Using Visualization Tools to Understand Drug Evidence Handling Processes

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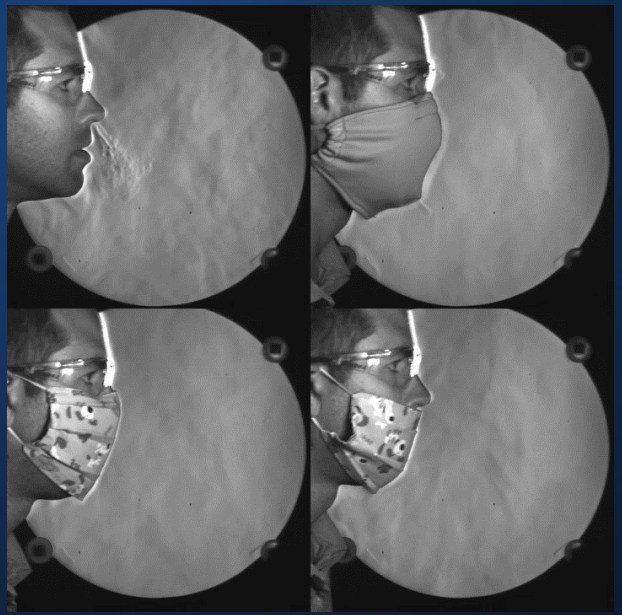
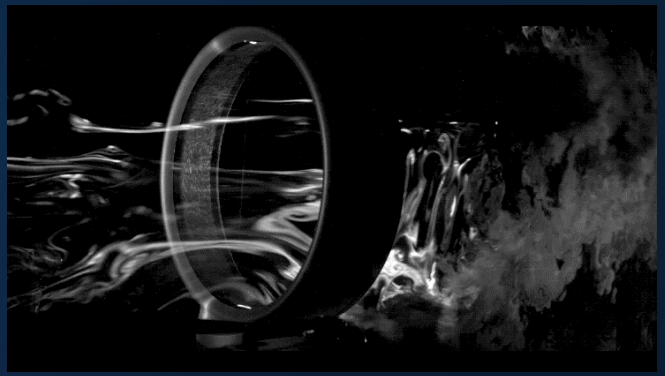
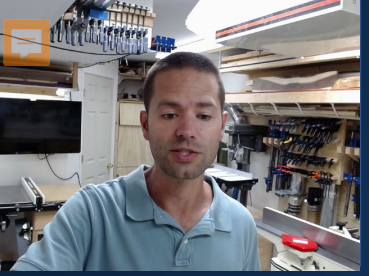
Quick background – flow visualization and scientific imaging

Many uses in the Surface and Trace Chemical Analysis Group, NIST



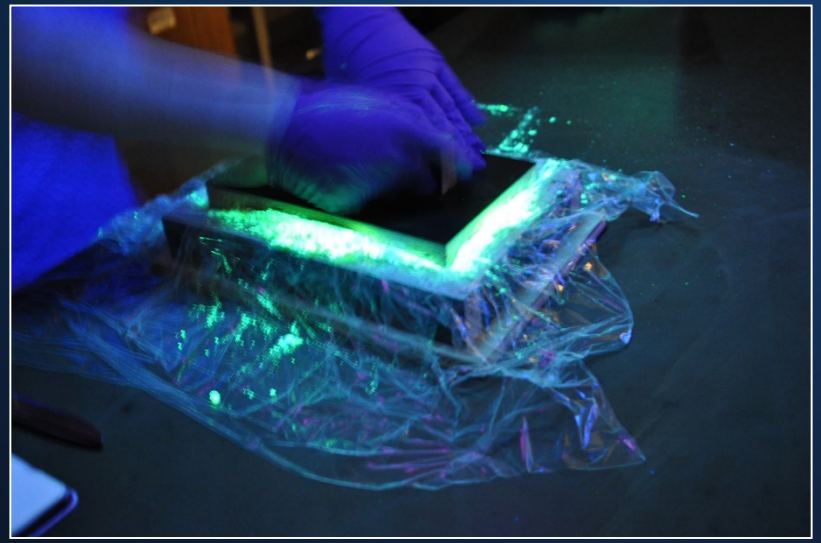


Schlieren imaging, high speed videography, laser-sheet imaging



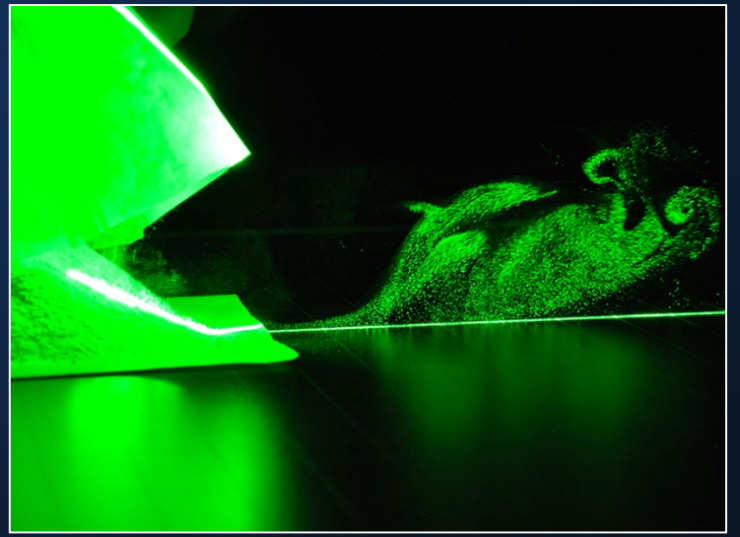
Two visualization methods

Fluorescent powder handling and visualization



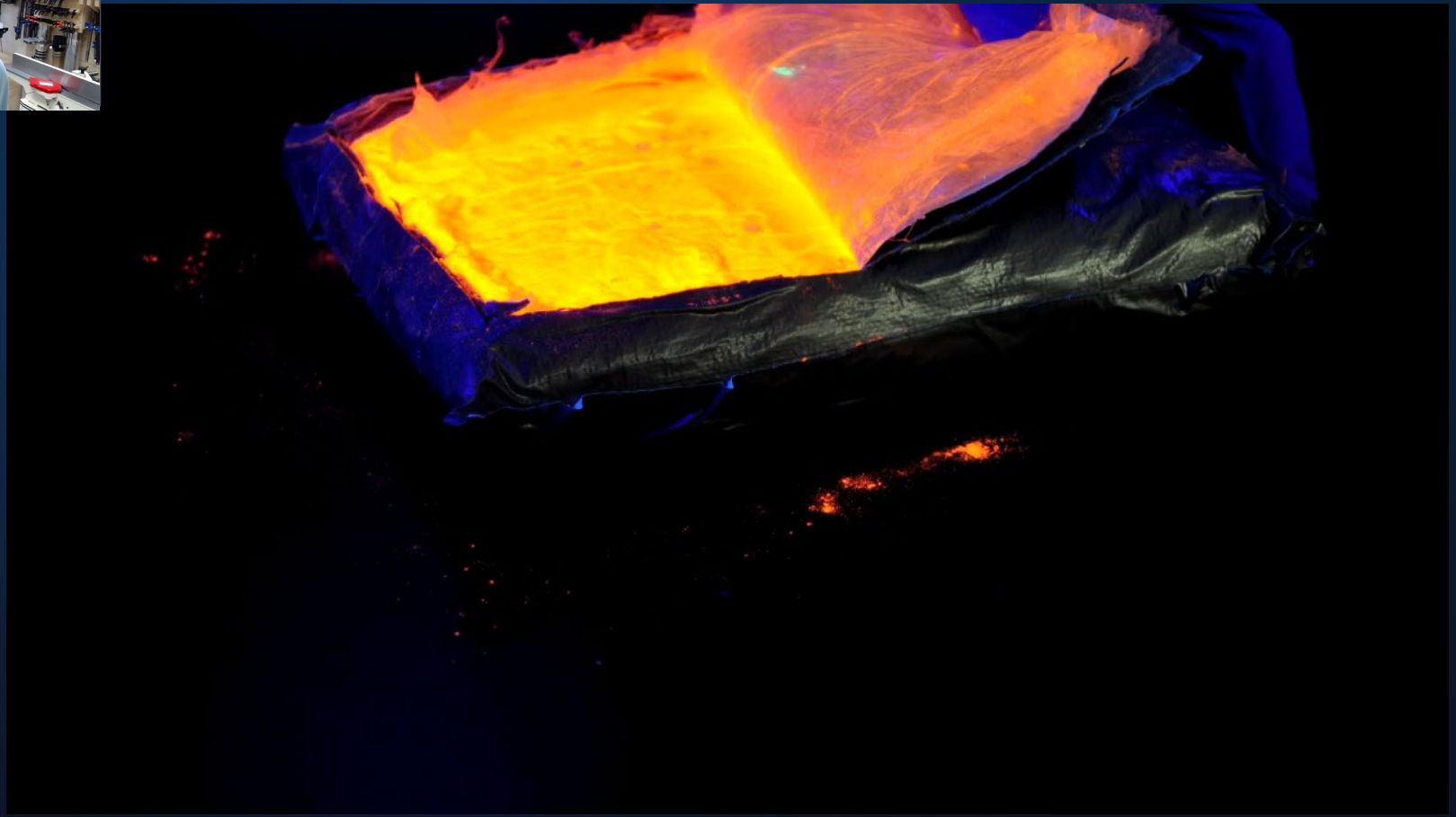
Created fluorescently tagged, mock drug evidence and had examiners handle it as they normally would. Recorded the entire process under a blacklight

Laser-sheet visualization



Lasers and optics help illuminate microparticles during net-weight operations. Provides 2D slice of the transport of particles during these activities.

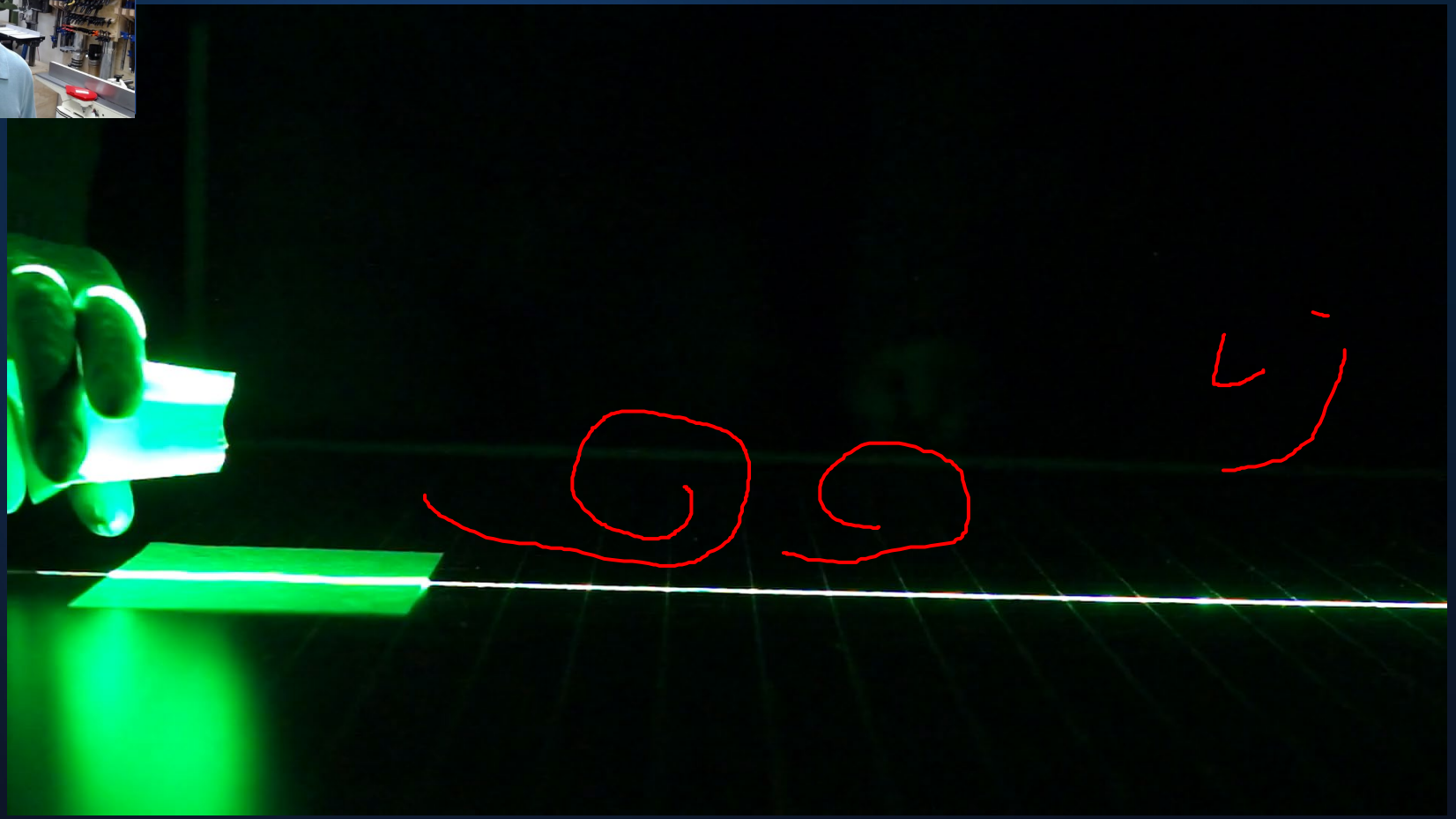
Fluorescent powder visualization



Take-aways from fluorescent power experiments

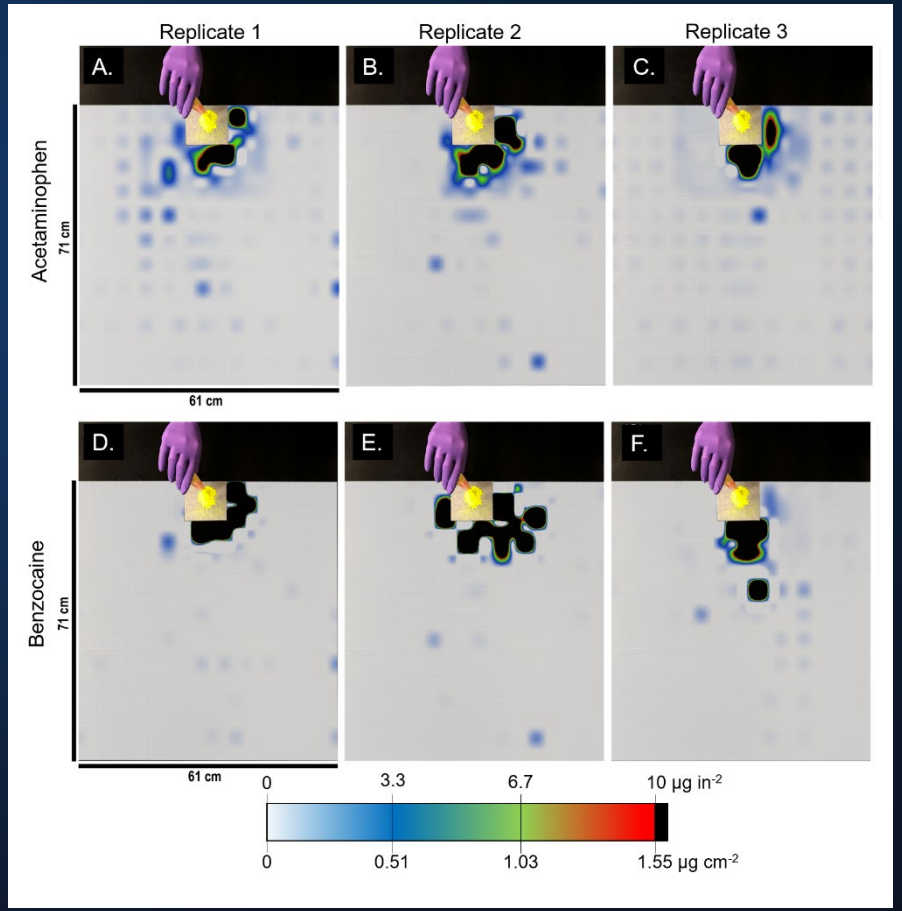
- Net weights were quickly identified as one of the most concerning practices
  - Emptying the entire contents of the drug evidence to obtain the weight of the material (powder) without the packaging
  - Required for prosecution based on weight
- Repackaging of evidence also of concern

Laser-sheet visualization ~2 g powder

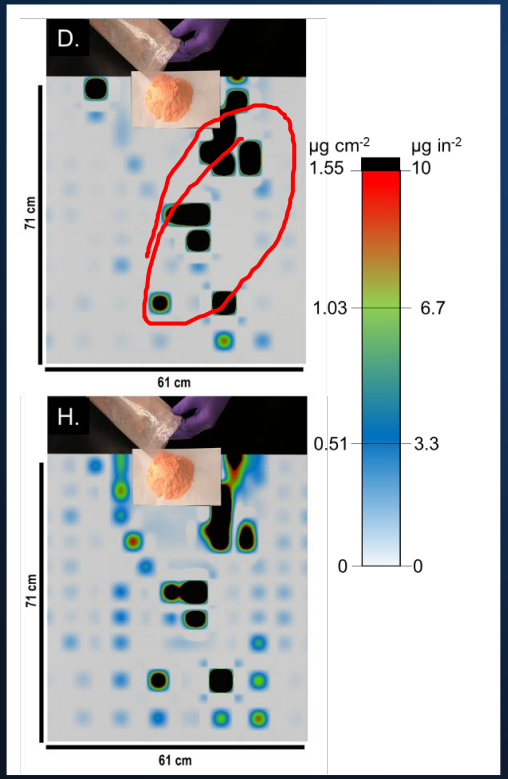


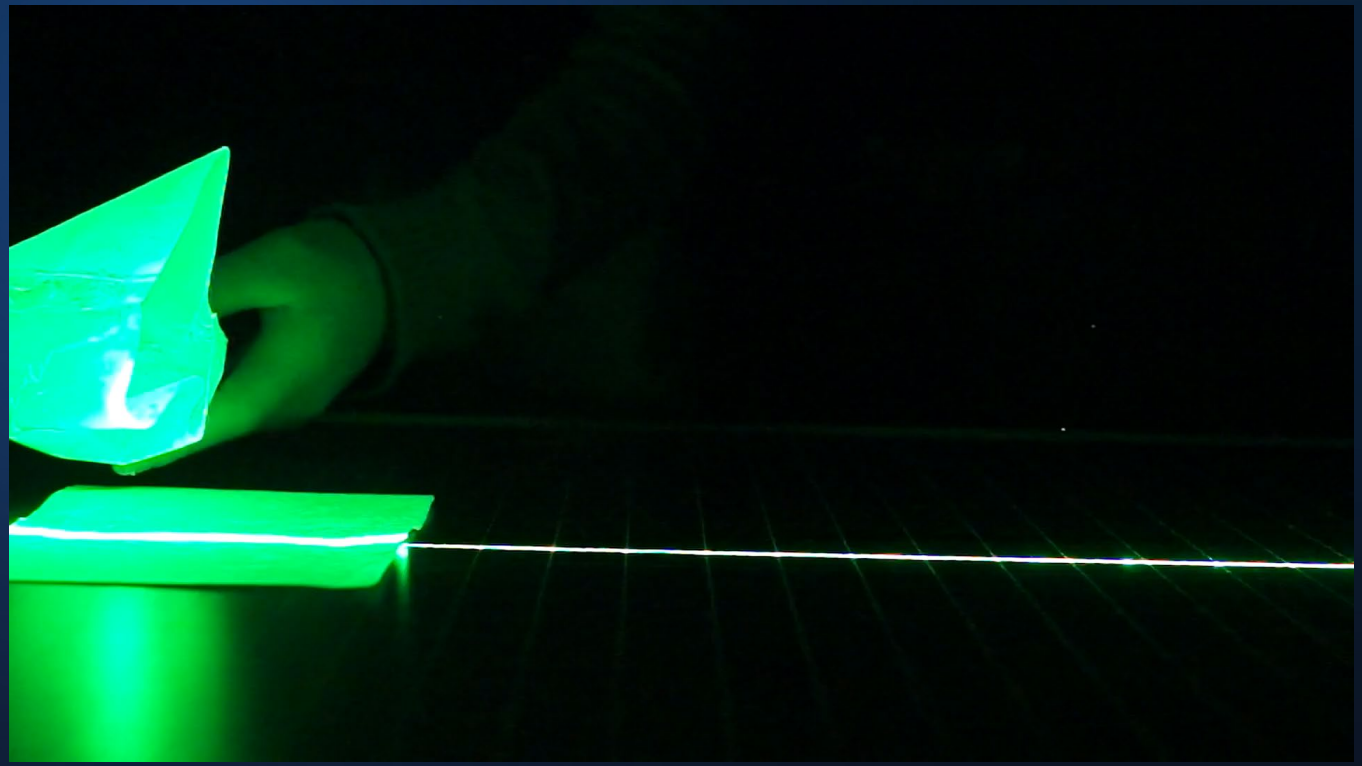
Measuring the Distribution

- Wet swabbing was completed in a grid-pattern to collect residue that settled onto the bench after several minutes
- As expected, the highest background was observed in area immediately surrounding the weigh paper
- Surface concentrations in excess of 10 µg/in^2 observed
- Airflow was not controlled in these experiments



Laser-sheet visualization ~100 g powder





New contamination visualization laboratory

New facility that improves visualization and imaging techniques

Current efforts are focused on:

- Particulate transport in the third dimension?
- Expanding studies to other workplace processes
- Visualize process modifications that minimize exposure risks

Summary

- Our goal is to increase the safety of drug chemists due to the increasing presence of extremely toxic substances
- We are developing imaging tools and techniques that help visualize the processes that increase exposure risk, and evaluate the efficacy of process modifications
- Collaborations with other agencies have aided in interpretation of analyst risk and development of best practices
- While the current focus is on seized drugs these processes and approaches could easily be translated to other areas

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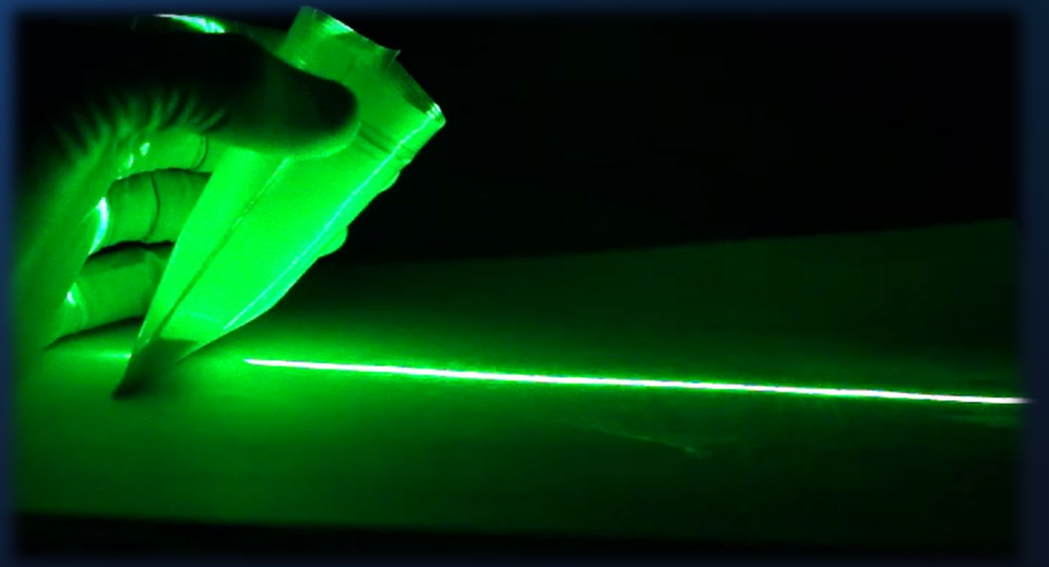
Thanks for listening!

Questions or Comments?

Many thanks to Amber Burns (Maryland State Police) and Ed Sisco (NIST)!

- A snapshot of drug background levels
- A multi-laboratory investigation of drug background levels
- Visualizing particle spread
- Net weights: Visualizing and quantifying
- Cleaning agents removing drugs



Development of Novel Workflows for Seized Drug Analysis

Edward Sisco - NIST

Amber Burns – MSP-FSD

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Certain commercial products are identified in order to adequately specify the procedure; this does not imply endorsement or recommendation by NIST, nor does it imply that such products are necessarily the best available for the purpose.

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Novel Workflows

Sample Handling and Preparation

Sample Analysis

Data Analysis & Interpretation

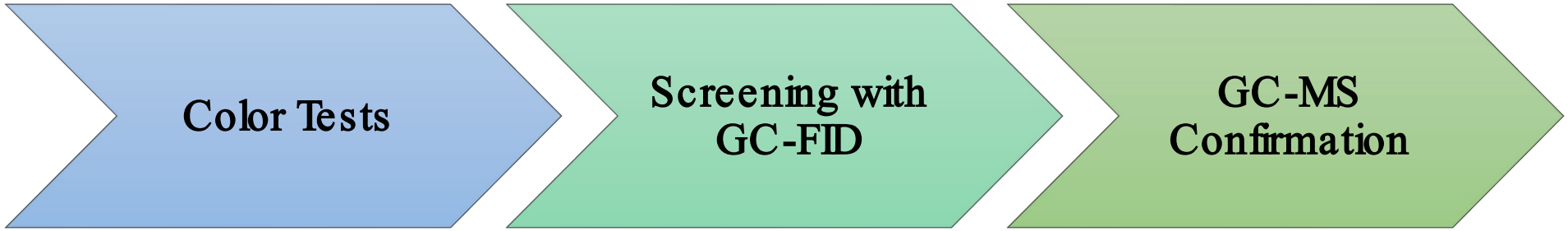
Screening Approaches – Expanding DART-MS Capabilities

Confirmatory Analyses – Targeted GC-MS Methods

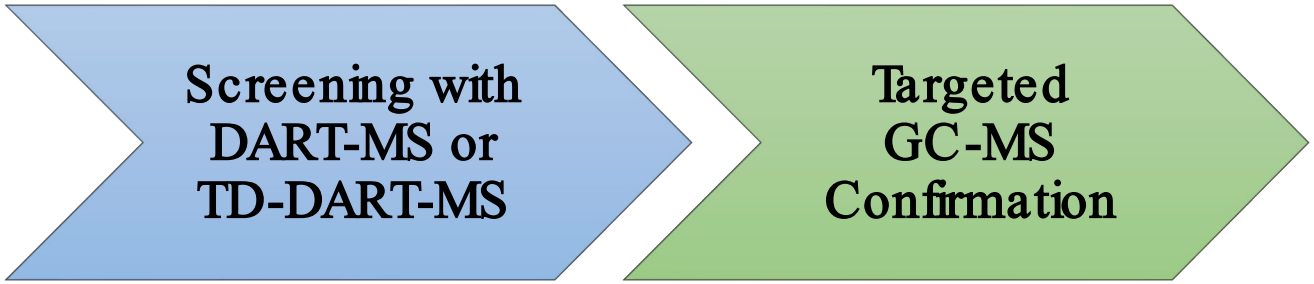
Workflow Shift

A large part of the development and implementation of this work has been done in collaboration with Maryland State Police, Forensic Sciences Division

Current Approach



New Approach



Expanding DART-MS Capabilities

NIST National Institute of Standards and Technology
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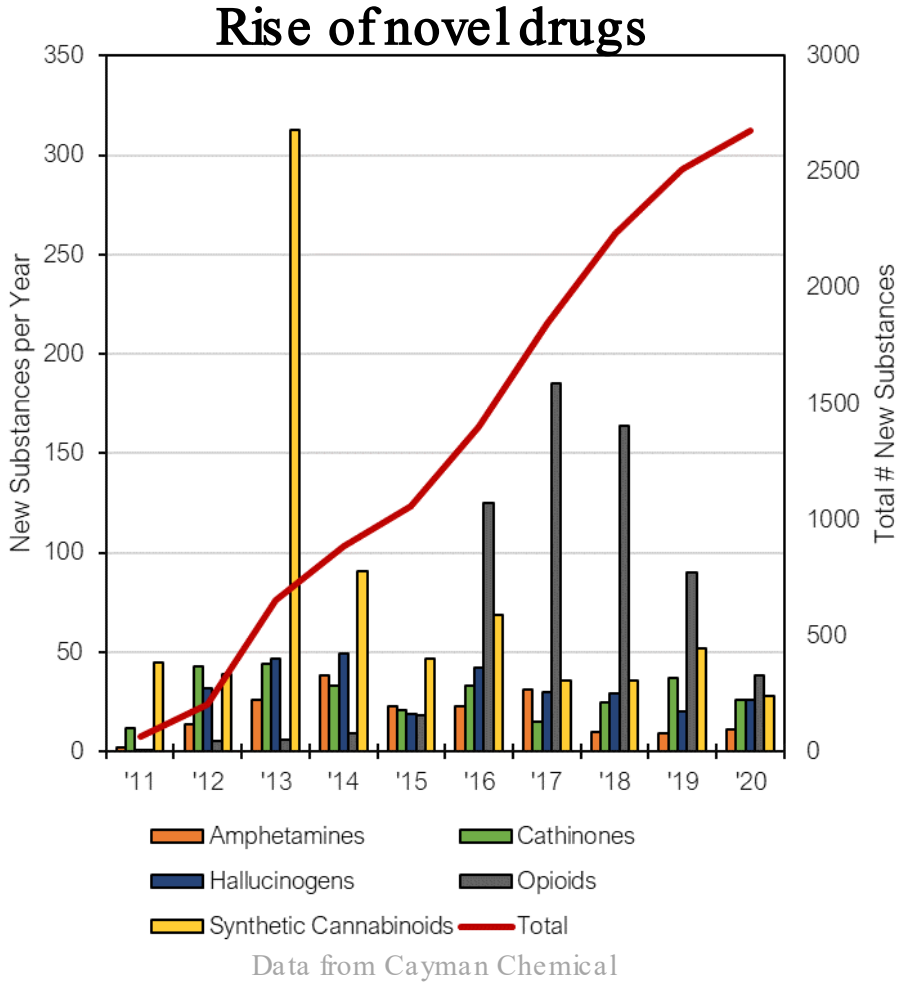
DART-MS in Forensics

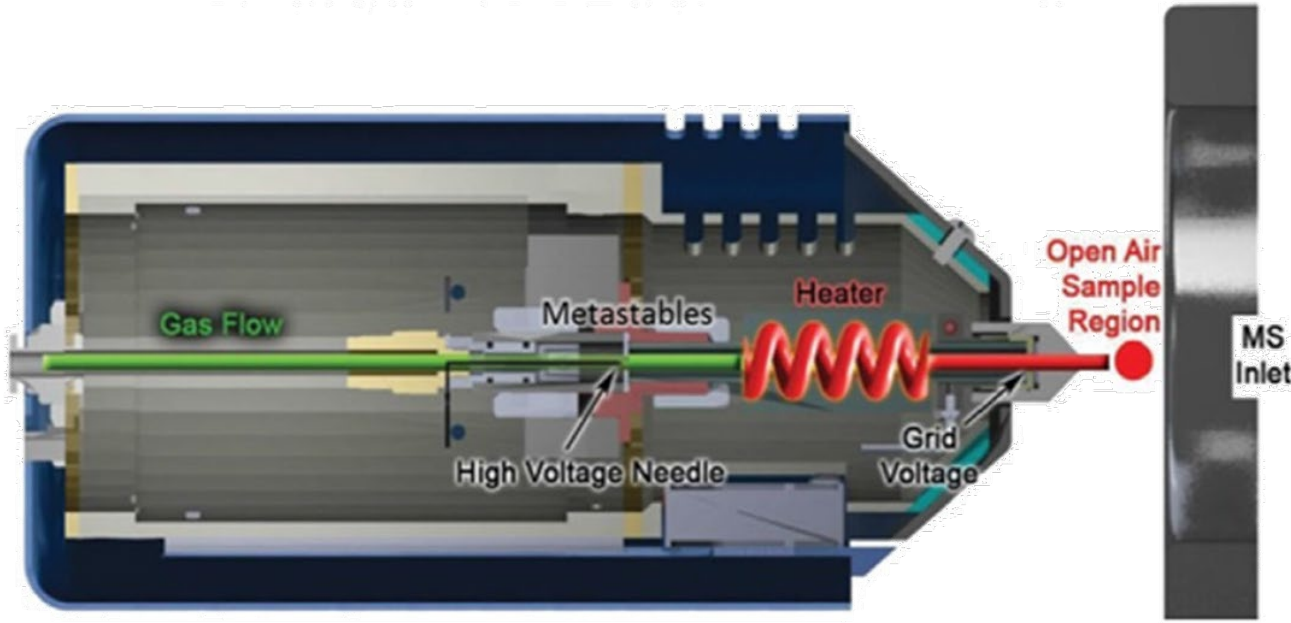
With the growing presence of novel drugs and increased complexity in cases, some labs are searching for technologies to aid in rapid screening

- DART-MS has been demonstrated as a powerful tool for this purpose
- Provides presumptive information in seconds with no sample preparation
- More specific than other presumptive tests
- Significant research effort at NIST surrounding DART-MS and its applications in the field



What is DART-MS?

