# FACILITATING THE ROUTINE ANALYSIS OF PEPTIDE MAPS WITH LIMITED SAMPLE AMOUNTS USING AN INTEGRATED NANOSCALE LC/MS PLATFORM

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## OVERVIEW

- **Advances such as microscale cell culture, and the desire to routinely analyze small amounts of purified product variants, have increased demand for microscale and nanoscale online separations in biopharma laboratories.**
- **It has been challenging to execute such studies with the same levels of routine success and robustness experienced with analytical LC/MS.**
- **A novel high-pressure ceramic microfluidic device is discussed that has been engineered to permit routine biotherapeutic LC/MS characterization studies at the nanoscale and microscale regimes.**

### WHY SCALE DOWN CHROMATOGRPHIC SEPARATIONS?

The primary reason to use microscale or nanoscale online separations is to address limitations in the amount of sample required for analysis. This is not a sensitivity issue. With modern electrospray source design, mass spectrometry behaves as a concentration sensitive detector, and good analytical scale chromatography can produce more concentrated peaks (and thus better signal) than a proportionally loaded capillary-scale experiment. In each case, once you have injected a sufficient amount to achieve the upper limits of MS response, the scale of chromatography has no effect on the dynamic range of the MS analysis.

Most biotherapeutic LC/MS characterization studies are not sample limited, and can be readily accomplished using microbore and analytical scale chromatography. However, advances such as high throughput microscale cell culture, or the desire to routinely analyze small amounts of a purified low-level product variant have raised the number of analytical groups employing microscale and nanoscale online separations. The challenge for most of these groups has been executing such studies with the same levels of routine success and robustness experienced with larger scale studies.

### WHY ARE CERAMIC MICROFLUIDIC DEVICES DESIRABLE OVER CAPILLARY-BASED SEPARATIONS?

- An integrated microfluidic device eliminates user-made fluidic connections, which are a primary cause of poor separation quality and system to system variability.
- Eliminating pre-column and post-column dead volumes maximizes chromatographic performance by reducing chromatographic dispersion.
- Monolithic ceramic construction is capable of withstanding high pressures and temperatures, allowing the full capabilities of BEH based sub-2 µm UPLC chemistries to be utilized.
- Reproducible separations require fine control of column temperature, which is enabled by the ability to integrate sensory and temperature control components into the ceramic matrix.
- Production techniques for a ceramic nanoTile allow rapid fabrication of multiple fluidic configurations and separation scalability (currently 75 µm to 300 µm)

## ENGINEERING A HIGH RESOLUTION UPLC MICROFLUIDIC DEVICE

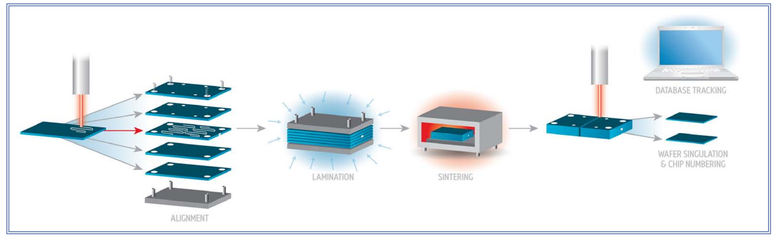


Figure 1. Fluidic pathways and passages are laser etched into thin layers of a ceramic tape. Through the application of heat and pressure, the compressed layers form a monolithic glass-like ceramic tile. Surface mounted fluidic connectors and electronics permit external fluidic connections, temperature management, run information storage, and support for integrated online detection technologies.

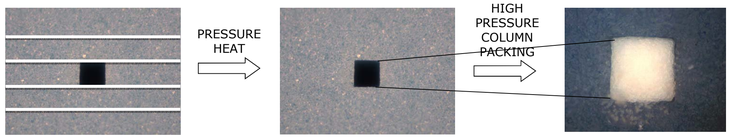


Figure 2. Following the lamination and sintering of the ceramic, the monilithic tile can be packed with sub 2u particles using high pressure packing techniques that yield consistent UPLC separations across tiles. The example above (RIGHT) is a 95 x 95 micron channel crossection packed with 1.2 micron BEH UPLC particles.

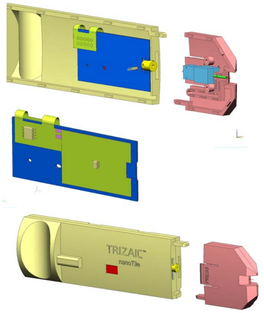


Figure 3. Nanotiles (blue) are mated with external fluidic connectors, and surface mounted electronics (green) that permit zonal temperature control, and recording of historical usage information for that separation unit. The fluidic enclosure (yellow) mates with an interchangable snap-on electrospray emitter module (red).

