Temperature and pH-dependent Stability: Identifying Fentalog Degradation Pathways

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Introduction

- Fentanyl Analogs
  - Synthetic Opioids
  - μ (mu) receptor agonists
  - Cause severe respiratory depression
- Sub ng/mL concentrations
  - Higher potencies
  - Loss of parent analyte to metabolism
- Fentanyl pKa = 8.4
  - pK_as range from 7.5 (alfentanil) - 9.1 ((+)-Cis-3-methylfentanyl)

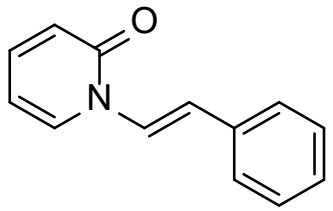
Fentalog Stability

Author	Year	Matrix	Fentalogs*	Conditions	Stability
Guerrieri	2017	blood	furanylfentanyl	RT (48 h), 4°C (7 days)	Stable
Fogarty	2018	blood	fentanyl, norfentanyl, furanylfentanyl, butyrylfentanyl, 4-ANPP, p-FIBF, isobutyrylfentanyl, o/p-fluorofentanyl, carfentanil,	-80°C, 20°C (30 days)	Stable except NF
Kahl	2018	blood	β-hydroxythiofentanyl, acetylfentanyl, furanylfentanyl, carfentanil, butyrylfentanyl, p-FIBF	4°C (9 mo)	Stable
Moody	2018	blood, serum, urine	β-hydroxythiofentanyl, 4-ANPP, butyrylfentanyl, valerylfentanyl, o/p-fluorofentanyl	RT, refrig, frozen (30 days)	Stable 2 wks except Acryl
Nan	2019	blood	fentanyl, alfentanil, acetylfentanyl, 4-ANPP, butyrylfentanyl, furanylfentanyl, p-FBF, o/p-fluorofentanyl, p-FIBF, β-hydroxythiofentanyl, remifentanil, carfentanil, sufentanil, valerylfentanyl, norcarfentanil, norfentanyl	-20°C (1 mo) FT x3 (-20->25°C)	Stable
Jung	2020	blood, urine	alfentanil, carfentanil, 4-ANPP, fentanyl, furanylfentanyl, norfentanyl, remifentanil, sufentanil, valerylfentanyl	4°C, -20°C (30 days)	Stable except Remi (5 days, 4°C)
Palmquist	2021	blood	3-methylfentanyl, 4-ANPP, p-FIBF, acetylfentanyl, butyrylfentanyl, carfentanil, fentanyl, furanylfentanyl, p-fluorofentanyl, valerylfentanyl	-20°C, 4°C, 25°C, 35°C (9 mo)	RT, 4°C stable except acryl 35°C - degradation all analytes

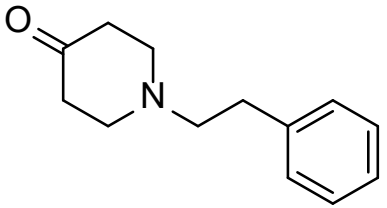
*Fentalogs also included in this study

Forced Degradation of Fentanyl

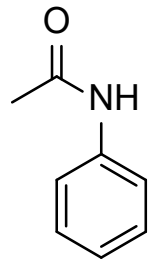
- Lambropoulos et al. 1999
  - 0.5 N HCl & NaOH, 24 hr, 80°C
    - Acidic → 4-ANPP
    - Basic → three products + 4-ANPP
- Garg et al. 2007
  - 5 N HCl & NaOH, 24 hr
    - Acidic → 4-ANPP
    - Basic → no degradation
  - Thermal, 350°C 5 min
    - Nor, 1-PPO, 1-PEP, 1-SPO, N-phenylpropanamide
- Ciesielski et al. 2022
  - Headspace analysis
  - 125°C, 72 h
    - NPP, 4-ANPP, 1-P-4-POP, acetylfentanyl, N-phenylpropanamide, N-phenylacetamide



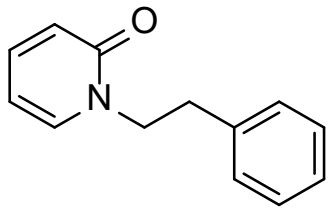
1-SPO



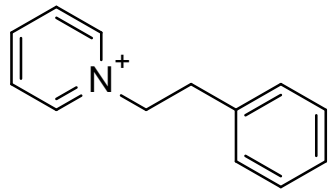
NPP



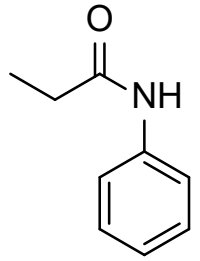
N-phenylacetamide



1-PPO



1-PEP



N-phenylpropanamide

Urinary pH

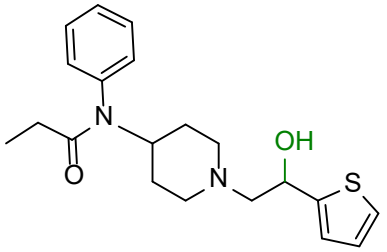
- Urine pH ~4-8
  - Diet, medication, overall health
- Acceptance Criteria: pH 4-9
  - Mandatory Guidelines for Federal Workplace Drug Testing (2017)

- Fura et al. 2003
  - Rat urine pH shift
  - Incubation at 37°C and RT
  - ↑ pH over time, pH >8.5 by 24 hr
- Cook et al. 2007
  - -20°C relatively stable
  - 4°C pH >8 (14 days)
  - ≥ RT resulted in pH >9 (1 day)

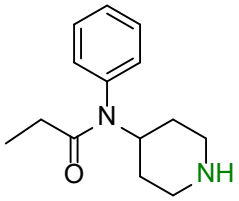
Urine is susceptible to pH changes driven by temperature which can in turn affect the stability of pH labile analytes.

Fentalogs

phenethyl

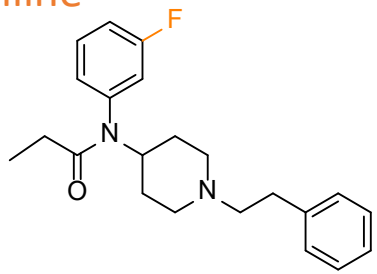


β-Hydroxythiofentanyl

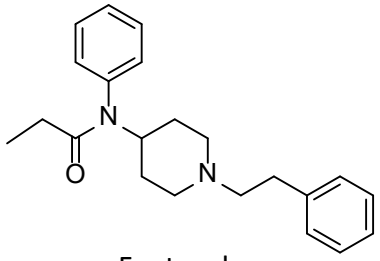


Norfentanyl

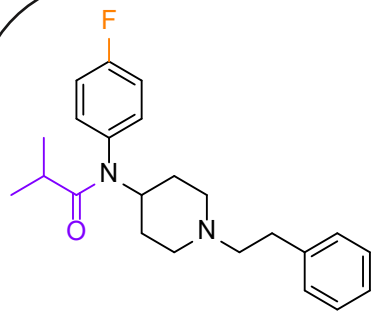
aniline



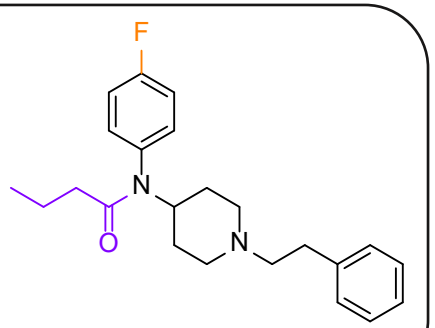
o-Fluorofentanyl



Fentanyl

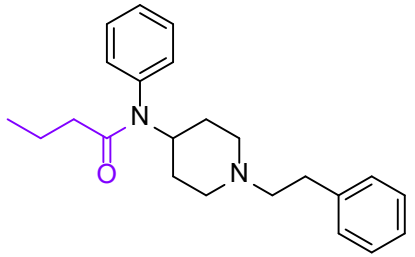


p-Fluoroisobutyrylfentanyl

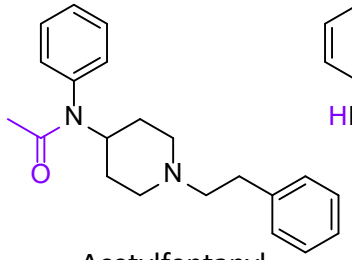


p-Fluorobutyrylfentanyl

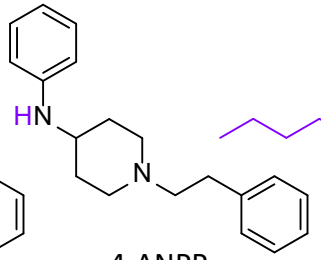
amide



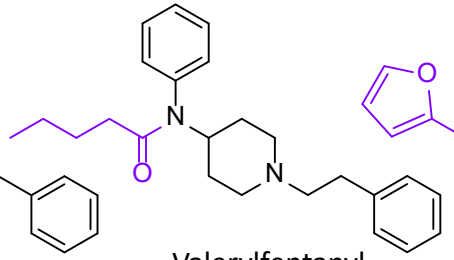
Butyrylfentanyl



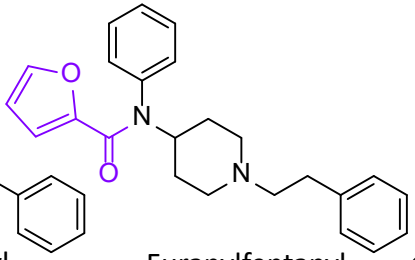
Acetylfentanyl



4-ANPP

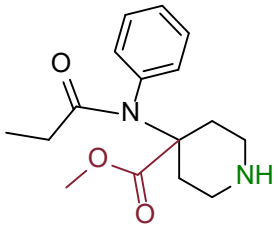


Valerylfentanyl

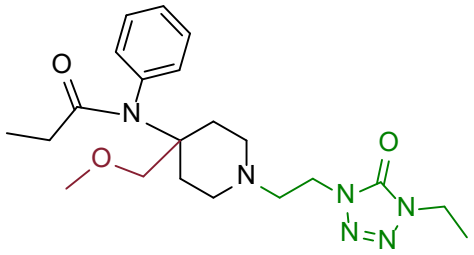


Furanylfentanyl

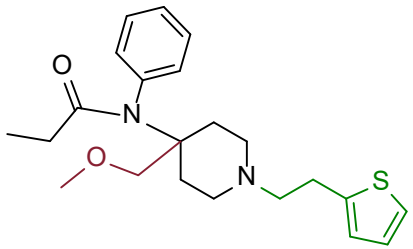
piperidine



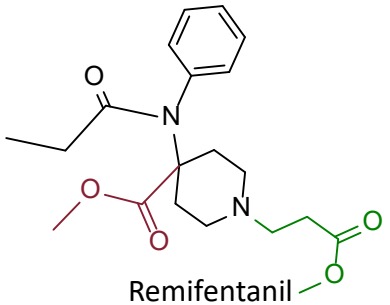
Norcarfentanil



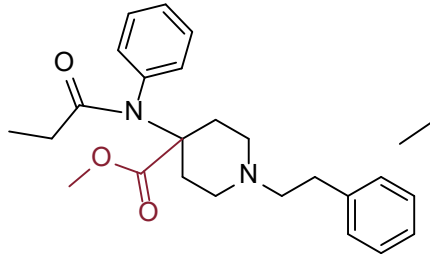
Alfentanil



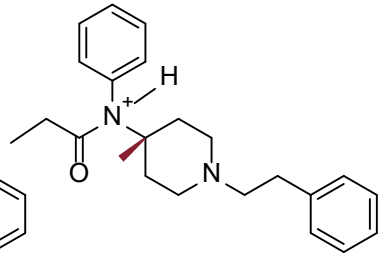
Sufentanil



Remifentanil



Carfentanil



(+)-Cis-3-methylfentanyl

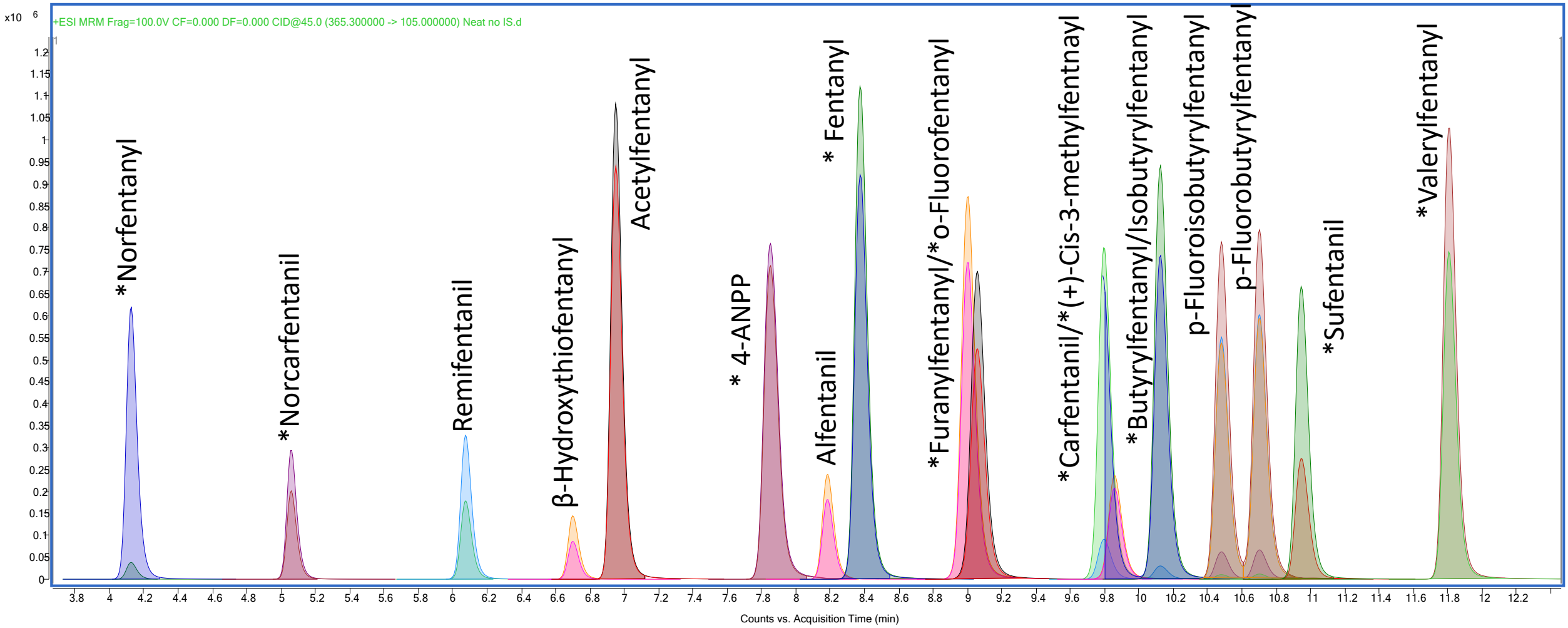
Experimental Conditions - LC

- Agilent Technologies 1290 Infinity Binary LC System
  - 6470 Triple Quadrupole Mass Spectrometer
  - 6530 Accurate-Mass Quadrupole-Time of Flight Mass Spectrometer
- Separation
  - Poroshell EC-C18 column (2.1 x 100 mm, 2.7 um)
  - Poroshell EC-C18 guard (2.1 x 5 mm, 2.7 um)
- Injection Volume:
  - 1 µL (QQQ)
  - 5 µL (QTOF)
- Validated to ANSI/ASB 036

