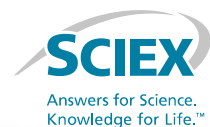


For Research Use Only. Not for use in diagnostic procedures  
RUO-



It's Time to  
See the Future  
Differently

**The Simpler and Faster Solution for Routine  
Biopharmaceutical Characterisation**

Alexandre Paccou, Sr Support Manager, EMEA



## Overview

- Defining the goals for ADC characterisation
- Routine Workflow Overview
  - Intact Mass (ADC-DAR)
  - Subunit
  - Peptide Map
- Hardware and software that enable these workflows
- Summary



Answers for Science.  
Knowledge for Life.™

© 2017 AB Sciex

## ADC Complexity – analysed routinely

- Assess and ensure the product
  - Quality in and after process of conjugation
  - Stability *in vitro* and *in vivo*
  - Conjugation sites – site occupancy and payload
  - Linker chemistry – stability

### Need to know your mAb – for every batch

- Three Main Workflows for ADC Characterisation
  - Intact protein fingerprint of the whole product
  - Subunit analysis – insight on localisation – simpler data
  - Peptide mapping – drilling deeper on the site occupancy
- Easy to use – get the results – make the right conclusions
  - Inviting you to analyse – you can handle this



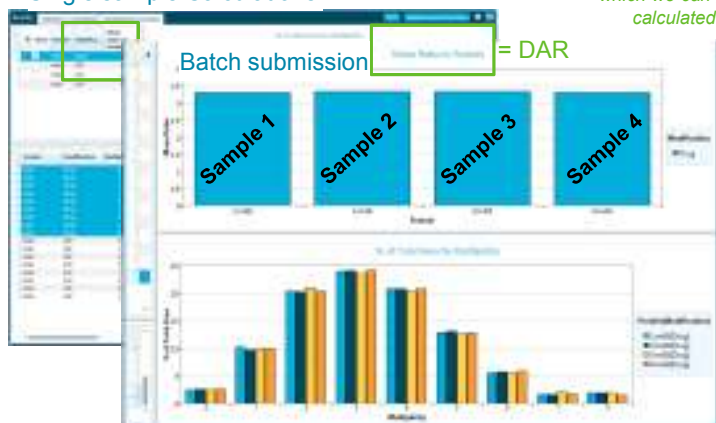
The need for information – a lot of it!

*Complexity is brought upon the antibody heterogeneity (glycosylation etc.) and the **multiple** possible sites of conjugation*

## ADCs – DAR demystified

- Drug-to-Antibody Ratio
- Compound Complexity is not an issue

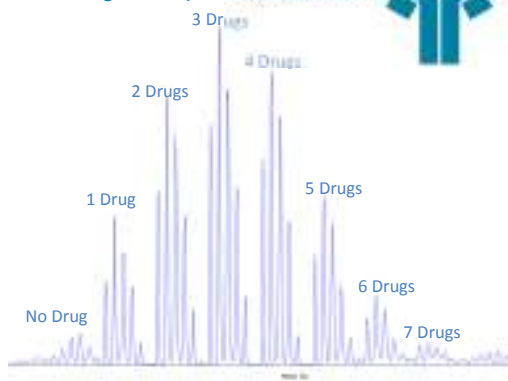
Single sample Calculations



*Sample here is non-deglycosylated Lys-linked Antibody-Drug-conjugate, from which we can get the DAR automatically calculated - for multiple samples*



Single sample reconstruction



## ADC characterisation by Mass Spectrometry made simple

SCIEX X500B QTOF System powered by SCIEX OS Software



SCIEX X500B QTOF system

SCIEX ExionLC™ AC system

SCIEX OS software



Answers for Science.  
Knowledge for Life.™

© 2017 AB Sciex

## SCIEIX X500B QTOF System- Inviting you to analyse

The Beauty – in appearance and in the data quality



**Engineered for simplicity**  
Optimal performance delivered through adjustment of only 2 voltage variables

**TwinSpray**  
An independent calibrant delivery path for reliable auto-calibration

**Service accessibility**  
Easy QJet® ion guide access for fast and efficient maintenance and single three stage split flow pump for increased system uptime

