[TECHNOLOGY BRIEF]

Reduced System Dispersion and Advanced Features to Minimize Gradient Delay for Achieving the Highest Peak Capacity in Ballistic Gradients

GOAL

To demonstrate the importance of reducing extra-column band spread in achieving the highest peak capacity, and, therefore, the greatest resolution for a given separation with the ACQUITY UPLC® I-Class System.

BACKGROUND

It is extremely important to manage system dispersion in order to maintain the high levels of resolution associated with separations on sub-2-µm columns. Instrumentation tubing lengths and inner diameters must be examined closely, as they may contribute significantly to extra-column band spread. In an effort to increase flow rate capabilities, some UHPLC systems have increased tubing inner diameters which negatively impacts system dispersion. This results in reduced peak capacities and loss of resolution. To compensate for this, a separation must run on a longer column to achieve the same resolution as a UPLC® System designed for low dispersion. Alternatively, a column with a larger inner diameter can be used on these systems to increase the peak volumes, which also compensates for the higher system dispersion. But, this results in increased cost per sample analysis as greater run time and/or greater solvent consumption is required, which undermines the impact of this high resolution technology. Another contributing factor to overall peak capactiy in gradient separations is system volume. The larger the gradient delay, the greater the impact on the peak width of early eluting peaks; thus, resulting in lower peak capacity and sensitivity. This is particularly evident in ballistic gradients where peak shapes are very narrow.

The low dispersion characteristics of the ACQUITY UPLC I-Class System provide the highest resolution separations of any UHPLC system.

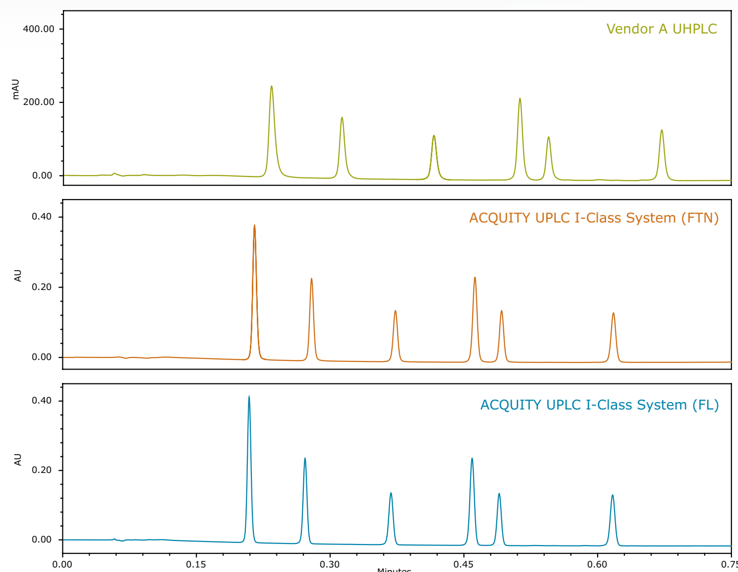

Figure 1. Ballistic gradients run on the ACQUITY UPLC I-Class System (both the fixed-loop and flow-through needle design) compared to a commercially available UHPLC system.

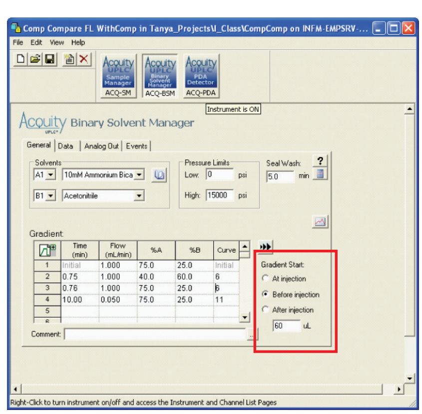

Figure 2. New software functionality minimizes gradient delay volumes for ballistic gradients.

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THE SOLUTION

The ACQUITY UPLC I-Class System was designed to deliver ultra-low dispersion for the ultimate in high resolution separations. Every connection and fluidic component was optimized to minimize extra-column band spread so that the low peak volumes inherent to UPLC separations are maintained. The ACQUITY UPLC I-Class System has two different options for sample injection, fixed-loop (FL), and flow-through needle (FTN), each offering low dispersion performance.