- One of many ambient ionization mass spectrometry sources
- Conventional DART-MS uses a heated helium metastable gas stream for sample desorption and ionization
- Allows for analysis of samples with minimal preparation or pre-treatment
- Analysis time 1 s to 5 s
- Typical LODs ppm to ppb
- Can be coupled to a range of mass spectrometers

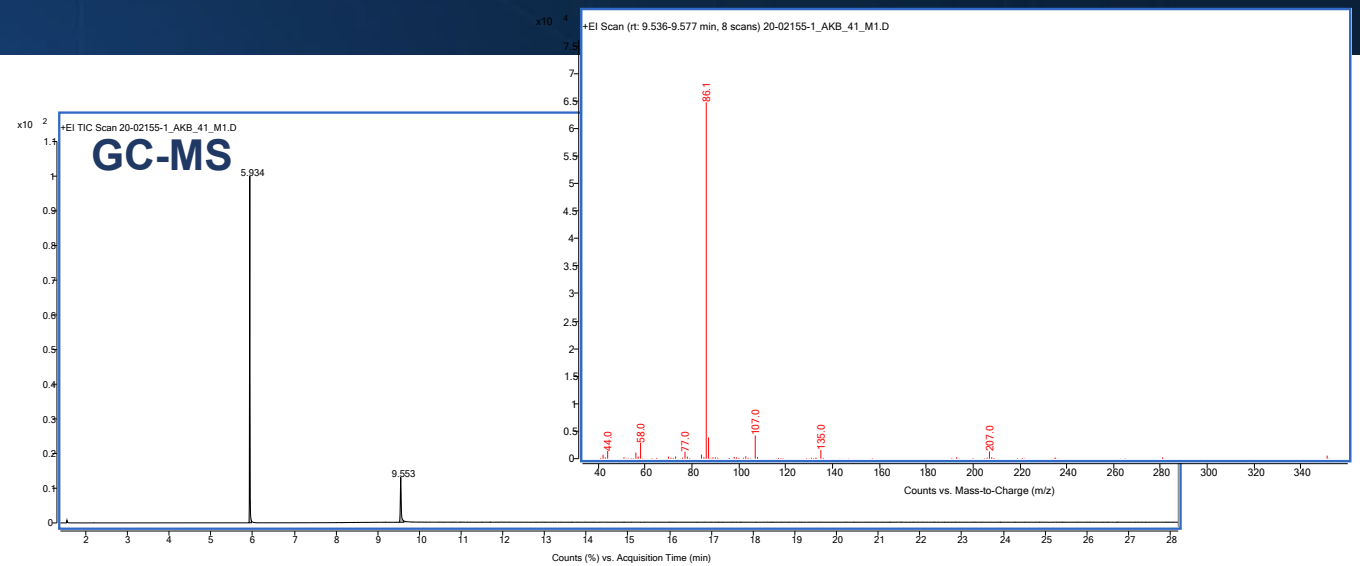


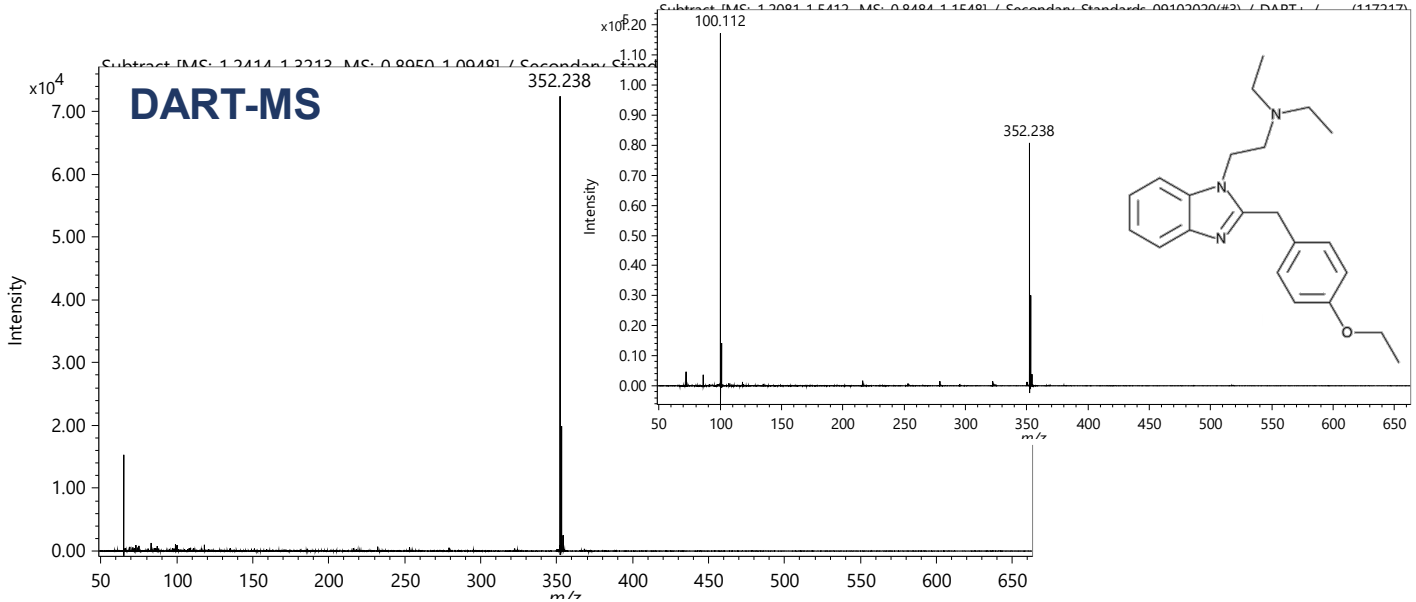
www.ionsense.com

DART-MS – Direct Analysis in Real Time Mass Spectrometry

DART-MS Use Cases

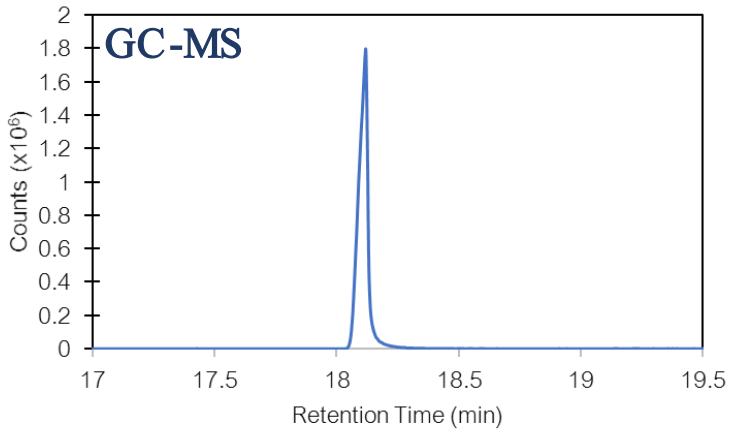
- We have been working with labs to identify unique use cases for DART-MS
- Utilizing GC-MS & DART-MS data can help identify unknowns
- Allows for determination of fragmentation and molecular ion of the compound
- Used to identify multiple unknown fentanyls and other NPSs

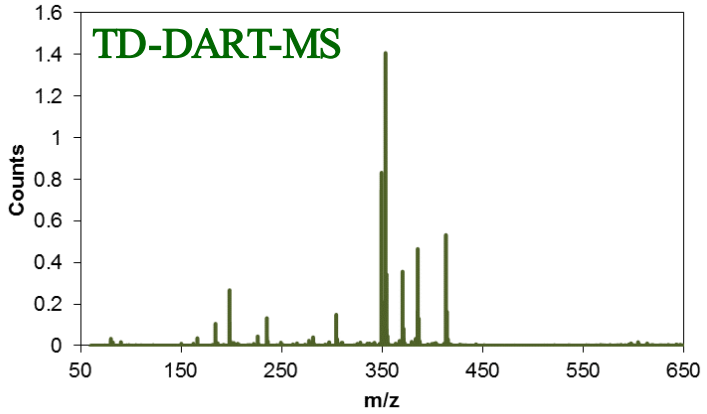


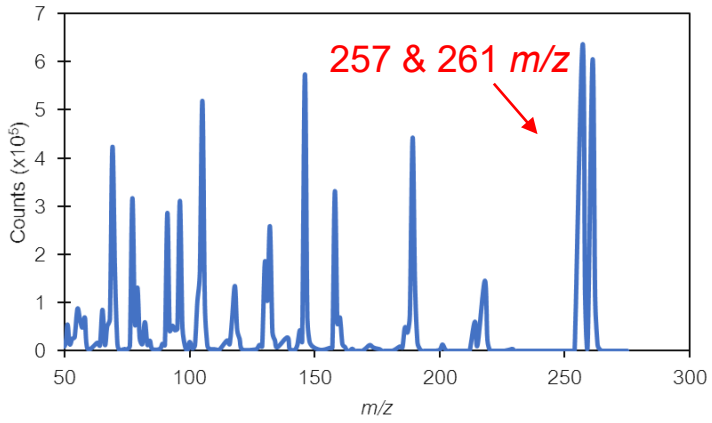


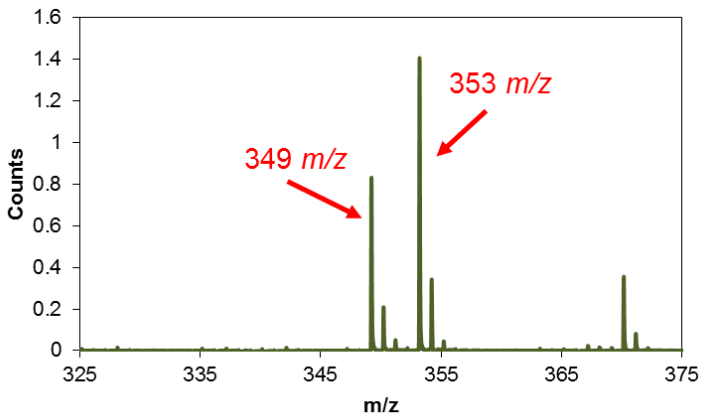
DART-MS Use Cases

Utilize DART-MS to identify compounds that were completely not resolvable in the GC chromatograph



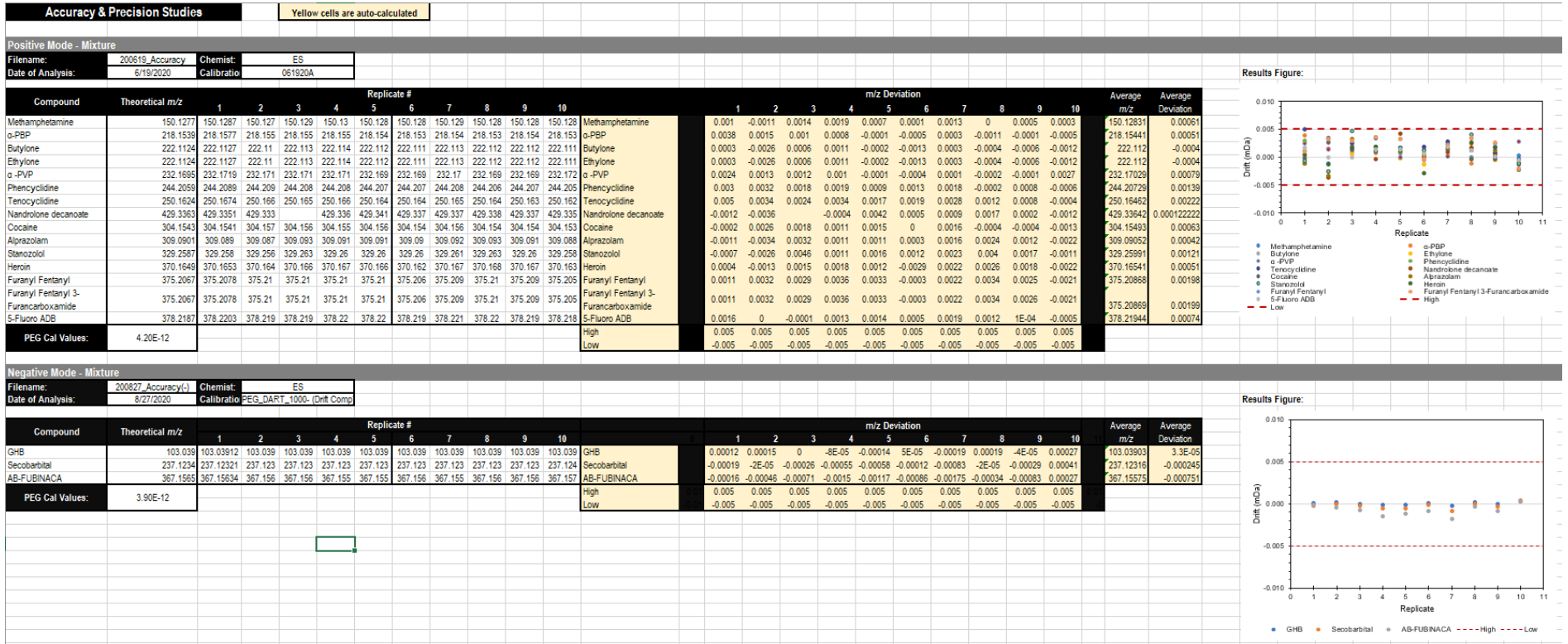






Validation Package Development

- Ongoing efforts to develop a DART-MS Validation package
- Includes validation plan, data workup document, SOPs, maintenance manuals, search lists, and training questions
- Available to labs who are interested



Accuracy & Precision Studies

Yellow cells are auto-calculated

Positive Mode - Mixture

Filename:	200619_Accuracy	Chemist:	ES
Date of Analysis:	6/19/2020	Calibratio	061920A

Compound	Theoretical *m/z*	Replicate # 1	2	3	4	5	6	7	8	9	10
Methamphetamine	150.1277	150.1287	150.127	150.129	150.13	150.128	150.128	150.129	150.128	150.128	150.128
α-PBP	218.1539	218.1577	218.155	218.155	218.155	218.154	218.153	218.154	218.153	218.154	218.153
Butylone	222.1124	222.1127	222.11	222.113	222.114	222.112	222.111	222.113	222.112	222.112	222.111
Ethylone	222.1124	222.1127	222.11	222.113	222.114	222.112	222.111	222.113	222.112	222.112	222.111
α -PVP	232.1695	232.1719	232.171	232.171	232.171	232.169	232.169	232.17	232.169	232.169	232.172
Phencyclidine	244.2059	244.2089	244.209	244.208	244.208	244.207	244.207	244.208	244.206	244.207	244.205
Tenocyclidine	250.1624	250.1674	250.166	250.165	250.166	250.164	250.164	250.165	250.164	250.163	250.162
Nandrolone decanoate	429.3363	429.3351	429.333		429.336	429.341	429.337	429.337	429.338	429.337	429.335
Cocaine	304.1543	304.1541	304.157	304.156	304.155	304.156	304.154	304.156	304.154	304.154	304.153
Alprazolam	309.0901	309.089	309.087	309.093	309.091	309.091	309.09	309.092	309.093	309.091	309.088
Stanozolol	329.2587	329.258	329.256	329.263	329.26	329.26	329.26	329.261	329.263	329.26	329.258
Heroin	370.1649	370.1653	370.164	370.166	370.167	370.166	370.162	370.167	370.168	370.167	370.163
Furanyl Fentanyl	375.2067	375.2078	375.21	375.21	375.21	375.21	375.206	375.209	375.21	375.209	375.205
Furanyl Fentanyl 3-Furancarboxamide	375.2067	375.2078	375.21	375.21	375.21	375.21	375.206	375.209	375.21	375.209	375.205
5-Fluoro ADB	378.2187	378.2203	378.219	378.219	378.22	378.22	378.219	378.221	378.22	378.219	378.218
PEG Cal Values:	4.20E-12										

Compound	m/z Deviation 1	2	3	4	5	6	7	8	9	10	Average *m/z*	Average Deviation
Methamphetamine	0.001	-0.0011	0.0014	0.0019	0.0007	0.0001	0.0013	0	0.0005	0.0003	150.12831	0.00061
α-PBP	0.0038	0.0015	0.001	0.0008	-0.0001	-0.0005	0.0003	-0.0011	-0.0001	-0.0005	218.15441	0.00051
Butylone	0.0003	-0.0026	0.0006	0.0011	-0.0002	-0.0013	0.0003	-0.0004	-0.0006	-0.0012	222.112	-0.0004
Ethylone	0.0003	-0.0026	0.0006	0.0011	-0.0002	-0.0013	0.0003	-0.0004	-0.0006	-0.0012	222.112	-0.0004
α -PVP	0.0024	0.0013	0.0012	0.001	-0.0001	-0.0004	0.0001	-0.0002	-0.0001	0.0027	232.17029	0.00079
Phencyclidine	0.003	0.0032	0.0018	0.0019	0.0009	0.0013	0.0018	-0.0002	0.0008	-0.0006	244.20729	0.00139
Tenocyclidine	0.005	0.0034	0.0024	0.0034	0.0017	0.0019	0.0028	0.0012	0.0008	-0.0004	250.16462	0.00222
Nandrolone decanoate	-0.0012	-0.0036		-0.0004	0.0042	0.0005	0.0009	0.0017	0.0002	-0.0012	429.33642	0.000122222
Cocaine	-0.0002	0.0026	0.0018	0.0011	0.0015	0	0.0016	-0.0004	-0.0004	-0.0013	304.15493	0.00063
Alprazolam	-0.0011	-0.0034	0.0032	0.0011	0.0011	0.0003	0.0016	0.0024	0.0012	-0.0022	309.09052	0.00042
Stanozolol	-0.0007	-0.0026	0.0046	0.0011	0.0016	0.0012	0.0023	0.004	0.0017	-0.0011	329.25991	0.00121
Heroin	0.0004	-0.0013	0.0015	0.0018	0.0012	-0.0029	0.0022	0.0026	0.0018	-0.0022	370.16541	0.00051
Furanyl Fentanyl	0.0011	0.0032	0.0029	0.0036	0.0033	-0.0003	0.0022	0.0034	0.0025	-0.0021	375.20868	0.00198
Furanyl Fentanyl 3-Furancarboxamide	0.0011	0.0032	0.0029	0.0036	0.0033	-0.0003	0.0022	0.0034	0.0026	-0.0021	375.20869	0.00199
5-Fluoro ADB	0.0016	0	-0.0001	0.0013	0.0014	0.0005	0.0019	0.0012	1E-04	-0.0005	378.21944	0.00074
High	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005		
Low	-0.005	-0.005	-0.005	-0.005	-0.005	-0.005	-0.005	-0.005	-0.005	-0.005		

Results Figure:

Negative Mode - Mixture

Filename:	200827_Accuracy(-)	Chemist:	ES
Date of Analysis:	8/27/2020	Calibratio	PEG_DART_1000- (Drift Comp

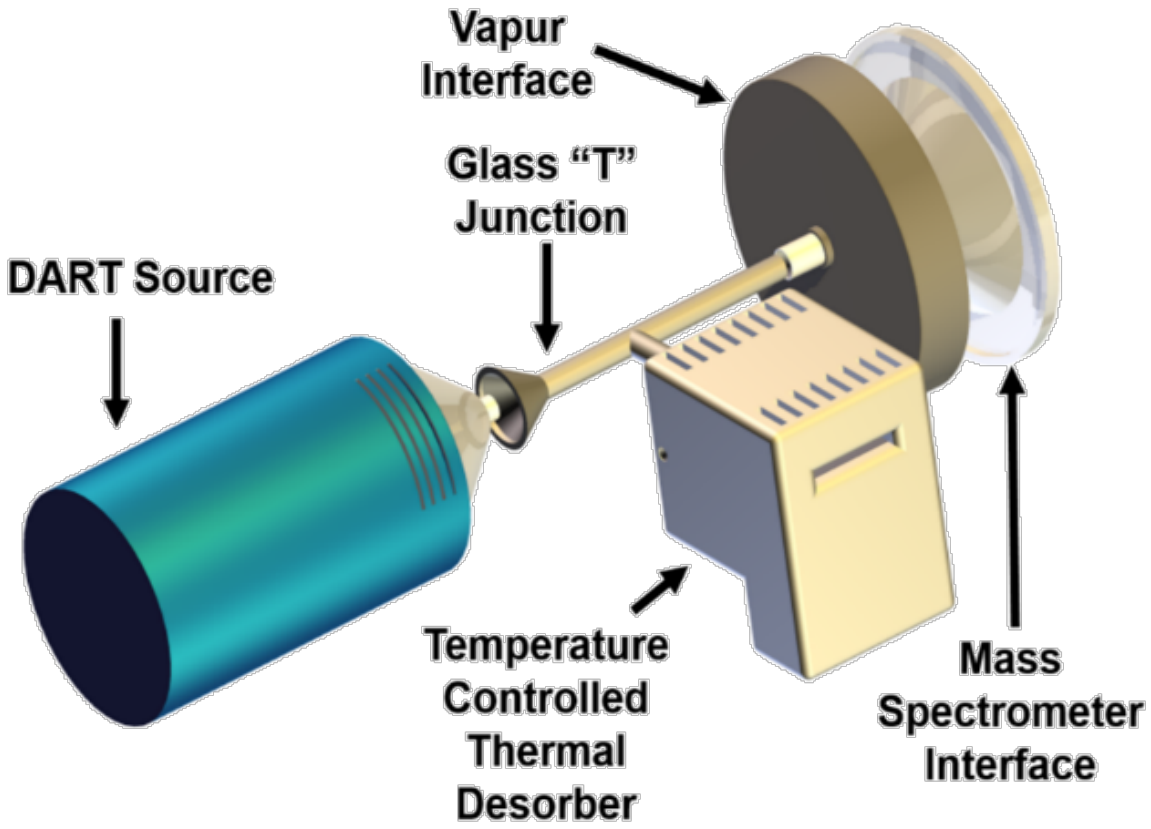
Compound	Theoretical *m/z*	Replicate # 1	2	3	4	5	6	7	8	9	10
GHB	103.039	103.03912	103.039	103.039	103.039	103.039	103.039	103.039	103.039	103.039	103.039
Secobarbital	237.1234	237.12321	237.123	237.123	237.123	237.123	237.123	237.123	237.123	237.123	237.124
AB-FUBINACA	367.1565	367.15634	367.156	367.156	367.155	367.155	367.156	367.155	367.156	367.156	367.157
PEG Cal Values:	3.90E-12										

Compound	m/z Deviation 1	2	3	4	5	6	7	8	9	10	Average *m/z*	Average Deviation
GHB	0.00012	0.00015	0	-8E-05	-0.00014	5E-05	-0.00019	0.00019	-4E-05	0.00027	103.03903	3.3E-05
Secobarbital	-0.00019	-2E-05	-0.00026	-0.00055	-0.00058	-0.00012	-0.00083	-2E-05	-0.00029	0.00041	237.12316	-0.000245
AB-FUBINACA	-0.00016	-0.00046	-0.00071	-0.0015	-0.00117	-0.00086	-0.00175	-0.00034	-0.00083	0.00027	367.15575	-0.000751
High	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005		
Low	-0.005	-0.005	-0.005	-0.005	-0.005	-0.005	-0.005	-0.005	-0.005	-0.005		

Results Figure:

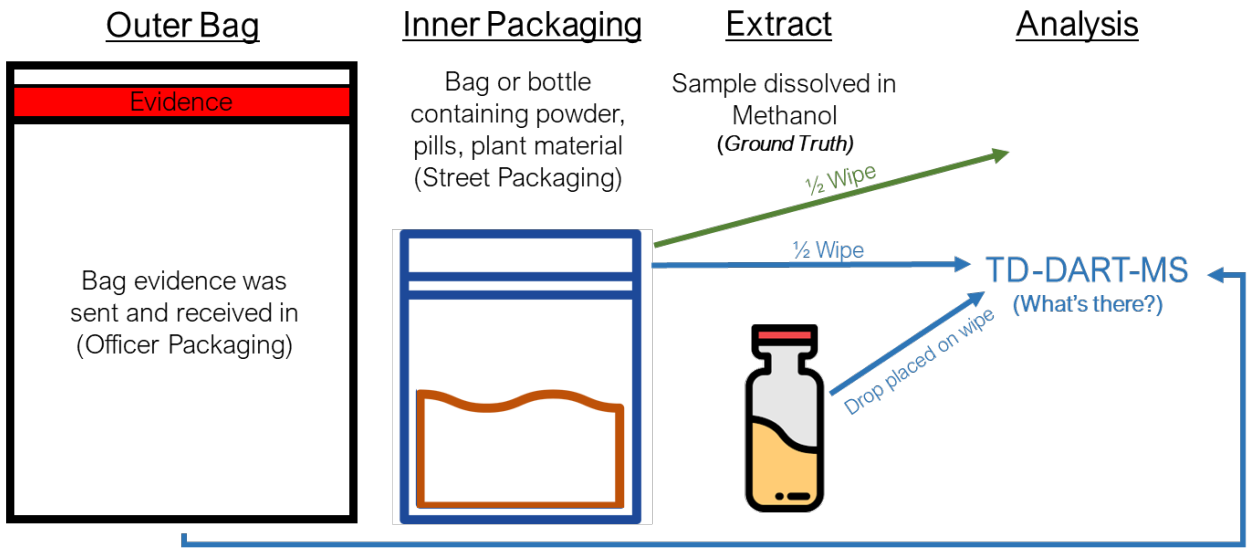
Non-Traditional TD-DART-MS

- Many recent research projects have used a TD-DART-MS configuration
- Glass T-junction mounted coupled with Vapur interface
  - Used to pull analyte towards mass spectrometer
- Thermal desorber attached to T-junction
  - Allows for wipe-based sample insertion
- Entire set-up can be removed and switched to traditional DART-MS in under 1 minute
- Increase sensitivity, reproducibility, safety
- Use nitrogen as the source gas



