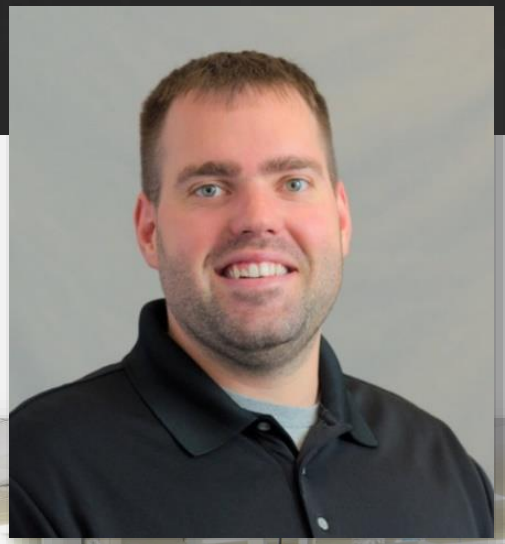
Theory – Data Dependent Acquisition (DDA) and Data Independent Acquisition (DIA)

Implementation Workshop: TOF/QTOF for Forensic Toxicology and Chemistry Planning/Implementation – Wednesday October 18, 2023

Alex J. Krotulski, Ph.D. – Center for Forensic Science Research and Education (CFSRE)

INTRODUCTION



- **Center for Forensic Science Research & Education**
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    - Toxicology & Chemistry
  - Program Manager
    - NPS Discovery

- **Thomas Jefferson University**
  - Assistant Program Director
    - MS in Forensic Toxicology
  - Faculty / Lecturer

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Waters Xevo® G2-S LC-QTOF-MS

Sciex X500R LC-TOF-MS

Sciex TripleTOF® 5600+ LC-TOF-MS

Agilent 6495 LC-QQQ-MS

Agilent 6430 LC-QQQ-MS

Waters TQS LC-QQQ-MS

Waters TQD LC-QQQ-MS

Agilent 5975 GC-MS

Agilent 5975 GC-MS

INTRODUCTION

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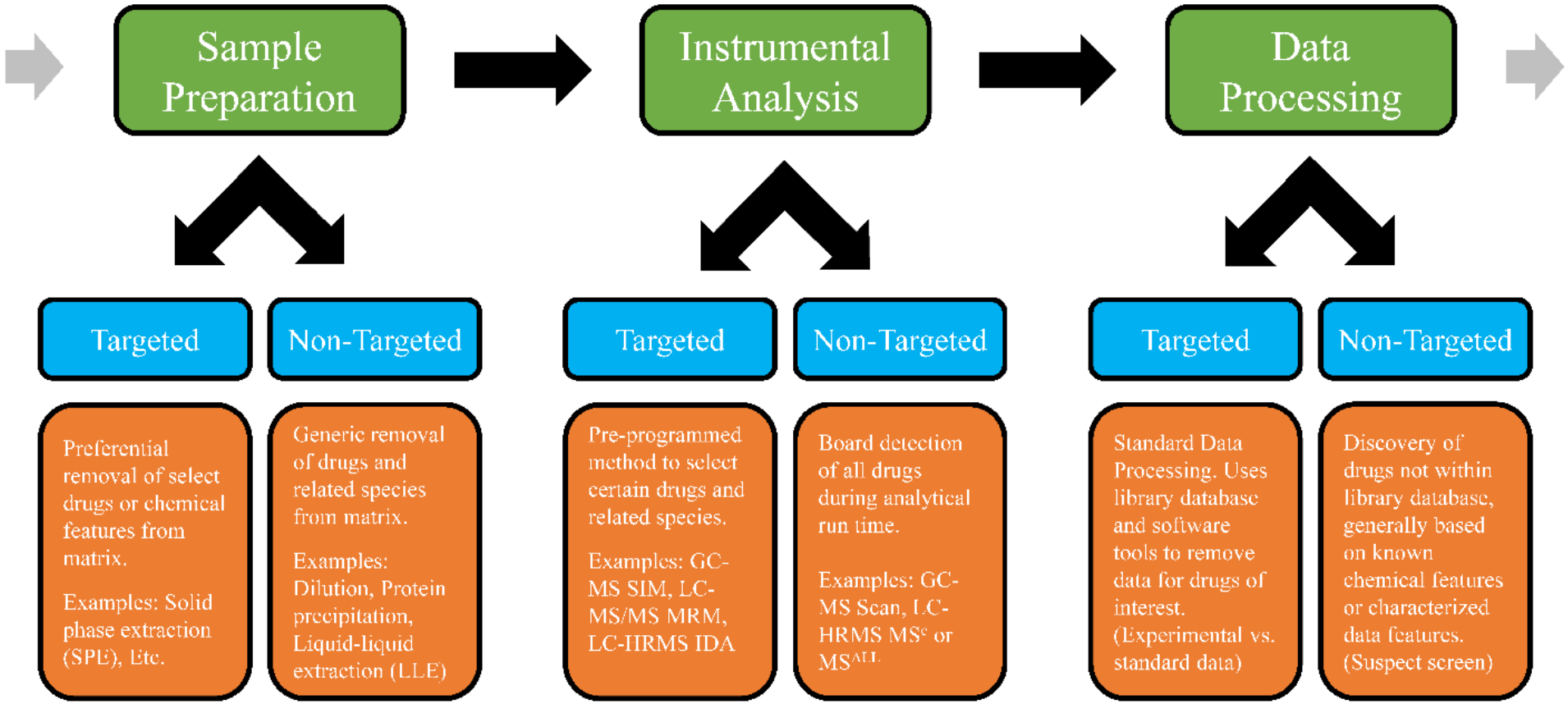
TARGETED VS. NON-TARGETED

- **Definitions:**
  - Targeted: Directed at a particular group or activity [or analyte]
  - Non-Targeted: Not directed at a particular group or activity [or analyte]

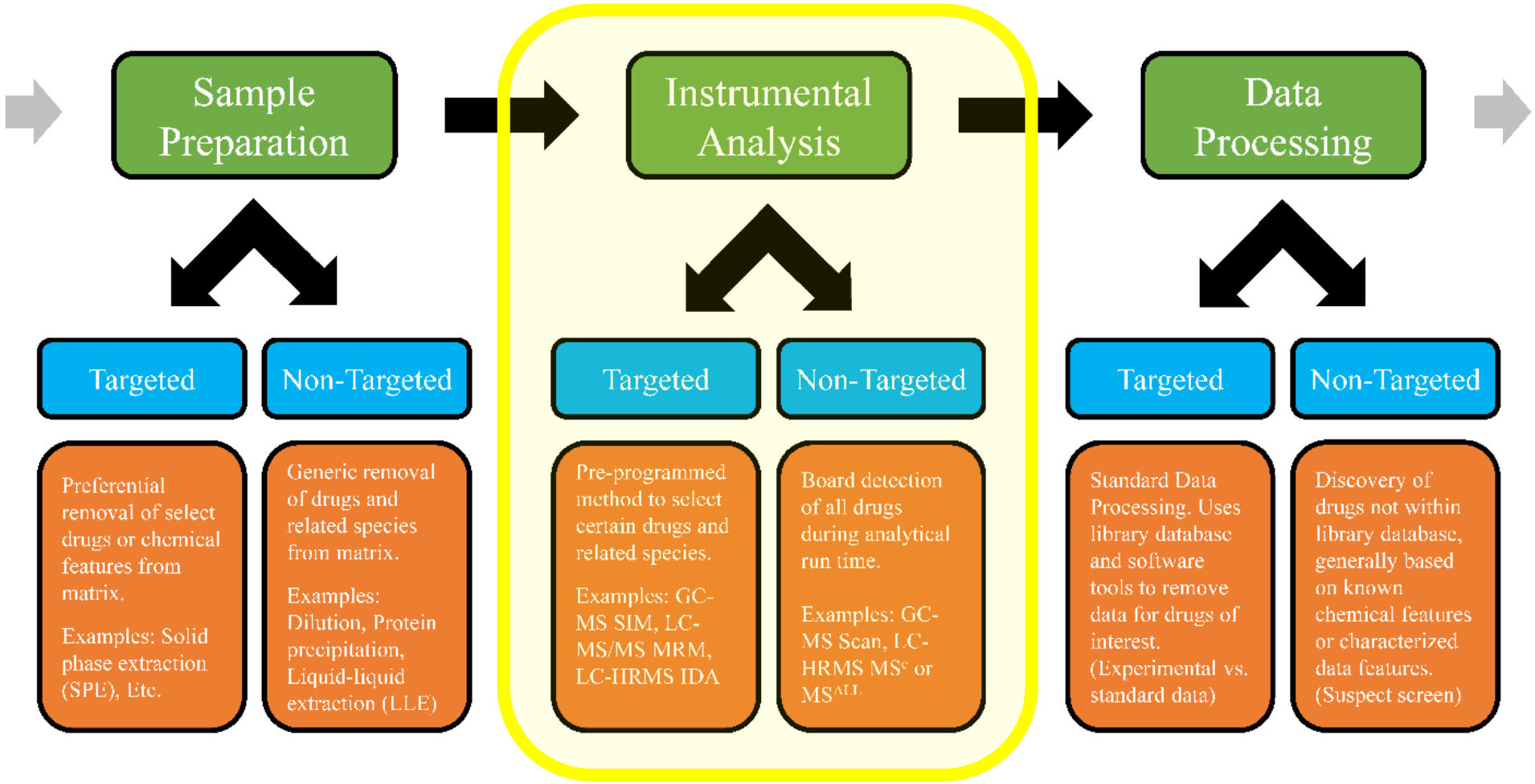
- **What are the benefits?**
  - Targeted: Specific, Direct, Intended, Focused, Aimed, Straightforward
  - Non-Targeted: Non-specific, Comprehensive, Indirect, Broad, Complicated

