Handling NPS Isomers – From Analytical Separation to Reporting

Society of Forensic Toxicologists (SOFT) Annual Meeting – Tuesday October 31, 2023

Workshop 11: Forensic Interpretation of Novel Psychoactive Substances in Challenging Cases

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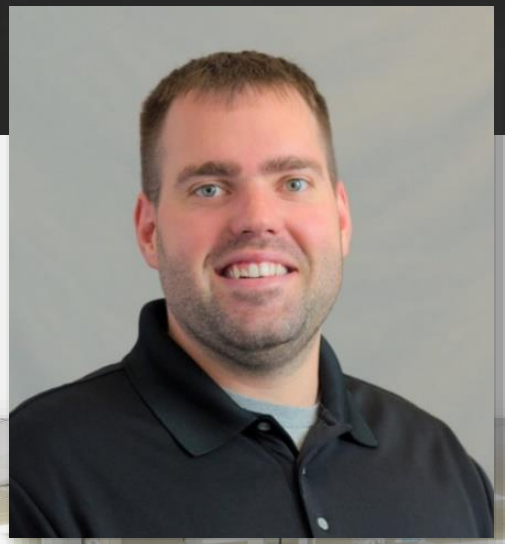
INTRODUCTION



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DISCLOSURES

- I have no conflicts of interest to disclose.
- I am a scientist and employee of FRFF / CFSRE, a 501(c)(3) non-profit research and educational facility.
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STRENGTHEN SCIENCE. ADVANCE JUSTICE.

PRESENTATION OUTLINE

- Types of Isomers
- Significance of Isomers
- Ways to Separate
- Examples

TYPES OF ISOMERS

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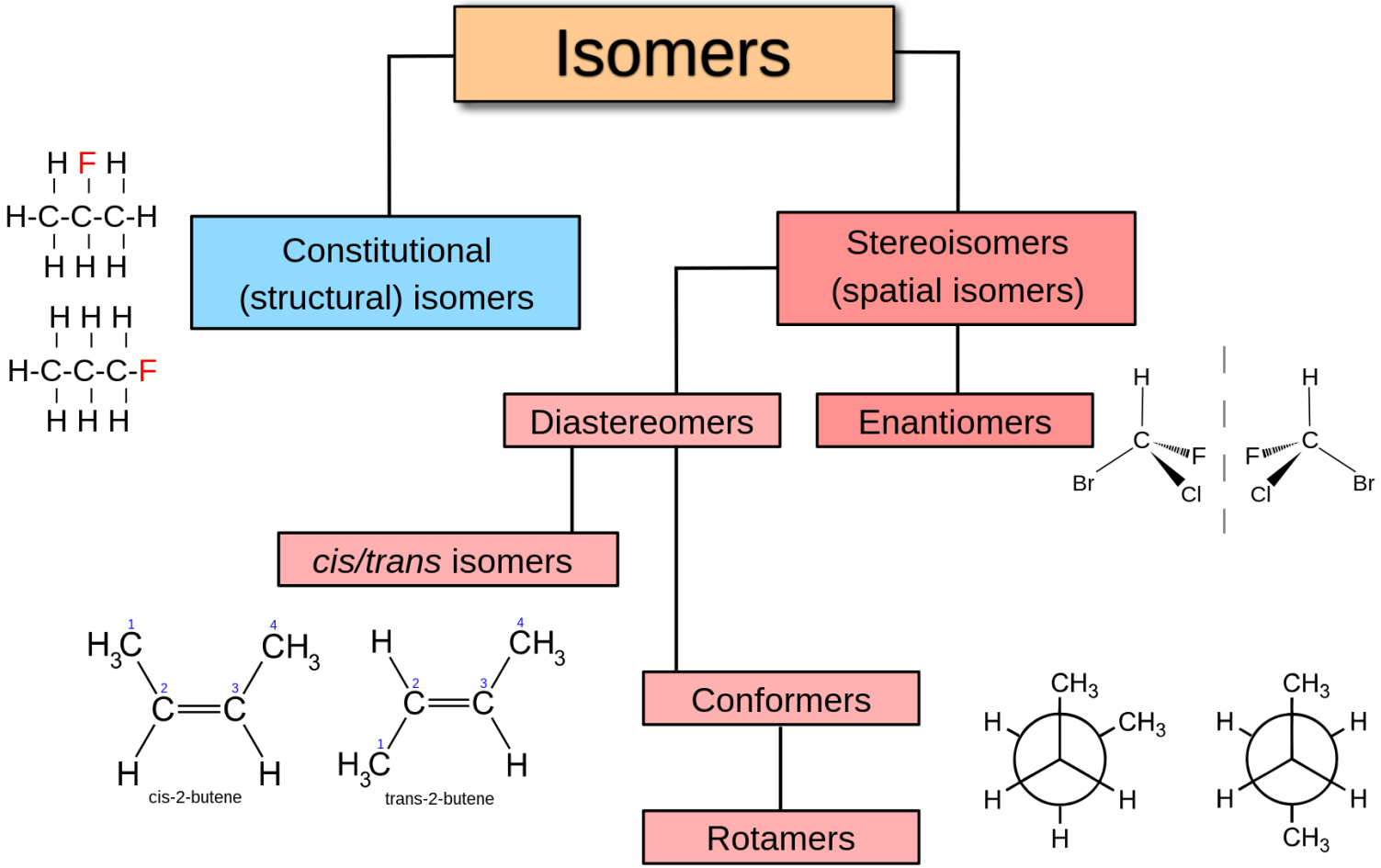
TYPES OF ISOMERS

- **Positional Isomers**
  - Movement of atoms or functional groups to other positions of the molecule
  - More common***
  - Generally, somewhat **easier** to differentiate during analysis
- **Stereoisomers**
  - Varying three-dimensional orientation / positioning
  - Generally, much **more difficult** to differentiate during analysis



A BRIEF GUIDE TO

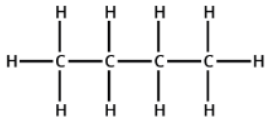
• TYPES OF ISOMERISM IN ORGANIC CHEMISTRY •

A GUIDE TO THE FIVE MAIN TYPES OF ISOMERISM THAT CAN BE EXHIBITED BY ORGANIC COMPOUNDS

AN ISOMER OF A MOLECULE IS A MOLECULE WITH THE **SAME MOLECULAR FORMULA** BUT A **DIFFERENT STRUCTURAL OR SPATIAL ARRANGEMENT** OF ATOMS. THIS VARIATION CAN LEAD TO A DIFFERENCE IN PHYSICAL OR CHEMICAL PROPERTIES.

STRUCTURAL ISOMERISM

CHAIN



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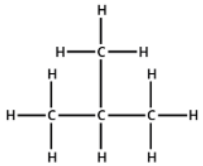
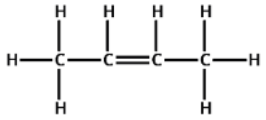


2-METHYL PROPANE

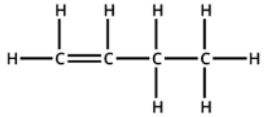
DIFFERENT ARRANGEMENT OF A MOLECULE'S CARBON SKELETON

The positions of the carbon atoms in the molecule can be rearranged to give 'branched' carbon chains coming off the main chain. The name of the molecule changes to reflect this, but the molecular formula is still the same.

POSITION



BUT-2-ENE

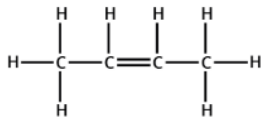


BUT-1-ENE

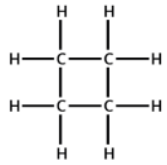
THE DIFFERING POSITION OF THE SAME FUNCTIONAL GROUP IN THE MOLECULE

The molecular formula remains the same; the type of functional group also remains the same, but its position in the molecule changes. The name of the molecule changes to reflect the new position of the functional group.

FUNCTIONAL



BUT-2-ENE



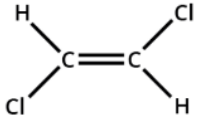
CYCLOBUTANE

DIFFERING POSITIONS OF ATOMS GIVE A DIFFERENT FUNCTIONAL GROUP

Also referred to as functional group isomerism, these isomers have the same molecular formula but the atoms are rearranged to give a different functional group. The name of the molecule changes to reflect the new functional group.

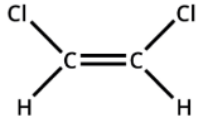
STEREOISOMERISM

GEOMETRIC



(E)-1,2-DICHLOROETHENE

E = opposite side



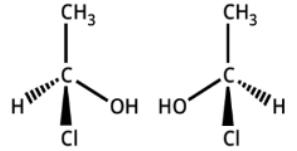
(Z)-1,2-DICHLOROETHENE

Z = same side

DIFFERENT SUBSTITUENTS AROUND A BOND WITH RESTRICTED ROTATION

Commonly exhibited by alkenes, the presence of two different substituents on both carbon atoms at either end of the double bond can give rise to two different, non-superimposable isomers due to the restricted rotation of the bond.

OPTICAL



L: (S)-1-CHLOROETHANOL

R: (R)-1-CHLOROETHANOL

NON-SUPERIMPOSABLE MIRROR IMAGES OF THE SAME MOLECULE

Optical isomers differ by the placement of different substituents, around one or more atoms in a molecule. Different arrangements of these substituents can be impossible to superimpose - these are optical isomers.