Evidence Screening Study

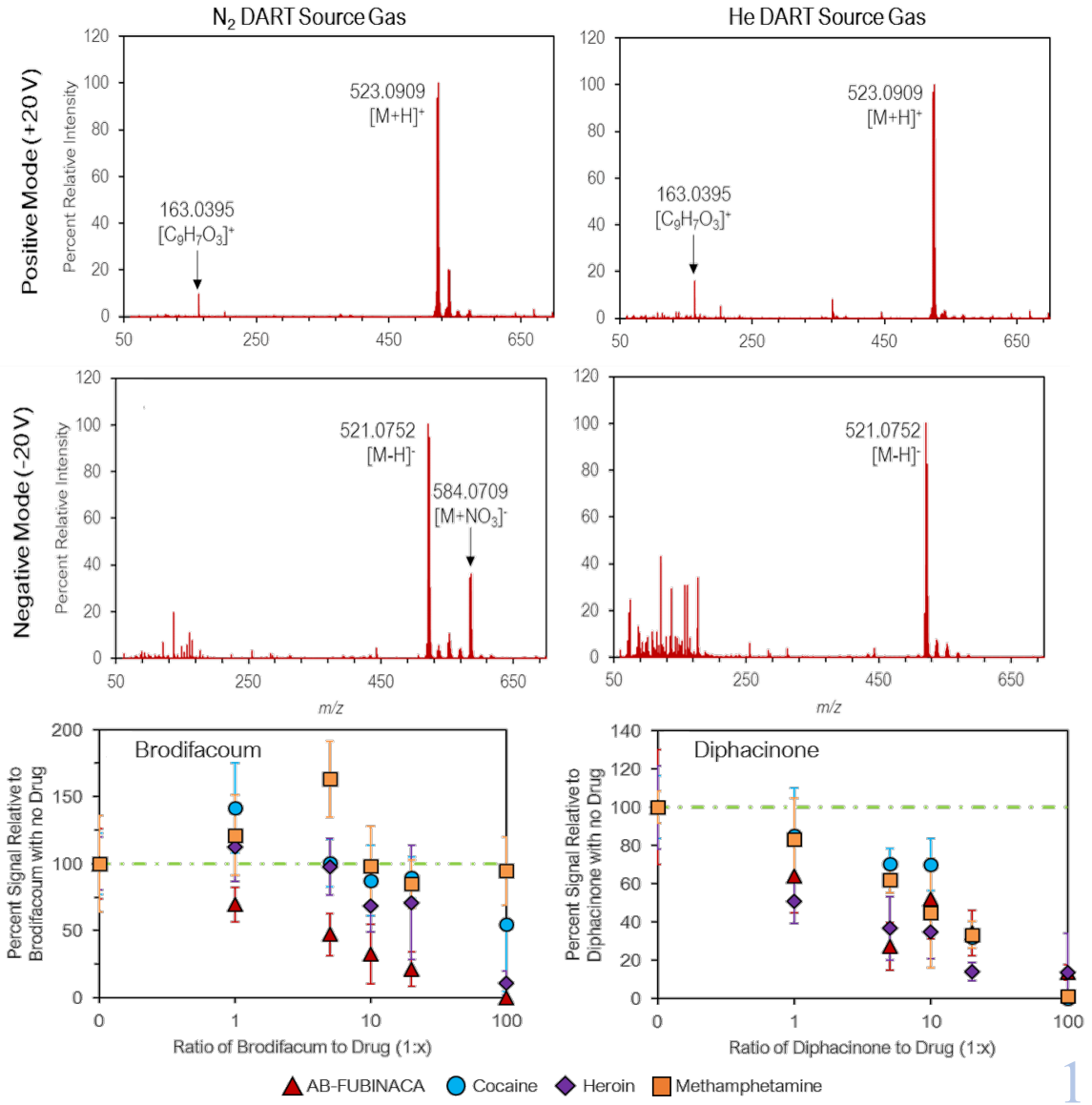
- To date >200 items sampled
- Inner packaging found to be the most representative (92 % accuracy)
- ***100 % so far in determining the presence of synthetic opioids***
- Typically enough material to saturate the MS or IMS
- False identifications attributed to plant material in foil bags or cases with large amounts of cocaine



Inner Packaging	Extract	Percent Occurrence	Result Type
Drug Detected	Same Drug Detected	79 % (n = 151)	True Positive
Drug Detected	No Drug Detected	1.5 % (n =3)	False Positive
Drug Detected	Different Drug Detected	2.5 % (n = 5)	False Positive
No Drug Detected	Drug Detected	4 % (n = 7)	False Negative
No Drug Detected	No Drug Detected	13 % (n = 25)	True Negative
Overall Accuracy:	92 %		

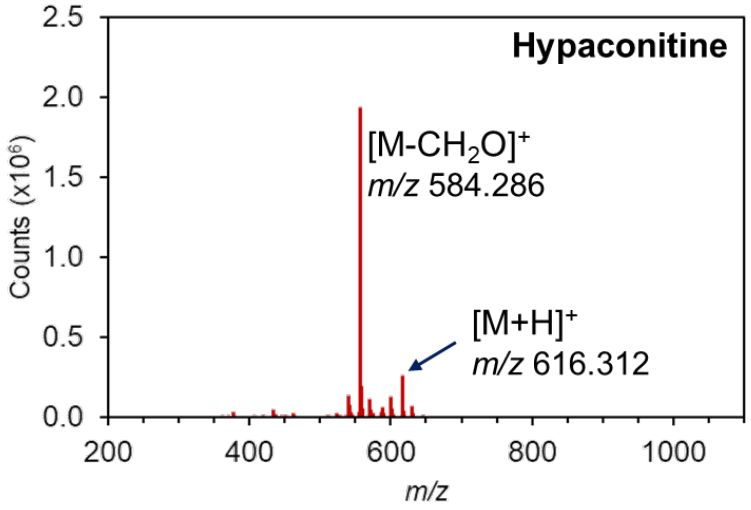
Recent Application: Rodenticides in Drugs

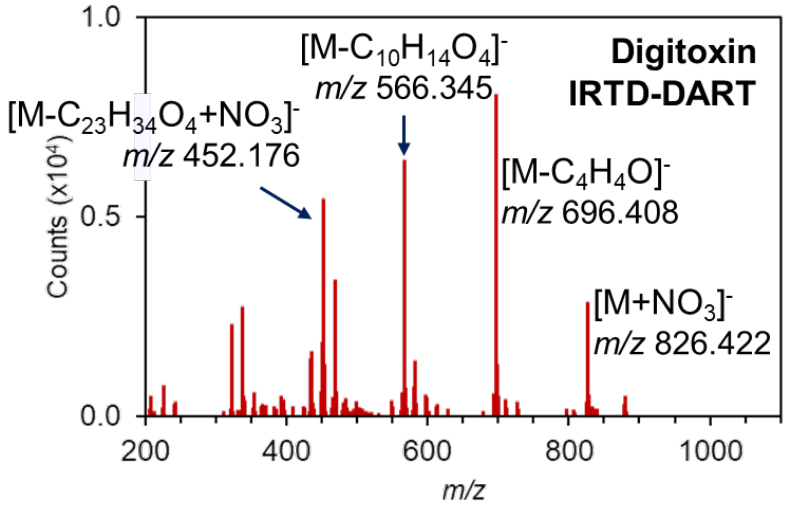
- Investigated if DART-MS could detect rodenticides (anti-coagulants) in illicit drug mixtures
- Six common compounds were easily detected by TD-DART-MS
  - Form both positive and negative ions
  - LODs in the 10's ng range
- In binary mixtures, competitive ionization with less volatile drugs was observed
  - Analysis in negative ionization mode eliminates competitive ionization concerns

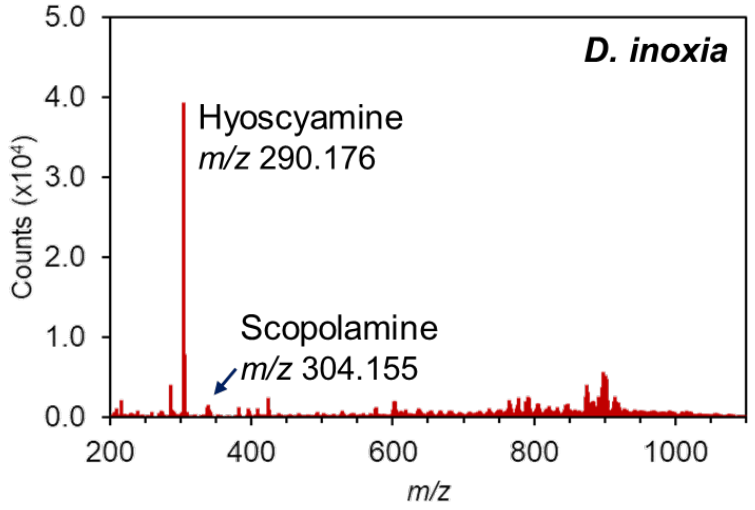


Recent Application: Seed-based Toxins

- Investigated the detection of seed-based toxins such as scopolamine, oleandrin, hyoscyamine, and digitoxin
- Several toxins (oleandrin, digoxin, digitoxin) performed better in negative ionization mode
- Compared different platforms (DART, TD-DART, IRTD-DART) to identify the most useful approach for this application







Targeted GC-MS Methods

National Institute of Standards and Technology
U.S. Department of Commerce

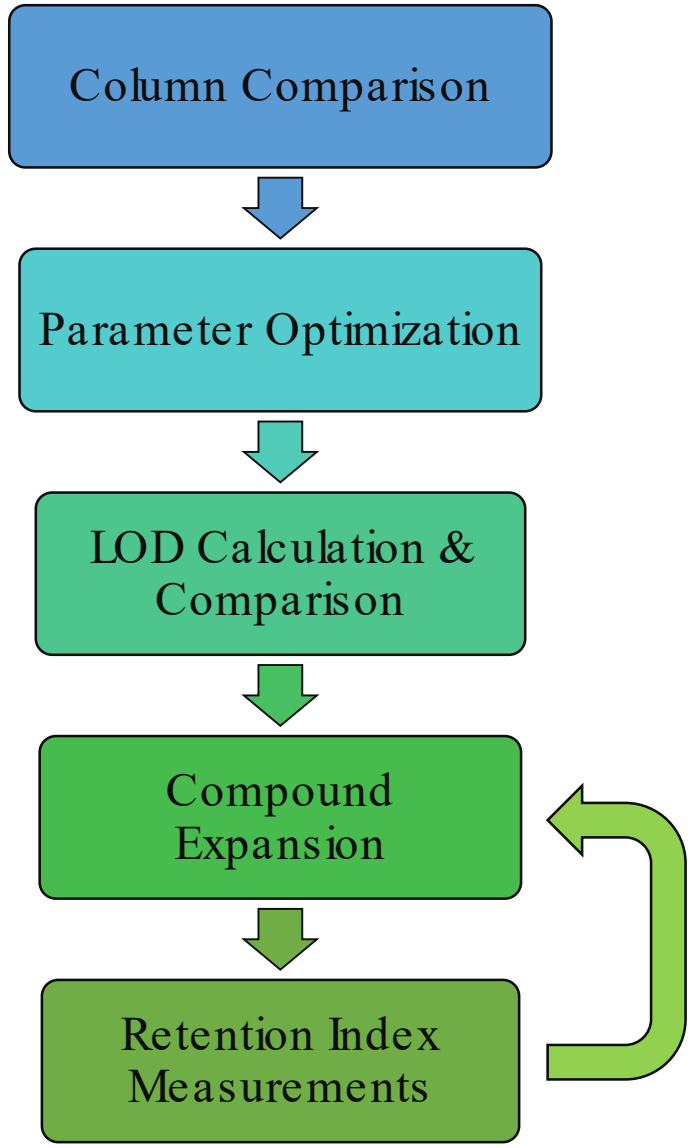
Targeted GC-MS Methods

Working with MSP-FSD to develop targeted GC-MS methods for different compound classes.

The goal is to develop methods that:

1) Enhance separation of isomers
2) Increase sensitivity
3) If possible, shorten runtimes
4) Standardize reporting / methods across labs

Methods also build in retention time locking and retention indices to improve rigor



Test Mixtures

- Worked with Cayman Chemical to develop custom text mixtures for each class
- Span range of elution times within class
- Include isomers to be able to measure resolution

Opioids	Cathinones	Cannabinoids
m-FIBF	Phentermine	FUB-AMB
p-FIBF	Methamphetamine	MDMB-FUBINACA
Cyclopropyl Fentanyl	Dimethylone	EMB-FUBINACA
Crotonyl Fentanyl	Butylone	MMB2201
Carfentanil	Ethylone	ADB-FUBINACA
Methoxyacetyl Fentanyl	Dibutylone	AB-FUBINACA
Furanyl Fentanyl	Pentylone	5F-ADBICA
Etizolam	Dimethylpentylone	5F-ABICA
Noscapine	Ethylpentylone	
Benzodioxole Fentanyl		

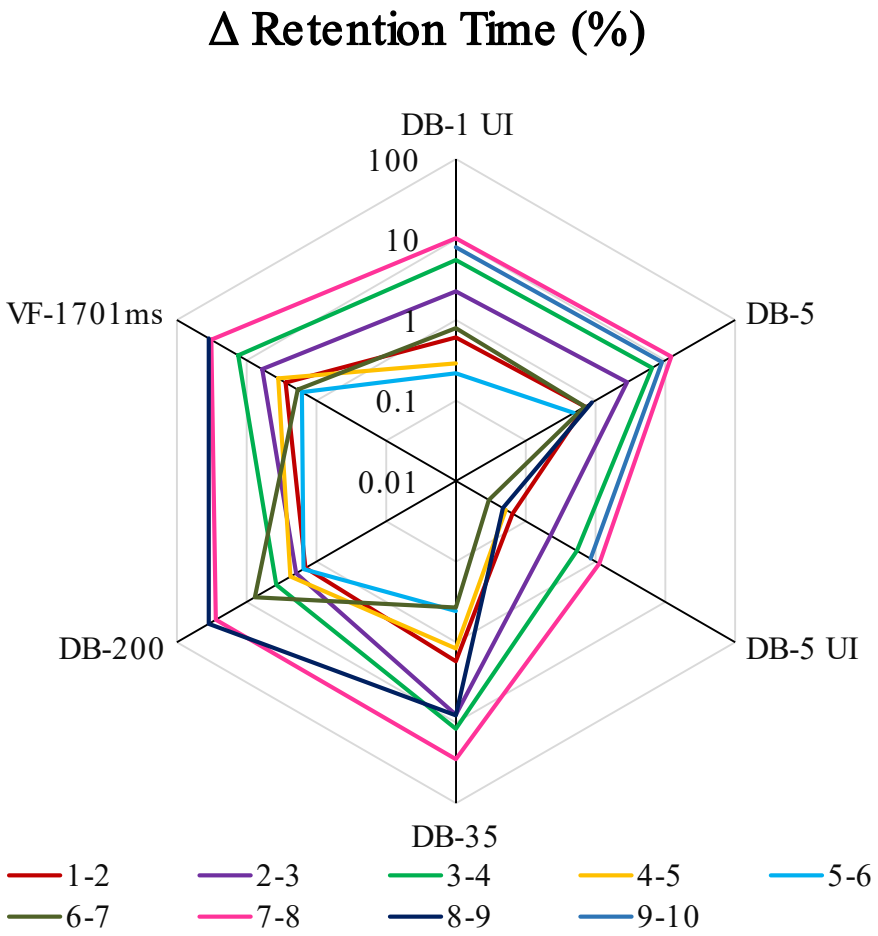
Column Comparison

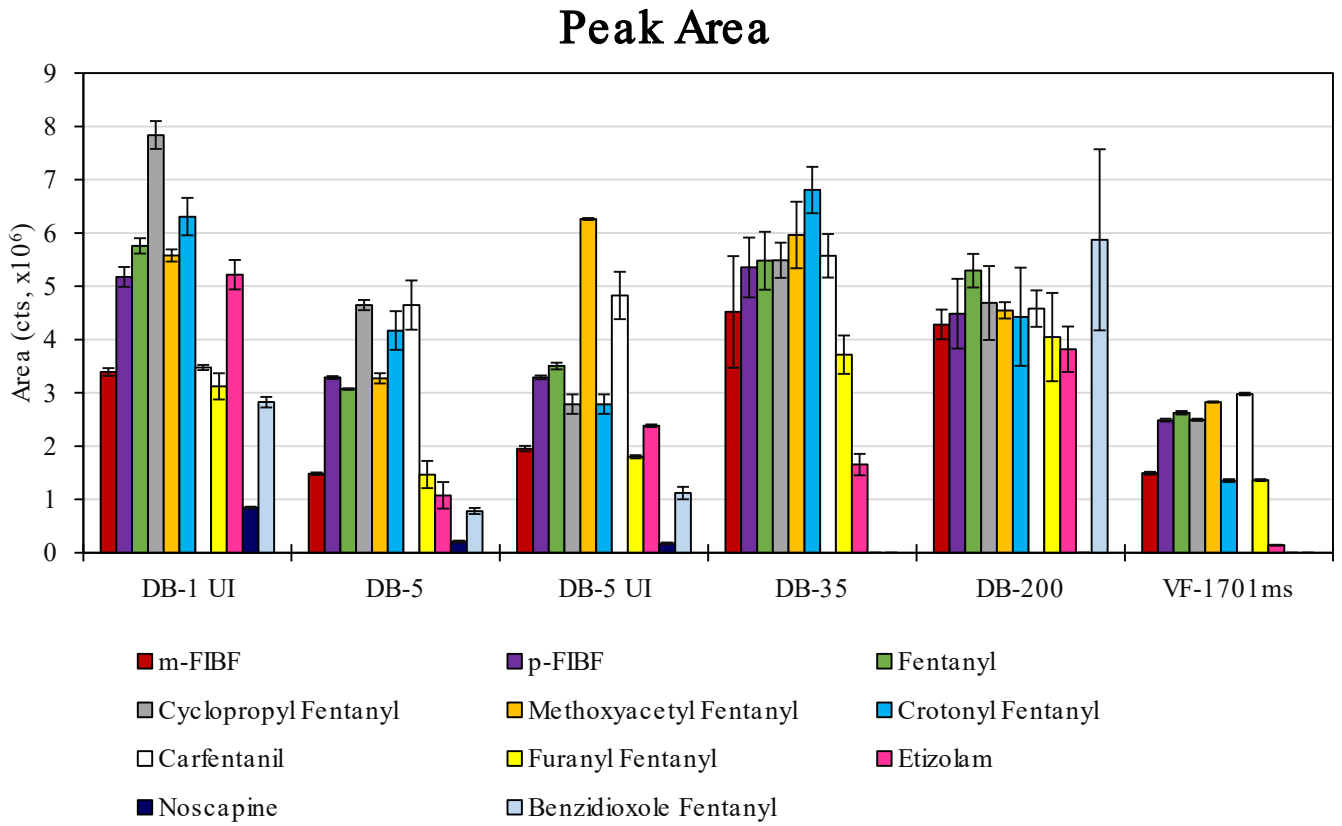
- First portion of study looked to identify the effect of different columns on test mixture response
- Evaluated six different columns
  - DB1UI, DB5, DB5UI, DB35, DB200, and VF1701ms
- Utilized a uniform method across all columns to keep other parameters fixed

Uniform method

Temperature Program	1) 100 °C for 0 min 2) Ramp at 30 °C/min to 300 °C 3) Hold for 24 min
Flow Rate	1.8 mL/min (Constant Flow)
Injection Volume	1 µL
Inlet Temperature	275 °C
Split Ratio	30:1
Transfer Line	300 °C
Quad Temperature	150 °C
Source Temperature	230 °C
Tune Mode	stune
Solvent Delay	1.30 min
Mass Scan Range	m/z 40 – m/z 550
Threshold	150
Scan Speed	N = 2
Total Run Time	30.667 min

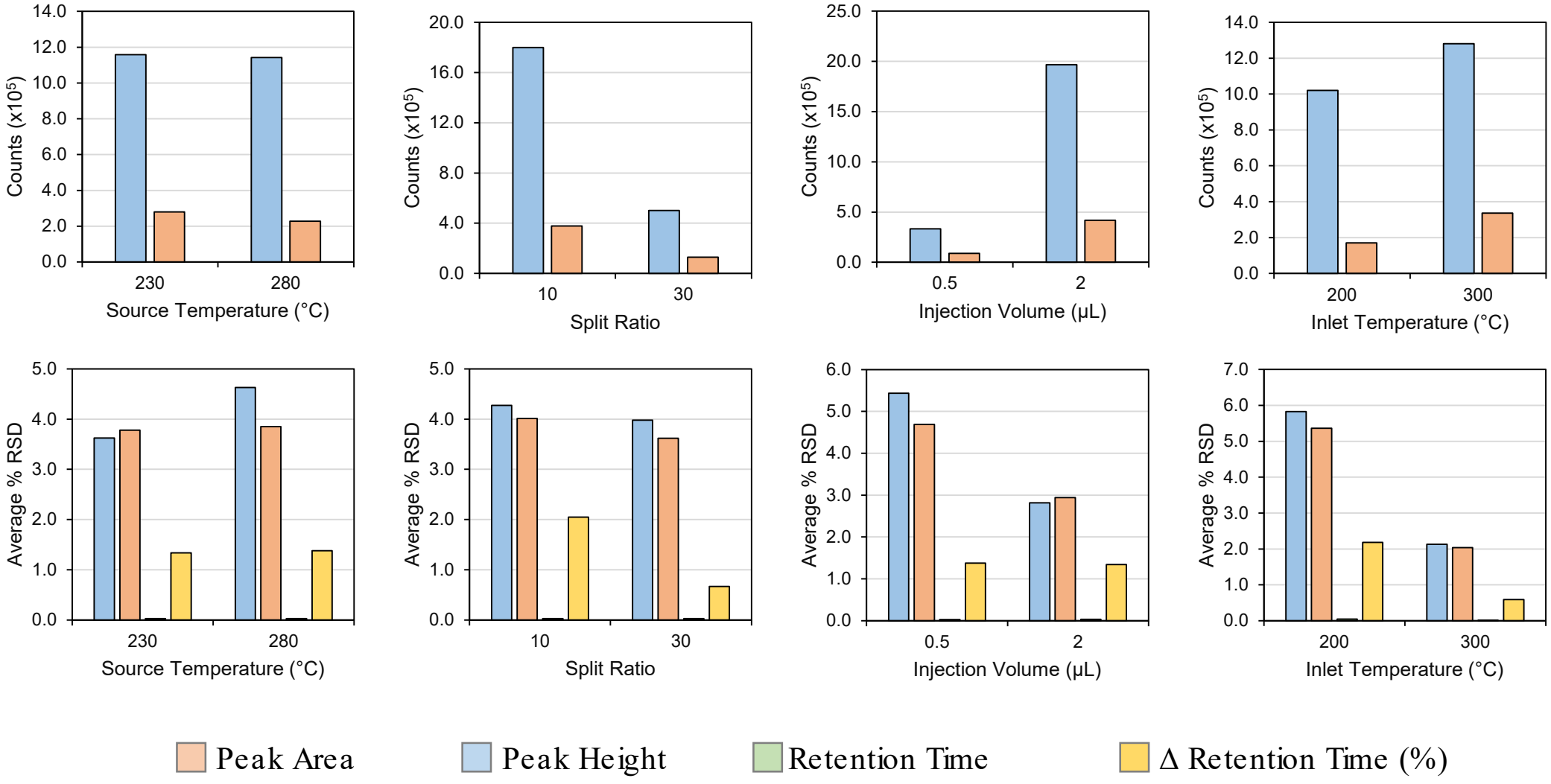
Column Comparison





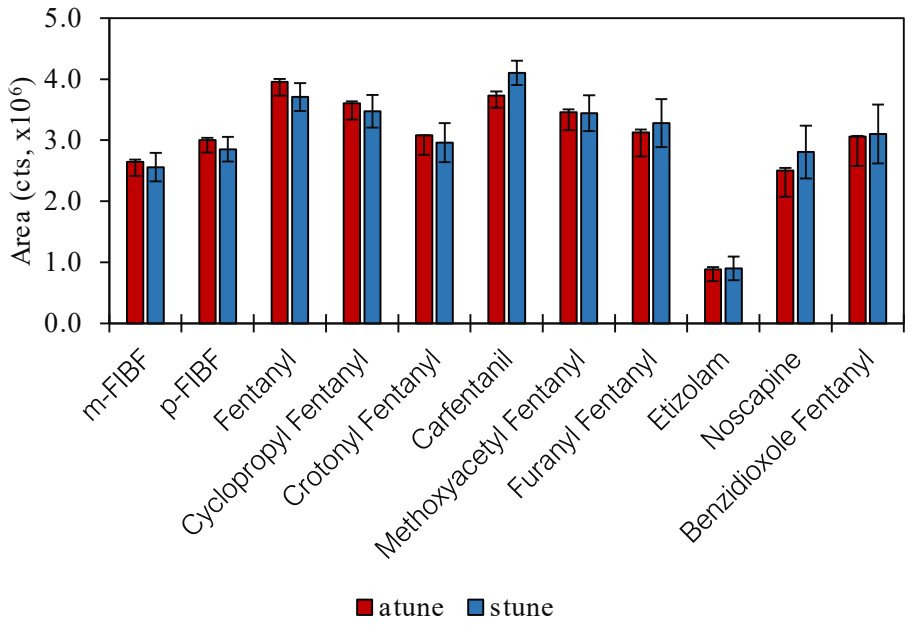
Once a column was chosen, studies were completed to optimize temperature and flow programs.

