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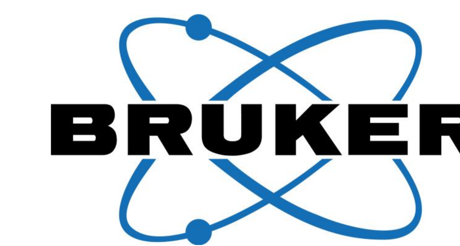
Turbocharging Discovery: Crafting a High-Speed, High-Efficiency Monolithic Column for Breakneck Single-Cell Analysis



Ruben Shrestha[1], Michael Krawitzky[1], Simion Kreimer, Goran Mitulovic[2], Ali Haghani; Jennifer E. Van Eyk, Gary Kruppa[2]

[1]Bruker Daltonics Inc., Billerica, USA; [2]Bruker Switzerland, Fallanden, Switzerland; [3]Cedars-Sinai Medical Center, Los Angeles, United States

Introduction

The frontier of single-cell proteomics aims to decode distinctive protein expression patterns within individual cells, a departure from conventional methods. Analyzing aggregated samples offers averaged profiles, while single-cell proteomics navigates unique challenges, demanding heightened sensitivity and resolution. Traditional mass spectrometry methods face complexity at minute scales. This study explores an innovative, high-sensitivity column, high throughput aspect for single-cell analysis. Challenging existing approaches and advancing the potential for precise insights into individual cellular protein expression intricacies.

Methods

We designed a prototype monolithic silica capillary column tailored for effectively separating low concentration samples, including single cells. This was achieved by harnessing the power of the nanoElute 2 UHPLC system in conjunction with the timsTOF ULTRA platform and incorporating gradients between 1.5 and 11 minutes utilizing the speed of dia-PASEF. Our approach involves a window schema comprising 26 Da windows, spanning a mobility range from 0.7 to 1.3, and a mass range from 300 to 1200 m/z. Parameters such as ramp and accumulation times were set at 25 milliseconds, resulting in a total cycle time of 0.21 seconds. Tryptic peptides from human lysate digest were used and data analysis was performed on Bruker Proteoscape software.

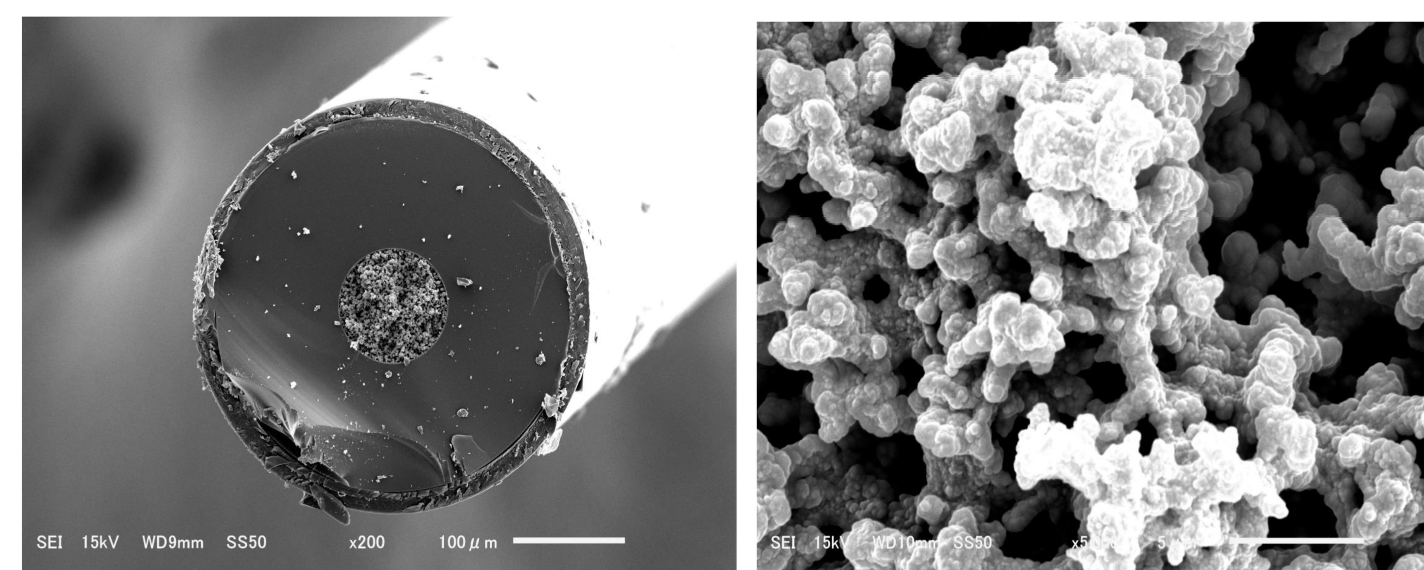


Figure 1: Scanning electron microscope images of monolithic column (A) x200 (B) x 5000

