Column Diameter's Role in the Performance of Small Scale Preparative Columns

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Abstract

The need for higher purity starting materials has led to the development of high-throughput preparative chromatography as the standard practice in drug discovery laboratories. Maximizing sample load is the goal for chemists preparing starting materials and intermediate compounds. This purification need has resulted in the widespread adoption of open-access systems, utilizing shorter bed length columns (50-100 mm), and operating generic gradients at very high flow rates. This work explores the options available to a chemist to improve throughput by increasing the column diameter or increasing the column length.

Introduction

Silica gel is considered rigid but this porous media compresses under routine slurry packing conditions. **Figure 1** shows the major problem with traditionally slurry packed 21.2 mm and larger diameter columns. At the end of the packing process the system pressure must be released to remove the packing bomb and the media extrudes from the end of the column. One attempt to solve this problem was awarded a patent (**Figure 2**) but this technique still disturbs the packed bed and allows the media to extrude from the column. The operator must scrape off this extruded media and then physically force the packed bed back into the column. The SEM of the inlet frit indicates that this technique results in the media crushing at the frit surface.

To produce stable preparative columns that withstand high flow rates, the pressure on the media must be maintained after the column is packed and never released. In addition, reducing the number of times the bed is disturbed or disrupted improves the reliability and repeatability of the process. The patent pending Axia technology packing process was awarded the R&D 100 award in 2006 for one of the top 100 innovations (**Figure 3**). The Axia packing process produces high bed density without physically crushing the media. The pressure on the piston is never released, the bed is not disturbed and the piston is locked into place before the hydraulic arm is retracted. The force applied to the media is adjusted based on the mechanical strength and porosity of the media, eliminating crushed media and providing more flexibility to pack less rigid media (Figure 4).

Results and Discussion

The Axia micro-process controlled packing technology has now been expanded providing the same performance with increased bed stability for larger diameter columns and also for longer columns. The Axia family of products is shown in **Figure 5** and illustrates that the 21.2 mm columns are now available in 50, 100, 150 and 250 mm lengths. **Figure 6** shows the performance comparison between traditional slurry packed columns and Axia packed 21.2, 30 and 50 mm diameter columns. The Axia packed columns provide higher efficiency, less peak tailing, and improved consistency indicated by the lower percent RSD for each value. **Figure 7** shows how a very difficult Suzuki reaction mixture is easily separated on 4.6, 21.2, 30 and 50 mm diameter columns without loss of resolution. These new Axia packed column sizes allow chemists greater flexibility in column choices to quickly purify larger quantities of compound on shorter columns without sacrificing performance as the column diameter increases.

Flexibility in Media Choices

The Axia packing process is a microprocessor controlled fully automated process that is customized based on the media characteristics.

For isocratic preparative separations such as chiral separations, higher theoretical plates are very important, and this is achieved by increasing the column length, but large porosity media have traditionally been difficult to pack since the media is more fragile. Since Chiralpak® AD columns are typically operated in an isocratic mode, therefore, column length is important. **Figure 8** illustrates how increasing the column length improves resolution - the TSO impurity and the optical isomer are better resolved as the column length increases. Performance is not sacrificed as the column diameter or length increases since the Axia process produced the same high performance for both the 21.2 and 30 mm diameter columns. With Axia packed columns, the chemist can now choose the correct length based on the resolution required and then scale up to the larger diameter for higher throughput.

Added Flexibility for Increased Throughput

To increase throughput (sample load), there are two options—increase column diameter or increase column length. If throughput, instrument time, and laboratory productivity are the critical factors, then increasing the column diameter provides a path to higher loading without sacrificing cycle times (**Figure 9**). Increasing the column diameter does not create higher backpressure and only requires HPLC systems with higher flow rate capability.

The second option to increase sample load is to increase the column length requiring adjustments to the chromatographic conditions. Since backpressure is directly proportional to column length, as the column length increases the backpressure also increases. (Pressure is proportional to L_2/L_1). In **Figure 10** to maintain the same flow rate when the column length increased from 100 mm to 250 mm the backpressure increased by 2.5X. The second adjustment required when changing column length is that the gradient time is increased proportional to the column length to maintain the same gradient slope for both columns. The 100 mm column separation was achieved with a 10-minute gradient and the 250 mm column required a 25-minute gradient. Even for a separation that has only an $\alpha = 1.1$ the sample loaded onto the 250 mm length column was 2.5-3X the load on the 100 mm column. There was no performance or resolution loss with the longer Axia packed column, and the compounds could be purified to the same percent yield and purity value.

