

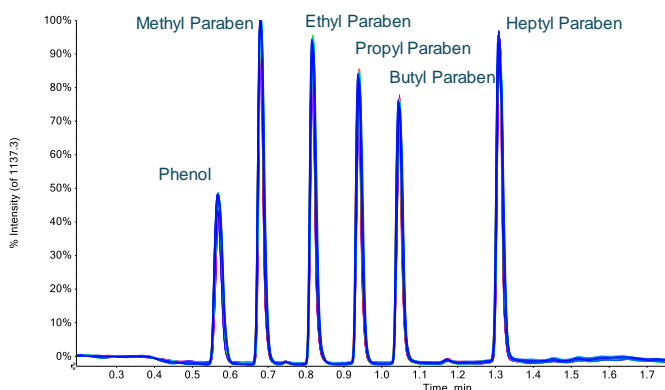
# Performance attributes of the ExionLC 2.0+ system ultrahigh pressure liquid chromatography system

## Flow stability and injection performance

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Increasing demands on the modern-day laboratory require finding ways to increase analytical throughput and expanding the lab's sample capacity. Mass spectrometry has become a popular analytical tool in many labs and liquid chromatography is still the most common sample introduction technology for high-quality up-front analyte separation prior to mass spectrometry. Ultrahigh pressure liquid chromatography, using sub 2  $\mu\text{m}$  column particle packing, provides the ability to run fast baseline separation of compounds with minimal equilibration times, aiding in both time and cost reductions over conventional liquid chromatography.

As the sensitivity, speed and selectivity of mass spectrometers continually improves, faster, more powerful LC-MS workflows are achievable when the full potential of UHPLC is leveraged. In this technical note, the key performance attributes of the SCIEX ExionLC 2.0+ system were investigated, specifically the flow rate precision and resulting retention time reproducibility for fast separation analysis. As is typical, the injection linearity and precision were studied. A SCIEX Triple Quad 5500+ system coupled with an ExionLC 2.0+ system was chosen as the test LC-MS/MS platform. To allow for direct LC pump and autosampler performance investigations, an integrated (optional) ExionLC 2.0+ diode array detector HS was used for ultraviolet-visible (UV-Vis) detection experiments.



**Figure 1. Retention time precision for high pressure analysis.** Overlaid ultraviolet-visible traces from 100 repeat injections of the same standard Supelco HPLC gradient diagnostic mix, using the ExionLC 2.0+ system operating at 1100 bar (16,000 psi). The retention time stability for this test across the 100 injections was <0.15 % RSD.



## Key features of the ExionLC 2.0+ system

- Dual high-pressure serial piston pumps, rated to 1240 bar (18,000 psi) at flow rates of 0.001 to 5 mL/min, for maximum flexibility in running fast gradient separations and maximizing laboratory throughput
- The ability to accommodate a wide range of UHPLC columns, notably those with sub 2- $\mu\text{m}$  column particle packing, making it possible to run fast baseline separations of compounds with minimal equilibration times, when compared to conventional liquid chromatography
- Novel camshaft geometry pumps providing precise, durable and stable solvent flow delivering less than 0.15% relative standard deviation (RSD) retention time variation, maximizing result consistency and assay uptime
- A fully automated piston pump simplifying method setup with linear movement that is adjusted automatically, in real time, and reduces pulsation to very low levels that are independent of flow rate, backpressure and eluent type
- A function for automatically purging the flow lines with mobile phase, allowing streamlined changeover between different methods

- Accurate and precise quantification results, with injection linearity performance of  $r > 0.999$  and precision  $<1\%$  CV for low injection volumes from 0.5 to 5  $\mu\text{L}$  for confidence in reporting compound amounts present

## Methods

**Material and solutions:** Flow rate and injection precision, as well as injection linearity, of the SCIEX ExionLC 2.0+ system, 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.

