^{19}F-NMR based determination of the absorption, metabolism and excretion of the oral phosphatidylinositol-3-kinase (PI3K) delta inhibitor leniolisib (CDZ173) in healthy volunteers

Supplementary Methods

Sample collection and preparation

Samples of blood, urine, and feces were collected over a 5-day period. Plasma obtained by centrifugation of blood samples (10-20 mL) collected at time points 0 (predose), 0.25, 0.5, 0.75, 1, 2, 3, 4, 8, 12, 16, 24, 48, and 96 h post-dose was analyzed. After drug administration all urine and feces were collected completely for 5 days. Urine samples were pooled per subject and day and accurately weighed. The individual feces sample containers were stored in a refrigerator (0-4°C) until transfer to the analysis laboratory, then were stored at -70°C pending processing. Fecal samples were processed as follows: pooling of feces per 24 hour collection period and subject, dilution of the pools with 1-2 volumes of water containing carboxymethylcelluose, determination of the total weight of each diluted sample, thorough homogenizing of each diluted sample. Processed sample aliquots were stored at -70°C.

The NMR solvent used for quantification was as follows: 70:30 d_4-methanol / 10 mM formic acid - ammonium formate buffer at pH 3.0 in deuterated water, containing 10 µM internal standard 3,5-bis(trifluoromethyl)phenol.

A single replicate was analysed for each sample, except for the calibration standards for total drug related material quantification, which were analysed in duplicate.

Individual plasma samples for quantification of total drug related material were extracted as follows: A 1 mL aliquot from each subject and chosen time point (Predose, 1, 2, 4, 8, 16, 24 h) was weighed, and then extracted by adding 4 volumes of acetonitrile. Samples were agitated at 15°C and 1000 rpm for at least 1 h before centrifugation at 13000 × g for 15 min. The supernatant was recovered and concentrated to dryness at 37°C using a nitrogen flow concentrator. The pellet from centrifugation was resuspended in 0.5 mL of water and 4 volumes of acetonitrile were added. The resulting mixture was agitated overnight at 15°C and 1000 rpm and then centrifuged at 10000 × g for 20 min. The supernatant was recovered and added to the (dried) first supernatant, then concentrated to dryness at 37°C using a nitrogen

flow concentrator. The residue was reconstituted in 800 µL of NMR solvent containing internal standard. After vortex-mixing and agitation at 15°C and 1000 rpm for 1h, samples were centrifuged at 13000 × g for 15 min. A volume of 600 µL of each supernatant was then transferred to an NMR tube.

Individual urine samples for quantification of total drug related material were extracted as follows: 2 mL urine samples from each subject and time interval were weighed and concentrated to dryness at 60°C using a nitrogen flow concentrator. The residue was reconstituted in 800 µL of NMR solvent containing internal standard. After vortex-mixing and agitation at 15°C and 1000 rpm for 1h, samples were centrifuged at 13000 × g for 15 min. A volume of 600 µL of each supernatant was then transferred to an NMR tube.

Individual feces samples for quantification of total drug related material were extracted as follows: A 1 mL aliquot of feces homogenate from each subject and time interval was weighed, and then extracted by adding 3 volumes of acetonitrile. Samples were agitated at 15°C and 1000 rpm for at least 1 h before centrifugation at 13000 × g for 15 min. The supernatant was recovered and concentrated to dryness at 37°C using a nitrogen flow concentrator. The pellet from centrifugation was resuspended in 0.5 mL of water and 4 volumes of acetonitrile were added. The resulting mixture was agitated overnight at 15°C and 1000 rpm and then centrifuged at 10000 × g for 20 min. The supernatant was recovered and added to the (dried) first supernatant, then concentrated to dryness at 37°C using a nitrogen flow concentrator. The residue was reconstituted in 800 µL of NMR solvent containing internal standard. After vortex-mixing and agitation at 15°C and 1000 rpm for 1h, samples were centrifuged at 13000 × g for 15 min. A volume of 600 µL of each supernatant was then transferred to an NMR tube.

A plasma pool (0-24 h) for metabolite profile determination was prepared by combining plasma aliquots of volume proportional to the time interval used for AUC calculation (using the linear trapezoidal method, Hamilton RA et al., 1981) for each individual subject. The

resulting plasma pool was extracted as follows: 3 volumes of acetonitrile was added to an aliquot of plasma pool (6 mL) and the sample was mixed at 15°C and 1000 rpm for at least 1 hour. The suspension was then centrifuged at 13000 × g for 15 min. The supernatant was separated from the pellet. Subsequently, 3 mL of water was added to the pellet, and the mixture was vortex mixed to resuspend the pellet. 4 volumes of acetonitrile were added to the suspension and the mixture was mixed at 15°C and 1000 rpm overnight. The suspension was then centrifuged at 13000 × g for 20 min. The supernatant was removed and combined with the first supernatant, then concentrated at 37°C under a stream of nitrogen to approximately 9 mL. The concentrate was then separated by preparative LC as described below.

A urine pool (0-120 h) for metabolite profile determination was prepared by combining urine aliquots of weight proportional to the total weight of urine excreted during each sample interval for each individual subject. The urine pool was extracted as follows: A 12 mL aliquot of the urine pool was centrifuged at 13000 × g for 15 min, then the supernatant was separated from the pellet then separated by preparative LC as described below.

A feces homogenate pool (0-120 h) for metabolite profile determination was prepared by combining feces aliquots of weight proportional to the total weight of urine excreted during each sample interval for each individual subject. The feces homogenate pool was then extracted as follows: 3 volumes of acetonitrile were added to an aliquot of feces homogenate pool (3 mL) and the sample was mixed at 15°C and 1000 rpm for 1 hour. The suspension was then centrifuged at 13000 × g for 15 min. The supernatant was separated from the pellet. 1.5 mL of water was added to the pellet and the mixture was vortex mixed to resuspend the pellet. 4 volumes of acetonitrile were added to the suspension and the mixture was mixed at 15°C and 1000 rpm overnight. The suspension was then centrifuged at 13000 × g for 20 min. The supernatant was removed from the pellet and combined with the first supernatant, then concentrated at 37°C under a stream of nitrogen to approximately 4 mL. A

10mM formic acid / ammonium formate buffer (pH 3.0) was added to reach approximately 9mL. The concentrate was separated by preparative LC as described below.

Quantification standards for total drug related material quantification were prepared by spiking predose urine with leniolisib, or by spiking the acetonitrile used for the initial feces or plasma extraction with leniolisib. Sample processing was then performed as described above for the test samples. Quantification standards for metabolite profiles were prepared in the NMR solvent containing internal standard. The following standard concentrations were prepared in plasma: 0.5, 1, 2, 5, 20, 50 and 200 µM, urine: 1, 2, 5, 20, 50 and 200 µM, feces: 2, 5, 20, 50, 200, 500, 2000 and 5000 µM, metabolite profiles: 1, 2, 5, 20, 50, 200, 500 and 2000 µM.

Preparative LC separation for metabolite profiles

Extracts from plasma, urine and feces pools (described above) were separated using a Waters autopurification system equipped with a 2545 binary gradient pump, a 2767 sample manager, a System fluidic organizer, a photodiode array detector (PDA) and a single quadrupole MS (QDa). The MS detector was operated with the following settings: positive ion mode, capillary voltage 1.5 kV, Cone voltage 32 V, probe temperature 600 °C, Source temperature 120 °C. Separations were performed over a Atlantis dC18 column (10 × 150 mm, 5 µm particle size; Waters) with a corresponding guard column (10 × 10 mm) using mobile phases A (10 mM Ammonium formate adjusted to pH 3.0 with formic acid) and B (acetonitrile) with a flow rate of 6.0 mL/min. The gradient was as follows: 0 min 3.5% B; 2 min 3.5% B; 3 min 12% B; 19 min 30% B; 23 min 95% B; 26 min 95% B; 27 min 3.5% B; 30 min 3.5% B. After the column, the effluent was split in a ratio of approximately 1:100. The smaller part of the effluent (~60 µL/min) was directed into the electrospray MS interface. The remaining effluent was fractionated as follows: one single fraction was taken from 0 to 1.5 min; between 1.5 and 25.5 min, 96 fractions of 0.25 min were taken on a 96 well plate; between 25.5 and 30 min, 3 fractions of 1.5 min were taken.

Quantification of total drug related material, leniolisib and metabolites by ^{19}F-NMR

Total drug related material in metabolite profile fractions and in extracts from individual plasma, urine, and feces samples was quantified by ^{19}F-NMR.

One dimensional ^{19}F NMR spectra were acquired at a temperature of 300 K using a Bruker 600MHz Avance III NMR spectrometer equipped with a 5 mm 1H/19F-13C/15N/D CryoProbe with a z-gradient system. A total of 128 scans were accumulated for each sample using a standard proton inverse-gated decoupling pulse sequence with a relaxation delay of 7 seconds. The relaxation delay was set to be on the relaxation plateau of the internal standard, which had a slower longitudinal relaxation than leniolisib. The T1 value of leniolisib is 766 ms (5*766 ms = 3.8s). As a result the use of 7 seconds provided a safety margin in the event metabolites relaxed more slowly. Complex points (262144) covering 56818.2 Hz were recorded at a transmitter frequency offset of -100 ppm. An exponential window function was applied with a line-broadening factor of 1.0 Hz. The spectra were manually phase and baseline corrected using Topspin v3.1 (Bruker) and referenced to the internal standard 3,5-bis(trifluoromethyl)phenol (–64.0 ppm).

For each NMR spectrum, all peaks were integrated manually with no fixed frequency area after a baseline correction and the integral of the internal standard as well as the sum of all other peak integrals were recorded. The ^{13}C satellites were most of the time not observed due to the concentration and were excluded when observed. Calibration curves were created using the integrals determined from the calibration standards samples with $1/x^2$ weighting (Supplementary Figure 1). The concentration of total drug related material in each NMR sample was determined using these calibration curves.

Mass balance calculations were performed using the total drug related material in individual urine and feces samples. The amount of drug related material was determined by multiplying the concentration of drug related material by the original sample weight/volume and any

relevant concentration factor. Subsequently, the amount of drug related material in mg was converted to percent of dose by dividing by the dose (estimated at 388 mg based on measured average drug content of 97% of nominal dose for leniolisib doses used in this clinical study).

Subsequent to NMR analysis, metabolite profile samples determined to contain drug related material were reanalyzed by LC-MS/MS to assign metabolites.

LC-MS/MS for structural Characterization of Metabolites

Extracts from plasma, urine and feces pools (described above) and NMR samples containing drug related material were analyzed by LC-MS/MS. A Waters Acquity LC system equipped with FTN sample manager, BSM pump, PDA detector and column heater/cooler was used in line with an Orbitrap XL mass spectrometer (ThermoFisher Scientific). The MS detector was operated with the following settings: positive ion mode, spray capillary 3.5 kV, transfer capillary 25 V; Tube lens 176 V, capillary temperature 275 °C, Sheath gas 15, Auxiliary gas 0, MS/MS collision mode HCD, collision energy 35-60% (normalized). Separations were performed over a Acquity HSS T3 column (2.1 × 150 mm, 1.7 µm particle size; Waters) with a corresponding guard column (2.1 × 3 mm) using mobile phases A (10 mM Ammonium formate adjusted to pH 3.0 with formic acid) and B (acetonitrile) with a flow rate of 0.5 mL/min. The gradient was as follows: 0 min 2% B; 2 min 2% B; 2.1 min 12% B; 20 min 30% B; 24 min 95% B; 25.5 min 95% B; 25.6 min 2% B; 28 min 2% B.

References

Hamilton RA, Garnett WR, Kline BJ, et al. (1981), Determination of mean valproic acid serum level by assay of a single pooled sample. Clin. Pharmacol. Ther; 29(3):408-413.

