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## Reducing HPLC/UHPLC System Noise and Volume with High Performance Static Mixers

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## Abstract

A revolutionary new inline static mixer has been developed and specifically tailored to meet the exacting demands of high performance liquid chromatography (HPLC) and ultra-high performance liquid chromatography (UHPLC) systems. Poor mixing of two or more mobile phase solvents leads to increased noise in chromatographic applications, limiting the sensitivity of analyte detection and quantification. Minimizing the volume devoted to mixing is critically important for achieving the fast gradient delay times required for high performance separations. The homogenous static mixing of two or more solvents, while utilizing the minimal internal volume and physical size of a static mixer, represents the ultimate criteria for the ideal static mixer. The new static mixer accomplishes this goal via use of a novel 3D printing technology to create a unique 3D structure that achieves improved hydrodynamic static mixing with the highest percentage reduction in baseline sine wave per unit of internal mixture volume. Greater than 95% reduction in baseline ripple was achieved using up to 1/2 the internal volume of some commonly available mixers. This mixer consists of interconnected 3D flow passageways that have varying cross-sectional areas and varying path lengths as the fluid transverses across and through complex 3D geometric shapes. The mixing in the multitude of tortuous flow paths is coupled with localized turbulent flow and eddies to create mixing on the micro-, meso-, and macro-scale. Computational fluid dynamic (CFD) modeling was employed in the design of this unique mixer. The test data presented demonstrates that superior mixing is achieved while minimizing the internal volume in various gradient test conditions, such as unparalleled mixing for Trifluoroacetic acid (TFA) and water/acetonitrile gradients.

## Introduction

Liquid chromatography has been the work horse analytical methodology for many industries such as pharmaceuticals, crop protection, environmental, forensics, and chemical analysis for 30+ years. The ability to measure down to the part per million (ppm) levels and lower is critical to innovative development processes that lay the groundwork for tomorrow's drug discovery and the protection of human and environmental health. Low mixing efficiency, resulting in poor signal to noise ratios, has plagued the chromatography world when it comes to limits of detection and sensitivity. When combining two solvents for HPLC testing, it is sometimes necessary to induce mixing by external means to homogenize the two solvents as some solvents do not mix easily. If poor mixing is present, baseline noise will appear as a sine wave (rise and fall) of the detector signal versus time. At the same time, poor mixing will both broaden and create asymmetrical peaks leading to reduced analytical efficiency and peak resolution. The ideal static mixer will combine the advantages of high mixing efficiency, low dead volume and low pressure drop, while minimizing the volume and maximizing the throughput of the system.

### PerfectPeak® Static Mixers from Mott

Mott recently developed a new line of PerfectPeak® in-line static mixers with five different internal volumes: 25  $\mu\text{L}$ , 50  $\mu\text{L}$ , 100  $\mu\text{L}$ , 150  $\mu\text{L}$ , and a prototype 300  $\mu\text{L}$ . These sizes cover the range of volumes and mixing performance needed for the majority of HPLC testing where enhanced mixing with low dispersion is required. All five models are 0.5 inches in diameter and have corresponding lengths of 1.4, 1.7, 2.4, 2.7 and 4.5 inches. They are fabricated in 316L stainless steel and passivated for inertness. These mixers are also available in Titanium and other corrosion resistant and chemically inert alloys. The maximum operating pressure is 20,000 psig.

Presented in Figure 1a is a photograph of the Mott 100  $\mu\text{L}$  static mixer developed for maximum mixing efficiency while utilizing a smaller internal volume comparable to standard mixers in this category. This new static mixer design utilizes a novel additive manufacturing technology to create a unique 3D structure that achieves high performance mixing. This mixer consists of interconnected three-dimensional flow passageways that have varying cross-sectional areas and varying path lengths as the fluid transverses through and across internal complex geometric obstacles. Shown in Figure 1b is a schematic representation of this new mixer utilizing industry standard 10-32 threaded HPLC compression fittings for the inlet and outlet, with the boundary of the patent pending internal flow path of the mixer shaded in

blue. The varying cross-sectional areas of the internal flow path and directional flow changes within the internal flow volume produce regions of turbulent and laminar flow that create mixing on the micro-, meso- and macro-scales. Computational Fluid Dynamic (CFD) modeling was employed in the design of this unique mixer to analyze flow patterns and to improve designs prior to fabrication of prototypes for internal analytical testing and customer beta site evaluations.



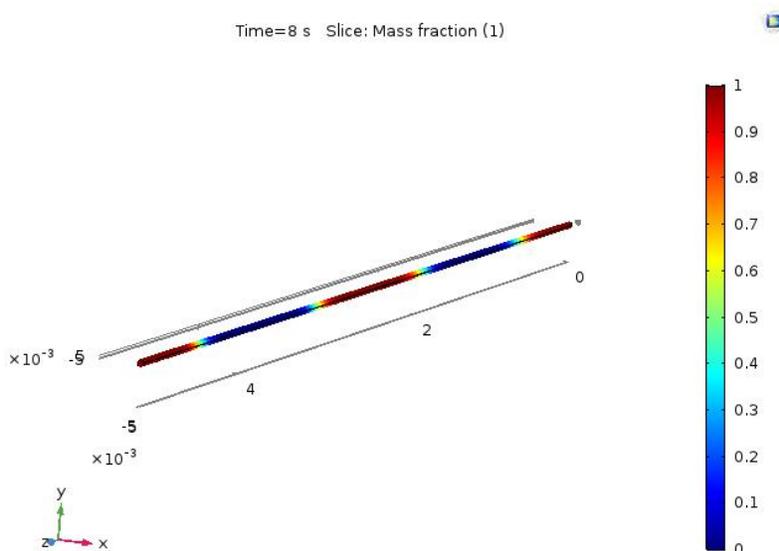
**Figure 1.** Photograph of a Mott 100  $\mu\text{L}$  static mixer (a) and a schematic representation showing a cross-section view with the mixer fluid flow path shaded in blue (b).

### CFD Modeling

Computational Fluid Dynamics (CFD) simulations of the static mixer performance were performed during the design stage to assist in the development of efficient designs and to reduce trial and error experimentation, which can be time consuming and expensive. CFD modeling of the Mott static mixer designs was performed using COMSOL Multiphysics package. Modeling was performed using flowrate-driven laminar flow fluid mechanics to understand the fluid velocity, pressure and turbulent nature within the part. The fluid mechanics interface was coupled with the chemical transport of mobile phase compounds to help understand the mixing of two different concentrated liquids. The model was studied under time dependent specifications of 30 seconds for ease of computing while still finding a comparable solution. Theoretical data was generated in the time dependent study using the point probe projection tool where a point in the middle of the outlet was selected to gather data. The data was then compared to the wave function that was specified at the inlet and a relative mixing efficiency was computed.

