

# Advantages of a High Pressure Hybrid Flash-Preparative System

Chromatography Technical Note  
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## Abstract

When purifying mixtures containing closely eluting peaks, the use of columns containing smaller particle sizes allows improved retention and resolution. This improved resolution leads to improved purity and higher sample loading.

Smaller particle sizes also lead to increased back-pressure. Most hybrid flash-preparative systems are unable to run columns with particle sizes smaller than 10  $\mu$  due to this increased back pressure.

## Experimental and Results

A crude mixture of capsaicins was run on a CombiFlash EZ Prep hybrid prep-flash chromatography system using a water/methanol gradient with C18 columns packed with either 5  $\mu$  or 10  $\mu$  particle sizes. Identical samples (100 mg) were dissolved in 1 mL DMSO and the entire sample injected on the column. Other than the particle size, the column dimensions, flow rates, and chemistry were identical.

The following differences were observed:

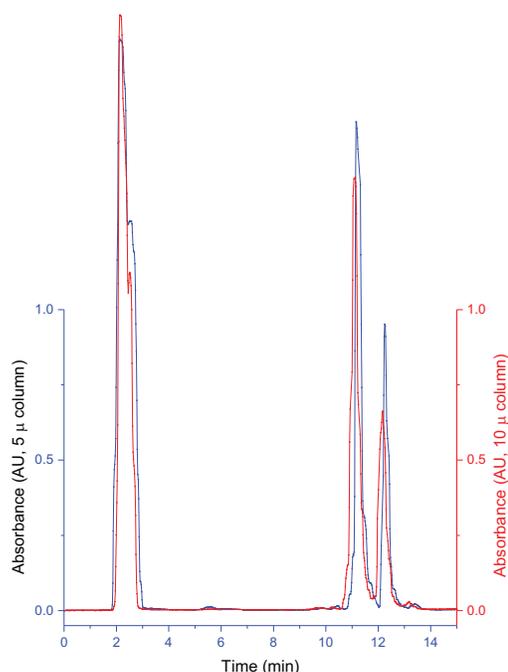
- The front of the peaks for the 10  $\mu$  column eluted earlier than those on the 5  $\mu$  column. This is most likely due to the reduced surface area of the column packing in the 10  $\mu$  material.

- The end of the peaks for the 10  $\mu$  packing was coincident with end of the peaks eluting from the 5  $\mu$  column packing. Combining this result with the above point means the peaks were wider, with reduced resolution.

- The column with the 5  $\mu$  packing achieved baseline resolution of the two closely eluting peaks. The 10  $\mu$  column failed to achieve baseline resolution. This result is consistent with earlier results on flash columns<sup>1</sup> where it was demonstrated that columns with smaller particle size exhibited higher loading capacities.

To obtain the increased resolution and loading capacities for the 5  $\mu$  packing, a back pressure of 2000 PSI (138 Bar) was observed, compared to 600 PSI (41 Bar) for the 10  $\mu$  column packing. Many hybrid prep-flash systems are unable to achieve the back pres-

ures needed to fully utilize 5  $\mu$  column packings. They may need to compromise on solvent mixtures, or are limited to running more expensive acetonitrile solvent systems to keep the below the maximum back pressure allowed by these systems. They may need to run at slower flow rates, extending run times and reducing the resolution advantage provided by 5  $\mu$  particles due to longitudinal diffusion of bands.



**Figure 1: Capsaicin purification with a 5  $\mu$  and 10  $\mu$  C18 column**

## Conclusion

The use of 5  $\mu$  particle sizes allow better retention, improved resolution, and thus improved purity for preparative chromatography of closely eluting peaks. The higher resolution also allows increased sample loading and more efficient use of the purification system. The CombiFlash EZ Prep is able to run up to 3500 PSI (241 Bar) and so is able to make use of the advantages of these columns without compromising on solvent choice or flow rate.

1. Silver, J.E.; Bilger, E.; Crea, T.R.; Pipes, R. Spherical silica shows increased loading capacity compared to irregular silica in MPLC. Presented at The 238th ACS National Meeting, Washington, DC, August 16, 2009, MEDI 172.

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