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

By

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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 and ultra-high performance liquid chromatography (HPLC and UHPLC) systems. Poor mixing of two or more mobile phases results in higher signal to noise ratio and, thus, decreased sensitivity. The homogenous static mixing of two or more fluids, while utilizing the minimal internal volume and physical size of the 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 99% reduction in baseline sine wave was achieved using 1/3 the internal volume of 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 TFA and water/acetonitrile gradients.

Introduction

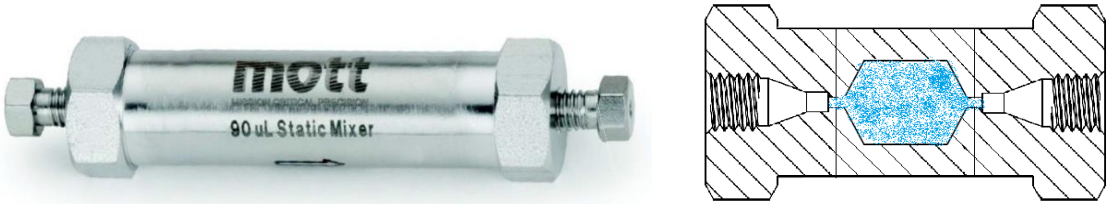
Liquid chromatography has been the work horse instrument for many industries such as pharmaceuticals, pesticides, environmental, forensics, and chemical analysis for 30+ years. The ability to measure down to the part per million (ppm) levels and lower is crucial to the development of technologies for each industry. 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 complete mixing of the solvents is not performed, degradation of the HPLC chromatogram may occur as observed by excessive baseline noise and/or poor peak shapes. 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, peak shape and peak resolution. The industry has recognized that inline and tee type static mixers are a means to improve on these limitations and allow the user to achieve lower limits of detection (sensitivity). 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. Furthermore, as analyses become more challenging, analysts are having to use more polar and difficult to mix solvents on a regular basis. This means that better mixing is a necessity for future testing, thereby further driving the need for superior mixer designs and performance.

Mott Static Mixer

Mott recently developed a new line of patent-pending PerfectPeak™ in-line static mixers with four different internal volumes: 30 µL, 60 µL, 90 µL and a prototype 180 µL. 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 four models are 0.5 inches in diameter and have corresponding lengths of 1.4, 1.7, 2.1, and 3.0 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 90 µL 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 3D printing technology to create a unique structure that achieves homogenous mixing, while using less internal flow volume than any mixer with comparable baseline noise

reduction currently used in the chromatography industry. 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 create 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.



(a) (b)

Figure 1. *Photograph of a Mott 90 µL 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 static mixer and standard tubing (to simulate no mixer) was performed using COMSOL Multiphysics package. Modeling was performed using pressure-driven laminar flow fluid mechanics to understand the fluid velocity and pressure within the part. These fluid mechanics were 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 10 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 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. These solvents were then subsequently mixed in the static mixer.

Modeling simulations for flow through a standard tubing (to simulate no mixer) and the Mott static mixer are shown in Figures 2 and 3, respectively. 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, shown in Figure 2, without the presence of a static mixer. The exact tube and mixer design dimensions and a flow rate of 0.3 ml/min were used in the simulations. Figure 3 shows the CFD mixing simulation for the 30 µL mixer.

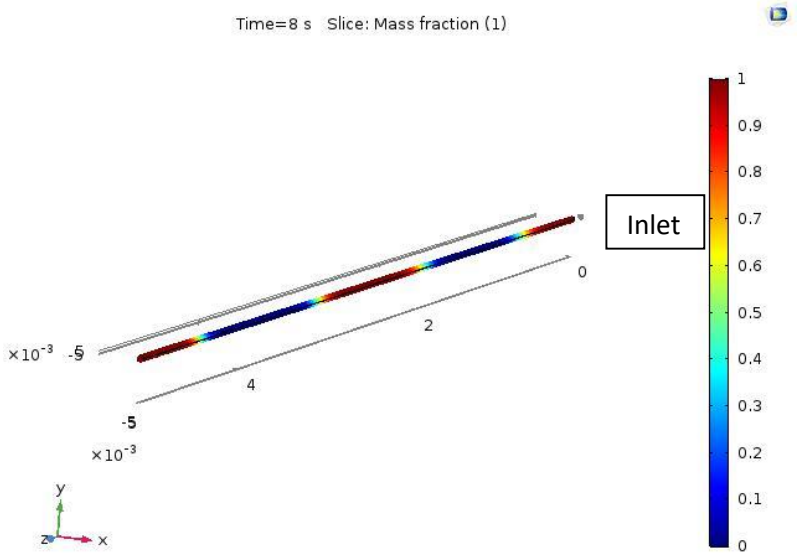


Figure 2. *Shows CFD modeling on flow in a 5 cm long by 0.25 mm ID tube to represent what is happening in the HPLC tubing, i.e., if no mixer is in place. The full red represents water as a mass fraction. The blue represents the lack of water, which is pure acetonitrile. A diffusion region can be seen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