Figure 1. Limitations of Conventional Slurry Packing

During disassembly, the packing pressure is released from the column, the bed "relaxes" and extrudes from column. Extrusion occurs inside the packing bomb and continues when the column is removed from the packing hardware. Although the column is capped as quickly as possible this extrusion causes:

1.) Disruption of the packed bed

2.) Reduced packing density

3.) Non-uniform packed column with a lower density at the column inlet

Bed expansion and extrusion is inherent in all slurry packed columns.

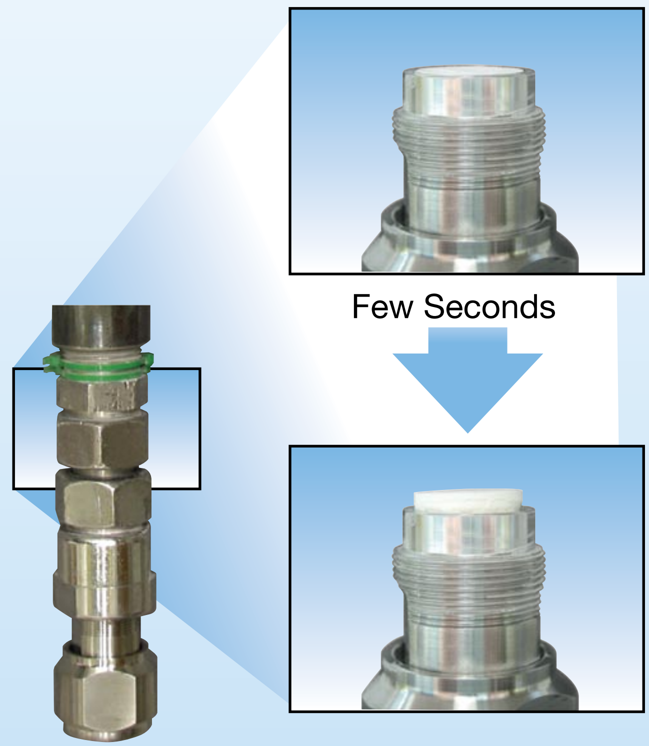
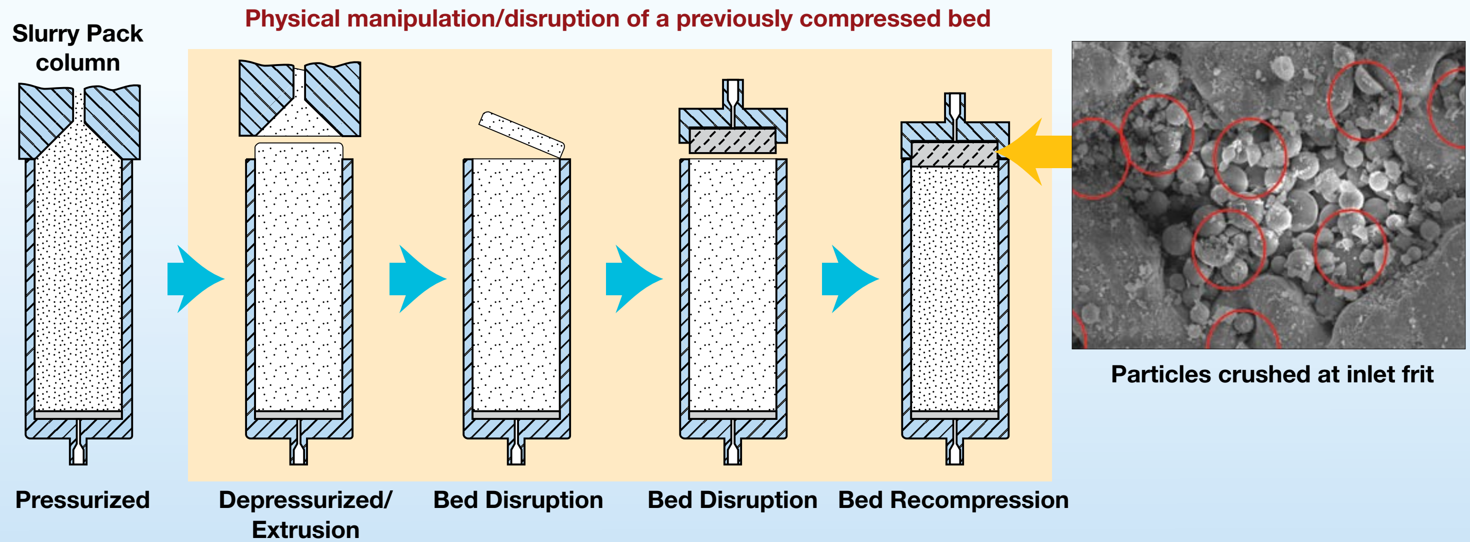


