

## ACQUITY UPLC I-Class: Optimized System Dispersion for Ultimate UPLC Performance

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This is an Application Brief and does not contain a detailed Experimental section.

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### Abstract

This application brief demonstrates that the ACQUITY UPLC I-Class System is optimized for lowest extra-column band spread, enhancing the separation quality for both high resolution and high throughput UPLC separations. The importance of these improvements will be shown for both impurity analysis and ballistic gradients.

## Benefits

The low dispersion characteristics of the ACQUITY UPLC I-Class System allows you to take full advantage of sub-2- $\mu\text{m}$  particles, whether you require higher throughput for your analysis or higher resolution in your separations.

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## Introduction

The improved peak capacity and resolution possible for separations utilizing columns packed with sub-2- $\mu\text{m}$  particles has been demonstrated for many applications, resulting in dramatic improvements in resolution and throughput. However, achieving the maximum resolution possible for a given separation requires that system dispersion be minimized. Any fluidic line or connection that is part of the system flow path post-injection will contribute to extra-column band spread. This includes the injection valve, solvent pre-heating assembly, connecting tubing, fittings, and the optical flow cell. Many vendors have tried to improve UHPLC system dispersion compared to HPLC predecessors, but with limited success. While dispersion may have been reduced, it still has not been optimized to take full advantage of narrowbore UPLC columns (2.1 mm I.D.). The lower flow rate requirements of these columns deliver greater return on investment against the cost of analysis per sample, providing that efficient separation with sufficient resolution can be obtained.

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## Results and Discussion

The ACQUITY UPLC I-Class System was optimized to reduce extra-column band spread in each of the contributing components post-injection. Newly designed UV Detector flow cells use the same optical path as their ACQUITY UPLC predecessors so the same high sensitivity is achieved; however, fluidic lines and connections have been re-designed to further reduce band spread. The use of active solvent pre-heaters is necessary to minimize the temperature gradients that contribute to on-column dispersion effects. Therefore,

the volume of the solvent pre-heaters has been minimized to ensure that the sample plug is delivered to the head of the column with minimal distortion while still providing excellent solvent heating characteristics even at high temperature and high flow.

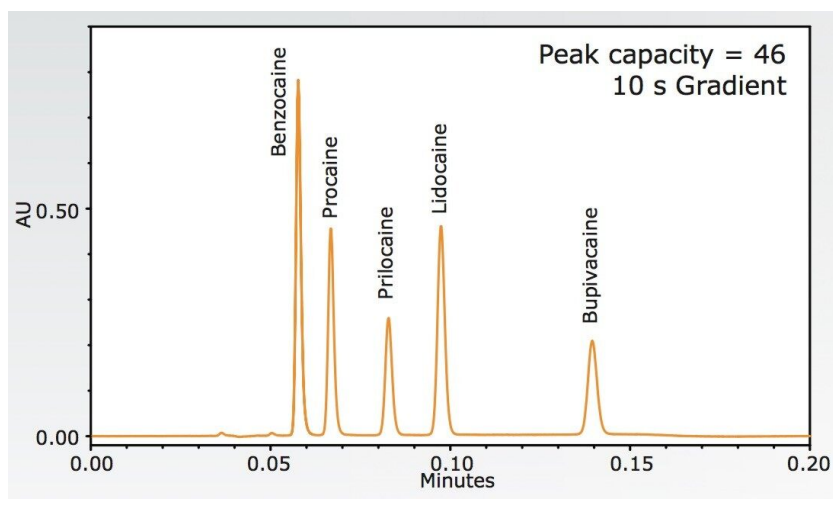
The ACQUITY UPLC I-Class System can be configured with two Sample Manager options depending upon your laboratory's needs. Whether using the fixed-loop (SM-FL) or flow-through needle (SM-FTN) injector, dispersion in the injection mechanism has been minimized with low volume needle ports, connection lines, and internal valve channels. Typically, less extra-column band spread is observed on fixed-loop injector designs due to a reduction in the volume of the injector flow path. By optimizing every component, extra-column band spread is significantly lower than any other commercially available LC system. Table 1 summarizes the band spread values obtained across multiple vendors' systems (including UHPLC systems).