The CFD model and experimental testing utilized two different solvents through a proportional sampling valve and pumping system, thereby resulting in alternative plugs of each solvent in the sample line. Prior to performing simulations through a static mixer, simulations were performed on simple tubing to validate the model. This initial modeling was performed on a 5 cm long by 0.25 mm ID straight tube to demonstrate the concept of alternating plugs of water and pure acetonitrile entering the tube with

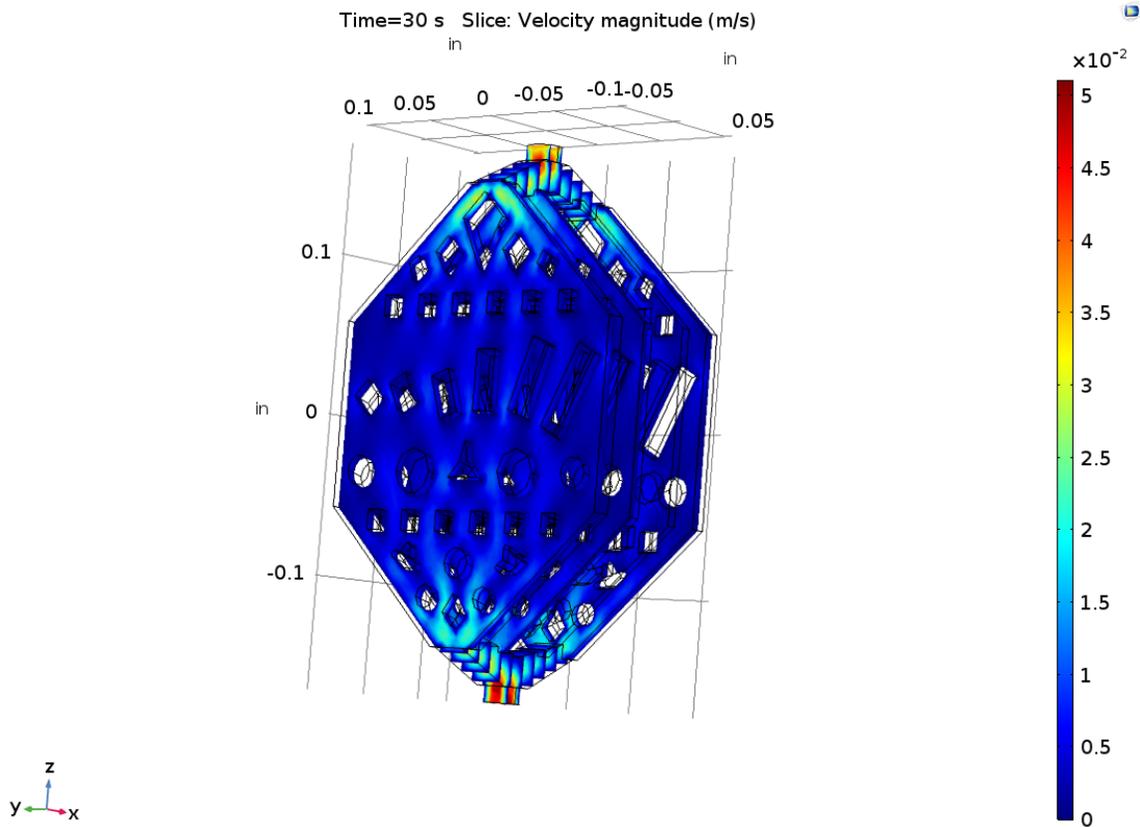
minimal evidence of mixing. These initial simulation results are shown in Figure 2 with a simulated flow rate of 0.3 ml/min.



**Figure 2.** Shows CFD modeling on flow in a 5 cm long by 0.25 mm ID tube to represent what is happening in the HPLC tubing, i.e., if no mixer is in place. The full red represents water as a mass fraction. The blue represents the lack of water, which is pure acetonitrile. A diffusion region can be seen between the alternating plugs of the two distinct liquids.

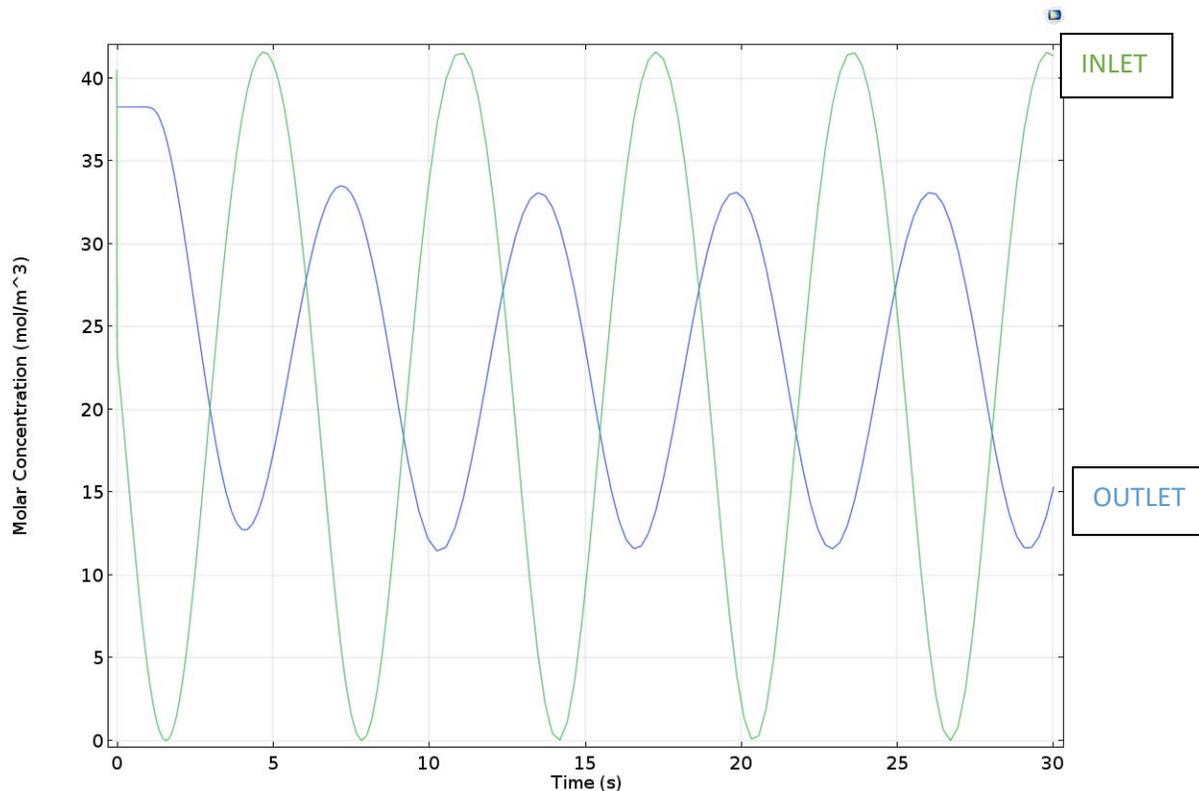
Numerous static mixer designs were tested using CFD modeling to reduce development time. The designs that showed the best performance in CFD modeling were then constructed as prototypes for testing purposes.

In addition to the reduction in prototyping time, CFD modeling gives the user the ability to look at the liquid profile as it traverses through the part. The benefit of this is being able to understand how the part is creating mixing. The goal was to maximize the phase mixing in the static mixer to completely utilize the full body of the mixer. Figure 3 shows the velocity profile through one of the top three Mott prototype static mixer designs. Evident in this figure are the streaks of lighter color through the middle of the part, indicating an offset in velocity through each channel. This is indicative of phase mixing throughout the part as plugs of fluid enter the part they will be diverted into the channels and pass through the part at different velocities before recombining at the bottom of the part in an offset phase.



**Figure 3.** Velocity profile of liquid in a Mott Static Mixer design. Blue indicates low velocity in the part, red indicates high velocity. It is important to have an offset of velocity throughout the part to induce phase mixing.

