HyperShear™ HPLC and UHPLC Mixers

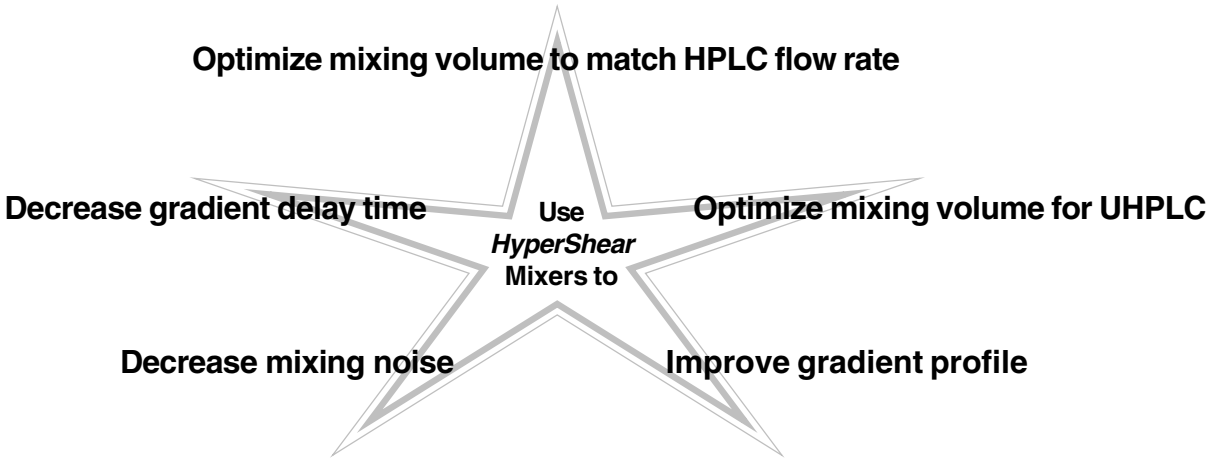
HyperShear™ HPLC and UHPLC Mixer Features:

- ❑ **Micro Flow, Low Flow, Analytical Flow, High Flow and Combo Series**
- ❑ **Reduce baseline noise, increase sensitivity and improve gradient accuracy**
- ❑ **UHPLC mixers are available in volumes ranging from 0.5 µL to 1.5 mL and pressure to 15,000 PSI**
- ❑ **Available for In-line, Binary and Ternary formats in Stainless Steel (SS) or PEEK**
- ❑ **Ideal for microbore HPLC, UHPLC and LC/MS**
- ❑ **Compact design is easily integrated into any HPLC and UHPLC system**
- ❑ **Increased reaction efficiency for post column derivatization**
- ❑ **Decreased mixing and delay volume without sacrificing mixing efficiency**
- ❑ **Mixing volume optimization is easy with interchangeable mixer cartridges**

HyperShear™ HPLC and UHPLC Mixers

ASI manufactures a wide range of Static Mixers to solve the most demanding high pressure mixing problems. *ASI HyperShear* Mixers incorporate a highly efficient cross-flow shearing mechanism which produces vortex shear mixing over a wide flow range. This mixing technology typically delivers between 25% to 200% better mixing efficiency compared to conventional packed bed or tortuous path mixers. *HyperShear* Mixers are available in a variety of housing formats including: inline, binary, and ternary inlets. Mixers are constructed from stainless steel or Biocompatible PEEK with volumes ranging from 0.5 µL to 1.5 mL.

Within a given mixer flow series, mixer cartridges are interchangeable. The ability to swap mixer cartridges within a given mixer series makes selecting the optimum mixer volume easy and economical. This is not the case with UHPLC mixers. Since we warranty the pressure rating of 15,000 PSI on the factory tested mixer assembly (housing and cartridge), UHPLC mixers are only sold as complete mixer assemblies.



Specifications

	Standard HPLC	UHPLC	Biocompatible PEEK
Pressure Rating	6,000 PSI	15,000 PSI	3,000 PSI
Female Port Geometry	10/32 Parker(1/16 CPI)	10/32 Parker(1/16 CPI)	10/32 Parker(1/16 CPI)
Wetted Materials	Stainless Steel and PEEK	Stainless Steel and PEEK	PEEK
Micro Flow Series 0.5, 1, 2, 5, 10 and 25 µL	available	available	available
Low Flow Series 50, 150 and 250 µL	available	available	available
Analytical Flow Series 350 and 500 µL	available	available	available (In-Line)
High Flow Series 800 µL, 1.0 and 1.5 mL	available	available	available (In-Line)
Combo Series 1 to 100 µL	available (In-Line)	available (In-Line)	available (In-Line)

Static Mixer Application Notes

Selecting the Right Size Mixer Cartridge

ASI offers static mixers with volumes that range from 0.5 microliters to 1,500 microliters. Choosing the right size mixer is a trade off between delay volume, mixing noise, gradient fidelity and chromatographic performance. ***Please refer to the Charts on pages 64 and 65 for more information on gradient accuracy as a function of flow rate and mixing volume.***

The following observations will provide some guidelines to help choose the right size mixer.

- For any given flow rate, the more the mixing volume the better the mixing, and the lower the baseline noise.
- The smaller the mixing volume, the better the definition and sharpness of linear gradients.
- Multi-pump high pressure gradient systems typically require far less mixing volume than low pressure single pump gradient systems when running linear gradients.
- An *ASI* 150 µL in-line static mixer can be added in addition to the standard onboard mixer to further reduce mixing noise.

