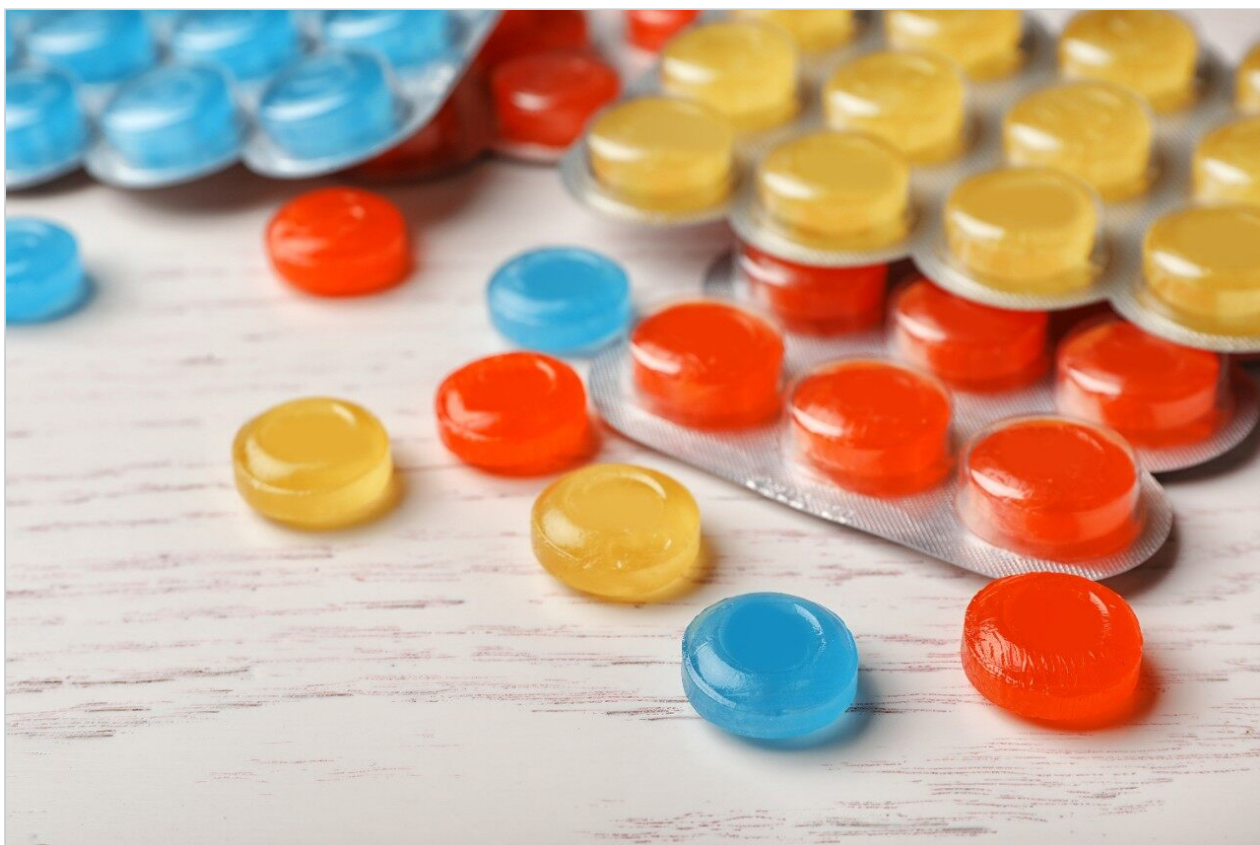


Application Note

Reliable and Robust Sample Injection with the Alliance HPLC System

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

Abstract

This application brief demonstrates that the Alliance HPLC System delivers linear and highly repeatable sample injections across the range of analytical HPLC injection volumes.

Benefits

The Alliance HPLC System uses a flow-through-needle injector design to achieve a wide injection volume range, maximize sample recovery, and provide accurate, precise, and linear injection performance.

Introduction

The overall quality of a quantitative HPLC method depends on minimizing any errors in the sample injection that can impact the accuracy of measurement. This quality must extend over a wide linear range and provide the flexibility to run multiple different methods on the same analytical system. In addition to precision and linear range, the accuracy of sample delivery is important for quantification. Ensuring that the requested sample volume and undistorted composition is delivered to the column for analysis decreases the susceptibility to method-induced precision issues. Systems that utilize an autosampler with a needle-in-flow-path design for complete transfer of the sample to the analytical column combined with accurate and precise sample metering provide the greatest performance, flexibility, and ease-of-use. The needle-in-flow-path style of sample delivery typically offers the widest injection volume range without the need for configuration changes. Most importantly, it requires very little method development for optimal performance.

Results and Discussion

The Alliance HPLC System uses a flow-through-needle injector design that delivers high injection precision across the range of analytical injection volumes. To demonstrate injection linearity, a mixture of three anesthetic standards was injected onto an analytical scale column (4.6 x 50 mm) at different injection volumes ranging from 5 to 80 μL , as shown in Figure 1. The correlation co-efficient (R^2) for peak height versus injection volume gives a measure of the linearity range of the injection volume of the autosampler. The

R² value across this injection volume range exceeded 0.999 for all three components. The same mixture was used to evaluate the repeatability of six replicate injections at each of the injection volumes. The %RSD across this entire injection volume range for these anesthetic standards was below 0.2% for all components and typically below 0.1%, well below the instrument specification of 0.5%, as shown in Figure 2.

