# Flash Method Development in a Flash

TELEDYNE ISCO
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**Chromatography Application Note**
**TN65**


## Abstract

The Focus Gradient Generator allows users to quickly create efficient preparative gradient methods using flash columns on the Teledyne ISCO Combi*Flash*® system. The resulting preparative gradients are 12 column volumes long, are focused for increased resolution around the peak of interest, and have a wash step at the end.

Being able to quickly determine a preparative method with increased resolution around the target peak enables methods that offer better separation between the target compound and other impurities. Such methods offer increased purity from preparative flash runs or the ability to increase sample loading for preparative runs. Performing more efficient flash chromatography helps decrease overall solvent usage and generates less waste solvent.

The first step to determine the optimized focused gradient is to run a scouting gradient using a small amount of sample. The scouting run typically requires the same time to run as a single thin layer chromatography (TLC) plate. Unlike a single TLC plate, which can only be run at a specific isocratic solvent mixture, the entire solvent composition range is tested at the same time during the scouting run. In addition to providing a retention time to calculate a focused gradient, the scouting gradient answers the following questions:

- Will the compound elute with a particular column or solvent system?
- Does it need a modifier such as trifluoroacetic acid or triethylamine?
- Can it be purified by flash? Is there enough resolution? Changing the column or solvent system may allow more resolution.

It also provides additional information:

- Estimated sample loading for a column and solvent system. Peaks separated by ~3% B solvent in a focused gradient or less will be subject to a light loading.


## Overview of Flash Method Development

### General Methods

The workflow for creating focused gradients on the Combi*Flash* system is the same for both normal and reverse phase. The first step is to select a column size and then choose the "Scout" method.

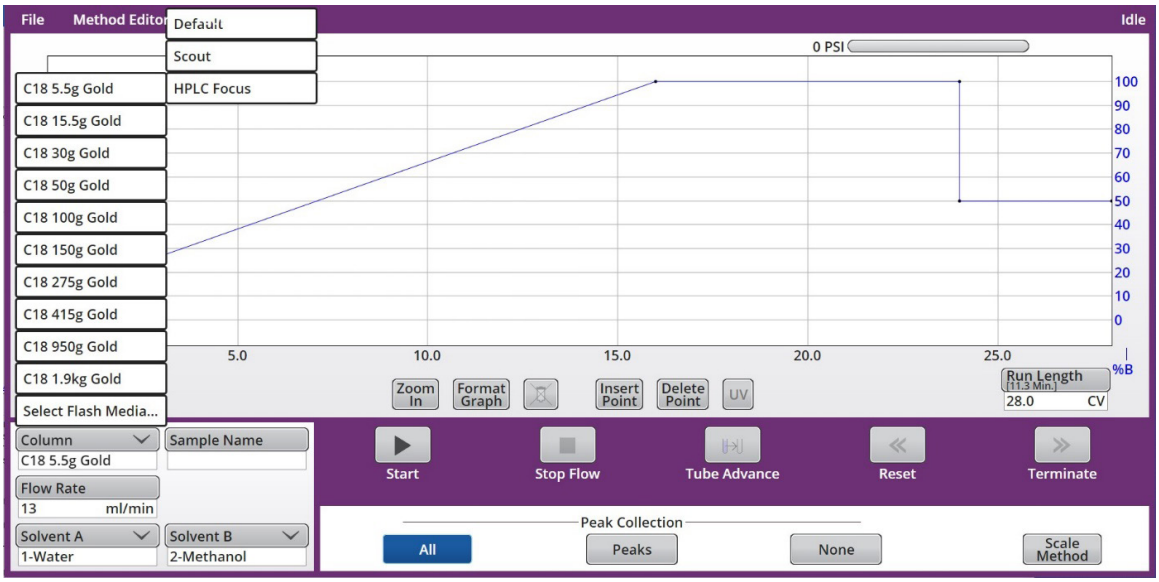


**Figure 1.** Column and method selection.

After selecting the scouting run, the system loads a pre-calibrated scouting run. Many of the parameters are locked, but some method variables can be changed as needed. In the Method Editor, the wavelength values can be changed as needed to maximize detection. The signal gain can also be increased to allow detection of as little as one milligram of compound.

