

Take A Deep Breath

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



+

8

+



+



Peptide Mapping (RP)

Aggregate Analysis (SEC)

NEW Charge Variant Analysis (IEX)

Glycan Analysis (HILIC)

Peptide Quantitation (RP)

Intact and Fragment Analysis (RP)

Intact Mass (RP)

Drug Antibody Ratio (RP)

NEW Glycan Sample Prep (SPE)

 **phenomenex**
...breaking with traditionSM

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?

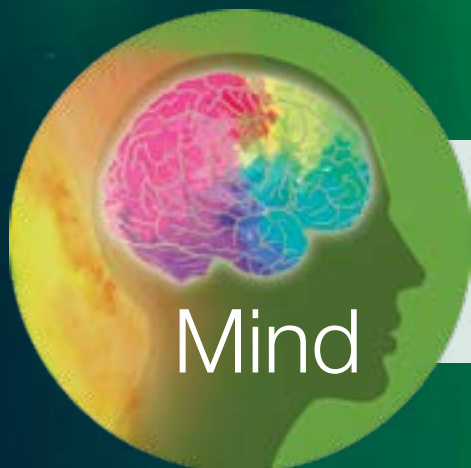
YES

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

YES

And that's not all. Welcome to bioZen.



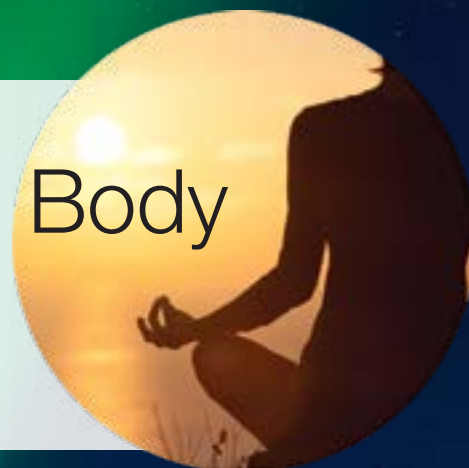


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Balanced pathway to bioseparations success...20-21

The bioZen™ Flow – Easy Column Selection

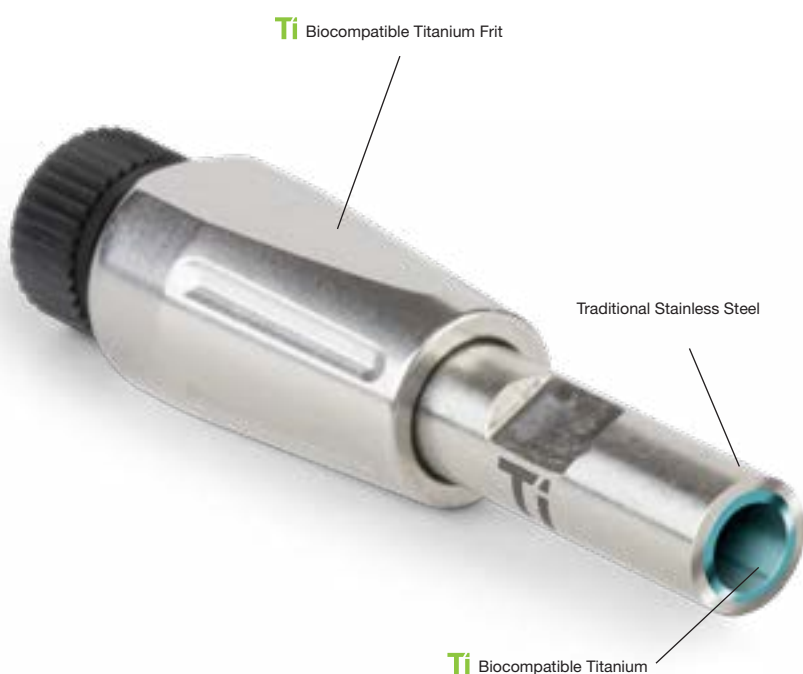
Engaged gurus to expand your reach 22-27

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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!



- Charge Variant Analysis
- 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

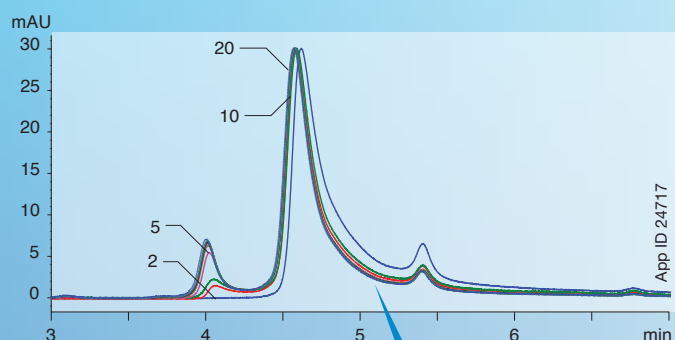
The new biocompatible SecurityGuard™ Standard and ULTRA cartridge systems remove unwanted contaminants before they clog your column or system. Each bioZen™ column has a matching guard to ensure workflow applicability. Learn more on page 26.

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.

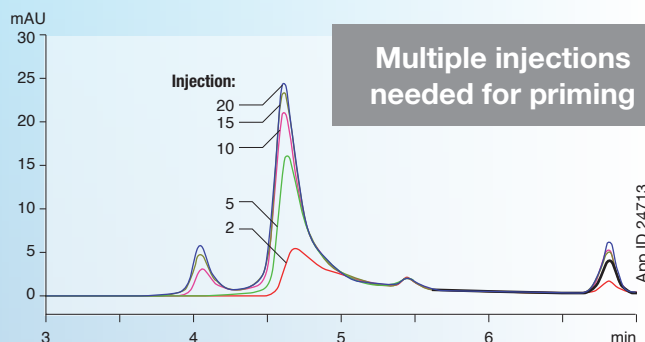


Overlaid Successive Injections – Protein Priming Comparison

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

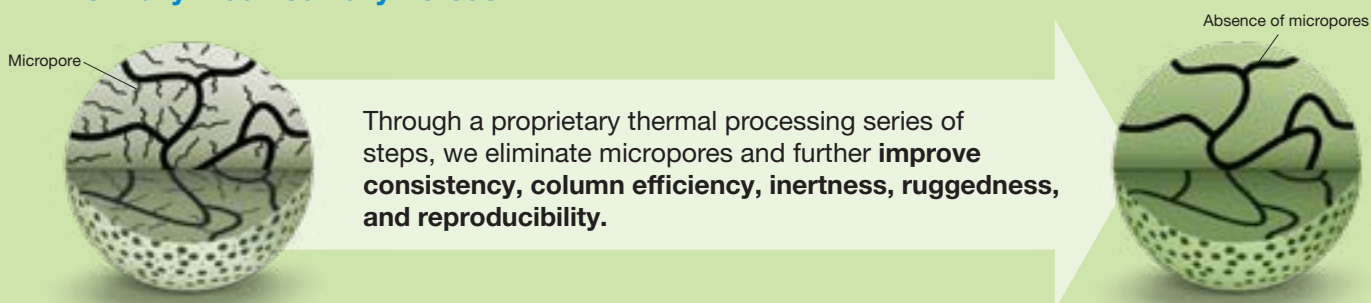
Conditions for both columns:

Column: bioZen 1.8 μ m SEC-3
Dimension: 150 x 4.6 mm
Mobile Phase: 50 mM Dipotassium Phosphate + 100 mM Sodium Sulfate, pH 5.0
Flow Rate: 0.3 mL/min
Temperature: Ambient
Detection: UV @ 280 nm
Sample: 1. γ -Globulin, 5 mg/mL
2. Ovalbumin, 1 mg/mL

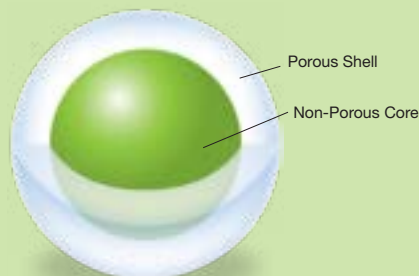
3 Advanced Particle Platforms

All three of the new bioZen™ particle platforms were individually designed and built by Phenomenex to take advantage of integral levels of performance, ruggedness, and reproducibility for protein characterization applications. Individually, each platform differs in the proprietary processing techniques used to control particle size and morphology. With such MINDfulness towards particle details, just imagine what our labs look like!

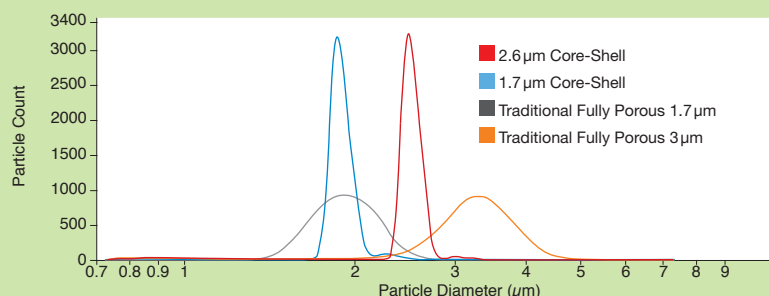
Thermally Modified Fully Porous



Core-Shell Technology

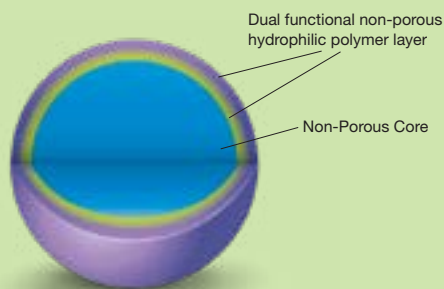


Uniform Particle Size Distribution

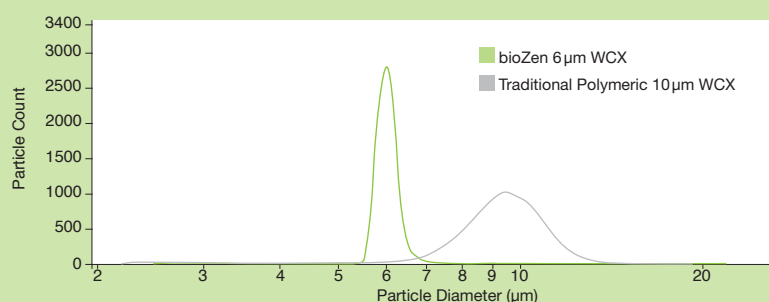


Using sol-gel processing techniques that incorporate nano structuring technology, a durable, homogeneous porous shell is grown on a solid silica core. This highly optimized process combined with industry leading column packing technology produces **highly reproducible columns that generate extremely high efficiencies and sensitivity.**

Monosized Polymeric Non-Porous



Uniform Particle Size Distribution



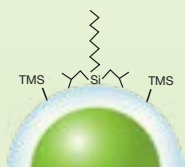
Meticulously controlled monosized particle technology secures **incredible particle consistency that leads to improved and reliable efficiency.** This innovative non-porous particle serves as the perfect backbone for complex ion-exchange chemistries.

