NEW OBJECTIVE

Evaluation of Fluoropolymer Frit Performance in High-pH nano-ESI Applications

Jeffrey Wynn, Adam W. Perala, Christopher J. Toher, Gary A. Valaskovic
New Objective, Inc., Woburn, MA

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[1]. 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 ß-casein. Fluoropolymer frits offer the identical uncompromising sensitivity and superior chromatographic peak separation associated with integral silica frits plus enhanced 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_6 (Concorde Specialty Gases, Inc.)
- Nanospray source (Digital PicoView® 150, New Objective, Inc.)
- PicoFrit® columns (360 µm OD, 75 µm ID, 15 µm tip ID) with integral silica or fluoropolymer-fritted tips and 10 cm sorbent beds containing one of the following:
  - ProteoPep™ II, C18, 5 µm, 300 Å (New Objective)
  - XBridge™, C18, 5 µm, 138 Å (Waters)

Sample Preparation

- A commercially available peptide mixture (186002337, Waters Corporation) was diluted to 500 fmol/µL in 98% water, 2% Methanol, 20 mM TEA
- Commercially available ß-casein was prepared via an overnight tryptic digest at 37° C and diluted to 500 fmol/µL 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

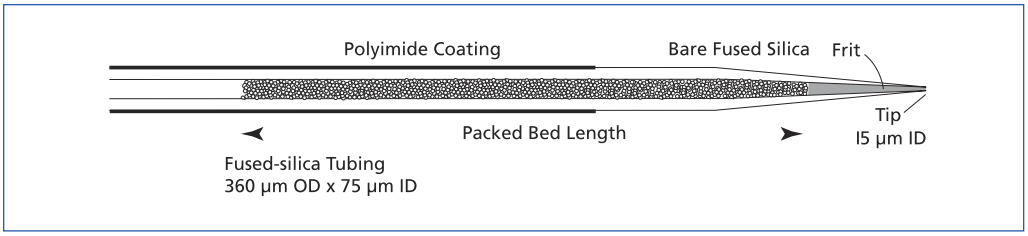

FIGURE 1 *PicoFrit® column*

Results

Columns with fluoropolymer frits displayed increased resolution for a peptide standard than columns containing silica frits (Figure 2). For a tryptic digest of ß-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^6 sheath gas successfully sustained stable electrospray in negative ion mode (Figure 5) and yielded data of outstanding analytical caliber.

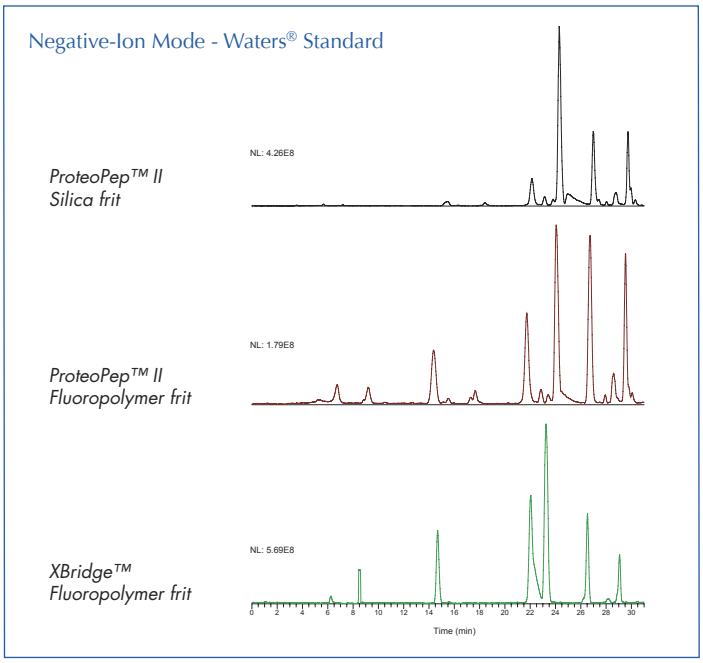

FIGURE 2 *Base-peak chromatograms of peptide standard evaluated via silica-fritted PicoFrit® columns containing 10 cm beds of ProteoPep™ II or XBridge™ in negative-ion mode.*