Multi-pump High Pressure Gradient Systems

Linear Gradients

If a larger mixing volume can be tolerated for a particular flow rate, the larger the volume will lower the mixing noise. The upper limits to mixing volume will be the maximum delay time that can be tolerated, and possible distortion (tailing) of the gradient at the beginning and end of the gradient. The lower limit will be defined by the amount of mixing noise that can be tolerated. ***Please refer to a table, page 59 for specific recommendations.***

Binary or Ternary – Steady State Composition

Always select the largest volume that will still provide an acceptable delay volume. In general, the more mixing volume, the better the mixing will be. For most pump systems, a 150 µL cartridge will provide adequate mixing.

Examples of this type of pump system include:

Shimadzu LC-10AD and LC-10ADvp
Beckman System Gold®
Gilson Model 305
Agilent Model 1100

Single-pump Low Pressure Mixing Gradient Systems

Linear Gradients

These systems generally require more mixing volume to perform linear gradients than multi-pump high pressure systems. The following will explain why this is the case. In a low pressure system the composition can only be changed once every pump stroke. Since the pump stroke volume of most pumps is 100 µL, and it takes a mixer volume that is about 3 times the batch volume to provide adequate mixing, we need 350 µL of mixer volume, at least, to do adequate mixing. More insoluble combinations may require even more mixing volume.

In general, choose the largest size mixer cartridge that will still provide an acceptable delay volume. For most applications this will be at least 350 µL.

Binary or Ternary – Steady State Composition

Always select the largest volume that will still provide an acceptable delay time. In general, the more mixing volume, the better the mixing will be. For most applications this will be at least 350 µL.

Examples of this type of pump system include:

Agilent 1090
Perkin Elmer series 200
TSP Spectra Vision®
Waters model 626
Varian Star® 9000

Mixer Cartridge Selection Guide for High Pressure Mixing

Linear Gradients - High Pressure Mixing

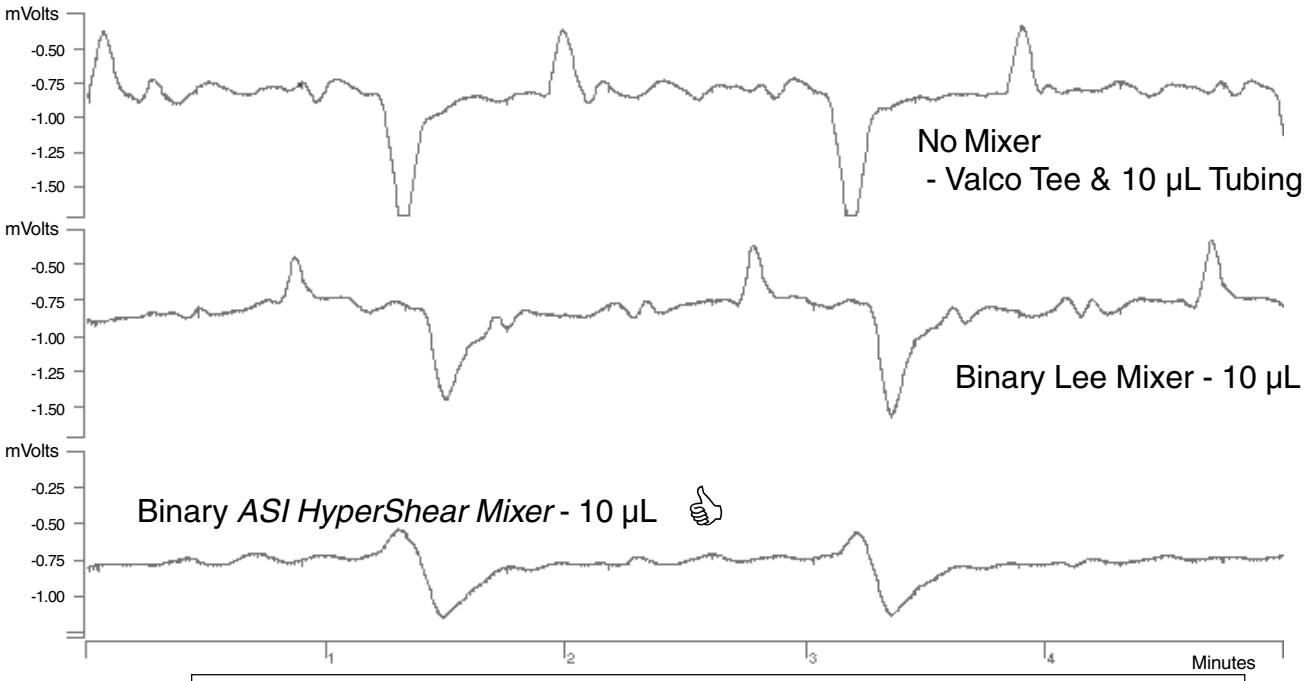
Larger mixing volume can be tolerated for a particular flow rate, with the larger the volume the lower the mixing noise. The upper limits to mixing volume will be the maximum delay time that can be tolerated, and possible distortion (tailing) of the gradient at the beginning and end of the gradient. The lower limit will be defined by the amount of mixing noise that can be tolerated. The following cartridge volumes are a compromise between these two limits.

Table 1.