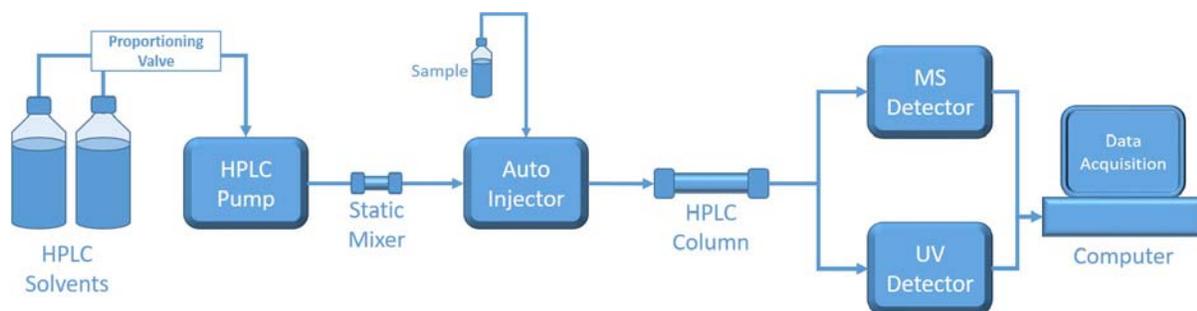
The experimental testing utilized two different solvents alternating through a proportional sampling valve and pumping system, thereby resulting in alternative plugs of each solvent in the sample line. These solvents were then subsequently mixed in the static mixer. To mimic this in the CFD model, the assumption was made that the specified inlet function was a representation of no mixing occurring, and then compared to the wave function from the outlet of the mixer where a mixing efficiency was calculated. This is best represented in Figure 4 where the green curve is the function specified at the inlet of the part. The blue curve represents the molar concentration at the outlet of the part, where the peaks and valleys of the curves are shallower than the specified inlet function. Similar to the experimental data collection, the amplitudes of the waves are computed, and then the efficiency of the mixer is calculated.



**Figure 4.** A representation of concentration as a function of time through the Mott static mixer. The green represents the concentration at the inlet of the part while the blue represents the concentration at the outlet of the part.

### Experimental Procedure

The following HPLC conditions and test setup were used to measure the baseline sine wave to compare the relative performance of various static mixers. Presented in Figure 5 is a schematic diagram showing a typical layout of a HPLC/UHPLC system. Testing of static mixers was performed by locating the mixer immediately downstream of the pump and upstream of the sample injector and HPLC column. Most background sinusoid measurements (case study 1 & 2) were performed by bypassing the sample injector and column using a capillary tube between the static mixer and the UV detector. When analysis of signal to noise ratios and/or peak shape were evaluated, the system was configured as shown in Figure 5.



**Figure 5.** Schematic diagram of the low-pressure gradient experimental test system.

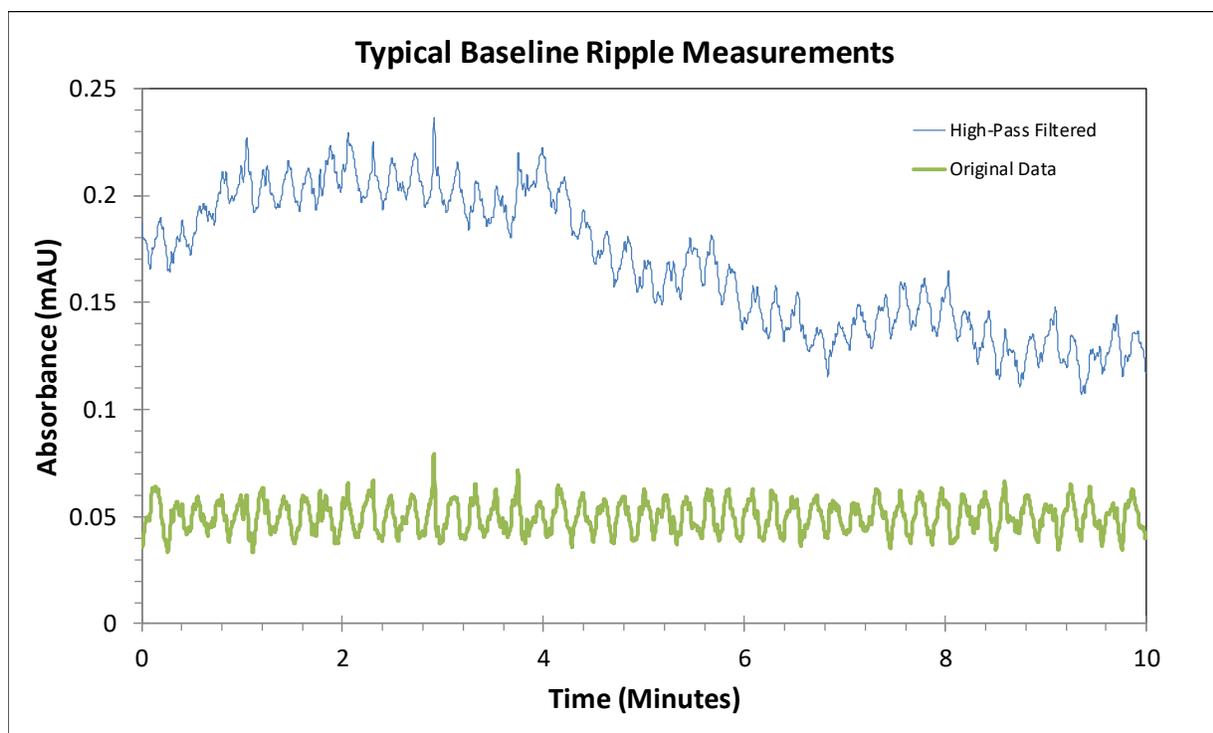
The HPLC system utilized for this testing was an Agilent 1260 Series HPLC with a UV detector controlled using the Agilent Chemstation Software. Presented in Table I are the typical setup conditions for measuring mixer efficiency by monitoring baseline sinusoid in two HPLC gradient case studies and TFA/Water:TFA/acetonitrile gradient mixing.

### HPLC Ripple Reduction Case Studies

Experimental tests were conducted for two different solvent case studies as outlined in Table I below. The two solvents mixed in Case 1 were Solvent A (20 Millimolar solution of ammonium acetate in deionized (DI) water) and Solvent B (80% acetonitrile (ACN) / 20% DI water). In Case 2 study, Solvent A was a solution of 0.05% acetone (tracer) in DI water and Solvent B was an 80/20% mixture of methanol and DI water. The pump was at a constant flow rate ranging from of 0.25 ml/min to 4.0 ml/min in Case 1 and at a constant flow rate of 1 mL/min for Case 2. In both cases the mixing ratio of Solvents A and B was 20% A / 80% B. The detector was set at 220 nm in Case 1 and the maximum absorbance of acetone, 265 nm wavelength for Case 2.

Table I HPLC Configurations for Case 1 & 2		
	Case 1	Case 2
Pump Speed	0.25 ml/min through 1.0 ml/min	1.0 ml/min
Solvent A	20 Millimolar ammonium acetate in DI water	0.05% Acetone in DI Water
Solvent B	80% Acetonitrile (ACN) / 20% DI water	80% Methanol / 20% DI Water
Solvent Ratio	20% A / 80% B	20% A / 80% B
Detector	220 nanometers	265 nanometers

Presented in Figure 6 is a typical example of mixing baseline noise for Case 1 appearing as a repeating sinusoidal pattern superimposed over baseline drift. Baseline drift is a slow increase or decrease of the background signal. It typically appears to be decreasing if the system was not allowed to equilibrate long enough but can appear as random drift even when the system is fully stabilized. The amount of this baseline drift tends to increase when the system is operating under steep gradient conditions or at higher back pressures. It is difficult to compare sample to sample results when this baseline drift is present, and this was overcome by applying a high-pass filter to the raw data to filter out these low frequency variations providing oscillation plots with flat baselines. Also, shown in Figure 6 is a plot of the mixer baseline noise after the high-pass filter was applied.



