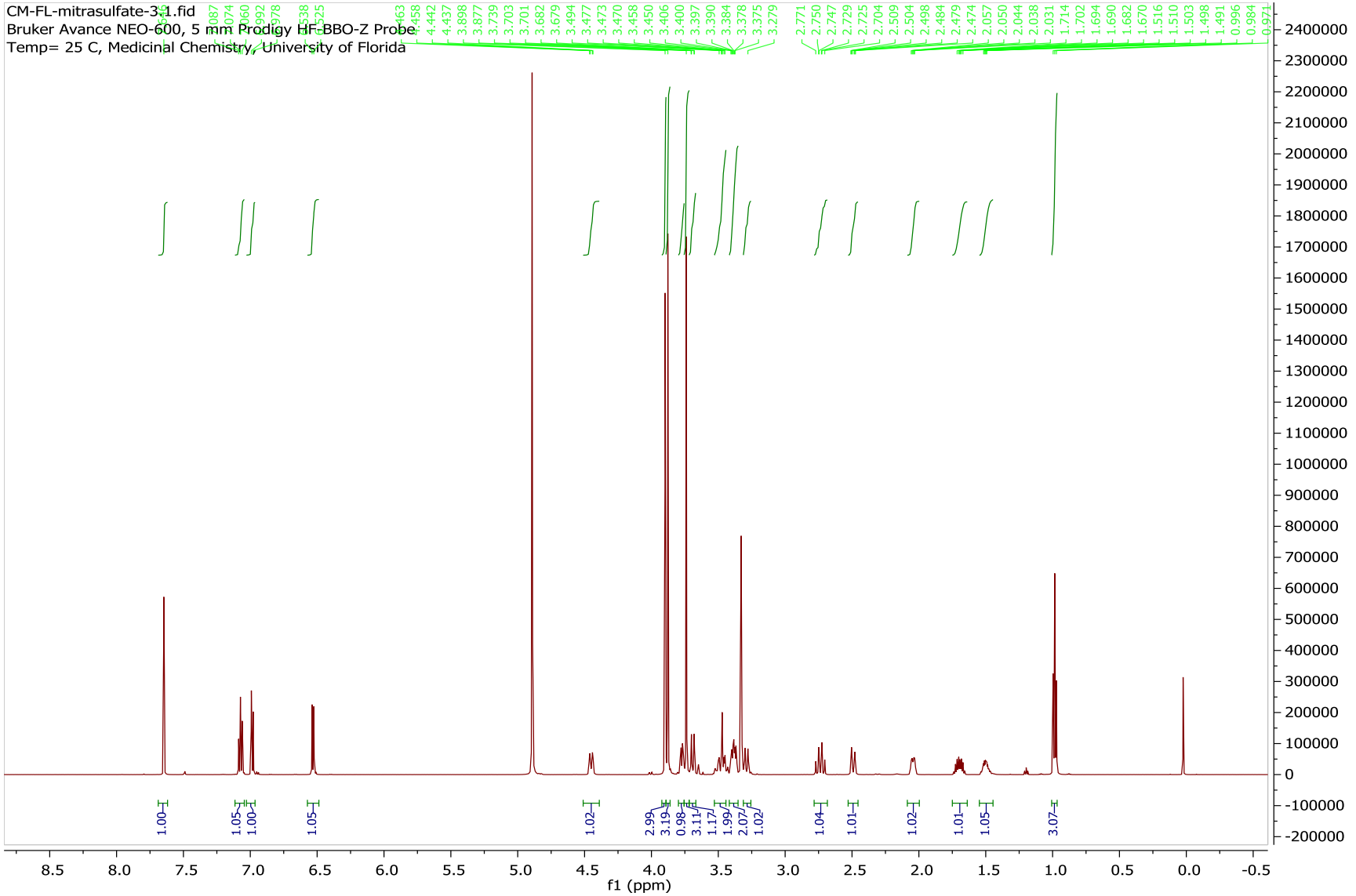

Fig. 4S ^{1}H NMR spectrum mitragynine sulfate in CD_3OD, 600 MHz

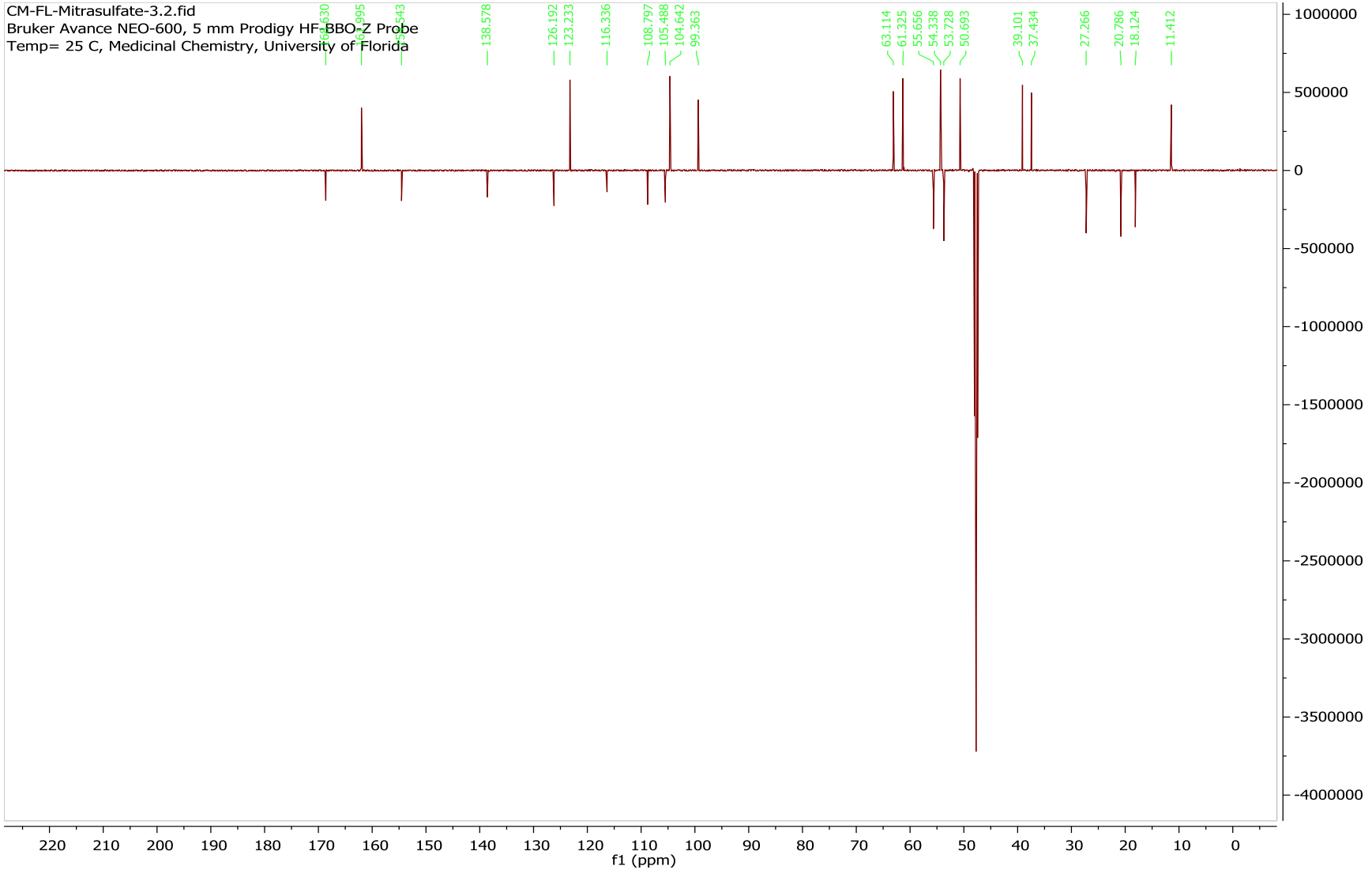

Fig. 5S ^{13}C NMR APT experiment of mitragynine sulfate in CD_3OD, 150 MHz

