Simple Solutions to Complex Workflows Innovation for Biotherapeutic Peptide Mapping

PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNAL SOLAVIEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSS SFNRGECTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV GLSSPVTKSFNRGECTVAAPSVFIFPPSDEQLKSGTASVVCLL KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADY CEVTHQGLSSPVTKSFNRGECTVAAPSVFIFPPSDEQLKSGTA FYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS



Answers for Science. Knowledge for Life.™



Simplify Biotherapeutic Peptide Mapping for Better Information, Faster

Accelerate your peptide mapping workflows with SCIEX QTOF platforms, separation systems and software. The compact and user-friendly X500B QTOF system speeds your standard mapping workflow, and powerful BioPharmaView[™] software automates data processing to get you answers faster.

Streamlined analyses help you make better decisions about your biologic development and speed your time to market.

Comprehensive peptide mapping of biologics is not a simple task. Usually, a mass spec expert needs to develop protocols and analyze the data to ensure the biotherapeutic is being produced as expected. The easy, point-and-click interface of SCIEX OS, exclusively on X500 series QTOF systems, makes setup of your peptide mapping workflow rapid and simple. In fact, even novice mass spec users should have no trouble getting up and running guickly.









Peptide Mapping for Every Mass Spec User

No-Worry Sample Queue:

X500B calibration seamlessly integrates into your sample queue for rock-solid, accurate performance

Fast Peptide Mapping MS Method Setup

It's easier than ever with point-and-click parameter definition and intuitive layout, even for novice users

Easy-to-Learn User Interface:

Simply build and optimize high-performance peptide mapping methods with SCIEX OS

Low Abundance Peptides and PTMs Can't Hide from SWATH[®] Acquisition

In addition to traditional information dependent acquisition (IDA) methods, the X500B QTOF supports proprietary SWATH[®] Acquisition for peptide mapping, which provides comprehensive data collection and eliminates the need for IDA criteria set-up and traditional method development.

With SWATH Acquisition, high-resolution MS/MS are acquired for all precursor ions, providing truly comprehensive and unbiased data collection. The unbiased approach enables acquisition of high-resolution, accurate mass MS/MS spectra of all low abundance peptides and post translational modifications (PTMs) that could be missed by information dependent peptide map workflows. Furthermore, a standard, generic SWATH method can be used for almost every biotherapeutic peptide mapping analysis, further simplifying your workflow setup and helping you get answers faster.



Four Easy Steps from Data to Answers

Data acquisition by an IDA or SWATH Acquisition method is only the first part of the story. To get faster answers to your peptide mapping questions, you need powerful data processing. BioPharmaView[™] Software, connected to the X500B system and SCIEX OS, is a rapid and intuitive package for the analysis of your peptide mapping data.



biotherapeutic on the X500B system

Modification Percent

4.2% ±3.1 (Oxidation@4(256) : None@4(256))

95.8% ±3.0 (None@4(256) : None@4(256))

4.2% ±3.1 (Oxidation@4(256) : None@4(256

95.8% ±3.0 (None@4(256) : None@4(256))

70.8% ±3.0 (Oxidation@4(256) : None@4(256

29.2% ±0.7 (None@4(256) : None@4(256))

29.2% ±0.7 (None@4(256) : None@4(256))

70.8% ±3.0 (Oxidation@4(256) : None@4(256)

See It All, Fast

Comprehensive SWATH Acquisition with high-resolution MS/MS data at lightning speeds means you won't miss low level peptides and PTMs. You'll guickly and easily identify changes in modification state using advanced visualization and automated PTM ratio calculations in BioPharmaView Software.

Identify changes in modification state for important peptides using the clear visualization and reporting functions in BioPharmaView Software

Peptide Results Matched Unmatched

treated_SWATH.wiff2 DTLMISR

treated_SWATH.wiff2_DTLMISR

treated_SWATH.wiff2 DTLMISR

treated_SWATH.wiff2 DTLMISR

Filename

SWATH.wiff2

SWATH.wiff2

SWATH.wiff2

SWATH.wiff2

Sequence

DTLMISR

DTLMISR

DTLMISR

DTLMISR

Modifications

Oxidation@4(256)

Oxidation@4(256)

Oxidation@4(256)

Oxidation@4(256)

Accurately and completely	y identify	v modified	peptides
from the SWATH Acquisiti	on data		

Mono m/z

851.4316

835,4347

426,2184

418.2206

851.4308

835.4360

2 418.2206 418.2207

2 426.2182

Charge

Speed analysis time and comparisons between samples with automated calculation of modification levels from multiple charge states of peptides

Observed Theoretical Error

Mono m/z

851,4291

835.4342

426,2182

418.2207

851.4291

835.4342

426.2182

Focus in on the altered levels of peptide oxidation between samples in the Explorer view

2 EEQYNSTYR 3 EEQYNSTYR **4 EEQYNSTYR** 5 EEQYNSTYR 6 EEQYNSTYR 7 EEQYNSTYR 8 EEQYNSTYR TOF MS Graph +IDA TOF



Peptide Results

Sequence

1 EEQYNSTYR

By monitoring glycan species at the peptide level, you can reduce some of the difficulty often associated with glycan release and labeling. BioPharmaView Software presents clear glycopeptide information in table format, links directly to the MS data, and shows high-resolution MS/MS for structural confirmation for reversed phase and HILIC separations.



