A Metabolic Profile Determination of 2F-Viminol, A Novel Synthetic Opioid (NSO) Identified in Forensic Investigations

Aracelis A. Velez, B.S.

Alex J. Krotulski, PhD; Donna M. Papsun, MS; Karen S. Scott, PhD

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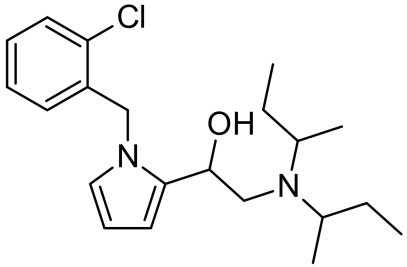
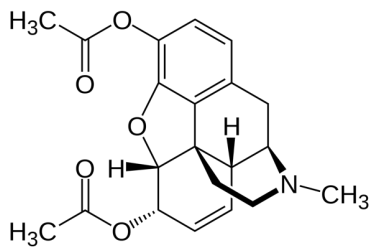
Viminol

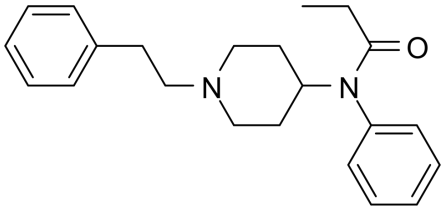
- Marketed as Dividol® in Italy and Brazil
- ~5.5x more potent than morphine
- Has shown little liability for development of dependence
- Racemic mixture of 6 different stereoisomers
  - 1S-(R,R)-disecbutyl isomer is a μ-opioid full agonist
  - 1S-(S,S)-disecbutyl isomer is an antagonist
- Structurally different from other opioids
- Not FDA approved or scheduled in the US



Viminol



Heroin



Fentanyl

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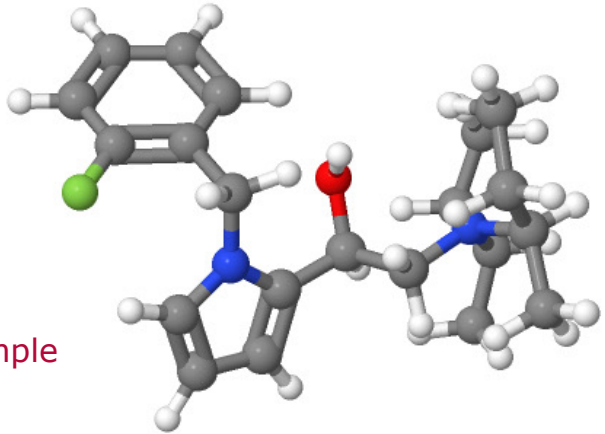
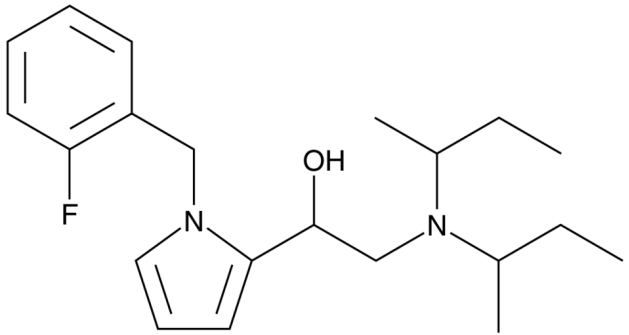
2F-Viminol

- Fluorine replaces Viminol's chlorine
  - Considerable stability of the C-F bond
  - Increased lipophilicity
- Classified as a novel opioid
- No literature available
- No pharmacokinetic studies
- 2 cases identified at CFSRE
  - One seized powder
  - One pending toxicological sample



2F-Viminol 3D model



2F-Viminol Structure

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Aims & Objectives

- Primary goal: To incubate and elucidate the major and minor metabolites of 2F-viminol
- Achieved by:

Incubating 2F-viminol in vitro with human liver microsomes (HLMs)

Analyzing the metabolite mixtures via LC-QTOF-MS

Using Metabolite Pilot software to identify metabolites and to elucidate their structures

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Methods: Sample Prep

- Phosphate buffer: pH 7.4
- Pooled HLMs
  - Vesicles of the hepatocyte endoplasmic reticulum containing a variety of enzymes
    - 50 donor, mixed gender
  - Requires addition of Nicotinamide Adenine Dinucleotide Phosphate (NADPH) for catalytic activation of enzymes
- Diazepam was used as a control drug

Sample ID	Phosphate Buffer (µL)	Drug (µL)	NADPH (µL)	HLM (µL)
Standard	595	5	0	0
Control	570	5	0	25
Reaction Mixture	520	5	50	25
Reaction Mixture	520	5	50	25

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Methods: Incubations

- Incubate samples at 37^{0}C with agitation for 2hrs
- Stop reaction by adding 500 µL ACN
- Centrifuge at 10,000 rpm to separate microsomes and cellular material
- Partial dry-down of supernatant
- Transfer to autosampler vials for LC-QTOF-MS analysis

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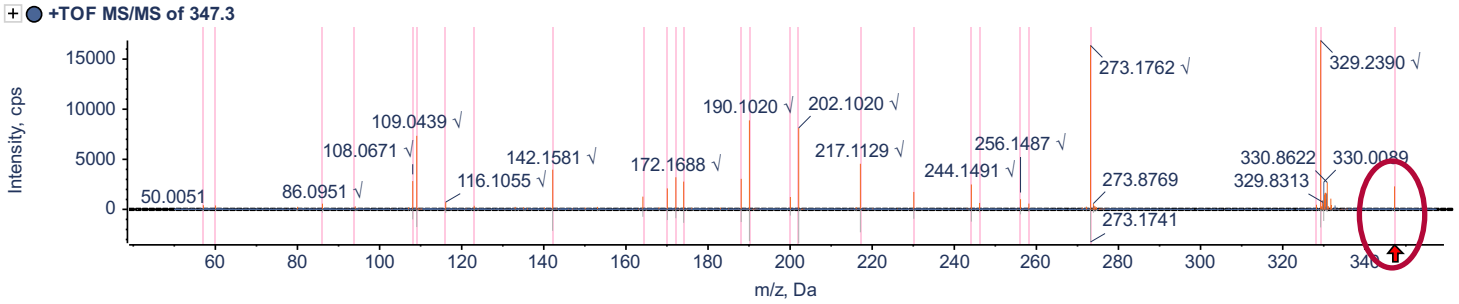
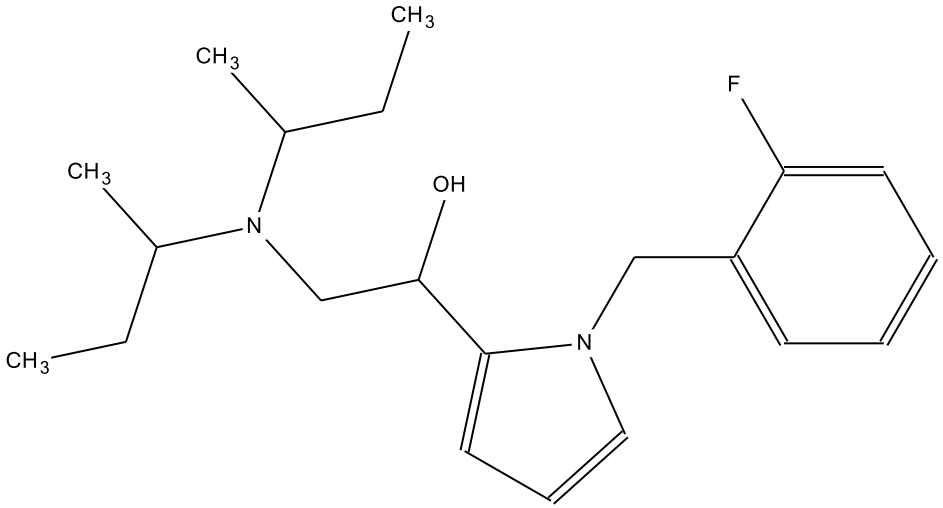
MS/MS Fragmentation



