

ChemMedChem

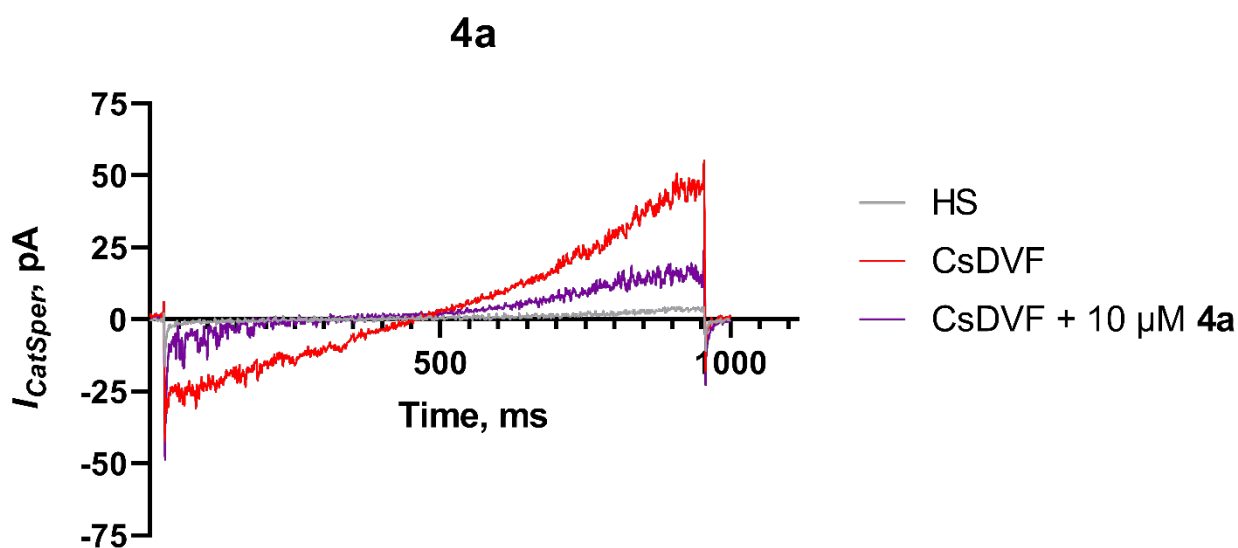
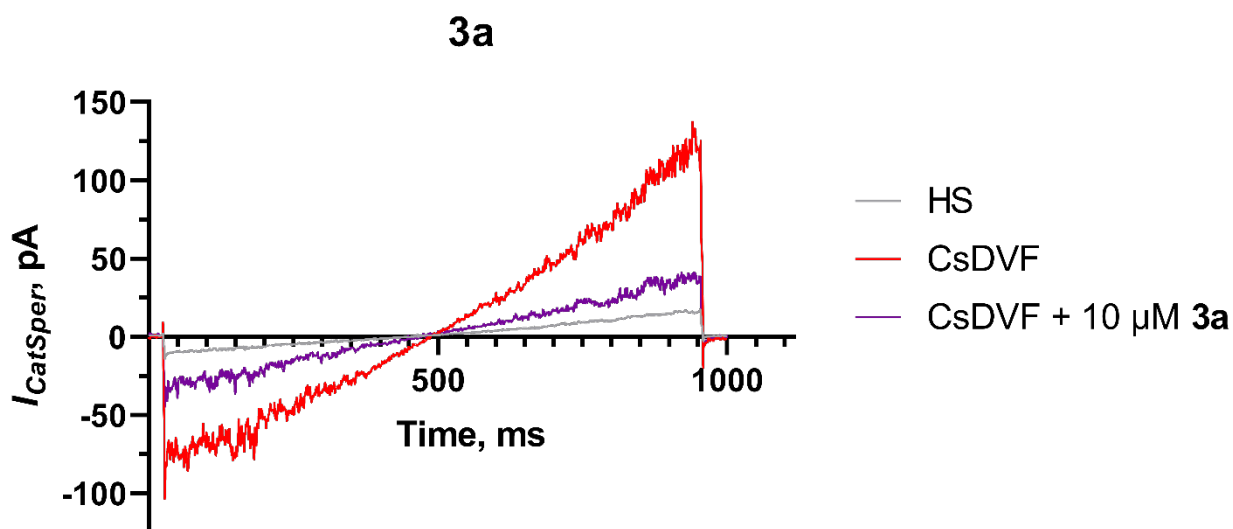
Supporting Information

Discovery and Characterization of Multiple Classes of Human CatSper Blockers

Erick J. Carlson⁺, Rawle Francis⁺, Yutong Liu, Ping Li, Maximilian Lyon, Celia M. Santi, Derek J. Hook, Jon E. Hawkinson,* and Gunda I. Georg*

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5f

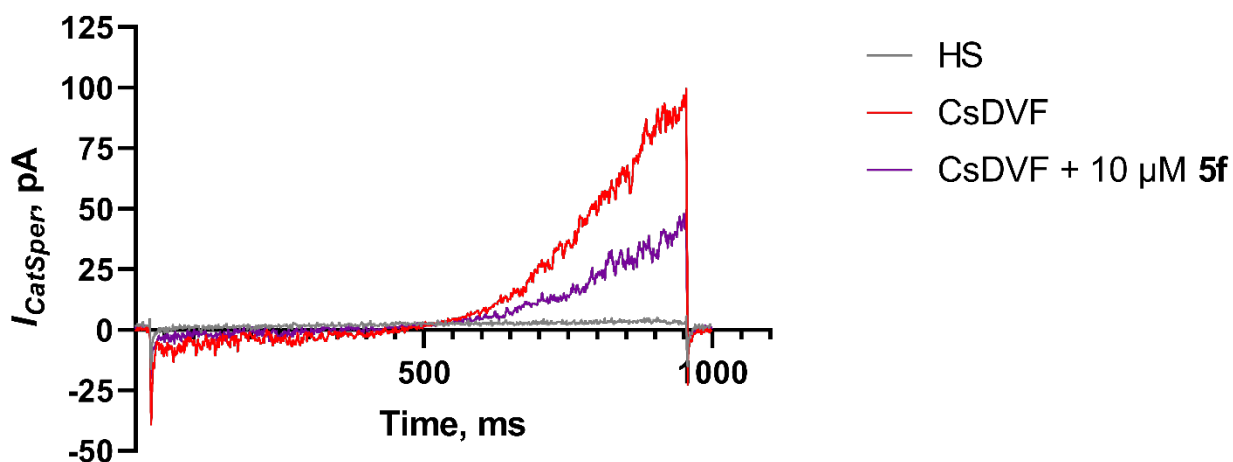


Figure S3. Representative $I_{CatSper}$ recordings from healthy human sperm in the absence (red) and presence (purple) of test compound **5f**. Baseline signal in high HS buffer shown in grey. $I_{CatSper}$ currents recorded in CsDVF elicited by the -80 mV (negative scale) or +80 mV (positive scale) voltage ramp.

6b

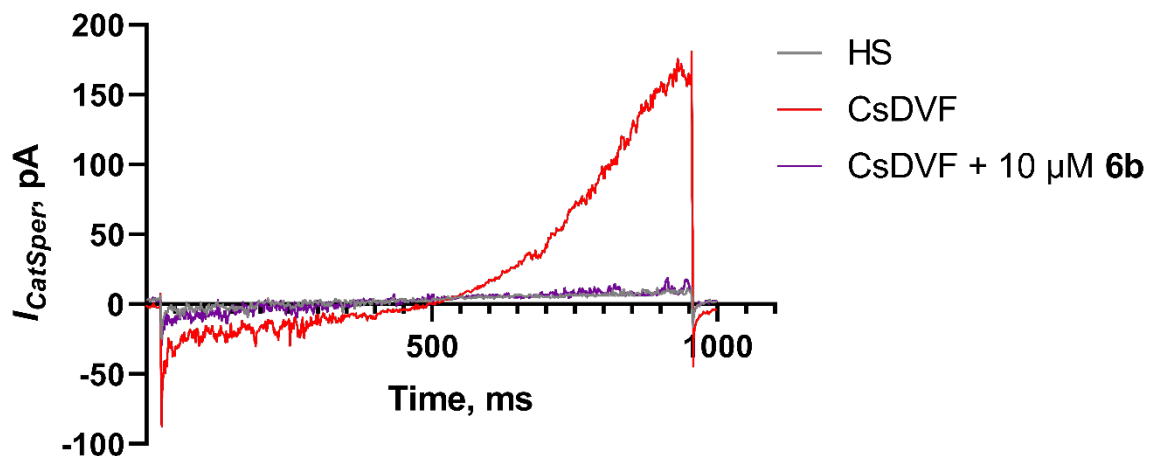


Figure S4. Representative $I_{CatSper}$ recordings from healthy human sperm in the absence (red) and presence (purple) of test compound **6b**. Baseline signal in high saline (HS) buffer shown in grey. $I_{CatSper}$ currents recorded in CsDVF elicited by the -80 mV (negative scale) or +80 mV (positive scale) voltage ramp.

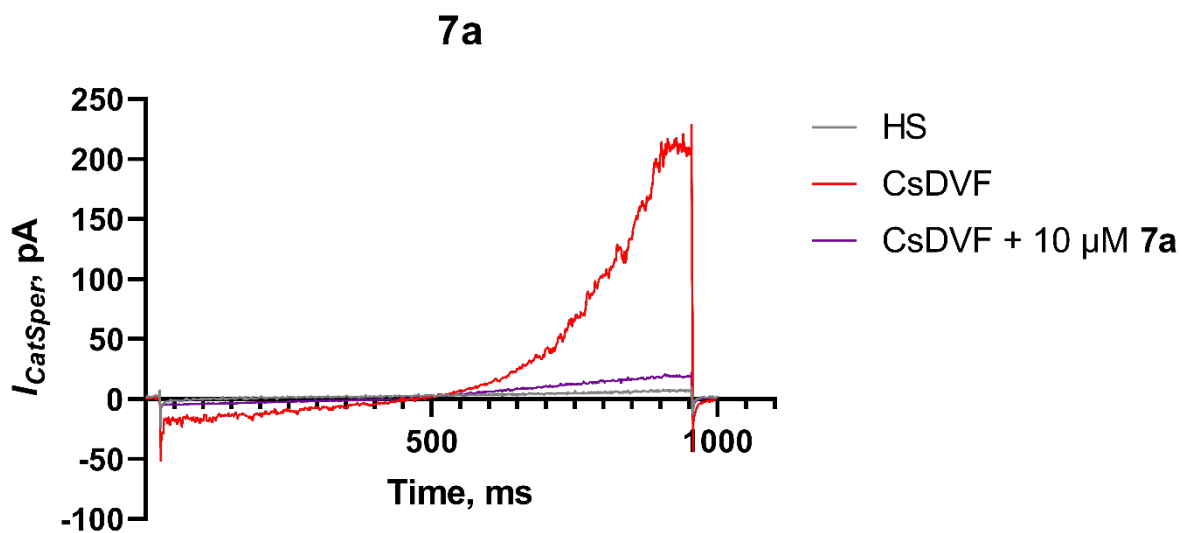
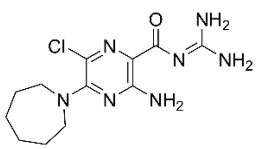
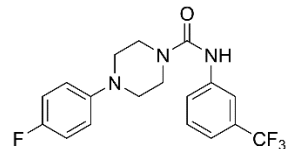
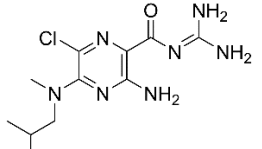
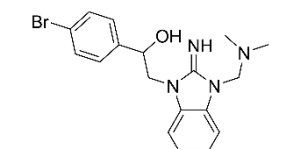
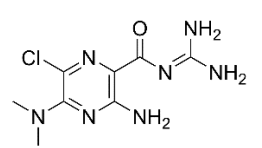
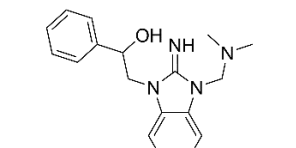
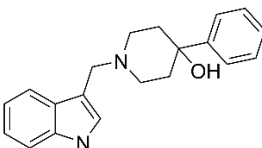
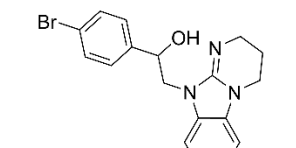
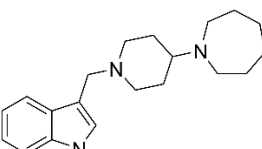
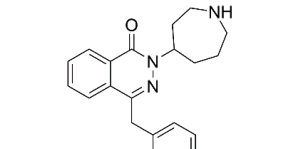
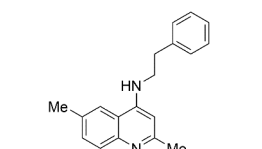
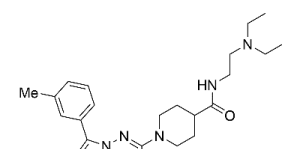
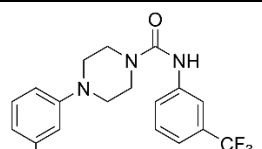


Figure S5. Representative $I_{CatSper}$ recordings from healthy human sperm in the absence (red) and presence (purple) of test compound **7a**. Baseline signal in HS buffer shown in grey. $I_{CatSper}$ currents recorded in CsDVF elicited by the -80 mV (negative scale) or +80 mV (positive scale) voltage ramp.