- **How do these two fit into forensic practice?**
  - Does non-targeted sound like the opposite of what we know in the forensic world???
  - Targeted and non-targeted can be used complementary to each other

TARGETED VS. NON-TARGETED



TARGETED VS. NON-TARGETED

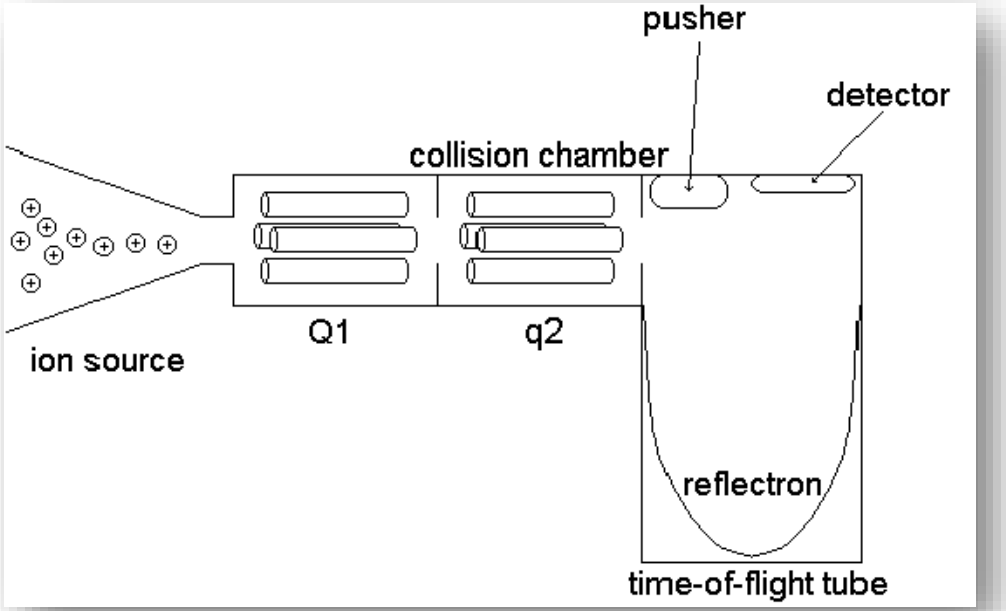


TOF/QTOF

TARGETED VS. NON-TARGETED

- **Targeted vs. Non-targeted in TOF-QTOF terms:**
  - TOF methods are generally all non-targeted (akin to GC-MS full scan)
  - QTOF methods can be targeted and/or non-targeted

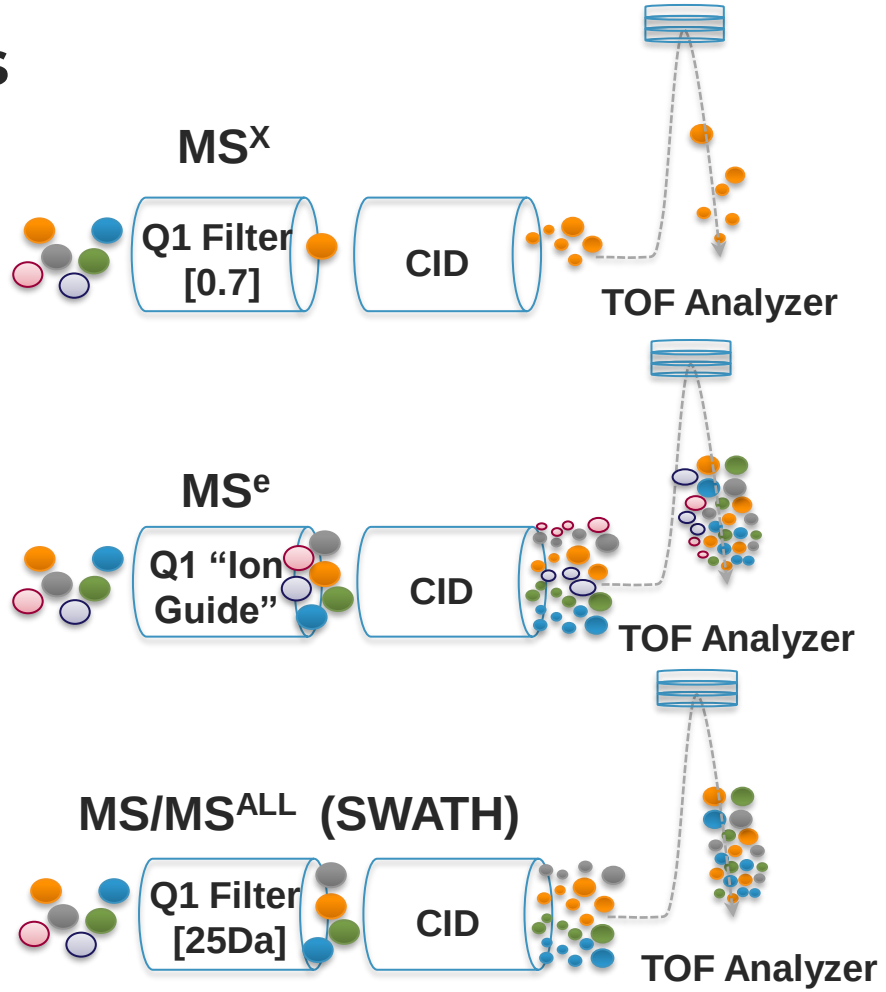
- **Primary components of a TOF/QTOF method:**
  - Source parameters
  - TOF-MS scan – produces mass spectrum #1
  - Quadrupole – results in mass spectrum #2 (QTOF only)
  - Collision cell set points (QTOF only)
  - *[TOF Analyzer]*
  - *[Detector]*



TARGETED VS. NON-TARGETED

- **Ion Filtering and Fragmentation Techniques**
  - Pass one mass – MS^X
  - Pass all masses – MS^{ALL} (or MS^e)
  - Pass range/pocket of masses – MS/MS^{ALL}

- **Acquisition mode dictates what data is produced and possible outcomes**



ACQUISITION MODES

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DATA DEPENDENT VS. DATA INDEPENDENT

TARGETED ACQUISITION MODES

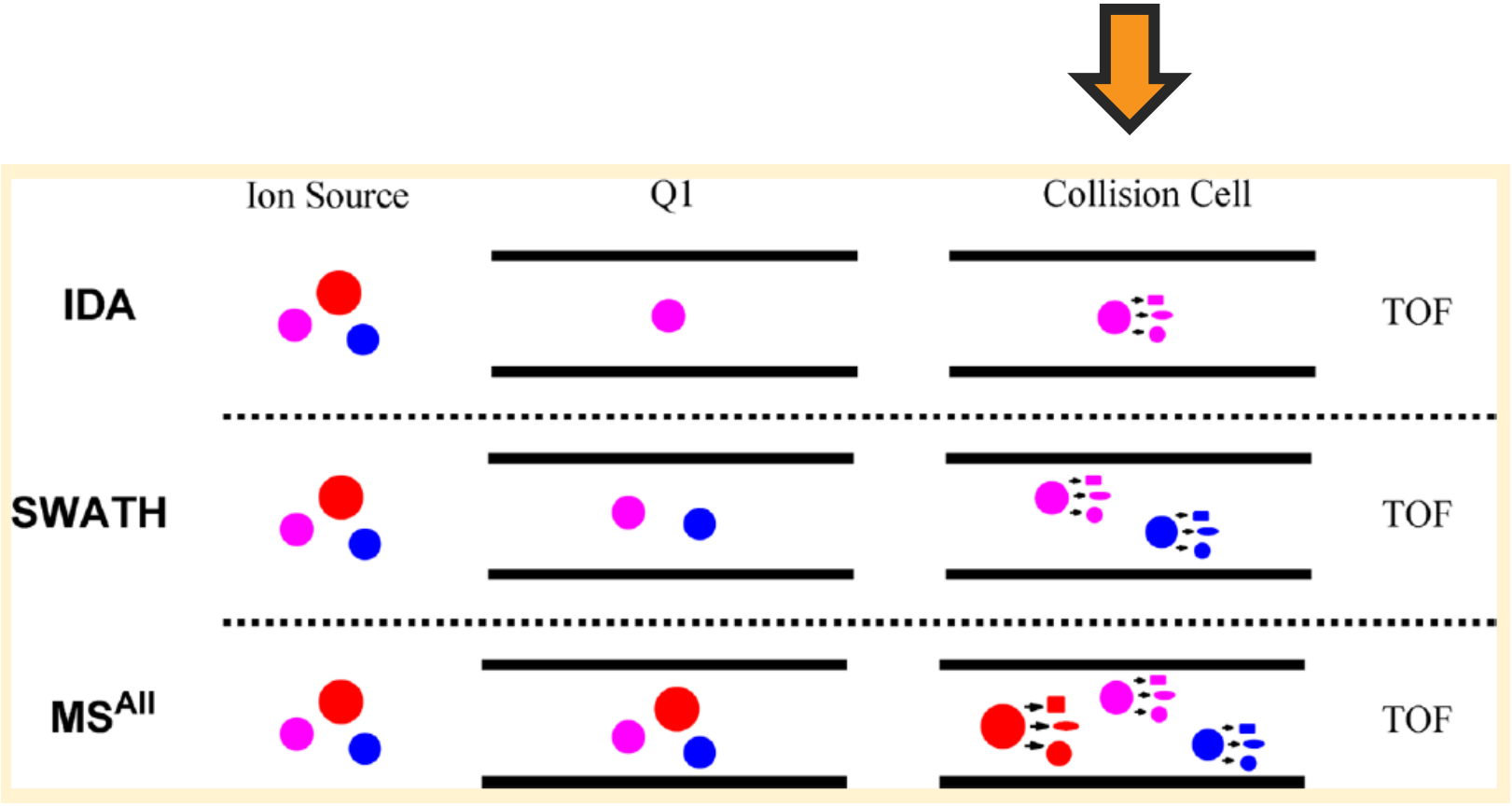
- **Data Dependent Acquisition (DDA)**
  - *Information Dependent Acquisition (IDA)*
  - Specific, non-inclusive, cycling acquisition
- MS/MS acquisition dependent on "sample"
- "If x → then y" (x = mass, intensity, etc.)
- *Multiple reaction monitoring (MRM^{HR}, MS^{X})*

