



# Removal Of PEG Contaminants From Peptides Through An Off-Line Ion-Exchange Spin Column

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# Abstract

## Introduction

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Consistent and reproducible sample preparation techniques are key to optimal mass spectrometric data acquisition. Contaminating substances such as polyethylene glycol (PEG) from plastic sources can obscure sample peaks in LC-MS analysis. The ability to perform sample clean up before loading the sample for analysis can ease bottlenecks and prevent column fouling. Here, we present a method for off-line ion-exchange clean up of samples using a modification of the ProTrap XG. The ProTrap XG is a two-stage filtration cartridge that facilitates sample precipitation and detergent removal. The filtration cartridge offers up to 99.8% purity and 95% recovery through acetone precipitation. An optional solid-phase extraction cartridge further cleans up the sample.

## Results

3

The use of the IEX cartridge resulted in a minimum of 90% recovery of the sample loaded as analyzed by LC-UV. Analysis of the samples by LC-MS showed no statistical difference in samples with and without ion-exchange treatment.

## Methods

2

The spin-on reversed-phase chromatography cartridge from the ProTrap XG was replaced with a well-characterized, commercial strong cation exchange media. This proof of concept analysis used trypsin digested bovine serum albumin (BSA). The resin was primed, and the peptides in 15% acetonitrile (ACN), 0.1% trifluoroacetic acid were loaded by centrifugation. After a wash step, the peptides were eluted with 50 mM ammonium acetate pH 10, 100 mM KCl, 5% ACN and subjected to LC-MS. To prove the utility of the ion exchange column in sample clean up, samples were purposely spiked with PEG 400 then loaded onto the ion exchange cartridge, eluted and compared via LC-MS analysis to the same sample with and without PEG contamination.

## Conclusions

4

The use of an off-line ion-exchange chromatography step before mass spectrometric analysis results in a cleaner sample. This modification of the ProTrap XG can simply and reliably remove PEG from a processed sample.

# Introduction



Few mass spectrometry results are more frustrating than observing contaminated samples.

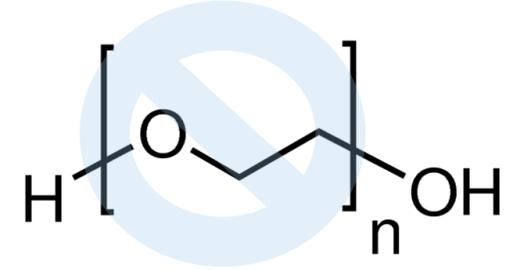


You can be using a state-of-the-art analyzer, but if your sample has 'garbage' in it, you get garbage out. Your spectrum is all but useless.



ProTrap XG is a single-use sample preparation device<sup>1</sup>. The SPE cartridge was modified from Reversed-Phase resin to Ion Exchange (IEX) resin.

# PEG



Polyethylene glycol is a ubiquitous chemical found in detergents to hand creams<sup>2</sup>



Mass spectra that include PEG have a 44 Da signature repeat



Outcompetes peptides for ionization, obscuring the peptide signal<sup>2,3</sup>— and you only find it once you run your sample



Effective removal of PEG from peptide solutions, when present, is necessary

# Methods

For this proof of concept, bovine serum albumin(BSA) was digested with trypsin (50:1)

Resulting mixture (25 µg) of peptides and intact protein spiked with variable amounts of PEG 400; 1 mg/mL to 5 ng/mL

Subjected to IEX using a repurposed ProTrap XG cartridge

Cartridge was primed with 15% acetonitrile (ACN), then 0.1% trifluoroacetic acid (TFA)