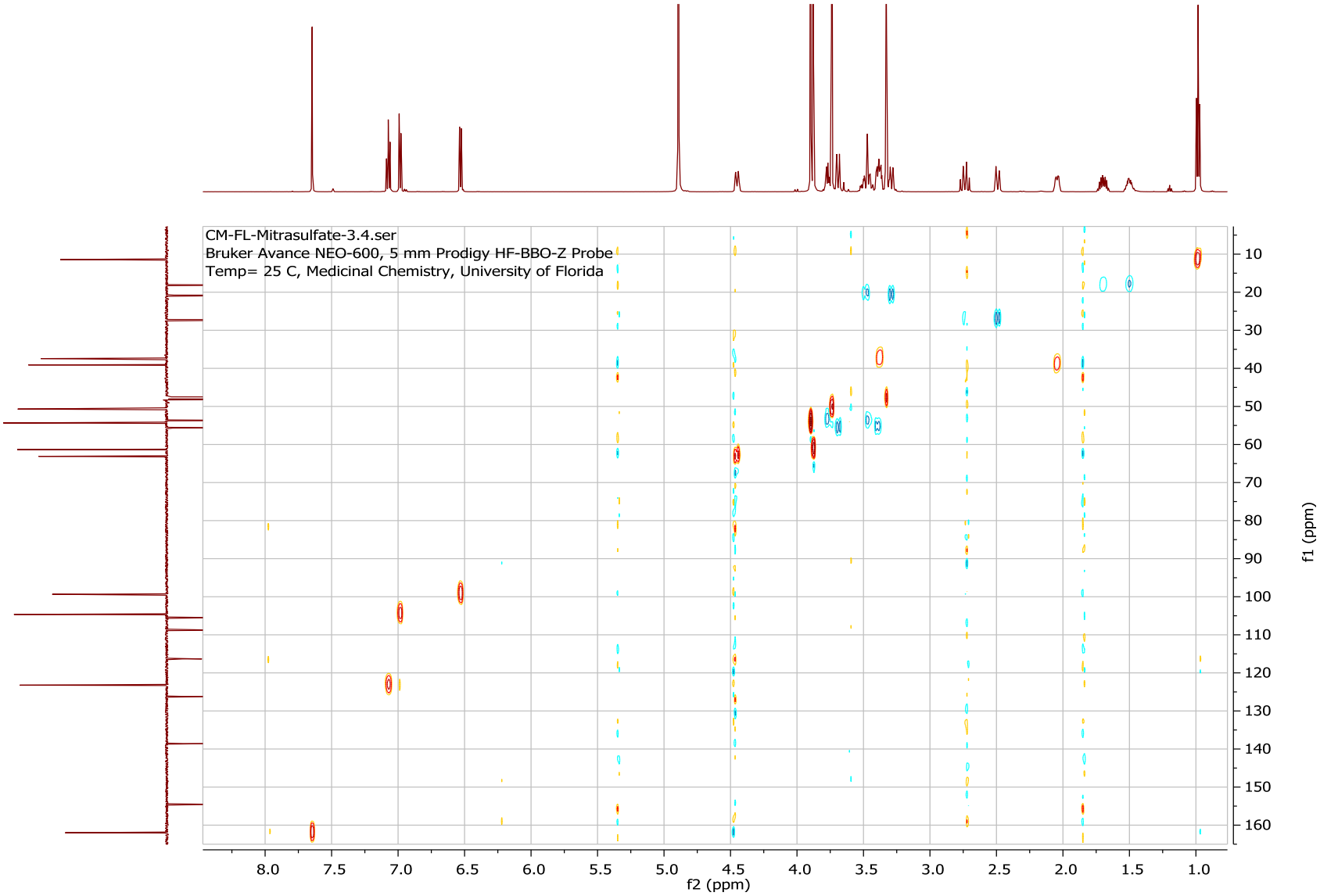

Fig. 6S 2D HSQC experiment of mitragynine sulfate in CD_3OD

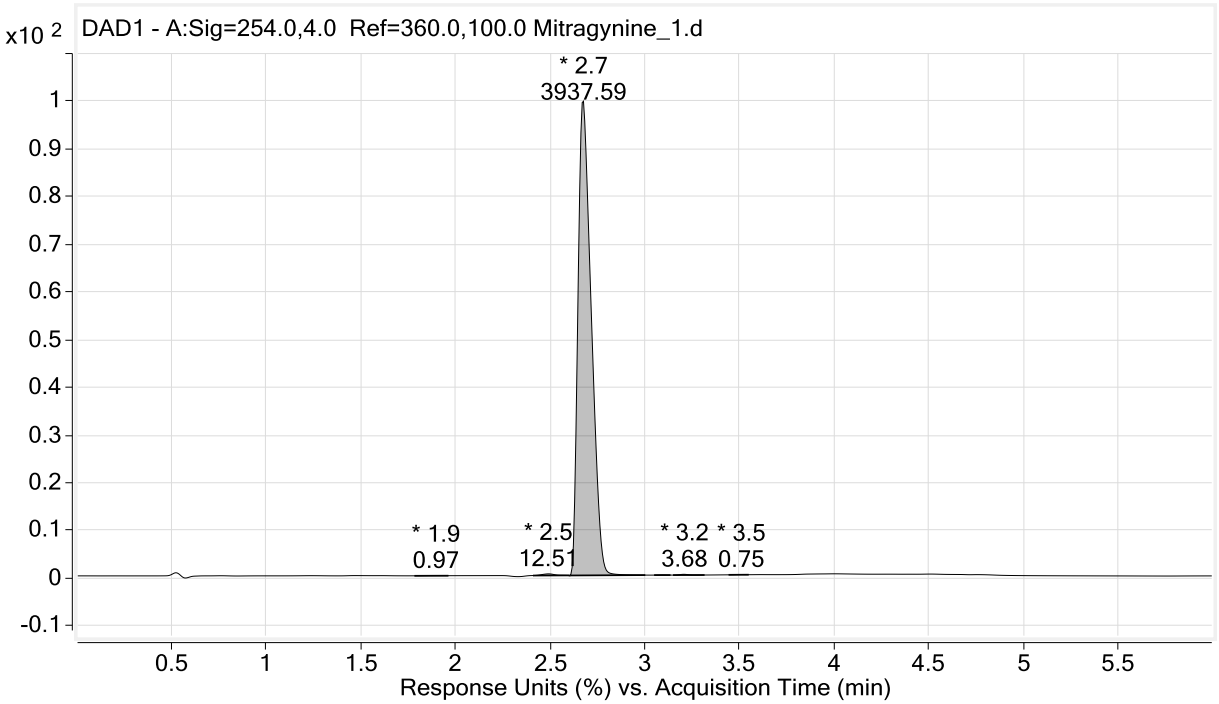

Fig. 7S UHPLC-PDA chromatogram and peak purity of mitragynine sulfate at 254 nm.

Table 1S Peak purity of mitragynine sulfate

Peak	RT	Area	Area Sum %	Height	Width	Compound
1	1.9	0.97	0.02	0.15	0.2	
2	2.5	12.51	0.32	2.71	0.2	
3	2.7	3937.59	99.53	806.42	0.4	Mitragynine
4	3.1	0.69	0.02	0.24	0.1	
5	3.2	3.68	0.09	1.23	0.2	
6	3.5	0.75	0.02	0.24	0.1	

