

Chromatography

Reducing HPLC/UHPLC System Baseline Noise and Increasing Sensitivity Using Novel 3D Printed, High Performance Static Mixers

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A revolutionary new inline static mixer has been developed and specifically tailored to meet the exacting demands of high performance and ultrahigh 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 utilising 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. Up to 98% 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 localised turbulent flow and eddies to create mixing on the micro-, meso-, and macro-scale. Computational fluid dynamic (CFD) modelling was employed in the design of this unique mixer. The test data presented demonstrates that superior mixing is achieved while minimising the internal volume.

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 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 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 minimising the volume and maximising 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 has recently developed a new line of patent-pending PerfectPeak™ inline static mixers with three internal volumes: 30 μL , 60 μL , and 90 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 three models are 0.5 inches in diameter and have industry leading performance in a compact engineered design. They are fabricated in 316L stainless steel which is passivated for inertness but could also be available in Titanium and other corrosion resistant and chemically inert metal alloys. The maximum operating pressure for these mixers is up to 20,000 psi.

Presented in *Figure 1a* is a photograph of the Mott 60 μL static mixer developed for maximum mixing efficiency while utilising a smaller internal volume comparable to standard mixers in this category. This new static mixer design utilises a novel additive manufacturing technology to create a unique 3D structure that achieves static mixing using less internal flow volume than any mixer 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 utilising 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) modelling was employed in the design of this unique mixer to analyse flow patterns and to improve designs prior to fabrication of prototypes for internal analytical testing and customer beta site evaluations.

Additive manufacturing is a process where 3D geometrical components can be printed directly from a CAD drawing without conventional machining (mills, lathes, etc.). These new static mixers were designed to be manufactured utilising this process where the mixer housing was generated from a CAD drawing and the part(s) manufactured (printed) one layer at a time utilising additive manufacturing. Here, a layer of metal powder is laid down roughly 20 μm thick and a computer controlled laser selectively melts and fuses the powder into a solid form. Another layer is applied over that layer and laser sintering is applied. This process is repeated until the part(s) are fully fabricated. The powder is then removed from the part where no laser bonding had occurred leaving behind a 3D printed part matching the original CAD drawing. The final products are somewhat comparable to micro-fluidic processes, with the main difference being that micro-fluidic components are generally 2D (planar) while when using additive manufacturing, one can create complex flow patterns in 3D geometries. The mixers can currently be fabricated as 3D printed parts in 316L stainless steel and in titanium. Most metallic alloys, polymers, and some ceramics can be used to fabricate components using this method and their use will be considered for future developments/products.

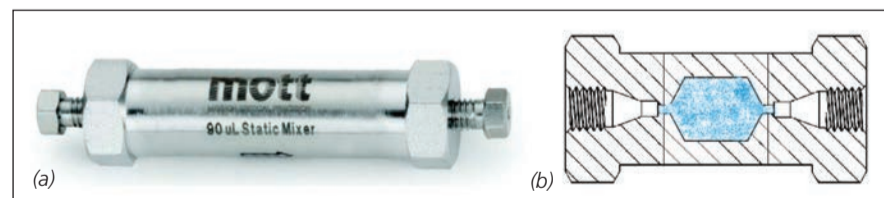


Figure 1. Photograph of a Mott 90 μL static mixer (a) and a schematic representation (b) showing a cross-section view with the mixer fluid flow path shaded in blue.

CFD Modelling

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 modelling of the static mixer and standard tubing (to simulate no mixer) was performed using COMSOL Multiphysics package. Modelling 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 utilised 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.

Modelling simulations for flow through a standard tubing (to simulate no mixer) and the Mott static mixer are shown in Figures 2 and 3, respectively. Modelling 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 mL mixer.