Flow	Mixer Cartridge Volume
0-5 micro liter/min.	5 micro liter
5-10	10
10-20	25
20-150	50
150-500	150
500+	250

Binary Static Mixer Comparison

Less Noise - Lower Detection Limits

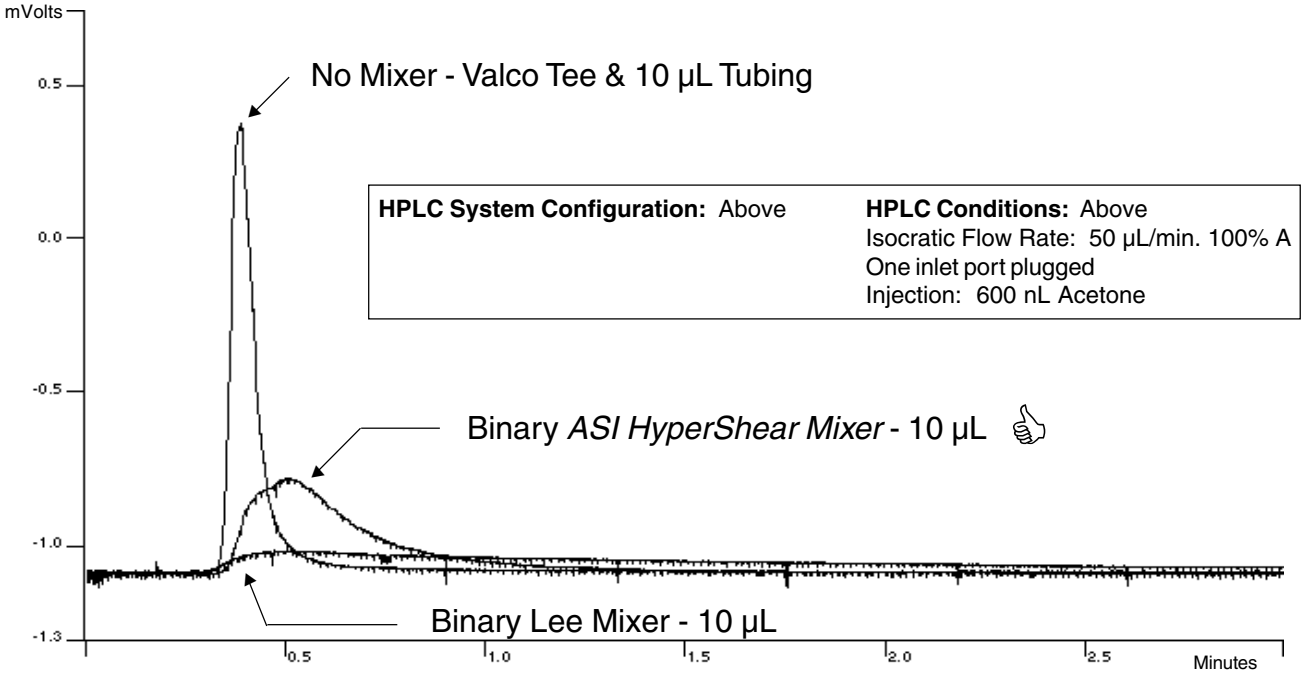


HPLC System Configuration:
ASI Model 500G Gradient System
ABI Model 785 UV/VIS @254 on-column
Varian Star Data Acquisition

HPLC Conditions:
MP: A = H_2O, B = H_2O/Acetone
Flow Rate: 50 µL/min. 50% B
Pressure: 1,700 PSI with *ASI* resistor cartridge

Figure 21

Superior Mixing with Less Gradient Dispersion

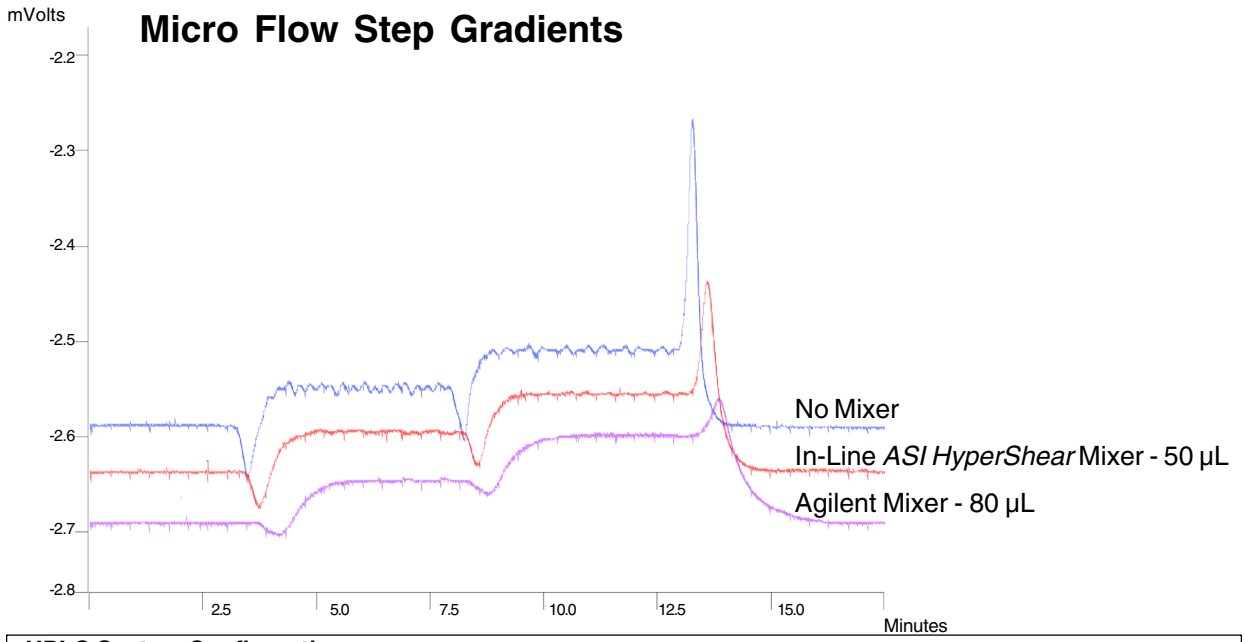


HPLC System Configuration: Above

HPLC Conditions: Above
Isocratic Flow Rate: 50 µL/min. 100% A
One inlet port plugged
Injection: 600 nL Acetone

