

Take A Deep Breath

Set down that
multi-channel pipette

And let us take
you to a new state
of biologics Zen



Peptide Mapping • Peptide Quantitation • Intact Mass •
Intact and Fragment Analysis • Aggregate Analysis •
Glycan Analysis • Drug Antibody Ratio

phenomenex[®]
...breaking with tradition[®]

www.phenomenex.com/bioZen



Focus on the hum of your instrumentation.

Notice the clicking of your autosampler.

Watch closely as the next peak on your chromatogram gets created.



We've been busy.

From the minds of protein chemists, chromatographers, and mass spec gurus, we've forged something new.

A comprehensive blend of innovative and acclaimed separation materials?

YES

A new titanium hardware to minimize priming?

YES

A product QC testing program to reflect customer applications?

YES

A team of savvy protein and separation scientists to back your endeavors?

YES

A promise to drive successful bioseparations and fulfill the needs of our customers worldwide?

YES

And that's not all. Welcome to bioZen.



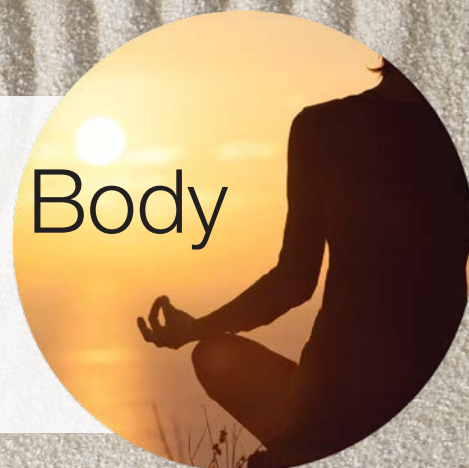


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MIND

Biocompatible Flow Path

Keep your **MIND at ease** knowing that we've **minimized the need for priming** with a new titanium infused biocompatible hardware and frit that doesn't interfere with protein or peptide integrity!



- Peptide Mapping
- **Aggregate Analysis**
- Glycan Analysis
- Peptide Quantitation
- Drug Antibody Ratio
- Intact Mass
- Intact and Fragment Analysis



Extend Column Lifetime with Biocompatible Guard Cartridge Systems

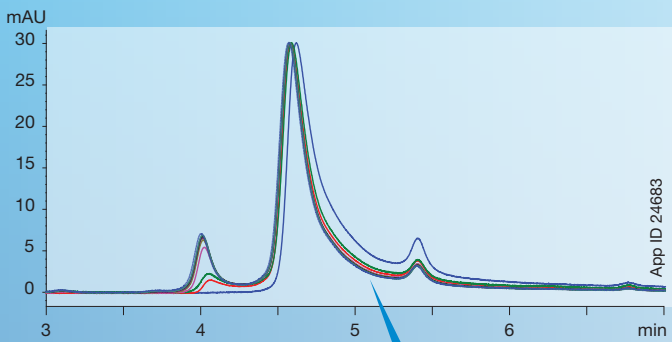
The new biocompatible SecurityGuard™ Standard and ULTRA cartridge systems remove unwanted contaminants before they clog your column or system. Each bioZen™ column has a matching guard to ensure workflow applicability. Learn more on page 22.

Proteins gave us a **piece of their MIND** and we **listened**. bioZen™ titanium BioTi™ HPLC/UHPLC hardware is designed to curtail unwanted secondary interactions, problematic carryover, and recovery issues between injection to detection.

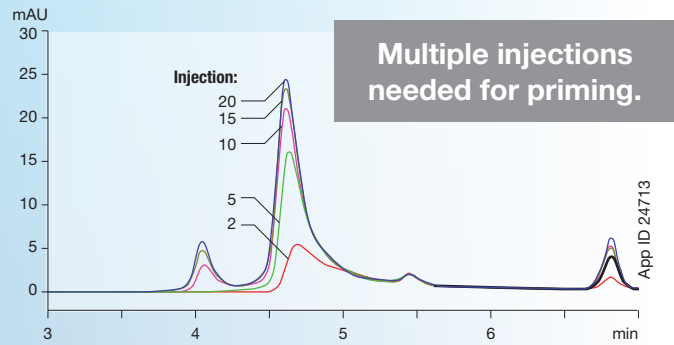


Overlaid Successive Injections – Protein Priming Comparison

bioZen Titanium BioTi™ Hardware



Traditional Stainless Steel



We engineered our new titanium BioTi biocompatible hardware to give you back the hours, days, and weeks typically spent on column priming.

—Jason Anspach, Ph.D.
Senior Scientist

Conditions for both columns:

- Column:** bioZen 1.8 μm SEC-3
- Dimension:** 150 x 4.6 mm
- Mobile Phase:** 100 mM Sodium Phosphate Buffer (pH 6.8)
- Flow Rate:** 0.3 mL/min
- Temperature:** Ambient
- Detection:** UV @ 280 nm
- Sample:** 1. γ-Globulin
2. Ovalbumin

7 Particle Chemistries and Growing

With a single innovative product line spanning major biologics workflows, you can now gain some reprieve from juggling multiple catalogs, bookmarks, and vendors. **Give your MIND a break** with high quality particle chemistries designed and tested for biologics.

Two Particle Platforms



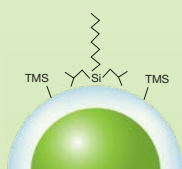
Thermally Modified Fully Porous

High Efficiency
Excellent Inertness
Increased Sensitivity
Exceptional Quality and Robustness



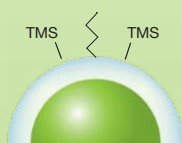
Core-Shell Technology

Intact



bioZen™ Intact XB-C8 3.6 μm

Large pore core-shell particle for fast intact biologic entry. C8 provides highly useful moderate hydrophobic selectivity.



bioZen Intact C4 3.6 μm

Large pore core-shell particle for fast intact biologic entry. C4 stationary phase provides highly sought after low hydrophobic retention, especially important for highly retentive biologics.

Size Exclusion (SEC)



bioZen SEC-2 1.8 μm

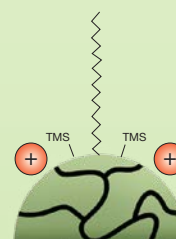
Extremely inert, high density fully porous particle with high efficiency and low molecular weight (LMW) separation range of 1 K–450 kDa.



bioZen SEC-3 1.8 μm

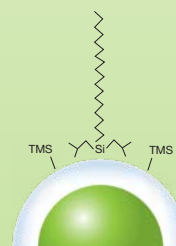
Extremely inert, high density fully porous particle with high efficiency and high molecular weight (HMW) separation range of 10K–700 kDa.

Peptide



bioZen Peptide PS-C18 1.6 μm and 3 μm

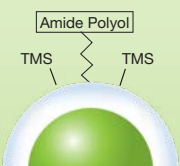
Excellent retention by combined positively charged surface ligand and C18 ligand.



bioZen Peptide XB-C18 1.7 μm and 2.6 μm

Overall retention of both acidic and basic peptides through C18 stationary phase with di-isobutyl side chains.

Glycan



bioZen Glycan 2.6 μm

Provides optimal combination of high efficiency and selectivity for released glycans.

We have 3 batches in stock of each material to ensure ease in method validation!

