Comprehensive LC-QTOF-MS Analysis for NPS in a Complex Forensic Toxicology World

SCIEX Lunch & Learn Lightning Talks – SOFT Annual Meeting – Tuesday October 31, 2023

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DISCLOSURES

- I have no conflicts of interest to disclose.
- I am a scientist and employee of FRFF / CFSRE, a 501(c)(3) non-profit research and educational facility.
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  - The opinions, findings, conclusions and/or recommendations expressed in this presentation are those of the author(s) and do not necessarily represent the official position or policies of the U.S. Department of Justice.

NIJ | National Institute of Justice

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INTRODUCTION

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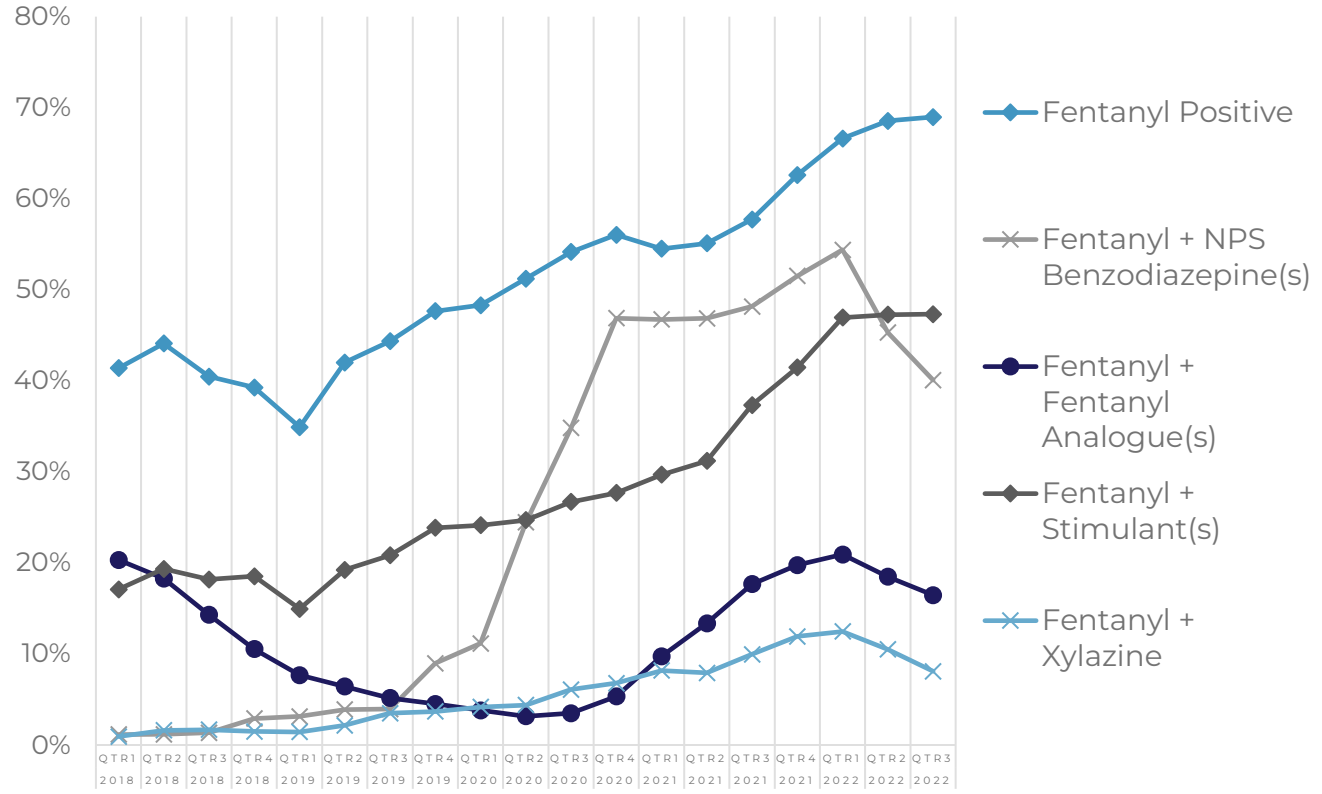
COMPLEX FORENSIC TOXICOLOGY WORLD

- **Dynamic and volatile** drug markets
- Increasing **polydrug use**
  - Single drug intoxications are becoming more rare
- **Scopes of testing** are being strained due to evolving drug markets
- Forensic toxicologists are being asked to **interpret complex cases** now more than ever

Fentanyl Co-Positivity ("Nested Waves")



WHAT ARE “NPS”?

- **Novel (or new) psychoactive substances (NPS) is a catch-all term that captures most drugs outside traditional and therapeutic categories, also referred to as “designer drugs”**
  - Newly synthesized or discovered
  - New to the drug supply
  - Used in a new way or manner
  - Altered toxicological effect profile
- **Five major subclasses of NPS**
  - Benzodiazepines
  - Opioids
  - Stimulants
  - Hallucinogens
  - Cannabinoids

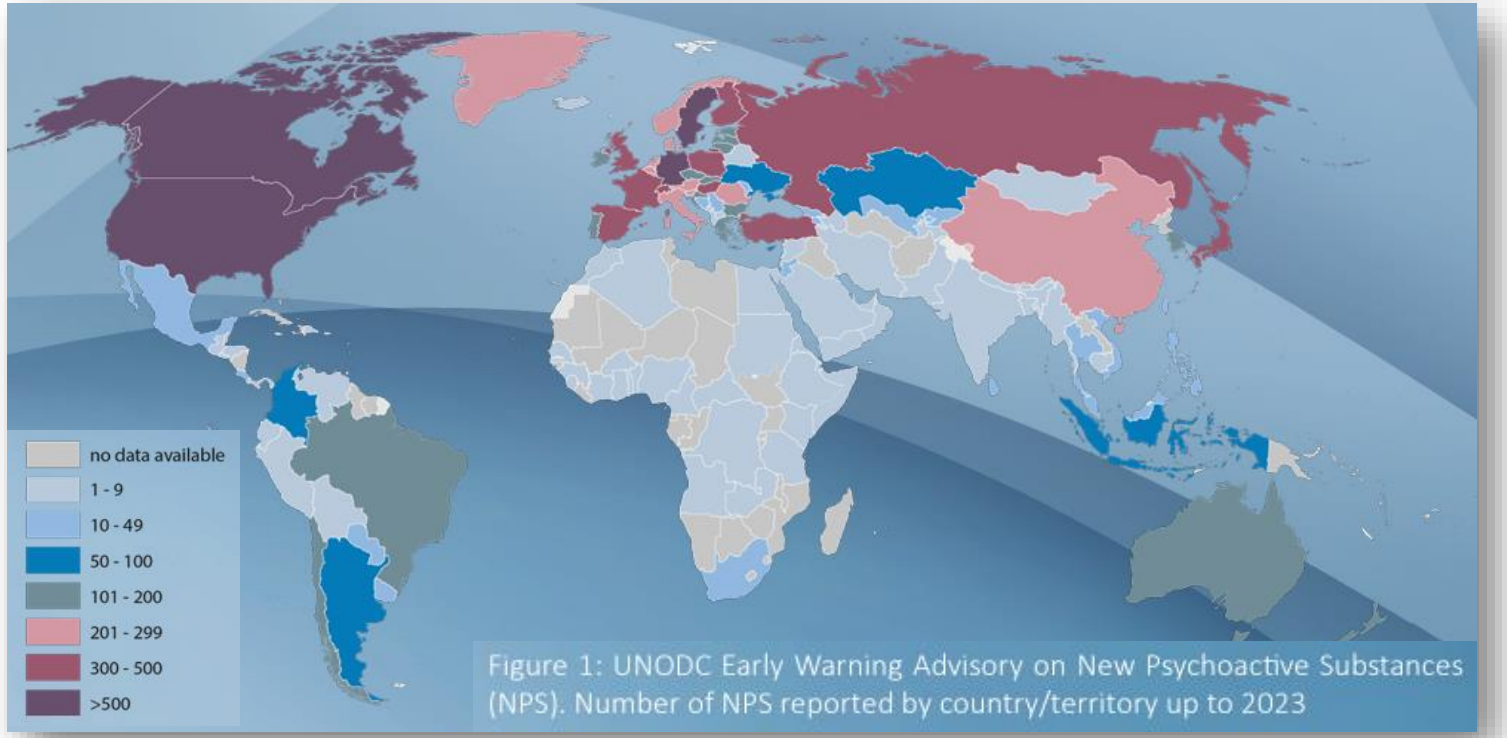


Figure 1: UNODC Early Warning Advisory on New Psychoactive Substances (NPS). Number of NPS reported by country/territory up to 2023

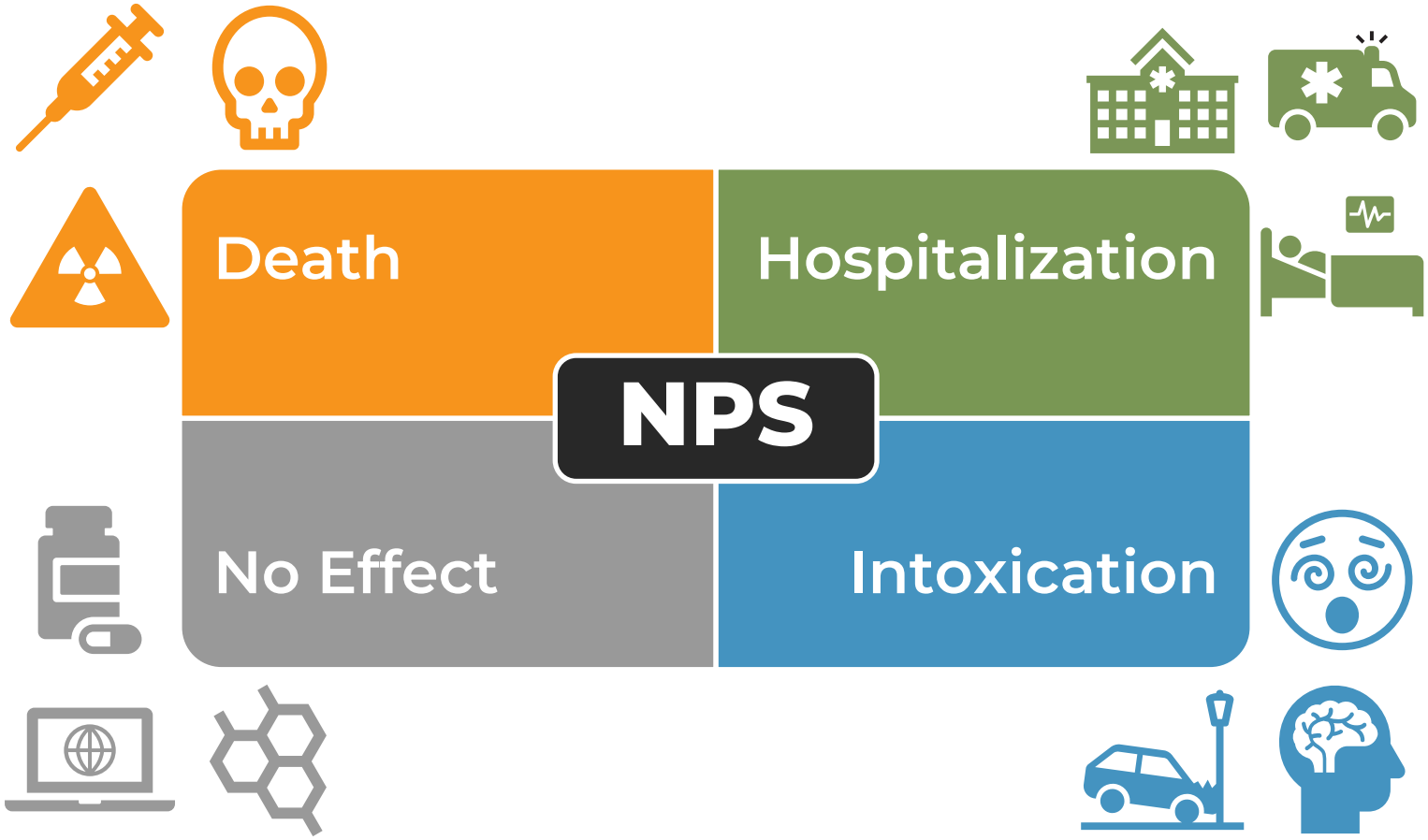
WHY ARE NPS POPULAR?

