

# Evaluation of Fluoropolymer Frit Performance in High-pH Nano-ESI Applications

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## Introduction

Nanoelectrospray applications at high pH require emitters of superior robustness under alkaline conditions. Conventional methodology mandates frequent emitter and column replacement due to deterioration of silica via base-induced hydrolysis<sup>1</sup>. Columns with an integral fluoropolymer frit combine the advantages of optimum sensitivity, band-broadening elimination, and exceptional longevity with enhanced resistance to high-pH conditions.

Benefits of fluoropolymer-fritted nanobore columns of hybrid-particle and traditional reverse-phase sorbents are evinced in the analyses of commercially available peptide mixture standard and tryptic digests of  $\beta$ -casein. Fluoropolymer frits offer the identical uncompromising sensitivity and superior chromatographic peak separation associated with integral silica frits plus enhanced column longevity and durability.

## Methods & Materials

### Instrumentation and Components

- Ion trap mass spectrometer (LCQ Deca™, Thermo Electron)
- Capillary HPLC Pump (1100 Series, Agilent) with 20:1 flow-splitter (Resulting flow rate of 250 nL/min)
- Water / methanol gradient, each containing 20 mM TEA
- Sulfur Hexafluoride, SF<sub>6</sub> (Concorde Specialty Gases, Inc.)
- Nanospray source (Digital PicoView® 150, New Objective, Inc.)
- PicoFrit® columns (360  $\mu$ m OD, 75  $\mu$ m ID, 15  $\mu$ m tip ID) with integral silica or fluoropolymer fritted tips and 10 cm sorbent beds containing one of the following:
  - ProteoPep™ II, C18, 5  $\mu$ m, 300 Å (New Objective)
  - XBridge™, C18, 5  $\mu$ m, 138 Å (Waters)

### Sample Preparation

- A commercially available peptide mixture (186002337, Waters Corporation) was diluted to 500 fmol/ $\mu$ L in 98% water, 2% Methanol, 20 mM TEA
- Commercially available  $\beta$ -casein was prepared via an overnight tryptic digest at 37° C and diluted to 500 fmol/ $\mu$ L in 98% water / 2% Methanol / 20 mM TEA
- Samples were analyzed at high pH using online nanobore ESI-MS in negative- and positive- ion modes

## Results

Columns with fluoropolymer frits displayed increased resolution for a peptide standard than columns containing silica frits (Figure 2). For a tryptic digest of  $\beta$ -casein, dramatically different chromatographic results were collected. Using a fluoropolymer-fritted column, analyte peaks absent from chromatograms collected with silica-fritted columns eluted early in both positive- (Figure 3) and negative- (Figure 4) ion modes. SF<sub>6</sub> sheath gas successfully sustained stable electrospray in negative ion mode (Figure 5) and yielded data of outstanding analytical caliber.

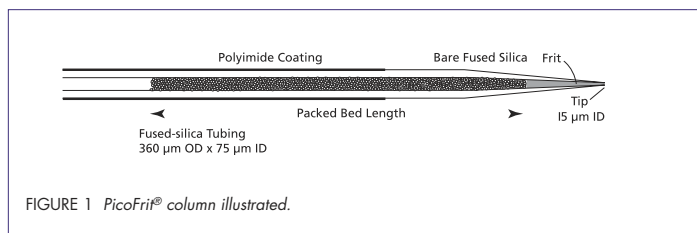
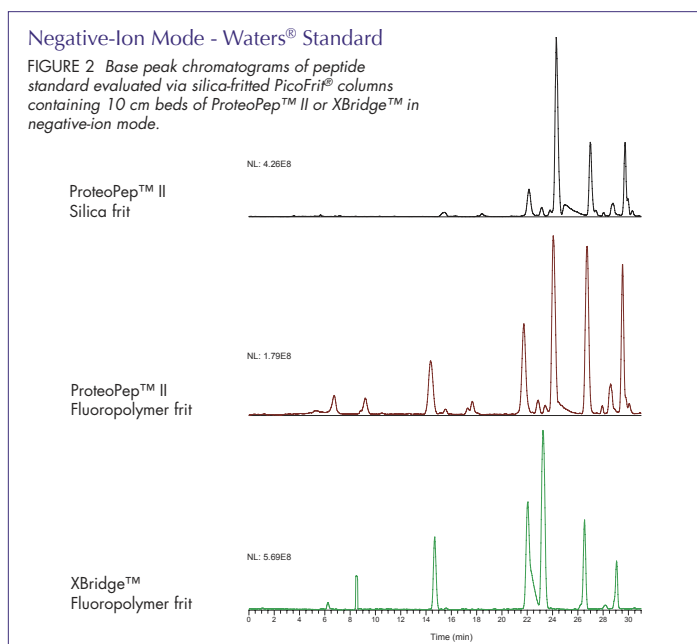


FIGURE 1 PicoFrit® column illustrated.

