Purification of Optically Active Pharmaceutical Compounds using Axial Compressed Columns

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A major improvement in preparative chiral column performance has been achieved by adapting axial compression to manufacture Lux® chiral preparative columns. This paper demonstrates the advantages of combining the Lux media and Axia™ packing technology to produce high performance stable preparative chiral columns. This process produces preparative columns packed with 5 µm media with the same efficiency (plates per meter) in prep columns as found in analytical columns and peak symmetry independent of column length and internal diameter.

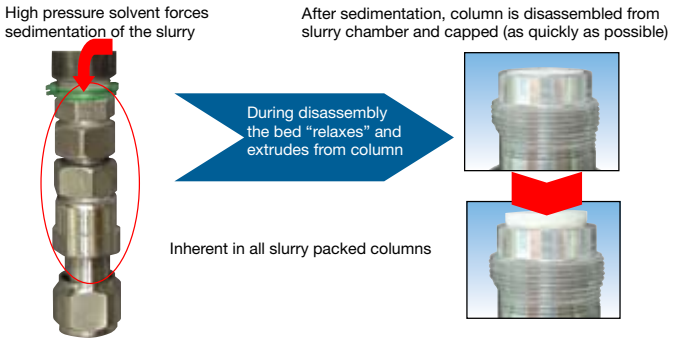
Introduction

Previous limitations with preparative chiral columns

Historically, it has been a limitation of chiral columns that performance and lifetime decrease as the column's internal diameter increases from analytical to preparative dimensions, despite being packed with the same particle size media. Column stability was also inherently less for the preparative chiral columns compared to analytical columns. This lower initial performance and/or loss of performance is inherent in all slurry packed chiral preparative columns, and is 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. Packing density not uniform throughout the column
4. Media extrudes from the packed bed during final hardware assembly (**Figure 1**)

Figure 1.
Conventional Slurry Packed Preparative Chiral Column



With slurry packed columns, the packing hardware must be disassembled before the end fitting is placed on the column. During this procedure the pressure on the media must be released, the packed bed is disturbed and the media begins to extrude from the column creating non-uniform density.

Recent improvements in chiral purification technology

A major improvement in preparative chiral column performance has been achieved by adapting axial compression to manufacture Lux preparative columns. For the last six years the Axia packing technology (explained in **Figure 2**) has been utilized to produce high performance stable achiral preparative columns. This same technology is now employed to produce preparative chiral columns packed with 5 µm chiral stationary phases (CSP).

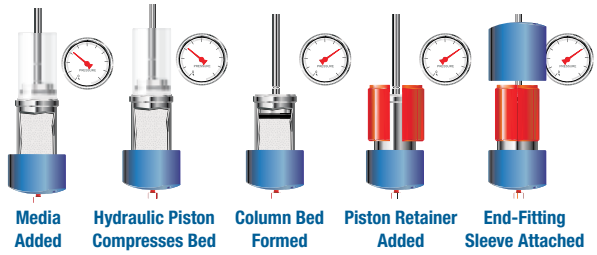
How Axia packed columns perform better

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. The Lux media is engineered to be mechanically stronger than previous chiral media allowing higher packing pressures to be applied (**Figure 3**). 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.

Creating efficient, more productive chiral purification methods

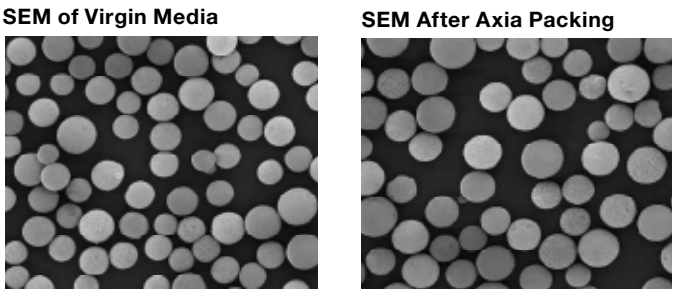
This paper demonstrates the advantages of combining the new Lux media and Axia packing technology to produce high performance stable preparative chiral columns. This process produces preparative columns packed with 5 µm media with the same efficiency (plates per meter) and peak symmetry independent of column length and internal diameter (**Figure 4**). Axia packed preparative columns are manufactured with 5 µm media in 100, 150, and 250 mm lengths with 21.2, 30, and 50 mm internal diameters (**Figure 5**).

