AdvanceBio Peptide Plus

Peptide Characterization

Agilent
Trusted Answers

RA.5079050926

What is the AdvanceBio Peptide Plus Column ?

AdvanceBio Peptide Plus columns feature a hybrid endcapped C-18 stationary phase on a Poroshell 120 2.7µm superficially porous particle modified to have a charged surface

AdvanceBio Peptide Plus
Stainless Steel Columns

Part Number	Column Size (mm)
699775-949	2.1 x 50
695775-949	2.1 x 150
693775-949	2.1 x 250
693975-349	3.0 x 150
693975-949	4.6 x 150

Fast Guards

Part Number	Column Size (mm)
821725-954	2.1 x 5
823750-952	3.0 x 5
820750-940	4.6 x 5

Method Validation Kit

Part Number	Colum Size (mm)
695775-949K	2.1 x 150

AdvanceBio Peptide Plus
PEEK Lined Columns

Part Number	Column Size (mm)
coming in Q2	2.1 x 50
coming in Q2	2.1 x 150

Realize the performance benefits of charged surface C18 chemistry across LC instrument platforms

AdvanceBio Peptide Plus

Poroshell 120, 2.7 µm — UHPLC performance at lower pressure

+

HPH Chemistry — Provides pH stability 1 to 11

+

Charged surface/C18 — Good peak shape with formic acid
Alternate selectivity

=

To make **AdvanceBio Peptide Plus** that provides sensitivity and resolution for accurate and robust identification and quantitation of biotherapeutic PTMs and synthetic peptide impurities when using formic acid as the mobile phase additive

Why choose FA versus TFA mobile phases?

	Formic Acid	TFA
LC separation	Moderate Resolution Poor shape for some peaks	Best Resolution Nearly all peaks have good peak shape
UV detection	Sloped baseline Low signal-to-noise	Flatter baseline Medium signal-to-noise
MS detection	Strong MS signals	Weak MS signals

There is not consensus, but there are strong generalizations

Formic acid is preferred for LC-MS
. . . but some still use TFA for better peak shape and retention.

TFA is preferred for LC-UV applications
. . . but some do LC-UV-MS with formic acid.

LC-UV is more common for routine and QC environments.

LC-MS is more common in development labs and places where unexpected peaks must be analyzed.

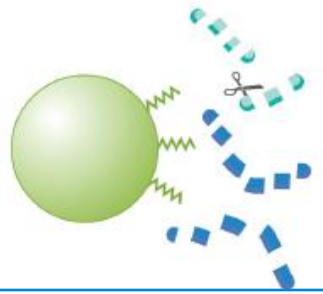
LC-MS is becoming more common over time.

Key Benefits of AdvanceBio Peptide Plus

1. **Good peak shape with formic acid**
   Increased analytical sensitivity with MS detection
2. **Excellent peak shape at high mass load**
   Identification of low level impurities
3. **Alternative selectivity**
   Improved resolution of critical pairs
4. **Quality assurance**
   Tested with Agilent peptide standard mix

The Agilent AdvanceBio Peptide Plus columns with the new charge surface provide improved peak shape with formic acid and alternative selectivity when compared to conventional C18 columns

Applications



Biotherapeutic PTM Analysis - Critical Pair Separation	Compared to traditional C18 columns, AdvanceBio Peptide Plus columns often provide better resolution for critical PTMs like deamidation due to the charged surface
Synthetic Peptide - Impurity Analysis	A single LC method can be run with either MS or UV detection to separate synthetic peptide impurities using Formic Acid as a mobile phase additive
Peptide Analysis - High Mass Load	The good peak shape at high mass load makes the AdvanceBio Peptide Plus the columns of choice when the peptides of interest are at lower levels compared to the rest of the peptides

NOT targeted for comprehensive high sequence coverage peptide mapping as may miss small hydrophilic peptides