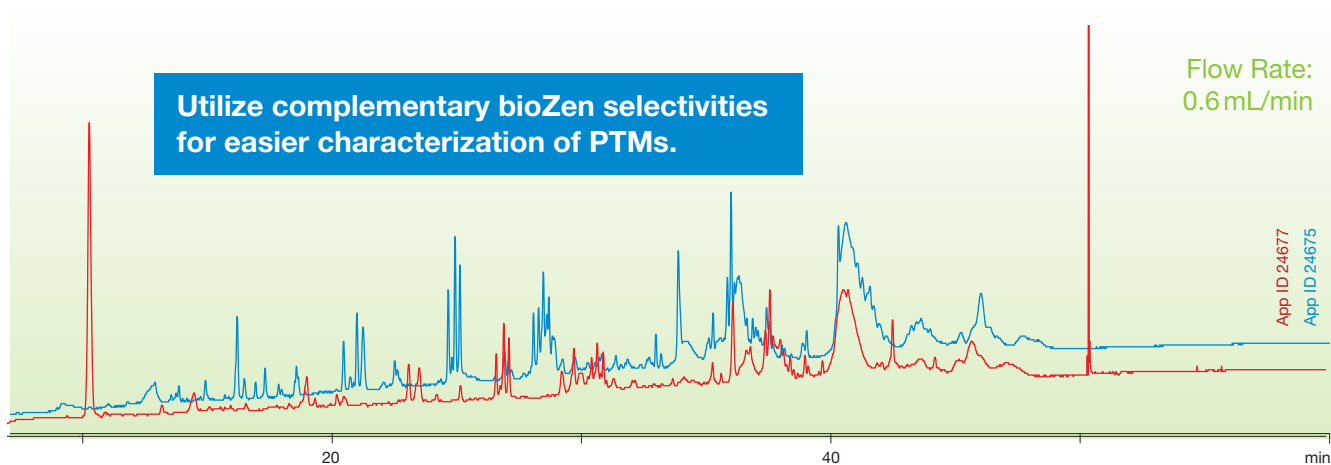


Peptide Mapping

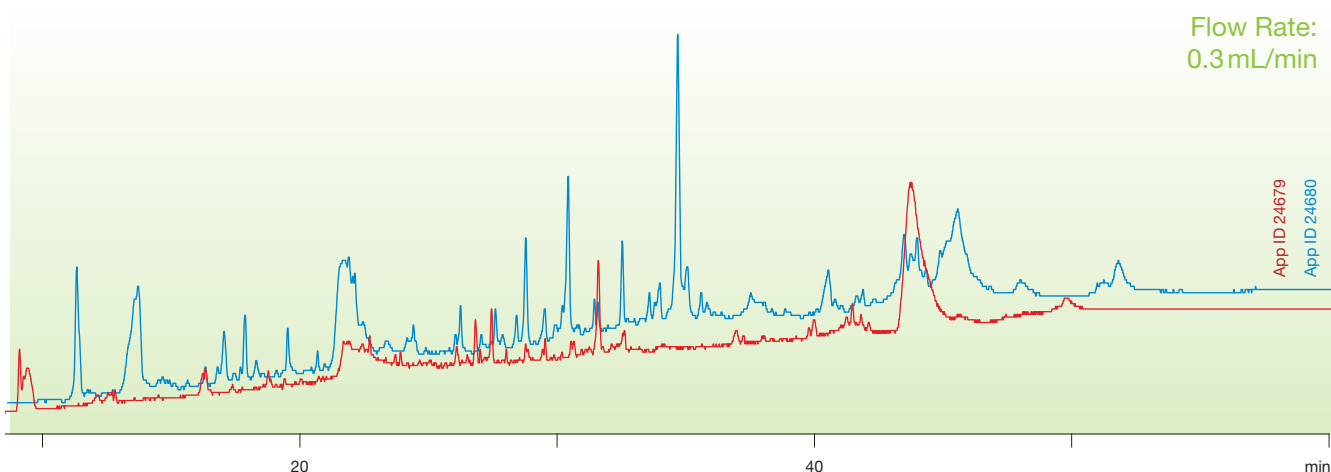
Digested mAbs or ADCs typically include a **large BODY of compounds** which are crucial to understanding post translation modifications. So we designed two bioZen Peptide columns to offer **highly useful and unique retention profiles**. Each allows for fast and effective elution windows by utilizing either high efficiency core-shell or thermally modified fully porous particles to gain sharper peaks, better peak capacities, and **overall higher sensitivity**.



Cetuximab Peptide Map



Anti Rituximab Fab Peptide Map



Conditions for all columns:

Columns: ■ bioZen 1.6 μm Peptide PS-C18
■ bioZen 1.7 μm Peptide XB-C18
Dimension: 150 x 2.1 mm
Part No.: 00F-4770-AN
 00F-4774-AN
Mobile Phase: A: 0.1% TFA in Water
 B: 0.1% TFA in Acetonitrile

Gradient:	Time (min)	% B
	0	1
	5	1
	60	50
	65	50
	70	95
	75	95
	78	95

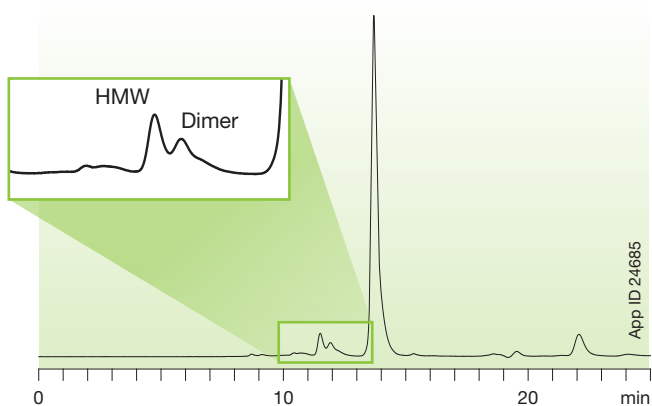
Flow Rate: as noted
Temperature: 40 °C
Detection: UV @ 214

Aggregate Analysis

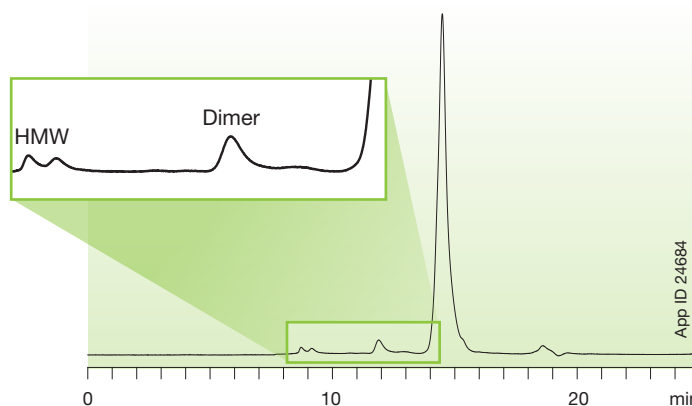
B
O
D
Y

With **mAb aggregate often at very low levels** (<0.1 % by peak area compared to monomer) and fragment separation a requirement, adequate resolution and peak shape have become even more crucial method outcomes. To address this need, the robust set of bioZen™ SEC columns were developed with a **combination of UHPLC efficiency and higher sensitivity**, to drive resolution and identification of even lower level targets.

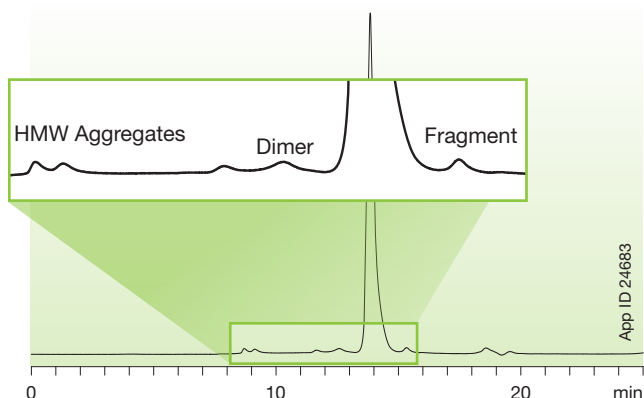
Adalimumab



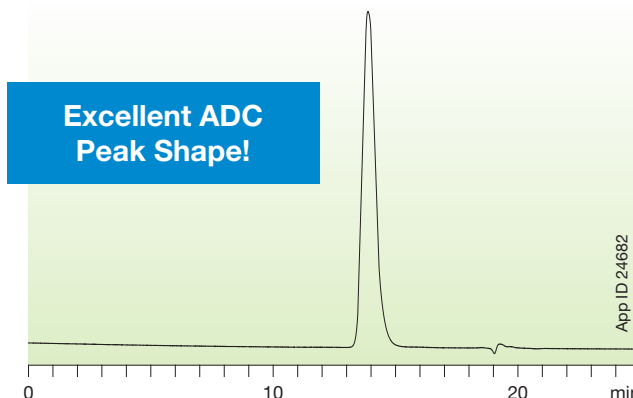
Trastuzumab



Rituximab



Herceptin - mcMMAF



Conditions same for all samples:

Column: bioZen 1.8 μm SEC-3
Dimension: 300 x 4.6 mm
Part No.: 00H-4772-E0
Mobile Phase: 50 mM Dipotassium phosphate + 100 mM Sodium Sulfate (pH 6.8)
Flow Rate: 0.2 mL/min
Temperature: 25 °C
Detection: UV @ 280 nm
Sample: As Noted



Acknowledgment

We would especially like to thank Colin McKee and ADC Biotechnology LTD for their support and ADC samples for this application.

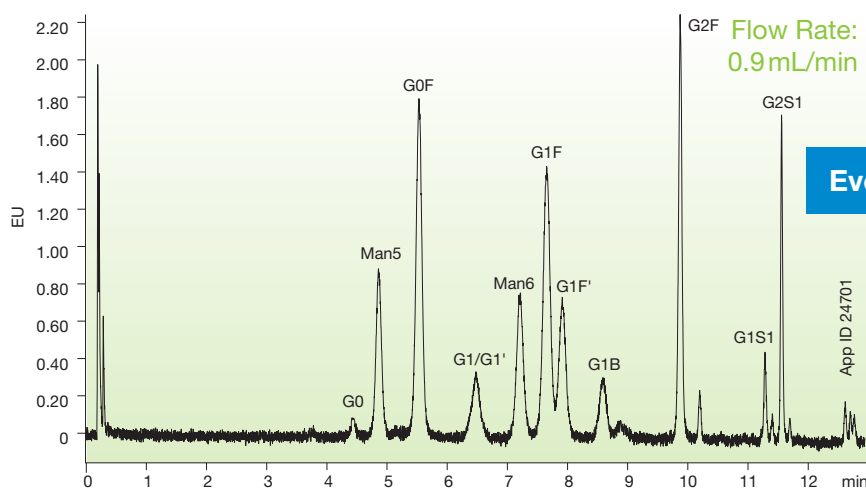
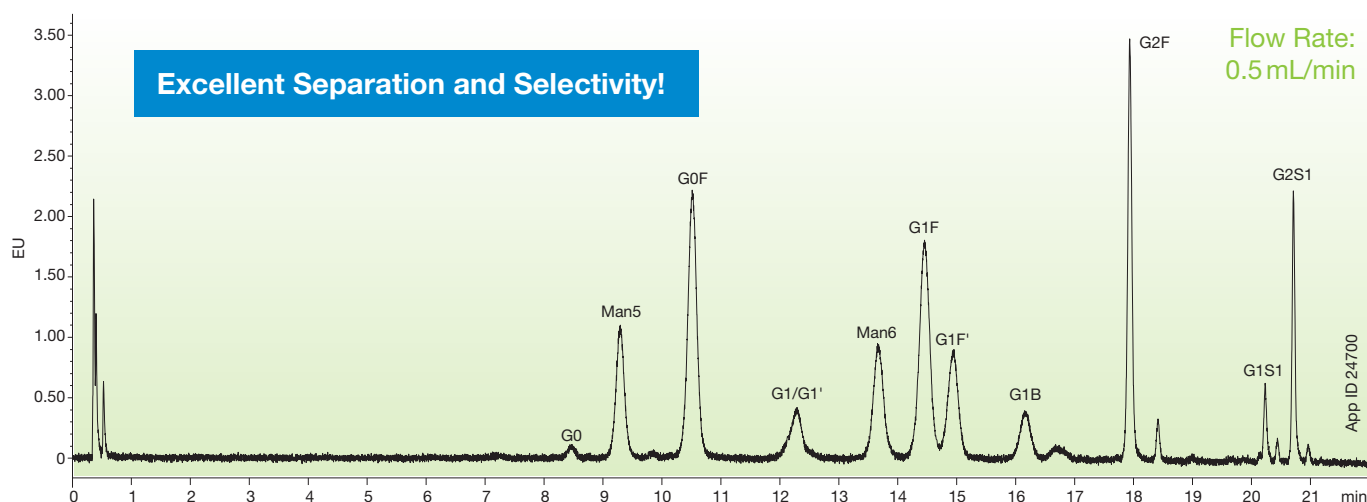


Glycan Analysis

The unique selectivity of the bioZen Glycan was designed to **provide higher order separations of released and labeled glycans**. With a 2.6 μm core-shell particle size, customers using either HPLC or UHPLC systems can draw upon a high efficiency bioZen Glycan particle run at higher linear velocities to easily provide sharper peak shapes and **faster elution windows**, without high UHPLC pressures. Under HILIC-FLR or HILIC-MS conditions, the bioZen Glycan excels with increased polar retention and selectivity.



2-AB Labeled Glycans from Standard Solution



Conditions for both columns:

Column: bioZen 2.6 μm Glycan
Dimensions: 100 x 2.1 mm
Part No.: 00D-4773-AN
Mobile Phase: A: 100 mM Ammonium Formate, pH 4.5
B: Acetonitrile

Gradient:	Time (min)	% B
	0	76
	16	72
	25.9	40
	27.2	40
	27.3	76
	30	76

Flow Rate: As noted
Temperature: 50 $^{\circ}\text{C}$
Detection: FLD ex/em 330/420 nm
Sample: Human IgG Glycan Library

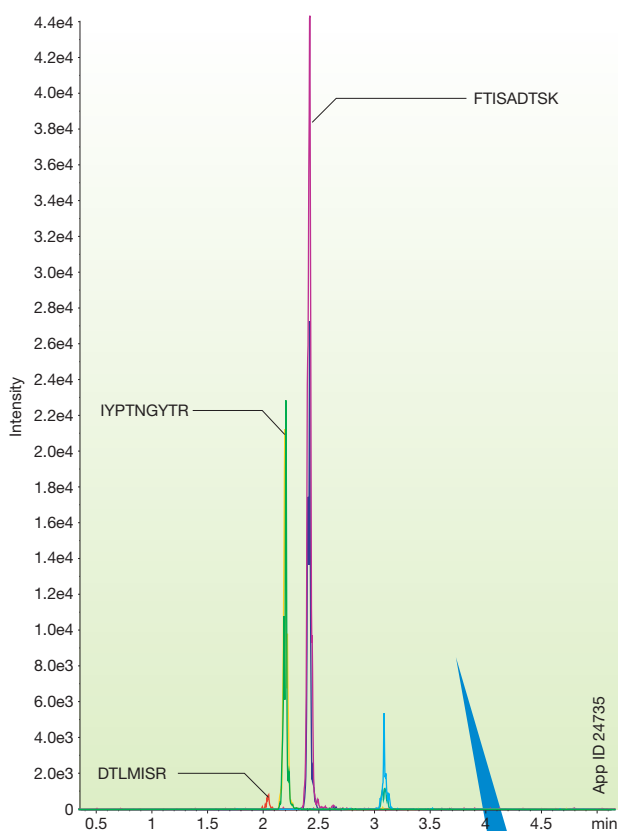
Peptide Quantitation

When quantitating signature peptides from biological matrices, you need sharp peak shape and sufficient retention of hydrophilic peptides to prevent any signal loss from matrix suppression regions. Both bioZen™ Peptide columns were developed to **deliver excellent selectivity for even closely related peptides**. Additionally, they build on this **BODY of valuable characteristics** with unique ways of delivering sharper peak shape for basic peptides; bioZen Peptide XB-C18 blocks secondary surface interactions via isobutyl side chains, while the bioZen Peptide PS-C18 contains a positively charged weak base that repels other basic species.



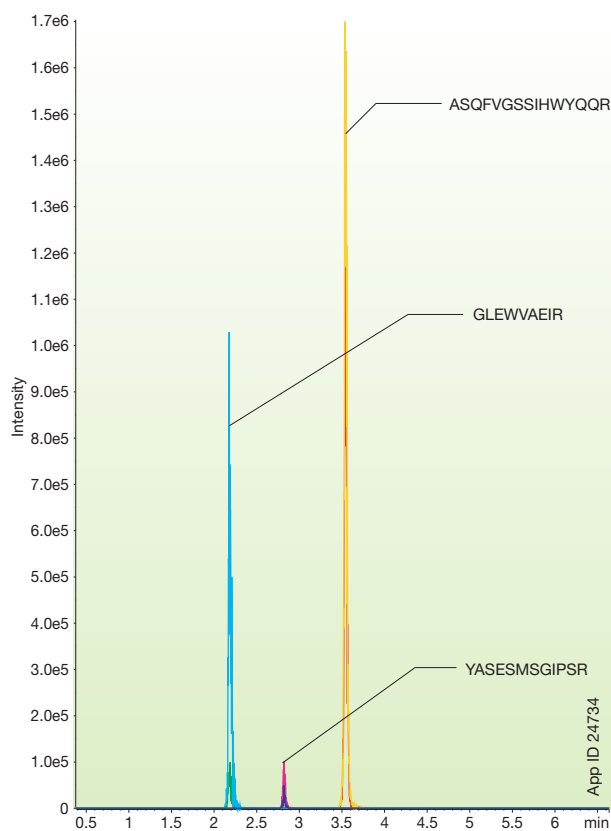
Kadcyla

(4 Signature Peptides)



Infliximab

(3 Signature Peptides)



Beautiful peak shape and height make quantitation with the PS-C18 a little like Lab Zen!