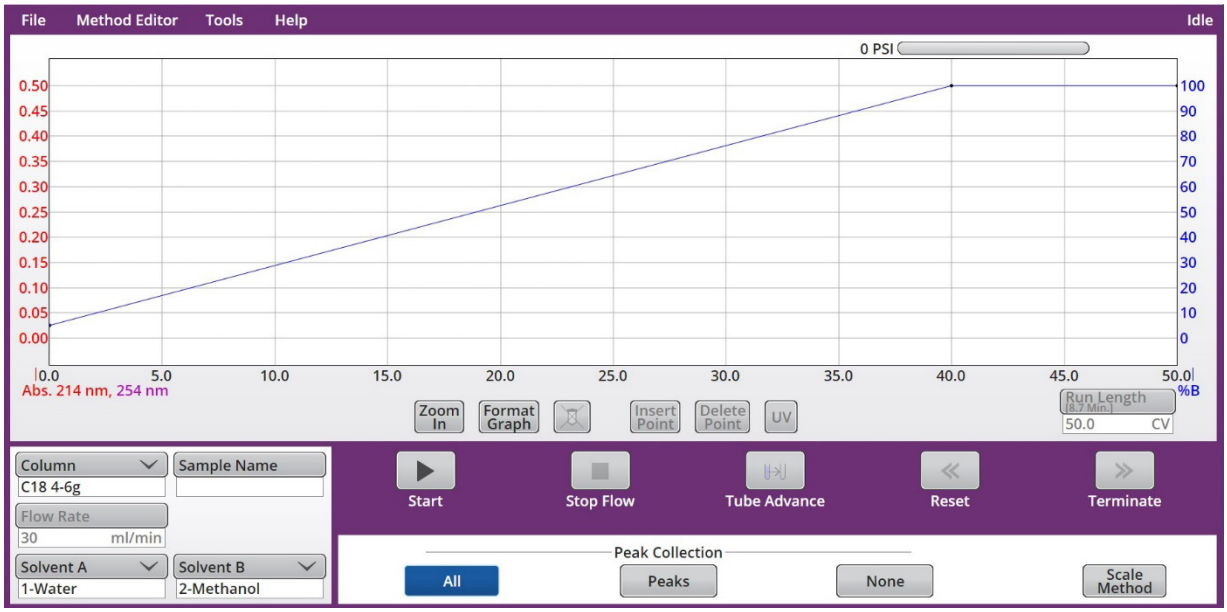


**Figure 2.** Example scouting run.

After setting up the detection parameters, press the Start button which opens the Minimum Run Requirements window where you can set the rack and tube location for fraction collection and the method of sample loading.

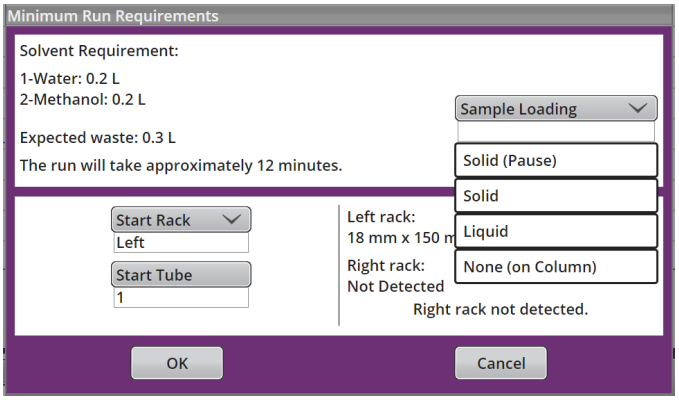


**Figure 3.** Run requirement screen

## Sample loading

Do not use None (on Column) for scouting run sample loading. If using a solid sample loading with smaller columns, 12 g (24 g alumina, 15.5 g reverse phase/amine) or smaller, use a 5 g solid load cartridge with 0.5 g silica (1 g alumina if you are making alumina solid load cartridges) or less since the silica in the cartridge alters the scouting gradient retention.

