

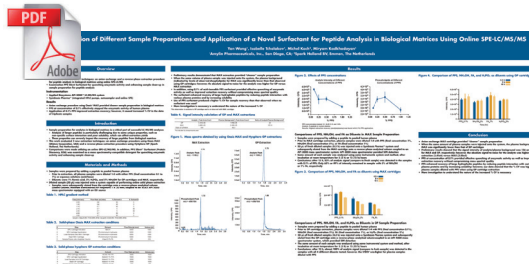
APPLICATION NOTE

Optimizing Hydrophobic Peptide Extraction Recovery from Human Plasma Without Compromising Mass Spectral Quality

INTRODUCTION

As a developer of peptide hormone-based medications, Amylin Pharmaceuticals, Inc., has an ongoing strategic interest in optimizing sample preparation of peptide amyloid protein analogues from biological matrices, such as human plasma. Preparing an amyloid peptide extract of sufficient concentration and purity for LC-MS/MS analysis presents a number of challenges, including poor solubility relating to amyloid hydrophobicity, non-specific surface interactions, and the enzymatic activity of human plasma.

One way to address all of these challenges simultaneously is to add a detergent to the sample preparation. However, conventional detergents are well-known for their deleterious effects on the quality of peptide mass spectra. This application note describes the evaluation and utilization of PPS Silent® Surfactant to increase amyloid peptide recovery and increase mass spectrometry analyte detection sensitivity.

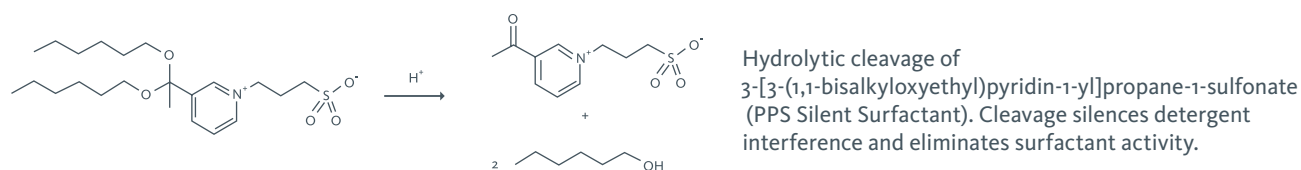


Yan Wang, Isabelle Tcholakov, Michel Koch, and Miryam Kadkhodayan. *Evaluation of Different Sample Preparations and Applications of a Novel Surfactant for Peptide Analysis in Biological Matrices Using Online SPE-LC/MS/MS*. Presented at the 55th Annual Conference of the American Society for Mass Spectrometry (2007). A complete version of this poster can be downloaded from www.proteindiscovery.com. Protein Discovery gratefully acknowledges the permission granted by Amylin Pharmaceuticals to reproduce and distribute this information.

BACKGROUND

Research at Amylin into peptide extraction optimization has included an evaluation of PPS Silent[®] Surfactant as a sample preparation reagent. PPS Silent Surfactant is an acid-cleavable detergent that disrupts cell membranes and solubilizes hydrophobic proteins. PPS is readily hydrolyzed at low pH into soluble by-products which do not produce mass spectrometry signals within the m/z range of proteins and peptides. PPS-based peptide extraction protocols are also compatible with downstream HPLC separations, and the soluble by-products of PPS cleavage have no residual detergent properties.¹

PPS CLEAVAGE REACTION



OBJECTIVE

Information in this application note was derived from the poster presentation referenced above. With respect to PPS Silent Surfactant, the objective of the work described in the poster presentation was to evaluate the reagent as a quenching agent against human plasma enzymatic activity, and as an enhancing agent for sample clean-up in advance of peptide analysis by LC-MS/MS.

METHOD

Samples were prepared by adding a peptide to pooled human plasma.

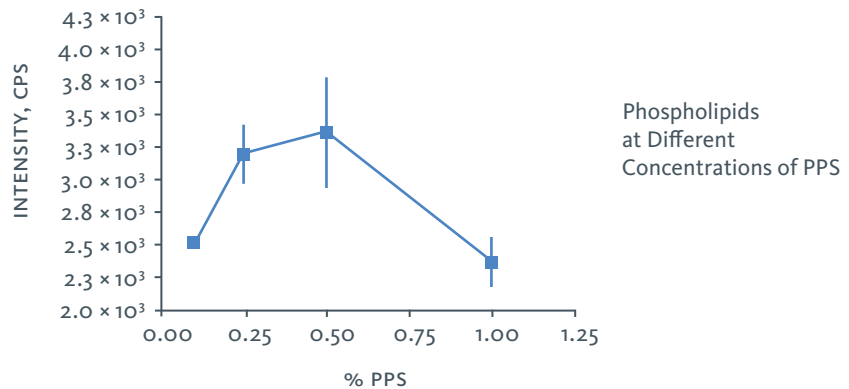
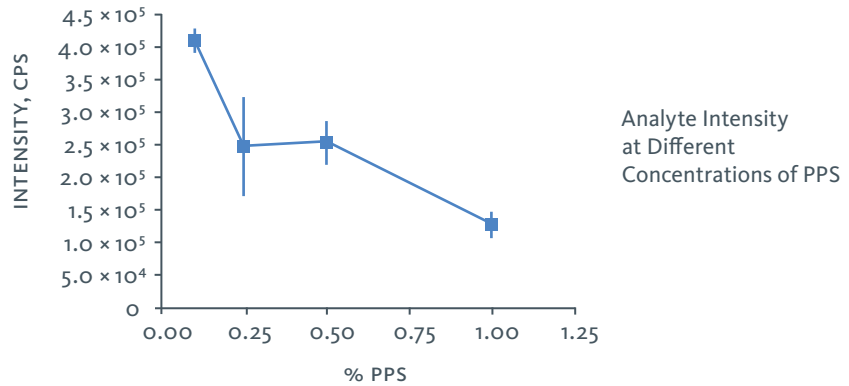
- Prior to extraction, all plasma samples were diluted 1:4 with either PPS (final concentration 0.1 to 1%) or aqueous solutions (acid/base)
- Aqueous diluents were 1% formic acid, and 5% ammonium hydroxide

