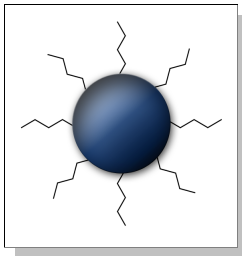


# MagSi-proteomics C18 magnetic beads for desalting applications in a typical proteomics workflow.

## Comparison of three different desalting/clean up methods

### Introduction

MagSi-proteomics beads are magnetic silica beads. The surface of the beads has been modified with C4, C8 and C18 Alkyl groups typical for reversed phase applications.



The MagSi-proteomics C18 beads are an ideal tool for the purification, desalting and concentration of peptides and protein digests.

Peptides bind to MagSi-proteomics C18 via hydrophobic adsorption interactions between the peptide and the hydrophobic surface of the beads. This

interaction is usually so strong that organic solvents (e.g. acetonitrile) are needed to break it. Peptides can therefore be separated according to their relative hydrophobicities using stepwise desorption in increasing concentrations of organic solvents.

In this application note, the performance of the MagSi-proteomics C18 beads is tested for the desalting of a BSA digest. This is then compared with two other known methods. The three methods compared are:

- MagSi magnetic beads with C18 material
- Microcolumn in gel loading tip using C18 'mesh' ('3M Empore Extraction Disks for Environmental Analysis with C18')
- ZipTips C18 (Millipore)

### Method

A BSA (Swiss-prot entry P02769) tryptic digest was labeled with iTRAQ (Applied Biosystems) according to the manufacturer's instructions and desalted with an Oasis column (Waters, Millford).

The iTRAQ labelled BSA digest (78.4 µg, ratio 114:115:116:117 = 1:10:27:81) was Speed Vaced to dryness, then redissolved in 100 µl 0.1 % TFA, then Speed Vaced again to dryness.

The dried down sample was redissolved in 350 µl IEF buffer (IEF buffer: 4 M urea, 1% ampholytes pH 4-7). An IEF strip (Immobiline Dry Strip GE Healthcare) pH 4-7, 18 cm was rehydrated 14 hrs. with the IEF buffer containing the labelled BSA digest sample.

Isoelectric focusing: 20 °C, 50 µA per strip (1 hr at 500 V gradient, 1 hr 1000 V gradient, 4 hrs at 8000 step-n-hold).

IEF strip was then rinsed in diethyl ether and cut up into 36 fractions. Peptide extraction from these IEF-strip fractions was done by 0.1% TFA; 50% ACN/0.1% TFA and final 0.1% TFA treatment. Each fraction was SpeedVaced almost to dryness.

### C18 peptide clean-up methods

Samples tested: fractions 15-20 from IEF (should contain peptides with pI ranging from about 5.16 to 5.67). Each sample ('pellet' of urea and peptides) was redissolved in 35 µl 0.1 % TFA and then desalted according to manufacturer's protocol:

#### MagSi-proteomics C18 beads

1. Wash sample with 100 µl 0.1 % TFA three times
2. Sample loading: mix 10 µl sample with 10 µl bead-suspension-in-0.1% TFA
3. Wash sample with 50 µl 0.1% TFA twice
4. Elute in 10 µl 80% acetonitrile, 0.1% TFA
5. SpeedVac to dryness
6. Redissolve sample in 10 µl 0.1% TFA

#### Microcolumn in gel loading tip using C18 'mesh'

('3M Empore Extraction Disks for Environmental Analysis with C18')

1. Equilibrate sample with 20 µl 0.1 % TFA
2. Wash with 20 µl 0.1 % TFA
3. Sample loading: 10 µl sample onto column
4. Wash Sample with 10 µl 0.1 % TFA
5. Elute in 10 µl 80% acetonitrile, 0.1% TFA
6. SpeedVac to dryness
7. Redissolve sample in 10 µl 0.1% TFA

#### ZipTips C18

1. Equilibrate sample in 10 µl 80% acetonitrile, 0.1% TFA
2. Wash with 0.1 % TFA
3. Sample loading: 10 µl sample (pipetting up and down in 10 µl sample)
4. Wash sample with 0.1 % TFA
5. Elute in 10 µl 80% acetonitrile, 0.1% TFA
6. SpeedVac to dryness
7. Redissolve sample in 10 µl 0.1% TFA

