Development of a Intergrated Microscale Ceramic Separation Device to Address Limited Sample Volumes in Bioanalysis

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INTRODUCTION

Small sample volumes from tail-bled rodents or pediatric studies place an extra challenge on the bioanalytical scientist; how to achieve the desired levels of sensitivity from limited sample volumes. The use of microscale separations have shown potential for the high-sensitivity analysis of limited-volume samples in the fields of proteomics. However, they have traditionally required a very experienced analyst and specialized instrument configurations. In this presentation, we will discuss the use of capillary-scale (300 µm) UPLC coupled to tandem quadrupole MS to achieve pg/mL levels of sensitivity from just a few microliters of sample. The analysis was performed on a standard tandem quadrupole high-sensitivity mass spectrometer with an easy-to-configure and exchange dedicated nano-spray source using a ceramic tile-based separations with integrated emitter. The design of the source and nanoTile correctly positions the separations device and emitter such that no user intervention is required.

In this work utilizing samples derived from rat plasma, the separations device showed excellent robustness with greater than 1,000 injections obtained, maintaining peak widths similar or superior to standard microscale LC/MS. Retention time variation of test analytes between different ceramic tiles was less than 0.6 % CV and resolution between critical pairs was between 1.2 and 1.5. Last, a bioassay was successfully developed for alprazolam and the hydroxylated metabolite with a LLOQ of 100 pg/mL utilizing only 1 µL of injected sample.

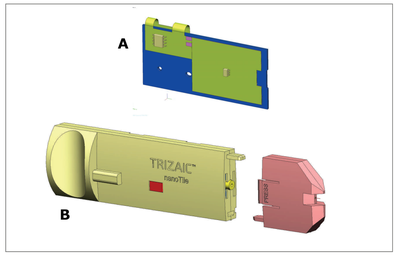
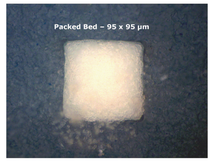


***Figure 1A**. A nanoTile (blue) with temperature control performed by an integrated circuit with on board temperature sensing (green).*
***B**. The nanoTile is placed within a protective housing (yellow). The electrospray emitter is connected to the channel using a snap-on fitting (pink).*

***Figure 2**. A custom ion source developed to affix the microfluidic device to a Q-Tof mass spectrometer. All of the fluidic and electronic connections (heater and electrospray voltage) are made within the ion source.*

Development of the nanoTile

1. **Laser Processing:** The ceramic process begins with a tape consisting of glass and alumina particles cast in an organic binder. A precision UV laser micromachining system creates the microfluidic channels and vias in the tape. Three or five layers of tape are used to create the entire structure with the center layer comprising the fluidic channel. The thickness of the channel layer defines the ultimate height of the microfludic channel.
2. **Lamination:** The layered tape is laminated using elevated temperature and pressure.
3. **Sintering:** The tape is placed in a furnace programmed with a temperature ramp to remove the organic binder and sinter the material. The sintering temperature allows the glass to flow and coat the alumina substrate. The resultant device is a monolithic substrate.
4. **Packing:** The microfluidic devices are packed with 1.7 BEH C18 particles. (**Right**)



Pressure Capability

The upper pressure capability is inversely proportional to the channel width. The greatest stress concentration within the channel occurs at the corners ultimately limiting the device. The glass within the ceramic substrate flows during sintering process and creates a radius at the corners enhancing the pressure limit. The limit can further be enhanced by adding layers encasing the channel layer. (**Below**)

Channel Size	3 Layer	5 Layer
75µm	20k	>32k
300µm (Initial Prototype)	7-9k	12k

RESULTS AND DISCUSSION

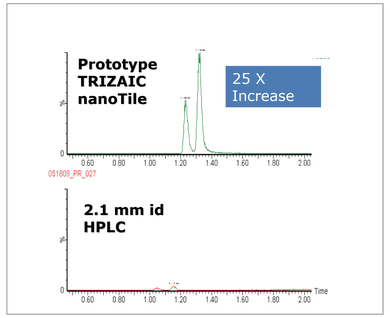


***Figure 3.** Comparison of MS response generated from equal injection volumes of a 10 ng/mL sample of alprazolam and its associated OH metabolite. A 25 x increase in signal was realized with the nanoTile over that of the conventional 2.1 mm id column. Both chromatographic formats were packed with 1.7 µm BEH C18 particles.*