XIC Area

9.81e3

8.12e5

1.43e5

1.87e6

1.61e5

6.43e4

8.81e5

(PPM)

2.8

0.6

0.5

-0.3

2.0

2.1

-0.1

-0.3 3.77e5

Identify Glycopeptides with Confidence

I	Matched	Unmatched						
	Modificat	tions	Modification Percent	Charge	Observed Mono m/z	Theoretical Mono m/z	Error (PPM)	XIC Area
	G0F-GlcN/	Ac@5(301)	42.3% ±4.6 (G0F-GlcNAc@5(301) : G0@5(301))	2	1215.9850	1215.9869	-1.5	4.13e5
	G2F@5(30)1)	1.7% ±0.2 (G2F@5(301) : G0@5(301))	3	986.7199	986.7220	-2.1	7.00e3
	G0F@5(30)1)	39.0% ±9.7 (G0F@5(301) : G0@5(301))	2	1317.5244	1317.5266	-1.6	2.94e5
	G0F@5(30)1)	39.0% ±9.7 (G0F@5(301) : G0@5(301))	3	878.6853	878.6868	-1.7	2.02e5
	G1F@5(30)1)	11.7% ±6.4 (G1F@5(301) : G0@5(301))	2	1398.5518	1398.5530	-0.8	5.25e4
	G1F@5(30)1)	11.7% ±6.4 (G1F@5(301) : G0@5(301))	3	932.7033	932.7044	-1.3	7.60e4
	G0@5(301	L)	5.4% ±0.6 (G0@5(301) : G0@5(301))	2	1244.4956	1244.4976	-1.6	5.25e4
	G0@5(301	L)	5.4% ±0.6 (G0@5(301) : G0@5(301))	3	829.9975	830.0008	-4.0	2.28e4

Confidently identify glycopeptides using high-resolution accurate mass TOF-MS data. High-resolution MS/MS data can be used for structural confirmation









Recognize Deamidation Instantly

Deamidation is a common and important PTM which is often monitored at the peptide level to detect and localize susceptible sites in the biologic. You can easily identity and localize even low level peptide modifications using SWATH Acquisition along with BioPharmaView Software, which provides automated ratio calculations.

High-resolution MS and MS/MS makes identification and confirmation of peptide deamidation sites straightforward

Ре	ptide Results	Matched	Unmatche	ed					
	Sequence	Modificati	ions	Modification Percent	Charge	Observed Mono m/z	Theoretical Mono m/z	Error (PPM)	XIC Area
1	IYPTNGYTR			<u>10.6% (None@5(55) : None@5(55))</u>	2	542.7747	542.7747	0.0	2.80e5
2	IYPTNGYTR	Deamidate	d@5(55)	89.4% (Deamidated@5(55) : None@5(55))	2	543.2666	543.2667	-0.3	2.35e6

Reveal deamidated peptides and obtain relative quantitation levels in a simple format with the peptide results table in BioPharmaView Software



Trastuzumab sample analyzed using SWATH acquisition on the X500B QTOF System. IYPTNGYTR peptide was identified in unmodified as well as in deamidated state, with confirmation available at both the MS1 peptide level as MS/MS fragment ion level.

Automatically Map Disulfide Bonds

Disulfide bond localization and confirmation is now even simpler, because BioPharmaView Software intuitively presents high-resolution, annotated MS/MS spectra, including multiple charge state identification.



Let the software quickly and accurately map disulfide bond locations to simplify your data analysis

ılts	Matched	Unmatched							Filt	e
			Disulfide Bonds	Theoretical Mono m/z	Observed Mono m/z	Error (PPM)	Char	XIC Area	Peptide	
YYC	QWTSNPPT	FGGGTK	(1,4)T2@5(23)=(1,4)T5@11(87)	882.8931	882.8929	-0.2	4	7.0457e5	T2 T5	
YYC	QQWTSNPPT	FGGGTK	(1,4)T2@5(23)=(1,4)T5@11(87)	1176.8550	1176.8570	1.7	3	1.1517e5	T2 T5	_

Gain confidence from multiple charge state identification of the disulfide bond location



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



Unreduced, trypsin digested therapeutic mAb analysis using SWATH acquisition on the X500B QTOF System

Take Your Analysis to the **Next Level with TripleTOF®** 6600 systems

When you have more advanced and complex questions about your biotherapeutic, the TripleTOF 6600 System can help you get the answers you need.

Increased sensitivity and dynamic range help you see deeper into your peptide maps. You can also use orthogonal separation technologies for extra clarity to make better decisions, with confidence.



Find the Right Solution For Your Lab	TripleTOF [®] 6600	X500B QTOF
Sensitive HRAM at Industry Leading Acquisition Rates	• •	•
Enhanced Linear Dynamic Range	• •	•
Sequence Variant Identification using ProteinPilot [™] Software	•	
Host Cell Protein Analysis using ProteinPilot Software	•	
User-friendly SCIEX OS Interface for Simplified Setup and Use		•

Expand the coverage of your TripleTOF System with CESI-MS

Enhance your peptide mapping with the high-efficiency separation of capillary electrophoresis on the CESI 8000 Plus High Performance Separation-ESI Module, coupled to the TripleTOF 6600 system. High resolution separations enable a more thorough characterization of your digested biologic product—especially short peptides, long hydrophobic peptides, drug-linked peptides, glycopeptides, and deamidated peptides—which can be challenging for conventional HPLC. Additionally, CESI-MS requires only limited sample volumes, making it a powerful option for ADCs and mAbs in early discovery.



Up to 5 orders of linear dynamic range for large molecules in TOF-MS and MS/MS



CESI-MS offers efficient separation and relative quantitation of

Achieve high resolution of and iso-aspartyl

Identify Unknown Sequence Variants:

at low ppm levels on the

TripleTOF 6600 System

Minimize the number of false positives by using the unique ProteinPilot software algorithm on a TripleTOF 6600 System



Your Success is Our Success We take it personally

As a SCIEX 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.

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Headquarters 500 Old Connecticut Path Framingham, MA 01701 USA Phone 508-383-7700 sciex.com

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