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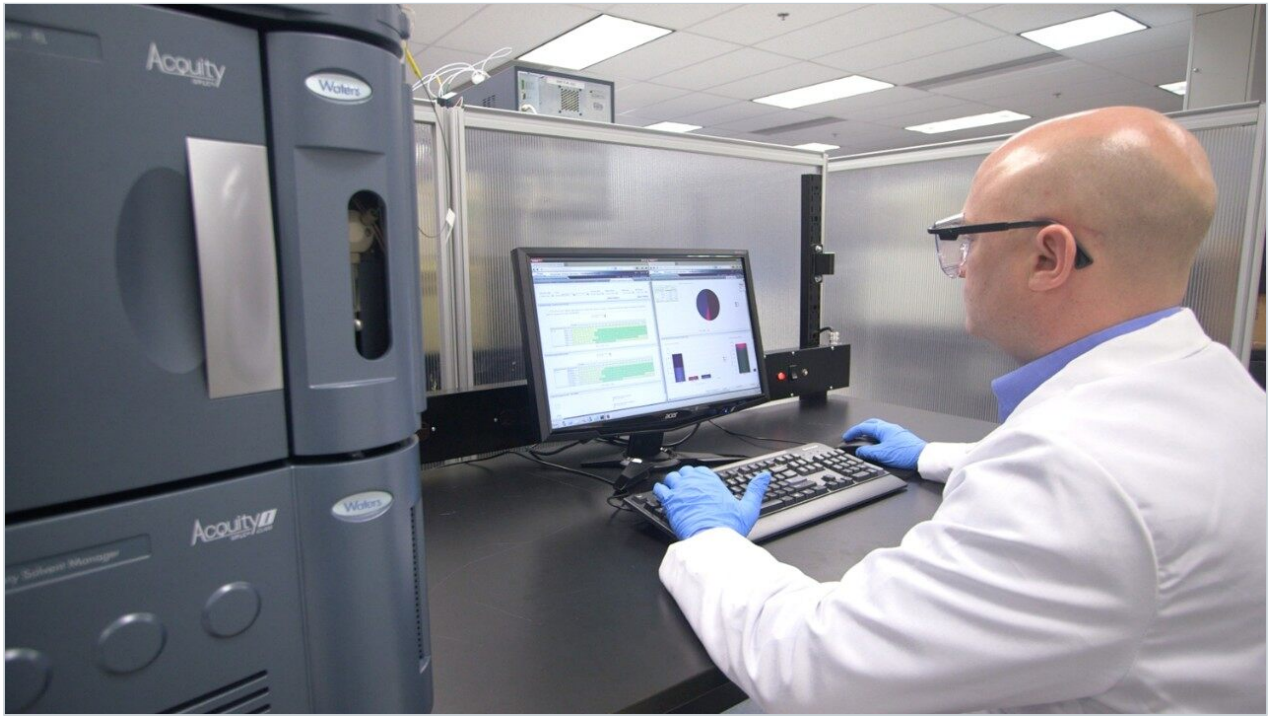
ACQUITY UPLC I-Class: Minimizing Carryover to Enhance the LC-MS/MS Quantitative Range

Waters Corporation



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

Abstract

This application brief demonstrates that carryover for LC-MS/MS applications can be reduced below a measurable level when quantification is required across greater than four orders of magnitude.

Benefits

During the analysis of omeprazole, the ACQUITY UPLC I-Class System reduced carryover below 0.0005% with a linear quantification range across greater than four orders of magnitude on the Xevo TQ-S.

Introduction

Today's mass spectrometers have become sufficiently sensitive for detection across five orders of magnitude with lower limits of quantification that can reach into the attogram on column range. Increasing the sensitivity of the highest performing mass spectrometers requires an LC system that can minimize carryover to optimize assay performance. Regulations that govern the validation of bioanalytical methods across multiple orders of magnitude typically require that carryover after the highest concentration calibrant be no greater than 20% of the lowest concentration calibrant.[1] Therefore, to achieve a calibration range across four orders of magnitude, the carryover must be reduced to below 0.002%. If the calibration range is to extend greater than four orders of magnitude, carryover must be reduced to an even lower level. Generally, as carryover requirements for mass injected on column become lower, system contamination becomes critical. The LC system and method must be able to repeatedly remove the analyte from the injector, tubing, and chromatographic column to achieve a clean injection each time.

