Investigating the performance attributes of an ultra-high pressure liquid chromatography system

Flexibility, performance and robustness of the ExionLC 2.0 system

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An important aspect of a successful mass spectrometry (MS) experiment is combining it with some form of high-quality upfront sample separation. In majority of cases, this is achieved using liquid chromatography (LC). As the sensitivity, speed and selectivity of mass spectrometers continually improves, faster, more powerful LC-MS workflows are achievable. LC-MS users also desire workflows with reduced sample preparation. They require detection levels to be attainable with less sample injected onto the LC-MS system. This puts pressure on the LC system to be able to handle increasingly complex matrices and provide good injection reproducibility for sub-5 µL volumes. In addition, more sensitive MS instruments with detection capabilities at lower levels have requirements for even lower carryover on the LC system, to reduce the risk of false positive results.

In this technical note, the key performance attributes of the SCIEX ExionLC 2.0 system were investigated, specifically the injection linearity and precision. Injection routines were also investigated to assess the system carryover with the various approaches. As is typical, flow rate precision and resulting retention time reproducibility was studied. Either a SCIEX Triple Quad 5500 system or the integrated optional ExionLC 2.0 diode array detector HS were used for detection, depending on the specific test performed.

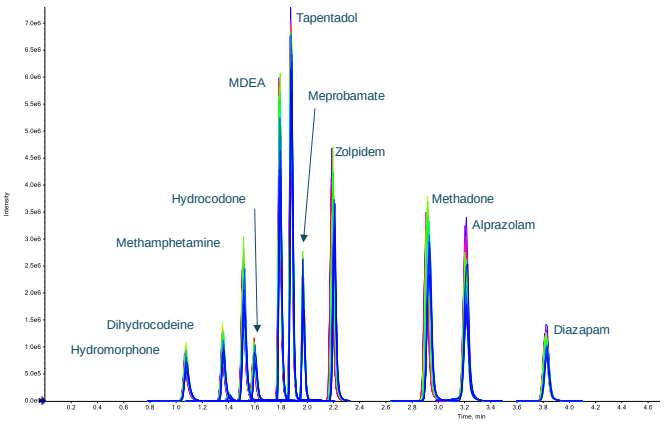


Figure 1. Retention time precision. Overlaid extracted ion chromatograms of select compounds from 100 repeat injections of the same standard solution of forensic drug compounds using the ExionLC 2.0 system. Typical retention time stability achieved is <0.3% RSD.

Key features of the ExionLC 2.0 system

- High-pressure, dual, serial piston pump rated to 860 bar at flow rates of 0.001 to 2 mL/min for maximum flexibility
- Precise and stable solvent flow delivering less than 0.3% RSD retention time variation
- Accurate and precise quantification results with injection linear coefficient of determination performance (r^2) > 0.999 and precision <1% coefficient of variation for injection volumes from 0.5 to 50 µL for most compounds studied; 0.5 to 25 µL for all compounds
- Multiple wash solutions and extended needle wash capabilities to minimize carryover and reduce the false positive rate and the need for repeat extractions and re-injections
- Ability to accommodate a wide range of UHPLC columns for fast separations and high throughput
- Achieve high peak resolution and capacity at lower pressures using fused core columns with particles in the 2-3 µm range

Methods

Material and solutions: Injection linearity and precision were investigated using a Supelco HPLC gradient system diagnostic mix (P/N 4-8271) containing uracil (dead volume retention marker), phenol, methyl paraben, ethyl paraben, propyl paraben, butyl paraben and heptyl paraben. For carryover determination, caffeine (Millipore Sigma P/N C0750), chlorhexidine (Millipore Sigma Pharmaceutical Secondary Standard; Certified Reference Material P/N: PHR1421) and amitriptyline (Cerilliant P/N: A-923 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material) were used. The following solutions were prepared: caffeine, 4 mg/mL and 10 µg/mL in methanol/water (20%/80%; v/v); chlorhexidine, 500 ng/µL and 1.25 ng/µL in water + 0.1% formic acid; amitriptyline, 285 ng/mL and 1 ng/mL in methanol/water (20%/80%; v/v). For flow rate precision determination, a total of 18 drugs and 11 deuterated internal standards were obtained from Cerilliant and combined in one solution.