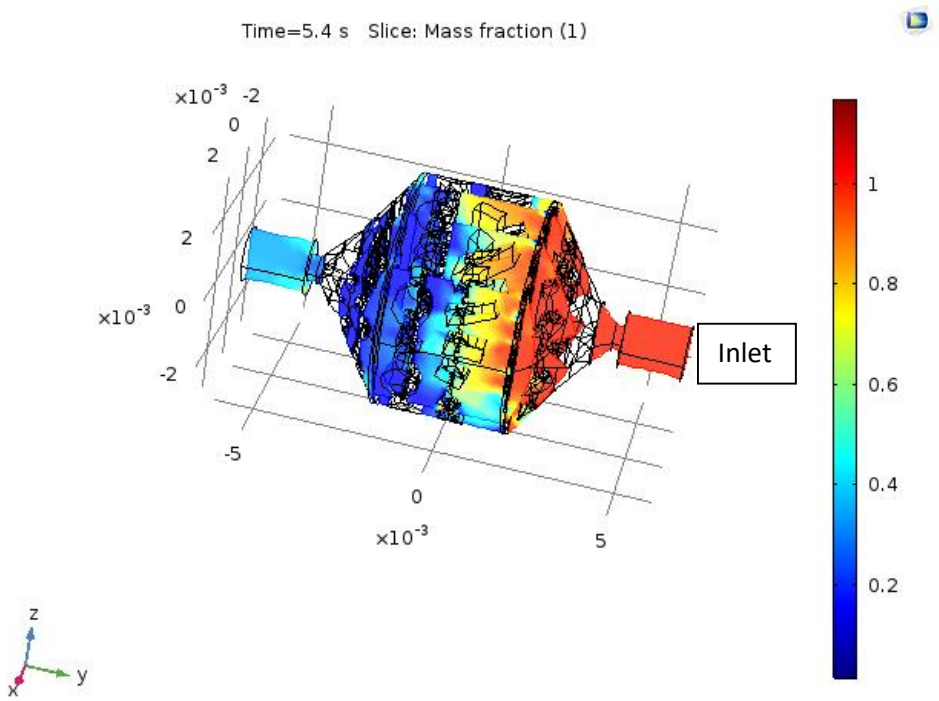


Figure 3. *The 30 µL static mixer modeled in COMSOL CFD software package. The legend represents the mass fraction of water within the mixer. Pure water is represented by red while pure acetonitrile is represented by blue. As the two fluids mix the color changes, to simulate the changing mass fraction of water.*

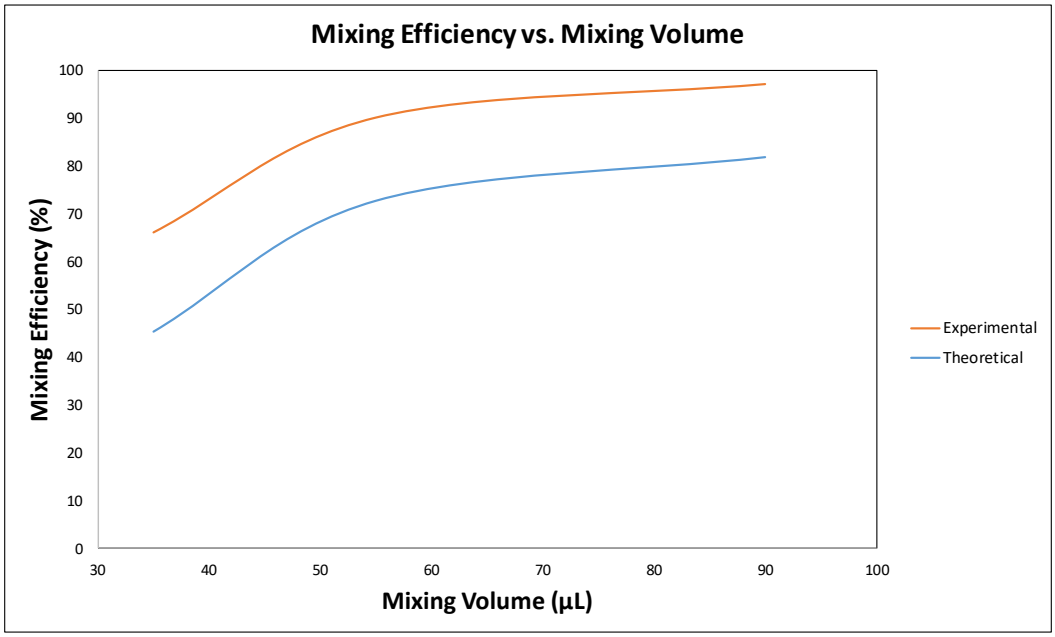


Figure 4. *Graph of mixing efficiency versus mixing volume of the static mixer series. The theoretical mixing follows the same trend of experimental mixing data validating the CFD modeling.*

Figure 4 is a validation study of the model relating mixing efficiency to mixing volume. As the mixing volume increases the mixing efficiency will increase. It is understood by the authors that there are other complex physical forces acting within the mixer that were unable to be captured in this CFD model, thereby resulting in greater mixing efficiency when the experimental testing was performed. The experimental mixing efficiency was measured as a percentage reduction in baseline sine wave. Furthermore, increased back pressure generally results in a higher level of mixing, something the modeling also does not consider.

Experimental Procedure

The following HPLC conditions and test setup were used to measure baseline sine wave to compare the relative performance for 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 separation 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.

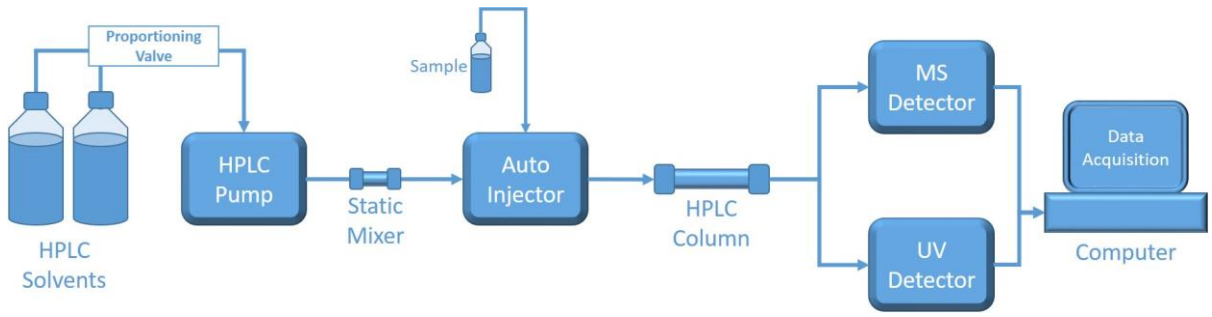


Figure 5. *Schematic diagram of the low pressure gradient experimental test system.*

