

# Click. Compare. Report.

## Innovation for Biotherapeutic Comparability with BioPharmaView™ Software



# Comparability is Critical

Comparability is at the core of biologics characterization. You need to understand how the attributes of your biotherapeutic may have changed as it moves through your pipeline, or how a biosimilar stacks up to an innovator. And the faster you can assess the comparability of your biologic, the faster you can take the next step.

SCIEX understands how critical it is to confirm comparability. That's why we've built a fast and simple automated tool to help you keep your biotherapeutic moving. BioPharmaView™ Software enables you to quickly assess intact mass and peptide mapping analyses, and easily identify differences from site-to-site and from lot-to-lot, even with large data sets.

Gain a comprehensive view by analyzing the intact protein or protease digested forms in reduced or non-reduced states. Now with the ability to process data-independent SWATH® Acquisition

Easily add custom modifications like proprietary linkers and drug conjugates

## Everything You Need in One View

Getting started with BioPharmaView software is easy. From the main window, you can input your assay information and save your batch processing parameters. You can also define the biotherapeutic sequence and choose from common post-translational modifications, or easily add custom modifications of interest.

### Compare it and Share it

When it comes time to report your results, BioPharmaView software provides you with comprehensive reports in multiple output formats. You'll be able to quickly show the products that passed or failed your custom flagging criteria, and easily transfer your processed data export to electronic notebook applications.

The screenshot displays the BioPharmaView software interface for Trastuzumab. The main window is titled "Trastuzumab" and includes a sidebar with navigation options like "Assay Information", "Intact Protein", "Peptide Mapping", and "System". The "Assay Information" tab is active, showing a "Summary" section with fields for "Protein Name" (Trastuzumab) and "Description" (Trastuzumab two chains). Below this, the "Protein Sequence" section shows two chains: "Chain 1 Light Chain" and "Chain 2 Heavy Chain", each with its corresponding amino acid sequence. The "Modifications" section features a table with columns for Chains, Type, Name, Position, Maximum Mods per Chain, Modified AA, Applies To, Workflow Usage, and Mass Shift. A "Disulfide Bonds" table is also present, showing bond counts between chains and cysteines. At the bottom, there are buttons for "Add modifications...", "Delete selected modifications...", "Add bonds...", and "Delete selected bonds...".

Chains	Type	Name	Position	Maximum Mods per Chain	Modified AA	Applies To	Workflow Usage	Mass Shift
1	1-2 Internal	Deamidated	*	3	n/a	NQ	Peptide Mapping	0.9940
2	1-2 Internal	Oxidation	*	1	n/a	M	Peptide Mapping	15.9949
3	2 N-terminal	Glu->pyro-Glu	-	-	E	E	Both	-18.0106
4	2 Internal	GD	300	-	N	N	Both	128.0450
5	2 Internal	G1I	300	-	N	N	Both	146.5388
6	2 Internal	G1F	300	-	N	N	Both	1606.5867
7	2 Internal	G2	300	-	N	N	Both	1622.5816
8	2 Internal	G2F	300	-	N	N	Both	1768.6395
9	2 Internal	G0F	300	-	N	N	Both	1444.5339

From Chain	To Chain	From Cysteine	To Cysteine
1	1	1	23
1	1	1	134
1	1	2	214
2	1	1	134
2	1	2	214
2	2	2	22
2	2	2	147
2	2	2	264
2	2	2	370

Save assay information and parameters for batch processing

Simply define your biotherapeutic sequence and modifications of interest

# Quick Look Intact Mass Comparability is Now a Reality

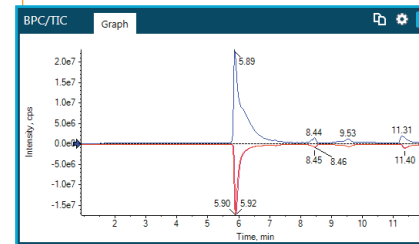
## Perform accurate intact protein deconvolution in seconds.

The hardest part of Intact Mass Analysis is processing the data and comparing biologic product characteristics. BioPharmaView Software makes it easy with one click batch processing, protein form matching, and automated ratio calculations for post translational modifications, including glycosylation. The multi-pane view in the main window allows you to see the processed and raw data from multiple samples side by side, so you can be confident about your comparability conclusions.

