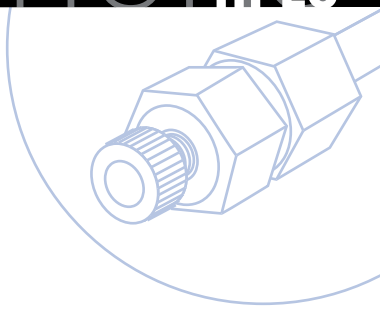

Improving Preparative HPLC Column Lifetime in High-Throughput Open-Access Purification Environments

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Introduction

Drug Discovery labs today are under pressure to produce larger amounts of purified drug intermediates and final product while reducing fraction size and solvent usage. This requirement has led to the widespread adoption of low aspect ratio preparative HPLC columns (large internal diameters for increased loading, shorter lengths for speed and reduced solvent usage) on open-access systems operated using generic gradients at very low or high pH, the majority of samples dissolved in 100 % DMSO or 50:50 DMSO:MeOH. With very little sample preparation, and under high speed chromatographic conditions, the media bed can compress, shift, or channel when subjected to viscous samples containing catalysts and reaction impurities or by-products, resulting in rapidly decaying column performance that can often result in system shut down and incomplete runs. A new preparative column packing process and hardware design, used in conjunction with a reliable, easy-to-use guard column can improve column lifetime and productivity.

Experimental Conditions

Gilson 845Z Preparative HPLC System

Column:	Luna® 5 µm C18(2) Axia packed
Dimensions:	50 x 21.2 mm
Mobile Phase:	A = 0.1 % TFA in Water
	B = 0.1 % TFA in Acetonitrile
Gradient:	1 minute at 98:2 (A/B), to 25:75 (A/B) over 5 minutes
Flow Rate:	60 mL/min
Detection:	UV @ 270 nm
Injection Volume:	400 µL (34 mg)
Sample:	Dissolved in 15 mL DMSO
	1. Nadolol (524 mg)
	2. Metoprolol (516 mg)
	3. Propranolol (276 mg)

Results and Discussion

The solution to producing mechanically stable preparative columns, which withstand high flow rates, fast gradients, and viscous DMSO injections is to ensure the packing pressure on the media is maintained after the column is packed and never released. Single bed compression is achieved using the new patent pending detachable, lockable piston system. This unique force-transfer design allows the piston to be locked in place and detached from the hydraulic ram after the end of the column is fixed. Compression force is maintained on the column bed and never released or relaxed. The media is not disturbed or allowed to expand after compression. The Axia™ packing technology eliminates post-packing manipulation including bed disruption, resulting in higher density, and more uniform, packed beds compared to conventional slurry packed preparative columns. While column failure due to column bed compression, shifting, or channeling has been solved with the Axia technology, the performance of these stable columns can still be compromised by poorly prepared samples.

Unfortunately the need for faster sample throughput does not allow chemists time to effectively filter their samples or perform more extensive sample cleanup prior to loading on the column. **Figure 1** shows the physical damage that has occurred to columns due to inherent particulates, precipitated catalyst, and reaction impurities or by-products. This contamination significantly shortens the lifetime of the preparative column by contaminating the column packing and physically damaging the hardware components causing frits to plug or bend. **Figure 2** shows the effect of a contaminated frit on chromatographic performance – the multiple peak splitting indicates the flow path through the frit was compromised.

This type of contamination will result in column failure which can be eliminated by the judicious use of the new SecurityGuard™ PREP guard cartridge system **Figure 3**. The new guard holder has been ergonomically designed for ease of use, the chemist easily inserts the guard cartridge and hand tightens the two ends together with no need for additional tools. The new SecurityGuard PREP system provides a leak-free seal up to 3000 psi backpressure.

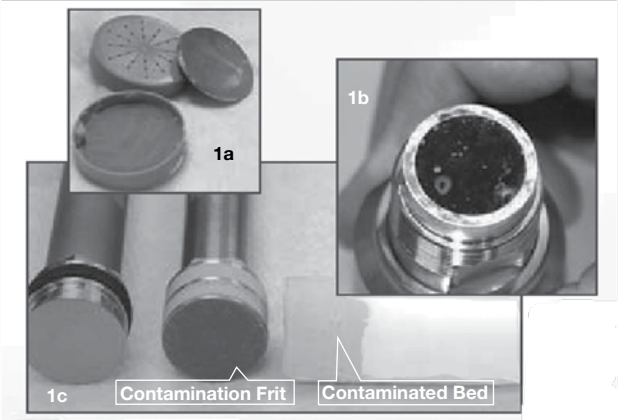
To illustrate how this guard cartridge system combines with the Axia™ column technology to increase preparative column lifetime, a 50 x 21.2 mm column was subjected to extremely high flow rates of 60 mL/min and a mixture of nadolol, metoprolol and propranolol dissolved in DMSO was repeatedly injected **Figure 4**. After 240 cycles, the chromatographic performance degraded yielding split peaks. Retesting the column after removing the guard column shows 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 **Figure 4A**.