Other Settings – Design of Experiments



Final Optimization

- Results of relevant parameters from the DOE were furthered refined
- Final optimization looked at tune type
- After optimization, ran expanded panel of drugs to ensure method parameters worked

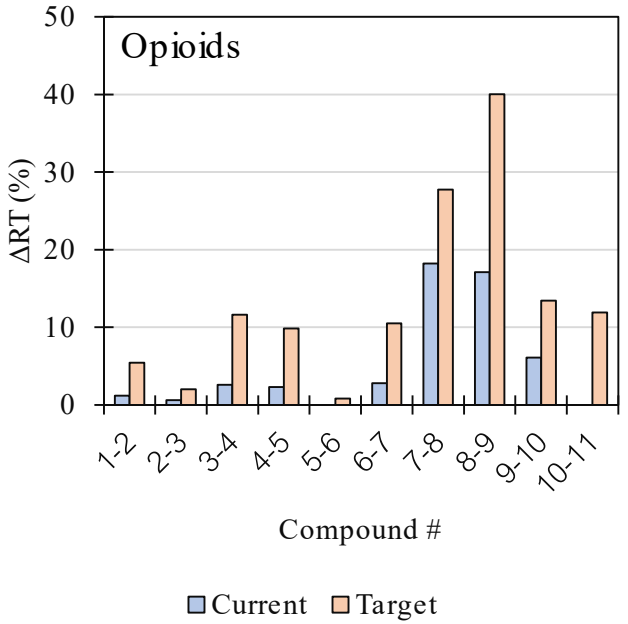


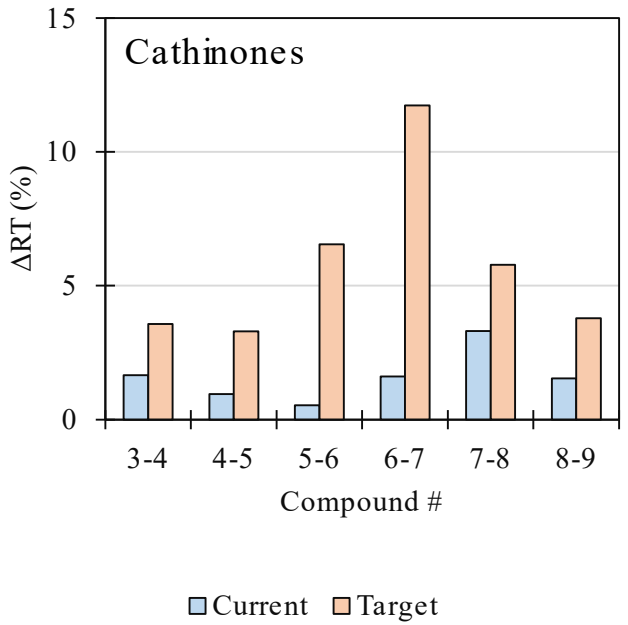
Approximate LODs

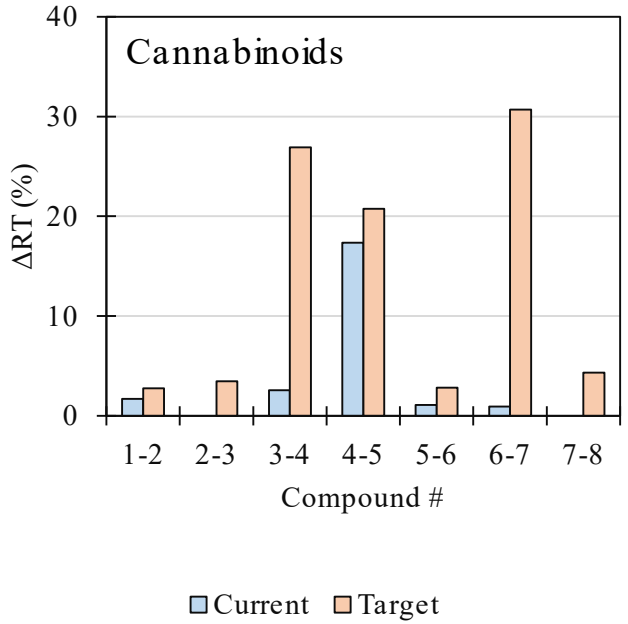
Opioids	LOD (µg/mL)	Cathinones	LOD (µg/mL)	Cannabinoids	LOD (µg/mL)
m-FIBF	1	Phentermine	0.5	FUB-AMB	1
p-FIBF	1	Methamphetamine	0.5	MDMB-FUBINACA	1
Fentanyl	1	Dimethylone	0.5	EMB-FUBINACA	5
Cyclopropyl Fent.	1	Butylone	0.5	MMB2201	1
Carfentanil	10	Ethylone	0.5	ADB-FUBINACA	10
Crotonyl Fentanyl	10	Dibutylone	0.5	AB-FUBINACA	10
Methoxyacetyl Fent.	10	Pentylone	0.5	5F-ADBICA	10
Furanyl Fentanyl	1	Dimethylpentylone	0.5	5F-ABICA	10
Etizolam	25	Ethylpentylone	0.5		
Noscapine	25				
Benzodioxole Fent.	10				

Comparison to Current Method

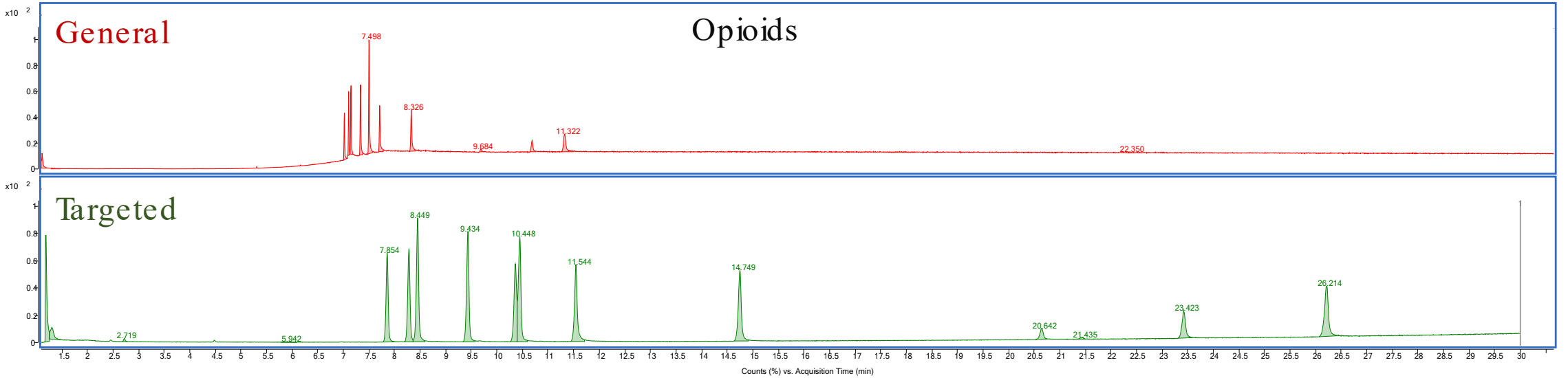
% Change (Average)	Area	Height	Delta RT	% RSD (RT)
Opioids	327 %	37 %	135 %	93 %
Cathinones	66 %	-19 %	262 %	0 %
Cannabinoids	6518 %	4045 %	220 %	537 %

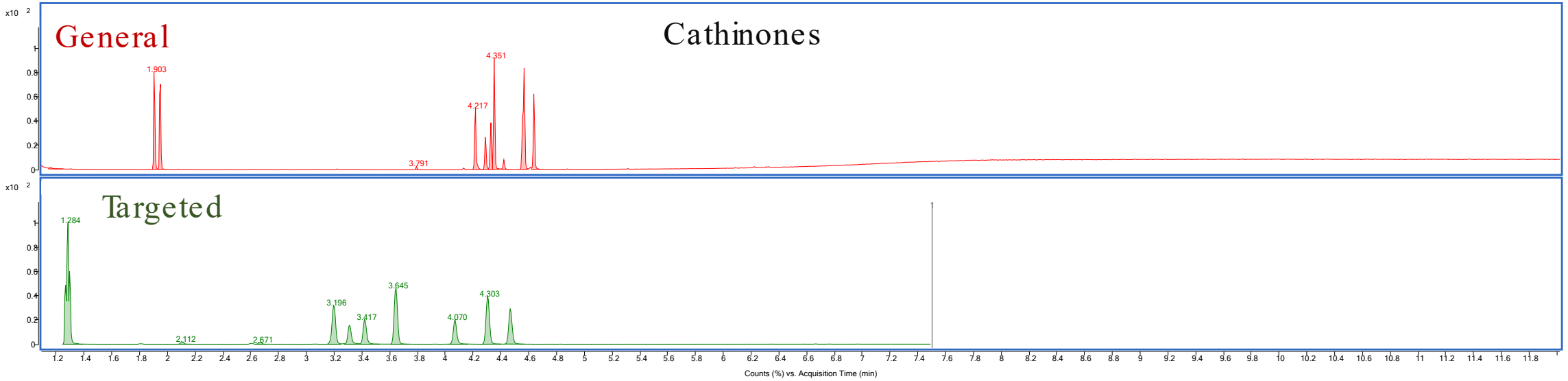




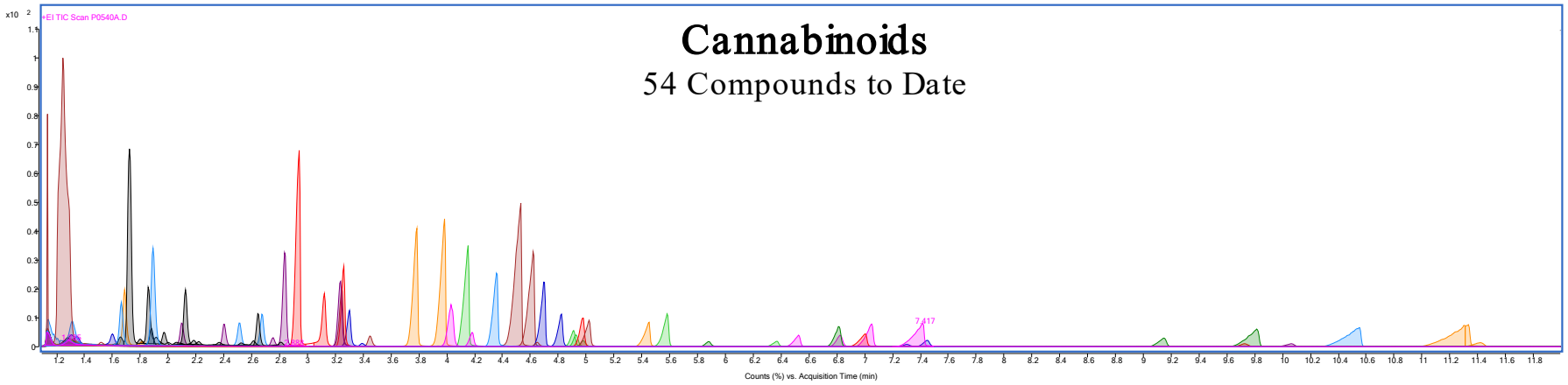


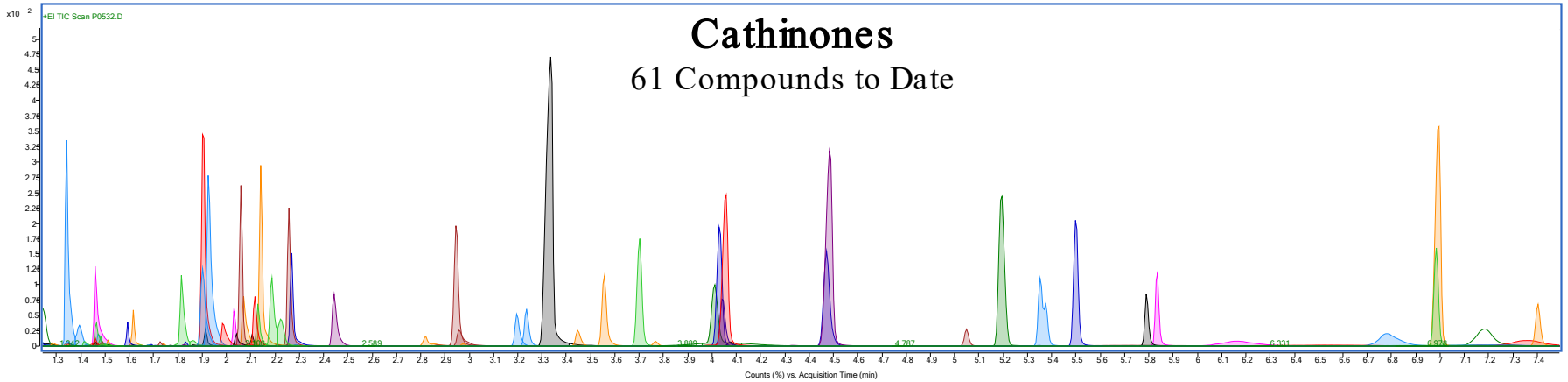
Comparison to Current Methods





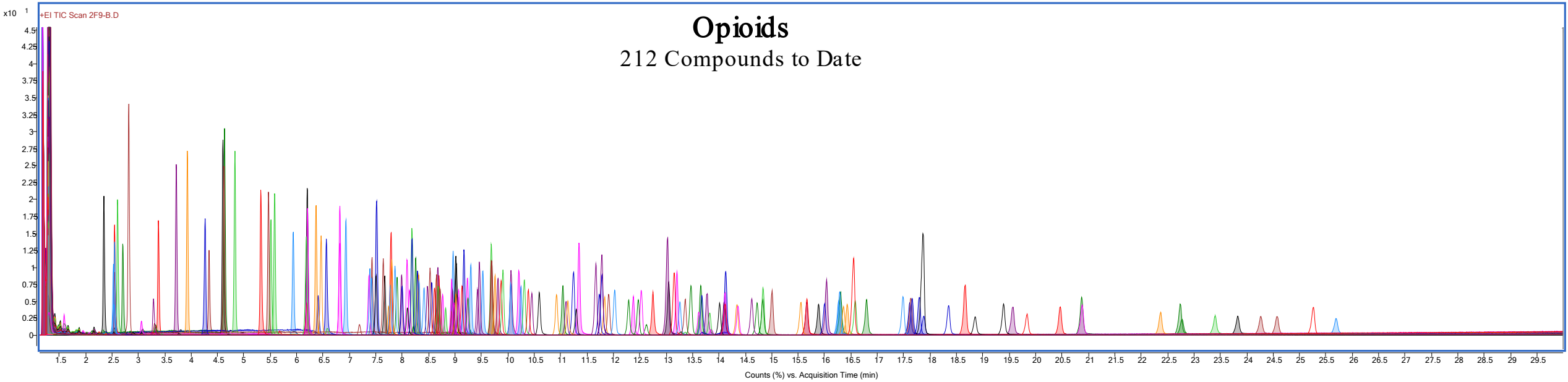
Compound Expansion





- Once developed, additional compounds were analyzed
  - Made adjustments to methods as needed
- Replicate analyses to evaluate locked RT and RI
  - Build library with RT and RI information
- All compounds had >1% RT difference or differentiable MS

Compound Expansion



- Utilized Fentanyl Analog Screening kit for expansion of opioid method
- Method has 8 pairs of compounds that have similar MS with <1 % RT difference
  - Six sets were ortho / meta isomer pairs
- Currently building out automated data analysis and reporting features

Workflow Comparison

The next step of this work is looking to quantify a comparison between the current workflow and a novel workflow.

- Take a subset of cases and have drug chemists analyze using one of the workflows
- Evaluate the level of detail gained at each step
- Quantify the time taken for each step
- Identify strengths and weaknesses in the novel workflow

Thank you.

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DARTdata@nist.gov

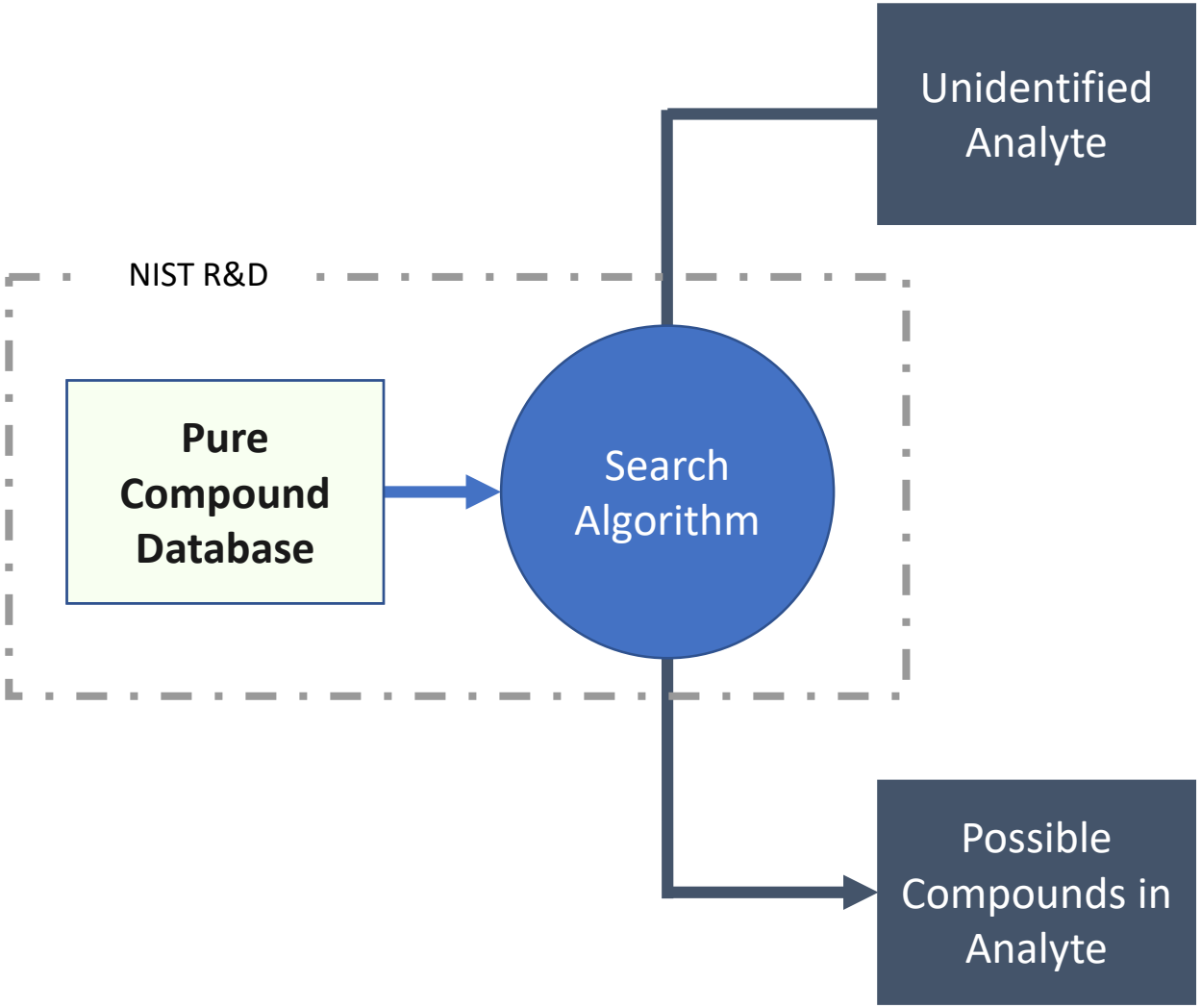
National Institute of Standards and Technology
U.S. Department of Commerce

NIST Mass Spectral Libraries and Search Tools for Seized Drug Analysis

Arun S. Moorthy
National Institute of Standards and Technology
Gaithersburg, MD, USA 20899

November 6th, 2020.

NIST National Institute of Standards and Technology U.S. Department of Commerce | Mass Spectrometry Data Center


Unidentified Analyte
NIST R&D
Pure Compound Database
Search Algorithm
Possible Compounds in Analyte

NIST20 EI MS Library

Why Upgrade to NIST20

- 350,704 spectra (44,082 new)
- 306,643 compounds (39,729 new)

Library Growth Concentrated in

- Human & plant metabolites
- Legal & illicit drugs
- General analytical interest

Gas Chromatography Retention Index and Methods Library

- 447,289 RI values
- 139,382 compounds

NIST20 Tandem MS Library

Comprehensive

- 30,999 compounds (17,191 new)
- 185,602 precursor ions (67,520 new)
- 1,320,464 spectra (745,638 new)
- Instruments Used: Ion Trap, Collision Cell

Wide Coverage

- Metabolites
- Pharmaceuticals
- Industrial Surfactants
- Glycans-Lipids-Sugars
- Pesticides
- Amino Acids, Di- & Tryptic Tri-Peptides

MS Software

- Every new spectrum reviewed by two analysts.
- New compounds chosen for wide analytical interest.
- MS Search v. 2.4 with hybrid search
- AMDIS (GC-MS)
- MS Interpreter Major Revision

Email massspec@nist.gov

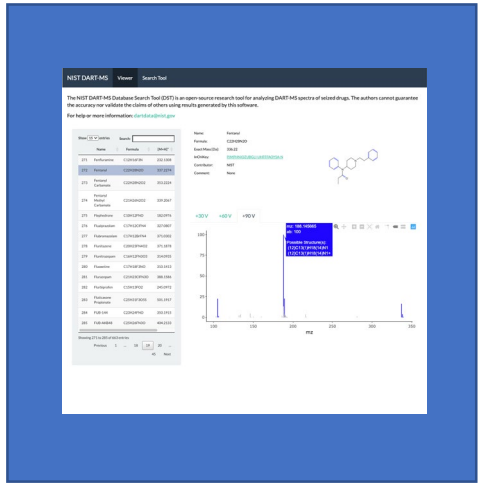
Web chemdata.nist.gov

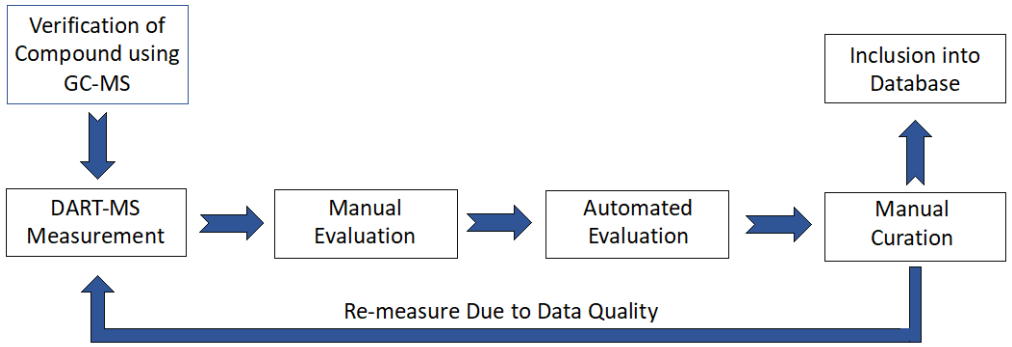
NIST National Institute of Standards and Technology U.S. Department of Commerce | Mass Spectrometry Data Center

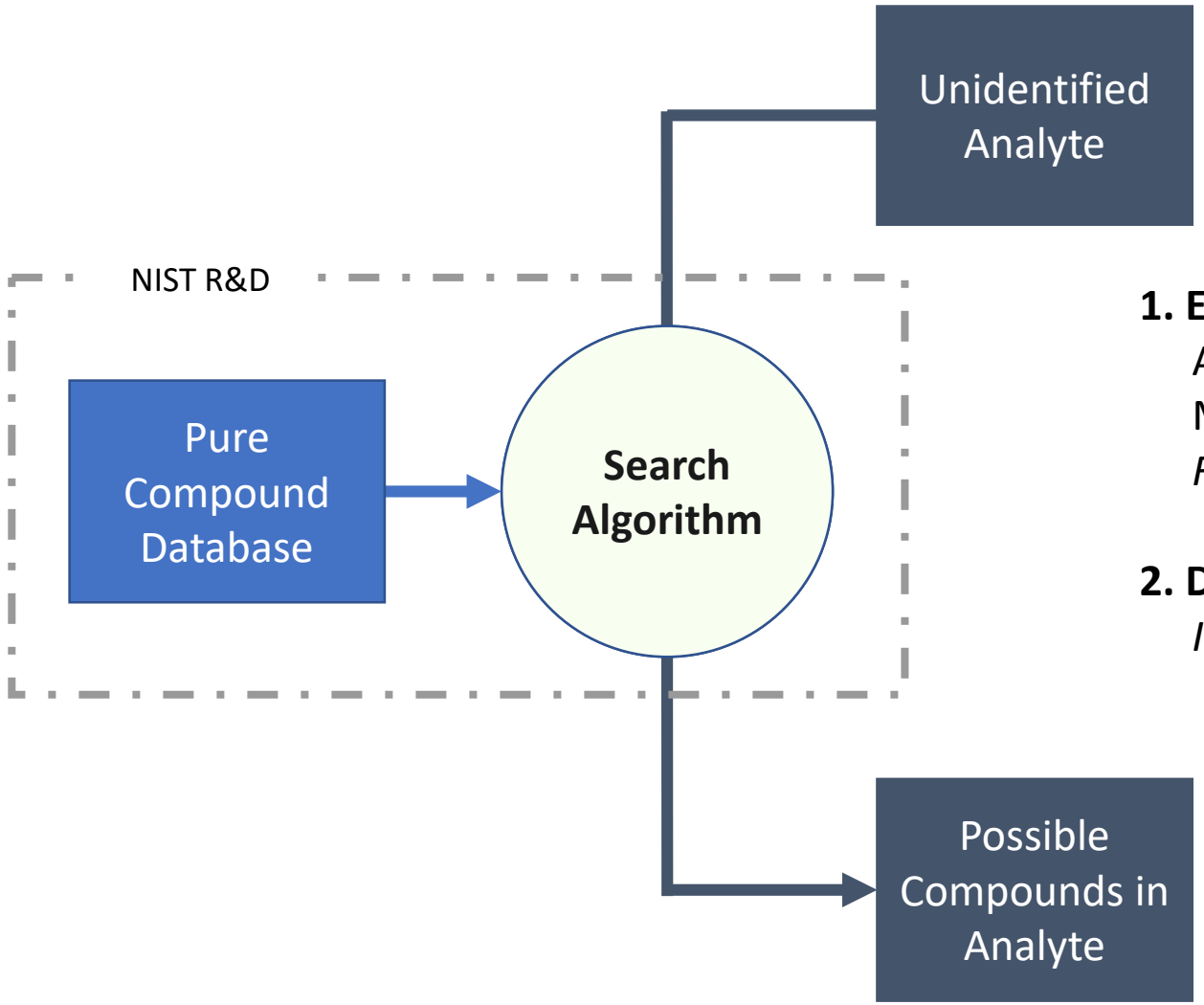
DART-MS Forensics Database

- A new database **available now**
  - focus on NPS's, synthetic opioids, cutting agents
  - spectra measured at multiple orifice energies
- Developed new manual and **automated** evaluation workflow
- Implemented workflow to facilitate rapid updating of database
  - open-source software
- Database and workflow available from DARTdata@nist.gov

