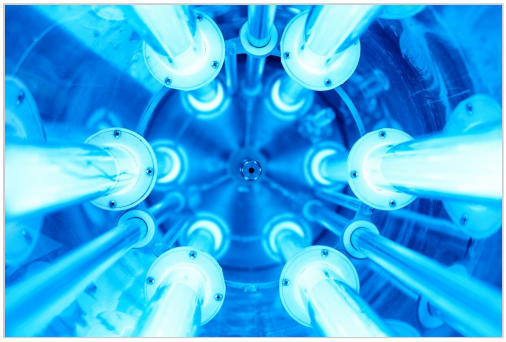
Waters™

Application Note

Instrument Mixer Considerations to Improve Assay Reproducibility and Sensitivity

David Dao, Robert E. Birdsall, Ying Qing Yu

Waters Corporation



This is an Application Brief and does not contain a detailed Experimental section.

Abstract

As part of a pharmaceutical quality system, biopharmaceutical companies rely on robust methods that deliver consistent and accurate results in the production of drug-products. Determining acceptance criteria for biopharmaceutical products can be challenging when operating detectors at or near their limit of detection. This is particularly true for LC-based ultra-violet detectors which are sensitive to the optical activity and heterogeneity of solvents flowing through them. LC-based mixers represent a hardware component that can be readily changed with minimal system downtime and impact to gradient while having a measurable impact on assay reproducibility and accuracy by reducing baseline noise due to solvent composition heterogeneity. Recently, Waters released a 340 µL biocompatible mixer (P/N 700011554) that has been evaluated and found to significantly decrease baseline noise with respect to optical performance while increasing peak area response and integration precision. In this study, identical peptide mapping samples were run on a dedicated LC system using a stock 50 µL mixer and the 340 µL biocompatible mixer. Signal-to-noise increased up to 50% with experimental values >3, and area %RSD reduced more than 5-fold to <2.0% for selected peaks, differences that could impact dynamic range and acceptance criteria for assays. This work demonstrates the advantages of using the Waters 340 µL biocompatible mixer to optimize chromatographic performance and detector response with minimal change to instrument configuration.

Benefits

- Higher volume mixers reduce optical baseline noise for increased assay performance
- Lower baseline noise improves peak integration and quantitative precision

Introduction

Optical-based assays that incorporate ultra-violet (UV) detection are commonly used in labs throughout the biopharmaceutical industry. This is in part due to both the intrinsic absorption/emission properties exhibited by protein-based molecules which are well suited for UV-detection and UV-detectors represent a cost-effective technology that can be readily scaled and deployed to address organizational needs. In instances where sample is limited or trace impurities are being monitored, it is important to maximize detector response to ensure assays are delivering consistent and accurate results. For LC-based assays, parameters such as gradient, temperature, mobile phase composition, and ion-pairing type are often evaluated as part of the optimization process. Apart from columns, evaluation of LC hardware contributions to assay performance are often kept to a minimum to maintain productivity. This is particularly true in regulated environments where methods are restricted to parameters and hardware configurations defined during the validation process. Considering this, solvent mixers represent a hardware component that can be readily changed without requiring method re-validation or verification while having measurable impact on assay performance.

Optical activity and the degree of heterogeneity in mobile phase composition as it flows through an optical detector can result in increased baseline noise which can negatively impact assay performance in terms of limit of detection (LOD) and quantitative precision. This phenomenon is exacerbated when using lower UV acquisition wavelengths with methods that incorporate shallow gradients with slow flow rates as in the case of peptide analyses. One mitigation strategy to reduce baseline noise in gradient-based separations is to incorporate solvent mixing chambers with increased volume to reduce heterogeneity in mobile phase composition. As part of their design, Waters LC instruments offer users the ability to select from a portfolio of mixers to facilitate efficient method development and maximize assay performance. The objective of this study is to demonstrate how mixers with increased volume can be used to mitigate baseline noise to improve detector response and method robustness.