SIGNIFICANCE OF ISOMERS

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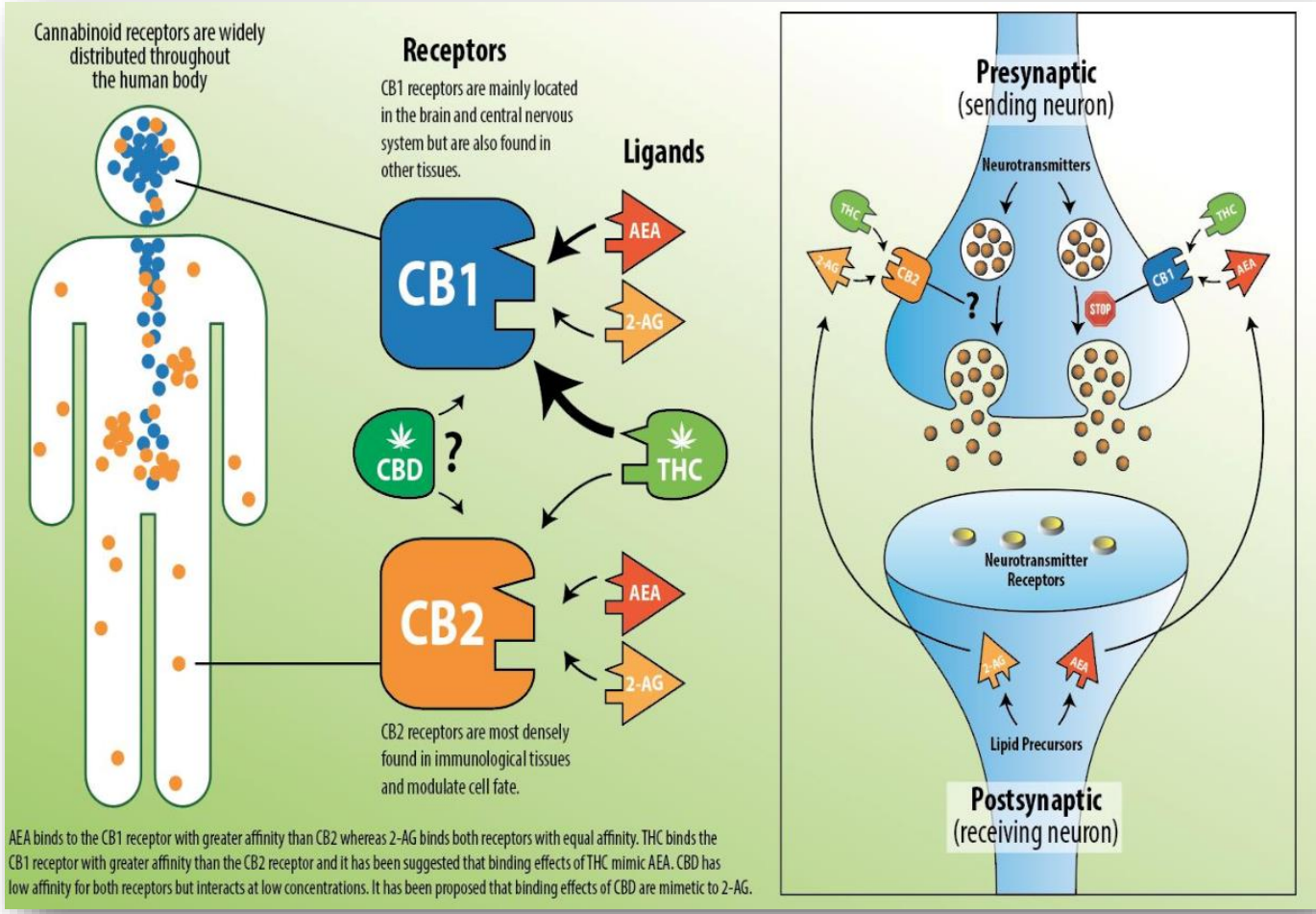
SIGNIFICANCE OF ISOMERS

- **Pharmacology**
  - Activity
  - Potency
  - **Receptor System**
    - E.g., cannabinoid CB1 vs. CB2
    - Agonist (full vs. partial)
    - Antagonist
  - **Metabolism**
    - Sites of biotransformation
    - Location of functional groups



SIGNIFICANCE OF ISOMERS

- Pharmacology
  - Activity
  - Potency
- **Legal Status**
  - Explicit Language
  - Isomer / Analogue Laws
  - *o-FF – Schedule I*
  - *m-FF – Not Scheduled*
  - *p-FF – Schedule I*

SUBSTANCE	CSCN	CSA SCH	NARC	OTHER NAMES
Alpha-methylfentanyl	9814	I	Y	China White, fentanyl
Alpha-methylthiofentanyl	9832	I	Y	China White, fentanyl
Alpha-methyltryptamine	7432	I	N	AMT (Positional Isomer: N-Methyltryptamine)
Alphaprodine	9010	II	Y	Nisentil
alpha-pyrrolidinobutiophenone (α-PBP)	7546	I	N	1-phenyl-2-(pyrrolidin-1-yl)butan-1-one)
alpha-pyrrolidinoheptaphenone (PV8)	7548	I	N	PV8; 1-phenyl-2-(pyrrolidin-1-yl)heptan-1-one)
alpha-pyrrolidinohexanophenone (α-PHP)	7544	I	N	α-PHP; α-pyrrolidinohexanophenone; 1-phenyl-2-(pyrrolidin-1-yl)hexan-1-one)
alpha-pyrrolidinopentiophenone (α-PVP)	7545	I	N	α-pyrrolidinovalerophenone, 1-phenyl-2- (pyrrolidin-1-yl)pentan-1-one) (Positional Isomers: 4-methyl-α-pyrrolidinobutiophenone (4-MePBP), 1-phenyl-2-(piperidin-1-yl)butan-1-one)
Para-chloroisobutyryl fentanyl	9826	I	Y	N-(4-chlorophenyl)-N-(1-phenethylpiperidin-4-yl)isobutyramide
Para-Fluoro furanyl fentanyl (N-(4-fluorophenyl)-N-(1-phenethylpiperidin-4-yl)furan-2-carboxamide)	9854	I	Y	
Para-fluorobutyryl fentanyl	9823	I	Y	N-(4-fluorophenyl)-N-(1-phenethylpiperidin-4-yl)butyramide
Para-Fluorofentanyl	9812	I	Y	China White, fentanyl

SIGNIFICANCE OF ISOMERS

- **Pharmacology**
  - Activity
  - Potency
- **Legal Status**
  - Explicit Language
  - Isomer / Analogue Laws
- **Downstream Impacts**
  - Public health → CDC death certificate data
  - Public safety → DEA drug scheduling efforts

NFLIS Public DQS

BASE_DESCRIPTION	DRUG_CATEGORY_DESCRIPTION	YYYY PERIOD	2022 2022	Totals
5F-EMB-PICA	Synthetic Cannabinoids		15	15
5F-MDMB-PICA	Synthetic Cannabinoids		97	97
Fluoro-MDMB-PICA	Synthetic Cannabinoids		32	32
		Totals	144	144

Table 2.5 *SYNTHETIC CANNABINOIDS*
Number and percentage of synthetic cannabinoid reports in the United States, 2020[1]

Synthetic Cannabinoid Reports	Number	Percent
MDMB-4en-PINACA	4,521	30.19%
5F-MDMB-PICA	2,888	19.28%
Fluoro-MDMB-PICA	1,466	9.79%
4F-MDMB-BUTINACA	1,010	6.74%
Fluoro-MDMB-BUTINACA	283	1.89%
Fluoro-EMB-PICA	263	1.76%
5F-ADB	218	1.46%
ADB-BUTINACA	210	1.40%
4F-MDMB-BUTICA	210	1.40%
5F-EMB-PICA	195	1.30%
5F-EDMB-PINACA	121	0.81%
Fluoro-MDMB-BUTICA	118	0.79%
FUB-AMB	116	0.77%
FUB-144	107	0.72%
5F-CUMYL-PINACA	74	0.50%
Other synthetic cannabinoids	3,176	21.20%
Total Synthetic Cannabinoid Reports[2]	14,978	100.00%
Total Drug Reports	1,283,971	

WAYS TO DIFFERENTIATE

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WAYS TO DIFFERENTIATE

- **Chromatography**
  - Gas Chromatography (GC)
  - Liquid Chromatography (LC)
- **Mass Spectrometry (MS)**
  - Single Dimensional vs. Multi-Dimensional
  - High Resolution (HRMS)
- **Complementary Techniques**
  - Infrared Spectroscopy (IR)
  - Nuclear Magnetic Resonance (NMR)
  - Others

EXAMPLES

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3-METHYL FENTANYL

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3-METHYL FENTANYL

- Two diastereomers → *cis*-3-methyl fentanyl and *trans*-3-methyl fentanyl
- Potency → ***cis*-3-MF is ~8x greater than fentanyl** and *trans*-3-MF similar to fentanyl

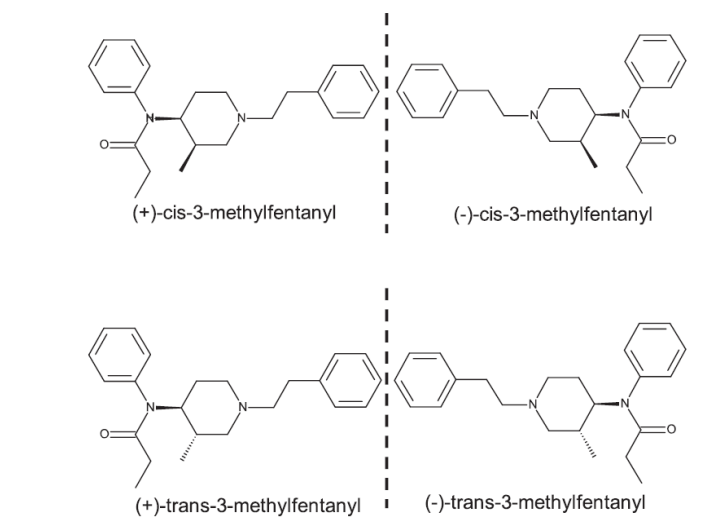
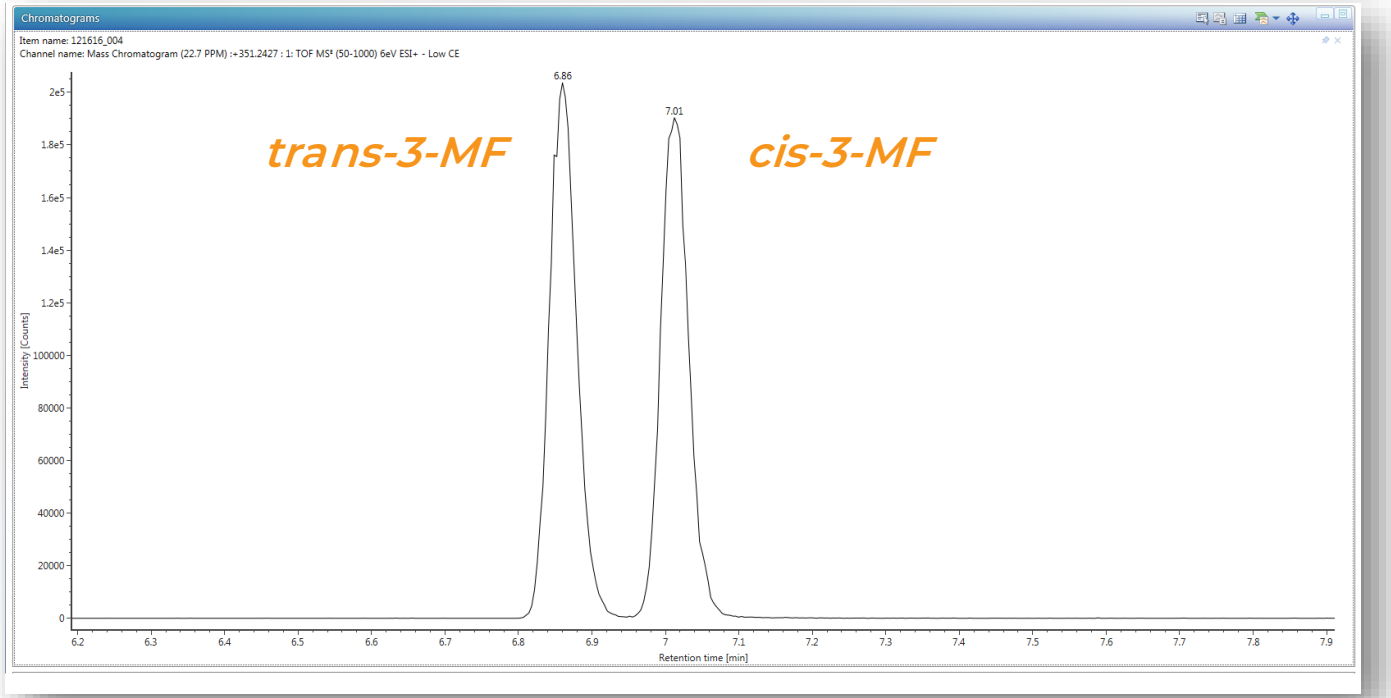