347.2493

$C_{21}H_{31}FN_2O$



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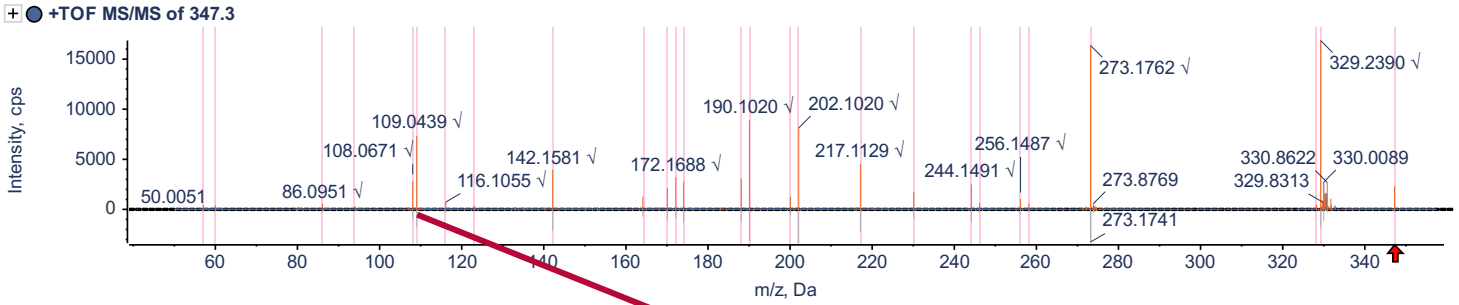
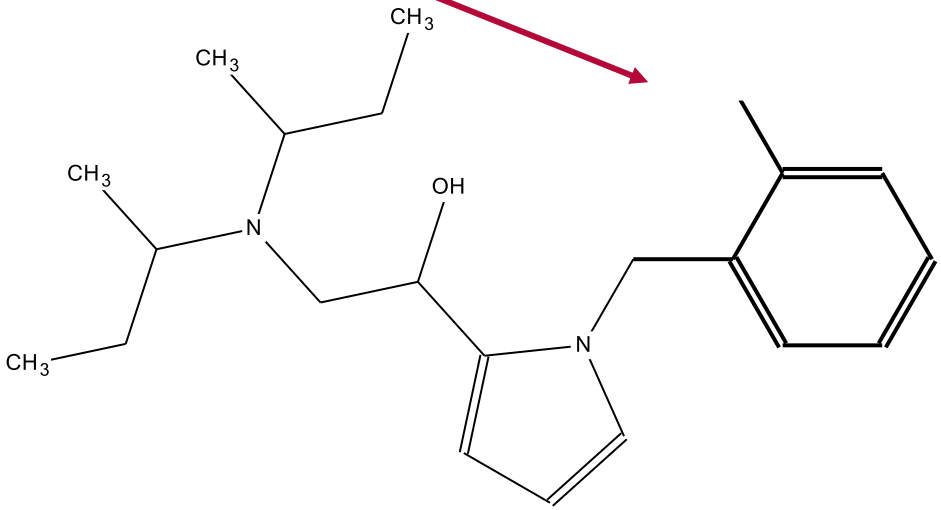
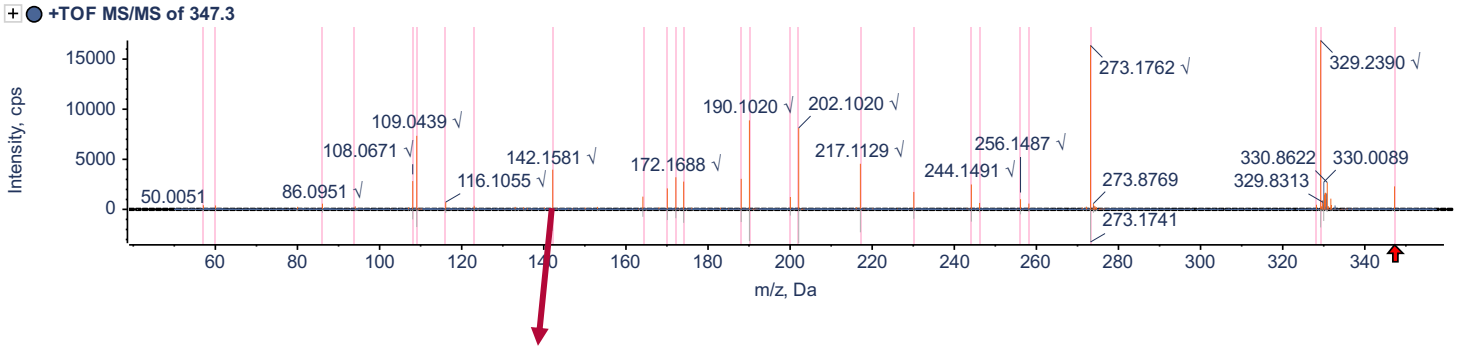




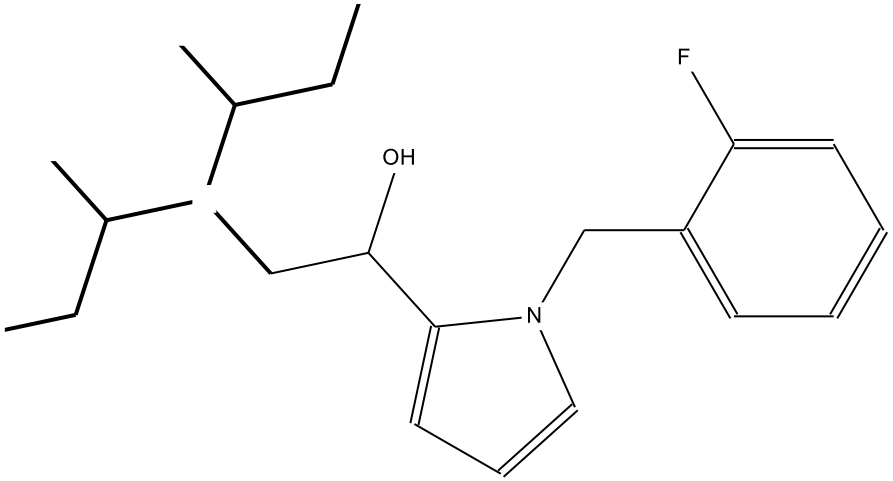
109.0439
C_7H_6F

MS/MS Fragmentation



142.1581

$C_9H_{20}N$



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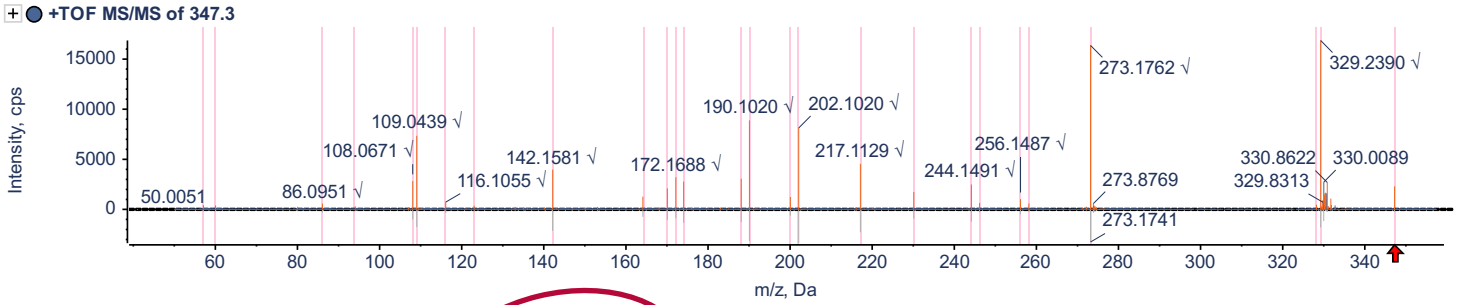
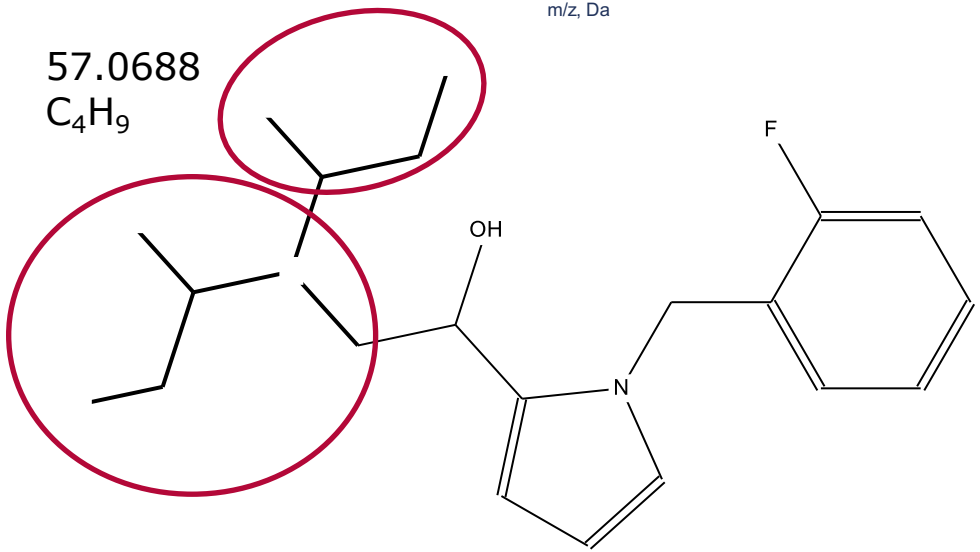