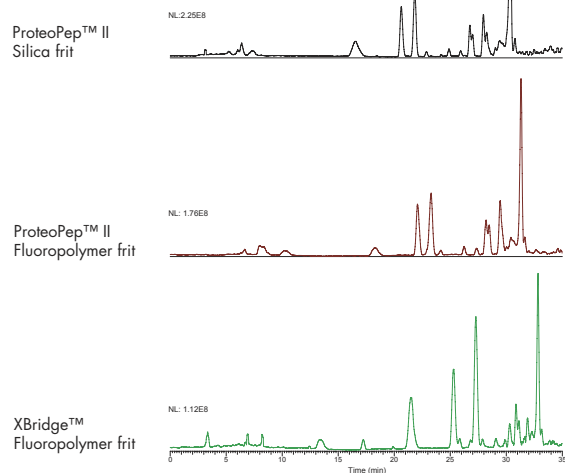


### Negative-Ion Mode - Waters® Standard

FIGURE 2 Base peak chromatograms of peptide standard evaluated via silica-fritted PicoFrit® columns containing 10 cm beds of ProteoPep™ II or XBridge™ in negative-ion mode.

### Positive-Ion Mode - $\beta$ -Casein

FIGURE 3 Base peak chromatograms of  $\beta$ -casein digest evaluated via fluoropolymer and silica-fritted PicoFrit® columns containing 10 cm beds of ProteoPep™ II or XBridge™ in positive-ion mode



### Negative-Ion Mode - $\beta$ -Casein

FIGURE 4 Base peak chromatogram of  $\beta$ -casein digest evaluated via silica-fritted PicoFrit® columns containing 10 cm beds of ProteoPep™ II or XBridge™ in negative-ion mode.

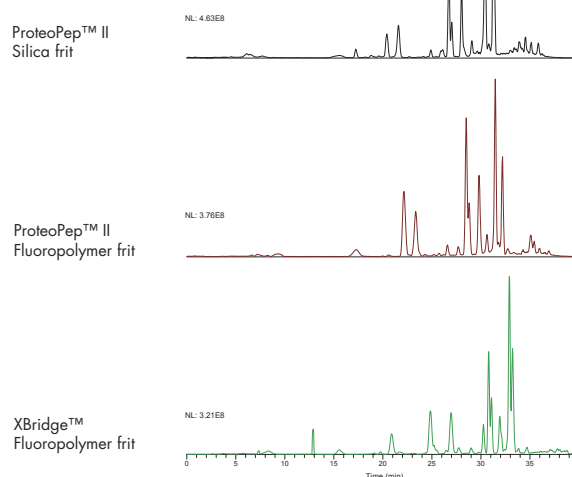


FIGURE 5 Base peak chromatogram of  $\beta$ -casein Digest evaluated with and without SF<sub>6</sub> sheath gas in negative-ion mode



## Conclusions

- Absence of hydrolyzable silica offers extended lifetimes for fluoropolymer-fritted columns in high-pH applications
- Fluoropolymer-fritted columns provide better peak shape and resolution than columns containing silica frits
- Paired with new hybrid C18 sorbents, fluoropolymer-fritted columns provide enhanced resistance to high-pH conditions and superior longevity
- Fluoropolymer-fritted columns facilitate detection of early-eluting peaks in a tryptic digest of  $\beta$ -casein
- In negative-ion mode, SF<sub>6</sub> sheath gas enhances spray stability for mobile phases containing high aqueous modifier concentrations
- Spray stability at high-aqueous conditions was facilitated using SF<sub>6</sub> sheath gas. Figure 5 illustrates a comparison between a  $\beta$ -casein sample analyzed with and without SF<sub>6</sub> sheath gas.

## References

1. Snyder, L.R., Kirkland, J.J., Glajch, J. L. *Practical HPLC Method Development*. John Wiley & Sons, Inc.: New York, NY, 1997.