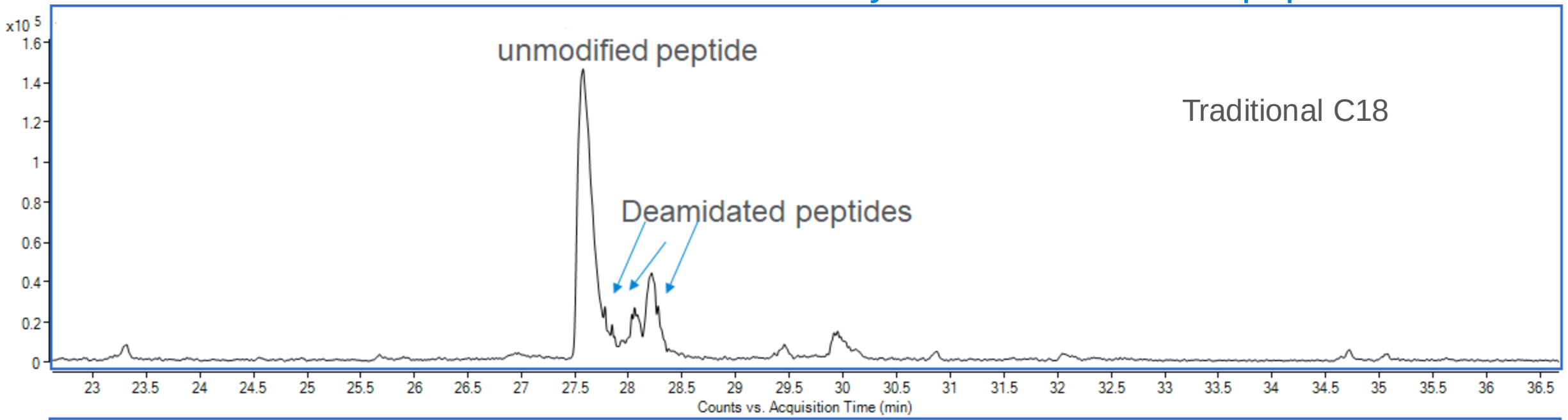
How Does Agilent AdvanceBio Peptide Plus Deliver your Need

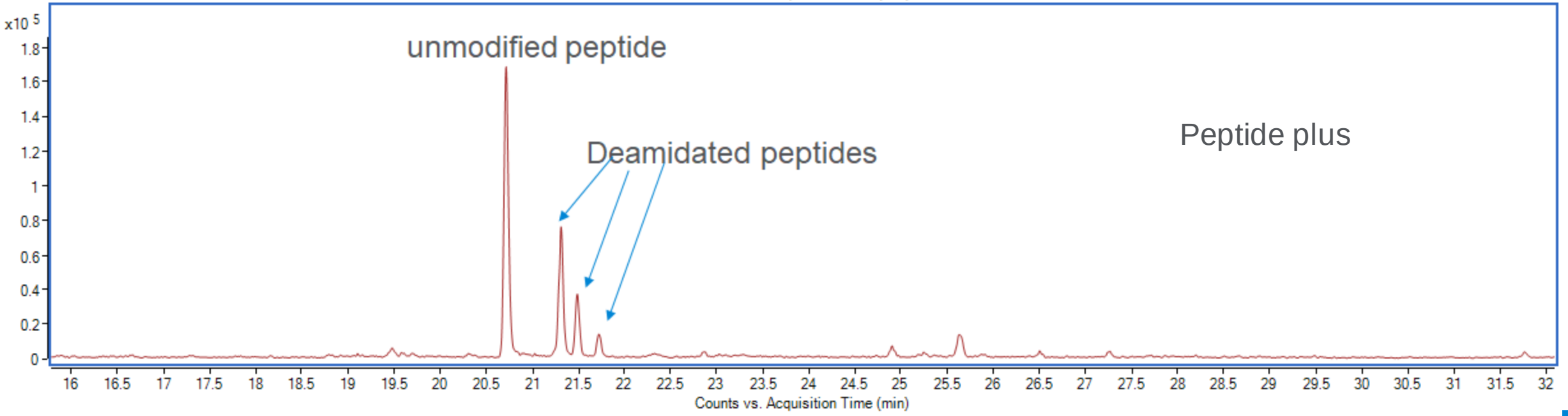
Identification of critical PTMs or impurities (Deamidation/oxidation)	Data accuracy	Identification of low abundant peptides	Data quality
Alternative selectivity to conventional C18 with a positively charged surface C18	Charge surface enables good peak shape when using formic acid	Charge surface C18 phase more tolerant of high mass loads	Based on proven Poroshell 120 HPH particle and application specific quality tested
Resolution of critical pairs	Good peak shape and high sensitivity with LC/MS	No peak tailing with higher mass loads	Reproducibility