NON-TARGETED ACQUISITION MODES

- **Data Independent Acquisition (DIA)**
  - Generic, comprehensive, cycling acquisition
- MS^{e} (or MS^{ALL})
  - E.g., Waters, Agilent All Ions MS/MS
- MS/MS^{ALL}
  - E.g., Sciex SWATH Acquisition

ACQUISITION MODES

- **Quadrupole**
  - "Ion Guide" or Mass Filter
- **Collision Cell**
  - Fragmentation
- **Time-of-Flight**
  - Accurate Mass
  - TOF MS & MS/MS

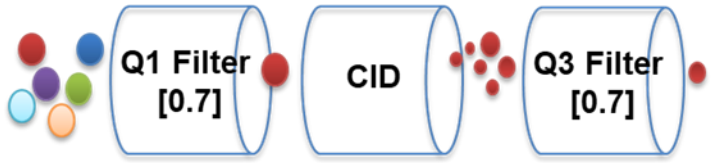


Source: https://pubs.acs.org/doi/10.1021/ac403385y

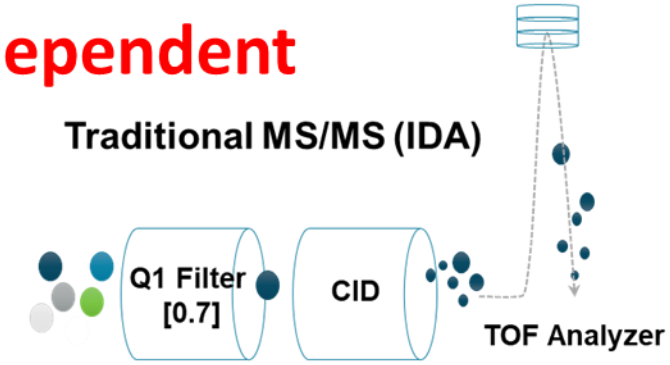
ACQUISITION MODES

Targeted / Data Dependent

Traditional "Triple Quad"



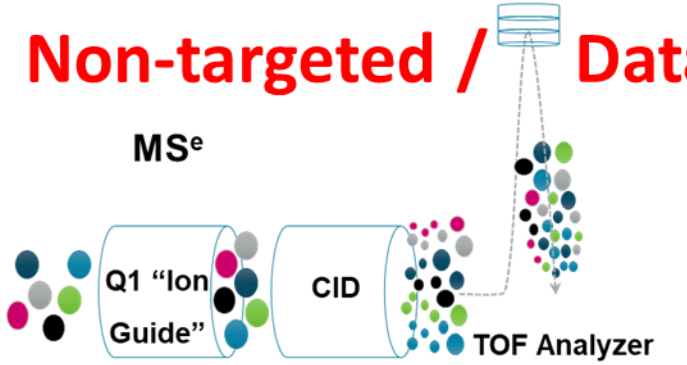
Traditional MS/MS (IDA)



