Using Visualization Tools to Understand Drug Evidence Handling Processes

Matthew Staymates and Edward Sisco
Material Measurement Laboratory
National Institute of Standards and Technology
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
• 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/in² observed

• Airflow was not controlled in these experiments
The spread of material is linked to the amount of material present.

Laser-sheet visualization ~100 g powder
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.
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
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.

A portion of this work was supported by Award No. 2018-DU-BX-0165, awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication/program/exhibition are those of the author(s) and do not necessarily reflect those of the Department of Justice.
Novel Workflows

Sample Handling and Preparation

Data Analysis & Interpretation

Sample Analysis

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

Color Tests -> Screening with GC-FID -> GC-MS Confirmation

New Approach

Screening with DART-MS or TD-DART-MS -> Targeted GC-MS Confirmation
Expanding DART-MS Capabilities
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
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.
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
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

<table>
<thead>
<tr>
<th>Inner Packaging</th>
<th>Extract</th>
<th>Percent Occurrence</th>
<th>Result Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug Detected</td>
<td>Same Drug Detected</td>
<td>79% (n = 151)</td>
<td>True Positive</td>
</tr>
<tr>
<td>Drug Detected</td>
<td>No Drug Detected</td>
<td>1.5% (n = 3)</td>
<td>False Positive</td>
</tr>
<tr>
<td>Drug Detected</td>
<td>Different Drug Detected</td>
<td>2.5% (n = 5)</td>
<td>False Positive</td>
</tr>
<tr>
<td>No Drug Detected</td>
<td>Drug Detected</td>
<td>4% (n = 7)</td>
<td>False Negative</td>
</tr>
<tr>
<td>No Drug Detected</td>
<td>No Drug Detected</td>
<td>13% (n = 25)</td>
<td>True Negative</td>
</tr>
</tbody>
</table>

**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
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

<table>
<thead>
<tr>
<th>Opioids</th>
<th>Cathinones</th>
<th>Cannabinoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>m-FIBF</td>
<td>Phentermine</td>
<td>FUB-AMB</td>
</tr>
<tr>
<td>p-FIBF</td>
<td>Methamphetamine</td>
<td>MDMB-FUBINACA</td>
</tr>
<tr>
<td>Cyclopropyl Fentanyl</td>
<td>Dimethylylone</td>
<td>EMB-FUBINACA</td>
</tr>
<tr>
<td>Crotonyl Fentanyl</td>
<td>Butylone</td>
<td>MMB2201</td>
</tr>
<tr>
<td>Carfentanil</td>
<td>Ethylone</td>
<td>ADB-FUBINACA</td>
</tr>
<tr>
<td>Methoxyacetyl Fentanyl</td>
<td>Dibutylone</td>
<td>AB-FUBINACA</td>
</tr>
<tr>
<td>Furanyl Fentanyl</td>
<td>Pentylylone</td>
<td>5F-ADBICA</td>
</tr>
<tr>
<td>Etizolam</td>
<td>Dimethylpentylylone</td>
<td>5F-ABICA</td>
</tr>
<tr>
<td>Noscapine</td>
<td>Ethylpentylylone</td>
<td></td>
</tr>
<tr>
<td>Benzodioxole Fentanyl</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Temperature Program</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1)</td>
<td>100 °C for 0 min</td>
</tr>
<tr>
<td>2)</td>
<td>Ramp at 30 °C/min to 300 °C</td>
</tr>
<tr>
<td>3)</td>
<td>Hold for 24 min</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow Rate</td>
<td>1.8 mL/min (Constant Flow)</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>1 µL</td>
</tr>
<tr>
<td>Inlet Temperature</td>
<td>275 °C</td>
</tr>
<tr>
<td>Split Ratio</td>
<td>30:1</td>
</tr>
<tr>
<td>Transfer Line</td>
<td>300 °C</td>
</tr>
<tr>
<td>Quad Temperature</td>
<td>150 °C</td>
</tr>
<tr>
<td>Source Temperature</td>
<td>230 °C</td>
</tr>
<tr>
<td>Tune Mode</td>
<td>stune</td>
</tr>
<tr>
<td>Solvent Delay</td>
<td>1.30 min</td>
</tr>
<tr>
<td>Mass Scan Range</td>
<td>m/z 40 – m/z 550</td>
</tr>
<tr>
<td>Threshold</td>
<td>150</td>
</tr>
<tr>
<td>Scan Speed</td>
<td>N = 2</td>
</tr>
<tr>
<td>Total Run Time</td>
<td>30.667 min</td>
</tr>
</tbody>
</table>
Once a column was chosen, studies were completed to optimize temperature and flow programs.
• 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