57.0688
C_4H_9

86.0951
$C_5H_{12}N$



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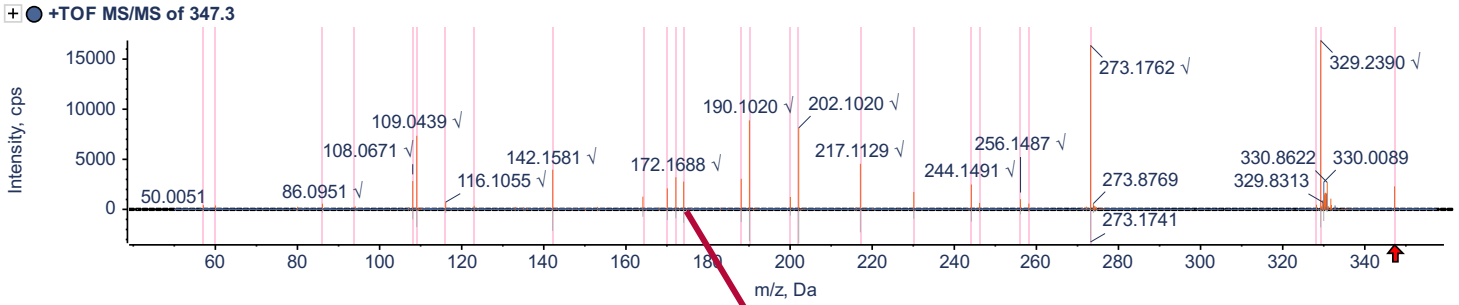
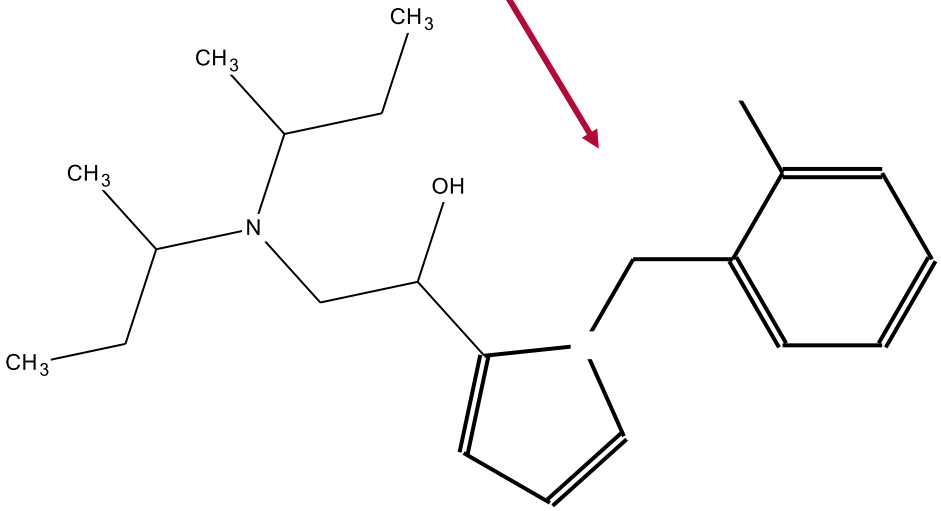
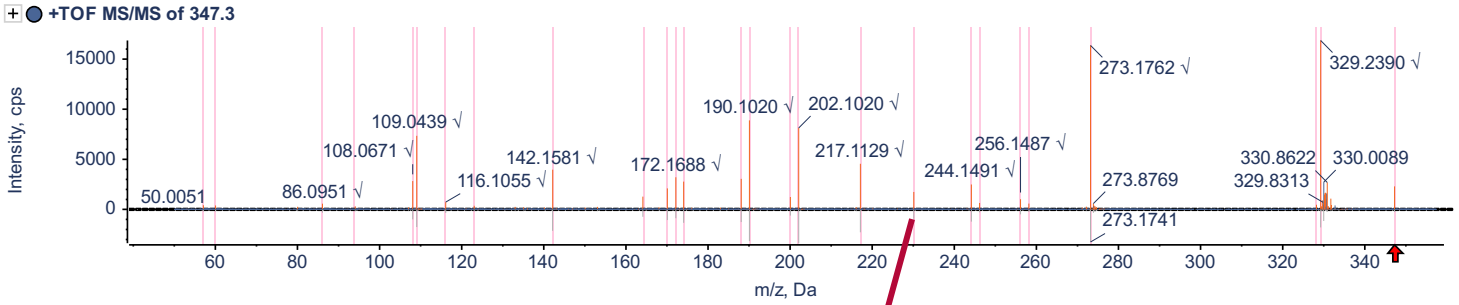
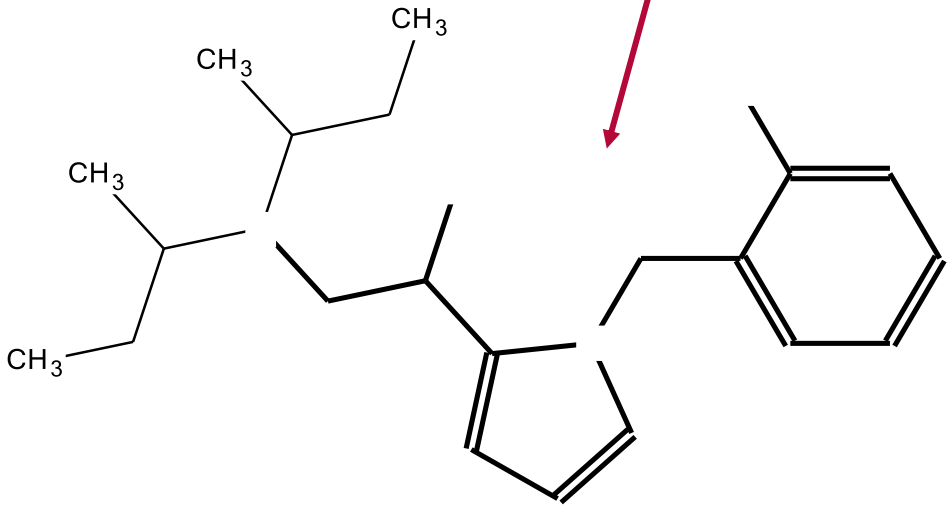




174.0704
$C_{11}H_9FN$

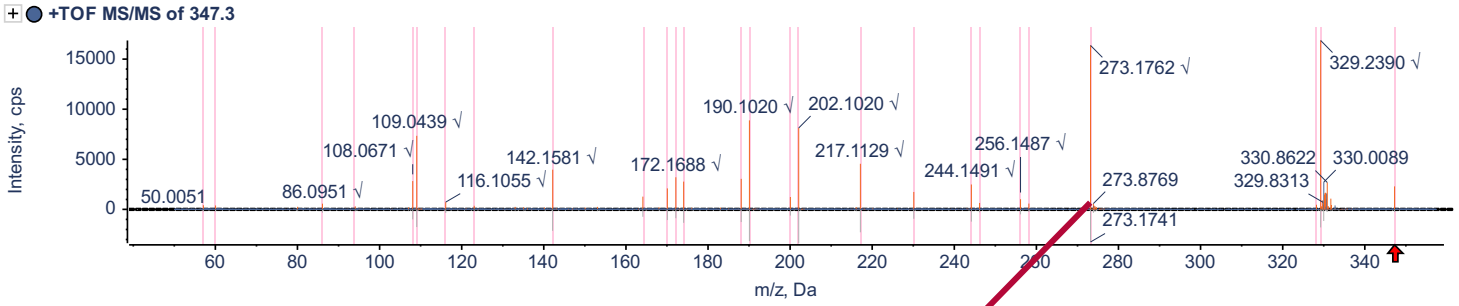
MS/MS Fragmentation

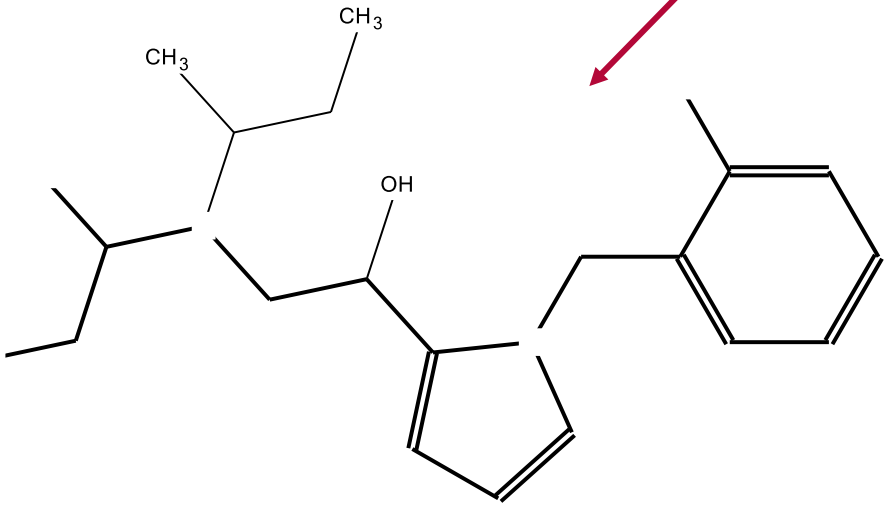




233.1012
$C_{13}H_{14}FN_2O$

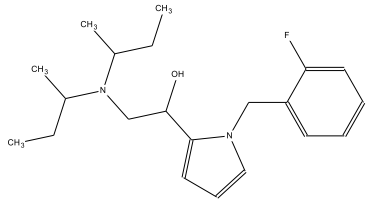
MS/MS Fragmentation





273.1762

$C_{17}H_{22}FN_2$



Results

ID	Biotransformation	RT (min)	Formula	[M+H]$^+$	Error (ppm)	Product Ions
Parent	2F-Viminol	7.15	$C_{21}H_{31}FN_2O$	347.2497	1.2	109.0439 142.1581 174.0704 273.1762
M1	Loss of C_7H_5F	4.72	$C_{14}H_{26}N_2O$	239.2120	1.1	142.193 172.1700
M2	N-dealkylation (sec-butyl)	6.36	$C_{17}H_{23}FN_2O$	291.1872	1.7	109.0435 174.0714 273.1753
M3	N-dealkylation (sec-butyl) + Hydroxylation	4.18	$C_{17}H_{23}FN_2O_2$	307.1824	2.6	57.0685 109.0456 190.0641 289.1688

