# Optimization of Preparative Purification Using Axia™ for Increased Purity, Yield, and Productivity

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*Axia column technology provides the same high efficiency chromatographic performance for preparative scale columns (21.2, 30, and 50 mm ID) as obtained in 4.6 mm ID analytical columns. This improvement in preparative column performance across all lengths and internal diameters makes it easier for chemists involved in compound purification to select the appropriate column size to achieve the desired purity and yield without having to compromise on performance.*

## Introduction

In recent years the trend to maximize throughput has resulted in the adoption of shorter bed length preparative columns (50 – 100 mm) operated on open access systems using generic gradients as a standard approach to purify intermediates and final products. As these compounds continue their development cycle, greater quantities of purified material are required, resulting in the need for larger scale preparative separations. Depending on the quantity of material required, either a laboratory chemist or the process chemist performs the purification. When purifying larger quantities, each lab has different requirements for purity and yield, as well as different limitations in their preparative HPLC equipment that influences their decisions to change the column length and inner diameter (ID). This technical note explores the impact column length and ID have on purity and yield when purifying larger quantities of these compounds.

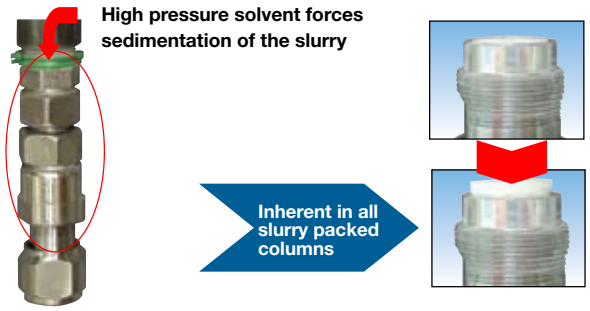
## Materials and Methods

All preparative separations were performed on a Gilson GX281 Preparative HPLC System that includes the Binary 333 and 334 HPLC pumps and a UV/VIS 155 variable wavelength detector. Trilution LC Software version 1.4 was used for the data analysis. The analytical analyses were performed using an Agilent 1100 LC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a quaternary pump, in-line degasser, multi-wavelength detector, and autosampler. HP Chemstation software (Version A.09.01) was used for the data analysis. The HPLC columns used were Luna® 5 µm C18(2) 50 x 4.6 mm, 50 x 21.2 mm, 50 x 30 mm, 50 x 50 mm (Phenomenex, Torrance, CA, USA). The mobile phase used in these separations was aqueous mobile phase 0.5 % TFA in Water and organic mobile phase was 0.5 % TFA in Acetonitrile. The gradient used was 5 % to 95 % B over 5 min with flow rates of 1.5 mL/min on 4.6 mm ID column, 30 mL/min on 21.2 mm ID column, 60 mL/min on 30 mm ID column, and 150 mL/min on 50 mm ID column. The UV detector was set at 254 nm. HPLC grade acetonitrile and water were obtained from Fisher Scientific (Springfield, NJ, USA). For purification samples a Suzuki Reaction Mixture was generously provided by Dr. Shahnaz Ghassemi from Biotage. Another sample was Propranolol and Diphenhydramine (Sigma Chemical, St. Louis, MO, USA). Fractions from preparative separations were analyzed using a Luna C18(2) 5 µm 50 x 4.6 mm column.

## Results and Discussion

Historically HPLC column performance has decreased as the column ID increased even though the same media was packed into the columns. Analytical columns always exhibited high efficiency and good peak symmetry but the preparative columns generally showed lower efficiency and greater peak tailing. This loss in performance is inherent in all slurry packed columns and was caused by the media extruding from the packed bed while the final hardware was assembled (**Figure 1**).

