

Biotherapeutic subunit mass analysis

Routine high-resolution accurate mass analysis of biotherapeutic subunits on the X500B QTOF System

Method details for the routine characterization of trastuzumab biotherapeutic protein following an IdeS subunit digestion strategy. Analysis performed by high-resolution accurate mass detection using HPLC coupled with the X500B QTOF System, powered by SCIEX OS Software.



Sample Prep

A generic sample preparation strategy is shown for IdeS subunit digestion and clean-up of an intact biotherapeutic prior to LC-MS analysis.



LC Method

Column	Agilent Zorbax SB300 C-8 1mm X 75mm						
Mobile Phase A	Water, 0.1% Formic acid						
Mobile Phase B	Acetonitrile, 0.1% Formic acid						
Flow rate	200 µL/min						
Column temperature	80 [°] C						
Injection volume	10 μL, ~1 μg total protein						
Gradient profile	Time (min)	% B					
	2.0	25					
	6.0	60					
	7.0	60					
	7.1 80						
	9.0 80						
	9.5 25						
	10.5	25					



MS Method

Suggested starting MS method parameters for routine mAb subunit 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.

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ட Subunit protein an	alysis MS					
Method Overview Device: X500 QTOF Ion Source: TurboSpray	Method duration Estimated cycles:	10 📦 min 1137	Total scan time: Intact protein mode:	0.527524 sec ON	✓ Large proteins (>70 kE	Da) Add Experiment ¥
TOF MS 0 min - 10 min	▼ Source and Gas Par Ion source gas 1 Ion source gas 2	45 psi 45 psi 5 psi	Curtain gas CAD gas	30 3	Temperature	450 ≎ °C
	• Experiment TOF MS Polarity TOF start mass TOF stop mass Accumulation time	Positive 900 Da 4500 Da 0.5 \$	Spray voltage Declustering potential DP spread	5500 \$ V 200 \$ V 0 \$ V	Collision energy CE spread	10 V 0 V
	Advanced Experiment S Time bins to sum Channel 3	settings 80 🗘	Channel 1 Channel 4	✓	Channel 2	
Data Acquisition	из				Start 👻 🔳 Stop	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.

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	Sample Name	MS Method		LC Method	Rack code	Vial position	Data File	â
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1/			Bovine Insulin					
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20			ESI Positive Calibration Sol	ution	OK	Calicer		
21			Glu-fibrinopeptide B					
22			PPG Negative Calibration S	olution				
23			PPG Positive Calibration So	lution				
24			X500 ESI Negative Calibrat	ion Solution				
25			X500 ESI Positive Calibratio	on Solution				
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Batch - Automatic (Calibration Editor	X
Provide ion reference and	দ্ব d calibrant delivery settings to be applied automatically, at the correct frequency during acquisition	
Ion reference table	X500 ESI Positive Calibration Solu 💙 Edit	
Calibrate every	3 samples	
Calibrant delivery	CDS CDS channel 1	
	OK Cancel	

For Research Use Only. Not For Use In Diagnostic Procedures.

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.

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	Assay Infor	mation	Sequence Features	Intact Prot	ein Peptic	le Mapping									
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Mapping	111-2	14 AAPSV	FIFPPSDEOLKSGTAS	VVCLLNNF	YPREAKV	OWKVDNA	LOSGNSOE	SVTEODSKI	STYSLSSTLTI	SKAD	YEKH	VYACEV	THOGLS	SSPVTKS	FNRGEC
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Navigate to the 'Intact Protein' tab complete processing parameters and to generate the protein forms for matching.

