

## Intact antibody drug conjugate (ADC) analysis

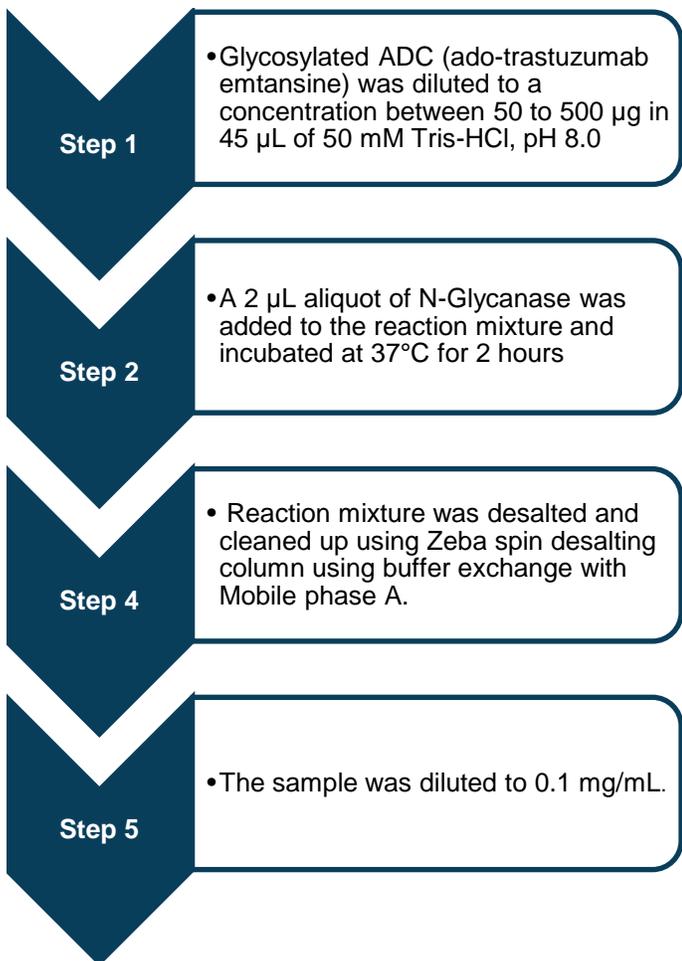
*Routine high-resolution accurate mass analysis of intact ADCs on the X500B QTOF System*

Method details for the routine characterization of an ADC biotherapeutic by high-resolution intact mass analysis using HPLC coupled with the X500B QTOF System, powered by the SCIEX OS Software.



### Sample Prep

A generic sample preparation strategy is shown for deglycosylation and general clean-up of an intact ADC biotherapeutic prior to LC-MS analysis.



### LC Method

<i>Column</i>	Waters Acquity UPLC BEH C4, 2.1mm x 50mm, 300A, 1.7 µm	
<i>Mobile Phase A</i>	Water, 0.1% Formic acid	
<i>Mobile Phase B</i>	Acetonitrile, 0.1% Formic acid	
<i>Flow rate</i>	200 µL/min	
<i>Column temperature</i>	80° C	
<i>Injection volume</i>	10 µL, 1 µg total protein	
<i>Gradient profile</i>	<b>Time (min)</b>	<b>% B</b>
	2.0	10
	6.0	90
	7.0	90
	7.1	10
	10	10

## MS Method

Suggested starting MS method parameters for routine intact ADC analysis as displayed in SCIEX OS. For best sensitivity and resolution, the declustering potential (DP) and collision energy (CE) parameters should be optimized for each individual biotherapeutic.

The screenshot shows the 'MS Method' configuration window for an 'Intact ADC method'. The interface includes a top navigation bar with 'New', 'Open...', 'Save', 'Print...', and 'Advanced' buttons. The main area is divided into several sections:

- Method Overview:** Shows 'Device: X500 QTOF' and 'Ion Source: TurboSpray'.
- TOF MS:** A sub-section for TOF MS parameters, currently set to 'TOF MS'.
- Source and Gas Parameters:**
  - Method duration: 10 min
  - Estimated cycles: 1137
  - Total scan time: 0.527524 sec
  - Intact protein mode: ON
  - Large proteins (>70 kDa):
  - Decrease detector voltage:
  - Ion source gas 1: 40 psi
  - Ion source gas 2: 40 psi
  - Curtain gas: 30 psi
  - CAD gas: 7 psi
  - Temperature: 400 °C
- Experiment:**
  - Polarity: Positive
  - Spray voltage: 5000 V
  - TOF start mass: 900 Da
  - Declustering potential: 250 V
  - TOF stop mass: 4500 Da
  - DP spread: 0 V
  - Accumulation time: 0.5 s
  - Collision energy: 20 V
  - CE spread: 0 V
- Advanced Experiment Settings:**
  - Time bins to sum: 120
  - Channel 1:
  - Channel 2:
  - Channel 3:
  - Channel 4:

The bottom status bar shows 'Data Acquisition' with 'MS' selected, and buttons for 'Start', 'Stop', and 'Save...'.

