

ADC Quantification using HRMS

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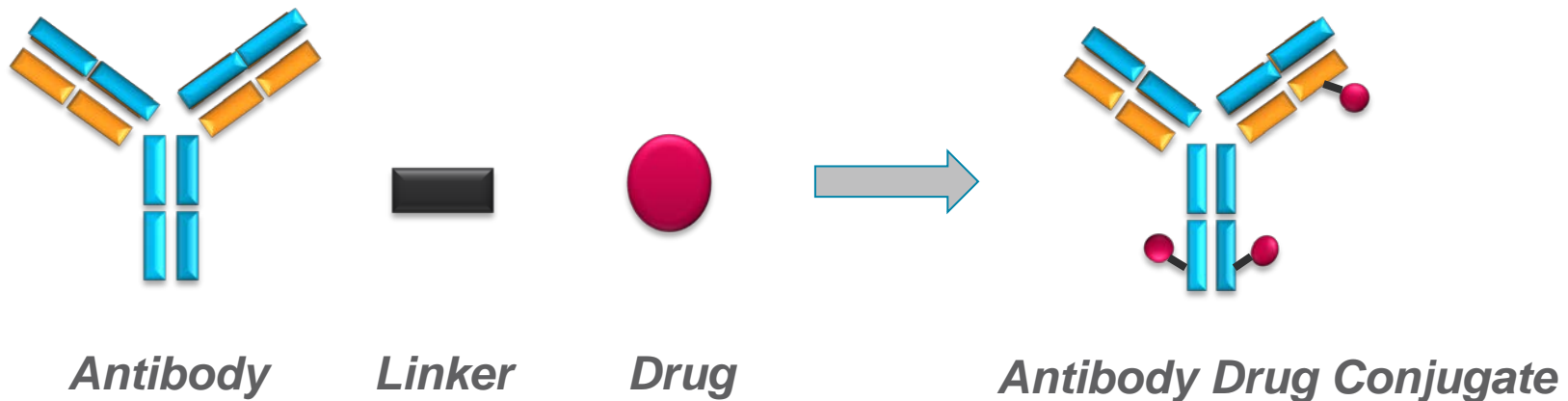


OUTLINE

1. Antibody Drug Conjugates- Few Facts
2. Bioanalytical Assay Platforms-Benefits & Limitations
3. HRMS Workflow-SWATH™ Acquisition for ADC Quant
 - T-DM1 Data Examples-Qual & Quant
4. Role of Differential Mobility for ADC Quantification
 - T-DM1 Data Examples using SelexION™ Technology

Antibody Drug Conjugates- Some Facts

- BEST OF BOTH WORLDS- **ANTIBODY** AND **SMALL MOLECULE**
- *Ultimate benefit-Targeted drug delivery*



- But, linker chemistry, site specific conjugation, payload, heterogeneity may pose unique analytical challenges for both characterization and quantification

Most Important ADC Exposure Studies

No.	ADC Form	Analyte Details	Assay Type	Purpose
1	Total Antibody (conjugated, partially conjugated & unconjugated)	DAR \geq 0	LBA	Exposure of protein component of ADC
2	Conjugated Antibody	DAR \geq 1	LBA/Affinity LC/MS/MS	ADC PK analyses
3	Cytotoxic Drug (Free/ Bound)	SM drug	LC/MS/MS	Assessment of safety & efficacy

Future Perspective

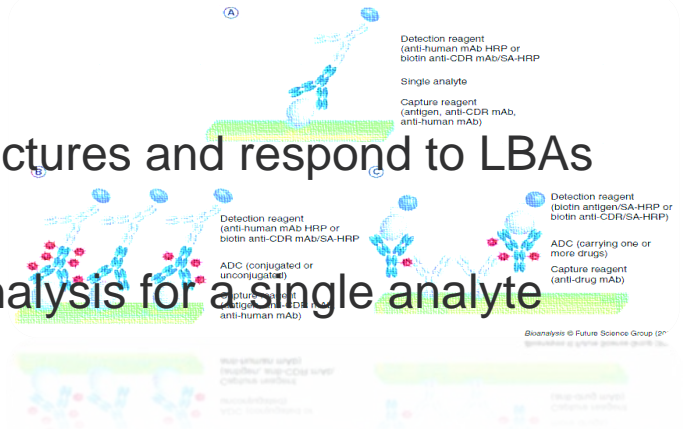
- ***Can we move all 3 assay's onto an LC/MS/MS System? and***
- ***Can we track metabolites/catabolites simultaneously during PK study?***

Bioanalytical Platform for ADC Quant

Need Best of Both Worlds

■ LBA Assays

- Large molecules have well defined tertiary structures and respond to LBAs (ELISA)
- ELISA is commonly used for large molecule analysis for a single analyte



■ Hybrid binding/MS based methods

- Large molecules can be quantified by MS methods
- Small molecule BA is predominately performed by LC/MS methods



Guidance for Industry (Bioanalytical Method Validation, Biopharmaceuticals) September 2013 Rev 1

- When possible, the LBA should be compared with a validated reference method (such as LC-MS) using incurred samples and predetermined criteria to assess the accuracy of the LBA method.

LBA vs. LC/MS/MS Assay Platform

LBA and LC/MS/MS-Both are complementary to each other

- Choice of platform depends on
 - Ease of use
 - Cost effectiveness
 - Turn around time
 - **Specificity, selectivity and sensitivity**
 - **Retrospective data analysis**-what else can we learn from the existing data?

Should we go for triple quad or HRMS?

- Important factors that can influence MS instrumentation
 - **Selectivity** and sensitivity requirement- Discovery vs. GLP bioanalysis
 - **Generic MS methods**
 - **Turn around time**

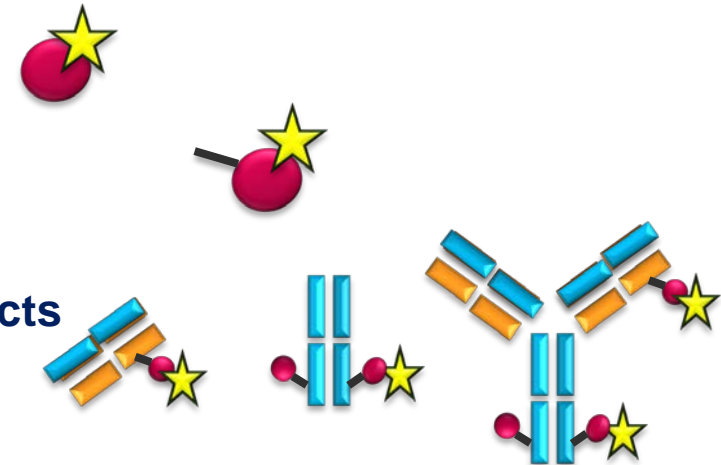
Bioanalytical Strategies for ADC PK Profile

Quantify All Possible Circulating Species with Cytotoxic Drug

“Typically, efficacy/ safety of ADC can be attributed to the overall systemic exposure of multiple moieties of ADC”

1. Drug Phase I, II and GSH adducts
2. Drug + Linker + Phase I, II, GSH adducts
3. Peptide + Drug + Linker Phase I, II GSH adducts

Many others....



Could many of these questions be addressed using

- a) **Single LC/MS platform and**
- b) **Most importantly using single acquisition method**

TripleTOF SWATH™ Acquisition Strategy

ADC Quantification



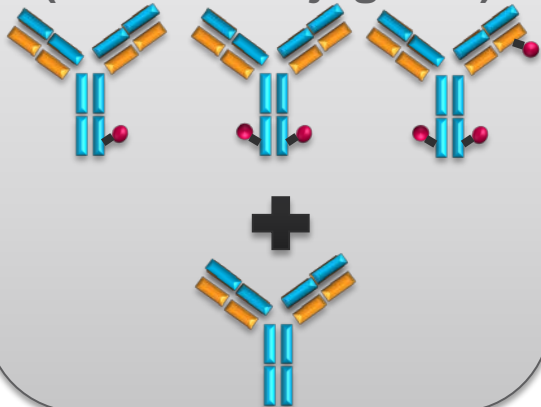
In vivo sample
Time point (x hr)

TripleTOF SWATH™ Acquisition

Single Raw Data file



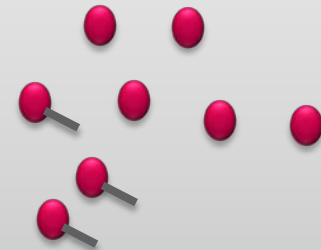
Total Antibody
(naked+conjugated)