8 Particle Chemistries

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.

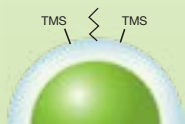


Intact



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

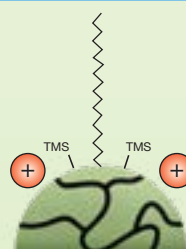
Extremely inert, high density fully porous particle with high efficiency and low molecular weight (LMW) separation range of 1 k–450 kDa.



bioZen SEC-3
1.8 μm

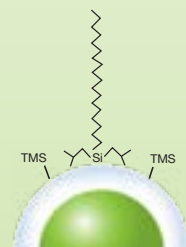
Extremely inert, high density fully porous particle with high efficiency and high molecular weight (HMW) separation range of 10 k–700 kDa.

Peptide



bioZen Peptide PS-C18
1.6 μm and 3 μm

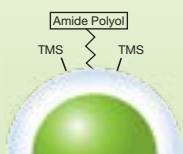
Excellent retention by combined positively charged surface ligand and C18 ligand.



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.

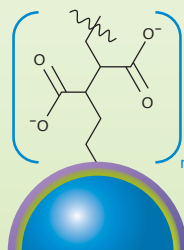
Glycan



bioZen Glycan
2.6 μm

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

Ion-Exchange



bioZen WCX
6 μm

Monosized particles grafted with linear polycarboxylate chains to envelop and separate proteins from acidic/basic variants

MIND Protein Meets Separation

We decided to **keep in MIND** that biologics prefer it if Biochemists and Chromatographers combine forces. All jokes aside, **our talent is at your disposal** and we have an incredible array of experience in all areas of protein chemistry, conjugation, sample preparation, analysis, and detection.

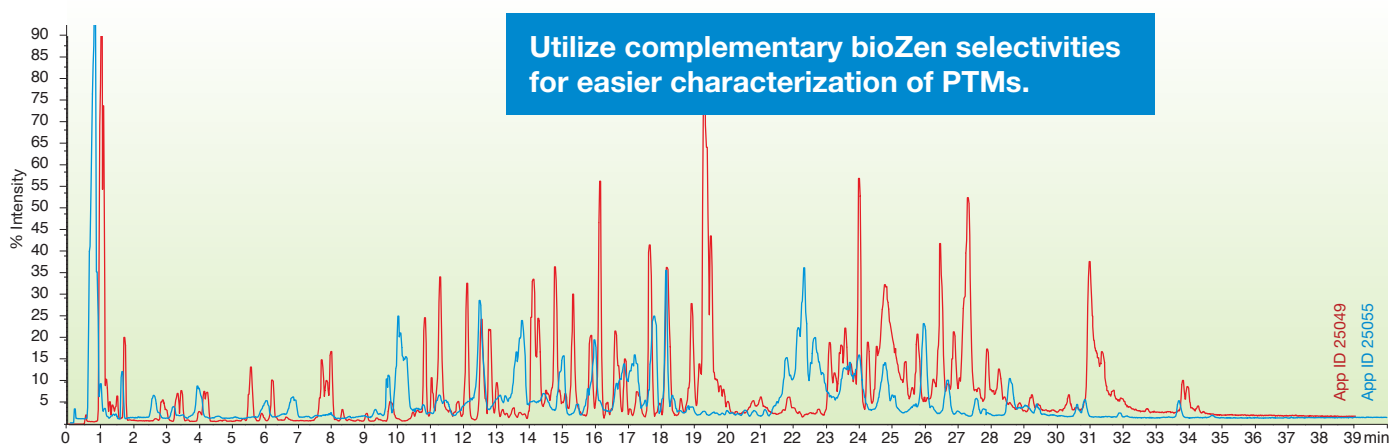


Peptide Mapping

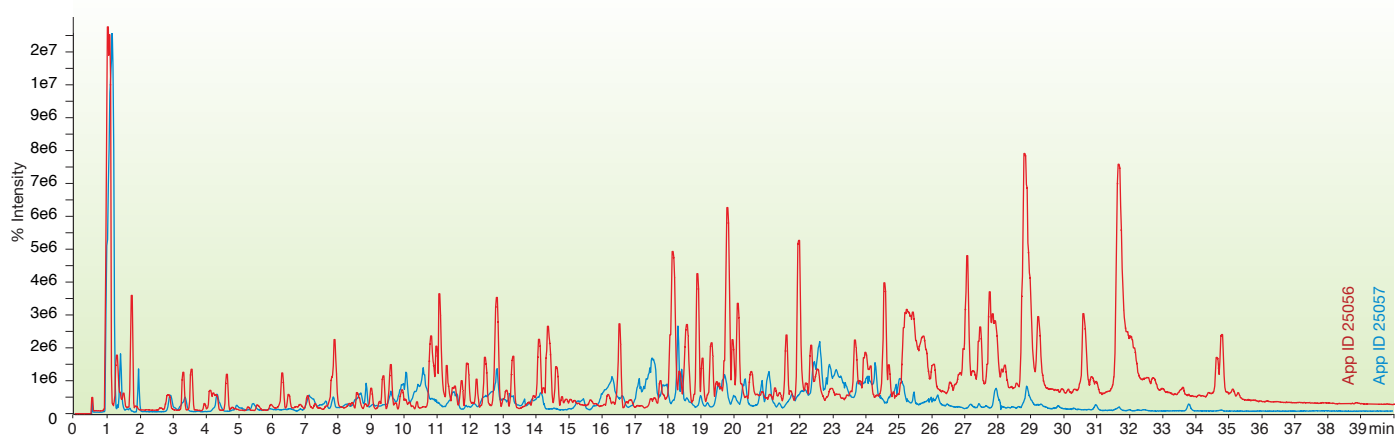
Digested mAbs or ADCs typically include a **large BODY of compounds** which are crucial to understanding post translation modifications. So we designed two bioZen™ Peptide columns to offer **highly useful and unique retention profiles**. Each allows for fast and effective elution windows by utilizing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

BODY

Trastuzumab Biosimilar Peptide Map



Infliximab Biosimilar Peptide Map



Conditions for all columns:

Columns: bioZen 1.6µm Peptide PS-C18
bioZen 2.6µm Peptide XB-C18
Dimension: 150 x 2.1 mm
Part No.: 00F-4770-AN
00F-4768-AN
Mobile Phase: A: 0.1 % Formic Acid in Water
B: 0.1 % Formic Acid in Acetonitrile

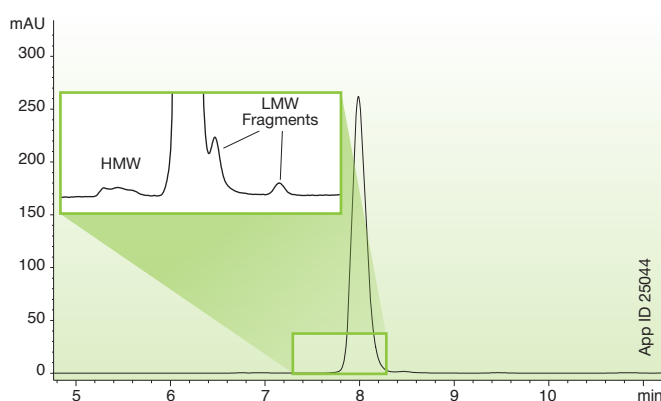
Gradient: Time (min) % B
0 1
0.5 1
50 50
55 50
56 95
Flow Rate: 0.3 mL/min
Temperature: 40 °C
Detection: QTOF (SCIEX® X500B)

Aggregate Analysis

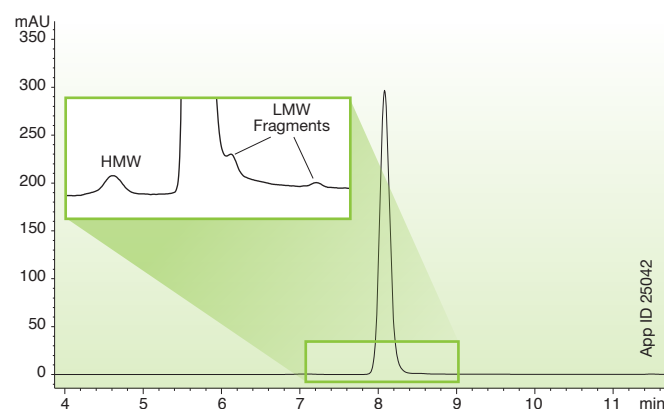
With **mAb aggregate often at very low levels** (<0.1 % by peak area compared to monomer) and fragment separation a requirement, adequate resolution and peak shape have become even more crucial method outcomes. To address this need, the robust set of bioZen™ SEC columns were developed with a **combination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