Supplementary Table 1: MS data for leniolisib and metabolites

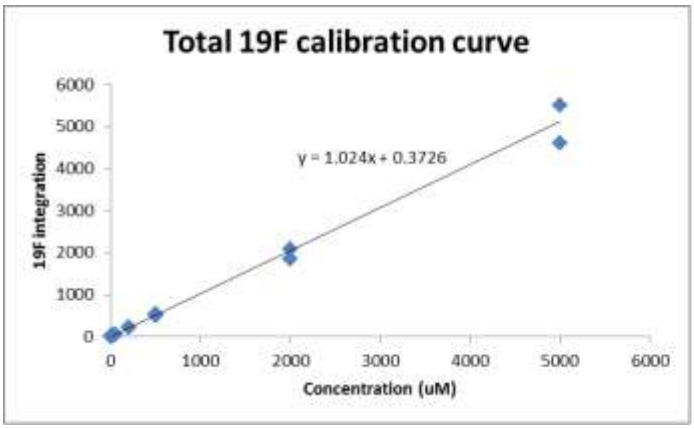
Component	Elemental composition of $[M+H]^+$	Exact mass (Da)[a]	Δmass (ppm)[b]
M1	$C_{20}H_{24}F_3N_6O_2$	437.1907	0.1
M3	$C_{21}H_{26}F_3N_6O_4$	483.1958	0.9
M4	$C_{21}H_{28}F_3N_6O_3$	469.2169	0.0
M5	$C_{21}H_{26}F_3N_6O_4$	483.1959	0.7
M6	$C_{21}H_{26}F_3N_6O_3$	467.2014	0.2
M7	$C_{21}H_{26}F_3N_6O_3$	467.2015	0.4
M9	$C_{21}H_{26}F_3N_6O_3$	467.2018	1.2
M10	$C_{21}H_{26}F_3N_6O_3$	467.2016	0.6
M14	$C_{20}H_{24}F_3N_6O_3$	453.1862	1.1
M16	$C_{20}H_{24}F_3N_6O_3$	453.1862	1.2
M18	$C_{20}H_{26}F_3N_6O_3$	455.2010	0.6
M20	$C_{20}H_{26}F_3N_6O_3$	455.2018	1.1
M21	$C_{21}H_{28}F_3N_6O_3$	469.2172	0.5
M34	$C_{20}H_{24}F_3N_6O_4$	469.1809	0.7
M43	$C_{14}H_{15}F_3N_5O$	326.1224	0.3
M48	$C_{13}H_{12}F_3N_5O$	312.1067	0.0
M52	$C_{21}H_{28}F_3N_6O_4$	485.2126	1.4
M53	$C_{21}H_{28}F_3N_6O_4$	485.2131	2.5
M54	$C_{21}H_{28}F_3N_6O_4$	485.2125	1.2
M55	$C_{21}H_{28}F_3N_6O_4$	485.2124	1.1
M56	$C_{21}H_{28}F_3N_6O_4$	485.2123	1.0
M57	$C_{20}H_{24}F_3N_6O_4$	469.1811	1.1
leniolisib	$C_{21}H_{26}F_3N_6O_2$	451.2063	0.3

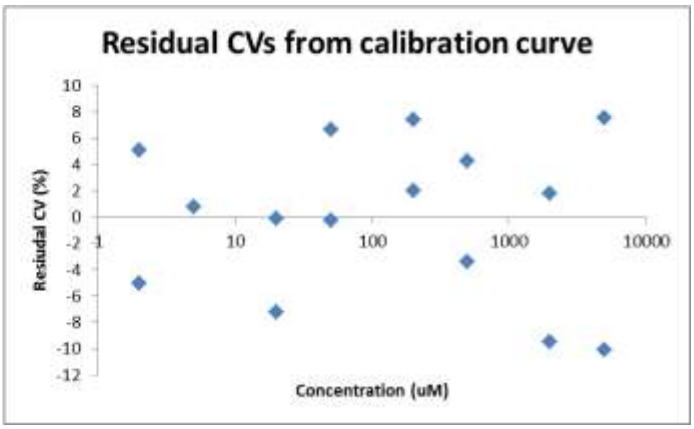
a: experimental mass

b: difference between measured and calculated mass of $[M+H]^+$

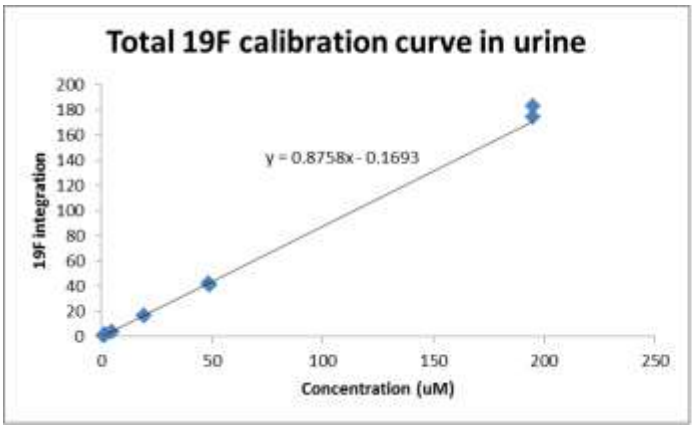
Supplementary Figure 1: ^{19}F-NMR Calibration curves and residual coefficients of variance (CV)