Deamidated peptides

- Sometimes you need to do determine deamidated variants of peptides in their sample.
- On uncharged C18 columns, deamidated peptide variants sometimes have poor resolution with the non-deamidated form.
- Since these forms only have ~1 Da mass difference, chromatographic resolution is important.
- Charged surface columns have higher selectivity for deamidated forms versus native forms.

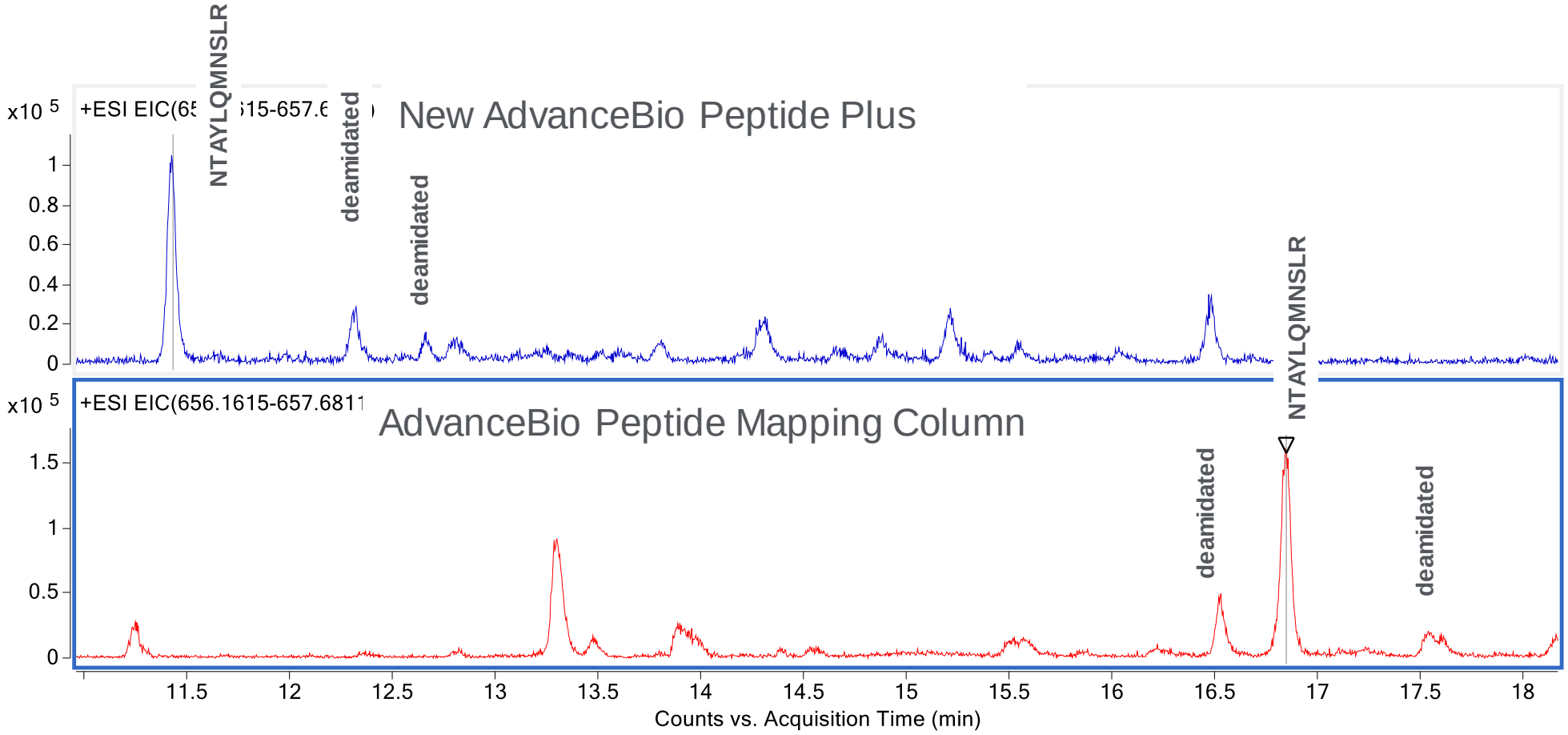
Excellent resolution of critical PTMs is mostly about deamidated peptides





