Generate Reverse Phase Flash Focused Gradients at Lightning Speed

TELEDYNE ISCO
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Chromatography Application Note AN119

Abstract

With an automated Focus Gradient Generator now available on ACCQ*Prep*® HP150 systems, it is no longer necessary to spend time manually developing or optimizing flash focused gradient methods. The tool automatically calculates the focused gradient from the results of a single analytical scouting run. It can be used to determine whether a particular purification will run effectively on a Combi*Flash*® NextGen system, and to calculate focused gradients for preparative HPLC or flash purifications. When using Redi*Sep*® Prep HPLC columns with matching selectivity to Redi*Sep* Gold® columns, determination of sample purity and calculation of the flash focused gradient is completed within 7 to 12 minutes, depending on the HPLC system and column used. Analytical HPLC systems can also be employed to calculate flash chromatography methods using the ACCQ*Prep* Focus Gradient Generator (see Tech note TN52 on the Teledyne ISCO web site).

Background

Reverse phase flash chromatography has been hindered by the lack of rapid method development techniques. Normal phase flash chromatography method development can be done with thin layer chromatography (TLC) plates, but if the sample elutes in a solvent system containing more than 60% water, reverse phase plates take a very long time to run. Another approach is to develop the flash method directly on the flash system, but this consumes large amounts of solvent and sample. Or the method can be developed on an analytical HPLC system and then transferred to the flash system, which typically involves multiple attempts and requires analytical columns with chemistry matching the flash columns (as described in AN118). Using Redi*Sep* Prep columns in conjunction with the Focus Gradient Generator tool in the ACCQ*Prep* avoids all these problems, saving time, sample, and solvent. Further, using the flash system for the easier purifications will free up the ACCQ*Prep* system to run the more difficult purifications that cannot be run with flash.

Experimental and Results

Use the following procedure to develop flash focused gradients from a scouting run.

1. Run the Focus Gradient Generator with the sample to be purified, on the ACCQ*Prep*. Either the built-in focus gradient generator can be used, or the tool that calculates a focused gradient from an analytical HPLC. If using the ACCQ*Prep* for the scouting run, use procedure “a”. Procedure “b” uses an analytical HPLC system calibrated with the ACCQ*Prep*.

   a. Use a 20x150 mm Redi*Sep* Prep column to calculate the focused gradient for the compound to be purified. Note that Redi*Sep* Prep columns match the column chemistry of Redi*Sep* reverse phase columns, thereby avoiding differences in column selectivity. Record the starting and ending points of the focused gradient; these values will be used on the flash chromatography system.

   b. Make sure the ACCQ*Prep* and analytical HPLC have been calibrated according to Tech Note TN52. Use a 2 x 50 mm or a 4.6x150 mm Redi*Sep* Prep analytical column that matches the flash column chemistry to be used, such as Redi*Sep* Gold C8, C18, or C18AQ. Enter the retention time for the peak of interest into the ACCQ*Prep* to calculate a focused gradient. Record the starting and ending points of the focused gradient; these values will be used on the flash chromatography system.

2. Determine whether there is enough resolution between the compound to be purified and the closest impurity. The easiest way to determine this is to calculate a focused gradient for both the impurity and the desired compound and then note the focused gradient starting points for each method. If the solvent composition for the gradient starting point for the impurity is more than 3% B away from that of the desired compound, the purification can be run on a flash column; otherwise the ACCQ*Prep* system will be required. The difference in solvent composition for the starting point of the gradient is used because this value will be the same whether the ACCQ*Prep* internal calculation or an external HPLC calculation tool is used. Another way to assess the required resolution is by the difference in retention time of the peaks in the scouting gradient. Determine

the scouting gradient slope m = (Ending %B – Start %B)/Gradient time. For the example in Figure 1, this is (100 - 5)/6 = 15.8. Since we require 3% resolution to resolve the peaks on a flash column, divide 3 by the slope. The equation is: Δ%B = 3/m. For the 6-minute gradient used in Figure 1, 3/15.8 = 0.19, which rounds to ≈0.2 minutes. This means peaks need to be resolved by at least 0.2 minutes on this scouting gradient to be resolved and purified on the CombiFlash NextGen system.

3. Load a column of the same type used for the scouting gradient on the Combi*Flash* NextGen Using the method editor, create a focused gradient with time units expressed in column volumes. Select the same solvents as used for the scouting run, including any modifiers. The starting and ending %B for the focused gradient are the same as that determined by the ACCQ*Prep*, and the gradient length, in column volumes, as listed in Table 1. If a solid load cartridge is used, the gradient length will need to be extended.

Redi*Sep* Gold Reverse Phase Column size (g)	Focused gradient length (column volumes)
5.5	20
15.5	14
30	14
50 and larger	12

Table 1– Focused gradient lengths for various flash column sizes.

The example below used a mixture of capsaicins, chosen to be at the limit of resolution for flash chromatography. The first step is to perform a scouting run that allows a gradient to be determined for the first eluting peak in the mixture at ~7.5 minutes. A Redi*Sep* Prep 20 x 150 mm column was used for the scouting run (PN 69-2203-810) with a water/methanol gradient.

