

# Supelco Preparative HPLC products for Pharmaceutical Development and Production

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There is an increasing need for purified materials in the development and production of pharmaceutical products. FDA and other regulatory agencies continue to require more stringent purity requirements on active pharmaceutical ingredients (API), including most recently chiral purity. Studies are often executed concurrently with impurities, degradants, and metabolites to determine if their potency or toxicity is a concern for the API in development. As the demand for purification increases, tools beyond classic crystallization are often needed. Some choices include filtration, distillation, solid phase extraction, thin-layer chromatography (TLC), low-pressure liquid chromatography (LPLC), and high-performance liquid chromatography (HPLC). In many instances, preparative HPLC is the most powerful and versatile method for the challenging purification tasks in the pharmaceutical industry.

## Stationary Phase Selection

The first important criterion for preparative chromatography is that the proper stationary phase is selected. A consideration beyond phase applicability is the commercial availability in both analytical and preparative dimensions as well as bulk packing material. Worldwide availability may be a consideration depending on final intended use. Typically, packing materials with 10 micron or greater particle size are used for preparative columns. Supelco offers three major product lines for preparative applications that are summarized in **Table 1**.

Product Line	Application	Particle Size (µm)	Multiple Phases	Bulk Available
Ascentis	Small Molecule	3, 5, 10	Yes	Yes
Astec	Chiral	5, 10, 16	Yes	Yes
Discovery® BIO	Peptide/Protein	3, 5, 10	Yes	Yes

**Table 1** Summary of Supelco HPLC Product Lines for Preparative Applications

Although >80% of analytical HPLC separations are performed on reversed-phase silica phases, a majority of small molecule preparative chromatography separations are performed on normal-phase silica phases. Normal phase methods are often the first choice because:

- Removing organic solvents typically used in normal-phase are easier and more cost effective than removing aqueous solutions used in reversed-phase chromatography
- Easier method transfer from normal-phase TLC
- Cost of bare silica for normal-phase is typically less than the cost of C18 phases for reversed-phase

## Preparative HPLC Scale-Up

Analytical conditions are typically developed on a 25 cm x 4.6 mm I.D. column with 5 micron particles. If excessive resolution is available, a 10 cm x 4.6 mm I.D. column should be considered. Once chromatographic conditions are optimized, a loading study on the analytical dimension is recommended. At this point, successful scale-up from analytical to preparative work is quickly and reliably obtained by using two simple formulas. The formulas are displayed in **Table 2**.

$$\text{Loading Capacity: } I_p = I_a \times (D_p/D_a)^2 \times L_p/L_a$$

Where:

$I_p$  = Injection load of preparative column

$I_a$  = Injection load of analytical column

$L_p$  = Length of preparative column

$L_a$  = Length of analytical column

$D_p$  = Internal diameter of preparative column

$D_a$  = Internal diameter of analytical column

$$\text{Flow Rate: } F_p = F_a \times (D_p/D_a)^2$$

Where:

$F_p$  = Flow rate of preparative column

$F_a$  = Flow rate of analytical column

$D_p$  = Internal diameter of preparative column

$D_a$  = Internal diameter of analytical column

**Table 2** Formulas for Scaling Analytical to Preparative Applications

## Ascentis for Small Molecule Purification

The Ascentis HPLC product line has several features that make it the ideal platform for small molecule HPLC purification. The benefits are outlined below.

Shown in **Figure 1** are the results of a loading experiment. The analytes used were quinidine and dihydroquinidine, two closely-related compounds that are typical of the type of preparative separation in which Ascentis might be utilized.

column: Ascentis C18, 15 cm x 21.2 mm I.D., 10 µm particles (S81358-U)