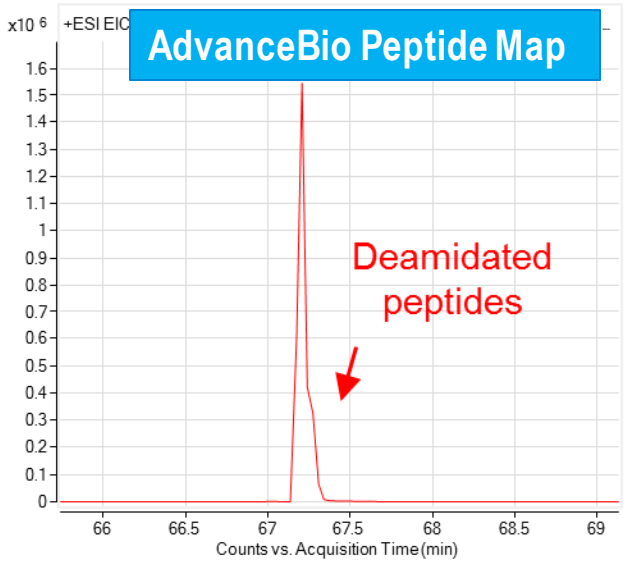
Deamidated Peptides

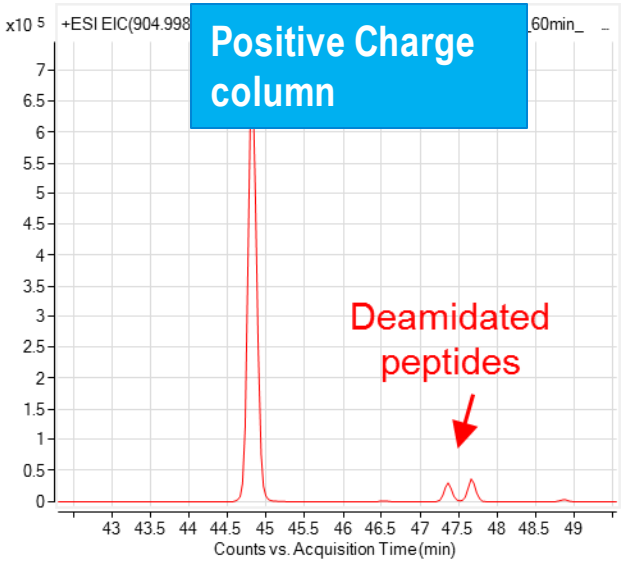
- Deamidated peptides tend to elute later on charged surface columns
- On standard C18 it is less predictable



Deamidated Peptides

VVSVLTVLHQDWLnGK
904.9984 m/z , 2+





Sometimes, the increased selectivity for deamidated peptides will resolve a critical pair which was preventing successful analysis on a standard C18 column.

Mass spec itself cannot resolve these pairs so separation is crucial.