Figure 2.
Axia Packing Process Integrates Axial Compression Technology into Prepacked Chiral Preparative Columns



The Axia process uses highly controlled pneumatic mechanical pressure to drive the piston into the column to produce a uniformly packed bed. Once the bed is formed the pressure on the piston and bed is not released, the bed is not disturbed and the piston is locked in place leaving the chiral media under pressure. This packing process won the 2006 R&D 100 Award for its innovation.

Figure 3.
Controlled Axia Packing Process for Lux Cellulose-2 Prevents Crushing the Media

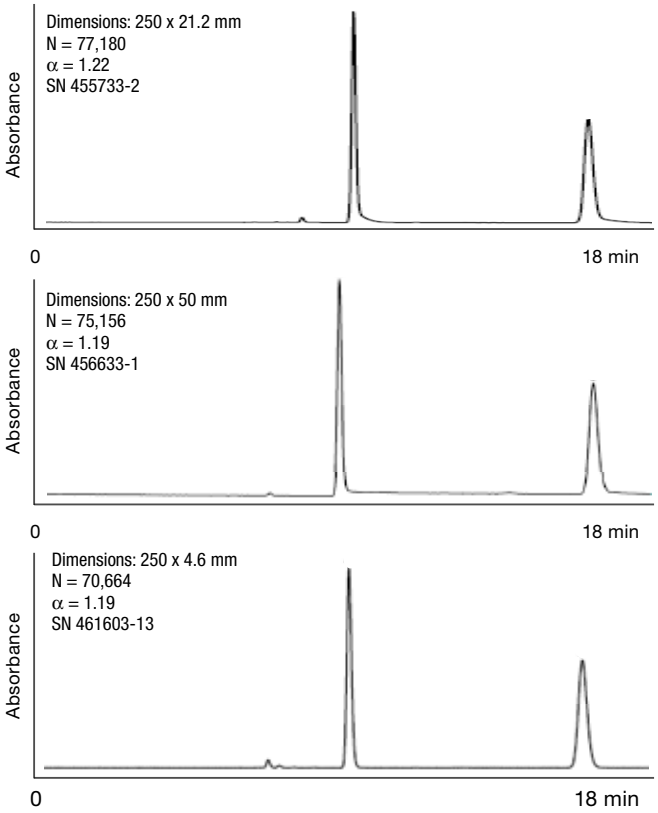


Lux media is mechanically stronger allowing higher packing pressures than previous chiral media.

The SEM of virgin media and after Axia packing proves that Axia's computer controlled process does not crush the Lux high porosity media that is engineered to be mechanically strong.

APPLICATIONS

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



Conditions for all columns:
Column: Lux 5 µm Cellulose-2
Mobile Phase: Hexane:IPA (90:10)
Detection: UV @ 220 nm
Sample: Trans-Stilbene oxide

Axia technology has the highest process control and produces reproducible, stable, high efficiency columns with the same plates per meter and peak asymmetry independent of column length and ID.

Figure 5.
Axia packed Lux Product Family Available in Three Diameters and Three Lengths

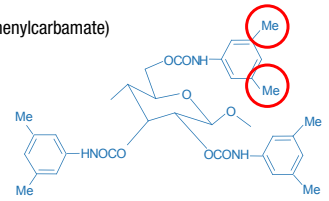
The Axia packing process is utilized to produce the Lux preparative columns in 100, 150 and 250 mm lengths and in the three diameters 21.2, 30 and 50 mm.

Experimental

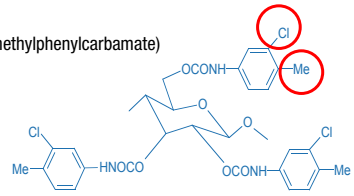
Lux is a media engineered to provide a straightforward approach to enantiomeric recognition and separation by HPLC and Supercritical Fluid Chromatography (SFC). Two Lux phases have been developed using a coated derivatized cellulose material as the chiral selector (**Figure 6**). Lux Cellulose-1 features the classical tris(3,5-dimethylphenylcarbamate) cellulose derivative used industry-wide for many enantiomer separations. This particular chiral selector has well-established enantiomeric abilities to resolve a wide range of racemates.