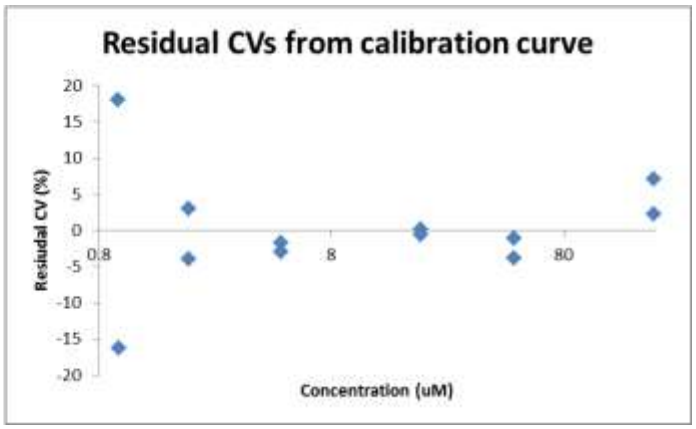
A) Individual feces samples



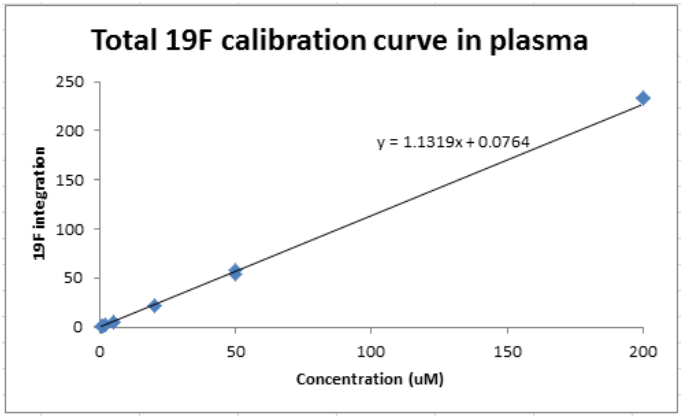


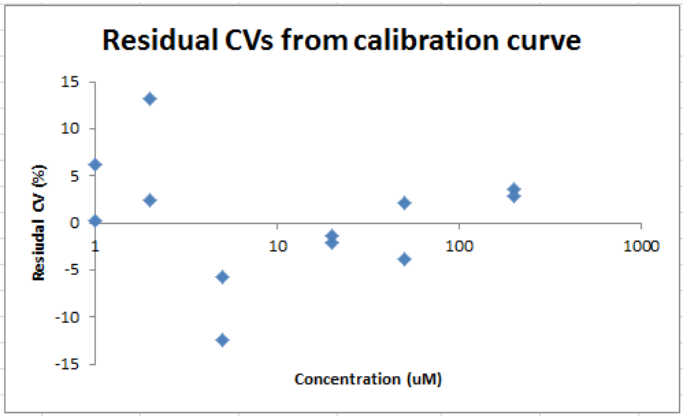
B) Individual urine samples



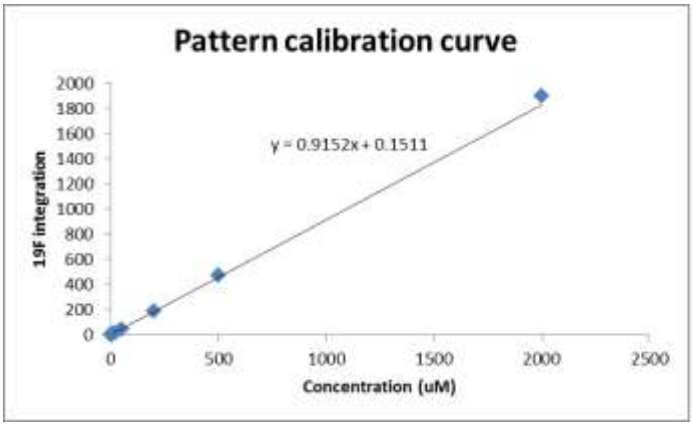


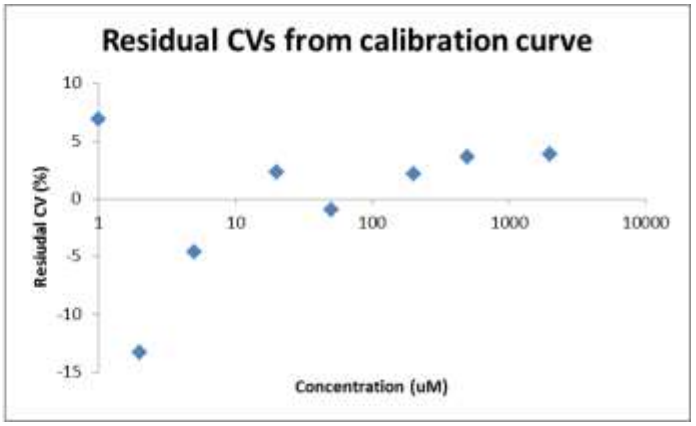
C) Individual plasma samples



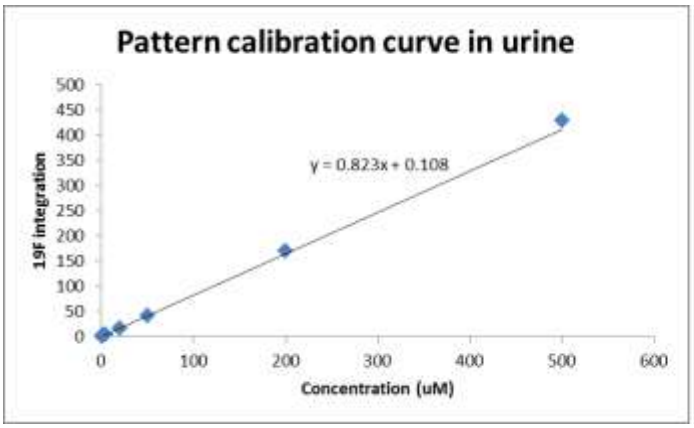


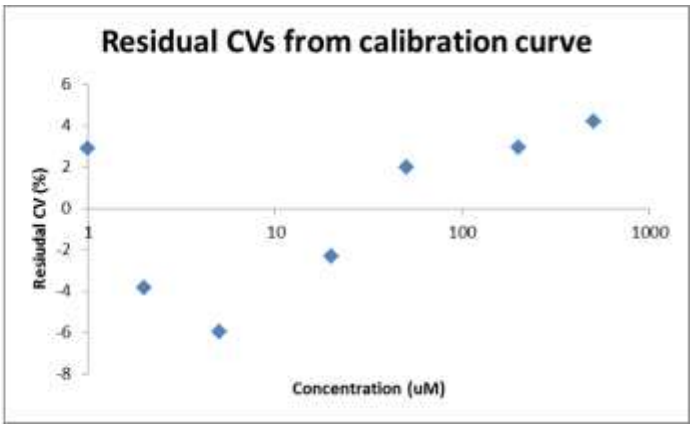
D) Feces metabolite profiles



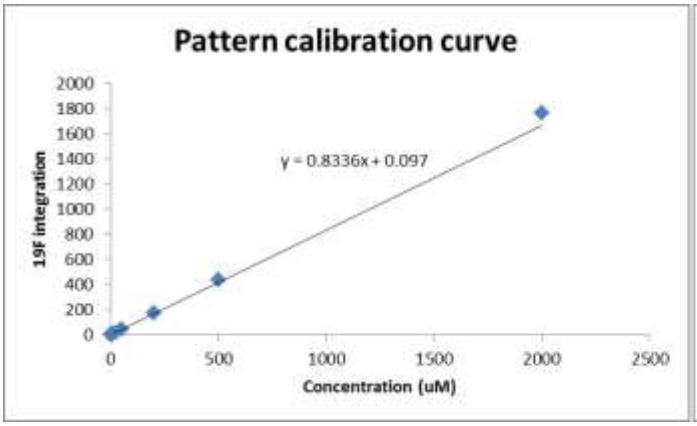


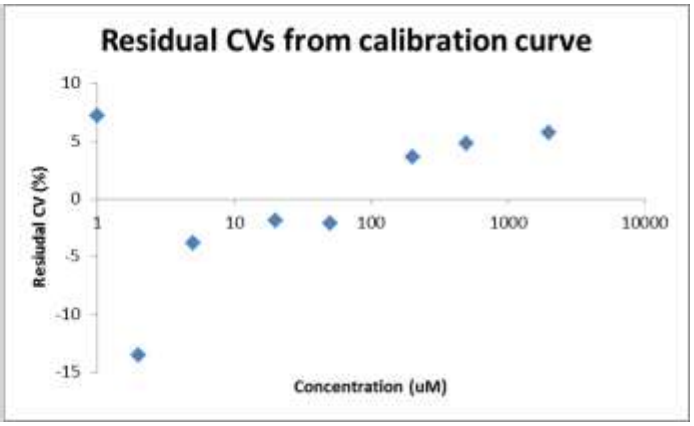
E) Urine metabolite profiles





F) Plasma metabolite profiles

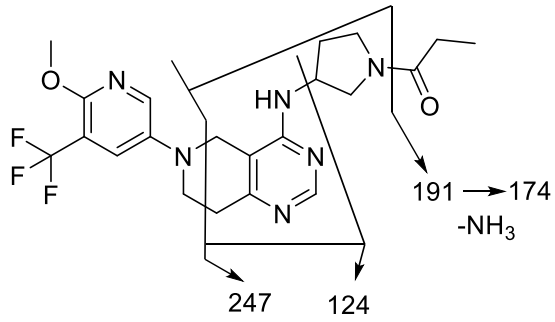


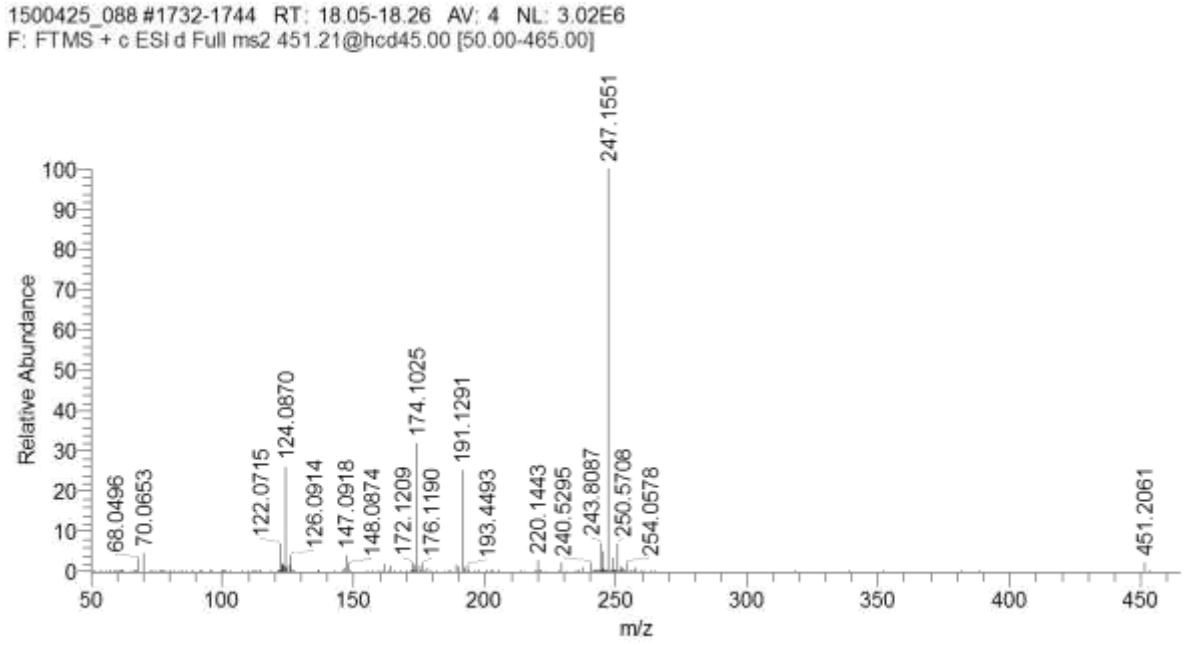


CVs were calculated as the percent difference between the actual and predicted values of ^{19}F-NMR peak integrations based on the linear calibration curve

Supplementary Figure 2: MS/MS data and structure elucidation for leniolisib and metabolites

Leniolisib





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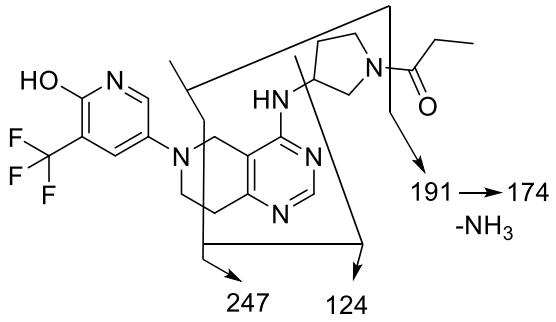

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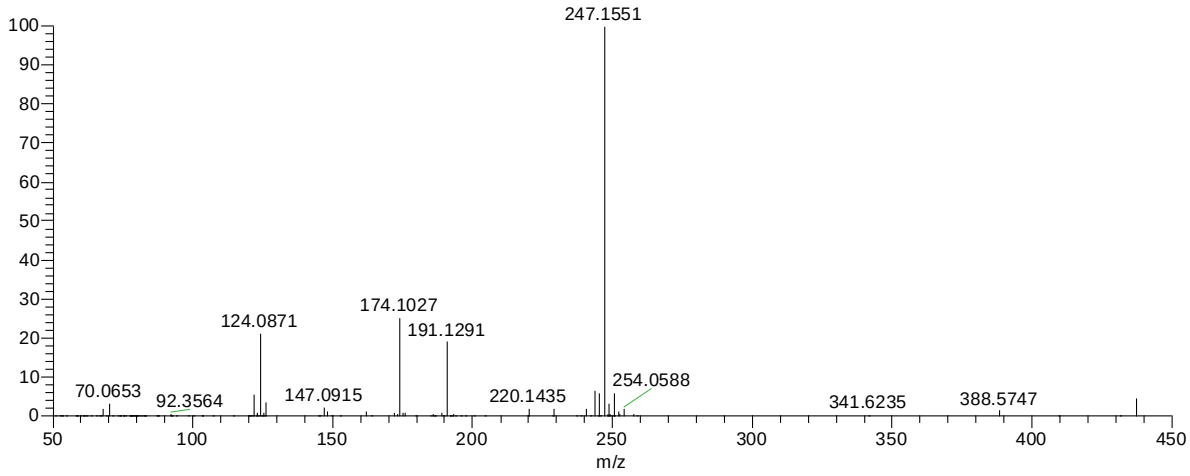
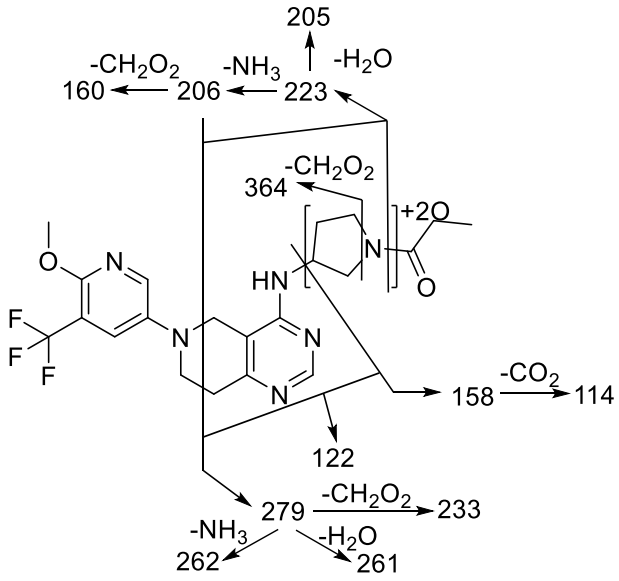


M3 (*m/z* 483)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206
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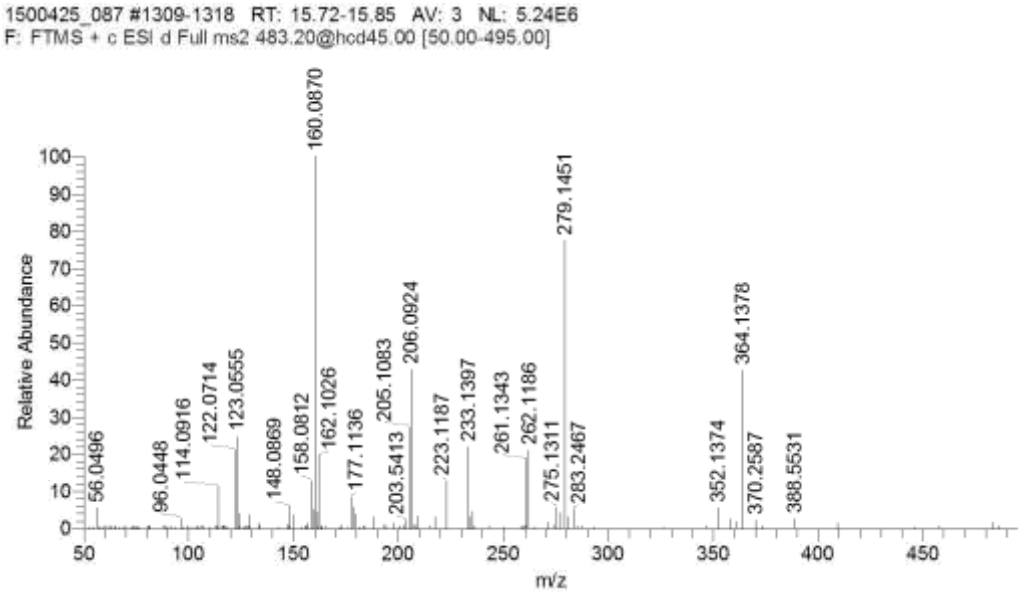
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Relative Abundance
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96.0448
56.0496
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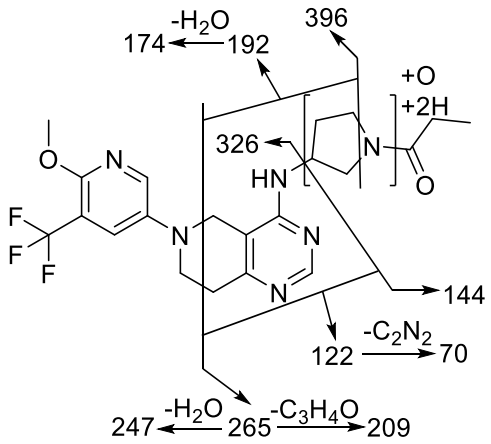
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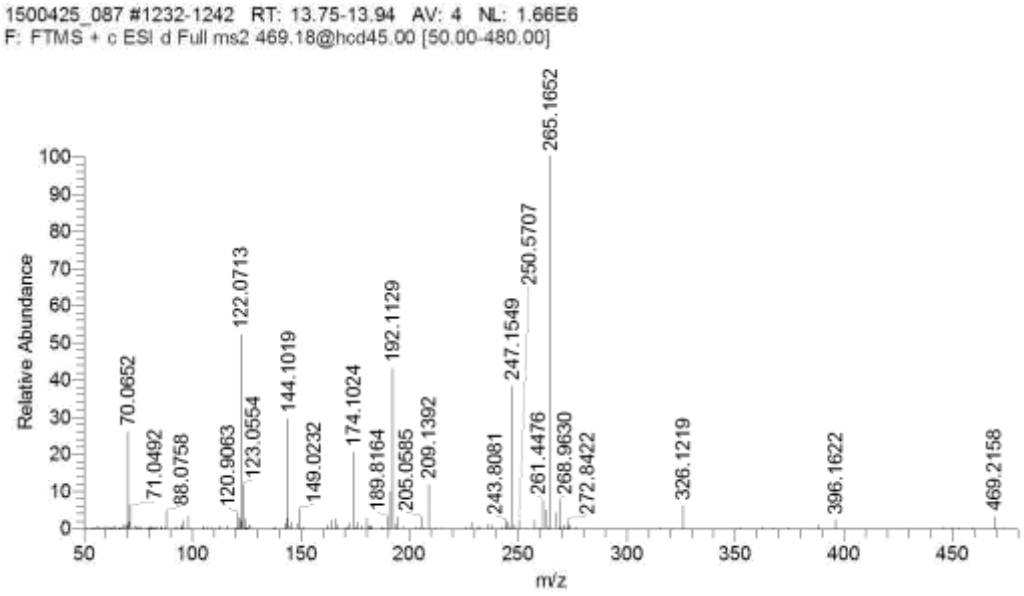
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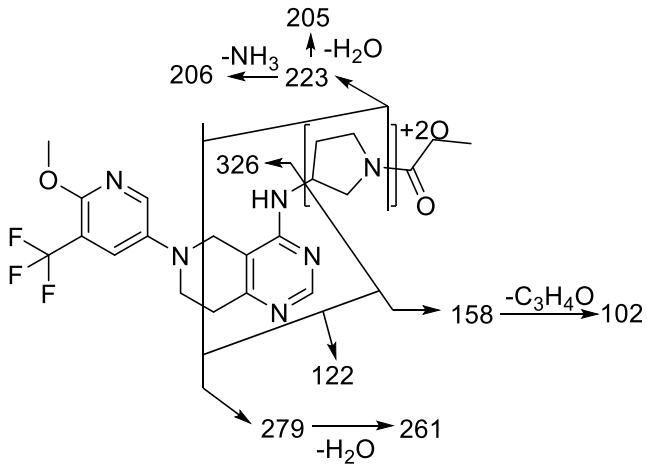
M5 (*m/z* 483)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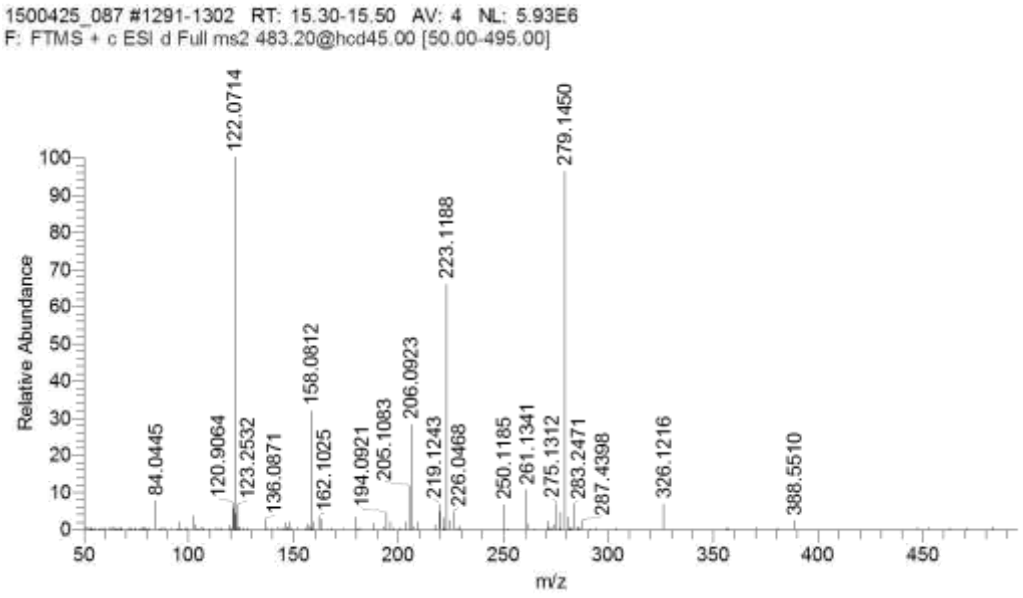
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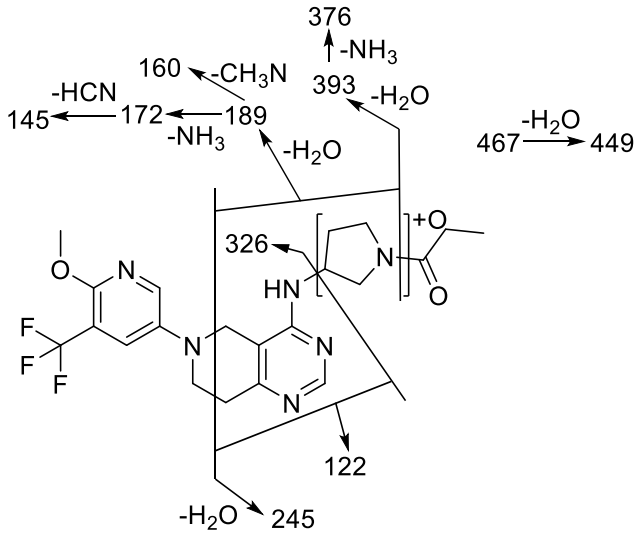
M6 (*m/z* 467)