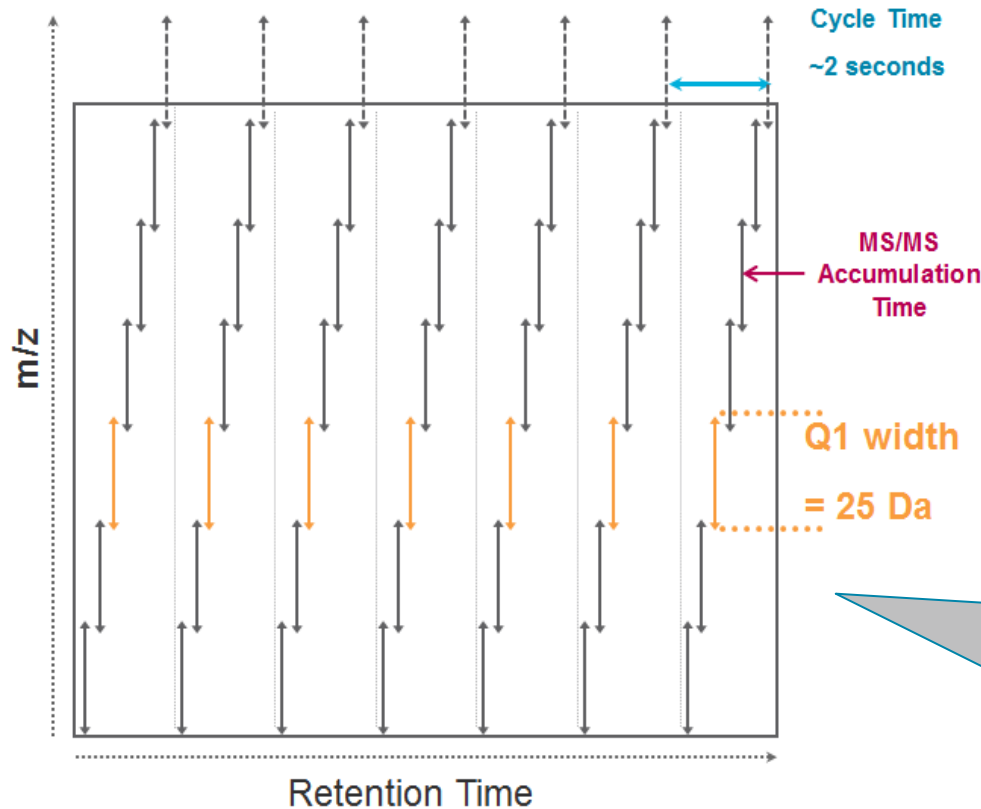
Conjugated Antibody/Drug



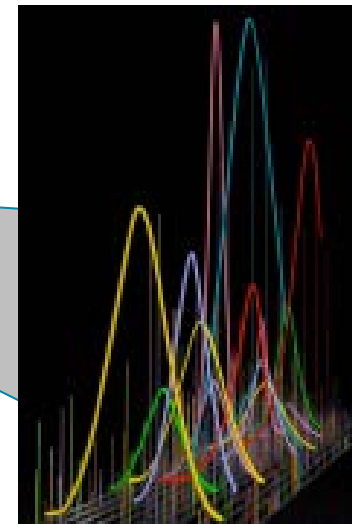
Free Drug/(+Linker)



Comprehensive DIA Quantitation-SWATH™



- *Sequential Q1 isolation stepped over mass range of interest (e.g. 25 Da, customizable)*
- *TripleTOF® 5600+ system speed allows full coverage of mass range in LC time scale*
- *High resolution XIC data for all fragment ions*



Key Benefits of SWATH for ADC Analysis

Benefits of SWATH Acquisition

1. **Generic MS method specially in Discovery and Development Phase**
2. **Comprehensive qualitative and quantitative analysis**
3. **Ultimate safety net** for capturing both predicted and unpredicted metabolites / catabolites
4. **Easy and retrospective** data analysis for ADC catabolite and metabolite ID

Unique Features of SWATH Acquisition

1. **Selective MS/MS Quantification**-Sum product ions
2. **Less complex MS/MS spectrum** than traditional DIA techniques
3. Wider Q1 selection **retains isotope pattern for each fragment**
 - Good for C14/SIL ADC metabolism studies
4. **100% MS/MS for low level** metabolite/catabolite identification

TripleTOF SWATH™ & HPLC Conditions

- TripleTOF™ 5600+ system with the Turbo V™ Source and Shimadzu Nexera
- SWATH- TOF MS- m/z 300-1250
- 24 SWATH windows each 30 Da wide
- TOF MS/MS-m/z 100-1500
- **Column Phenomenex Aeris Peptide (2.1mm X 100 mm, 3 μm)**
 - Flow: 250 μL/min
 - Column Oven at 30 °C
- Solvent A: 2%ACN 0.1% FA
- Solvent B: 98%ACN 0.1% FA
- Injection: 5 μL

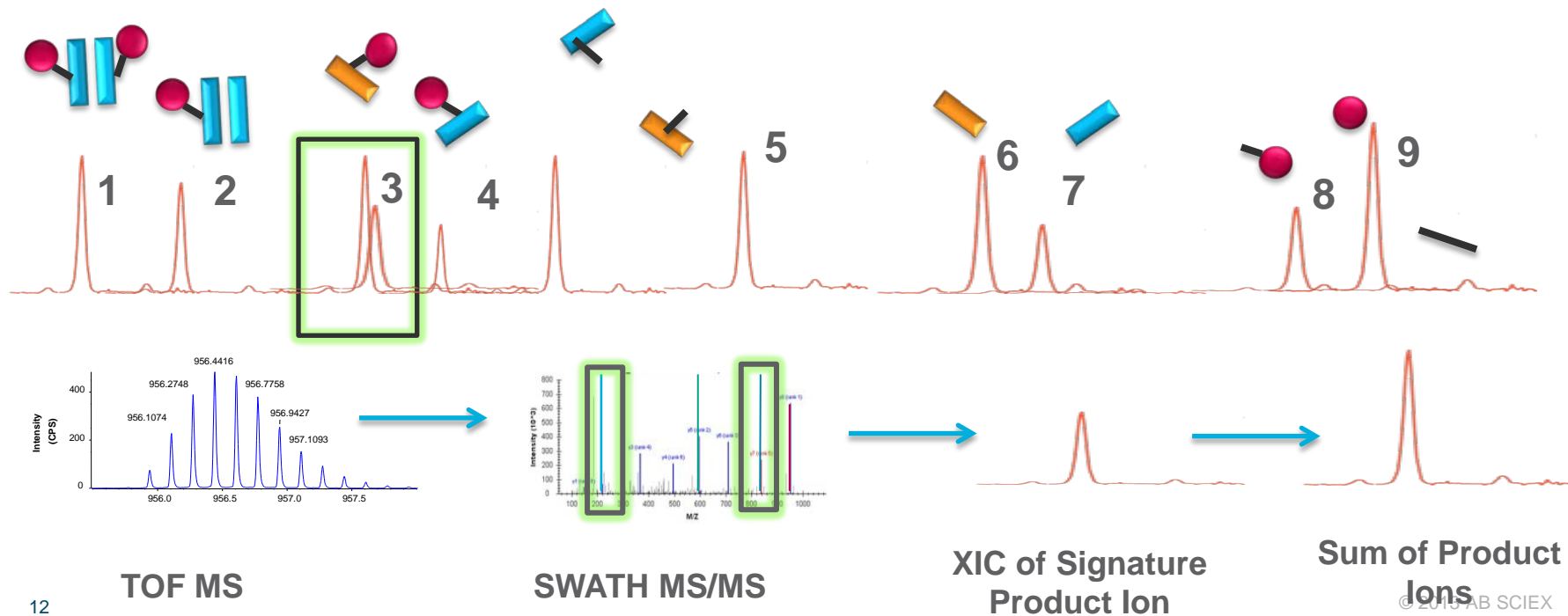
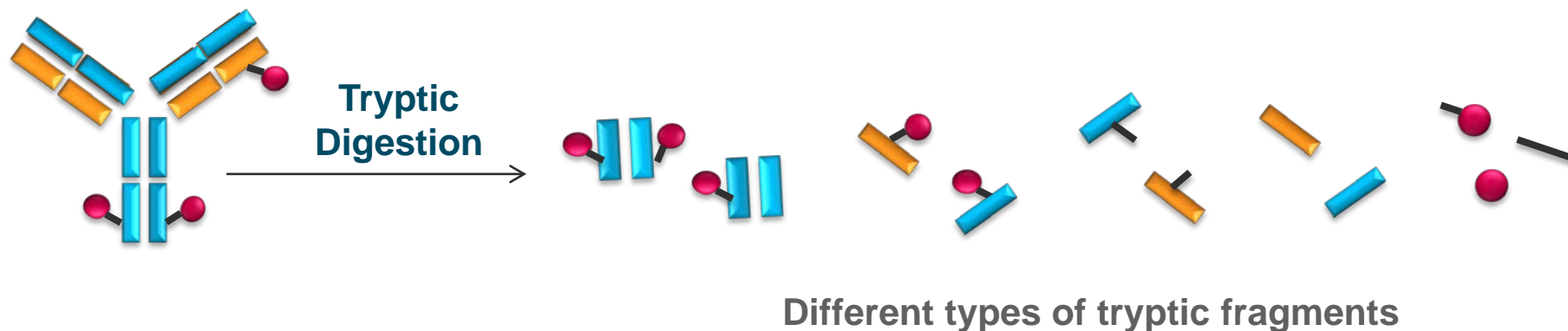


