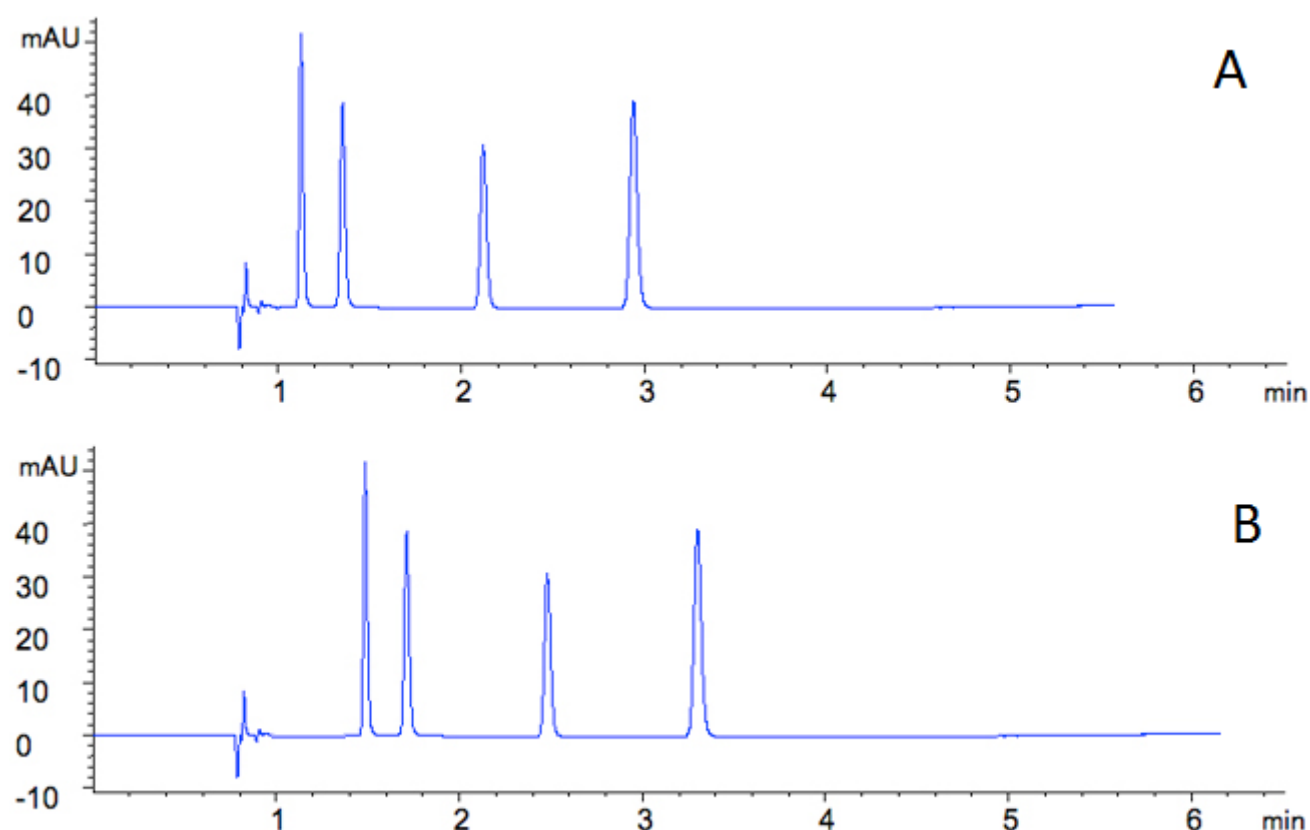


## Dwell volume effect on HPLC analyte retention in gradient methods – Primer

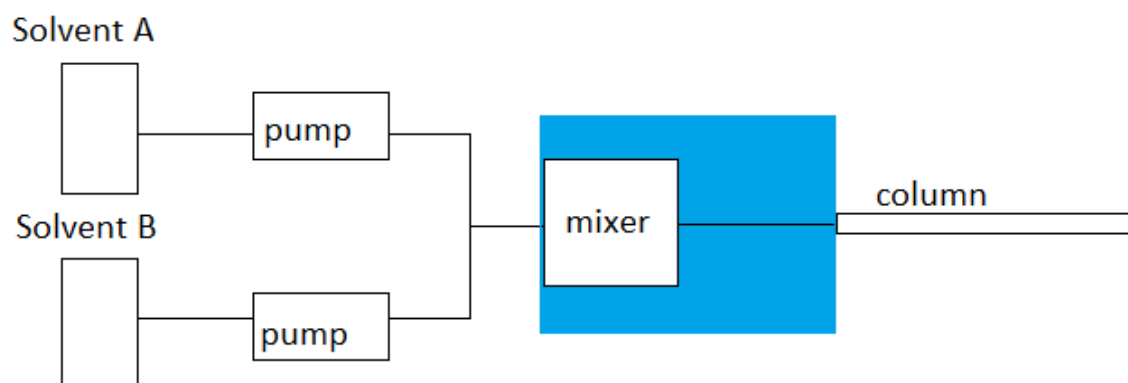
Dwell volume is the volume between the point where the solvents are mixed and the HPLC column. Suppose we have a method with a hypothetical gradient as follows:

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

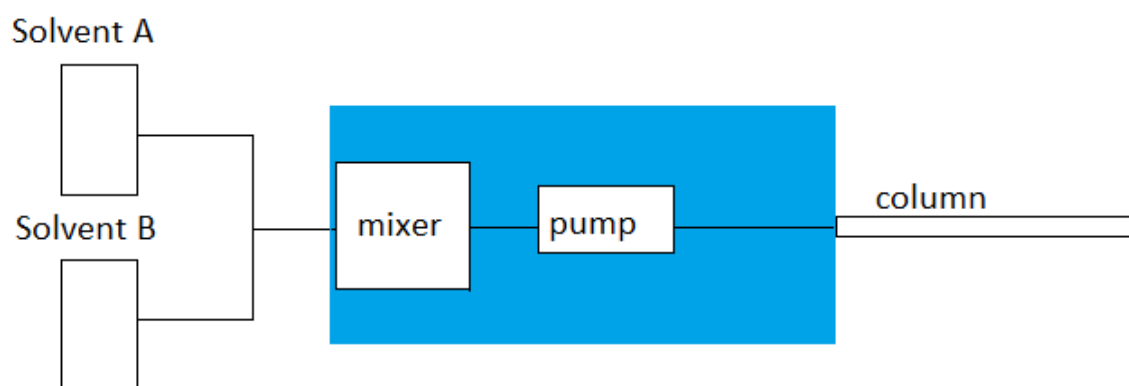
From the table, we can see that the gradient begins immediately, but in practice this is not what really happens. It takes a certain amount of time for the mixed solvents to reach the column, so there is a delay in when the gradient actually starts. The amount of delay is determined by how large the 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:



High Pressure Mixing



Low Pressure Mixing

 dwell volume