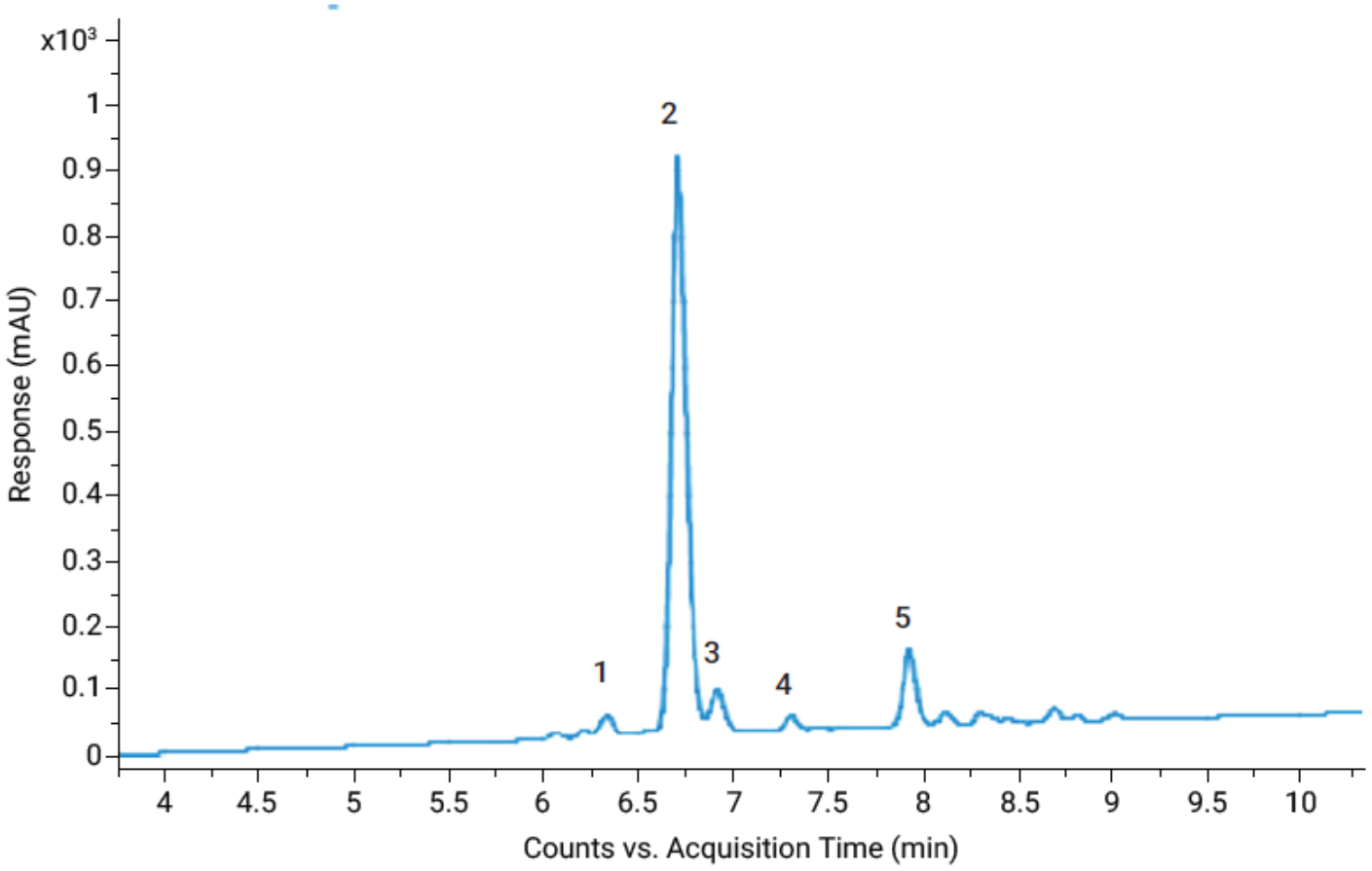
Synthetic Peptides

With AdvanceBio Peptide Plus, a single liquid chromatography (LC) method can be run with either UV or MS detection to separate synthetic peptide impurities using FA as a mobile phase additive.

This LC/MS method can be used in discovery and early development for impurity identification and later move to UV for quantitation of critical pairs, eliminating the need for costly, time-consuming method redevelopment.

