Performance PerSPECtives

Gradient HPLC Solvent Delivery System

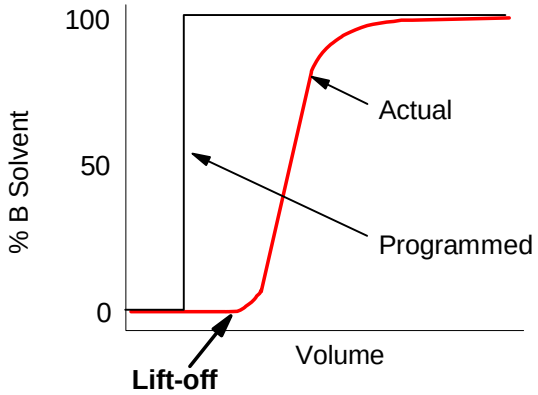
System volume

What is system volume?

System volume in a gradient solvent delivery system is the volume (time) required for the gradient to pass through the HPLC system. It is the actual volume of the fluid path, where the solvent flows, from the point where the A and B solvents first come together until the mixture enters the column. It applies to all gradient systems, either a single pump (low pressure) or a multipump (high pressure) system. Other terms that are used for this volume are delay volume and dead volume. When measuring or comparing system volumes, one must understand what is being measured, i.e. is it just the pump? Does it include the autosampler?

Why is system volume important?

The system volume will affect the gradient separation. The transfer of a chromatographic method from one HPLC system to another, especially from different manufacturers, may be difficult when the system volumes are different. The separation will change because the gradient shape can change.



- Programmed step 100%A to 100% B
- Actual response
- Lift-off equals system volume

How can the system volume be measured?

The solvent A is methanol and the solvent B is methanol containing a UV absorbing compound, for example 9 mg/L propylparaben. A UV detector is connected directly to the outlet of the gradient system (no column). A small amount of backpressure should be applied to the system at the outlet of the detector. (Be sure not to exceed the pressure limit of the detector.) A system volume for today's gradient HPLC system should be <600 uL and <400 uL for the pump portion. This may require the installation of a low volume option in the autosampler.

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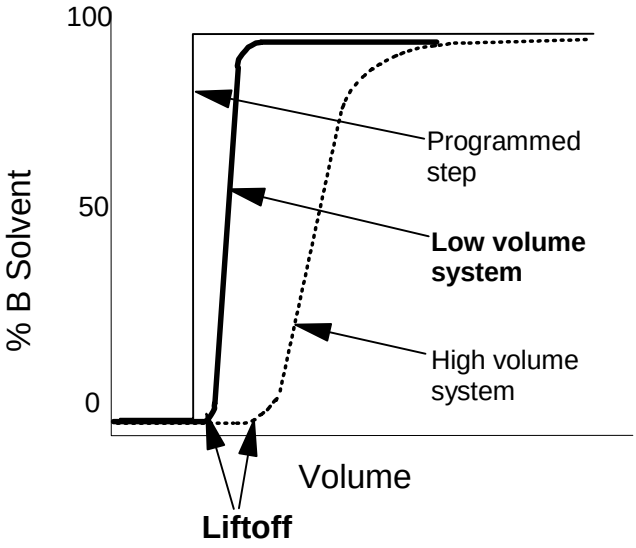
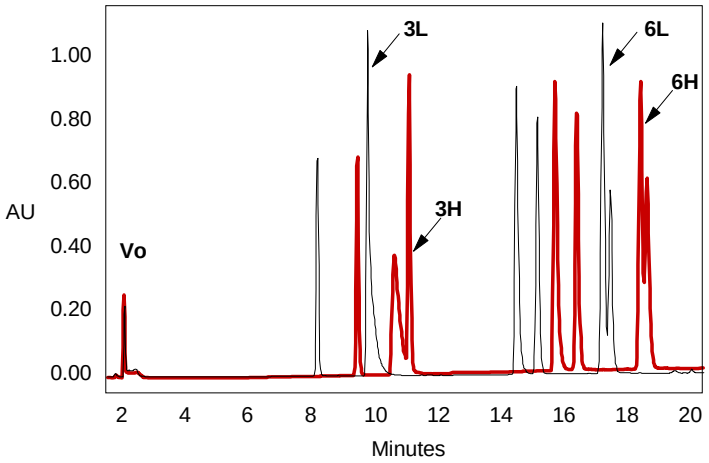
Effect of System Volume



A **step gradient** from 100% A to 100% B solvent is the steepest gradient .

Liftoff is the actual system volume. However, the effect of 100% Solvent B on the separation occurs when 100%B reaches the column, not when the first trace of B reaches the column at liftoff. This may be several mL (or minutes) greater than liftoff.

Manufacturers specifications for delay volume are usually the liftoff point. For a low volume or low dispersion, gradient pump, this should be <400 uL. For a low volume HPLC system, without column, this should be <600 uL.



- ►Linear gradient 0 - 80%B in 40 min
- ►Mobile phases: A = water B = acetonitrile; +0.015% phosphoric acid
- ►Column: Nova-Pak C18, 3.9x150cm, at 30C
- ►Flow rate: 1 mL/min
- ►Detection: UV at 254 nm
- ►Standards of ionic and neutral compounds

Low system volume
High system volume

Affect of Delay Volume on Chromatography

The figure above is an overlay of the same chromatographic separation performed on two systems one with different system volumes.

- ►The first peak, the **Vo** marker, is in the same place in both separations because it is unretained and is a measure of only the column volume.
- ► All other peaks in the HPLC with lower system volume elute sooner because the blended solvents of the gradient reaches the column sooner.
- ►Peak 3L has a back shoulder caused by a coeluting peak. In the higher volume system Peak 3H is separated into the two components because the shape of the linear gradient has been distorted to make it flatter at the beginning (see upper figure).
- ►Peaks 6 and 7 are better separated in the low volume system than in the higher volume system because the gradient shape is different.
- ►**Ideally, the system volume should be as low as possible (<600 uL).**

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