Time	%A	%B
0	90	10
1	90	10
7	74	26
8	74	26
11.1	65	35
12.4	60	40
13.4	10	90
14	90	10

Post time: 1 min Flow: 0.4 mL/min
A: 0.1% FA diH_2O B: 0.1% FA ACN

Chromatography



Experimental Conditions - MS

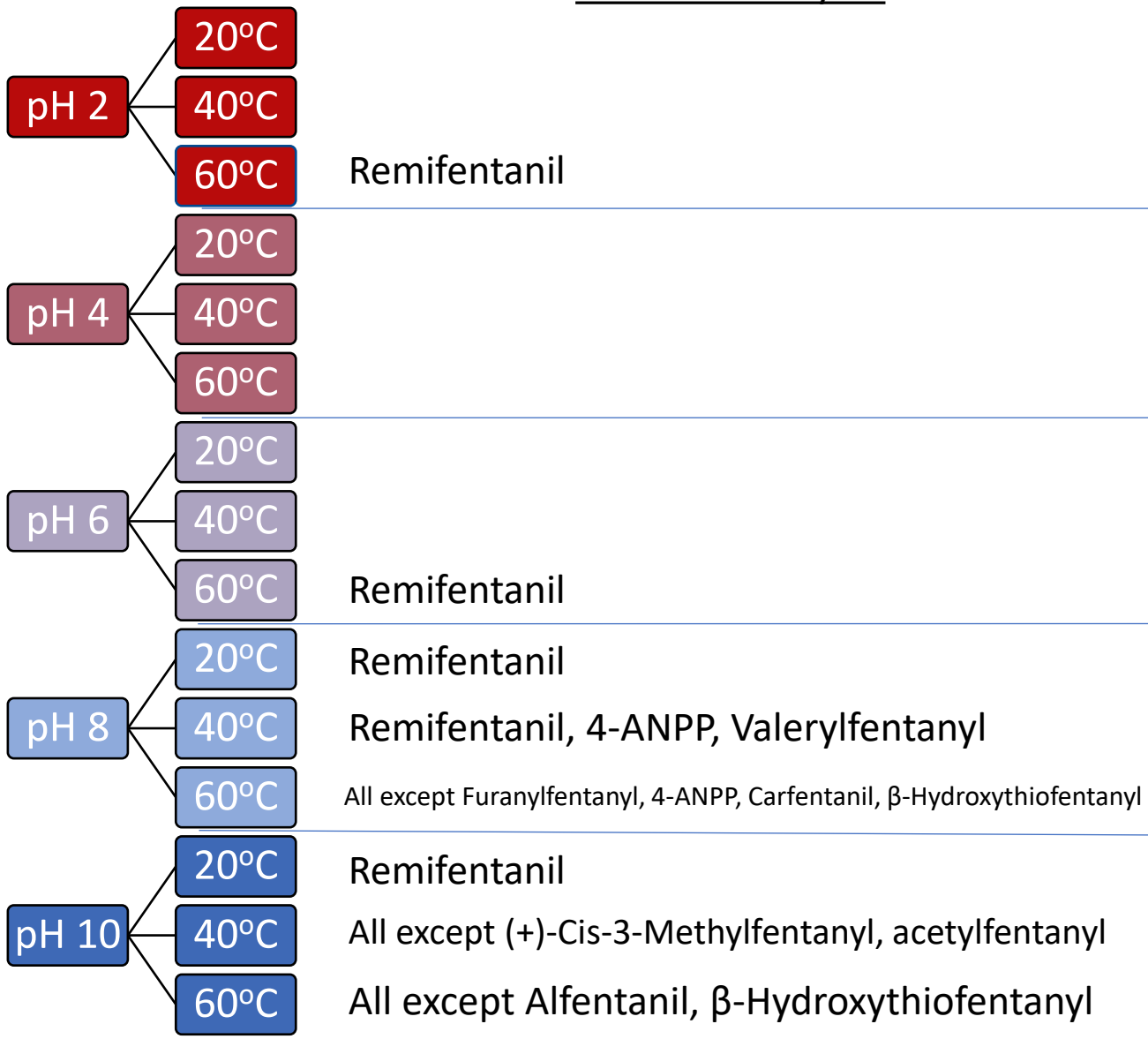
- Agilent Technologies 1290 Infinity Binary LC System
  - 6470 Triple Quadrupole Mass Spectrometer
  - 6530 Accurate-Mass Quadrupole-Time of Flight Mass Spectrometer
- Acquisition – QQQ
  - Dynamic Multiple Reaction Monitoring
- Acquisition - QTOF
  - Auto MS/MS
  - Preferred list (precursor ions)
  - CE 0, 10, 20, 30 V
  - ESI Mode: Positive

	Scan Rate	Scan Range
MS	3	40-1000
MS/MS	8	40-500

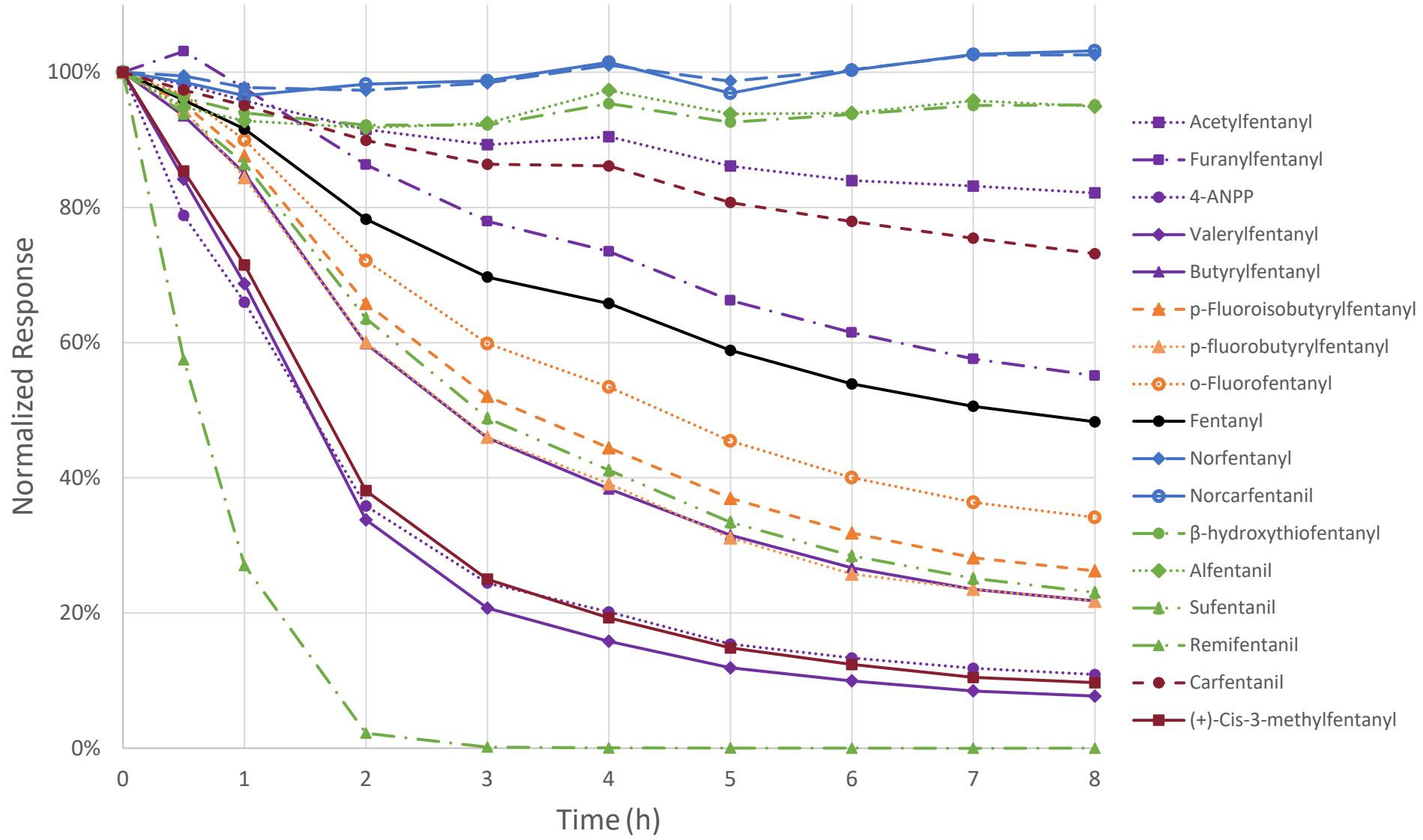
Parameter	Value
Gas Temp (°C)	350
Gas Flow (l/min)	9
Nebulizer (psi)	35
Sheath Gas Temp	290
Sheath Gas Flow	9
Capillary	3000

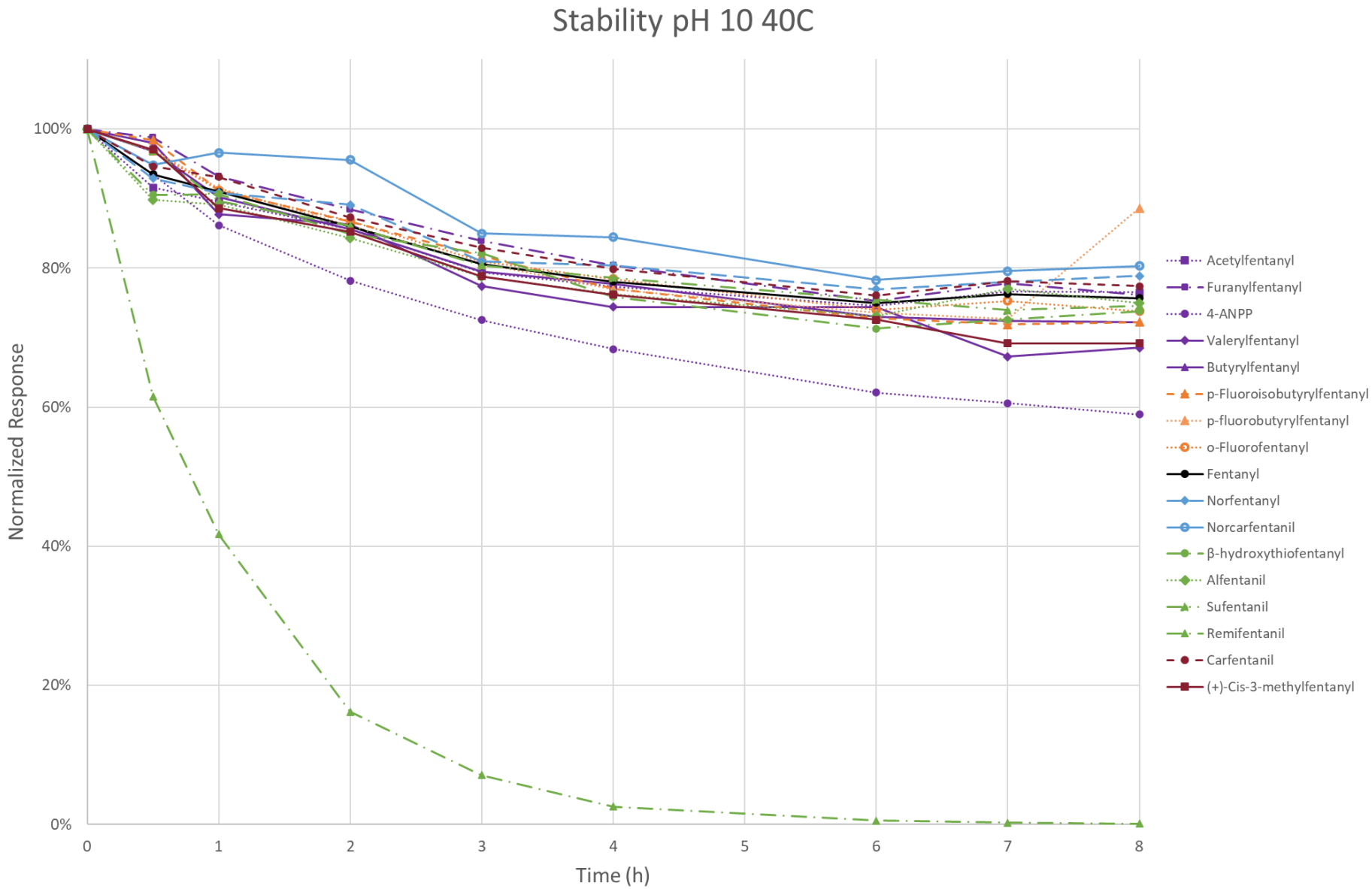
Accelerated Stability Study

- Buffers:
  - pH 2: 10 mM HCl
  - pH 4: 10 mM Ammonium Acetate
  - pH 6: 10 mM Ammonium Formate
  - pH 8 & 10: 10 mM Ammonium Bicarbonate
- Temperature
  - 20, 40, 60°C
- Time
  - t0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 24 h