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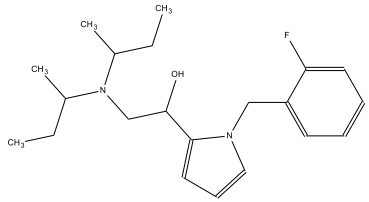
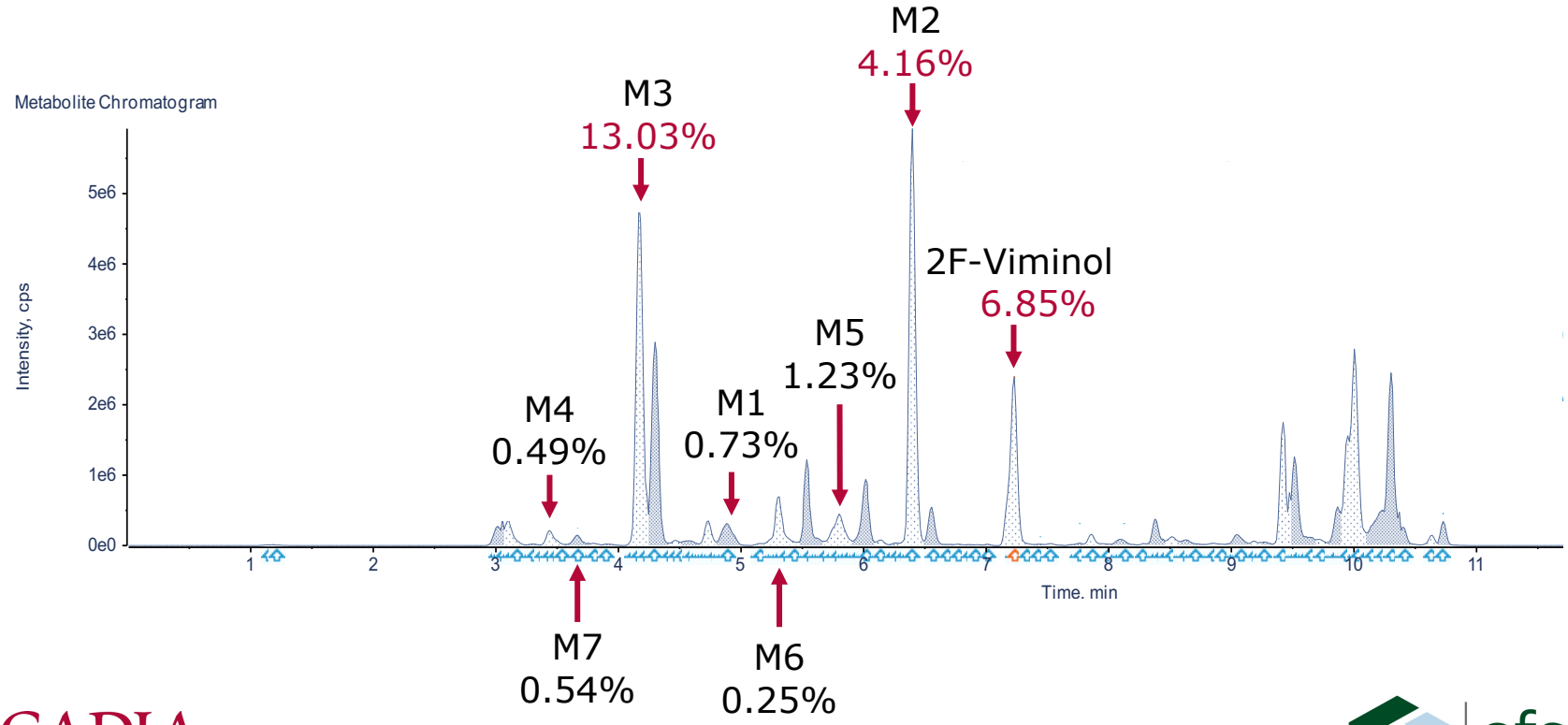


Results

ID	Biotransformation	RT (min)	Formula	$[M+H]^+$	Error (ppm)	Product Ions
M4	N,N-didealkylation (both sec-butyls) + Hydroxylation	3.39	$C_{13}H_{15}FN_2O_2$	251.1193	1.1	109.0434 190.0640
M5	Hydroxylation	5.81	$C_{21}H_{31}FN_2O_2$	363.2444	0.6	109.0447 142.1586 190.0669
M6	Di-hydroxylation	5.29	$C_{21}H_{31}FN_2O_3$	379.2387	-0.72	109.0450 100.0704 156.1384 190.0663
M7	N-dealkylation (sec-butyl) + Di-hydroxylation	3.67 4.12	$C_{17}H_{23}FN_2O_3$	323.1765	0.03	109.0444 190.0699 249.1046

Results



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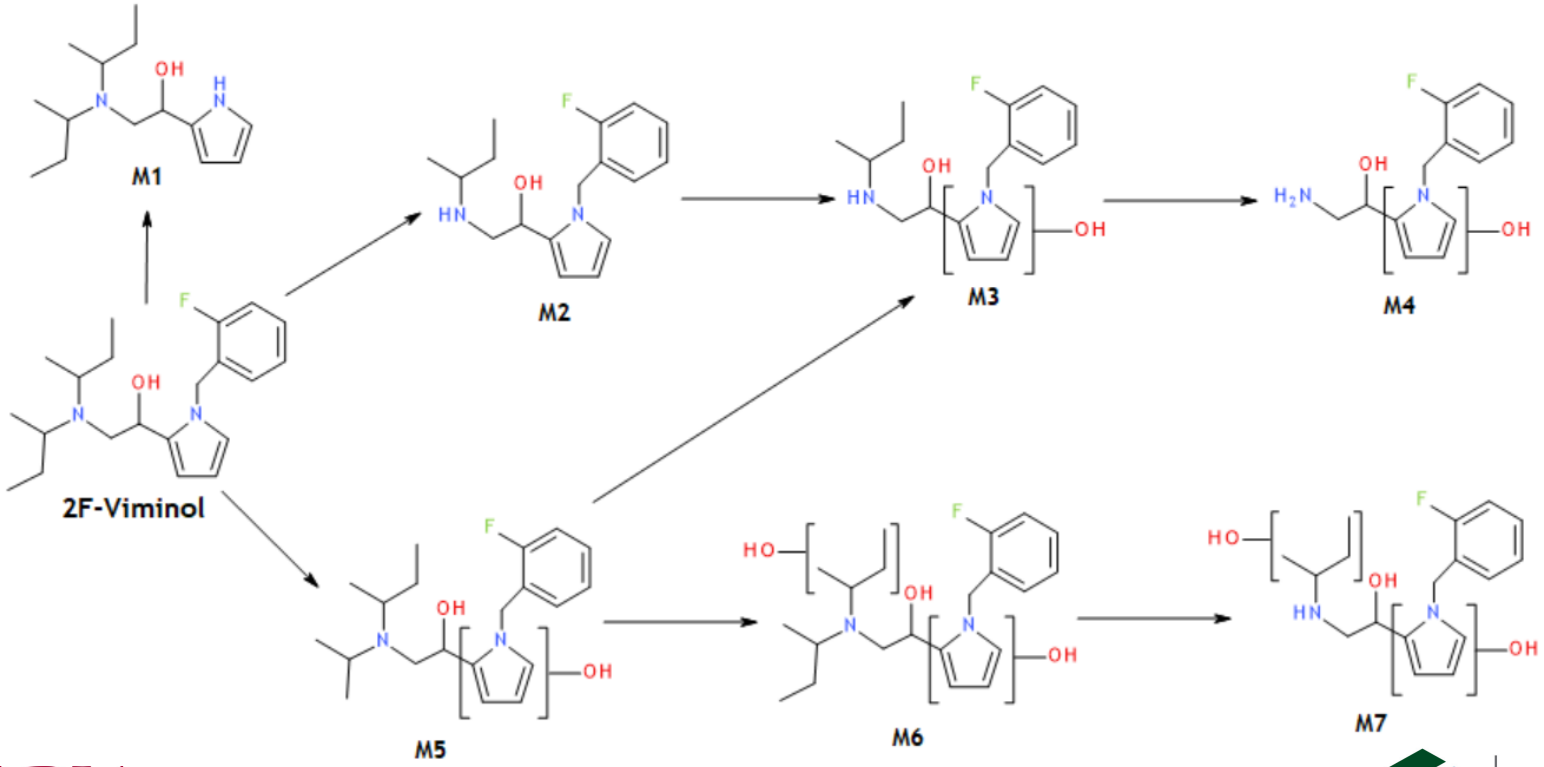
Proposed Metabolic Pathway