**Minimized footprint**  
The benchtop stature (110 x 57 x 112 cm)\* occupies less lab space than any other HRMS system on the market

**Integrated calibration**  
Maintains mass calibration through long runs without effect on sample flow

## SCIEX OS - inviting you to analyse



## SCIEX OS - Users



**Basic - For Routine User**

Start-up page provides easy access to features based on user roles



**Advanced - For Method Developer / Lab Manager**





## BioPharmaView™ 2.0 Software for characterisation



Comparability

### Inviting you to process

- Fast – intact mass deconvolution and peptide mapping in minutes
- Designed for processing and reviewing **batches**
- Automated calculations for **modification ratios**
- Hundreds of built-in modifications, easy to add **custom modifications**
- Complete support for IDA and SWATH® data
- Key routine workflows supported:



### Intact Protein

- Subunits



### Peptide Map

- Reduced
- Non-reduced
- Disulfide bond mapping



### ADC DAR



Answers for Science.  
Knowledge for Life.™

© 2017 AB Sciex

## Easily Define Your Experiment – with ease


The screenshot displays the SCIEX Trastuzumab 2017 software interface. The interface is divided into several sections:

- Left Panel:** A vertical navigation menu with options such as "Method Editor", "Data Review", "Data Acquisition", "Data Processing", "Data Analysis", "Data Reporting", "System", and "Help".
- Top Panel:** A header area with the SCIEX logo and the text "Trastuzumab 2017".
- Main Content Area:** A configuration form with various input fields and buttons. A green box highlights a "Data Review" button in the top right of this section.
- Table:** A data table at the bottom of the main content area, containing columns for "Sample Type", "Sample", "Injection", "Retention Time", "Peak Area", "Peak Height", "Peak Width", "Peak Position", "Peak Shape", "Peak Quality", "Peak Label", "Peak Color", "Peak Size", "Peak Position", "Peak Shape", "Peak Quality", "Peak Label", "Peak Color", "Peak Size".


## Easily Define Your Experiment for your ADC

The screenshot shows the 'Custom Modifications' window in the SCIEX Trastuzumab 2017 software. The window is divided into two main sections: 'Customized Standard Modifications' and 'User-Defined Modifications'. Both sections contain tables with columns for Name, Symbol, Mass Shift, Applied %, Formula, Neutral Loss, and Inhibitor. The 'Inhibitors' row in the 'User-Defined Modifications' table is highlighted with a green box. The 'SCIEX' logo and 'Trastuzumab 2017' title are visible at the top of the window.

Name	Symbol	Mass Shift	Applied %	Formula	Neutral Loss	Inhibitor
Deuterated	D4	38.040	100			
13C	13C	1.00335	100			
15N	15N	2.00635	100			
18O	18O	2.00426	100			
13C	13C	1.00335	100			
15N	15N	2.00635	100			
18O	18O	2.00426	100			
Inhibitors						



## Intact Mass and Subunit Analysis

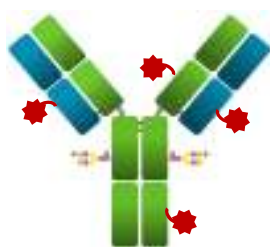


**SCIEX** Answers for Science.  
Knowledge for Life.™

© 2016 AB Sciex

## Intact Protein Analysis via MS

### Main Workflows for Routine Characterisation



#### WHY?

- Determination of intact mass (observing truncations, and glycosylation)
  - *is everything alright before conjugation?*
- Determination of drug-antibody-ratio (DAR) for an ADC

