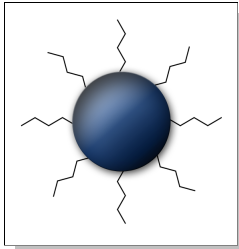


MagSi-proteomics C18 magnetic beads for fractionation applications in a typical proteomics workflow.

Comparison of three different beads in peptide profiling

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, protein profiles in saliva samples were measured using solid phase extraction (SPE) and subsequent analysis with MALDI-TOF. The MagSi-proteomics C18 beads were compared with two beads from other suppliers.

Method

For the saliva profiling, 10 μ l of a 5-fold diluted saliva sample was added to 10 μ l beads with 100 μ l of 5% acetonitrile/0.1% TFA in a 96-well plate (200 μ l well volume). This was shaken and the beads were collected with a flat plate magnet. Supernatant was removed and beads were washed 4 times with the above binding buffer.

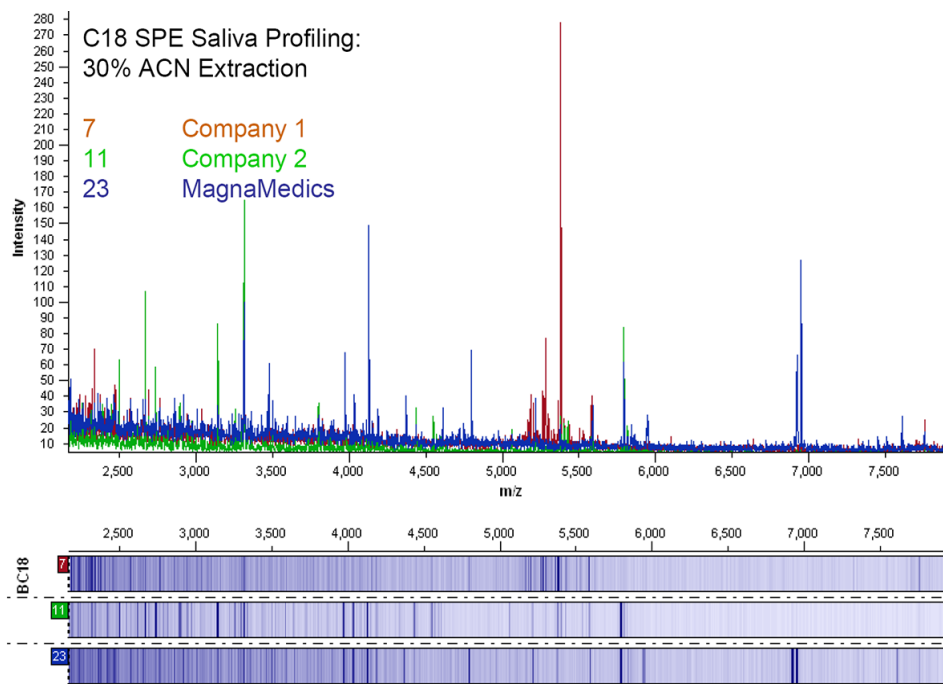


Figure 1: Spectra of peptides after elution with 30% acetonitrile.

MagnaMedics C18 beads show 23 annotated proteins (blue) as compared to the two reference beads, 7 (red) and 11 (green) respectively.

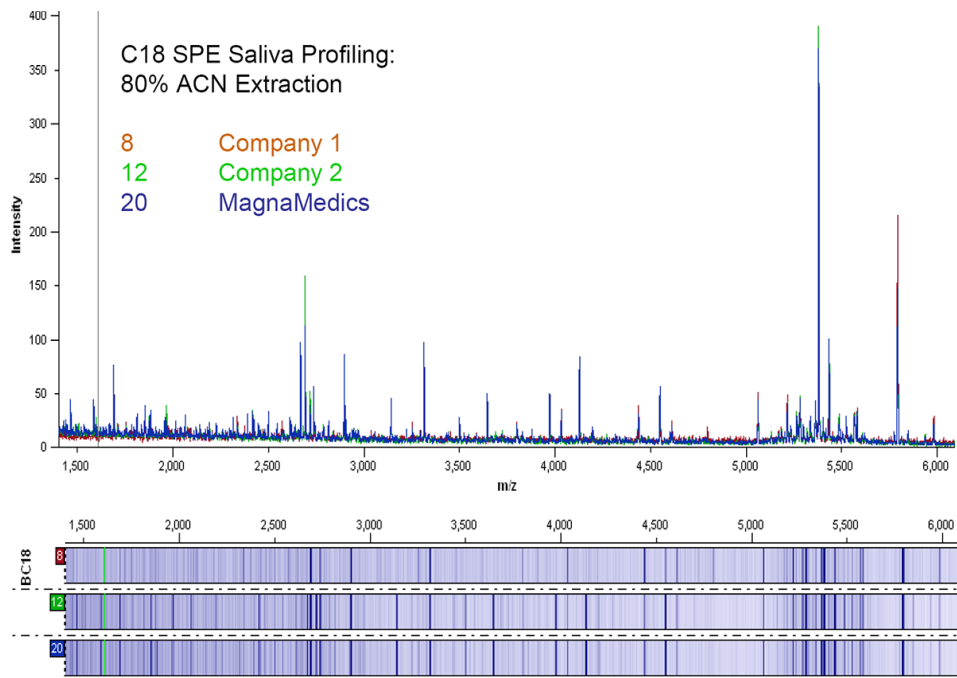


Figure 2: Spectra of peptides after elution with 80% acetonitrile.

MagnaMedics C18 beads show 20 annotated proteins (blue) as compared to the two reference beads, 8 (red) and 12 (green) respectively.

Proteins were eluted by adding 12 μ l elution buffer 4 times with different concentrations of acetonitrile. 8 μ l was removed and mixed with an equal volume of 5 mg/ml CHCA* and then 2 μ l is spotted onto a MALDI target allowing the matrix to dry between spotting. The plates were read with two different laser intensities with a Perkin-Elmer/Sciex prOTOF 2000 reflectron MALDI-TOF mass spectrometer.

Results

Spectra in fig. 1 and fig. 2 show the elution of magnetic beads at 30% and 80% of acetonitrile respectively. The MagSi-proteomics C18 beads show in both graphs the most annotated peptides as compared to the other beads, 23 versus 7 and 11 with 30% acetonitrile and 20 versus 8 and 12 with 80% acetonitrile. This shows that our MagSi-proteomics C18 beads can efficiently bind and elute peptides from a complex mixture.

Conclusion

MagSi-proteomics C18 beads are applicable in various applications e.g. fractionation of complex mixtures. This fractionation can be accomplished by eluting peptides from the beads by using different concentrations of acetonitrile. The results presented here show that our C18 beads can handle acetonitrile concentrations up to 80%.

Our MagSi-proteomics beads have twice the amount and number of annotated peptides isolated from saliva sample compared to reversed phase magnetic beads from competitors

Acknowledgements

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*CHCA: alpha-cyano-4-hydroxy cinnamic acid