

Direct Comparison of HPLC and SFC for the Milligram to Gram Scale Purification of Enantiomers

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High efficiency Axia™ preparative columns packed with Lux® polysaccharide-based chiral stationary phases are utilized to compare chiral SFC and HPLC purification of several pharmaceutical compounds.

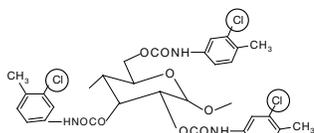
Introduction

High-throughput analysis and purification of enantiomers are important in drug discovery. With today's regulations to improve safety and efficacy of drugs, the pharmaceutical industry needs to provide high quality pure enantiomers for pharmacological testing. Historically, chiral purification has been achieved with normal phase HPLC, and more recently, with reversed phase separations. In recent years, SFC has gained acceptance as a very effective complementary tool for other chiral separation modes to produce pure enantiomeric compounds.

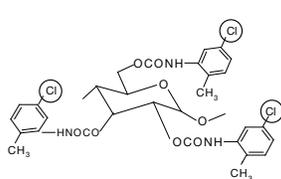
This technical note compares the throughput (grams per hour) for the same chiral preparative separations performed under HPLC conditions and SFC conditions. In this work, three different polysaccharide-based chiral stationary phases (CSPs) are utilized (**Figure 1**): Lux Cellulose-1, Lux Cellulose-2, and Lux Amylose-2. The same 5 μm media was used to produce both the analytical columns and the Axia packed preparative columns. This work also demonstrates the capability to directly scale analytical chiral separations to preparative chiral separations when both column sizes are packed with 5 μm media.

Figure 1.
Structures of Polysaccharide-based Chiral Phases

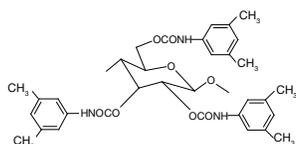
Lux® Cellulose-2
Cellulose tris
(3-chloro-4-methylphenylcarbamate)



Lux® Amylose-2
Amylose tris
(5-chloro-2-methylphenylcarbamate)



Lux® Cellulose-1
Cellulose tris
(3,5-dimethylphenylcarbamate)

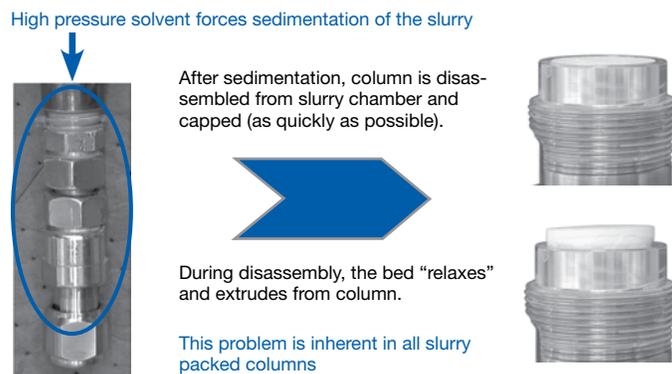


Background

In the past, the same particle size chiral media packed in 4.6 mm ID columns was packed into preparative columns but the column performance and lifetime decreased as the column internal diameter increased. Column stability was also inherently less for the preparative chiral columns compared to analytical columns. The lower initial performance and/or loss of performance are inherent in all slurry packed chiral preparative columns and caused by:

1. Packed bed structure being disturbed after the media is packed
2. Media fracture, and or fines, created by packing media in large diameter columns
3. Non-uniform packing density throughout the column
4. Media extrusion from the packed bed during final hardware assembly (**Figure 2**).

