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Nota de aplicación

Chromatographic Reproducibility of TRIZAIC nanoTile Technology

Waters Corporation

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief evaluates chromatographic performance and lot-to-lot reproducibility of the nanoACQUITY UPLC System paired with Xevo QTof and TRIZAIC UPLC nanoTile. The experiment tested the reproducibility of peak width (FWHM) and retention time over a sample of 40 TRIZAIC UPLC nanoTiles using a MassPREP Enolase Digest with Phosphopeptide mix (186003287).

Benefits

During this lot-to-lot reproducibility test, the TRIZAIC UPLC System and Xevo QTof yielded standard deviations of 0.3 for relative retention time and less than 0.06 minutes for FWHM.

Introduction

The TRIZAIC UPLC nanoTile replaces traditional fittings, capillary columns, and emitters typically used for nanoscale separations. A nanoTile can be dedicated to one analysis or analyst, confining any effects of the sample or operator to an easily interchangeable chromatographic setup.

The TRIZAIC UPLC System overcomes the challenges of nanoscale chromatography with a convenient plug-and-play approach while achieving UPLC performance, and has an 85 µm x 100 mm analytical channel packed with sub-2-µm chromatographic particles. The in-board trapping channel (180 µm x 20 mm) allows for sample focusing and desalting by utilizing 5 µm reversed phase particles. The nanoTile design integrates an analytical column, trap column, and electrospray emitter into a thermally controlled, highly usable microfluidic device. TRIZAIC nanoTile Technology enables a true UPLC microfluidic separation device that provides highly reproducible chromatographic results at nanoflow.

Results and Discussion

This study was conducted with 40 individual TRIZAIC UPLC nanoTiles using a nanoACQUITY UPLC System with a Xevo QTof Mass Spectrometer and TRIZAIC UPLC source. A MassPREP Enolase Digest with Phosphopeptide mix was run with a linear gradient from 3% ACN to 40% ACN at 450 nL/min at 35 °C as shown in Figure 1.

