Injection linearity, precision, and carryover for ExionLC™ AD system

Outstanding UHPLC capabilities, performance, and robustness

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For the most demanding and challenging UHPLC analyses, the SCIEX ExionLC AD System has been engineered to meet and surpass the most exacting standards of analytical LC-MS laboratories. A high pressure dual piston system rated to 1300 bar at flow rates of up to 3 mL/min allowing users to access almost any UHPLC column or conditions. The use of small particle columns in the range of 1.5 to 3.0µ for UHPLC dramatically improves peak resolution and capacity while reducing overall analytical run times.

Figure 1. SCIEX ExionLC AD System coupled to SCIEX QTRAP® 4500 System (UV Vis detector not shown)

In this application note we highlight the injection linearity, precision, and carryover using a commercially available HPLC test mixture.(Supelco HPLC Gradient System Diagnostic Mix, Product # 4-8271). The benefits of using a commercial mixture is direct performance comparisons between different LC systems and troubleshooting causes of poor instrument performance. A SCIEX QTRAP® 4500 coupled with a ExionLC AD system with a UV-Vis detector was chosen as the test LC-MS platform

For rapid chromatographic analysis a 2.6 µ Phenomenex Kinetix C-18 column (2.1 x 50 mm) was chosen as the LC column using a simple gradient of Water and Acetonitrile both containing 0.1% Formic acid. The analytical run including equilibration was 10 minutes to ensure maximum reproducibility (Figure 2.)

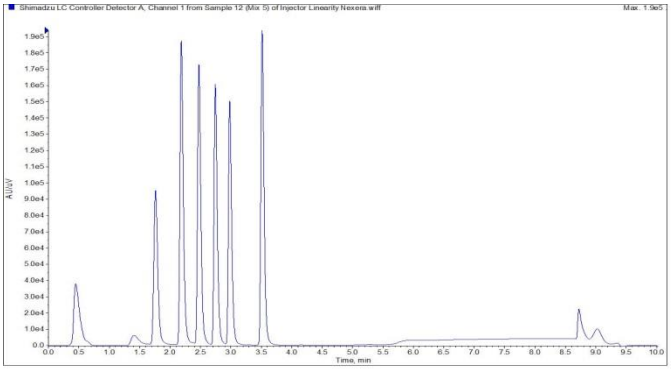

Figure 2. UV-Vis trace at 254nm showing elution of Diagnostic Mix components. Order of elution major peaks from 0.50 to 4.40 minutes: Uracil, Phenol, Methyl paraben, Ethyl paraben , Propyl paraben, Butyl paraben, Heptyl paraben.

UV-Vis detection was performed using an Exion LC UV detector (not shown) operating at 254nm.

Injection Linearity calculation were performed using 5 replicate injections at volumes between 0.50 to 50.0µL. Area counts for Phenol (Figure 3).and Heptyl paraben were calculated and plotted against corresponding injection volumes to determine linearity.

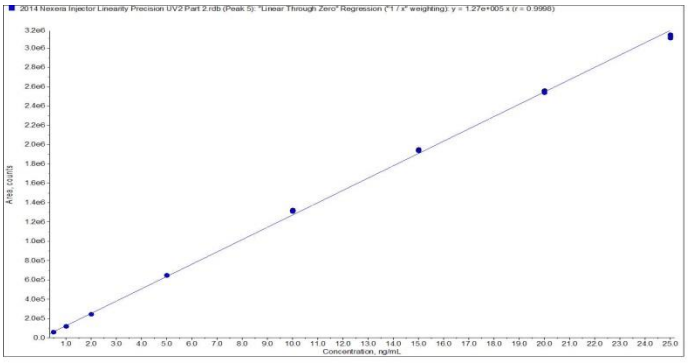

Figure 3. Injection linearity for replicate injections of HPLC Gradient System Diagnostic Mix (Phenol). Linearity using a linear regression analysis was > 0.9996

Using the individual coefficients of variation from the replicate injection the %CV at various injection volumes could be

determined. Figure 4. Shows that the %CVs are typically below 0.5% except at very small injection volumes.