TripleTOF™ 5600+System

Time	Solvent A	Solvent B
0	100	0
3	100	0
15	68	32
18	10	90
20	10	90
25	100	0

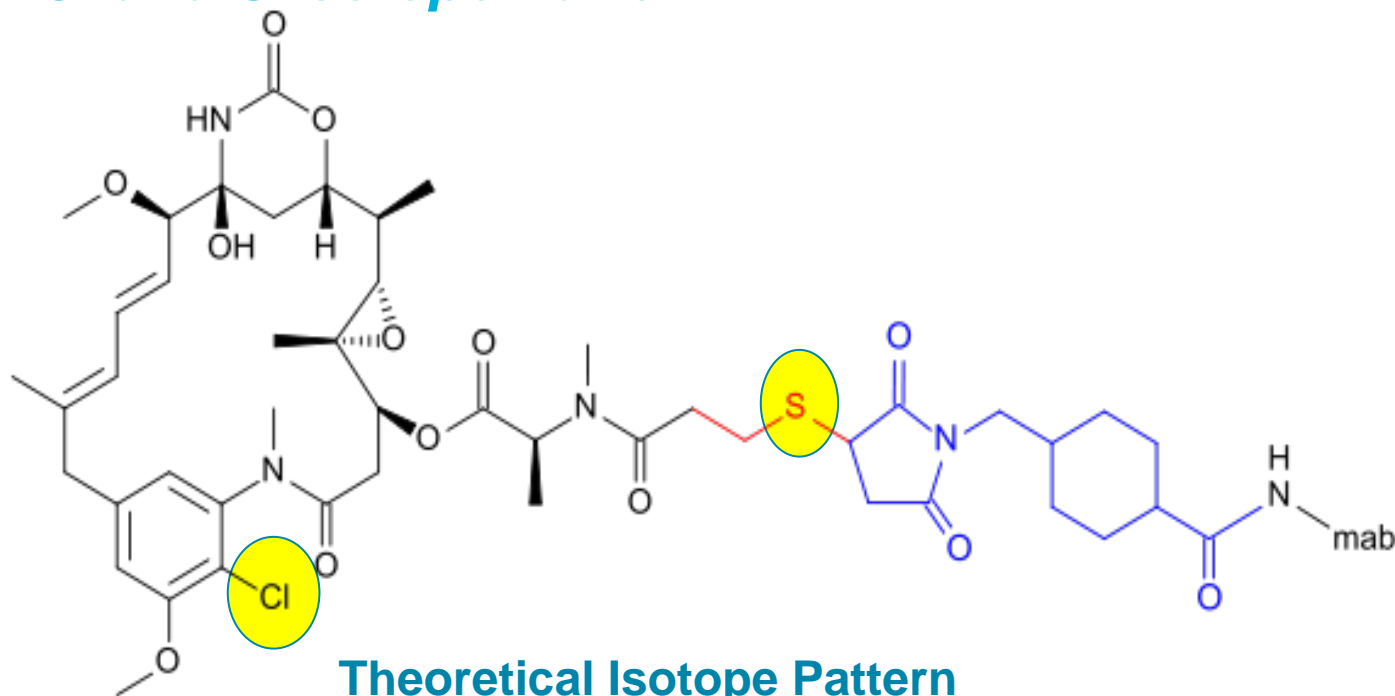


ADC PK Profile- Multicomponent Quantification in Single Inj. *SWATH Acquisition on TripleTOF+ 5600 System*



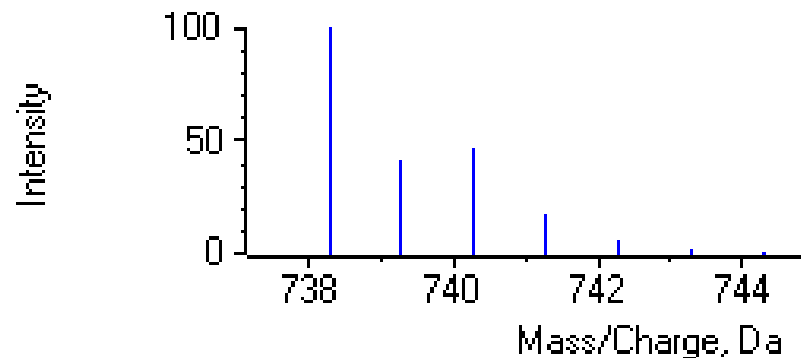
Data Processing Strategies

T-DM1- Cl and S Isotope Pattern



Theoretical Isotope Pattern

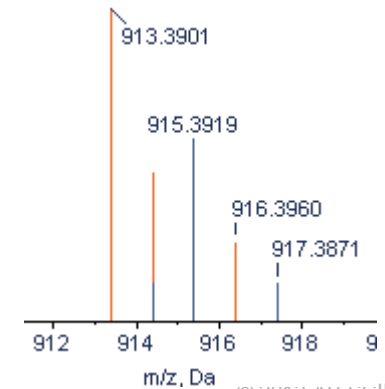
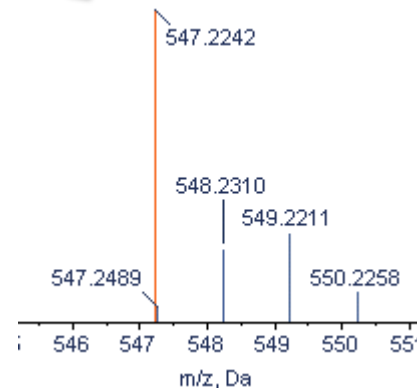
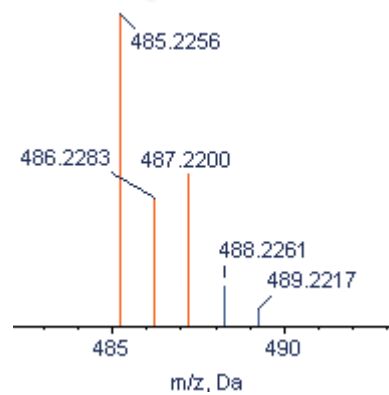
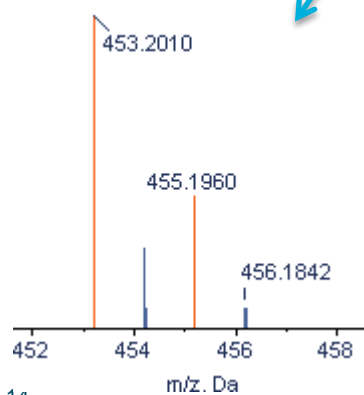
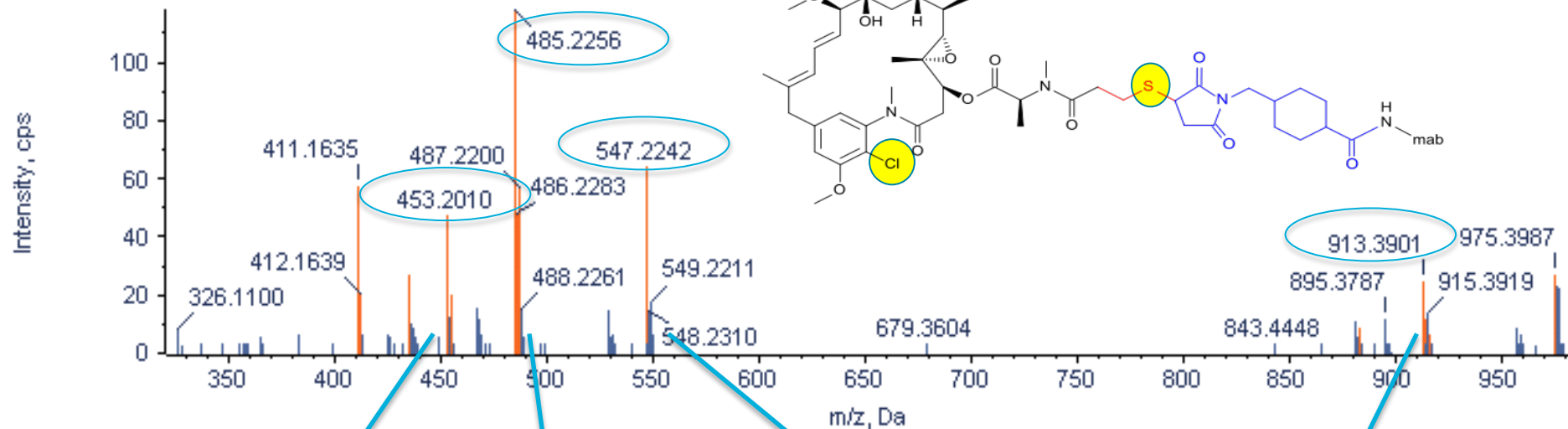
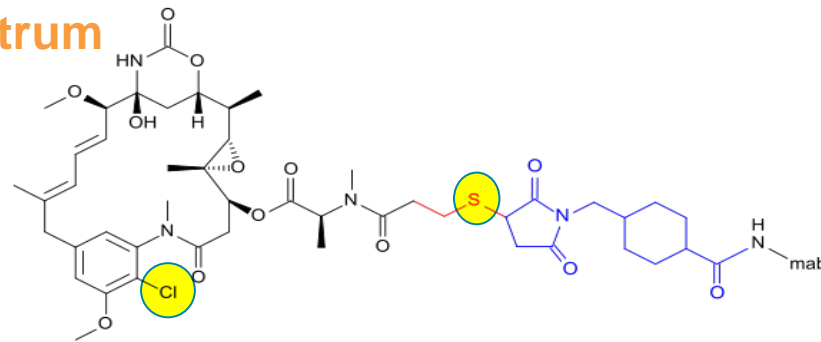
Isotopic Distribution for C₃₅H₄₈ClN₃O₁₀S H⁺