If using liquid injection, it is best to inject directly onto the column instead of through the column shuttle. A small column (4 g silica, 8 g alumina, 5.5 g amine or reverse phase) runs well with a 0.1 mL injection. Injecting 0.1 mL allows the sample to be dissolved in the strong solvent with minimal effects on the chromatography. Injecting through the top column holder requires 2 mL of chase solution to ensure the sample is loaded onto the column and so isn't recommended.

Press "OK" to start the scout run.

## Calculating a focused gradient

After the scouting run is complete, a chromatogram will appear. Pressing the Reset button closes the run, but a focused gradient may be calculated later by opening the file in the system run file viewer.

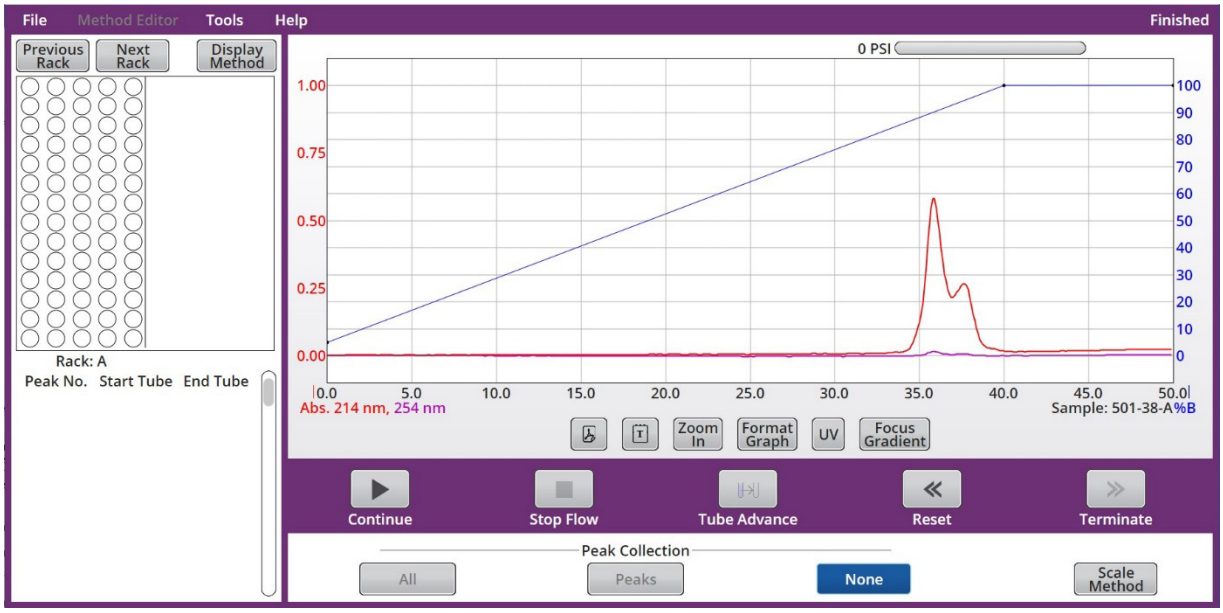


**Figure 4.** Completed scouting run.

Pressing the Focus Gradient button opens the Focused Gradient Definition window.

