Improving Preparative Column Lifetime for High-Throughput Open-Access Systems

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Introduction

High speed, high-throughput separations significantly increase stress on the media bed resulting in rapidly decaying column performance. These high-speed chromatographic conditions cause the media bed to compress, shift or channel when subjected to the higher operating pressures and viscous samples. This results in overall shorter column lifetime due to bed failure and physical damage to column components.

For low aspect ratio columns (large diameter and short column length) conventional slurry packing technology produces columns with an improper packing density that is either too low or non-uniform throughout the packed bed. The slurry packing technique has inherent disadvantages and is the major limiting factor in producing consistent, uniformly packed preparative columns. During disassembly of slurry packed columns, a necessary step in the procedure, the packing pressure is released from the column, the bed "relaxes" and the media extrudes from the column. Media extrusion occurs inside the packing bomb and continues when the column is removed from the packing hardware (**Figure 1**). Although the column is capped as quickly as possible, this extrusion causes disruption of the packed bed and reduced packing density, producing a non-uniformly packed column with a lower density at the column inlet.

Axia™ Packed Columns

Axia packed columns use a unique patent pending manufacturing process that completely departs from conventional slurry packing. Axia columns are packed using a computer controlled automated hydraulic compression system to axially compress the bed. Single bed compression is achieved using a new patent pending detachable, lockable piston system. Media is not disturbed or allowed to expand after compression (**Figure 2**). This unique force-transfer design (patent pending) allows the piston to be locked in place and detached after the end of the column is fixed. Once the bed is compressed, the compression force is maintained on the column bed and never released or relaxed, achieving ideal uniform packing density. The Axia pioneering packing process and hardware have proven to produce extremely long lifetimes as illustrated (**Figure 3**). In studies the columns have lasted well over 1000 gradient cycles while being subjected to large volume injections of high viscosity samples dissolved in DMSO.

Failure due to column bed failure has been solved with the Axia technology but these stable columns can still be destroyed by poorly prepared samples. The need for larger quantities of highly purified intermediates and final products has led medicinal and combinatorial chemists to increase mass throughput using smaller diameter columns reducing fraction size and solvent usage. Today's open-access systems often operate preset generic gradient conditions using either pH 2 or pH 10 buffers and the majority of samples are dissolved in 100 % DMSO or DMSO: MeOH. Unfortunately the need for higher productivity does not allow the chemists time to effectively filter the sample prior to loading on the column. **Figure 4** shows the physical damage that has occurred to columns due to inherent particulates, precipitated catalyst, and reaction impurities or by-products from reactions. This contamination significantly shortens the preparative column's lifetime by physically damaging the hardware components causing frits to plug or bend. Frits can be bent due to partial blockage caused by precipitated samples or particulates. The chromatographic performance is compromised by this contamination as shown in (**Figure 4**) where a contaminated frit was inserted into a new column bed and the chromatogram shows multiple

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peak splitting indicating the flow path through the frit was compromised.

Axia SecurityGuard

Column failure due to contaminated frits can be eliminated by the judicious use of a guard cartridge. The new Axia guard column and cartridge is designed to prevent this chromatographic failure (**Figure 5**). The new guard holder is easy to use and the cartridge is conveniently changed without tools. The two halves are ergonomically designed allowing the chemist to easily twist the two parts and insert a new cartridge and then to hand tighten. The new SecurityGuard system will seal against 3000 psi backpressure.

To illustrate how this guard cartridge system combines with the Axia column technology to increase column lifetime, a 21.2 mm column was subjected to extremely high flow rates of 60 mL/min and a mixture of nadolol, metoprolol and propranolol dissolved in DMSO was repeatedly injected. After 240 cycles, the column's chromatographic performance degraded yielding split peaks. Re-testing the column after removing the guard column shows that the preparative column was still good and only the guard cartridge needed replacing. After a total of 520 cycles, the guard cartridge was again contaminated but the preparative column itself still performed as new. This work was repeated one more time with a new guard cartridge but first the sample was filtered. The guard cartridge lifetime was greater than 360 cycles and only the guard cartridge was replaced when the backpressure exceeded the preparative HPLC pressure limit. The guard cartridge did its job—it protected the preparative column from particulates, dust and other debris.

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.