Chromatography: LC separations were performed using the SCIEX ExionLC 2.0 system. For injection linearity and precision determination a 2.6 µm Phenomenex Kinetex® C18 column (2.1 x 50 mm, P/N: 00B-4462-AN) was chosen, and a simple gradient of water and acetonitrile, both containing 0.1% formic acid, was used. The analytical run including equilibration was 10 minutes to ensure maximum reproducibility. The syringe speed was set to low and the speed factor to 0.1. For caffeine and chlorhexidine carryover determinations, a flow restrictor (15 m yellow PEEK tubing, 0.18 mm ID) with 2 low dead volume unions was used to ensure measurements are made with a back pressure of about 140 bar. For amitriptyline carryover and flow-rate precision determinations (using 18 compound drug mixture), a 2.6 µm Phenomenex Kinetex Phenyl-Hexyl column (4.6 x 50 mm, P/N: 00B-4495-EO) was chosen. Mobile phase A was ammonium formate in water. Mobile phase B was formic acid in methanol. The LC flow rate was 1 mL/min and the LC run-time was 6.5 minutes. The SCIEX ExionLC 2.0 system autosampler was used in the standard configuration consisting of a 250 µL syringe, 100 µL sample loop, 250 µL buffer tubing and 15 µL needle tubing. For all injections, the µL pick-up plus mode was selected in order to minimize the injection cycle time whilst optimizing sample consumption.

Mass spectrometry and diode array detector conditions: Ultraviolet-visible detection was performed using an ExionLC 2.0 system with integrated ExionLC 2.0 diode array detector HS, equipped with a 10 mm, 10 µL, 300 bar flow-cell. For the Supelco HPLC gradient system diagnostic mix, the detector was operated at 254 nm and, for the caffeine experiments, at 272 nm.

All diode array detector data collection was collected with a data rate of 10 Hz.

Mass spectrometry used a SCIEX Triple Quad 5500 system. The ionization source was operated using electrospray ionization (ESI) in positive mode. Three MRM transitions were monitored for chlorhexidine and two for amitriptyline.

Data acquisition was performed using Analyst software 1.7.1 with Components for the ExionLC 2.0 system. It is worth noting that The ExionLC 2.0 system is also fully supported for instruments in which data acquisition is performed using SCIEX OS software.

Data processing: Data processing of both mass spectrometry and diode array detector acquired data was performed using SCIEX OS software 2.0.1 in which calibration curves, precision and accuracy statistics were generated.

Injection linearity and precision

First a mixture of compounds was analyzed using the integrated ExionLC 2.0 diode array detector HS for detection. Very good separation of the components was achieved (Figure 2).

Replicate injections (n=5) were performed across a broad injection volume range of 0.5 and 25 µL using the µL pick-up plus injection mode. The area counts were measured and plotted against corresponding injection volumes for each component in the Sulpelco HPLC gradient system diagnostic mix.

Figure 3 shows that the linear coefficient of determination (r^2) is > 0.999.

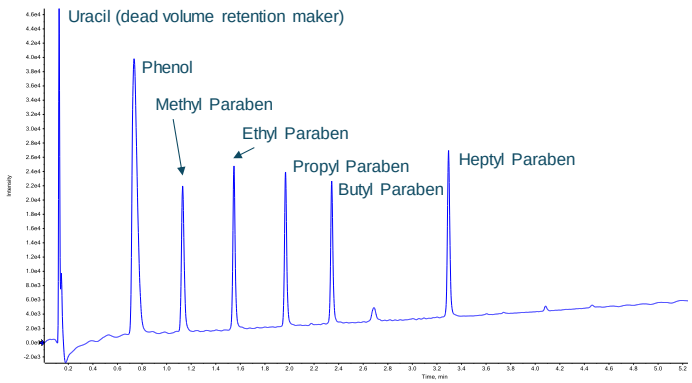


Figure 2. High quality separation of the diagnostic mix components. UV-Vis trace at 254 nm with elution order of major peaks from earliest to latest: uracil, phenol, methyl paraben, ethyl paraben, propyl paraben, butyl paraben and heptyl paraben.

