Tips from our Protein Separation ZenMasters

Intact Mass

Chad Eichman, Ph.D.
BioPharm Global Marketing Manager

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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 as an online desalting device. 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.