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

Highly reliable HPLC system with UHPLC capabilities, performance, and robustness

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Highest quality components and focused design considerations give rise to exceptional data quality. The SCIEX ExionLC AC system has been engineered to meet the demanding standards of modern analytical LC/MS laboratories. A high pressure dual piston system rated to 660 bar at flow rates of up to 2mL/min allowing users to access a wide range of UHPLC columns using fused-core particles in the 2-3 um range. These fused-core particles dramatically improve peak resolution and capacity while maintaining lower pressures that conventional UHPLC systems.

Figure 1. SCIEX ExionLC AC system coupled to SCIEX QTRAP® 4500 system (UV 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. An AB SCIEX QTRAP® 4500 system coupled with a ExionLC AC system with an optional UV 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.)

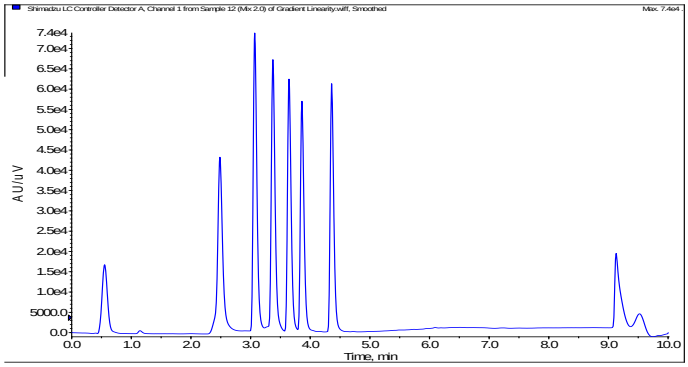

Figure 2. UV-Vis trace at 254nm showing elution of Diagnostic Mix components. Order of elution major peaks from 0.50 to 4.40 minutes: Uracil, Phenol, Methyl paraben, Ethyl paraben , Propyl paraben, Butyl paraben, Heptyl paraben.

UV-Vis detection was performed using an ExionLC AC 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.

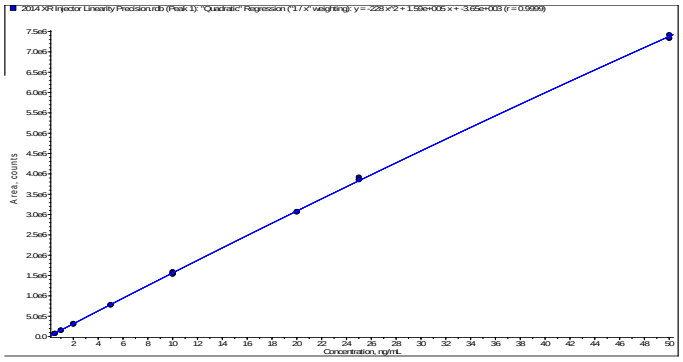

Figure 3. Injection linearity for replicate injections of HPLC Gradient System Diagnostic Mix (Phenol). Linearity using a linear regression analysis was > 0.9996

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 2% except at very small injection volumes.

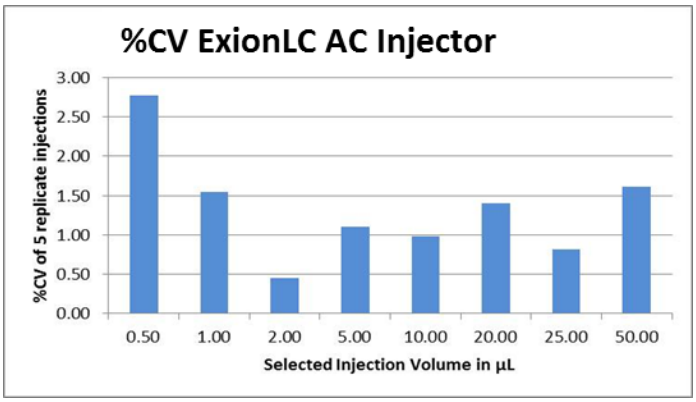

Figure 4. Schematic representation of typical %CVs obtained using the ExionLC AC autosampler. Nominal %RSD are typically below 2% in practice.