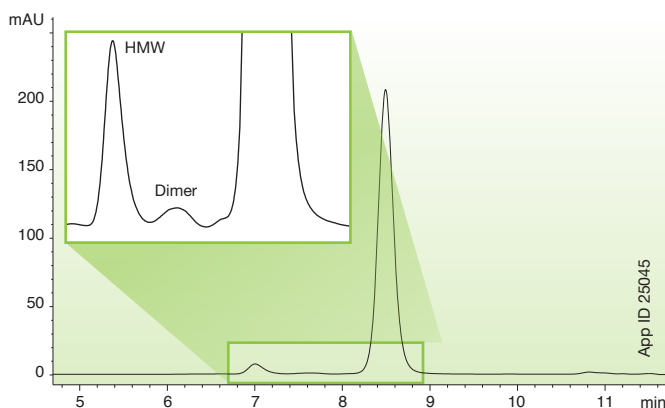
Cetuximab



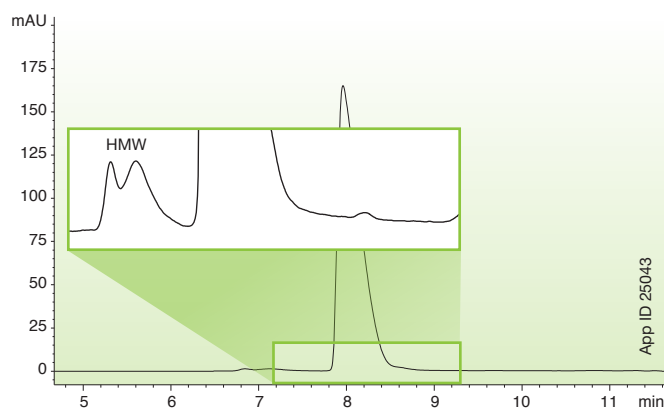
Trastuzumab



Rituximab



Infliximab – abda



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 Potassium Phosphate +
 250 mM Potassium Chloride (pH 6.8)
 Flow Rate: 0.35 mL/min

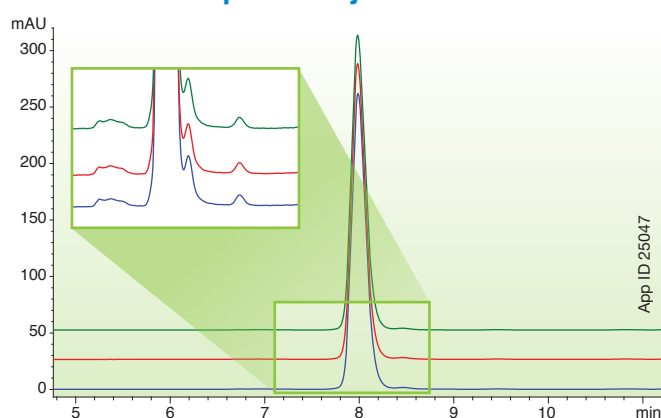
Temperature: 30 °C
 Detection: UV @ 280 nm
 Sample: As noted

Aggregate Analysis

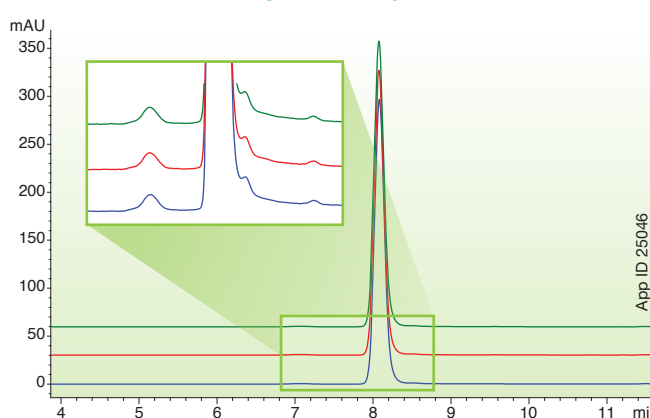
High inertness and particle consistency of both bioZen™ SEC columns drives greater reproducibility from injection to injection. Combine this with the bioinert BioTi™ hardware and good aggregate recovery is no longer something that you're missing out on.

BODY

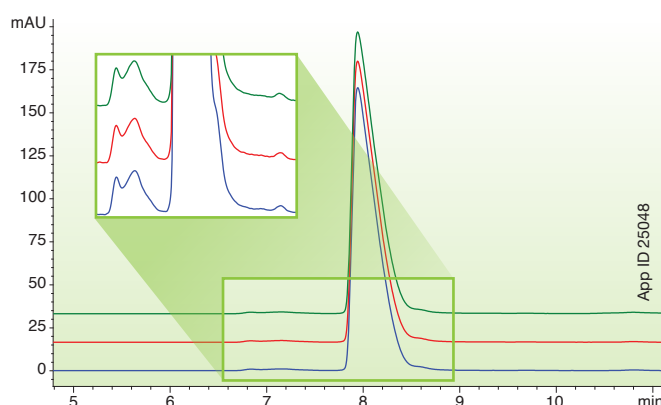
Cetuximab Triplicate Injections



Trastuzumab Triplicate Injections



Infliximab-dyyb Triplicate Injections

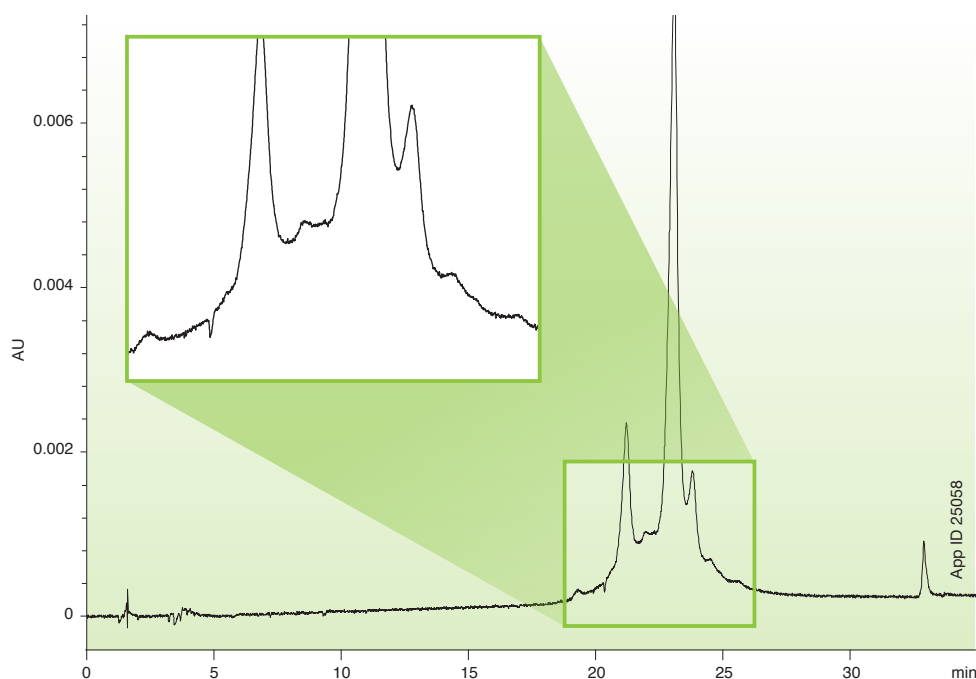


Conditions same for all samples:
Column: bioZen 1.8 μ m SEC-3
Dimension: 300 x 4.6 mm
Part No.: 00H-4772-E0
Mobile Phase: 50 mM Potassium Phosphate +
250 mM Potassium Chloride (pH 6.8)
Flow Rate: 0.35 mL/min
Temperature: 30 °C
Detection: UV @ 280 nm
Sample: As noted

Charge Variant Analysis

bioZen WCX was crafted to consistently decipher between native protein variants that arise from PTMs within a therapeutics creation and development. The linear polycarboxylate chains grafted to monosized non-porous polymeric particles, envelop and separate proteins from acidic and basic protein variants. With such a highly tuned and controlled manufacturing process, bioZen WCX media affords scientists a way to reproducibly characterize heterogeneity while taking advantage of excellent recovery through high particle inertness and bioinert titanium BioTi column hardware.

Trastuzumab (MES Salt Gradient)

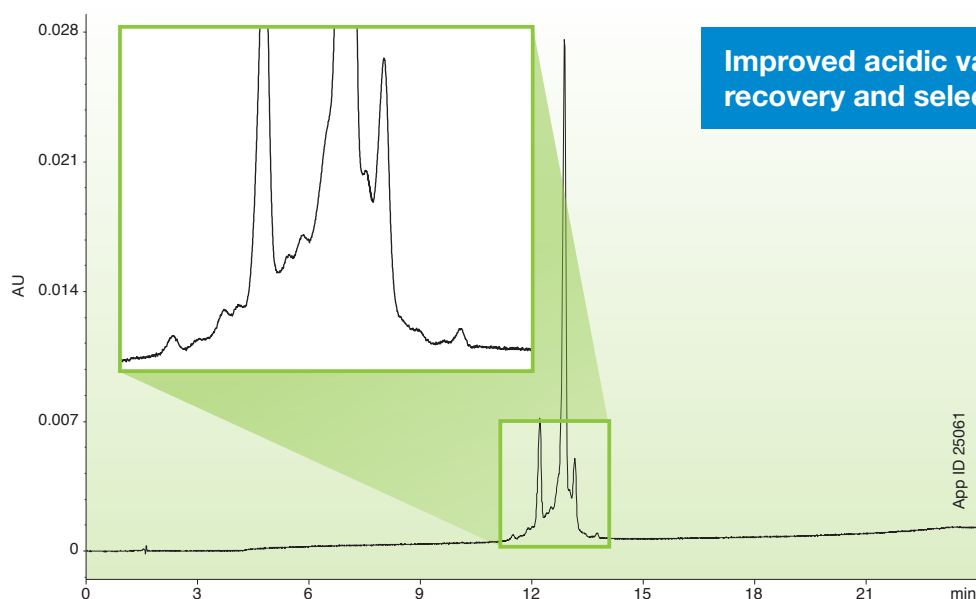


Column: bioZen 6 μ m WCX
Dimension: 250 x 4.6 mm
Part No.: 00G-4777-E0
Mobile Phase: A: 20 mM MES (pH 5.6)
 B: 20 mM MES + 300 mM NaCl (pH 5.6)
Gradient:

Time (min)	% B
0	15
1	15
31	45
31.1	100
34	100
35	15

Flow Rate: 1 mL/min
Temperature: 30 °C
Detection: UV @ 280 nm
Sample: Trastuzumab

Trastuzumab (pH Gradient Buffer)



Improved acidic variant recovery and selectivity!

