Tips from our Protein Separation ZenMasters

Intact-RP Mass

Chad Eichman, Ph.D.
Biopharm Global Market Manager

I need to perform intact mass experimentation, but often I have no idea of what my protein is formulated in. Should I perform a dialysis or spin filtration before analysis?

Whether looking at mAbs or other proteins, formulation buffers often contain matrix interferences. These include the buffer salts themselves (Tris, HEPES, TBS, PBS, CHAPS, etc.) which are all very common storage buffers, and are not compatible with ESI-MS.

Typically, a buffer exchange using dialysis or a spin filtration device is performed. However, one can simply use the reversed phase column for intact mass to perform desalting on-line For example, start your method with a 3 minute isocratic wash that goes to waste, or use a diverter valve. This can rid most of the buffer salts that would potentially interfere with the MS analysis.

My spectra for intact mass is horrible and my MS vendor said that it might be chromatography related! What do I do?

Unlike other forms of chromatography, like impurity profiling by reversed phase, for intact mass the goal is for good peak shape, allowing the high-resolution MS to scan under the peak. Although this might seem straightforward, any separation with proteins is never as easy as it seems, so optimization might be necessary for proteins that behave in unexpected ways.

Because intact mass works optimally with proteins unfolded and denatured, high heat is mandatory for optimal peak shape. 70-90 °C is required for most applications, especially mAbs which require a fair amount of heat for denaturation.

The other consideration is solvent composition. For most silica-based columns, even a low surface area core-shell media, isopropanol (IPA) might be required for optimal peak shape. Not only does IPA have a stronger elution strength in reversed phase than the commonly used acetonitrile, but alcohols do better at destabilizing the hydrophobic interactions that keep proteins folded.

Finally, if a clean spectra still cannot be obtained, try deglycosylation of the protein, as this will simplify the spectra. If spectra still cannot be obtained, this may be sample related and the protein may be too degraded for MS analysis.

What should I do about carryover in my intact mass separation?

Some proteins, especially mAbs and ADCs, can be quite hydrophobic and “memory effect” might be observed even with an optimized gradient and a column with good surface chemistry and particle morphology for intact mass. Carryover by memory effect is more pronounced in very sensitive techniques like MS.

One effective way to reduce carryover is to implement a “zig zag” gradient (e.g. short, repeated gradients of 15-85% B) after the initial wash to ensure no carryover is observed. If carryover is still observed, it could be system related.

I’ve heard DFA is a better mobile phase for intact analysis. How does this mobile phase perform on the bioZen WidePore C4?

Historically, researchers opted for formic acid (FA) for MS analysis over trifluoroacetic acid (TFA) to maintain instrument source integrity and reduce ion suppression. Utilizing FA, however, results in poor peak shape chromatographically compared to TFA due to TFA’s ability to minimize protein secondary interactions. In recent years, difluoroacetic acid (DFA) has been reported to match the chromatographic performance of TFA but maintain the ion sensitivity of FA. Indeed, DFA has clear advantages on the MS as previously reported, but optimal peak shape is still acquired on TFA when using the bioZen WidePore C4 column, though in certain instances the peak shape between DFA and TFA may be nominal. If you are currently using FA as the mobile phase for intact analysis, we recommend a switch to DFA. However, if your method is performing well under TFA conditions, this option will still give the best chromatographic performance. Finally, it should be noted that FA and TFA are ubiquitous in the laboratory and method development is more easily initiated with these two options.

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	100 x 4.6	150 x 4.6	250 x 4.6	for 2.1 mm /3pk	for 4.6 mm /10pk	Holder ea
bioZen 2.6 µm Glycan	00B-4773-AN	00D-4773-AN	00F-4773-AN	—	—	—	—	AJ0-9800	—	AJ0-9000
bioZen 1.6 µm Peptide PS-C18	00B-4770-AN	00D-4770-AN	00F-4770-AN	—	—	—	—	AJ0-9803	—	AJ0-9000
bioZen 3 µm Peptide PS-C18	00B-4771-AN	—	00F-4771-AN	00B-4771-E0	—	00F-4771-E0	—	AJ0-7605	AJ0-7606	KJ0-4282
bioZen 1.7 µm Peptide XB-C18	00B-4774-AN	00D-4774-AN	00F-4774-AN	—	—	—	—	AJ0-9806	—	AJ0-9000
bioZen 2.6 µm Peptide XB-C18	00B-4768-AN	00D-4768-AN	00F-4768-AN	00B-4768-E0	—	00F-4768-E0	—	AJ0-9806	AJ0-9808	AJ0-9000
bioZen 2.6 µm WidePore C4	00B-4786-AN	00D-4786-AN	00F-4786-AN	00B-4786-E0	00D-4786-E0	00F-4786-E0	00G-4786-E0	AJ0-9809	AJ0-9811	AJ0-9000
bioZen 3.6 µm Intact XB-C8	00B-4766-AN	00D-4766-AN	00F-4766-AN	00B-4766-E0	—	00F-4766-E0	—	AJ0-9812	AJ0-9814	AJ0-9000

	50 x 2.1	100 x 2.1	150 x 2.1	250 x 2.1	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	300 x 4.6	for 4.6 mm /3pk	Holder ea
bioZen 1.8 µm SEC-2	00B-4769-AN	—	00F-4769-AN	—	—	—	00F-4769-E0	—	00H-4769-E0	AJ0-9850	AJ0-9000
bioZen 1.8 µm SEC-3	00B-4772-AN	—	00F-4772-AN	—	—	00D-4772-E0	00F-4772-E0	—	00H-4772-E0	AJ0-9851	AJ0-9000

										for 4.6 mm /10pk	Holder ea
bioZen 6 µm WCX	00B-4777-AN	00D-4777-AN	00F-4777-AN	00G-4777-AN	00B-4777-E0	00D-4777-E0	00F-4777-E0	00G-4777-E0	—	AJ0-9400	KJ0-4282

Have Questions? Visit:
www.phenomenex.com/bioZen
We're here to help!

BE-HAPPY™ guarantee

Your happiness is our mission. Take 45 days to try our products. If you are not happy, we'll make it right.
www.phenomenex.com/behappy

Australia
t: +61 (0)2-9428-6444
auinfo@phenomenex.com

Austria
t: +43 (0)1-319-1301
anfrage@phenomenex.com

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
info@phenomenex.com

China
t: +86 400-606-8099
cninfo@phenomenex.com

Denmark
t: +45 4824 8048
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
anfrage@phenomenex.com

India
t: +91 (0)40-3012 2400
indiainfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
italiainfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
nlinfo@phenomenex.com

Mexico
t: 01-800-844-5226
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
nordicinfo@phenomenex.com

Poland
t: 0-0-800-4911952
pl-info@phenomenex.com

Portugal
t: +351 221 450 488
ptinfo@phenomenex.com

Singapore
t: +65 800-852-3944
sginfo@phenomenex.com

Spain
t: +34 91-413-8613
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
nordicinfo@phenomenex.com

Switzerland
t: +41 (0)61 692 20 20
swissinfo@phenomenex.com

Taiwan
t: +886 (0) 0801-49-1246
twinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
ukinfo@phenomenex.com

USA
t: +1 (310) 212-0555
info@phenomenex.com

All other countries/regions
Corporate Office USA
t: +1 (310) 212-0555
info@phenomenex.com

phenomenex® ...breaking with tradition℠