- **Consumers**
  - New highs / more desirable effects
  - Easier to source than tradition drugs
  - Legal status (or avoid illegal status)
  - Beat traditional drug tests

- **“Manufacturers” / Processers**
  - Cheaper alternatives
  - Desirable combined drug effects
  - Legal status (or avoid illegal status)

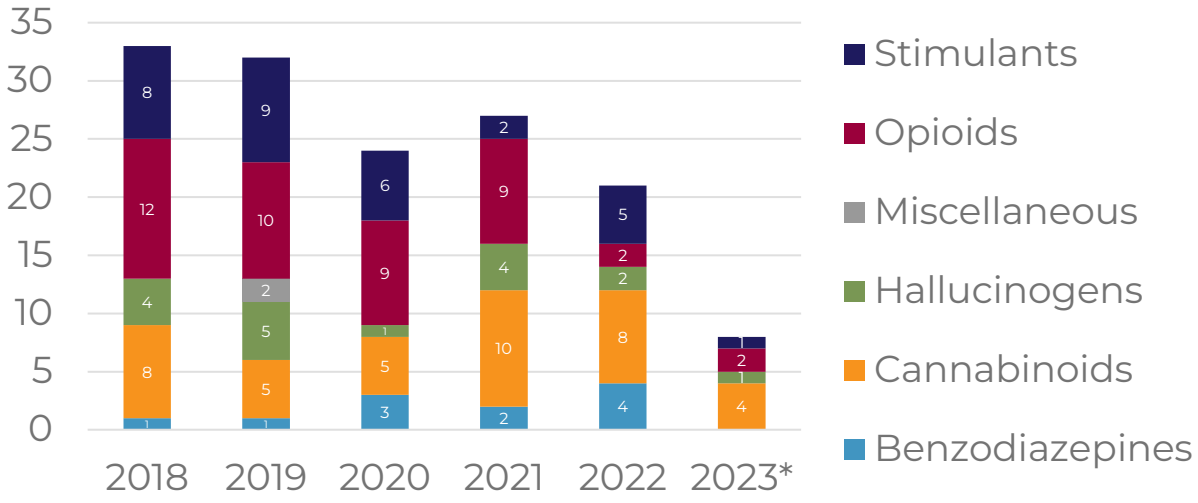
- **NPS are often unknown to consumer / processors**

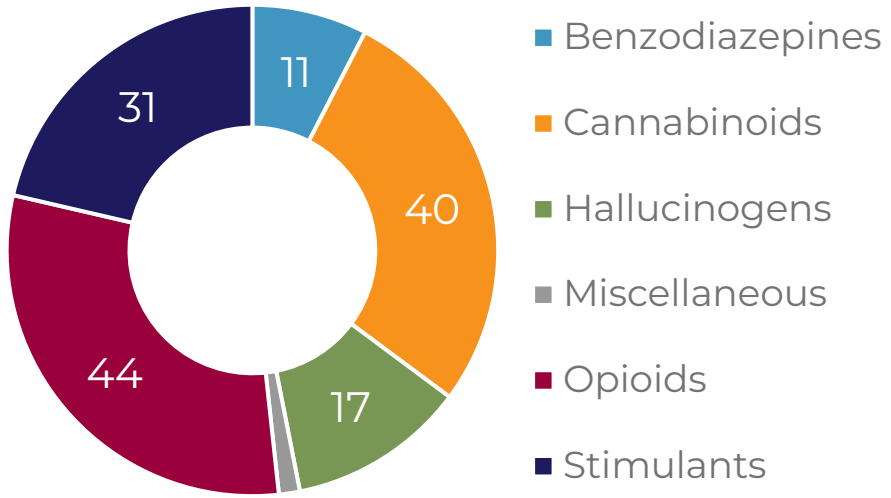
WHERE DO NPS APPEAR?



EMERGENCE OF NPS IN THE U.S.

- Since 2018, NPS Discovery has reported **145** newly discovered NPS (and counting)
- **NPS opioids** remain the largest subclass of newly emerging drugs encountered
- As of June 2023, NPS Discovery has reported **8** NPS for the first time this year





LANDSCAPE OF NPS IN THE U.S.

- Since 2018, NPS Discovery has identified **more than 225** NPS in forensic samples
- **NPS opioids**, **stimulants**, and **cannabinoids** represent the largest subclasses observed

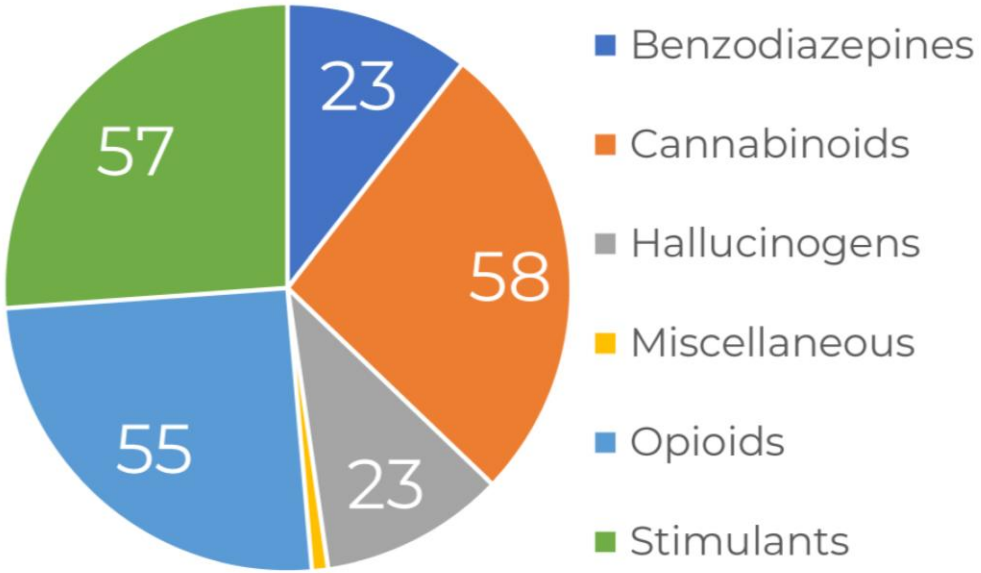


Figure 3: Breakdown by subclass of individual NPS detected, 2018-2022.

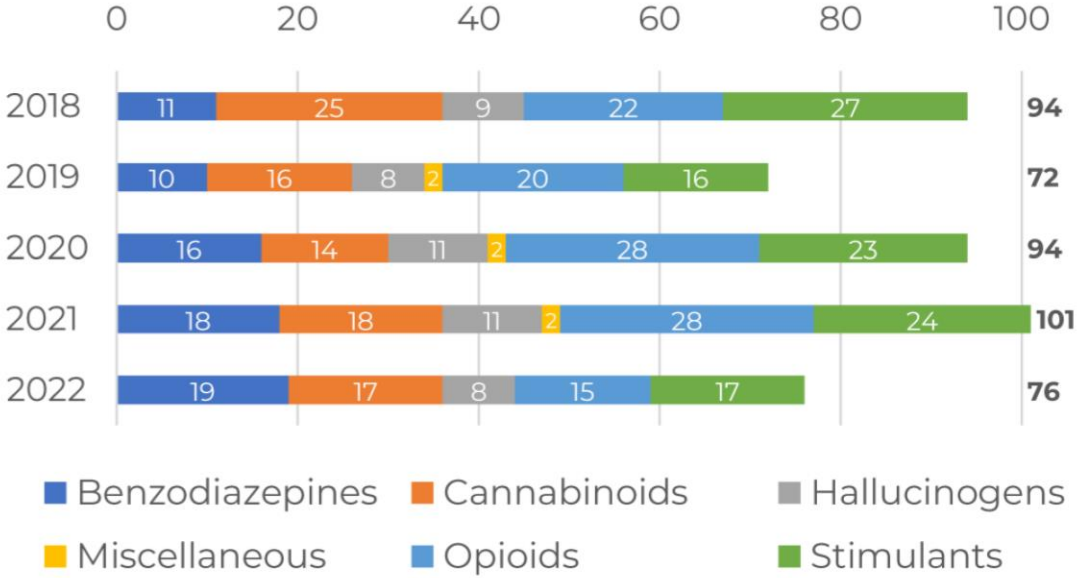


Figure 4: Individual NPS detected each year, cumulative since 2018.

WORKFLOWS & METHODS

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FORENSIC LABORATORY

- The Center for Forensic Science Research and Education (CFSRE)
  - 501(c)(3) non-profit research and educational facility
  - *Surveillance & Casework*

Waters Xevo® G2-S LC-QTOF-MS

Sciex X500R LC-QTOF-MS

Sciex TripleTOF® 5600+ LC-QTOF-MS

Agilent 6495 LC-QQQ-MS

Agilent 6430 LC-QQQ-MS

Waters TQS LC-QQQ-MS

Waters TQD LC-QQQ-MS

Agilent 5975 GC-MS

Agilent 5975 GC-MS

LC-QTOF-MS ASSAYS

Sample Preparation | Chromatography | Mass Spectrometry | Data Processing

Basic Liquid-Liquid Extraction

- *Broad-based drug screening for NPS (excluding synthetic cannabinoids)*

- **Procedure:**
  - Add 1 mL of Borax buffer, pH 10.4
  - Add 3 mL of 70:30 *N*-butyl chloride and ethyl acetate
  - Cap and rotate for 10 mins at 40%
  - Centrifuge 4600 rpm for 15 minutes
  - Freeze pour and transfer supernatant
  - Drydown in TurboVap at 35 °C, 10 psi, for 30 mins
  - Reconstitute for LC-MS analysis

Acidic Liquid-Liquid Extraction

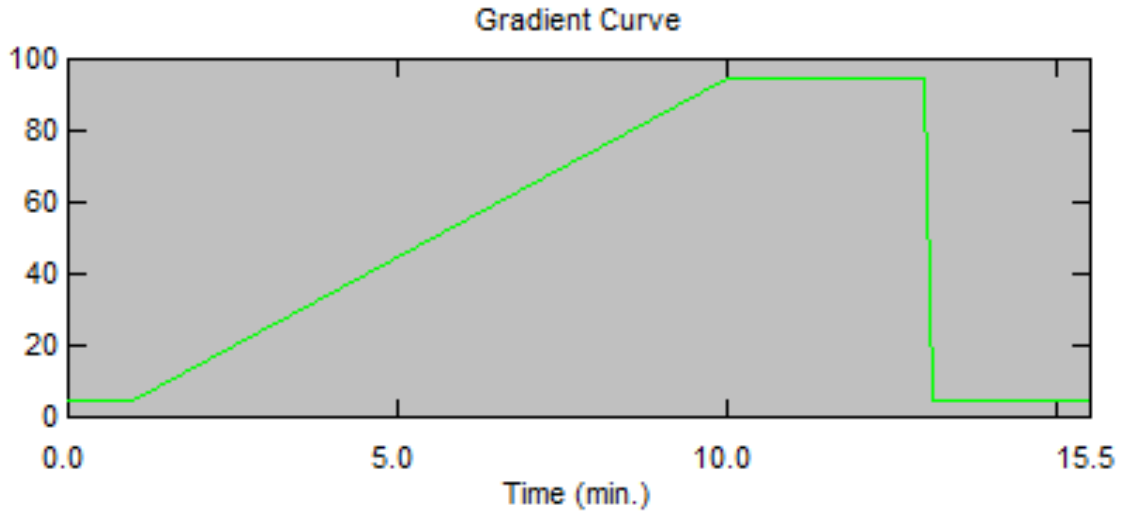
- *Capture synthetic cannabinoids, their metabolites, and other acidic drugs*