Figure 2.
Conventional Slurry Packed Preparative Chiral Column



A major improvement in preparative chiral column performance has been achieved by adapting Axia packing technology to manufactured Lux chiral preparative columns. Axia packing technology (**Figure 3**) has been utilized to produce stable, high performance achiral preparative columns. This same technology is now employed to produce preparative chiral columns packed with 5 μm chiral stationary phases. The Lux media is engineered to be mechanically stronger than previous chiral media, allowing higher packing pressures to be applied. A computerized mechanical process packs the column bed. The force applied to the column is carefully controlled during the packing process to prevent crushing or cracking of the media. Once the column bed forms, the media is never allowed to expand or extrude from the column and the internal packing force is maintained on the column during final hardware assembly. The advantages of combining the new Lux media and Axia packing technology to produce high performance stable preparative chiral columns are

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illustrated in this technical note. The Axia packing technology produces preparative columns packed with 5 μm media having the same efficiency and peak symmetry as analytical columns (**Figure 4**). Axia packed preparative columns are manufactured with Lux 5 μm chiral media in 100, 150 and 250 mm lengths with 21.2, 30 and 50 mm internal diameters.

Figure 3.
Axia™ Packing Process Integrates Axial Compression Technology into Pre-packed Chiral Preparative Columns

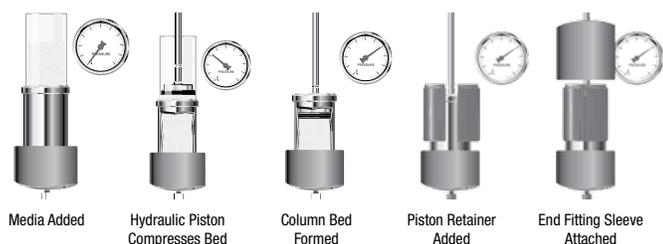
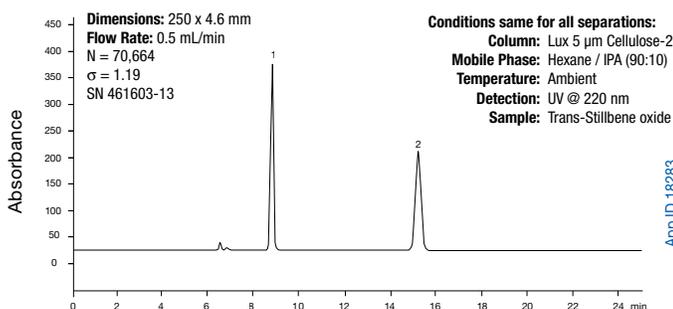
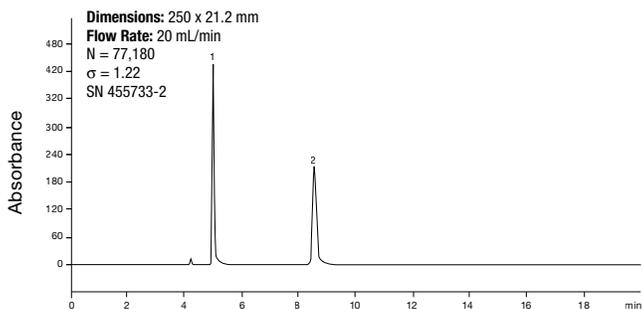


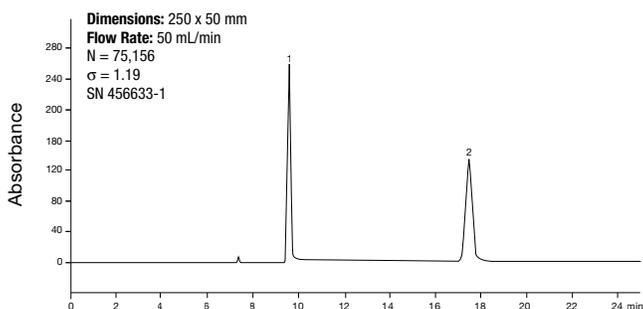
Figure 4.
Axia™ Packed Lux® Preparative Columns Provide the Same High Performance Independent of Column Diameter