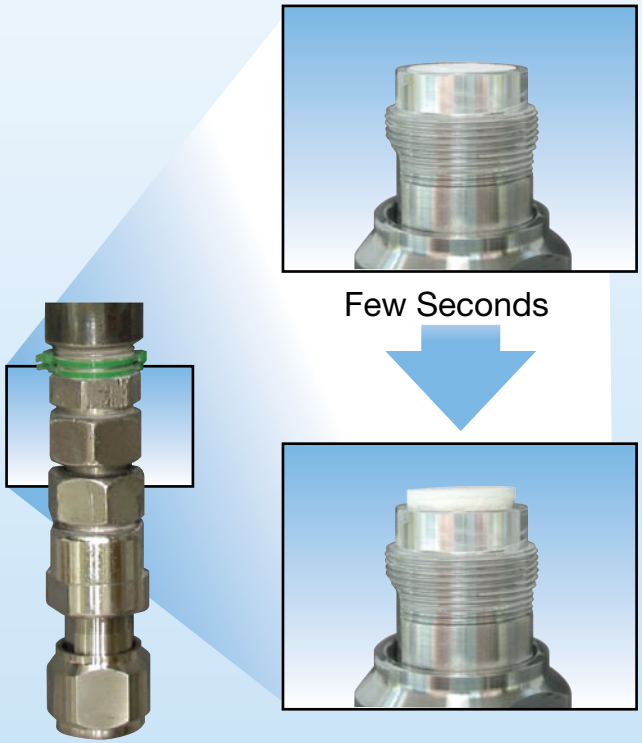


Figure 2. A New Paradigm

Axia™ Hydraulic Piston Compression

- Integrate Axial compression technology into pre-packed short prep columns.
- For low aspect ratio columns, a newly developed, automated hydraulic compression system axially compresses the bed.
- Axia™ packed columns use a unique manufacturing process that completely departs from conventional slurry packing.

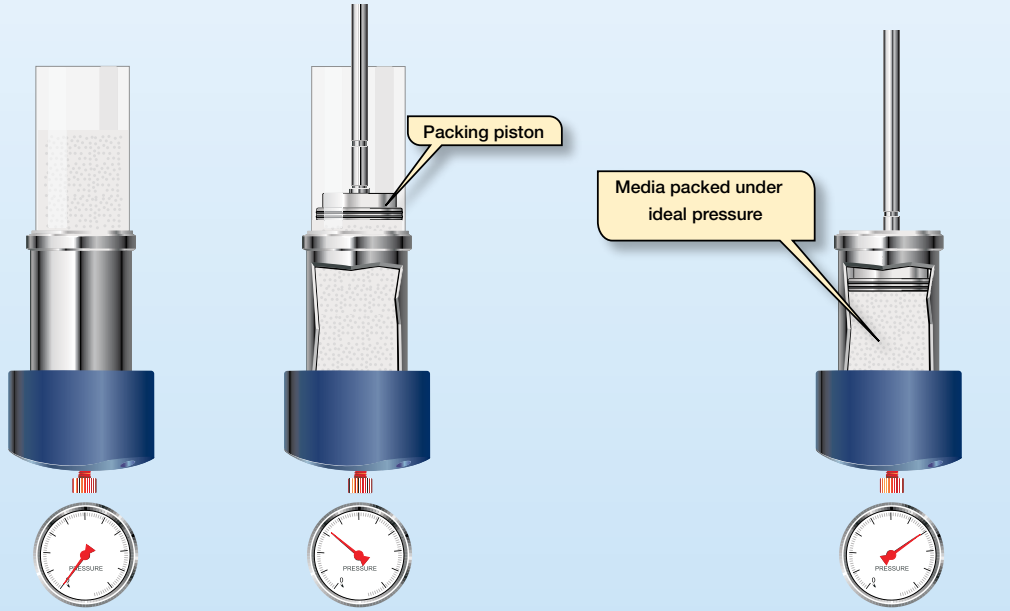


Figure 2. A New Paradigm

Single bed compression is achieved using a new patent pending detachable, lockable piston system. The media is not disturbed or allowed to expand after compression.

This unique force-transfer design (patent pending) allows the piston to be locked in place and detached after the end of the column is fixed (patent pending). Once the bed is compressed, the compression force is maintained on the column bed and never released or relaxed.