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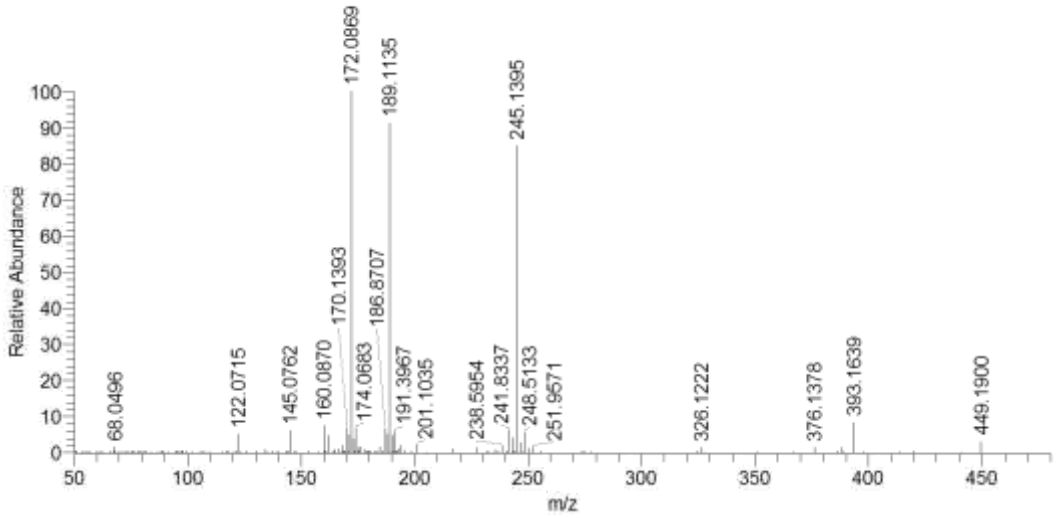
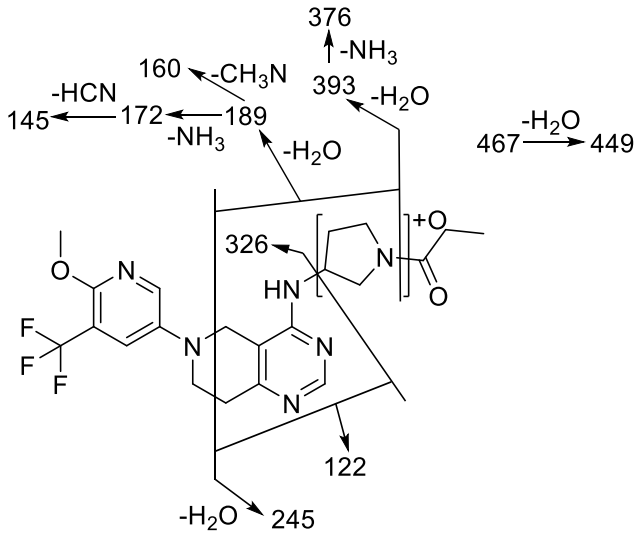
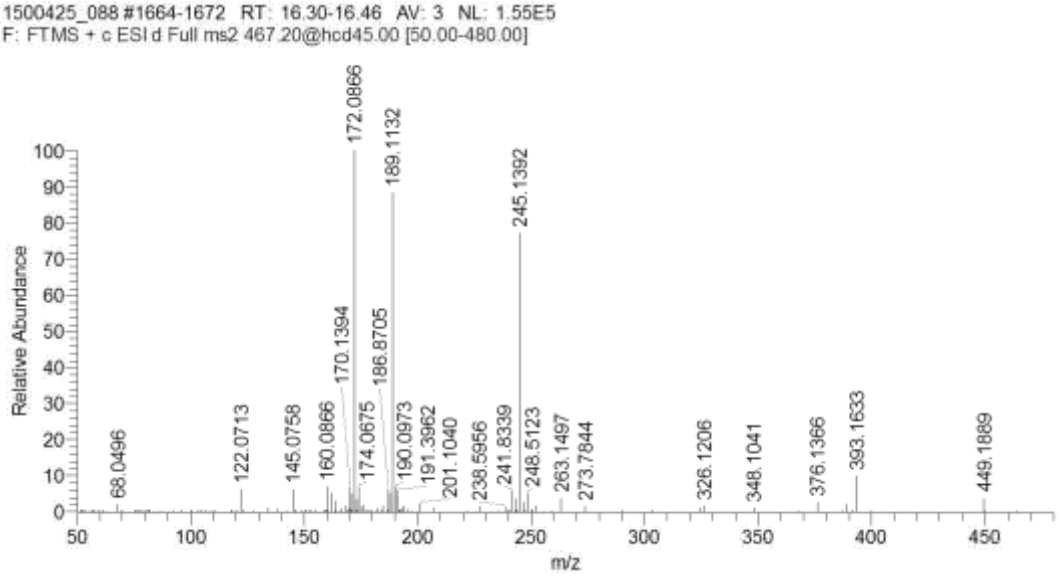
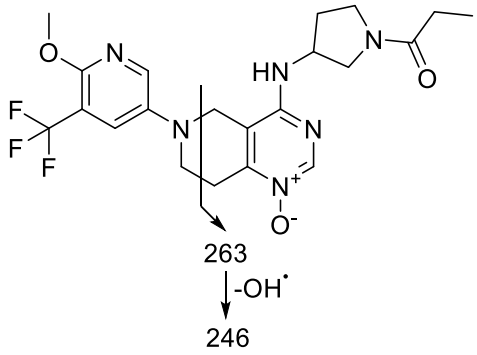


M7 (*m/z* 467)

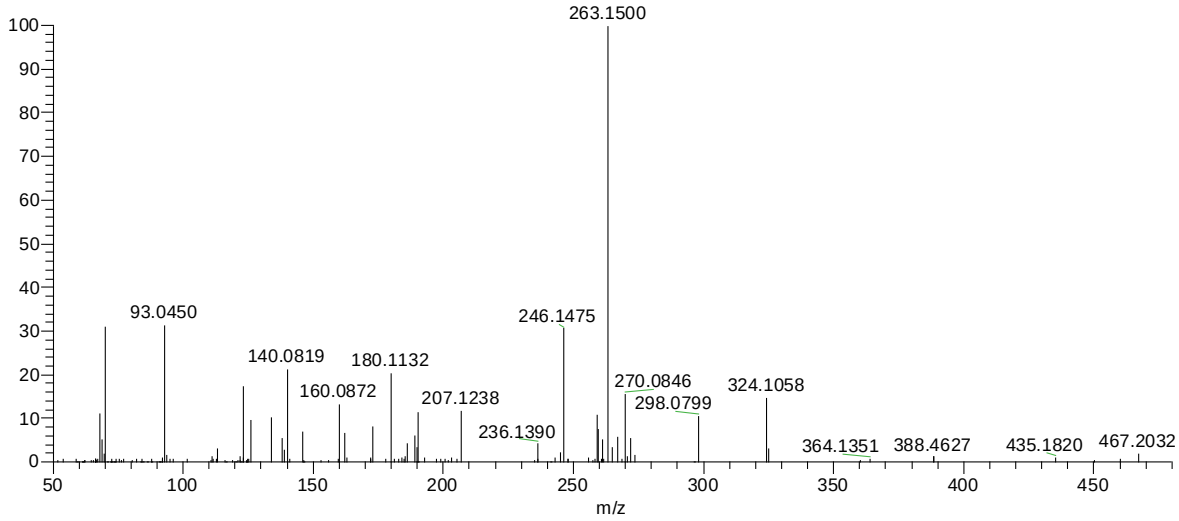

376
-NH3
160
-CH3N
393
-H2O
-HCN
145
172
189
-NH3
-H2O
467
-H2O
449
326
+O
O
N
HN
F
F
F
N
N
N
122
-H2O
245


1500425_088 #1664-1672 RT: 16.30-16.46 AV: 3 NL: 1.55E5
F: FTMS + c ESI d Full ms2 467.20@hcd45.00 [50.00-480.00]
Relative Abundance
m/z
68.0496
122.0713
145.0758
160.0866
170.1394
172.0866
174.0675
186.8705
189.1132
190.0973
191.3962
201.1040
238.5956
241.8339
245.1392
248.5123
263.1497
273.7844
326.1206
348.1041
376.1366
393.1633
449.1889

