APPLICATION NOTE 22116

A comparison of stationary phases for preparative chromatography

Benefits of Thermo Scientific Hypersil PREP HS columns

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Goal

1. Review factors that are important for preparative separations
2. Demonstrate the enhanced selectivity provided by the Thermo Scientific™ Hypersil™ PREP HS column vs. competitor columns at analytical scale
3. Review loadability of the columns for the compound mixture tested

Introduction

The primary goal of a preparative liquid chromatography is the purification of target compounds through fractionation. A major secondary goal for laboratories is the method economy of this analysis. To save money, chromatographers often begin their method screening at the analytical scale, to determine the optimum separation gradient and column chemistry that delivers the resolution of their target components. Through this method design, conscious decisions are made, such as selecting methanol over acetonitrile, minimizing the use of expensive buffers, etc., to design a method that saves material cost for the laboratory.

In this application note, a compound mixture of voriconazole and several known impurities was used as a model to replicate raw pharmaceutical synthesis. Method design and column screening were performed to demonstrate the considerations a chromatographer may take as they develop a method for preparative scale chromatography.

Experimental

Instruments

- Thermo Scientific™ Vanquish™ UHPLC system with the following modules:
  - Vanquish Column Compartment H (P/N VH-C10-A)
  - Vanquish Diode Array Detector HL (P/N VH-D10-A)
  - Vanquish Split Sampler FT (P/N VF-A10-A)
  - Vanquish Quaternary Pump F (P/N VF-P20-A)

Chromatographic conditions

- Mobile phase A: Water/formic acid (100/0.1, v/v)
- Mobile phase B: Methanol/formic acid (100/0.1, v/v)
- Gradient mode: Isocratic at 68% B

Table 1. Compounds

No.	Compound name	Concentration in mix (mg/mL)
1	2,4-difluoro-α-(1H-1,2,4-triazolyl) acetophenone	20
2	voriconazole n-oxide	1
3	4-ethyl-5-fluoropyrimidine HCl	20
4	voriconazole EP impurity B	20
5	voriconazole	20

Table 2. Characteristics of columns used in this application note

Column name	Particle size (um)	Length (mm)	Inner diameter (mm)	Pore size (Å)
Thermo Scientific™ Hypersil™ PREP HS C18	5	250	4.6	100
Competitor P C18	5	250	4.6	100
Competitor P Solid Core C18	5	250	4.6	100
Competitor K C18	5	250	4.6	100
Competitor Y C18	5	250	4.6	120
Thermo Scientific™ Acclaim™ 120 C18	5	250	4.6	120
PREP BDS C18	5	250	4.6	145
Thermo Scientific™ Hypersil GOLD™ C18	5	250	4.6	175

Results and discussion

Importance of pore size

One of the important considerations for selecting a column for preparative scale chromatography is the impact that pore size has on the performance of the column.

Comparing the Hypersil PREP HS column (100 Å) to the Hypersil PREP BDS column (145 Å), greater hydrophobic retention is noted on the Hypersil PREP HS column. The smaller pore size of the Hypersil PREP HS media accommodates more pores on the particle surface, yielding a higher overall column surface area. While actual loading capacity for compounds needs to be experimentally determined, a general rule of thumb is columns with higher surface areas typically offer chromatographers greater loading capacity.

The compounds to be fractionated will affect column choice. For example, Figure 1 shows the Hypersil PREP HS column resolves all the compounds, while there is some co-elution with the Hypersil PREP BDS column. If the aim were only to purify the last eluting peak, the user may still select the Hypersil PREP BDS column, as it separates the last component nearly a minute earlier than the Hypersil PREP HS column. This would represent a significant saving of time, and hence solvent costs, at prep scale. However, if the chromatographer were interested in a higher resolution separation of the earlier components, the Hypersil PREP HS column would be the better option, demonstrating why multiple phase types should be considered for new method development.