- **Procedure:**
  - Add 1 mL of 5% phosphoric acid in water (v:v)
  - Add 3 mL of 80:10:10 hexane, EtOAc, and MTBE
  - Cap and rotate for 10 mins at 40%
  - Centrifuge 4600 rpm for 15 minutes
  - Freeze pour and transfer supernatant
  - Drydown in TurboVap at 35 °C, 10 psi, for 30 mins
  - Reconstitute for LC-MS analysis

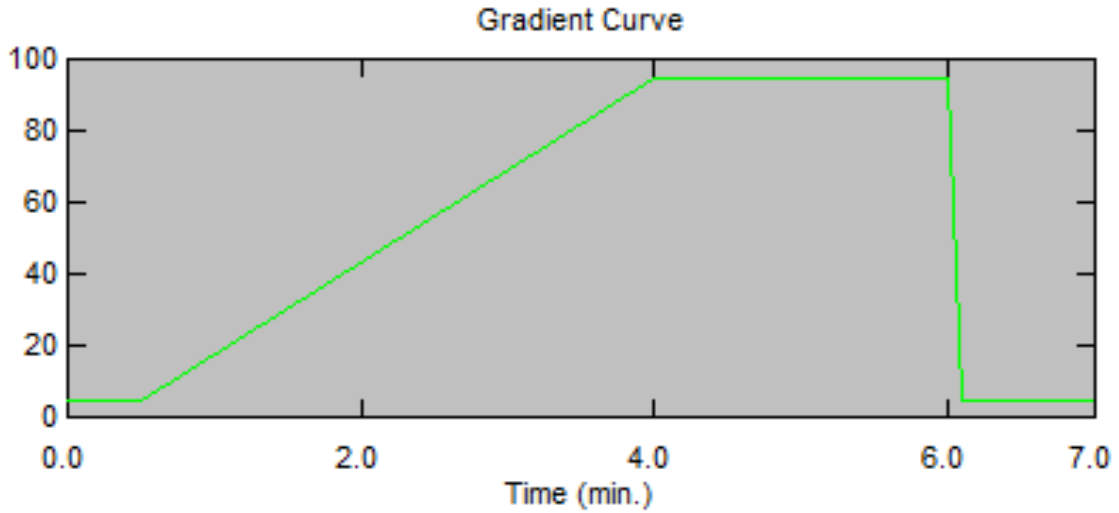
LC-QTOF-MS ASSAYS

Sample Preparation | **Chromatography** | Mass Spectrometry | Data Processing

15.5-minute General Screening Method



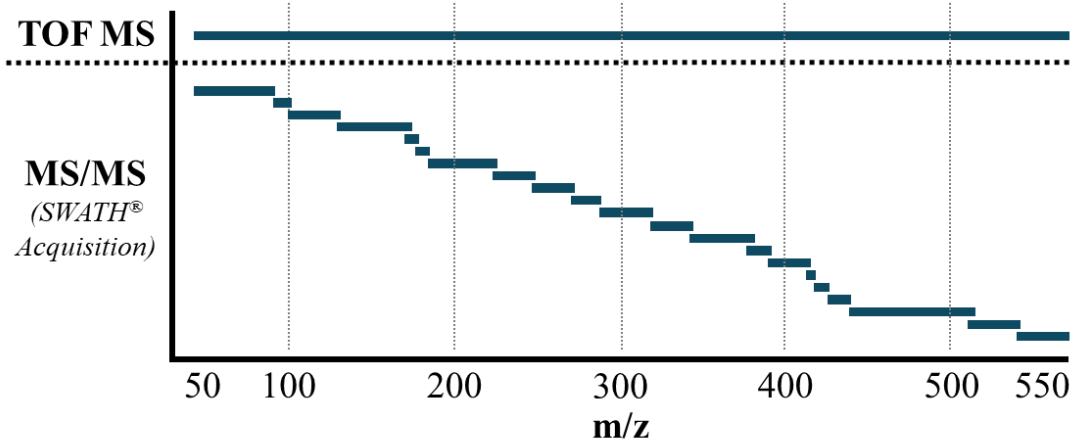
7-minute Synthetic Cannabinoid Method

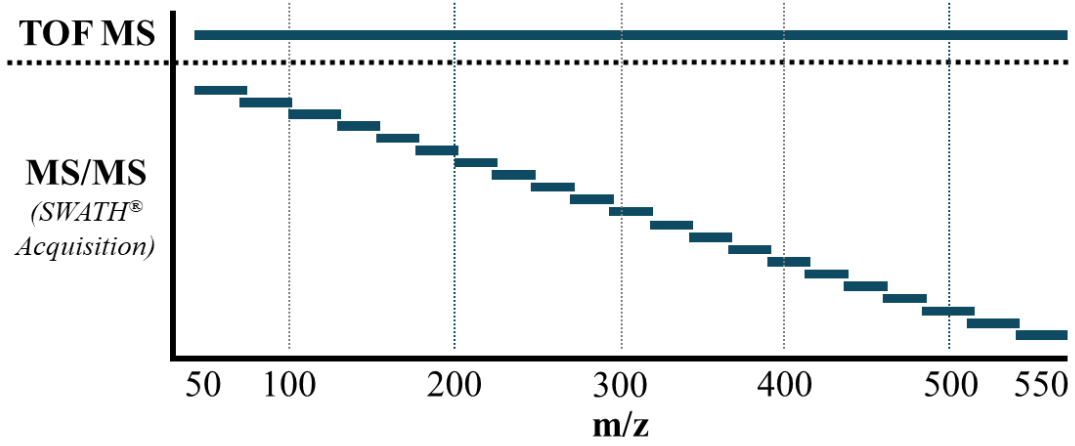


LC-QTOF-MS ASSAYS

Sample Preparation | Chromatography | **Mass Spectrometry** | Data Processing

- **Liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS)**
- Information Dependent Acquisition (IDA)
- **SWATH Acquisition (DIA) – Primarily Used**





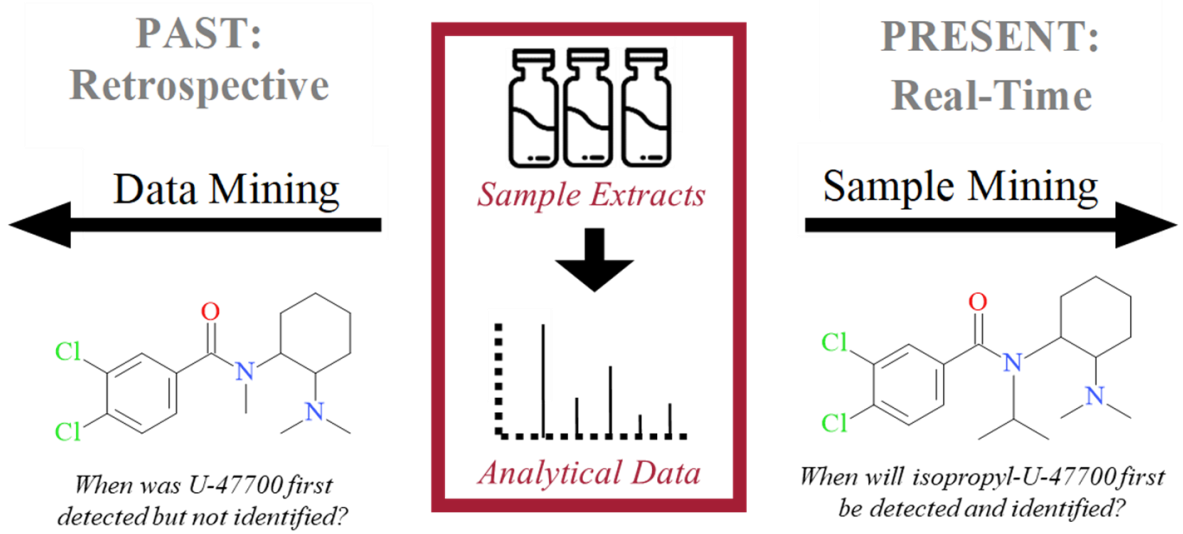
LC-QTOF-MS ASSAYS

Sample Preparation → Chromatography → Mass Spectrometry → **Data Processing**

- Data Processing
  - Targeted (In-Scope)
    - Routine Criteria
  - Targeted (Monitoring)
    - Standard → Mass, RT, Library
    - Formula → Mass
  - Non-Targeted
    - "Suspect Screening" **
    - Peak Finding Algorithms
    - Unknown Searching

WORKFLOWS TO DISCOVERY NEW NPS

- **Sample-Mining (Prospective):**
  - Use of discarded authentic sample extract vials
  - High-*ish* volume, higher rate of incidence
  - Best for trend data
- **Data-Mining (Retrospective):**
  - Use of electronic mass spectrometry datafiles
  - Gives historical perspective
  - Not ideal way to discover NPS
- **Real-Time Surveillance / Case Analysis:**
  - Extraction and analysis of biological samples from forensic investigations
  - Individual case basis but often can give insights into trending information



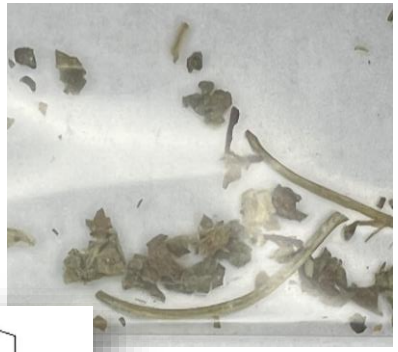
EXAMPLES OF NEW DISCOVERIES

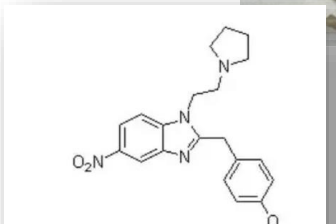
- **Important** → Assessing the right populations paired with good intelligence

- **Toxicology:**
  - Collaboration with medical examiner / coroner offices → Assist in determining MOD and COD
  - Initial toxicology testing negative but “suspected opioid death”

- **Drug Material:**
  - Plant-like material submitted from PDPH / contained peak not in library database
  - Structural elucidation → new synthetic cannabinoid: ADB-5’Br-BINACA

- **Intelligence:**
  - Monitor online surface web gray market sites → new nitazene analogues
  - Purchase to confirm identity before first forensic case???





