CDS Empore™

# Evaluation of Empore™ C18 Spin Columns for Peptide Desalting and Proteomic Analysis

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**Application Note**
Life Science

## Abstract

This application note describes evaluation of spin columns packed with C18 Empore™ membrane to load and desalt peptides for the purpose of proteomics analysis by LC-MS. Spin columns are shown to exhibit the same desalting ability as popular Empore™ Stage Tips with the advantage of higher loading capacity enabling the ability to process large sample sizes without the need to split sample and risk loss.

## Experiment Setup

1. Materials and Equipment


(1) Empore™ Spin Column: C18 (CDS Analytical, #70-2019-2001-0).
(2) Microtubes and Pipette tips: Low binding or maximum recovery microcentrifuge tubes (1.5 mL and 2.0 mL), Low binding tips (20 µL, 200 µL, and 1 mL).
(3) Peptide sample for analysis: BSA digest or sample of choice.
(4) Solutions:
Activation solution: 100% methanol (MeOH).
Equilibration and wash solution: 0.5% acetic acid (HAc) in water.
Elution solution: 80% acetonitrile (ACN), 0.5% HAc, and 19.5% water.
(5)Dried BSA digest prepared via in solution digestion starting with Pierce BSA standard (Thermo Scientific, #23210)
(6) UV-Vis Spectrophotometer (Lab Tech, BlueStar A)
(7) Quartz cuvette 100 µL (Cuvet,co #SLB100)

2. General Desalting Procedure

BSA digest was resuspended into 0.5% HAc to a concentration of about 1 µg/µL. For desalting procedure 100 µg was loaded. For capacity test 400 µg of peptides were tested. All steps utilized fixed angle rotor with centrifugation at 500 x g.

(1) Spin column activation: Load 400 µL MeOH, spin for 30 seconds. Note: Do not spin to dryness. Drying will affect binding ability. Discard flow through.
(2) Equilibration: Load 400 µL 0.5% HAc, spin for 30 seconds. Discard flow through.
(3) Loading: Place spin column into low binding microcentrifuge tube for elutant collection. Load sample solution at volume less than 500 µL, spin until all solution has passed through. For improved loading repeat loading one more time with the eluted solution. Upon completion of loading discard flow through.
(4) Wash: Load 400 µL 0.5% HAc, spin for 1 minute. Repeat wash 1-2 more times if high concentration of salts present. Discard flow through.
(5) Elute: Place spin column into another clean low binding microcentrifuge tube for elutant collection. Load 200 µL 80% ACN, 0.5% HAc, 19.5% water, spin for 1 minute. Repeat elution 1-2 times for higher recovery.

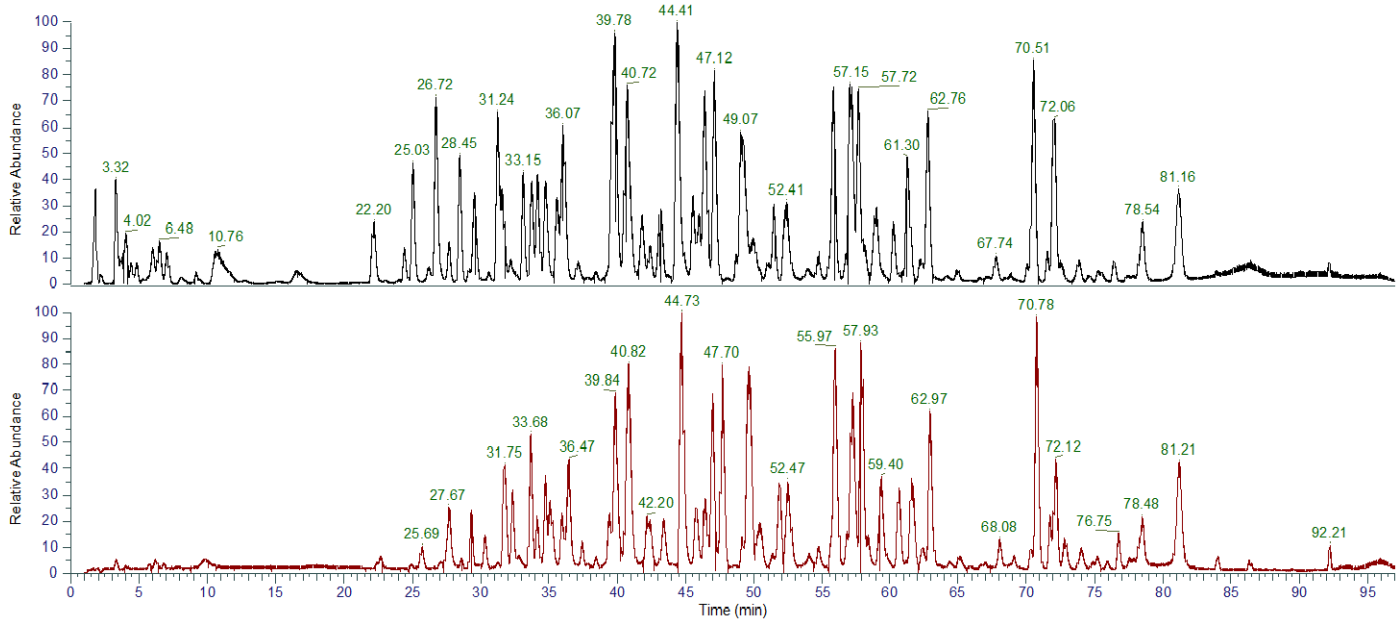


**Figure 1.** TIC chromatogram showing BSA peptides not desalted (top) and desalted via Empore™ C18 Spin Column (bottom).