Peptides + PEG loaded by centrifuging at 350 g

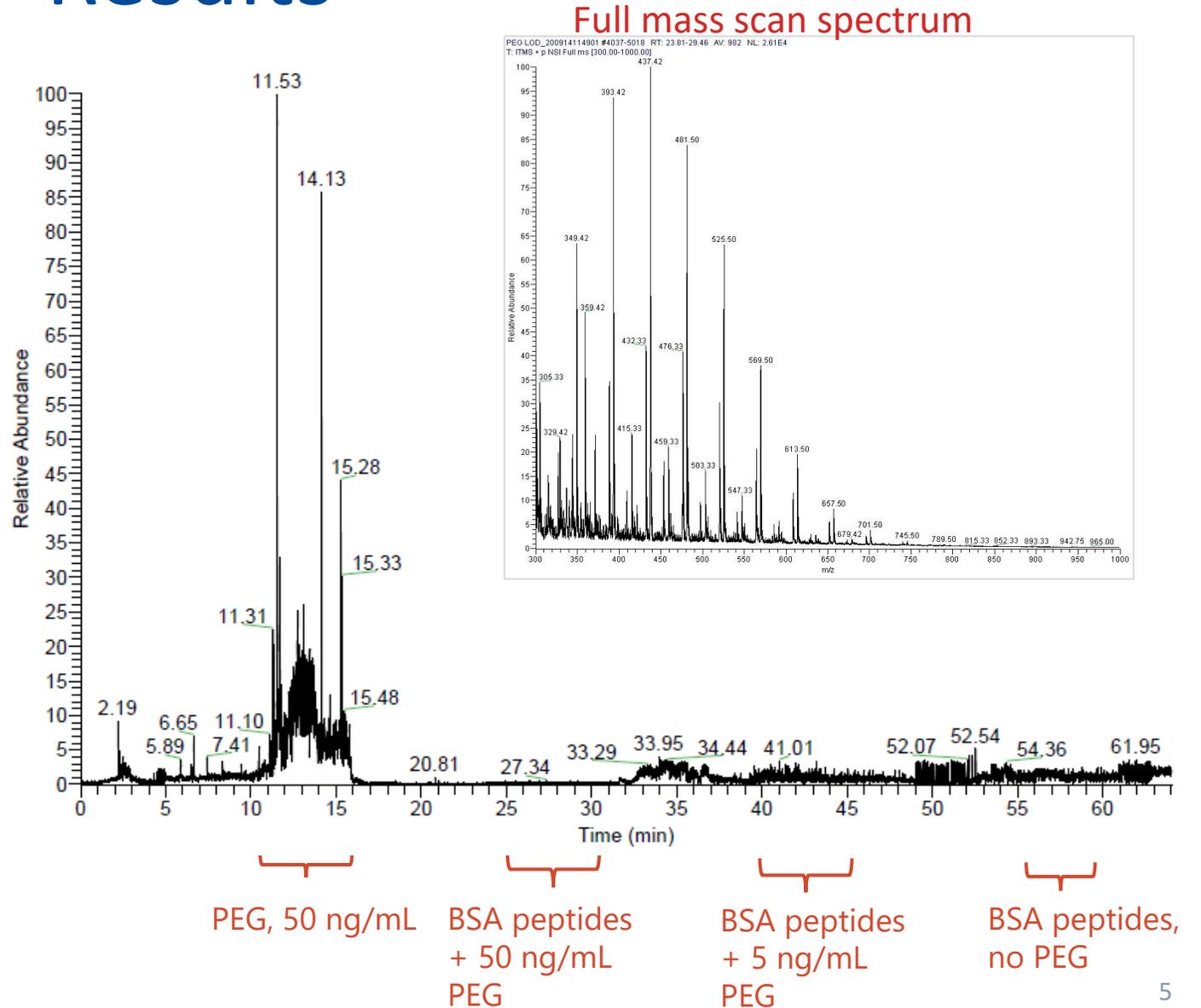
Wash with 5% ACN/0.1% TFA

Elute with 50 mM ammonium acetate pH 10, 100 mM KCl, 5% ACN, centrifuge 350 g

Total clean up time: 21 minutes