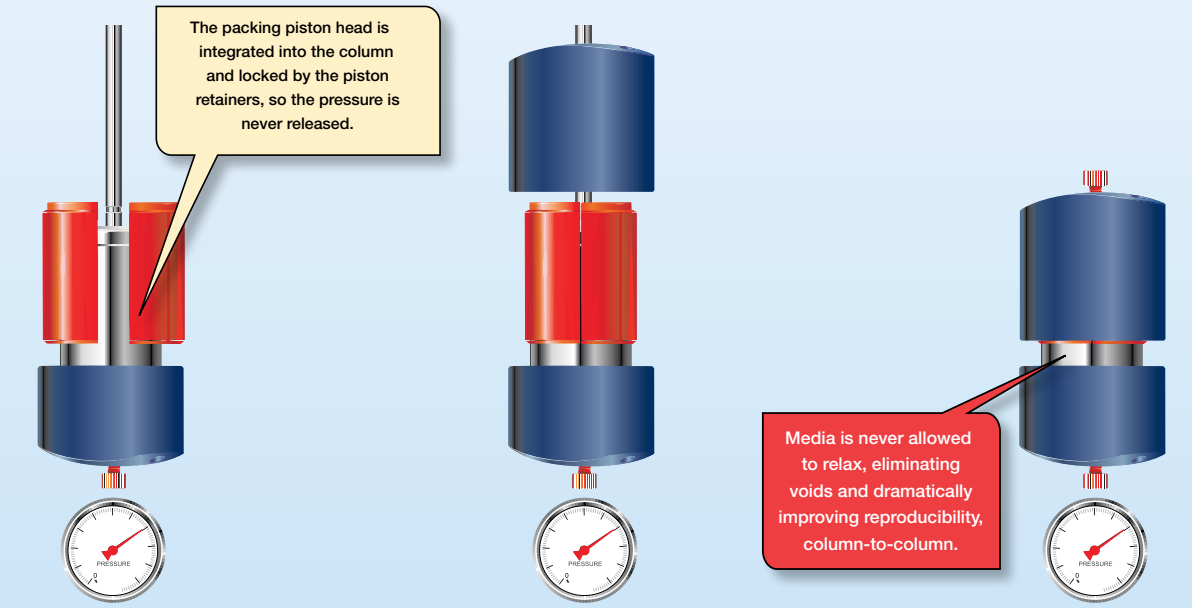


Figure 3. Axia™ Gradient Lifetime Study

Less than 1.5 % change in performance

Column Performance

Cycle 1	Plates	Asymmetry
1	3950	1.19
150	3943	1.19
300	3940	1.22
450	4059	1.14
600	4100	1.15
900	4000	1.12
1040	3888	1.11
1130	3523	1.12

Overlay of 1, 800, 1300 chromatograms

Conditions

Column: Luna® 5µm C18(2) Axia™ Packed
Dimensions: 50 x 21.2 mm
Mobile Phase: A: 0.5 % TFA in Water
B: 0.5 % TFA in Acetonitrile
Gradient: 95:5 (A/B) to 5:95 (A/B) over 5 min
Flow Rate: 30 mL/min
Detection: UV @ 254 nm
Injection Volume: 0.5 mL DMSO
Sample: 1. Tripolidine
2. Methacycline
3. Amitriptyline