Figure 22

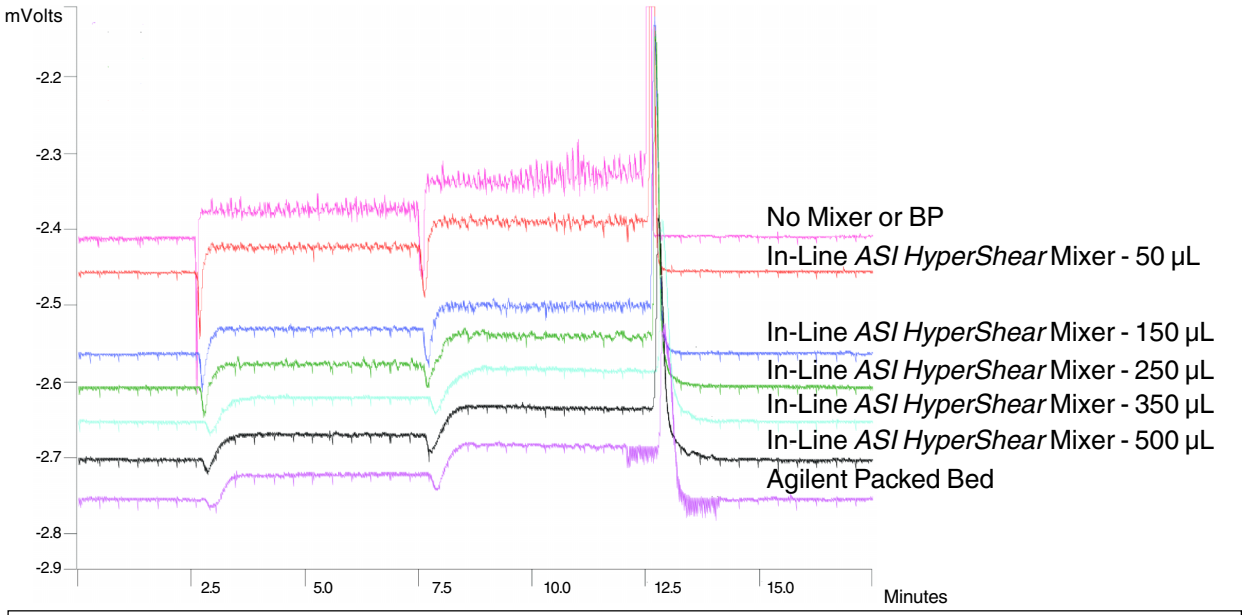
Agilent Static Mixer Optimization



HPLC System Configuration:
Agilent® Binary 1100 Pump
Packed bed mixer (approximate volume: 500 µL) replaced with union and/or *ASI HyperShear* In-Line Static Mixer
Varian Star Data Acquisition

HPLC Conditions:
MP: A = H_2O, B = ACN doped with 0.01% Acetone
Flow Rate: 250 µL/min.
Pressure: 2,000 PSI with *ASI* resistor cartridge
Gradient: 0.0 = off%B 7.5 = 20%B
2.5 = 10%B 12.4 = 20%B
7.4 = 10%B 12.5 = 0.0%B and hold

Figure 23



HPLC System Configuration: Above
Agilent® Binary 1100 Pump
Packed bed mixer (approximate volume: 500 µL) replaced with union and/or *ASI HyperShear* In-Line Static Mixer
Varian Star Data Acquisition

HPLC Conditions:
MP: A = H_2O, B = IPA doped with 0.01% Acetone
Flow Rate: 1.0 mL/min.
Pressure: 2,000 PSI with *ASI* resistor cartridge
Gradient: Above

Figure 24

Shimadzu Static Mixer Optimization

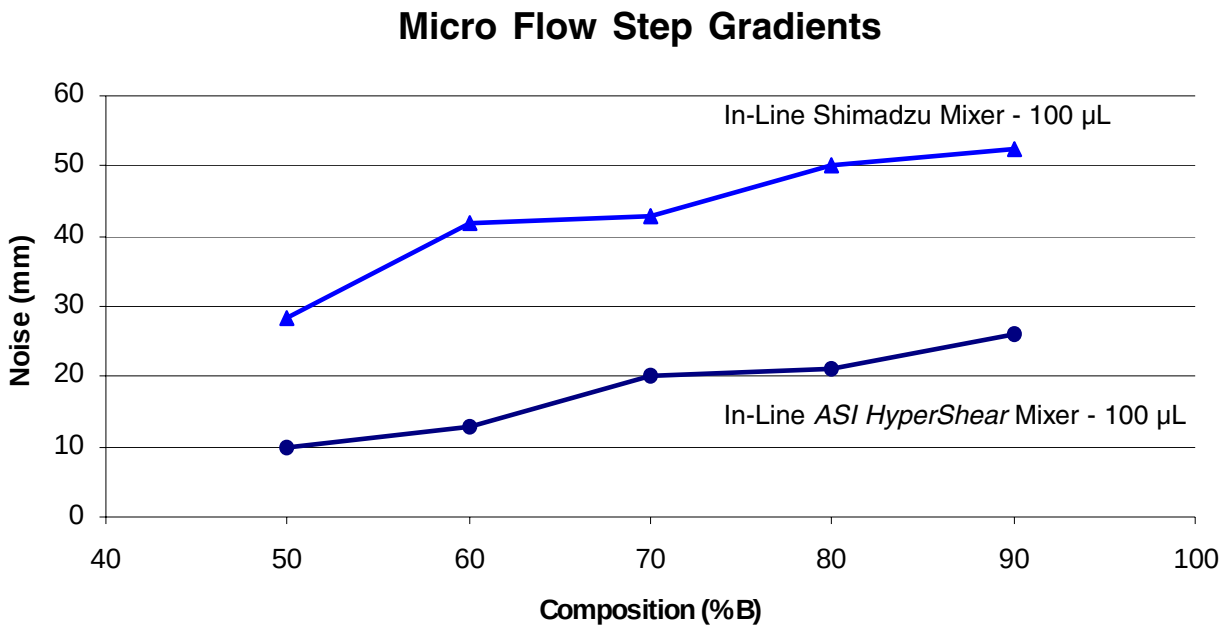
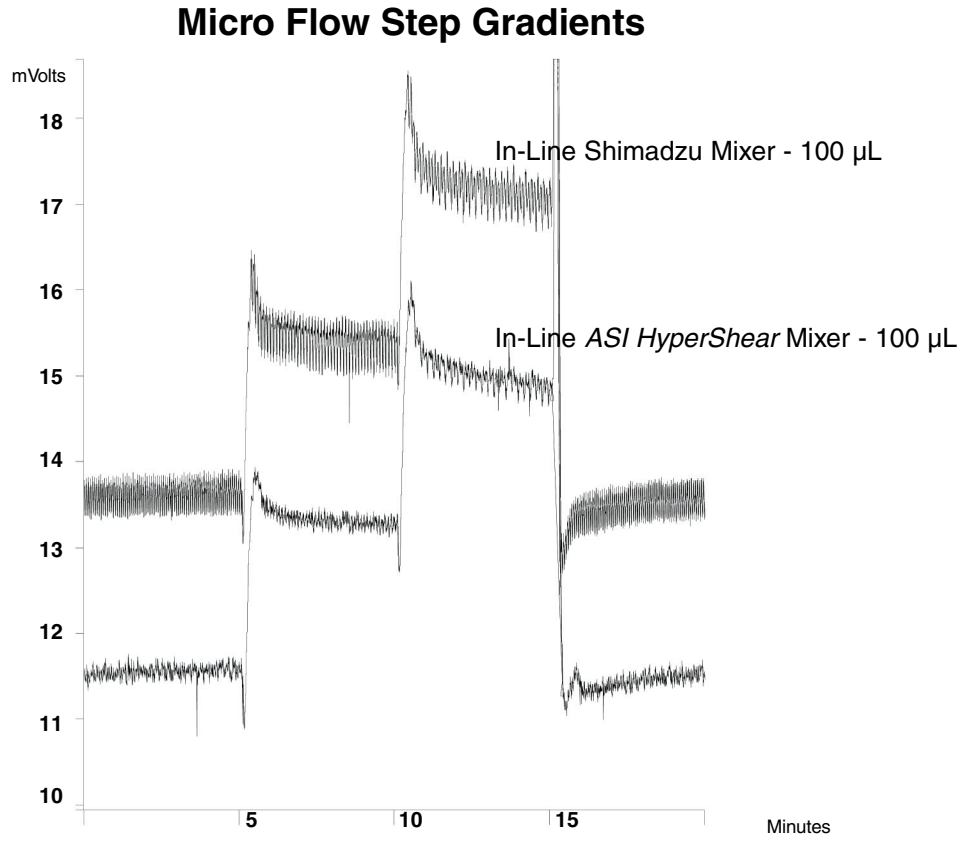


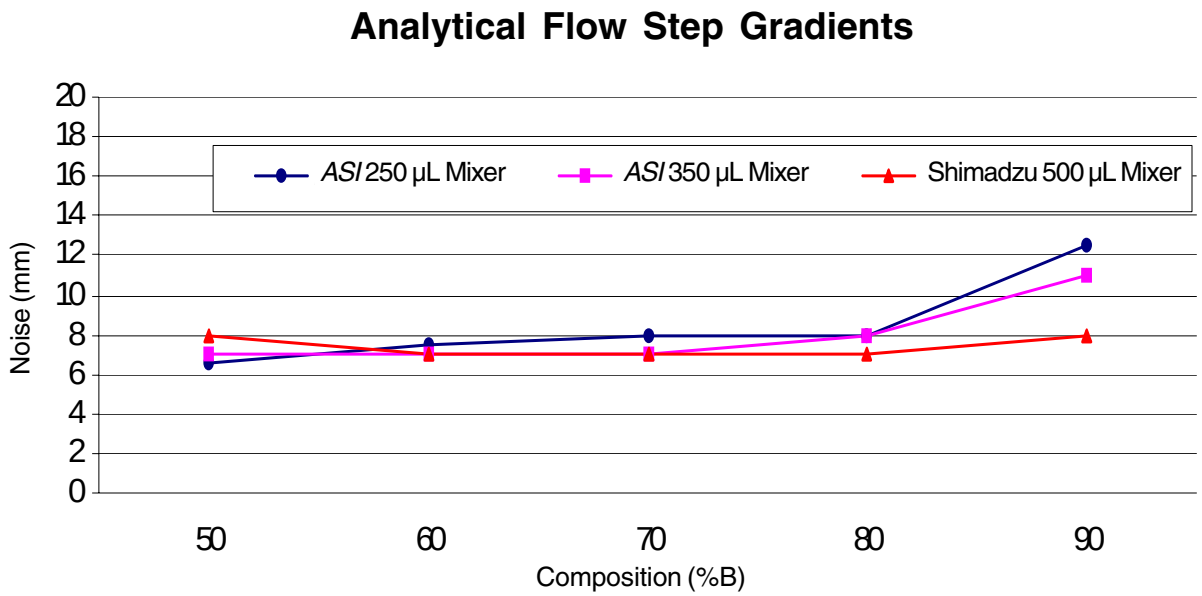
Figure 25



HPLC System Configuration:	**HPLC Conditions:**
Shimadzu: LC-10ADvp	MP: A = H_2O, B = H_2O + 35% 2-Propanol + 0.003% Acetone
Mixer: Connected at Shimadzu Tee	Flow Rate: 250 µL/min.
Detector: Knauer 2501 @254	Pressure: 2,000 PSI with *ASI* resistor cartridge

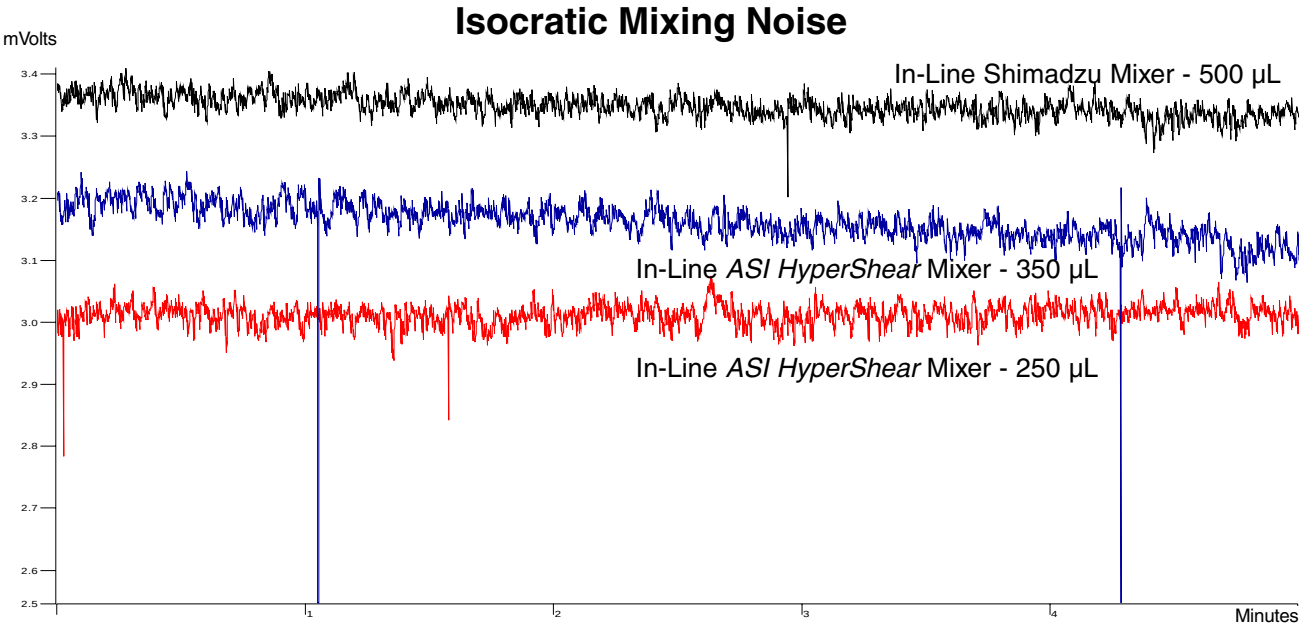
Figure 26

Shimadzu Static Mixer Optimization



HPLC System Configuration:	HPLC Conditions:
Shimadzu: LC-10ADvp	MP: A = H_2O, B = H_2O + 35% 2-Propanol + 0.003% Acetone
Mixer: Connected at Shimadzu Tee	Flow Rate: 1.0 mL/min.
Detector: Knauer 2501 @254	Pressure: 2,000 PSI with *ASI* resistor cartridge