Column: bioZen 6 μ m WCX
Dimension: 250 x 4.6 mm
Part No.: 00G-4777-E0
Mobile Phase: A: CX -1 (pH 5.6) pH Gradient Buffer*
 B: CX -1 (pH 10.2) pH Gradient Buffer*
Gradient:

Time (min)	% B
0	0
1	0
21	100
23	100
24	0

Flow Rate: 1 mL/min
Temperature: 30 °C
Detection: UV @ 280 nm
Sample: Trastuzumab

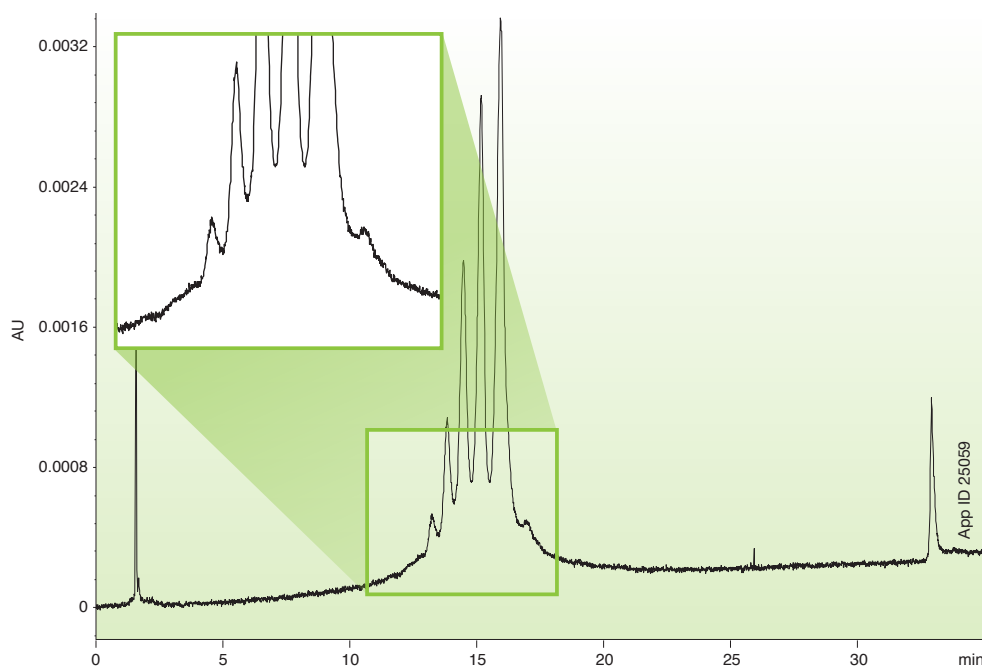
* From Thermo Fisher Scientific® Inc.

Charge Variant Analysis

While the monosized non-porous polymer particles serve as a perfect vehicle for the polycarboxylate chains, these rugged particles also ensure that the bioZen™ WCX is stubborn in face of typically harsh solvent pH and salt gradient systems. This ruggedness and high stability in combination with consistent selectivity allows scientists to not settle for either pH or salt gradients when needing to separate and quantitate charge variants of proteins, innovators, or biosimilars. Sometimes, it's nice to know you have a choice.

BODY

Cetuximab (MES Salt Gradient)

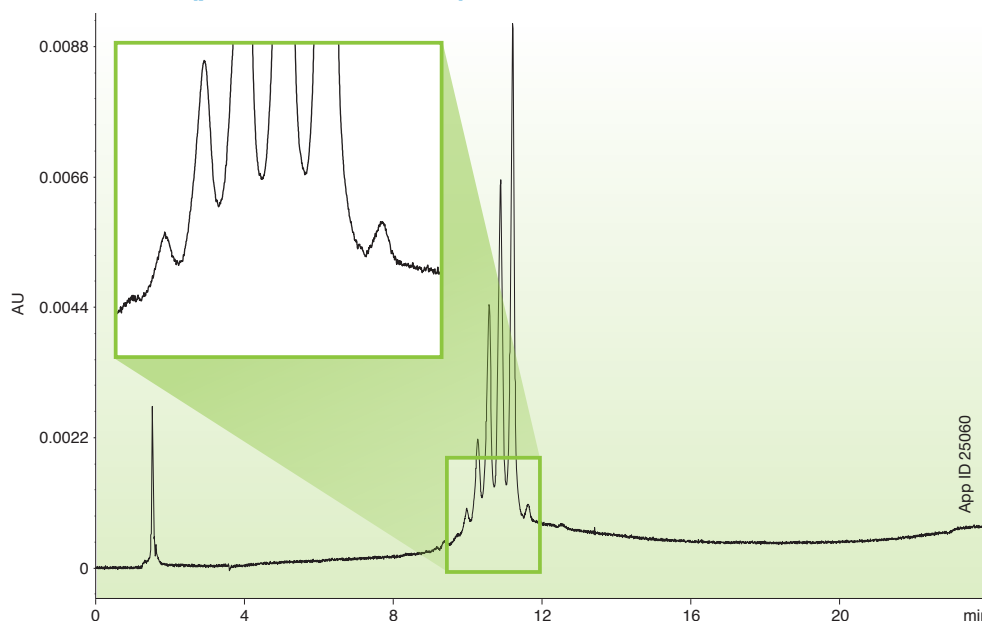


Column: bioZen 6 μ m WCX
Dimension: 250 x 4.6 mm
Part No.: 00G-4777-E0
Mobile Phase: A: 20 mM MES (pH 5.6)
 B: 20 mM MES + 300 mM NaCl (pH 5.6)
Gradient:

Time (min)	% B
0	15
1	15
31	45
31.1	100
34	100
35	15

Flow Rate: 1 mL/min
Temperature: 30 °C
Detection: UV @ 280 nm
Sample: Cetuximab, biosimilar expressed in HEK
Acknowledgment: Sample graciously gifted by Catherine Bladen, Absolute Antibody

Cetuximab (pH Gradient Buffer)



Column: bioZen 6 μ m WCX
Dimension: 250 x 4.6 mm
Part No.: 00G-4777-E0
Mobile Phase: A: CX -1 (pH 5.6) Gradient Buffer*
 B: CX -1 (pH 10.2) Gradient Buffer*
Gradient:

Time (min)	% B
0	0
1	0
21	100
23	100
24	0

Flow Rate: 1 mL/min
Temperature: 30 °C
Detection: UV @ 280 nm
Sample: Cetuximab, biosimilar expressed in HEK
Acknowledgment: Sample graciously gifted by Catherine Bladen, Absolute Antibody
 * From Thermo Fisher Scientific Inc.