FIGURE 1 Structures of 3-MF isomers



Fogarty et al. (2018) https://doi.org/10.1002/dta.2414

FLUOROFENTANYLS

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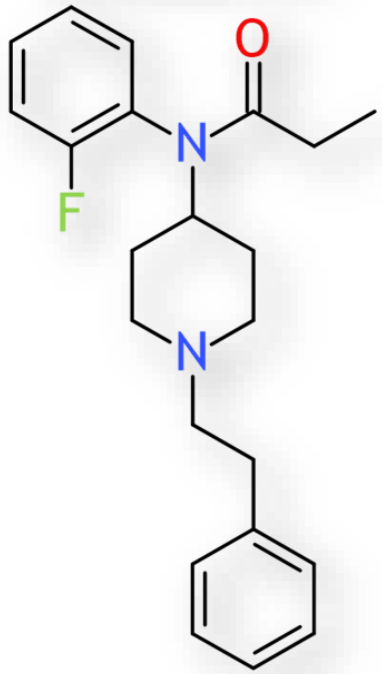
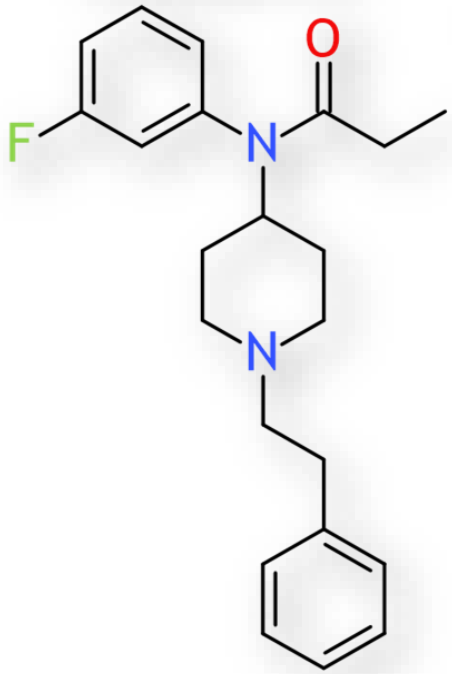
FLUOROFENTANYL (FF) ISOMERS

- "Fluorofentanyl" can exist in many forms but substitution on the aniline ring is most common
  - *ortho*-Fluorofentanyl
  - *meta*-Fluorofentanyl
  - ***para*-Fluorofentanyl**
- *o*-FF and p-FF are Schedule I
- Reported potencies (comp. to fent.):
  - *o*-FF → approx. 4x more potent
  - *m*-FF → approx. 6x less potent
  - *p*-FF → approx. 3x less potent