3. LC-MS Conditions

Analysis of BSA peptides was performed by Vanquish Horizon LC and Orbitrap Q Exactive Plus MS system coupled to a HESI source (all components Thermo Scientific). LC separation utilized Acclaim™ PepMap™ 100 C18 1 mm ID x 150 mm. Mobile phase A: 0.1% formic acid (FA) in water, B: 0.1% FA in 100% acetonitrile. Gradient: 107 minutes, 0% B for 7 minutes, 0-25% B for 65 minutes, 25-80% B for 10 minutes, 80% B for 10 minutes, 80-0% B for 5 minutes, 0% B for 10 minutes. Constant flow rate of 0.06 mL/min. Data was collected from minute 1-97. The MS survey scans were acquired at a resolution of 35,000 over a mass range of 350-1500 m/z. At each cycle the top ten most intense ions were subjected to high-energy collisional dissociation (HCD), applying a normalized collision energy of 30%. MS/MS scans were captured at a 17,500 resolution with a dynamic exclusion of 20 seconds. Charges 1, 7, 8, and >8 were excluded form fragmentation. Raw data was analyzed by Proteome Discoverer 2.5 using the basic workflows for Q Exactive with default parameters.

## Results and Discussion

We first analyzed the Empore™ C18 Spin Column ability to desalt peptides by desalting 100 µg BSA digest following the above general protocol with slight modifications, loading sample through the Spin Column twice and desalting washing four times totaling 520 µL. Sample was dried via vacuum centrifugation and resuspended in 0.1% FA for LC-MS analysis. Two runs of 5 µg total peptide was injected for LC-MS analysis for both desalted and non-desalted BSA peptide for comparison.

Figure 1 shows the total ion count (TIC) chromatogram comparison. The results show loss of contaminant salt peaks. Salt peaks are believed to be located in the first 20 minutes of the run based on the lack of retention by C18 column and lack of peptide peak identification during this time.

Analysis of the four LC-MS runs by Proteome discover are shown in figure 2. Results show that the identification of the average sum of peptides and PSM are very similar (Figure

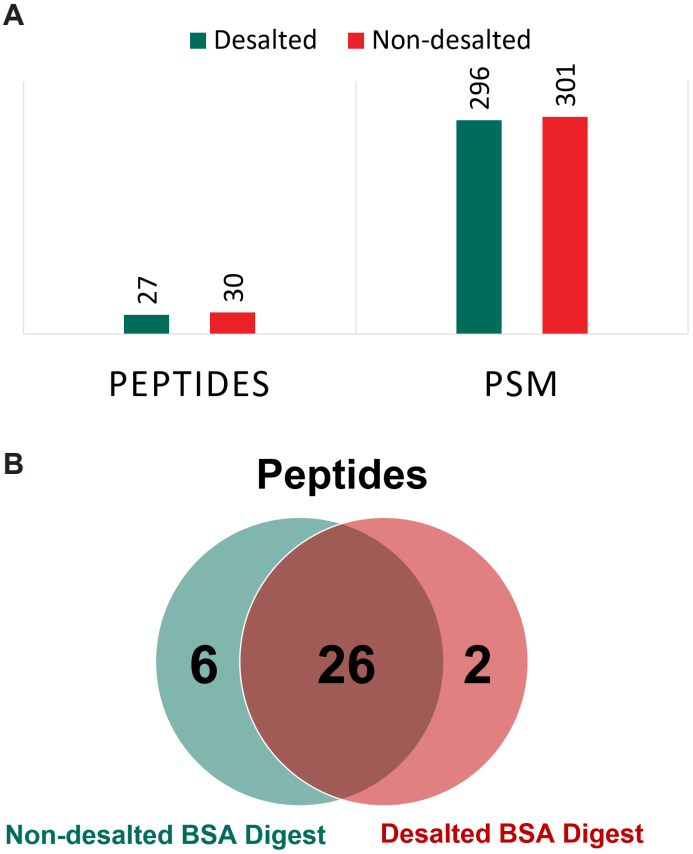


**Figure 2.** Proteome Discoverer analysis of LC-MS runs comparson desalted vs non-desalted BSA digest. **(A)** Bar chart showing average number of peptides and PSMs. **(B)** Venn diagram showing unique and common peptides identified between conditions.