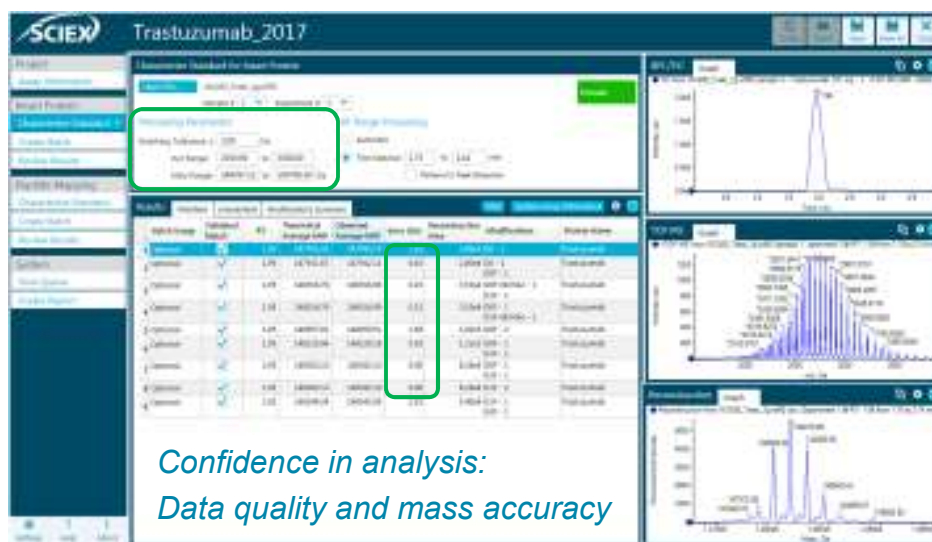


Answers for Science.  
Knowledge for Life.™

© 2017 AB Sciex

## Intact Protein Analysis – Characterise One...

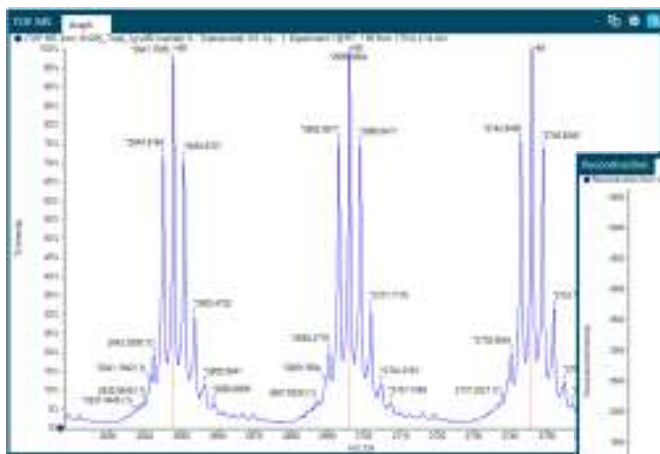
The example: an unconjugated, glycosylated mAb



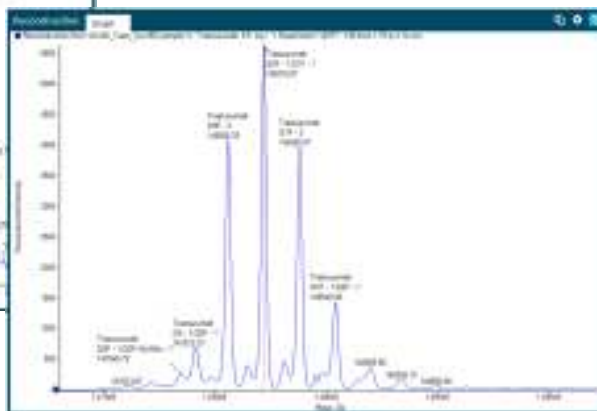
Confidence in analysis:  
Data quality and mass accuracy