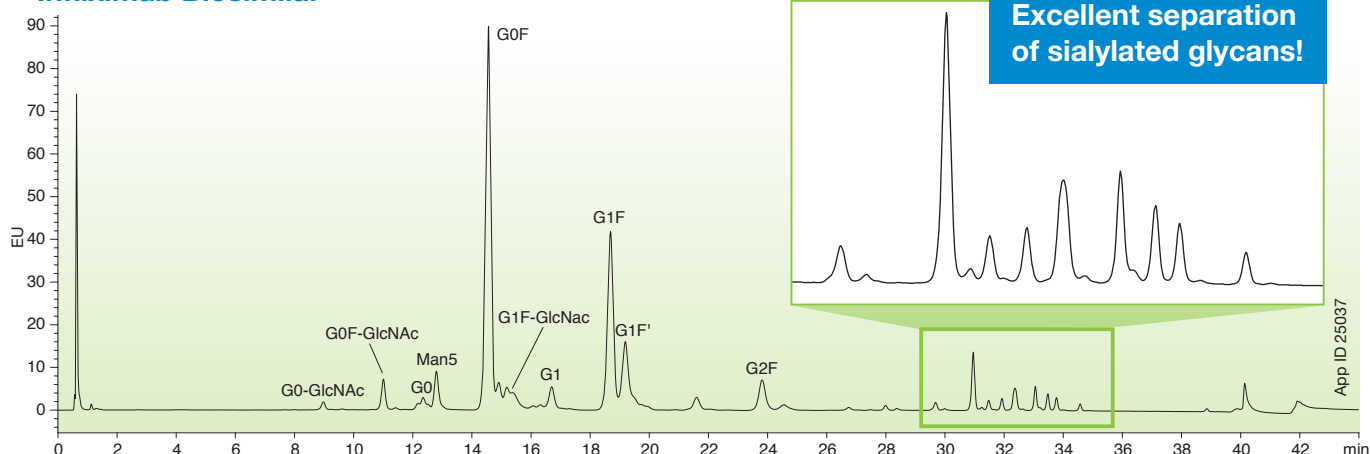


Glycan Analysis

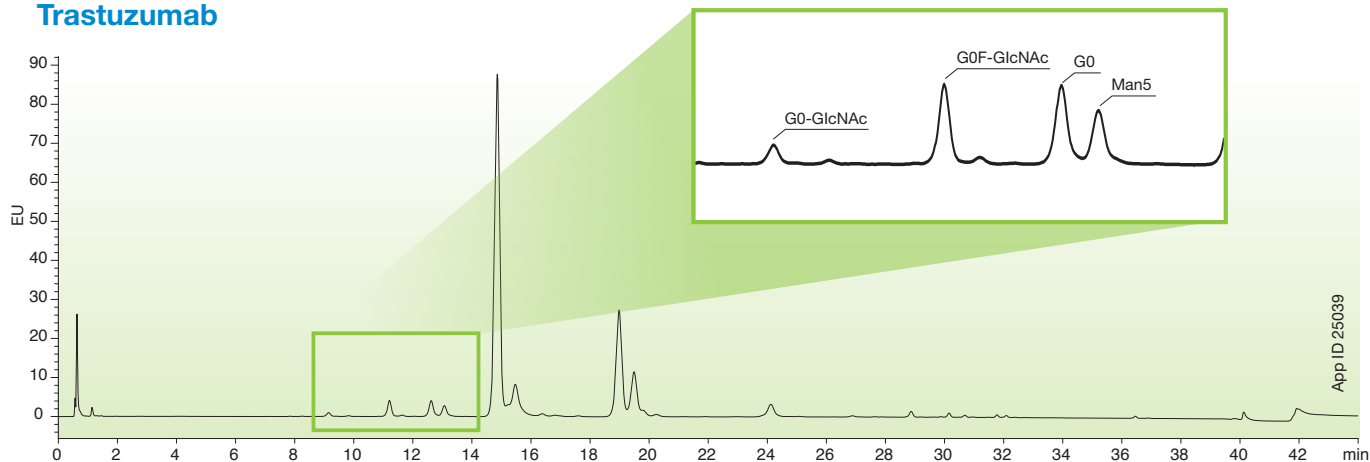
The unique selectivity of the bioZen Glycan was designed to **provide higher order separations of released and labeled glycans**. With a 2.6 μm core-shell particle size, customers using either HPLC or UHPLC systems can draw upon a high efficiency bioZen Glycan particle run at higher linear velocities 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.



Infliximab Biosimilar



Trastuzumab

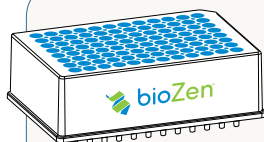


Conditions for both columns:

Column: bioZen 2.6 μm Glycan
 Dimensions: 150 x 2.1 mm
 Part No.: 00F-4773-AN
 Mobile Phase: A: 100 mM Ammonium Formate, pH 4.5
 B: Acetonitrile

Gradient: Time (min)	% B
0	78
10	74.5
24	72
38.5	55.9
38.6	40
40.6	40
40.7	78
48	78

Flow Rate: 0.5 mL/min
 Temperature: 50 °C
 Detection: FLD ex/em 285/345 nm
 Sample: As noted



bioZen N-Glycan Clean-Up

Novel solid phase extraction (SPE) HILIC stationary phase that excels at retention and recovery of labeled, released N-glycans! Available in microelution 96-well plate format that works extremely well for processing and clean-up of small sample volumes.

www.phenomenex.com/GlycanSPE

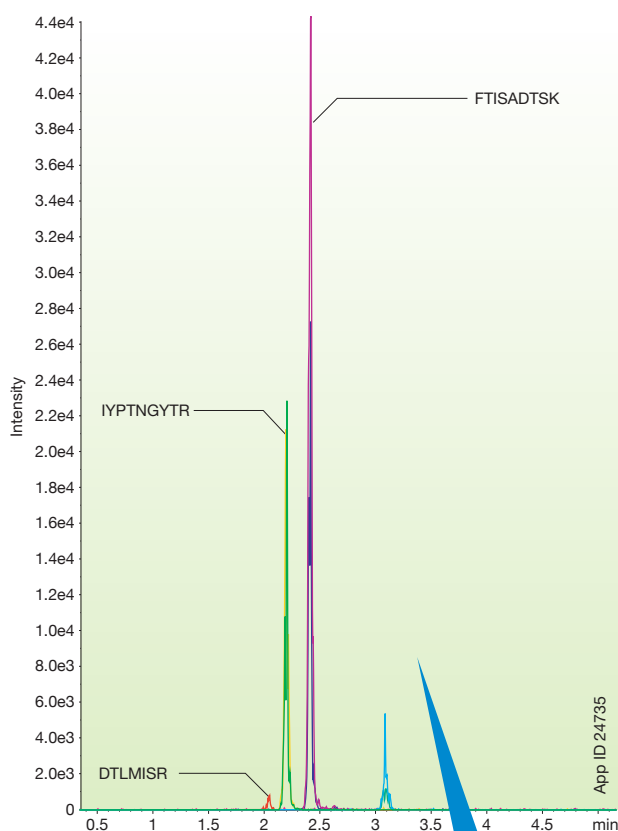
Peptide Quantitation

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.

BODY

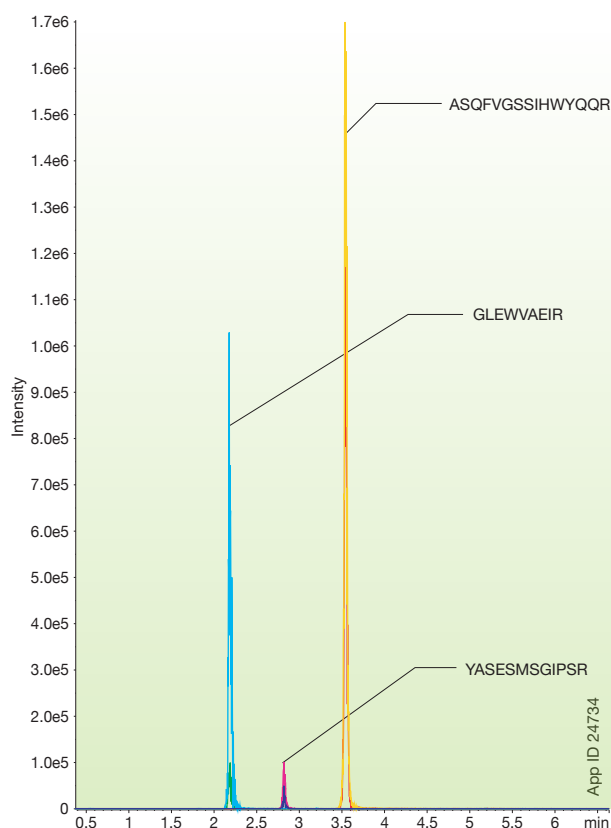
Kadcyla

(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:

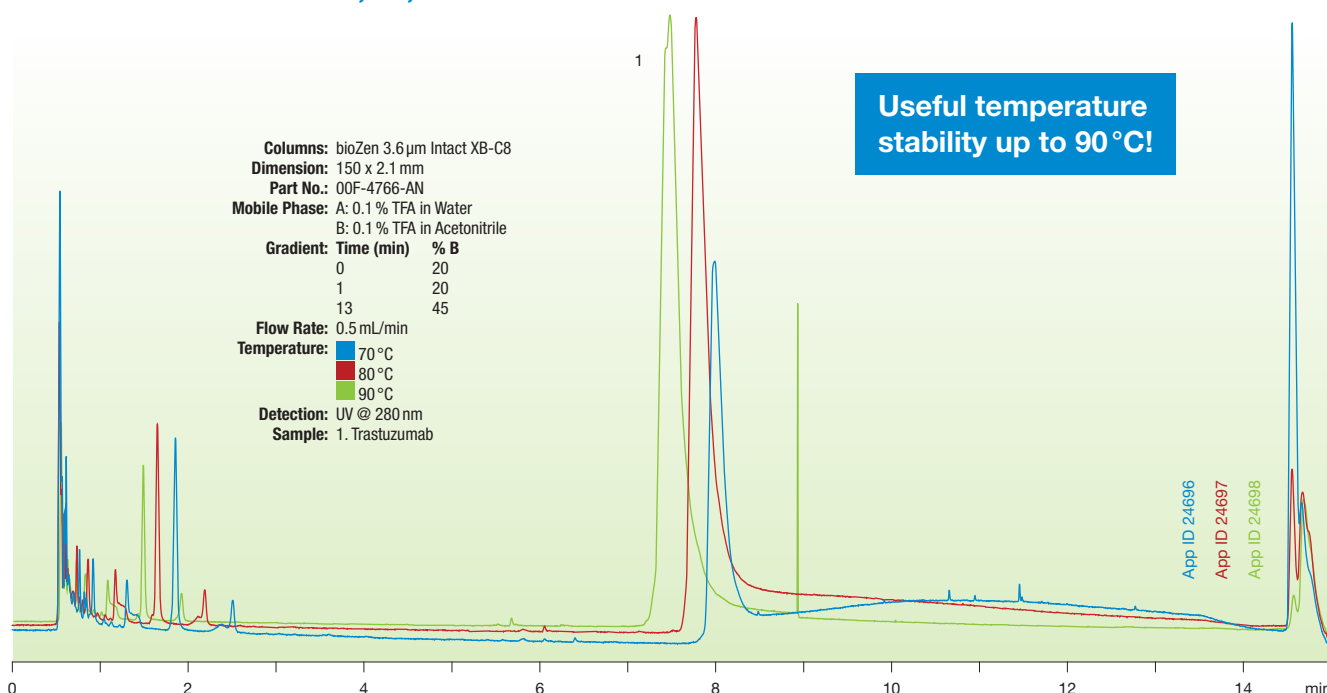
- Column: bioZen 3µm Peptide PS-C18
- Dimensions: 50 x 2.1 mm
- Part No.: 00B-4771-AN
- Mobile Phase: A: 0.1 % Formic Acid in Water
B: 0.1 % Formic Acid in Acetonitrile
- Gradient:

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

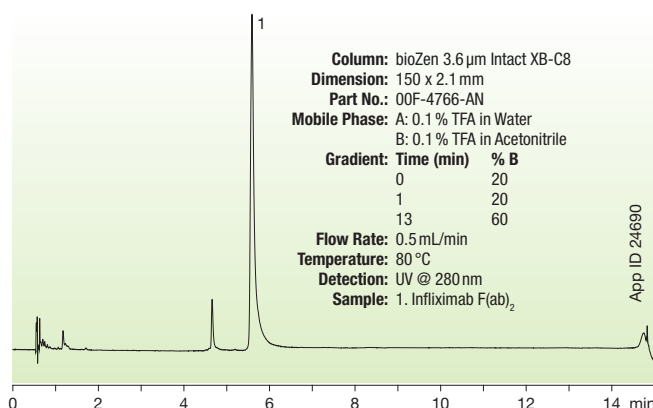
Intact & Fragment Analysis

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**.