Metonitazepyne

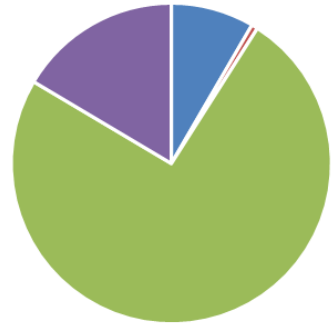
CONTINUAL DEVELOPMENT OF DATABASE

Cayman CHEMICAL

Sciex X500R LC-QTOF-MS

Sciex TripleTOF® 5600+ LC-QTOF-MS

LB



EXAMPLE OF NPS IDENTIFICATIONS

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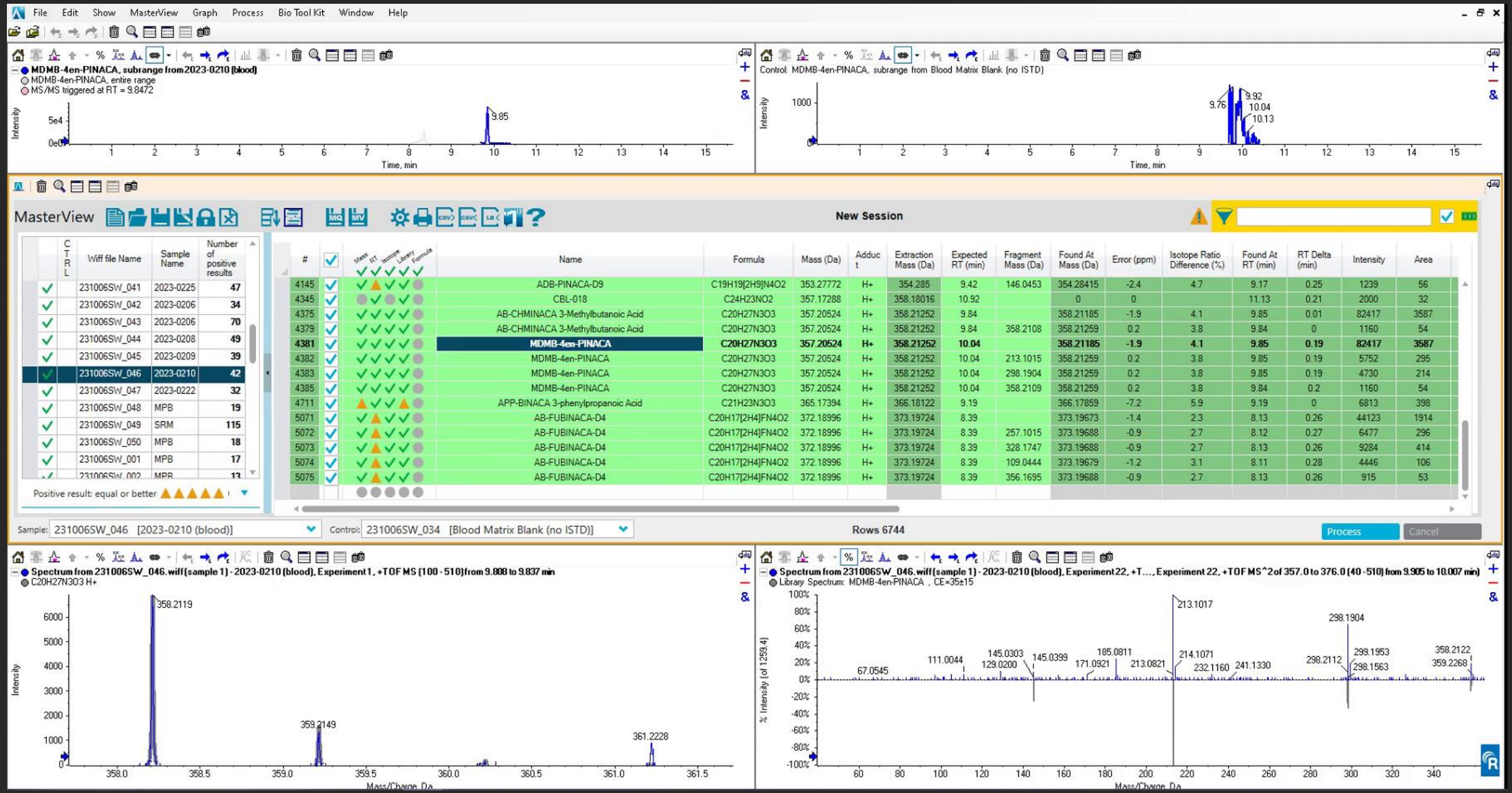
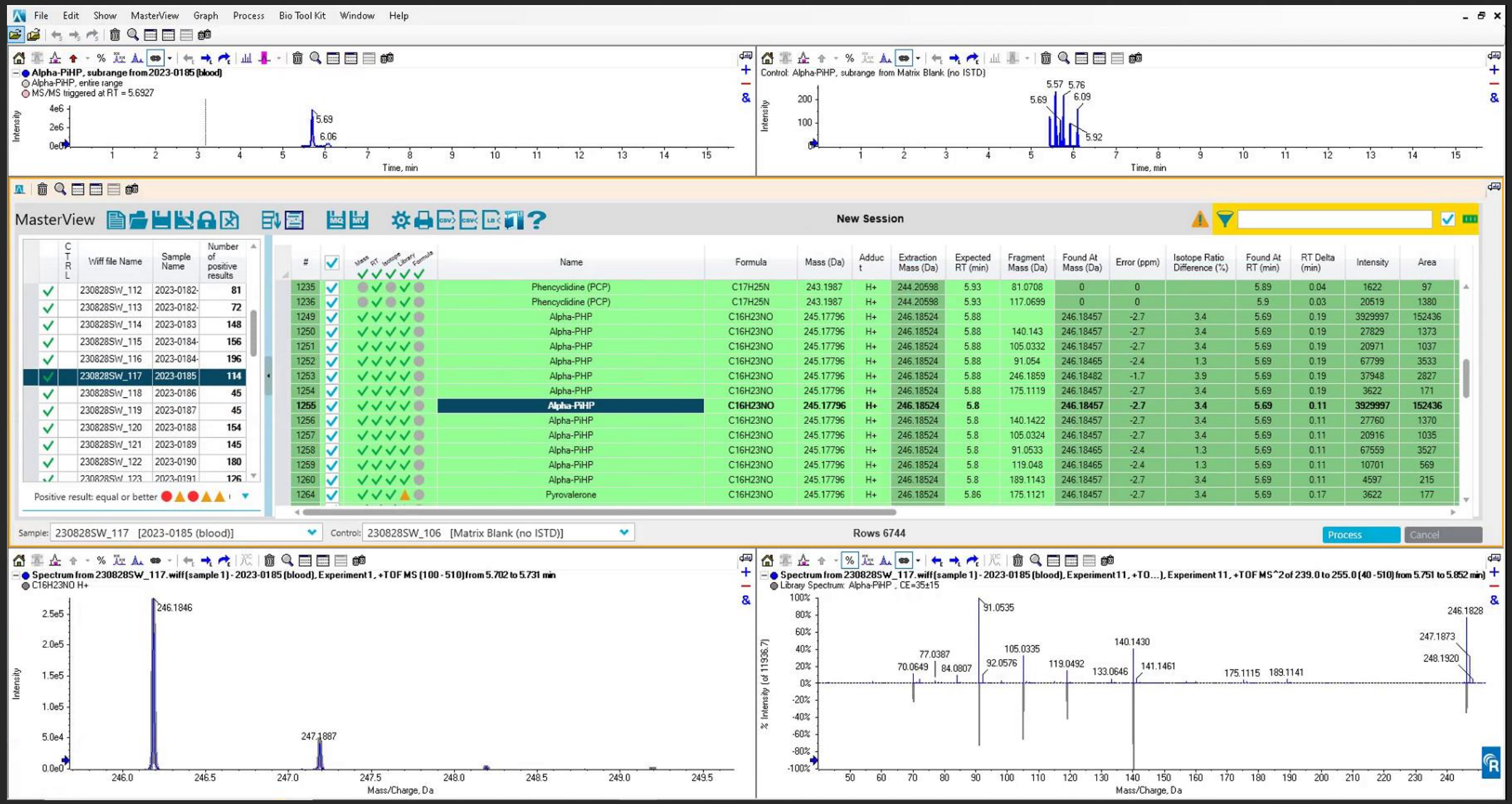
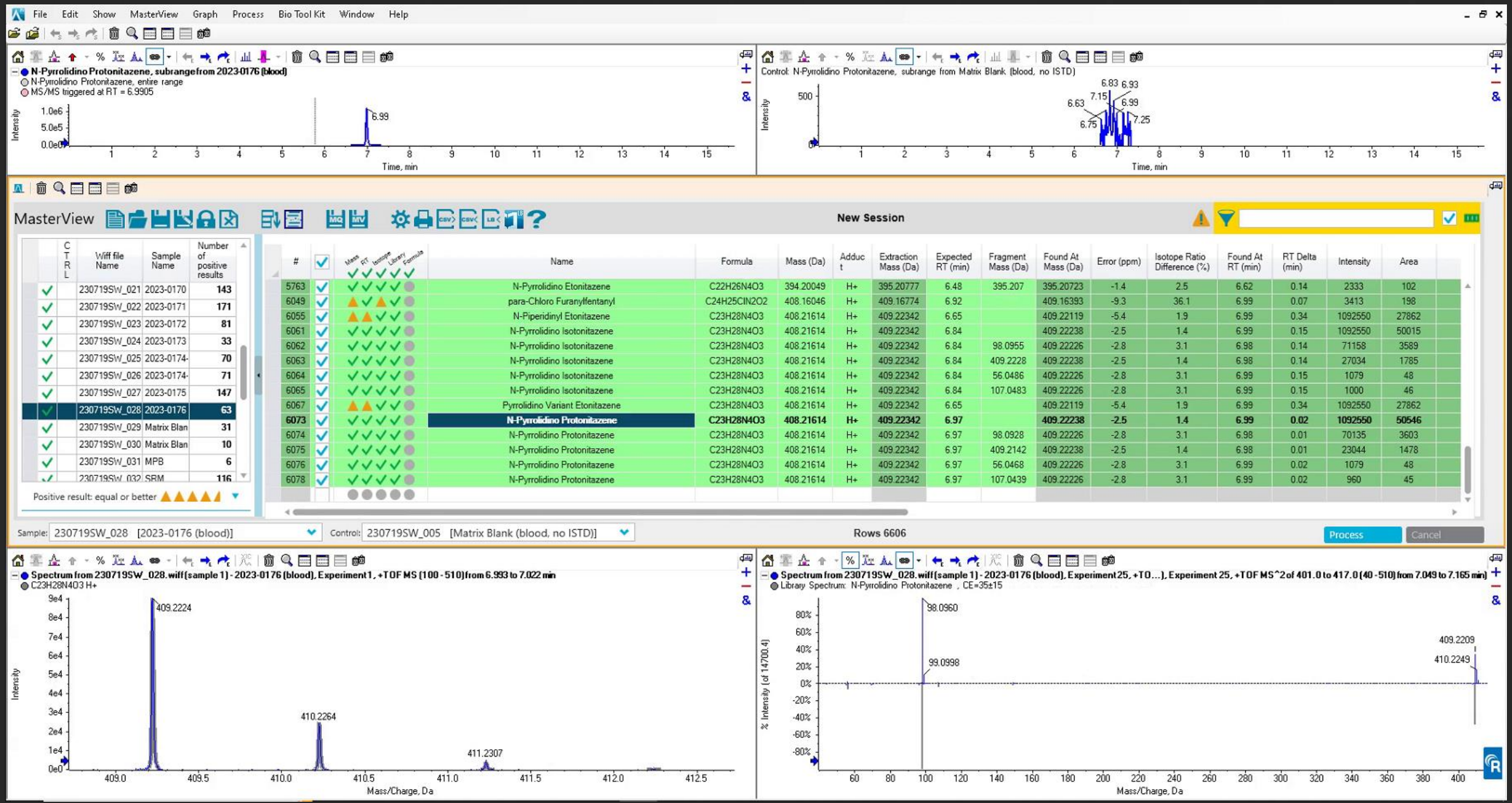
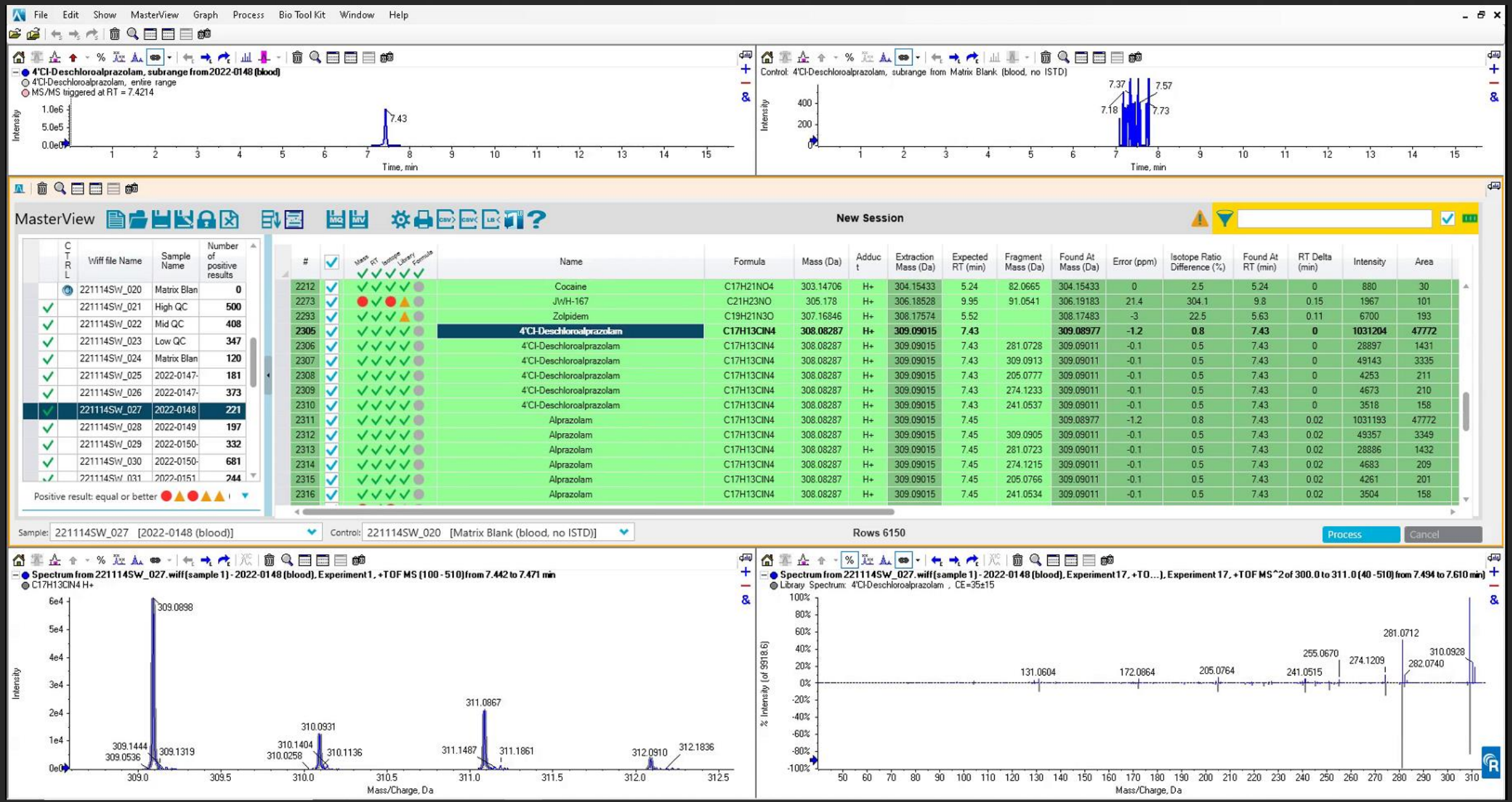
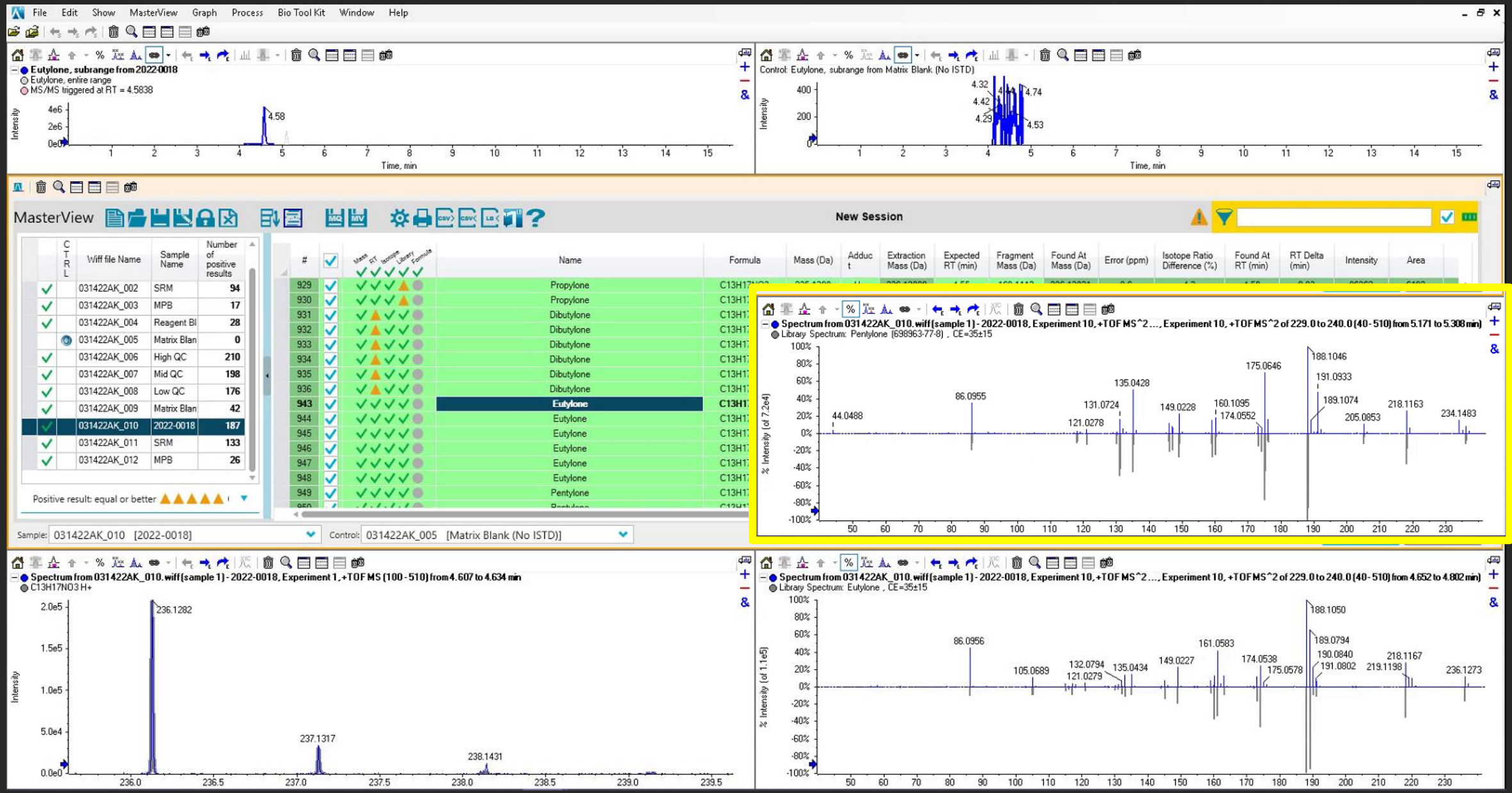