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 **(Figure 4)**. This demonstrates how simply filtering the sample prior to injection can contribute to extended lifetime of the guard cartridge – in this example by 50 %. Both experiments clearly demonstrated that the guard cartridge had done its job by protecting the preparative column from particulates, dust and other debris, and pneumatic shock.

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.

Figure 1: Physical Damage to Prep Columns In a High-Throughput Environment



- Frits shown were bent due to partial blockage caused by precipitated samples or particulates. Figure 1a
- Media contamination by irreversibly absorbed samples and catalysts also leads to lower lifetimes as shown in Figure 1b.
- Gross column contamination causes peak splitting and cross contamination.

Figure 2: Chromatogram shows the impact a blocked frit has on column performance.

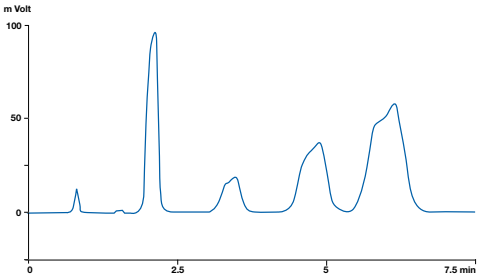
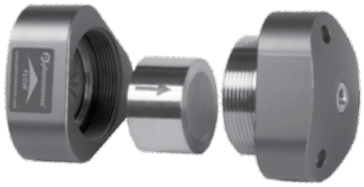
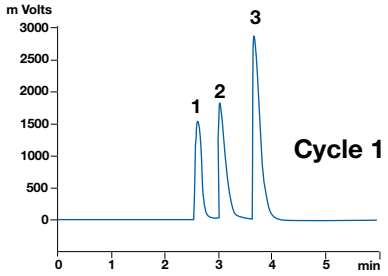


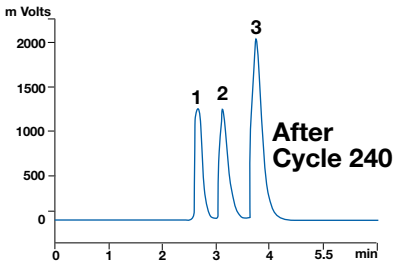
Figure 3: SecurityGuard PREP Holder and Cartridge



21.2 and 30 mm diameter holders and cartridges available to protect columns.

Figure 4: Axia Packed Column with Guard





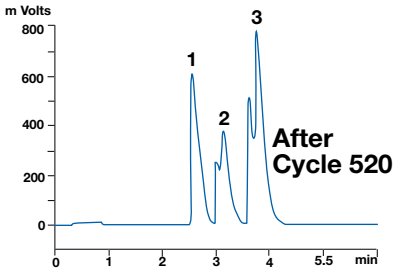
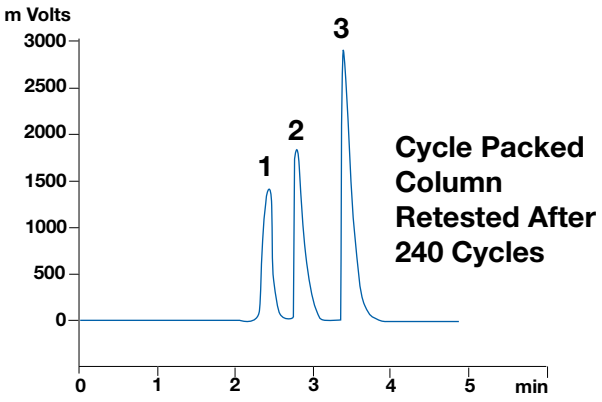
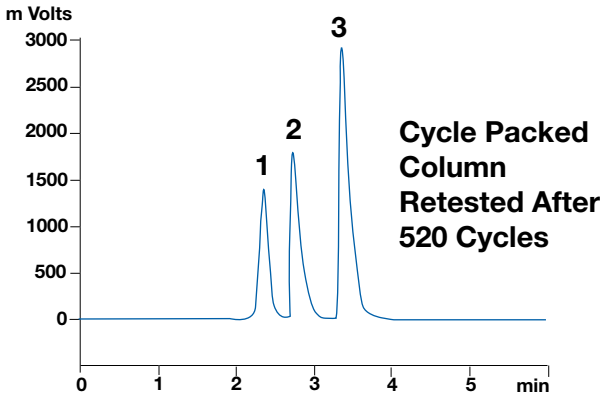


