ExionLC™ series Gradient Delay Volume Considerations

Understanding the differences in gradient delay volumes between various HPLC and UHPLC systems

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Contemporary LC/MS analysis can require the use of a wide variety of analytical columns and mobile phase gradients for the effective analysis of pharmaceutical and biological samples, foods, and beverages. Typically users can operate and traditional HPLC pressures (< 400 bar, 6000 psi), high pressures (~ 600 bar, 10000 psi) or UHPLC modes (> 900 bar, > 10000 psi). For each of these application spaces, SCIEX offers an HPLC or UHPLC system designed to maximize throughput and flexibility.

For LC/MS/MS applications involving lower pressures and diverse analytes, the ExionLC 100 with a quaternaryHPLC pump, offers a single easily configurable LC platform. More advanced or demanding application will benefit from the capabilities of the ExionLC AC (660 bar limit) or AD systems (1300 bar limit).

Figure 1. SCIEX ExionLC systems, from left to right: ExionLC 100, AC, and AD

In order to effectively compare analytical systems and their resulting chromatographic performance, an assessment of "gradient delay volume" is usually considered. The gradient delay volume is a portion of the total column "dead volume" which includes delay volumes associated with the pump, fittings, tubing, mixer, and interstitial column volumes. A simple approximation for the column volume (Vm) is given by:

$$Vm \approx 0.5\ L\ d_c^2$$

Where d_c is the column diameter in mm and L is the column length in mm. The resulting column void volume (in mL) provides a rough estimate of the column void volume.

In practice we wish to compare gradient delay volumes accross varios LC systems for the purpose of comparing and transferring various analytical methods. For for a more practical illustartion of gradient delay, a step gradient experiment is performed. In this case a 2.6 µ Phenomenex Kinetix C-18 column (2.1 x 50 mm) was chosen as the LC column. A step gradient experiment is performed using 90% water / 10% methanol mixture in mobile phase A and a solution of 0.1% caffeine dissolved in 90% methanol / 10% water for mobile phase B. The concentration of caffeine (monitored by a UV/Vis detector at 260 nm) corresponds to the gradient formation and the delay volume

The resulting volume delay between the observed jump in UV absorbance and the programmed "step" time provides the user with a complete picture of the total system "gradient delay volume" which includes the pump, tubing, mixer, and column delay volumes. It provides a very useful comparative measure of the gradient delay volume of various LC systems. In the example below, each gradient delay trace was repeated 10 times and the results averaged to provide a comprehensive illustration of the gradient delay and reproducibility of each ExionLC system. (Figure 2.).

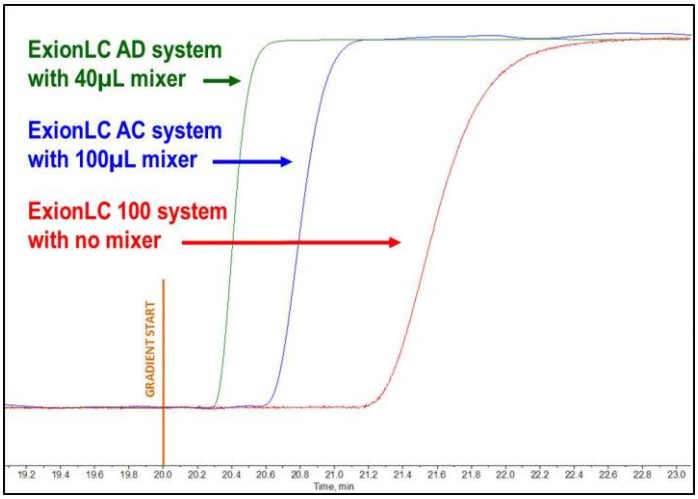

Figure 2. Gradient Delay Volume comparisons between ExionLC systems.

The ExionLC 100 system is a single unit standard pressure HPLC comprising a quaternary pump, a 218 sample capacity autosampler, and a column oven all in one compact unit. As is typical for quaternary systems the dwell volume is larger than a corresponding binary system, but the design of the ExionLC 100 keeps the dwell volume to less than 500µL. The increased gradient delay volumes of the ExionLC 100 are typically offset by the increased analytical flexibility of a quaternary solvent system. For both the ExionLC AC and AD series, the observed gradient delay volumes are typically quite small. The gradient delay volume for the ExionLC AD system being a little under 120µL using a 40µL mixer module. Using the standard 20µL mixer module for LC/MS/MS applications the ExionLC AD system with provide a gradient delay volume of less than 100µL (excluding the analytical column “dead volume”).

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

Publication number: RUO-MKT-02-1805-A

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