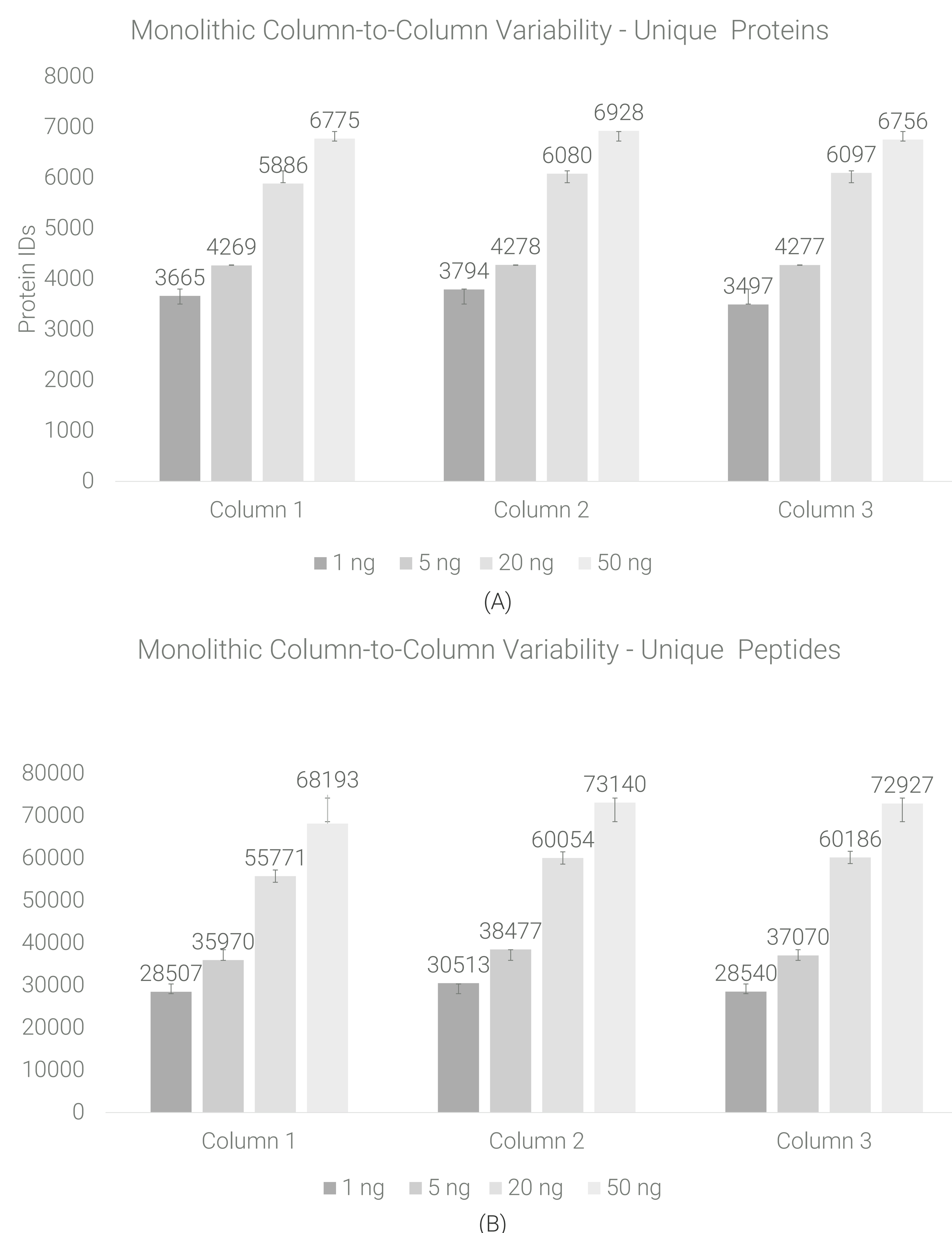


Figure 2: Analysis of commercial K562 digest using Monolithic columns (15 cm x 75 µm), coupled to the nanoElute2® and timsTOF ULTRA. A dia-PASEF method with 25 variable (width and mobility) windows, using 3 steps was used. One-hundred samples-per-day throughput was achieved with an 11.5-minute gradient. Ion mobility (IM) was set at 0.7 (1/k0 start) to 1.3 (1/k0 end). Data analysis was conducted using Bruker Proteoscape (no MBR), and triplicate injections were performed per concentration per column. Unique Proteins (A) and Unique Peptides identifications are reported.

