

APPLICATIONS

Determining Trap Loadability using the bioZen™ 3 μm Polar C18 Nano Column in Conjunction with the bioZen RP1 Nano Trap Column

Dr. Jason Anspach, Roxana Eggleston-Rangel, and Dr. Bryan Tackett
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA



Dr. Jason Anspach
Global Product Manager – HPLC

When not advancing the forefront of HPLC, Jason enjoys scuba diving with his wife and being the tickle monster for his daughter.

Introduction

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. By decreasing the column inner diameter (ID), the optimum column flow rate decreases, which in turn decreases the sample dilution due to the chromatographic process. By reducing the dilution, the sample concentration increases in the electrospray source and thereby the ionization efficiency, which in turn increases MS sensitivity.

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 time-consuming process. To substantially reduce injection time, as well as provide sample cleanup and column protection, frequently a trap and elute injection mode is employed. A key consideration in trap and elute injections is the loading capacity of the trap column. A trap with inadequate sample loadability will result in the sample not being captured on the trap and going to waste. Thus, in this technical note we explore the effects of trap loadability using a bioZen 3 μm Polar C18 nano column in conjunction with the bioZen RP1 (C18) nano trap column.

Results and Discussion

A mixture of four peptides of varying properties were chosen as model compounds and injected at a range of masses on the trap, and subsequently the analytical column. The injection mass range chosen was well within the limits of detection for this method (**Figure 1**). The peak area and peak width 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. A non-linear peak width response to mass load would be indicative of surpassing the loadability of the analytical column. None of the peptides reached the capacity threshold as the peak area was consistently increasing (**Figure 2a**) and the peak width remained unchanged (**Figure 2b**).

LC Conditions

Column: bioZen 3 μm Polar C18
Dimensions: 150 x 0.075 mm
Part No.: [00F-4782-AW-21](#)
Trap: bioZen RP1 (C18)
Dimensions: 10 x 0.075 mm
Part No.: [05N-4252-AW](#)
Pressure: 200 bar
Mobile Phase: A: 0.1 % Formic Acid in Water
 B: 0.1 % Formic Acid in Acetonitrile

Gradient	Time (min)	% B
	0	3
	15	40
	20	70
	26	80
	26	3
	30	3

Flow Rate: 250 nL/min
Temperature: 25 °C
LC System: NanoLC™ 425 (SCIEX®)
Detection: MS/MS
Detector: 6500 QTRAP® (SCIEX)
Injection Volume: 1 μL
Sample: 1. 10 pg peptide
 2. 50 pg peptide
 3. 100 pg peptide
 4. 200 pg peptide
 5. 500 pg peptide
 6. 1000 pg peptide

MS Conditions

Ion Source: Positive
CUR: 30
IHT: 150
DP: 80
EP: 10
CXP: 15
GS1: 35
GS2: 0
IS: 2500

SIM Scan

ID	Q1	Dwell
VYV	380	50
NRVYIHPF	524	50
YGGFM	574	50
YGGFL	556	50

Figure 1.
Detection of increasing quantities of peptide.