**Chromatography:** LC separations were performed using the SCIEX ExionLC 2.0+ system. For injection linearity and precision determination, a 2.6  $\mu\text{m}$  Phenomenex Kinetex® C18 column (2.1 x 50 mm, P/N: 00B-4462-AN) was chosen, and a simple gradient of water and acetonitrile, containing 0.1% formic acid, was used. The syringe speed was set to low and the speed factor to 0.1. For determining the flow rate precision at high pressure, an Acquity UPLC HSS T3 1.8  $\mu\text{m}$  2.1 x 50 mm was selected. Mobile phase was water/acetonitrile. The LC flow rate was 1.2 mL/min and the LC run-time was 3.25 minutes, with a maximum pressure of 1,100 bar (16,000 psi). The 1.8  $\mu\text{m}$  particle column was replaced with a Phenomenex Synergi 4  $\mu\text{m}$  Fusion-RP 2 x 50 mm column (PN 00B-4424-B0) to investigate the low-pressure method performance on this high-pressure system with a minimum pressure of 110 bar (1,600 psi). The SCIEX ExionLC 2.0+ system autosampler was used with a configuration consisting of a 250  $\mu\text{L}$  syringe, 250  $\mu\text{L}$  buffer tubing and 15  $\mu\text{L}$  needle tubing. A 10  $\mu\text{L}$  sample loop was used to test the partial loop injection mode with low injection volume injections. In order to optimize sample consumption and minimize the injection cycle time, the  $\mu\text{L}$  pick-up plus injection mode was selected for the 100 injections retention time and area reproducibility experiments, which required using a 100  $\mu\text{L}$  sample loop.

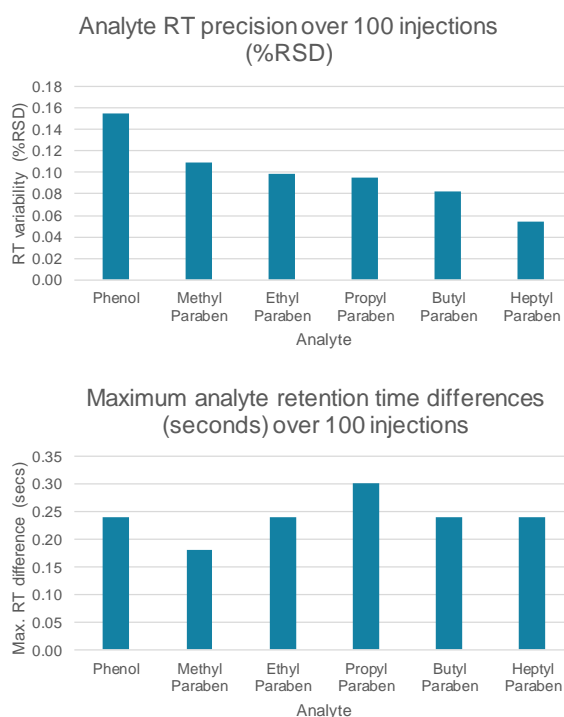
**Diode array detection:** Ultraviolet-visible detection was performed using an ExionLC 2.0+ system with integrated ExionLC 2.0 diode Array detector HS, equipped with a 10 mm, 10  $\mu\text{L}$ , 300 bar flow cell. For the Supelco HPLC Gradient System Diagnostic Mix, the detector was operated at 254 nm. All diode array detector data collection was performed with a data rate of 10 Hz.

**Mass spectrometry:** 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 diode array detector acquired data was performed using SCIEX OS software 2.0.1 in which calibration curves, precision and accuracy statistics were generated.

## Flow rate precision

Figure 1 shows overlaid chromatograms from 100 consecutive fast, 78 second gradient separations and the retention time stability of the SCIEX ExionLC 2.0+ system running with a pressure of 1100 bar. As shown in Figure 2, the retention time precision of each of the analytes eluting at different stages of the gradient for these injections is less than 0.15% RSD. For compounds tested, the maximum retention time difference over 100 injections was less than 0.3 seconds.