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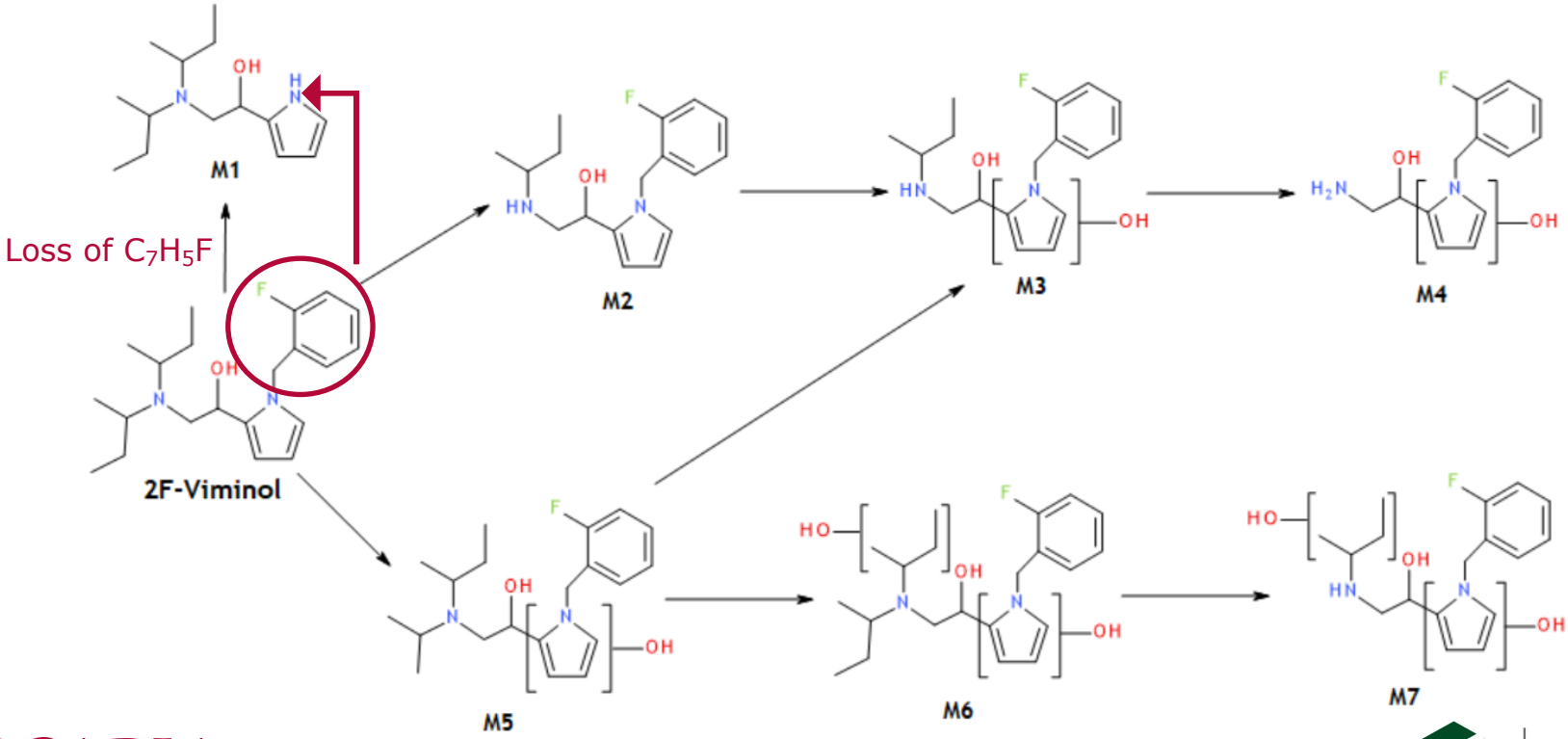

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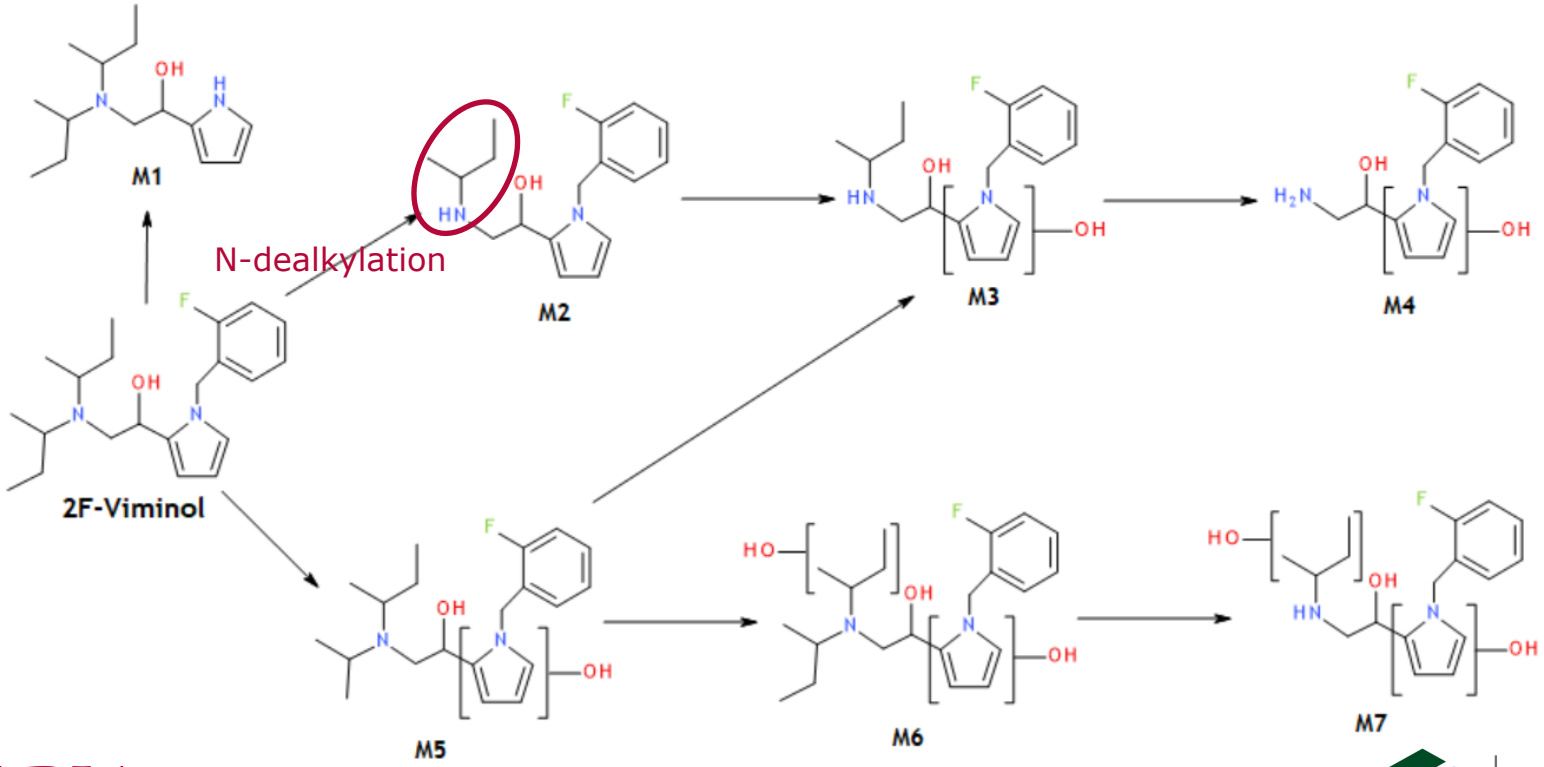
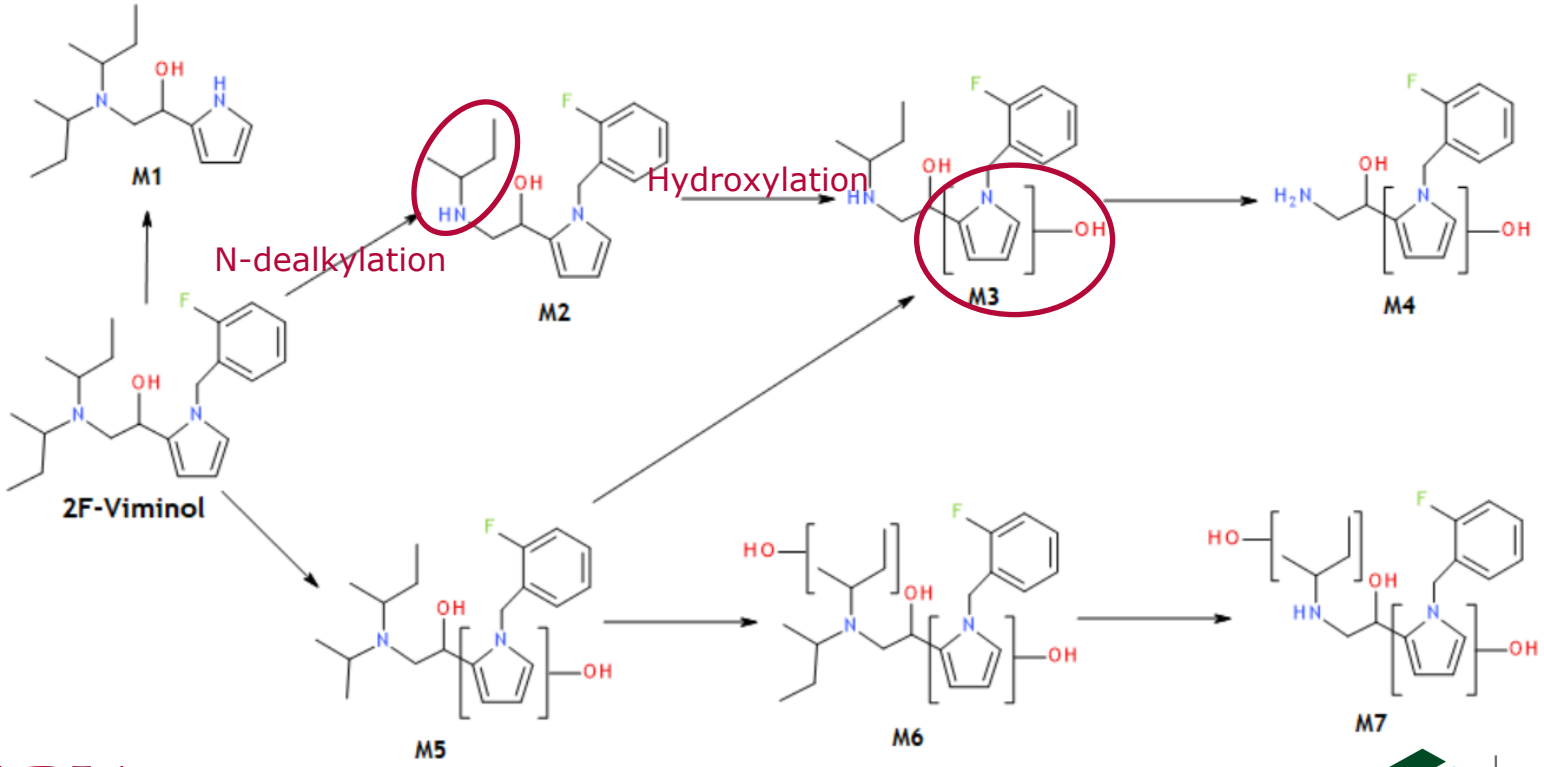