Table S1. Inhibition of potassium-activation of CatSper by compounds from each hit series

ID	Structure	IC ₅₀ , μM	ID	Structure	IC ₅₀ , μM
1a		9.6	4b		6.9
1b		12	5a		6.3
1c		59	5d		33
2a		12	5k		18
2d		12	6b		5.1
3a		7.8	7a		11
4a		9.2			

CatSper activated by high potassium/high pH buffer. IC₅₀ values calculated from single experiments.

Table S2. Reduction of $I_{CatSper}$ current density in human sperm by compounds from series 2 through 7 .		
Compound	Current Density (pA/pF)	
	Inward (-80 mV)	Outward (+80 mV)
CsDVF	-20 ± 3	86 ± 12
2a	-5.0 ± 0.5	23 ± 4
3a	-4.0 ± 0.3	3.3 ± 2.9
4a	-3.4 ± 0.2	15 ± 2.4
5f	-15 ± 3	35 ± 4
6b	-2.5 ± 1.1	0.1 ± 1.4
7a	-1.2 ± 0.4	5.9 ± 1.4

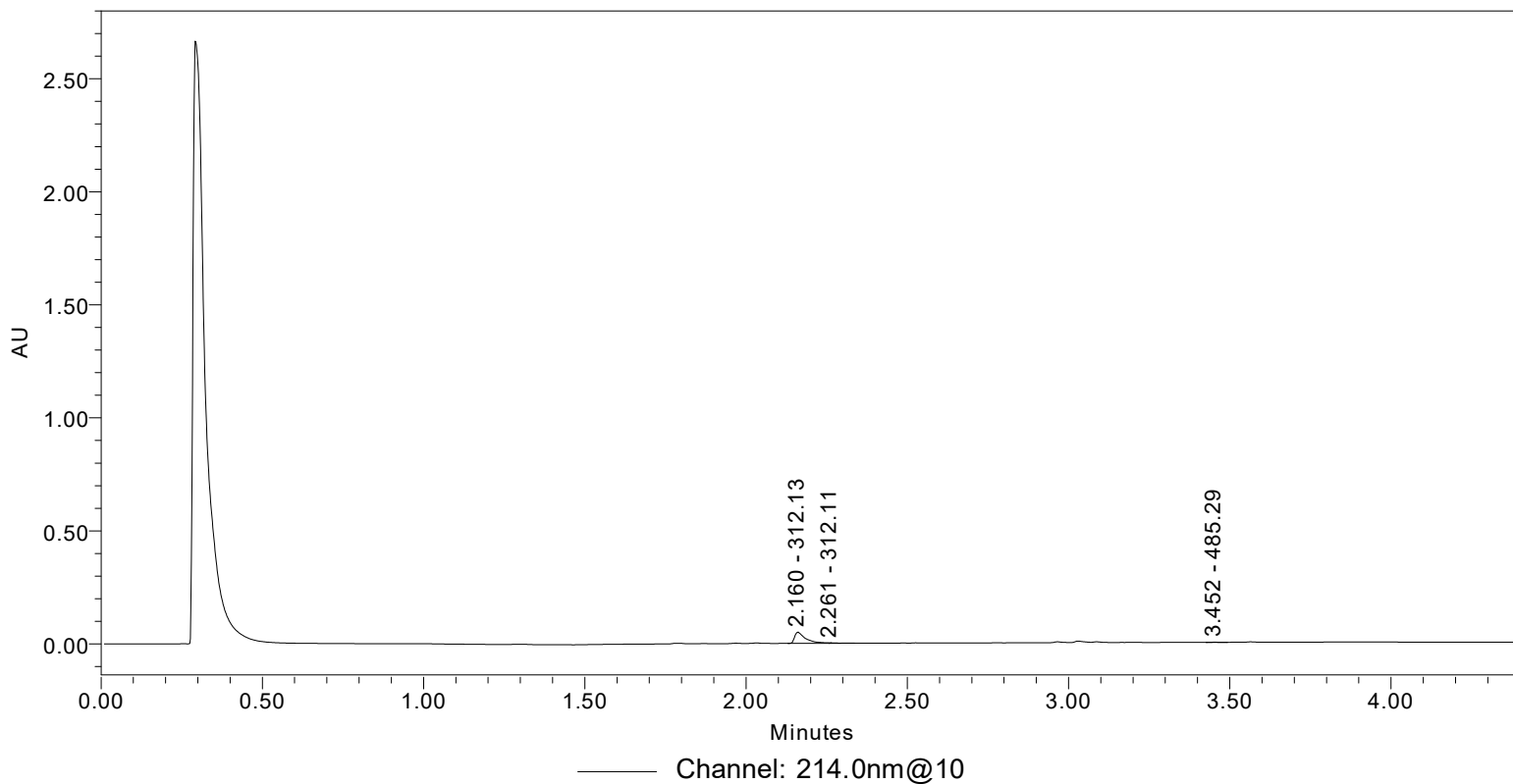
CsDVF = Cesium divalent free media. Values are the mean ± SEM of at least 3 independent experiments.

Mass Analysis Report

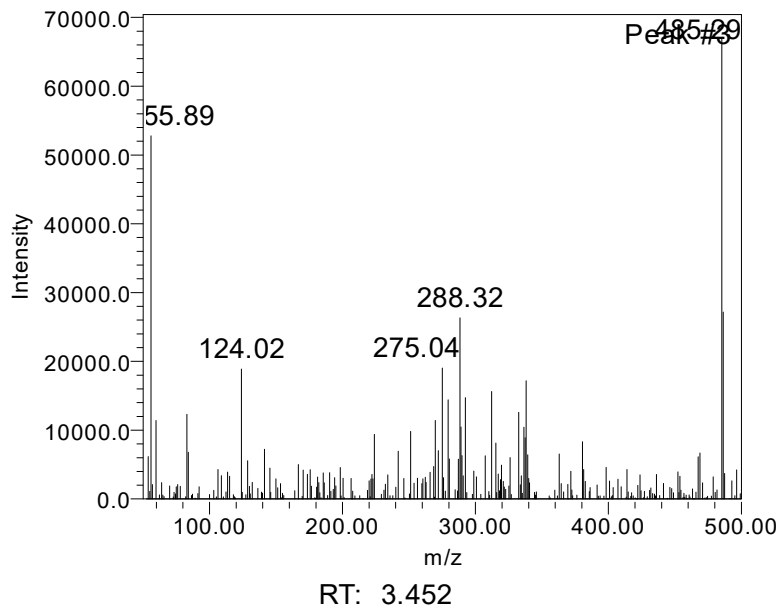
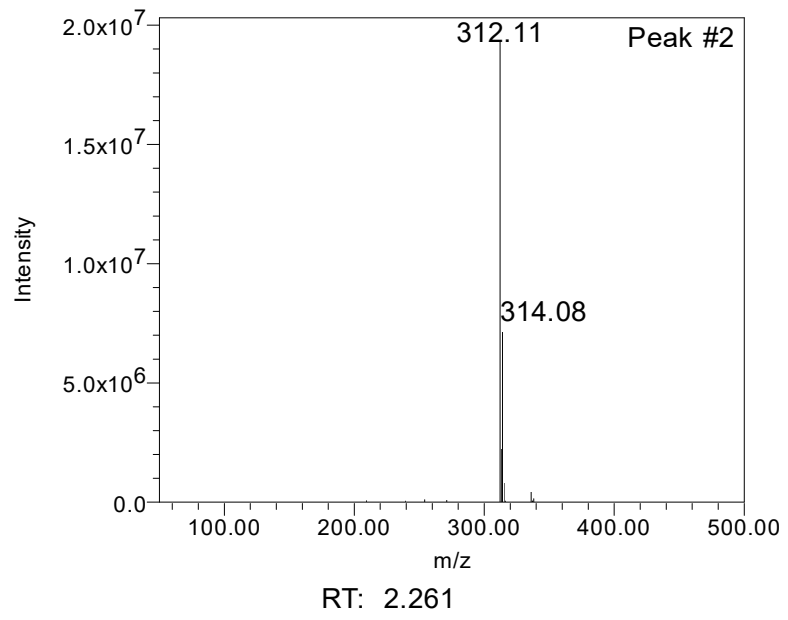
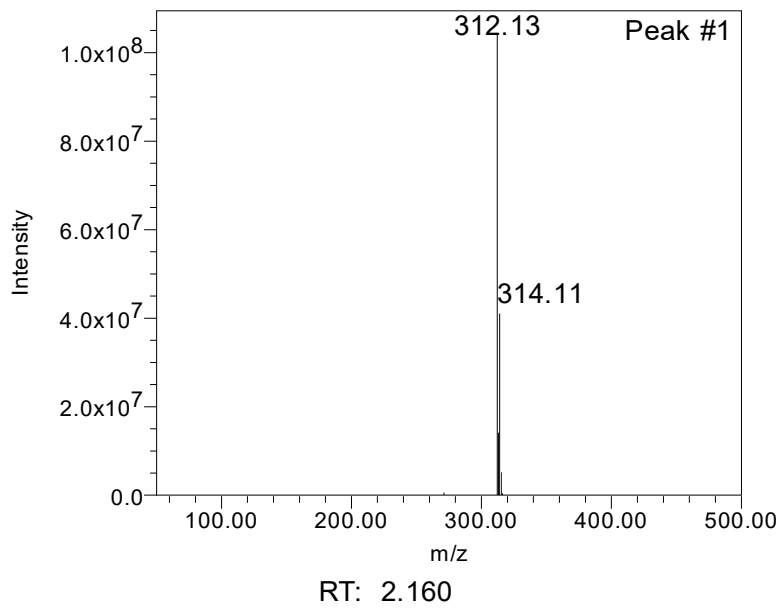
SAMPLE INFORMATION

Sample Name: 1a
Acq Method Set: Col2_50to500_PosOnly

Acquired: 1/28/2022 8:12:07 PM CST
InjVol: 3.00 uL



	RT	Area	% Area	Height	Base Peak (m/z)
1	2.160	116430	96.48	48975	312.13
2	2.261	3612	2.99	1843	312.11
3	3.452	636	0.53	389	485.29

