

APPLICATIONS

bioZen[™] 3 µm NX-C18 Fractionation Column has Improved Loading Capacity in Comparison to Competitor Brand

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Overview

High pH fractionation is a strategy commonly used to increase proteome coverage and its main purpose is to maximize protein identification in complex sample matrices. In proteomics it is often the situation where the amount of sample that is available for analysis is limited. This presents the analyst with a challenge to successfully collect all the information needed to advance their research. A powerful tool that can, and often is, used to increase the effectiveness of the MS efficiency is to miniaturize the LC separation. While the mass of the sample available is often small in proteomics applications, the volume that the sample is present in typically is not. The injection of a large volume on a small ID column directly is a very timeconsuming process. Thus, in this technical note we explore the loading capacity of the bioZen 3 µm NX-C18 fractionation column and how it compares to the competitor column.

A mixture of Angiotensin III Peptide (RVYIHPF) was injected at a range of masses on the columns. The injection mass range chosen was well within the limits of detection for this method (Figure 1). The peak area response to the mass load on the column was monitored to investigate the loadability. A non-linear response of peak area to mass would indicate that the loadability of the trap had been exceeded. None of the masses reached the capacity threshold for the bioZen NX-C18 fractionation column as the peak area was consistently increasing, but the Waters XBridge column did not reach the same amount of angiotensin before reaching the same peak area (Figure 2). This data suggests that the bioZen NX-C18 fractionation column has a 50 % higher loading capacity than the Waters XBridge column.

LC-MS/MS Conditions

Column:	bioZen 3 μm NX-C18 Fractionation			
	Waters [®] XBridge [®] 3.5 µm BEH C18			
Dimension:	150 x 2.1 mm			
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Part No.:	<u>00F-4793-AN</u> (bioZen)			
Pressure (bar):	220 bar			
Mobile Phase:	A: 10 mM Ammonium Formate pH 10 in Water			
	B: 10 mM Ammonium Formate pH 10 in 90 %			
	Acetonitrile, 10 % Water			
Gradient:	Time (min)	%A	%В	
	0	50	50	
	7	50	50	
Flow Rate:	300 μL/min			
Temperature:	Ambient			
LC System:	EASY-nLC™ 1200			
Detection:	UV @ 220 nm			
Injection Volume:	10 μL			



Figure 1. Chromatograms of loading capacity of (A) bioZen[™] NX-C18 Fractionation and (B) Waters[®] XBridge[®] BEH C18.

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