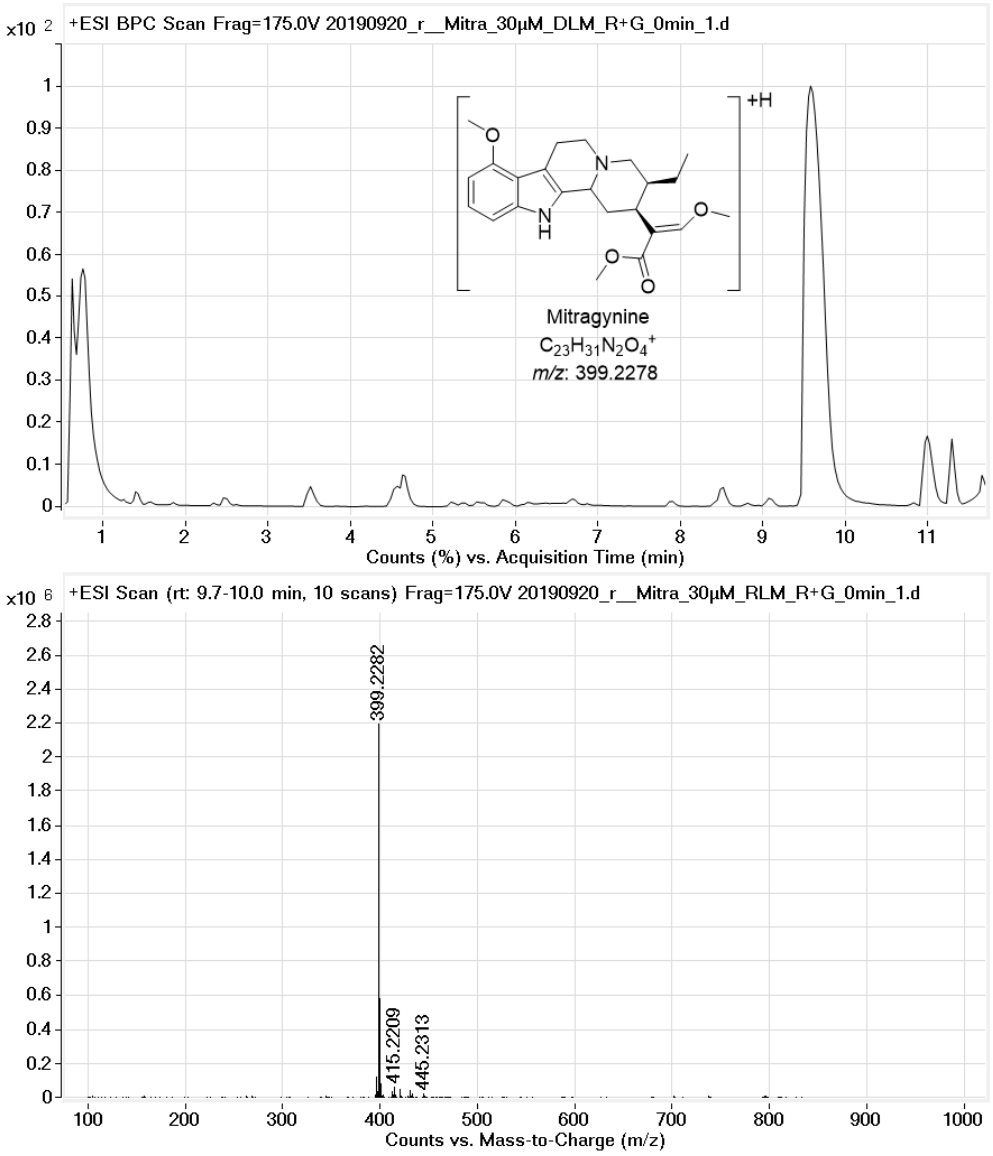

Fig. 8S UPLC-Q-TOF chromatogram and corresponding MS spectrum of mitragynine

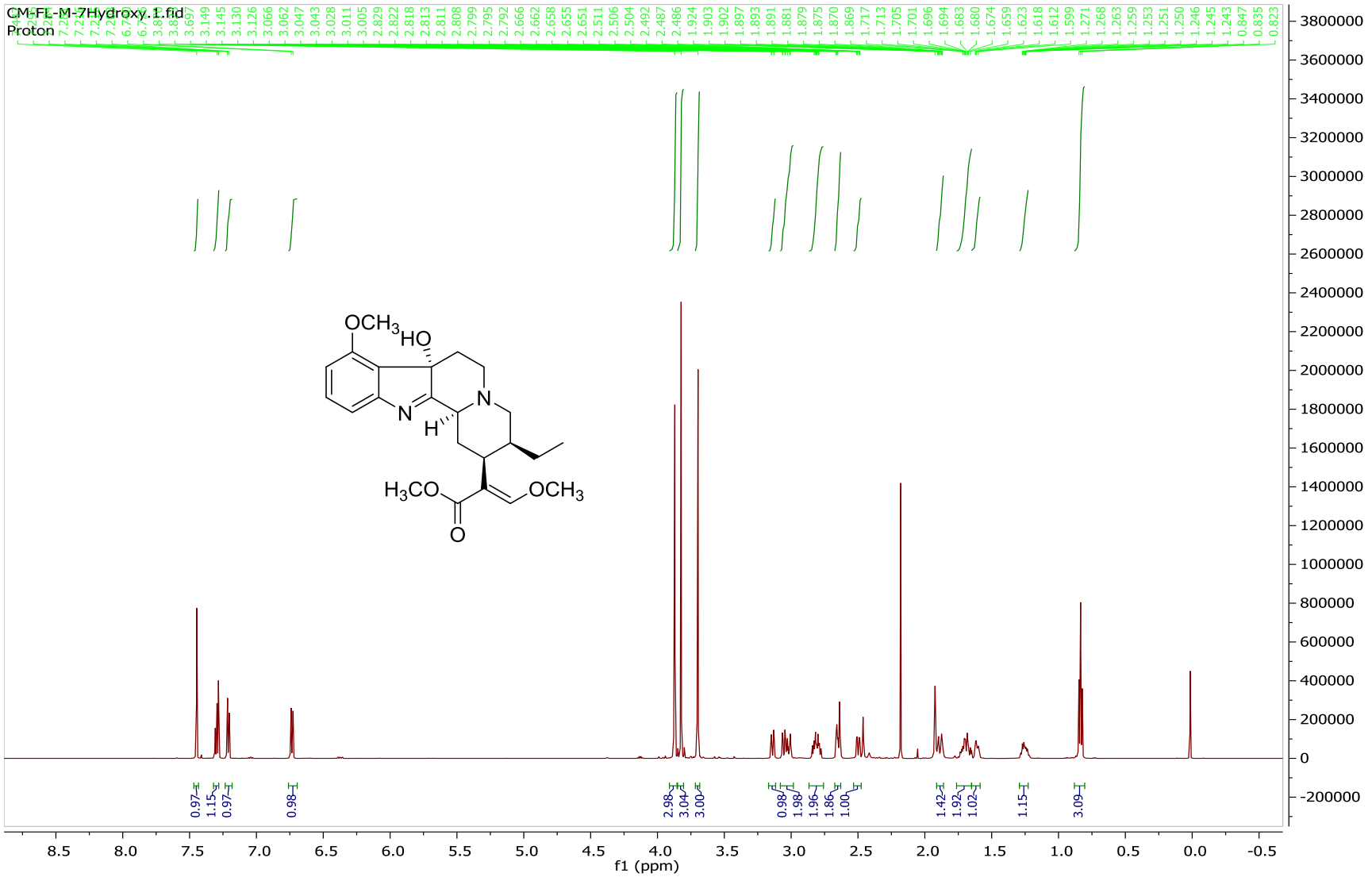

Fig. 9S ^{1}H NMR spectrum 7-hydroxymitragynine in $CDCl_3$, 600 MHz

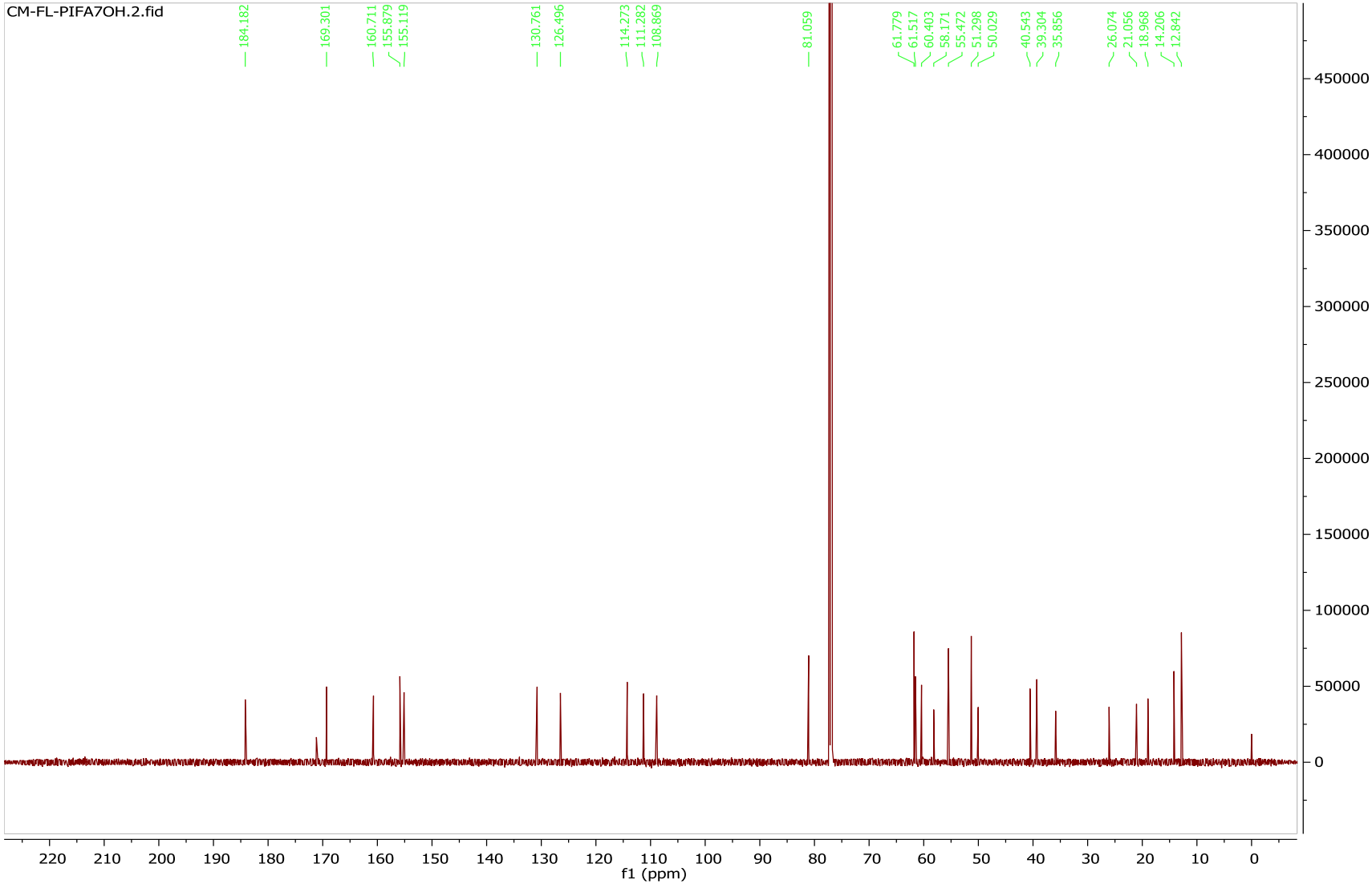