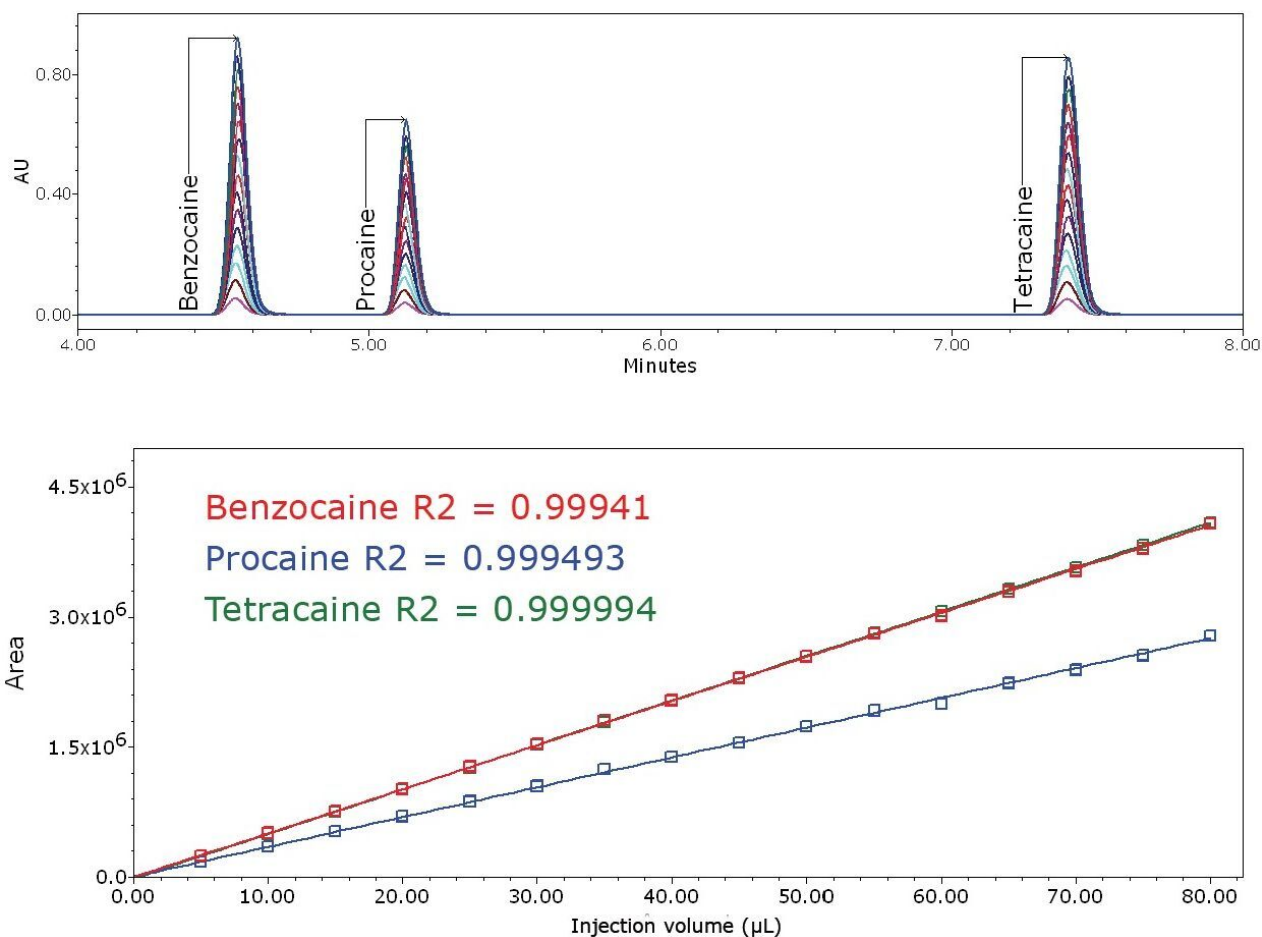
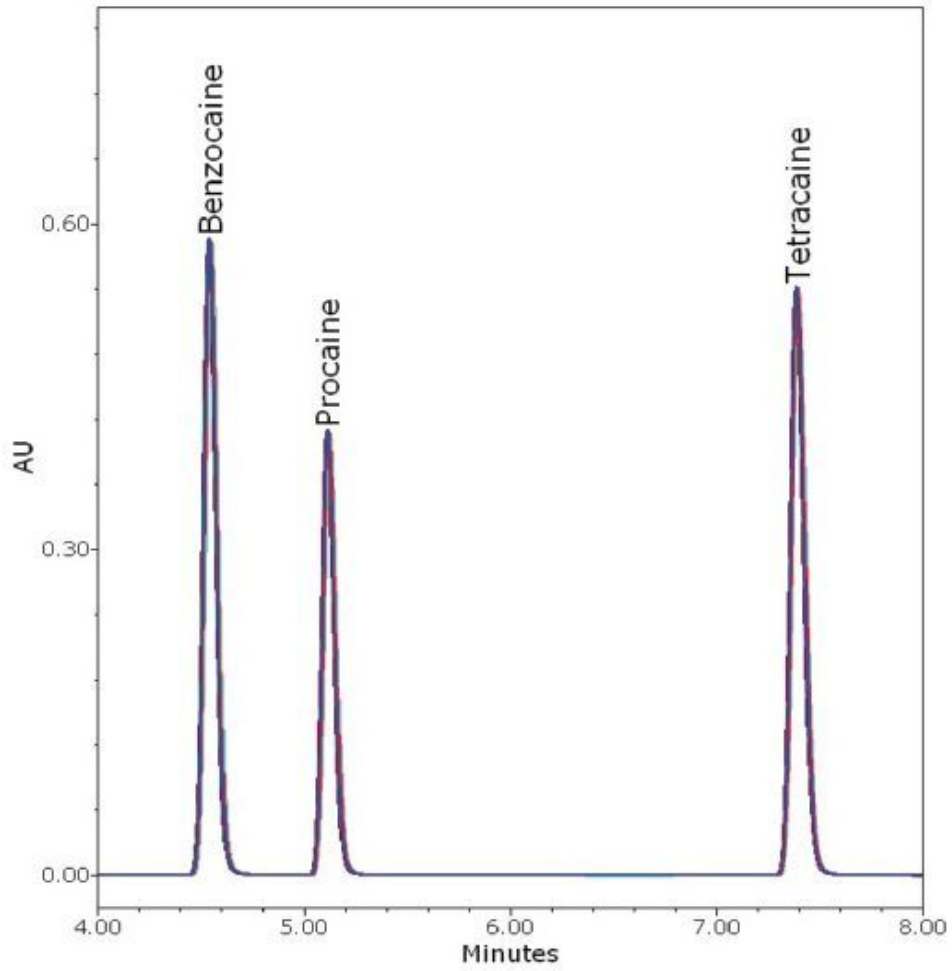