The HPLC system utilized for this testing was an Agilent 1100 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 Gradient Mixing Case Studies

Experimental tests were conducted for two different solvent case studies. The two solvents mixed in Case 1 were Solvent A (20 Millimolar solution of Ammonium Acetate in 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. Solvent B was an 80/20% mixture of methanol and DI water. The pump was set to ramp from 0.25 ml/min to 1.0 ml/min in Case 1 and to a constant flowrate 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

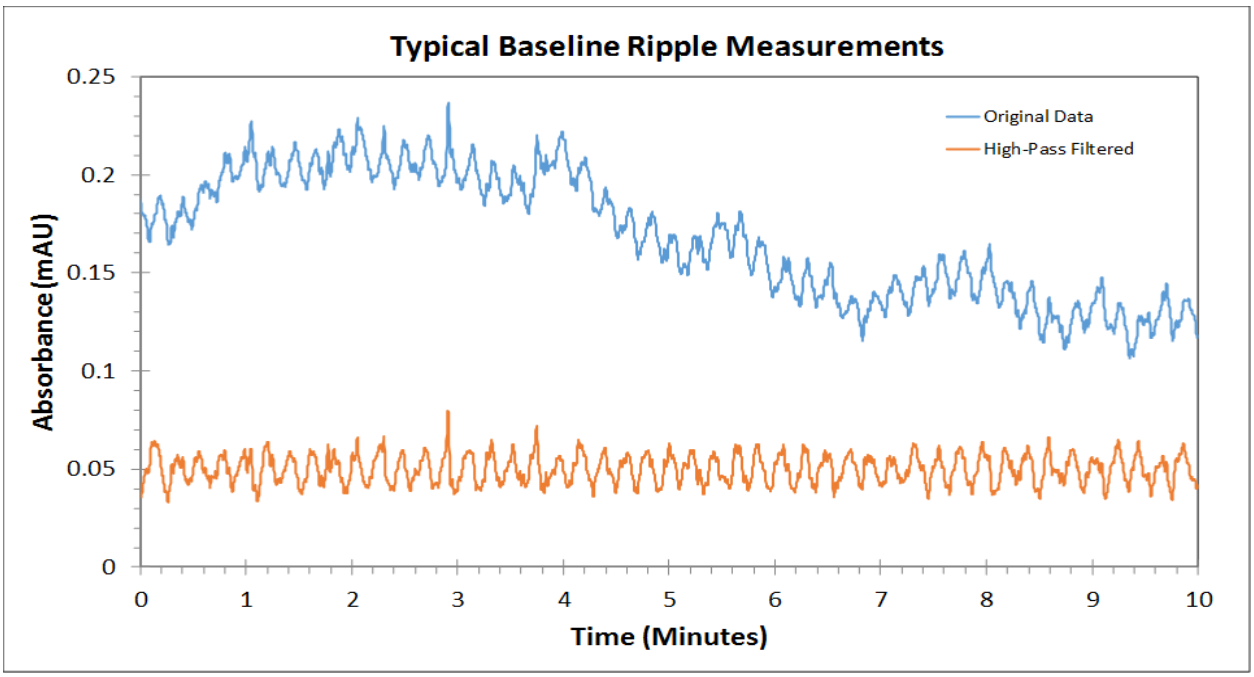


Figure 6. *Plots of measured mixing sinusoid before and after a high-pass filter was applied to remove the baseline drift component of the signal.*

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.

HPLC Gradient Mixing Case Studies Test Results

Upon completion of CFD modeling and initial experimental testing, four separate static mixers were subsequently developed utilizing the internal structures noted above with four internal volumes, 30 µL, 60 µL, 90 µL, and 180 µL. 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 the three 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 the 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 0.221 mAu with the amplitude dropping to 0.077, 0.017, 0.004, and 0.002 mAu for the Mott 30 µL, 60 µL, 90 µL, and 180 µL static mixers, respectively.

Presented in Figure 7 is the relative performance of Mott static mixers compared to no mixer. The data presented in Figure 8 is the same data as in Figure 7, but with comparison to commonly available competitive mixers on the market today.