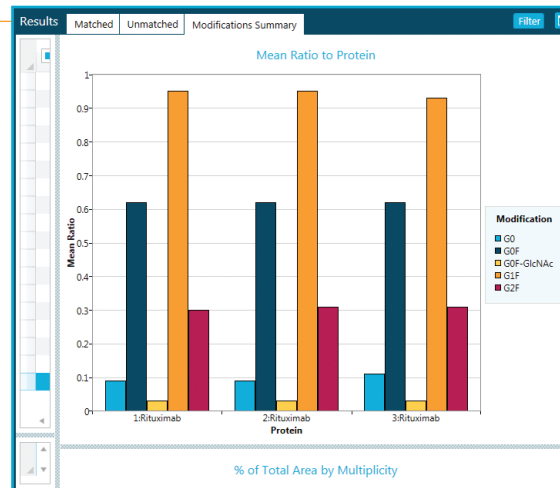
One click batch processing and automated flagging on Pass/Fail comparability criteria

Filename	Experiment #	#	Pass/Fail	Details
160322_2_10_Ritu_1p0ug.wiff.2	2	1	Fail	Reconstruction Area criteria not met
160322_2_28_Ritu_0p4ug.wiff.2	2	2	Pass	-
160322_2_29_Ritu_0p4ug.wiff.2	2	3	Pass	-

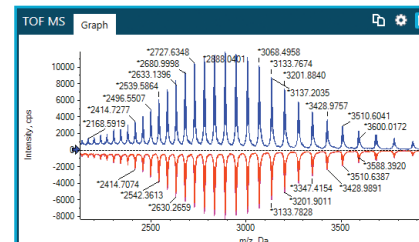
Mirror plot or overlays for easy visual comparison between samples



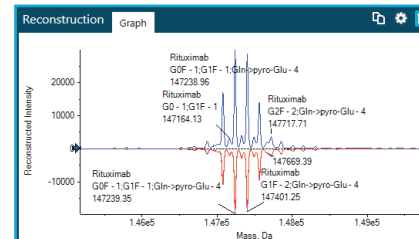
Powerful automated ratio calculations for modifications, including glycosylation



Increase confidence in the final output with direct link to raw data



View of deconvoluted and matched protein samples



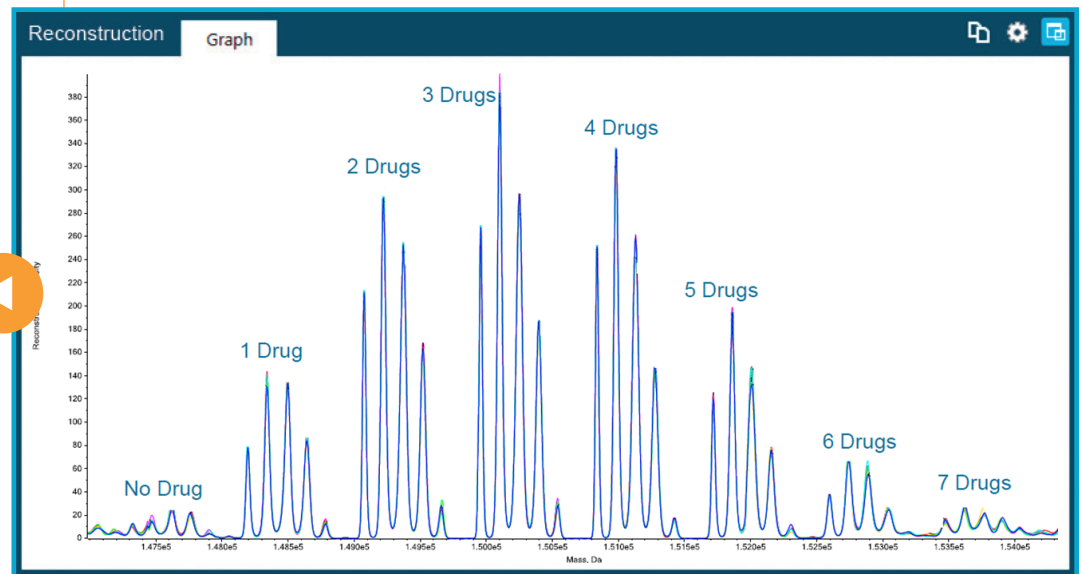
Intact analysis of 3 lots of therapeutic mAb protein. The first lot has significantly higher intensity than the assay standard, and is flagged by the Pass/Fail parameters, while the next two lots show comparable MS intensity. The graphical view of the glycosylation levels indicates all three mAb samples have comparable glycosylation patterns.

