INTRODUCTION

The greater sensitivity provided by Mass Detection as well as regulatory pressure to detect ever-lower levels of impurities are driving a need for improved carryover mitigation in LC applications. Carryover is sample left over from a previous injection that may interfere or co-elute with analytes of interest, often interfering with accurate quantitation. Furthermore, carryover that would otherwise be undetected in UV will often be evident with Mass Detection. While carryover has many potential sources, autosampler design plays a critical role in controlling instrument-related carryover and is the focus of this study. A key attribute of the autosampler is its ability to effectively remove sample from all surfaces the sample comes in contact with. In this study, an UHPLC system employing a flow through needle (FTN) design and configured with both Photodiode Array and Mass Detector will be evaluated for how well the FTN effectively manages both volumetric (caffeine) and adsorptive (chlorhexidine) carryover.

SAMPLING

Caffeine: Samples prepared in Water:Acetonitrile (ACN), 90:10. Low Calibrator: 0.08 µg/mL, Challenge Sample: 4000 µg/mL
Chlorhexidine: Samples prepared in water containing 0.1% TFA. Low Calibrator: 0.13 µg/mL, Challenge Sample: 2600 µg/mL
Post Challenge Blank: Sample diluent

METHODS

LC System: ACQUITY® Arc™ System
Detection: 2998 PDA Detector
Mobile Phase
- Water:ACN, 20:80
- Water:ACN, 50:50
- Water:ACN, 90:10 (premixed)
Column
- Cortecs C18+ 2.7 µm; 4.6 x 100 mm
- ACQUITY® UPLC® C18+ 2.7 µm; 2.1 x 100 mm
Injection Vol.: 1 µL
Flow Rate (mL/min)
- 1
- 1.2
- 4
- 5
Gradient
- 65%A-35%B
- 70%A-30%B
- 50%A-50%B
- 30%A-70%B
Needle Wash
- Water:ACN, 20:80
- 0.1% TFA in Water
- 0.1% TFA in ACN
PDA (A)
- 273 nm
- 299 nm
- 350 nm
Cone Voltage (V)
- 15
- 30
- 45
Probe:
- 600°C

Table 1. Volumetric Carryover: Caffeine is very polar, highly soluble and demonstrates low surface activity, it is frequently used to evaluate auto-samplers for volumetric carryover. This test measures the effectiveness of the injector wash cycle to completely flush the sample out of the autosampler. The challenge sample is followed by 5 injections of sample diluent, each from separate vials. These injections are used to evaluate carryover from the challenge sample and determine if there are un-swept volumes in the autosampler. Un-swept volumes will show up as carryover detected in subsequent blank injections following the challenge sample. The low volume design of the combined wash/injection port of ACQUITY Arc FTN-R™ (Fig. 1) is flushed with @ 25 wash/injection port volumes (1 mL) of wash solvent during the Default 6s post injection wash mode without impact to injection cycle time. Inadequate washing of wash port within an autosampler can increase the likelihood of cross contamination and inaccurate quantitation.

Table 2. Adsorptive Carryover: Chlorhexidine is known to have surface activity; it “sticks” to components of the autosampler and can be difficult to remove. This type of carryover is chemical in nature and is known as adsorptive carryover. This tests the ability of the autosampler wash cycle to manage a wide range of sample types. Chlorhexidine carry over undetected in the UV is detectable with MS but the ACQUITY Arc FTN-R is able to effectively remove the chlorhexidine in all wash modes.

INVESTIGATION OF NEEDLE WASH SOLVENT CHEMISTRY

To demonstrate how the wash solvent chemistry can impact adsorptive carryover the load of chlorhexidine was increased 3X and the type of organic, organic/aqueous ratio and pH were modified in order to determine the optimal wash solvent for the application. In this case unmodified Water:ACN (20:80) provided the best carryover performance. Wash solvent chemistry should be investigated and optimized as part of LC method development.

CONCLUSION

The efficient design of the ACQUITY Arc FTN-R easily manages both volumetric and adsorptive/chemical carryover providing greater flexibility to manage carryover for multiple sample types and increased reliability of quantitation for both UV and MS applications.