## Intact Protein Analysis – Characterize One...

The example: an unconjugated, glycosylated mAb

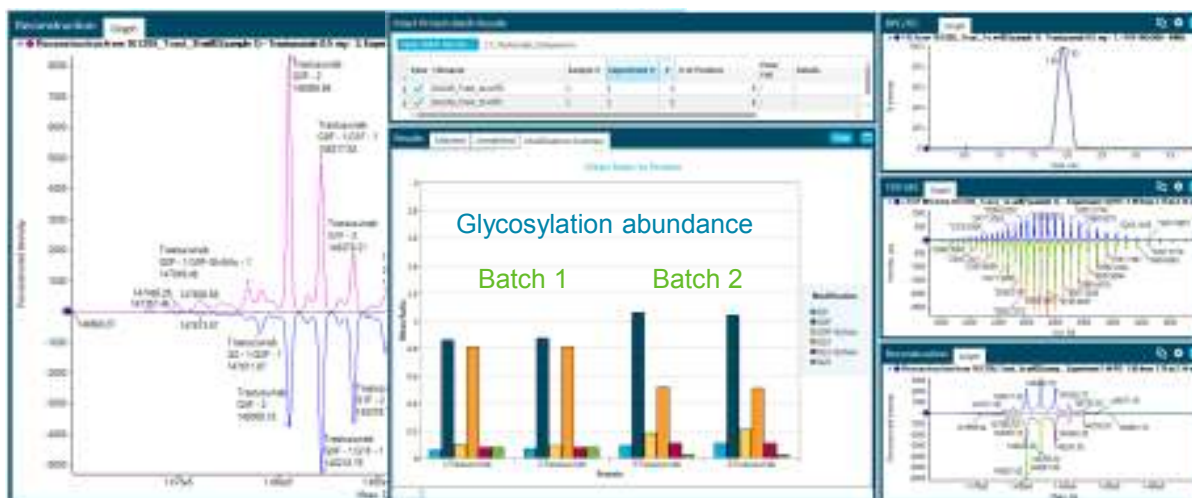


Confidence in analysis:  
Data quality and mass accuracy



# Intact Protein Analysis ... Batch Analyse Many

Information - Comparability - Calculations



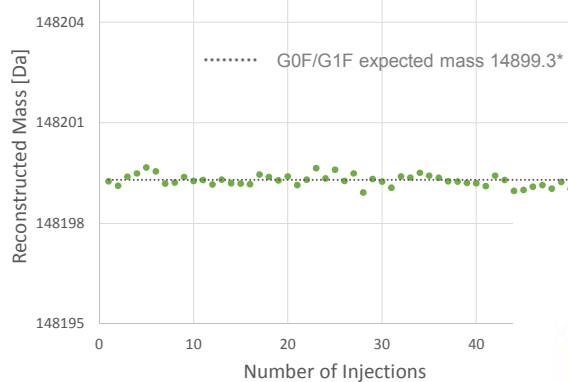
The example: two batches of an unconjugated, glycosylated mAb in duplicate injection

