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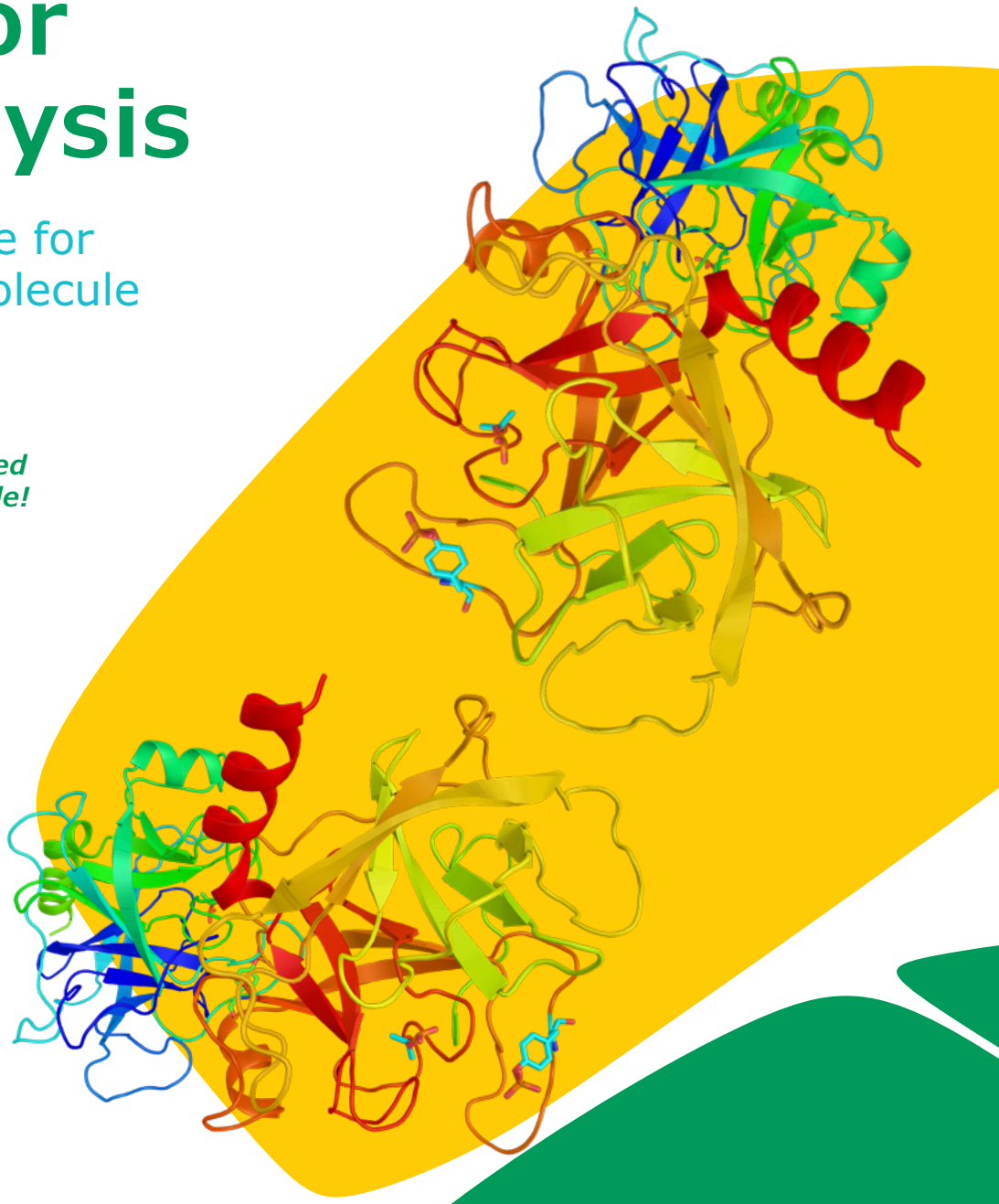

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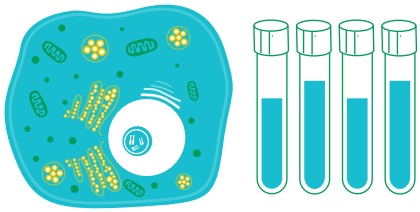

