

Introduction

For sample preparation in the field of proteomics, a clean up procedure is essential before analyzing peptides. Solid Phase Extraction (SPE) is the most commonly used procedure to isolate peptides from the chemicals utilized during digestion. Detergents are often used to solubilize hydrophobic proteins, therefore Strong Cation Exchange (SCX) SPE needs to be used to remove these detergents. We tested three SCX SPE methods on a peptide digest from *Shewanella oneidensis* containing a detergent. The methods used for comparison were called Standard, OMIX and KIM. We were trying to find the method that gave the best recovery, reproducibility and was the most consistent in results..

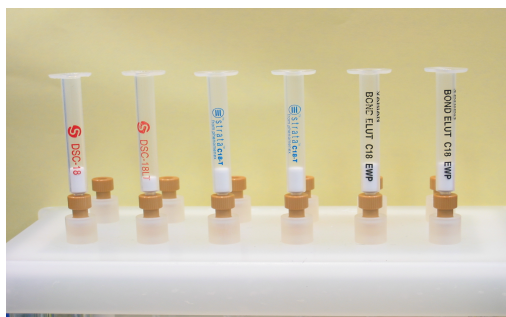


Figure 1: Close up of SPE columns on a vacuum manifold.

Methods

	Conditioning	Sample Loading	Wash	Elution	# of Steps
Standard	MeOH, Buffer A, Buffer B	Acidify sample to 1% TFA, Centrifuge	Buffer A	80:15:5 MeOH:H ₂ O: NH ₄ OH, pH 10	15
Omix	MeOH, 0.1%TFA, 1.0% TFA	No Treatment	0.1% TFA in 70%MeOH	65:30:5 H ₂ O:MeOH:NH ₄ OH	9
KIM	MeOH, Buffer A	Acidify sample to pH 3.0 w/ Formic Acid, Centrifuge	Buffer A	80:15:5 MeOH:H ₂ O: NH ₄ OH, pH 10	7

Table 1. Boxes don't accurately reflect the number of steps for each procedure. Number of steps is shown to highlight the desire to simplify procedures.

Buffer A: 10mM Ammonium Formate in 25% ACN, pH 3.0.

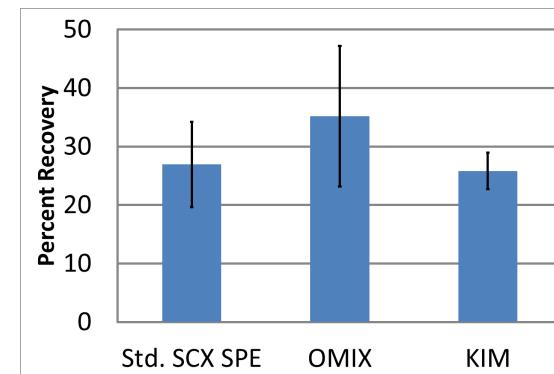
Buffer B: 500mM Ammonium Formate in 25% ACN, pH 6.8

Results

Sample ID	% Recovery	Mean	SD
Standard SCX SPE	36.0	29.5	7.3
	26.6		
	31.1		
	24.4		
	16.7		
OMIX	15.0	40.2	12
	40.0		
	46.9		
	36.0		
	38.0		
KIM	27.9	25.8	3.1
	26.8		
	25.0		
	28.6		
	20.8		

Table 2. Observed recovery from 5 replicate samples tested.

Figure 2 : Graphic representation of Table 2, with mean % recovery and SD (error bars).



Conclusion

The data suggests that all of the methods have relatively the same % recovery when compared to each other taking into account the standard deviation from the mean. The OMIX method showed a higher mean % recovery, but an unacceptable standard deviation. The KIM method had lower overall % recovery with the least amount of variation compared to other two methods. Both the standard method and OMIX methods had one point that might be able to be excluded. However, after performing a Grubbs test to determine if they were outliers, those points were not able to be removed from the sets. It is highly likely that if there were more replicates in the dataset, those points would have been removed.

Further experiments must be conducted to investigate whether a method could be developed to yield higher recoveries, be more time efficient, and is more reproducible.

Reference

Supelco (1998), Guide to Solid Phase Extraction.

Acknowledgements

Ann Wright-Mockler and Heather Brewer

Contact

For more information on the science you see here, please contact:

Heather Brewer

Pacific Northwest National Laboratory

P.O. Box 999, MS-IN: K8-98

Richland, WA 99352

(509) 371-6566

Heather.brewer@pnnl.gov