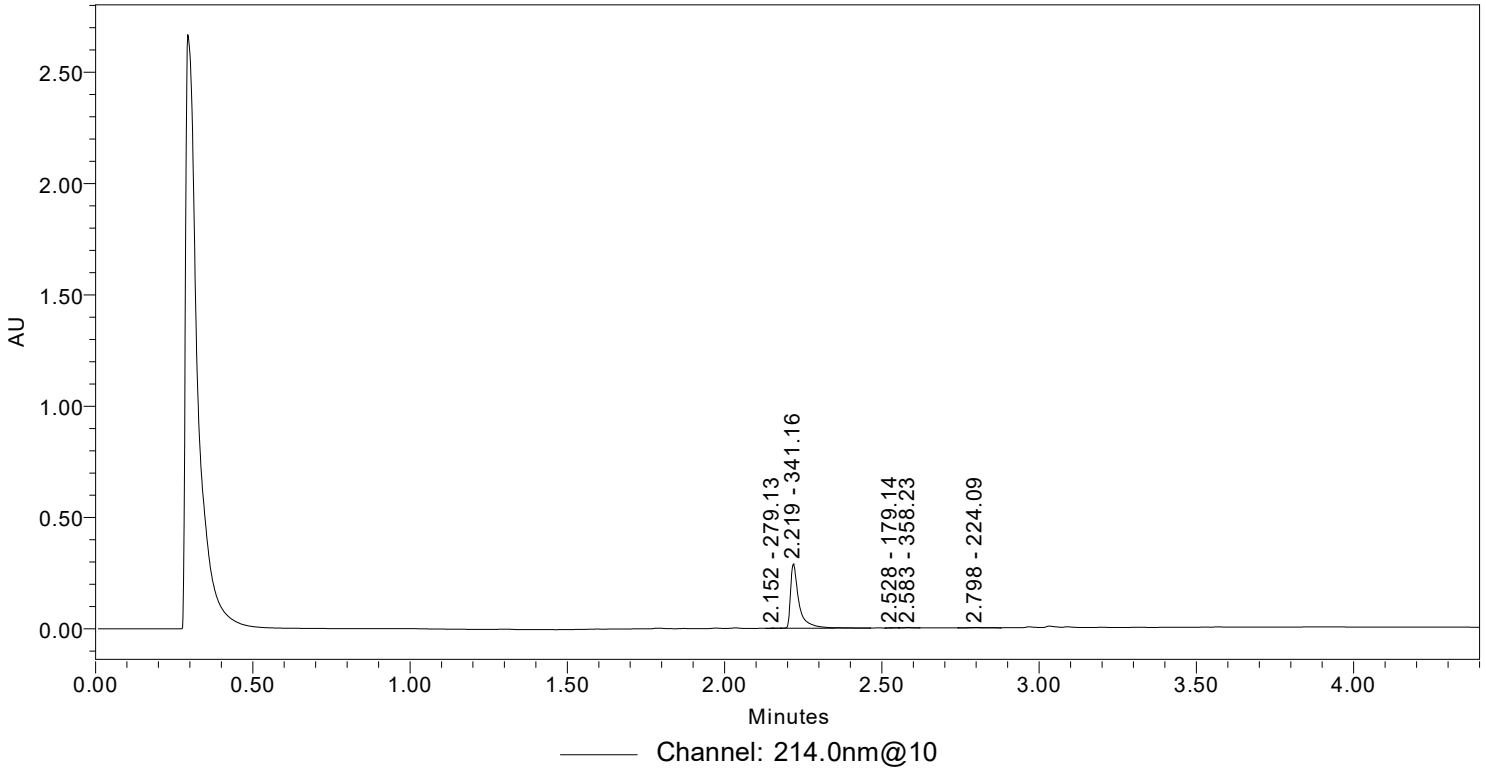


Mass Analysis Report

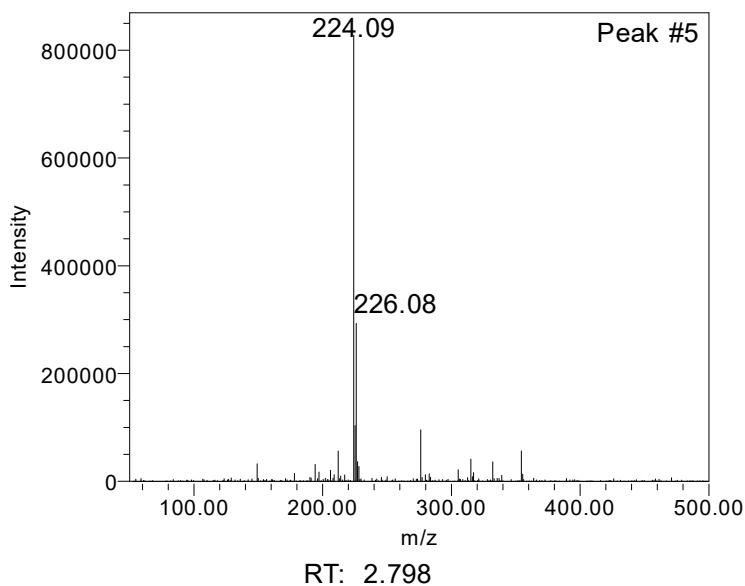
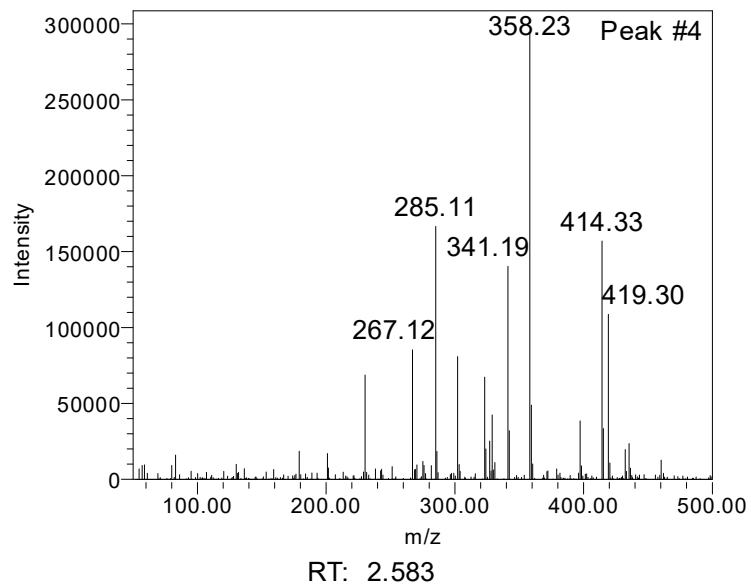
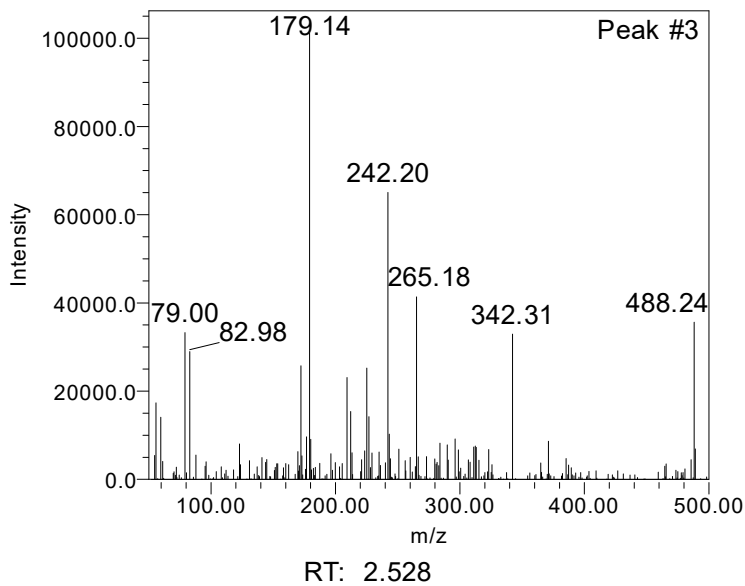
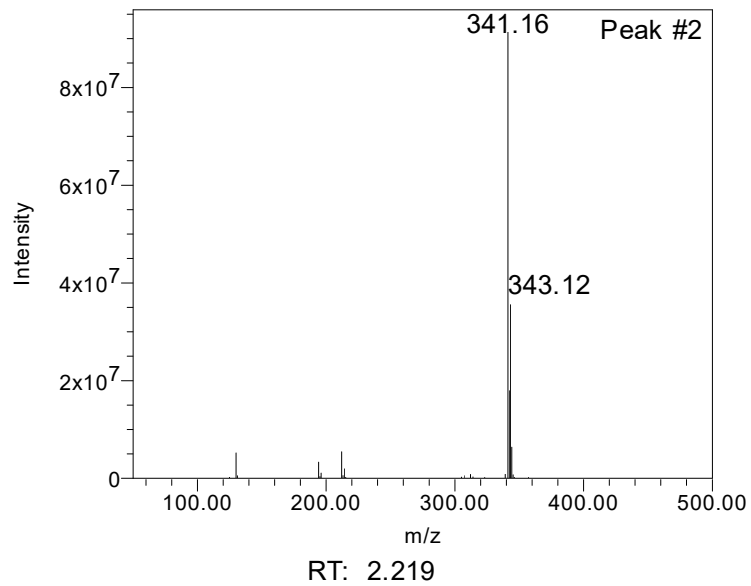
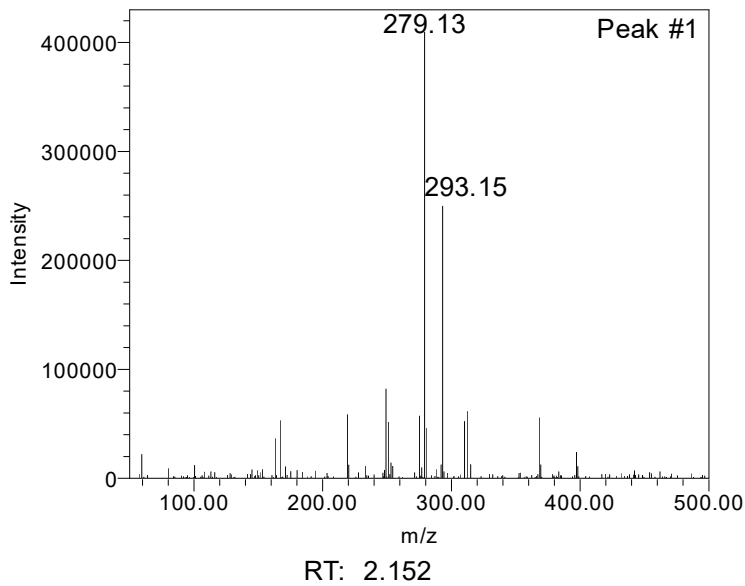
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Acq Method Set: Col2_50to500_PosOnly

Acquired: 1/28/2022 8:19:07 PM CST
InjVol: 3.00 uL



	RT	Area	% Area	Height	Base Peak (m/z)
1	2.152	2054	0.37	1309	279.13
2	2.219	541358	98.20	288613	341.16
3	2.528	1156	0.21	944	179.14
4	2.583	2494	0.45	1661	358.23
5	2.798	4191	0.76	1555	224.09