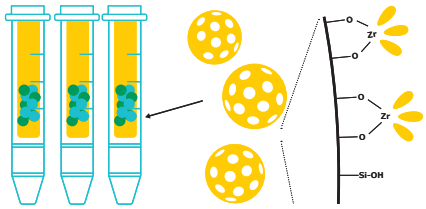

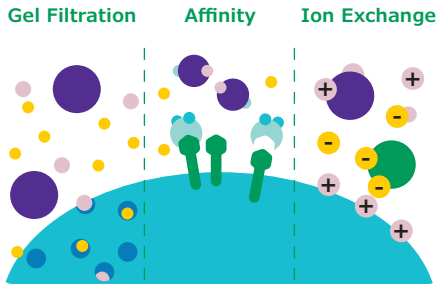

Sample Source

- Blood
- Plasma
- Tissues
- Cell Line

Sample Preparation

- Supelclean™ LC-4 (Wide Pore) SPE
- Empore™ SPE Disks
- ZipTip Pipette Tips
- Supel™-Select Polymeric SPE (HLB, SAX; SCX)
- Supel™ Swift HLB - SPE Made Easy
- Discovery (SCX, WCX, SAX, Glycan)
- PTM_Discovery® Glycan SPE
- BioSPME
- HybridSPE®-Phospholipid Technology
- Matrix Substances for MALDI-MS
- MS Standards
- Ultra-Pure MALDI Matrices
- Supel™ BioSPME 96-Pin Devices

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- Affinity Chromatography
- Ion Exchange Chromatography
- Size Exclusion Chromatography
- Gel Filtration Chromatography
- Hydrophobic interaction Chromatography

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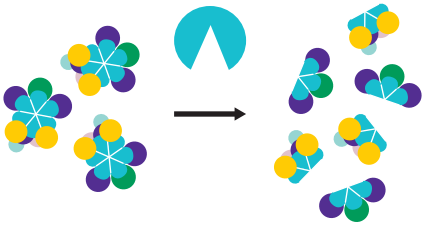
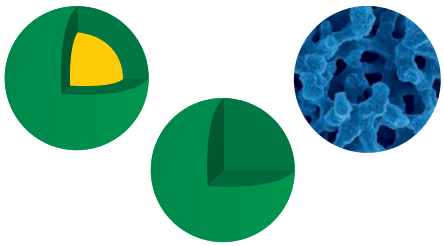


Digestion

- Trypsin proteomics grade
- SOLu-Trypsin
- PNGase Fast
- Other proteases



Analysis & Quantification

- Bioshell™U/HPLC columns
- Chromolith® WidePore 300
- TSKgel® HPLC and UHPLC columns
- Cytiva gel filtration chromatography
- BIOshell™Glycan
- Ascentis Express™ U/HPLC columns
- Chromolith® U/HPLC monolithic columns
- SeQuant® ZIC-HILIC U/HPLC columns
- Supel™ Carbon LC U/HPLC Columns
- Metabolite MSMLS library
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Appendix: New released N-glycan application guide

Sample preparation for Biomolecules

Supel™ Swift HLB SPE

- Directly load the sample without conditioning and equilibration steps
- Excellent recovery

Description	Item Code
Supel™ Swift HLB SPE Tubes bed wt. 60 mg, volume 3 mL, pk of 54	57492-U
Supel™ Swift 96-well SPE bed wt. 60 mg/well, pk of 1	57494-U

More phases and bed weights available here

Solid Phase Extraction Products

Improve Sensitivity, Increase Throughput and Ensure Reliability

Supelclean™ LC-4 SPE Tube

- Larger pore size to accommodate macromolecules (e.g. proteins and peptides)
- Commonly used for desalting and extracting proteins/peptides in aqueous samples

Description	Item Code
Supelclean™ LC-4 SPE Tube Wide Pore, bed wt. 500 mg, volume 3 mL	57089

Discovery® Glycan SPE

- Polyamide Resin: Particle Size: 50-160 µm, Surf pH: 4.5-7.5, Density: 0.2-0.3 cm3/g, Water Content: < 5 %
- Useful for extracting gylcans from aqueous solutions.