## Batch

In Batch setup, open the 'Automated Calibration Editor' window in order to select the use of the autocalibration function. Designate use of the 'X500 ESI Positive Calibration Solution', and then determine how often you would like the system to perform a fast, automated calibration. These short calibrations will be added automatically to your queue once you have submitted a sample batch.

The screenshot shows the 'Batch' software interface. At the top, there is a navigation bar with a home icon, a grid icon, and a 'Running' status indicator. Below the navigation bar is a toolbar with buttons for 'Auto-Calibrate...', 'Plate Layout...', 'New', 'Open', 'Save', 'Print...', 'Manage', and 'Submit'. The main area contains a table titled 'Untitled' with the following columns: Sample Name, MS Method, LC Method, Rack code, Vial position, and Data File. The table has 26 rows, with the first row containing 'Intact protein', 'intact protein analysis MS', 'Intact\_10min', '1.5mL (105 vial)', '1', and 'Intact protein file'. Overlaid on the table is a dialog box titled 'Batch - Automatic Calibration Editor'. The dialog box contains the following fields and options:

- Ion reference table: X500 ESI Positive Calibration Solu... (dropdown menu)
- Calibrate every: 3 samples (spin box)
- Calibrant delivery: CDS (dropdown menu)
- CDS channel: 1 (dropdown menu)

Buttons for 'Edit...', 'OK', and 'Cancel' are also visible in the dialog box.

This is a close-up view of the 'Batch - Automatic Calibration Editor' dialog box. It contains the following settings:

- Ion reference table: X500 ESI Positive Calibration Solu... (dropdown menu)
- Calibrate every: 3 samples (spin box)
- Calibrant delivery: CDS (dropdown menu)
- CDS channel: 1 (dropdown menu)

Buttons for 'Edit...', 'OK', and 'Cancel' are also visible.

# Pharma and Biopharma



## Data Processing

### Process intact biotherapeutic data in BioPharmaView™ Software 2.0.

Input the protein sequence, and assign potential modifications in the 'Assay Information' window. Click on the 'Add Modifications' tab to insert the drug conjugate mass information, as well as expected location. In this case the DM1/Emtansine drug conjugate and linker is conjugated to lysine residues within the protein. The DM1/Emtansine has an average mass of 957.37 Da.

The screenshot displays the BioPharmaView software interface. The 'Assay Information' window is active, showing the 'Sequence Features' tab. The protein is identified as 'Trastuzumab emtansine (ADC)'. The protein sequence is displayed in four chains (LC1, HC1, HC2, LC2). The 'Modifications' section shows a table of modifications and a detailed view of disulfide bonds.

Chains	Type	Name	Position	Maximum Mods per Chain	Modified AA	Applies To	Workflow Usage	Mass Shift
1	1-4	Internal Emtansine	*	2	n/a	K	Both	957.3723
2	2-3	Internal GGF	300	-	N	N	Both	1444.5339
3	2-3	Internal GIF	300	-	N	N	Both	1606.5867
4	2-3	Internal G2F	300	-	N	N	Both	1768.6395

Disulfide Bonds - (16)		From Chain	To Chain	From Cysteine	To Cysteine
1	1	1	1	23	88
2	1	1	1	134	194
3	4	4	4	23	88
4	4	4	4	134	194
5	1	2	2	214	223
6	4	3	3	214	223
7	2	2	2	22	96
8	2	2	2	147	203
9	3	3	3	22	96
10	3	3	3	147	203
11	3	2	2	229	229
12	3	2	2	232	232
13	3	3	3	264	324
14	3	3	3	370	428
15	2	2	2	264	324
16	2	2	2	370	428

# Pharma and Biopharma



Navigate to the 'Intact Protein' tab complete processing parameters and to generate the protein forms for matching.