NEW DART-MS Forensic database: 663 compounds, 1989 spectra







1. EI-MS

AMDIS

MS Search + Interpreter

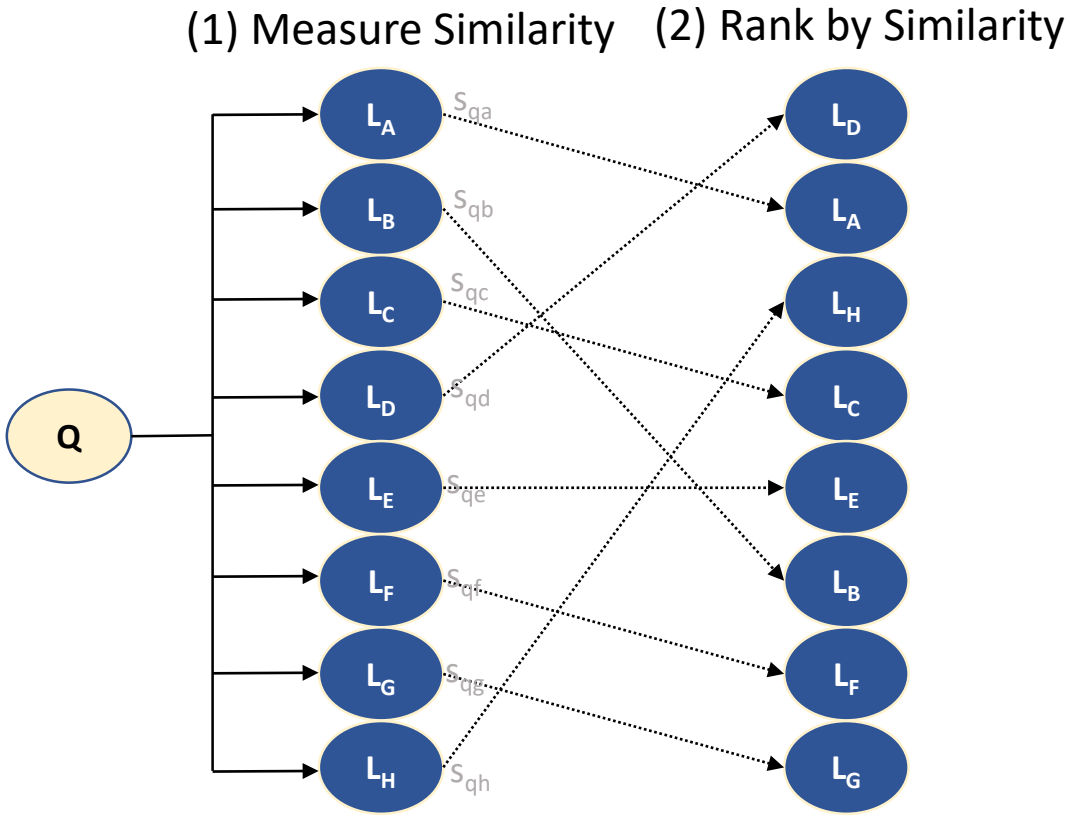
Fentanyl Classifier

2. DART-MS

Inverted Search Algorithm

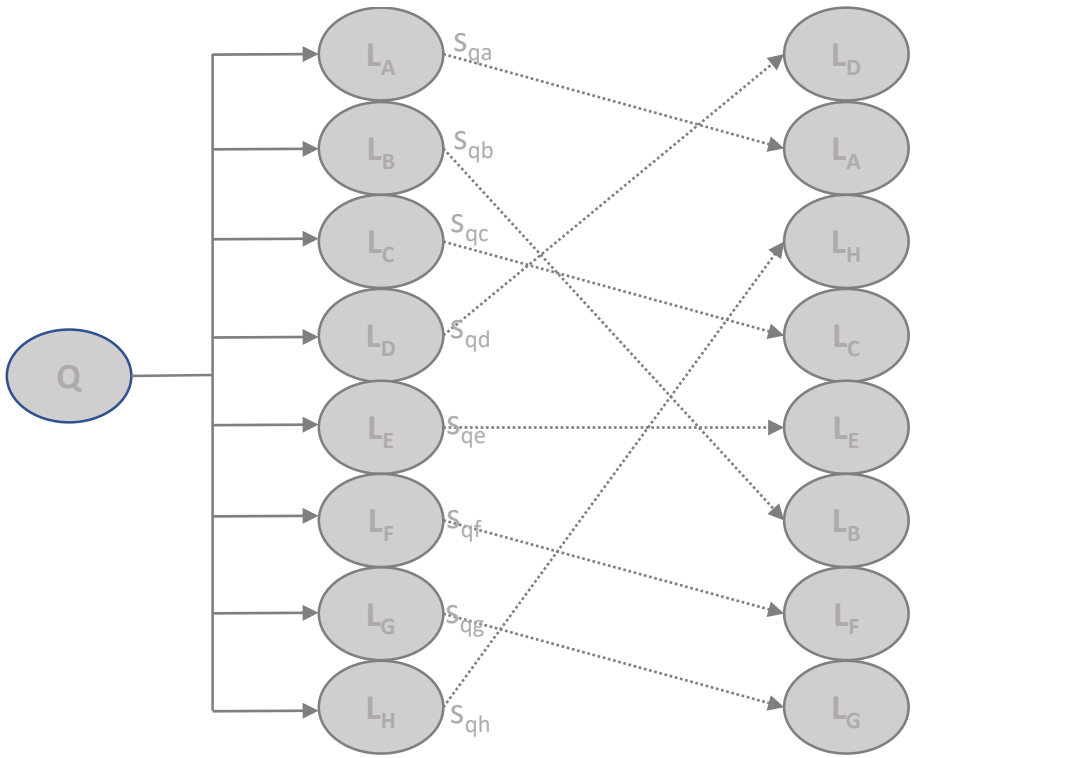
EI-MS: Fentanyl Classifier

Mass spectral library searching



EI-MS: Fentanyl Classifier

Mass spectral library searching

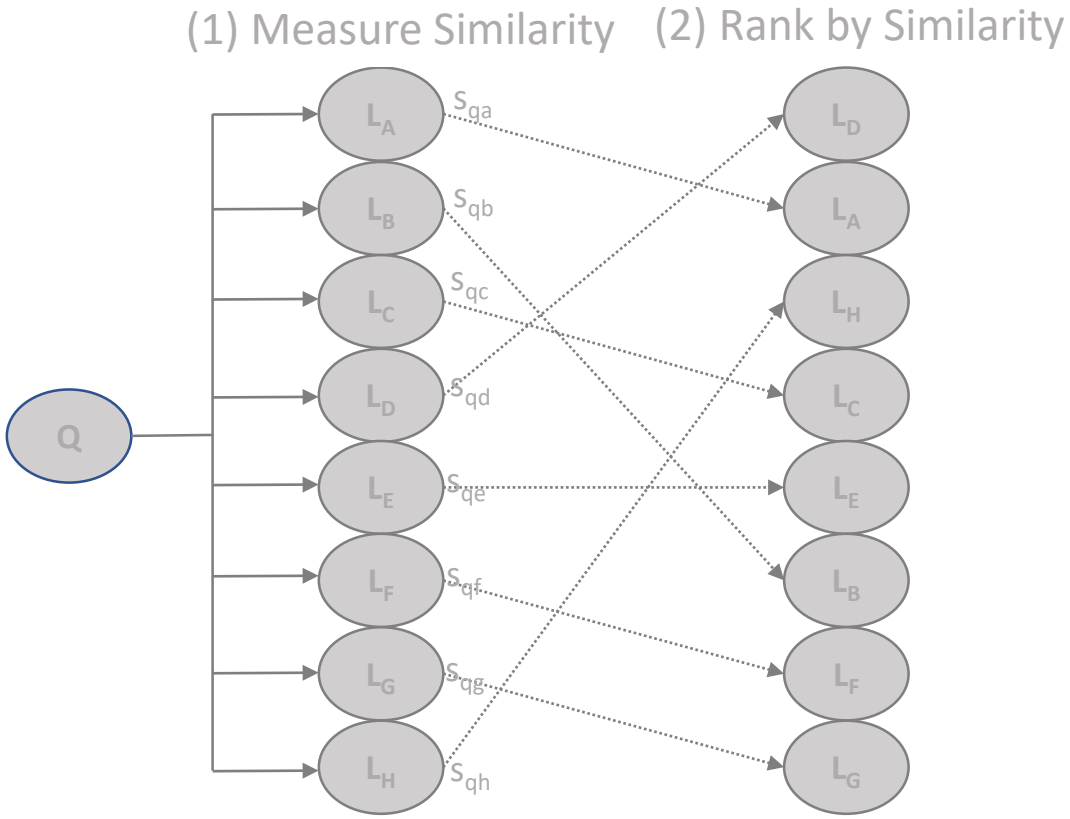


Mass spectral similarity mapping

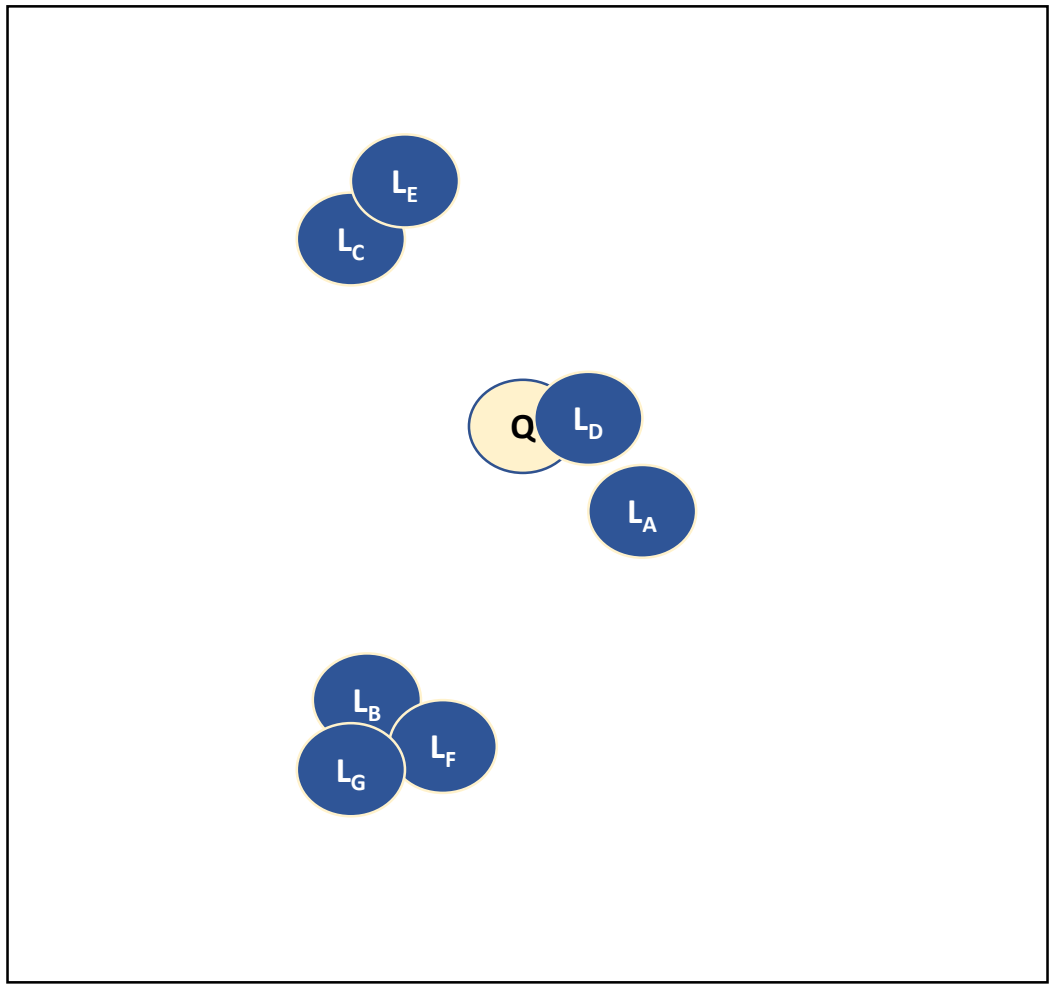
	Q	L_A	L_B	L_C	L_D	L_E	L_F	L_G
Q	1	s_{aq}	s_{bq}	s_{cq}	s_{dq}	s_{eq}	s_{fq}	s_{gq}
L_A	s_{qa}	1	s_{ba}	s_{ca}	s_{da}	s_{ea}	s_{fa}	s_{ga}
L_B	s_{qb}	s_{ab}	1	s_{cb}	s_{db}	s_{eb}	s_{fb}	s_{gb}
L_C	s_{qc}	s_{ac}	s_{bc}	1	s_{dc}	s_{ec}	s_{fc}	s_{gc}
L_D	s_{qd}	s_{ad}	s_{bd}	s_{cd}	1	s_{ed}	s_{fd}	s_{gd}
L_E	s_{qe}	s_{ae}	s_{be}	s_{ce}	s_{de}	1	s_{fe}	s_{ge}
L_F	s_{qf}	s_{af}	s_{bf}	s_{cf}	s_{df}	s_{ef}	1	s_{gf}
L_G	s_{qg}	s_{ag}	s_{bg}	s_{cg}	s_{dg}	s_{eg}	s_{fg}	1

EI-MS: Fentanyl Classifier

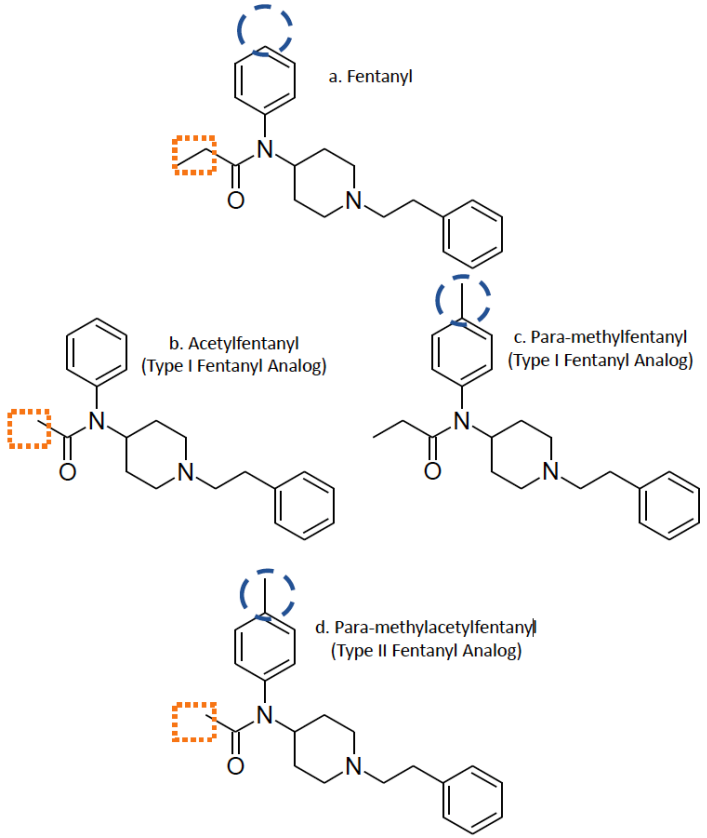
Mass spectral library searching



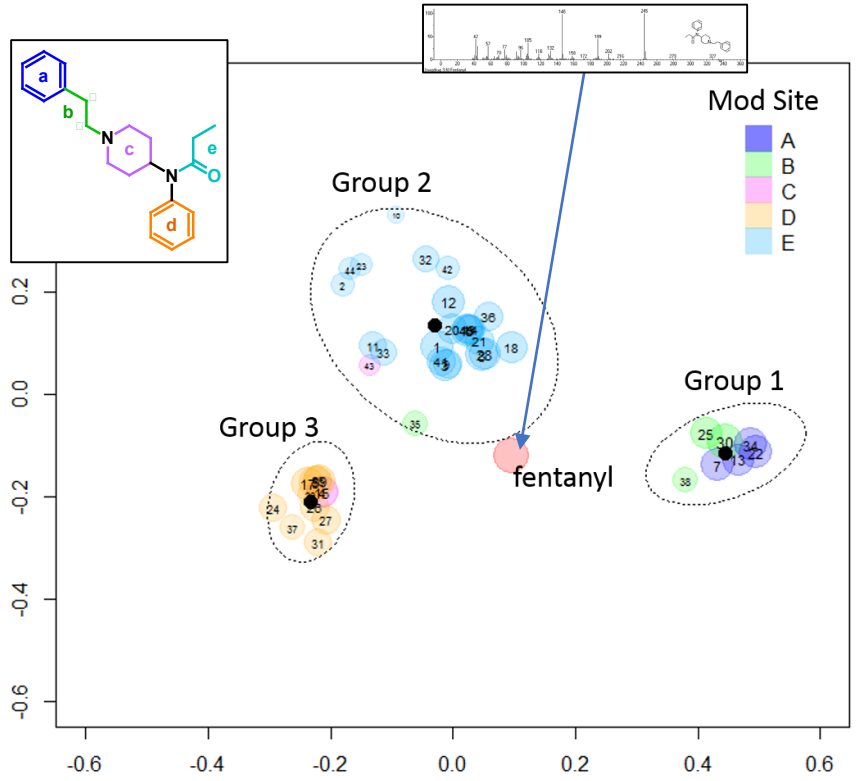
Mass spectral similarity mapping



EI-MS: Fentanyl Classifier



Examples of fentanyl and fentanyl analogs, with colored shapes demonstrating the sites at which the analogs differ from the fentanyl



Example of 2D mass spectral similarity map created by the NIST Fentanyl Classifier. Each circle represents a mass spectrum. Based on where a query spectrum lands in this space, an analyst can determine whether it is a fentanyl analog (with up to two modifications) or not.

Software Availability:

1. NIST Fentanyl Classifier (2020): http://github.com/asm3-nist/FentanylClassifier

Relevant Publications:

1. Moorthy et. al. "Combining fragment-ion and neutral-loss matching during mass spectral library searching: A new general purpose algorithm applicable to illicit drug identification." *Analytical chemistry* 89, no. 24 (2017): 13261-13268.

2. Moorthy et. al. "Mass spectral similarity mapping applied to fentanyl analogs." *Forensic Chemistry* 19 (2020): 100237.

3. Moorthy & Kearsley. ”Pattern similarity measures applied to mass spectra”. To appear in “Progress in Industrial Mathematics” (2021)

4. Kearsley & Moorthy. “Mathematics and Mass Spectra: Model problems to study the Fentanyl epidemic”. Submitted July 2021.

Search Results | Instructions

Fentanyl Classifier

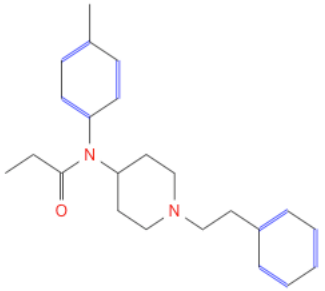
The Fentanyl Classifier is a prototype implementation of "augmented mass spectral library searching". The software was designed for demonstration purposes. The authors cannot guarantee the accuracy of results generated using the Fentanyl Classifier, and cannot validate claims of others using this software.

Choose Query Spectrum (MSP File)

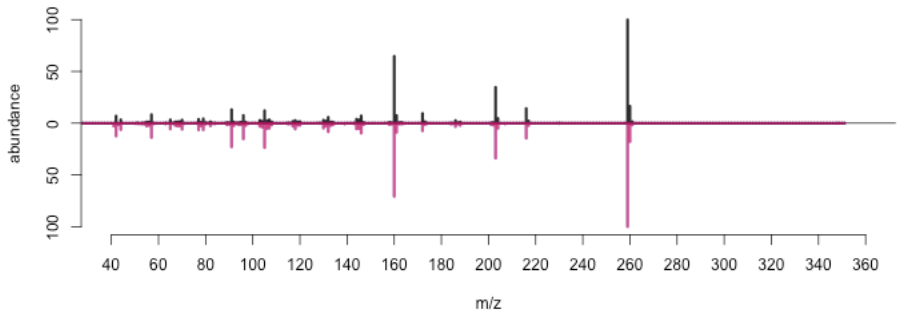
Browse... 248327.MSP

Upload complete

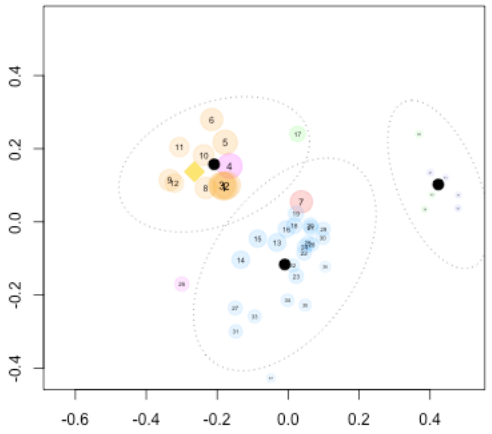
Potential structure based on library search results. **Disclaimer: The authors do not guarantee the accuracy of this result or claims of others based on results generated using this tool.**



No molecular weight information available from Query MSP. An estimate of 350 Da was generated by adding 91 Da to the highest mass with intensity greater than 800.

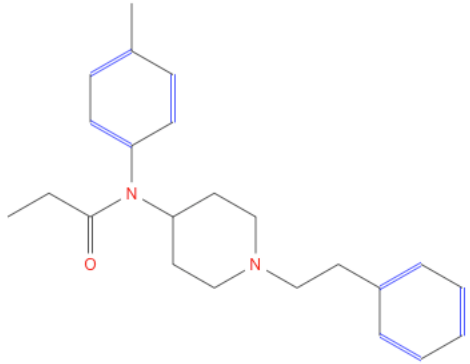


Hit List | Hit Map | Fentanyl Structure Definition



Library Compound:

Name:	para-Methylfentanyl
Formula:	C23H30N2O
Exact Mass:	350.236
MW:	350
InChIKey:	XHWYYMNEJCMADF-UHFFFAOYSA-N



Search Results | Instructions

Fentanyl Classifier

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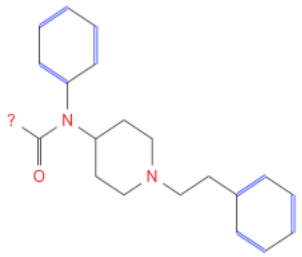
Choose Query Spectrum (MSP File)

Browse... synthType1E.MSP

Upload complete

Potential structure based on library search results. **Disclaimer: The authors do not guarantee the accuracy of this result or claims of others based on results generated using this tool.**



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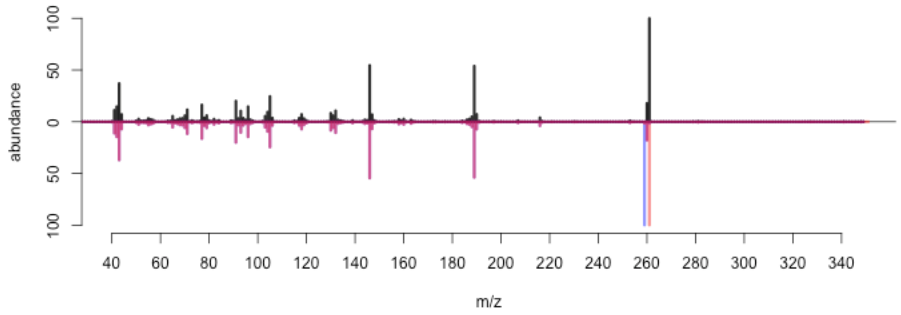
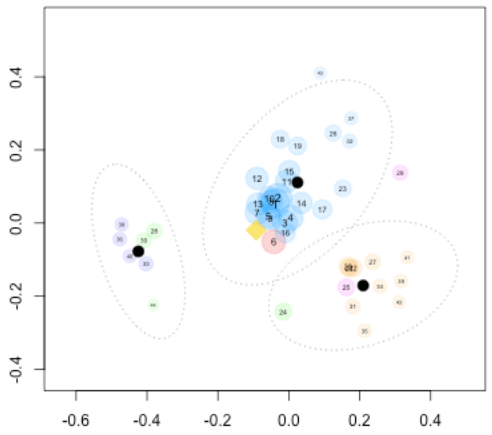
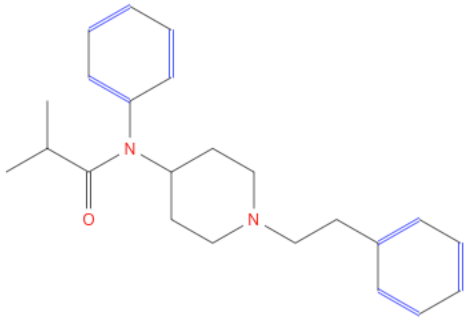


Hit List | Hit Map | Fentanyl Structure Definition



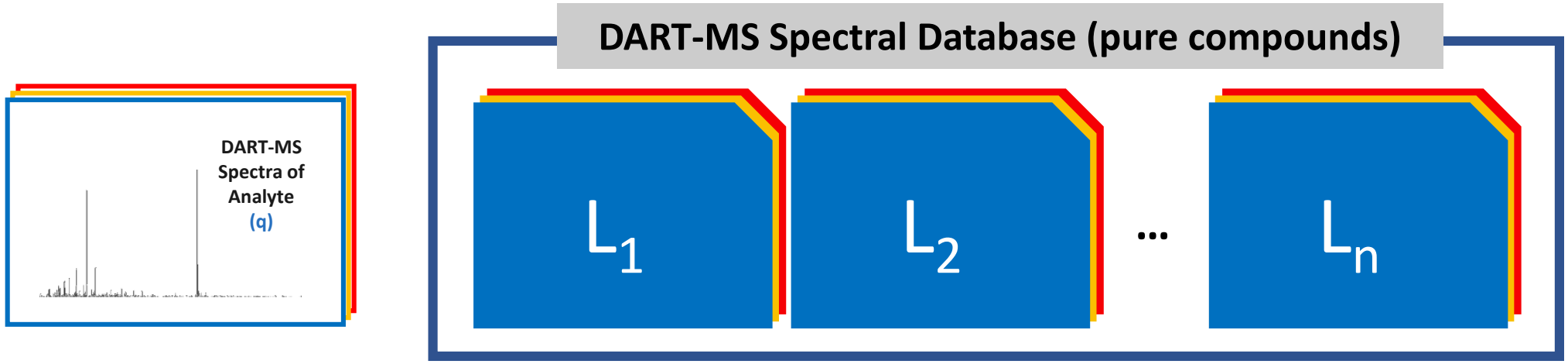
Library Compound:

Name:	Isobutyryl fentanyl
Formula:	C23H30N2O
Exact Mass:	350.236
MW:	350
InChIKey:	WRPFPNIHTOSMKU-UHFFFAOYSA-N



DART-MS: Inverted Search Procedure

GIVEN:

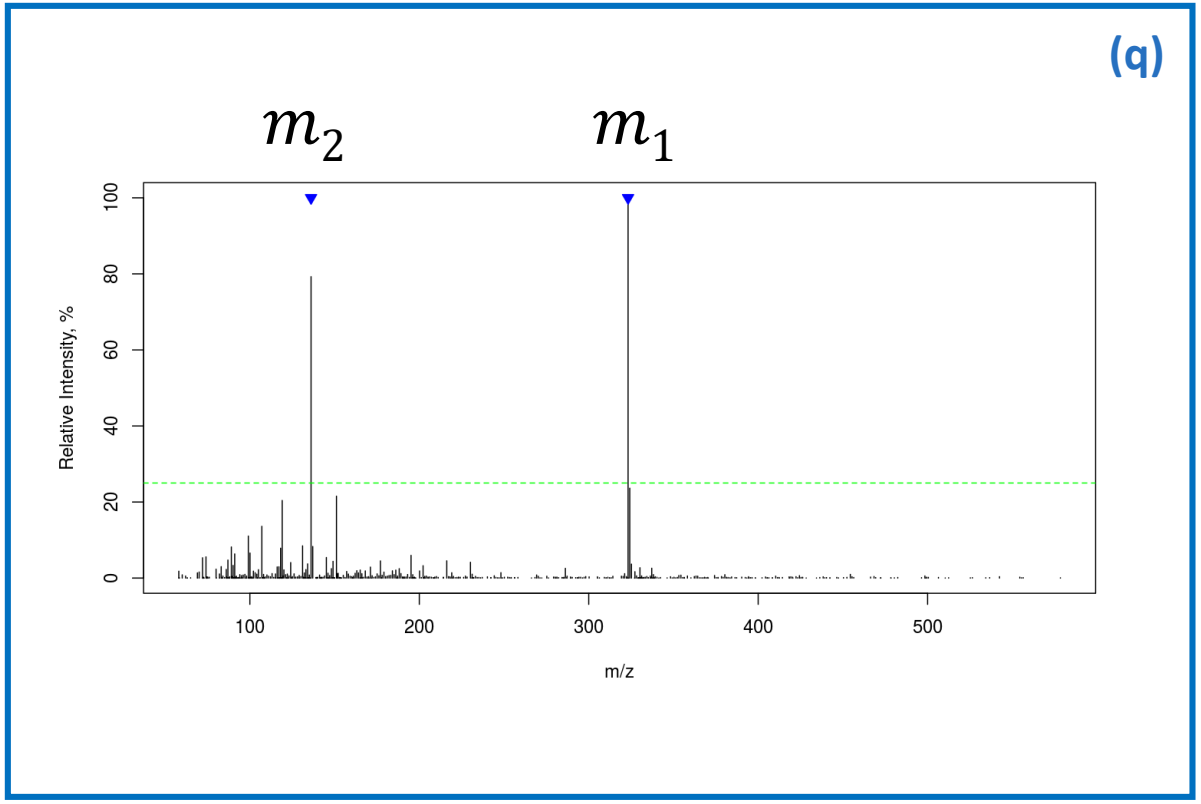


Assumption 1: The component molecules contained in a mixture will each present an $[M + H]^+$ peak in the low energy spectrum and the relative intensity of these peaks will be greater than a threshold intensity.

DART-MS: Inverted Search Procedure

Assumption 2a:

Reference mass spectra of the component molecules contained in the analyte are available in a searchable database.

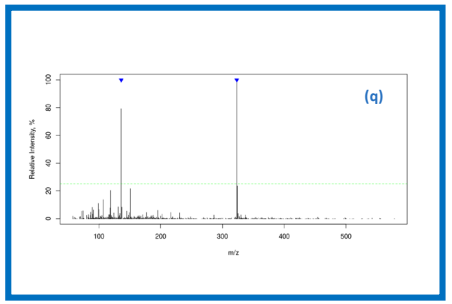


Assumption 2b:

The difference between protonated molecule *m/z* values of database entries and those observed in the query is accurate to a known resolution $\pm \epsilon_0$.

DART-MS: Inverted Search Procedure

Target: m_1



q

q

L_4

L_4

L_4

$$\phi_{m_1,L_4} = g(f_1(q, L_4, q, L_4, q, L_4, P), f_2(q, L_4, q, L_4, q, L_4, P), f_3(q, L_4))$$

weighted fraction of abundance explained

weighted mass bias

mass difference

NIST DART-MS | Viewer | Search Tool

The NIST DART-MS Database Search Tool (DST) is an open-source research tool for analyzing DART-MS spectra of seized drugs. The authors cannot guarantee the accuracy nor validate the claims of others using results generated by this software.

For help or more information: dartdata@nist.gov

Search Mode:

○ Pure Compound ◉ Mixture Analysis

Query Spectra | Advanced

These settings can be adjusted to address expected variations in MS sensitivity and resolution.

min abundance of targets (mixture analysis)

0.01 0.25 1

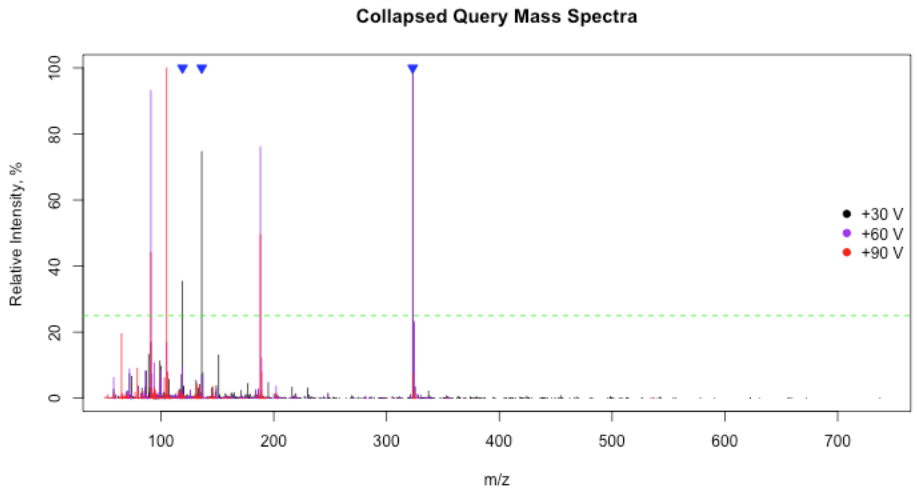
0.01 0.11 0.21 0.31 0.41 0.51 0.61 0.71 0.81 0.91 1

m/z tolerance

☐ Integer resolution spectra.