Description	Item Code
Discovery® Glycan SPE Tube bed wt. 50 mg, volume size 1 mL, pk of 108	55465-U

Discovery® DPA-6S SPE

- Polyamide Resin: Particle Size: 50-160µm, pH: 4.5-7.5, Water Content: < 5 %
- For extraction of polar compounds (-OH groups, esp. phenolic compounds) from aqueous or methanolic solution.

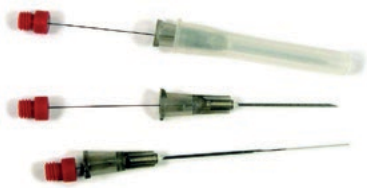
Description	Item Code
Discovery® DPA-6S SPE Tube bed wt. 50 mg, volume 1 mL, pk of 108	52624-U

Discovery C18

- Polymerically bonded, octadecyl (C18), endcapped
- Higher % of C18 loading for increased binding capacities and higher recoveries

Description	Item Code
DISCOVERY DSC-18 PK/30 6ML TUBE 500MG	52604-U

More Discovery SPE products available here



BioSPME

- BioSPME offers an innovative approach for biological microsampling and sample preparation. The new Supel™ BioSPME Pin Device enables fast and reproducible measurement of free small molecule analyte fraction for protein binding studies.

Description	Item Code
SPME-LC Pipette Tips, functional group C18, 96-tip array	57234-U
SPME-LC Fiber Needle Probe, functional group C18, package of 5 probes	57281-U
Supel™ BioSPME C18 96-Pin Devices, 1 pack	59680-U

More selectivities available here

ZipTips

- Single-step desalting, concentration, and purification
- Ideal for peptides, proteins, nucleic acids, no sample loss

Description	Item Code
ZipTip with 0.6 µL C18 resin	ZTC18S096

More choices available here

Empore™ SPE Disks

- Empore membrane SPE technology comprises of SPE particles tightly enmeshed within a network of inert PTFE fibrils (90% SPE particles : 10% PTFE, by weight).

Description	Item Code
Empore™ SPE Disks, matrix active group C18, diam. 47 mm, pk of 20	66883-U

More selection available here

Purification and Enrichment

Tosoh columns*

- TSKgel SW Size Exclusion Chromatography columns contain silica-based, hydrophilic bonded phase packings that permit minimal interaction with protein samples and high resolution.

Description	Item Code
TSKgel® G3000 SWXL 30 cm × 7.8 mm, 5 µm particle size	808541
TSKgel® UP-SW3000 30 cm × 4.6 mm, 2 µm particle size	80023448
TSKgel® UP-SW2000 30 cm × 4.6 mm, 2 µm particle size	823514

- Use TSKgel® G5000PWXL columns for analysis of polymers, including oligosaccharides with molecular weights up to 1 million Da.

Description	Item Code
TSKGEL G5000 PWXL 30 cm × 7.8 mm, 10 µm particle size	808023

Cytiva columns

- Pre-packed with small rigid agarose beads for high flow rates and pH stability.

Description	Item Code
SUPERDEX 200 INCREASE 10/300 GL	GE28-9909-44

- Superose 6 Increase 10/300 GL is a versatile column for high-resolution size exclusion chromatography, preparative purification, as well as for characterization and analysis of proteins and other biomolecules.

Description	Item Code
SUPEROSE 6 INCREASE 10/300 GL	GE29-0915-96

Navigate the workflow to learn more

Discover the full portfolio on Biopolymer separation here

* Tosoh products are only available in North America, South America, Europe, and Africa.

Digestion

- SOLu-Trypsin (EMS0004) is our exclusive, Advanced Proteomics Grade enzyme that is solution stable for mass spectrometry. Designed to be stable in solution when refrigerated, it can be used immediately without preparation.

Description	Item Code
SOLu-Trypsin, Proteomics Grade, recombinant	EMS0004
SOLu-Trypsin Dimethylated, Proteomics Grade, recombinant	EMS0005
Trypsin, Proteomics Grade	T6567
Trypsin Spin Column, Proteomics Grade	TT0010
PNGase Fast	EMS0001

More selection available here

Chromatographic Separation

BIOshell™ columns

- BIOshell™ columns deliver maximum speed and efficiency for the separation of biomolecules.
- Fused-Core® superficially porous silica particles ranging from 90 Å up to 1000 Å allows superior separation of glycans as well as very large proteins.