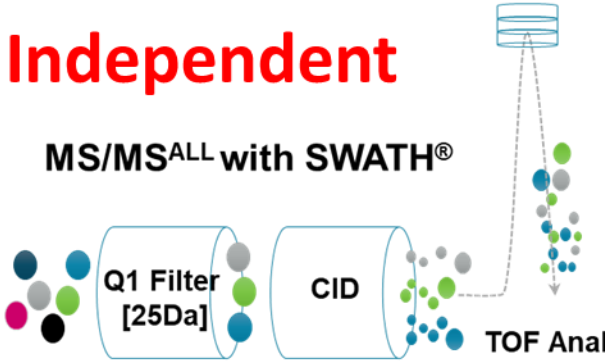
Non-targeted / Data Independent

"Single Quad" / Scan

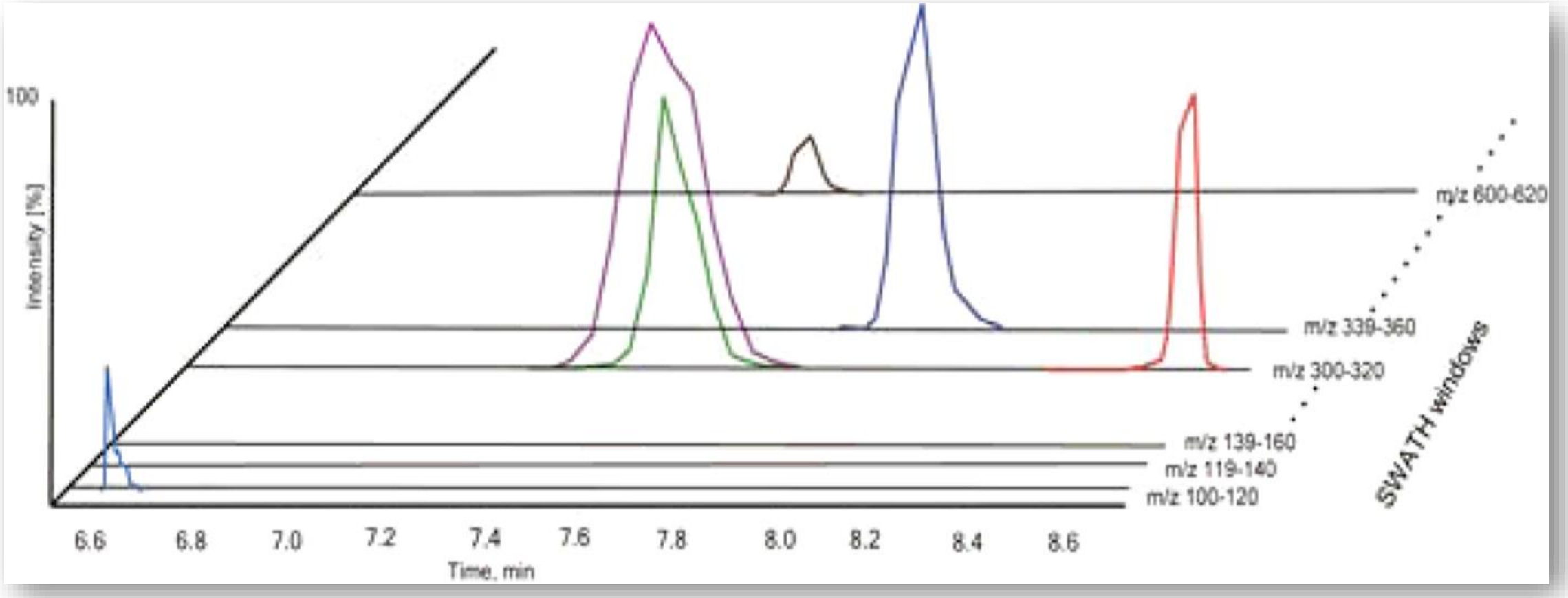
MS^e



MS/MS^{ALL} with SWATH®



3D VIEW OF DATA ACQUISITION

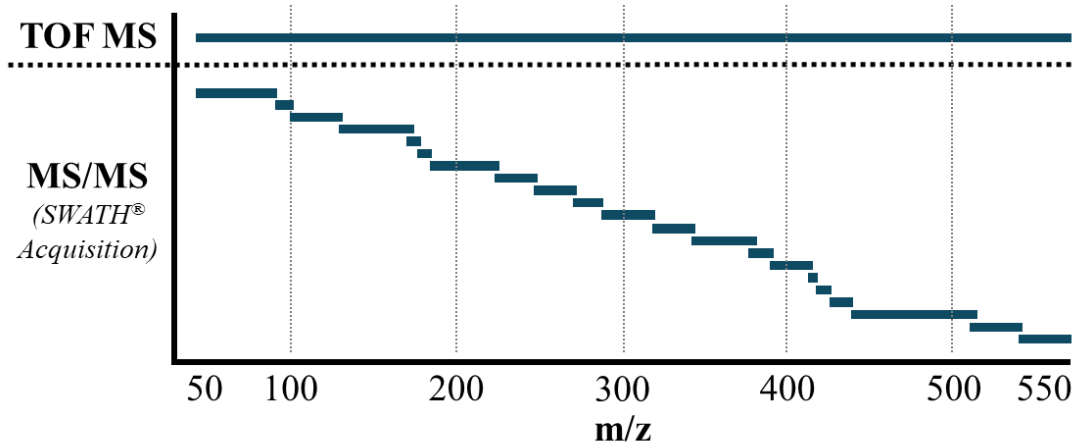


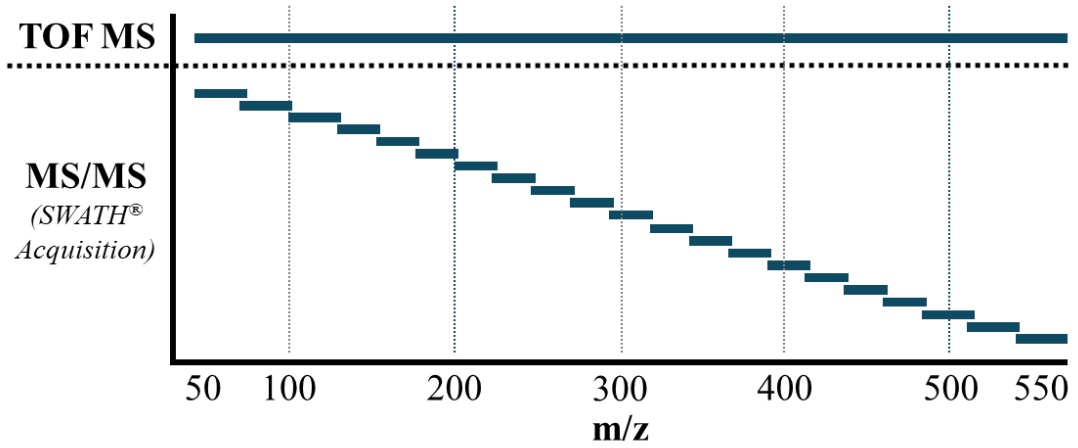
Source: https://pubs.acs.org/doi/10.1021/ac503144p

2D VIEW OF DATA ACQUISITION

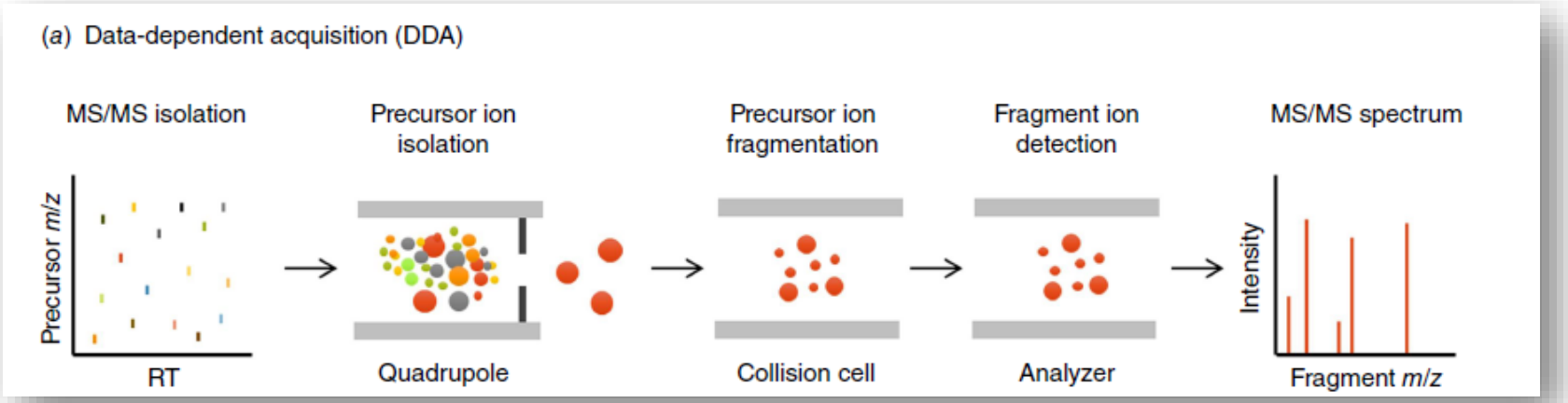
- **There is constant acquisition of data on a cycling basis**
  - Each cycle consists of a TOF-MS acquisition (generally spanning totally range of masses of interest)
  - Each cycle consists of sequential QTOF-MS (or MS/MS) acquisition(s) (variable)

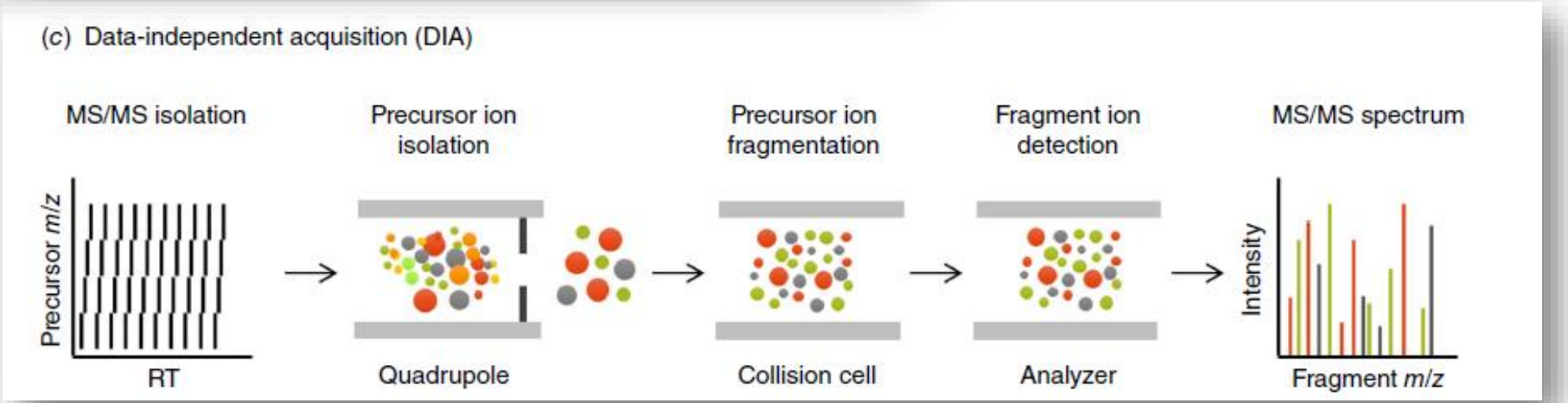
- **Examples for SWATH Acquisition:**





ACQUISITION MODES





Source: https://www.publish.csiro.au/CH/CH23039

STRENGTHS OF DDA VS. DIA

- **DDA (Targeted)**
  - High specificity
  - Certainty in fragment-to-precursor correlation
  - Isotopes discernable through accurate mass
  - May be ideal for structural elucidation
- **Both**
  - Perfectly valid approaches to data acquisition
  - Employed successfully in many toxicology labs (and chemistry)
  - Library search capabilities
- **DIA (Non-Targeted)**
  - Lower specificity (but remember chromatography)
  - Isotope patterns more obvious
  - Generally, more manageable for comprehensive screening
  - Extracted ion chromatograms

DATA EXAMPLES

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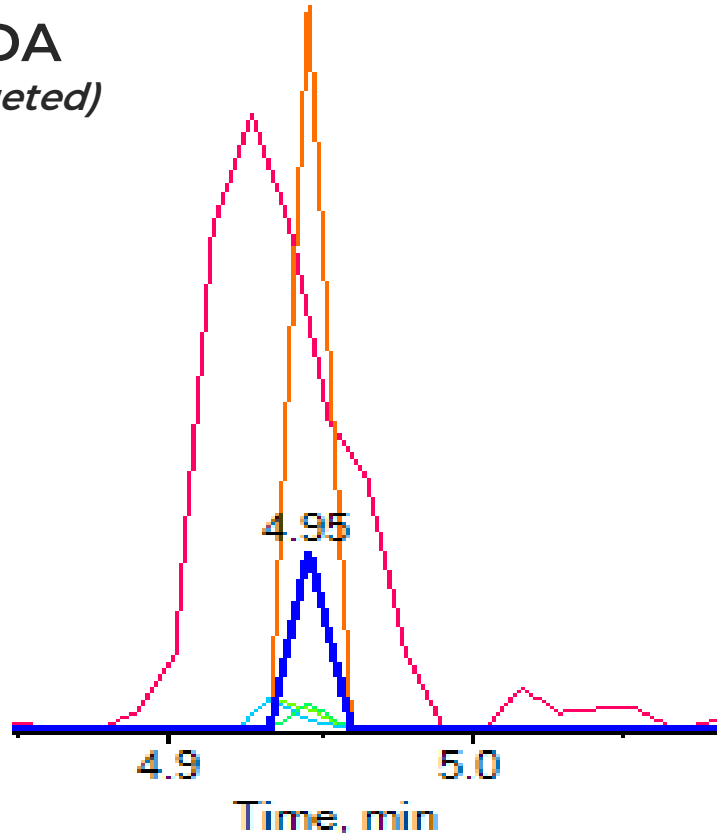
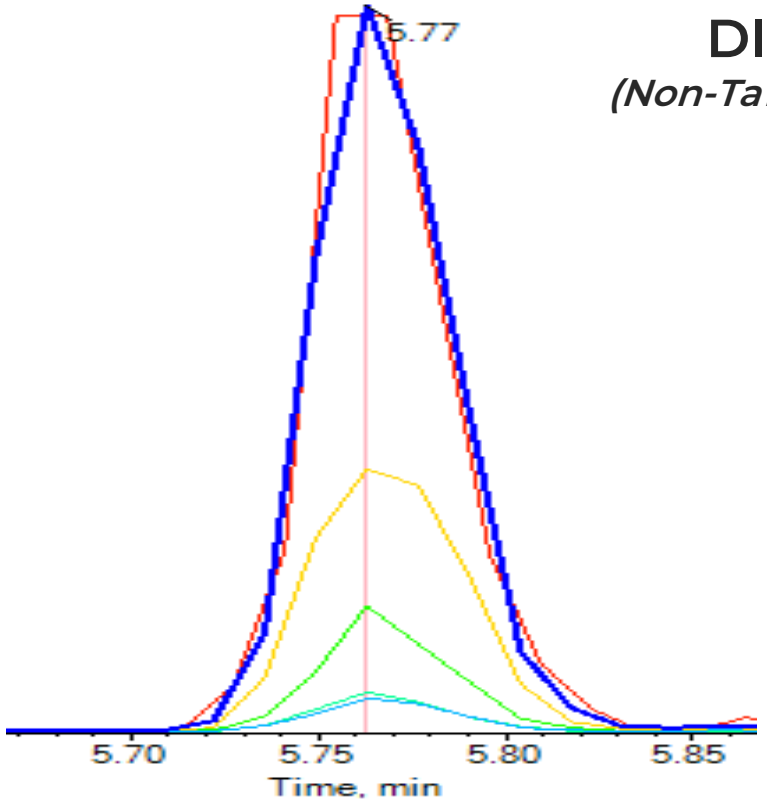
IMPACTS ON CHROMATOGRAPHY

