

Application Note

## Reliable and Robust Injector Precision and Linearity with the Arc HPLC System

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

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## Abstract

Precision and linearity are two measurable characteristics that determine how well an HPLC injector operates under various chromatographic conditions. Analytical methods that have 100% organic sample diluent can have a large impact on the performance of the sample delivery system. The work presented here demonstrates that the new Arc HPLC System delivers linear and highly reproducible sample injections across the range of analytical HPLC injection volumes even when the sample diluent is 100% organic.

### Benefits

- The correlation coefficient ( $R^2$ ) observed for injection linearity for caffeine and acenaphthene is  $>0.999$  for injection volumes 0.5 to 20  $\mu\text{L}$
- Area %RSD of injection precision across all injection volumes from 0.5 to 20  $\mu\text{L}$ , with aqueous sample diluent and with 100% organic sample diluent, for caffeine and acenaphthene respectively are less than 0.73%

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## Introduction

The results of an HPLC analysis critically depend on the performance of the sample delivery system. It is necessary to verify that the performance of the instrument is maintained within a set of pre-determined criteria to ensure the quality of the data generated by the system is reliable. The performance can be verified by testing the functionality of the sample manager (injector). The effects of two key performance characteristics, such as injector precision and injector linearity, on the chromatographic results are discussed in this application brief.

The Arc HPLC System uses a flow-through needle (FTN) injector design that delivers high injection precision across the range of analytical injection volumes. The Arc HPLC System helps in achieving high-efficiency separations and quality data to meet the most stringent regulatory requirements with confidence. The Arc HPLC System allows you to easily replicate and improve the performance of existing LC methods without compromising data quality, eliminating the burdens of older or less efficient LC systems for routine use.

Dependent upon the in-house protocols of the pharmaceutical company, the quality control lab would make five to six replicate injections to check instrument precision. As per USP General Chapter 621, the acceptance criteria for RSD of five replicates, unless otherwise specified, should be less than (or equal to) 0.73%.



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*Figure 1. The Waters Arc HPLC System.*

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## Results and Discussion

The Arc HPLC System uses a flow-through needle injector design that delivers high injection precision across the range of analytical injection volumes, irrespective of the sample diluent. To demonstrate injection precision and linearity, caffeine standard and acenaphthene standard were injected repeatedly onto an analytical column (4.6 x 50 mm) at different injection volumes ranging from 0.5 to 20  $\mu\text{L}$ . Injection precision characteristics of the Arc HPLC System sample manager, configured with the standard 100- $\mu\text{L}$  syringe and 50- $\mu\text{L}$  sample loop, were determined by injecting 0.5 to 20  $\mu\text{L}$  of standard solution of caffeine in aqueous diluent and acenaphthene in 100% organic diluent. The area %RSD for the caffeine and acenaphthene peaks at each injection volume was evaluated ( $n=5$ ). As shown in Figures 2 and 3, area %RSD were less than 0.73% across the range of injection volumes.

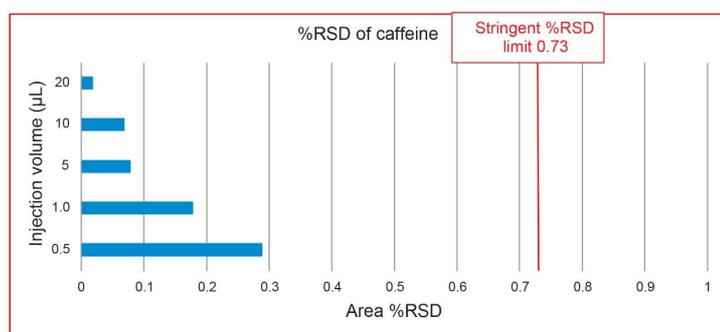


Figure 2. Area %RSD of caffeine peak for injection volume ranges from 0.5 to 20  $\mu\text{L}$ .

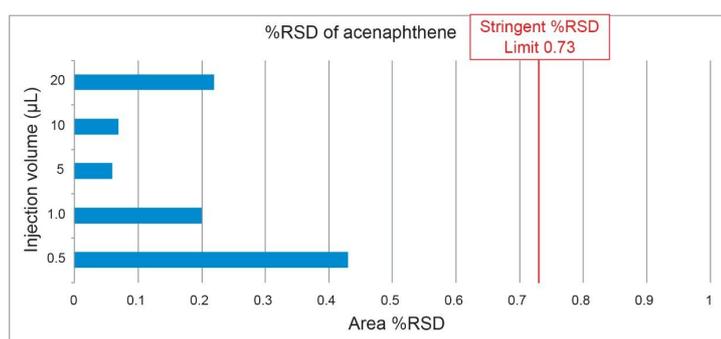


Figure 3. Area %RSD of acenaphthene peak for injection volume ranges from 0.5 to 20  $\mu\text{L}$ .

The experiments performed in this application brief were designed to confirm the Arc HPLC System sample manager delivers the precise amount of sample when programmed to deliver different

injection volumes. The average area of caffeine and acenaphthene were plotted against injection volume (n=6) as shown in Figures 4 and 5 and excellent injector linearity was obtained. The correlation co-efficient ( $R^2$ ) for peak area versus injection volume gives a measure of the linearity range of the injection volume of the autosampler. The  $R^2$  value across this injection volume range exceeded 0.999.