Stability pH 10 60C

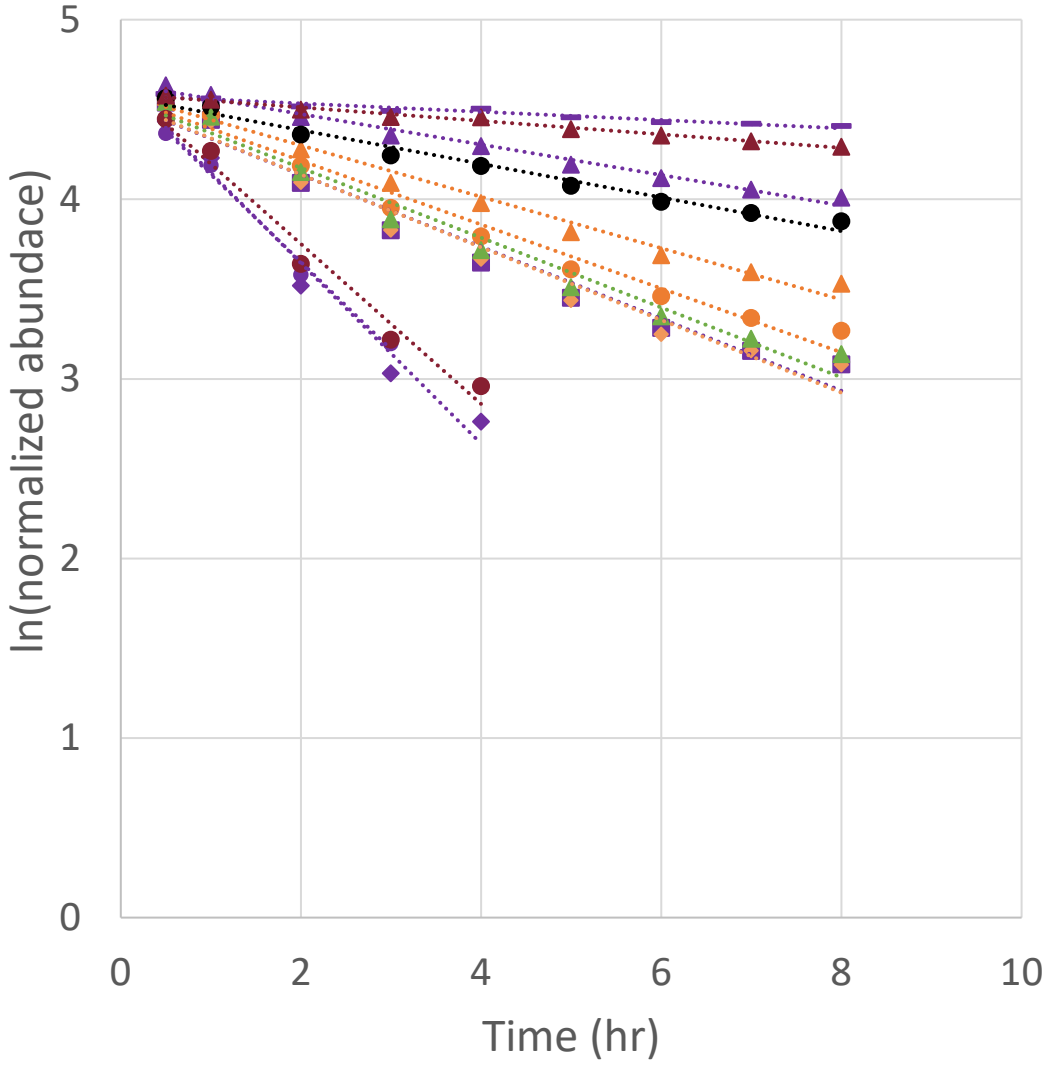



Stability pH 10 40C
Normalized Response
100%
80%
60%
40%
20%
0%
0
1
2
3
4
5
6
7
8
Time (h)
Acetylfentanyl
Furanylfentanyl
4-ANPP
Valerylfentanyl
Butyrylfentanyl
p-Fluoroisobutyrylfentanyl
p-fluorobutyrylfentanyl
o-Fluorofentanyl
Fentanyl
Norfentanyl
Norcarfentanil
β-hydroxythiofentanyl
Alfentanil
Sufentanil
Remifentanil
Carfentanil
(+)-Cis-3-methylfentanyl

Stability >20% Loss (h)

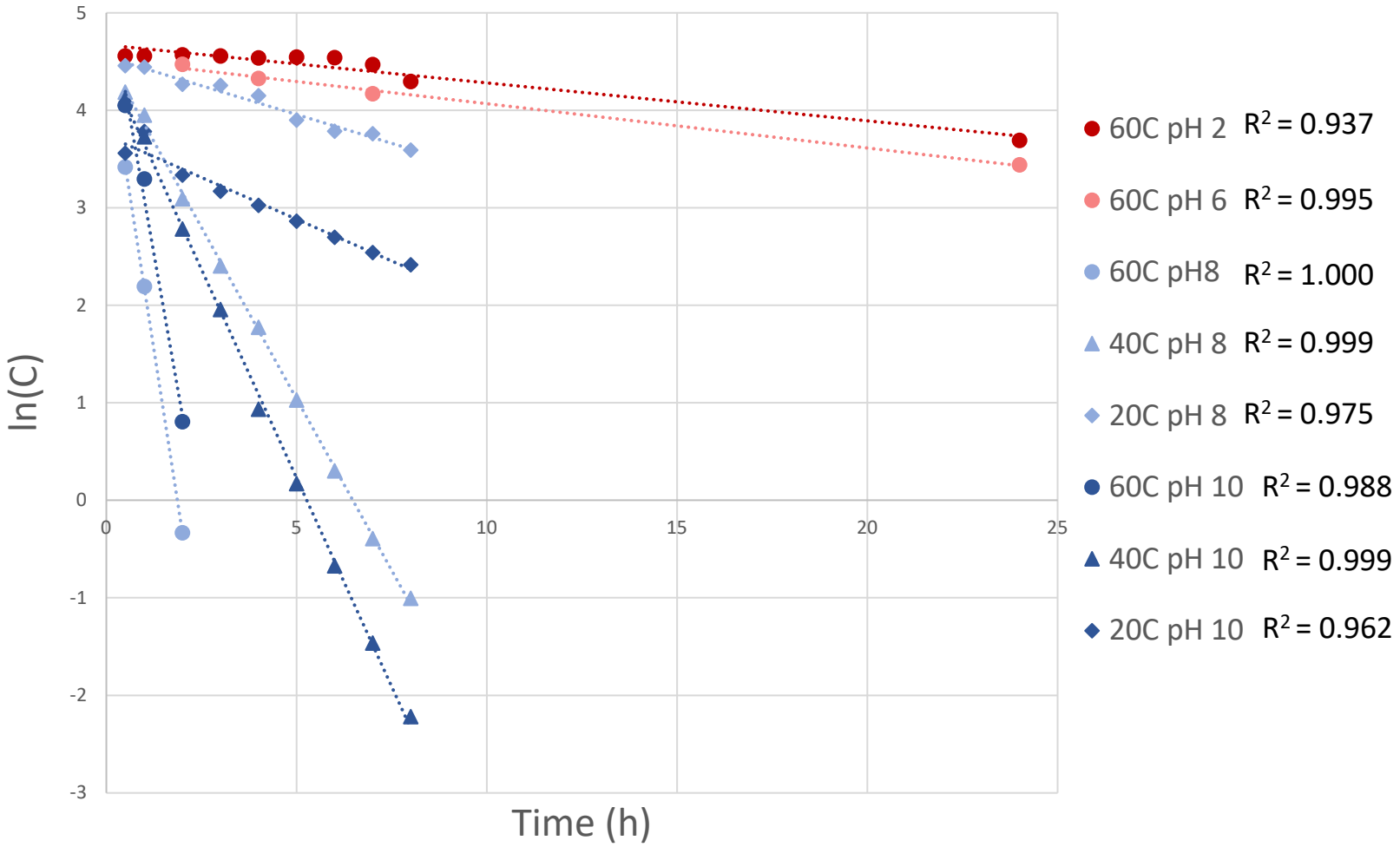
	pH 10			pH 8			pH 6			pH 4			pH 2		
	20	40	60	20	40	60	20	40	60	20	40	60	20	40	60
Fentanyl	--	4	2	--	--	20	--	--	--	--	--	--	--	--	--
Acetylfentanyl	--	--	10	--	--	2	--	--	--	--	--	--	--	--	--
Butyrylfentanyl	--	3	2	--	--	6	--	--	--	--	--	--	--	--	--
Furanylfentanyl	--	6	3	--	--	--	--	--	--	--	--	--	--	--	--
Valerylfentanyl	--	3	1	--	17	2	--	--	--	--	--	--	--	--	--
4-ANPP	--	2	0.5	--	20	--	--	--	--	--	--	--	--	--	--
Remifentanil	0.5	0.5	0.5	2	0.5	0.5	--	--	4	--	--	--	--	--	16.5
Sufentanil	--	4	2	--	--	6	--	--	--	--	--	--	--	--	--
Alfentanil	--	3	--	--	--	6	--	--	--	--	--	--	--	--	--
β-hydroxythiofentanyl	--	4	--	--	--	--	--	--	--	--	--	--	--	--	--
Carfentanil	--	4	2	--	--	--	--	--	--	--	--	--	--	--	--
(+)-Cis-3-methylfentanyl	--	--	1	--	--	--	--	--	--	--	--	--	--	--	--
o-Fluorofentanyl	--	4	2	--	--	2	--	--	--	--	--	--	--	--	--
p-fluorobutyrylfentanyl	--	4	2	--	--	6	--	--	--	--	--	--	--	--	--
p-Fluoroisobutyrylfentanyl	--	4	2	--	--	6	--	--	--	--	--	--	--	--	--
Norfentanyl	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Norcarfentanil	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

pH 10 60°C Rate Plot



- Acetylfentanyl $R^2 = 0.946$
- Furanylfentanyl $R^2 = 0.985$
- 4-ANPP $R^2 = 0.990$
- Valerylfentanyl $R^2 = 0.976$
- Butyrylfentanyl $R^2 = 0.968$
- p-Fluoroisobutyrylfentanyl $R^2 = 0.975$
- p-fluorobutyrylfentanyl $R^2 = 0.967$
- o-Fluorofentanyl $R^2 = 0.979$
- Fentanyl $R^2 = 0.980$
- Sufentanil $R^2 = 0975$
- Carfentanil $R^2 = 0.987$
- (+)-Cis-3-methylfentanyl $R^2 = 0.978$

