Performance PerSPECtives

Waters® Alliance® System

Accuracy and Carryover 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 from chromatographic separations. Previous Performance PerSPECtives demonstrated how solvent management affects the quality of HPLC collected data. Sample management is of equal importance in any HPLC system. Performance characteristics of the Alliance HPLC System sample manager in the areas of injector accuracy and carryover are addressed in this PerSPECtive. A complementary PerSPECtive details precision and linearity characteristics of the sample manager contained in the Alliance HPLC System (See WPP 221).

Accuracy of Alliance System Sample Manager:

The tolerance in microliters (µL) with which an injector delivers the requested volume is its accuracy. The accuracy of an injector helps ensure reproducible chromatograms when the same sample is analyzed on different chromatographs. The accuracy of the injection volume was evaluated as weight of the solution injected. In the study detailed below, maximum recovery vials (Waters Part No. 186000327) were filled with HPLC grade water and capped with pre-slit silicon septa. The weight difference of the vials before and after the injection series was used to determine the average volume injected and the deviation from the programmed amount. The test was repeated six times at each injection volume. Accuracy, expressed as absolute deviation from the programmed injection volume, ranged from + 0.21 µL to - 0.21 µL at 50 µL (Figure 1) and from - 0.02 µL to + 0.11 µL at 25 µL (Figure 2). Instrument specification for injection accuracy on the 2690 Separations Module is +/- 1 µL at 50 µL.

Materials :

- Sample: HPLC grade water in Alliance System maximum recovery vials capped with a pre-slit PTFE silicon septa
- Mobile Phase: HPLC grade water (filtered and degassed)
- Flow rate: 1.0 mL/min
- Column: Symmetry® C_{18} 3.9 x 150 mm (30°C) to create normal system backpressure

Experimental Protocol :

- The weight of each HPLC grade water filled vial was measured to four places on an analytical balance (Note: Balance accuracy verified using an ASTM Class 1 traceable stainless steel weight)
- For 50 µL series: Six consecutive injections of 50 µL were made from each vial
- For 25 µL series: Ten consecutive injections of 25 µL were made from each vial
- The vials were re-weighted after the injection series
- The vial weight difference before and after the test was used to determine the deviation from the programmed injection volume

Figure 1: Measured accuracy in µL at 50 µL injection volume

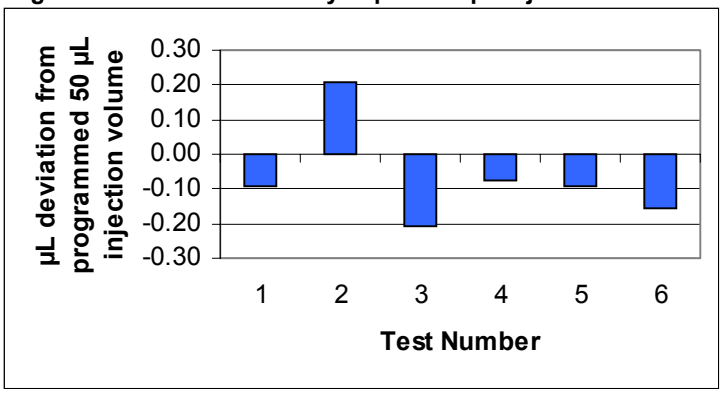
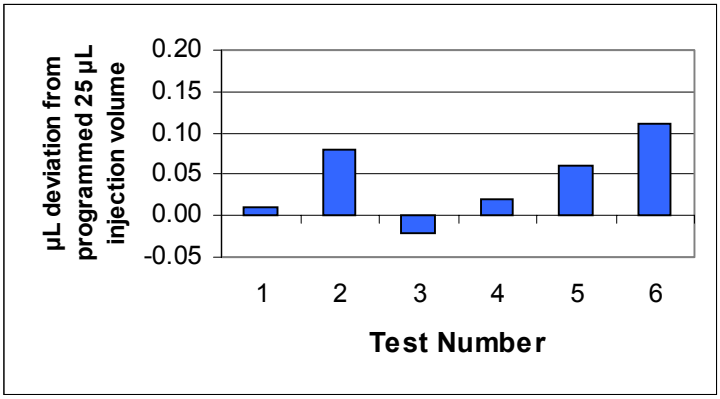


Figure 2: Measured accuracy in µL at 25 µL injection volume



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Measurement of Alliance System carryover performance in HPLC applications:
The "chromatographic memory" resulting from a previous injection is frequently referred to as carryover. It can be expressed as a percent of the mass or volume from the previous injection. When the mass is small, carryover has minimal impact on results obtained during a subsequent sample analysis. When injector carryover mass is large, poor precision and linearity result with obvious legal and financial ramifications.

Sample carryover on the Alliance System was evaluated using an aggressive standard method developed by Waters to evaluate autosampler carryover specifications. A "system blank injection" of 25 µL of system mobile phase (water/methanol: 40/60) was made onto a Waters Symmetry® C_{18} column (3.9 x 150 mm). Column effluent was monitored at 257 nm with a Waters 2487 Dual Wavelength Absorbance Detector and the signal processed using a Millennium®32 Chromatography Manager Workstation. After the system blank run, injection precision was verified and a calibration created using a 1 µg/mL standard solution of propyl paraben. The system was then stressed with six consecutive 25 µL injections of a 1 mg/mL (1000 µg/mL) solution of propyl paraben. Between injections, the needle wash system automatically flushes the needle in the sample manager with the needle wash solvent (in this experiment 40%water/60%methanol). Prior to the final analysis, the Symmetry C_{18} column was washed with 100% methanol to remove residual propyl paraben material. After the column was reequilibrated to initial conditions, a single blank injection of 25 µL methanol/water was made to monitor the carryover. Sample carryover was expressed as both % carryover and nL carryover as indicated below:

• % Carryover = (Area of propyl paraben in "blank injection for carryover") / (Average area (N=6) of 1 µg/mL propyl paraben standard injections) * 0.1

• nL Carryover = (Area of propyl paraben in "blank injection for carryover") / (Average area (N=6) of 1 µg/mL propyl paraben standard injections) * 25

Assay conditions:

Injection volume: 25 µL (22°C)
Column: Symmetry C_{18} 3.9 x 150 mm (30°C)
Detection: 2487: 257 nm Data rate: 10 pts./sec.
Data System: Millennium 32 Software 3.05.01

Mobile Phase: 40% water/60% methanol on-line mix
Flow Rate: 1.0 mL/min.
Needle Wash Solvent: 40% water/60% methanol
Purge Solvent: 40% water/60% methanol

Amount of carryover detected in "carryover injection" after six, 25 µL injections of 1 mg/ml (1000 µg/mL) of propyl paraben

% Carryover	0.0380
nL Carryover	9.61

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

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