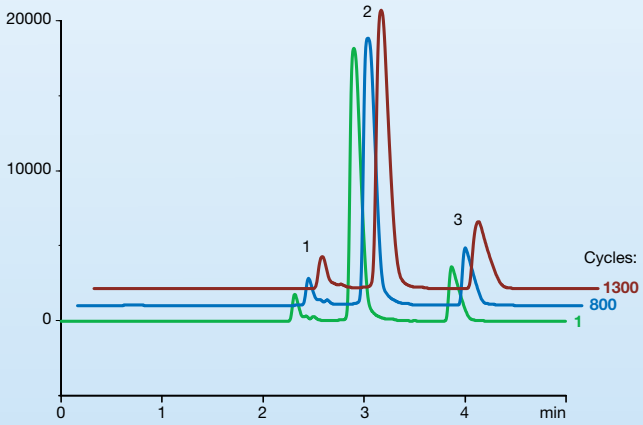
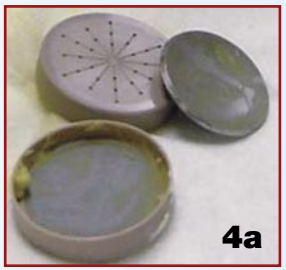
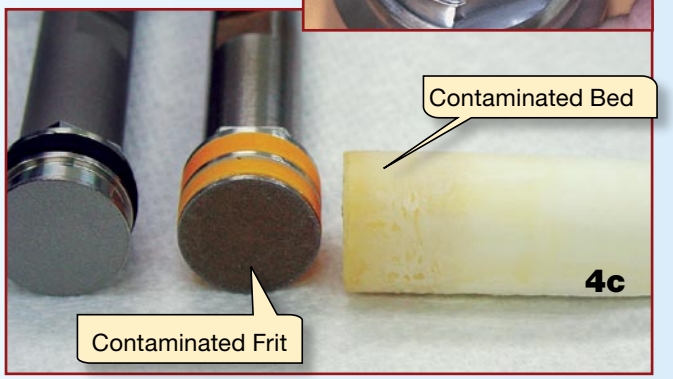
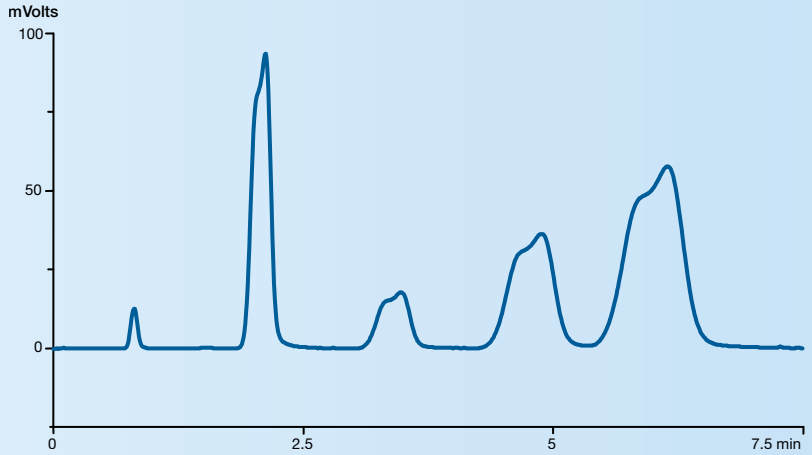


Figure 4. Why Protect Your Axia™ Packed Column from Unpredictable Sample Precipitation Particulates and Highly Viscous Samples?

- Frits were bent due to partial blockage caused by precipitated samples or particulates. **(Figure 4a)**
- Media contamination by irreversibly adsorbed samples and catalysts also lead to lower lifetimes as shown in the second picture. **(Figure 4b)**
- Gross column contamination causes peak splitting and cross contamination between samples **(Figure 4c)**. Chromatogram shows poor column performance due to contamination blocking the frit resulting in peak splitting.
- SecurityGuard™ column protects Axia™ packed column and frits from sample and mobile phase contaminants and extends the column lifetime.







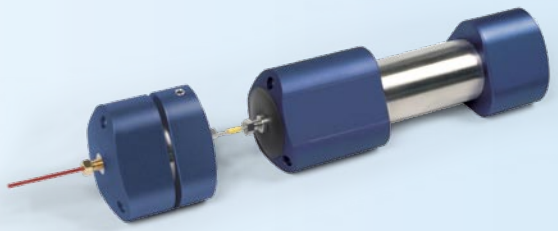


Figure 5. **Protecting Axia™ Packed Columns at Extreme Flow Rates and Pressures**

New Axia SecurityGuard Holder and Cartridge

- Hand tighten, no tools required
- Easily change cartridge in 30 seconds
- Guard column protects against precipitated samples, particulates, and media contamination
- **21.2 mm and 30 mm diameter holders and cartridges now available**