Proposed Metabolic Pathway



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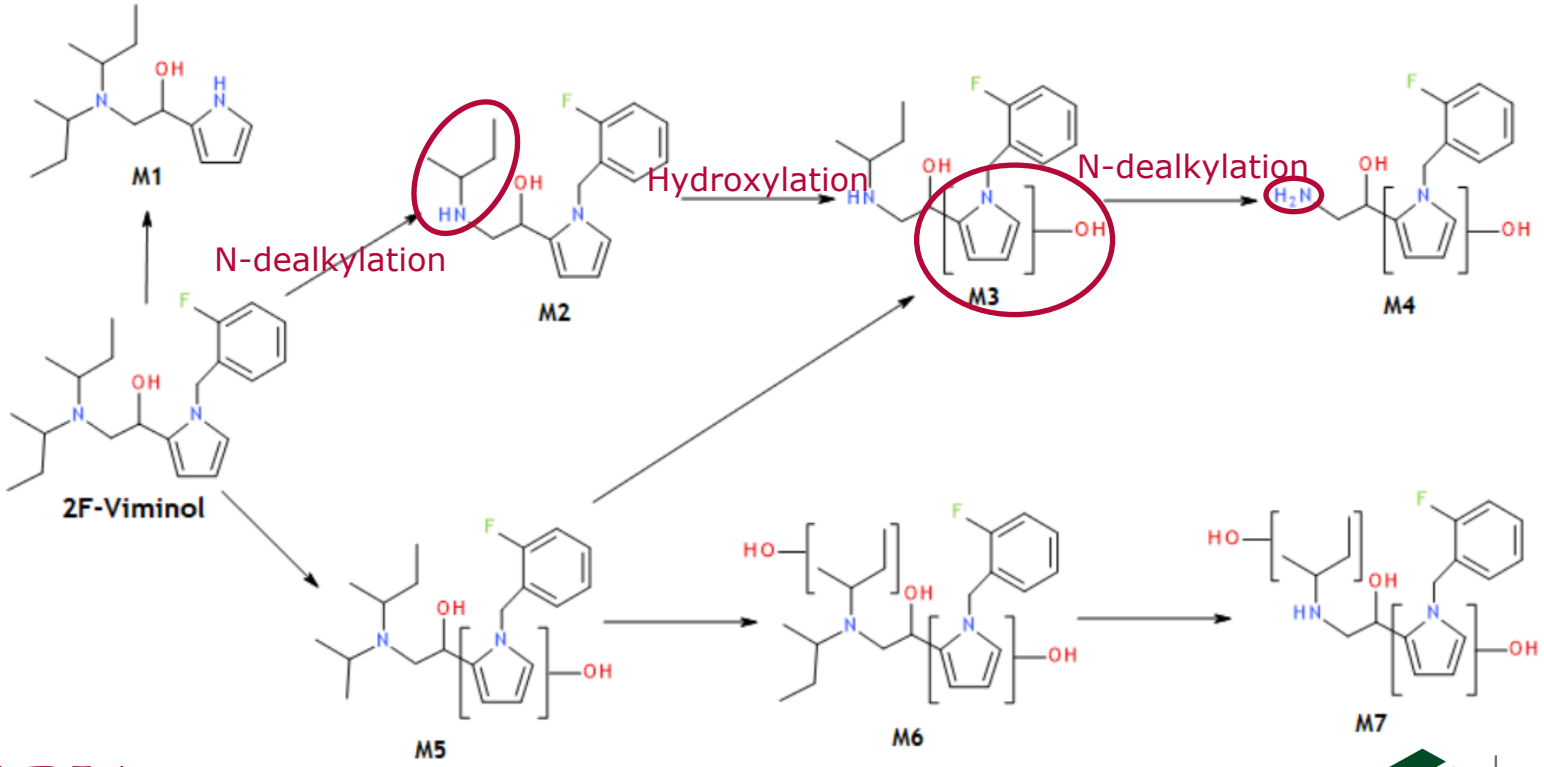