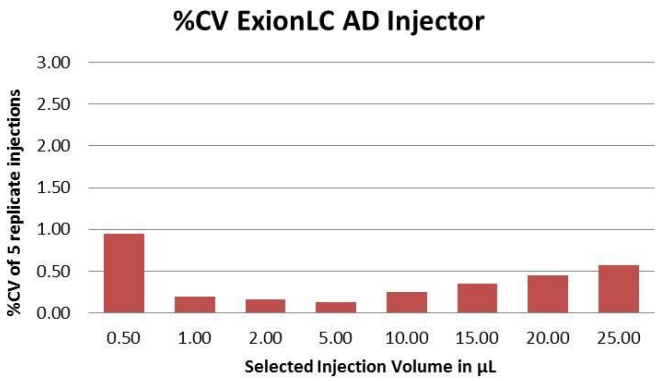

Figure 4. Schematic representation of typical %CVs obtained using the ExionLC 300 autosampler. Nominal %RSD are typically below 0.5% in practice.

For testing carryover, a solution of Amitriptyline (285 ng/mL) was prepared in 10% acetonitrile to provide approximately 20-30 million area counts of response for the analyte (Figure 6.). Ten series of 5µL injections were performed. Each injection series consisted of 2 clean system blank vials containing a solution of water and 10% acetonitrile with 0.1% formic acid. Following a single injection of the high level analytes, carryover was determined using a series of blank samples of 10% acetonitrile.

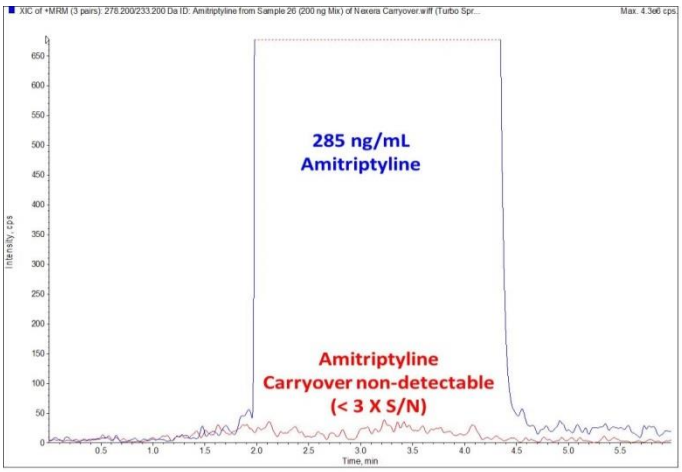

Figure 6. Extracted Ion Chromatogram (XIC) showing the 3 carryover test analyes, Diphenhydramine, Amitryptiline, and Clomipramine.

Rinsing options, rinsing time, and total flush time were selected to minimize total wash time while provide minimal carryover. The total time increase is dependent on the number of washes selected, the time, and the total volume used during each wash step (Figure 7.)

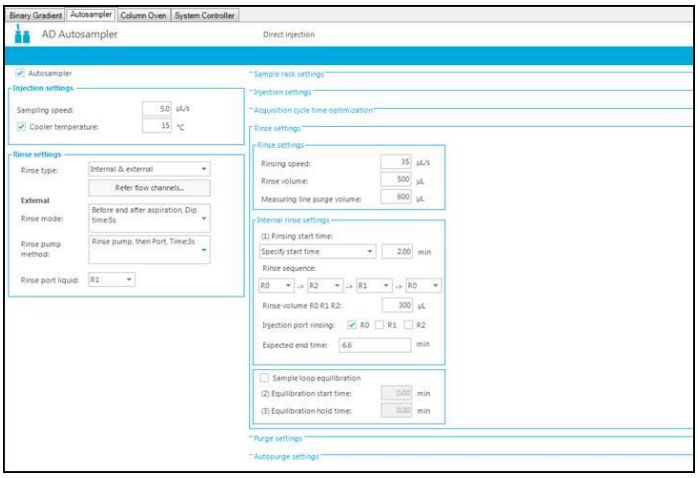

Figure 7. Injector wash program showing Rinsing Mode, Rinse Dip Time, and Flush Volume.

In general a mixture of 20% Acetonitrile, 20% Methanol, 20% Isopropanol in Water with 0.1% formic acid is usually effective for a broad range of analytes. For purposes of quantitation, changes to the solvent wash composition may be needed for low carryover components. Improvements in carryover performance are usually obtained by increasing the rinse dip times and volumes rather than trying to use more aggressive wash solvent compositions.

In conclusion, the ExionLC AD UHPLC system can be optimized with a series of wash solutions to minimize carryover for target analytes to virtually non-detectable levels. Typically, one must adjust both the solvent strength and the ionic properties to obtain minimal carryover under experimental conditions.

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