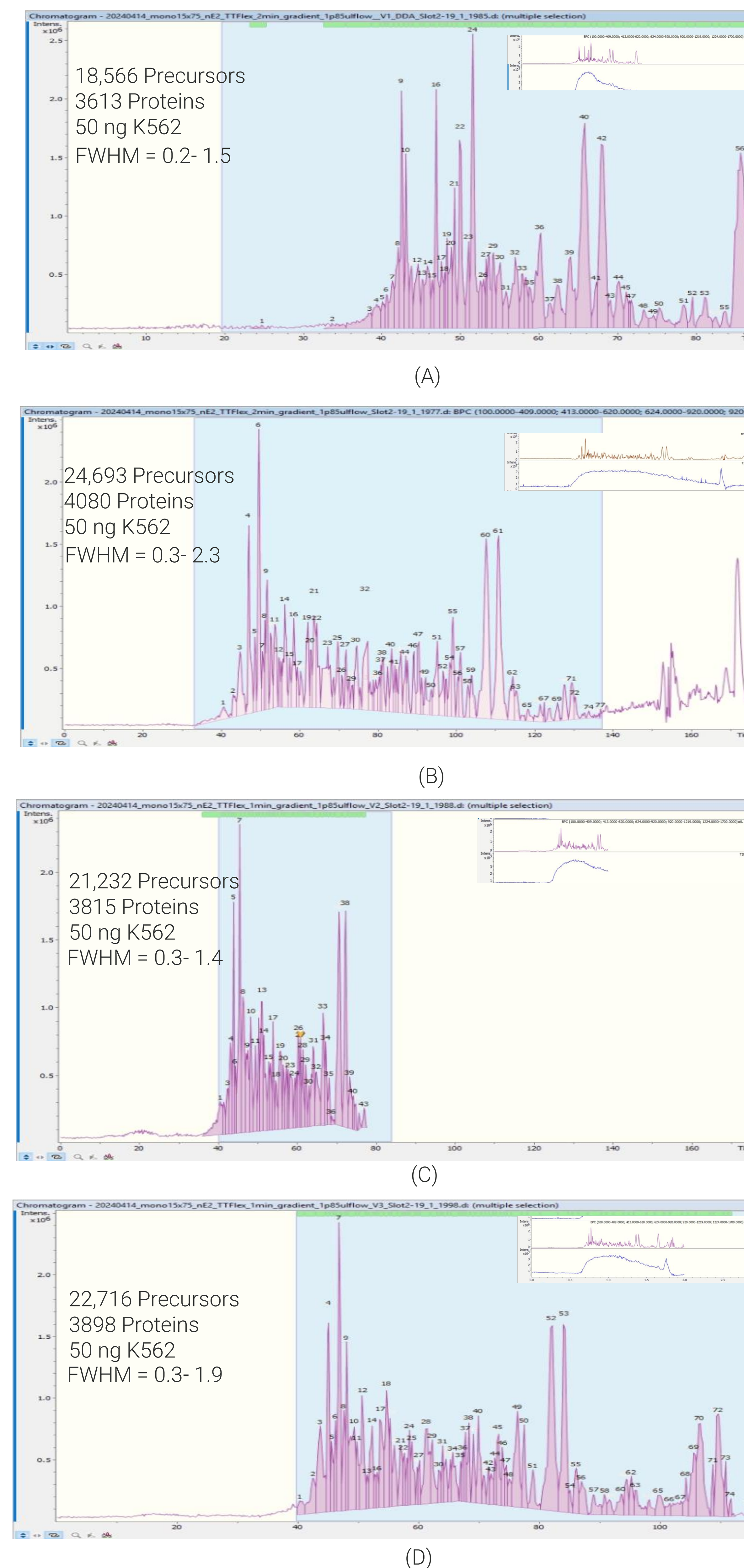


Figure 3: Base Peak Chromatograms of commercial K562 digest using a prototype monolithic columns (15 cm x 75 µm), coupled to the nanoElute2® and timsTOF Flex. 90 (A), 180 (B), 80 (C), 120 (D) second gradients were optimized with flow rates as high as 1.85 ul/min. A dia-PASEF method with 25 variable (width and mobility) windows, using 3 steps was used. Ion mobility (IM) was set at 0.7 (1/k0 start) to 1.3 (1/k0 end). The total cycle time was approximately 0.2 sec. Peak full width at half maximum and additional peak information was calculated in Bruker Compass DataAnalysis software. DIA results were determined in DIA-NN 1.8.1.