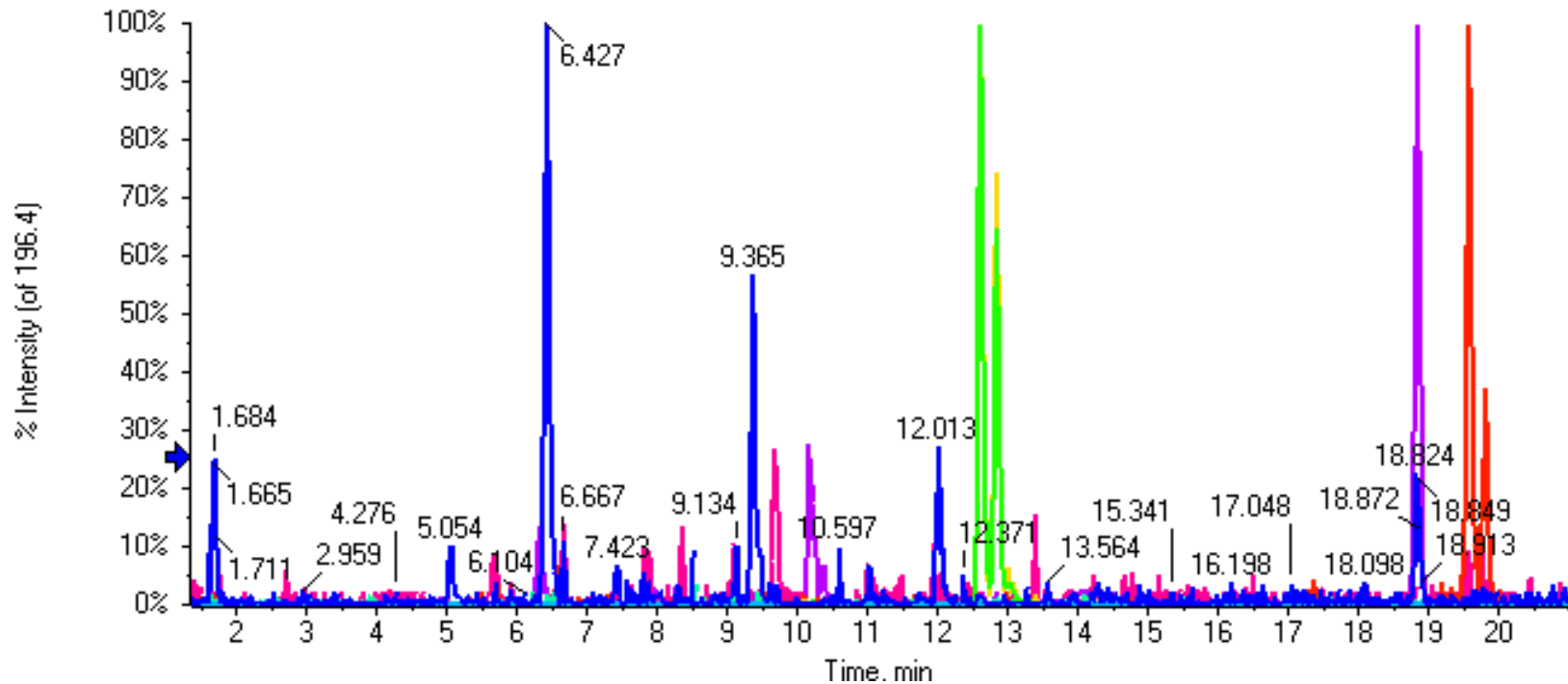
Data Processing Strategies

T-DM1- Common Product Ion

DM1+SMCC+NH2 Product Ion Spectrum



Extracted Ion Chromatogram (XIC) Common Product Ions

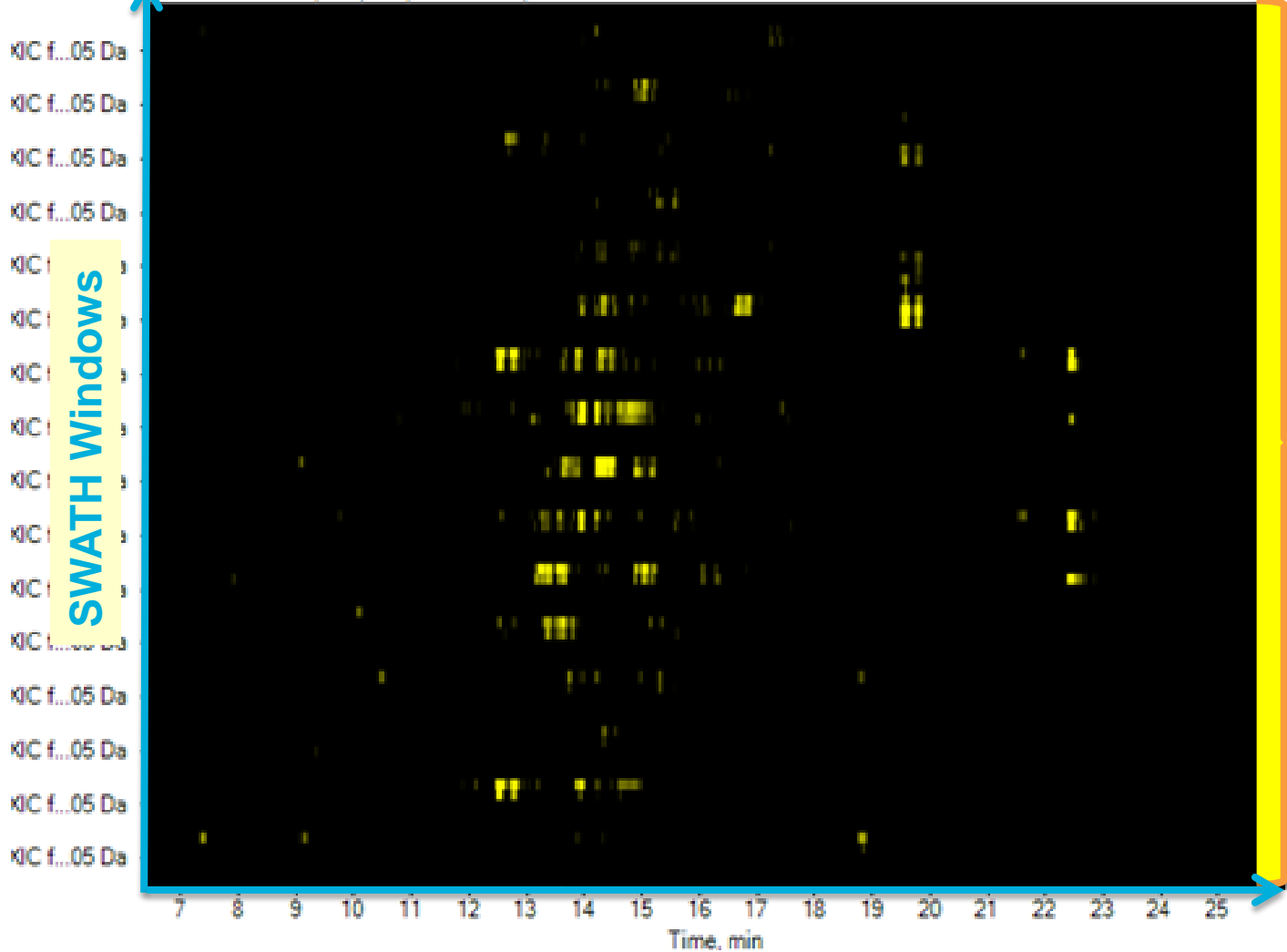


Using regular TOF MS approach, we see XIC associated with common product ions

All in the same XIC window leads to more complexity

SWATH Acquisition Heat Map Showing All Potential Species with Drug

DataSET-SWATH TDM D-1.wiff (sample 1) - Test sample-tdmd SWATH



SWATH Windows

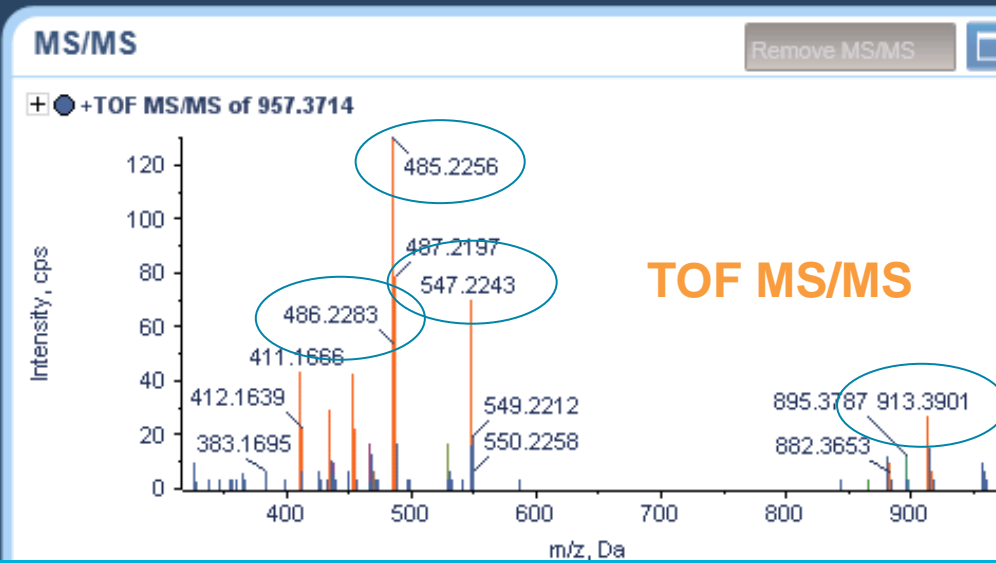
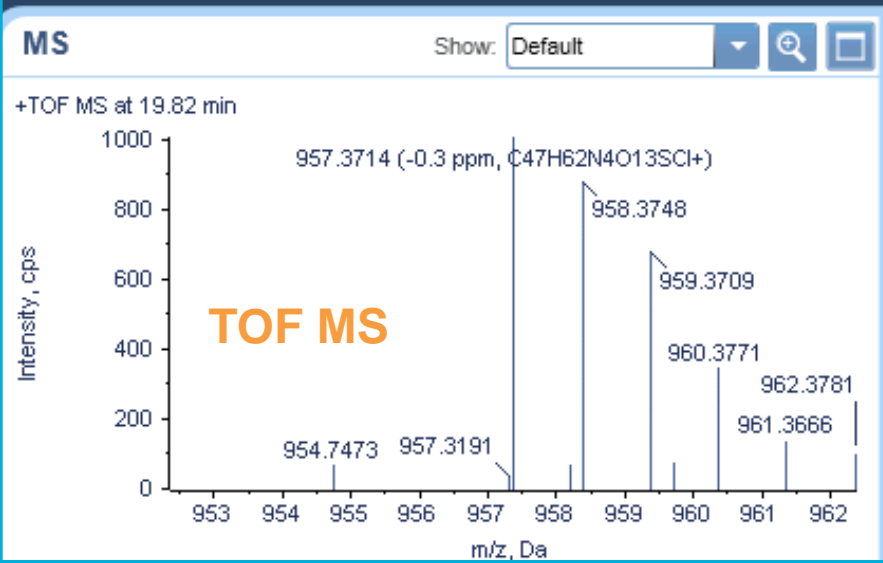
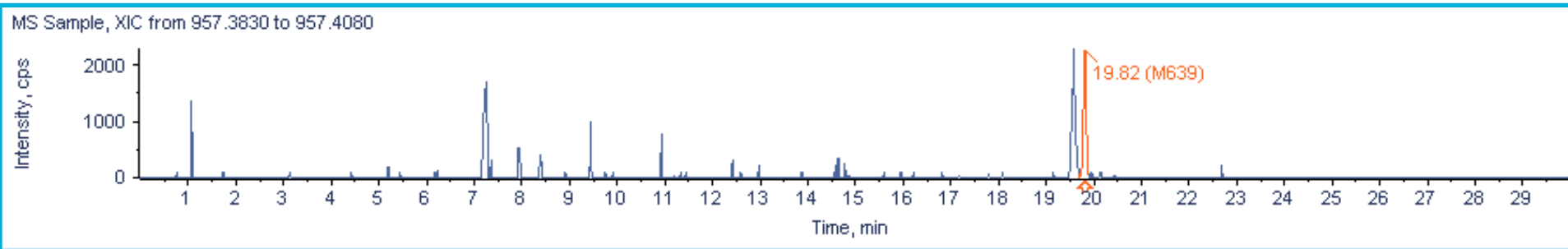
Time (min)

