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Direct On-Plate Desalting and Enrichment for Improved Low Molecular Weight Marker Detection

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NanoMedical Systems

nHance™ PCS

Direct On-Plate Desalting and Enrichment for Improved Low Molecular Weight Marker Detection

Novel Aspects

A fast depletion and clean-up platform that is ideally suited for MALDI-TOF MS-based biomarker investigation.

Introduction

Research in the biomarker field has often been compared to the saying: "finding a needle in a haystack". When investigating low-level markers in body fluids using mass spectrometry, we are limited by a strong background signal resulting from the detection of highly abundant proteins. In order to increase the sensitivity of the detection of a marker, one can choose to either selectively remove it from the initial fluid or exclude abundant proteins. We present herein novel plates that feature a proprietary mesoporous silica surface (MPS) that excludes high abundant proteins based on their size, charge, and hydropathy index. This technique coupled with MALDI-TOF analysis provides biomarker researchers a unique platform combining: throughput, speed, reproducibility and ease of use.

Methods

Experiment A: proof of concept. Enrichment was performed on two solutions. Working Solution 1 (WS1) contains BSA 0.5 nmol/µl and ACTH (18-39) and angiotensin I at 33 fmol/µl. Working Solution 2 (WS2) contains BSA at 0.5 pmol/µl and ACTH, angiotensin I at 33 fmol/µl. A volume of 5 µl of sample was deposited on the nHance ™ Peptide Capture System (PCS) plate (NanoMedical Systems, Austin, TX, USA) and was incubated for 30 min. Remaining sample solution was removed and a 5 µl wash solution (100% ddH_2O) was applied on each well and repeated 6 times. Peptides were eluted from the wells using 5 µl of 50% ACN / 50% H_2O 0.1%TFA and 0.5 µl of the solution was mixed with 0.5 µl of matrix on a MALDI plate and analyzed using a MALDI-TOF/TOF (Shimadzu Kratos, Manchester, UK).

Experiment B: evaluation of pH effect on peptide retention. Table 1 summarizes the composition of Working Solution 3 (WS3). A pH of 1.5 was measured for the initial conditions. A solution of NaOH 1M was used in order to increase the basicity of WS3. In order to achieve pH values of 5, 7.5 and 12, volumes of 2 µL, 3 µL and 5 µL of 1M NaOH were respectively added to three tubes containing WS3 (200 µL). Prior to nHance™ PCS enrichment, pH of each tube was confirmed.

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Table 1. Composition of WS3

Peptide / Protein	MW (Da)	pI	Concentration (fmol/µL)
Bradykinin (1-7)	756	11.18	80
ACTH Clip (7-38)	3657	10.04	30
Angiotensin II	1046	7.95	20
Angiotensin I	1296	8.04	20
[Glu[1]]-Fibrinopeptide B (Glu-Fib)	1570	3.47	20
N-Acetyl-Renin	1800	7.99	20
ACTH Clip (1-17)	2093	10.96	20
ACTH Clip (18-39)	2465	3.82	20
Insulin Human	5808	5.24	80
Cytochrome c	12351	n/a	15000
Myoglobin	16952	n/a	12000
Bovine Serum Albumin	66430	n/a	50000

