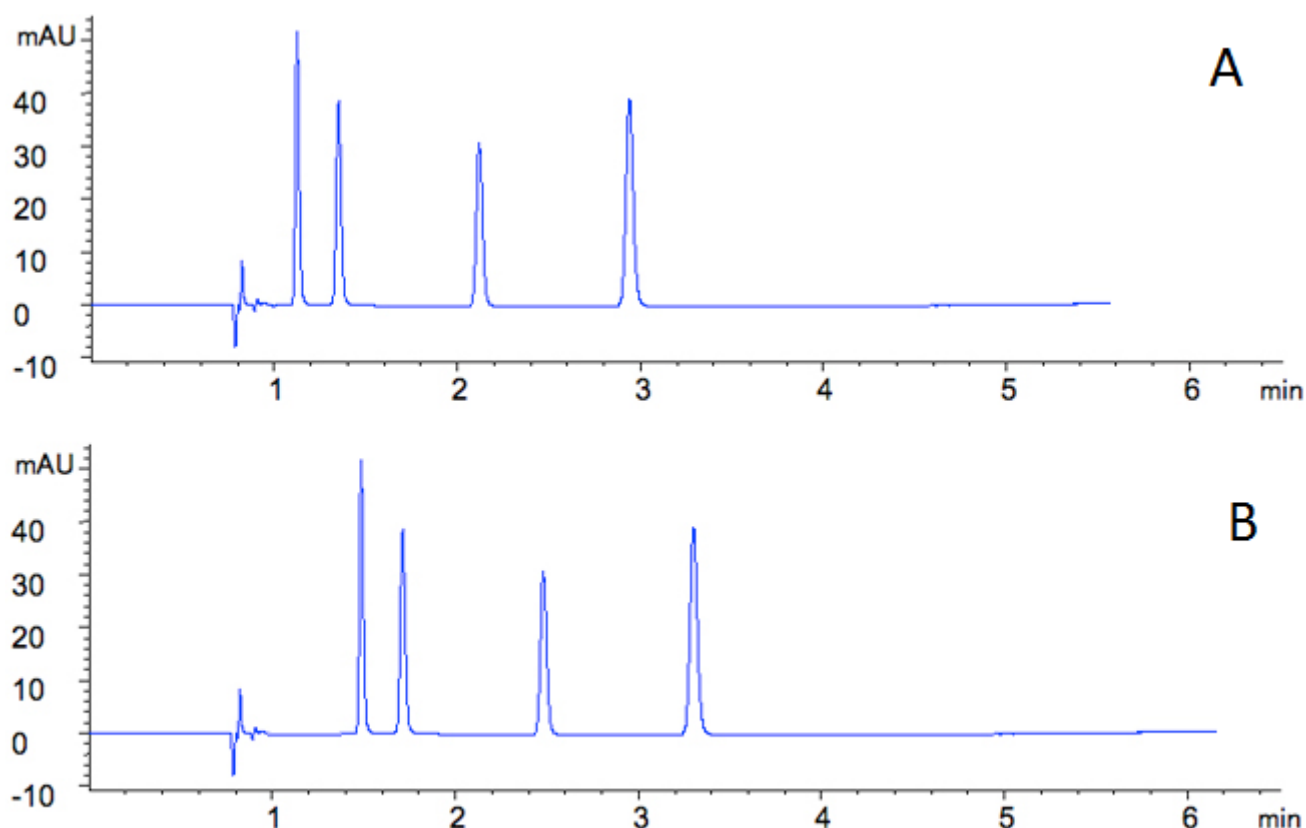


Dwell volume effect on HPLC analyte retention in gradient methods – Primer

Dwell volume is the volume between the point where the solvents are mixed and the HPLC column. Suppose we have a method with a hypothetical gradient as follows:

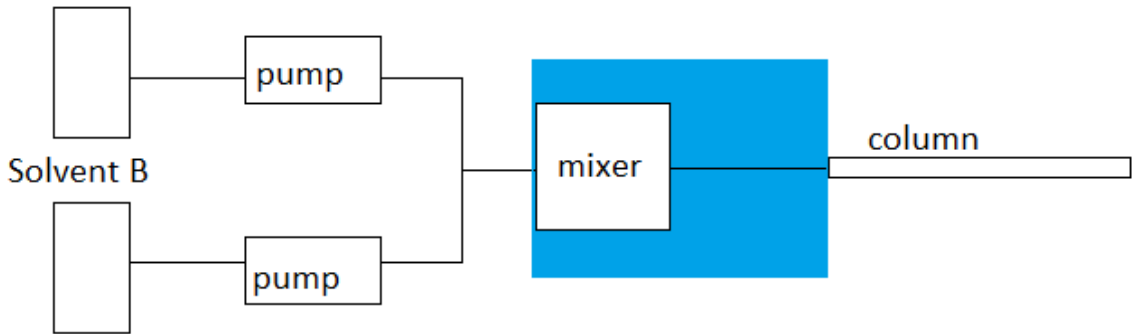
| Time (min) | % B Solvent |
|------------|-------------|
| 0 | 20 |
| 9 | 60 |
| 10 | 20 |

From the table, we can see that the gradient begins immediately, but in practice this is not what really happens. It takes a certain amount of time for the mixed solvents to reach the column, so there is a delay in when the gradient actually starts. The amount of delay is determined by how large the dwell volume is. An example in data between a system with a lower (A) and higher (B) dwell volume could look something like this:



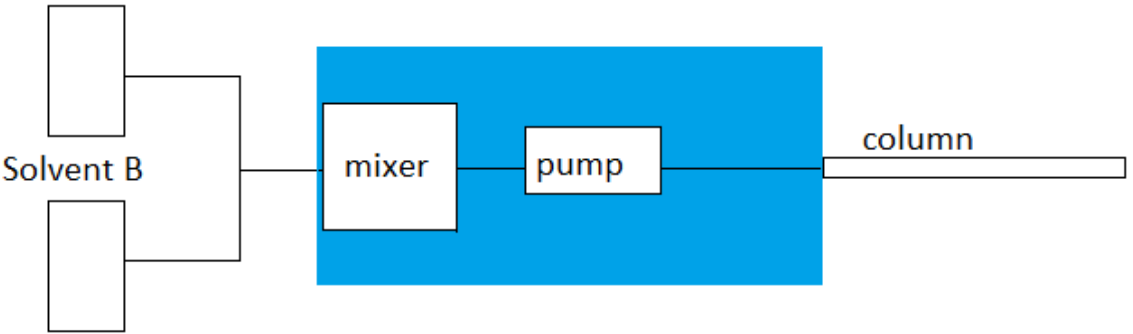
The main contribution to dwell volume is whether high or low pressure mixing is used. Here is a simplified schematic showing the difference in dwell volume between the two systems:

Solvent A




High Pressure Mixing

Solvent A



Low Pressure Mixing

 dwell volume