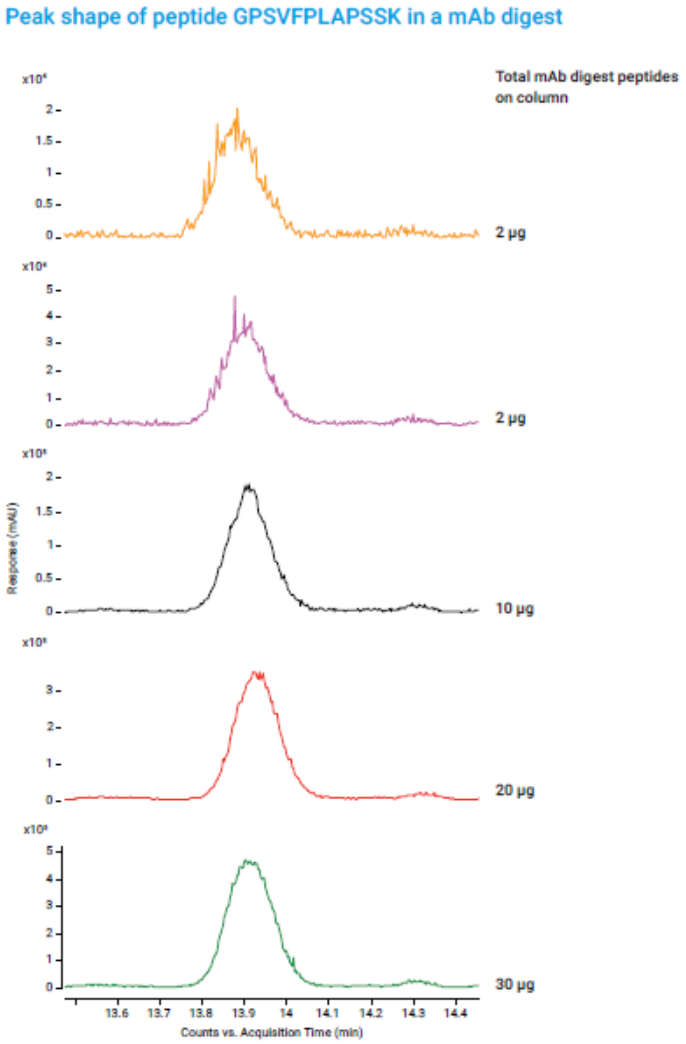
Separation of synthetic peptide and impurities under FA conditions

Excellent separation of bivalirudin peptide and impurities



Parameter	Value	
Column:	AdvanceBio Peptide Plus 2.1 x 150 mm	
Column temp:	60° C	
Eluent A:	0.1% formic acid in water	
Eluent B:	0.1% formic acid in water	
Gradient:	Time (min)	B%
	0 2 22 24 26 26.1	17 17 37 95 95 17
Post Time:	5 minutes	
Peak: 1 2 3 4 5	Peak ID: Deletion of Glu Product Deletion of Gly Loss of H20 Deamidation	