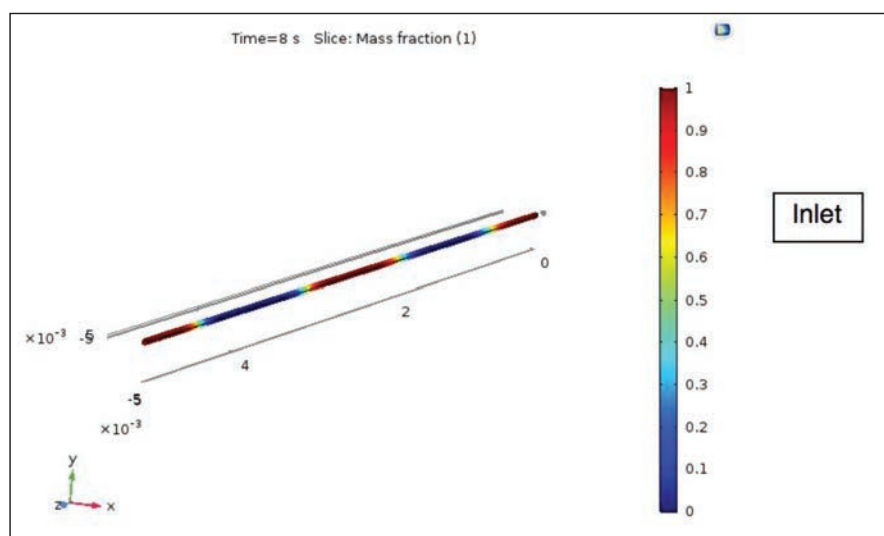


Figure 2. Shows CFD modelling on flow in a 5 cm long by 0.25 mm ID tube to represent what is happening in the HPLC tubing, i.e., if no mixer is in place. The full red represents water as a mass fraction. The blue represents the lack of water, which is pure acetonitrile. A diffusion region can be seen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

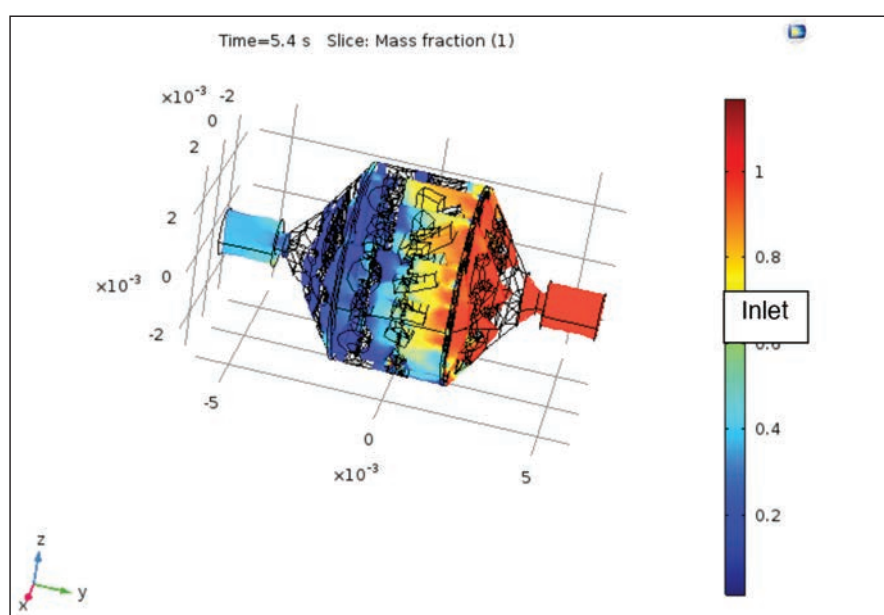


Figure 3. The 30 mL static mixer modelled in COMSOL CFD software package. The legend represents the mass fraction of water within the mixer. Pure water is represented by red while pure acetonitrile is represented by blue. As the two fluids mix the colour changes, to represent a simulate the changing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

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 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.

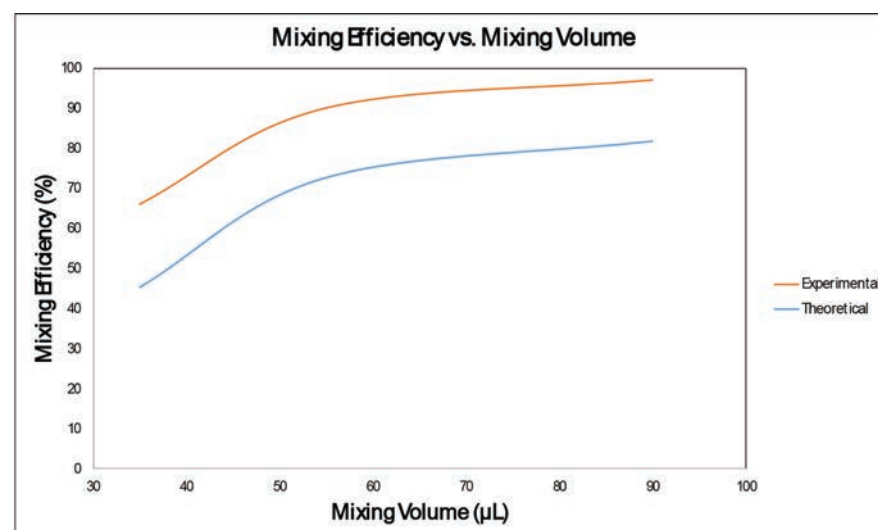


Figure 4. Graph of mixing efficiency versus mixing volume of the static mixer series. The theoretical mixing follows the same trend of experimental mixing data validating the CFD modelling.