SCIEX?	Trastuzumab	subunit								Create	pen Save	Save As Close
Project	Assay Information s	equence Features Intac	ct Protein	Peptide Mappi	ng							
Assay Information Intact Protein Characterize Standard Create Batch Review Results Peptide Mapping Characterize Standard	Processing Parameters Matching Tolerance: ± 5.00 D Start m/2: 600.00 Stop m/2: 3000.00 Start Mass: 18786.94 D Stop Mass: 102629.28 D Maximum Number of Combined Maximum Number of Combined	A RT Range P Perfor Start RT: 3. Stop RT: 4. Modifications per Prot	Processing: T n LC Peak De 93 min 98 min 98 min	ime Selection tection	Batc	h Proces Retention h Proces Reconstr Required Restricted	ising Paramete In Time Tolerance: Ising Pass / Fai Juction Area Limits Form Minimum: d Form Maximum:	rs ± 1.00 Criteria ± 10.0 ≥ 80 ≤ 0	min % %			
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Review Results	Batch Usage Protein Nam	e Modifications	User Defined	Mono. Mass	Avg. Mass	Match	Reconstruction Area	Retention Time				Î
System	1 Optional Trastuzumab- cterm	HC Protein Terminal Lys- loss - 1		23771.8983	23786.94				<u></u>			
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Create Report	3 Optional Trastuzumab- cterm	HC dHex - 1 Protein Terminal Lys- loss - 1		23917.9562	23933.08				•			
	4 Optional Trastuzumab-	HC dHex - 1		24046.0511	24061.25				*			
	Optional Trastuzumab- 5 cterm	HC GOF-GlcNAc - 1 Protein Terminal Lys- loss - 1		25013.3527	25029.09							
	6 Optional Trastuzumab- cterm	HC G0 - 1 Protein Terminal Lys- loss - 1		25070.3742	25086.14				-			
	7 Optional Trastuzumab-	HC GOF-GICNAC - 1		25141.4477	25157.26		<i>.</i>		۰			
	Optional Trastuzumab- 8 cterm	HC G1F-GlcNAc - 1 Protein Terminal Lys- loss - 1		25175.4056	25191.23				-			
	9 Optional Trastuzumab-	HC G0 - 1		25198.4692	25214.32				•			
	Optional Trastuzumab- 10 cterm	HC GOF - 1 Protein Terminal Lys- loss - 1		25216.4321	25232.28				-			
	Optional Trastuzumab- 11 cterm	HC G1 - 1 Protein Terminal Lys- loss - 1		25232.4270	25248.28		*		*			
	12 Optional Trastuzumab- cterm	HC G1F-GlcNAc - 1		25303.5005	25319.41				-			Ŧ
Settings Help About								Import		Delete	Reset Char	acterized Proteins



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

BioPharmaView Settings	All Assess Researchers			×				
Custom Modifications Intact Protein Settings	Intact Protein Settings							
Peptide Mapping 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:	≥[50.00					
	Resolution:		3000					
	Gaussian Smoothing:		1.00					
	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				



Protein deconvolution of each subunit can be performed in seconds, on either a single datafile, or on multiple samples using the batch processing function. Below is shown the deconvolution results for the Fc domain of the biotherapeutic, as well as the Fab2 domain of the biotherapeutic (non-reduced).

📔 BioPharmaView		
SCIEX		Create Open Save As Close
Project	Characterize Standard for Intact Protein	BPC/TIC Graph 🖸 🌣 🗖
Assay Information	Open File 160412_SubUnit_03_Trastuz_IdeZ_10ul_2.wiff2	TIC from 160412_SubUnit_03_Trastuz_IdeZ_10ulIdeZ_10ul, Experiment 2, +TOF MS (600 - 3000) 12e7.4
Intact Protein	Sample # 1 V Experiment # 2 V	0.31
Characterize Standard 🔸	Processing Parameters RT Range Processing	x 8.0e6
Create Batch	Matching Tolerance: ± 5.00 Da Automatic	4.21 ★ 6.0e6
Review Results	m/z Range: 1800.00 to 2600.00 • Time Selection 3.94 to 4.92 min	4.0e6
Peptide Mapping	Mass Range: 18786.94 to 102629.28 Da Perform LC Peak Detection	2.0e6 -
Characterize Standard	Results Matched Unmatched Modifications Summary Filter Update Assay Information 🌣 🗖	0.0ea
Create Batch	RT Theoretical Observed Error (Da) Reconstruction Modifications Protein Name	1 2 3 4 5 6 7 8 9 10 Time, min
Review Results	Average MW Average MW Area Area 4.43 25232.28 25231.90 -0.38 1.02e5 G0F - 1 Trastuzumab-HC cterm	TOF MS Graph
System	1 Protein Terminal Lys- loss - 1	+TOF MS from 160412_SubUnit_03_Trastuz_IdeZExperiment 2 @ RT: 4.43 from 3.94 to 4.92 min
View Queue	4.43 25394.43 25394.07 -0.35 2.32e4 G1F - 1 Trastuzumab-HC cterm	6000 - 1202.5166 *1328.9883
Create Report	055 - 1 055 - 1 7627 01 - 1 27 - 4116 Textury mob UC steep	5000 *1147.9084 *1402.7640 *1485.2351 *2170.5070 *2271.4260
	3 443 9702320 9702731 1.37 4.129 Insudument from the method	5 4000 *1098.0535 *1578.0047 2219.8205 3000 *1052.2453 *1341.9296 *2325.4903 2000 *1050.0956 *2325.1910 2382.1910 1000 *1000 2000 *270.3881 1000 1500 2000 2500 1000 1500 2000 2500
* ? !		Reconstruction Graph C • Reconstruction from 160412_SubUnit_03_Trastuz_I Experiment 2 @ RT: 4.43 from 3.94 to 4.92 min • Reconstruction from 160412_SubUnit_03_Trastuz_I Experiment 2 @ RT: 4.43 from 3.94 to 4.92 min • Optimized and the state of the

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

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