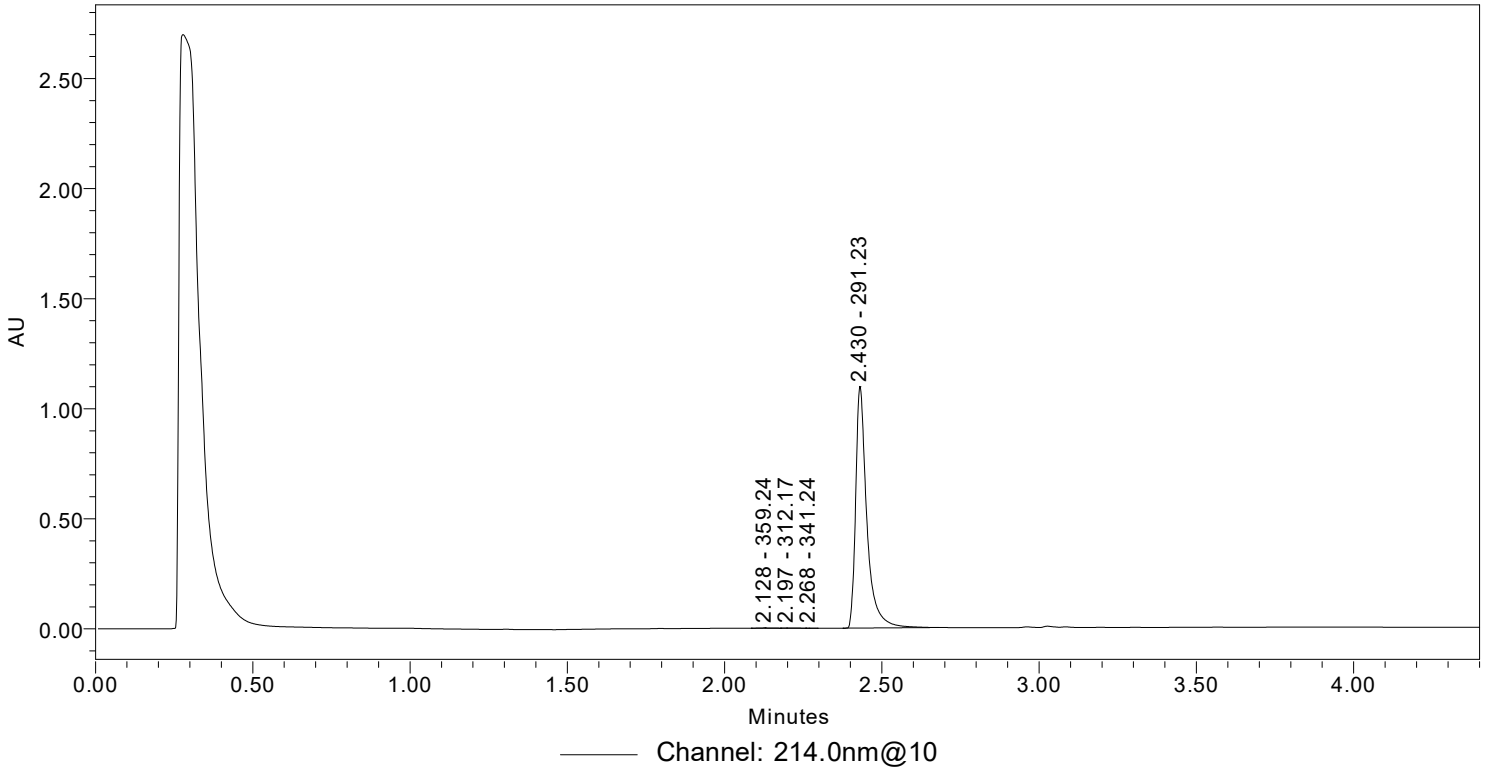


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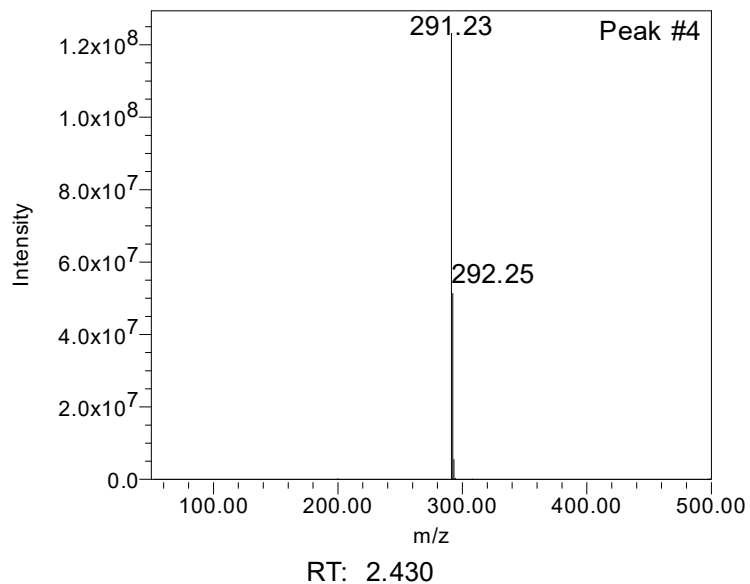
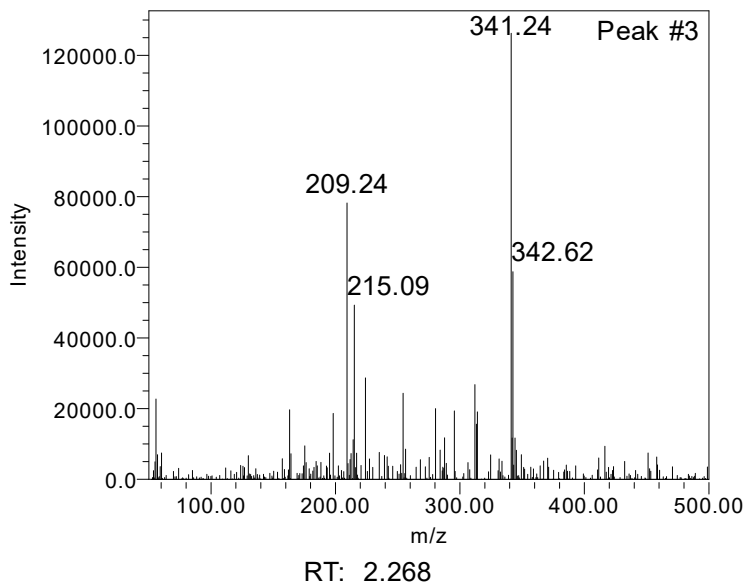
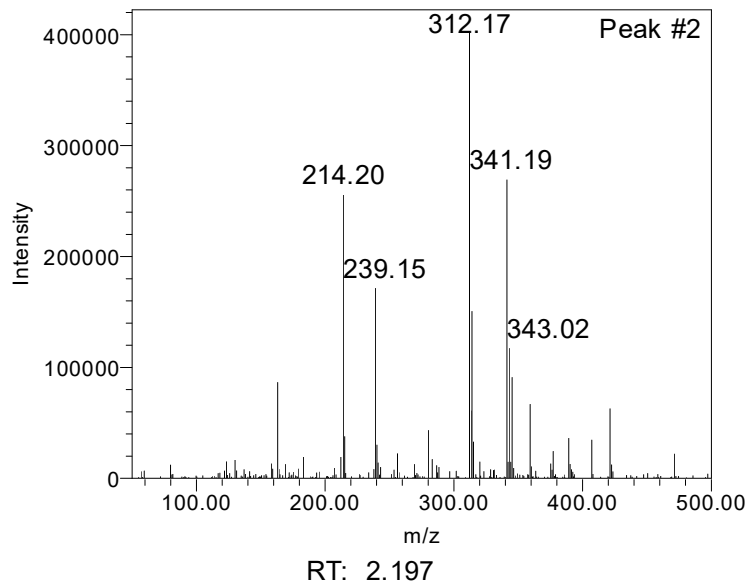
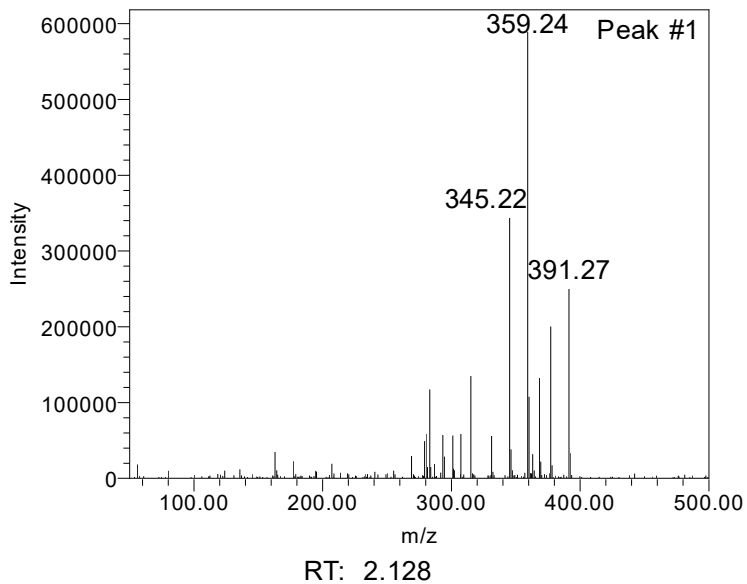
SAMPLE INFORMATION

Sample Name: 3a
Acq Method Set: Col2_50to500_PosOnly

Acquired: 1/28/2022 10:27:28 PM CST
InjVol: 7.50 uL



	RT	Area	% Area	Height	Base Peak (m/z)
1	2.128	4919	0.18	1590	359.24
2	2.197	3898	0.14	1234	312.17
3	2.268	364	0.01	288	341.24
4	2.430	2714937	99.66	1098416	291.23