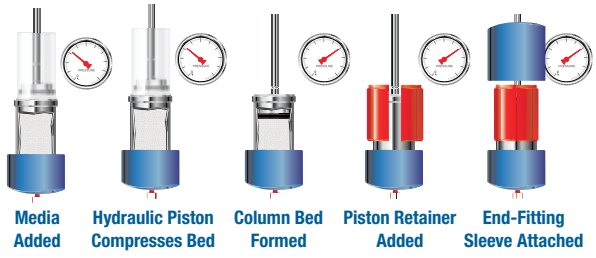
**Figure 1.**
Limitations of Conventional Slurry Packed Preparative Columns




After packing, the solvent pressure is released to remove the column from the packing station and the media extrudes from the column.

A major improvement in preparative column performance has been achieved by adapting axial compression to manufacture laboratory scale preparative HPLC columns. A computerized mechanical process packs the column bed and the media is never allowed to expand or extrude from the column and the internal packing force is maintained on the column (**Figure 2**). This new technology produces higher performance preparative columns yielding the same plates per meter independent of length and ID. This new technology was granted the R&D 100 award in 2006 for the innovation resulting in the Axia™ product line. Axia has recently been extended to include additional column lengths resulting in all 50, 100, 150 and 250 mm length columns being made in 21.2, 30 and 50 mm IDs.

**Figure 2.**
Axia™ Packing Process

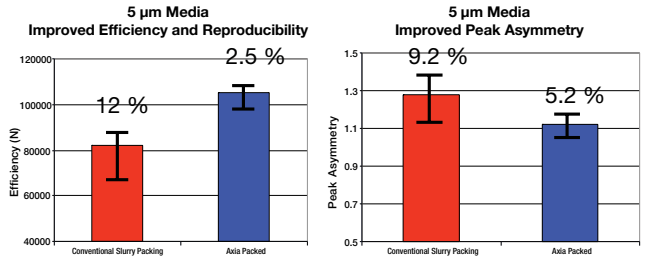



Axial compression technology is incorporated into manufacturing pre-packed short prep columns. Once the column is formed, the column hardware is fully assembled leaving the piston in place and the media fully compressed before the pneumatic ram is removed.

The results from packing four different types of media via the Axia packing process are shown in **Figure 3**. The improved performance for 21.2, 30 and 50 mm ID columns graphically illustrates the increased efficiency (>15 %), improved reproducibility in efficiency from column to column (% RSD decreased by 4X), with peak asymmetry reduced by 2-6 fold.

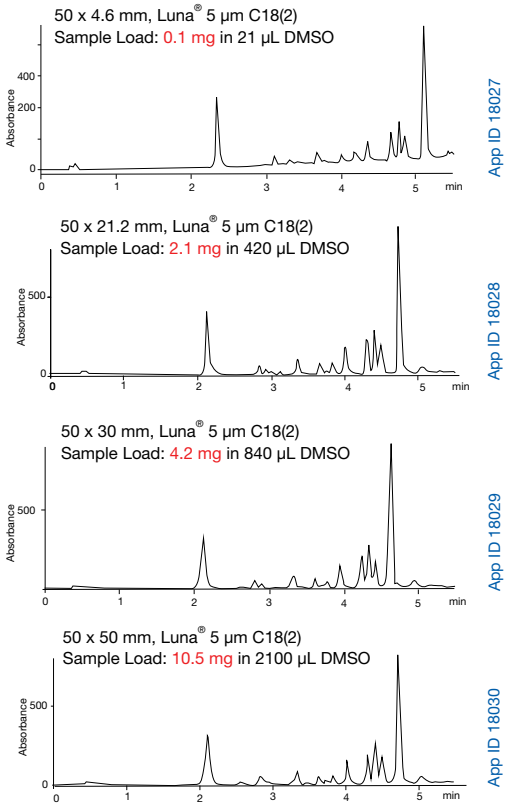
To illustrate that the column performance is independent of the column ID, the same Suzuki reaction mixture was separated on 4.6,

**Figure 3.**
Axia™ Packing Process




21.2, 30 and 50 mm ID columns (**Figure 4**). These chromatograms show the same high efficiency separation is achieved on the analytical (4.6 mm ID) and on the Axia packed 21.2, 30, and 50 mm ID columns. No loss in performance is observed as column ID is increased. Since these new preparative columns have the same plates/meter (efficiency and asymmetry factors) independent of ID and length, the chemist has more options to scale up a separation without sacrificing purity or yield.