Figure 6.
Structures of Lux Cellulose-1 and Lux Cellulose-2 Chiral Phases

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



Lux 5 µm Cellulose-2
Cellulose tris(3-chloro-4-methylphenylcarbamate)

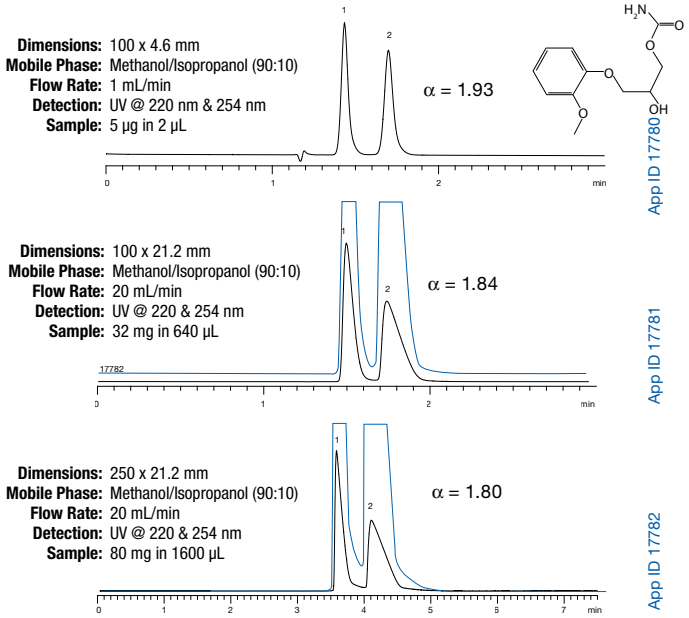


Lux Cellulose-1 and Lux Cellulose-2 are derivatized phenyl carbamates with different functional groups substituted on the aromatic rings. The substitution of the chlorine molecule with Lux Cellulose-2 provides unique selectivity compared to the traditional Lux Cellulose-1 structure.

Lux Cellulose-2 incorporates an advanced halogenated derivative, leading to unique enantioselectivity compared to previously commercialized cellulose phases. The unique selectivity of Lux Cellulose-2 makes it an ideal CSP providing excellent complementary selectivity to Lux Cellulose-1, and any improvement in the alpha value is extremely important for preparative separations. The two Lux phases are compatible with a wide range of solvent systems including normal phase, polar organic, reversed phase, and SFC.

Methocarbamol represents an important class of compounds routinely separated and purified by HPLC with CSP columns. The initial separation was developed on a 100 x 4.6 mm column using MeOH:IPA (90:10) and the response monitored at 220 and 254 nm. Increasing the column diameter from 4.6 to 21.2 mm provides higher throughput (32 mg) for each run without increasing the overall purification time (**Figure 7**). By increasing the column length to 250 mm the load can be further increased to 80 mg per run. The methocarbamol separation demonstrates that the separation scales up linearly based on the column length with no loss of resolution. An important factor to consider when performing these higher mass loading separations is the UV detector response is not linear making it difficult to determine where the major mass is located based solely on the UV signal. Although de-tuning a detector will keep the peaks on scale, the UV signal is not the best indicator for purity and resolution when column overloading occurs. It is crucial to first evaluate the purity and yield for the collected fractions to determine the maximum load per run that could be achieved.

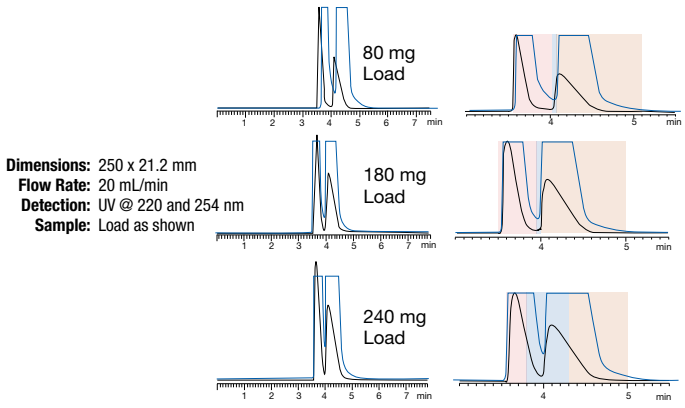
Figure 7.
Direct Scale Up of Methocarbamol Purification on Lux® 5 µm Cellulose-1



Separation scales up directly based on column length. With the 100 mm length column a 32 mg/load separation was achieved and a higher sample load required the longer 250 mm length column. As expected when increasing the load, the peak width and tailing increased but there was no loss of resolution. For the higher sample loads, the detector sensitivity was decreased by monitoring at 254 nm.