File Edit Show MasterView Graph Process Bio Tool Kit Window Help
MDMB-4en-PINACA, subrange from 2023-0210 (blood)
MDMB-4en-PINACA, entire range
MS/MS triggered at RT = 9.8472
Intensity
Time, min
9.85
Control: MDMB-4en-PINACA, subrange from Blood Matrix Blank (no ISTD)
9.76
9.92
10.04
10.13
MasterView
New Session
Wiff file Name
Sample Name
Number of positive results
231006SW_041 2023-0225 47
231006SW_042 2023-0206 34
231006SW_043 2023-0206 70
231006SW_044 2023-0208 49
231006SW_045 2023-0209 39
231006SW_046 2023-0210 42
231006SW_047 2023-0222 32
231006SW_048 MPB 19
231006SW_049 SRM 115
231006SW_050 MPB 18
231006SW_001 MPB 17
Positive result: equal or better
Name Formula Mass (Da) Adduct Extraction Mass (Da) Expected RT (min) Fragment Mass (Da) Found At Mass (Da) Error (ppm) Isotope Ratio Difference (%) Found At RT (min) RT Delta (min) Intensity Area
4145 ADB-PINACA-D9 C19H19[2H9]N4O2 353.27772 H+ 354.285 9.42 146.0453 354.28415 -2.4 4.7 9.17 0.25 1239 56
4345 CBL-018 C24H23NO2 357.17288 H+ 358.18016 10.92 0 0 11.13 0.21 2000 32
4375 AB-CHMINACA 3-Methylbutanoic Acid C20H27N3O3 357.20524 H+ 358.21252 9.84 358.21185 -1.9 4.1 9.85 0.01 82417 3587
4379 AB-CHMINACA 3-Methylbutanoic Acid C20H27N3O3 357.20524 H+ 358.21252 9.84 358.2108 358.21259 0.2 3.8 9.84 0 1160 54
4381 MDMB-4en-PINACA C20H27N3O3 357.20524 H+ 358.21252 10.04 358.21185 -1.9 4.1 9.85 0.19 82417 3587
4382 MDMB-4en-PINACA C20H27N3O3 357.20524 H+ 358.21252 10.04 213.1015 358.21259 0.2 3.8 9.85 0.19 5752 295
4383 MDMB-4en-PINACA C20H27N3O3 357.20524 H+ 358.21252 10.04 298.1904 358.21259 0.2 3.8 9.85 0.19 4730 214
4385 MDMB-4en-PINACA C20H27N3O3 357.20524 H+ 358.21252 10.04 358.2109 358.21259 0.2 3.8 9.84 0.2 1160 54
4711 APP-BINACA 3-phenylpropanoic Acid C21H23N3O3 365.17394 H+ 366.18122 9.19 366.17859 -7.2 5.9 9.19 0 6813 398
5071 AB-FUBINACA-D4 C20H17[2H4]FN4O2 372.18996 H+ 373.19724 8.39 373.19673 -1.4 2.3 8.13 0.26 44123 1914
5072 AB-FUBINACA-D4 C20H17[2H4]FN4O2 372.18996 H+ 373.19724 8.39 257.1015 373.19688 -0.9 2.7 8.12 0.27 6477 296
5073 AB-FUBINACA-D4 C20H17[2H4]FN4O2 372.18996 H+ 373.19724 8.39 328.1747 373.19688 -0.9 2.7 8.13 0.26 9284 414
5074 AB-FUBINACA-D4 C20H17[2H4]FN4O2 372.18996 H+ 373.19724 8.39 109.0444 373.19679 -1.2 3.1 8.11 0.28 4446 106
5075 AB-FUBINACA-D4 C20H17[2H4]FN4O2 372.18996 H+ 373.19724 8.39 356.1695 373.19688 -0.9 2.7 8.13 0.26 915 53
Sample: 231006SW_046 [2023-0210 (blood)]
Control: 231006SW_034 [Blood Matrix Blank (no ISTD)]
Rows 6744
Process
Cancel
Spectrum from 231006SW_046.wiff (sample 1) - 2023-0210 (blood), Experiment 1, +TOF MS (100 - 510) from 9.808 to 9.837 min
C20H27N3O3 H+
358.2119
359.2149
361.2228
Mass/Charge, Da
Spectrum from 231006SW_046.wiff (sample 1) - 2023-0210 (blood), Experiment 22, +T..., Experiment 22, +TOF MS^2 of 357.0 to 376.0 (40 - 510) from 9.905 to 10.007 min
Library Spectrum: MDMB-4en-PINACA , CE=35±15
% Intensity (of 1259.4)
213.1017
298.1904
67.0545
111.0044
145.0303
129.0200
145.0399
171.0921
185.0811
213.0821
214.1071
232.1160
241.1330
298.2112
299.1953
298.1563
358.2122
359.2268