Fig. 10S ^{13}C NMR spectrum of 7-hydroxy mitragynine in $CDCl_3$, 150 MHz

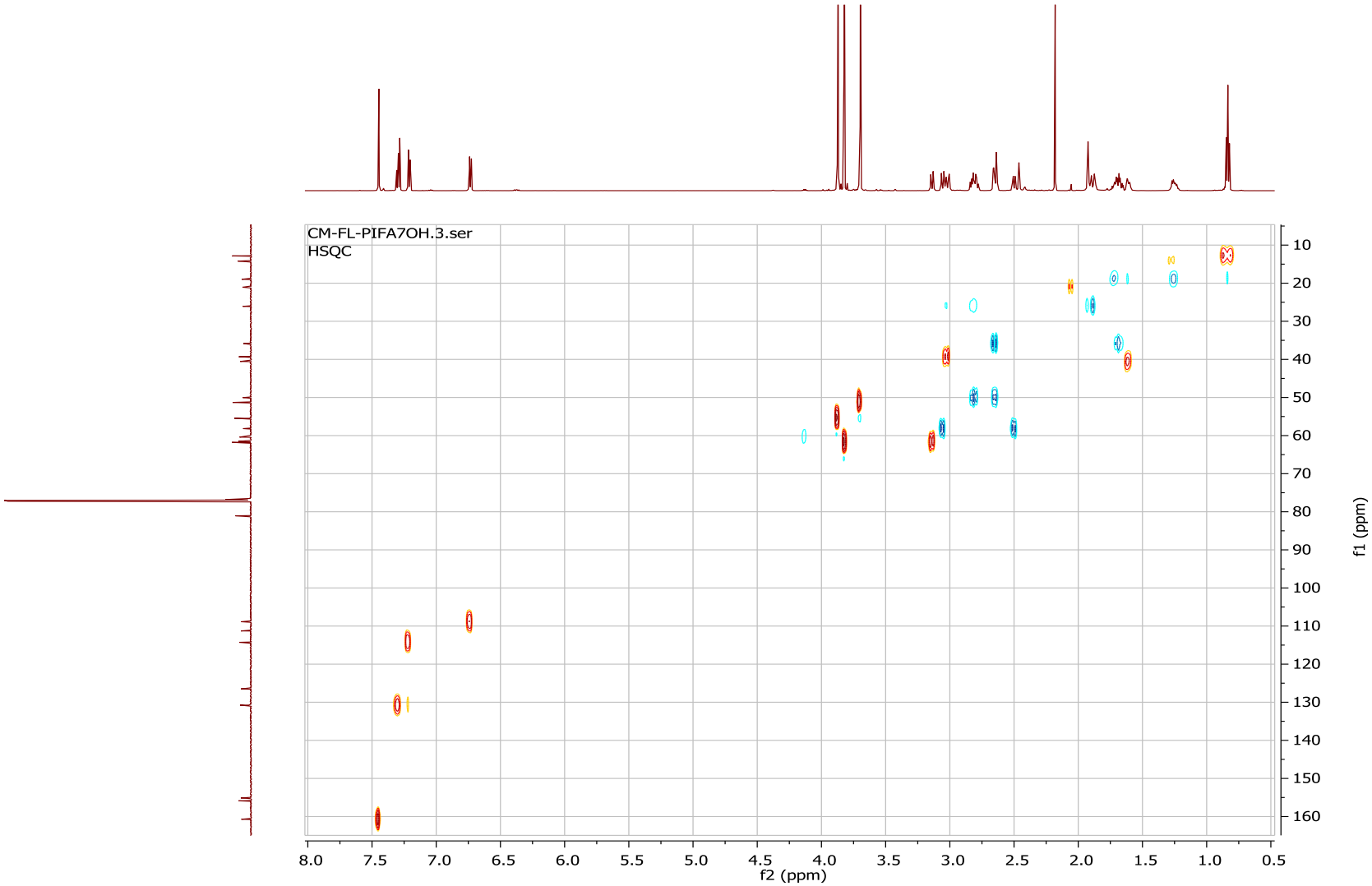

Fig. 11S 2D HSQC experiment of 7-hydroxymitragynine $CDCl_3$

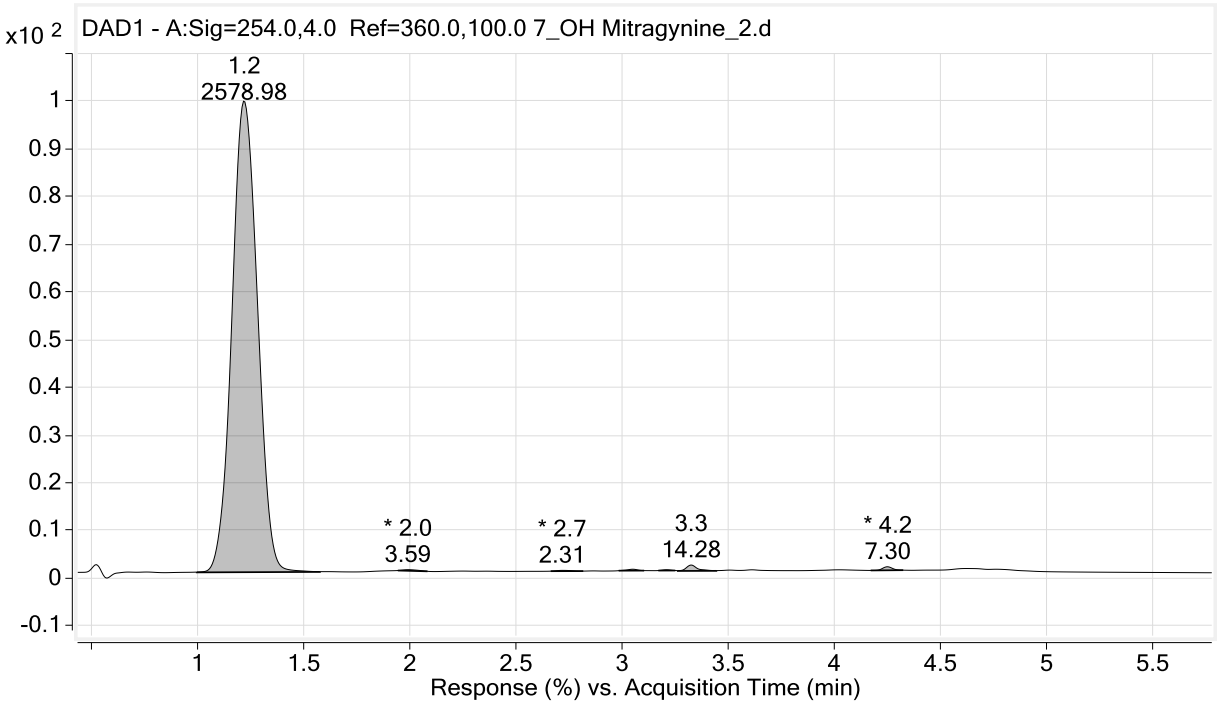

Fig. 12S UHPLC-PDA chromatogram and peak purity of 7-hydroxymitragynine at 254 nm.

Table 2S Peak purity of 7-hydroxymitragynine

Peak	RT	Area	Area Sum %	Height	Width	Compound
1	1.2	2578.98	98.78	307.85	0.1	7-hydroxymitragynine
2	2	3.59	0.14	0.72	0.1	
3	2.7	2.31	0.09	0.5	0.2	
4	3	3.07	0.12	0.92	0.1	
5	3.2	1.34	0.05	0.51	0.1	
6	3.3	14.28	0.55	3.86	0.1	
7	4.2	7.3	0.28	2.32	0.2	

