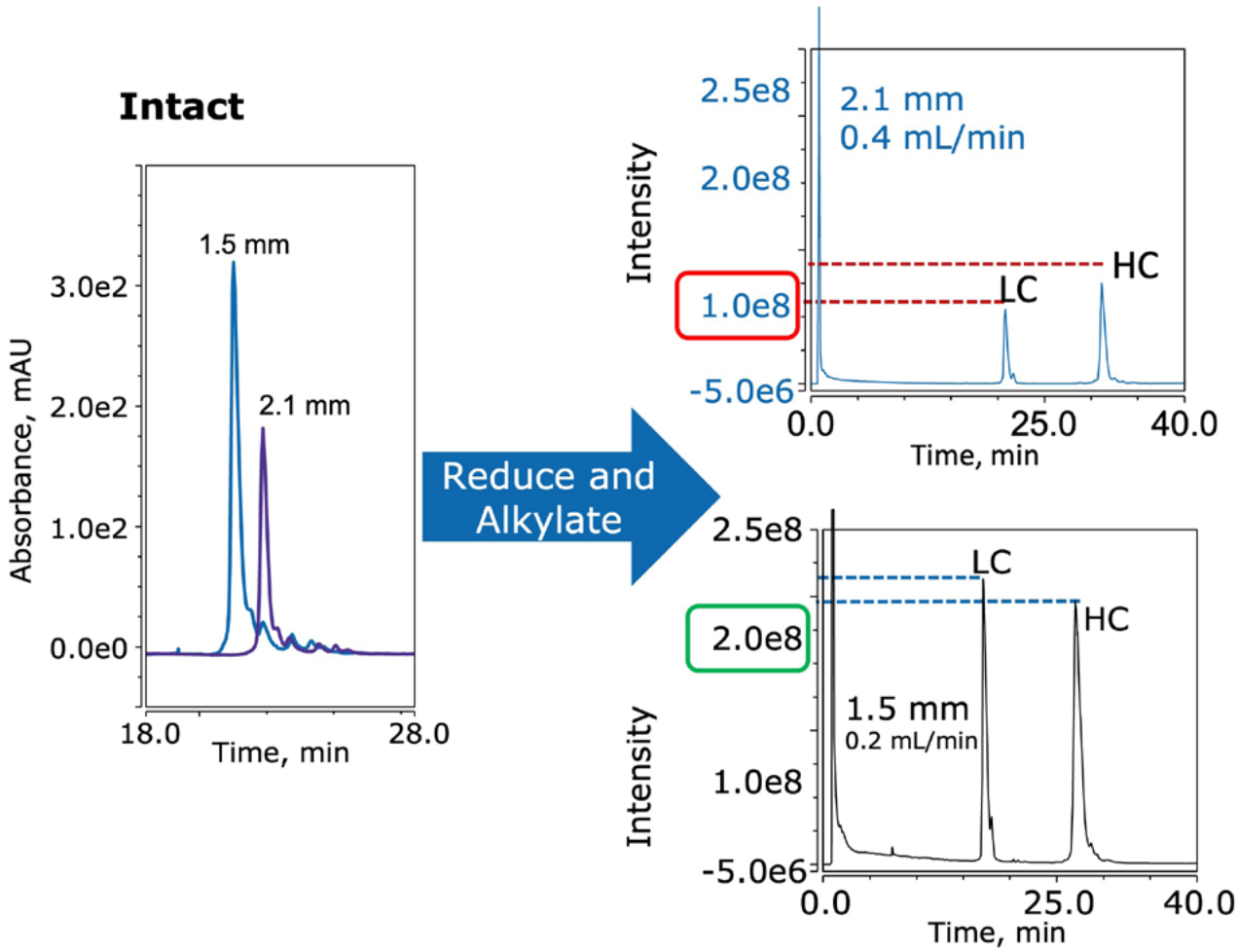
Supelco®
Analytical Products

Application Note

UHPLC-MS Middle-Up and Intact Analysis of Trastuzumab on a BIOshell™ IgG 1000 Å Diphenyl Column

Introduction:

Sensitivity and recovery are two desirable traits in any method, especially in methods for characterizing monoclonal antibodies (mAbs). Having a method that can provide sharp, well-resolved peaks with sufficient area counts allows the analyst to better quantitate the analyte of interest and determine if any degradation products are in the sample. This application note demonstrates the advantage of using a smaller I.D. column (1.5 mm) versus a conventional 2.1 mm I.D. column. In the intact analysis of trastuzumab, double the area for the mAb was obtained on the 1.5 mm I.D. column as compared to the 2.1 mm I.D. column. In addition, in the reduced and alkylated assay, the area counts and sensitivity were improved significantly (2.7-fold increase in area count for the light chain (LC) and 2.3-fold increase in area count for the heavy chain (HC)) when using a 1.5 mm I.D. column as compared to a 2.1 mm I.D. column.



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Conditions:

Column:	BIOshell™ IgG 1000 Å Diphenyl, 15 cm x 2.1 or 1.5 mm I.D., 2.7 µm
Mobile phase:	[A] Water (0.1% (v/v) DFA); [B] 50:50 Acetonitrile (0.1% (v/v) DFA): n-Propanol (0.1% (v/v) DFA)
Gradient:	27 – 36% B in 40 min
Flow rate:	As indicated
Column temp.:	60 °C
Detector:	MSD, ESI-(+)
Injection:	3.0 µL
Sample:	Trastuzumab, 1.0 mg/mL, 100 mM Ammonium bicarbonate

Conclusion:

This application note describes the use of a 1.5 mm I.D. column in analyzing both intact and reduced trastuzumab. Improvements in both recovery and sensitivity were observed with the use of a 1.5 mm I.D. column over a conventional 2.1 mm I.D. column.

No special equipment or modifications to existing UHPLC systems were needed to achieve the benefits of the 1.5 mm I.D. column, which allows for any analyst to reap the benefits of this new geometry.

Materials:

Product Part Number	Description
577450-U	BIOshell™ IgG 1000 Å Diphenyl, 15 cm x 1.5 mm I.D., 2.7 µm
577421-U	BIOshell™ IgG 1000 Å Diphenyl, 15 cm x 2.1 mm I.D., 2.7 µm
900682	Water, for UHPLC, suitable for MS
900667	Acetonitrile, for UHPLC, suitable for MS
1.01024	1-Propanol, for liquid chromatography, LiChrosolv®
00922	Difluoroacetic acid, for LC-MS, LiChropur™
09830	Ammonium bicarbonate, BioUltra

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