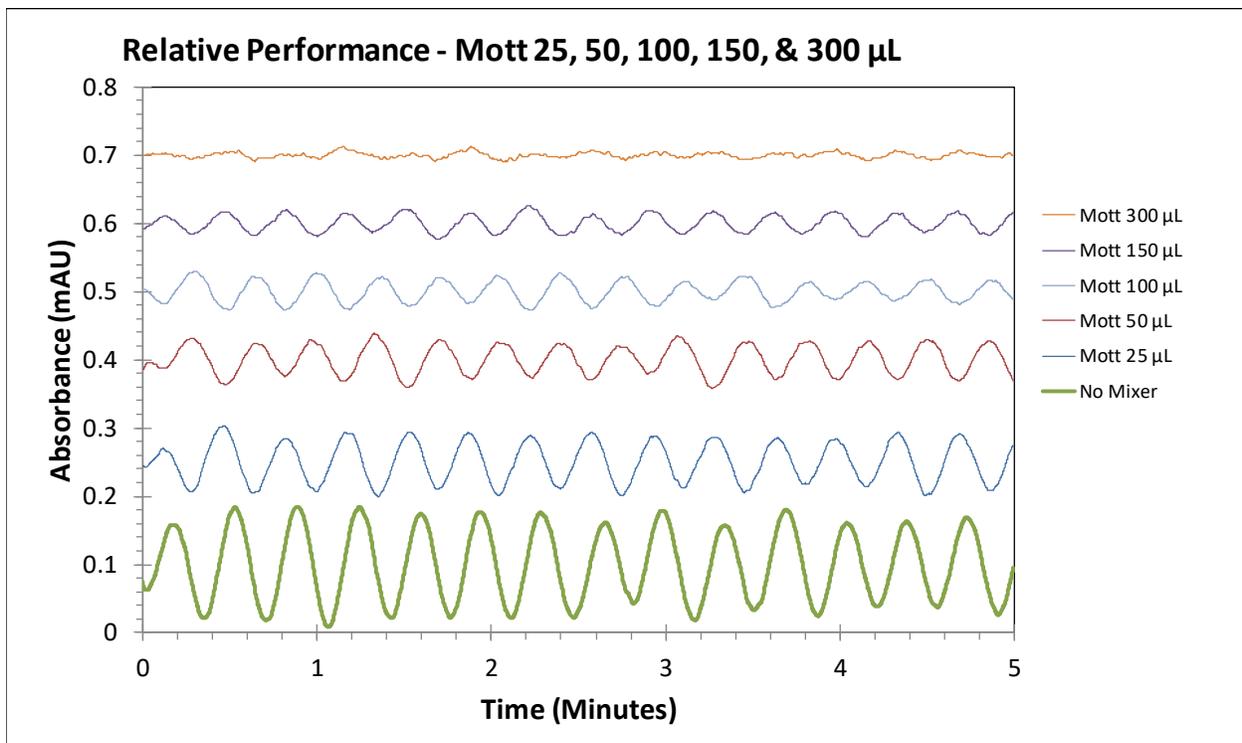
**Figure 6.** Plots of measured mixing sinusoid before and after a high-pass filter was applied to remove the baseline drift component of the signal.

### HPLC Gradient Mixing Case Studies Test Results

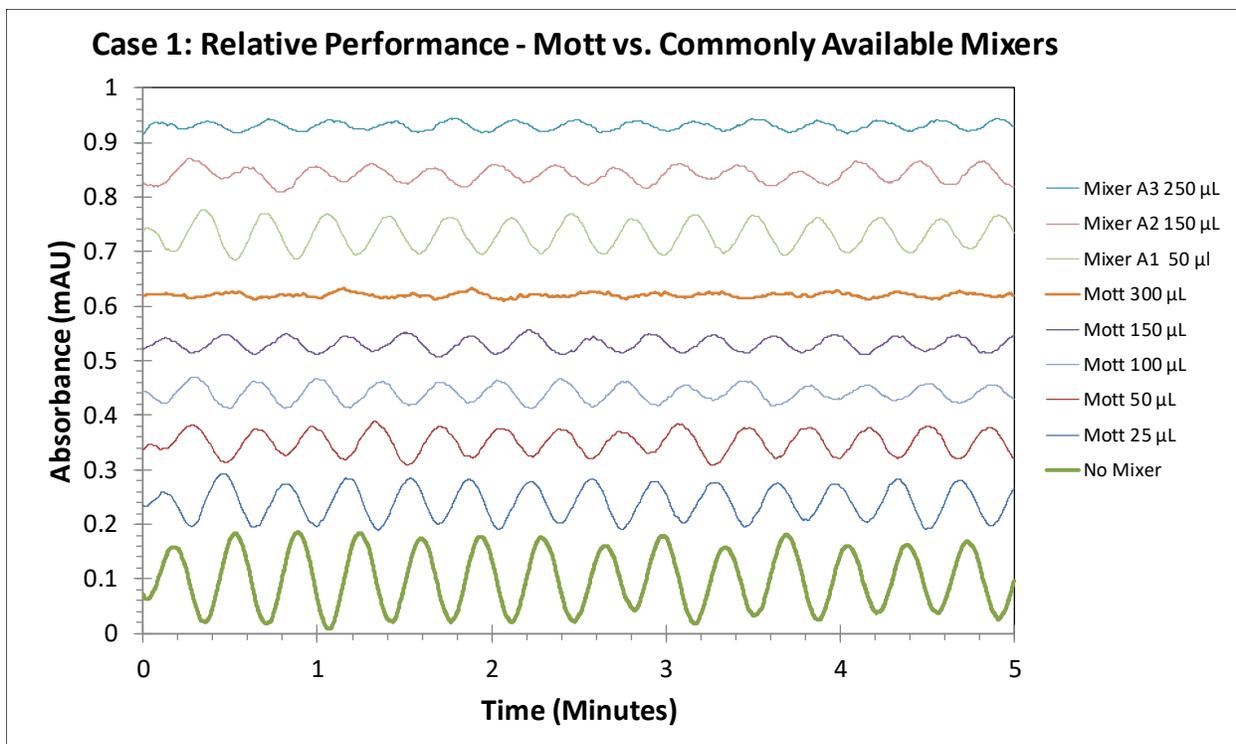
Upon completion of CFD modeling and initial experimental testing, five separate static mixers were subsequently developed utilizing the internal structures noted above with five internal volumes, 25  $\mu\text{L}$ , 50  $\mu\text{L}$ , 100  $\mu\text{L}$ , 150  $\mu\text{L}$ , and 300  $\mu\text{L}$ . This range covers the range in volumes and mixing performance needed for the majority of low level analyte HPLC testing where enhanced mixing with low dispersion is required to produce a low amplitude baseline.

## Water/Acetonitrile Data and Results

Presented in Figure 7 are the results of baseline sine wave measurements taken from the test system for Case 1 (Acetonitrile with ammonium acetate as a tracer) shown using Mott's standard volumes of static mixers along with no mixer installed. The experimental test conditions for the results shown in Figure 7 were held constant for all 4 tests following procedure outlined in Table I with a solvent flow rate of 0.5 ml/min. Offset values were applied to the data set so they could be displayed next to each other without signal overlap. The offset does not affect the amplitude of the signal which is used to rate the mixer performance levels. The average amplitude of the sine wave with no mixer installed was 0.18 mAu with the amplitude dropping to 0.10, 0.06, 0.05, 0.03 and 0.01 mAu for the Mott 25  $\mu$ L, 50  $\mu$ L, 100  $\mu$ L, 150  $\mu$ L and 300  $\mu$ L static mixers, respectively. Presented in Figure 8 is the same data as in Figure 7, but with additional data showing a comparison to commonly available competitive mixers on the market today.