Conditions same for both samples:

Column: bioZen™ 3 μm Peptide PS-C18
Mobile Phase: A: 0.1% Formic Acid in Water
B: 0.1% Formic Acid in Acetonitrile
Gradient:

Time (min)	% B
0	3
1	3
4.5	25

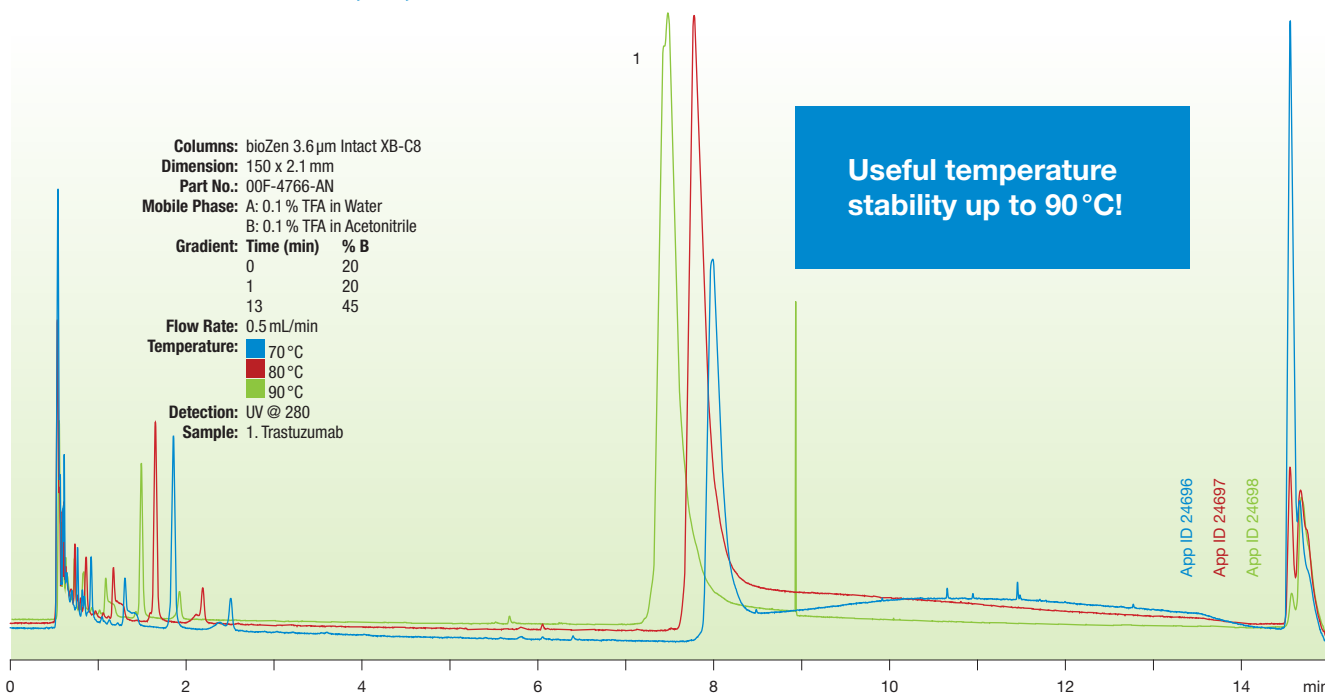
Flow Rate: 0.5 mL/min
Temperature: 22 °C
LC System: ExionLC™ AD HPLC
Detection: MS/MS
Detector: SCIEX QTRAP® 5500
Sample: As noted above



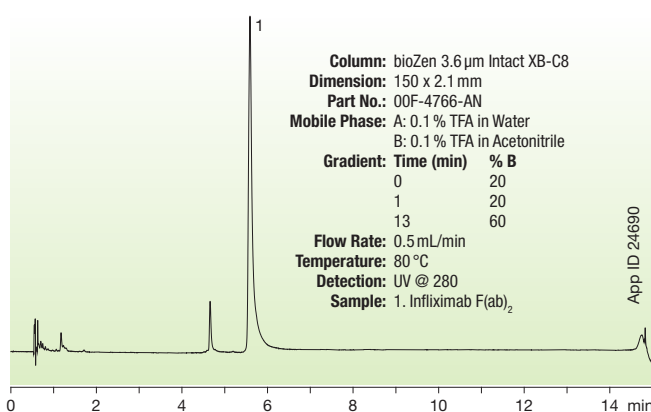
Intact & Fragment Analysis

Impurity profiling and characterization of intact biologic fragments is a challenging undertaking because of the need to identify very small differences between variants. Both bioZen Intact columns contain skillfully manufactured large pore core-shell particles that **provide narrower, taller peaks** in conjunction with **higher resolution between the target HC/LC, Fc/Fab, or isoforms**.

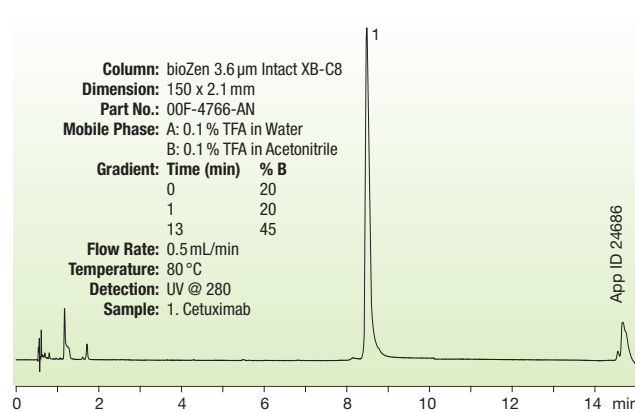
Intact Trastuzumab at 70, 80, and 90 °C



Infliximab F(ab)₂



Cetuximab



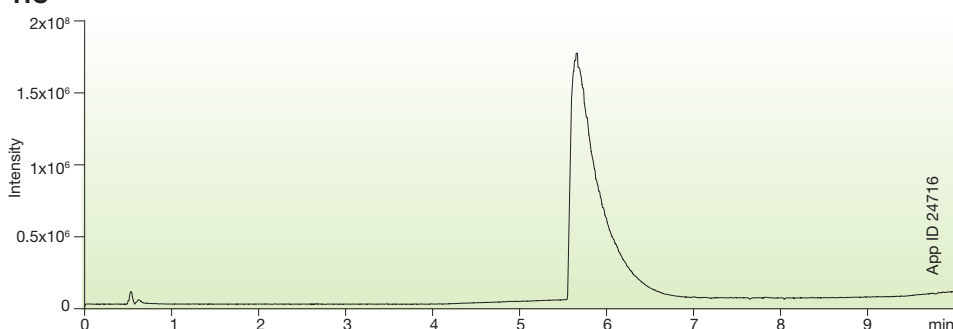
Intact Mass

Intact Mass can give indications not only of relative abundance of glycoforms, but also stability as degraded mAbs will not give good charge envelope by ESI-MS. Intact Mass with a high resolution MS to identify PTMs, especially relative abundance of glycoforms, **combines extremely well with the fast run times and tight peak shapes** provided by the bioZen™ Intact C4 and XB-C8.

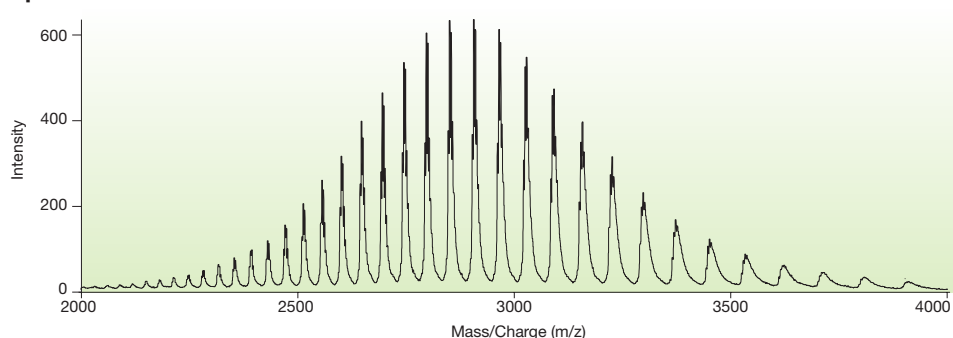
BIODATA

Intact Mass of NIST mAb using a bioZen Intact C4 and SCIEX® X500B

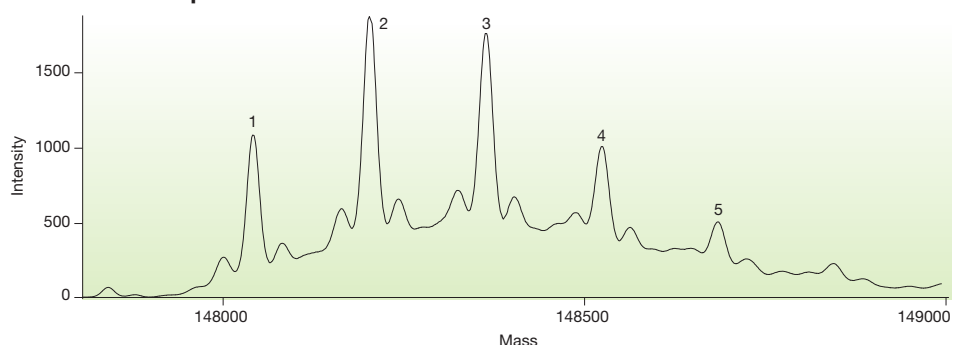
TIC



Spectra NIST mAb



Deconvoluted Spectra NIST mAb



Columns: bioZen 3.6 µm Intact C4
Dimension: 150 x 2.1 mm
Part No.: 00F-4767-AN
Mobile Phase: A: 0.1 % Formic Acid in Water
B: 0.1 % Formic Acid in Acetonitrile

Gradient:	Time (min)	% B
	0	15
	3	15
	9	90
	11.4	90
	11.5	85
	15	85

Flow Rate: 0.5 mL/min
Temperature: 80 °C
Detection: QTOF (SCIEX® X500B)

Sample:	mAb Glycoform	MW
1.	G0F/G0F	148038.5
2.	G0F/G1F	142801.5
3.	G1F/G1F	148363.6
	G0F/G2F	
4.	G1F/G2F	148524.8
5.	G2F/G2F	148685.5

Simplified Biologics Characterization Workflows on the X500B QTOF System

Accelerate your throughput with this easy-to-use benchtop QTOF system that combines robust instrumentation with powerful and intuitive software to get your characterization answers faster and easier.

