PROTEOMIC/BIOMARKER

Onyx" Monolithic Capillary-LC Solutions Specifically for Proteomic and Biomarker Applications

Is carryover a problem?
Is it affecting your
ICAT results?

Do you have problems setting up 2D LC/MS/MS applications? Do you have problems with "peak parking" on your peptide of interest?

Would you like to spend less time re-equilibrating your column and more time analyzing your sample? Do you spend too much time injecting and loading sample on to your capillary column? Would you like to identify more peptides from your proteomics samples?

STOP wishing you could successfully achieve the results and solve the problems listed above and start actually doing it. The capillary solution (150 x 0.1mm (100 μ m)) offered within the Onyx portfolio gives those in proteomic and biomarker laboratories capabilities not had before with common silica particle capillary columns.

Onyx C18 150 x 0.1mm: A unique solution for proteomic applications

Onyx capillary HPLC columns are specially engineered by directly forming a C18 bonded monolithic silica gel rod within a 100µm I.D. glass capillary tube. This process creates a column that is highly suited for proteomic applications that require low flow rates, high peak capacities, and low sample-to-sample carryover.

Applications:

- Complex proteomic samples with a large number of peptides
- Protein digests with very low-level peptides for LC/MS analysis
- Complex clinical biomarker identification samples
- Differential display analysis using ICAT or other sample comparison technologies
- Two-dimensional LC-LC/MS/MS applications that use reversed phase for the second dimension

Improved results:

- More peptide identifications due to the greater peak capacity resulting from high efficiency
- Minimal background noise and longer column lifetimes due to lower carryover
- **More method flexibility** due to the wider flow rate range made possible by lower backpressures
- Easier coupling to 2-D chromatography methods due to Onyx's enhanced resistance to salts and other contaminants interfering at the inlet of the column

Tryptic digest of β -Amylase

Background: β-Amylase is digested by Trypsin to generate a complex peptide map.

No desalting or trap column is used, demonstrating Onyx's resistance to

matrix contaminants.

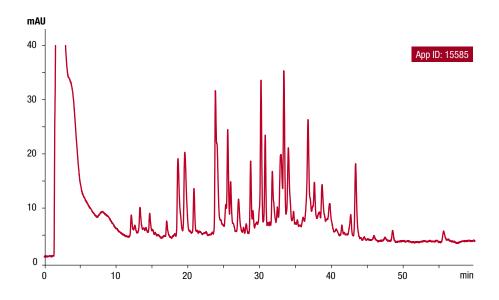
#1 Challenge: Resolve large numbers of different peptides from a protein digest in a micro capillary format.

Solution: The monolithic phase of Onyx delivers high peak efficiency and good

peak shape even in a capillary format.

#2 Challenge: Maintain separation of peptides when loading in the presence of buffer salts.

Solution: The pore structure of Onyx's monolithic phase allows buffer salts to pass quickly through the column while retaining and separating peptides efficiently across the large surface area of the column.



 Column:
 Onyx Monolithic C18

 Dimensions:
 150 x 0.1mm

 Order no.:
 CH0-7646

Mobile phase A: 0.1% TFA in Water

B: 0.08% TFA#/ 95% Acetonitrile

Gradient: 5-65% B in 60 minutes

Sample: 1. β-Amylase Tryptic Digest



High volume loading of a cytochrome C digest

Background: A large volume of a protein digest is directly loaded onto the Onyx column

at a high flow rate (10µL/min) without the use of a trapping column.

Flow rate is reduced during analysis to improve sensitivity.

#1 Challenge: Load large volumes of sample quickly without the use of a trap column.

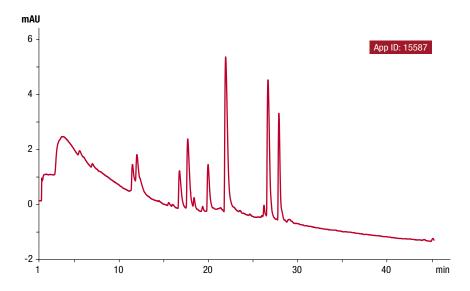
Solution: Onyx monolithic silica allows for wide flow rate flexibility resulting in

loading at 10µL/min flow rates. A 20µL sample can be directly loaded in

less than 2 minutes without the use of a trap column.

#2 Challenge: Maintain good chromatography at varying flow rates to minimize loading time and maximize analysis time.

Solution: The low backpressure and high efficiency of the Onyx monolithic format delivers high efficiency separations across a wide flow rate range (200nL/min-4µL/min). With Onyx, you can use flow rate to optimize the speed and sensitivity of your application.



 Column:
 Onyx Monolithic C18

 Dimensions:
 150 x 0.1mm

 Order no.:
 CH0-7646

Loading:

 Mobile phase:
 0.1% TFA/5% Acetonitrile/Water

 Flow rate:
 10 μL/min for 2 minutes

 Elution:

Mobile Phase:

A: 0.1% TFA in Water

B: 0.08% TFA/ 95% Acetonitrile

Gradient: 5 - 65% B in 60 minutes

 Flow rate:
 400 nL/min

 Temperature:
 Ambient

 Detection:
 UV @ 210nm



Key Onyx™ capillary features & benefits for proteomic and biomarker applications

1) High peak capacity

 Onyx columns have high efficiency due to the improved flow characteristics of the monolithic material. Higher efficiency separations deliver greater peak capacity resulting in an increase in peptide identifications.

2) Flow rate flexibility

 High flow rate flexibility (200nL/min – 10μL/min) allows for rapid loading of sample directly on to the column at high flow rates, while still maintaining good efficiency at both extremes.

3) Low carryover

- Trap loading is unnecessary with the flow rate flexibility and low carryover of Onyx capillary columns. Eliminating use of a trap columns, removes an additional source of carryover and peak broadening.
- Compared to typical particle-based columns, Onyx has decreased run-to-run carryover due to the monolithic nature.

4) Low backpressure

 Low backpressures of the monolithic Onyx capillary column make "peak parking" easier. This is because flow rate changes occur rapidly with less likelihood of losing the peak of interest by pressure drops forcing the peak through.

Onyx[™] products for proteomic and biomarker applications

Order No.	Description
CH0-7646	Onyx Monolithic C18 150 x 0.1 mm



Phenomenex product based on monolithic technology under license from Merck KGaA, Darmstadt, Germany.



www.phenomenex.com

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