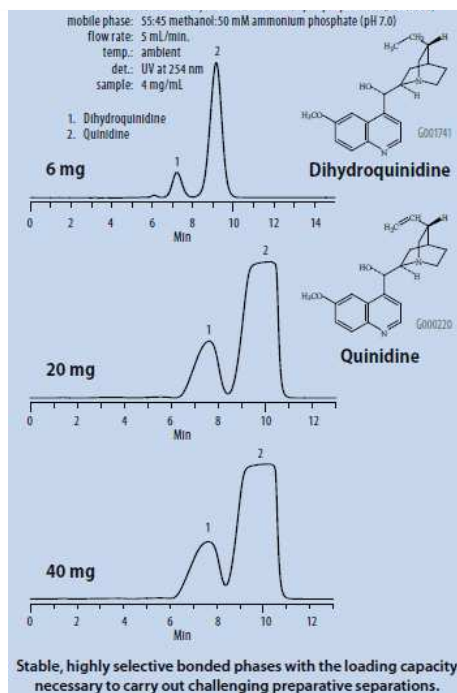


Figure 1 High Loading Capacity (581358-U)

This study utilized a 15 cm x 21.2 mm I.D. column packed with 10 micron particles. The separation was performed in the reversed-phase mode with C18 as the stationary phase. The high surface area Ascentis columns provided maximum loading capacity for this small molecule application.

## Astec CHIROBIOTIC for enantiomeric purification

The Astec CHIROBIOTIC product line has several features that make it useful for enantiomeric purification.

CHIROBIOTIC columns can be used in all preparative HPLC techniques, including simulated moving bed (SMB), supercritical fluid chromatography (SFC), and mass-directed prep.

Prep separations on CHIROBIOTIC are reproducible and scalable, as shown in **Figure 2**. **Figure 2** shows the separation of a racemic mixture of phenylalanine on columns packed with 5, 10, and 16 micron particles of CHIROBIOTIC T.

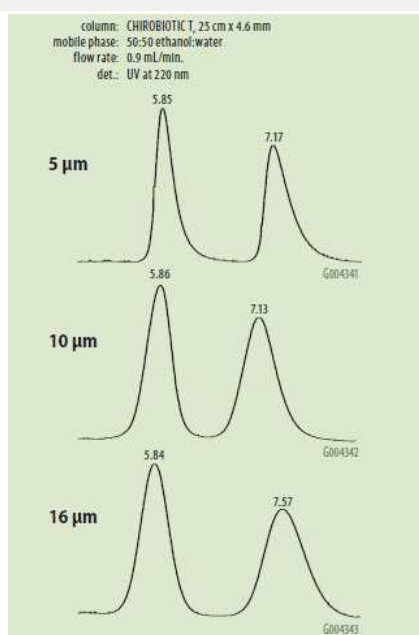


Figure 2 Scalability Across CHIROBIOTIC CSP Particle Sizes

Another advantage of CHIROBIOTIC for preparative applications is that the mobile phase flexibility can be utilized to optimize sample solubility. **Figure 3** shows the analytical and preparative separation of thalidomide on CHIROBIOTIC V. The analytical scale method showed a high selectivity with a 100% methanol mobile phase. However, since thalidomide is poorly soluble in pure

methanol, it was possible to add 20% dioxane to the mobile phase to increase solubility three-fold while still achieving the necessary separation.