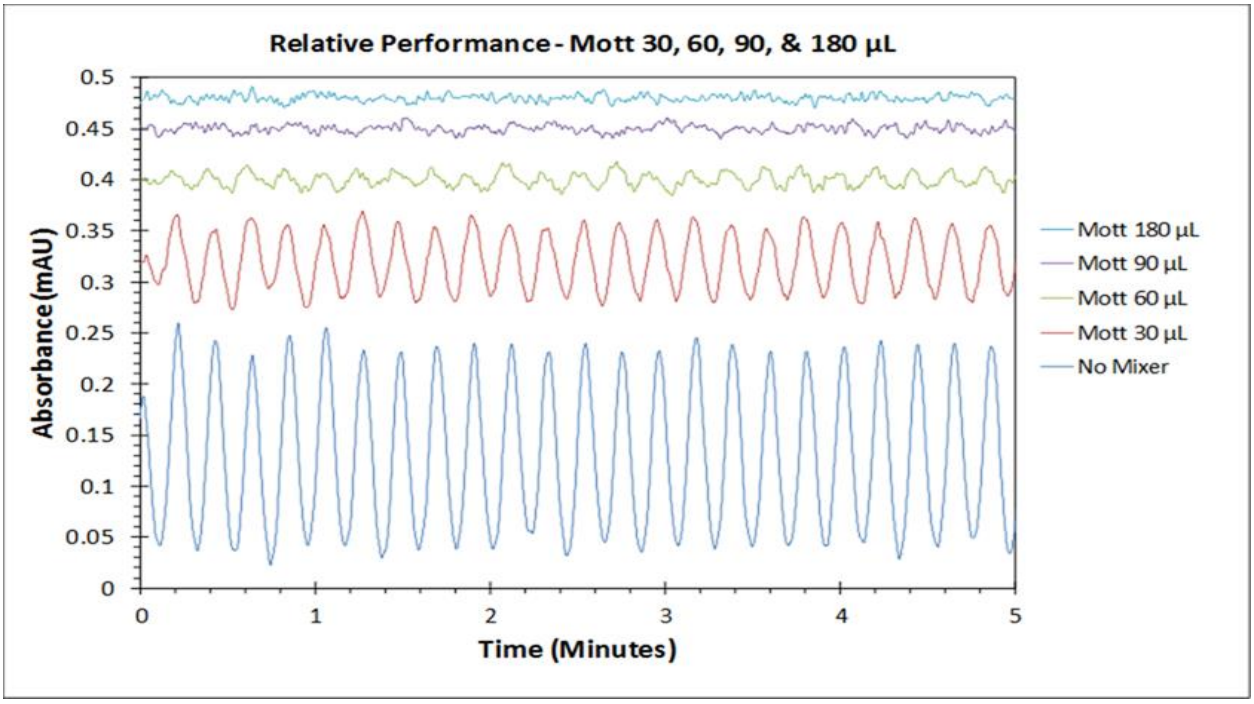


Figure 7. *Plots showing offset HPLC UV detector signal versus time for Case 1 (Acetonitrile with ammonium acetate tracer) showing solvent mixing with no mixer, and Mott 30 µL, 60 µL, 90 µL and 180 µL mixers installed showing improved mixing (smaller signal amplitudes) as the volume of the static mixer is increased.*

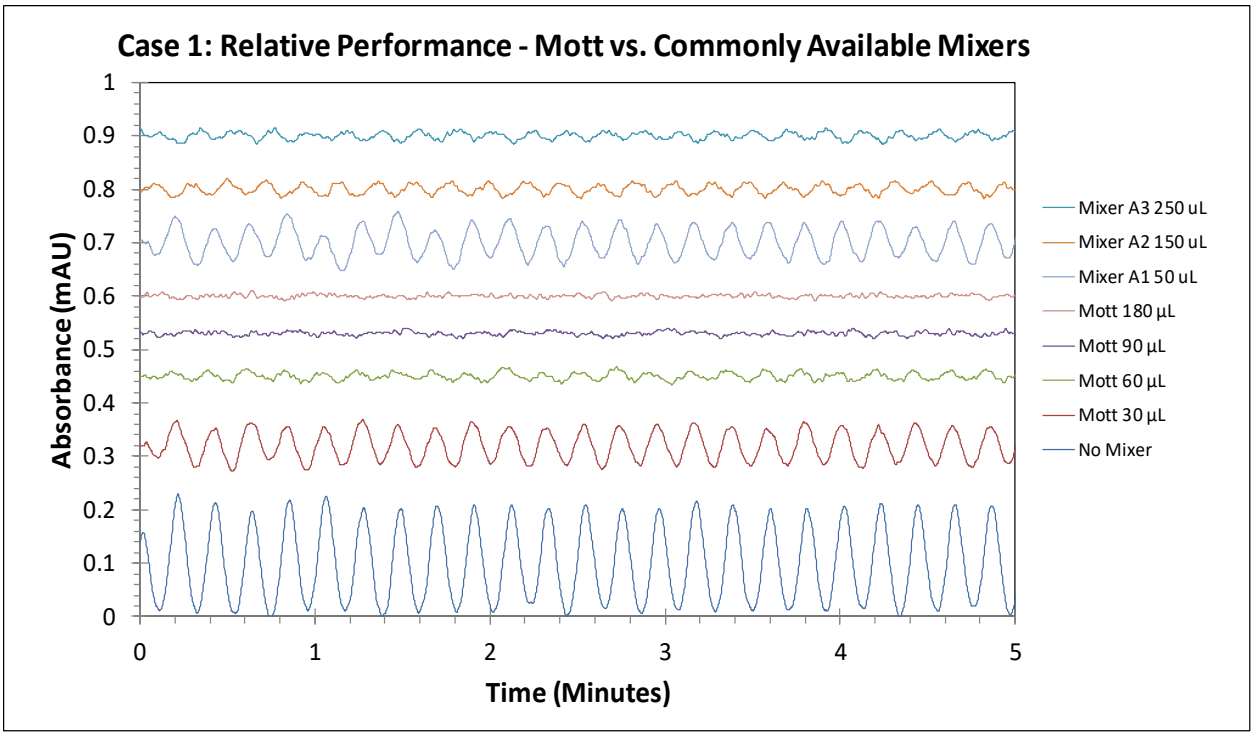


Figure 8. *Plots showing offset HPLC UV detector signal versus time for Case 1 (Acetonitrile with ammonium acetate as a tracer) showing solvent mixing with no static mixer, new line of Mott static mixers and three commonly available mixers.*