Description	Item Code
BIOshell™ Glycan, 15 cm x 2.1 mm, 2.7 µm	50994-U
BIOshell™ A160 Peptide , 15 cm × 2.1 mm, 2.7 µm	66905-U
BIOSHELL A400 PROTEIN C4, 3.4UM, 15CM X&	66826-U
BIOSHELL 1000A IgG C4 2.7UM 5CM X 2.1MM&	63283-U

More choices available here

Chromolith® columns

- Completely bioinert column hardware
- Very low column backpressure and high-speed separations

Description	Item Code
Chromolith® WidePore 300 10 cm x 4,6 mm	1522700001

More choices available here

SeQuant® columns

- SeQuant® ZIC-HILIC is the ideal choice for separation of polar and hydrophilic compounds. The zwitterionic stationary phase, ensures reproductible retention of compounds that are difficult to separate on reversed-phase HPLC columns

Description	Item Code
SeQuant® ZIC-HILIC 3.5µm, 200Å 100 x 2.1 mm	1504470001

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- Maximize speed with sharp peaks even at ultra-high flow rates
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Description	Item Code
ASCENTIS EXPRESS C18 2.7UM 15CM X 4.6MM	53829-U

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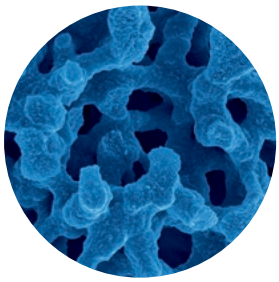

Chromolith® C18

- Truly speed up your separations.
- Revolutionary monolithic technology provides separations in a fraction of the time required by conventional particulate columns.

Description	Item Code
Chromolith® Performance RP-18e 100-4.6	1021290001

More product available here

Supel™ Carbon LC column

- Compatible with mobile phases in the pH range of 1 – 14 up to 250 degree C.
- Unique retention mechanism for polar or charged compounds without HILIC conditions. The mechanism also allows the resolution of geometric isomers.

Description	Item Code
Supel™ Carbon LC, 2.7 µm HPLC Column column L × I.D. 10 cm × 4.6 mm	59998-U

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UHPLC-MS NEW Grade

Description	Item Code
Acetonitrile for UHPLC-MS LiChrosolv®	1037251002
Methanol for UHPLC-MS LiChrosolv®	1037261002
Water for UHPLC-MS LiChrosolv®	1037281002

LC-MS Grade Solvents

Description	Item Code
Acetonitrile LC-MS Hypergrade	1000292500
Methanol LC-MS Hypergrade	1060352500
Water LC-MS Hypergrade	1153332500
Isopropanol LC-MS Hypergrade	1027812500
Water with 0.1% Formic Acid LC-MS	1590132500

HPLC Grade Solvents

Description	Item Code
Acetonitrile HPLC Grade	34851-2L
Methanol HPLC Grade	34885-2L-R
Water HPLC Grade	34877-4L
Isopropanol HPLC Grade	34863-2L

Visit our solvent center for more information here

Screw Thread (Certified kits glass, screw thread vials and cap)

Description	Item Code
0.3 mL, clear glass vial, PTFE/silicone septum (bonded), thread for 9 mm	29391-U
2 mL, clear glass vial, PTFE/silicone septum	29378-U
2 mL, clear glass vial, PTFE/silicone septum (with slit)	29379-U
2 mL, amber glass vial, PTFE/silicone septum	29385-U
2 mL, amber glass vial, graduated, PTFE/silicone pre-slit septum	29387-U

Crimp (Crimp vial kits with cap/septa)

Description	Item Code
2 mL, clear glass vial, wide opening, crimp top PTFE/silicone septum	29125-U
2 mL, amber glass vial, wide opening, crimp top PTFE/silicone septum	29128-U

Snap (Kit of vials and caps)

