INTRODUCTION
Characterization of glycoproteins entails analysis of the primary structure of the protein and analysis of the attached oligosaccharides. Oligosaccharides are routinely characterized by mapping after release from the protein. Peptide maps have the advantage of providing information about the site-specific micro heterogeneity of the glycosylations. The enhanced chromatographic resolution associated with Ultra Performance Liquid Chromatography™ has been demonstrated for peptide mapping.1 The technique improves resolution by a factor of three or more. The Peptide Separation Technology Columns for UPLC™ include both 130Å and 300Å pore sizes. The separation of two protein digests into these pore sizes are compared here. One digested protein contains large highly sialylated N-linked and O-linked structures. A mouse monoclonal antibody fragment contains modestly sialylated glycopeptides. Coupling UPLC™ chromatography to on-line liquid mass spectrometry allows for identification of glycopeptides by deconvoluted exact mass measurement. Optimized chromatographic resolution of individual glycopeptides of glycoproteins provides excellent in mass spectral quality, and may provide a simple UV method of glycoform quantitation.4

METHODS
UPLC Conditions
Injection Volume: 10 µL of 1 mg/ml digested solution
Columns: ACQUITY™ ULTIMA™ UPLC C8 130Å, 1.7 µm, 2.1 x 100 mm
ACQUITY™ UPLC™ C18 300Å, 1.7 µm, 2.1 x 100 mm
Temperature: 40°C (unless otherwise noted)
Flow Rate: 0.30–0.90 ml/min
Mobile Phase: 99% A (0.1% TFA in water) / 1% B (99% CH3CN/0.1% TFA)
Gradient Table: Time (min) %A %B
0 100 0
1 100 0
Temperature: 40°C (unless otherwise noted)
Flow Rate: 0.30–0.90 ml/min
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MS Conditions for Glycan Detection
Glycopeptides were detected using the classic method of source fragmentation to create diagnostic glycan ions.5 MS/MS scans were performed at 23.99 eV (m/z 1000) and 44.78 eV (m/z 200). The fragment ion, m/z 100, identifies the presence of N-Linked or O-Linked oligosaccharides. Subtracted protein ions, both N- and O-linked, produce m/z 200 (Figure 3) and m/z 857 (Figure 5b,Glu-CyanoGlu).

RESULTS
Figure 1. Composition of the separation of tryptic peptides of fetuin digested with trypsin. Both the 200Å and 130Å pura pure UPLC™ mixed system. 

Figure 2. The intensity of the total ion chromatograms from the fetuin trypsin digests is comparable whether the source is tuned to molecular ion or fragments. Response is not affected by changing the energy from 15 to 20 eV. m/z 200 (unlabeled peptides are depicted in Table 2).

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8

Figure 9

Figure 10

DISCUSSION