To demonstrate the high peak capacities achieved on the ACQUITY UPLC I-Class System, a series of anesthetics were analyzed with ballistic gradient separations. The separations on both platforms were compared to that on another commercially available UHPLC system as shown in Figure 1. Significantly higher peak capacities were achieved on both ACQUITY UPLC I-Class Systems. The narrower peak widths on the ACQUITY UPLC I-Class System also resulted in significantly higher peaks and better sensitivity. The longer elution times on the UHPLC system resulted from lower temperatures, due to less effective column heating and passive solvent pre-heating; compared to the highly-efficient active pre-heating of the ACQUITY UPLC I-Class System. To further improve peak capacity performance, new software functionality that minimizes the gradient was enabled as seen in Figure 2. This functionality reduces the isocratic hold imparted by the system; therefore, the elution times of the peaks decrease and the widths of the early eluting peaks improve. This results in higher peak capacity for ballistic gradients. When a similar functionality is enabled on the UHPLC system, the peak capacities minimally improve but are still significantly lower than those achieved on the ACQUITY UPLC I-Class System as shown in Figure 3. To demonstrate the impact of the high dispersion associated with injection on the UHPLC system, the ratio of the peak widths of the early and late eluting peaks are compared in Table 1. The better controlled the injection dispersion and gradient delay in a system, the lower the ratio. It is significantly lower on the ACQUITY UPLC I-Class System.

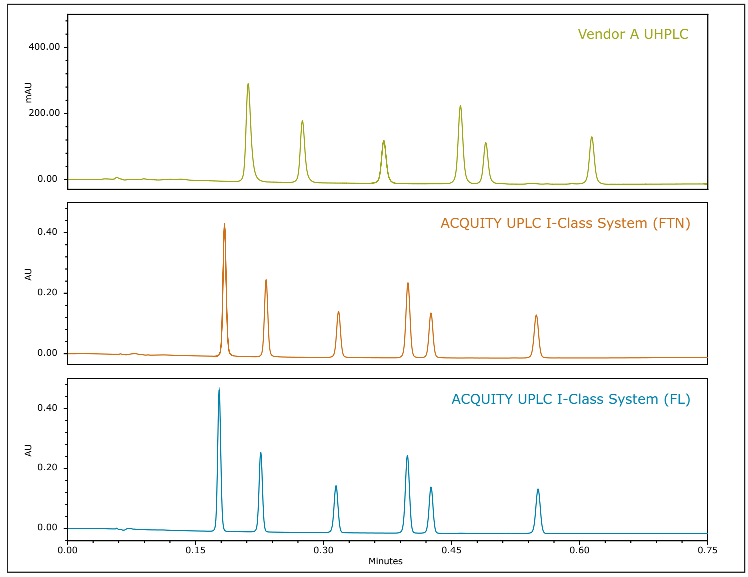

Figure 3. Ballistic gradients run on the ACQUITY UPLC I-Class System (both the FL and FTN design) compared to a commercially available UHPLC system. All systems have reduced gradient delay volumes through software capabilities.

System	Gradient delay minimized	Peak capacity	First peak width (ms)	Last peak width (ms)	First/Last peak width ratio	Retention time of last peak (s)
Vendor A UHPLC System	No	**63**	804	720	**1.13**	40.3
	Yes	**65**	721	710	**1.01**	37.0
ACQUITY UPLC I-Class System (FTN)	No	**81**	510	630	**0.81**	37.1
	Yes	**84**	445	623	**0.71**	33.0
ACQUITY UPLC I-Class System (FL)	No	**83**	475	619	**0.77**	37.0
	Yes	**86**	426	615	**0.69**	33.1

Table 1. Summary of data demonstrating the impact of dispersion on the ACQUITY UPLC I-Class systems and another UHPLC system.

SUMMARY

The low dispersion characteristics of the ACQUITY UPLC I-Class System result in the highest peak capacities for ballistic gradient separations. New system functionality helps to further improve this performance. Peak capacities achieved are significantly higher than those possible on other UHPLC systems.

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