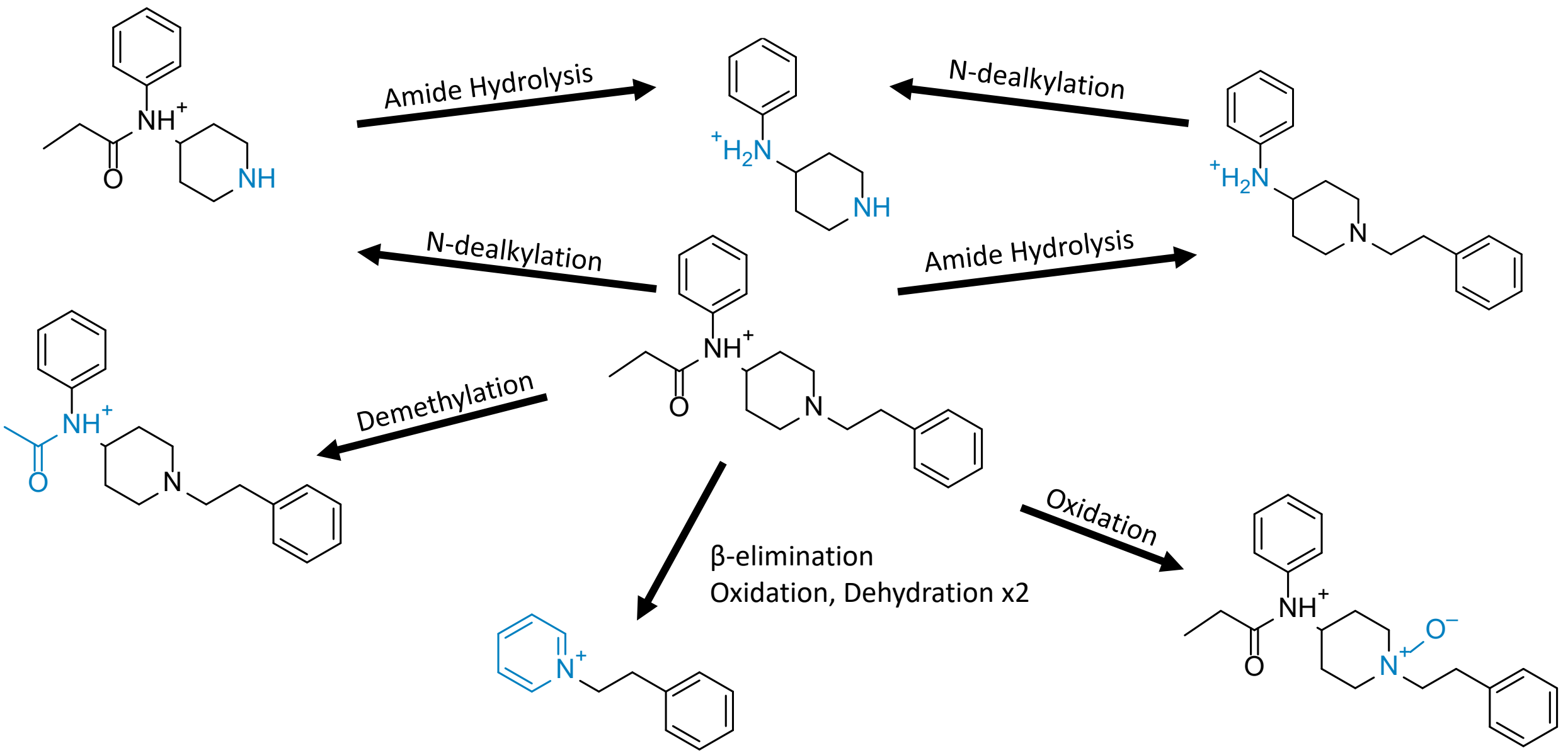
Remifentanil Rate Plot



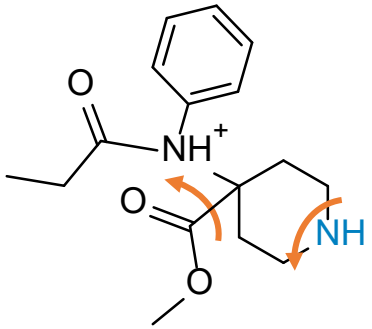
Half-life $t_{1/2}$ (h)

	pH 10			pH 8			pH 6			pH 4			pH 2		
	20	40	60	20	40	60	20	40	60	20	40	60	20	40	60
Fentanyl	--	17	7	--	--	90	--	--	--	--	--	--	--	--	--
Acetylfentanyl	--	--	30	--	--	--	--	--	--	--	--	--	--	--	--
Butyrylfentanyl	--	9	4	--	--	20	--	--	--	--	--	--	--	--	--
Furanylfentanyl	--	15	8	--	--	--	--	--	--	--	--	--	--	--	--
Valerylfentanyl	--	14	1	--	--	9	--	--	--	--	--	--	--	--	--
4-ANPP	--	10	1	--	--	11	--	--	--	--	--	--	--	--	--
Remifentanil	--	0.8	0.3	4	1	0.3	--	--	15	--	--	--	--	--	18
Sufentanil	--	16	4	--	--	22	--	--	--	--	--	--	--	--	--
Alfentanil	--	17	--	--	--	--	--	--	--	--	--	--	--	--	--
Beta hydroxythiofentanyl	--	15	--	--	--	--	--	--	--	--	--	--	--	--	--
Carfentanil	--	17	18	--	--	--	--	--	--	--	--	--	--	--	--
(+)-Cis-3-methylfentanyl	--	14	2	--	--	13	--	--	--	--	--	--	--	--	--
o-Fluorofentanyl	--	15	9	--	--	--	--	--	--	--	--	--	--	--	--
p-fluorobutyrylfentanyl	--	13	3	--	--	23	--	--	--	--	--	--	--	--	--
p-Fluoroisobutyrylfentanyl	--	14	4	--	--	26	--	--	--	--	--	--	--	--	--
Norfentanyl	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Norcarfentanil	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

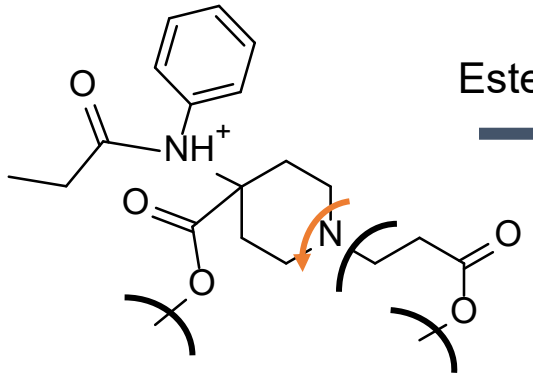
Breakdown Products



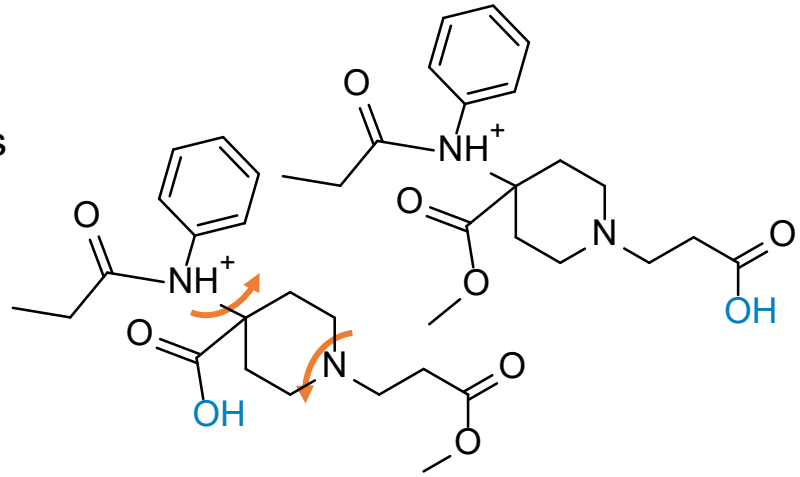
Remifentanil



N-dealkylation



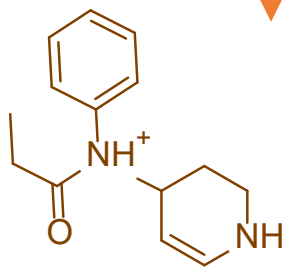
Ester Hydrolysis

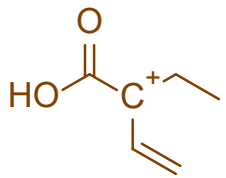


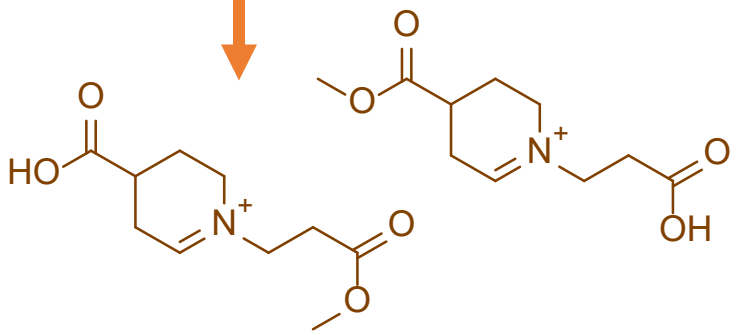
$C_{16}H_{23}N_2O_3^+$ 291.1691 *m/z*

$C_{20}H_{29}N_2O_5^+$ [M+H] 377.2071 *m/z*

$C_{19}H_{27}N_2O_5^+$ 363.1915 *m/z*





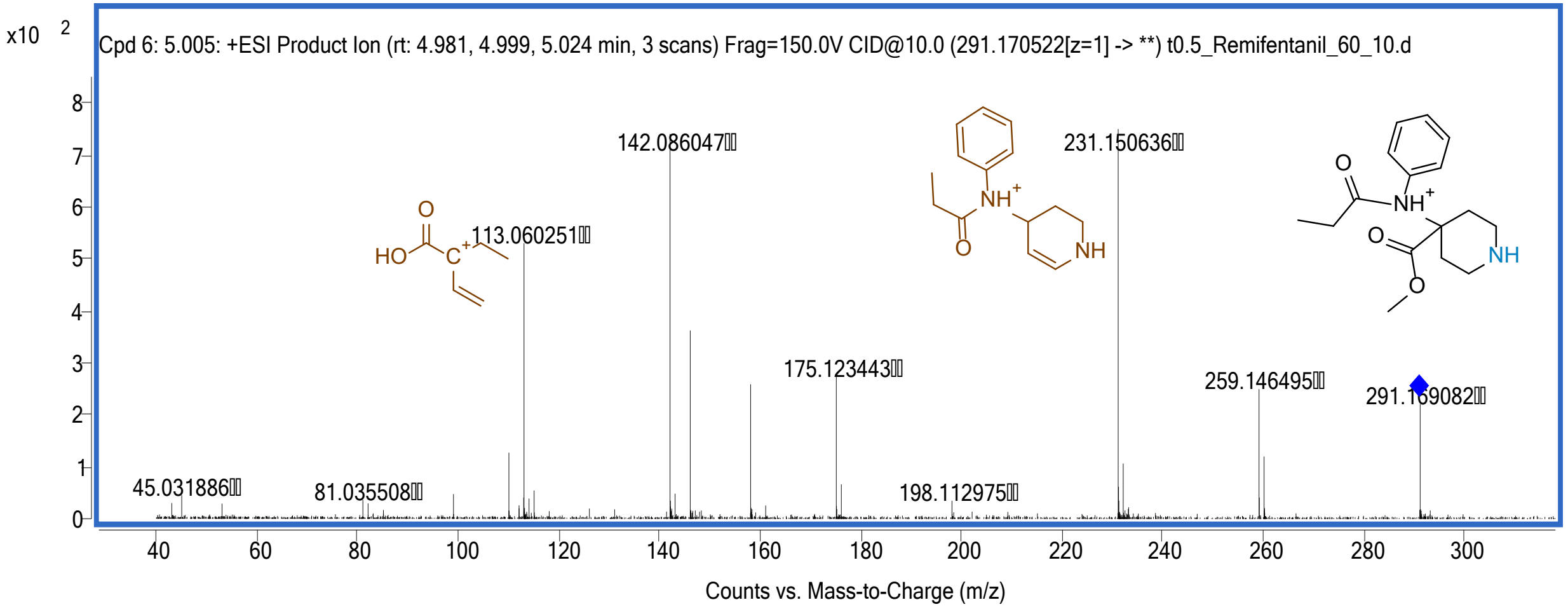


$C_{14}H_{20}N_2O^+$ 231.1507 *m/z*

$C_8H_9O_2^+$ 113.0603 *m/z*

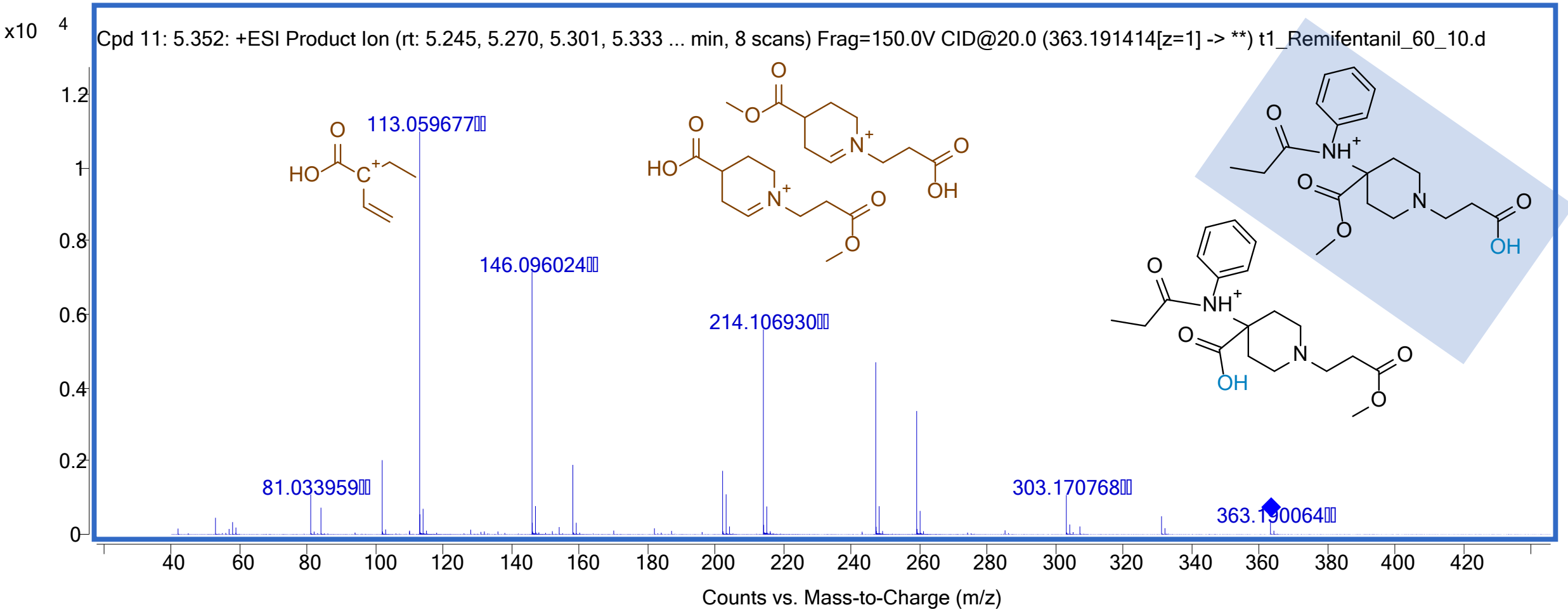
$C_{10}H_{16}NO_4^+$ 214.1079 *m/z*

Remifentanil N-Dealkylation (291 *m/z*)