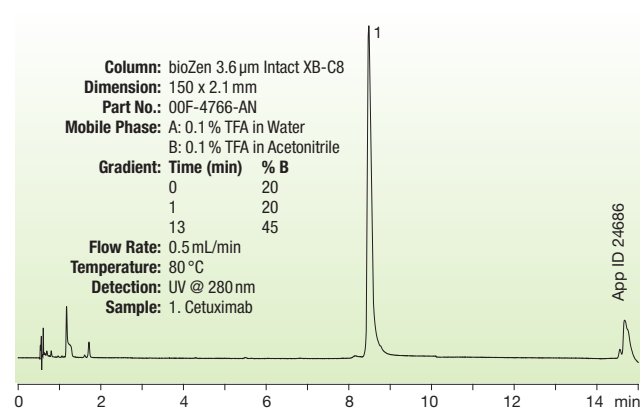
Intact Trastuzumab at 70, 80, and 90 °C



Infliximab F(ab)₂



Cetuximab



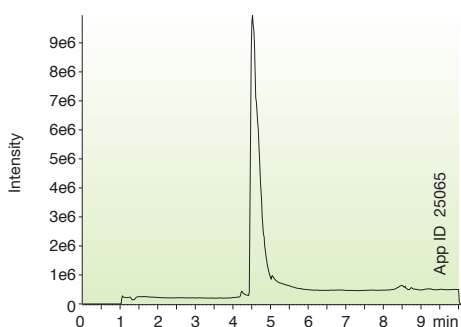
Intact Mass

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.

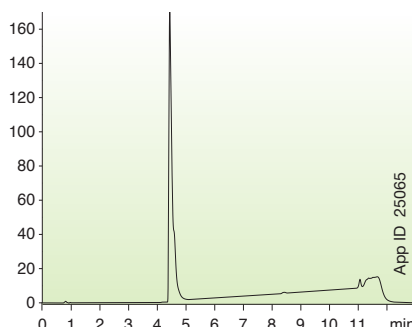
BODY

Intact Mass of Trastuzumab using a bioZen Intact XB-C8 and SCIEX® X500B

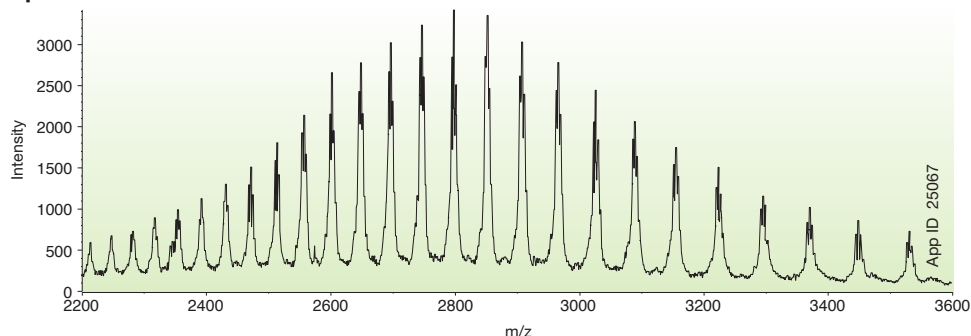
TIC



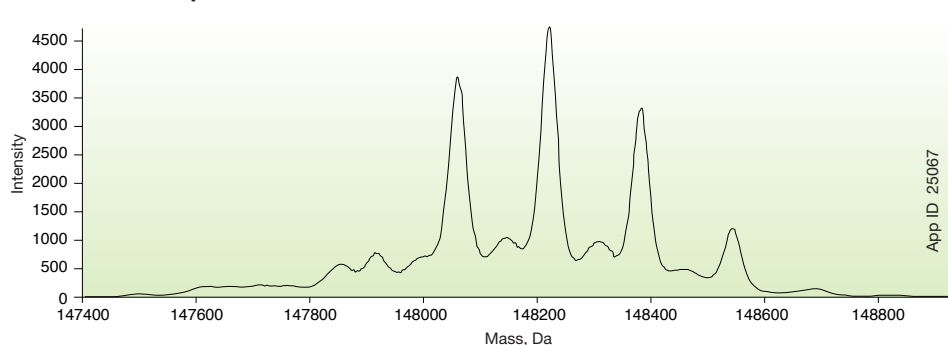
UV



Spectra



Deconvoluted Spectra



Columns: bioZen 3.6µm Intact XB-C8
 Dimension: 150 x 2.1 mm
 Part No.: 00F-4766-AN
 Mobile Phase: A: 0.1 % Formic Acid in Water
 B: 0.1 % Formic Acid in Acetonitrile /
 Isopropyl alcohol (50:50)
 Gradient:

Time (min)	% B
2.5	20
10	65
10.1	95

 Flow Rate: 0.3 mL/min
 Temperature: 90 °C
 Detection: QTOF (SCIEX X500B)
 Sample: Trastuzumab

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.