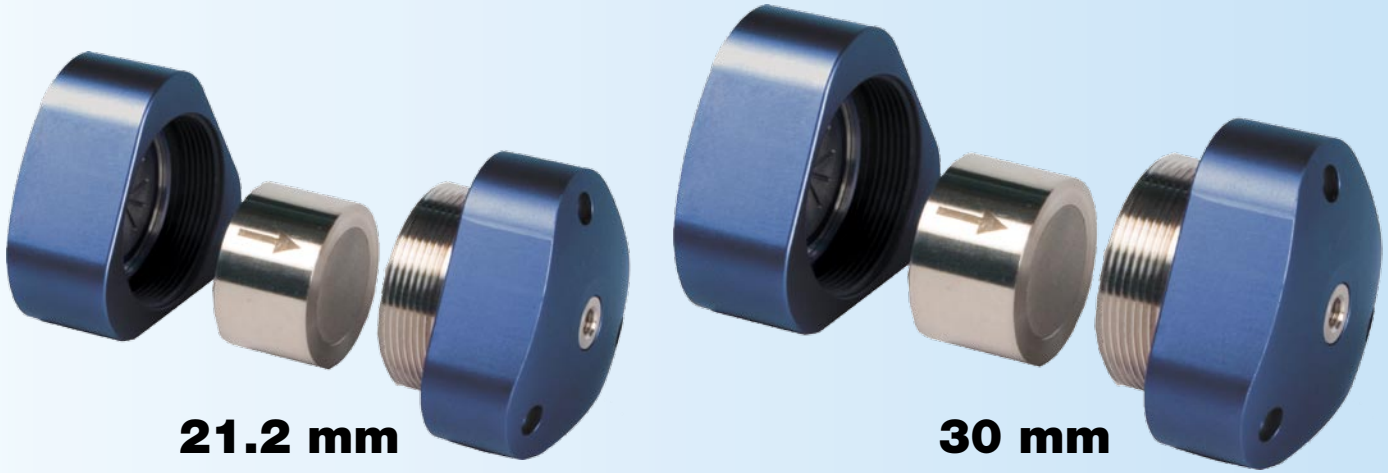


Figure 6. Lifetime of 50 x 21.2 mm Axia™ Packed Columns at Extreme Flow Rates and Pressures, 60 mL/min Flow Rate

Lifetime Test Conditions

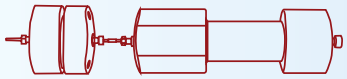
- 9 isocratic cycles with 75:25 Acetonitrile/Water with 0.1 % TFA
- **Flow Rate: 60 mL/min**
- 1 mL DMSO injections made each cycle
- Every 10th cycle separate 1) nadolol, 2) metoprolol and 3) propranolol dissolved in DMSO under gradient conditions

Conditions

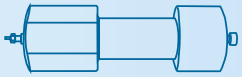
Injection Volume: 400 µL (34 mg)
Gradient: 1 minute initial hold at 98:2 Water/Acetonitrile with 0.1 % TFA, 5 minutes to 25:75 Water/Acetonitrile with 0.1 % TFA

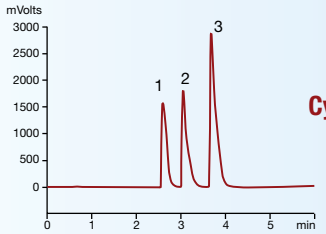
Flow Rate: 60 mL/min
Detection: UV @ 270 nm
Sample Dissolved in 15 mL DMSO
1. Nadolol (524 mg)
2. Metoprolol (516 mg)
3. Propranolol (276 mg)