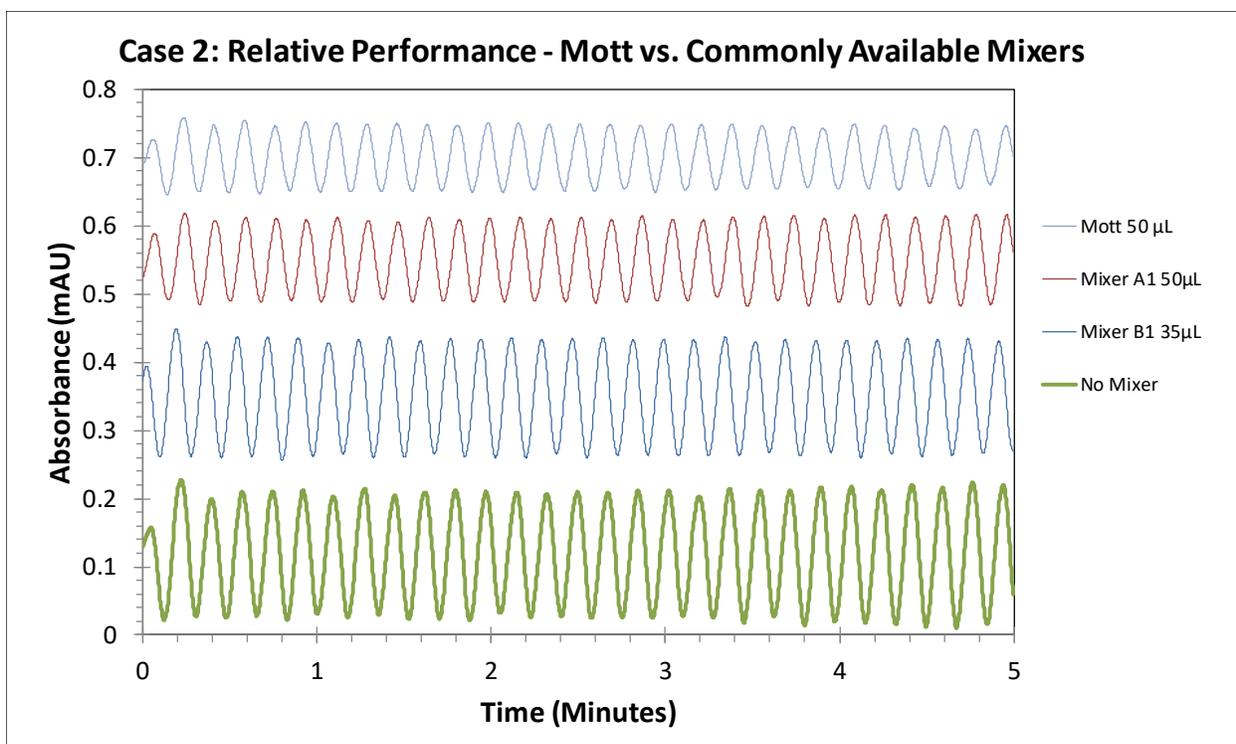


**Figure 7.** Plots showing offset HPLC UV detector signal versus time for Case 1 (Acetonitrile with ammonium acetate tracer) showing solvent mixing with no mixer, and Mott 25  $\mu$ L, 50  $\mu$ L, 100  $\mu$ L, 150  $\mu$ L, and 300  $\mu$ L mixers installed showing improved mixing (smaller signal amplitudes) as the volume of the static mixer is increased.



**Figure 8.** Plots showing offset HPLC UV detector signal versus time for Case 1 (Acetonitrile with ammonium acetate as a tracer) showing solvent mixing with no static mixer, new line of Mott static mixers and three commonly available mixers.

The percentage reduction in baseline sine wave was computed by taking the ratio of the sinusoid amplitudes to the amplitude with no mixer installed. Presented in Table II are the measured percentage sinusoid reduction, for Case 1 and 2, and internal volumes for the new static mixers along with several standard mixers commonly used in the industry. The data in Figures 8 and 9, and the calculated results presented in Table II, show that the Mott static mixers achieve greater than 95% reduction baseline sine wave, significantly outperforming commonly available mixers in use for the HPLC industry under these test conditions. Nine commonly available mixers in the industry were also evaluated. These included three mixers of different internal volumes from each of Company A (labeled Mixer A1, A2 and A3) and Company B (labeled Mixer B1, B2 and B3) and Company C (labeled C1, C2, and C3).



**Figure 9.** Plots showing offset HPLC UV detector signal versus time for Case 2 (Methanol with acetone as a tracer) showing solvent mixing with no static mixer (union), new line of Mott static mixers and two commonly available mixers.

Table II Static Mixer Mixing performance and Internal Volumes		
Static Mixer	Case 1: Sinusoid Reduction: Acetonitrile testing (Efficiency)	Case 2: Sinusoid Reduction: Methanol Water test (Efficiency)
Mott 25 µL	27.3%	21.3%
Mott 50 µL	56.0%	47.3%
Mott 100 µL	71.3%	70.4%
Mott 150 µL	78.4%	79.9%
Mott 300 µL	94.5%	95.9%
Mixer A1 (50 µL)	48.0%	49.7%
Mixer A2 (150 µL)	68.8%	77.7%
Mixer A3 (250 µL)	84.0%	88.7%
Mixer B1 (35 µL)	31.4%	27.9%
Mixer B2 (100 µL)	57.8%	67.6%
Mixer B3 (370 µL)	87.1%	87.4%
Mixer C1 (100 µL)	34.0%	25.7%
Mixer C2 (250 µL)	83.1%	79.1%
Mixer C3 (380 µL)	93.0%	86.5%

Examination of the results in Figure 8 and Table II show that the Mott 50  $\mu\text{L}$  static mixer has a similar mixing efficiency to the Mixer B2 100  $\mu\text{L}$  for the Case 1 study, noting that the Mott 50  $\mu\text{L}$  has  $\frac{1}{2}$  the internal volume while achieving similar performance. When the Mott 50  $\mu\text{L}$  mixer was compared to the Mixer A1 50  $\mu\text{L}$ , a significant improvement in mixing efficiency is observed 56% versus 48%. The performance of the Mott 100  $\mu\text{L}$  mixer compared to the Mixer A2 150  $\mu\text{L}$ , shows a higher ripple reduction efficiency with a value of 71.3% versus 68.8% again achieving improved performance at a lower internal volume. Examination of the Mott 300  $\mu\text{L}$  compared to the A3 250  $\mu\text{L}$  mixer clearly shows significantly greater mixing efficiency, 94.5% versus 84%, with only a marginal increase in internal volume. Similar results and comparisons can be observed with Mixers B and C. Thus, the new line of Mott PerfectPeak<sup>®</sup> static mixers achieve improved mixing efficiencies over comparable competitors' mixers, but with smaller internal volumes, thereby providing improved background noise, better signal to noise ratios, better analyte sensitivity, peak shapes, and peak resolution. Similar trends in the mixing efficiency were observed in both Case 1 and Case 2 studies.

For the Case 2 study (Figure 9) using (Methanol with acetone as a tracer) testing was performed to compare the mixing efficiencies of the Mott 50  $\mu\text{L}$ , the comparable Mixer A1 (also with a 50  $\mu\text{L}$  internal volume) and comparable Mixer B1 (with a lower 35  $\mu\text{L}$  internal volume). As expected the performance when no mixer installed was poor but is used for a baseline of analysis. The Mott 50  $\mu\text{L}$  showed comparable mixer performance to the A1 mixer with significant improvement over the smaller volume B1 mixers. The ripple reduction efficiency for the Mott 50  $\mu\text{L}$  mixer was 47.3%, for the A1 50  $\mu\text{L}$  mixer, the efficiency was 49.7% and for the B1 35  $\mu\text{L}$  mixer, the mixing efficiency was lower being 27.9%.