DDA
(Targeted)

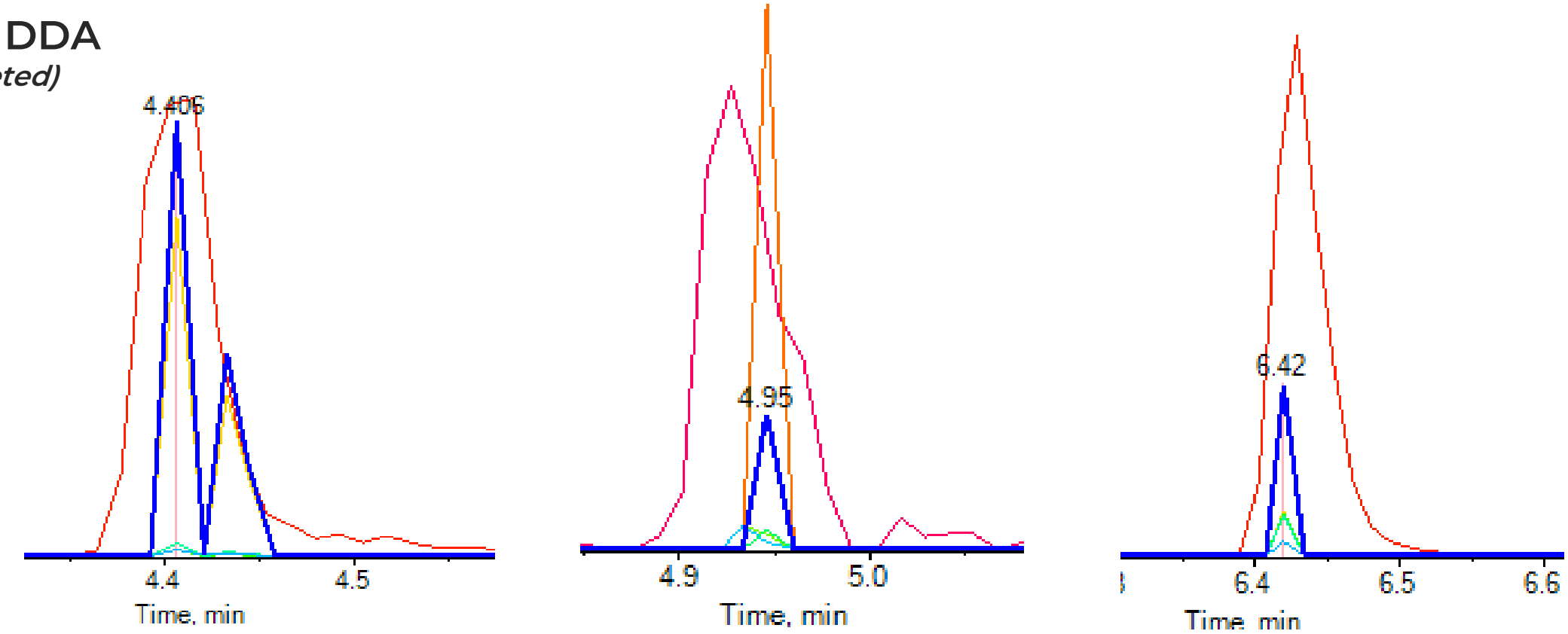


DIA
(Non-Targeted)



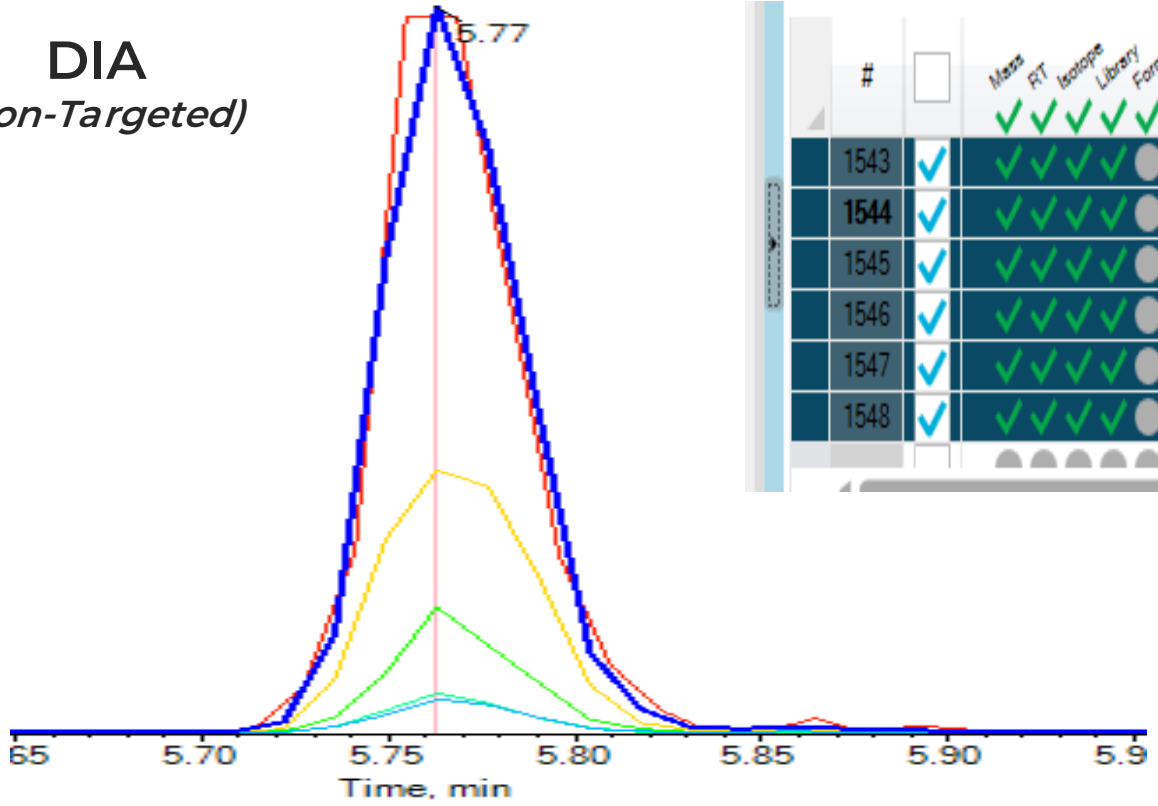
IMPACTS ON CHROMATOGRAPHY

All are DDA
(Targeted)



IMPACTS ON CHROMATOGRAPHY

DIA
(Non-Targeted)



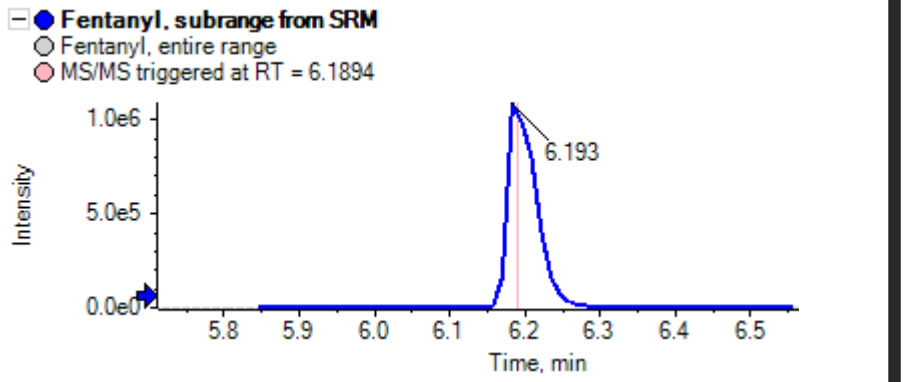
#	Mass	RT	Isotope	Library	Formula	Name	Formula	Mass (Da)
1543	✓	✓	✓	✓	✓	Cocaethylene	C18H23NO4	317.16271
1544	✓	✓	✓	✓	✓	**Cocaethylene**	**C18H23NO4**	**317.16271**
1545	✓	✓	✓	✓	✓	Cocaethylene	C18H23NO4	317.16271
1546	✓	✓	✓	✓	✓	Cocaethylene	C18H23NO4	317.16271
1547	✓	✓	✓	✓	✓	Cocaethylene	C18H23NO4	317.16271
1548	✓	✓	✓	✓	✓	Cocaethylene	C18H23NO4	317.16271

