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應用手冊

Increasing Sample Throughput Using the ACQUITY UPLC System with 2D Technology and Parallel Column Regeneration

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

Abstract

This application brief demonstrates to increase sample throughput using the ACQUITY UPLC System with 2D Technology configured for parallel column regeneration.

Benefits

Laboratories constantly challenged to analyze more samples in less time now have a tool to easily increase sample throughput while maintaining high quality chromatographic results – the ACQUITY UPLC System with 2D Technology.

Introduction

A typical gradient LC analysis is comprised of the following general steps:

- Sample aspiration and injection
- Gradient separation
- Regeneration (column is rinsed)
- Re-equilibration (column is returned to initial conditions)

These are sequential events that involve the passage of the sample and gradient through specific volumes of the system and the column. The time required to perform these steps is related to the flow rate set for the analysis. Increasing flow rate, using a shorter column (decreasing the volume), and shortening the re-equilibration time can all be used to minimize the total analysis time. There are, however, limits to the extent that these parameters, especially the last two, can be modified to the point when chromatographic performance is compromised. Parallel column regeneration offers an additional means to reduce the total analysis time.

In parallel column regeneration, sample analysis is alternated between two identical columns with identical flow paths. One sample is injected, separated, and regenerated on one column, while the other column undergoes re-equilibration, allowing more samples to be analyzed in a given time period. The time advantage of this approach is shown in Figure 1.

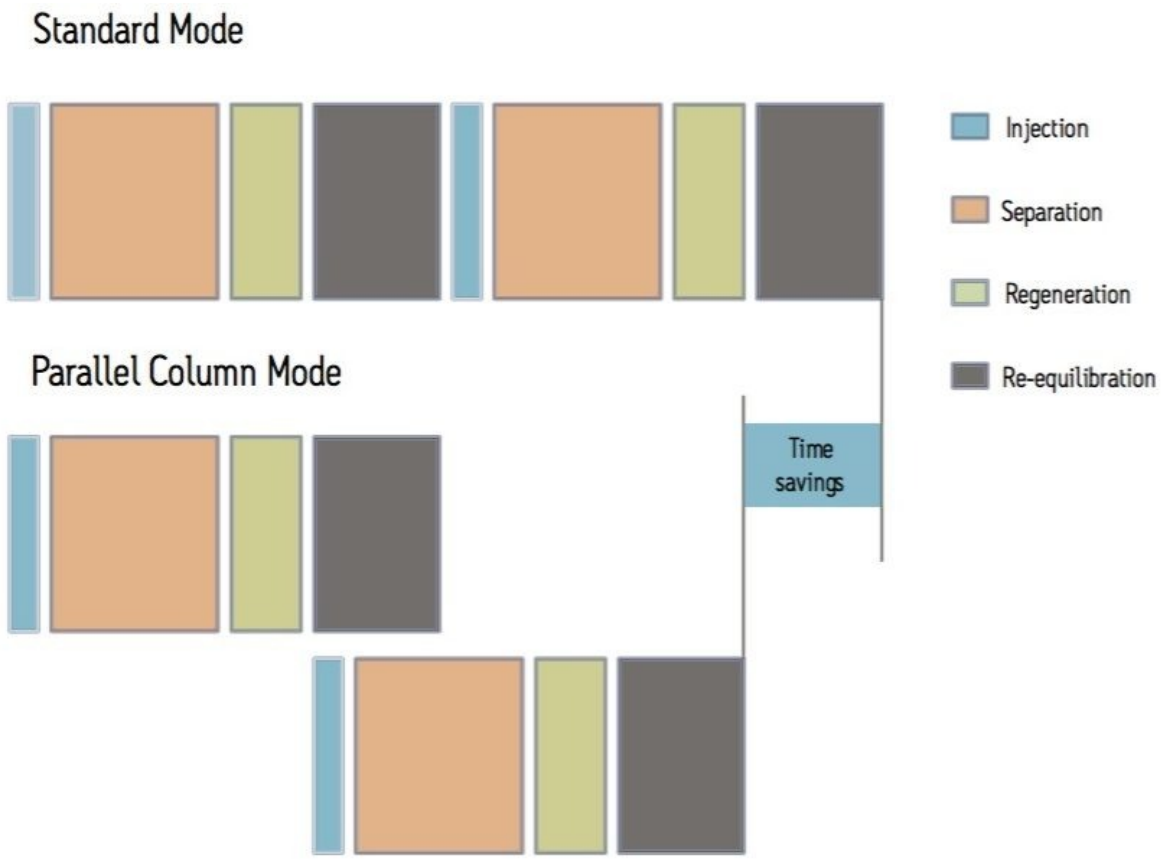

Figure 1. Comparison of the sequence of events between the standard 1D UPLC and parallel column regeneration UPLC.

