Note d'application

Optimized System Dispersion for UPLC Performance in a Versatile LC Design

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

Abstract

This application brief demonstrates the effect of extra-column band broadening on UPLC resolution.

Benefits

The ACQUITY UPLC H-Class System's ultralow dispersion delivers the highest efficiency separations of any commercially available LC system in a flexible and easy-to-use platform.

Introduction

The high-efficiency separations achieved on sub-2-μm columns require a system that exhibits very low extra-column band spread. There are now several commercial LC systems modified to deliver fluid at high system pressures with reduced gradient delay volumes (compared to traditional HPLC systems). However, most have not developed solutions to manage extra-column band spread to the levels required by high-resolution separations. Attempting improvements such as tightening connections for high-pressure operation and/or increasing the pump delivery power to be deemed compatible with UPLC are simply not enough.

In achieving high-resolution separations, systems cannot sacrifice the flexibility or ease-of-use required by laboratories. Ideally, a system designed for method development and/or routine analysis will include quaternary mixing capabilities, a needle-in-flow-path injector, and multi-column configurations while preserving low system dispersion for UPLC. This configuration allows for the greatest diversity of methods in a single system without adding extra complexity, which can make method optimization more challenging. A system designed with this versatility can be used across many different functional laboratories, ranging from R&D to QC.

Results and Discussion

The ACQUITY UPLC H-Class System is a flexible platform that enables the user to run HPLC and UPLC applications without reconfiguring the system. This same design provides the low extra-column band spread required for high-efficiency UPLC separations and is typically 8 to 9 μL at 5-Sigma peak width. The system's sample manager (SM-FTN) is designed for maximum flexibility with minimized dispersion. The low volume injection valve delivers reliable performance at the pressures required by UPLC. The injection port is located close to the inject valve to reduce the extra-column band spread within the injector. The newly designed column heater (CH-A) with active solvent preheating adds minimal dispersion and achieves excellent column heating. This ensures that solvent entering the chromatographic column has reached the set point which reduces temperature gradients across the column (a source of on-column band spread). Post-column system dispersion is managed with a detector flow cell design that has a 500-nL volume but still provides high energy throughput for enhanced sensitivity. With all the components of the ACQUITY UPLC H-Class System so diligently designed for minimal dispersion, it is also important to minimize gradient delay to avoid the isocratic elution of the more polar sample components. The system has advanced control capabilities for gradient delay reduction. Although the ACQUITY UPLC H-Class System was designed as a low-pressure quaternary gradient system for maximum flexibility, it can deliver the gradient to the head of the column at the same time as the sample.

The result is unparalleled UPLC performance as shown in Figure 1. This 10 s ballistic gradient generated narrow, Gaussian peaks that were less than 30 ms at 5-Sigma peak width. The flow rate for this separation was 1.75 mL/min, which translates to a peak volume of 52.5 µL.

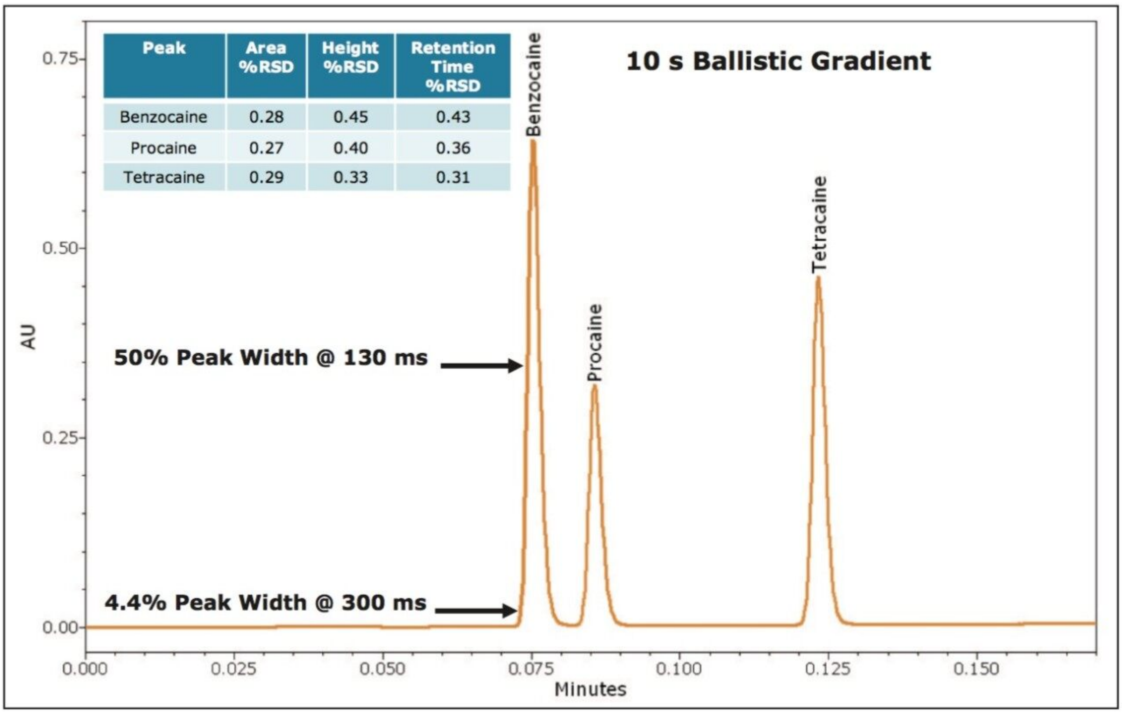

Figure 1. 10 s ballistic gradient on the ACQUITY UPLC H-Class System for some common anesthetics. The system's minimized extra-column band spread maintains the extremely narrow peaks generated on the ACQUITY UPLC sub-2-µm Column.