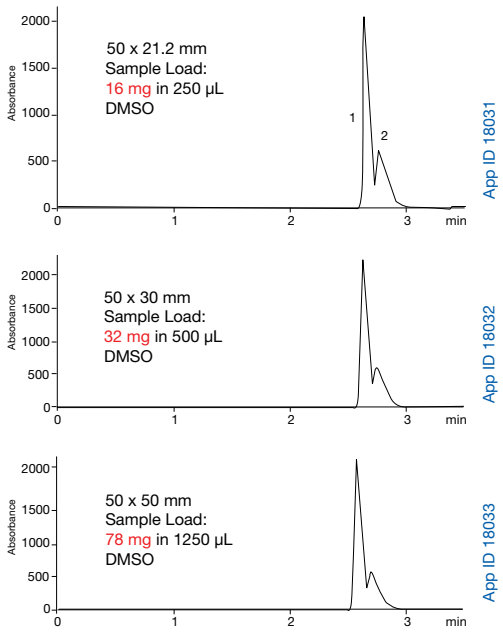
**Figure 4.**
Suzuki Reaction Purified on 21.2, 30 and 50 mm Diameter Axia Packed Columns




The needs and requirements of a laboratory chemist purifying 50 –100 mg compared to the process chemist producing 500 mg to multiple grams of a purified product are very different. The laboratory chemist is always under a restraint to produce multiple products or compounds and cannot spend the time developing and optimizing purification methods. The laboratory chemist has a large number of samples that must be purified and will continue to use generic gradients and UV or Mass based collection system. Process chemists purify fewer compounds per year but the purified compounds must be well above 95 % pure in 100 gram to kilogram quantities. To achieve these higher mass and purity requirements the separations must be optimized and repetitive runs and UV based fraction collection are common. Since the requirements of the two labs are very different, their choices and approaches in scaling up a separation are also very different. How the choices of column length and ID affect these different labs are shown in the following examples.

Initially a mixture of two compounds (propranolol and diphenhydramine) were separated on a 50 x 4.6 mm column using a generic gradient, the maximum load was determined and the separation was directly scaled up to the larger ID Axia packed columns – 21.2, 30 and 50 mm (**Figure 5**). By increasing only the column ID, the sample load increases exponentially [SL $\alpha$ $(D_2/D_1)^2$]. For the smaller 21.2 and 30 mm ID columns, the load was only 16 mg and 32 mg, respectively, but the purity of each fraction was the same as achieved on the larger 50 mm ID column for which the load was 78 mg. For the laboratory chemist who needs higher yields and still needs to separate a large number of compounds, increasing the column ID is recommended since the separation time and gradient conditions do not change.

**Figure 5.**
Increased Productivity and Throughput with No Loss of Performance with Axia Packed Columns




**Column:** Luna® 5 µm C18(2)
**Gradient:** 5 min, 5 % to 95 % Acetonitrile, 0.5 % TFA
**Flow Rate:** 30 mL/min on 21.2 mm column; 60 mL/min on 30 mm column; 150 mL/min on 50 mm column
**Detection:** UV @ 254 nm
**Sample:** 1. Propranolol 2. Diphenhydramine

The scale-up factors from a 4.6 mm diameter column are:

21.2 mm SF = 20X

30 mm SF = 40X

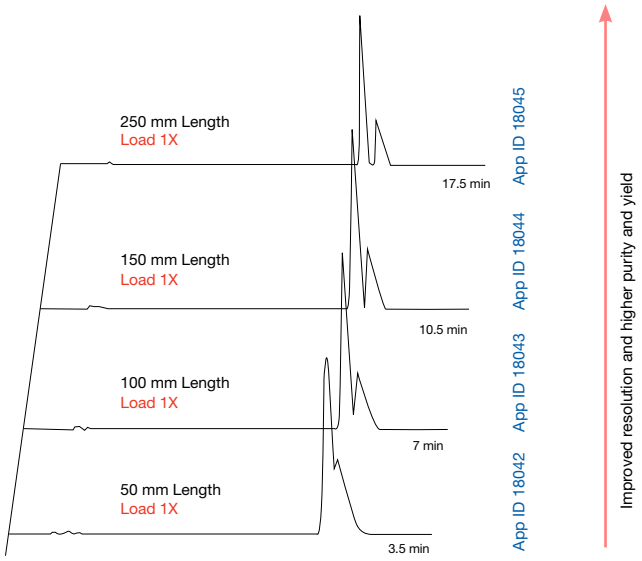
50 mm SF = 118X

The maximum load for each column must be adjusted based on the column diameter but each column provides the same purification capability and performance.

# APPLICATIONS

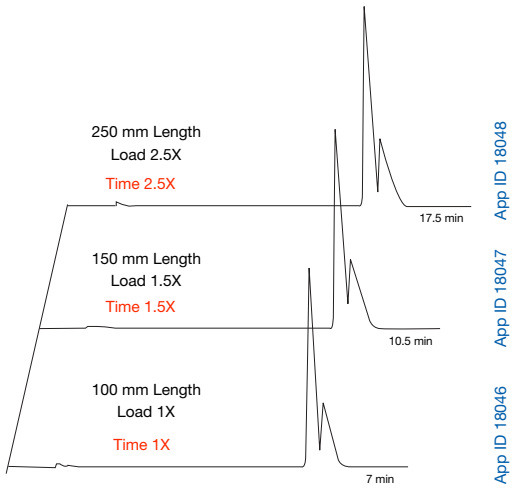
If higher purity product is required, then increasing the column length will achieve this goal as shown in **Figure 6**. The sample load was maintained as the column length increased. Increasing the column length improves resolution but the purification time and backpressure increases directly proportional to column length (Time = $L_2/L_1$ and Pressure = $L_2/L_1$). Since the gradient time must change proportionally to the increase in column length this results in an increase in the separation time for each sample. Increasing the column length may be a hindrance for the laboratory chemist as the instrumentation may not be capable of operating at the required higher backpressure and high flow rate.

**Figure 6.**
Impact of Column Length for Gradient Separations




For the kilo lab where purity and overall yield are critical requirements, optimizing gradient conditions along with increasing the column length allows higher sample load per run and reduces the number of cycles required to produce the needed material (**Figure 7**). In addition, the combination of fewer runs and higher sample loads results in fewer fractions to evaluate and pool. Although throughput (grams/hour) remains the same with increased column length, the more important requirements of very high purity and overall yield are achieved. Increasing the column length has the additional advantage to allow larger sample volumes before the dissolution solvent degrades the separation.

**Figure 7.**
Improving Productivity and Throughput–Relationship of Sample Load and Column Length




Increasing the column length and increasing the sample load proportional to the length reduces the number of runs or cycles and generates fewer fractions to evaluate. The longer column is also more forgiving with the injection volume. The throughput remains constant (grams/hour).

## Conclusion

**Table 1** summarizes the impact of changing column ID and length for the two different laboratories. Given the limitations typical in a chemist's lab environment it is always better to increase the column ID to achieve higher purity and higher yield. With the same length column, the separation time, gradient conditions and backpressure do not change and a large number of samples can still be processed per day. For the process chemist performing fewer separations, optimizing the gradient conditions is well worth the time and effort. Multiple runs are always required to produce the larger quantities of material and yield and higher purity are critical success factors. Increasing the column ID and length will help achieve the higher purity and yield while decreasing the number of runs required. This higher purity and yield can now be achieved without sacrificing performance as the columns increase in length or ID.