**Processing Parameters**

Matching Tolerance: ± 15.00 Da  
 Start m/z: 2000.00  
 Stop m/z: 4500.00  
 Start Mass: 140167.12 Da  
 Stop Mass: 161374.75 Da

RT Range Processing: Time Selection  
 Perform LC Peak Detection  
 Start RT: 4.11 min  
 Stop RT: 5.24 min

Maximum Number of Combined Modifications per Protein: 20

**Batch Processing Parameters**

Retention Time Tolerance: ± 1.00 min

**Batch Processing Pass / Fail Criteria**

Reconstruction Area Limits: ± 10.0 %  
 Required Form Minimum: ≥ 80 %  
 Restricted Form Maximum: ≤ 120 %

**Characterized Proteins**  Reduced Protein Form

Batch Usage	Protein Name	Modifications	User Defined	Mono. Mass	Avg. Mass	Match...	Reconstruction Area	Retention Time
1 Optional	Deglycosylated			145075.6702	145167.12			
2 Optional	Deglycosylated	Emtansine - 1		146033.0425	146125.67			
3 Optional	Deglycosylated	G0F - 1		146520.2041	146612.47			
4 Optional	Deglycosylated	G1F - 1		146682.2569	146774.61			
5 Optional	Deglycosylated	G2F - 1		146844.3097	146936.75			
6 Optional	Deglycosylated	Emtansine - 2		146990.4147	147084.21			
7 Optional	Deglycosylated	Emtansine - 1		147477.5763	147571.01			
8 Optional	Deglycosylated	G0F - 1		147639.6292	147733.16			
9 Optional	Deglycosylated	Emtansine - 1		147801.6820	147895.30			
Optional	Deglycosylated	G2F - 1		147947.7870	148042.76			

Navigate to the 'Settings' icon and review your global 'Intact Protein Settings'

**Intact Protein Settings**

**Chromatographic Data Processing**

Peak Threshold: ≥ 5.00 %  
 Gaussian Smoothing: 0.90 points  
 Number of TOFMS Spectra to Combine: 3 ± scans

**Reconstruction Processing**

Iterations: 20  
 Signal To Noise Threshold: ≥ 20.00  
 Resolution: 1000  
 Gaussian Smoothing: 0.00

**Protein Results**

Relative Result Threshold: ≥ 5.00 %

**Chromatogram Peaks Labeling**

Label Matching Tolerance: 0.10 Minutes  
 Display Labels For: All Peaks

Reset to Default

OK Cancel

# Pharma and Biopharma

Intact protein deconvolution can be performed in seconds, on either a single datafile, or on multiple samples using the batch processing function. Review your intact ADC protein deconvolution results in the BioPharmaView Software window. Annotated reconstruction mass graph (bottom right) hyperlinks to the raw spectra (middle right) to confirm peak identity and show fidelity between raw and deconvoluted data. Detailed information on drug load can be found on the bottom left, with automated calculation of the drug to antibody ratio (DAR) presented clearly in the window.

**Characterize Standard for Intact Protein**

Sample # 1 Experiment # 1

**Processing Parameters**

Matching Tolerance: ± 15.00 Da

m/z Range: 2000.00 to 4500.00

Mass Range: 140167.12 to 157835.49 Da

**RT Range Processing**

Automatic

Time Selection  4.11 to 5.24 min

Perform LC Peak Detection

*Processing settings have changed since characterization was performed. Please reprocess the data to get current results.*

**Results** Matched Unmatched Modifications Summary

View	Protein	Modification	Mean Ratio To Protein
<input checked="" type="checkbox"/>	Deglyco	Emtansine	3.66

**% of Total Area by Multiplicity**

Protein	Modificati...	Multiplicity	% of Total Area	Summed Area
Deglyco	Emtansine	0	1.61	1.26e4
Deglyco	Emtansine	1	7.70	6.02e4
Deglyco	Emtansine	2	16.41	1.28e5
Deglyco	Emtansine	3	21.74	1.70e5
Deglyco	Emtansine	4	21.47	1.68e5
Deglyco	Emtansine	5	16.62	1.30e5
Deglyco	Emtansine	6	10.75	8.40e4
Deglyco	Emtansine	7	2.82	2.20e4
Deglyco	Emtansine	8	0.88	6.91e3

**BPC/TIC Graph**

Intensity, cps vs Time, min. Peak at 4.55 min.

**TOF MS Graph**

Intensity, cps vs m/z, Da. Peaks labeled with m/z values.

**Reconstruction Graph**

Reconstructed Intensity vs Mass, Da. Peaks labeled with m/z values and modifications.

[Learn more at sciex.com/X500B](http://sciex.com/X500B)

AB Sciex is doing business as SCIEX.

© 2016 AB Sciex. For Research Use Only. Not for use in diagnostic procedures. The trademarks mentioned herein are the property of AB Sciex Pte. Ltd. or their respective owners. AB SCIEX™ is being used under license.

Document number: RUO-MKT-02-4522-A



**Headquarters**  
 500 Old Connecticut Path | Framingham, MA 01701 USA  
 Phone 508-383-7700  
[sciex.com](http://sciex.com)

**International Sales**  
 For our office locations please call the division headquarters or refer to our website at [sciex.com/offices](http://sciex.com/offices)