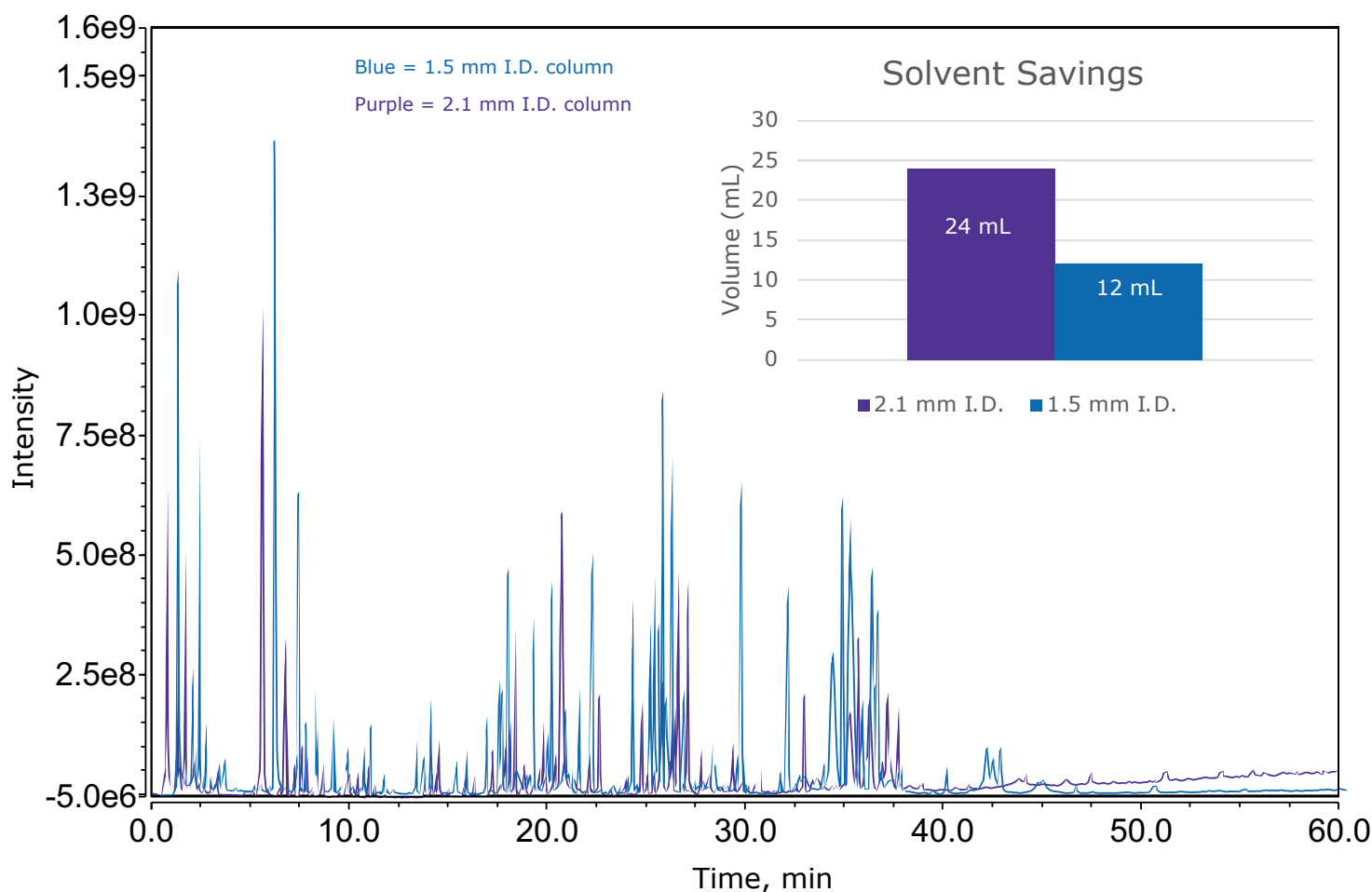


UHPLC-MS Bottom-Up Analysis of Trastuzumab on a BIOshell™ A160 Peptide C18 Column

Introduction:

Bottom-up analysis (also called peptide mapping) is a routine assay performed by analysts in the biopharmaceutical industry as determining the primary structure of a biotherapeutic is a critical quality attribute (CQA). Narrow I.D. columns with 15 cm lengths are typically employed for this analysis in order to achieve high resolution and sensitivity. However, peptide mapping methods require a long run time and,

therefore, utilize larger volumes of solvent than shorter methods. This requirement leads to higher costs of the method in terms of higher volumes of solvent used as well as additional expense in removing the solvent from the laboratory. This application note demonstrates the use of a new, 1.5 mm I.D. column in reducing solvent consumption for peptide mapping assays without a compromise in method performance.



Conditions:

Column:	BIOshell™ A160 Peptide C18, 15 cm x 2.1 or 1.5 mm I.D., 2.7 µm
Mobile phase:	[A] Water (0.1% (v/v) DFA); [B] Acetonitrile (0.1% (v/v) DFA)
Gradient:	2 – 50% B in 60 min
Flow rate:	0.2 mL/min (1.5 mm I.D.) or 0.4 mL/min (2.1 mm I.D.)
Column temp.:	60 °C
Detector:	MSD, ESI-(+)
Injection:	2.0 µL
Sample:	Trastuzumab tryptic digest, 1.25 mg/mL, 1.5 M Guanidine hydrochloride, 0.5% (v/v) formic acid

MS Conditions:

Spray voltage:	3.8 kV
Capillary temp:	320 °C
Sheath gas:	35
Aux gas:	10
RF lens:	50

Conclusion:

This application note described the use of a new 1.5 mm I.D. column to reduce solvent consumption in peptide mapping workflows without a compromise in efficiency. As noted, 50% less solvent was consumed, as compared to a 2.1 mm I.D. column, using the 1.5 mm I.D. column as the optimum flow rate for this column is 0.2 mL/min. This observation translates to only 12 mL of solvent being used in this assay versus

24 mL using a 2.1 mm I.D. column. By using less solvent, the cost per sample is reduced as well as the cost of waste disposal, making this a truly “green” method. Finally, sensitivity, in general, was improved using the 1.5 mm I.D. column versus the 2.1 mm I.D. column, enabling more accurate quantitation of signature peptides as well as the detection of post-translational modifications.

Materials:

Product Part Number	Description
66922-U	BIOshell™ A160 Peptide
66905-U	BIOshell™ A160 Peptide C
900682	Water, for UHPLC, suitable for MS
900667	Acetonitrile, for UHPLC, suitable for MS
G4505	Guanidine hydrochloride, ≥99% (titration), organic base and chaotropic agent
00922	Difluoroacetic acid, for LC-MS, LiChropur™
00940	Formic acid, for LC-MS LiChropur™, 97.5-98.5% (T)

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