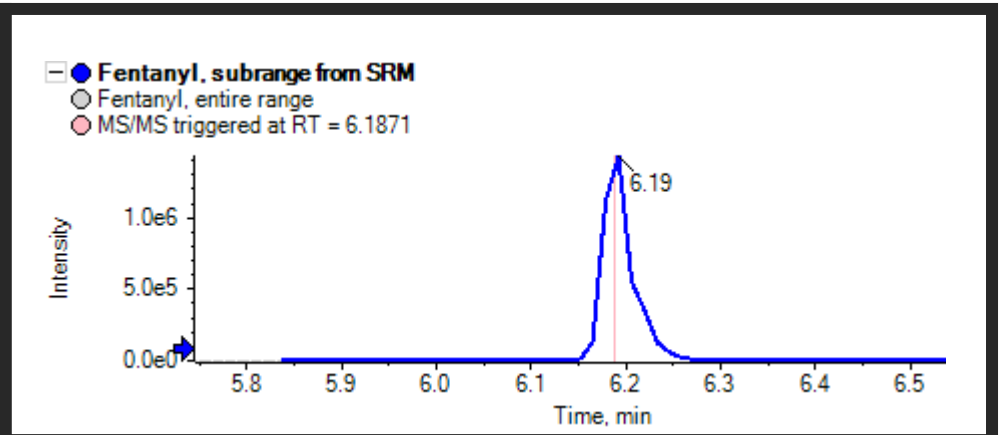
IMPACTS ON CHROMATOGRAPHY

DDA *(Targeted)*

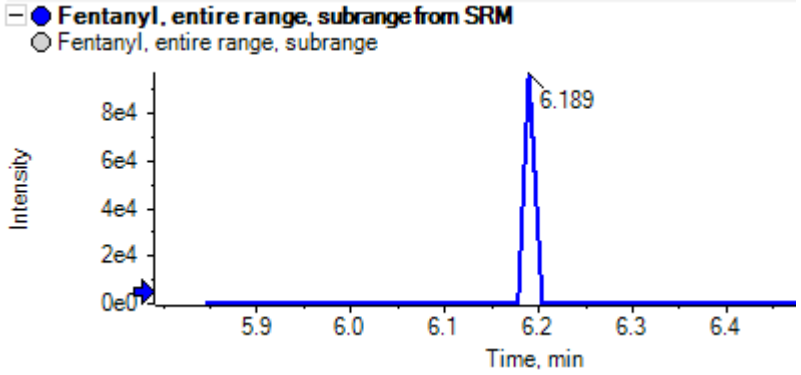
DIA *(Non-Targeted)*

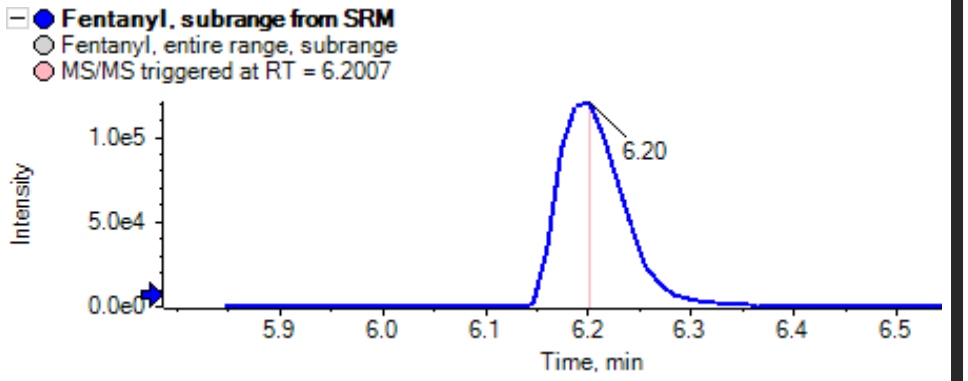
TOF-MS





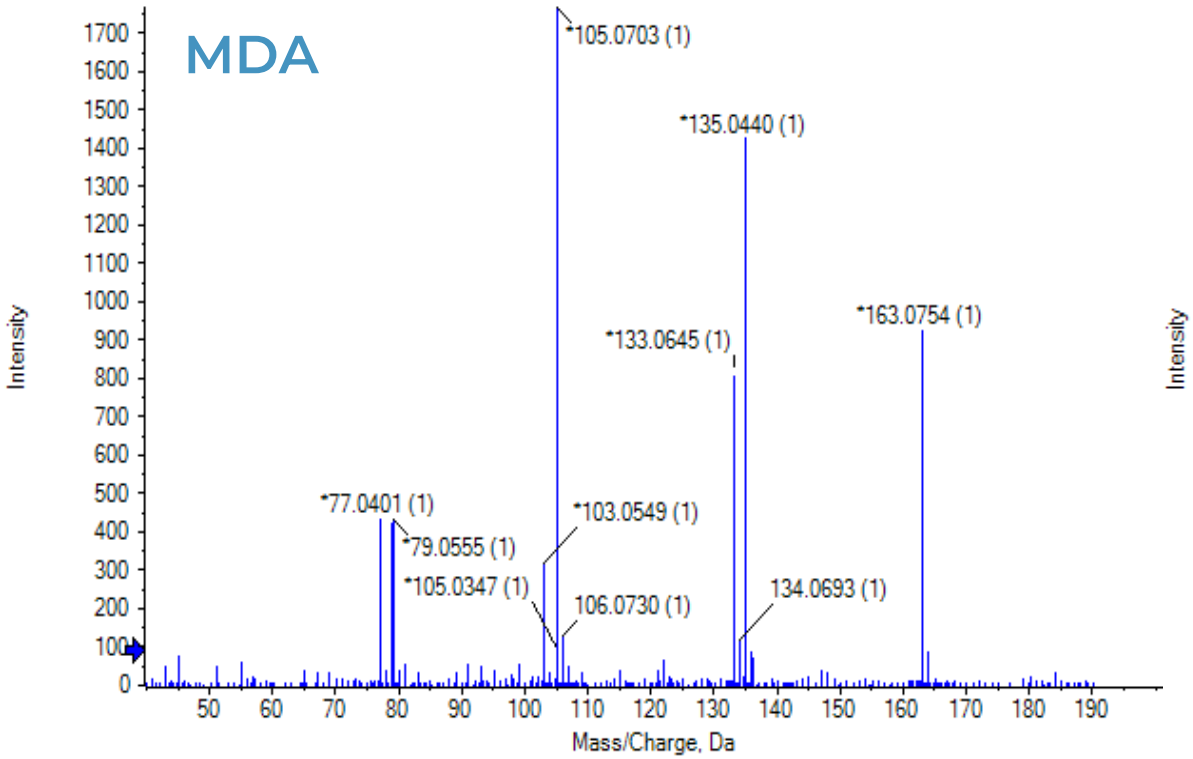
MS/MS



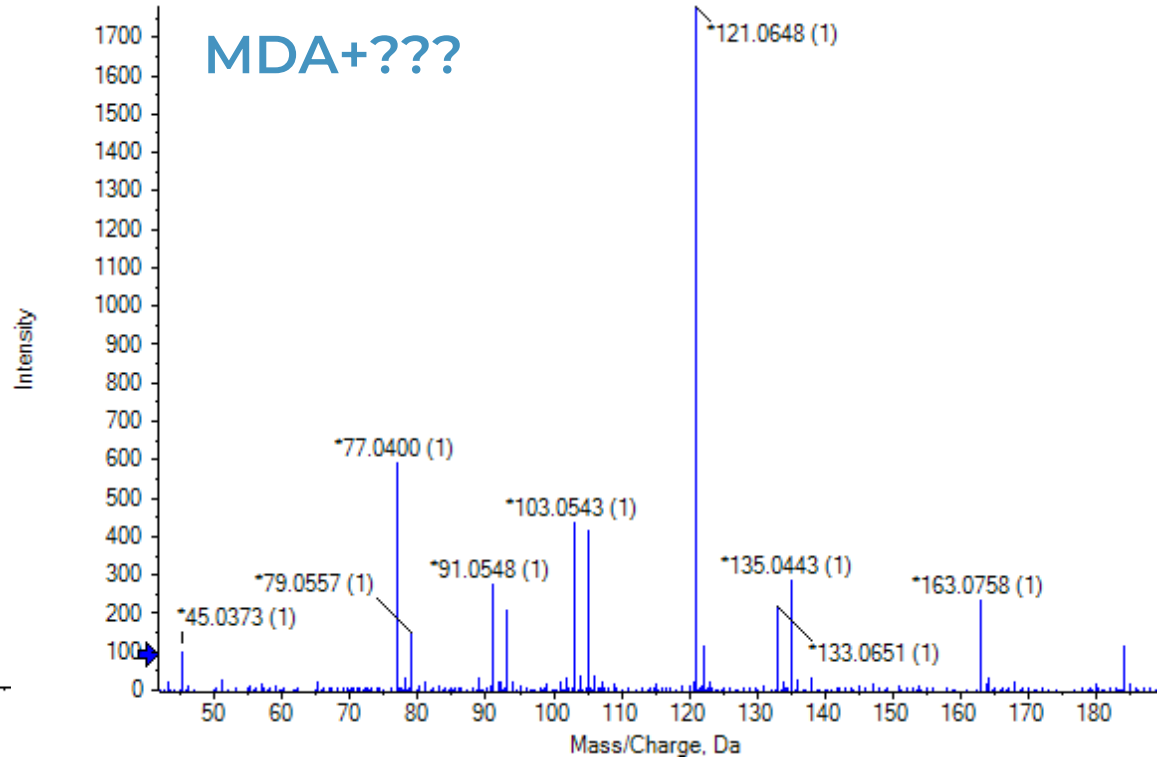


INTERFERING SUBSTANCES

DDA *(Targeted)*

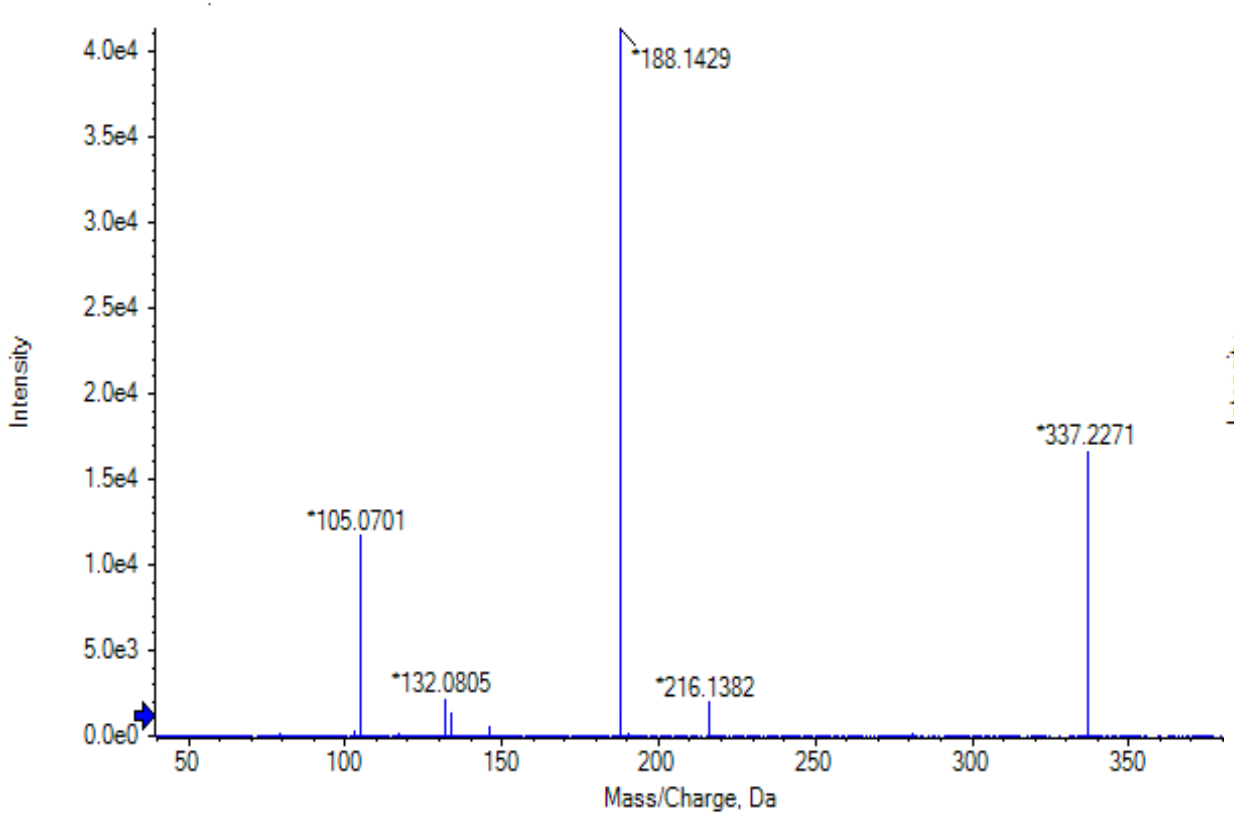


DIA *(Non-Targeted)*

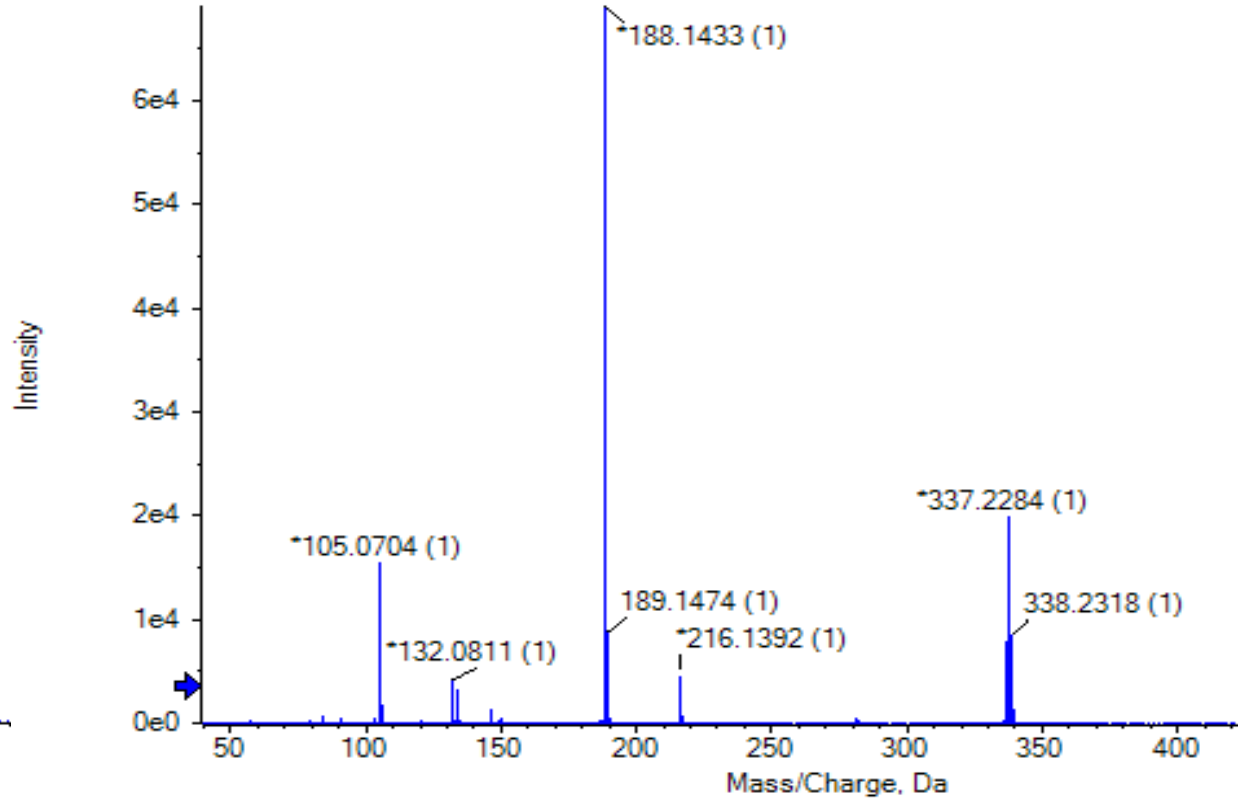


MS/MS DATA DIFFERENCES

DDA *(Targeted)*



DIA *(Non-Targeted)*



MS/MS DATA DIFFERENCES

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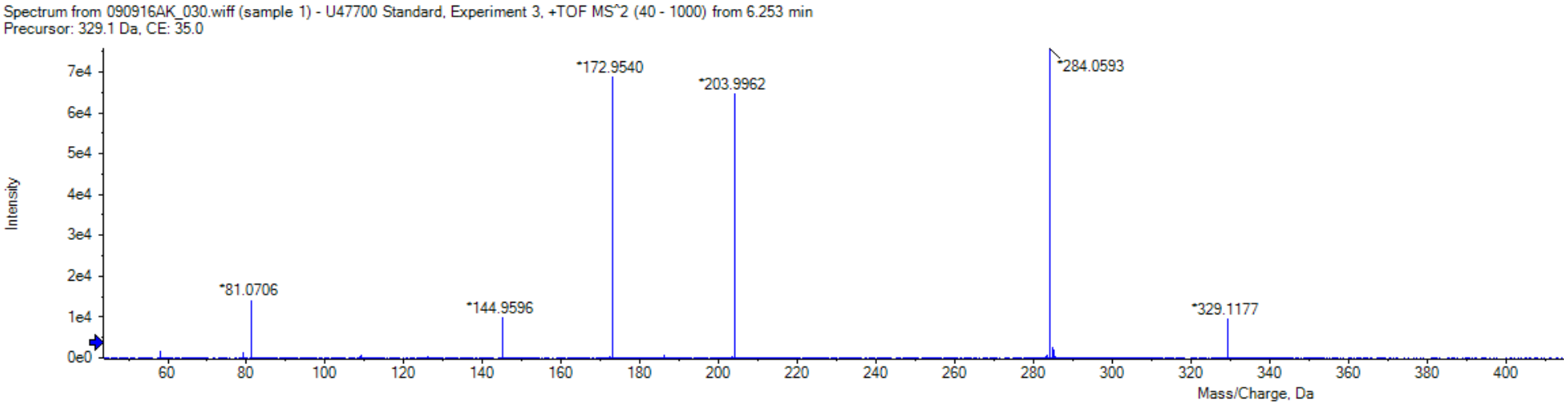
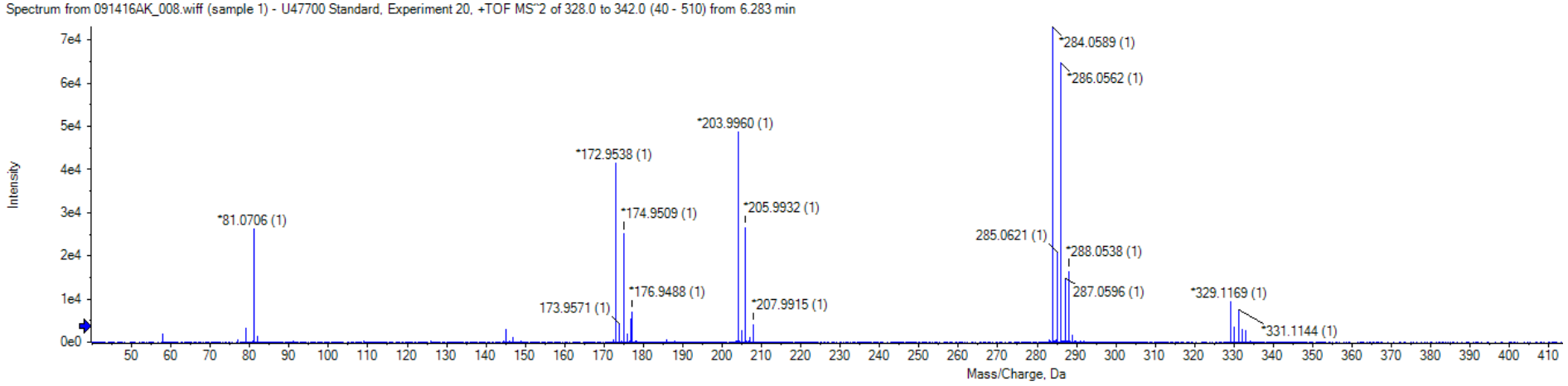


DIA *(Non-Targeted)*



DATA PROCESSING COMPLEXITIES

DDA *(Targeted)*

Name	Extraction Mass (Da)	Expected RT (min)	Found At Mass (Da)
Acetaminophen (P	152.07061	2.44	152.07075
Methcathinone	164.10699	3.15	164.10682
PMA (para-methox	166.12264	3.99	166.12248
Levetiracetam	171.1128	3.11	171.1126
MDA (3,4-Methyler	180.10191	3.83	180.10171
Phensuximide	190.08626	5.63	190.0862
MDMA-D5	199.14894	3.98	199.14911