## No Fear of Antibody Drug Conjugates

Intact analysis of antibody-drug conjugates (ADCs) can be challenging because of the size and complexity of the molecules. There's no need to worry about your ADCs with automated drug-antibody ratio (DAR) calculation and visualization, as well as a simplified—yet highly accurate—view of protein deconvolution in BioPharmaView Software. Comparing ADC products is much easier when you can see drug load and DAR across multiple samples, so you'll be clear on just what you've got—and how much.

Quickly visualize drug load on an Antibody Drug Conjugate and compare average DAR across multiple samples with the automatic DAR calculator

Simplified protein deconvolution to view ADC drug states with high precision



Intact analysis of four lots of antibody drug conjugate therapeutic samples. The precise overlay of the deconvoluted spectra on the right indicates highly similar samples across the lots, for all drug states. The graphical view of the drug states and calculated drug to antibody ratio (DAR) on the left shows highly similar proportions of drug-protein form between all four ADC samples analyzed.

# Peptide Maps in a Flash

Comparison of peptide sequences across samples is much faster and easier than before in BioPharmaView Software. Everything you need to see for peptide map comparability is available in one view: from comprehensive sequence coverage and PTM ratios, to the raw MS/MS data you need to see to confirm modifications, BioPharmaView Software provides an easy to use dashboard for Peptide Mapping. At a glance, you can compare peptides, PTMs, PTM ratios and disulfide bond localization.

**Processing Parameters**

Sample # 1 Experiment # 1

m/z Tolerance, ppm: ± 5.5 ppm

Minimum Score for Auto Validation: 3.0

MS/MS Matching Tolerance: 0.03 Da

**Peptide Results**

Batch Usage	Validated Match	Review Required	RT	Theoretical Mono m/z	Observed Mono m/z	Error (PPM)	Score	Charge	XIC Area	User Defined	Sequence	Modifications
Optional	✓		15.61	593.8270	593.8274	0.8	10.256	2	6.1577e7		GPSVFPLAPSSK	
Optional	✓		14.91	581.3184	581.3196	2.1	10.123	2	5.8605e7		NQVSLTLVK	Carbamidomethyl@7(371)
Optional	✓		17.80	713.6807	713.6810	0.4	18.934	3	4.9543e7		TPEVTGVVDVSHEDPEVK	Carbamidomethyl@6(265)
Optional	✓		13.07	661.3427	661.3435	1.4	11.902	2	4.9474e7		STSGGTAALGCLV	Carbamidomethyl@11(148)
Optional	✓		10.75	625.9805	625.9816	1.8	12.091	3	4.2051e7		VYACEVTHQGLSSPVTK	Carbamidomethyl@4(194)
Optional	✓		16.63	559.9388	559.9394	1.0	15.359	3	3.8471e7		FNWYVDGVEVHNAK	
Optional	✓		25.33	603.3403	603.3411	1.2	10.188	3	3.7969e7		VVSVLTLHQDWLNGK	

**Sequence Coverage 100.0 %**

Chain 2 - HC Sequence Coverage 100.0 %

```
EVQLVESGGGLVQPGRSRLRSCAASGFTFDYAMHWVRQAPGKLEWVSA
ITWNSGHIADYADSVGEGRFTISRDNAKNSLYLQMNLSRAEDTAVYYCAKVS
YLSTASSLDYWGQGTLLVTSASATKPSVPEPLAPSSKSTSGGTAALGCLV
KDYFPEPVTVSWNSGALTSGVHTFPAVLPQSSGSLYSLSSVVPSSSLGTQ
TYICNVNHKPSNTKRVDDKVEPKSCDKHTHTCPCPAPELGGPSVFLPFPK
PRDLMISRTEPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQV
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIISKAKGQPREP
QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPP
VLDSDDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKLSLSLSPG
K
```

Chain 3 - LC Sequence Coverage 100.0 %

```
DIQMTQSPFSSLSASVGRVITTCRASQGIIRNYLAWYQQKPKAPKLLIYA
ASTLQSGVPSRFSGSGSGTDFTLTISLQLQPEDVATYYCQRYNRAPYTFGQ
GTRKVEIKRITVAAPSVFIFPPSDEQLKSGTASVAVCLLNNFYPREAKVQWKV
DNALQSGNSQESVTEQDSKDSYLSSTLTLSKADYERKHKVYACEVTHQG
LSSPVTKSFNRGEC
```

Displaying 130 unique peptides

Quick look visualization of peptide mapping sequence coverage across all mAb chains

Comprehensive list of identified peptides and modifications

Direct visualization of raw MS level data (left) and annotated MS/MS data (right)