0.005 0.1

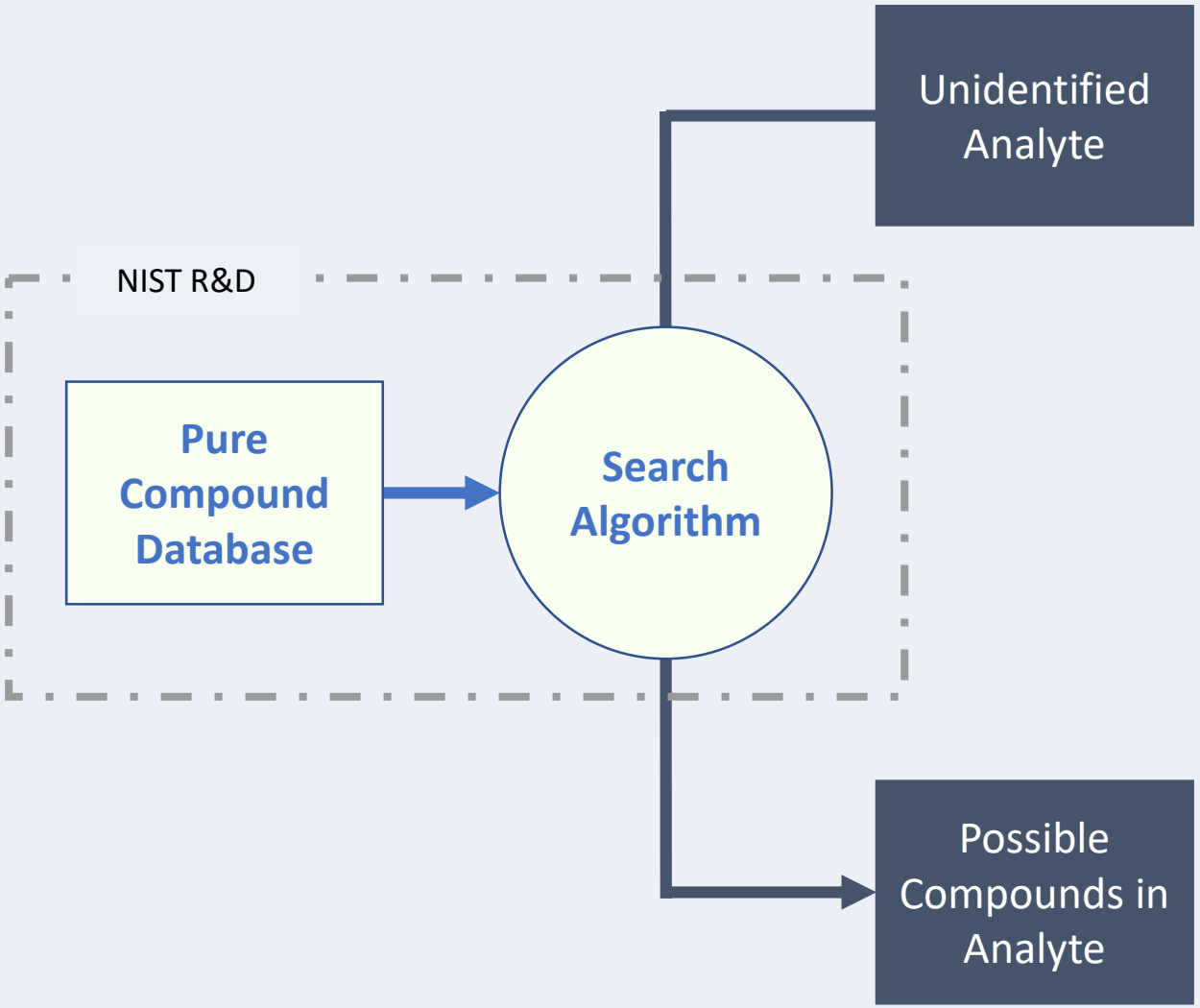
0 0.01 0.02 0.03 0.04 0.05 0.06 0.07 0.08 0.09 0.1



Target 1 | Target 2 | Target 3

Mass-to-charge: 119.085
Relative intensity: 35.4 %.

No matches in database.



Summary of Tools

AMDIS: Automates extraction of GC-MS data files to generate consistent/reproducible mass spectra.

- Built-in “standard” library search procedure

MS SEARCH/Interpreter: A comprehensive tool for interacting with mass spectral libraries, including a variety of useful search algorithms and data interpretation tools.

Fentanyl Classifier: A tool specifically for interacting with mass spectra of potential fentanyl analogs, attempting to localize the site of modification.

Available: https://github.com/asm3-nist/FentanylClassifier

Inverted Search Algorithm: A new method currently in preparation for identifying components in DART-MS.

For status updates: DARTdata@nist.gov

Questions?

arun.moorthy@nist.gov

Benchtop NMR for Forensic Drug Analysis

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National Institute of Standards & Technology
Gaithersburg, Maryland USA

NIST National Institute of Standards and Technology U.S. Department of Commerce

FORENSIC SCIENCES

MATERIAL MEASUREMENT LABORATORY

Outline

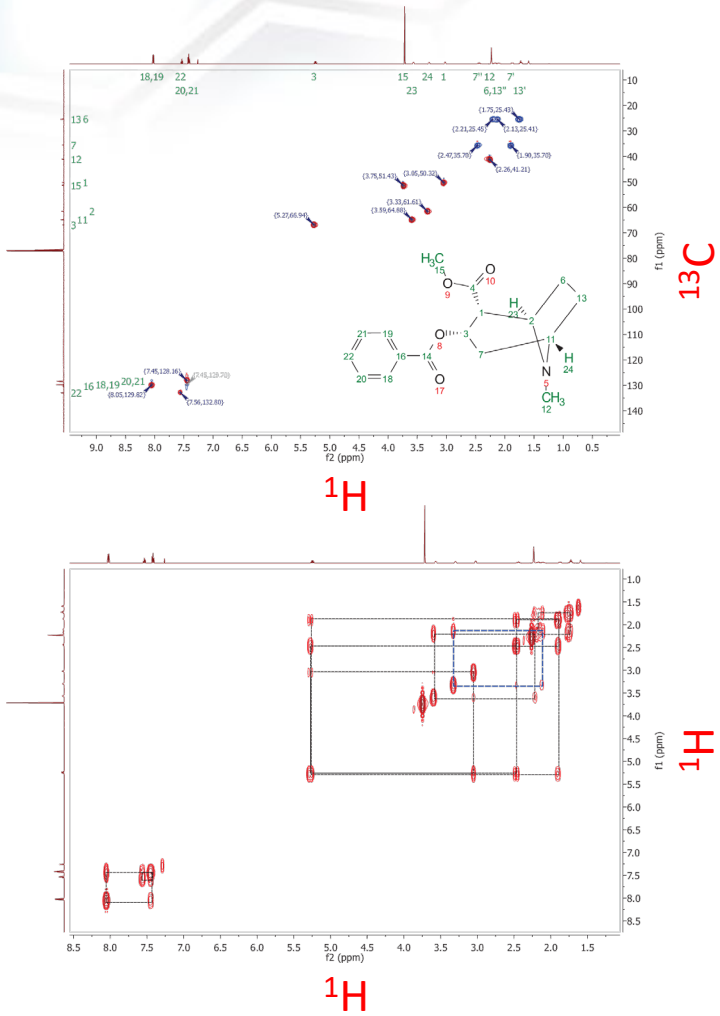
- NMR at a Glance
- Benchtop NMR
- Fentanyl Analog Differentiation with ^{1}H low-field/benchtop NMR Spectra
- Fluorine (^{19}F) low-field/benchtop NMR
- Quantum Mechanic Spectral Analysis (QMSA) of ^{1}H NMR Spectra and Translation of ^{1}H Spectra Across Magnet Field Strengths
- Recent Sample Investigations
- Conclusion & Acknowledgements

NMR at a Glance

❑ Powerful Structure Elucidation Tool

- NMR Active Nuclei (Spin ½)
  - ^{1}H, ^{13}C, ^{15}N ^{19}F, ^{31}P mainly
- 2D experiments offer a wealth of connectivity information
  - COSY : ^{1}H-^{1}H single bond correlations
  - TOCSY : ^{1}H-^{1}H multi-bond correlations
  - HSQC : ^{1}H-X single-bond single bond connectivity
  - HMBC, HMQC : ^{1}H-X multi-bond single bond connectivity

There are **MANY** more methods including variants of these and others.



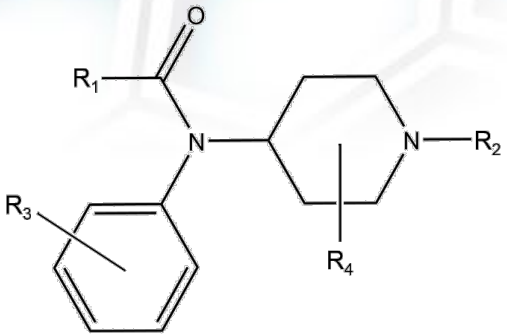
❑ Analytical Tool

- Quantification
  - Absolute purity determinations against a reference material
  - Quantification of multiple compounds from a single internal (or external) standard
- Powerful screening method for unknowns
  - In most cases, if it's soluble and has a proton you can see it

Benchtop NMR

- 40 – 90 MHz Permanent Magnet Systems
- Range from ~ $40K - $100K
- No cryogens, little maintenance
- Easy to Use
- Portable to varying extents
- Some 2D spectral capabilities
- Drawbacks
  - Sensitivity & Resolution

Fentanyl Analog Benchtop NMR Evaluation



General fentanyl structure labeling functional groups and opportunity for modification

In the case of fentanyl:

R1) N-propionyl group
R2) phenethyl group
R3) aniline ring
R4) piperidine ring

Duffy J, Urbas A, Niemitz M, Lippa K, Marginean I, "Differentiation of fentanyl analogues by low-field NMR spectroscopy." *Anal Chim Acta,* **2019**, 1049:161-169

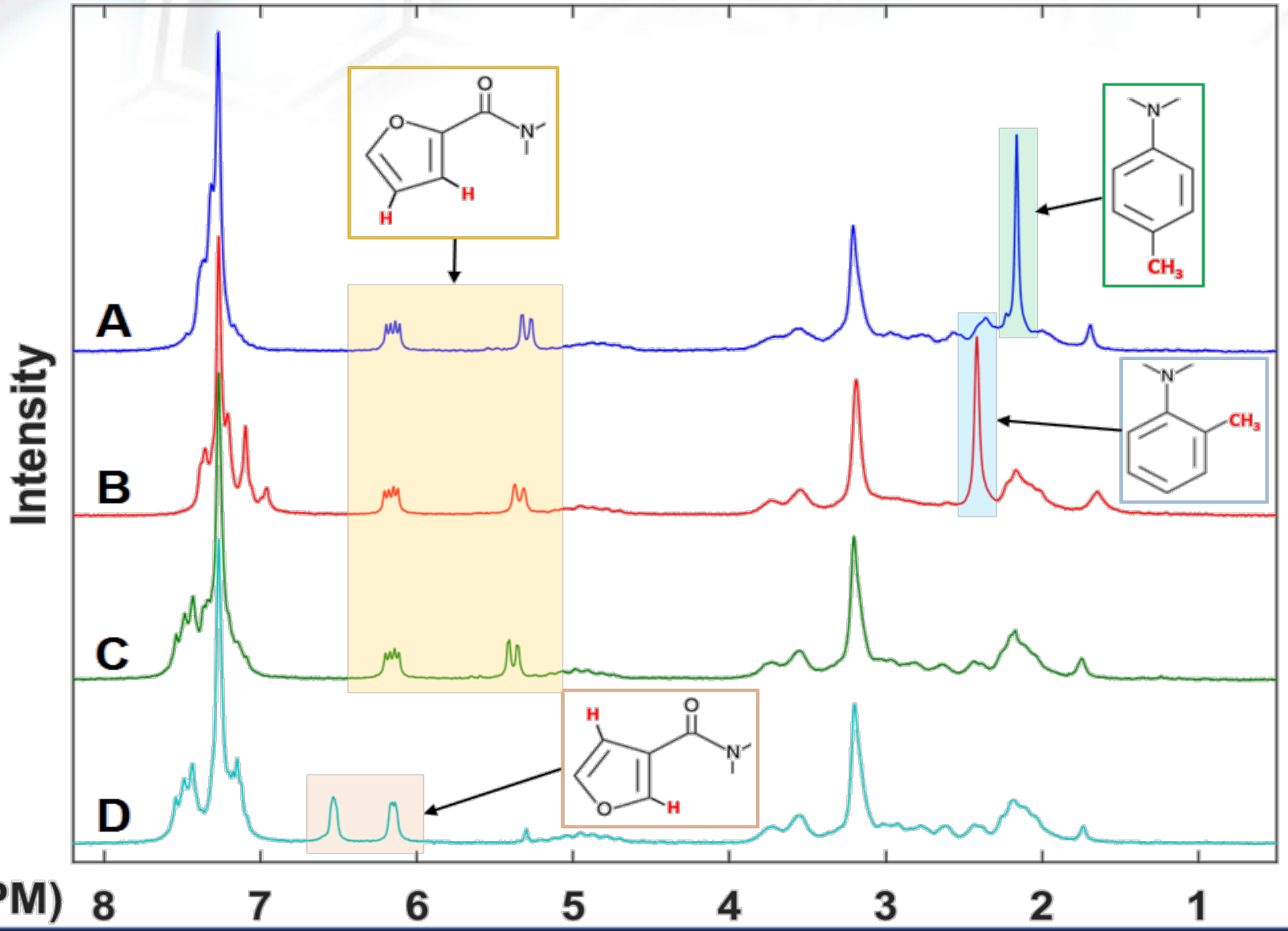
65 fentanyl analogs and related compounds were examined

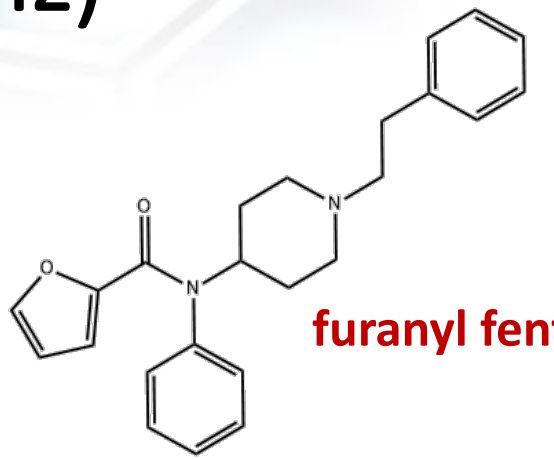
All samples were prepared in $CDCl_3$ (~5 mg in 0.6-0.7 mL)

Name	MW	R1	R2	R3
Fentanyl HCl	372.9	$-CH_2CH_3$	$-CH_2CH_2Ph$	
Fentanyl	336.5	$-CH_2CH_3$	$-CH_2CH_2Ph$	
Norfentanyl	232.3	$-CH_2CH_3$	$-H$	
α-Methyl Fentanyl HCl	387.0	$-CH_2CH_3$	$-CH(CH_3)CH_2Ph$	
β-Methyl Fentanyl HCl	387.0	$-CH_2CH_3$	$-CH_2CH(CH_3)Ph$	
ortho-Methylfentanyl HCl	387.0	$-CH_2CH_3$	$-CH_2CH_2Ph$	-2-CH_3
meta-Methylfentanyl HCl	387.0	$-CH_2CH_3$	$-CH_2CH_2Ph$	-3-CH_3
para-Methylfentanyl HCl	387.0	$-CH_2CH_3$	$-CH_2CH_2Ph$	-4-CH_3
β-hydroxy Fentanyl HCl	388.9	$-CH_2CH_3$	$-CH_2CH(OH)Ph$	
3-Fluorofentanyl HCl* [a]	390.9	–	–	–
ortho-Fluorofentanyl HCl	390.9	$-CH_2CH_3$	$-CH_2CH_2Ph$	-2-F
meta-Fluorofentanyl HCl	390.9	$-CH_2CH_3$	$-CH_2CH_2Ph$	-3-F
para-Fluorofentanyl HCl	390.9	$-CH_2CH_3$	$-CH_2CH_2Ph$	-4-F
para-Chlorofentanyl HCl	407.4	$-CH_2CH_3$	$-CH_2CH_2Ph$	-4-Cl
Despropionyl *ortho*-Fluorofentanyl	298.4	$-H$	$-CH_2CH_2Ph$	-2-F
Despropionyl *meta*-Fluorofentanyl	298.4	$-H$	$-CH_2CH_2Ph$	-3-F
Despropionyl *para*-Fluorofentanyl	298.4	$-H$	$-CH_2CH_2Ph$	-4-F
Butyryl Fentanyl HCl	387.0	$-CH_2CH_2CH_3$	$-CH_2CH_2Ph$	
α-Methyl Butyryl Fentanyl HCl	401.0	$-CH_2CH_2CH_3$	$-CH(CH_3)CH_2Ph$	
ortho-Fluorobutyryl Fentanyl HCl	405.0	$-CH_2CH_2CH_3$	$-CH_2CH_2Ph$	-2-F
meta-Fluorobutyryl Fentanyl HCl	405.0	$-CH_2CH_2CH_3$	$-CH_2CH_2Ph$	-3-F
para-Fluorobutyryl Fentanyl HCl	405.0	$-CH_2CH_2CH_3$	$-CH_2CH_2Ph$	-4-F
para-Chlorobutyryl Fentanyl HCl	421.4	$-CH_2CH_2CH_3$	$-CH_2CH_2Ph$	-4-Cl
para-methoxy Butyryl Fentanyl HCl	417.0	$-CH_2CH_2CH_3$	$-CH_2CH_2Ph$	-4-OCH_3
Isobutyryl Fentanyl HCl	387.0	$-CH(CH_3)CH_3$	$-CH_2CH_2Ph$	

the list goes on….

Furanyl Fentanyl Analogs (1H NMR, 62 MHz)





furanyl fentanyl

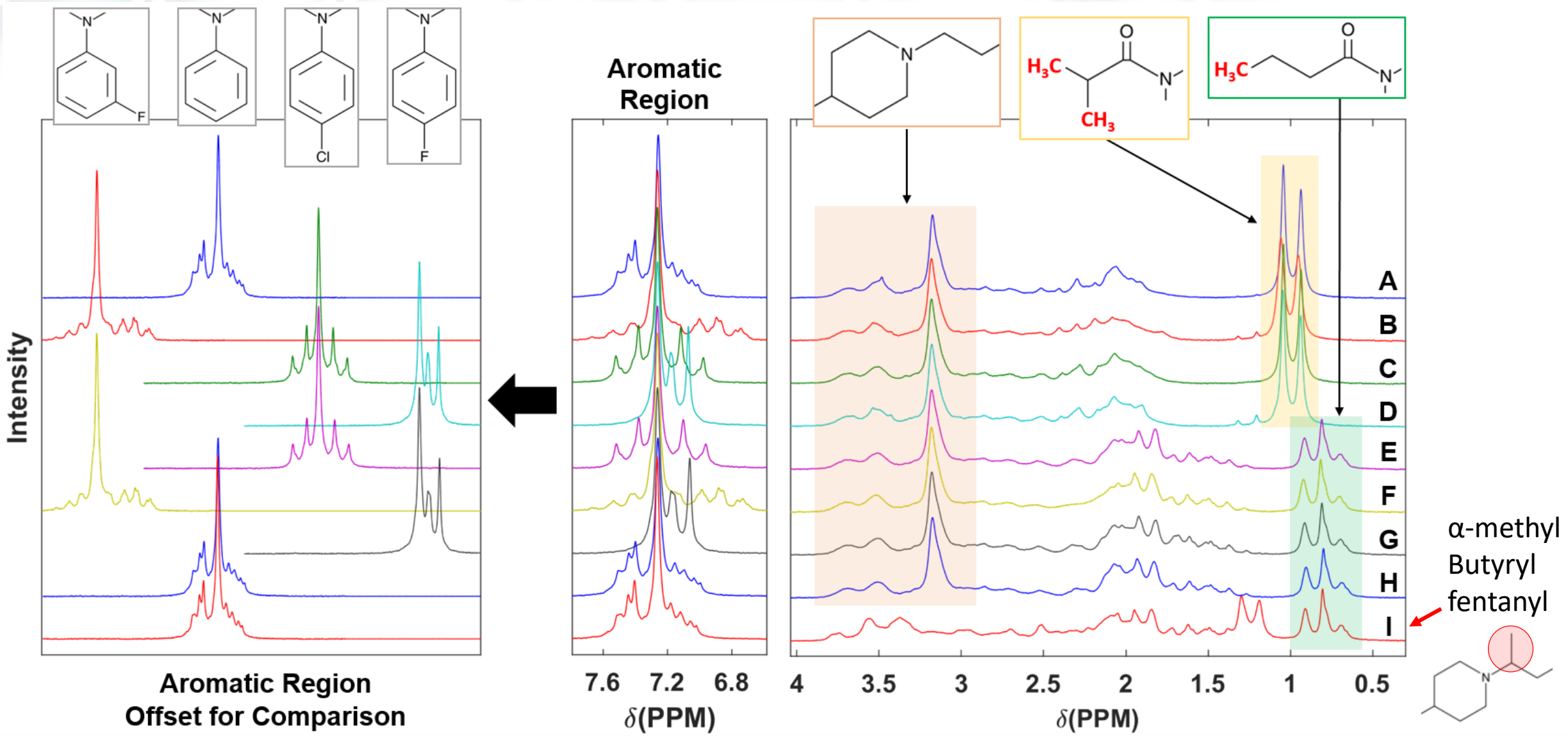
A) para-methyl furanyl fentanyl

B) ortho-methyl furanyl fentanyl

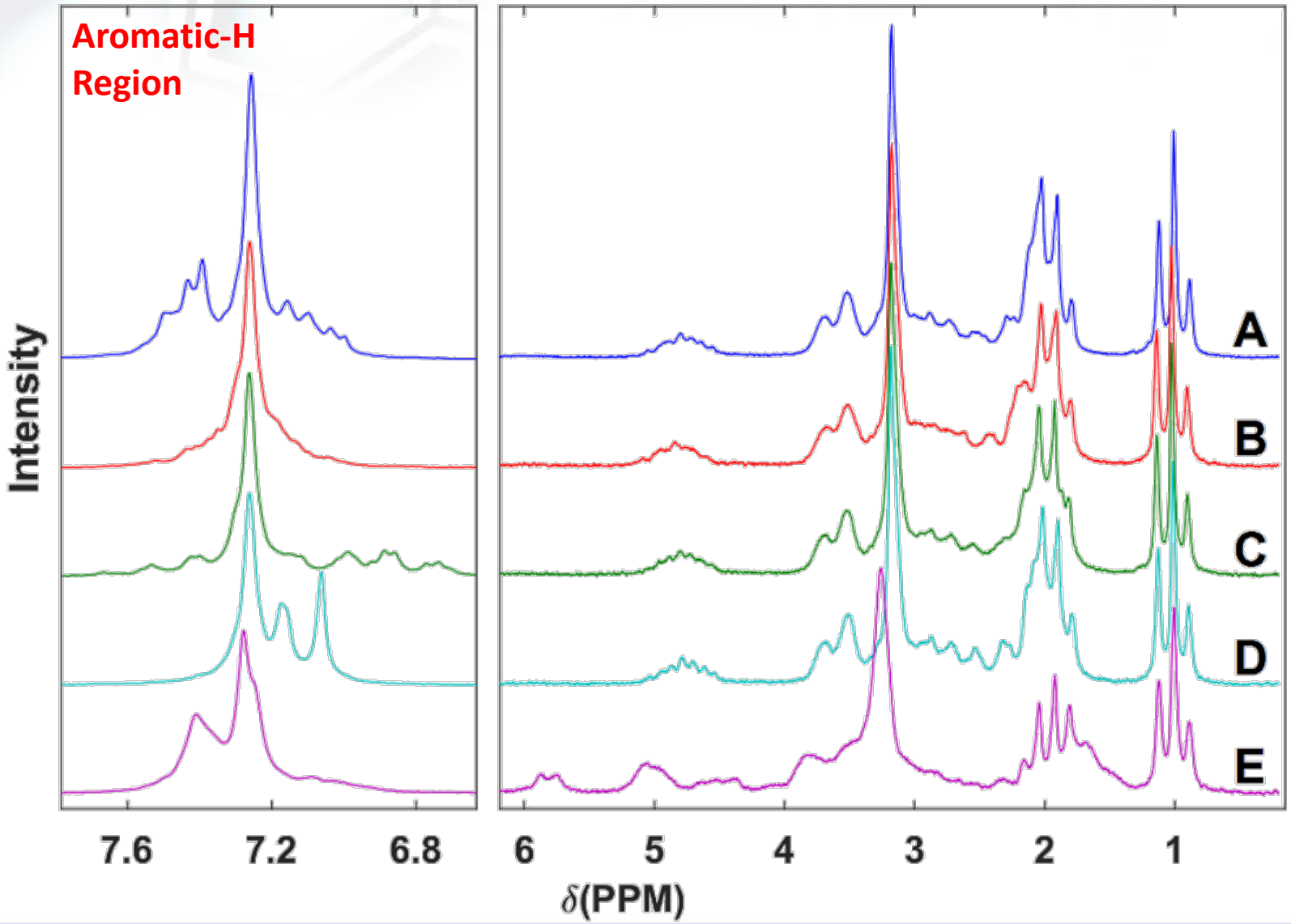
C) furanyl fentanyl

D) furanyl fentanyl 3-furancarboxamide isomer

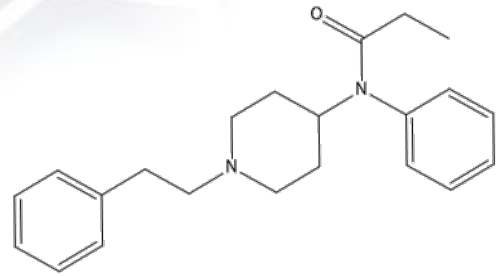
Butyrl Fentanyl Analogs (1H NMR, 62 MHz)



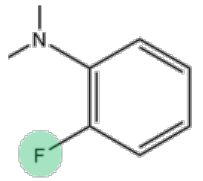
Fluorofentanyl Analogs (1H NMR, 62 MHz)