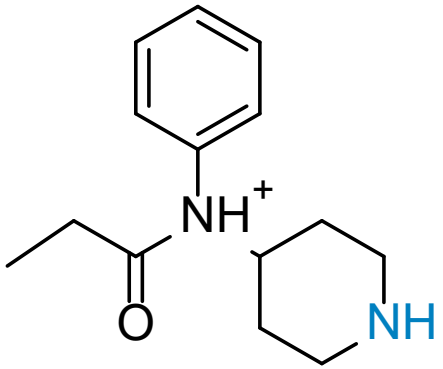
m/z	113	231	291
Mass Error (ppm)	-0.04	3.9	-4.3

Remifentanil Ester Hydrolysis (363 *m/z*)



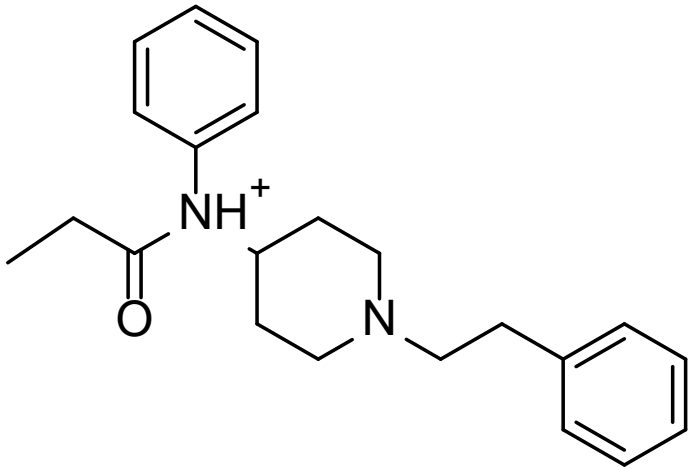
m/z	113	214	363
Mass Error (ppm)	-5.1	-4.7	-3.8

Fentanyl

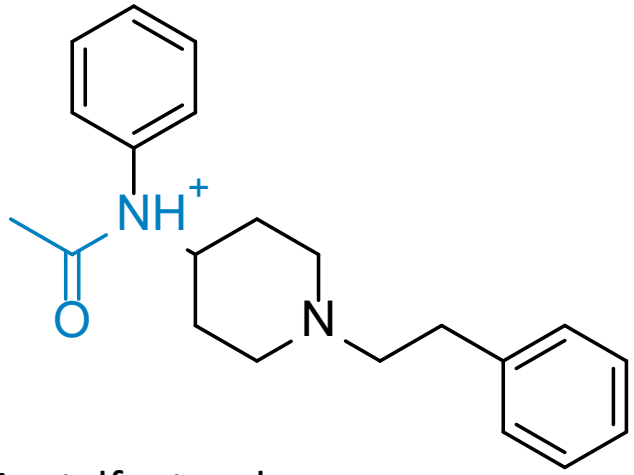


Norfentanyl

$C_{14}H_{21}N_2O^+$ 233.1648 *m/z*

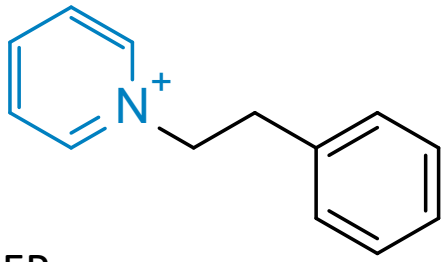


$C_{20}H_{29}N_2O^+$ [M+H] 337.2274 *m/z*



Acetylfentanyl

$C_{21}H_{27}N_2O^+$ 323.2118 *m/z*



1-PEP

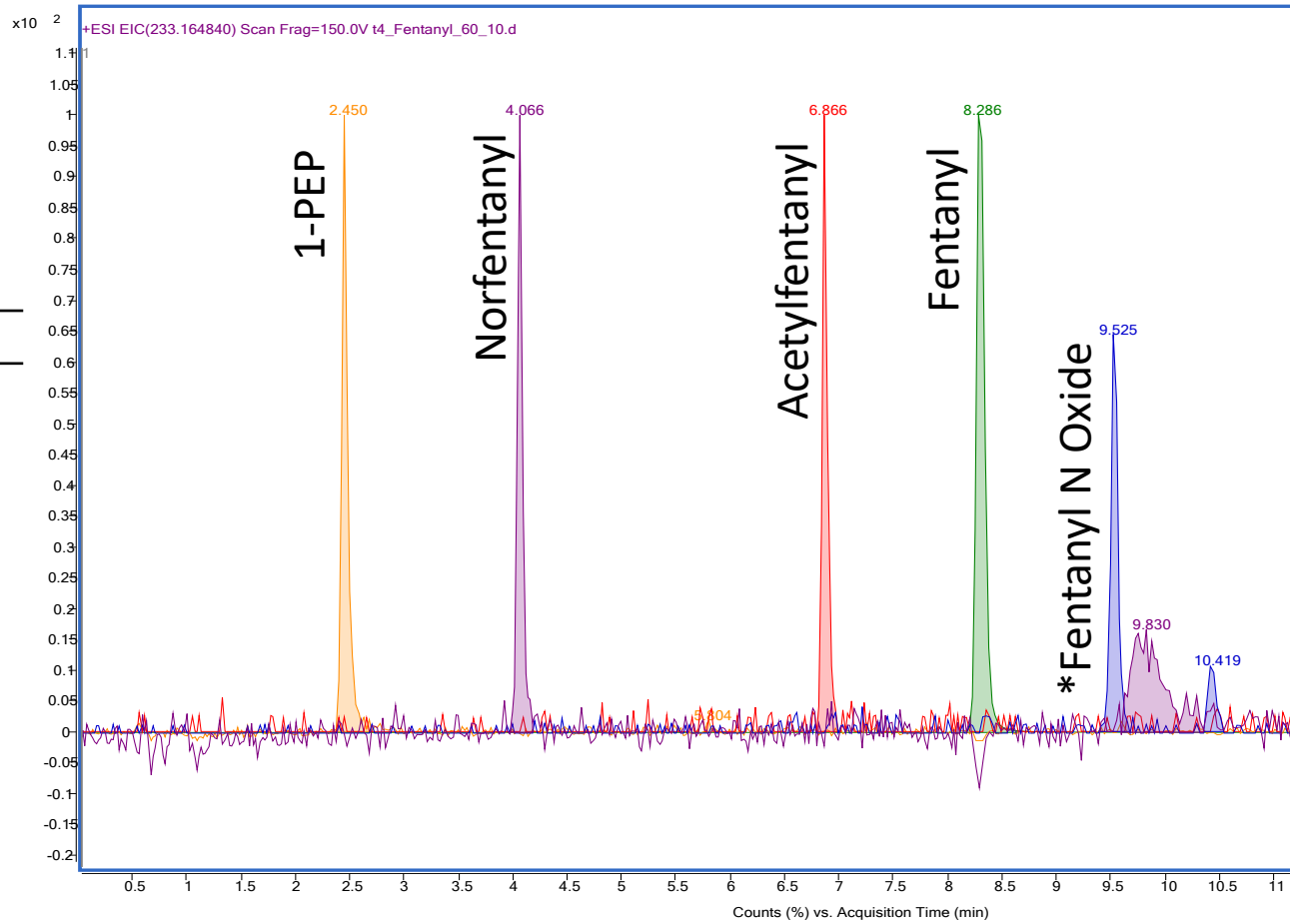
$C_{13}H_{19}N^+$ 184.1126 *m/z*

Fentanyl N oxide

$C_{22}H_{29}N_2O_2^+$ 353.2224 *m/z*

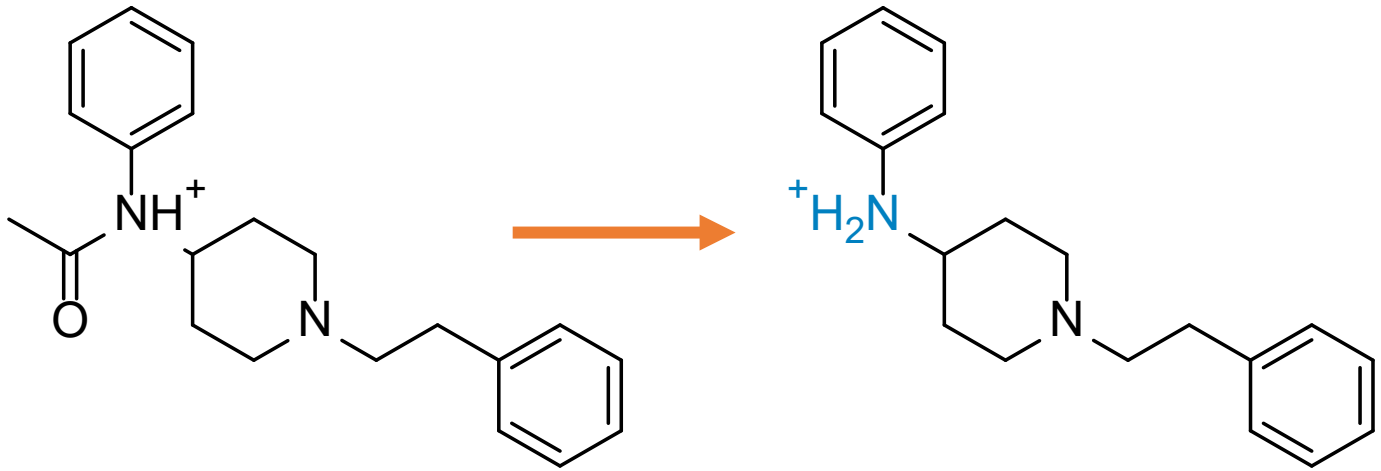
Fentanyl

Analyte	RT	m/z (ppm error) M+H	Products	
Fentanyl	8.286	337 (1.1)	188 (0.9)	105 (-2.2)
Fentanyl N Oxide	9.525	353 (-5.5)	146 (-11.8)	105 (-12.9)
Acetylfentanyl	6.866	337 (0.3)	188 (-3.2)	105 (-3.2)
Norfentanyl	4.066	233 (-0.6)	--	84 (-3.2)
1-PEP	2.450	184 (-3.0)	--	--



Acetylfentanyl

Analyte	RT	m/z (ppm error) M+H	Products	
Acetylfentanyl	6.813	365 (-1.4)	188 (-4.2)	105 (-3.3)
4-ANPP	7.742	381 (-0.7)	--	105 (-3.9)



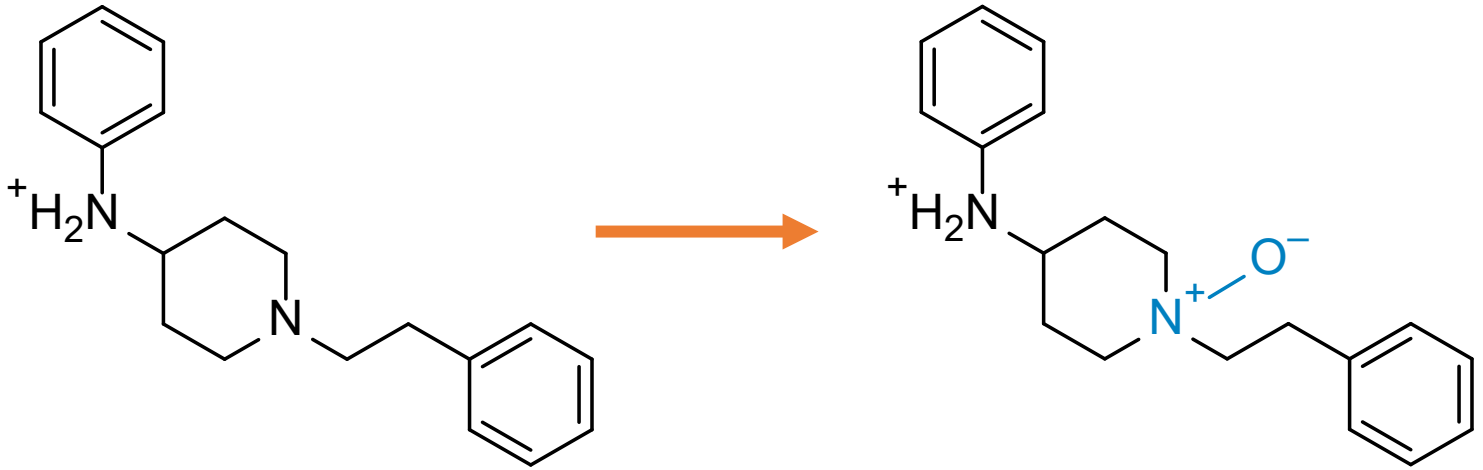
$C_{21}H_{27}N_2O^+$ [M+H] 323.2118 *m/z*

4-ANPP

$C_{19}H_{25}N_2^+$ 281.2012 *m/z*

4-ANPP

Analyte	RT	m/z (ppm error) M+H	Products	
4-ANPP	7.705	365 (-1.4)	188 (-4.2)	105 (-3.3)
4-ANPP N Oxide	8.232	297 (-2.9)	--	--

