Waters Alliance[®] Separations Module System Volume Definitions & Comparisons

In gradient chromatography, the system volume, also known as the gradient delay or dead volume, causes difficulties in gradient reproducibility and methods transfer. The system volume is the fluid volume from the point the eluents are proportioned (from the gradient proportioning valve or mixing tee) to the inlet of the column. System volume is shown in the following figures (white box) for traditional HPLC systems and the new Waters Alliance systems.

Traditional Single Pump Gradient HPLC System



Some systems have a pulse dampener/mixer, some have mixers, others have both.

Traditional Multi-Pump Gradient HPLC System



Some systems have a mixer, some have a pulse dampener/mixer, others have both.

Waters Alliance System



Waters Alliance requires no mixers or pulse dampeners



Of the wide variety of HPLC systems, only the Waters Alliance Separation Module was designed to minimize volume by the elimination of mixers, pulse dampeners and minimizing the internal tubing of the Alliance.

Some of the effects of system volume were discussed in Performance PerSPECtives WPP10. A large system volume will delay the arrival of the gradient at the column. What is acceptable system volume at 1 mL/min, (e.g. 1.5 mL or 1.5 min) is too large at 50 mL/min (30 min). This results in longer gradient run times (gradient delay) and longer re-equilibration times. For adequate re-equilibration, a general rule of thumb is 3 system volumes plus 5 column volumes. The smaller the system volume the shorter this time can be, especially at low flow rates.

Changes in the shape of the programmed gradient occur with higher system volumes because there is bandspreading of the gradient. The bandspreading makes the actual gradient shallower at the beginning and end of the curve. The differences in the gradient shapes make methods transfer from HPLC system to system difficult.



Binary high pressure systems without mixers may have low system volumes, but they often suffer from poor retention time reproducibility and baseline ripple. The Waters Alliance Separations Module has a low total system volume, <650 mL, with excellent performance. This system produces accurate gradients with good eluent mixing, and excellent retention time reproducibility. Unit to unit consistency has been designed into the Alliance, making gradient methods transfer easier.

| Component | Mixers | Pulse | Component | Total System | Total System |
|-----------------|--------|--------|------------|--------------|-----------------|
| or System | μL | Dampen | Volume | Volume µL | Volume µL |
| | | er | μL | with mixer | no mixer |
| Waters Alliance | None | None | | | < 650 |
| A: Autosampler | | | <1600 | | |
| Quaternary pump | | Yes | <2300 | | <3900 |
| Binary pumps | 500 | Yes | <550 | <2150 | Not recommended |
| B: Autosampler | | | <380 | | |
| Quaternary pump | 300 | No | <650 | <1030 | <730 |
| Binary pumps | 500 | Yes | <550 | <930 | Not recommended |
| C: Autosampler | | | 300 +loop | | |
| Quaternary pump | None | Yes | 800 - 1100 | | >1100 - >1400 |
| Binary pumps | 420 | Yes | 600 - 900 | >900 - >1200 | >480 - >780 |