Learn More at www.sciex.com/X500B

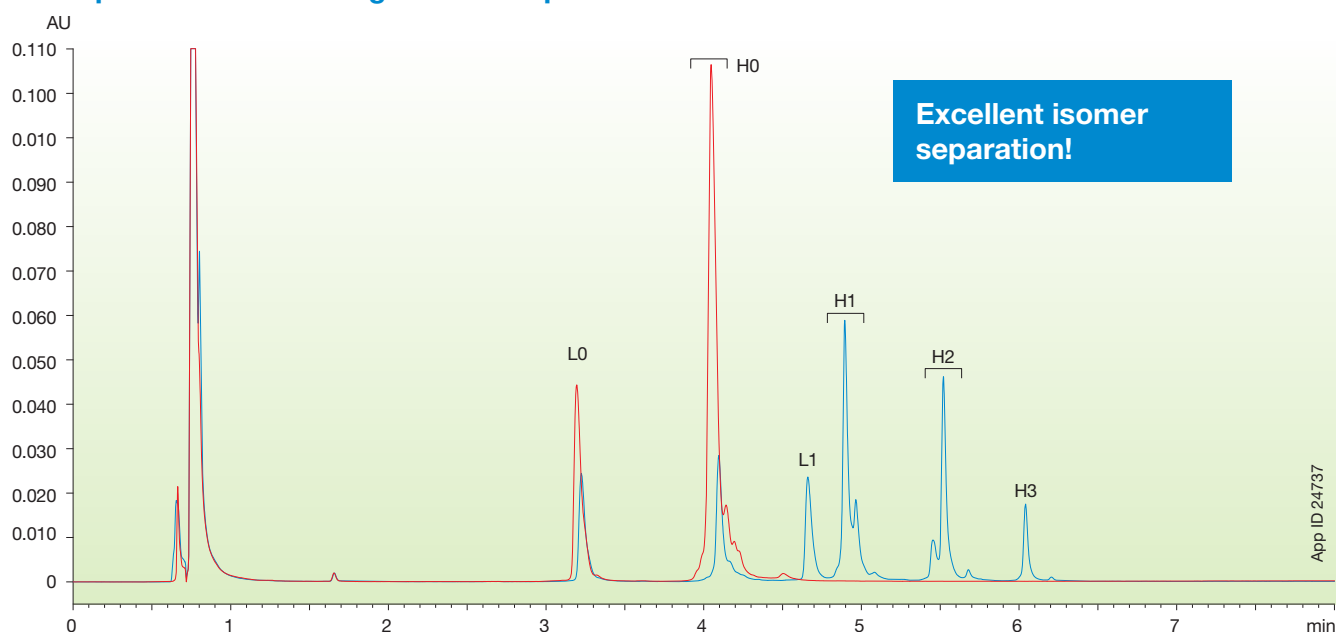
SCIEX



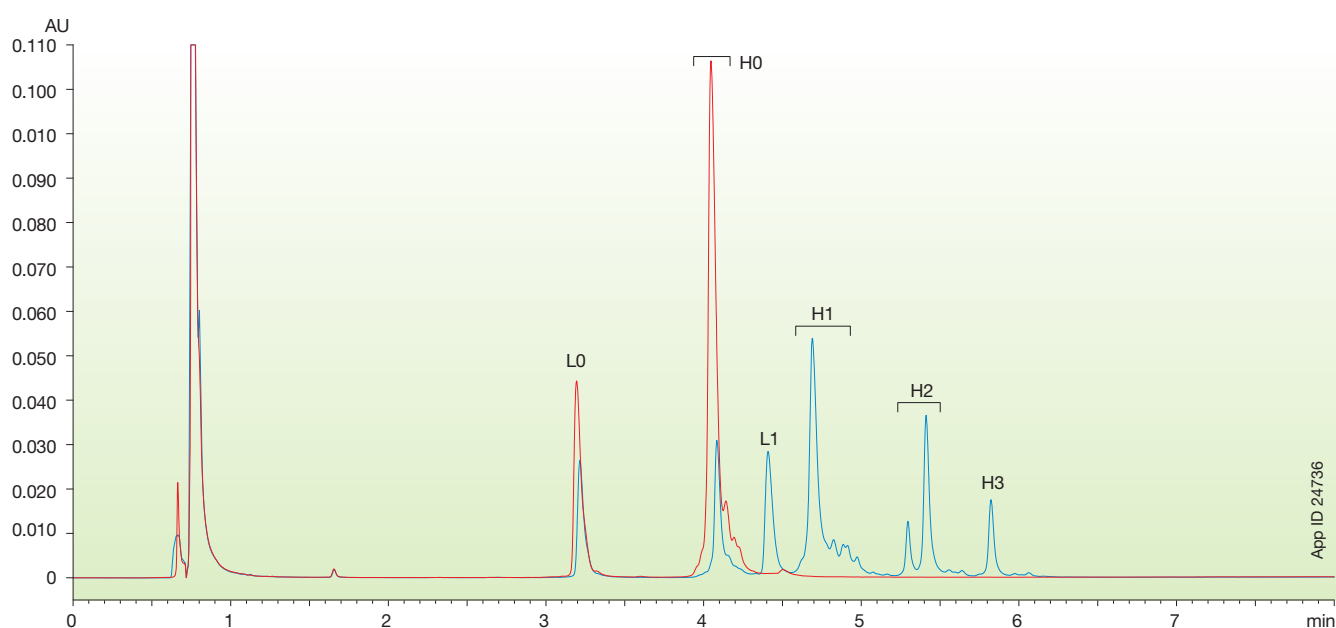
Drug Antibody Ratio (DAR)

With a direct effect on efficacy and safety, **conjugation for each ADC must be well understood**. The bioZen Intact XB-C8 provides an excellent vehicle for determining drug load distribution and DAR for ADCs. Its large pore size allows intact ADCs to interact with a moderately retentive stationary phase while the core-shell particle supplies increased efficiency to **deliver the required resolution between ADC species with differing drug loads**.

Herceptin—vcMMAE using bioZen 3.6µm Intact XB-C8



Herceptin—mcMMAF using bioZen 3.6µm Intact XB-C8



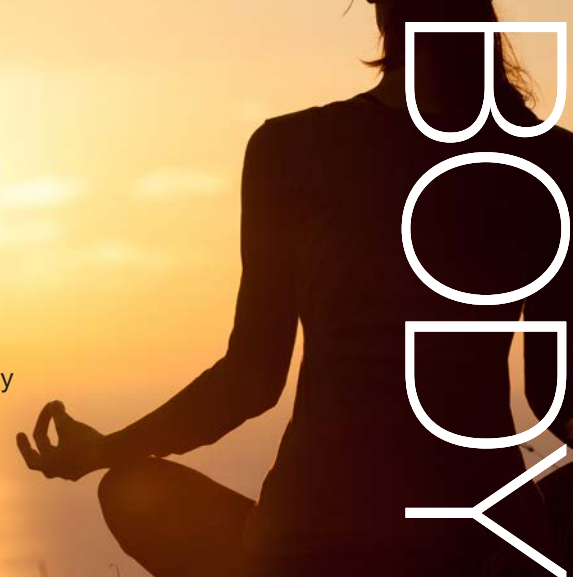
Acknowledgment
We would especially like to thank Colin McKee and ADC Biotechnology LTD for their support and ADC samples for this application.