For testing carryover, 2 different experiments were performed. As a general surrogate for HPLC compounds an injection of the HPLC Gradient Diagnostic Test Mix (25µL) was performed on a 2.1 x 50 mm Kinetex 2.6µ C-18 column. Elution was performed using a conventional Water:Acetonitrile gradient. Following the injection of the high standard, carryover was monitored using blank water solutions for 4 successive injections. As shown in Figure 5. carryover was below 0.005% for all compounds in both UV and MS monitoring modes.

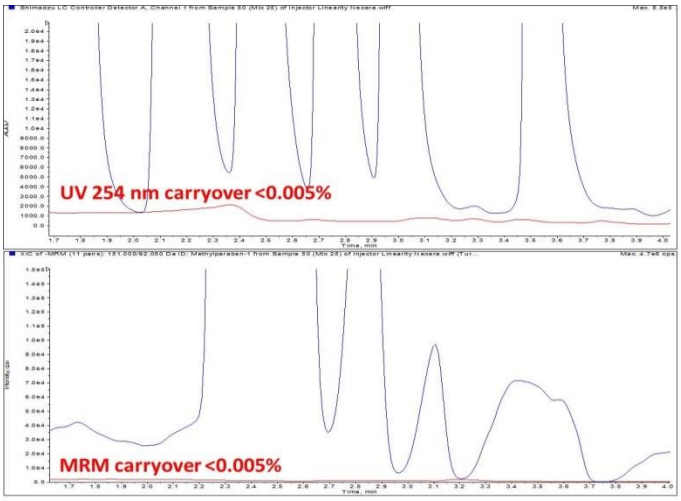

Figure 5. Carryover example showing negligible carryover by either UV absorbance or MRM MS monitoring.

For additional carryover testing, a solution of Amitriptyline was prepared in 10% acetonitrile to provide approximately 20-30 million area counts of response for main MRM transition (Figure 6.). The final concentration was approximately 285 ng/mL Amitriptyline. 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 5 blank samples of 10% acetonitrile. Each series contained unique sample material to prevent any carryover from successive vials. The % carryover from the first blank following the high level sample was calculated as indicated below:

- % carryover = (Analyte area in first carryover blank) / (Analyte area in high level standard) * 100

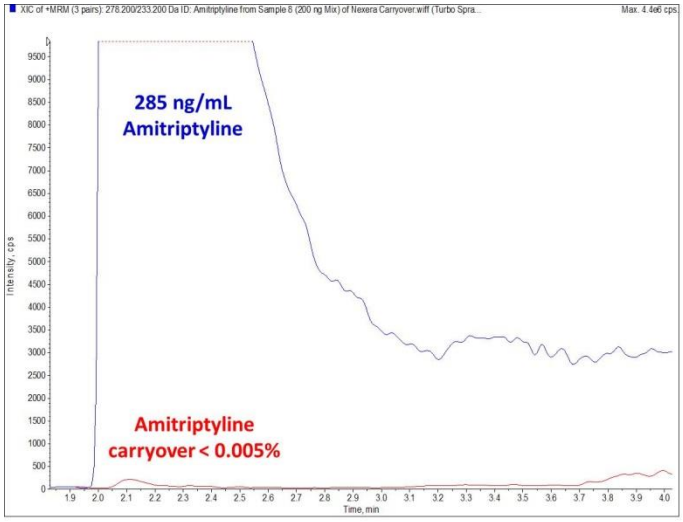

Figure 6. Extracted Ion Chromatogram (XIC) showing the carryover results for Amitriptyline at 285 ng/mL.

Using a simple rinse solvent composition of 20% Acetonitrile, 20% Methanol, 20% Isopropanol in Water with 0.2% formic acid the carryover levels could be reduced to very low levels. In practice, a carryover level of 0.005% easily translates into a linear dynamic range of greater than 4 orders of magnitude in a conventional analytical method.

The results of linearity, precision, and carryover clearly show the ExionLC AC series as an excellent choice for a versatile, expandable UHPLC capable HPLC system.

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