App ID 18283



App ID 18284



App ID 18285

Experimental Conditions

Analytical HPLC separations were developed using an Agilent® 1100 system with diode array detector (Agilent, Palo Alto, CA). The Gilson 845ZPREP™ HPLC system (Gilson, Middleton, WI) was used for the preparative HPLC separations and fraction collection. For SFC separations, a Berger preparative SFC system was utilized consisting of the pumping system, variable UV and PDR-Chiral detectors, and a 6-port fraction collector capable of collecting hundreds of milliliters of eluent. The advanced laser polarimeter (ALP) detector (PDR-Chiral, Lake Park, FL) measures the rotation of plane-polarized 660 nm laser beam passing through the flow cell and indicates the optical rotation of each enantiomer. Using a mobile phase consisting of 25 % polar modifier (methanol or ethanol) added to the carbon dioxide (CO₂), a flow rate of 50 mL/min through the 21.2 mm diameter columns was easily achieved without exceeding the 200 bar pressure limits of the SFC instrument.

Results and Discussion

Figures 5A – 5D contain the SFC and HPLC chromatograms, purification conditions, and results for each sample. **Figure 5A** compares the Atenolol separation for SFC and HPLC. The SFC conditions required 25 % methanol while the HPLC conditions yielded the best separation with 20 % ethanol. The PDR-Chiral detector (ALP) indicates the optical rotation of each enantiomer. The SFC cycle time was 4 minutes and the HPLC cycle time was 6 minutes. In addition to a faster cycle time the total load on the column was also 1.8 times higher for SFC (102 mg for SFC vs. 60).

Figure 5B compares the Terfenadine HPLC and SFC separations. The Lux Cellulose-1 column using 25 % methanol as the polar modifier provided the best SFC separation. The Lux Cellulose-1 column with the polar organic mobile phase of 3 % isopropanol and 97 % acetonitrile provided the best HPLC separation. SFC sample load per cycle was 105 mg compared to 12 mg/cycle for the HPLC runs in the same 7 minute cycle time for SFC and for HPLC. This results in a significantly higher throughput for SFC (840 mg/hour) compared with HPLC (102 mg/hour) and a significantly smaller volume collected per gram of product. The overall purity and recovery for HPLC and SFC were the same.

Figure 5C compares the two Propranolol chiral separations. For SFC, the Lux Cellulose-1 column with 25 % methanol polar modifier provided the best separation with a sample load of 120 mg per cycle. The HPLC conditions also utilized the Lux Cellulose-1 column with 20 % isopropanol as the polar solvent (hexane as the non-polar solvent) and a sample load of 60 mg per cycle. Since the load per cycle was higher for SFC, the throughput for SFC was 450 mg/hour compared to 553 mg/hour for HPLC and the total volume collected for 1 gram of product was 508 mL for SFC compared to 799 mL for HPLC.

The Propafenone separations using Lux Amylose-2 are shown in **Figure 5D**. Lux Cellulose-1 and Lux Cellulose-2 could not resolve these enantiomers in HPLC or SFC but a 50 mm long Lux Amylose-2 provided the best resolution possible although the resolution is on the low end for a preparative purification. The SFC conditions utilized 20 % ethanol as the polar solvent with a load of 15 mg per 7 minute cycle. Under HPLC conditions, 50 % isopropanol was required to achieve the separation on the Lux Amylose-2 column with a load of 18 mg per 4 minute cycle. The throughput is limited by the resolution between the two compounds, which is minimal

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for a preparative separation. A longer column could be used to improve resolution, but the overall throughput would remain the same. A larger diameter column is required for higher throughput.