Find the conditions online at:
www.phenomenex.com/bioZen

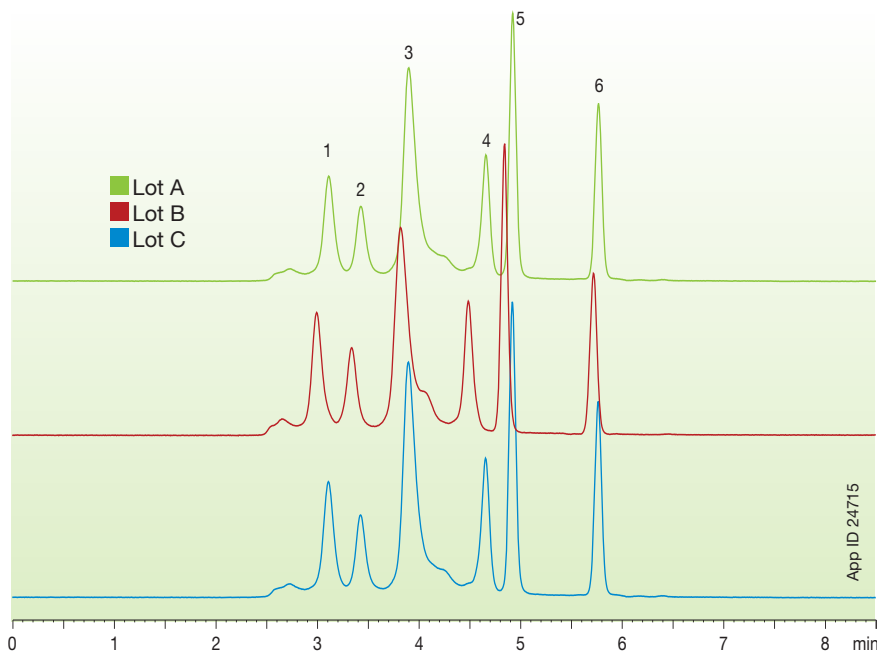
Bio QC Testing

At every stage of our manufacturing and quality testing we keep you and your biologics analysis in mind. We initially focus on innovative products that will enhance workflows, then we work tirelessly to ensure that those products are reliably made time and time again. To further enrich the quality of these products, we assign very specific application-oriented testing protocols that properly mimic the conditions that you and other customers ultimately require.

Each batch of media and each column goes through a gambit of testing to ensure that you're getting our highest level of science, so that you can kick down the door of progress.

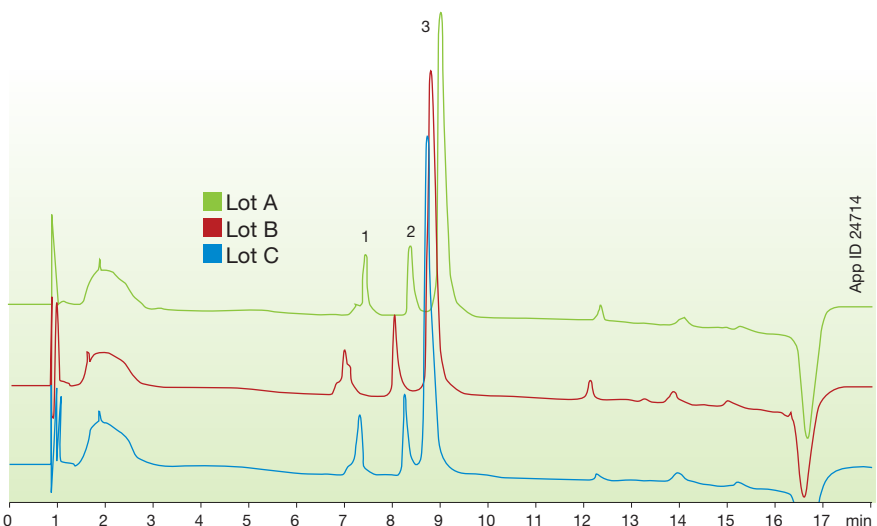


Batch-to-Batch Results—bioZen™ 1.8 µm SEC-3



Column: bioZen 1.8 µm SEC-3
Dimensions: 150 x 4.6 mm
Part No.: 00F-4772-E0
Mobile Phase: 100 mM Sodium Phosphate in Water pH 6.8
Flow Rate: 0.3 mL/min
Temperature: Ambient
Detection: UV @ 280 nm
Sample: 1. Thyroglobulin (669 kDa)
2. IgA (300 kDa)
3. IgG (150 kDa)
4. Ovalbumin (44 kDa)
5. Myoglobin (17 kDa)
6. Uridine

Batch-to-Batch Results—bioZen 3.6 µm Intact C4



Columns: bioZen 3.6 µm Intact C4
Dimension: 150 x 2.1 mm
Part No.: 00F-4767-AN
Mobile Phase: A: 0.1 % TFA in Water
B: 0.1 % TFA in Acetonitrile
Gradient:

Time (min)	% B
0	10
1	30
20	50
20.1	10

Flow Rate: 1.5 mL/min
Temperature: 40 °C
Detection: UV @ 280 nm
Sample: 1. Light Chain
2. Heavy Chain
3. Intact mAb

The bioZen Flow— Column Selection

We wanted to copy your dedication to biologics assays, so we **put our hearts and SOULS** into the **development** of the bioZen™ portfolio. Throughout the development of a biologic, bioZen separation products provide enhanced characterization over an incredibly wide range of techniques.

Screening / Early Development mAb

Peptide Mapping (RP-MS)

- Whole mAb
 - Fab region
- bioZen Peptide PS-C18
bioZen Peptide XB-C18

Aggregation (SEC)

bioZen SEC-3

Aggregation (High-Throughput SEC)

bioZen SEC-3



Average DAR ADC (RP-UV)

bioZen Intact XB-C8

Glycan Analysis (HILIC-FL)

bioZen Glycan

Glycan Analysis (HILIC-MS)

bioZen Glycan



Biocompatible Titanium Hardware:

Better recovery and reproducibility for all workflows!



**Preclinical
mAb**

**Formulation
(SEC)**

bioZen™ SEC-2
bioZen SEC-3

**Total mAb
(RP-UV)**

bioZen Intact C4
bioZen Intact XB-C8

**Intact Mass
(RP-MS)**

bioZen Intact C4
bioZen Intact XB-C8

**Peptide Quantitation
(RP-MS)**

bioZen Peptide PS-C18
bioZen Peptide XB-C18

**Total mAb
(SEC-UV)**

bioZen SEC-2
bioZen SEC-3



Size Exclusion and a Well Salted Buffer



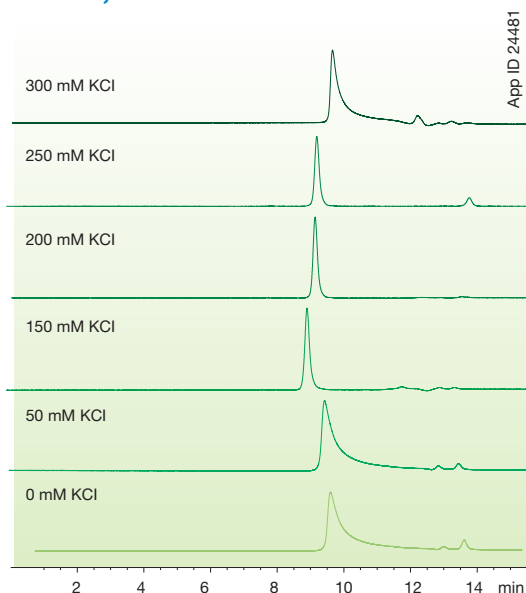
Dani Xing

Technical Guru - Bioseparations

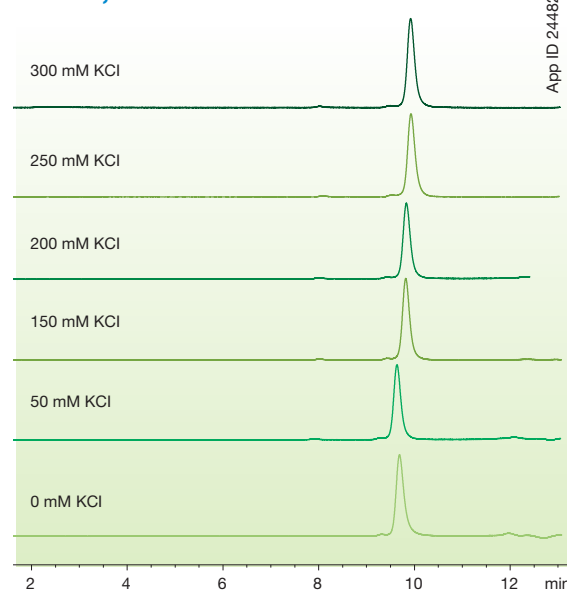
When developing a method for aggregate analysis of mAbs by SEC, it is critical to optimize mobile phase conditions to prevent non-specific secondary interactions. Below, you can see the effect of altering salt concentration in the mobile phase for two different biosimilar mAbs. The first mAb required a moderate amount of salt for acceptable peak shape. The second mAb performed well even with no salt. However, increases in salt showed incremental improvements in peak shape.