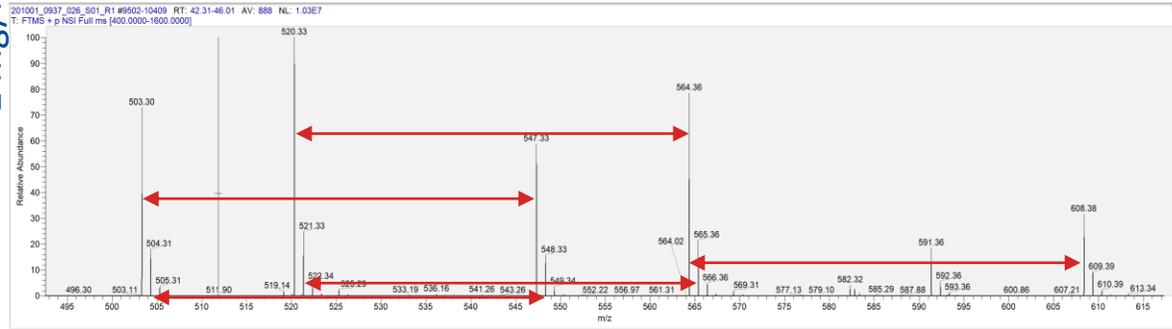
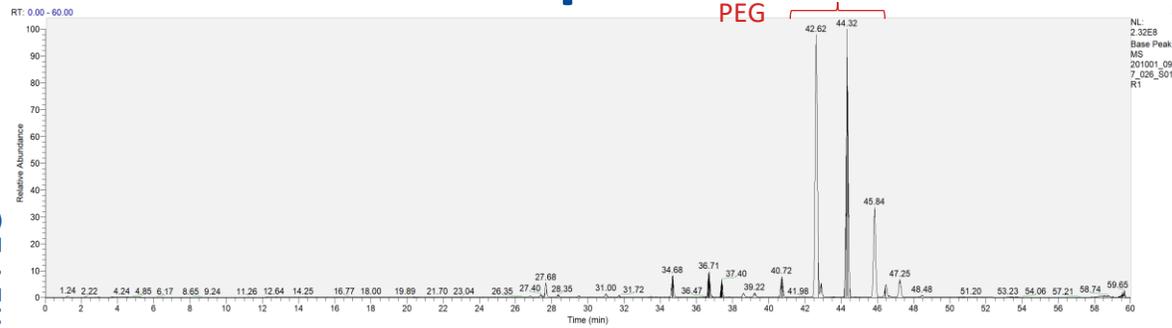
Eluted peptides subjected to LC-MS

# Results

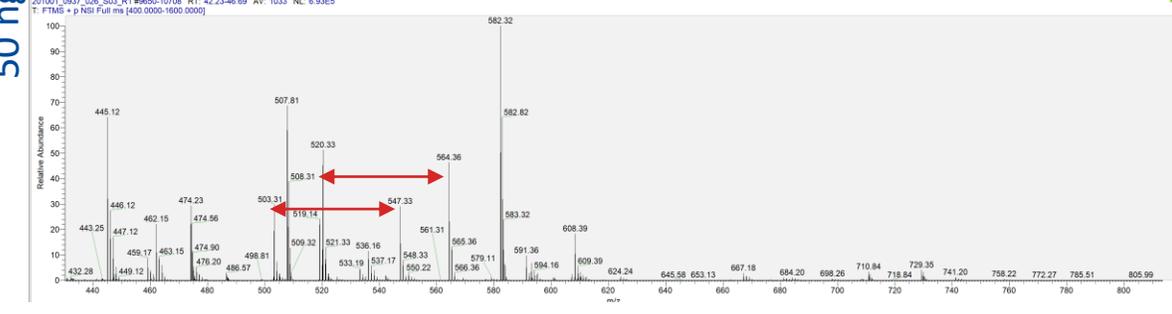
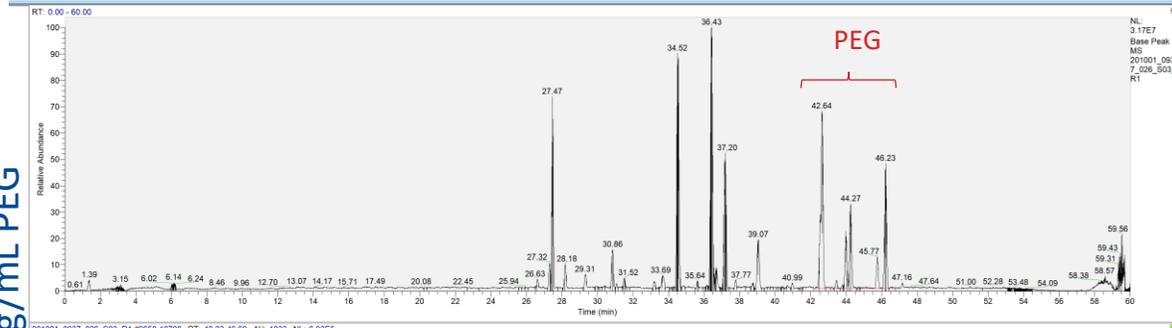


