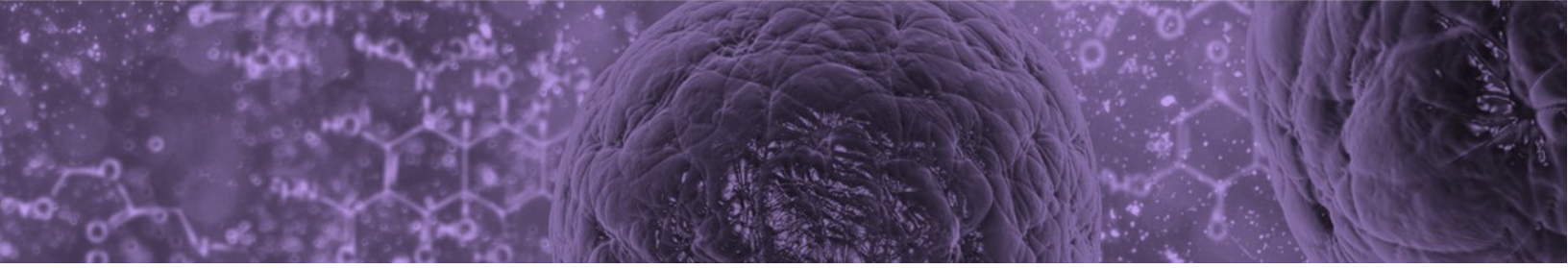

nHance™ PCS

Next Generation Peptide Extraction

Case Study

Simultaneous Peptide Detection

Benefits

- Detect more than one peptides simultaneously with one workflow.
- Fast, simple and effective workflow.
- Reduces ion-suppressing contaminants such as salts, detergents, and clinically irrelevant proteins from the sample.

Keywords

Sample cleanup, Peptide enrichment, LCMS, Simultaneous analyte detection, Post-translational modification, Size exclusion, electrostatic interactions, mesoporous surface, peptides, small proteins, mass spectrometry, bioanalytical analysis

Introduction

Many times, in bioanalysis you are asked to monitor more than one biological molecule or therapeutic at once. This can be challenging if the analytes of interest have very different properties such as differing molecular size and charge. Traditional methods of bioanalysis are unable to meet these requirements. Enzyme-Linked Immunosorbent Assay (ELISA) assays are very selective and are only suited for monitoring one analyte. Similarly, Liquid-Liquid Extraction (LLE) assays cannot distinguish between two peptides with large differences in hydrophobicity or charge. The chemistry of the bed of Solid Phase Extraction (SPE) assays is not adaptable enough to retain more than one biological molecule at a time. The SPE assay can either be set up to capture both or multiple analytes but this results in a high background. A secondary form of cleanup such as a trap column is then required to capture the other analyte. All of these methods would then require an additional assay or additional steps in order to monitor more than one analyte. These approaches waste time, money and sample volume. The benefit of the nHance™ Peptide Capture System (PCS) is the ability to switch back and forth with one workflow to monitor multiple analytes.

Sample Preparation Workflow
Sample Pre-Treatment (Optional)
As needed (peptide specific. Refer to WFPCS1001)
Loading
Add and cover 20µL-100µL of sample solution to each well
Incubate 30 mins at room temp on slow-moving shaker/ rotation table
Wash
Without removing loaded sample, add 50µL washing solution to each well (total volume 100µL)
Remove 50µL of solution from each well and discard to waste
Repeat addition and removal of wash solution an additional four (4) more times
After final wash, remove all solution from the wells so that all wells are empty prior to elution
Elution
Add eluent solution to each well
Pipet up and down 30 times over 30 seconds and withdraw eluent solution for analysis
Samples ready for LCMS

The nHance™ Peptide Capture System: Method

The nHance™ PCS device is shown to be a multi-dimensional sample clean-up and small protein/ peptide enrichment technique. The inherent surface charge of the nHance™ coating is negative, therefore by adjusting the pH of the sample to drive the target analyte to a positive charge state can significantly improve overall recovery of the molecule(s) of interest. The pores of the nHance™ PCS system were specifically engineered to capture molecules ranging in size from approximately 500 to 10,000 Daltons. Simple surface chemistry modifications of the nHance™ coating can be employed for the extraction of negative and/or hydrophobic peptide targets and can be performed at the bench, or integrated into the nHance™ manufacturing process.

The nHance™ PCS workflow consists of three critical steps: 1) Loading, 2) Washing, & 3) Elution. The loading step consists of employing the appropriate pH sample modulation for your target analyte, and the introduction of an organic to "loosen" the peptide structure to encourage loading into the nHance™ nanoporous coating. The washing steps are employed to remove any uncaptured proteins, salts, and other extraneous components commonly contained in complex biological matrices. Washing solutions are intended to remove unwanted sample material without impacting the retention of the target analytes loaded within the nHance™ nanoporous coating. During the elution step, peptide extraction is achieved by employing the same principals and strategy used when loading the peptides from a complex matrix. The elution solution will have the appropriate pH and organic modifier to elicit peptide release from the nHance™ material to promote optimal peptide recovery. The acidified sample will have a pH of 3 – 5 and along with a low percent of organic modifier the peptides will be gently denatured. This makes them more linear which facilitates interaction with the nanoporous layer.