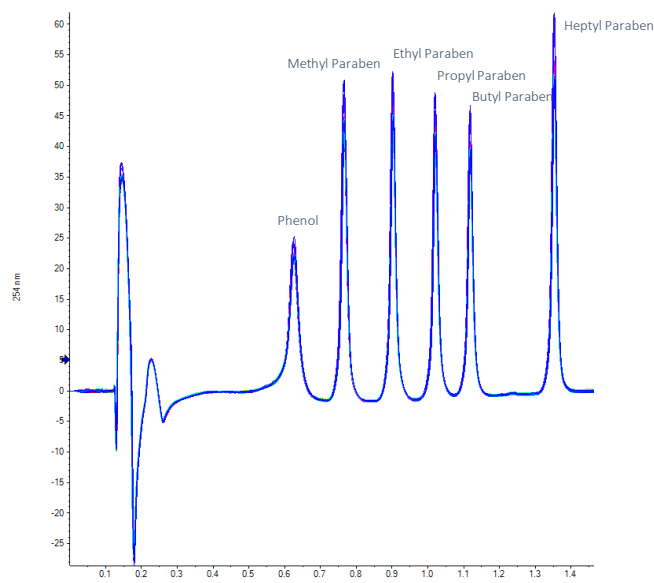
**Figure 2. High-pressure method flow rate precision.** Retention time variability (%RSD) for 100 injections is plotted per analyte and all are below 0.15% RSD (top). This translates to retention time differences of less than 0.3 seconds over 100 injections for these analytes (bottom) for this high-pressure method (1100 bar).

The flow rate precision experiment was repeated using the same gradient but replacing the 1.8  $\mu\text{m}$  particle column with a Phenomenex Synergi 4  $\mu\text{m}$  Fusion-RP 2 x 50 mm column to produce lower pressure conditions. This was to allow investigation of the low-pressure method performance on this high-pressure system, running with a minimum pressure of 110 bar (1,600 psi).

**Table 1. Retention time precision observed for the low-pressure test.**

|                            | Phenol | Methyl Paraben | Ethyl Paraben | Propyl Paraben | Butyl Paraben | Heptyl Paraben |
|----------------------------|--------|----------------|---------------|----------------|---------------|----------------|
| Average Retention Time     | 0.62   | 0.77           | 0.90          | 1.02           | 1.12          | 1.35           |
| Retention Time Std. Dev.   | 0.0008 | 0.0007         | 0.0007        | 0.0006         | 0.0006        | 0.0006         |
| Retention Time % Std. Dev. | 0.1236 | 0.0959         | 0.0730        | 0.0632         | 0.0544        | 0.0462         |
| Max. Difference (sec)      | 0.22   | 0.22           | 0.19          | 0.24           | 0.18          | 0.24           |

Figure 3 shows overlaid chromatograms from 100 consecutive injections and the retention time stability obtained from the SCIEX ExionLC 2.0+ system running at conventional, lower pressure conditions [110 bar(1,600 psi)].



**Figure 3. Retention time precision at low pressure.** Overlaid ultraviolet-visible traces from 100 repeat injections of the same standard Supelco HPLC Gradient Diagnostic Mix using the ExionLC 2.0+ system but operating at 110 bar (1,600 psi). Here the retention time precision was also very good,  $\leq 0.12\%$  RSD across the 100 injections.

Table 1 shows retention time variability results for these 100 injections, and the flow rate precision of the SCIEX ExionLC 2.0+ system when running at 110 bar (1,600 psi). The retention time precision of each of the analytes eluting at different stages of the gradient for these injections is  $\leq 0.12\%$  RSD. For compounds tested, the maximum retention time difference over 100 injections was less than 0.3 seconds.

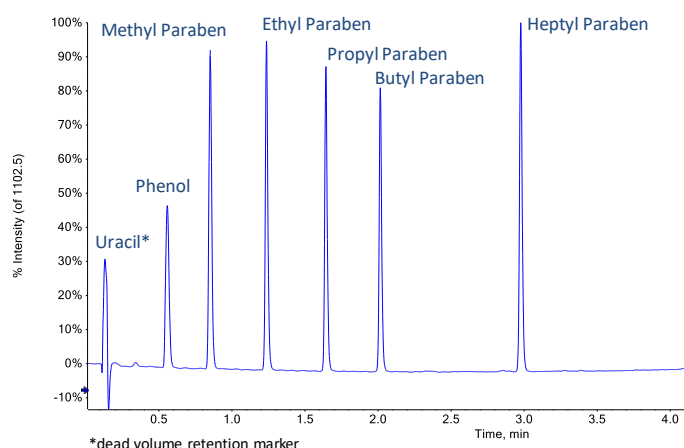
The results show that there is no detrimental effect on LC pump stability with respect to retention time when using the SCIEX ExionLC 2.0+ system at pressures as low as 110 bar. This demonstrates that the SCIEX ExionLC 2.0+ system can be used for both ultrahigh pressure liquid chromatography workflows,

using sub 2  $\mu\text{m}$  column particle packing, and conventional low-pressure methods with columns of larger particle size.