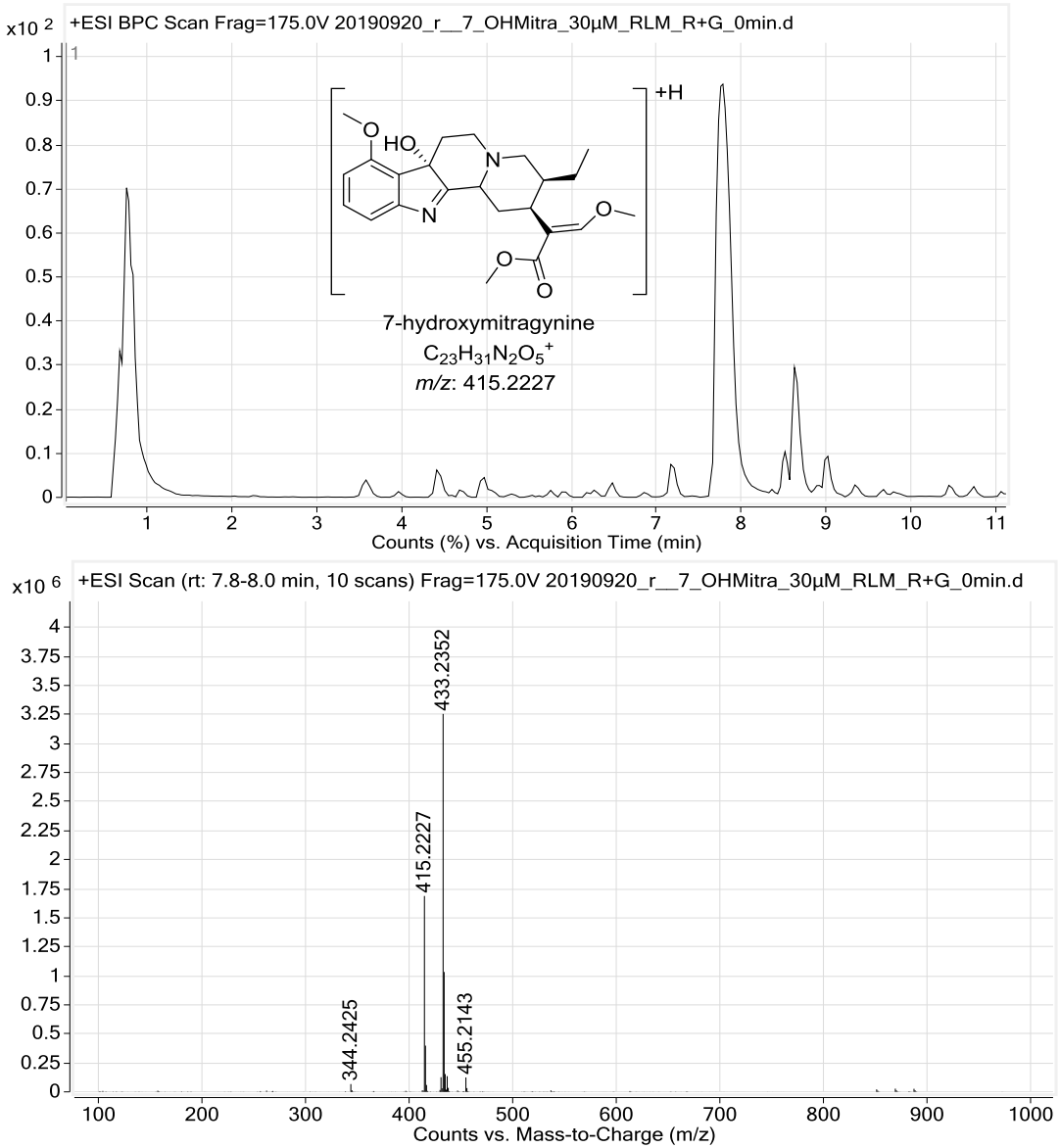

Fig. 13S UPLC-Q-TOF chromatogram and corresponding MS spectrum of 7-hydroxymitragynine and its water adduct

