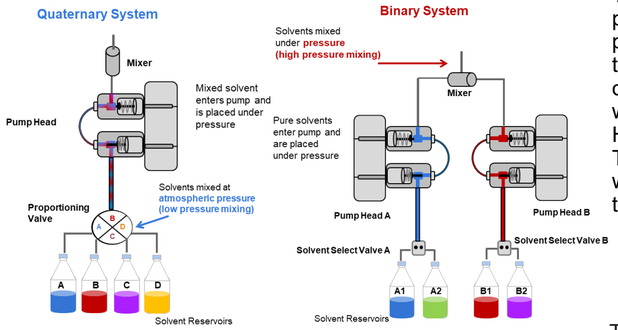
COMPARISON OF MIXER PERFORMANCE FOR HPLC METHODS UTILIZING TFA GRADIENTS

Authors: Lise Gauthier, Kimberly Martin, Elom Pedanou, Paula Hong, and Jennifer Simeone
Affiliations: Waters Corporation, 34 Maple Street, Milford, MA 01757

INTRODUCTION

Solvent mixing is critical to obtaining optimal LC separations. Currently, there are two commonly available pump mixing designs for reversed phase gradient HPLC separations - high pressure (typically binary) and low pressure (all quaternary) systems. High pressure systems use two independent pumps to deliver different solvents. Mixing occurs at high pressures with solvent composition controlled by the flow rates of the two pump heads. In low pressure systems, solvent composition is controlled by a gradient proportioning valve. Each solvent is delivered in packets (based on the gradient specified in the method) which are mixed as they go through the pump head. Both high and low pressure systems are subject to mobile phase composition fluctuations. Mixers of various volumes and designs are utilized to help minimize these composition fluctuations by reducing baseline noise and oscillations in mobile phase composition.



Trifluoroacetic acid (TFA) is a commonly used ion-pairing reagent used in combination with acetonitrile for many gradient reversed phase applications. TFA absorbs strongly at wavelengths below 250 nm. Additionally, TFA is slightly retained on reversed phase columns which results in fluctuations in TFA concentration as the acetonitrile gradient passes through the column. In combination, these factors result in significant baseline disturbances (ripples) when TFA - acetonitrile gradients are used at low wavelengths. These baseline ripples may make peak integration difficult, and can impact the sensitivity and retention time precision of the method.

Most modern HPLC systems include a standard mixer in the pump design. Additionally, different mixers may be available to improve mixing performance for specific applications. In this study, mixers of varying volumes and design were evaluated.

	System X	Alliance™ iS HPLC System	
Pump Type	High Pressure (Binary)	Low Pressure (Quaternary)	
Mixer Type	Standard	Standard	Optional
Mixer Material	Stainless Steel/ Static	Stainless Steel/Packed Bead	Titanium/Microfluidic Channel
Mixer Volume	400 µL	675 µL	690 µL

Table 1. Systems and Mixers

METHOD

USP Tryptophan Organic Impurities					
Mobile Phase A	0.1% Trifluoroacetic Acid in Water				
Mobile Phase B	0.1% Trifluoroacetic Acid in 80:20 Acetonitrile:Water				
Flow Rate	1 mL/min				
Gradient Table	Time	%A	%B	Curve	
	0.00	95.0	5.0	Initial	
	2.00	95.0	5.0	6	
	37.00	35.0	65.0	6	
	42.00	0.0	100.0	6	
	47.00	0.0	100.0	6	
	50.00	95.0	5.0	6	
	60.00	95.0	5.0	6	
Run Time	60 minutes				
Injection Volume	20.0 µL				
Column Temperature	30.0ºC				
Sample Temperature	15.0ºC				
Column	XBridge™ C18 Column: 4.6 x 250 mm, 5µm (P/N 186003117)				
Detector	UV: λ = 220 nm; 5 Hz				
Seal Wash	90:10 Acetonitrile:Water				

SYSTEMS

Two HPLC systems were used for the evaluation of mixer performance. The first system was a competitor's high pressure binary system – System X. The second system was the Waters Alliance iS HPLC System, a low pressure quaternary system. The mixing performance of each system was evaluated with their standard mixer. The Alliance iS HPLC System was also evaluated with an optional mixer, the Ti Diffusion Bonded Mixer (Waters Part # 205002590), which was designed to reduce baseline noise. Table 1 summarizes the systems and mixers used in this study.

RESULTS & DISCUSSION

The USP method for organic impurities in tryptophan was run on a competitor's high pressure system with their standard 400 µL static mixer (System X). The analysis was repeated on the Alliance iS HPLC System with the standard 675 µL (stainless steel) packed bead mixer in place, and then with the 690 µL (titanium) microfluidic channel mixer installed. Mixer performance was evaluated from the retention time precision and signal to noise ratio for Tryptophan Related Compound B in the system suitability solution. Results are summarized in Table 2.