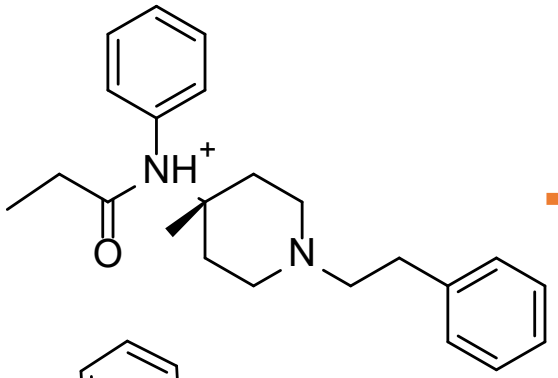


$C_{19}H_{25}N_2^+$ [M+H] 281.2012 *m/z*

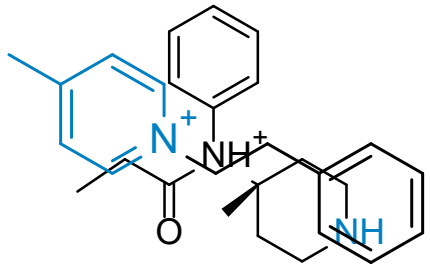
4-ANPP N Oxide

$C_{19}H_{25}N_2O^+$ 297.1691 *m/z*

(+)-Cis-3-methylfentanyl•Butyrylfentanyl•Furanylfentanyl

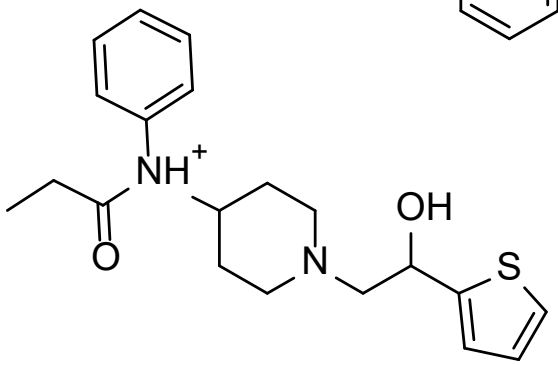


$C_{23}H_{31}N_2O^+$ [M+H] 351.2431 *m/z*

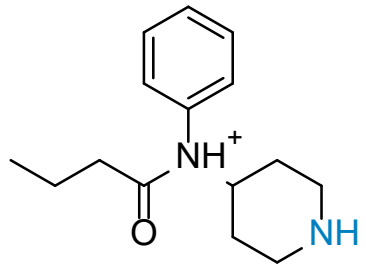


(+)-Cis-3-methyl-1-PEP norfentanyl

$C_{15}H_{23}N_2O^+$ 247.1805 *m/z*

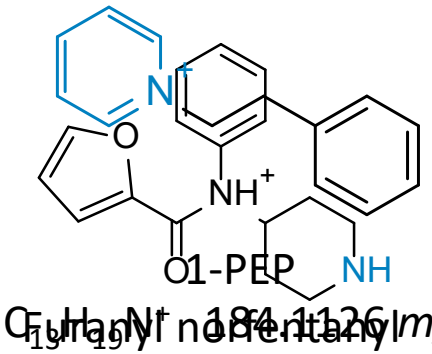


$C_{22}H_{27}N_2O_2^+$ [M+H] 375.2067 *m/z*



Butyryl norfentanyl

$C_{15}H_{23}N_2O^+$ 247.1805 *m/z*



1-PEP

$C_{16}H_{19}N_2O_2^+$ 271.1441 *m/z*

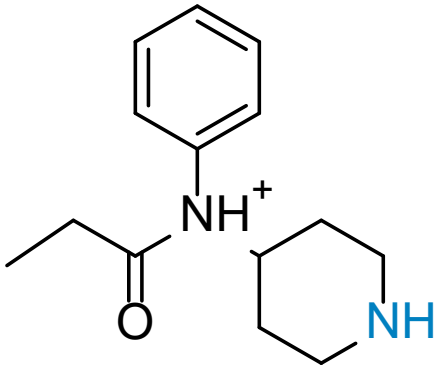
Analyte	RT	m/z (ppm error) M+H	Products	
(+)-Cis-3-methylfentanyl	9.745	351 (-2.8)	202 (-4.1)	105(-0.1)
(+)-Cis-3-methyl norfentanyl	5.010	247 (-1.0)	98 (-0.8)	--
4-methyl-1-PEP	8.246	198 (-1.5)		

Analyte	RT	m/z (ppm error) M+H	Products	
Butyrylfentanyl	9.932	355 (-1.0)	188 (-4.5)	105 (-3.9)
Butyryl norfentanyl	5.583	247 (-4.2)	--	
1-PEP	2.400	184 (-0.6)	--	--

Analyte	RT	m/z (ppm error) M+H	Products	
Furanylfentanyl	8.955	365 (-0.5)	188 (-1.6)	105 (-4.8)
Furanyl norfentanyl	4.463	271 (3.3)	--	--
1-PEP	2.448	184 (-1.8)	--	--

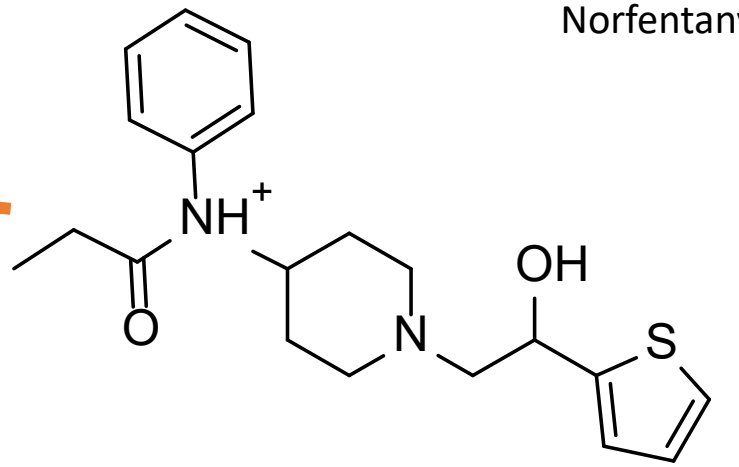
β-Hydroxythiofentanyl

Analyte	RT	m/z (ppm error) M+H	Products	
β-Hydroxythiofentanyl	6.578	359 (-2.5)	192 (-6.0)	111 (-3.1)
β-Hydroxythiofentanyl N oxide	7.364	375 (-3.2)	--	--
Norfentanyl	4.402	233 (7.4)	84 (4.0)	55 (-7.6)

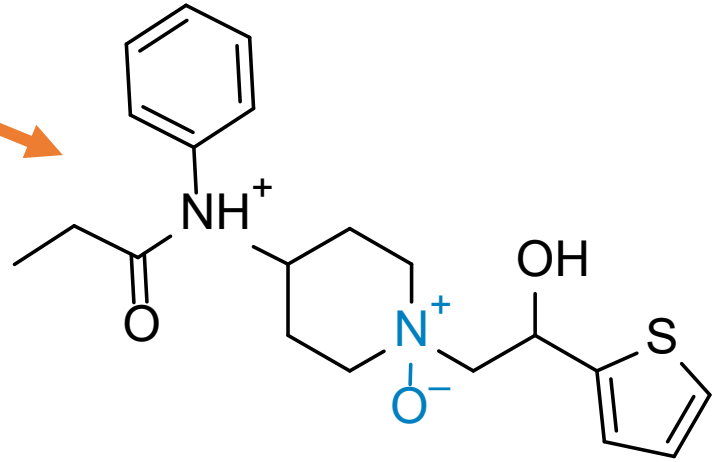


Norfentanyl

$C_{14}H_{21}N_2O^+$ 233.1648 *m/z*



$C_{22}H_{31}N_2SO_2^+$ [M+H] 359.1788 *m/z*

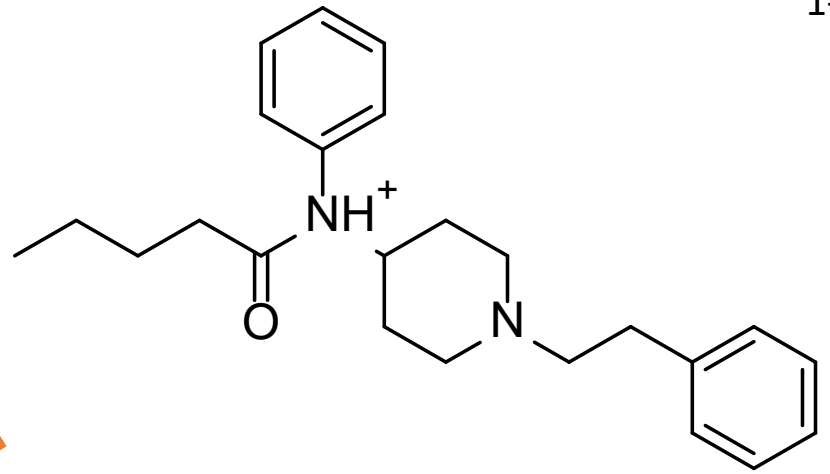


β-Hydroxythiofentanyl N oxide

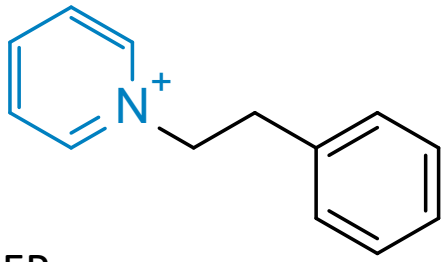
$C_{20}H_{27}N_2SO_3^+$ 375.1737 *m/z*

Valerylfentanyl

Analyte	RT	m/z (ppm error) M+H	Products	
Valerylfentanyl	11.627	365 (-1.1)	188 (-4.4)	105 (-4.1)
Valerylfentanyl N oxide	12.285	381 (1.5)	146 (-9.4)	105 (-2.9)
1-PEP	2.395	184 (-3.2)	--	--