Results and Discussion

To investigate the impact of mixer volume on UV detector baseline noise some considerations were given with respect to LC platform and technique used in this study. The ACQUITY UPLC H-Class PLUS Bio Binary System was selected as the LC platform because it delivers the highest compositional accuracy in gradient-based separations. As part of its design, the ACQUITY UPLC H-Class PLUS Bio Binary System performs solvent mixing directly from two separate pumps by combining flow via a tee assembly which is then introduced to a mixing chamber prior to the column for increased compositional accuracy of mobile phases. To minimize system variability, the stock 50 µL mixer shipped with the ACQUITY UPLC H-Class Binary Bio PLUS Bio System was compared against a 340 µL mixer (P/N 700011554) using the same chromatographic system running the same mobile phases, column, and sample where only the mixer was changed for each data set. Reversed phase chromatography was selected for this study as a commonly encountered technique in industry that uses gradients. Mobile phases (MP) were prepared as water (MP: A) and acetonitrile (MP: B) with 0.1% (v/v) formic acid. A standard 135 min peptide mapping assay was performed using a gradient of 1.7 %B/min at a flow rate of 0.200 mL/min using the Waters Tryptic Digest Standard (P/N 186009126) separated on an ACQUITY UPLC Peptide CSH C_{18} Column (130Å, 1.7 µm, 2.1 mm x 100 mm, P/N 186006937) with UV detection performed at a wavelength of 214 nm. To ensure baseline response was stable after hardware changes, sample sets consisting of 4 blank injections were performed followed by 5 injections of the tryptic digest standard.

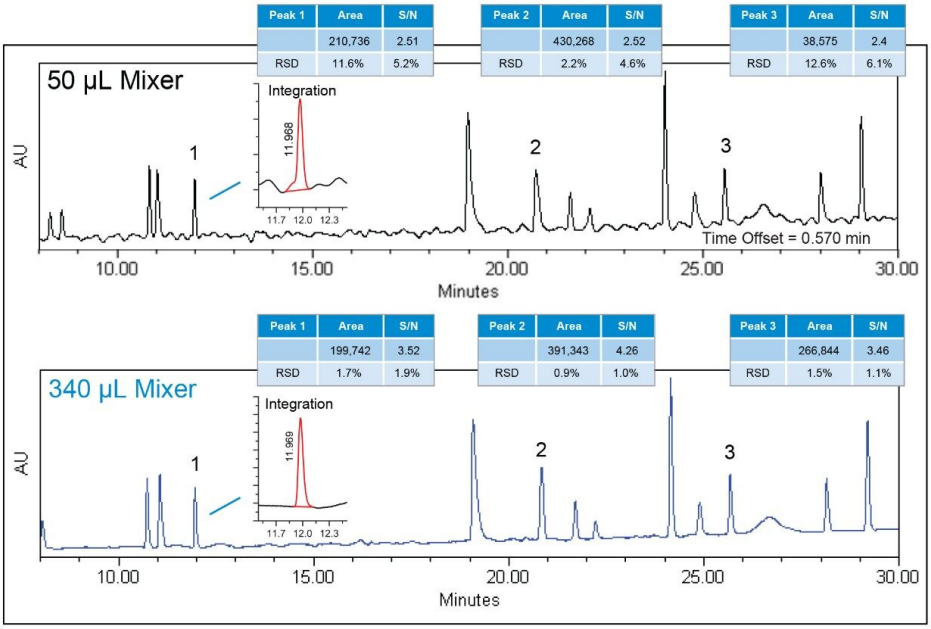


Figure 1. Chromatogram overlay of the two mixer volumes showcasing increased baseline noise levels when using a 50 µL mixer vs a 340 µL mixer running the same sample under the same chromatographic conditions. Resulting S/N measurements and reproducibility are higher as a result of the lower baseline noise.

As shown in Figure 1, baseline noise has visibly been reduced by using the larger volume mixer (bottom chromatogram). Using a selection of peaks, peak area and signal-to-noise ratio (S/N) were calculated to determine quantitative impact of mixer volume on assay performance. As shown in the top chromatogram the selected peaks were calculated to have S/N <3 when using the lower volume 50 µL mixer, S/N values considered to be below the analytical threshold to be considered “real”. However, these same peaks were observed to have a calculated S/N >3 when using the larger volume 340 µL mixer, a difference that could impact dynamic range or defining acceptance criteria for assays. In addition, area reproducibility of the assay was improved up to 8-fold with the larger volume mixer due to increased precision in peak integration across the injection series (inset figures). These results demonstrate how increasing mixing volume can mitigate baseline noise and maximize UV detector response for increased assay performance with minimal change to chromatographic systems.

Conclusion

As part of method development, optimizing system performance is necessary to obtain reliable and consistent results. High baseline noise due to increased optical noise or mobile phase compositional heterogeneity can negatively impact chromatographic performance with respect to peak signal to noise and area reproducibility. Waters offers a portfolio of mixers options that can be used to increase system performance and address application needs with minimal instrument changes enabling the development of more robust and reproducible methods.

Featured Products

ACQUITY UPLC H-Class PLUS Bio System

ACQUITY UPLC Tunable UV Detector

Empower Chromatography Data System

720007011, September 2020