Results and Discussion

The Xevo TQ-S is a highly sensitive mass spectometer for LC-MS/MS analysis. It requires a UPLC inlet that can manage carryover to match the instrument's broad, linear dynamic range. The ACQUITY UPLC I-Class System has two Sample Manager options: fixed-loop (SM-FL) or flow-through needle (SM-FTN) injector, both of which were designed to deliver excellent carryover performance. In the analysis of omeprazole, the SM-FTN design was used. With this style of injector, the interior of the needle is washed by the mobile phase (gradient) during the analysis. The FTN uses a single solvent to clean the exterior of the needle in the injection port and was designed to prevent the wash solvent from coming in contact with the sample or

mobile phase. Washing both the needle and the seal together at the sealing surface reduces the chance for contamination. The duration of the wash is programmable in the method and can be configured for both pre-injection and post-injection wash. The composition of the wash solvent is sample dependent and should be formulated to easily solubilize the analyte. For omeprazole, which has a pKa of 8.8, a wash solvent containing ammonium hydroxide was used to adequately clean the injector. Additionally, the system carryover was lower when ammonium hydroxide was used in the mobile phase. The ionization efficiency of omeprazole was also significantly higher under basic conditions. To assess carryover, the highest concentration standard at 10 ng/mL or 10 pg on column was injected. The first blank injection after the highest standard had no observable carryover as shown in Figure 1.

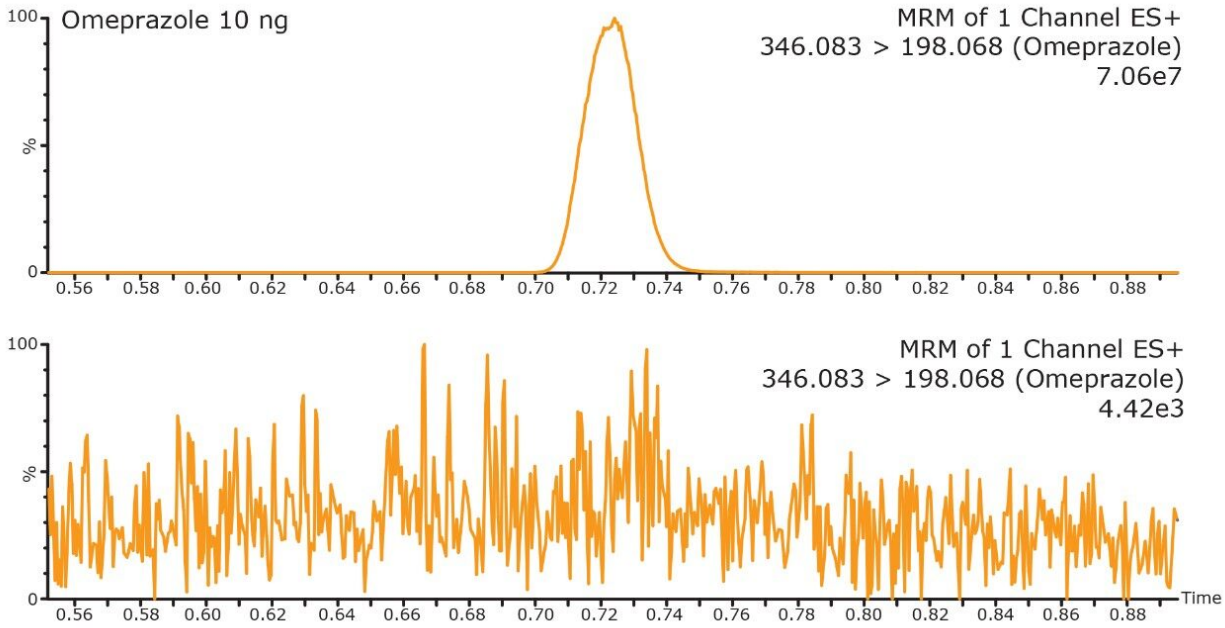


Figure 1. No carryover was observed for the first blank injection after the highest concentration standard at 10 ng/mL.