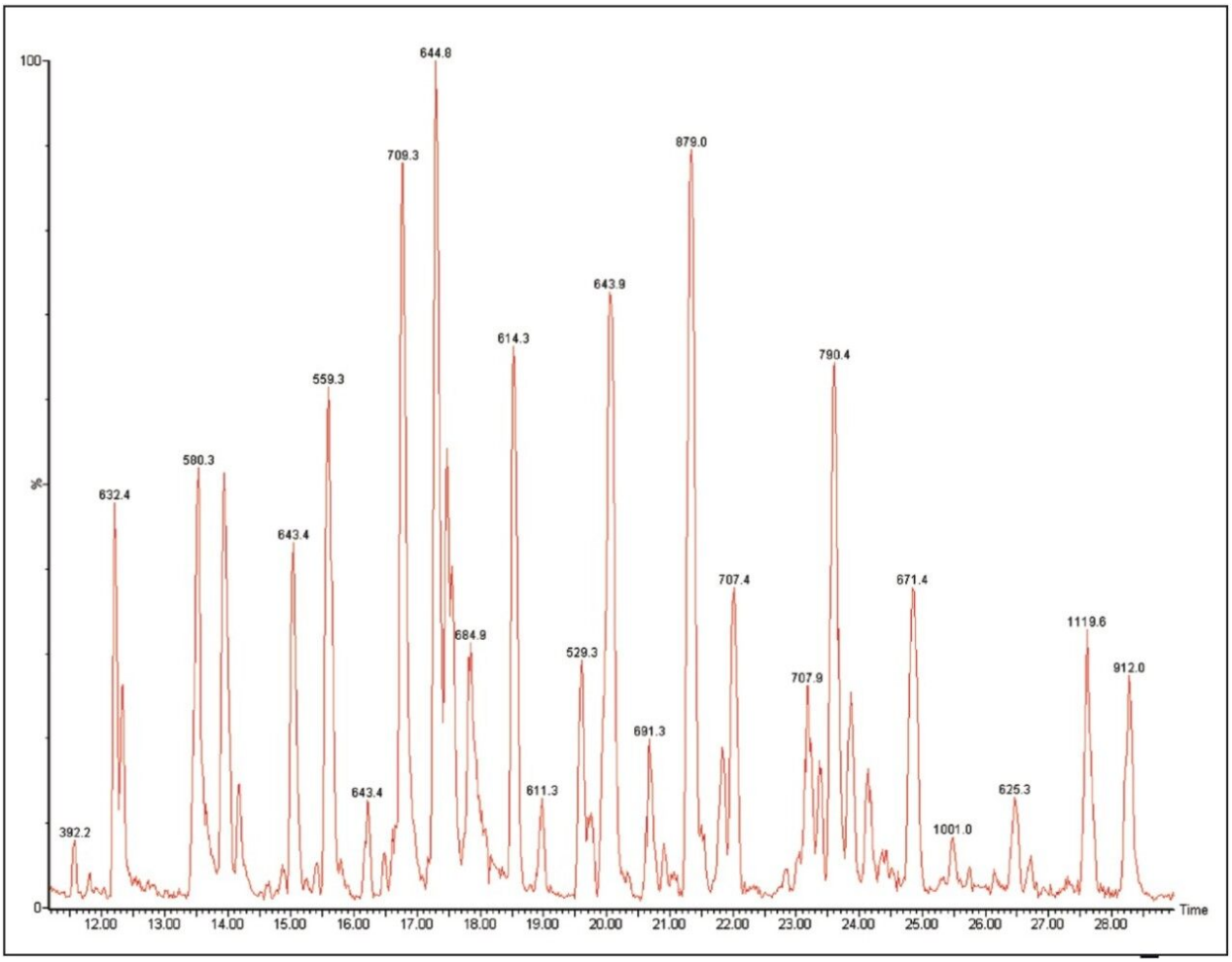

Figure 1. Enolase separation on the TRIZAIC UPLC nanoTile.

From this separation, six (6) peptides, representing hydrophilic-, hydrophobic-, phosphopeptide-, and histidine-containing peptide peaks were quantified for retention time and full width half maximum (FWHM) to determine the consistency of the manufacturing processes on 40 separate TRIZAIC UPLC nanoTiles from several batches.

Figure 2 shows the plot of the retention times of two tryptic peptides over the course of 40 separations. When these retention time data were normalized to the first eluting peptide, IATAIEK, and the relative retention time (RRT) averaged for the later eluting peptides, the resultant standard deviation indicates that retention time is highly reproducible with 40 separate nanoTiles over an 18-week time period and several batches.

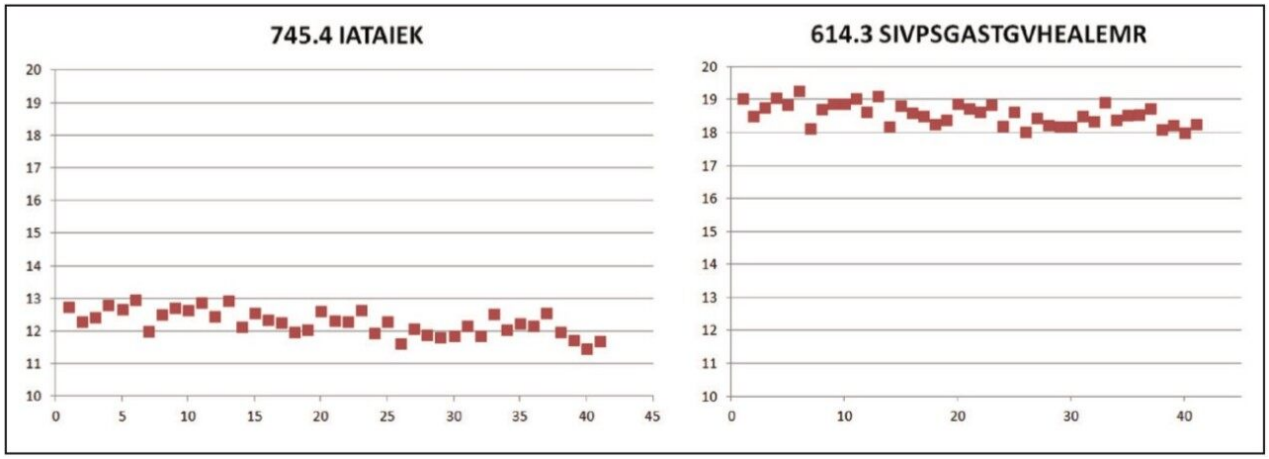

Figure 2. Plot of the retention time for two representative enolase tryptic peptides.