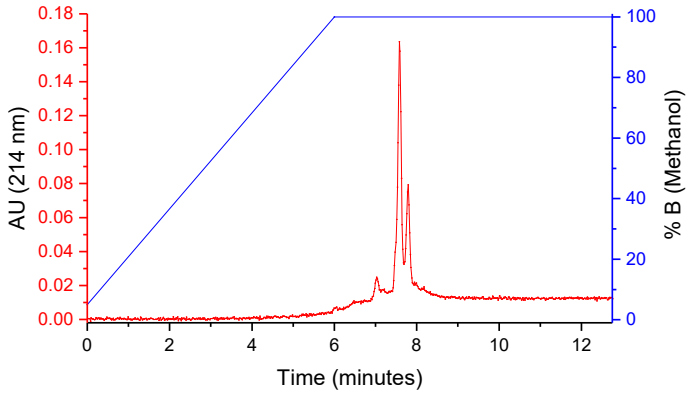

Figure 1–Scouting run of capsaicins run on a Redi*Sep* Prep 20x150 mm C18 column

The next step is to calculate a focused gradient for the first eluting peak. The chromatogram is run only as an example. The peaks in the scouting run are 0.2 minutes apart, corresponding to a difference of 3 %B in the calculated gradients of the two compounds.

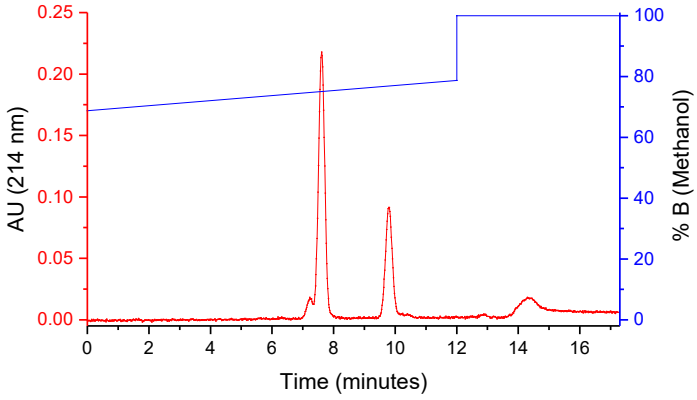

Figure 2– Calculated ACCQ*Prep* gradient for the first peak in Figure 1.

A 50 g Redi*Sep* Gold C18 column (PN 69-2203-336) is inserted into the Combi*Flash* NextGen. After the column is recognized by the system, the run parameters for the column are loaded. The solvent system is the same used for the scouting run. The gradient is modified to a focused gradient with the same starting and ending solvent composition as for the ACCQ*Prep*. The column is washed with at least 2.5 CV of B solvent at the end of the run to purge strongly retained compounds.

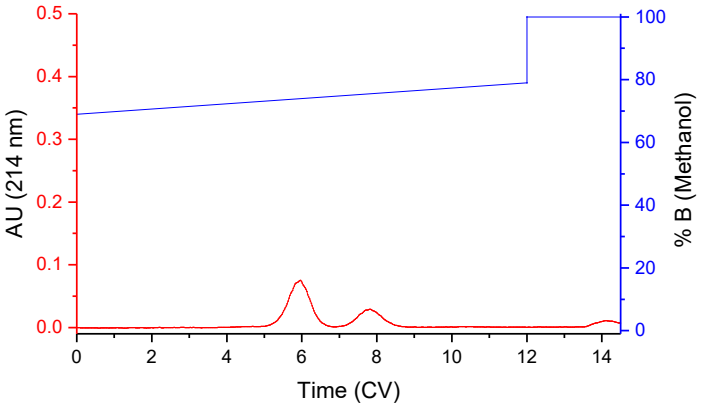

Figure 3– Flash focused gradient using the ACCQ*Prep* gradient in Figure 2, with a 50 g Redi*Sep* Gold C18 column.

The same gradient range is used on a 5.5 g Redi*Sep* C18 column, but the gradient length is changed to 20 CV.

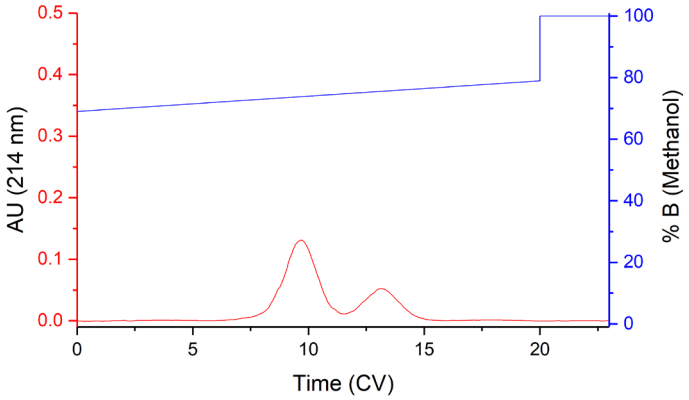

Figure 4– Flash focused gradient using the ACCQ*Prep* gradient in Figure 2, with a 5.5 g Redi*Sep* Gold C18 column.

Conclusion

Using the Focus Gradient Generator built into the ACCQ*Prep* allows an optimized flash method to be determined within 12 minutes, using only the data from the scouting run used to evaluate sample purity. This tool also allows a user to determine in advance whether or not there is sufficient resolution between the compound of interest and the nearest impurity for it to be run on a flash column, or if the higher resolution of the ACCQ*Prep* is required. Given sufficient resolution, the gradient method calculated by the ACCQ*Prep* can be used for flash purification; otherwise it is run on the ACCQ*Prep*.

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