

Choose an appropriate re-equilibration or post time for gradient HPLC - How To

When using gradient methods in HPLC or LCMS, the mobile phase and column must be allowed to equilibrate back to its initial conditions before starting the next run in order to ensure consistent chromatography.

This portion of the gradient is called the **Post Time**. It is best to use as low a post time as possible without compromising retention time precision in order to increase throughput, save time and also reduce solvent use and disposal.

The best way to find the optimal post time is to use a large or excess time during the method development stage and reduce the post time for your method with each run. As soon as precision becomes compromised to an extent that the acceptance criteria of the method are not met, use the next longer post time.

Also, check this for robustness by reducing the post time on different instruments and different days. Alternatively, a "quick and dirty" technique that helps to find a suitable post time is to simply observe when the pressure returns to its initial value and levels off.

The number of column volumes needed for the post time will depend on several factors such as the difference between the initial and final %B, the dominating retention mechanism (*function of LC operating conditions and the analytes' physico/chemical properties*). With Ion Exchange phases, for example, up to 100 column volumes may be required. On the other hand, retention in many methods that use Cogent TYPE-C Silica™ columns was found to be unaffected when using as low as 3-5 column volumes for the post time re-equilibration.