From the calibration plot, carryover was determined to be below 0.0005% as it was below the detection limits of the mass spectrometer. With measurable carryover eliminated from the analysis, the linearity across the calibration range was assessed. With a 1/x weighting used for the response across the range of 500 ag to 10 pg on column, a correlation coefficient of 0.99997 was achieved as seen in Figure 2, demonstrating the excellent linear calibration of omeprazole on the ACQUITY UPLC I-Class System coupled with the Xevo TQ-S.

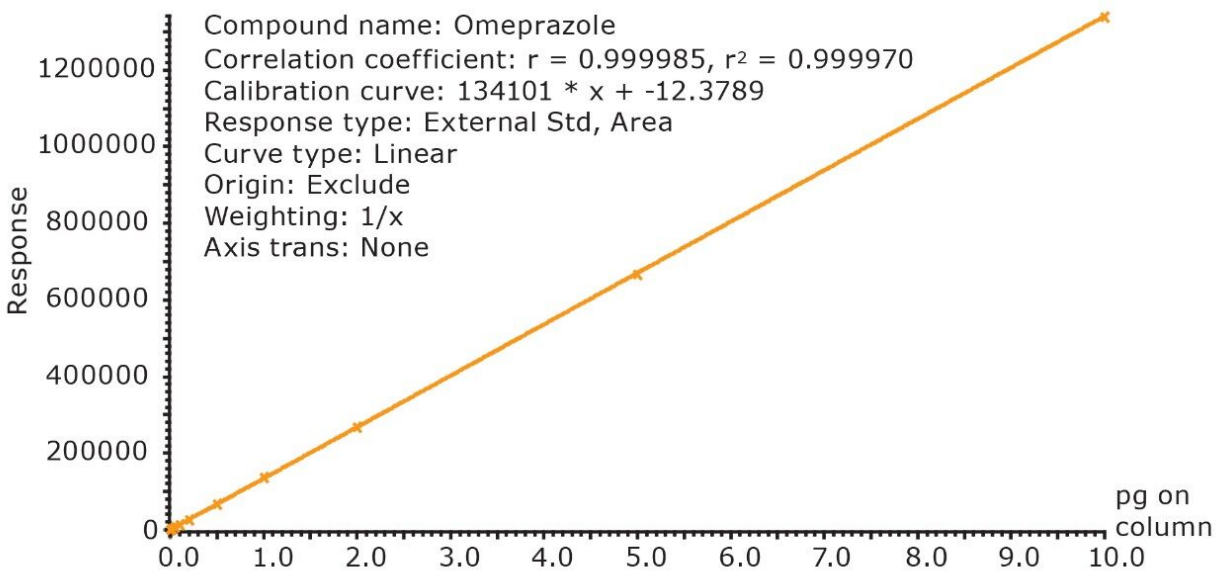


Figure 2. The calibration plot for omeprazole was linear from 500 fg/mL to 10 ng/mL or 500 ag to 10 pg on column.

Conclusion

The ACQUITY UPLC I-Class System is ideally suited for managing the challenging carryover requirements of sensitive LC-MS/MS methods requiring quantification across greater than four orders of magnitude. During the analysis of omeprazole, carryover was not detected on the Xevo TQ-S and was, therefore, reduced to below 0.0005%. Because sample carryover was minimized, calibration was achieved from 500 fg/mL to 10 ng/mL or 500 ag to 10 pg on column.

References

1. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070107.pdf

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ACQUITY UPLC I-Class PLUS System <https://www.waters.com/134613317>

Xevo TQ-S <https://www.waters.com/10160596>

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