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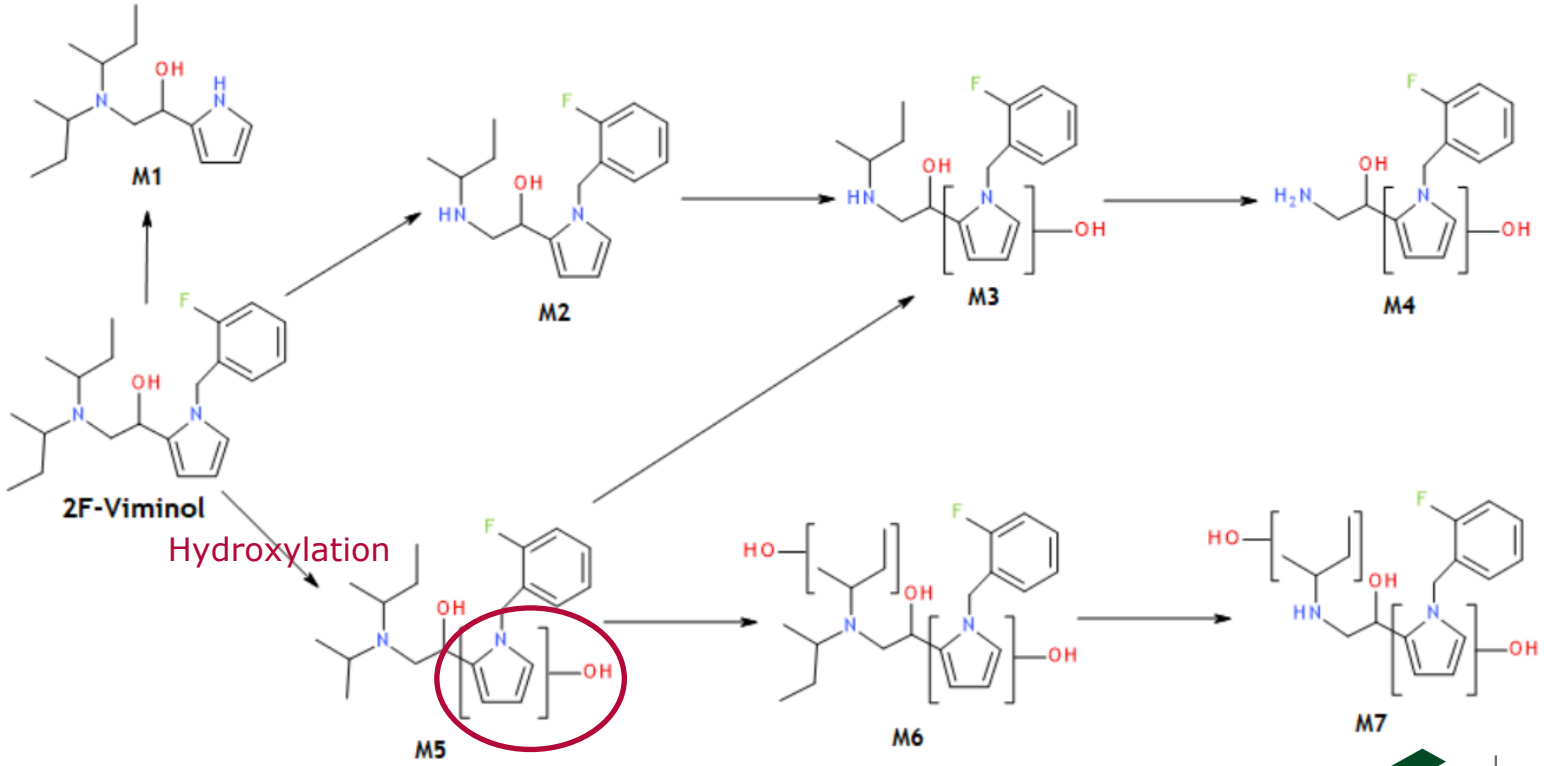

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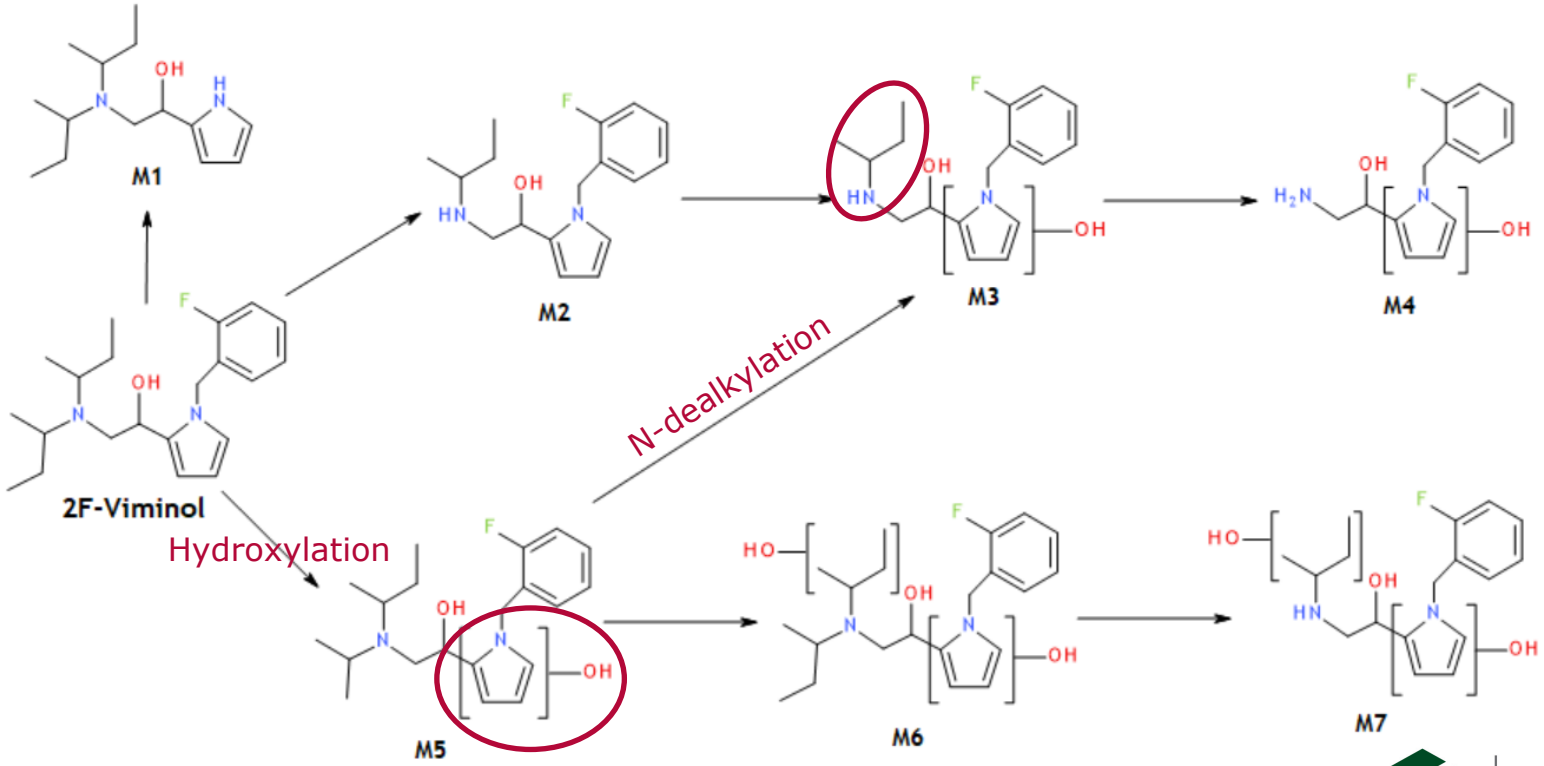

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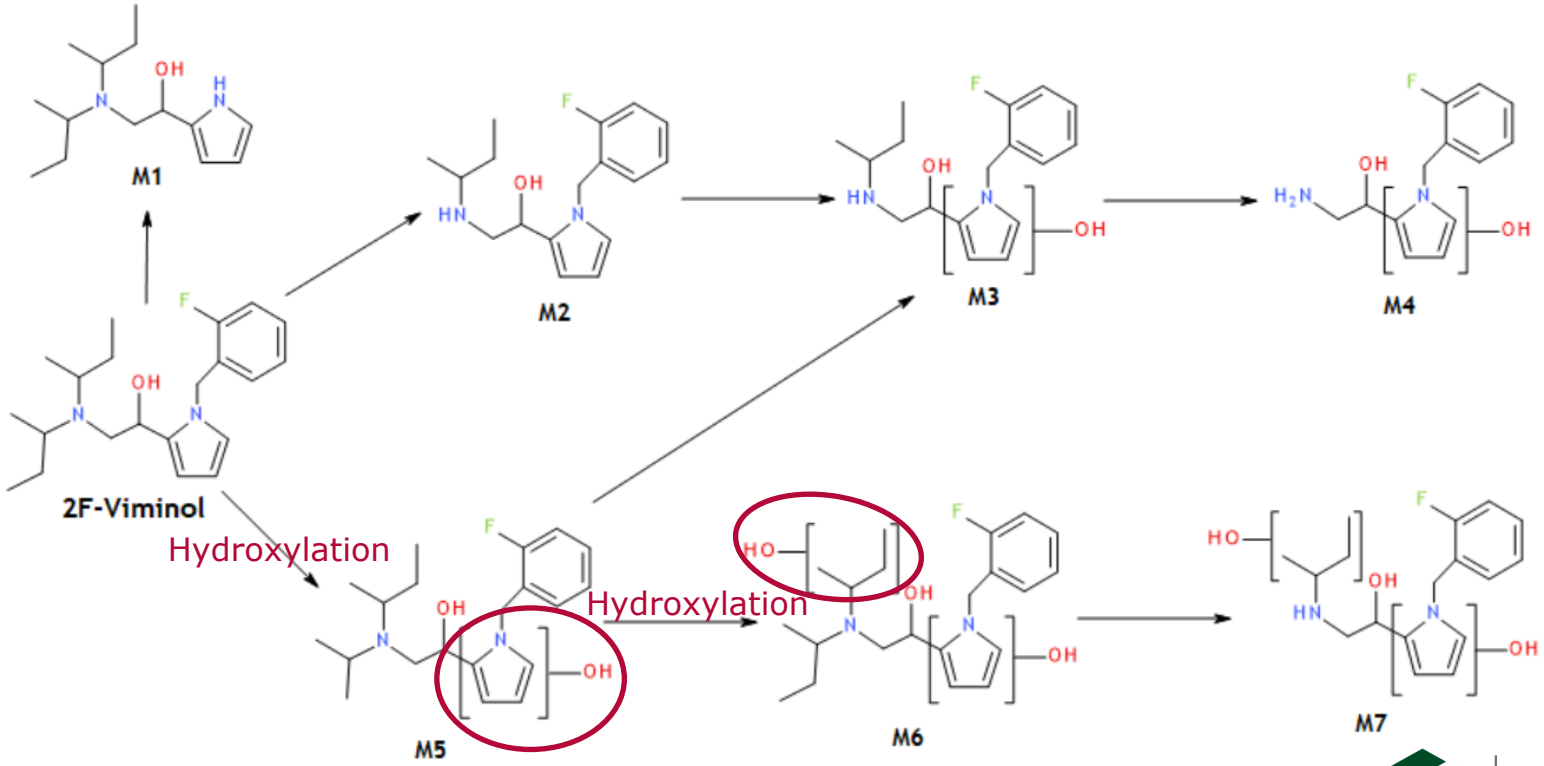