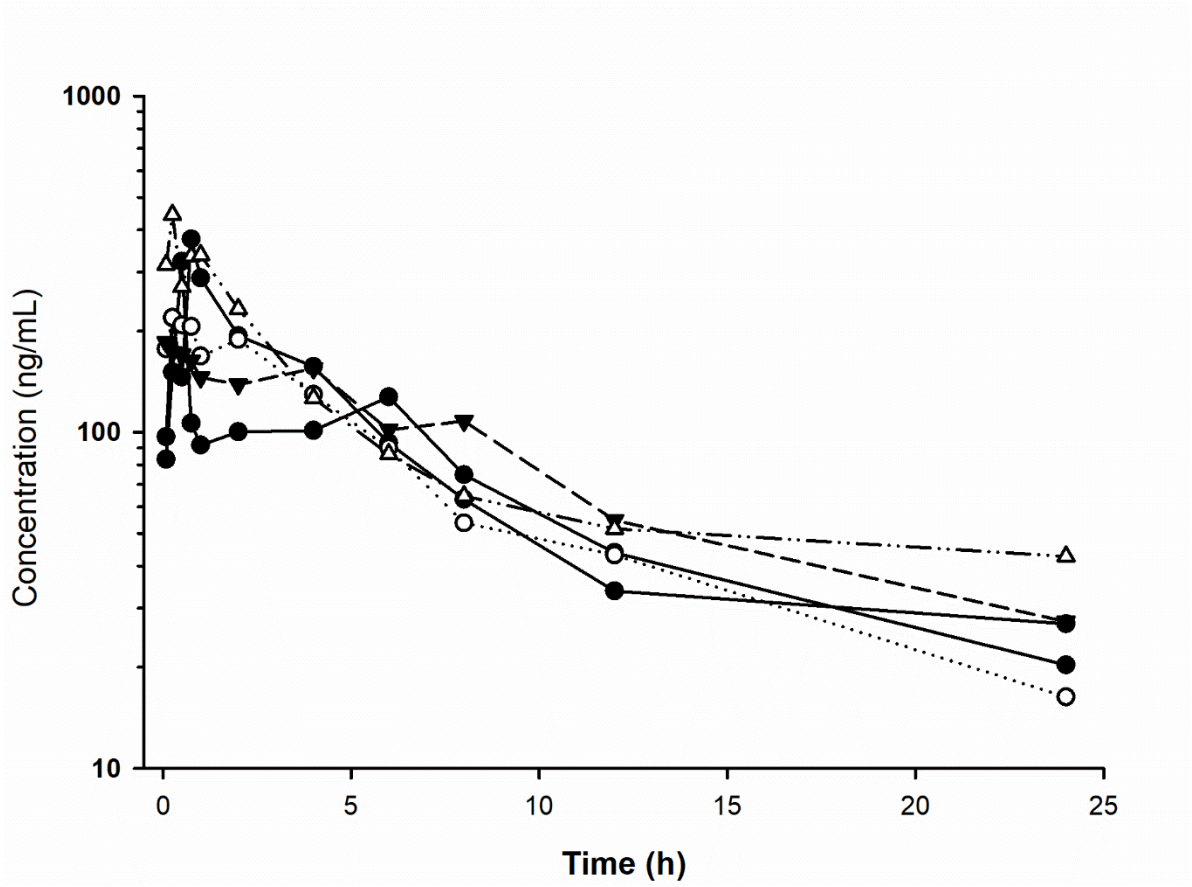

Fig. 14S Mean concentration-time profile of mitragynine after single oral dose (5 mg/kg) of mitragynine in female beagle dogs

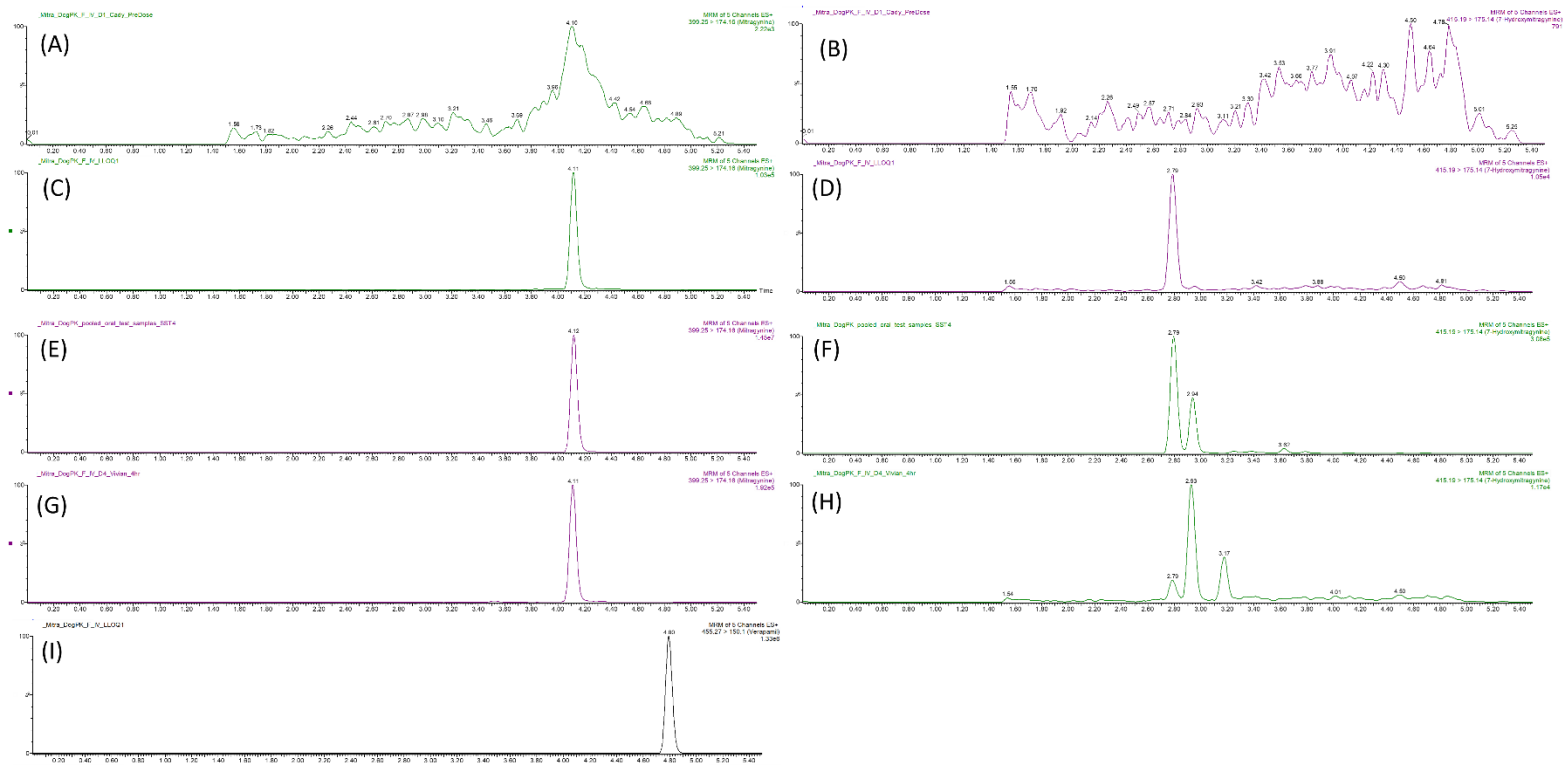

Fig. 15S Representative MRM chromatograms of pre-dose test sample before intravenous study for (**A**) mitragynine, and (**B**) 7-hydroxymitragynine; spiked dog plasma (**C**) mitragynine (1 ng/mL; LLOQ), and (**D**) 7-hydroxymitragynine (1 ng/mL; LLOQ); pooled plasma from oral study test samples for (**E**) mitragynine and (**F**) 7-hydroxymitragynine; test sample collected at 4 h from intravenous study (mitragynine, 0.1 mg/kg) for (**G**) mitragynine and (**H**) 7-hydroxymitragynine, (**I**) internal standard.