All potential species from the drug-linker

- Isotope Pattern
- Common PI

MetabolitePilot™ Software Showing DM1+SMCC

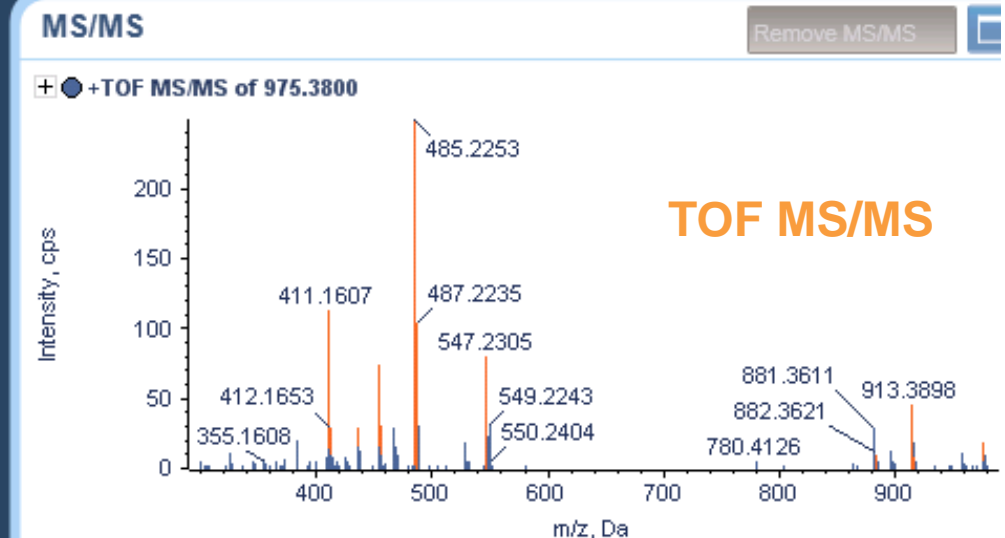
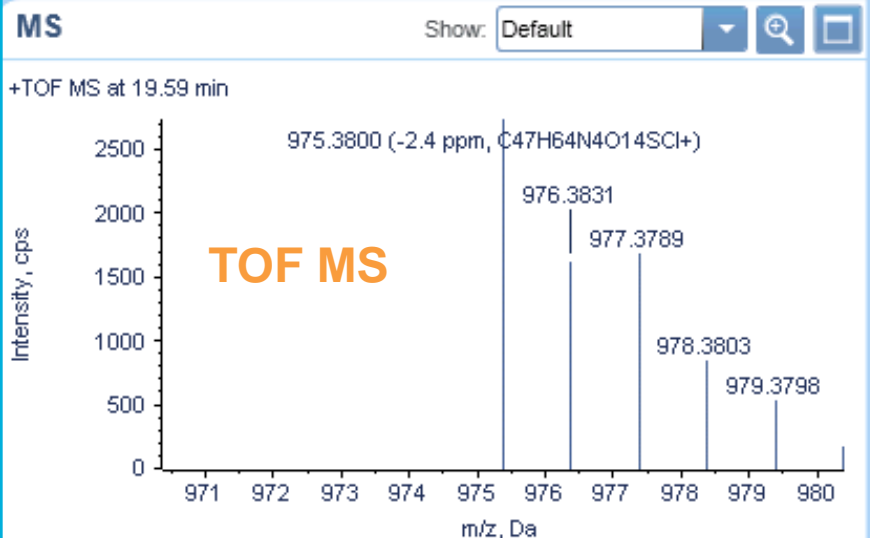
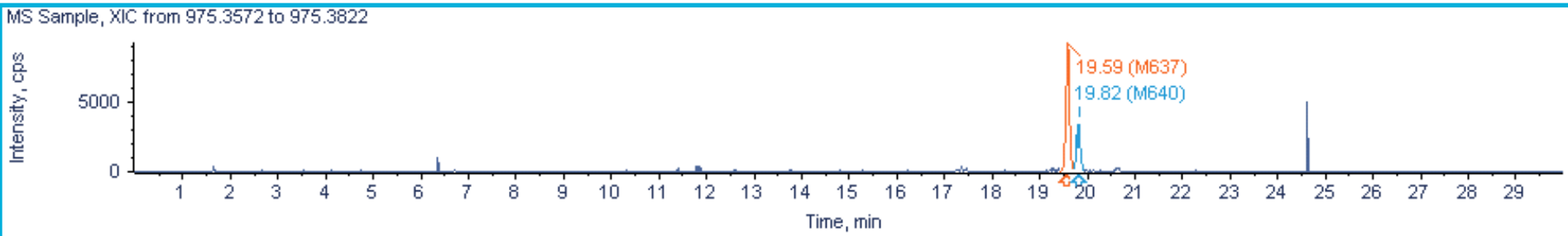
Extracted Ion Chromatogram of m/z 957.3714



TOF MS/MS showing fragment ions m/z 913, 547, 485, 453, 411

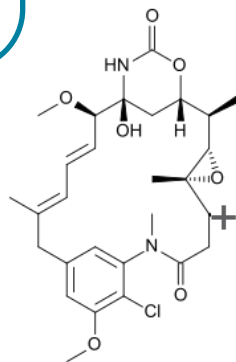
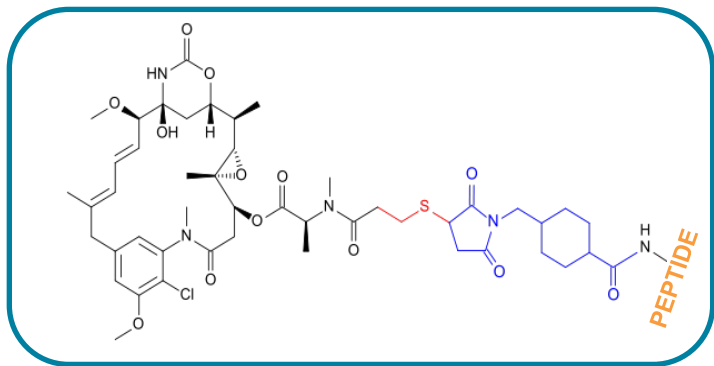
MetabolitePilot™ Software Showing DM1+SMCC+NH2

Extracted Ion Chromatogram of m/z 975.3800



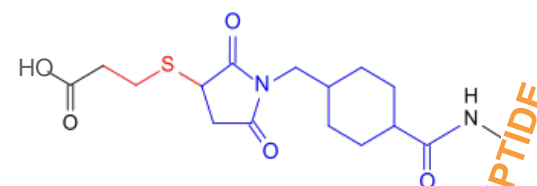
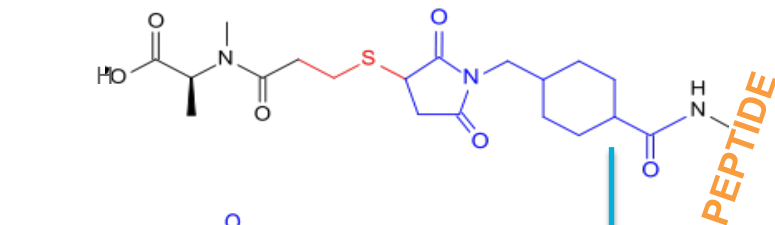
TOF MS/MS showing fragment ions m/z 913, 547, 485, 453, 411