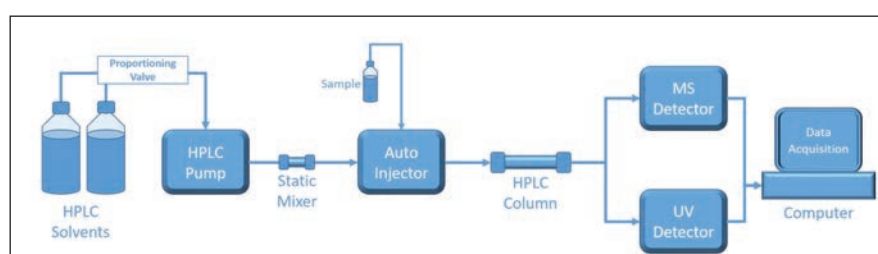


Figure 5. Schematic diagram of the low pressure gradient experimental test system.

The HPLC system utilised for this testing was an Agilent 1100 Series HPLC with a UV detector controlled using a PC with Chemstation Software. Presented in Table 1 are the typical setup conditions for measuring mixer efficiency by monitoring baseline sinusoid in two 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 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 1. 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

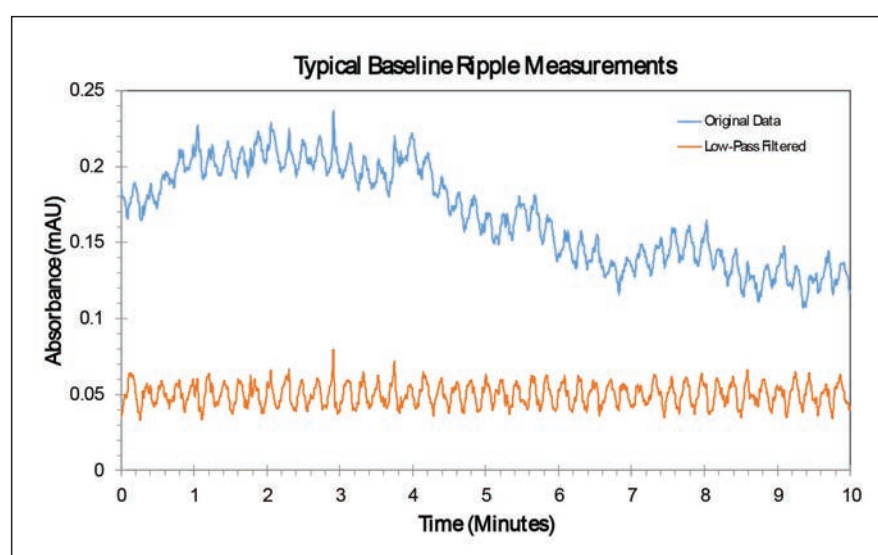


Figure 6. Plots of measured mixing sinusoid before and after a low-pass filter was applied to remove the baseline drift component of the signal.

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 stabilised. 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 low-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 low-pass filter was applied.

Test Results

Upon completion of CFD modelling and initial experimental testing, three separate static mixers were subsequently developed utilising the internal structures noted above with three internal volumes, 30 μL , 60 μL , and 90 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. 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 1* 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, and 0.004 mAu for the Mott 30 μL , 60 μL , and 90 μL static mixers, respectively.