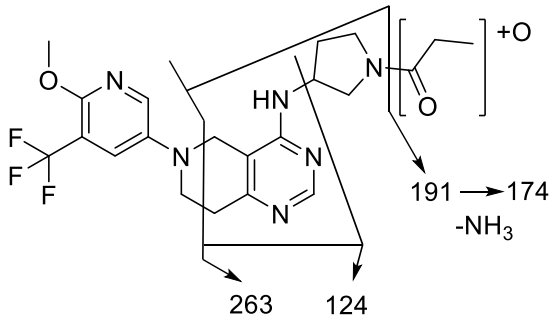
M9

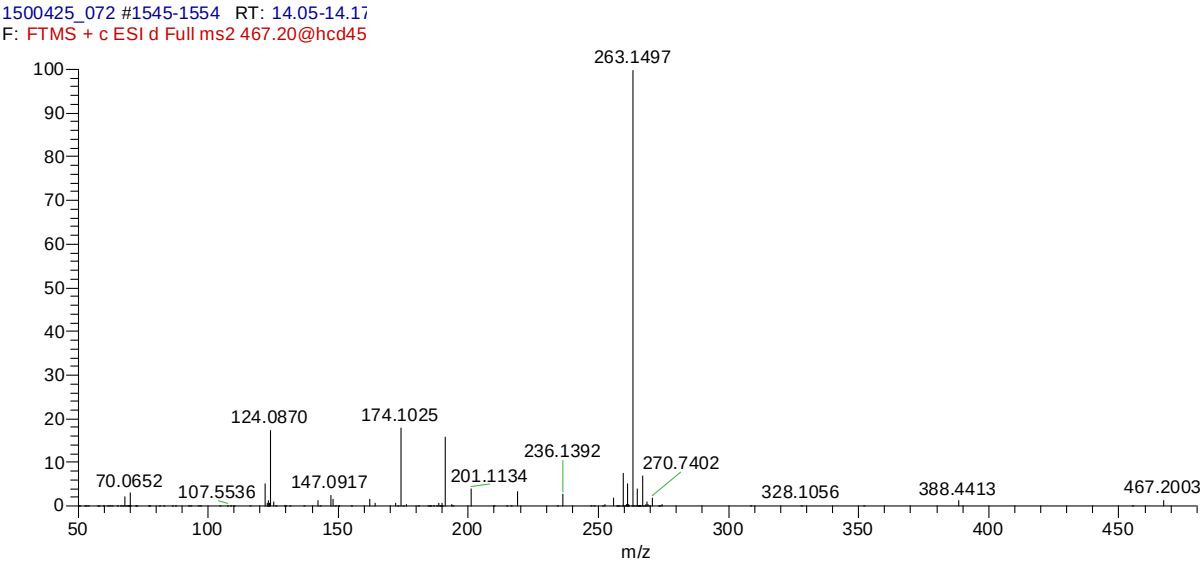


1500425_084 #2296-2303 RT: 20.52-20.62
F: FTMS + c ESI d Full ms2 467.20@hcd45

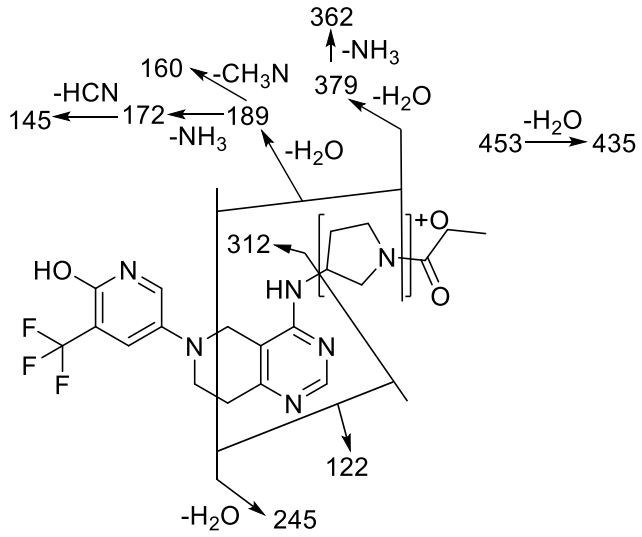


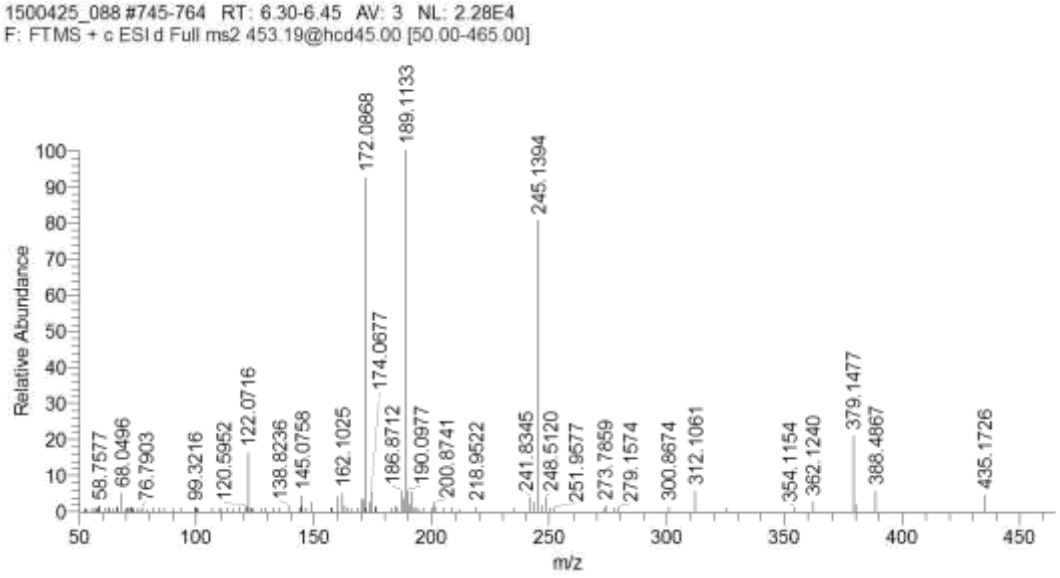
M10 (m/z 467)


O
N
F
F
F
N
HN
N
N
N
+O
O
191 → 174
-NH3
263
124

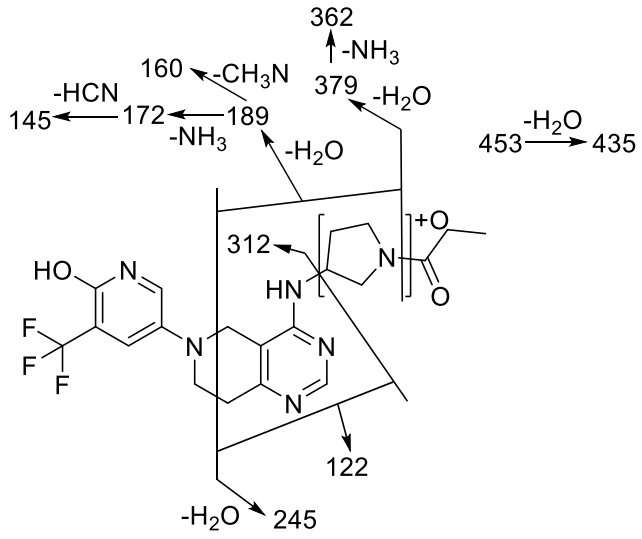

1500425_072 #1545-1554 RT: 14.05-14.17
F: FTMS + c ESI d Full ms2 467.20@hcd45
263.1497
124.0870
174.1025
236.1392
270.7402
70.0652
107.5536
147.0917
201.1134
328.1056
388.4413
467.2003
m/z

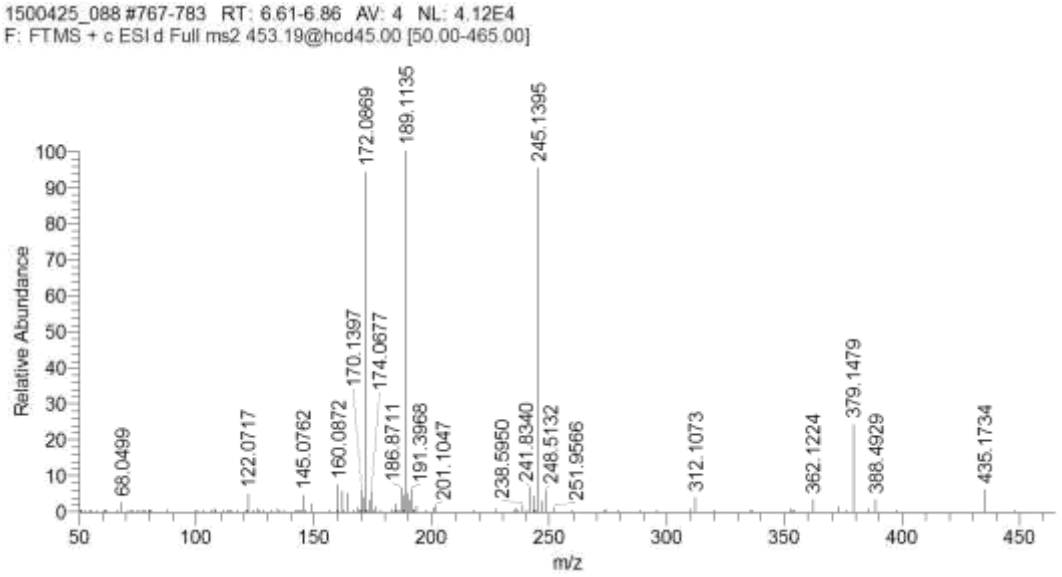
M14 (*m/z* 453)


362
-NH3
160
-CH3N
379
-H2O
-HCN
145
172
189
-NH3
-H2O
-H2O
453
435
+O
312
HO
N
HN
F
F
F
N
N
N
O
122
-H2O
245

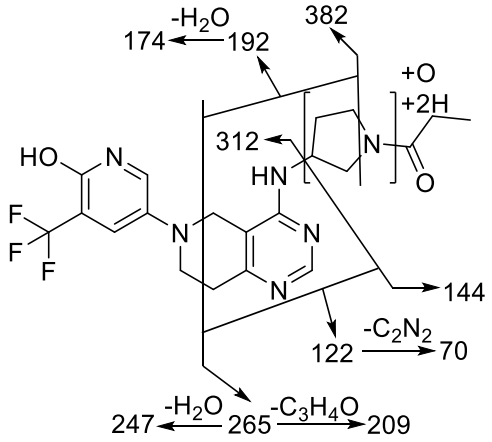

1500425_088 #745-764 RT: 6.30-6.45 AV: 3 NL: 2.28E4
F: FTMS + c ESI d Full ms2 453.19@hcd45.00 [50.00-465.00]
Relative Abundance
m/z
172.0868
189.1133
245.1394
174.0677
122.0716
58.7577
68.0496
76.7903
99.3216
120.5952
138.8236
145.0758
162.1025
186.8712
190.0977
200.8741
218.9522
241.8345
248.5120
251.9577
273.7859
279.1574
300.8674
312.1061
354.1154
362.1240
379.1477
388.4867
435.1726

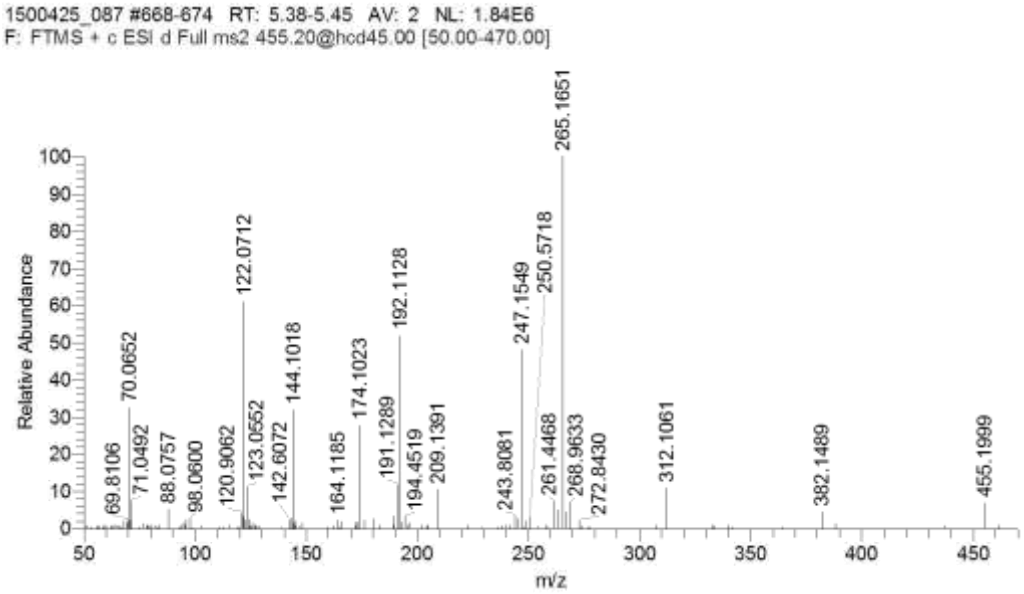
M16 (*m/z* 453)


362
-NH3
160
-CH3N
379
-H2O
-HCN
145
172
189
-NH3
-H2O
453
-H2O
435
312
+O
HO
N
HN
N
O
F
F
F
N
N
N
122
-H2O
245

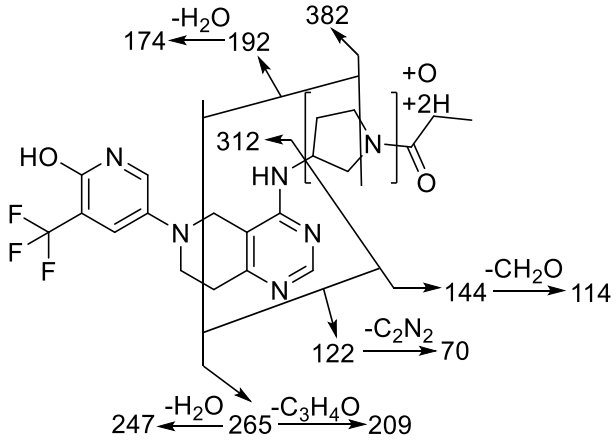

1500425_088 #767-783 RT: 6.61-6.86 AV: 4 NL: 4.12E4
F: FTMS + c ESI d Full ms2 453.19@hcd45.00 [50.00-465.00]
Relative Abundance
m/z
68.0499
122.0717
145.0762
160.0872
170.1397
172.0869
174.0677
186.8711
189.1135
191.3968
201.1047
238.5950
241.8340
245.1395
248.5132
251.9566
312.1073
362.1224
379.1479
388.4929
435.1734

