Depending on your system and application, developing ultra-high performance methods on an HPLC may require small modifications to reduce extra-column volume.

Measuring Extra-Column Volume

Isocratic Methods

Measuring system volume for isocratic methods is rather easy and can be performed on a system without having to change the mobile phase on the system. First, remove the HPLC column and replace the column with a zero dead volume union. Then start the HPLC at a fixed flow rate and inject a small volume of Acetone or other UV adsorbing component on the system and monitor at either 270 nm or 220 nm. The time delay between injection of the Acetone and the start of the peak deflection in the chromatogram indicates the extra-column volume of the system. The calculation is straightforward:

Adjusting the flow path between the injector and the inlet of the column, as well as the post-column flow path, can reduce this volume.

Gradient Methods

While measuring extra-column volume for gradient methods is not as simple as for isocratic methods, it is still straightforward. First, remove the HPLC column and replace the column with a zero dead volume union. Switching mobile phase is required where one mobile phase is water and the other is Acetonitrile with some amount of Acetone spiked into the mobile phase. UV should be monitored at an appropriate wavelength to detect Acetone (220 nm or 270 nm will both work). A gradient method should be programmed with a system hold at 100 % A (for 1-2 minutes) followed by a 0 % B to 100 % B gradient over the course of a few minutes. The delay from the gradient start time to a baseline rise in the UV signal gives you the total system volume. The calculation is also straightforward:

The gradient extra-column volume testing method can be a powerful tool in optimizing an HPLC system to use Kinetex core-shell columns. Alternating the injector position (bypass vs. main-pass) during extra-column volume testing can reveal the volume contributions that the injector loop can have on the entire HPLC system.

Summary

Calculating the extra-column volume of your HPLC system will allow you to determine if you need to make system modifications for ultra-high performance results. For instructions on how to reduce system volume, please view the "System Optimization Manual" or talk to our Technical Chat Support.

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