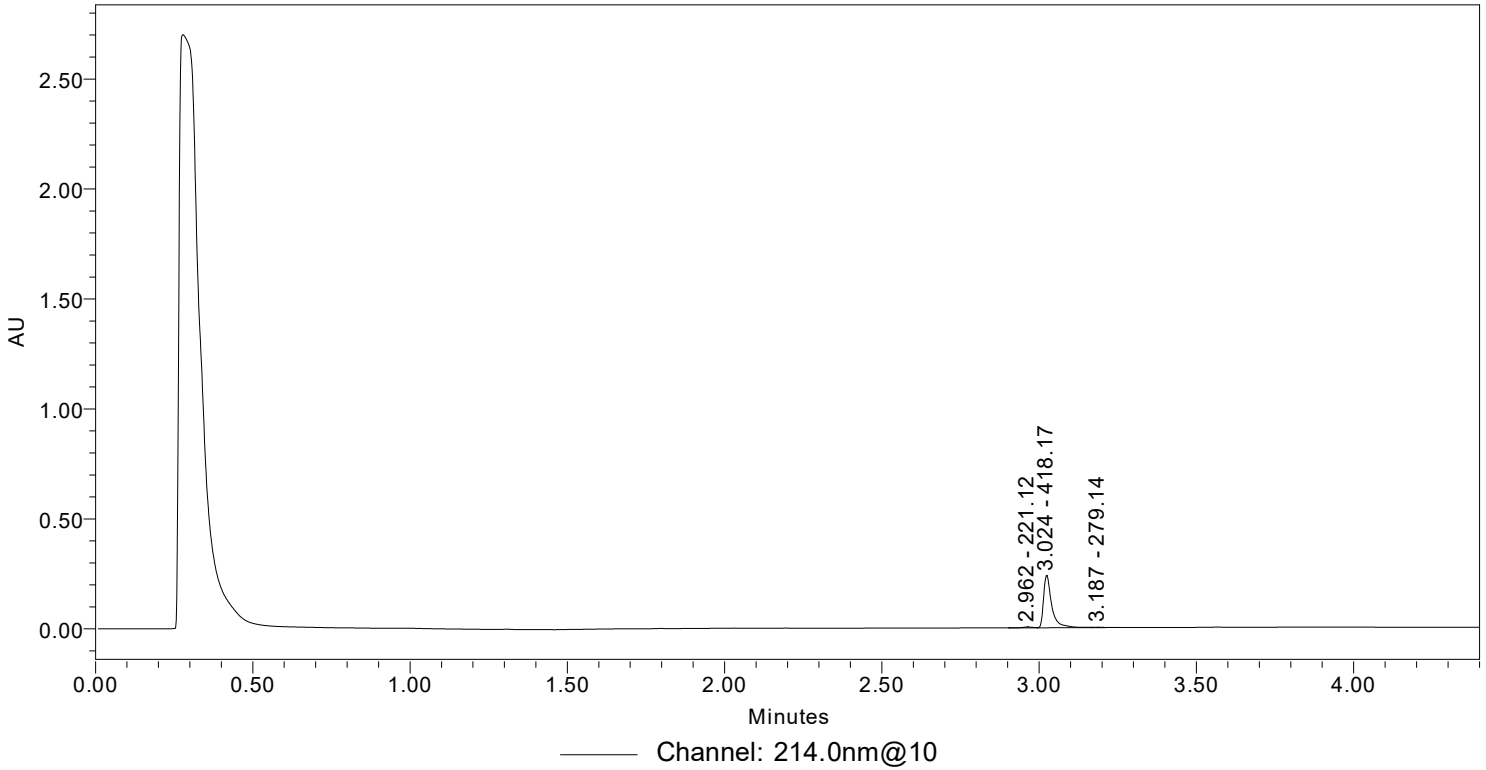


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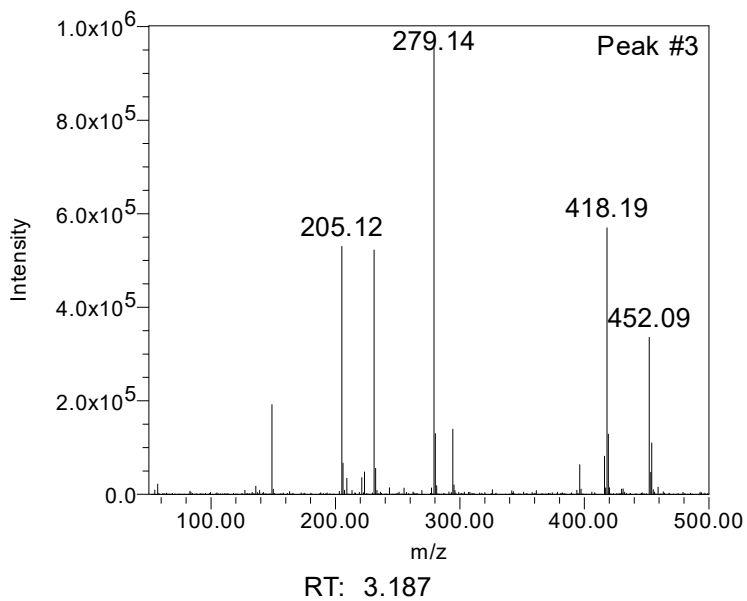
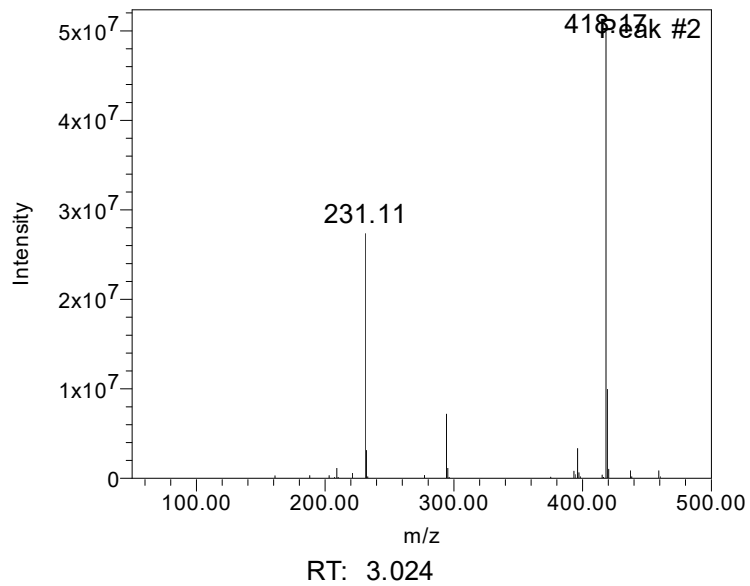
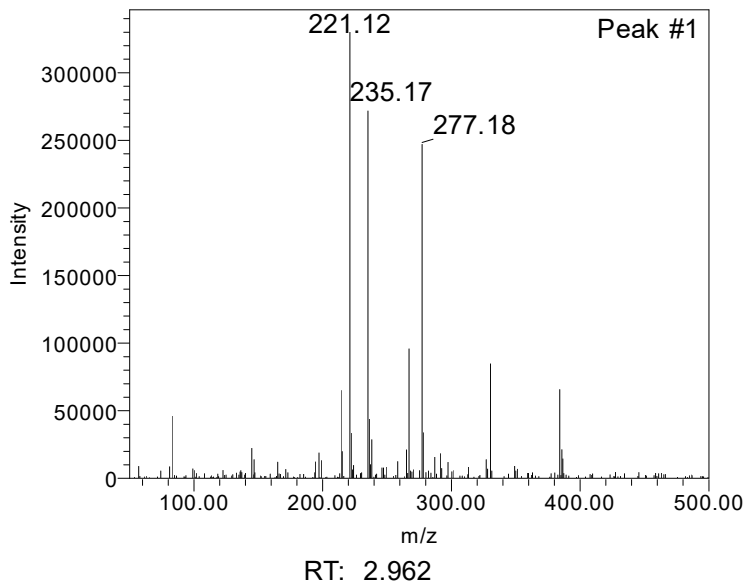
SAMPLE INFORMATION

Sample Name: 4a
Acq Method Set: Col2_50to500_PosOnly

Acquired: 1/28/2022 10:34:35 PM CST
InjVol: 7.50 uL



	RT	Area	% Area	Height	Base Peak (m/z)
1	2.962	8491	1.91	3932	221.12
2	3.024	432574	97.18	238038	418.17
3	3.187	4053	0.91	1755	279.14

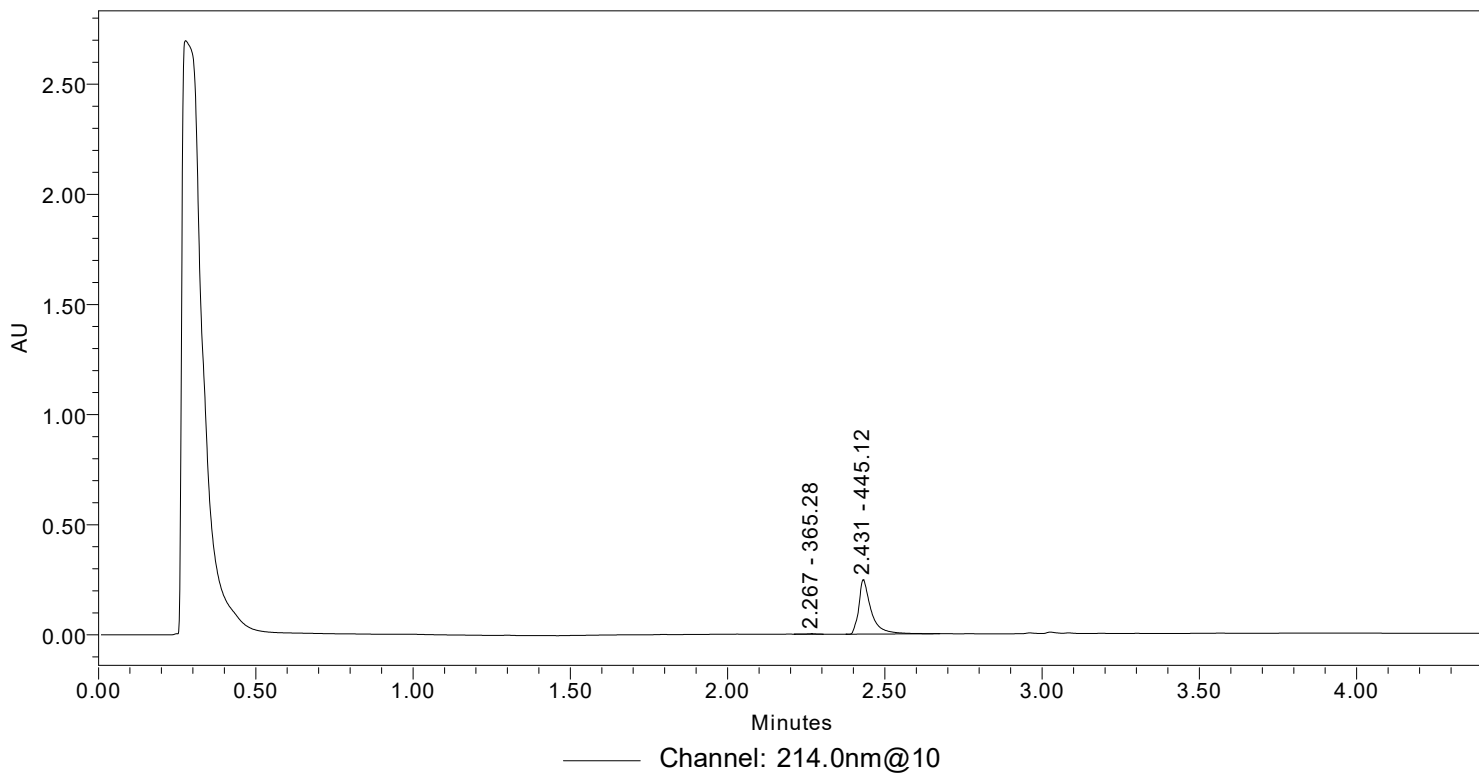


Mass Analysis Report

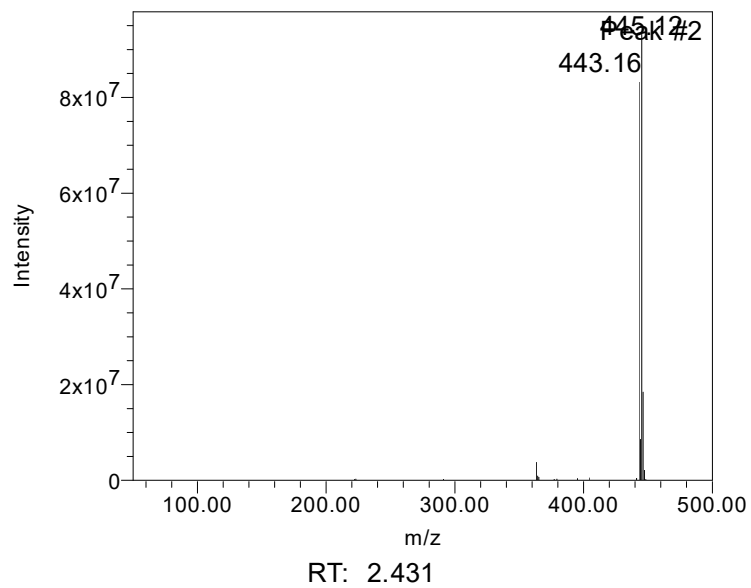
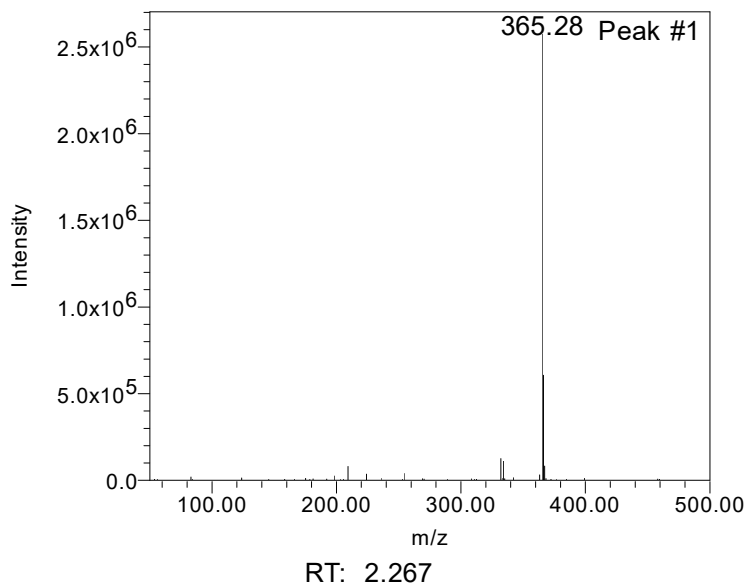
SAMPLE INFORMATION

Sample Name: 5f
Acq Method Set: Col2_50to500_PosOnly

Acquired: 1/28/2022 10:41:39 PM CST
InjVol: 7.50 uL



	RT	Area	% Area	Height	Base Peak (m/z)
1	2.267	4324	0.64	1859	365.28
2	2.431	674377	99.36	246673	445.12