### **Mixer Back Pressure versus Flow Rate**

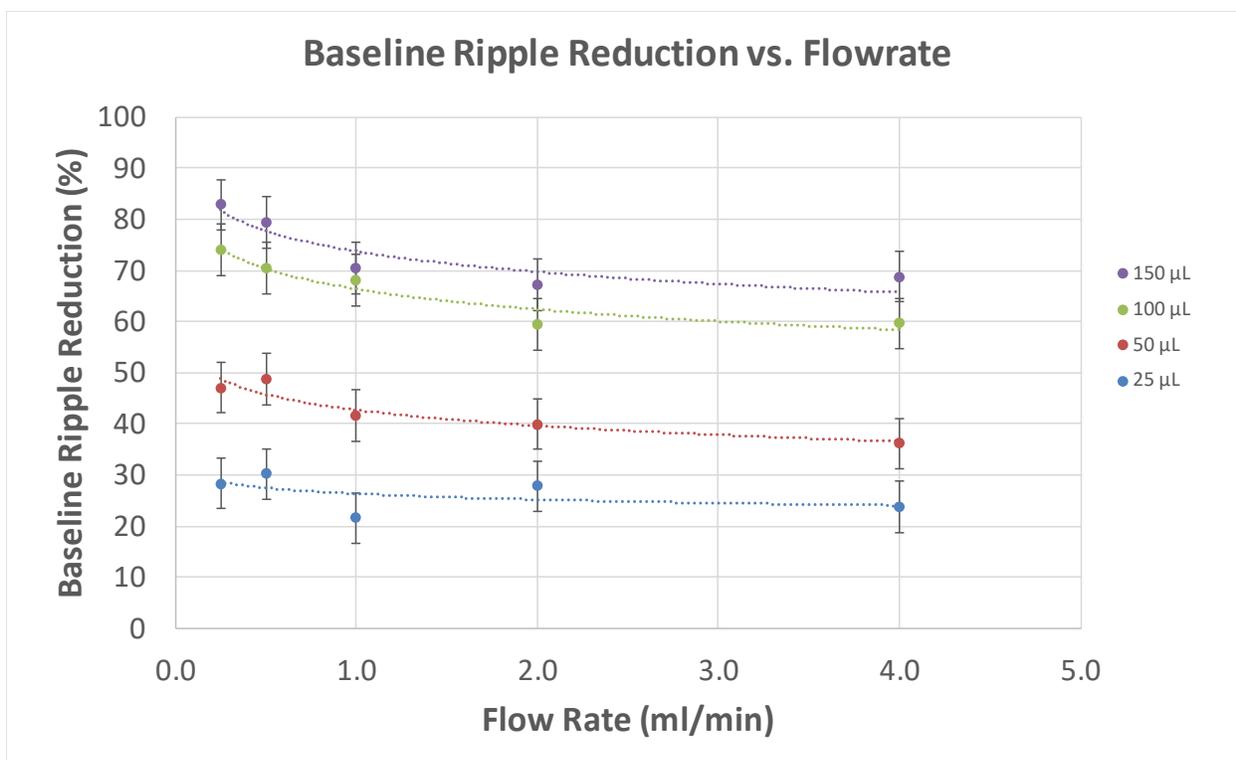
To investigate the differences in back pressure created by the new line of Mott mixers versus the competitor mixers previously studied, an experiment was performed by installing an external pressure sensor immediately upstream of the mixer with no fluid lines connected to the mixer outlet and the pressure was recorded versus flow rate. The solution used for this test was an isocratic mix of 50% isopropyl alcohol in DI water. Presented in Table III are the results of this study. Here, it is observed in all cases, the back pressure increases with flow rate as expected. The Mott line of mixers in nearly all cases create the lowest back pressures of all mixers tested. The one exception is Mixer A3 250  $\mu\text{L}$  mixer which measured nearly the same back pressures as Mott's 300  $\mu\text{L}$  mixer for all flow rates tested. A big advantage of the Mott design having significantly lower back pressures is being able to run higher viscosity mobile

phases at higher flow rates and continue to maintain the lowest overall system pressure possible. If the back pressure is compared at 4 ml/min for the Mott 300  $\mu\text{L}$  versus the Mixer C2 250  $\mu\text{L}$  mixer in this test, the back pressure for the Mott mixer is nearly 100 times lower.

Mixer type and volume	Flowrate (mL/min)				
	0.25	0.5	1	2	4
Mott 25 $\mu\text{L}$	0.01 psi	0.02 psi	0.03 psi	0.08 psi	0.24 psi
Mott 50 $\mu\text{L}$	0.01 psi	0.02 psi	0.04 psi	0.12 psi	0.31 psi
Mott 100 $\mu\text{L}$	0.02 psi	0.02 psi	0.05 psi	0.16 psi	0.44 psi
Mott 150 $\mu\text{L}$	0.02 psi	0.03 psi	0.06 psi	0.18 psi	0.51 psi
Mott 300 $\mu\text{L}$	0.03 psi	0.04 psi	0.09 psi	0.30 psi	1.08 psi
Mixer A1 (50 $\mu\text{L}$ )	0.47 psi	0.91 psi	1.77 psi	3.51 psi	7.20 psi
Mixer A2 (150 $\mu\text{L}$ )	0.53 psi	0.82 psi	1.23 psi	2.24 psi	4.67 psi
Mixer A3 (250 $\mu\text{L}$ )	0.02 psi	0.06 psi	0.12 psi	0.25 psi	0.51 psi
Mixer B1 (35 $\mu\text{L}$ )	4.60 psi	8.55 psi	14.45 psi	31.25 psi	62.80 psi
Mixer B2 (100 $\mu\text{L}$ )	1.35 psi	2.60 psi	4.75 psi	10.60 psi	21.10 psi
Mixer B3 (380 $\mu\text{L}$ )	1.60 psi	3.015 psi	5.70 psi	12.15 psi	23.50 psi
Mixer C1 (100 $\mu\text{L}$ )	2.70 psi	4.93 psi	9.72 psi	17.45 psi	33.25 psi
Mixer C2 (250 $\mu\text{L}$ )	2.65 psi	5.46 psi	10.70 psi	21.50 psi	42.10 psi
Mixer C3 (380 $\mu\text{L}$ )	16.20 psi	30.5 psi	59.50 psi	115.5 psi	235.0 psi

### Mixing Efficiency versus Flow Rate

Baseline ripple reduction testing as a function of flowrate was conducted on the Mott mixers under the same conditions as the Case 1 tests previously described, changing only the flowrate. The system flow rates used for this study were 0.25, 0.5, 1, 2, and 4 ml/min. Presented in Figure 10 are the results of this study for all four, standard sized Mott PerfectPeak® static mixers. Over the flow rate range of 0.25 to 4 ml/min, the data shows a gradual increase at lower flow rates for all four mixer volumes. This increase in efficiency at lower flow rates is expected due to the increased residence time of the solvents within the mixer allowing for greater diffusional mixing.



**Figure 10.** Ripple reduction efficiency as a function of flowrate for Case 1 with flow rates from 0.25 through 4 ml/min.