Figure 2. **Modifications of Conventional Slurry Packing**



Waters Corporation, Patent WO 2004/024285 A1

Figure 3. A New Paradigm to Pack Disposable Preparative Columns

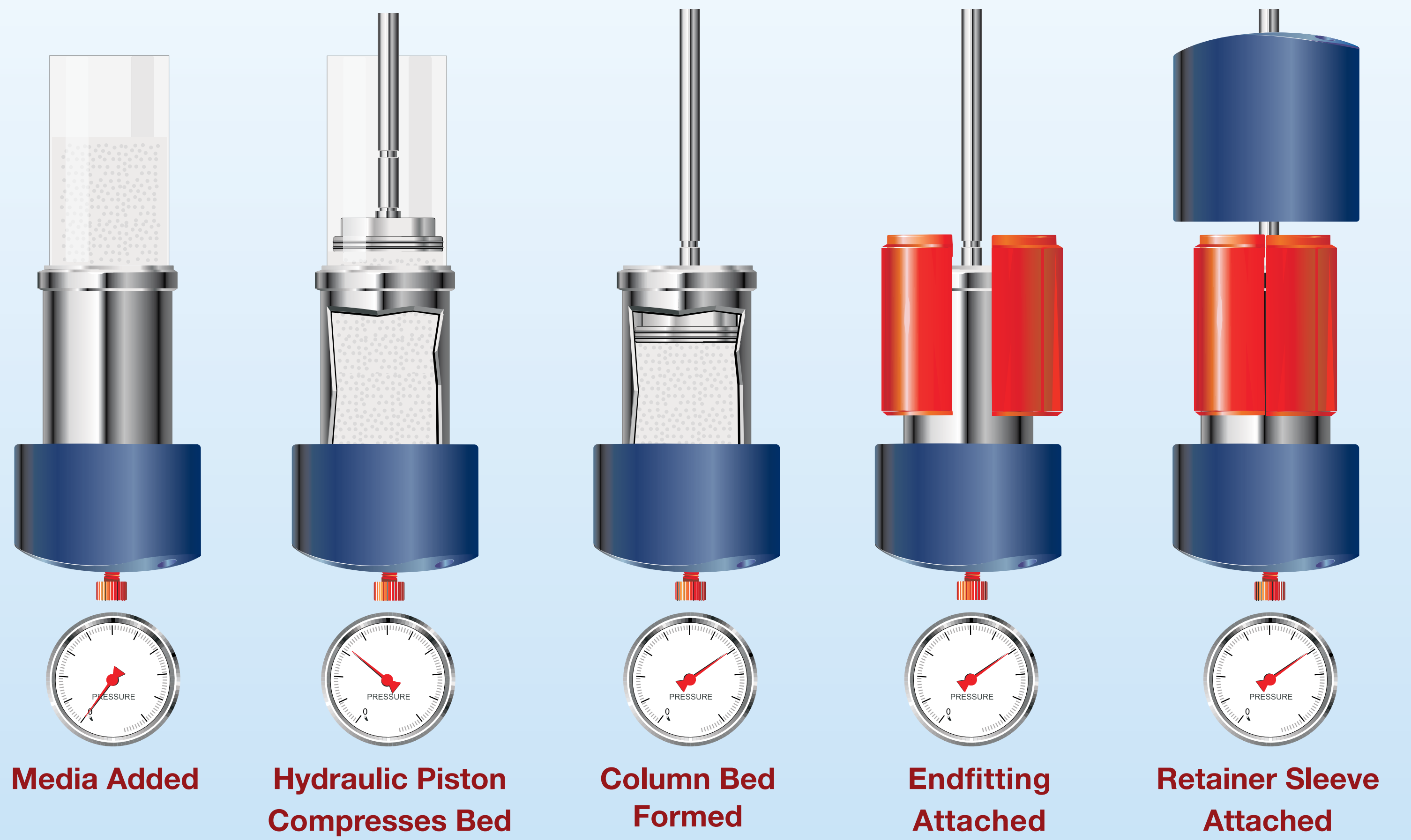
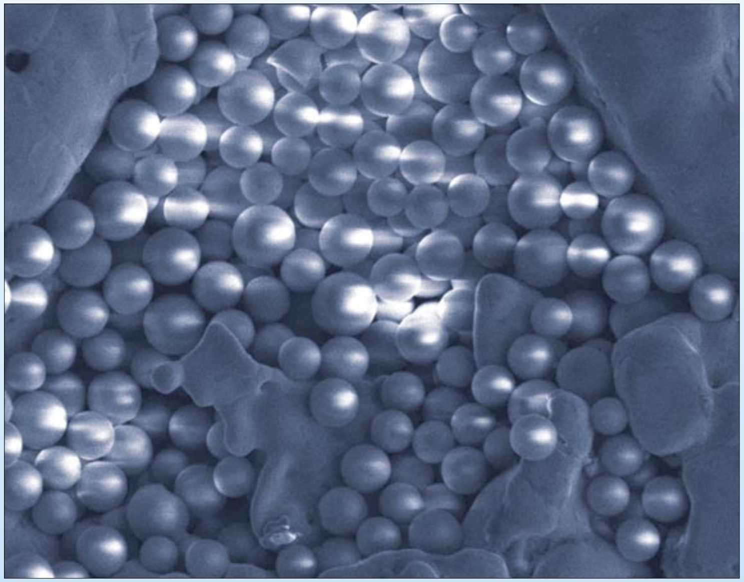