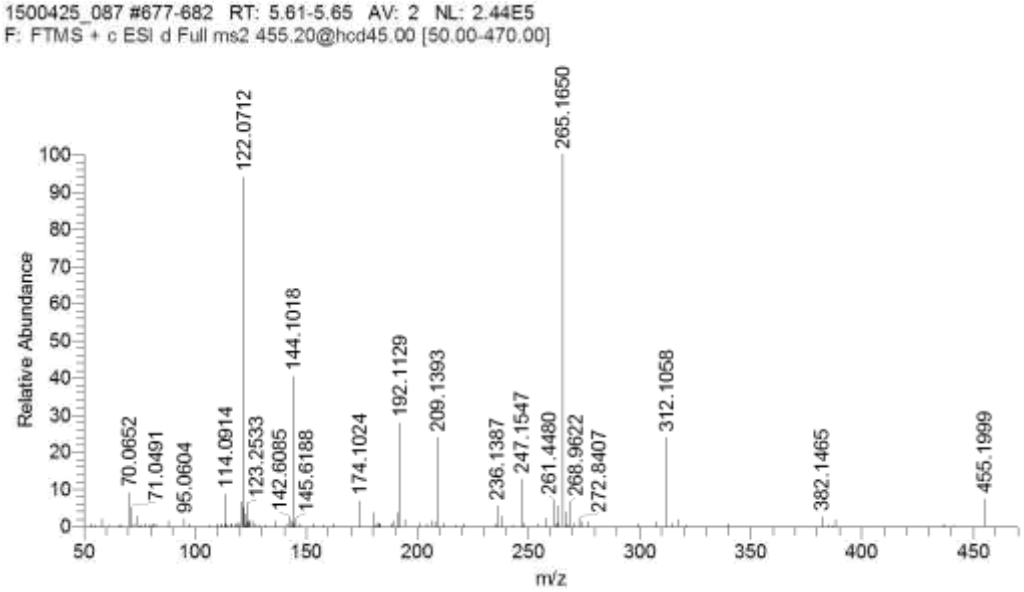
M18 (*m/z* 455)


-H2O
174
192
382
+O
+2H
312
HO
N
HN
N
F
F
F
N
N
N
O
144
-C2N2
122
70
-H2O
247
265
-C3H4O
209

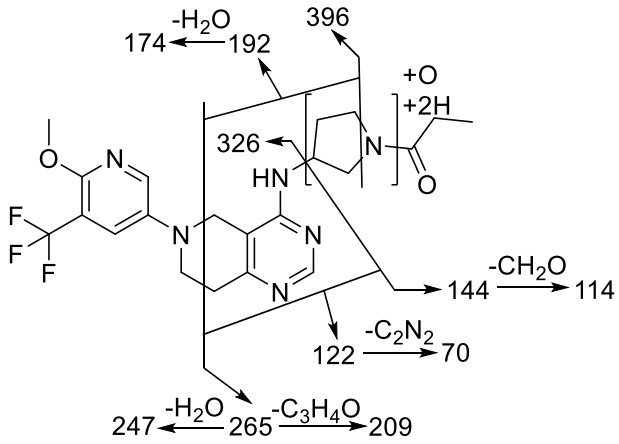

1500425_087 #668-674 RT: 5.38-5.45 AV: 2 NL: 1.84E6
F: FTMS + c ESI d Full ms2 455.20@hcd45.00 [50.00-470.00]
Relative Abundance
m/z
265.1651
122.0712
70.0652
192.1128
247.1549
250.5718
144.1018
174.1023
123.0552
191.1289
142.6072
69.8106
71.0492
88.0757
98.0600
120.9062
164.1185
194.4519
209.1391
243.8081
261.4468
268.9633
272.8430
312.1061
382.1489
455.1999

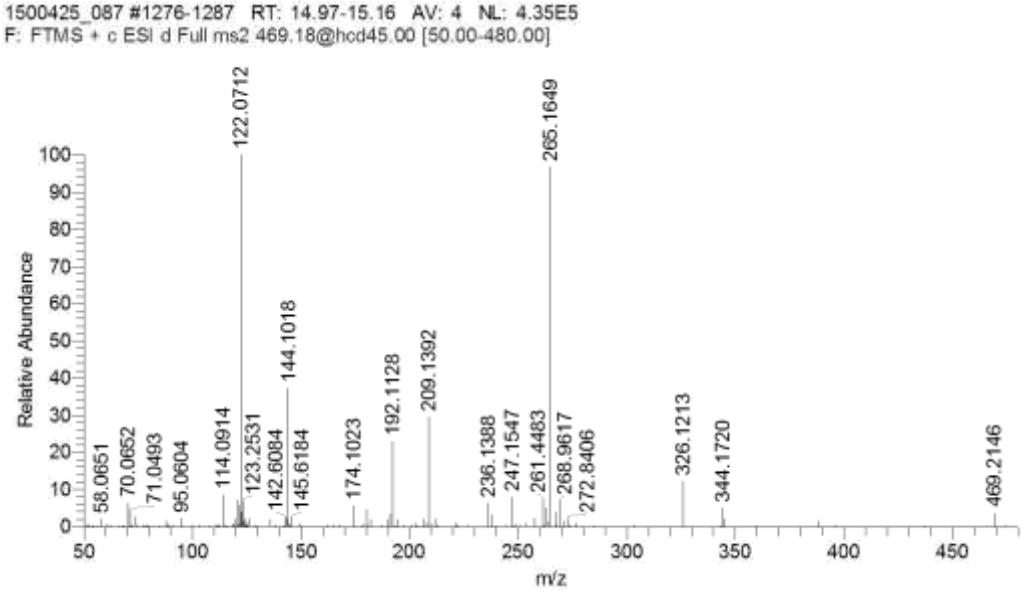
M20 (*m/z* 455)


-H2O
174
192
382
+O
+2H
312
HO
N
HN
N
O
F
F
F
N
N
N
-CH2O
144
114
-C2N2
122
70
-H2O
247
265
-C3H4O
209

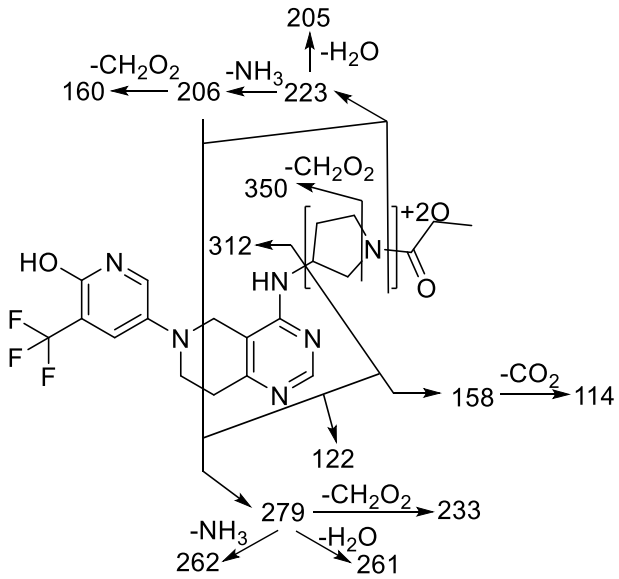

1500425_087 #677-682 RT: 5.61-5.65 AV: 2 NL: 2.44E5
F: FTMS + c ESI d Full ms2 455.20@hcd45.00 [50.00-470.00]
Relative Abundance
m/z
122.0712
265.1650
144.1018
192.1129
209.1393
312.1058
70.0652
71.0491
95.0604
114.0914
123.2533
142.6085
145.6188
174.1024
236.1387
247.1547
261.4480
268.9622
272.8407
382.1465
455.1999

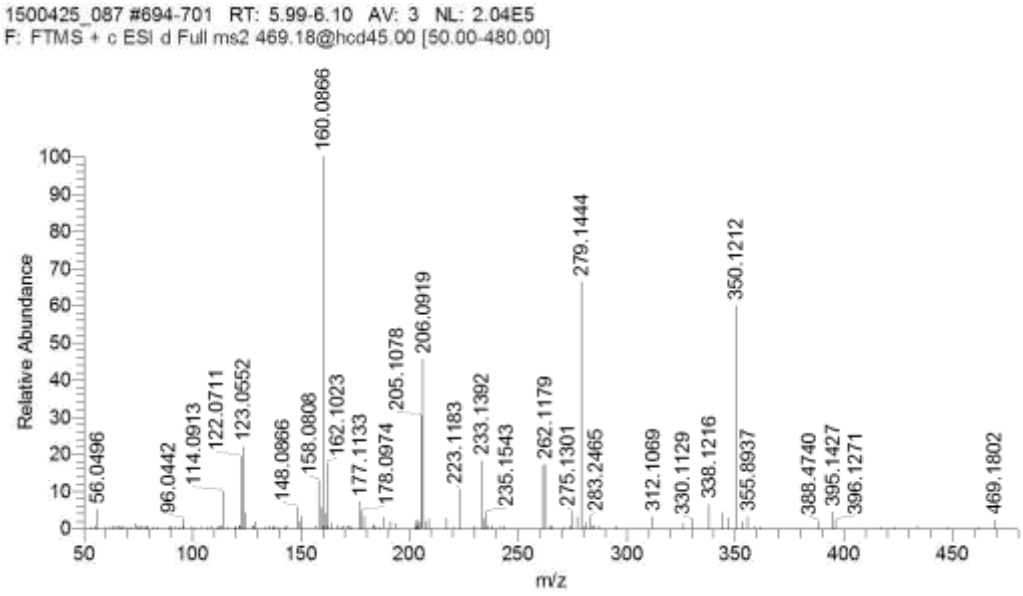
M21 (*m/z* 469)


-H2O
174
192
396
+O
+2H
326
HN
O
N
F
F
F
-CH2O
144
114
-C2N2
122
70
-H2O
247
265
-C3H4O
209

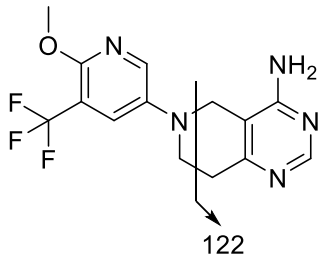

1500425_087 #1276-1287 RT: 14.97-15.16 AV: 4 NL: 4.35E5
F: FTMS + c ESI d Full ms2 469.18@hcd45.00 [50.00-480.00]
Relative Abundance
m/z
122.0712
265.1649
144.1018
209.1392
192.1128
114.0914
123.2531
142.6084
145.6184
174.1023
236.1388
247.1547
261.4483
268.9617
272.8406
326.1213
344.1720
469.2146
58.0651
70.0652
71.0493
95.0604

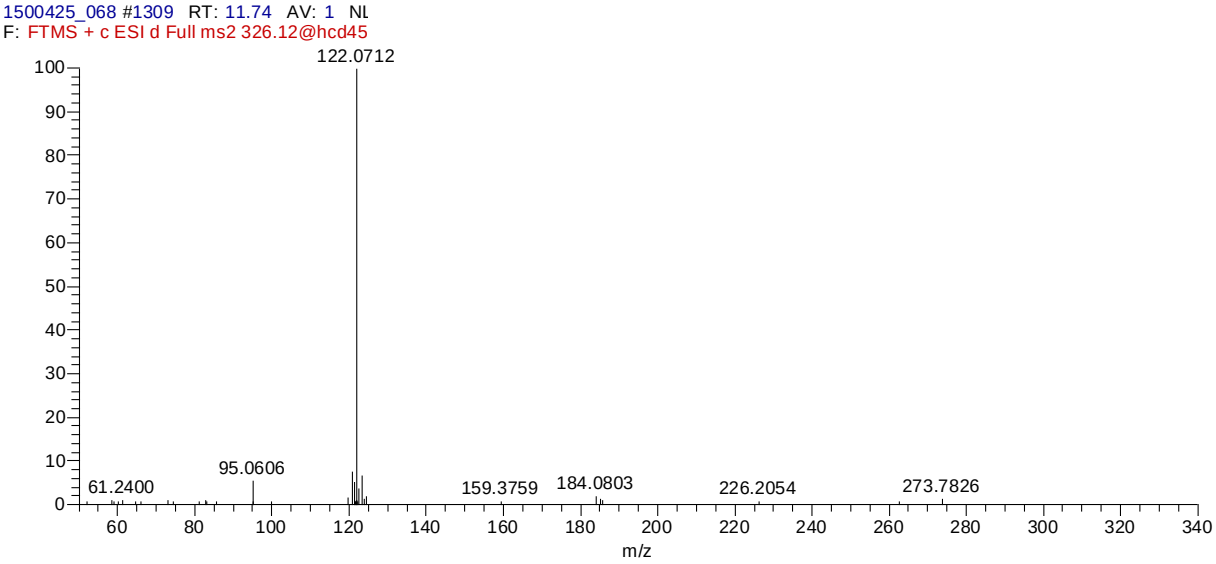
M34 (*m/z* 469)