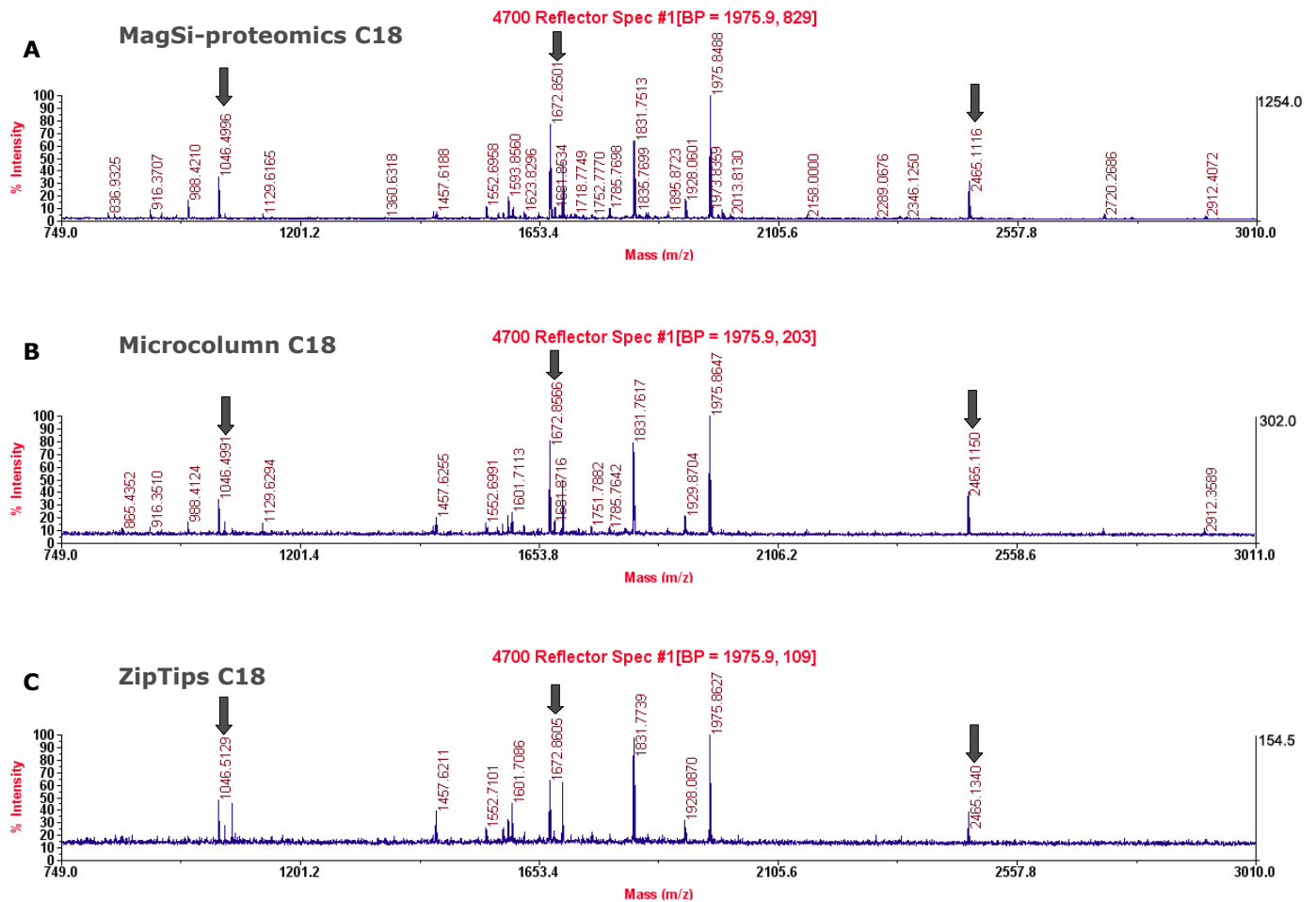


Figure 1: The samples (fraction 19 from IEF) were analyzed by direct spotting of 0.5  $\mu$ l onto MALDI-target and after drying 0.5  $\mu$ l matrix solution (5 mg/ml *a*-cyano-4-hydroxycinnamic acid, 70% acetonitrile, 0.1% TFA, 25 mM citric acid) was added. For internal calibration 10 fmol/ml standard peptides 1046.542, 1672.918, 2465.199 (Angiotensin II, Neutrosin and ACTH clip 18-39 resp.) were added to the matrix solution (arrows). A: MagSi-proteomics C18 beads, B: Microcolumn C18, C: ZipTips.

## Results

Samples were analyzed with MALDI-TOF-TOF MS (4700 Proteomics Analyzer, Applied Biosystems). In fig.1 the MALDI TOF spectra using three clean up techniques are shown using IEF fraction 19 as sample. The direct spotting of eluate from MagSi-proteomics C18 beads (**fig. 1A**), eluate from C18 microcolumn (**fig. 1B**) and eluate from ZipTips (**fig. 1C**) are shown respectively.

## Conclusion

Considering background and peak height, the MALDI spectra performed with the MagSi-proteomics C18 beads resulted in the best quality MALDI-TOF spectra compared to samples cleaned using ZIPTip C18 or micro column. As sample, an iTRAQ labelled BSA

tryptic digest was used for desalting. The MALDI spectra generated using MagSi-proteomics C18 magnetic beads showed a 4 - 8 times better sensitivity compared to the other tested methods.

## Acknowledgements

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