Figure 4. Axia™ Tuned Packing Process Eliminates Crushed Media

- No media crushing
- Automated process tuned for different media based on mechanical strength and porosity
  - Improved process control
  - Infinite tuning of packing density
  - Optimum chromatographic performance based on peak efficiency and asymmetry



*The images are believed to be representative, but individual columns may vary.

Figure 5. Axia™ Column Hardware

Hydraulic Piston Compression Incorporated into Three Diameters

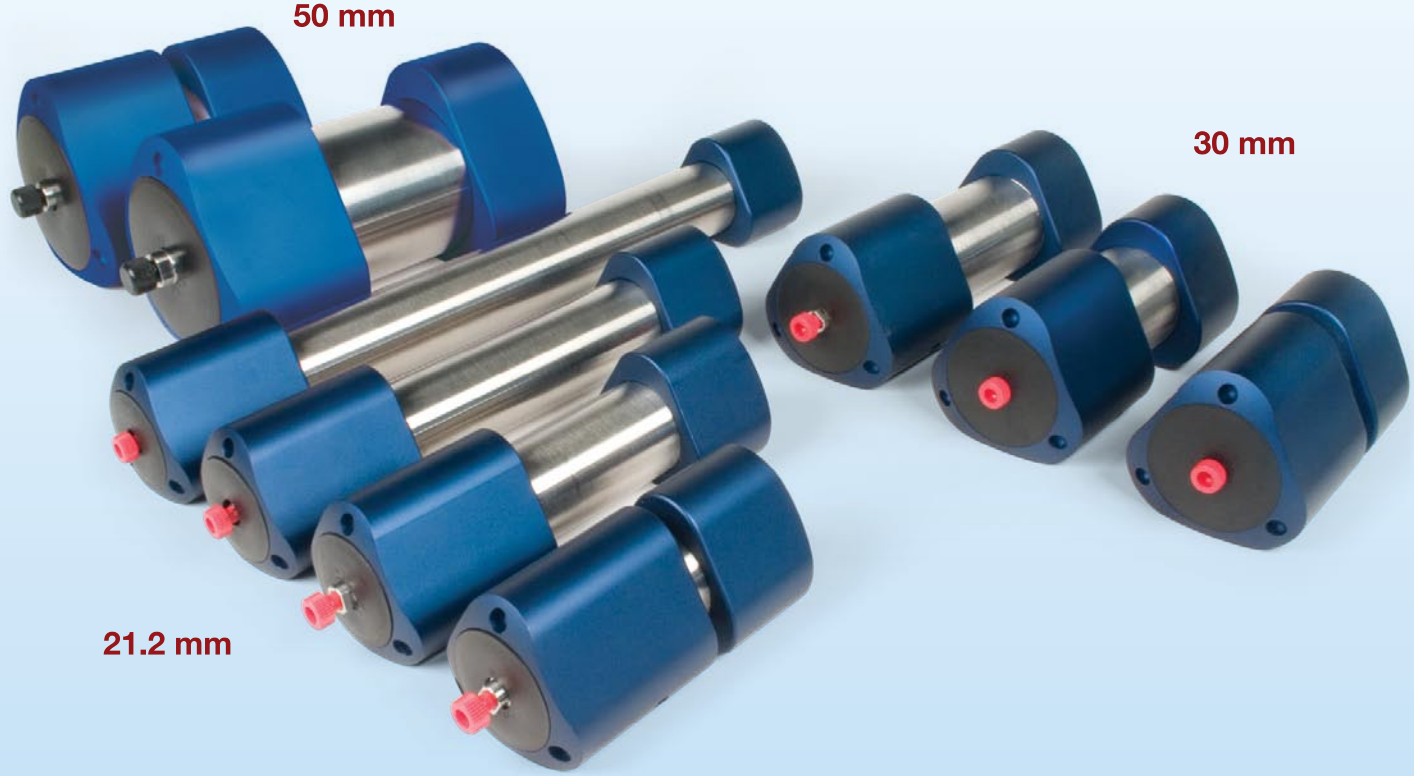
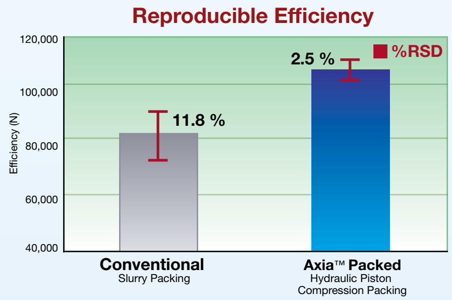
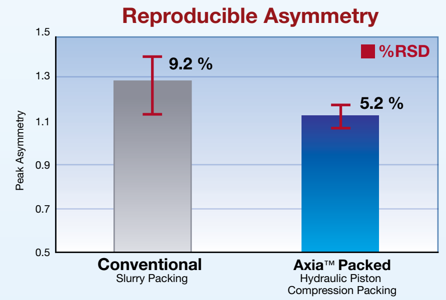


Figure 6. **Improved Performance and Reproducibility for Axia™ Packed Prepartive Columns**

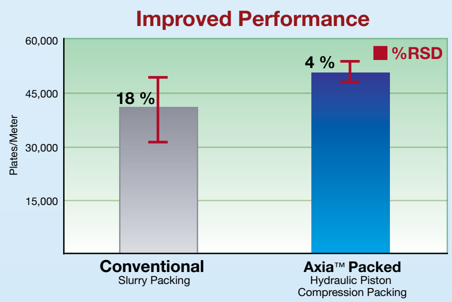
Summary of Eight Different Media in 21.2 and 30 mm Diameter Columns

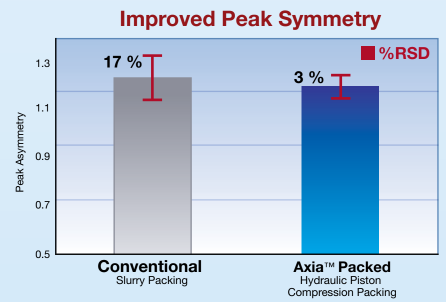




- Average efficiency increased by 27 %
- Peak asymmetry improved by 13 %
- Improved process control yields reproducible columns
  - Efficiency % RSD improved 4x
  - Asymmetry % RSD decreased 2x

Summary of Different 10 μm Media Packed in 50 mm Diameter Columns in Both 50 mm and 100 mm Lengths





- Average efficiency increased by 25 %
- Peak asymmetry improved by 3 %
- Improved process control yields reproducible columns
  - Efficiency % RSD improved 4x
  - Asymmetry % RSD decreased 6x

Figure 7. Suzuki Reaction Purified on 21.2, 30 and 50 mm Diameter Axia™ Packed Columns

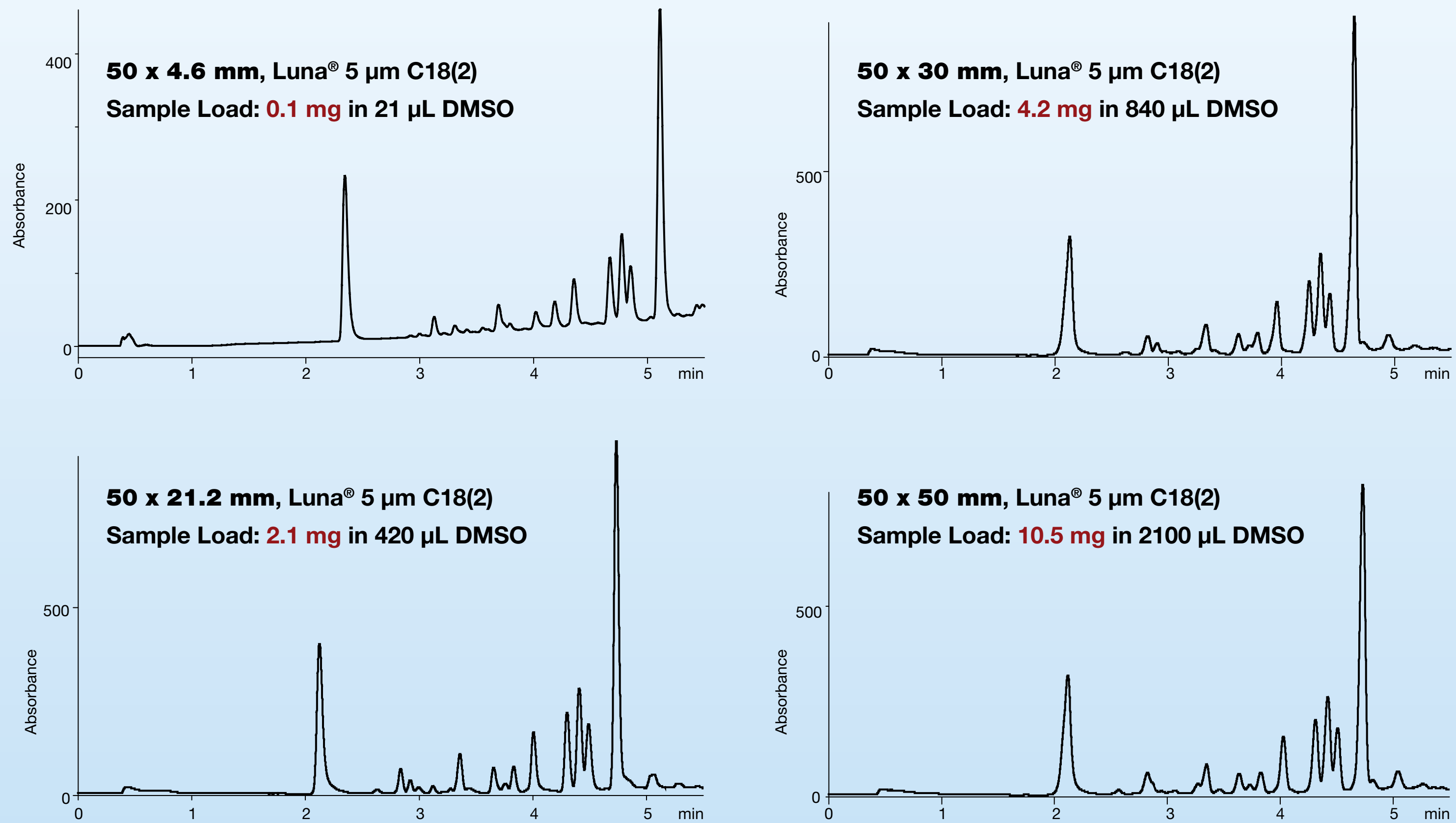


Figure 8. Optimize Performance by Either Column Length or Column Diameter for CSP Media

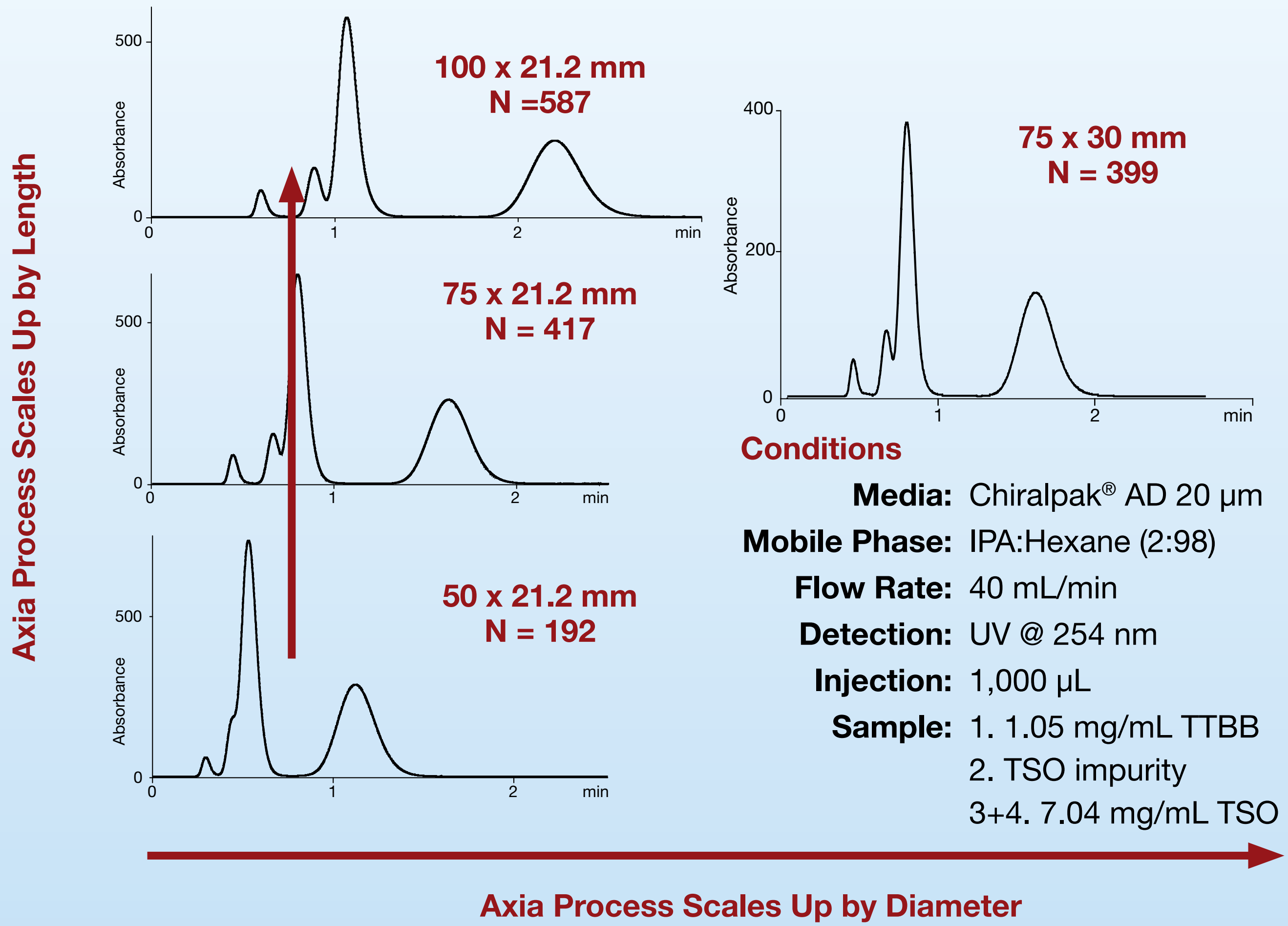
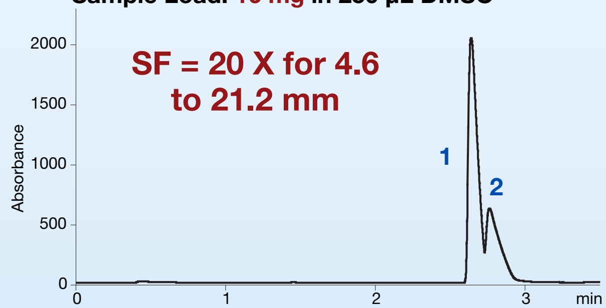


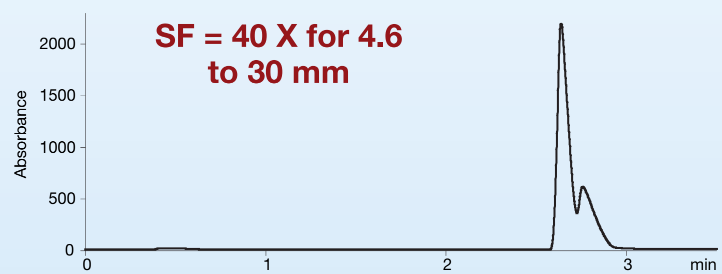
Figure 9. Direct Scale Up Benefits with Axia™ Design

- Axia Packed 21.2, 30 mm and 50 mm diameter columns provide same purification capability and performance

50 x 21.2 mm, Luna® 5 µm C18(2) Axia Packed
Sample Load: 16 mg in 250 µL DMSO



50 x 30 mm, Luna 5 µm C18(2) Axia Packed
Sample Load: 32 mg in 500 µL DMSO



Conditions

Gradient: 5 min, 5 % to 95 % Acetonitrile, 0.5 % TFAA

Flow Rate: 30 mL/min on 21.2 mm column
60 mL/min on 30 mm column
50 mL/min on 50 mm column

Detection: UV @ 254 nm

Sample: 1. Propranolol
2. Diphenhydramine

50 x 50 mm, Luna 5 µm C18(2) Axia Packed
Sample Load: 78 mg in 1250 µL DMSO

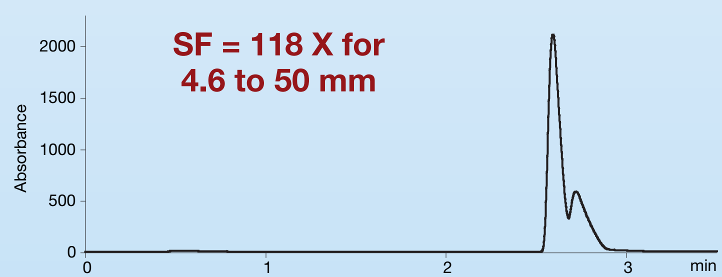
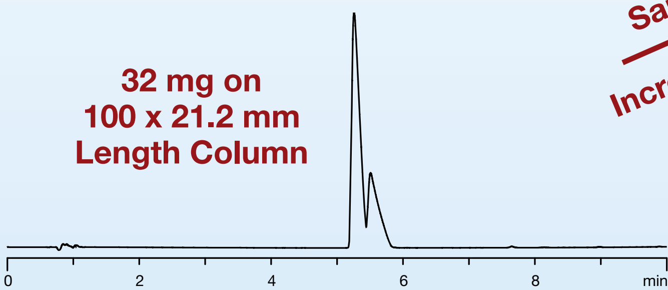
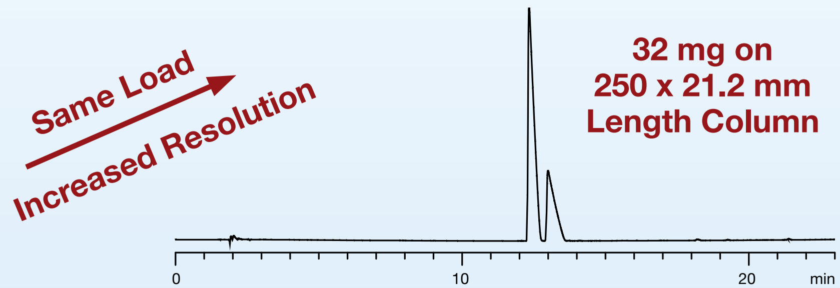
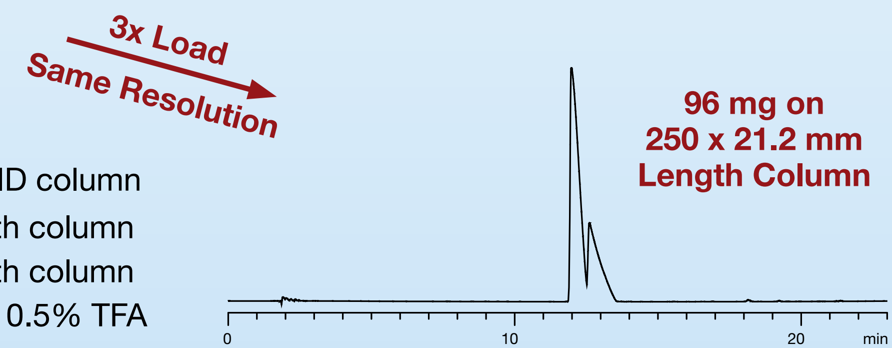


Figure 10. Separation Capability in Longer Axia™ Columns

- Load increases directly proportional to column length but separation time increases, throughput remains constant







Conditions

Flow Rate: 30 mL/min on 21.2 mm ID column

Gradient: 10 min on 100 mm length column
25 min on 250 mm length column
5% to 95% Acetonitrile, 0.5% TFA

Detection: UV @ 254 nm

Sample: 1. Propranolol
2. Diphenhydramine

Conclusions

This work demonstrates the improvement in preparative column performance for Axia packed columns.

1. This new packing process with its high level of process control produces highly efficient, robust 21.2, 30 and 50 mm diameter columns. This work reports the results for a new series of preparative columns providing the chemist the ability to directly scale-up from the 4.6 mm analytical scale column to 50 mm diameter preparative columns that yield 10-250 mg of purified product.

2. The Axia technology is an easily adaptable and controllable process to produce preparative columns for highly compressible polymeric materials as well as fragile larger porosity silica media such as chiral stationary phases (CSP).

3. Axia technology is now also available in 150 and 250 mm lengths for the 21.2 mm diameter columns.

4. The chemist now has the option to increase productivity by increasing the column diameter and/or increasing the column length without sacrificing performance.

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