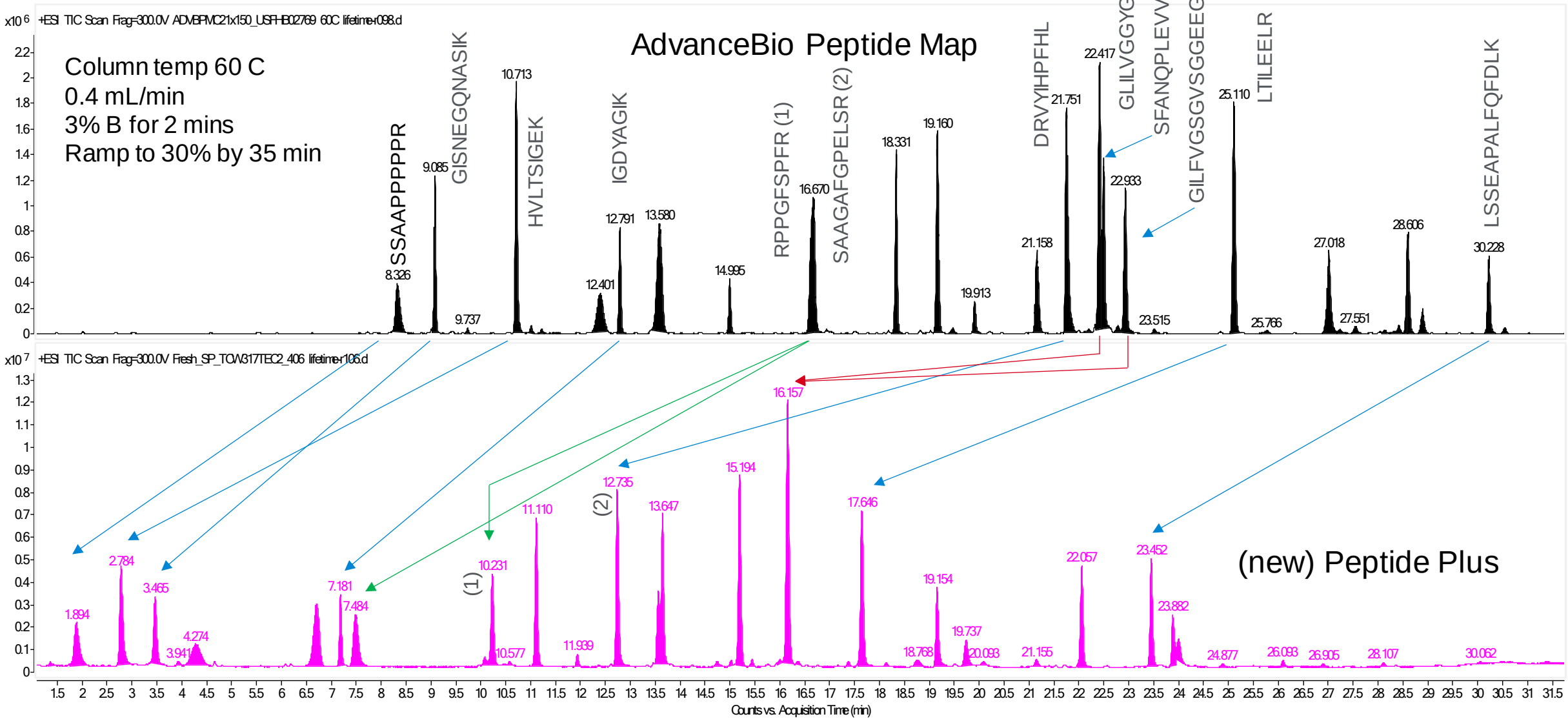
Optimize your separations with high column loadability



Peak shape comparison in 0.1% FA modified mobile phase at increasing sample loads

The AdvanceBio Peptide Plus column is designed to maintain narrow, symmetrical peak shapes and stable retention times in formic acid even as sample load is increased to very high levels. Excellent performance even with high sample loads can facilitate LC/MS determination of minor components such as peptides from host cell proteins in biologics.

Alternate Selectivity: AdvanceBio Peptide Map versus new AdvanceBio Peptide Plus



Rule of thumb for porting methods

- Expect peptide elution at 5 – 10 % lower acetonitrile versus standard C18 column (AdvanceBio Peptide Mapping Column)
- Start at lower % acetonitrile to mitigate loss of small hydrophilic peptides*