<table>
<thead>
<tr>
<th>Opioids</th>
<th>LOD (µg/mL)</th>
<th>Cathinones</th>
<th>LOD (µg/mL)</th>
<th>Cannabinoids</th>
<th>LOD (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>m-FIBF</td>
<td>1</td>
<td>Phentermine</td>
<td>0.5</td>
<td>FUB-AMB</td>
<td>1</td>
</tr>
<tr>
<td>p-FIBF</td>
<td>1</td>
<td>Methamphetamine</td>
<td>0.5</td>
<td>MDMB-FUBINACACA</td>
<td>1</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>1</td>
<td>Dimethyline</td>
<td>0.5</td>
<td>EMB-FUBINACACA</td>
<td>5</td>
</tr>
<tr>
<td>Cyclopropyl Fent.</td>
<td>1</td>
<td>Butylone</td>
<td>0.5</td>
<td>MMB2201</td>
<td>1</td>
</tr>
<tr>
<td>Carfentanil</td>
<td>10</td>
<td>Ethylene</td>
<td>0.5</td>
<td>ADB-FUBINACACA</td>
<td>10</td>
</tr>
<tr>
<td>Crotonyl Fentanyl</td>
<td>10</td>
<td>Dibutylone</td>
<td>0.5</td>
<td>AB-FUBINACACA</td>
<td>10</td>
</tr>
<tr>
<td>Methoxyacetyl Fent.</td>
<td>10</td>
<td>Pentylone</td>
<td>0.5</td>
<td>5F-ABICACA</td>
<td>10</td>
</tr>
<tr>
<td>Furanyl Fentanyl</td>
<td>1</td>
<td>Dimethylpentyline</td>
<td>0.5</td>
<td>5F-ABICACA</td>
<td>10</td>
</tr>
<tr>
<td>Etizolam</td>
<td>25</td>
<td>Ethylpentyline</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noscapine</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzodioxole Fent.</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Comparison to Current Method

<table>
<thead>
<tr>
<th>% Change (Average)</th>
<th>Area</th>
<th>Height</th>
<th>Delta RT</th>
<th>% RSD (RT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opioids</td>
<td>327 %</td>
<td>37 %</td>
<td>135 %</td>
<td>93 %</td>
</tr>
<tr>
<td>Cathinones</td>
<td>66 %</td>
<td>-19 %</td>
<td>262 %</td>
<td>0 %</td>
</tr>
<tr>
<td>Cannabinoids</td>
<td>6518 %</td>
<td>4045 %</td>
<td>220 %</td>
<td>537 %</td>
</tr>
</tbody>
</table>

### Graphs

**Opioids**

- Compound #: 1-2, 2-3, 3-4, 4-5, 5-6, 6-7, 7-8, 8-9, 9-10, 10-11
- ΔRT (%): 0, 10, 20, 30, 40

**Cathinones**

- Compound #: 3-4, 4-5, 5-6, 6-7, 7-8, 8-9
- ΔRT (%): 0, 5, 10, 15

**Cannabinoids**

- Compound #: 1-2, 2-3, 3-4, 4-5, 5-6, 6-7, 7-8
- ΔRT (%): 0, 5, 10, 15
Comparison to Current Methods

Counts (%) vs. Acquisition Time (min)

General

Opioids

Targeted

General

Cathinones

Targeted
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

Cannabinoids
54 Compounds to Date

Cathinones
61 Compounds to Date
• 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
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.

edward.sisco@nist.gov
DARTdata@nist.gov

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

November 6\textsuperscript{th}, 2020.
Unidentified Analyte

Possible Compounds in Analyte

Pure Compound Database

Search Algorithm

NIST R&D
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

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

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
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
Unidentified Analyte