Results and Discussion

The AQUITY UPLC System with 2D Technology is comprised of two Binary Solvent Managers (BSMs), a Sample Manager with Flow-Through Needle design, and a Column Manager with two six-port, two-position valves. When configured for parallel column regeneration, the two pumps must be identical BSMs. A schematic of the plumbing for this configuration is shown in Figure 2. In this configuration, the alpha pump delivers the analytical gradient, while the beta pump regenerates the column.

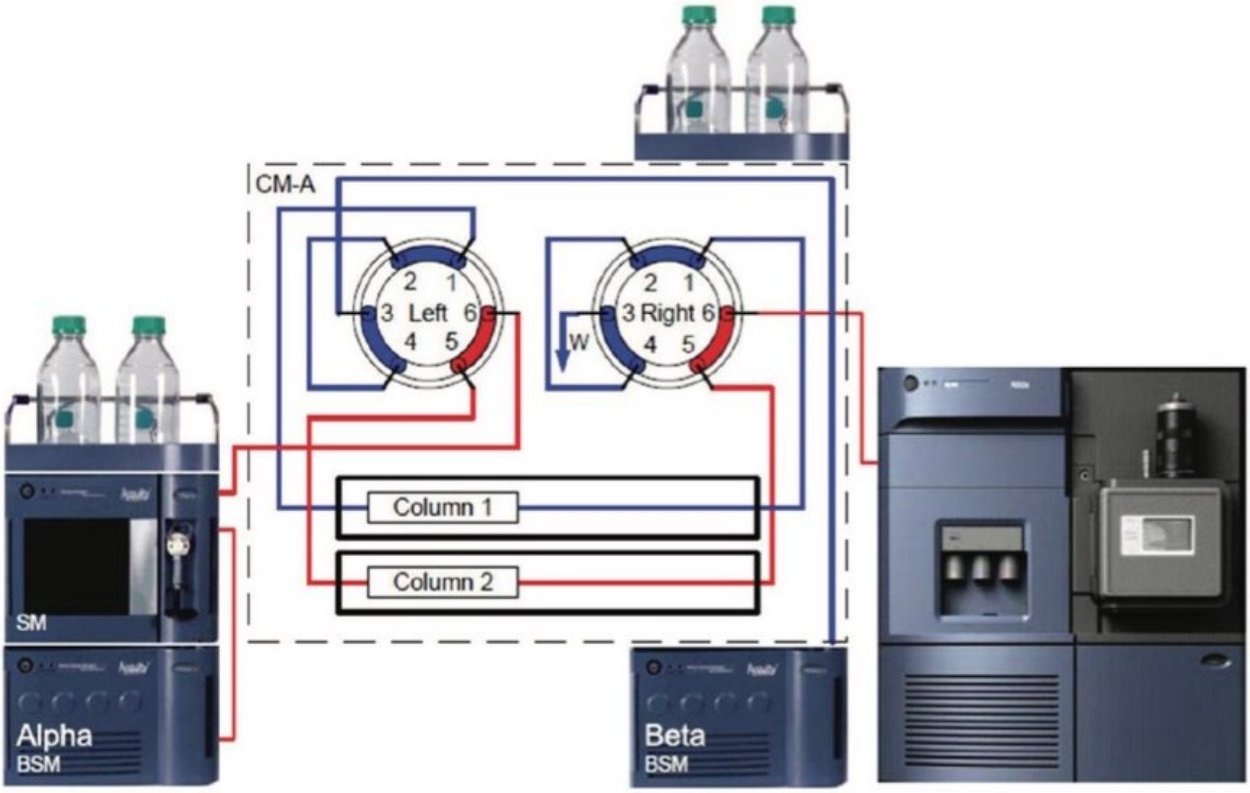

Figure 2. A schematic plumbing diagram of the ACQUITY UPLC with 2D Technology configured for parallel column regeneration.

A UPLC method for the bioanalysis of the benzodiazepine alprazolam in plasma was analyzed by standard mode UPLC and compared to the parallel column regeneration mode of the ACQUITY UPLC System with 2D Technology. The separation has been optimized to most effectively separate the analyte of interest from the matrix effects associated with protein precipitated plasma in as short a run time as possible. Identical columns from identical lots were employed and the MRMs, shown in Figure 3a, reveal the similar chromatography attained from each.