Figure 4A: Axia Packed Column tested without Guard

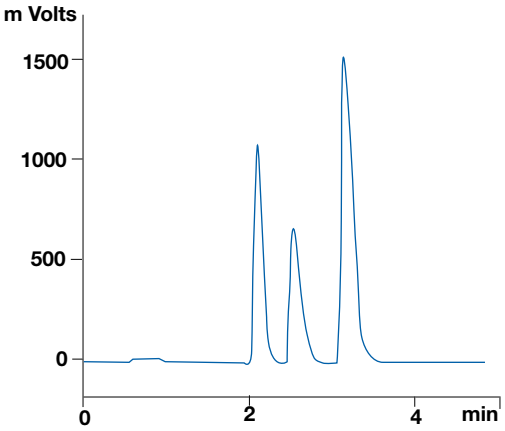


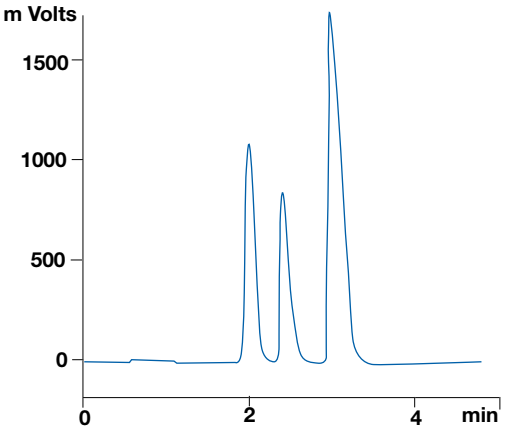


- 9 isocratic cycles with 75:25 Acetonitrile/Water with 0.1 % TFA
- Flow Rate: 60 mL/min
- 1 mL DMSO injections made each cycle
- Every 10th cycle separate sample dissolved in DMSO under gradient conditions

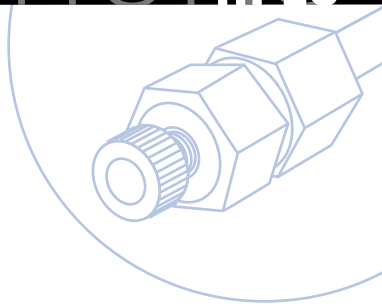
Peak assignments:
1. Nadolol
2. Metoprolol
3. Propranolol

Figure 5: Luna® C18(2) 5 µm Axia™ Column Performance Comparison after 200 and 360 Cycles at 60 mL/min with SecurityGuard PREP Attached





- Preparative SecurityGuard column (21.2 mm diameter) protected Axia packed column for over 360 cycles
- Protects column from particulates, dust, precipitation and pneumatic shock
- At 360 cycles, backpressure exceeded preparative system limit due to particulates build up on SecurityGuard column. It did its job!
- No degradation of preparative column performance



Conclusions

While Axia packed column technology has been shown to eliminate column voiding or channeling as a source of column failure, it cannot prevent column failure due to contamination by the sample. A guard column is recommended for use in both open-access and standard preparative environments to maintain the overall preparative column performance by eliminating contamination or fouling due to precipitated samples or particulates.

The SecurityGuard PREP 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 in conjunction with Axia packed preparative columns - even when operating the columns at extremely high flow rates. Results shown here clearly demonstrate that the use of the SecurityGuard PREP system can improve preparative column lifetimes even under the high flow rate, high-pressure conditions used in high-throughput, open-access preparative HPLC systems.

Ordering Information

Part No.	Description
00B-4252-P0-AX-TN	Luna 5 µm C18(2) Axia Packed column, 50 x 21.2 mm
AJ0-8223-TN	SecurityGuard Holder Kit for 21.2 mm ID cartridge
AJ0-7839-TN	SecurityGuard Cartridge, C18, 15 x 21.2 mm

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