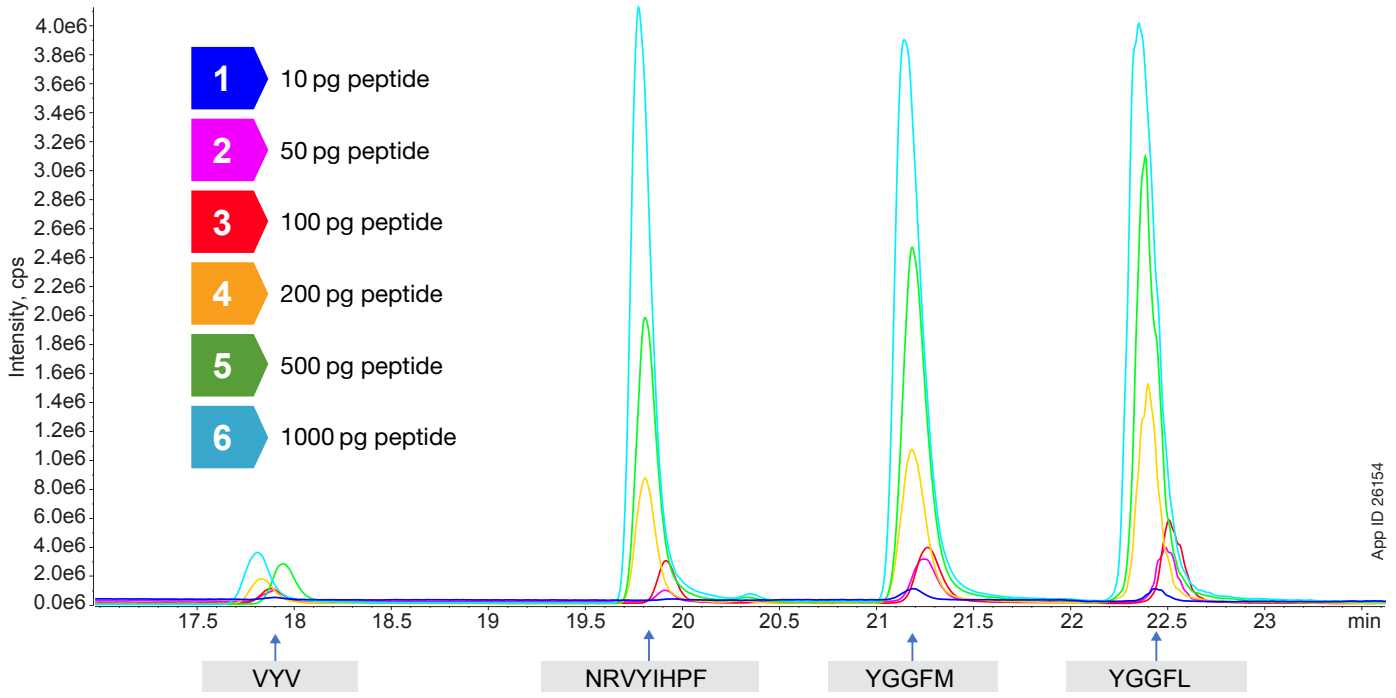


Figure 2a.
Peak area threshold not met with increasing amounts of peptide.