# Results – spectra after IEX treatment

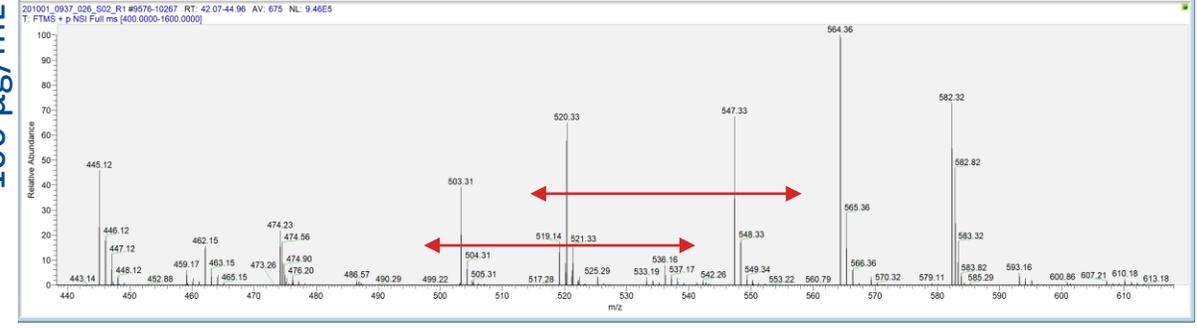
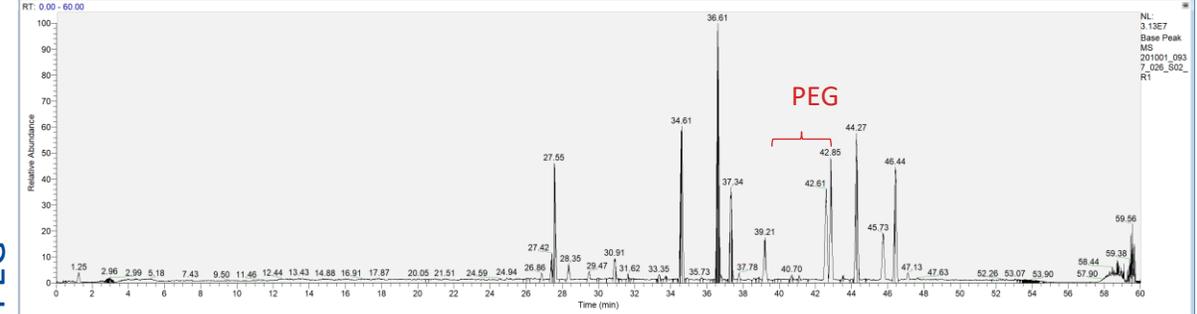
1 mg/mL PEG