Results

Proof of Concept

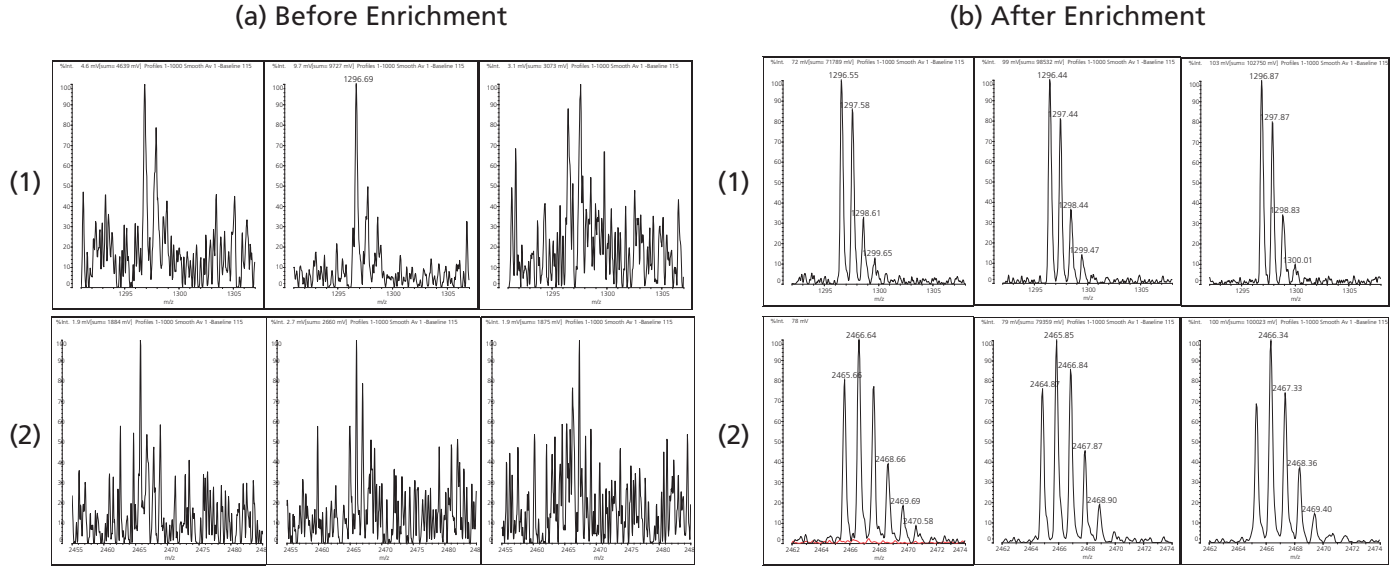

Figure 1. MALDI MS spectra of triplicate measurements of angiotensin I (row (1)) and ACTH (18- 39) (row (2)) in WS1. Peak absolute intensity comparison before and after enrichment saw an increase of 18x for Angio I and 40x for ACTH (18-39).

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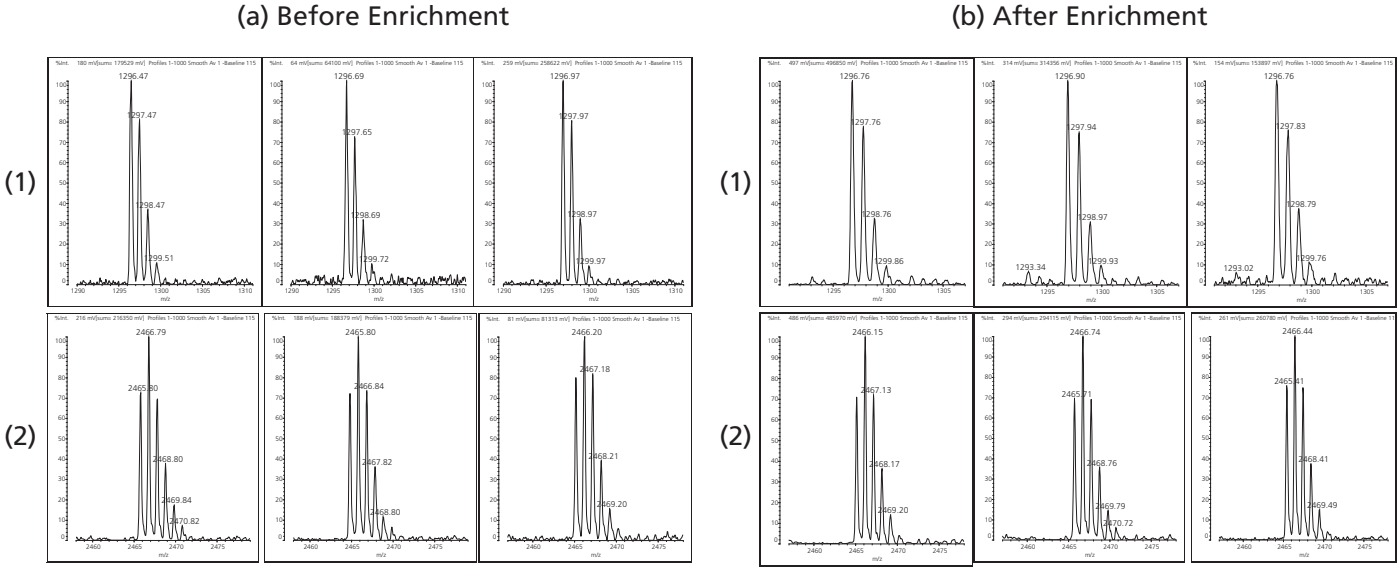

Figure 2. MALDI MS spectra of triplicate measurements of angiotensin I (row (1)) and ACTH (18- 39) (row (2)) in WS1. Peak absolute intensity comparison before and after enrichment saw an increase of 2x for angiotensin I and ACTH (18-39).