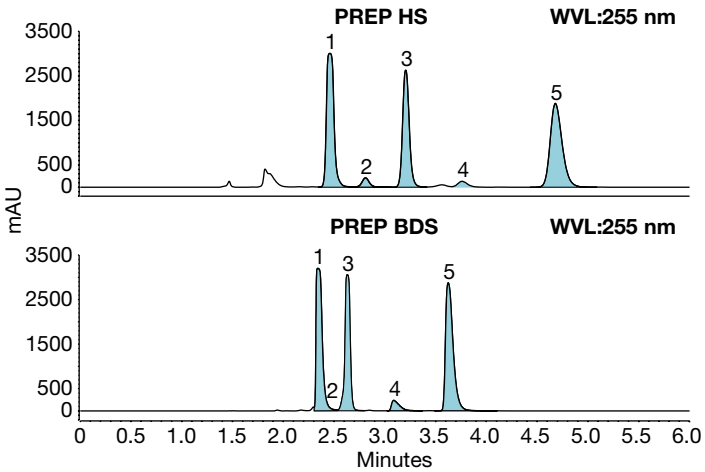

Figure 1. Comparison of a Hypersil PREP HS column with a Hypersil PREP BDS column using an identical method

Importance of selectivity

For this mix, the compounds had poor aqueous solubility, so the goal was to maintain the highest organic percentage mobile phase, ensuring the compounds remained dissolved in the mobile phase. Even at these higher organic percentages, the Hypersil PREP HS column showed excellent retention of the compounds, allowing baseline separation of all the compounds.

When comparing popular preparative LC chemistries (Figure 2), it is noticed that Thermo Scientific columns deliver increased hydrophobic retention for the most polar compound as well as better resolution between peaks 2 and 3.

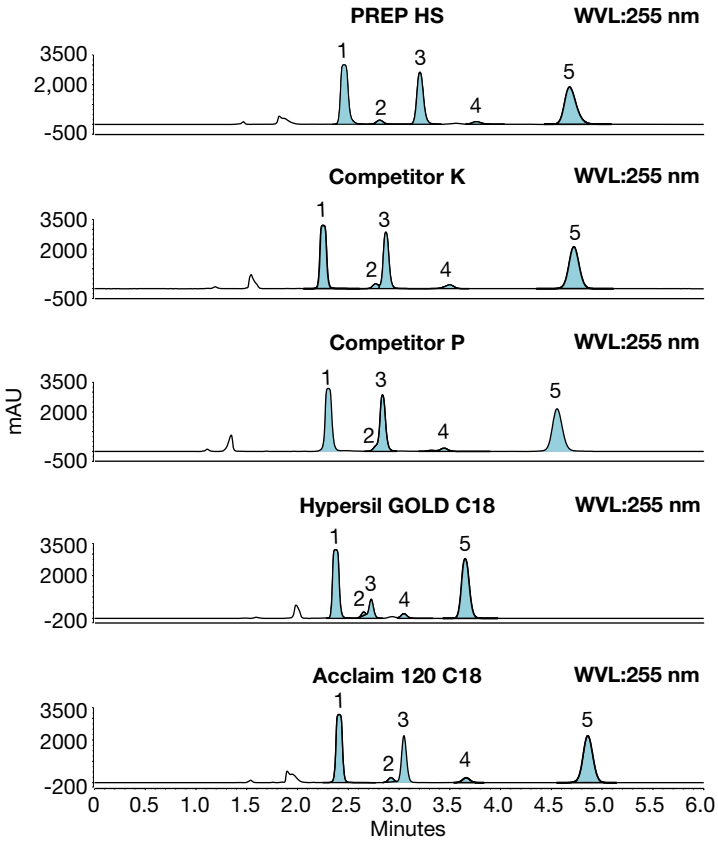

Figure 2. Comparison of PREP HS and popular LC chemistries

As previously mentioned, achieving resolution between the target compound and any interferences is one of the key requirements of the assay. The difference for prep scale is that when the column is overloaded, the peak width will increase significantly. If the resolution is low at analytical scale, this will transfer identically to the prep separation and the increase in peak widths ensures resolution is lost quicker. The result is reduced purity of the fraction or a reduction in the yield per injection.

