Zip…Tips…

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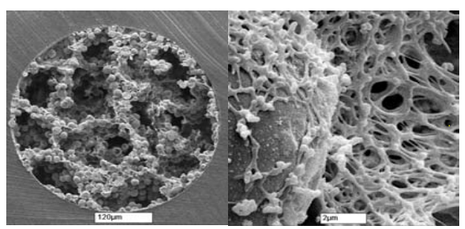
Abstract

ABRF 2003 marks the 5th year anniversary of ZipTip® pipette tips for the micro-volume sample preparation of peptides prior to MALDI TOF MS. Over the last five years, researchers have experimented with the devices developing creative ways to use them. In this poster, we will summarize the most notable of these "tricks of the trade" that protein chemists have employed to solve their sample preparation challenges. Such "tips" include: how does one load a large sample volume? How can the sample be eluted in the highest concentration ? How can the tip be eluted into an electrospray needle? When to use C4 vs. C18 ? When to use SCX vs. C18 ? How can ZipTip sample processing be automated ? This information should serve as a useful contribution regarding how microscale solid phase extraction has been adapted to solve a variety sample handling issues in protein chemistry.

Introduction

ZipTip is a 10 µl pipette that contains 0.6 µl immobilized chromatography media in the distal end. The beads are immobilized in a membrane scaffold that has been formed within the pipette tip housing. The device serves as a miniature chromatography column for microscale solid phase extraction (µSPE). Although several types of chromatography beads have been immobilized and commercialized, undoubtedly the use of C18 for desalting and concentrating peptides prior to MS analysis is most common. Typically, a sample containing 300 fmol of peptide(s) in 10 µl of solution can be prepared in about 1 minute. If the sample is intended for MALDI TOF MS analysis, bound peptides can be eluted with matrix and spotted directly on the target. Alternatively, for direct infusion ESI MS, peptides can be eluted with 0.1 % formic acid/50% methanol and loaded directly into a nanoelectrospray needle. The following figures address a few of more popular questions and adaptations of the technology. More information can be found at www.millipore.com/ziptip.

Miniaturization of Chromatography

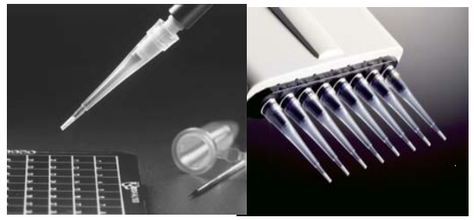


Membrane Technology allows the immobilization of chromatography beads in a highly porous polymer lattice.

Types of Chromatography

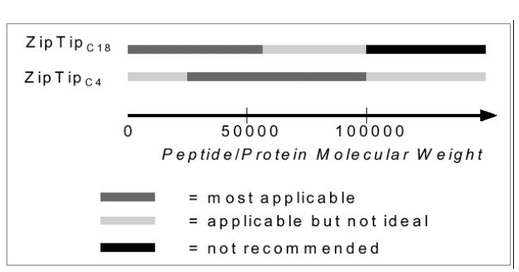
- Reversed-Phase (C4/C18)
- Ion Exchange (SCX)
- Affinity (IMAC)

Single or Multi-Channel Operation



ZipTip is compatible with most single channel 10 µl pipettors. The Biohit Proline® electronic multi-channel pipettor is recommended for simultaneous processing of eight samples.

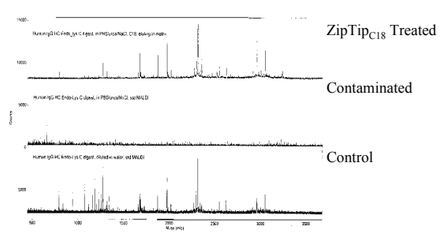
When to use C4 or C18 ?



Note: the addition of guanidine-HCL can improve polypeptide binding.

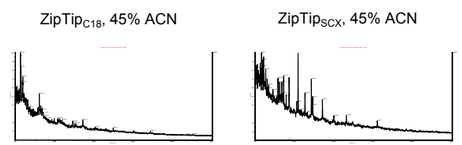
Peptide Desalting with ZipTip$_{C18}$

Prior to MALDI TOF MS



ZipTip desalting and concentration is most effective on samples that contain less than ca. 500 fmol of protein or peptide.

Sample Contains Solvent



ZipTip$_{SCX}$ concentrates peptides out of acetonitrile eliminating the need for a dry down step. Peptides can then be eluted with 5% ammonium hydroxide/30% methanol (volatile). Analysis by MALDI TOF MS.

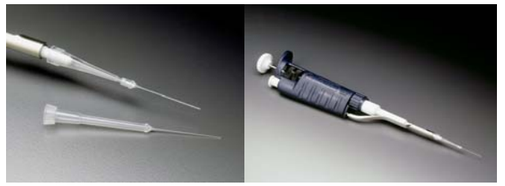
How to Achieve the Highest Concentration

Square-end HPLC needles press fit snuggly above the C18 membrane. When ca. 1-2 µl of 50% ACN/0.1% TFA is preloaded into the syringe, it can be slowly pushed through the membrane in a single downward flow. The highest concentration of peptides will be in the first 0.5 µl of eluant. HPLC syringes can also be used for loading higher volumes.

Sample Size is Greater Than 10 µl

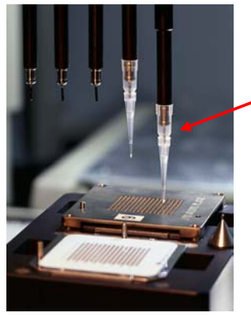
ZipTip can be press-fit onto most disposable 200 µl pipette tips and subsequently operated on a common 200 µl pipettor (left). For larger volumes (ca. 1 ml), an adaptor can be fashioned out of a 200 µl pipette tip that connects the ZipTip to a syringe (right).

Elution into a Nanoelectrospray Needle



The end of an Eppendorf GELoader® tip can be severed with a razor blade and press fit onto a ZipTip. With this adapter, eluted peptides can be delivered directly into a nanoelectrospray needle.

Automation



Micromass Mass*PREP*™ and PE MultiPROBE® compatibility. Integrated device containing an adapter collar press fit into a ZipTip$_{µ\text{-}C18}$ containing a 200 nl bed of C18 allowing elution in minimal volumes.

Additional Automation Protocols:

- ABI Symbiot™
- Bruker MAP™ II and MAP II/8
- Genomic Solution ProMS™
- Tecan Genesis

Automation reduces the labor involved with sample processing and target spotting.

Conclusions

- C18 and C4 are somewhat interchangeable. C18 is most applicable for peptides and proteins less than ca. 50 Kd. C4 works well for 50Kd and greater.
- ZipTip$_{SCX}$ concentrates peptides out of acetonitrile/DIW solutions.
- Using a variety of adaptations, milliliter volumes can be loaded onto a tip.
- The highest concentration of eluted peptides can be obtained with a single downward elution using an HPLC syringe.
- Peptides can be directly eluted into a nanoelectrospray needle using an Eppendorf gel loader tip adaptation.
- ZipTip pipette tips are compatible with a range of automation platforms.