DM1+SMCC+Peptide Spectrum

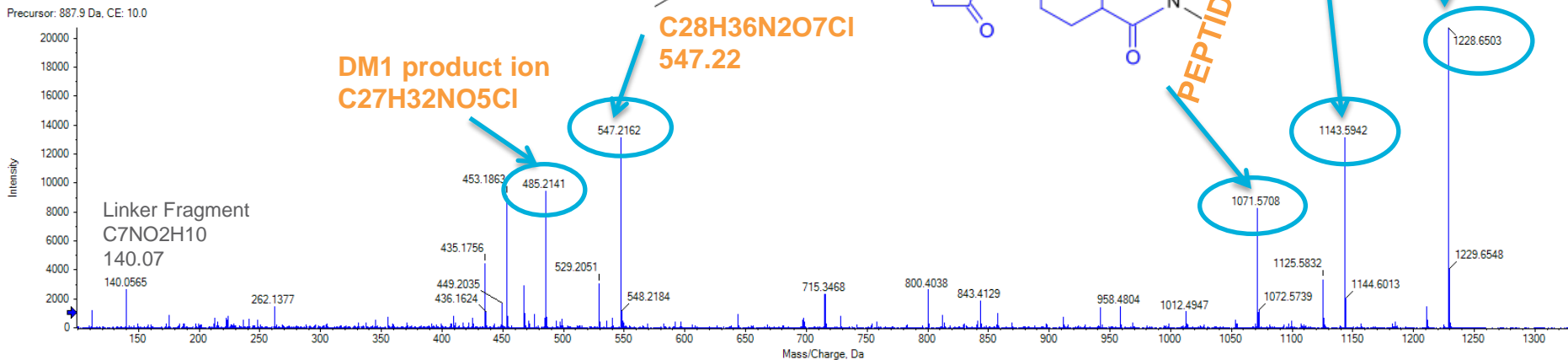
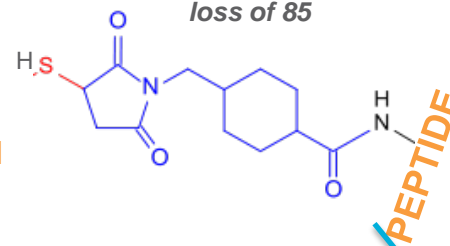


C₂₈H₃₆N₂O₇Cl
547.22

DM1 product ion
C₂₇H₃₂N₂O₅Cl

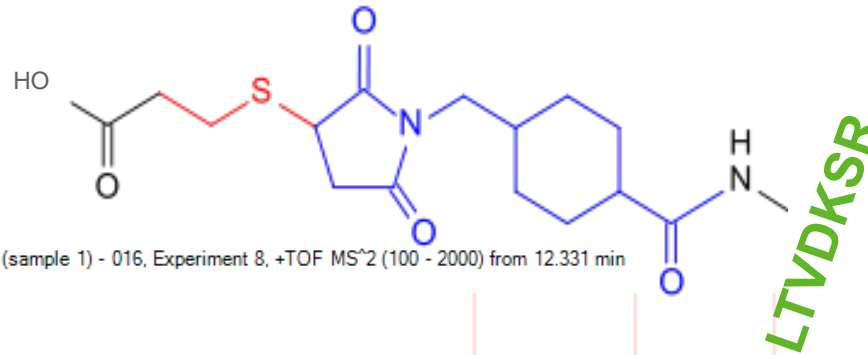


Speculative: cyclization then fragmentation gives observed loss of 85

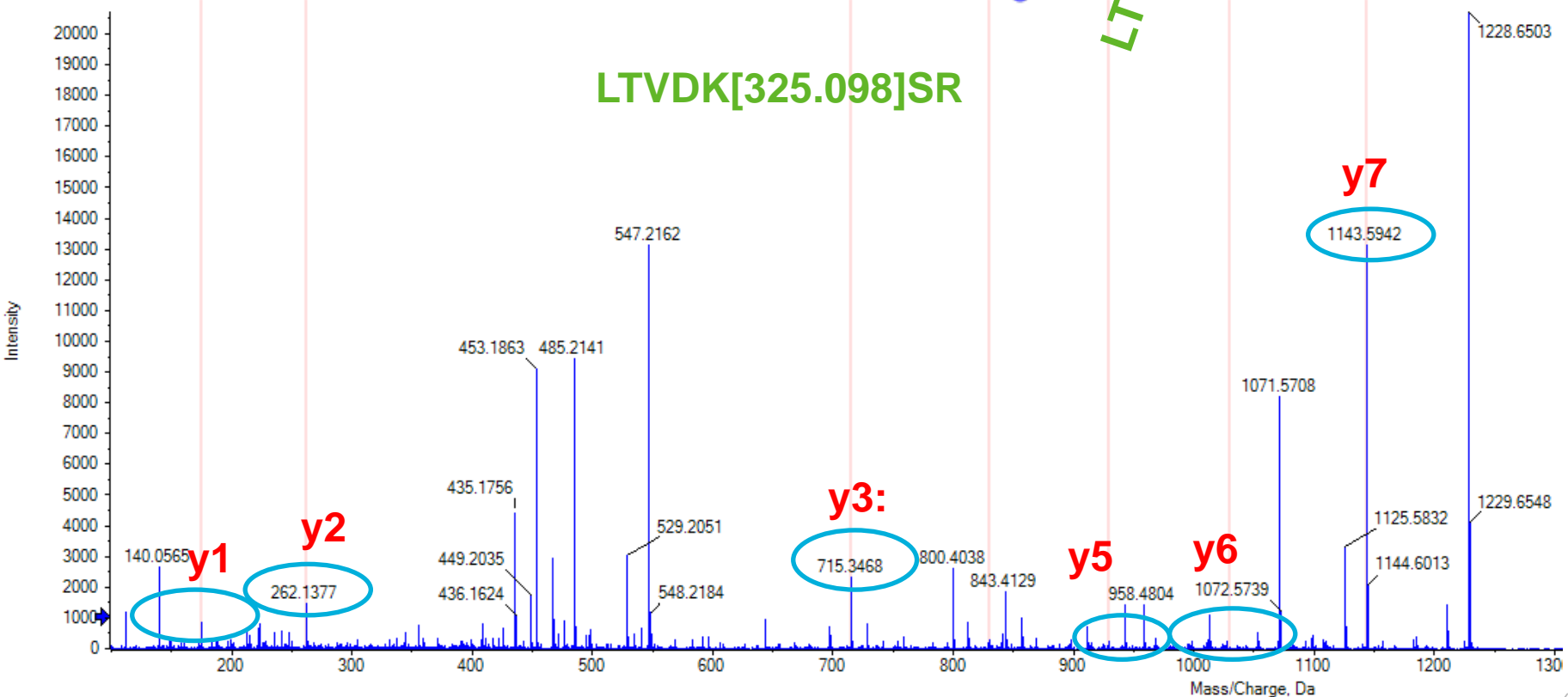


DM1+SMCC+Peptide Spectrum

y-ions with linker modification- Mod delta mass: 325.098

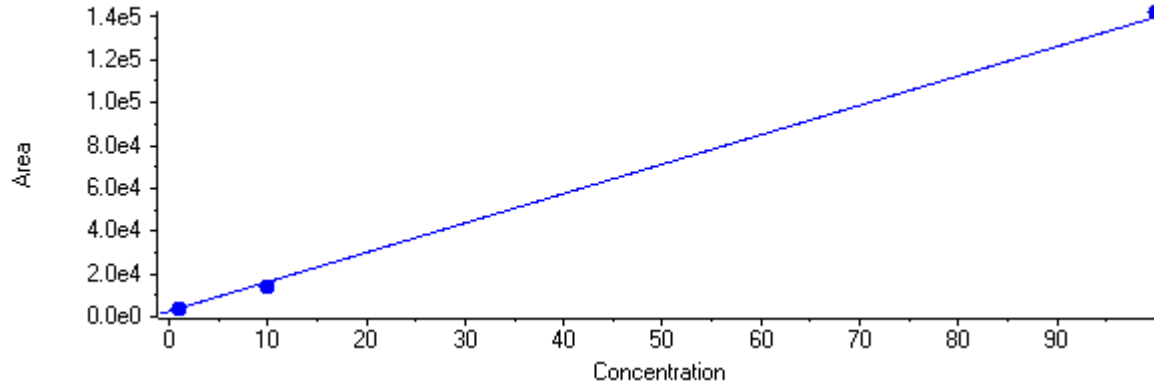


Spectrum from TDM1C8_LowDP_higher_gradient_end10.wiff (sample 1) - 016, Experiment 8, +TOF MS² (100 - 2000) from 12.331 min
Precursor: 887.9 Da, CE: 10.0



Calibration Curve- m/z 784-DM1+46

Calibration for 784: $y = 1373.99890 x + 2583.37620$ ($r = 0.99809$) (weighting: $1 / x$)

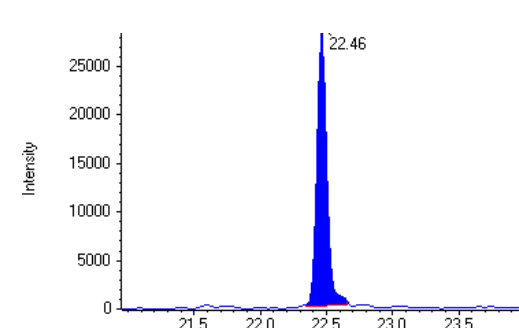
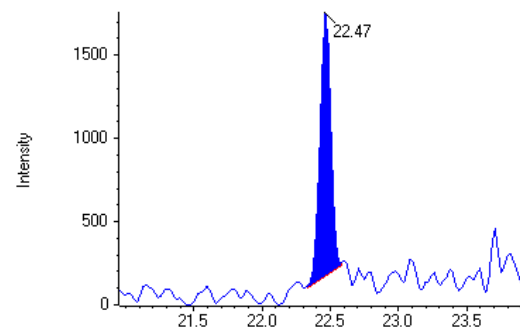
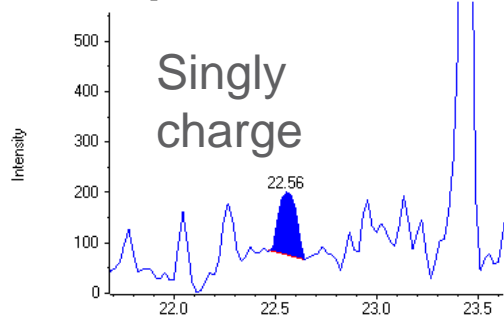