File Edit Show MasterView Graph Process Bio Tool Kit Window Help
Alpha-PiHP, subrange from 2023-0185 (blood)
Alpha-PiHP, entire range
MS/MS triggered at RT = 5.6927
Intensity
Time, min
5.69
6.06
Control: Alpha-PiHP, subrange from Matrix Blank (no ISTD)
5.57
5.76
6.09
5.92
MasterView
New Session
Wiff file Name
Sample Name
Number of positive results
230828SW_112 2023-0182- 81
230828SW_113 2023-0182- 72
230828SW_114 2023-0183 148
230828SW_115 2023-0184- 156
230828SW_116 2023-0184- 196
230828SW_117 2023-0185 114
230828SW_118 2023-0186 45
230828SW_119 2023-0187 45
230828SW_120 2023-0188 154
230828SW_121 2023-0189 145
230828SW_122 2023-0190 180
Positive result: equal or better
Name Formula Mass (Da) Adduct Extraction Mass (Da) Expected RT (min) Fragment Mass (Da) Found At Mass (Da) Error (ppm) Isotope Ratio Difference (%) Found At RT (min) RT Delta (min) Intensity Area
1235 Phencyclidine (PCP) C17H25N 243.1987 H+ 244.20598 5.93 81.0708 0 0 5.89 0.04 1622 97
1236 Phencyclidine (PCP) C17H25N 243.1987 H+ 244.20598 5.93 117.0699 0 0 5.9 0.03 20519 1380
1249 Alpha-PHP C16H23NO 245.17796 H+ 246.18524 5.88 246.18457 -2.7 3.4 5.69 0.19 3929997 152436
1250 Alpha-PHP C16H23NO 245.17796 H+ 246.18524 5.88 140.143 246.18457 -2.7 3.4 5.69 0.19 27829 1373
1251 Alpha-PHP C16H23NO 245.17796 H+ 246.18524 5.88 105.0332 246.18457 -2.7 3.4 5.69 0.19 20971 1037
1252 Alpha-PHP C16H23NO 245.17796 H+ 246.18524 5.88 91.054 246.18465 -2.4 1.3 5.69 0.19 67799 3533
1253 Alpha-PHP C16H23NO 245.17796 H+ 246.18524 5.88 246.1859 246.18482 -1.7 3.9 5.69 0.19 37948 2827
1254 Alpha-PHP C16H23NO 245.17796 H+ 246.18524 5.88 175.1119 246.18457 -2.7 3.4 5.69 0.19 3622 171
1255 Alpha-PiHP C16H23NO 245.17796 H+ 246.18524 5.8 246.18457 -2.7 3.4 5.69 0.11 3929997 152436
1256 Alpha-PiHP C16H23NO 245.17796 H+ 246.18524 5.8 140.1422 246.18457 -2.7 3.4 5.69 0.11 27760 1370
1257 Alpha-PiHP C16H23NO 245.17796 H+ 246.18524 5.8 105.0324 246.18457 -2.7 3.4 5.69 0.11 20916 1035
1258 Alpha-PiHP C16H23NO 245.17796 H+ 246.18524 5.8 91.0533 246.18465 -2.4 1.3 5.69 0.11 67559 3527
1259 Alpha-PiHP C16H23NO 245.17796 H+ 246.18524 5.8 119.048 246.18465 -2.4 1.3 5.69 0.11 10701 569
1260 Alpha-PiHP C16H23NO 245.17796 H+ 246.18524 5.8 189.1143 246.18457 -2.7 3.4 5.69 0.11 4597 215
1264 Pyrovalerone C16H23NO 245.17796 H+ 246.18524 5.86 175.1121 246.18457 -2.7 3.4 5.69 0.17 3622 177
Sample: 230828SW_117 [2023-0185 (blood)]
Control: 230828SW_106 [Matrix Blank (no ISTD)]
Rows 6744
Process
Cancel
Spectrum from 230828SW_117.wiff (sample 1) - 2023-0185 (blood), Experiment 1, +TOF MS (100 - 510) from 5.702 to 5.731 min
C16H23NO H+
246.1846
247.1887
Mass/Charge, Da
Spectrum from 230828SW_117.wiff (sample 1) - 2023-0185 (blood), Experiment 11, +TO...), Experiment 11, +TOF MS^2 of 239.0 to 255.0 (40 - 510) from 5.751 to 5.852 min
Library Spectrum: Alpha-PiHP , CE=35±15
% Intensity (of 11936.7)
91.0535
246.1828
247.1873
248.1920
105.0335
140.1430
77.0387
70.0649
84.0807
92.0576
119.0492
133.0646
141.1461
175.1115
189.1141
Mass/Charge, Da


File Edit Show MasterView Graph Process Bio Tool Kit Window Help
N-Pyrrolidino Protonitazene, subrange from 2023-0176 (blood)
N-Pyrrolidino Protonitazene, entire range
MS/MS triggered at RT = 6.9905
Intensity
Time, min
Control: N-Pyrrolidino Protonitazene, subrange from Matrix Blank (blood, no ISTD)
MasterView
New Session
Wiff file Name
Sample Name
Number of positive results
Name
Formula
Mass (Da)
Adduct
Extraction Mass (Da)
Expected RT (min)
Fragment Mass (Da)
Found At Mass (Da)
Error (ppm)
Isotope Ratio Difference (%)
Found At RT (min)
RT Delta (min)
Intensity
Area
Positive result: equal or better
Sample: 230719SW_028 [2023-0176 (blood)]
Control: 230719SW_005 [Matrix Blank (blood, no ISTD)]
Rows 6606
Process
Cancel
Spectrum from 230719SW_028.wiff (sample 1) - 2023-0176 (blood), Experiment 1, +TOF MS (100 - 510) from 6.993 to 7.022 min
C23H28N4O3 H+
409.2224
410.2264
411.2307
Mass/Charge, Da
Spectrum from 230719SW_028.wiff (sample 1) - 2023-0176 (blood), Experiment 25, +TO...), Experiment 25, +TOF MS^2 of 401.0 to 417.0 (40 - 510) from 7.049 to 7.165 min
Library Spectrum: N-Pyrrolidino Protonitazene , CE=35±15
98.0960
99.0998
409.2209
410.2249
% Intensity (of 14700.4)
Mass/Charge, Da


File Edit Show MasterView Graph Process Bio Tool Kit Window Help
4'Cl-Deschloroalprazolam, subrange from 2022-0148 (blood)
4'Cl-Deschloroalprazolam, entire range
MS/MS triggered at RT = 7.4214
Intensity
Time, min
Control: 4'Cl-Deschloroalprazolam, subrange from Matrix Blank (blood, no ISTD)
MasterView
New Session
Wiff file Name
Sample Name
Number of positive results
Positive result: equal or better
Name
Formula
Mass (Da)
Adduct
Extraction Mass (Da)
Expected RT (min)
Fragment Mass (Da)
Found At Mass (Da)
Error (ppm)
Isotope Ratio Difference (%)
Found At RT (min)
RT Delta (min)
Intensity
Area
Sample: 221114SW_027 [2022-0148 (blood)]
Control: 221114SW_020 [Matrix Blank (blood, no ISTD)]
Rows 6150
Process
Cancel
Spectrum from 221114SW_027.wiff (sample 1) - 2022-0148 (blood), Experiment 1, +TOF MS (100 - 510) from 7.442 to 7.471 min
C17H13ClN4 H+
Mass/Charge, Da
Spectrum from 221114SW_027.wiff (sample 1) - 2022-0148 (blood), Experiment 17, +TO...), Experiment 17, +TOF MS^2 of 300.0 to 311.0 (40 - 510) from 7.494 to 7.610 min
Library Spectrum: 4'Cl-Deschloroalprazolam , CE=35±15
% Intensity (of 9918.6)


File Edit Show MasterView Graph Process Bio Tool Kit Window Help
Eutylone, subrange from 2022-0018
Eutylone, entire range
MS/MS triggered at RT = 4.5838
Intensity
Time, min
Control: Eutylone, subrange from Matrix Blank (No ISTD)
MasterView
New Session
Wiff file Name
Sample Name
Number of positive results
031422AK_002 SRM 94
031422AK_003 MPB 17
031422AK_004 Reagent Bl 28
031422AK_005 Matrix Blan 0
031422AK_006 High QC 210
031422AK_007 Mid QC 198
031422AK_008 Low QC 176
031422AK_009 Matrix Blan 42
031422AK_010 2022-0018 187
031422AK_011 SRM 133
031422AK_012 MPB 26
Positive result: equal or better
Name
Formula
Mass (Da)
Extraction Mass (Da)
Expected RT (min)
Fragment Mass (Da)
Found At Mass (Da)
Error (ppm)
Isotope Ratio Difference (%)
Found At RT (min)
RT Delta (min)
Intensity
Area
929 Propylone
930 Propylone
931 Dibutylone
932 Dibutylone
933 Dibutylone
934 Dibutylone
935 Dibutylone
936 Dibutylone
943 Eutylone
944 Eutylone
945 Eutylone
946 Eutylone
947 Eutylone
948 Eutylone
949 Pentylone
Spectrum from 031422AK_010.wiff (sample 1) - 2022-0018, Experiment 10, +TOF MS^2 ..., Experiment 10, +TOF MS^2 of 229.0 to 240.0 (40-510) from 5.171 to 5.308 min)
Library Spectrum: Pentylone (698963-77-8) , CE=35±15
% Intensity (of 7.2e4)
Sample: 031422AK_010 [2022-0018]
Control: 031422AK_005 [Matrix Blank (No ISTD)]
Spectrum from 031422AK_010.wiff (sample 1) - 2022-0018, Experiment 1, +TOF MS (100 - 510) from 4.607 to 4.634 min
C13H17NO3 H+
Spectrum from 031422AK_010.wiff (sample 1) - 2022-0018, Experiment 10, +TOF MS^2 ..., Experiment 10, +TOF MS^2 of 229.0 to 240.0 (40-510) from 4.652 to 4.802 min)
Library Spectrum: Eutylone , CE=35±15
% Intensity (of 1.1e5)

Index	Component Name	Mass Error...	RT Confi...	Isotope Confi...	Library Confi...	Formula	Add... / Ch...	Precursor Mass	Fragment Mass	Expected RT	Found At Mass	Mass Error (...	Isotope Ratio...	Retent... Time	Retenti... Time D...	Height	Area	Libr...
6264	5/6-APB	✓	✓	✓	✓	C11H13NO	[M+H]+	176.1070	N/A	4.62	176.1069	-0.6	4.8	4.74	0.12	3.880e5	1.644e6	6-APB [Smart
6265	5/6-APB_1	✓	✓	✓	✓	C11H13NO	[M+H]+	176.1070	131.0494	4.62	176.1071	0.4	3.8	4.74	0.12	1.931e5	7.966e5	6-APB [Smart
6266	5/6-APB_2	✓	✓	✓	✓	C11H13NO	[M+H]+	176.1070	91.0550	4.62	176.1071	0.4	3.8	4.74	0.12	5.634e4	2.249e5	6-APB [Smart
6267	5/6-APB_3	✓	✓	✓	✓	C11H13NO	[M+H]+	176.1070	159.0805	4.62	176.1071	0.4	3.8	4.75	0.13	2.598e4	1.332e5	6-APB [Smart
6268	5/6-APB_4	✓	✓	✓	✓	C11H13NO	[M+H]+	176.1070	116.0625	4.62	176.1071	0.4	3.8	4.74	0.12	1.638e4	6.496e4	6-APB [Smart
6269	5/6-APB_5	✓	✓	✓	✓	C11H13NO	[M+H]+	176.1070	115.0547	4.62	176.1071	0.4	3.8	4.74	0.12	1.644e4	6.263e4	6-APB [Smart
6378	Theophylline	▲	✓	✓	▲	C7H8N4O2	[M+H]+	181.0720	N/A	3.32	181.0730	5.3	14.1	3.42	0.10	8.631e2	2.579e3	Theophylline
6402	Fluoroethamphe...	✓	✓	✓	✓	C11H16FN	[M+H]+	182.1340	N/A	4.50	182.1347	3.9	2.4	4.63	0.13	1.393e3	4.493e3	Fluoroethamp