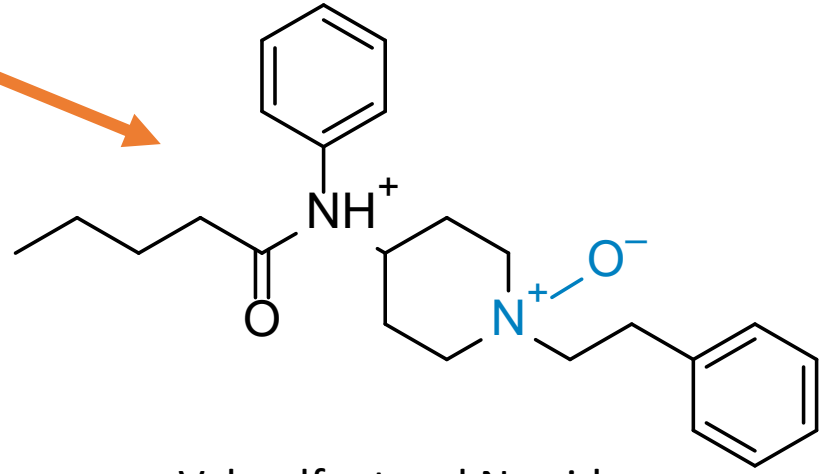


$C_{24}H_{33}N_2O^+$ [M+H] 365.2587 *m/z*



1-PEP

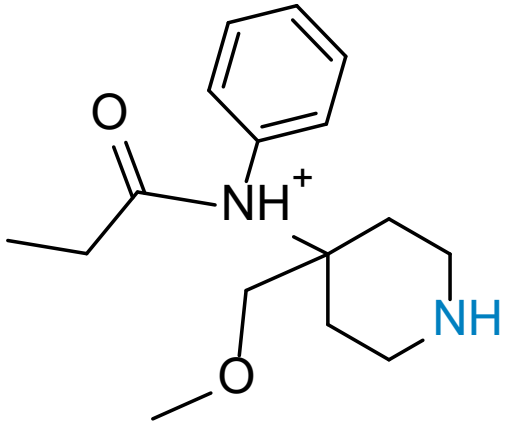
$C_{13}H_{19}N^+$ 184.1126 *m/z*



Valerylfentanyl N oxide

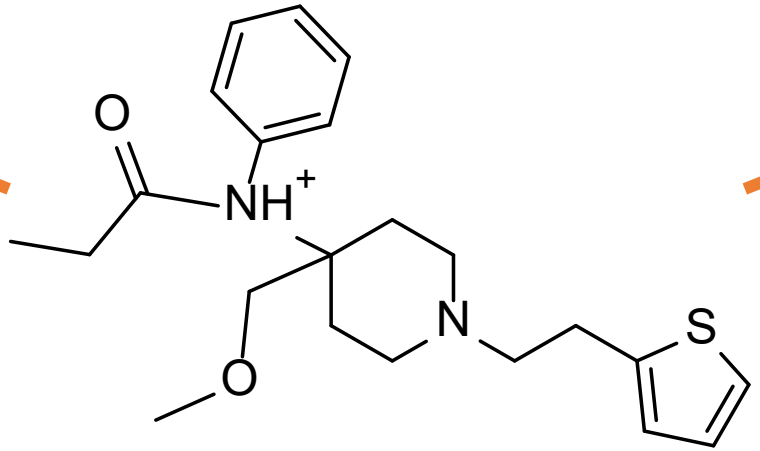
$C_{24}H_{32}N_2O_2^+$ 381.2537 *m/z*

Sufentanil

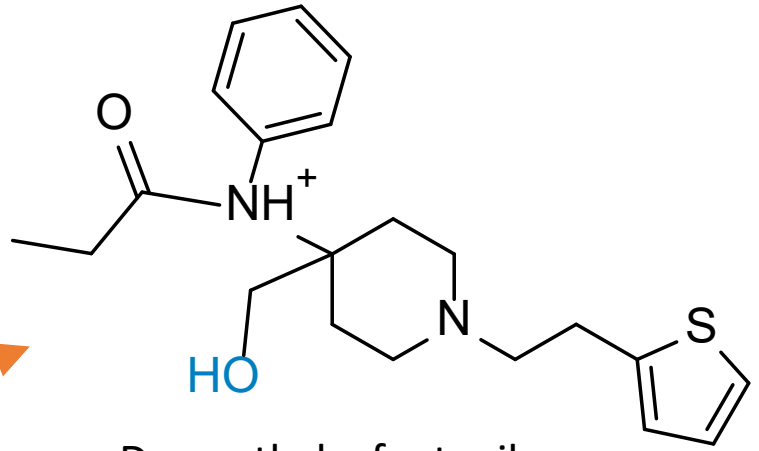


Norsufentanil

$C_{16}H_{25}N_2O_2^+$ 277.1911 *m/z*

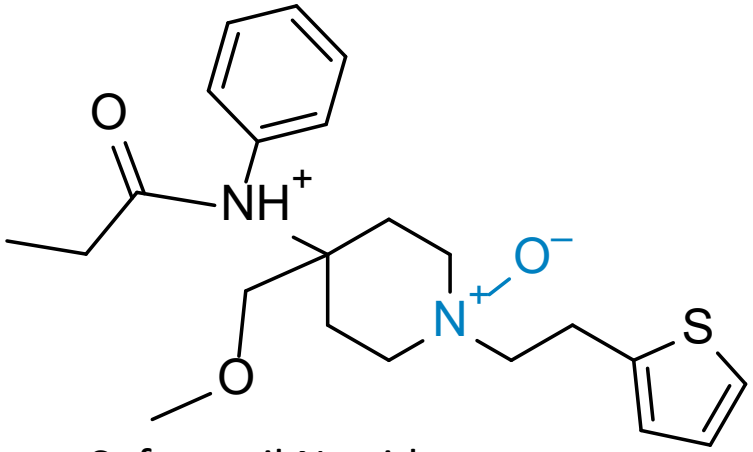


$C_{22}H_{31}N_2O_2S^+$ [M+H] 387.2101 *m/z*



Desmethylsufentanil

$C_{21}H_{29}N_2O_2S^+$ 373.1944 *m/z*

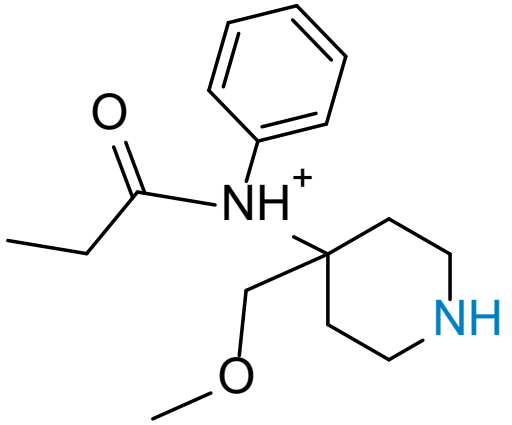


Sufentanil N oxide

$C_{22}H_{31}N_2O_3S^+$ 403.2050 *m/z*

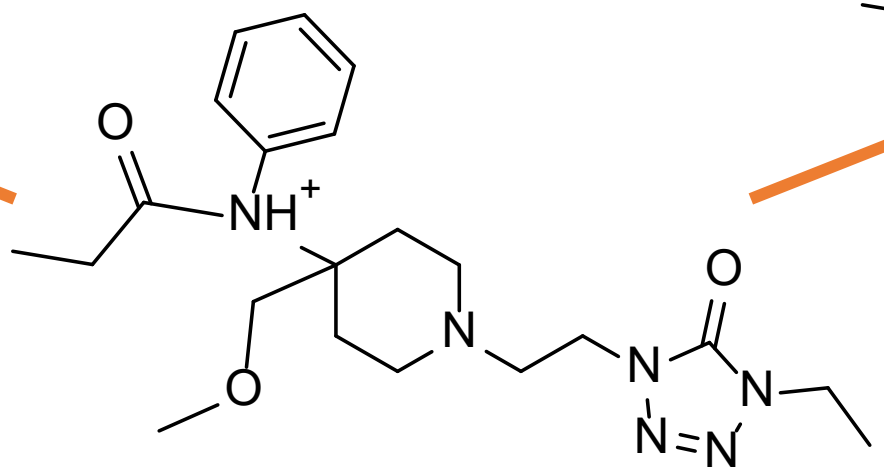
Analyte	RT	m/z (ppm error) M+H	Products	
Sufentanil	10.768	387 (-0.4)	238 (-2.7)	111 (-4.2)
Norsufentanil	6.193	277(3.1)	--	--
Desmethylsufentanil	8.893	373 (1.3)	--	--
Sufentanil N oxide	11.470	403 (-4.0)	111 (-3.8)	--

Alfentanil

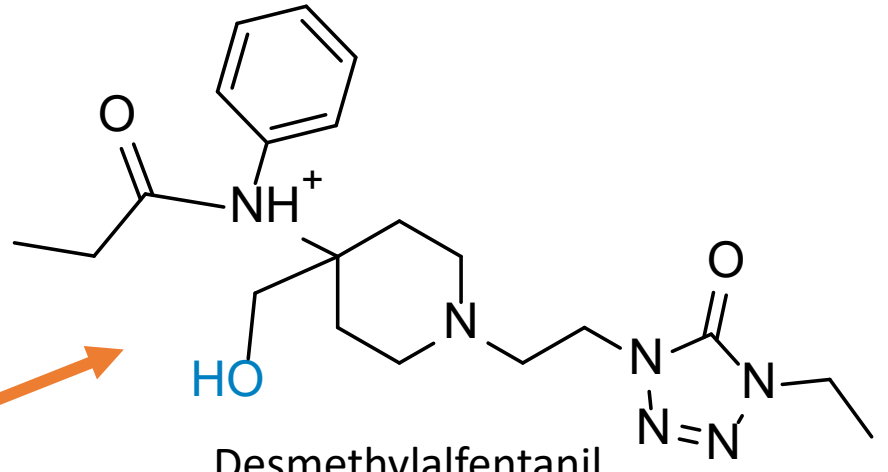


Norsufentanil

$C_{16}H_{25}N_2O_2^+$ 277.1911 *m/z*

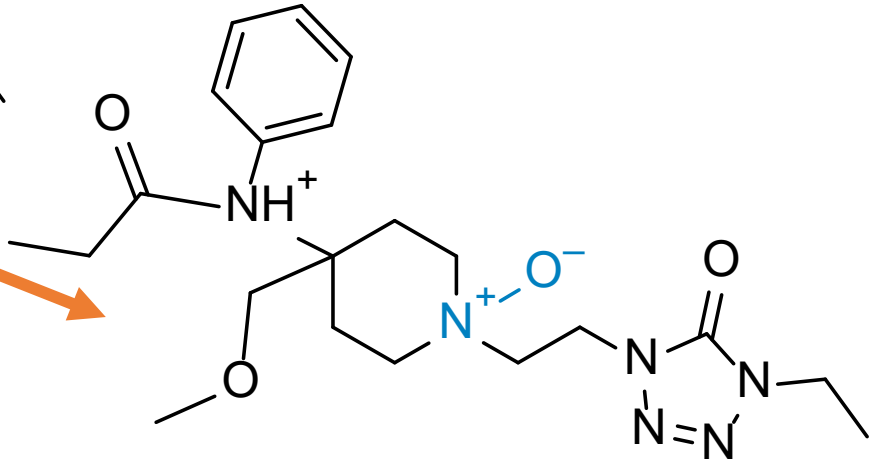


$C_{21}H_{33}N_6O_3^+$ [M+H] 417.2609 *m/z*



Desmethylalfentanil

$C_{20}H_{31}N_6O_3^+$ 403.2452 *m/z*



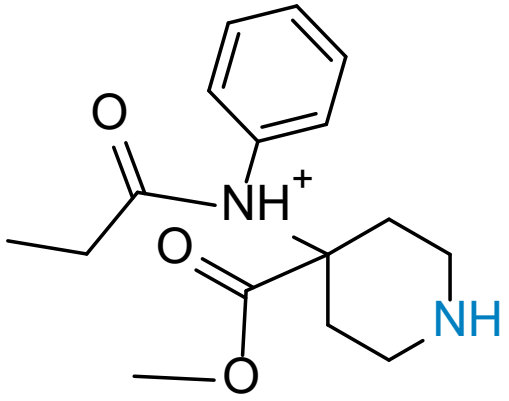
Alfentanil N oxide

$C_{20}H_{33}N_6O_4^+$ 433.2558 *m/z*

Analyte	RT	m/z (ppm error) M+H	Products	
Alfentanil	8.806	417 (-0.7)	111 (-4.3)	--
Norsufentanil	6.186	277 (-1.7)	--	--
Desmethylalfentanil	9.035	403 (-1.1)	--	--
Alfentanil N oxide	6.566	433 (1.8)	--	--

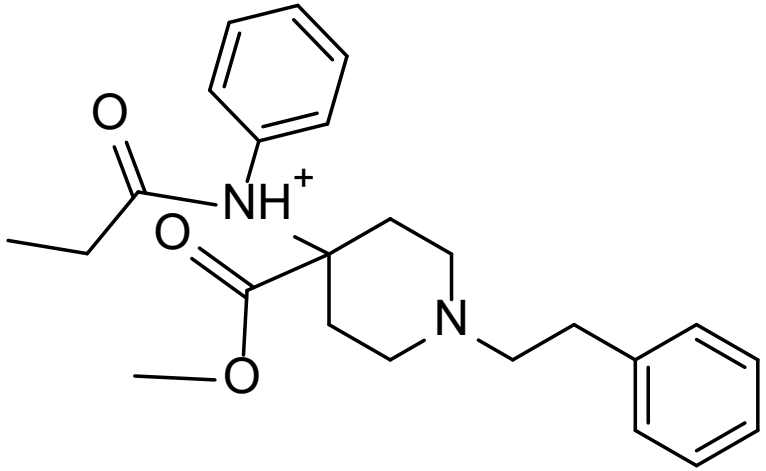
Carfentanil

Analyte	RT	m/z (ppm error) M+H	Products	
Carfentanil	9.759	395 (1.1)	335 (-0.6)	113 (-1.7)
4-carboxy-1-(2-phenylethyl)pyridinie-1-ium	2.377	228 (2.1)	105 (-0.1)	77 (5.4)
Norcarfentanil	4.998	291 (0.7)	--	--
Carfentanil N oxide	10.639	411 (0.7)	--	--

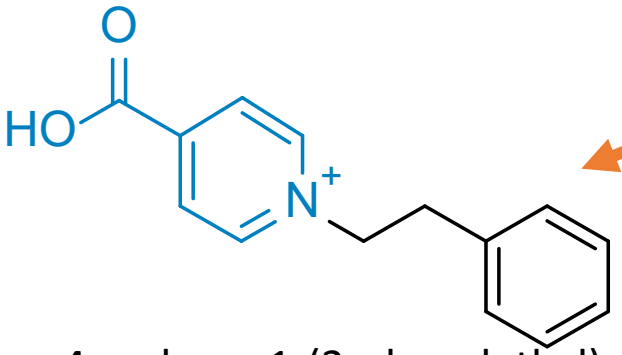


Norcarfentanil

$C_{16}H_{23}N_2O_3^+$ 291.1703 *m/z*

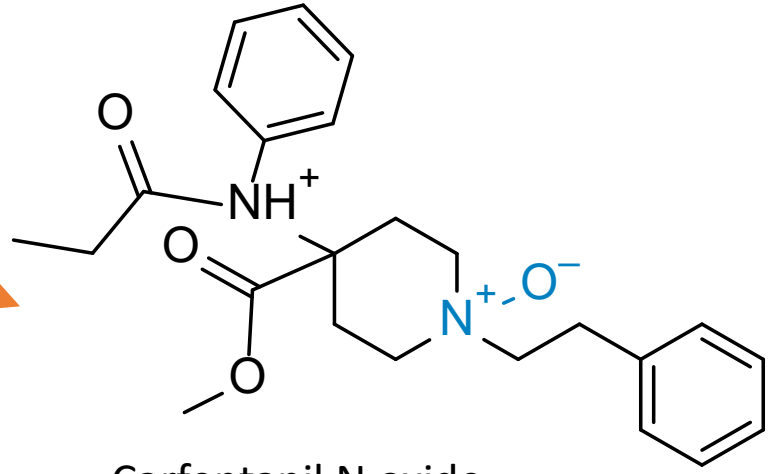


$C_{20}H_{29}N_2O^+$ [M+H] 395.2067 *m/z*



4-carboxy-1-(2-phenylethyl)pyridine-1-ium

$C_{14}H_{14}NO_2^+$ 228.1025 *m/z*

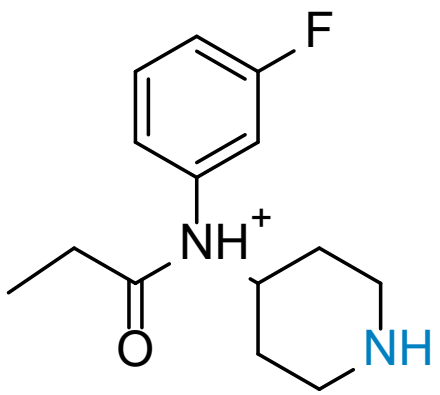


Carfentanil N oxide

$C_{24}H_{31}N_2O_4^+$ 411.2278 *m/z*

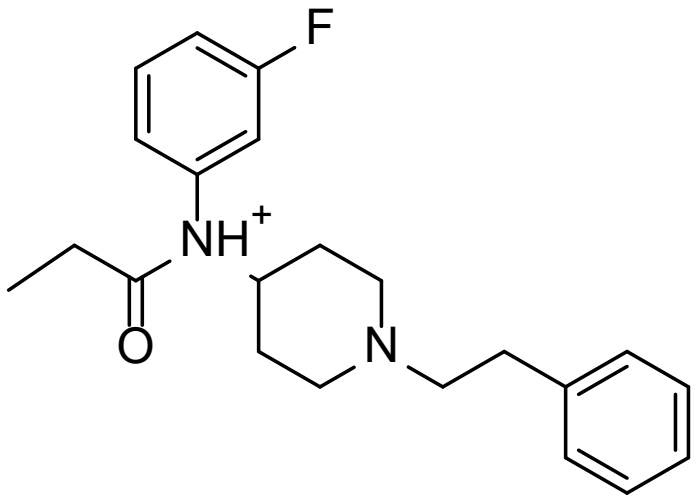
o-Fluorofentanyl

Analyte	RT	m/z (ppm error) M+H	Products	
o-Fluorofentanyl	9.000	355 (0.7)	188 (-3.3)	105 (-1.5)
o-Fluorofentanyl N Oxide	10.039	371 (4.1)	--	--
o-Fluoronorfentanyl	4.066	251 (0.0)	--	84 (-13.3)
1-PEP	2.460	184 (-5.3)	--	--