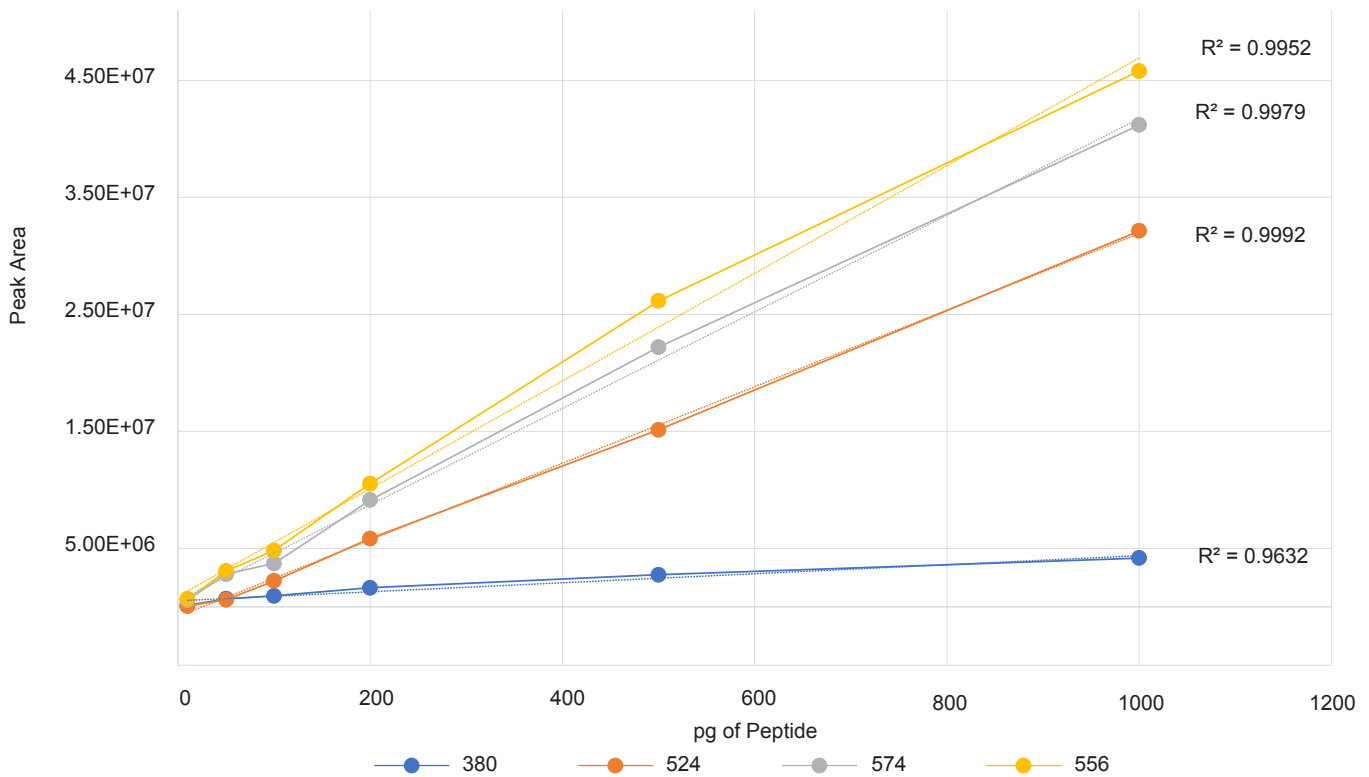
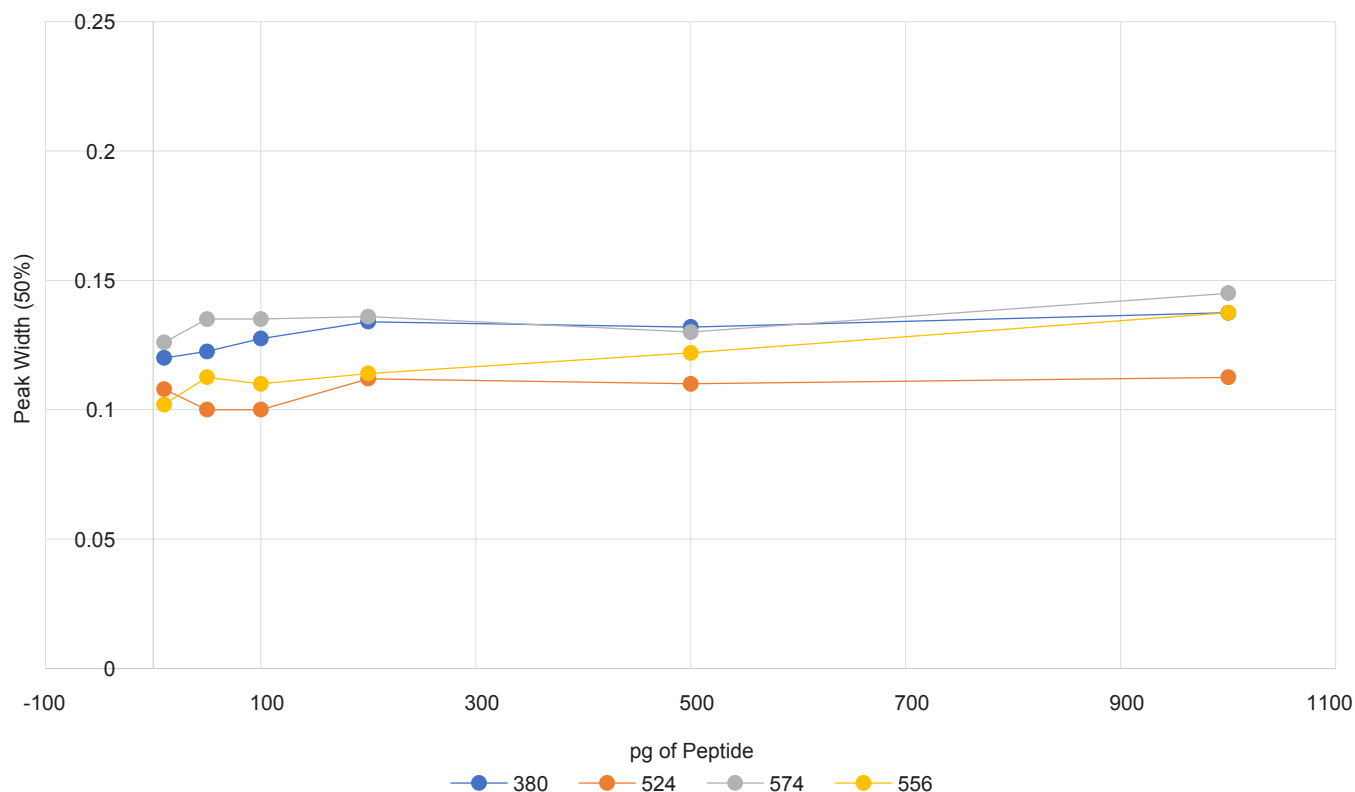


Figure 2b.
Peak width unchanged with increasing amount of peptide.



Conclusions

In trap and elute injection mode, inadequate loadability of the trap or analytical column can lead to sample loss or peak distortions leading to loss of sensitivity. In this investigation we saw a linear peak area response to mass loads of 1-1000 pg per peptide (4-4000 pg total mass loads) showing that 4 µg mass loads are easily achievable with the bioZen 3 µm Polar C-18 column and bioZen RP1 trapping column system.