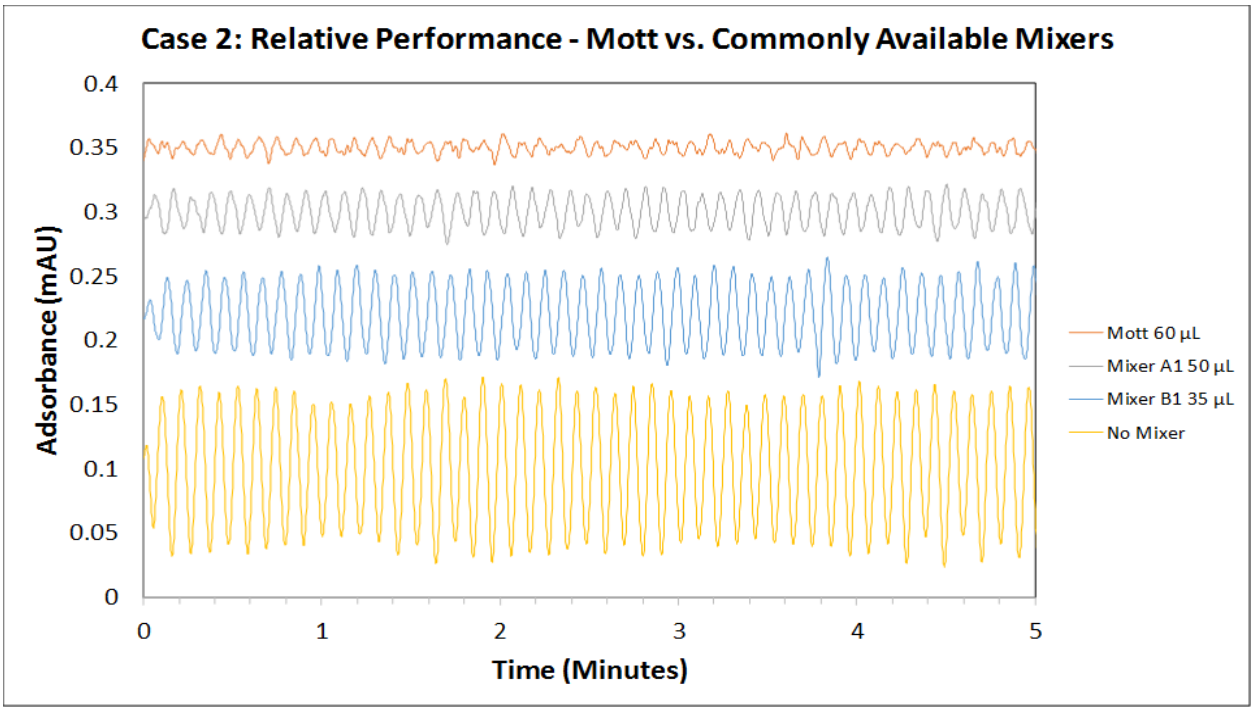


Figure 9. *Plots showing offset HPLC UV detector signal versus time for Case 2 (Methanol with acetone as a tracer) showing solvent mixing with no static mixer (union), new line of Mott static mixers and two commonly available mixers.*

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 seven 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 99% reduction baseline sine wave, significantly outperforming commonly available mixers in use for the HPLC industry under these test conditions.

Seven 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). Only one size was evaluated from Company C.

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)
No Mixer	-	-
Mott 30 µL	65%	67.2%
Mott 60 µL	92.2%	91.3%
Mott 90 µL	98.1%	97.5%
Mott 180 µL	99.4%	-
Mixer A1 (50 µL)	66.4%	73.7%
Mixer A2 (150 µL)	89.8%	91.6%
Mixer A3 (250 µL)	92.2%	94.5%
Mixer B1 (35 µL)	44.8%	45.7%
Mixer B2 (100 µL)	93.2%	84.5%
Mixer B3 (370 µL)	96.9%	96.2%
Mixer C (250 µL)	97.2%	97.4%

Table III shows the backpressure of each static mixer at a given flowrate along with a range of competitive mixers. This test was done using a 50/50 mixture of acetonitrile and water. The column employed was a ES Industries Chromegabond WR C18 10 µ, 120 Å, 10 cm X 4.6 mm. The pump functionality is dependent on pressure and that is why it is critical to control backpressure in your system when adding new components. Every user should monitor backpressure and its repeatability as part of method validation. The different sizes of Mott Mixers prove repeatability throughout a wide flow range.

Table III. Static Mixer Flowrate vs. backpressure

Mixer type and volume	Flowrate (mL/min)				
	0.25	0.5	1	2	4
No Mixer	8 bar	22 bar	47 bar	95 bar	190 bar
Mott 30 µL	7 bar	20 bar	45 bar	93 bar	190 bar
Mott 60 µL	8 bar	21 bar	46 bar	94 bar	191 bar
Mott 90 µL	8 bar	21 bar	47 bar	95 bar	191 bar
Mott 180 µL	8 bar	22 bar	48 bar	97 bar	195 bar
Mixer A1 (50 µL)	7 bar	18 bar	43 bar	95 bar	213 bar
Mixer A2 (150 µL)	6 bar	18 bar	41 bar	88 bar	178 bar
Mixer A3 (250 µL)	6 bar	18 bar	41 bar	88 bar	185 bar
Mixer B1 (35 µL)	7 bar	20 bar	44 bar	93 bar	190 bar
Mixer B2 (100 µL)	7 bar	19 bar	44 bar	93 bar	191 bar
Mixer B3 (370 µL)	7 bar	19 bar	43 bar	91 bar	190 bar
Mixer C (50 µL)	7 bar	19 bar	43 bar	92 bar	191 bar