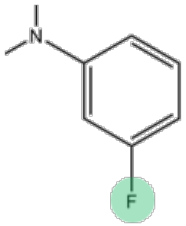
A) fentanyl



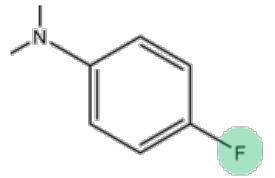
B) o-fluorofentanyl



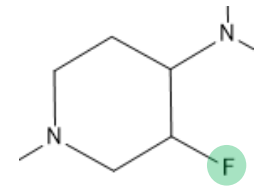
C) m-fluorofentanyl



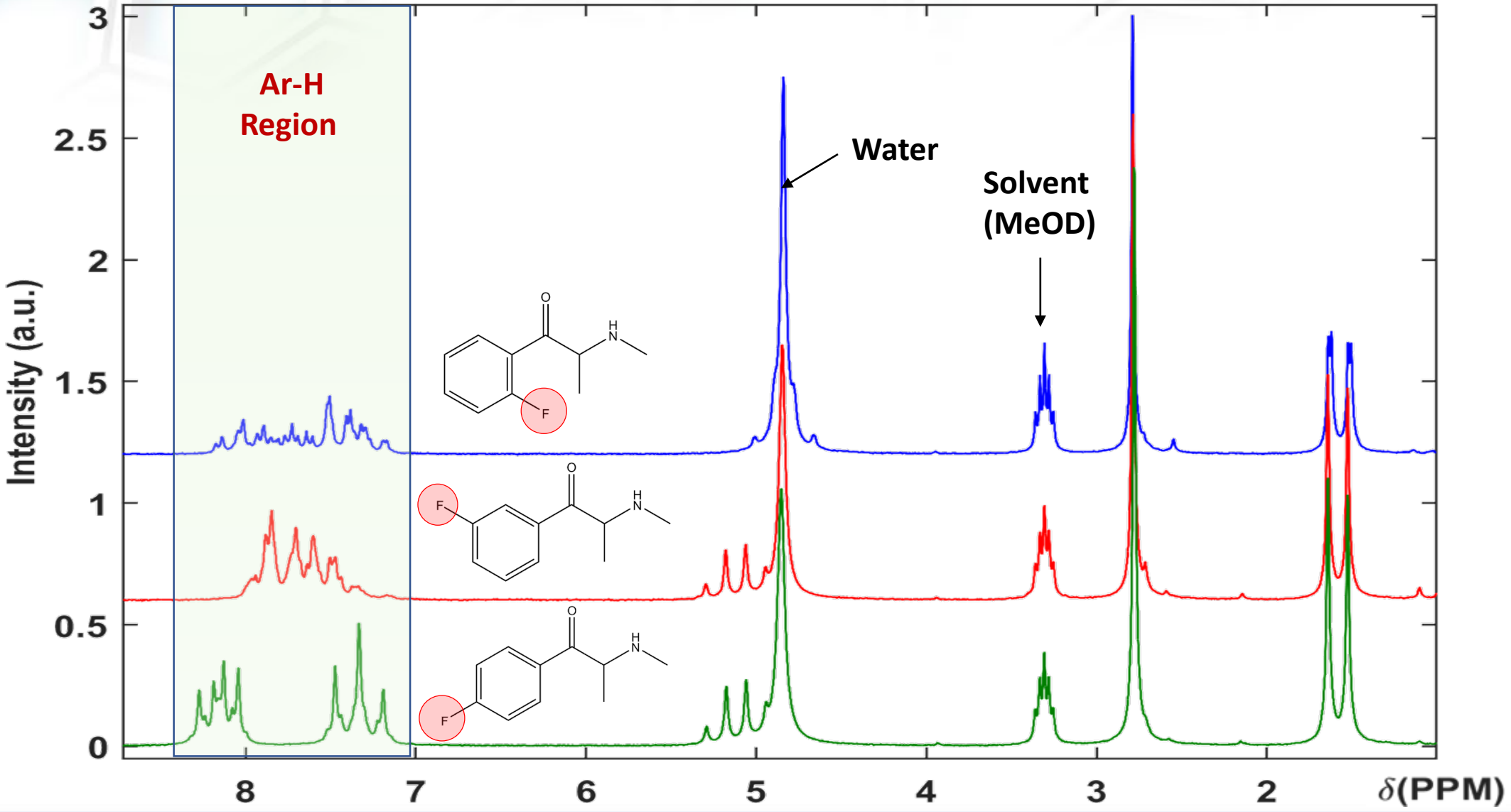
D) p-fluorofentanyl



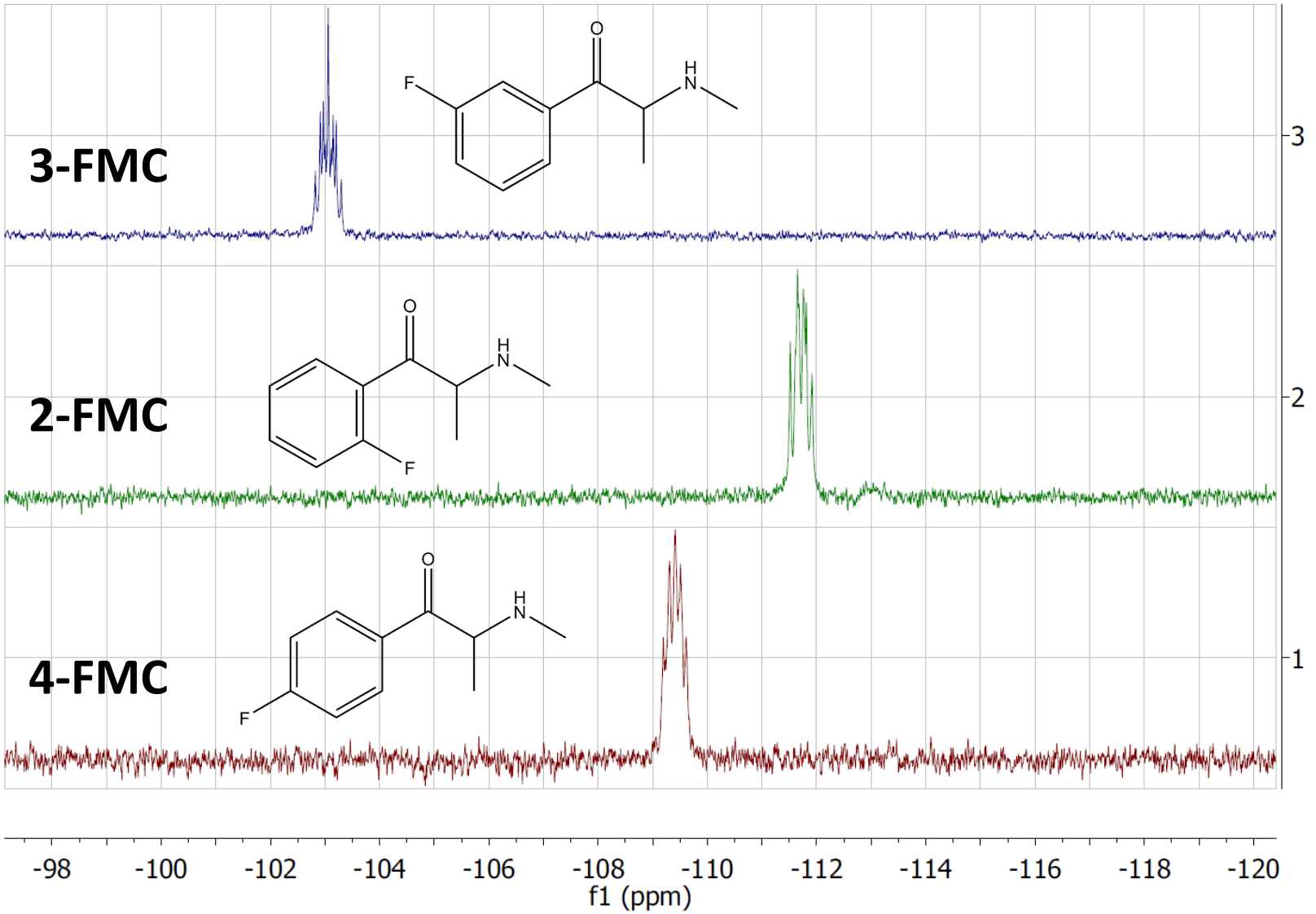
E) 3-fluorofentanyl

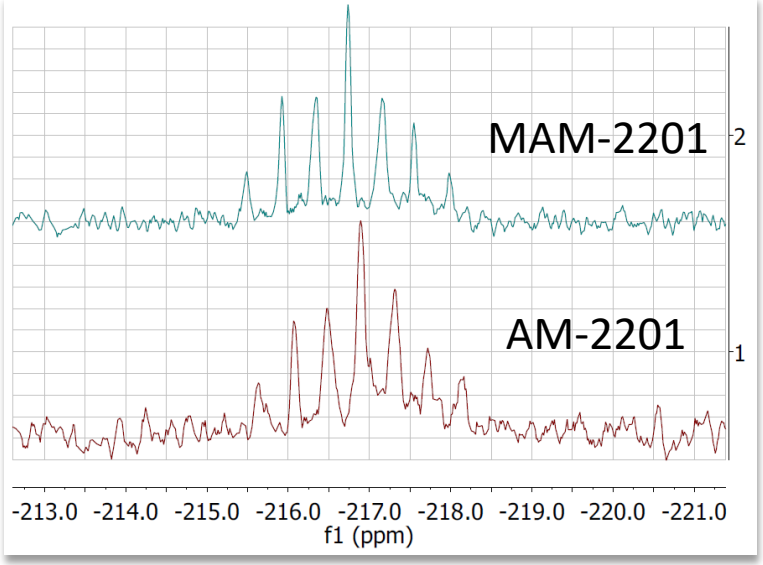


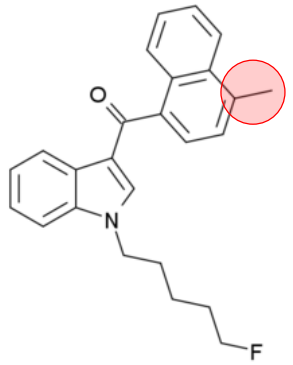
Fluoromethcathinone Isomers (^{1}H, 62 MHz, MeOD)



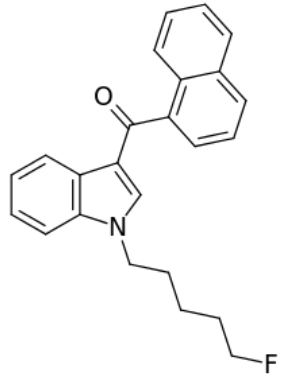
^{19}F NMR Spectra (~58 MHz)





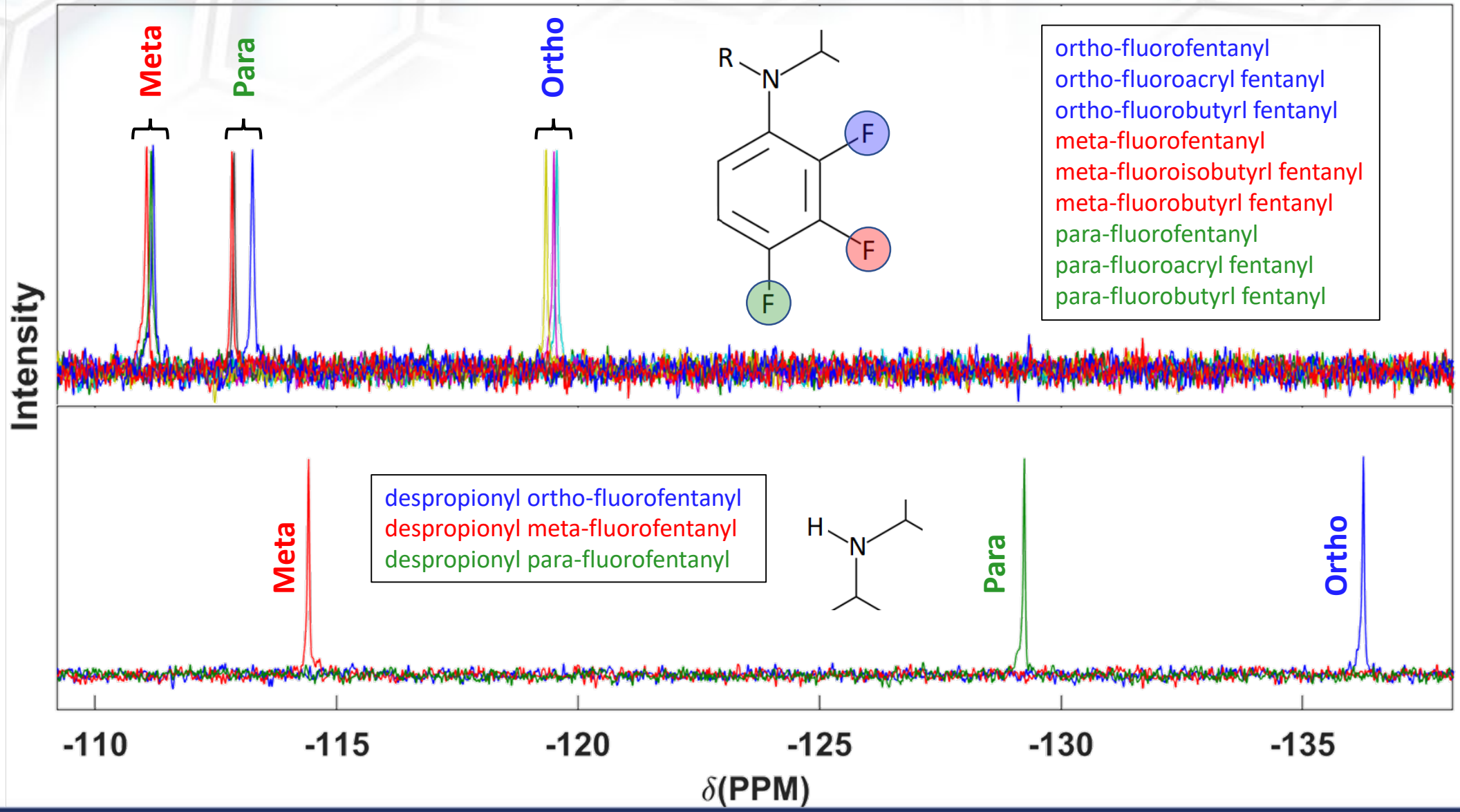


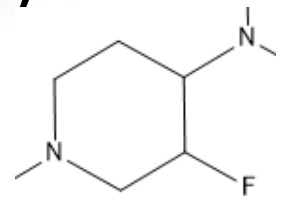
MAM-2201



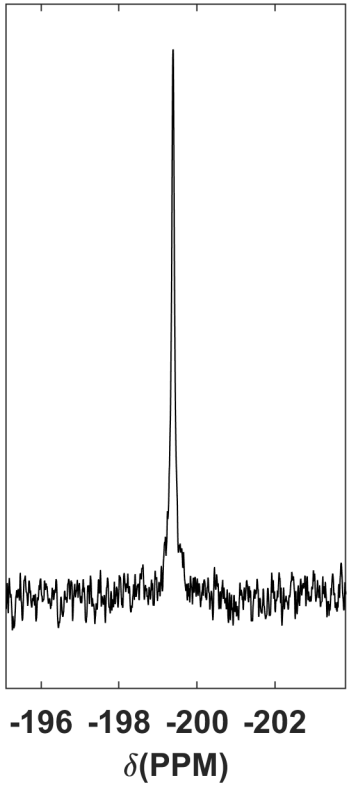
AM-2201

^{19}F NMR of Fluorinated Fentanyl Analogs (1H Decoupled)





3-fluorofentanyl



Outline

- NMR at a Glance
- Benchtop NMR
- Fentanyl Analog Differentiation with ^{1}H low-field/benchtop NMR Spectra
- Fluorine (^{19}F) low-field/benchtop NMR
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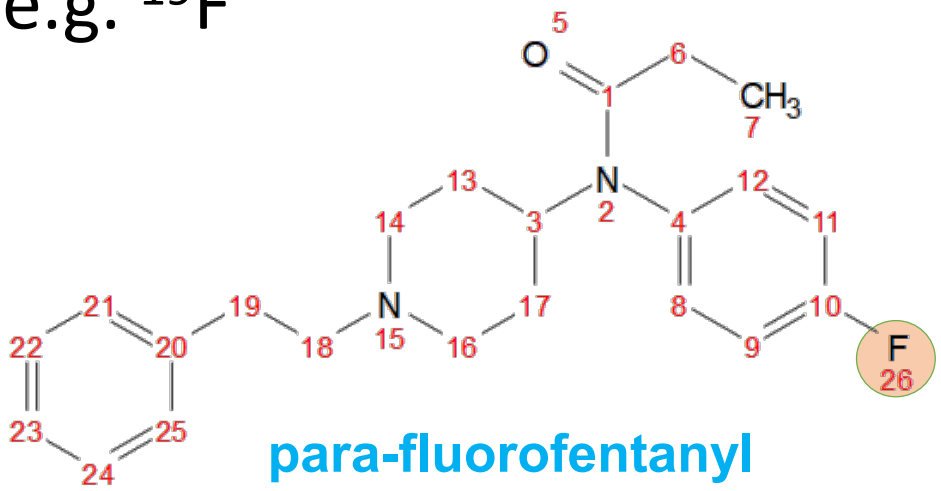
- Conclusion & Acknowledgements

Can We Better Utilize ^{1}H Spectra?

- Wealth of structural information available
- Proton counts
- Chemical shift structure correlations
- Connectivity via couplings and coupling constants
- Indirect heteronuclear information through coupling, e.g. ^{19}F



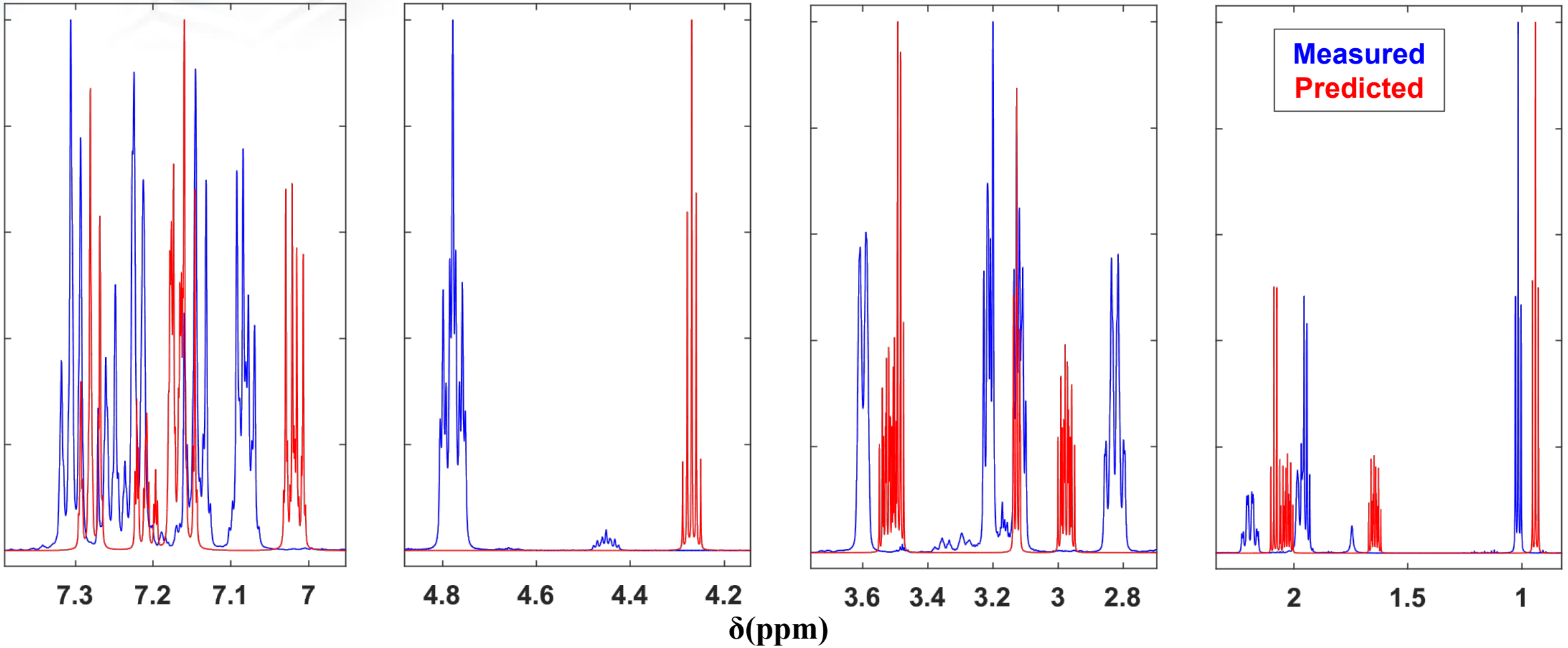
para-fluorofentanyl

Predicted Chemical Shifts & Coupling Constants for para-fluorofentanyl

Atom	Shift (ppm)	J (Hz)	
3 CH	4.27	J(3-13')	5.78
		J(3-13'')	5.78
		J(3-17')	5.78
		J(3-17'')	5.78
6 CH2	2.082	J(6)	14.56
		J(6-7)	7.89
7 CH3	0.94	J(7-6)	7.89
		J(7)	6.99
8 CH	7.019	J(8-9)	8.43
		J(8-12)	1.5
		J(8-26)	5
9 CH	7.156	J(9-8)	8.43
		J(9-11)	1.5
		J(9-26)	8
11 CH	7.156	J(11-9)	1.5
		J(11-12)	8.43
		J(11-26)	8
12 CH	7.019	J(12-8)	1.5
		J(12-11)	8.43
		J(12-26)	5
13' CH2	1.645	J(13'-3)	5.78
		J(13'-13'')	12.29
		J(13'-14')	8.01
		J(13'-14'')	5.65
13'' CH2	2.031	J(13''-3)	5.78
		J(13''-13')	12.29
		J(13''-14')	5.65
		J(13''-14'')	8.01

Predicting ^{1}H NMR Spectra

Measured vs Predicted Para-Fluorofentanyl ^{1}H NMR Spectra (600 MHz)



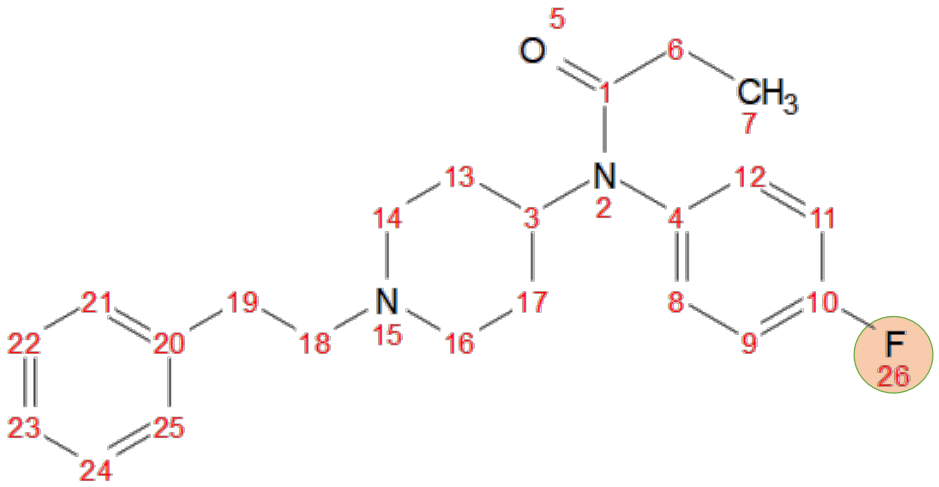
While predicted ^{1}H spectra can be useful for spectral interpretation they often differ quite considerably from observed spectra in both chemical shifts and coupling constants.

Quantum Mechanic Spectral Analysis (QMSA)

Predicted Chemical Shifts & Coupling Constants

Atom	Shift (ppm)	J (Hz)	
3 CH	4.27	J(3-13')	5.78
		J(3-13'')	5.78
		J(3-17')	5.78
		J(3-17'')	5.78
6 CH2	2.082	J(6)	14.56
		J(6-7)	7.89
7 CH3	0.94	J(7-6)	7.89
		J(7)	6.99
8 CH	7.019	J(8-9)	8.43
		J(8-12)	1.5
		J(8-26)	5
9 CH	7.156	J(9-8)	8.43
		J(9-11)	1.5
		J(9-26)	8
11 CH	7.156	J(11-9)	1.5
		J(11-12)	8.43
		J(11-26)	8
12 CH	7.019	J(12-8)	1.5
		J(12-11)	8.43
		J(12-26)	5
13' CH2	1.645	J(13'-3)	5.78
		J(13'-13'')	12.29
		J(13'-14')	8.01
		J(13'-14'')	5.65
13'' CH2	2.031	J(13''-3)	5.78
		J(13''-13')	12.29
		J(13''-14')	5.65
		J(13''-14'')	8.01

Long story short….



Para-fluorofentanyl

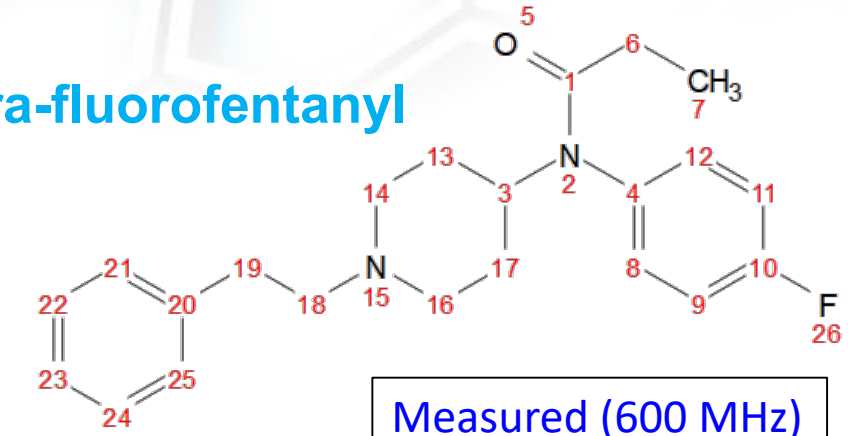
There are a total of 117 chemical shifts and couplings in the spin system utilized for this molecule, the tables only represent a subset.

Fit Chemical Shifts & Coupling Constants

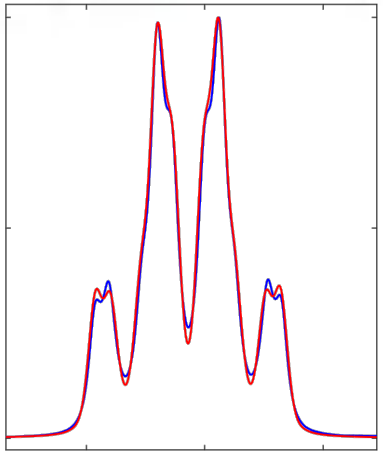
Atom	Shift (ppm)	J (Hz)	
3 CH	4.778	J(3-13')	12.3336
		J(3-13'')	3.6189
		J(3-17')	12.3336
		J(3-17'')	3.6189
6 CH2	1.9495	J(6)	14.56
		J(6-7)	7.4367
7 CH3	1.0157	J(7-6)	7.4367
		J(7)	6.99
8 CH	7.0817	J(8-9)	8.663
		J(8-12)	3.1175
		J(8-26)	4.7923
9 CH	7.1451	J(9-8)	8.663
		J(9-11)	2.6866
		J(9-26)	8.0205
11 CH	7.1451	J(11-9)	2.6866
		J(11-12)	8.663
		J(11-26)	8.0205
12 CH	7.0817	J(12-8)	3.1175
		J(12-11)	8.663
		J(12-26)	4.7923
13' CH2	2.1927	J(13'-3)	12.3336
		J(13'-13'')	-13.6442
		J(13'-14')	13.0136
		J(13'-14'')	4.2744
13'' CH2	1.9738	J(13''-3)	3.6189
		J(13''-13')	-13.6442
		J(13''-14')	3.1651
		J(13''-14'')	3.1276

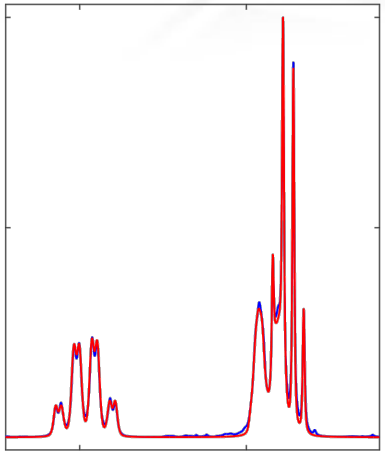
Quantum Mechanic Spectral Analysis (QMSA)