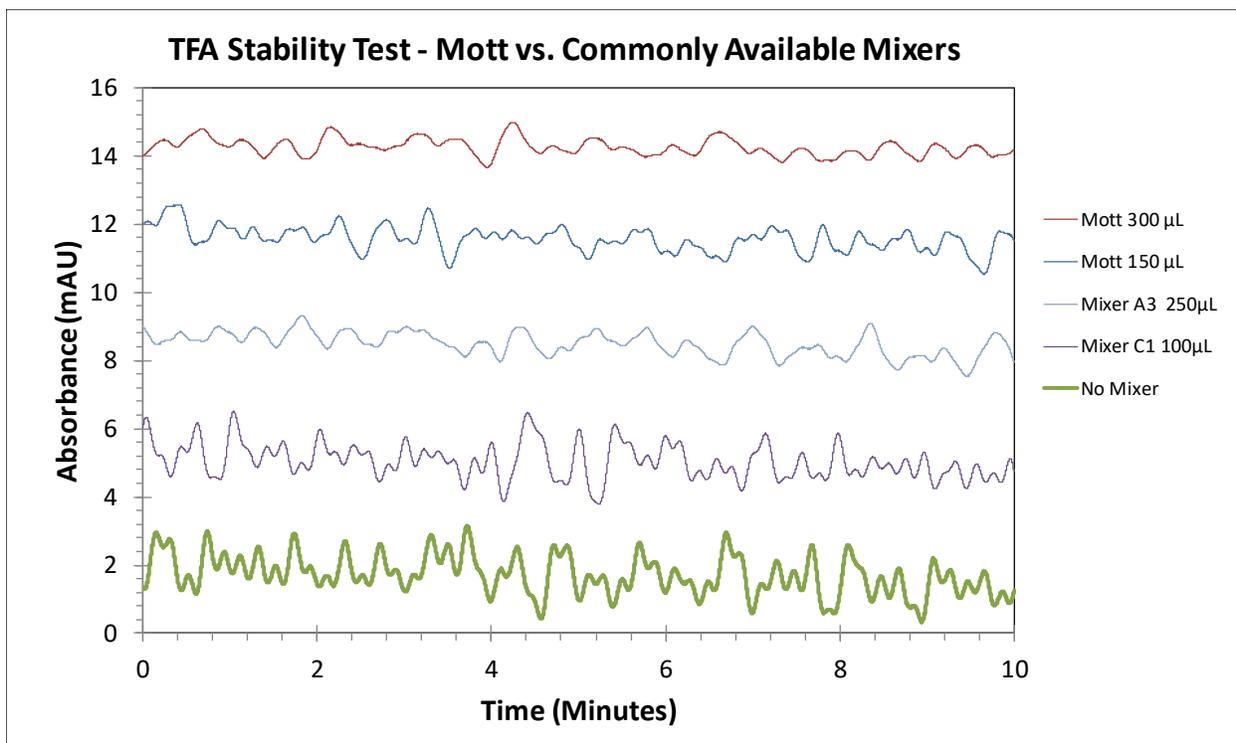
### TFA Noise Reduction Experiment

The Mott static mixer line was evaluated under conditions that push mixing efficiency to the limit. TFA is a volatile liquid that is unstable under certain conditions and commonly used as a mobile phase for HPLC and UHPLC in the pharmaceutical industry. Gradient mobile phases using TFA/water and TFA/ACN are difficult to mix and often require very large static mixers or a dynamic mixer to produce stable baselines (one mixer supplier recommends a 1.0 – 1.5ml mixer be employed). The designed test is tailored to evaluate larger volume mixers (180, 270 and 360 µL) in order to achieve the mixing efficiency needed for a stable baseline.

HPLC testing using (TFA/water: TFA/Acetonitrile) in gradient conditions is often very difficult to perform due to excessive noise present as a result of poor mixing when using these types of mobile phases. To evaluate mixer performance simulating TFA conditions, a method was developed utilizing constant mixing conditions to evaluate the various mixers. Mobile phase A was prepared with 0.1% TFA in HPLC grade water and mobile phase B was prepared with 0.1% TFA in acetonitrile. The HPLC system used for this

analysis was an Agilent 1260 with a binary pump module. The pumps were set to flow at 1 mL/min with a 95% mobile phase A and 5% mobile phase B mixing ratio. Once the static mixer to be tested was installed and stabilized, the signal was recorded for 10 minutes. Pump conditions and solution compressibility factors were set to automatic for this testing. The column was a Waters Symmetry® C18, 5  $\mu$ m, 3.9 x 150 mm, heated to 30°C. The detector, an Agilent 1100 WVD G1314A, was set at 210 nm wavelength for analysis.

### TFA Data and Results

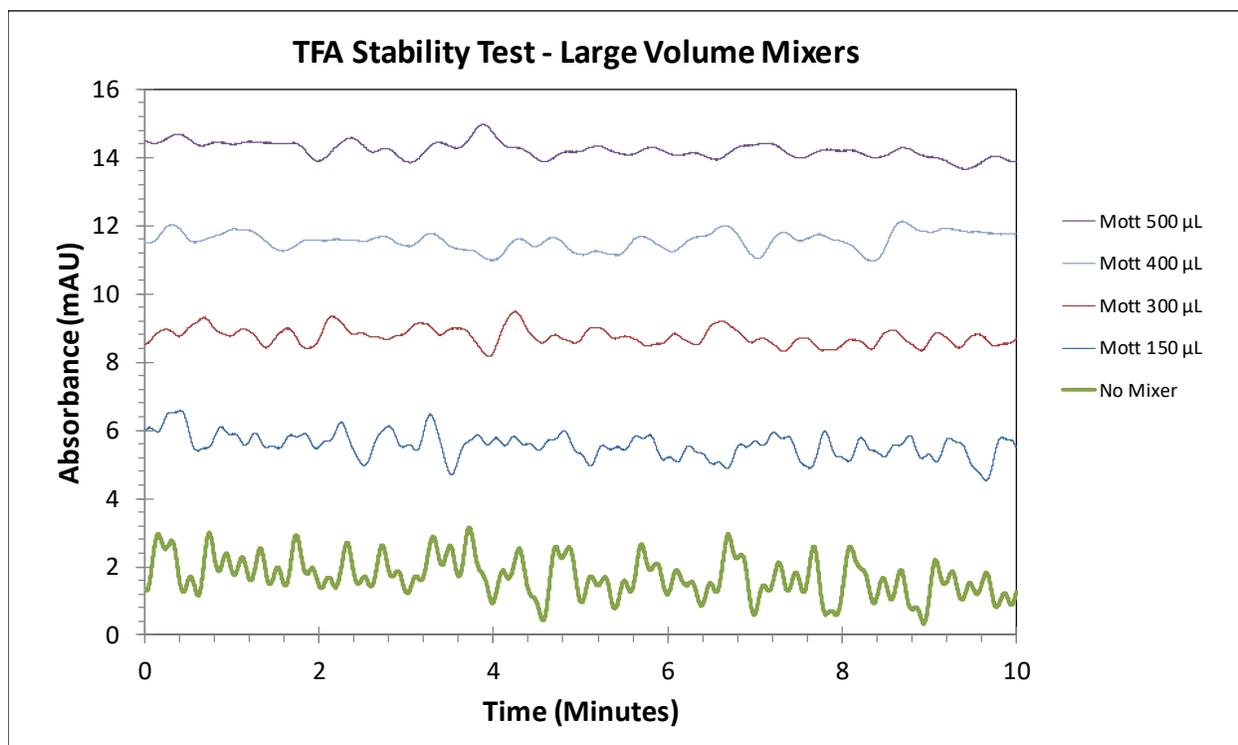


**Figure 11.** TFA Stability test data. The given test conditions are as follows: Mobile phase A: 0.1% TFA in DI water, Mobile phase B: 0.1% TFA in acetonitrile, A:B ratio of 95%:5% Flowrate: 1 mL/min, Column: Symmetry® C18, 5  $\mu$ m, 3.9 x 155 mm at 30°C, Detector: 210 nm.