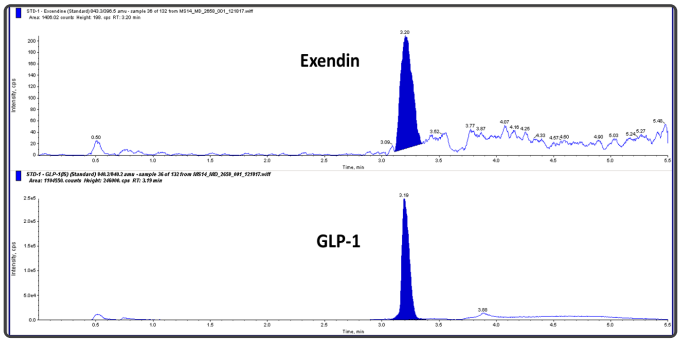

Figure 1: Representative Chromatogram of GLP-1 and Exendin in dog plasma at 125 ng/mL, single sample treatment and clean up method developed for both analytes (using the other analyte as the internal standard, depending on quantitation requirements).

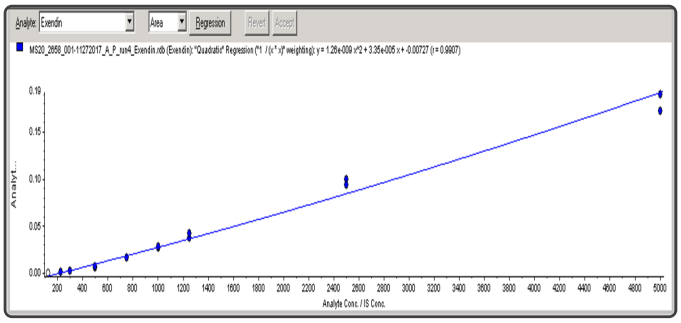

Figure 2: Representative Calibration Curve of Exendin using GLP-1 as the internal standard, separation on nHance™ PCS demonstrating linear response across calibration curve range.

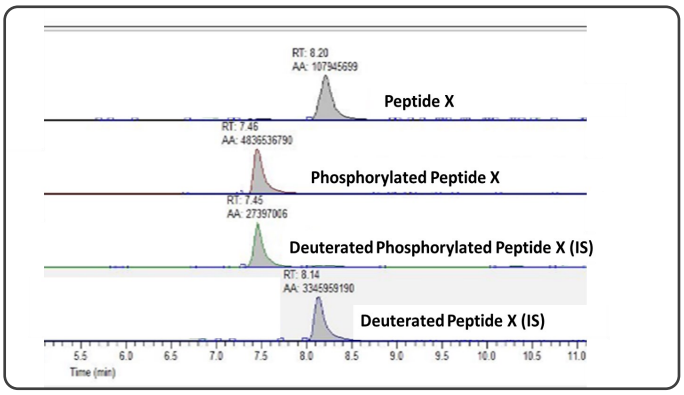

Figure 3: Representative Chromatogram of Peptide X, Phosphorylated Peptide X, and their respective stable labeled internal standards (IS) in cell lysate, single sample treatment and clean up method developed for both analytes.

Experimental Results

The results below from the analysis of GLP-1 and Exendin illustrate the ability of the nHance™ PCS to monitor two analytes using a single workflow. The biomarker, GLP-1 is usually included when monitoring for Exendin. Exendin is a biosimilar for GLP-1. GLP-1 is typically monitored using an ELISA assay. The nHance™ PCS is able to monitor both GLP-1 and Exendin using the same assay and pretreatment. This reduces development time, analysis time, required sample volume and costs.

The following example illustrates the ability of the nHance™ PCS to differentiate between a phosphorylated and non-phosphorylated peptide phosphorylation. Phosphorylation of peptides are forms of post-translational modifications (PTMs) that can play critical roles in the setting of disease. The subtle structural differences between a phosphorylated and non-phosphorylated peptides, for example, can be so minute that standard extraction methods and/or ligand binding assay strategies are incapable of differentiating, let alone quantifying. Prior to the advent of the nHance™ PCS there was no single assay that could differentiate between phosphorylated and non-phosphorylated peptides. There is no reagent for ELISA assays with the specificity to distinguish the two and SPE assays are not able to retain both peptides at one time. The nHance™ PCS plate achieved a >80% recovery for both forms of the peptide in a surrogate matrix (3% BSA solution) and a >60% recovery in a cell lysate solution with minimal process optimization and method development time.

Conclusion

Utilizing the three key attributes, electrostatic interaction, size exclusion and ion pairing/hydrophobicity, the nHance™ PCS is able to monitor multiple analytes using one workflow. It also has the sensitivity to distinguish between peptides with very minor structural difference and the ability to distinguish between two peptides with large differences in hydrophobicity and differing charges. Traditional assays require additional steps, additional assays and can lack the sensitivity to detect the analytes of interest. All of this leads to additional time, money and the need for larger sample volumes. The nHance™ PCS has the selectivity, sensitivity and the flexibility to analyze multiple analytes when the analytes of interest are very different or when they are very similar and do so with minimal development and fewer steps in sample processing.

nHance™ PCS Quality Assurance

The nHance™ PCS 96-well plate brings the precision, accuracy, and reproducibility of the semiconductor manufacturing industry to the bioanalytical community. Respectful to its integrated circuit origins, the nHance™ PCS plates feature unparalleled control of all manufacturing processes resulting in tight product specification ranges and miniscule well-to-well, plate-to-plate, batch-to-batch variation. Each purchase of nHance™ products will include a certificate of analysis (COA) featuring critical product performance criteria with a detailed explanation of our quality control testing procedures.

Application Support

Contact us today at appsupport@nhancetechnology.com to see how nHance™ PCS can simplify your peptide and protein sample preparation for LC/MS analysis. Our application team of seasoned bioanalytical chemists have significant experience in the use of LC/MS techniques for the detection and quantification of peptides and proteins.

Contact Information

Contact us today at info@nhancetechnology.com or visit us at www.nhancetechnology.com.

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