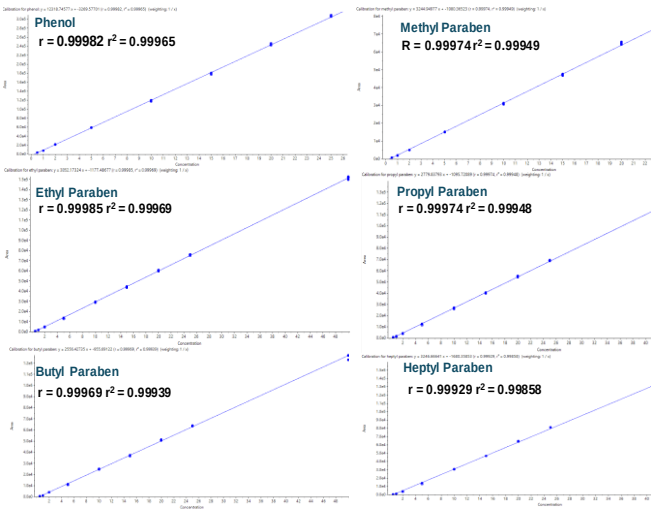


Figure 3. Injection linearity. Replicate injections (n=5) of HPLC gradient system diagnostic mix for injection volumes between 0.5 and 50 µL. Linearity using a linear coefficient of determination (r^2) was > 0.999 for most compounds 0.5 to 50 µL; 0.5 to 25 µL for all compounds.

The injection reproducibility was also computed from this same data, for all the various injection volumes. Very low variance was observed, even for the very low injection volumes. Figure 4 shows that the %CVs for all injection volumes are below 1%.

When optimizing sample consumption, the µL pickup plus injection mode is the preferred option as no excess sample is utilized. The injection sequence has been optimized so that the time to injection is not compromised over other injection modes which can be made in less than 17 seconds.

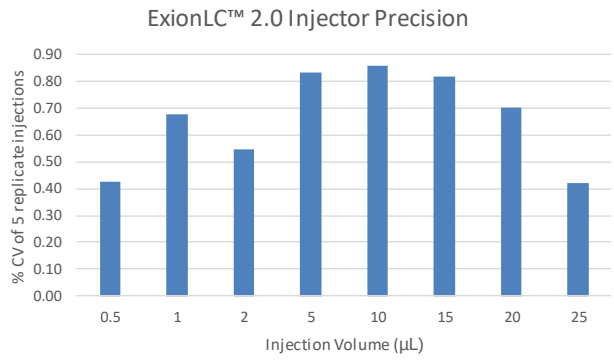


Figure 4. Injection precision. Replicate injections of gradient system diagnostic mix were performed across a range of injection volumes and the injection reproducibility was computed. Measured %CVs were found to be <1% for all injection volumes.

Injection carryover determination

For determining carryover, 3 different compounds were selected that are prone to adsorption: caffeine, chlorohexidine and amitriptyline. For each compound, carryover was determined using the following sequence of 6 injections: Blank 1, Low concentrated standard, High concentration (highly concentrated standard, exceeding the linear range of the detector), Blank 2, Blank 3, Blank 4. The following equation was used to calculate carryover.

$$Carryover = \frac{A_{Blank2} - A_{Blank1}}{A_{low} * (\frac{C_{high}}{C_{low}})} * 100$$

A_{Blank1}	*Area of first blank injection*
A_{Blank2}	*Area of second blank injection*
A_{low}	*Area of the standard with low concentration*
C_{high}	*Concentration of sample with high concentration*
C_{low}	*Concentration of sample with low concentration*

As shown in Figure 5, carryover for caffeine was 0.0006% with a simple wash cycle in the UV experiment.