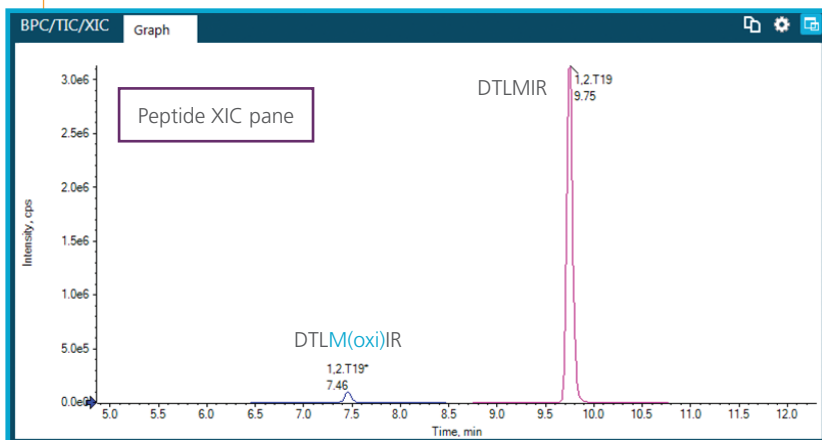
Peptide mapping using SWATH® analysis with 100% sequence coverage of adalimumab biotherapeutic on a TripleTOF system. Now with dramatically improved processing speed.

## Low Level Modifications Can't Hide from SWATH® Acquisition

When performing Peptide Mapping of biotherapeutics, you have to determine where post-translational modifications are. Using SWATH® Acquisition and BioPharmaView Software processing, you can detect low level modifications—as well as confirm them in the high-resolution MS/MS spectra—all in a single run. Plus, automated calculation of PTM ratios means you can spend more time understanding your data, and less time crunching the numbers.

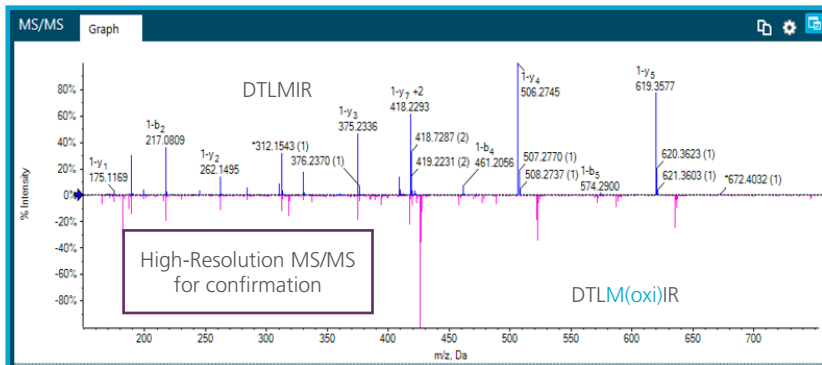
Acquire complete high-resolution MS/MS data in a single run with SWATH Acquisition to ensure important peptide and PTM information is not missed.

Save time and energy with the automated PTM ratio calculation.



Peptide Details							
Validated Match	RT	Observed Mono m/z	Charge	XIC Area	Sequence	Modifications	Modification Percent
1	7.45	851.4295	1	6.7285e4	DTLMISR	Oxidation@4(256)	2.2% ±1.1 (Oxidation@4(256) : None@4(256))
2	7.46	426.2202	2	4.7631e5	DTLMISR	Oxidation@4(256)	2.2% ±1.1 (Oxidation@4(256) : None@4(256))
3	9.75	418.2221	2	1.3938e7	DTLMISR		97.8% ±1.1 (None@4(256) : None@4(256))
4	9.75	835.4351	1	5.9211e6	DTLMISR		97.8% ±1.1 (None@4(256) : None@4(256))

Detailed % modification is automatically calculated between the modified form of the peptide and the unmodified form. For the DTLMISR peptide 2.2% contains oxidation of the M residue



Trypsin digested therapeutic mAb analyzed by SWATH Acquisition. Top pane shows high resolution XIC extraction of the MS1 level for the modified and unmodified peptide. Bottom pane displays the high-resolution MS/MS data for the unmodified peptide in blue, with alignment to the modified peptide on bottom in pink, allowing for simple and fast confirmation of the peptide identification