Description	Item Code
2 mL, clear glass vial, PTFE/silicone septum, black PP cap	29142-U
2 mL, amber glass vial, PTFE/silicone septum, black PP cap	29145-U

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Released N-Glycan Analysis of a Therapeutic Antibody Using BIOshell™ Glycan Column

Maricar Dube, Analytical Sciences Liaison
Cory Muraco, HPLC Product Manager
Judy Boland, Senior R&D Scientist
Amber Henry, R&D Scientist

Introduction

Therapeutic monoclonal antibodies (mAbs) have seen an explosive growth since the first mAb was approved by the US FDA over thirty years ago. In fact, over the past five years, therapeutic antibodies have become the best-selling drugs,[1] and they continue to grow in terms of new approvals and targets.[2]

Monoclonal antibodies are target specific, which means they have high efficacy and exhibit few side effects. However, compared to chemically synthesized small molecule therapies, mAbs are considerably more complex owing to their size and the nature of their development and production. These mAbs are expressed using recombinant technologies in mammalian cell lines or other expression systems, giving rise to heterogeneity mainly through post-translational modifications (PTMs).[3] These PTMs need to be characterized because they affect the efficacy, stability, half-life and safety of mAbs.

Glycosylation is one of the most common and important PTM for mAbs. Glycosylation involves the attachment of glycans at specific sites on a protein, most commonly at asparagine (Asn) (*N*-linked) or serine/threonine (Ser/Thr) (*O*-linked) amino acid residues.[4] There are four levels of analytical approaches to *N*-glycan analysis: intact glycoproteins, glycopeptides, released glycans, and monosaccharide analyses. This article focuses on the analysis of released *N*-glycans by HPLC.

The steps in released N- glycan analysis are outlined in **Figure 1**. The N-linked glycans are released by an amidase such as peptide-N-glycosidase F (PNGase F). The released glycans are then labeled with a fluorescent tag, like aminobenzamide (2-AB) or Procainamide (4-amino-N-[2-(diethylamino)ethyl] benzamide; ProA). Prior to HPLC analysis, a cleanup step is needed to remove excess tags and salts. Hydrophilic interaction liquid chromatography (HILIC) is a proven technique for the separation and quantitation of glycans over other HPLC methods (*e.g.* reverse phase, anion exchange).[5]

Figure 1. Workflow for released N-glycan analysis by HPLC.

In this article, a BIOshell™ Glycan HPLC column is used to analyze Cetuximab (Erbitux®) *N*-glycans labeled with ProA. BIOshell™ Glycan HPLC columns are specifically engineered to deliver fast, high resolution, reproducible glycan identification using HILIC.

Experimental

Glycan Release and Labeling: PNGase Fast Kit was used for glycan release with FASP (filter aided sample prep). The released glycans were labeled using procainamide with reductive amination.

Sample Cleanup. **Table 1** shows the SPE conditions.

Table 1. SPE Conditions

SPE	Discovery® Glycan SPE Tubes
equilibrant, diluent and wash	99% Acetonitrile
Eluent	20% Acetonitrile

The eluted, labeled glycans were dried by vacuum centrifugation.

HPLC Analysis: The glycans were solubilized by dissolving in 50 µL of 75% ACN / 25% 75 mM ammonium formate pH 4.4, vortexed for 2 min, and centrifuged at 16,000 x g for 2 mins. **Table 2** shows the chromatographic conditions.

Table 2. HPLC condition for the analysis of procainamide labeled Cetuximab N-glycans

Column:	BIOshell™ Glycan; 15 cm x 2.1 mm I.D., 2.7 µm
column temp.:	58 °C
mobile phase:	[A]: 75 mM ammonium formate pH 4.4 (50 mM ammonium hydroxide, adjusted to pH 4.4 with formic acid)
	[B]: Acetonitrile
flow rate:	0.37 mL/min
gradient:	75% B to 59% B in 75 min
Injection:	10 µL
fluorescence detection parameters:	308 nm excitation
	359 nm emission
sample:	Dried procainamide labeled Cetuximab reconstituted in 50 µL 25% 75 mM ammonium formate pH 4.4 and 75% ACN