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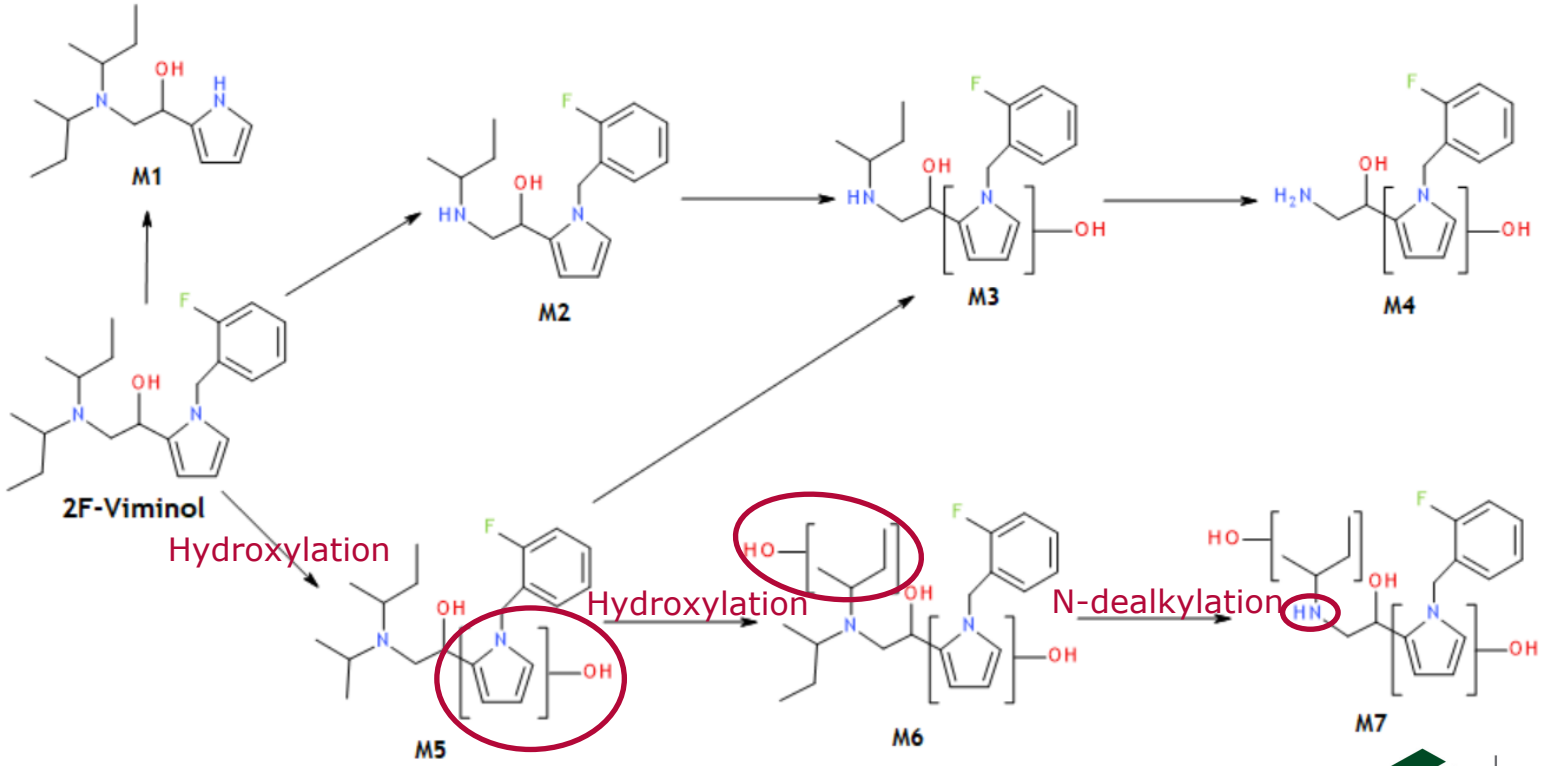
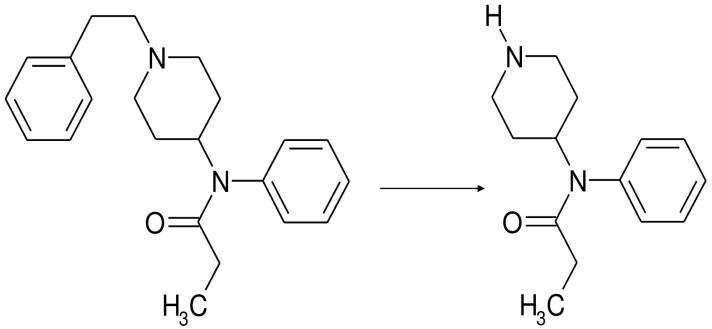


Conclusions

- 7 potential metabolites of 2F-Viminol identified
  - Main biotransformation pathways: hydroxylation and N-dealkylation
- Hydroxylation and N-dealkylation are commonly seen as metabolic pathways in opioids
  - N-dealkylation of fentanyl → norfentanyl
  - Hydroxylation of brorphine



Fentanyl Norfentanyl

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Future Work

- Other isomers
- Development of an extraction method
  - Determine stability of 2F-Viminol in biological specimens
  - Authentic sample analysis
  - Relatively quantify metabolites

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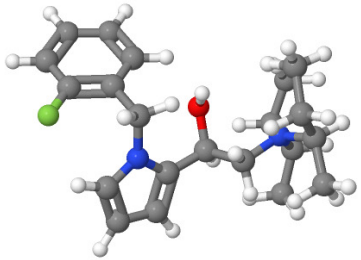
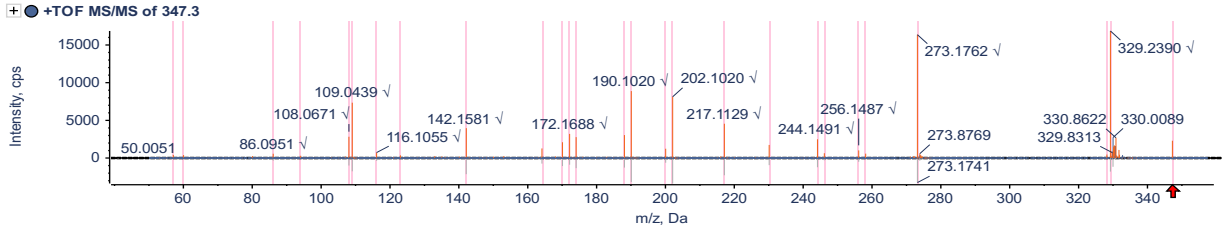
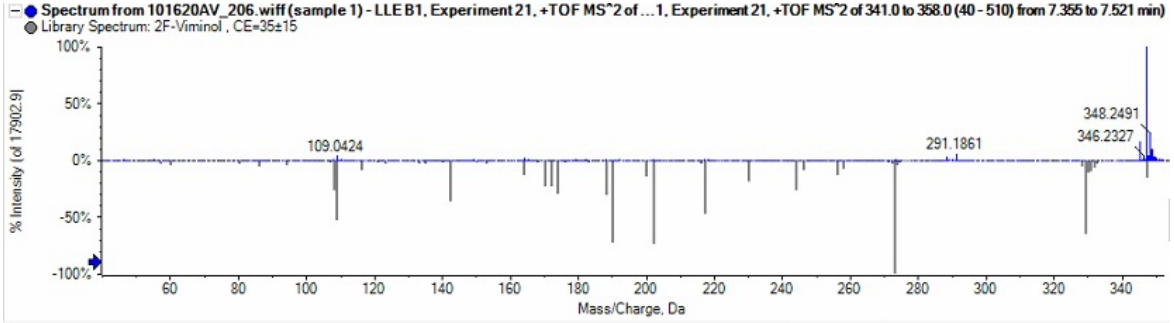
Future Work



2F-Viminol MS/MS Spectrum:



2F-Viminol Extracted from Blood:



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Acknowledgements

- Sara Walton and the staff at the CFSRE
- My class of 2021 cohort

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Thank you for watching!

Any questions?

avelez@arcadia.edu