

Waters® Alliance® System

Precision and Linearity Considerations in a Sample Manager

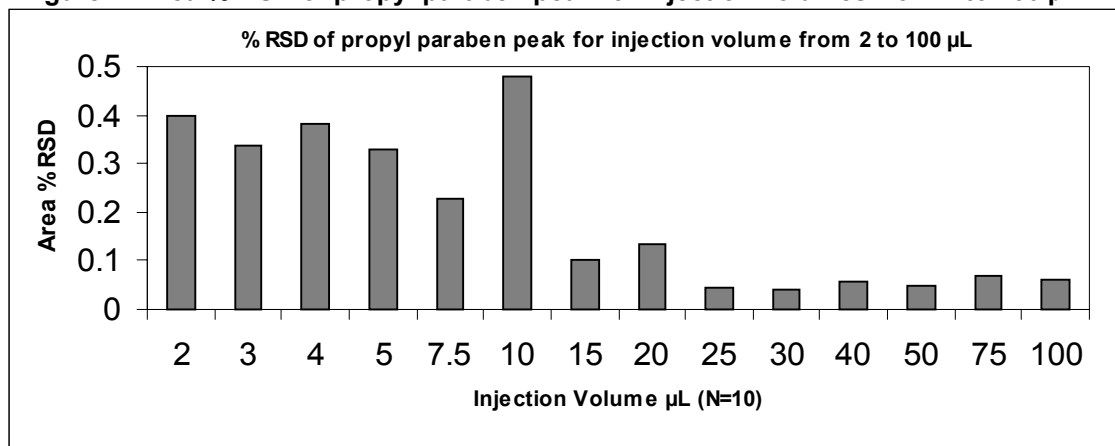
The Waters Alliance System is the first HPLC system to combine innovative solvent and sample management technologies to deliver high quality results for chromatographic separations. Previous Performance PerSPECTives have demonstrated how solvent management affects the quality of HPLC collected data. Sample management is of equal importance in any HPLC system. The performance characteristics of the Alliance HPLC System sample manager in the areas of precision and linearity are addressed in this PerSPECTive. A complementary PerSPECTive details injection accuracy and carryover characteristics of the sample manager contained in the Alliance HPLC System (See WPP 222).

Sample Delivery Precision of Alliance System Sample Manager:

The ability of an autosampler to deliver the requested injection volume repeatedly is its sample delivery precision. Precision can be expressed as the standard deviation (in absolute units) or as the relative standard deviation expressed as a percentage of the mean (% RSD). A small relative standard deviation (less than 1%) ensures that the sample manager does not significantly contribute to the area precision of the analytical method, which is affected by sampling, sample preparation, chromatographic resolution, and the signal-to-noise ratio.

Injection precision characteristics of the Alliance System sample manager, configured with the standard 250- μ L syringe and 100- μ L sample loop, were determined by injecting 2 to 100 μ L of a standard solution containing 10 μ g/mL of propyl paraben in system mobile phase. The area % RSDs for the propyl paraben peak at each injection volume were evaluated (N=10). As shown in Figure 1, area % RSDs were less than 0.5%.

Figure 1: Area % RSD of propyl paraben peak for injection volumes from 2 to 100 μ L

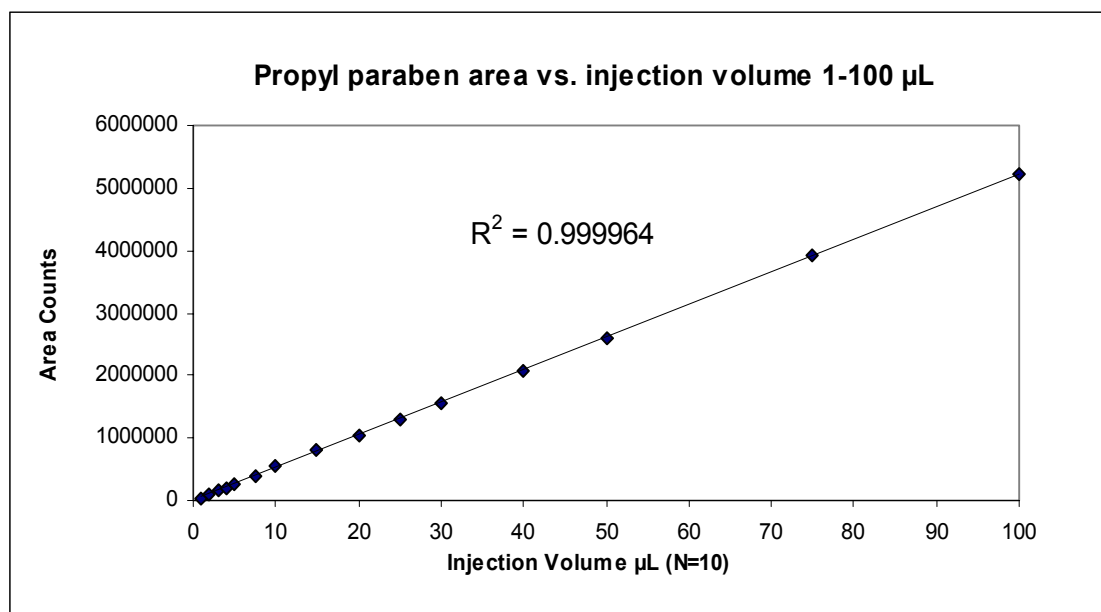


System:	Waters Alliance HPLC System with 2487 Dual Wavelength Detector set at 257 nm
Data:	Waters Millennium® 32 Chromatography Manager Software. Data collection rate: 10 pts/sec
Column:	Waters Symmetry® C ₁₈ , 100Å, 5 μ m, 3.9 mm x 150 mm at 35° C
Sample:	Propyl paraben at 10 mcg/mL in HPLC Eluent
Mobile Phase:	40% water/60% methanol. On-line mixing
Degasser:	Continuous
Flow:	1 mL/min
Needle Wash:	40% water/60% methanol. Mixed and filter degassed under vacuum
Seal Wash:	40% water/60% methanol. Mixed and filter degassed under vacuum
Vials:	Maximum recovery vials with pre-slit PTFE silicon septa (Waters Part No. 186000327)
μ L injected:	2, 3, 4, 5, 7.5, 10, 15, 20, 25, 30, 35, 40, 50, 75, and 100 μ L
Injections:	Ten per volume. Sample compartment set to 20° C

Linearity of Alliance System Sample Manager:

The ability of an injector to vary the injected volume in direct proportion to the requested volume is its linearity. The “goodness of fit” of the resulting peak areas to a linear equation is usually described by the correlation coefficient or R^2 . This value approaches unity for highly linear data. When injector linearity is excellent, calibration curves can be constructed from a minimum number of standard solutions by simply varying the injection volume. The experiments detailed in this study were designed to determine whether the Alliance System sample manager delivered the proportionately correct amount of sample when programmed to deliver volumes from 1 through 100 μL . The average area of the propyl paraben peak was determined and plotted against injection volume ($N=10$). As indicated in Figure 2, excellent injector linearity was obtained. (Note: This experiment was designed so that the amount of injected material was within the linear range of the Waters 2487 detector.)

Figure 2: Injection Volume Linearity of Alliance System Sample Manager



Data collected using conditions as described in Figure 1

Summary:

- The quality of results obtained from HPLC separations can be significantly impacted by HPLC injector performance.
- Precision and linearity are two measurable characteristics that determine how well an HPLC injector operates under various chromatographic conditions.
- The Waters Alliance HPLC System sample manager provides a high level of injection precision and linearity to enhance the chromatographer's confidence in the data collected.

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