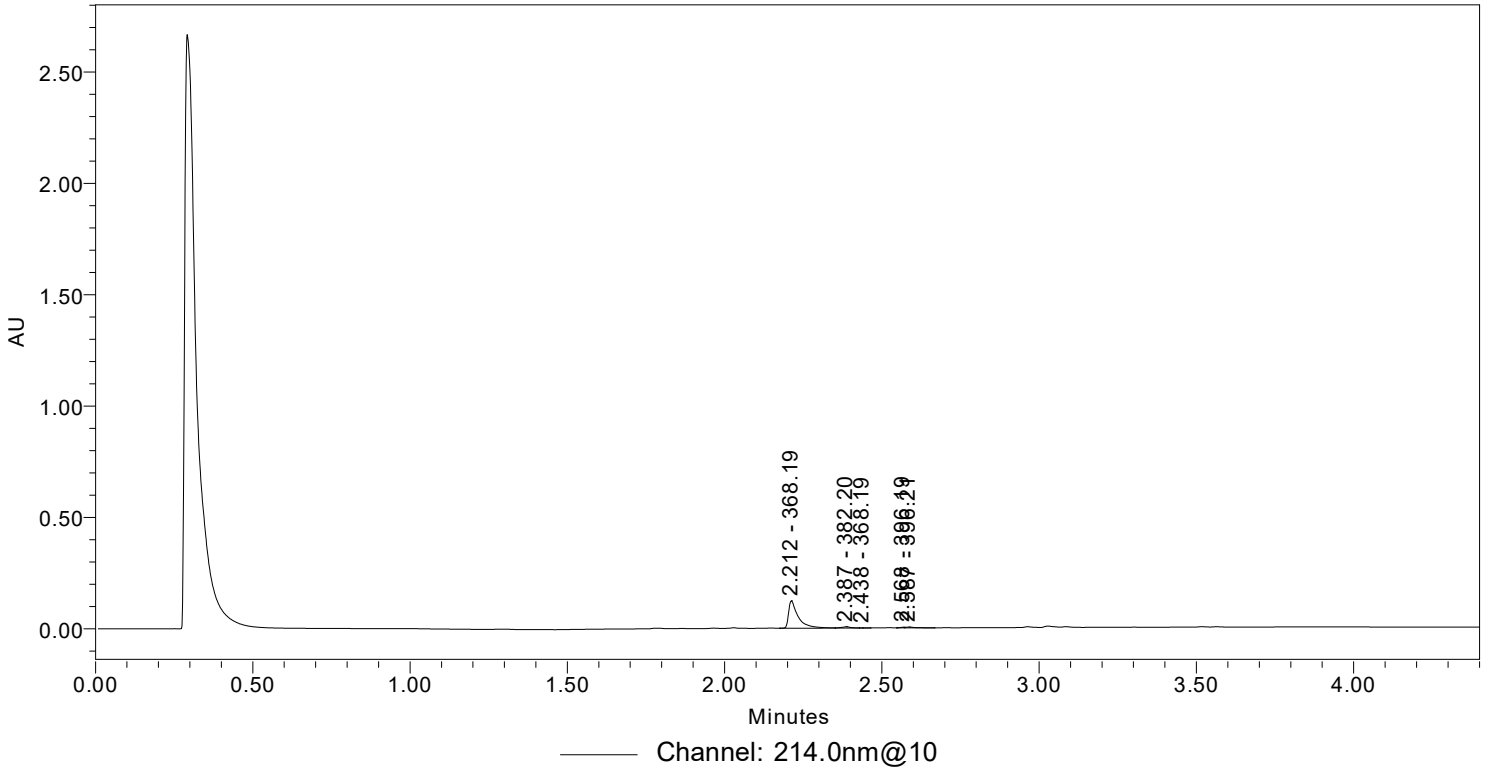


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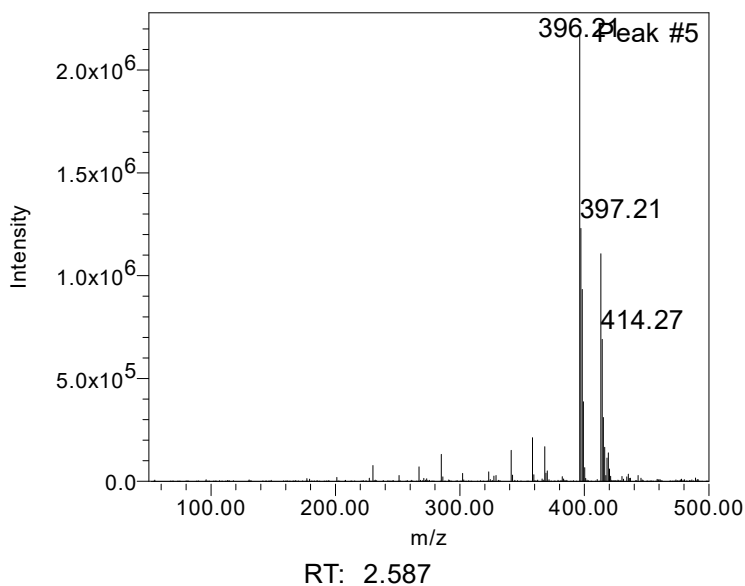
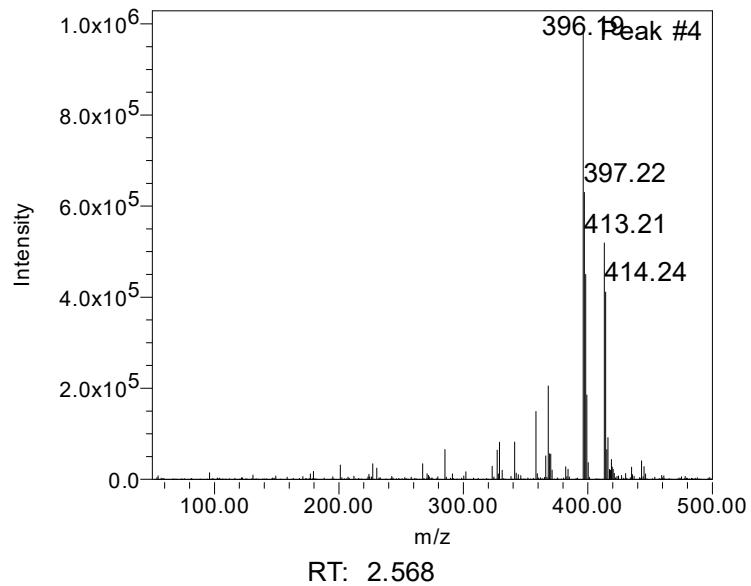
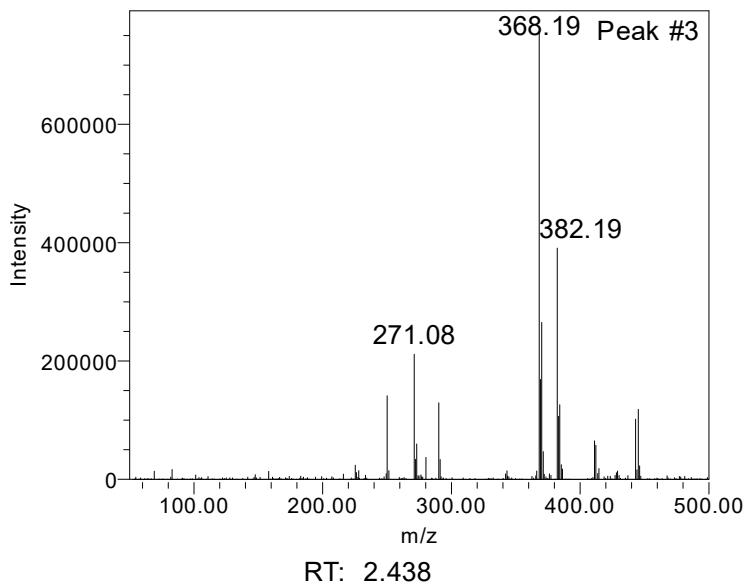
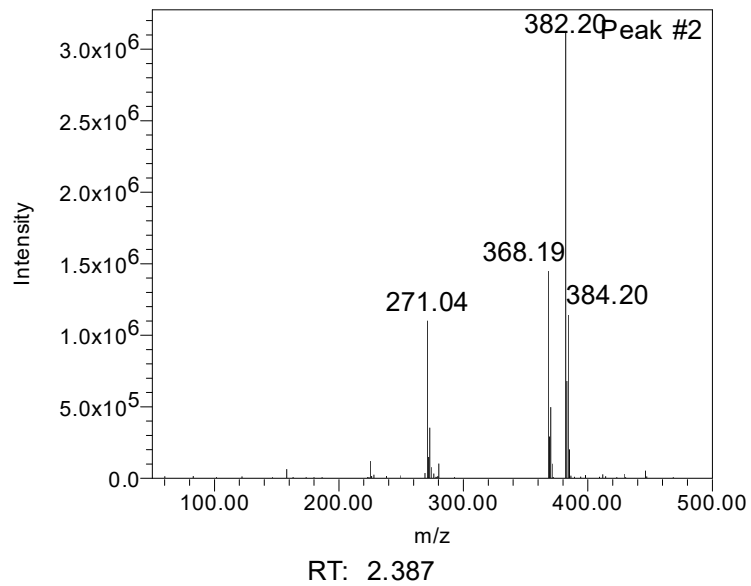
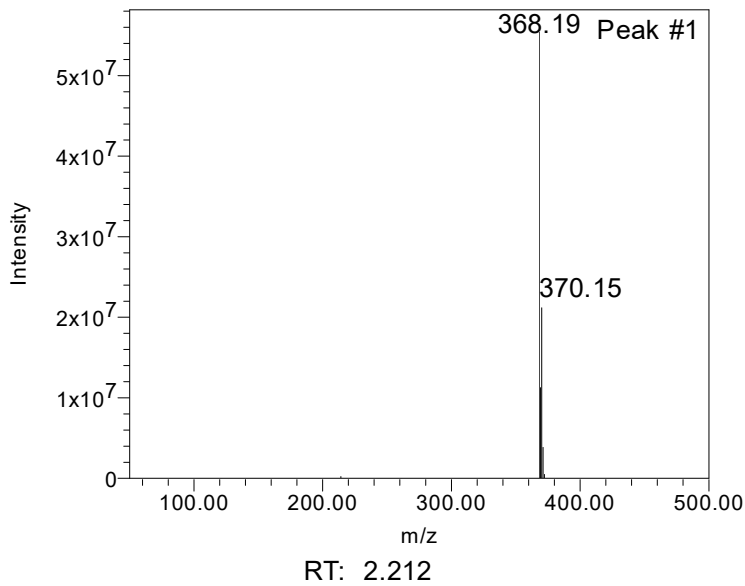
SAMPLE INFORMATION

Sample Name: 6b
Acq Method Set: Col2_50to500_PosOnly

Acquired: 1/28/2022 8:47:06 PM CST
InjVol: 3.00 uL



	RT	Area	% Area	Height	Base Peak (m/z)
1	2.212	261797	91.79	123446	368.19
2	2.387	12417	4.35	5882	382.20
3	2.438	1349	0.47	759	368.19
4	2.568	2820	0.99	2823	396.19
5	2.587	6844	2.40	3792	396.21



LCMS Analysis Report

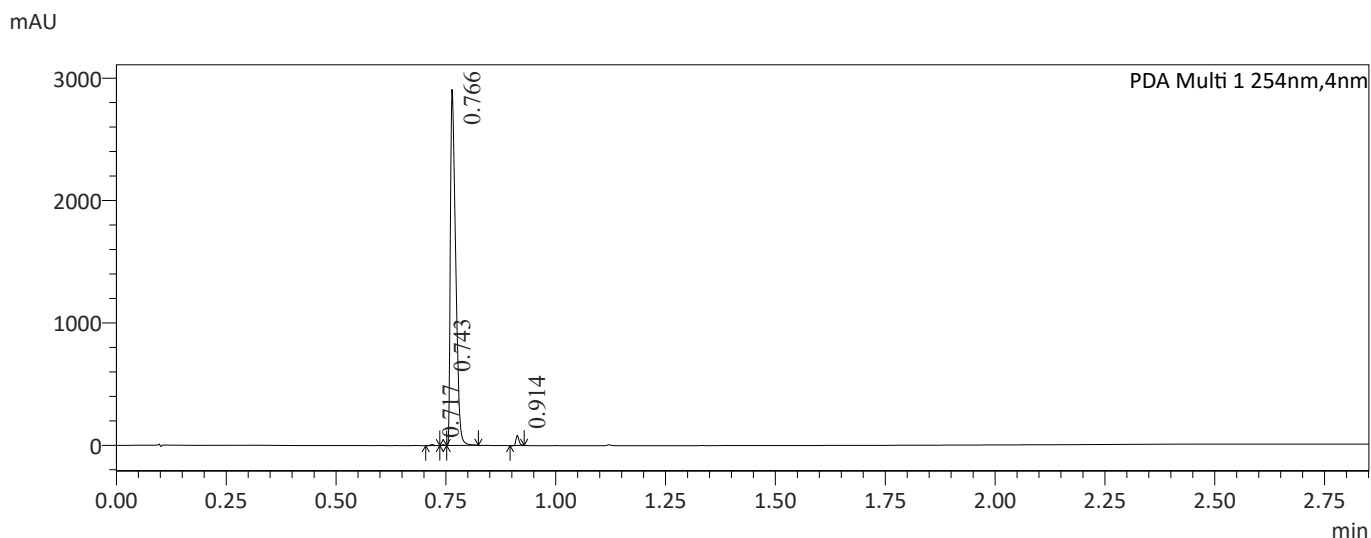
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 Data File : YTL-12-6-2-1.lcd
 Method File : ACN-Water-0.05%TFA-5%B-1.5-3.0MIN(90-900).lcm
 Date Acquired : 2022/2/22 17:24:34
 Comment : Mobile phaseA:water/0.05%TFA
 Mobile phaseB:ACN/0.05%TFA