205
-H2O
-CH2O2
-NH3
160
206
223
-CH2O2
350
+2O
312
HO
N
HN
F
F
F
N
N
N
O
-CO2
158
114
122
279
-CH2O2
233
-NH3
-H2O
262
261

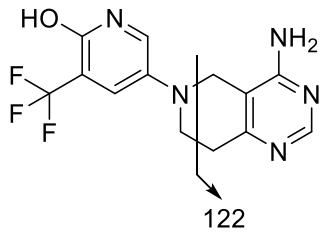

1500425_087 #694-701 RT: 5.99-6.10 AV: 3 NL: 2.04E5
F: FTMS + c ESI d Full ms2 469.18@hcd45.00 [50.00-480.00]
Relative Abundance
m/z
160.0866
279.1444
350.1212
206.0919
205.1078
123.0552
122.0711
114.0913
96.0442
56.0496
148.0866
158.0808
162.1023
177.1133
178.0974
223.1183
233.1392
235.1543
262.1179
275.1301
283.2465
312.1069
330.1129
338.1216
355.8937
388.4740
395.1427
396.1271
469.1802

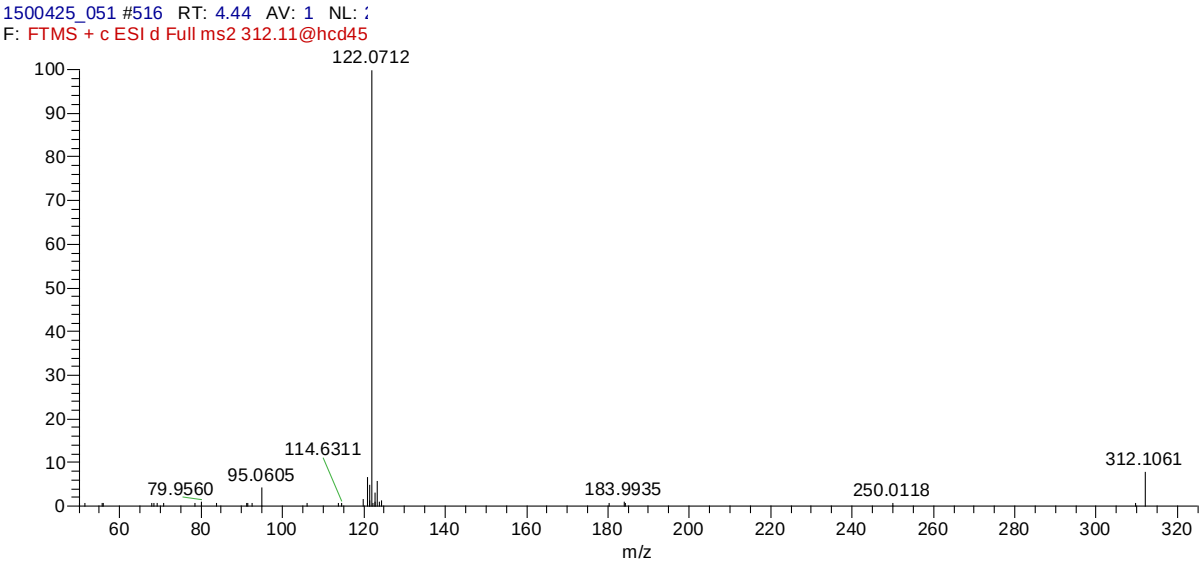
M43 (*m/z* 326)


O
N
NH2
F
F
F
N
N
N
122

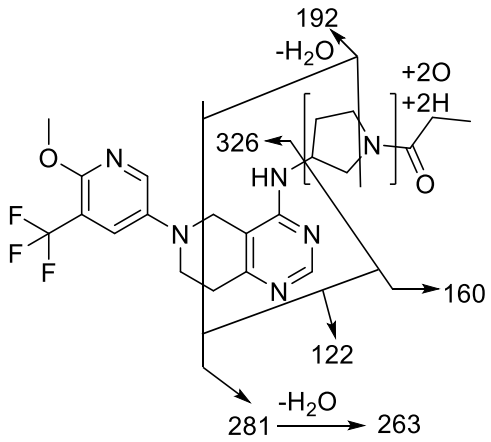

1500425_068 #1309 RT: 11.74 AV: 1 NL
F: FTMS + c ESI d Full ms2 326.12@hcd45
122.0712
95.0606
61.2400
159.3759
184.0803
226.2054
273.7826
m/z

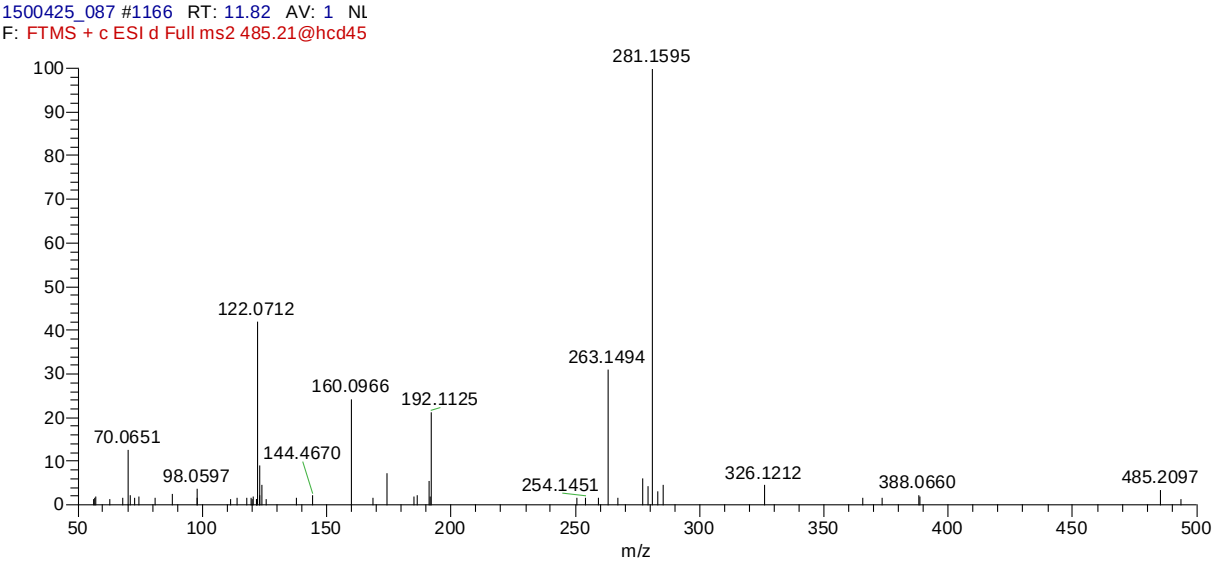
M48 (*m/z* 312)


HO
N
NH2
F
F
F
N
N
N
122

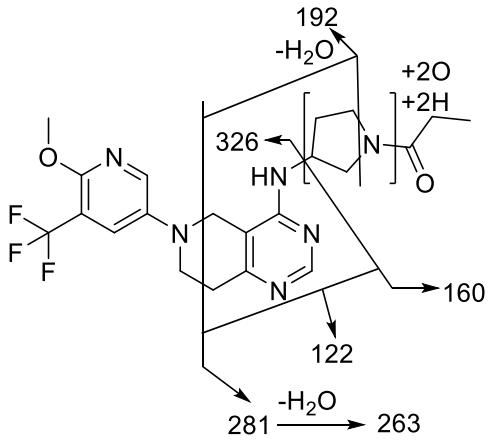

1500425_051 #516 RT: 4.44 AV: 1 NL:
F: FTMS + c ESI d Full ms2 312.11@hcd45
122.0712
114.6311
95.0605
79.9560
183.9935
250.0118
312.1061
m/z

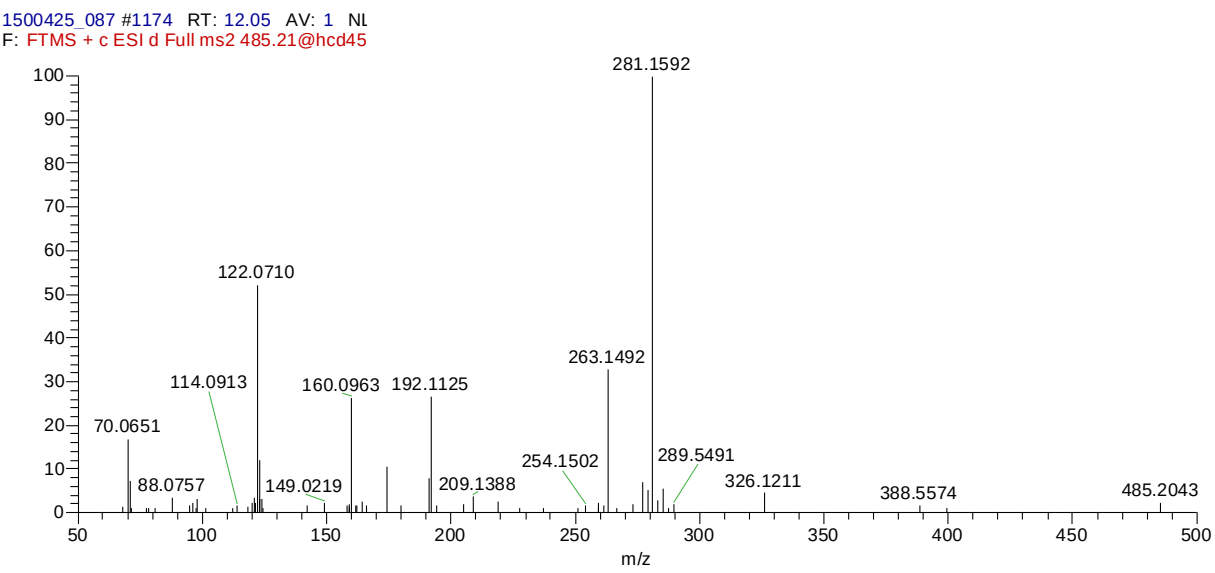
M52 (*m/z* 485)


192
-H2O
+2O
+2H
326
HN
O
N
F
F
F
N
N
N
160
122
-H2O
281
263

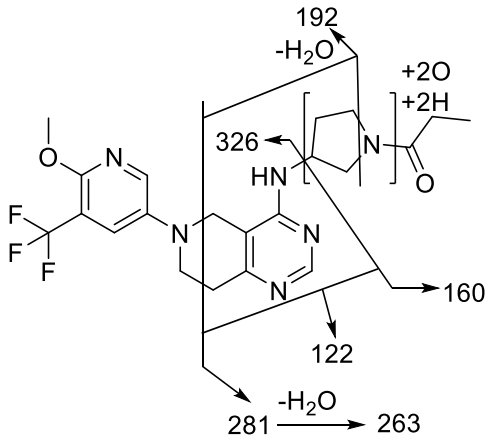

1500425_087 #1166 RT: 11.82 AV: 1 NL
F: FTMS + c ESI d Full ms2 485.21@hcd45
281.1595
122.0712
263.1494
160.0966
192.1125
70.0651
144.4670
98.0597
254.1451
326.1212
388.0660
485.2097
m/z

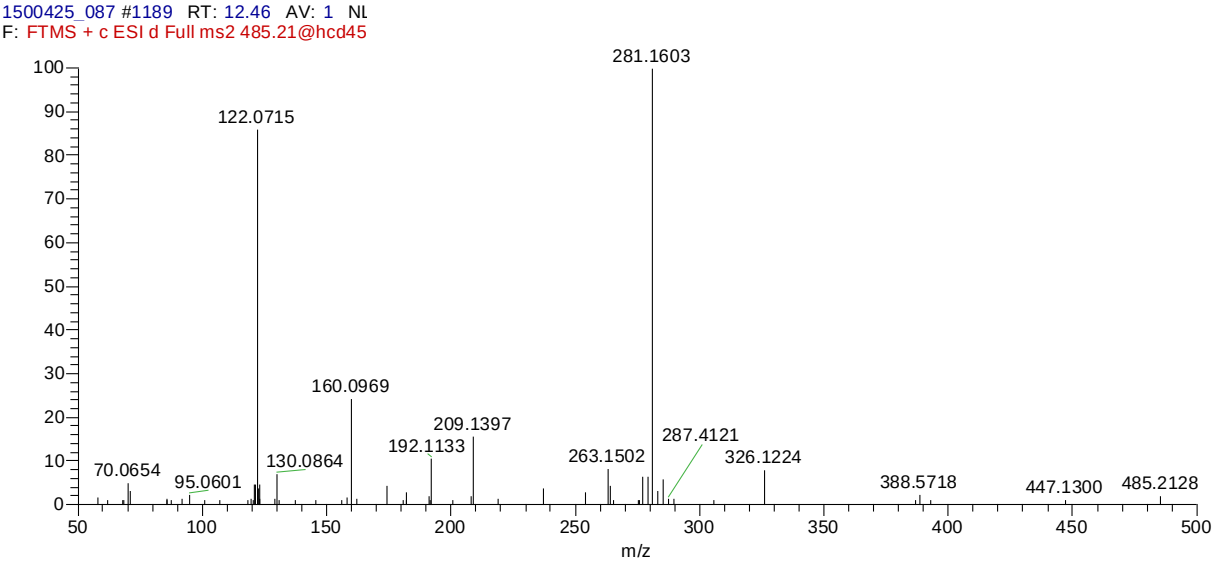
M53 (*m/z* 485)