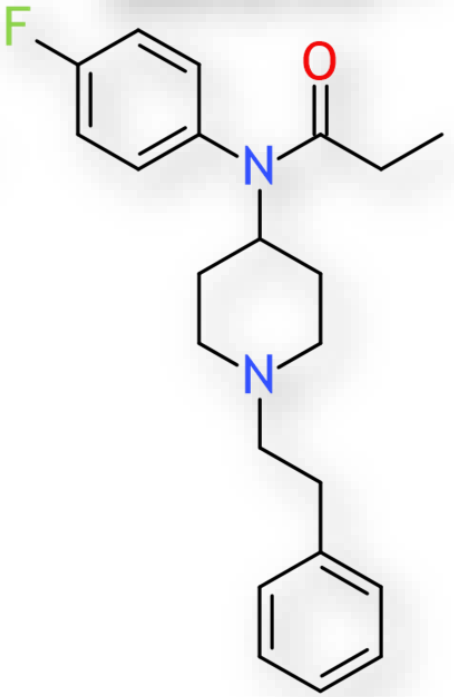
***ortho*-FF**



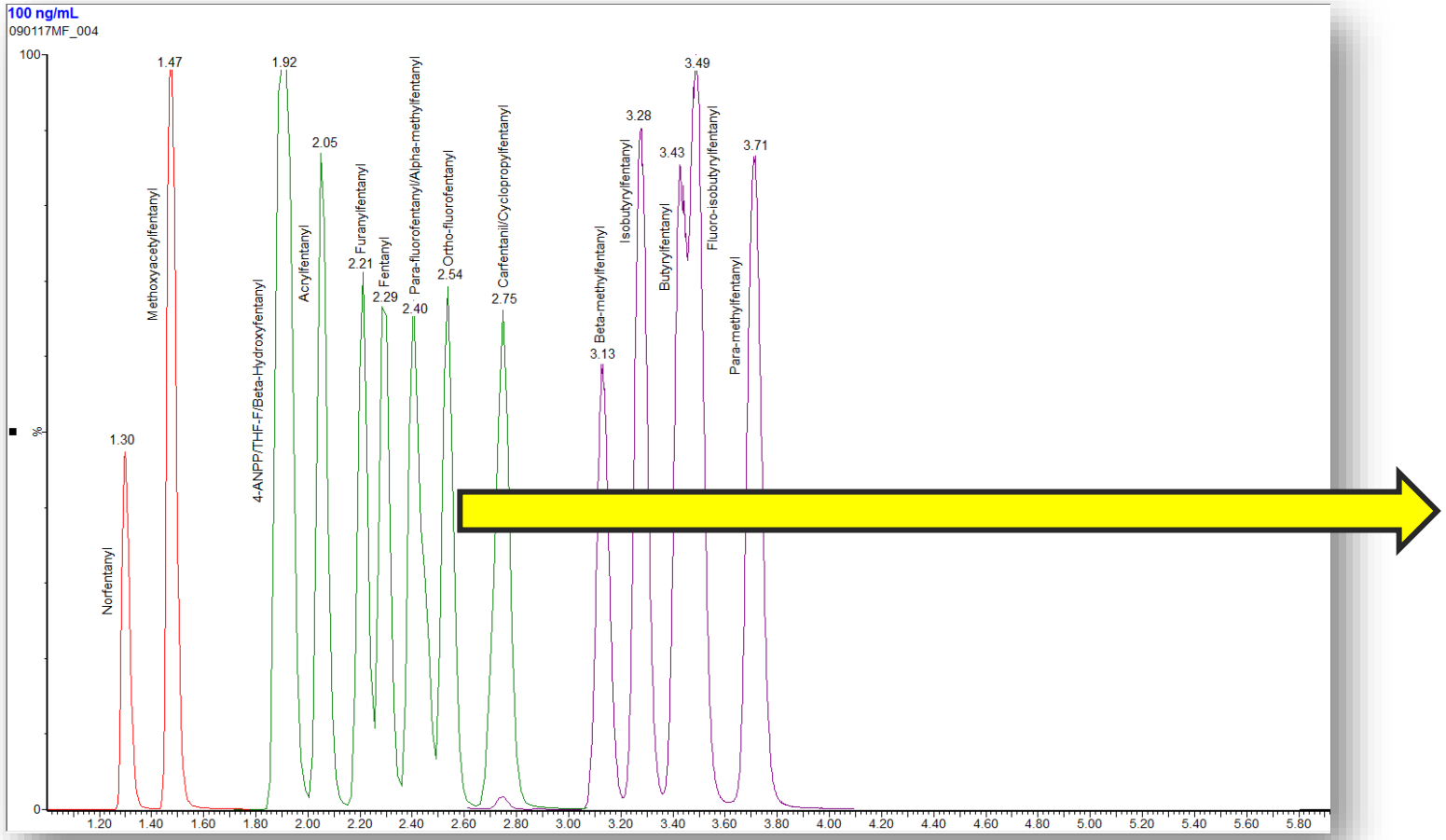
***meta*-FF**

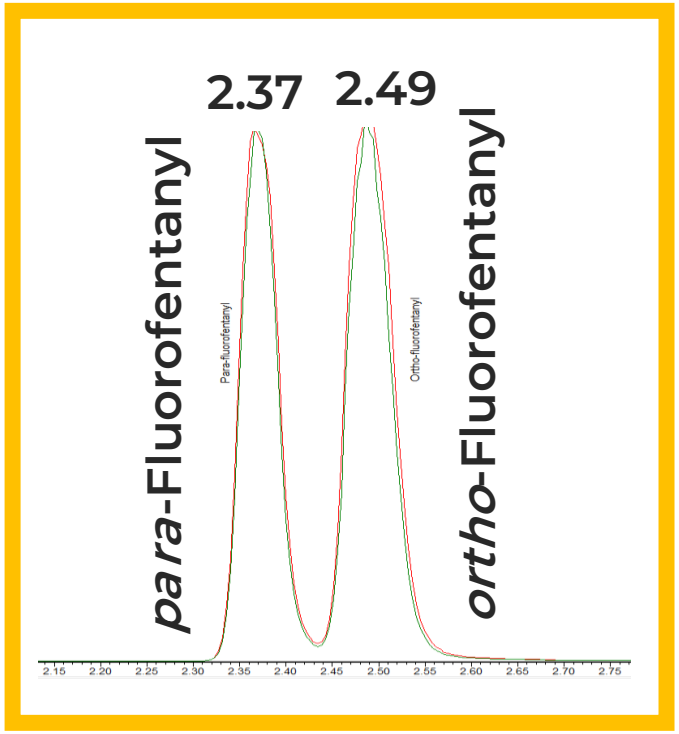


***para*-FF ***



PARA-FF VS. *ORTHO*-FF CHROMATOGRAPHY





NITAZENE ANALOGUES

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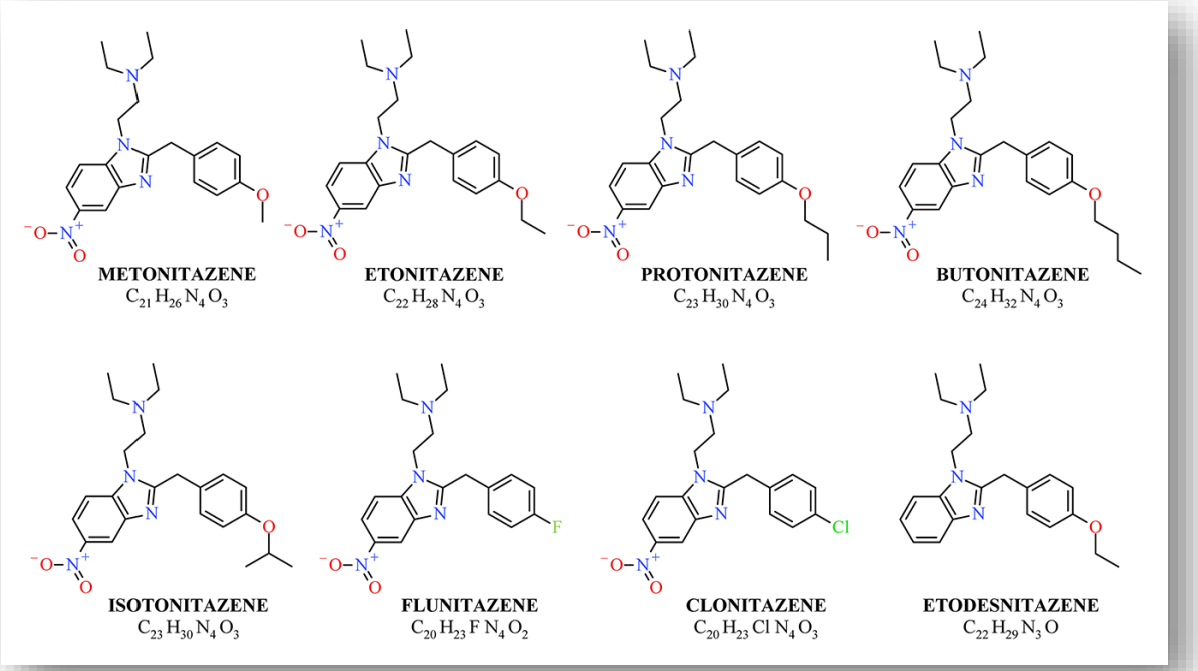
NITAZENE ANALOGUES

- Like all drug analogue series, there are various isomer pairs (+) that exist (or could exist)
  - Some are distinguishable by **mass spectrometry** alone
  - Some are identical by mass spectrometry but distinguishable by **chromatography**
  - Lack of separation/differentiation could lead to data issues or anomalies



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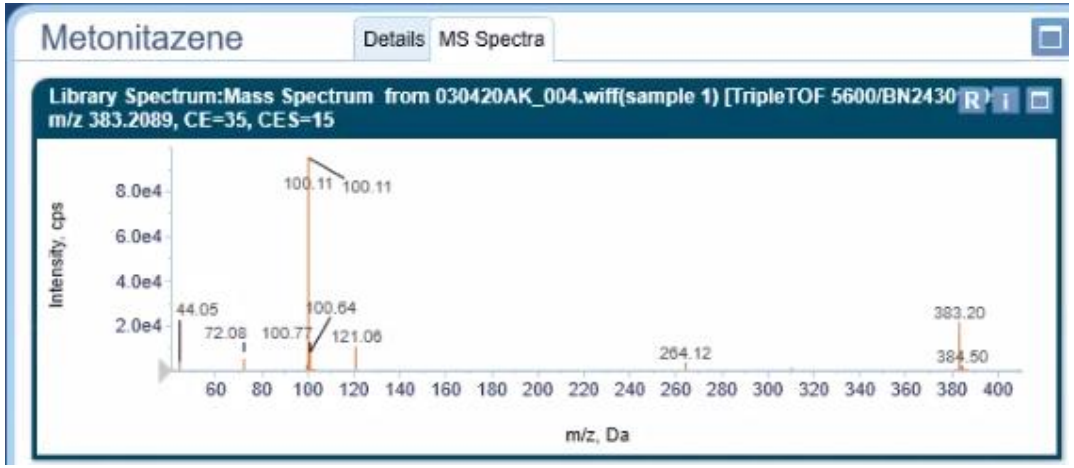