Relative RT	745.4	644.9	684.8	614.3	643.9	878.5
Average RRT	1.000	1.412	1.453	1.514	1.635	1.739
STD	0.031	0.031	0.029	0.027	0.032	0.030
RSD	3.13%	2.16%	1.96%	1.79%	1.97%	1.72%

Table 1. Relative retention time (RRT) using an early eluting peptide as the reference peak.

FWHM is a measure of chromatographic efficiency, since narrow peaks indicate an efficient separation with a higher peak capacity. In methods development, FWHM can be used to determine if separations are equivalent. As shown in Figure 3 and Table 2, the 40 separate TRIZAIC UPLC nanoTiles yield a consistent FWHM for the selected peptides.

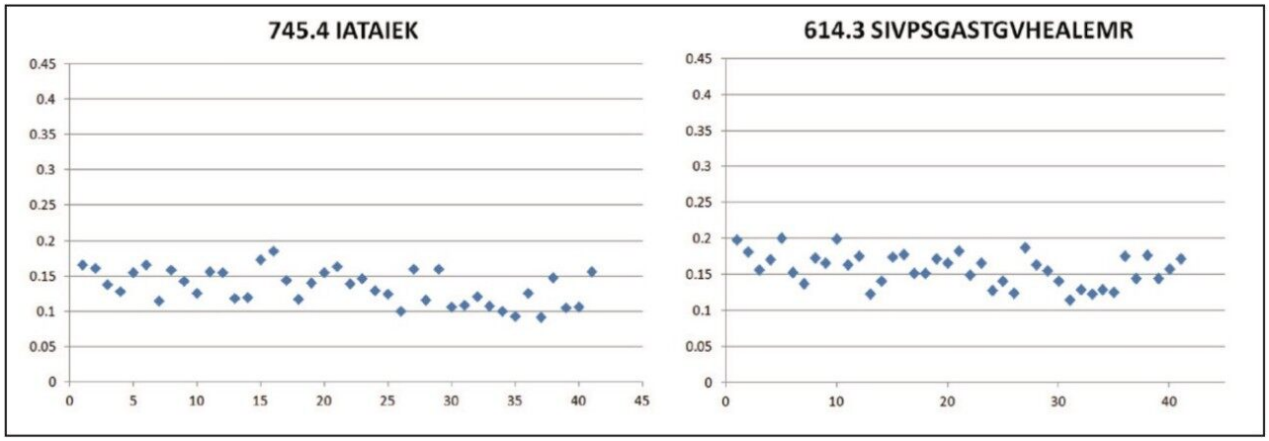

Figure 3. Peak width (FWHM) reproducibility.

FWHM	745.4	644.9	684.8	614.3	643.9	878.5
Avg. (min)	0.135	0.165	0.282	0.157	0.202	0.173
STD (min)	0.024	0.038	0.054	0.023	0.032	0.028
RSD	18.10%	23.14%	19.14%	14.53%	15.83%	16.06%

Table 2. Full width half maximum (FWHM) for six enolase peptides.

Conclusion

This study demonstrated that the chromatographic performance of the TRIZAIC UPLC nanoTiles was highly reproducible. The 40 TRIZAIC UPLC nanoTiles that were run during this study were obtained from multiple manufacturing lots run over the course of several weeks, and yielded a standard deviation of 0.3 for relative retention time and less than 0.06 min for FWHM. In conclusion, the processes used to manufacture the TRIZAIC UPLC nanoTiles are robust, and provide reproducible chromatographic separations.

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ACQUITY UPLC M-Class System <https://www.waters.com/134776759>

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