

RediSep Gold® C18– from UHPLC to 8.6 kg



Chromatography Application Note
AN139

Abstract

When large amounts of sample need to be purified, the CombiFlash® Torrent® system is the ideal tool for the task, especially when combined with 7.0 kg RediSep Gold and Silver Silica or 8.6 kg RediSep Gold alternative media columns. However, because of the scale involved, getting to an optimized and efficient method is even more important, while maintaining sufficient resolution to purify compounds and maximize loading and throughput

- minimizes the amount of solvent used, and waste generated,
- minimizes the time of the method to allow for increased throughput, and
- increases concentration of sample fractions, which decreases evaporation time per gram of material.

This application note describes an example of method development using the Focus Gradient Generator feature of the CombiFlash® NextGen system's PeakTrak® software. This feature allows for the fast and easy calculation of a gradient that is easily transferred to the Torrent.

Experimental and results

Vanillin with an early eluting contaminant was run on a RediSep® Prep (PN 692203854) UHPLC column on an Agilent 1290 analytical system using a pre-calibrated scouting gradient (see Technical Note 62) (Figure 2, top). The media in the RediSep Prep C18 UHPLC column matches the media used in the flash column, except for particle size. The Focus Gradient Generator tool in PeakTrak on the CombiFlash NextGen 300+ was used to generate a focused gradient (Figure 2, bottom) using a 50 g RediSep Gold C18 column (PN 692203336). The preparative focused gradient was 12 column volumes (CV) long and showed good resolution.



Figure 1. UHPLC column (circled) and an 8.6 kg C18 column

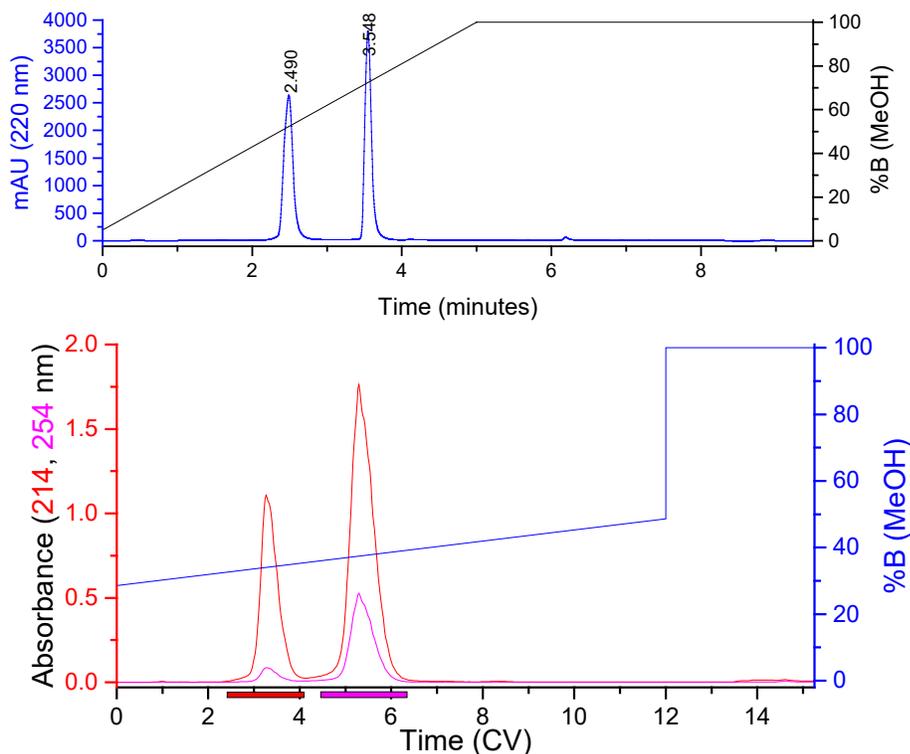


Figure 2. Analytical UHPLC of contaminated vanillin (top) and calculated flash gradient (bottom) for a 50 g RediSep Gold C18 column

The preparative method was transferred to the Torrent and to the 8.6 kg RediSep Gold C18 column (PN 692203900). As the focused preparative method proposed on the NextGen is in column volumes, the same gradient length (in CVs) and the same starting and ending solvent composition can be used on the Torrent as on the NextGen. The 8.6 kg RediSep Gold C18 column was conditioned with methanol and equilibrated. A PeriXus[®] peristaltic pump (PN 681240962; Figure 4) was used to load the sample (130 g, dissolved in 1:1 methanol: water, 800 mL). The pump was run at 300 rpm using Masterflex[®] PharmaPure[®] 06435-16 tubing. This mass of sample is a 1.5% column load, suggested by resolution on the UHPLC scout and the retention difference on the flash column. The sample was run at 850 mL/minute (Figure 3). After the vanillin eluted, the run was terminated, as both the UHPLC and NextGen runs showed that nothing else eluted after the vanillin.

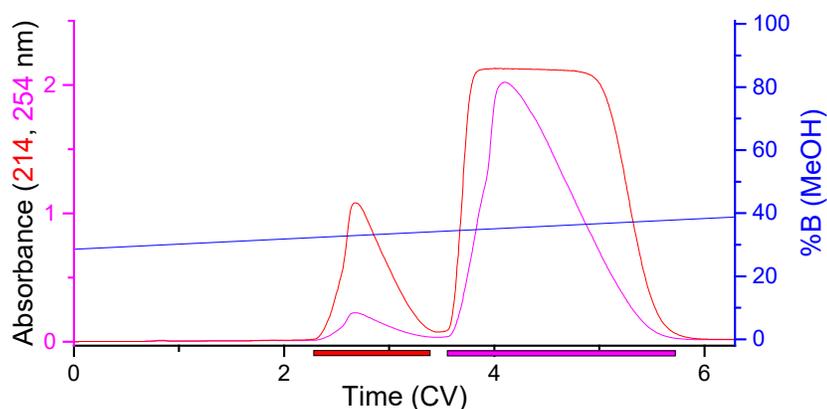


Figure 3. 8.6 kg C18 run on the Torrent.

Conclusions

A new workflow for reverse phase columns using PeakTrak's predictive Focus Gradient Generator feature was demonstrated. This workflow can be used to create efficient focused gradients for columns ranging from 5.5 g through 8.6 kg using the same UHPLC scouting run. The calculated gradient was easily scaled from the small flash column to the larger Torrent column. Additionally, the utility of the PeriXus pump to load the large volume of liquid sample onto the column was demonstrated.



Figure 4. A Teledyne ISCO PeriXus pump.

Teledyne ISCO

P.O. Box 82531, Lincoln, Nebraska, 68501 USA
Toll-free: (800) 228-4373 • Phone: (402) 464-0231 • Fax: (402) 465-3022
Email: Isco.Service@teledyne.com

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