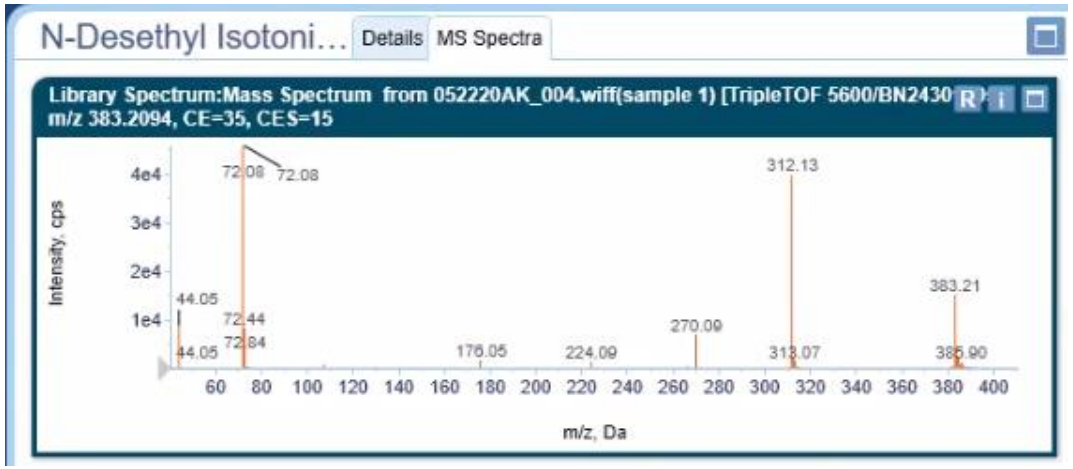

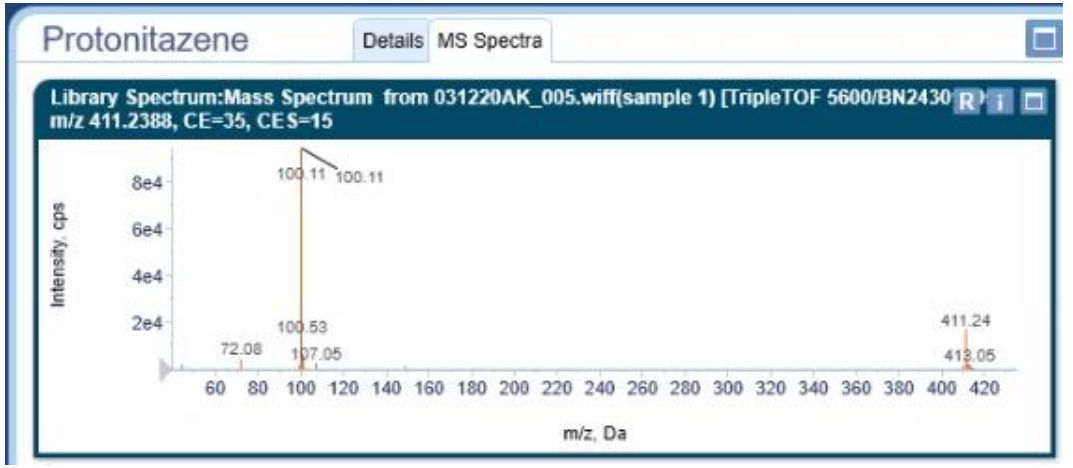

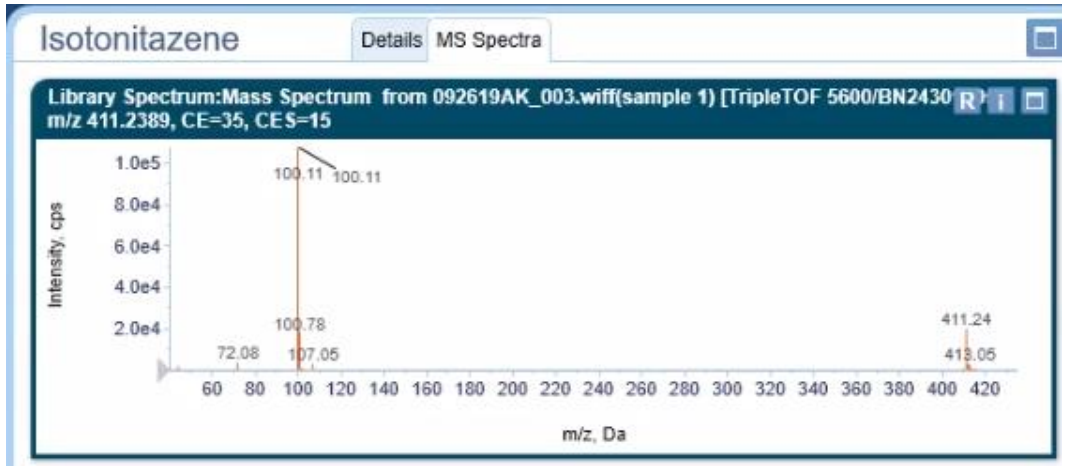

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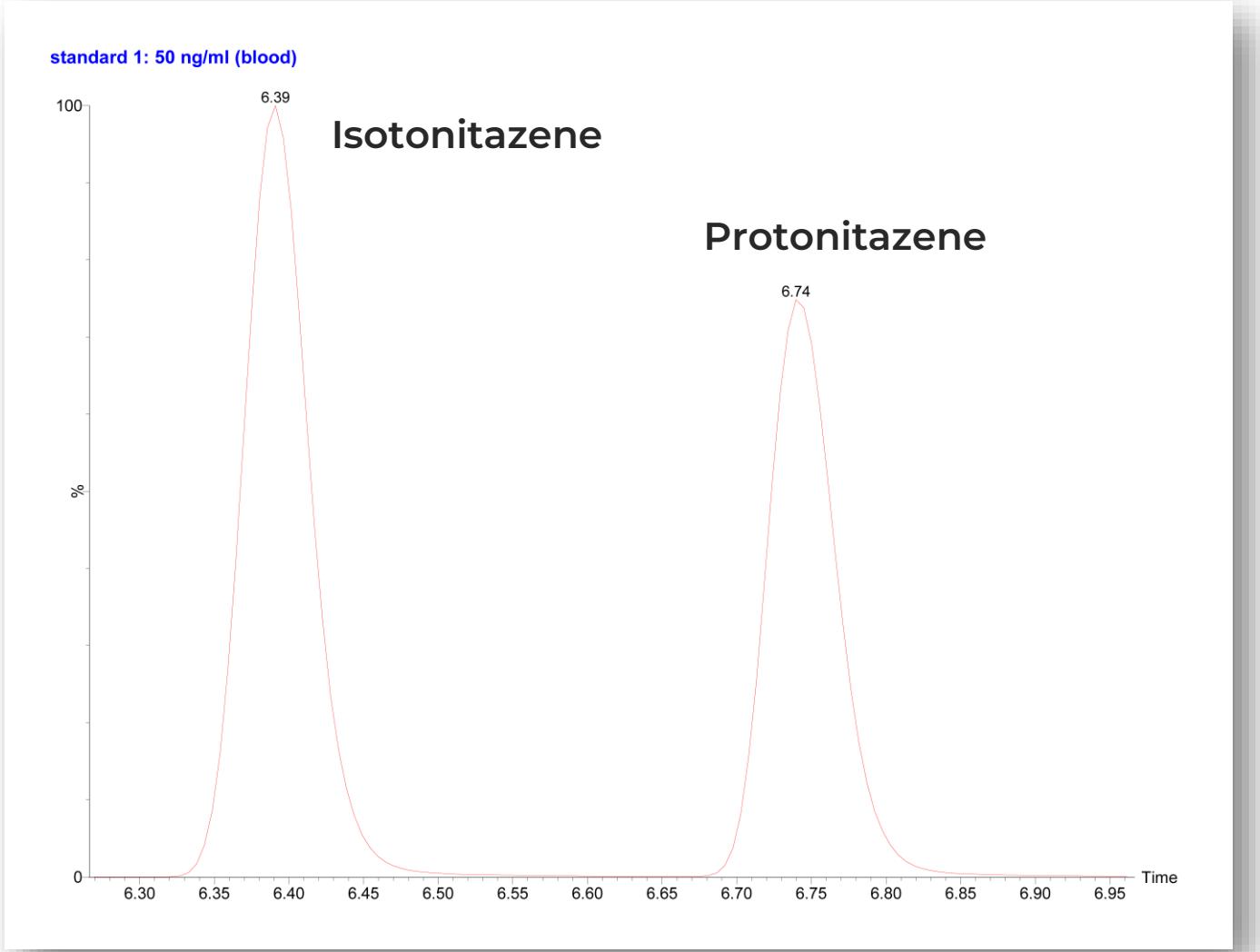

N,N-DIMETHYLPENTLYONE

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N,N-DIMETHYLPENTLYONE

- Like all drug analogue series, there are various isomer pairs (+) that exist (or could exist)
  - Some are distinguishable by **mass spectrometry** alone
  - Some are identical by mass spectrometry but distinguishable by **chromatography**
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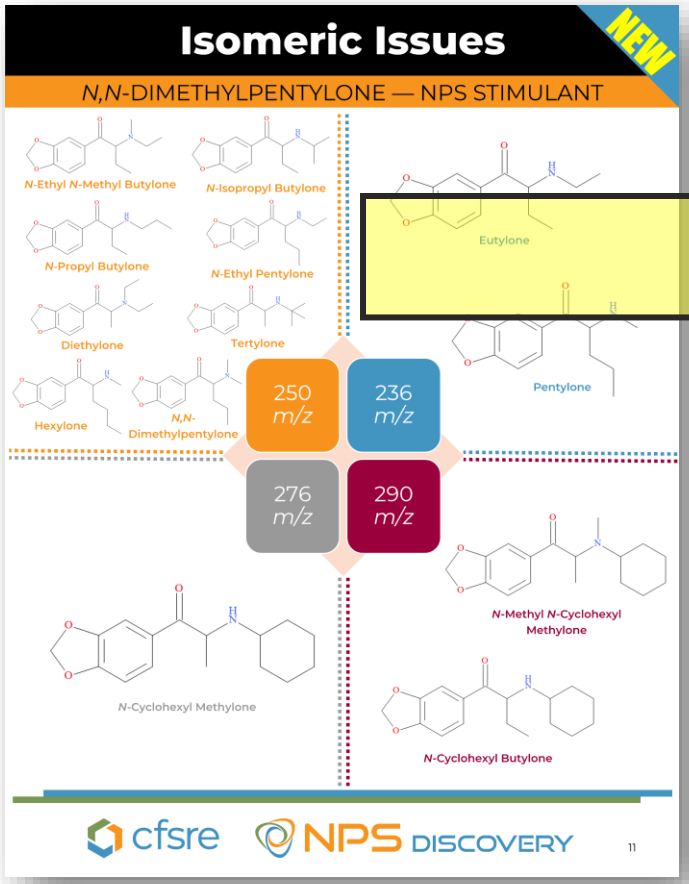
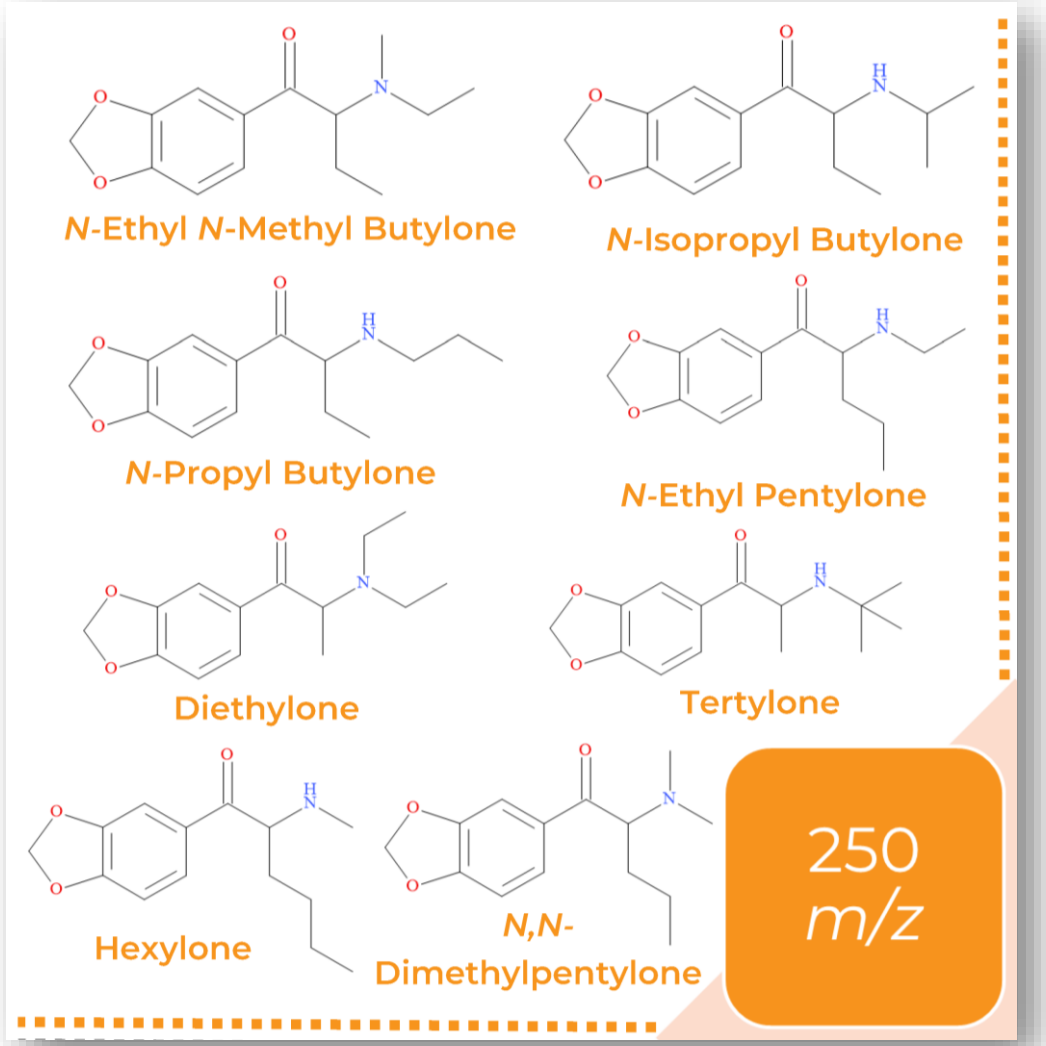
“-YLONE” M/Z 250 ISOMERS