1. EI-MS
   AMDIS
   MS Search + Interpreter
   *Fentanyl Classifier*

2. DART-MS
   *Inverted Search Algorithm*

Possible Compounds in Analyte

Pure Compound Database

Search Algorithm
EI-MS: Fentanyl Classifier

Mass spectral library searching

(1) Measure Similarity   (2) Rank by Similarity
EI-MS: Fentanyl Classifier

**Mass spectral library searching**

(1) Measure Similarity  (2) Rank by Similarity

**Mass spectral similarity mapping**

<table>
<thead>
<tr>
<th></th>
<th>( Q )</th>
<th>( L_A )</th>
<th>( L_B )</th>
<th>( L_C )</th>
<th>( L_D )</th>
<th>( L_E )</th>
<th>( L_F )</th>
<th>( L_G )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>( s_{qa} )</td>
<td>1</td>
<td>( s_{bq} )</td>
<td>( s_{cd} )</td>
<td>( s_{eq} )</td>
<td>( s_{fq} )</td>
<td>( s_{gq} )</td>
<td></td>
</tr>
<tr>
<td>( s_{qb} )</td>
<td>( s_{ba} )</td>
<td>( s_{cb} )</td>
<td>1</td>
<td>( s_{eb} )</td>
<td>( s_{fb} )</td>
<td>( s_{gb} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( s_{qc} )</td>
<td>( s_{ac} )</td>
<td>( s_{bc} )</td>
<td>1</td>
<td>( s_{ec} )</td>
<td>( s_{fc} )</td>
<td>( s_{gc} )</td>
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<td></td>
</tr>
<tr>
<td>( s_{qd} )</td>
<td>( s_{ad} )</td>
<td>( s_{bd} )</td>
<td>1</td>
<td>( s_{ed} )</td>
<td>( s_{fd} )</td>
<td>( s_{gd} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( s_{qe} )</td>
<td>( s_{ae} )</td>
<td>( s_{be} )</td>
<td>( s_{ce} )</td>
<td>1</td>
<td>( s_{fe} )</td>
<td>( s_{ge} )</td>
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<td></td>
</tr>
<tr>
<td>( s_{qf} )</td>
<td>( s_{af} )</td>
<td>( s_{bf} )</td>
<td>( s_{cf} )</td>
<td>( s_{df} )</td>
<td>1</td>
<td>( s_{gf} )</td>
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<td></td>
</tr>
<tr>
<td>( s_{qg} )</td>
<td>( s_{ag} )</td>
<td>( s_{bg} )</td>
<td>( s_{cg} )</td>
<td>( s_{dg} )</td>
<td>( s_{eg} )</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
EI-MS: Fentanyl Classifier

Mass spectral library searching

1. Measure Similarity
2. Rank by Similarity

Mass spectral similarity mapping
EI-MS: Fentanyl Classifier

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

Relevant Publications:

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.
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]

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 the highest mass with intensity greater than 800.

Library Compound:
Name: para-Methylfentanyl
Formula: C12H10N2O
Exact Mass: 350.236
Mw: 350
InChIKey: OAWM9H99C9S8-MW3Y050S4U/6C/9
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)

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 352 Da was generated by adding 51 Da to the highest mass with intensity greater than 600.

Library Compound:
Name: Inolubrystyfentanyl
Formula: C12H13ON2O
Exact Mass: 352.236
InChI: WEDITG17S5Q4U1
InChIKey: LIFEBBSQ5V15555A4
DART-MS: Inverted Search Procedure

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.
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 $\pm \epsilon_0$. 
Target: $m_1$

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

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

min abundance of targets (mixture analysis)

m/z tolerance

[Checkbox] Integer resolution spectra.