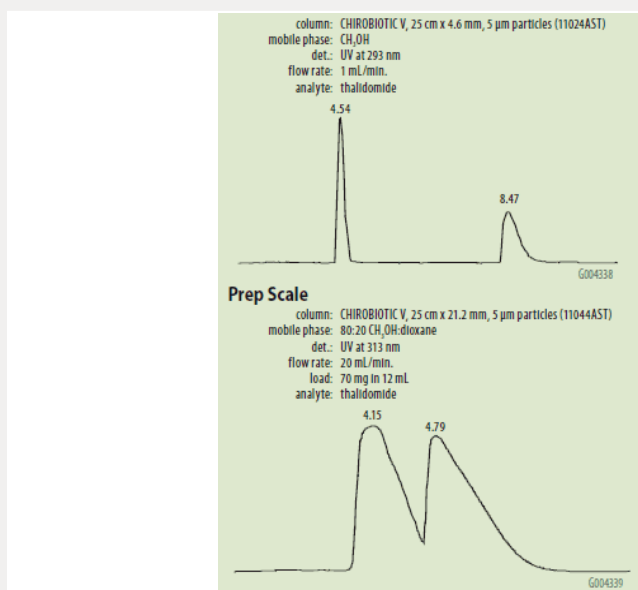


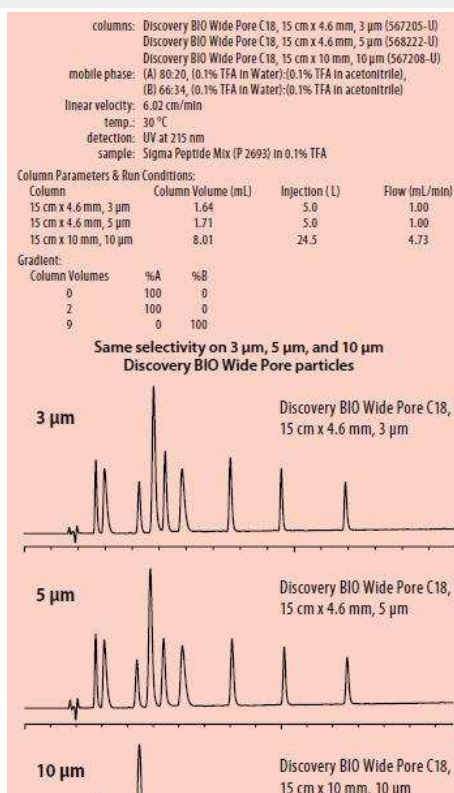
Figure 3 Sample Solubility Considerations in Preparative Analytical Scale

## Discovery BIO for Peptide and Protein Purification

The Discovery BIO HPLC product line has several features that make it the ideal platform for peptide and protein purification. The benefits are outlined below.

Reversed-phase chromatography is often used in the final polishing steps of oligonucleotides and peptides, and is ideal for scaling from analytical to preparative purification. While reversed-phase chromatography can be used for protein purification, it is typically not recommended if recovery of activity or correct tertiary structure are required, since many proteins are denatured when exposed to reversed-phase solvents. Discovery BIO HPLC columns are available in three different phase hydrophobicities (C18, C8 and C5) as well as three different particle sizes (3, 5 and 10 micron).

Shown in **Figure 4** is the separation of a peptide mix on all three available particle sizes. The same selectivity is obtained on all three particles making scale up quite easy and predictable. Although peaks were not identified for this study, the peptide mix contains *bradykinin*, *bradykinin fragment 1-5*, *substance P*, *[Arg8]-vasopressin*, *luteinizing hormone releasing hormone*, *bombesin*, *leucine enkephalin*, *methionine enkephalin* and *oxytocin*.



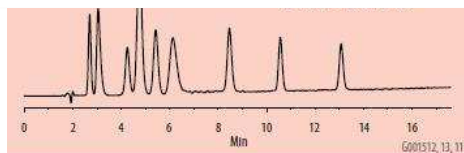


Figure 4 Matched Selectivity from Analytical to Preparative on Discovery BIO Wide Pore C18 (567205-U)(568222-U)(567208-U)

## Conclusions

Sigma-Aldrich offers a complete line of HPLC purification products for small molecule, chiral, and peptide/protein separations. Beyond HPLC purification methods, Sigma-Aldrich offers other purification products such as Flash, bulk silica, TLC, glass columns, and solvents.

## Materials

Product #	Image	Description	Molecular Formula	Add to Cart
581358-U		Ascentis® C18 HPLC Column 10 µm particle size, L × I.D. 15 cm × 21.2 mm		<a href="#">pricing</a>
567205-U		Discovery® BIO Wide Pore C18 HPLC Column 3 µm particle size, L × I.D. 15 cm × 4.6 mm		<a href="#">pricing</a>
568222-U		Discovery® BIO Wide Pore C18 HPLC Column 5 µm particle size, L × I.D. 15 cm × 4.6 mm		<a href="#">pricing</a>
567208-U		Discovery® BIO Wide Pore C18 HPLC Column 10 µm particle size, L × I.D. 15 cm × 10 mm		<a href="#">pricing</a>