Con (nM)	% Accuracy
1	101.17
10	87.14
100	111.69

STD1

STD 10

STD 100



SWATH™ Acquisition to MRM Workflow

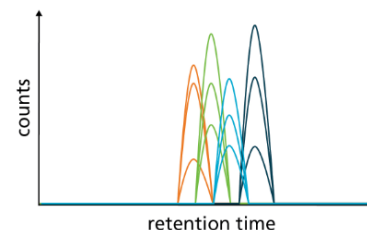
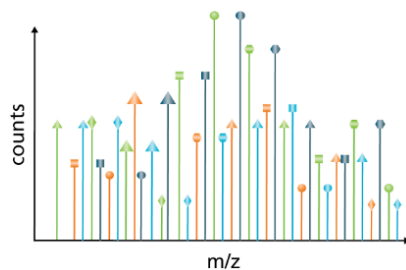
Discovery

Development

GLP
Bioanalysis

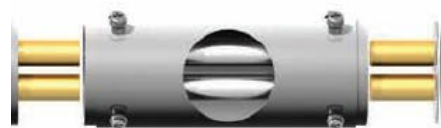
SWATH™
Acquisition

Multiple Reaction
Monitoring (MRM)



High Res XICs

Q2



Easy Method Transfer with LINAC® Collision Cell



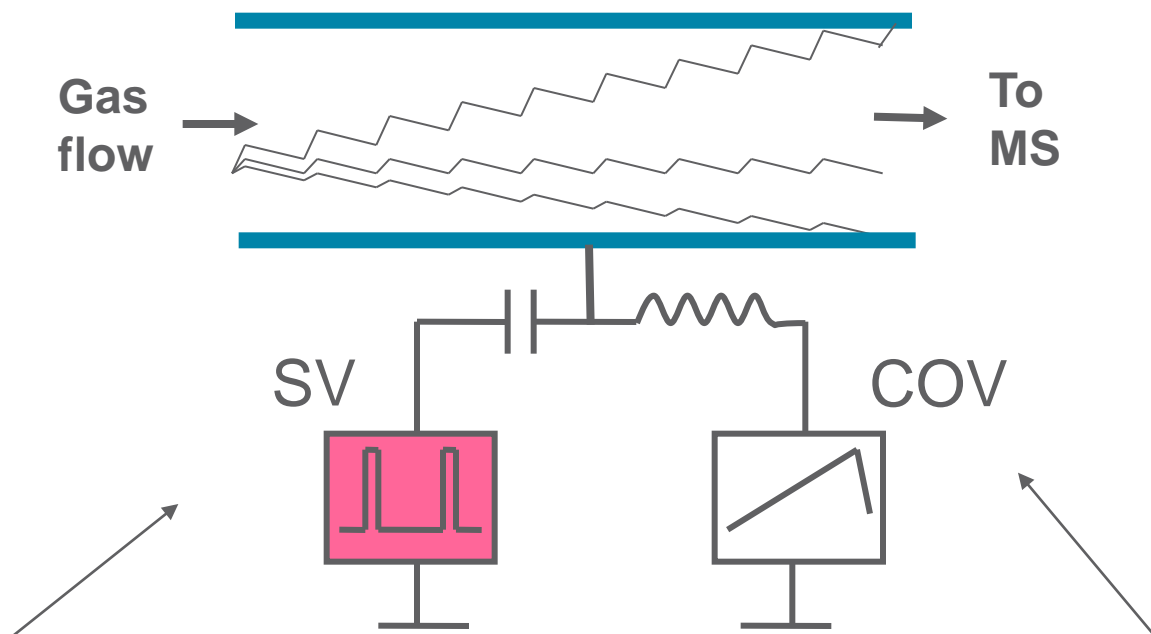
TripleTOF® System



QTRAP® System



Differential Mobility Spectrometry (DMS) is the term used for planar geometry



Separation waveform (SV):
Radially displaces ions towards one or the other electrode, depending upon high and low field mobility characteristics

Compensation voltage (COV):
Restores the trajectory for a given ion to allow them to transmit through the DMS device and enter the mass spectrometer

Benefits of SelexION for ADC Analysis

Benefits of SelexION

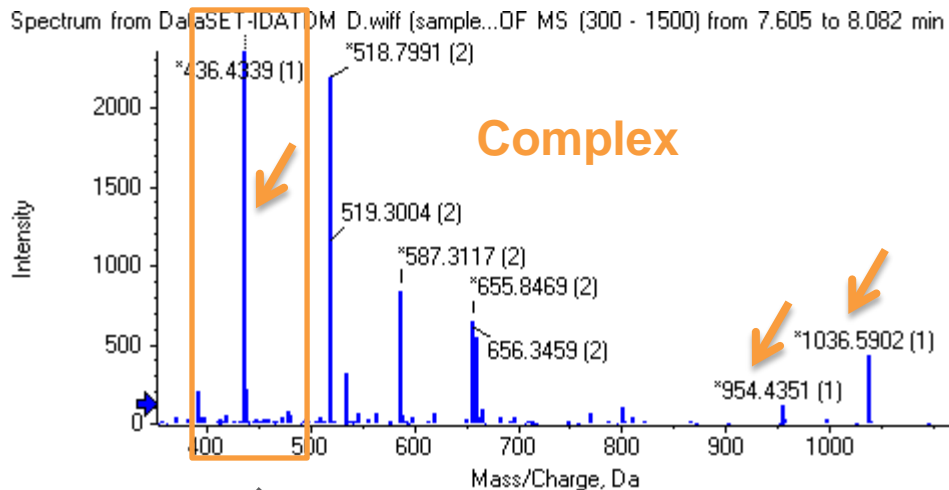
1. Enhanced specificity/selectivity by eliminating background interference
2. High quality MS/MS data for accurate identification
3. Simultaneous Quantification and Qualitative Analysis

Unique Features of SelexION

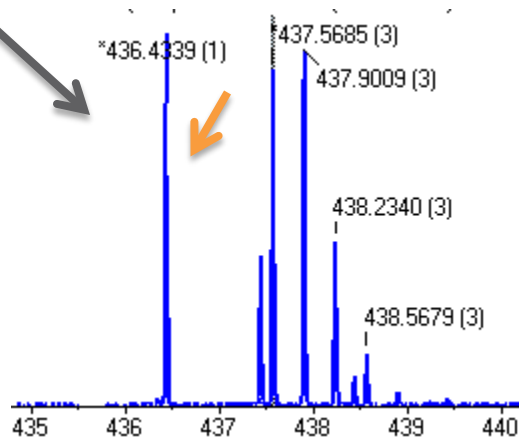
1. **SelexION is Quant driven** with the benefit of Qual
SelexION is in the ion source-enables precursor selection and fragmentation
2. **Chemical modifiers** gives the flexibility to try various options for better separation
3. **SelexION is compatible with both QQQ/QTRAP and TripleTOF**
4. **Easy switch between DMS ON and OFF modes without breaking vacuum**

Elimination of Singly Charge Interference with SelexION

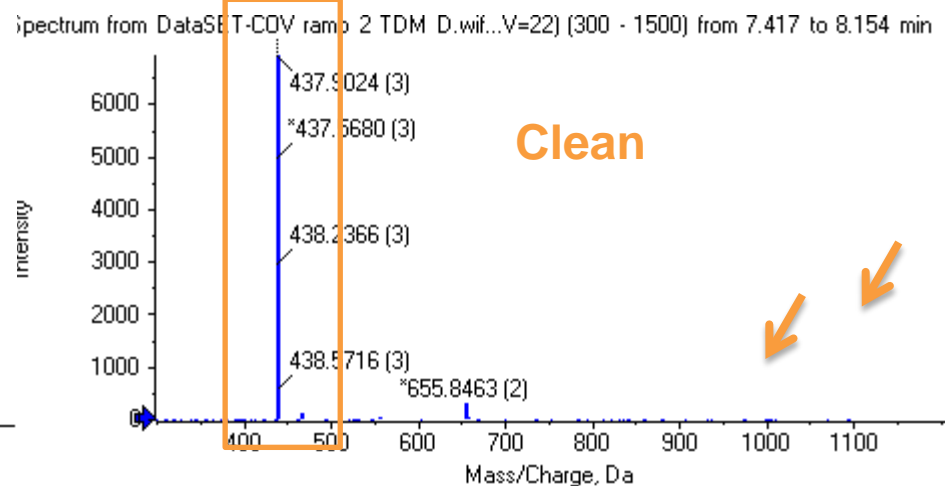
TOF MS Spectrum DMS OFF



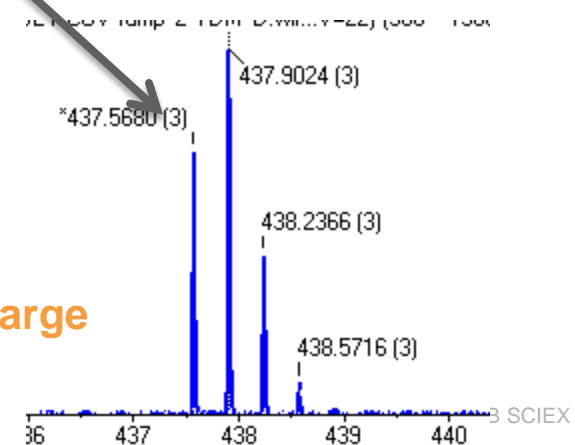
**ZOOM IN
DMS OFF**
Singly charge
interferences