Examination of the results in Figure 8 and Table II show that the Mott 30 µL static mixer has a similar mixing efficiency to the Mixer A1, with 50 µL; however, Mott 30 µL has a 30% smaller internal volume. When the Mott 60 µL mixer was compared to the Mixer A2, with 150 µL internal volume, a slight improvement in mixing efficiency is observed - 92% versus 89%, but more importantly, this higher level of mixing is performed with 1/3 the volume of the comparable Mixer A2. The performance of the Mott 90 µL and Mott 180 µL mixer compared to the Mixer A3, with 250 µL internal volume follows a similar trend. Improved mixing performance of 98% and 99% versus 92% is also observed along with an internal volume that is nearly 3 times smaller. Similar results and comparisons can be observed with Mixers B and C. Thus, the new line of Mott PerfectPeak™ static mixers achieves 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 using (Methanol with acetone as a tracer) testing was performed to compare the mixing efficiencies of the Mott 60 μL, the comparable Mixer A1 (with 50 μL internal volume) and comparable Mixer B1 (with 35 μL internal volume). As expected the performance when no mixer installed was poor but is used for a baseline of analysis. The Mott 60 μL mixer was the best performing mixer of the test group with a 90% increase in mixing efficiency. The comparable Mixer A1 mixer followed with a 75% increase in mixing efficiency followed by the comparable Mixer B1 with 45% improvement.

Baseline sine wave reduction testing as a function of flowrate was conducted on the mixer series under the same conditions as the Case 1 sinusoid tests, changing only the flowrate. Over the flow rate range of 0.25 to 1 ml/min, the data shows that the baseline sine wave reduction remains relatively consistent for all four mixer volumes. For the two smaller volume mixers, there is a small rise in sinusoid reduction with decreasing flow rate, which is expected due to the increased residence time of the solvents within the mixer allowing for greater diffusional mixing. It is anticipated that the sinusoid deduction will further increase as the flow rates are further reduced. However, for the largest mixer volume, which had the highest baseline sine wave reduction, the baseline sine wave reduction was basically unchanged (within the limits of experimental uncertainty) with values ranging from 95% to greater than 99%.

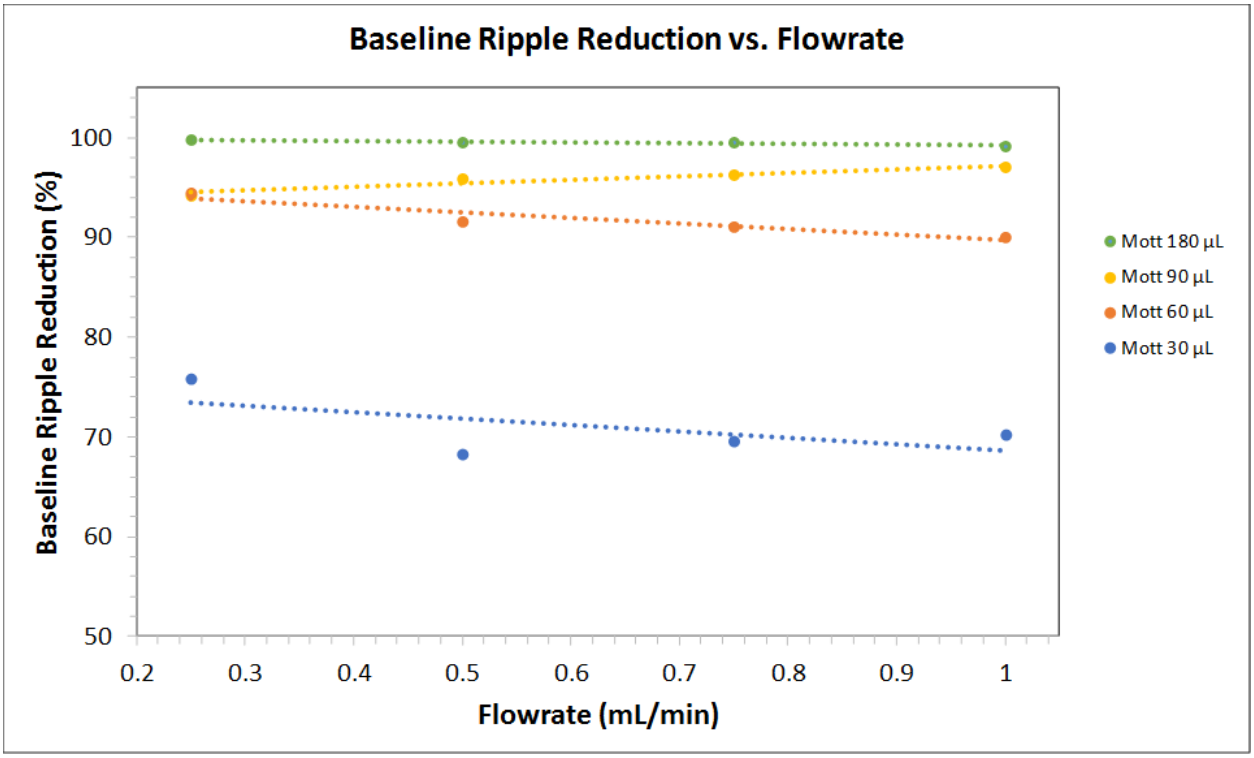


Figure 10. *Reduction in baseline sine wave as a function of flowrate for Case 1. This testing was performed using similar conditions as the sinusoid test, variable flowrate, introducing 80% of an 80/20 mixture of acetonitrile and water, and 20% 20 mM ammonium acetate.*

TFA Gradient Experiment

Testing was performed to evaluate performance of the mixer using hard to mix gradients using mobile phases such as TFA/water:TFA/Acetonitrile. Mobile phase A was prepared with 0.1% TFA in water. Mobile phase B was prepared with 0.1% TFA in acetonitrile. The pump was set to flow at 1 mL/min with a gradient from 5% to 40% B in 35 minutes. The column was a Waters Symmetry® C18, 5µm, 3.9 x 150 mm, heated to 35 °C. The detector, an Agilent 1100 WVD G1314A, was set at 214 nm wavelength for analysis.