Monoethylglycinex	207.14919	3.76	207.14917
MDEA (3,4-Methyl	208.13321	4.34	208.13299
Methylone-D3	211.11565	3.5	211.11547
Meprobamate	219.13393	5.66	219.13373
Tapentadol	222.18524	5.16	222.18542
Norketamine	224.08367	4.45	224.08378
Naproxen	231.10157	7.79	231.10173
Alpha-PVP	232.16959	5.1	232.16967
Methylphenidate	234.14886	5.05	234.14898
Lidocaine	235.18049	4.28	235.18062
Procainamide	236.17574	1.97	236.17574

DIA *(Non-Targeted)*

Name	Extraction Mass (Da)	Expected RT (min)	Fragment Mass (Da)	Found At Mass (Da)
Alpha-PVP	232.16959	5.1		232.17008
Alpha-PVP	232.16959	5.1	232.1703	232.16958
Alpha-PVP	232.16959	5.1	91.0556	232.17003
Alpha-PVP	232.16959	5.1	126.1281	232.17003
Alpha-PVP	232.16959	5.1	105.0344	232.17008
Alpha-PVP	232.16959	5.1	161.0958	232.17003
Ketamine	238.09932	4.54		238.09953
Ketamine	238.09932	4.54	125.0155	238.09953
Ketamine	238.09932	4.54	179.0621	238.09953
Ketamine	238.09932	4.54	238.0999	238.09953
Ketamine	238.09932	4.54	220.0892	238.09953
Ketamine	238.09932	4.54	207.0571	238.09953
2C-B	260.02807	5.36		260.02789