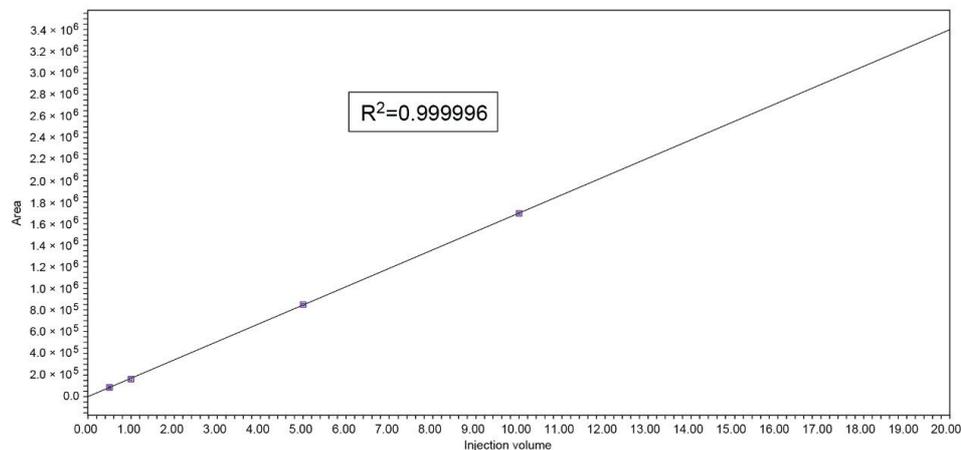


Figure 4. Injection volume linearity of caffeine peak from 0.5 to 20  $\mu\text{L}$ .

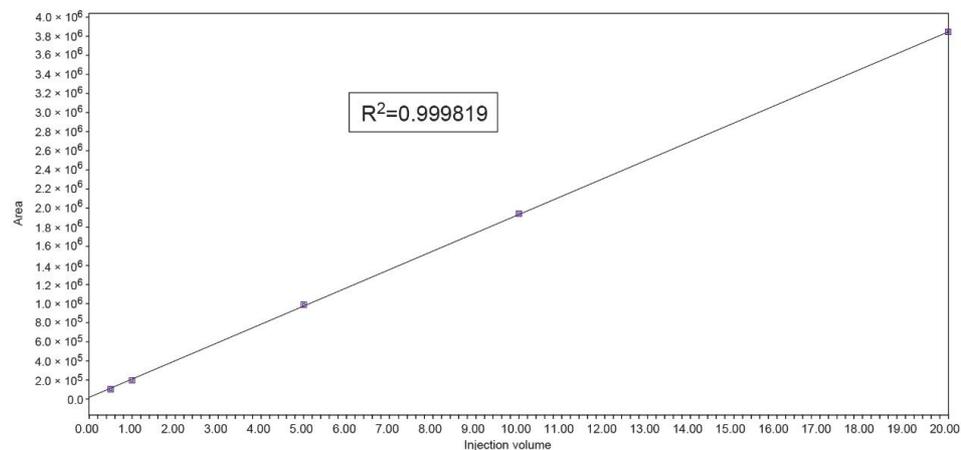


Figure 5. Injection volume linearity of acenaphthene peak from 0.5 to 20  $\mu\text{L}$ .

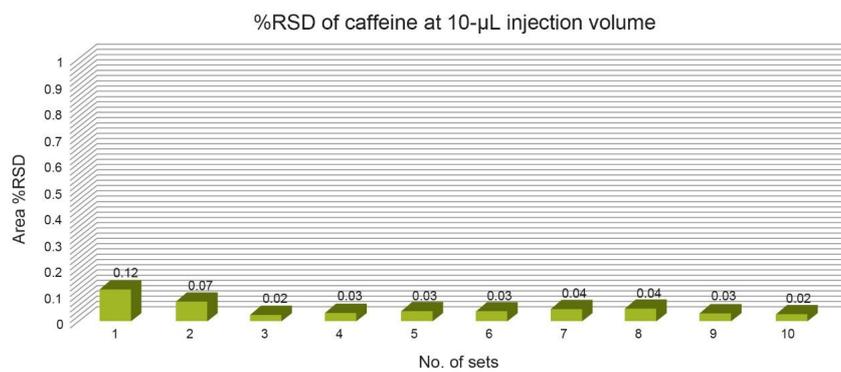


Figure 6. Area %RSD of caffeine peak for an injection volume of 10  $\mu$ L.

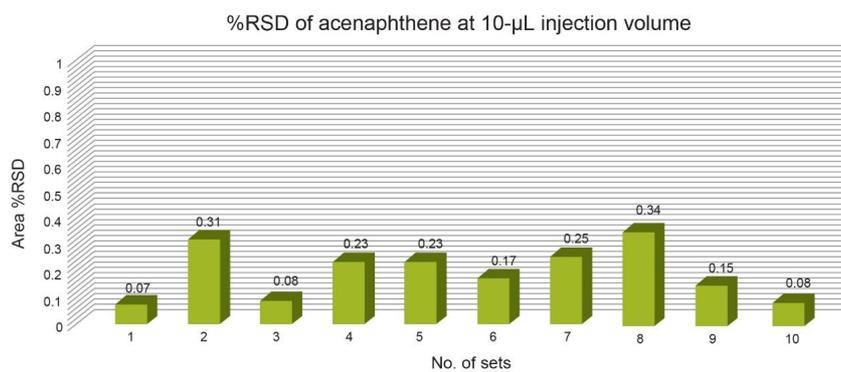


Figure 7. Area %RSD of acenaphthene peak for an injection volume of 10  $\mu$ L.

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## Conclusion

In routine analysis, laboratories require an autosampler that can deliver a sample that is highly reproducible and linear across the injection volumes for accurate quantification. The Arc HPLC System meets the stringent system suitability requirement for challenging analytical methods and demonstrates performance efficiency required in a highly regulated environment.

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