Instrument Name	: Shimadzu LCMS-2020	<<Interface>>	
<<Pump>>		Interface	: ESI
Mode	: Binary gradient	DL Temperature	: 250 C
Pump A	: LC-20ADXR	Nebulizing Gas Flow	: 1.50 L/min
Pump B	: LC-20ADXR	Heat Block	: 250 C
Total Flow	: 1.5000 mL/min	Drying Gas	: On
B Conc.	: 5.0 %		: 15.00 L/min
<<MS Parameter>>			
<<Oven>>		Initial Valve Position	: -
Oven Temperature	: 40 C	--Segment 1 Event 1--	
		Start Time	: 0.00 min
<<PDA>>		End Time	: 2.85 min
PDA Model	: SPD-M20A	Acquisition Mode	: Scan
Lamp	: D2	Polarity	: Positive
Start Wavelength	: 190 nm	Event Time	: 0.30 sec
End Wavelength	: 400 nm	Detector Voltage	: +0.90 kV
		Threshold	: 0
<<Column>>		Start m/z	: 90.00
Column Name	: HALO C18	End m/z	: 900.00
Length	: 30 mm	Scan Speed	: 3000 u/sec
Internal Diameter	: 3.0 mm	Interface Volt.	: Use the Data in the Tuning File
		DL Volt.	: Use the Data in the Tuning File
		Qarray DC Voltage	: Use the Data in the Tuning File
		Qarray DC Voltage	: Use the Data in the Tuning File

<<LC Time Program>>

Time	Module	Command	Value
0.01	Pumps	B.Conc	5
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2.70	Pumps	B.Conc	100
2.75	Pumps	B.Conc	5
3.00	Controller	Stop	

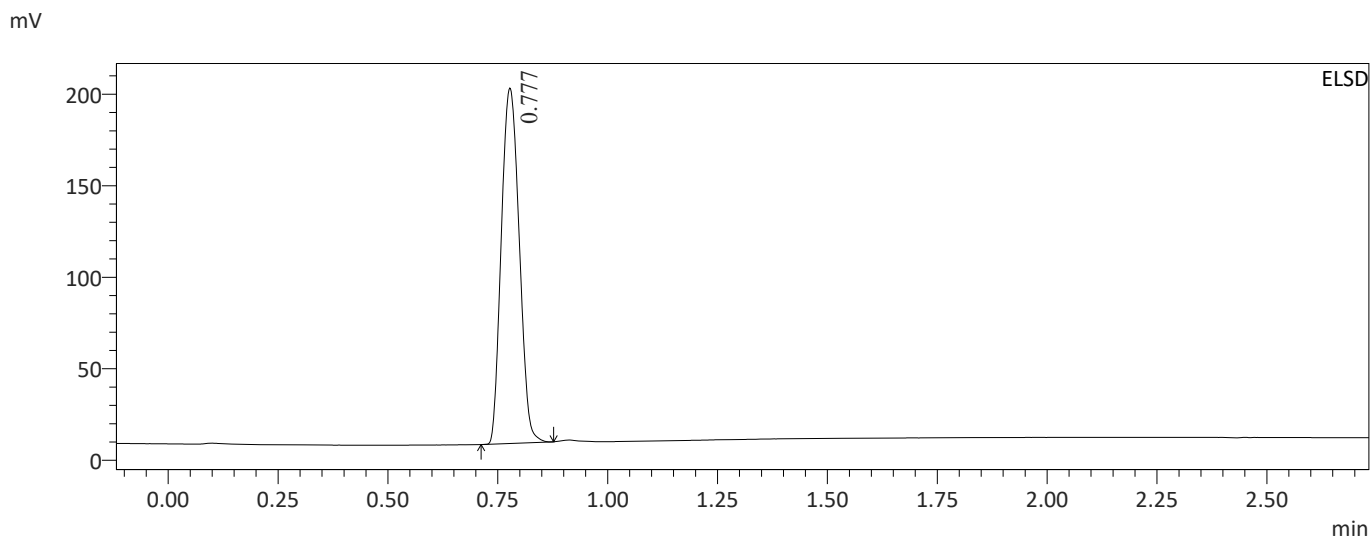
Chromatogram



Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Height	Height%	Area	Area%
1	0.717	6809	0.254	4945	0.201
2	0.743	3550	0.132	3076	0.125
3	0.766	2633161	98.096	2417257	98.173
4	0.914	40753	1.518	36961	1.501
Total		2684272	100.000	2462239	100.000

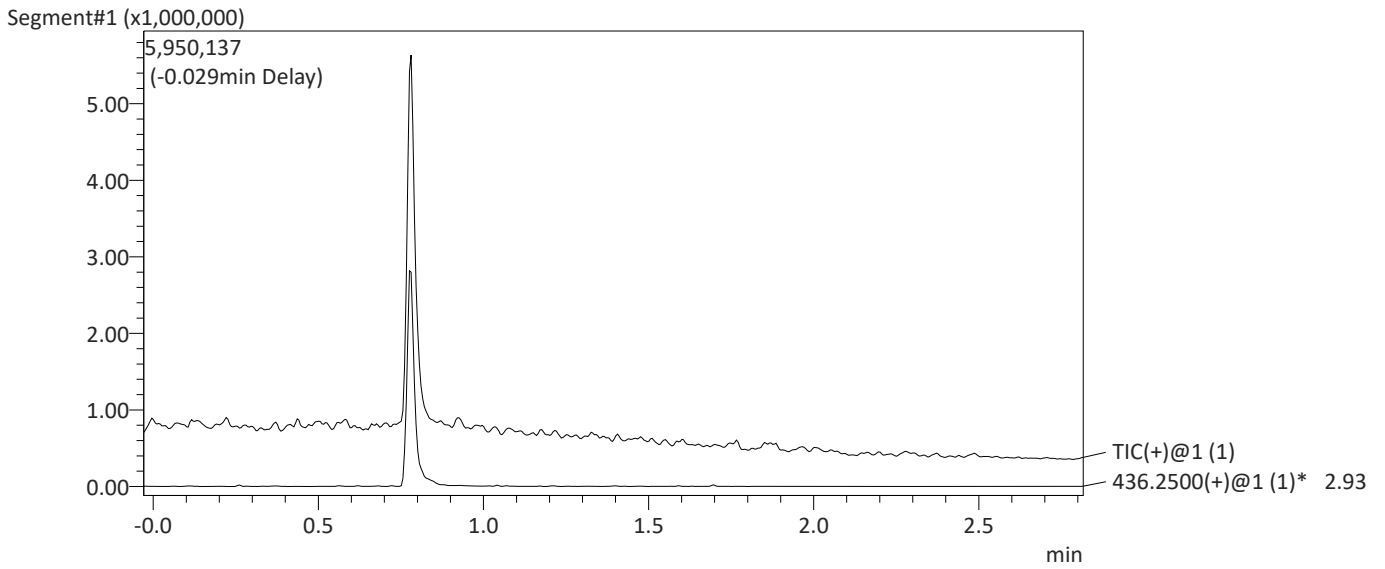


Peak Table

ELSD

Peak#	Ret. Time	Height	Height%	Area	Area%
1	0.777	192746	100.000	551055	100.000
Total		192746	100.000	551055	100.000