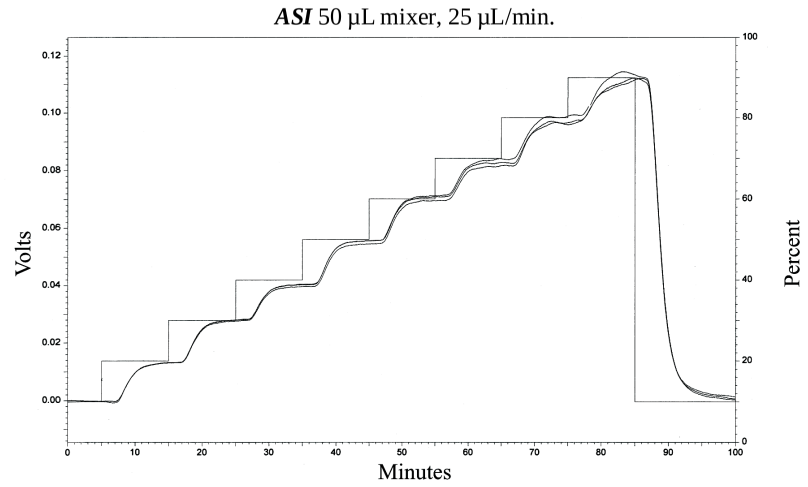
Figure 27

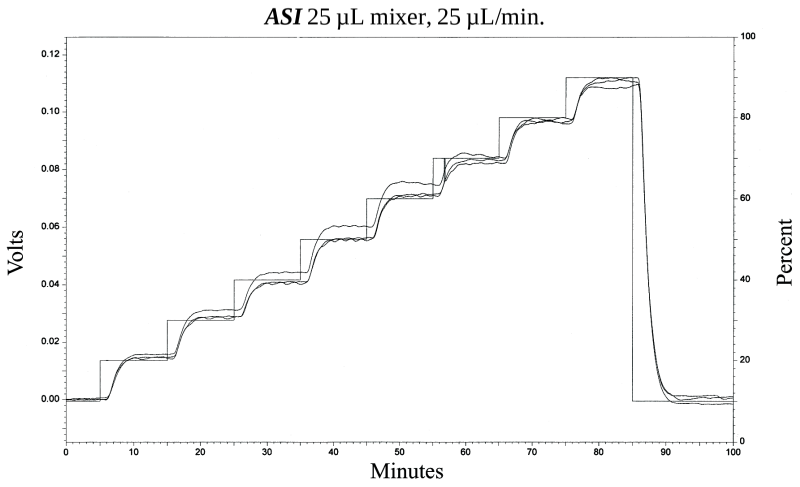


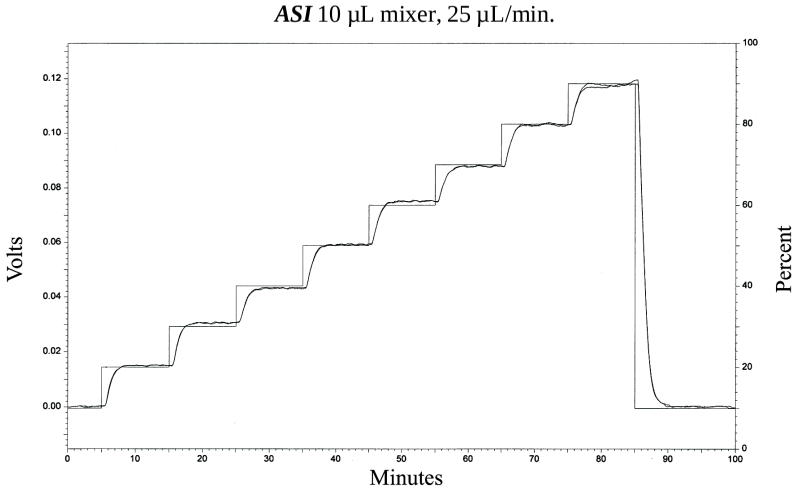
HPLC System Configuration:	HPLC Conditions:
Shimadzu: LC-10ADvp	MP: A = H_2O, B = H_2O + 35% 2-Propanol + 0.003% Acetone
Mixer: Connected at Shimadzu Tee	Flow Rate: 1.0 mL/min.
Detector: Knauer 2501 @254	Pressure: 2,000 PSI with *ASI* resistor cartridge