Ideally, buffer and salt concentration are optimized based upon the requirements for the method or analysis. However, when there is a need for a platform method, like when needed to evaluate several different mAbs, a good starting point for method development is 50mM monopotassium phosphate, 250mM potassium chloride, pH 6.8.

mAb 1, SEC Profiles



mAb 2, SEC Profiles



Conditions same for both samples, except where noted:

Column: bioZen 1.8 μ m SEC-3
Dimensions: 300 x 4.6 mm
Part No.: 00H-4772-E0
Mobile Phase: 50 mM KH_2PO_4 , pH 6.8
 KCl (as indicated)
Flow Rate: 0.3 mL/min
Detection: UV @ 280 nm
Temperature: Ambient

Deglycosylation Topics

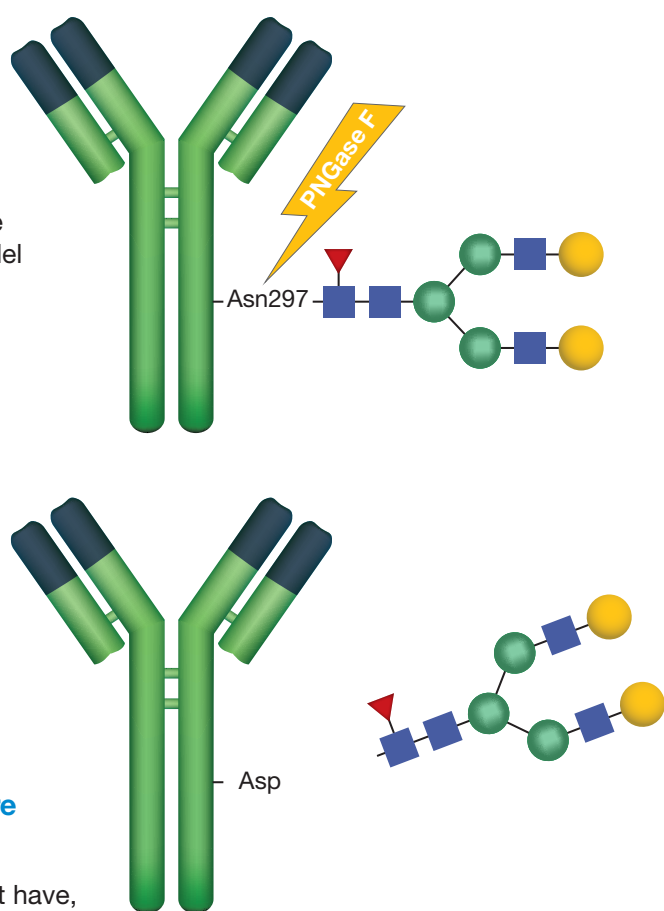


How should I deglycosylate my antibody?

PNGase F is an endoglycosidase that cleaves N-glycans without bias, except for any that are core fucosylated $\alpha(1-3)$ —might I add if you're working with insects and plants, congrats, you're doing some rather interesting work in the world of glycobiology.

Most protocols for PNGase F were originally developed to deglycosylate complex glycoproteins; i.e. proteins with multiple glycosylation sites. For example, bovine fetuin, a common model glycoprotein, has 18 glycosylation sites.¹ As such, most protocols are developed using overnight deglycosylation to ensure deglycosylation to completion.

But if you need your answers tomorrow, what do you do? For a less complex glycoprotein like an IgG1 (2 glycosylation sites in the conserved region at Asn297), a shorter digestion time is acceptable. In fact, most vendors sell PNGase F formulated for faster deglycosylation, in some cases ten minutes or less. Furthermore, because the glycosylation sites are easily accessible, no denaturation is required.²



Why should I deglycosylate my ADC or antibody before intact mass?

Depending on how many different glycoforms the sample might have, a high degree of complexity in glycosylation could lead to some pretty messy spectra, which is especially difficult with ADCs.

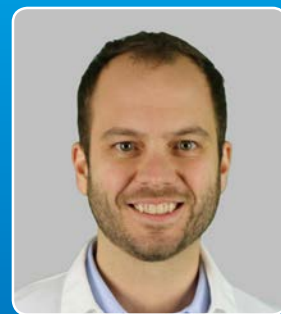
As such, deglycosylation should be able to provide much nicer spectra, thus better assessment of relative quantitation of different DAR species, as well as average DAR.

One thing to always keep in mind—deglycosylation of the N-linked glycan yields an aspartic acid (Asp), resulting in a mass shift of 1 Da. Also to bear in mind—PNGase F reactions buffer is typically a Tris buffer, i.e. relatively high pH. Deamidation might be observed, commonly with the N-G motif; faster deglycosylation protocols might thus be desired.

1. Nwosu, Charles C., et al. "Simultaneous and Extensive Site-Specific N- and O-Glycosylation Analysis in Protein Mixtures." *Journal of Proteome Research*, vol. 10, no. 5, June 2011, pp. 2612–2624. doi:10.1021/pr2001429

2. Hosfield, C., Engel, L., Pagueio, A., Surowy, T., Jones, R., Ford, M., Urh, M., Rosenblatt, M. Recombinant PNGase F for Glycoprotein Analysis. Promega Corporation Web site. <http://www.promega.com/resources/pubhub/recombinant-pngase-f-for-glycoprotein-analysis-article/> Updated 2013. Accessed January 29, 2018.

Loading Capacity for SEC and RP



Chad Eichman, Ph.D.
BioPharm Global Marketing Manager

How do I determine the loading capacity of a SEC column?

For size exclusion, there are two considerations—sample volume and sample concentration.

As a general rule, load no more than 5% of the column volume. Theoretically, a 300 x 4.6 mm column, with a column volume of ~5 mL, would limit injection volume to 200 μ L. In practice, volumes of 10-30 μ L are common.

Another important consideration is sample concentration; the higher the concentration of protein, the higher the viscosity of the sample, and this difference in viscosity can lead to peak shape distortion (either through exclusion effects or a solvent front referred to as “viscous fingering”). A good starting point is 1 mg/mL, though optimal concentrations must be determined experimentally.

What is the loading capacity of bioZen™ Intact and Peptide columns?

For bioZen Peptide columns, similar loads as other RP-LC columns can be used: 5-20 μ g of digest or peptide mixture on a 4.6 mm ID column will give good sensitivity (especially for LC-MS) for peptide separations. Up to 50 μ g can be loaded of a digest without increasing peak width too severely. For 2.1 mm ID columns, load should be scaled accordingly.

Because bioZen Intact columns have lower surface area, loading can drastically effect peak shape and must be determined experimentally for optimal results. For 4.6 mm ID's, 5 μ g is a good starting point. For 2.1 mm ID's, 1 μ g is a good starting point. Increasing in load may increase peak tailing and peak width significantly.