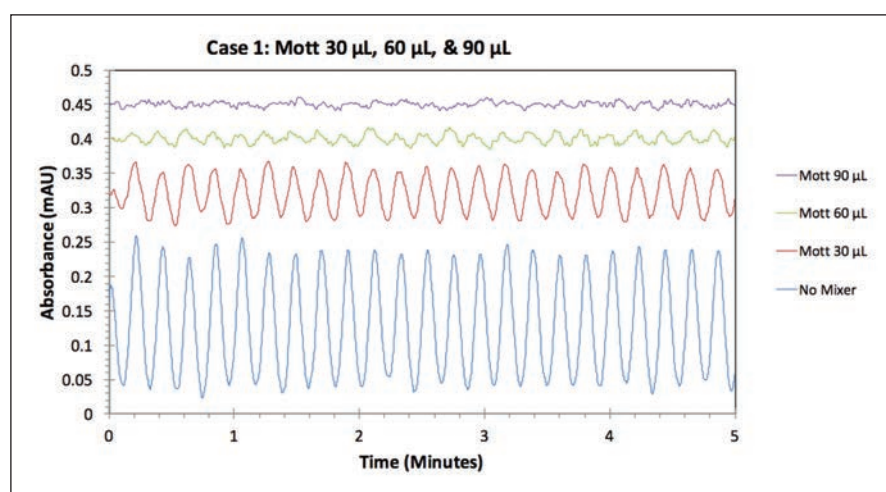


Figure 7. Plots showing offset HPLC UV detector signal versus time for Case 1 (Acetonitrile with ammonium acetate tracer) showing solvent mixing with no mixer, and Mott 30 μL , 60 μL , and 90 μL mixers installed showing improved mixing (smaller signal amplitudes) as the volume of the static mixer is increased (actual data offset by 0.13 (No Mixer), 0.32, 0.4, 0.45 mAu respectively to best display results).

Presented in *Figure 8* is the same data displayed from *Figure 7* but this time including the results for three commonly available HPLC static mixers, which have internal volumes of 50 μL , 150 μL , and 250 μL .

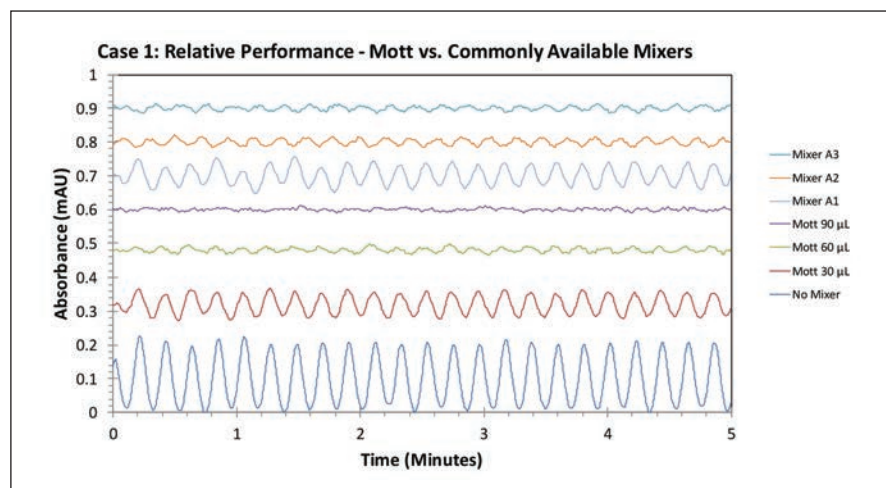


Figure 8. Plots showing offset HPLC UV detector signal versus time for Case 1 (Acetonitrile with ammonium acetate as a tracer) showing solvent mixing with no static mixer, new line of Mott static mixers and a three commonly available mixers (actual data offset by 0.1 (No Mixer), 0.32, 0.48, 0.6, 0.7, 0.8, 0.9 mAu respectively to best display results).

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 2* 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 2*, show that the Mott

static mixers achieve up to 98.1% reduction baseline sine wave, significantly outperforming commonly available mixers in use for the HPLC industry under these test conditions.