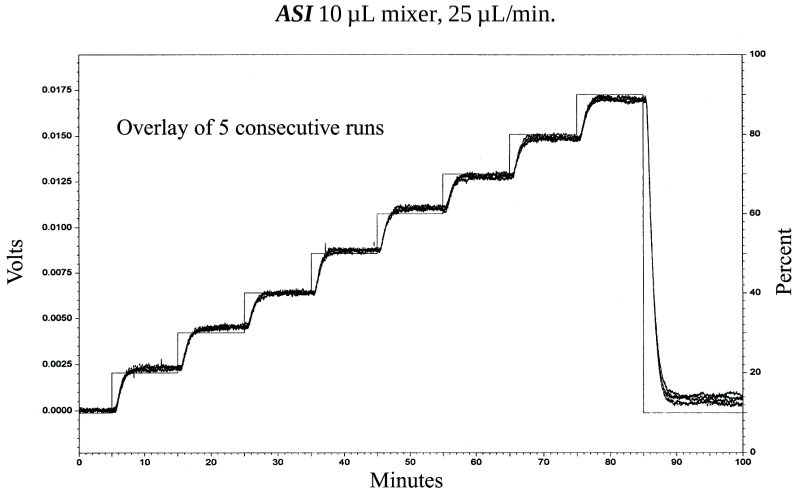
Figure 28

Effect of Mixer Volume on Gradient Accuracy with a Constant Flow Rate









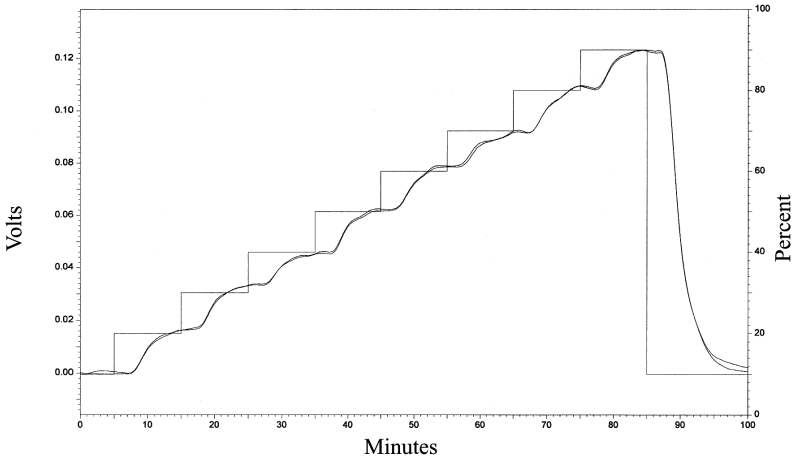
HPLC System configuration:
Shimadzu LC-10ADVP pumps with micro-flow modification
Shimadzu SPD-10AVP UV-VIS detector
Shimadzu Class-VP v5.03 software

HPLC conditions:
Gradient: 10-90% B in 10 min. steps
A = H_2O
B = 0.3% Acetone in H_2O

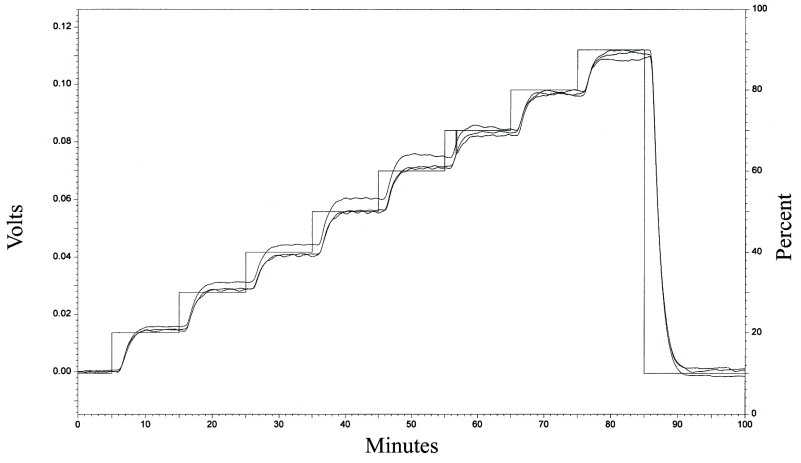
Figure 29

Effect of Flow Rate on Gradient Accuracy with a Constant Mixing Volume

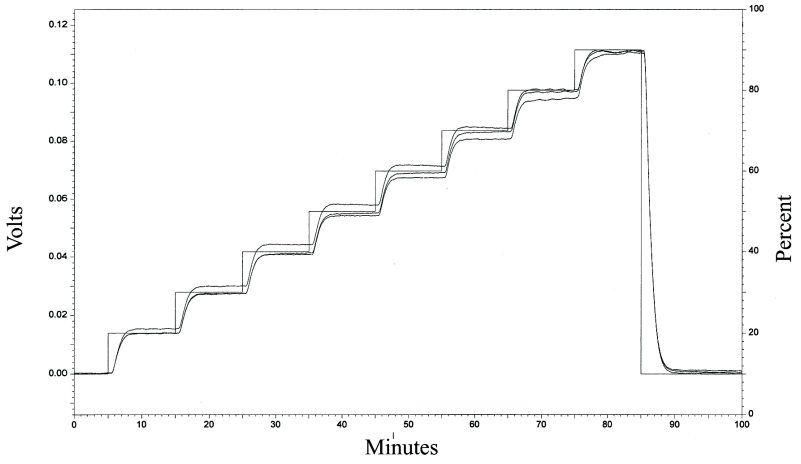
ASI 25 µL mixer, 10 µL/min.



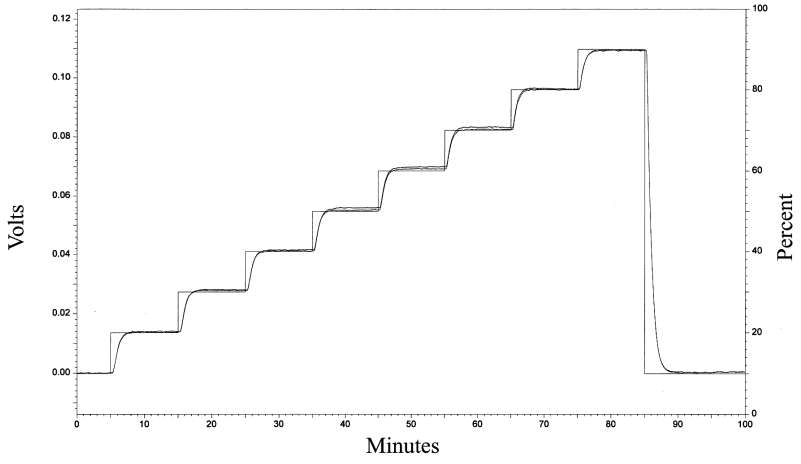
ASI 25 µL mixer, 25 µL/min.



ASI 25 µL mixer, 50 µL/min.



ASI 25 µL mixer, 100 µL/min.



HPLC System configuration:
Shimadzu LC-10ADVP pumps with micro-flow modification
Shimadzu SPD-10AVP UV-VIS detector
Shimadzu Class-VP v5.03 software

HPLC conditions:
Gradient: 10-90% B in 10 min. steps
A = H_2O
B = 0.3% Acetone in H_2O

Figure 30