Presented in Figure 11 are the results comparing Mott’s 150  $\mu$ L and 300  $\mu$ L prototype volume mixers to industry standard Mixer A3 250  $\mu$ L and Mixer C1 100  $\mu$ L mixers along with no mixer installed. It is visually evident that the Mixer B1 100  $\mu$ L mixer did not perform as well as the other mixers showing little to no improvement to when no mixer was installed. Mott 150  $\mu$ L mixer performs very similarly to the Mixer A3 250  $\mu$ L mixer doing so with about 100  $\mu$ L less internal volume. The Mott 300  $\mu$ L mixer provided the lowest

and smoothest noise amplitude outperforming the Mixer A3 250  $\mu\text{L}$  mixer with only a minimal increase in internal volume.

One of the benefits of the Mott PerfectPeak<sup>®</sup> line of static mixers is their modularity giving you the ability to achieve different volume combinations. The stackability of the mixers allows for further noise reduction. Figure 12 shows the baseline stability when stacking our 100  $\mu\text{L}$  and 150  $\mu\text{L}$  mixers in series to larger internal mixing volumes of 300  $\mu\text{L}$ , 400  $\mu\text{L}$ , and 500  $\mu\text{L}$ . The data in Figure 12 clearly shows a progression of improved performance as the internal volume increases. The 150  $\mu\text{L}$  Mott mixer shows an average reduction in amplitude of about 30% and the Mott 300  $\mu\text{L}$  mixer shows a reduction in amplitude of about 50%. Examination of the larger volume mixers (400  $\mu\text{L}$  and 500  $\mu\text{L}$ ) shows further reductions in noise amplitude with smoother sinusoid waves along with a lower frequency (number of background ripple humps).

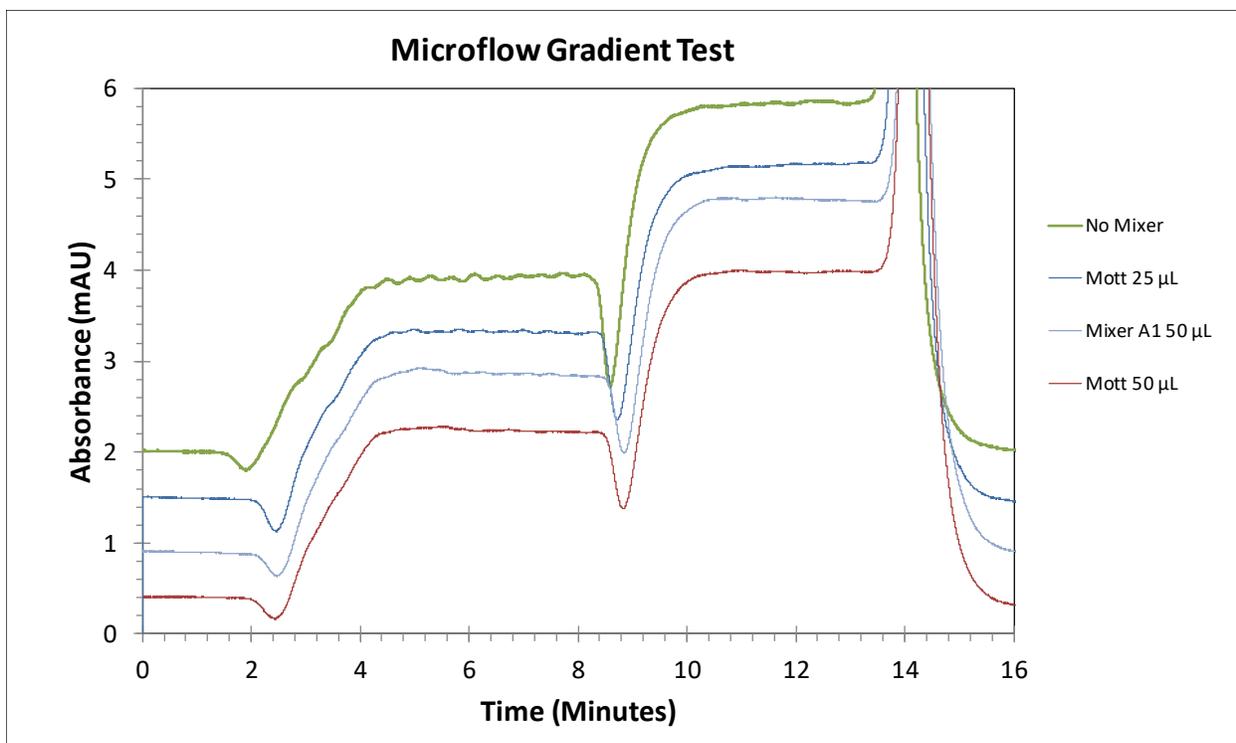


**Figure 12.** TFA gradient test data. Same test method as stated in Figure 11. Mott 100  $\mu\text{L}$  and 150  $\mu\text{L}$  mixers tested in series to achieve higher internal volume mixing.

## Microflow Gradient Test

A microflow gradient test was performed to mimic low flow, low throughput applications where dead volume can be critical. The same Agilent 1260 system was used for this test. Mobile phase A was HPLC grade water, while mobile phase B was acetonitrile containing 0.01% acetone. The pump flow was set at 0.25 mL/min and the gradient test run following the step program listed in Table IV. A 2,000 psi pressure resistor was used in place of a column downstream of the mixer, and the UV detector was set to 265 nm.

Time	%B	Time	%B
0	0%B	7.5	20%B
2.5	10%B	12.4	2-%B
7.4	10%B	12.5	0%B



**Figure 13.** Microflow gradient test performed comparing a competitor low volume mixers to Mott's 25 µL and 50 µL static mixers

Presented in Figure 13 are gradient profiles for this test with no mixer installed, Mott's 25  $\mu\text{L}$  and 50  $\mu\text{L}$  mixers, and the competitor's Mixer A1 50  $\mu\text{L}$ . When the traces are examined for the Mott 50  $\mu\text{L}$  and Mixer A1 50  $\mu\text{L}$  mixers, it can be seen they lag in time behind the others with no difference in their dwell times (dip in traces near 9 minutes) indicating they have the same internal volumes. The Mott 25  $\mu\text{L}$  mixer has a shorter dwell time than both 50  $\mu\text{L}$  mixers and is roughly midway between both 50  $\mu\text{L}$  mixers and the scan with no mixer installed. Less baseline ripple is also observed when mixers are installed with the Mott 25  $\mu\text{L}$  and competitor's Mixer A1 50  $\mu\text{L}$  showing similar reductions in baseline ripple. The Mott 50  $\mu\text{L}$  mixer shows the lowest ripple amplitude of the mixers tested.

## Summary

The recently developed line of patent-pending Mott PerfectPeak® inline static mixers with five internal volumes, 25  $\mu\text{L}$ , 50  $\mu\text{L}$ , 100  $\mu\text{L}$ , 150  $\mu\text{L}$ , and prototype 300  $\mu\text{L}$  cover the range in volumes and mixing performance needed for a majority of HPLC analyses, including difficult to mix gradients using TFA as an additive, where enhanced mixing with low dispersion is required. The new static mixer accomplishes this goal via use of a novel 3D printing technology to create a unique structure that achieves improved hydrodynamic static mixing with the highest percentage reduction in baseline noise per unit of internal mixture volume. Greater than 95% reduction in baseline noise was achieved using up to 1/2 the internal volume of some commonly available mixers. This mixer consists of parallel and interconnected three-dimensional flow passageways that have varying cross-sectional areas and path lengths as the fluid transverses through and across internal complex geometric obstacles. The new line of static mixers achieves improved performance over comparable competitors' mixers, but with smaller internal volumes and lower back pressures. This provides increased sensitivity through better signal to noise ratios and lower limits of quantitation with improved peak shape, efficiency, and resolution even for difficult to mix gradients employing TFA as an additive.



**Figure 14.** 150  $\mu$ L, 100  $\mu$ L, 50  $\mu$ L and 25  $\mu$ L mixers manufactured by Mott Corporation,



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