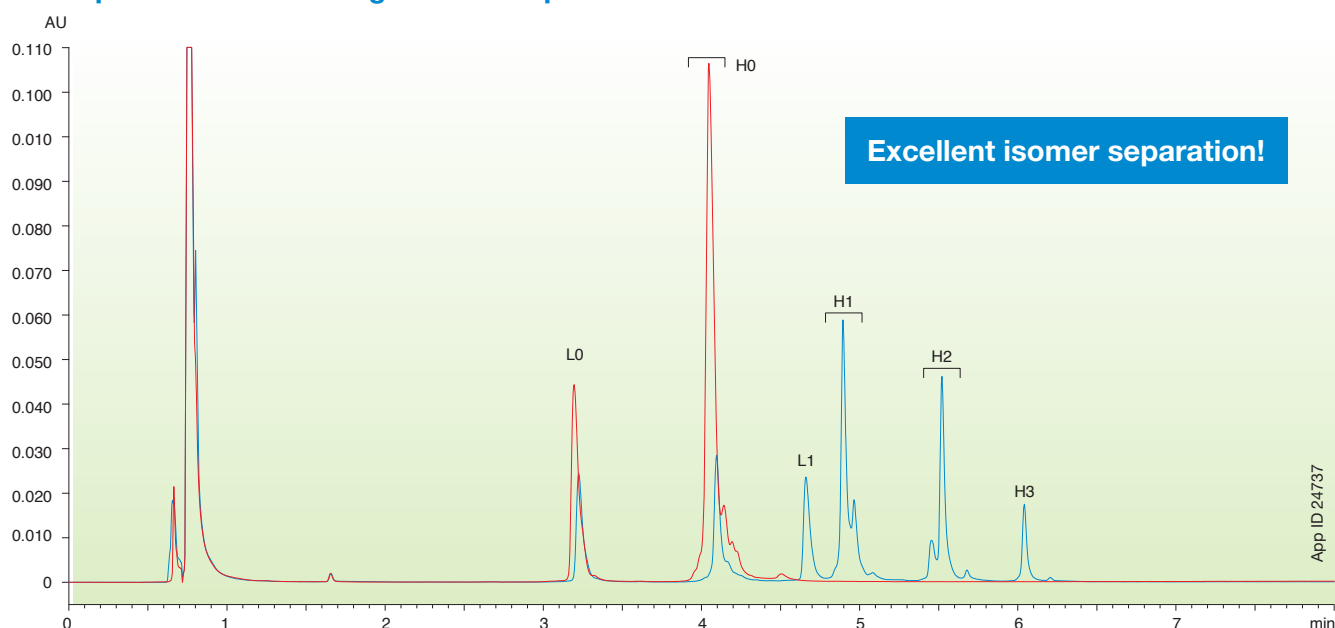
Learn More at www.sciex.com/X500B



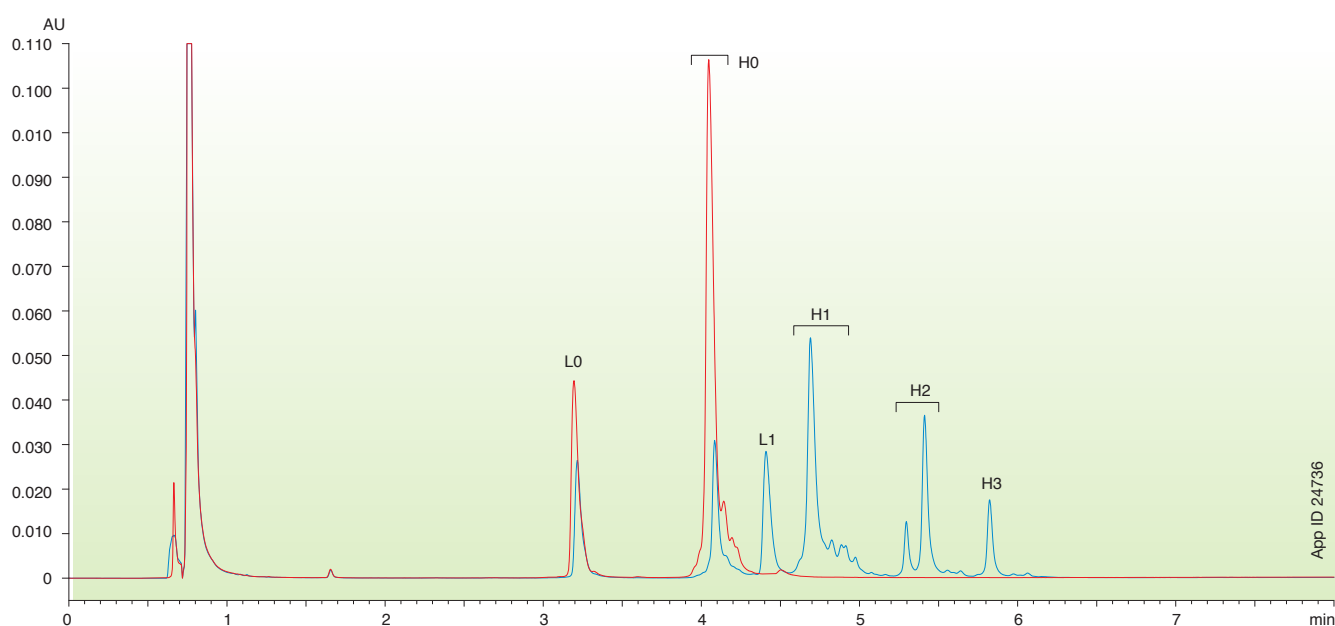
Drug Antibody Ratio (DAR)

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

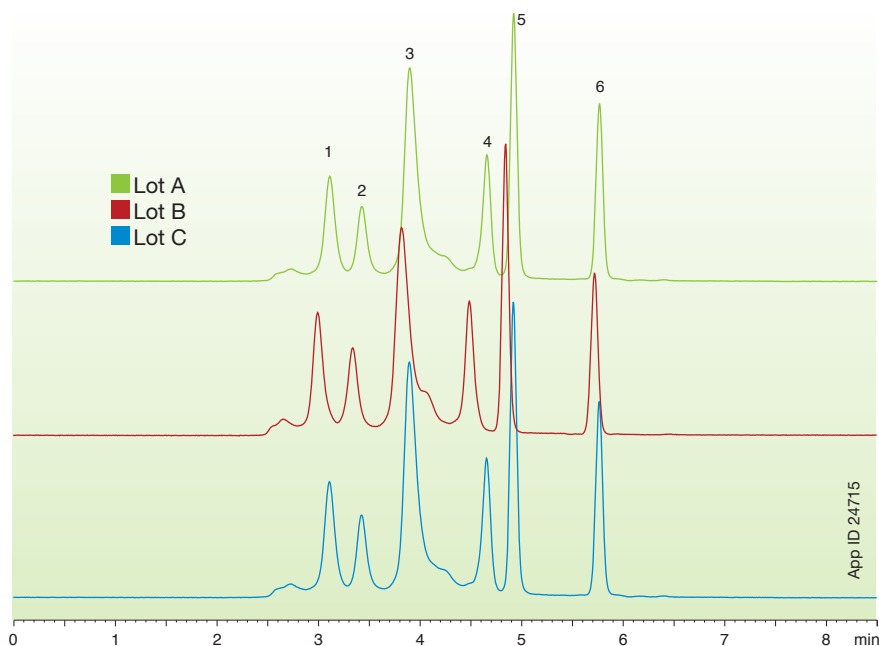
Bio QC Testing

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.

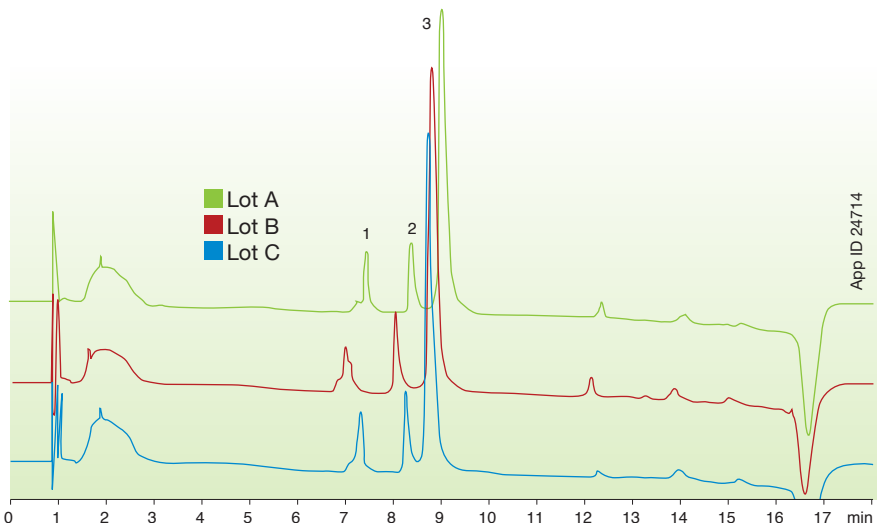
BIO
BODY

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



Column: bioZen 1.8µm 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 3.6µm Intact C4



Columns: bioZen 3.6µm 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

Charge Variant (IEX)

bioZen WCX

Aggregation (SEC)

bioZen SEC-3

Aggregation (High-Throughput SEC)

bioZen SEC-3

Average DAR ADC (RP-UV)

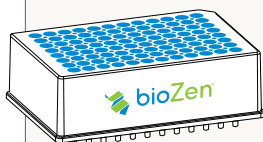
bioZen Intact XB-C8

Glycan Analysis (HILIC-FL)

bioZen Glycan

Glycan Analysis (HILIC-MS)

bioZen Glycan



bioZen N-Glycan Clean-Up

Novel solid phase extraction (SPE) HILIC stationary phase that excels at retention and recovery of labeled, released N-glycans! Available in microelution 96-well plate format that works extremely well for processing and clean-up of small sample volumes.

www.phenomenex.com/GlycanSPE

Preclinical mAb

Formulation (SEC)

bioZen™ SEC-2
bioZen SEC-3

Charge Variant (IEX)

bioZen WCX

Total mAb (RP-UV)

bioZen Intact C4
bioZen Intact XB-C8

Intact Mass (RP-MS)

bioZen Intact C4
bioZen Intact XB-C8

Total mAb (SEC-UV)

bioZen SEC-2
bioZen SEC-3

Peptide Quantitation (RP-MS)

bioZen Peptide PS-C18
bioZen Peptide XB-C18



Biocompatible Titanium Hardware:

Better recovery and reproducibility for all workflows!

Size Exclusion and a Well Salted Buffer

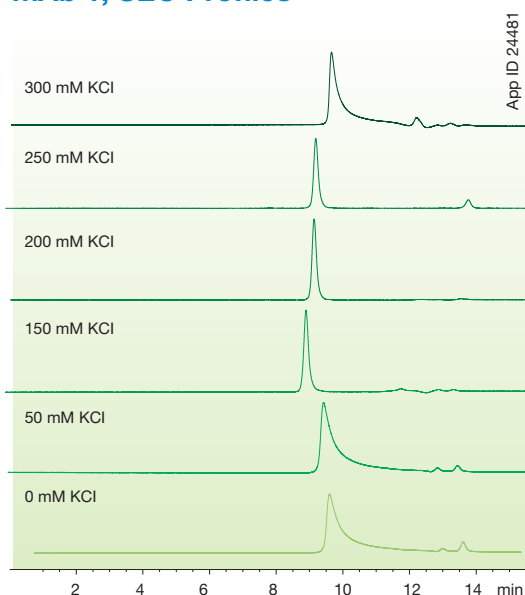
**Dani Xing**

Technical Guru - Bioseparations

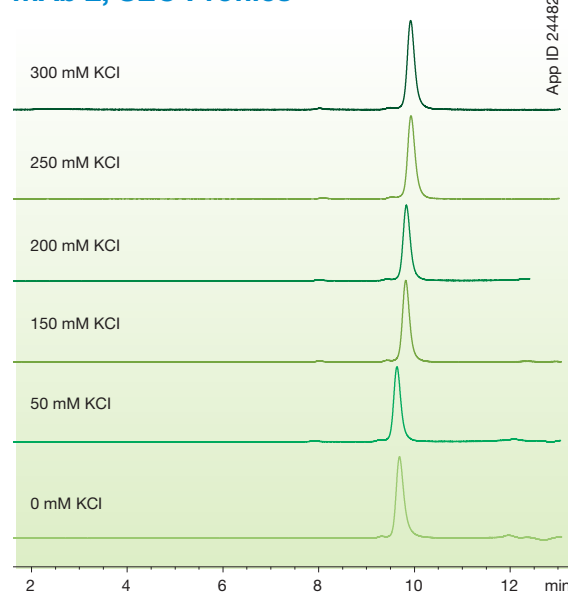
When developing a method for aggregate analysis of mAbs by SEC, it is critical to optimize mobile phase conditions to prevent non-specific secondary interactions. Below, you can see the effect of altering salt concentration in the mobile phase for two different biosimilar mAbs. The first mAb required 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 50 mM potassium phosphate, 250 mM 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 µm 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