Results

In this study, Cetuximab was used as a model therapeutic mAb to analyze N-released glycans. It is a chimeric mouse-human IgG1 monoclonal antibody against the epidermal growth factor receptor (EGFR). Cetuximab is used to treat head and neck as well as colorectal cancers. The antibody is N-glycosylated both in the fragment crystallizable (Fc) and fragment antigen binding (Fab) regions. There are numerous studies and reports showing how attached N-glycans on mAbs affect biological and physicochemical processes leading to safety and quality issues.[6,7] Some of the processes that are affected by glycosylation are enhancement of the structural integrity of the mAb, serum half-life, antibody-dependent cellular toxicity (ADCC), anti-inflammatory activities, immunity, and antigen recognition. Clearly, understanding glycosylation patterns is exceptionally important.

BIOshell™ HPLC columns are based on Fused-Core® particles (also called core-shell or superficially porous particles (SPPs)) which are characterized as having a thin, porous shell of high-purity silica surrounding a solid, silica core. This design allows for shorter diffusion path compared to traditional fully porous particles, as illustrated in Figure 2. The short diffusion path increases mass transfer of solutes (“C” term in the van Deemter equation), concomitantly resulting in high column efficiency.

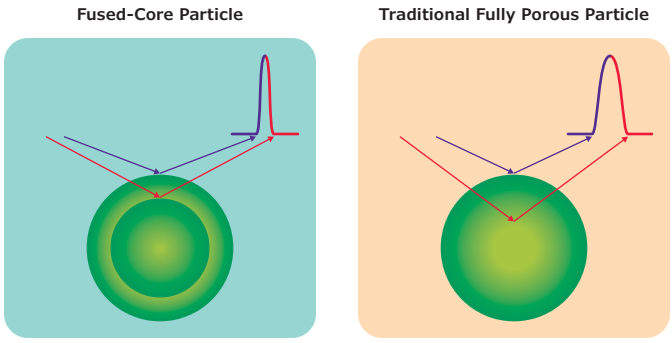

Figure 2. Fused-core particles have shorter diffusion paths compared to traditional fully porous particles.

The stationary phase in the BIOshell™ Glycan column is a highly polar ligand that possesses five hydroxyl groups tethered to the silica via a novel, proprietary chemical linkage. This unique column chemistry is suitable for analysis of oligosaccharides, particularly for protein-linked glycans using the typical mobile phases for hydrophilic interaction liquid chromatography (HILIC) of oligosaccharides.

A fluorescence chromatogram of procainamide-labeled Cetuximab glycans is shown in **Figure 3**. The BIOshell™ Glycan column was able to elucidate the complex glycosylation of this mAb, with 36 peaks identified.

Successful analysis of N-linked glycans by HPLC requires an efficient and reproducible glycan release step. The traditional protocol involves multiple wash steps and an overnight digestion of the native or denatured mAb. The protocol described in the Experimental section of this article is a fast protocol that uses a proprietary detergent-based buffer for rapid deglycosylation of N-linked glycans using PNGase F. In this fast protocol, complete release of N-glycans is achieved in a 15-minute incubation, compared to the traditional overnight digestion.

In another experiment, the BIOshell™ Glycan column was used to compare released glycans from Cetuximab using three glycan release protocols:

- Traditional overnight protocol, denatured using guanidine hydrochloride
- Fast protocol, non-reduced (rapid deglycosylation)
- Fast protocol, reduced (rapid deglycosylation under reducing conditions using 2-mercaptoethanol)

The results are shown in **Figure 4**.

With this particular analyte (Cetuximab), there were glycan species where the three protocols were equally efficient, for example G0F-N, Man5, G0F, G1(F1,6), Man5G0F Hybrid, and G1F(1,3). But there were some glycans that were not efficiently released with the fast protocol when a reducing step using 2-mercaptoethanol was included, for example G1F5′, G2FG2, FA3G, FA3GF, G2F2S′, and G2FGS′. Indeed, the use of 2-mercaptoethanol in the denaturing step is not required for most proteins when using the fast protocol. There are proteins, like RNAse B, that seem to require it. It is recommended that as part of optimizing a method for released glycan analysis, the fast protocol must be tested with and without 2-mercaptoethanol to see which gives the best results.