Figure 1. Excellent linearity across a large injection volume range provides the user with maximum versatility without the need to change instrument configuration. The chromatographic overlay and calibration curves demonstrate the large, linear injection volume range of the Alliance HPLC System.



- Benzocaine Peak Area %RSD
- Procaine Peak Area %RSD
- Tetracaine Peak Area %RSD

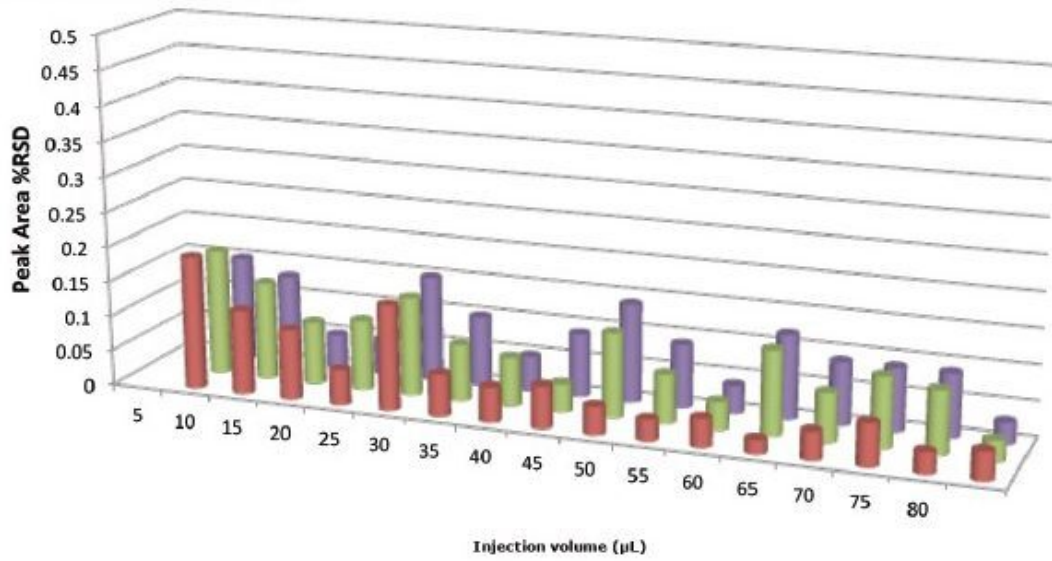


Figure 2. An overlay of six replicate 50- μ L injections demonstrates the injection repeatability of the Alliance HPLC System. For this application, the e2695 Separations Module consistently performs below the injection precision specification (<0.5 %RSD) and typically below 0.2 %RSD across a large injection range providing accurate and repeatable quantification with maximum versatility.

Conclusion

Laboratories that perform routine analysis applications require a sample injector design that is highly repeatable and linear for accurate quantification. The Alliance HPLC System fully satisfies this performance requirement in a flexible and robust format, making it well-suited for quality control applications.

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[Alliance HPLC System <https://www.waters.com/534293>](https://www.waters.com/534293)

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