Organic Solvent and Size Exclusion

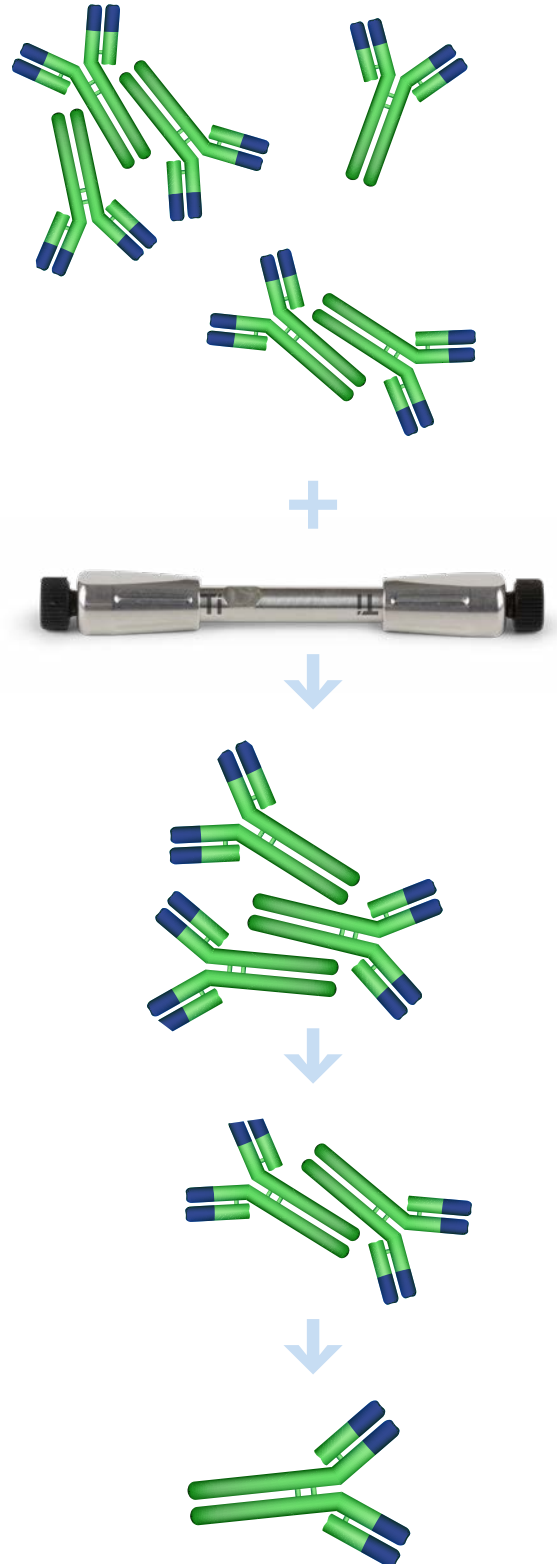


Organic Solvent and Size Exclusion

In order to get an “ideal” SEC separation (i.e. purely entropic separation, with no interaction of analyte with stationary phase), oftentimes some organic modifier, 5-15 % isopropanol or acetonitrile, might be necessary.

However, the question now is whether the protein is in a truly native state; one of the main contributors to aggregation are the hydrophobic interactions between monomers and fragments.

Most methods for ADCs use some organic, with 15 % IPA being the most common. This is widely accepted as appropriate for assessing aggregate, though results might need to be confirmed with an orthogonal sedimentation velocity analytical ultracentrifugation (SV-AUC).



How should a column be cleaned if it is typically used to analyze protein samples?

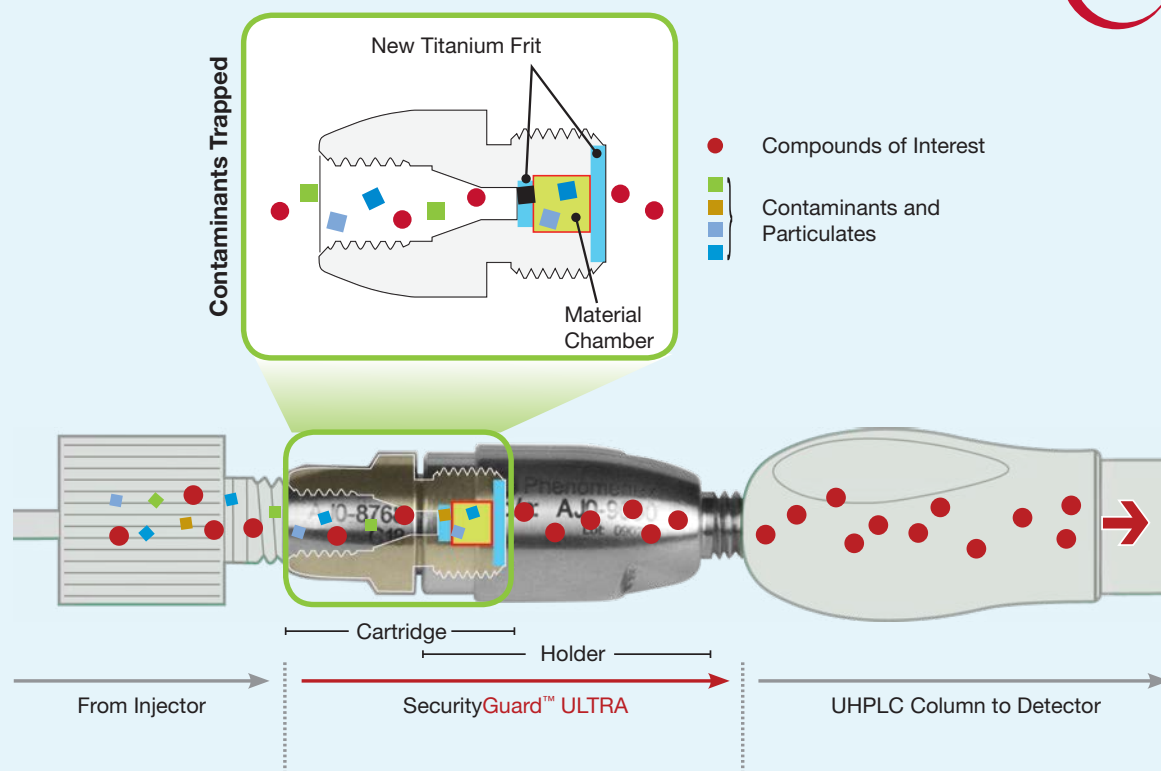
If strong ionic interactions between proteins and the stationary phase are suspected, then start cleaning with a denaturant such as 6 M guanidine hydrochloride or 10 % DMSO. If the protein is relatively hydrophobic, start by flushing out buffer with 95-100 % water, then clean out the hydrophobic proteins with a gradient from 95 % water/5 % acetonitrile up to 5 % water/95 % acetonitrile over 3-5 column volumes. During each step, be mindful that backpressures do not exceed the recommended limits; adjust flow rates as necessary.

Biocompatible Column/System Protection



The easiest way to extend column performance and minimize costly system and detector upkeep is to prevent contaminants and particulates from getting into and past your LC column with a guard system. SecurityGuard guard cartridge systems provide this protection and truly make it easy to acquire this benefit on both HPLC and UHPLC systems.

SecurityGuard ULTRA Guard Cartridge System with Titanium



* Cartridge schematic not drawn to scale



Sensitive Clean-Up for Small Sample Volumes



Without the need for dry-down and reconstitution, Strata-X Microelution SPE 96-well plates provide consistent sample preparation results with two big benefits: Better absolute recovery and greater time savings.

www.phenomenex.com/microelution

Product Ordering Information



bioZen™ Products - Powered by Biocompatible Hardware

bioZen Columns (mm)						Biocompatible Guard Cartridges		
	100 x 2.1	150 x 2.1				for 2.1 mm	Holder	
						/3pk		ea
bioZen 2.6 µm Glycan	00D-4773-AN	00F-4773-AN				AJO-9800		AJO-9000
	50 x 2.1	150 x 2.1				for 2.1 mm	Holder	
						/3pk		ea
bioZen 1.6 µm Peptide PS-C18	00B-4770-AN	00F-4770-AN				AJO-9803		AJO-9000
	50 x 4.6	150 x 4.6				for 4.6 mm	Holder	
						/10pk		ea
bioZen 3 µm Peptide PS-C18	00B-4771-E0	00F-4771-E0				AJO-7606		KJO-4282
	50 x 2.1	150 x 2.1				for 2.1 mm	Holder	
						/3pk		ea
bioZen 1.7 µm Peptide XB-C18	00B-4774-AN	00F-4774-AN				AJO-9806		AJO-9000
	50 x 2.1	150 x 2.1	250 x 2.1	50 x 4.6	150 x 4.6	for 2.1 mm	for 4.6 mm	Holder
						/3pk	/3pk	ea
bioZen 2.6 µm Peptide XB-C18	00B-4768-AN	00F-4768-AN	00G-4768-AN	00B-4768-E0	00F-4768-E0	AJO-9806	AJO-9808	AJO-9000
	50 x 2.1	150 x 2.1	50 x 4.6	150 x 4.6		for 2.1 mm	for 4.6 mm	Holder
						/3pk	/3pk	ea
bioZen 3.6 µm Intact C4	00B-4767-AN	00F-4767-AN	00B-4767-E0	00F-4767-E0		AJO-9809	AJO-9811	AJO-9000
bioZen 3.6 µm Intact XB-C8	00B-4766-AN	00F-4766-AN	00B-4766-E0	00F-4766-E0		AJO-9812	AJO-9814	AJO-9000
	150 x 4.6	300 x 4.6				for 4.6 mm	Holder	
						/3pk		ea
bioZen 1.8 µm SEC-2	00F-4769-E0	00H-4769-E0				AJO-9850		AJO-9000
bioZen 1.8 µm SEC-3	00F-4772-E0	00H-4772-E0				AJO-9851		AJO-9000

guarantee

If bioZen columns in this brochure do not provide at least equivalent separations to a competing column of the same phase, particle size, and dimensions, return the Phenomenex column with comparative data within 45 days for a FULL REFUND.

Ensure Protein Recovery with Biocompatible Accessories!



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Strata-X is patented by Phenomenex. U.S. Patent No. 7,119,145

SecurityGuard is patented by Phenomenex. U.S. Patent No. 6,162,362

CAUTION: this patent only applies to the analytical-sized guard cartridge holder, and does not apply to SemiPrep, PREP or Ultra holders, or to any cartridges.

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