MS Chromatogram

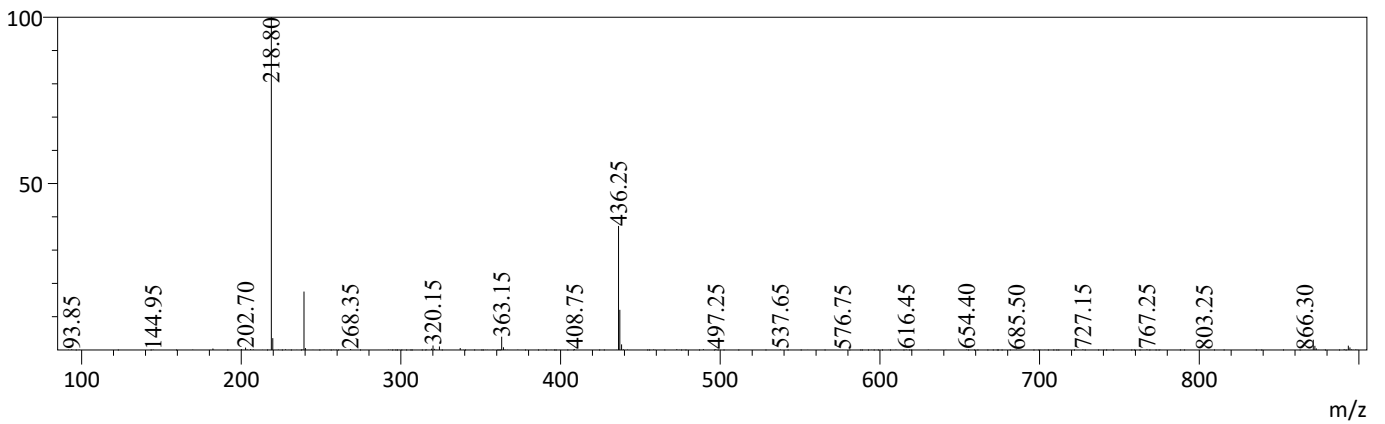


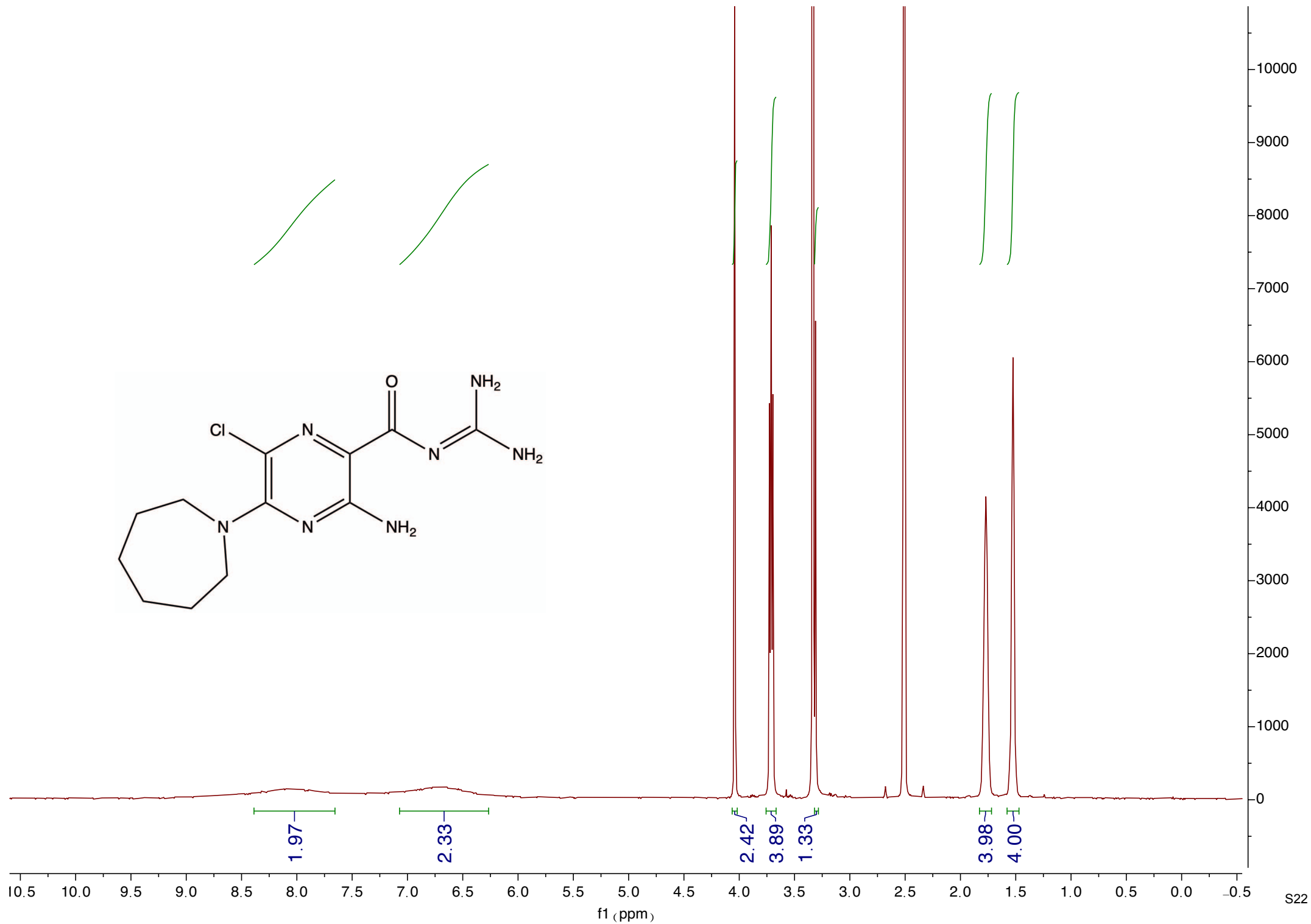
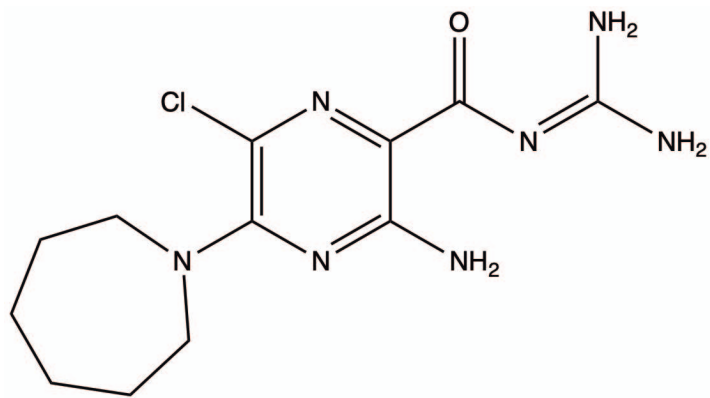
Mass Spectrum

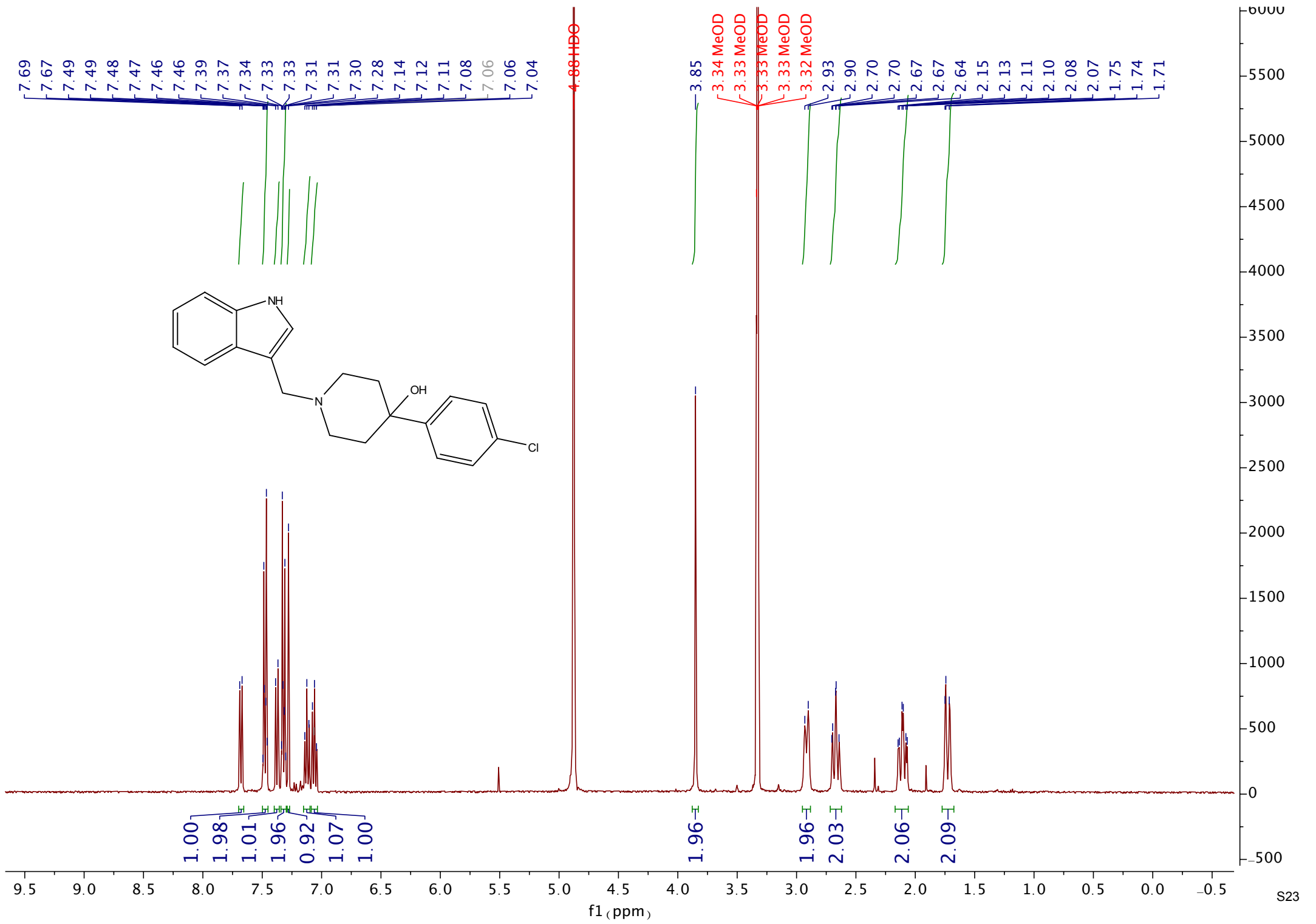
Retention time: 0.781

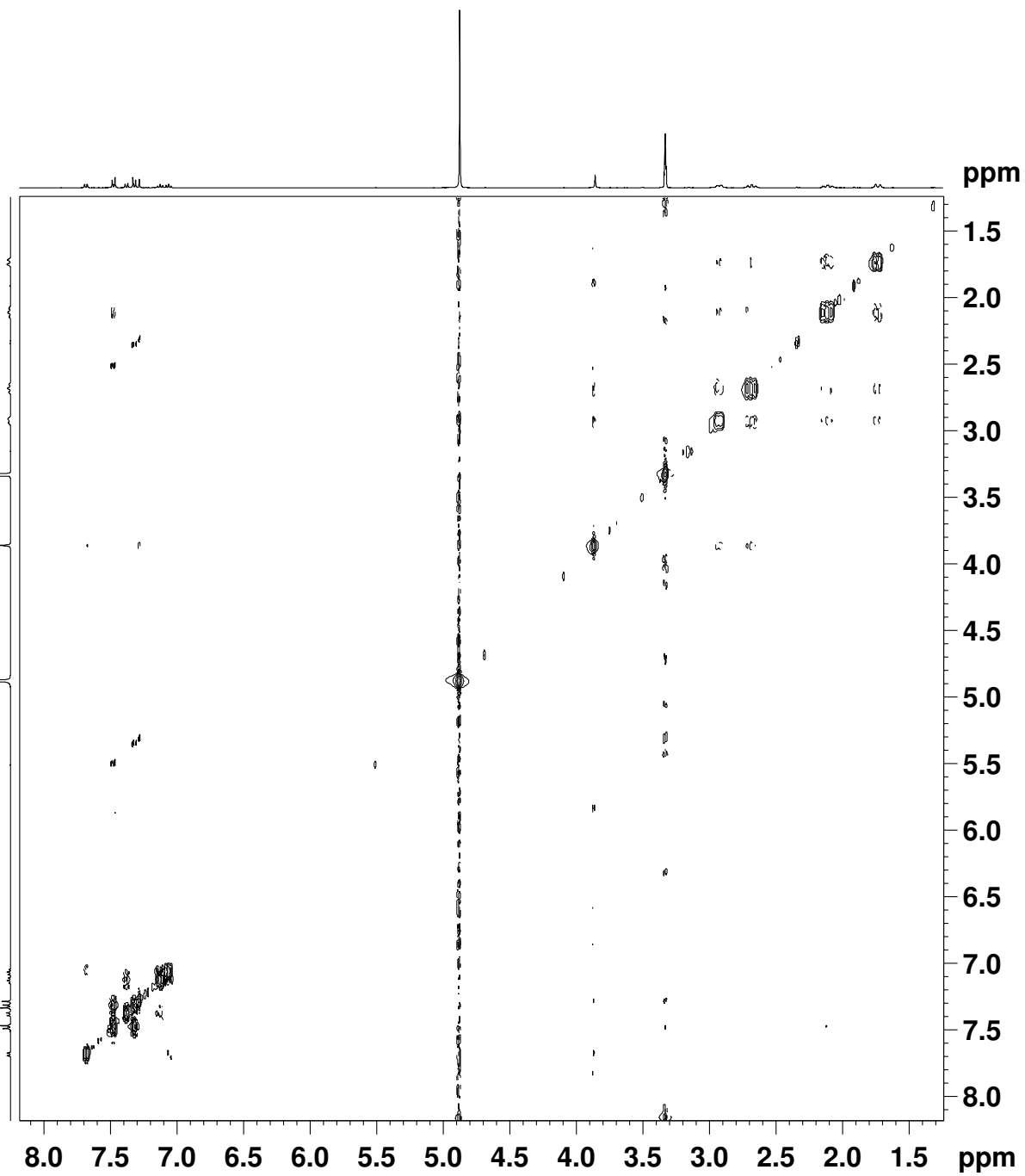
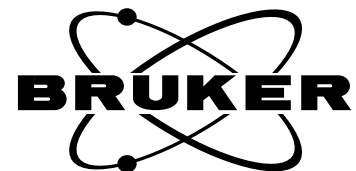
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BG Mode:Averaged 0.431-1.301(93-267) Segment 1 - Event 1









NAME 2NOESY
EXPNO 2
PROCNO 1
Date_ 20220422
Time 22.06
INSTRUM spect
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PULPROG noesyph
TD 2048
SOLVENT MeOD
NS 16
DS 4
SWH 2777.778 Hz
FIDRES 1.356337 Hz
AQ 0.3686900 sec
RG 203
DW 180.000 usec
DE 6.50 usec
TE 298.0 K
D0 0.00015976 sec
D1 1.88203502 sec
D8 0.30000001 sec
IN0 0.00036000 sec

===== CHANNEL f1 =====
NUC1 1H
P1 15.90 usec
PL1 -3.00 dB
PL1W 18.64416504 W
SFO1 400.1318852 MHz
ND0 1
TD 256
SFO1 400.1319 MHz
FIDRES 10.850695 Hz
SW 6.942 ppm
FnMODE States-TPPI
SI 1024
SF 400.1300000 MHz
WDW QSINE
SSB 2
LB 0.00 Hz
GB 0
PC 1.00
SI 1024
MC2 States-TPPI
SF 400.1300000 MHz
WDW QSINE
SSB 2
LB 0.00 Hz
GB 0