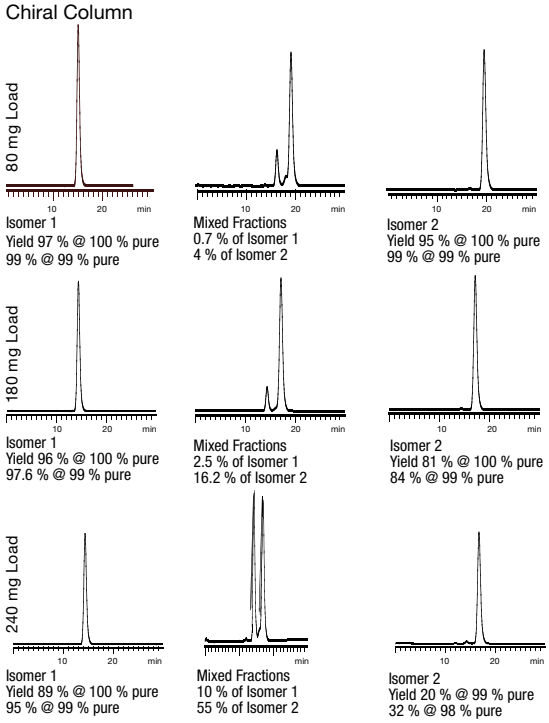
To further improve sample throughput and productivity, the sample load on the column was increased from 80 mg to 180 mg and then finally to 240 mg (**Figure 8a**). The UV signal was used to determine the starting and ending collection points, but time based fractions were collected across the peaks including the valley area. After the fractions were collected, each fraction was first analyzed using a 100 x 4.6 mm Lux Cellulose-1 column on an analytical HPLC and then the fractions were pooled together and the overall yield and purity were assessed (**Figure 8b**).

Figure 8a.
Resolution Change with Increased Load for Chiral Separations



Fractions were collected across the peak and evaluated for purity at 220 nm using a 100 x 4.6 mm Lux Cellulose-1 column. The purity for each pooled fraction was determined. The 254 nm detector trace for the preparative separation shows the presence of each material but cannot be used to predict the purity of the fraction.

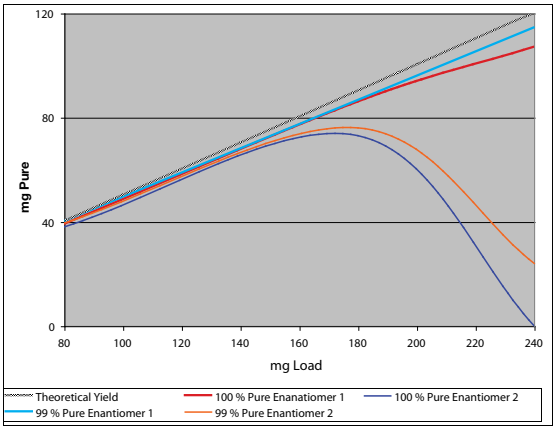
Figure 8b.
Comparison of Yield and Purity for Different Loads on Lux® Cellulose-1 Chiral Column



Each fraction was individually evaluated and then pooled providing three fractions that were then evaluated for the overall purity and yield. At 80 mg load both enantiomer 1 and 2 were obtained in high yield and purity. When 180 mg load was purified, the yield for pure enantiomer 1 was not significantly affected but enantiomer 2's yield decreased to 81 %. With the 240 mg load, the column overload was too great and the capability to collect pure enantiomer 2 was lost. Even if a 2 % enantiomer impurity was acceptable only 32 % of the load was recovered at this purity level.

The dramatic effect increasing sample load has on throughput, purity, and productivity is illustrated in **Figure 9.**

Figure 9.
Effect on Purity and Yield When Increasing Methocarbamol Load to Improve Throughput



The quantity of 100 % pure enantiomer 1 collected increases with larger sample loads with a slight sacrifice in yield. The later eluting enantiomer's purity and yield dramatically drops when the load is above 180 mg on the 250 x 21.2 mm column. A larger diameter column with the same load would provide increased throughput for the second enantiomer without sacrificing purity.

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Enantiomer 1 – Even when 240 mg was loaded onto the column, the purity of the first enantiomer was not greatly affected and 107 mg (89 % of this enantiomer) was determined to be 100 % pure. Increasing the mass loading per run is a tremendous advantage when larger quantities of this enantiomer are required. If the enantiomer purity requirement was 99 %, then additional fractions could be pooled and a total of 114 mg (95 %) of the first enantiomer mass was collected.