Figure 4. The device is inserted and locked within a new universal TRIZAIC source (Xevo and Synapt G2 Instruments). High pressure fluidic connections are made to the nanoACQUITY system without user intervention.

| Channel Size | 3 Layer Tile | 5 Layer Tile |
|---|---|---|
| 75 µm | 20k PSI | >32k PSI |
| 300 µm | 7-9k PSI | 12k PSI |

Figure 5. The pressure capability of a ceramic nanoTile is inversely proportional to column channel width, and is enhanced by encasing fluidic channels with additional layers of ceramic. This allows engineering of microfluidic devices that operate in excess of the pressure regimes associated with higher resolution UPLC bioseparations.

| Channel Size (Col i.d.) | Theoretical Load scaling factor | mAb Peptide LC/MS Tp Map Load |
|---|---|---|
| 2.1 mm | 1 | ~30 pmol |
| 1.0 mm | 0.23 (~1/4) | |
| 300 µm | .02 ( ~1/50) | ~1 pmol |
| 75 µm | .0013 (~1/800) | |

Figure 6. The amount of sample applied to achieve a comparable peptide mapping result is inversely proportional to the square of column channel width (column diameter). Experiments (300 µm vs. 2.1 mm) showed a practical load scaling (in pmol) of 1:30 for a humanized monoclonal Ab map, and 1:40 for a human interferon map.

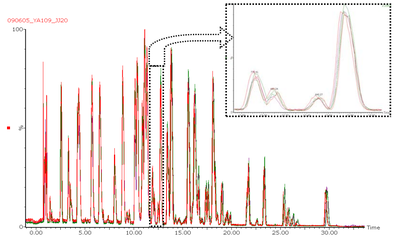


Figure 7. Reproducibility of 20 LC/MS$^E$ tryptic peptide maps, displaying every 5th mass chromatogram. Sample: Enolase

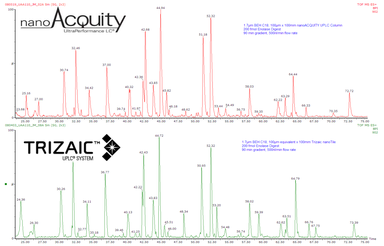


Figure 8. Chromatography with a TRIZAIC based separation will be of equal or greater quality than conventional capillary based UPLC separations.

## TRIZAIC PERFORMANCE FOR BIOTHERAPEUTICS AND COMPLEX SAMPLES

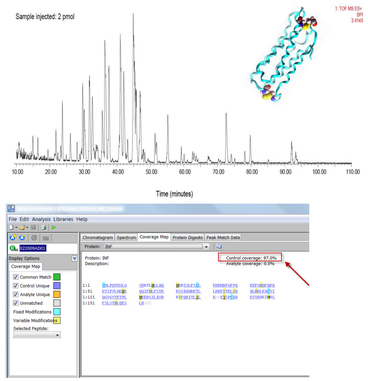


Figure 9. LC/MS$^E$ Tryptic map analysis (Top) of a human interferon on a 300 µm x 150 mm TRIZAIC nanoTile packed with 1.7 µm ACQUITY BEH $C_{18}$ particles. BiopharmaLynx 1.2 processing of the data confirms the high sequence coverage obtained by the mapping experiment.

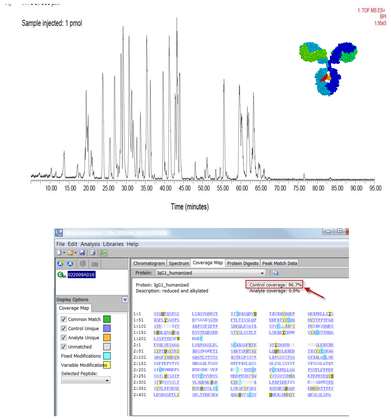


Figure 10. LC/MS$^E$ Tryptic map analysis of a humanized mAb on a 300 µm x 150 mm TRIZAIC nanoTile packed with 1.7 µm ACQUITY BEH $C_{18}$ particles. BiopharmaLynx 1.2 processing of the data confirms the high sequence coverage obtained by the mapping experiment.

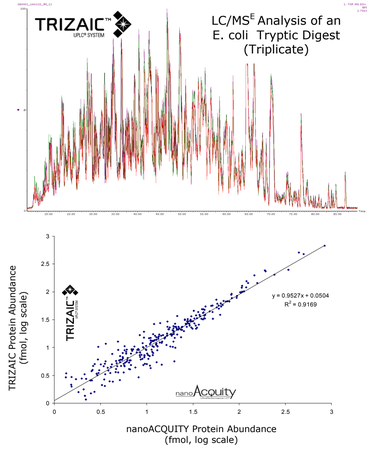


Figure 11. BPI chromatograms demonstrate the reproducibility LC/MS$^E$ analysis (Triplicate. TRIZAIC 75 µm x 150 mm) of a global E. coli digest (Top Panel). The results were processed by PLGS 2.4 (Identity$^E$ and Expression modules) to determine absolute concentration of all identified proteins (Bottom Panel) using the Hi$^3$ nonlabeled protein quantitation approach and was shown to be comparable to a capillary based separation on the nanoACQUITY. This workflow is being employed to identify and quantitate host cell proteins within intermediate process samples during biotherapeutic production.

## CONCLUSIONS

- TRIZAIC is a ceramic-based microfluidic LC separations device that improves the analytical reproducibility of nano- and microscale LC/MS by eliminating user-mediated fluidic connections, and maintaining tight control over key chromatographic parameters.
- Exceptional pressure tolerance allows extended columns packed with sub-2 um chemistries to provide high resolution peptide and biomolecule separations.
- The ability to maintain fine control over column temperature and swiftly replace a deteriorated spray tip facilitates routine high performance LC/MS biotherapeutic characterization studies.
- Separation performance of the TRIZAIC nanoTile is independent of sample complexity, allowing simple maps and complex sample analysis without system alterations.
- TRIZAIC offers bioanalytical R&D organizations the opportunity to achieve greater productivity through more effective use of scientific and capital resources.

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