The resolution between all compounds on the Hypersil PREP HS column is by far the greatest. For a prep separation, the Hypersil PREP HS column could take the highest load before the resolution between compounds would be lost; regardless of which compound is the target.

Solid core vs. fully porous

Modern chromatographers often develop methods on solid core particles. The solid center yields a reduction in resistance to mass transfer, eddy diffusion, and longitudinal diffusion of the analytes (Figure 3), making solid core columns desirable for high resolution separations.

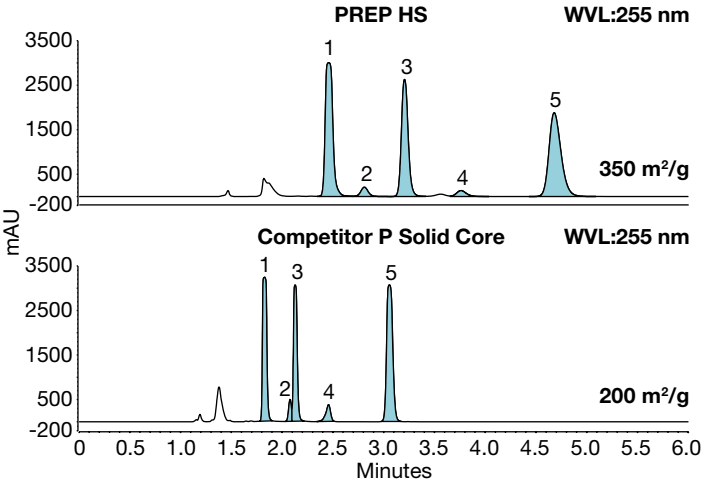

Figure 3. Comparison of the Hypersil PREP HS column and the competitor P solid core C18 column

However, the impact of these columns having a solid center is a significant reduction in surface area (200 m^2/g vs. 350 m^2/g), meaning a reduced maximum load. Solid core columns cannot separate as much material as fully porous particle columns, and this significantly reduces the amount that can be purified per injection, requiring additional injections to be made.

To demonstrate this experimentally, 3.5 mg of total load was injected onto both the solid core and the Hypersil PREP HS column (Figure 4). On the solid core column, peaks 1, 2, and 4 have merged, whereas all five peaks are still clearly visible and could be fraction collected using the Hypersil PREP HS column.

Scaling up

The importance of pore size, surface area, and selectivity were described in the previous section. Bringing these elements together is what dictates the effectiveness of a preparative separation.

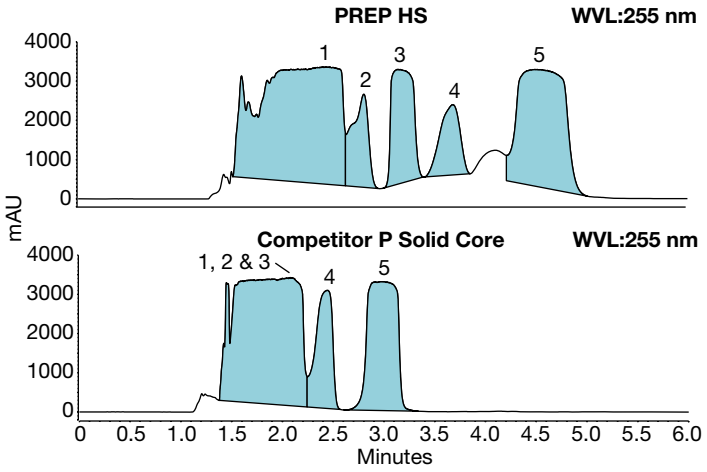

Figure 4. 3.5 mg loaded on to the Hypersil PREP HS column and the competitor P solid core C18 column

When gradually overloading the analytical Hypersil PREP HS column to determine the maximum that can be loaded before resolution is lost (Figure 5), for this compound mixture, the combination of the high surface area and novel selectivity between compounds 2 and 3 show that 3.5 mg can be loaded and adequate resolution maintained. Injecting the same 3.5 mg on column onto the competitor