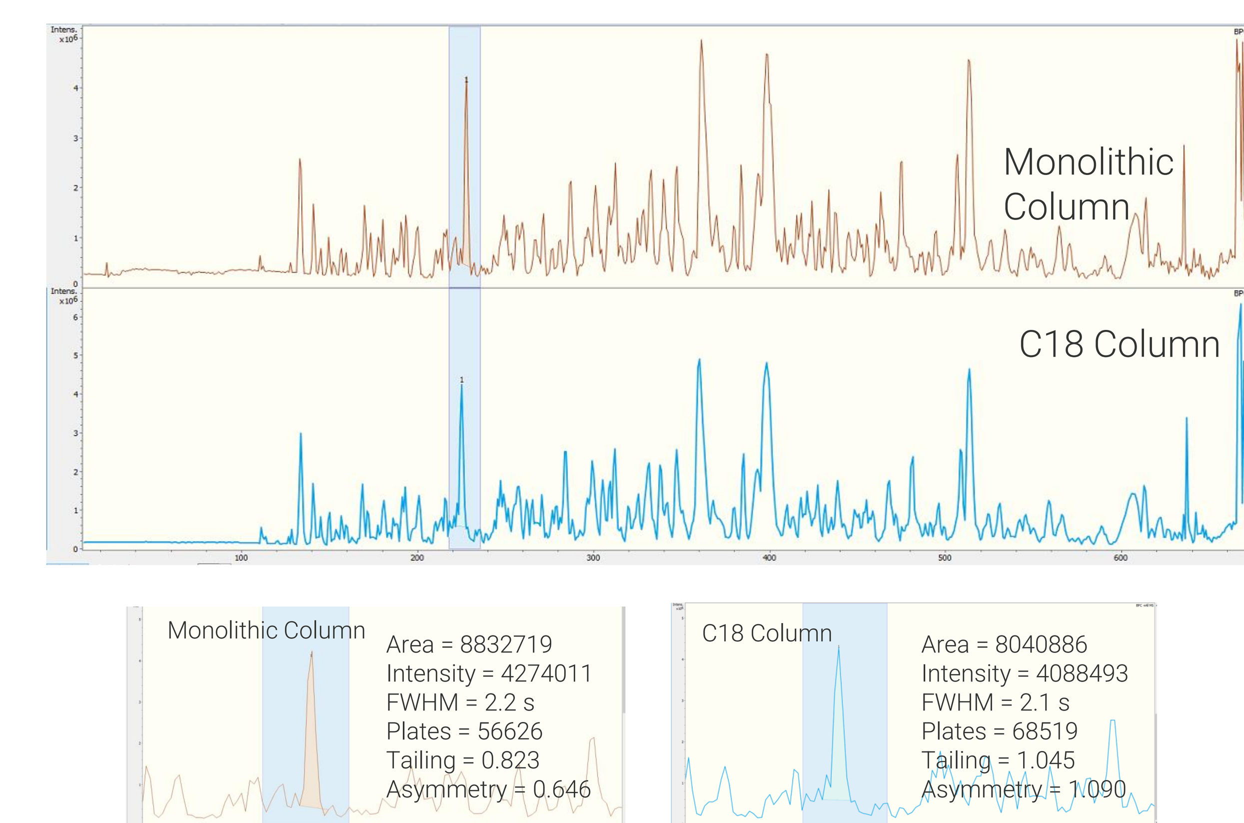


Figure 4: Base peak chromatogram comparison between conventional packed c18 column vs Monolithic Column running 50 ng K562 on 11.5 min gradients

Results

We obtained peptide and protein results that are on par with those achieved using conventional packed 8 cm packed C18 columns. Notably, we sustained lower pressures consistently across all injections, underscoring the versatility of our approach for diverse UHPLC systems. In sum, our analysis, facilitated by Proteoscape software, led to the identification of more than 3500 proteins and 18,500 precursors.

Our data showcases the remarkable proteomic depth that can be achieved when utilizing monolithic silica capillary columns in single-cell applications. The results indicate the potential for a wider application of these columns in LC-MS and multi-omics applications.

Conclusion

- **Lower pressures observed across all injections, underscoring the versatility of our approach for diverse UHPLC systems**
- **Potential for High throughput profiling of many samples (600-1000 SPD) without carryover**
- **Comparable peak width, FWHM and improved asymmetry and tailing vs conventional C18**

Technology

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