Figure 5A.
Purification of Atenolol

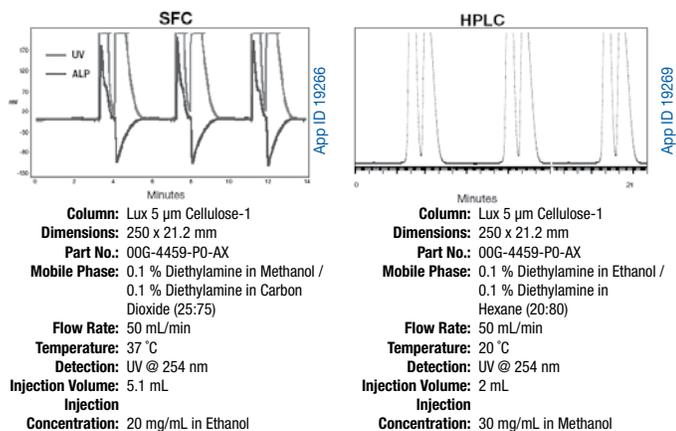


Figure 5B.
Purification of Terfenadine

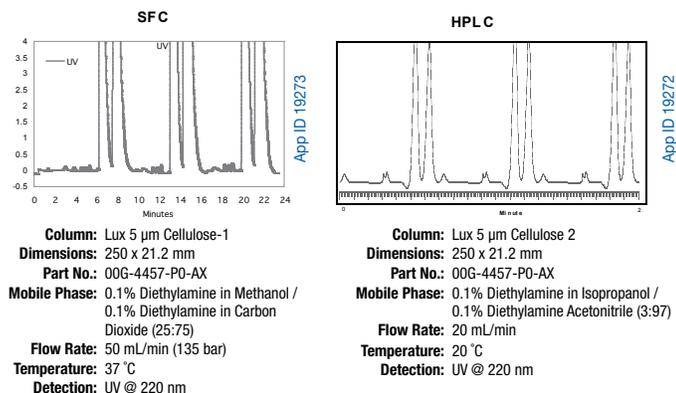


Figure 5C.
Purification of Propranolol

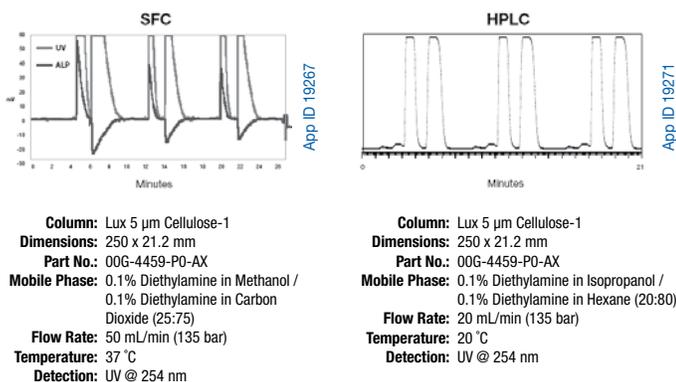


Figure 5D.
Purification of Propafenone

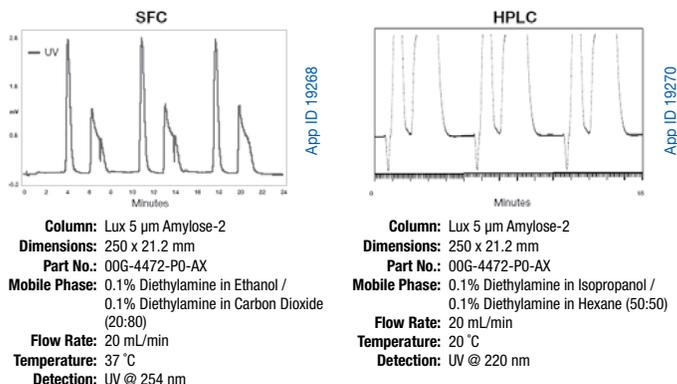
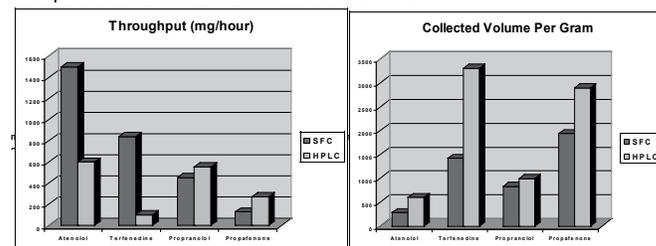


Figure 6 graphically compares the SFC and HPLC results to obtain 1 gram of purified product. The total volume collected was always less for SFC, in some cases as much as one-half the volume collected for HPLC. However, since significant amounts of polar solvent modifiers were required for SFC, the difference in volume was not as dramatic as expected. However, the lower volume of solvent collected for SFC would result in less time to remove the solvent and recover the desired product. With the exception of the propafenone separation, the SFC yields (mg/hour) were significantly greater than the HPLC yields.