TFA Data and Results

The Mott static mixer line was evaluated under conditions that push mixing efficiency to the limit. Trifluoroacetic acid (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.

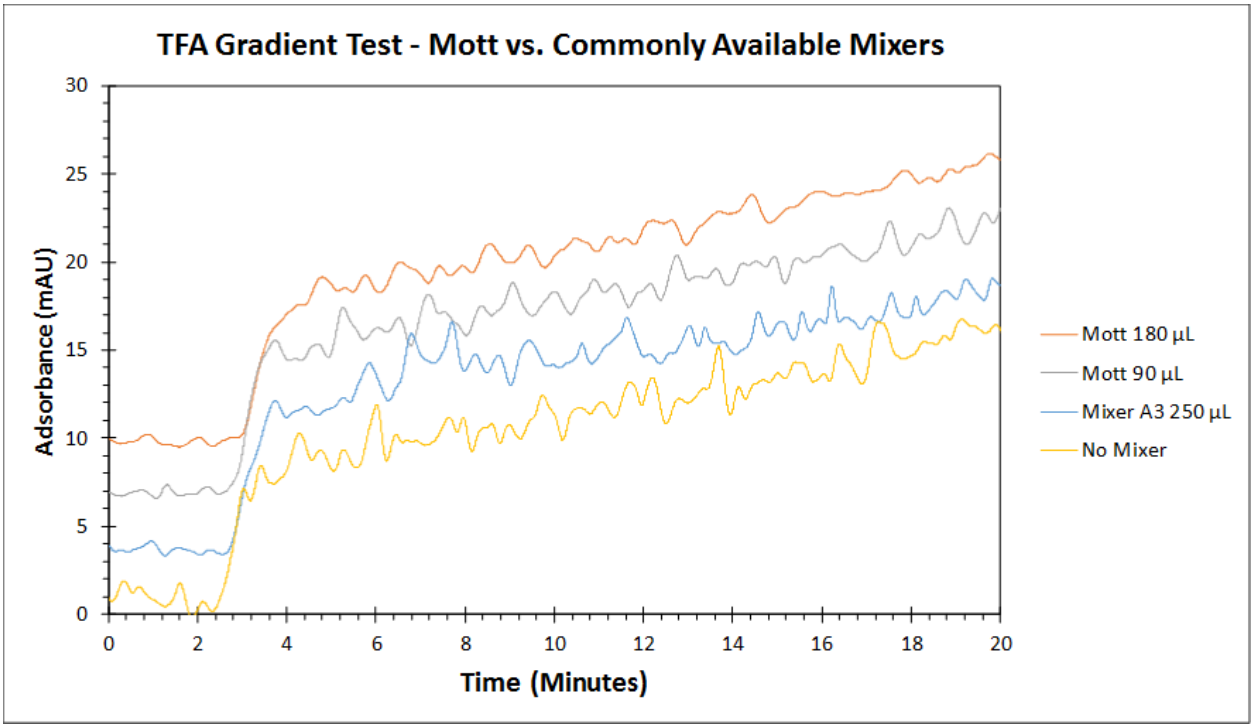


Figure 11. *TFA gradient test data. The given test conditions are as follows: Mobile phase A: 0.1% TFA in DI water, Mobile phase B: 0.1% TFA in acetonitrile, with a gradient 5- 40 % B in 35 minutes, Flowrate: 1 mL/min, Column: Symmetry® C18, 5 µm, 3.9 x 155 mm at 35°C, Detection: 214 nm.*

Presented in Figure 11 are the results comparing Motts larger prototype volume mixers to an industry standard 250 µL mixer. It is visually evident that the Mott mixers outperform the competition. The Mott 90 µL achieved a 25% improvement in baseline stability while the Mott 180 µL prototype mixer achieved greater than 50% improvement in baseline stability. The competitor mixer visually had no improvement in baseline stability. One of the benefits of the Mott static mixer is the modularity giving you the ability to achieve different volume combinations. The stackability of the mixer allows for further noise reduction. Figure 12 shows the baseline stability when stacking our 180 µL and 90 µL mixer for a total volume of 270 µL as well as 2, 180 µL mixers in series for a total volume of 360 µL. The increase in baseline over time is expected since the absorbance of TFA at 214 nm increases as concentration of acetonitrile increases.