© 2017 AB Sciex

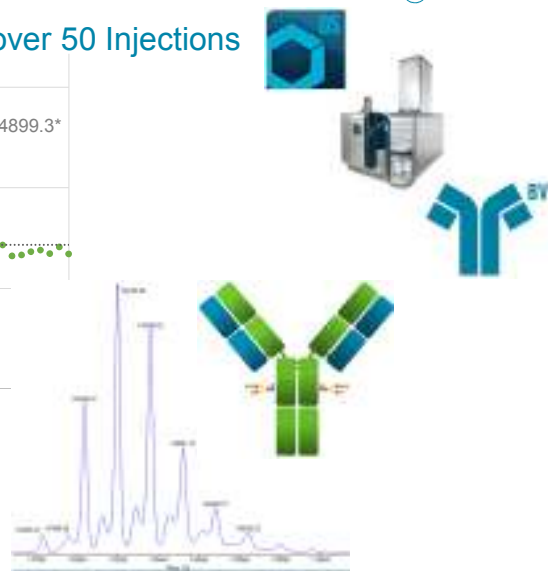


## Excellent Mass Accuracy for Highly Reproducible Results

Reconstructed Masses of Glycoform G0F/G1F over 50 Injections



\*NIST Monoclonal Antibody Reference Material 8671

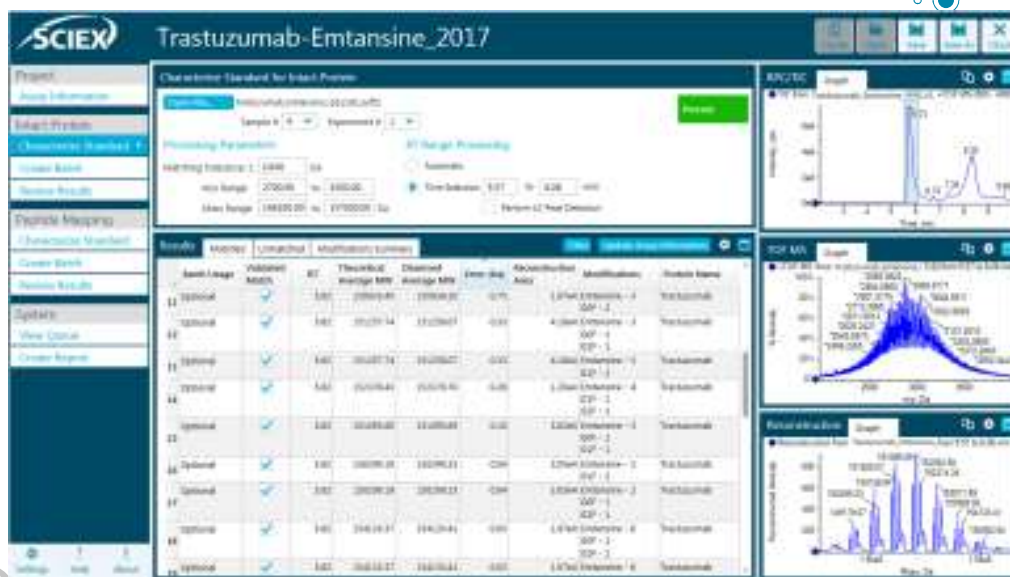


Answers for Science.  
Knowledge for Life.™

© 2017 AB Sciex

## Ado-trastuzumab emtansine (glycosylation on)

Example here a Lysine-linked non-deglycosylated ADC



## Ado-trastuzumab emtansine (glycosylation on)

Example here a Lysine-linked non-deglycosylated ADC



## Ado-trastuzumab emtansine – Batch processing

Example here multiple injections of a  
Lysine-linked non-deglycosylated ADC



Answers for Science.  
Knowledge for Life.™

© 2017 AB Sciex

## Ado-trastuzumab emtansine – Batch processing

Example here multiple injections of a  
Lysine-linked non-deglycosylated ADC



## Subunit Analysis via MS

### Main Workflows for Routine Characterisation

- A) Enzymatic (IdeS/ FabRICATOR) digestion into large junks  
 - Optional followed by: Denaturation and Reduction



Figures here: Lys-linked ADC

- B) Denaturation & Reduction into heavy and light chains




#### WHY sub-unit analysis?

- Deeper insight into modifications (inc. Location)
- In the case Cys-Linked: RP can be used, as the non-covalently bound complex no-longer needs to be held together (better sensitivities)


## Subunit Analysis – mAb Heavy Chain

The example: two batches of an unconjugated, glycosylated mAb in duplicate injection





**Peptide Mapping**



© 2016 AB Sciex

The image shows a laboratory setup for peptide mapping. On the left is a multi-well sample tray. In the center is a liquid handling robot with a white top and blue base. On the right is a computer monitor displaying a software interface with several data panels. The entire scene is framed by a black border with decorative blue lines and dots.



## Peptide Mapping via MS

### Main Workflows for Routine Characterisation

- Denaturation and optional Reduction & Alkylation
- Enzymatic digestion into peptides