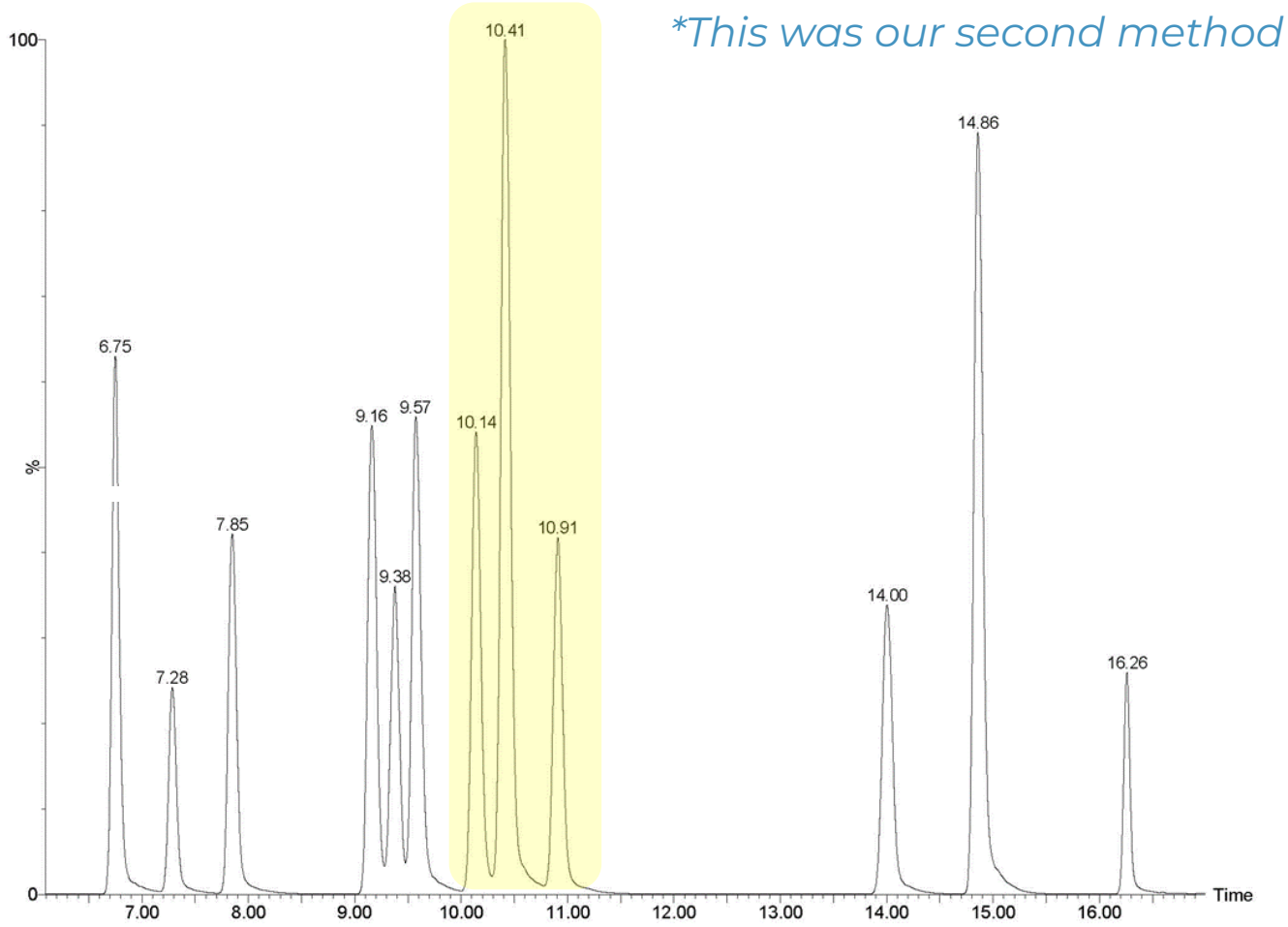




N,N-DIMETHYLPENTLYONE

- Some of the m/z 250s are distinguishable by MS alone
- Chromatographic separation might be required
  - Not necessarily for identification but for **confirmation and quantitation** when multiple isomers might be present
  - 10.14 min – *N*-Propyl Butylone
  - 10.41 min – *N,N*-Dimethylpentylone
  - 10.91 min – *N*-Ethyl Pentylone

**This was our second method*



Publication pending

ALPHA-PHP VS. ALPHA-PIHP

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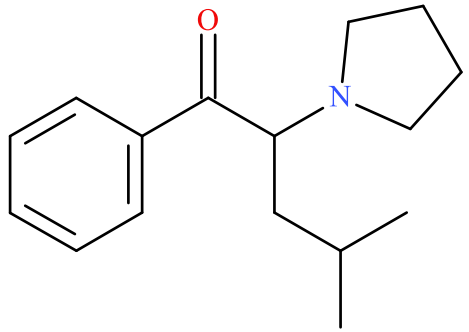
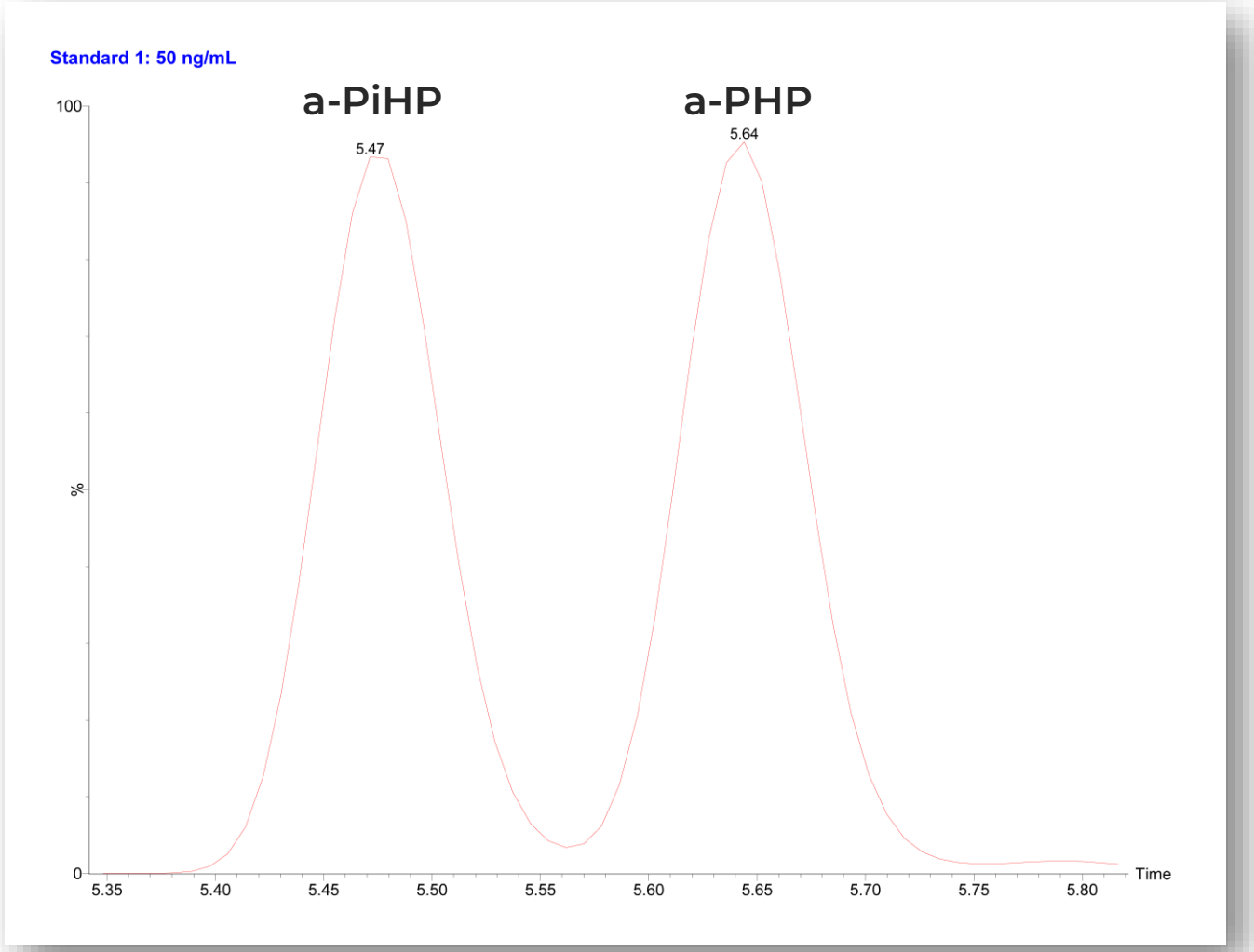
ALPHA-PHP VS. ALPHA-PIHP

- Not distinguishable by MS data
- A retention time shift (compared to standards) could indicate that a **new isomer** is appearing
- Split / co-eluting peaks could affect testing and reporting





FXE – 2- & 3-FLUORO ISOMERS

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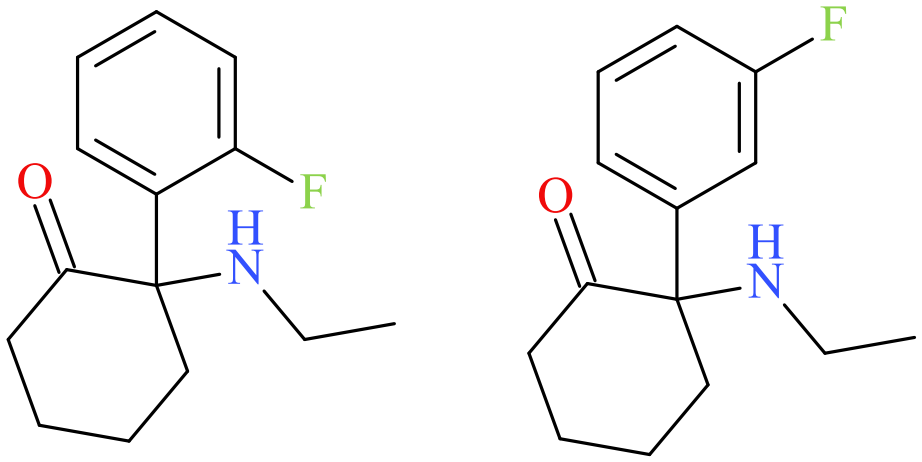
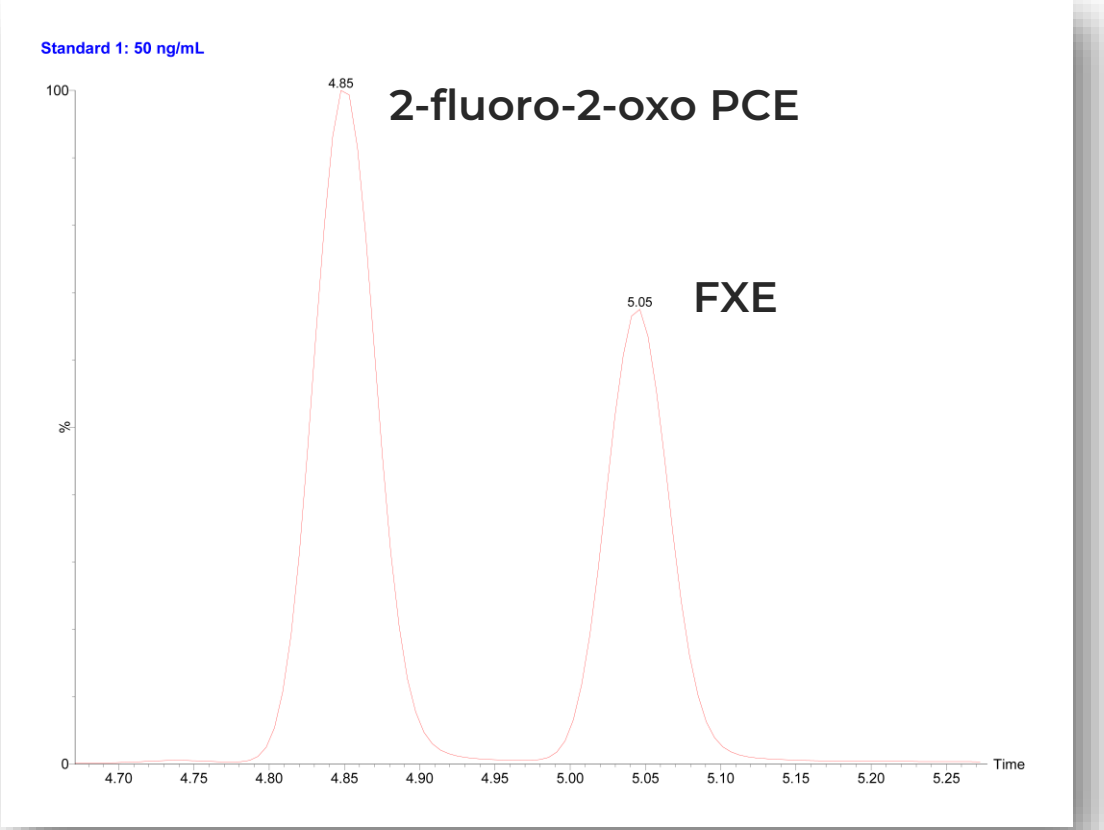
FLUOROEXETAMINE (FXE)