EXAMPLE OF “TRUE” UNKNOWNS

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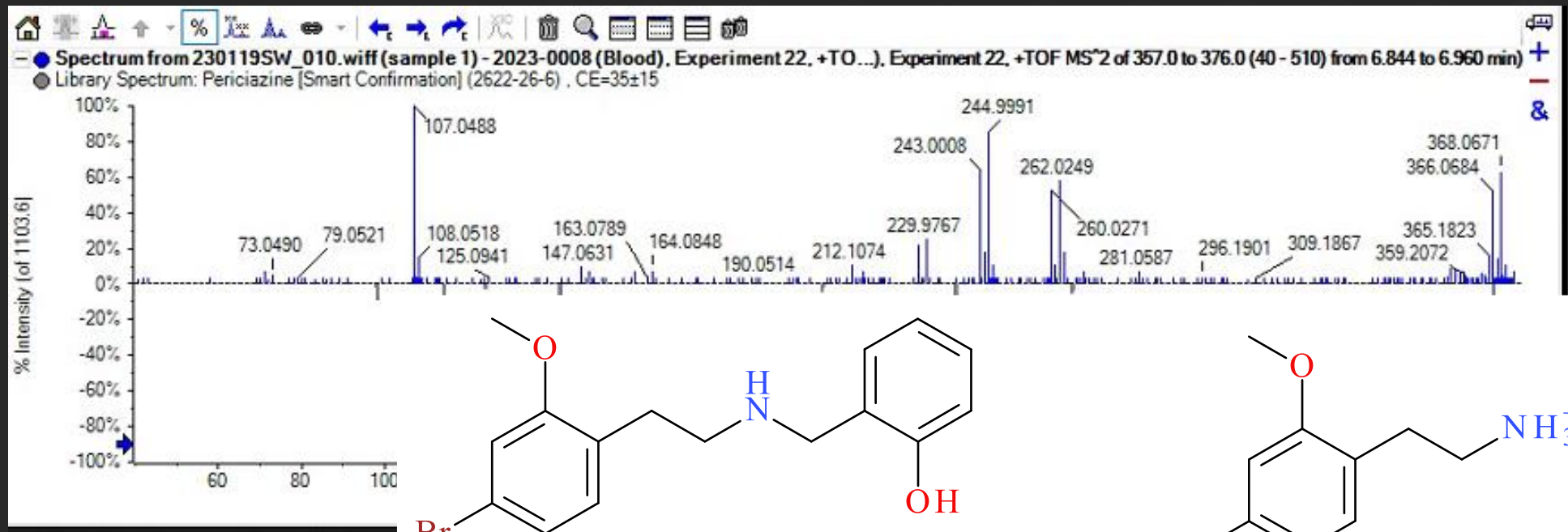
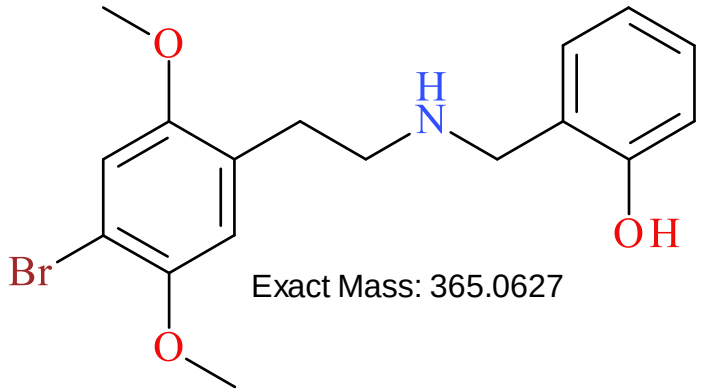
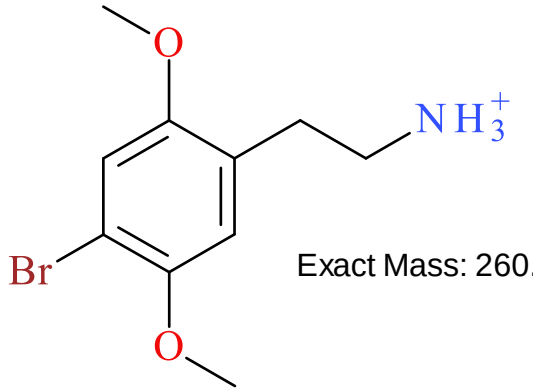
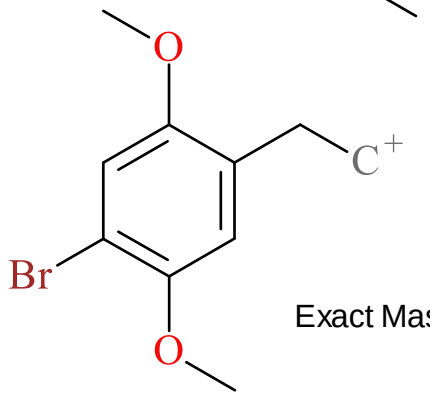