It is also worth noting that some proteins are not amenable to fast deglycosylation techniques. When working with mAbs without established protocols for glycan analysis, it is best to compare the results of the traditional overnight digest to the fast/rapid digestion protocol.[8]

Conclusion

Characterizing and monitoring the glycosylation pattern of a therapeutic mAb is required by regulatory authorities to ensure efficacy and safety of the drug. While analysis and identification of glycans can be challenging because of their structural complexity, this article has shown that a BIOshell™ Glycan HPLC column was able to elucidate the complex glycosylation of Cetuximab after an appropriate glycan release and labeling protocol. Another key consideration in glycan analysis is the deglycosylation protocol. While there is a fast method that significantly saves time, it is recommended to compare the results with the traditional overnight digestion and pick the one that gives more efficient deglycosylation.

Featured Products

Glycan Release	
PNGase Fast Kit	EMS0001-1KT
30 kDa MWCO Centrifugal Filtration Units, 0.5 mL	MRCF0R030
Labeling	
Procainamide HCl	SML2088 or PHR1252
Cleanup	
Discovery® Glycan SPE Tube	55465-U or 52624-U
Vacuum Manifold	VM20
Acetonitrile, HPLC or LC-MS grade	34851 or 1.00029
HPLC	
BIOshell™Glycan, 15 cm x 2.1 mm I.D., 2.7 µm	50994-U
Acetonitrile, LC-MS grade	1.00029
Ammonium formate, LC-MS grade	70221
Formic acid, LC-MS grade	5.33002

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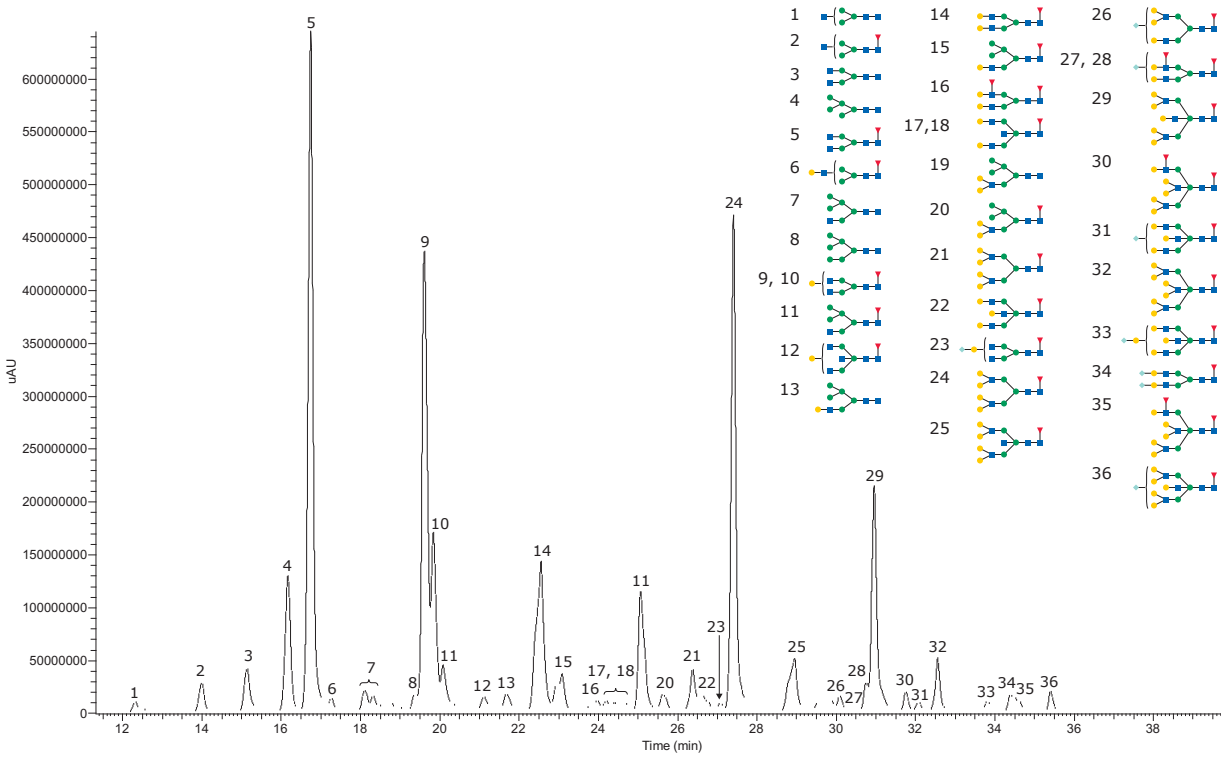