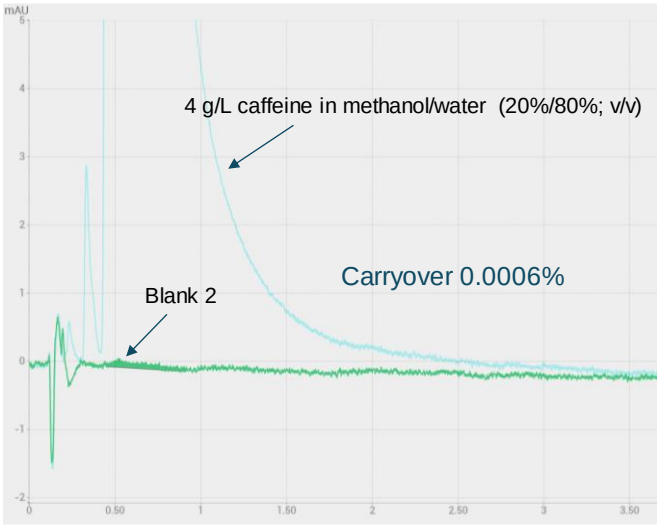


Figure 5. Caffeine carryover. UV-Vis trace at 272 nm of the high standard overlaid with the subsequent blank. Very low carryover was observed with a minimal wash method.

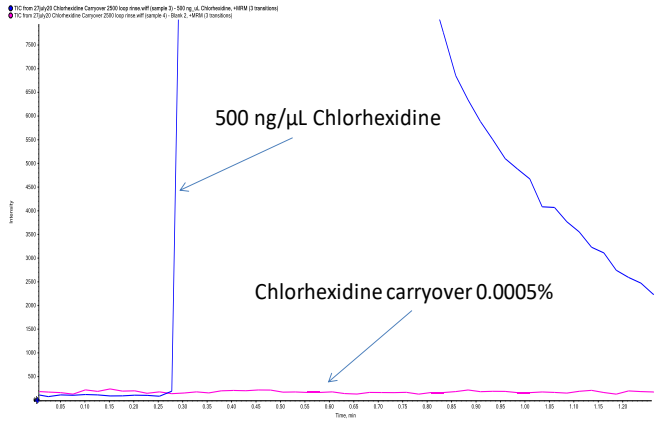


Figure 6. Chlorhexidine carryover. Extracted ion chromatograms showing a challenging carryover example in which negligible carryover was achieved.

The more challenging experiment is using the chlorhexidine compound, which is notoriously 'sticky'. Here, carryover was measured using the more sensitive MS experiment. Multiple wash solutions and extended needle washes were required to achieve desired results in this case, but the flexibility of the autosampler enabled construction of an optimized wash method. The total wash time is dependent on the total volume used during each wash step. Varying wash solvent and transport solvent conditions were investigated to minimize total wash time while providing minimal carryover. Figure 6 shows the chlorhexidine carryover was calculated to be 0.0005% using the optimized wash volumes.

For additional carryover testing, 1 ng/mL and 285 ng/mL amitriptyline solutions were prepared in 10% acetonitrile, the latter providing approximately 20 million area counts for the most intense MRM transition in the MS experiment.