2C-B	260.02807	5.36	227.9777	260.02789
2C-B	260.02807	5.36	243.0018	260.02789
2C-B	260.02807	5.36	212.9543	260.02789
2C-B	260.02807	5.36	164.0825	260.02789
2C-B	260.02807	5.36	134.073	260.02789

CONCLUSIONS

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CONCLUSIONS

- It's important to understand:
  - **Targeted vs. non-targeted** in terms of analytical workflows
  - Data dependent acquisition (DDA) vs. data independent acquisition (DIA)

- **Instruments, hardware, and software** vary by vendor
  - MS^e vs. MS^{ALL} vs. MS/MS^{ALL}

- There is no **"right way"** to TOF/QTOF acquisition
  - Highly dependent on purpose of method

- **More and more forensic labs using HRMS!**

ACKNOWLEDGEMENTS

- **CFSRE Team**
  - Barry Logan
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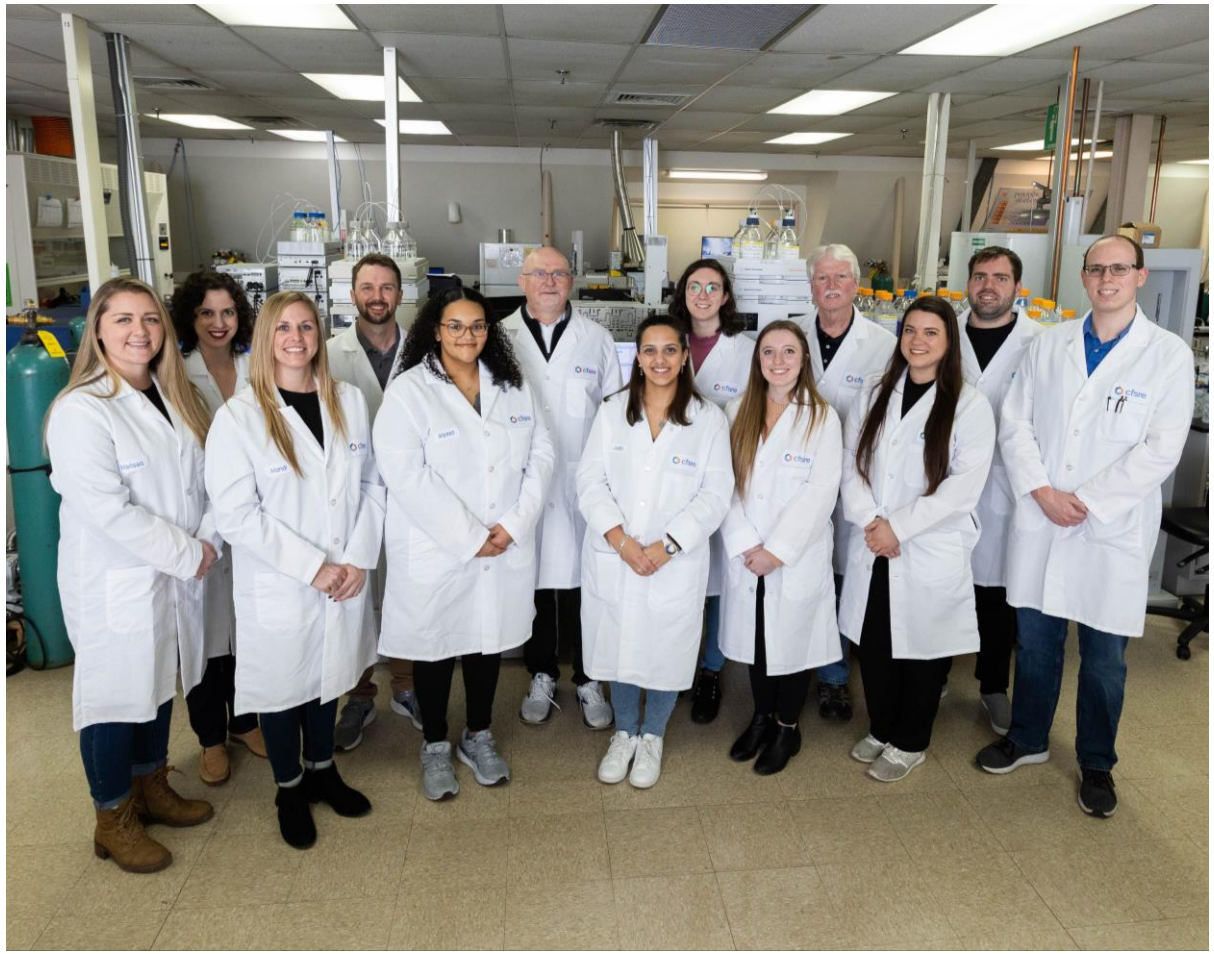
  - Brianna Stang
  - Alexis Quinter
  - Max Denn
  - Many others!
- **MS Collaborators**
  - Sciex
  - Waters
  - Agilent



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THANK YOU! QUESTIONS?

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