

# Post Separation Pause I: Why Use a Post Separation Pause? I-Optimizing Gradient

## Abstract

The Post Separation Pause function implemented in the ACCQPrep and EZ Prep automation allows fine-tuning of preparative chromatography. The Post Separation Pause is designed to be used during multiple injections of the same sample. After a purification is complete, the system pauses before starting the next injection to allow adjustment of the gradient, run length, and sample loading. The pause allows verification that the chromatography is running as expected and to make adjustments if desired.

Typically, a small amount of sample is run and the elution verified, minimizing potential sample loss while fine-tuning the purification. After choosing an appropriate gradient, a maximum sample load is estimated to reduce the number of injections that minimizes run time and solvent usage.

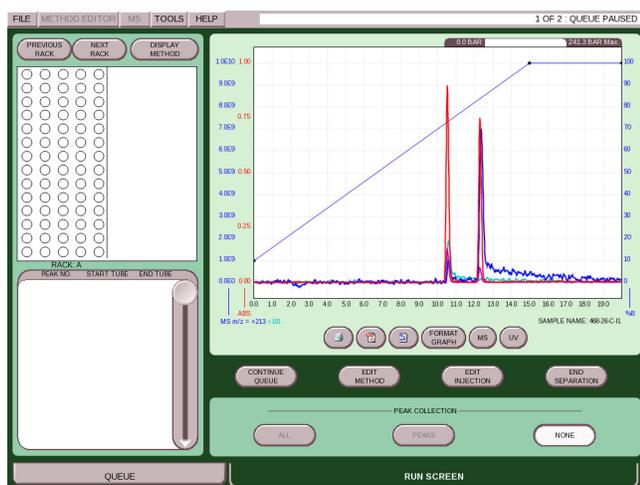
## Overview

It is important to minimize solvent usage and purification time when running preparative chromatography. One way to minimize time is to optimize the gradient so that the desired compound elutes quickly while still eluting away from the impurities. A focused gradient allows maximum resolution while minimizing run time and solvent usage. Also, a focused gradient allows for some variation in solvent delivery between an analytical system and a preparative system. The Post Separation Pause allows fine-tuning of the gradient.

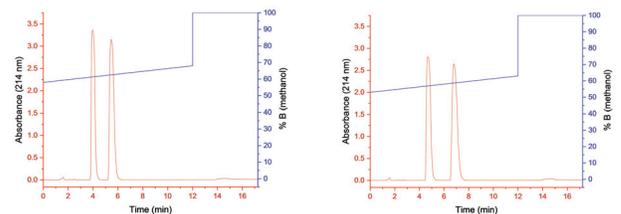
If the Post Separation Pause is enabled, a screen similar to that in Figure 1 is displayed. The CONTINUE QUEUE button runs the next injection with no changes. The EDIT METHOD button allows any method parameter to be changed prior to the next injection. This includes detection parameters as well as gradient and solvent parameters, but not injection parameters. The EDIT INJECTION button allows the volume to be changed of an injected sample. The button on the far right, END SEPARATION, ends all injections for this sample.

## Gradient Adjustment

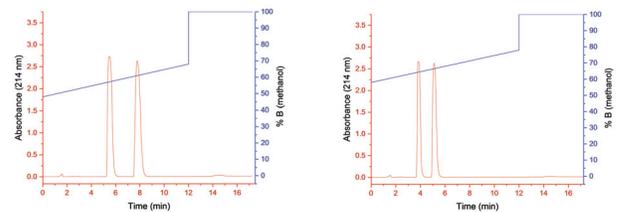
In Figure 2, the gradient is defined by two points followed by a wash at 100% B solvent. The gradient increases the concentration of the “strong” solvent, which causes the sample to elute down the column more quickly. The strong solvent is usually the “B” solvent.



**Figure 1: Display of Post Separation Pause after an injected purification is complete**



Original chromatogram (58-68% methanol) Lower start and end of gradient by 5% B



Lower start of gradient by 5% B Raise end of gradient by 5% B

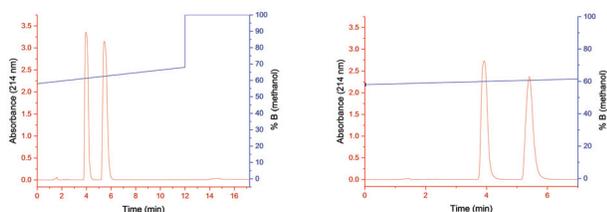
**Figure 2: Adjusting the gradient to control retention time and resolution. All changes are relative to the original chromatogram.**

The elution of compounds may be adjusted and optimized by adjusting the gradient end points. Increasing the percentage of strong solvent causes faster elution. Changing the slope of the gradient changes resolution. For example, increasing the gradient slope reduces resolution.

It is also possible to shorten the run. To keep the current gradient starting point and slope, the following *equation can be used* to determine the ending gradient for a reduced run time:

$$\text{End \%B} = \left( \frac{\text{Original } \Delta\%B}{\text{Original run time}} \right) * (\text{New End Time}) + (\text{Start \%B})$$

For the original chromatogram in Figure 1, both peaks eluted before 7 minutes, so it is possible to reduce the run time by 5 minutes, thus eliminating the remaining “unused” gradient. The new end %B =  $(10/12)*7+58 = 63.8$ , which will be rounded up to 64% B. The new gradient is complete in 7 minutes. As the mixture was clean, the 5 minute wash step was also eliminated to save a total of 10 minutes per run. The column should be washed before running other samples to avoid possible cross contamination and ghost peaks. The column should also be washed after use to remove any solvent modifiers.



**Figure 3: Original chromatogram; chromatogram modified to reduce run time**

**✓ Note**

All of the gradient changes are done using the method editor accessed with the EDIT METHOD button in Figure 1.

## Conclusion

The Post Separation Pause is useful to adjust gradients to improve resolution and reduce run times. Gradients can be fine-tuned to improve resolution while minimizing run times. The Post Separation Pause feature allows users to easily adjust gradient and injection parameters while minimizing sample use until the ideal purification parameters are set.

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