

ACQUITY UPLC I-Class: A High-Throughput, High-Sensitivity Platform for Quantitative Bioanalysis

GOAL

To demonstrate that fast bioanalytical assays of 45 to 60 seconds that retain high chromatographic fidelity are now possible with the ACQUITY UPLC® I-Class System.

BACKGROUND

Successful quantitative bioanalysis requires a high-sensitivity analytical methodology. The sensitivity and selectivity of modern LC technologies such as sub-2- μ m particle UPLC® has resulted in analysis times in the 1.5 to 2 minute range. These high-throughput assays allow several batches to be analysed per day, maximizing instrument utilization and thus their profitability. Even faster analysis times have been possible – but at the expense of chromatographic resolution and assay performance. As assay sensitivity requirements continue to become more challenging – single-figure pg/mL – the bioanalytical scientist must develop assays that are free from matrix interference and metabolite coelution, while still maintaining the acceptable throughput.

In bioanalysis, where the balance between data quality, sensitivity, and productivity must be carefully weighed, the ACQUITY UPLC I-Class System provides unprecedented levels of performance and productivity.

In the last five years, regulatory requirements on the quality of bioanalytical assays have become more rigorous. Components such as incurred Sample Reanalysis (ISR), metabolites In Safety Testing (MIST), and matrix factor are all becoming more prominent considerations for the bioanalyst.

To meet these requirements, it has been necessary to improve the sample preparation process and to develop more selective and specific separations methods. This results in increased analysis times. Analytical systems are now being challenged to execute high-throughput methods that require low-volume chromatographic instrumentation, a rapid injection-to-injection autosampler, and fast detection capabilities as well as the ability to address the backpressure developed by modern sub-2- μ m chromatography columns.

THE SOLUTION

The ACQUITY UPLC I-Class System has been specifically designed to deliver rapid gradients with low system delay volume and a fast, flow-through-needle autosampler. This, combined with its binary solvent manager's ability to deliver high-fidelity gradients extremely rapidly, makes the ACQUITY UPLC I-Class the ideal chromatographic solution for bioanalysis.

The UPLC/MS/MS analysis shown in Figure 1 illustrates the quantification of fluticasone propionate. The separation in Figure 1 shows the use of a 60-second gradient. The fluticasone propionate peak elutes at a time of 0.67 min. The effect of reducing the gradient time from 90 seconds to 45 seconds, in these examples the same chromatography column is employed a 2.1 x 50 mm ACQUITY UPLC C18, 1.7- μ m column with a 50:85 methanol/aqueous ammonium hydroxide gradient at 500 μ L/min.

In this case, we were able to achieve an extremely rapid analysis due to the high resolution of the analyte from the matrix. Matrix interferences always need to be accounted for in ballistic gradients.

These results indicate that with ACQUITY UPLC I-Class System, analysis times as low as 45 seconds can be achieved without adversely affecting chromatographic performance. Apart from the increase in throughput achieved with this new system, there is also an associated increase in peak height as a result of the reduced residence time of the peak on the column and, thus, less band broadening.

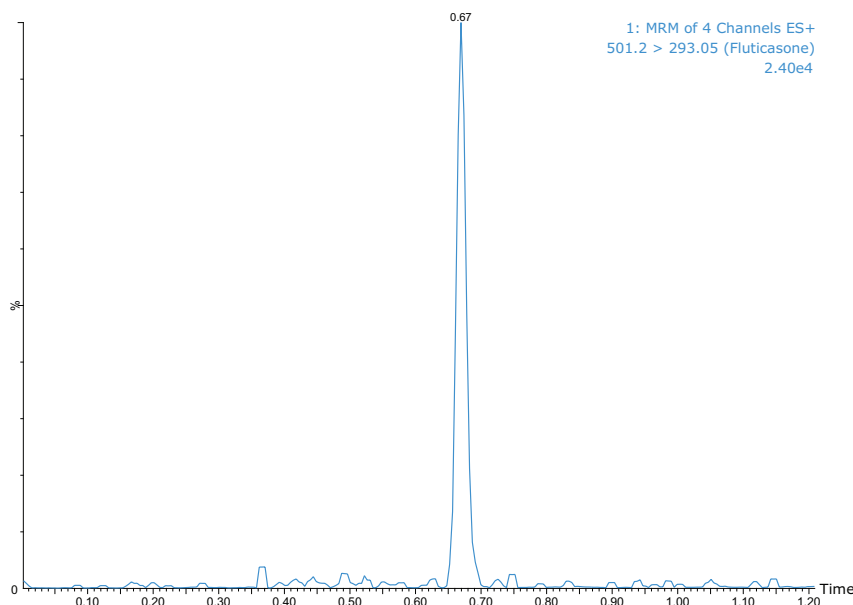


Figure 1. Fluticasone propionate from a dried blood spot is successfully resolved in a very fast quantitative assay with the ACQUITY UPLC I-Class System.

SUMMARY

The ACQUITY UPLC I-Class System provides the highest levels of chromatographic performance, providing excellent dispersion characteristics and the ability to use longer columns due to the increased back pressure capability. The use of high-resolution UPLC for bioanalysis delivers sharp analyte peaks for maximum sensitivity and highest resolution that minimizes coelution with endogenous material in the sample. The system's high back pressure facilitates the use of long, high resolution, columns with run times of just 5 to 10 minutes.

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