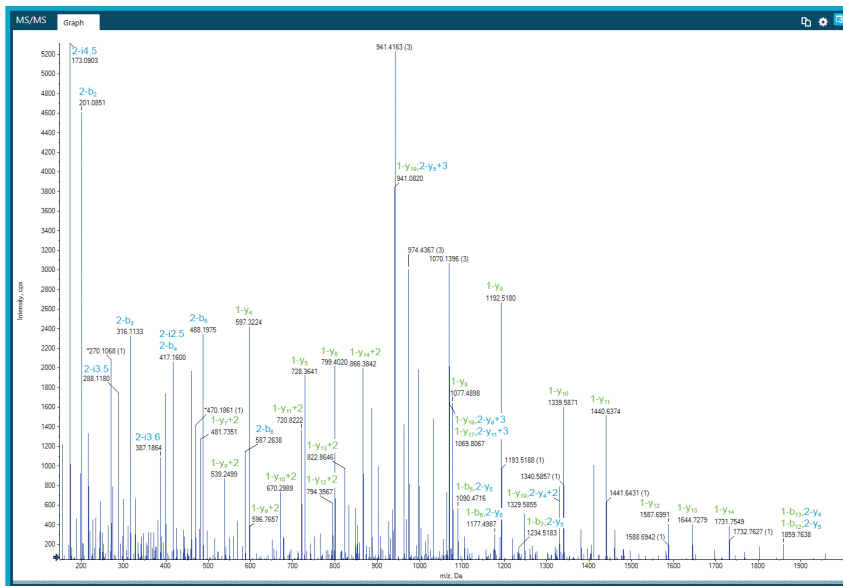


## Where are the Disulfide Bonds?

Localizing disulfide bonds is no easy task. But the algorithms in BioPharmaView software make quick work of defining the bond locations and presenting the high-resolution, annotated MS/MS spectra for confirmation.

- Quickly and accurately map disulfide bond locations in an automated fashion to simplify your data analysis. Confidently assign bond localization using the high-resolution, annotated MS/MS spectral data.

Peptide Results		Matched	Unmatched	Filter	Protein Sequence Coverage = 99.1 %		View Sequence	Optimize Matching Parameters	Update Assay Information		
RT	Theoretical Mono m/z	Observed Mono m/z	Error (PPM)	Score	Charge	XIC Area	User Defined	Sequence	Disulfide Bonds	Notes	Peptide
19	21.47	1186.2570	1186.2567	-0.2	15.366	3	5.6358e5	SGTASVWCLLNFFYPR VYACEVTHQGLSSPVTK	(3,4)T20@4(194)=(3,4)T13@8(134)	Validated match	T13 T20
20	22.45	852.6256	852.6272	1.8	16.824	4	9.7560e5	LSCAASGFTFDDYAMHWVR AEDTAVYYCAK	(1,2)T3@3(22)=(1,2)T9@9(96)	Validated match	T3 T9
21	24.57	764.7626	764.7643	2.2	6.016	5	9.4508e5	VTITCR FSGSGSGTDFLTITSSLPEDVATY...	(3,4)T2@5(23)=(3,4)T7@27(88)	Validated match	T2 T7
22	24.57	1273.9329	1273.9346	1.3	13.438	3	1.0835e6	VTITCR FSGSGSGTDFLTITSSLPEDVATY...	(3,4)T2@5(23)=(3,4)T7@27(88)	Validated match	T2 T7
25.65	916.2351	916.2350	-0.1	5.185	5	8.2285e2	LSCAASGFTFDDYAMHWVR	(1,2)T3@3(22)=(1,2)T9@9(96)	Validated match	T3 T9	



Fast view confirmation with high-resolution, annotated MS/MS data for both peptides involved in the disulfide bond.

Unreduced, trypsin digested therapeutic mAb analysis. Disulfide bond locations are automatically determined and output with corresponding MS/MS for confirmation.

# Your Success is Our Success

## We take it personally

As a SCIEEX customer you have access to a world-class customer support organization. Wherever you are, we're there with you as a trusted partner to answer questions, provide solutions, and maximize lab productivity.

Our customer support organization has access to the latest product updates, software revisions, methods and repair procedures to make sure that you stay on top of your game.

When you have questions, we have answers.

Learn more at [sciex.com/customersupport](http://sciex.com/customersupport), or locate your local account representative at [sciex.com/contactus](http://sciex.com/contactus)

For more information on SCIEEX biologics characterization solutions, please visit [sciex.com/biologics](http://sciex.com/biologics)

Answers for Science.  
Knowledge for Life.™

AB Sciex is doing business as SCIEEX.

© 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 SCIEEX™ is being used under license.

RUO-MKT-03-1737-A 05/2016

---

#### Headquarters

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

#### International Sales

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