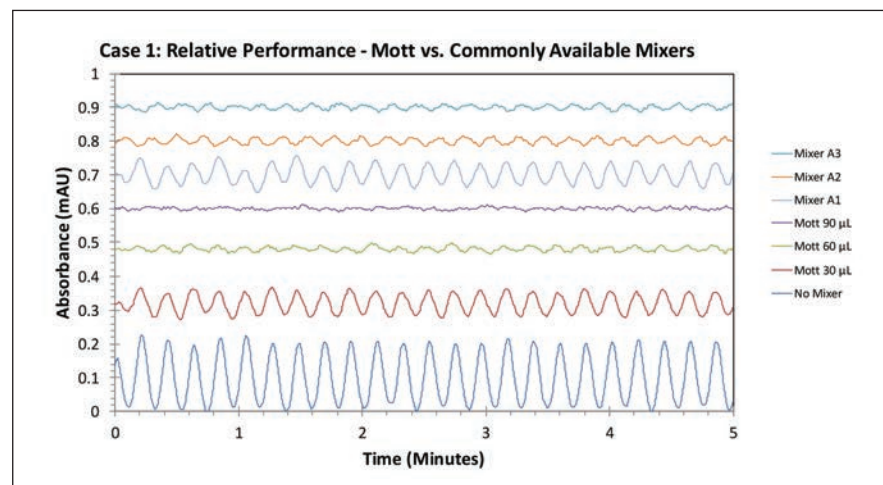


Figure 9. Plots showing offset HPLC UV detector signal versus time for Case 2 (Methanol with acetone as a tracer) showing solvent mixing with no static mixer (union), new line of Mott static mixers and a two commonly available mixers (actual data offset by 0.11 (No Mixer), 0.22, 0.3, 0.35 mAu respectively to best display results).

Seven commonly available mixers in the industry were also evaluated. These included three mixers of different internal volumes from each of Company A (labelled Mixer A1, A2 and A3) and Company B (labeled Mixer B1, B2 and B3). Only one size was evaluated from Company C.

Table 2. Static Mixer Mixing performance and Internal Volumes

Static Mixer	Case 1 Sinusoid Reduction: Acetonitrile testing (Efficiency)	Case 2 Sinusoid Reduction: Methanol Water test (Efficiency)	Internal Volume (μL)
No Mixer	-	-	0
Mott 30	65%	67.2%	30
Mott 60	92.2%	91.3%	60
Mott 90	98.1%	97.5%	90
Mixer A1	66.4%	73.7%	50
Mixer A2	89.8%	91.6%	150
Mixer A3	92.2%	94.5%	250
Mixer B1	44.8%	45.7%	35
Mixer B2	93.2%	84.5%	100
Mixer B3	96.9%	96.2%	370
Mixer C	97.2%	97.4%	250

Examination of the results in *Figure 8* and *Table 2* 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 mixer compared to the Mixer A3, with 250 μL internal volume follows a similar trend. Improved mixing performance of 98% versus 92% is also observed along with an internal volume that is 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 mL, 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 mL 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 three 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 reduction 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 98%.

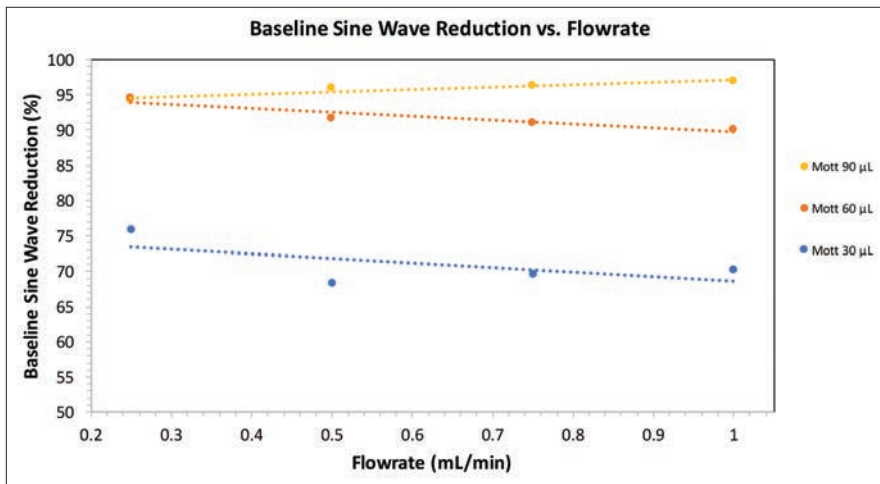


Figure 10. Reduction in baseline sine wave as a function of flowrate for Case 1. This testing was performed using similar conditions as the sinusoid test, variable flowrate, introducing 80% of an 80/20 mixture of acetonitrile and water, and 20% 20 mM ammonium acetate.

Summary

The recently developed line of patent-pending PerfectPeak™ inline static mixers with three internal volumes, 30 µL, 60 µL, and 90 µL cover the range in volumes and mixing performance needed for the majority of HPLC analyses 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 3D structure that achieves improved hydrodynamic static mixing with the highest percentage reduction in baseline noise per unit of internal mixture volume. Up to 98% reduction in baseline noise was achieved using 1/3 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, thereby providing increased sensitivity through better signal to noise ratios and lower limits of quantitation with improved peak shape, efficiency, and resolution.



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