Enantiomer 2 – When 180 mg was loaded onto the column, 73 mg (81 %) of the enantiomer was obtained at 100 % purity level which is only a slight change from enantiomer 1 results where 86 mg was collected for the same purification. Whereas when the mass loading was increased to 240 mg the purity for enantiomer 2 dramatically decreased. In fact, at the 240 mg load, the highest purity achieved was 99 % for enantiomer 2, but only 24 mg was recovered representing only about 20 % of the initial load. With the 240 mg load, the first enantiomer contaminates the second enantiomer and 0 % was obtained with a 100 % purity level. The extent of the peak overlap is very evident in the preparative chromatograms in **Figure 8a**. Although the UV trace indicates there is still resolution between the two compounds the amount of overlap between enantiomer 1 and enantiomer 2 is very significant. If the desired enantiomer is the later eluting compound on the chiral column, the sample load and throughput must be carefully monitored by evaluating the fractions to ensure an acceptable enantiomer purity is achieved.

Conclusions

Axia technology is the industry standard for consistency and robustness in preparative columns with the same performance achieved from 4.6 mm analytical columns to 50 mm ID preparative columns. Over the last several years the Axia technology with its high level of process control has been proven to produce columns with the same performance (plates per meter) independent of length and diameter. The 5 µm Lux preparative columns are available in 100, 150, and 250 mm lengths with 21.2, 30, and 50 mm diameters. There has been a significant improvement in the asymmetry and efficiency across all lengths and IDs for the Axia packed preparative columns allowing chemists more flexibility to achieve their goals for increased purity and yield for their preparative purifications.

Since these preparative Lux columns packed with 5 µm have the same plates/meter (efficiency and asymmetry factors) independent of ID and length, the chemist has more options to quickly scale up a separation to obtain higher quantities of purified enantiomer without sacrificing purity or yield. Many times the shorter column provides sufficient resolution for the required compounds resulting in faster turn-around times and higher productivity.

Selectivity is still the most critical factor for chiral separations. Screening and having a choice of multiple chiral phases is important to increase resolution prior to performing the preparative separations.

This work has shown that sample load scales up directly based on column length, but the separation time increases as the column length increases. Whereas, sample load increases exponentially with column diameter without increasing the separation time. The judicious choice of overall column length and column diameter of the Axia packed Lux columns will have a major affect on a laboratory's overall throughput.

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

	150 x 21.2	250 x 21.2	250 x 30	250 x 50	SecurityGuard™ Cartridges (mm) 15 x 21.2**	15 x 30.0+
Phases					/ea	/ea
Cellulose-1*	00F-4459-P0-AX	00G-4459-P0-AX	00G-4459-U0-AX	00G-4459-V0-AX	AJ0-8405	AJ0-8406
Cellulose-2*	00F-4457-P0-AX	00G-4457-P0-AX	00G-4457-U0-AX	00G-4457-V0-AX	AJ0-8400	AJ0-8401
Cellulose-3	00F-4493-P0-AX	00G-4493-P0-AX	00G-4493-U0-AX	00G-4493-V0-AX	AJ0-8624	AJ0-8625
Cellulose-4	00F-4491-P0-AX	00G-4491-P0-AX	00G-4491-U0-AX	00G-4491-V0-AX	AJ0-8629	AJ0-8630
Amylose-2	00F-4472-P0-AX	00G-4472-P0-AX	00G-4472-U0-AX	00G-4472-V0-AX	AJ0-8473	AJ0-8474
					for ID: 18–29 mm	30–49 mm

*Inquire for Lux 10 µm Cellulose-1 and Cellulose-2 columns.
**PREP SecurityGuard Cartridges require holder, Part No. : AJ0-8223
+PREP SecurityGuard Cartridges require holder, Part No. : AJ0-8277

Bulk Media

Phases	100 g	1 kg
10 µm		
Cellulose-1	04G-4501	04K-4501
Cellulose-2	04G-4502	04K-4502
20 µm		
Cellulose-1	04G-4473	04K-4473
Cellulose-2	04G-4464	04K-4464
Cellulose-3	04G-4504	04K-4504
Cellulose-4	04G-4503	04K-4503

Please inquire for 20 µm Lux Amylose-2 media

guarantee

If Axia packed columns do not provide at least an equivalent separation as compared to a competing preparative column of the same particle size, same phase and dimensions, return the column with comparative data within 45 days for a FULL REFUND. Only applies to 21.2 mm ID columns.

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