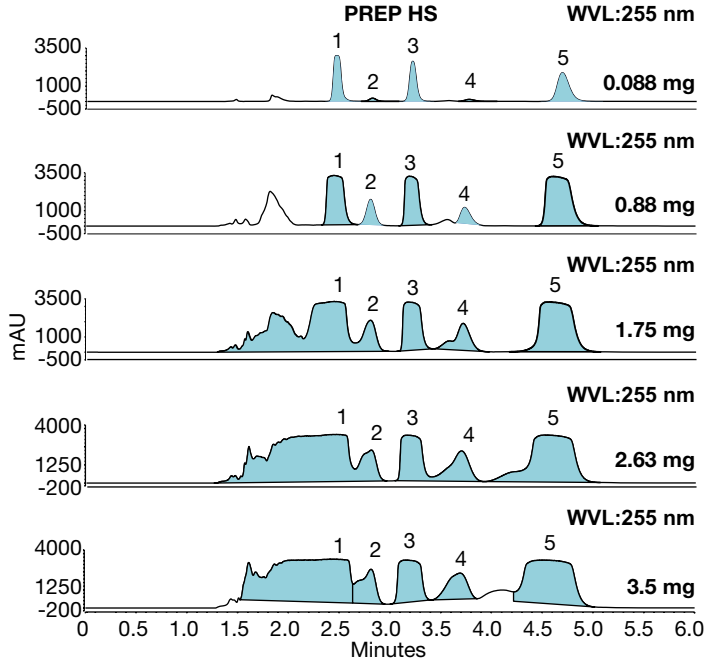

Figure 5. Increasing total load (mg) on the Hypersil PREP HS column

columns shows that resolution is not maintained, meaning reduced quantities of sample could be loaded, having a direct impact on assay economy (Figure 6).

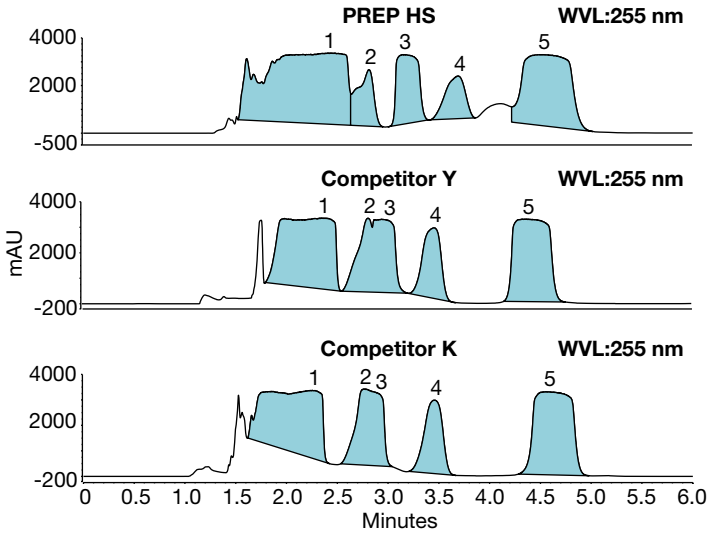

Figure 6. 3.5 mg total load on column; Hypersil PREP HS column vs. competitor columns

Conclusion

We have reviewed what factors are important in optimizing scale up from analytical to preparative scale separation. Two closely retaining compounds (peaks 2 and 3) were poorly resolved on the competitor phases with the Hypersil PREP HS media offering alternative selectivity for this compound set.

For challenging separations that require increased retention and alternative selectivity, the Hypersil PREP HS column is the column of choice. For this compound set, the Hypersil PREP HS column allowed more compound to be loaded before resolution was lost, resulting in fewer injections at prep scale. Fewer injections means less mobile phase used and less time to achieve experimental objectives.

Solid core media are often looked at for prep separations given their proven characteristics for analytical separations. The reduced surface area, and hence lower loadability, as a result of the solid core mean repeated injections to achieve the same fraction purity obtained using fully porous media.

Find out more at **thermofisher.com/prepLC**

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