- Drug exists in two isomeric forms: 2-fluoro-2-oxo PCE and 3-fluoro-2-oxo PCE (FXE)
- Two isomers looks identical by MS
- Does differentiation matter for a tox case???
  - Consistency with crime lab





SYNTHETIC CANNABINOIDS

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SYNTHETIC CANNABINOIDS

4’CL-DESCHLOROALPRAZOLAM

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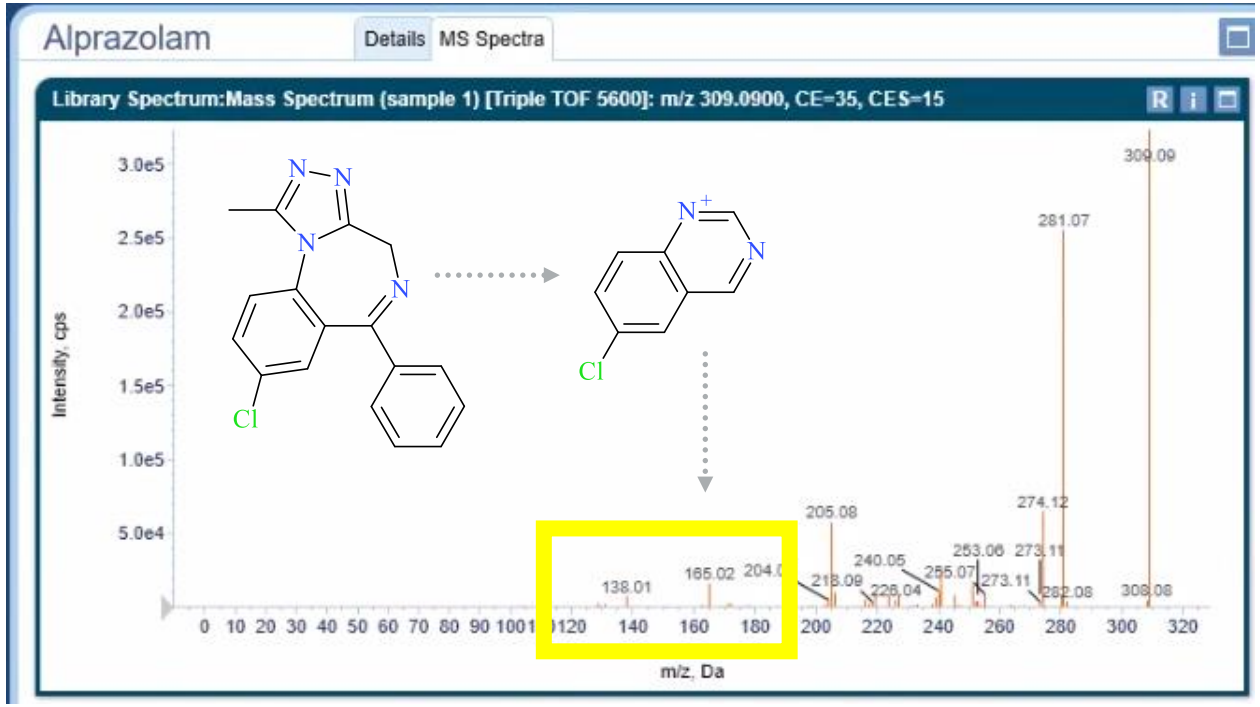
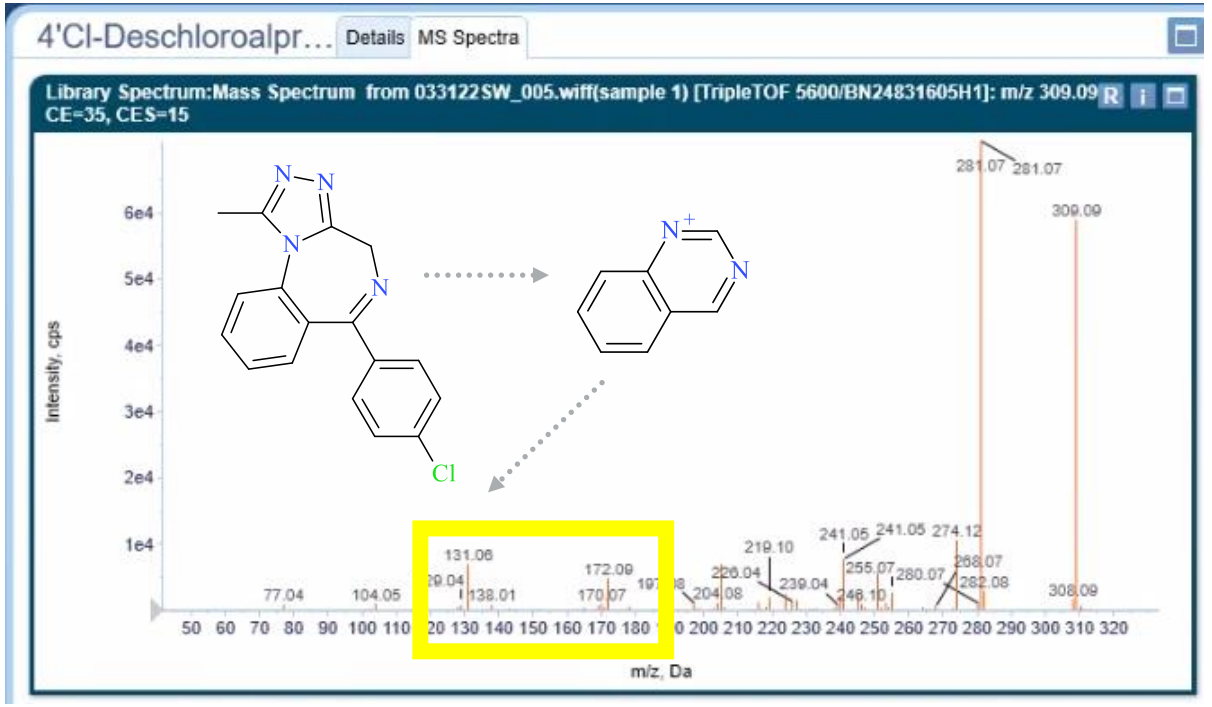
ALPRAZOLAM VS 4'CL-DESCHLOROALPRAZOLAM

- The two isomer *can* be differentiated by MS data if intensity is high enough
- Alprazolam positive screens but failing confirm criteria