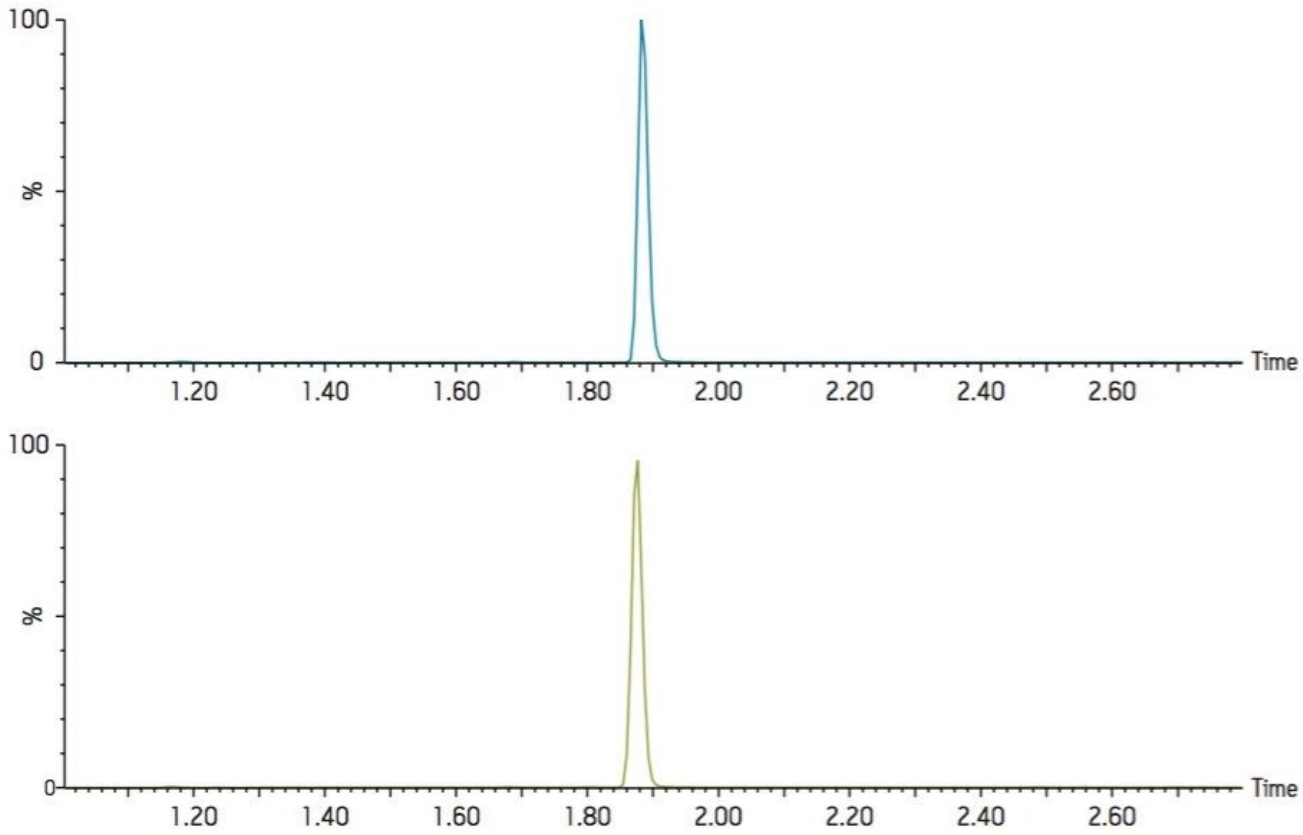

Figure 3a. Similar chromatography is attained between the columns used in parallel column regeneration mode on the ACQUITY UPLC System with 2D Technology.

The 5.5-min injection-to-injection cycle time of the standard mode UPLC run was easily reduced to 4.1 min without making any changes to the instrument method. Extrapolating to a 24-hour period for comparison, the parallel column mode analyzed 30% more samples for this method, as shown in Figure 3b. The actual increase in throughput in this method was a result of overlapping the method equilibration time with the next sample injection and analysis. Time savings will be dependent on the degree of overlap that is possible in an analytical method, however.

Mode	Injection-to- injection cycle time	Samples/h	Total samples in 24 h	Difference (%)
Single column	5.5	11	264	–
Parallel column	4.1	14.6	350	87 (30%)

Figure 3b. Parallel column mode increased sample throughput by 30%.

Conclusion

The ACQUITY UPLC System with 2D Technology configured for parallel column regeneration provides a simple tool to increase sample throughput while maintaining high quality chromatographic results. No changes in the chromatographic parameters of an analytical method are needed to benefit from this approach. The ACQUITY UPLC System with 2D Technology can address a broad range of application needs with flexible configurations for various scientific and business challenges.

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- ACQUITY UPLC Systems with 2D LC Technology <https://www.waters.com/10203030>

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