Collapsed Query Mass Spectra

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](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

Aaron Urbas
Chemical Sciences Division, Material Measurement Laboratory
National Institute of Standards & Technology
Gaithersburg, Maryland USA
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)
    - $^1\text{H}$, $^{13}\text{C}$, $^{15}\text{N}$, $^{19}\text{F}$, $^{31}\text{P}$ mainly
  - 2D experiments offer a wealth of connectivity information
    - COSY: $^1\text{H}-^1\text{H}$ single bond correlations
    - TOCSY: $^1\text{H}-^1\text{H}$ multi-bond correlations
    - HSQC: $^1\text{H}$-$X$ single-bond single bond connectivity
    - HMBC, HMQC: $^1\text{H}$-$X$ 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

65 fentanyl analogs and related compounds were examined. All samples were prepared in CDCl₃ (~5 mg in 0.6-0.7 mL)

<table>
<thead>
<tr>
<th>Name</th>
<th>MW</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fentanyl HCl</td>
<td>372.9</td>
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<td>Fentanyl</td>
<td>336.5</td>
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<td>Norfentanyl</td>
<td>232.3</td>
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<td>−H</td>
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<tr>
<td>α-Methyl Fentanyl HCl</td>
<td>387.0</td>
<td>−CH₂CH₃</td>
<td>−CH(CH₃)CH₂Ph</td>
<td></td>
</tr>
<tr>
<td>β-Methyl Fentanyl HCl</td>
<td>387.0</td>
<td>−CH₂CH₃</td>
<td>−CH₂CH(CH₃)Ph</td>
<td></td>
</tr>
<tr>
<td>Ortho-Methylfentanyl HCl</td>
<td>387.0</td>
<td>−CH₂CH₃</td>
<td>−CH₂CH₃</td>
<td>−2-CH₃</td>
</tr>
<tr>
<td>Meta-Methylfentanyl HCl</td>
<td>387.0</td>
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<td>−CH₂CH₂Ph</td>
<td>−3-CH₃</td>
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<td>−H</td>
<td>−CH₂CH₂Ph</td>
<td>−2-F</td>
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<tr>
<td>Despropionyl meta-Fluorofentanyl</td>
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<td>−2-F</td>
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<tr>
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<td>−CH₂CH₂Ph</td>
<td>−3-F</td>
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<tr>
<td>Para-Fluorobutyl Fentanyl HCl</td>
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<td>−CH₂CH₂Ph</td>
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<td>−CH₂CH₂Ph</td>
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</tr>
</tbody>
</table>

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

Furanyl Fentanyl Analogs (1H NMR, 62 MHz)

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)

Aromatic Region

Intensity

Aromatic Region Offset for Comparison

$\delta$(PPM)

$\alpha$-methyl Butyryl fentanyl
Fluorofentanyl Analogs (1H NMR, 62 MHz)

Aromatic-H Region

Intensity

δ(PPM)

7.6  7.2  6.8

6  5  4  3  2  1

A) fentanyl
B) o-fluorofentanyl
C) m-fluorofentanyl
D) p-fluorofentanyl
E) 3-fluorofentanyl
Fluoromethcatinone Isomers (\(^1\text{H}, 62\text{ MHz}, \text{MeOD}\))
$^{19}$F NMR Spectra (~58 MHz)
$^{19}$F NMR of Fluorinated Fentanyl Analogs (1H Decoupled)

- Ortho-fluorofentanyl
- Ortho-fluoroacryl fentanyl
- Ortho-fluorobutyrl fentanyl
- Meta-fluorofentanyl
- Meta-fluoroisobutyrl fentanyl
- Meta-fluorobutyrl fentanyl
- Para-fluorofentanyl
- Para-fluoroacryl fentanyl
- Para-fluorobutyrl fentanyl
- Despropionyl ortho-fluorofentanyl
- Despropionyl meta-fluorofentanyl
- Despropionyl para-fluorofentanyl

Intensity vs. $\delta$(PPM)

$\delta$(PPM): -110 to -135

3-fluorofentanyl

NIST
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
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

<table>
<thead>
<tr>
<th>Atom</th>
<th>Shift (ppm)</th>
<th>J (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 CH</td>
<td>4.27</td>
<td>J[3-13']</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>J[8-26]</td>
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<td>J[9-26]</td>
</tr>
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<td>11 CH</td>
<td>7.156</td>
<td>J[11-12]</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>J[13''-14’’]</td>
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</table>
Predicting $^1$H NMR Spectra

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.
### Predicted Chemical Shifts & Coupling Constants