Ordering Information

bioZen Nano LC Columns with Integrated SecurityLINK™ Fingertight Fitting

Phases	150 x 0.075 mm	250 x 0.075 mm	500 x 0.075 mm
bioZen 3 µm Peptide PS-C18	00F-4771-AW-21	00G-4771-AW-21	-
bioZen 2.6 µm Peptide XB-C18	00F-4768-AW-21	00G-4768-AW-21	-
bioZen 3 µm Polar C18	00F-4782-AW-21	00G-4782-AW-21	-
bioZen 5 µm Peptide XB-C18	-	-	00J-4605-AW-21

bioZen Nano LC Columns with Open Fused-Silica Inlet Fitting

Phases	150 x 0.075 mm	250 x 0.075 mm	500 x 0.075 mm
bioZen 3 µm Peptide PS-C18	00F-4771-AW-11	00G-4771-AW-11	-
bioZen 2.6 µm Peptide XB-C18	00F-4768-AW-11	00G-4768-AW-11	-
bioZen 3 µm Polar C18	00F-4782-AW-11	00G-4782-AW-11	-
bioZen 5 µm Peptide XB-C18	-	-	00J-4601-AW-11

bioZen Trap Columns

Nano Trap Columns		
Phases	10 x 0.075 mm	Unit
RP1 (General RP)	05N-4252-AW	3/pk
RP2 (Aqueous Stable RP)	05N-4754-AW	3/pk

PEEKlok™ Trap Fittings

Trap Fittings		
Part No.	Description	Unit
AQO-7602	PEEKlok fittings with 6-40 thread for 1/32" OD tubing (2 x fittings, 6 x ferrules and 1 x tightening tool)	ea
AQO-7603	PEEKlok fittings with 6-32 thread for 1/32" OD tubing (2 x fittings, 6 x ferrules and 1 x tightening tool)	ea
AQO-7600	PEEKlok fittings with 10-32 thread for 1/32"OD tubing with low profile hex head (2 x fittings, 6 x ferrules and 1 x wrench)	ea

Australia

t: +61 (0)2-9428-6444
 auinfo@phenomenex.com

Austria

t: +43 (0)1-319-1301
 anfrage@phenomenex.com

Belgium

t: +32 (0)2 503 4015 (French)
 t: +32 (0)2 511 8666 (Dutch)
 beinfo@phenomenex.com

Canada

t: +1 (800) 543-3681
 info@phenomenex.com

China

t: +86 400-606-8099
 cninfo@phenomenex.com

Czech Republic

t: +420 272 017 077
 cz-info@phenomenex.com

Denmark

t: +45 4824 8048
 nordicinfo@phenomenex.com

Finland

t: +358 (0)9 4789 0063
 nordicinfo@phenomenex.com

France

t: +33 (0)1 30 09 21 10
 franceinfo@phenomenex.com

Germany

t: +49 (0)6021-58830-0
 anfrage@phenomenex.com

India

t: +91 (0)40-3012 2400
 indiainfo@phenomenex.com

Ireland

t: +353 (0)1 247 5405
 eireinfo@phenomenex.com

Italy

t: +39 051 6327511
 italiainfo@phenomenex.com

Luxembourg

t: +31 (0)30-2418700
 nlinfo@phenomenex.com

Mexico

t: 01-800-844-5226
 tecnicomx@phenomenex.com

The Netherlands

t: +31 (0)30-2418700
 nlinfo@phenomenex.com

New Zealand

t: +64 (0)9-4780951
 nzinfo@phenomenex.com

Norway

t: +47 810 02 005
 nordicinfo@phenomenex.com

Poland

t: +48 22 104 21 72
 pl-info@phenomenex.com

Portugal

t: +351 221 450 488
 ptinfo@phenomenex.com

Singapore

t: +65 800-852-3944
 sginfo@phenomenex.com

Slovakia

t: +420 272 017 077
 sk-info@phenomenex.com

Spain

t: +34 91-413-8613
 espinfo@phenomenex.com

Sweden

t: +46 (0)8 611 6950
 nordicinfo@phenomenex.com

Switzerland

t: +41 (0)61 692 20 20
 swissinfo@phenomenex.com

Taiwan

t: +886 (0) 0801-49-1246
 twinfo@phenomenex.com

Thailand

t: +66 (0) 2 566 0287
 thaiinfo@phenomenex.com

United Kingdom

t: +44 (0)1625-501367
 ukinfo@phenomenex.com

USA

t: +1 (310) 212-0555
 info@phenomenex.com

🌐 **All other countries/regions
 Corporate Office USA**

t: +1 (310) 212-0555
 info@phenomenex.com

