About Guest Author: Dr. J Preston

Dr. J Preston is a Senior Scientist and Group Leader for HPLC, UHPLC, and Prep projects within the **PhenoLogix™ group at Phenomenex**. This group is a full-service support laboratory within Phenomenex that specializes in method development and optimization for HPLC, UHPLC, GC, GCMS, LC-MS/MS, and Sample Preparation. He has several publications on a variety of topics and has participated on multiple industrial advisory boards. Prior to joining Phenomenex, Dr. Preston worked for Amgen in Thousand Oaks, California where he was the technical manager during the creation of their Small Molecule Process Analytical Group. Later, he managed their Small Molecule GMP/ Research prep chromatography facility. Dr. Preston has also worked in an early development analytical group for Searle/ Pharmacia/ Pfizer in Skokie, Illinois and in a late development GMP analytical group for Abbott Laboratories in North Chicago, Illinois.

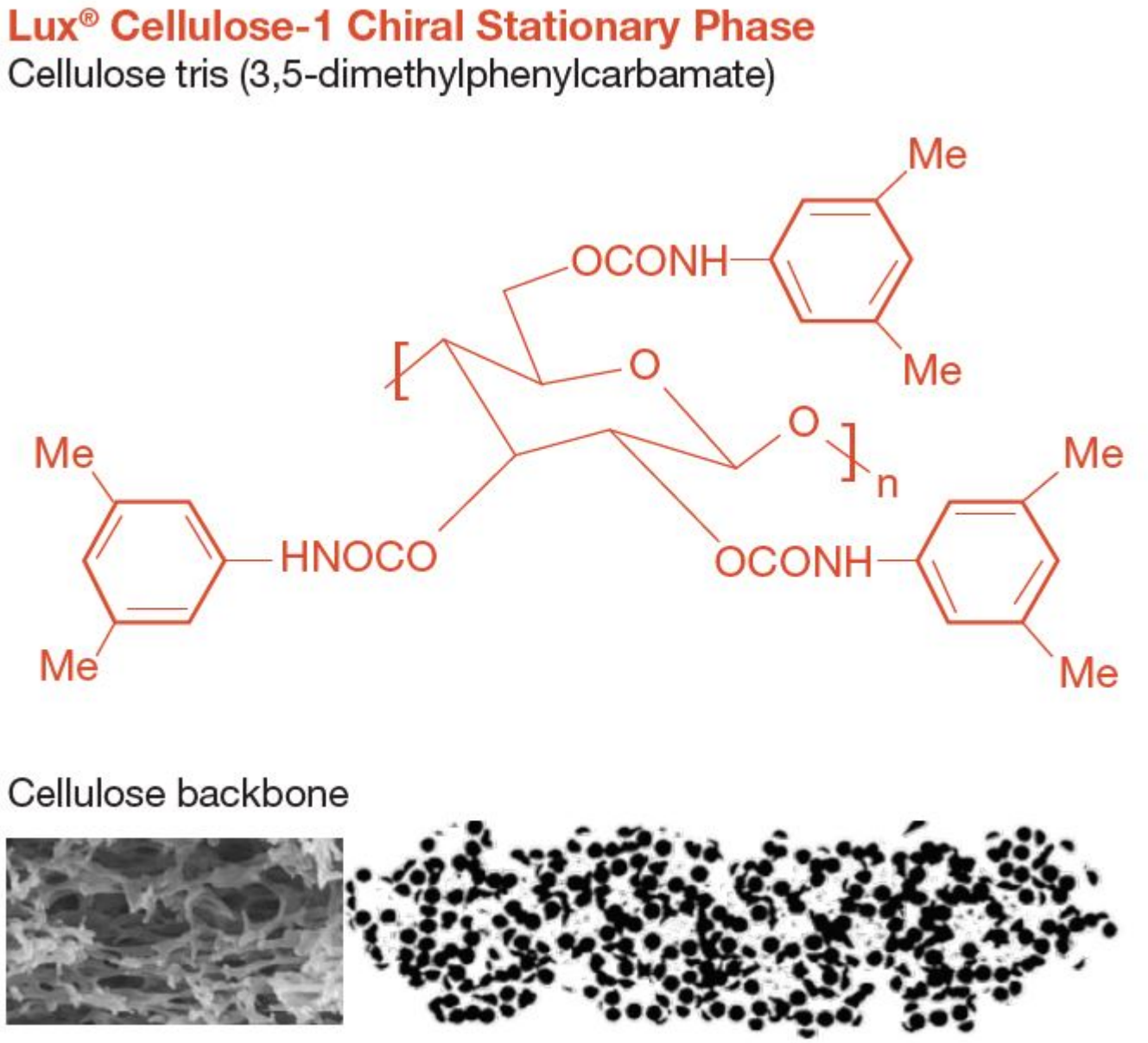
Dr. Preston will be one of our esteemed speakers at this year's **North American PhenoPrep Seminar** in Princeton, NJ, October 19th, discussing how to pick the right tools for preparative chromatography. Get a sneak into his insights in the following article, that can also be found in the recent edition of our **Purify Newsletter, Vol. 2**!

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 straightforward 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 supercritical 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, and 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.



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

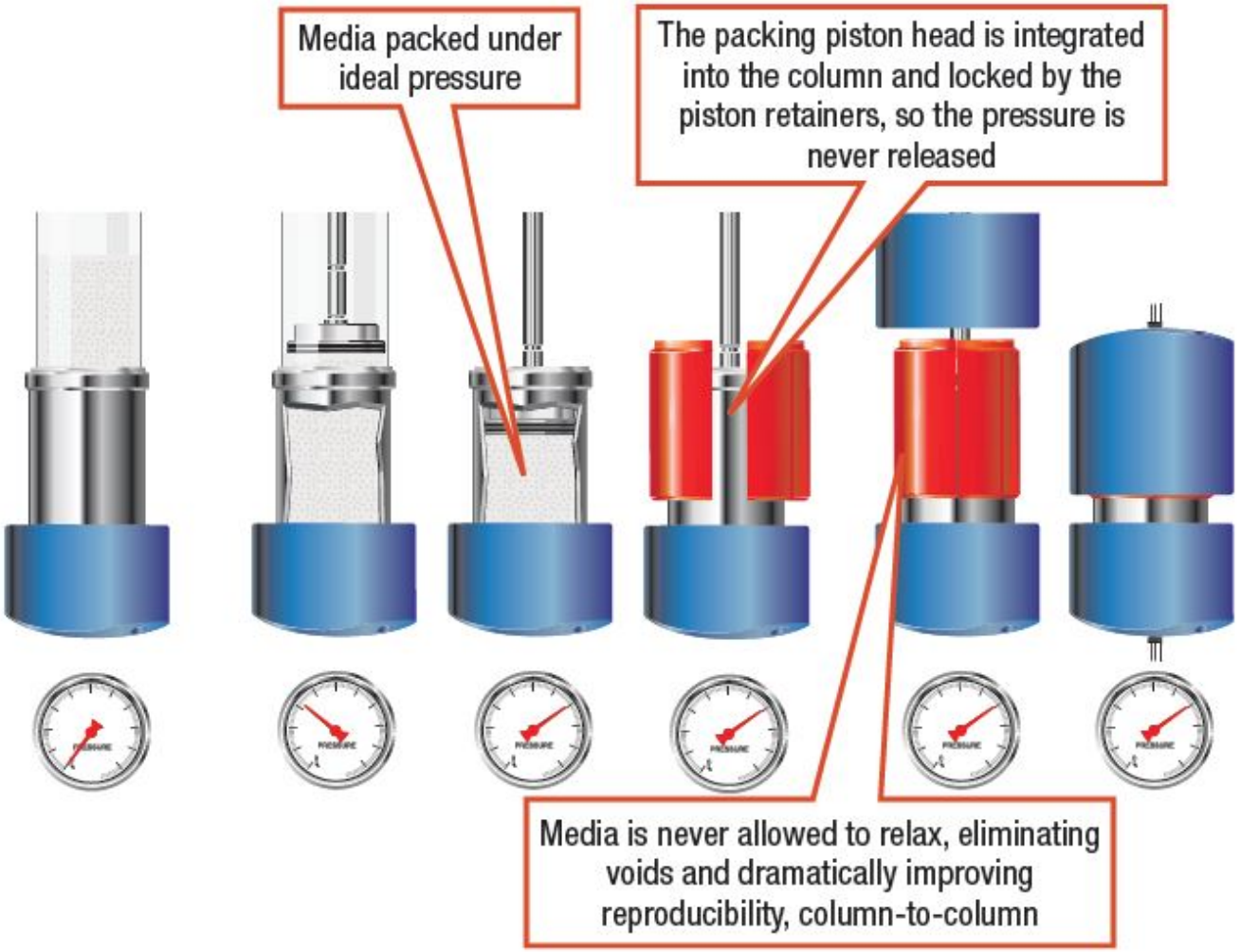
Shimadzu® LC20 Prep HPLC system, with an LC-10 autosampler and fraction collector. SFC purifications were performed on a Berger Automated PrepSFC™ system (Mettler-Toledo, USA) consisting of a Bohdan automated injection/collection robot, Berger SCM-250 (separator control module), Berger ECM-2500 (electronic control module), KNAUER K-2500 UV variable detector, VarianR SD-1 methanol and CO2 delivery systems, JULABOR 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 columns 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. 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

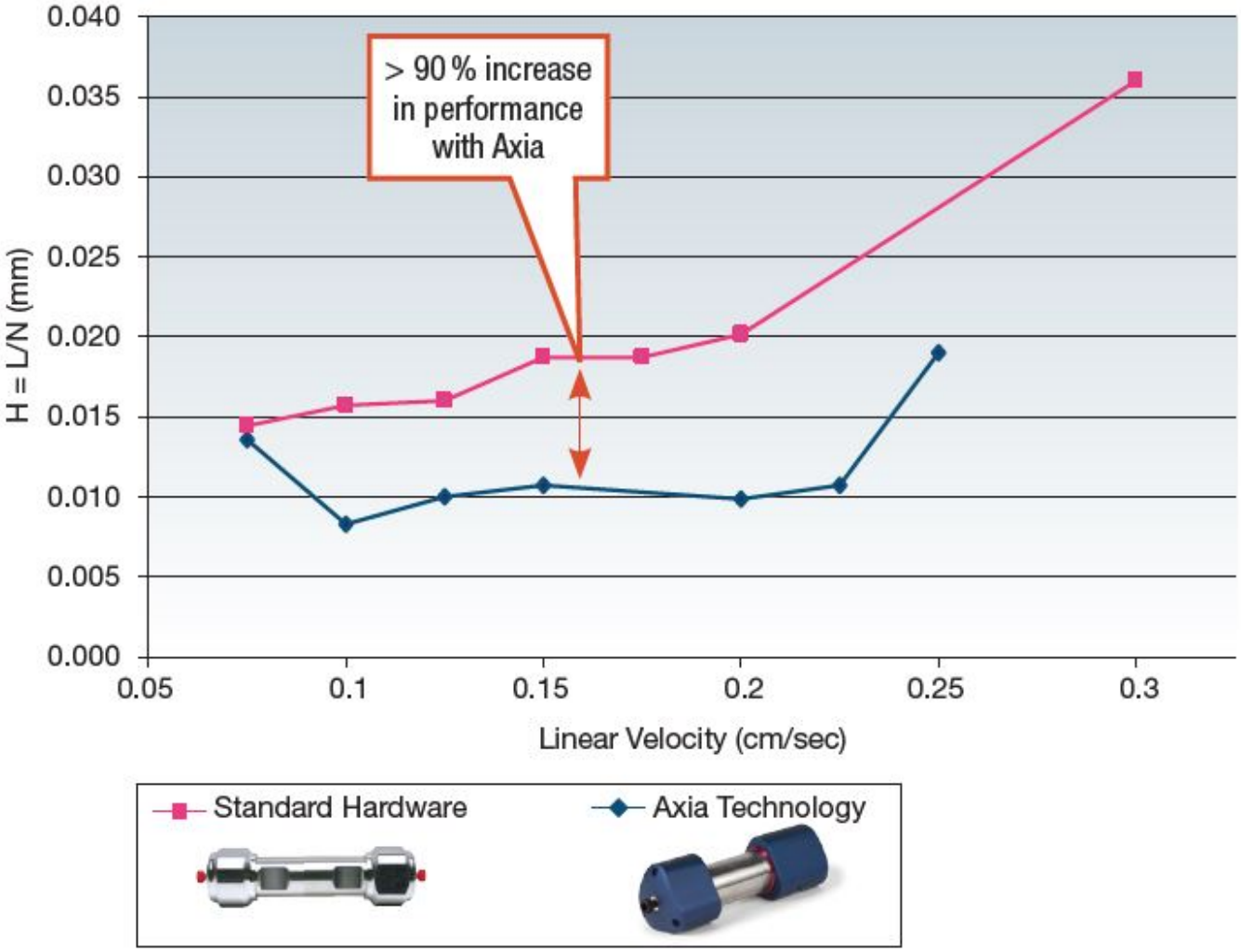
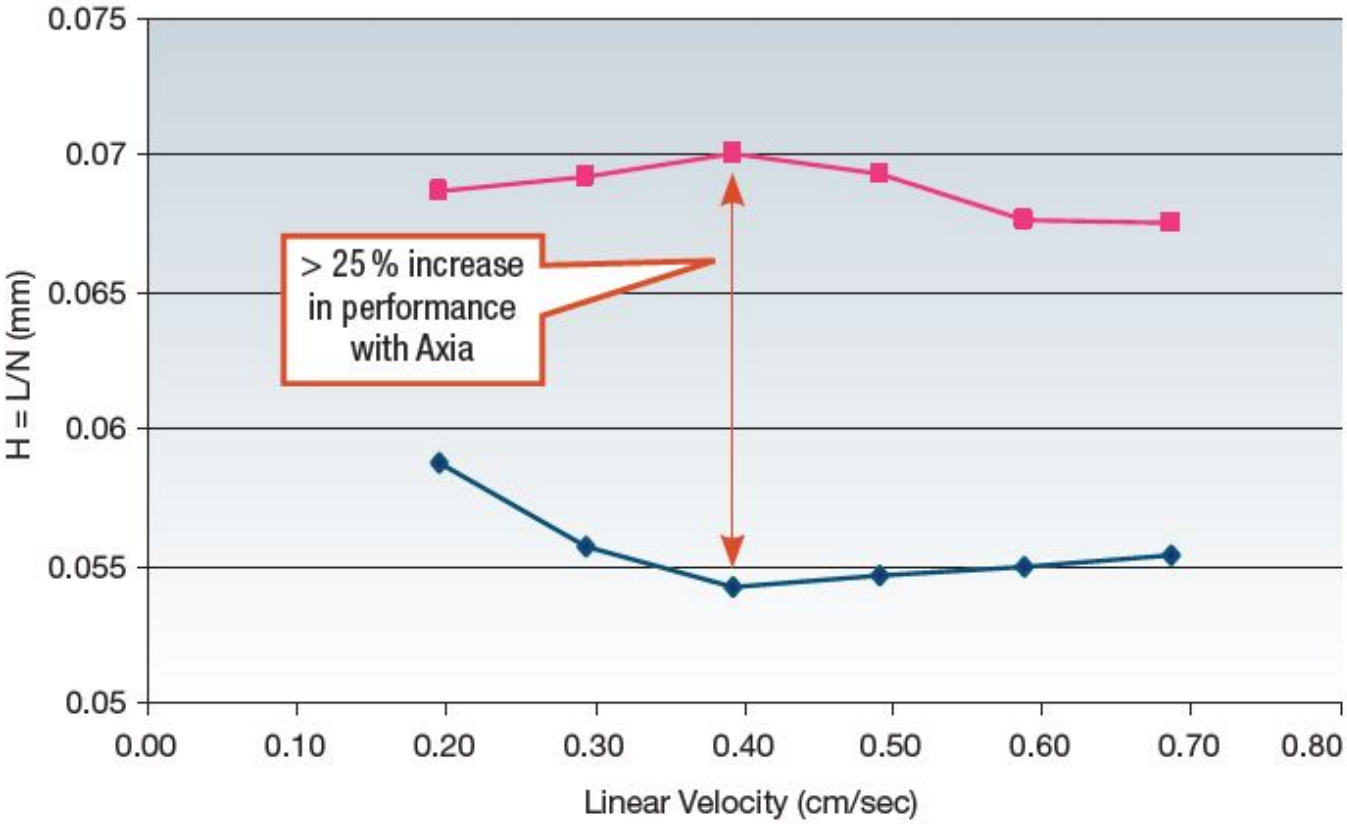


Figure 3. Van Deemter Plots - SFC Mode



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

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 Phase Mode

Column (mm)	Analytical 150 x 4.6	Standard 150 x 21.2	Axia 150 x 21.2
Mass Loaded (mg)	2	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

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.

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Chromatography Separation Newsletter: PURIFY

And if you are looking for more information on chiral or preparative and process scale chromatography, don't forget to sign up for the

North American PhenoPrep Seminar

on October 19th in Princeton, NJ. Discuss innovations and challenges of preparative chromatography and enjoy a lively exchange with industry leading experts. Learn from them how they are tackling challenges, overcoming obstacles, and using new technologies. **Register now**!

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, SPICA 2008

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Summary

Article Name

The Balancing Act—Purification of Chiral Compounds

Description

Dr. J Preston discusses methods, tips, and tools to use to increase throughput for purification of chiral compounds, while comparing SFC and HPLC conditions

Author

Dr. J Preston