<table>
<thead>
<tr>
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<th>Shift (ppm)</th>
<th>(J) (Hz)</th>
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<tbody>
<tr>
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<td>4.27</td>
<td>J(3-13') 5.78, J(3-13'') 5.78, J(3-17') 5.78, J(3-17'') 5.78</td>
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<tr>
<td>6 CH2</td>
<td>2.082</td>
<td>J(6) 14.56, J(6-7) 7.89</td>
</tr>
<tr>
<td>7 CH3</td>
<td>0.94</td>
<td>J(7-6) 7.89, J(7) 6.99</td>
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<td>8 CH</td>
<td>7.019</td>
<td>J(8-9) 8.43, J(8-12) 1.5, J(8-26) 5</td>
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<tr>
<td>9 CH</td>
<td>7.156</td>
<td>J(9-8) 8.43, J(9-11) 1.5, J(9-27) 8</td>
</tr>
<tr>
<td>11 CH</td>
<td>7.156</td>
<td>J(11-9) 1.5, J(11-12) 8.43, J(11-26) 8</td>
</tr>
<tr>
<td>12 CH</td>
<td>7.019</td>
<td>J(12-8) 1.5, J(12-11) 8.43, J(12-26) 5</td>
</tr>
<tr>
<td>13' CH2</td>
<td>1.645</td>
<td>J(13'-3) 5.78, J(13'-13'') 12.29</td>
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<td>13'' CH2</td>
<td>2.031</td>
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### Fit Chemical Shifts & Coupling Constants

<table>
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---

**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.

---

**Quantum Mechanic Spectral Analysis (QMSA)**
Quantum Mechanic Spectral Analysis (QMSA)

Para-fluorofentanyl

\[
\begin{array}{c}
\text{Measured (600 MHz)} \\
\text{QMSA Model}
\end{array}
\]

\[
\begin{array}{c}
\delta(\text{ppm}) \\
7.3 \\
7.2 \\
7.1 \\
4.81 \\
4.77 \\
4.73 \\
3.65 \\
3.6 \\
3.55 \\
3.2 \\
3.1
\end{array}
\]
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)

- Fentanyl (HCl), $r^2 = 0.9952$
- Butyryl fentanyl (HCl), $r^2 = 0.99041$
- Para-fluorofentanyl (HCl), $r^2 = 0.99522$
- Acryl fentanyl (HCl), $r^2 = 0.99315$
- Isobutyryl fentanyl (HCl), $r^2 = 0.99376$
- FIBF (HCl), $r^2 = 0.99032$

Measured vs Modeled
QMSA Sub-Systems as Spectral Building Blocks

Facilitates building new QMSA models and predicting spectra of unknowns

Measured para-fluorobutyryl fentanyl
62 MHz $^1$H

para-fluorofentanyl
QMSA model

butyrl fentanyl
QMSA model
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

δ(ppm)
Outline

• NMR at a Glance
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• Recent Sample Investigations
• Conclusion & Acknowledgements
Synthetic Tryptamine Analog Example 1

Sample Spectrum Compared to 62 MHz QMSA Simulations

- 4-methoxy MiPT (HCl) (62 MHz Simulation)
- 5-methoxy MiPT (62 MHz Simulation)
- 5-methoxy DET (62 MHz Simulation)
Synthetic Tryptamine Analog Example 1

62 MHz QMSA Model of Sample Spectrum

- Sample
- 4-methoxy MiPT (HCl) (62 MHz Simulation, Optimized)
- MeOD (62 MHz Simulation)

Intensity vs. δ(PPM)
Synthetic Tryptamine Analog Example 2

Sample Spectrum Compared to 62 MHz QMSA Simulations

62 MHz QMSA Model of Sample Spectrum

N,N-Dipropyltryptamine (DPT)
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.
Acknowledgements

• Support from the NIST Special Programs Office

• George Washington University (collaboration on the fentanyl analog project)
  • Ioan Marginean and Jonathan Duffy

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  • Maryland State Police Forensic Science Division (Amber Burns)
  • DEA Special Testing and Research Laboratory (Charlotte Corbett)
  • US Postal Inspection Service (Mike Hitchcock)

• QMSA Assistance
  • Matthias Niemitz (NMR Solutions)
  • Pekka and Reino Laatikainen (Spin Discoveries) for assistance with QMSA

Disclaimer

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Thank You!