*Peptides < 1000 mass with few or zero hydrophibic amino acids

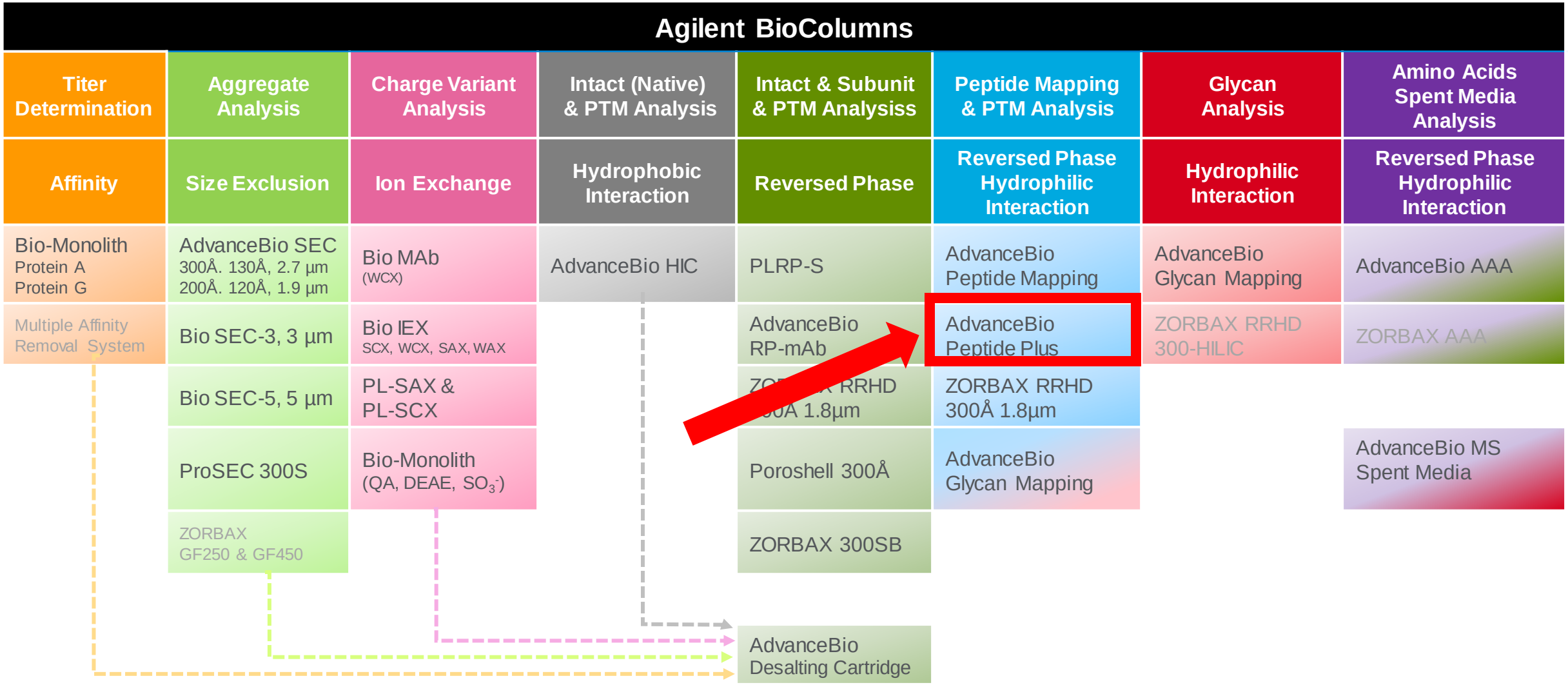
Smaller peptides which elute *near* the void on AdvanceBio Peptide Map may elute *in the void* on AdvanceBio Peptide Plus.

Example: If your AdvanceBio Peptide Mapping Column analytical gradient was:

5% → 40% → 95%,

then switch this to 3% → 30% → 95%

How does it fit into the AdvanceBio Portfolio?



Unfortunately, no one-size-fits-all solution

Peptide Mapping & PTM Analysis	Primary Application	Exceptions
AdvanceBio Peptide Mapping Silica 100Å 2.7 µm	• Protein identification via primary sequence peptide mapping using LC/MS with TFA or FA with low mass loads • Synthetic peptide identification using LC/MS with TFA or FA at low mass	1. Where higher mass load is needed to identify low abundance peptides - FA 2. When critical pairs are not resolved
AdvanceBio Peptide Plus 100Å, 2.7 µm	• For protein purity, PTMs, via analysis of protein digest for specific peptide critical pairs using LC/MS with FA • For identification of synthetic peptide impurity profile using LC/MS with FA	1. For comprehensive high sequence coverage peptide mapping as small hydrophilic peptides may be missed 2. When critical pairs are not resolved
ZORBAX RRHD SB-C18 Silica 300Å 1.8 µm	• For LC analysis of protein digests containing large peptide fragments or hydrophobic core • When alternative selectivity is required	1. Where higher mass load is needed to identify low abundance peptides – FA 2. When critical pairs are not resolved
AdvanceBio Glycan Mapping Silica HILIC-amide	• For LC analysis of hydrophilic/glycopeptides	1. Where the peptides of interest are hydrophobic

Summary

- Hybrid endcapped C-18 stationary phase on a 100 Å 2.7µm superficially porous Poroshell particle modified to have a charged surface C18
- Target application peptide identification and quantitation
  Protein PTMs and synthetic peptides
- Good peak shape with formic acid
  – increased analytical sensitivity with MS detection
- Excellent peak shape at high mass load
  – identification of low abundant peptides
- Alternative selectivity
  – improved resolution of critical pairs