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Analytical Products

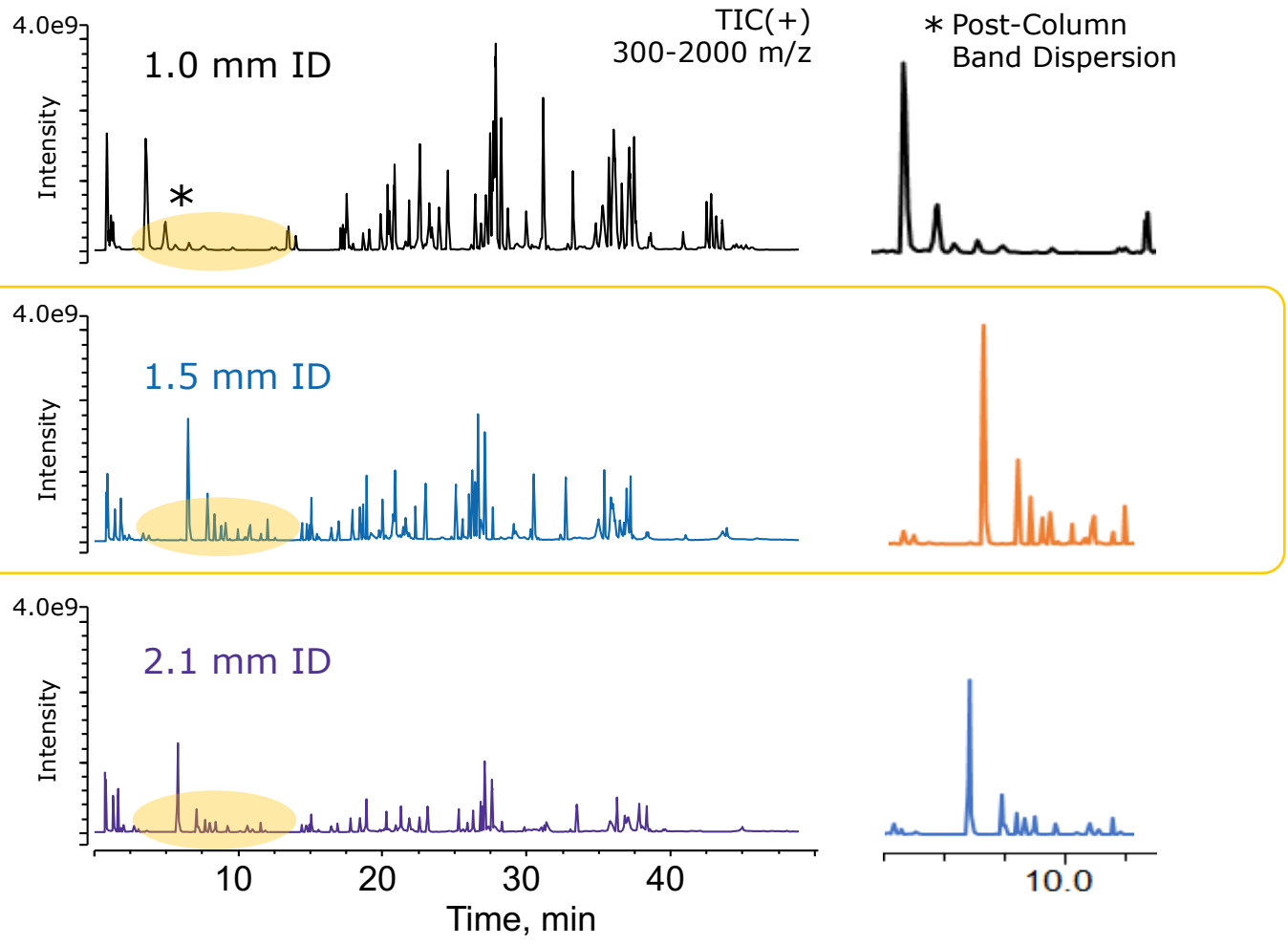
Application Note

Effect of Extracolumn Dispersion on Chromatographic Performance Using Narrow Bore UHPLC Columns

Introduction:

Extracolumn dispersion is defined as any dead volume that comes from sources outside the column which can lead to band broadening of chromatographic peaks. The extracolumn dispersion can come from the injector, pre-column tubing, heat exchanger, post-column tubing, and the detector. For isocratic separations, all these sources of extracolumn dispersion contribute to the loss of efficiency from the method.

For gradient separations, only the post-column tubing and the detector affect the efficiency. As the I.D. of the column becomes narrower, the band broadening due to extracolumn dispersion becomes more pronounced. The purpose of this application note is to demonstrate how a new 1.5 mm I.D. column can minimize the effects of band broadening due to extracolumn dispersion and yield superior performance over a 1.0 mm I.D. and 2.1 mm I.D. column.



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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 importance of the effects extracolumn dispersion had on chromatographic performance of a method, especially with those methods employing narrow I.D. columns. As observed in the spectra, the 1.0 mm I.D. column showed broader peak shape due to the pronounced effect extracolumn dispersion had going from the column to the MS. The new 1.5 mm I.D. column geometry outperformed both the 2.1 mm I.D. column (in terms of sensitivity) and the 1.0 mm I.D. column (in terms of efficiency).

Materials:

Product Part Number	Description
66922-U	BIOshell™ A160 Peptide C18, 15 cm x 1.5 mm I.D., 2.7 µm
66905-U	BIOshell™ A160 Peptide C18, 15 cm x 2.1 mm I.D., 2.7 µm
67099-U	BIOshell™ A160 Peptide C18, 15 cm x 1.0 mm I.D., 2.7 µm
900682	Water, for UHPLC, suitable for MS
900667	Acetonitrile, for UHPLC, suitable for MS
G4505	Guanidine hydrochloride, ≥99% (titration), organic base and chaeotropic agent
00922	Difluoroacetic acid, for LC-MS, LiChropur™
00940	Formic acid, for LC-MS LiChropur™, 97.5-98.5% (T)

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