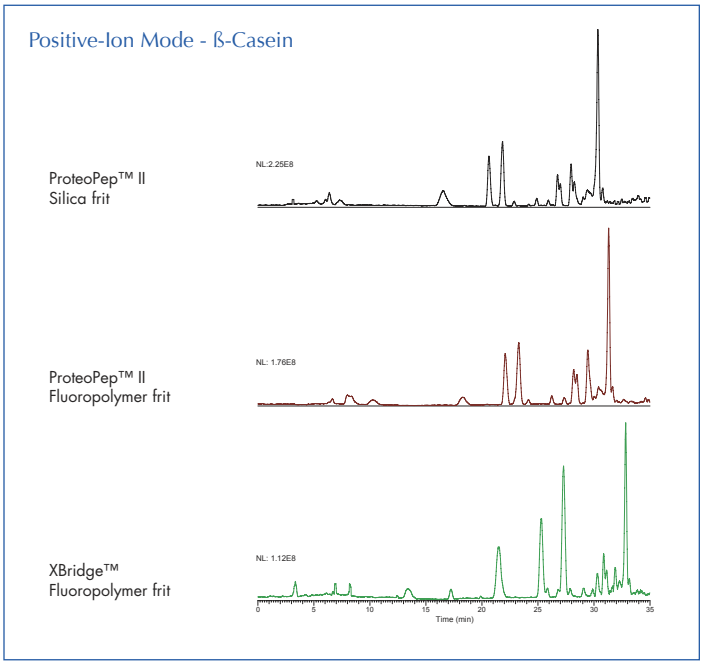

FIGURE 3 *Base-peak chromatograms of ß-casein digest evaluated via fluoropolymer and silica-fritted PicoFrit® columns containing 10 cm beds of ProteoPep™ II or XBridge™ in positive-ion mode*

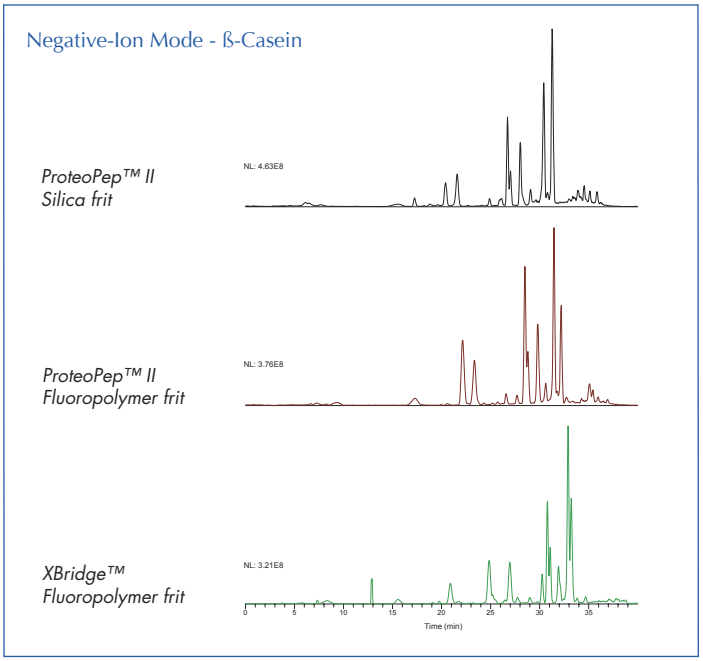

FIGURE 4 *Base-peak chromatogram of ß-casein digest evaluated via silica-fritted PicoFrit® columns containing 10 cm beds of ProteoPep™ II or XBridge™ in negative-ion mode.*

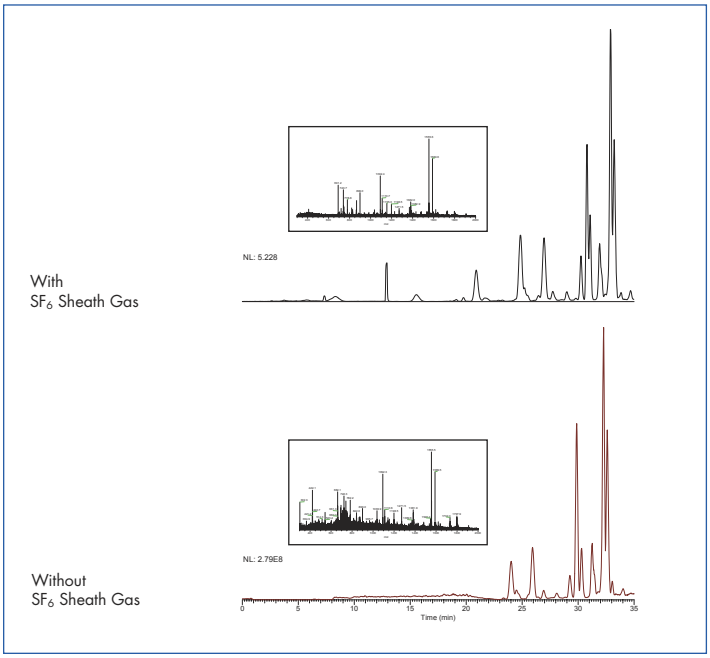

FIGURE 5 *Base-peak chromatogram of ß-casein Digest evaluated with and without SF_6 sheath gas in negative-ion mode*

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 ß-casein
- In negative-ion mode, SF_6 sheath gas enhances spray stability for mobile phases containing high aqueous modifier concentrations
- Spray stability at highly aqueous conditions was facilitated using SF_6 sheath gas. Figure 5 illustrates a comparison between a ß-casein sample analyzed with and without SF_6 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.

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New Objective, Inc.
2 Consitution Way
Woburn, MA 01801 USA
781 933 9560 tel
781 933 9564 fax
www.newobjective.com