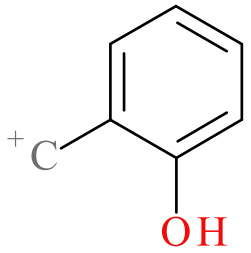
Exact Mass: 365.0627



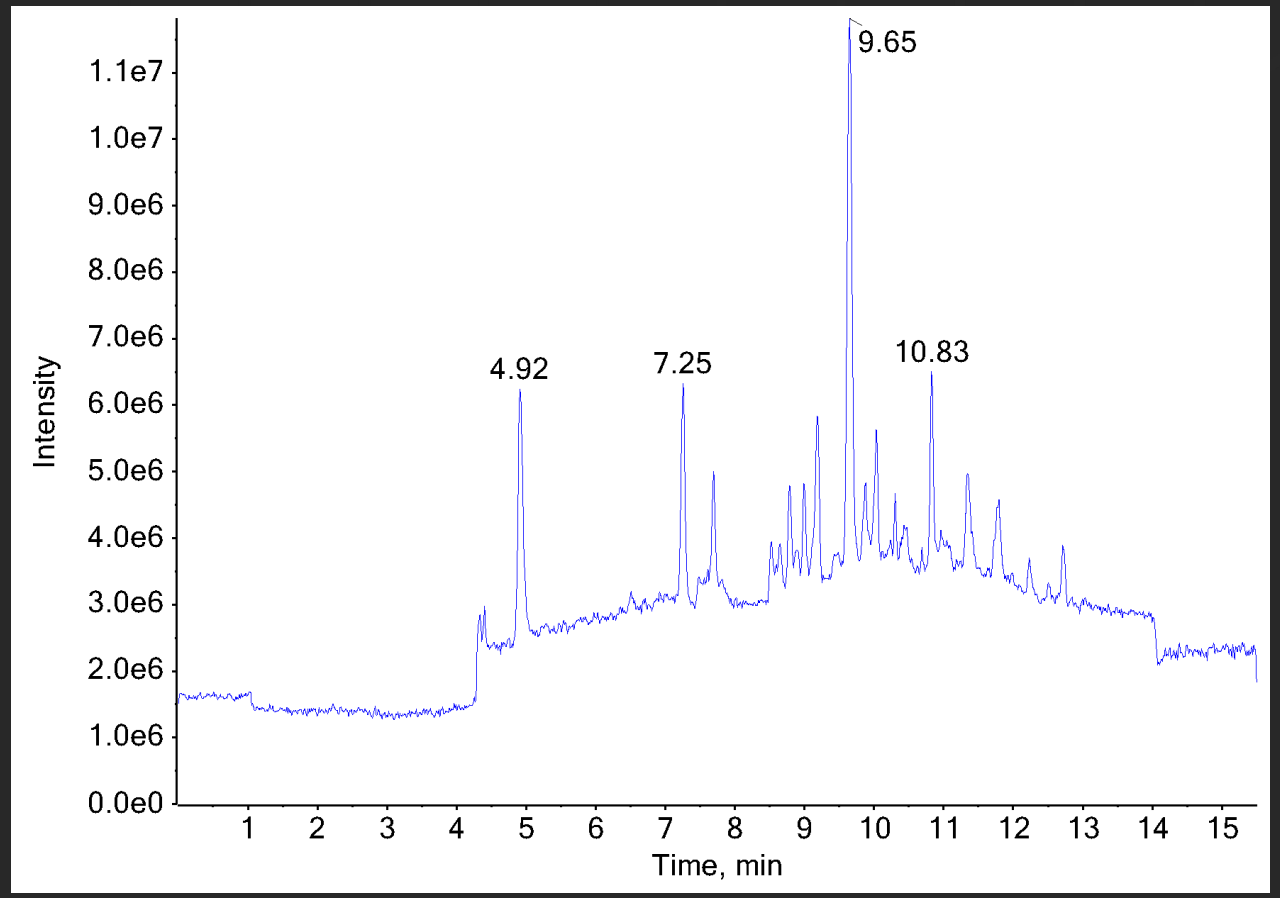
Exact Mass: 260.0286

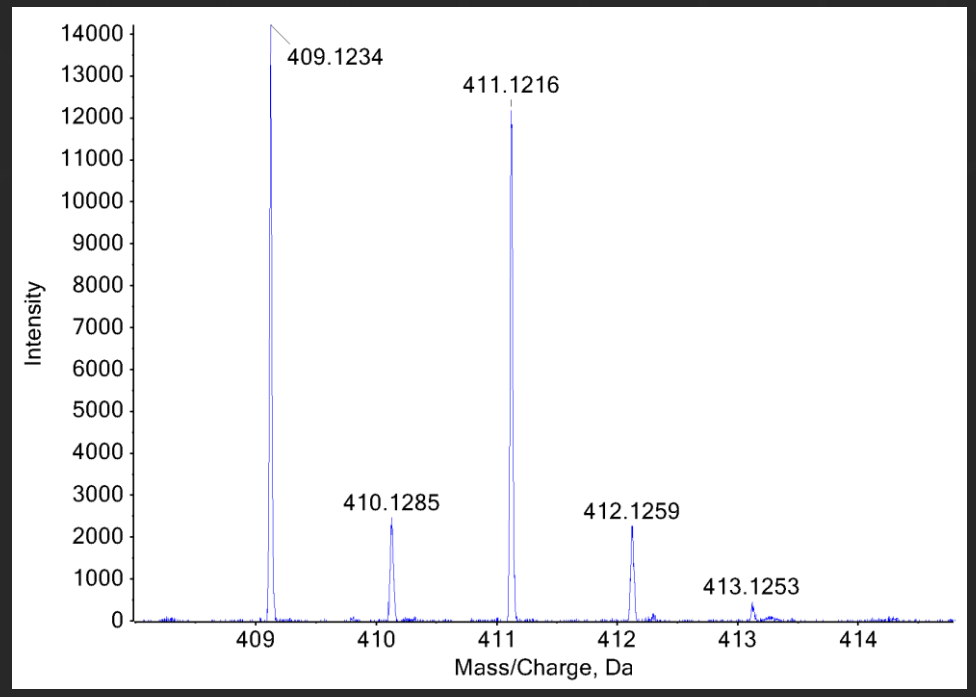


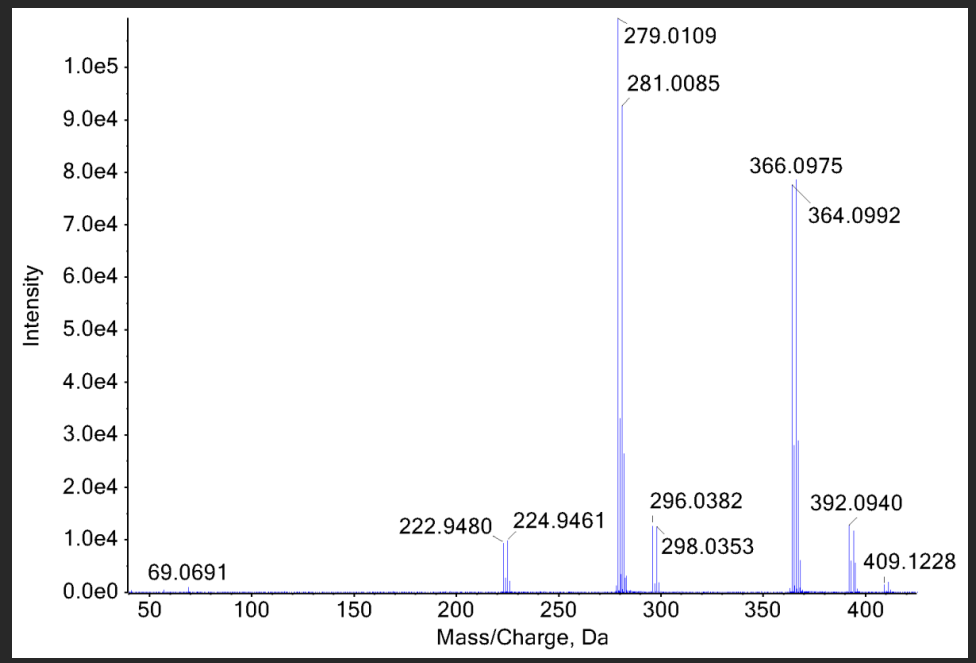
Exact Mass: 243.0021



Exact Mass: 107.0497







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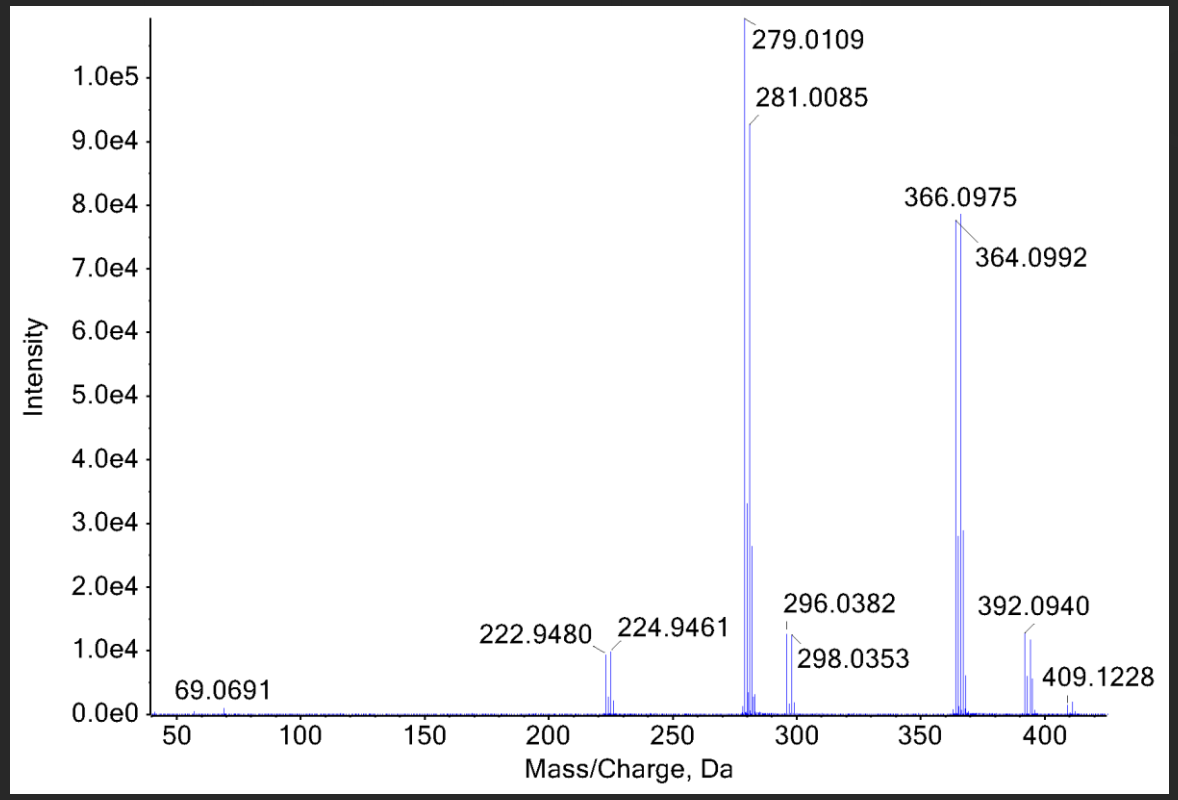
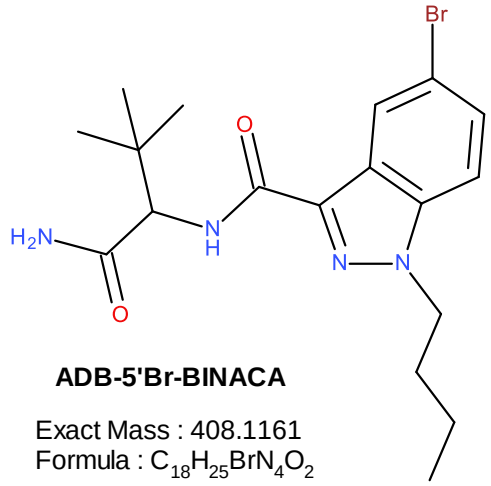
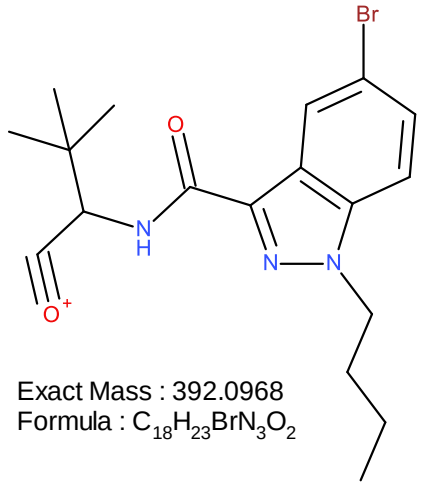


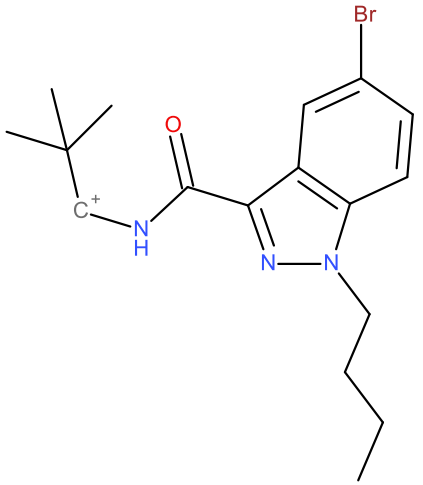


ADB-5'Br-BINACA

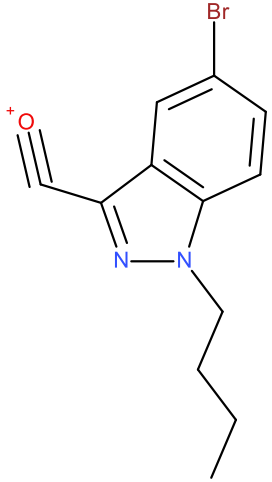
Exact Mass : 408.1161
Formula : $C_{18}H_{25}BrN_4O_2$



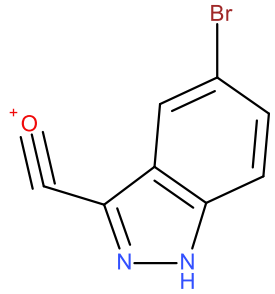
Exact Mass : 392.0968
Formula : $C_{18}H_{23}BrN_3O_2$



Exact Mass : 364.1019
Formula : $C_{17}H_{23}BrN_3O$



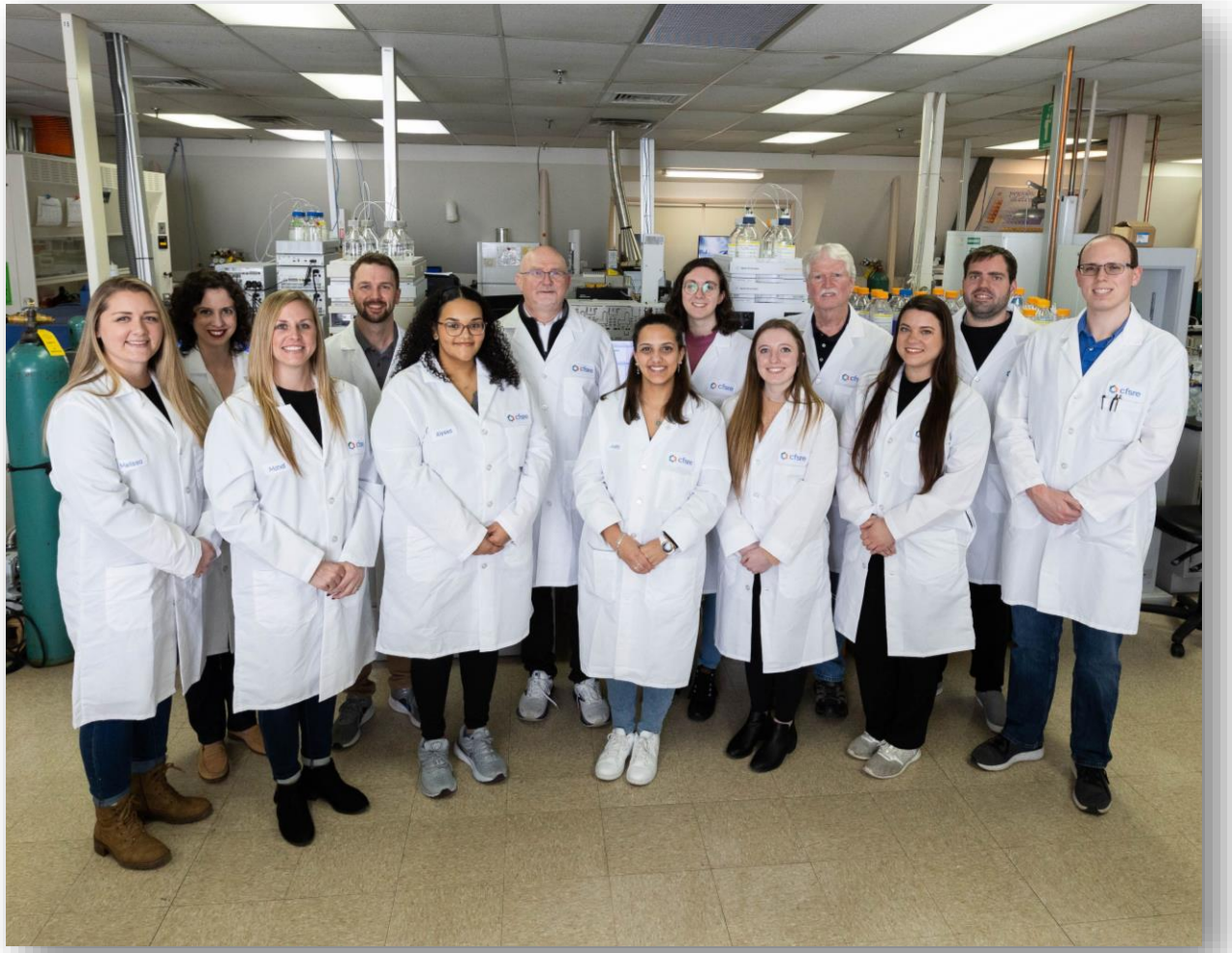
Exact Mass : 279.0128
Formula : $C_{12}H_{12}BrN_2O$



Exact Mass : 222.9502
Formula : $C_8H_4BrN_2O$

ACKNOWLEDGEMENTS

- SCIEX
- CFSRE Team
- Funding Agencies
  - NIJ
- Collaborators & Partners



HAPPY
HALLOWEEN

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THANK YOU! QUESTIONS?

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Associate Director – CFSRE
Program Manager – NPS Discovery
alex.krotulski@cfsre.org