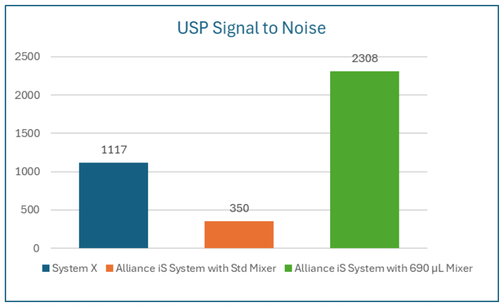


Table 2. Results -Tryptophan Related Compound B

RESULTS & DISCUSSION

The results showed good retention time precision across the systems/mixers. A comparison of the baseline of blank injections obtained with each mixer is shown in Figure 1. The standard mixers produced the characteristic baseline ripple typical of TFA-acetonitrile gradients at low wavelengths. The Alliance iS HPLC System with the 690 µL (titanium) microfluidic channel mixer produced a much smoother baseline.

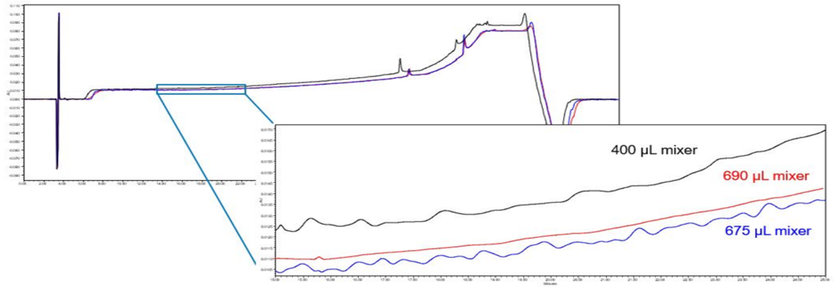


Figure 1. Blank injections from System X with 400 µL mixer; the Alliance iS HPLC System with the 675 µL mixer; and the 690 µL mixer

The effect of the different mixers on the signal to noise ratio is shown in Figures 2-3 which contain the system suitability solution injections and signal to noise ratios obtained with each system/mixer.

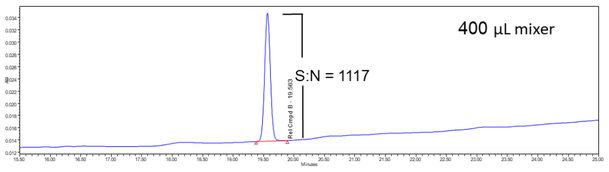


Figure 2. Signal to Noise ratio for Tryptophan Related Compound B obtained on System X with the standard 400 µL mixer

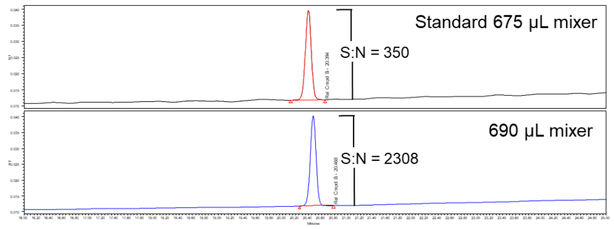


Figure 3. Signal to Noise ratio for Tryptophan Related Compound B obtained on the Alliance iS HPLC System with the standard 675 µL and optional 690 µL mixers

System X with its standard 400 µL mixer produced a higher signal to noise ratio than the Alliance iS HPLC System with the standard 675 µL mixer installed. As can be seen in Figure 3, with the 690 µL mixer installed on the Alliance iS HPLC System the signal to noise ratio increased 7X over that obtained on the same system with the standard 675 µL mixer installed. The value obtained with 690 µL mixer was also 2X greater than the with System X. This is due to a reduction in noise, not because the peak signal was larger.

CONCLUSION

Mixing performance is critical to obtaining optimal LC separations. In this study, several HPLC systems and mixers were evaluated using a TFA-Acetonitrile gradient method at a low wavelength which is known to produce baseline ripples. The USP signal to noise ratio was used to determine the impact of the different mixers.

The results demonstrate that the sensitivity of the method is impacted by the system/mixer used, and consideration should be given to mixer selection, especially when running methods which are subject to increased baseline noise.

References

1. Hong, P and J. Simeone. (2019) Deciding Between Quaternary and Binary Systems. How to Select the Best UPLC System For Your Application Needs? Waters Corporation
2. United States Pharmacopeia (2024). *USP Monographs, Tryptophan.* USP-NF. Rockville, MD:USP.
3. Ti Diffusion Bonded Mixer – 690 µL Kit Installation Guide, 715009251 V 00, Waters Corporation, Milford, MA, USA, 2024.

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS