Scaling from Analytical to Preparative Chiral Chromatography While Balancing Purity, Yield, and Throughput under HPLC and SFC Conditions

J Preston, J.T. Presley III, Michael McCoy, Michael Klein, Marc Jacob et al.
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Axia™ preparative column technology along with Axia specilized hardware shows higher performance than traditionally packed standard hardware preparative columns. The Axia packing technology is compatible with both SFC and HPLC conditions. In this application, we will demonstrate how the Axia packed columns with the Lux® Cellulose-1 polysaccharide-based chiral stationary phase can be a tool to increase throughput for purification of chiral compounds.

Introduction

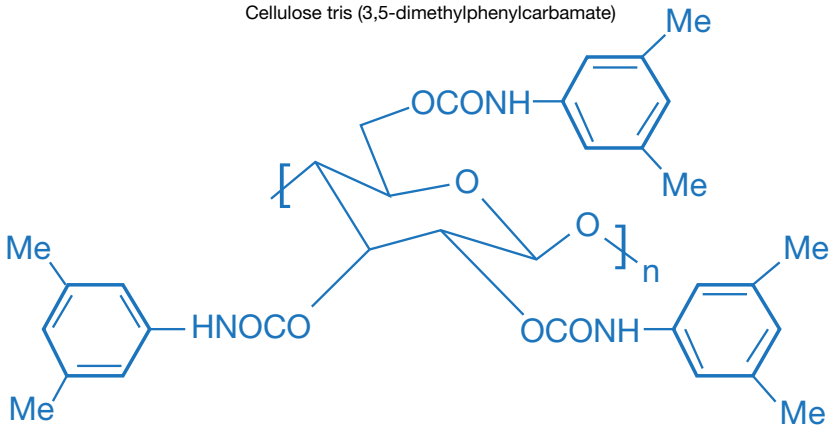
HPLC has been extensively studied since the late 1960's and there have been numerous theoretical models developed to describe, explain, and predict the results of chromatographic experiments. The typical goal of chromatography is to separate compounds from each other, and the most straight forward way to evaluate a separation is to calculate the resolution between two peaks of interest. Resolution of two peaks will be a function of numerous factors, including mobile phase composition, stationary phase selectivity, and running conditions. In practical terms, the resolution is predicted by how far apart the two peaks are separated in time and how broad the peaks are shaped. Thus, optimal resolution is provided by obtaining narrower peaks, as this allows them to be more easily resolved from one another in any given time frame.

One particular method of chromatography known as supercritical fluid chromatography (SFC) has become increasingly popular in the last several years. In contrast to traditional liquid chromatography, the SFC mobile phase consists of a mixture of liquid carbon dioxide and organic solvent, such as Methanol. The principle advantages of SFC over conventional HPLC techniques are increased speed, reduced waste generation and for preparative purifications, minimized post-chromatography sample manipulation. For chiral separations in particular, SFC is increasing in popularity because it is often very simple to convert an existing normal phase HPLC method into an SFC method. The use of preparative chiral chromatography has increased significantly over the past 5-10 years, and SFC has been a significant driver for this increase.

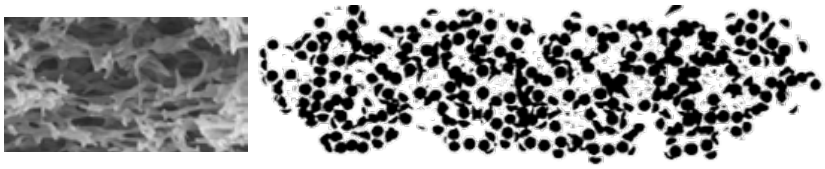
It is well known that chromatography can be directly scaled from very small columns to very large columns when the eluent composition remains consistent. The work presented in this application will address the relationship between both normal phase and SFC chiral methodologies at the analytical and preparative scale. The impact on resolution at both scales due to flow rates will be evaluated and compared between SFC and normal phase. The effect of preparative column hardware technology along with resulting purity and throughput from related SFC and normal phase purification methodologies will also be evaluated.

Lux Cellulose-1 Chiral Stationary Phase

Cellulose tris (3,5-dimethylphenylcarbamate)



Cellulose backbone



Material and Methods

Analytical HPLC separations were developed using an Agilent® 1100 system with diode array detector (Agilent, Palo Alto, CA). SFC analytical was performed on a Waters® ACQUITY® UPC²® system (Waters, Milford, MA USA) consisting of a convergence manager, sample manager, binary solvent manager, PDA detector, column manager with 6 positions, and a Waters 3100 mass spectrometer. Data analysis was performed using MassLynx® software (Version 4.1).

Normal phase preparative scale separations were performed on a Shimadzu® LC20 Prep HPLC system, with an LC-10 autosampler and fraction collector. SFC purifications were performed on a Berger Automated PrepSFC™ system (Mettler-Toledo, USA) consisting of a Bohdan automated injection/collection robot, Berger SCM-250 (separator control module), Berger ECM-2500 (electronic control module), KNAUER K-2500 UV variable detector, Varian® SD-1 methanol and CO2 delivery systems, JULABO® chiller, and SFC ProNTo™ control software (Version 1.5.305.15) with SFC Automation Controller add-on (Version 1.5.92.3).

Compounds were evaluated using a Phenomenex Lux® 5 µm Cellulose-1 column, dimensions are as noted in each Figure. HPLC conditions and injection amounts are as noted in each Figure. Warfarin test solutions were prepared at 20 mg/mL in ethanol and used for all testing.

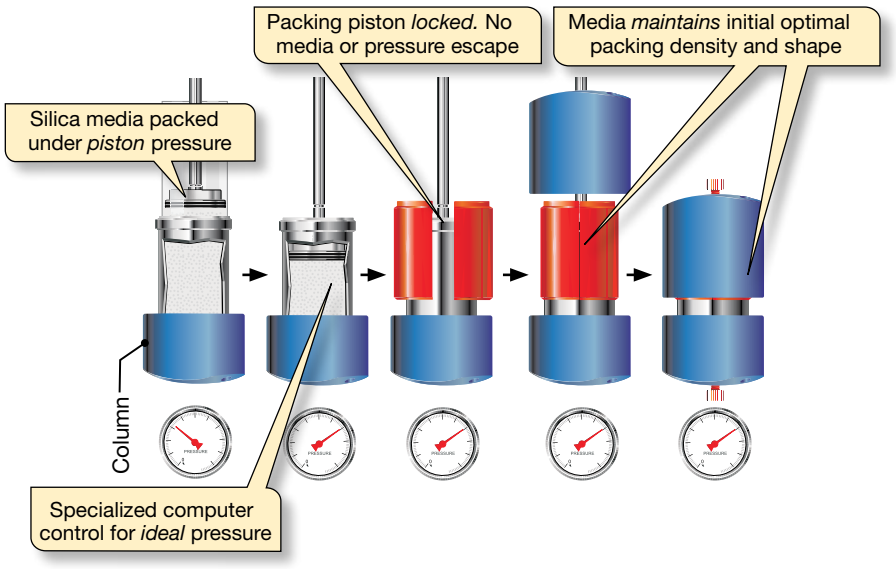
Results and Discussion

When scaling up from analytical sized columns (4.6 mm I.D.) to traditionally packed larger I.D. columns (>10 mm I.D.), there has historically been some loss in efficiency and performance that is attributed to the packing quality with these larger I.D. columns. The reasons for this change in performance are complex but include non-uniform packing density throughout the column, the bed structure being disturbed after the media is packed, media fracture and/or fines created during the packing process, and media extrusion from the packed bed during final hardware assembly.

The standard hardware column packing process is complicated and there are many opportunities for a loss in column performance. To address this issue, Phenomenex developed a unique column packing technology and hardware, AXIA™, to maintain analytical-like column performance in preparative column dimensions. The Axia technology, patented by Phenomenex, is an advanced column packing and column hardware design that incorporates Hydraulic Piston Compression technology that mimics axial compression columns. This results in Axia preparative columns outperforming column packed using traditional packing methods.

Axia packing technology uses a computerized mechanical process to pack the column bed (**Figure 1**). 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 packing during final hardware assembly and into the final product.

Figure 1. Axia Patented Packing Technology



Previous work by Jan Priess et. al. demonstrated increased column efficiency and resolution for polysaccharide-based chiral stationary phase (CSP) media packed using Axia™ columns.[1] To better understand how much this hardware technology improves column performance we packed the same 5 µm Lux® Cellulose-1 chiral media into two different 150 x 21.2 mm I.D. columns. The Lux media is engineered to be mechanically stronger than previous chiral media, allowing higher packing pressures to be applied; thus increasing the column plate count and column performance. One column was packed using a traditional HPLC column packing process with standard hardware and the other column was packed using Axia technology with Axia hardware. The QC data for the Axia column showed 73,000 plates per meter, which was a > 22 % increase over the standard hardware column.

The 150 x 21.2 mm traditionally packed standard hardware preparative column and Axia packed preparative column were first evaluated by generating Van Deemter curves for trans-Stilbene Oxide (TSO) to find out if there was any difference in column efficiency versus linear velocity. The normal phase data indicated the Axia packing technology had a substantial 91.6 % increase in column efficiency over traditionally packed columns at a 0.1 cm/sec linear flow as depicted in **Figure 2**. The difference in performance was less pronounced in SFC, but still showed a 26.8 % increase in efficiency for the Axia packed column at 0.4 cm/sec (**Figure 3**). As expected, the decrease in column efficiency as linear velocity increased was less under SFC conditions.

Figure 2. Van Deemter Plots - Normal Phase Mode

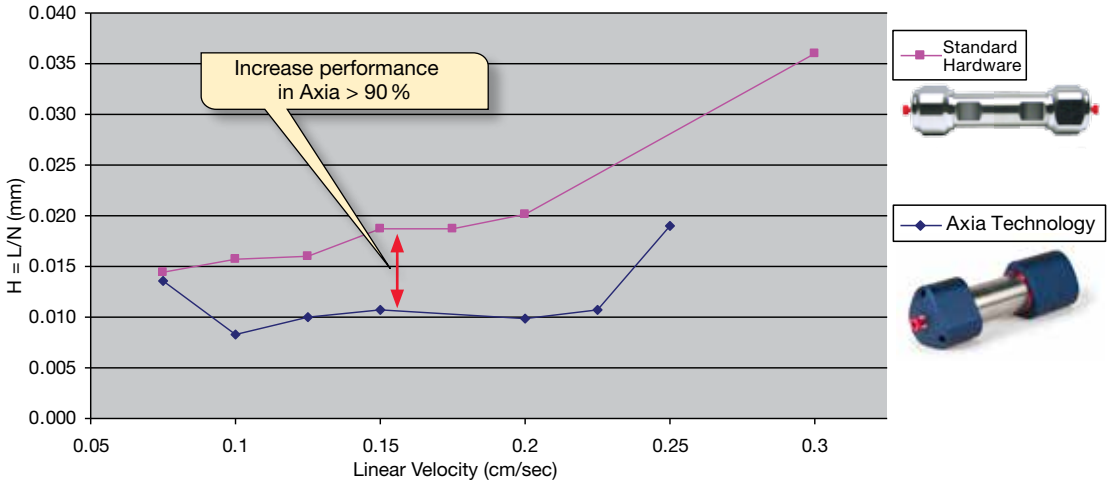
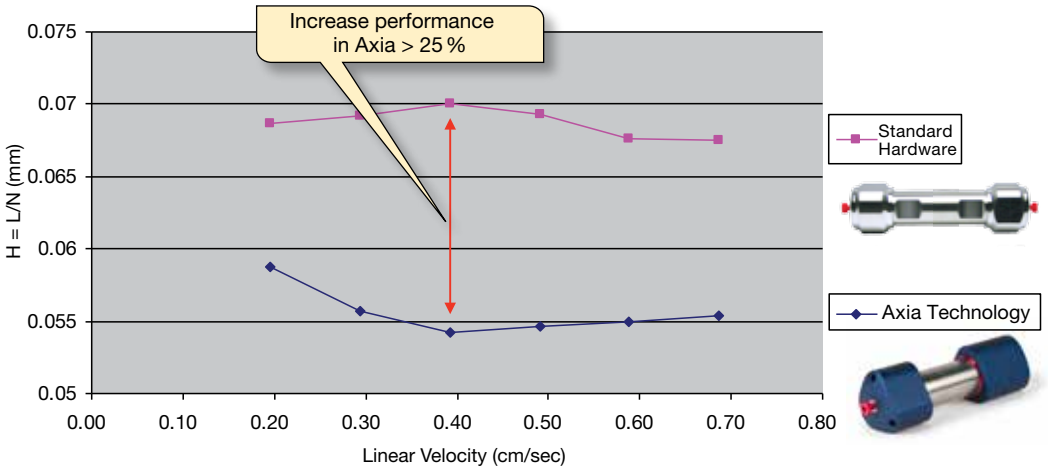


Figure 3. Van Deemter Plots - SFC Mode



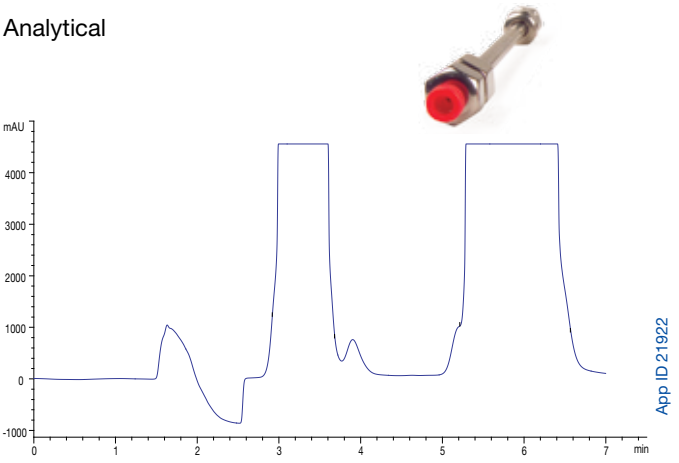
To understand what advantage this would provide for a high-throughput purification laboratory, we performed a scale up experiment using Warfarin. Analytical separations were first developed in normal phase on a 150 x 4.6 mm column and loading was increased until a reasonable loading capacity was achieved. The injection volume was then directly scaled up (geometrically) twenty fold for both of the 150 x 21.2 mm preparative columns. The resolution and efficiency for the second peak were measured. Again, the preparative column packed using Axia™ technology showed roughly a 30 % increase in resolution and 42 % increase in efficiency over the traditionally packed standard hardware column. (**Figure 4**).

Figure 4.
Warfarin Purification in Normal Phase Mode

Column (mm)	Analytical 150 x 4.6	Standard 150 x 21.2	Axia 150 x 21.2
Mass Loaded (mg)	2	40	40
Resolution*	1.5	2.85	3.72
Plates (N)	117	535	760

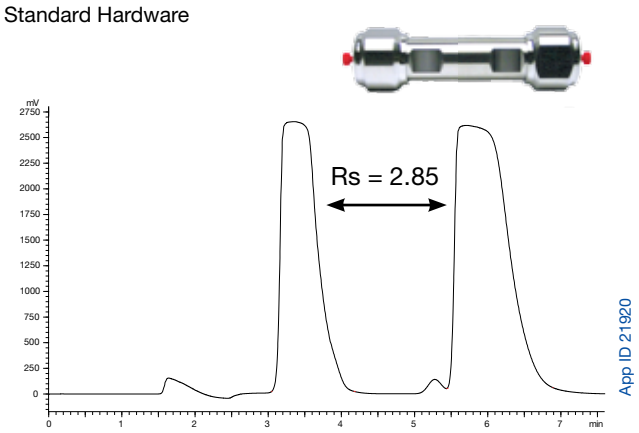
* Resolution calculated with peak width at baseline and center retention time due to the overloaded peaks being off-scale

Analytical

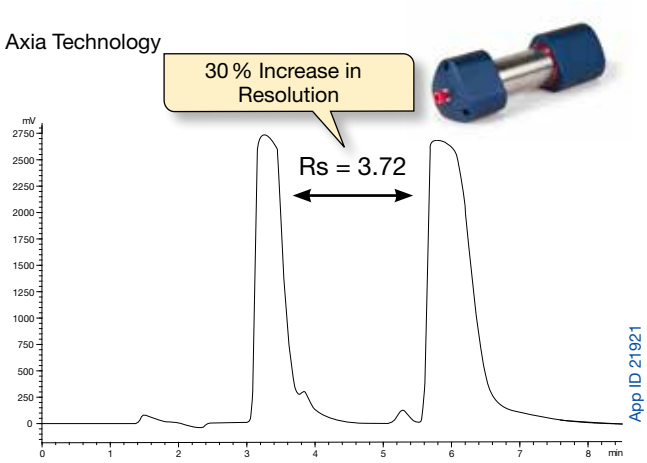


Column: Lux® 5 µm Cellulose-1
Dimensions: 150 x 4.6 mm
Mobile Phase: Hexane/Ethanol (75:25)
Flow Rate: 1 mL/ min
Temperature: Ambient
Inj. Volume: 100 µL

Standard Hardware



Axia Technology



Conditions for both columns:
Column: Lux 5 µm Cellulose-1
Dimensions: 150 x 21.2 mm
Mobile Phase: Hexane / Ethanol (75:25)
Flow Rate: 20 mL/ min
Temperature: Ambient
Inj. Volume: 2 mL

The separation was then adapted to SFC to provide a reduced run time, while maintaining suitable resolution. Due to the SFC systems injection volume limitation, direct loading comparisons were not possible. We did attempt to make a more concentrated solution of Warfarin, but reached a saturation point. However, when comparing loads of 36 mg on-column, the Axia™ columns again showed a 25 % increase in resolution and a 14 % increase in efficiency for the second peak (**Figure 5**) when compared with the traditionally packed standard hardware column.

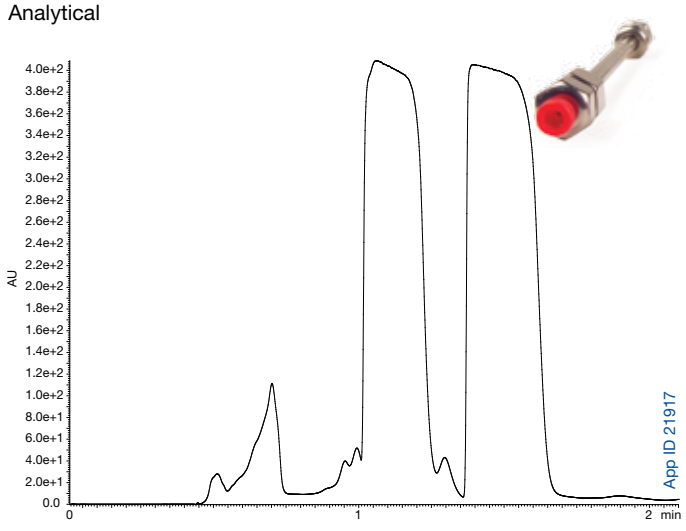
Fractions were collected for both normal phase and SFC runs and yielded similar masses collected with similar purity profiles. This was to be expected since the peaks were still well resolved at this load.

Figure 5.
Warfarin Purification in SFC Mode

Column (mm)	Analytical 150 x 4.6	Standard 150 x 21.2	Axia 150 x 21.2
Mass Loaded (mg)	1.5	36	36
Resolution*	1.39	1.87	2.33
Plates (N)	206	441	503

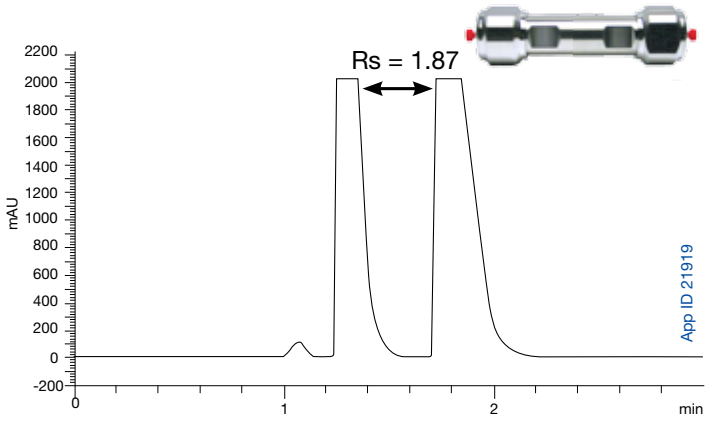
* Resolution calculated with peak width at baseline and center retention time due to the overloaded peaks being off-scale

Analytical

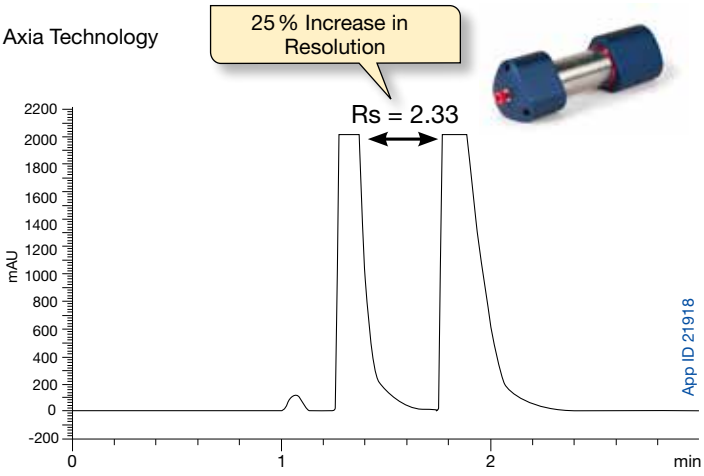


Column: Lux® 5 µm Cellulose-1
Dimensions: 150 x 4.6 mm
Mobile Phase: CO_2/Methanol (65:35)
Flow Rate: 3.5 mL/min
Temperature: 55 °C
Inj. Volume: 75 µL

Standard Hardware



Axia Technology



Conditions for both columns:
Column: Lux 5 µm Cellulose-1
Dimensions: 150 x 21.2 mm
Mobile Phase: CO_2/Methanol (65:35)
Flow Rate: 70 mL/min
Temperature: 55 °C
Inj. Volume: 1.8 mL

Conclusion

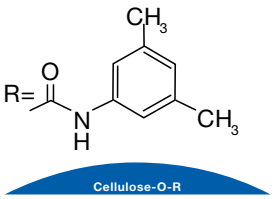
Axia™ preparative columns packed with 5 µm Lux® polysaccharide-based media gives higher performance than traditionally packed standard hardware columns. The Axia technology is compatible with both SFC and HPLC separation conditions and can be a tool to increase throughput for purification.

References

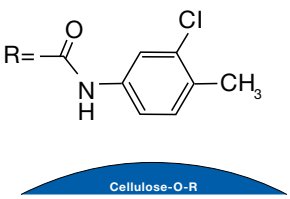
1. Evaluation of Chiral Stationary Phase Packed Axia HPLC Column, J. Priess, C. Valente, G. Diehl and E. Francotte, Novartis Institutes for Biomedical Research, Basel, Switzerland, Poster 2137, SPICA 2008

Acknowledgements

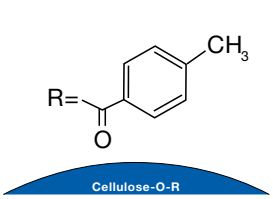
The authors would like to acknowledge Bill Ferrell at Pfizer, La Jolla, CA for his contribution to this work.



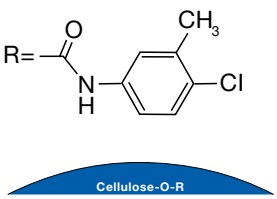
Lux Cellulose-1
Cellulose tris (3.5-dimethylphenylcarbamate)



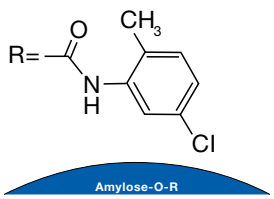
Lux Cellulose-2
Cellulose tris (3-chloro-4-methylphenylcarbamate)



Lux Cellulose-3
Cellulose tris (4-methylbenzoate)



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



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

Lux Ordering Information

3 µm Analytical Columns (mm)							SecurityGuard™ Cartridges (mm)	
Phases	**50 x 2.0**	**150 x 2.0**	**50 x 4.6**	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**4 x 2.0***	**4 x 3.0***
							/10pk	/10pk
Cellulose-1	00B-4458-B0	00F-4458-B0	00B-4458-E0	00D-4458-E0	00F-4458-E0	00G-4458-E0	AJ0-8402	AJ0-8403
Cellulose-2	00B-4456-B0	00F-4456-B0	00B-4456-E0	00D-4456-E0	00F-4456-E0	00G-4456-E0	AJ0-8398	AJ0-8366
Cellulose-3	00B-4492-B0	00F-4492-B0	00B-4492-E0	00D-4492-E0	00F-4492-E0	00G-4492-E0	AJ0-8621	AJ0-8622
Cellulose-4	00B-4490-B0	00F-4490-B0	00B-4490-E0	00D-4490-E0	00F-4490-E0	00G-4490-E0	AJ0-8626	AJ0-8627
Amylose-2	00B-4471-B0	00F-4471-B0	00B-4471-E0	00D-4471-E0	00F-4471-E0	00G-4471-E0	AJ0-8471	AJ0-8470
						for ID:	2.0–3.0 mm	3.2–8.0 mm

5 µm Analytical Columns (mm)						SecurityGuard Cartridges (mm)	
Phases	**50 x 2.0**	**50 x 4.6**	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**4 x 2.0***	**4 x 3.0***
						/10pk	/10pk
Cellulose-1	00B-4459-B0	00B-4459-E0	00D-4459-E0	00F-4459-E0	00G-4459-E0	AJ0-8402	AJ0-8403
Cellulose-2	00B-4457-B0	00B-4457-E0	00D-4457-E0	00F-4457-E0	00G-4457-E0	AJ0-8398	AJ0-8366
Cellulose-3	00B-4493-B0	00B-4493-E0	00D-4493-E0	00F-4493-E0	00G-4493-E0	AJ0-8621	AJ0-8622
Cellulose-4	00B-4491-B0	00B-4491-E0	00D-4491-E0	00F-4491-E0	00G-4491-E0	AJ0-8626	AJ0-8627
Amylose-2	00B-4472-B0	00B-4472-E0	00D-4472-E0	00F-4472-E0	00G-4472-E0	AJ0-8471	AJ0-8470
					for ID:	2.0–3.0 mm	3.2–8.0 mm

5 µm Semi-Prep Columns (mm)			SecurityGuard Cartridges (mm)
Phases	**150 x 10.0**	**250 x 10.0**	**10 x 10.0‡**
			/3pk
Cellulose-1†	00F-4459-N0	00G-4459-N0	AJ0-8404
Cellulose-2†	00F-4457-N0	00G-4457-N0	AJ0-8399
Cellulose-3	00F-4493-N0	00G-4493-N0	AJ0-8623
Cellulose-4	00F-4491-N0	00G-4491-N0	AJ0-8628
Amylose-2	00F-4472-N0	00G-4472-N0	AJ0-8472
		for ID:	9–16 mm

†Inquire for 10 µm Cellulose-1 and Cellulose-2 columns.

*SecurityGuard Analytical Cartridges require holder, Part No. : KJ0-4282
‡SemiPrep SecurityGuard™ Cartridges require holder, Part No.: AJ0-7220

guarantee

If Lux analytical columns (≤ 4.6 mm ID) do not provide at least an equivalent or better separation as compared to a competing column of the same particle size, similar phase and dimensions, return the column with comparative data within 45 days for a FULL REFUND.

Lux® Ordering Information *cont'd*

5 µm Axia™ Packed Preparative Columns (mm)					SecurityGuard™ Cartridges (mm)	
Phases	**150 x 21.2**	**250 x 21.2**	**250 x 30**	**250 x 50**	**15 x 21.2****	**15 x 30.0♦**
					/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

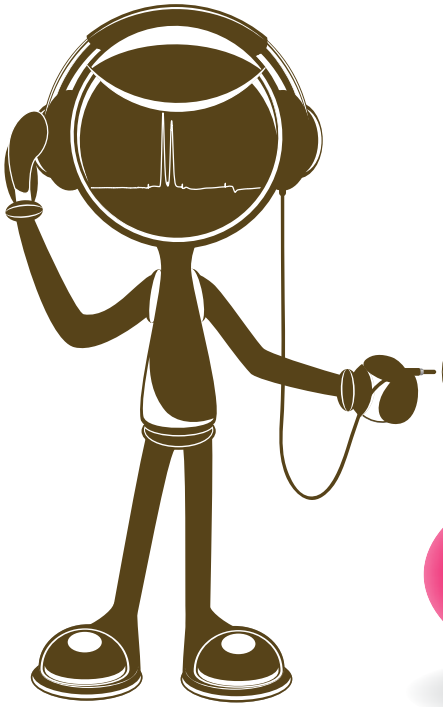
†Inquire for Lux 10 µm Cellulose-1 and Cellulose-2 columns

**HPLC PREP SecurityGuard Cartridges require holder, Part No. : AJ0-8223
SFC PREP SecurityGuard Cartridges require holder, Part No. : AJ0-8617

♦ HPLC PREP SecurityGuard Cartridges require holder, Part No. : AJ0-8277
SFC PREP SecurityGuard Cartridges require holder, Part No. : AJ0-8618

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



APPLICATIONS

Australia
t: 02-9428-6444
f: 02-9428-6445
auinfo@phenomenex.com

Austria
t: 01-319-1301
f: 01-319-1300
anfrage@phenomenex.com

Belgium
t: 02 503 4015 (French)
t: 02 511 8666 (Dutch)
f: +31 (0)30-2383749
beinfo@phenomenex.com

Canada
t: (800) 543-3681
f: (310) 328-7768
info@phenomenex.com

Denmark
t: 4824 8048
f: +45 4810 6265
nordicinfo@phenomenex.com

Finland
t: 09 4789 0063
f: +45 4810 6265
nordicinfo@phenomenex.com

France
t: 01 30 09 21 10
f: 01 30 09 21 11
franceinfo@phenomenex.com

Germany
t: 06021-58830-0
f: 06021-58830-11
anfrage@phenomenex.com

India
t: 040-3012 2400
f: 040-3012 2411
indiainfo@phenomenex.com

Ireland
t: 01 247 5405
f: +44 1625-501796
eireinfo@phenomenex.com

Italy
t: 051 6327511
f: 051 6327555
italiainfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

Mexico
t: 001-800-844-5226
f: 001-310-328-7768
tecnicomx@phenomenex.com

The Netherlands
t: 030-2418700
f: 030-2383749
nlinfo@phenomenex.com

New Zealand
t: 09-4780951
f: 09-4780952
nzinfo@phenomenex.com

Norway
t: 810 02 005
f: +45 4810 6265
nordicinfo@phenomenex.com

Puerto Rico
t: (800) 541-HPLC
f: (310) 328-7768
info@phenomenex.com

Sweden
t: 08 611 6950
f: +45 4810 6265
nordicinfo@phenomenex.com

United Kingdom
t: 01625-501367
f: 01625-501796
ukinfo@phenomenex.com

United States
t: (310) 212-0555
f: (310) 328-7768
info@phenomenex.com

All other countries:
Corporate Office USA
t: (310) 212-0555
f: (310) 328-7768
info@phenomenex.com

www.phenomenex.com

Phenomenex products are available worldwide. For the distributor in your country, contact Phenomenex USA, International Department at international@phenomenex.com

TN15980813_W