Competitive systems claim to offer 'UPLC-like' capabilities, however if extra-column band spread is compared across these systems, the values indicate their system dispersion is not adequately managed as shown in Table 1. When compared to the ballistic gradient peak volume, competitive systems will add an additional 40% up to 100% dispersion to these UPLC peaks. Figure 2 demonstrates the effect of this additional dispersion on a typical UPLC separation. The same resolution cannot be achieved, due to the higher extra-column band spread. Although competitive systems may achieve high-pressure with such high system dispersion, they cannot offer UPLC performance.

System	Band Spread (μL)
Alliance® HPLC	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 with Column Heater	**9**
ACQUITY UPLC H-Class with Column Manager	**12**

Table 1. Extra-column band spread values measured across multiple vendors' HPLC, UHPLC, and UPLC systems at 5-σ (4.4% peak height).

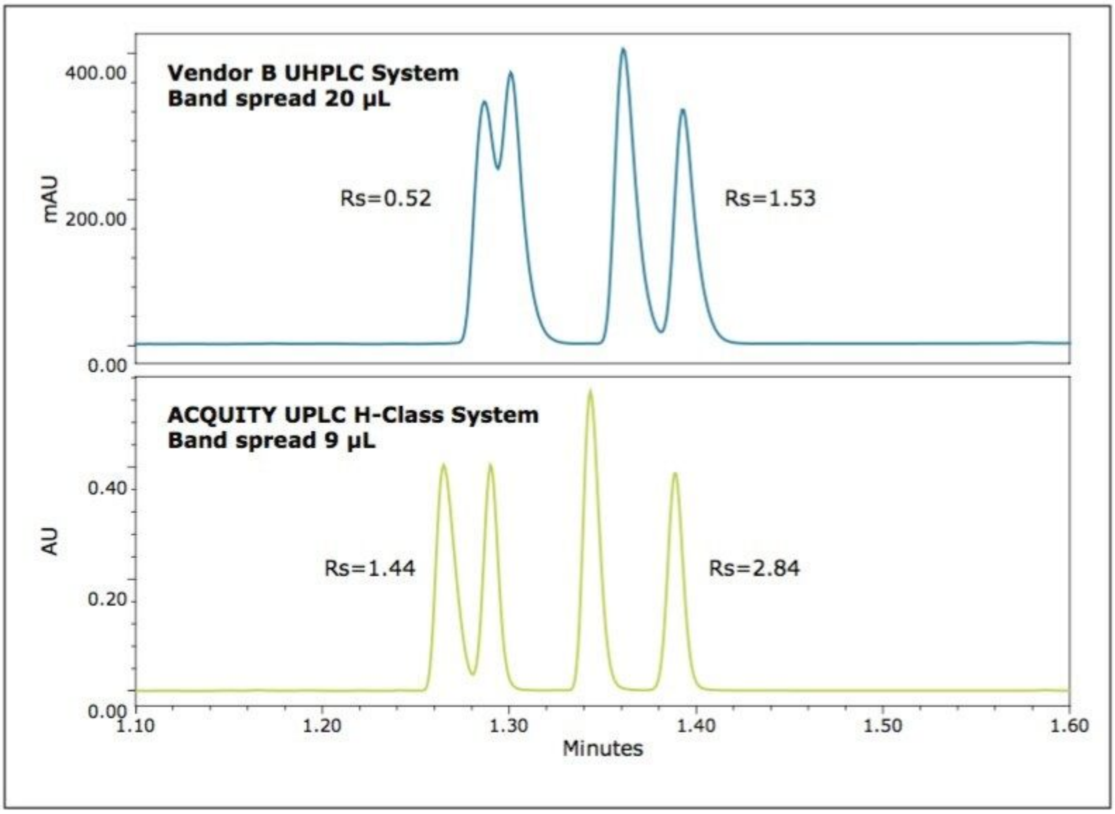

Figure 2. Comparison of a typical UPLC separation on the ACQUITY UPLC H-Class System and Vendor B's UHPLC System.

Conclusion

There are a number of contributing factors in achieving high-resolution UPLC separations. System design must be considered in order to achieve true UPLC performance. Although operating pressure and system volume are enablers of UPLC, the ultimate performance is only realized when system dispersion is optimized to match the resolving power of the chromatographic column.

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ACQUITY UPLC H-Class PLUS System <https://www.waters.com/10138533>

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