METHYLMETHCATHINONE

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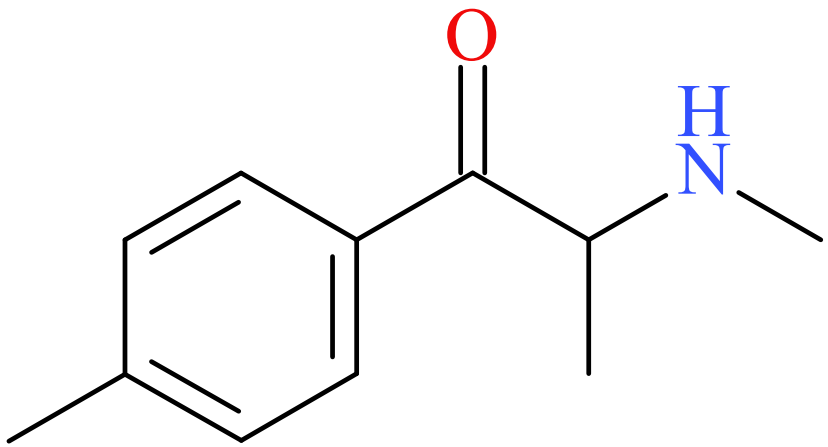
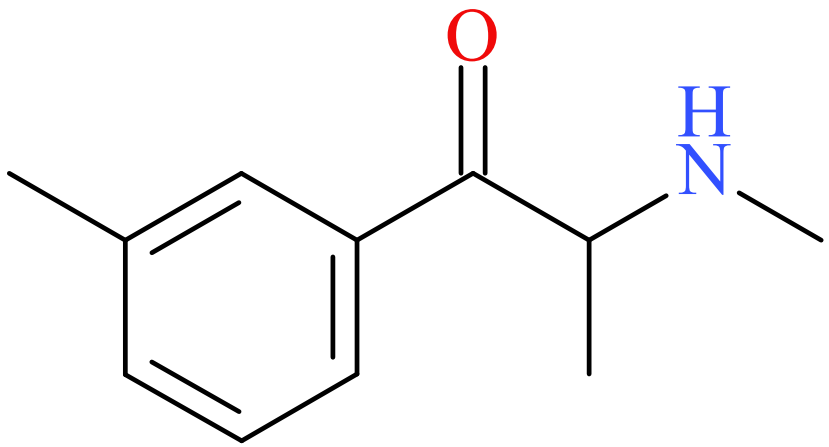
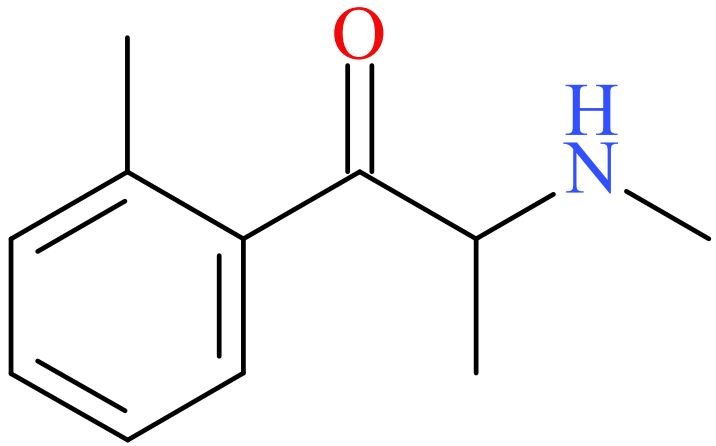
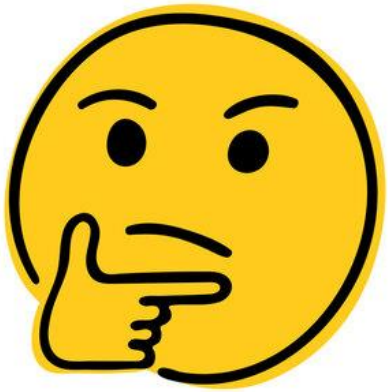
METHYLMETHCATHINONE









DISCUSSION & CONCLUSIONS

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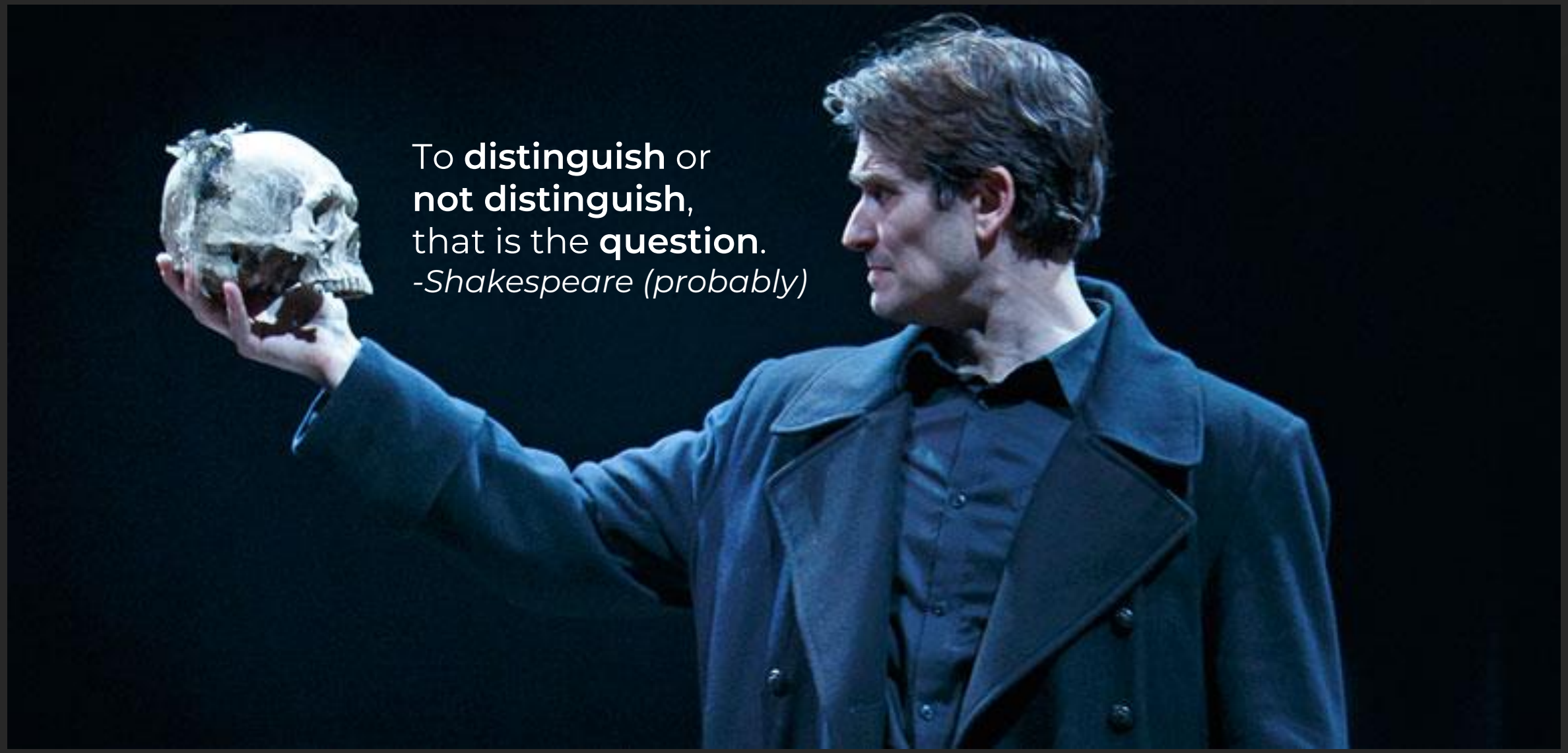
DISCUSSION & CONCLUSIONS

- There is **no "one-size-fits-all" solution** to NPS isomers
  - The tools exist but it's a balance of time and resources
- **Absolute determination** can be significant
- Isomer differentiation can influence **interpretation** (e.g., potency) and **expert opinions** (e.g., legal status)
- **Communicate** and **collaborate** (when possible)

- ***AK's Opinion:*** *Reporting unspecified isomer is still better than not reporting the drug at all…*
  - *The key is reporting properly*


To **distinguish** or
not distinguish,
that is the **question**.
-Shakespeare (probably)

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  - Barry Logan
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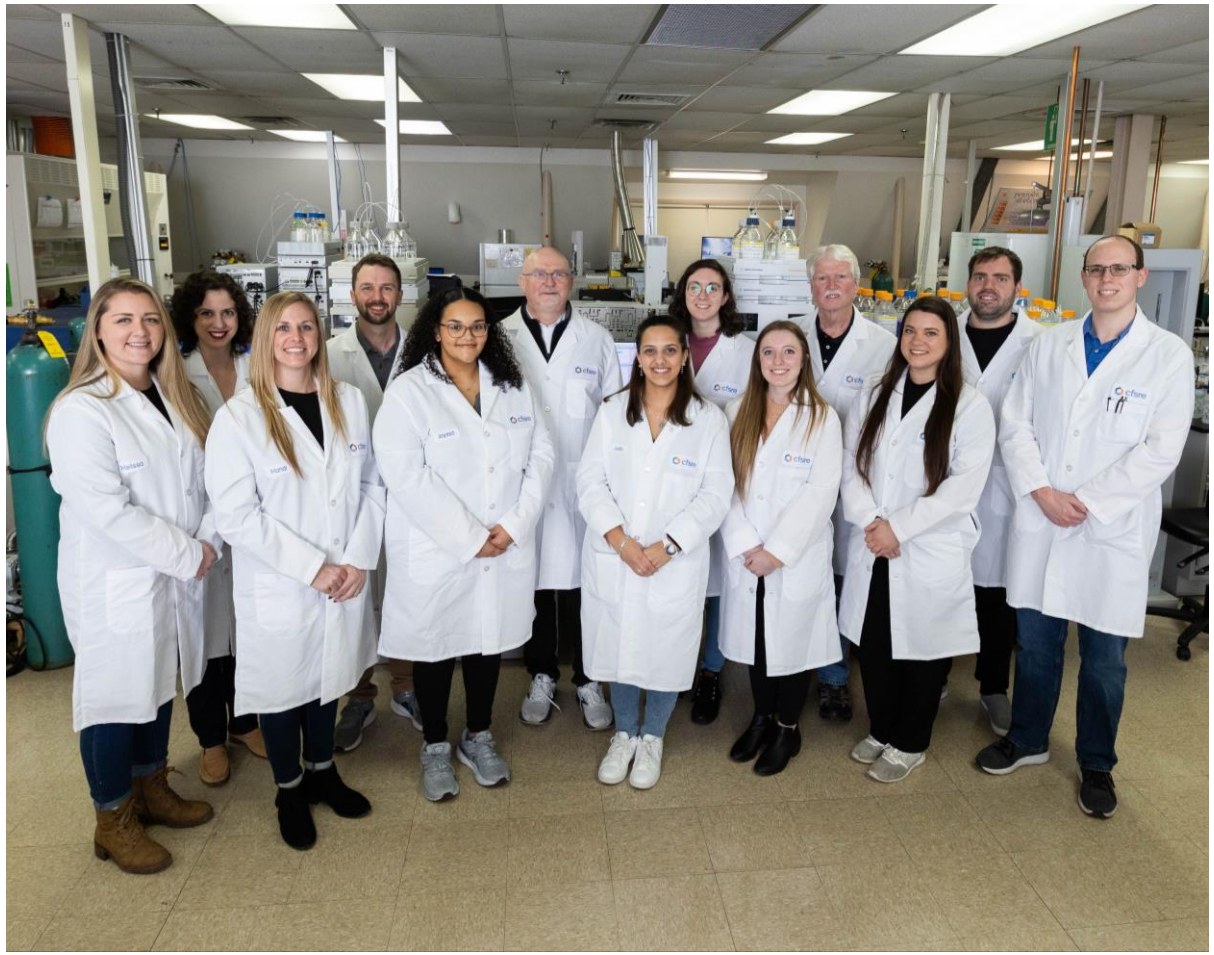
  - NIJ
- **Collaborators & Partners**
  - Forensic
  - Clinical
  - Medical Examiners
  - Coroners
  - Crime Labs
  - Etc.



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THANK YOU! QUESTIONS?

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