2A). This suggests a small loss of peptides occurred during desalting. Analysis of unique peptides between conditions show apparent disappearance of 6 peptides and appearance of 2 peptides after desalting via Empore™ C18 Spin Column, shown in figure 2 B. Unique peptides in the non-desalted sample are believed to have weak or no association with C18 functional chain as these peptides were identified early on in the analysis with 4 of the 6 peptides identified before the 30 minute mark. This could explain the loss of these peptides when desalting as they would not be strongly captured by the C18 membrane.

Next, we tested the maximum binding capacity and recovery of the Empore™ C18 Spin Column again using BSA digest. This time 400 µg of BSA digest was loaded onto the Empore™ Spin Column. UV-Vis absorbance at 280 nm was measured during the loading, wash, and elution steps to monitor the binding and recovery. Peptide mass was estimated using our own BSA standard curve.

Figure 3 shows the observed binding and recovery results. Loading of BSA sample was performed two times with a volume of 200 µL at a concentration of 2 mg/mL. Most of the peptides bound the first time with 73 µg found in the unbound. This was further decreased to 62 µg after a repeat loading using the unbound peptide solution. Of the 400 µg, 338 µg was observed to bind the Empore™ C18 Spin Column. Washing the spin column with 150 µL of wash solution showed a peptide loss of 4 µg after the first wash but no further observable loss after the second wash suggesting a loading capacity of about 334 µg (Figure 3 A).

To test the recovery of loaded BSA peptides, sequential elutions by elution buffer used 100 µL volumes and were measured by UV-Vis at 280 nm. Figure 3 B shows the sum of total eluted peptide after different elution volumes. Most peptides (82%) are eluted after 400 µL total, and 90% of the 334 µg has been recovered after 700 µL. At the end, all elutions were combined and concentrated by vacuum centrifugation and measured a final time with an estimated recovery of 98%. This data suggests a recovery >90% should be easily and consistently achievable. Further optimization should provide an efficient and timely elution step.

## Conclusions

This application note has demonstrated the ability of Empore™ C18 Spin Columns for a easy and efficient peptide desalting in preparation for LC-MS analysis. Additionally, we show a binding capacity of at least 300 µg for BSA peptides with a high recovery of >90% after 700 µL elution volume. It should be noted that these conditions will vary for the sample size and composition. Therefore, for absolute best results users should find their own optimal conditions. With minimal loss of peptides, this product will prepare your samples to provide cleaner data and prolong the life of the LC-MS systems by avoiding issues related to salt contamination.

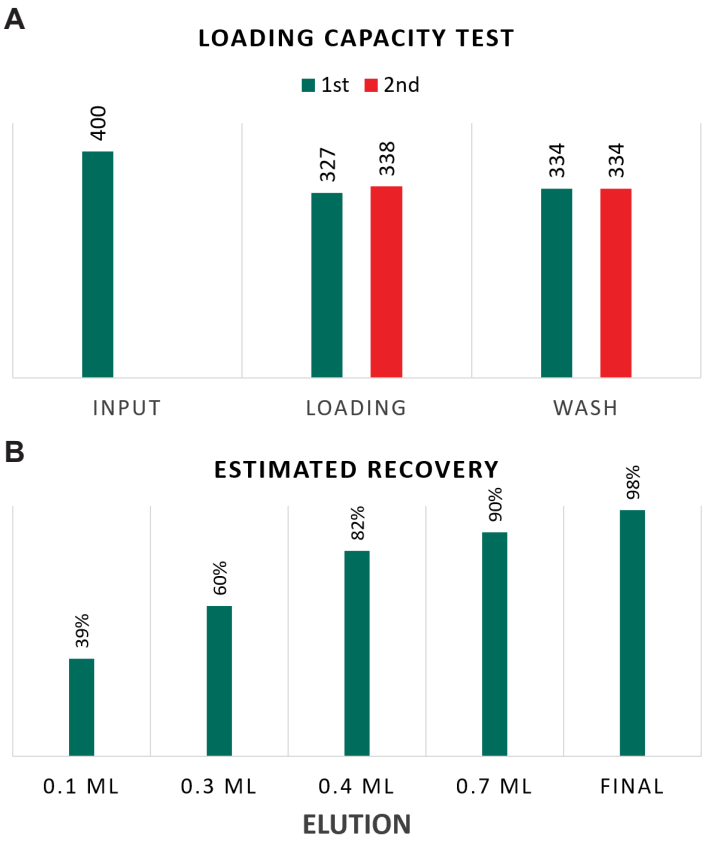


**Figure 3.** Empore™ C18 Spin Column loading capacity and recovery bar charts measured by UV-Vis 280 nm. **(A)** Observed binding capactiy of 400 µg BSA digest loaded and washed two times. **(B)** Estimated recovery of BSA digest summing the observed peptide mass at each elution volume. The final recovery was the estimated mass after all elutions were combined, concentrated by vacuum centrifugation, and measured.

## Empore™ C18 Spin Column Order Information

| | Part Number |
|---|---|
| CDS Analytical | 70-2019-2001-0 |