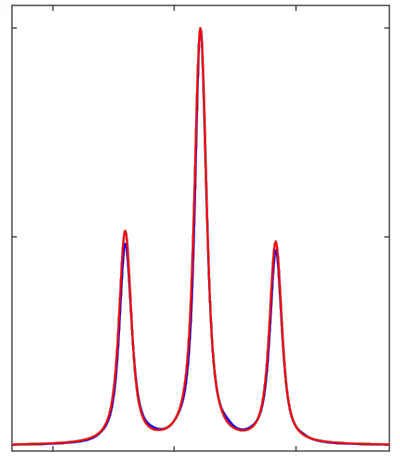
Para-fluorofentanyl

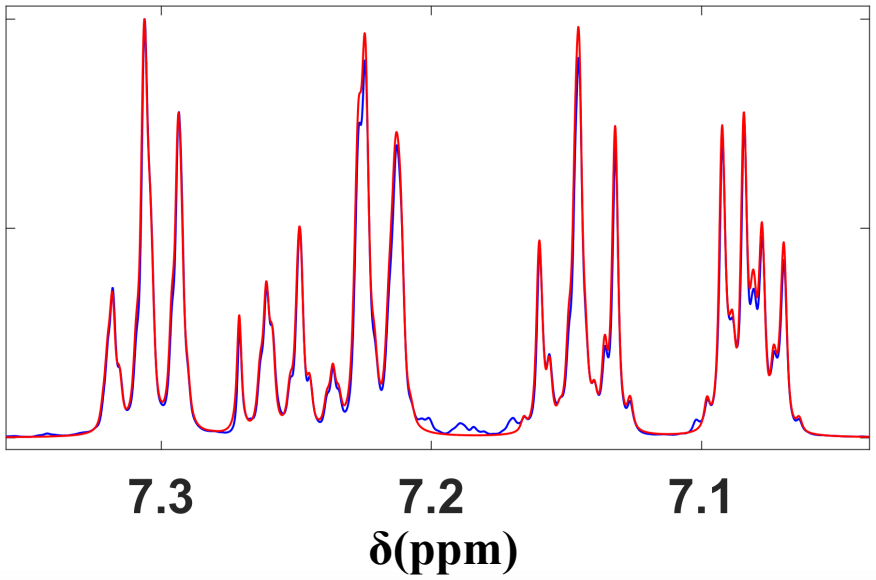


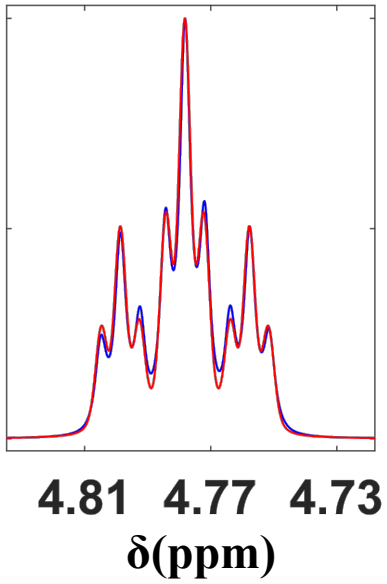
Measured (600 MHz)
QMSA Model

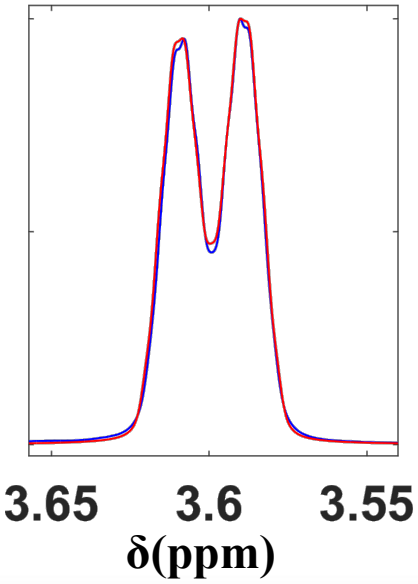


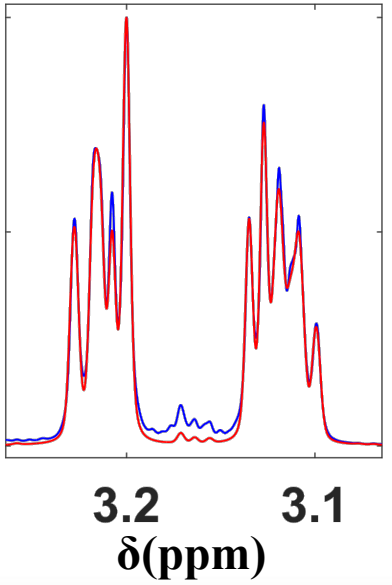






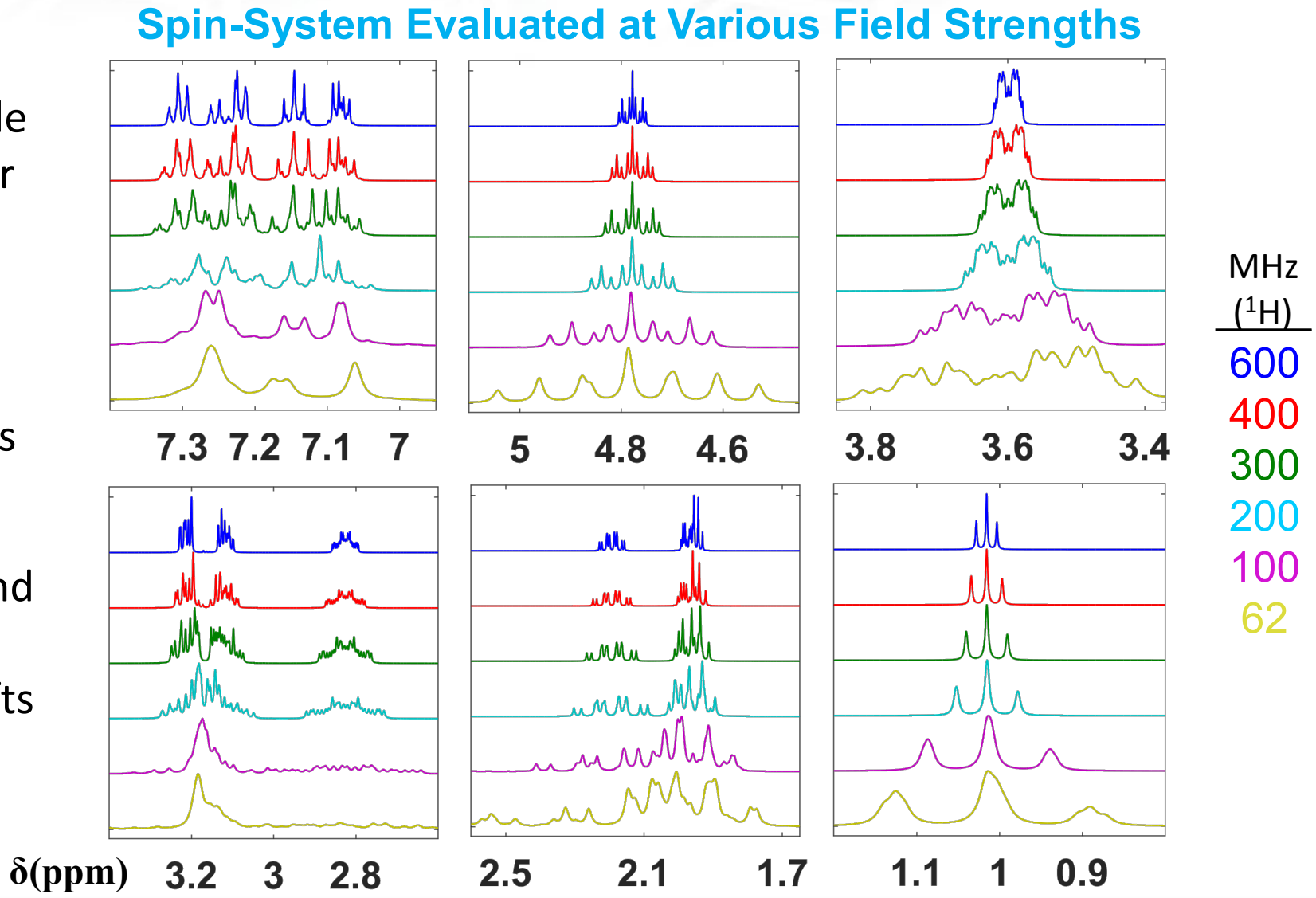




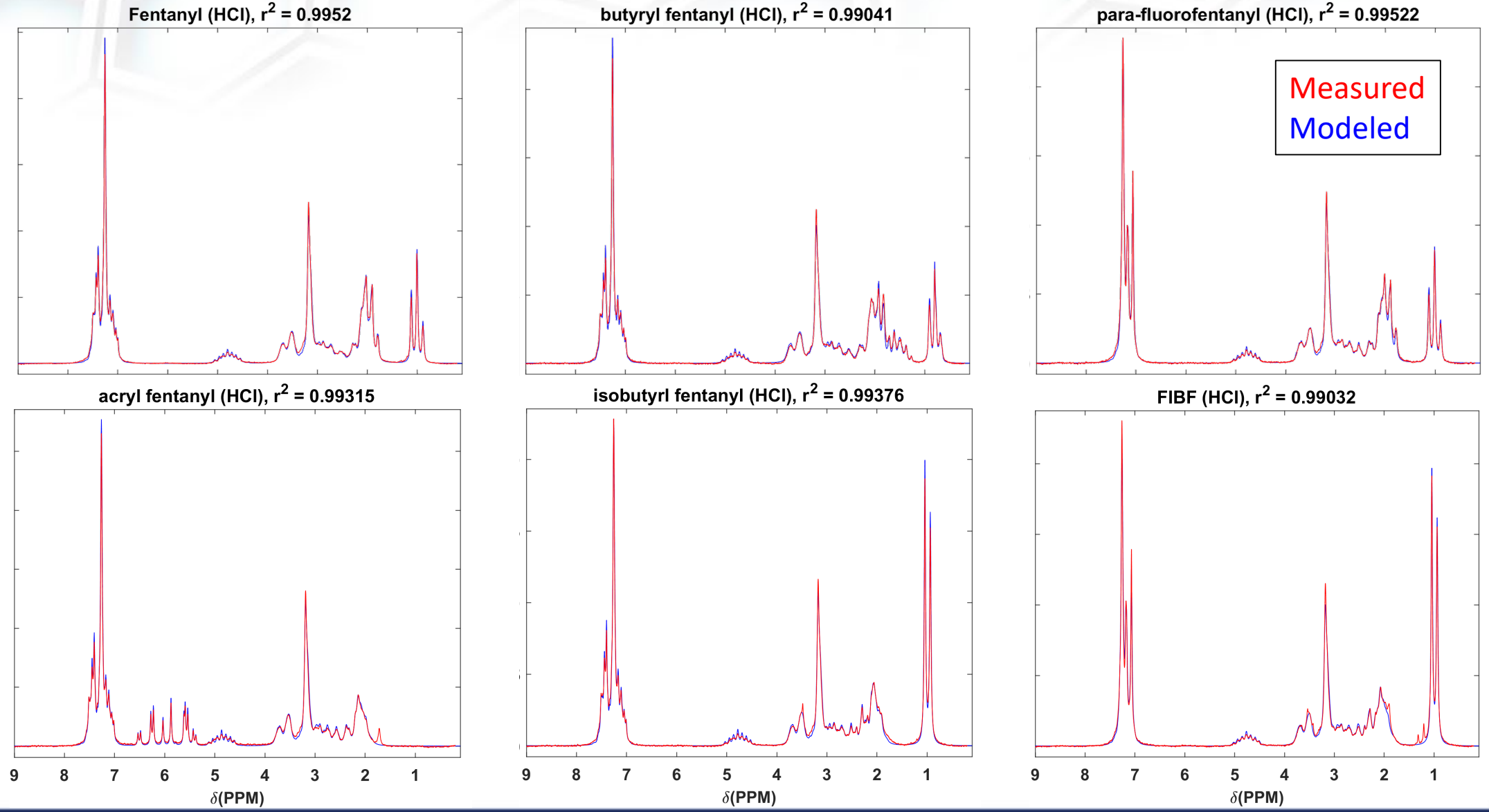


Field Translation of ^{1}H NMR Spectra using Spin-System Models

- QMSA models are field independent and thus portable to different magnetic fields for reproducing spectral information.
- QMSA models are free of solvent and impurity signals as well as instrumental artifacts
- QMSA models are adaptive and enable handling of small perturbations in chemical shifts and coupling constants between samples.

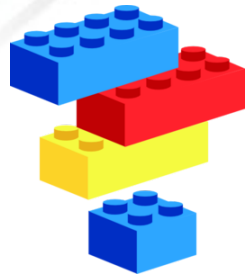


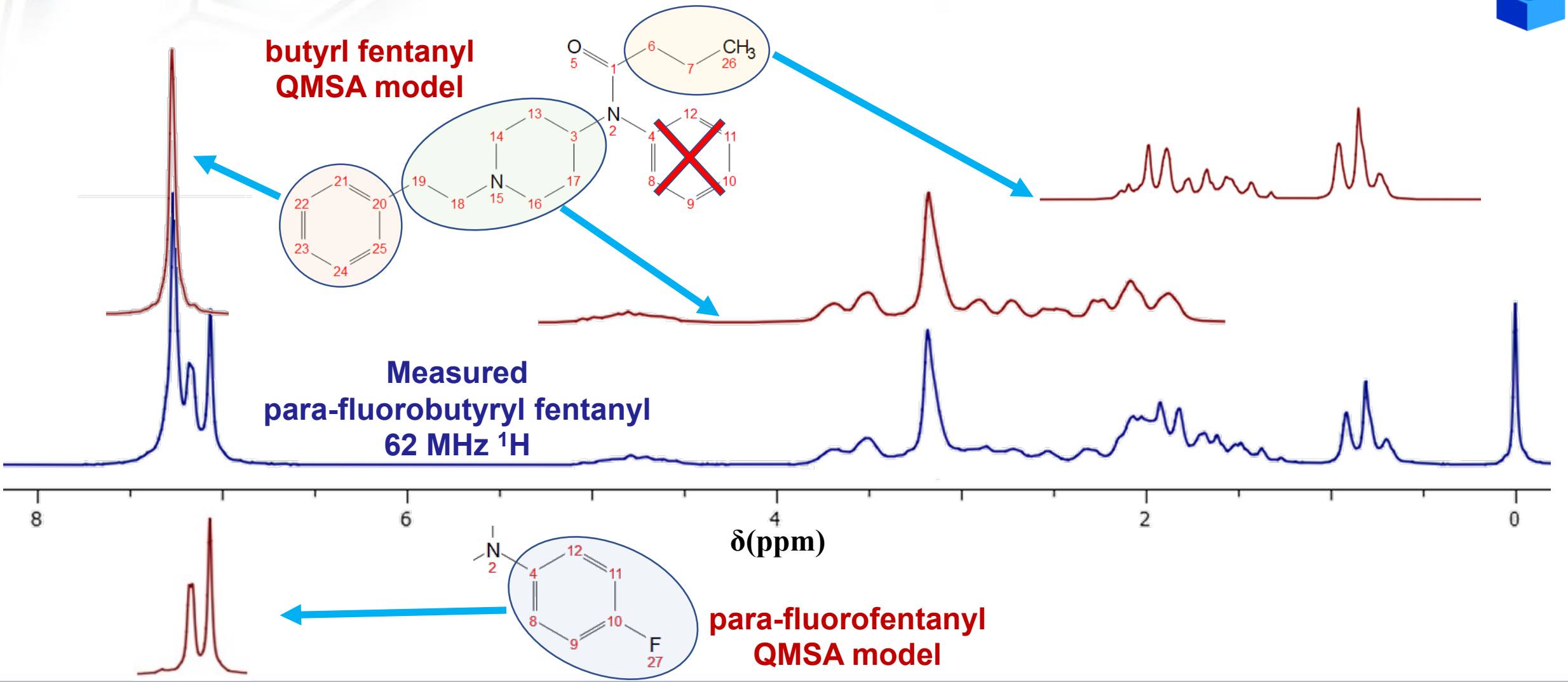
QMSA Fentalog Translation Examples (600 to 62 MHz)



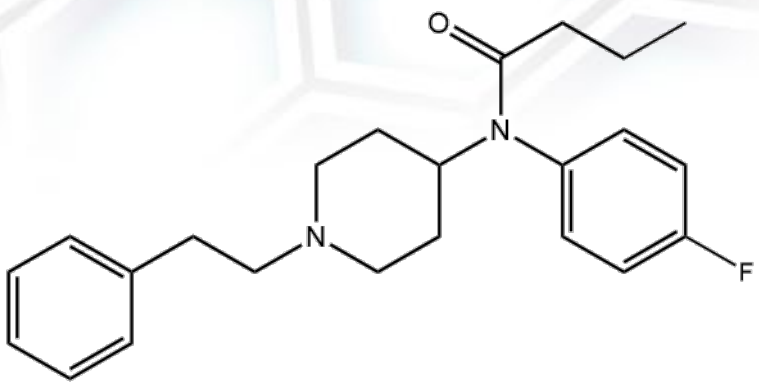
QMSA Sub-Systems as Spectral Building Blocks

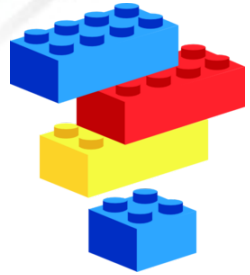
Facilitates building new QMSA models and predicting spectra of unknowns





Bringing it all together…..





Simulated para-fluorobutyryl fentanyl
62 MHz ^{1}H Spectrum
from
butyrl fentanyl & para-fluorofentanyl
QMSA Models

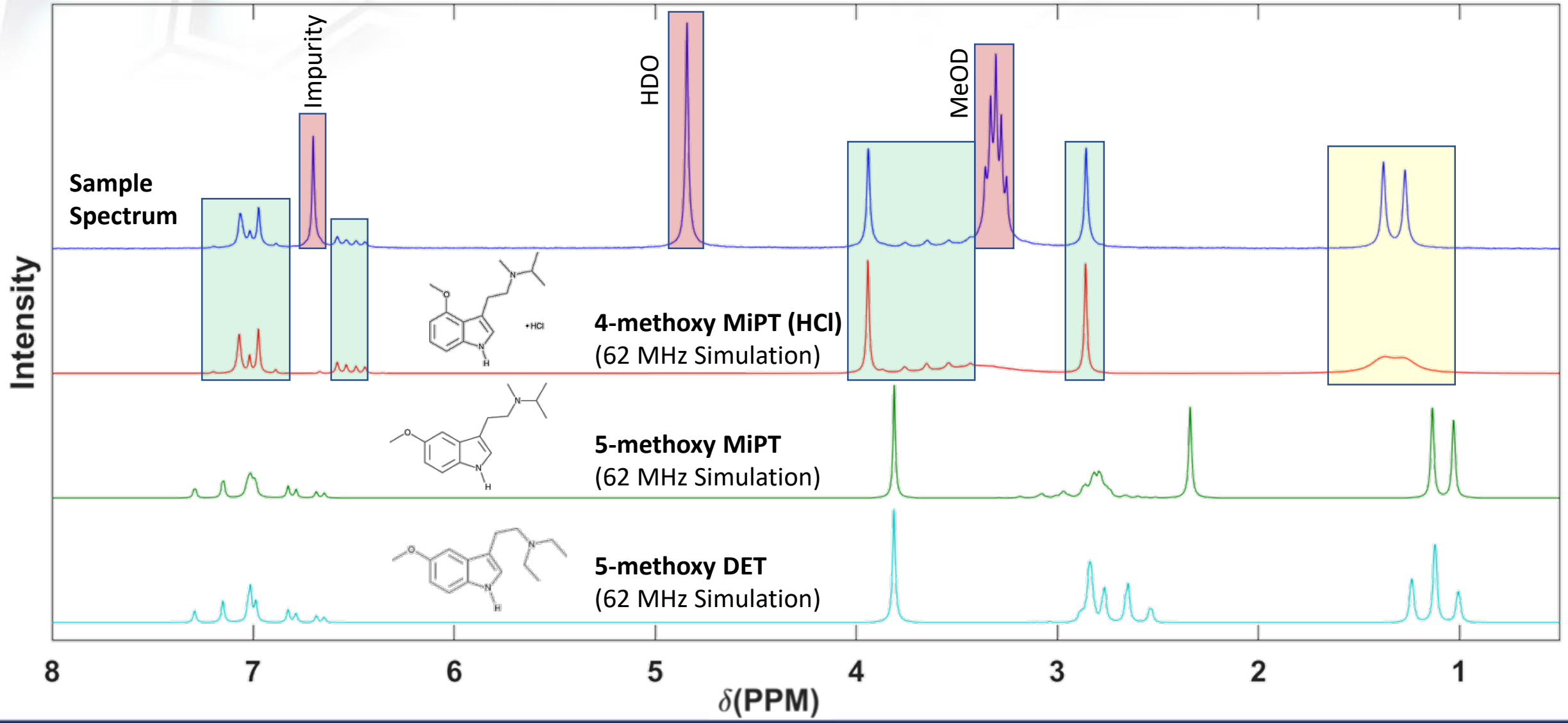
Measured para-Fluorobutyryl fentanyl
62 MHz ^{1}H Spectrum

Outline

- NMR at a Glance
- Benchtop NMR
- Fentanyl Analog Differentiation with ^{1}H low-field/benchtop NMR Spectra
- Fluorine (^{19}F) low-field/benchtop NMR
- Quantum Mechanic Spectral Analysis (QMSA) of ^{1}H NMR Spectra and translation of ^{1}H Spectra Across Magnet Field Strengths
- Recent Sample Investigations
- Conclusion & Acknowledgements

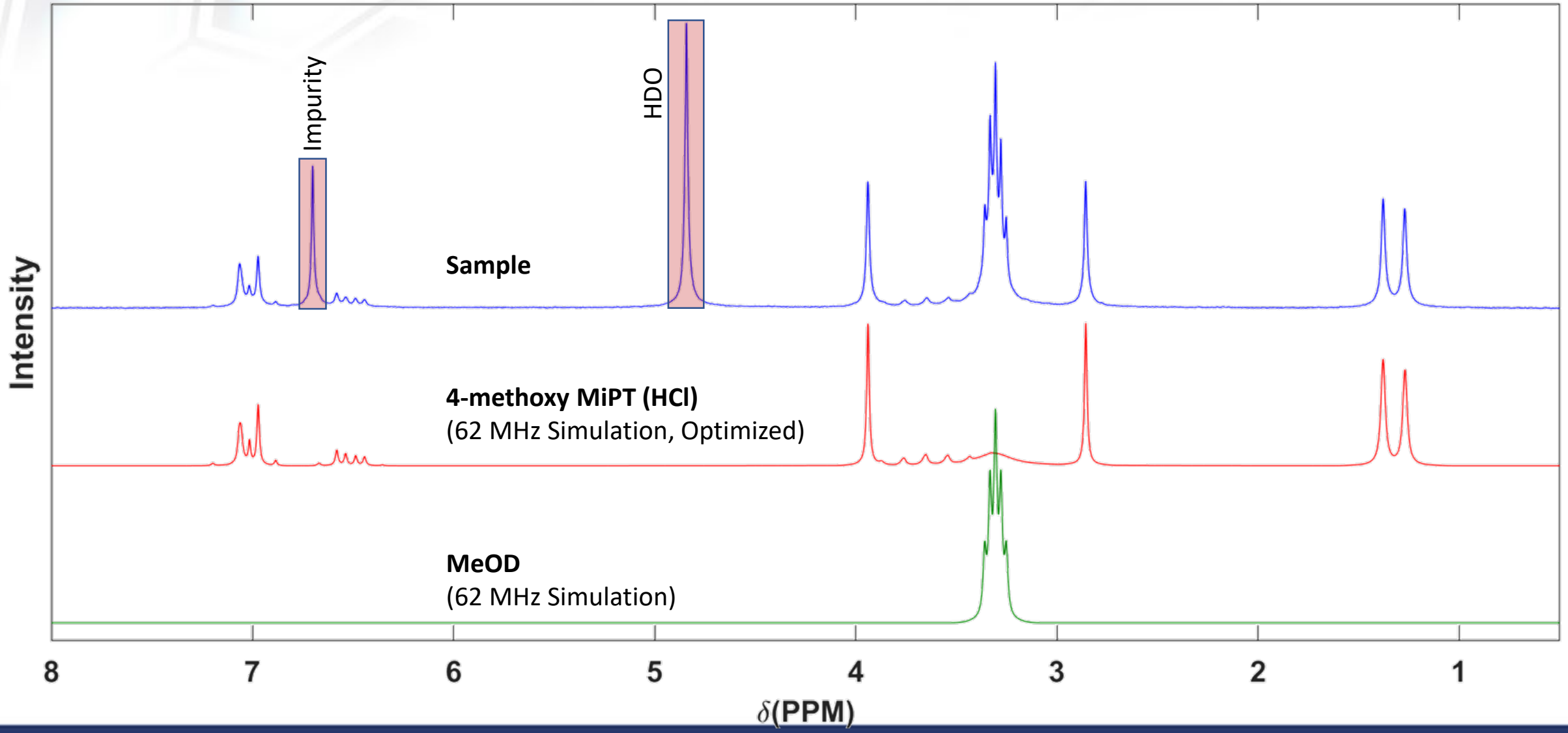
Synthetic Tryptamine Analog Example 1

Sample Spectrum Compared to 62 MHz QMSA Simulations



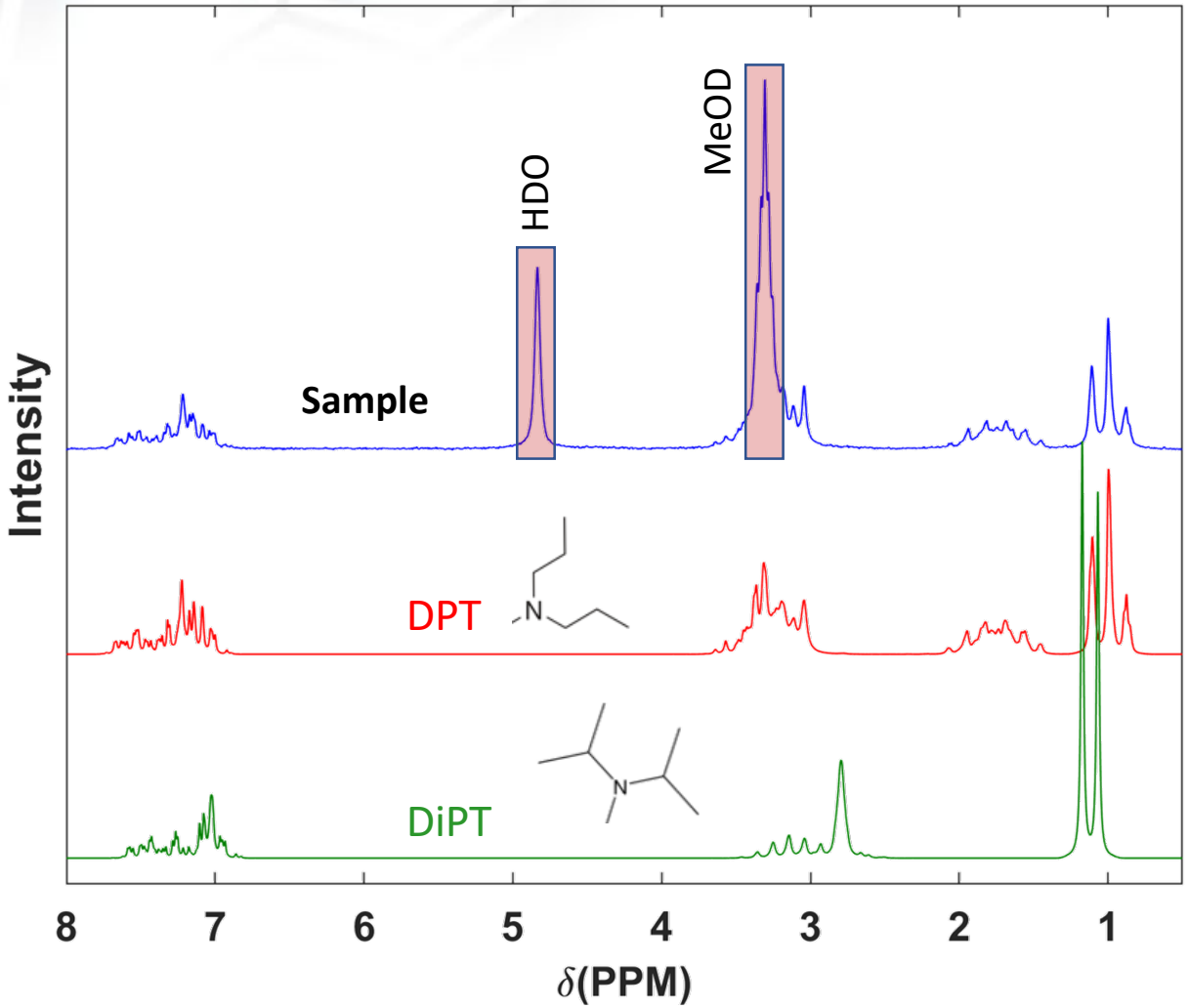
Synthetic Tryptamine Analog Example 1

62 MHz QMSA Model of Sample Spectrum

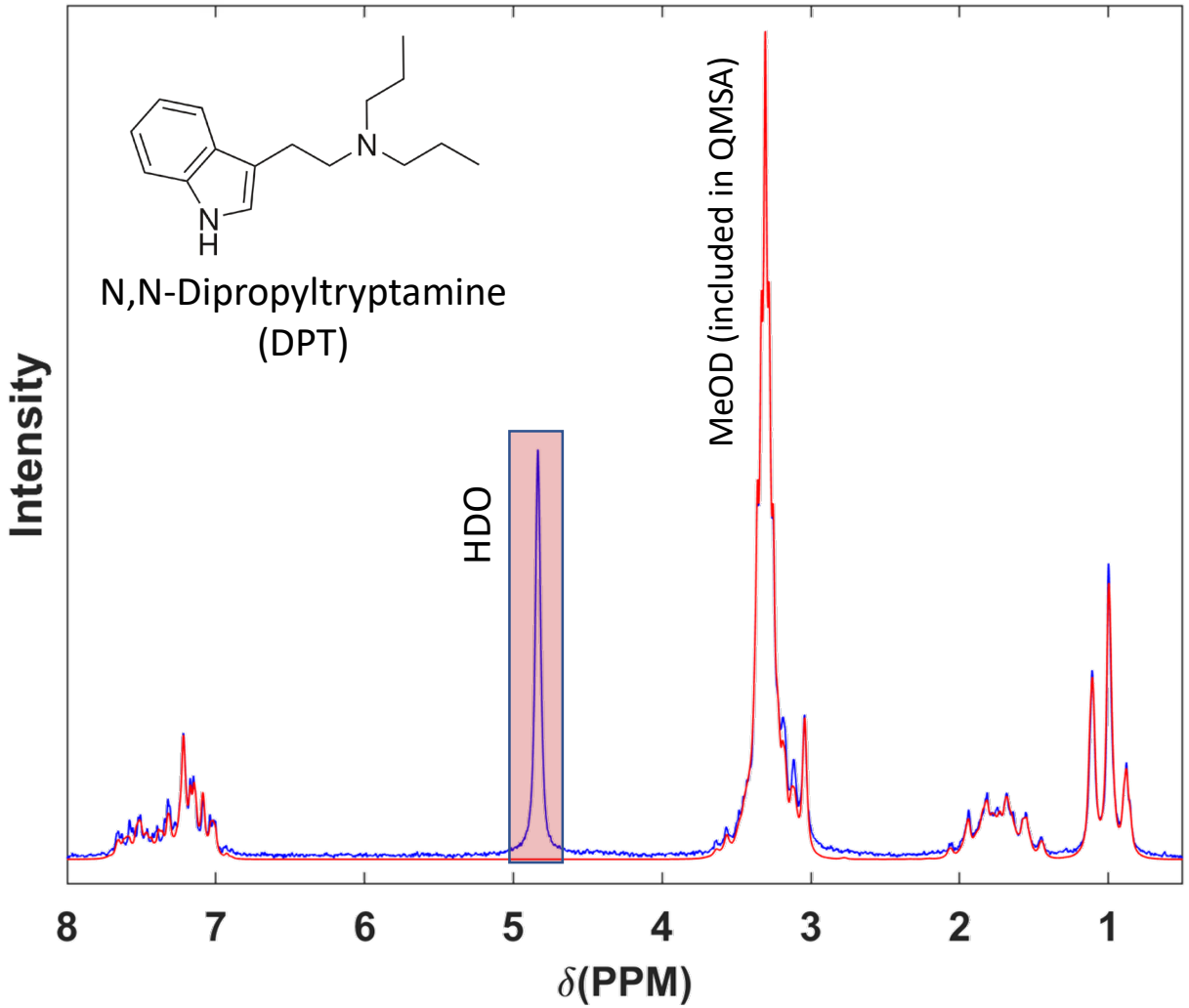


Synthetic Tryptamine Analog Example 2

Sample Spectrum Compared to 62 MHz QMSA Simulations



62 MHz QMSA Model of Sample Spectrum



Conclusions & Future Efforts

- Demonstrated that analogs and isomers of fentanyl and some other classes of compounds were readily differentiated using low-field NMR spectroscopy
- Showed how ^{19}F NMR might be useful in the analysis of fluorinated compounds
- Demonstrated the potential utility of quantum mechanic spectral analysis (QMSA) to enable exchange of ^{1}H spectra between NMR instruments of different field strengths.

  Going Forward….

  - Broaden effort to develop QMSA libraries by enlisting collaborators.
- Resolution and sensitivity are significantly reduced at lower magnetic fields. Mixtures are anticipated to be challenging.

  Going Forward….

  - Explore whether the use of Quantum Mechanic Spectral Analysis (QMSA) will permit effective mixture analysis with low-field NMR. Low-level components (< 5%-10%) would likely be difficult in many situations, though.
  - Continue work with forensic lab partners to evaluate “real-world” samples.

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  - US Postal Inspection Service (Mike Hitchcock)
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Thank You!