#### WHY?

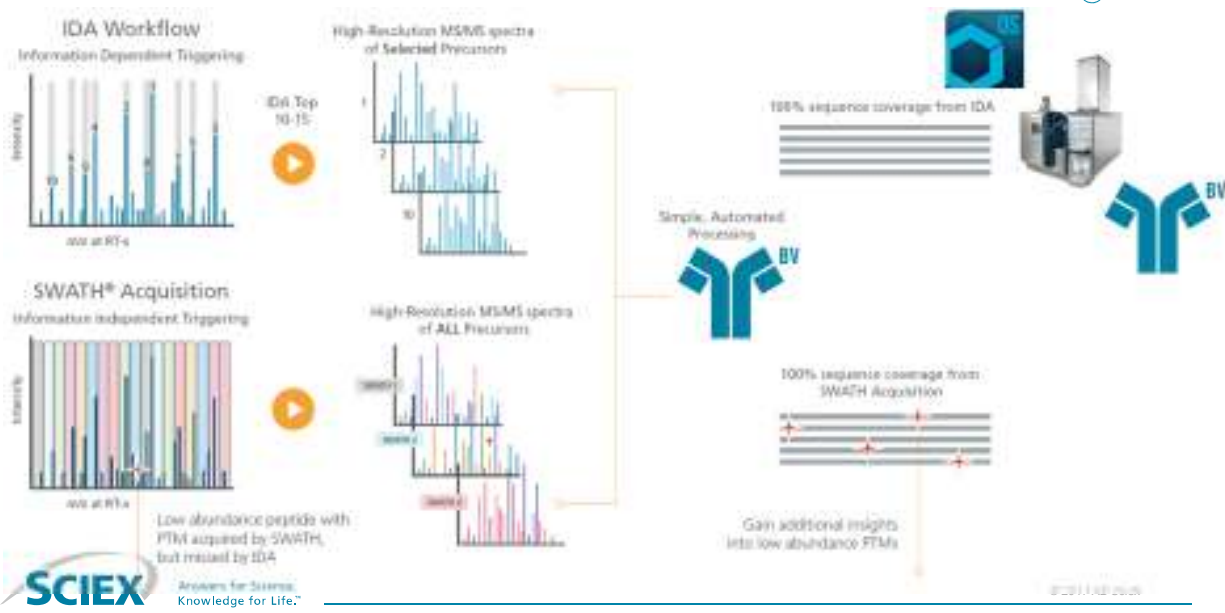
- Determination of modifications: conjugated drug location and quality attributes (QA) which have an impact on safety on efficacy
- Determination of remaining disulfide bonds for Cys –linked ADCs



Answers for Science.  
Knowledge for Life.™

© 2017 AB Sciex

## Peptide Mapping Acquisition Modes



# Peptide maps in a flash

The example: an unconjugated, glycosylated mAb



## SWATH Acquisition and Processing for Ultimate Confidence



The example: an unconjugated, glycosylated mAb

© 2017 AB Sciex

# Modification ratios

phosphorylated mAb

**Characterize Standard for Peptide Mapping**

Processing Parameters:  Range Processing,  Maximize,  Theoretical

**Peptide Details**

Validated Match	RT	Observed Mono isotope	Charge	XIC Area	Sequence	Modifications	Modification Percent	Use for Quant	Use for ID	Notes
1	7.40	851.4290	1	6.7185e4	QTLMRIS	Oxidation@4(256)	2.2% ±1.1 (Oxidation@4(256) - None@4(256))	Use	Use	
2	7.48	426.2202	2	4.7651e7	QTLMRIS	Oxidation@4(256)	2.2% ±1.1 (Oxidation@4(256) - None@4(256))	Use	Use	validated match
3	9.75	426.2222	2	1.3925e7	QTLMRIS		97.8% ±1.1 (None@4(256) - None@4(256))	Use	Use	validated match
4	9.75	852.4355	1	5.8521e4	QTLMRIS		97.8% ±1.1 (None@4(256) - None@4(256))	Use	Use	

**MS/MS**

Scan	RT	Observed	Charge	XIC Area	Sequence	Modifications	Modification Percent	Use for Quant	Use for ID	Notes
46	7.46	851.4294	1	6.7185e4	QTLMRIS	Oxidation@4(256)	2.2% ±1.1 (Oxidation@4(256) - None@4(256))	Use	Use	
48	7.48	426.2219	2	4.7651e7	QTLMRIS	Oxidation@4(256)	2.2% ±1.1 (Oxidation@4(256) - None@4(256))	Use	Use	
51	9.75	426.2222	2	1.3925e7	QTLMRIS		97.8% ±1.1 (None@4(256) - None@4(256))	Use	Use	
52	9.75	852.4355	1	5.8521e4	QTLMRIS		97.8% ±1.1 (None@4(256) - None@4(256))	Use	Use	