Figure 3. Fluorescence chromatogram of procainamide-labeled Cetuximab glycans on BIOshell™ Glycan column. LC-MS was used to characterize each peak (MS data not shown). A rapid release glycan protocol was used.

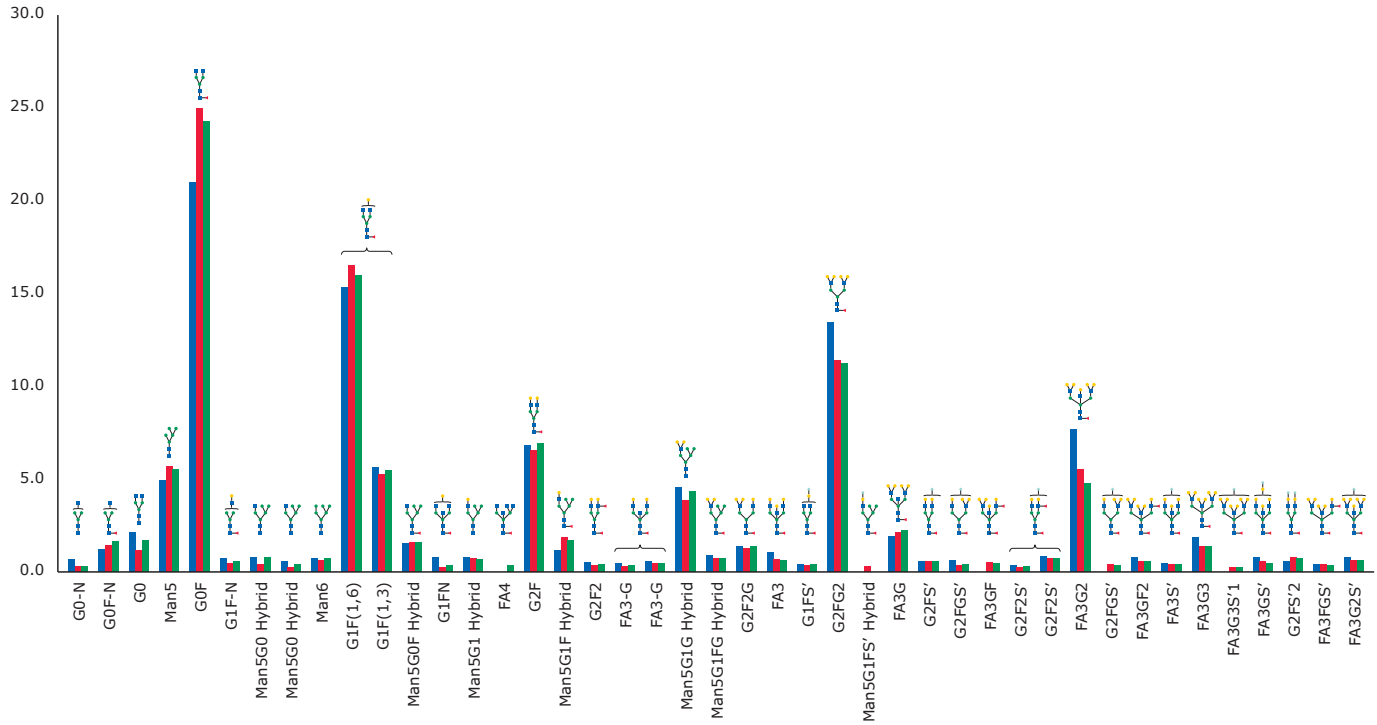

Figure 4. Comparison of Cetuximab glycan distribution using three glycan release protocols:
■ Fast (reduced), ■ Fast (non-reduced), and ■ Traditional overnight protocol. A BIOshell™ Glycan column was used in all three samples.

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