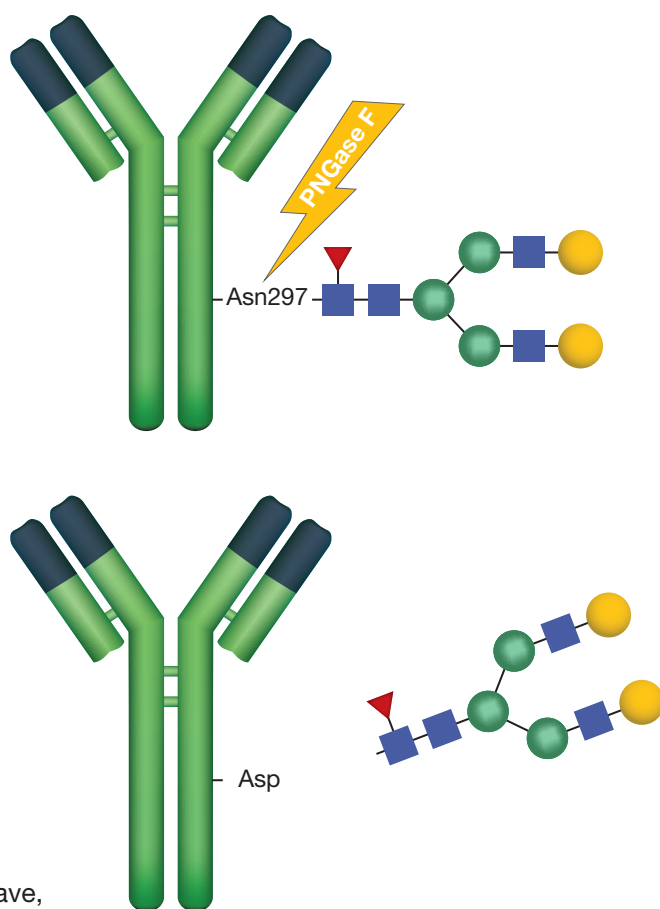


How should I deglycosylate my antibody?

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., Paguio, 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

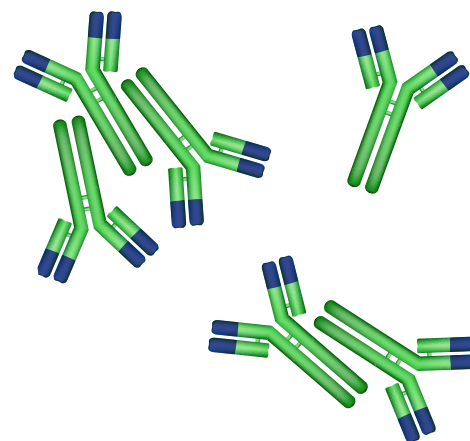


Organic Solvent and Size Exclusion

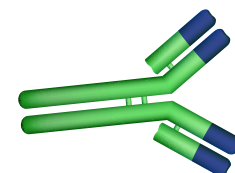
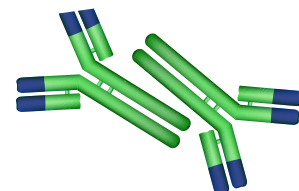
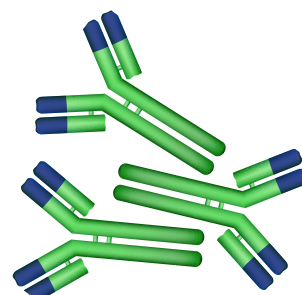
In order to get an “ideal” SEC separation (i.e. purely entropic separation, with no interaction of analyte with stationary phase), oftentimes some organic modifier, 5-15 % isopropanol or acetonitrile, might be necessary.

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?

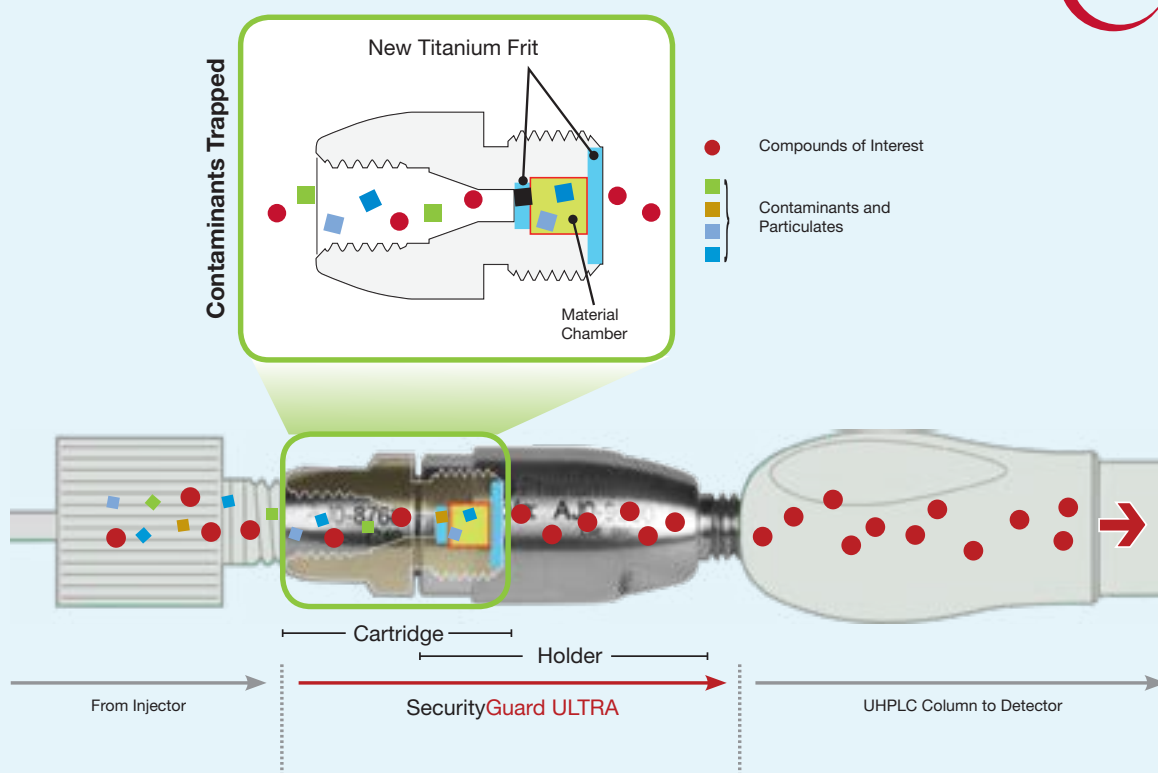
If strong ionic interactions between proteins and the stationary phase are suspected, then start cleaning with a denaturant such as 6 M guanidine hydrochloride or 10 % DMSO. If the protein is relatively hydrophobic, start by flushing out buffer with 95-100 % water, then clean out the hydrophobic proteins 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

With the microelution plate format, both Strata-X Polymeric SPE and bioZen N-Glycan Clean-Up offer two big benefits: better absolute recovery and greater time savings.



N-Glycan Clean-Up SPE

HILIC stationary phase that excels at retention and recovery of labeled, released N-glycans.

www.phenomenex.com/GlycanSPE



Strata-X Polymeric SPE

De-salt your sample before injection onto your column for more accurate results and longer column lifetimes.

www.phenomenex.com/StrataX

Product Ordering Information

bioZen™ Products - Powered by Biocompatible Hardware

bioZen Columns (mm)						Biocompatible Guard Cartridges		
	50 x 2.1	100 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	—	ea
bioZen 2.6 µm Glycan	—	00D-4773-AN	00F-4773-AN	—	—	AJO-9800	—	AJO-9000
	—	—	—	—	—	/3pk	—	ea
bioZen 1.6 µm Peptide PS-C18	00B-4770-AN	00D-4770-AN	00F-4770-AN	—	—	AJO-9803	—	AJO-9000
	—	—	—	—	—	/10pk	/10pk	ea
bioZen 3 µm Peptide PS-C18	00B-4771-AN	—	00F-4771-AN	00B-4771-E0	00F-4771-E0	AJO-7605	AJO-7606	KJO-4282
	—	—	—	—	—	/3pk	—	ea
bioZen 1.7 µm Peptide XB-C18	00B-4774-AN	00D-4774-AN	00F-4774-AN	—	—	AJO-9806	—	AJO-9000
	—	—	—	—	—	/3pk	/3pk	ea
bioZen 2.6 µm Peptide XB-C18	00B-4768-AN	00D-4768-AN	00F-4768-AN	00B-4768-E0	00F-4768-E0	AJO-9806	AJO-9808	AJO-9000
	—	—	—	—	—	/3pk	/3pk	ea
bioZen 3.6 µm Intact C4	00B-4767-AN	00D-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	00D-4766-AN	00F-4766-AN	00B-4766-E0	00F-4766-E0	AJO-9812	AJO-9814	AJO-9000

	50 x 4.6	100 x 4.6	150 x 4.6	250 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	—	00D-4772-E0	00F-4772-E0	—	00H-4772-E0	—	AJO-9851	AJO-9000
	—	—	—	—	—	—	/10pk	ea
bioZen 6 µm WCX	00B-4777-E0	00D-4777-E0	00F-4777-E0	00G-4777-E0	—	—	AJO-9400	KJO-4282

Sample Preparation

bioZen Solid Phase Extraction	Format	Sorbent Mass	Part Number	Unit	Price
bioZen N-Glycan Clean-Up	Microelution 96-Well Plate	5 mg/well	8M-S009-NGA	1/box	



guarantee

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

Ensure Protein Recovery with Biocompatible Accessories!



Phenex Syringe Filters

PES membranes offer fast-flow and ultra-low protein binding

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Verex Vials, Caps, and Kits

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

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