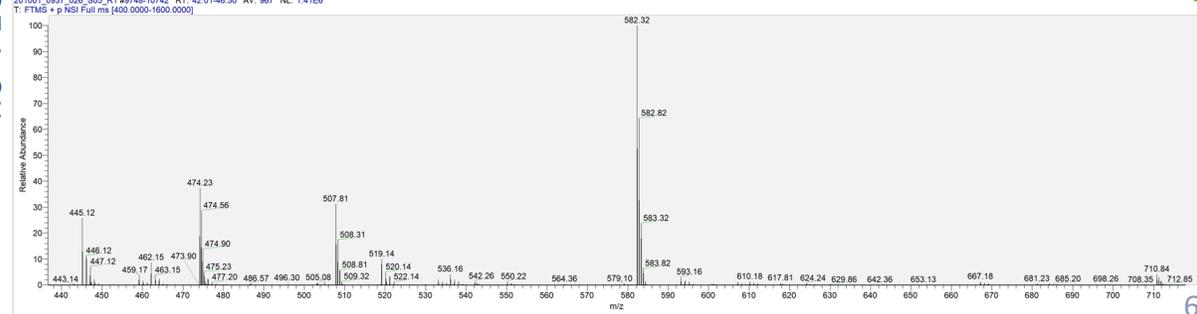
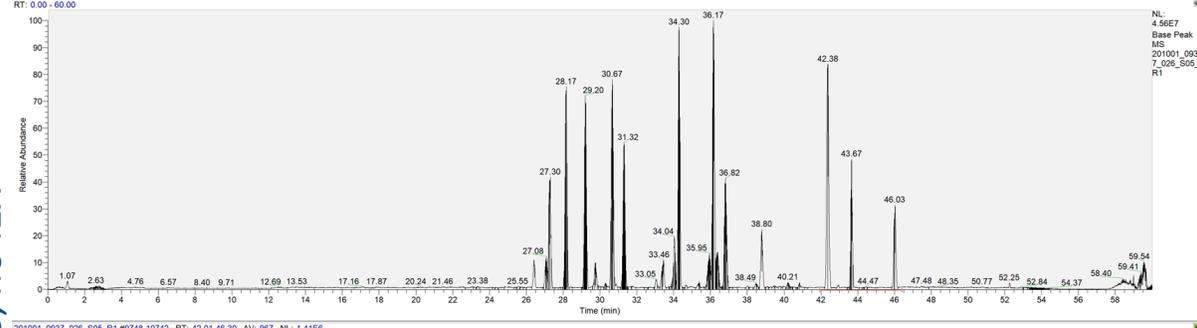
50 ng/mL PEG