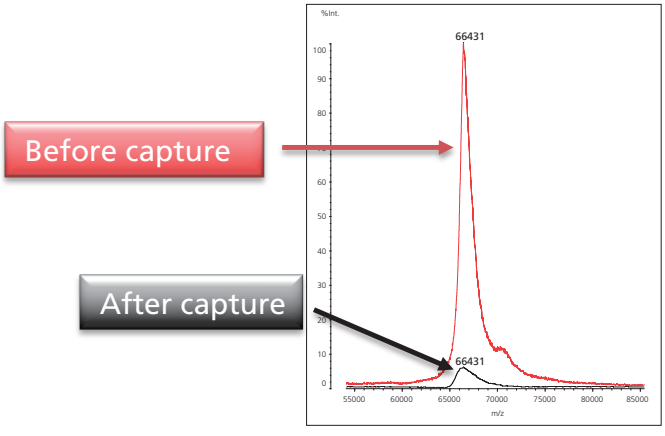

Figure 3. MALDI MS spectra of BSA in the linear mode. BSA signal intensity decreased 16x after sample processing on nHance™ PCS plate.

Table 1. Ratio of MALDI-MS peak intensity between post and pre nHance™ PCS sample processing for Angio I, ACTH (18-39) and BSA.

	angio I	ACTH (18-39)	BSA
WS1	16x	40x	15x
WS2	2x	2x	0.06x

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Evaluation of pH Effect on Peptide Capture on nHance PCS™