**MS/MS** (Left): Spectrum showing peaks at 426.2219 and 852.4355.

**MS/MS** (Right): Spectrum showing peaks at 426.2222 and 852.4355.

The example: an



Answers for Science. Knowledge for Life.™

© 2017 AB Sciex

## Modification ratios

glycosylated mAb



Yielded Mass	#1	Observed Mass (ppm)	Charge	KE Area	Sequence	Modifications	Modifications Percent	View for Details	View for ID	Notes
1	141	451.470	1	4.73E+07 (100%)	YKLNKQK	YKLNKQK	2.7% 41.1 (YKLNKQK) None (4250)	Use	Use	
2	140	495.130	1	4.76E+07 (100%)	YKLNKQK	YKLNKQK	2.7% 41.2 (YKLNKQK) None (4250)	Use	Use	glycosylated mAb
3	139	418.122	2	1.38E+07 (21.8%)	YKLNKQK	YKLNKQK	0.7% 41.1 (YKLNKQK) None (4250)	Use	Use	glycosylated mAb
4	138	384.122	1	1.42E+07 (23.8%)	YKLNKQK	YKLNKQK	0.7% 41.1 (YKLNKQK) None (4250)	Use	Use	

The example:



Answers for Science.  
Knowledge for Life.™

© 2017 AB Sciex

# Click. Compare. Report.

## Intact Protein Batch Report

Project	MS0004PCB
Batch Name	MS001_20161104_1
Method/Injection Applied Filter	
Downloaded Protein Applied Filter	

### Acqy Information Settings

FullScan MS/MS Scan Type	MS
Resolved Protein Form	MS

### Processing Settings

Maxing Tolerance	10.000%
Max MS1	2000.0
Max MS2	4000.0
Max Mass	40000.00 Da
Max Mass	20000.00 Da
MS1 Charge Processing	True (Default)
Max Time (min)	5.00
Max Time (min)	4.00
Perform a Peak Selection	MS

### Chromatographic Data Processing

Peak Threshold	1.00%
Collision Monitoring	2.00 points
Number of TIC/MS Spectra to Compare	1.0 column

### Summary

File Name	Sample #	Component #	#	# of Proteins	Pass/Fail	Details
MS001_2_20161104_1.ms	1	1	1	0	Fail	Resolution MS MS 10.0
MS001_2_20161104_1.ms	1	2	2	0	Fail	
MS001_2_20161104_1.ms	1	2	3	0	Fail	

### Protein Results

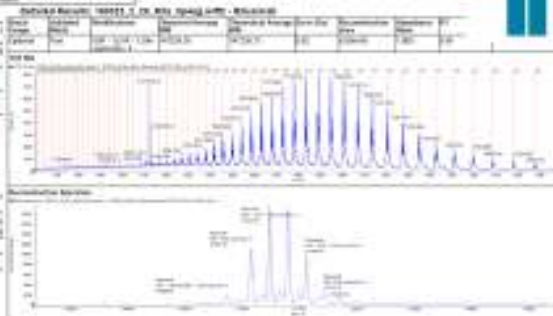
Resolution Report Threshold	4.0 0.0%
Maximum Allowed Modifications for MS Proteins	20

### Chromatography Peak Labeling

Labeling Method	1: 10 Minutes
Display Label For	All Peaks

### Batch Processing Pass/Fail Criteria

Criteria	Value
MS1 Tolerance	1.00%
MS2 Resolution Above Tolerance	40.00
MS2 Resolution Below Tolerance	0.00
MS2 Resolution Protein Tolerance	0.00



## ADC Complexity – analysed routinely

- Assess and ensure the product
  - Quality in and after process of conjugation
  - Stability *in vitro* and *in vivo*
  - Conjugation sites – site occupancy and payload
  - Linker chemistry – stability

Need to know your mAb – for every batch

- Three Main Workflows for ADC Characterisation
- Easy to use – *Inviting you to acquire and process*
- Fits to your lab – a Benchtop accurate mass instrument





## Unique Solutions to Meet Biologics Characterization Challenges



© 2017 AB Sciex

## X500 – Next Generation Mass Spectrometer



- Next generation platform Architecture. Most complex project since the API 300-(1995)
- 70% of HW R&D worked on the project, 60% of SW R&D on SCIEX OS
- Used predictive modeling and testing for enhanced reliability
- Significant investment in industrial design and usability testing with customers



© 2017 AB Sciex



# SCIEX OS

Single Software Platform for MS Control, Data Quantitation and Reporting



© 2017 AB Sciex