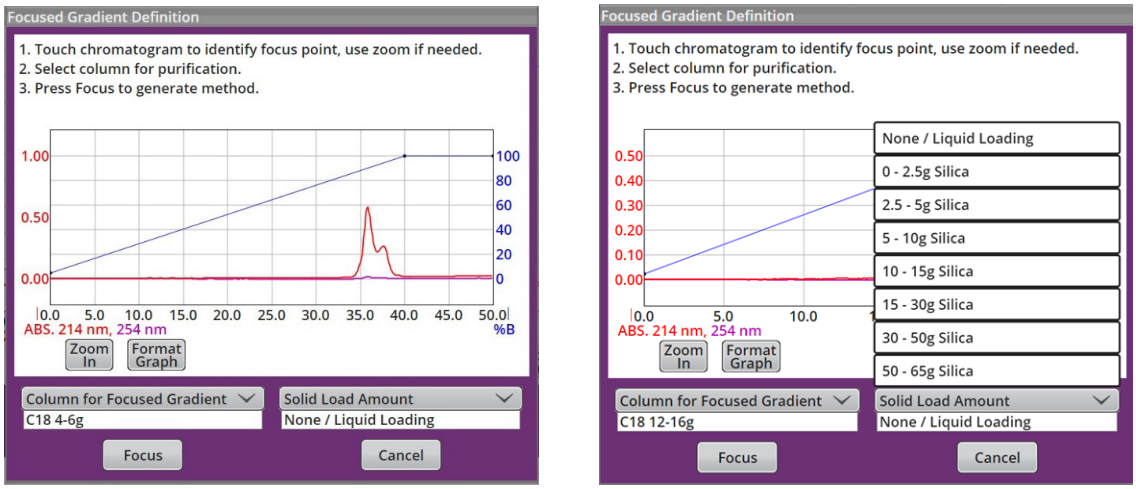


**Figure 5.** Focused Gradient Definition window and solid load selection.

The Focused Gradient Definition window shows the results of the scouting run, allowing the user to touch the chromatogram to identify the focus point. Select the size of the column to be used for the preparative focused gradient method using the drop-down list, and then select how the sample will be loaded for the preparative run. When choosing the Solid Load Amount, choose the amount of silica used in the solid load cartridge or choose Liquid Loading. The system will create a focused gradient method that you can run in the same manner as a method entered by a user.

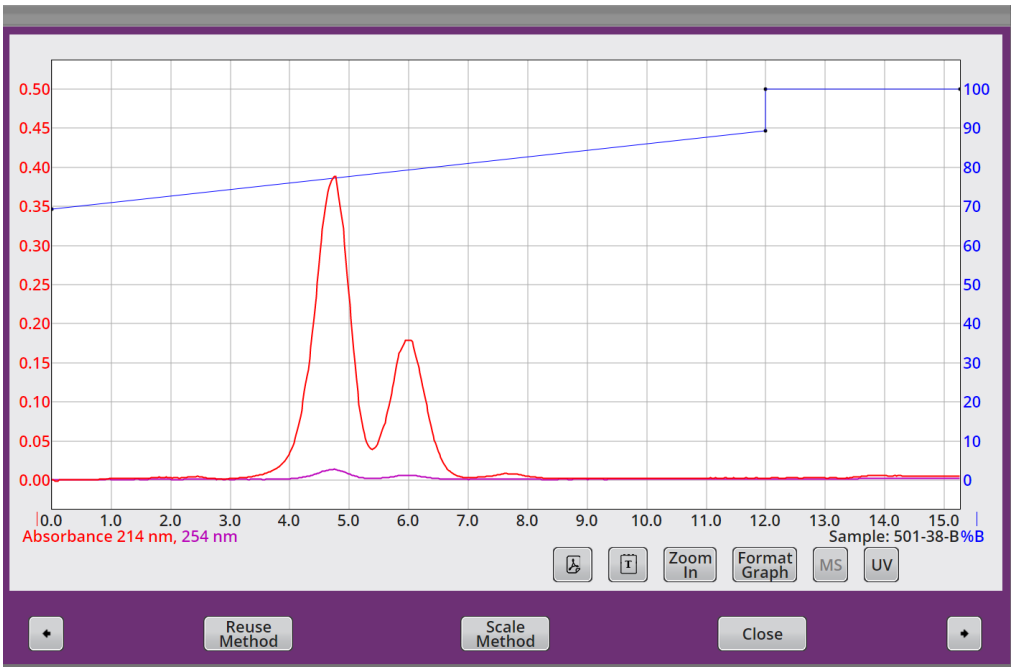


**Figure 6.** Gradient focused on second eluting peak in Figure 5.

The desired compound elutes in a range of 2 to 10 column volumes, offering a fast and efficient method to increase resolution around your peak of interest.

## Conclusion

The flash Focus Gradient Generator is a quick means to determine whether a compound can run on a given column and solvent system. We can see if the compound elutes, determine if it will have good peak shape, and is sufficiently resolved from impurities to run as a focused gradient. The calculated focused gradients are efficient because they run quickly and use little solvent. The scouting runs allow method development for all types of Teledyne ISCO columns without setting up multiple TLC solvent conditions or using multiple TLC plates, even if they are available to a lab.

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