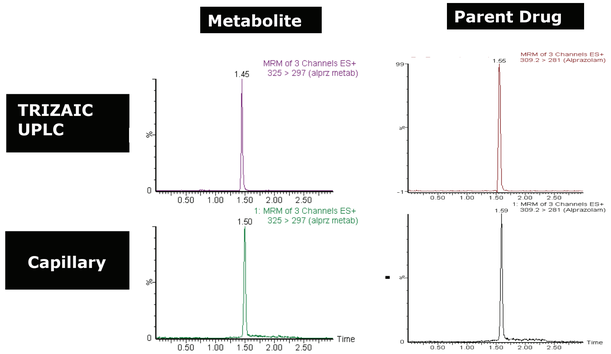


***Figure 4**. Comparison of chromatographic peak shape for alprazolam and OH metabolite analyzed by nanoTile and conventional capillary LC. Observed is comparable peak shape between the two chromatographic formats.*

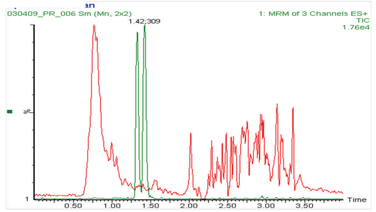
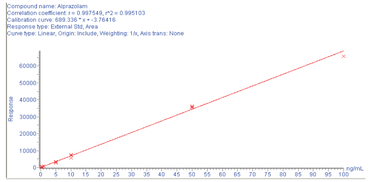


***Figure 5A.** Separation of alprazolam, D5 and OH metabolite in rat plasma (green trace) from matrix (red trace) . Gradient 35-95%B in 2 minutes, flow rate of 10µl/min, temp 45°C. **B** Calibration line of alprazolam from 0.1 to 100 ng/mL.*



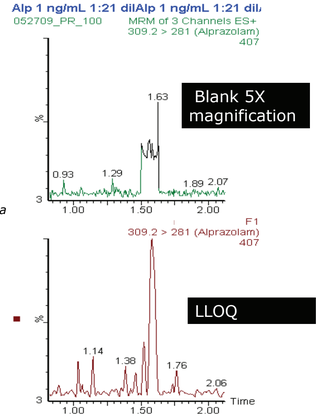


***Figure 6.** LLOQ and Blank for alprazolam assay. A LLOQ of 100 pg/mL was accomplished utilizing a 1µL injection onto the TRIZAIC nanoTile coupled with tandem quadrupole MS.*

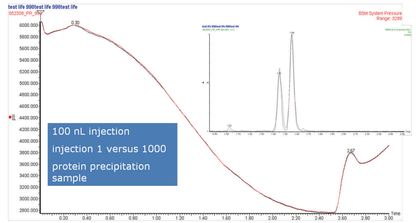


***Figure 7**. Column pressure overlay of injection 1 and 1000 of 100nl injection of protein precipitated rat plasma. (Equivalent to a 5µl injection on a 2.1mm ID UPLC column) The table **below** shows reproducibility between nanoTiles*

	Tile 1		Tile 2	
	Avg RT (min)	CV	Avg RT (min)	CV
Aprazolam	1.55	0.33	1.57	0.00
Metabolite	1.45	0.28	1.48	0.55
Rs (5% height)	1.5		1.2	

CONCLUSION

- **A high-pressure microfluidic platform capable of UPLC pressures has been developed based upon a modified co-fired ceramic manufacturing process.**
- **The TRIZAIC nanoTile technology greatly simplifies the implementation of capillary scale chromatography.**
- **Increases in detection limit on the order of 25x over traditional 2.1 mm HPLC was observed**
- **>1000 injections of a model pharmaceutical and OH metabolite in protein precipitated plasma was successfully achieved.**
- **A bioanalytical assay was successfully developed using only 1 µL injections.**
- **Analyte retention time and resolution was maintained between assays run between TRIZAIC nanoTiles**

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