192
-H2O
+2O
+2H
326
HN
O
N
F
F
F
N
N
N
N
O
160
122
-H2O
281
263

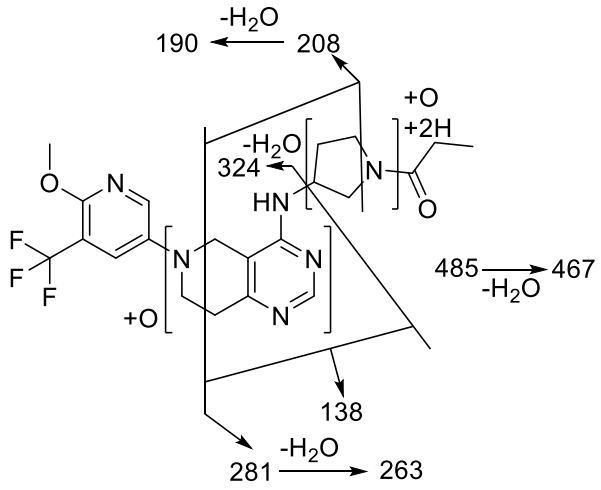

1500425_087 #1174 RT: 12.05 AV: 1 NL
F: FTMS + c ESI d Full ms2 485.21@hcd45
281.1592
122.0710
263.1492
114.0913
160.0963
192.1125
70.0651
254.1502
289.5491
88.0757
149.0219
209.1388
326.1211
388.5574
485.2043
m/z

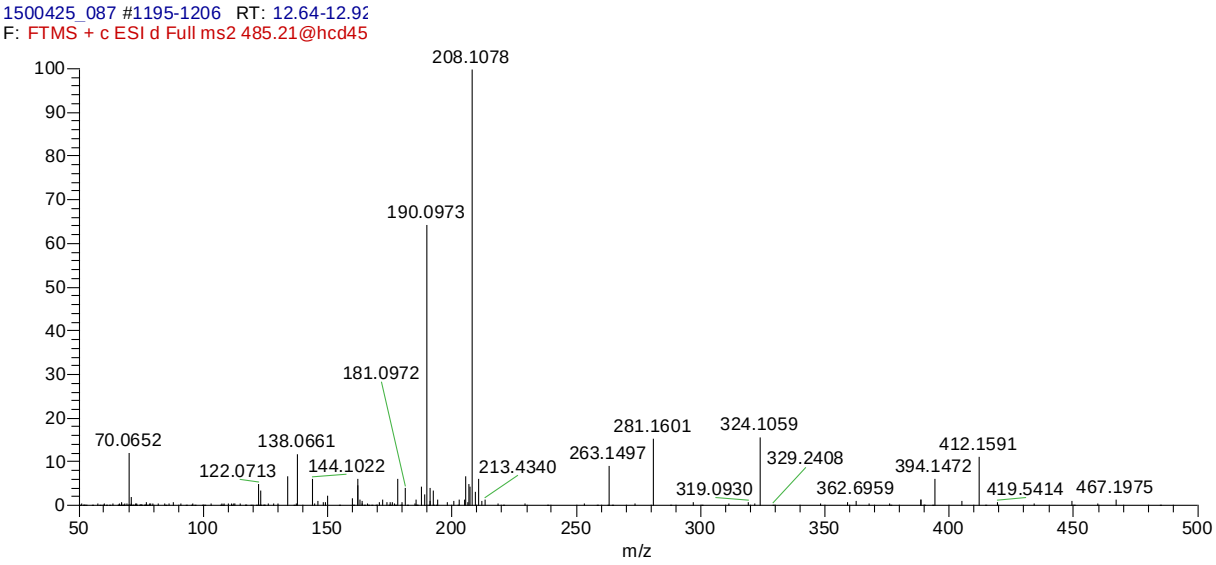
M54 (*m/z* 485)


192
-H2O
+2O
+2H
326
HN
O
N
F
F
F
N
N
N
160
122
-H2O
281
263

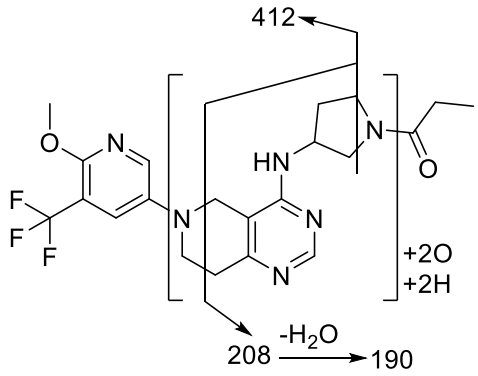

1500425_087 #1189 RT: 12.46 AV: 1 NL
F: FTMS + c ESI d Full ms2 485.21@hcd45
281.1603
122.0715
160.0969
209.1397
192.1133
287.4121
326.1224
263.1502
130.0864
70.0654
95.0601
388.5718
447.1300
485.2128
m/z

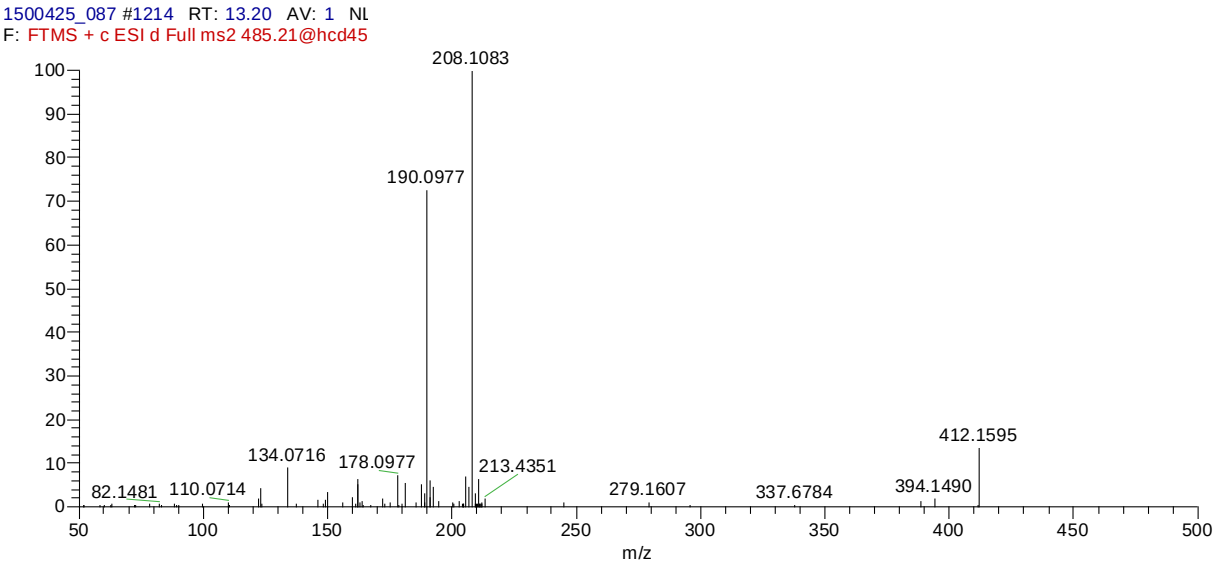
M55 (*m/z* 485)


-H2O
190
208
+O
+2H
-H2O
324
O
N
N
HN
O
F
F
F
N
N
N
+O
485
467
-H2O
138
-H2O
281
263

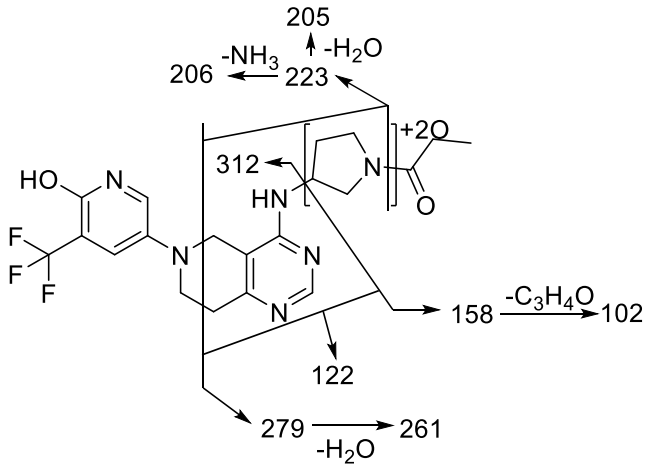

1500425_087 #1195-1206 RT: 12.64-12.92
F: FTMS + c ESI d Full ms2 485.21@hcd45
208.1078
190.0973
181.0972
281.1601
324.1059
263.1497
329.2408
412.1591
394.1472
70.0652
138.0661
122.0713
144.1022
213.4340
319.0930
362.6959
419.5414
467.1975
100
90
80
70
60
50
40
30
20
10
0
50
100
150
200
250
300
350
400
450
500
m/z

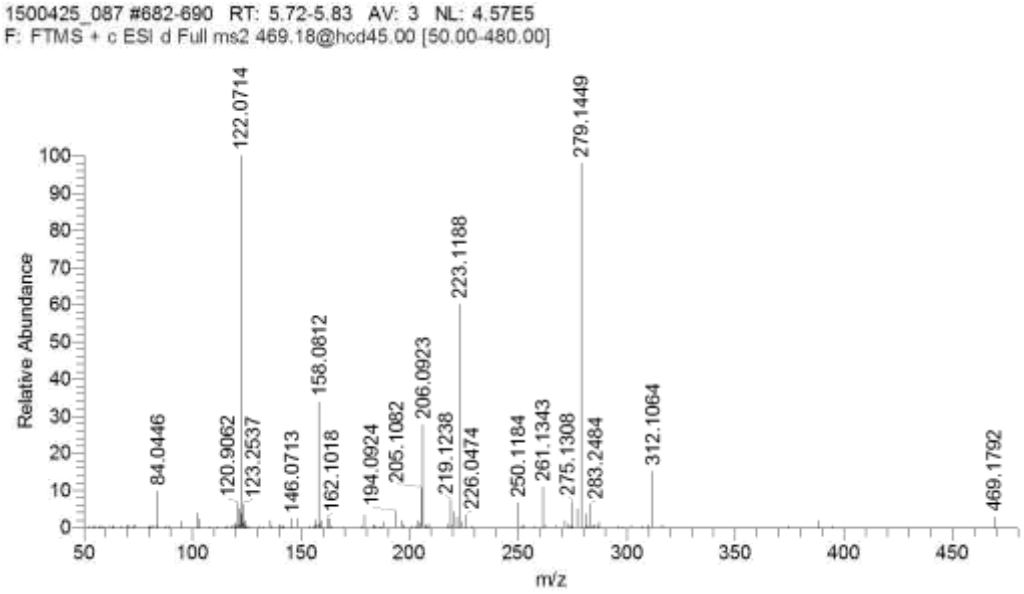
M56 (*m/z* 485)


412
O
N
HN
N
O
F
F
F
N
N
N
+2O
+2H
208
-H2O
190


1500425_087 #1214 RT: 13.20 AV: 1 NL
F: FTMS + c ESI d Full ms2 485.21@hcd45
208.1083
190.0977
134.0716
178.0977
213.4351
82.1481
110.0714
279.1607
337.6784
394.1490
412.1595
m/z

M57 (*m/z* 469)


205
-NH3
-H2O
206
223
+2O
312
HO
N
HN
F
F
F
N
N
N
O
158
-C3H4O
102
122
279
261
-H2O


1500425_087 #682-690 RT: 5.72-5.83 AV: 3 NL: 4.57E5
F: FTMS + c ESI d Full ms2 469.18@hcd45.00 [50.00-480.00]
Relative Abundance
m/z
122.0714
279.1449
223.1188
158.0812
206.0923
84.0446
120.9062
123.2537
146.0713
162.1018
194.0924
205.1082
219.1238
226.0474
250.1184
261.1343
275.1308
283.2484
312.1064
469.1792