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FAQs for WidePore C4

bioZen 2.6 µm WidePore C4 is an intact core-shell reversed phase column that provides good peak shape and selectivity for both intact monoclonal antibodies (mAbs) and subunit analyses. Its robust surface grafting and optimal particle and pore size morphology ensures high reproducibility for analytical methods

To learn more about bioZen WidePore C4 go to:

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For impurity profiling and intact characterization, what is one of the biggest separation issues and how would you resolve this?

For impurity profiling and characterization of large molecules the biggest separation issue would be the resolution between closely related peaks. For example, the separation of a deamidated variant from the main peak can be challenging to get good separation.

As such, one must rely on selectivity as the primary driver for the separation. With intact reversed phase liquid chromatography (RP-LC) methods, the method development tool is gradient optimization - essentially, shallowing the gradient slope as much as is practically possible. However other method development levers: temperature, concentration of acidic modifier, and type of organic solvent (e.g. isopropanol) are other method development parameters to consider when optimizing an intact RP-LC method.

With a 400 Å (or larger) pore size, are there any chromatography or backpressure issues that using a column with a larger pore size would cause?

There is some liberty in disclosing the nominal pore size of "wide-pore" reversed phase LC columns. This may be due to discrepancies in measurement (e.g. nitrogen adsorption by BET or mercury intrusion by BJH can lead to very different results). However, the more problematic challenge that any column vendor faces when disclosing pore size is that it is not one nominal pore size but a pore size distribution that is contributing to the overall separation of the large molecules.

That said, provided there is some level of verity in the reporting of the pore size, pores from 200-500 Å are appropriate for the separation of intact monoclonal antibodies. Denatured, intact mAbs have a hydrodynamic radius of ~6 nm or so, in which, ~200-300 Å pore size should suffice. However, this is not to say that any smaller pores in the pore size distribution won't contribute to the separation of smaller fragments (e.g. Heavy Chain and Light Chain).

Regarding "backpressure issues" with a larger pore size - ultimately, backpressure is a function of particle size and not pore size per se. A common misunderstanding is that smaller pores will get "clogged." In reality, intact proteins - especially monoclonal antibodies - won't clog pores and instead can be excluded from the pores, thus eluting in the void. Or, if pore size is large enough to accommodate for the protein (in reality, a pore 120 or so angstrom in diameter will likely retain a protein), the protein will still retain and will elute later but with a very bad peak shape due to poor mass transfer due to smaller pore size.

For the selectivity of the column, how does the C4 particle chemistry change the chromatography compared to other columns on the market?

The C4 stationary phase itself actually does not impart too much selectivity in intact RPLC. If one thinks about it, reversed phase is driven by methylene selectivity. A butyl chain, being a relatively small alkyl chain, has very little methylene selectivity. However, the reason C4s are ubiquitous in protein separations is because alkyl chains can present challenges for separation of intact proteins…not the least of which is that getting enough of them on the surface of the silica without having entirely too hydrophobic of a particle is a fine balancing act.

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Selectivity in intact RPLC is more mediated by three factors - organic solvent (i.e. gradient slope and optimization), temperature, and pH of mobile phase, all of which modulate the interaction of the buried hydrophobic regions of the proteins with the moderately hydrophobic stationary phase. Indeed, those three factors can be much more meaningful to the separation.

So why the C4, then? This is simply because the low hydrophobicity phases allow for the modulation of the method parameters, allowing for a wide enough experimental design space because it isn't too retentive. That is to say, you have much more room to "play" with a moderately hydrophobic phase than one that is too hydrophobic.

We have a lot of different large pore columns to choose from, when would you advise using a bioZen vs. an Aeris column?

The bioZen WidePore C4 particle is a different particle platform - it is also a low surface area but has a substantially larger rho - the solid core to core-shell ratio. This is to accommodate for the larger pore size distribution. To have sufficiently high enough surface area, a thicker "shell" is necessary.

That said, the Aeris WIDEPORE particle is still very useful for smaller proteins (mAb fragments come to mind) but also larger polypeptides. So it certainly still has niche utility in that regards.

If someone is trying to separate Intact proteins or peptides and not achieving the resolution that they want, what are some changes to the chromatography that they could make?

In general, small changes to the HPLC conditions such as temperature, gradient, and flow rates could have large improvements to the peak shapes and overall separation. For the bioZen WidePore, these conditions have been evaluated and optimized and technical notes are available at www.phenomenex.com/biozenwidepore to learn more.

How does the bioZen WidePore C4 affect carry over?

In intact reversed phase separations, carryover is an issue that is best resolved with a practical method - so one that utilized a proper wash gradient, along with a possible "zig zag" or "shark tooth" gradient, essentially cycling up and down with the gradient, that should remove any "memory effect;" that is, an overly retentive protein that elutes as a broad peak earlier in the chromatogram.

There should be some acceptable amount (0.2% by peak area) carryover associated with the injection itself and this might be column related but can also be related to system (anything in the injection flow path). Otherwise, with an optimized core-shell surface chemistry, such as the bioZen WidePore, one should expect a minimum amount of carryover.

How does the reproducibility on the WidePore C4 column look, what in the column chemistry makes it better batch-to-batch?

bioZen WidePore columns undergo a PEAK manufacturing process, ensuring that batch-to-batch the results are as similar as possible to reduce any reworks or reproducibility issues.

Any applications that you do not recommend using the bioZen WidePore C4 for?

mRNA exceeds the current materials science and spectrometric analytical space but other nucleic acid therapeutics (plasmids, 100-200mer oligos) have a decent chance of working reasonably well. I would preface this with as much as our column is stable under low pH, nucleic acid therapeutics are run at moderately basic conditions. This is still a silica particle so column lifetime might be an issue but otherwise, most proteins of reasonable size should have a chance to work on bioZen WidePore C4.

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