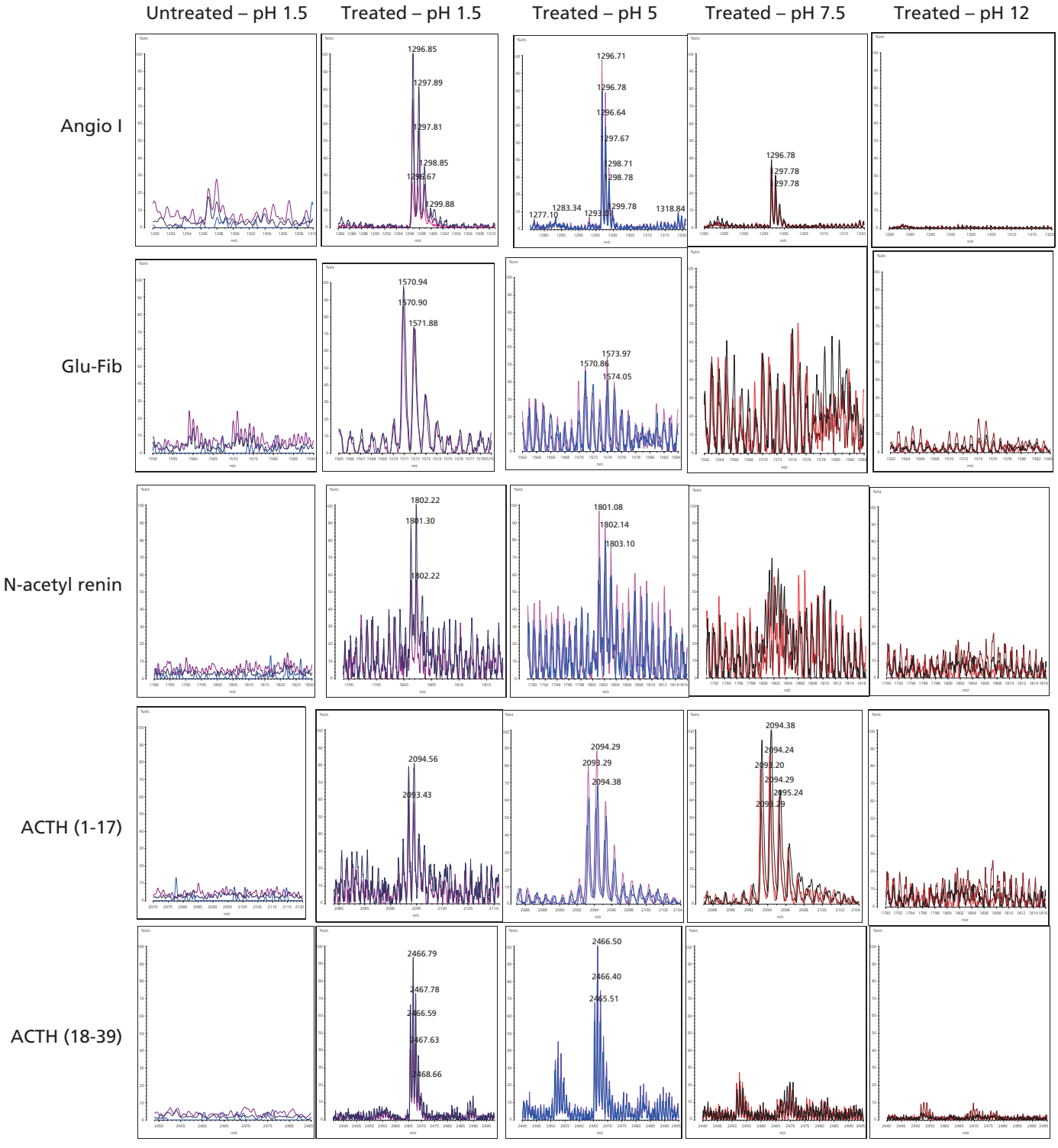

Figure 4. MALDI-TOF MS spectra of peptides present in WS3. Overlay of triplicate measurements at various initial pH conditions are shown. Intensity variation was evaluated and correlated with peptide on-plate capture, results are summarized in table 2.

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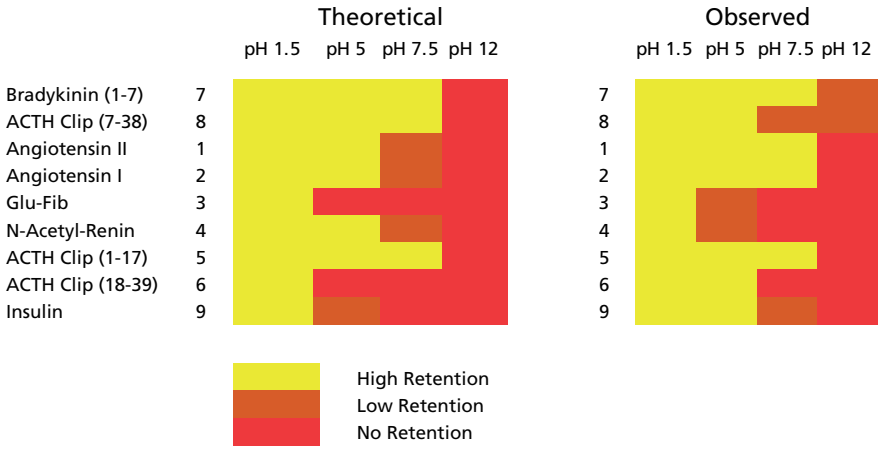

Table 2. Comparison of theoretical sample retention to the nHance™ PCS plate and the observed retention pattern with respect to pH variation. The theoretical table for retention was based on values of pI for a given peptide. When the working solution pH is below the pI value of the peptide, its charge enables it to migrate into the nanopores. The peptide is subsequently eluted and detected. If the WS pH is higher than the pI, the peptide charge prohibits its migration into the nanopores, which will hinder subsequent detection.

Discussion

Experiment A: Two solutions were submitted to nHance™ PCS sample treatment. WS1 contained the highest amount of BSA and this resulted in signal suppression of targeted peptides. Treatment with nHance™ PCS and removal of large amounts of BSA improved greatly the intensities of the peptides. In WS2, the amount of BSA was much lower (1000x more dilute), resulting in weaker signal suppression. Yet, nHance™ PCS treatment increased the signal by a factor of 2 for peptides and lowered the intensity of BSA detection by a factor of 16.

Experiment B. The result from experiment B showed good correlation between the theoretical model of adsorption and the observed data. Peptide net charge plays an important role in its interaction with the nHance™ PCS surface, and by varying the solution pH from acidic to basic we were able to affect negatively the retention of the peptides onto the plate. Some of the discrepancies seen between the theoretical and observed data may be attributed to other types of interactions that may come into play besides the electrostatic ones that were modulated via peptide net charge.

Conclusion

nHance™ PCS enables fast and easy sample preparation for MALDI MS analysis. Removal of highly abundant proteins and desalting improve noticeably the detection of markers of interest

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