Axia Packed Column with guard

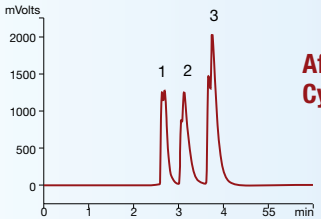


Axia Packed Column Tested without guard

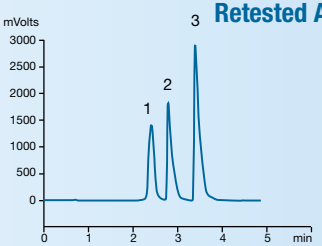




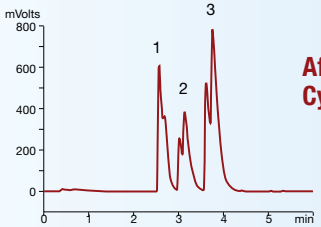
Cycle 1



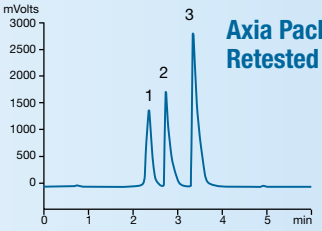
After Cycle 240



Axia Packed Column Retested After 240 Cycles



After Cycle 520



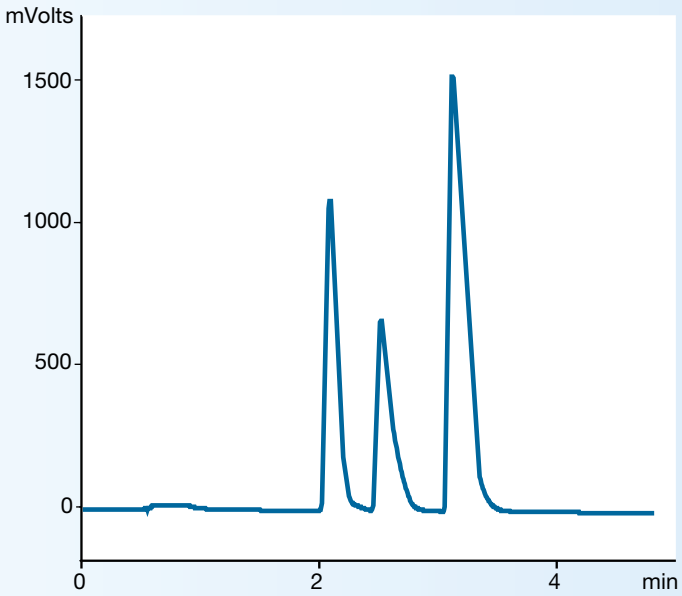
Axia Packed Column Retested After 520 Cycles

Figure 7. Luna® C18(2) 10 µm Axia™ Column Performance Comparison after 200 and 360 Cycles at 60 mL/min with Guard Column Attached

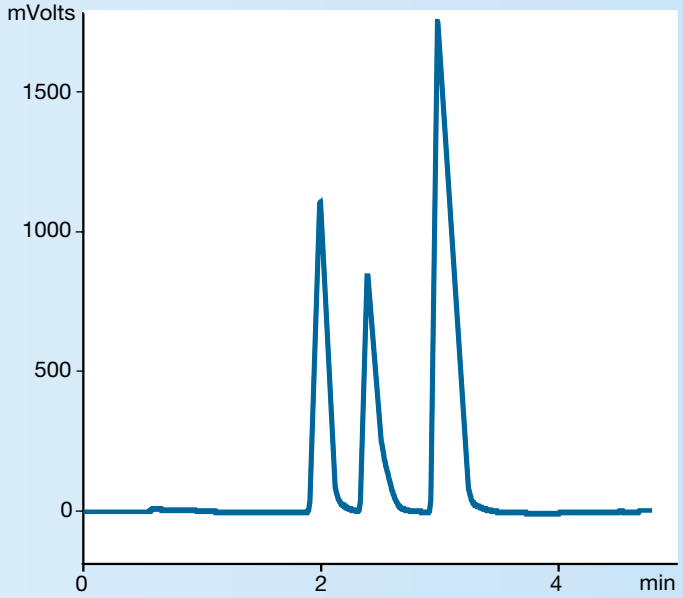
Even at extremely high flow rates (60 mL/min) the 21.2 mm preparative SecurityGuard™ column protected the Axia packed column for over 360 cycles from particulates, dust, precipitation and pneumatic shock.

After 360 cycles, particulates built up on the SecurityGuard™ cartridge causing high backpressure that exceeded the preparative HPLC system limit, but there is no degradation of the preparative column itself as shown in the chromatograms.

Initial Separation



After 360 Gradient/Isocratic Cycles



Conclusion

This work demonstrates the improvements in column performance achieved by changing the paradigm for packing preparative columns. Axia incorporates a computer controlled packing process using axial compression, that eliminates the disruption or expansion of the packed bed and produces an ideal packing density throughout the column. Column voiding or channeling is eliminated as a source of column failure. A guard column is recommended for open-access systems to maintain the overall preparative column performance by eliminating contamination or fouling due to precipitated samples or particulates. Results from operating the Axia™ columns under extreme flow rates demonstrated that the column bed withstands and maintains its performance under high linear velocities and pressures.

In this work we have also shown how the new Axia SecurityGuard system combined with the Axia™ preparative column provides a complete solution to improving column lifetime. The Axia SecurityGuard system protects the preparative column from particulates, reduces premature column failure, and significantly extends column lifetime. This work demonstrates the dramatic increase in column lifetime achievable with this easy to use guard column system even when operating the columns at extremely high flow rates.

Since chemists using open-access systems have seen a dramatic increase in column lifetime using the 21.2 mm preparative SecurityGuard system, this technology has now been scaled up for use with the 30 mm Axia™ preparative columns. This new holder and cartridge have 2x the capacity compared to the 21.2 mm cartridge and are designed to protect the 30 mm diameter Axia™ packed columns from particulates, catalysts, and precipitated samples.