TOF MS Spectrum DMS ON

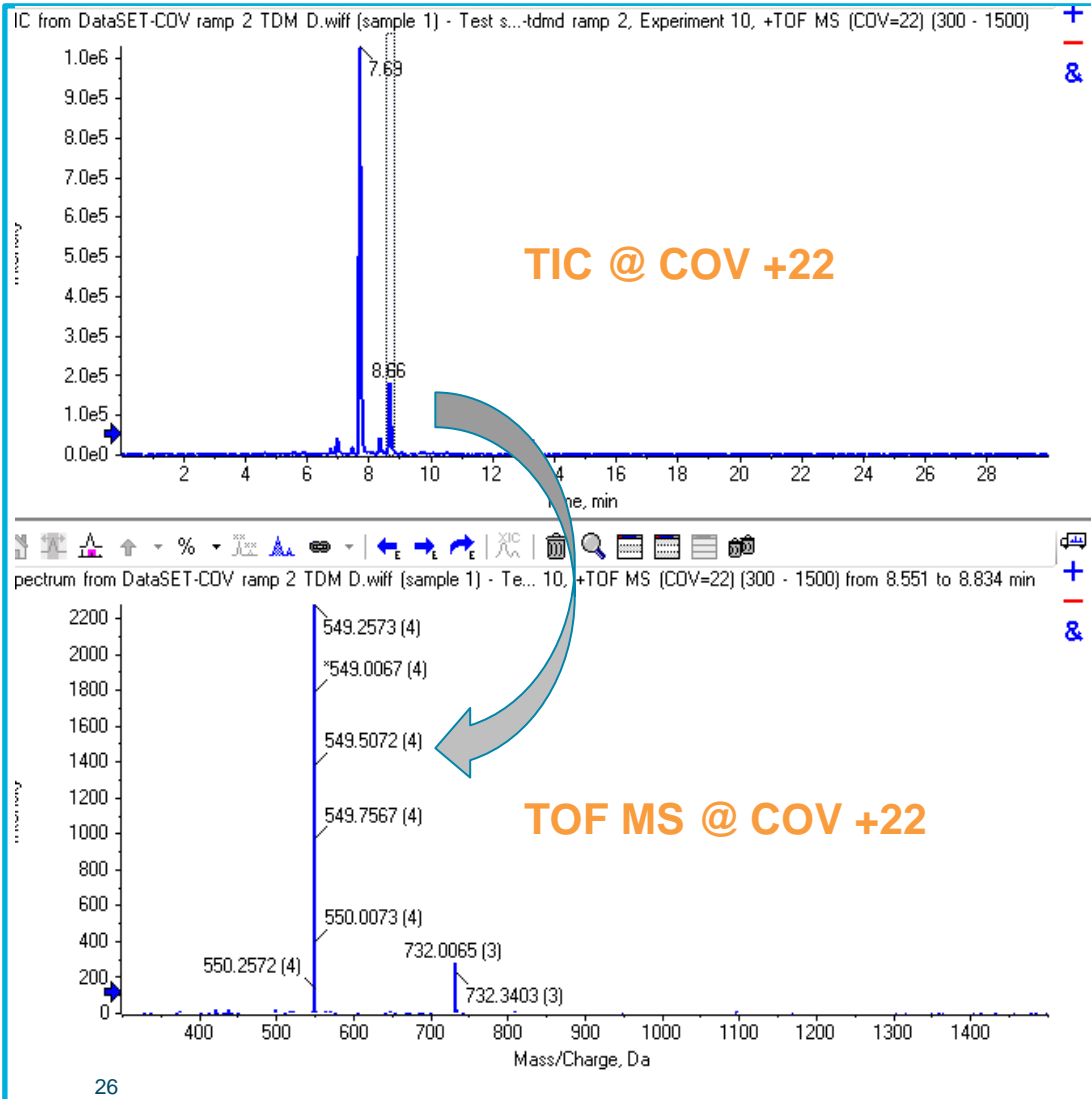


**ZOOM IN
DMS ON**
Clean spectrum
only multiply charge



Difference in Chromatographic Profile using SelexION

TIC of T-DM1 Digest at +22 COV value

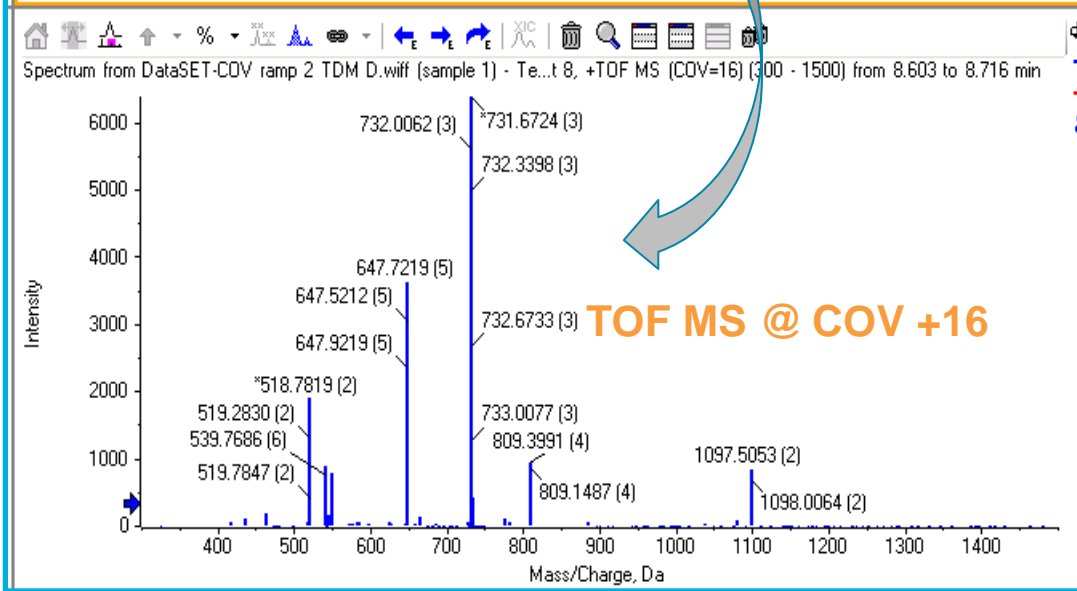
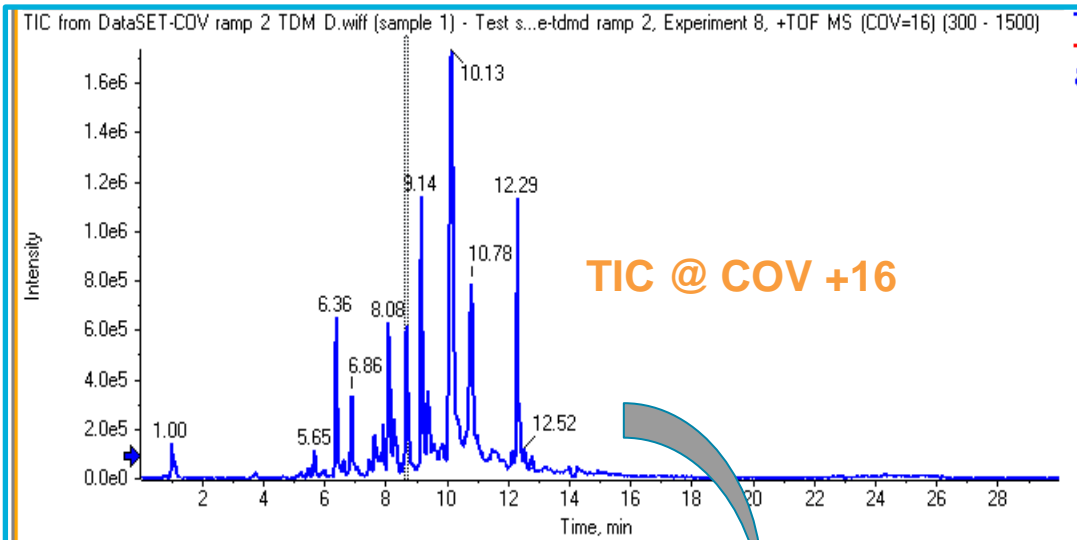


- **Additional Selectivity (Orthogonal separation)**
- **Less complex MS and MS/MS spectrum**
- **No singly charged interferences**

Difference in Chromatographic Profile using SelexION

TIC of T-DM1 Digest at +16 COV value

- **Additional Selectivity (Orthogonal separation)**
- **Less complex MS and MS/MS spectrum**
- **No singly charged interferences**



Conclusion

- HRMS SWATH™ Acquisition can be complementary to LBA Assay
- SWATH and SelexION provide options for greater selectivity
 - SWATH acquisition allows 100% MS/MS coverage for qualitative analysis
 - SWATH has the benefit of quantitative analysis
 - Quant and Qual all in one method
 - SelexION technology provides selectivity orthogonal to MS
 - Can greatly aid in cleaning up interferences (singly charged) and background
- Common product ion & isotope pattern assisted with initial data processing
- Looks promising to move all three assays (total, conjugated & SM) on to LC/MS/MS platform

Will be interesting to analyse real in-vivo samples to highlight full benefits of HRMS.....

Acknowledgements

AB SCIEX Team

- Justin Blethrow
- Steve Tate
- Yves Le Blanc
- Eva Duchoslav
- Eric Johansen
- Gary Impey
- Joe Fox

ADC Biotransformation-AB SCIEX Webinar



Keyang Xu
Senior Scientist
Genentech, USA

Nov 20th 2013- 11.00 AM EST/4.00PM BST

"Biotransformation of Antibody-Drug Conjugates: Current Perspective and Analytical Approaches "

In this webinar, you will learn:

- The unique challenges involved in analysis of biotransformation's of antibody-drug conjugates
- Analytical strategies and rationale employed in real-world case studies
- Characterization studies outcomes that can guide the development of effective quantitative bioanalytical strategies