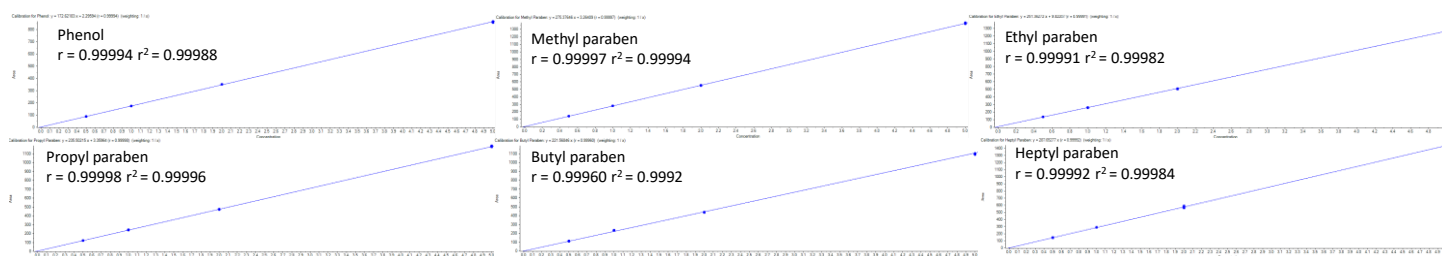
Utilizing the  $\mu\text{L}$  pickup plus injection mode in these experiments ensures no excess sample is used. The injection sequence has been optimized so that the time to injection is not compromised over other injection modes which can be made in less than 17 seconds.

## Injection linearity and precision

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 4). Replicate injections ( $n=5$ ) were performed for small injection volumes between 0.5 and 5  $\mu\text{L}$  to investigate the use of a 10  $\mu\text{L}$  sample loop and partial loop injection mode. The area counts were measured and plotted against corresponding injection volumes for each component in the Supelco HPLC Gradient System Diagnostic Mix.



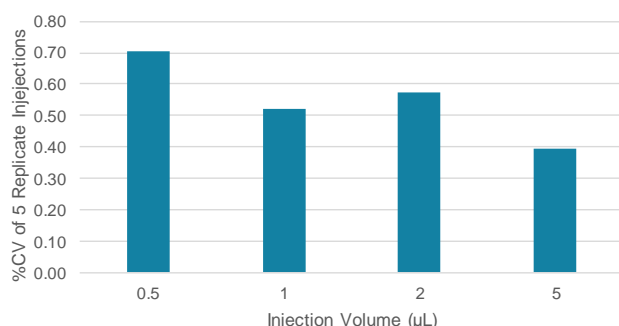
**Figure 4. High quality separation of the HPLC diagnostic mix components.** Ultraviolet-visible trace at 254 nm is shown for the seven compounds in this injection test (5  $\mu\text{L}$  injection volume).



**Figure 5. Injection linearity.** Replicate injections (n=5) of HPLC Gradient System Diagnostic Mix for injection volumes between 0.5 and 5  $\mu$ L are shown. Linearity using a linear correlation coefficient (r) regression analysis was > 0.999.

Figure 5 shows that the linear correlation coefficient (r) regression analysis for all compounds is > 0.999.

The injection reproducibility was also computed from these same data, for all the various injection volumes. Very low variance was observed, even for the very low injection volumes. Figure 6 shows that the %CVs for all injection volumes are below 1%.



**Figure 6. Injection precision.** Replicate injections of Gradient System Diagnostic Mix were performed across a range of low injection volumes and the injection reproducibility was computed. Measured %CVs were found to be <1% for all injection volumes.

## Conclusions

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

- Retention time precision is critical when running multi-analyte panels and time scheduled MRM assays. High flow rate precision of the ExionLC 2.0+ system, using fast gradient separations (sub 90 seconds), provided RSD of <0.15% for the analytes tested here.

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