100 µg/mL PEG

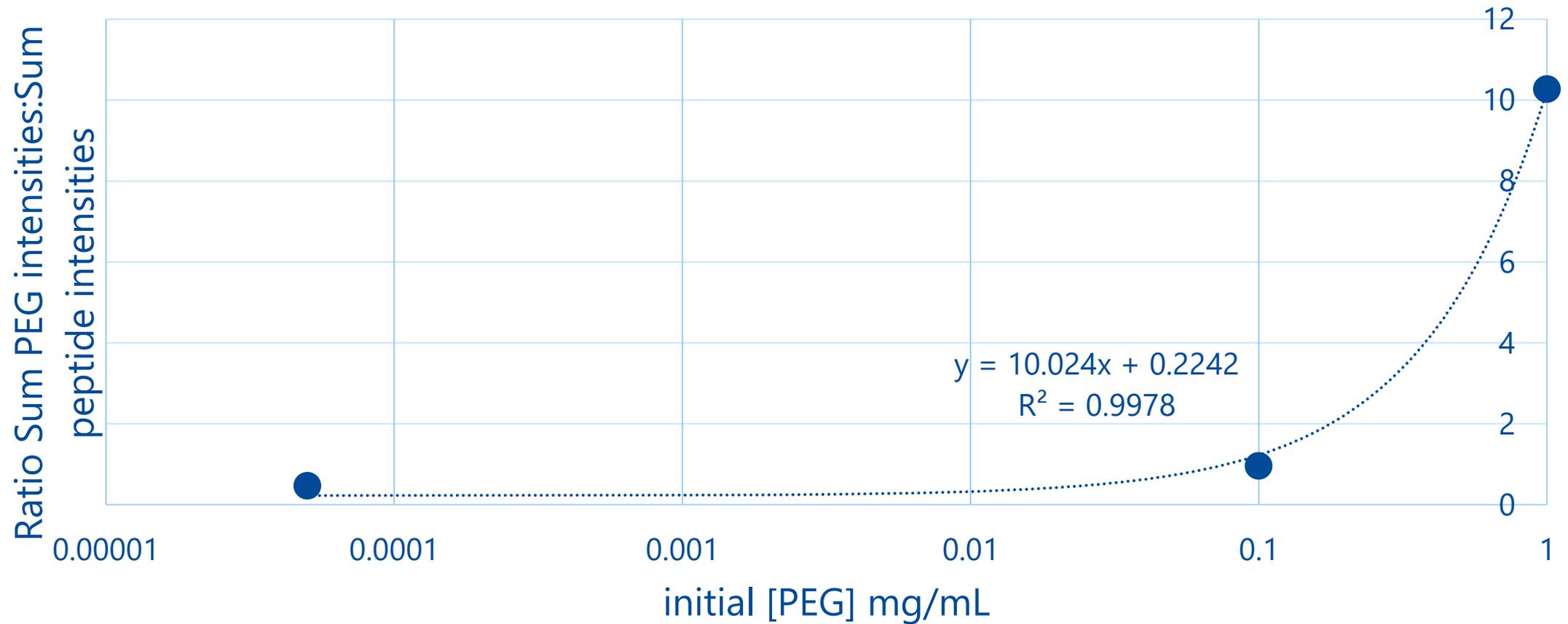


no PEG, no IEX

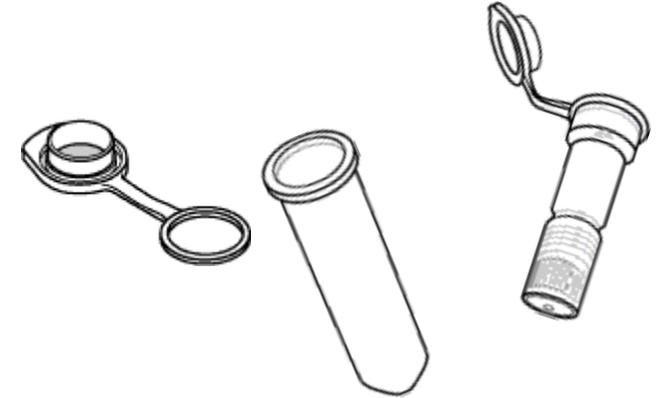


# Results

The sum of the relative abundance for each peak identified as PEG was divided by the sum of the relative abundance for each peptide peak. The resulting ratio (PEG/peptide) was plotted against initial PEG concentration.



# Discussion



Study to be repeated with a full proteome to demonstrate utility over range of peptides, including hydrophobicity, iso-electric point and a full range of peptide sizes, including intact proteins.

Illustration of capture processed peptides – utility for reflex clean up

Demonstration of utility to decrease the amount of PEG present. Removal of trace amount of PEG was effective, higher levels of PEG may require further washes.

This potential new offering increases the diversity of the ProTrap family for providing the optimal sample for LC-MS/MS.



# Conclusions



Ion-exchange modification of the ProTrap XG results in a decrease in the amount of PEG remaining over a range of PEG concentrations, and could prove to be a useful tool to deplete PEG to acceptable level



Further study is required to determine parameters for optimal use with proteome



ProTrap XG modified to include an off-line IEX cartridge could have utility in sample preparation other than PEG removal, for example fractionation of peptides

# References

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- (3) Zhao, C.; O'Connor, P. B. Removal of Polyethylene Glycols from Protein Samples Using Titanium Dioxide. *Anal Biochem* **2007**, *365* (2), 283–285. <https://doi.org/10.1016/j.ab.2007.03.024>.

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# Acknowledgments

Thank you to Dr Alejandro Cohen, Biological Mass Spectrometry Core, Dalhousie University for running a portion of the IEX treated samples