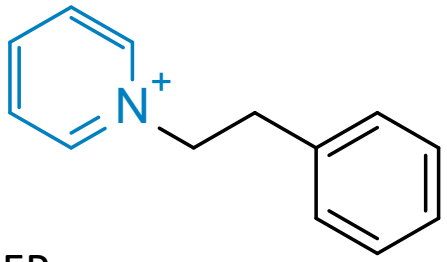


o-Fluoronorfentanyl

$C_{14}H_{20}FN_2O^+$ 251.1528 *m/z*

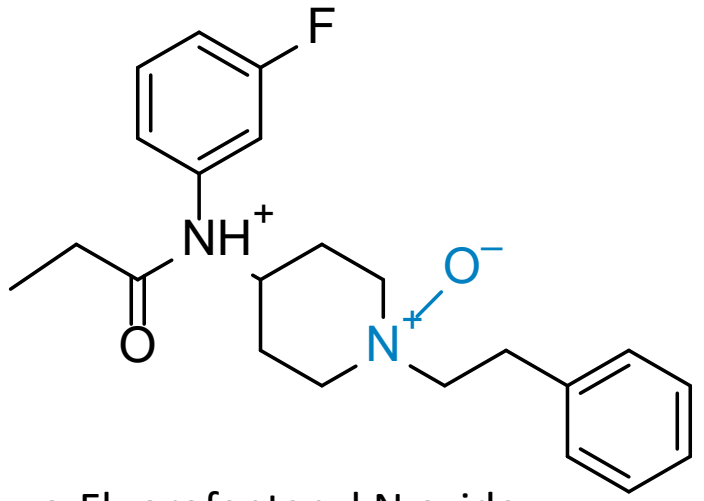


$C_{22}H_{28}FN_2O^+$ [M+H] 355.21801 *m/z*



1-PEP

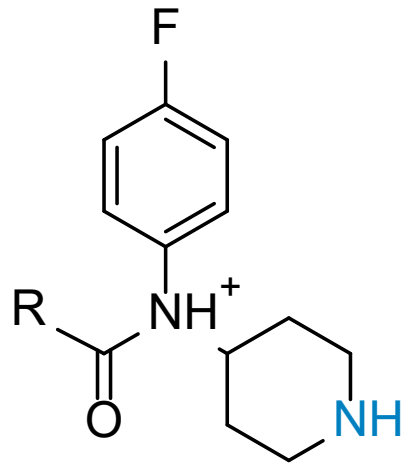
$C_{13}H_{19}N^+$ 184.1126 *m/z*



o-Fluorofentanyl N oxide

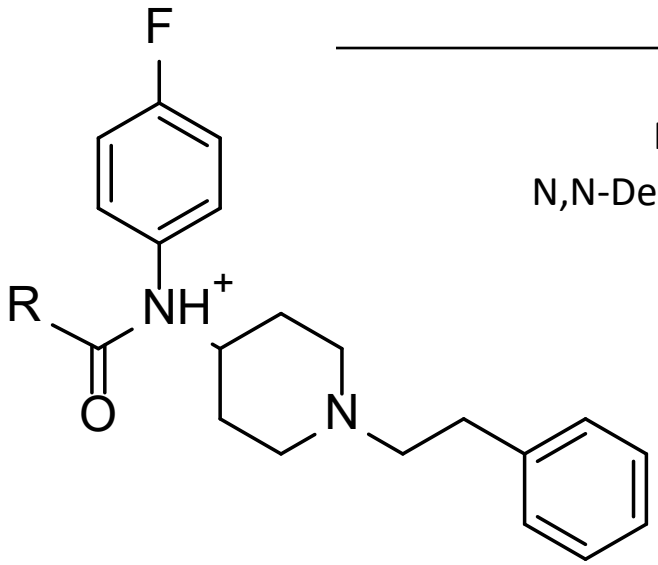
$C_{22}H_{28}FN_2O_2^+$ 371.2129 *m/z*

P-Fluorobutyrylfentanyl/isobutyrylfentanyl



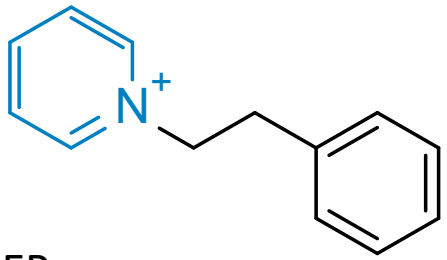
Nor p-FBF/Nor p-FIBF

$C_{15}H_{22}FN_2O^+$ 265.1711 *m/z*



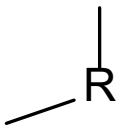
$C_{20}H_{29}FN_2O^+$ [M+H] 369.2337 *m/z*

Analyte	RT	m/z (ppm error) M+H	Products	
p-FIBF	10.305	369 (0.2)	188 (-3.9)	105 (-3.4)
Nor p-FIBF	6.090	265 (-3.3)	--	--
N,N-Dealkylation	1.024	195 (3.9)	--	--
1-PEP	2.386	184 (-4.9)	--	--
P-FBF	10.501	369 (-0.4)	188 (-4.7)	105 (-2.9)
Nor p-FBF	6.190	265 (-4.8)	84 (7.7)	55 (-1.1)
N,N-Dealkylation	0.986	195 (1.9)	--	--
1-PEP	2.389	184 (-0.6)	--	--

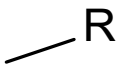


1-PEP

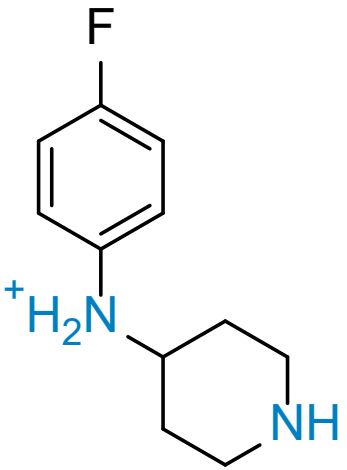
$C_{13}H_{19}N^+$ 184.1126 *m/z*



p-FIBF



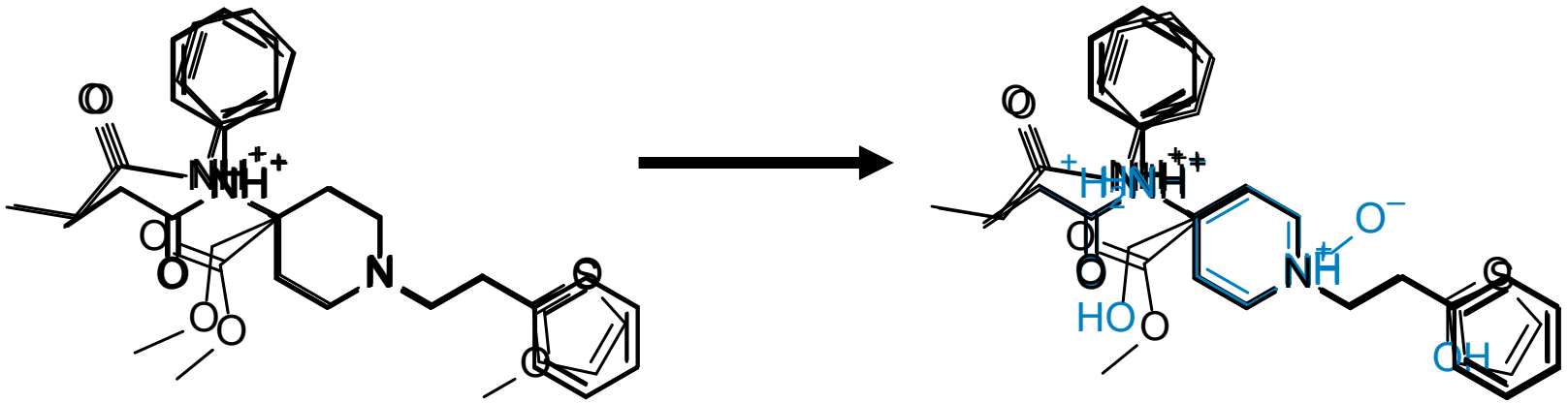
p-FBF



N,N-Dealkylation

$C_{11}H_{16}FN_2^+$ 195.1292 *m/z*

Analyte	Demethylation O-Demethylation		Amide Hydrolysis Ester Hydrolysis		N-Dealkylation N,N-Dealkylation		Oxidation	β-Elimination, Oxidation + Dehydration x2
Fentanyl	X				X		X	X
Acetylfentanyl			X					
Butyrylfentanyl					X			X
Furanylfentanyl					X			X
Valerylfentanyl							X	X
4-ANPP							X	
β-hydroxythiofentanyl					X		X	
Remifentanil				X	X			
Sufentanil		X			X		X	
Alfentanil		X			X		X	
Carfentanil					X		X	X
(+)-Cis-3-methylfentanyl					X			X
o-Fluorofentanyl					X		X	X
p-fluorobutyrylfentanyl					X	X		X
p-Fluoroisobutyrylfentanyl					X	X		X



Authentic

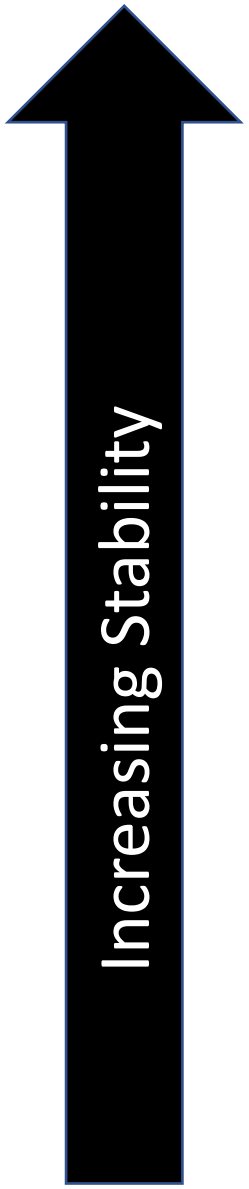
	A		B		C	
	Reported	Tested	Reported	Tested	Reported	Tested
Fentanyl	↑POS		231.5 ng/mL		↑POS	
Norfentanyl	↑POS		↑POS		↑POS	
Acetylfentanyl	0.9 ng/mL		--		POS	
Valerylfentanyl	POS		--		--	
Carfentanil	--		--		POS	
Fluorofentanyl	--		POS		--	
4-ANPP	--		--		POS	
Norcarfentanil	--		--		--	

↑ POS = beyond dynamic range

Discussion

- Fentanyl analog degradation was more rapid and extensive in alkaline environments
- Norfentanyl and norcarfentanil (N-dealkylation) analogs stable at all conditions tested
- N-Dealkylation and piperidine nitrogen oxidation were most common products followed by 1-PEP formation
- 4-ANPP instability & lack of amide hydrolysis product
- Fluorinated analogs were similar in stability to the base analog
- Rapid remifentanil degradation

Stability Comparison



Norfentanyl & Norcarfentanil
Acetylfentanyl
β-Hydroxythiofentanyl
Alfentanil
Carfentanil
Fentanyl
o-Fluorofentanyl
Furanylfentanyl
Sufentanil
P-Fluoroisobutyrylfentanyl
P-Fluorobutyrylfentanyl
Butyrylfentanyl
(+)-Cis-3-methylfentanyl
Valerylfentanyl
4-ANPP
Remifentanil

Conclusion

- Temperature and pH-dependent stability importance:
  - Pre-analytical
    - Storage, transportation, benchtop
  - Analytical
    - Extraction
  - Post-analytical
    - Qualitative ID and quantitative results

- Target identification for pH and temperature labile analytes

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Questions?

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