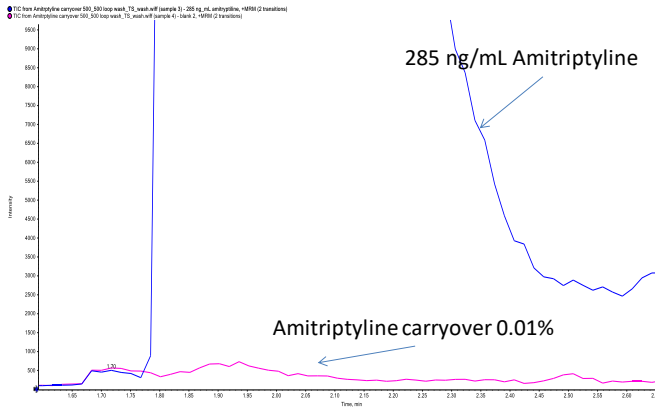
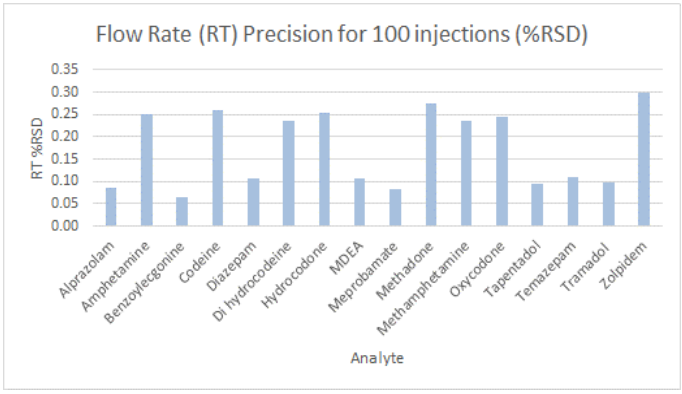


Figure 7. Amitriptyline carryover. Extracted ion chromatograms showing the carryover results for amitriptyline at 285 ng/mL.

Figure 7 shows the amitriptyline % carryover from the first blank following the high concentrated standard was determined to be 0.01% using a minimal volume wash solvent composed of 20% acetonitrile, 20% methanol, 40% water and 20% isopropanol by volume with 0.1% formic acid.

Flow rate precision

Figure 1 shows the retention time stability with overlaid chromatograms from 100 consecutive injections. As shown in Figure 8 (top), the retention time precision of each of the analytes across a range of retention times for these injections is less than 0.3% RSD. For most compounds tested, the maximum retention time difference over 100 injections was <1 second (Figure 8, bottom).



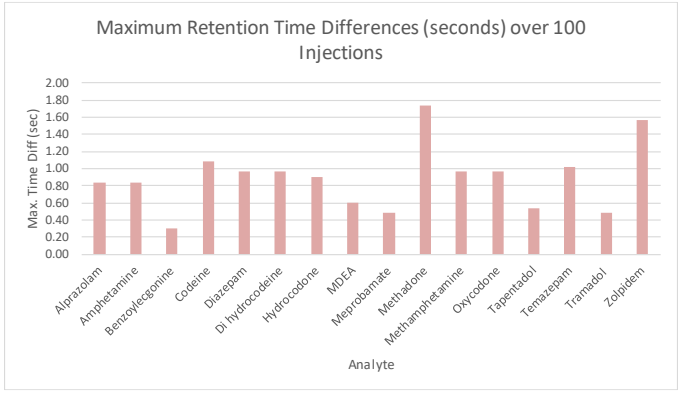


Figure 8. Flow rate precision. Retention time variability (%RSD) for select analytes over 100 injections is plotted per analyte and all are below 0.3% RSD (top). This translates to retention time differences of less than 2 seconds over 100 injections for these analytes.

Conclusions

The ExionLC 2.0 system is a flexible and robust UHPLC system that is suitable for today's challenging LC-MS workflows.

- High autosampler precision and accuracy are essential for quantitative experiments. As demonstrated here, this system can achieve <1% CV across the full range of injection volumes tested (0.5 – 50 µL) with excellent linearity (linear correlation coefficient (r) regression analysis was > 0.999 for all analytes).
- Low carryover is also critical in many LC-MS applications. The flexible autosampler wash options (with multiple wash solutions and extended needle wash capabilities) were shown to reduce carryover, even for a very challenging analyte, down to 0.0005%.
- Retention time precision can also be critical when running multi-analyte panels and time scheduled MRM assays. High flow rate precision of the ExionLC 2.0 system provided RSD of <0.3% for the analytes tested here.

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