**Table 1.**
Purification Needs Comparison for Different Laboratories

| Variable | Combinatorial Chemist Medicinal Chemist | Pilot Scale Up Process Chemist |
|---|---|---|
| **Yield** | 10 – 20 mg | 100 mg – 1 kg |
| **Purity** | >80 % | >95 % |
| **Method Development** | None / Generic Gradient | Optimize Separation |
| **Speed** | Very Important | Less Important |
| **Throughput (g/hour)** | Less Important | Very Important |
| **Number of Samples** | High | Low |
| **Higher Yield** | Increase Column ID | Increase Column Length / ID |
| **Increase Purity** | Increase Column ID | Increase Column Length / ID |

# APPLICATIONS

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

The Axia™ technology has become the new industry standard for consistency in preparative columns with the same performance achieved from 4.6 mm analytical columns to 50 mm ID preparative columns. The high level of process control has resulted in columns with the same performance characteristics (plates per meter) for columns between 50 and 250 mm length. There has been a significant improvement in the asymmetry and efficiency across all lengths and IDs allowing chemists more flexibility to choose the appropriate column size to achieve their goals for increased purity and yield for their preparative purifications.

## Ordering Information

**5 µm Luna® C18(2) Axia™ Packed Columns**

| Part No. | Dimensions (mm) | Unit |
|---|---|---|
| 00B-4252-P0-AX | 50 x 21.2 | ea |
| 00D-4252-P0-AX | 100 x 21.2 | ea |
| 00F-4252-P0-AX | 150 x 21.2 | ea |
| 00G-4252-P0-AX | 250 x 21.2 | ea |
| 00B-4252-U0-AX | 50 x 30.0 | ea |
| 00D-4252-U0-AX | 100 x 30.0 | ea |
| 00F-4252-U0-AX | 150 x 30.0 | ea |
| 00G-4252-U0-AX | 250 x 30.0 | ea |
| 00B-4252-V0-AX | 50 x 50.0 | ea |

**10 µm Luna C18(2) Axia Packed Columns**

| Part No. | Dimensions (mm) | Unit |
|---|---|---|
| 00G-4253-P0-AX | 250 x 21.2 | ea |
| 00G-4253-U0-AX | 250 x 30.0 | ea |
| 00G-4253-V0-AX | 250 x 50 | ea |

Additional part numbers and media available upon request.

## SecurityGuard™ PREP System

*(Highly recommended for extending column lifetime)*
Protect your Axia Packed column and prolong its lifetime with SecurityGuard, the advanced HPLC guard cartridge system.

- Get full protection with minimal impact on your chromatographic results
- Contaminants are retained by an inexpensive, 15 x 21.2 or 15 x 30 mm ID disposable cartridge

## Ordering Information

**SecurityGuard™ PREP System**

| Part No. | Description | Unit |
|---|---|---|
| AJ0-8223 | SecurityGuard PREP HPLC Guard Cartridge Holder Kit, 21.2 mm ID, includes column coupler | ea |
| AJ0-8277 | SecurityGuard PREP HPLC Guard Cartridge Holder Kit, 30.0 mm ID, includes column coupler | ea |

**SecurityGuard™ PREP Cartridges**

| Part No. | Description | Unit |
|---|---|---|
| AJ0-7839 | SecurityGuard PREP Cartridge, C18 15 x 21.2 mm ID | ea |
| AJ0-8301 | SecurityGuard PREP Cartridge, C18 15 x 30 mm ID | ea |

guarantee

If Axia packed columns do not provide LONGER LIFETIME when used with SecurityGuard PREP, as compared to a competing column of the same particle size, phase and dimensions, send in your comparative data and the column within 45 days for a FULL REFUND.



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