Diluted sample (50 μ L) was injected onto a system capable of performing online solid phase extraction.

- Samples were subsequently eluted from the SPE cartridge onto a reverse-phase analytical column (Guard Column, Michrom Bioresources/RP Polymeric 1 \times 20 mm) coupled to an SCIEX API 3000 mass spectrometer equipped with an ESI source

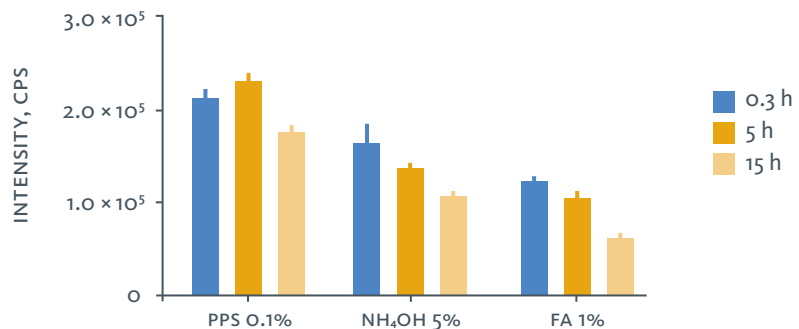
FINDINGS

For improving analyte extraction recovery, a 0.1% concentration of PPS Silent Surfactant was optimum. Plasma phospholipid extraction was minimized at a 0.1% concentration of PPS Silent Surfactant.



Comparison of PPS, Ammonium Hydroxide, and Formic Acid Diluents in Sample Preparation for MAX Cartridge Solid Phase Extraction

Initial plasma sample dilution with PPS Silent Surfactant, rather than ammonium hydroxide or formic acid, improves hydrophobic peptide analyte recovery. After 15 hours, 83% of analyte signal (compared to fresh sample) was detected in the samples with 0.1% PPS; only 65% or 49% of analyte signal intensity remained in samples with 5% ammonium hydroxide or 1% formic acid, respectively.



“PPS enhanced recovery of large hydrophobic peptides by reducing peptide interactions with plasma proteins and increasing solubility. PPS allows us to use a more sensitive protocol for analyte measurement.”



Yan Wang, PhD
Amylin Pharmaceuticals

CONCLUSION

Plasma sample dilution in 0.1% PPS Silent Surfactant provided effective quenching of enzymatic activity and improved hydrophobic peptide analysis recovery without compromising mass spectral quality.

This finding is consistent with those of other investigators who have published or submitted information pertaining specifically to PPS Silent Surfactant. In a comparison of surfactants for protein extraction and in-solution trypsin digestion, Emily Chan, *et al.*, from the Scripps Research Institute found that an aqueous solution digest enhanced by PPS Silent Surfactant yielded more identified proteins than similar digests using other mass spectrometry surfactants. PPS was also unique among the surfactants tested in that a comparative hydrophobicity analysis of the identified proteins revealed that the peptide population prepared using PPS elicited the highest average hydrophobicity.ⁱⁱ

Separately, noting that the by-products of PPS cleavage need not be removed prior to downstream sample analysis, Thomas Conrads and contributing researchers at the University of Pittsburgh Cancer Institute developed a single-tube protocol for high-yield extraction of peptides from cells selected by laser capture microdissection. Their gene ontology (GO) analysis of the resulting peptides revealed that more than half of the peptides prepared using PPS were classified as membrane proteins.ⁱⁱⁱ

ORDERING INFORMATION



PPS Silent Surfactant is available in 1 mg and 10 mg vials. To place an order for PPS Silent Surfactant, please contact Protein Discovery by phone, fax, or e-mail.

Tel: (865) 521-7400
Fax: (865) 521-3548
E-mail: sales@proteindiscovery.com

DESCRIPTION	PART NUMBER
PPS Silent Surfactant, 1 mg vial, 5-pack	21011
PPS Silent Surfactant, 10 mg vial	21010

REFERENCES

- i Norris, J.R.; Porter, N; Caprioli, R. *Anal Chem.* 2003, 75, 666-675.
- ii Chen, E.I.; Cociorva, D; Norris, J.L.; Yates, J.R., III. *J. Proteome Res.* 2007, 6, 2529-2538.
- iii Protein Discovery, Inc. Application Note. NanoLC-MS/MS Analysis of Proteins from Microdissected Cells. 2007.



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