Figure 6.
Comparison of Yield and Total Collected Volume



Conclusions

Axia preparative columns packed with 5 μ m Lux polysaccharide-based media are compatible with SFC and HPLC conditions and gave the same high performance as the analytical columns, allowing for scale up under both SFC and HPLC separation conditions. Larger diameter Axia packed preparative columns are available if higher flow rate SFC systems are utilized.

Sample solubility is a major problem for both SFC and HPLC. The use of polar organic solvents such as methanol, ethanol and isopropanol to improve solubility can have an adverse effect on peak shape, retention time, and resolution. If the polar solvent in the sample is too high, the strong polar solvent will cause sample breakthrough and reduce resolution. For these examples the column capacity was higher than the actual load achieved because the polar solvent limited the total volume that could be injected. The use of polar solvents also directly impacts the column operating pressure and limits the overall flow rate on the SFC system. The polar solvents also create higher backpressure on the HPLC systems but generally these systems have higher pressure limits.

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Conclusions (cont'd)

Advantages of SFC relative to HPLC are dependent on the analyte, the CSP, and the amount of polar solvent required. Generally, higher load and higher flow rates were achieved with SFC but the volume collected per gram of product was not as different as other achiral separations previously reported. This is a result of the higher percentage of polar mobile phase solvent used in the SFC separations.

Ordering Information

Lux® 5 µm Axia™ Packed Preparative Columns (mm)

Phases	Lux® 5 µm Axia™ Packed Preparative Columns (mm)				SecurityGuard™ Cartridges (mm)	
	150 x 21.2	250 x 21.2	250 x 30	250 x 50	15 x 21.2**	15 x 30.0*
Cellulose-1	00F-4459-P0-AX	00G-4459-P0-AX	00G-4459-U0-AX	00G-4459-V0-AX	AJO-8405	AJO-8406
Cellulose-2	00F-4457-P0-AX	00G-4457-P0-AX	00G-4457-U0-AX	00G-4457-V0-AX	AJO-8400	AJO-8401
Cellulose-3	00F-4493-P0-AX	00G-4493-P0-AX	00G-4493-U0-AX	00G-4493-V0-AX	AJO-8624	AJO-8625
Cellulose-4	00F-4491-P0-AX	00G-4491-P0-AX	00G-4491-U0-AX	00G-4491-V0-AX	AJO-8629	AJO-8630
Amylose-2	00F-4472-P0-AX	00G-4472-P0-AX	00G-4472-U0-AX	00G-4472-V0-AX	AJO-8473	AJO-8474

for ID: 18–29 mm 30–49 mm

**PREP SecurityGuard Cartridges require holder, Part No. : AJO-8223

*PREP SecurityGuard Cartridges require holder, Part No. : AJO-8277

For analytical and semi-prep sizes and pricing, please contact us.



If Axia™ packed columns do not provide LONGER LIFETIME when used with SecurityGuard PREP, as compared to a competing column of the same particle size, phase and dimensions, send in your comparative data and the column within 45 days for a FULL REFUND.

Terms and Conditions

Subject to Phenomenex Standard Terms and Conditions which may be viewed at <http://www.phenomenex.com/TermsAndConditions>.

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Disclaimer

The research presented in this technical note was a joint effort between Phenomenex, Inc. and PDR-Chiral Inc. Comparative separations may not be representative of all applications.

Axia is patented by Phenomenex. U.S. Patent No. 7,674,383

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