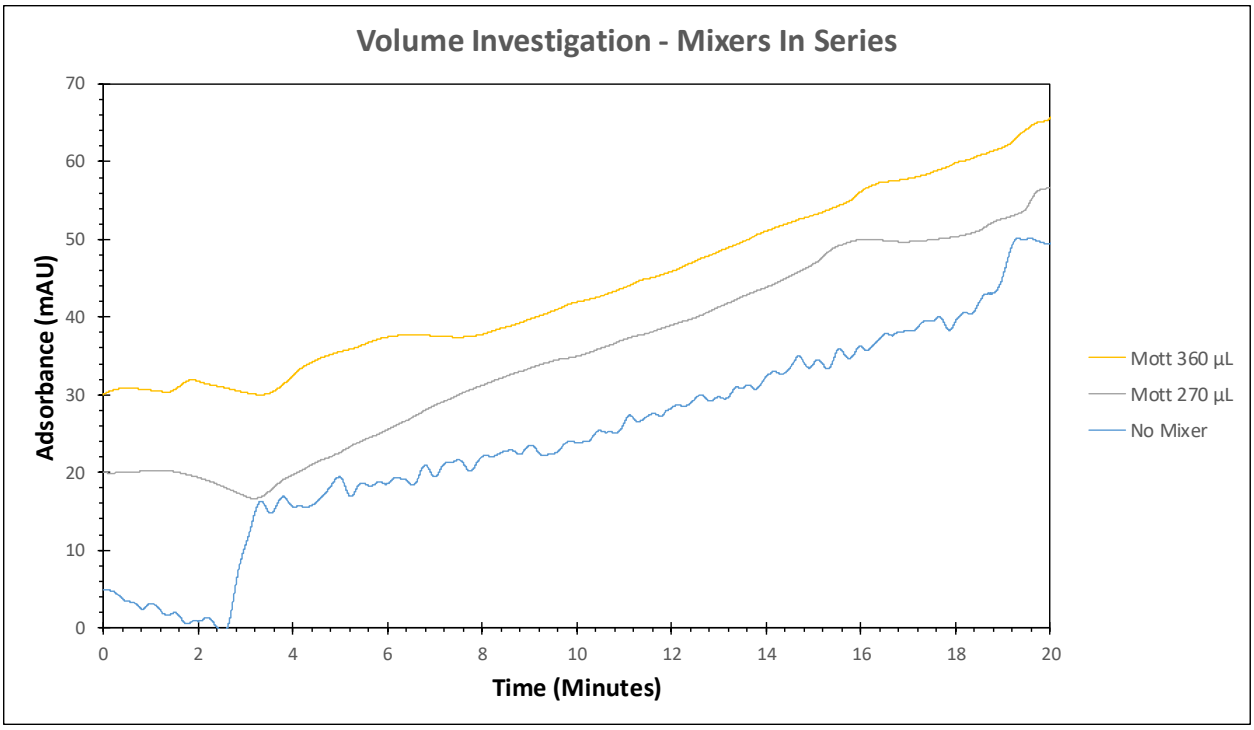


Figure 12. *TFA gradient test data. Same test method as stated in Figure 11. Mott mixers tested in series to achieve higher volume targets.*

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 1100 system was used for this test. Mobile phase A was water, while mobile phase B was acetonitrile containing 0.01% acetone. The pump flow was set at 0.25 mL/min with a gradient step program, listed in Table IIII. A 2,000 PSI pressure resistor was used in place of a column.

Table IIII. Microflow Gradient Formation

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

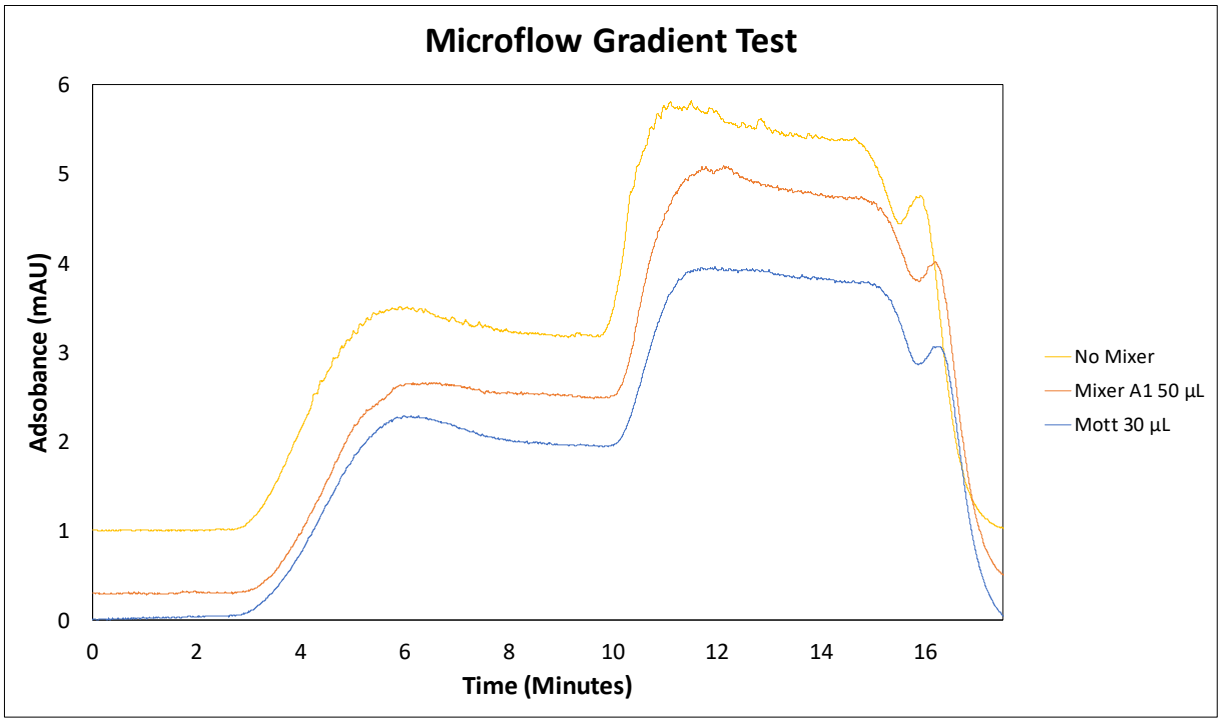


Figure 13. *Microflow gradient test performed comparing competitor low volume mixer to Mott low volume mixer.*

Evident by the results in Figure 13 the Mott 30 µL mixer provides maximum stability of the baseline at less volume than current small volume mixers. This will enhance your peak response while still maintaining a flat baseline.

Summary

The recently developed line of patent-pending PerfectPeak™ inline static mixers with four internal volumes, 30 µL, 60 µL, 90 µL, and prototype 180 µL cover the range in volumes and mixing performance needed for the 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 99% reduction in baseline noise was achieved using 1/3rd the internal volume of commonly available mixers. 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. The new line of static mixers achieves improved performance over comparable competitors' mixers, but with smaller internal volumes and system pressure changes. 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. *90 µL, 60 µL, and 30 µL mixers manufactured by Mott Corporation*

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