System	Band Spread (μL)
Alliance® HPLC System	29
Vendor A HPLC	41
Vendor B1 UHPLC	28
Vendor B2 UHPLC Configured for Single Column	20
Vendor B2 UHPLC Configured for Dual Column	23
Vendor C UHPLC	21
Vendor D UHPLC	17
ACQUITY UPLC H-Class	9
ACQUITY UPLC H-Class with Column Manager	12
<b>ACQUITY UPLC I-Class (SM-FTN)</b>	<b>7.5</b>
<b>ACQUITY UPLC I-Class (SM-FL)</b>	<b>5.5</b>

*Table 1. The extra-column band spread measured on the ACQUITY UPLC family of products is significantly less than that of competitive UHPLC systems, with the ACQUITY UPLC I-Class having the lowest dispersion.*

The ACQUITY UPLC family outperforms all other systems when it comes to maintaining the integrity of high resolution separations by reducing band spread to 9 μL on the ACQUITY UPLC H-Class System and as low as 5.5 μL on the ACQUITY UPLC I-Class System. The reduced system dispersion can be directly translated into increased resolution on the ACQUITY UPLC I-Class System. Separations can be pushed to ballistic gradients that maintain the resolution found in typical analytical gradients. The separation of a series of anesthetics analyzed in 10 s with a 4 Sigma peak capacity of 46 is shown in Figure 1. Even with these rapid

analysis times, performance is maintained with a standard deviation for retention time as low as 12 ms. The reduced system dispersion is also realized in high resolution separations such as impurity analysis. Figure 2 shows an impurity analysis of tetracaine. This separation, run under the same conditions on the ACQUITY UPLC I-Class System and the UHPLC system from Vendor B, achieved significantly better resolution on the ACQUITY UPLC I-Class. Vendor B's system, configured with their recommended 60 mm pathlength flow cell, showed such significant band spread that the shoulder peak could not be detected.



*Figure 1. A series of anesthetics was separated by a ballistic 10 s gradient on the ACQUITY UPLC I-Class System. The optimized dispersion delivered a separation with a peak capacity of 46. Benzocaine had a peak width of 88 ms at 1/2 height while still maintaining a retention time standard deviation of 12 ms for six replicate injections.*

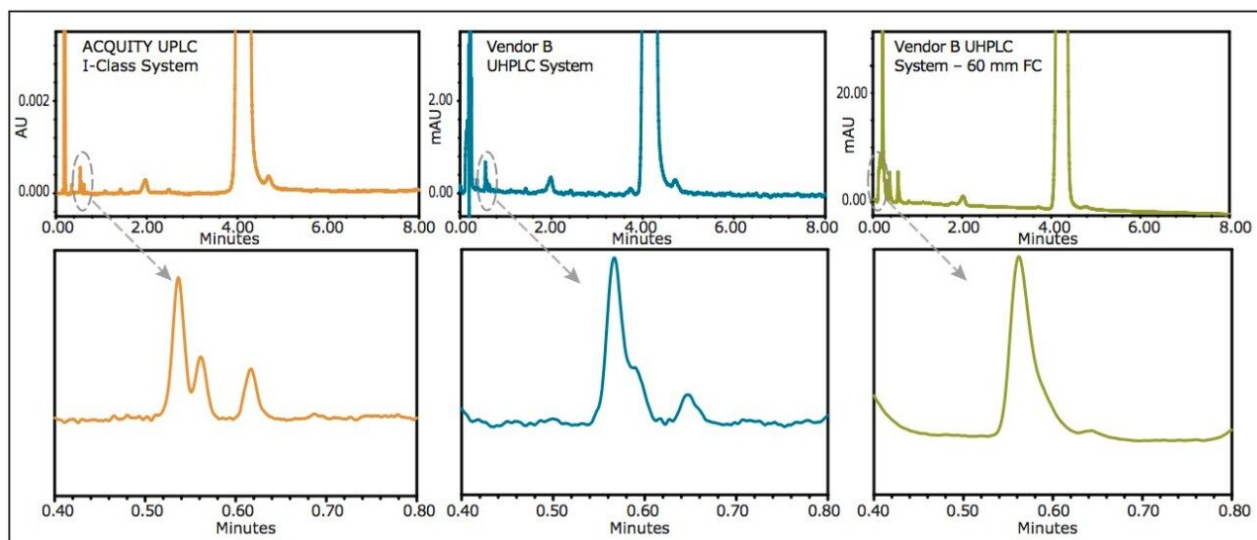


Figure 2. The impurity analysis of tetracaine was performed on the ACQUITY UPLC I-Class System (band spread 5.5  $\mu$ L) and the UHPLC system from Vendor B (band spread 20  $\mu$ L). The full scale separation is shown at the inset to the right with the expanded region marked on the traces. Significantly better resolution was observed on the ACQUITY UPLC I-Class System. When Vendor B's high sensitivity 60 mm flow cell was installed on their system, resolution was entirely lost for the critical pair.

## Conclusion

The ACQUITY UPLC I-Class System delivers unparalleled performance for today's most challenging separations. The flexible system configurations have minimized extracolumn band spread to achieve optimal separation performance on UPLC columns, whether your laboratory's separations require increased resolution or increased sample throughput.

## Featured Products

ACQUITY UPLC I-Class PLUS System <<https://www.waters.com/134613317>>

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